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Análogos Monocetônicos de Curcumina:

Planejamento, Síntese e Atividades Antibacteriana e Antifúngica

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Tese apresentada como parte dos requisitos para obtenção do título de Doutor em Química, junto ao Programa de Pós-Graduação em Química, do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista “Júlio de Mesquita Filho”, Câmpus de São José do Rio Preto.

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“Se Deus te ajudou até aqui, por que Ele te abandonaria agora?”

Leandro Santos

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“Ando devagar
Porque já tive pressa
E levo esse sorriso
Porque já chorei demais
Hoje me sinto mais forte
Mais feliz, quem sabe
Só levo a certeza
De que muito pouco sei
Ou nada sei”

(SATER & TEIXEIRA, 1990)

RESUMO

A curcumina é um dicetônico natural com efeitos antimicrobianos promissores. Contudo, limitações farmacocinéticas e farmacodinâmicas dificultam os seus estudos clínicos. Uma das estratégias para otimizar as suas propriedades negativas é a substituição da β -dicetona pelo grupo monocarbonila, gerando curcuminoides monocetônicos (**MACs**). Nesse contexto, monocetônicos foram planejados, sintetizados e investigados contra bactérias e fungos de importância clínica, e contra uma bactéria de interesse agrônômico. Primeiro, **MACs 1–8** foram sintetizados e avaliados contra bactérias patogênicas humanas. **MAC 4** apresentou atividade potente contra *Mycobacterium tuberculosis* (concentração inibitória mínima (CIM) de 5,6 $\mu\text{mol/L}$). A avaliação citogenotóxica contra células humanas (MRC-5 e A549) demonstraram **MAC 4** como potencialmente seguro (índices de seletividade (IS) de 15,6 e 14,2, respectivamente). Além disso, **MAC 4** foi mais estável do que a curcumina (pH 7,4 e 37 °C) e foi capaz de perturbar o divisomo de *Bacillus subtilis*. Segundo, na busca por curcuminoides com acessibilidade sintética maior, monocetônicos simplificados (**SMACs 1–40**) foram planejados a partir de **MAC 4** e avaliados contra *M. tuberculosis*. **SMACs 30 e 34** (CIM 7,2 e 4,5 $\mu\text{mol/L}$, respectivamente) apresentaram atividade similar a **MAC 4**, porém eles demonstraram valores de IS maiores (IS 27,2 e 23,3, respectivamente) do que **MAC 4** e também foram ativos contra isolados resistentes de *M. tuberculosis* (3,8 $\mu\text{mol/L} \leq \text{CIM} \leq 81,4 \mu\text{mol/L}$). Terceiro, os efeitos dos **MACs 1–8** e **SMACs 1, 2, 4 e 5** foram avaliados contra o fitopatôgeno *Xanthomonas citri* subsp. *citri*. **MAC 4** foi o curcuminóide mais ativo (CIM 330 $\mu\text{mol/L}$) e ele reduziu a habilidade do *X. citri* induzir lesões de cancro cítrico sobre as folhas de *Citrus sinensis*. **MAC 4** disruptou o divisomo e diminuiu a atividade GTPásica da proteína FtsZ de *X. citri*. Quarto, na busca por curcuminoides mais potentes do que **MAC 4**, monocetônicos (**MACs 9–23**) baseados na estrutura de **MAC 4** foram sintetizados e avaliados contra *X. citri*. **MAC 22** (CIM 135 $\mu\text{mol/L}$) foi cerca de 2,4 vezes mais potente do que **MAC 4** e foi capaz de perturbar a membrana de *X. citri*. Quinto, **MACs 1–8 e 24–34** foram sintetizados e investigados contra fungos do gênero *Candida*. **MAC 8** apresentou atividade anti-*Candida* potente (7,8 $\mu\text{mol/L} \leq \text{CIM} \leq 31,2 \mu\text{mol/L}$), reduziu a adesão de *C. albicans* às células humanas, bem como inibiu o seu biofilme e demonstrou toxicidade baixa no modelo *Galleria mellonella* (DL₅₀ 300 $\mu\text{mol/kg}$). Sexto, na busca por curcuminoides mais potentes do que **MAC 8**, monocetônicos (**MACs 35–48**) baseados na estrutura de **MAC 8** foram sintetizados e avaliados contra *Candida*. **MAC 43** (CIM 15,2, 7,4, 15,2 e 7,4, $\mu\text{mol/L}$, respectivamente) apresentou atividade mais potente do que **MAC 8** contra *C. albicans*, *C. tropicalis*, *C. parapsilosis* e *C. krusei*, e

inibiu o biofilme de *C. albicans*. Em conjunto, os resultados indicaram o potencial antimicrobiano dos monocetônicos, com atividades potentes, bem como potencialmente seguros e estáveis. Além disso, esse trabalho corroborou o potencial da curcumina como uma estrutura privilegiada no planejamento de substâncias bioativas para o tratamento de infecções bacterianas e fúngicas.

Palavras-chave: Curcumina. Simplificação molecular. Tuberculose. Cancro cístico. Candidíase.

ABSTRACT

Curcumin is a natural dicarbonyl with promising antimicrobial effects. However, pharmacokinetic and pharmacodynamic limitations hinder its clinical studies. One of the strategies to overcome its negative properties is the replacement of β -diketone by the monocarbonyl group, providing the monocarbonyl curcuminoids (**MACs**). In this context, monocarbonyl curcuminoids were designed, synthesized and investigated against clinically important bacteria and fungi, and also against a phytopathogenic bacteria. First, **MACs 1–8** were synthesized and evaluated against human pathogenic bacteria. **MAC 4** showed potent activity against *Mycobacterium tuberculosis* (minimum inhibitory concentration (MIC) 5.6 $\mu\text{mol/L}$). Cyto-genotoxic evaluation against human cells (MRC-5 and A549) demonstrated **MAC 4** as potentially safe (selectivity indexes (SI) 15.6 and 14.2, respectively). In addition, it was more stable than curcumin (pH 7.4 and 37 °C), and it disrupted the *Bacillus subtilis* divisome. Second, in the search for curcuminoids with greater synthetic accessibility, simplified monocarbonyl analogs (**SMACs 1–40**) were designed from **MAC 4** framework and evaluated against *M. tuberculosis*. **SMACs 30** and **34** (MIC 7.2 and 4.5 $\mu\text{mol/L}$, respectively) showed similar activity to **MAC 4**, but they showed higher SI values (IS 27.2 and 23.3, respectively) than **MAC 4**, and they were also active against drug-resistant *M. tuberculosis* isolates (3.8 $\mu\text{mol/L} \leq \text{MIC} \leq 81.4 \mu\text{mol/L}$). Third, the **MACs 1–8** and **SMACs 1, 2, 4** and **5** effects were evaluated against *Xanthomonas citri* subsp. *citri*. **MAC 4** was the most active curcuminoid (MIC 330 $\mu\text{mol/L}$), and it was able to reduce the ability of *X. citri* to induce citrus canker lesions on the *Citrus sinensis* leaves. **MAC 4** has the ability to perturb *X. citri* cell division by disrupting the divisome and inhibiting the GTPase activity of FtsZ. Fourth, in the search for curcuminoids more potent than **MAC 4**, monocarbonyl analogs (**MACs 9–23**) based on the **MAC 4** structure were synthesized and evaluated against *X. citri*. **MAC 22** (MIC 135 $\mu\text{mol/L}$) was about 2.4 times more potent than **MAC 4**, and it was able to disturb the *X. citri* membrane. Fifth, **MACs 1–8** and **24–34** were synthesized and investigated against *Candida*. **MAC 8** showed potent anti-*Candida* activity (7.8 $\mu\text{mol/L} \leq \text{MIC} \leq 31.2 \mu\text{mol/L}$), it decreases *C. albicans* cell adhesion and biofilm, and it demonstrated low toxicity in *Galleria mellonella* model (LD₅₀ 300 $\mu\text{mol/kg}$). Sixth, in the search for curcuminoids more potent than **MAC 8**, monocarbonyl analogs (**MACs 35–48**) based on the **MAC 8** structure were synthesized and evaluated against *Candida*. **MAC 43** (MIC 15.2, 7.4, 15.2 and 7.4 $\mu\text{mol/L}$, respectively) showed more potent activity than **MAC 8** against *C. albicans*, *C. tropicalis*, *C. parapsilosis* and *C. krusei*, and it inhibited *C. albicans* biofilm. Taken together, the results indicated that monocarbonyl curcuminoids were potent,

safe and stable. In addition, this work confirmed the potential of curcumin as a privileged structure for the design of bioactive compounds for the treatment of bacterial and fungal infections.

Keywords: Curcumin. Molecular simplification. Tuberculosis. Citrus canker. Candidiasis.

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LISTA DE ABREVIATURAS E SÍMBOLOS

δ_H : deslocamento químico de hidrogênio

δ_C : deslocamento químico de carbono treze

Ab: *Acinetobacter baumannii*

AMP B: anfotericina B (*amphotericin B*)

ATCC: *American Type Culture Collection*

BBBP: *blood-brain barrier penetration*

BMI: *bacterial metabolism inhibition*

Bs: *Bacillus subtilis*

Ca: *Candida albicans*

CBM (MBC): concentração bactericida mínima (*minimum bactericidal concentration*)

CFU: *colony forming unit*

Cg: *Candida glabrata*

CIM (MIC): concentração inibitória mínima (*minimum inhibitory concentration*)

Ck: *Candida krusei*

Cp: *Candida parapsilosis*

CNS: *central nervous system*

Ct: *Candida tropicalis*

CUR: curcumina (*curcumin*)

d: duplete

dd: duplo duplete

ddd: duplo duplo duplete

Ec: *Escherichia coli*

Ef: *Enterococcus faecalis*

FCZ: fluconazol (*fluconazole*)

FDA: *Food and Drug Administration*

FICI: *fractional inhibitory concentration index*

FtsZ: *filamentous temperature-sensitive protein Z*

GDF: *guanosina difosfato*

GFP: *green fluorescent protein*

GRAS: *generally recognized as safe*

GTP: *guanosine triphosphate*

HBA: *number of hydrogen bond acceptors*

HBD: *number of hydrogen bond donors*

HIB: *human intestinal absorption*

HLB: *huanglongbing*

Hz: *Hertz*

IC₅₀: *concentration required in order to reduce cell viability at 50%*

IMPS: *invalid metabolic panaceas*

IS (SI): *índice de seletividade (selectivity index)*

IP: *propidium iodide*

J: *constante de acoplamento*

Log P: *lipophilicity*

Log S: *water solubility*

MAC: *análogos monocetônicos de curcumina (monocarbonyl analogs of curcumin)*

MDR: *multidrug resistant*

MOM: *grupo protetor metoximetila*

MR: *molar refractivity*

Mt: *Mycobacterium tuberculosis*

MW: *molecular weight*

NA: *number of atoms*

NC: *negative control*

NHA: *number of heteroatoms*

NR: *number of rings*

NROTB: *number of rotatable bonds*

NYG: *nitrogen yeast glicerol*

OD: *optical density*

Pa: *Pseudomonas aeruginosa*

PAINS: *pan-assay interference compounds*

PC: *positive control*

ppm: *parte por milhão*

REMA: *resazurin microtiter assay plate*

RMN (NMR): *ressonância nuclear magnética (nuclear magnetic resonance)*

s: *simpleto*

SAR: *structure-activity relationship*

SARM (MRSA): *Staphylococcus aureus* resistente à meticilina (*methicillin-resistant Staphylococcus aureus*)

SASM (MSSA): *Staphylococcus aureus* sensível à meticilina (*methicillin-sensitive Staphylococcus aureus*)

SCUR: *curcumina simplificada (simplified curcumin)*

Se: *Salmonella enterica*

SMAC: *análogos monocetônicos simplificados de curcumina (simplified monocarbonyl analogs of curcumin)*

t: *triplete*

TPSA: *topological polar surface area*

VC: *vehicle control*

ZapA: *Z ring associated protein A*

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1. CAPÍTULO I

Considerações Iniciais, Revisão Bibliográfica e Objetivos

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1.1. CONSIDERAÇÕES INICIAIS

Os produtos naturais são fontes de protótipos para a pesquisa e o desenvolvimento de fármacos e substâncias bioativas. Nesse contexto, o Laboratório de Antibióticos e Quimioterápicos (LAQ) do Instituto de Biociências, Letras e Ciências Exatas – UNESP/São José do Rio Preto, inspira-se na estrutura de produtos naturais derivados de plantas para o desenvolvimento de novas substâncias bioativas. A curcumina, uma substância dicetônica, foi utilizada nesse trabalho como um protótipo para o planejamento de novos agentes antimicrobianos. Ela é isolada dos rizomas de *Curcuma longa* e apresenta um espectro amplo de atividades biológicas, com destaque para os seus efeitos antibacteriano e antifúngico. Contudo, fatores como instabilidade química e fotoquímica, solubilidade aquosa baixa e biodisponibilidade oral baixa dificultam o seu avanço em estudos farmacêuticos e clínicos. Uma das estratégias utilizadas para superar essas limitações é o planejamento de análogos simplificados monocetônicos. Essa classe de substâncias tem demonstrado parâmetros físico-químicos e farmacocinéticos adequados, além de apresentar atividade biológica superior à curcumina em vários estudos.

Nessa perspectiva, monocetônicos foram planejados, sintetizados e avaliados contra bactérias de interesse clínico, incluindo *Mycobacterium tuberculosis*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus*, *S. epidermidis* e *Enterococcus faecalis*, e contra uma bactéria de interesse agrônomo, *Xanthomonas citri* subsp. *citri* (*X. citri*). Além disso, os monocetônicos também foram avaliados contra fungos do gênero *Candida*, incluindo as espécies *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis* e *C. krusei*.

A tese foi organizada em oito Capítulos (I–VIII). O **Capítulo I** contém uma revisão bibliográfica para contextualizar a importância da simplificação molecular de produtos naturais no desenvolvimento de fármacos e substâncias bioativas, bem como um levantamento sobre a curcumina, o qual inclui suas atividades e modo de ação contra bactérias e fungos, as limitações que impedem o seu avanço em estudos clínicos e as estratégias utilizadas para otimizar suas propriedades negativas, como o planejamento de análogos monocetônicos. Foram apresentados, no final desse capítulo, o objetivo geral da tese e os objetivos específicos dos Capítulos II–VII.

Os **Capítulos II e III** contêm os manuscritos obtidos a partir dos estudos de monocetônicos contra bactérias de interesse clínico, enquanto os **Capítulos IV e V** abrangem as investigações dos monocetônicos contra o fitopatógeno *X. citri*. Já os **Capítulos VI e VII**

são compostos pelos trabalhos resultantes das avaliações dos análogos monocetônicos contra fungos do gênero *Candida*. Por fim, o **Capítulo VIII** apresenta as conclusões gerais dos estudos com os curcuminoides monocetônicos contra bactérias e fungos, além de conter as referências e apêndices dos Capítulos II, III, V, VI e VII.

1.2. REVISÃO BIBLIOGRÁFICA

1.2.1. Simplificação Molecular de Produtos Naturais

Os produtos naturais, em especial os derivados de plantas, são fontes de protótipos para a descoberta e o desenvolvimento de novos fármacos e substâncias bioativas (BERNARDINI et al., 2018; THOMFORD et al., 2018). Um levantamento sobre as fontes dos fármacos aprovados no período de 1981 a 2019 indicou que mais de 65 % dos antimicrobianos (antibacterianos, antifúngicos, antiparasitários e antivirais) foram originados de produtos naturais e seus derivados, bem como de substâncias sintéticas baseadas em naturais (farmacóforos de produtos naturais), confirmando a importância da pesquisa em Química de Produtos Naturais para o desenvolvimento de novos agentes antimicrobianos (NEWMAN & CRAGG, 2020). Essa relevância foi reconhecida recentemente pelo laureamento da pesquisadora Youyou Tu com o Prêmio Nobel de Fisiologia e Medicina de 2015. Ela estudou a ação da artemisinina, uma substância isolada da *Artemisia annua*, contra o parasita causador da malária, *Plasmodium falciparum* (SU & MILLER, 2015).

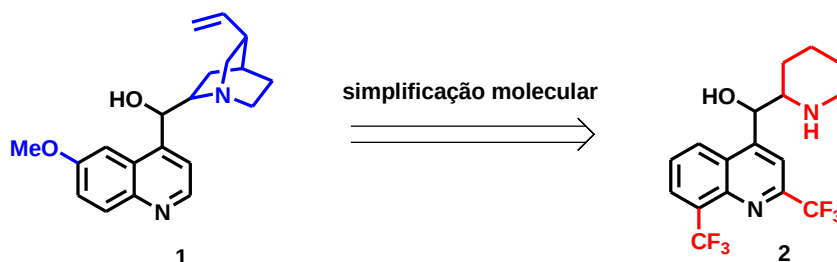
Durante o desenvolvimento de fármacos a partir de substâncias naturais, pode ser necessário a otimização de parâmetros farmacocinéticos, propriedades físico-químicas, estabilidade, toxicidade e potência dos protótipos (BAUER & BRÖNSTRUP, 2014; ATANASOV et al., 2015). Além disso, a complexidade estrutural de alguns produtos naturais dificulta o avanço nas etapas de desenvolvimento, uma vez que a obtenção sintética dessas substâncias pode requerer rotas com muitas etapas (PETERSON & OVERMAN, 2004; ROCHE & PORCO JR, 2011; BUESCHLEB et al., 2016).

Uma das estratégias utilizadas pela Química Medicinal na otimização de produtos naturais é a simplificação molecular (BARREIRO, 2002). Esta visa à remoção de fragmentos estruturais que podem estar relacionados à toxicidade, labilidade, centros estereogênicos ou sítios de metabolização nos protótipos (MAGEDOV et al., 2008; MAGEDOV et al., 2012; LIU et al., 2018a; MORÃO et al., 2019). Essa ferramenta também permite a identificação de subunidades essenciais para a atividade biológica, possibilitando o planejamento de análogos que podem ser sintetizados empregando rotas mais simples (BARREIRO, 2002; MIGUELEZ et al., 2013).

A simplificação, antes empregada empiricamente, hoje é uma das principais ferramentas de planejamento racional na “domesticação” de produtos naturais, visando, sobretudo, a obtenção de substâncias estruturalmente mais simples (BARREIRO, 2002; HE et al., 2017; STEADMAN et al., 2017; LIU et al., 2018b). Um exemplo de sucesso no uso dessa

estratégia é o fármaco mefloquina, que foi obtido por meio da simplificação molecular da quinina (**1**), um alcaloide quinolínico isolado da casca de *Cinchona officinalis* (GRIGG et al., 2016; LILES et al., 2016). O antimalárico mefloquina (**2**) foi obtido a partir da substituição do anel rubânico da quinina pelo piperidínico, estruturalmente mais simples (Figura 1). Além disso, o grupo metoxila foi retirado e inserido dois grupos trifluorometila no anel quinolínico (BARREIRO et al., 2002).

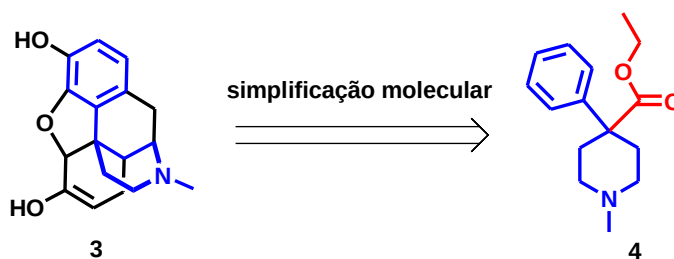
Figura 1. Simplificação molecular da quinina (**1**) para a obtenção da mefloquina (**2**).



Fonte: Elaborado pelo autor

Outro fármaco concebido pela simplificação de uma substância natural foi o analgésico petidina (BURCHELL, COSTER & NORMAN, 2016). Este foi obtido a partir da morfina (**3**), um alcaloide isolado da *Papaver somniferum* (SCHUURMANS et al., 2015). A petidina (**4**) apresenta um anel piperidínico *N*-metilado similar àquele contido na morfina, que é o farmacóforo dos analgésicos opiáceos (Figura 2).

Figura 2. Simplificação molecular da morfina (**3**) para a obtenção da petidina (**4**).

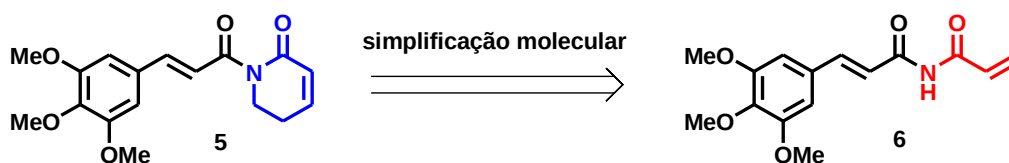


Fonte: Elaborado pelo autor

Além de fármacos, há diversas substâncias bioativas planejadas pela simplificação de produtos naturais (STEADMAN et al, 2017; VALLI et al., 2017; MORÃO et al., 2019). O análogo simplificado **6** foi planejado a partir da abertura do anel piperidônico da piperlongumina (**5**), uma alcalamida isolada de várias espécies de *Piper* (Piperaceae) (Figura 3).

A substância **6** apresentou atividade citotóxica potente contra linhagens celulares de câncer de mama, além de demonstrar seletividade alta (VALLI et al., 2017).

Figura 3. Simplificação molecular da piperlongumina (**5**) para a obtenção do análogo **6** (VALLI et al., 2017).

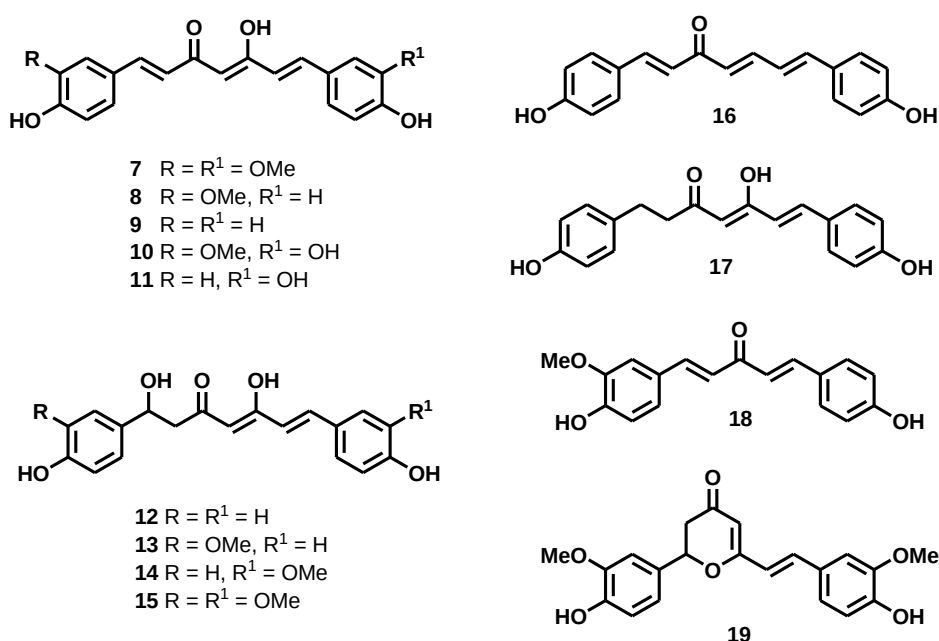


Fonte: Elaborado pelo autor

1.2.2. Curcumina e seus Análogos Simplificados Monocetônicos

O rizoma da *Curcuma longa* (Zingiberaceae), que é conhecido popularmente como açafrão, tumérico ou cúrcuma, tem sido amplamente utilizado como condimento alimentar, pois confere sabor levemente apimentado e coloração amarela intensa (HOSSEINI & HOSSEINZADEH, 2018). Os principais constituintes químicos do rizoma incluem sesquiterpenos oxigenados e curcuminoides (**7–19**) (Figura 4), que são predominantes nas frações volátil e não-volátil, respectivamente (SUETH-SANTIAGO et al., 2015).

Figura 4. Estrutura de alguns curcuminoides (**7–19**) isolados do rizoma de *C. longa* (ANAND et al., 2008; LI et al., 2009).

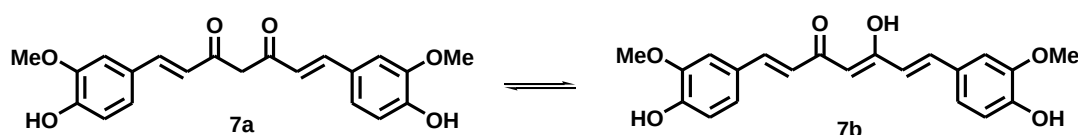


Fonte: Elaborado pelo autor

O rizoma de *C. longa* apresenta diversas propriedades medicinais. Na medicina tradicional indiana e chinesa, esse material vegetal tem sido empregado no tratamento de distúrbios hepáticos, doenças reumáticas e infecções cutâneas (MIQUEL et al., 2002). No Brasil, o fitoterápico Motore[®] é produzido com o extrato seco do rizoma (ACHÉ, 2020). Esse medicamento possui curcuminoides que formam complexos com a fostatidilcolina, o que melhora a sua biodisponibilidade (MIRZAEI et al., 2017). As indicações terapêuticas do Motore[®] incluem o tratamento da osteoartrite e artrite reumatoide, além de apresentar ação anti-inflamatória e antioxidante (ACHÉ, 2020). Estudos correlacionam a polifarmacologia do extrato seco à presença do conjunto de curcuminoides, especialmente, à curcumina (**7**), substância majoritária no rizoma, que equivale, aproximadamente, a 2 % do seu peso seco (JURENKA et al., 2009; PULIDO-MORAN et al., 2016; HEWLINGS & KALMAN, 2017; KOCAADAM & ŞANLIER, 2017).

A curcumina foi isolada primeiramente em 1815 e sua estrutura determinada em 1910 (HESARI et al., 2018). Essa substância é caracterizada por dois anéis guaiacólicos espaçados por uma β -dicetona *bis- α,β'* -insaturada, com um sistema de elétrons π conjugado, que é responsável por sua capacidade de absorver luz visível na região de 408 a 500 nm (KHOR et al., 2019). O fragmento β -dicetônico exibe tautomerismo ceto-enólico (Figura 5). Embora a predominância do tautômero cetônico (**7a**) em soluções ácidas e neutras tenha sido reportada (ANAND et al., 2008), estudos de Ressonância Magnética Nuclear (RMN) utilizando vários solventes (DMSO-*d*₆, D₂O:DMSO-*d*₆ [3:7], CD₃OD, CD₃COOD e CDCl₃) e diferentes valores de pH (3 a 9), indicaram que a forma enólica (**7b**) foi a predominante em todas as condições avaliadas (PAYTON, SANDUSKY & ALWORTH, 2007). O tautômero **7b** prevalece devido à sua estabilidade maior, uma vez que apresenta uma estrutura planar que permite a conjugação entre as nuvens de elétrons π das duas unidades vinil-guaiacólicas (KOLEV et al., 2005; PRIYADARSINI, 2009; JAGANNATHAN, ABRAHAM & PODDAR, 2012).

Figura 5. Tautomerismo ceto-enólico da curcumina: forma cetônica (**7a**) e forma enólica (**7b**).



Fonte: Elaborado pelo autor

A curcumina tem sido reconhecida pelos seus efeitos pleiotrópicos devido à sua capacidade de regular diversas vias de sinalização celular (WANG et al., 2015; LUTHRA & LAL, 2016), resultando em um espectro amplo de atividades biológicas, incluindo antitumoral (DOELLO et al., 2018), anti-inflamatório (TIZABI et al., 2014), antioxidante (MALIK & SINGH, 2017) e antiviral, incluindo potencial ação contra coronavírus 2 (SARS-CoV-2) (CHEN et al., 2020; ZAHEDIPOUR et al., 2020). Além disso, estudos clínicos reportaram a sua segurança para humanos em doses elevadas (12 g/dia) (LAO et al., 2006; JAMES et al., 2015), sendo classificada como GRAS (*generally recognized as safe*) pela FDA (*Food and Drug Administration*) (FADUS et al., 2017). O grande potencial farmacológico e a toxicidade baixa fazem desse produto natural um esqueleto privilegiado na pesquisa e no desenvolvimento de novos fármacos e substâncias bioativas (DI MARTINO et al., 2016; KUNNUMAKKARA et al., 2017).

Dentre as atividades biológicas, a ação antimicrobiana da curcumina se destaca, sobretudo contra bactérias (MOGHADAMTOUSI et al., 2014). Vários estudos *in vitro* descreveram o seu espectro abrangente contra espécies Gram-positivas, Gram-negativas e micobacterianas (MOGHADAMTOUSI et al., 2014; MARINI et al., 2018). Além disso, investigações *in vivo* têm comprovado os seus efeitos, corroborando o potencial da curcumina como um agente antibacteriano promissor. De e colaboradores relataram que uma dose diária de 25 mg/kg desse produto natural ao longo 7 dias eliminou a bactéria *Helicobacter pylori* do estômago de camundongos doentes (DE et al., 2009). Já a administração subcutânea de curcumina (100 mg/kg) a cada 8 h durante 3 dias diminuiu a mortalidade de ratos infectados com cepas de *Staphylococcus aureus* sensível e resistente à meticilina (SASM e SARM, respectivamente) (WANG et al., 2016).

O modo de ação da curcumina tem sido relatado como uma combinação entre a inibição da divisão celular (RAI et al., 2008) e a ruptura da membrana citoplasmática (TYAGI et al., 2015). A capacidade de afetar a divisão celular foi correlacionada com a sua habilidade de inibir a montagem dos protofilamentos e aumentar a atividade GTPásica da proteína FtsZ (*filamentous temperature-sensitive protein Z*) (RAI et al., 2008). O curcumina também foi capaz de se ligar a essa proteína, alterando a sua estrutura secundária (RAI et al., 2008; KAUR et al., 2010). Já a ação sobre a membrana foi atribuída a sua anfipatia e lipofilia alta, características que permitem a sua inserção entre as bicamadas lipídicas, aumentando a permeabilidade e causando danos às biomembranas (VARSHNEY et al., 2013; TYAGI et al., 2015).

A curcumina também apresentou atividade contra gêneros fúngicos leveduriformes e filamentosos que causam infecções em humanos, como *Candida*, *Cryptococcus*, *Aspergillus*, *Trichophyton* e *Microsporum* (MARTINS et al., 2008; BRASCH et al., 2017). Khan e colaboradores demonstraram o efeito da curcumina contra espécies de *Candida* sensíveis e resistentes ao fluconazol, apresentando valores de CIM (concentração inibitória mínima) entre 250–650 µg/mL (KHAN et al., 2012). Além disso, a curcumina (200 µg/mL) reduziu a atividade metabólica do biofilme pré-formado de *C. albicans* (ALALWAN et al., 2017), apresentou efeito sinérgico com o voriconazol contra espécies de *Aspergillus* (PASRIJA & KUNDU, 2018), e reduziu a viabilidade do biofilme de *C. dubliniensis* quando utilizada como agente fotossensibilizante (20 µmol/L) na terapia fotodinâmica (SANITÁ et al., 2018).

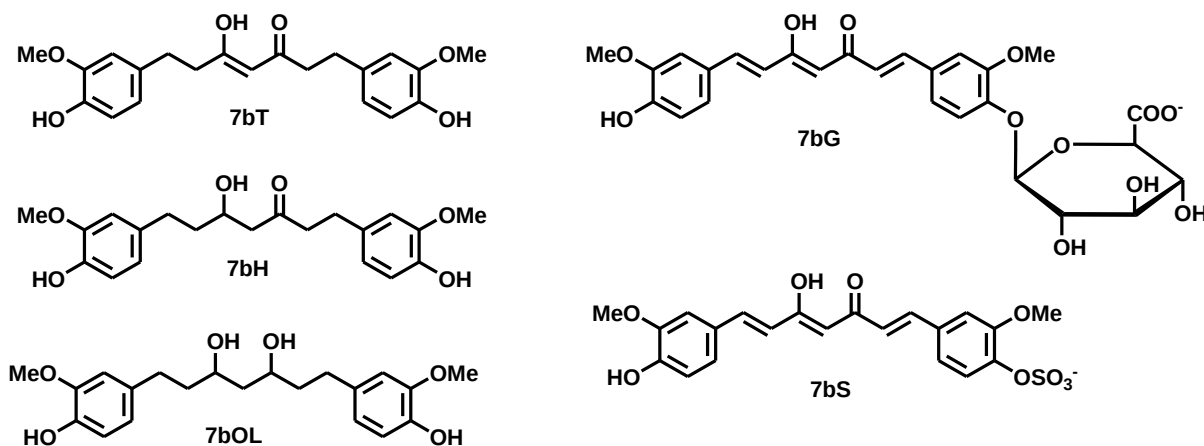
Fernandes e colaboradores reportaram em um estudo pré-clínico que a aplicação da curcumina incorporada em um creme vaginal (0,01%, m/m) foi capaz de reduzir o número de ratas infectadas com *C. albicans* após 6 dias de tratamento. Os resultados também mostraram que a formulação foi estável, e a análise histológica demonstrou que as células do epitélio vaginal não foram danificadas pelo tratamento, corroborando que o creme vaginal contendo curcumina pode ser considerado um antifúngico eficaz e potencialmente seguro no tratamento da candidíase vulvovaginal (FERNANDES et al., 2018).

As investigações sobre o modo de ação desse curcuminoide contra fungos vêm descrevendo uma ação multialvos, incluindo (i) a indução de apoptose pela geração de espécies reativas de oxigênio (SHARMA et al., 2010), (ii) aumento na permeabilidade e dissipação do potencial da membrana citoplasmática (LEE & LEE, 2014), (iii) efeito sobre a biossíntese do ergosterol por meio da inibição da esterol- $\Delta^{5,6}$ -desaturase (ERG3) (SHARMA et al., 2012), e (iv) a interferência na biossíntese e integridade da parede celular (KUMAR et al., 2014).

Embora a curcumina seja um agente antibacteriano e antifúngico promissor, fatores como a sua solubilidade em água baixa e má absorção, dificultam o avanço dos estudos farmacêuticos e clínicos (ANAND et al., 2007; NELSON et al., 2017; TODEN & GOEL, 2017). Além disso, esse curcuminoide sofre metabolismo de primeira passagem no fígado, contribuindo, sobremaneira, para a sua eliminação sistêmica rápida e, consequentemente, biodisponibilidade oral baixa (SHARMA, GESCHER & STEWARD, 2005). De acordo com Siviero e colaboradores, os metabólitos reduzidos da curcumina foram os principais após as reações de fase I, entre eles a tetrahidrocurcumina (**7bT**), hexahidrocurcumina (**7bH**) e o hexahidrocurcuminol (**7bOL**), os quais, incluindo a curcumina, foram facilmente conjugados

com glicuronídeo (**7bG**) ou sulfato (**7bS**) nas reações de fase II do metabolismo (Figura 6) (SIVIERO et al., 2015).

Figura 6. Principais metabólitos da curcumina após as reações de fase I e II do metabolismo (ASAI & MIYAZAWA, 2000; ANAND et al., 2007).

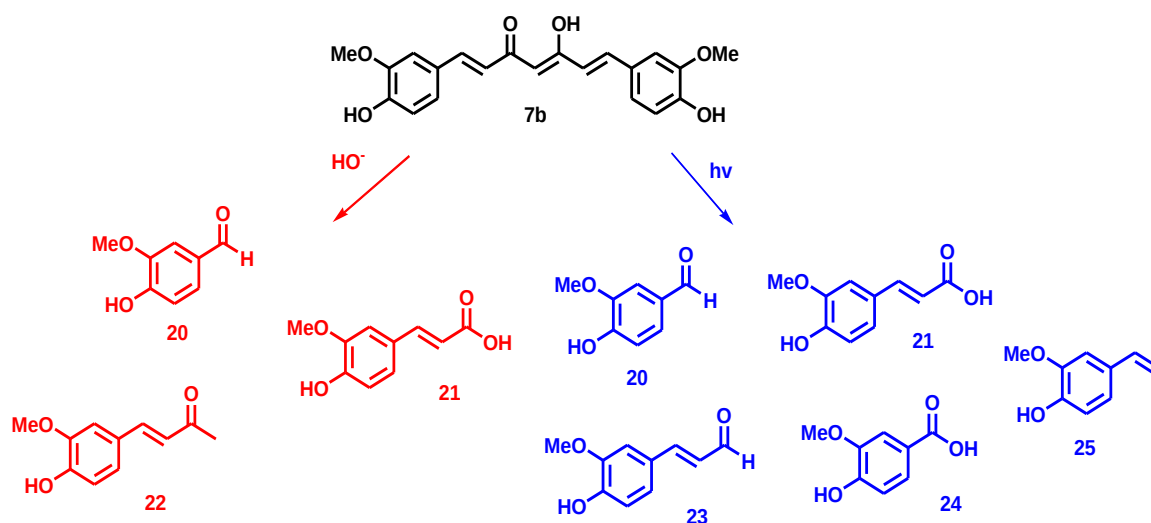


Fonte: Elaborado pelo autor

Outros fatores que limitam o uso farmacêutico da curcumina são as suas instabilidades química e fotoquímica (KHARAT et al., 2017). Embora seja relativamente mais estável em meio ácido (GRIESSER et al., 2011), ela é degradada rapidamente em soluções alcalinas (NELSON et al., 2017). O nosso grupo demonstrou que em soluções tamponadas de pH 5,0 e 7,4 (37 °C), a concentração de curcumina foi reduzida cerca de 52 e 63 %, respectivamente, após 24 h de monitoramento (POLAQUINI et al., 2019). Wang e colaboradores descreveram que os principais produtos de degradação desse curcuminoide sob condição alcalina (pH 7,2 e 37 °C) foram a vanilina (**20**), ácido ferúlico (**21**) e a desidrozingerona (**22**) (Figura 7) (WANG et al., 1997).

Já a cinética e a abundância relativa dos produtos gerados pela sua fotodegradação foram dependentes da temperatura e dos solventes utilizados na extração, cristalização e armazenamento, quando em solução. As principais substâncias formadas nesse processo foram **20**, **21**, aldeído ferúlico (**23**), ácido vanílico (**24**) e 4-vinil-guaiacol (**25**) (Figura 7) (HEGER et al., 2014; CASTILLO et al., 2015). Algumas revisões atribuem os efeitos pleiotropicos da curcumina ao conjunto de substâncias formadas durante o seu metabolismo ou oriundas da sua degradação (HEGER et al., 2014; NELSON et al., 2017).

Figura 7. Principais produtos de degradação (**20–25**) da curcumina (HEGER et al., 2014; NELSON et al., 2017).



Fonte: Elaborado pelo autor

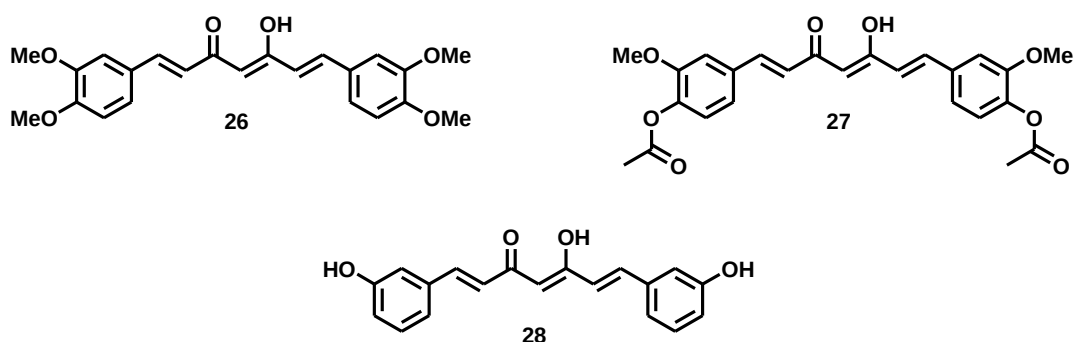
Estratégias vêm sendo empregadas para otimizar a potência, solubilidade em água, biodisponibilidade oral e a estabilidade da curcumina, como o seu encapsulamento em lipossomas, micelas e nanoemulsões (HUSSAIN et al., 2017; ARTIGA-ARTIGAS, LANJARI-PÉREZ & MARTÍN-BELLOSO, 2018). Esses carreadores melhoram a estabilidade, solubilidade aquosa, absorção e segurança (MAHRAN et al., 2017). Outra abordagem é o uso do curcuminoide com adjuvantes (MOORTHY & KATHIRESAN, 2013; MAHRAN et al., 2017), como a quercetina, resveratrol ou a piperina. Esta inibe a glicuronidação em células hepáticas e intestinais, aumentando a biodisponibilidade oral da curcumina (SHOBA et al., 1998; SRINIVASAN, 2007). Sharma e colaboradores mostraram que ratos infectados com *C. albicans* apresentaram uma redução maior na carga fúngica com o tratamento oral combinado de curcumina (100 mg/kg) e piperina (20 mg/kg) do que apenas com a administração intraperitoneal do curcuminoide (100 mg/kg) (SHARMA et al., 2010).

Além das estratégias citadas anteriormente, o planejamento de derivados e análogos sintéticos tem sido investigado extensamente para otimizar parâmetros que limitam o uso da curcumina (BADAVATH et al., 2015; JORDAN et al., 2018; RAMYA et al., 2018). Estudos têm reportado o aumento na potência antitumoral e na estabilidade metabólica da curcumina pela *O*-metilação das suas hidroxilas *p*-fenólicas (**26**) (Figura 8) (TAMVAKOPOULOS et al., 2007; PATWARDHAN et al., 2011; TEYMOURI et al., 2018). Motivados pelos resultados das pesquisas com o análogo **26**, bem como pelas investigações que descreveram a potencialização

de substâncias fenólicas a partir da sua acetilação (BEAUMONT et al., 2003), o nosso grupo vem estudando a ação antibacteriana do derivado diacetilcurcumina (**27**) (Figura 8). Nós descrevemos que essa substância foi mais potente do que a curcumina contra células planctônicas de *Streptococcus mutans*, SASM e SARM. Além disso, o derivado apresentou ação equivalente à vancomicina contra os biofilmes de SASM e SARM, reduziu significativamente o biofilme de *S. mutans* quando empregado como agente fotossensibilizante na terapia fotodinâmica, e ele demonstrou toxicidade baixa contra células de fibroblastos humano e larvas de *Galleria mellonella* (SARDI et al., 2017a; SANCHES et al., 2019).

Nós também identificamos que o análogo 3,3'-dihidroxicurcumina (**28**) (Figura 8) foi mais estável do que a curcumina em soluções tamponadas de pH 5,0 e 7,4 (37 °C), além de apresentar potência superior ao produto natural contra *Enterococcus faecalis*, *S. aureus* e *Mycobacterium tuberculosis*, sem exibir citogenotoxicidade significativa contra células humanas (POLAQUINI et al., 2019). Em conjunto, nossos resultados anteriores corroboram a hipótese de que a retirada do grupo *p*-hidroxila da curcumina, bem como a sua *O*-metilação ou *O*-acetilação resultam em substâncias mais potentes e/ou estáveis do que o curcuminóide natural.

Figura 8. Derivados e análogos difenileptanóides (**26–28**) mais potentes e/ou estáveis do que a curcumina (SARDI et al., 2017a; TEYMOURI et al., 2018; POLAQUINI et al., 2019).



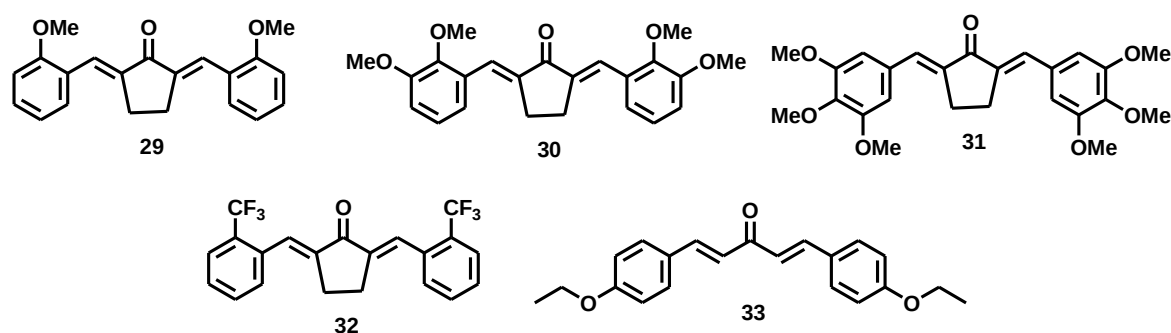
Fonte: Elaborado pelo autor

Ainda que alguns derivados ou análogos contendo o fragmento β -dicetônico, o qual é chamado de “coração da curcumina” (SHETTY et al., 2015), tenham demonstrado vantagens em relação ao protótipo natural, muitos estudos *in vitro* e *in vivo* relacionam essa subunidade à instabilidade química e metabólica da curcumina (ZHAO, LIU & LIANG, 2013; SHETTY et al., 2015). A β -dicetona é instável em pH $\geq 6,5$, sendo hidrolisada rapidamente (HEGER et al., 2014). Além disso, estudos *in vivo* reportaram que essa porção é um substrato específico de

várias enzimas aldo-ceto redutases (ROSEMOND et al., 2004; GROGAN et al., 2005). A subunidade β -dicetona também confere à curcumina características relacionadas a PAINS (*pan-assay interference compounds*) e IMPS (*invalid metabolic panaceas*), como a quelação de metais e a degradação rápida (BAELL et al., 2016; GLASER & HOLZGRABE, 2016; NELSON et al., 2017). Essas denominações são atribuídas a protótipos que apresentam um desempenho baixo como substâncias líderes, uma vez que suas atividades em vários ensaios biológicos podem estar relacionadas à interferência no método de análise, não pela interação específica com um alvo (NELSON et al., 2017).

Vários estudos reportaram que análogos simplificados monocetônicos, os quais são planejados pela substituição da porção β -dicetona pelo grupo monocarbonila, foram mais estáveis *in vitro* e apresentaram parâmetros farmacocinéticos melhores do que a curcumina (ZHAO, LIU & LIANG, 2013; SHETTY et al., 2015). Liang e colaboradores monitoraram durante 75 h a estabilidade química de análogos monocetônicos em solução alcalina (pH 7,4 e 37 °C). Os vinte análogos avaliados foram mais estáveis do que a curcumina, com destaque para as substâncias **29–33** (Figura 9), que apresentaram um decaimento em relação à concentração inicial menor do que 20 %, enquanto o curcuminóide natural reduziu 64 % (LIANG et al., 2009).

Figura 9. Análogos simplificados monocetônicos (**29–33**) mais estáveis do que a curcumina (LIANG et al., 2009).

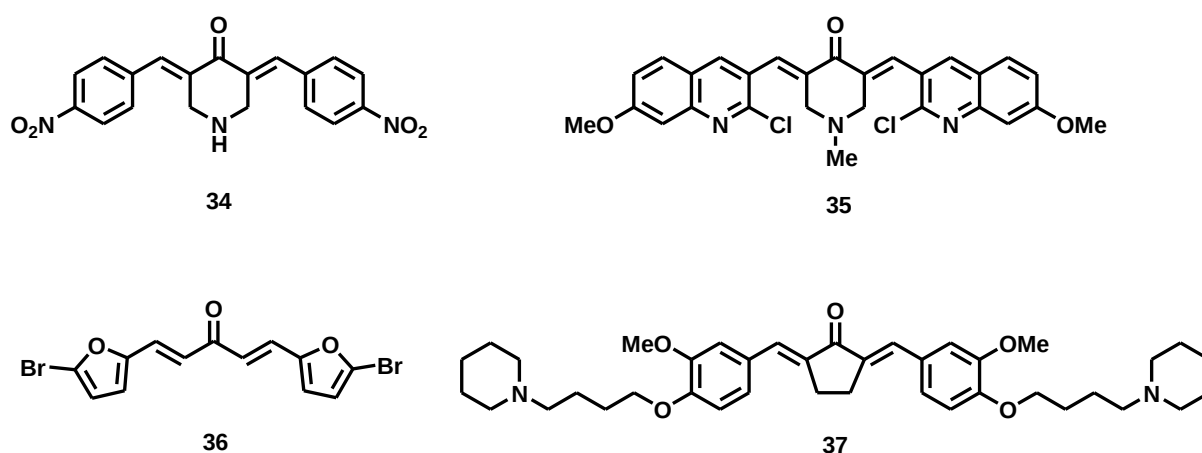


Fonte: Elaborado pelo autor

Além de apresentarem propriedades físico-químicas e parâmetros farmacocinéticos adequados, os análogos monocetônicos também demonstraram efeitos antibacterianos promissores. Os estudos descritos até o momento foram concentrados em monocetônicos simétricos. Investigações têm reportado a atividade antimicobacterina de análogos monocetônicos simétricos derivados da piperidona contra *M. tuberculosis*, *M. bovis* e *M.*

marinum (DAS et al., 2008; BALDWIN et al., 2015; SUBHEDAR et al., 2017; DESHMUKH et al., 2019). Por outro lado, os efeitos de monocetônicos simétricos derivados da acetona, ciclopentanona e ciclohexanona têm sido mais descritos contra espécies bacterianas Gram-positivas e Gram-negativas, tal como *S. aureus*, *S. epidermidis*, *S. mutans*, *Enterobacter cloacae* and *E. coli* (LIANG et al., 2008a; LIANG et al., 2008b; ŞEKER et al., 2018; VIEIRA et al., 2018; KUMAR et al., 2019; KUMAR et al., 2020). A Figura 10 sumarizou as estruturas dos monocetônicos com atividades antimicobacteriana (**34** e **35**) e antibacteriana (**36** e **37**) mais promissoras.

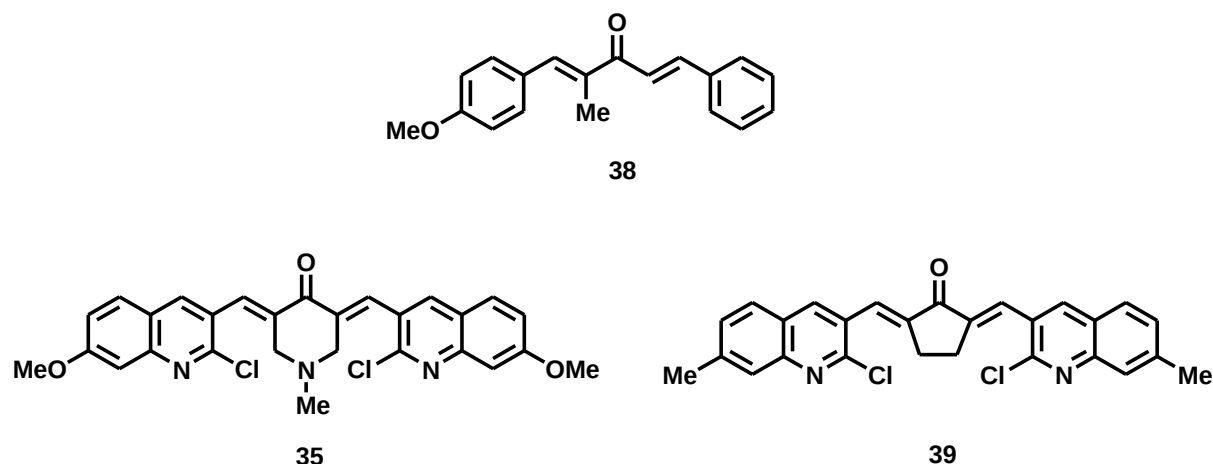
Figura 10. Análogos monocetônicos com atividades antimicobacteriana (**34** e **35**) e antibacteriana (**36** e **37**) (DAS et al., 2008; LIANG et al., 2008b; SUBHEDAR et al., 2017; KUMAR et al., 2019).



Fonte: Elaborado pelo autor

Diferente dos estudos contra bactérias, os efeitos de monocetônicos contra fungos têm sido pouco explorados. Há apenas alguns trabalhos que descreveram a atividade antifúngica de análogos monocetônicos de curcumina (DIN et al., 2017; NAGARGOJE et al., 2019). Din e colaboradores investigaram a atividade de monocetônicos assimétricos derivados da acetona contra as espécies *C. albicans* e *C. krusei*. O monocetônico **38** (Figura 11) foi o mais potente, com valor de CIM de 62,5 µg/mL (DIN et al., 2017). Nagargoje e coautores avaliaram o potencial antifúngico de monocetônicos simétricos contra *C. albicans*, *Fusarium oxysporum*, *Aspergillus flavus*, *A. niger* e *Cryptococcus neoformans*. As substâncias mais potentes (**35** e **39**) (Figura 11) apresentaram valores de CIM variando de 6,2 a 25 µg/mL (NAGARGOJE et al., 2019).

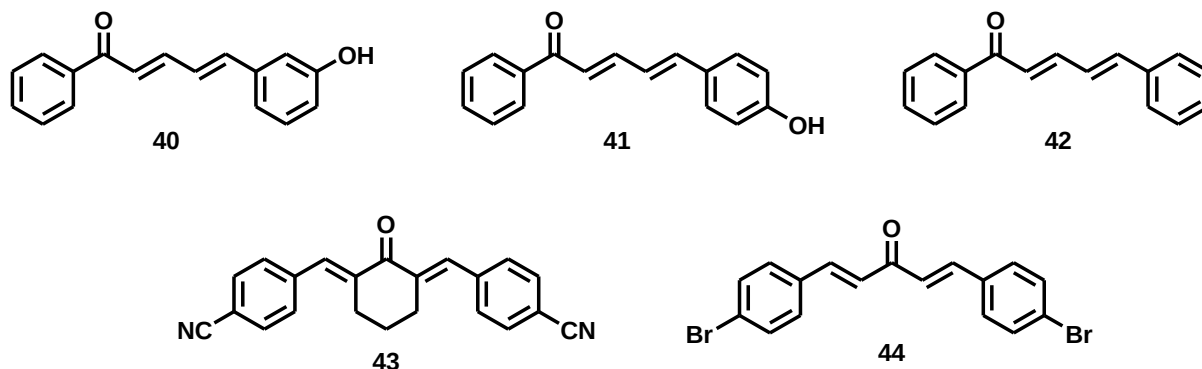
Figura 11. Monocetônicos com atividade antifúngica (**35**, **38** e **39**) (DIN et al., 2017; NAGARGOJE et al., 2019).



Fonte: Elaborado pelo autor

O nosso grupo vem estudando as atividades antibacteriana, antimicobacteriana e antitumoral de análogos monocetônicos (**40–44**) (Figura 12) (POLAQUINI et al., 2017; LIMA et al., 2018; OLIVEIRA et al., 2018; SILVA et al., 2018; MORÃO et al., 2019). As substâncias monocetônicas assimétricas **40** e **41** inibiram a atividade metabólica de *S. aureus*, *S. mutans* e *S. sanguinis* (CIM 77,9 a 156 $\mu\text{mol/L}$), enquanto **42** apresentou atividade contra *M. tuberculosis* (CIM 57,2 $\mu\text{mol/L}$) (POLAQUINI et al., 2017). O monocetônico cíclico simétrico **43** induziu apoptose, diminuiu a migração e reduziu a invasão das células de câncer gástrico HGC-27 pela inibição da expressão e da atividade da metaloproteinase de matriz 2 (SILVA et al., 2018). Em investigação posterior, nós também demonstramos a atividade de **43** contra as linhagens de osteossarcoma humano U2OS, MG-63 e Saos-2 pelo aumento dos níveis da p53 e redução da Sp1, as quais são proteínas relacionadas à indução de apoptose (LIMA et al., 2018). Já o análogo monocetônico simétrico *p*-bromado **44** induziu à morte células de carcinoma cervical CasKi pelo aumento na atividade das caspases 3 e 7 (OLIVEIRA et al., 2018).

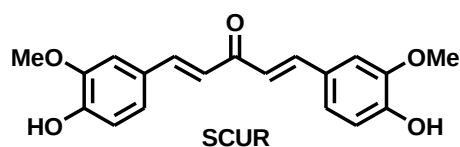
Figura 12. Análogos monocetônicos descritos pelo nosso grupo (40–44) com atividades antibacteriana, antimicobacteriana e antitumoral (POLAQUINI et al., 2017; LIMA et al., 2018; OLIVEIRA et al., 2018; SILVA et al., 2018).



Fonte: Elaborado pelo autor

Nós também descrevemos a atividade antibacteriana do análogo simplificado de curcumina **SCUR** (Figura 12) contra o fitopatógeno *Xanthomonas citri* subsp. *citri* (*X. citri*) (MORÃO et al., 2019), que é o agente etiológico do cancro cítrico Asiático, uma doença que acomete plantações citrícolas (DAS, 2003). A substância **SCUR** demonstrou atividade anti-*X. citri* superior à curcumina. Além disso, investigações sobre o seu modo de ação indicaram a sua capacidade de perturbar a membrana citoplasmática de *Bacillus subtilis* (MORÃO et al., 2019). Essa substância também apresentou parâmetros farmacocinéticos melhores do que a curcumina, corroborando o seu potencial como um análogo de curcumina promissor na pesquisa de novas substâncias bioativas (LIANG et al., 2009).

Figura 13. Análogo simplificado de curcumina (**SCUR**) com atividade anti-*X. citri* (MORÃO et al., 2019).



Fonte: Elaborado pelo autor

1.3. OBJETIVOS

1.3.1. Objetivo Geral

Planejar, sintetizar e avaliar o potencial de análogos monocetônicos de curcumina contra espécies bacterianas e fúngicas de importância clínica, além de investigar os seus efeitos contra uma bactéria de interesse agrônômico.

1.3.2. Objetivos Específicos dos Capítulos II e III

- A. Sintetizar, purificar e identificar uma série de análogos monocetônicos difenilpentanoides (**MACs 1–8**) baseados na estrutura da curcumina (Figura 14);
- B. Avaliar a atividade antibacteriana da curcumina e seus análogos monocetônicos (**SCUR** e **MACs 1–8**) contra *M. tuberculosis*, *A. baumannii*, *P. aeruginosa*, *E. coli*, *S. aureus*, *S. epidermidis* e *E. faecalis*;
- C. Avaliar a toxicidade dos monocetônicos mais potentes (**MACs 4, 6 e 8**) contra as linhagens celulares de pulmão humano MRC-5 e A549 e determinar os seus índices de seletividade;
- D. Investigar a genotoxicidade do monocetônico mais seletivo (**MAC 4**) contra a linhagem celular A549;
- E. Avaliar a estabilidade química de **MAC 4** (pH 7,4 e 37 °C);
- F. Analisar os efeitos de **MAC 4** sobre o divisomo de *B. subtilis*;
- G. Estudar os efeitos de **MAC 4** sobre a atividade GTPásica da proteína FtsZ de *B. subtilis*;
- H. Sintetizar, purificar e identificar uma série de análogos monocetônicos arilbutanoides (**SMACs 1–40**) baseados na estrutura de **MAC 4** (Figura 14);
- I. Avaliar a atividade antimicobacteriana dos monocetônicos arilbutanoides (**SMACs 1–40**) contra *M. tuberculosis* H₃₇Rv;

J. Investigar *in silico* as propriedades farmacocinéticas e de *drug-likeness* dos monocetônicos mais potentes (**SMACs 3, 4, 9–11, 13, 15, 18, 19, 21–26, 29, 30, 34–36, 38 e 39**);

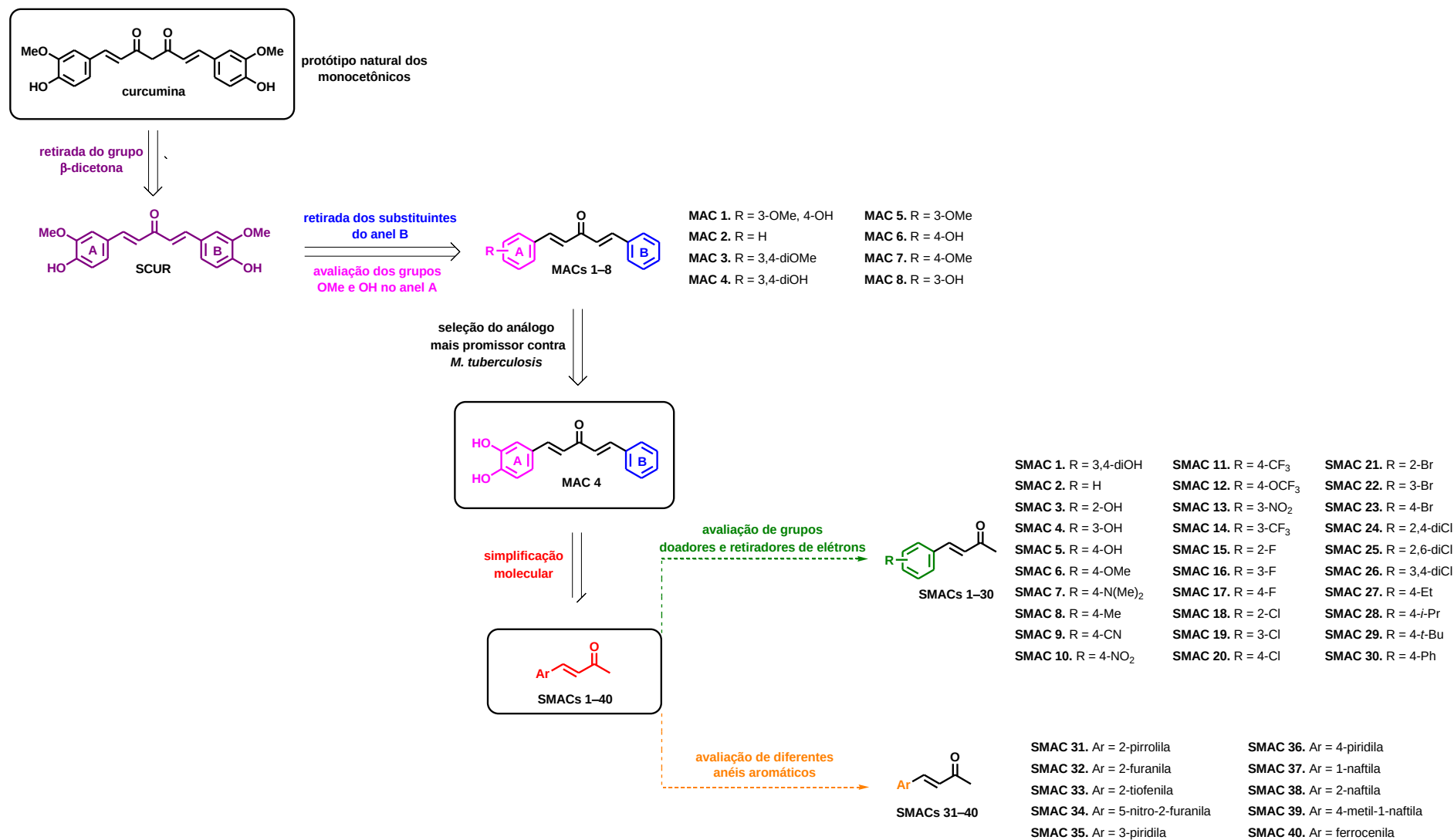
K. Avaliar a toxicidade dos monocetônicos mais potentes (**SMACs 3, 4, 9–11, 13, 15, 18, 19, 21–26, 29, 30, 34–36, 38 e 39**) contra a linhagem celular de pulmão humano MRC-5 e determinar os seus índices de seletividade;

L. Analisar a estabilidade química dos monocetônicos mais seletivos (**SMACs 30 e 34**) (pH 7,4 e 37 °C);

M. Investigar a atividade de **SMACs 30 e 34** contra isolados clínicos de *M. tuberculosis* com resistência à múltiplos fármacos (cepas MDR);

N. Estudar os efeitos de **SMACs 30 e 34** contra espécies bacterianas Gram-negativas e Gram-positivas, incluindo *P. aeruginosa*, *S. enterica*, *A. baumannii*, *S. aureus*, *E. faecalis* e *B. subtilis*.

Figura 14. Planejamento estrutural dos análogos monocetônicos contra espécies bacterianas de interesse clínico.

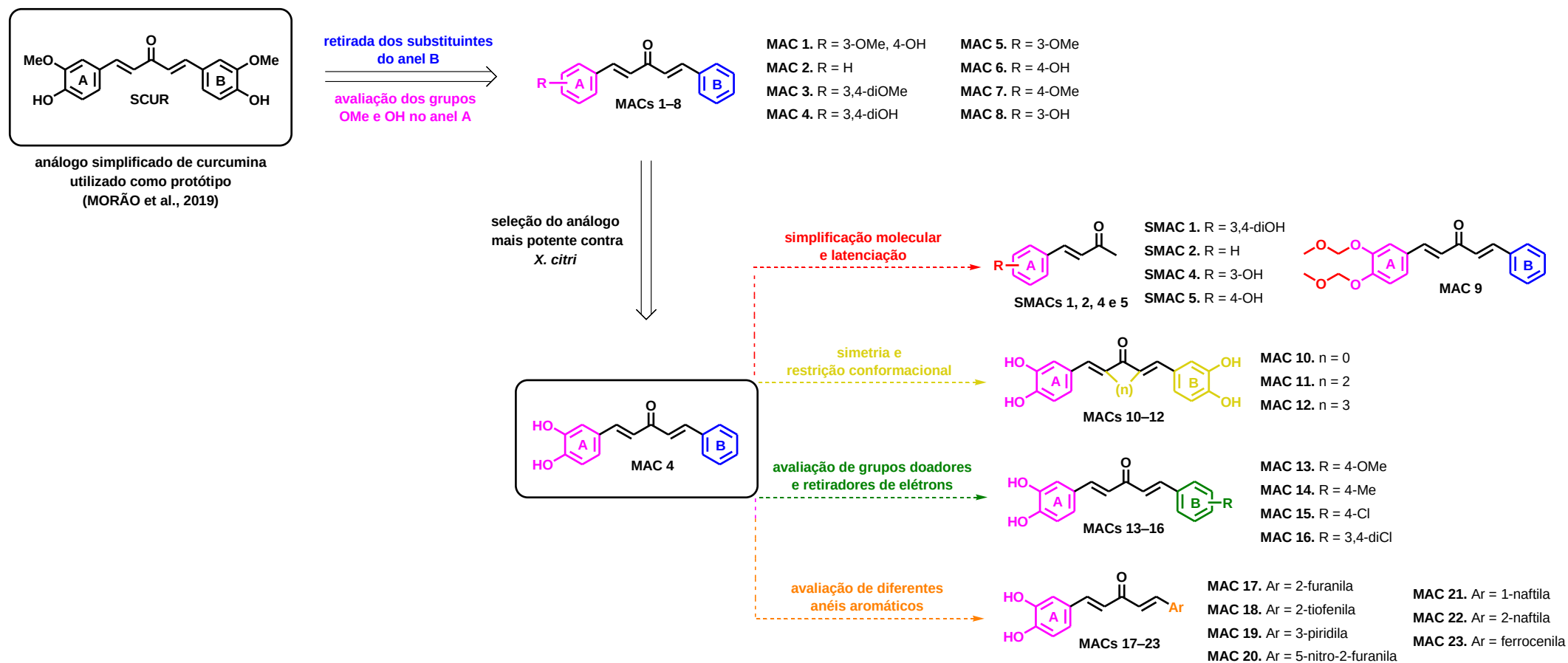


Fonte: Elaborado pelo autor

1.3.3. Objetivos Específicos dos Capítulos IV e V

- A.** Avaliar a atividade anti-*X. citri* dos análogos monocetônicos difenilpentanoides (**MACs 1–8**) e fenilbutanoides (**SMACs 1, 2, 4 e 5**) (Figura 15);
- B.** Investigar os efeitos do monocetônico mais potente (**MAC 4**) sobre o crescimento de *X. citri*;
- C.** Analisar a capacidade de *X. citri* desenvolver resistência ao monocetônico **MAC 4**;
- D.** Investigar a capacidade de *X. citri* pré-exposta ao monocetônico **MAC 4** desenvolver os sintomas de cancro cítrico sobre as folhas de *Citrus sinensis*;
- E.** Analisar os efeitos de **MAC 4** sobre o divisomo de *X. citri*;
- F.** Estudar os efeitos de **MAC 4** sobre a atividade GTPásica da proteína FtsZ de *X. citri*;
- G.** Sintetizar, purificar e identificar uma série de análogos monocetônicos diarilpentanoides (**MACs 9–23**) baseados na estrutura de **MAC 4** (Figura 15);
- H.** Avaliar a atividade anti-*X. citri* dos análogos monocetônicos diarilpentanoides (**MACs 9–23**);
- I.** Investigar os efeitos do monocetônico mais potente (**MAC 22**) sobre o crescimento de *X. citri*;
- J.** Analisar os efeitos de **MAC 22** sobre a membrana de *X. citri*.

Figura 15. Planejamento estrutural dos análogos monocetônicos contra *X. citri*.

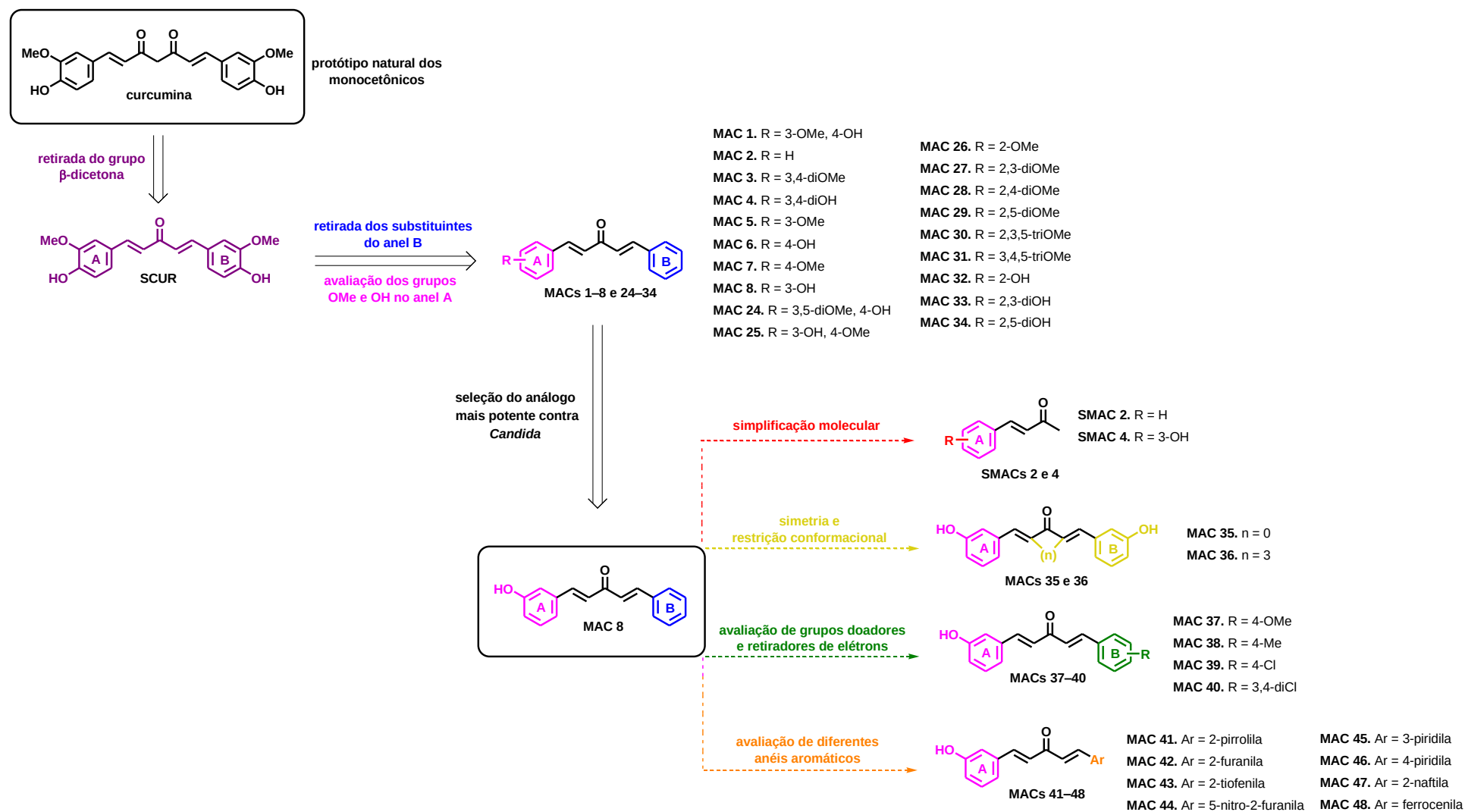


Fonte: Elaborado pelo autor

1.3.4. Objetivos Específicos dos Capítulos VI e VII

- A. Sintetizar, purificar e identificar uma série de análogos monocetônicos difenilpentanoides (**MACs 24–34**) baseados na estrutura da curcumina (Figura 16);
- B. Avaliar a atividade antifúngica da curcumina e seus análogos monocetônicos (**SCUR**, **MACs 1–8** e **24–34**) contra *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis* e *C. krusei*;
- C. Investigar a atividade antifúngica do monocetônico mais potente (**MAC 8**) contra isolados clínicos de *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis* e *C. krusei*;
- D. Analisar os efeitos de **MAC 8** sobre a adesão de *C. albicans* às células humanas de queratinócito (HaCaT) e fibroblasto gengival (HGF-1);
- E. Avaliar os efeitos de **MAC 8** sobre os biofilmes em formação e pré-formado de *C. albicans*;
- F. Estudar os efeitos das combinações de **MAC 8** com os fármacos antifúngicos anfotericina B e fluconazol contra *C. albicans*;
- G. Investigar os efeitos de **MAC 8** sobre a membrana e a parede celular de *C. albicans*;
- H. Avaliar a estabilidade química de **MAC 8** (pH 7,4 e 37 °C);
- I. Avaliar a toxicidade sistêmica de **MAC 8** em larvas de *Galleria mellonella*;
- J. Sintetizar, purificar e identificar uma série de análogos monocetônicos diarilpentanoides (**MACs 35–48**) baseados na estrutura de **MAC 8** (Figura 16);
- K. Avaliar a atividade antifúngica dos monocetônicos (**MACs 35–48** e **SMACs 2 e 4**) contra *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis* e *C. krusei*;
- L. Avaliar os efeitos do monocetônico mais potente (**MAC 43**) sobre os biofilmes em formação e pré-formado de *C. albicans*;
- M. Estudar os efeitos das combinações de **MAC 43** com os fármacos antifúngicos anfotericina B e fluconazol contra *C. albicans*.

Figura 16. Planejamento estrutural dos análogos monocetônicos contra espécies fúngicas do gênero *Candida*.



Fonte: Elaborado pelo autor

2. CAPÍTULO II

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2. CAPÍTULO II

Antibacterial Activity of a New Unsymmetrical Monocarbonyl Analog of Curcumin (MAC 4) is Associated with Divisome Disruption

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ABSTRACT

Curcumin (**CUR**) is a symmetrical dicarbonyl compound with promising antibacterial action. However, pharmacokinetic limitations hinder its therapeutic application. Some monocarbonyl analogs of curcumin (**MACs**) have been shown to overcome these problems. Thus, we synthesized and investigated the antimicrobial activity of a series of unsymmetrical **MACs** derived from acetone against *Mycobacterium tuberculosis*, and also against Gram-negative and Gram-positive bacterial species. **MACs 4, 6 and 8** showed a broad spectrum and potent activity, mainly against *M. tuberculosis*, *Acinetobacter baumannii* and methicillin-resistant *Staphylococcus aureus*, with MIC (minimum inhibitory concentration) values ranging from 4.4 to 62.3 $\mu\text{mol/L}$. The investigation regarding toxicity on human lung cells (MRC-5 and A549 lines) revealed that **MAC 4** was more selective than **MACs 6 and 8**, with SI (selectivity index) values ranging from 5.4 to 15.6. In addition, **MAC 4** did not demonstrate genotoxic effects on A549 cells, and it was more stable than **CUR** in phosphate buffer (pH 7.4) for 24 h at 37 °C. Fluorescence and phase contrast microscopies indicated that **MAC 4** had the ability to disrupt the *Bacillus subtilis* divisome without causing damage to the membrane. However, biochemical investigation demonstrated that **MAC 4** did not affect the GTPase activity of the cytoskeletal protein FtsZ from *B. subtilis*. These results indicated that **MAC 4** is a promising compound with antitubercular and antibacterial activities.

2.1. INTRODUCTION

Curcumin (**CUR**) is a symmetrical dicarbonyl compound which is considered safe for humans (LAO et al., 2006) and recognized for its antibacterial effects, with moderate activity against Gram-positive, Gram-negative and mycobacterial species (MOGHADAMTOUSI et al., 2014; MARINI et al., 2018). Studies have shown that bacterial division is one of the targets of this natural product (RAI et al., 2008; MORÃO et al., 2019). Microscopy analyses demonstrated that the treatment of *Bacillus subtilis* with **CUR** caused the divisome disruption (MORÃO et al., 2019), which is a multiprotein complex operating on cell division (HURLEY et al., 2016). This effect has been correlated with the ability of **CUR** to inhibit the assembly of the cytoskeletal protein FtsZ in protofilaments by stimulating its GTPase activity (RAI et al., 2008). *In silico* investigations have elucidated the specific interactions of **CUR** with FtsZ from *B. subtilis*, *Escherichia coli* (KAUR et al., 2010) and *Mycobacterium tuberculosis* (FUJIMORI et al., 2018).

Despite the promising antibacterial effects *in vitro* and *in vivo* (DE et al., 2009; WANG et al., 2016), **CUR** has poor chemical stability, low absorption and fast metabolism (NELSON et al., 2017), requiring repetitive doses, which hinders its use as a therapeutic agent. One of the strategies to overcome these problems is the design of synthetic analogs. Among them, monocarbonyl compounds constitute one of the main classes of **CUR** analogs which have been investigated, especially for their antitumoral and anti-inflammatory activities (SHETTY et al., 2015; NOUREDDIN, EL-SHISHTAWY & AL-FOOTY, 2019). These compounds are designed by replacing the β -diketone moiety by a monocarbonyl group, since the β -diketone is easily hydrolyzed under basic conditions (HEGER et al., 2014), and it appears to be a specific substrate of a series of aldo-keto reductases (ROSEMOND et al., 2004). Several monocarbonyl analogs of curcumin (**MACs**) not only improved chemical and metabolic stability (YUAN et al., 2014; ZHANG et al., 2014), but they also showed an increase in intestinal permeability (ZENG et al., 2017) and water solubility (WANG et al., 2017).

In addition to presenting better physical-chemical properties and pharmacokinetic parameters than **CUR**, **MACs** also have potent activity against several bacterial species. Studies have demonstrated the antimycobacterial activity of various symmetrical **MACs** derived from piperidone against *M. tuberculosis*, *M. bovis* and *M. marinum* (DAS et al., 2008; BALDWIN et al., 2015; SUBHEDAR et al., 2017; DESHMUKH et al., 2019). Regarding this class, Subhedar and co-authors described its specificity against mycobacterial species (SUBHEDAR et al., 2017). On the other hand, the effects of symmetrical **MACs** derived from cyclopentanone

and cyclohexanone have been more frequently reported against Gram-positive and Gram-negative bacterial species, including *Staphylococcus aureus*, *S. epidermidis*, *Enterobacter cloacae* and *E. coli* (LIANG et al., 2008a; LIANG et al., 2008b; ŞEKER et al., 2018; KUMAR et al., 2019; KUMAR et al., 2020).

In a previous study, our group investigated the antibacterial action mode of simplified curcumin (**SCUR**), a symmetrical **MAC** derived from acetone. In that study, we reported that **SCUR** lost its ability to perturb the *B. subtilis* cell division, but it demonstrated action on the membrane (MORÃO et al., 2019), which is also a target for **CUR** (TYAGI et al., 2015). Although some studies have described the antibacterial activity of symmetrical **MACs**, investigations on unsymmetrical **MACs** are rare. In this context, we synthesized a series of unsymmetrical **MACs** **1–8** derived from acetone and investigated the effects of OMe and OH substituents on antitubercular and antibacterial activities. The toxicity on human pulmonary cells and the selectivity indexes of most active compounds (**MACs** **4**, **6** and **8**) were determined. The most selective compound (**MAC** **4**) was investigated regarding its genotoxic effects and chemical stability. Additionally, we also evaluated the ability of **MAC** **4** to perturb the cell division concerning its effects on the divisome and on the FtsZ GTPase activity of *B. subtilis*.

2.2. RESULTS AND DISCUSSION

2.2.1. Chemistry

MACs **1–3** and **5–8** were synthesized in two steps (Figure 1). First, benzylideneacetone (**1**) was prepared by mono-condensation of acetone with benzaldehyde using NaOH as a catalyst, resulting in a yield of 89%. The intermediate **1** was condensed again with methoxylated and/or hydroxylated benzaldehyde derivatives in the presence of H₂SO₄, providing **MACs** **1–3** and **5–8** in 26–83% yields.

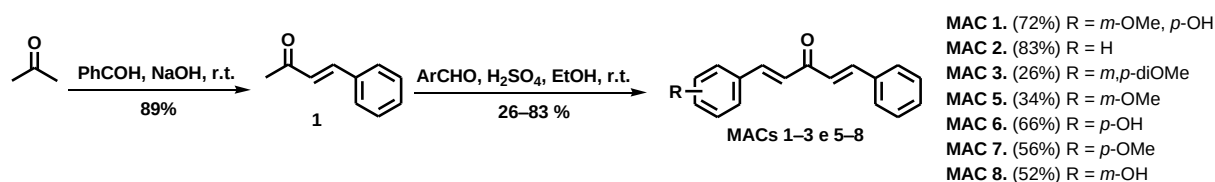


Figure 1. Synthesis of **MACs** **1–3** and **5–8**.

Fonte: Elaborado pelo autor

In addition to the benzylideneacetone (**1**) preparation stage, three more steps were necessary to synthesize **MAC 4** (Figure 2). The methoxymethyl protecting group (MOM) was required due to difficulties in obtaining diphenolic compounds via aldol condensation reaction (KIM et al., 2008; KIM et al., 2015). First, 3,4-bis(methoxymethoxy)benzaldehyde (**2**) was prepared in 73% yield from the protection of 3,4-dihydroxybenzaldehyde with methoxymethyl chloride (MOMCl) in dry acetone and K₂CO₃. In the second step, MOM-protected benzaldehyde (**2**) was condensed with benzylideneacetone (**1**) in order to provide MOM-diphenylpentanoid (**3**) in 42% yield. Lastly, MOM protecting groups were removed by treatment with HCl, providing **MAC 4** in a 26% yield.

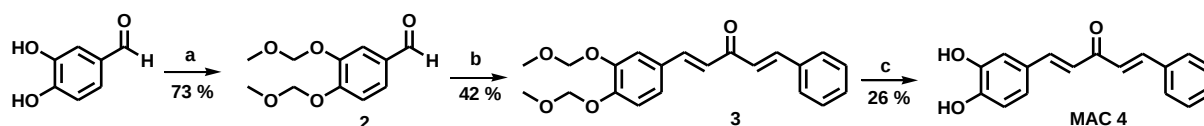


Figure 2. Synthesis of **MAC 4**. Reagents and conditions: (a) MOMCl, K₂CO₃, dry acetone, N₂ atmosphere, room temperature, 1 h; (b) benzylideneacetone (**1**), NaOH, EtOH, N₂ atmosphere, room temperature, 8 h; (c) HCl, EtOH, N₂ atmosphere, room temperature, 48 h.

Fonte: Elaborado pelo autor

The structures of synthesized compounds were confirmed by their melting point and ¹H and ¹³C NMR spectra data analyses. For all compounds, NMR data, including chemical shifts, integrations multiplicities and coupling constants, corresponded to the proposed structures. Additionally, ESI-MS spectra data analyses of the new compound **MAC 4** were carried out. Detailed spectral analyses were presented in the Supplementary Material.

2.2.2. Antitubercular and antibacterial activities

The antitubercular activity of curcumin (**CUR**), simplified curcumin (**SCUR**) and unsymmetrical monocarbonyl analogs (**MACs 1–8**) was evaluated against *M. tuberculosis* H₃₇Rv (ATCC 27294). The antibacterial activity was tested against Gram-negative bacteria, including *Acinetobacter baumannii* (ATCC 19606), *Pseudomonas aeruginosa* (ATCC 27853) and *E. coli* (ATCC 43895), as well as Gram-positive bacteria species, such as methicillin-sensitive *S. aureus* (MSSA) (ATCC 25923), methicillin-resistant *S. aureus* (MRSA) (ATCC 33591), *S. epidermidis* (ATCC 12228) and *Enterococcus faecalis* (ATCC 29212). Antimicrobial potency was expressed as minimum inhibitory concentration (MIC) and

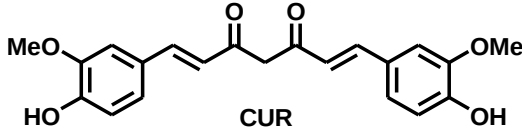
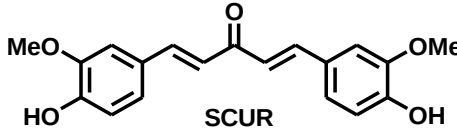
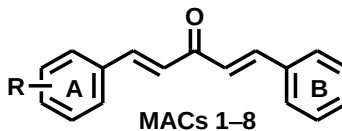
minimum bactericidal concentration (MBC) values in $\mu\text{mol/L}$ (Table 1). Isoniazid, gentamicin and vancomycin were used as reference antibiotics.

CUR showed activity against MSSA, *S. epidermidis* and *E. faecalis*, with MIC value of $340 \mu\text{mol/L}$. However, it displayed MIC $>500 \mu\text{mol/L}$ against *M. tuberculosis*, *P. aeruginosa*, *E. coli* and MRSA. These findings corroborate a previous study carried out by our group which described similar MIC values against *M. tuberculosis*, *P. aeruginosa*, *E. coli*, MSSA and *E. faecalis* (POLAQUINI et al., 2019). **CUR** also demonstrated activity against *A. baumannii*, with MIC value of $170 \mu\text{mol/L}$. *A. baumannii* belongs to the ESKAPE pathogens, a bacterial group that also includes *E. faecium*, *S. aureus*, *Klebsiella pneumoniae*, *P. aeruginosa* and *Enterobacter* spp (SANTAJIT & INDRAWATTANA, 2016). These microorganisms are recognized as the leading cause of nosocomial infections worldwide (MULANI et al., 2019). In addition, the carbapenem resistant *A. baumannii* figures in the “critical” category of World Health Organization’s priority pathogens list for the development of new antibiotics (TACCONELLI et al., 2018), which reinforces the importance of the search for agents against *A. baumannii*.

In order to assess the effects of replacing β -diketone by monocarbonyl moiety on antitubercular and antibacterial activities, we compared the MIC values of **CUR** and **SCUR**. **SCUR** displayed higher potency than the **CUR** one against *M. tuberculosis*, *A. baumannii*, *P. aeruginosa*, MSSA and MRSA (MIC 25.3, 47.8, 191, 191 and $383 \mu\text{mol/L}$, respectively), indicating that the **CUR** molecular simplification increased the activity against these species. However, this replacement reduced **SCUR** activity against *S. epidermidis* and *E. faecalis* (MIC $>500 \mu\text{mol/L}$).

We also investigated the effects of removing substituents on the **SCUR** aromatic ring B. **MAC 1** (unsubstituted ring B) was more active against *M. tuberculosis*, *A. baumannii*, MSSA and MRSA (MIC 12.3, 27.8, 114 and $223 \mu\text{mol/L}$, respectively) than **SCUR** (MIC 25.3, 47.8, 191 and $383 \mu\text{mol/L}$, respectively), indicating that the withdrawal of *m*-OMe and *p*-OH groups on the aromatic ring B resulted in an increased activity. In order to evaluate the preliminary relationships between OMe and OH substituents on the aromatic ring A and antitubercular/antibacterial activities, the unsymmetrical **MAC 1** was used as a framework.

Table 1. Antitubercular and antibacterial activities of curcumin (**CUR**), simplified curcumin (**SCUR**) and unsymmetrical monocarbonyl analogs (**MACs 1–8**) expressed as MIC and MBC values in $\mu\text{mol/L}$.

  																
Compounds	R	Mt	Ab		Pa		Ec		MSSA		MRSA		Se		Ef	
		MIC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
CUR	–	>500	170	170	>500	>500	>500	>500	340	>500	>500	>500	340	>500	340	>500
SCUR	–	25.3	47.8	95.7	191	383	>500	>500	191	191	383	>500	>500	>500	>500	>500
MAC 1	<i>m</i> -OMe, <i>p</i> -OH	12.3	27.8	27.8	>500	>500	>500	>500	114	223	223	223	>500	>500	>500	>500
MAC 2	H	3.5	267	267	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
MAC 3	<i>m,p</i> -diOMe	46.6	425	425	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
MAC 4	<i>m,p</i> -diOH	5.6	14.6	14.6	117	235	>500	>500	479	479	58.6	58.6	235	479	235	479
MAC 5	<i>m</i> -OMe	19.4	59.1	59.1	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
MAC 6	<i>p</i> -OH	4.4	31.2	31.2	>500	>500	>500	>500	125	250	62.3	62.3	125	125	>500	>500
MAC 7	<i>p</i> -OMe	32.2	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
MAC 8	<i>m</i> -OH	4.6	15.6	15.6	>500	>500	>500	>500	31.2	62.3	31.2	62.3	31.2	62.3	>500	>500
isoniazid	–	0.7	–	–	–	–	–	–	–	–	–	–	–	–	–	–
gentamicin	–	–	2.0	–	0.4	–	1.0	–	–	–	–	–	–	–	–	–
vancomycin	–	–	–	–	–	–	–	–	0.7	–	1.4	–	1.4	–	1.4	–

MIC = minimal inhibitory concentration; MBC = minimum bactericidal concentration; Mt = *Mycobacterium tuberculosis* H₃₇Rv (ATCC 27294); Ab = *Acinetobacter baumannii* (ATCC 19606); Pa = *Pseudomonas aeruginosa* (ATCC 27853); Ec = *Escherichia coli* (ATCC 43895); MSSA = methicillin-sensitive *Staphylococcus aureus* (ATCC 25923); MRSA = methicillin-resistant *Staphylococcus aureus* (ATCC 33591); Se = *Staphylococcus epidermidis* (ATCC 12228); Ef = *Enterococcus faecalis* (ATCC 29212); – not determined.

Fonte: Elaborado pelo autor

First, we assayed **MAC 2** in order to determine the effects of eliminating substituents on the aromatic rings A and B. **MAC 2** activity against *A. baumannii*, MSSA and MRSA (MIC 267, >500 and >500 $\mu\text{mol/L}$, respectively) was reduced when compared to **MAC 1** (MIC 27.8, 114 and 223 $\mu\text{mol/L}$, respectively). On the other hand, **MAC 2** (MIC 3.5 $\mu\text{mol/L}$) showed higher antitubercular activity than **MAC 1** (MIC 12.3 $\mu\text{mol/L}$). In a previous study with $\alpha,\beta,\gamma,\delta$ -unsaturated ketones (cinnamylideneacetophenones), we also found that the unsubstituted analog (MIC 57.2 $\mu\text{mol/L}$) was more active against *M. tuberculosis* than the substituted analogs (MIC 66.6 to >200 $\mu\text{mol/L}$) (POLAQUINI et al., 2017). *M. tuberculosis* is the main etiological agent of tuberculosis, a communicable lung disease that is one of the top 10 causes of death worldwide and the leading cause of death from a single infectious agent (WHO, 2019).

Second, we evaluated the activities of **MAC 3** (*m,p*-diOMe) and **MAC 4** (*m,p*-diOH), and compared them to **MAC 1** (*m*-OMe, *p*-OH). The comparison between the MIC values of **MACs 3** and **1** revealed that the replacement of OH by OMe at *para* position on the aromatic ring A reduced both spectrum and potency. On the other hand, **MAC 4** demonstrated potent activity against *M. tuberculosis*, *A. baumannii*, *P. aeruginosa*, MSSA, MRSA, *S. epidermidis* and *E. faecalis* ($5.6 \mu\text{mol/L} \leq \text{MIC} \leq 479 \mu\text{mol/L}$), indicating that the replacement of OMe by OH at *meta* position increased the action spectrum and the potency when compared with **MAC 1**. In addition, the comparison between the MIC values of **MAC 4** against MSSA and MRSA (MIC 479 and 58.6 $\mu\text{mol/L}$, respectively) indicated that the resistant strain was more sensitive to the **MAC 4**. MRSA is a leading cause of infection worldwide, including a wide array of both hospital and community acquired infections, such as bacteremia, respiratory tract infection, skin and soft tissue infection, osteomyelitis and septic arthritis (SMITH et al., 2019; MEHTA et al., 2020).

Third, the effects of *m*-OMe and *p*-OH substituents on the aromatic ring A were assessed separately, testing **MACs 5** and **6**, respectively. **MAC 5** (*m*-OMe) was active only against *M. tuberculosis* and *A. baumannii* (MIC 19.4 and 59.1 $\mu\text{mol/L}$, respectively). On the other hand, **MAC 6** (*p*-OH) demonstrated activity against *M. tuberculosis*, *A. baumannii*, MSSA, MRSA and *S. epidermidis* ($4.4 \mu\text{mol/L} \leq \text{MIC} \leq 125 \mu\text{mol/L}$). These results suggest that the *p*-OH substituent was more relevant for the activity than the *m*-OMe group. Furthermore, the comparison between the MIC values of **MAC 1** (*m*-OMe, *p*-OH) and **MAC 6** (*p*-OH) indicated that the presence of *m*-OMe group did not affect the activity against *A. baumannii* and MSSA, but its removal increased the potency against *M. tuberculosis*, MRSA and *S. epidermidis*.

Fourth, we investigated the activities of **MAC 7** (*p*-OMe) and **MAC 8** (*m*-OH), which are regioisomers of **MAC 5** (*m*-OMe) and **MAC 6** (*p*-OH), respectively. **MAC 7** was inactive (MIC >500 $\mu\text{mol/L}$) against Gram-negative and Gram-positive species, and it showed less activity (MIC 32.2 $\mu\text{mol/L}$) than the regioisomer **MAC 5** (MIC 19.4 $\mu\text{mol/L}$) against *M. tuberculosis*. On the other hand, **MAC 8** (MIC 4.6 $\mu\text{mol/L}$) demonstrated similar antitubercular activity to the regioisomer **MAC 6** (MIC 4.4 $\mu\text{mol/L}$). Regarding Gram-negative and Gram-positive species, **MAC 8** displayed higher activity than **MAC 6** against *A. baumannii*, MSSA, MRSA and *S. epidermidis*. In general, these results indicated that the position of OMe and OH substituents affects the bioactivity, suggesting that the *meta* position was more relevant for antibacterial and antitubercular activities.

In order to determine whether the active **MACs** displayed bactericidal or bacteriostatic activity against Gram-negative and Gram-positive species, the ratios among the MBC and MIC values were calculated. Antibacterial agents are generally regarded as bactericidal if MBC/MIC ratio < 4 (SARDI et al., 2017a; SALEM et al., 2020). All active **MACs** exhibited MBC/MIC ratios between 1 and 2, indicating that the curcuminoids have bactericidal activity against these species.

2.2.3. Assessment of **MACs 4, 6 and 8** toxicity on human lung cells, and determination of the selectivity indexes

The evaluation of the effects against human eukaryotic cells is a relevant step to investigate the selectivity and safety of new antibacterial agents. We selected the curcuminoids **MACs 4, 6 and 8**, which demonstrated a broad spectrum and potent activity, in order to evaluate their toxicity on human cells. Since *M. tuberculosis* and *A. baumannii*, which were the most sensitive species to the selected compounds, cause pulmonary infection (CARUGATI et al., 2020; MOULE & CIRILLO, 2020), we investigated the toxicity against human lung cells, including fibroblast (MRC-5) and epithelial (A549) cell lines. **MACs 4, 6 and 8** toxic effects were expressed according to the concentration required in order to reduce cell viability at 50% (IC₅₀) (Table 2). Doxorubicin was used as a reference cytotoxic agent.

MAC 4 was less toxic on MRC-5 and A549 cells (IC₅₀ 87.6 and 79.4 $\mu\text{mol/L}$, respectively) than **MACs 6 and 8** (17.5 $\mu\text{mol/L}$ $\leq$ IC₅₀ $\leq$ 52.4 $\mu\text{mol/L}$). In addition, **MAC 4** was about 120 and 50 times less toxic on MRC-5 and A549 cells, respectively, than doxorubicin, a compound which is known for its cytotoxic properties (MOHAJERI & SAHEBKAR, 2018). In order to compare the effects on prokaryotic and eukaryotic cells, the selectivity indexes (SI) of

selected compounds were determined as the ratio among the IC₅₀ values against MRC-5 and A549, and MIC values against *M. tuberculosis* and *A. baumannii* (Table 2). **MAC 4** was more selective than **MACs 6** and **8**, with SI values ranging from 5.4 to 15.6. Furthermore, the comparison among the SI values of **MAC 4** suggests that it was more promising as an anti-*M. tuberculosis* agent, since it had higher SI values for this species (SI 15.6 and 14.2 for MRC-5 and A549 cells, respectively). According to Orme and collaborators, candidates for new antitubercular agents must have a SI value ≥ 10 (ORME et al., 2001).

Table 2. Toxicity on human lung cells and selectivity indexes of selected **MACs**.

Compounds	R	MRC-5	SI		A549	SI	
		IC ₅₀	Mt	Ab	IC ₅₀	Mt	Ab
MAC 4	<i>m,p</i> -diOH	87.6 $\pm$ 2.1	15.6	6.0	79.4 $\pm$ 1.3	14.2	5.4
MAC 6	<i>p</i> -OH	52.4 $\pm$ 1.2	11.9	1.7	50.5 $\pm$ 2.0	11.5	1.6
MAC 8	<i>m</i> -OH	17.5 $\pm$ 0.9	3.8	1.1	23.3 $\pm$ 1.8	5.1	1.5
CUR	–	42.1 $\pm$ 0.7	–	–	87.6 $\pm$ 0.2	–	–
doxorubicin	–	0.7 $\pm$ 0.04	–	–	1.6 $\pm$ 0.2	–	–

IC₅₀ (in $\mu\text{mol/L}$) = concentration required in order to reduce cell viability at 50%; SI (IC₅₀/MIC) = selectivity index; MRC-5 = lung fibroblast cells; A549 = lung epithelial cells; Mt = *Mycobacterium tuberculosis*; Ab = *Acinetobacter baumannii*; – not determined.

Fonte: Elaborado pelo autor

2.2.4. Genotoxic evaluation of **MAC 4**

In order to further investigate the safety of **MAC 4**, we evaluated its genotoxic effects on human cells by the single cell gel electrophoresis assay (alkaline comet assay). This method is able to measure the damage induced by oxidizing, alkylating or intercalating agents in the DNA of individual cells (TICE et al., 2000; GUNASEKARANA, RAJ & CHAND, 2015). A549 cells were exposed to **MAC 4** at $\frac{1}{4}\text{IC}_{50}$ and $\frac{1}{2}\text{IC}_{50}$ (19.8 and 39.7 $\mu\text{mol/L}$, respectively) for 24 h, and their nuclei were submitted to electrophoresis. The A549 line was selected because it was the most sensitive to **MAC 4**, and concentrations lower than IC₅₀ values were used in order to guarantee high percentages of metabolically viable cells. Hydrogen peroxide was used as a reference genotoxic compound.

Figure 3 presents the **MAC 4** genotoxic parameters, including the tail moment and the percentage of DNA in tail, which are directly proportional to the DNA break (AZQUETA & COLLINS, 2013). A549 cells exposed to **MAC 4** at $\frac{1}{4}\text{IC}_{50}$ and $\frac{1}{2}\text{IC}_{50}$ did not show significant

genotoxic effects compared to the negative control (untreated cells). In addition, the comparison with the genotoxic parameters of cells treated with H₂O₂ (positive control) corroborated the weak genotoxic effect of **MAC 4**. Furthermore, **MAC 4** genotoxic parameters were similar to the **CUR** ones, and **CUR** was considered safe for humans (HEWLINGS & KALMAN, 2017).

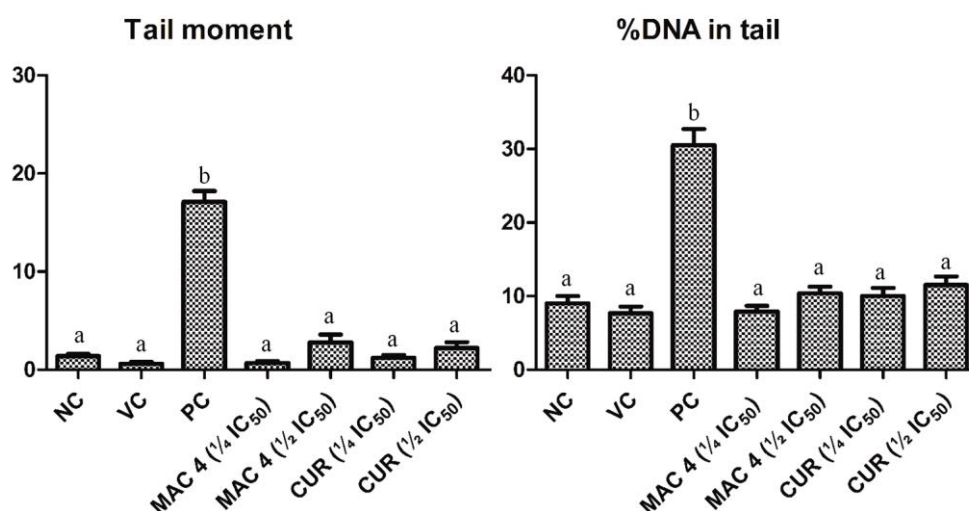


Figure 3. Genotoxic parameters of **MAC 4** against A549 cell line measured by tail moment and percentage of DNA in tail using the alkaline comet assay. **NC** = negative control (untreated cells); **VC** = vehicle control (cells treated with 1% DMSO); **PC** = positive control (cells treated with H₂O₂ at 1 mmol/L). 1/4IC₅₀ values of **MAC 4** and **CUR** = 19.8 and 21.9 µmol/L, respectively; 1/2IC₅₀ values of **MAC 4** and **CUR** = 39.7 and 43.8 µmol/L, respectively. ANOVA with Tukey's post-test NC versus treatment. The same letters do not differ significantly at 5% level in comparison to all groups (a), different letters significantly differ at 5% level in comparison to all groups (b).

Fonte: Elaborado pelo autor

2.2.5. Evaluation of **MAC 4** chemical stability

One of the main factors that hinder the clinical use of **CUR** is its poor stability under physiological conditions (NELSON et al., 2017). In order to assess whether the new monocarbonyl analog has overcome this limitation, we investigated **MAC 4** stability at pH 7.4 and 37 °C by monitoring the decrease in its UV–Vis absorption for 24 h (Figure 4). The initial concentration of **MAC 4** reduced only 28% after 24 h, while **CUR** initial concentration reduced 57%. This indicates that **MAC 4** was more stable than its parent compound. Our findings corroborated other investigations that also identified an increase in the chemical stability of

monocarbonyl analogs when compared with **CUR** (ZHANG et al., 2014; ARSHAD et al., 2017), supporting the hypothesis that the β -diketone moiety is correlated with the poor chemical stability of this natural product (SHETTY et al., 2015).

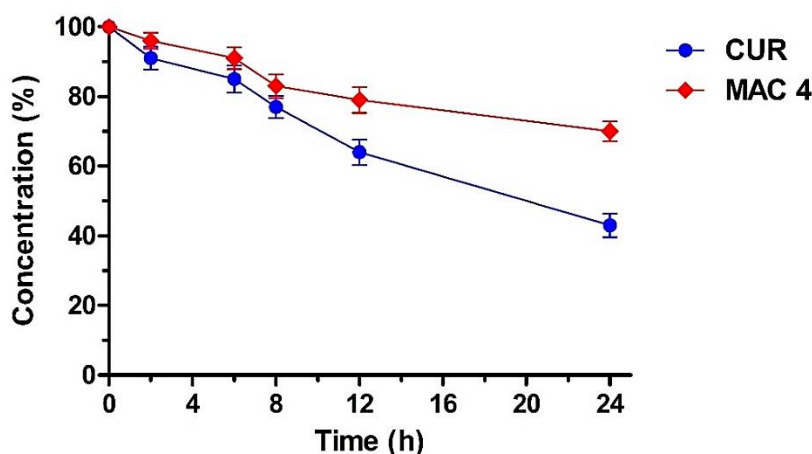


Figure 4. Chemical stability of **MAC 4** and **CUR** (pH 7.4 and 37 °C) by monitoring the decrease in their maximum absorbance for 24 h. Data were represented as means $\pm$ SEMs and expressed as percentage.

Fonte: Elaborado pelo autor

2.2.6. Effects of **MAC 4** on the divisome of *B. subtilis*

In order to investigate whether cell division is a target for the new curcumin analog, we evaluated the effects of **MAC 4** on the divisome using *B. subtilis* strain expressing FtsZ-GFP (labeled for divisome). *B. subtilis* was selected because this species is commonly used in studies on the antibacterial action mode of new agents (KRÓL et al., 2015; KUNAL et al., 2020). *B. subtilis* was treated with **MAC 4** at $\frac{1}{2}$ MIC (73.6 μ mol/L) for up to 30 min and analyzed under the microscope. Hexyl gallate was used as a reference compound for divisome disruption (KRÓL et al., 2015).

Figure 5 depicts phase contrast and fluorescence microscopy representative images of the negative and positive controls, and treatment with **MAC 4** after 15 and 30 min. Untreated *B. subtilis* (negative control) showed septation normal pattern (Figure 5A). The divisome (FtsZ-GFP) was located in the middle of the cells and shown as fluorescent bars perpendicular to the long axis of the rods. The same pattern was observed for *B. subtilis* exposed to 1% DMSO (vehicle control) (data not shown). In Figure 5B, *B. subtilis* treated with hexyl gallate (positive control) had complete divisome disruption, with accumulation of fluorescence in the cytoplasm.

When *B. subtilis* was exposed to **MAC 4** at $\frac{1}{2}$ MIC after 15 min there was a divisome formation and stability interference (Figure 5C). Although some divisomes were detected, there was a change in the septation pattern. After 30 min of treatment with **MAC 4** at $\frac{1}{2}$ MIC, the divisomes were completely unstructured (Figure 5D). Previous investigations demonstrated that **CUR** completely disrupted the *B. subtilis* divisome after 15 min (RAI et al., 2008; MORÃO et al., 2019).

The cell division machinery is very complex, and it makes the localization of each protein involved in this process crucial for the performance of their functions. FtsZ protein constitutes the scaffold at the center of the dividing cells, and it recruits and coordinates several other proteins to accomplish the tasks of proper chromosome segregation, membrane invagination and remodeling, and cell wall synthesis (HURLEY et al., 2016). Since the cell division apparatus is associated with the membrane, membrane perturbations may alter the spatial organization of proteins involved in bacterial division (STRAHL & HAMOEN, 2010). Thus, we investigated the ability of **MAC 4** at $\frac{1}{2}$ MIC to perturb the membrane of *B. subtilis* (strain 168). Nisin was used as a reference compound for membrane disruption (SUN et al., 2017).

Figure 6 shows phase contrast and fluorescence microscopy representative images of *B. subtilis* stained with the nucleic acid dyes SYTO9 and propidium iodide (IP). Both of them stain the nucleoid of cells with damaged membrane (in red) and intact membrane (in blue). Most untreated *B. subtilis* cells were stained in blue (Figure 6A). The same pattern was observed when *B. subtilis* was exposed to 1% DMSO (data not shown). However, *B. subtilis* treated with nisin were stained in red (Figure 6B). Exposure to **MAC 4** at $\frac{1}{2}$ MIC, in both periods of 15 and 30 min (Figure 6C and 6D, respectively), led to a prevalence of blue stained cells, indicating membrane integrity, which corroborated the effect of **MAC 4** on the *B. subtilis* divisome.

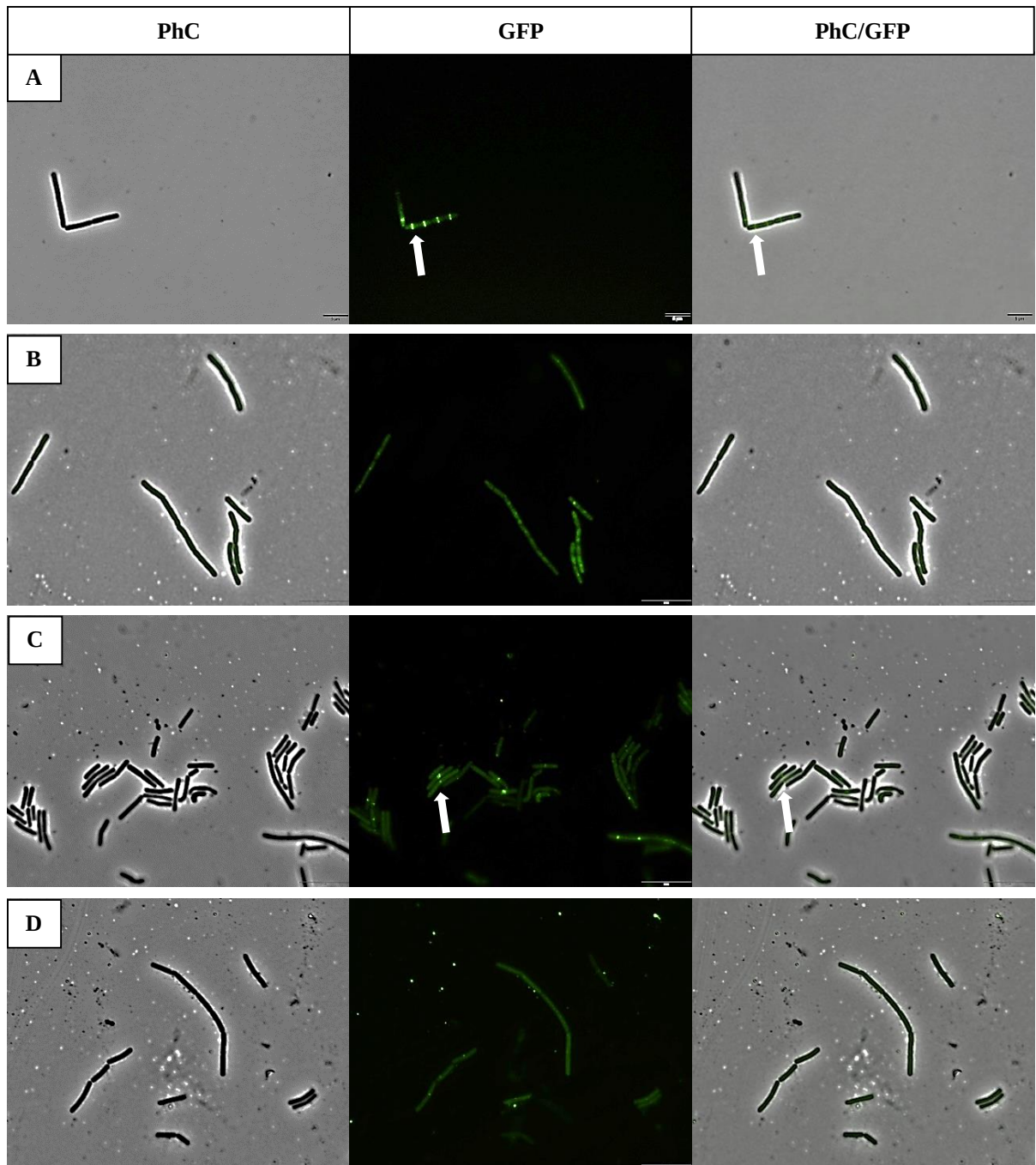


Figure 5. Effects of **MAC 4** on the divisome of *B. subtilis* expressing FtsZ-GFP. **(A)** Untreated *B. subtilis* (negative control); **(B)** *B. subtilis* treated with hexyl gallate at 255 $\mu\text{mol/L}$ after 30 min (positive control); **(C)** *B. subtilis* treated with **MAC 4** at $\frac{1}{2}\text{MIC}$ (73.6 $\mu\text{mol/L}$) after 15 min; **(D)** *B. subtilis* treated with **MAC 4** at $\frac{1}{2}\text{MIC}$ after 30 min. Magnification: 100 $\times$; bar 5 μm . The arrows indicate the intact divisome.

Fonte: Elaborado pelo autor

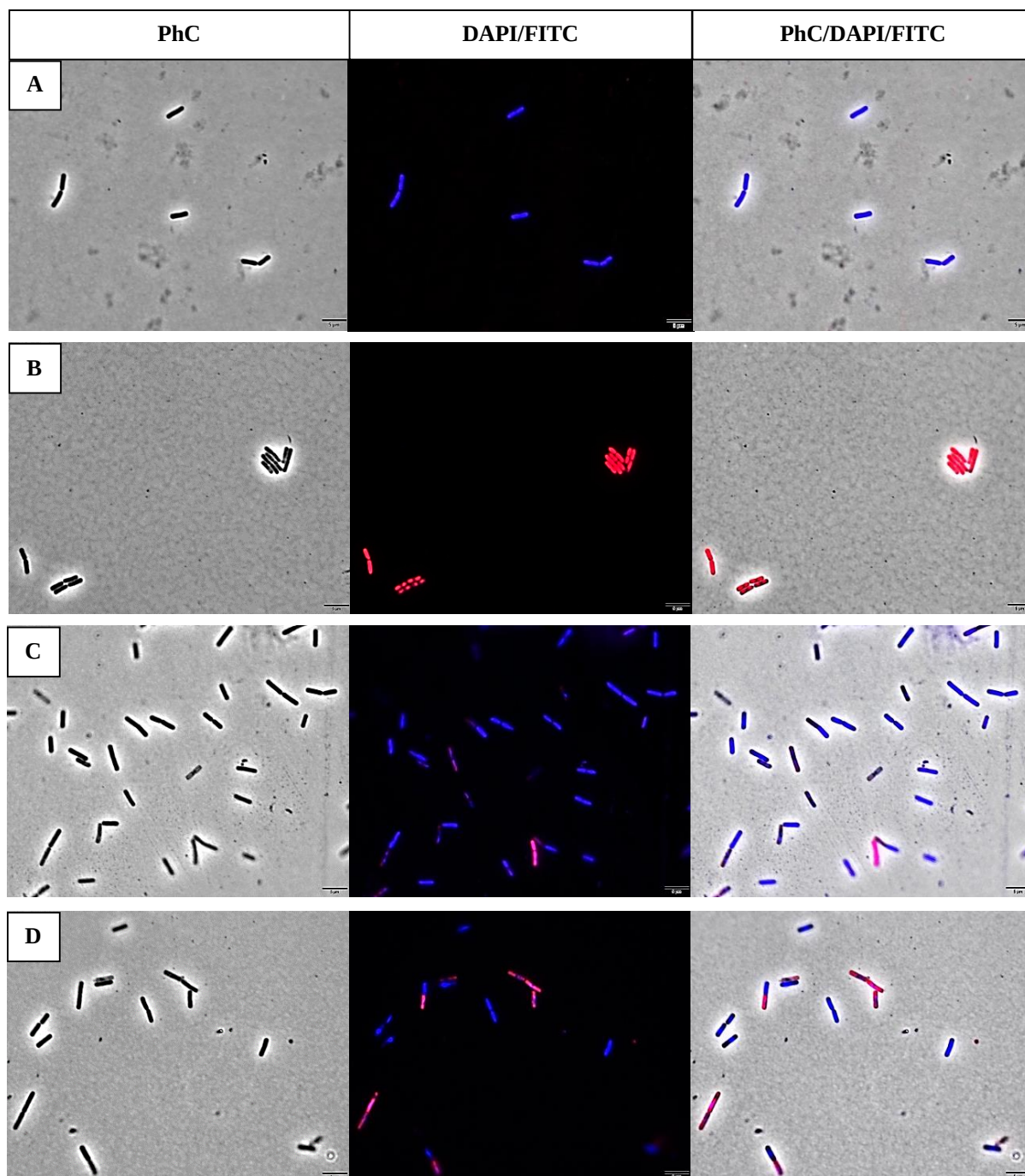


Figure 6. Effects of **MAC 4** on the membrane of *B. subtilis* (strain 168) stained with Live/Dead Baclight kit. **(A)** Untreated *B. subtilis* (negative control); **(B)** *B. subtilis* treated with nisin at 1.5 $\mu\text{mol/L}$ after 30 min (positive control); **(C)** *B. subtilis* treated with **MAC 4** at $\frac{1}{2}\text{MIC}$ (73.6 $\mu\text{mol/L}$) after 15 min; **(D)** *B. subtilis* treated with **MAC 4** at $\frac{1}{2}\text{MIC}$ after 30 min. Magnification: 100 $\times$; bar 5 μm . Blue cells: whole membrane. Pink-red cells: damaged membrane.

Fonte: Elaborado pelo autor

2.2.7. Effects of **MAC 4** on the GTPase activity of *B. subtilis* FtsZ

In order to verify whether the divisome disruption after treatment with **MAC 4** is related to a direct action on FtsZ protein, we evaluated its ability to affect the GTPase activity of FtsZ from *B. subtilis*. The GTPase activity regulates the assembly dynamics of FtsZ in protofilaments. Therefore, compounds that affect the GTP hydrolysis are potential inhibitors of this protein (SUN et al., 2014, GROUNDWATER et al., 2017). We incubated FtsZ with **MAC 4** at MIC and 2×MIC (147 and 294 µmol/L, respectively), and its GTPase activity was measured. The protein incubated with 1% DMSO was used as a negative control. The GTPase activity of FtsZ was weakly increased by exposure to **MAC 4** at MIC and 2×MIC (15% and 23%, respectively) when compared to the negative control. This increase was not significant, and it indicates that the ability of **MAC 4** to disrupt the divisome is not related to a direct action on the GTPase activity of FtsZ from *B. subtilis*.

In a previous study with **SCUR**, a monocarbonyl curcuminoid, we also did not identify the ability of this compound to alter the FtsZ GTPase activity from *B. subtilis* (MORÃO et al., 2019). It suggests that the β-diketone moiety was important for the **CUR** action on the FtsZ GTPase activity. This hypothesis was supported by a previous *in silico* investigation that demonstrated the β-diketone in specific interactions with Glycine residues 21 and 22 of FtsZ from *B. subtilis* (KAUR et al., 2010). Our current study with **MAC 4** corroborated this hypothesis. However, the β-diketone moiety does not appear to be fundamental for the action on FtsZ, since the curcuminoid 3,3'-dihydroxycurcumin, which maintains the β-diketone, was also not able to affect the GTPase activity of FtsZ from *B. subtilis* (POLAQUINI et al., 2019).

2.3. CONCLUSION

In summary, we synthesized a series of unsymmetrical monocarbonyl curcuminoids derived from acetone as part of our ongoing search for antibacterial agents based on the **CUR** structure. **MACs 4, 6** and **8** showed potent activity against Gram-negative and Gram-positive species, and *M. tuberculosis*. The assessment of toxicity on human cells revealed **MAC 4** as the most selective curcuminoid. In addition, preliminary investigations of genotoxicity and chemical stability indicated that **MAC 4** is potentially safe and stable. **MAC 4** action mode involved divisome disruption, which is an innovative target for the development of new antibacterial agents. Altogether, our results corroborate the **CUR** potential as a privileged framework for the discovery of compounds for bacterial infection treatments.

2.4. EXPERIMENTAL

2.4.1. Chemistry

2.4.1.1. Material and instruments

CUR and all reagents (Merck, Kenilworth, USA), solvents and catalysts (Synth, Diadema, Brazil) were purchased from commercial sources. Reactions were monitored using thin-layer chromatography (TLC) (Merck, Kenilworth, USA). Silica-gel (200–300 mesh) and LH-20 (Sephadex) (Merck, Kenilworth, USA) were used for column chromatography. Melting points (MP) were determined in Tecnopon PFM-IIV apparatus (MS Tecnopon Instrumentação, Piracicaba, Brazil) and were uncorrected. Nuclear magnetic resonance spectra (^1H and ^{13}C NMR) were carried out on Bruker Avance III 14.1 T (600 MHz) and Bruker Avance III 9.4 T (400 MHz) spectrometers (Bruker, Billerica, USA), using CDCl_3 or $\text{DMSO}-d_6$ as solvents and TMS as internal standard (Merck, Kenilworth, USA). Chemical shifts (δ) and coupling constants (J) were expressed in ppm and Hz, respectively. Multiplicities were reported as singlet (s), doublet (d) and doublet of doublet (dd). Electrospray ionization mass spectrometry data (ESI-MS) were obtained on a Thermo LCQ Fleet spectrometer (Thermo Fisher Scientific, Waltham, USA).

2.4.1.2. Synthesis of SCUR

SCUR was synthesized by reaction between acetone and vanillin as previously described by Morão and co-authors (MORÃO et al., 2019).

2.4.1.3. Synthesis of MACs 1–3 and 5–8

MACs 1–3 and 5–8 were synthesized in two steps.

2.4.1.3.1. Synthesis of benzylideneacetone (1). A solution of benzaldehyde (8 mmol) in acetone (15 mL) was cooled in an ice bath for 10 min. Acetonic suspension of NaOH (1 mL, 0.5 mol/L) was added dropwise to the reaction mixture. The suspension was stirred at room temperature for 1 h. Reaction medium was poured onto crushed ice. The crude product was partitioned with ethyl acetate (3×25 mL), and the combined organic phase was concentrated under reduced pressure. Compound **1** was recrystallized in cold EtOH.

2.4.1.3.2. Synthesis of MACs 1–3 and 5–8. To a solution of benzylideneacetone (**1**) (5.2 mmol) in EtOH (10 mL) was added the respective benzaldehyde derivative (5.0 mmol).

Catalytic amounts of H₂SO₄ were added to the reaction mixture, and the solution was stirred at room temperature for 24–48 h. Reaction medium was poured onto crushed ice. The crude product was partitioned with ethyl acetate (3 × 25 mL), and the combined organic phase was concentrated under reduced pressure. **MACs 1, 6 and 8** were purified by gel filtration column chromatography (LH-20) using EtOH as eluent. **MAC 2** was recrystallized in EtOH. **MACs 3, 5 and 7** were purified by column chromatography over silica gel using mixtures of hexane and ethyl acetate [8:2], [9:1] and [9:1], respectively, as eluents.

2.4.1.4. Synthesis of MAC 4

MAC 4 was synthesized in three steps (KIM et al., 2008), in addition to the benzylideneacetone preparation.

2.4.1.4.1. Synthesis of 3,4-bis(methoxymethoxy)benzaldehyde (2). A suspension of K₂CO₃ (50 mmol) in dry acetone (25 mL) was cooled in an ice bath for 10 min. After this period, 3,4-dihydroxybenzaldehyde (5 mmol) and methoxymethyl chloride (MOMCl) (20 mmol) were added sequentially. The reaction mixture was stirred at room temperature for 1 h under N₂ atmosphere. The suspension was diluted with deionized water (25 mL), and the crude product extracted with ethyl acetate (3 × 25 mL). The combined organic layer was concentrated under reduced pressure. Compound **2** was purified by column chromatography over silica gel using mixture of hexane and ethyl acetate [7:3] as eluent.

2.4.1.4.2. Synthesis of compound 3. A solution of compound **2** (2 mmol) and benzylideneacetone (**1**) (2.2 mmol) in EtOH (7 mL) was cooled in an ice bath for 10 min. Ethanolic solution of NaOH (1 mL, 1 mol/L) was added dropwise to the reaction mixture. The solution was stirred at room temperature for 8 h under N₂ atmosphere. Reaction medium was poured onto crushed ice. The crude product was partitioned with ethyl acetate (3 × 15 mL), and the combined organic phase was concentrated under reduced pressure. Compound **3** was purified by column chromatography over silica gel using mixture of hexane and ethyl acetate [6:4] as eluent.

2.4.1.4.3. Synthesis of MAC 4. A solution of compound **3** (1 mmol) in EtOH (5 mL) and 33% HCl (0.3 mL) was stirred at room temperature for 48 h under N₂ atmosphere. Reaction medium was poured onto crushed ice. Precipitated crude product was filtered, washed with cold water and dried at room temperature. **MAC 4** was purified by gel filtration column chromatography (LH-20) using EtOH as eluent.

2.4.2. Antibacterial assay

Microorganisms used in this study were *A. baumannii* (ATCC 19606), *P. aeruginosa* (ATCC 27853), *E. coli* (ATCC 43895), methicillin-sensitive *S. aureus* (ATCC 25923), methicillin-resistant *S. aureus* (ATCC 33591), *S. epidermidis* (ATCC 12228), *E. faecalis* (ATCC 29212) and *B. subtilis* (strain 168). The MIC and MBC values were determined by the microdilution method according to the Clinical and Laboratory Standards Institute (CLSI, 2012), with minor modifications described in previous work (MARQUES et al., 2019a). **CUR**, **SCUR** and **MACs 1–8** were diluted in DMSO and assayed in concentrations ranging from 500 to 1.0 $\mu\text{mol/L}$. Gentamicin and vancomycin were used as reference antibiotics against Gram-negative and Gram-positive bacterial species, respectively. Three independent assays were performed. The MIC value was defined as the lowest concentration of compound that inhibited visible microbial growth. The MBC value was defined as the lowest concentration of compound that allowed no visible growth on the solid medium.

2.4.3. Antitubercular assay

The antitubercular activity was evaluated against *M. tuberculosis* H₃₇Rv (ATCC 27294). The MIC values were determined by Resazurin Microtiter Assay (PALOMINO et al., 2002), with minor modifications described in previous work (POLAQUINI et al., 2017). **CUR**, **SCUR** and **MACs 1–8** were prepared in DMSO and assayed in concentrations ranging from 500 to 1.0 $\mu\text{mol/L}$. Isoniazid was used as a reference antitubercular drug. Three independent assays were performed. The MIC value was defined as the lowest compound concentration at which 90% of the cells are infeasible relative to the control.

2.4.4. Cytotoxicity assay

The cell lines used in this study were MRC-5 (normal human lung fibroblast cell line, ATCC CCL-171) and A549 (human lung adenocarcinoma epithelial cell line, ATCC CCL-185). The IC₅₀ values were determined as described by Silva and co-authors (SILVA et al., 2017). Cells were treated with **CUR** and **MACs 4, 6 and 8** in concentrations ranging from 100 to 1 $\mu\text{mol/L}$. Doxorubicin was used as a reference cytotoxic drug. Three independent assays were performed. The IC₅₀ value was calculated from an analytical curve through a regression analysis and it represents the concentration required to reduce the viability of cells at 50%.

2.4.5. Genotoxicity assay

The cell line used in this investigation was the A549 (human lung adenocarcinoma epithelial cell line, ATCC CCL-185). The genotoxicity was assessed using the alkaline version of the comet assay (single cell gel electrophoresis) (TICE et al., 2000). Minor modifications in this protocol and their detailed procedures were reported in previous work by Polaquini and co-authors (POLAQUINI et al., 2019). Cells were treated with **CUR** and **MAC 4** at $\frac{1}{2}IC_{50}$ and $\frac{1}{4}IC_{50}$ values. Hydrogen peroxide (1 mmol/L) was used as a reference genotoxic compound. The level of DNA damage was assessed by image analysis system (TriTekCometScore™ 1.5, 2006) and the percentages of DNA in the tail and tail moment were calculated. The results were expressed as the mean $\pm$ standard error of three independent experiments performed in duplicate.

2.4.6. Chemical stability assay

Chemical stability of **MAC 4** (at λ_{max} 375 nm) and **CUR** (at λ_{max} 426 nm) at 35 μ mol/L was analyzed by monitoring their UV–visible absorptions in phosphate buffer (pH 7.4) at 37 °C. Minor modifications in this protocol and their detailed procedures were reported in our previous work (POLAQUINI et al., 2019).

2.4.7. Phase contrast and fluorescence microscopy assays

The effect of **MAC 4** on the divisome of *B. subtilis* was investigated according to Morão and co-authors (MORÃO et al., 2019). *B. subtilis* (*amy::Pspac-ftsZ-gfpmut1*) was cultivated in presence of 0.02 mmol/L IPTG (isopropyl β -D-1-thiogalactopyranoside) to induce the expression of FtsZ-GFP (green fluorescent protein) from the *Pspac* promoter. The cells were exposed to **MAC 4** at $\frac{1}{2}MIC$ (73.6 μ mol/L) for 15 and 30 min at 29 °C. Hexyl gallate (255 μ mol/L) was used as a reference divisome disruptor (KRÓL et al., 2015). The membrane disruption assay was performed as described by Polaquini and co-authors (POLAQUINI et al., 2019). *B. subtilis* (strain 168) was treated with **MAC 4** at the $\frac{1}{2}MIC$ for 15 and 30 min at 29 °C. Membrane integrity was assessed using the Live/Dead BacLight Bacterial Viability kit (Thermo-Scientific L7012). Nisin (1.5 μ mol/L) was used as a positive control for membrane permeability. All visualizations were carried out using an Olympus BX61 microscope (Olympus, Melville, USA) equipped with a monochromatic camera OrcaFlash 2.8

(Hamamatsu, Shizuoka, Japan). Image documentation and processing were done using the software CellsSen Dimension (Olympus, Melville, USA).

2.4.8. Anti-FtsZ assay

FtsZ of *B. subtilis* was expressed and purified using the ammonium sulfate precipitation method as described by Król and Scheffers (KRÓL & SCHEFFERS, 2013). FtsZ GTP hydrolysis rate was determined using the malachite green phosphate assay as described by Król and co-authors (KRÓL et al., 2015).

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3. CAPÍTULO III

Antimycobacterial Activity of Simplified Monocarbonyl Curcuminoids

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ABSTRACT

According to the World Health Organization, tuberculosis is one of the top 10 causes of death in the world. It is an infectious disease caused by some mycobacterial species and it most often affects the lungs. Nowadays, there is an increasing need for the discovery of new antitubercular agents due to the emergence of multidrug-resistant strains. In this context, we synthesized and investigated the antimycobacterial activity of two series of simplified monocarbonyl analogs (**SMACs**) based on the **MAC 4** framework, an unsymmetrical monocarbonyl curcuminoid with promising antitubercular effects. **SMACs 30** and **34** showed potent activity against *Mycobacterium tuberculosis* H₃₇Rv, with MIC (minimum inhibitory concentration) values of 7.2 and 4.5 $\mu\text{mol/L}$, respectively. *In silico* predictions showed that **SMACs 30** and **34** had suitable physico-chemical properties and pharmacokinetic parameters. In addition, these compounds did not violate the drug-likeness rules proposed by Lipinski, Veber and Egan. The investigation regarding their toxicity on human lung cells (MRC-5 line) revealed that **SMACs 30** and **34** had low toxicity and were more selective than **MAC 4**, with SI (selectivity index) values of 27.2 and 23.3, respectively. Besides, the two simplified analogs were more stable than **MAC 4** in phosphate buffer (pH 7.4) for 24 h at 37 °C. **SMACs 30** and **34** were also active against *M. tuberculosis* clinical isolates with multidrug resistance, with MIC values ranging from 3.8 to 81.4 $\mu\text{mol/L}$. The assessment of **SMACs 30** and **34** effects against Gram-negative and Gram-positive bacterial species indicated high specificity towards *M. tuberculosis*. Taken together, these data indicated that the molecular simplification provided new candidates for the future development of antitubercular agents.

3.1. INTRODUCTION

Tuberculosis is an infectious disease caused by some mycobacterial species, especially by the *Mycobacterium tuberculosis* (YATHURSAN et al., 2019). It has caused the death of approximately 1 billion people in the past two centuries, and for 2020, the World Health Organization estimates the death of more than 1 million individuals (WHO, 2019). Nowadays, tuberculosis is among the top 10 causes of death in the world and it is the leading cause of death from a single infectious agent (WHO, 2019). Furthermore, co-infection with both HIV or SARS-CoV-2 may increase the severity of tuberculosis (BELL & NOURSADEGHI, 2018; LIU et al., 2020).

The first-line drugs used to treat tuberculosis patients have been the same since the 70's (SOUZA & VASCONCELOS, 2005). The treatment consists of two stages: for 2 months, a combination of isoniazid, pyrazinamide, rifampicin and ethambutol, followed by the association of isoniazid and rifampicin for another 4 months (CAMPANIÇO, MOREIRA & LOPES, 2018). Nevertheless, the treatment period can be extended to 18–24 months for patients infected with resistant strains, with the inclusion of some second-line drugs, such as moxifloxacin and amikacin (FALZON et al., 2017; KOCH, COX & MIZRAHI, 2018). The long period of treatment and its adverse effects are factors that contribute to reducing patients' adherence to treatment (WHO, 2019). In 2010, two new anti-tuberculosis drugs were approved, bedaquiline and delamanid (ESPOSITO, BIANCHINI & BLASI, 2015), ending a period of more than 4 decades without the approval of a new drug against tuberculosis. However, the adaptive capacity of *M. tuberculosis* has already led to the emergence of resistant strains to the two new antimycobacterial drugs (BLOEMBERG, GAGNEUX & BÖTTGER, 2015). Considering these problems, the development of new agents against *M. tuberculosis*, which can especially combat multidrug-resistant strains, is crucial.

Our group has been researching new therapeutic agents for tuberculosis treatment. We have been engaged in the study of unsaturated ketones based on the structure of bioactive natural products, such as cinnamaldehyde and curcumin (**CUR**) (POLAQUINI et al., 2017; POLAQUINI et al., 2019). We recently reported the antimycobacterial effects of a new unsymmetrical monocarbonyl analog of **CUR** (**MAC 4**) (Chapter II). It showed potent activity against *M. tuberculosis*, and it also demonstrated to be a potentially safe and stable compound. However, its synthesis required four reaction steps (Chapter II), a factor that hinders its preparation in large scale.

In the search for compounds with higher synthetic accessibility, but maintaining **MAC 4** antitubercular effects, we synthesized two series of simplified analogs (arylbutanoids) based on **MAC 4** framework (diphenylpentanoid). In series I, we investigated the effects of electron-donating and electron-withdrawing groups on the benzene ring, while in series II, we evaluated the effects of replacing the benzene ring with other aromatic rings concerning the activity against *M. tuberculosis* H₃₇Rv. The most potent analogs (MIC $\leq$ 50 μ mol/L) were subjected to pharmacokinetic and drug-likeness filters, and their toxicity on human pulmonary cells and the selectivity indexes were determined. The most promising compounds (**SMACs 30** and **34**) were investigated regarding their chemical stability, and their effects against clinical isolates of *M. tuberculosis* with multidrug resistance. In addition, we also evaluated **SMACs 30** and **34** activities against Gram-negative and Gram-positive bacterial species.

3.2. RESULTS AND DISCUSSION

3.2.1. Chemistry

SMACs 1–30 (series I) were synthesized by aldol condensation reactions (TORREZAN et al., 2020), with yields of 29–89%. The acetone was mono-condensed with benzaldehyde derivatives substituted by electron-donating (OH, OMe, N(Me), Me, Et, *i*-Pr, *t*-Bu and Ph) and electron-withdrawing (CN, NO₂, CF₃, OCF₃, F, Cl and Br) groups under basic catalysis and at room temperature (Figure 1).

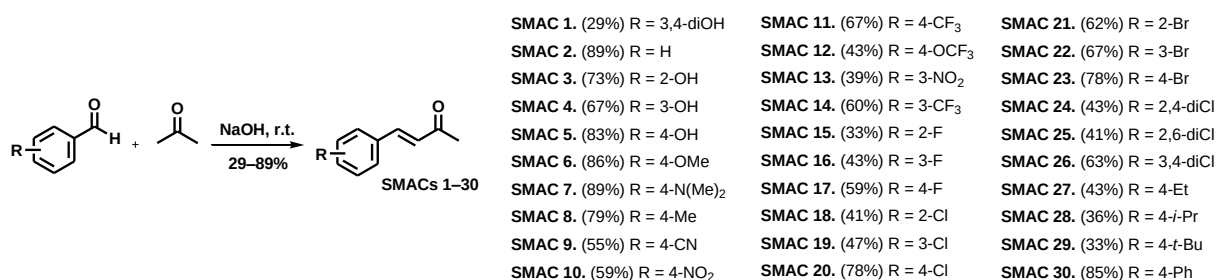


Figure 1. Synthesis of **SMACs 1–30** (series I).

Fonte: Elaborado pelo autor

Simplified curcuminoids of series II (**SMACs 31–40**) were also synthesized by aldol condensation reactions, under basic catalysis and at room temperature, with yields of 36–92%. However, acetone was mono-condensed with other aromatic aldehydes, including

pyrrolecarboxaldehyde, furaldehyde, thiophenecarboxaldehyde, pyridinecarboxaldehyde, naphthaldehyde and ferrocenecarboxaldehyde (Figure 2).

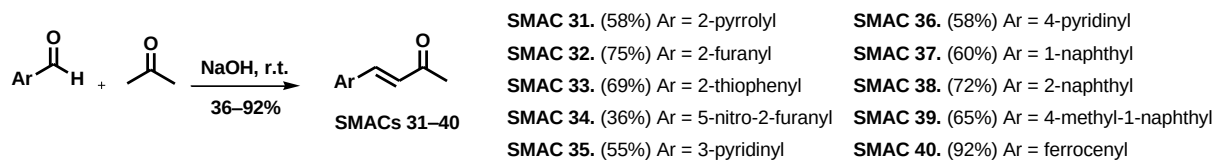


Figure 2. Synthesis of **SMACs 31–40** (series II).

Fonte: Elaborado pelo autor

The structures of the synthesized compounds were confirmed by their melting point and ^1H and ^{13}C NMR spectra data analyses. They were then compared to previous data. All spectra were presented in Supplementary Material. NMR parameters, including hydrogen and carbon chemical shifts (δ_{H} and δ_{C} in ppm), integrations, multiplicities, and coupling constants (J in Hz), corresponded to the proposed structures of **SMACs 1–40**.

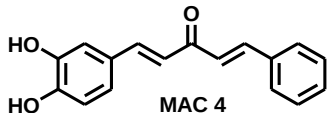
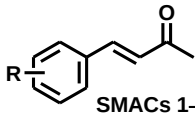
3.2.2. Anti-*M. tuberculosis* activity and structure-activity relationship (SAR)

The antitubercular activities of simplified monocarbonyl curcuminoids of series I (**SMACs 1–30**) and series II (**SMACs 31–40**) were evaluated against *M. tuberculosis* H₃₇Rv (ATCC 27294). Isoniazid was used as a reference antitubercular drug. The anti-*M. tuberculosis* potency of compounds was expressed as minimum inhibitory concentration (MIC) values in $\mu\text{mol/L}$ (Tables 1 and 2). The *n*-octanol/water partition coefficient theoretical values (*miLog P*) were predicted using the Molinspiration Cheminformatics property explorer (Tables 1 and 2) (MOLINSPIRATION CHEMINFORMATICS, 2020).

Previously, we described the **MAC 4** antitubercular activity, a new unsymmetrical monocarbonyl curcuminoid (diphenylpentanoid) designed from **CUR** (diphenylheptanoid). It had promising activity against *M. tuberculosis*, with MIC value of $5.6 \mu\text{mol/L}$ (Chapter II). In order to search for compounds with greater synthetic accessibility, but which maintain the **MAC 4** antitubercular effects, we evaluated two simplified monocarbonyl analogs (**SMACs 1** and **2**) designed from **MAC 4** framework. Phenylbutanoids **SMAC 1** (3,4-diOH) and **SMAC 2** (unsubstituted benzene ring) showed MIC values of 118 and $>150 \mu\text{mol/L}$, respectively. From these results, it was possible to confirm the importance of the presence of substituents on the benzene ring for antitubercular activity. Based on this fact, we investigated the preliminary

relationships regarding the substituents on the benzene ring of phenylbutanoids (series I) and their activity against *M. tuberculosis* H₃₇Rv (Table 1).

Table 1. Anti-*M. tuberculosis* H₃₇Rv activity of simplified monocarbonyl curcuminoids of series I (SMACs 1–30).

 MAC 4				 SMACs 1–30			
Series I	R	MIC	milog P*	Series I	R	MIC	milog P*
MAC 4**	–	5.6	–	SMAC 16	3-F	72.2	2.35
SMAC 1	3,4-diOH	118	1.24	SMAC 17	4-F	94.4	2.37
SMAC 2	H	>150	2.21	SMAC 18	2-Cl	26.7	2.66
SMAC 3	2-OH	26.6	1.97	SMAC 19	3-Cl	35.5	2.86
SMAC 4	3-OH	39.2	1.71	SMAC 20	4-Cl	55.8	2.89
SMAC 5	4-OH	52.3	1.73	SMAC 21	2-Br	29.5	2.79
SMAC 6	4-OMe	117	2.27	SMAC 22	3-Br	21.2	2.99
SMAC 7	4-N(Me) ₂	>150	2.31	SMAC 23	4-Br	9.2	3.02
SMAC 8	4-Me	>150	2.66	SMAC 24	2,4-diCl	12.1	3.29
SMAC 9	4-CN	12.1	1.96	SMAC 25	2,6-diCl	17.2	3.31
SMAC 10	4-NO ₂	9.9	2.17	SMAC 26	3,4-diCl	18.1	3.49
SMAC 11	4-CF ₃	31.7	3.10	SMAC 27	4-Et	>150	3.12
SMAC 12	4-OCF ₃	72.3	3.18	SMAC 28	4- <i>i</i> -Pr	57.9	3.72
SMAC 13	3-NO ₂	9.6	2.14	SMAC 29	4- <i>t</i> -Bu	23.6	3.92
SMAC 14	3-CF ₃	52.5	3.08	SMAC 30	4-Ph	7.2	4.00
SMAC 15	2-F	27.3	2.15	isoniazid	–	0.7	–

*Theoretical lipophilicity (MOLINSPIRATION CHEMINFORMATICS, 2020); **Chapter II; MIC (in μmol/L) = minimum inhibitory concentration; – not determined.

Fonte: Elaborado pelo autor

First, we assessed the effects of the number and the position of the hydroxyl group concerning the simplified analogs activity, since this substituent is present in the parent compound (MAC 4). Monohydroxylated analogs (SMACs 3–5, 26.6 μmol/L ≤ MIC ≤ 52.3 μmol/L) were more potent than the dihydroxylated analog (SMAC 1, MIC 118 μmol/L), suggesting that the reduction in the number of hydroxyl groups on the aromatic ring increases the antitubercular activity. Additionally, the comparison among monohydroxylated analogs indicated the following order of antitubercular potency: SMAC 5 (4-OH) < SMAC 4 (3-OH) < SMAC 3 (2-OH). Taken together, the results demonstrated that the presence of only one

weak-acidic, hydrophilic and electron-donating substituent at position 2 on the aromatic ring of phenylbutanoids enhanced the activity against *M. tuberculosis*.

Second, we compared the MIC values of **SMACs 5–8**, which are analogs substituted by the OH, OMe, N(Me)₂ and Me groups, respectively, at position 4 on the benzene ring. It was done in order to investigate the importance of electron-donating groups for the bioactivity. **SMACs 5–8** showed MIC values of 52.3, 117, >150 and >150 µmol/L, respectively. The comparison among **SMACs 5–8** indicated that the electron donation effect on the benzene ring was not relevant for the antitubercular activity. However, our findings confirmed the importance of the presence of more hydrophilic groups for the antitubercular activity, since the analogs substituted by OH and OMe groups (*miLog* P 1.73 and 2.27, respectively) were more active than the analogs substituted by N(Me)₂ and Me groups (*miLog* P 2.31 and 2.66, respectively). In addition, bioactivity also seems to be related to the acidity of the OH group, since neutral (OMe and Me) and basic (N(Me)₂) groups did not improve antitubercular potency.

Third, we tested a set of phenylbutanoids carrying CN, NO₂, CF₃ and OCF₃ groups at position 4 on the benzene ring (**SMACs 9–12**, respectively), in order to establish the effect of hydrophilicity on the bioactivity of simplified curcuminoids substituted by electron-withdrawing groups. **SMACs 9–12** showed MIC values of 12.1, 9.9, 31.7 and 72.3 µmol/L, respectively. The analogs substituted by CN and NO₂ groups (*miLog* P 1.96 and 2.17, respectively) were more active than the analogs substituted by CF₃ and OCF₃ groups (*miLog* P 3.10 and 3.18, respectively). This fact corroborated the relevance of the hydrophilicity for the antitubercular activity. Nevertheless, the comparison among **SMACs 5–8** (electron-donating groups) and **SMACs 9–12** (electron-withdrawing groups) suggested that, although hydrophilicity has been an important parameter, the electron-withdrawing effect was pivotal for the phenylbutanoids activity against *M. tuberculosis*.

Fourth, we investigated the effect of the electron-withdrawing groups position concerning the phenylbutanoids bioactivity. For regioisomers **SMAC 10** (4-NO₂) and **SMAC 13** (3-NO₂), the position of the NO₂ group did not affect the antitubercular activity (MIC 9.9 and 9.6 µmol/L, respectively). On the other hand, **SMAC 11** (4-CF₃) was more active than its regioisomer **SMAC 14** (3-CF₃) (MIC 31.7 and 52.5 µmol/L, respectively). In order to further investigate the effect of the electron-withdrawing groups position, we evaluated a set of monohalogenated analogs (**SMACs 15–23**). For both fluorinated or chlorinated curcuminoids, the antitubercular potency order regarding the halogen position on the benzene ring was as follows: 4 < 3 < 2. These analogs showed the same potency order as monohydroxylated

curcuminoids (**SMACs 3–5**). On the other hand, for brominated analogs, the antitubercular potency order regarding the halogen position on the aromatic ring was as follows: $2 < 3 < 4$. This change in the potency order may be due to the steric effect of the bromine atom.

Fifth, we also investigated the effect of the number of electron-withdrawing groups on antitubercular activity by comparing the MIC values of the mono- (**SMACs 18–20**) and dichlorinated (**SMACs 24–26**) analogs. Dichlorinated curcuminoids ($12.1 \mu\text{mol/L} \leq \text{MIC} \leq 18.1 \mu\text{mol/L}$) showed higher potency than monochlorinated analogs ($26.7 \mu\text{mol/L} \leq \text{MIC} \leq 55.8 \mu\text{mol/L}$). This finding may be related to the intensification of the electron withdrawing effect due to the insertion of a second electron-withdrawing group. Furthermore, the increase in lipophilicity of dichlorinated analogs may also have corroborated the improvement in the bioactivity.

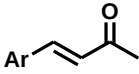
Sixth, although analogs substituted by more hydrophilic groups (OH, CN and NO_2) appear to be important for the bioactivity, some curcuminoids carrying lipophilic groups showed potent antitubercular activity, such as **SMACs 23** (4-Br), **24** (2,4-diCl), **25** (2,6-diCl) and **26** (3,4-diCl) (*miLog P* 3.02, 3.29, 3.31 and 3.49, respectively). Fernandes and co-authors reported that lipophilicity is an important physico-chemical property in the development of new antitubercular agents. They described that the most compounds which were active against *M. tuberculosis* had Log P values ranging from 2.5 to 6.0. The lipophilicity increase facilitates the permeation of compounds through *M. tuberculosis* mycolic acid membrane (FERNANDES et al., 2015). Based on this fact, we hypothesized that the substantial increase in lipophilicity could lead to more active compounds. Thus, we tested a set of analogs substituted by Me, Et, *i*-Pr, *t*-Bu and Ph groups (**SMACs 8** and **27–30**), with *miLog P* values of 2.66, 3.12, 3.72, 3.92 and 4.00, respectively. **SMACs 8** and **27–30** showed MIC values of >150, >150, 57.9, 23.6 and 7.2 $\mu\text{mol/L}$, respectively. The results confirmed that a substantial increase in lipophilicity resulted in improved potency against *M. tuberculosis*.

In general, the series I SAR data indicated that the electron-withdrawing effect on the benzene ring was pivotal for the activity against *M. tuberculosis*. Moreover, although hydrophilicity appears to be relevant for the bioactivity of phenylbutanoids, the substantial increase in the analogs lipophilicity improves antitubercular potency. In addition, preliminary data on the effect of the substituents position on the benzene ring suggest that position 2 was the most relevant for the activity against *M. tuberculosis*.

The analogs of series II were designed in order to investigate the effects of replacing the benzene ring with other aromatic rings (**SMACs 31–40**) (Table 2). First, comparisons were

made in order to determine the effect of replacing the phenyl ring (**SMAC 2**) with 5-membered heteroaryl rings with six π -electrons, including 2-pyrrolyl (**SMAC 31**), 2-furanyl (**SMAC 32**) and 2-thiophenyl (**SMAC 33**). **SMACs 2** and **31–33** were inactive against *M. tuberculosis* (MIC >150 $\mu\text{mol/L}$). The results indicated that the substitution of the benzene ring by the pyrrole, furan or thiophene rings did not affect the curcuminoids bioactivity.

Table 2. Anti-*M. tuberculosis* H₃₇Rv activity of simplified monocarbonyl analogs of series II (**SMACs 31–40**).

 SMACs 31–40			
Series II	Ar	MIC	<i>m</i> log P*
SMAC 31	2-pyrrolyl	>150	1.18
SMAC 32	2-furanyl	>150	1.29
SMAC 33	2-thiophenyl	>150	1.93
SMAC 34	5-nitro-2-furanyl	4.5	1.37
SMAC 35	3-pyridinyl	26.7	0.79
SMAC 36	4-pyridinyl	31.2	0.92
SMAC 37	1-naphthyl	75.9	3.19
SMAC 38	2-naphthyl	46.7	3.39
SMAC 39	4-methyl-1-naphthyl	21.1	3.59
SMAC 40	ferrocenyl	>150	–
isoniazid	–	0.7	–

*Theoretical lipophilicity (MOLINSPIRATION CHEMINFORMATICS, 2020); MIC (in $\mu\text{mol/L}$) = minimum inhibitory concentration; – not determined.

Fonte: Elaborado pelo autor

Second, we previously described that the insertion of a NO₂ group on the furanyl ring increased the antitubercular activity of an unsymmetrical monocarbonyl curcuminoid (diphenylpentanoid) (AYUSSO, 2019). Based on this result, we hypothesized that the insertion of a NO₂ group at position 5 on the furanyl ring could lead to a more potent arylbutanoid. **SMAC 34** (5-nitro-2-furanyl) showed potent activity against *M. tuberculosis*, with MIC value of 4.5 $\mu\text{mol/L}$. The presence of a NO₂ group was essential for the furanyl analog activity, corroborating our previous data (AYUSSO, 2019). The nitrofuran moiety is present in some antibacterial drugs, including nitrofurazone, nifuroxazide and nitrofurantoin (VASS, HRUSKA & FRANEK, 2008; PRICE et al., 2016). Studies have correlated their action mode with the

ability of the aromatic nitro group to be reduced, which leads to the production of reactive radical species (VIOUÉ et al., 1999; WIJMA et al., 2019). This fact may also have corroborated the potent activity of **SMAC 10** (4-NO₂) and **SMAC 13** (3-NO₂) (MIC 9.9 and 9.6 µmol/L, respectively).

Third, comparisons were made in order to assess the effects of replacing the phenyl ring (**SMAC 2**) with 6-membered heteroaryl rings with six π -electrons, such as 3-pyridinyl (**SMAC 35**) and 4-pyridinyl (**SMAC 36**). Curcuminoids **SMACs 35** and **36** had MIC values of 26.7 and 31.2 µmol/L, respectively. The results demonstrated that the replacement of the phenyl ring by pyridinyl rings improved the potency against *M. tuberculosis*, and the nitrogen position was not relevant for the activity of pyridinyl analogs. Ayusso also studied the antitubercular effects of replacing the phenyl ring with pyridinyl rings in a series of unsaturated ketones (curcuminoids). For that class of compounds, the pyridinyl analogs were also more potent than the phenyl analog. In addition, the 3- and 4-pyridinyl regioisomers showed similar activity against *M. tuberculosis* (AYUSSO, 2019), corroborating our results.

Fourth, we tested two analogs replaced by homoaryl rings with ten π -electrons. **SMAC 37** (1-naphthyl) and **SMAC 38** (2-naphthyl) had MIC values of 75.9 and 46.7 µmol/L, respectively, suggesting that the naphthyl ring position was relevant for the bioactivity. Additionally, the increase in π -electron density improved the antitubercular activity of arylbutanoids, since the naphthyl analogs (**SMACs 37** and **38**) were more potent than phenyl, pyrrolyl, furanyl and thiophenyl analogs (**SMACs 2** and **31–33**). In addition, the high π -electron density of **SMAC 30** (biphenyl, twelve π -electrons) may also have corroborated its activity against *M. tuberculosis*.

Fifth, since lipophilicity is an important parameter for antitubercular agents (FERNANDES et al., 2015), we investigated the effect of the insertion of a Me group at position 4 on the 1-naphthyl ring. **SMAC 39** (4-methyl-1-naphthyl, *miLog P* 3.59) showed activity about four times more potent than **SMAC 37** (1-naphthyl, *miLog P* 3.19), suggesting that the lipophilicity increase improved the antitubercular activity. This fact may be related to the facility of the compound to permeate the mycolic acid membrane of *M. tuberculosis* (FERNANDES et al., 2015).

Sixth, we tested an analog carrying the ferrocenyl ring (**SMAC 40**). This organometallic ring was selected due to its antimycobacterial effects (MAGUENE et al., 2011; SINGH et al., 2018). However, **SMAC 40** was inactive (MIC >150 µmol/L) against *M.*

tuberculosis, indicating that the organometallic ring was not relevant for the antitubercular activity of arylbutanoids.

In general, the series II SAR data indicated that the increase in π -electron density, together with the enhance in lipophilicity, were important for the bioactivity. In addition, the replacement of the phenyl ring with pyridinyl rings, as well as the insertion of a NO₂ on the furan ring increased the potency against *M. tuberculosis*.

3.2.3. ADME and drug-likeness predictions of selected SMACs

Undesirable physico-chemical and pharmacokinetic properties are the main causes of new drug candidates failure in the clinical trial phase (HONORIO, MODA & ANDRICOPULO, 2013). Therefore, selecting compounds with a good balance of potency, along with adequate absorption, distribution, metabolism and excretion (ADME) can increase the chance of a new candidate entering and passing in clinical trials. Because of that, we performed an *in silico* study with the most potent **SMACs** ($\text{MIC} \leq 50 \mu\text{mol/L}$) in order to predict some physico-chemical and pharmacokinetic parameters, including water solubility (Log S), human intestinal absorption (HIB) and blood-brain barrier penetration (BBBP) (Table 3).

Water solubility is one of the most important physico-chemical parameters in the development of new drugs. It has impact on pharmacokinetic properties, such as absorption, distribution and oral bioavailability (BERGSTRÖM & LARSSON, 2018). Thus, we investigated the water solubility of the selected **SMACs** by the Log S values, which were predicted according to the Ali model (ALI et al., 2012), using the SwissADME property explorer (DAINA, MICHIELIN & ZOETE, 2017). Log S values are used to classify compounds as insoluble ($\text{Log S} \leq -10$), poorly soluble ($\text{Log S} > -10$ to -6), moderately soluble ($\text{Log S} > -6$ to -4), soluble ($\text{Log S} > -4$ to -2), very soluble ($\text{Log S} > -2$ to 0) or highly soluble ($\text{Log S} > 0$) (SWISSADME, 2020). The selected **SMACs** showed Log S values ranging from -4.18 to -1.18 . Two analogs were classified moderately soluble (**SMACs 29 and 39**), eighteen compounds were classified as soluble (**SMACs 3, 4, 9–11, 13, 15, 18, 19, 21–26, 30, 34 and 38**), and two analogs were classified as very soluble (**SMACs 35 and 36**).

The human intestinal absorption of the selected **SMACs** was predicted according to the Zhao model (ZHAO et al., 2001), using the PreADMET property explorer (PREADMET, 2020). HIB values are used to classify compounds as poorly absorbed (HIB 0% to 20%), moderately absorbed (HIB 20% to 70%) or well absorbed (HIB 70% to 100%) (YEE, 1997).

All selected **SMACs** were classified as well absorbed compounds, with HIB values ranging from 81% to 100%.

We also predicted the blood-brain barrier penetration of the selected **SMACs**, since it is a crucial property to combat central nervous system tuberculosis (SCHALLER et al., 2019). BBBP is represented as the ratio between the [brain] and [blood] values, where [brain] and [blood] are the steady-state concentration of compounds in brain and peripheral blood, respectively (MA, CHEN & YANG, 2005). BBBP values were predicted using the PreADMET property explorer (PREADMET, 2020), and they were used to classify the compounds absorption to central nervous system (CNS) as high absorption ($\text{BBBP} > 2.0$), middle absorption ($0.1 \leq \text{BBBP} \leq 2.0$) and low absorption ($\text{BBBP} < 0.1$) (MA, CHEN & YANG, 2005). All selected **SMACs** were classified as middle absorption to CNS, with BBBP values ranging from 1.8 to 0.6.

The concept of drug-likeness can be defined as the sum of the molecular and physico-chemical properties that are characteristic of chemicals known as drugs. Indeed, drug-likeness filters are often used to describe compounds with desirable ADMET properties (JIA et al., 2020). There are several drug-likeness rules based on simple molecular properties, including molecular weight (MW), lipophilicity (Log P), number of hydrogen bond donors (HBD), number of hydrogen bond acceptors (HBA), number of rotatable bonds (NROTB), topological polar surface area (TPSA), molar refractivity (MR), number of atoms (NA), number of rings (NR), number of carbons (NC) and number of heteroatoms (NHA). Here, we performed an *in silico* study using the SwissADME property explorer (DAINA, MICHIELIN & ZOETE, 2017) in order to verify the number of violations of the rules proposed by Lipinski (LIPINSKI et al., 1997), Veber (VEBER et al., 2002), Egan (EGAN, MERZ & BALDWIN, 2000), Ghose (GHOSE, VISWANADHAN & WENDOLOSKI, 1999) and Muegge (MUEGGE, HEALD & BRITTELLI, 2011) (Table 3).

No one of the selected **SMACs** violated Lipinski, Veber and Egan rules, corroborating their drug-likeness properties. These rules established the following parameters; Lipinski: (a) $\text{MW} \leq 500 \text{ g/mol}$, (b) $\text{Log P} \leq 5$, (c) $\text{HBD} \leq 5$ and (d) $\text{HBA} \leq 10$ (LIPINSKI et al., 1997); Veber: (a) $\text{NROTB} \leq 10$ and (b) $\text{TPSA} \leq 140 \text{ \AA}^2$ (VEBER et al., 2002); and Egan: (a) $\text{Log P} \leq 5.88$ and (b) $\text{TPSA} \leq 131.6 \text{ \AA}^2$ (EGAN, MERZ & BALDWIN, 2000).

According to Ghose and Muegge rules, some simplified curcuminoids did not have suitable MW. The Ghose rule establishes the following parameters: (a) $160 \text{ g/mol} \leq \text{MW} \leq 480 \text{ g/mol}$, (b) $-0.4 \leq \text{Log P} \leq 5.6$, (c) $40 \leq \text{MR} \leq 130$ and (d) $20 \leq \text{NA} \leq 70$ (GHOSE,

VISWANADHAN & WENDOLOSKI, 1999). The Muegge rule establishes the following parameters: (a) $200 \text{ g/mol} \leq \text{MW} \leq 600 \text{ g/mol}$, (b) $-2 \leq \text{Log P} \leq 5$, (c) $\text{TPSA} \leq 150 \text{ \AA}^2$, (d) $\text{NR} \leq 7$, (e) $\text{NC} > 4$, (f) $\text{NHA} > 1$, (g) $\text{NROTBS} \leq 15$, (h) $\text{HBD} \leq 5$ and (i) $\text{HBA} \leq 10$ (MUEGGE, HEALD & BRITTELLI, 2011). **SMACs 35** and **36** have $\text{MW} = 147.2 \text{ g/mol}$, violating a parameter of both Ghose and Muegge rules. On the other hand, **SMACs 3, 4, 9, 10, 13, 15, 18, 19, 34** and **38** violated only one parameter of Muegge rule, since they have $\text{MW} < 200 \text{ g/mol}$. Although these rules can quickly filter out drug-like compounds, considering simple boundaries for individual molecular properties might be excessively strict. As a consequence, a possibly successful compound may be missed if only one or two parameters fall outside the criteria boundaries (YUSOF & SEGALL, 2013).

Table 3. ADME and drug-likeness predictions of selected **SMACs**.

Series I	R	Log S	HIA	BBBP	Lipinski's Violations	Veber's Violations	Egan's Violations	Ghose's Violations	Muegge's Violations
SMAC 3	2-OH	-2.61	95	0.7	0	0	0	0	1; MW < 200
SMAC 4	3-OH	-2.19	95	0.7	0	0	0	0	1; MW < 200
SMAC 9	4-CN	-2.11	98	1.8	0	0	0	0	1; MW < 200
SMAC 10	4-NO ₂	-2.84	94	1.2	0	0	0	0	1; MW < 200
SMAC 11	4-CF ₃	-3.16	100	1.1	0	0	0	0	0
SMAC 13	3-NO ₂	-2.82	94	1.2	0	0	0	0	1; MW < 200
SMAC 15	2-F	-2.34	100	1.2	0	0	0	0	1; MW < 200
SMAC 18	2-Cl	-2.37	100	1.1	0	0	0	0	1; MW < 200
SMAC 19	3-Cl	-2.83	100	1.1	0	0	0	0	1; MW < 200
SMAC 21	2-Br	-2.95	100	1.1	0	0	0	0	0
SMAC 22	3-Br	-3.04	100	1.1	0	0	0	0	0
SMAC 23	4-Br	-3.05	100	1.1	0	0	0	0	0
SMAC 24	2,4-diCl	-3.15	100	1.2	0	0	0	0	0
SMAC 25	2,6-diCl	-3.32	100	1.2	0	0	0	0	0
SMAC 26	3,4-diCl	-3.75	100	1.2	0	0	0	0	0
SMAC 29	4- <i>t</i> -Bu	-4.02	100	1.3	0	0	0	0	0
SMAC 30	4-Ph	-3.75	100	1.1	0	0	0	0	0
Series II	Ar								
SMAC 34	5-nitro-2-furanyl	-2.64	81	0.6	0	0	0	0	1; MW < 200
SMAC 35	3-pyridinyl	-1.18	100	1.4	0	0	0	1; MW < 160	1; MW < 200
SMAC 36	4-pyridinyl	-1.18	100	1.4	0	0	0	1; MW < 160	1; MW < 200
SMAC 38	2-naphthyl	-3.66	100	1.8	0	0	0	0	1; MW < 200
SMAC 39	4-methyl-1-naphthyl	-4.18	100	1.5	0	0	0	0	0

Log S = water solubility; **HIA** (in %) = human intestinal absorption; **BBBP** ([brain]/[blood]) = blood-brain barrier penetration; **Lipinski rule**: (a) MW ≤ 500 g/mol, (b) Log P ≤ 5.0, (c) HBD ≤ 5 and (d) HBA ≤ 10; **Veber rule**: (a) NROTB ≤ 10 and (b) TPSA ≤ 140 Å²; **Egan rule**: (a) Log P ≤ 5.88 and (b) TPSA ≤ 131.6 Å²; **Ghose rule**: (a) 160 g/mol ≤ MW ≤ 480 g/mol, (b) -0.4 ≤ Log P ≤ 5.6, (c) 40 ≤ MR ≤ 130 and (d) 20 ≤ NA ≤ 70; **Muegge rule**: (a) 200 g/mol ≤ MW ≤ 600 g/mol, (b) -2 ≤ Log P ≤ 5, (c) TPSA ≤ 150 Å², (d) NR ≤ 7, (e) NC > 4, (f) NHA > 1, (g) NROTB ≤ 15, (h) HBD ≤ 5 and (i) HBA ≤ 10. MW = molecular weight; Log P = lipophilicity; HBD = number of hydrogen bond donors; HBA = number of hydrogen bond acceptors; NROTB = number of rotatable bonds; TPSA = topological polar surface area; MR = molar refractivity; NA = number of atoms; NR = number of rings; NC = number of carbons; NHA = number of heteroatoms.

Fonte: Elaborado pelo autor

3.2.4. Assessment of selected SMACs toxicity on human lung cell line, and determination of the selectivity indexes

In order to investigate preliminary data on curcuminoids toxicity, we evaluated the effects of the most potent **SMACs** ($\text{MIC} \leq 50 \mu\text{mol/L}$) against human eukaryotic cells. The pulmonary line (MRC-5) was selected based on its importance as host site for *M. tuberculosis* infections (RAVIMOHAN et al., 2018). Selected **SMACs** toxic effects were expressed according to the concentration required in order to reduce cell viability at 50% (IC_{50}) (Table 4). Doxorubicin was used as a reference cytotoxic agent. The selected **SMACs** had IC_{50} values ranging from 104.4 to 569.5 $\mu\text{mol/L}$. Our results indicated that the selected curcuminoids showed low toxicity against human lung line when compared to doxorubicin (IC_{50} 0.7 $\mu\text{mol/L}$), a compound which is known for its cytotoxic properties (MOHAJERI & SAHEBKAR, 2018).

Table 4. Toxicity on human lung cell line (MRC-5) and selectivity indexes of selected **SMACs**.

Series I	R	IC_{50}	SI
SMAC 3	2-OH	306.3 ± 11.8	11.5
SMAC 4	3-OH	271.8 ± 10.1	6.7
SMAC 9	4-CN	179.3 ± 5.9	14.8
SMAC 10	4- NO_2	175.7 ± 4.5	17.7
SMAC 11	4- CF_3	188.5 ± 8.8	5.9
SMAC 13	3- NO_2	163.3 ± 5.3	16.9
SMAC 15	2-F	514.6 ± 18.5	18.8
SMAC 18	2-Cl	516.9 ± 23.2	19.4
SMAC 19	3-Cl	375.2 ± 4.3	10.6
SMAC 21	2-Br	351.1 ± 12.9	11.9
SMAC 22	3-Br	303.5 ± 13.1	14.3
SMAC 23	4-Br	141.4 ± 6.2	15.3
SMAC 24	2,4-diCl	241.4 ± 12.1	19.8
SMAC 25	2,6-diCl	234.5 ± 11.6	13.6
SMAC 26	3,4-diCl	179.8 ± 9.7	9.9
SMAC 29	4- <i>t</i> -Bu	247.6 ± 12.3	10.5
SMAC 30	4-Ph	196.6 ± 4.4	27.2
Series II	Ar		
SMAC 34	5-nitro-2-furanyl	104.4 ± 5.2	23.3
SMAC 35	3-pyridinyl	512.3 ± 26.2	19.3
SMAC 36	4-pyridinyl	569.5 ± 29.1	17.8
SMAC 38	2-naphthyl	313.8 ± 19.7	6.7
SMAC 39	4-methyl-1-naphthyl	314.3 ± 21.9	14.9
MAC 4*	—	87.6 ± 2.1	15.6
doxorubicin	—	0.7 ± 0.04	—

IC_{50} (in $\mu\text{mol/L}$) = concentration required in order to reduce cell viability at 50%; SI ($\text{IC}_{50}/\text{MIC}$) = selectivity index; MRC-5 = lung fibroblast cells; — not determined. *Chapter II. Fonte: Elaborado pelo autor.

In order to compare the effects on prokaryotic and eukaryotic cells, the selectivity indexes (SI) of selected **SMACs** were determined as the ratio among the IC₅₀ and MIC values (Table 4). According to Orme and collaborators, candidates for new antitubercular agents must have a SI value ≥ 10 (ORME et al., 2001). Selected **SMACs** exhibited SI values ranging from 5.9 to 27.2. Four analogs showed SI value < 10 , sixteen compounds showed $10 \leq \text{SI} \leq 20$, and two analogs had SI value > 20 . The two most selective curcuminoids were **SMAC 30** (4-Ph) and **SMAC 34** (5-nitro-2-furanyl), with SI values of 27.2 and 23.3, respectively. Although **SMACs 30** and **34** (MIC 7.2 and 4.5 $\mu\text{mol/L}$, respectively) have similar antitubercular activity to **MAC 4** (MIC 5.6 $\mu\text{mol/L}$), they were more selective than its parent compound (SI 15.6) (Chapter II).

Based on both *in silico* predictions and toxicity against human cells, we selected **SMACs 30** and **34** for further assays, aiming to evaluate their chemical stability, and their effects against *M. tuberculosis* with multidrug resistance. In addition, we also evaluated **SMACs 30** and **34** activities against Gram-negative and Gram-positive bacterial species.

3.2.5. Evaluation of **SMACs 30** and **34** chemical stability

Investigating the chemical stability of a hit compound is an important step in drug development, since this parameter can affect its safety and efficacy (BLESSY et al., 2014). One of the main factors that hinder the clinical use of **CUR** is its poor stability under physiological conditions (NELSON et al., 2017). We demonstrated that **MAC 4** showed higher chemical stability than **CUR**, overcoming this limitation (Chapter II). In order to investigate whether the simplified analogs maintained the **MAC 4** chemical stability, we evaluated the **SMACs 30** and **34** stabilities at pH 7.4 and 37 °C by monitoring the decrease in their UV–Vis absorption for 24 h (Figure 3). The initial concentrations of **SMACs 30** and **34** reduced only 11% and 23%, respectively, after 24 h. The **MAC 4** simplification not only improved the selectivity index, but also resulted in more stable analogs, since **MAC 4** initial concentration decreased by 28% after 24 h, at pH 7.4 and 37 °C (Chapter II).

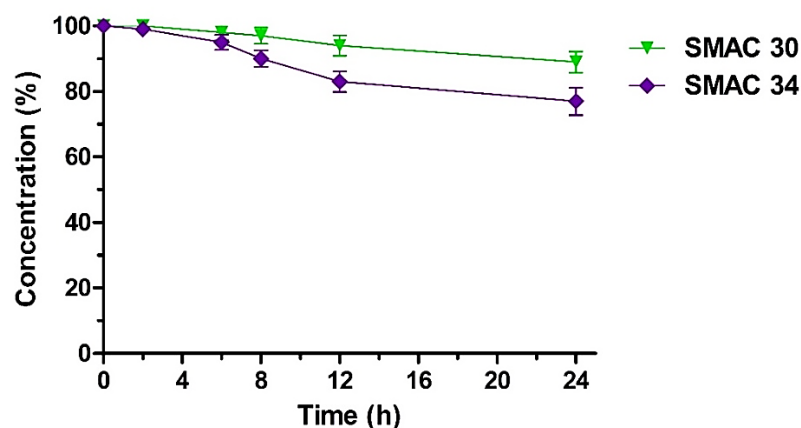


Figure 3. Chemical stability of **SMACs 30** and **34** (pH 7.4 and 37 °C) by monitoring the decrease in their maximum absorbance for 24 h. Data were represented as means $\pm$ SEMs and expressed as percentage.

Fonte: Elaborado pelo autor

3.2.6. Anti-*M. tuberculosis* activity of **SMACs 30** and **34** against drug resistant clinical isolates

We investigated the **SMACs 30** and **34** effects against two *M. tuberculosis* clinical isolates with resistance to different antitubercular drugs, including *M. tuberculosis* 48 (resistant to isoniazid, rifampicin and amikacin) and *M. tuberculosis* 169 (resistant to isoniazid and rifampicin). Both multidrug resistant strains were classified as MDR strains (resistant at least to isoniazid and rifampicin) (MIYATA, 2011). Antimycobacterial potency was expressed as MIC values in $\mu\text{mol/L}$ (Table 5). Isoniazid, rifampicin, moxifloxacin and amikacin were used as reference antitubercular drugs. **SMACs 30** and **34** were active against the two MDR strains, with MIC values ranging from 3.8 to 81.4 $\mu\text{mol/L}$. However, **SMAC 34** was more potent than **SMAC 30**.

3.2.7. Evaluation of **SMACs 30** and **34** effects against Gram-positive and Gram-negative bacterial species

Tuberculosis is a chronic infection, the treatment of which requires a drug with a narrow spectrum, such as isoniazid (FERNANDES et al., 2017). In order to check the **SMACs 30** and **34** specificities for *M. tuberculosis*, we evaluated their antibacterial activities against Gram-negative and Gram-positive species, including *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella enterica* serovar Typhimurium (ATCC 13311), *Acinetobacter baumannii* (ATCC

19606), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212) and *Bacillus subtilis* (strain 168). Antibacterial potency was expressed as MIC values in $\mu\text{mol/L}$ (Table 6). Gentamicin and vancomycin were used as reference antibiotics.

SMAC 30 was inactive against all species (MIC >150 $\mu\text{mol/L}$), while **SMAC 34** showed activity against *S. enterica* and *S. aureus*, with MIC values of 18.7 and 150 $\mu\text{mol/L}$, respectively. The inhibitory effects of **SMACs 30** and **34** were smaller against Gram-positive and Gram-negative bacterial species than against *M. tuberculosis* H₃₇Rv, indicating that these compounds showed higher specificity towards *M. tuberculosis*. However, **SMAC 34** action spectrum was broader than the one of **SMAC 30**.

Table 5. Anti-*M. tuberculosis* activity of **SMACs 30** and **34** against drug resistant clinical isolates expressed as MIC values in $\mu\text{mol/L}$.

Compounds	Mt 48	Mt 169
SMAC 30	42.9	81.4
SMAC 34	7.5	3.8
isoniazid	R	R
rifampicin	R	R
moxifloxacin	3.1	R
amikacin	R	0.3

MIC = minimal inhibitory concentration; Mt = *Mycobacterium tuberculosis* (MDR strains); R = resistant.

Fonte: Elaborado pelo autor

Table 6. Antibacterial activity of **SMACs 30** and **34** against Gram-negative and Gram-positive bacterial species expressed as MIC values in $\mu\text{mol/L}$.

Compounds	Pa	Se	Ab	Sa	Ef	Bs
SMAC 30	>150	>150	>150	>150	>150	>150
SMAC 34	>150	18.7	>150	150	>150	>150
gentamicin	0.4	1.0	2.0	—	—	—
vancomycin	—	—	—	0.7	1.4	0.1

MIC = minimal inhibitory concentration; Pa = *Pseudomonas aeruginosa* (ATCC 27853); Se = *Salmonella enterica* serovar Typhimurium (ATCC 13311); Ab = *Acinetobacter baumannii* (ATCC 19606), Sa = *Staphylococcus aureus* (ATCC 25923); Ef = *Enterococcus faecalis* (ATCC 29212); Bs = *Bacillus subtilis* (strain 168); — not determined.

Fonte: Elaborado pelo autor

3.3. CONCLUSION

In summary, we described the antimycobacterial activity of two series of simplified monocarbonyl curcuminoids based on **MAC 4** framework, an unsymmetrical monocarbonyl curcuminoid with promising antitubercular effects, but its synthesis required four reaction steps. The **MAC 4** molecular simplification led to analogs (**SMACs 30** and **34**) with antitubercular activity similar to the parental compound, but these analogs were synthesized in only one reaction step. Furthermore, the two simplified analogs were less toxic and more stable than **MAC 4**, and they presented high specificity for *M. tuberculosis*. In addition, **SMACs 30** and **34** were also active against MDR strains. Preliminary investigations on SAR data have suggested that the electron withdrawing effect, as well as the lipophilicity together with the π -electron density, were important factors for the simplified curcuminoids bioactivity. Altogether, our data can serve as a template for the design of future agents against *M. tuberculosis* which aims the successful clinical control of the disease.

3.4. EXPERIMENTAL

3.4.1. Chemistry

3.4.1.1. Material and instruments (Chapter II, item 2.4.1.1)

3.4.1.2. Synthesis of SMACs 1–40

A solution of aromatic aldehyde (5 mmol) in acetone (15 mL) was cooled in an ice bath for 10 min. Acetonic suspension of NaOH (1 mL, 0.5 mol/L) was added dropwise to the reaction mixture. The suspension was stirred at room temperature for 1–8 h. Reaction medium was poured onto crushed ice. The crude product was partitioned with ethyl acetate (3 × 25 mL), and the combined organic phase was concentrated under reduced pressure. **SMACs 2, 4, 6** and **40** were recrystallized in EtOH. **SMACs 1** and **34** were purified by gel filtration column chromatography (LH-20) using EtOH as eluent. **SMACs 3, 5, 7–33** and **35–39** were purified by column chromatography over silica gel using mixtures of hexane and ethyl acetate as eluents

3.4.2. Antitubercular assay

The antitubercular activity was evaluated against *M. tuberculosis* H₃₇Rv (ATCC 27294), and against two multi-drug resistant clinical isolates (*M. tuberculosis* 48 and 169). The clinical isolates were obtained from tuberculosis patients at Clemente Ferreira Institute, a national reference center in the tuberculosis treatment, located in São Paulo city, São Paulo

State, Brazil (MIYATA et al., 2011). The MIC values were determined by Resazurin Microtiter Assay (PALOMINO et al., 2002), with minor modifications described in previous work (POLAQUINI et al., 2017). **SMACs 1–40** were prepared in DMSO and assayed in concentrations ranging from 150 to 1.2 $\mu\text{mol/L}$. Isoniazid was used as a reference antitubercular drug. Three independent assays were performed. The MIC value was defined as the lowest compound concentration at which 90% of the cells are infeasible relative to the control.

3.4.3. Cytotoxicity assay

The cell line used in this study was MRC-5 (normal human lung fibroblast cell line ATCC CCL-171). The IC_{50} values were determined as described by Silva and co-authors (SILVA et al., 2017). Cells were treated with selected **SMACs** in concentrations ranging from 600 to 50 $\mu\text{mol/L}$. Doxorubicin was used as a reference cytotoxic drug. Three independent assays were performed. The IC_{50} value was calculated from an analytical curve through a regression analysis and it represents the concentration required to reduce the viability of cells at 50%.

3.4.4. Chemical stability assay

Chemical stability of **SMACs 30** and **34** at 35 $\mu\text{mol/L}$ were analyzed by monitoring their UV–visible absorptions in phosphate buffer (pH 7.4) at 37 °C. Minor modifications in this protocol and their detailed procedures were reported in our previous work (POLAQUINI et al., 2019).

3.4.5. Antibacterial assay

Microorganisms used in this study were *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella enterica* serovar Typhimurium (ATCC 13311), *Acinetobacter baumannii* (ATCC 19606), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212) and *Bacillus subtilis* (strain 168). The MIC values were determined by the microdilution method according to the Clinical and Laboratory Standards Institute (CLSI, 2012). **SMACs 30** and **34** were diluted in DMSO and assayed in concentrations ranging from 150 to 1.2 $\mu\text{mol/L}$. Gentamicin and vancomycin were used as reference antibiotics against Gram-negative and Gram-positive species, respectively. Three independent assays were performed. The MIC value was defined as the lowest concentration of compound that inhibited visible microbial growth.

4. CAPÍTULO IV

4. CAPÍTULO IV: Catecholic monocarbonyl curcuminoid (MAC 4) interferes with cell division and the ability of <i>Xanthomonas citri</i> subsp. <i>citri</i> to induce citrus canker lesions	98
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4. CAPÍTULO IV

Catecholic Monocarbonyl Curcuminoid (MAC 4) Interferes with Cell Division and the Ability of *Xanthomonas citri* subsp. *citri* to Induce Citrus Canker Lesions

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ABSTRACT

Citrus canker is a plant disease caused by *Xanthomonas citri* subsp. *citri* (*X. citri*). Nowadays, the chemical control of this phytopathogen requires recurrent sprays of copper-based formulations that accumulate in soil. In addition, several copper-resistant *X. citri* strains have been identified. In this work, we assessed the anti-*X. citri* activity of monocarbonyl curcuminoids (**MACs**) designed from simplified curcumin (**SCUR**), which is a potent anti-*X. citri* compound. Cathecolic curcuminoid (**MAC 4**) exhibited MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) values of 330 and 375 $\mu\text{mol/L}$, respectively, against *X. citri*. Our investigations showed that **MAC 4** did not induce resistance in *X. citri*. Furthermore, artificial inoculation tests demonstrated reduction of citrus canker lesions on *Citrus sinensis* leaves infiltrated with **MAC 4** pre-exposed *X. citri*. Both microscopy and biochemical assays revealed that **MAC 4** had the ability to perturb *X. citri* cell division by disrupting the divisome and inhibiting the GTPase activity of FtsZ. Together, our results suggest that **MAC 4** is a promising anti-*X. citri* agent.

4.1. INTRODUCTION

One of the world's leading fruit crops, citrus contributes to the economy of several countries, including China, Brazil, USA, India and Mexico (XU et al., 2018). However, some diseases, such as citrus canker (LANZA et al., 2018) and HLB (huanglongbing) (ALVAREZ et al., 2016), have been causing damage to the production. Citrus canker affects commercially important species, including orange, grapefruit, lemon and tangerine, but the severity of symptoms varies according to the citrus species and the climate (DAS, 2003). The appearance of necrotic lesions on the leaves, fruits and stems, as well as defoliation and premature fruit drop are the most common symptoms of this disease (DUAN et al., 2018).

Xanthomonas citri subsp. *citri* (*X. citri*) is the etiological agent of the Asiatic citrus canker, the most severe form of the disease (GONÇALVES-ZULIANI et al., 2016). This Gram-negative bacterium belongs to the Xanthomonadaceae family, which is one of the main groups of bacterial phytopathogens (ASSIS et al., 2017). *X. citri* can enter the plants through natural openings, such as stomata, or through wounds caused by insect larvae or during orchard management operations (GOTTWALD, GRAHAM & SCHUBERT, 2002).

Some of the measures taken in order to control the spread of *X. citri* include the use of windbreaks, eradication of diseased trees and application of copper-based formulations (FERENCE et al., 2018). However, the chemical control of this phytopathogen with copper-based compounds only has led to the emergence of resistant *X. citri* strains (BEHLAU et al., 2011; RICHARD et al., 2017). In addition, the continuous application of copper formulations results in environmental damage due to their accumulation in soil and leaching to water bodies (ADREES et al., 2015). In this context, our research group has been engaged in the study of new environmentally friendly compounds which are able to combat *X. citri* (SILVA et al., 2013; NAZARÉ et al., 2018; SAVIETTO et al., 2018; ZAMUNER et al., 2020).

Curcumin (**CUR**), a diphenolic compound from *Curcuma longa* (Zingiberaceae), has been used as a template for the design of new antibacterial agents (SARDI et al., 2017a; POLAQUINI et al., 2019; SANCHES et al., 2019) due to its low toxicity in humans (LAO et al., 2006) and its broad spectrum against bacteria (MOGHADAMTOUSI et al., 2014). Furthermore, **CUR** has the ability to act directly on the FtsZ protein. It inhibits the FtsZ assembly in protofilaments by increasing its GTPase activity (RAI et al., 2008; MORÃO et al., 2019). The **CUR** effects on FtsZ support the efforts regarding the search for new compounds based on **CUR** structure, since FtsZ plays a key role in bacterial cell division. The FtsZ protein initiates cell division from its polymerization, leading to the Z-ring formation (ESWARA &

RAMAMURTHI, 2017). This ring acts as a scaffold for other proteins that participate in cell division. The macromolecular complex formed by Z-ring and other proteins is called divisome (ADAMS & ERRINGTON 2009; ERICKSON, ANDERSON & OSAWA, 2010).

In a previous study, we reported the anti-*X. citri* activity of a simplified analog of **CUR** (**SCUR**). It was designed by replacing the β -diketone moiety for the monocarbonyl group, since the β -diketone has been associated with the poor chemical stability of **CUR** (SHETTY et al., 2015). **SCUR** was more stable and active against *X. citri* than **CUR**, and it demonstrated action on the *Bacillus subtilis* membrane (MORÃO et al., 2019). In the current work, we investigated the anti-*X. citri* effects of monocarbonyl curcuminoids (**MACs 1–8** and **SMACs 1, 2, 3** and **5**) designed from **SCUR** framework. The most active curcuminoid (**MAC 4**) was investigated regarding its effects on the bacterial growth and the ability to induce resistance in *X. citri*. In addition, the ability of *X. citri* pre-exposed to **MAC 4** to induce citrus canker lesions on the *Citrus sinensis* leaves was evaluated. We also assessed **MAC 4** ability to disrupt the bacterial cell division by its effects on the divisome and on the GTPase activity of FtsZ from *X. citri*.

4.2. RESULTS AND DISCUSSION

4.2.1. Anti-*X. citri* activity

The anti-*X. citri* activity of monocarbonyl curcuminoids (**MACs 1–8**) was evaluated by the resazurin microtiter assay (REMA) (MORÃO et al., 2019). This method measures bacterial metabolic activity by the reduction of resazurin to resorufin (CHEN, STEELE & STUCKEY, 2018). The curcuminoids effect was determined at 300 $\mu\text{mol/L}$, and the results were expressed as percentage of bacterial metabolism inhibition (BMI) (Table 1).

In a previous study of our group, **SCUR** at 300 $\mu\text{mol/L}$ (100 $\mu\text{g/mL}$) showed BMI value of 61% against *X. citri*, while **CUR** was inactive (BMI <10%). These data confirmed that the replacement of the β -diketone by monocarbonyl moiety resulted in increased potency. On the other hand, the replacement of *m*-OMe and *p*-OH groups on the **SCUR** aromatic rings by other electron-donating (*p*-Me or *p*-N(Me)₂) or electron-withdrawing (*p*-F or *p*-Br) groups, resulted in reduction of anti-*X. citri* activity (MORÃO et al., 2019).

In order to further investigate the relationships between the substituents on the aromatic rings of monocarbonyl analogs and the anti-*X. citri* activity, we first compared the **SCUR** and the **MAC 1** activities. **MAC 1** (BMI 77.5%), which contains the unsubstituted ring B, demonstrated higher activity than **SCUR** (BMI 61%). This result may be related to the increase of lipophilicity due to the removal of hydrophilic substituents on the aromatic ring,

which facilitates permeation through biomembranes. Recent studies have elucidated the importance of this physico-chemical parameter in phenolic natural product derivatives concerning anti-*X. citri* potency, such as gallates (SILVA et al., 2013; SAVIETTO et al., 2018), protocatechuates, gentisates and β -resorcyates (NAZARÉ et al., 2018).

Table 1. Anti-*X. citri* activity of monocarbonyl curcuminoids (**MACs 1–8**) at 300 $\mu\text{mol/L}$ expressed as BMI values (%).

Compounds	R	BMI
CUR	—	<10*
SCUR	—	61*
MAC 1	<i>m</i> -OMe, <i>p</i> -OH	77.5 $\pm$ 2.4
MAC 2	H	<10
MAC 3	<i>m,p</i> -diOMe	<10
MAC 4	<i>m,p</i> -diOH	88.3 $\pm$ 0.5
MAC 5	<i>m</i> -OMe	<10
MAC 6	<i>p</i> -OH	55.2 $\pm$ 1.6
MAC 7	<i>p</i> -OMe	<10
MAC 8	<i>m</i> -OH	69.3 $\pm$ 1.9

*MORÃO et al., 2019; **CUR** = curcumin; **SCUR** = simplified curcumin; BMI (%) = bacterial metabolism inhibition.

Fonte: Elaborado pelo autor

Second, we assayed **MAC 2**, which contains the unsubstituted rings A and B. **MAC 2** was not able to inhibit the *X. citri* metabolism (BMI <10%). Taken together, the BMI values of **SCUR** and **MACs 1** and **2** (BMI 61, 77.5 and <10%, respectively) suggest that when there was only one substituted aromatic ring by *m*-OMe and *p*-OH groups (guaiacol moiety) it was ideal for the anti-*X. citri* activity of monocarbonyl analogs. The guaiacol moiety is present in various natural products with antibacterial activity, including **CUR**, eugenol, dehydrozingerone and ferulic acid (MARCHESE et al., 2017; PERNIN et al., 2019). Preliminary structure-activity relationship data of symmetrical dicarbonyl analogs demonstrated the importance of guaiacol moiety for **CUR** antibacterial activity against Gram-positive species (POLAQUINI et al., 2019).

Third, we investigated the effects of **MAC 3** (*m,p*-diOMe) and **MAC 4** (*m,p*-diOH), and compared them to **MAC 1** (*m*-OMe, *p*-OH). **MAC 3** (BMI <10%) was inactive, but **MAC 4** (BMI 88.3%) showed a higher activity against *X. citri* than **MAC 1** (BMI 77.5%). These

results indicated that the replacement of OH by OMe at *para* position on the aromatic ring A reduced the activity, but the replacement of OMe by OH at *meta* position increased the potency against *X. citri*. These findings corroborated **MACs 3** and **4** investigations against human pathogenic bacteria (Chapter II), demonstrating the same effect on the bioactivity.

Fourth, the effects of *m*-OMe and *p*-OH substituents on the aromatic ring A were evaluated separately, assessing **MAC 5** (*m*-OMe) and **MAC 6** (*p*-OH). Curcuminoids **MAC 5** (BMI <10%) and **MAC 6** (BMI 55.2%) showed reduction in activity when compared with **MAC 1** (BMI 77.5%), indicating that the anti-*X. citri* potency can be correlated to the simultaneous presence of OMe and OH at *meta* and *para* positions, respectively. On the other hand, these results suggest that the *p*-OH substituent was more relevant for activity against the phytopathogen than the *m*-OMe group.

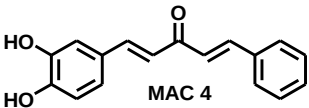
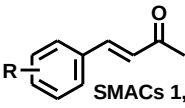
Fifth, we evaluated the two regioisomers of **MAC 5** (*m*-OMe) and **MAC 6** (*p*-OH), which were designed by the replacement of OMe and OH positions, resulting in **MAC 7** (*p*-OMe) and **MAC 8** (*m*-OH). The position of OMe did not affect anti-*X. citri* activity, since **MACs 5** and **7** were inactive (BMI <10%). On the other hand, the position of OH affected the bioactivity. **MAC 8** demonstrated BMI value of 69.3%, while its regioisomer **MAC 6** showed BMI value of 55.2%. Hydroxyl group at *meta* position was more relevant for anti-*X. citri* activity than OH at *para* position.

We selected **MAC 4**, which was the most active compound (BMI 88.3%), for subsequent anti-*X. citri* assays. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values against *X. citri* were determined. Kanamycin and copper oxychloride, a commercial active compound which is often used to combat citrus canker, were used as reference anti-*X. citri* compounds. **MAC 4** showed MIC value of 87.9 µg/mL (330 µmol/L), while kanamycin and copper oxychloride demonstrated MIC values of 19.9 and 43.1 µg/mL, respectively (Table 2). Furthermore, **MAC 4** exhibited MBC value of 375 µmol/L.

We also investigated anti-*X. citri* activity of simplified monocarbonyl analogs (phenylbutanoids) (**SMACs 1, 2, 4** and **5**) based on the **MAC 4** structure (diphenylpentanoid) (Table 2). **SMAC 1** (*m,p*-diOH) and **SMAC 2** (unsubstituted ring) had MIC values of 531 and >680 µmol/L, respectively. Therefore, it confirms the importance of OH groups on the aromatic ring for anti-*X. citri* activity. The effects of OH substituents at *meta* and *para* positions were evaluated separately, assessing **SMAC 4** (*m*-OH) and **SMAC 5** (*p*-OH). The position of hydroxyl group was not relevant for activity against *X. citri*, since **SMACs 4** and **5**

demonstrated similar activity (MIC 608 and 615 $\mu\text{mol/L}$, respectively). The comparison among the MIC values of **SMACs 1, 4 and 5** (MIC 531, 608 and 615 $\mu\text{mol/L}$, respectively) corroborated the relevance of simultaneous presence of the two hydroxyl groups on the aromatic ring for increased activity. Nevertheless, the **MAC 4** simplification reduced the potency against the phytopathogen, revealing that the diphenylpentanoid framework was better for activity than the phenylbutanoid framework.

Table 2. MIC values of **MAC 4** and its simplified analogs (**SMACs 1, 2, 4 and 5**) against *X. citri*.

 MAC 4  SMACs 1, 2, 4 and 5		
Compounds	R	MIC in $\mu\text{g/mL}$ (MIC in $\mu\text{mol/L}$)
MAC 4	—	87.9 (330)
SMAC 1	<i>m,p</i> -diOH	94.6 (531)
SMAC 2	H	>100 (>680)
SMAC 4	<i>m</i> -OH	98.6 (608)
SMAC 5	<i>p</i> -OH	99.7 (615)
kanamycin	—	19.9 (40)
copper oxychloride	—	43.1

MIC = minimum inhibitory concentration.

Fonte: Elaborado pelo autor

4.2.2. Effects of **MAC 4** on the *X. citri* growth

MAC 4 effects at $\frac{1}{2}$ MIC and MIC (165 and 330 $\mu\text{mol/L}$, respectively) on the *X. citri* growth pattern were evaluated. Growth curves were constructed by measuring the OD₆₀₀ (optical density) every 30 min for 72 h (Figure 1). The untreated *X. citri* growth curve (negative control) reached the stationary phase after a period of 15 h. The same pattern was obtained for *X. citri* exposed to 1% DMSO (vehicle control). On the other hand, the bacterial growth profile changed when *X. citri* was exposed to **MAC 4**. Treatment with **MAC 4** at $\frac{1}{2}$ MIC inhibited the early growth, delaying the exponential phase start and prolonging the required time for the culture to reach the stationary phase (~24 h). The effect during the first few hours of exposure was probably due to the bacteriostatic action of **MAC 4** at $\frac{1}{2}$ MIC, a dynamic process that was minimized as time passed. However, treatment with **MAC 4** at MIC completely inhibited the *X. citri* growth during the assessed period (72 h).

Nazaré and colleagues also investigated the effects of heptyl dihydroxybenzoates on the *X. citri* growth (NAZARÉ et al., 2018). Tests using subinhibitory concentrations ($\frac{1}{2}$ MIC) of heptyl dihydroxybenzoates demonstrated similar effects to **MAC 4** at $\frac{1}{2}$ MIC on the *X. citri* growth, inhibiting the early growth and delaying the exponential phase start. On the other hand, treatment with heptyl hydroxybenzoates in MIC did not completely inhibit the growth, while the exposure to **MAC 4** at MIC completely inhibited the *X. citri* growth.

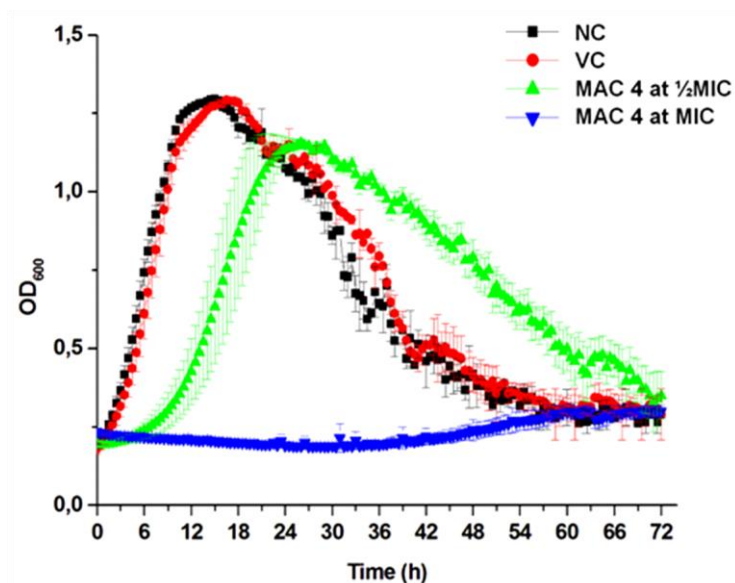


Figure 1. Effects of **MAC 4** at $\frac{1}{2}$ MIC and MIC (165 and 330 $\mu\text{mol/L}$, respectively) on the *X. citri* growth. NC = negative control (untreated *X. citri*); VC = vehicle control (*X. citri* treated with 1% DMSO).

Fonte: Elaborado pelo autor

4.2.3. Evaluation of resistance development to **MAC 4**

A major challenge in treatment of bacterial infections is the rapid appearance of resistant pathogens. In this context, we evaluated the *X. citri* ability to develop resistance to **MAC 4**. *X. citri* was subcultured for twenty-one passages over three weeks in order to determine if a shift at the initial MIC value of **MAC 4** (330 $\mu\text{mol/L}$) would be observed (Figure 2). Resistance is usually defined as a >4 -fold increase from the original MIC value (KOSOWSKA-SHICK et al., 2009). For kanamycin, no increase at MIC value (40 $\mu\text{mol/L}$) was observed after twenty-one passages. For **MAC 4**, the MIC value remained unchanged over six passages. A one-fold increase at the MIC value (660 $\mu\text{mol/L}$) of **MAC 4** was observed after the

seventh passage. Nevertheless, no additional increase at MIC value was observed up to the twenty-first passage. This fact indicated that **MAC 4** did not induce resistance in *X. citri*.

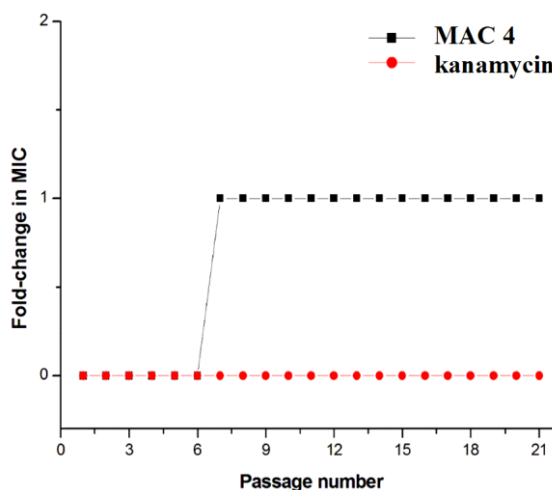


Figure 2. Propensity of the development of bacterial resistance towards **MAC 4** by *X. citri*. The original MIC values of **MAC 4** and kanamycin against *X. citri* were 330 and 40 $\mu\text{mol/L}$, respectively. Resistance was defined as a >4-fold increase in the original MIC value (KOSOWSKA-SHICK et al., 2009).

Fonte: Elaborado pelo autor

4.2.4. Pathogenicity assessment of *X. citri* pretreated with **MAC 4**

We investigated whether a prior exposure of *X. citri* to **MAC 4** affects its ability to induce citrus canker symptoms on the *C. sinensis* leaves (sweet orange, cultivar Pera Rio). The *C. sinensis* leaves abaxial surface was artificially infiltrated with *X. citri* pretreated with **MAC 4** at $\frac{1}{2}\text{MIC}$ (165 $\mu\text{mol/L}$), ensuring that after treatment there would be metabolically viable bacteria. A representative image of a *C. sinensis* leaf after four weeks of inoculation was shown in Figure 3. The infiltration process did not damage the leaf tissues, since the control using only sterile water (negative control) did not cause lesions on the leaf surface. Untreated *X. citri* (positive control) induced the appearance of citrus canker symptoms, characterized by cellular hyperplasia and hypertrophy. Exposure to 1% DMSO (vehicle control) did not affect the ability of *X. citri* to induce disease symptoms. On the other hand, treatment with **MAC 4** at $\frac{1}{2}\text{MIC}$ reduced the appearance of citrus canker symptoms, developing only isolated pustules, without more severe lesions.

Silva and colleagues also investigated the effects of a homologous series of alkyl gallates on the pathogenicity of *X. citri* pretreated with subinhibitory concentrations (MIC_{80}). Among the thirteen compounds, only seven reduced the appearance of citrus canker symptoms on the *C. sinensis* leaves (SILVA et al., 2013). These results indicated that, despite the anti-*X. citri* activity *in vitro*, some compounds did not demonstrate the ability to reduce the *X. citri* pathogenicity *in planta*, corroborating the potential of **MAC 4** as an agent against citrus canker.

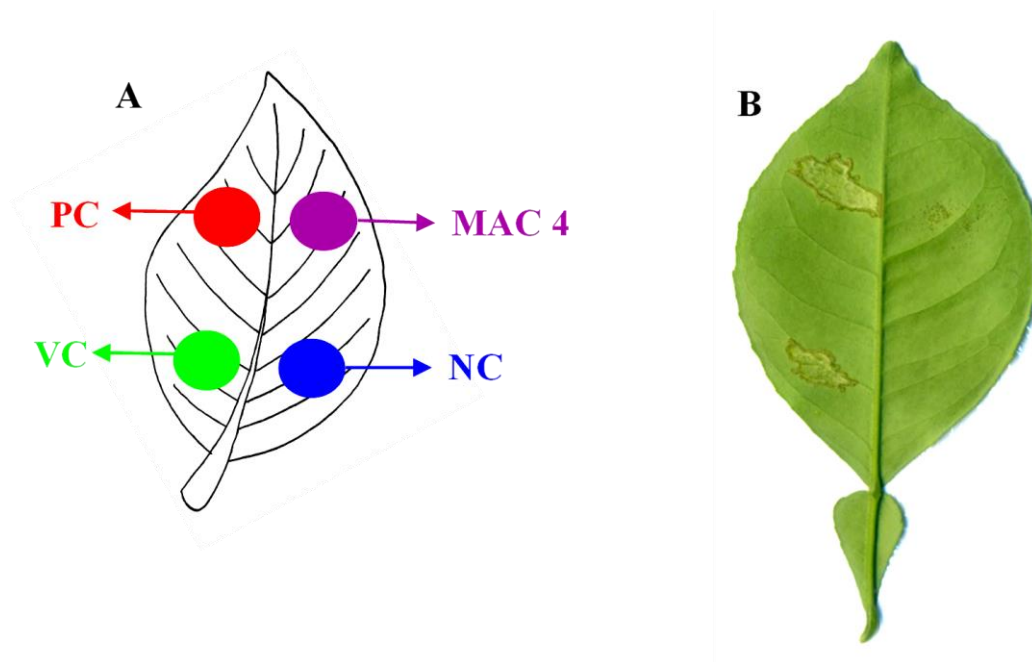


Figure 3. Effect of **MAC 4** at $\frac{1}{2}MIC$ (165 $\mu\text{mol/L}$) on the ability of *X. citri* to induce citrus canker symptoms. **(A)** Schematic representation of infiltration points on the *C. sinensis* leaves (sweet orange, cultivar Pera Rio); **(B)** Representative image from three independent tests after four weeks of infiltration. **NC** = negative control (sterile water infiltration); **PC** = positive control (untreated *X. citri*); **VC** = vehicle control (*X. citri* treated with 1% DMSO).

Fonte: Elaborado pelo autor

4.2.5. Effects of **MAC 4** on the *X. citri* divisome

In order to assess whether the bacterial cell division would be a target for **MAC 4**, we evaluated its effects on the divisome of an *X. citri* strain which express the ZapA-GFP (*X. citri amy::pPM2a-zapA*), a FtsZ accessory protein that labels the divisome. *X. citri* was treated with **MAC 4** at $\frac{1}{2}MIC$ (165 $\mu\text{mol/L}$) for 15 and 30 min and analyzed under the microscope. Hexyl gallate was used as a reference compound for divisome disruption (KRÓL et al., 2015).

Figure 4 depicts phase contrast and fluorescence microscopy representative images of the negative and positive controls, as well as of the treatment with **MAC 4** after 15 and 30 min. Untreated *X. citri* (negative control) demonstrated the septation normal pattern, a green fluorescent band perpendicular to the long axis of the cell and positioned at middle of cell, which represents the divisome localization (Figure 4A). The same pattern was observed when *X. citri* was treated with 1% DMSO (data not shown). *X. citri* exposed to hexyl gallate (positive control) exhibited a diffuse fluorescence, which is consistent with divisome perturbation (Figure 4B). Treatment with **MAC 4** at $\frac{1}{2}$ MIC for 15 min also perturbed the divisome, as observed by the lack of mid-cell septum localization and the diffuse fluorescence pattern (Figure 4C). Although some intact divisomes could be observed, this could be a consequence of the **MAC 4** concentration used. After 30 min of treatment with **MAC 4** at $\frac{1}{2}$ MIC, the divisomes were completely unstructured (Figure 4D).

Since the divisome is associated with the bacterial membrane, membrane perturbations may alter the spatial organization of proteins involved in division (KRETSCHMER & SCHWILLE, 2016). Thus, we evaluated the effects of **MAC 4** at $\frac{1}{2}$ MIC on the *X. citri* membrane. Two fluorescent dyes propidium iodide (PI) and SYTO9 were used. They stain cells with damaged membrane (red) and intact membrane (blue). Phase contrast and fluorescence microscopy representative images of the negative control (untreated *X. citri*), positive control (*X. citri* after a heat shock treatment) and treatment with **MAC 4** after 15 and 30 min were shown in Figure 5.

Most untreated *X. citri* cells were stained blue (Figure 5A). The same pattern was observed when *X. citri* was exposed to 1% DMSO (data not shown). However, *X. citri* cells after a heat shock treatment for 2 min at 55 °C were stained red (Figure 5B). Exposure to **MAC 4** at $\frac{1}{2}$ MIC, in both periods of 15 and 30 min (Figure 5C and 5D, respectively), led to a prevalence of blue stained *X. citri* cells, indicating membrane integrity, which corroborated the effect of **MAC 4** on the *X. citri* divisome. Furthermore, investigations concerning **MAC 4** effects on the *B. subtilis* divisome also demonstrated its ability to disrupt the cell division apparatus, without causing significant damage on the bacterial membrane (Chapter II). Together, the results corroborate that bacterial cell division is a target for **MAC 4**.

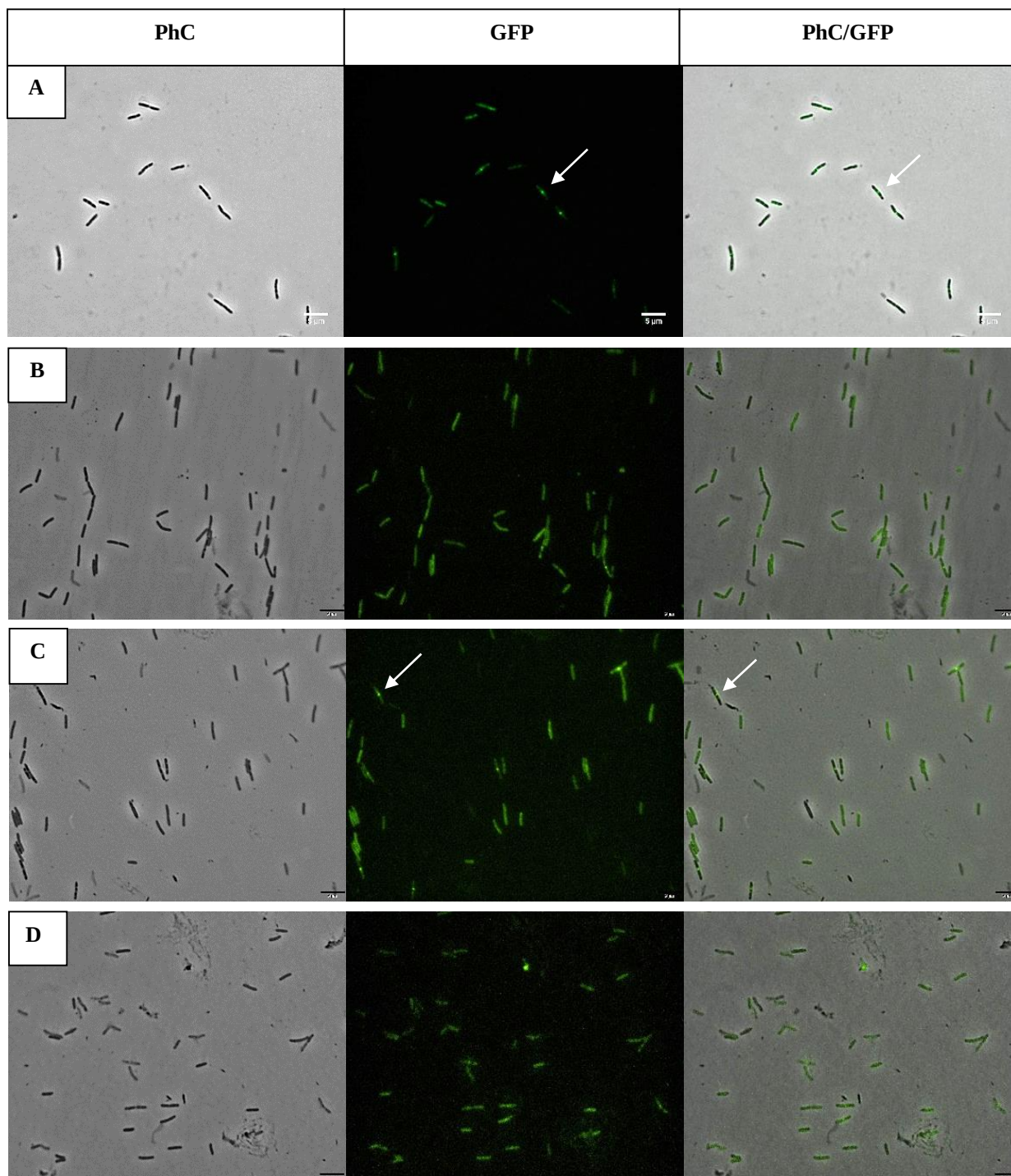


Figure 4. Effects of **MAC 4** on the divisome of *X. citri* expressing ZapA-GFP. **(A)** Untreated *X. citri* (negative control); **(B)** *X. citri* treated with hexyl gallate at 255 $\mu\text{mol/L}$ after 30 min (positive control); **(C)** *X. citri* treated with **MAC 4** at $\frac{1}{2}\text{MIC}$ (165 $\mu\text{mol/L}$) after 15 min; **(E)** *X. citri* treated with **MAC 4** at $\frac{1}{2}\text{MIC}$ after 30 min. Magnification: 100 $\times$; bar 5 μm . The arrows indicate the intact divisome.

Fonte: Elaborado pelo autor

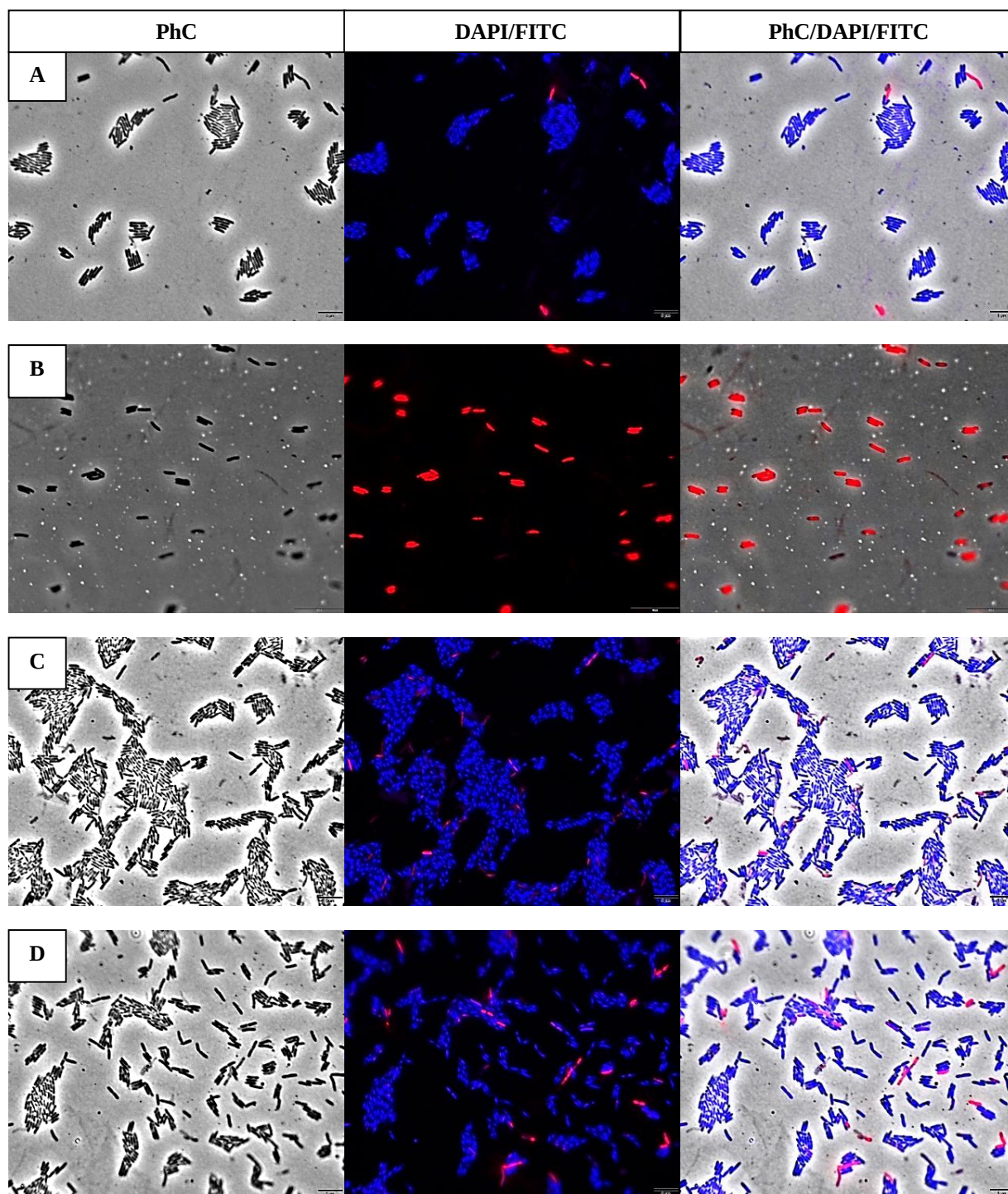


Figure 5. Effects of **MAC 4** on the *X. citri* membrane stained with Live/Dead BacLight kit. **(A)** Untreated *X. citri* (negative control); **(B)** *X. citri* after a heat shock treatment for 2 min at 55 °C (positive control); **(C)** *X. citri* treated with **MAC 4** at $\frac{1}{2}$ MIC (165 μ mol/L) after 15 min; **(D)** *X. citri* treated with **MAC 4** at $\frac{1}{2}$ MIC after 30 min. Magnification: 100 $\times$; bar 5 μ m. Blue cells: whole membrane. Pink-red cells: damaged membrane.

Fonte: Elaborado pelo autor

4.2.6. Effects of MAC 4 on the FtsZ GTPase activity of *X. citri*

In order to verify whether the disruption of the *X. citri* divisome is related to FtsZ inhibition, we evaluated **MAC 4** ability to affect the GTPase activity of FtsZ from *X. citri*. Cell division is dependent of the FtsZ polymerization, a process that requires GTP hydrolysis (GROUNDWATER et al., 2017). Therefore, compounds that alter the FtsZ GTPase activity can block bacterial cell division. The rate of GTP hydrolysis was measured during FtsZ exposure to **MAC 4** at MIC and 2×MIC (330 and 660 µmol/L, respectively). The results revealed that **MAC 4** significantly inhibited the GTPase activity of FtsZ in a concentration-dependent manner (Figure 6). GTP hydrolysis rate was reduced about 65% and 85% during treatment at MIC and 2×MIC, respectively, when compared with the negative control (FtsZ exposed to 1% DMSO). Investigations concerning **MAC 4** effects on the GTPase activity of FtsZ from *B. subtilis* indicated a weak increase in the GTP hydrolysis rate (Chapter II). Although the increase was not significant, **MAC 4** had the same effect as **CUR**, stimulating the GTPase activity of FtsZ from *B. subtilis* (RAI et al., 2008; MORÃO et al., 2019). However, **MAC 4** significantly reduced the GTPase activity of FtsZ from *X. citri*.

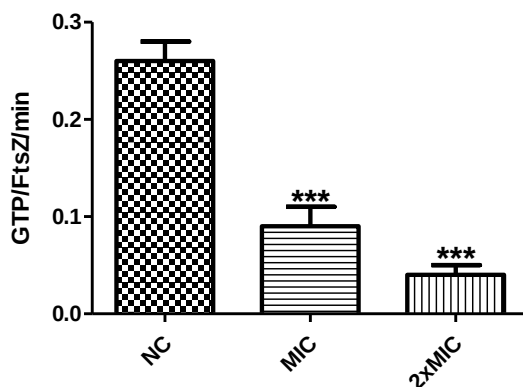


Figure 6. Effects of **MAC 4** at MIC and 2×MIC (330 and 660 µmol/L, respectively) on the GTPase activity of FtsZ from *X. citri*. NC = negative control (FtsZ exposed to 1% DMSO) (*** $p < 0.0001$, ANOVA with Dunnett's post-test).

Fonte: Elaborado pelo autor

4.3. CONCLUSION

In conclusion, we investigated the effects of monocarbonyl curcuminoids against *X. citri* as part of our search for new compounds which are able to combat citrus canker. **MAC 4** was considered to be an important hit with both its anti-*X. citri* activity *in vitro* and its ability to reduce the *X. citri* pathogenicity on the *C. sinensis* leaves. Furthermore, **MAC 4** did not induce *X. citri* resistance. Investigations on **MAC 4** action mode indicated that *X. citri* cell division is one of its targets. **MAC 4** had the ability to disrupt the divisome and inhibit the GTPase activity of FtsZ. Therefore, **MAC 4** is a promising agent to combat against citrus canker. Nevertheless, its potential risks and beneficial effects should be confirmed further by *in planta* approaches.

4.4. EXPERIMENTAL

4.4.1. Synthesis of MACs 1–8 and SMACs 1, 2, 4 and 5

Compounds **MACs 1–8** and **SMACs 1, 2, 4 and 5** were synthesized as described previously in Chapters II and III, respectively.

4.4.2. Anti-*X. citri* activity assay

The *Xanthomonas citri* subsp. *citri* used in this study was the sequenced strain 306 (IBSBF-1594) (DA SILVA et al., 2002). Susceptibility tests of *X. citri* to compounds were performed as described by Morão and co-authors (MORÃO et al., 2019). Stock solutions of compounds were prepared in DMSO. First, **MACs 1–8** were assayed at 300 µmol/L in order to determine the percentage of bacterial metabolism inhibition (BMI). The selected compound (**MAC 4**) and its simplified analogs (**SMACs 1, 2, 4 and 5**) were tested in concentrations ranging from 680 to 3.9 µmol/L to determine MIC and MBC values. Kanamycin and copper oxychloride were used as reference anti-*X. citri* compounds. Three independent assays were performed. MIC values were calculated from an analytical curve through a regression analysis and it represents the concentration required to reduce the viability of *X. citri* at 90%. The MBC values were defined as the lowest compound concentration that allowed no visible growth on the solid medium.

4.4.3. Growth curve assay

The growth curve assay was performed as previously reported by our group (NAZARÉ et al., 2018), with minor modifications. An isolated colony of *X. citri* was cultivated at 29 °C during a time period of 14 h. Then, the OD₆₀₀ of the bacterial culture was adjusted to ~0.1, and *X. citri* was treated with **MAC 4** at ½MIC and MIC (165 and 330 µmol/L, respectively). The experiments were performed in a 24-well plate in a total volume of 1.5 mL. The OD₆₀₀ was measured on a Synergy H1 microplate reader (BioTek, Winooski, USA) every 30 min for 72 h. Untreated *X. citri* cells and those treated with 1% DMSO were used as negative and vehicle controls, respectively. Three independent assays were performed.

4.4.4. Bacterial resistance induction assay

MAC 4 ability to induce resistance in *X. citri* was assessed according to Oz and co-authors (OZ et al., 2014), with modifications. First, *X. citri* was cultivated in nitrogen yeast glycerol (NYG) medium in the presence of a subinhibitory concentration of **MAC 4** (½MIC, as determined above) at 29 °C for 12 h. After incubation, 100 µL of the bacterial suspension was plated on NYG-agar to check for cell viability. A fraction of the same culture was then diluted so to give an OD₆₀₀ of ~0.1, and then exposed to a subinhibitory concentration of **MAC 4** (½MIC). The process was repeated for 21 passages over a period of three weeks. Untreated *X. citri* cells and those treated with 1% DMSO were used as negative and vehicle controls, respectively. Kanamycin was used as a reference antibacterial drug. Three independent assays were performed.

4.4.5. Bacterial leaf infiltration assay

The pathogenicity test was performed according to Silva and co-authors (SILVA et al., 2013), with minor modifications. The susceptible host used was *C. sinensis* (sweet orange, cultivar Pera Rio). Plants were cultivated under greenhouse conditions (15–35 °C). *X. citri* was exposed to **MAC 4** at ½MIC (165 µmol/L) for 6 h at 29 °C. After this period, 100 µL of the bacterial suspension were infiltrated on the abaxial surface of the *C. sinensis* leaves using a hypodermic syringe without the needle. The leaves were monitored every 7 days for four weeks. At the end of this period, leaves were detached and digitalized for citrus canker severity analyses. Infiltrated leaves with untreated *X. citri* were used as a positive control, whereas those infiltrated with *X. citri* exposed to 1% DMSO were used as a vehicle control. Infiltrated leaves

with sterile water (100 µL) were used as a negative control. The assay was performed in triplicate.

4.4.6. Microscopy assays

MAC 4 effects on the *X. citri* divisome were investigated according to Savietto and co-authors (SAVIETTO et al., 2018), with minor modifications. Strain “*X. citri amy::pPM2a-zapA*” was cultivated in the presence of 1% xylose to induce the expression of ZapA-GFP, which labels the divisome (MARTINS et al., 2010). *X. citri* was exposed to **MAC 4** at ½MIC (165 µmol/L) for 15 and 30 min at 29 °C. Hexyl gallate (at 255 µmol/L) was used as a reference compound for divisome disruption (KRÓL et al., 2015). The membrane disruption assay was performed as described by Nazaré and co-authors (NAZARÉ et al., 2018), with minor modifications. *X. citri* was treated with **MAC 4** at ½MIC for 15 and 30 min at 29 °C. Membrane integrity was assessed using the Live/Dead BacLight Bacterial Viability kit (Thermo-Scientific L7012). *X. citri* after a heat shock treatment for 2 min at 55 °C was used as a positive control for membrane permeability. All visualizations were carried out using an Olympus BX61 microscope (Olympus, Melville, USA) equipped with a monochromatic camera Orca Flash 2.8 (Hamamatsu, Shizuoka, Japan). Image documentation and processing were done using the software Cells Sen Dimension (Olympus, Melville, USA).

4.4.7. FtsZ GTPase activity assay

FtsZ of *X. citri* was expressed and purified as described by Kopacz and co-authors (KOPACZ et al., 2019). The FtsZ GTPase activity was determined using the Malachite Green Phosphate Assay Kit (Sigma MAK307-1KT), as described by Król and co-authors (KRÓL & SCHEFFERS, 2013). Minor modifications in this protocol and its procedures were reported in a previous work by Polaquini and co-authors (POLAQUINI et al., 2019). 1% DMSO was used as a negative control. The assay was performed in duplicate in two independent assays.

5. CAPÍTULO V

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5. CAPÍTULO V

New Unsymmetrical Monocarbonyl Curcuminoid (MAC 22) Disrupts *Xanthomonas citri* subsp. *citri* Membrane

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ABSTRACT

Xanthomonas citri subsp. *citri* (*X. citri*) is a phytopathogen that affects citrus plants, causing the Asian citrus canker. In a previous work, we reported the promising activity of an unsymmetrical monocarbonyl curcuminoid (**MAC 4**) against *X. citri*. It reduced the ability of *X. citri* to induce citrus canker lesions on *Citrus sinensis* leaves. In the current work, we synthesized two new series of monocarbonyl curcuminoids (**MACs 9–23**) based on the **MAC 4** framework in order to search for analogs with higher potency against *X. citri*. **MAC 22** (2-naphthyl analog) showed potency about 2.4 times higher than **MAC 4** (phenyl analog), with MIC (minimum inhibitory concentration) value of 135 $\mu\text{mol/L}$. In addition, our investigations demonstrated that **MAC 22** caused a delay in the *X. citri* exponential growth phase. Fluorescence and phase contrast microscopies indicated that **MAC 22** had the ability to disrupt the *X. citri* membrane. Taken together, these data indicated that the ring bioisosterism provided a promising candidate for the development of a new anti-*X. citri* agent.

5.1. INTRODUCTION

The genus *Xanthomonas* comprises about 30 species of plant-associated bacteria, many of which are pathogens of economically important crops, including rice (QUIBOD et al., 2020), sugarcane (NTAMBO et al., 2019) and corn (HARTMAN et al., 2020). The member *Xanthomonas citri* subsp. *citri* (*X. citri*) infects all the commercial citrus plants at different levels, causing the Asian citrus canker (GONÇALVES-ZULIANI et al., 2016). The main consequences of this disease are the defoliation of trees, which compromises the plant photosynthetic area, and the depreciation of production to the fresh fruit market due to the appearance of necrotic lesions on the fruits. Nevertheless, the citrus canker greatest impact is related to the fruit drop before harvest, which results in more serious economic losses (GOCHEZ et al., 2020). Despite the deleterious effects on the citriculture, *X. citri* is harmless to humans and other animals, and it has been used as a model organism in pathogenesis and phylogeny studies (GOCHEZ et al., 2018; FERENCE et al., 2018; BARCAROLO et al., 2019).

X. citri is easily dispersed through the combined action of wind and rain (DAS, 2003), and it employs several strategies in order to survive in complex and changing environments. Its ability to form biofilms not only makes the epiphytic survival possible but it also increases its protection against the environmental stress. (DUNGER et al., 2007; SENA-VÉLEZ et al., 2016). In addition, the biofilm provides resistance to damage induced by oxidative stress (FERENCE et al., 2018). Studies have also demonstrated that *X. citri* has a secretion system that injects antibacterial proteins into neighboring Gram-negative species. This mechanism confers an obvious fitness benefit, since it reduces interbacterial competition, increasing the availability of nutrients (SOUZA et al., 2015; SGRO et al., 2019; CENENS et al., 2020). These factors make the chemical or biological control of *X. citri* even more difficult.

In citrus canker endemic areas, the only viable option to control this phytopathogen has been the concomitant use of a set of agricultural management measures, such as the adoption of windbreaks, the control of citrus leafminer (*Phyllocnistis citrella*) and the application of copper-based bactericides (FERENCE et al., 2018). However, spraying with copper-based formulations should be used with caution. Copper accumulates in the soil and it can negatively affect root growth and nutrient uptake by the citrus plants (FAN et al., 2011). Moreover, high copper concentrations may cause fruit blemish due to its phytotoxicity (FERENCE et al., 2018). Another negative factor regarding the chemical control of *X. citri* with copper-based compounds only is the emergence of copper-resistant strains (BEHLAU et al., 2011; BEHLAU et al., 2013).

Our research group has been engaged in the study of new compounds which are able to combat *X. citri* (SILVA et al., 2013; NAZARÉ et al., 2018; SAVIETTO et al., 2018; ZAMUNER et al., 2020). Previously, we described the anti-*X. citri* effects of a monocarbonyl analog of curcumin (**MAC 4**). It affected the *X. citri* cell division by disrupting the divisome and inhibiting the GTPase activity of FtsZ. Furthermore, it reduced the ability of *X. citri* to induce citrus canker lesions on *Citrus sinensis* leaves. However, **MAC 4** showed moderate potency against the phytopathogen (Chapter IV). In the search for compounds with higher potency, we synthesized two series of monocarbonyl analogs (**MACs 9–23**), which were based on the **MAC 4** framework. The effects of **MACs 9–23** against *X. citri* were investigated. The most active analog (**MAC 22**) was investigated regarding its effects on the *X. citri* growth. Additionally, we evaluated its effects on the *X. citri* membrane.

5.2. RESULTS AND DISCUSSION

5.2.1. Chemistry

MACs 10–12 were prepared in three steps as summarized in Figure 1. First, 3,4-bis(methoxymethoxy)benzaldehyde (**1**) was prepared in 86% yield from the protection of 3,4-dihydroxybenzaldehyde with methoxymethyl chloride (MOMCl) in dry acetone and K₂CO₃. In the second step, ketones (acetone, cyclopentanone or cyclohexanone) were di-condensed with MOM-protected benzaldehydes (**1**) in order to provide MOM-diphenylpentanoids **2–4** in 45–67% yields. Lastly, MOM protecting groups were removed by treatment with HCl, providing **MACs 10–12** in 12–19% yields.

MACs 13–23 were prepared in four steps as summarized in Figure 1. The intermediate **1** was mono-condensed with acetone under basic catalysis and at room temperature, providing compound **5** in a yield of 68%. Ketone **5** was condensed with different aromatic aldehydes in order to synthesize MOM-diphenylpentanoids **6–16**. Finally, the compounds **6–16** were unprotected under acid catalysis, providing **MACs 13–23** in 12–36% yields.

The structures of synthesized curcuminoids **MACs 10–23** were confirmed by ¹H and ¹³C NMR spectra data analyses. Out of the 14 monocarbonyl curcuminoids, **MACs 14–23** were identified as new chemical entities (SCIFINDER, 2020). For all compounds, NMR data, including chemical shifts, integration multiplicities and coupling constants, corresponded to the proposed structures. In ¹H NMR spectrum, hydrogens α/α' and β/β' resonated as doublets and clearly indicated the formation of two olefins. Coupling constants values of olefin hydrogens

signals ranged from 15 to 16 Hz, corroborating their *trans* configuration. Spectra were presented as Supplementary Material.

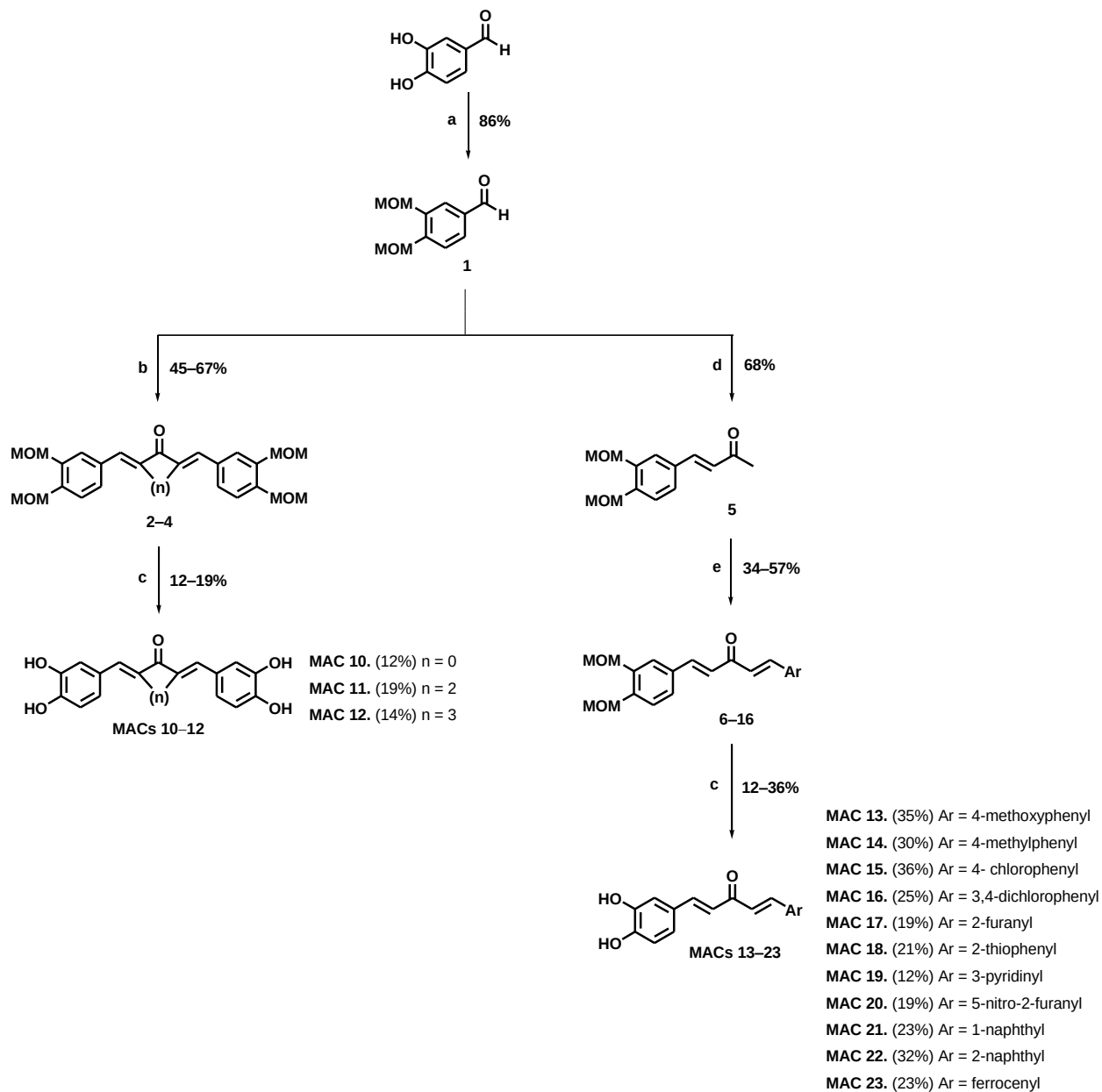


Figure 1. Synthesis of **MACs 10–23**. Reagents and conditions: **(a)** MOMCl, K₂CO₃, dry acetone, N₂ atmosphere, room temperature, 1 h; **(b)** ketone (acetone, cyclopentanone or cyclohexanone), NaOH, EtOH, N₂ atmosphere, room temperature, 6–8 h; **(c)** HCl, EtOH, N₂ atmosphere, room temperature, 48–72 h; **(d)** acetone, NaOH, N₂ atmosphere, room temperature, 1 h; **(e)** aromatic aldehyde, NaOH, EtOH, N₂ atmosphere, room temperature, 10–24 h.

Fonte: Elaborado pelo autor

5.2.2. Anti-*X. citri* activity

The effects of monocarbonyl curcuminoids of series I (**MACs 9–16**) and series II (**MACs 17–23**) were evaluated against *X. citri* subsp. *citri* (strain 306, IBSBF-1594) (DA SILVA, 2002). Kanamycin and copper oxychloride, which is a commercial active compound often employed to combat citrus canker, were used as reference anti-*X. citri* compounds. The anti-*X. citri* potency of the compounds was expressed as minimum inhibitory concentration (MIC) values (Tables 1 and 2).

We previously reported the **MAC 4** anti-*X. citri* activity, an unsymmetrical monocarbonyl curcuminoid designed from curcumin. It had promising effects against this phytopathogen, but it showed moderate potency, with MIC value of 330 $\mu\text{mol/L}$ (Chapter IV). In order to search for compounds with higher potency against *X. citri*, we evaluated a set of analogs (**MACs 9–23**) designed from **MAC 4** framework.

First, in a previous study, we described that monoacetylated alkyl gallates were more active than non-acetylated alkyl gallates against *X. citri* (SAVIETTO et al., 2018). Thus, we hypothesized that the **MAC 4** etherification with a labile group could lead to a more potent analog due to the increase of lipophilicity, which is a factor that facilitates permeation through biomembranes (RAUTIO et al., 2008; RAUTIO et al., 2018). However, **MAC 9** (3,4-diMOM) was inactive (MIC >350 $\mu\text{mol/L}$), corroborating the relevance of OH groups (phenol moiety) for the anti-*X. citri* activity. The phenol moiety appears to be pivotal for the activity against this phytopathogen, since this fragment was present in all compounds that we previously described against *X. citri* (SILVA et al., 2013; SAVIETTO et al., 2018, NAZARÉ et al., 2018; MORÃO et al., 2019).

Second, based on the importance of the OH groups for the bioactivity, we investigated the effect of the insertion of two OH groups on the **MAC 4** benzene ring B. The tetrahydroxylated analog, **MAC 10** (MIC 290 $\mu\text{mol/L}$), showed more potent activity than **MAC 4** (MIC 330 $\mu\text{mol/L}$). This finding indicated that the presence of a second diphenolic fragment improved the anti-*X. citri* activity of monocarbonyl curcuminoids.

Third, we evaluated the conformational restriction effect on the tetrahydroxylated analogs activity. **MACs 11** and **12** (cyclopentanone and cyclohexanone derivatives, respectively) had MIC values of 196 and 213 $\mu\text{mol/L}$, respectively. They were more active than **MAC 10** (acetone derivative, MIC 290 $\mu\text{mol/L}$), what indicates the importance of the compact conformation for the activity against this phytopathogen.

Table 1. Anti-*X. citri* activity of monocarbonyl curcuminoids of series I (**MACs 9–16**).

Compounds	R	n	MIC (μmol/L)
MAC 4*	—	—	330
MAC 9	—	—	>350
MAC 10	<i>m,p</i> -diOH	0	290
MAC 11	<i>m,p</i> -diOH	2	196
MAC 12	<i>m,p</i> -diOH	3	213
MAC 13	<i>p</i> -OMe	0	321
MAC 14	<i>p</i> -Me	0	>350
MAC 15	<i>p</i> -Cl	0	>350
MAC 16	<i>m,p</i> -diCl	0	>350
kanamycin	—	—	40
copper oxychloride	—	—	43**

MIC = minimum inhibitory concentration; *Chapter IV; **MIC in μg/mL.

Fonte: Elaborado pelo autor

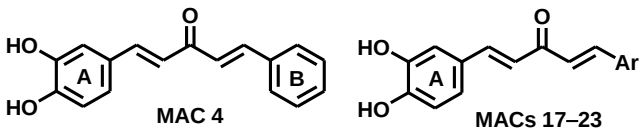
Fourth, we also evaluated the effects concerning the insertion of electron-donating (**MACs 13** [4-OMe] and **14** [4-Me]) and electron-withdrawing (**MACs 15** [4-Cl] and **16** [3,4-diCl]) groups on the **MAC 4** benzene ring B. **MAC 13** (MIC 321 μmol/L) showed similar activity to **MAC 4** (MIC 330 μmol/L), while **MACs 14–16** were inactive against *X. citri* (MIC >350 μmol/L). These results suggest that the presence of electron-donating and electron-withdrawing groups on the benzene ring B was not relevant or reduced the anti-*X. citri* activity.

The series II analogs were designed in order to evaluate the effects of replacing the **MAC 4** benzene ring with other aromatic rings (Table 2). First, in order to investigate the hypothetical ring bioisosterism (LIMA & BARREIRO, 2005), comparisons were made to determine the effects of replacing benzene ring B with heteroaryl rings with six π-electrons, such as 2-furanyl (**MAC 17**), 2-thiophenyl (**MAC 18**) and 3-pyridinyl (**MAC 19**). **MAC 17** was inactive (MIC >350 μmol/L). On the other hand, **MACs 18** and **19** showed MIC values of 167 and 242 μmol/L, respectively. It suggests that substitution of the benzene ring for both thiophene and pyridine rings improved the activity against this phytopathogen.

Second, we previously described that the insertion of a NO₂ group on the furan ring increased the antibacterial activity of monocarbonyl curcuminoids against *Mycobacterium tuberculosis* (AYUSSO, 2019; Chapter III). Based on this result, we hypothesized that the insertion of a NO₂ group at position 5 on the furan ring could lead to a more potent analog

against *X. citri*. **MAC 20** (5-nitro-2-furanyl) showed potent activity against *X. citri*, with MIC value of 197 $\mu\text{mol/L}$. The presence of a NO_2 group was essential for the antibacterial activity of furanyl analog against *X. citri*, corroborating our previous data (AYUSSO, 2019; Chapter III).

Table 2. Anti-*X. citri* activity of monocarbonyl curcuminoids of series II (**MACs 17–23**).

		
Compounds	Ar	MIC ($\mu\text{mol/L}$)
MAC 4*	—	330
MAC 17	2-furanyl	>350
MAC 18	2-thiophenyl	167
MAC 19	3-pyridinyl	242
MAC 20	5-nitro-2-furanyl	197
MAC 21	1-naphthyl	322
MAC 22	2-naphthyl	135
MAC 23	ferrocenyl	>350
kanamycin	—	40
copper oxychloride	—	43**

MIC = minimum inhibitory concentration; *Chapter IV; **MIC in $\mu\text{g/mL}$.

Fonte: Elaborado pelo autor

Third, we also evaluated the effects of replacing benzene with homoaryl rings with ten π -electrons, including 1-naphthyl (**MAC 21**) and 2-naphthyl (**MAC 22**). **MACs 21** and **22** had MIC values of 322 and 135 $\mu\text{mol/L}$, respectively, demonstrating that the naphthalene ring position was pivotal for the bioactivity. In Chapter III, we also showed that the naphthalene ring position was relevant for the antimycobacterial activity of simplified monocarbonyl curcuminoids. The 2-naphthyl analog was also approximately two times more potent than the 1-naphthyl analog against *M. tuberculosis* (Chapter III).

Fourth, we evaluated an analog carrying the ferrocenyl ring (**MAC 23**). This organometallic ring was selected due to its antibacterial effects (LIU et al., 2018; HENRY et al., 2020). However, **MAC 23** was inactive (MIC >350 $\mu\text{mol/L}$) against *X. citri*, indicating that the organometallic ring was not relevant for antibacterial activity of monocarbonyl curcuminoids.

In general, preliminary structure-activity relationships indicated that conformational restriction and ring bioisosterism increased the activity of monocarbonyl curcuminoids against

X. citri. We selected **MAC 22** (MIC 135 $\mu\text{mol/L}$), which was about 2.4 times more potent than **MAC 4** (MIC 330 $\mu\text{mol/L}$), for subsequent anti-*X. citri* assays. The minimum bactericidal concentration (MBC) value against *X. citri* was determined. **MAC 22** showed MBC value of 316 $\mu\text{mol/L}$, while **MAC 4** had MBC value of 375 $\mu\text{mol/L}$ (Chapter IV), confirming the potent effect of **MAC 22** against *X. citri*.

5.2.3. Effects of MAC 22 on the *X. citri* growth

We investigated the **MAC 22** effects at MIC (135 $\mu\text{mol/L}$) on the *X. citri* growth pattern. Culture optical density (OD_{600}) was measured every 30 min for 72 h in order to construct the growth curves (Figure 2). The untreated *X. citri* culture reached the stationary phase after a period of 14 h (negative control). The *X. citri* culture treated with 1% DMSO (vehicle control) showed the same profile as the negative control. However, treatment with **MAC 22** at MIC inhibited the early growth, delaying the exponential phase start and prolonging the required time for the culture to reach the stationary phase (~ 34 h). The effect during the first few hours of exposure was probably due to the bacteriostatic action of **MAC 22** at MIC.

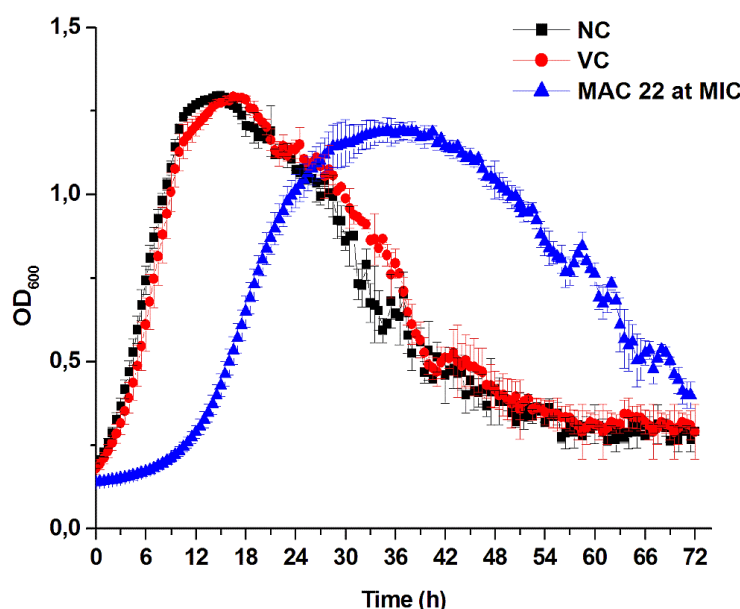


Figure 2. Effects of **MAC 22** at MIC (135 $\mu\text{mol/L}$) on the *X. citri* growth. NC = negative control (untreated *X. citri*); VC = vehicle control (*X. citri* treated with 1% DMSO).

Fonte: Elaborado pelo autor

In Chapter IV, **MAC 4** effects on the *X. citri* growth pattern were assessed. Unlikely **MAC 22**, the treatment with **MAC 4** at MIC completely inhibited the *X. citri* growth during the assessed period (72 h). This difference may be related to the bactericidal effect of **MAC 4** at MIC, since its MIC and MBC (330 and 375 $\mu\text{mol/L}$, respectively) values were similar (Chapter IV).

5.2.4. Effects of **MAC 22** on the *X. citri* membrane

We evaluated the **MAC 22** effects on the *X. citri* membrane by phase contrast and fluorescence microscopies. *X. citri* was treated with **MAC 22** at $\frac{1}{2}\text{MIC}$ (67.5 $\mu\text{mol/L}$) for 15 and 30 min. The fluorescent dyes propidium iodide (PI) and SYTO9 were used. They stain cells with damaged membrane (in pink-red) and intact membrane (in blue). Phase contrast and fluorescence microscopy representative images of the negative control (untreated *X. citri*), positive control (*X. citri* after a heat shock treatment) and treatment with **MAC 22** after 15 and 30 min were shown in Figure 3. The untreated *X. citri* cells were stained mostly in blue (Figure 3A). The same pattern was observed when *X. citri* was exposed to 1% DMSO (data not shown). However, *X. citri* cells after a heat shock treatment for 2 min at 55 °C were stained red (Figure 3B). Exposure to **MAC 22** at $\frac{1}{2}\text{MIC}$, in both periods of 15 and 30 min (Figure 3C and 3D, respectively), led to a prevalence of pink-red stained cells, indicating membrane permeability.

Percentages of cells with damaged membrane were quantified from the microscope images and were shown in Figure 4. The untreated *X. citri* had approximately 7% and 13% of the cells with permeabilized membrane after 15 and 30 min, respectively. On the other hand, *X. citri* after a heat shock treatment showed 72% and 87% of the cells with permeabilized membrane after 15 and 30 min, respectively. Treatment with **MAC 22** at $\frac{1}{2}\text{MIC}$ for 15 and 30 min showed, respectively, 82% and 96% of the cells with permeabilized membrane. It was an evidence that the *X. citri* membrane disruption by **MAC 22** was a time-dependent effect.

Previously, we also investigated the **MAC 4** action mode against *X. citri* (Chapter IV). It had the ability to perturb *X. citri* cell division by disrupting the divisome and inhibiting the GTPase activity of FtsZ, and it did not significantly damage the *X. citri* membrane. However, the replacement of the benzene ring (**MAC 4**) by the β -naphthalene ring (**MAC 22**) affected the action mode. This change may be correlated with the increase in amphiphilicity due to the presence of the naphthyl ring, a parameter that allows the compound insertion more easily into phospholipid bilayers, resulting in increased membrane permeability (HERZOG & FRIDMAN, 2014; KOH et al., 2016).

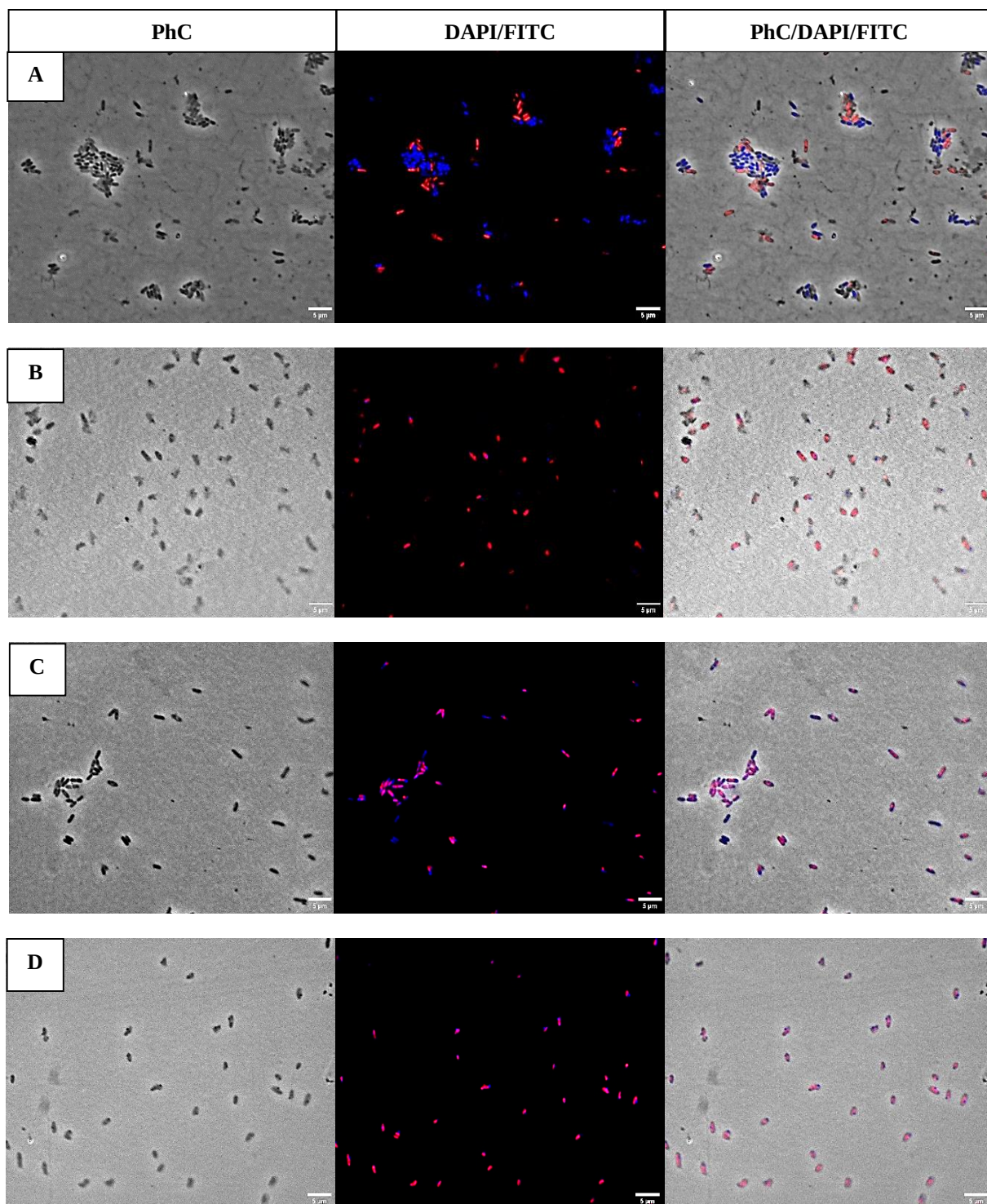


Figure 3. Effects of **MAC 22** on the *X. citri* membrane stained with Live/Dead BacLight kit. (A) Untreated *X. citri* (negative control); (B) *X. citri* after a heat shock treatment for 2 min at 55 °C (positive control); (C) *X. citri* treated with **MAC 22** at $\frac{1}{2}$ MIC (67.5 μ mol/L) after 15 min; (D) *X. citri* treated with **MAC 22** at $\frac{1}{2}$ MIC after 30 min. Magnification: 100 $\times$; bar 5 μ m. Blue cells: whole membrane. Pink-red cells: damaged membrane.

Fonte: Elaborado pelo autor

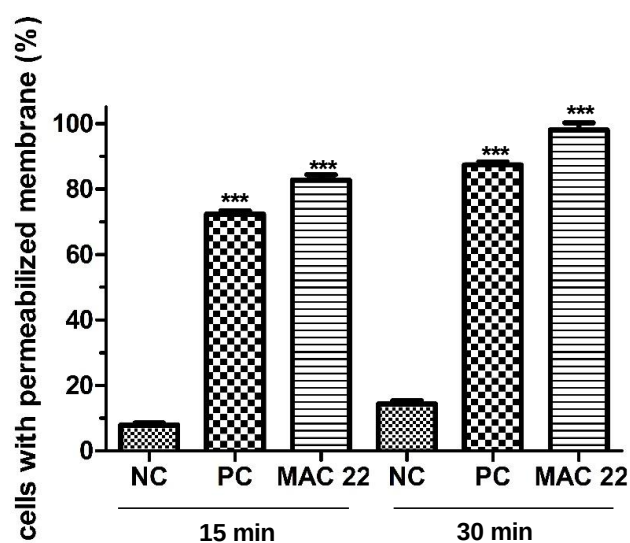


Figure 4. Percentage of *X. citri* cells with permeabilized membrane. **NC**: negative control (untreated *X. citri*); **PC**: positive control (*X. citri* after a heat shock treatment for 2 min at 55 °C); **MAC 22** (*X. citri* treated with **MAC 22** at $\frac{1}{2}$ MIC [67.5 μ mol/L]). *** $p < 0.05$, ANOVA with Dunnett's post-test.

Fonte: Elaborado pelo autor

5.3. CONCLUSION

In summary, we synthesized two new series of unsymmetrical monocarbonyl curcuminoids based on the **MAC 4** structure. We demonstrated that replacing the benzene ring (**MAC 4**) with a β -naphthalene ring (**MAC 22**) increased the potency against *X. citri*. Therefore it indicates the importance of ring bioisosterism for the activity against the phytopathogen. **MAC 22** action mode involved *X. citri* membrane disruption, which is an important target for the development of new antibacterial agents. **MAC 22** is a promising agent to combat against *X. citri*. Nevertheless, its potential beneficial effects should be confirmed by *in planta* approaches.

5.4. EXPERIMENTAL

5.4.1. Chemistry

5.4.1.1. Material and instruments (Chapter II, item 2.4.1.1.)

5.4.1.2. Synthesis of **MAC 9**

MAC 9 was synthesized as described previously in Chapter II, item 2.4.1.4.2.

5.4.1.3. Synthesis of MACs 10–12

MACs 10–12 were synthesized in three steps.

5.4.1.3.1. Synthesis of 3,4-bis(methoxymethoxy)benzaldehyde (1).

Chapter II, item 2.4.1.4.1.

5.4.1.3.2. Synthesis of compounds 2–4. A solution of compound **1** (4 mmol) and ketone (acetone, cyclopentanone or cyclohexanone) (1.5 mmol) in EtOH (5 mL) was cooled in an ice bath for 10 min. Ethanolic solution of NaOH (2 mL, 1 mol/L) was added dropwise to the reaction mixture. The solution was stirred at room temperature for 6–8 h under N₂ atmosphere. Reaction medium was poured onto crushed ice. The crude product was partitioned with ethyl acetate (3 × 15 mL), and the combined organic phase was concentrated under reduced pressure. Compounds **2–4** were purified by column chromatography over silica gel using mixtures of hexane and ethyl acetate (7:3) as eluents.

5.4.1.3.3. Synthesis of MACs 10–12. A solution of compounds **2–4** (1 mmol) in EtOH (5 mL) and 33% HCl (0.4 mL) was stirred at room temperature for 72 h under N₂ atmosphere. Reaction medium was poured onto crushed ice. Precipitated crude product was filtered, washed with cold water and dried at room temperature. MACs 10–12 were purified by gel filtration column chromatography (LH-20) using EtOH as eluent.

5.4.1.4. Synthesis of MACs 13–23

MACs 13–23 were synthesized in four steps.

5.4.1.4.1. Synthesis of 3,4-bis(methoxymethoxy)benzaldehyde (1).

Chapter II, item 4.1.4.1.

5.4.1.4.2. Synthesis of compound 5. A solution of compound **1** (2 mmol) in acetone (8 mL) was cooled in an ice bath for 10 min. Acetonic suspension of NaOH (0.8 mL, 1 mol/L) was added dropwise to the reaction mixture. The suspension was stirred at room temperature for 1 h under N₂ atmosphere. Reaction medium was poured onto crushed ice. The crude product was partitioned with ethyl acetate (3 × 15 mL), and the combined organic phase was concentrated under reduced pressure. Compound **5** was purified by column chromatography over silica gel using mixture of hexane and ethyl acetate (8:2) as eluent.

5.4.1.4.3. Synthesis of compounds 6–16. A solution of compound **5** (1 mmol) and aromatic aldehydes (1.5 mmol) in EtOH (5 mL) was cooled in an ice bath for 10 min. Ethanolic solution of NaOH (1 mL, 1 mol/L) was added dropwise to the reaction mixture. The solution was stirred at room temperature for 10–24 h under N₂ atmosphere. Reaction medium was poured onto crushed ice. The crude product was partitioned with ethyl acetate (3 × 15 mL), and the combined organic phase was concentrated under reduced pressure. Compounds **6–16** were purified by gel filtration column chromatography (LH-20) using EtOH as eluent.

5.4.1.4.4. Synthesis of MACs 13–23. A solution of compounds **6–16** (1 mmol) in EtOH (5 mL) and 33% HCl (0.4 mL) was stirred at room temperature for 48 h under N₂ atmosphere. Reaction medium was poured onto crushed ice. Precipitated crude product was filtered, washed with cold water and dried at room temperature. **MACs 13–23** were purified by gel filtration column chromatography (LH-20) using EtOH as eluent.

5.4.2. Anti-*X. citri* activity assay

Chapter IV, item 4.4.2.

5.4.3. Growth curve assay

Chapter IV, item 4.4.3.

5.4.4. Microscopy assay

Chapter IV, item 4.4.6.

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6. CAPÍTULO VI

Unsymmetrical Monocarbonyl Curcuminoid (MAC 8) Decreases *Candida albicans* Cell Adhesion and Biofilm

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ABSTRACT

Infections caused by *Candida* species have increased considerably in recent decades. However, antifungal agents available for treatment are restricted to a few classes of drugs that present several problems, including toxicity and narrow spectrum. In this context, we synthesized and investigated the anti-*Candida* activity of unsymmetrical monocarbonyl curcuminoids (**MACs**) designed from curcumin (**CUR**), which is a promising phytochemical against *Candida*. **MAC 8** showed potent activity against *C. albicans* and non-*albicans Candida* species, with MIC (minimum inhibitory concentration) values ranging from 7.8 to 31.2 $\mu\text{mol/L}$. In addition, **MAC 8** was active against clinical *Candida* isolates ($62.5 \mu\text{mol/L} \leq \text{MIC} \leq 250 \mu\text{mol/L}$). It was also able to reduce *C. albicans* adhesion to human fibroblast and epithelial cells (HGF-1 and HaCaT lines, respectively). Our investigations have demonstrated its ability to inhibit *C. albicans* biofilm. Furthermore, **MAC 8** combinations with amphotericin B or fluconazole showed synergistic effects against *C. albicans*. Preliminary studies on its action mode indicated a combination of its effects on the *C. albicans* membrane and cell wall. Finally, **MAC 8** was more stable than **CUR** in phosphate buffer, and it also demonstrated low systemic toxicity in *Galleria mellonella* model, with LD₅₀ (dose required in order to kill 50% of the larvae) value of 300 $\mu\text{mol/kg}$. These findings open new avenues for the study of this curcuminoid as an excellent prototype with anti-*Candida* activity.

6.1. INTRODUCTION

The incidence of fungal infections in humans has increased considerably in the last three decades. Various factors have led to this escalation, including an increment of both invasive surgical procedures and immunocompromised individuals, as well as the indiscriminate use of broad spectrum antibiotics (STAMATIADES et al., 2018). Of the fungi regarded as human pathogens, the members of the genus *Candida* are the most frequently recovered from infection, especially *C. albicans* (NAGLIK, RICHARDSON & MOYES, 2014). However, the number of diseases caused by non-*albicans Candida* species, such as *C. tropicalis*, *C. glabrata*, *C. parapsilosis* and *C. krusei*, has increased significantly in recent years (KOŁACZKOWSKA & KOŁACZKOWSKI, 2016). *Candida* pathogenicity is mediated by a number of virulence factors, including adherence and biofilm formation on host tissues, as well as on implantable medical devices (STANISZEWSKA, 2020). Nowadays, the available treatment for fungal infections is restricted mainly to azoles, polyenes and echinocandins (BELLMANN & SMUSZKIEWICZ, 2017). Nevertheless, factors such as the increase in the number of resistant strains, the narrow spectrum due to intrinsic resistance, the toxicity and the low bioavailability affect the antifungal drugs efficacy (FISHER et al., 2018). Therefore, therapeutic options become limited.

Our research group has been engaged in the study of natural products and synthetic compounds based on natural products in order to develop new anti-*Candida* agents (LIMA et al., 2016; SINGULANI et al., 2017; MARQUES et al., 2019a). Curcumin (**CUR**) is a bioactive phytochemical which is considered safe for humans (LAO et al., 2006). In addition to its *in vitro* moderate activity against several *Candida* species (NEELOFAR et al., 2011; KHAN et al., 2012), a preclinical study reported that the application of a vaginal cream containing **CUR** reduced the number of rats infected with *C. albicans* after 6 days of treatment (FERNANDES et al., 2018). Investigations regarding the **CUR** action mode have described an activity involving fungal multi-targets. It acts on both the biosynthesis and the integrity of the *C. albicans* membrane and cell wall (KUMAR et al., 2014; LEE & LEE, 2014). Moreover, it also inhibits important virulence factors, including adhesion (ALALWAN et al., 2017), biofilm (DOVIGO et al., 2011) and yeast-to-hyphae transition (SHARMA et al., 2010).

Although **CUR** has a promising anti-*Candida* activity, some factors hinder its clinical use, such as its poor chemical and metabolic stability (NELSON et al., 2017). Studies have correlated these factors with the presence of the β -diketone moiety in the **CUR** structure (SHETTY et al., 2014). The β -diketone fragment appears to be a specific substrate of a series

of aldo-keto reductases (ROSEMOND et al., 2004), and it is also rapidly degraded under alkaline conditions (HEGER et al., 2014). Studies have demonstrated that the **CUR** pharmacokinetic limitations can be overcome when the β -diketone is replaced by a monocarbonyl moiety, providing the monocarbonyl curcuminoids (**MACs**) (SHETTY et al., 2015). In addition, investigations have reported that **MACs** had more potent antibacterial and antitumor effects than **CUR** (SILVA et al., 2018; MORÃO et al., 2019). However, researches into the antifungal effects of **MACs** are scarce (DIN et al., 2017; NAGARGOJE et al., 2019).

In this context, we synthesized a series of monocarbonyl curcuminoids and evaluated their antifungal effects against *Candida* species. The most potent compound (**MAC 8**) was investigated regarding its activity against clinical *Candida* isolates, as well as with regard to its effects on *C. albicans* cell adhesion and biofilm. Furthermore, **MAC 8** effects combined with antifungal drugs against *C. albicans* were analyzed and preliminary studies on its action mode were carried out. We also investigated its chemical stability and systemic toxicity in *Galleria mellonella* model.

6.2. RESULTS AND DISCUSSION

6.2.1. Chemistry

MACs 24–31 were prepared in two steps by aldol condensation reactions (TORREZAN et al., 2020). First, the benzylideneacetone intermediate (**1**) was synthesized by the mono-condensation reaction between acetone and benzaldehyde under basic catalysis and at room temperature, resulting in a yield of 89%. Then, the benzylideneacetone was condensed again with benzaldehyde derivatives under acid catalysis and at room temperature, providing **MACs 24–31** in 25–79% yields (Figure 1).

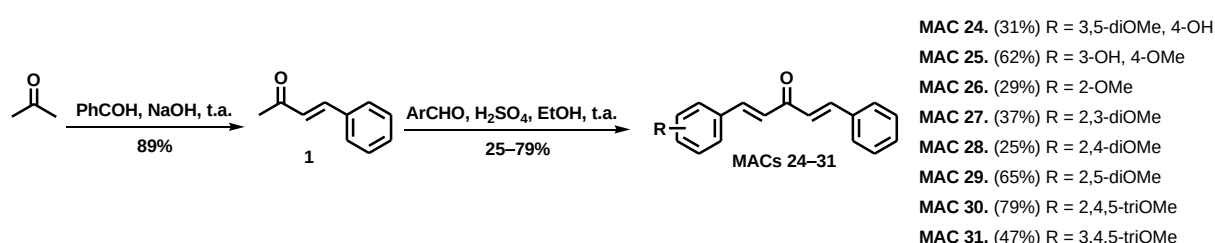


Figure 1. Synthesis of **MACs 24–31**.

Fonte: Elaborado pelo autor

MACs 32–34 were synthesized as summarized in Figure 2 (KIM et al., 2008; KIM et al., 2015). First, hydroxybenzaldehydes (2-hydroxybenzaldehyde, 2,3-dihydroxybenzaldehyde and 2,5-dihydroxybenzaldehyde) were protected with the MOM (methoxymethyl) group using K_2CO_3 as a catalyst, with yields ranging from 73 to 90%. MOM-benzaldehydes (**2a–c**) were condensed with benzylideneacetone (**1**) in order to provide MOM-diphenylpentanoids (**3a–c**) in 53–66% yields. MOM groups were removed by treatment with HCl, providing **MACs 32–34** in 10–21% yields.

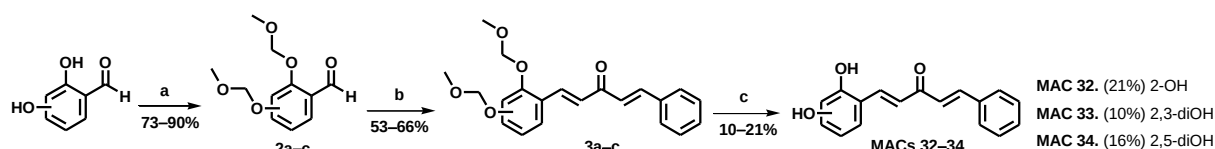


Figure 2. Synthesis of **MACs 32–34**. Reagents and conditions: (a) MOMCl, K_2CO_3 , dry acetone, N_2 atmosphere, room temperature, 1 h; (b) benzylideneacetone (**1**), NaOH, EtOH, N_2 atmosphere, room temperature, 8 h; (c) HCl, EtOH, N_2 atmosphere, room temperature, 48 h.
Fonte: Elaborado pelo autor

The structures of synthesized compounds were confirmed by their melting point and 1H and ^{13}C NMR spectra data analyses. Out of the 11 monocarbonyl curcuminoids, **MACs 24**, **25**, **27**, **29**, **30**, **33** and **34** were identified as new chemical entities (SCIFINDER, 2020). For all compounds, NMR data, including chemical shifts, integration multiplicities and coupling constants, corresponded to the proposed structures. In 1H NMR spectrum, hydrogens α/α' and β/β' resonated as doublets and clearly indicated the formation of two olefins. Coupling constants values of olefin hydrogens signals ranged from 15 to 16 Hz, corroborating their *trans* configuration. Spectra were presented as Supplementary Material.

6.2.2. Anti-*Candida* activity

The anti-*Candida* activity of curcumin (**CUR**), simplified curcumin (**SCUR**) and unsymmetrical monocarbonyl analogs (**MACs 1–8** and **24–34**) was evaluated against *C. albicans* (ATCC 90028), *C. tropicalis* (ATCC 750), *C. glabrata* (ATCC 90030), *C. parapsilosis* (ATCC 22019) and *C. krusei* (ATCC 6258). The antifungal potency of the compounds was expressed as minimum inhibitory concentration (MIC) and minimum

fungicidal concentration (MFC) values in $\mu\text{mol/L}$ (Table 1). Fluconazole and amphotericin B were used as standard antifungal drugs.

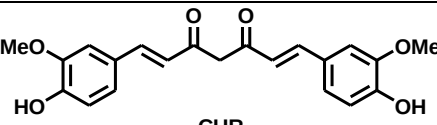
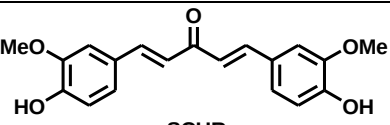
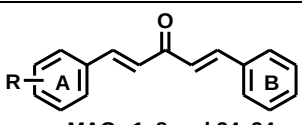
CUR showed MIC values $>250 \mu\text{mol/L}$ against all *Candida* species. Our results corroborated with other studies (NEELOFAR et al., 2011; KHAN et al., 2012). Neelofar and co-authors described MIC values of **CUR** ranging from 678 to 1357 $\mu\text{mol/L}$ against *C. albicans* (ATCC10261 and ATCC 44829), *C. tropicalis* (ATCC 750) and *C. glabrata* (ATCC 90030) (NEELOFAR et al., 2011). However, we did not evaluate concentrations higher than 250 $\mu\text{mol/L}$ due to its moderate solubility in the culture medium, which could lead to ambiguous measurements of MIC values.

First, in order to investigate the effects of replacing the β -diketone moiety by a monocarbonyl group, we compared **CUR** and **SCUR** anti-*Candida* activities. The simplified analog **SCUR** showed MIC values of 95.7 $\mu\text{mol/L}$ against all tested *Candida* species, indicating that the **CUR** simplification increased the antifungal potency. Moreover, the replacement of β -diketone by a monocarbonyl fragment also improved the **SCUR** bioactivity against Gram-negative and Gram-positive bacteria species, and against *Mycobacterium tuberculosis* (Chapter II). Taken together, the **CUR** and **SCUR** antitubercular, antibacterial and antifungal effects confirmed the importance of removing this moiety in order to have a higher antimicrobial potency.

Second, comparisons were made between the MIC values of **SCUR** and **MAC 1** in order to assess the effects of removing substituents on the aromatic ring B. **MAC 1** (unsubstituted benzene ring B) had similar activity to **SCUR** against *C. parapsilosis* (MIC 111 and 95.7 $\mu\text{mol/L}$, respectively). On the other hand, **MAC 1** (MIC 27.8 $\mu\text{mol/L}$) showed higher activity against *C. albicans*, *C. tropicalis*, *C. glabrata* and *C. krusei* when compared to **SCUR** (MIC 95.7 $\mu\text{mol/L}$). In general, the results indicated that the removal of 3-OMe and 4-OH substituents on the aromatic ring B improved the antifungal activity.

Third, we also assayed **MAC 2**, which contains the unsubstituted aromatic rings A and B. **MAC 2** activity (MIC $\geq 133 \mu\text{mol/L}$) was less potent than **SCUR** and **MAC 1** activities ($27.8 \mu\text{mol/L} \leq \text{MIC} \leq 111 \mu\text{mol/L}$). Taken together, MIC values of **SCUR** and **MACs 1** and **2** suggest that, when there was only one substituted aromatic ring by 3-OMe and 4-OH groups, it was ideal for the monocarbonyl curcuminoids anti-*Candida* activity. The same relationship was also found for the antibacterial activity of **SCUR** and **MACs 1** and **2** against *Acinetobacter baumannii*, *Staphylococcus aureus* (Chapter II) and *Xanthomonas citri* subsp. *citri* (Chapter IV).

Table 1. Anti-*Candida* activity of curcumin (**CUR**), simplified curcumin (**SCUR**) and unsymmetrical monocarbonyl analogs (**MACs 1–8** and **24–34**) expressed as MIC and MFC values in $\mu\text{mol/L}$.

 CUR  SCUR  MACs 1–8 and 24–34											
Compound	R	Ca		Ct		Cg		Cp		Ck	
		MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
CUR	–	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250
SCUR	–	95.7	192	95.7	192	95.7	192	95.7	192	95.7	192
MAC 1	3-OMe, 4-OH	27.8	55.7	27.8	55.7	27.8	55.7	111	223	27.8	55.7
MAC 2	H	>250	>250	>250	>250	133	133	>250	>250	133	>250
MAC 24	3,5-diOMe, 4-OH	101	201	201	201	101	201	201	201	201	>250
MAC 25	3-OH, 4-OMe	111	223	55.7	111	111	223	55.7	111	27.8	55.7
MAC 26	2-OMe	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250
MAC 5	3-OMe	237	237	118	237	>250	>250	>250	>250	118	237
MAC 7	4-OMe	>250	>250	237	237	>250	>250	>250	>250	237	>250
MAC 27	2,3-diOMe	212	212	106	212	>250	>250	>250	>250	106	212
MAC 28	2,4-diOMe	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250
MAC 29	2,5-diOMe	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250
MAC 3	3,4-diOMe	>250	>250	106	212	>250	>250	>250	>250	>250	>250
MAC 30	2,4,5-triOMe	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250
MAC 31	3,4,5-triOMe	193	193	>250	>250	>250	>250	>250	>250	193	193
MAC 32	2-OH	62.3	62.3	62.3	125	15.6	31.2	62.3	125	62.3	62.3
MAC 8	3-OH	31.2	62.3	31.2	62.3	7.8	15.6	31.2	62.3	15.6	31.2
MAC 6	4-OH	62.3	62.3	125	250	31.2	62.3	62.3	125	31.2	62.3
MAC 33	2,3-diOH	58.6	58.6	117	235	29.3	29.3	117	117	58.6	117
MAC 34	2,5-diOH	117	235	235	235	58.6	58.6	235	235	117	235
MAC 4	3,4-diOH	117	117	235	235	29.3	58.6	117	235	58.6	117
FCZ	–	52.2	–	6.5	–	6.5	–	3.2	–	–	–
AMP B	–	0.5	–	0.5	–	0.5	–	0.3	–	0.5	–

MIC = minimum inhibitory concentration; MFC = minimum fungicidal concentration; Ca = *C. albicans* (ATCC 90028); Ct = *C. tropicalis* (ATCC 750); Cg = *C. glabrata* (ATCC 90030); Cp = *C. parapsilosis* (ATCC 22019); Ck = *C. krusei* (ATCC 6258); FCZ = fluconazole; AMP B = amphotericin B; – not determined.

Fonte: Elaborado pelo autor

Fourth, we evaluated **MAC 24** (3,5-diOMe, 4-OH), and its antifungal effects were compared with **MAC 1** (3-OMe, 4-OH). **MAC 24** had MIC values ≥ 101 $\mu\text{mol/L}$, while **MAC 1** showed MIC values ≥ 27.8 $\mu\text{mol/L}$. The presence of an OMe group at position 5 on the benzene ring A decreased **MAC 24** antifungal activity against all *Candida* species.

Fifth, we investigated anti-*Candida* activity of **MAC 25** (3-OH, 4-OMe), which is a regioisomer of **MAC 1** (3-OMe, 4-OH). **MAC 25** effects against *C. albicans*, *C. tropicalis* and *C. glabrata* were reduced when compared to **MAC 1**. On the other hand, **MAC 25** showed similar potency against *C. krusei* (MIC 27.8 $\mu\text{mol/L}$) and higher activity against *C. parapsilosis* (MIC 55.7 $\mu\text{mol/L}$) when compared to **MAC 1** (MIC 27.8 and 111 $\mu\text{mol/L}$, respectively). *C. parapsilosis* is the second or third most frequently isolated *Candida* species from patients (TÓTH et al., 2019). It causes infections mainly in premature neonates, with a mortality rate of approximately 10% (ASBECK, CLEMONS & STEVENS, 2009; PAMMI et al., 2013).

Sixth, the effects of the number and the position of the OMe group concerning the anti-*Candida* activity of monocarbonyl curcuminoids were investigated by testing a set of mono- (**MACs 5, 7 and 26**), di- (**MACs 3 and 27–29**) and trimethoxylated (**MACs 30 and 31**) analogs. The MIC values of the methoxylated curcuminoids indicated that the number of OMe substituents on the aromatic ring A was not relevant, but their position affected the antifungal activity. All methoxylated analogs that had an OMe group at position 3 on the aromatic ring A (**MACs 5** [3-OMe], **27** [2,3-diOMe], **3** [3,4-diOMe] and **29** [3,4,5-triOMe]) were active against at least one *Candida* species. Marques and co-authors also investigated the effects of the OMe group on anti-*Candida* activity of α,β -unsaturated ketones (chalcones). For this class of compounds, the number and the position of OMe substituents on the aromatic ring were important for the antifungal activity. The decrease in the number of OMe groups, as well as the presence of an OMe group at position 2 on the aromatic ring, increased the chalcones activity against *Candida* species (MARQUES et al., 2019a).

Seventh, the methoxylated analogs activity (**MACs 3, 5, 7 and 26–31**) was lower than the activity of the curcuminoids carrying both the OH and the OMe groups (**MACs 1, 24 and 25**), suggesting that the OH group was important for bioactivity. Thus, we also tested a set of mono- (**MACs 6, 8 and 32**) and dihydroxylated (**MACs 4, 33 and 34**) analogs in order to investigate the effects of the number and the position of the OH group concerning the anti-*Candida* activity. The number of OH groups affected the monocarbonyl curcuminoids activity. In general, monohydroxylated **MACs** ($7.8 \mu\text{mol/L} \leq \text{MIC} \leq 62.3 \mu\text{mol/L}$) showed higher potency than dihydroxylated **MACs** ($29.3 \mu\text{mol/L} \leq \text{MIC} \leq 235 \mu\text{mol/L}$). This result may be

related to the decrease in lipophilicity due to the insertion of a second hydrophilic substituent on the aromatic ring, which hinders the permeation through biomembranes. These findings did not encourage us to investigate the trihydroxylated analogs. The position of the OH group was also important for the curcuminoids activity. **MAC 8** (3-OH) showed higher activity than its regioisomers **MACs 27** (2-OH) and **6** (4-OH), indicating that the OH at position 3 on the aromatic ring A increased the anti-*Candida* activity.

In order to determine whether the active **MACs** showed fungistatic or fungicidal activity against *Candida* species, the ratios among the MFC and MIC values were calculated. Antifungal agents are generally regarded as fungicidal if MFC/MIC ratio < 4 (SARDI et al, 2017b). All active **MACs** exhibited MFC/MIC ratios between 1 and 2, indicating that the monocarbonyl curcuminoids showed fungicidal activity against these *Candida* species.

In general, the **MACs** carrying the OH group had potent anti-*Candida* activity, notably **MAC 8** (3-OH). It showed broad spectrum against *Candida* species, with MIC values ranging from 7.8 to 31.2 $\mu\text{mol/L}$. In addition, **MAC 8** had higher potency than fluconazole against *C. albicans*, and it showed similar potency to fluconazole against *C. glabrata*. Of all infections caused by non-*albicans Candida* species, the mortality rate associated with *C. glabrata* is the highest. However, unlike the *C. parapsilosis*, the *C. glabrata* incidence is higher in adults than in children or neonates (SILVA et al., 2012; RODRIGUES, SILVA & HENRIQUES, 2014).

As far as we know at the present, there are no studies which have investigated **MAC 8** antifungal activity. Thus, we selected **MAC 8** for further assays, aiming to evaluate its effects against clinical *Candida* isolates, as well as its chemical stability and safety. Also, we investigated its effects on *C. albicans* virulence factors. *C. albicans* was selected because this species is the most frequently recovered from human fungal infection by *Candida* (BASSETTI et al., 2015; WHALEY et al., 2017).

6.2.3. Anti-*Candida* activity of **MAC 8** against clinical isolates

We investigated the effects of **MAC 8**, the most active monocarbonyl curcuminoid, against a panel of clinical *Candida* isolates, including *C. albicans* 6293, *C. albicans* 6326, *C. tropicalis* 6477, *C. tropicalis* 6459, *C. glabrata* 5136, *C. glabrata* 5371, *C. parapsilosis* 6175, *C. parapsilosis* 6186, *C. krusei* 6473 and *C. krusei* 6347. Clinical isolates were recovered from different parts of the human body, such as nail (7), genital tract (2) and skin (1). The **MAC 8** antifungal potency against clinical isolates was expressed as MIC values in $\mu\text{mol/L}$ (Table 2). Fluconazole and amphotericin B were used as the standard antifungal drugs. **MAC 8** was active

against *C. albicans* 6293, *C. tropicalis* 6477, *C. tropicalis* 6459, *C. glabrata* 5371 and *C. parapsilosis* 6175, with MIC values ranging from 62.5 to 250 µmol/L. Furthermore, **MAC 8** showed lower MIC values than fluconazole against *C. tropicalis* 6477, as well as similar potency to fluconazole against *C. glabrata* 5371, corroborating its antifungal potential against *Candida* species.

Table 2. Anti-*Candida* activity of **MAC 8** against clinical isolates expressed as MIC values in µmol/L.

Clinical isolate	Body side	MAC 8	FCZ	AMP B
Ca 6293	genital tract	250	3.9	–
Ca 6326	nail	>250	7.8	–
Ct 6477	nail	125	250	–
Ct 6459	skin	125	7.8	–
Cg 5136	genital tract	>250	125	–
Cg 5371	nail	62.5	62.5	–
Cp 6175	nail	125	15.6	–
Cp 6186	nail	>250	7.8	–
Ck 6473	nail	>250	–	0.5
Ck 6347	nail	>250	–	1.0

MIC = minimum inhibitory concentration; Ca = *C. albicans*; Ct = *C. tropicalis*; Cg = *C. glabrata*; Cp = *C. parapsilosis*; Ck = *C. krusei*; FCZ = fluconazole; AMP B = amphotericin B; – not determined.

Fonte: Elaborado pelo autor

6.2.4. Effects of MAC 8 on the *C. albicans* adhesion to human cells

Fungal adhesion to host cells is the first step in the development of an infectious process (MODRZEWSKA & KURNATOWSKI, 2015). This step is mediated by specific mechanisms, such as the fungal adhesins interaction with receptors on host cells, and non-specific mechanisms, such as *Candida* cell surface hydrophobicity (MUADCHEINGKA & TANTIVITAYAKUL, 2015; HÖFS, MOGAVERO & HUBE, 2016). Inhibiting *Candida* adhesion may be an efficient strategy to reduce its pathogenicity (OLIVEIRA et al., 2016). Therefore, we evaluated the effects of **MAC 8** at ¼MIC (7.8 µmol/L) on the *C. albicans* (ATCC 90028) adhesion to human keratinocyte cells (HaCaT) and human gingival fibroblast cells (HGF-1). The selected concentration (¼MIC) did not affect the growth and survival of the human and fungal cells.

As shown in Figure 3, treatment with **MAC 8** at $\frac{1}{4}$ MIC significantly inhibited *C. albicans* adhesion to HaCaT and HGF-1 cells when compared to the negative control (untreated *C. albicans*). In addition, the inhibitory effects of **MAC 8** on *C. albicans* adhesion to keratinocyte and fibroblast cells were similar, suggesting that the human cell type did not affect its anti-adhesion activity. Akerey and co-authors also found no difference in the nisin Z effects on *C. albicans* adhesion to human gingival fibroblast and epithelial cells (AKEREY et al., 2009). **MAC 8** anti-adhesion effects were also not affected by exposure period (1 or 2h). Nani and colleagues also described that treatment time was not relevant to the effects of an ethanol extract of propolis on the *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. krusei* and *C. glabrata* adhesion to HaCaT cells (NANI et al., 2020).

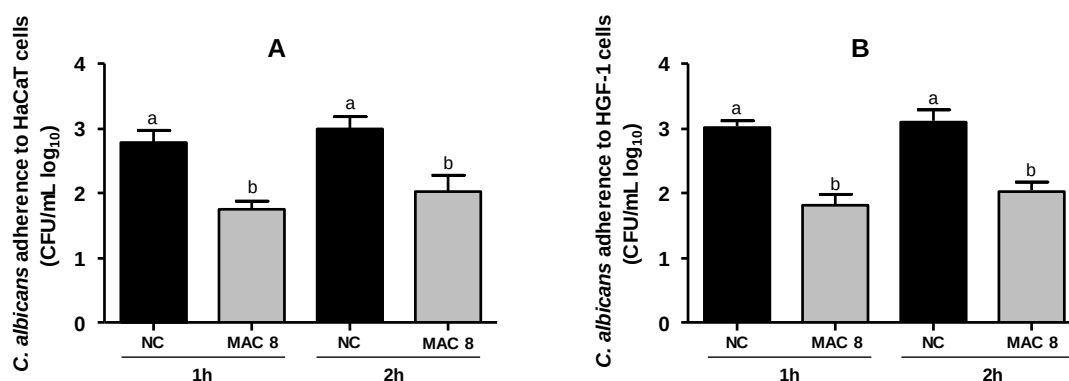


Figure 3. Effects (mean±SD) of **MAC 8** at $\frac{1}{4}$ MIC (7.8 μ mol/L) on the *C. albicans* (ATCC 90028) adhesion to (A) human keratinocyte cells (HaCaT) and (B) human gingival fibroblast cells (HGF-1). NC = negative control (untreated *C. albicans*). Different letters indicate statistical difference between treatments ($p < 0.05$; ANOVA with Tukey's post hoc test).

Fonte: Elaborado pelo autor

6.2.5. Effects of MAC 8 on the *C. albicans* biofilm

The ability to form biofilms on biotic and abiotic surfaces is one of *Candida* main virulence factors (NOBILE & JOHNSON, 2015). *C. albicans* biofilm formation has various steps, including initial adhesion (0–2h), germination, micro-colony formation, filamentation, monolayer development, proliferation (2–24h) and biofilm maturation (24–48h) (RAMAGE et al., 2001; ZOYSA, GLOSSOP & SAROJINI, 2018). **MAC 8** reduced *C. albicans* adhesion to human cells, the initial stage which occurs in the first hours of incubation. In order to investigate whether **MAC 8** also affects other biofilm formation steps, we evaluated its effects at MIC and

10×MIC (31.2 and 312 µmol/L, respectively) on *C. albicans* (ATCC 90028) biofilm formation (2–24 h) and preformed biofilm (24–48h). Amphotericin B was used as a standard antifungal drug.

As seen in Figure 4A, treatment with **MAC 8** at MIC and 10×MIC significantly inhibited the *C. albicans* biofilm formation when compared to the negative control (untreated biofilm). In addition, the inhibitory effects of **MAC 8** on the biofilm formation were similar to the amphotericin B ones. **MAC 8** at MIC and 10×MIC also significantly reduced the preformed biofilm when compared to the negative control (Figure 4B). However, its inhibitory effects on the *C. albicans* preformed biofilm were weaker than the amphotericin B ones.

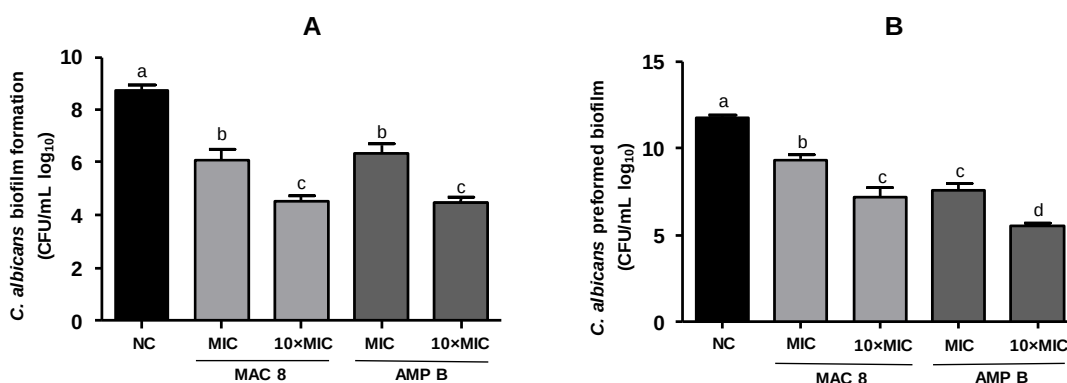


Figure 4. Effects (mean±SD) of **MAC 8** at MIC and 10×MIC (31.2 and 312 µmol/L, respectively) on the *C. albicans* (ATCC 90028) biofilm formation (A) and preformed biofilm (B). NC = negative control (untreated biofilm). Amphotericin B (AMP B) at MIC and 10×MIC (0.5 and 5.0 µmol/L, respectively) was used as a standard antifungal drug. Different letters indicate statistical difference between treatments ($p < 0.05$; ANOVA with Tukey's post hoc test).

Fonte: Elaborado pelo autor

6.2.6. Effects of MAC 8 combined with amphotericin B or fluconazole

The treatment of fungal infections using drug combination therapy may enhance its effectiveness, mitigate adverse effects and reduce the emergence of resistant strains (SUN, ZHANG & LI, 2012). On account of that, we evaluated the effects of **MAC 8** against *C. albicans* (ATCC 90028) in combination with antifungal drugs, including amphotericin B and fluconazole. We used the checkerboard assay in order to determine fractional inhibitory concentration index (FICI) values, which are used to classify the effects of the combinations as

synergistic (FICI ≤ 0.5), additive (FICI >0.5 to 1), indifferent (FICI >1 to 4) or antagonistic (FICI >4) (DOERN, 2014).

Both **MAC 8** associations, with amphotericin B and with fluconazole, showed synergistic effects, with FICI values of 0.5 and 0.2 respectively (Table 3). Synergism indicates that the combined effect was more potent than the isolated effect of each compound. However, the **MAC 8** combination with fluconazole was more effective than its association with amphotericin B. This result may be related to **MAC 8** action mode. Studies have correlated the antimicrobial activity of some phenolic compounds, including **CUR**, with their ability to disrupt the cytoplasmic membrane (GYAWALI & IBRAHIM, 2014; PISOSCHI et al., 2018). As amphotericin B also causes membrane damage (NAKAGAWA et al., 2016), and its association with **MAC 8** showed FICI value at the threshold between synergistic/additive effects, it is possible that the *C. albicans* membrane is a target for **MAC 8**. The FICI value of **MAC 8** combination with fluconazole corroborated this hypothesis. As fluconazole inhibits ergosterol biosynthesis (SAGATOVA et al., 2015; BONGOMIN et al., 2018), its combination with a compound that increases the membrane permeability facilitates its permeation through this barrier.

Table 3. FICI values and effects of **MAC 8** combinations with amphotericin B or fluconazole against *C. albicans* (ATCC 90028).

Combination	FICI	Effect
MAC 8 + AMP B	0.5	synergistic
MAC 8 + FCZ	0.2	synergistic

FICI = fractional inhibitory concentration index; AMP B = amphotericin B; FCZ = fluconazole. FICI ≤ 0.5 = synergy; FICI >0.5 to 1 = additive; FICI >1 to 4 = indifference; FICI >4 = antagonism (DOERN, 2014).

Fonte: Elaborado pelo autor

6.2.7. Preliminary studies on the **MAC 8** action mode against *C. albicans*

In order to investigate whether the *C. albicans* membrane is a target for **MAC 8**, we assessed its effect on the ergosterol, a sterol that regulates the fungal membrane fluidity and integrity (ERMAKOVA & ZUEV, 2017; RODRIGUES, 2018). If **MAC 8** action mode is related to its ability to interact with ergosterol, the MIC value of **MAC 8** will increase with the addition of exogenous ergosterol in the culture medium (STANISZEWSKA et al., 2018; ŁUKOWSKA-CHOJNACKA et al., 2019). This is because exogenous ergosterol reduces **MAC 8** effects on the membrane ergosterol, requiring a higher concentration of curcuminoid

in order to inhibit the fungal growth. For this reason, we evaluated the effect of the presence of exogenous ergosterol (100 $\mu\text{mol/L}$) on **MAC 8** activity against *C. albicans* (ATCC 90028) (Table 4). The addition of exogenous ergosterol in the culture medium did not affect the *C. albicans* growth.

As shown in Table 4, amphotericin B, an antifungal which is known for its ability to interact with ergosterol for membrane disruption (CIESIELSKI et al., 2016), showed a 62 times increase in the MIC value against *C. albicans* in the presence of exogenous ergosterol. For **MAC 8**, the addition of exogenous ergosterol increased its MIC value against *C. albicans* about 4 times. Although it was less effective than amphotericin B, **MAC 8** was able to interact with ergosterol, corroborating the hypothesis that its action mode involves membrane.

Table 4. MIC values ($\mu\text{mol/L}$) of **MAC 8** in the absence and presence of ergosterol (100 $\mu\text{mol/L}$) or sorbitol (0.8 $\mu\text{mol/L}$) against *C. albicans* (ATCC 90028).

Compound	Ergosterol		Sorbitol	
	Absence	Presence	Absence	Presence
MAC 8	31.2	125	31.2	250
amphotericin B	0.5	31.2	–	–
caspofungin	–	–	0.05	1.8

– not determined.

Fonte: Elaborado pelo autor

We also investigated whether *C. albicans* cell wall is a target for **MAC 8** by the sorbitol assay. Sorbitol is an osmotic protector used to stabilize fungal cells with damaged cell wall. Therefore, cell wall disruptors have their potency reduced in the presence of sorbitol (FROST et al., 1995; LEITE et al., 2014). This effect has been measured by the increase in the MIC value of antifungal agents in the presence of this polyol (GUERRA et al., 2015; LEITE et al., 2015). Thus, we evaluated the effect of the presence of sorbitol (0.8 $\mu\text{mol/L}$) on the **MAC 8** activity against *C. albicans* (ATCC 90028) (Table 4). Sorbitol addition in the culture medium did not affect the *C. albicans* growth.

Caspofungin showed a 36 times increase in the MIC value against *C. albicans* in the presence of sorbitol. This echinocandin inhibits β -1,3-glucan biosynthesis, a cell wall component that provides the fungal cell integrity and shape (SONG & STEVENS, 2016). The MIC value of **MAC 8** against *C. albicans* increased about 8 times in the presence of sorbitol, suggesting that cell wall is also a targets for **MAC 8**. Taken together, preliminary studies on

the **MAC 8** action mode indicated a combined effect between membrane and cell wall disruptions.

6.2.8. Evaluation of **MAC 8** chemical stability

One of the main factors that hinder the clinical use of **CUR** is its poor stability under physiological conditions (NELSON et al., 2017). In order to assess whether the monocarbonyl curcuminoid has overcome this limitation, we investigated **MAC 8** stability at pH 7.4 and 37 °C by monitoring the decrease in its UV–Vis absorption for 24 h (Figure 5). The initial concentration of **MAC 8** reduced only 17% after 24 h, while the **CUR** initial concentration reduced 55%. These results indicated that **MAC 8** was more stable than its parent compound. Together, our results demonstrated that the replacement of the β -diketone by a monocarbonyl fragment not only improved **MAC 8** anti-*Candida* activity, but also enhanced its chemical stability. In addition, **MAC 8** (3-OH) showed higher stability than **MAC 4** (3,4-diOH). The initial concentration of **MAC 4** decreased 28% after 24 h at pH 7.4 and 37 °C (Chapter II). This finding suggested that the removal of 4-OH substituent on the aromatic ring increases the chemical stability of monocarbonyl analogs, corroborating a previous study by our group with symmetrical dicarbonyl curcuminoids (POLAQUINI et al., 2019).

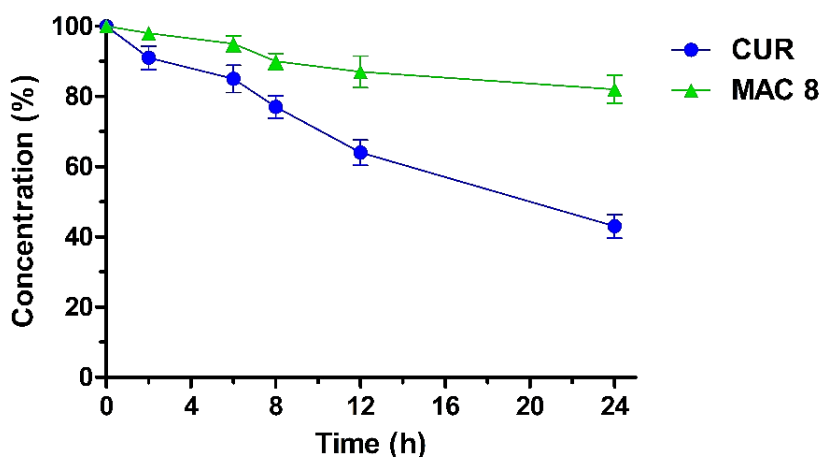


Figure 5. Chemical stability of **MAC 8** and **CUR** (pH 7.4 and 37 °C) by monitoring the decrease in their maximum absorbance for 24 h. Data were represented as means $\pm$ SEMs and expressed as percentage.

Fonte: Elaborado pelo autor

6.2.9. Systemic toxicity of MAC 8 in *G. mellonella* larvae

In order to investigate **MAC 8** safety, we evaluated its systemic toxicity in *G. mellonella* model. The larvae of the greater wax moth *G. mellonella* have been used as an alternative animal model in order to assess the *in vivo* toxicity of new antifungal agents (KAVANAGH & SHEEHAN, 2018). Therefore, **MAC 8** was injected into the larvae in doses ranging from 12.5 to 100 mg/kg, and the larvae survival percentage was monitored up to 96 h (Figure 6). Larvae groups treated with saline (negative control) or DMSO (vehicle control) showed high survival after 96 h (100% and 90% survival, respectively). **MAC 8** injection in doses ≤ 50 mg/kg resulted in $\geq 60\%$ survival. However, *G. mellonella* groups treated with **MAC 8** in doses ≥ 75 mg/kg showed $\leq 50\%$ survival. From these results, the LD₅₀ (dose required in order to kill 50% of the larvae) value of **MAC 8** in *G. mellonella* was estimated to be 75 mg/kg (300 μ mol/kg) after 96 h of treatment.

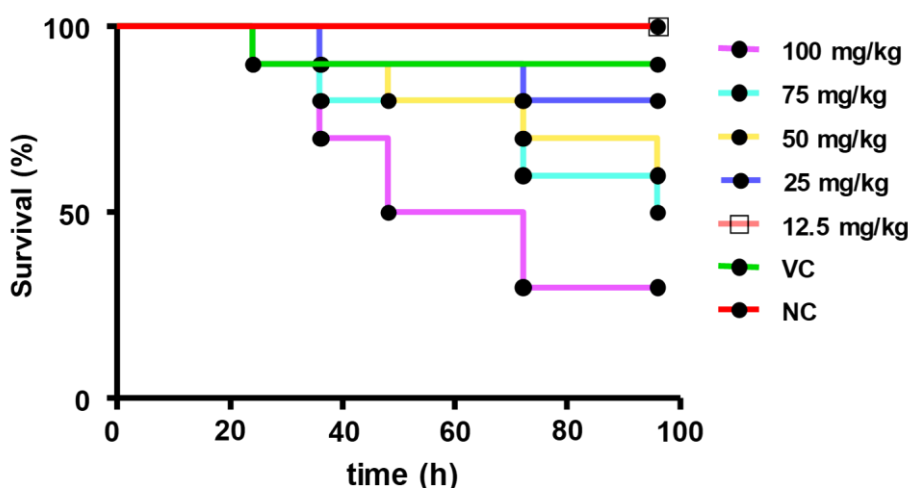


Figure 6. Effects of **MAC 8** on *G. mellonella* survival. Larvae groups were monitored up to 96 h. **NC** = negative control (larvae group treated with 0.9% saline); **VC** = vehicle control (larvae group treated with 12.5% DMSO); ($p < 0.05$, log-rank Mantel–Cox test).

Fonte: Elaborado pelo autor

Merigo and authors reported 80% survival of *G. mellonella* larvae treated with **CUR** at 2.85 μ mol/kg after 96 h (MERIGO et al., 2017). **MAC 8** at 50 μ mol/kg (12.5 mg/kg), the lowest dose assessed in our study, showed 100% survival after 96 h. The comparison between the survival percentage values of the larvae treated with **CUR** and **MAC 8** suggested that the monocarbonyl analog was less toxic than its parent compound, which is a natural product

considered safe for humans in high doses (12 g/day) (LAO et al., 2006; JAMES et al., 2015). Furthermore, the LD₅₀ value of **MAC 8** (300 µmol/kg) in *G. mellonella* larvae was higher than the LD₅₀ values of other antimicrobial monocarbonyl analogs described by our group previously (190 µmol/kg ≤ LD₅₀ ≤ 290 µmol/kg) (AYUSSO, 2019; MARQUES, 2019b), corroborating the low toxicity of **MAC 8** in *G. mellonella* model.

6.3. CONCLUSION

In summary, we synthesized a series of unsymmetrical monocarbonyl curcuminoids as part of our ongoing search for anti-*Candida* agents based on natural products. As far as we know, we are the first group to describe the unsymmetrical **MACs** effects on the *C. albicans* cell adhesion and biofilm. The **MAC 8** showed a broad spectrum against *Candida* species, including clinical *Candida* isolates. It inhibited important virulence factors, such as cell adhesion and biofilm. In addition, its combination with antifungal drugs showed synergistic effects. Preliminary investigations have indicated that the **MAC 8** action mode is a combination of its effects on fungal membrane and cell wall. Furthermore, **MAC 8** has been shown to be a potentially safe and stable compound.

6.4. EXPERIMENTAL

6.4.1. Chemistry

6.4.1.1. Material and instruments

Chapter II, item 2.4.1.1.

6.4.1.2 Synthesis of SCUR and MACs 1–8

SCUR was synthesized according to Morão and co-authors (MORÃO et al., 2019). **MACs 1–8** were prepared as described previously in Chapter II.

6.4.1.3 Synthesis of MACs 24–31

MACs 24–31 were synthesized in two steps.

6.4.1.3.1. Synthesis of benzylideneacetone (1). Chapter II, item 2.4.1.3.1.

6.4.1.3.1. Synthesis of MACs 24–31. To a solution of benzylideneacetone (**1**) (5.2 mmol) in EtOH (10 mL) the respective benzaldehyde derivative (5.0 mmol) was added.

Catalytic amounts of H₂SO₄ were added to the reaction mixture, and the solution was stirred at room temperature for 48 h. Reaction medium was poured onto crushed ice. The crude product was partitioned with ethyl acetate (3 × 25 mL), and the combined organic phase was concentrated under reduced pressure. **MACs 24** and **25** were purified by gel filtration column chromatography (LH-20) using EtOH as eluent. **MACs 26–29** and **30/31** were purified by column chromatography over silica gel using mixtures of hexane and ethyl acetate, (9:1) and (8:2), respectively, as eluents.

6.4.1.4. Synthesis of MACs 32–34

MACs 32–34 were synthesized in three more steps (KIM et al., 2008; KIM et al., 2015) in addition to the benzylideneacetone (**1**) preparation.

6.4.1.4.1. Synthesis of MOM-benzaldehydes 2a–c. A suspension of K₂CO₃ (50 mmol) in dry acetone (25 mL) was cooled in an ice bath for 10 min. After this period, the respective hydroxybenzaldehyde derivative (5 mmol) and methoxymethyl chloride (MOMCl) (20 mmol) were added sequentially. The reaction mixture was stirred at room temperature for 1 h under N₂ atmosphere. The suspension was diluted with deionized water (25 mL) and the crude product extracted with ethyl acetate (3 × 25 mL). The combined organic layer was concentrated under reduced pressure. Compounds **2a** and **2b/2c** were purified by column chromatography over silica gel using mixtures of hexane and ethyl acetate, (9:1) and (8:2), respectively, as eluents.

6.4.1.4.2. Synthesis of MOM-diphenylpentanoids 3a–c. A solution of respective MOM-benzaldehyde (2 mmol) and benzylideneacetone (**1**) (2.2 mmol) in EtOH (7 mL) was cooled in an ice bath for 10 min. Ethanolic solution of NaOH (1 mL, 1 mol/L) was added dropwise to the reaction mixture. The solution was stirred at room temperature for 8 h under N₂ atmosphere. Reaction medium was poured onto crushed ice. The crude product was partitioned with ethyl acetate (3 × 15 mL), and the combined organic phase was concentrated under reduced pressure. Compounds **3a** and **3b/3c** were purified by column chromatography over silica gel using mixtures of hexane and ethyl acetate, (8:2) and (7:3), respectively, as eluents.

6.4.1.4.3. Synthesis of MACs 32–34. A solution of respective MOM-diphenylpentanoid (1 mmol) in EtOH (5 mL) and 33% HCl (0.3 mL) was stirred at room temperature for 48 h under N₂ atmosphere. Reaction medium was poured onto crushed ice. The crude product was partitioned with ethyl acetate (3 × 15 mL), and the combined organic phase

was concentrated under reduced pressure. **MAC 32–34** were purified by preparative thin-layer chromatography on a silica-gel plate using mixtures of hexane and ethyl acetate (9:1), 0.01% EtOH and 0.01% AcOH as eluent.

6.4.2. Anti-*Candida* assay

Five ATCC biological standards were used in this study, including *C. albicans* (ATCC 90028), *C. tropicalis* (ATCC 750), *C. glabrata* (ATCC 90030), *C. parapsilosis* (ATCC 22019) and *C. krusei* (ATCC 6258). In addition, ten clinical *Candida* isolates were also used in this study, including *C. albicans* 6293, *C. albicans* 6326, *C. tropicalis* 6477, *C. tropicalis* 6559, *C. glabrata* 5136, *C. glabrata* 5361, *C. parapsilosis* 6175, *C. parapsilosis* 6186, *C. krusei* 6473 and *C. krusei* 6347. All clinical isolates were obtained from the collection of Laboratory of Mycology, Department of Dermatological, Infectious and Parasitic Diseases, São José do Rio Preto Medical School (FAMERP), São José do Rio Preto, Brazil. The MIC and MFC values were determined by the microdilution method according to Clinical & Laboratory Standards Institute (CLSI) protocols. Minor modifications in this protocol and their detailed procedures were reported in a previous work by Marques and co-authors (MARQUES et al., 2019a). Stock solutions of **CUR**, **SCUR** and **MACs 1–8** and **24–34** were prepared in DMSO and tested in concentrations ranging from 250 to 1.9 $\mu\text{mol/L}$. Amphotericin B and fluconazole were used as standard antifungal drugs. Three independent assays were performed. The MIC values were visually determined as the lowest concentration of the compound that did not produce any visual turbidity in the wells. The MFC values were defined as the lowest concentration of the subculture at which no visible growth was observed on solid media.

6.4.3. Human cell adhesion assay

The effects of **MAC 8** on *C. albicans* (ATCC 90028) adhesion to human cells were evaluated according to Sardi and co-authors (SARDI et al., 2017a), with minor modifications described in a previous work (NANI et al., 2020). The HaCaT (human keratinocyte cell line) and HGF-1 (human gingival fibroblast cell line) cell lines were obtained from the Cell Bank of Rio de Janeiro (Brazil). The **MAC 8** concentration ($\frac{1}{4}$ MIC 7.8 $\mu\text{mol/L}$) was chosen because it ensured that the growth and survival of human and fungal cells were not affected by the treatment with this curcuminoid. The assays were carried out in triplicates of three independent experiments.

6.4.4. Antibiofilm assays

The effects of **MAC 8** on biofilm formation and preformed biofilm of *C. albicans* (ATCC 90028) were evaluated according to Sardi and co-authors (SARDI et al., 2017a), with modifications.

6.4.4.1. Biofilm formation assay

An aliquot of 100 μ L *C. albicans* suspension (10^8 CFU/mL) in yeast nitrogen base medium (YNB) was added to the wells of 96-well plates. The plates were incubated at 37 °C for 2 h to allow for *C. albicans* adhesion. The supernatant was removed, and the wells were washed in order to remove non-adherent cells. The biofilm in formation was treated with **MAC 8** at MIC and 10×MIC (31.2 and 312 μ mol/L, respectively) for 24 h at 37 °C. After that, the supernatants were removed, and the wells were washed in order to remove non-adhered or killed cells. The biofilm was homogenized, serially diluted, and plated on sabouraud dextrose agar medium (SDA). The plates were incubated at 37 °C for 24 h and colonies were counted. The assays were carried out in triplicates of three independent experiments. Untreated biofilm was used as a negative control. Amphotericin B was used as a standard antifungal drug.

6.4.4.2. Preformed biofilm assay

An aliquot of 100 μ L *C. albicans* suspension (10^8 CFU/mL) in YNB was added to the wells of 96-well plates. The plates were incubated at 37 °C for 24 h in order to allow biofilm formation. The supernatant was removed, and the wells were washed to remove non-adherent cells. The preformed biofilm was treated with **MAC 8** at MIC and 10×MIC (31.2 and 312 μ mol/L, respectively) for 24 h at 37 °C. After that, the supernatants were removed, and the wells were washed in order to remove non-adhered or killed cells. The biofilm was homogenized, serially diluted, and plated on SDA. The plates were incubated at 37 °C for 24 h and colonies were counted. The assays were carried out in triplicates of three independent experiments. Untreated biofilm was used as a negative control. Amphotericin B was used as a standard antifungal drug.

6.4.5. Checkerboard Assay

The effects of **MAC 8** combined with amphotericin B or fluconazole against *C. albicans* (ATCC 90028) were determined according to Sardi and co-authors (SARDI et al., 2016). The FICI values were interpreted as described by Doern (DOERN, 2014).

6.4.6. Ergosterol and sorbitol assays

The MIC values of **MAC 8** against *C. albicans* (ATCC 90028) in the presence of ergosterol (100 $\mu\text{mol/L}$) or sorbitol (0.8 $\mu\text{mol/L}$) were determined by the microdilution technique, as previously described by Sardi and colleagues (SARDI et al., 2017b). Amphotericin B and caspofungin were used as standard antifungal drugs. Three independent assays were performed. The MIC values were visually determined as the lowest concentration of the **MAC 8** that did not produce any visual turbidity in the wells.

6.4.7. Chemical stability assay

Chemical stability of **MAC 8** (at λ_{max} 362 nm) and **CUR** (at λ_{max} 426 nm) was analyzed by monitoring their UV–visible absorptions in phosphate buffer (pH 7.4) at 37 °C. Minor modifications in this protocol and their detailed procedures were reported in our previous work (POLAQUINI et al., 2019).

6.4.8. Systemic toxicity assay in *G. mellonella* larvae

The systemic toxicity of **MAC 8** in *G. mellonella* model was determined according to Sardi and co-authors (SARDI et al., 2017a), with minor modifications. Ten larvae (weighing 0.2–0.3 g) with no signs of melanization were randomly selected for each group. The larvae were chilled on ice for 20 min, and 10 μL of **MAC 8** (2.0, 1.5, 1.0, 0.5 and 0.25 mg/mL) were injected into the hemocoel of each larva via the last left proleg by a trained operator using a 25 μL syringe. All groups were incubated at 37 °C, and their survival was monitored for up to 96 h. The larvae showing high melanization and absence of movements upon touch were counted as dead. The tests were performed in three independent assays. Larvae groups treated with 12.5% DMSO or 0.9% saline were used as vehicle and negative controls, respectively.

7. CAPÍTULO VII

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7. CAPÍTULO VII

Antifungal Activity of a New Unsymmetrical Monocarbonyl Curcuminoid (MAC 43) Results in Decreased *Candida Albicans* biofilm

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ABSTRACT

In a previous work, we reported the promising activity of an unsymmetrical monocarbonyl curcuminoid (**MAC 8**) against *Candida* species. It decreased *C. albicans* cell adhesion and biofilm. In the current work, we synthesized a new series of monocarbonyl curcuminoids (**MACs 35–48**) based on the **MAC 8** framework in order to search for analogs with higher potency against *Candida*. **MAC 43** (2-thiophenyl analog) showed higher activity than **MAC 8** (phenyl analog) against *C. albicans*, *C. tropicalis*, *C. parapsilosis* and *C. krusei*, with MIC (minimum inhibitory concentration) values ranging from 7.4 to 15.2 $\mu\text{mol/L}$. Our investigations also demonstrated the ability of **MAC 43** to decrease *C. albicans* biofilm. Furthermore, **MAC 43** combinations with amphotericin B or fluconazole showed synergistic effects against *C. albicans*. These results indicated that **MAC 43** is a promising compound with anti-*Candida* activity.

7.1. INTRODUCTION

Most fungi-related deaths result from species belonging to the genera *Candida*, *Aspergillus* and *Cryptococcus* (BROWN et al., 2012; GARCIA-RUBIO et al., 2020). *Candida* is the leading cause of invasive fungal infections, especially in intensive care patients (SOBEL & AKINS, 2017). In the USA, *Candida* species were the fourth most common health care-associated infection (MAGILL et al., 2018). Among *Candida* species, *C. albicans* is the most frequently recovered from human fungal infection (WHALEY et al., 2017). Its prevalence is particularly strong in the increased risk populations, including elderly patients, diabetics, premature neonates and immunocompromised individuals (MARTINS et al., 2014). Non-*albicans* *Candida* species, such as *C. glabrata*, *C. parapsilosis*, *C. krusei* and *C. tropicalis*, also represent an emerging risk to human health, due to the increase in the number of infections associated with these species (KOŁACZKOWSKA & KOŁACZKOWSKI, 2016).

The treatment of fungal infections depends on a very limited number of drugs belonging to three main classes: azoles, polyenes and echinocandins. These antifungal drugs differ in their action spectrum, pharmacokinetic properties, toxicity and cost (BELLMANN & SMUSZKIEWICZ et al., 2017). One of the main resistance mechanisms to antifungal drugs is the biofilm formation by *Candida* species. Persistent biofilms make fungi less susceptible to drugs compared to their planktonic forms (WU et al., 2017). Moreover, *Candida* biofilms lead to recurring infections and in some cases death (GULATI & NOBILE, 2016). Therefore, there is a strong need for the develop new agents against *Candida* with anti-biofilm activity.

In a previous study, we described the anti-*Candida* activity of a monocarbonyl curcuminoid (**MAC 8**). It was able to reduce the *C. albicans* adhesion to human fibroblast and epithelial cells. Additionally, **MAC 8** affected both the biofilm formation and preformed biofilm of *C. albicans* (Chapter VI). Based on its promising effects, we synthesized new monocarbonyl curcuminoids designed from **MAC 8** framework, and we evaluated their effects against *Candida* species. The most active analog (**MAC 43**) was investigated regarding its effects on *C. albicans* biofilm formation and preformed biofilm. Furthermore, **MAC 43** effects combined with antifungal drugs against *C. albicans* were analyzed.

7.2. RESULTS AND DISCUSSION

7.2.1. Chemistry

MACs 35 and **36** were prepared as summarized in Figure 1. Ketones (acetone or cyclohexanone) were di-condensed with 3-hydroxybenzaldehyde under acid catalysis, in order to provide **MACs 35** and **36** with yields of 27% and 38%, respectively. **MACs 37–48** were prepared in two steps as summarized in Figure 1. The acetone was mono-condensed with 3-hydroxybenzaldehyde under basic catalysis and at room temperature, providing **SMAC 4** in a yield of 84%. **SMAC 4** was condensed with different aromatic aldehydes in order to synthesize **MACs 37–48**, with yields ranging from 23 to 65%.

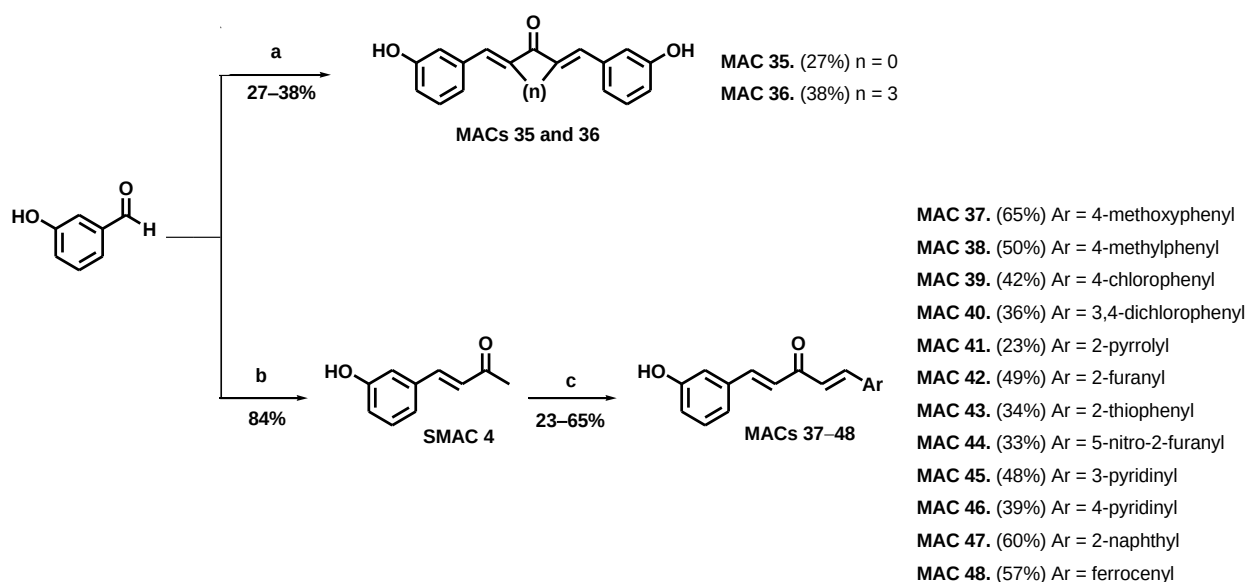


Figure 1. Synthesis of **MACs 35–48**. Reagents and conditions: (a) ketone (acetone, or cyclohexanone), H_2SO_4 , EtOH, room temperature, 24 h; (b) acetone, NaOH, room temperature, 2 h; (c) aromatic aldehyde, NaOH, EtOH, room temperature, 10–48 h.

Fonte: Elaborado pelo autor

The structures of synthesized curcuminoids **MACs 35–48** were confirmed by ^1H and ^{13}C NMR spectra data analyses. Out of the 14 monocarbonyl curcuminoids, **MACs 38–48** were identified as new chemical entities (SCIFINDER, 2020). For all compounds, NMR data, including chemical shifts, integration multiplicities and coupling constants, corresponded to the proposed structures. In ^1H NMR spectrum, hydrogens α/α' and β/β' resonated as doublets and clearly indicated the formation of two olefins. Coupling constants values of olefin hydrogens

signals ranged from 15 to 16 Hz, corroborating their *trans* configuration. Spectra were presented as Supplementary Material.

7.2.2. Anti-*Candida* activity

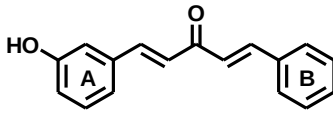
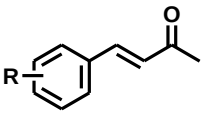
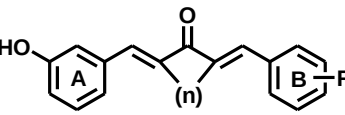
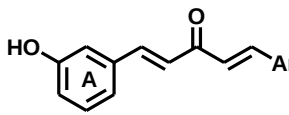
The anti-*Candida* activities of simplified curcuminoids (**SMAC 2** and **4**) and unsymmetrical monocarbonyl curcuminoids of series I (**MACs 35–40**) and series II (**MACs 41–48**) were evaluated against *C. albicans* (ATCC 90028), *C. tropicalis* (ATCC 750), *C. glabrata* (ATCC 90030), *C. parapsilosis* (ATCC 22019) and *C. krusei* (ATCC 6258). The antifungal potency of the compounds was expressed as minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values in $\mu\text{mol/L}$ (Table 1). Fluconazole and amphotericin B were used as standard antifungal drugs.

We previously reported the **MAC 8** anti-*Candida* activity, an unsymmetrical monocarbonyl curcuminoid designed from curcumin. It had promising antifungal effects against *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis* and *C. krusei*, with MIC values ranging from 7.8 to 31.2 $\mu\text{mol/L}$ (Chapter VII). In order to search for new compounds with higher potency against *Candida* species, we evaluated a set of analogs (**SMAC 2** and **4**, and **MACs 35–48**) designed from **MAC 8** framework.

First, we evaluated two simplified monocarbonyl analogs (**SMACs 2** and **4**) based on the **MAC 8** structure. Phenylbutanoids **SMAC 2** (unsubstituted benzene ring) was inactive (MIC >250 $\mu\text{mol/L}$), while **SMAC 4** (*m*-OH) showed activity against *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. krusei* (MIC 193, 48.2, 193 and 96.5 $\mu\text{mol/L}$, respectively). From these results, it was possible to confirm the importance of the presence of the *m*-OH group on the benzene ring for the anti-*Candida* activity. Nevertheless, **SMAC 4** was less active than its parent compound (**MAC 8**), revealing that the diphenylpentanoid framework was better for the bioactivity than the phenylbutanoid framework.

Second, based on the importance of the *m*-OH group for the bioactivity, we also investigated the effects of the insertion of an OH group at *meta* position on the **MAC 8** benzene ring B. The dihydroxylated analog, **MAC 35**, was more potent against *C. tropicalis* and *C. krusei* (MIC 14.6 and 7.1 $\mu\text{mol/L}$, respectively) than **MAC 8** (MIC 31.2 and 15.6 $\mu\text{mol/L}$, respectively). On the other hand, the presence of a second *m*-OH group resulted in a reduction of **MAC 35** activity against *C. albicans*, *C. glabrata* and *C. parapsilosis*.

Table 1. Anti-*Candida* activity of simplified curcuminoids (**SMACs 2 and 4**) and monocarbonyl curcuminoids of series I (**MACs 35–40**) and series II (**MACs 41–48**) expressed as MIC and MFC values in $\mu\text{mol/L}$.

   												
Compounds			Ca		Ct		Cg		Cp		Ck	
			MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
MAC 8*			31.2	62.3	31.2	62.3	7.8	15.6	31.2	62.3	15.6	31.2
Simplified	R											
SMAC 2	H		>250	>250	>250	>250	>250	>250	>250	>250	>250	>250
SMAC 4	<i>m</i> -OH		193	193	>250	>250	48.2	96.5	193	193	96.5	96.5
Series I	R	n										
MAC 35	<i>m</i> -OH	0	58.6	117	14.6	29.3	58.6	58.6	58.6	117	7.1	14.6
MAC 36	<i>m</i> -OH	3	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250
MAC 37	<i>p</i> -OMe	0	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250
MAC 38	<i>p</i> -Me	0	29.5	59.1	29.5	29.5	237	237	237	237	59.1	59.1
MAC 39	<i>p</i> -Cl	0	110	220	>250	>250	54.9	110	110	110	54.9	110
MAC 40	<i>m,p</i> -diCl	0	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250
Series II	Ar											
MAC 41	2-pyrrolyl		>250	>250	>250	>250	>250	>250	>250	>250	>250	>250
MAC 42	2-furanyl		64.9	64.9	64.9	64.9	250	250	250	250	16.2	16.2
MAC 43	2-thiophenyl		15.2	30.4	7.4	15.2	7.4	15.2	15.2	15.2	7.4	15.2
MAC 44	5-nitro-2-furanyl		>250	>250	>250	>250	>250	>250	>250	>250	>250	>250
MAC 45	3-pyridinyl		62.1	62.1	15.5	15.5	62.1	62.1	62.1	124	31.1	62.1
MAC 46	4-pyridinyl		62.1	124	31.1	62.1	62.1	62.1	15.5	31.1	31.1	62.1
MAC 47	2-naphthyl		>250	>250	>250	>250	>250	>250	>250	>250	>250	>250
MAC 48	ferrocenyl		>250	>250	>250	>250	>250	>250	>250	>250	>250	>250
FCZ	–		52.2	–	6.5	–	6.5	–	3.2	–	–	–
AMP B	–		0.5	–	0.5	–	0.5	–	0.3	–	0.5	–

MIC = minimum inhibitory concentration; MFC = minimum fungicidal concentration; Ca = *C. albicans* (ATCC 90028); Ct = *C. tropicalis* (ATCC 750); Cg = *C. glabrata* (ATCC 90030); Cp = *C. parapsilosis* (ATCC 22019); Ck = *C. krusei* (ATCC 6258); FCZ = fluconazole; AMP B = amphotericin B; – not determined.

Fonte: Elaborado pelo autor

Third, we evaluated the conformational restriction effect on the dihydroxylated analog activity. **MAC 36** (cyclohexanone derivative) was inactive against all *Candida* species (MIC >250 $\mu\text{mol/L}$). This result indicated that the compact conformation reduced antifungal activity. In Chapter V, we also investigated the conformational restriction effect on the anti-*Xanthomonas citri* subsp. *citri* (*X. citri*) activity of unsymmetrical monocarbonyl curcuminoids. Curcuminoids derived from cyclopentanone and cyclohexanone were more active than the analog derived from acetone, suggesting that for this bacterium the compact conformation improves activity.

Fourth, we evaluated the effects of the insertion of electron-donating groups on the **MAC 8** benzene ring B. **MAC 37** (*p*-OMe) was inactive (MIC >250 $\mu\text{mol/L}$), but **MAC 38** (*p*-Me) showed activity against all *Candida* species, with MIC values ranging from 29.5 to 237 $\mu\text{mol/L}$. However, **MAC 38** was less active than **MAC 8** against *C. glabrata*, *C. parapsilosis* and *C. krusei*. In general, these results indicated that the presence of electron-donating groups on the benzene ring B decreased the anti-*Candida* activity of monocarbonyl curcuminoids.

Fifth, comparisons were made in order to evaluate the effects of the presence of electron-withdrawing groups on the **MAC 8** benzene ring B. **MACs 39** (*p*-Cl) and **40** (*m,p*-diCl) showed less potency than **MAC 8**, suggesting that the presence of electron-withdrawing groups on the benzene ring B also reduced the antifungal activity.

The series II analogs were designed in order to evaluate the effects of replacing the **MAC 8** benzene ring B with other aromatic rings (Table 1). First, in order to investigate the hypothetical ring bioisosterism (LIMA & BARREIRO, 2005), comparisons were made to determine the effects of replacing benzene ring B with heteroaryl rings with six π -electrons, such as 2-pyrrolyl (**MAC 41**), 2-furanyl (**MAC 42**), 2-thiophenyl (**MAC 43**). **MAC 41** was inactive (MIC >250 $\mu\text{mol/L}$), while **MAC 42** showed activity against all *Candida* species (16.2 $\mu\text{mol/L} \leq \text{MIC} \leq 250 \mu\text{mol/L}$), but it had less potency than **MAC 8**. On the other hand, **MAC 43** (7.4 $\mu\text{mol/L} \leq \text{MIC} \leq 15.2 \mu\text{mol/L}$) was more potent activity than **MAC 8** against *C. albicans*, *C. tropicalis*, *C. parapsilosis* and *C. krusei*, indicating that replacement of benzene by thiophene improved the antifungal activity of curcuminoids.

Second, we previously described that the insertion of a NO₂ group on the furan ring increased the antimicrobial activity of monocarbonyl curcuminoids against *Mycobacterium tuberculosis* and *X. citri* (AYUSSO, 2019; Chapters III and V). Based on this result, we hypothesized that the insertion of a NO₂ group at position 5 on the furan ring could lead to a more potent analog against *Candida*. However, **MAC 44** (5-nitro-2-furanyl) had MIC values

>250 $\mu\text{mol/L}$. The presence of a NO_2 group decreased the antifungal activity of the furanyl analog, corroborating the hypothesis that the insertion of an electron-withdrawing group on the aromatic ring B decreases the anti-*Candida* activity.

Third, comparisons were made in order to assess the effects of replacing the phenyl ring with pyridine rings (six π -electrons). **MAC 45** (3-pyridinyl) and **MAC 46** (4-pyridinyl) had less potency than **MAC 8**. The results demonstrated that the replacement of the phenyl ring by pyridinyl rings decreased the potency against *Candida*. Furthermore, the nitrogen position on the pyridine ring was not relevant for the activity of the pyridinyl analogs.

Fourth, we also evaluated the effects of replacing benzene with naphthalene. **MAC 47** (2-naphthyl) had MIC values >250 $\mu\text{mol/L}$ against all *Candida* species, suggesting that the increase in π -electron density reduced antifungal activity.

Fifth, we evaluated an analog carrying the ferrocenyl ring (**MAC 48**). This organometallic ring was selected due to its antifungal effects. However, **MAC 48** was inactive against all *Candida* species (MIC >250 $\mu\text{mol/L}$). In Chapters III and V, we also investigated the antimicrobial effects of monocarbonyl curcuminoids carrying the ferrocenyl ring. Ferrocenic curcuminoids were also inactive against *M. tuberculosis* and *X. citri*, corroborating the hypothesis that the presence of a ferrocene ring in monocarbonyl curcuminoids is not relevant for the bioactivity.

In order to determine whether the active **MACs** showed fungistatic or fungicidal activity against *Candida* species, the ratios among the MFC and MIC values were calculated. Antifungal agents are generally regarded as fungicidal if MFC/MIC ratio < 4 (SARDI et al, 2017a). All active **MACs** exhibited MFC/MIC ratios between 1 and 2, indicating that the monocarbonyl curcuminoids showed fungicidal activity against these *Candida* species.

7.2.3. Effects of **MAC 43** on the *C. albicans* biofilm

The ability to form biofilms on biotic and abiotic surfaces is one of *Candida* main virulence factors (NOBILE & JOHNSON, 2015). We evaluated the **MAC 43** effects at MIC and 10 $\times$ MIC (15.2 and 152 $\mu\text{mol/L}$, respectively) on *C. albicans* (ATCC 90028) biofilm formation and preformed biofilm. Amphotericin B was used as a standard antifungal drug.

As seen in Figure 2A, treatment with **MAC 43** at MIC and 10 $\times$ MIC significantly inhibited the *C. albicans* biofilm formation when compared to the negative control (untreated biofilm). **MAC 43** at MIC and 10 $\times$ MIC also significantly reduced the preformed biofilm when

compared to the negative control (Figure 2B). However, its inhibitory effects on the *C. albicans* biofilm formation and preformed biofilm were weaker than amphotericin B.

In Chapter VI, we also assessed the effects of **MAC 8** at MIC and 10×MIC on the *C. albicans* (ATCC 90028) biofilm. Unlike **MAC 43**, the inhibitory effects of **MAC 8** on the biofilm formation were similar to the amphotericin B ones. This indicated that although **MAC 43** had a lower MIC value than **MAC 8**, its anti-biofilm effects were reduced.

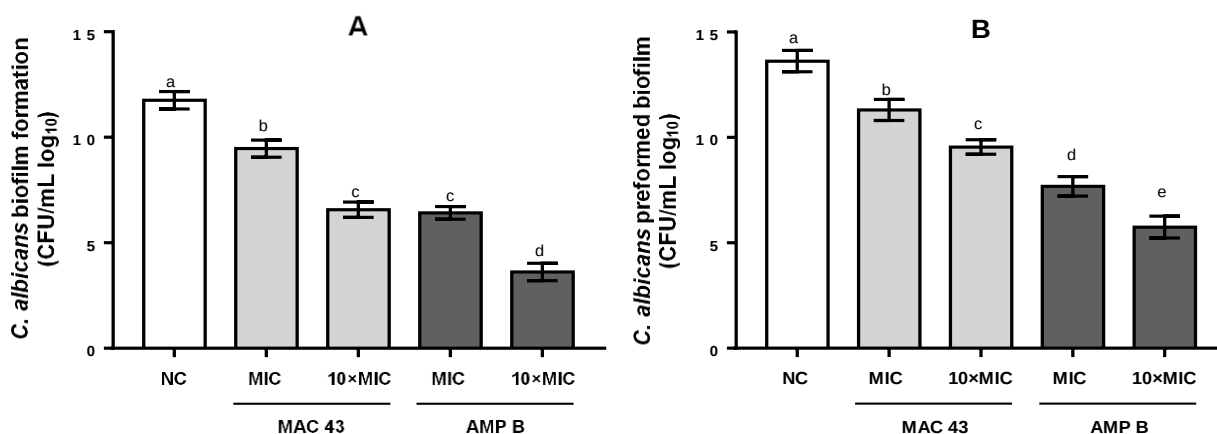


Figure 2. Effects (mean±SD) of **MAC 43** at MIC and 10×MIC (15.2 and 152 μmol/L, respectively) on the *C. albicans* (ATCC 90028) biofilm formation (A) and preformed biofilm (B). NC = negative control (untreated biofilm). Amphotericin B (AMP B) at MIC and 10×MIC (0.5 and 5.0 μmol/L, respectively) was used as a standard antifungal drug. Different letters indicate statistical difference between treatments ($p < 0.05$; ANOVA with Tukey's post hoc test).

Fonte: Elaborado pelo autor

7.2.4. Effects of MAC 43 combined with amphotericin B or fluconazole

The treatment of fungal infections using drug combination therapy may enhance its effectiveness, mitigate adverse effects and reduce the emergence of resistant strains (SUN, ZHANG & LI, 2012). On account of that, we evaluated the effects of **MAC 43** against *C. albicans* (ATCC 90028) in combination with antifungal drugs, including amphotericin B and fluconazole. We used the checkerboard assay in order to determine fractional inhibitory concentration index (FICI) values, which are used to classify the effects of the combinations as synergistic ($FICI \leq 0.5$), additive ($FICI > 0.5$ to 1), indifferent ($FICI > 1$ to 4) or antagonistic

(FICI >4) (DOERN, 2014). Both **MAC 43** associations, with amphotericin B and with fluconazole, showed synergistic effects, with FICI values of 0.5 and 0.2 respectively (Table 2). **MAC 8** also showed synergistic effects with amphotericin B and fluconazole against *C. albicans* (ATCC 90028), with FICI values of 0.5 and 0.2, respectively (Chapter VI).

Table 2. FICI values and effects of **MAC 43** combinations with amphotericin B or fluconazole against *C. albicans* (ATCC 90028).

Combination	FICI	Effect
MAC 43 + AMP B	0.4	synergistic
MAC 43 + FCZ	0.1	synergistic

FICI = fractional inhibitory concentration index; AMP B = amphotericin B; FCZ = fluconazole. FICI ≤ 0.5 = synergy; FICI >0.5 to 1 = additive; FICI >1 to 4 = indifference; FICI >4 = antagonism (DOERN, 2014).

Fonte: Elaborado pelo autor

7.3. CONCLUSION

In summary, we synthesized a series of monocarbonyl analogs of curcumin as part of our ongoing search for antifungal agents based on curcumin. **MAC 43** was found to be an important hit with antifungal activity *C. albicans* and non-*albicans Candida* species. In addition, **MAC 43** reduced *C. albicans* biofilm, and it showed synergistic effects with antifungal drugs. Altogether, our results corroborate the potential of monocarbonyl curcuminoids in the development of new antifungal agents.

7.4. EXPERIMENTAL

7.4.1. Chemistry

7.4.1.1. Material and instruments

Chapter II, item 2.4.1.1.

7.4.1.2. Synthesis of MACs 35 and 36

A solution of 3-hydroxybenzaldehyde (4 mmol) and ketone (acetone or cyclohexanone) (1.5 mmol) in EtOH (5 mL) was cooled in an ice bath for 10 min. Catalytic amounts of H₂SO₄ were added dropwise to the reaction mixture. The solution was stirred at room temperature for 24 h. Reaction medium was poured onto crushed ice. The crude product was partitioned with ethyl acetate (3 × 15 mL), and the combined organic phase was

concentrated under reduced pressure. **MACs 35** and **36** was purified by column chromatography over silica gel using mixture of hexane and ethyl acetate (6:4) as eluent.

7.4.1.3. Synthesis of MACs 37–48

MACs 37–48 were synthesized in two steps.

7.4.1.3.1. Synthesis of SMAC 4.

Chapter III, item 4.1.2.

7.4.1.3.2. Synthesis of MACs 37–48. A solution of **SMAC 4** (1 mmol) and aromatic aldehydes (1.5 mmol) in EtOH (5 mL) was cooled in an ice bath for 10 min. Ethanolic solution of NaOH (1 mL, 1 mol/L) was added dropwise to the reaction mixture. The solution was stirred at room temperature for 10–48 h. Reaction medium was poured onto crushed ice. The crude product was partitioned with ethyl acetate (3 × 15 mL), and the combined organic phase was concentrated under reduced pressure. **MACs 37–48** were purified by gel filtration column chromatography (LH-20) using EtOH as eluent.

7.4.2. Anti-*Candida* assay

Chapter VI, item 6.4.2

7.4.3. Antibiofilm assays

Chapter VI, item 6.4.4

7.4.4. Checkerboard Assay

Chapter VI, item 6.4.5

8. CAPÍTULO VIII

Conclusões Gerais, Referências e Apêndices

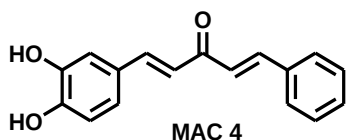
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8.1. CONCLUSÕES GERAIS

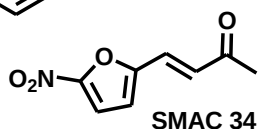
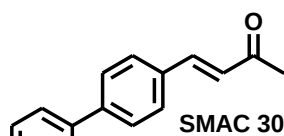
Esse trabalho utilizou a curcumina como protótipo para o planejamento de análogos monocetônicos, os quais foram desenhados pela permutação da subunidade β -dicetona pelo fragmento monocarbonila. Foram descritas as sínteses de 110 substâncias, entre intermediários (22) e substâncias-alvo (88), sendo 48 inéditas. As substâncias-alvo, curcuminoides monocetônicos (**MACs 1–48**) e curcuminoides monocetônicos simplificados (**SMACs 1–40**), foram preparadas por meio de reações de condensação aldólica entre aldeídos e cetonas, sobre catálise básica ou ácida. Contudo, para a obtenção de alguns análogos hidroxilados foi necessária a proteção da hidroxila fenólica com o grupo protetor MOM, devido às dificuldades que esse substituinte impôs à síntese pelo metodologia convencional.

As avaliações dos monocetônicos **MACs 1–8** contra bactérias de importância clínica revelaram os efeitos promissores de **MAC 4**, uma substância inédita na literatura, contra *M. tuberculosis*. As investigações demonstraram que **MAC 4** foi potencialmente seguro e estável. Além disso, o seu modo de ação envolveu a divisão celular bacteriana, um alvo inovador para o desenvolvimento de novos agentes antibacterianos. Contudo, a síntese de **MAC 4** requereu quatro etapas reacionais. Na busca por substâncias com acessibilidade sintética maior, os monocetônicos simplificados **SMACs 1–40** foram planejados a partir da estrutura de **MAC 4**. As avaliações demonstraram que os monocetônicos **SMACs 30** e **34**, que foram preparados em apenas uma etapa reacional, apresentaram atividade similar ao análogo **MAC 4** contra *M. tuberculosis*. Entretanto, os dois monocetônicos simplificados apresentaram índices de seletividade maiores e foram quimicamente mais estáveis do que **MAC 4**. Além disso, **SMACs 30** e **34** foram ativos contra isolados clínicos de *M. tuberculosis* com resistência à múltiplos fármacos.

Curcuminoides monocetônicos com efeitos promissores contra *M. tuberculosis*



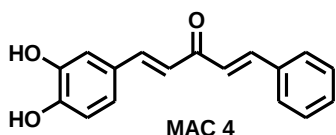
- ✓ CIM = 5,6 $\mu\text{mol/L}$
- ✓ IS = 15,6 e 14,2 (linhagens celulares MRC-5 e A549, respectivamente)
- ✓ Não demonstrou efeitos genotóxicos contra a linhagem A549
- ✓ Quimicamente mais estável do que a curcumina
- ✓ Desestrutou o septo divisional de *B. subtilis*



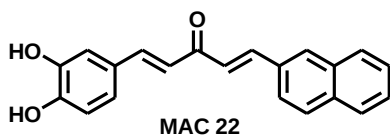
- ✓ Obtenção em apenas uma etapa reacional
- ✓ CIM = 7,2 e 4,5 $\mu\text{mol/L}$ (**SMACs 30** e **34**, respectivamente)
- ✓ IS = 27,2 e 23,3 para a linhagem MRC-5 (**SMACs 30** e **34**, respectivamente)
- ✓ Quimicamente mais estáveis do que **MAC 4**
- ✓ Ativos contra cepas MDR de *M. tuberculosis* ($3,8 \mu\text{mol/L} \leq \text{CIM} \leq 81,4 \mu\text{mol/L}$)

As investigações dos monacetônicos **MACs 1–8** e **SMACs 1, 2, 4 e 5** contra a bactéria fitopatogênica *X. citri* também revelaram os efeitos promissores de **MAC 4**. Esse curcuminóide foi capaz de reduzir a habilidade de *X. citri* induzir lesões de cancro cítrico sobre as folhas de *Citrus sinensis*. Além disso, as investigações sobre o seu modo de ação indicou a sua capacidade de afetar a divisão celular de *X. citri*, perturbando o septo divisional e inibindo a atividade GTPásica da proteína FtsZ. Entretanto, a potência de **MAC 4** contra *X. citri* foi moderada. Na busca por substâncias com atividade maior, os monacetônicos **MACs 9–23** foram planejados a partir da estrutura de **MAC 4**. O curcuminóide **MAC 22**, uma substância inédita na literatura, apresentou potência cerca de 2,4 vezes maior do que **MAC 4**. As investigações demonstraram que modo de ação de **MAC 22** envolveu a perturbação da membrana de *X. citri*.

Curcuminóides monacetônicos com efeitos promissores contra *X. citri*



- ✓ CIM e CBM = 330 e 375 µmol/L, respectivamente
- ✓ *X. citri* não desenvolveu resistência
- ✓ Reduziu a patogenicidade *in planta*
- ✓ Desestrutou o septo divisional de *X. citri*
- ✓ Inibiu a atividade GTPásica da proteína FtsZ

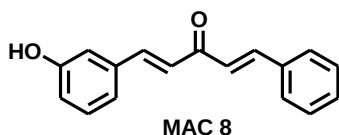


- ✓ CIM e CBM = 135 e 316 µmol/L, respectivamente
- ✓ Mais potente do que **MAC 4**
- ✓ Inibiu a fase exponencial do crescimento de *X. citri*
- ✓ Pertubou a membrana de *X. citri*

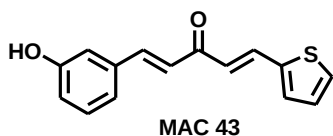
Fonte: Elaborado pelo autor

As avaliações dos monacetônicos **MACs 1–8** e **24–34** contra fungos do gênero *Candida* revelaram os efeitos promissores de **MAC 8**. Esse curcuminóide apresentou espectro largo e atividade potente contra *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis* e *C. krusei*. Além disso, **MAC 8** inibiu a adesão celular e o biofilme de *C. albicans*, e apresentou toxicidade sistêmica baixa no modelo *G. mellonella*. Na busca por novos análogos, os monacetônicos **MACs 35–48** foram planejados a partir da estrutura de **MAC 8**. O curcuminóide **MAC 43**, uma substância inédita na literatura, apresentou atividade mais potente do que **MAC 8** contra *C. albicans*, *C. tropicalis*, *C. parapsilosis* e *C. krusei*. Além disso, **MAC 43** foi capaz de inibir o biofilme de *C. albicans* e apresentar efeitos sinérgicos quando associado com os fármacos anfotericina B e fluconazol.

Curcuminoides monocetônicos com efeitos promissores contra espécies de *Candida*



- ✓ $7,8 \mu\text{mol/L} \leq \text{CIM} \leq 31,2 \mu\text{mol/L}$
- ✓ $15,6 \mu\text{mol/L} \leq \text{CBM} \leq 62,3 \mu\text{mol/L}$
- ✓ Reduziu a adesão de *C. albicans* às células humanas
- ✓ Inibiu o biofilme de *C. albicans*
- ✓ Quimicamente mais estável do que a curcumina
- ✓ Toxicidade sistêmica baixa em *G. mellonella*



- ✓ $7,4 \mu\text{mol/L} \leq \text{CIM} \leq 15,2 \mu\text{mol/L}$
- ✓ $15,2 \mu\text{mol/L} \leq \text{CBM} \leq 30,4 \mu\text{mol/L}$
- ✓ Mais potente do que **MAC 8** contra *C. albicans*, *C. tropicalis*, *C. parapsilosis* e *C. krusei*
- ✓ Inibiu o biofilme de *C. albicans*
- ✓ Sinergismo com anfotericina B e fluconazol

Fonte: Elaborado pelo autor

Em conclusão, esse trabalho corroborou a relevância da curcumina como uma estrutura privilegiada para o desenvolvimento de substâncias potencialmente bioativas. Os seus análogos monocetônicos demonstraram efeitos antibacterianos e antifúngicos potentes, além de serem potencialmente seguros e estáveis. Esse estudo também trouxe luz às relações estrutura-atividade de curcuminoides monocetônicos, fornecendo caminhos para o desenvolvimento de novos análogos bioativos para o tratamento de infecções bacterianas e fúngicas.

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APÊNDICE A

Material Suplementar do Capítulo II

Antibacterial Activity of a New Unsymmetrical Monocarbonyl Analog of Curcumin (MAC 4) is Associated with Divisome Disruption

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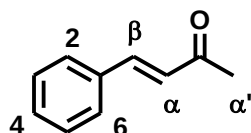
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1. SPECTROSCOPY DATA ANALYSES

1.1. (*E*)-4-phenylbut-3-en-2-one (**1**) (AHLSTEN & MARTÍN-MATUTE, 2011; NAIK, KUMAR & SWETHA, 2011).



Yellow solid

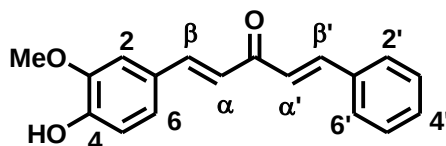
Yield: 89%

Melting point: 43–44 °C

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 2.40 (s; H-α'), 6.73 (d; 16.2; H-α), 7.41–7.42 (H-3, H-4 and H-5), 7.53 (d; 16.2; H-β), 7.56 (dd; 3.0 and 6.3; H-2 and H-6) (Figure S1).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 27.5 (C-α'), 127.2 (C-α), 128.3 (C-2 and C-6), 129.0 (C-3 and C-5), 130.5 (C-4), 134.4 (C-1), 143.5 (C-β), 198.4 (C=O) (Figure S2).

1.2. (1*E*,4*E*)-1-(4-hydroxy-3-methoxyphenyl)-5-phenylpenta-1,4-dien-3-one (**MAC 1**) (WEBER et al., 2005).



Red oil

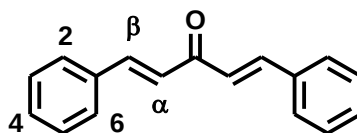
Yield: 72%

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 3.95 (s; 3-OMe), 6.95 (d; 15.6; H-α), 6.97 (d; 7.8; H-5), 7.12 (d; 15.6; H-α'), 7.13 (d; 1.8; H-2), 7.19 (dd; 1.8 and 7.8; H-

6), 7.42–7.43 (H-3', H-4' and H-5'), 7.63 (dd; 2.4 and 6.3; H-2' and H-6'), 7.71 (d; 15.6; H-β), 7.75 (d; 15.6; H-β') (Figure S3).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 56.0 (3-OMe), 109.7 (C-5), 114.9 (C-2), 123.4 (C-6), 123.6 (C-α), 125.3 (C-α'), 127.3 (C-1), 128.4 (C-2' and C-6'), 129.0 (C-3' and C-5'), 130.4 (C-4'), 134.9 (C-1'), 142.9 (C-β), 143.7 (C-β'), 146.8 (C-4), 148.3 (C-3), 188.9 (C=O) (Figure S4).

1.3. (1E,4E)-1,5-diphenylpenta-1,4-dien-3-one (MAC 2) (NAIK, KUMAR & SWETHA, 2011; DAI et al., 2015).



Yellow solid

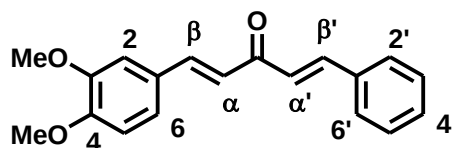
Yield: 83%

Melting Point: 110–112 °C

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 7.12 (d; 15.6; H-α), 7.44–7.45 (H-3, H-4 and H-5), 7.65 (dd; 1.8 and 7.2; H-2 and H-6), 7.77 (d; 15.6; H-β) (Figure S5).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 125.4 (C-α), 128.4 (C-2 and C-6), 129.0 (C-3 and C-5), 130.6 (C-4), 134.8 (C-1), 143.4 (C-β), 189.0 (C=O) (Figure S6).

1.4. (1*E*,4*E*)-1-(3,4-dimethoxyphenyl)-5-phenylpenta-1,4-dien-3-one (MAC 3).



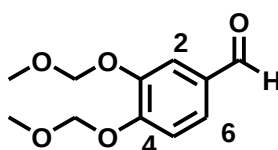
Yellow oil

Yield: 26%

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 3.95 (s; 3-OMe), 3.97 (s; 4-OMe), 6.92 (d; 8.4; H-5), 6.97 (d; 15.6; H-α), 7.13 (d; 16.2; H-α'), 7.17 (s; H-2), 7.23 (d; 8.4; H-6), 7.43–7.44 (H-3', H-4' and H-5'), 7.64 (dd; 1.2 and 5.7; H-2' and H-6'), 7.72 (d; 15.6; H-β), 7.76 (d; 16.2; H-β') (Figure S7).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 55.9 (3-OMe), 56.0 (4-OMe), 109.8 (C-5), 111.1 (C-2), 123.3 (C-6), 123.7 (C-α), 125.3 (C-α'), 127.7 (C-1), 128.4 (C-2' and C-6'), 129.0 (C-3' and C-5'), 130.4 (C-4'), 134.9 (C-1'), 143.0 (C-β), 143.5 (C-β'), 149.2 (C-4), 151.4 (C-3), 188.9 (C=O) (Figure S8).

1.5. 3,4-bis(methoxymethoxy)benzaldehyde (2) (KIM et al., 2008).



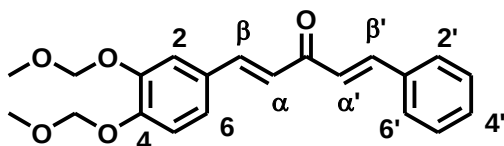
Colorless oil

Yield: 73%

¹H NMR (600 MHz; DMSO-*d*₆), δ_H in ppm (multiplicity; *J* in Hz): 3.41 (s; 3-OCH₂OCH₃), 3.42 (s; 4-OCH₂OCH₃), 5.28 (s; 3-OCH₂OCH₃), 5.33 (s; 4-OCH₂OCH₃), 7.30 (d; 9.0; H-5), 7.58–7.59 (H-2 and H-6), 9.85 (s; -CHO) (Figure S9).

¹³C NMR (150 MHz; DMSO-*d*₆), δ_C in ppm: 56.3 (3-OCH₂OCH₃), 56.5 (4-OCH₂OCH₃), 94.8 (3-OCH₂OCH₃), 95.1 (4-OCH₂OCH₃), 115.8 (C-2), 116.0 (C-5), 126.7 (C-6), 131.0 (C-1), 147.4 (C-3), 152.7 (C-4), 191.9 (C=O) (Figure S10).

1.6. (1*E*,4*E*)-1-(3,4-bis(methoxymethoxy)phenyl)-5-phenylpenta-1,4-dien-3-one (3).



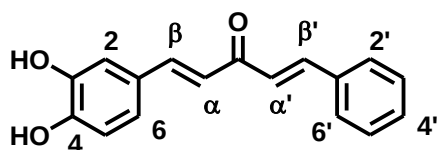
Yellow oil

Yield: 42%

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 3.52 (s; 3-OCH₂OCH₃), 3.55 (s; 4-OCH₂OCH₃), 5.28 (s; 3-OCH₂OCH₃), 5.29 (s; 4-OCH₂OCH₃), 6.96 (d; 16.2; H-α), 7.12 (d; 16.2; H-α'), 7.18 (d; 8.4; H-5), 7.25 (dd; 1.8 and 8.4; H-6), 7.40–7.41 (H-3', H-4' and H-5'), 7.46 (d; 1.8; H-2), 7.62 (dd; 2.4 and 7.2; H-2' and H-6'), 7.67 (d; 16.2; H-β), 7.72 (d; 16.2; H-β') (Figure S11).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 56.2 (3-OCH₂OCH₃), 56.3 (4-OCH₂OCH₃), 95.0 (3-OCH₂OCH₃), 95.4 (4-OCH₂OCH₃), 115.8 (C-2), 116.2 (C-5), 123.9 (C-6), 124.4 (C-α), 125.2 (C-α'), 128.3 (C-2' and C-6'), 128.9 (C-3' and C-5'), 129.1 (C-1), 130.4 (C-4'), 134.8 (C-1'), 142.9 (C-β), 143.0 (C-β'), 147.4 (C-4), 149.4 (C-3), 188.7 (C=O) (Figure S12).

1.7. (1*E*,4*E*)-1-(3,4-dihydroxyphenyl)-5-phenylpenta-1,4-dien-3-one (MAC 4).



Yellow solid

Yield: 26%

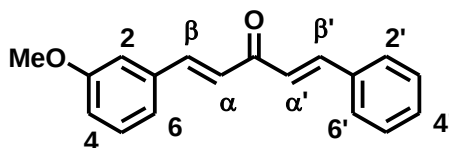
Melting point: 150–152 °C

ESI-MS (Mr 266.09): m/z 267.17 $[M+H]^+$ (Figure S13).

^1H NMR (600 MHz; DMSO- d_6), δ_{H} in ppm (multiplicity; J in Hz): 6.81 (d; 7.8; H-5), 7.01 (d; 15.6; H- α), 7.10 (dd; 2.4 and 7.8; H-6), 7.17 (d; 2.4; H-2), 7.36 (d; 16.2; H- α'), 7.45–7.46 (H-3', H-4' and H-5'), 7.66 (d; 15.6; H- β), 7.71 (d; 16.2; H- β'), 7.78 (dd; 2.4 and 7.8; H-2' and H-6') (Figure S14).

^{13}C NMR (150 MHz; DMSO- d_6), δ_{C} in ppm: 115.4 (C-2), 116.3 (C-5), 122.4 (C-6), 123.0 (C- α), 126.2 (C- α'), 126.6 (C-1), 128.9 (C-2' and C-6'), 129.4 (C-3' and C-5'), 130.8 (C-4'), 135.3 (C-1'), 142.3 (C- β), 144.3 (C- β'), 146.1 (C-4), 149.1 (C-3), 188.7 (C=O) (Figure S15).

1.8. (1*E*,4*E*)-1-(3-methoxyphenyl)-5-phenylpenta-1,4-dien-3-one (MAC 5) (DAI et al., 2015).



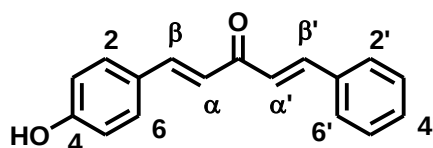
Yellow oil

Yield: 34%

^1H NMR (600 MHz; CDCl_3), δ_{H} in ppm (multiplicity; J in Hz): 3.87 (s; 3-OMe), 6.99 (ddd; 0.6, 2.4 and 8.2; H-4), 7.09 (d; 15.6; H- α), 7.12 (d; 16.2; H- α'), 7.16 (dd; 1.8 and 2.4; H-2), 7.24 (d; 7.8; H-6), 7.35 (dd; 7.8 and 8.2; H-5), 7.43–7.44 (H-3', H-4' and H-5'), 7.64 (dd; 1.8 and 6.6; H-2' and H-6'), 7.73 (d; 15.6; H- β), 7.77 (d; 16.2; H- β') (Figure S16).

^{13}C NMR (150 MHz; CDCl_3), δ_{C} in ppm: 55.4 (3-OMe), 113.3 (C-2), 116.4 (C-4), 121.1 (C-6), 125.3 (C- α), 125.7 (C- α'), 128.5 (C-2' and C-6'), 129.0 (C-3' and C-5'), 130.0 (C-4'), 130.6 (C-5), 134.8 (C-1'), 136.2 (C-1), 143.3 (C- β), 143.4 (C- β'), 159.9 (C-3), 189.0 (C=O) (Figure S17).

1.9. (1*E*,4*E*)-1-(4-hydroxyphenyl)-5-phenylpenta-1,4-dien-3-one (**MAC 6**) (NAIK, KUMAR & SWETHA, 2011; ZHOU et al., 2018).



Yellow solid

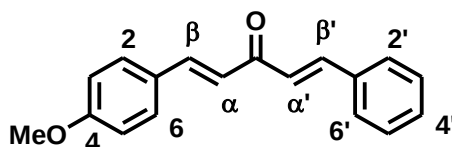
Yield: 66%

Melting Point: 135–136 °C

¹H NMR (600 MHz; DMSO-*d*₆), δ_H in ppm (multiplicity; *J* in Hz): 6.85 (d; 8.4; H-3 and H-5), 7.13 (d; 15.6; H-α), 7.35 (d; 16.2; H-α'), 7.45–7.47 (H-3', H-4' and H-5'), 7.65 (d; 8.4; H-2 and H-6), 7.73 (d; 15.6; H-β), 7.74 (d; 16.2; H-β'), 7.79 (dd; 1.8 and 8.1; H-2' and H-6') (Figure S18).

¹³C NMR (150 MHz; DMSO-*d*₆), δ_C in ppm: 116.4 (C-3 and C-5), 123.0 (C-α), 126.2 (C-α'), 128.7 (C-1), 128.9 (C-2' and C-6'), 129.4 (C-3' and C-5'), 130.8 (C-4'), 131.1 (C-2 and C-6), 135.3 (C-1'), 142.4 (C-β), 143.8 (C-β'), 160.5 (C-4), 188.7 (C=O) (Figure S19).

1.10. (1*E*,4*E*)-1-(4-methoxyphenyl)-5-phenylpenta-1,4-dien-3-one (**MAC 7**) (CHEN et al., 2004; NAIK, KUMAR & SWETHA, 2011).



Yellow solid

Yield: 56%

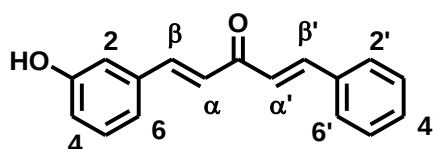
Melting Point: 91–92 °C

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 3.88 (s; 4-OMe), 6.96 (d; 9.0; H-3 and H-5), 6.99 (d; 16.2; H-α), 7.11 (d; 15.6; H-α'), 7.43–7.44 (H-3', H-4' and H-5'),

7.60 (d; 9.0; H-2 and H-6), 7.64 (dd; 1.8 and 6.9; H-2' and H-6'), 7.74 (d; 16.2; H- β), 7.75 (d; 15.6; H- β') (Figure S20).

^{13}C NMR (150 MHz; CDCl_3), δ_{C} in ppm: 55.4 (4-OMe), 114.5 (C-3 and C-5), 123.3 (C- α), 125.6 (C- α'), 127.5 (C-1), 128.4 (C-2' and C-6'), 129.0 (C-3' and C-5'), 130.2 (C-2 and C-6), 130.4 (C-4'), 134.9 (C-1'), 142.9 (C- β), 143.3 (C- β'), 161.7 (C-4), 188.9 (C=O) (Figure S21).

1.11. (1*E*,4*E*)-1-(3-hydroxyphenyl)-5-phenylpenta-1,4-dien-3-one (MAC 8) (DAI et al., 2015; SHEN et al., 2015).



Yellow solid

Yield: 52%

Melting Point: 130–131 °C

^1H NMR (600 MHz; CDCl_3), δ_{H} in ppm (multiplicity; *J* in Hz): 6.94 (ddd; 1.8, 2.4 and 7.8; H-4), 7.08 (d; 16.2; H- α), 7.10 (d; 16.2; H- α'), 7.16 (dd; 1.8 and 1.8; H-2), 7.20 (d; 7.2; H-6), 7.30 (dd; 7.2 and 7.8; H-5), 7.44–7.45 (H-3', H-4' and H-5'), 7.64 (dd; 2.4 and 6.6; H-2' and H-6'), 7.72 (d; 16.2; H- β), 7.77 (d; 16.2; H- β') (Figure S22).

^{13}C NMR (150 MHz; CDCl_3), δ_{C} in ppm: 114.9 (C-2), 117.9 (C-4), 120.9 (C-6), 125.3 (C- α), 125.5 (C- α'), 128.5 (C-2' and C-6'), 129.0 (C-3' and C-5'), 130.2 (C-5), 130.6 (C-4'), 134.7 (C-1'), 136.7 (C-1), 143.5 (C- β), 143.7 (C- β'), 156.5 (C-3), 189.3 (C=O) (Figure S23).

Figure S1. ^1H NMR spectrum of compound **1** (600 MHz; CDCl_3).

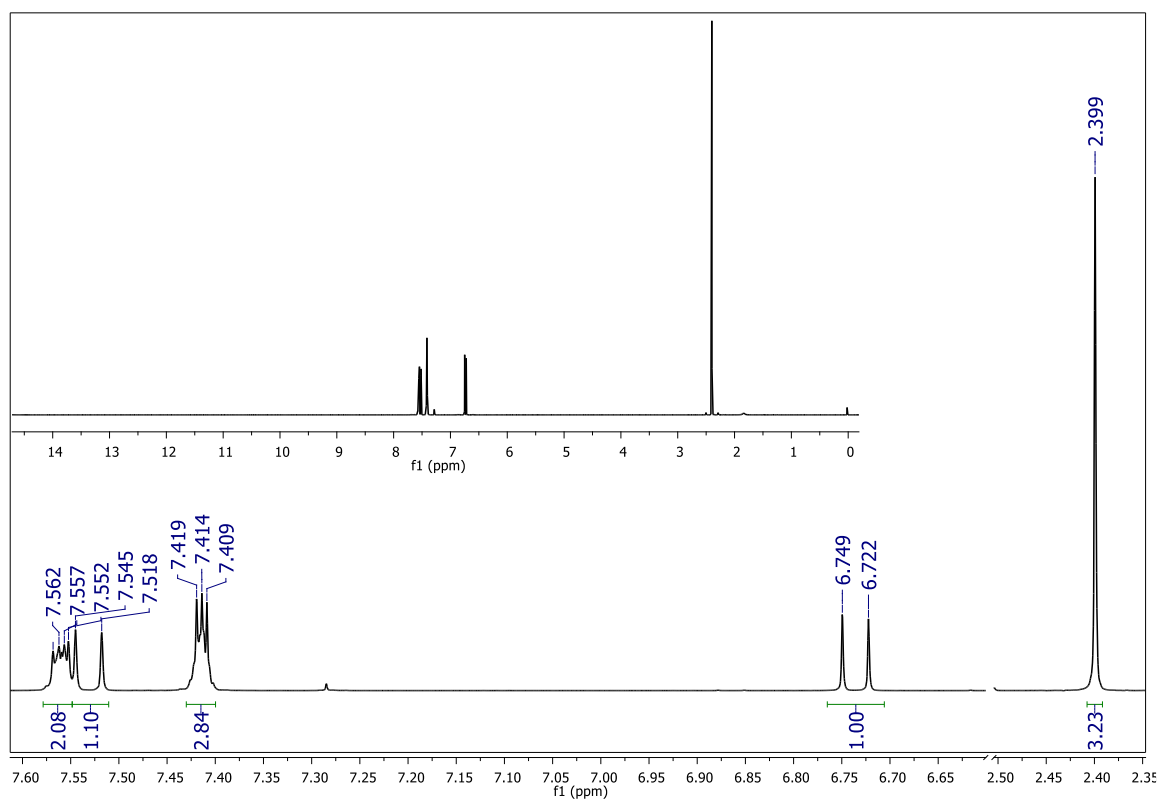


Figure S2. ^{13}C NMR spectrum of compound **1** (150 MHz; CDCl_3).

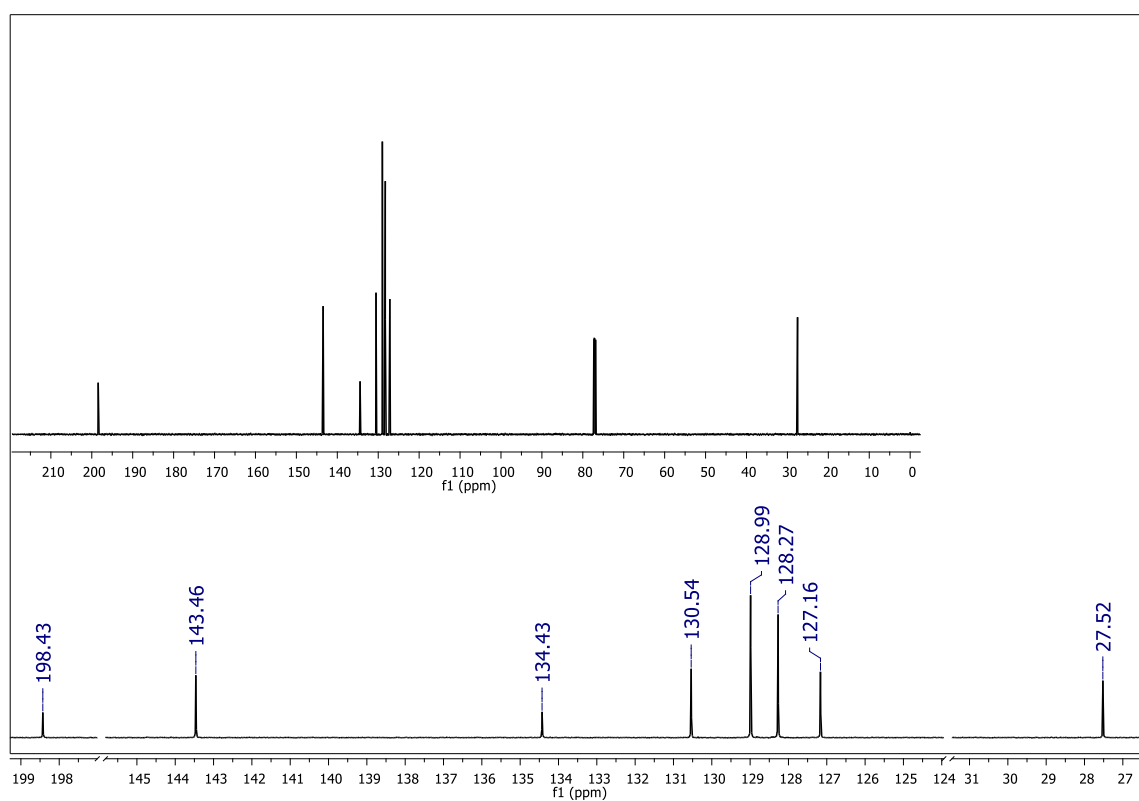


Figure S3. ^1H NMR spectrum of **MAC 1** (600 MHz; CDCl_3).

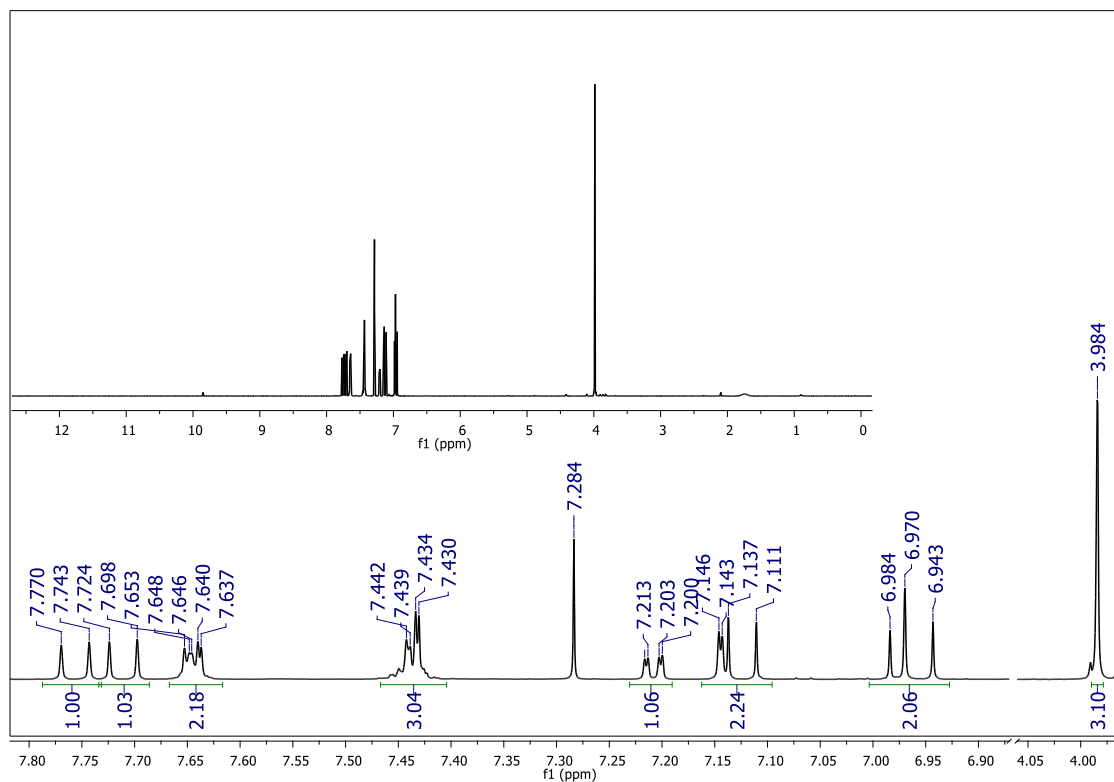


Figure S4. ^{13}C NMR spectrum of **MAC 1** (150 MHz; CDCl_3).

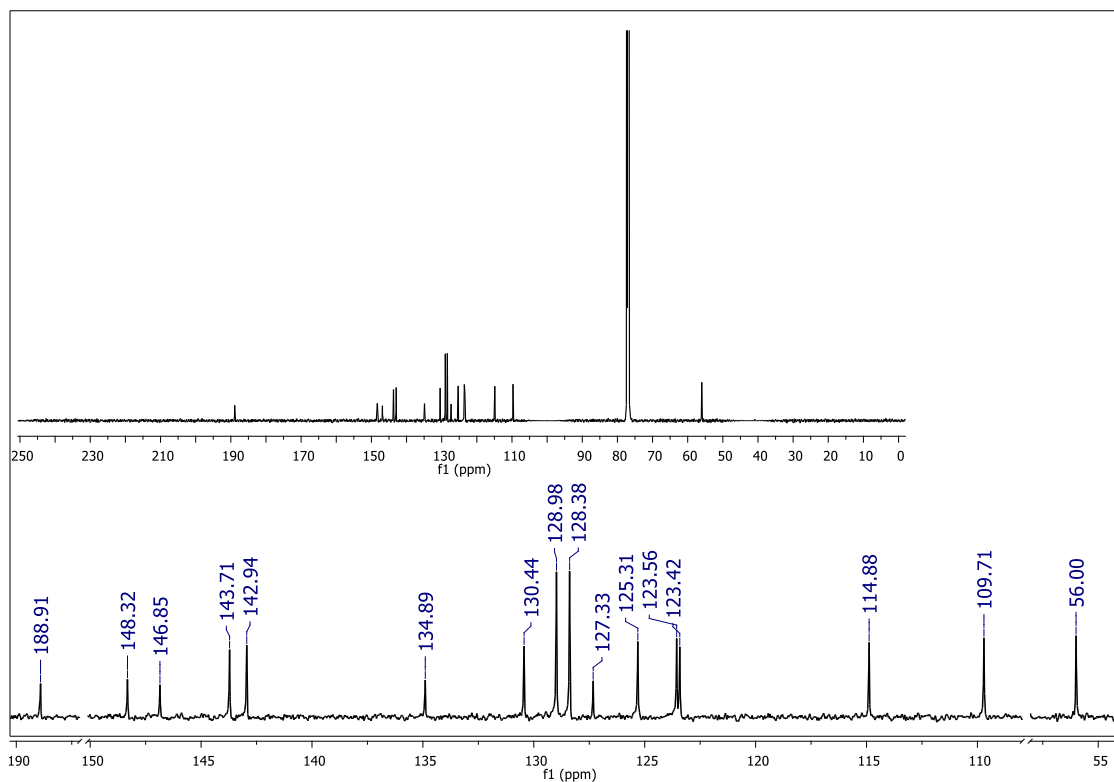


Figure S5. ^1H NMR spectrum of **MAC 2** (600 MHz; CDCl_3).

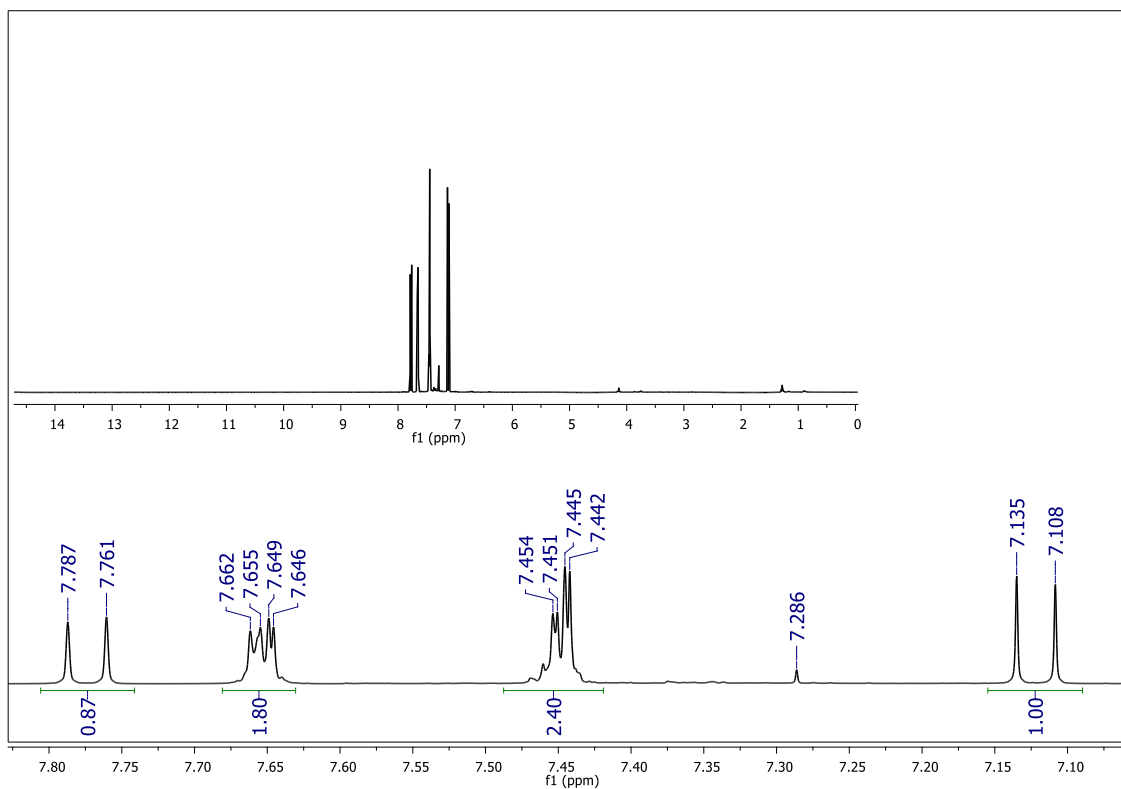


Figure S6. ^{13}C NMR spectrum of **MAC 2** (150 MHz; CDCl_3).

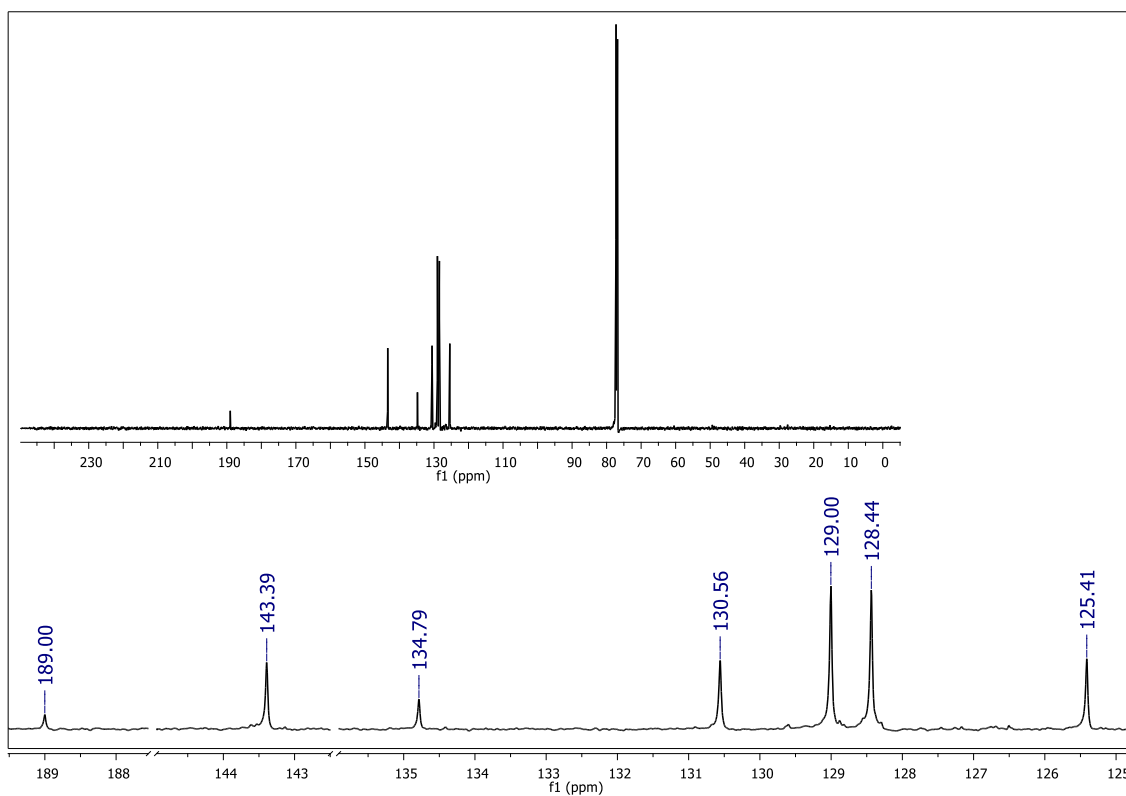


Figure S7. ^1H NMR spectrum of **MAC 3** (600 MHz; CDCl_3).

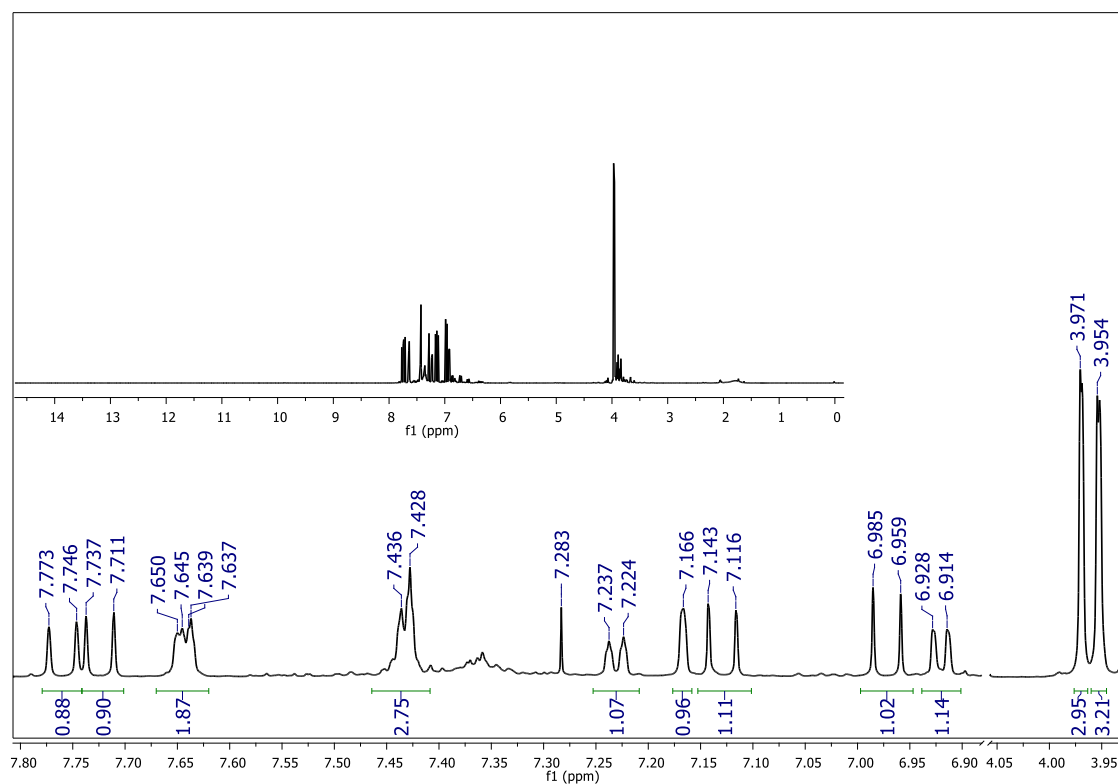


Figure S8. ^{13}C NMR spectrum of **MAC 3** (150 MHz; CDCl_3).

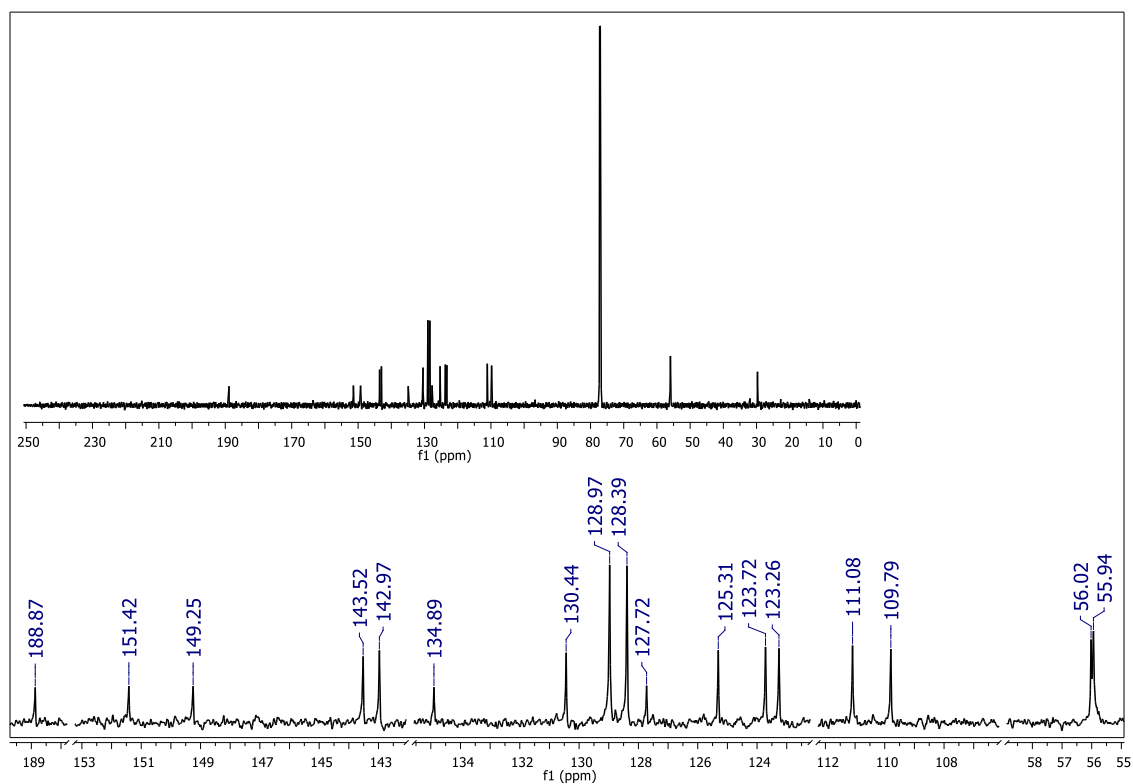


Figure S9. ^1H NMR spectrum of compound **2** (600 MHz; $\text{DMSO}-d_6$).

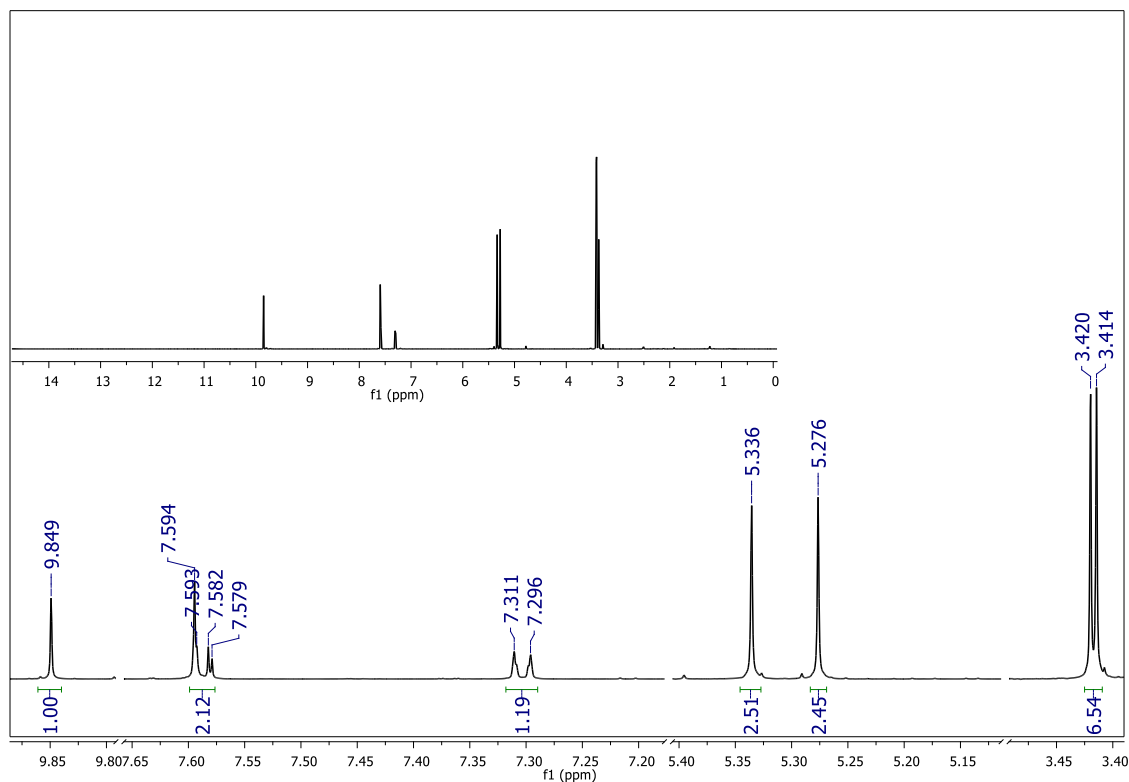


Figure S10. ^{13}C NMR spectrum of compound **2** (150 MHz; $\text{DMSO}-d_6$).

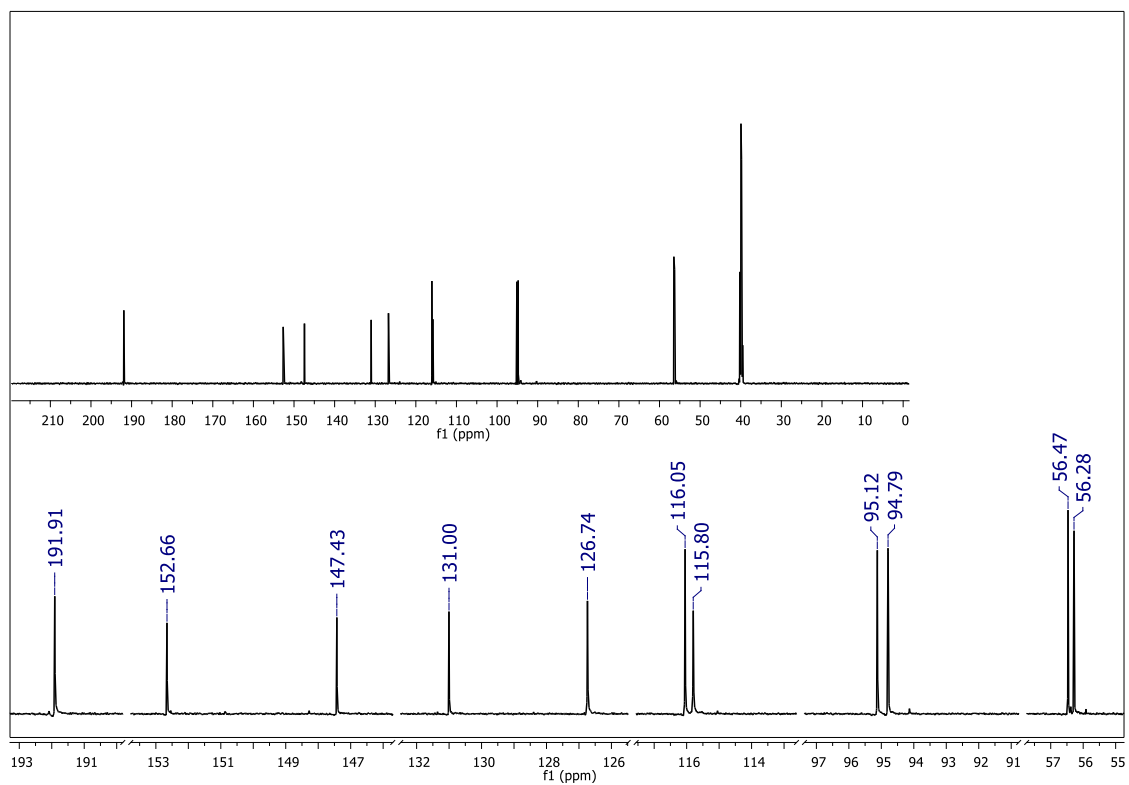


Figure S11. ^1H NMR spectrum of compound **3** (600 MHz; CDCl_3).

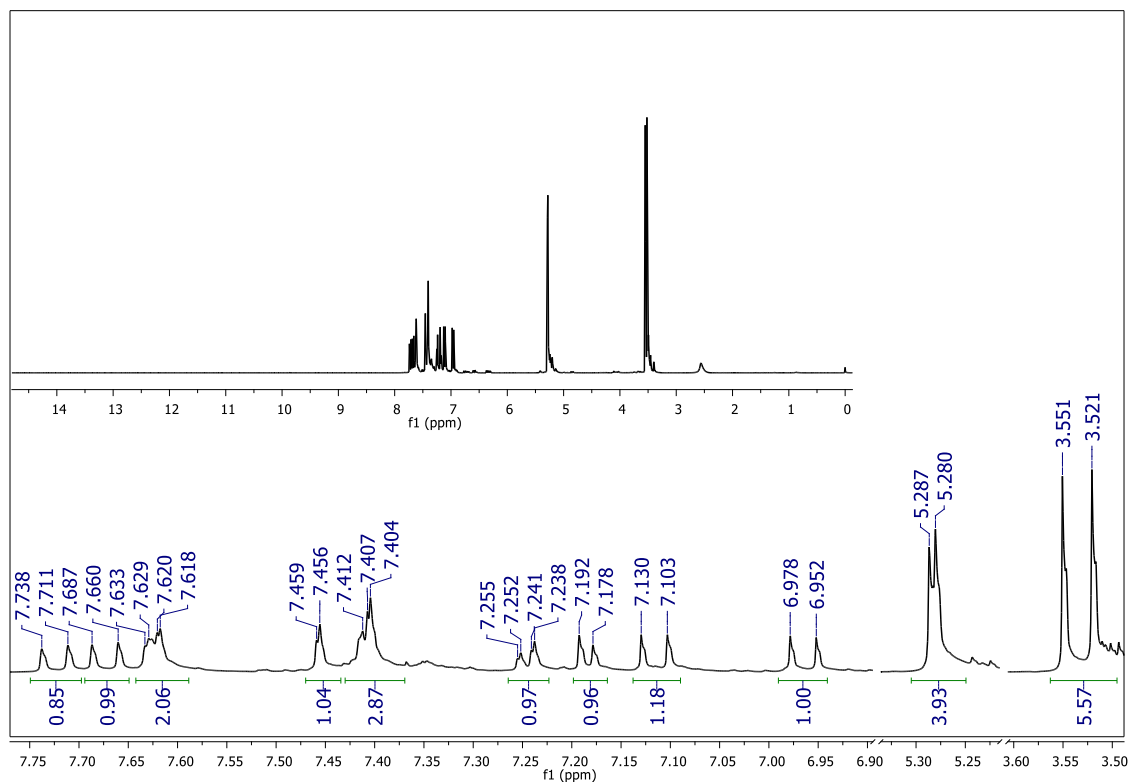


Figure S12. ^{13}C NMR spectrum of compound **3** (150 MHz; CDCl_3).

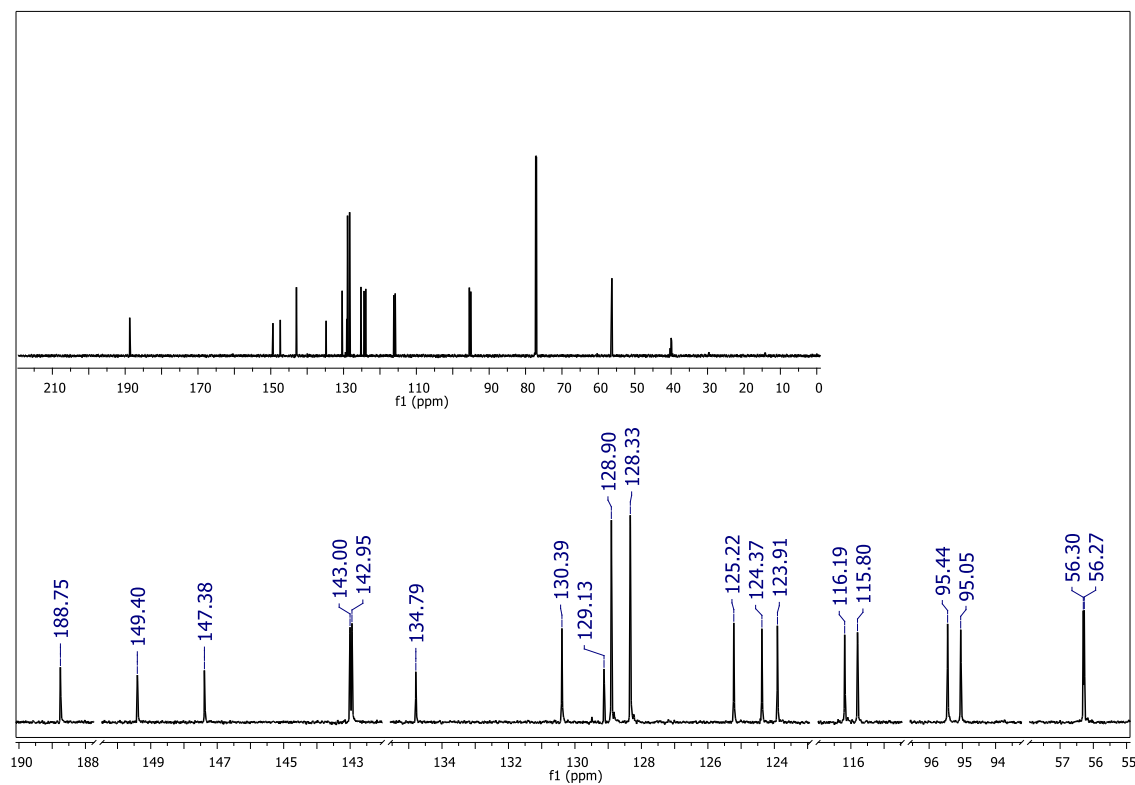


Figure S13. Mass spectrum of **MAC 4**.

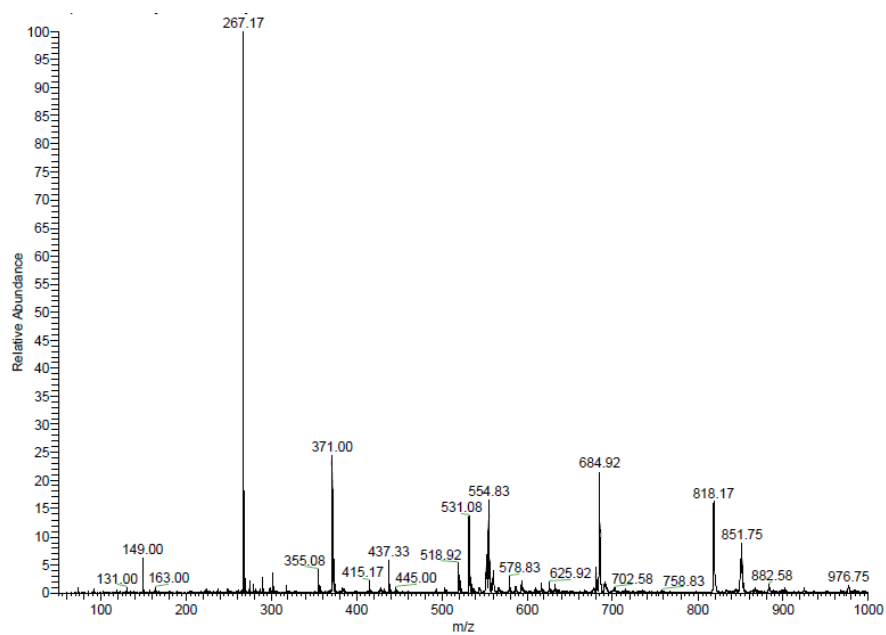


Figure S14. ^1H NMR spectrum of **MAC 4** (600 MHz; $\text{DMSO-}d_6$).

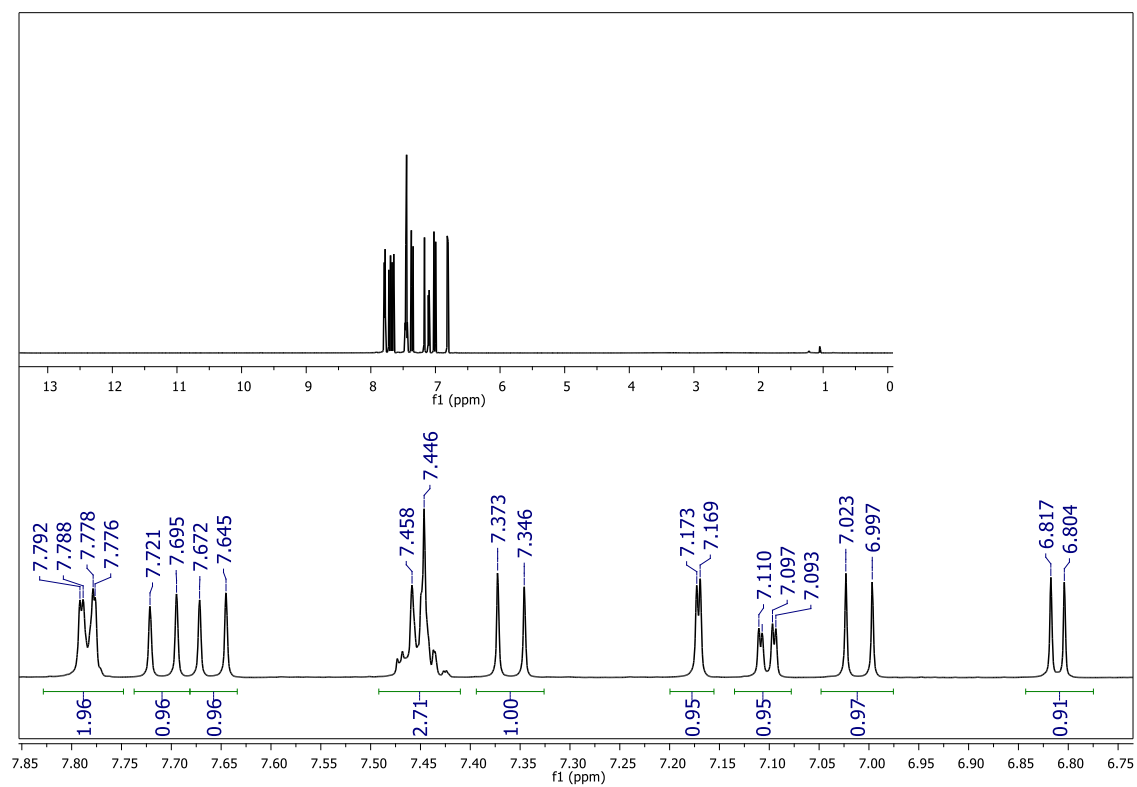


Figure S15. ^{13}C NMR spectrum of **MAC 4** (150 MHz; $\text{DMSO-}d_6$).

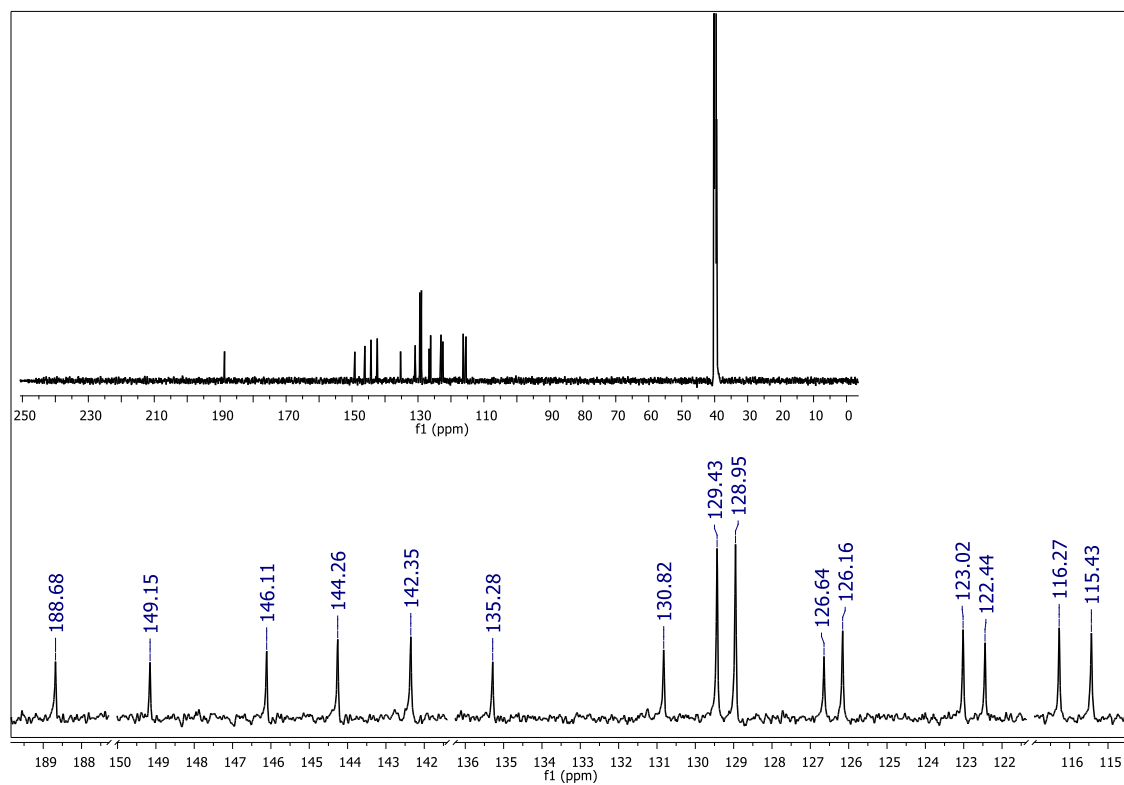


Figure S16. ^1H NMR spectrum of **MAC 5** (600 MHz; CDCl_3).

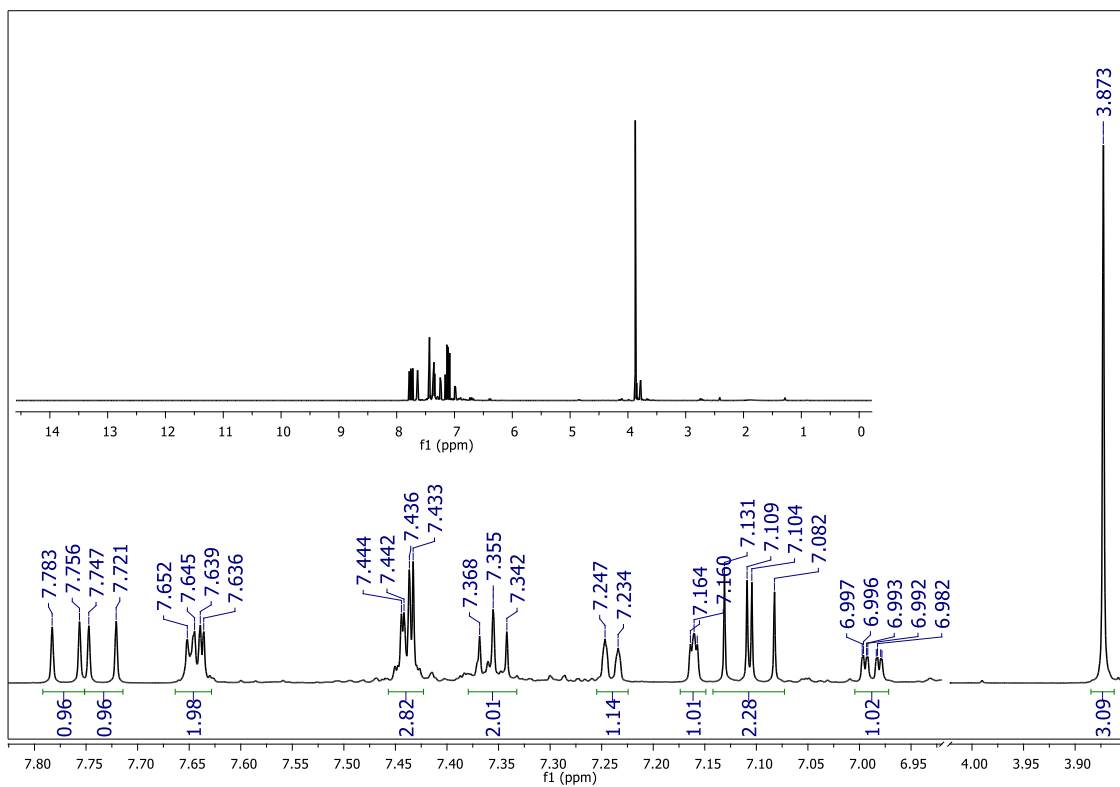


Figure S17. ^{13}C NMR spectrum of **MAC 5** (150 MHz; CDCl_3).

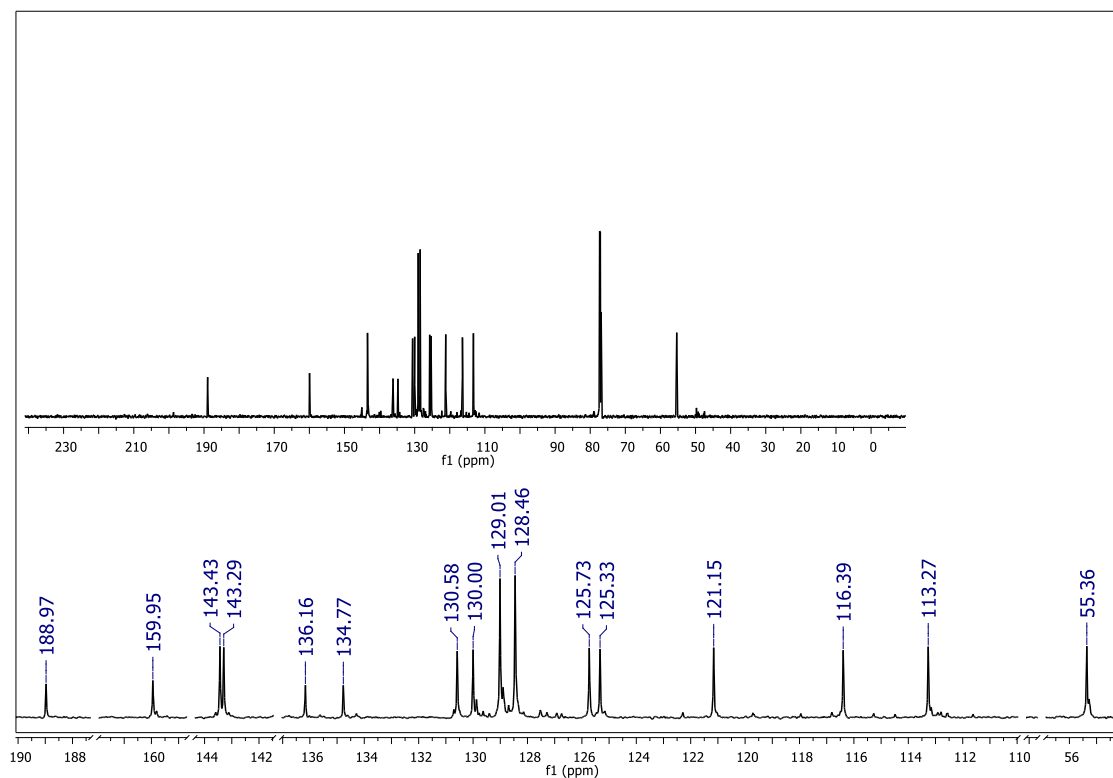


Figure S18. ^1H NMR spectrum of **MAC 6** (600 MHz; $\text{DMSO-}d_6$).

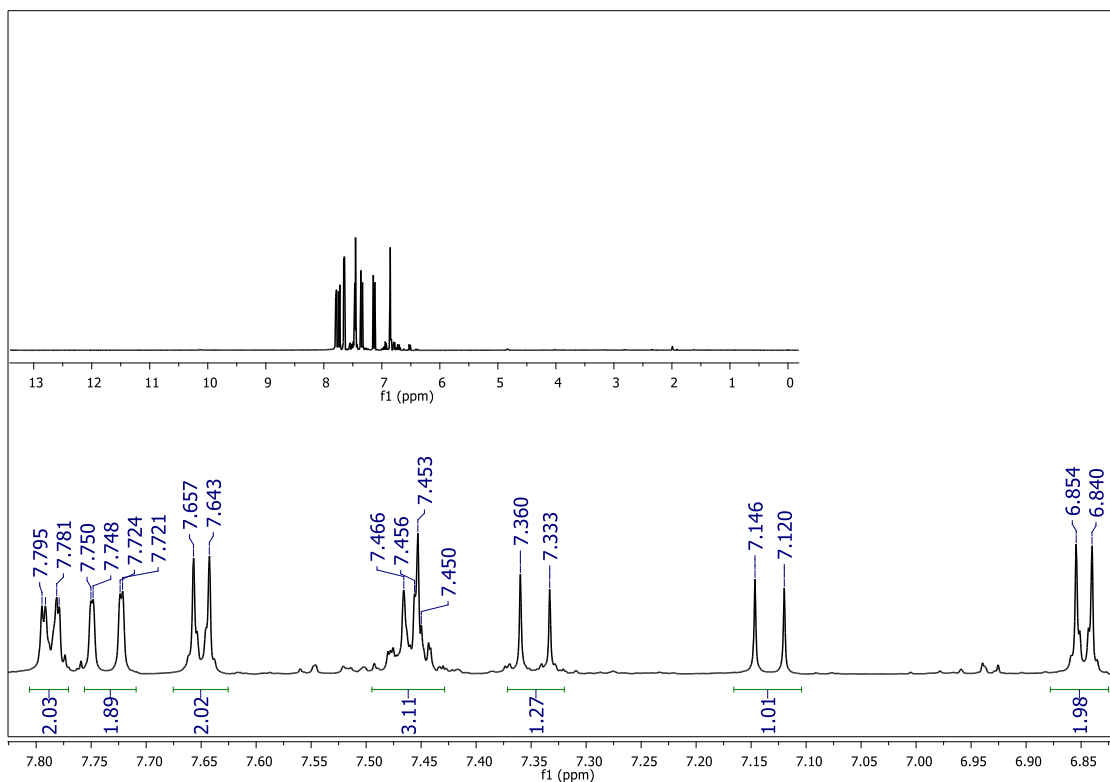


Figure S19. ^{13}C NMR spectrum of **MAC 6** (150 MHz; $\text{DMSO-}d_6$).

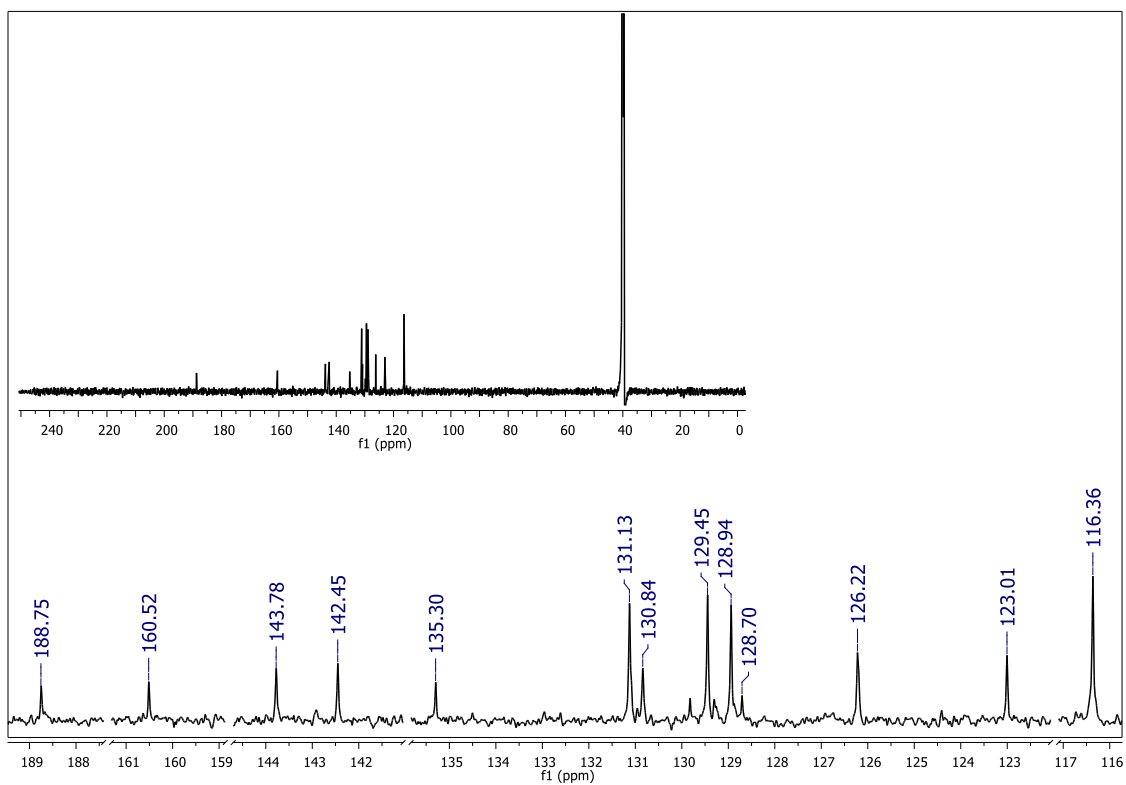


Figure S20. ^1H NMR spectrum of **MAC 7** (600 MHz; CDCl_3).

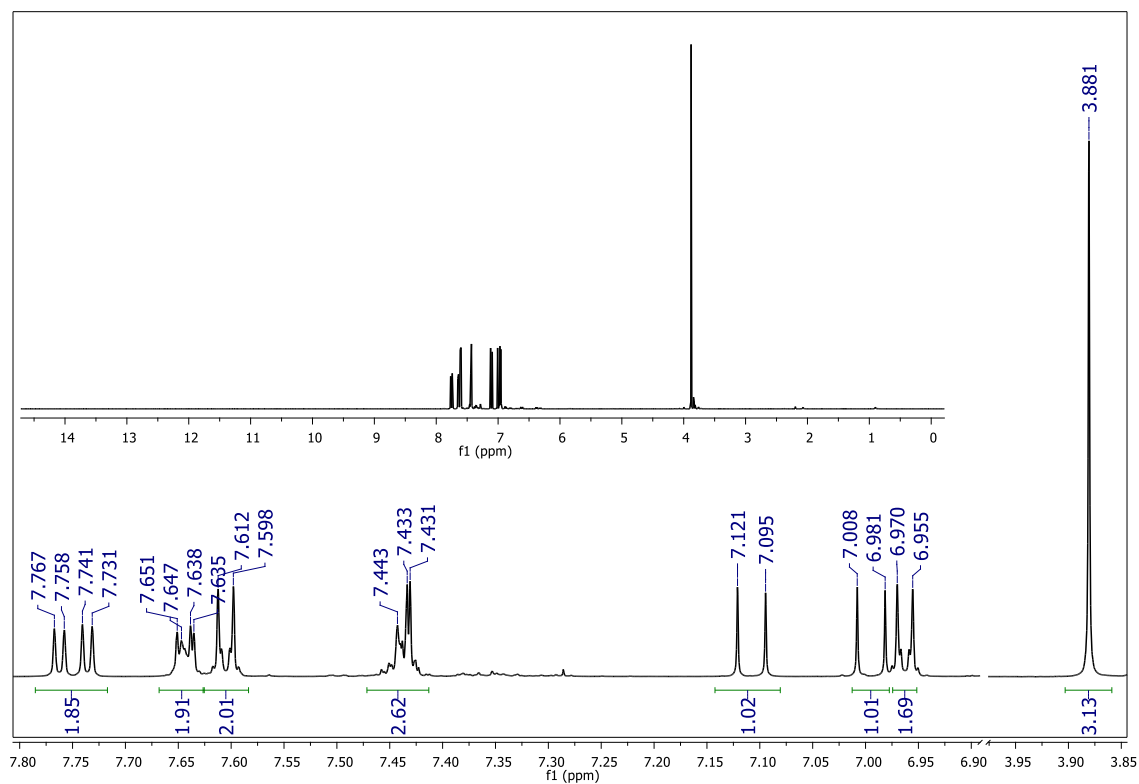


Figure S21. ^{13}C NMR spectrum of **MAC 7** (150 MHz; CDCl_3).

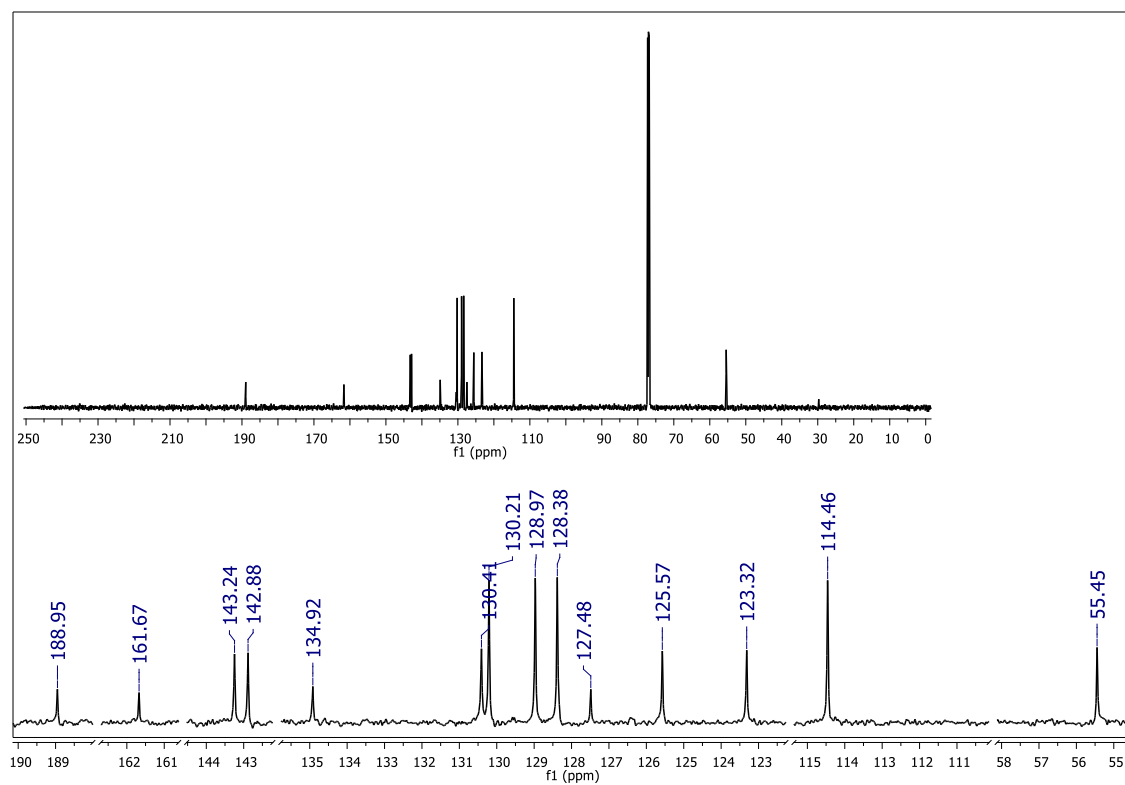


Figure S22. ^1H NMR spectrum of **MAC 8** (600 MHz; CDCl_3).

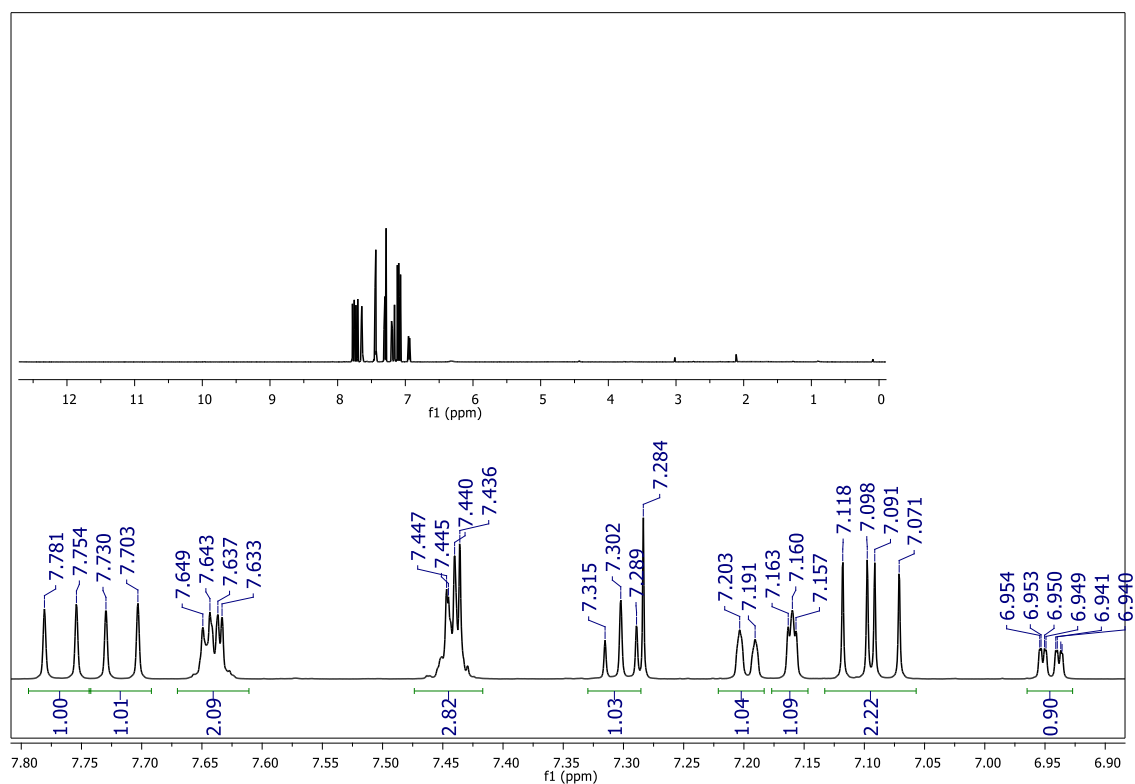
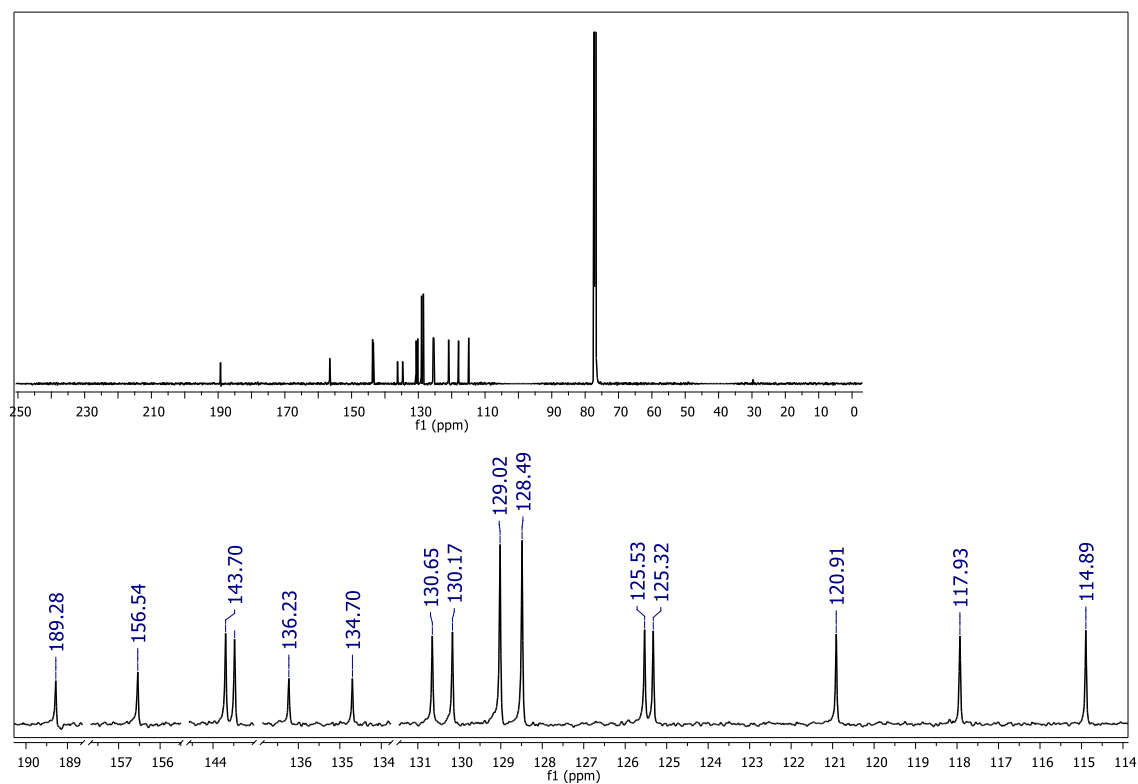


Figure S23. ^{13}C NMR spectrum of **MAC 8** (150 MHz; CDCl_3).



APÊNDICE B

Material Suplementar do Capítulo III

Antimycobacterial Activity of Simplified Monocarbonyl Curcuminoids

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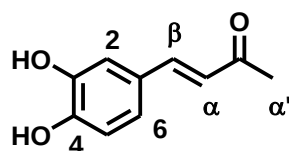
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1. SPECTROSCOPY DATA ANALYSES

1.1 (*E*)-4-(3,4-dihydroxyphenyl)but-3-en-2-one (**SMAC 1**) (YOON & PAIK, 2010; GUNJIMA et al., 2014; PATIVADA et al., 2019).



Yellow solid

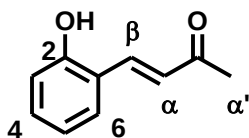
Yield: 29%

Melting point: 175–176 °C

¹H NMR (600 MHz; DMSO-*d*₆), δ_H in ppm (multiplicity; *J* in Hz): 2.28 (s; H-α'), 6.49 (d; 16.2; H-α), 6.78 (d; 7.8; H-5), 7.01 (dd; 2.4 and 7.8; H-6), 7.07 (d; 2.4; H-2), 7.46 (d; 16.2; H-β) (Figure S1).

¹³C NMR (150 MHz; DMSO-*d*₆), δ_C in ppm: 27.9 (C-α'), 115.2 (C-2), 116.3 (C-5), 122.0 (C-6), 124.4 (C-α), 126.2 (C-1), 144.5 (C-β), 146.1 (C-4), 148.9 (C-3), 198.3 (C=O) (Figure S2).

1.2 (*E*)-4-(2-hydroxyphenyl)but-3-en-2-one (**SMAC 3**) (WEBER et al., 2005; SIDORYK et al., 2018).



White solid

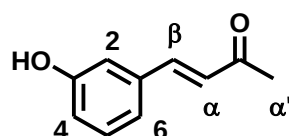
Yield: 73%

Melting point: 110–111 °C

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 2.43 (s; H-α'), 6.91–6.95 (H-3 and H-5), 7.03 (d; 16.8; H-α), 7.26 (ddd; 1.8, 7.6 and 8.4; H-4), 7.48 (dd; 1.8 and 7.5; H-6), 7.86 (d; 16.8; H-β) (Figure S3).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 27.0 (C-α'), 116.5 (C-3), 120.9 (C-5), 121.6 (C-1), 127.9 (C-α), 129.4 (C-6), 131.8 (C-4), 139.9 (C-β), 155.5 (C-2), 200.4 (C=O) (Figure S4).

1.3 (*E*)-4-(3-hydroxyphenyl)but-3-en-2-one (**SMAC 4**) (JAFARI & GHADAMI, 2016; SIDORYK et al., 2018).



White solid

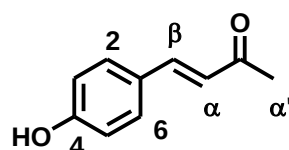
Yield: 67%

Melting point: 97–98 °C

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 2.41 (s; H-α'), 6.70 (d; 16.2; H-α), 6.95 (dd; 1.8 and 8.4; H-4), 7.09–7.10 (H-2 and H-6), 7.27 (dd; 7.8 and 8.4; H-5), 7.49 (d; 16.2; H-β) (Figure S5).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 27.5 (C-α'), 114.5 (C-2), 117.9 (C-4), 121.1 (C-6), 127.3 (C-α), 130.2 (C-5), 135.9 (C-1), 143.7 (C-β), 156.4 (C-3), 199.1 (C=O) (Figure S6).

1.4 (*E*)-4-(4-hydroxyphenyl)but-3-en-2-one (**SMAC 5**) (MOTOHASHI et al., 2005; CHEN et al., 2017; VARELA et al., 2019).



Yellow solid

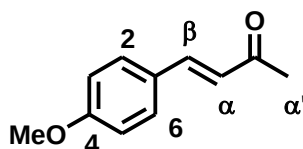
Yield: 83%

Melting point: 119–120 °C

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 2.39 (s; H-α'), 6.60 (d; 16.2; H-α), 6.91 (d; 9.0; H-3 and H-5), 7.43 (d; 9.0; H-2 and H-6), 7.50 (d; 16.2; H-β) (Figure S7).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 27.2 (C-α'), 116.2 (C-3 and C-5), 124.3 (C-α), 126.2 (C-1), 130.4 (C-2 and C-6), 144.7 (C-β), 159.3 (C-4), 199.8 (C=O) (Figure S8).

1.5 (*E*)-4-(4-methoxyphenyl)but-3-en-2-one (**SMAC 6**) (YAMAGAMI & MOTOHASHI, 2002; MCCONVILLE et al., 2009; JAFARI & GHADAMI, 2016).



White solid

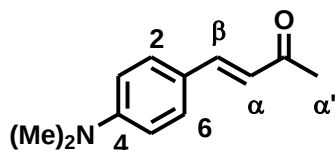
Yield: 86%

Melting point: 70–71 °C

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 2.38 (s; H-α'), 3.86 (s; 4-OMe), 6.63 (d; 16.2; H-α), 6.94 (d; 9.0; H-3 and H-5), 7.50 (d; 16.2; H-β), 7.52 (d; 9.0; H-2 and H-6) (Figure S9).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 27.4 (C-α'), 55.4 (4-OMe), 114.4 (C-3 and C-5), 125.0 (C-α), 127.0 (C-1), 130.0 (C-2 and C-6), 143.3 (C-β), 161.6 (C-4), 198.5 (C=O) (Figure S10).

1.6 (*E*)-4-(4-(dimethylamino)phenyl)but-3-en-2-one (**SMAC 7**) (MOTOHASHI et al., 2000; JAFARI & GHADAMI, 2016).



Orange solid

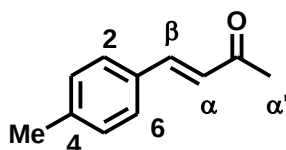
Yield: 89%

Melting point: 99–100 °C

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 2.34 (s; H-α'), 3.03 (s; 4-N(Me)₂), 6.54 (d; 15.6; H-α), 6.67 (d; 9.0; H-3 and H-5), 7.44 (d; 9.0; H-2 and H-6), 7.46 (d; 15.6; H-β) (Figure S11).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 27.2 (C-α'), 40.1 (4-N(Me)₂), 111.9 (C-3 and C-5), 122.0 (C-1), 122.4 (C-α), 130.1 (C-2 and C-6), 144.4 (C-β), 152.0 (C-4), 198.6 (C=O) (Figure S12).

1.7 (*E*)-4-(4-methylphenyl)but-3-en-2-one (**SMAC 8**) (MC CONVILLE et al., 2009; JAFARI & GHADAMI, 2016; GANDOMKAR et al., 2019).



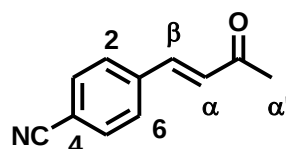
Yellow oil

Yield: 79%

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 2.35 (s; 4-Me), 2.36 (s; H-α'), 6.67 (d; 16.2; H-α), 7.19 (d; 8.4; H-3 and H-5), 7.43 (d; 8.4; H-2 and H-6), 7.48 (d; 16.2; H-β) (Figure S13).

^{13}C NMR (150 MHz; CDCl_3), δ_{C} in ppm: 21.5 (4-Me), 27.4 (C- α'), 126.2 (C- α), 128.3 (C-2 and C-6), 129.7 (C-3 and C-5), 131.6 (C-1), 141.0 (C-4), 143.6 (C- β), 198.6 (C=O) (Figure S14).

1.8 4-((*E*)-3-oxobut-1-enyl)benzonitrile (SMAC 9) (LEUNG, TENG & TOY, 2010; STERN et al., 2010; ZHANG et al., 2018).



White solid

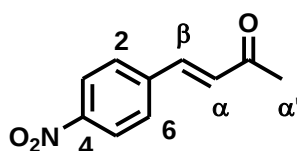
Yield: 55%

Melting point: 110–111 °C

^1H NMR (600 MHz; CDCl_3), δ_{H} in ppm (multiplicity; J in Hz): 2.42 (s; H- α'), 6.79 (d; 16.2; H- α), 7.50 (d; 16.2; H- β), 7.65 (d; 8.4; H-3 and H-5), 7.70 (d; 8.4; H-2 and H-6) (Figure S15).

^{13}C NMR (150 MHz; CDCl_3), δ_{C} in ppm: 28.0 (C- α'), 113.5 (C-4), 118.3 (4-CN), 128.6 (C-2 and C-6), 129.8 (C- α), 132.7 (C-3 and C-5), 138.8 (C-1), 140.6 (C- β), 197.7 (C=O) (Figure S16).

1.9 (*E*)-4-(4-nitrophenyl)but-3-en-2-one (SMAC 10) (LEUNG, TENG & TOY, 2010; STERN et al., 2010; JAFARI & GHADAMI, 2016).



White solid

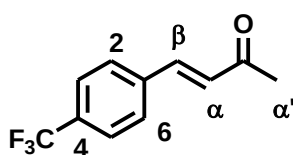
Yield: 59%

Melting point: 113–114 °C

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 2.45 (s; H-α'), 6.84 (d; 16.2; H-α), 7.56 (d; 16.2; H-β), 7.72 (d; 9.0; H-2 and H-6), 8.29 (d; 9.0; H-3 and H-5) (Figure S17).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 28.1 (C-α'), 124.2 (C-3 and C-5), 128.8 (C-2 and C-6), 130.4 (C-α), 134.1 (C-1), 140.1 (C-β), 142.0 (C-4), 197.5 (C=O) (Figure S18).

1.10 (*E*)-4-(4-(trifluoromethyl)phenyl)but-3-en-2-one (**SMAC 11**) (RAYAR, VEITÍA & FERROUD, 2015; TANEMURA, 2019; RIGOTTI, MAS-BALLESTÉ & ALEMÁN, 2020).



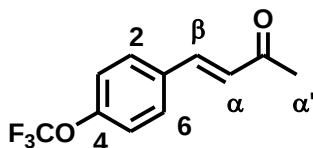
Colorless oil

Yield: 67%

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 2.41 (s; H-α'), 6.78 (d; 16.2; H-α), 7.52 (d; 16.2; H-β), 7.66 (H-2, H-3, H-5 and H-6) (Figure S19).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm (multiplicity; *J*_{C,F} in Hz): 27.8 (C-α'), 123.8 (d; 270; 4-CF₃), 125.9 (C-α), 128.4 (C-3 and C-5), 129.1 (C-2 and C-6), 131.9 (d; 31; C-4); 137.9 (C-1), 141.3 (C-β), 198.0 (C=O) (Figure S20).

1.11 (*E*)-4-(4-(trifluoromethoxy)phenyl)but-3-en-2-one (**SMAC 12**).



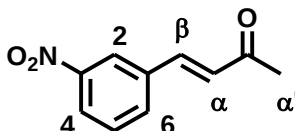
Colorless oil

Yield: 43%

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 2.39 (s; H-α'), 6.69 (d; 16.2; H-α), 7.25 (d; 8.4; H-3 and H-5), 7.49 (d; 16.2; H-β), 7.58 (d; 8.4; H-2 and H-6) (Figure S21).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm (multiplicity; *J*_{C,F} in Hz): 27.7 (C-α'), 118.6 (d; 256; 4-OCF₃), 121.2 (C-3 and C-5), 127.8 (C-α), 129.7 (C-2 and C-6), 133.0 (C-1), 141.5 (C-β), 150.5 (C-4), 198.1 (C=O) (Figure S22).

1.12 (*E*)-4-(3-nitrophenyl)but-3-en-2-one (**SMAC 13**) (JAFARI & GHADAMI, 2016).



White solid

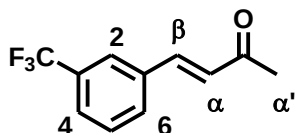
Yield: 39%

Melting point: 106–107 °C

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 2.44 (s; H-α'), 6.85 (d; 16.2; H-α), 7.56 (d; 16.2; H-β), 7.62 (dd; 7.8 and 8.1; H-5), 7.87 (d; 7.8; H-6), 8.25 (ddd; 1.8, 2.1 and 8.1; H-4), 8.41 (dd; 1.8 and 1.8; H-2) (Figure S23).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 28.1 (C-α'), 122.6 (C-4), 124.7 (C-2), 129.4 (C-α), 130.1 (C-5), 133.8 (C-6), 136.3 (C-1), 140.2 (C-β), 148.7 (C-3), 197.6 (C=O) (Figure S24).

1.13 (*E*)-4-(3-(trifluoromethyl)phenyl)but-3-en-2-one (**SMAC 14**) (MOTOHASHI et al., 2000; YAMAGAMI & MOTOHASHI, 2002).



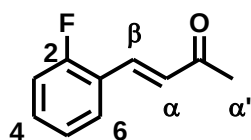
Colorless oil

Yield: 60%

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 2.41 (s; H-α'), 6.78 (d; 16.2; H-α), 7.53 (d; 16.2; H-β), 7.54 (dd; 7.8 and 7.8; H-5), 7.65 (d; 7.8; H-6), 7.72 (d; 7.8; H-4), 7.79 (H-2) (Figure S25).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm (multiplicity; *J*_{C,F} in Hz): 27.8 (C-α'), 123.7 (d; 271; 3-CF₃), 124.8 (d; 4.5; C-2), 126.8 (d; 3.0; C-4), 128.5 (C-α), 129.5 (C-6), 131.2 (C-5), 131.5 (d; 31.5; C-3), 135.3 (C-1), 141.4 (C-β), 197.9 (C=O) (Figure S26).

1.14 (*E*)-4-(2-fluorophenyl)but-3-en-2-one (SMAC 15) (PAN et al., 2018; SONG et al., 2019).



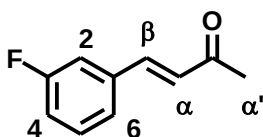
Colorless oil

Yield: 33%

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 2.40 (s; H-α'), 6.79 (d; 16.8; H-α), 7.11 (ddd; 1.2, 8.4 and 10.8; H-5), 7.18 (ddd; 1.2, 7.8 and 8.4; H-3), 7.38 (ddd; 1.8, 5.4 and 10.8; H-6), 7.57 (ddd; 1.8, 7.8 and 8.4; H-4), 7.68 (d; 16.8; H-β) (Figure S27).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm (multiplicity; *J*_{C,F} in Hz): 27.5 (C-α'), 116.2 (d; 16.5; C-3), 122.5 (d; 12.0; C-1), 124.6 (d; 3.0; C-5), 128.7 (d; 3.0; C-α), 129.2 (d; 6.0; C-6), 132.0 (d; 9.0; C-4), 135.7 (d; 4.5; C-β), 161.4 (d; 252; C-2), 198.5 (C=O) (Figure S28).

1.15 (*E*)-4-(3-fluorophenyl)but-3-en-2-one (SMAC 16) (HE et al., 2013; PAN et al., 2018).



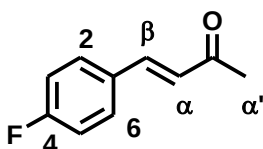
Colorless oil

Yield: 43%

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 2.39 (s; H-α'), 6.70 (d; 16.2; H-α), 7.10 (dddd; 1.8, 2.4, 8.0 and 8.2; H-4), 7.24 (ddd; 1.8, 2.4 and 9.0; H-2), 7.32 (d; 7.8; H-6), 7.37 (ddd; 5.4, 7.8 and 8.0; H-5), 7.47 (d; 16.2; H-β) (Figure S29).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm (multiplicity; *J*_{C,F} in Hz): 27.7 (C-α'), 114.4 (d; 22.5; C-2), 117.4 (d; 21.0; C-4), 124.3 (d; 3.0; C-6), 128.2 (C-α), 130.5 (d; 9.0; C-5), 136.7 (d; 7.5; C-1), 141.9 (d; 3.0; C-β), 163.0 (d; 246; C-3), 198.1 (C=O) (Figure S30).

1.16 (*E*)-4-(4-fluorophenyl)but-3-en-2-one (**SMAC 17**) (MC CONVILLE et al., 2009; LEUNG, TENG & TOY, 2010; STERN et al., 2010).



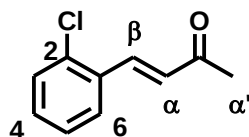
Yellow oil

Yield: 59%

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 2.38 (s; H-α'), 6.65 (d; 16.2; H-α), 7.09 (dd; 8.4 and 9.0; H-3 and H-5), 7.48 (d; 16.2; H-β), 7.54 (dd; 5.4 and 9.0; H-2 and H-6) (Figure S31).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm (multiplicity; *J*_{C,F} in Hz): 27.6 (C-α'), 116.1 (d; 22.5; C-3 and C-5), 126.8 (d; 3.0; C-α), 130.1 (d; 9.0; C-2 and C-6), 130.7 (d; 3.0; C-1), 142.1 (C-β), 164.0 (d; 250; C-4), 198.2 (C=O) (Figure S32).

1.17 (*E*)-4-(2-chlorophenyl)but-3-en-2-one (**SMAC 18**) (SONG et al., 2019).



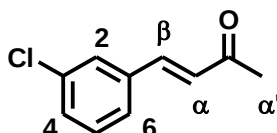
Colorless oil

Yield: 41%

^1H NMR (600 MHz; CDCl_3), δ_{H} in ppm (multiplicity; J in Hz): 2.43 (s; H- α'), 6.67 (d; 16.8; H- α), 7.30 (ddd; 1.8, 7.5 and 8.1; H-4), 7.33 (ddd; 1.8, 7.5 and 7.8; H-5), 7.43 (dd; 1.8 and 8.1; H-3), 7.64 (dd; 1.8 and 7.8; H-6), 7.94 (d; 16.8; H- β) (Figure S33).

^{13}C NMR (150 MHz; CDCl_3), δ_{C} in ppm: 27.2 (C- α'), 127.2 (C- α), 127.6 (C-5), 129.7 (C-6), 130.2 (C-3), 131.3 (C-4), 132.7 (C-2), 135.1 (C-1), 139.3 (C- β), 198.4 (C=O) (Figure S34).

1.18 (*E*)-4-(3-chlorophenyl)but-3-en-2-one (**SMAC 19**) (RAYAR, VEITÍA & FERROUD, 2015; PAN et al., 2018; SONG et al., 2019).



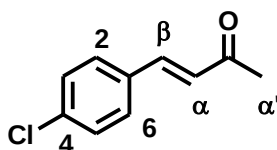
Colorless oil

Yield: 47%

^1H NMR (600 MHz; CDCl_3), δ_{H} in ppm (multiplicity; J in Hz): 2.39 (s; H- α'), 6.71 (d; 16.2; H- α), 7.34 (dd; 7.5 and 7.8; H-5), 7.37 (ddd; 1.5; 1.8, and 7.5; H-4), 7.42 (ddd; 1.5, 2.1 and 7.8; H-6), 7.44 (d; 16.2; H- β), 7.53 (dd; 1.8 and 2.1; H-2) (Figure S35).

^{13}C NMR (150 MHz; CDCl_3), δ_{C} in ppm: 27.8 (C- α'), 126.4 (C-6), 128.0 (C-2), 128.2 (C- α), 130.2 (C-4), 130.4 (C-5), 135.0 (C-3), 136.3 (C-1), 141.6 (C- β), 198.0 (C=O) (Figure S36).

1.19 (*E*)-4-(4-chlorophenyl)but-3-en-2-one (**SMAC 20**) (MC CONVILLE et al., 2009; JAFARI & GHADAMI, 2016; GANDOMKAR et al., 2019).



White solid

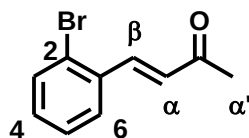
Yield: 78%

Melting point: 56–57 °C

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 2.40 (s; H-α'), 6.70 (d; 16.2; H-α), 7.39 (d; 9.0; H-3 and H-5), 7.45 (d; 16.2; H-β), 7.49 (d; 9.0; H-2 and H-6) (Figure S37).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 27.7 (C-α'), 127.5 (C-α), 129.3 (C-2 and C-6), 129.4 (C-3 and C-5), 132.9 (C-1), 136.4 (C-4), 141.9 (C-β), 198.2 (C=O) (Figure S38).

1.20 (*E*)-4-(2-bromophenyl)but-3-en-2-one (**SMAC 21**) (SONG et al., 2019).



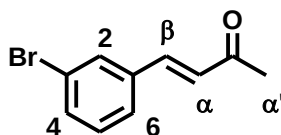
Colorless oil

Yield: 62%

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 2.43 (s; H-α'), 6.62 (d; 16.2; H-α), 7.25 (ddd; 1.2, 7.5 and 7.6; H-4), 7.34 (ddd; 1.2, 7.5 and 7.8; H-5), 7.62–7.63 (H-3 and H-6), 7.89 (d; 16.2; H-β) (Figure S39).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 27.2 (C-α'), 125.6 (C-2), 127.7 (C-α), 127.8 (C-5), 129.9 (C-6), 131.4 (C-4), 133.5 (C-3), 134.4 (C-1), 141.9 (C-β), 198.4 (C=O) (Figure S40).

1.21 (*E*)-4-(3-bromophenyl)but-3-en-2-one (**SMAC 22**) (ZHANG et al., 2011).



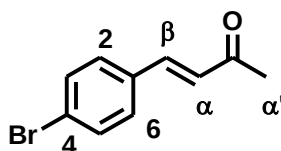
Colorless oil

Yield: 67%

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 2.38 (s; H-α'), 6.70 (d; 16.8; H-α), 7.27 (dd; 7.8 and 7.8; H-5), 7.43 (d; 16.8; H-β), 7.46 (d; 7.8; H-6), 7.52 (ddd; 1.2, 1.8 and 7.8; H-4), 7.69 (dd; 1.8 and 1.8; H-2) (Figure S41).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 27.8 (C-α'), 123.1 (C-3), 126.8 (C-6), 128.2 (C-α), 130.5 (C-2), 130.9 (C-5), 133.3 (C-4), 136.6 (C-1), 141.5 (C-β), 198.0 (C=O) (Figure S42).

1.22 (*E*)-4-(4-bromophenyl)but-3-en-2-one (**SMAC 23**) (STERN et al., 2010; ZHANG et al., 2018; RIGOTTI, MAS-BALLESTÉ & ALEMÁN, 2020).



Yellow solid

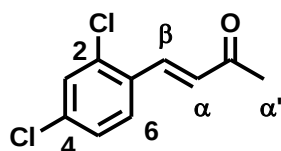
Yield: 78%

Melting point: 79–80 °C

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 2.38 (s; H-α'), 6.70 (d; 16.2; H-α), 7.41 (d; 9.0; H-2 and H-6), 7.44 (d; 16.2; H-β), 7.53 (d; 9.0; H-3 and H-5) (Figure S43).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 27.7 (C-α'), 124.8 (C-4), 127.5 (C-α), 129.6 (C-2 and C-6), 132.2 (C-3 and C-5), 133.3 (C-1), 142.0 (C-β), 198.2 (C=O) (Figure S44).

1.23 (*E*)-4-(2,4-dichlorophenyl)but-3-en-2-one (**SMAC 24**) (SONG et al., 2019).



White solid

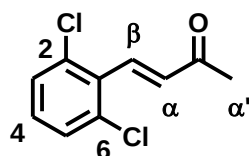
Yield: 43%

Melting point: 84–85 °C

^1H NMR (600 MHz; CDCl_3), δ_{H} in ppm (multiplicity; J in Hz): 2.42 (s; H- α'), 6.65 (d; 16.2; H- α), 7.29 (dd; 1.8 and 9.0; H-5), 7.46 (d; 1.8; H-3), 7.57 (d; 9.0; H-6), 7.85 (d; 16.2; H- β) (Figure S45).

^{13}C NMR (150 MHz; CDCl_3), δ_{C} in ppm: 27.4 (C- α'), 127.7 (C- α), 128.3 (C-5), 129.8 (C-6), 130.1 (C-3), 131.3 (C-1), 135.7 (C-2), 136.6 (C-4), 137.9 (C- β), 198.1 (C=O) (Figure S46).

1.24 (*E*)-4-(2,6-dichlorophenyl)but-3-en-2-one (**SMAC 25**) (LEUNG, TENG & TOY, 2010; SONG et al., 2019).



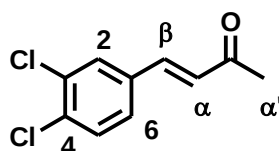
Yellow oil

Yield: 41%

^1H NMR (600 MHz; CDCl_3), δ_{H} in ppm (multiplicity; J in Hz): 2.43 (s; H- α), 6.81 (d; 16.8; H- α), 7.20 (dd; 7.8 and 8.1; H-4), 7.36 (d; 7.8; H-3 and H-5), 7.60 (d; 16.8; H- β) (Figure S47).

^{13}C NMR (150 MHz; CDCl_3), δ_{C} in ppm: 27.6 (C- α'), 128.8 (C-3 and C-5), 130.0 (C- α), 132.0 (C-1), 134.9 (C-4), 135.0 (C-2 and C-6), 136.9 (C- β), 198.2 (C=O) (Figure S48).

1.25 (*E*)-4-(3,4-dichlorophenyl)but-3-en-2-one (**SMAC 26**) (DIMMOCK et al., 1995; YAN et al., 2013).



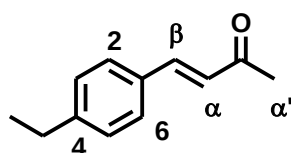
White oil

Yield: 63%

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 2.40 (s; H-α'), 6.70 (d; 16.2; H-α), 7.38 (dd; 1.8 and 8.4; H-6), 7.41 (d; 16.2; H-β), 7.49 (d; 8.4; H-5), 7.64 (d; 1.8; H-2) (Figure S49).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 27.9 (C-α'), 127.2 (C-α), 128.4 (C-6), 129.8 (C-2), 131.0 (C-5), 133.3 (C-1), 134.4 (C-4), 134.5 (C-3), 140.5 (C-β), 197.8 (C=O) (Figure S50).

1.26 (*E*)-4-(4-ethylphenyl)but-3-en-2-one (**SMAC 27**) (POLADURA et al., 2013; CHENG et al., 2016).



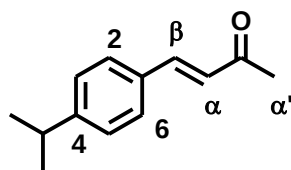
Colorless oil

Yield: 43%

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 1.25 (t; 7.8; 4-CH₂CH₃), 2.37 (s; H-α'), 2.67 (q; 7.8; 4-CH₂CH₃), 6.69 (d; 16.2; H-α), 7.23 (d; 7.8; H-3 and H-5), 7.47 (d; 7.8; H-2 and H-6), 7.50 (d; 16.2; H-β) (Figure S51).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 15.3 (4-CH₂CH₃), 27.4 (C-α'), 28.8 (4-CH₂CH₃), 126.3 (C-α), 128.4 (C-2 and C-6), 128.5 (C-3 and C-5), 131.9 (C-1), 143.6 (C-β), 147.4 (C-4), 198.6 (C=O) (Figure S52).

1.27 (*E*)-4-(4-isopropylphenyl)but-3-en-2-one (**SMAC 28**) (CHENG et al., 2016; GŁADKOWSKI et al., 2016).



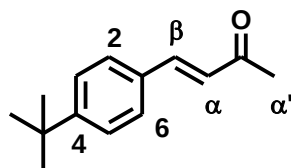
Colorless oil

Yield: 36%

^1H NMR (600 MHz; CDCl_3), δ_{H} in ppm (multiplicity; J in Hz): 1.26 (d; 6.6; 4-CH($\underline{\text{CH}}_3$) $_2$), 2.37 (s; H- α'), 2.93 (hep; 6.6; 4-CH($\underline{\text{CH}}_3$) $_2$), 6.69 (d; 16.2; H- α), 7.24 (d; 7.8; H-3 and H-5), 7.48 (d; 7.8; H-2 and H-6), 7.50 (d; 16.2; H- β) (Figure S53).

^{13}C NMR (150 MHz; CDCl_3), δ_{C} in ppm: 23.8 (4-CH($\underline{\text{CH}}_3$) $_2$), 27.4 (C- α'), 34.1 (4- $\underline{\text{CH}}(\text{CH}_3)_2$), 126.3 (C- α), 127.1 (C-3 and C-5), 128.4 (C-2 and C-6), 132.0 (C-1), 143.6 (C- β), 152.0 (C-4), 198.6 (C=O) (Figure S54).

1.28 (*E*)-4-(4-*tert*-butylphenyl)but-3-en-2-one (**SMAC 29**) (RAYAR, VEITÍA & FERROUD, 2015; CHENG et al., 2016).



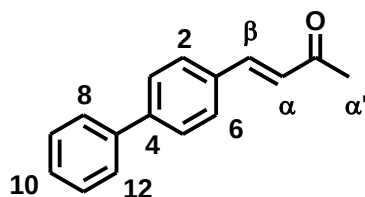
Colorless oil

Yield: 33%

^1H NMR (600 MHz; CDCl_3), δ_{H} in ppm (multiplicity; J in Hz): 1.33 (s; 4-C($\underline{\text{CH}}_3$) $_3$), 2.38 (s; H- α'), 6.69 (d; 16.2; H- α), 7.42 (d; 8.4; H-2 and H-6), 7.49 (d; 8.4; H-3 and H-5), 7.51 (d; 16.2; H- β) (Figure S55).

^{13}C NMR (150 MHz; CDCl_3), δ_{C} in ppm: 27.4 (C- α'), 31.5 (4-C($\underline{\text{CH}}_3$) $_3$), 34.9 (4- $\underline{\text{C}}(\text{CH}_3)_3$), 126.0 (C-3 and C-5), 126.5 (C- α), 128.1 (C-2 and C-6), 131.6 (C-1), 143.5 (C- β), 154.2 (C-4), 198.6 (C=O) (Figure S56).

1.29 (*E*)-4-[(1,1'-biphenyl)-4-yl]but-3-en-2-one (**SMAC 30**) (MUSSO et al., 2015).



White solid

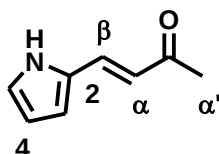
Yield: 85%

Melting point: 130–131 °C

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 2.40 (s; H-α'), 6.76 (d; 16.2; H-α), 7.38 (dddd; 1.2, 1.8, 7.2 and 7.8; H-10'), 7.46 (dd, 7.5 and 7.8; H-9' and H-11'), 7.55 (d; 16.2; H-β), 7.61–7.64 (H-2, H-3, H-5, H-6, H-8 and H-12) (Figure S57).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 27.6 (C-α'), 127.0 (C-α), 127.1 (C-2 and C-6), 127.6 (C-3 and C-5), 127.9 (C-10), 128.8 (C-8 and C-12), 128.9 (C-9 and C-11), 133.4 (C-1), 140.1 (C-4), 143.0 (C-β), 143.3 (C-7), 198.4 (C=O) (Figure S58).

1.30 (*E*)-4-(1*H*-pyrrol-2-yl)but-3-en-2-one (**SMAC 31**) (CHIMNI & MAHAJAN, 2005; SINGH et al., 2016).



White solid

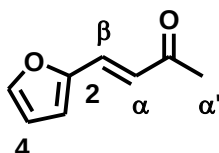
Yield: 58%

Melting point: 122–123 °C

¹H NMR (300 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 2.35 (s; H-α'), 6.30–6.33 (H-4), 6.45 (d; 16.2; H-α), 6.61–6.63 (H-3), 7.01–7.03 (H-5), 7.45 (d; 16.2; H-β), 9.55 (s; 1-NH) (Figure S59).

^{13}C NMR (75 MHz; CDCl_3), δ_{C} in ppm: 26.8 (C- α'), 111.2 (C-4), 115.9 (C-3), 120.7 (C-5), 123.7 (C- α), 128.4 (C-2), 134.0 (C- β), 198.8 (C=O) (Figure S60).

1.31 (*E*)-4-(furan-2-yl)but-3-en-2-one (**SMAC 32**) (CHIMNI & MAHAJAN, 2005; RIGOTTI, MAS-BALLESTÉ & ALEMÁN, 2020).



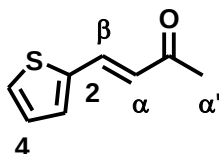
Red oil

Yield: 75%

^1H NMR (600 MHz; CDCl_3), δ_{H} in ppm (multiplicity; *J* in Hz): 2.33 (s; H- α'), 6.49 (dd; 1.8 and 3.6; H-4), 6.62 (d; 16.2; H- α), 6.67 (d; 3.6; H-3), 7.28 (d; 16.2; H- β), 7.51 (d; 1.8; H-5) (Figure S61).

^{13}C NMR (150 MHz; CDCl_3), δ_{C} in ppm: 27.9 (C- α'), 112.6 (C-3), 115.7 (C-4), 124.3 (C- β), 129.5 (C- α), 145.1 (C-5), 150.9 (C-2), 198.0 (C=O) (Figure S62).

1.32 (*E*)-4-(thiophen-2-yl)but-3-en-2-one (**SMAC 33**) (CHIMNI & MAHAJAN, 2005; WANG et al., 2018).



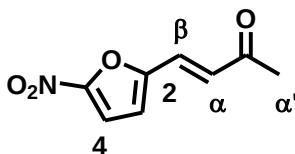
Red oil

Yield: 69%

^1H NMR (600 MHz; CDCl_3), δ_{H} in ppm (multiplicity; *J* in Hz): 2.32 (s; H- α'), 6.51 (d; 16.2; H- α), 7.05 (dd; 3.6 and 4.8; H-4), 7.27 (d; 3.6; H-5), 7.38 (d; 4.8; H-5), 7.62 (d; 16.2; H- β) (Figure S63).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 27.6 (C-α'), 125.8 (C-3), 128.3 (C-4), 129.0 (C-β), 131.6 (C-α), 135.8 (C-5), 139.7 (C-2), 197.8 (C=O) (Figure S64).

1.33 (*E*)-4-(5-nitrofuran-2-yl)but-3-en-2-one (**SMAC 34**) (SITKIN & KLIMENKO; 2005).



Yellow solid

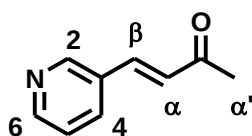
Yield: 36%

Melting point: 119–120 °C

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 2.40 (s; H-α'), 6.83 (d; 3.6; H-3), 6.94 (d; 16.2; H-α), 7.37 (d; 3.6; H-4), 7.28 (d; 16.2; H-β) (Figure S65).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 28.9 (C-α'), 113.1 (C-3), 116.0 (C-4), 126.7 (C-α), 129.4 (C-β), 152.6 (C-2) 152.7 (C-5), 196.7 (C=O) (Figure S66).

1.34 (*E*)-4-(pyridin-3-yl)but-3-en-2-one (**SMAC 35**) (REDDY et al., 2007; XIA & TOY, 2015).



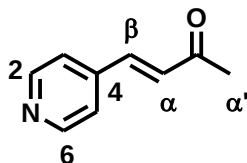
Yellow oil

Yield: 55%

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 2.43 (s; H-α'), 6.81 (d; 16.2; H-α), 7.37 (dd; 4.8 and 7.8; H-5), 7.53 (d; 16.2; H-β), 7.89 (ddd; 1.8, 1.8 and 7.8; H-4), 8.64 (dd; 1.8 and 4.8; H-6), 8.79 (d; 1.8; H-2) (Figure S67).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 29.7 (C-α'), 123.9 (C-5), 128.8 (C-α), 130.3 (C-3), 134.3 (C-4), 139.5 (C-β), 149.9 (C-6), 151.2 (C-2), 197.8 (C=O) (Figure S68).

1.35 (*E*)-4-(pyridin-4-yl)but-3-en-2-one (**SMAC 36**) (WANG et al., 2016; ZHANG et al., 2017).



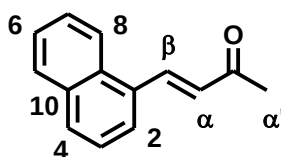
Yellow oil

Yield: 58%

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 2.40 (s; H-α'), 6.84 (d; 16.8; H-α), 7.41 (d; 16.8; H-β), 7.42 (d; 6.0; H-3 and H-5), 8.65 (d; 6.0; H-2 and H-6) (Figure S69).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 27.9 (C-α'), 122.2 (C-3 and C-5), 131.1 (C-α), 139.8 (C-β), 142.5 (C-1), 149.7 (C-2 and C-6), 197.7 (C=O) (Figure S70).

1.36 (*E*)-4-(naphthalen-4-yl)but-3-en-2-one (**SMAC 37**) (RAYAR, VEITÍA & FERROUD, 2015; RIGOTTI, MAS-BALLESTÉ & ALEMÁN, 2020).



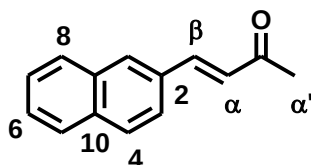
Yellow oil

Yield: 60%

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 2.47 (s; H-α'), 6.82 (d; 16.2; H-α), 7.48 (dd; 7.2 and 7.8; H-3), 7.53–7.60 (H-6 and H-7), 7.77 (d; 7.2; H-2), 7.88–7.91 (H-5 and H-8), 8.18 (d; 7.8; H-4), 8.37 (d; 16.2; H-β) (Figure S71).

^{13}C NMR (150 MHz; CDCl_3), δ_{C} in ppm: 27.9 (C- α'), 123.1 (C-2), 125.1 (C-8), 125.5 (C-3), 126.3 (C-6), 126.9 (C-7), 128.9 (C- α), 129.5 (C-4), 130.8 (C-5), 131.5 (C-10), 131.7 (C-9), 133.7 (C-1), 140.1 (C- β), 198.1 (C=O) (Figure S72).

1.37 (*E*)-4-(naphthalen-3-yl)but-3-en-2-one (**SMAC 38**) (NORDQVIST et al., 2011; LAO, ZHANG & TOY, 2019).



White solid

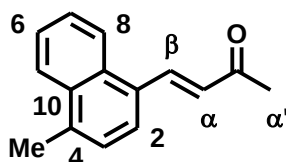
Yield: 72%

Melting point: 97–98 °C

^1H NMR (600 MHz; CDCl_3), δ_{H} in ppm (multiplicity; *J* in Hz): 2.44 (s; H- α'), 6.85 (d; 16.2; H- α), 7.52–7.55 (H-6 and H-7), 7.69 (dd; 1.5 and 9.0; H-3), 7.70 (d; 16.2; H- β), 7.84–7.88 (H-4, H-5 and H-8), 7.97 (s; H-1) (Figure S73).

^{13}C NMR (150 MHz; CDCl_3), δ_{C} in ppm: 27.6 (C- α'), 123.5 (C-3), 126.8 (C-1), 127.3 (C- α), 127.4 (C-6), 127.8 (C-7), 128.6 (C-5), 128.8 (C-8), 130.3 (C-4), 131.9 (C-9), 133.3 (C-10), 134.3 (C-2), 143.5 (C- β), 198.4 (C=O) (Figure S74).

1.38 (*E*)-4-(1-methylnaphthalen-4-yl)but-3-en-2-one (**SMAC 39**)



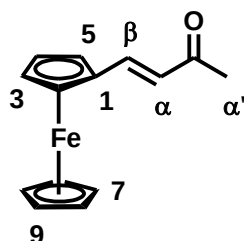
Red oil

Yield: 65%

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 2.44 (s; H-α'), 2.70 (s; 4-Me), 6.77 (d; 16.2; H-α), 7.32 (d; 7.8; H-3), 7.56–7.58 (H-6 and H-7), 7.66 (d; 7.8; H-2), 8.03 (dd; 2.4 and 6.0; H-8), 8.18 (dd; 2.4 and 6.9; H-5), 8.35 (d; 16.2; H-β) (Figure S75)..

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 19.9 (4-Me), 27.2 (C-α'), 123.7 (C-2), 124.9 (C-3), 125.0 (C-8), 126.1 (C-5), 126.5 (C-6), 126.6 (C-7), 128.8 (C-α), 130.0 (C-10), 131.6 (C-9), 132.8 (C-1), 137.7 (C-4), 140.4 (C-β), 198.3 (C=O) (Figure S76).

1.39 (*E*)-4-(ferrocen-1-yl)but-3-en-2-one (SMAC 40)



Red solid

Yield: 92%

¹H NMR (300 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 2.30 (s; H-α'), 4.16 (H-6 to H-10), 4.45–4.52 (H-2 to H-5), 6.35 (d; 15.9; H-α), 7.44 (d; 15.9; H-β) (Figure S77).

¹³C NMR (75 MHz; CDCl₃), δ_C in ppm: 27.2 (C-α'), 68.8 (C-3 and C-4), 69.7 (C-6 to C-10), 71.2 (C-2 and C-5), 78.5 (C-1), 124.7 (C-α), 145.2 (C-β), 197.7 (C=O) (Figure S78).

Figure S1. ^1H NMR spectrum of **SMAC 1** (600 MHz; $\text{DMSO-}d_6$).

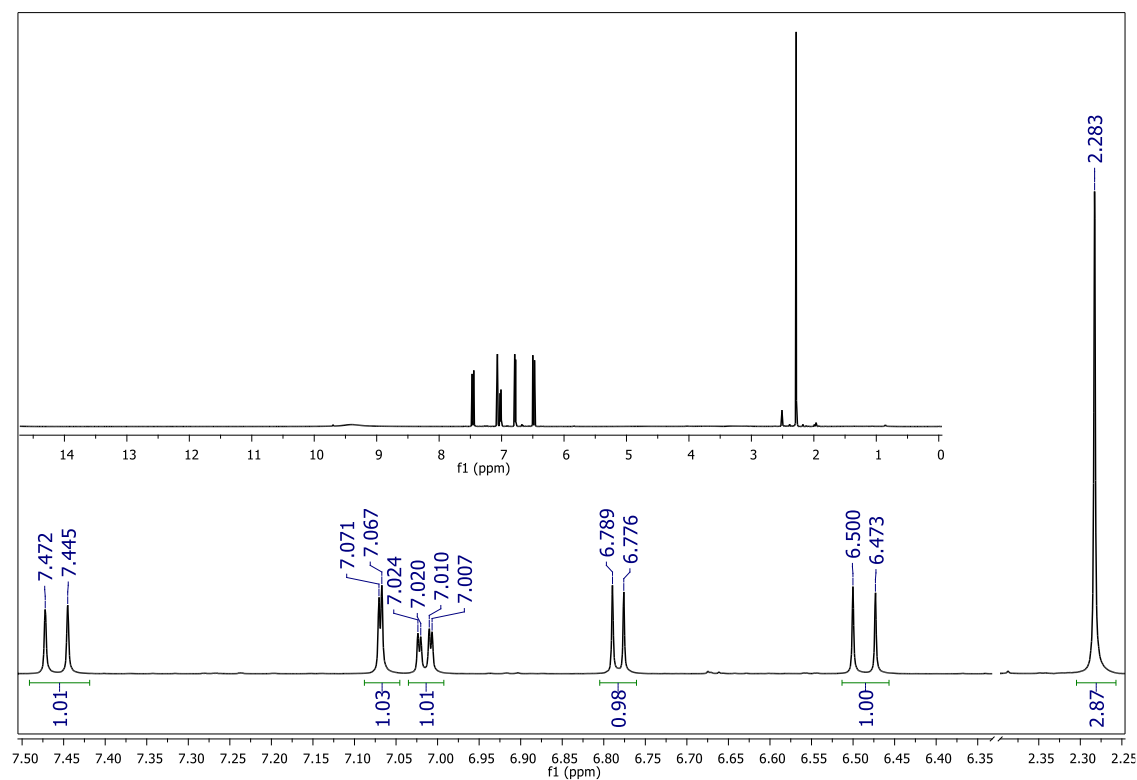


Figure S2. ^{13}C NMR spectrum of **SMAC 1** (150 MHz; $\text{DMSO-}d_6$).

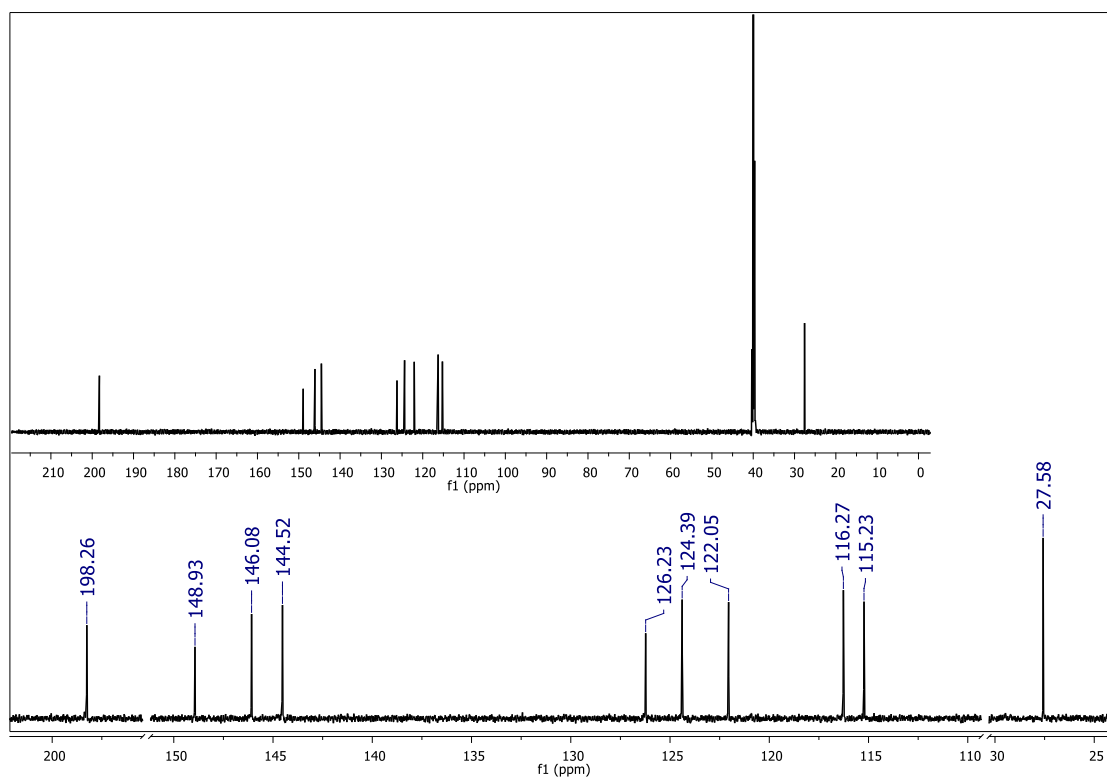


Figure S3. ^1H NMR spectrum of **SMAC 3** (600 MHz; CDCl_3).

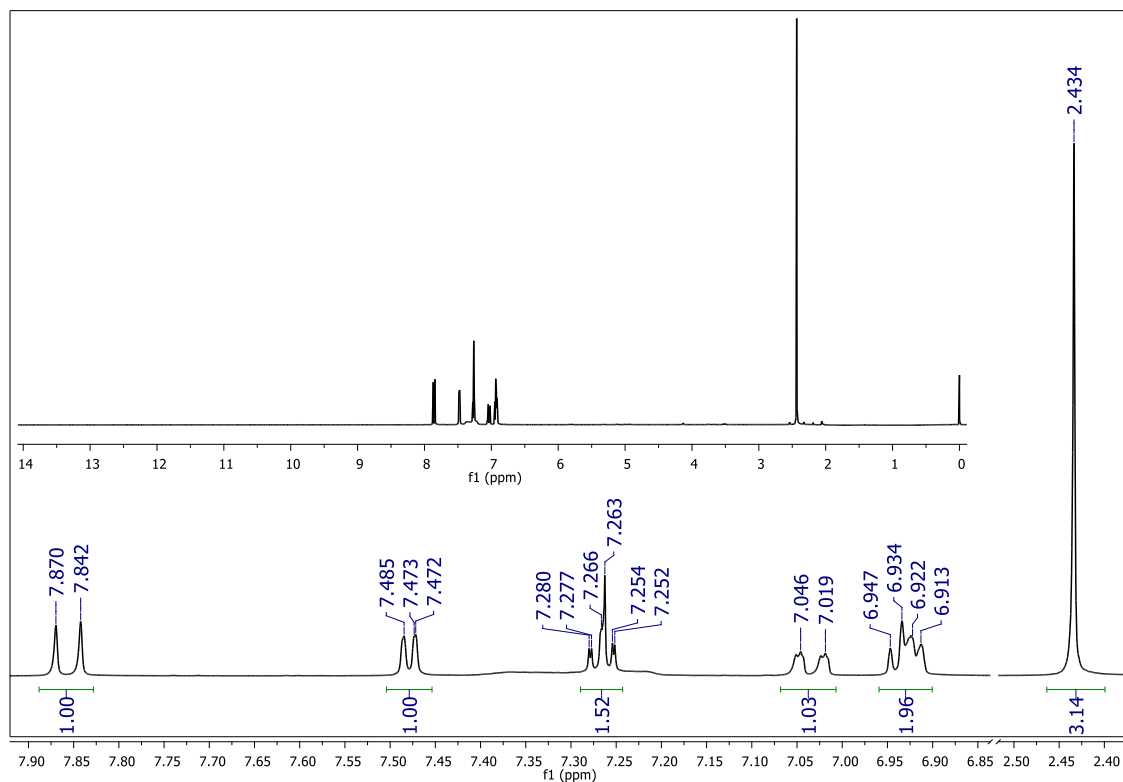


Figure S4. ^{13}C NMR spectrum of **SMAC 3** (150 MHz; CDCl_3).

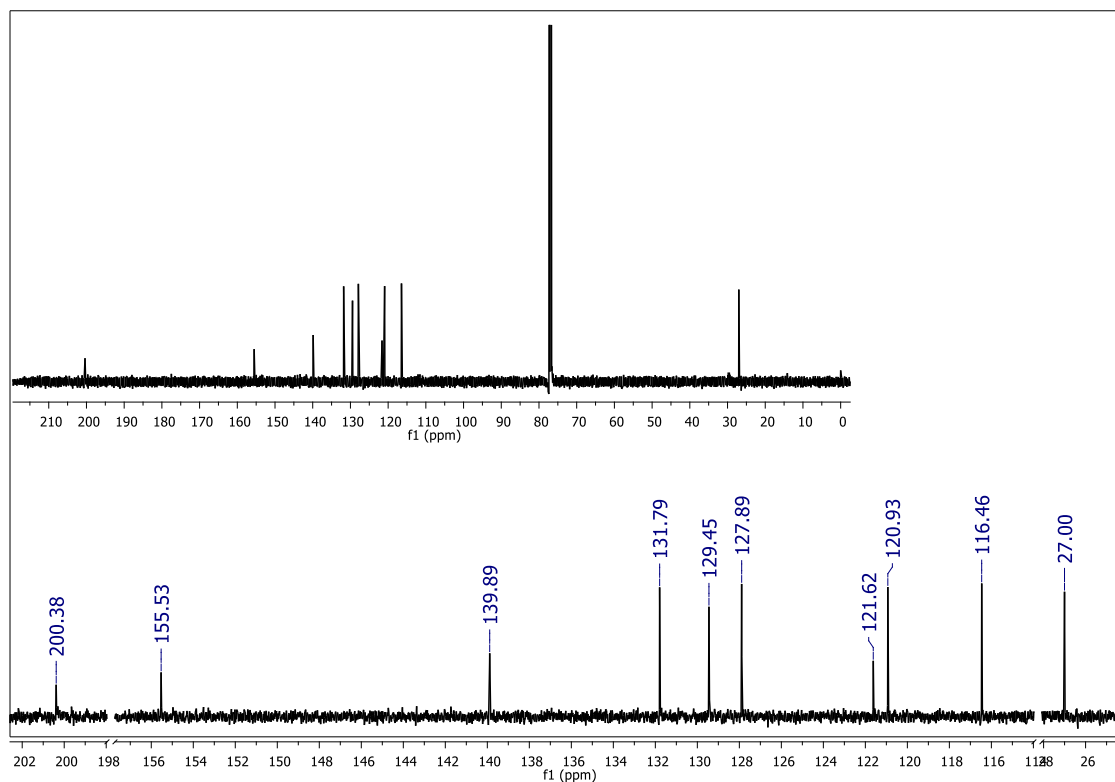


Figure S5. ^1H NMR spectrum of **SMAC 4** (600 MHz; CDCl_3).

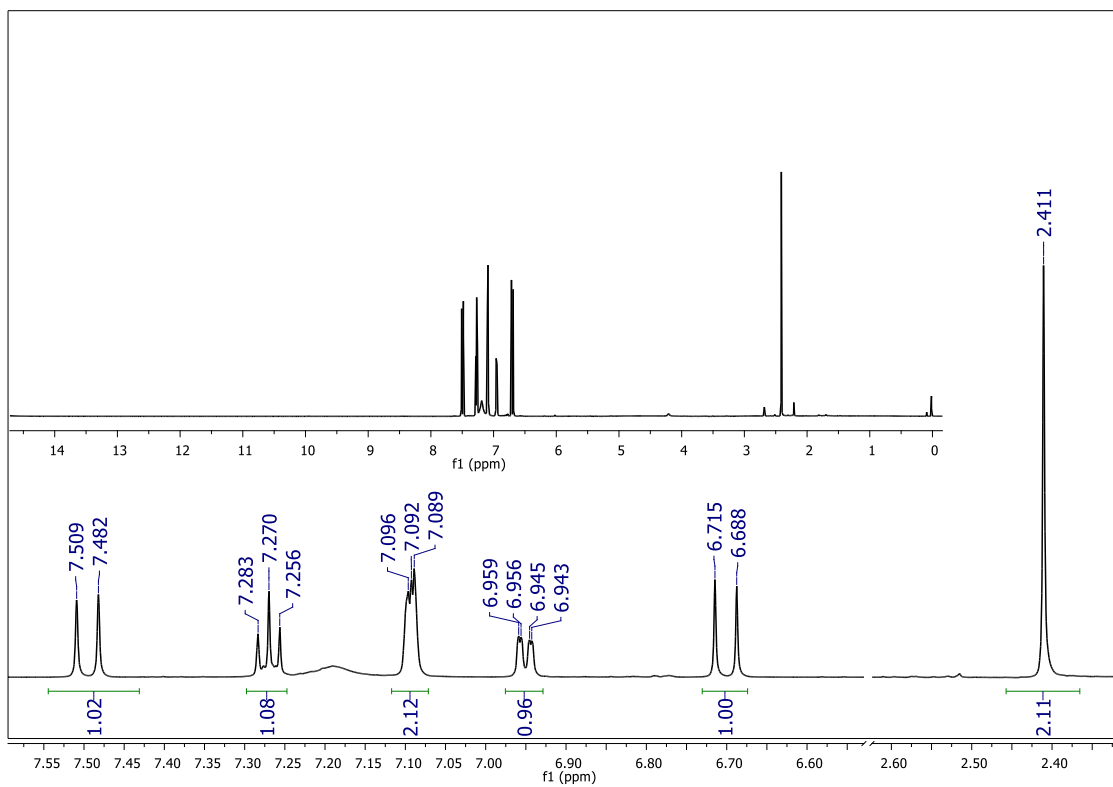


Figure S6. ^{13}C NMR spectrum of **SMAC 4** (150 MHz; CDCl_3).

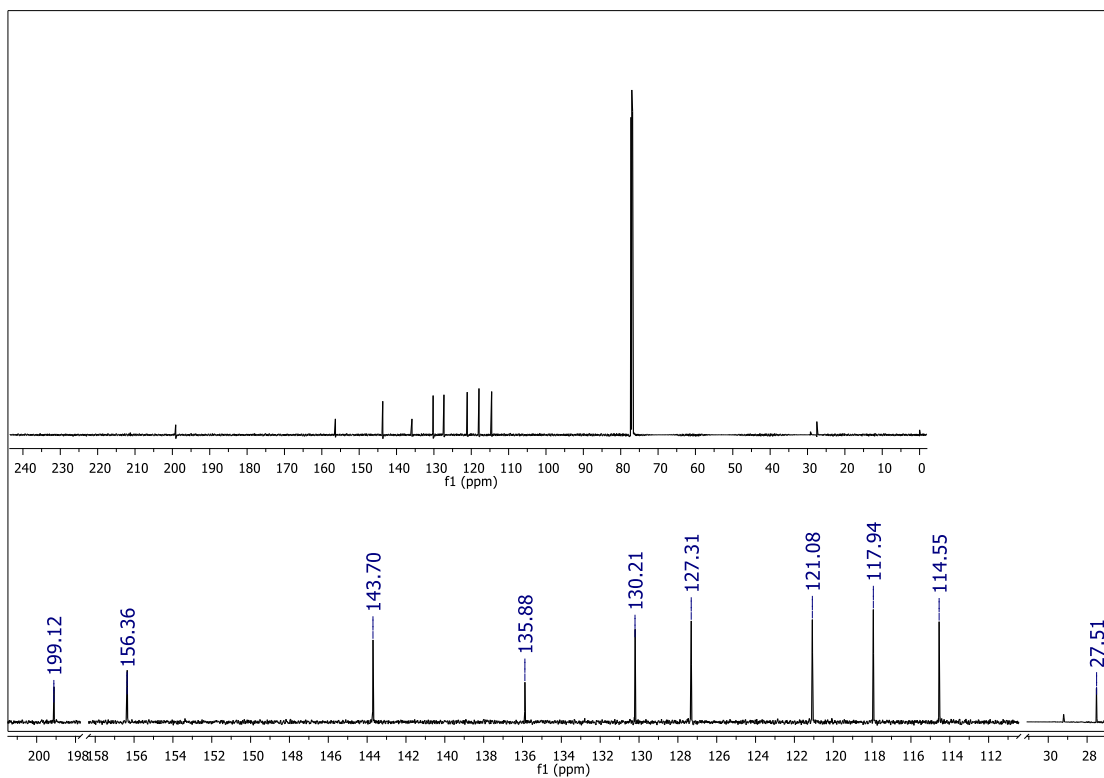


Figure S7. ^1H NMR spectrum of **SMAC 5** (600 MHz; CDCl_3).

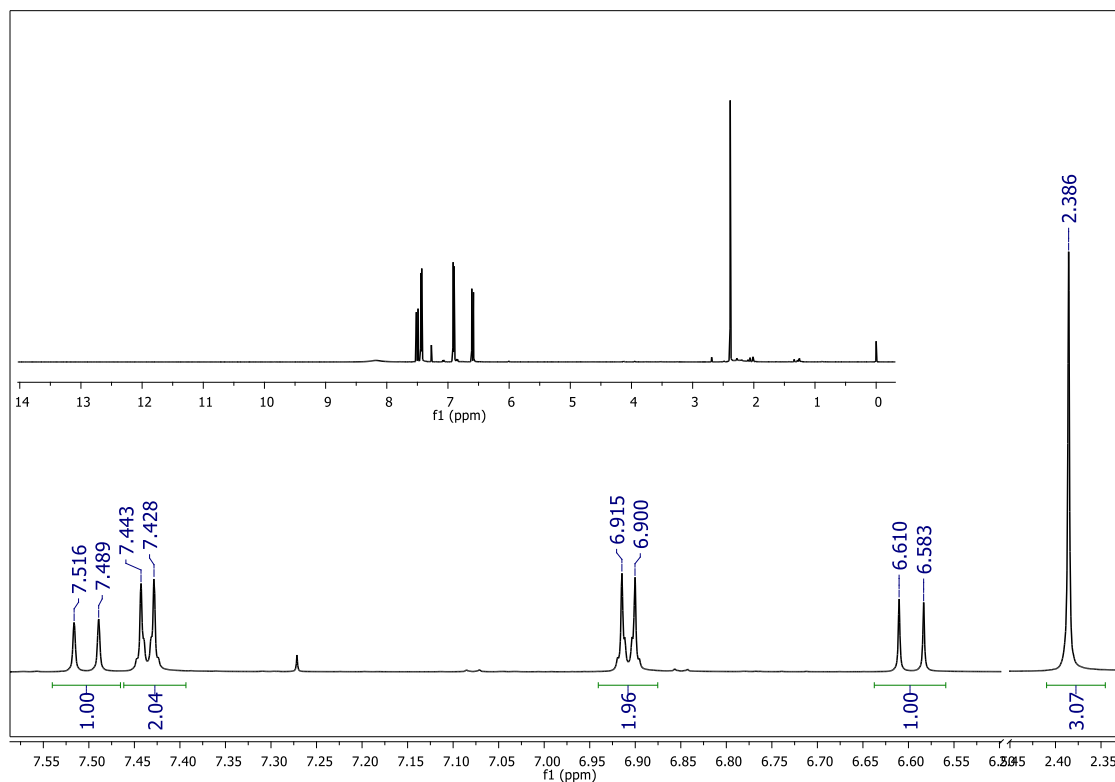


Figure S8. ^{13}C NMR spectrum of **SMAC 5** (150 MHz; CDCl_3).

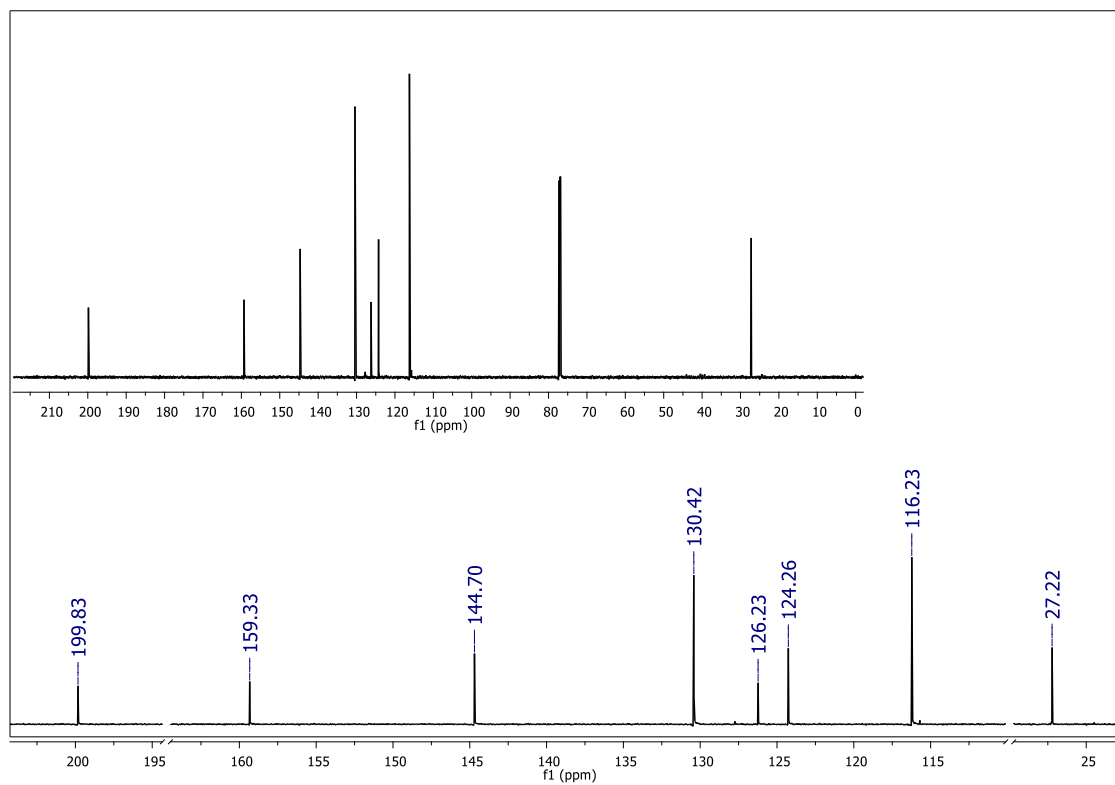


Figure S9. ^1H NMR spectrum of **SMAC 6** (600 MHz; CDCl_3).

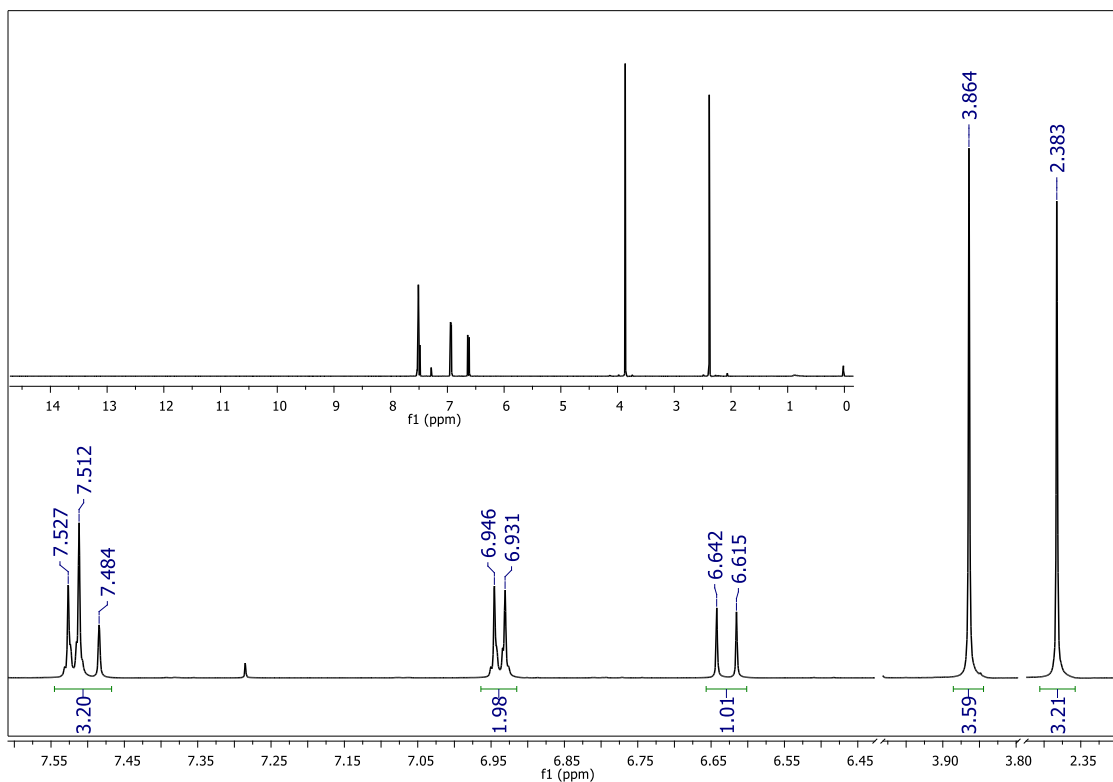


Figure S10. ^{13}C NMR spectrum of **SMAC 6** (150 MHz; CDCl_3).

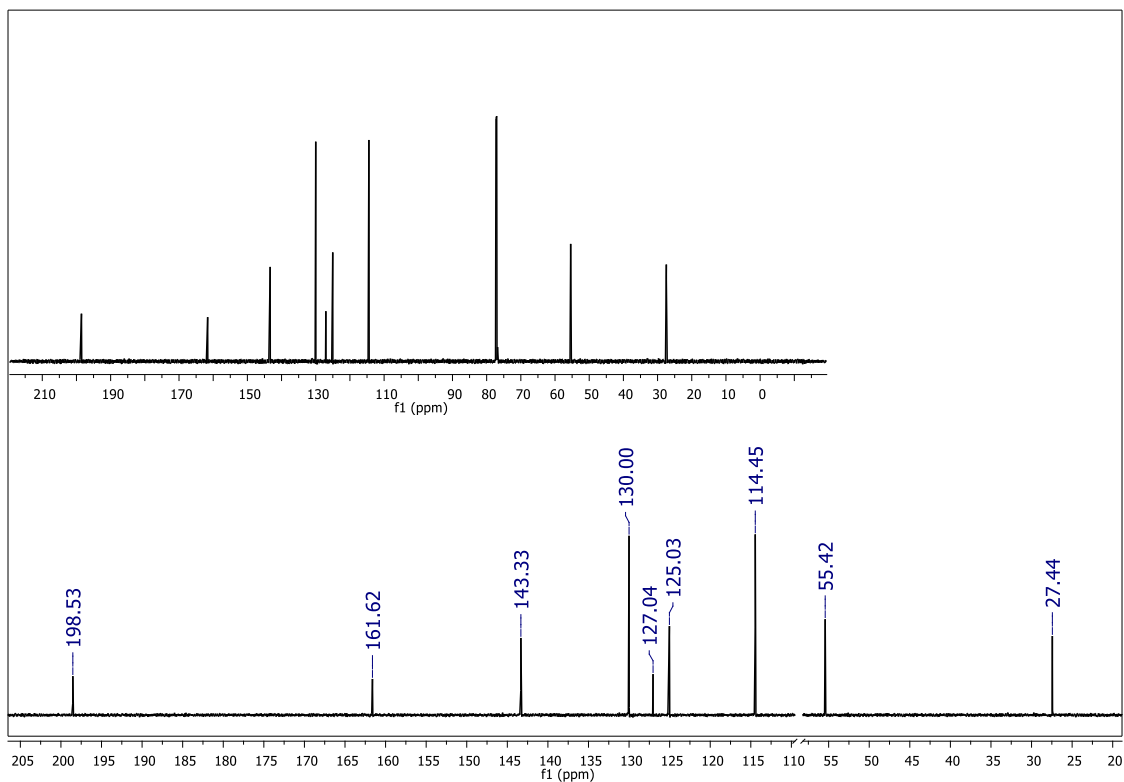


Figure S11. ^1H NMR spectrum of **SMAC 7** (600 MHz; CDCl_3).

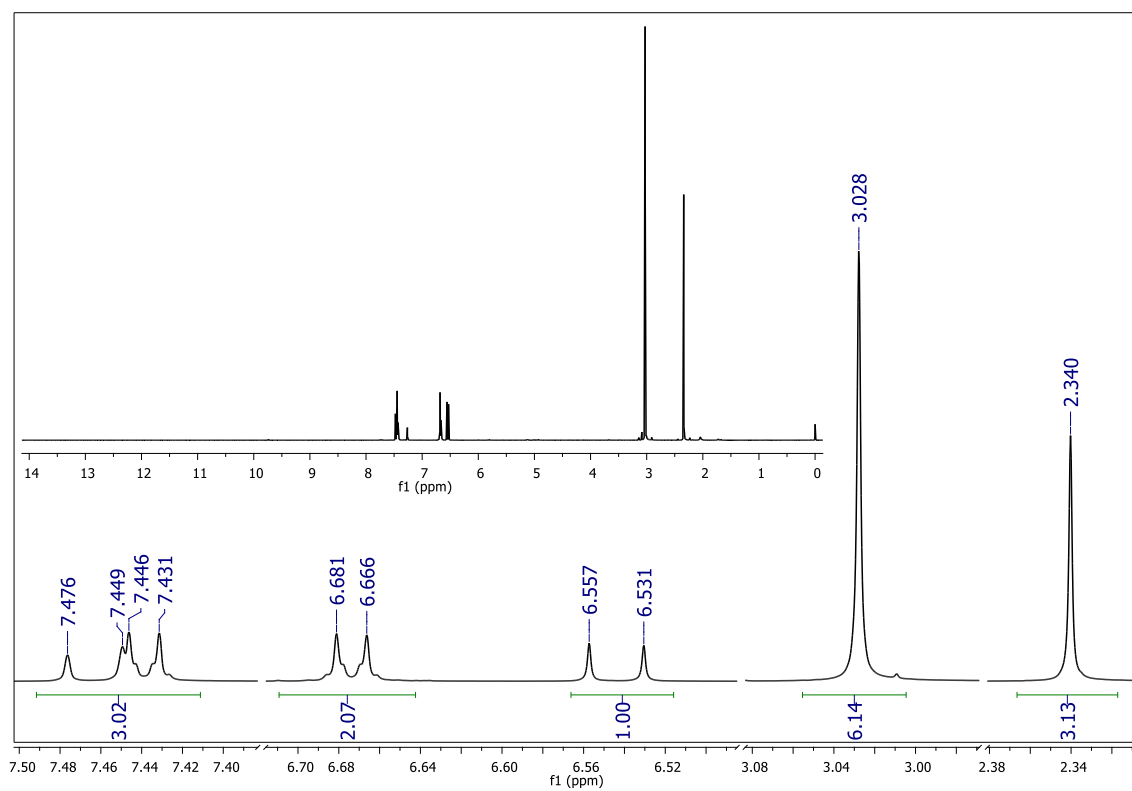


Figure S12. ^{13}C NMR spectrum of **SMAC 7** (600 MHz; CDCl_3).

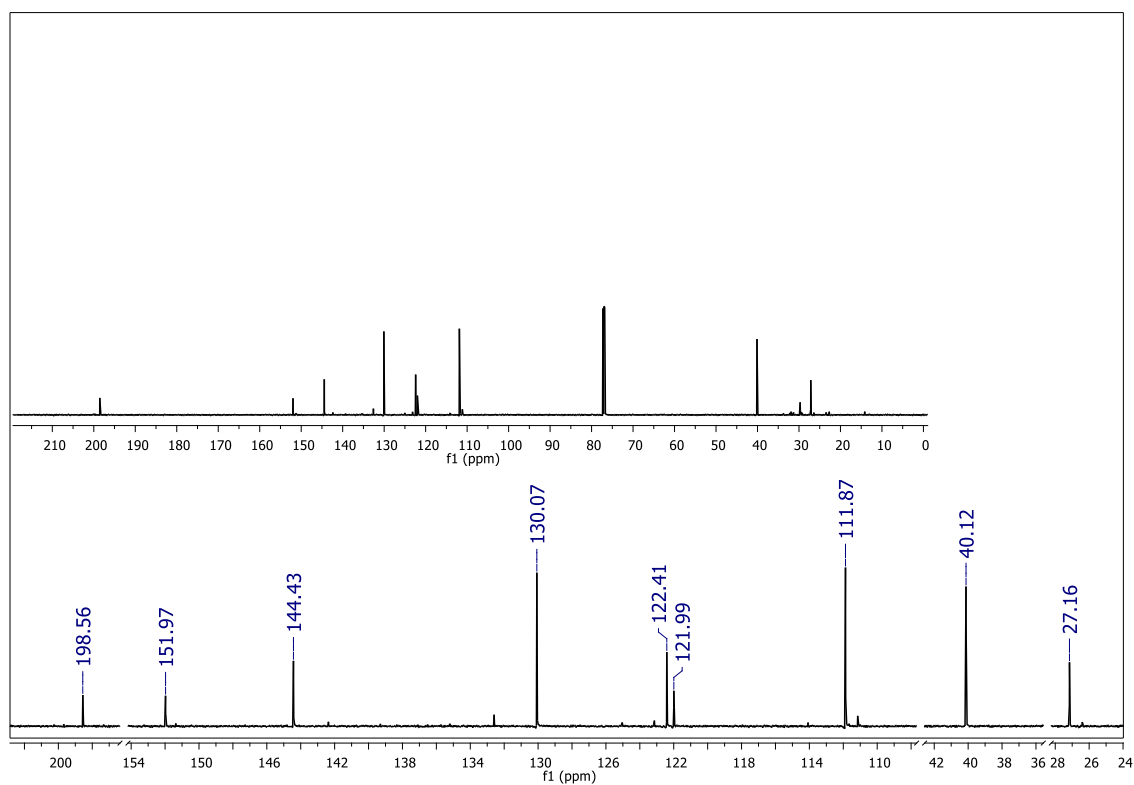


Figure S13. ^1H NMR spectrum of **SMAC 8** (600 MHz; CDCl_3).

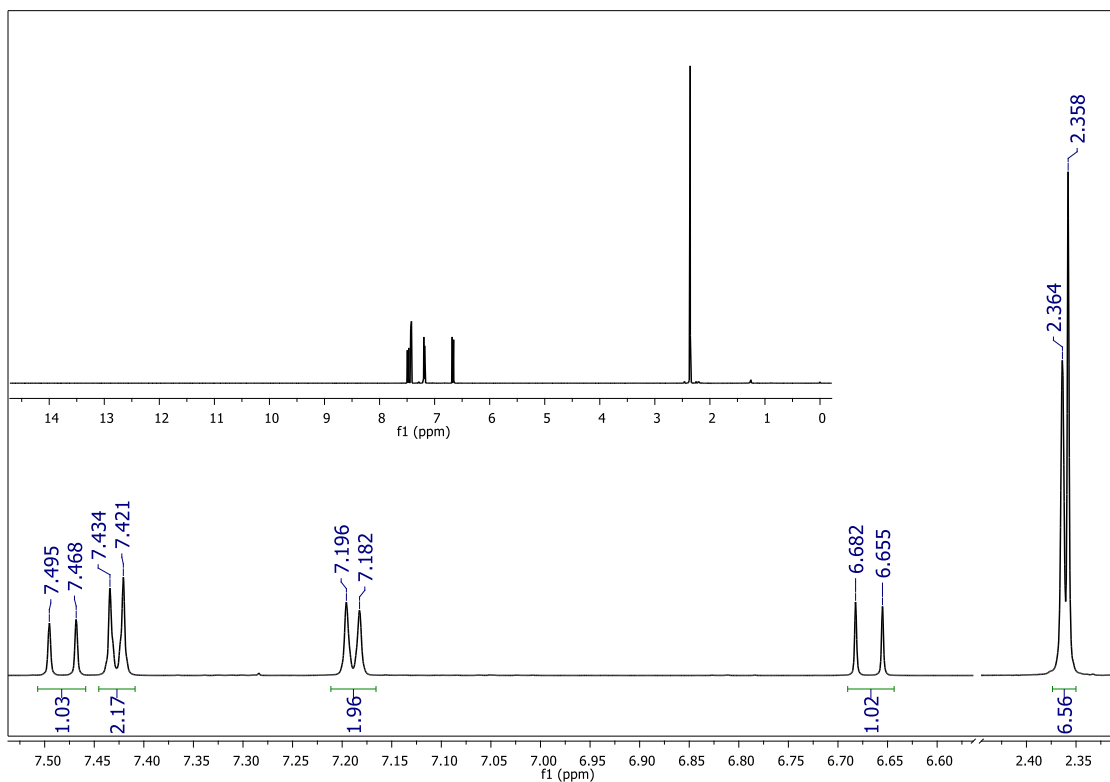


Figure S14. ^{13}C NMR spectrum of **SMAC 8** (150 MHz; CDCl_3).

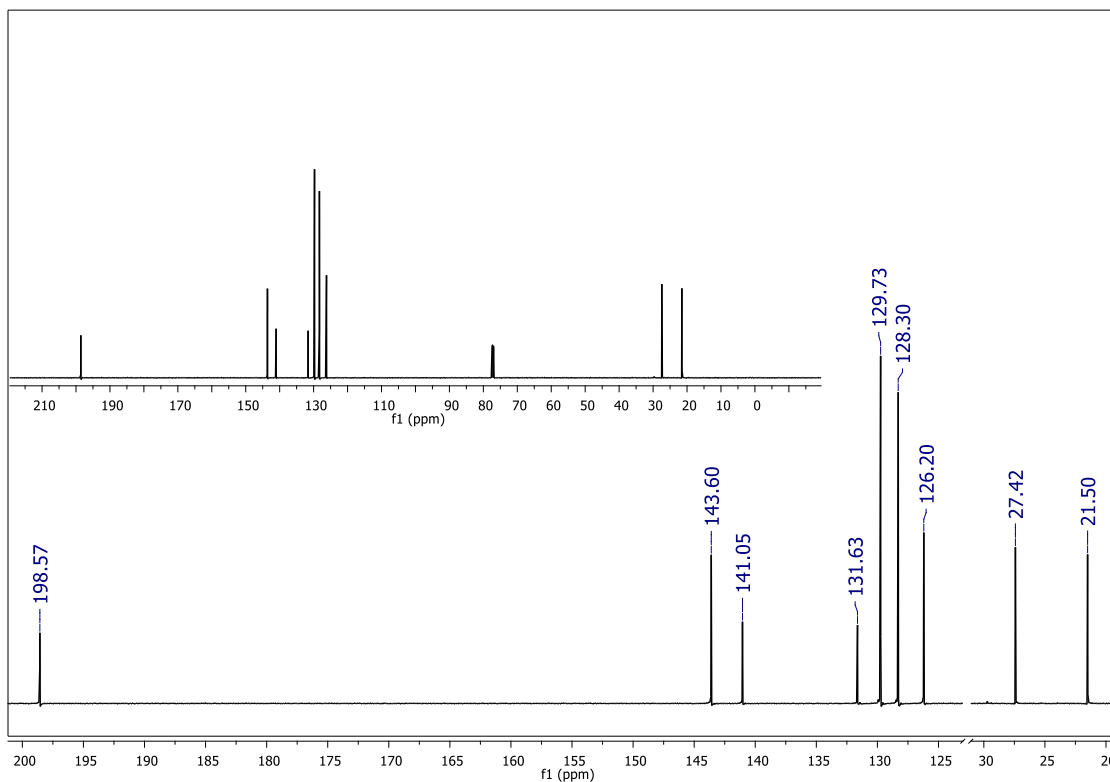


Figure S15. ^1H NMR spectrum of **SMAC 9** (600 MHz; CDCl_3).

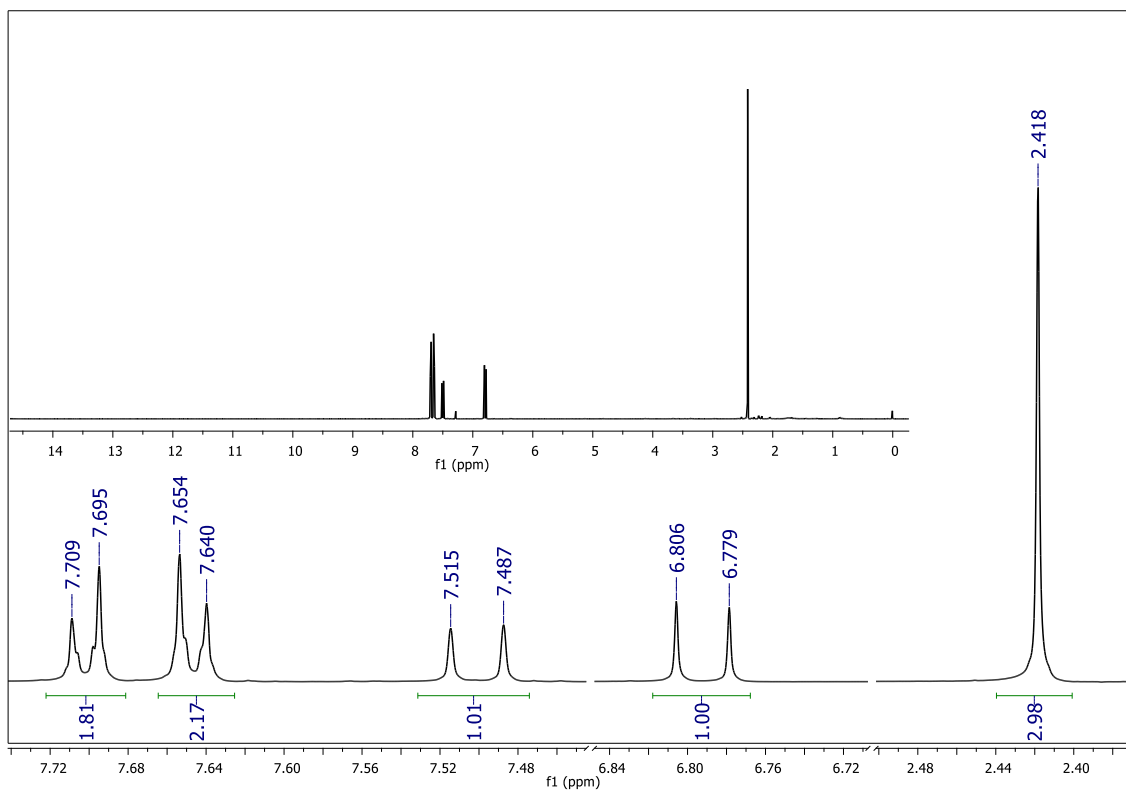


Figure S16. ^{13}C NMR spectrum of **SMAC 9** (150 MHz; CDCl_3).

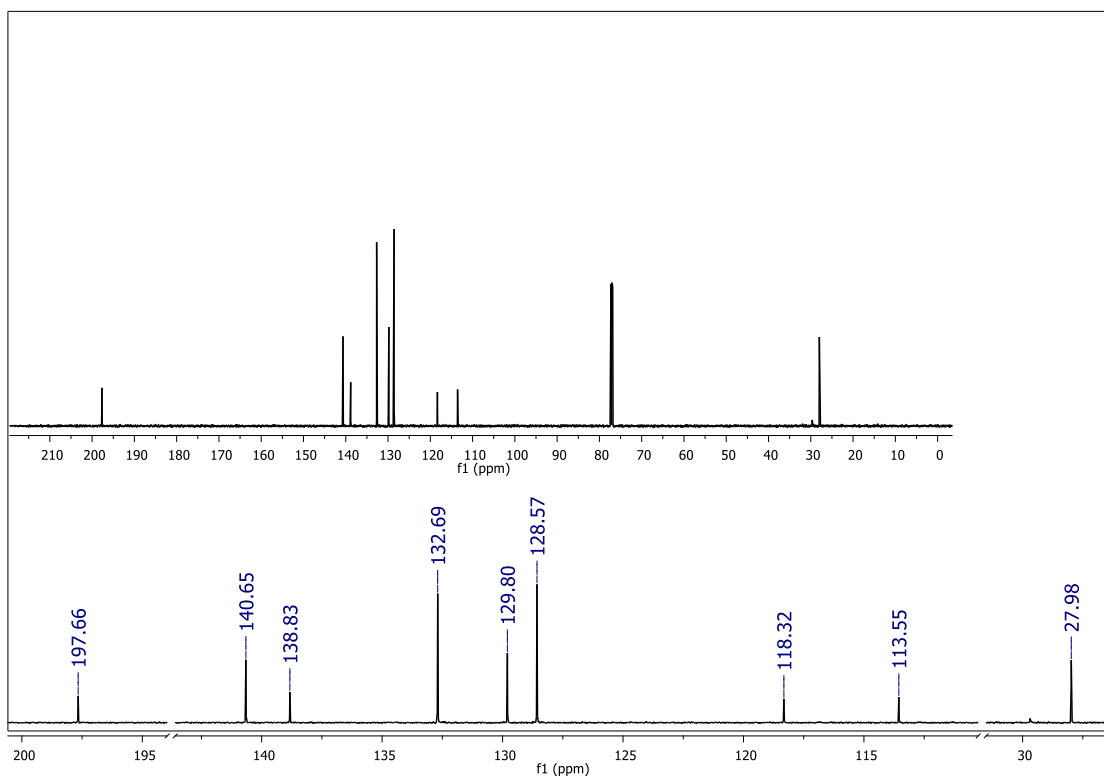


Figure S17. ^1H NMR spectrum of **SMAC 10** (600 MHz; CDCl_3).

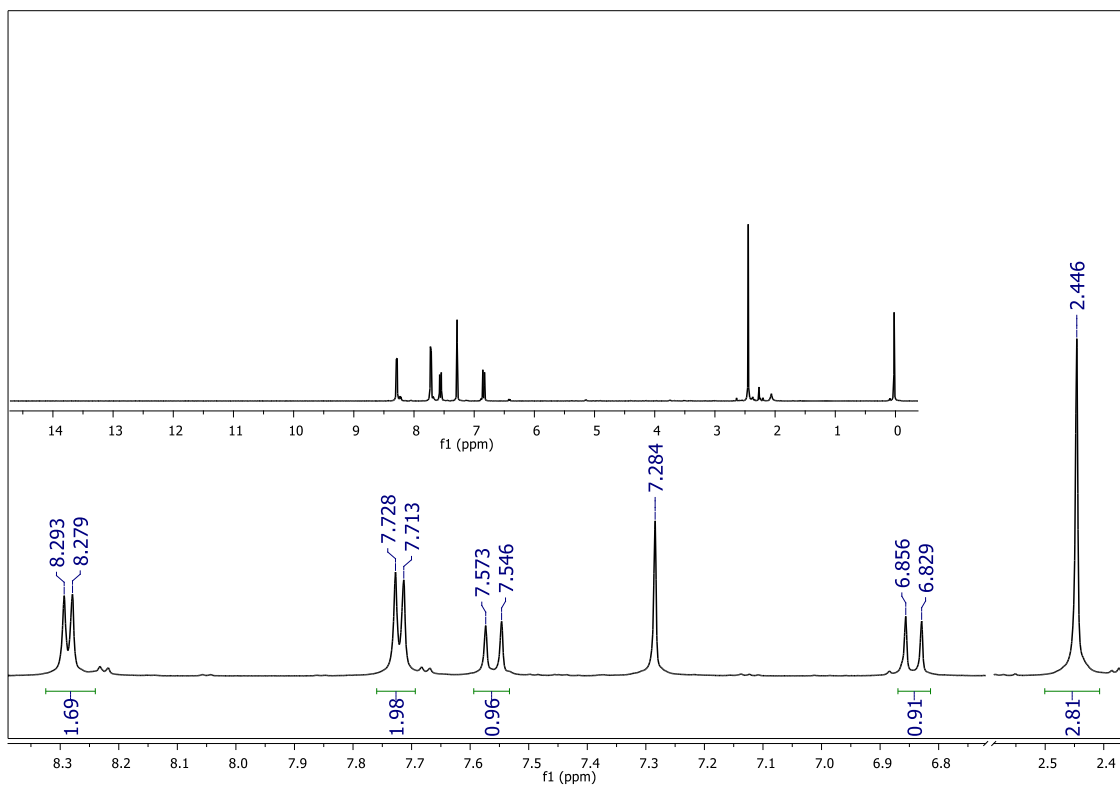


Figure S18. ^{13}C NMR spectrum of **SMAC 10** (150 MHz; CDCl_3).

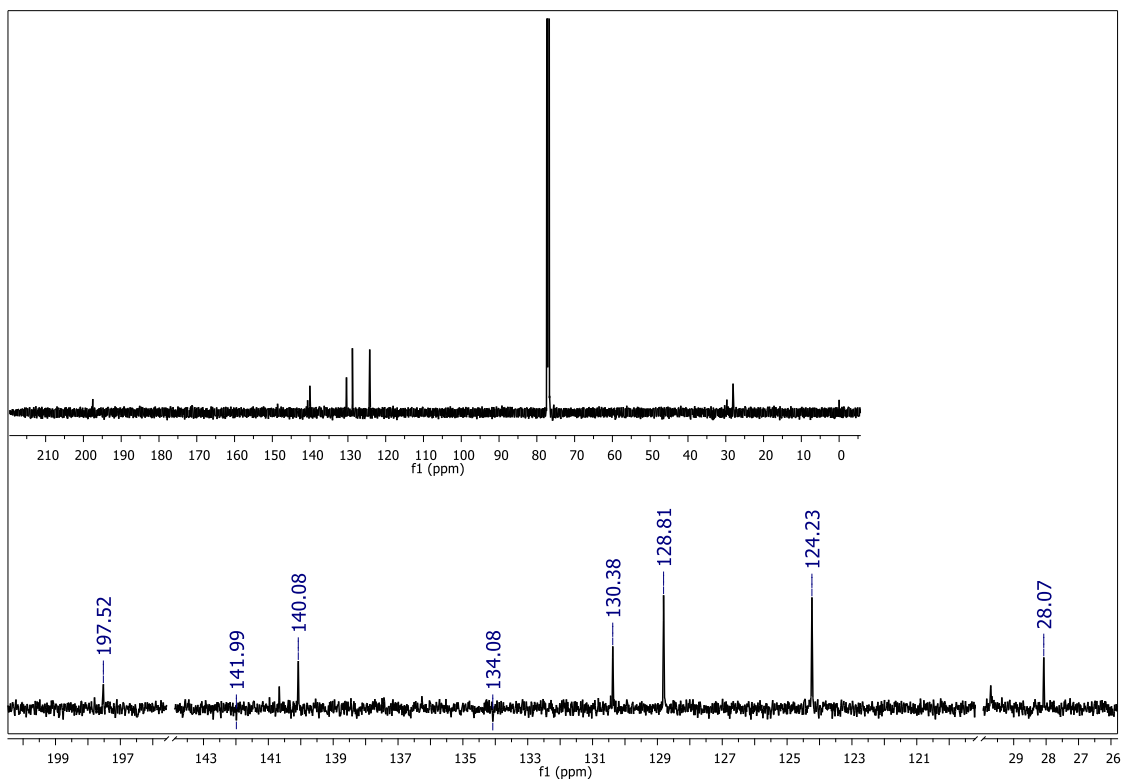


Figure S19. ^1H NMR spectrum of **SMAC 11** (600 MHz; CDCl_3).

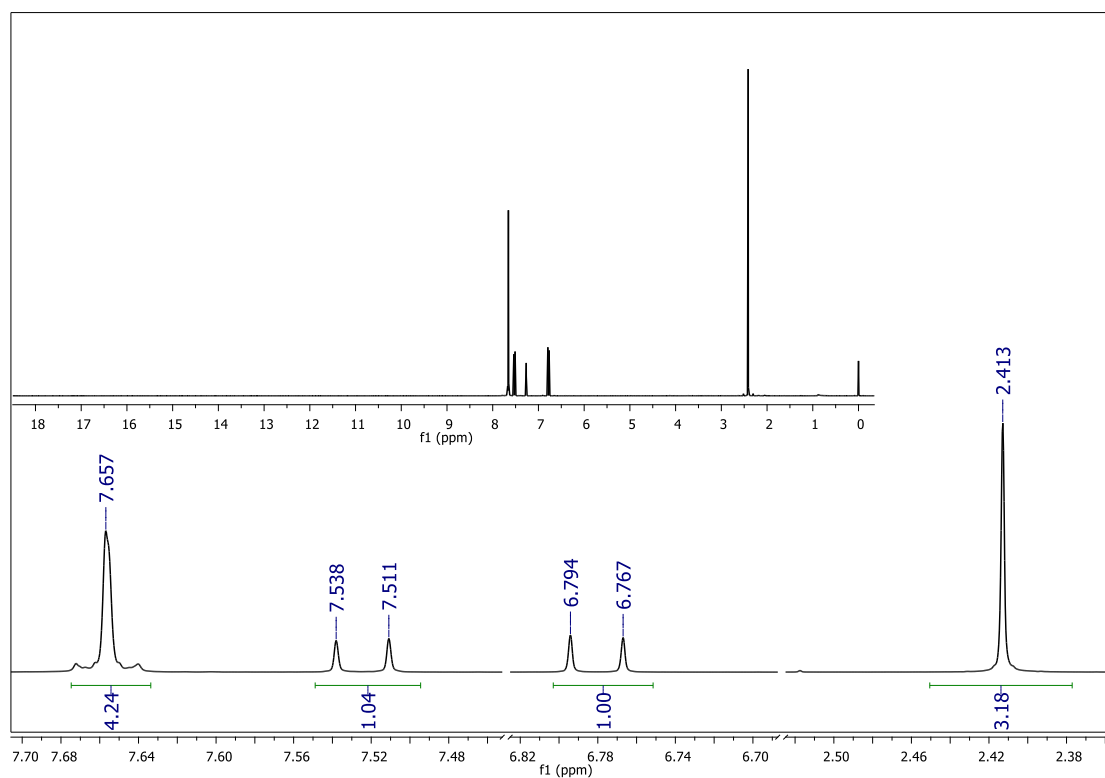


Figure S20. ^{13}C NMR spectrum of **SMAC 11** (150 MHz; CDCl_3).

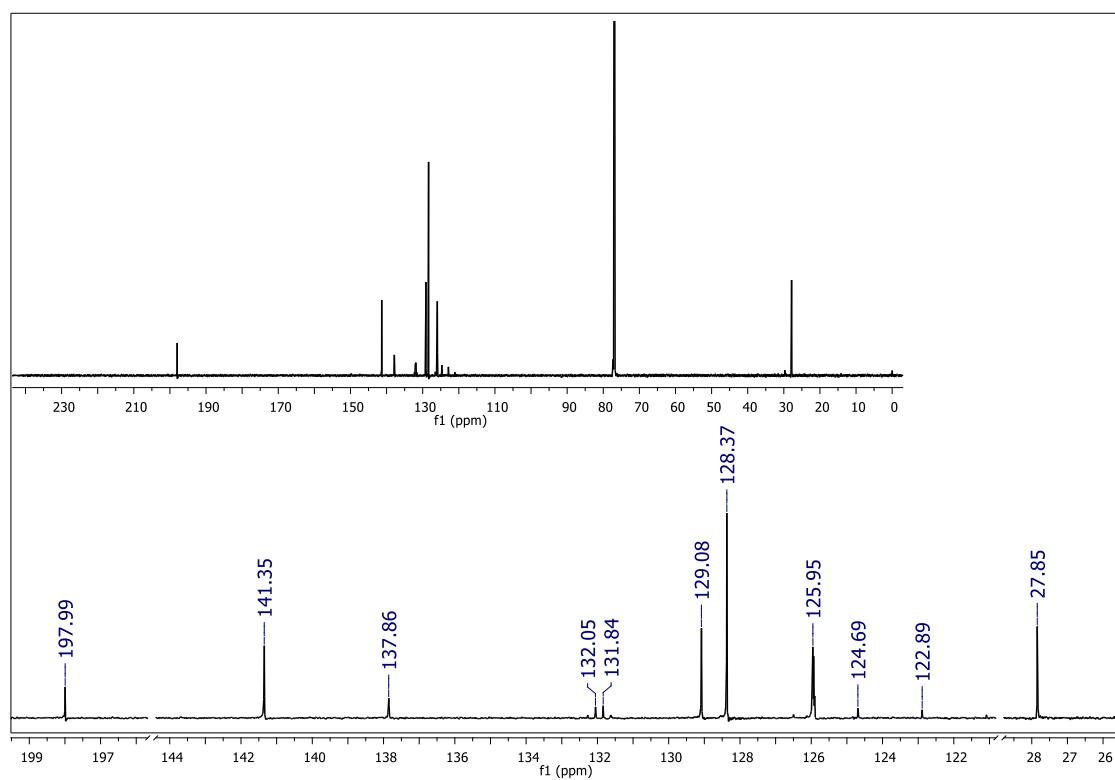


Figure S21. ^1H NMR spectrum of **SMAC 12** (600 MHz; CDCl_3).

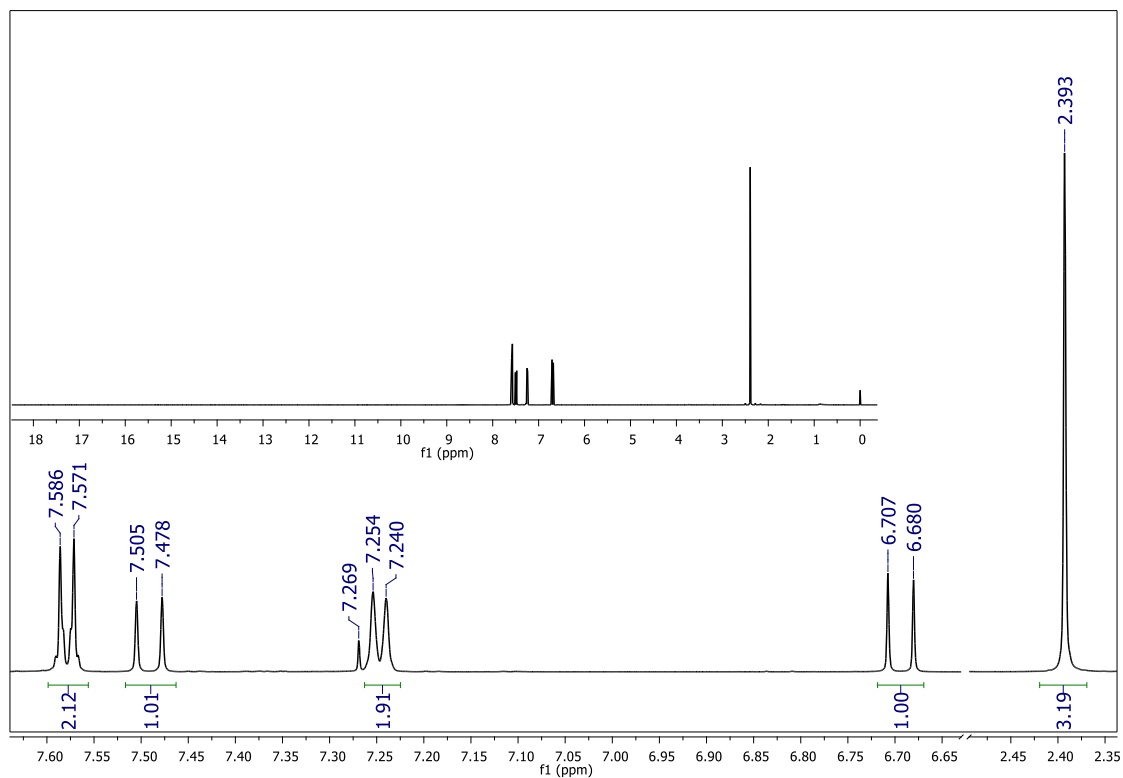


Figure S22. ^{13}C NMR spectrum of **SMAC 12** (150 MHz; CDCl_3).

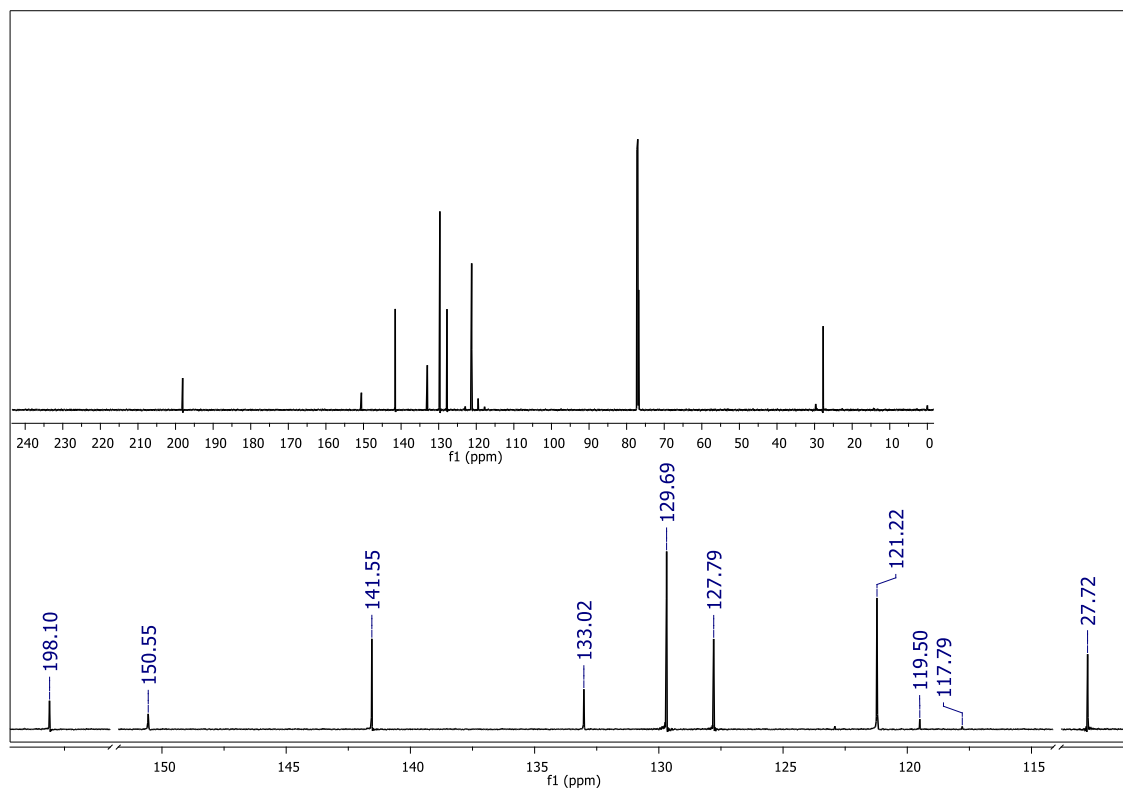


Figure S23. ^1H NMR spectrum of **SMAC 13** (600 MHz; CDCl_3).

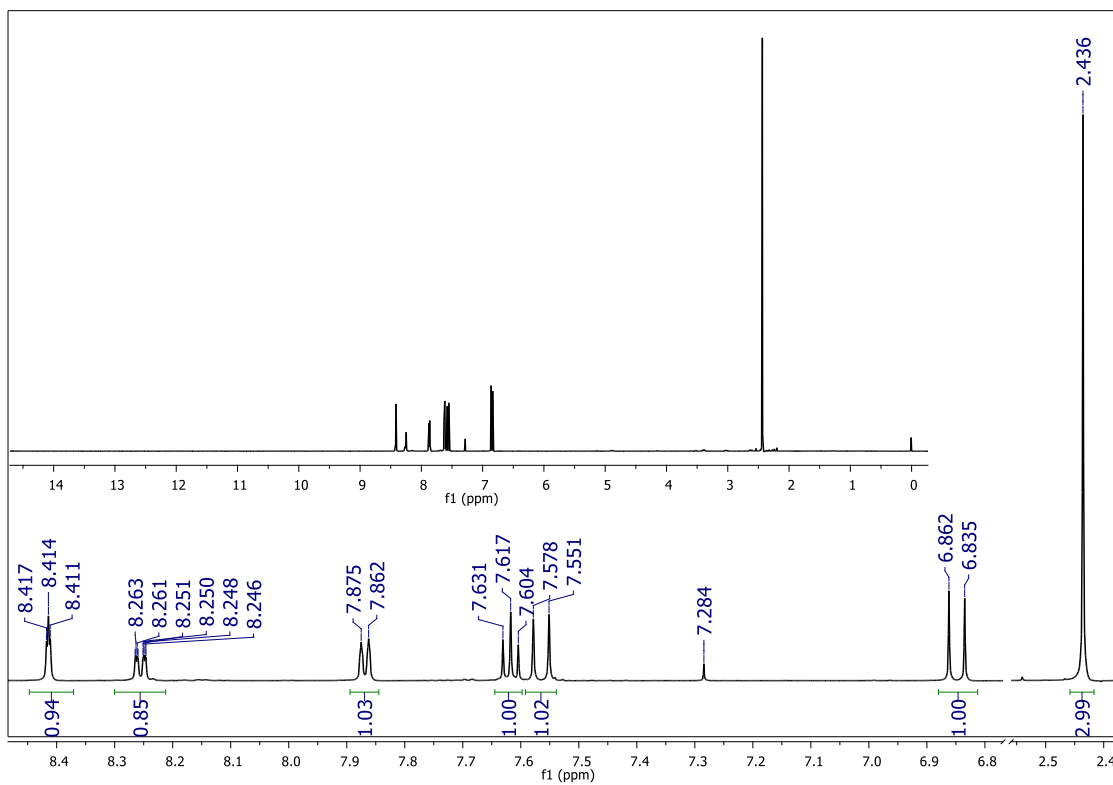


Figure S24. ^{13}C NMR spectrum of **SMAC 13** (150 MHz; CDCl_3).

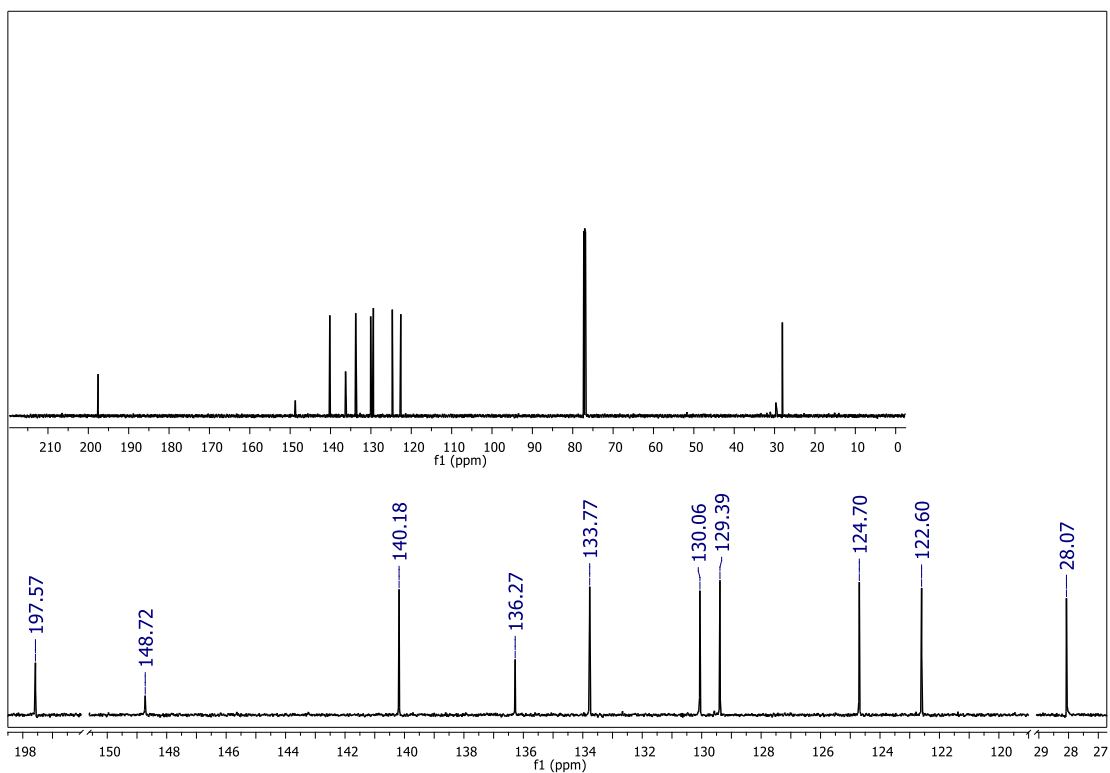


Figure S25. ^1H NMR spectrum of **SMAC 14** (600 MHz; CDCl_3).

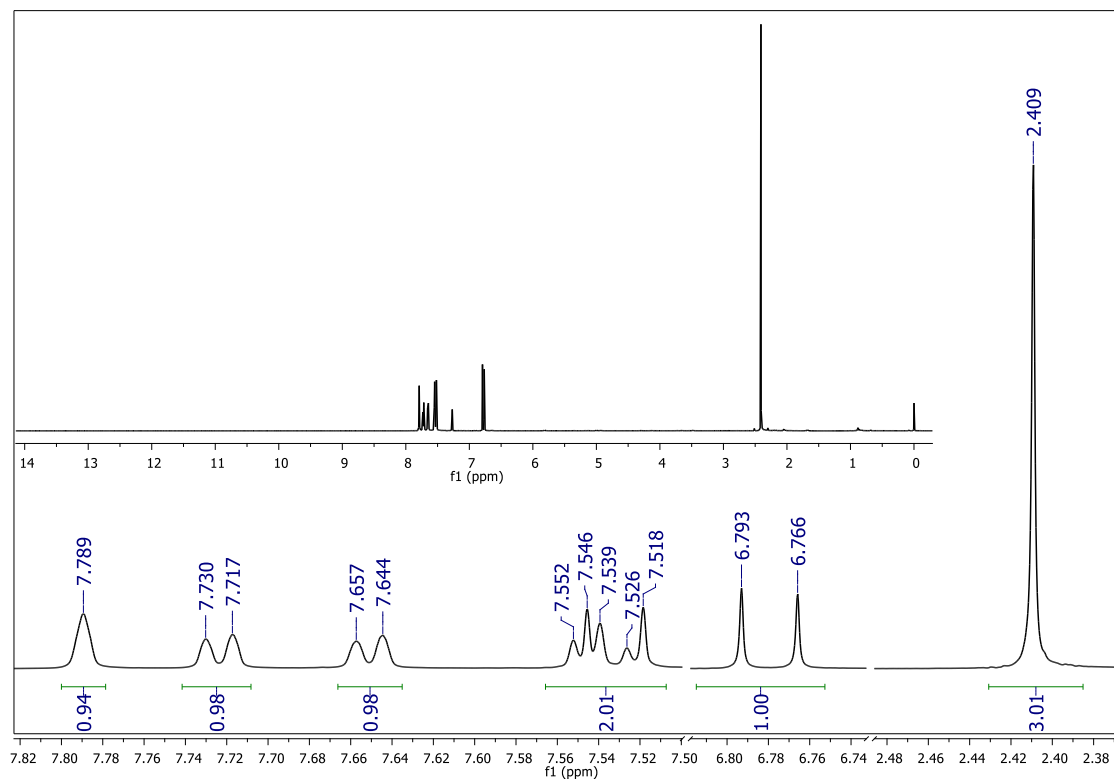


Figure S26. ^{13}C NMR spectrum of **SMAC 14** (150 MHz; CDCl_3).

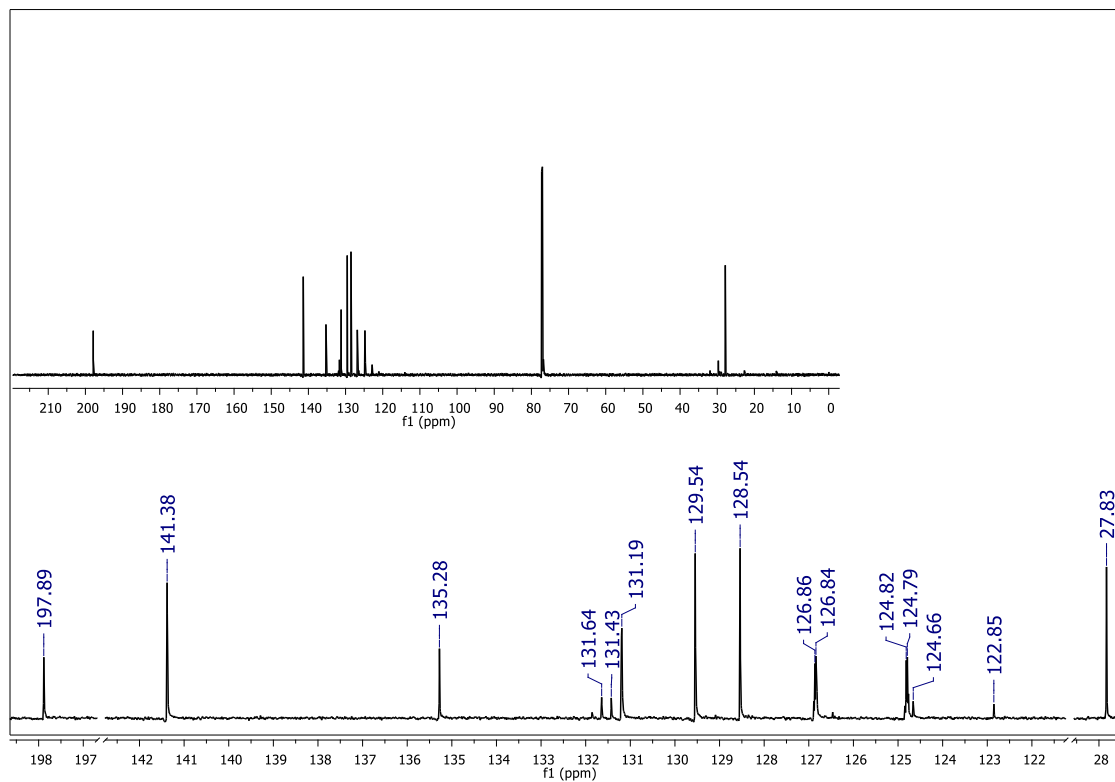


Figure S27. ^1H NMR spectrum of **SMAC 15** (600 MHz; CDCl_3).

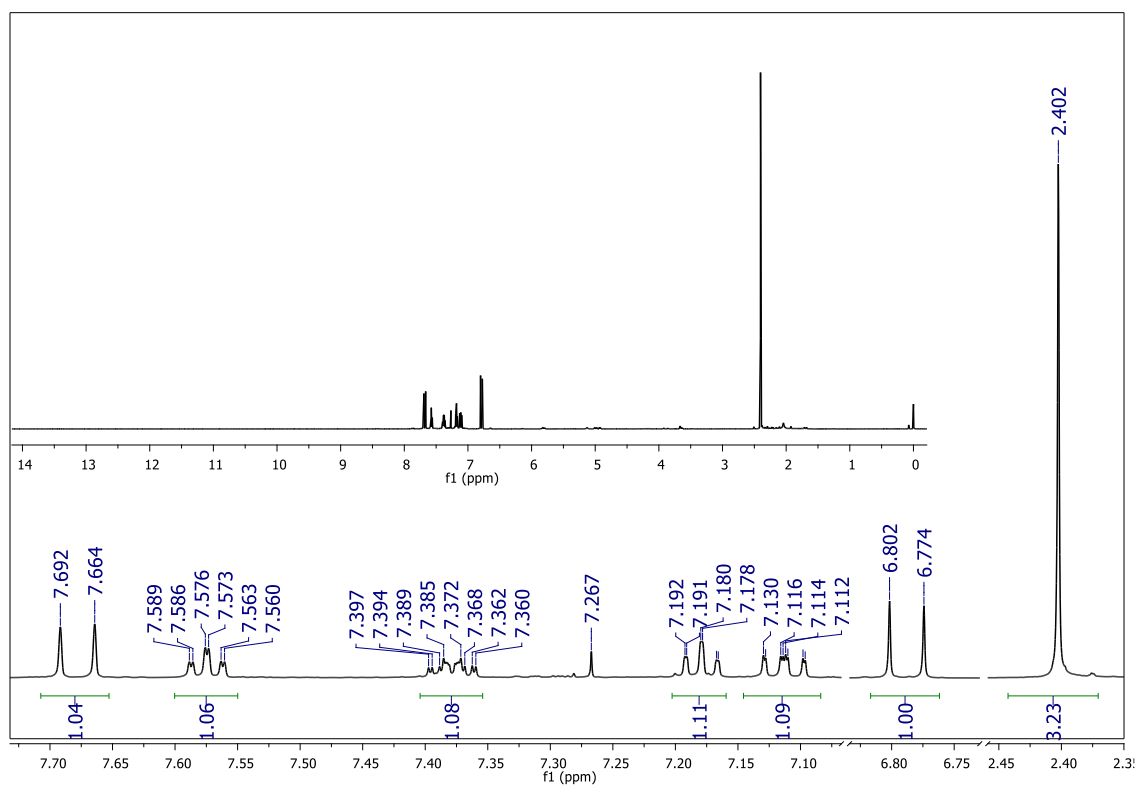


Figure S28. ^{13}C NMR spectrum of **SMAC 15** (150 MHz; CDCl_3).

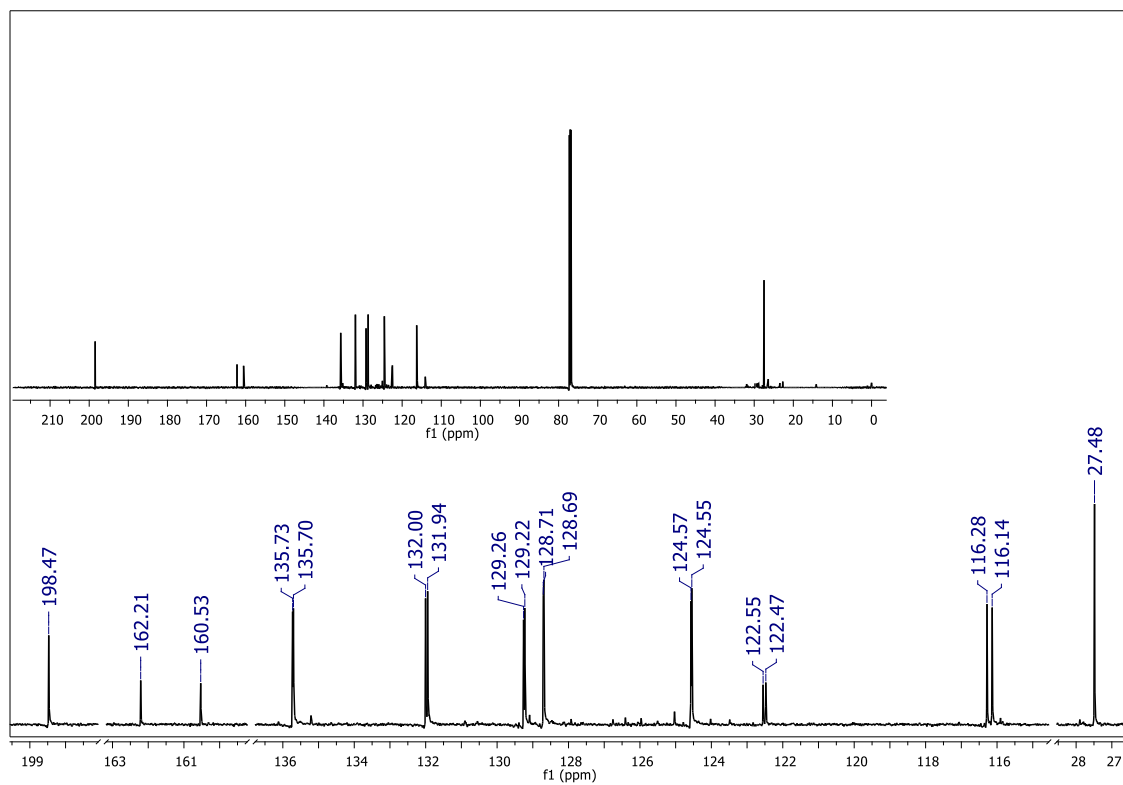


Figure S29. ^1H NMR spectrum of **SMAC 16** (600 MHz; CDCl_3).

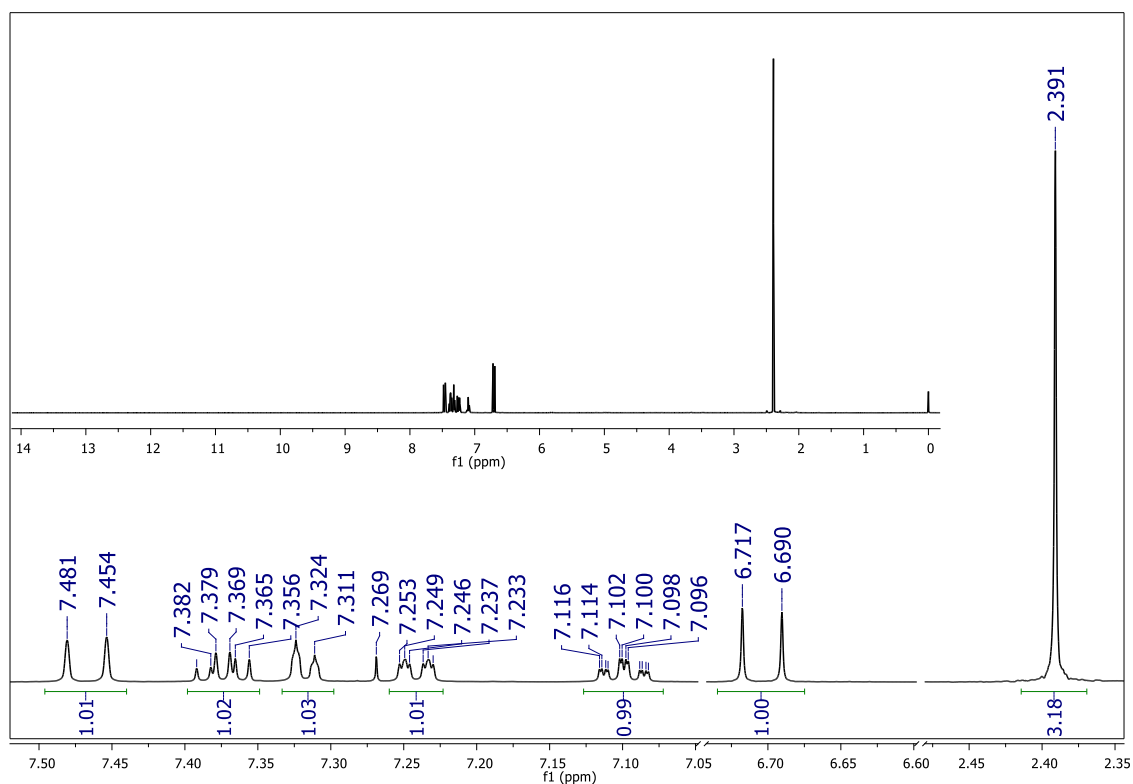


Figure S30. ^{13}C NMR spectrum of **SMAC 16** (150 MHz; CDCl_3).

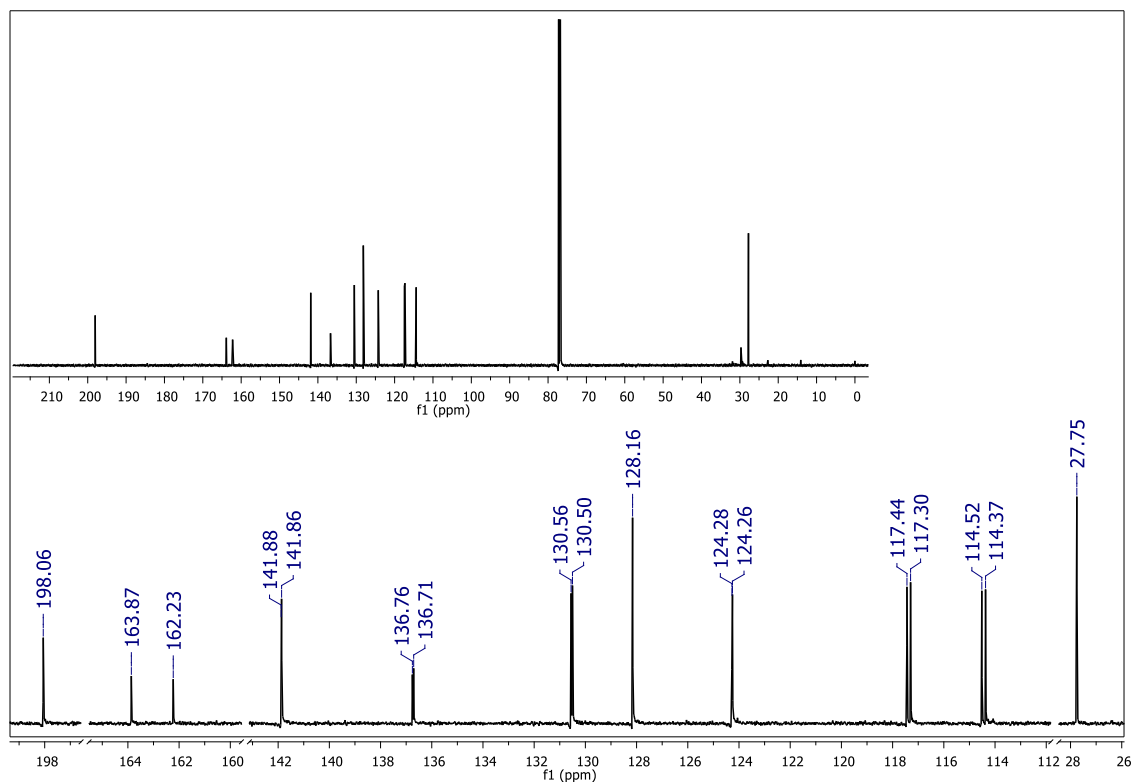


Figure S31. ^1H NMR spectrum of **SMAC 17** (600 MHz; CDCl_3).

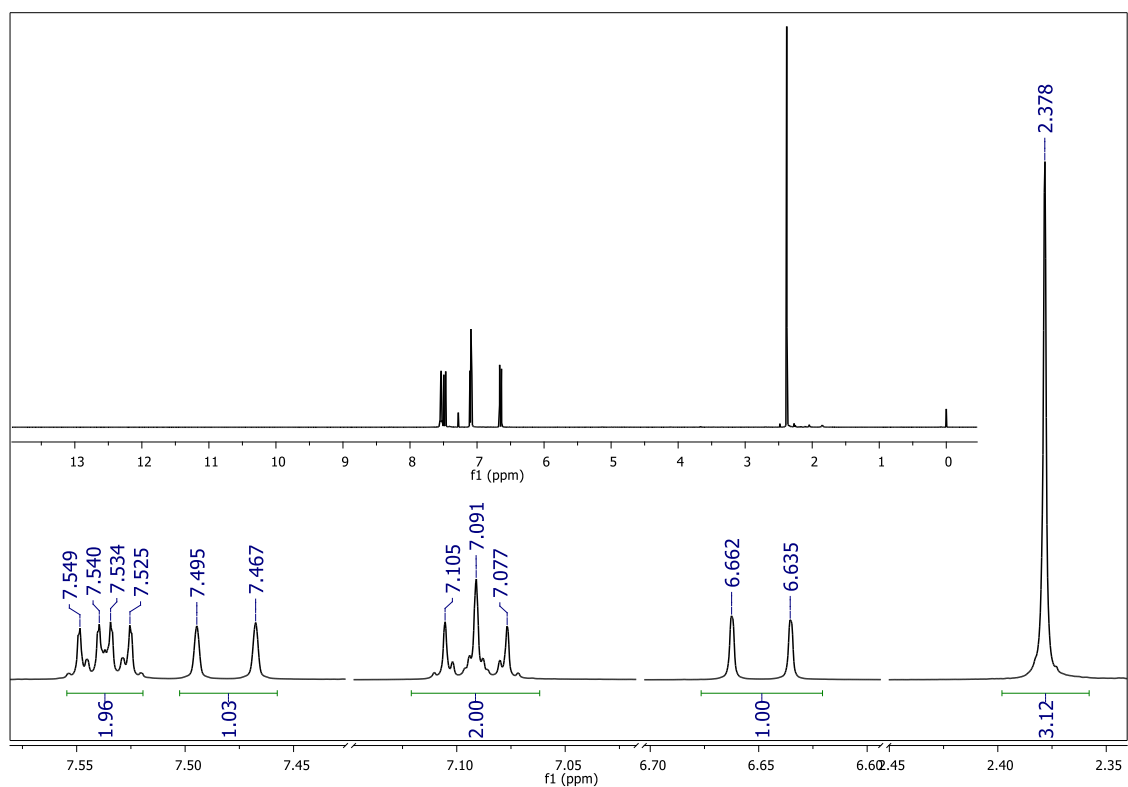


Figure S32. ^{13}C NMR spectrum of **SMAC 17** (150 MHz; CDCl_3).

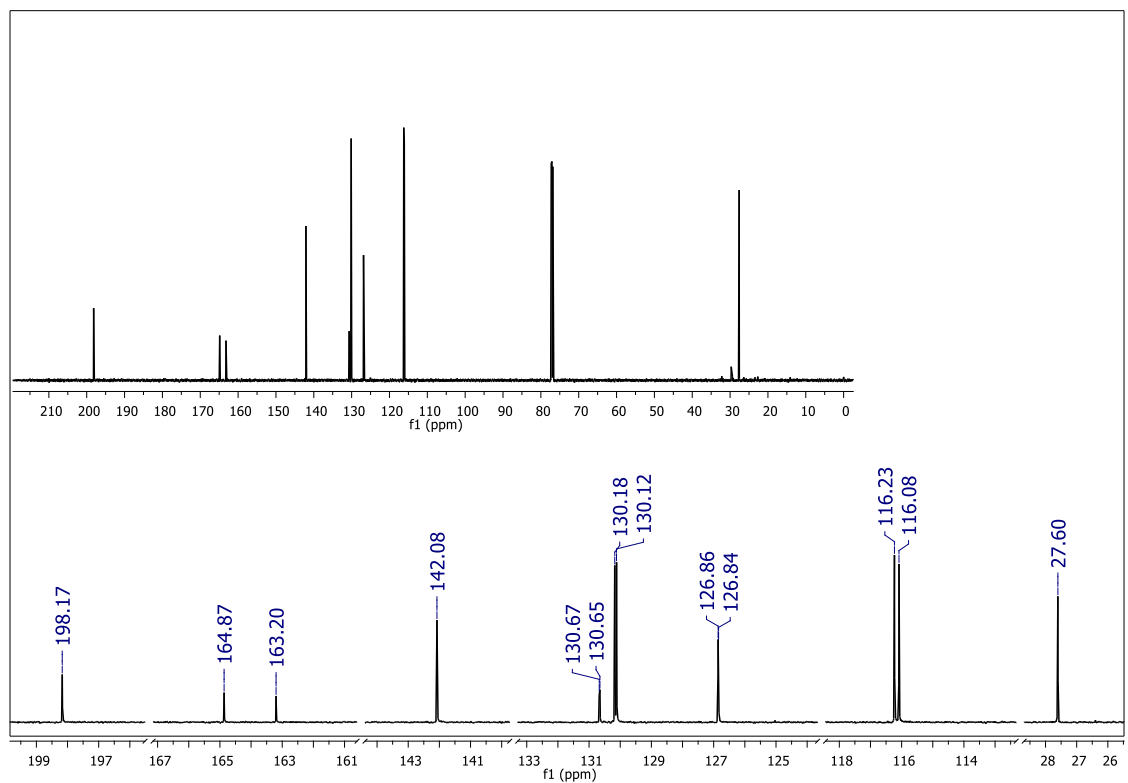


Figure S33. ^1H NMR spectrum of **SMAC 18** (600 MHz; CDCl_3).

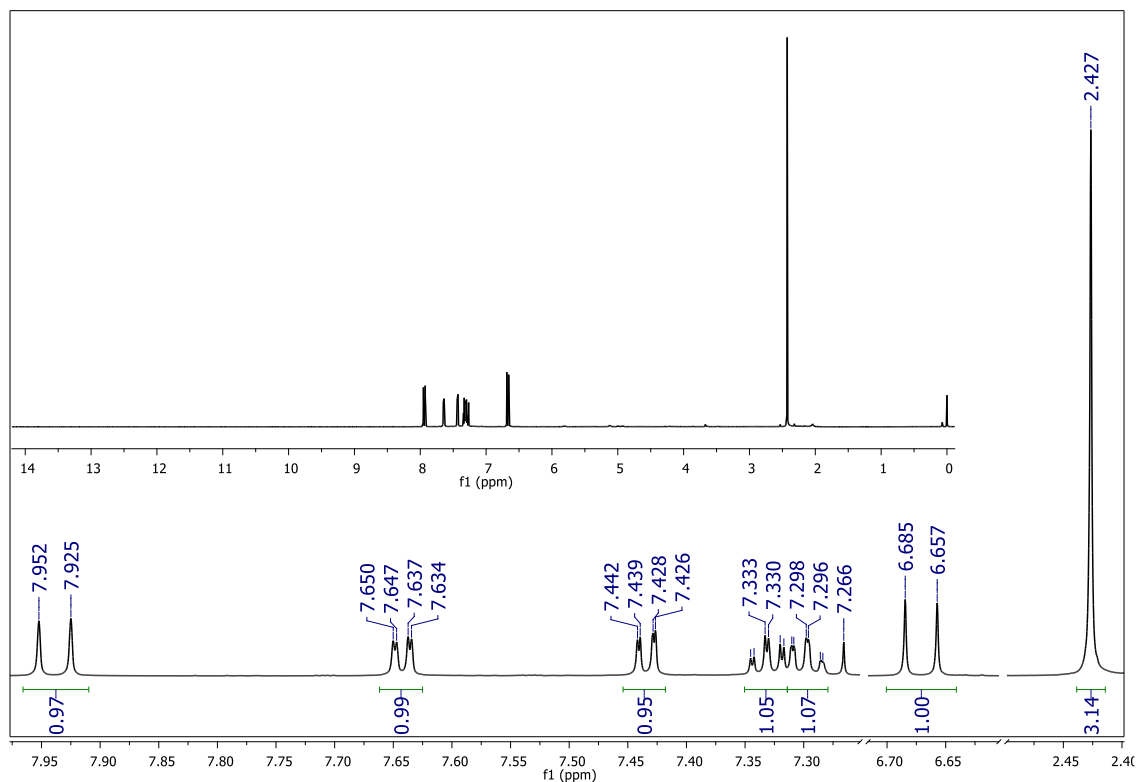


Figure S34. ^{13}C NMR spectrum of **SMAC 18** (150 MHz; CDCl_3).

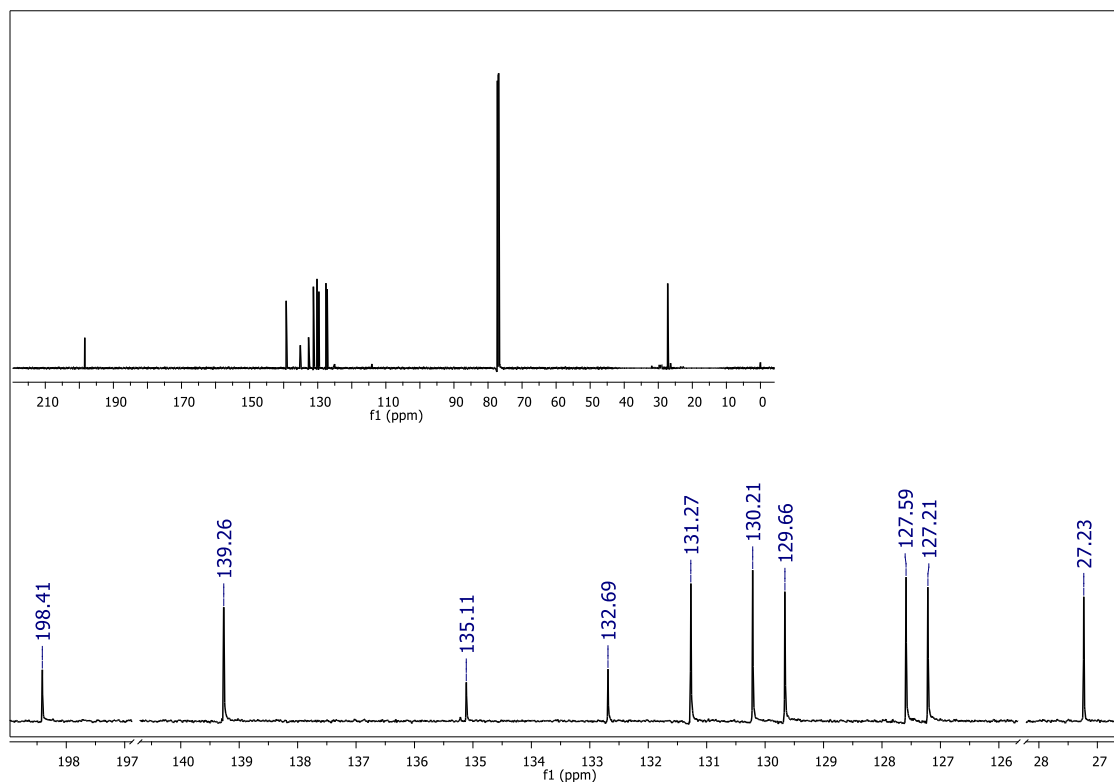


Figure S35. ^1H NMR spectrum of **SMAC 19** (600 MHz; CDCl_3).

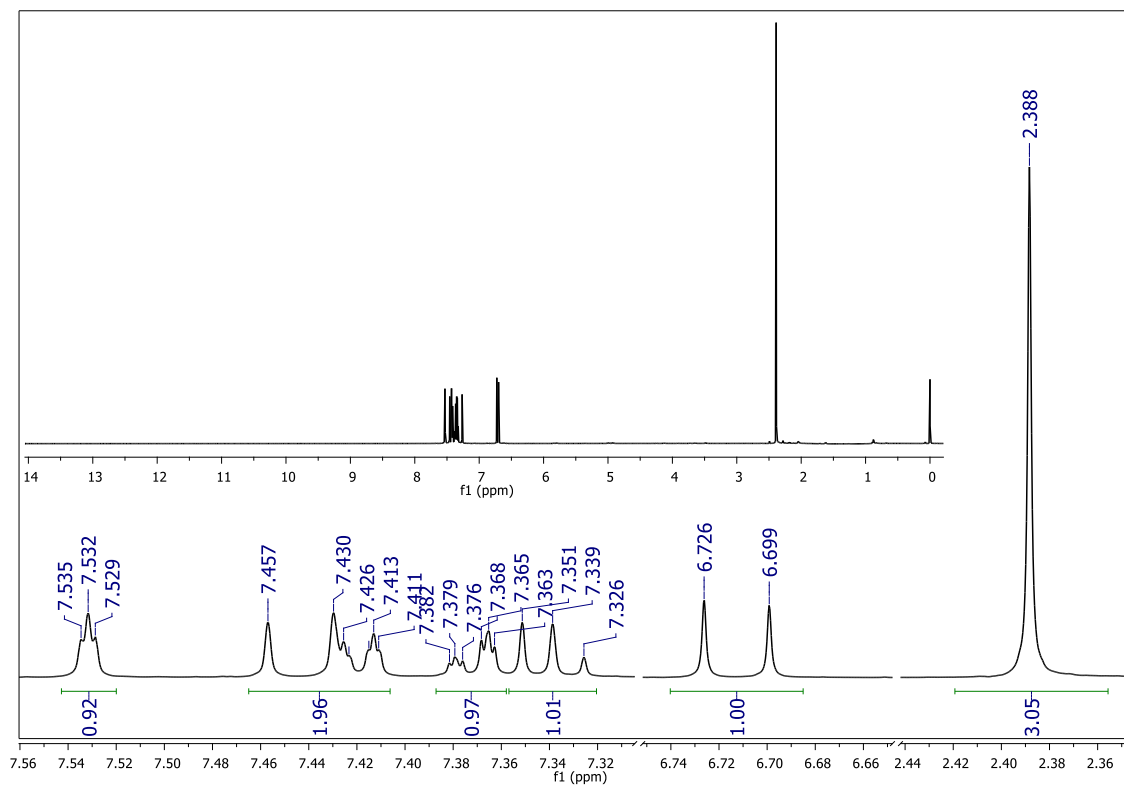


Figure S36. ^{13}C NMR spectrum of **SMAC 19** (150 MHz; CDCl_3).

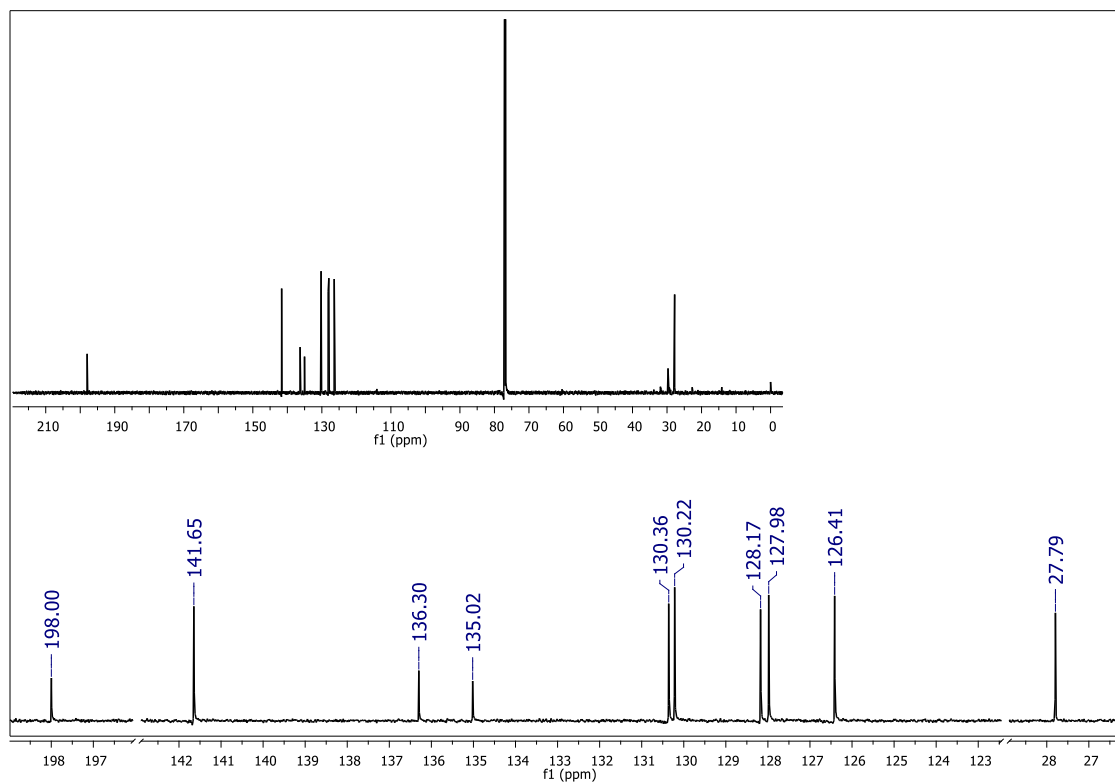


Figure S37. ^1H NMR spectrum of **SMAC 20** (600 MHz; CDCl_3).

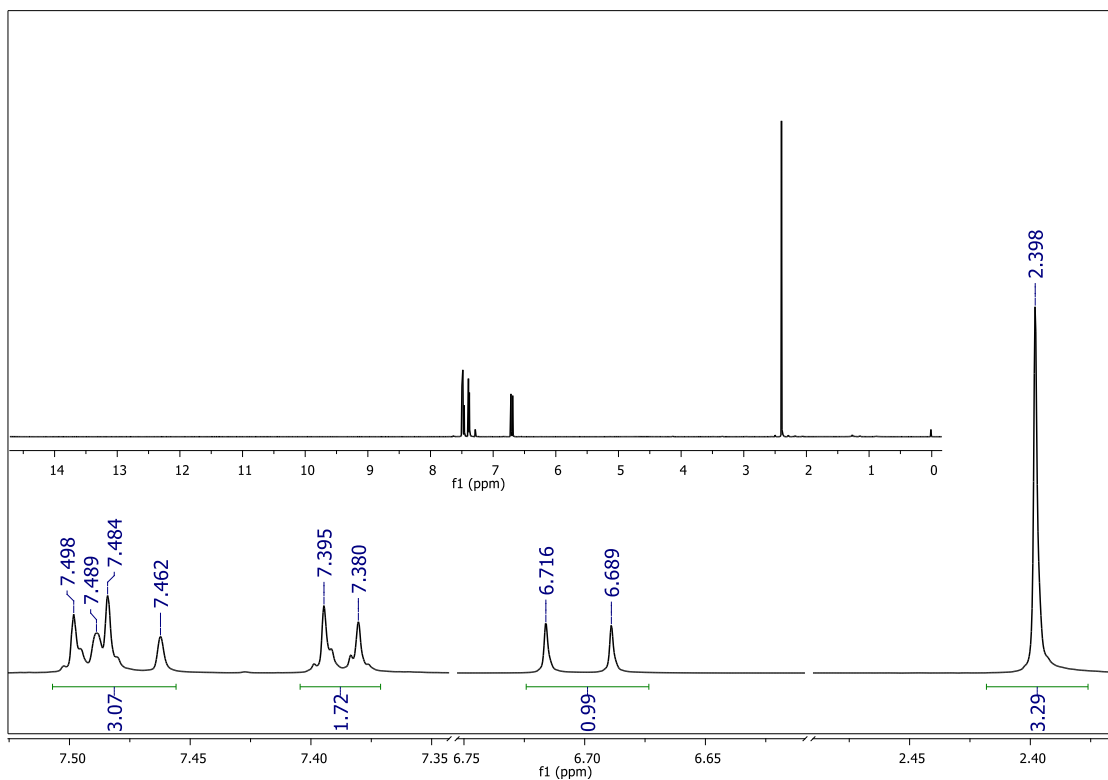


Figure S38. ^{13}C NMR spectrum of **SMAC 20** (150 MHz; CDCl_3).

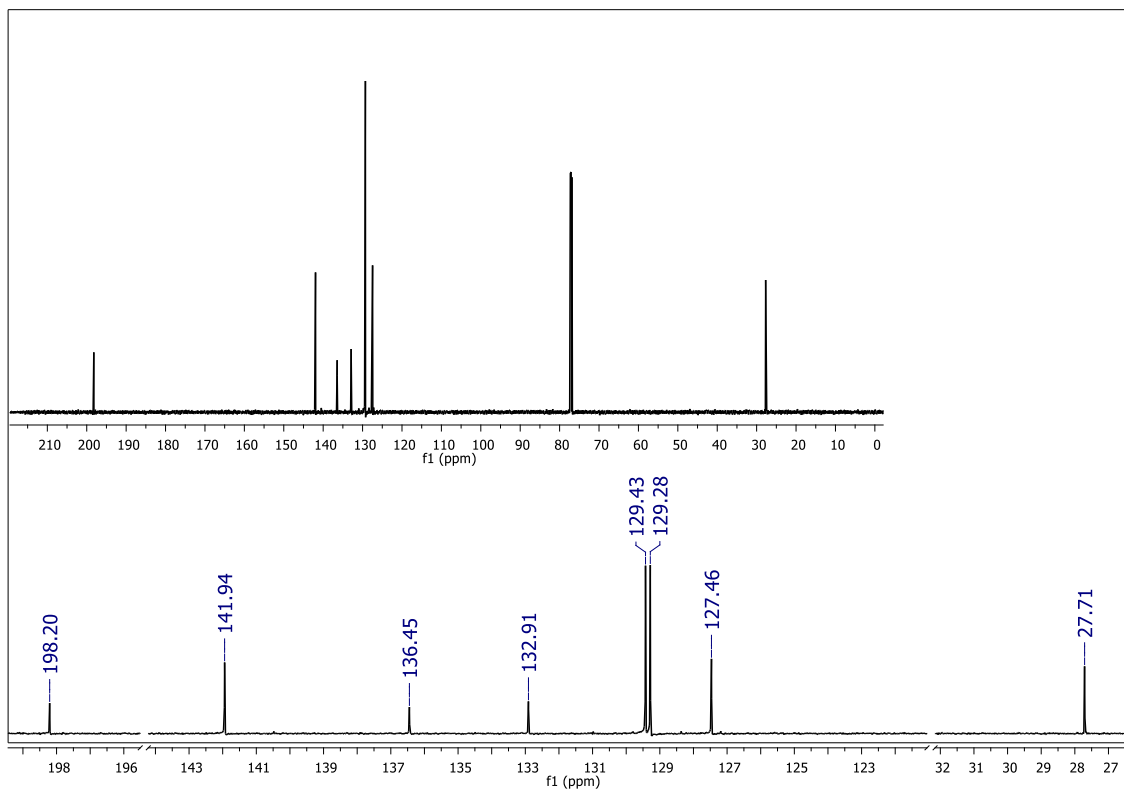


Figure S39. ^1H NMR spectrum of **SMAC 21** (600 MHz; CDCl_3).

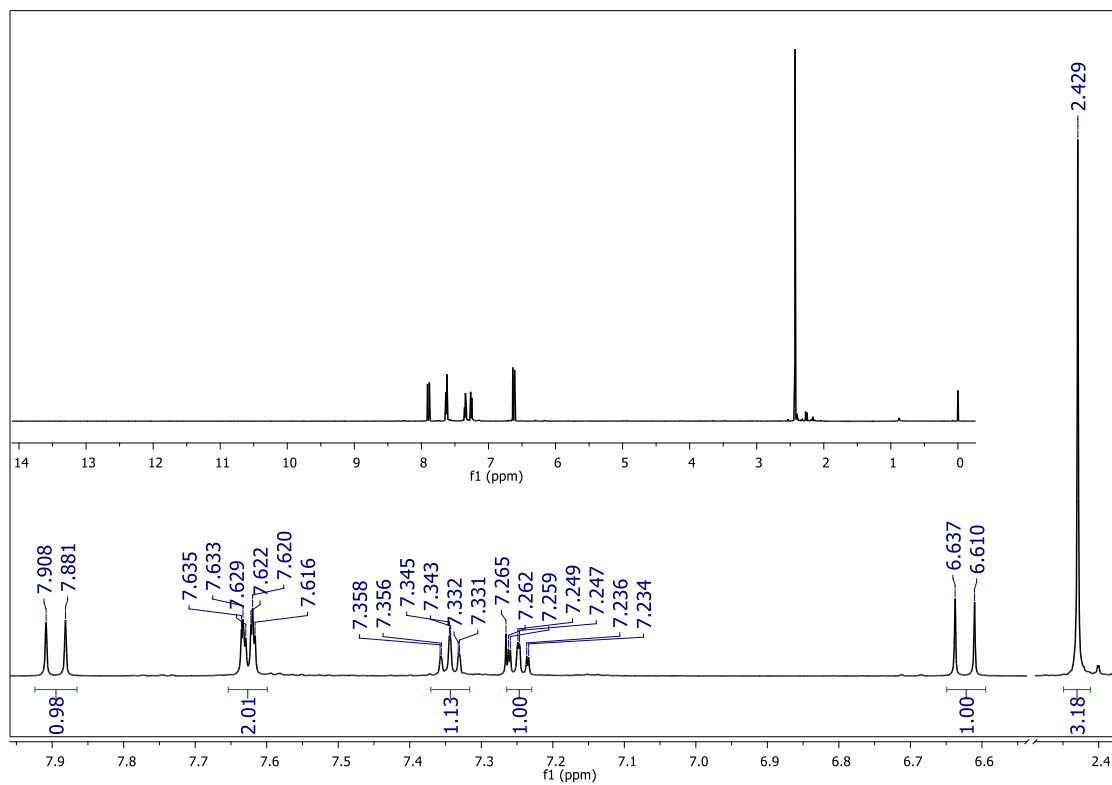


Figure S40. ^{13}C NMR spectrum of **SMAC 21** (150 MHz; CDCl_3).

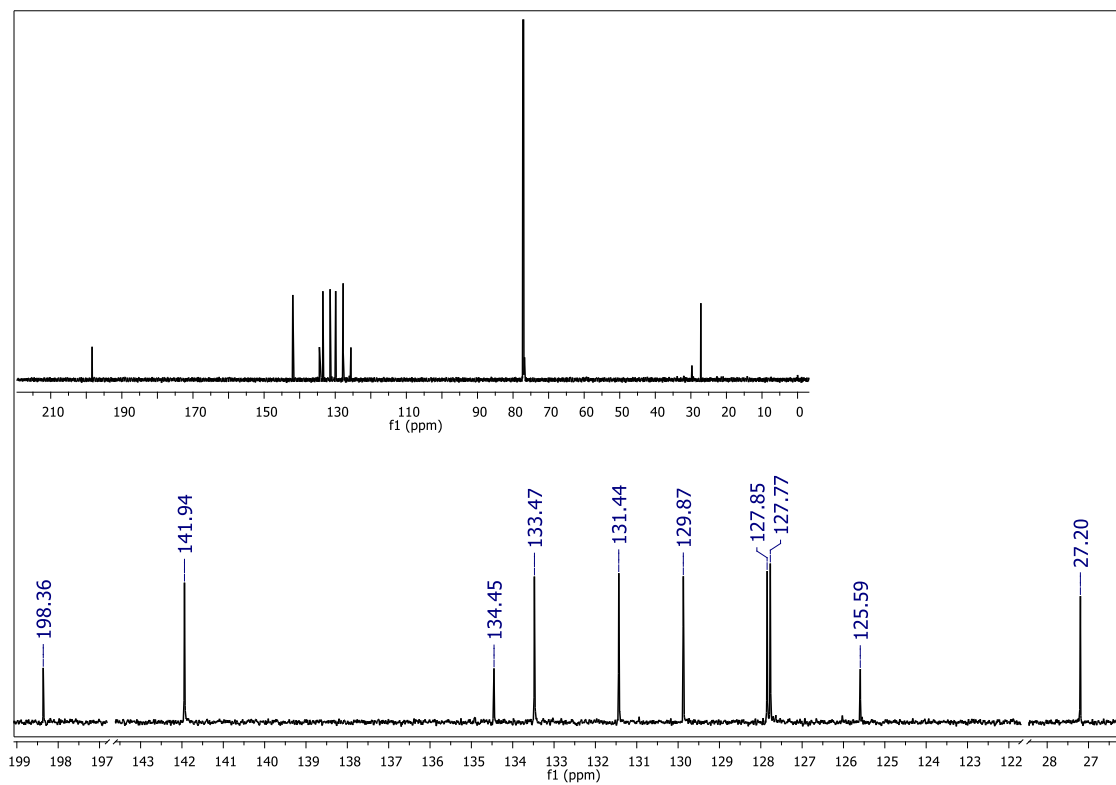


Figure S41. ^1H NMR spectrum of **SMAC 22** (600 MHz; CDCl_3).

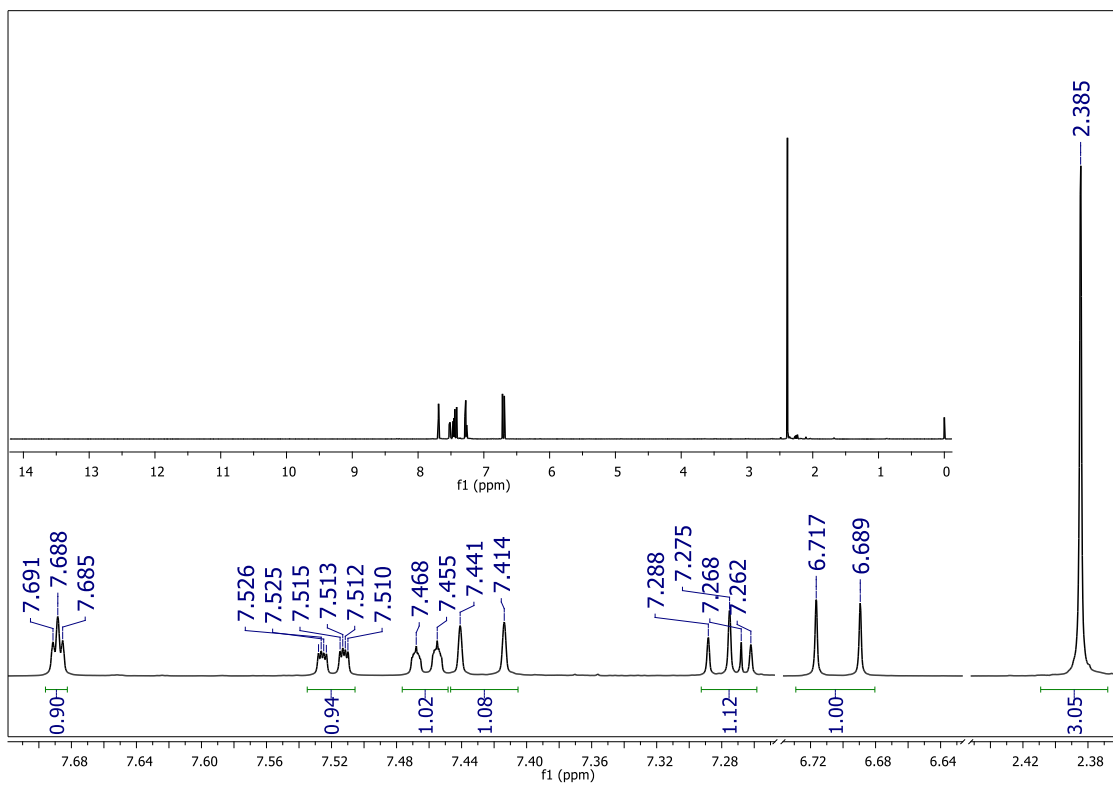


Figure S42. ^{13}C NMR spectrum of **SMAC 22** (150 MHz; CDCl_3).

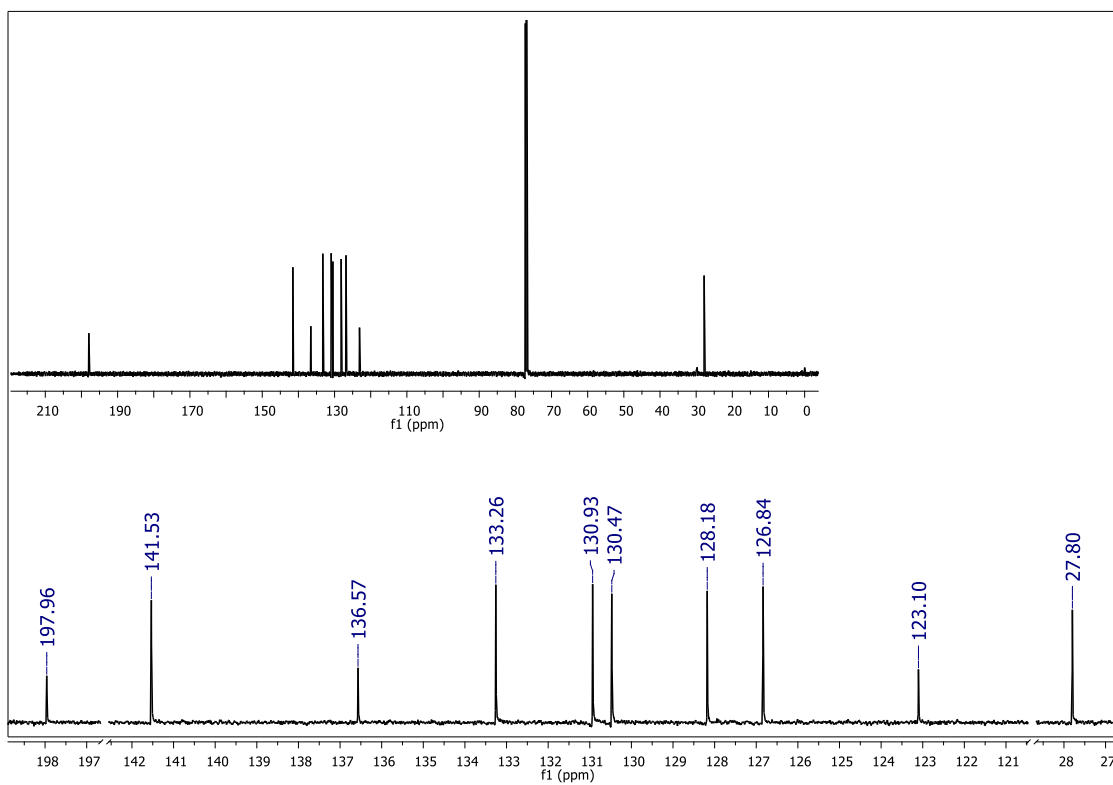


Figure S43. ^1H NMR spectrum of **SMAC 23** (600 MHz; CDCl_3).

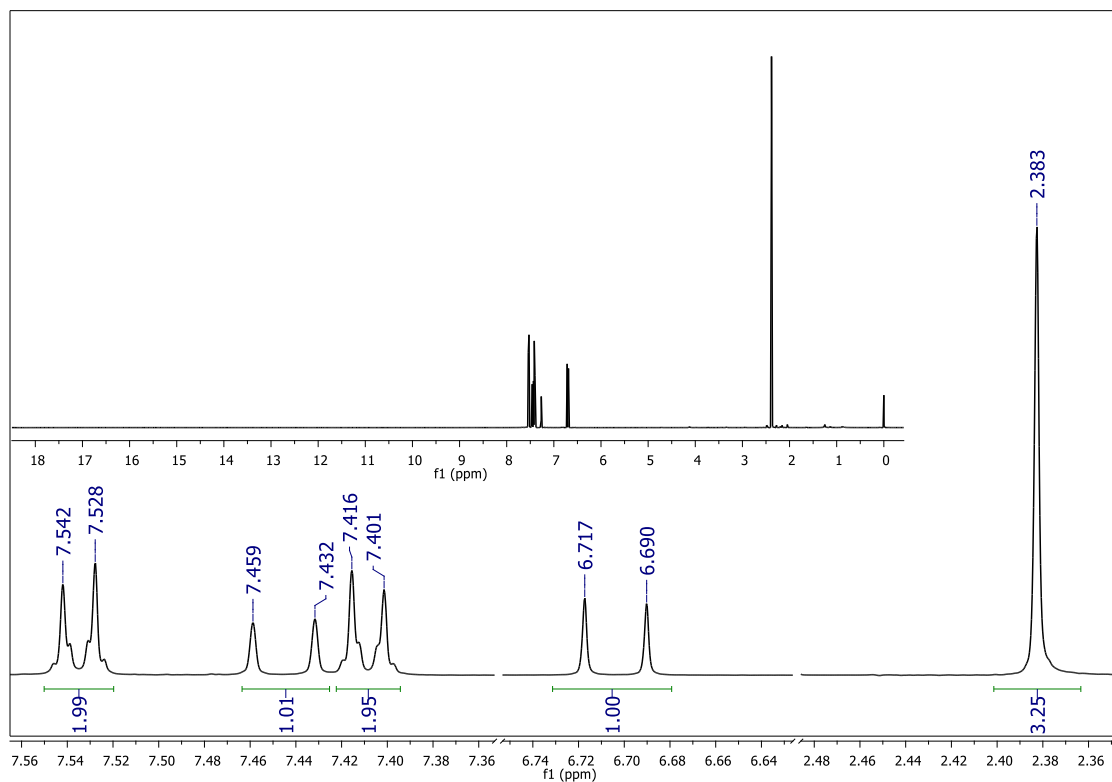


Figure S44. ^{13}C NMR spectrum of **SMAC 23** (150 MHz; CDCl_3).

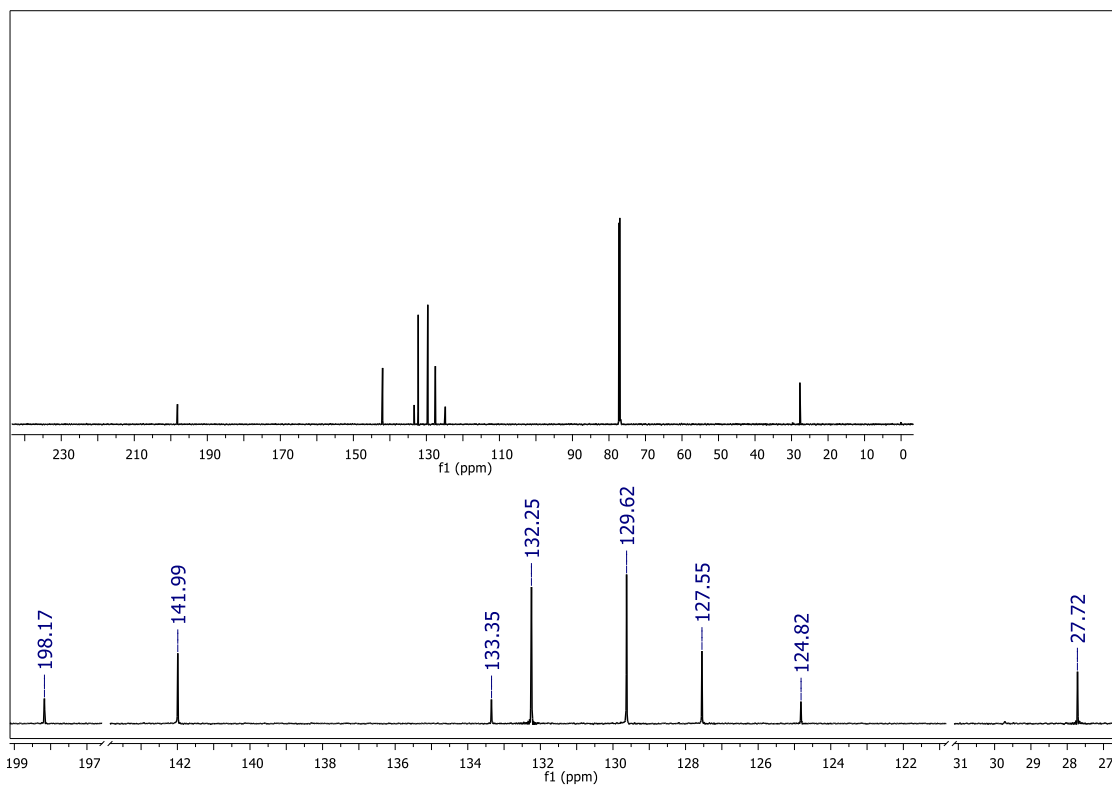


Figure S45. ^1H NMR spectrum of **SMAC 24** (600 MHz; CDCl_3).

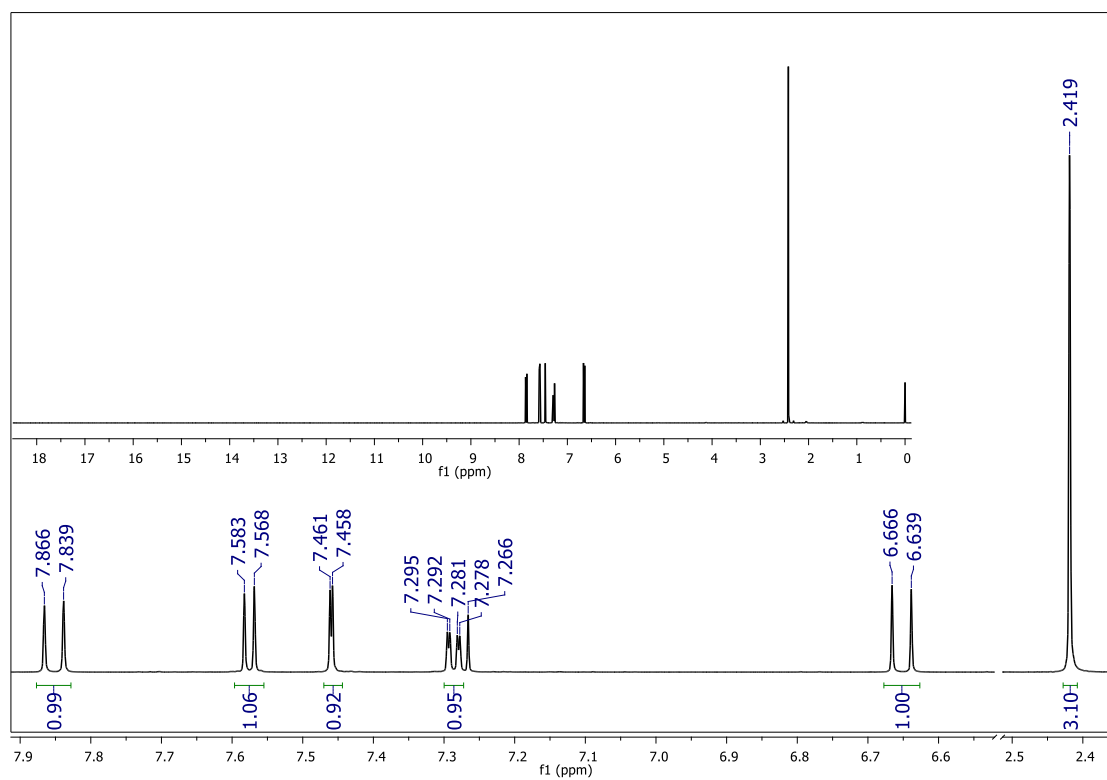


Figure S46. ^{13}C NMR spectrum of **SMAC 24** (150 MHz; CDCl_3).

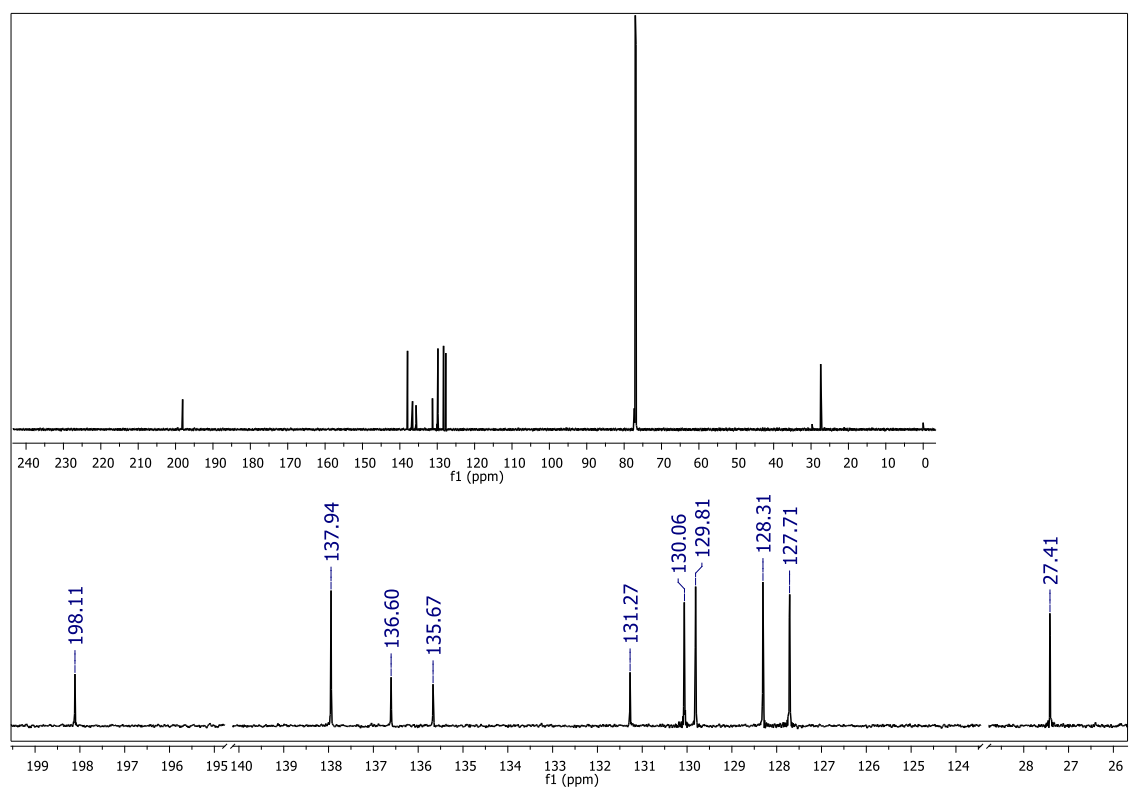


Figure S47. ^1H NMR spectrum of **SMAC 25** (600 MHz; CDCl_3).

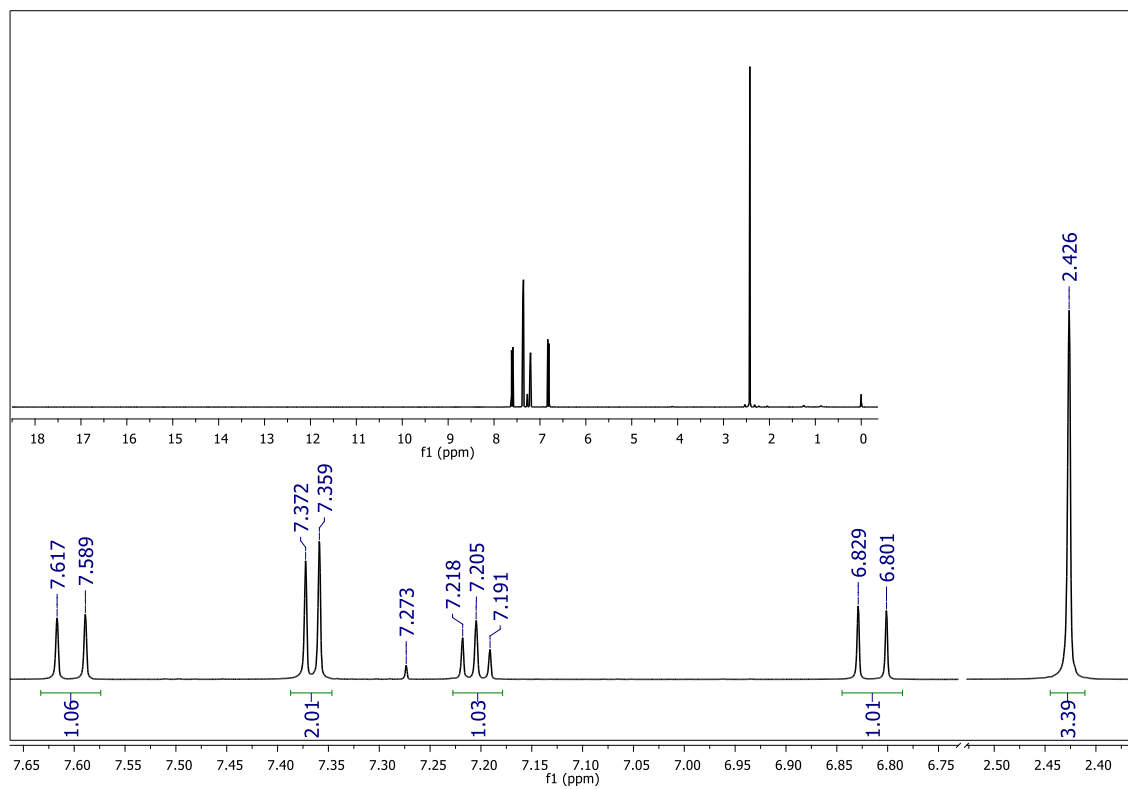


Figure S48. ^{13}C NMR spectrum of **SMAC 25** (150 MHz; CDCl_3).

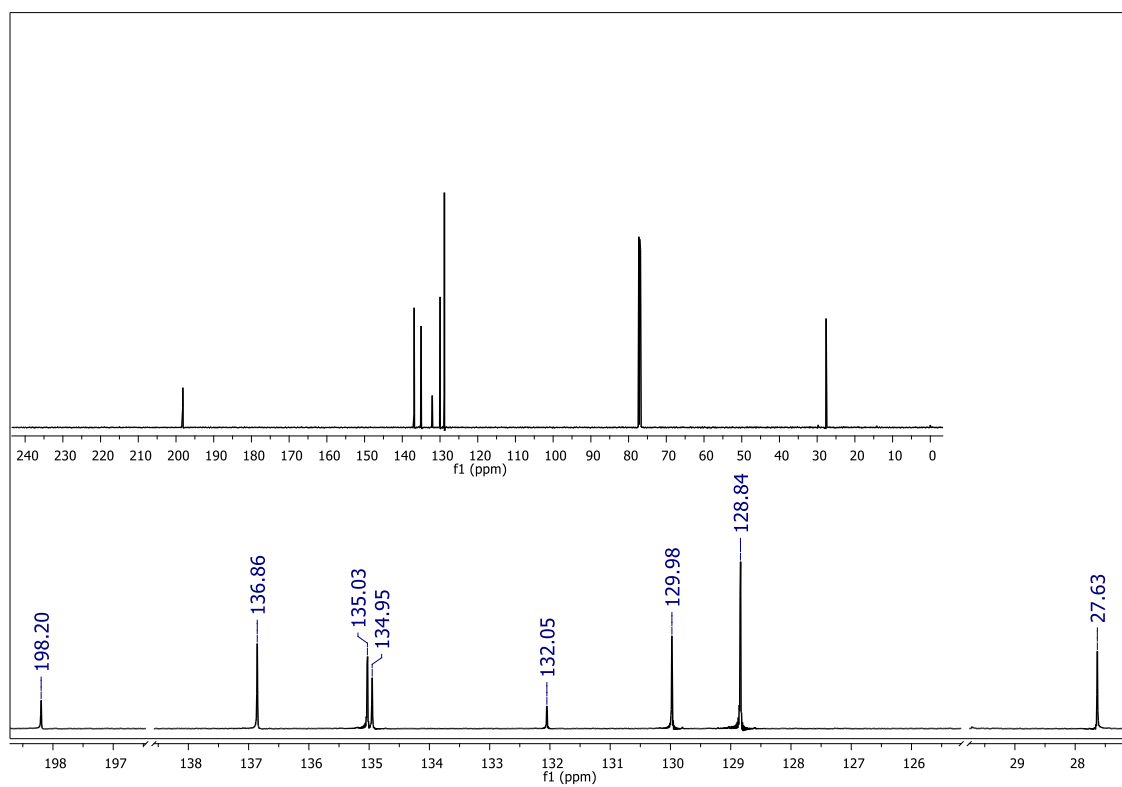


Figure S49. ^1H NMR spectrum of **SMAC 26** (600 MHz; CDCl_3).

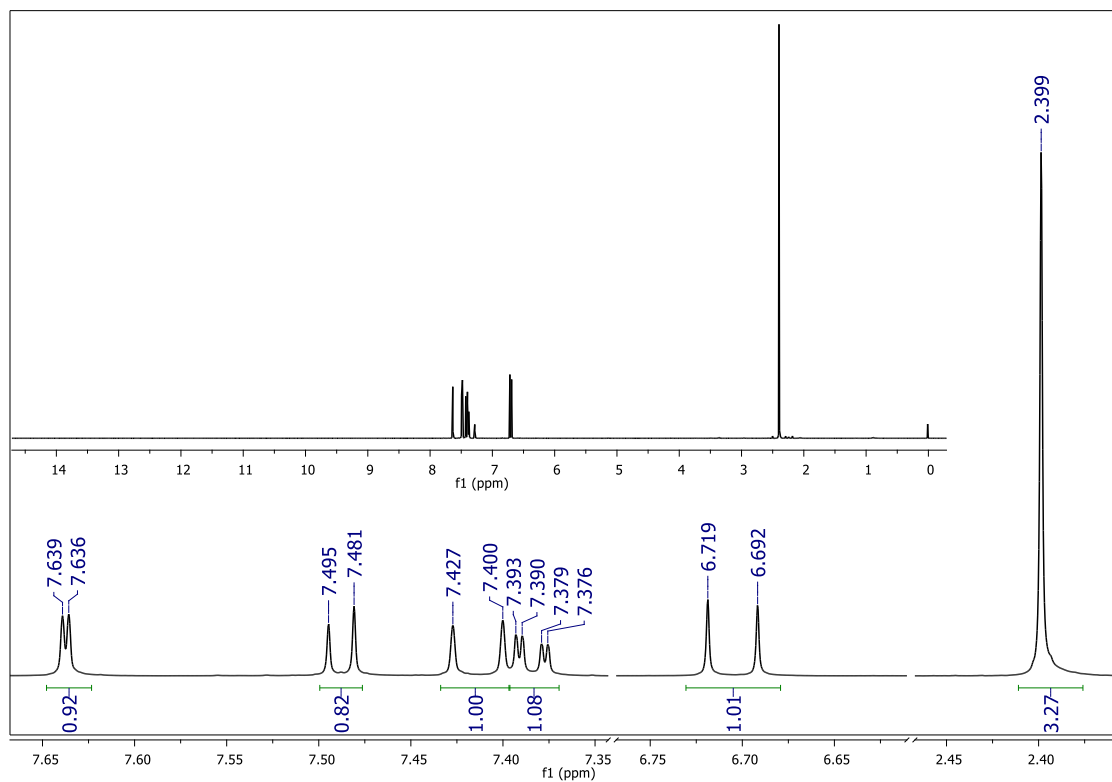


Figure S50. ^{13}C NMR spectrum of **SMAC 26** (150 MHz; CDCl_3).

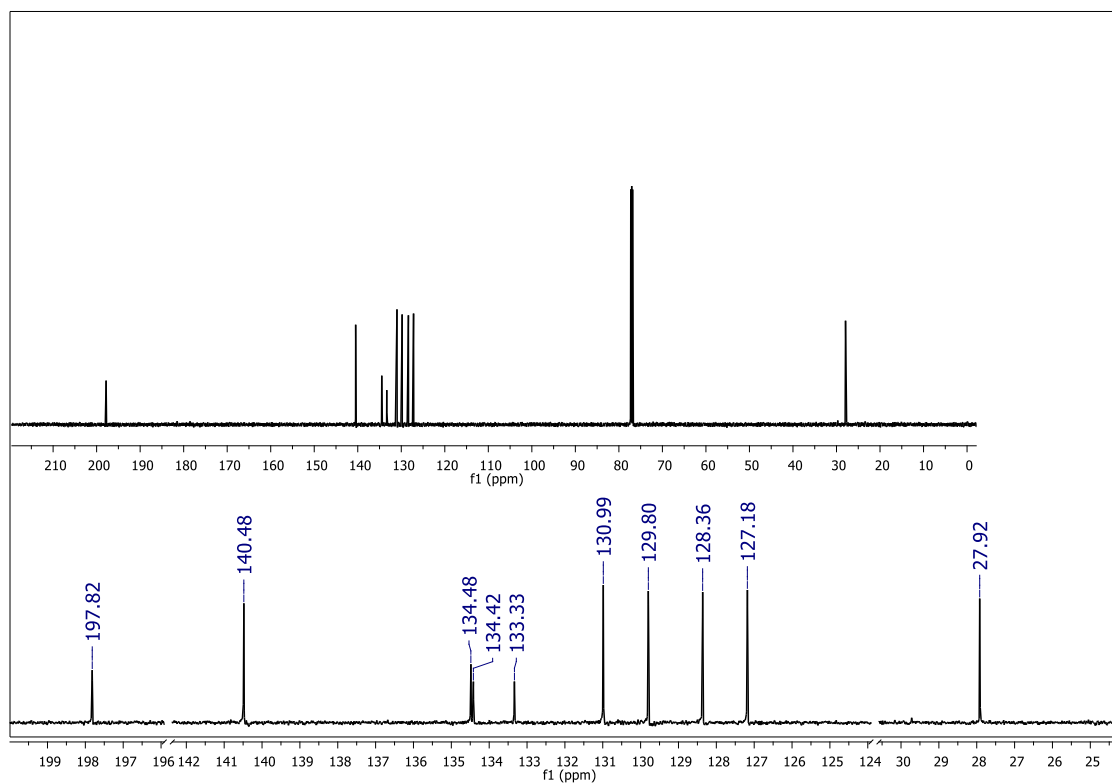


Figure S51. ^1H NMR spectrum of **SMAC 27** (600 MHz; CDCl_3).

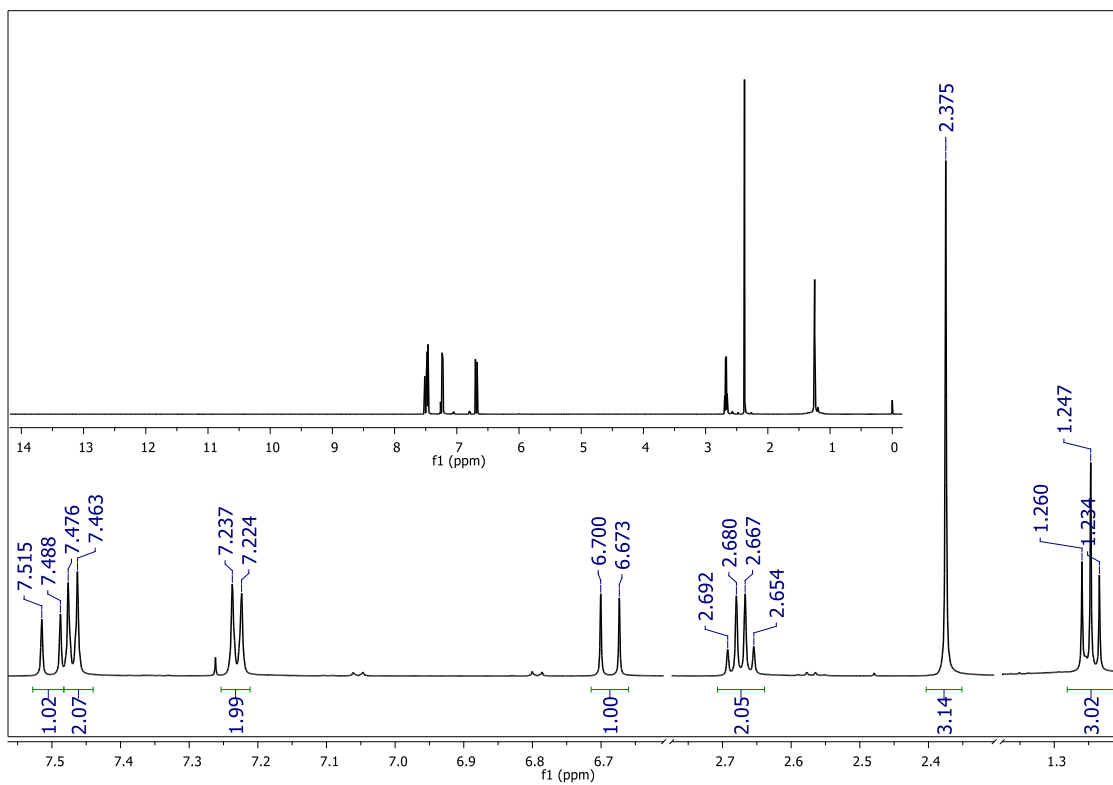


Figure S52. ^{13}C NMR spectrum of **SMAC 27** (150 MHz; CDCl_3).

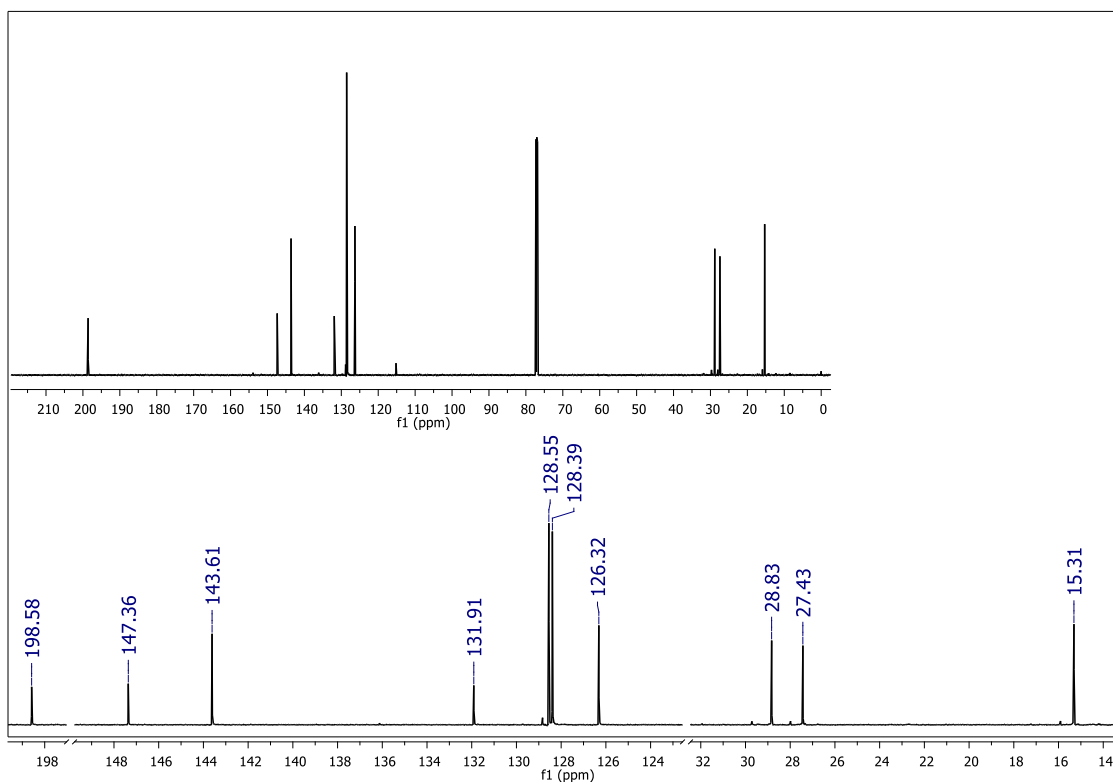


Figure S53. ^1H NMR spectrum of **SMAC 28** (600 MHz; CDCl_3).

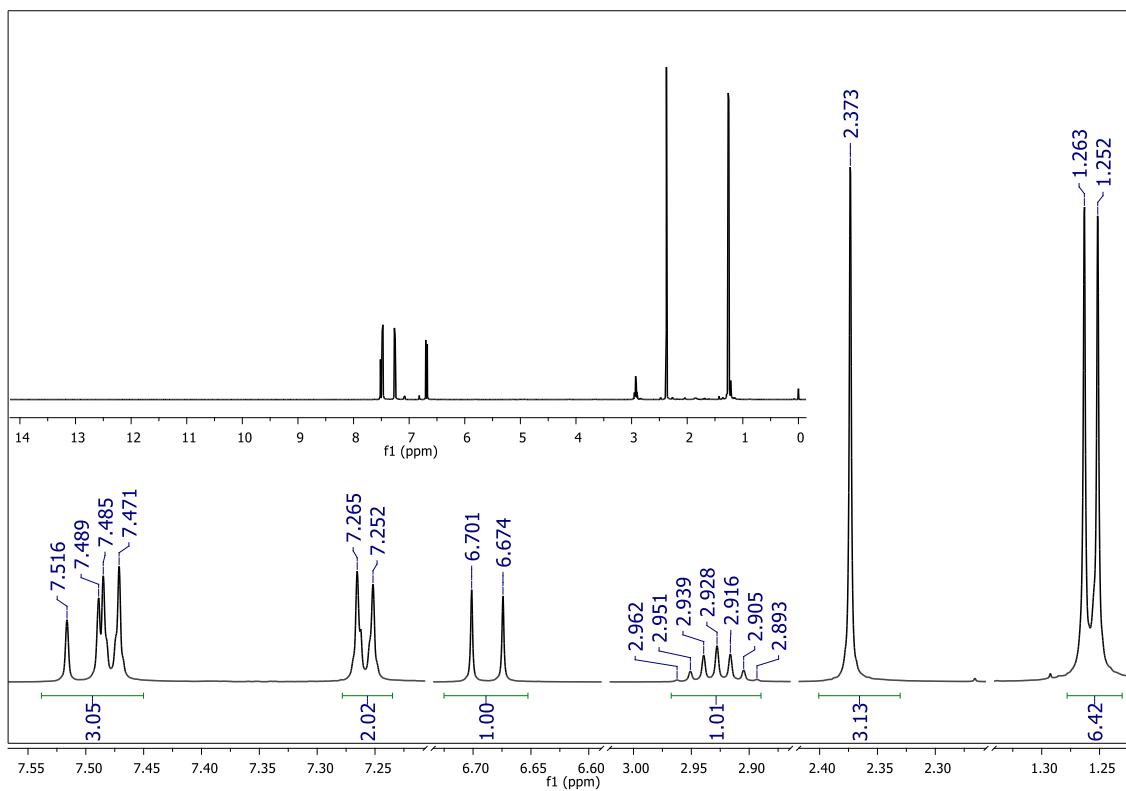


Figure S54. ^{13}C NMR spectrum of **SMAC 28** (150 MHz; CDCl_3).

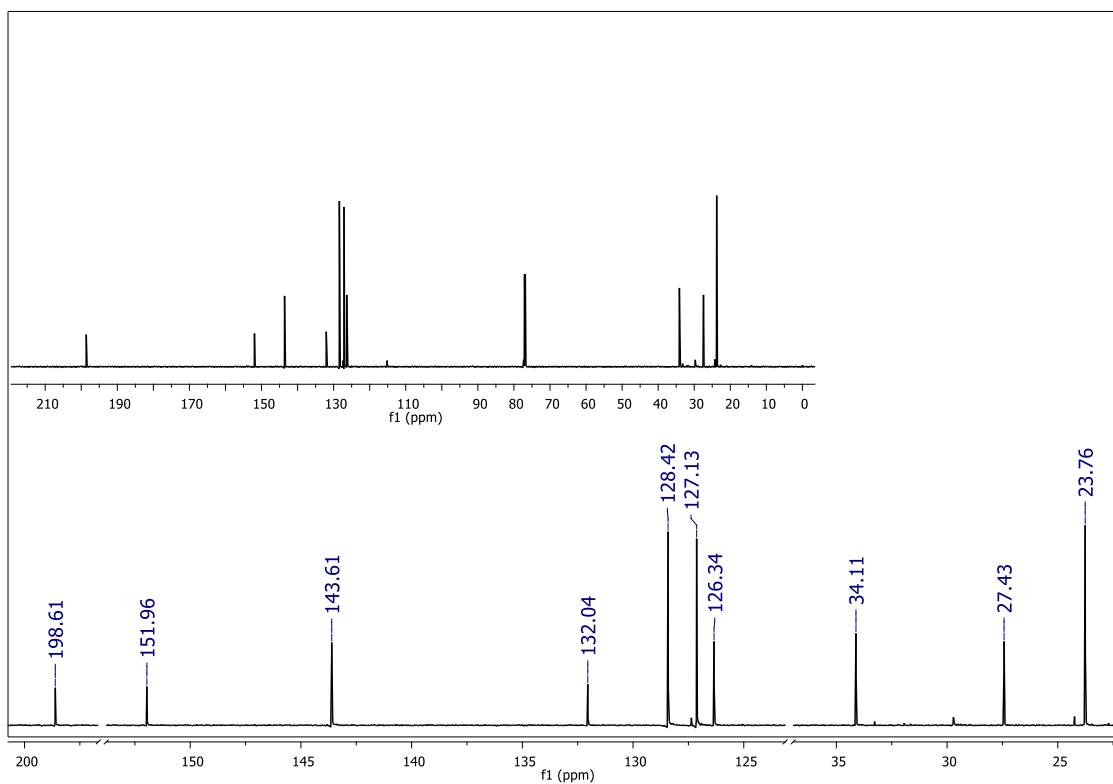


Figure S55. ^1H NMR spectrum of **SMAC 29** (600 MHz; CDCl_3).

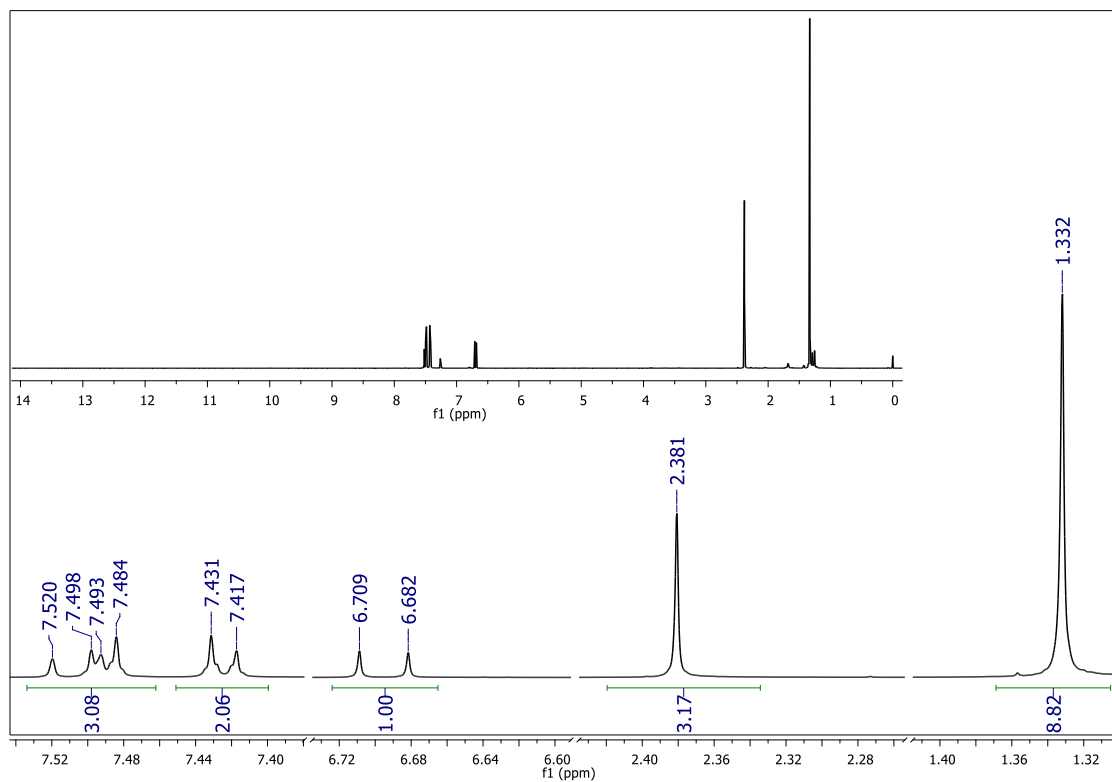


Figure S56. ^{13}C NMR spectrum of **SMAC 29** (150 MHz; CDCl_3).

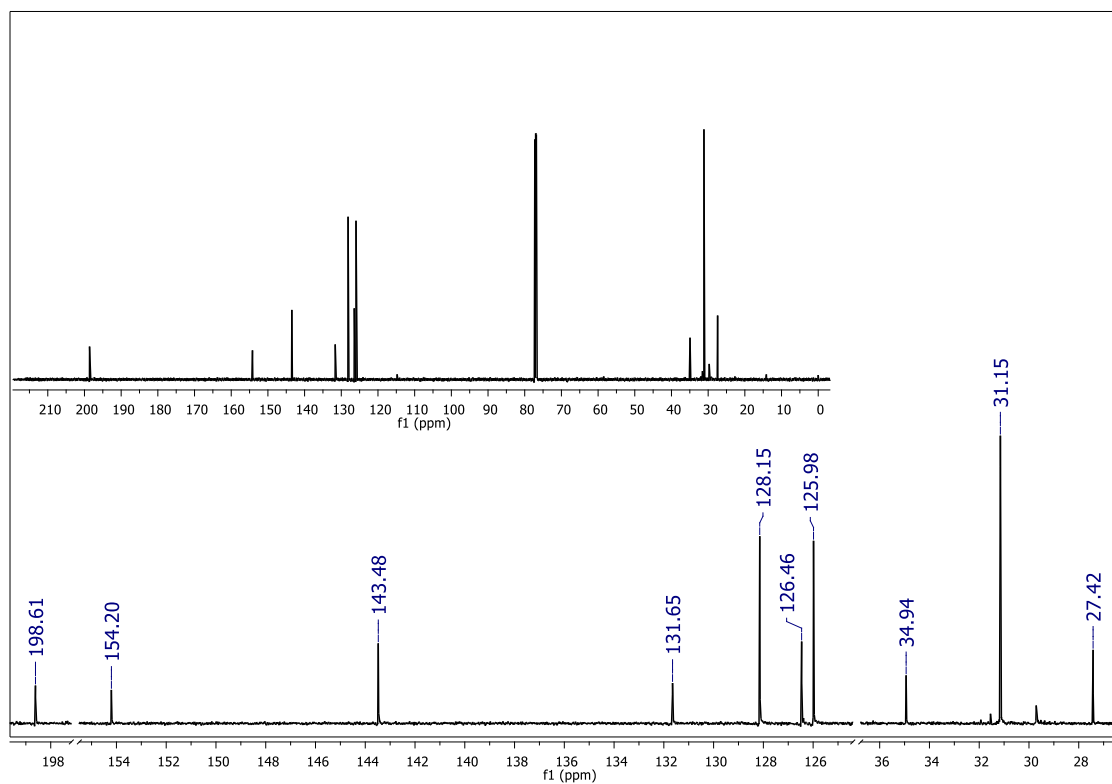


Figure S57. ^1H NMR spectrum of **SMAC 30** (600 MHz; CDCl_3).

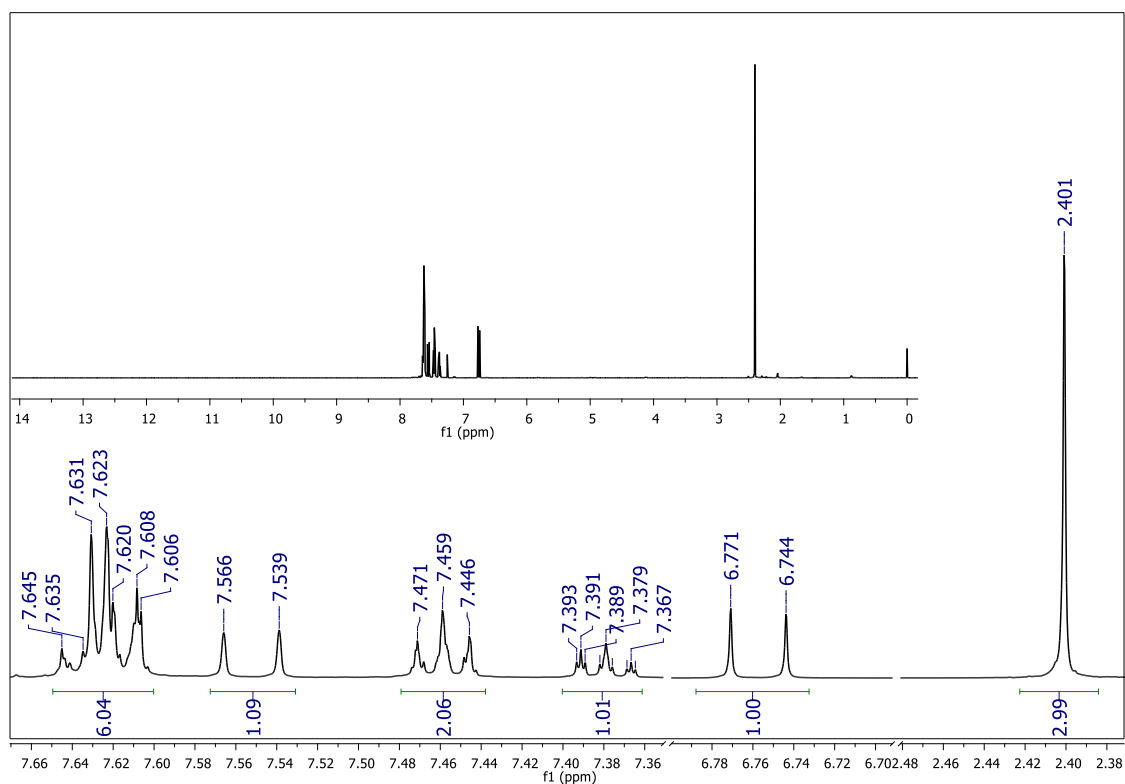


Figure S58. ^{13}C NMR spectrum of **SMAC 30** (160 MHz; CDCl_3).

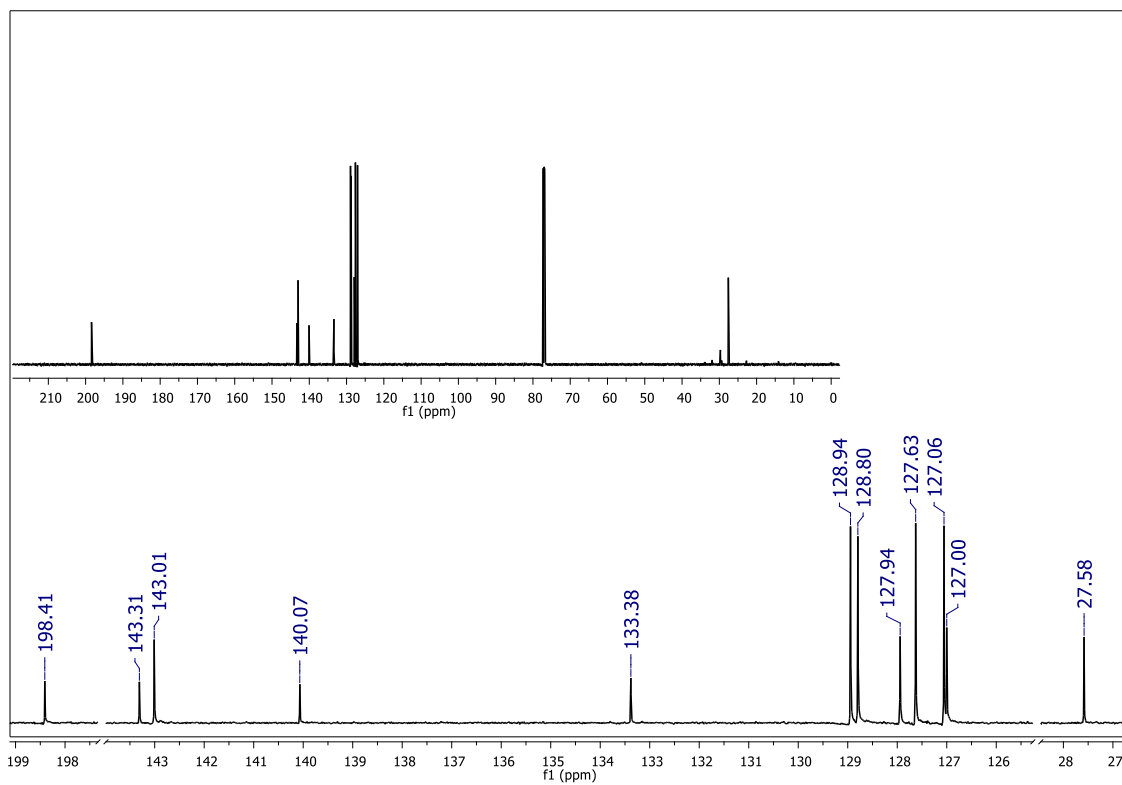


Figure S59. ^1H NMR spectrum of **SMAC 31** (300 MHz; CDCl_3).

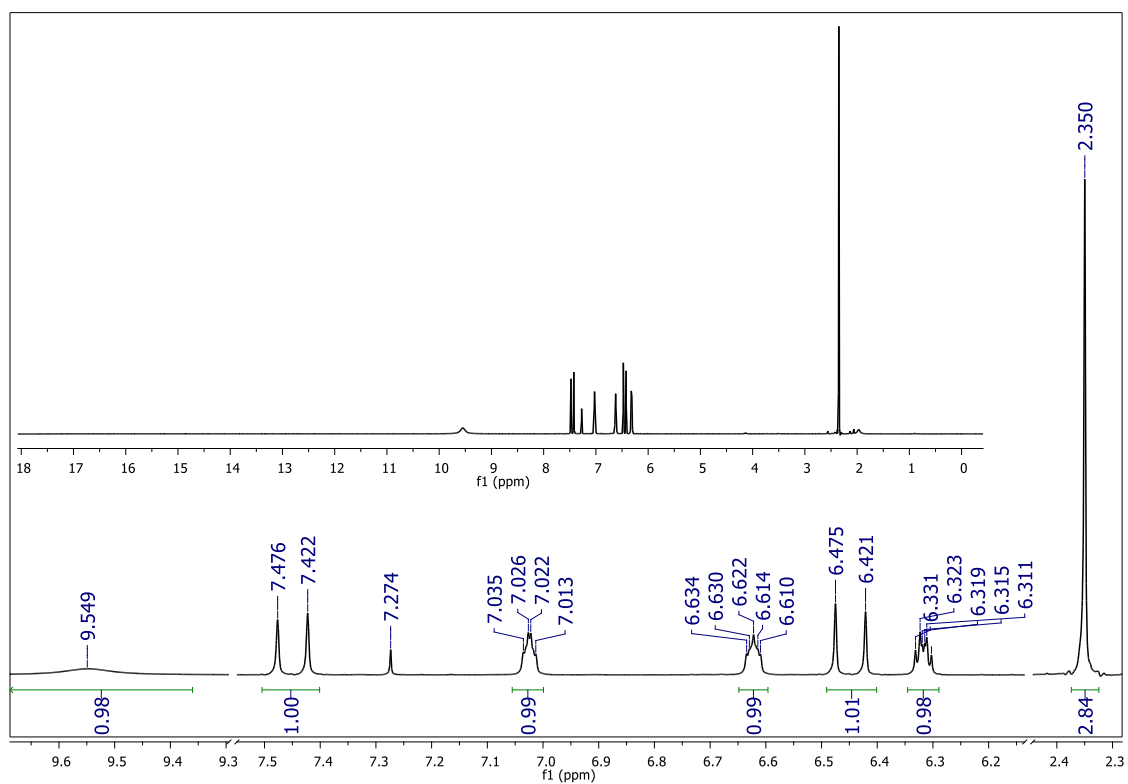


Figure S60. ^{13}C NMR spectrum of **SMAC 31** (75 MHz; CDCl_3).

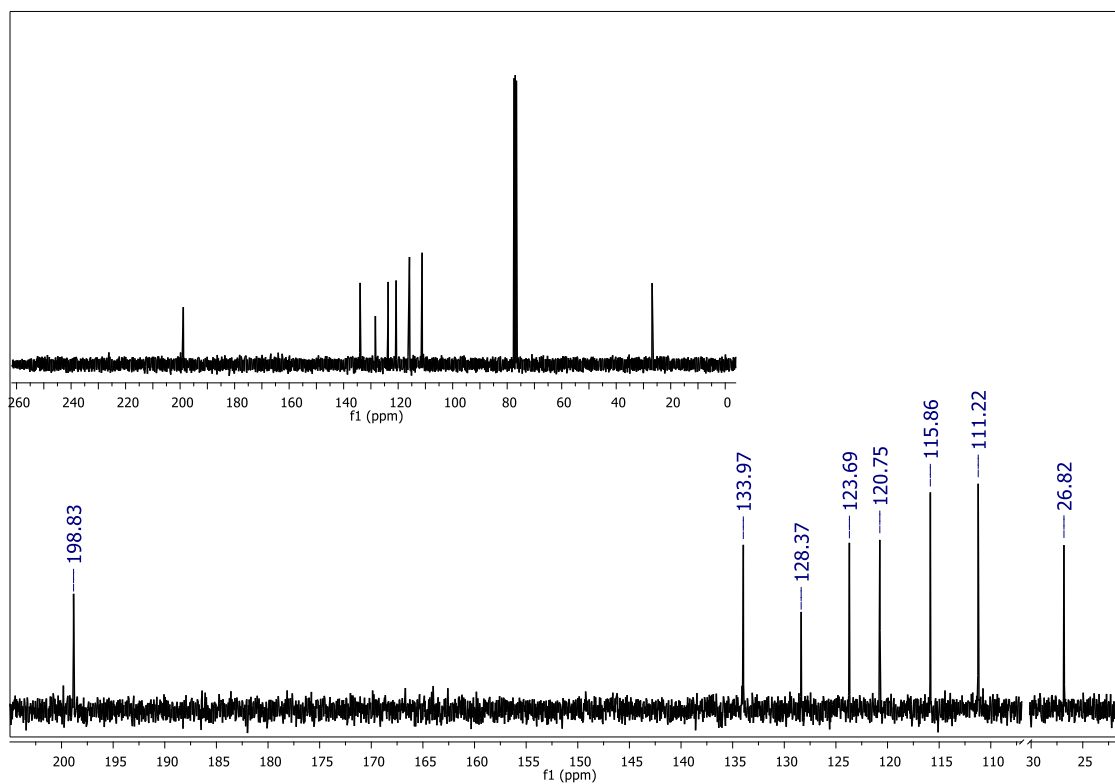


Figure S61. ^1H NMR spectrum of **SMAC 32** (600 MHz; CDCl_3).

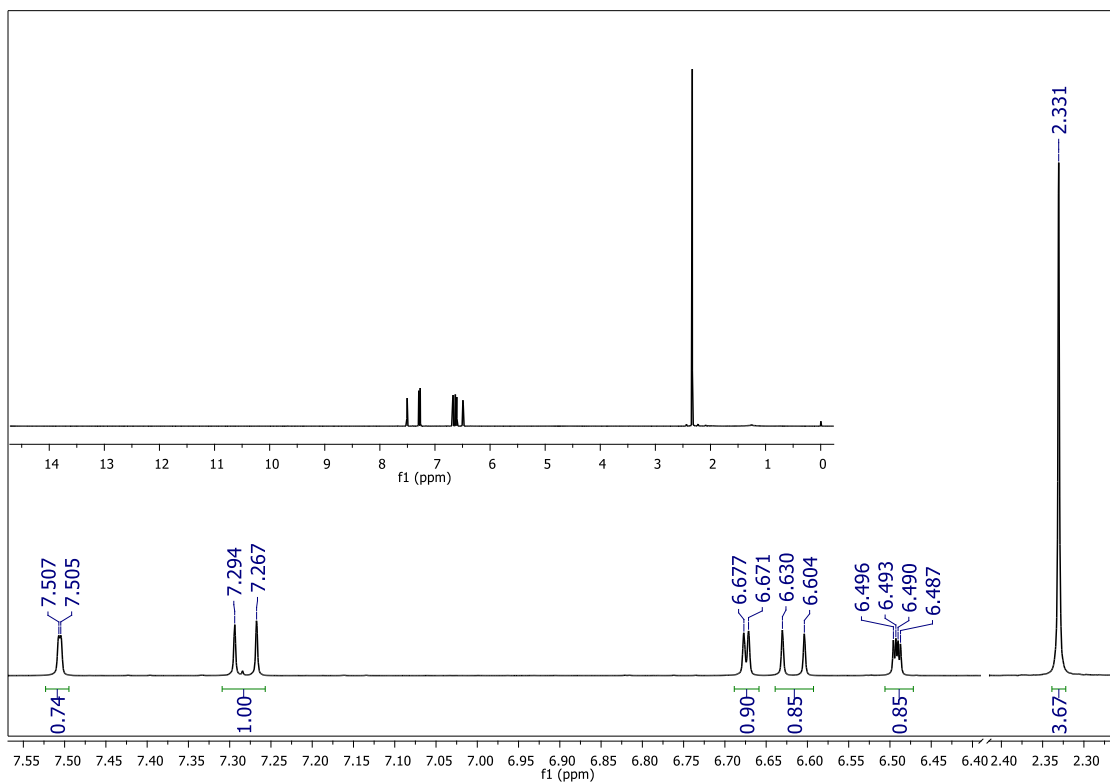


Figure S62. ^{13}C NMR spectrum of **SMAC 32** (150 MHz; CDCl_3).

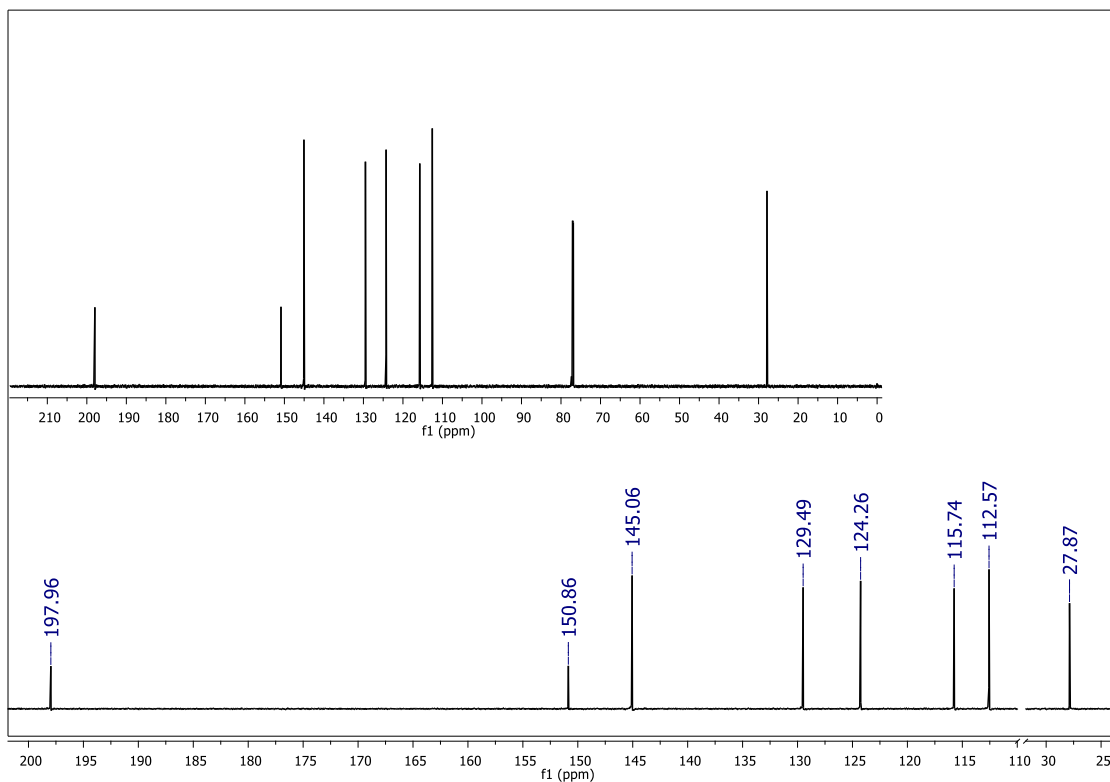


Figure S63. ^1H NMR spectrum of **SMAC 33** (600 MHz; CDCl_3).

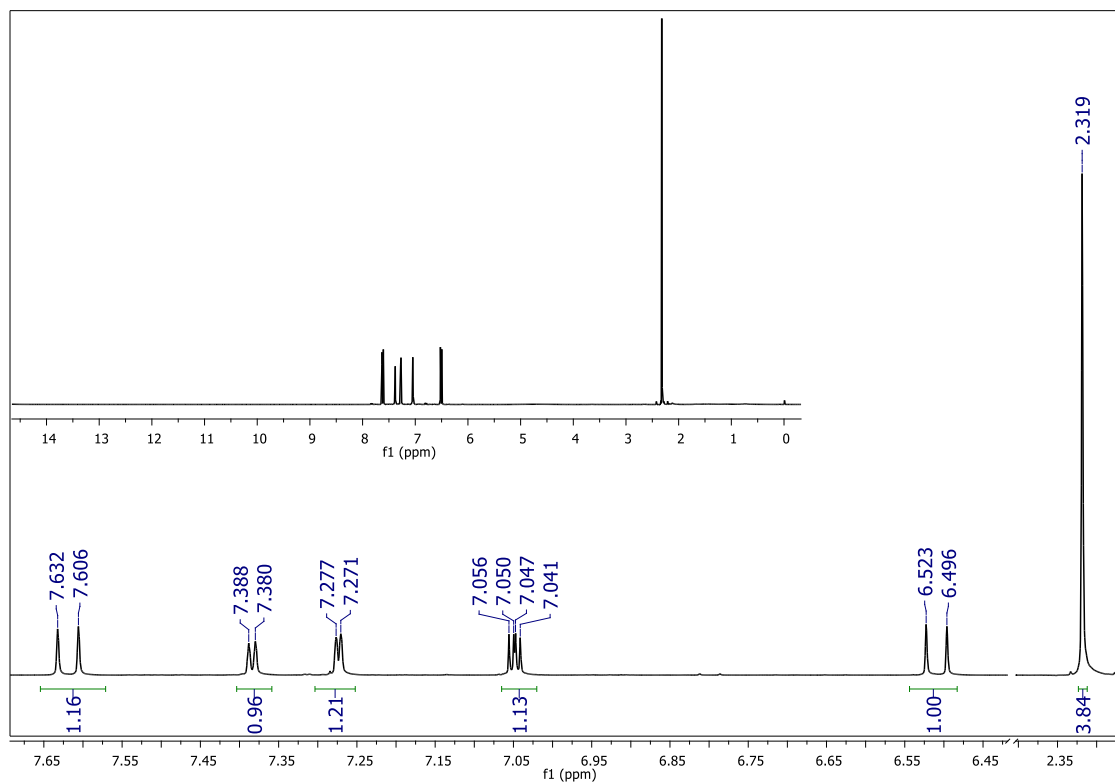


Figure S64. ^{13}C NMR spectrum of **SMAC 33** (150 MHz; CDCl_3).

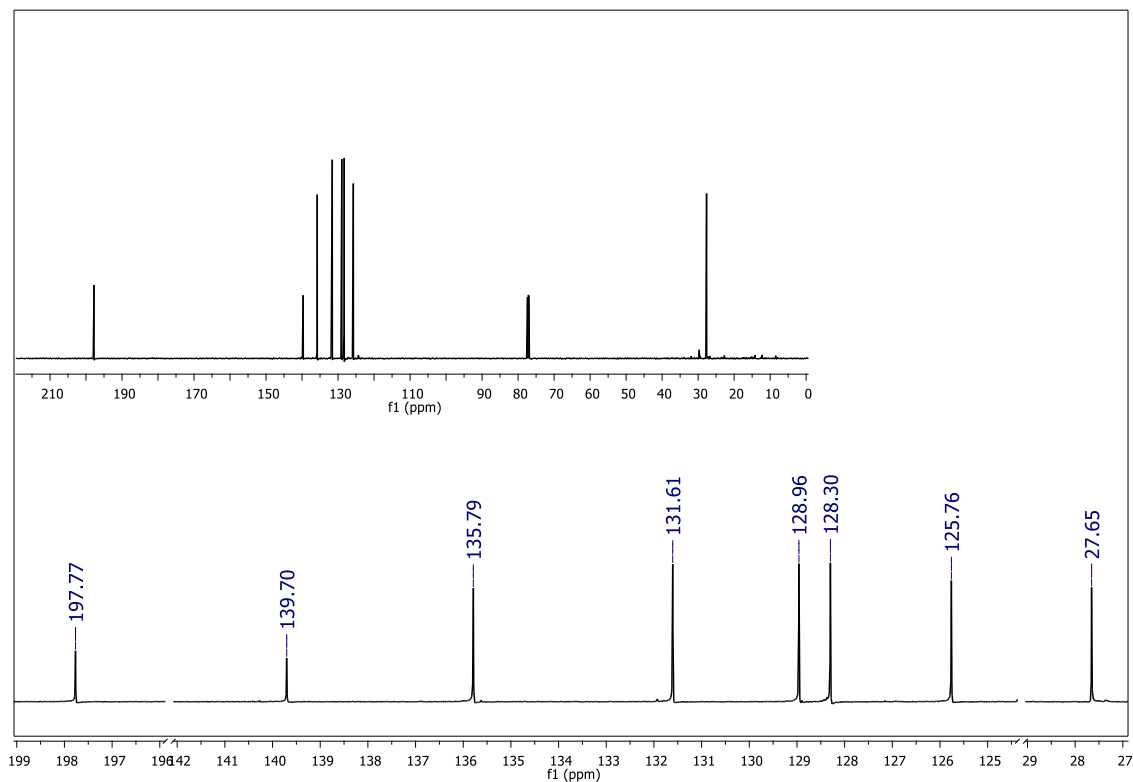


Figure S65. ^1H NMR spectrum of **SMAC 34** (600 MHz; CDCl_3).

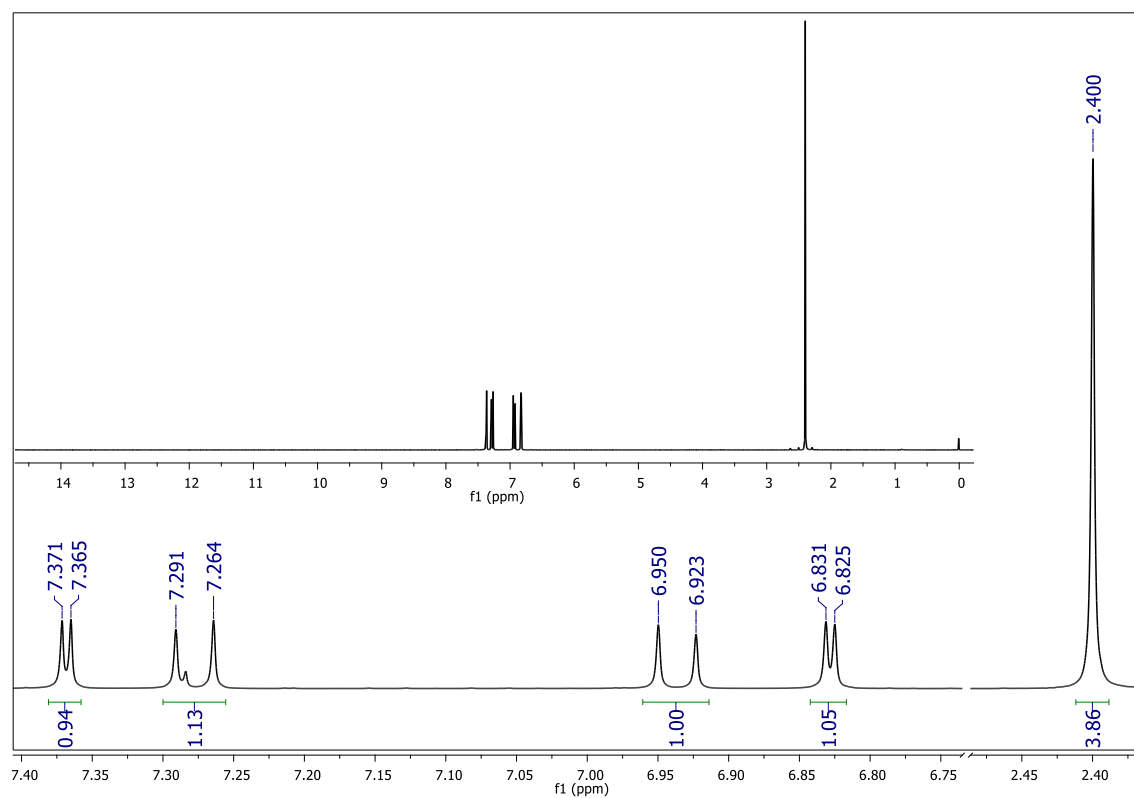


Figure S66. ^{13}C NMR spectrum of **SMAC 34** (150 MHz; CDCl_3).

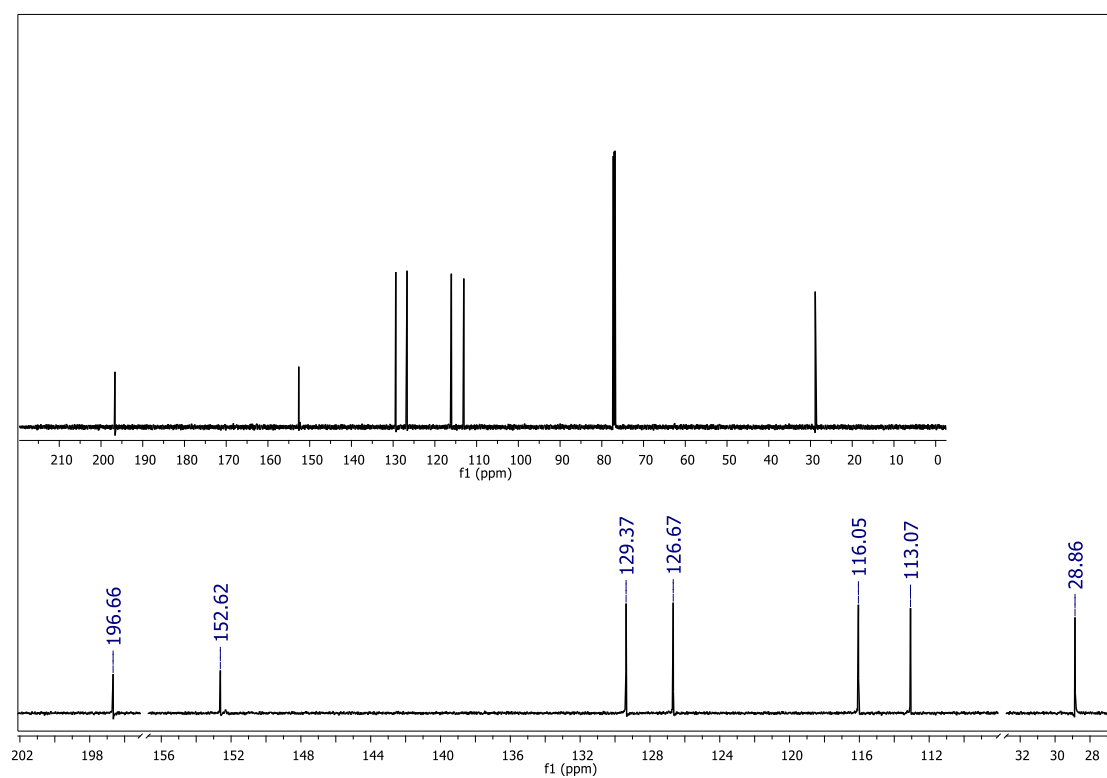


Figure S67. ^1H NMR spectrum of **SMAC 35** (600 MHz; CDCl_3).

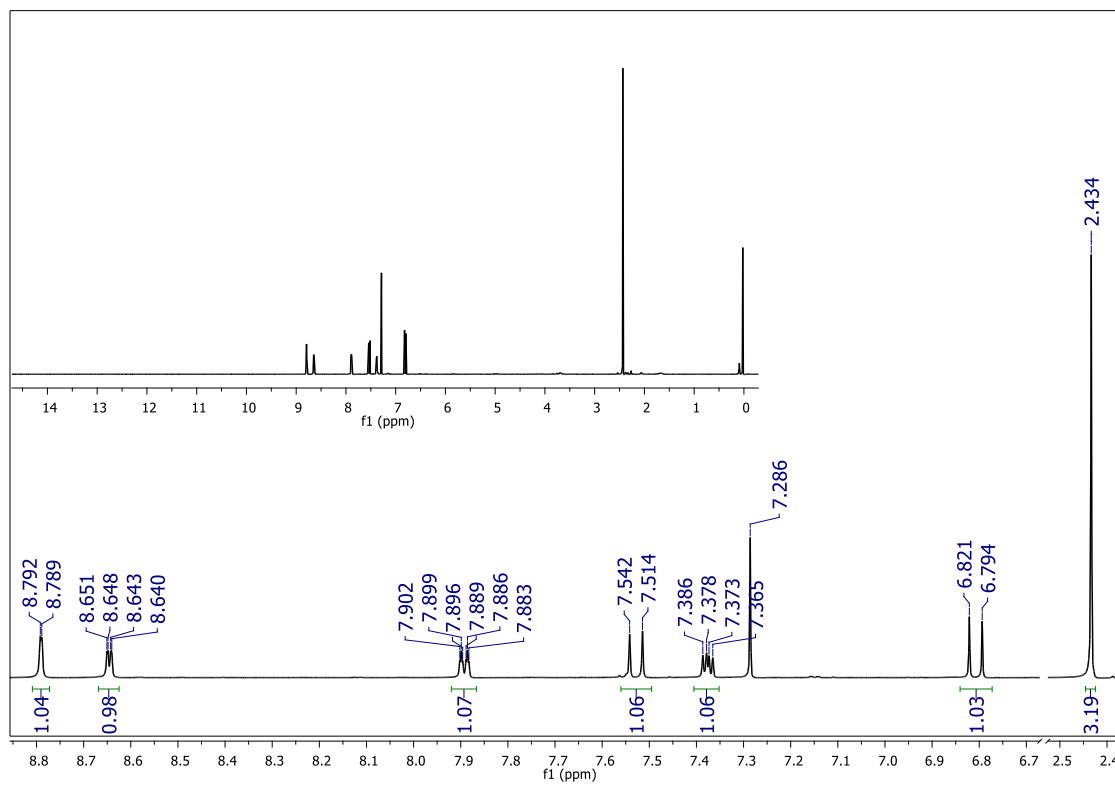


Figure S68. ^{13}C NMR spectrum of **SMAC 35** (150 MHz; CDCl_3).

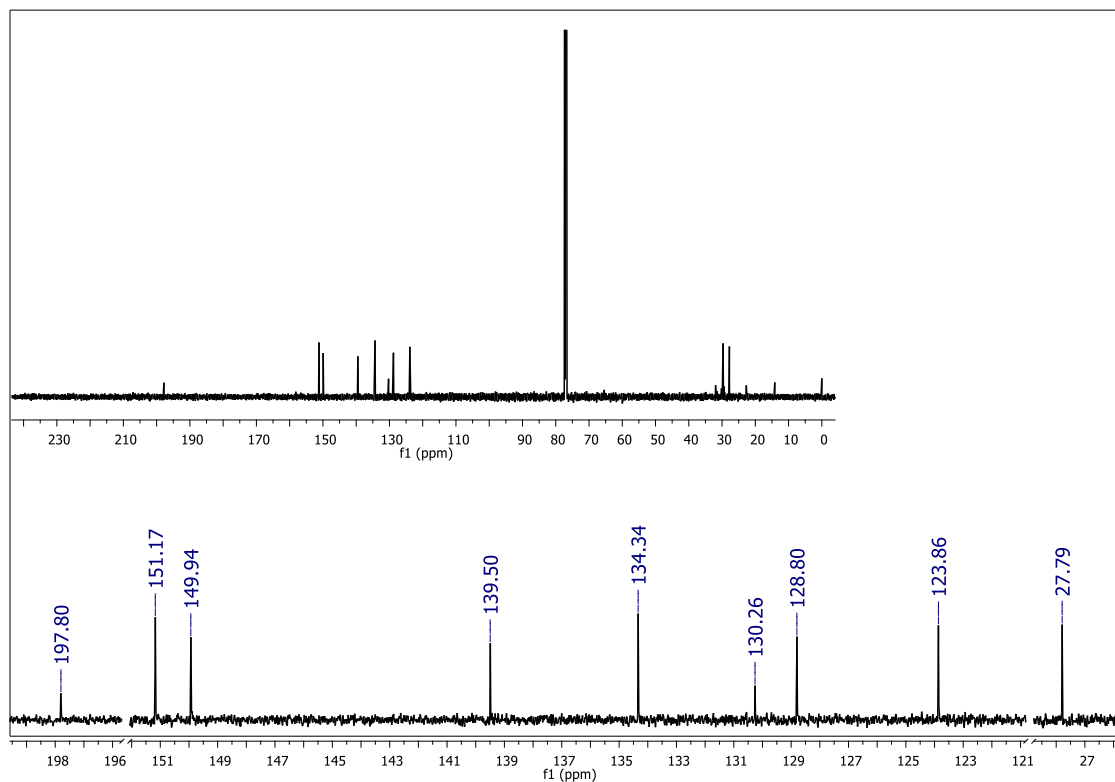


Figure S69. ^1H NMR spectrum of **SMAC 36** (600 MHz; CDCl_3).

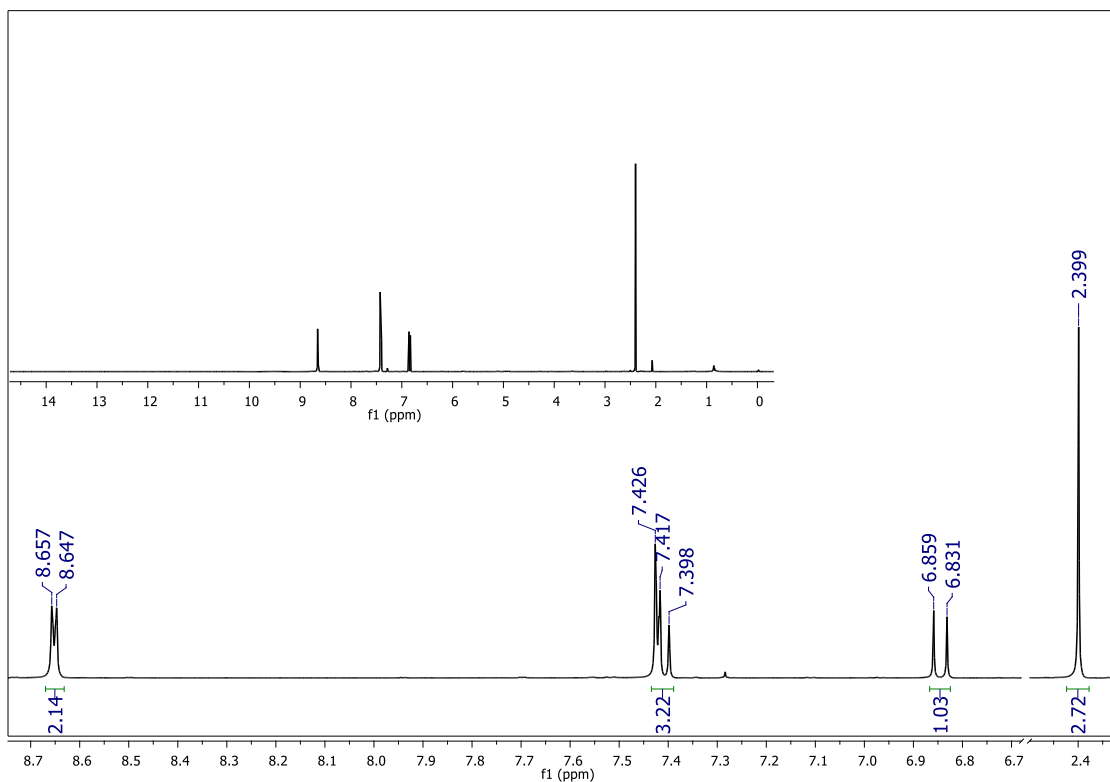


Figure S70. ^{13}C NMR spectrum of **SMAC 36** (150 MHz; CDCl_3).

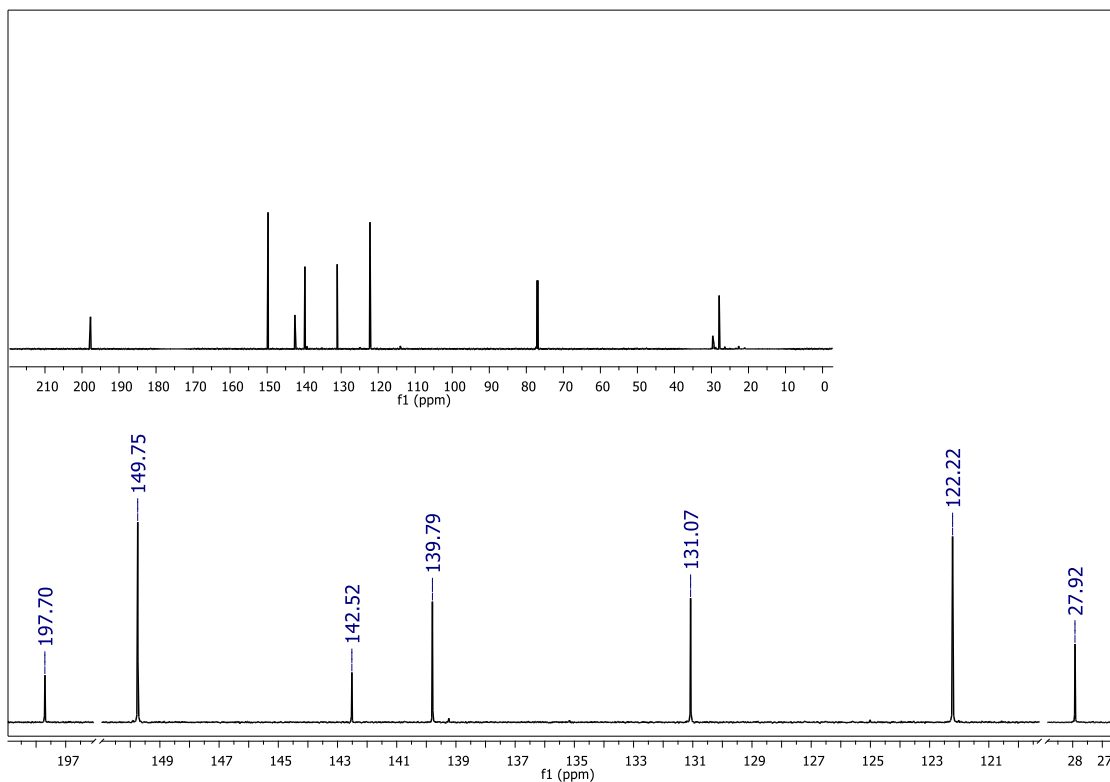


Figure S71. ^1H NMR spectrum of **SMAC 37** (600 MHz; CDCl_3).

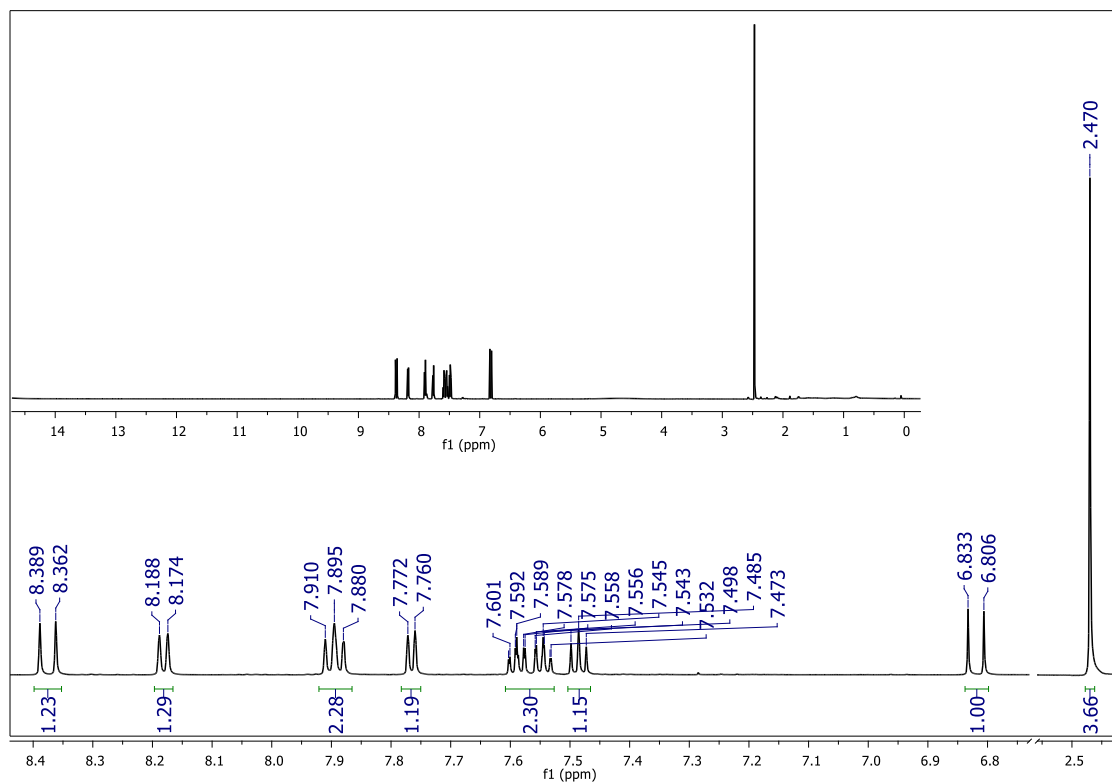


Figure S72. ^{13}C NMR spectrum of **SMAC 37** (150 MHz; CDCl_3).

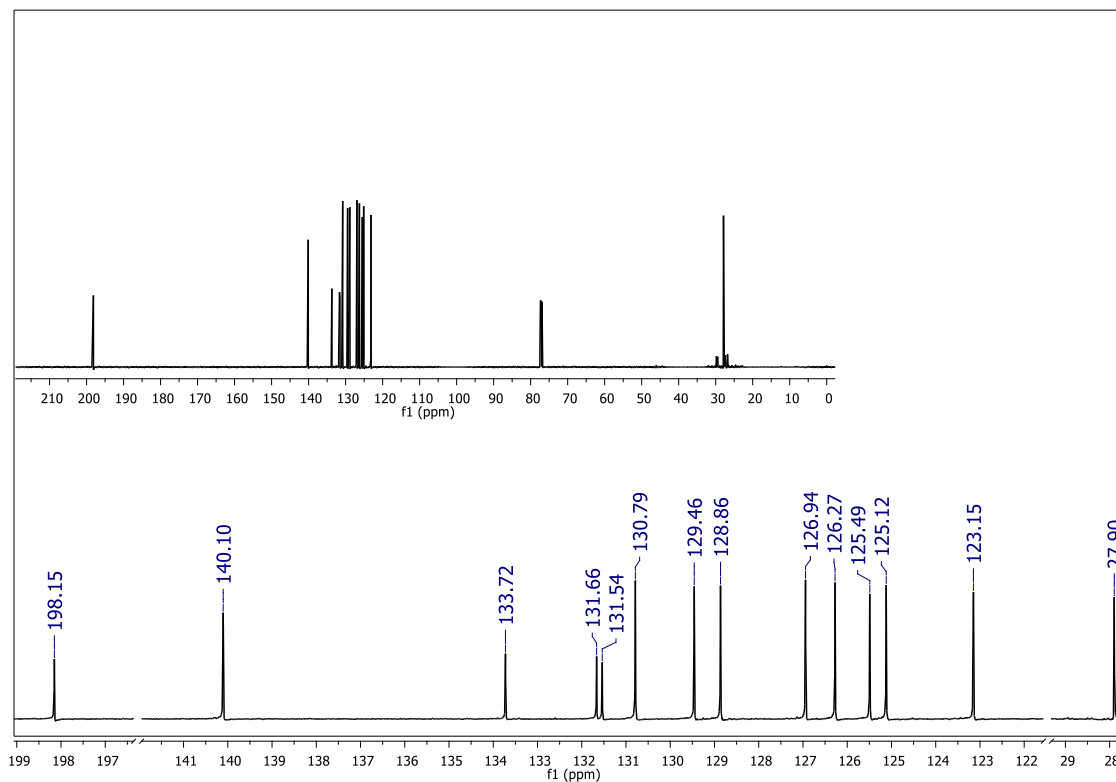


Figure S73. ^1H NMR spectrum of **SMAC 38** (600 MHz; CDCl_3).

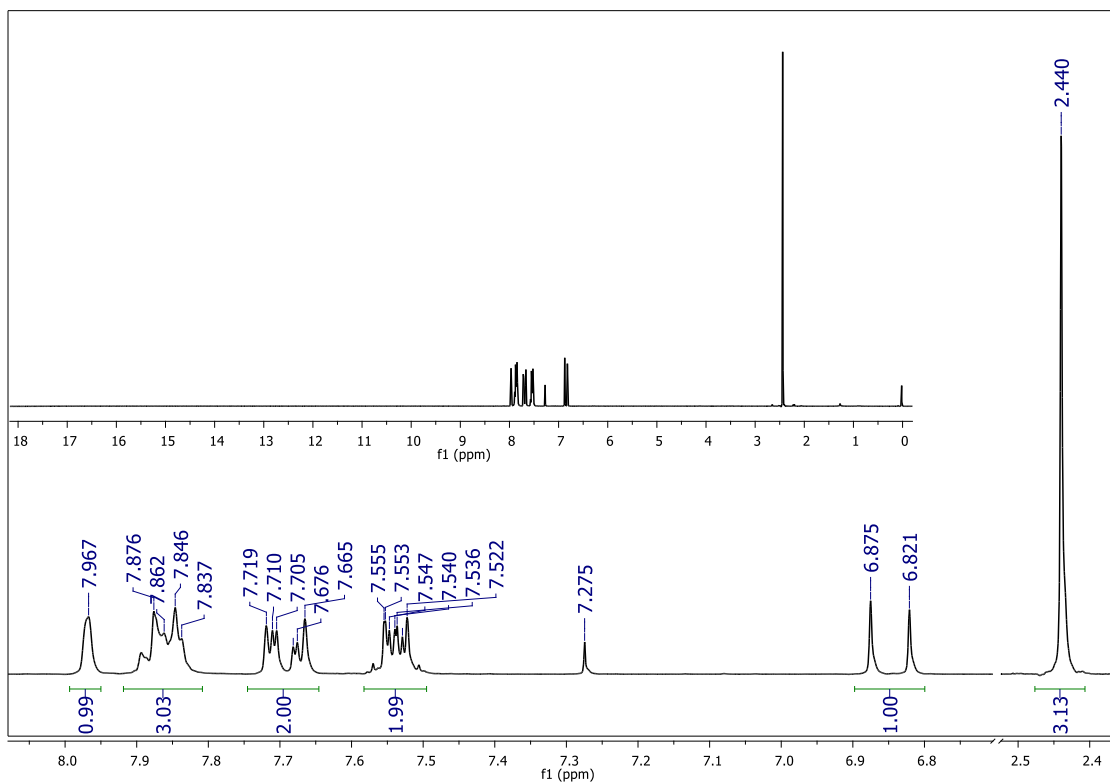


Figure S74. ^{13}C NMR spectrum of **SMAC 38** (150 MHz; CDCl_3).

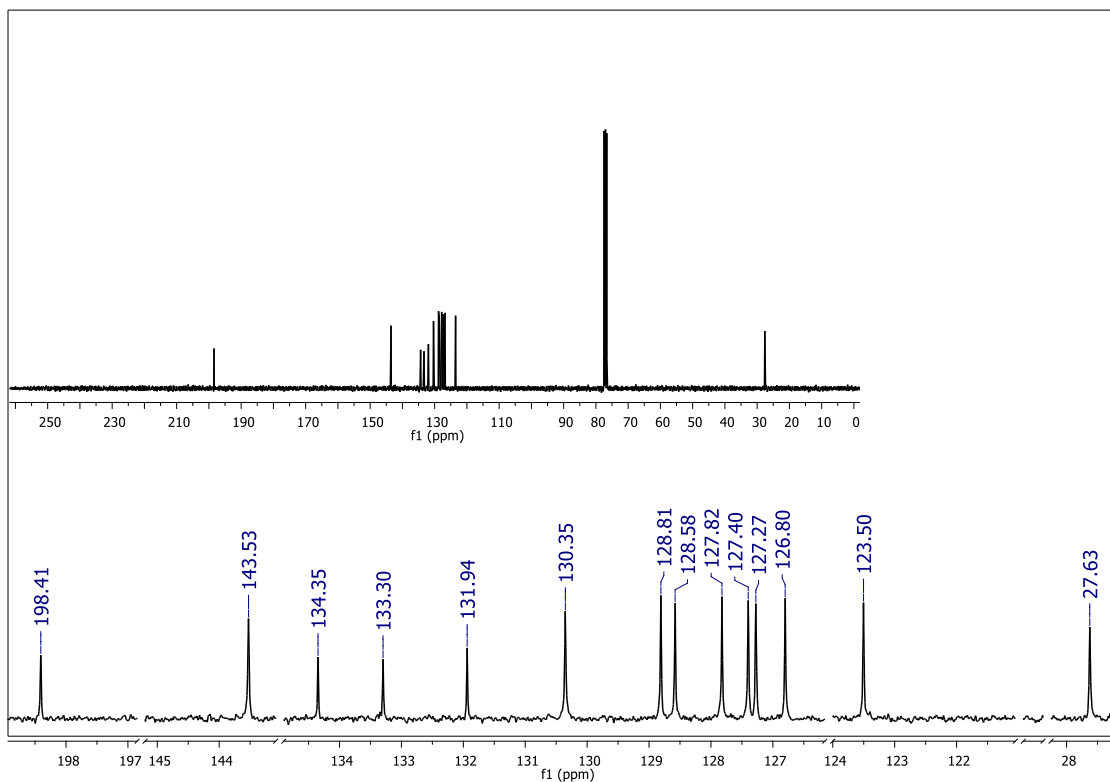


Figure S75. ^1H NMR spectrum of **SMAC 39** (600 MHz; CDCl_3).

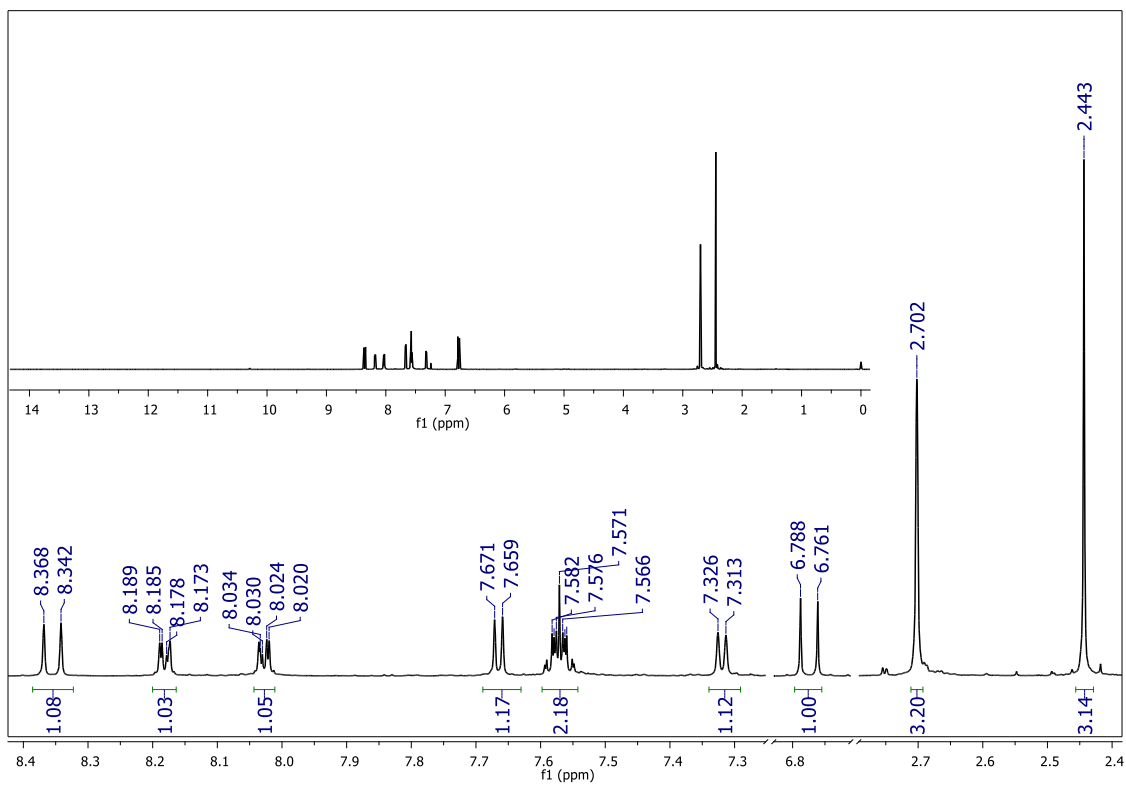


Figure S76. ^{13}C NMR spectrum of **SMAC 39** (150 MHz; CDCl_3).

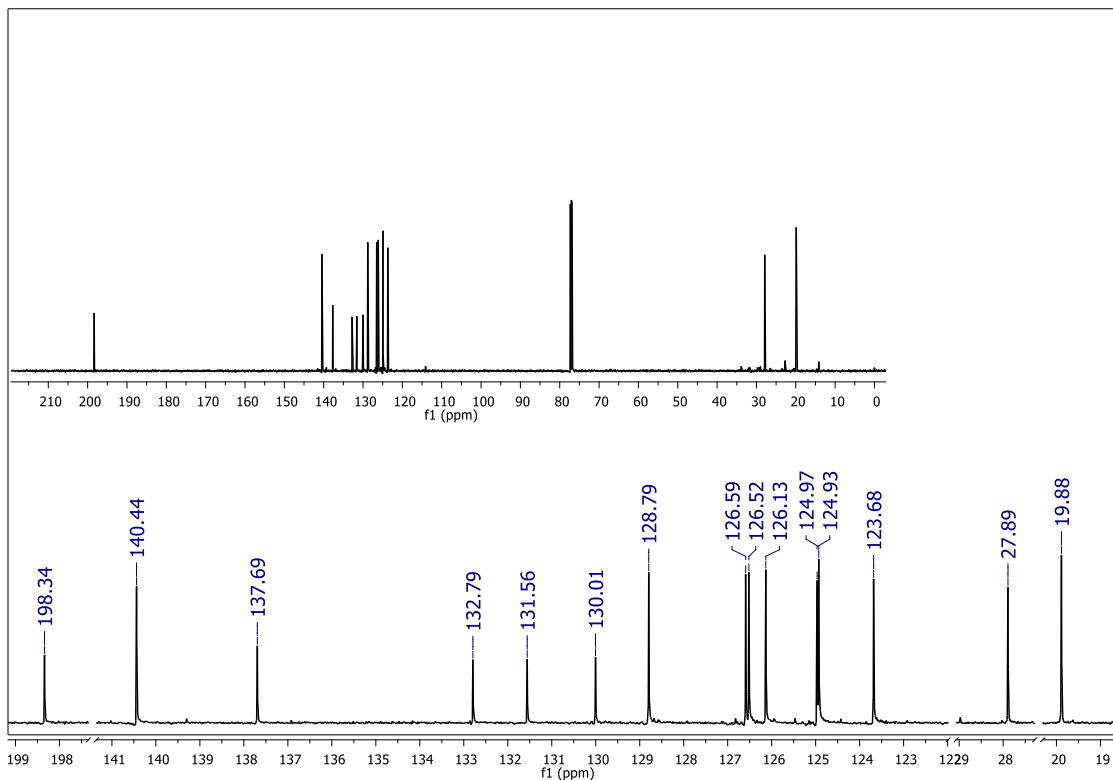


Figure S77. ^1H NMR spectrum of **SMAC 40** (300 MHz; CDCl_3).

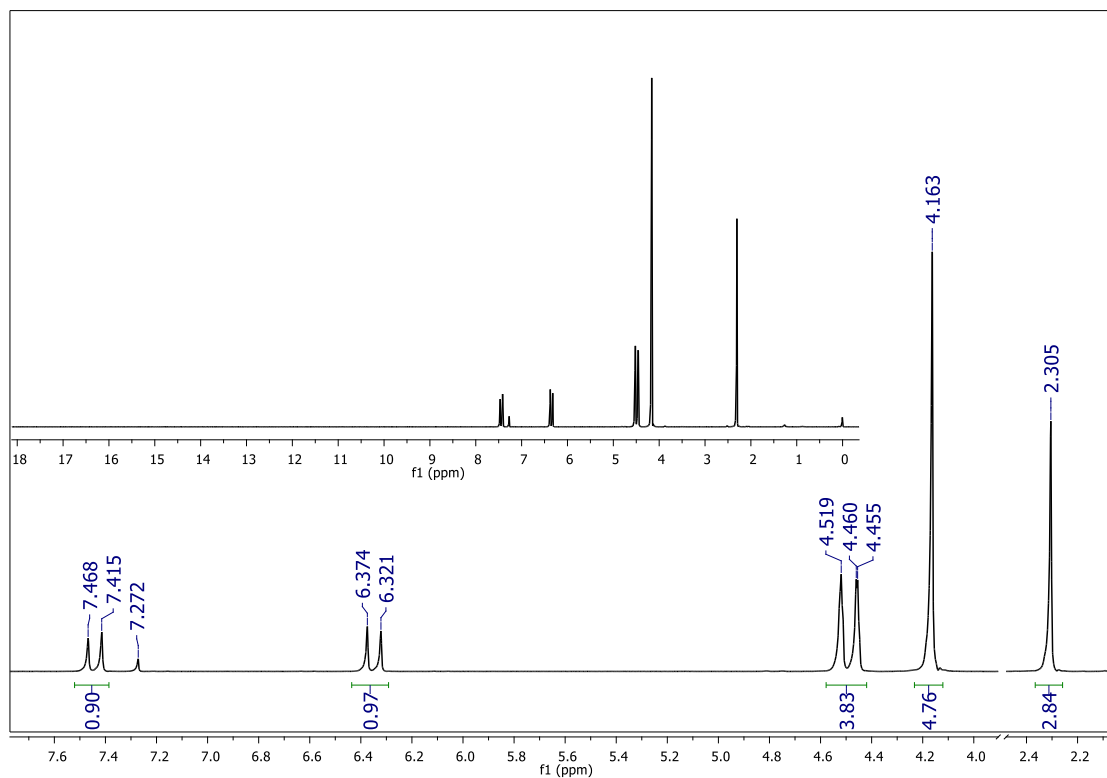
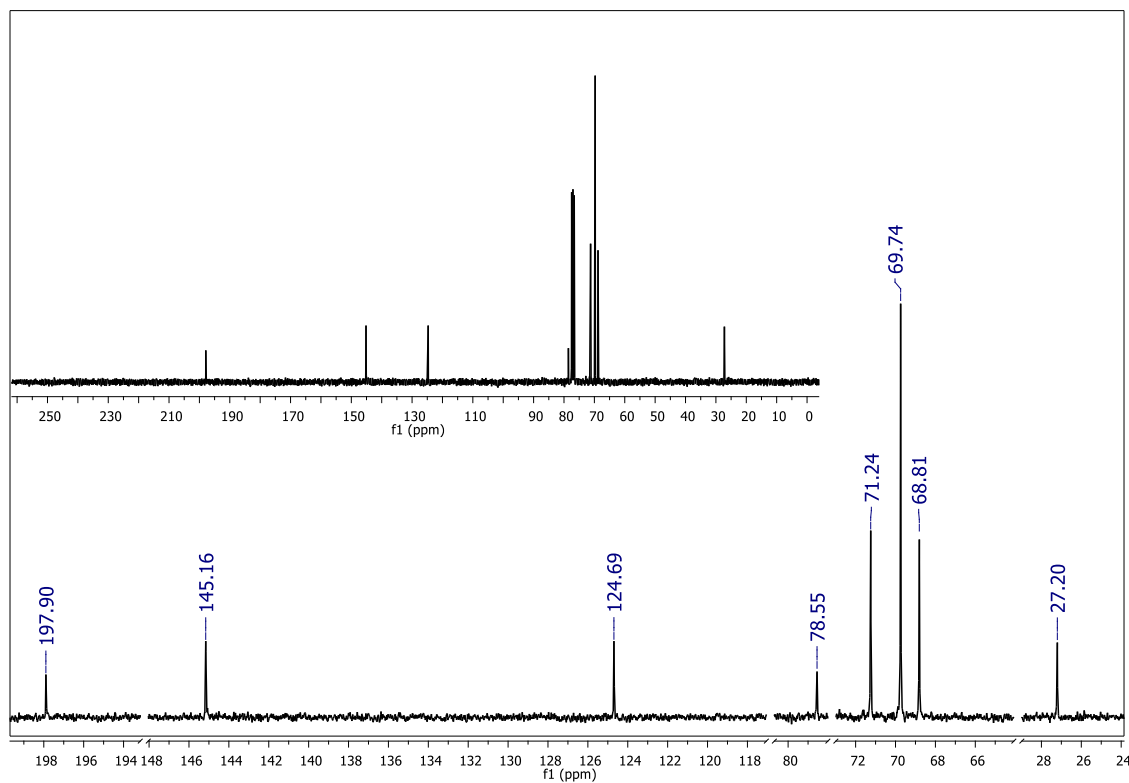


Figure S78. ^{13}C NMR spectrum of **SMAC 40** (75 MHz; CDCl_3).



APÊNDICE C

Material Suplementar do Capítulo V

New Unsymmetrical Monocarbonyl Curcuminoid (MAC 22) Disrupts *Xanthomonas citri* subsp. *citri* Membrane

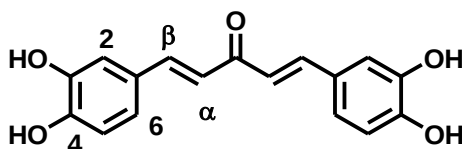
Carlos R. Polaquini^a, Thaíse C. Borges^a, Guilherme Dillari^b, Caio F. C. Zamuner^b, Mario N. Caccalano^b, Henrique Ferreira^{b,*}, Luis O. Regasini^{a,*}

^a Department of Chemistry and Environmental Sciences, Institute of Biosciences, Humanities and Exact Sciences, São Paulo State University (Unesp), São José do Rio Preto 15054-000, SP, Brazil.

^b Department of Biochemistry and Microbiology, Institute of Biosciences, São Paulo State University (Unesp), Rio Claro 13506-900, SP, Brazil.

1. SPECTROSCOPY DATA ANALYSES

1.1 (1*E*,4*E*)-1,5-bis(3,4-dihydroxyphenyl)penta-1,4-dien-3-one (**MAC 10**) (VERMA & THAREJA, 2017; HOTSUMI et al., 2019).



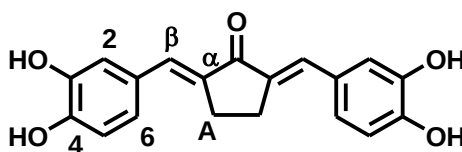
Brown oil

Yield: 12%

¹H NMR (600 MHz; DMSO-*d*₆), δ_{H} in ppm (multiplicity; *J* in Hz): 6.79 (d; 8.4; H-5), 7.00 (d; 15.6; H- α), 7.07 (dd; 1.8 and 8.4; H-6), 7.16 (d; 1.8; H-2), 7.57 (d; 15.6; H- β) (Figure S1).

¹³C NMR (150 MHz; DMSO-*d*₆), δ_{C} in ppm: 115.3 (C-2), 116.3 (C-5), 122.3 (C-6), 122.9 (C- α), 126.6 (C-1), 143.3 (C-4), 146.3 (C- β), 149.4 (C-3), 188.3 (C=O) (Figure S2).

1.2 (2*E*,5*E*)-2,5-bis(3,4-dihydroxybenzylidene)cyclopentanone (**MAC 11**) (LAM et al, 2012; VERMA & THAREJA, 2017).



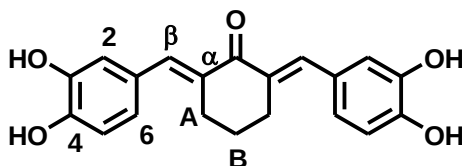
Yellow solid

Yield: 19%

¹H NMR (600 MHz; DMSO-*d*₆), δ_{H} in ppm (multiplicity; *J* in Hz): 3.00 (s; H-A), 6.83 (d; 8.4; H-5), 7.00 (dd; 1.8 and 8.4; H-6), 7.11 (d; 1.8; H-2), 7.24 (s; H- β) (Figure S3).

¹³C NMR (150 MHz; DMSO-*d*₆), δ_{C} in ppm: 26.4 (C-A), 116.4 (C-2), 117.8 (C-5), 124.4 (C-6), 127.5 (C-1), 133.2 (C- α), 134.9 (C- β), 145.9 (C-4), 148.0 (C-3), 195.3 (C=O) (Figure S4).

1.3 (2*E*,6*E*)-2,6-bis(3,4-dihydroxybenzylidene)cyclohexanone (**MAC 12**) (LAM et al., 2012; VERMA & THAREJA, 2017).



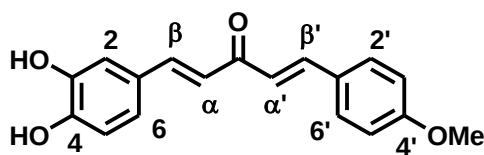
Yellow solid

Yield: 14%

¹H NMR (600 MHz; DMSO-*d*₆), δ_{H} in ppm (multiplicity; *J* in Hz): 1.72 (quint; 5.4; H-B), 2.85 (t; 5.4; H-A), 6.80 (d; 8.4; H-5), 6.88 (dd; 1.8 and 8.4; H-6), 6.99 (d; 1.8; H-2), 7.45 (s; H- β) (Figure S5).

¹³C NMR (150 MHz; DMSO-*d*₆), δ_{C} in ppm: 22.9 (C-B), 28.5 (C-A), 116.2 (C-2), 118.0 (C-5), 123.8 (C-6), 127.4 (C-1), 133.6 (C- α), 136.6 (C- β), 145.6 (C-4), 147.3 (C-3), 188.9 (C=O) (Figure S6).

1.4 (1*E*,4*E*)-1-(3,4-dihydroxyphenyl)-5-(4-methoxyphenyl)penta-1,4-dien-3-one (**MAC 13**) (JIANG et al., 2013).



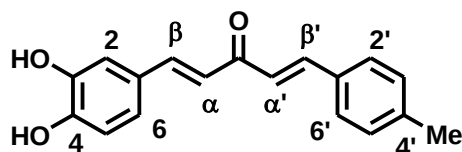
Yellow solid

Yield: 35%

¹H NMR (600 MHz; DMSO-*d*₆), δ_{H} in ppm (multiplicity; *J* in Hz): 3.82 (s; 4'-OMe), 6.79 (d; 7.8; H-5), 6.99 (d; 15.6; H- α), 7.02 (d; 8.4; H-3' and H-5'), 7.08 (dd; 1.8 and 7.8; H-6), 7.16 (d; 1.8; H-2), 7.20 (d; 15.6; H- α'), 7.61 (d; 15.6; H- β), 7.68 (d; 15.6; H- β'), 7.74 (d; 8.4; H-2' and H-6') (Figure S7).

^{13}C NMR (150 MHz; DMSO- d_6), δ_{C} in ppm: 55.8 (4'-OMe), 114.9 (C-3' and C-5'), 115.4 (C-2), 116.3 (C-5), 122.3 (C-6), 123.1 (C- α), 124.0 (C- α'), 126.7 (C-1'), 127.9 (C-1), 130.8 (C-2' and C-6'), 142.3 (C-4), 143.6 (C-3), 146.1 (C- β), 149.0 (C- β'), 161.6 (C-4'), 188.5 (C=O) (Figure S8).

1.5 (1E,4E)-1-(3,4-dihydroxyphenyl)-5-(4-methylphenyl)-1,4-dien-3-one (MAC 14).



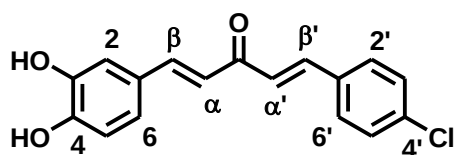
Yellow solid

Yield: 30%

^1H NMR (600 MHz; DMSO- d_6), δ_{H} in ppm (multiplicity; J in Hz): 2.35 (s; 4'-Me), 6.81 (d; 7.8; H-5), 6.99 (d; 15.6; H- α), 7.09 (dd; 1.8 and 7.8; H-6), 7.17 (d; 1.8; H-2), 7.27 (d; 8.4; H-3' and H-5'), 7.30 (d; 15.6; H- α'), 7.64 (d; 15.6; H- β), 7.68 (d; 8.4; H-2' and H-6'), 7.68 (d; 15.6; H- β') (Figure S9).

^{13}C NMR (150 MHz; DMSO- d_6), δ_{C} in ppm: 21.5 (4'-Me), 115.4 (C-2), 116.3 (C-5), 122.4 (C-6), 123.0 (C- α), 125.2 (C- α'), 126.6 (C-1), 129.0 (C-2' and C-6'), 130.0 (C-3' and C-5'), 132.6 (C-1'), 140.8 (C-4'), 142.4 (C- β'), 144.0 (C-4), 146.2 (C- β), 149.2 (C-3), 188.6 (C=O) (Figure S10).

1.6 (1E,4E)-1-(4-chlorophenyl)-5-(3,4-dihydroxyphenyl)penta-1,4-dien-3-one (MAC 15).



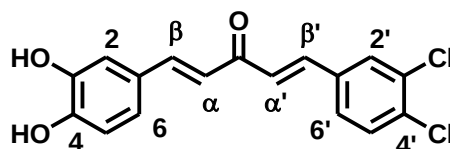
Yellow solid

Yield: 36%

¹H NMR (600 MHz; DMSO-*d*₆), δ_H in ppm (multiplicity; *J* in Hz): 6.81 (d; 7.8; H-5), 6.98 (d; 16.2; H-α), 7.09 (dd; 1.8 and 7.8; H-6), 7.17 (d; 1.8; H-2), 7.35 (d; 16.2; H-α'), 7.49 (d; 8.4; H-3' and H-5'), 7.66 (d; 16.2; H-β), 7.67 (d; 16.2; H-β'), 7.79 (d; 8.4; H-2' and H-6') (Figure S11).

¹³C NMR (150 MHz; DMSO-*d*₆), δ_C in ppm: 115.3 (C-2), 116.3 (C-5), 122.6 (C-6), 122.9 (C-α), 126.5 (C-1), 126.8 (C-α'), 129.4 (C-2' and C-6'), 130.6 (C-3' and C-5'), 134.2 (C-1'), 135.3 (C-4'), 140.9 (C-β), 144.6 (C-4), 146.2 (C-β'), 149.5 (C-3), 188.6 (C=O) (Figure S12).

1.7 (1*E*,4*E*)-1-(3,4-dichlorophenyl)-5-(3,4-dihydroxyphenyl)penta-1,4-dien-3-one (MAC 16).



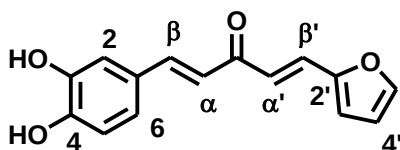
Yellow solid

Yield: 25%

¹H NMR (600 MHz; DMSO-*d*₆), δ_H in ppm (multiplicity; *J* in Hz): 6.80 (d; 8.4; H-5), 6.93 (d; 15.6; H-α), 7.09 (dd; 2.4 and 8.4; H-6), 7.15 (d; 2.4; H-2), 7.50 (d; 15.6; H-α'), 7.65 (d; 15.6; H-β), 7.71 (d; 15.6; H-β'), 7.72 (d; 8.4; H-5'), 7.79 (dd; 1.8 and 8.4; H-6), 8.13 (d; 1.8; H-2') (Figure S13).

¹³C NMR (150 MHz; DMSO-*d*₆), δ_C in ppm: 115.3 (C-2), 116.3 (C-5), 122.5 (C-6), 123.1 (C-α), 126.4 (C-1), 127.8 (C-α'), 129.0 (C-6'), 130.4 (C-2'), 131.5 (C-5'), 132.2 (C-4'), 132.8 (C-3'), 136.3 (C-1'), 139.4 (C-β), 144.9 (C-4), 146.2 (C-β'), 149.6 (C-3), 188.4 (C=O) (Figure S14).

1.8 (1*E*,4*E*)-1-(furan-2-yl)-5-(3,4-dihydroxyphenyl)penta-1,4-dien-3-one (**MAC 17**).



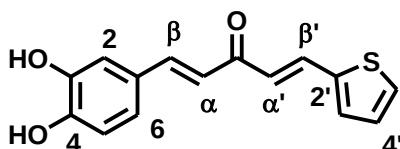
Red solid

Yield: 19%

¹H NMR (600 MHz; DMSO-*d*₆), δ_{H} in ppm (multiplicity; *J* in Hz): 6.65 (dd; 1.8 and 3.6; H-4'), 6.80 (d; 8.4; H-5), 6.98 (d; 16.2; H- α), 6.99 (d; 3.6; H-3'), 7.00 (d; 16.2; H- α'), 7.09 (dd; 1.8 and 8.4; H-6), 7.17 (d; 1.8; H-2), 7.54 (d; 16.2; H- β), 7.57 (d; 16.2; H- β'), 7.83 (d; 1.8; H-5') (Figure S15).

¹³C NMR (150 MHz; DMSO-*d*₆), δ_{C} in ppm: 113.5 (C-3'), 115.5 (C-2), 116.3 (C-5), 116.7 (C-4'), 122.5 (C-6), 122.8 (C- α'), 123.5 (C- α), 126.5 (C-1), 129.1 (C- β'), 143.9 (C-4), 146.2 (C- β), 146.3 (C-5'), 149.4 (C-3), 151.6 (C-2'), 188.0 (C=O) (Figure S16).

1.9 (1*E*,4*E*)-1-(3,4-dihydroxyphenyl)-5-(thiophen-2-yl)penta-1,4-dien-3-one (**MAC 18**).



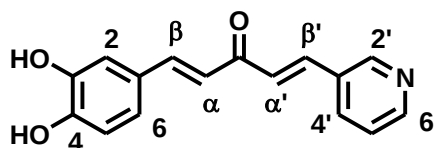
Yellow solid

Yield: 21%

¹H NMR (600 MHz; DMSO-*d*₆), δ_{H} in ppm (multiplicity; *J* in Hz): 6.81 (d; 8.4; H-5), 6.98 (d; 15.6; H- α), 7.03 (d; 16.2; H- α'), 7.10 (dd; 1.8 and 8.4; H-6), 7.17–7.18 (H-2 and H-4'), 7.57 (d; 4.8; H-5'), 7.58 (d; 15.6; H- β), 7.74 (d; 5.4; H-3'), 7.90 (d; 16.2; H- β') (Figure S17).

¹³C NMR (150 MHz; DMSO-*d*₆), δ_{C} in ppm: 115.6 (C-2), 116.3 (C-5), 122.4 (C-6), 122.7 (C- α), 125.2 (C- α'), 126.7 (C-1), 129.1 (C-3'), 130.3 (C-4'), 132.7 (C-5'), 135.2 (C- β'), 140.4 (C-2'), 143.9 (C-4), 146.1 (C- β), 149.2 (C-3), 188.0 (C=O) (Figure S18).

1.10 (1*E*,4*E*)-1-(3,4-dihydroxyphenyl)-5-(pyridin-3-yl)penta-1,4-dien-3-one (**MAC 19**).



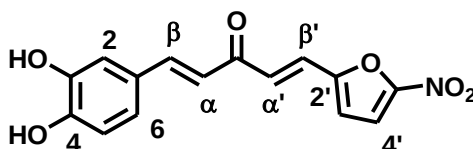
Yellow solid

Yield: 12%

¹H NMR (600 MHz; DMSO-*d*₆), δ_H in ppm (multiplicity; *J* in Hz): 6.82 (d; 8.4; H-5), 6.99 (d; 16.2; H-α), 7.10 (dd; 1.8 e 8.4; H-6), 7.17 (H-2), 7.48 (dd; 4.8 and 8.7; H-5'), 7.50 (d; 16.2; H-α'), 7.69 (d; 16.2; H-β), 7.71 (d; 16.2; H-β'), 8.22 (d; 8.7; H-4'), 8.59 (dd; 1.2 and 4.8; H-6'), 8.92 (H-2') (Figure S19).

¹³C NMR (150 MHz; DMSO-*d*₆), δ_C in ppm: 115.4 (C-2), 116.3 (C-5), 122.5 (C-6), 123.0 (C-5'), 124.5 (C-α), 126.6 (C-1), 127.7 (C-α'), 131.2 (C-3'), 135.2 (C-4'), 138.9 (C-β), 144.8 (C-4), 146.1 (C-β'), 149.3 (C-3), 150.4 (C-6'), 151.2 (C-2'), 188.5 (C=O) (Figure S20).

1.11 (1*E*,4*E*)-1-(3,4-dihydroxyphenyl)-5-(5-nitrofuran-2-yl)penta-1,4-dien-3-one (**MAC 20**).



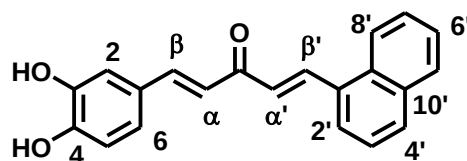
Red solid

Yield: 19%

¹H NMR (600 MHz; DMSO-*d*₆), δ_H in ppm (multiplicity; *J* in Hz): 6.81 (d; 8.4; H-5), 7.08 (d; 16.2; H-α), 7.13 (dd; 1.8 and 8.4; H-6), 7.18 (d; 1.8; H-2), 7.33 (d; 16.2; H-α'), 7.33 (d; 3.6; H-3'), 7.58 (d; 16.2; H-β), 7.65 (d; 16.2; H-β'), 7.80 (d; 3.6; H-4') (Figure S21).

¹³C NMR (150 MHz; DMSO-*d*₆), δ_C in ppm : 115.3 (C-2), 115.7 (C-3'), 116.3 (C-5), 117.8 (C-4'), 122.7 (C-6), 122.8 (C-α), 126.5 (C-1), 127.2 (C-α'), 129.4 (C-β'), 145.3 (C-4), 146.1 (C-β), 149.5 (C-3), 152.4 (C-2'), 153.9 (C-5'), 187.5 (C=O). (Figure S22).

1.12 (1E,4E)-1-(3,4-dihydroxyphenyl)-5-(naphthalen-1-yl)penta-1,4-dien-3-one (MAC 21).



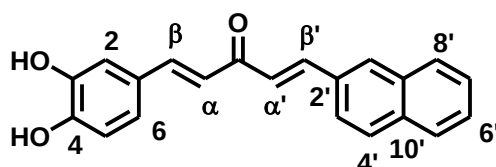
Yellow solid

Yield: 23%

¹H NMR (600 MHz; DMSO-*d*₆), δ_{H} in ppm (multiplicity; *J* in Hz): 6.81 (d; 8.4; H-5), 7.14 (d; 16.2; H- α), 7.15 (dd; 2.4 and 8.4; H-6), 7.22 (d; 2.4; H-2), 7.41 (d; 16.2; H- α'), 7.60–7.68 (H-6' and H-7'), 7.61 (dd; 7.2 and 8.4; H-3'), 7.70 (d; 16.2; H- β), 8.01–8.09 (H-2', H-5' and H-8'), 8.36 (d; 8.4; H-4'), 8.51 (d; 16.2; H- β') (Figure S23).

¹³C NMR (150 MHz; DMSO-*d*₆), δ_{C} in ppm: 115.5 (C-2), 116.3 (C-5), 122.6 (C-6), 122.7 (C-2'), 123.8 (C- α), 125.7 (C- α'), 126.2 (C-8'), 126.6 (C-1), 126.8 (C-3'), 127.6 (C-6'), 129.1 (C-7'), 129.2 (C-4'), 131.0 (C-5'), 131.6 (C-10'), 132.2 (C-9'), 133.8 (C-1'), 138.4 (C- β), 144.5 (C-4), 146.2 (C- β'), 149.6 (C-3), 188.6 (C=O) (Figure S24).

1.13 (1E,4E)-1-(3,4-dihydroxyphenyl)-5-(naphthalen-2-yl)penta-1,4-dien-3-one (MAC 22).



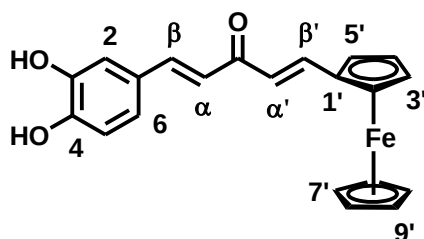
Yellow solid

Yield: 32%

¹H NMR (600 MHz; DMSO-*d*₆), δ_{H} in ppm (multiplicity; *J* in Hz): 6.84 (d; 7.8; H-5), 7.05 (d; 16.2; H- α), 7.13 (d; 1.8 and 7.8; H-6), 7.21 (d; 1.8; H-2), 7.50 (d; 16.2; H- α'), 7.56–7.58 (H-6' and H-7'), 7.72 (d; 16.2; H- β), 7.88 (d; 16.2; H- β'), 7.94–7.99 (H-3', H-4', H-5' and H-8'), 8.26 (H-1') (Figure S25).

¹³C NMR (150 MHz; DMSO-*d*₆), δ_C in ppm: 115.5 (C-2), 116.3 (C-5), 122.4 (C-6), 123.1 (C-α), 124.6 (C-α'), 126.5 (C-3'), 126.6 (C-1), 127.3 (C-1'), 127.8 (C-6'), 128.2 (C-7'), 128.9 (C-5'), 129.0 (C-8'), 130.6 (C-4'), 133.0 (C-9'), 133.5 (C-10'), 134.2 (C-2'), 142.3 (C-β'), 144.3 (C-4), 146.2 (C-β), 149.4 (C-3), 188.6 (C=O) (Figure S26).

1.14 (1*E*,4*E*)-1-(3,4-dihydroxyphenyl)-5-(ferrocen-1-yl)penta-1,4-dien-3-one (MAC 23)



Red oil

Yield: 23%

¹H NMR (600 MHz; DMSO-*d*₆), δ_H in ppm (multiplicity; *J* in Hz): 4.17 (s; H-6' to H-10'), 4.51 (s; H-3' and H-4'), 4.73 (s; H-2' and H-5'), 6.46–7.62 (H-2, H-5, H-6, H-α, H-α', H-β and H-β') (Figure S27).

¹³C NMR (150 MHz; DMSO-*d*₆), δ_C in ppm: 68.6 (C-3' and C-4'), 69.3 (C-6' to C-10'), 70.8 (C-2' and C-5'), 79.1 (C-1'), 114.7 (C-2), 115.6 (C-5), 121.4 (C-6), 122.2 (C-α), 123.2 (C-α'), 126.1 (C-1), 142.3 (C-β), 143.7 (C-β'), 145.4 (C-4), 148.2 (C-3), 187.0 (C=O) (Figure S28)

Figure S1. ^1H NMR spectrum of **MAC 10** (600 MHz; $\text{DMSO-}d_6$).

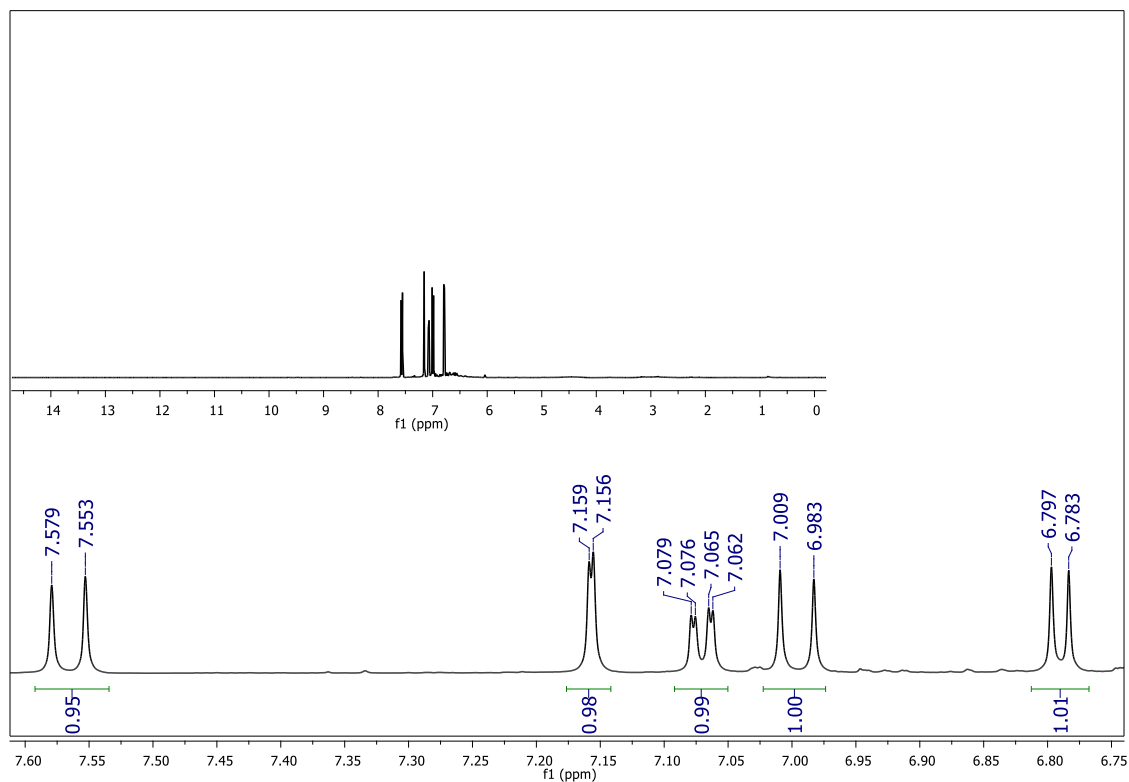


Figure S2. ^{13}C NMR spectrum of **MAC 10** (150 MHz; $\text{DMSO-}d_6$).

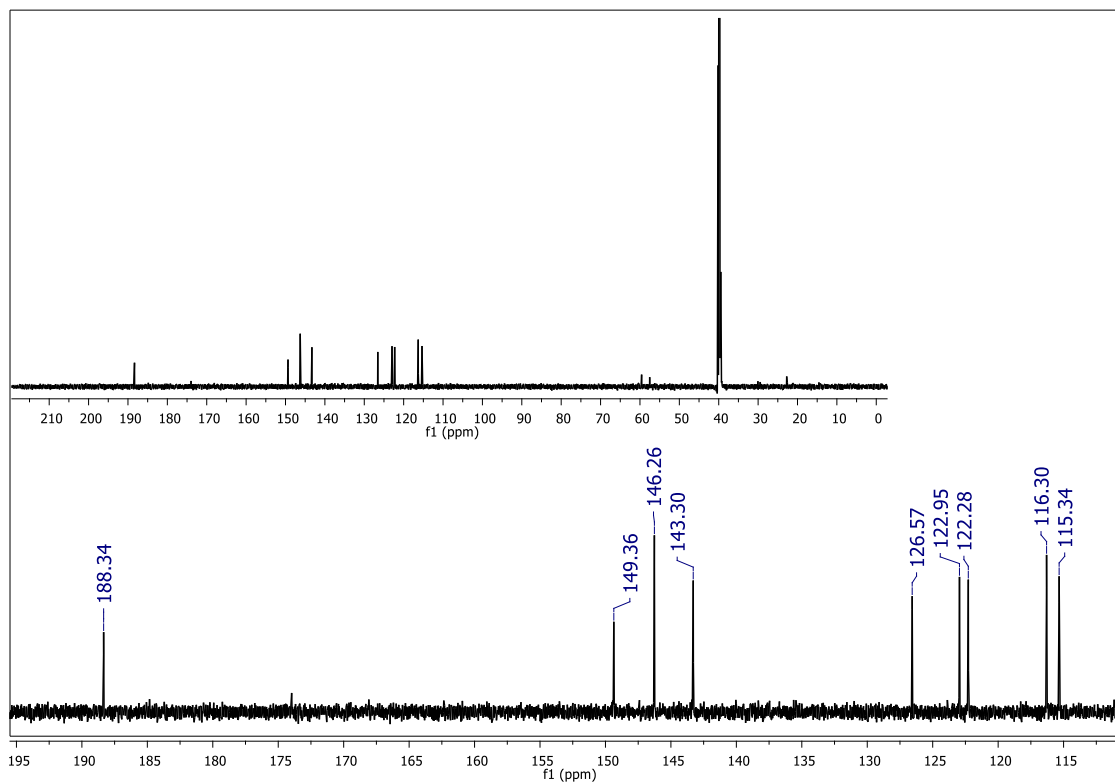


Figure S3. ^1H NMR spectrum of **MAC 11** (600 MHz; $\text{DMSO-}d_6$).

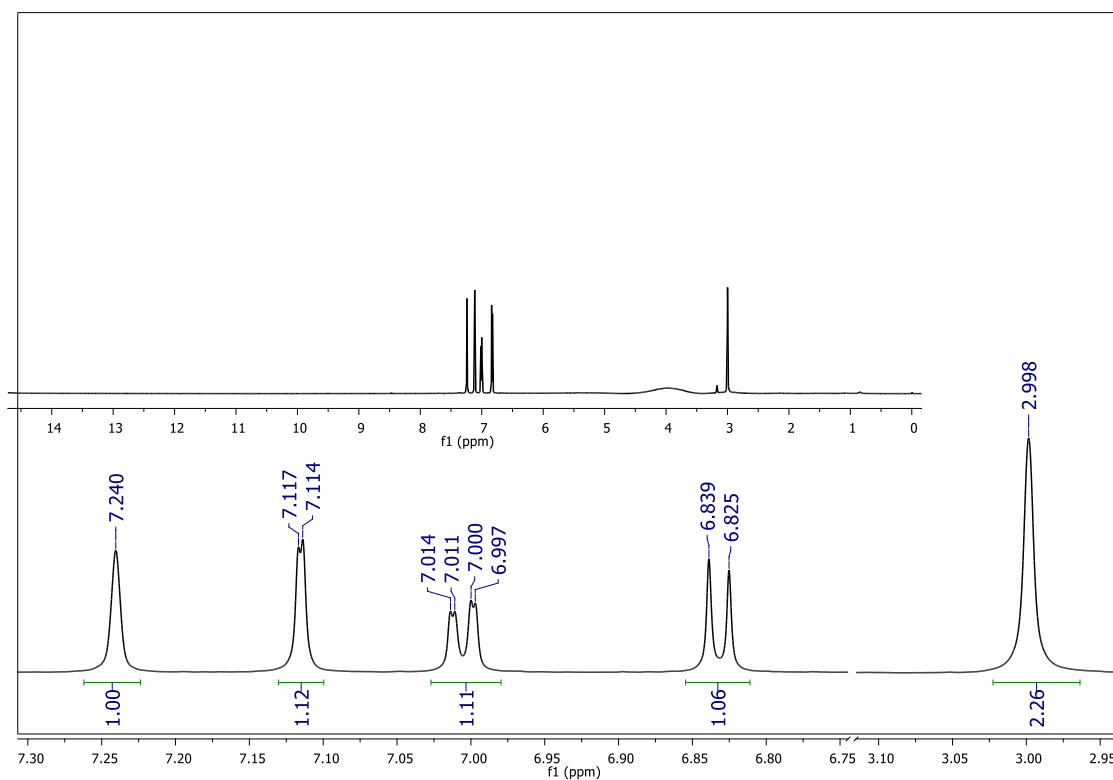


Figure S4. ^{13}C NMR spectrum of **MAC 11** (150 MHz; $\text{DMSO-}d_6$).

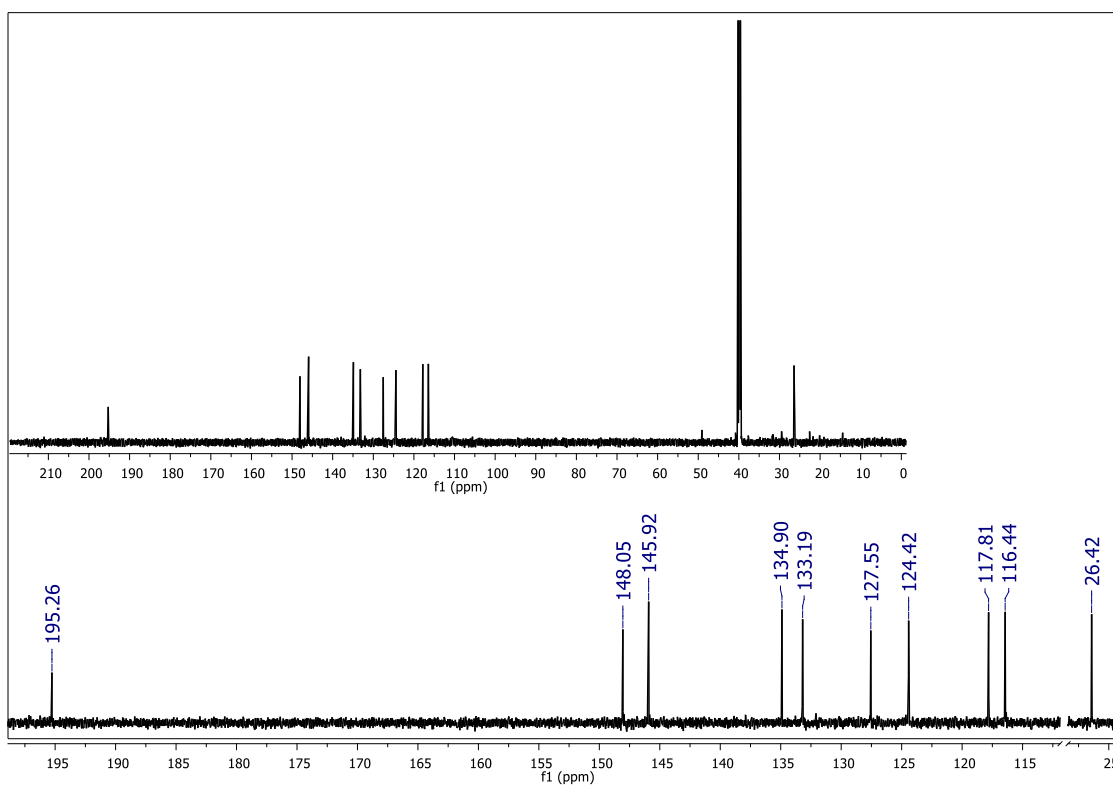


Figure S5. ^1H NMR spectrum of **MAC 12** (600 MHz; $\text{DMSO-}d_6$).

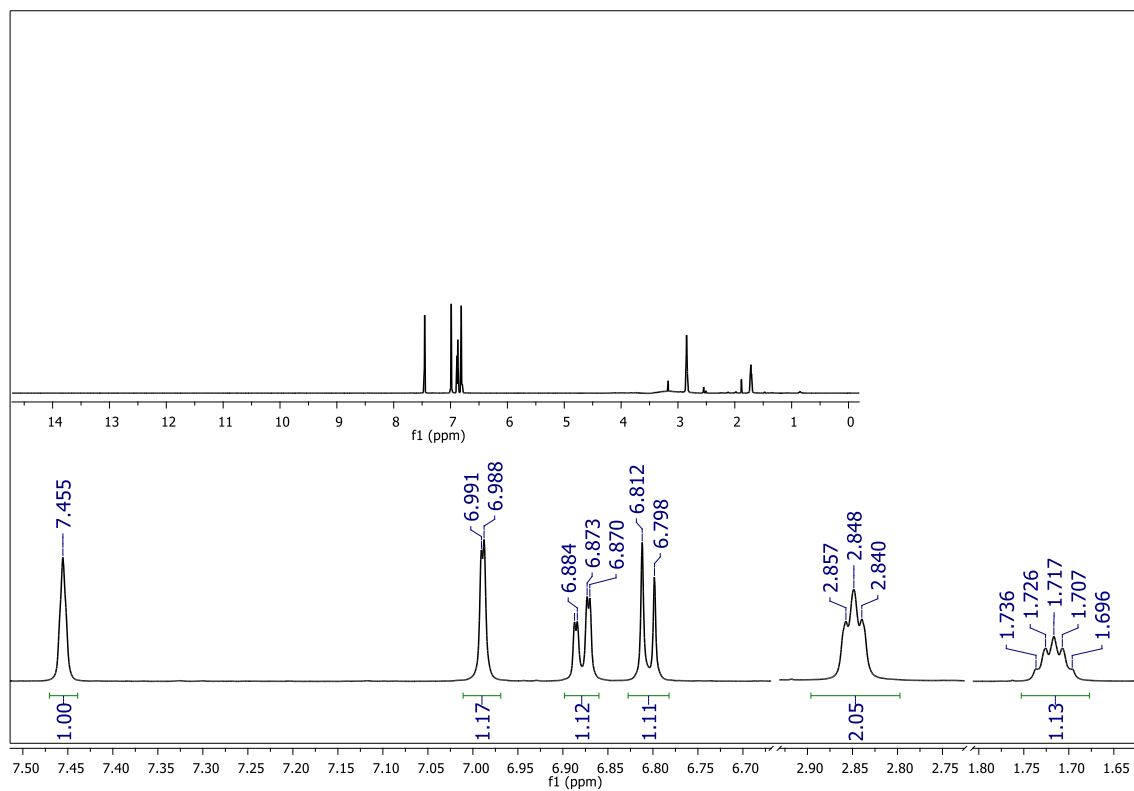


Figure S6. ^{13}C NMR spectrum of **MAC 12** (150 MHz; $\text{DMSO-}d_6$).

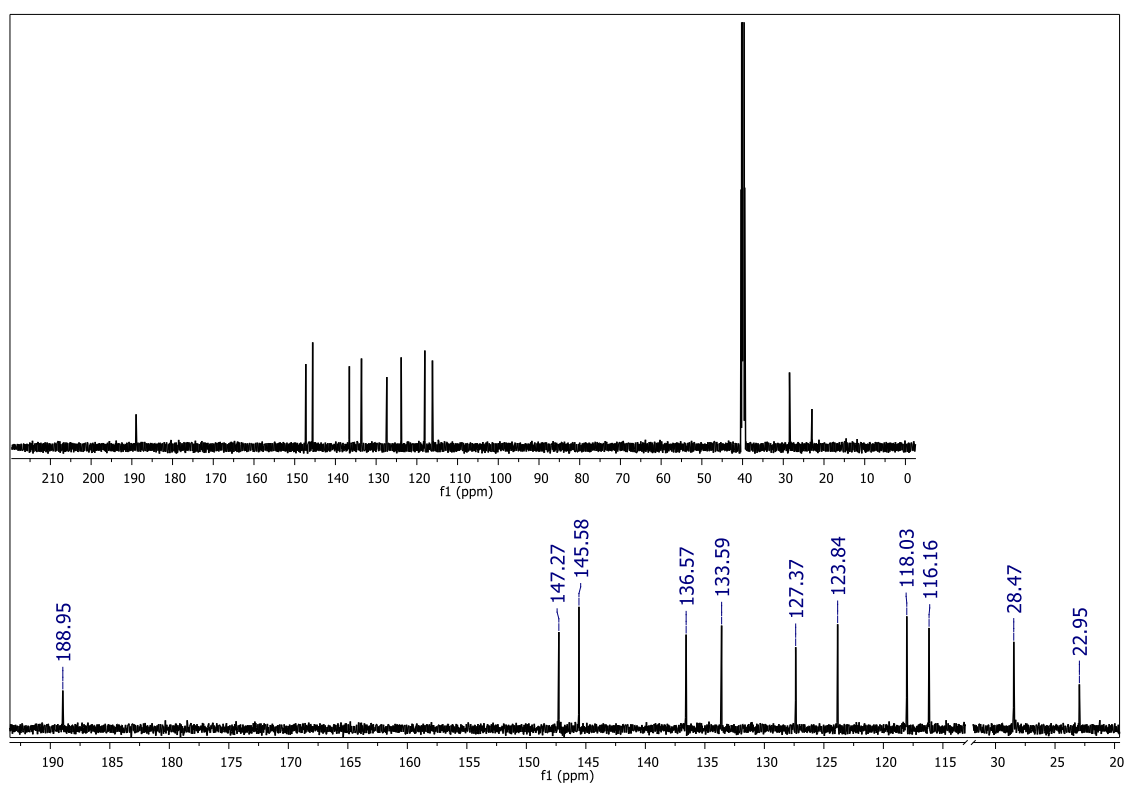


Figure S7. ^1H NMR spectrum of **MAC 13** (600 MHz; $\text{DMSO-}d_6$).

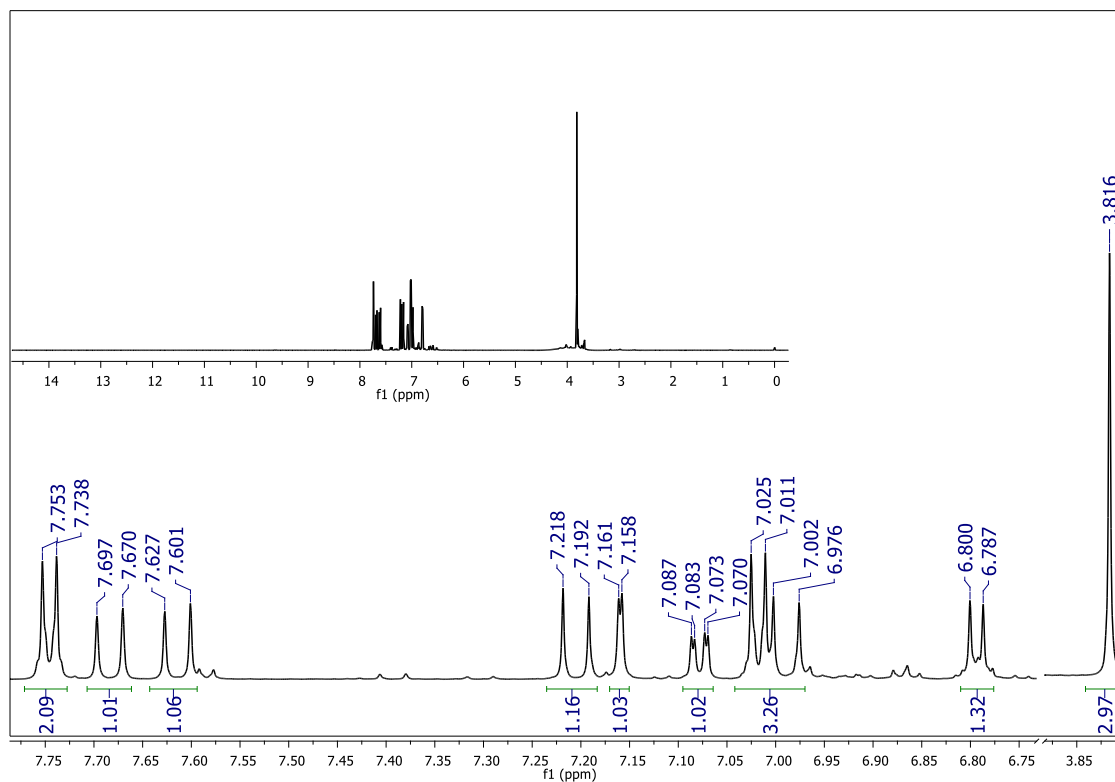


Figure S8. ^{13}C NMR spectrum of **MAC 13** (150 MHz; $\text{DMSO-}d_6$).

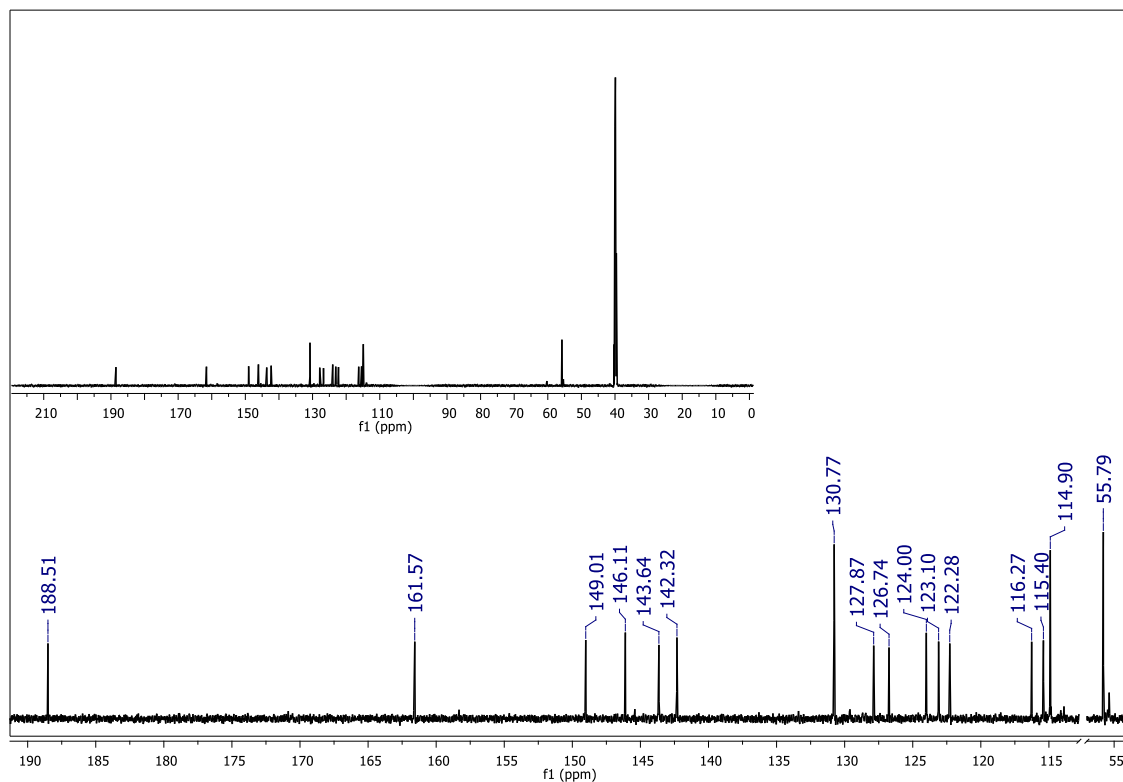


Figure S9. ^1H NMR spectrum of **MAC 14** (600 MHz; $\text{DMSO-}d_6$).

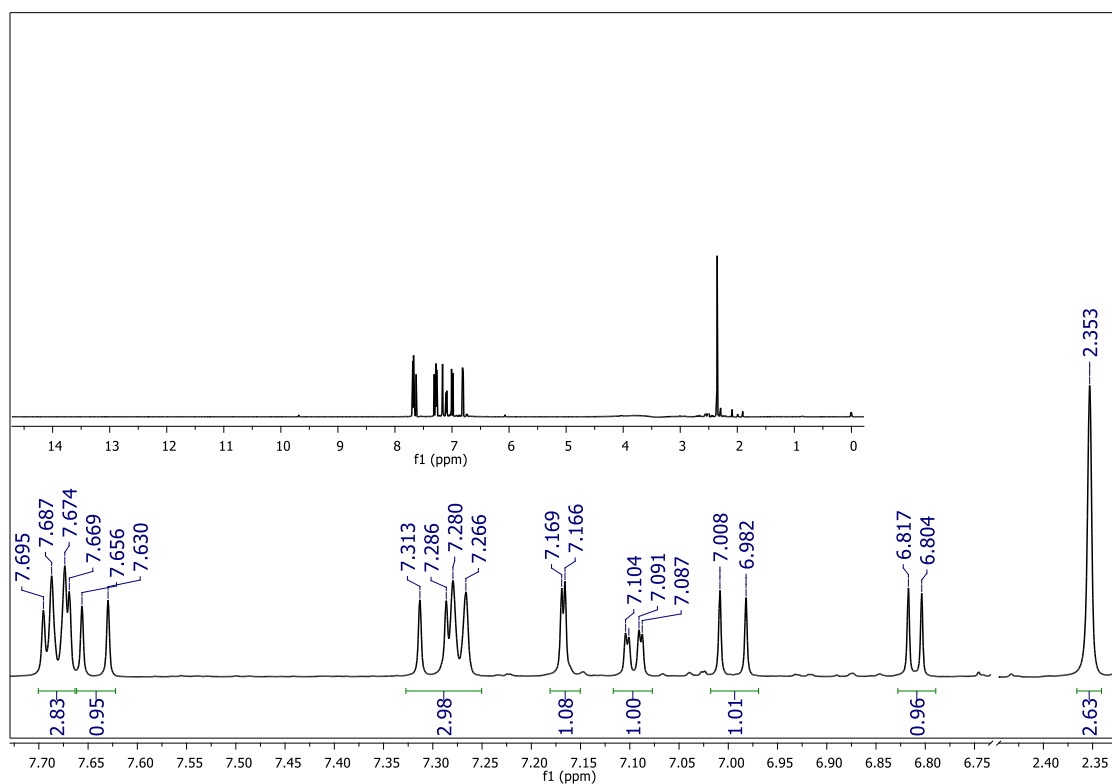


Figure S10. ^{13}C NMR spectrum of **MAC 14** (150 MHz; $\text{DMSO-}d_6$).

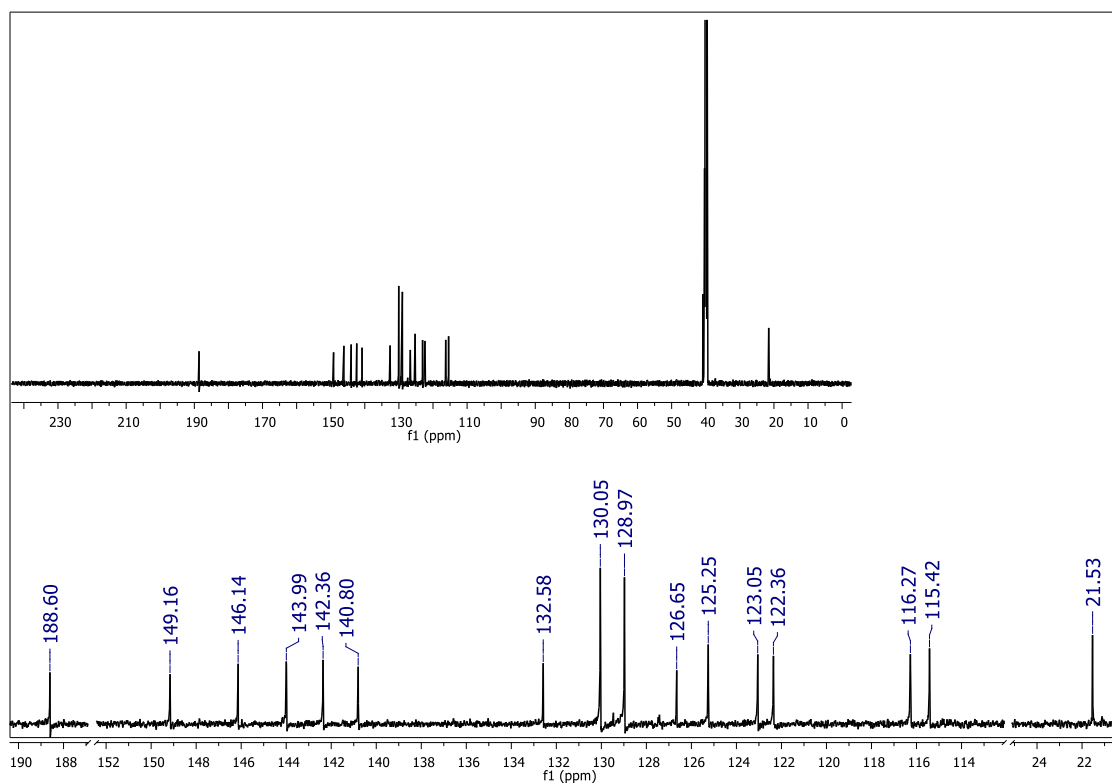


Figure S11. ^1H NMR spectrum of **MAC 15** (600 MHz; $\text{DMSO-}d_6$).

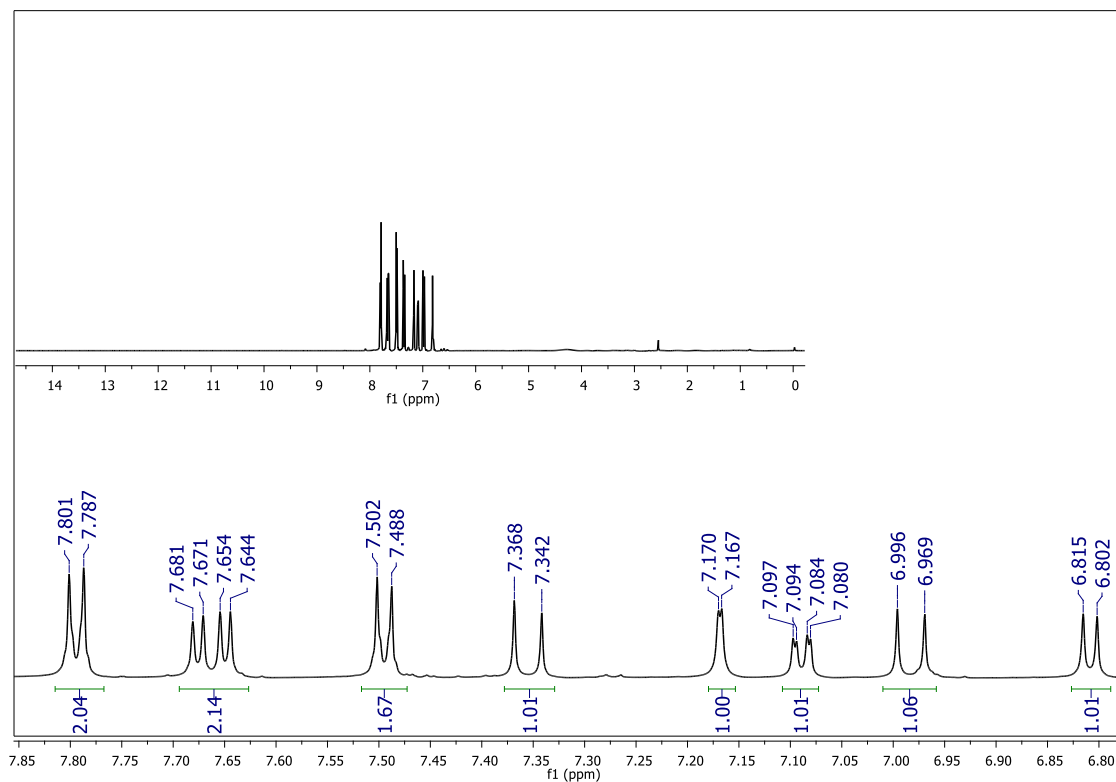


Figure S12. ^{13}C NMR spectrum of **MAC 15** (150 MHz; $\text{DMSO-}d_6$).

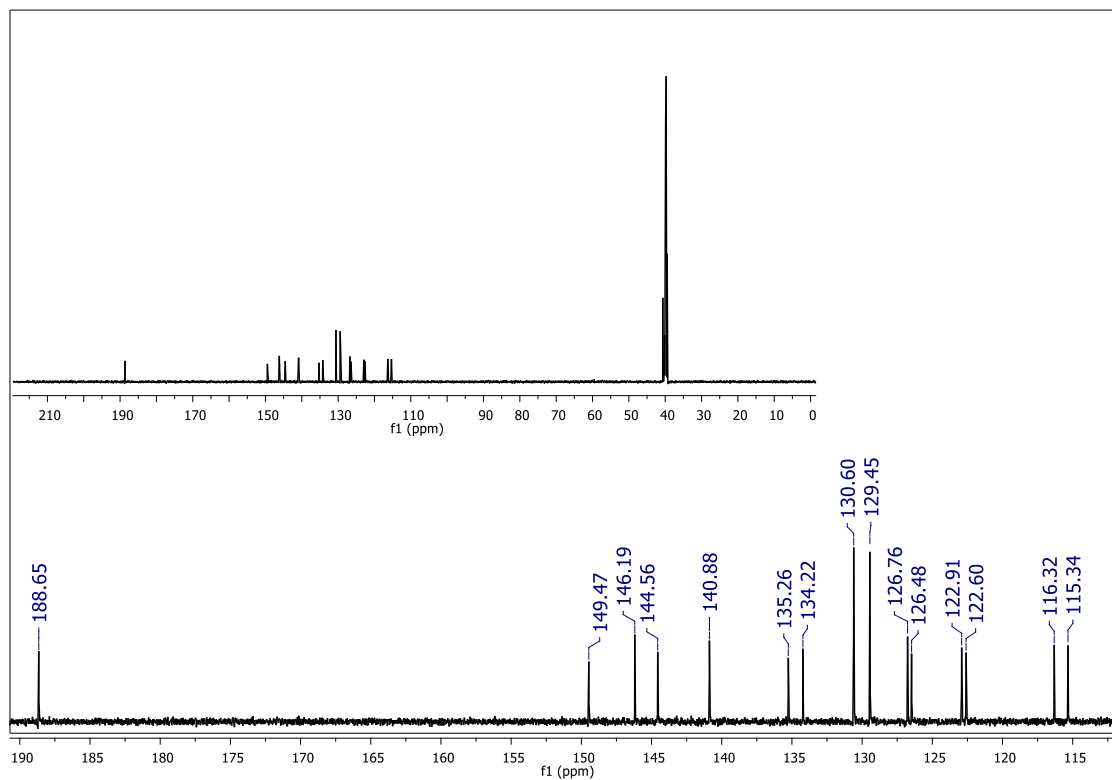


Figure S13. ^1H NMR spectrum of **MAC 16** (600 MHz; $\text{DMSO-}d_6$).

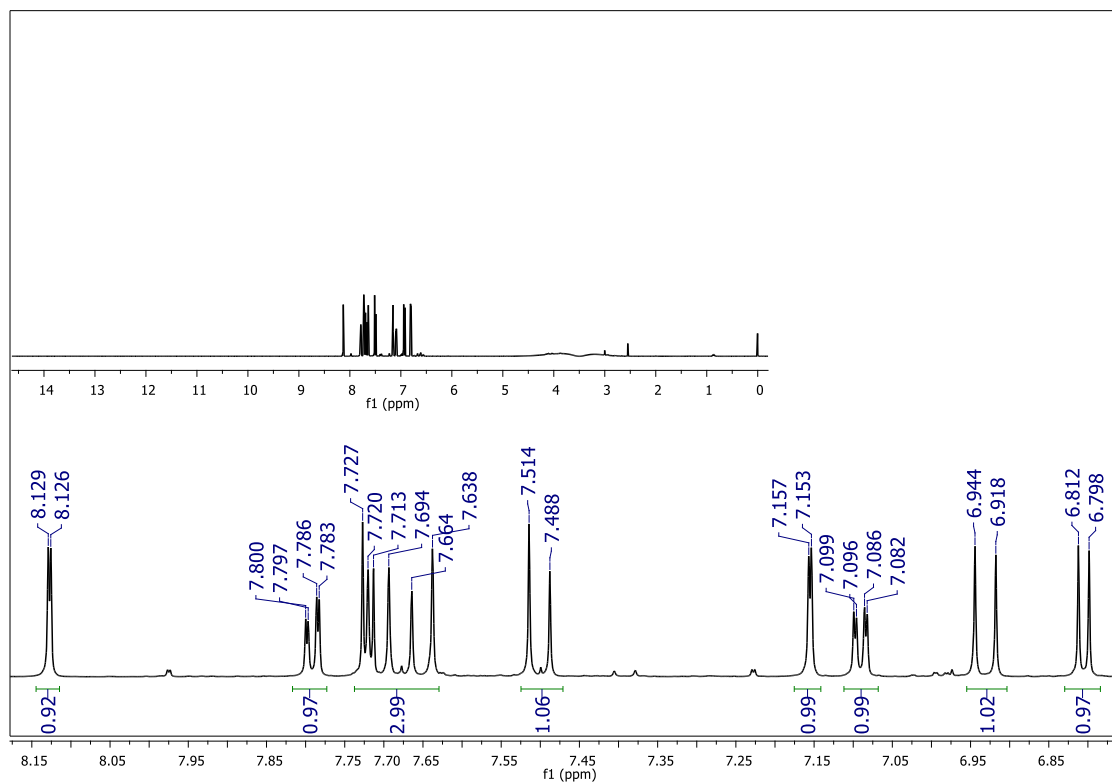


Figure S14. ^{13}C NMR spectrum of **MAC 16** (150 MHz; $\text{DMSO-}d_6$).

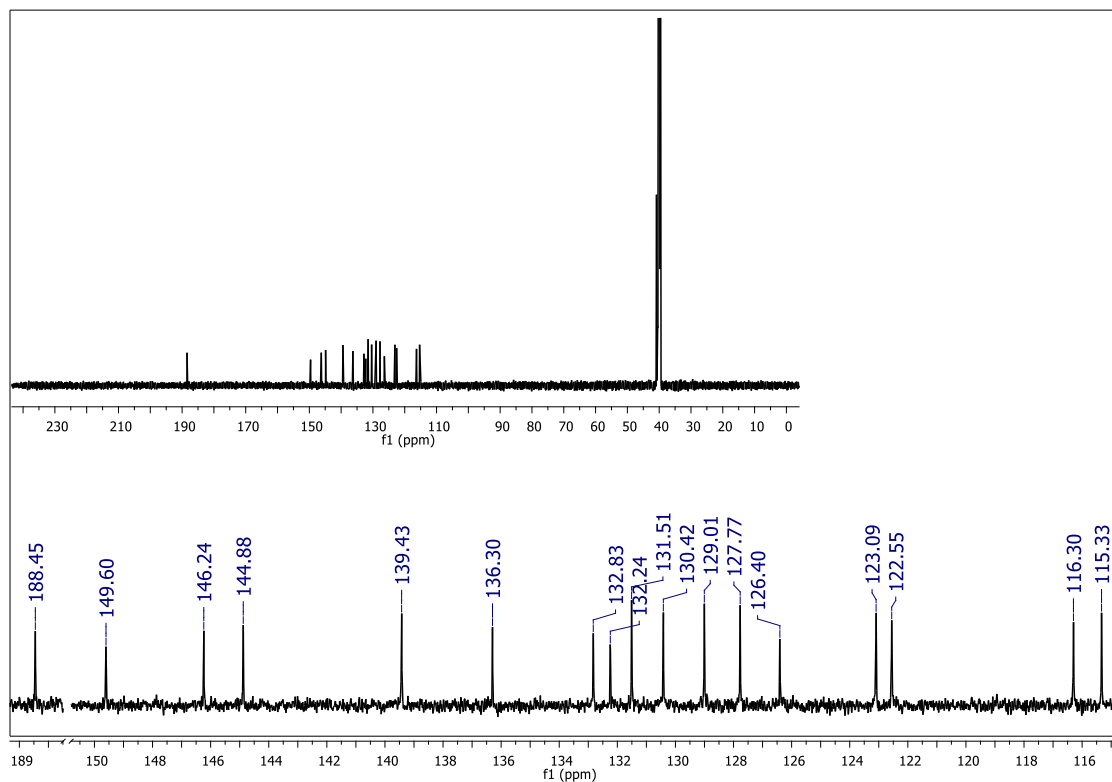


Figure S15. ^1H NMR spectrum of **MAC 17** (600 MHz; $\text{DMSO-}d_6$).

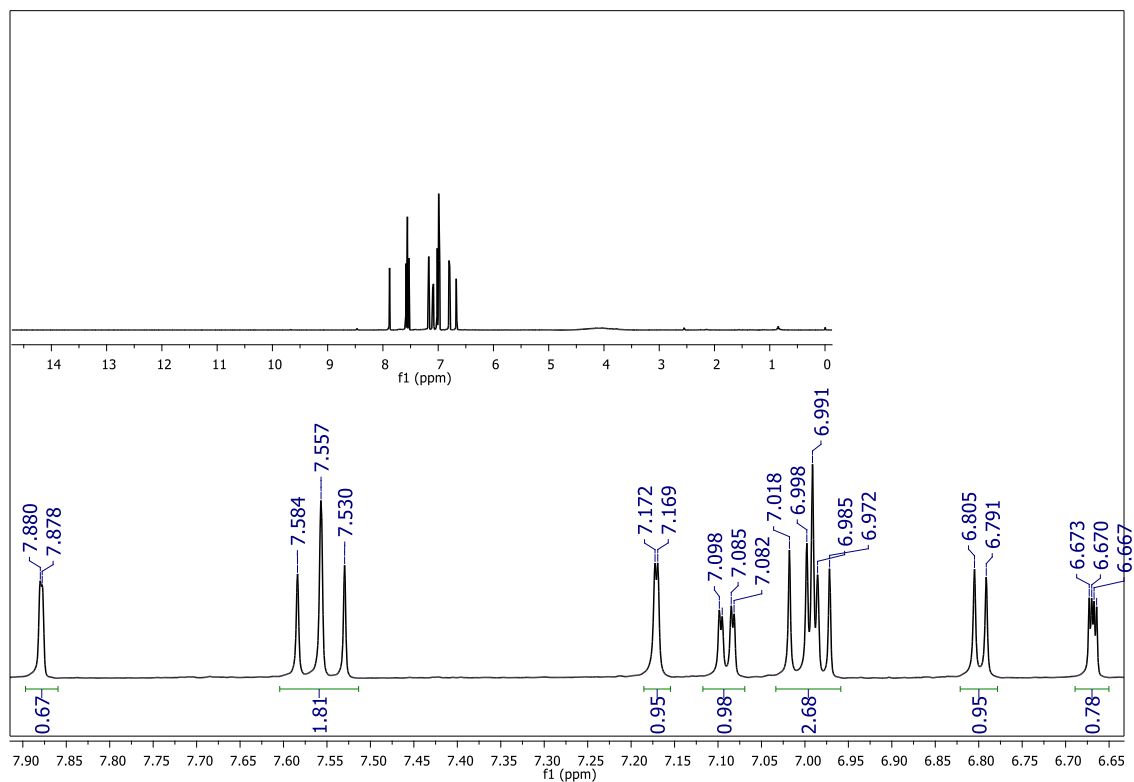


Figure S16. ^{13}C NMR spectrum of **MAC 17** (150 MHz; $\text{DMSO-}d_6$).

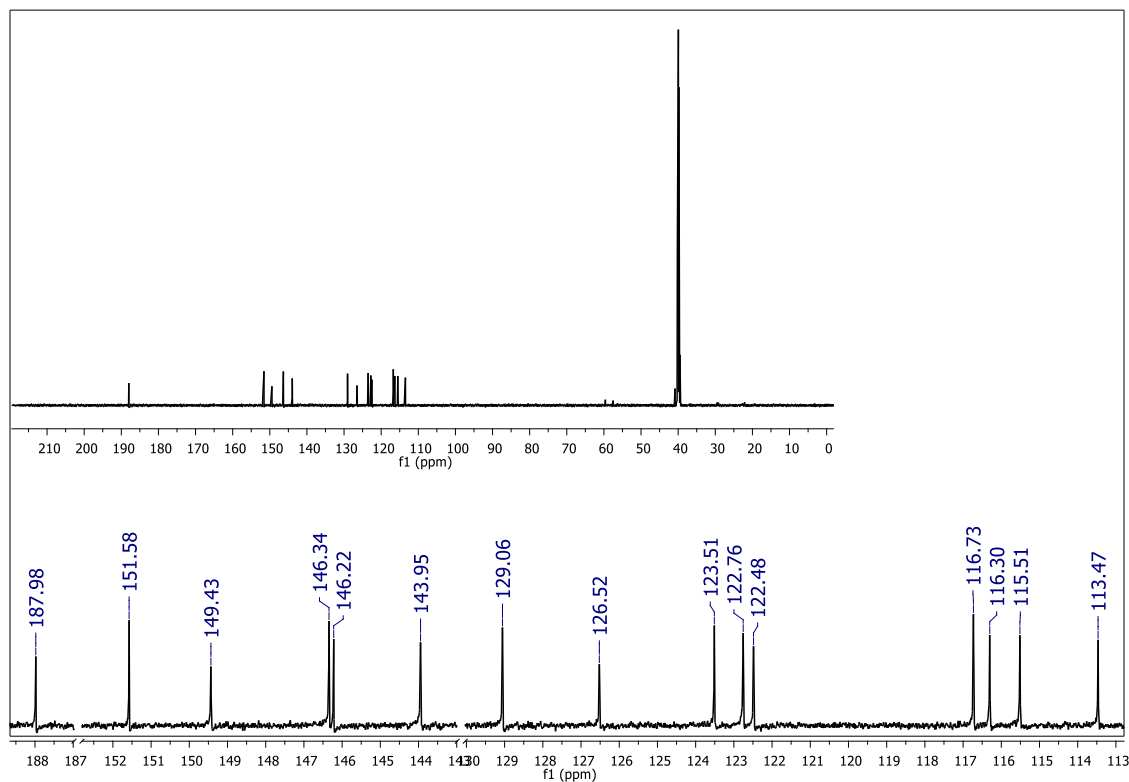


Figure S17. ^1H NMR spectrum of **MAC 18** (600 MHz; $\text{DMSO-}d_6$).

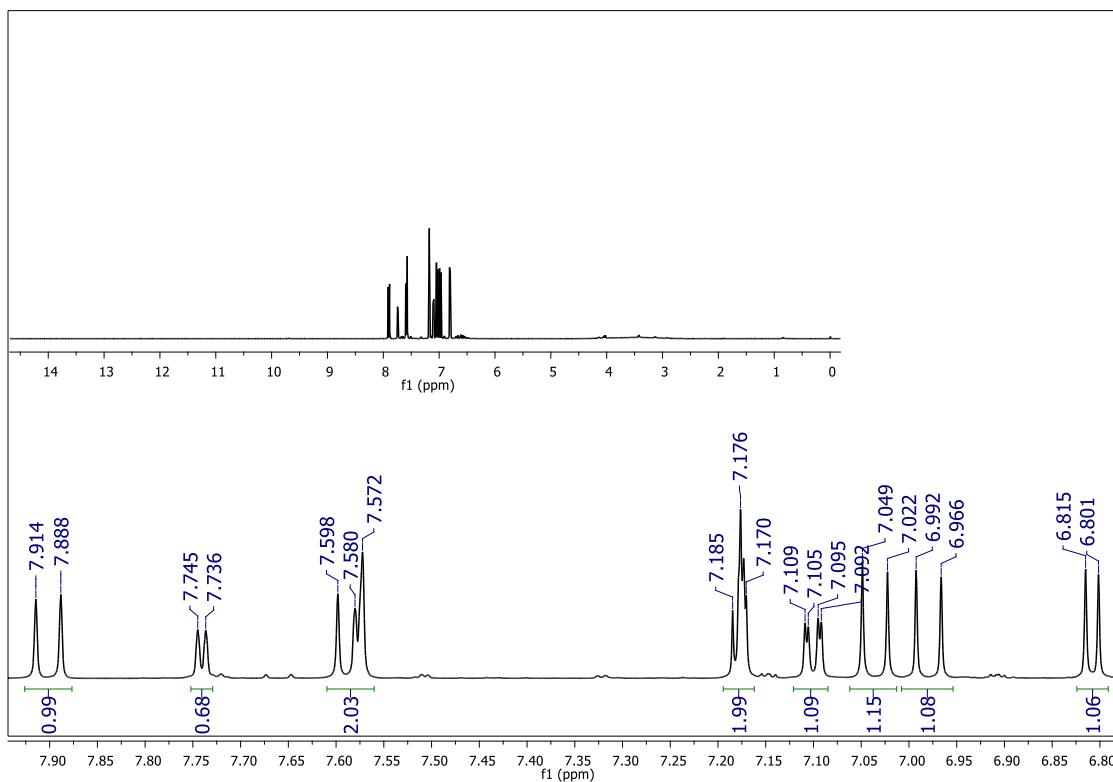


Figure S18. ^{13}C NMR spectrum of **MAC 18** (150 MHz; $\text{DMSO-}d_6$).

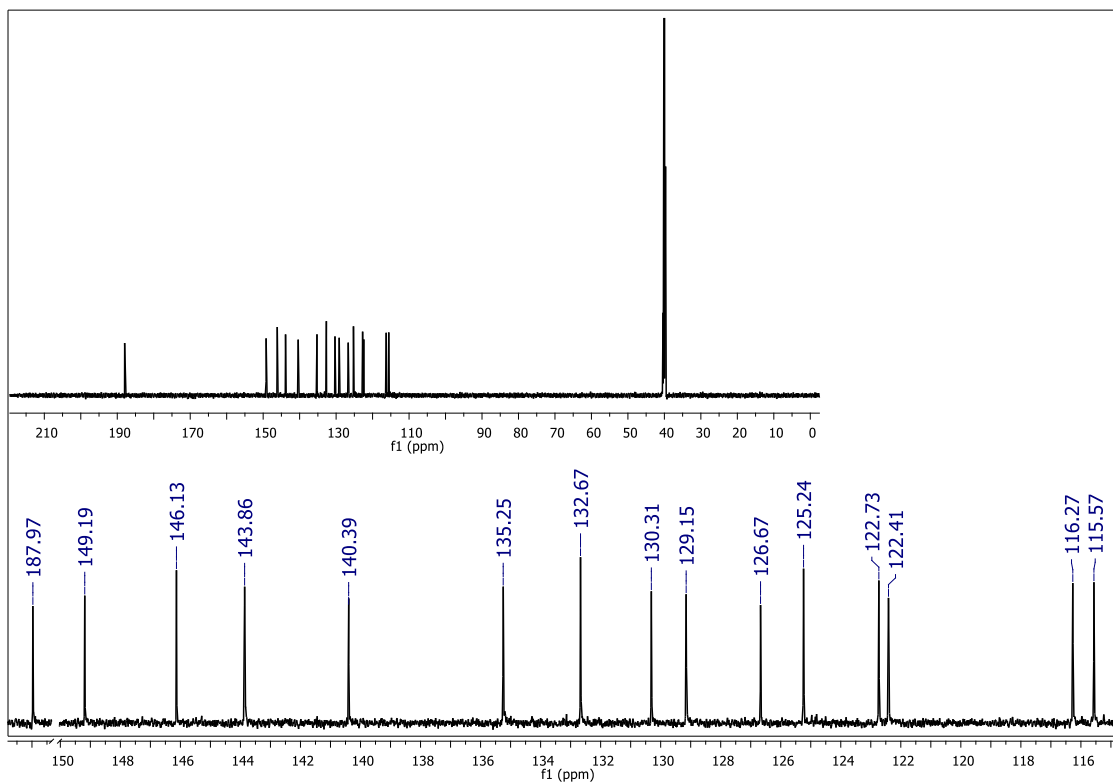


Figure S19. ^1H NMR spectrum of **MAC 19** (600 MHz; $\text{DMSO-}d_6$).

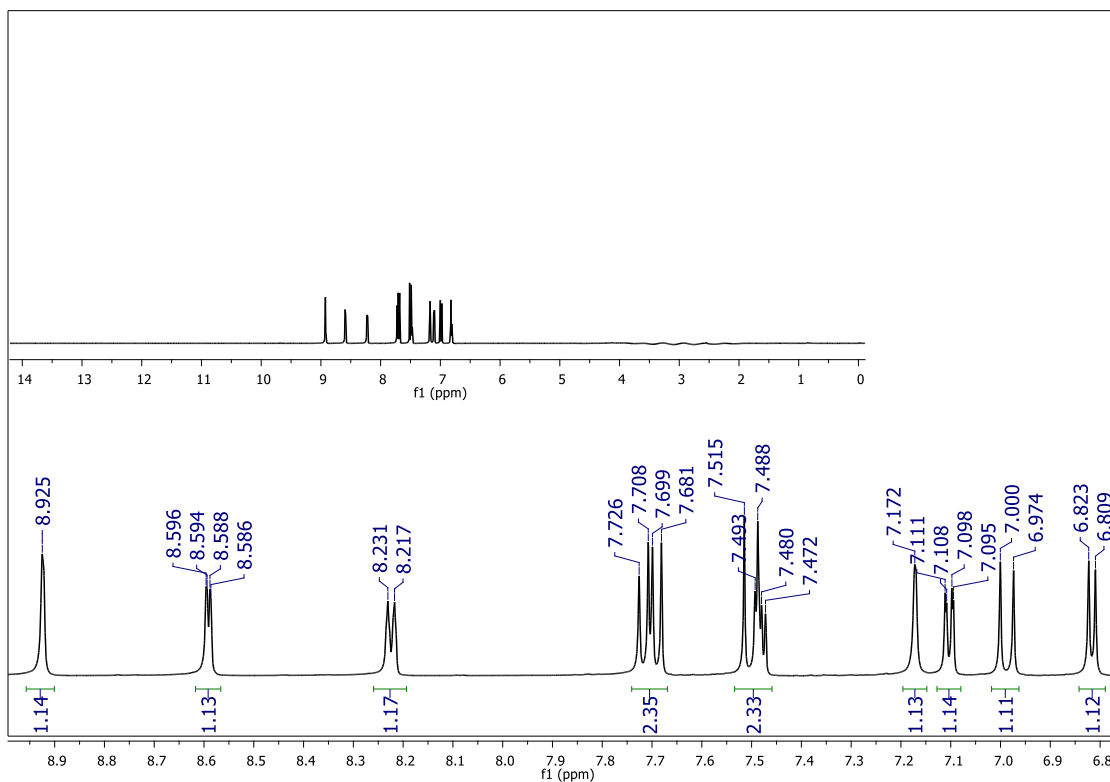


Figure S20. ^{13}C NMR spectrum of **MAC 19** (150 MHz; $\text{DMSO-}d_6$).

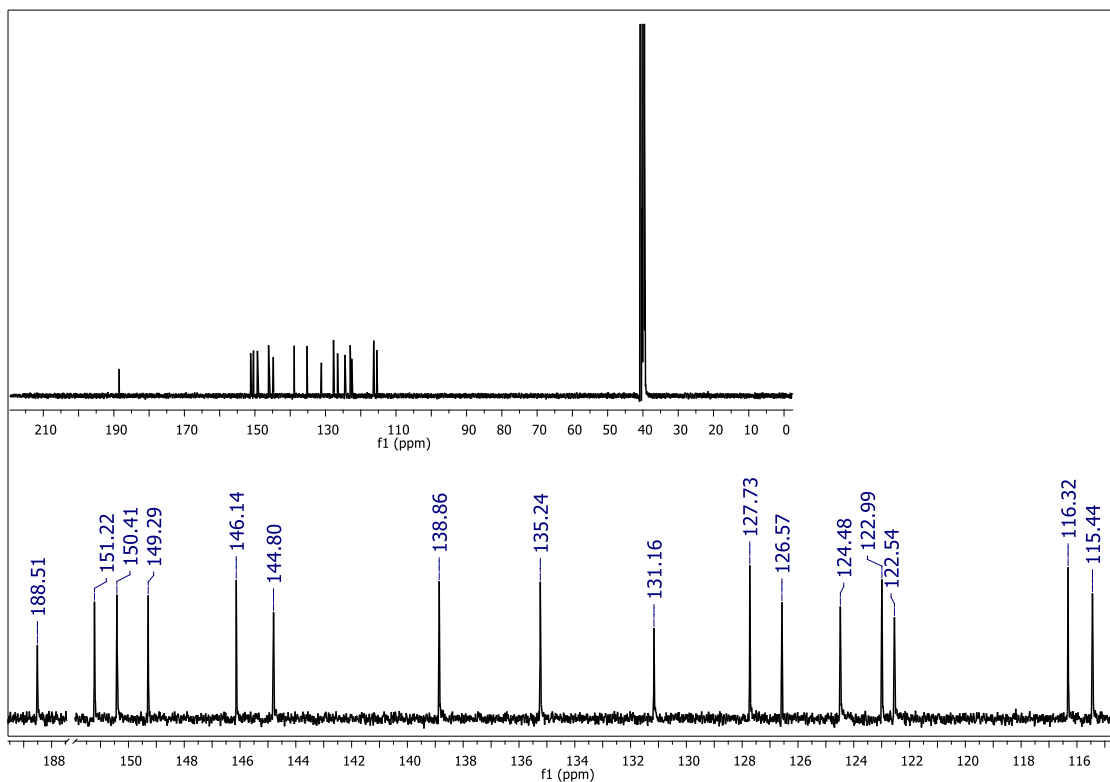


Figure S21. ^1H NMR spectrum of **MAC 20** (600 MHz; $\text{DMSO-}d_6$)

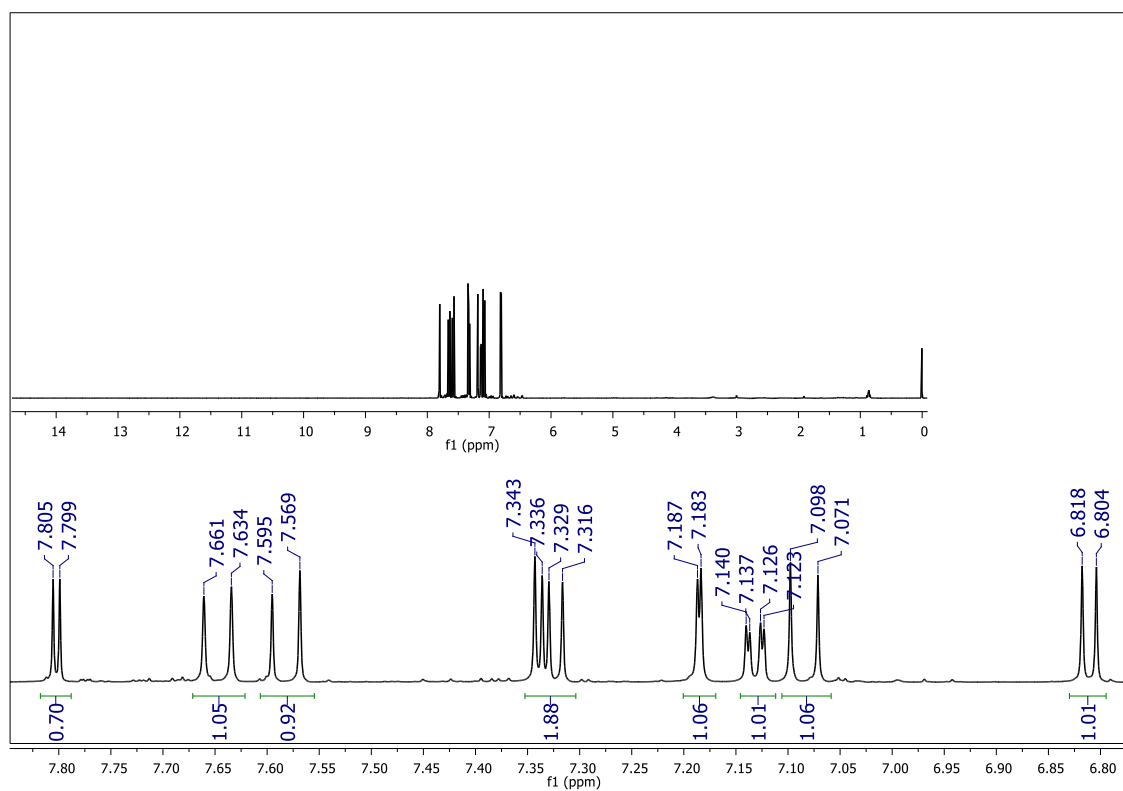


Figure S22. ^{13}C NMR spectrum of **MAC 20** (150 MHz; $\text{DMSO-}d_6$).

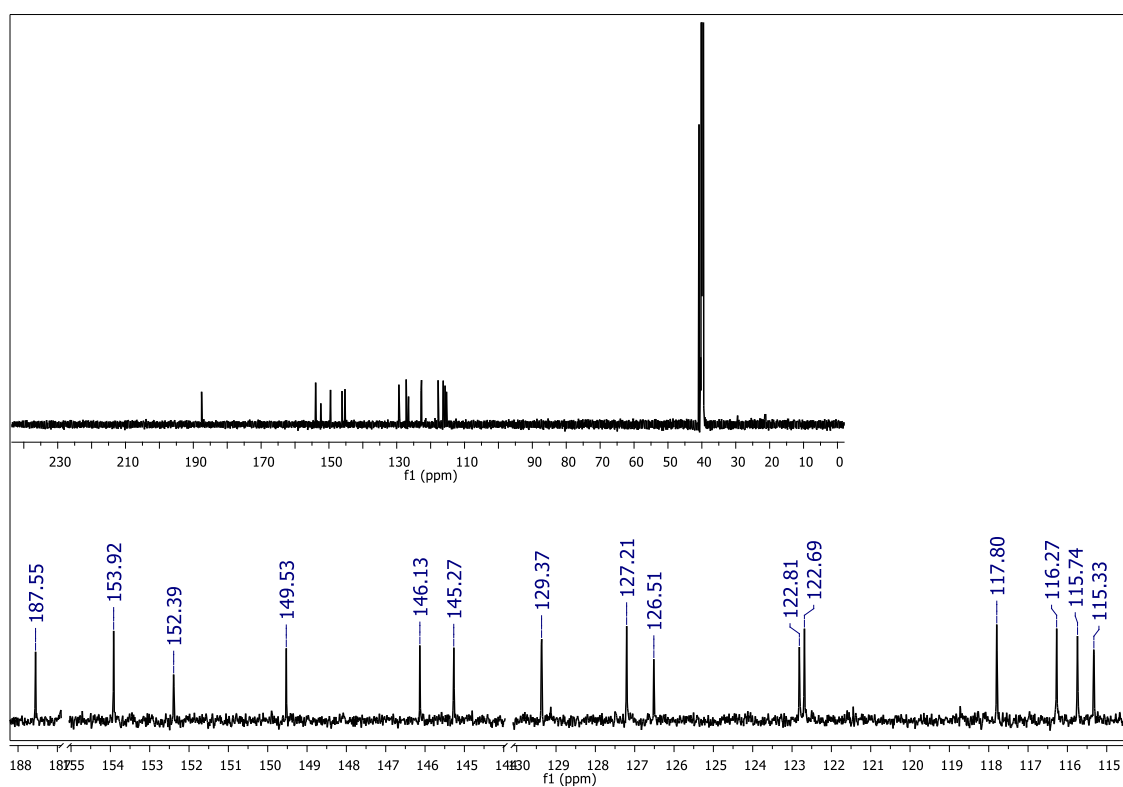


Figure S23. ^1H NMR spectrum of **MAC 21** (600 MHz; $\text{DMSO-}d_6$).

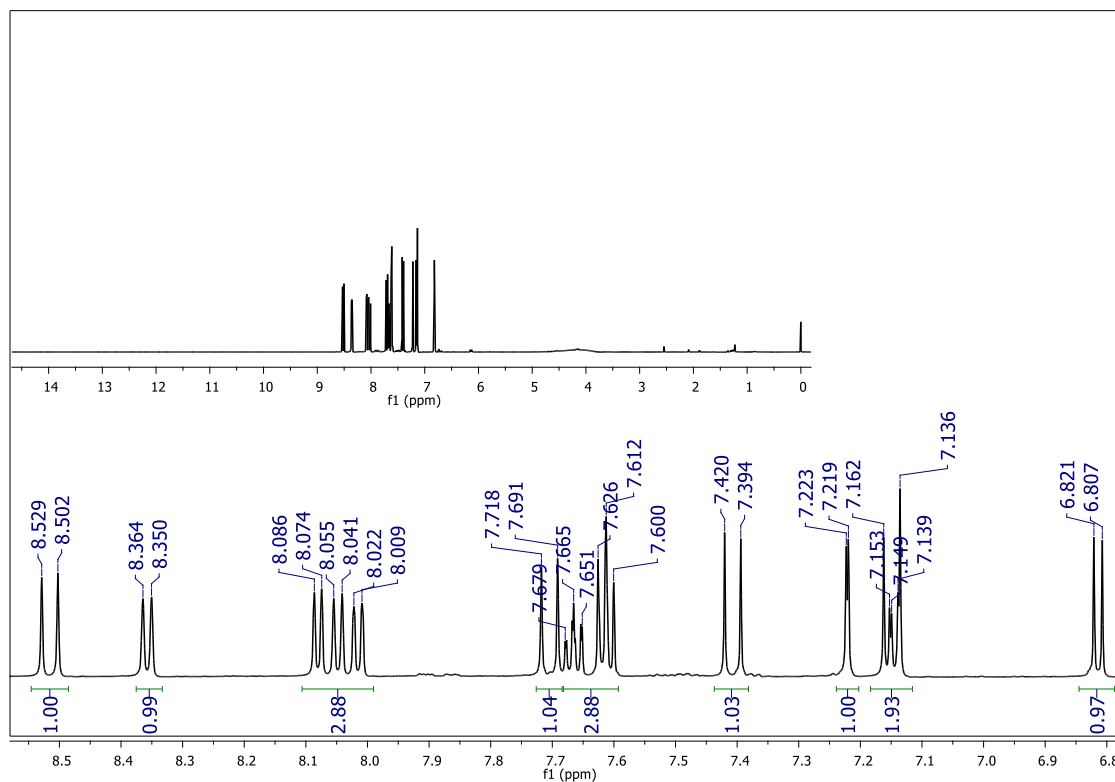


Figure S24. ^{13}C NMR spectrum of **MAC 21** (150 MHz; $\text{DMSO-}d_6$).

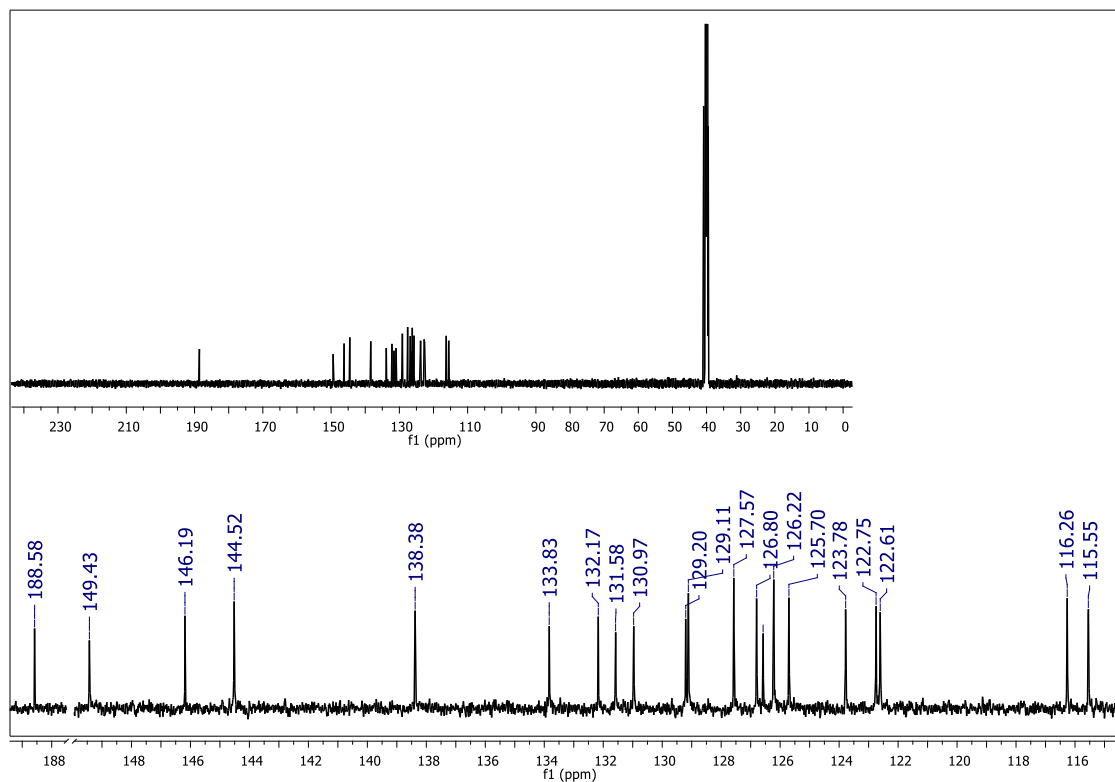


Figure S25. ^1H NMR spectrum of **MAC 22** (600 MHz; $\text{DMSO-}d_6$).

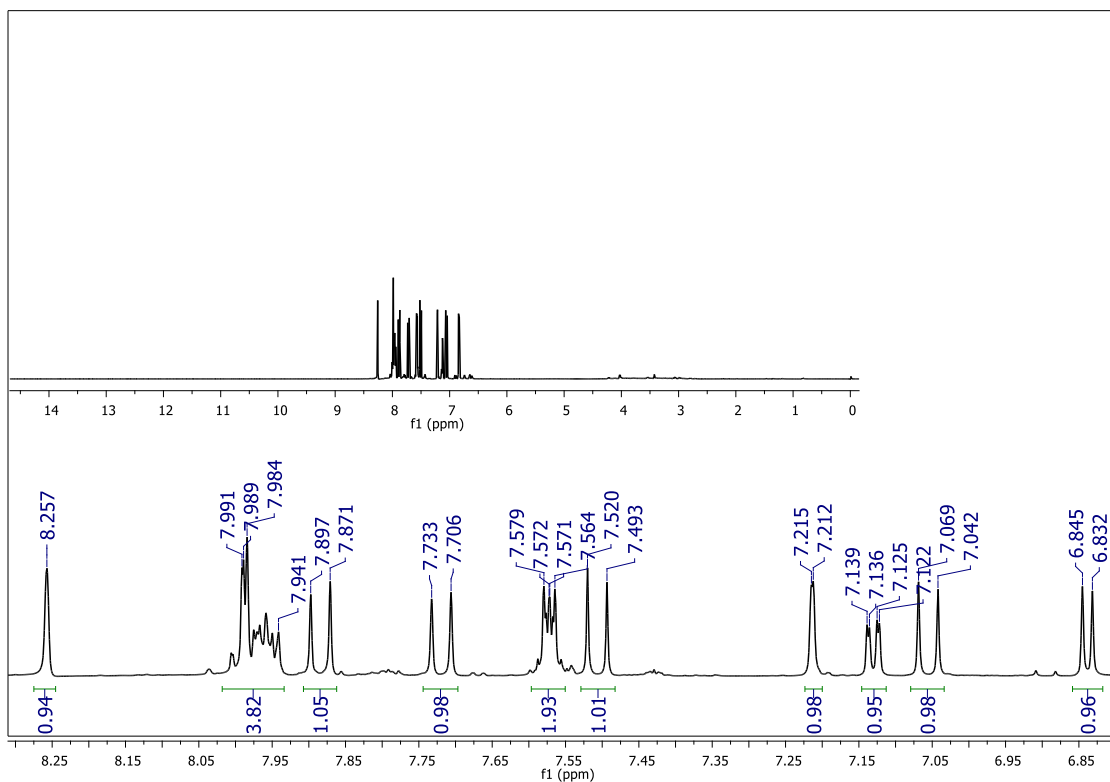


Figure S26. ^{13}C NMR spectrum of **MAC 22** (150 MHz; $\text{DMSO-}d_6$).

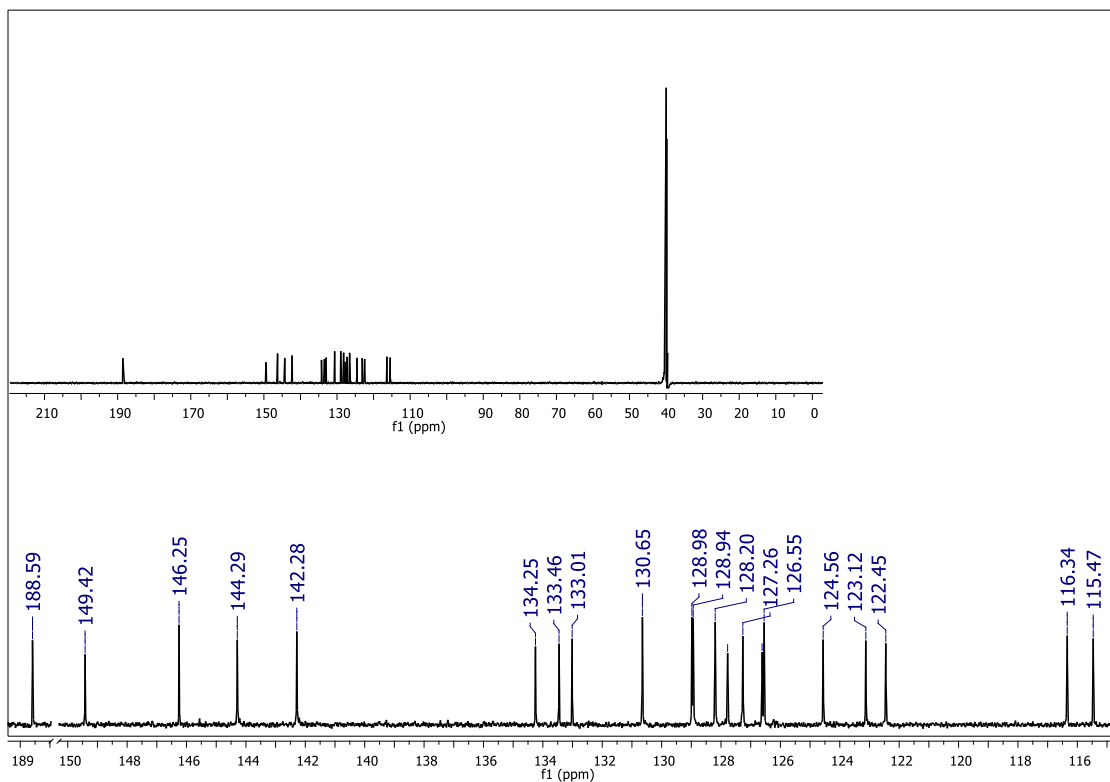


Figure S27. ^1H NMR spectrum of **MAC 23** (600 MHz; $\text{DMSO-}d_6$).

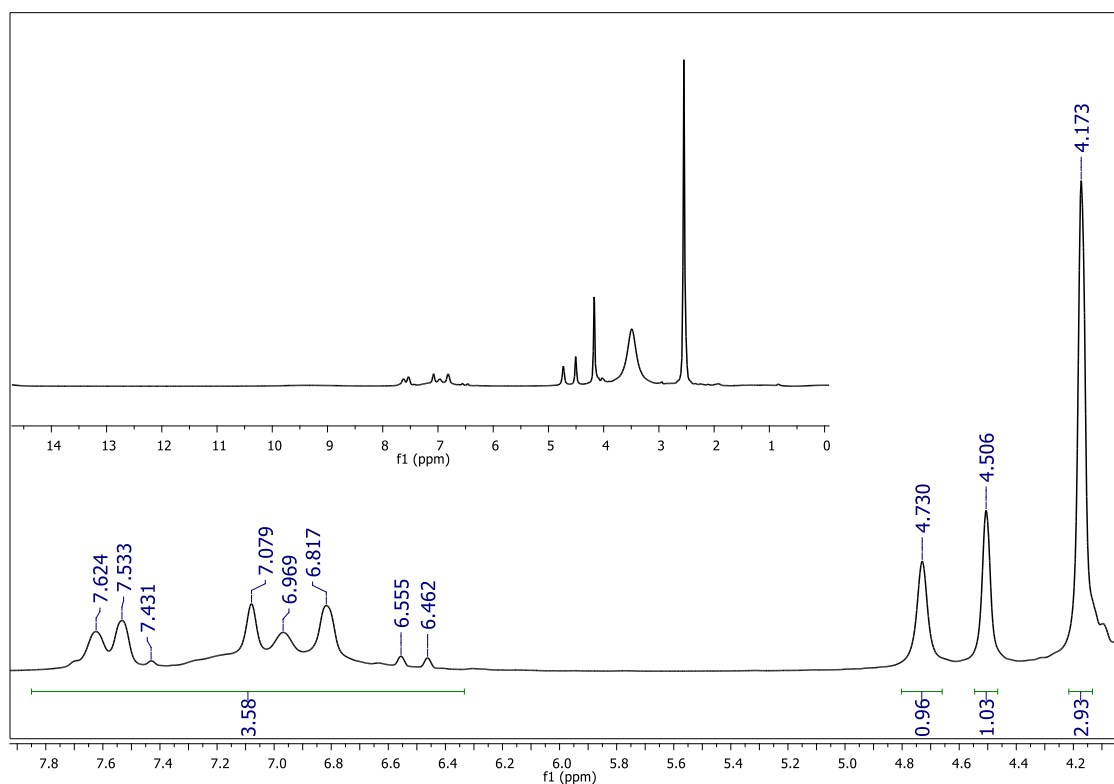
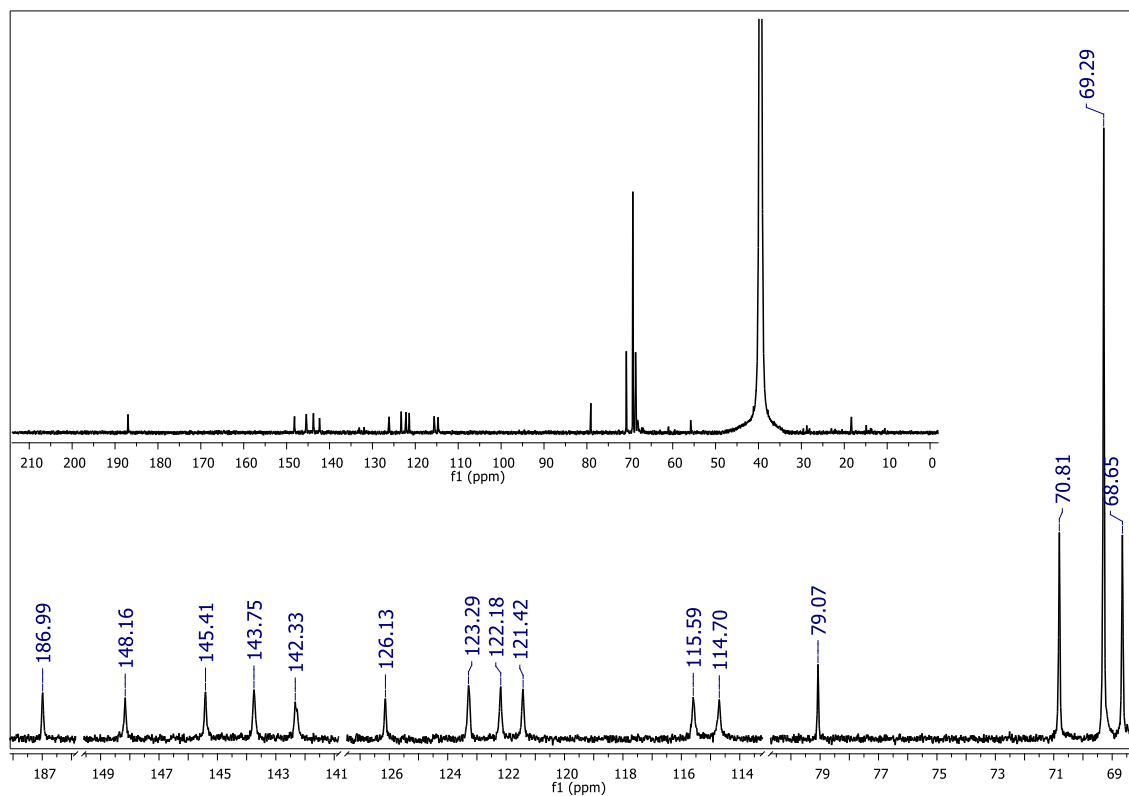


Figure S28. ^{13}C NMR spectrum of **MAC 23** (150 MHz; $\text{DMSO-}d_6$).



APÊNDICE D

Material Suplementar do Capítulo VI

Unsymmetrical Monocarbonyl Curcuminoid (MAC 8) Decreases *Candida albicans* Cell Adhesion and Biofilm

Carlos R. Polaquini^a, Janaina C. O. Sardi^b, Ana C. Nazaré^a, Veridianna C. Pattini^a, Margarete T. G. Almeida^c, Pedro L. Rosalen^b, Luis O. Regasini^a

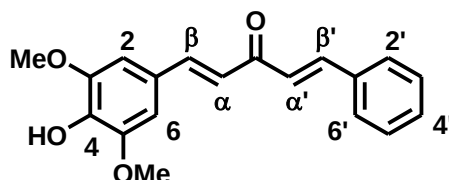
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1. SPECTROSCOPY DATA ANALYSES

1.1 (1E,4E)-1-(4-hydroxy-3,5-dimethoxyphenyl)-5-phenylpenta-1,4-dien-3-one (MAC 24).



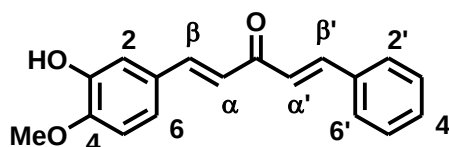
Red oil

Yield: 31%

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 3.97 (s; 3,5-diOMe), 6.89 (s; H-2 and H-6), 6.96 (d; 15.6; H-α), 7.13 (d; 15.6; H-α'), 7.42–7.44 (H-3', H-4' and H-5'), 7.64 (dd; 1.8 and 6.3; H-2' and H-6'), 7.69 (d; 15.6; H-β), 7.76 (d; 15.6; H-β') (Figure S1).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 56.4 (3,5-diOMe), 105.4 (C-2 and C-6), 123.8 (C-α), 125.2 (C-α'), 126.2 (C-1), 128.4 (C-2' and C-6'), 129.0 (C-3' and C-5'), 130.5 (C-4'), 134.9 (C-1'), 137.5 (C-4), 143.0 (C-β), 143.9 (C-β'), 147.3 (C-3 and C-5), 188.8 (C=O) (Figure S2).

1.2 (1E,4E)-1-(3-hydroxy-4-methoxyphenyl)-5-phenylpenta-1,4-dien-3-one (MAC 25).



Yellow solid

Yield: 62%

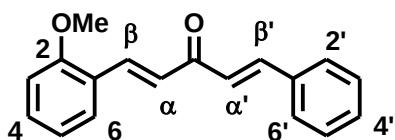
Melting point: 156–157 °C

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 3.97 (s; 4-OMe), 5.76 (s; 3-OH), 6.90 (d; 8.4; H-5), 6.98 (d; 15.6; H-α), 7.09 (d; 16.2; H-α'), 7.15 (dd; 1.8 e 8.4; H-6), 7.28

(d; 1.8; H-2), 7.43–7.44 (H-3', H-4' and H-5'), 7.64 (dd; 2.4 and 7.2; H-2' and H-6'), 7.69 (d; 15.6; H-β), 7.75 (d; 16.2; H-β') (Figure S3).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 56.0 (4-OMe), 110.6 (C-5), 113.0 (C-6), 122.6 (C-2), 123.7 (C-α), 125.7 (C-α'), 128.4 (C-1, C-2' and C-6'), 129.0 (C-3' and C-5'), 130.4 (C-4'), 134.9 (C-1'), 143.0 (C-β), 143.4 (C-β'), 145.9 (C-3), 148.8 (C-4), 188.9 (C=O) (Figure S4).

1.3 (1E,4E)-1-(2-methoxyphenyl)-5-phenylpenta-1,4-dien-3-one (MAC 26) (DAI et al., 2015; SHEN et al., 2015).



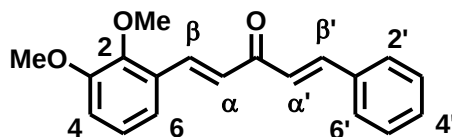
Yellow oil

Yield: 29%

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 3.95 (s; 2-OMe), 6.97 (d; 8.4; H-3), 7.02 (ddd; 0.6, 7.5 and 7.5; H-5), 7.16 (d; 16.2; H-α and H-α'), 7.39–7.46 (H-4, H-3', H-4' and H-5'), 7.64–7.66 (H-6, H-2' and H-6'), 7.76 (d; 16.2; H-β'), 8.10 (d; 16.2; H-β) (Figure S5).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 55.6 (2-OMe), 111.2 (C-3), 120.8 (C-5), 123.7 (C-1), 125.3 (C-α'), 126.4 (C-α), 128.4 (C-2' and C-6'), 128.8 (C-6), 128.9 (C-3' and C-5'), 130.4 (C-4'), 131.8 (C-4), 135.0 (C-1'), 138.8 (C-β'), 143.0 (C-β), 158.6 (C-2), 189.6 (C=O) (Figure S6).

1.4 (1*E*,4*E*)-1-(2,3-dimethoxyphenyl)-5-phenylpenta-1,4-dien-3-one (**MAC 27**).



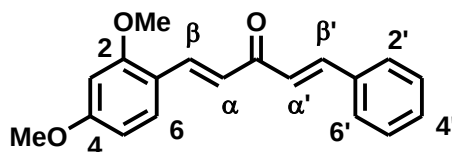
Yellow solid

Yield: 37%

¹H NMR (300 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 3.92 (2-OMe and 3-OMe), 6.99 (dd; 1.5 and 7.9; H-4), 7.11 (d; 16.2; H-α), 7.12 (dd; 7.5 and 7.9; H-5), 7.14 (d; 15.6; H-α'), 7.27 (dd; 1.5 and 7.5; H-6), 7.42–7.44 (H-3', H-4' and H-5'), 7.64 (dd; 2.1 and 6.7; H-2' and H-6'), 7.76 (d; 15.6; H-β'), 8.06 (d; 16.2; H-β) (Figure S7).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 55.9 (3-OMe), 61.5 (2-OMe), 114.1 (C-4), 119.3 (C-6), 124.3 (C-α), 125.3 (C-α'), 127.0 (C-1 and C-5), 128.4 (C-2' and C-6'), 129.0 (C-3' and C-5'), 130.5 (C-4'), 134.9 (C-1'), 138.0 (C-β), 143.3 (C-β'), 148.8 (C-3), 153.2 (C-2), 189.4 (C=O) (Figure S8).

1.5 (1*E*,4*E*)-1-(2,4-dimethoxyphenyl)-5-phenylpenta-1,4-dien-3-one (**MAC 28**)
(LAVRUSHIN et al., 1972).



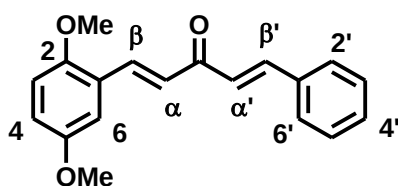
Yellow oil

Yield: 25%

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 3.88 (s; 4-OMe), 3.93 (s; 2-OMe), 6.50 (d; 1.8; H-3), 6.56 (dd; 1.8 and 8.4; H-5), 7.08 (d; 16.2; H-α), 7.15 (d; 15.6; H-α'), 7.42–7.43 (H-3', H-4' and H-5'), 7.59 (d; 8.4; H-6), 7.64 (dd; 2.4 and 7.5; H-2' and H-6'), 7.74 (d; 15.6; H-β'), 8.03 (d; 16.2; H-β) (Figure S9).

^{13}C NMR (150 MHz; CDCl_3), δ_{C} in ppm: 55.5 (4-OMe), 60.4 (2-OMe), 98.4 (C-3), 105.5 (C-5), 116.9 (C-1), 124.1 (C- α), 125.5 (C- α'), 128.3 (C-2' and C-6'), 128.9 (C-3' and C-5'), 130.2 (C-4'), 130.5 (C-6), 135.1 (C-1'), 138.9 (C- β), 142.4 (C- β'), 160.2 (C-2), 163.1 (C-4), 189.6 (C=O) (Figure S10).

1.6 (1E,4E)-1-(2,5-dimethoxyphenyl)-5-phenylpenta-1,4-dien-3-one (MAC 29).



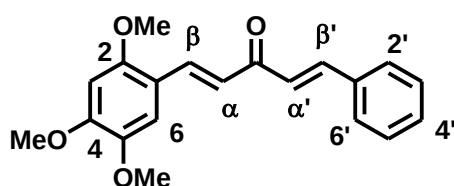
Yellow oil

Yield: 65%

^1H NMR (600 MHz; CDCl_3), δ_{H} in ppm (multiplicity; J in Hz): 3.82 (s; 5-OMe), 3.88 (s; 2-OMe), 6.88 (d; 9.0; H-3), 6.95 (dd; 3.0 and 9.0; H-4), 7.12 (d; 16.2; H- α), 7.15 (d; 15.6; H- α'), 7.16 (d; 3.0; H-6), 7.42–7.43 (H-3', H-4' and H-5'), 7.63 (dd; 2.4 and 7.5; H-2' and H-6'), 7.75 (d; 15.6; H- β'), 8.07 (d; 16.2; H- β) (Figure S11).

^{13}C NMR (150 MHz; CDCl_3), δ_{C} in ppm: 55.8 (5-OMe), 56.1 (2-OMe), 112.4 (C-6), 113.1 (C-4), 117.5 (C-3), 124.2 (C-1), 125.2 (C- α), 126.5 (C- α'), 128.4 (C-2' and C-6'), 129.0 (C-3' and C-5'), 130.4 (C-4'), 134.9 (C-1'), 138.4 (C- β), 143.0 (C- β'), 153.1 (C-2), 153.5 (C-5), 189.4 (C=O) (Figure S12).

1.7 (1E,4E)-1-(2,4,5-trimethoxyphenyl)-5-phenylpenta-1,4-dien-3-one (MAC 30).



Yellow solid

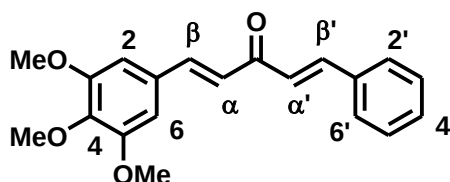
Yield: 79%

Melting point: 171–172 °C

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 3.92 (s; 5-OMe), 3.94 (s; 4-OMe), 3.97 (s; 2-OMe), 6.54 (s; H-3), 6.99 (d; 16.2; H-α), 7.14 (s; H-6), 7.18 (d; 16.2; H-α'), 7.43–7.44 (H-3', H-4' and H-5'), 7.65 (dd; 1.8 and 7.5; H-2' and H-6'), 7.75 (d; 16.2; H-β'), 8.09 (d; 16.2; H-β) (Figure S13).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 56.1 (5-OMe), 56.4 (4-OMe), 56.5 (2-OMe), 96.6 (C-3), 110.6 (C-6), 115.2 (C-1), 124.1 (C-α), 125.1 (C-α'), 128.4 (C-2' and C-6'), 128.9 (C-3' and C-5'), 130.3 (C-4'), 135.1 (C-1'), 138.4 (C-β), 142.5 (C-β'), 143.3 (C-5), 152.5 (C-4), 154.4 (C-2), 189.4 (C=O) (Figure S14).

1.8 (1*E*,4*E*)-1-(3,4,5-trimethoxyphenyl)-5-phenylpenta-1,4-dien-3-one (**MAC 31**)
(YAMAKOSHI et al., 2010; NAIK, KUMAR & SWETHA, 2011).



Yellow oil

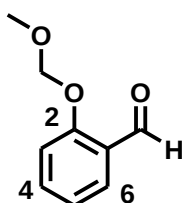
Yield: 47%

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 3.92 (s; 4-OMe), 3.94 (s; 3,5-diOMe), 6.87 (s; H-2 and H-6), 6.99 (d; 16.2; H-α), 7.14 (d; 15.6; H-α'), 7.43–7.44 (H-3', H-4' and H-5'), 7.65 (dd; 1.8 and 6.6; H-2' and H-6'), 7.69 (d; 15.6; H-β'), 7.77 (d; 16.2; H-β) (Figure S15).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 56.2 (3,5-diOMe), 61.0 (4-OMe), 105.5 (C-2 and C-6), 125.0 (C-α), 125.1 (C-α'), 128.4 (C-2' and C-6'), 129.0 (C-3' and C-5'), 130.3 (C-1),

130.6 (C-4'), 134.8 (C-1'), 140.3 (C-4), 143.3 (C-β), 143.5 (C-β'), 153.5 (C-3 and C-5), 188.8 (C=O) (Figure S16).

1.9 2-(methoxymethoxy)benzaldehyde (2a) (HOTSUMI et al., 2019).



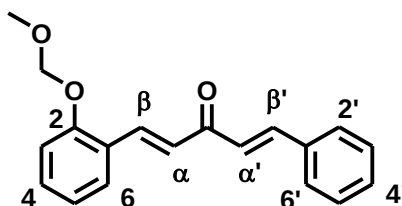
Colorless oil

Yield: 90%

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 3.49 (s; 2-OCH₂OCH₃), 5.27 (s; 2-OCH₂OCH₃), 7.04 (dd; 7.8 and 7.8; H-5), 7.19 (d; 8.4; H-3), 7.49 (ddd; 1.8, 7.8 and 8.4; H-4), 7.81 (dd; 1.8 and 7.8; H-6), 10.48 (s, -COH) (Figure S17).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 56.4 (2-OCH₂OCH₃), 94.5 (2-OCH₂OCH₃), 115.0 (C-3), 121.7 (C-5), 125.3 (C-1), 128.1 (C-6), 135.8 (C-4), 159.6 (C-2), 189.6 (C=O) (Figure S18).

1.10 (1E,4E)-1-(2-(methoxymethoxy)phenyl)-5-phenylpenta-1,4-dien-3-one (3a).



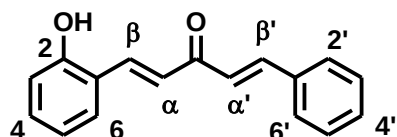
Yellow oil

Yield: 66%

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 3.55 (s; 2-OCH₂OCH₃), 5.32 (s; 2-OCH₂OCH₃), 7.08 (dd; 7.5 and 7.8; H-5), 7.14 (d; 16.2; H-α'), 7.16 (d; 16.2; H-α),

7.13–7.17 (H-3), 7.21 (d; 7.8; H-4), 7.44–7.45 (H-3', H-4' and H-5'), 7.65 (dd; 1.8 and 7.5; H-2' and H-6'), 7.68 (dd, 1.2 and 7.5, H-6), 7.77 (d; 16.2; H- β'), 8.15 (d; 16.2; H- β) (Figure S19).

1.11 (1*E*,4*E*)-1-(2-hydroxyphenyl)-5-phenylpenta-1,4-dien-3-one (**MAC 32**) (DAI et al., 2015; SHEN et al., 2015).



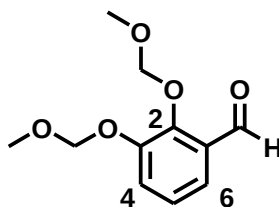
Yellow oil

Yield: 21 %

^1H NMR (600 MHz; CDCl_3), δ_{H} in ppm (multiplicity; J in Hz): 6.95–6.99 (H-3, H-4 and H-5), 7.18 (d; 15.6; H- α), 7.31 (d; 15.6; H- α'), 7.44–7.45 (H-3', H-4' and H-5'), 7.60 (dd; 1.2 and 7.5; H-6), 7.65 (dd; 1.8 and 6.6; H-2' and H-6'), 7.80 (d; 15.6; H- β'), 8.15 (d; 15.6; H- β) (Figure S20).

^{13}C NMR (150 MHz; CDCl_3), δ_{C} in ppm: 116.6 (C-3), 120.9 (C-5), 122.1 (C-1), 125.2 (C- α'), 126.2 (C- α), 128.5 (C-2' and C-6'), 129.0 (C-3' and C-5'), 129.3 (C-6), 130.5 (C-4'), 131.8 (C-4), 134.9 (C-1'), 139.5 (C- β'), 143.4 (C- β), 155.8 (C-2), 190.2 (C=O) (Figure S21).

1.12 2,3-bis(methoxymethoxy)benzaldehyde (**2b**) (HOTSUMI et al., 2019).



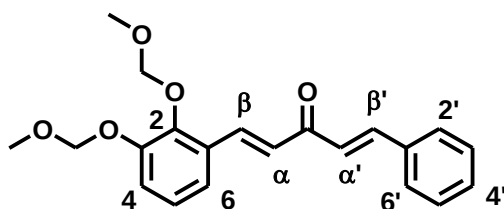
Colorless oil

Yield: 73%

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 3.51 (s; 3-OCH₂OCH₃), 3.57 (s; 2-OCH₂OCH₃), 5.23 (s; 3-OCH₂OCH₃), 5.25 (s; 2-OCH₂OCH₃), 7.14 (dd; 7.8 and 8.1; H-5), 7.40 (dd; 1.8 and 8.1; H-4), 7.50 (dd; 1.8 and 7.8; H-6), 10.46 (s; -COH) (Figure S22).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 56.4 (3-OCH₂OCH₃), 57.9 (2-OCH₂OCH₃), 95.2 (3-OCH₂OCH₃), 99.7 (2-OCH₂OCH₃), 120.9 (C-5), 122.3 (C-4), 124.6 (C-6), 130.6 (C-1), 149.9 (C-3), 150.1 (C-2), 190.3 (C=O) (Figure S23).

1.13 (1*E*,4*E*)-1-(2,3-bis(methoxymethoxy)phenyl)-5-phenylpenta-1,4-dien-3-one (3b).



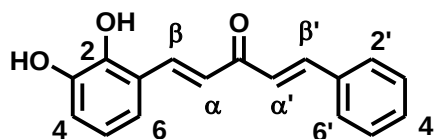
Yellow oil

Yield: 53%

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 3.54 (s; 3-OCH₂OCH₃), 3.65 (s; 2-OCH₂OCH₃), 5.22 (s; 3-OCH₂OCH₃), 5.24 (s; 2-OCH₂OCH₃), 7.11 (dd; 7.8 and 7.8; H-5), 7.12 (d; 16.2; H-α), 7.13 (d; 16.2; H-α'), 7.23 (d; 7.8; H-4), 7.37 (d; 7.8; H-6), 7.43–7.44 (H-3', H-4' and H-5'), 7.63 (dd; 1.2 and 6.6; H-2' and H-6'), 7.76 (d; 16.2; H-β'), 8.19 (d; 16.2; H-β) (Figure S24).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 56.3 (3-OCH₂OCH₃), 58.0 (2-OCH₂OCH₃), 95.1 (3-OCH₂OCH₃), 99.5 (2-OCH₂OCH₃), 118.3 (C-4), 120.5 (C-6), 124.6 (C-5), 125.3 (C-α'), 126.7 (C-α), 128.4 (C-2' and C-6'), 129.0 (C-3' and C-5'), 130.0 (C-1), 130.5 (C-4'), 134.8 (C-1'), 138.5 (C-β), 143.3 (C-β'), 146.4 (C-2), 150.3 (C-3), 189.1 (C=O) (Figure S25).

1.14 (1*E*,4*E*)-1-(2,3-dihydroxyphenyl)-5-phenylpenta-1,4-dien-3-one (**MAC 33**).



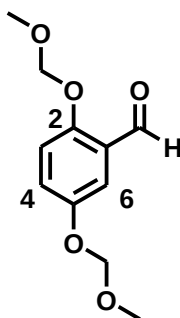
Red oil

Yield: 10%

¹H NMR (600 MHz; DMSO-*d*₆), δ_{H} in ppm (multiplicity; *J* in Hz): 6.70 (dd; 7.8 and 8.1; H-5), 6.86 (dd; 1.2 and 7.8; H-4), 7.19 (dd; 1.2 and 8.1; H-6), 7.28 (d; 16.2; H- α'), 7.30 (d; 16.2, H- α), 7.45–7.46 (H-3', H-4' and H-5'), 7.74 (d; 16.2; H- β'), 7.78 (dd; 2.4 and 7.5; H-2' and H-6'), 8.01 (d; 16.2; H- β) (Figure S26).

¹³C NMR (150 MHz; DMSO-*d*₆), δ_{C} in ppm: 117.4 (C-4), 119.0 (C-1), 119.5 (C-6), 122.3 (C-5), 125.0 (C- α), 126.7 (C- α'), 129.0 (C-2' and C-6'), 129.4 (C-3' and C-5'), 130.5 (C-4'), 135.2 (C-1'), 139.1 (C- β), 142.7 (C- β'), 146.4 (C-2), 146.6 (C-3), 189.0 (C=O) (Figure S27).

1.15 2,5-bis(methoxymethoxy)benzaldehyde (**2c**) (HOTSUMI et al., 2019).



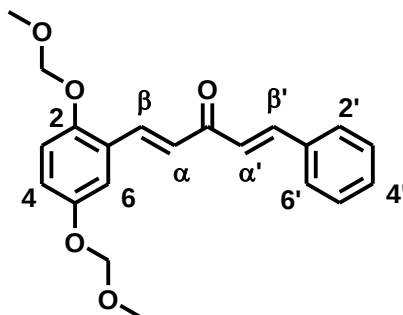
Colorless oil

Yield: 85%

¹H NMR (600 MHz; CDCl₃), δ_{H} in ppm (multiplicity; *J* in Hz): 3.41 (s; 5-OCH₂OCH₃), 3.46 (s; 2-OCH₂OCH₃), 5.09 (s; 5-OCH₂OCH₃), 5.20 (s; 2-OCH₂OCH₃), 7.12 (d; 9.0; H-3), 7.18 (dd; 3.0 and 9.0; H-4), 7.43 (d; 3.0; H-6), 10.40 (s; -COH) (Figure S28).

^{13}C NMR (150 MHz; CDCl_3), δ_{C} in ppm: 55.9 (5- OCH_2OCH_3), 56.3 (2- OCH_2OCH_3), 94.8 (5- OCH_2OCH_3), 95.1 (2- OCH_2OCH_3), 114.3 (C-3), 116.9 (C-4), 124.7 (C-6), 125.9 (C-1), 151.9 (C-5), 154.9 (C-2), 189.4 (C=O) (Figure S29).

1.16 (1*E*,4*E*)-1-(2,5-bis(methoxymethoxy)phenyl)-5-phenylpenta-1,4-dien-3-one (**3c**).

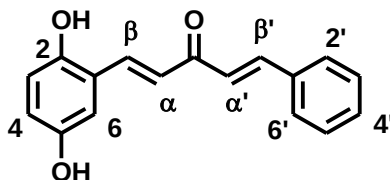


Yellow oil

Yield: 62%

^1H NMR (600 MHz; CDCl_3), δ_{H} in ppm (multiplicity; *J* in Hz): 3.53 (s; 5- OCH_2OCH_3), 3.54 (s; 2- OCH_2OCH_3), 5.19 (s; 5- OCH_2OCH_3), 5.25 (s; 2- OCH_2OCH_3), 7.08 (dd; 2.4 and 9.0; H-4), 7.11 (d; 16.2; H- α'), 7.13 (d; 16.2; H- α), 7.14 (d; 9.0; H-3), 7.35 (d; 2.4; H-6), 7.44–7.45 (H-3', H-4' and H-5'), 7.65 (dd; 2.4 and 7.5; H-2' and H-6'), 7.76 (d; 16.2; H- β'), 8.10 (d; 16.2; H- β) (Figure S30).

1.17 (1*E*,4*E*)-1-(2,5-dihydroxyphenyl)-5-phenylpenta-1,4-dien-3-one (**MAC 34**).



Red oil

Yield: 16%

¹H NMR (600 MHz; DMSO-*d*₆), δ_H in ppm (multiplicity; *J* in Hz): 6.74 (dd; 3.0 and 8.4; H-4), 6.78 (d; 8.4; H-3), 7.08 (d; 3.0; H-6), 7.23 (d; 16.2; H- α'), 7.28 (d; 15.6; H-α), 7.45–7.46 (H-3', H-4' and H-5'), 7.73 (d; 16.2; H-β'), 7.78 (dd; 1.8 and 7.5; H-2' and H-6'), 7.93 (d; 15.6; H-β) (Figure S31).

¹³C NMR (150 MHz; DMSO-*d*₆), δ_C in ppm: 113.7 (C-6), 117.6 (C-4), 120.2 (C-3), 122.0 (C-1), 124.8 (C- α'), 126.8 (C-α), 129.0 (C-2' and C-6'), 129.4 (C-3' and C-5'), 130.9 (C-4'), 135.2 (C-1'), 138.9 (C-β'), 142.7 (C-β), 150.4 (C-2), 150.8 (C-5), 189.0 (C=O) (Figure S32).

Figure S1. ^1H NMR spectrum of **MAC 24** (600 MHz; CDCl_3).

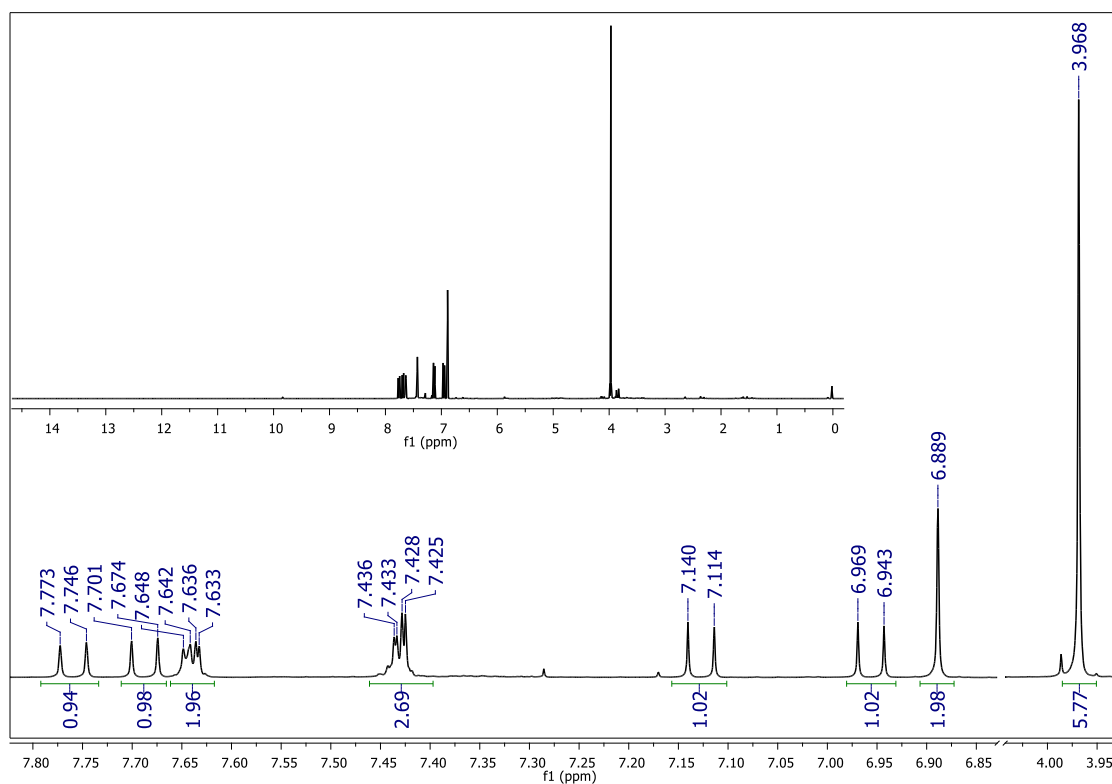


Figure S2. ^{13}C NMR spectrum of **MAC 24** (150 MHz; CDCl_3).

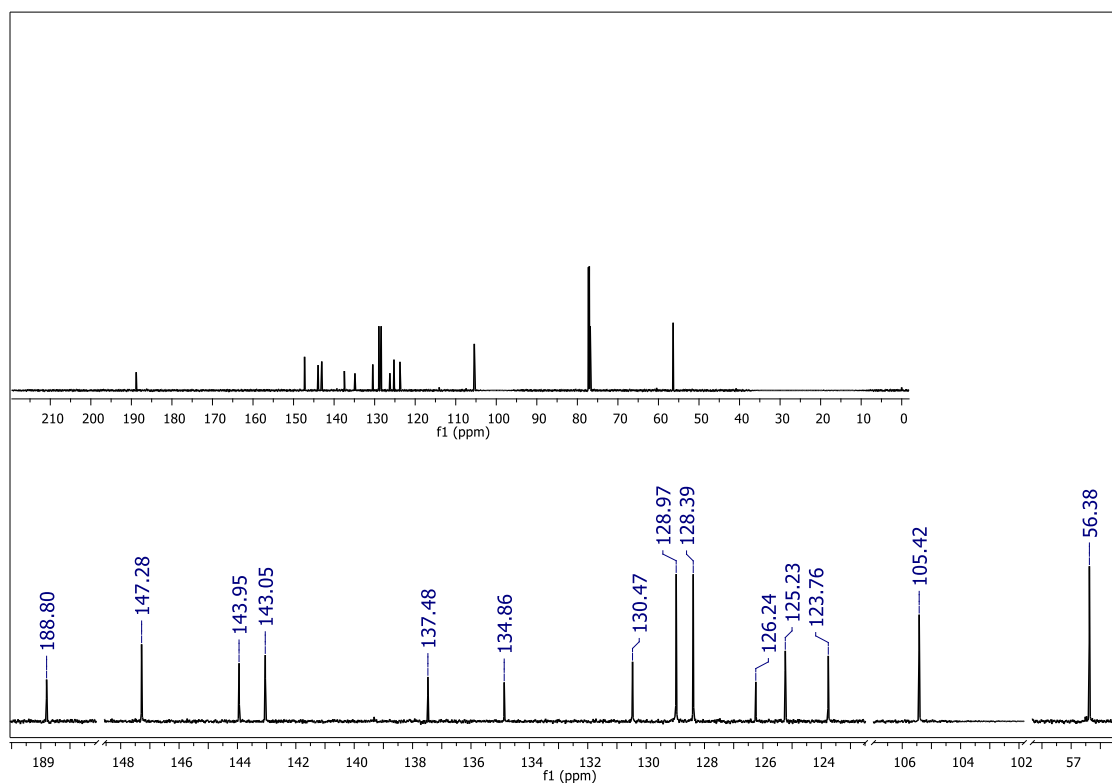


Figure S3. ^1H NMR spectrum of **MAC 25** (600 MHz; CDCl_3).

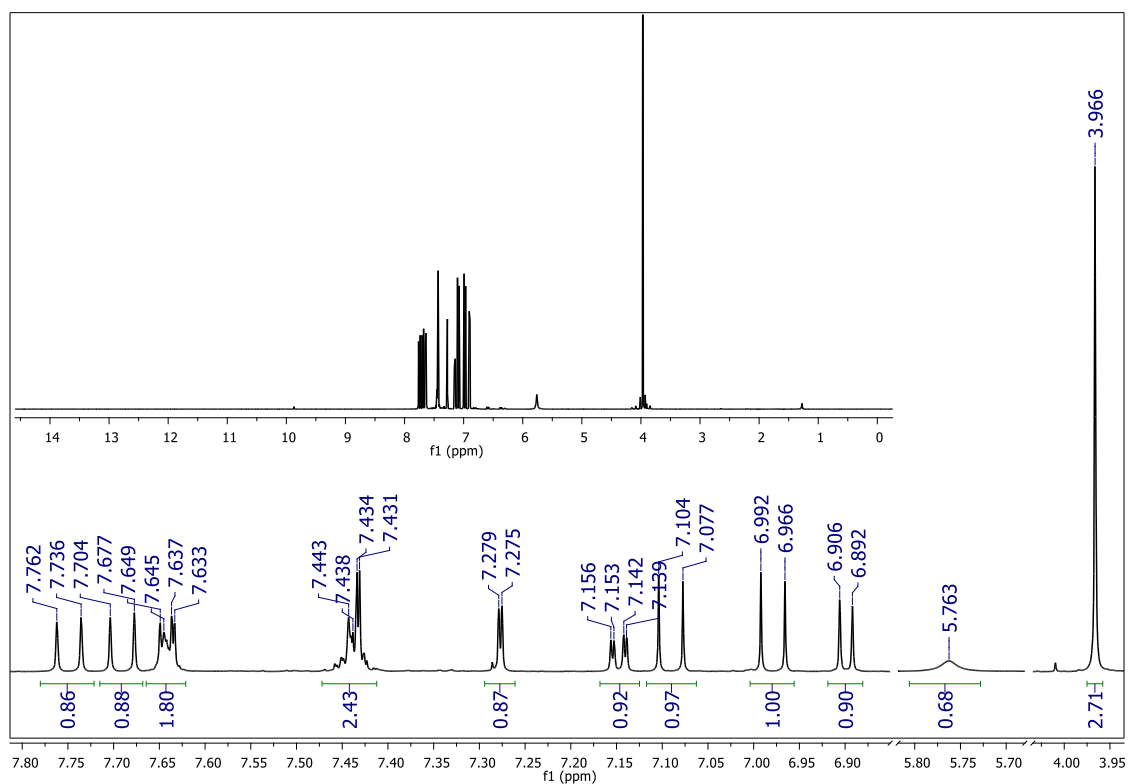


Figure S4. ^{13}C NMR spectrum of **MAC 25** (150 MHz; CDCl_3).

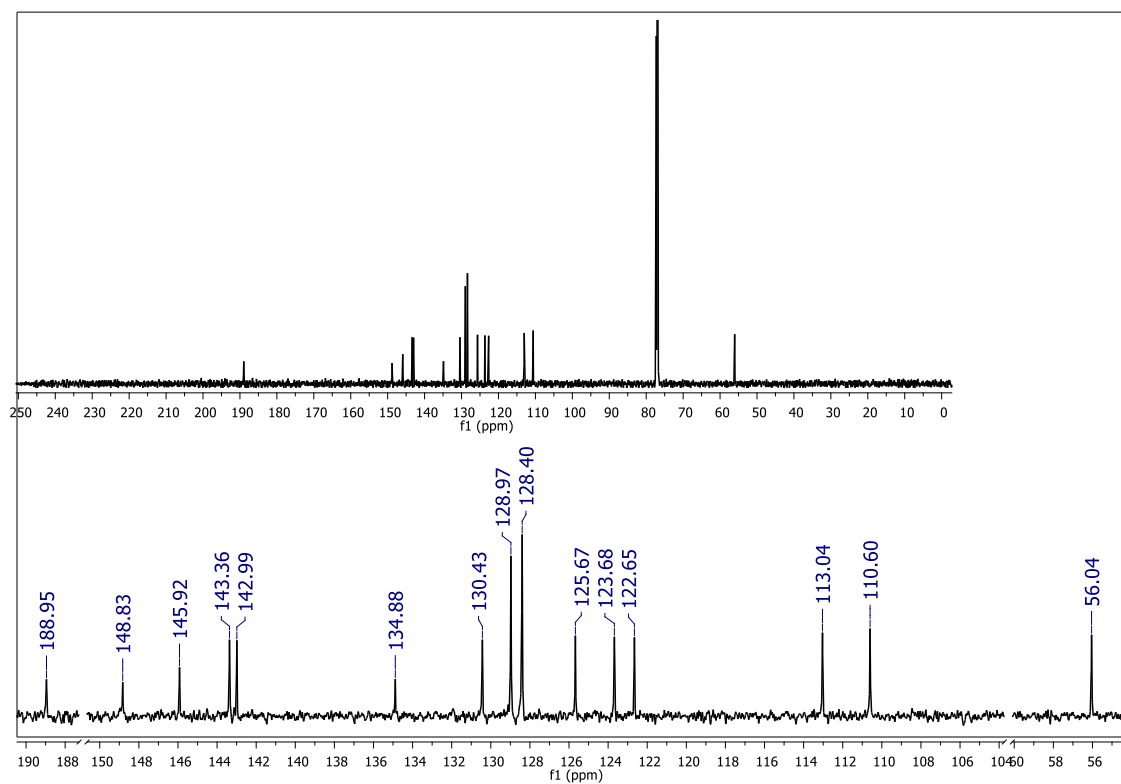


Figure S5. ^1H NMR spectrum of **MAC 26** (600 MHz; CDCl_3).

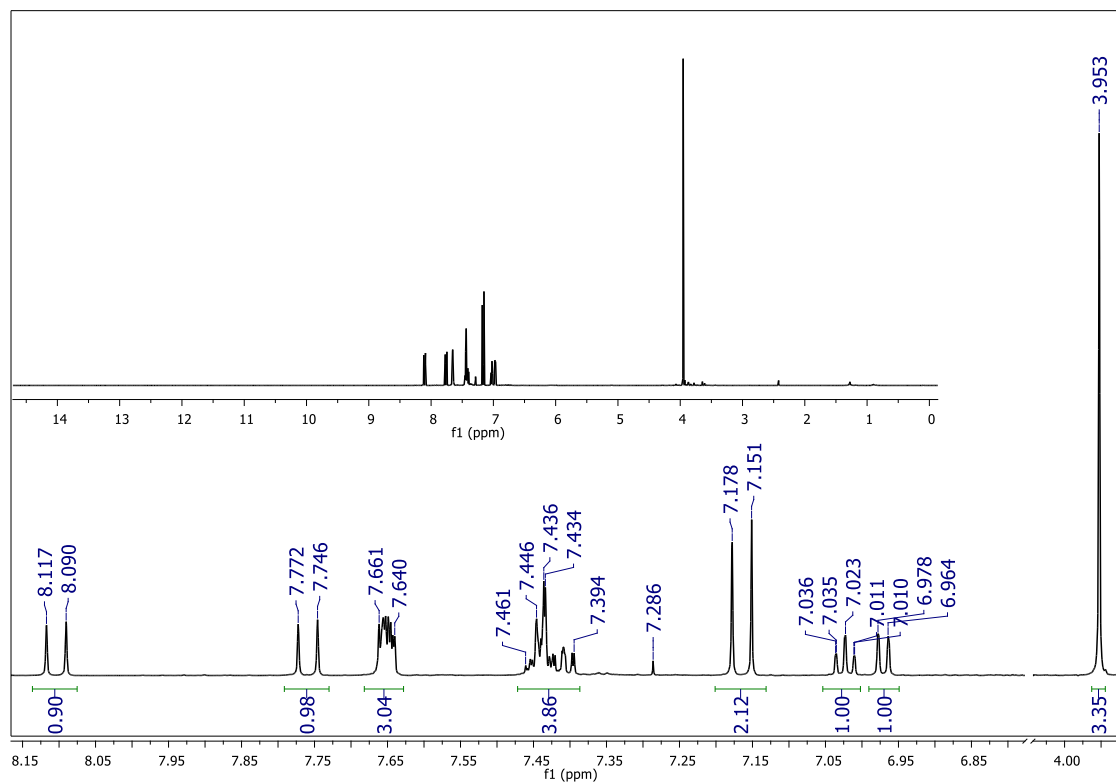


Figure S6. ^{13}C NMR spectrum of **MAC 26** (150 MHz; CDCl_3).

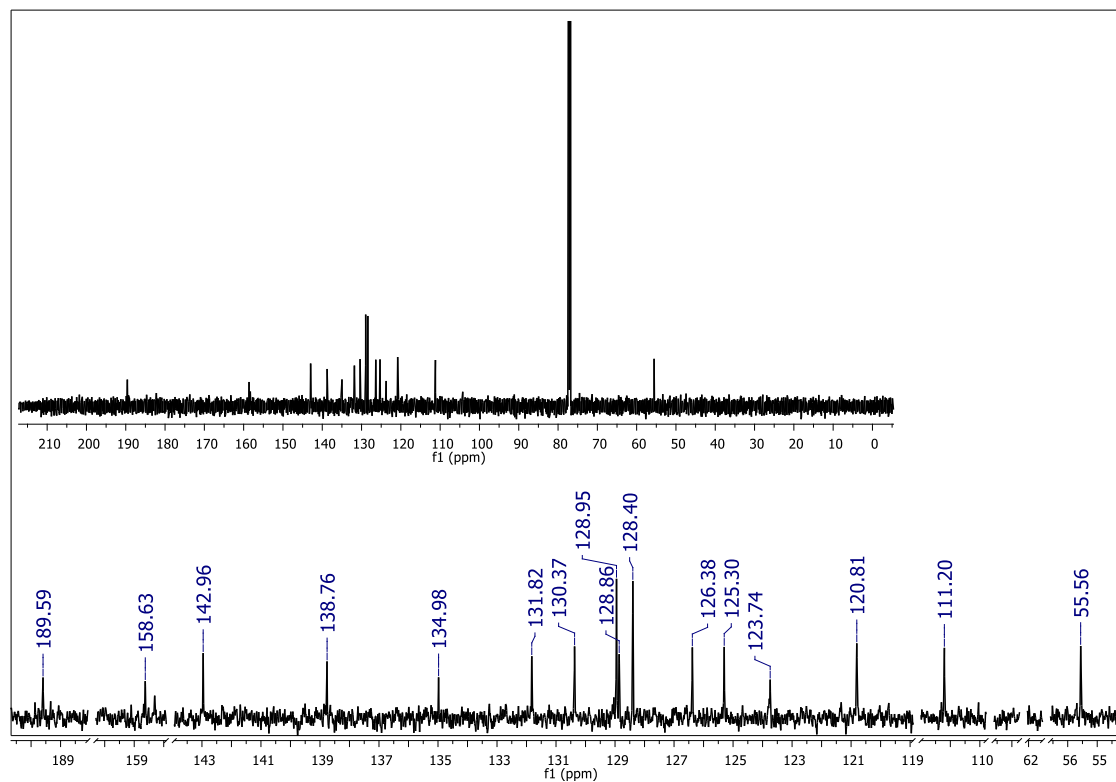


Figure S7. ^1H NMR spectrum of **MAC 27** (300 MHz; CDCl_3).

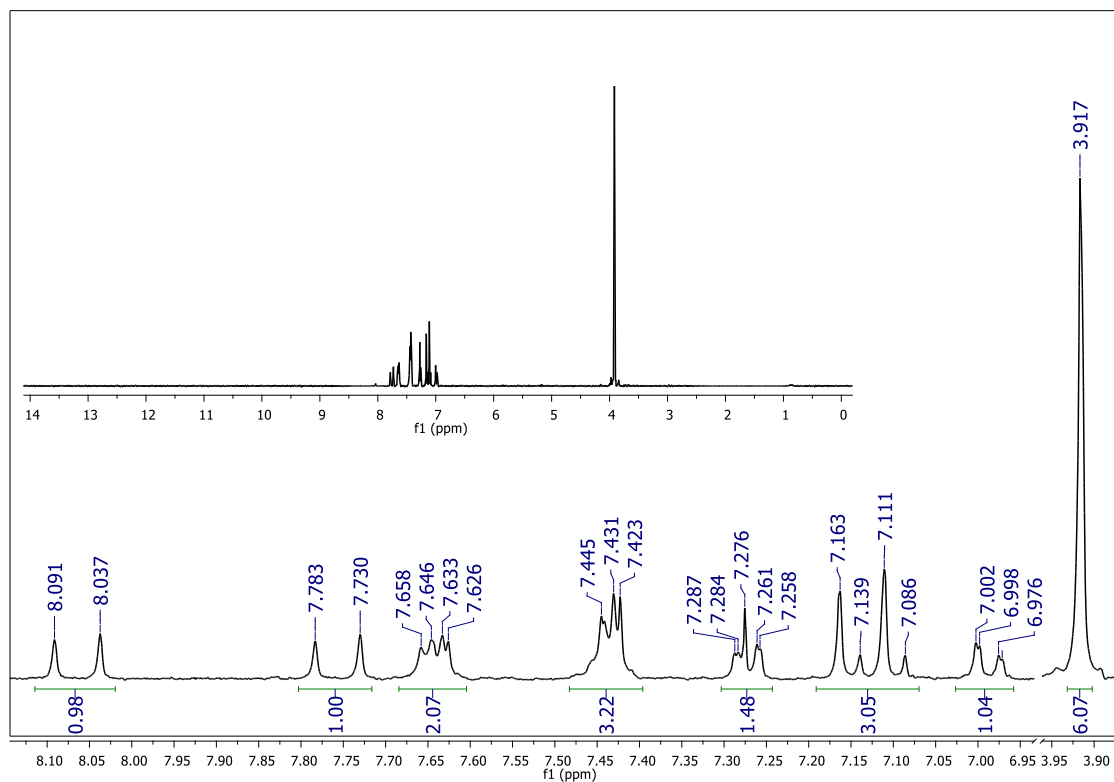


Figure S8. ^{13}C NMR spectrum of **MAC 27** (150 MHz; CDCl_3).

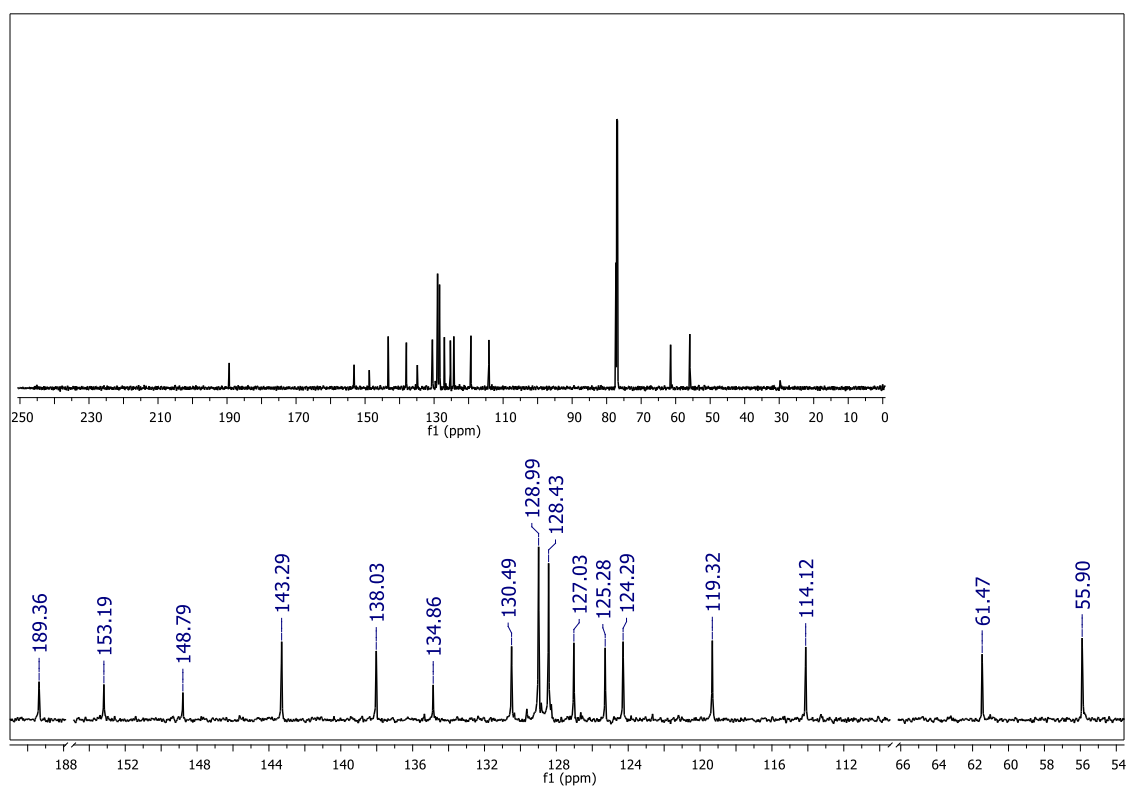


Figure S9. ^1H NMR spectrum of **MAC 28** (600 MHz; CDCl_3).

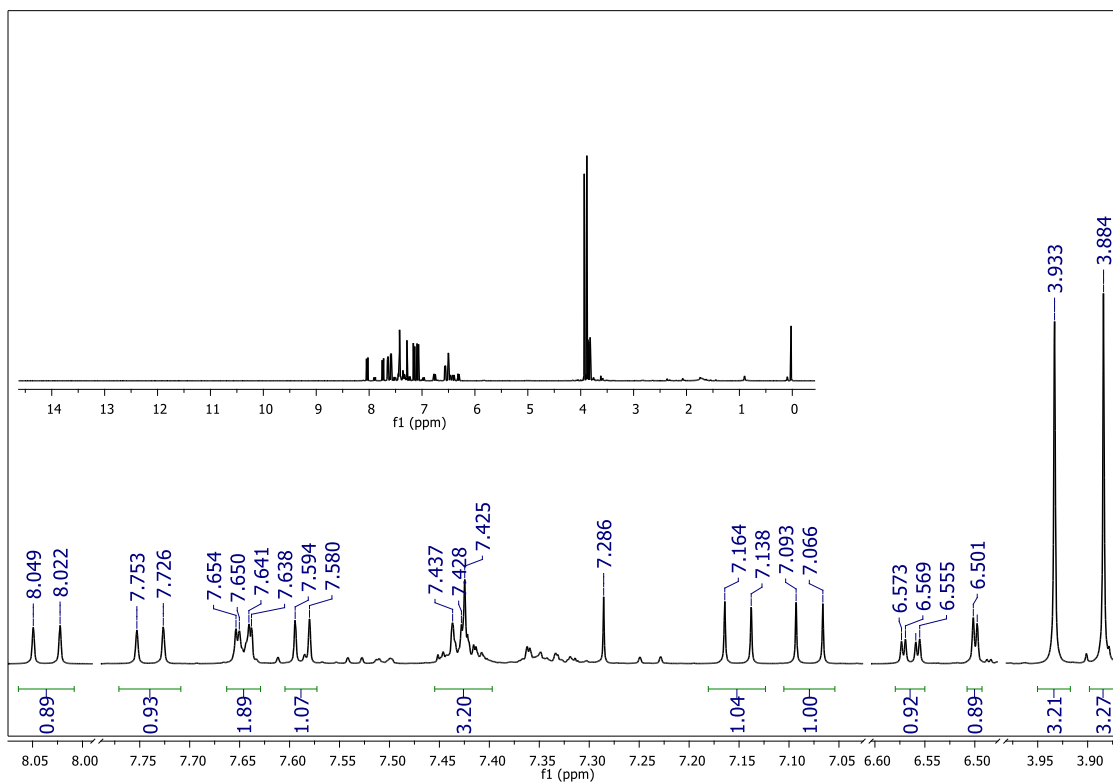


Figure S10. ^{13}C NMR spectrum of **MAC 28** (150 MHz; CDCl_3).

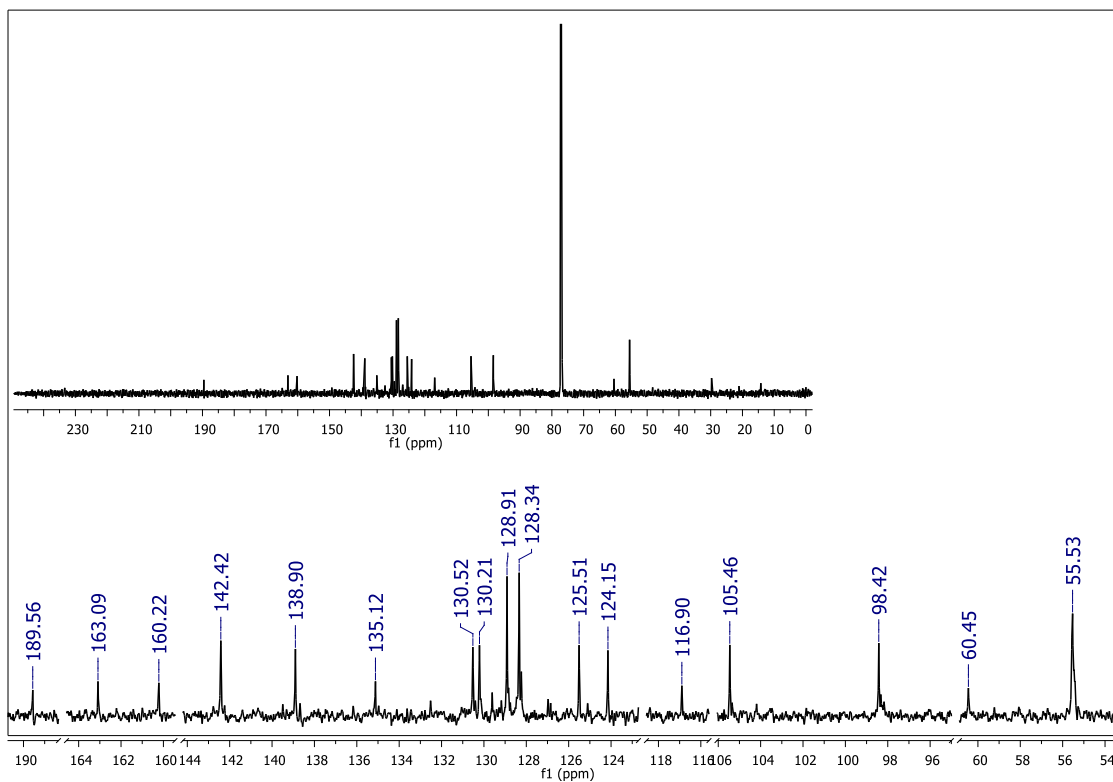


Figure S11. ^1H NMR spectrum of **MAC 29** (600 MHz; CDCl_3).

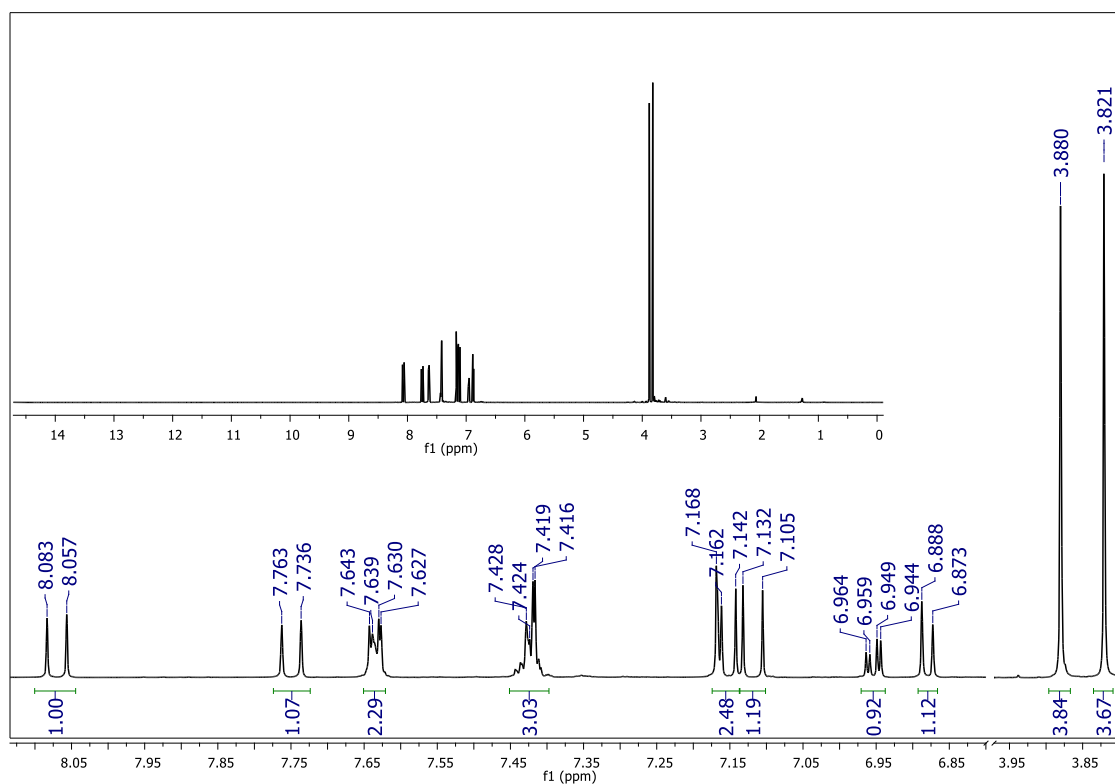


Figure S12. ^{13}C NMR spectrum of **MAC 29** (150 MHz; CDCl_3).

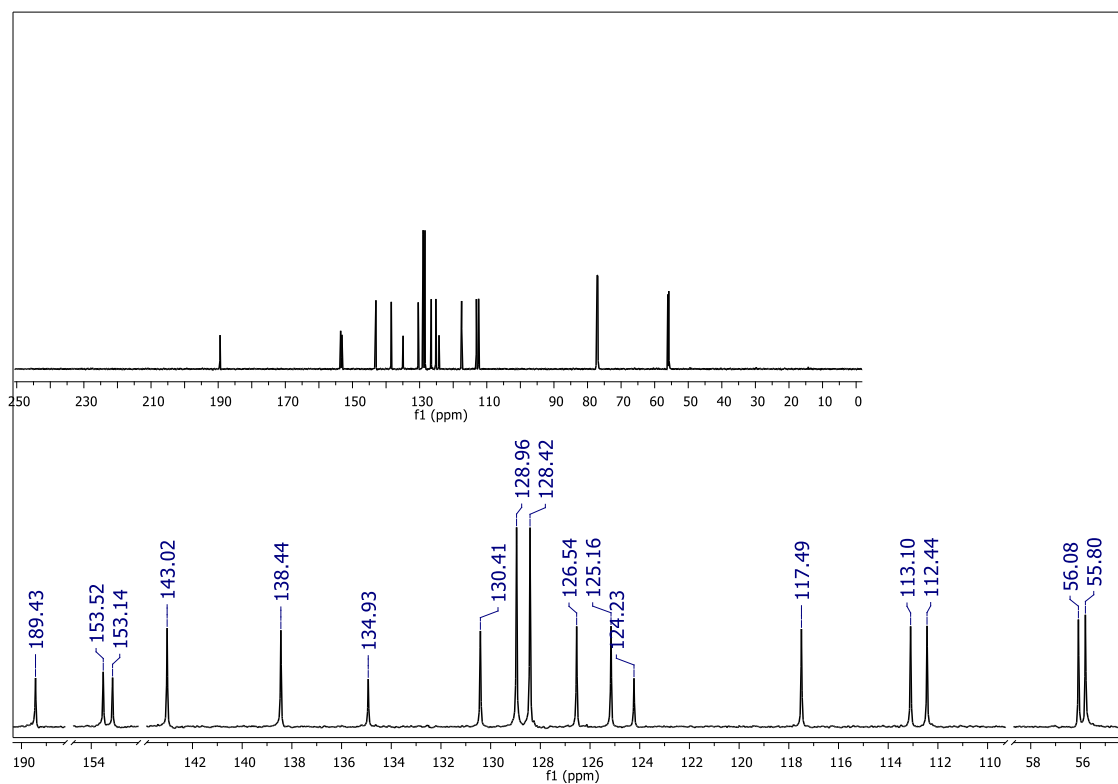


Figure S13. ^1H NMR spectrum of **MAC 30** (600 MHz; CDCl_3).

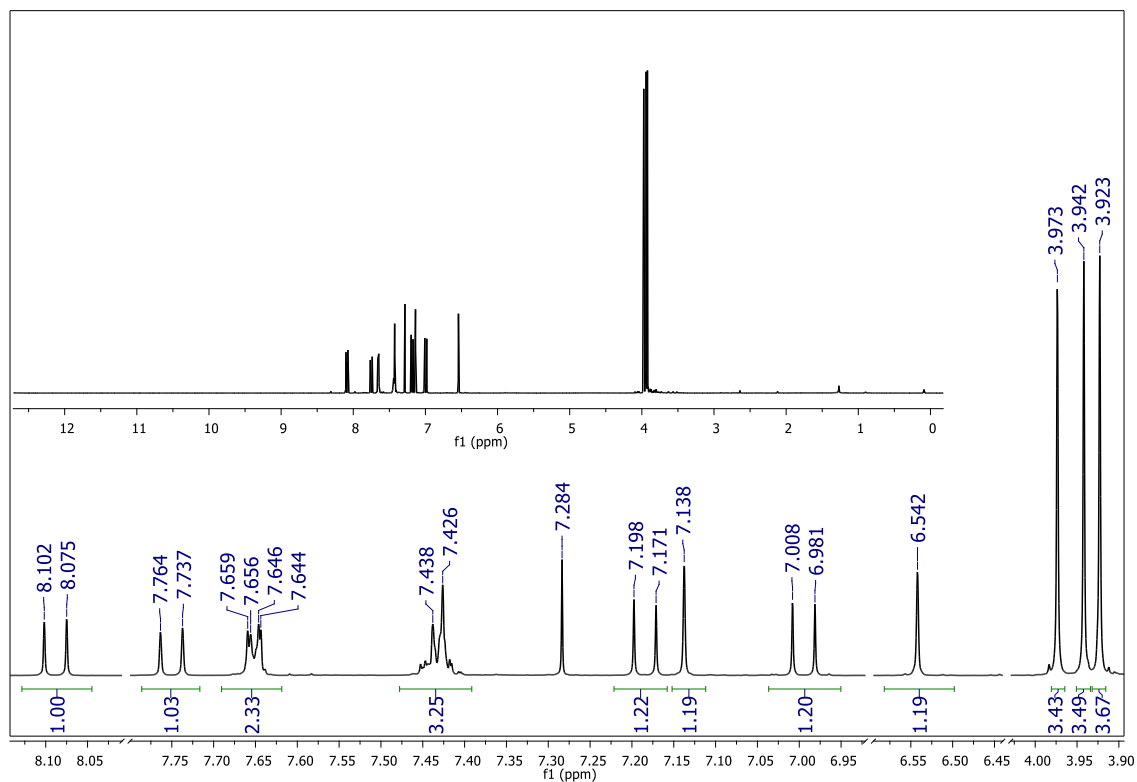


Figure S14. ^{13}C NMR spectrum of **MAC 30** (150 MHz; CDCl_3).

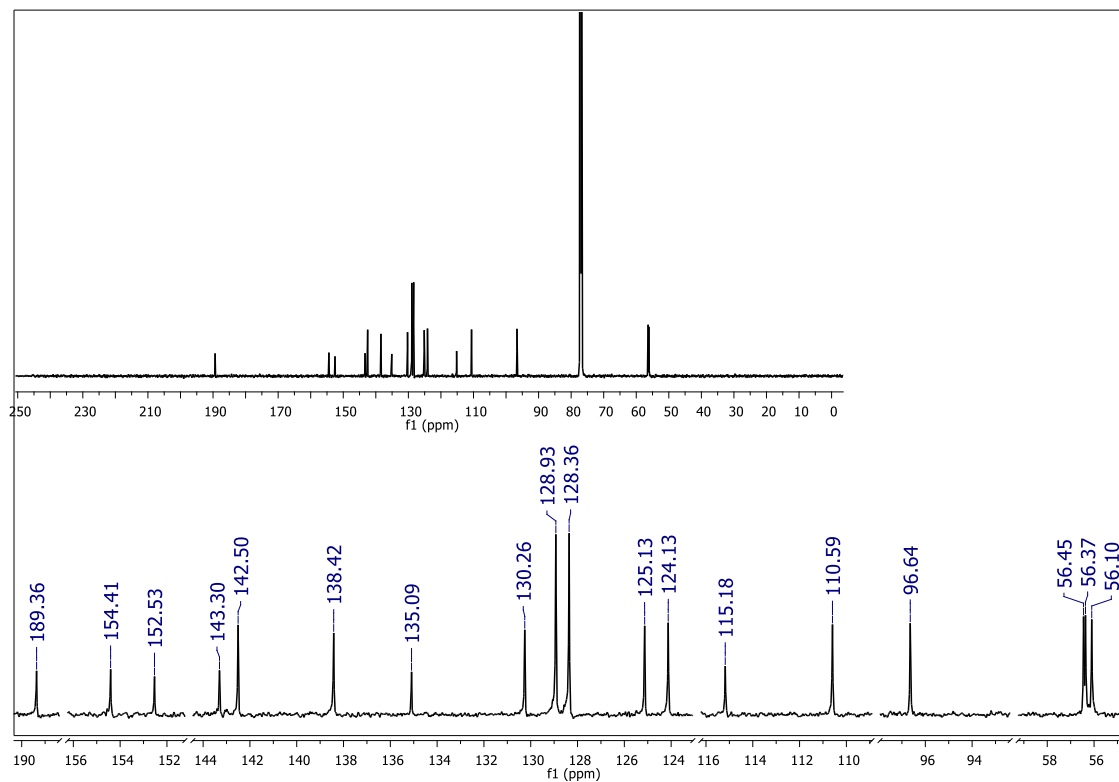


Figure S15. ^1H NMR spectrum of **MAC 31** (600 MHz; CDCl_3).

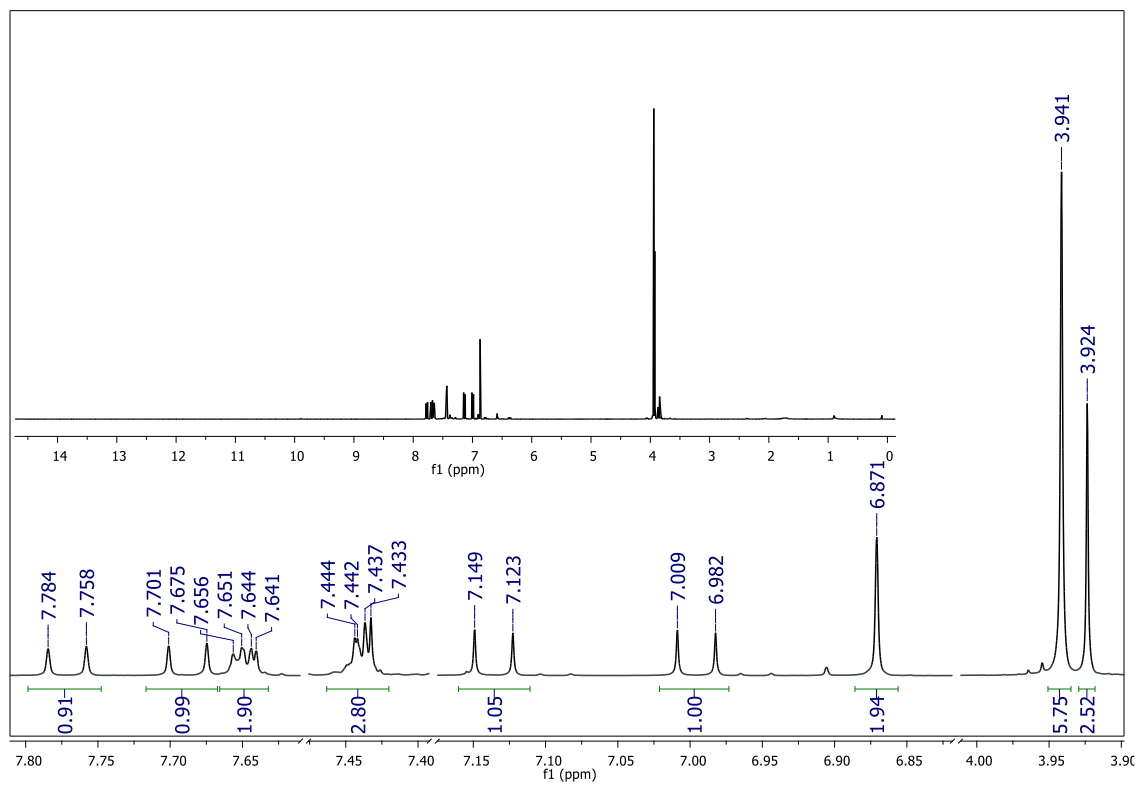


Figure S16. ^{13}C NMR spectrum of **MAC 31** (150 MHz; CDCl_3).

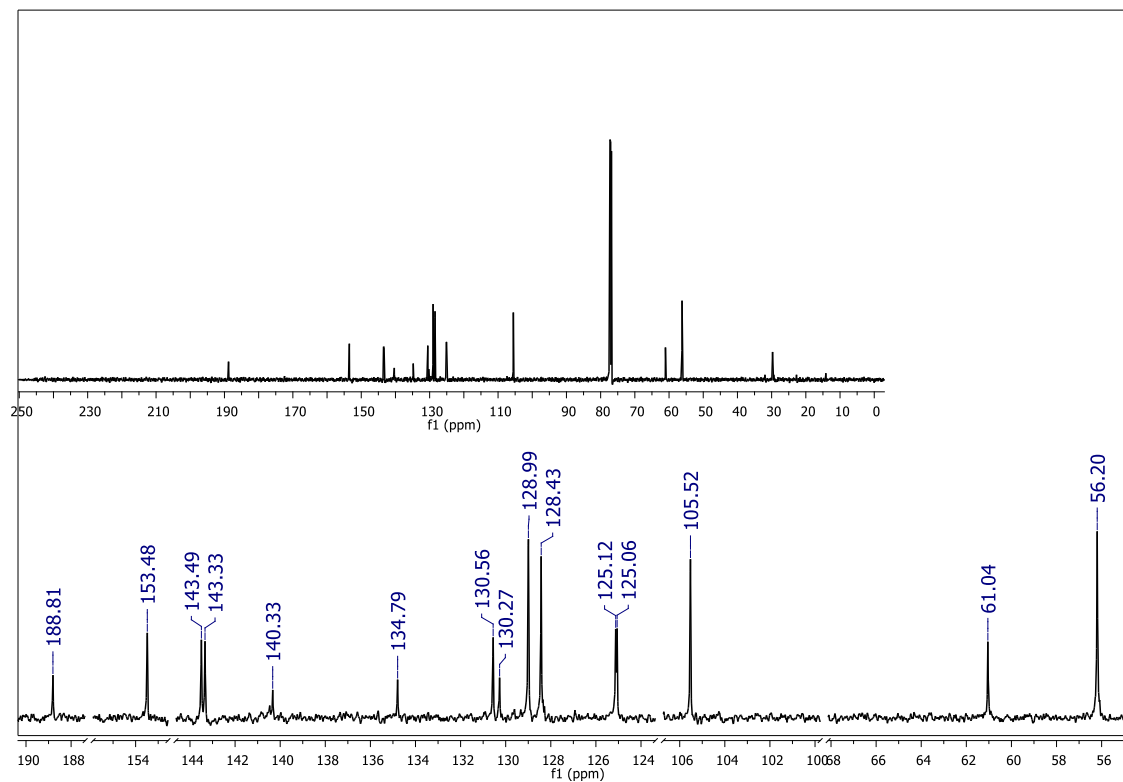


Figure S17. ^1H NMR spectrum of compound **2a** (600 MHz; CDCl_3).

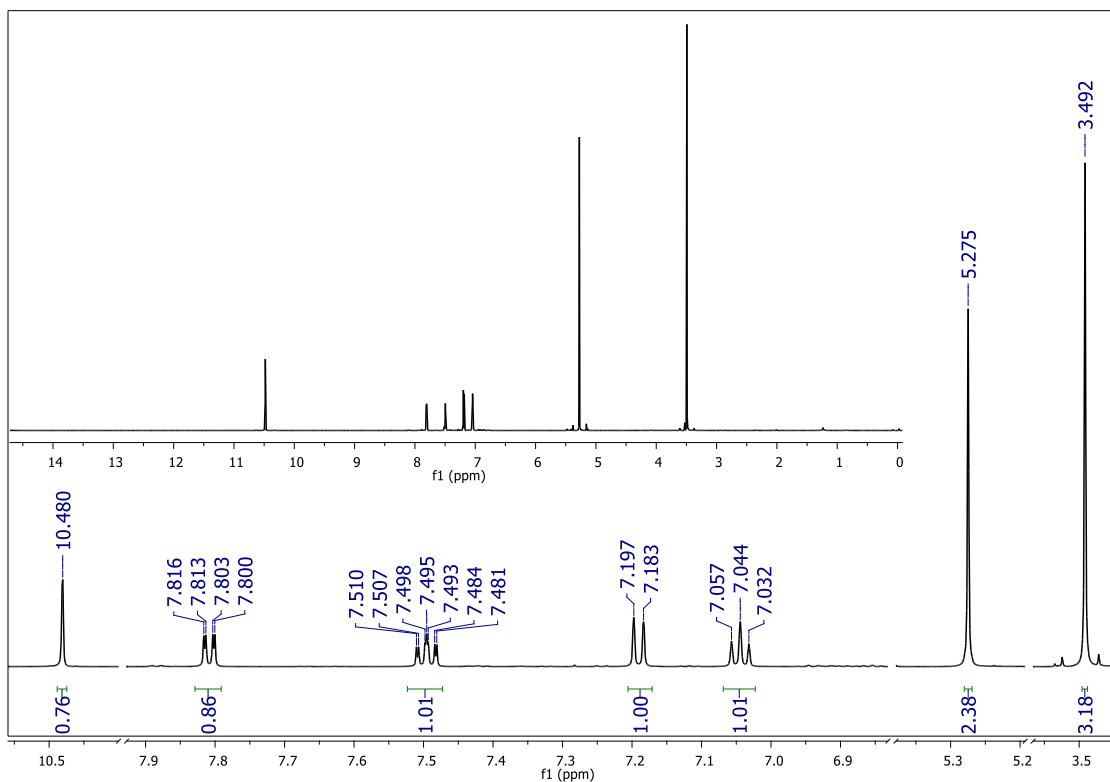


Figure S18. ^{13}C NMR spectrum of compound **2a** (150 MHz; CDCl_3).

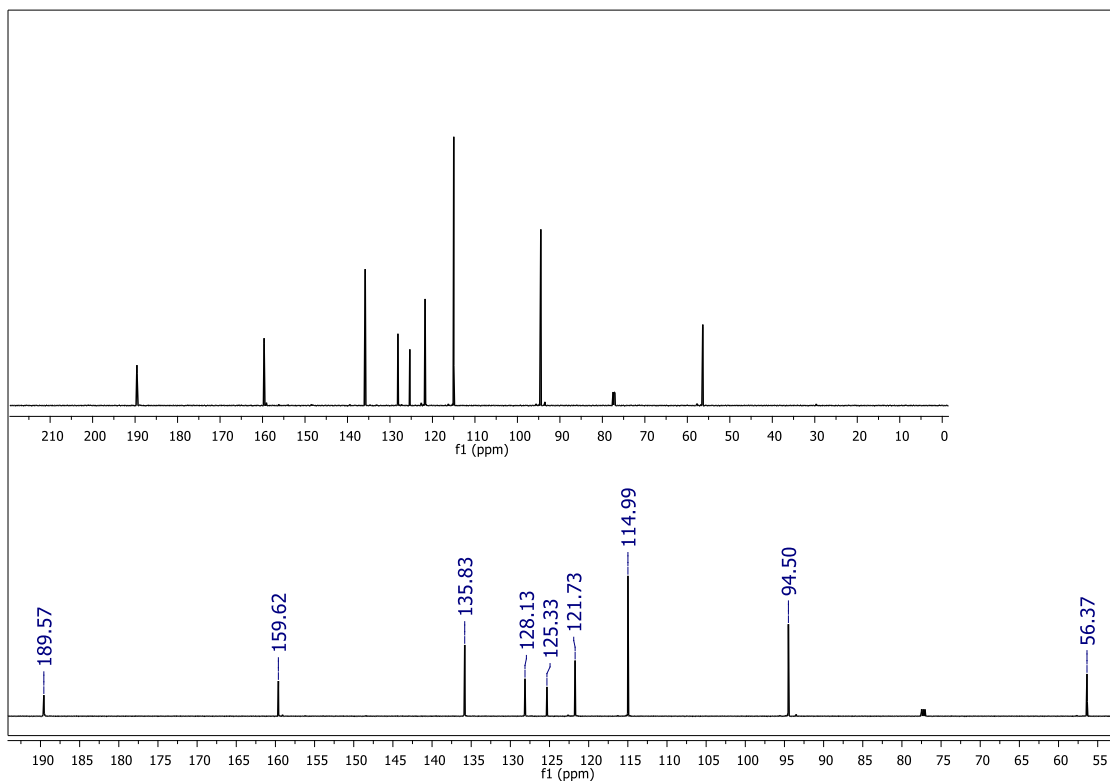


Figure S19. ^1H NMR spectrum of compound **3a** (600 MHz; CDCl_3).

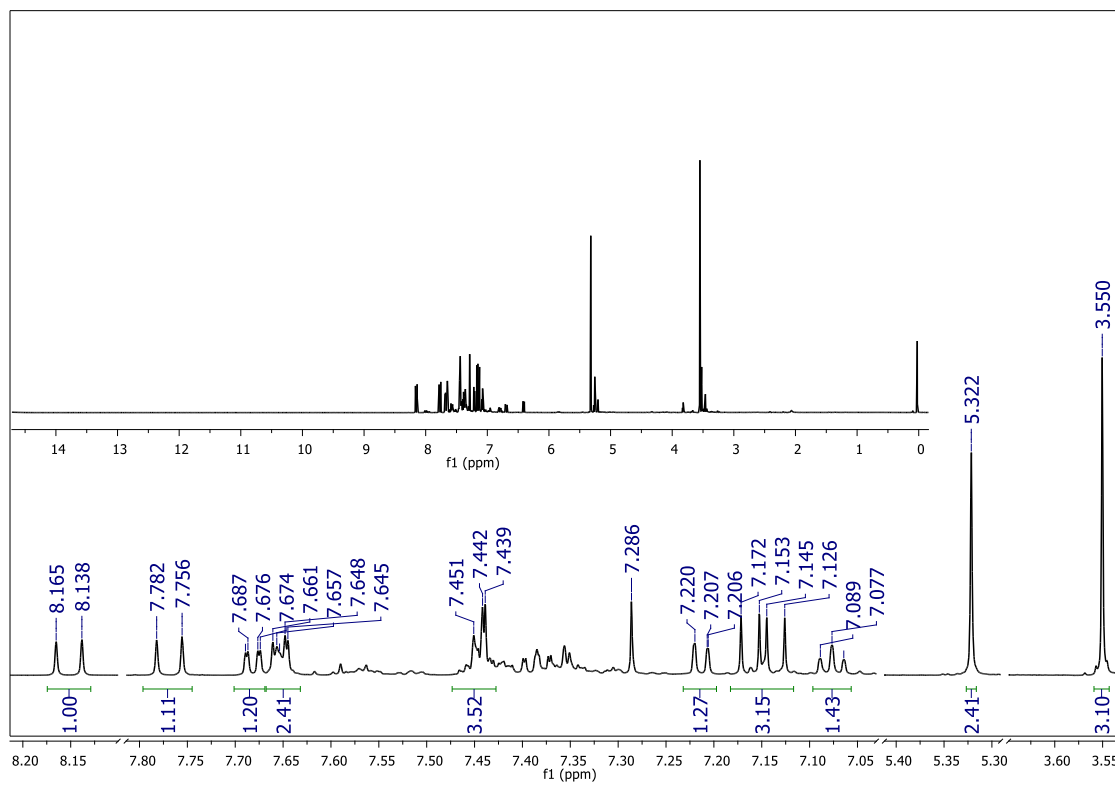


Figure S20. ^1H NMR spectrum of **MAC 32** (600 MHz; CDCl_3).

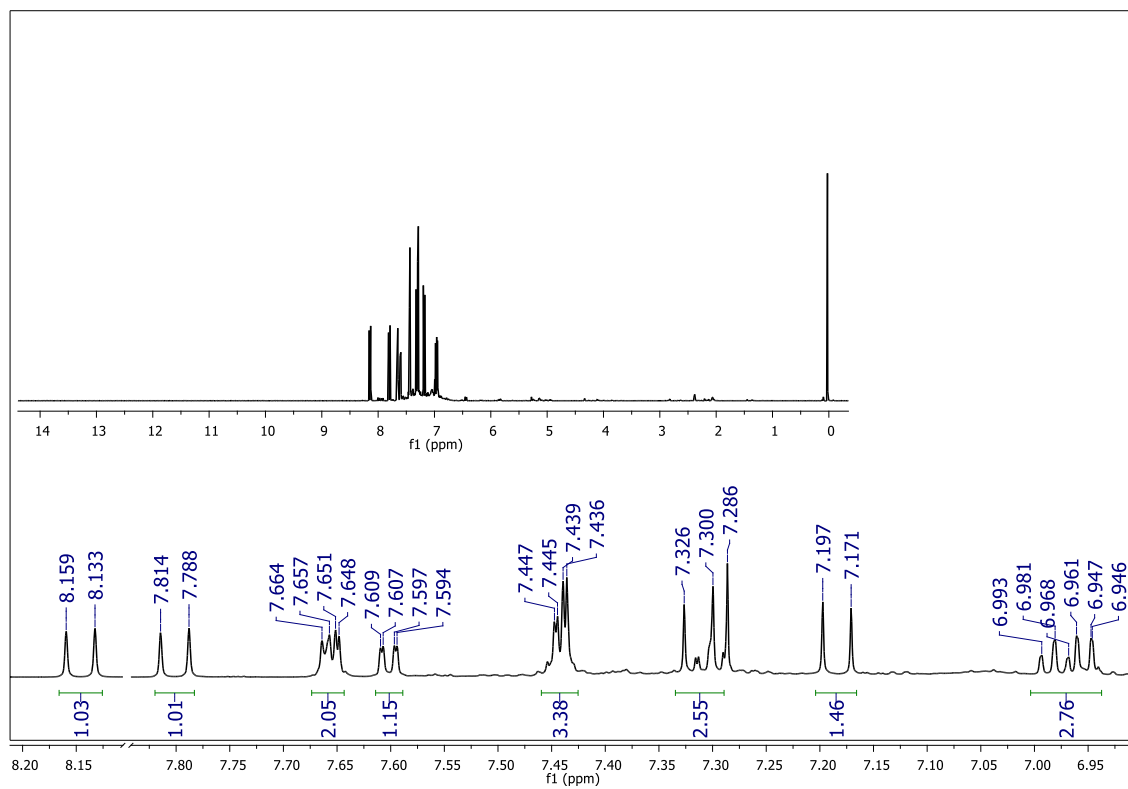


Figure S21. ^{13}C NMR spectrum of **MAC 32** (150 MHz; CDCl_3).

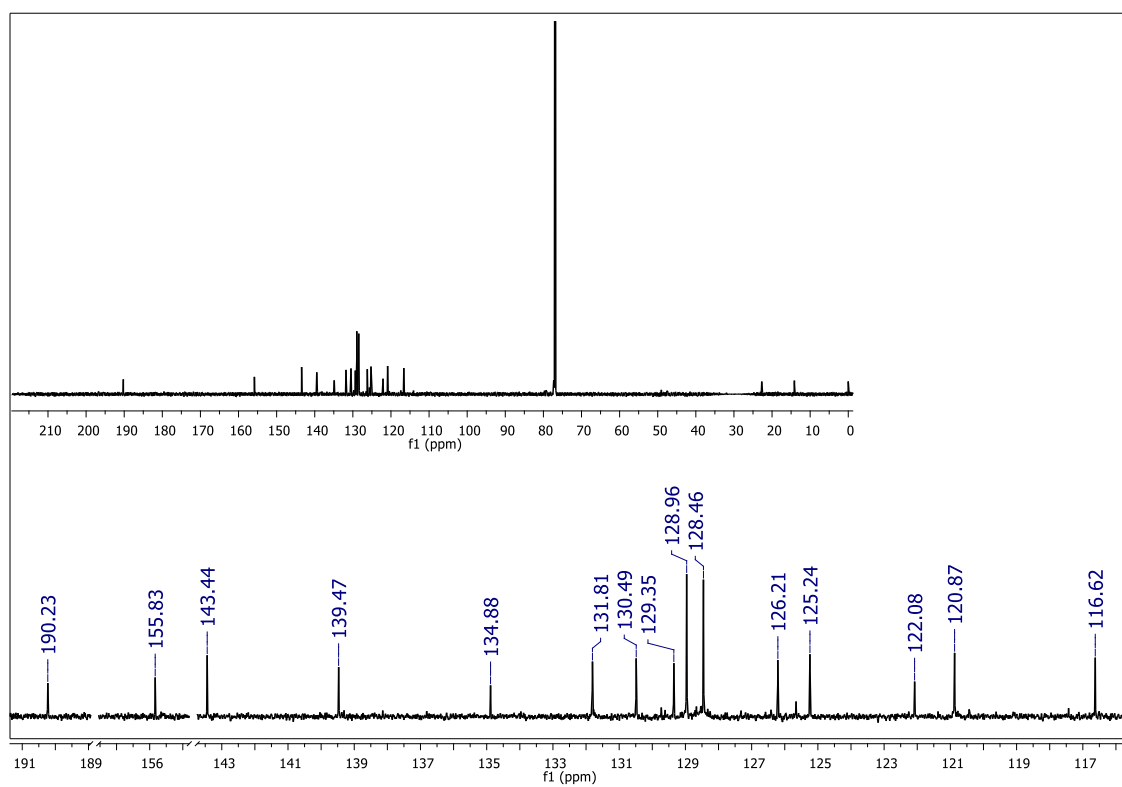


Figure S22. ^1H NMR spectrum of compound **2b** (600 MHz; CDCl_3).

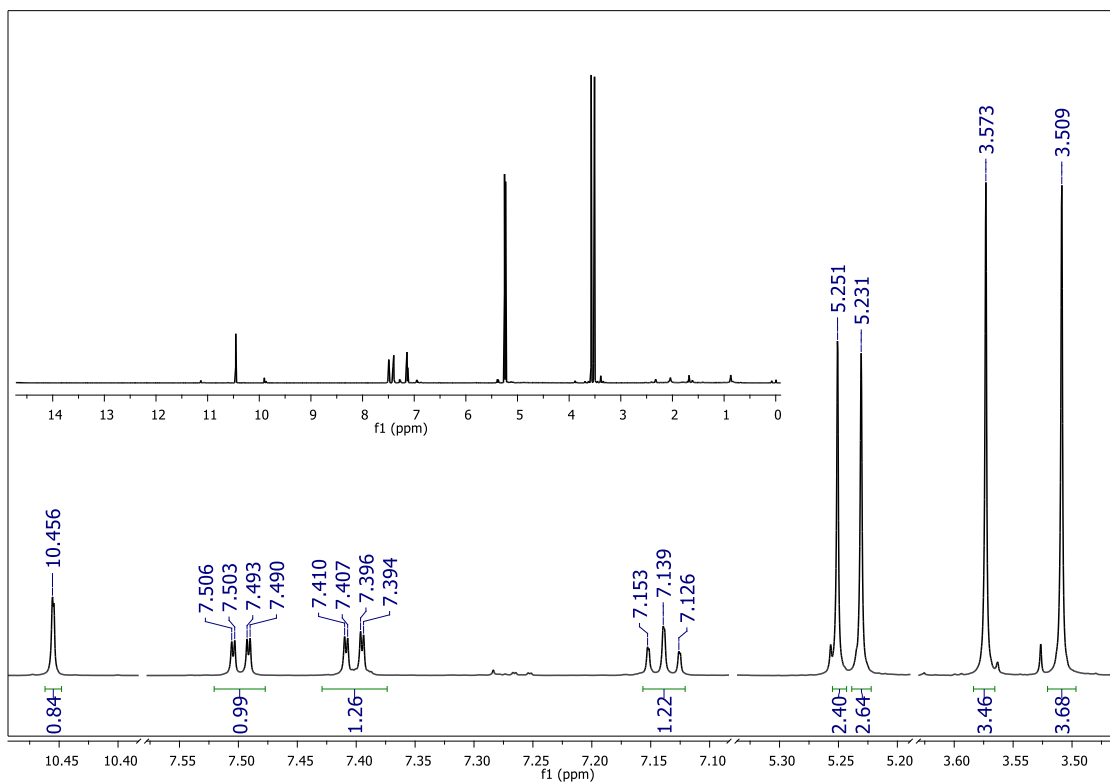


Figure S23. ^{13}C NMR spectrum of compound **2b** (150 MHz; CDCl_3).

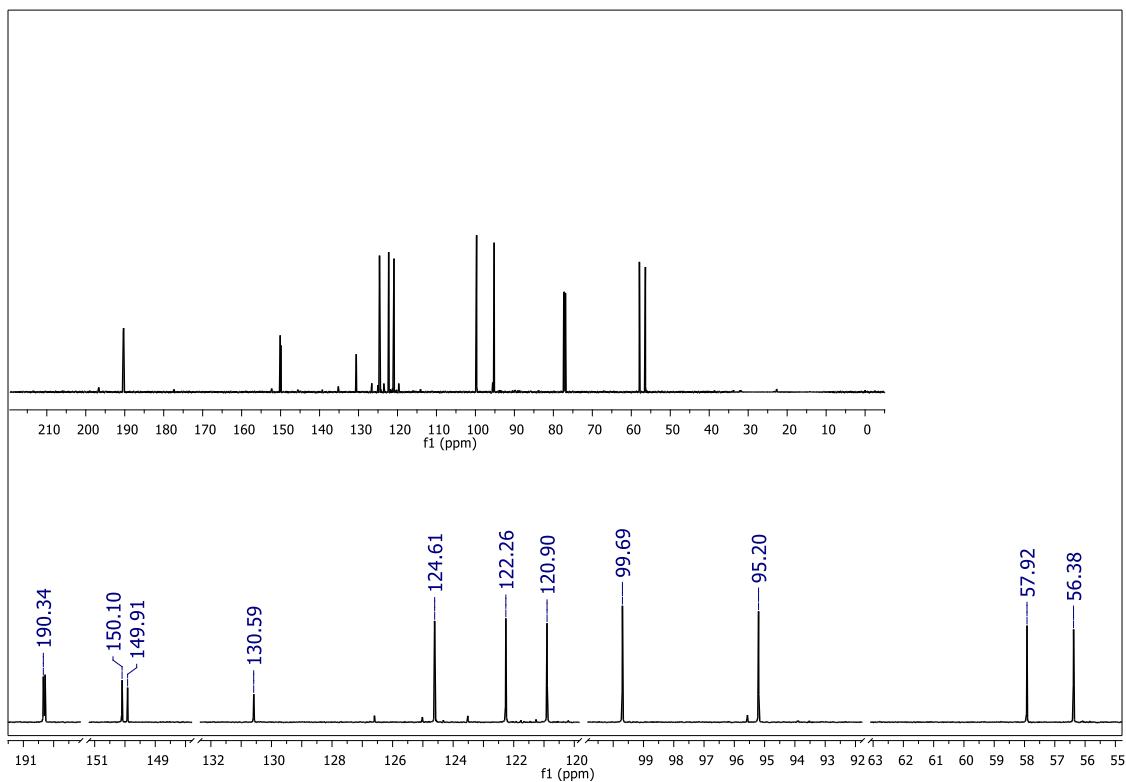


Figure S24. ^1H NMR spectrum of compound **3b** (600 MHz; CDCl_3).

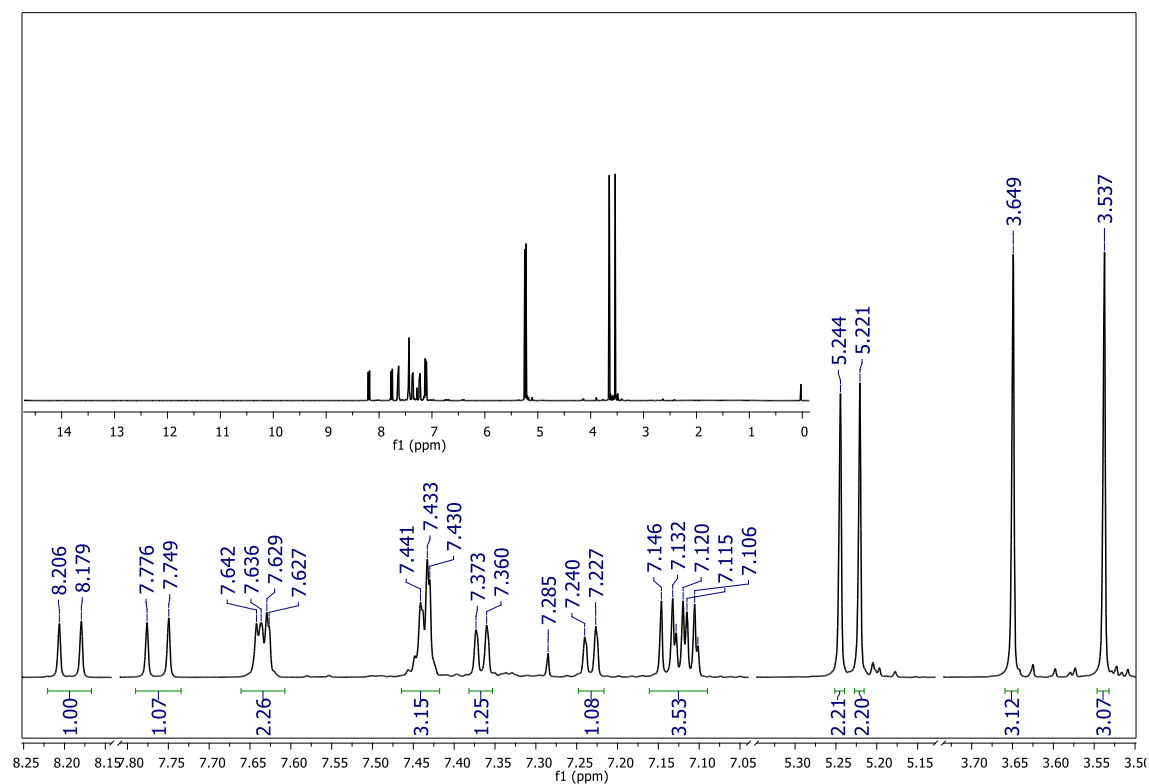


Figure S25. ^{13}C NMR spectrum of compound **3b** (150 MHz; CDCl_3).

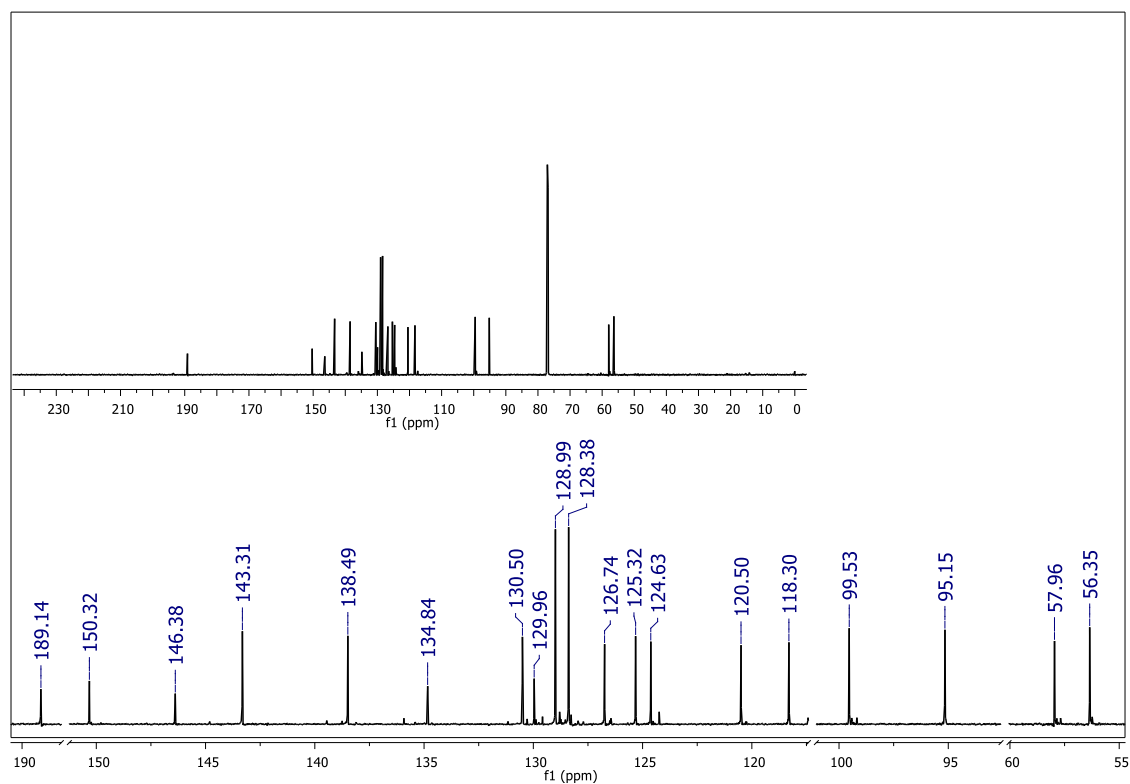


Figure S26. ^1H NMR spectrum of **MAC 33** (600 MHz; $\text{DMSO-}d_6$).

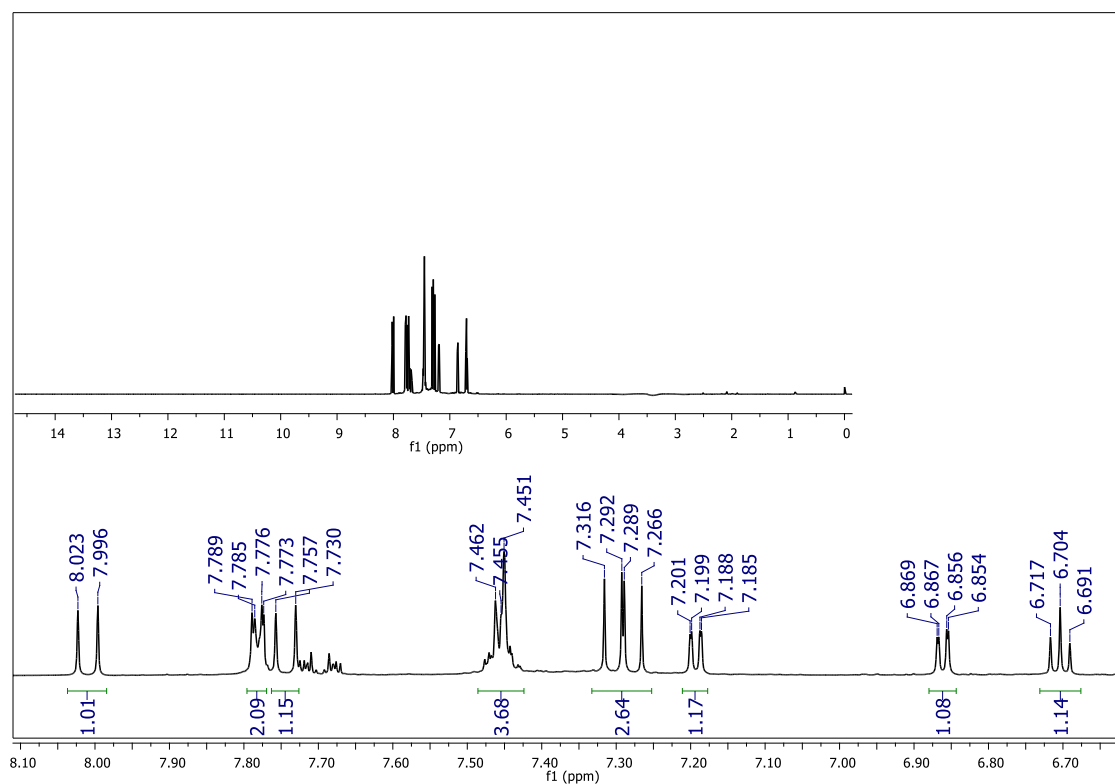


Figure S27. ^{13}C NMR spectrum of **MAC 33** (150 MHz; $\text{DMSO-}d_6$).

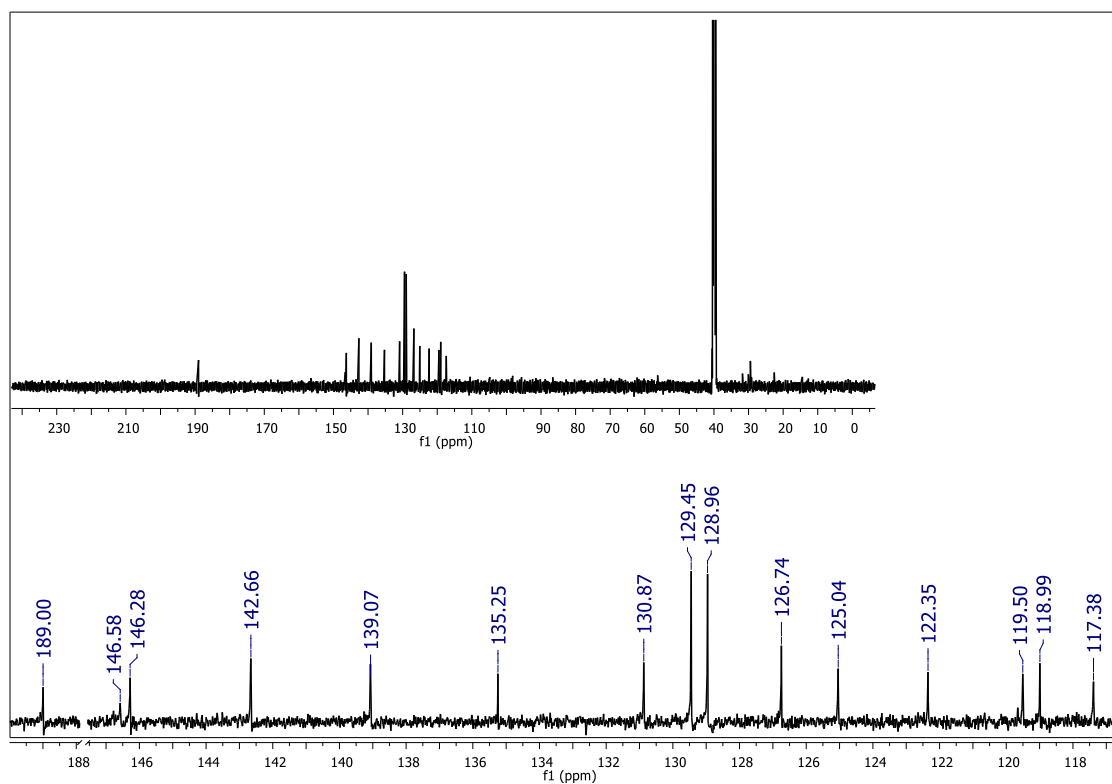


Figure S28. ^1H NMR spectrum of compound **2c** (600 MHz; CDCl_3).

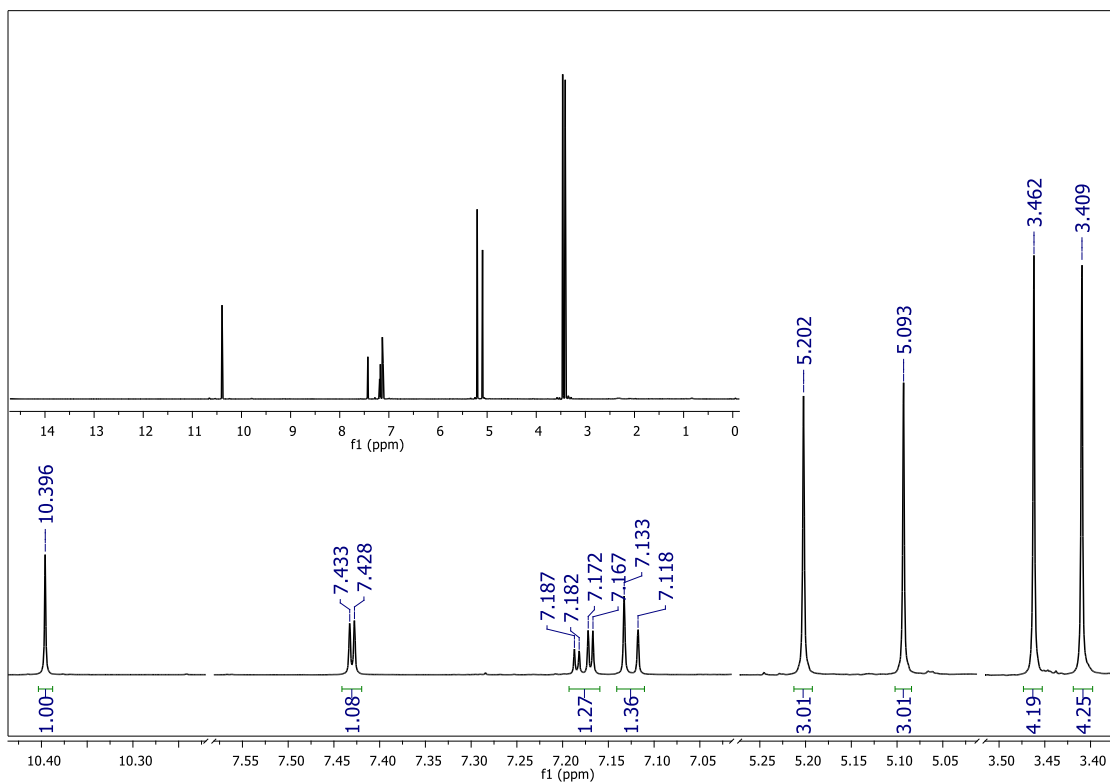


Figure S29. ^{13}C NMR spectrum of compound **2c** (150 MHz; CDCl_3).

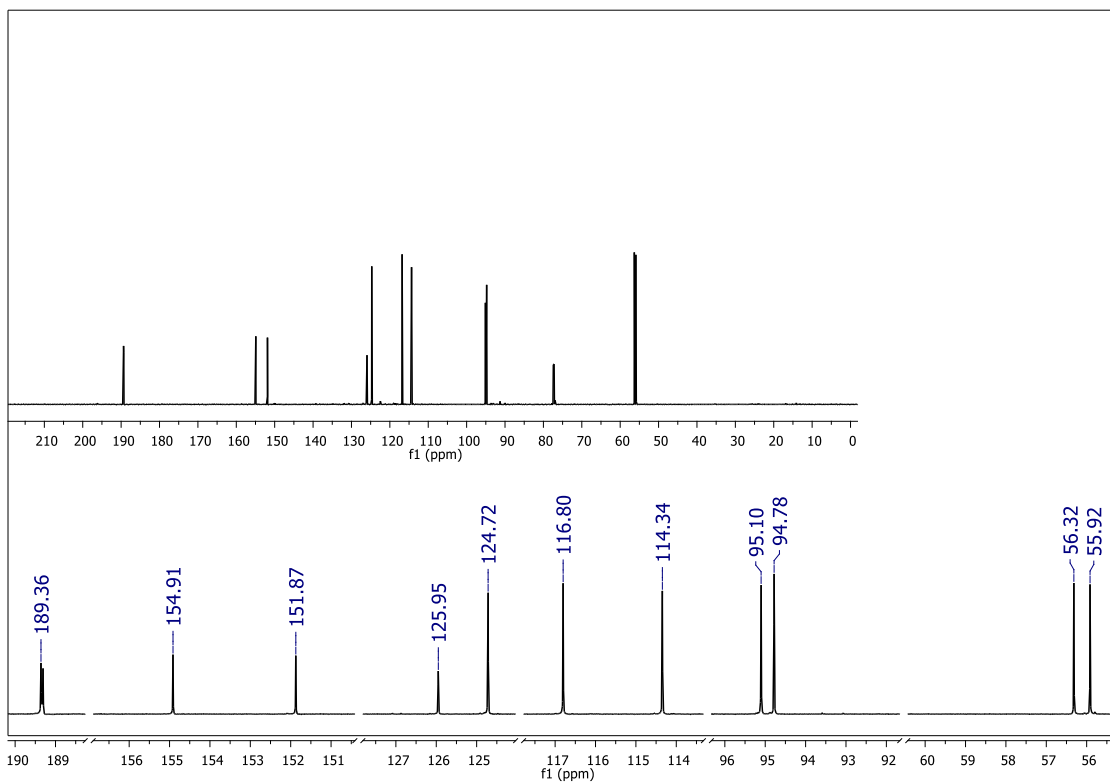


Figure S30. ^1H NMR spectrum of compound **3c** (600 MHz; CDCl_3).

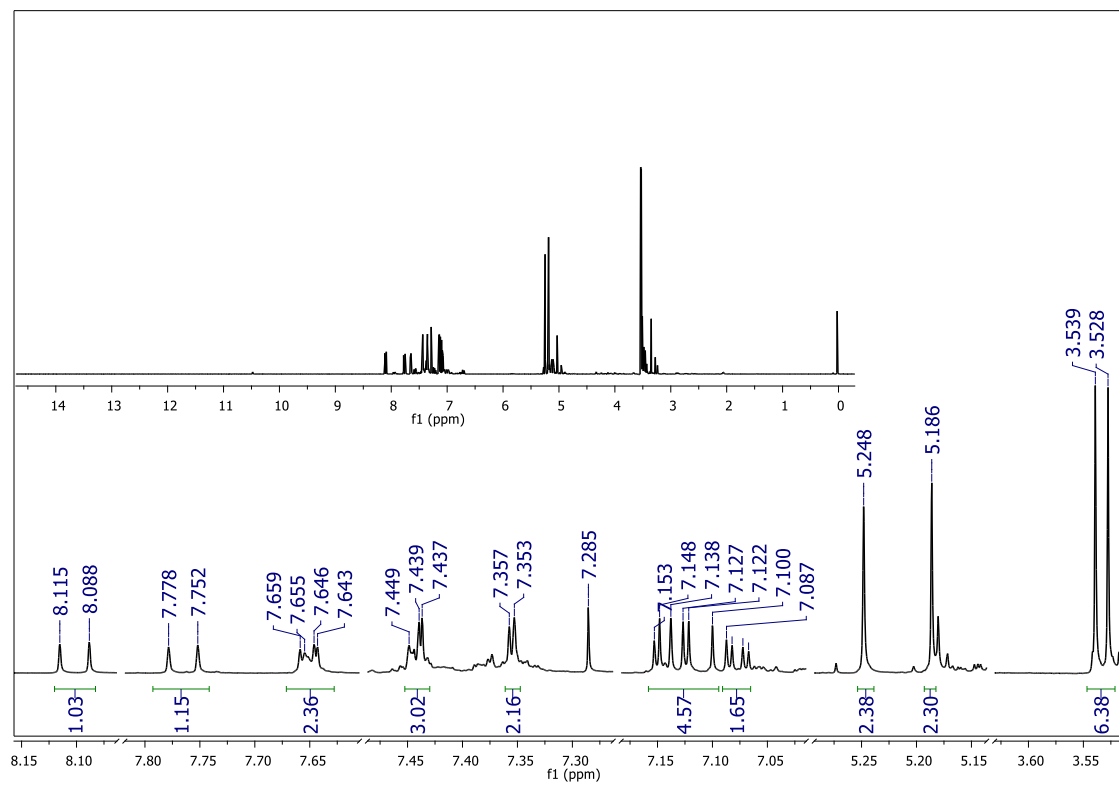


Figure S31. ^1H NMR spectrum of **MAC 34** (600 MHz; $\text{DMSO-}d_6$).

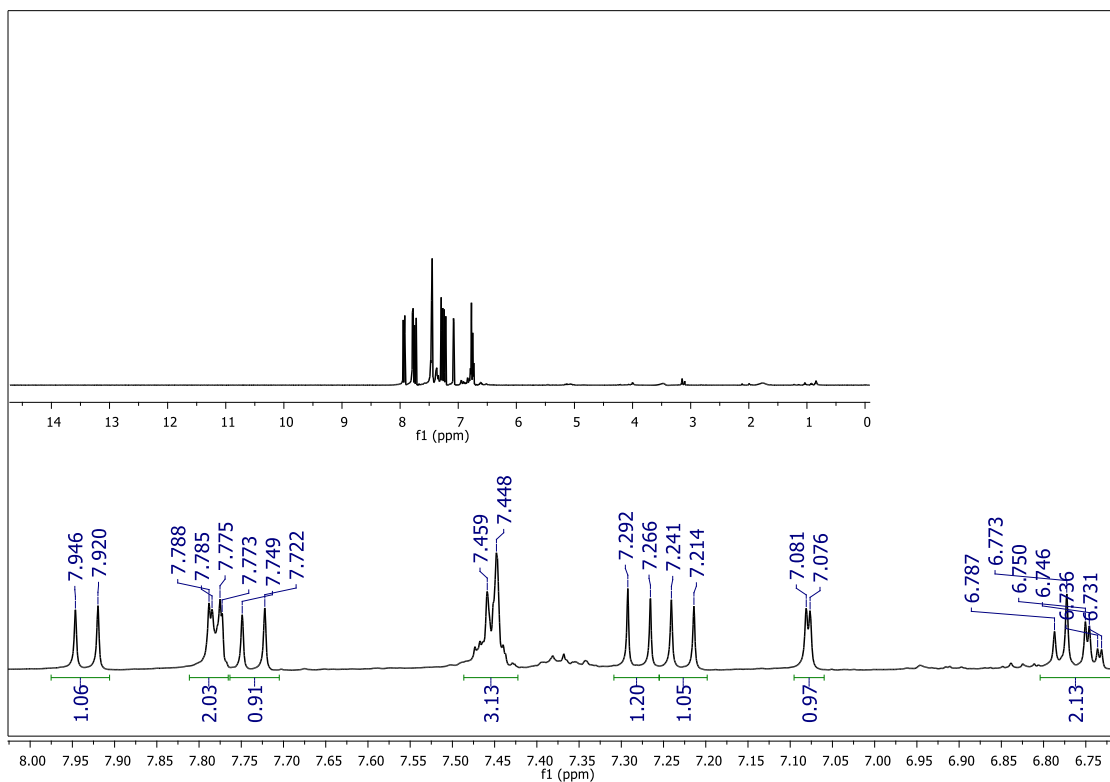
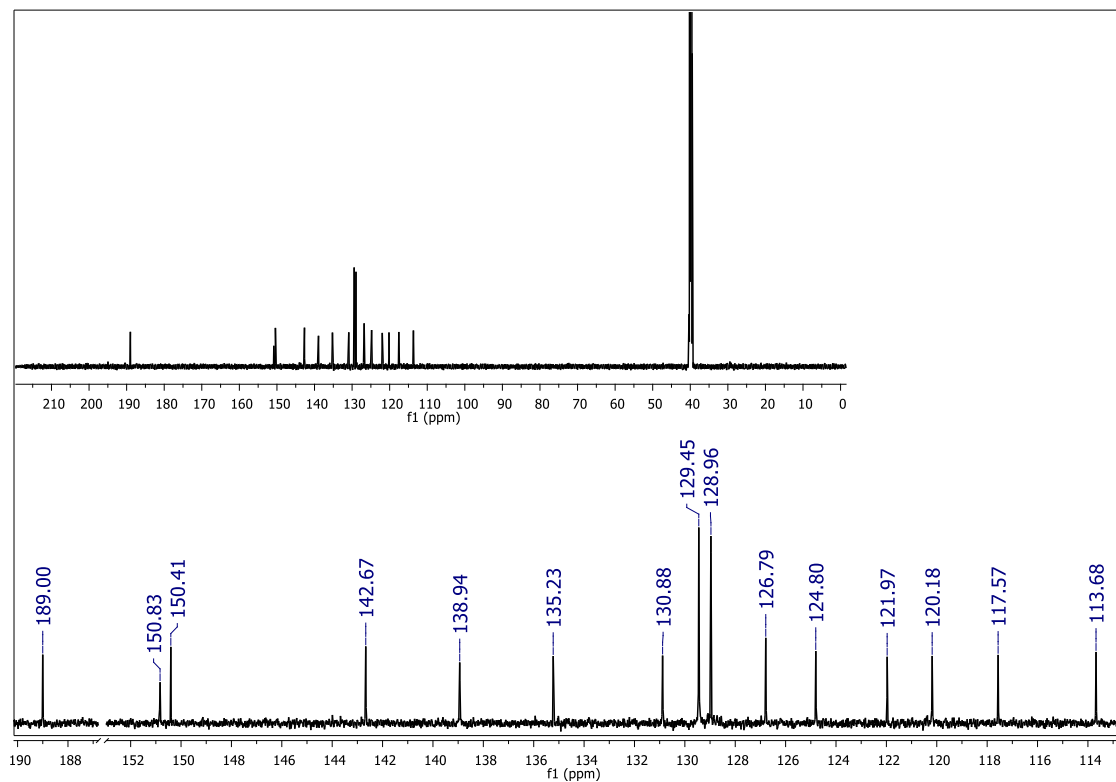


Figure S32. ^{13}C NMR spectrum of **MAC 34** (150 MHz; $\text{DMSO-}d_6$).



APÊNDICE E

Material Suplementar do Capítulo VII

Antifungal Activity of a New Unsymmetrical Monocarbonyl Curcuminoid (MAC 43) Results in Decreased *Candida Albicans* biofilm

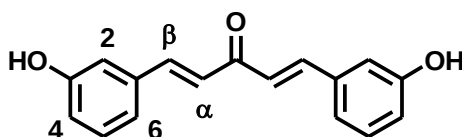
Carlos R. Polaquini^a, Janaina C. O. Sardi^b, Pedro L. Rosalen^b, Luis O. Regasini^a.

^a Department of Chemistry and Environmental Sciences, Institute of Biosciences, Humanities and Exact Sciences, São Paulo State University (Unesp), São José do Rio Preto 15054-000, SP, Brazil.

^b Department of Physiological Sciences, Piracicaba Dental School, University of Campinas (Unicamp), Piracicaba 13414-903, SP, Brazil.

1. SPECTROSCOPY DATA ANALYSES

1.1 (1E,4E)-1,5-bis(3-hydroxyphenyl)penta-1,4-dien-3-one (MAC 35) (DAI et al., 2015 DECK et al., 2018).



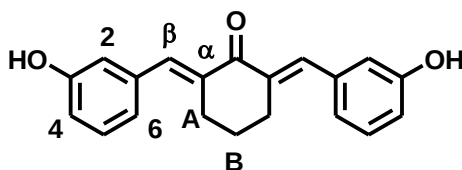
Yellow oil

Yield: 27%

¹H NMR (600 MHz; DMSO-*d*₆), δ_H in ppm (multiplicity; *J* in Hz): 6.87 (ddd; 1.8, 2.1 and 7.8; H-4), 7.15 (dd; 1.8 and 1.8; H-2), 7.22 (d; 7.8; H-6), 7.25 (d; 16.2; H-α), 7.26 (dd; 7.8 and 7.8; H-5), 7.69 (d; 16.2; H-β), 9.70 (s; 3-OH) (Figure S1).

¹³C NMR (150 MHz; DMSO-*d*₆), δ_C in ppm: 115.3 (C-2), 118.2 (C-4), 120.1 (C-6), 126.0 (C-α), 130.4 (C-5), 136.5 (C-1), 143.4 (C-β), 158.2 (C-3), 189.0 (C=O) (Figure S2).

1.2 (2E,6E)-2,6-bis(3-hydroxybenzylidene)cyclohexanone (MAC 36) (TAN et al., 2014; LEONG et al., 2019).



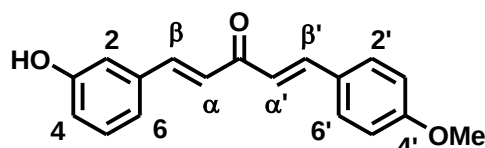
Yellow solid

Yield: 38%

¹H NMR (600 MHz; DMSO-*d*₆), δ_H in ppm (multiplicity; *J* in Hz): 1.72 (quint; 5.4; H-B), 2.88 (t; 5.4; H-A), 6.80 (dd; 1.8 and 8.4; H-4), 6.93–6.96 (H-2 and H-6), 7.25 (dd; 7.8 and 8.4; H-5), 7.53 (s; H-β) (Figure S3).

^{13}C NMR (150 MHz; DMSO- d_6), δ_{C} in ppm: 22.9 (C-B), 28.4 (C-A), 116.5 (C-2), 117.2 (C-4), 121.8 (C-6), 130.0 (C-5), 136.3 (C-1) 136.6 (C- α), 137.0 (C- β), 156.8 (C-3), 189.5 (C=O) (Figure S4).

1.3 (1E,4E)-1-(3-hydroxyphenyl)-5-(4-methoxyphenyl)penta-1,4-dien-3-one (MAC 37).



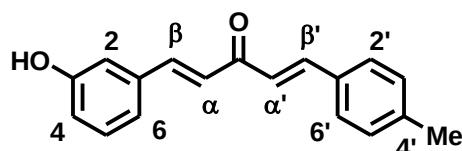
Yellow solid

Yield: 65%

^1H NMR (600 MHz; CDCl_3), δ_{H} in ppm (multiplicity; J in Hz): 3.88 (s; 4'-OMe), 6.94 (dd; 2.4 and 8.4; H-4), 6.96 (d; 8.4; H-3' and H-5'), 6.98 (d; 15.6; H- α), 7.07 (d; 15.6; H- α'), 7.15 (dd; 1.8 and 2.4; H-2), 7.20 (d; 7.8; H-6), 7.30 (dd; 7.8 and 8.4; H-5), 7.59 (d; 8.4; H-2' and H-6'), 7.70 (d; 15.6; H- β), 7.74 (d; 15.6; H- β') (Figure S5).

^{13}C NMR (150 MHz; CDCl_3), δ_{C} in ppm: 55.4 (4'-OMe), 114.5 (C-3' and C-5'), 115.0 (C-2), 117.9 (C-4), 120.8 (C-6), 123.2 (C- α'), 125.6 (C- α), 127.4 (C-1'), 130.1 (C-5), 130.3 (C-2' and C-6'), 136.3 (C-1), 143.2 (C- β'), 143.8 (C- β), 156.5 (C-3), 161.8 (C-4'), 189.4 (C=O) (Figure S6).

1.4 (1E,4E)-1-(3-hydroxyphenyl)-5-(4-methylphenyl)penta-1,4-dien-3-one (MAC 38).



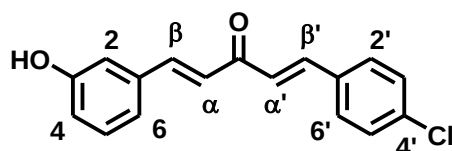
Yellow oil

Yield: 50%

¹H NMR (600 MHz; CDCl₃), δ_H in ppm (multiplicity; *J* in Hz): 2.41 (s; 4'-Me), 6.95 (ddd; 1.2, 2.1 and 7.8; H-4), 7.05 (d; 16.2; H-α), 7.07 (d; 16.2; H-α'), 7.17–7.18 (H-2 and H-6), 7.24 (d; 7.8; H-3' and H-5'), 7.29 (dd; 7.8 and 7.8; H-5), 7.53 (d; 7.8; H-2' and H-6'), 7.71 (d; 16.2; H-β), 7.75 (d; 16.2; H-β') (Figure S7).

¹³C NMR (150 MHz; CDCl₃), δ_C in ppm: 21.6 (4'-Me), 115.0 (C-2), 118.0 (C-4), 120.7 (C-6), 124.4 (C-α'), 125.5 (C-α), 128.5 (C-2' and C-6'), 129.8 (C-3' and C-5'), 130.1 (C-5), 131.9 (C-1'), 136.2 (C-1), 141.3 (C-4'), 143.6 (C-β'), 144.0 (C-β), 156.8 (C-3), 189.6 (C=O) (Figure S8).

1.5 (1*E*,4*E*)-1-(4-chlorophenyl)-5-(3-hydroxyphenyl)penta-1,4-dien-3-one (MAC 39).



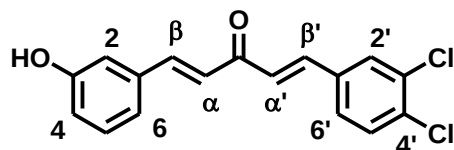
Yellow oil

Yield: 42%

¹H NMR (600 MHz; DMSO-*d*₆), δ_H in ppm (multiplicity; *J* in Hz): 6.88 (dd; 1.2, 2.4 and 8.1, H-4), 7.15 (dd; 1.2 and 1.8; H-2), 7.21 (d; 7.8; H-6), 7.22 (d; 16.2; H-α), 7.27 (dd; 7.8 and 8.1; H-5), 7.41 (d; 16.2; H-α'), 7.54 (d; 9.0; H-3' and H-5'), 7.73 (d; 16.2; H-β), 7.77 (d; 16.2; H-β'), 7.84 (d; 9.0; H-2' and H-6') (Figure S9).

¹³C NMR (150 MHz; DMSO-*d*₆), δ_C in ppm: 115.3 (C-2), 118.3 (C-4), 120.1 (C-6), 126.0 (C-α), 126.6 (C-α'), 129.5 (C-2' and C-6'), 130.5 (C-5), 130.7 (C-3' and C-5'), 134.2 (C-1'), 135.4 (C-4'), 136.4 (C-1), 141.7 (C-β), 143.7 (C-β'), 158.2 (C-3), 188.9 (C=O) (Figure S10).

1.6 (1E,4E)-1-(3,4-dichlorophenyl)-5-(3-hydroxyphenyl)penta-1,4-dien-3-one (MAC 40).



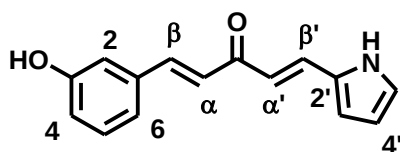
Yellow solid

Yield: 36%

¹H NMR (600 MHz; DMSO-*d*₆), δ_H in ppm (multiplicity; *J* in Hz): 6.80 (d; 8.4; H-5), 6.93 (d; 15.6; H-α), 7.09 (dd; 2.4 and 8.4; H-6), 7.15 (d; 2.4; H-2), 7.50 (d; 15.6; H-α'), 7.65 (d; 15.6; H-β), 7.71 (d; 15.6; H-β'), 7.72 (d; 8.4; H-5'), 7.79 (dd; 1.8 and 8.4; H-6), 8.13 (d; 1.8; H-2') (Figure S11).

¹³C NMR (150 MHz; DMSO-*d*₆), δ_C in ppm: 115.3 (C-2), 116.3 (C-5), 122.5 (C-6), 123.1 (C-α), 126.4 (C-1), 127.8 (C-α'), 129.0 (C-6'), 130.4 (C-2'), 131.5 (C-5'), 132.2 (C-4'), 132.8 (C-3'), 136.3 (C-1'), 139.4 (C-β), 144.9 (C-4), 146.2 (C-β'), 149.6 (C-3), 188.4 (C=O) (Figure S12).

1.7 (1E,4E)-1-(3-hydroxyphenyl)-5-(1H-pyrrol-2-yl)penta-1,4-dien-3-one (MAC 41).



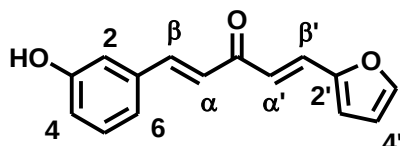
Brown solid

Yield: 23%

¹H NMR (600 MHz; DMSO-*d*₆), δ_H in ppm (multiplicity; *J* in Hz): 6.22–6.23 (H-4'), 6.67 (H-3'), 6.85 (ddd; 1.2, 2.1 and 7.8; H-4), 6.91 (d; 15.6; H-α), 7.11 (H-2 and H-5'), 7.15 (d; 15.6; H-α'), 7.18 (d; 7.8; H-6), 7.25 (dd; 7.8 and 7.8; H-5), 7.53 (d; 15.6; H-β'), 7.64 (d; 15.6; H-β), 9.68 (s; 3-OH), 11.64 (s; 1'-NH) (Figure S13).

¹³C NMR (150 MHz; DMSO-*d*₆), δ_C in ppm: 111.1 (C-4'), 115.1 (C-2), 116.3 (C-3'), 117.9 (C-5'), 119.5 (C-4), 119.9 (C-6), 124.7 (C-α), 126.1 (C-α'), 129.3 (C-1'), 130.4 (C-5), 133.9 (C-β'), 136.6 (C-1), 141.8 (C-β), 158.2 (C-3), 188.3 (C=O) (Figure S14).

1.8 (1*E*,4*E*)-1-(furan-2-yl)-5-(3-hydroxyphenyl)penta-1,4-dien-3-one (MAC 42).



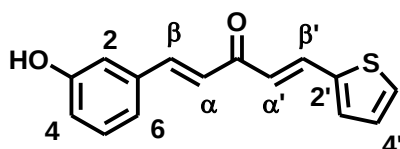
Red oil

Yield: 49%

¹H NMR (600 MHz; DMSO-*d*₆), δ_H in ppm (multiplicity; *J* in Hz): 6.68 (dd; 1.2 and 3.6; H-4'), 6.86 (ddd; 1.2, 1.8 and 7.8; H-4), 6.99 (d; 16.2; H-α), 7.02 (d; 3.6; H-3'), 7.14 (dd; 1.2 and 1.8; H-2), 7.21 (d; 7.8; H-6), 7.24 (d; 16.2; H-α'), 7.25 (dd; 7.8 and 7.8; H-5), 7.61 (d; 16.2; H-β), 7.62 (d; 16.2; H-β'), 7.90 (d; 1.2; H-5'), 9.68 (s; 3-OH) (Figure S15).

¹³C NMR (150 MHz; DMSO-*d*₆), δ_C in ppm: 113.6 (C-3'), 115.3 (C-2), 117.2 (C-4'), 118.2 (C-4), 120.1 (C-6), 123.3 (C-α'), 125.9 (C-α), 129.8 (C-5'), 130.4 (C-5), 136.4 (C-1), 143.6 (C-β), 146.6 (C-β'), 151.4 (C-1'), 158.2 (C-3), 188.3 (C=O) (Figure S16).

1.9 (1*E*,4*E*)-1-(3-hydroxyphenyl)-5-(thiophen-2-yl)penta-1,4-dien-3-one (MAC 43).



Red oil

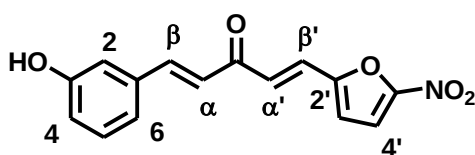
Yield: 34%

¹H NMR (600 MHz; DMSO-*d*₆), δ_H in ppm (multiplicity; *J* in Hz): 6.86 (ddd; 1.2, 2.4 and 7.2; H-4), 6.99 (d; 15.6; H-α), 7.15 (dd; 1.2 and 1.8; H-2), 7.19 (dd; 3.6 and 5.4; H-4'), 7.22 (d;

7.8; H-6), 7.26 (dd; 7.2 and 7.8; H-5), 7.27 (d; 15.6; H- α'), 7.60 (d; 3.6; H-5'), 7.63 (d; 15.6; H- β), 7.77 (d; 5.4; H-3'), 7.97 (d; 15.6; H- β'), 9.69 (s; 3-OH) (Figure S17).

^{13}C NMR (150 MHz; DMSO- d_6), δ_{C} in ppm: 115.4 (C-2), 118.2 (C-4), 120.1 (C-6), 125.0 (C- α), 125.8 (C-3'), 129.2 (C- α'), 130.4 (C-5), 130.7 (C-4'), 133.0 (C-5'), 136.1 (C-1'), 136.5 (C-1), 140.2 (C- β'), 143.1 (C- β), 158.2 (C-3), 188.3 (C=O) (Figure S18).

1.10 (1E,4E)-1-(3-hydroxyphenyl)-5-(5-nitrofuran-2-yl)penta-1,4-dien-3-one (MAC 44).



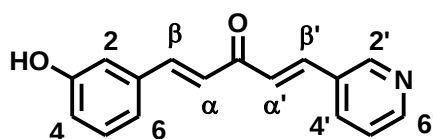
Red solid

Yield: 33%

^1H NMR (600 MHz; DMSO- d_6), δ_{H} in ppm (multiplicity; J in Hz): 6.88 (ddd; 1.8, 1.8 and 7.8; H-4), 7.16 (H-2), 7.24 (d; 7.8; H-6), 7.27 (dd; 7.8 and 7.8; H-5), 7.32 (d; 16.2; H- α), 7.33 (d; 16.2; H- α'), 7.35 (d; 4.2; H-3'), 7.65 (d; 16.2; H- β), 7.69 (d; 16.2; H- β'), 7.80 (d; 4.2; H-4'), 9.68 (s; 3-OH) (Figure S19).

^{13}C NMR (150 MHz; DMSO- d_6), δ_{C} in ppm: 115.3 (C-2), 115.5 (C-3'), 118.1 (C-4), 118.5 (C-4'), 120.3 (C-6), 125.7 (C- α), 127.1 (C- α'), 129.1 (C- β'), 130.4 (C-5), 136.3 (C-1), 144.5 (C- β), 152.5 (C-2'), 153.7 (C-5'), 158.2 (C-3), 188.0 (C=O) (Figure S20).

1.11 (1E,4E)-1-(3-hydroxyphenyl)-5-(pyridin-3-yl)penta-1,4-dien-3-one (MAC 45).



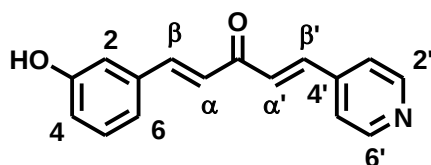
Yellow solid

Yield: 48%

¹H NMR (600 MHz; DMSO-*d*₆), δ_H in ppm (multiplicity; *J* in Hz): 6.88 (ddd; 1.8, 2.4 and 7.8; H-4), 7.15 (dd; 1.8 and 1.8; H-2), 7.21 (d; 16.2; H-α), 7.22 (d; 7.8; H-6), 7.27 (dd; 7.8 and 7.8; H-5), 7.49 (dd; 4.8 and 7.8; H-5'), 7.53 (d; 16.2; H-α'), 7.76 (d; 16.2; H-β), 7.80 (d; 16.2; H-β'), 8.24 (ddd; 1.8, 2.4 and 7.8; H-4'), 8.61 (dd; 1.8 and 4.8; H-6'), 8.96 (s; 2.4; H-2'), 9.72 (s, 3-OH) (Figure S21).

¹³C NMR (150 MHz; DMSO-*d*₆), δ_C in ppm: 115.3 (C-2), 118.3 (C-4), 120.1 (C-6), 124.5 (C-5'), 126.0 (C-α), 127.6 (C-α'), 130.5 (C-5), 131.0 (C-3'), 135.3 (C-4'), 136.4 (C-1), 139.7 (C-β'), 144.0 (C-β), 150.5 (C-6'), 151.4 (C-2'), 158.2 (C-3), 188.8 (C=O) (Figure S22).

1.12 (1*E*,4*E*)-1-(3-hydroxyphenyl)-5-(pyridin-4-yl)penta-1,4-dien-3-one (MAC 46).



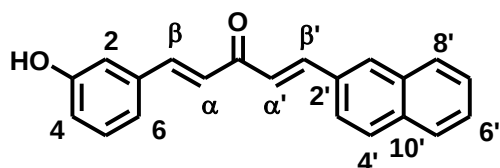
Yellow solid

Yield: 39%

¹H NMR (600 MHz; DMSO-*d*₆), δ_H in ppm (multiplicity; *J* in Hz): 6.88 (ddd; 1.8, 2.4 and 7.8; H-4), 7.16 (dd; 1.8 and 1.8; H-2), 7.22 (d; 16.2; H-α), 7.23 (d; 7.8; H-6), 7.28 (dd; 7.8 and 7.8; H-5), 7.60 (d; 16.2; H-α'), 7.72 (d; 16.2; H-β), 7.75 (d; 6.0; H-3' and H-5'), 7.77 (d; 16.2; H-β'), 8.67 (d; 6.0; H-2' and H-6'), 9.73 (s, 3-OH) (Figure S23).

¹³C NMR (150 MHz; DMSO-*d*₆), δ_C in ppm: 115.4 (C-2), 118.5 (C-4), 120.2 (C-6), 122.8 (C-3' and C-5'), 125.9 (C-α), 129.8 (C-α'), 130.5 (C-5), 136.3 (C-1), 140.2 (C-β'), 142.4 (C-4'), 144.5 (C-β), 150.8 (C-2' and C-6'), 158.2 (C-3), 189.0 (C=O) (Figure S24).

1.13 (1E,4E)-1-(3-hydroxyphenyl)-5-(naphthalen-2-yl)penta-1,4-dien-3-one (MAC 47).



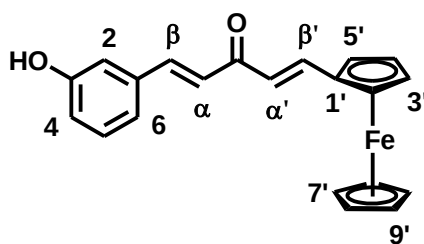
Yellow solid

Yield: 60%

¹H NMR (600 MHz; DMSO-*d*₆), δ_{H} in ppm (multiplicity; *J* in Hz): 6.88 (ddd; 1.8, 2.4 and 7.8; H-4), 7.17 (dd; 1.8 and 1.8; H-2), 7.24 (d; 7.8; H-6), 7.28 (dd; 7.8 and 7.8; H-5), 7.29 (d; 16.2; H- α), 7.51 (d; 16.2; H- α'), 7.58–7.59 (H-6' and H-7'), 7.75 (d; 16.2; H- β), 7.95 (d; 16.2; H- β'), 7.95–8.02 (H-3', H-4', H-5' and H-8'), 8.28 (H-1'), 9.73 (s, 3–OH) (Figure S25).

¹³C NMR (150 MHz; DMSO-*d*₆), δ_{C} in ppm: 115.3 (C-2), 118.2 (C-4), 120.1 (C-6), 124.5 (C-3'), 126.2 (C- α), 126.3 (C- α'), 127.3 (C-1'), 127.9 (C-6'), 128.2 (C-7'), 128.9 (C-5'), 129.0 (C-8'), 130.5 (C-5), 130.9 (C-4'), 132.9 (C-9'), 133.3 (C-2'), 133.4 (C-10'), 136.5 (C-1), 143.2 (C- β), 143.5 (C- β'), 158.2 (C-3), 189.0 (C=O) (Figure S26).

1.14 (1E,4E)-1-(3-hydroxyphenyl)-5-(ferrocen-1-yl)penta-1,4-dien-3-one (MAC 48)



Red solid

Yield: 57%

¹H NMR (600 MHz; DMSO-*d*₆), δ_{H} in ppm (multiplicity; *J* in Hz): 4.19 (s; H-6' to H-10'), 4.54 (s; H-3' and H-4'), 4.75 (s; H-2' and H-5'), 6.81–6.86 (H-4), 6.82 (d; 16.2; H- α), 7.13–7.21

(H- α' , H-2 and H-6), 7.25 (dd; 7.5 and 7.8; H-5), 7.58 (d; 15.6; H- β'), 7.71 (d; 16.2; H- β), 9.68 (s; 3-OH) (Figure S27).

^{13}C NMR (150 MHz; DMSO- d_6), δ_{C} in ppm: 69.5 (C-3' and C-4'), 70.0 (C-6' to C-10'), 71.7 (C-2' and C-5'), 79.6 (C-1'), 115.2 (C-2), 117.9 (C-4) 119.9 (C-6), 123.8 (C- α), 125.9 (C- α'), 130.4 (C-5), 136.6 (C-1), 142.3 (C- β), 145.6 (C- β'), 158.2 (C-3), 187.9 (C=O).

Figure S1. ^1H NMR spectrum of **MAC 35** (600 MHz; $\text{DMSO-}d_6$).

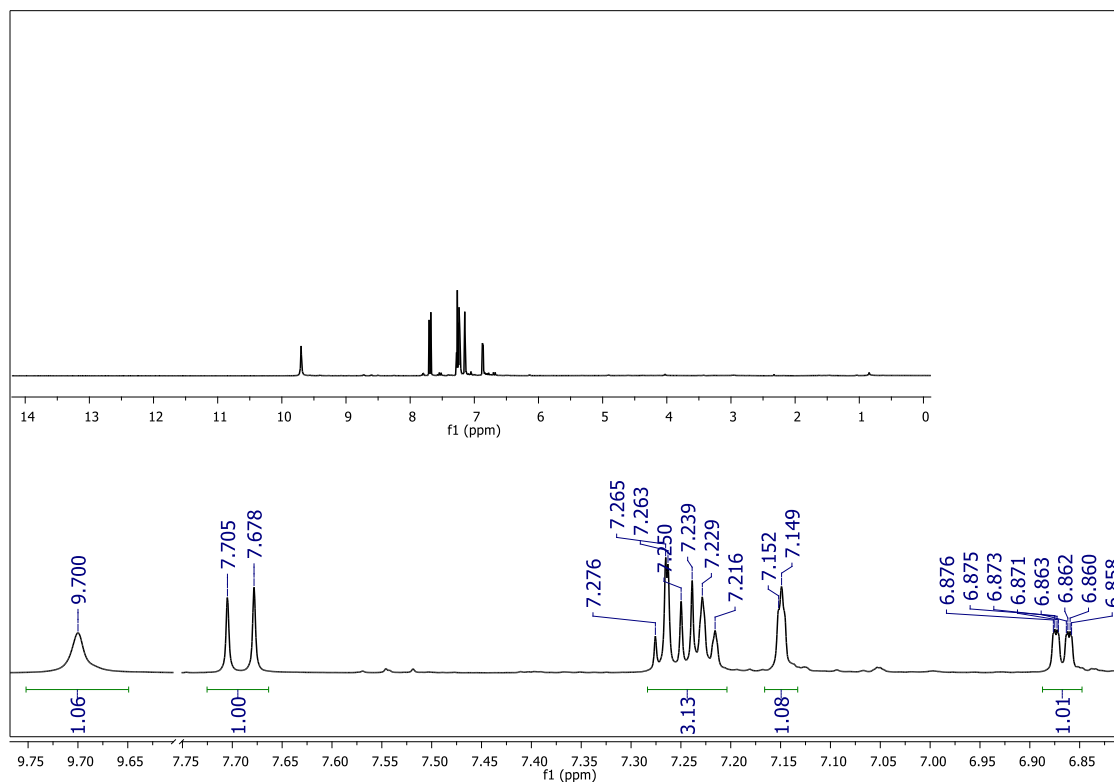


Figure S2. ^{13}C NMR spectrum of **MAC 35** (150 MHz; $\text{DMSO-}d_6$).

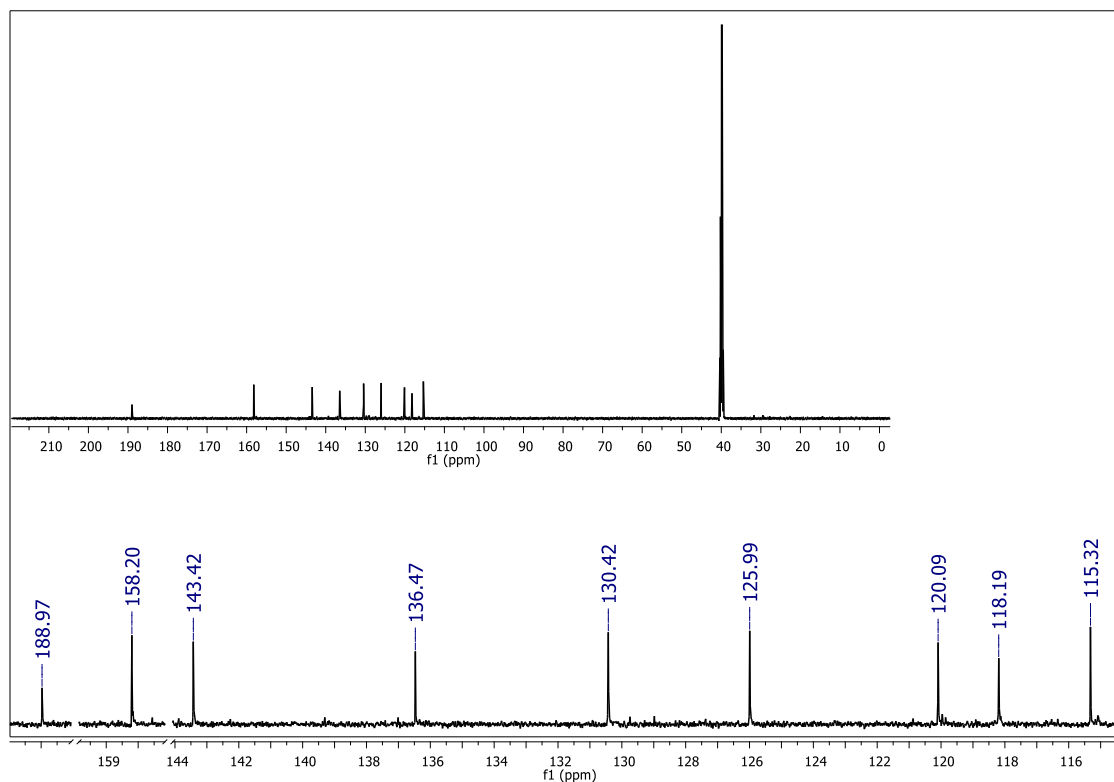


Figure S3. ^1H NMR spectrum of **MAC 36** (600 MHz; $\text{DMSO-}d_6$).

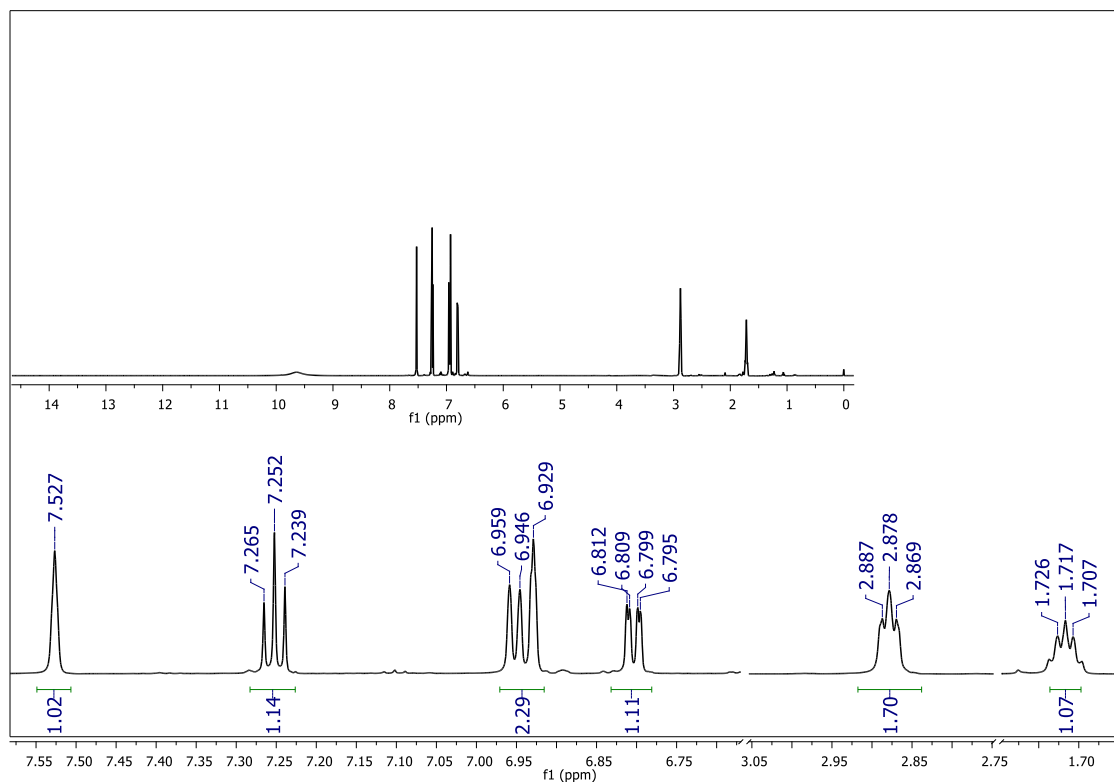


Figure S4. ^{13}C NMR spectrum of **MAC 36** (150 MHz; $\text{DMSO-}d_6$).

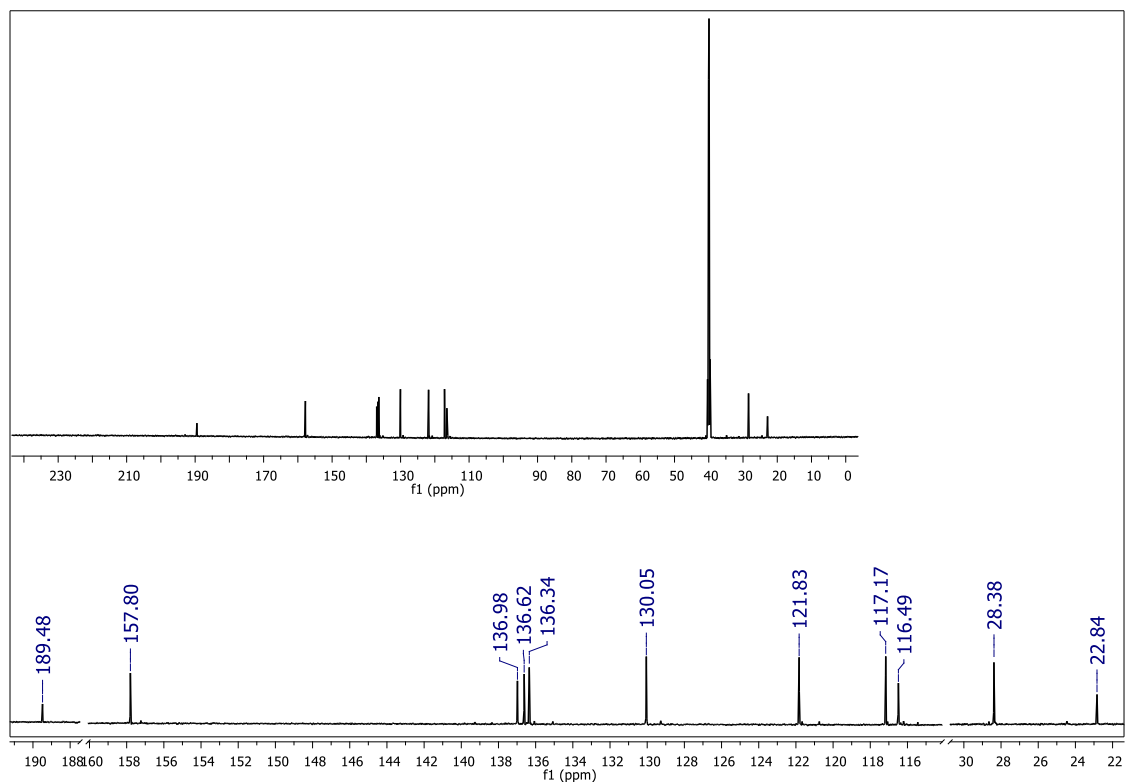


Figure S5. ^1H NMR spectrum of **MAC 37** (600 MHz; CDCl_3).

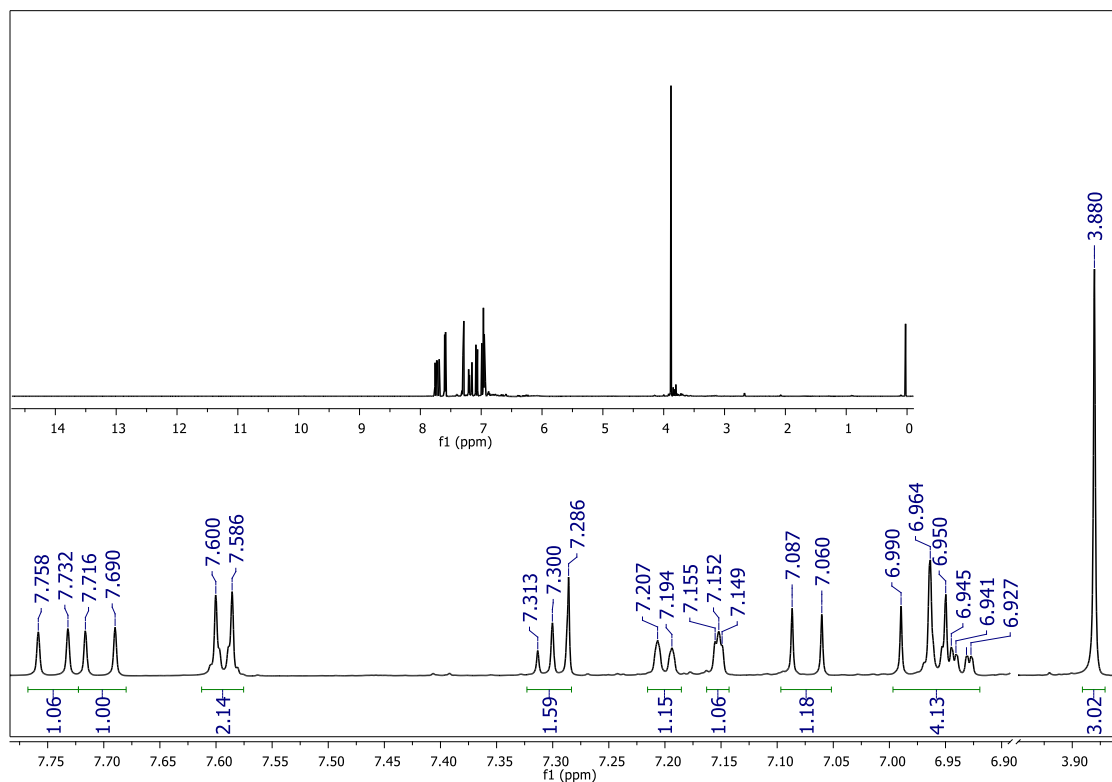


Figure S6. ^{13}C NMR spectrum of **MAC 37** (150 MHz; CDCl_3).

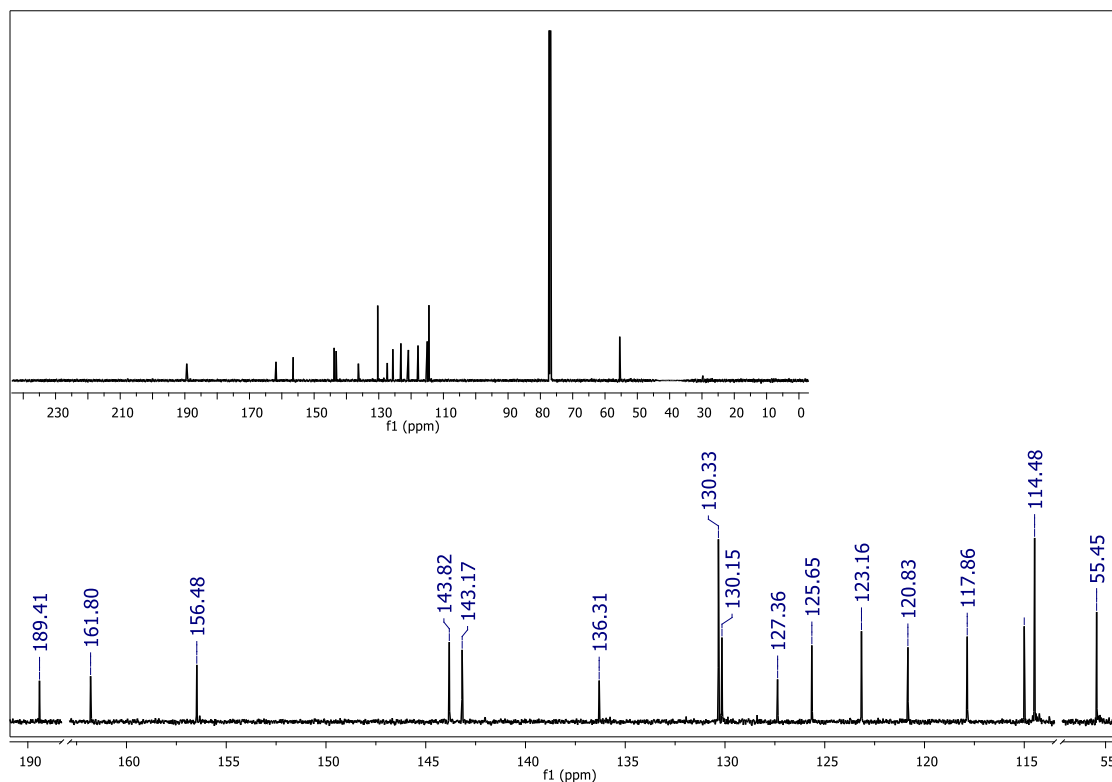


Figure S7. ^1H NMR spectrum of **MAC 38** (600 MHz; CDCl_3).

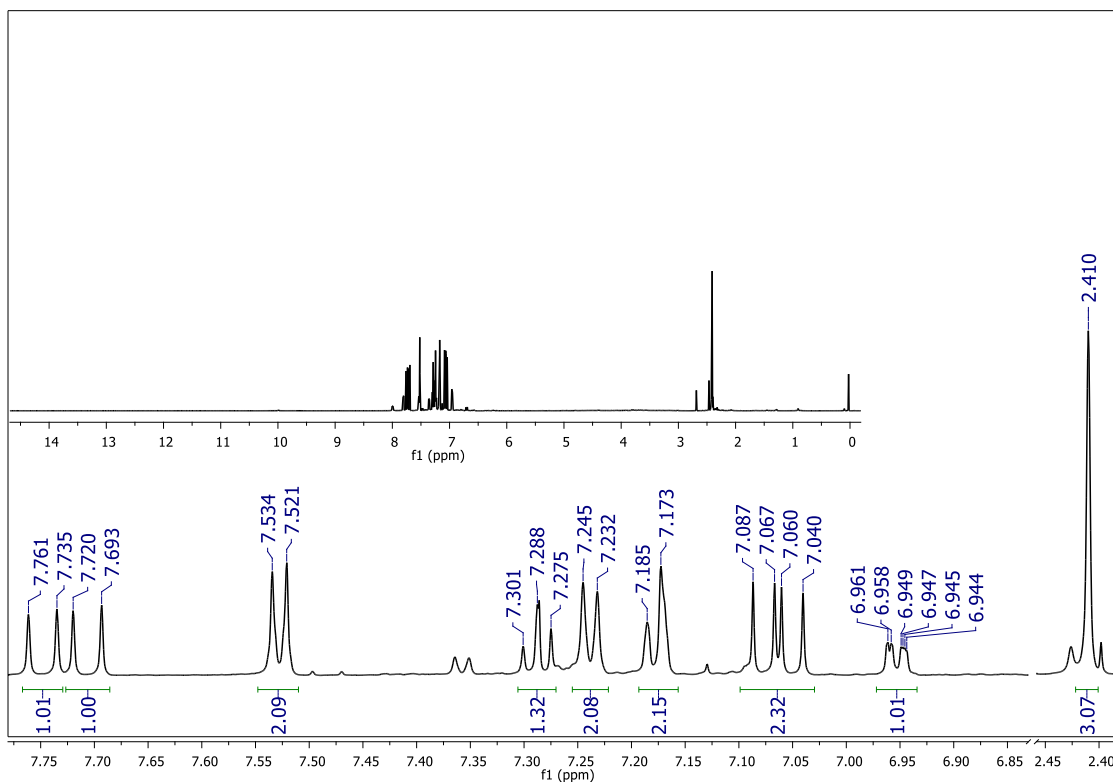


Figure S8. ^{13}C NMR spectrum of **MAC 38** (150 MHz; CDCl_3).

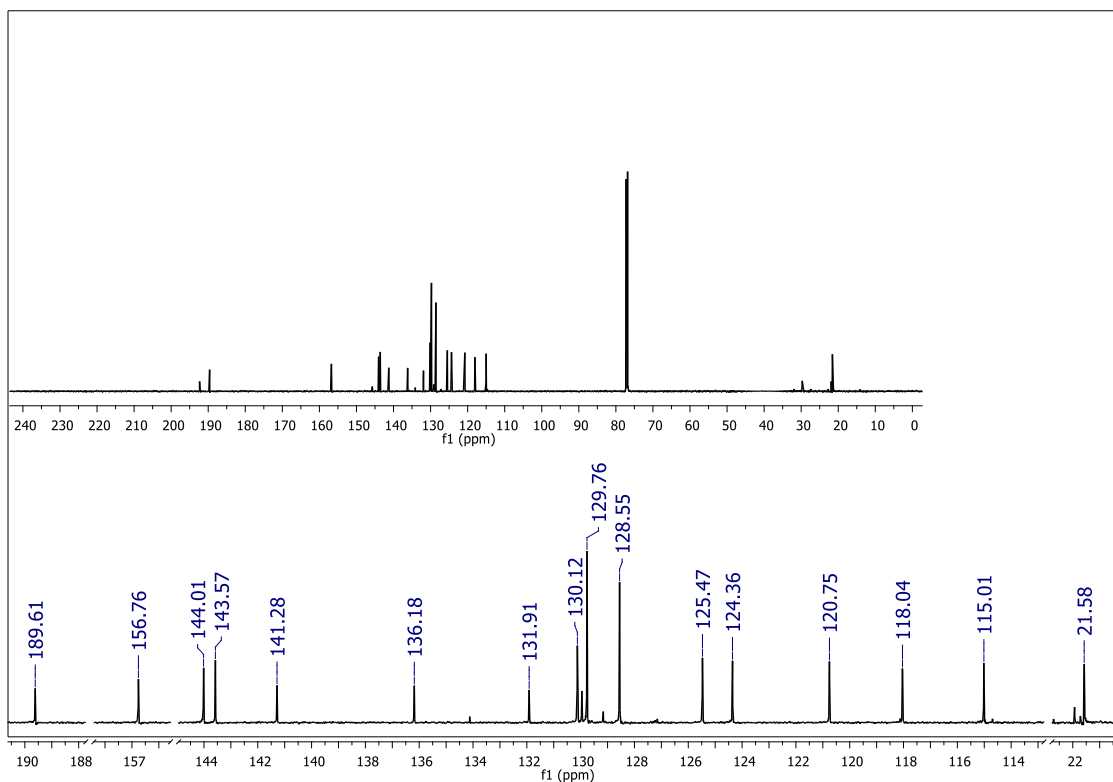


Figure S9. ^1H NMR spectrum of **MAC 39** (600 MHz; $\text{DMSO}-d_6$).

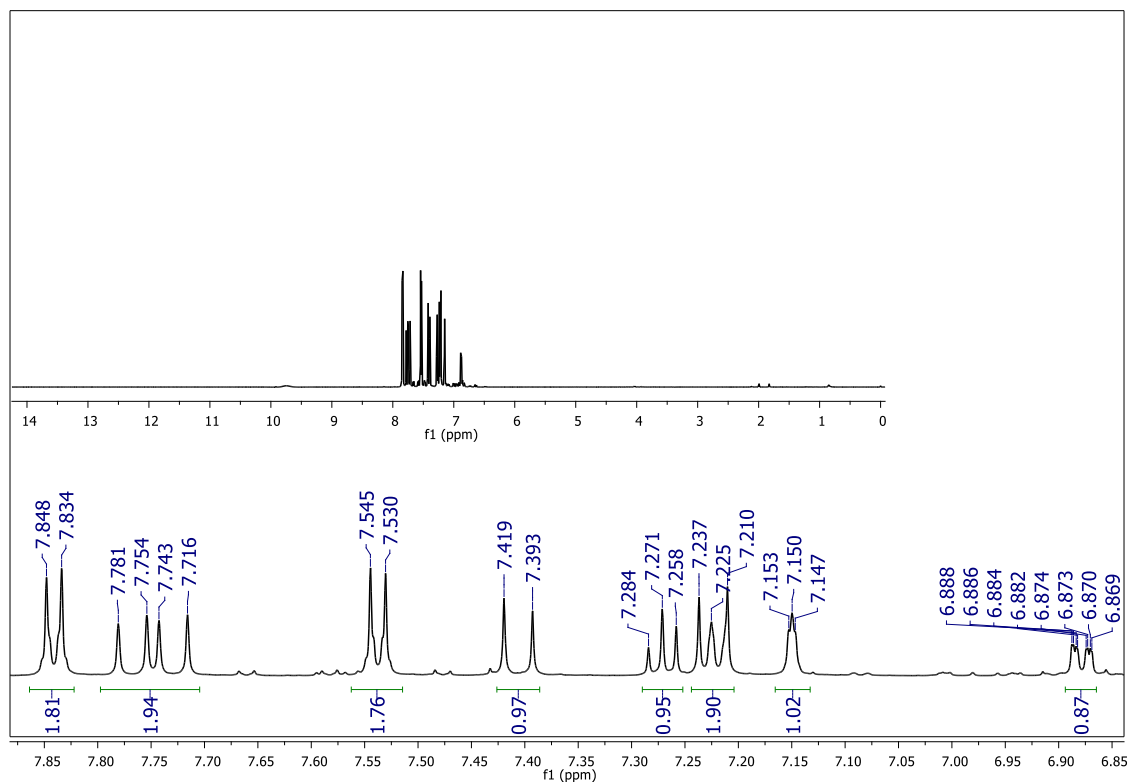


Figure S10. ^{13}C NMR spectrum of **MAC 39** (150 MHz; $\text{DMSO}-d_6$).

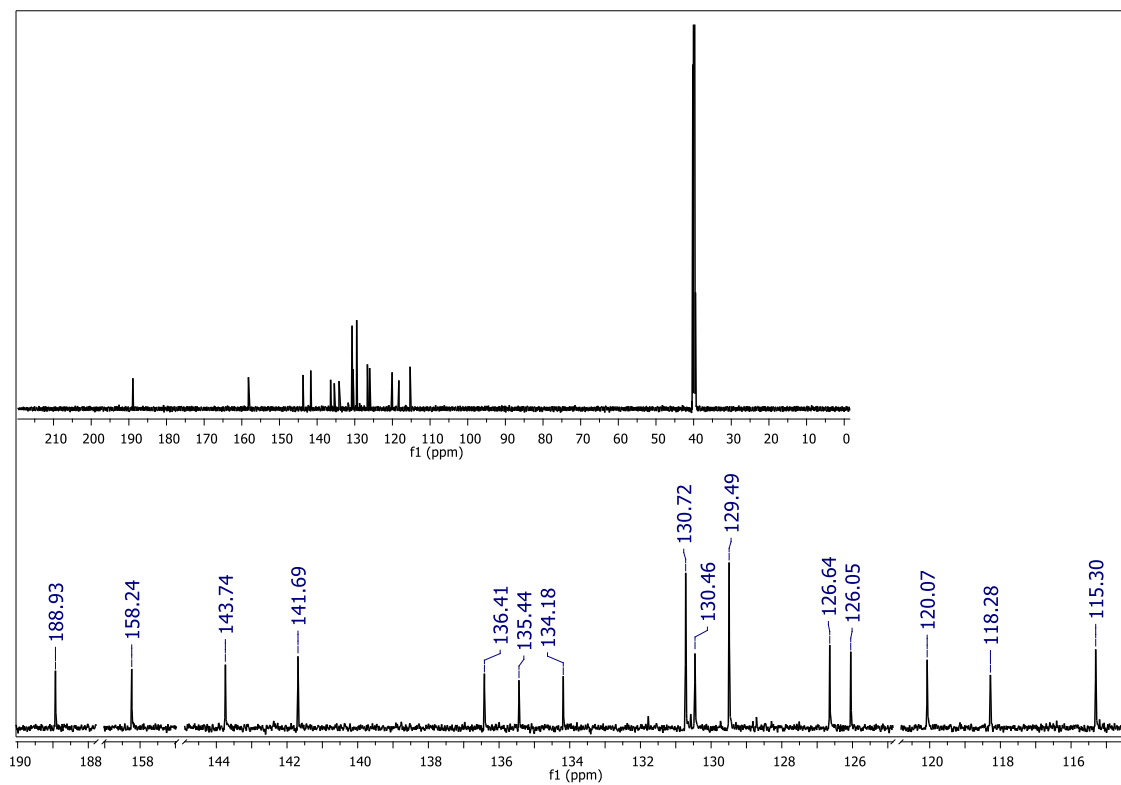


Figure S11. ^1H NMR spectrum of **MAC 40** (600 MHz; $\text{DMSO-}d_6$).

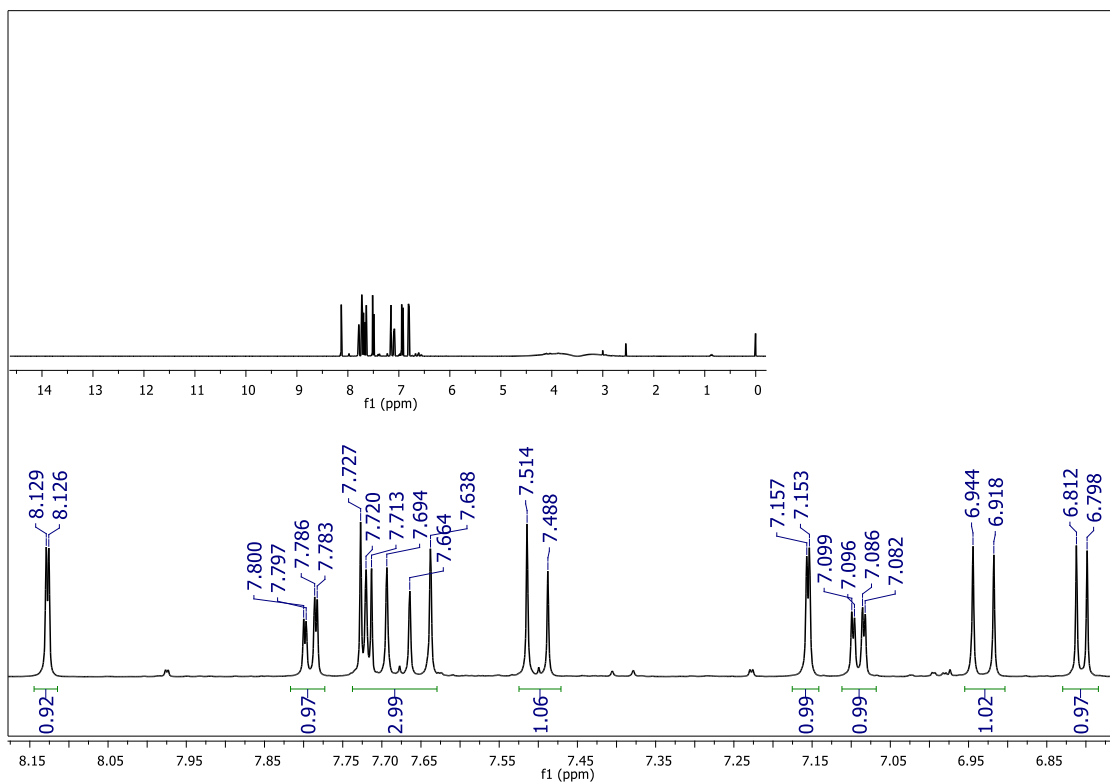


Figure S12. ^{13}C NMR spectrum of **MAC 40** (150 MHz; $\text{DMSO-}d_6$).

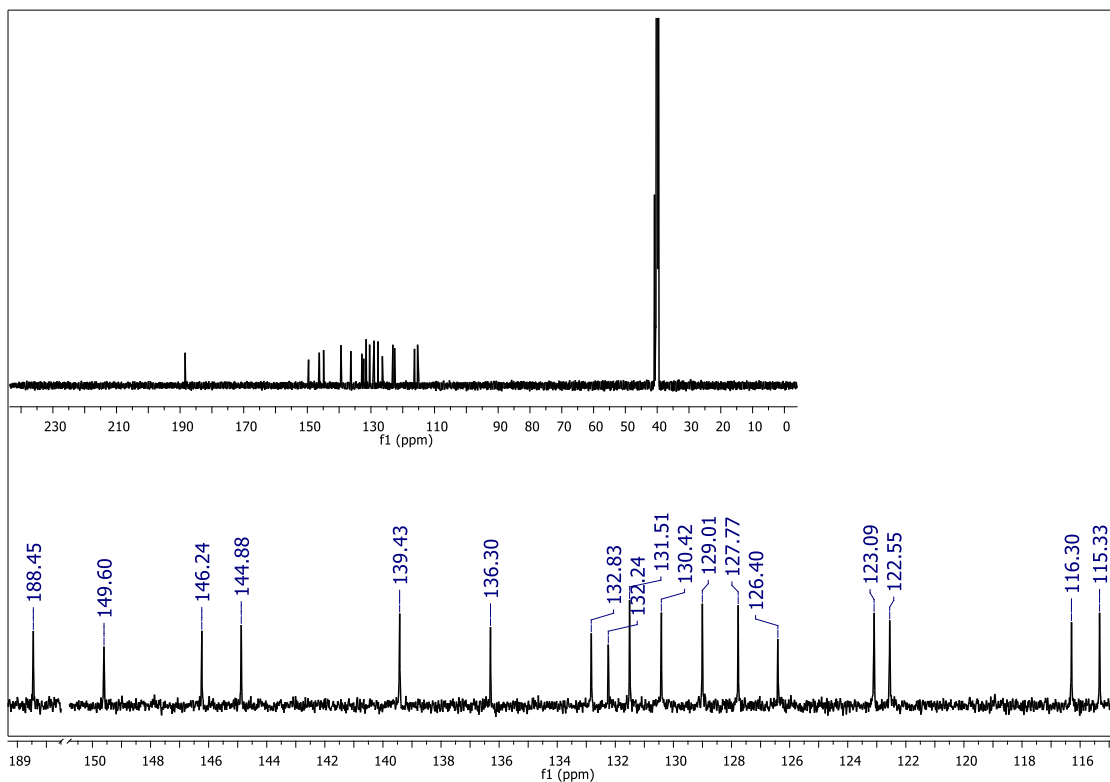


Figure S13. ^1H NMR spectrum of **MAC 41** (600 MHz; $\text{DMSO-}d_6$).

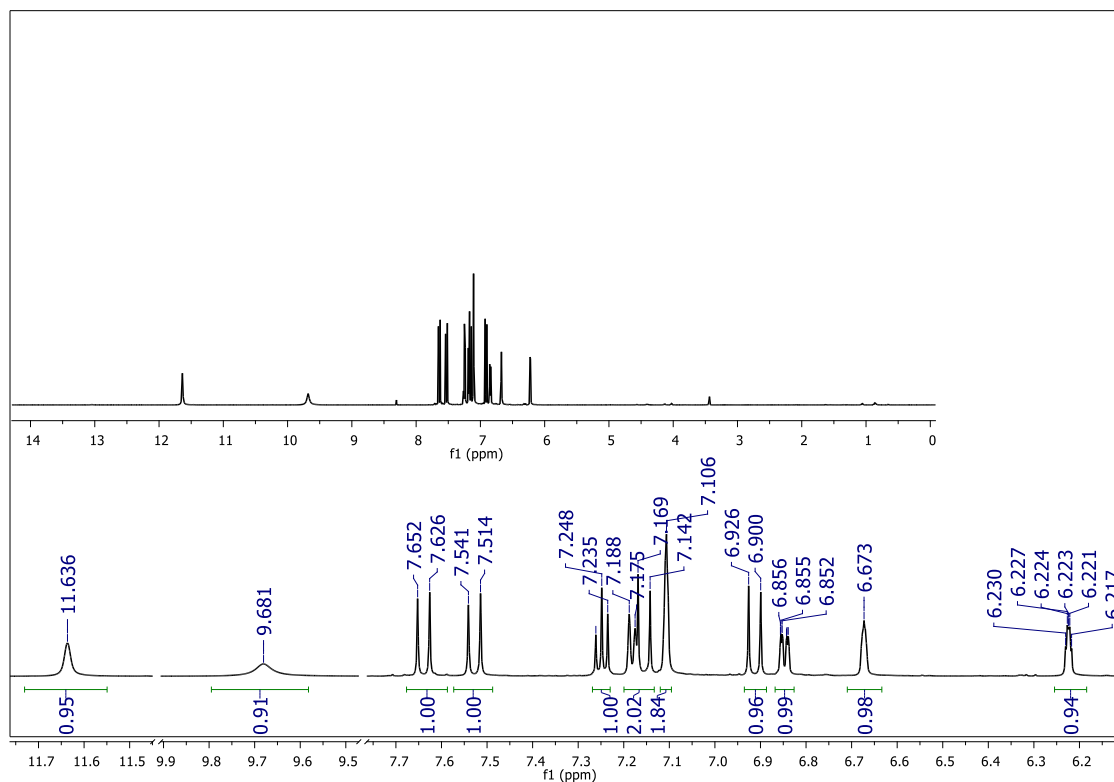


Figure S14. ^{13}C NMR spectrum of **MAC 41** (150 MHz; $\text{DMSO-}d_6$).

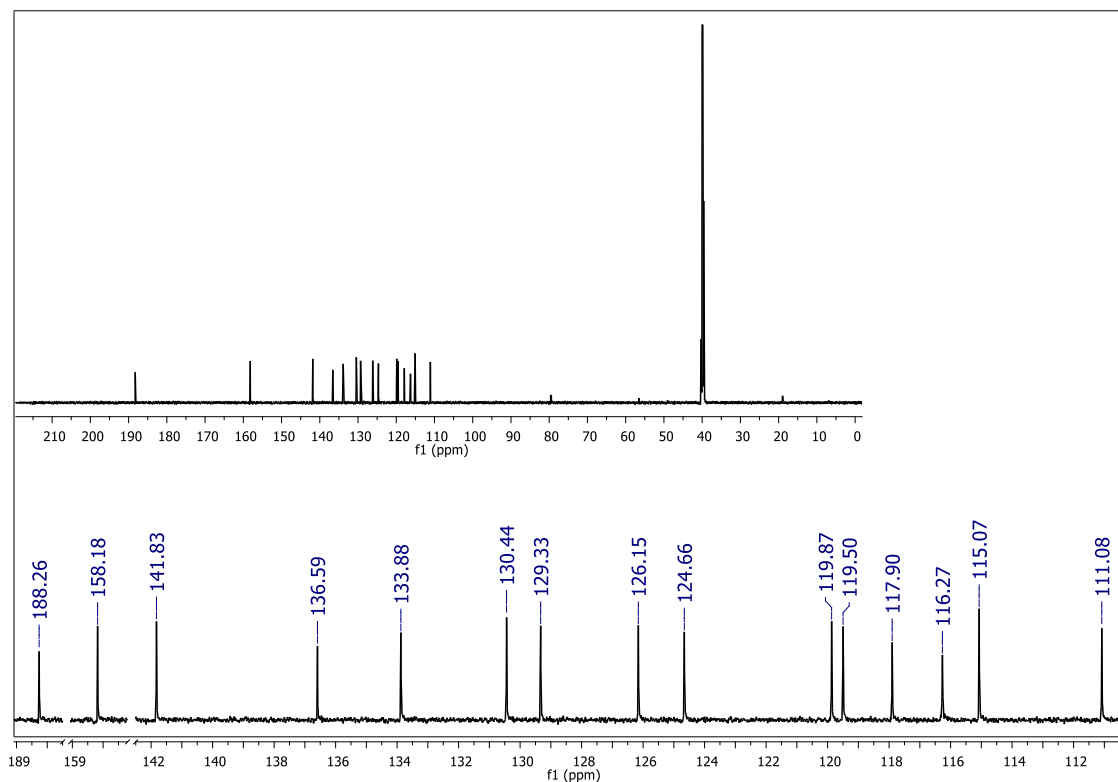


Figure S15. ^1H NMR spectrum of **MAC 42** (600 MHz; $\text{DMSO-}d_6$).

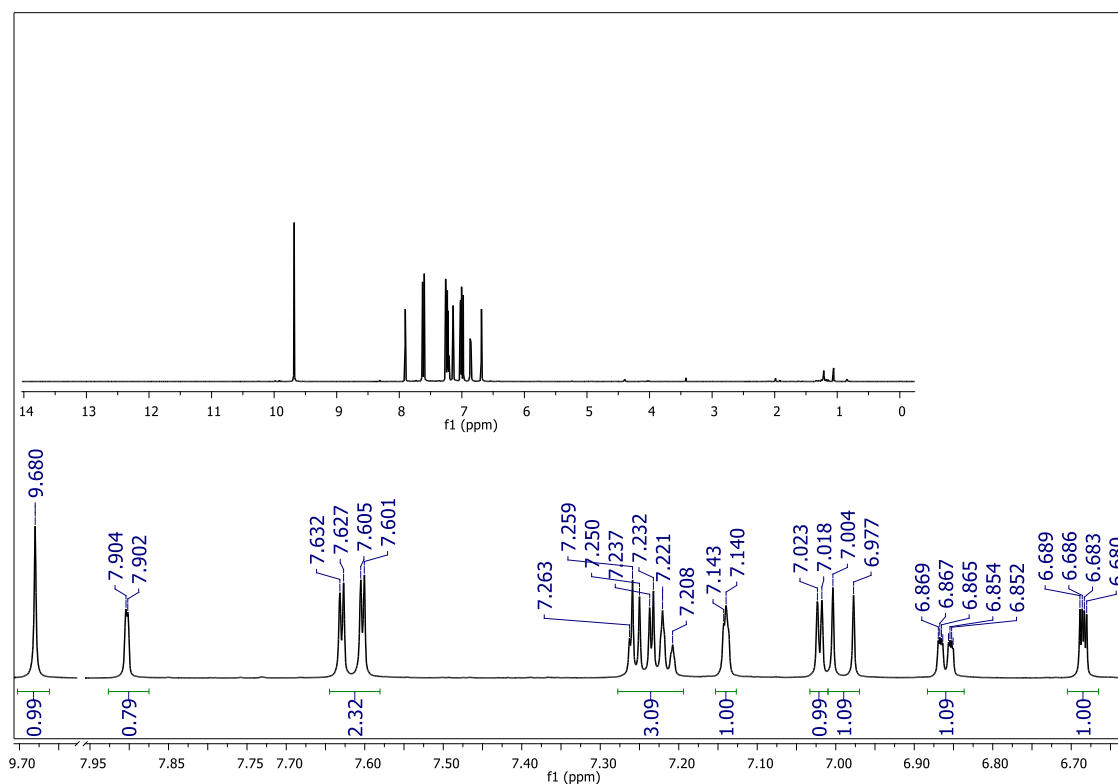


Figure S16. ^{13}C NMR spectrum of **MAC 42** (150 MHz; $\text{DMSO-}d_6$).

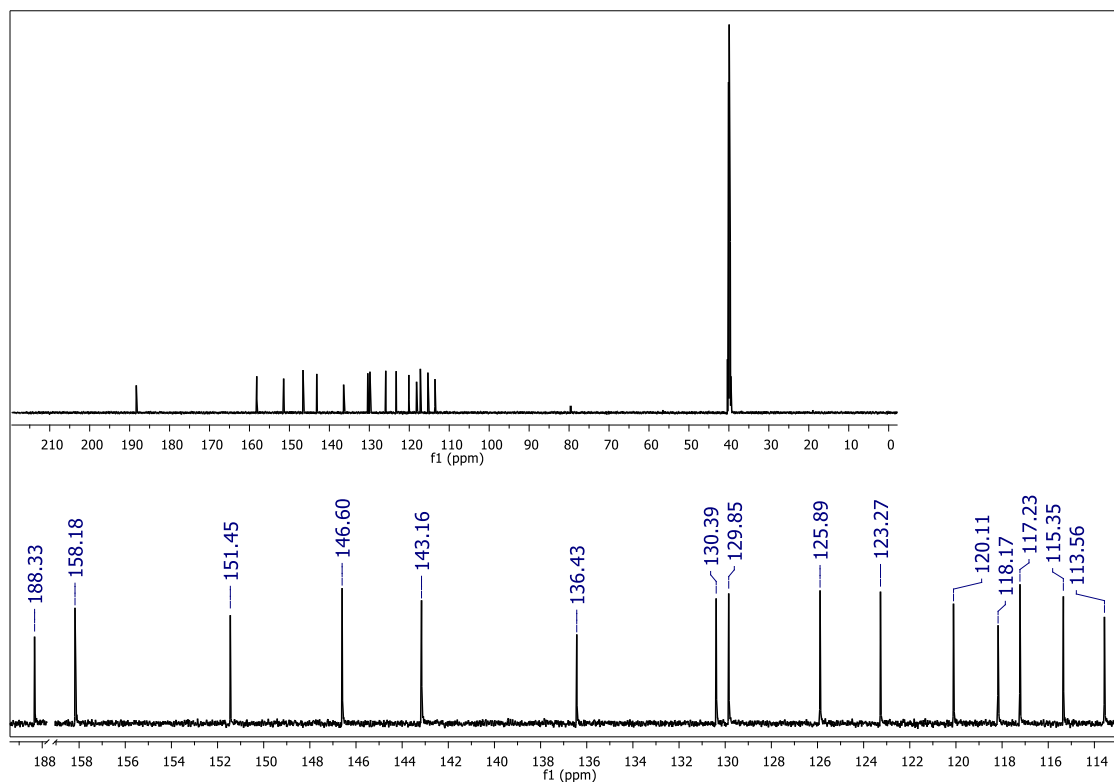


Figure S17. ^1H NMR spectrum of **MAC 43** (600 MHz; $\text{DMSO-}d_6$).

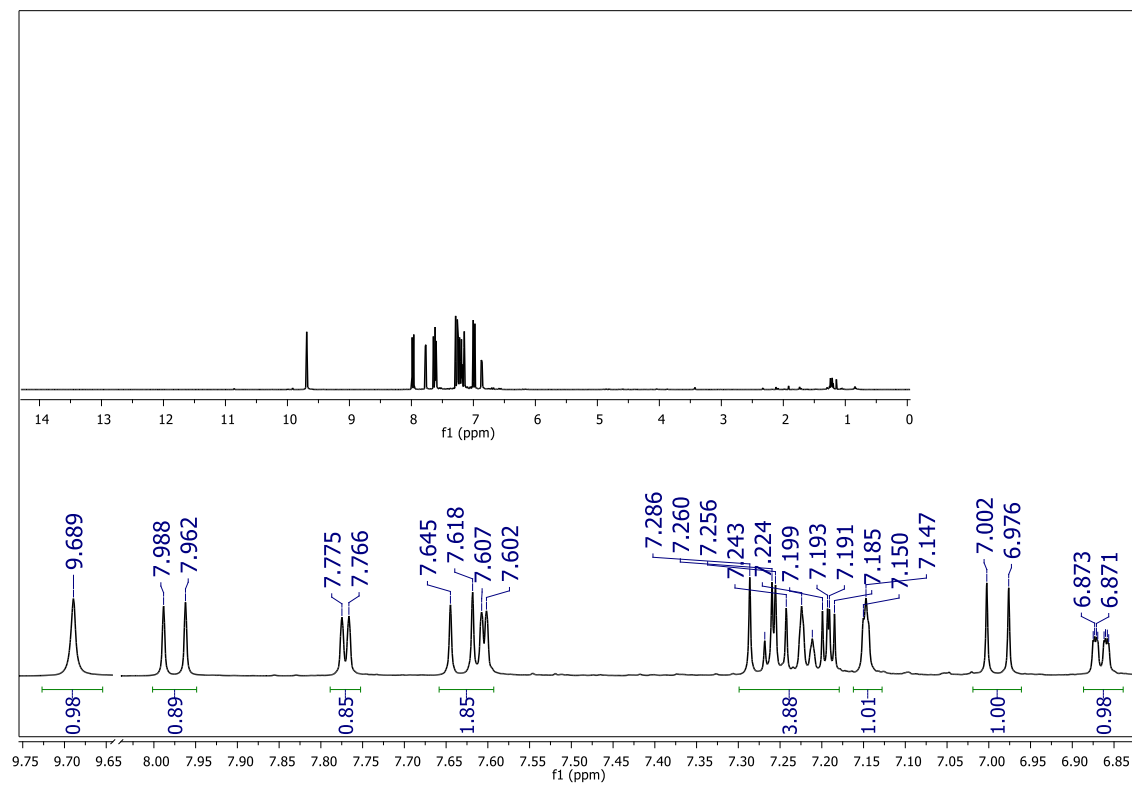


Figure S18. ^{13}C NMR spectrum of **MAC 43** (150 MHz; $\text{DMSO-}d_6$).

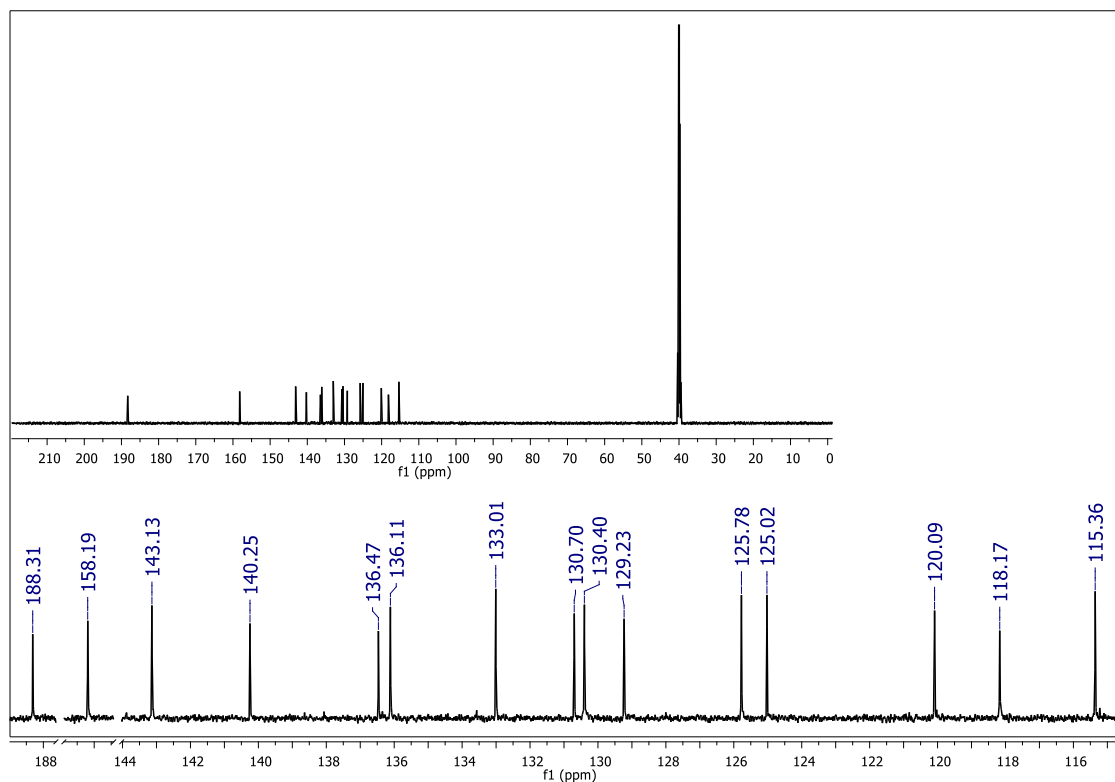


Figure S19. ^1H NMR spectrum of **MAC 44** (600 MHz; $\text{DMSO-}d_6$).

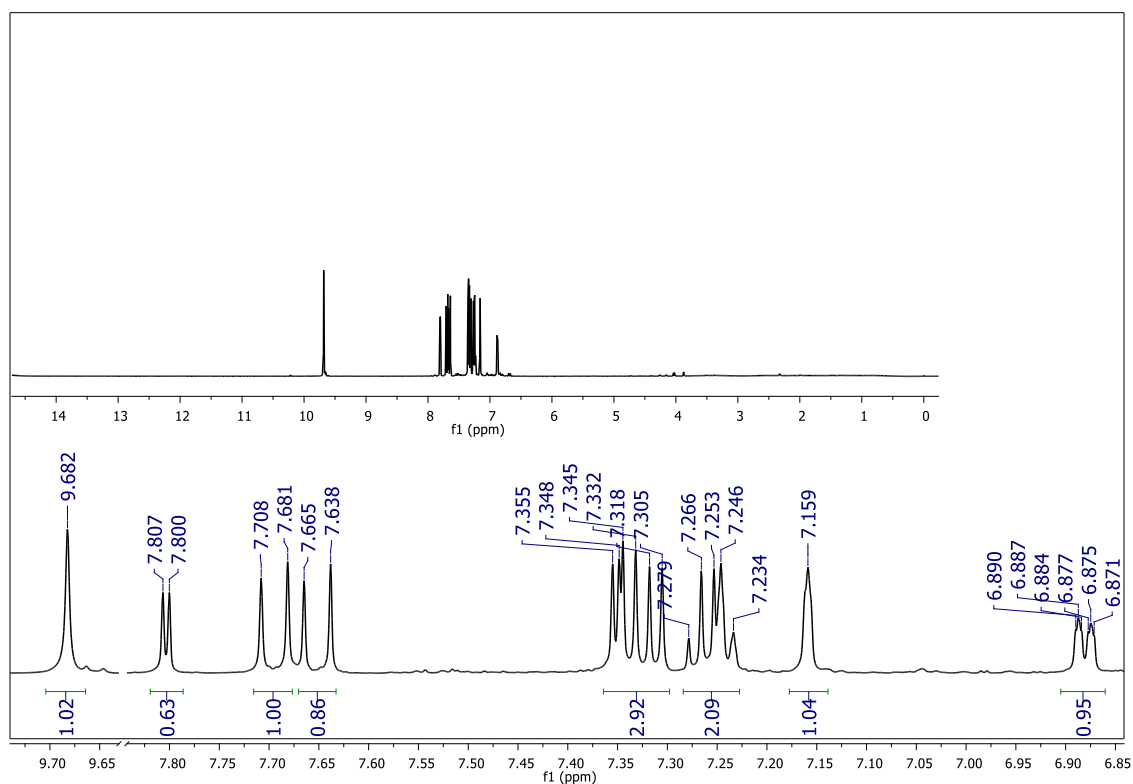


Figure S20. ^{13}C NMR spectrum of **MAC 44** (150 MHz; $\text{DMSO-}d_6$).

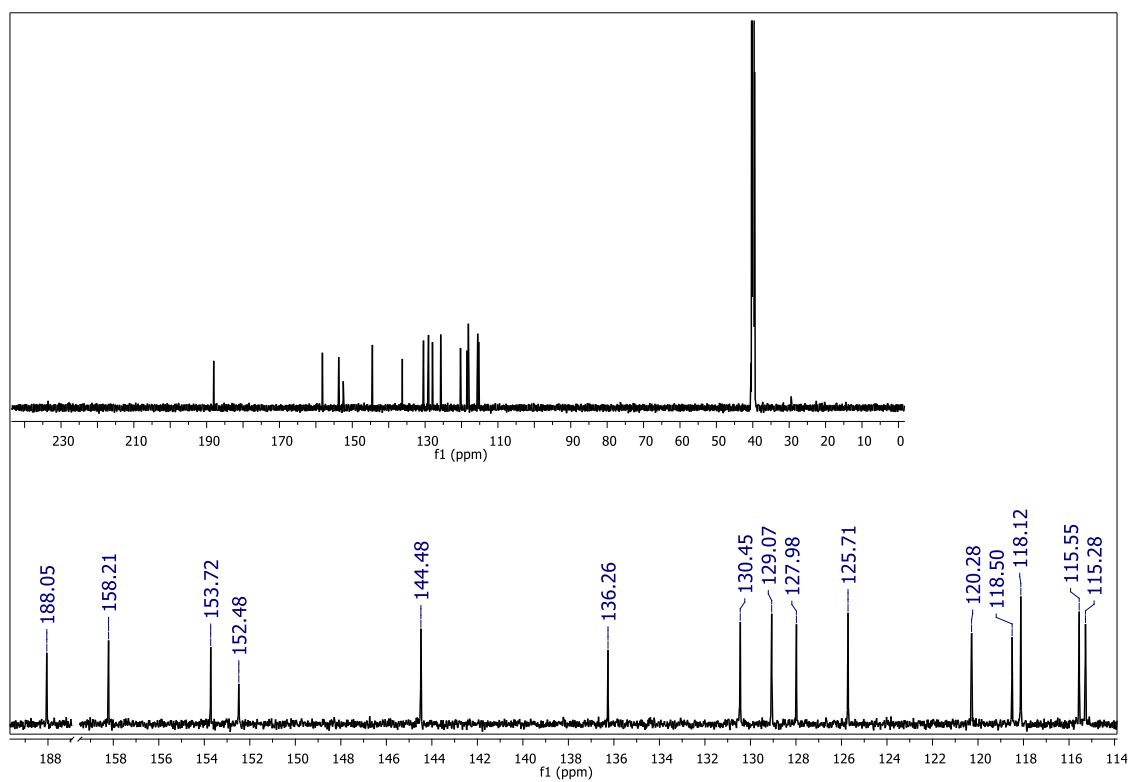


Figure S21. ^1H NMR spectrum of **MAC 45** (600 MHz; $\text{DMSO-}d_6$).

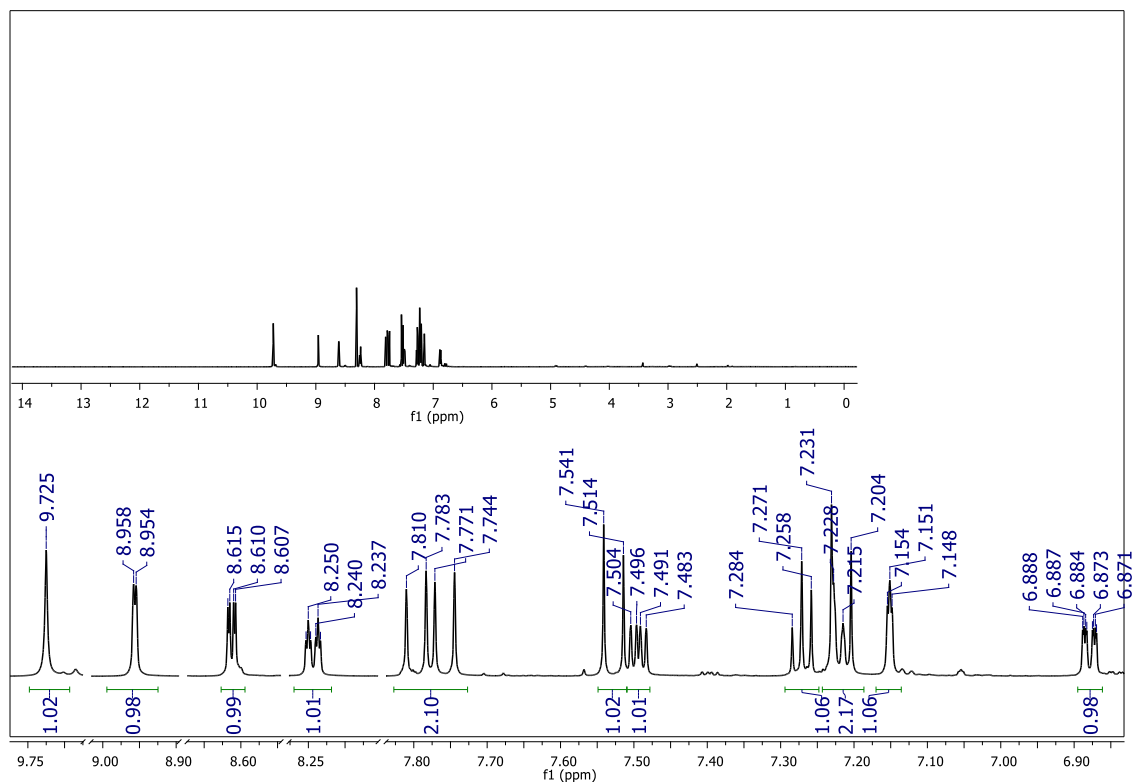


Figure S22. ^{13}C NMR spectrum of **MAC 45** (150 MHz; $\text{DMSO-}d_6$).

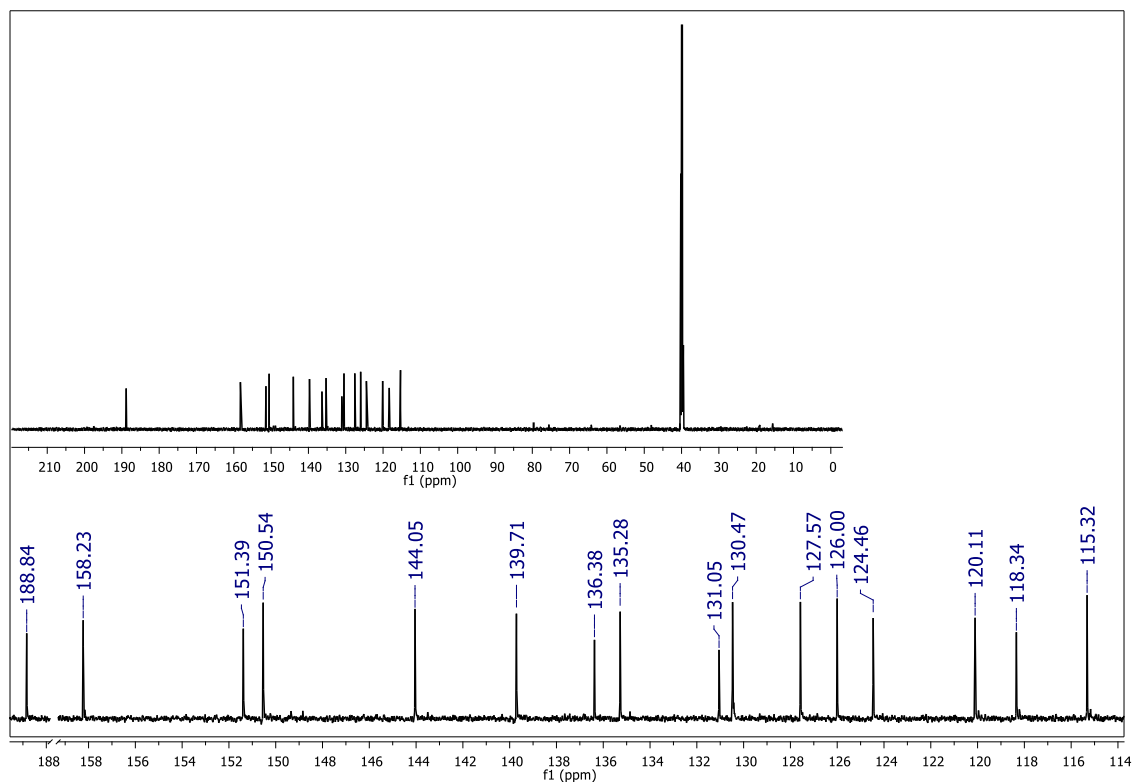


Figure S23. ^1H NMR spectrum of **MAC 46** (600 MHz; $\text{DMSO-}d_6$).

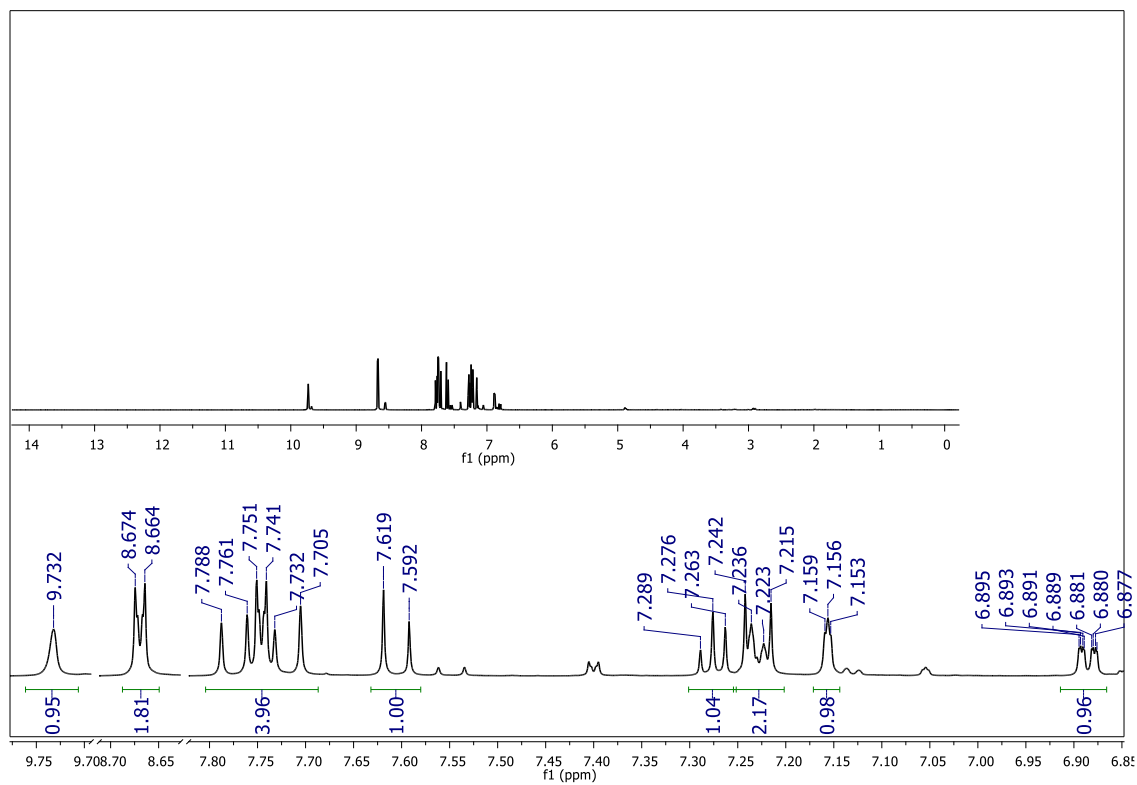


Figure S24. ^{13}C NMR spectrum of **MAC 46** (150 MHz; $\text{DMSO-}d_6$).

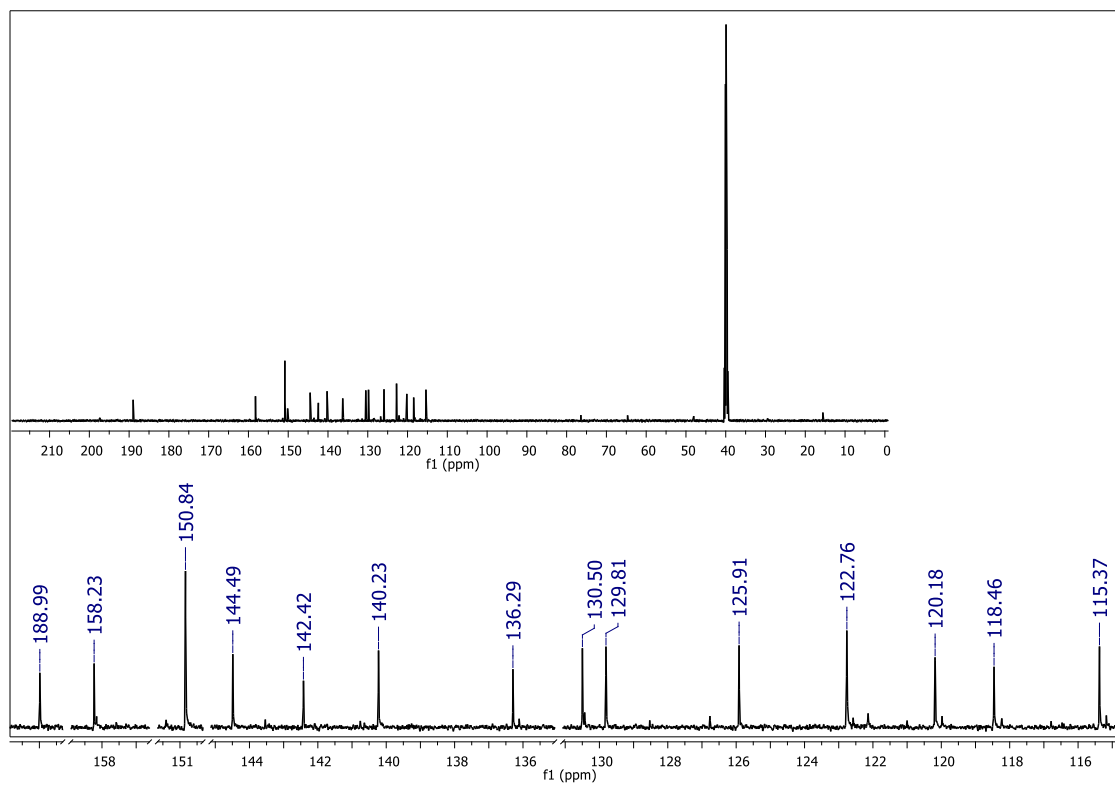


Figure S25. ^1H NMR spectrum of **MAC 47** (600 MHz; $\text{DMSO-}d_6$).

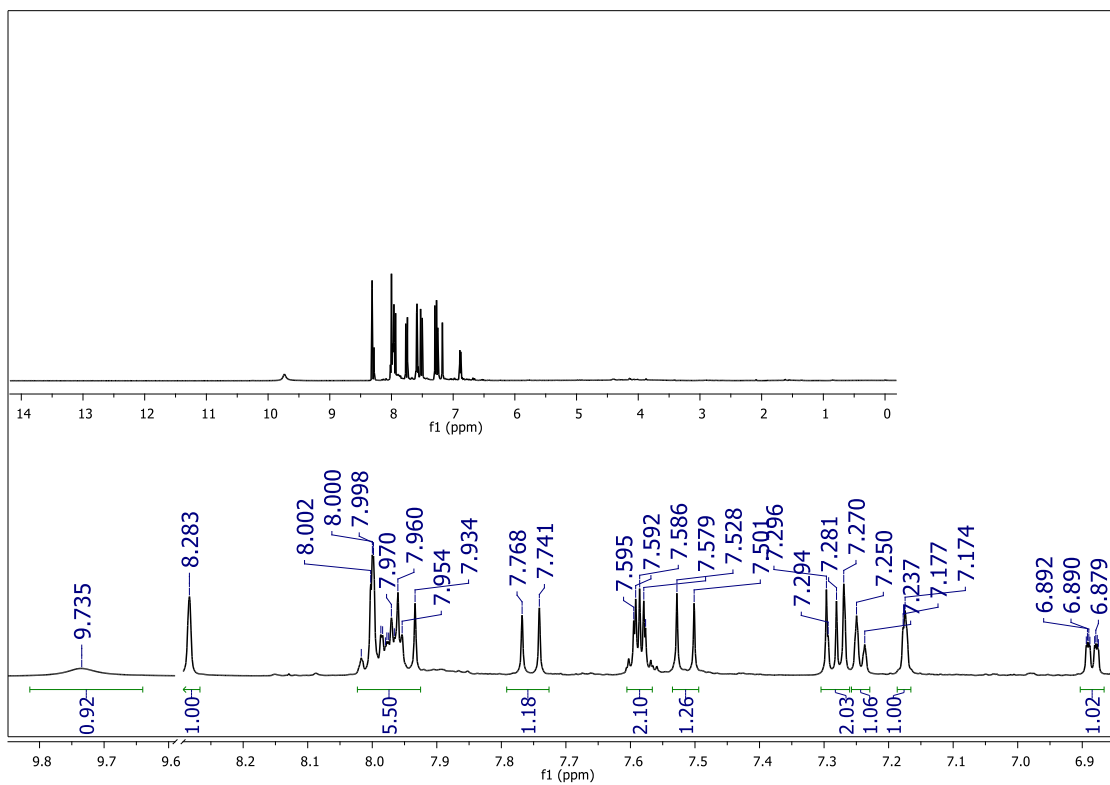


Figure S26. ^{13}C NMR spectrum of **MAC 47** (150 MHz; $\text{DMSO-}d_6$).

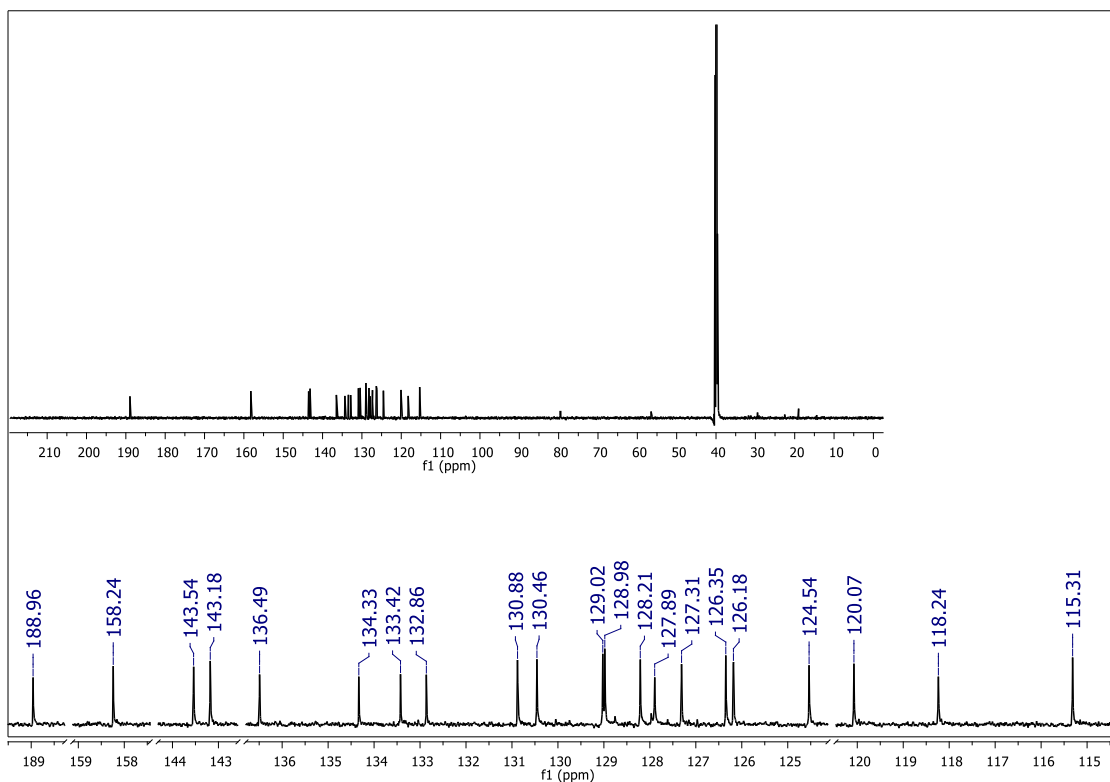


Figure S27. ^1H NMR spectrum of **MAC 48** (600 MHz; $\text{DMSO-}d_6$).

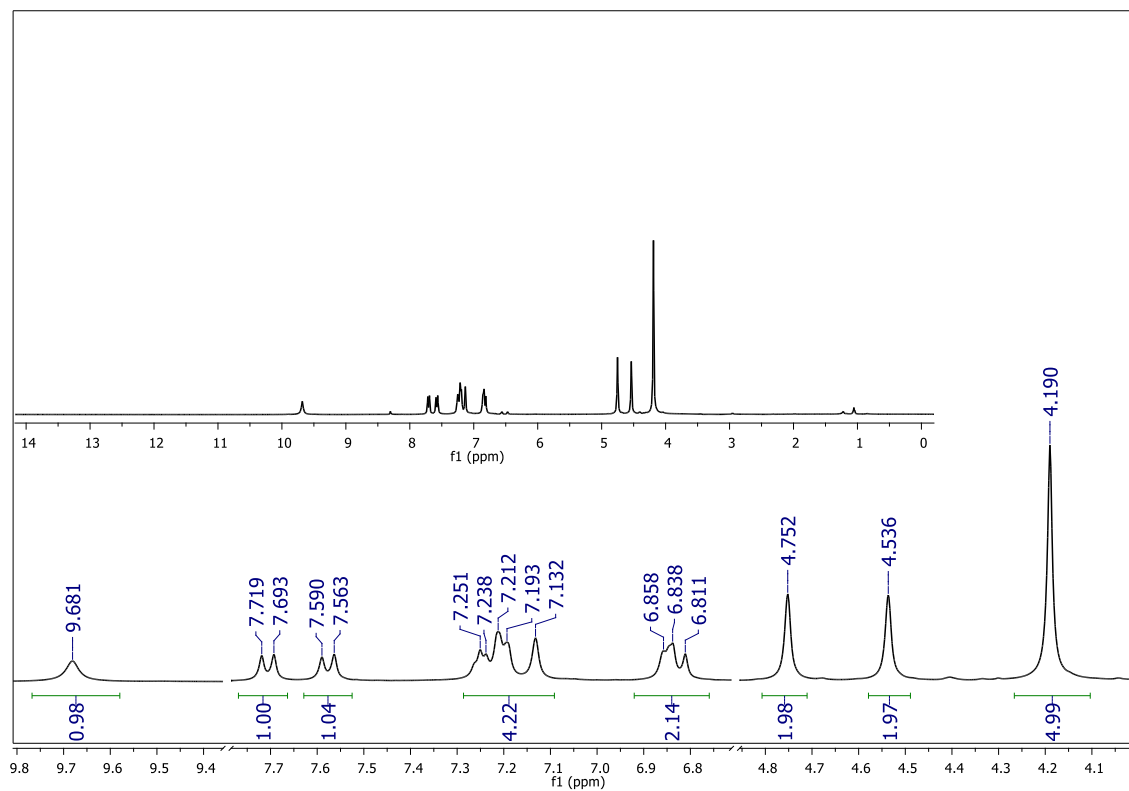


Figure S28. ^{13}C NMR spectrum of **MAC 48** (150 MHz; $\text{DMSO-}d_6$).

