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**Isolamento, elucidação estrutural e avaliação da atividade
antineuroinflamatória de novos sesquiterpenos de *Anaxagorea dolichocarpa***

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antineuroinflamatória de novos sesquiterpenos de *Anaxagorea dolichocarpa***

Tese apresentada ao Programa de Pós-Graduação em Produtos Naturais e Sintéticos Bioativos do Centro de Ciências da Saúde da Universidade Federal da Paraíba em cumprimento às exigências para obtenção do título de Doutor em Produtos Naturais e Sintéticos Bioativos.
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Ata da 381ª (trecentésima octogésima primeira) Tese de Doutorado do(a) aluno(a) do Programa de Pós-Graduação em Produtos Naturais e Sintéticos Bioativos **Rodrigo Silva de Andrade**, candidato(a) ao Título de “Doutor(a)” em Produtos Naturais e Sintéticos Bioativos na área de concentração Farmacoquímica.

Às oito horas e trinta minutos (8h30) do dia vinte e nove de agosto do ano dois mil e vinte e quatro (29/08/2024), em ambiente virtual de videoconferência através do aplicativo Google Meet, <https://meet.google.com/ttp-asgc-itd>, reuniram-se em caráter de Solenidade Pública os membros da Comissão designada para examinar o(a) discente **Rodrigo Silva de Andrade**, candidato(a) ao Título de “DOUTOR(A)” em Produtos Naturais e Sintéticos Bioativos na área de concentração Farmacoquímica. Foram componentes da Banca Examinadora os pesquisadores Mario Geraldo de Carvalho, Ph.D em Química Orgânica; Jorge Mauricio David, Ph.D em Química Orgânica; Sócrates Golzio dos Santos, Ph.D em Farmacoquímica; Francisco Jaime Bezerra Mendonça Junior, Ph.D em Química Orgânica e Josean Fachine Tavares, Ph.D em Farmacoquímica. Sendo o primeiro, integrante do corpo docente da Universidade Federal Rural do Rio de Janeiro, o segundo, integrante do corpo docente da Universidade Federal da Bahia, e os demais, integrantes do corpo docente da Universidade Federal da Paraíba. Dando início aos trabalhos, o(a) Presidente da Banca, professor(a) Josean Fachine Tavares, após declarar os objetivos da reunião, apresentou o(a) candidato(a) **Rodrigo Silva de Andrade**, a quem concedeu a palavra para que dissertasse oral e sucintamente sobre o tema apresentado e intitulado “Isolamento, elucidação estrutural e avaliação da atividade antineuroinflamatória de novos sesquiterpenos de *Anaxagorea dolichocarpa*”. Após discorrer sobre o referido tema durante cerca de cinquenta minutos, o(a) candidato(a) foi arguido(a) pelos Examinadores na forma Regimental. Em seguida, passou a comissão, em caráter secreto, a proceder à avaliação e julgamento do trabalho, concluindo por atribuir-lhe o conceito **APROVADO(A)**. Em face da aprovação, declarou o(a) Presidente, achar-se o(a) examinado(a) **Rodrigo Silva de Andrade**, legalmente habilitado(a) a receber o Título de “DOUTOR(A)” em Produtos Naturais e Sintéticos Bioativos, na Área de Concentração Farmacoquímica, cabendo a Universidade Federal da Paraíba, providências, como de direito, a expedição do Diploma que o(a) mesmo(a) faz jus. Nada mais havendo a tratar, foi lavrada a presente ata que é abaixo assinada pelos membros.

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RESUMO

ANDRADE, R. S. **Isolamento, elucidação estrutural e avaliação da atividade antineuroinflamatória de novos sesquiterpenos de *Anaxagorea dolichocarpa*.** João Pessoa, 2024. 254p. Tese de Doutorado - Programa de Pós-Graduação em Produtos Naturais e Sintéticos Bioativos. Centro de Ciências da Saúde, Universidade Federal da Paraíba.

O Brasil, por ser o país com a maior biodiversidade de plantas do mundo, desperta o interesse científico e tecnológico, pois a maioria dos medicamentos da indústria farmacêutica é proveniente de espécies vegetais. Dentre essas, *Anaxagorea dolichocarpa* Sprague & Sandwith ("paixinho"), destaca-se devido aos constituintes químicos isolados de importância farmacológica, como alcaloides e sesquiterpenos, tornando-a uma espécie promissora para a continuidade dos estudos. Assim, este trabalho teve como objetivo expandir o conhecimento fitoquímico e farmacológico desta espécie. Para isso, as raízes de *Anaxagorea dolichocarpa* foram coletadas em maio de 2021 no município de Cruz do Espírito Santo - PB e submetidas a processos de extração e isolamento por métodos cromatográficos clássicos e técnicas hifenadas. Dessa forma, o estudo fitoquímico resultou no isolamento e identificação de dezenove substâncias, as quais tiveram suas estruturas determinadas inequivocamente por meio da análise dos dados de RMN, EM e IV, juntamente com cálculos teóricos de RMN (DP4+) e ECD. Foram isolados dezoito sesquiterpenos macrocíclicos do tipo humuleno inéditos na literatura, denominados de dolichocarpols G-X (**1–18**) e um que já havia sido isolado previamente (nordine), porém neste trabalho foi feita a correção da sua estrutura bem como a determinação da sua estereoquímica absoluta por Raios-X e ECD. Ainda, as substâncias foram testadas para avaliar sua citotoxicidade e atividade antineuroinflamatória em uma linhagem celular BV2 estimulada com LPS/IFN- γ . Os compostos dolichocarpols G, K, N, O e T além de não apresentarem citotoxicidade aparente, reduziram significativamente os níveis de NO nas células BV2 em concentrações variando de 25 a 200 μ M, chegando a atingir um efeito máximo semelhante ao do controle (quercetina). Além disso, possíveis interações entre esses compostos e a enzima iNOS foram previstas por meio de estudos *in silico*, e os resultados demonstraram que os compostos apresentaram valores de probabilidade de atividade superiores a 0,5, indicando afinidade pelo alvo e que os complexos formados são estáveis, com baixas flutuações na estrutura terciária da enzima. Portanto, esses achados ampliam a compreensão sobre a diversidade estrutural e atividade biológica dos sesquiterpenos macrocíclicos humulenos da família Annonaceae e da espécie *Anaxagorea dolichocarpa*, contribuindo para o seu conhecimento fitoquímico e farmacológico.

Palavras-chave: *Anaxagorea dolichocarpa*, Annonaceae, antineuroinflamatório, humuleno

ABSTRACT

ANDRADE, R. S. **Isolation, structural elucidation and evaluation of the antineuroinflammatory activity of novel sesquiterpenes from *Anaxagorea dolichocarpa***. João Pessoa, 2024. 254p. PhD Thesis - Postgraduate Program in Natural and Synthetic Bioactive Products. Health Sciences Center, Federal University of Paraíba.

Brazil, being the country with the greatest plant biodiversity in the world, has drawn scientific and technological interest, since most of the pharmaceutical industry's drugs come from plant species. Among these, *Anaxagorea dolichocarpa* Sprague & Sandwith ("paixinho") stands out due to the isolated chemical constituents of pharmacological importance, such as alkaloids and sesquiterpenes, making it a promising species for continued studies. Thus, this work aimed to expand the phytochemical and pharmacological knowledge of this species. For this purpose, the roots of *Anaxagorea dolichocarpa* were collected in May 2021 in the municipality of Cruz do Espírito Santo – PB and were subjected to extraction and isolation processes by classical chromatographic methods and hyphenated techniques. The phytochemical study resulted in the isolation and identification of nineteen compounds, whose structures were unequivocally determined through the analysis of NMR, MS, and IR data, along with theoretical calculations of NMR (DP4+) and ECD. Eighteen novel macrocyclic humulene sesquiterpenoids, namely dolichocarpols G-X (1–18), were isolated, along with one previously known compound (nordine), whose structure was corrected in this work, and its absolute stereochemistry was determined by X-ray and ECD analysis. Furthermore, the compounds were tested for their cytotoxicity and antineuroinflammatory activity in the LPS/IFN- γ -stimulated BV2 cell line. Dolichocarpols G, K, N, O, and T, didn't presented any cytotoxicity and effectively reduced NO levels in BV2 cells at concentrations ranging from 25 to 200 μ M, reaching a maximum effect similar to that of the control (quercetin). Moreover, possible interactions between these compounds and the iNOS enzyme were predicted via *in silico* studies, and the results demonstrated that the compounds exhibited activity probability values greater than 0.5, which indicated their affinity for the target and that the formed complexes are stable, with low fluctuations in the tertiary structure of the enzyme. Therefore, these findings broadens the understanding of the structural diversity and biological activity of the macrocyclic humulene sesquiterpenoids from the Annonaceae family and the *Anaxagorea dolichocarpa* species, contributing to its phytochemical and pharmacological knowledge.

Keywords: *Anaxagorea dolichocarpa*, Annonaceae, antineuroinflammatory, humulene.

LISTA DE ABREVIATURAS E SIGLAS

AcOEt	Acetato de etila
APT	<i>Attached Proton Test</i>
BB	<i>Broadband</i>
C	Carbono
¹³C	Carbono 13
CC	Cromatografia em Coluna
CCDA	Cromatografia em Camada Delgada Analítica
COSY	<i>Correlation Spectroscopy</i>
CLAE	Cromatografia Líquida de Alta Eficiência
CLMP	Cromatografia Líquida de Média Pressão
d	Dupleto
dd	Duplo dupleto
ddd	Duplo duplo dupleto
dl	Dupleto largo
dq	Duplo quarteto
DCM	Diclorometano
DEPT	<i>Distortionless Enhancement by Polarization Transfer</i>
ECD	<i>Electronic Circular Dichroism</i>
EEB	Extrato Etanólico Bruto
EM	Espectrometria de massa
EMAR-IES	Espectrometria de massa de alta resolução com ionização por electrospray
EtOH	Etanol
g	Grama
¹H	Hidrogênio
HMBC	<i>Heteronuclear Multiple Bond Correlation</i>
HSQC	<i>Heteronuclear Single Quantum Correlation</i>
HPLC	<i>High performance liquid chromatography</i>
HRESIMS	<i>High resolution electrospray ionization mass spectroscopy</i>
Hz	Hertz
IV	Infravermelho
J	Constante de acoplamento

Kg	Quilograma
m	Multipeto
MeOH	Metanol
mg	Miligrama
m/z	Relação massa-carga
MHz	Megahertz
mL	Mililitro
NOESY	<i>Nuclear Overhauser Spectroscopy</i>
ppm	Partes por milhão
R_f	Fator de retenção
RMN	Ressonância Magnética Nuclear
s	Simpleto
sl	Simpleto largo
t	Tripleto
td	Triplo duplete
tl	Tripleto largo
UV	Ultravioleta
δ	Deslocamento químico
μm	Micrômetro

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1. INTRODUÇÃO

Os produtos naturais constituem um amplo grupo de substâncias químicas provenientes de bactérias, fungos, animais marinhos e plantas. Essas possuem uma vasta variedade de atividades biológicas, sendo utilizadas principalmente na medicina humana, veterinária e na agricultura (Katz; Baltz, 2016). Entre essas fontes, as plantas se destacam por serem amplamente aceitas e empregadas em inúmeros países para o tratamento de várias doenças, além de serem a principal fonte dos medicamentos da indústria farmacêutica. (Mustafa *et al.*, 2017; Newman; Cragg, 2020; Newman, 2022; Yun *et al.*, 2012).

A Organização Mundial de Saúde (OMS) estima que entre 65-80% da população dos países em desenvolvimento fazem uso de plantas medicinais para o tratamento de doenças (Mustafa, 2017). Portanto, com o objetivo de promover o desenvolvimento socioeconômico e sustentável da biodiversidade na área de plantas medicinais e fitoterápicos, além de proporcionar melhorias na qualidade de vida da população brasileira, a partir de 2006, foi instituído pelo Ministério da Saúde a Política Nacional de Plantas Medicinais e Fitoterápicos, por meio do decreto nº 5.813 (BRASIL, 2006), que determina prioridade nas investigações de plantas medicinais devido à tendência de utilização de produtos naturais pelas comunidades do nosso país.

O Brasil, por ser o país com a maior biodiversidade do mundo, com cerca de 170 a 210 mil espécies, possuindo mais de 50 mil espécies de plantas, desperta o interesse científico e tecnológico, pois a maioria dessas espécies ainda não foram estudadas (Costa; Peralta, 2015; Prado, 2015; Ribeiro *et al.*, 2018). Assim, uma diversidade de compostos orgânicos que podem ser candidatos a fármacos permanecem ocultos. Dessa forma, o aprofundamento do conhecimento sobre as espécies vegetais, desenvolvidos por estudos integrados nas áreas de química, farmacologia e etnobotânica se torna fundamental para subsidiar a pesquisa e uso do potencial natural existente no Brasil (Braz-Filho, 2010; Sganzerla *et al.*, 2022).

Diante disto, a Química de Produtos Naturais vem evoluindo frente a diversas técnicas e tecnologias inovadoras que em convergência com áreas como Bioquímica, Biologia Molecular e Imunologia, visam o isolamento, caracterização e avaliação farmacológica de novas substâncias provenientes de plantas e outras fontes de produtos naturais de modo a desenvolver novos medicamentos, gerando

benefícios para a população e lucro para a indústria farmacêutica (Berlinck *et al.*, 2017; David; Wolfender; Dias, 2015; Ferreira; Pinto, 2010).

Nesse contexto, a família Annonaceae destaca-se por ser uma das maiores entre as angiospermas, possuindo uma imensa riqueza em metabolitos secundários, tais como alcaloides, terpenoides e acetogeninas, os quais possuem diversas atividades biológicas, como citotóxica, anti-inflamatória, antitumoral, antiparasitária e antifúngica (Cascaes *et al.*, 2023; Castello-branco *et al.*, 2009; Castello-branco *et al.*, 2011; Costa *et al.*, 2006; Wu *et al.*, 2012). Dentre as Annonaceae, a espécie *Anaxagorea dolichocarpa* Sprague & Sandwith, nos últimos anos, vem sendo estudada pelos pesquisadores do Programa de Pós-graduação em Produtos Naturais e Sintéticos Bioativos (PPgPNSB). Nesta espécie, já foram isolados alguns constituintes químicos de importância farmacológica, como alcaloides do tipo azafenantreno (Lúcio *et al.*, 2011; Sales *et al.*, 2020), oxoaporfínico e protoberberínico (Tavares *et al.*, 2005) além de sesquiterpenos macrocíclicos do tipo humuleno (Sales *et al.*, 2020), destacando a espécie como promissora para a continuidade dos estudos.

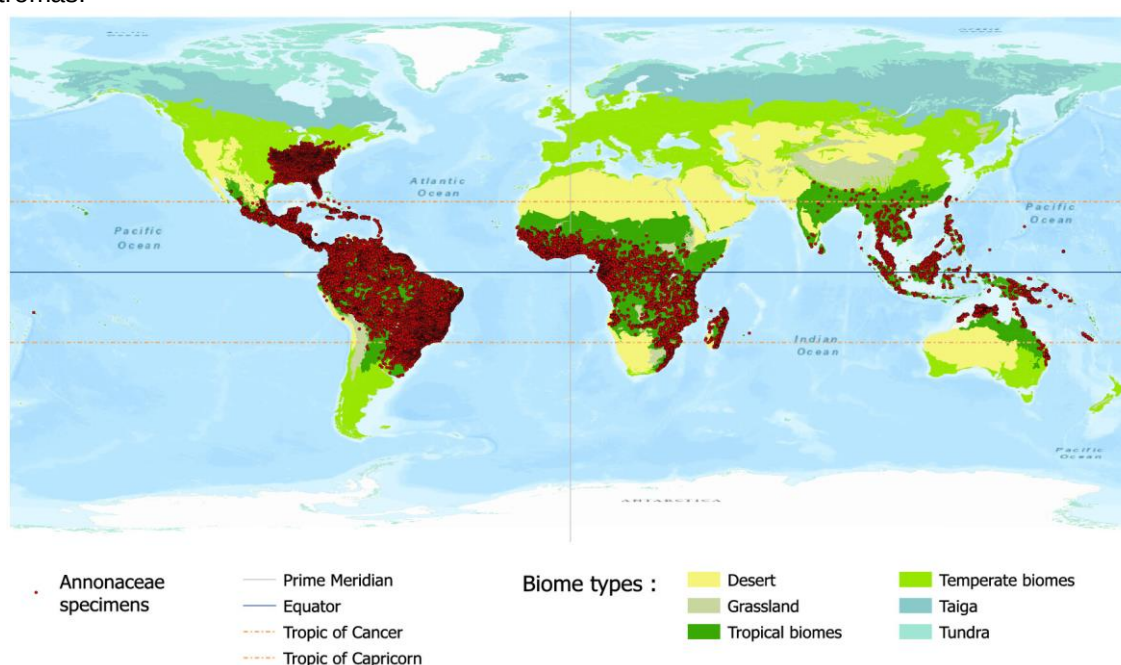
Dessa forma, percebendo os benefícios que os medicamentos de origem natural vêm trazendo para a sociedade, além de contemplar a importância dos estudos fitoquímicos, optou-se neste trabalho, dar continuidade ao estudo de *Anaxagorea dolichocarpa* por se tratar de uma espécie com um grande potencial em produzir metabólitos secundários de interesse farmacológico, buscando assim, isolar, identificar e realizar ensaios biológicos das substâncias químicas com o intuito de descobrir possíveis novos candidatos a medicamentos, contribuindo com o conhecimento químico e farmacológico da família Annonaceae e ampliando o trabalho já desenvolvido no PPgPNSB.

2. FUNDAMENTAÇÃO TEÓRICA

2.1 Família Annonaceae

A família Annonaceae é a maior dentro da ordem das Magnoliales com cerca de 122 gêneros e 2440 espécies de ocorrência pantropical com predominância em zonas equatoriais, florestas tropicais e subtropicais de planície (Figura 1), sendo composta principalmente por árvores, arbustos e cipós (Guo *et al.*, 2017; Rangel *et al.*, 2024). Além disso, está presente em quatro dos seis principais biomas terrestres, distribuída por cerca de 109 países, em biomas arbóreos e outros biomas associados (Erkens *et al.*, 2023). Estudos filogenéticos subdividiram essa família em quatro subfamílias: Anaxagoreoideae, Ambavioideae, Annonoideae e Malmeoideae, sendo divididas em 14 tribos (Chatrou *et al.*, 2012).

Figura 1. Mapa de distribuição geográfica da família Annonaceae através de seus biomas e antromas.



Fonte: (Erkens *et al.*, 2023).

As subfamílias Annonoideae e Malmeoideae são as mais representativas, possuindo cerca de 1515 e 783 espécies, respectivamente. Ambavioideae possui 56 espécies e em seguida aparece Anaxagoreoideae (30 espécies) como o grupo-irmão de todas as Annonaceae e possui apenas um gênero (Doyle; Le Thomas, 1996; Erkens *et al.*, 2023; Guo *et al.*, 2017; Richardson *et al.*, 2004; Stevens, 2016). Dentre

os gêneros mais representativos desta família estão *Guatteria* (177), *Annona* (170), *Uvaria* (199) e *Xylopia* (164) (Guo *et al.*, 2017). No Brasil, ocorrem 392 espécies distribuídas em 29 gêneros. A Amazônia abriga a maior parte da diversidade de Annonaceae, com 27 gêneros e 265 espécies, e a Mata Atlântica, vem em seguida com cerca de 16 gêneros e 95 espécies (Maas; Lobão; Rainer, 2015; BFG *et al.*, 2015).

Morfologicamente, suas espécies podem variar de arbustos a árvores, raramente atingindo o porte de árvores de dossel, e também podem ser lianas. Apresentam casca fibrosa que pode ser facilmente retirada em longas tiras e muitas vezes exala um aroma picante quando cortada. O caule e os ramos são cilíndricos, flexíveis quando jovens, e podem ser glabros ou pilosos. A presença de tricomas pode ser simples, escamiformes ou estrelados. As folhas são simples, alternas e dispostas de forma dística. As flores podem surgir em posições axilares, terminais, opositifólias e internodais. O cálice é frequentemente menor que as pétalas, com sépalas que podem ser livres, unidas na base ou completamente conatas, com antese variando de valvar a imbricada. Finalmente, os frutos são majoritariamente apocárpicos, constituídos por poucos a muitos carpódios, que podem ser sésseis ou estipitados, secos ou carnosos, e deiscentes ou indeiscentes (Oliveira, 2018).

Figura 2. Espécies da família Annonaceae.



A-C: *Annona pickelii*; **D:** *Annona sylvatica*; **E-F:** *Cymbopetalum brasiliense*.

Fonte: (Oliveira, 2018).

Figura 3. Espécies da família Annonaceae.

A-B: *Anaxagorea dolichocarpa*; **C-D:** *Annona coriacea*; **E:** *Annona exsucca*; **F-G:** *Annona glabra*; **H:** *Annona leptopetala*; **I-J:** *Annona montana*; **K:** *Annona mucosa*; **L:** *Annona paludosa*.
Fonte: (Oliveira, 2018).

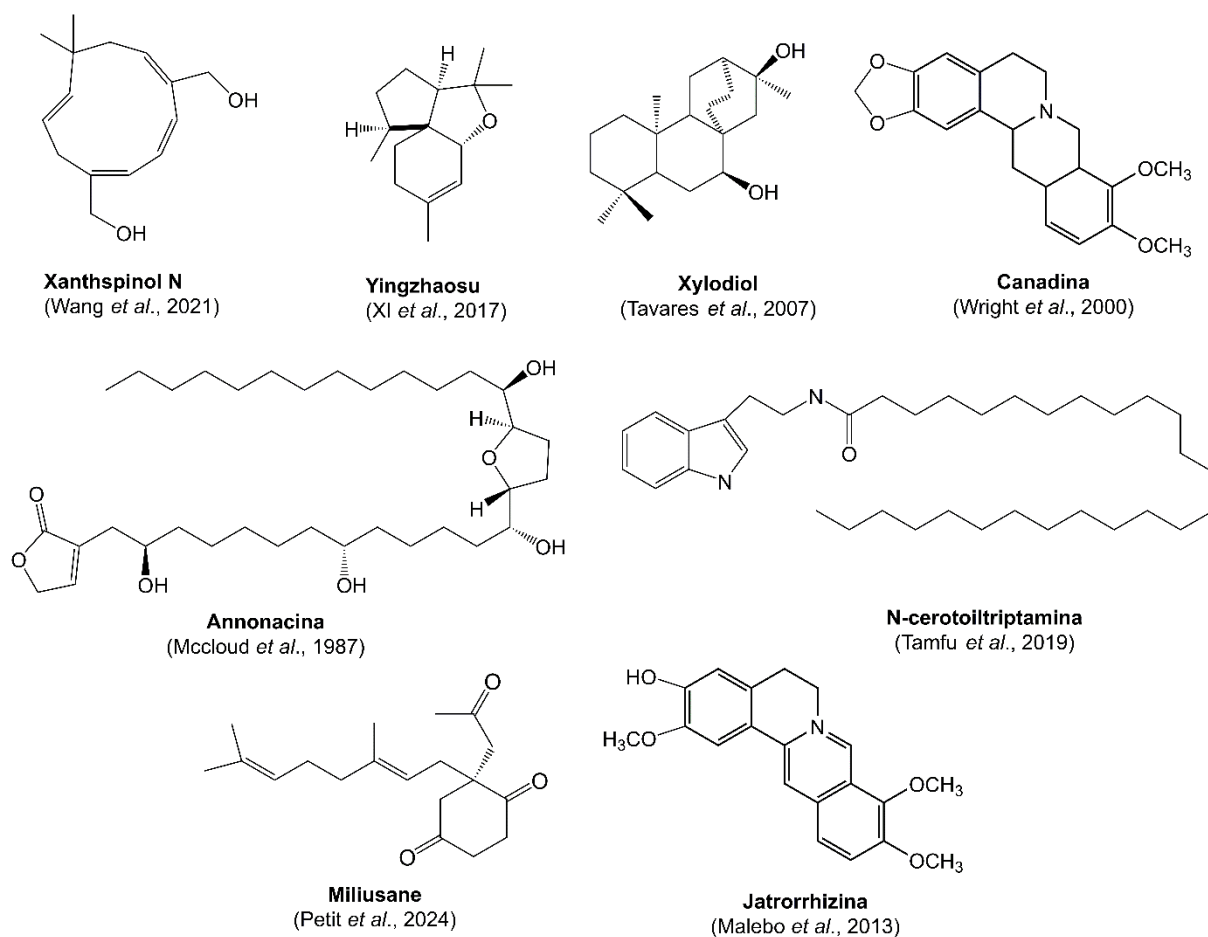
As espécies de Annonaceae possuem grande importância econômica. Exemplos notáveis incluem a “pinha” (*Annona squamosa* L.) e a “graviola” (*Annona muricata* L.), cujos frutos são amplamente consumidos pela população. Outros gêneros também despertam interesse econômico, como aqueles que fornecem essências para a perfumaria, como o óleo de *Cananga odorata*, popularmente conhecido como “ylang-ylang”. *Xylopia aromatica* “pindaíba” é utilizada na culinária como especiaria e *Polyalthia longifolia* é empregada em ornamentação (Brophy Goldsack; Forster, 2004; Mallavarapu; Gurudutt; Syamasundar, 2016; Oliveira *et al.*, 2012; Oliveira *et al.*, 2021; Rabelo *et al.*, 2016; Rinaldi *et al.*, 2017; Teixeira *et al.*, 2019; Thang *et al.*, 2013). Além disso, alguns gêneros são valorizados na produção de biocompostos, como *Annona*, *Duguetia* e *Xylopia* (Inoue *et al.* 2009; Martin *et al.*, 2019). A presença de acetogeninas em alguns táxons da família tem incentivado estudos para a produção de inseticidas, que oferecem vantagens como moléculas biodegradáveis, menor toxicidade para mamíferos e eficácia no controle de pragas. Entre os gêneros com potencial inseticida destacam-se *Annona*, *Duguetia*, *Guatteria*, *Oxandra* e *Xylopia* (Krinski *et al.*, 2014).

Entre os usos populares, o chá de *Duguetia cadavérica* é empregado para problemas intestinais, malária, dores de cabeça e reumatismo. *Annona muricata* e *Annona squamosa* são usadas como antitumorais, antiparasitárias, antidiarreicas, analgésicas e sedativas. *Annona hypoglauca* é utilizada também no tratamento de parasitas, diarreia e anemia (Barbalho *et al.*, 2012; Meira *et al.*, 2015; Rinaldi *et al.*, 2017). Ainda, as folhas *Annona glabra* são usadas contra reumatismo e as de *Annona Montana* são usadas contra picadas de cobra e a decocção é usada para combater a obesidade e auxiliar a perda de peso. *Rollinia leptopetala* e *Xylopia frutescens* são usadas como digestivo, sendo bebidas como chá após as refeições (Agra *et al.*, 2008).

A respeito dos estudos fitoquímicos, as classes de substâncias químicas encontradas nessa família são diversas, porém, podem-se citar três classes como as principais encontradas, sendo elas: acetogeninas, alcaloides e terpenoides (Figura 4), pois além da abundância na família, são responsáveis por importantes atividades farmacológicas, como citotóxica e antitumoral, antimalárica, antifúngica, antiprotozoária, antiviral, hipoglicemiante e anti-inflamatória (Fuentes, *et al.*, 2022; Kan *et al.*, 2019; Muganza *et al.*, 2016; Tavares *et al.*, 2007; Tamfu *et al.*, 2019;

Sumary; Mgina; Joseph, 2019; Sharma *et al.*, 2023; Petit *et al.*, 2024; Wang *et al.*, 2021; Xi *et al.*, 2017). Portanto, fica evidente a importância e relevância da família Annonaceae no campo de descoberta de novas moléculas ativas, justificada pelos estudos fitoquímicos e farmacológicos das espécies desta família, que visam descobrir mais a respeito, justificando a necessidade de se realizar novas pesquisas científicas.

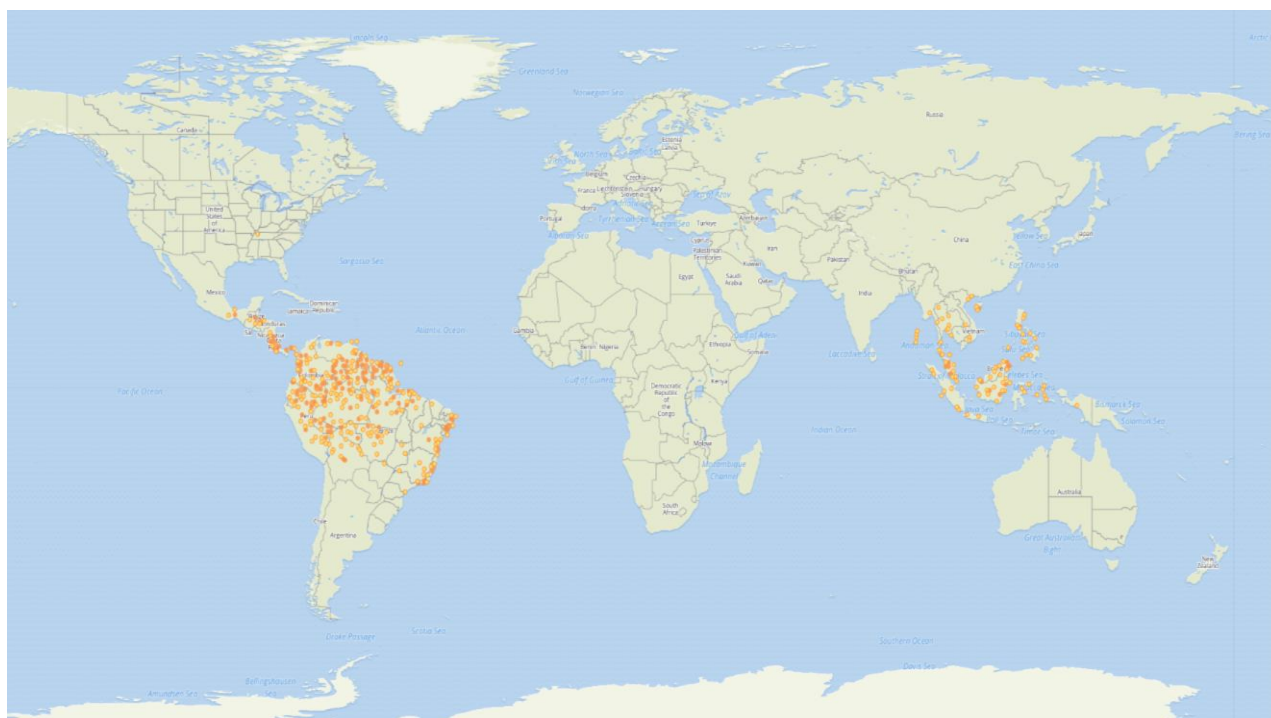
Figura 4. Exemplos de substâncias isoladas na família Annonaceae.



2.2 Gênero *Anaxagorea* e a espécie *Anaxagorea dolichocarpa* Sprague & Sandwith

O gênero *Anaxagorea* possui cerca de 30 espécies com ocorrência em regiões neotropicais e Sul da Ásia, sendo a grande maioria encontrada na América do Sul (Figura 5) (Guo *et al.*, 2017; Gottsberger, 2016). No Brasil, são catalogadas 14 espécies, a maior parte localizada nos biomas da Amazônia e Mata Atlântica (Maas; Lobão; Rainer, 2015). É o único gênero da subfamília Anaxagoreoideae, sendo considerado irmão taxonômico do resto da família Annonaceae (Guo *et al.*, 2017), porém apresenta uma particularidade por possui frutos com carpódios livres e deiscentes que são de rara ocorrência em sua família (Maas; Westra, 1984, 1985).

Figura 5. Mapa de distribuição geográfica do gênero *Anaxagorea*.

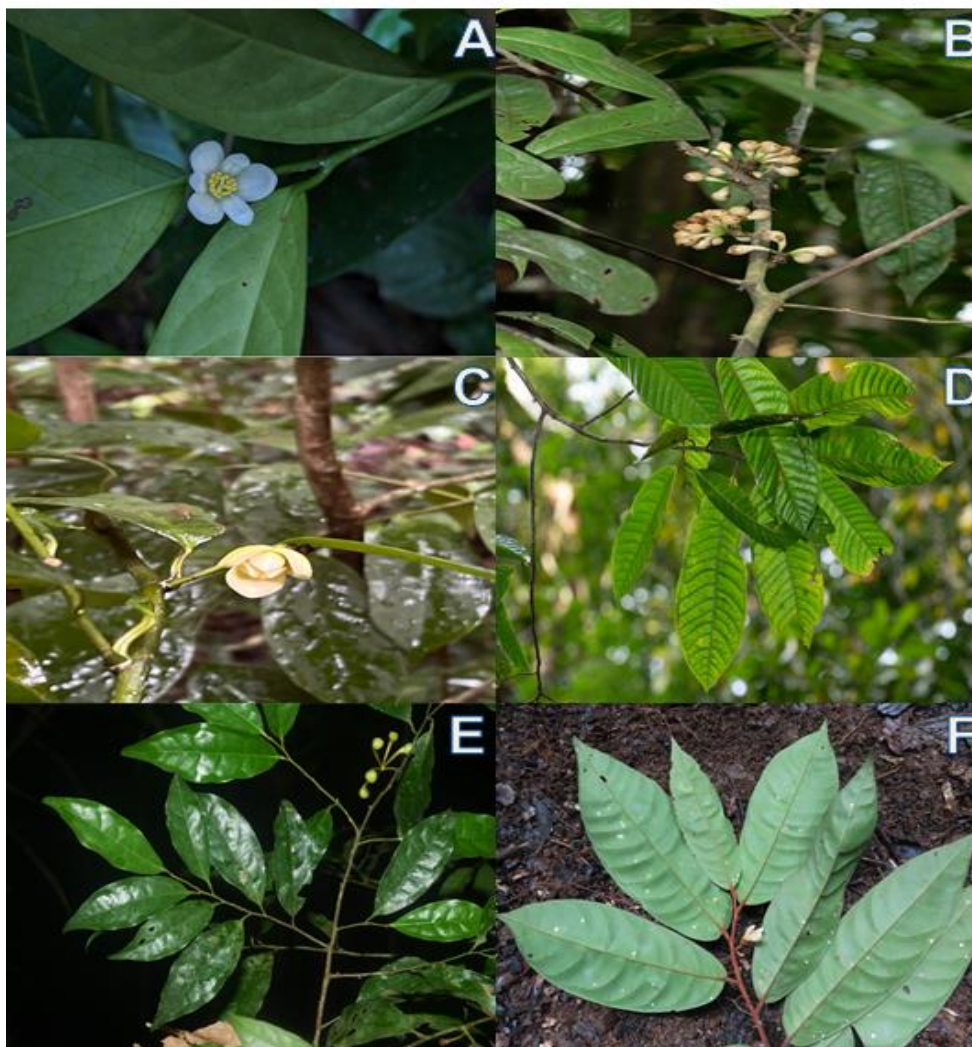


Fonte: gbif.org/occurrence/map

As espécies desse gênero (Figura 6) apresentam-se como árvores ou arbustos, com flores axilares ou, raramente, terminais e monóclinas. Geralmente possuem três sépalas e seis pétalas livres, estames numerosos e carpelos com dois óvulos. Esse gênero é considerado basal devido a suas características distintas e supostamente ancestrais, como estaminódios internos, pólen granular e monossulcado. Suas espécies ocorrem predominantemente em florestas tropicais de baixa altitude, com poucas espécies adaptadas a crescer em altitudes mais

elevadas. (Maas; Westra, 1984, 1985; Gottsberg, 2016; Endress; Armstrong, 2011). Na medicina popular são utilizadas como antipirético, antigripal, alívio de dores musculares e para melhorar o fluxo sanguíneo (Na-songkha, 1982; Gripp *et al.*, 2020).

Figura 6. Espécies do gênero *Anaxagorea*.



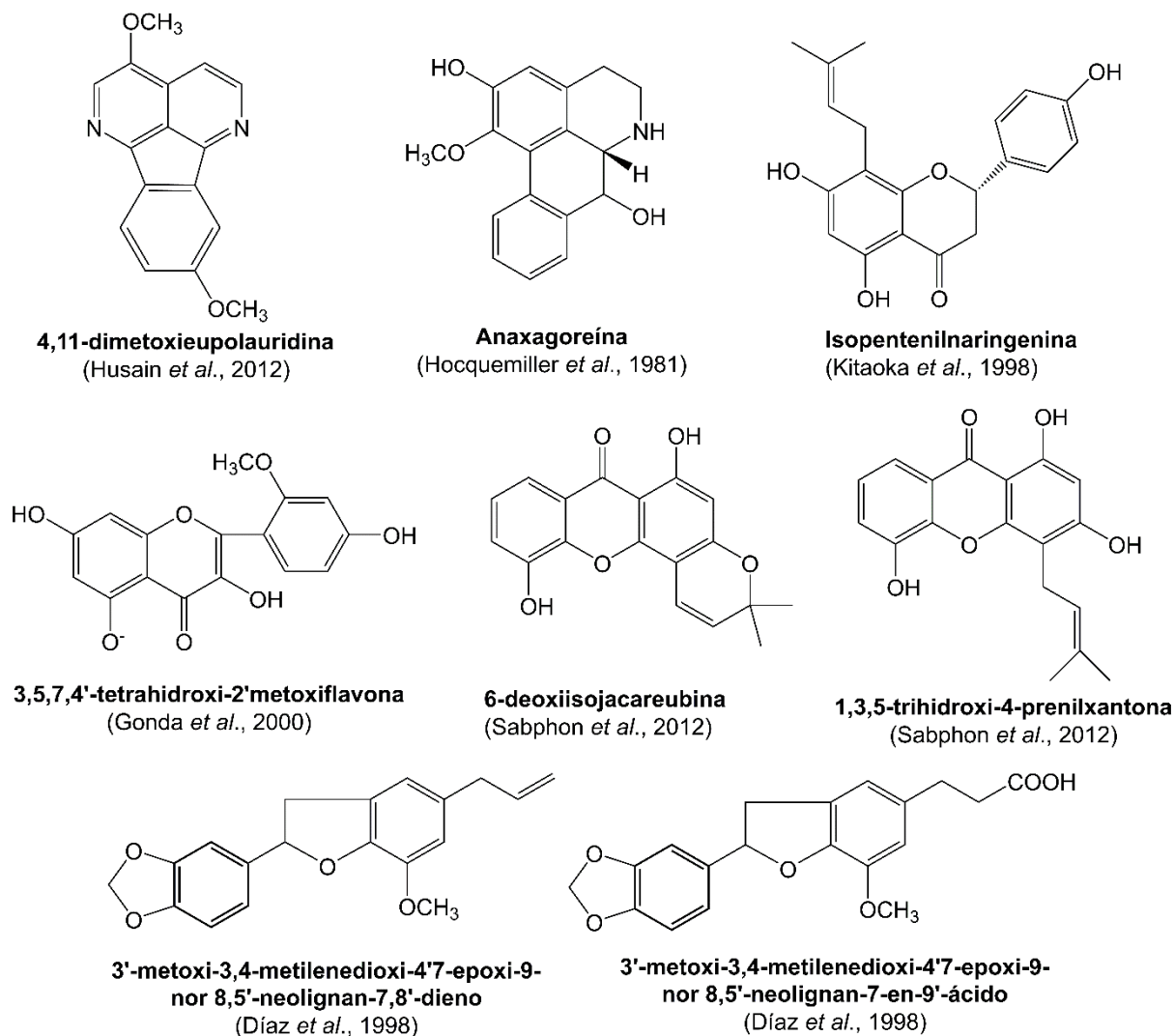
A: *Anaxagorea luzonensis*; **B:** *Anaxagorea brachycarpa*; **C:** *Anaxagorea javanica*; **D:** *Anaxagorea phaeocarpa*; **E:** *Anaxagorea prinoides*; **F:** *Anaxagorea crassipetala*.

Fonte: gbif.org/occurrence/gallery

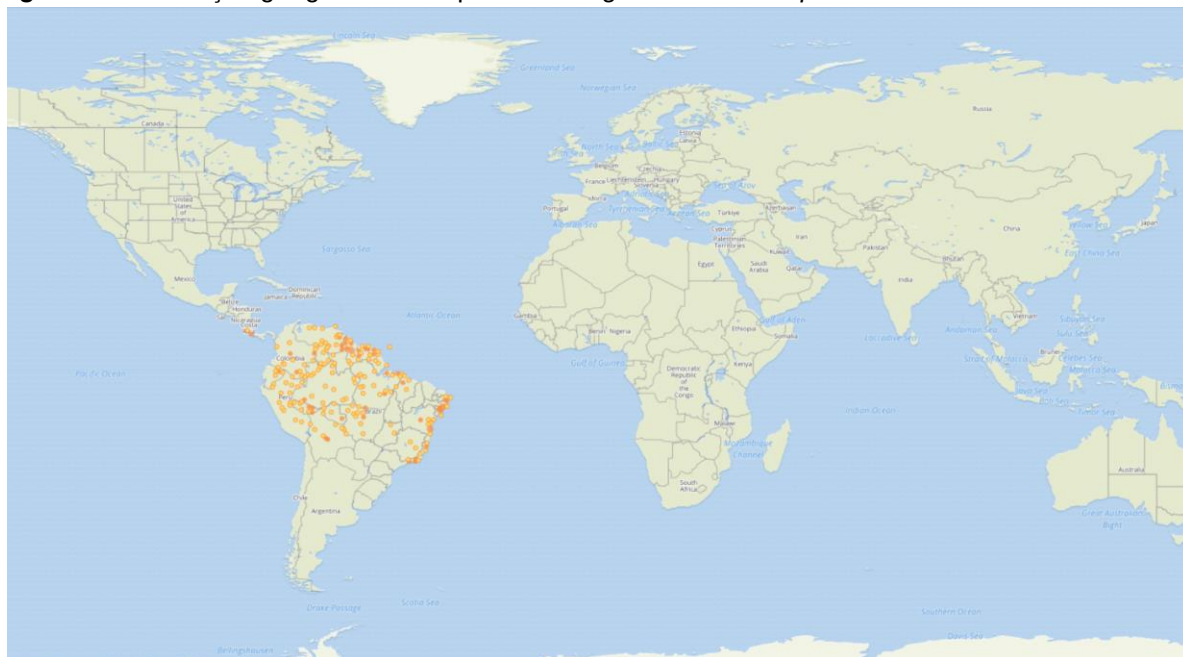
Estudos mostraram que as espécies deste gênero possuem vários efeitos farmacológicos, como atividade antinociceptiva, antimicrobiana, antiviral, antiproliferativa, anti-inflamatória, vasorelaxante em aorta de rato e inibição da acetilcolinesterase (Alencar, 2016; Brandão *et al.*, 2011; Gripp *et al.*, 2020; Tepareenan; Sawasdee, 2011; Tran *et al.*, 2024; Sabphon *et al.*, 2012). A respeito dos estudos fitoquímicos, foram relatados a presença de diversos metabólitos

secundários nas espécies deste gênero, como, flavonoides, xantonas, lignoides, terpenoides e alcaloides (Figura 7) (Díaz, 1997; Gonda *et al.*, 2000; Husain *et al.*, 2012; Kitaoka *et al.*, 1998; Sabphon *et al.*, 2012; Sabphon *et al.*, 2015).

Figura 7. Exemplos de substâncias isoladas no gênero *Anaxagorea*.



Anaxagorea dolichocarpa, popularmente conhecida como “paixinho”, é uma espécie de Annonaceae amplamente distribuída na região neotropical. É a espécie com a maior distribuição geográfica do gênero, sendo também a mais comum, ocorrendo na Colômbia, Venezuela, Costa Rica, nas Guianas e no Brasil (Figura 8). Neste último, ocorrem para as regiões Norte, Nordeste, Centro-Oeste e Sudeste, habitando florestas úmidas e de baixa altitude (Lopes; Mello, 2014; Maas; Westra 1984, 1985; Maas; Lobão; Rainer, 2015). Na Paraíba, foi referida pela primeira vez em 2004 onde foi encontrada em remanescentes de mata atlântica (Pontes *et al.*, 2004).

Figura 8. Distribuição geográfica da espécie *Anaxagorea dolichocarpa*.

Fonte: gbif.org/species/3156852

Lopes e Mello (2014) descreveram *Anaxagorea dolichocarpa* (Figura 9) como uma árvore ou arbusto de 4 a 7 m de altura. Os ramos, pecíolos e gemas são cobertos por tricomas simples e ferrugíneos. As folhas são estreitamente oblongas, com ápice e base variando entre agudos e obtusos. As flores possuem pedicelo de 9 mm e sépalas lanceoladas a ovadas. As pétalas externas são estreitamente oblongas ou elípticas, com a face abaxial densamente coberta por tricomas ferrugíneos. As pétalas internas são estreitamente elípticas, com a face adaxial glabra e a abaxial coberta por tricomas na porção central. Os estames são numerosos, assim como os carpelos. Os frutos são clavados, medindo cerca de 3,5 cm, com sementes obcônicas e pretas quando secas.

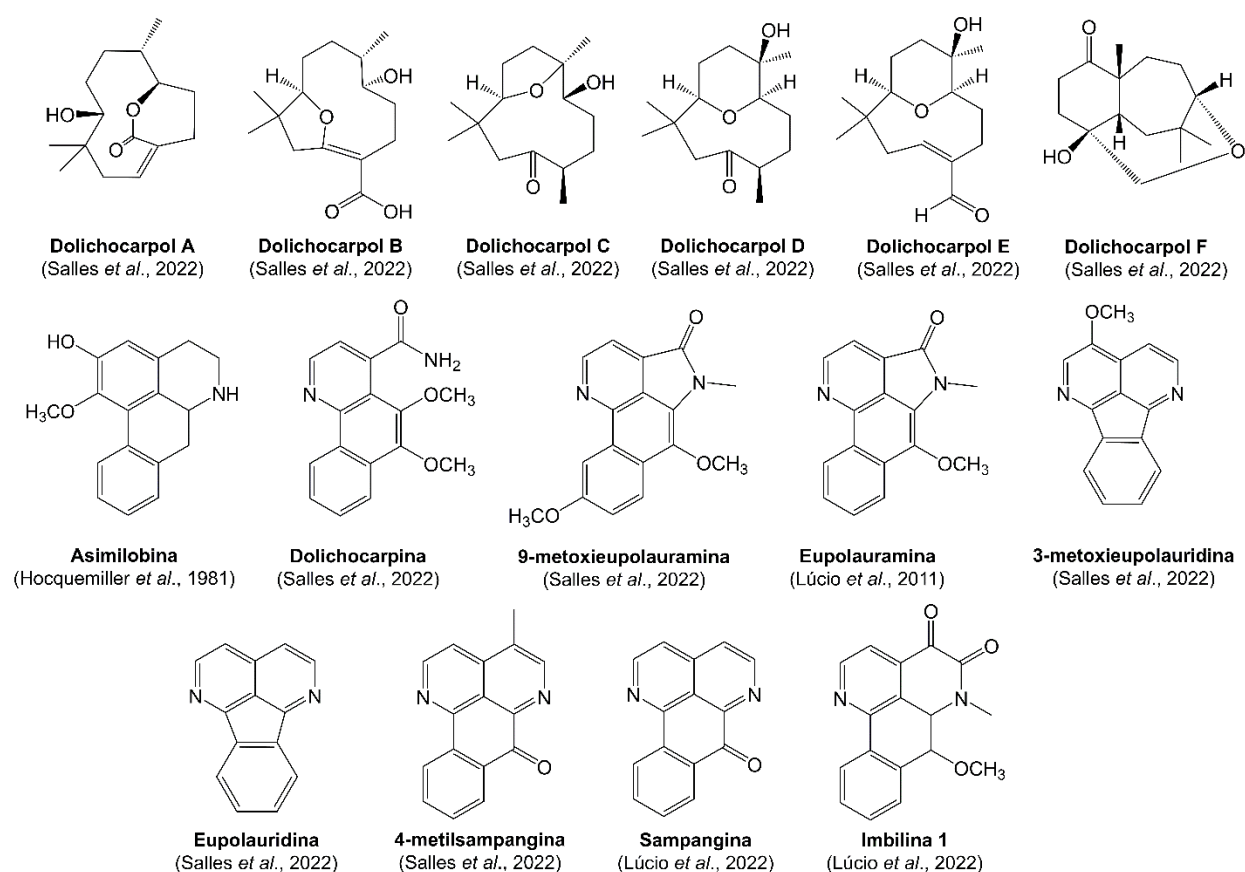
Figura 9. *Anaxagorea dolichocarpa* Sprague & Sandwith.

Fonte: gbif.org/occurrence/gallery?taxon_key=3156852

Na medicina popular, seus frutos, folhas e casca do caule são utilizados contra gripes e resfriados (Gripp *et al.*, 2020). Além disso, a casca é utilizada na Colômbia e Equador como um dos ingredientes do curare e na Guiana para fabricação de linha de pesca, fio dental, corda e cestas, demonstrando uma importância econômica para a espécie (Maas; Westra, 1984; Schultes; Raffaui, 1990). No que se refere aos estudos farmacológicos de seus extratos e substâncias isoladas, foram avaliados os potenciais antioxidante, antiviral, antifúngico, antinociceptivo, antitumoral, além da citotoxicidade. (Almeida *et al.*, 2011; Brandão *et al.*, 2011; Cascaes *et al.*, 2023; Gripp *et al.*, 2020; Pinheiro *et al.*, 2016; Lúcio *et al.*, 2011).

Em relação aos estudos fitoquímicos desta espécie, foram reportados o isolamento de alcaloides do tipo aporfínicos e azafenantrenos (Hocquemiller *et al.*, 1981; Lúcio *et al.*, 2011; Sales *et al.*, 2020b) além de sesquiterpenos macrocíclicos do tipo humuleno (Sales *et al.*, 2020a). Ainda, foi feita a avaliação da composição do óleo essencial de seus frutos, folhas e caule, sendo o espatulenol (29,88%) e o α -pineno (15,73%) os principais constituintes (Andrade; Oliveira; Zoghbi, 2007; Cascaes *et al.*, 2023; Fournier *et al.*, 1994).

Figura 10. Substâncias isoladas em *Anaxagorea dolichocarpa*.



2.3 Terpenoides

Os terpenoides, também conhecidos como isoprenoides, são uma família vasta e estruturalmente diversificada de substâncias químicas derivadas de unidades de isopreno unidas através do acoplamento cabeça com cauda. Com mais de 80.000 membros conhecidos, os terpenoides representam o maior grupo de metabolitos secundários. Muitos outros produtos naturais incorporam elementos terpenoides em suas moléculas, combinados com esqueletos de carbono derivados de outras vias biossintéticas, como as do acetato e do chiquimato. Eles desempenham papéis fundamentais no metabolismo de todos os organismos e são particularmente abundantes nas plantas, onde atuam como metabólitos secundários. Além disso, são cruciais para as interações ecológicas, agindo como aleloquímicos para atrair polinizadores, repelir herbívoros ou atrair predadores de herbívoros. (Dewick, 2009; Boutanaev *et al.*, 2015; Bohlmann *et al.*, 1998; Bergman; Davis; Phillips, 2019; Nagegowda; Gupta, 2020).

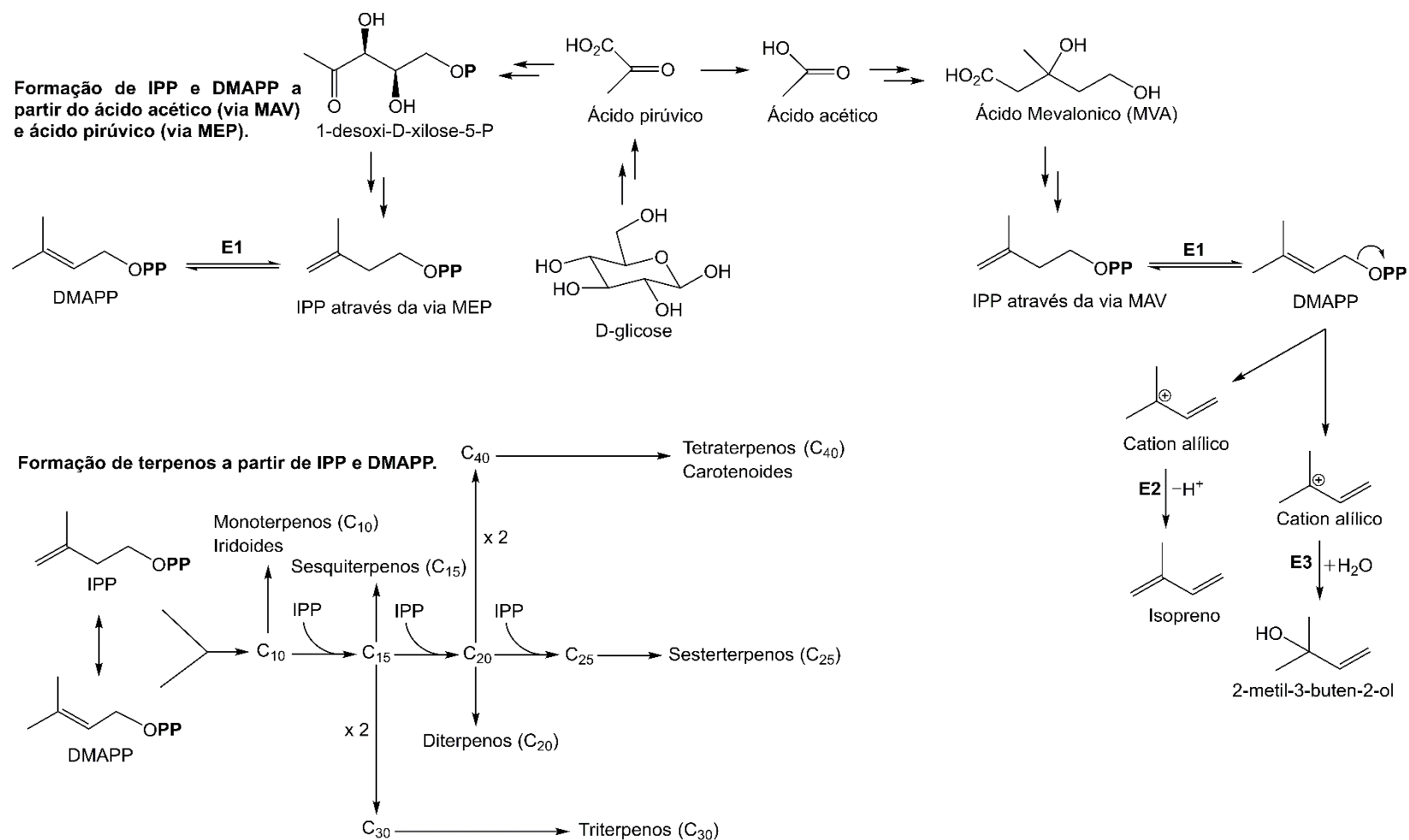
Os terpenoides são classificados com base no número de átomos de carbono em seus esqueletos: hemiterpenos (C-5), monoterpenos (C-10), sesquiterpenos (C-15), diterpenos (C-20), sesterterpenos (C-25), triterpenos (C-30) e tetraterpenos (C-40). Também existem polímeros maiores, como a borracha. Embora o próprio isopreno, um produto de decomposição de vários hidrocarbonetos cíclicos naturais, tenha sido sugerido como o bloco de construção fundamental, as unidades de isopreno bioquimicamente ativas foram posteriormente identificadas como os ésteres de dimetilalil difosfato (DMAPP) e isopentenil difosfato (IPP) (Dewick, 2009). A diversidade de estruturas de terpenoides reflete uma história marcada pelo estresse e outras pressões seletivas impostas por animais, levando a uma ampla gama de terpenoides funcionalizados nas plantas. Essa base evolutiva e bioquímica resultou em terpenoides com atividades biológicas potentes em relação aos animais (Salminen *et al.*, 2008).

Relativamente poucos terpenoides seguem exatamente o conceito simples de uma combinação linear de cabeça-cauda de unidades de isopreno. Por exemplo, o geraniol (C-10), farnesol (C-15) e geranilgeraniol (C-20) exibem essa configuração. Em contraste, esqualeno (C-30) e fitoeno (C-40) apresentam uma ligação cauda-cauda no centro de suas moléculas. A maioria dos terpenoides passa por modificações adicionais através de reações de ciclização, mantendo a disposição de

unidade cabeça-cauda, como visto em compostos como mentol, bisaboleno e taxadieno. No entanto, em muitas estruturas, como os esteroides, a disposição linear das unidades de isopreno torna-se difícil de reconhecer devido a reações de rearranjo e à perda de vários átomos de carbono. Apesar dessas complexidades, tais compostos originam-se de precursores terpenoides regulares (Dewick, 2009; Gershenzon; Croteau, 2018).

A unidade básica de isopreno é formada a partir de IPP ou DMAPP através de duas possíveis vias: ácido mevalônico (MVA) ou através da via do mevalonato independente (Metil-eritritol-fosfato ou MEP) (Figura 11). O ácido mevalônico é formado a partir de três moléculas de acetil-CoA seguido de reações de claisen, aldólica e reações de redução. Durante muitos anos, acreditou-se que as primeiras partes da via do mevalonato eram comuns a todos derivados terpenóides naturais em todos os organismos. No entanto, após investigação detalhada foi provado que existe uma via alternativa para formação do IPP e DMAPP, a via MEP, e que esta é provavelmente mais utilizada na natureza do que a via do mevalonato. Os intermediários da via glicolítica, ácido pirúvico e gliceraldeído-3-fosfato são utilizados na produção de IPP e DMAPP na via MEP. A via do MVA produz IPP e DMAPP em todos os organismos eucariotos e archaea, enquanto a via MEP é funcional em eubactérias, plastídios de algas, plantas superiores e protistas (Bergman; Davis; Phillips, 2019; Dewick, 2009; Liao *et al.*, 2014; Lombard; Moreira, 2010).

Figura 11. Biossíntese simplificada de terpenoides.



E1: IPP isomerase (isomerização alílica estereoespecífica)

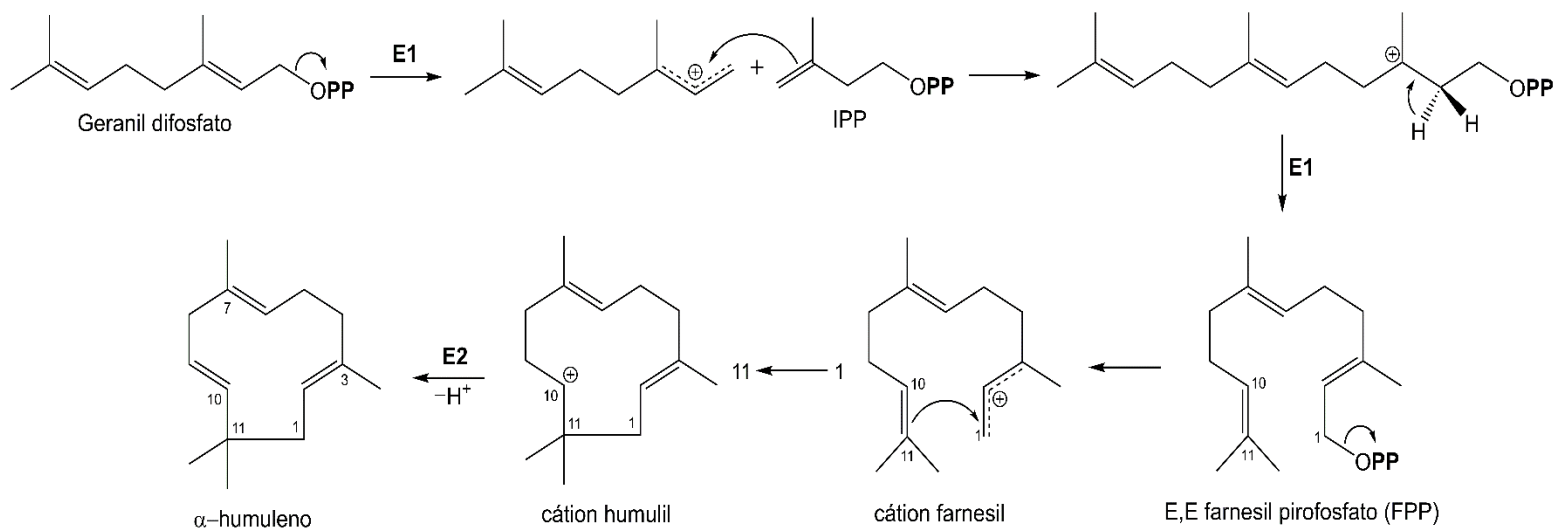
E2: isopreno sintase

E3: metilbutenol sintase

2.3.1 Sesquiterpenos humulenos

Os sesquiterpenos são formados por três unidades de C₅, a partir do geranyl difosfato (GPP) e uma unidade de isopreno. Embora seus mecanismos de formação sejam semelhantes aos dos monoterpenos, os sesquiterpenos são geralmente sintetizados pela via do MAV, e não pela via do MEP. A adição de uma unidade de IPP ao GPP, em uma extensão da reação da GPP sintase, resulta na formação do precursor fundamental dos sesquiterpenos, o farnesil difosfato (FPP). Este por sua vez, pode então originar sesquiterpenos lineares e cíclicos a partir do seu cátion farnesil. Devido ao aumento do comprimento da cadeia e à presença de uma ligação dupla adicional, o número de modos de ciclização possíveis aumenta, resultando em uma enorme variedade de estruturas mono, bi e tricíclicas (Dewick, 2009).

Os humulenos são formados após a produção do cátion farnesil e sua posterior ciclização anti-Markovnikov via C1-C11 formando o cátion humulil, que em seguida é estabilizado pela α -humuleno sintase com formação de uma dupla ligação entre C9-C10 para formar o α -humuleno (Figura 12). Esse composto macrocíclico de 11 membros é um dos sesquiterpenos mais comuns e amplamente distribuídos em plantas. Além disso, atua como precursor dessa classe, permitindo a formação de seus derivados através de reações de ciclização, migração de dupla ligação, hidrólise e oxidação (Chappell; Coates, 2010; Dewick, 2009).

Figura 12. Biossíntese α -humuleno.**E1:** farnesil difosfato sintase**E2:** α -humuleno sintase

Fonte: (Chappel; Coates, 2010; Dewick, 2009).

2.4 Inflamação e atividade antineuroinflamatória

A inflamação é um mecanismo de defesa do corpo humano que pode ser desencadeado por diversos fatores, incluindo trauma físico, exposição à alérgenos, estímulos químicos ou térmicos e infecções microbianas (Guo *et al.*, 2015). A inflamação ocorre por meio de uma interação complexa entre mediadores celulares, envolvendo quimiocinas, enzimas plasmáticas, lipídios e citocinas. Macroscopicamente, é caracterizada pela presença de rubor, edema, calor e dor na região inflamada, levando à interferência ou alteração da função (Ahmed, 2011). Embora seja uma resposta fisiológica e benéfica, a inflamação descontrolada e inadequada pode resultar na patogênese e progressão de vários distúrbios inflamatórios, como doenças cardiovasculares, respiratórias e neurológicas (Freitas *et al.*, 2019). A resposta inflamatória pode gerar três fases distintas: a fase aguda, caracterizada por vasodilatação local e aumento da permeabilidade vascular; a fase subcrônica, onde ocorre infiltração de leucócitos e células do sistema fagocitário mononuclear; e a fase crônica, caracterizada por presença de lesão permanente com substituição de células funcionais por células fibrosas e afuncionais (Freitas *et al.*, 2019).

A resposta inflamatória típica ocorre em quatro níveis principais, cada um correspondendo a um componente específico da via inflamatória: os indutores da inflamação, os sensores que detectam esses indutores, os mediadores inflamatórios gerados pelos sensores e os tecidos-alvo afetados pelos mediadores. Cada componente opera de maneiras diversas em diferentes vias inflamatórias, dependendo da natureza do evento que inicia o processo inflamatório (Medzhitov, 2010). Trata-se de um processo rigorosamente regulado, envolvendo estímulos pró-inflamatórios para iniciar e manter a inflamação, bem como sinais anti-inflamatórios que ajudam a encerrar o processo (Ziebell; Morganti-Kossmann, 2010). No entanto, diversos fatores internos ou externos podem perturbar essa rede regulatória de mediadores pró e anti-inflamatórios, resultando em várias condições inflamatórias, como doenças inflamatórias intestinais, artrite reumatoide, esclerose múltipla e asma crônica (Sahlmann; Ströbel, 2016). Dentre os principais mediadores pró-inflamatórios pode-se citar a PCR, IL-6, IL-1 β , TNF- α e indiretamente o óxido nítrico.

O óxido nítrico (NO) é um neurotransmissor com diversas atividades biológicas o qual é sintetizado a partir do aminoácido L-arginina e produzido pelos neurônios e células endoteliais no cérebro (Kuriyama; Ohkuma, 1995; Togo *et al.*, 2004). Três enzimas de NO facilitam a sua síntese em diferentes partes do corpo, sendo elas a sintase neuronal de NO (nNOS), sintase induzível de NO (iNOS) e sintase endotelial de NO (eNOS). De acordo com a localização dessas enzimas em células lisas, a síntese de NO ocorre em macrófagos, micróglia, astrócitos, neurônios e células endoteliais. O NO é importante para o fluxo sanguíneo cerebral e os metabolismos subjacentes (Toda; Ayajiki; Okamura, 2009) e também pode desempenhar um papel importante na memória e aprendizado, mediação da nocicepção, modulação de funções neuroendócrinas e atividade comportamental (Esplugues, 2002; Garthwaite, 2008).

Contudo, o excesso de NO pode causar vasodilatação dependente do endotélio, inibir a agregação plaquetária, induzir imunomodulação, inflamação e transmissão neuronal nos sistemas nervoso central e periférico. Portanto, as principais causas de distúrbios neurológicos geralmente observados após a alta produção de NO incluem a modulação imunológica mediada pelo NO, inflamação e neurotoxicidade (Forstermann; Sessa, 2012; Luiking *et al.*, 2010). Ele está envolvido em várias condições fisiopatológicas, incluindo danos oxidativos, neurodegeneração, complicações diabéticas, inibição plaquetária, relaxamento alterado dos músculos lisos e morte celular através da alteração das funções de diversas proteínas (Subedi *et al.*, 2021). Além disso, é um gasotransmissor que se difunde facilmente através das membranas e atua como vasodilatador, neuromodulador e mediador inflamatório (Balez; Ooi, 2016). Ainda, o NO exibe efeitos coordenados nas funções cerebrais, e há evidências substanciais sugerindo que a via do NO está associada a distúrbios neurodegenerativos como doença de Alzheimer (DA) e demência (Austin *et al.*, 2013; Montagne *et al.*, 2015; Togo *et al.*, 2004).

Vários endotoxinas e exotoxinas, como lipopolissacarídeos, TNF- α , IL-1 β , estrogênio, interferon gama, condições hipóxicas e o etanol, podem induzir a ativação da micróglia e macrófagos ou alterar a homeostase, resultando em produção excessiva de NO que causa danos ao sistema fisiológico, ao cérebro ou a outras partes do corpo. Nas últimas décadas, descobriu-se que o NO desempenha um papel em vários tipos de dores de cabeça, incluindo primárias e vasculares

(como enxaqueca) e o tratamento de distúrbios neuroinflamatórios com inibidores de NO pode proporcionar um alívio considerável (Ashina *et al.*, 2000; Olesen, 2008; Gupta *et al.*, 2011; Sharma *et al.*, 2007).

O NO é considerado envolvido na neuroinflamação porque produz radicais livres que afetam a integridade celular devido ao dano mitocondrial. Os mecanismos pelos quais ocorre o aumento da produção de NO ainda não estão totalmente esclarecidos, no entanto, o estresse oxidativo é uma das principais causas de alterações na função neuronal. Assim, controlar a produção excessiva de NO pode ser uma estratégia promissora para o tratamento de distúrbios neuroinflamatórios (Subedi *et al.*, 2021). Nessas condições, a inibição da produção de NO utilizando produtos naturais e seus derivados pode ajudar a desenvolver novas estratégias de tratamento. Portanto, é extremamente necessário realizar pesquisas adicionais sobre espécies de plantas com propriedades anti-inflamatórias para validar sua atividade terapêutica (Attig; Jalil; Husain, 2017; Gaire; Choi, 2021).

3. OBJETIVOS

3.1 Objetivo geral

Contribuir para o conhecimento fitoquímico e farmacológico da família Annonaceae através do estudo da espécie *Anaxagorea dolichocarpa* Sprague & Sandwith.

3.2 Objetivos específicos

- Preparar e particionar extratos das raízes de *Anaxagorea dolichocarpa* utilizando técnicas extrativas como maceração, percolação e partição líquido-líquido;
- Isolar, purificar, identificar e/ou elucidar as estruturas químicas dos constituintes químicos das raízes de *Anaxagorea dolichocarpa* por meio de métodos cromatográficos (CC, CLMP, CLAE, CLAE-EM), espectroscópicos (RMN, UV, IV) e espectrometria de massa (EM).
- Contribuir com o conhecimento quimiotaxonômico da família Annonaceae;
- Avaliar citotoxicidade e a atividade antineuroinflamatória das substâncias isoladas utilizando linhagem de células BV2.
- Realizar estudos *in silico* para avaliar a afinidade de interação dos compostos isolados com a enzima sintase induzível de NO (i-Nos) (PDB: 1NSI).

4. MATERIAIS E MÉTODOS

4.1 Materiais e equipamentos de isolamento e purificação

A análise, fracionamento, isolamento e a purificação dos constituintes químicos foram feitos por meio de técnicas de cromatografia em camada delgada analítica (CCDA), cromatografia em coluna (CC), cromatografia líquida de média pressão (CLMP) e cromatografia líquida de alta eficiência (CLAE). Para a realização da CCDA, utilizou-se placas de sílica gel 60 (F254 ART 7749) da Merck®, na espessura de 0,25 mm e como sistema eluente foram utilizados os solventes: hexano (Hex), clorofórmio (CHCl_3), acetato de etila (AcOEt) e metanol (MeOH), puros ou em misturas binárias em ordem crescente de polaridade. As revelações das substâncias nas placas de sílica gel foram executadas pela exposição das mesmas a uma lâmpada de irradiação ultravioleta (UV) sob dois comprimentos de onda (254 e 365 nm).

Para realização da CC foi utilizada como fase estacionária sílica gel SiliCycle®, com partículas de 60-200 μm , 70-230 mesh e como fase móvel os solventes hexano, clorofórmio, acetato de etila e metanol puros ou em misturas binárias em ordem crescente de polaridade. A CLMP foi realizada utilizando um cromatógrafo BUCHI® com um sistema de bomba e injetor de solventes e como fase estacionária sílica gel (SiliaFlash® - F60 SiliCycle®, 40-63 μm , 230-400 mesh) e como eluentes hexano, clorofórmio, acetato de etila e metanol puros ou em misturas binárias sob um fluxo de 20 mL/min.

A CLAE em escala analítica foi realizada utilizando um aparelho cromatográfico da marca Shimadzu Prominence®, equipado por uma coluna de fase reversa C-18 da marca Kromasil® modelo 100-5-C18 (250 mm x 4,6 mm e partículas de 10 μm) conectada a uma pré-coluna C18 (4 mm x 3,0 mm e partículas de 10 μm), controlador de sistema modelo CBM-20A, bomba LC-20AT, injetor automático SIL-20A_{HT} e detector de arranjo de diodos (DAD) SPD-M20A. Como fase móvel foi utilizada água Milli-Q e acetonitrila (ACN) grau HPLC. O fluxo empregado para as análises foi de 0,6 mL/min, o volume de injeção foi de 20 μL e as amostras foram preparadas na concentração de 1 mg/mL.

A CLAE em escala preparativa foi realizada utilizando um aparelho cromatográfico Shimadzu Prominence®, composto por controlador de sistema SLC-

10A vp, duas bombas LC-6AD, injetor manual e detector DAD SPD-M10A. Neste aparelho utilizou-se uma coluna de fase reversa C18 da marca YMC® modelo YMC-Actus Triart C18. Como fase móvel foi utilizada água Milli-Q e ACN grau HPLC. O fluxo empregado para as análises foi de 8,0 mL/min, o volume de injeção foi de 100 µl e as amostras foram preparadas na concentração de 100 mg/mL.

4.2 Equipamentos e procedimentos analíticos de caracterização estrutural

Os espectros de massa de alta resolução das substâncias foram obtidos por ionização por electrospray (IES) utilizando espectrômetros de massa de alta resolução da marca BRUKER®, modelo micro TOF II operando no modo positivo.

Os espectros de infravermelho foram adquiridos em um espectrômetro Shimadzu IRPrestige-21, bem como em um espectrofotômetro FTIR (Shimadzu IRSpirit-T) com um acessório QATR-S.

As rotações ópticas foram medidas em um polarímetro Jasco P-2000 a 20 °C em CHCl₃.

Os espectros experimentais de Dicroísmo Circular Eletrônico (ECD) foram registrados com um espectrômetro Jasco J-1100 (Jasco, Tóquio, Japão) na região de 200–400 nm, utilizando os seguintes parâmetros: largura de banda, 1 nm; tempo de resposta, 1 s; velocidade de varredura, 100 nm/min; número de acumulações, 3; temperatura ambiente (25 °C); amostra em solução de acetonitrila; comprimento do caminho óptico da célula, 0,1 cm; e concentração, 0,1 mg/ml.

Os espectros de RMN 1D e 2D foram obtidos em equipamentos da Bruker AVANCE III HD (400 e 100 MHz para ¹H e ¹³C, respectivamente) e Varian NMR (500 e 125 MHz para ¹H e ¹³C, respectivamente). Para dissolução das amostras foi utilizado o solvente clorofórmio deuterado (CDCl₃) referenciando o solvente residual de CHCl₃ em δ_H 7,26 e δ_C 77,0 como padrão interno. Os deslocamentos químicos (δ) foram expressos em partes por milhão (ppm) e as constantes de acoplamento *J* em Hz. Utilizaram-se as convenções s (simpleto), d (duplete), dd (duplo duplete), ddd (duplo duplo duplete), dq (duplo quarteto), td (triplo duplete), t (triplete) e m (multiplete) para indicar as multiplicidades dos sinais de ¹H.

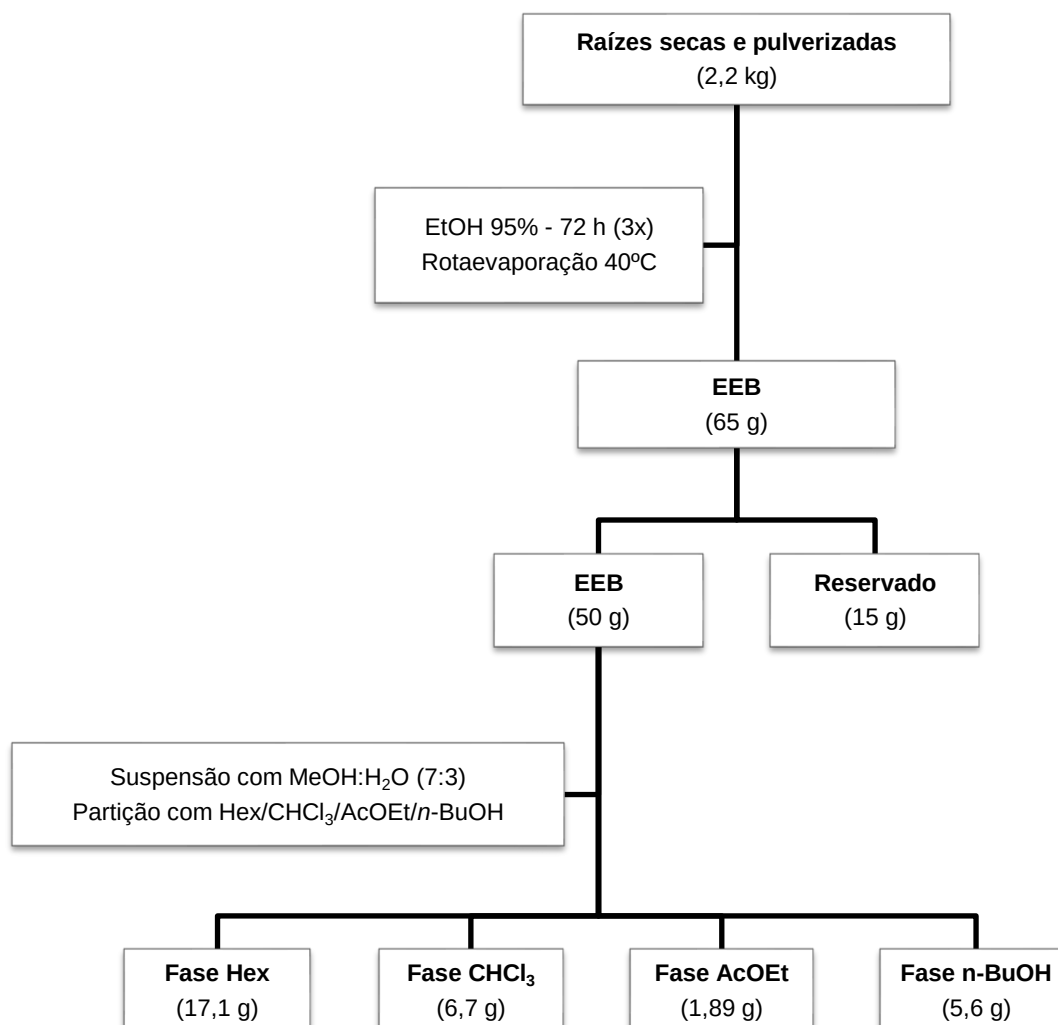
4.3 Material vegetal

As raízes de *anaxagorea dolichocarpa* foram coletadas no município de Cruz do Espírito Santo, estado da Paraíba (7°05'26.3"S, 35°04'32.6"W), em maio de 2021. A identificação botânica foi realizada pela Profa. Dra. Maria de Fátima Agra. Uma exsicata desta espécie encontra-se depositada no Herbário Prof. Lauro Pires Xavier do Centro de Ciências Exatas e da Natureza (CCEN / UFPB) sob o código (AGRA & GÓES 5543) e o estudo está registrado no Sistema Nacional de Gestão do Patrimônio Genético e Conhecimento Tradicional Associado (SISGEN) sob o número AE4B71A.

4.4 Obtenção e fracionamento do extrato etanólico bruto (EEB)

As raízes foram pesadas e desidratadas em estufa com ar circulante à temperatura média de 40°C durante 72 horas, em seguida foram submetidas a um processo de pulverização em moinho mecânico, obtendo-se 2,2 kg do pó da planta. O material vegetal seco e pulverizado foi submetido à maceração com etanol (EtOH) a 95% em um recipiente de aço inoxidável, durante 72 horas. Este processo foi repetido por três vezes, obtendo-se uma solução extrativa. A solução extrativa foi filtrada e em seguida concentrada em rotaevaporador sob pressão reduzida a uma temperatura média de 40°C, para obter 65 g do EEB (Esquema 1).

Uma parte do EEB (50 g) foi adicionada em uma solução de MeOH:H₂O (500 ml, 7:3 v/v) obtendo-se uma suspensão hidroalcóolica que foi submetida a uma cromatografia líquido-líquido em uma ampola de separação utilizando os solventes hexano, CHCl₃, AcOEt e *n*-butanol (*n*-BuOH) em ordem crescente de polaridade, obtendo-se as soluções extrativas referentes a cada solvente que foram tratadas com sulfato de sódio anidro para retirar o excesso de água e em seguida filtradas. Após isso, estas soluções foram concentradas em rotaevaporador sob pressão reduzida a uma temperatura média de 40°C, fornecendo 17,1 g da fase Hex; 6,7 g da fase CHCl₃; 1,89 g da fase AcOEt e 5,6 g da fase *n*-BuOH (Esquema 1).

Esquema 1. Obtenção e fracionamento do EEB das raízes de *Anaxagorea dolichocarpa*.

4.5 Isolamento e purificação dos constituintes químicos

4.5.1 Fracionamento da fase CHCl₃

Uma alíquota de 6,0 g da fase CHCl₃ foi submetida a uma CLMP utilizando cromatógrafo da BUCHI® e como fase estacionária sílica gel (SiliaFlash®- F60, 40-63 µm) em uma coluna de vidro cilíndrica de tamanho compatível com a quantidade de amostra utilizada. Como eluentes foram utilizados os solventes hexano, CHCl₃, AcOEt e MeOH puros ou em mistura binária. O fluxo da fase móvel foi de 20 mL/min, sendo coletadas 41 frações de 50 mL cada, denominadas ARC seguida da numeração que indica a ordem de coleta (01-41), as quais foram analisadas pelo

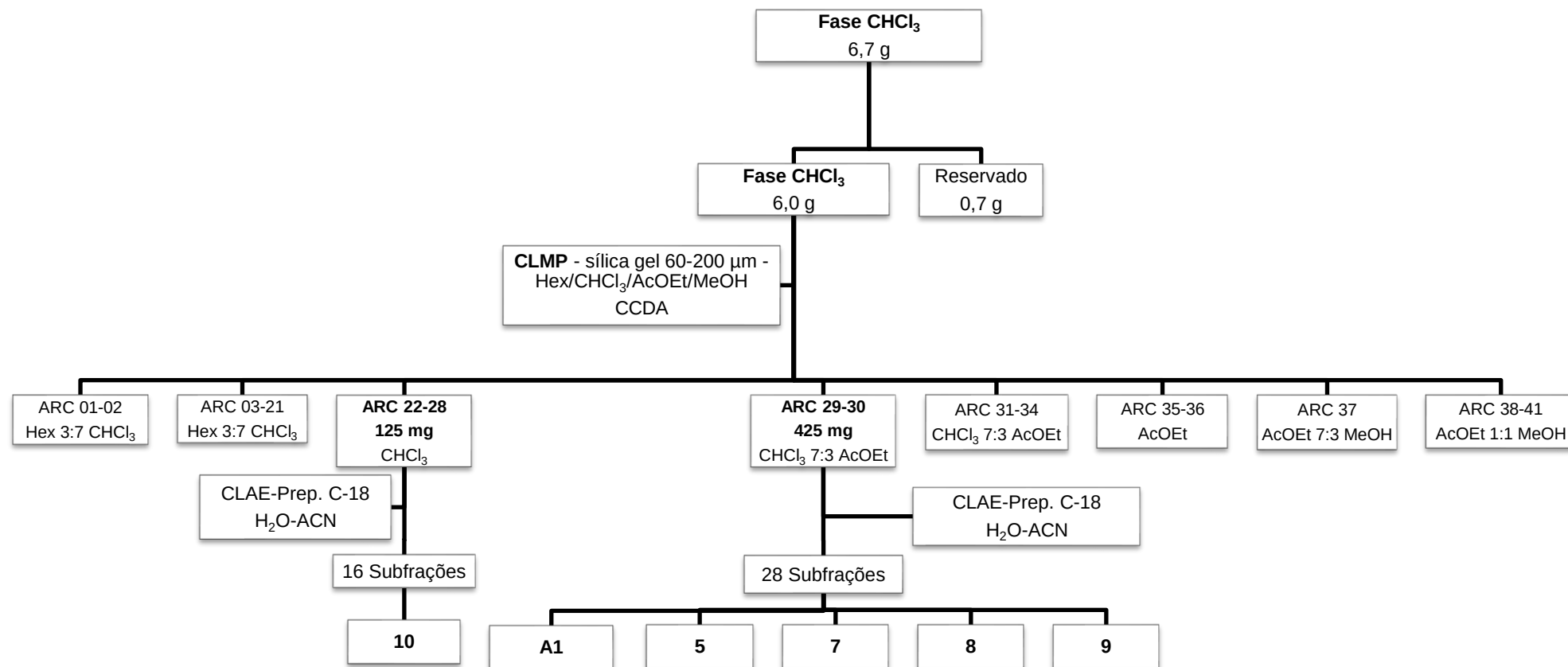
cromatograma do equipamento e por CCDA e agrupadas por similaridade em 08 frações (Tabela 1 e Esquema 2).

A fração **ARC 29-30** (425 mg) após ser analisada por RMN de ^1H foi possível observar sinais característicos de sesquiterpenos, portanto, foi submetida a uma CLAE em escala analítica que em seguida foi transposta para escala preparativa visando o isolamento desses compostos. O método de análise consistiu em um sistema gradiente dos eluentes água milli-Q e ACN, com leitura em 220 e 254 nm. O método gradiente teve um tempo total de 90 min, onde iniciou com 10% até 45% de ACN em 80 min, continuando a corrida com gradiente até 100% de ACN em 90 min. Essa análise permitiu a separação de 28 subfrações, das quais, a subfração **10**, pesando 49 mg, **19** (8,0 mg), **27** (5,0 mg), **23** (6,0 mg) e **26** (6,2 mg) foram codificadas como **composto A1**, **composto 5**, **composto 7**, **composto 8** e **composto 9**, e foram submetidas para análise por RMN (Esquema 2).

A fração **ARC 22-28** (125 mg) após ser analisada por RMN de ^1H também foi possível observar sinais característicos de sesquiterpenos, sendo submetida a uma CLAE em escala analítica que em seguida foi transposta para escala preparativa visando o isolamento desses compostos. O método de análise consistiu em um sistema gradiente dos eluentes água milli-Q e ACN, com leitura em 220 e 248 nm. O método gradiente teve um tempo total de 85 min, onde iniciou com 15% até 60% de ACN em 75 min, continuando a corrida com gradiente até 100% de ACN em 85 min. Essa análise permitiu a separação de 16 subfrações, das quais, a subfração **9**, pesando 12 mg foi codificada como **composto 10** e submetida a uma análise por RMN a fim de elucidar sua estrutura (Esquema 2).

Tabela 1. Dados do fracionamento da fase CHCl_3 em CLMP.

Sistema eluente	Frações
Hex 3:7 CHCl_3	01-02
CHCl_3 7:3 Hex	03-21
CHCl_3	22-28
CHCl_3 7:3 AcOEt	29-30
AcOEt 7:3 CHCl_3	31-34
AcOEt	35-36
AcOEt 7:3 MeOH	37
AcOEt 1:1 MeOH	38-41

Esquema 2. Fracionamento da fase CHCl_3 de *Anaxagorea dolichocarpa*.

4.5.2 Fracionamento da fase hexânica

Uma alíquota de 8,0 g da fase hexânica foi submetida a uma CC com sílica gel e como eluentes foram utilizados os solventes hexano, CHCl_3 , AcOEt e MeOH puros ou em mistura binária. Desta corrida foram coletadas 24 frações de 100 mL cada, denominadas ARH seguida da numeração que indica a ordem de coleta (01-24), as quais foram analisadas por CCDA e agrupadas por similaridade em 06 frações (Tabela 2 e Esquema 3).

Tabela 2. Dados do fracionamento da fase hexânica em CC.

Sistema eluente	Frações reunidas
Hex 9:1 CHCl_3	01-04
Hex 8:2 CHCl_3	05-06
Hex 7:3 CHCl_3	07-12
CHCl_3 8:2 AcOEt	13-18
AcOEt 1:1 MeOH	19-22
AcOEt 3:7 MeOH	23-24

A fração **ARH 07-12** (2,8 g) foi submetida a uma CLMP utilizando cromatógrafo da BUCHI® e como fase estacionária sílica gel (SiliaFlash®- F60, 40-63 μm) em uma coluna de vidro cilíndrica de tamanho compatível com a quantidade de amostra utilizada. Como eluentes foram utilizados os solventes hexano, CHCl_3 , AcOEt e MeOH puros ou em mistura binária. O fluxo da fase móvel foi de 20 mL/min, sendo coletadas 29 frações de 50 mL cada, denominadas ARH 07 seguida da numeração que indica a ordem de coleta (01-29). Essas foram analisadas pelo cromatograma do equipamento e por CCDA, sendo agrupadas por similaridade em 12 frações (Tabela 3 e Esquema 3).

Tabela 3. Dados do fracionamento da fração ARH 07-12 em CLMP.

Sistema eluente	Frações reunidas
Hex 9:1 CHCl_3	01
Hex 8:2 CHCl_3	02-04
Hex 9:2 CHCl_3	05-07
Hex 7:3 CHCl_3	08-09
Hex 6:4 CHCl_3	10-13
Hex 1:1 CHCl_3	14-21
CHCl_3 8:2 Hex	22-23

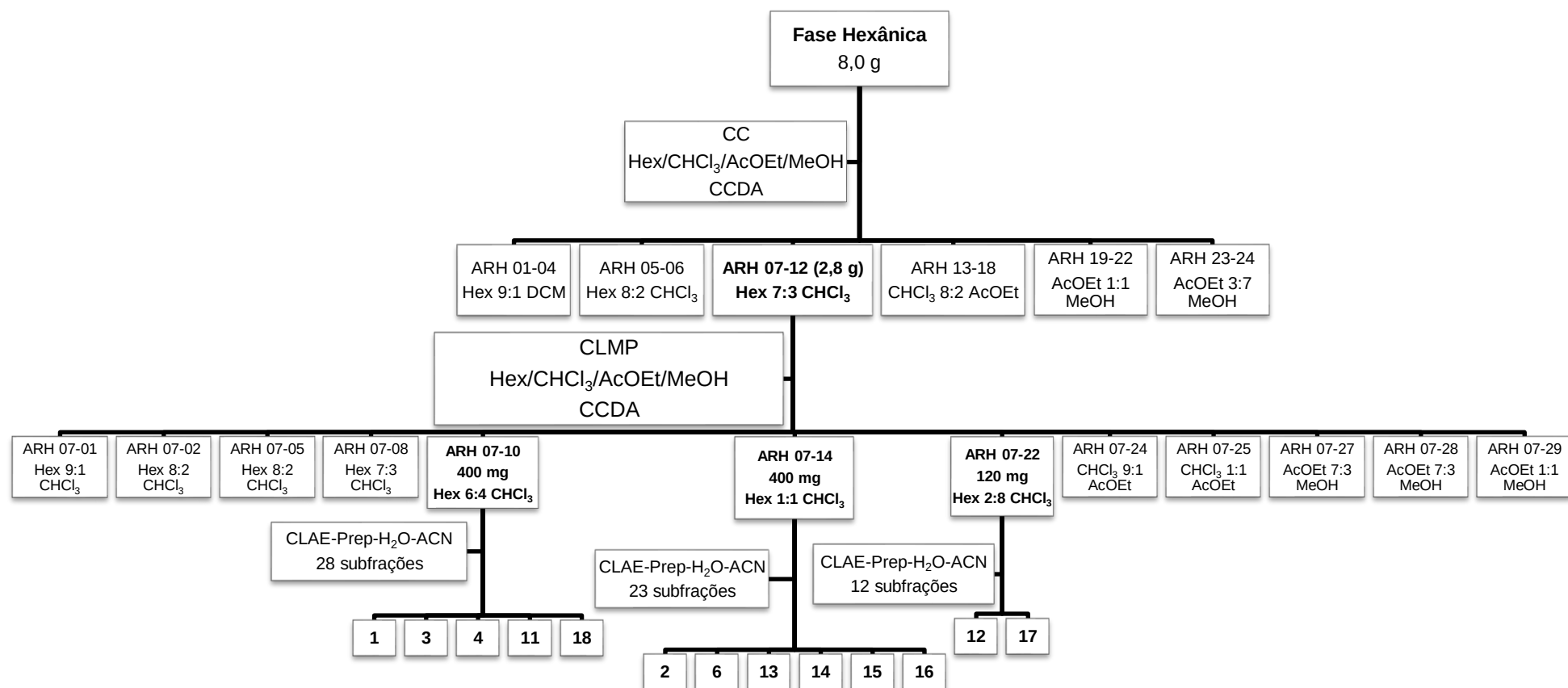
CHCl ₃ 9:1 AcOEt	24
CHCl ₃ 1:1 AcOEt	25-26
AcOEt 7:3 MeOH	27
AcOEt 7:3 MeOH	28
AcOEt 1:1 MeOH	29

A fração **ARH 07-10** (400 mg), após análise por RMN de ¹H, exibiu sinais característicos de sesquiterpenos. Dessa forma, foi submetida a uma CLAE em escala analítica, posteriormente transposta para escala preparativa, visando o isolamento desses compostos. O método de análise utilizou um sistema gradiente com os eluentes água milli-Q e ACN, com detecção em 220 e 254 nm. O método gradiente teve um tempo total de 75 minutos, iniciando com 25% até 70% de ACN em 60 minutos e prosseguindo com gradiente até 100% de ACN em 75 minutos. Essa corrida resultou na separação de 28 subfrações. As subfrações **23** (3,5 mg), **28** (18 mg), **19** (5,0 mg), **9** (4,0 mg) e **10** (5,1 mg) foram codificadas, respectivamente, como **Composto 1**, **Composto 3**, **Composto 4**, **Composto 11** e **Composto 18**, e submetidas à análise por RMN (Esquema 3).

A fração **ARH 07-14** (400 mg) foi analisada por RMN de ¹H, revelando sinais característicos de sesquiterpenos. Em seguida, a fração foi submetida a uma CLAE em escala analítica e, posteriormente, transposta para escala preparativa com o objetivo de isolar esses compostos. O método de análise utilizou um sistema gradiente de eluentes água milli-Q e ACN, com leituras a 220 e 254 nm. O método teve uma duração total de 100 minutos, começando com 10% de ACN e aumentando gradualmente até 54% em 90 minutos, seguido por um aumento até 100% de ACN nos 10 minutos finais. Essa análise resultou na separação de 23 subfrações. As subfrações **13** (4,5 mg), **7** (10,2 mg), **4** (4,0 mg), **5** (6,5 mg), **1** (4,0 mg) e **2** (4,2 mg) foram codificadas, respectivamente, como **Composto 2**, **Composto 6**, **Composto 13**, **Composto 14**, **Composto 15** e **Composto 16**, e submetidas à análise por RMN (Esquema 3).

A fração **ARH 07-24** (120 mg), após ser analisada por RMN de ¹H, apresentou sinais indicativos de sesquiterpenos. Assim, foi submetida a uma CLAE em escala analítica, seguida de escala preparativa para isolar esses compostos. A análise utilizou um sistema gradiente com eluentes água milli-Q e ACN, com detecção em 220 e 254 nm. O método durou 70 minutos, iniciando com 20% de ACN,

aumentando para 55% em 55 minutos, e atingindo 100% de ACN nos últimos 15 minutos. Essa análise resultou na separação de 12 subfrações. A subfração **4** (3,9 mg) e a subfração **5** (1,4 mg) foram denominadas como **Composto 12** e **Composto 17**, respectivamente, e submetidas à análise por RMN (Esquema 3).

Esquema 3. Fracionamento da fase hexânica de *Anaxagorea dolichocarpa*.

4.6 Cálculos teóricos de RMN e ECD.

Foram realizadas buscas conformacionais aleatórias para todos os possíveis estereoisômeros dos compostos **1–18** utilizando o algoritmo de Monte Carlo com um campo de força MMFF no software SPARTAN '14. Todos os confôrmeros dentro de uma janela de energia livre relativa de 10 kcal/mol foram selecionados para cálculos de otimização de geometria na fase gasosa, empregando o nível de teoria B3LYP/6-31 (d). Cálculos de frequência vibracional foram realizados no mesmo nível de teoria para confirmar que os pontos estacionários correspondem a mínimos na superfície de energia potencial. Para determinar a configuração relativa dos compostos (Albuquerque; Martorano; Santos-JR, 2024; Costa *et al.*, 2021), confôrmeros dentro de uma janela de energia relativa de 3 kcal/mol foram posteriormente selecionados para cálculos de constantes de blindagem magnética nuclear de ^{13}C e ^1H (σ) utilizando o nível de teoria GIAO-B3LYP/6-31G (d), juntamente com o modelo contínuo polarizável com o formalismo da equação integral (IEF-PCM) para simular a solvatação por acetonitrila (Miertus *et al.*, 1981).

As constantes de blindagem média da população foram obtidas assumindo-se a estatística de Boltzmann a uma temperatura de 298 K. O método DP4+, que se baseia na análise bayesiana, foi utilizado para estabelecer uma correlação estatística entre os deslocamentos químicos de ^{13}C e ^1H calculados e experimentais. Cada estrutura foi classificada com base da probabilidade, e probabilidades superiores a 90% indicaram um alto nível de confiança em qual candidato apresentou a melhor concordância com os dados experimentais (Grimblat; Zanardi; Sarotti, 2015). Sempre que a metodologia DP4+ apresentou uma probabilidade inferior a 90%, as constantes de blindagem de ^{13}C e ^1H foram recalculadas em um nível de teoria superior: GIAO-B3LYP/6-311+G (d,p). Uma vez estabelecidas as configurações relativas, as simulações de ECD foram conduzidas para determinar as configurações absolutas. Para os cálculos de ECD, um nível de teoria TD-DFT foi aplicado em CAM-B3LYP/TZVP para calcular a energia de excitação (em nm) e a força rotatória R na forma de velocidade do dipolo para as primeiras 30 transições eletrônicas singleto $\rightarrow$ singleto. O IEF-PCM foi aplicado para simular implicitamente a acetonitrila como solvente. Os espectros finais de ECD foram gerados com base nas estatísticas de Boltzmann dos confôrmeros selecionados e plotados utilizando o

software Origin 8. Todos os cálculos quânticos foram realizados utilizando o pacote de software Gaussian 16 (Frisch *et al.*, 2016)

4.7 Estudos *in silico*

4.7.1 Docking molecular

A difração de raios-X da enzima humana induzível óxido nítrico sintase (iNOS) (PDB: 1NSI) com resolução de 2,55 Å foi obtida da biblioteca Protein Data Bank (PDB) (<https://www.rcsb.org/structure/1NSI>) (Acessado em: 17 de janeiro de 2024). Inicialmente, todas as moléculas de água e cofatores foram removidas do espaço de trabalho do software. As configurações padrão foram aplicadas para realizar a simulação: função de pontuação: MolDock Score; avaliação do ligante: ES interna, ligação de hidrogênio interna, torções Sp2-Sp2; número de execuções: 30; algoritmo: MolDock SE; número de poses: 5. A simulação foi realizada com um raio de grade (GRID) de 15 Å e uma resolução de 0,30 Å. As interações entre os ligantes e os resíduos de aminoácidos da iNOS foram realizadas utilizando o BIOVIA Discovery Studio, 2021.

Os compostos em estudo foram desenhados no software Marvin Sketch v.23.10, ChemAxon (<https://chemaxon.com/marvin>) e, subsequentemente, a padronização das estruturas químicas em 3D e a minimização da energia dos compostos foram realizadas usando métodos de mecânica molecular e o método semi-empírico Austin Model 1 (AM1) utilizando o software Spartan 14, WaveFunction. O composto 1400-W foi utilizado como controle para comparação dos valores de energia de ligação, e o sítio ativo foi identificado visualizando os resíduos correspondentes ao sítio ativo nos artigos referentes a cada uma das proteínas no Protein Data Bank (PDB), assim como por meio de previsões na plataforma web de acesso livre para predição de sítio alvo (Bite Net - Skolteck I Molecule, 2022). (Garvey *et al.*, 1997; Jafarian *et al.*, 2005; Rajiv *et al.*, 2023).

4.7.2 Dinâmica molecular

As simulações de Dinâmica Molecular (MD) foram realizadas com o software GROMACS 5.0 (Abraham *et al.*, 2015; Berendsen *et al.*, 1995) para estimar a flexibilidade das interações entre proteínas e ligantes. A topologia dos ligantes foi

preparada com o Automated Topology Builder (ATB) (Malde *et al.*, 2011) utilizando o campo de força GROMOS43a1. As simulações utilizaram o modelo de água SPC em caixa cúbica (Bondi, 1964), com neutralização por íons (Cl^- e Na^+) e minimização para remoção de maus contatos. O sistema foi equilibrado a 300K, utilizando o algoritmo V-rescale de 100 ps, representado por NVT (número constante de partículas, volume e temperatura), seguido de uma equilibração à pressão de 1 atm, utilizando o algoritmo de Parrinello-Rahman como NPT (constante de partícula, pressão e temperatura) até 100 ps. As simulações duraram 100 ns, com 50 milhões de passos. A estabilidade e flexibilidade do complexo foram avaliadas pelos valores RMSD e RMSF, e os gráficos correspondentes foram gerados no software Grace (Pettersen *et al.*, 2020; Nachbagauer *et al.*, 2020).

4.8 Avaliação da citotoxicidade

A citotoxicidade dos compostos **1–18** foi avaliada por meio do ensaio de MTT (brometo de 4,5-dimetil-tiazol-2-il-2,5-difeniltetrazólio) (MOSMANN, 1983). A linhagem de células microgлияis BV2 foi obtida do Banco de Células do Rio de Janeiro (BCRJ), Brasil, e cultivada em meio Roswell Park Memorial Institute (RPMI; Sigma Aldrich, St. Louis, MO, EUA), suplementado com 10% de soro fetal bovino (SFB; GIBCO, Grand Island, NY, EUA) e 1% de penicilina-estreptomicina (Sigma Aldrich, St. Louis, MO, EUA) a 37 °C, 5% de CO_2 .

As células (1×10^5 células/mL) foram plaquedadas em placas de 96 poços e incubadas durante a noite. Em seguida, os compostos **1–18** foram adicionados em quatro concentrações (25, 50, 100 ou 200 μM) em cinco réplicas, e as placas foram incubadas por 24 h. Após o tratamento, o sobrenadante (110 μL) foi removido e 10 μL de solução de MTT foi adicionada (5 mg/mL) (Sigma Aldrich). As placas foram incubadas por mais quatro horas, e dodecil sulfato de sódio (100 μL /poço) foi adicionado para dissolver o formazan. As densidades ópticas foram medidas em um espectrofotômetro (leitor de microplacas BioTek Instruments, Sinergy HT, Winooski, VT, EUA) a um comprimento de onda de 570 nm.

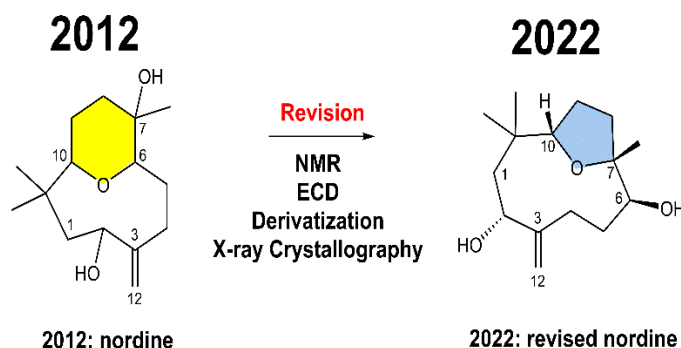
4.9 Avaliação da atividade antineuroinflamatória

Para a determinação de NO, as células BV2 (1×10^6 células/mL) foram semeadas em placas de cultura de 96 poços em meio RPMI suplementado com 10% de SFB e 1% de penicilina-estreptomicina em uma incubadora com 5% de CO₂ a 37 °C. Após um período de quatro horas, as células foram estimuladas com LPS (500 ng/mL, Sigma Aldrich) e IFN- γ (5 ng/mL, Thermofisher) na presença dos compostos **1–18**. No primeiro conjunto de experimentos, todos os compostos foram testados em uma concentração de 100 μ M em cinco réplicas. Posteriormente, os compostos **1**, **5**, **8**, **9** e **14** foram testados em concentrações variando de 25 a 200 μ M e a quercetina (20 μ M) foi usada como controle positivo. Adicionalmente, foram incluídos um grupo de repouso (não estimulado) e um grupo controle estimulado com LPS/IFN- γ . Após 24 horas, os sobrenadantes livres de células foram coletados para quantificação de NO usando o método de Griess (Griess, 1879).

Os dados foram analisados estatisticamente utilizando o GraphPad Prism 8.0.2 (GraphPad Software Inc., San Diego, CA, EUA) e os valores foram apresentados como média $\pm$ erro padrão da média (SEM). $n = 5$. As comparações entre os grupos foram realizadas usando a análise de variância de uma via (ANOVA), seguida pelo pós-teste de Tukey ($p < 0,05$).

5. RESULTADOS

5.1 Artigo 1: Structure Revision of the Sesquiterpene Nordine based on NMR-spectroscopic Analysis and X-ray crystallography



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ABSTRACT: Nordine was reported to be an unusual humulene-type macrocyclic sesquiterpenoid that contains an ether-bridged bicyclic ring between C-10 and C-6 with a hydroxy group at position 2. Here, we report the structure revision of nordine based on incongruities found for carbon chemical shifts in the originally proposed structure, in addition to formation of a diacetylated derivative. As expected, a single-crystal X-ray diffraction analysis unambiguously confirmed our proposal that the nordine (**A1**) structure contains an ether-bridged bicyclic ring between C-10 and C-7 and hydroxy groups at C-2 and C-6. Furthermore, the absolute configuration was determined by ECD spectroscopic analysis.

INTRODUCTION

Sesquiterpenoids play an important role in biological systems and are widely distributed in plants and microorganisms.¹ In addition, they have complex structures and exhibit extraordinary biological activities, such as anti-inflammatory, antitumor, antiparasitic, and antiviral activities.² Among sesquiterpenoids, macrocyclic humulene-type compounds possess wide structural diversity and are found in bicyclic and tricyclic models with different functional groups and substituents in regio- and stereospecific manners.³⁻⁷

The biological effects of these molecules are influenced by their three-dimensional structures and by the presence of different functional groups. Therefore, determining the correct structure is crucial, and elucidating the structure of these compounds is a major challenge in the field of medicinal chemistry. Thus, different approaches, including computational chemistry, structural derivatizations, NMR spectroscopic methods, X-ray crystallography techniques, and biosynthetic studies, have been used to reveal and resolve structural mistakes.⁸

In 2012,⁹ Husain and co-workers reported a new macrocyclic humulene-type sesquiterpenoid called nordine, which was obtained from the roots of *Anaxagorea javanica* Blume. Nordine was proposed to contain an ether-bridged bicyclic ring between C-10 and C-6 and bears a hydroxy group at C-2. Its structure determination was based on the analysis of 1D and 2D NMR, IR and HREIMS data.

In our continuing study of humulene-type sesquiterpenes from *Anaxagorea dolichocarpa* Sprague & Sandwith,¹⁰ we isolated nordine and realized that the values of chemical shifts for the carbons C-6 and C-7 may indicate another ether fusion. Thus, we suggest that the structure of nordine involves an ether cycle between carbons C-10 and C-7, which is also compatible with other reported compounds.¹⁰⁻¹² Therefore, with NMR data analysis and support by literature data, we propose the structure revision of nordine (Figure 1) by obtaining the diacetylated derivative and by X-ray crystallography.

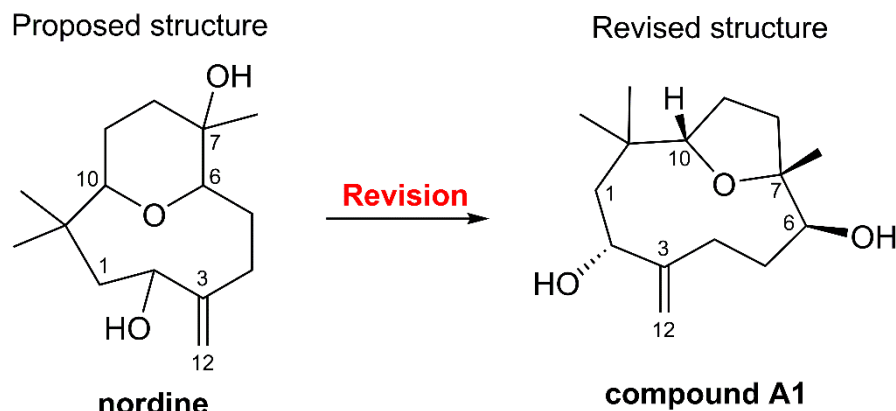


Figure 1. Proposed and revised structures of nordine.

RESULTS AND DISCUSSION

The air-dried and powdered roots of *Anaxagorea dolichocarpa* were extracted with EtOH at room temperature. The extract was partitioned with hexane, CHCl₃, and EtOAc. The CHCl₃ extract was subjected to MPLC, and eight fractions were obtained. Compound **A1** was obtained from fraction **4** through HPLC, and its structure was determined by 1D and 2D NMR, HRESIMS, electronic circular dichroism (ECD), chemical derivatization, and X-ray crystallography. Compound **A1** was isolated as colorless crystals with a positive optical rotation of $[\alpha]^{20}_D +20$ (c. 0.1, CHCl₃). The molecular formula was determined as C₁₅H₂₆O₃ by HRESIMS, corresponding to four indices of hydrogen deficiency.

The 1D and 2D NMR data for **A1** (Table 1) were identical to those described for nordine.⁹ However, according to the literature,¹⁰⁻¹² the chemical shifts at δ_C 87.8, 84.4 and 73.7, which were attributed to C-10, C-7, and C-6 are consistent with the ether fusion between C-10 and C-7 and not C-10 and C-6, as determined by Husain et al. In the HMBC spectrum, the signal at δ_H 4.42 (H-2) showed a correlation with the carbons at δ_C 46.1, 157.0, 35.8 and 112.6, which were assigned to C-1, C-3, C-11 and C-12, respectively. The signals at δ_H 5.29 and 5.00 (H₂-12) correlated with the carbons at δ_C 69.4 and 32.4, which were assigned to C-2 and C-4, respectively, confirming the insertion of a hydroxy group at C-2. Moreover, correlations of the signals at δ_H 0.77 (H₃-14) and 1.20 (H₃-15) were observed with the carbons at δ_C 46.1 (C-1), 35.8 (C-11) and δ_C 87.8 which was assigned to C-10, confirming oxygenation at this position. Additionally, the HSQC spectrum exhibited a correlation between the oxymethine proton at δ_H 3.64 (H-10) and carbon C-10 (δ_C 87.8). Lastly,

the positions of C-6 and C-7 were established by the HMBC correlations between δ_{H} 1.18 (H₃-13) and the signals at δ_{C} 73.7 and 84.4.

Table 1. ^1H and ^{13}C NMR Data of A1 and Nordine in CDCl_3 .

no.	A1 ^a		nordine ^b		HMBC
	δ_{C} , type	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	J_2/J_3
1	46.1, CH ₂	2.13, dd (15.0, 5.5) 1.51, dd (15.0, 3.5)	46.6	2.13, dd (15.4, 5.5) 1.51, dd (15.4, 3.3)	11, 2, 15, 10, 3, 14
2	69.4, CH	4.42, dd (5.5, 3.5)	69.5	4.42, dd (5.5, 3.3)	1, 3, 4, 11, 12
3	157.0, C	-	157.1	-	-
4	32.4, CH ₂	2.52, ddd (14.5, 10.5, 4.5) 2.40, ddd (14.5, 10.5, 4.5)	32.4	2.52, ddd (14.5, 5.5, 1.2) 2.40, ddd (15.4, 10.2, 4.6)	5, 3, 2, 6, 12
5	30.1, CH ₂	2.22, dddd (14.5, 10.5, 5.5, 4.5) 1.55, m	30.2	2.22, dddd (14.5, 10.2, 5.5, 4.6) 1.55, m	4, 6, 7, 3
6	73.7, CH	3.34, dd (5.5, 4.5)	73.7	3.34, dd (5.5, 4.6)	5, 7, 13, 4, 8
7	84.4, C	-	84.4	-	-
8	35.5, CH ₂	2.08, m 1.55, m	35.5	2.08, m 1.55, m	9, 7, 6, 10
9	26.6, CH ₂	1.66, m 1.59, m	26.6	1.68, m 1.60, m	8, 10, 11, 7
10	87.8, CH	3.64, dd (10.5, 5.5)	87.8	3.64, dd (9.3, 5.5)	9, 15, 1
11	35.8, C	-	35.8	-	-
12	112.6, CH ₂	5.29, s 5.00, s	112.6	5.30, s 5.01, s	3, 4, 2
13	20.5, CH ₃	1.18, s	20.5	1.18, s	7, 8, 6
14	28.5, CH ₃	0.77, s	28.5	0.78, s	11, 15, 10, 1
15	25.8, CH ₃	1.20, s	25.8	1.20, s	11, 14, 10, 1

^{a,b}Recorded at 500 MHz for ^1H and 125 MHz for ^{13}C .

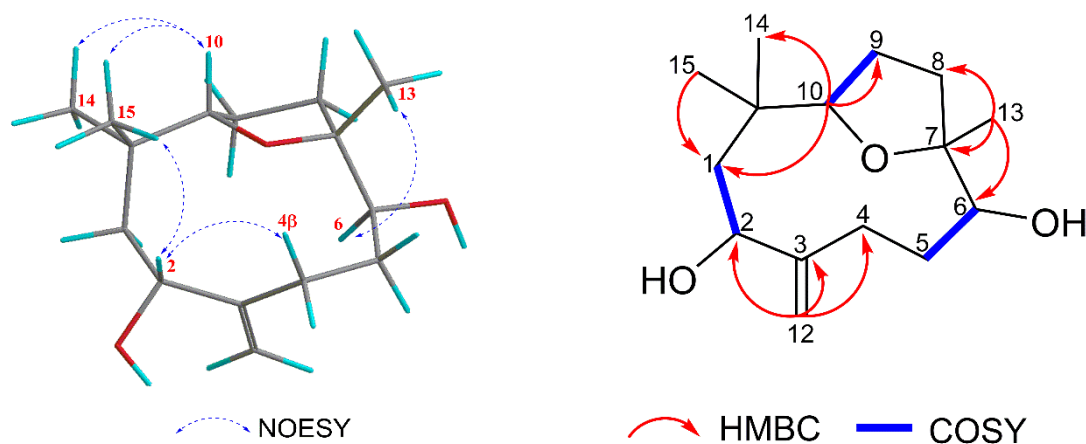


Figure 2. Key 2D NMR data of compound A1.

To confirm the ether bridge between C-10 and C-7, **A1** was acetylated. If our proposed structure was correct, two acetate esters should have formed. The result revealed the presence of two acetate esters, confirming the presence of two secondary hydroxy groups and showing that the original nordine structure was incorrect. In the HMBC spectrum of the acetylated derivative, the signals at δ_{H} 5.45 and 4.55 showed a correlation with the carbons at δ_{C} 33.5 (C-4), δ_{C} 35.9 (C-11), δ_{C} 152.6 (C-3), δ_{C} 115.3 (C-12), and δ_{C} 170.8 and with δ_{C} 83.3 (C-7), δ_{C} 21.3 (CH₃-13), and δ_{C} 170.9, respectively, confirming the acetylation of the hydroxy groups at positions 2 and 6. Furthermore, the monocrystal X-ray diffraction analysis of **A1** (Figure 3) was performed to definitively prove its exact structure. Thus, the molecular structure was confirmed, and the result showed a five-membered ring cyclized between C-10 and C-7 and hydroxyl groups at C-6 and C-2. In addition, the NMR data obtained from other sesquiterpenoids isolated from *A. dolichocarpa*¹⁰ and *Xanthium spinosum*¹¹ corroborated our proposal.

The absolute configuration was established as (2*R*,6*S*,7*R*,10*S*) by comparing a positive Cotton effect at λ 209 from the experimental ECD spectrum with the calculated spectra (Figure 4). Thus, based on detailed and careful analysis of spectroscopic data, chemical derivatization and X-ray crystallography, the structure of nordine was revised to (2*R*,6*S*,7*R*,10*S*)-7,11,11-trimethyl-3-methylene-12-oxabicyclo-[7.2.1]-dodecane-2,6-diol (**A1**).

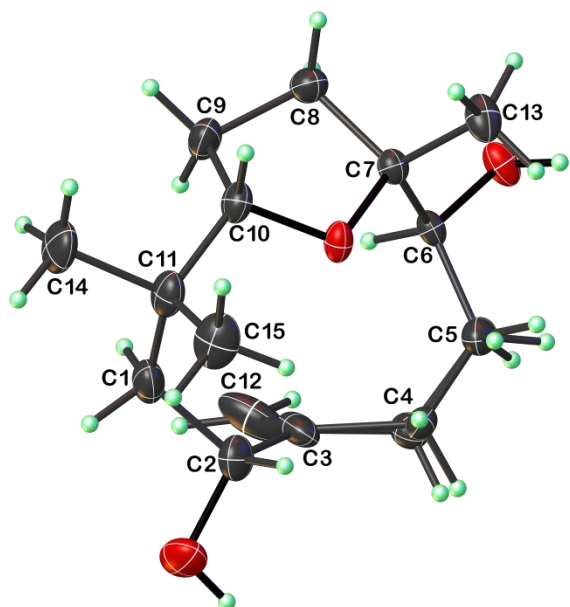


Figure 3. X-ray ORTEP drawing of compound **A1**

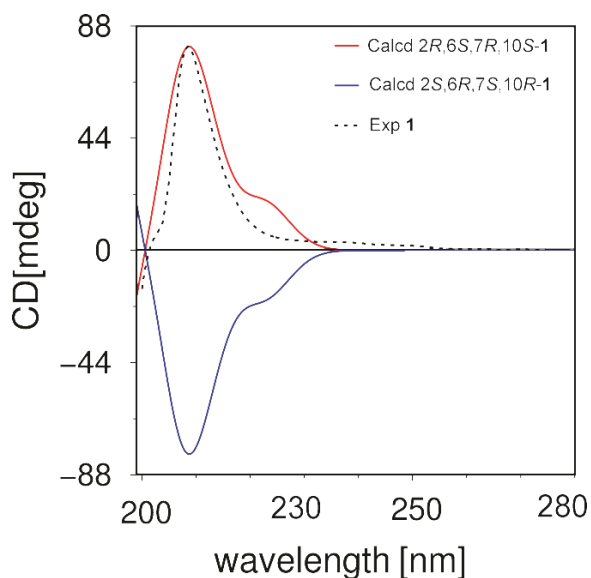


Figure 4. Experimental and calculated ECD spectra of compound **A1**.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Jasco P-2000 polarimeter at 20 °C in CHCl₃. FTIR spectra were acquired on a Shimadzu IRPrestige-21 spectrometer. 1D and 2D NMR spectra were recorded on a Bruker AVANCE III HD spectrometer (500 and 125 MHz for ¹H and ¹³C) using CHCl₃ (δ_{H} 7.26 and δ_{C} 77.0), the residual solvent, as an internal standard. HRESIMS analysis was carried out using a Bruker spectrometer model micrOTOFII operating in positive mode. Medium pressure liquid chromatography (MPLC) was performed on silica gel 60 (40-63 μm , 230-400 mesh, SiliCycle). Thin-layer chromatography (TLC) was carried out using precoated silica gel F-254 aluminum sheets (SiliCycle), and the compound spots were observed under UV light at 254 and 366 nm and stained with iodine vapor. For preparative high-performance liquid chromatography (HPLC), a Shimadzu apparatus with an SPD-M10A VP diode array detector and a YMC-Triart C₁₈ preparative column (250 mm $\times$ 10 mm and 5 μm particles) was used with a flow rate of 8.0 mL/min.

Plant Material and Extraction. The *Anaxagorea dolichocarpa* roots were collected and extracted as previously reported.¹⁰

Isolation. An aliquot of the CHCl₃ soluble fraction (5.8 g) was subjected to MPLC with silica gel and eluted with a gradient mixture of hexane, CHCl₃, EtOAc, and MeOH. The fractions obtained were combined according to the TLC profile to afford fractions 1-8. Fraction **4** (425 mg) was subjected to preparative HPLC using a C₁₈ column (YMC-Triart; 250 mm $\times$ 10 mm and 5 μm , flow rate of 8.0 mL/min) with the following elution gradient: solvent A = H₂O; solvent B = CH₃CN; elution profile = 0-50 min (10-45% B) and 50-60 min (45-100% B). Thus, Compound **A1** was isolated (t_{R} 43 min) (49 mg).

Compound (A1): colorless crystals, mp 113-114 °C; $[\alpha]_{\text{D}}^{20}$ +20 (c. 0.1, CHCl₃); IR ν_{max} 3354, 2972, 2927, 2872, 1641, 1458, 1365, 1124, 1060, 1014, 970, 898 cm⁻¹; ¹H and ¹³C NMR data, Table 1; HRESIMS m/z 255.1961 [M + H]⁺ (calcd for C₁₅H₂₇O₃, 255.1955).

Acetylated Derivatization of Compound A1. A 5 mg aliquot of **A1** was dissolved in 3 mL of a 1:2 mixture of acetic anhydride-pyridine, and the reaction stood for 24 h at 25 °C. The reaction mixture was diluted with H₂O (30 mL) and extracted with CHCl₃ (3 x 30 mL). The CHCl₃ extract was washed successively with 5% hydrochloric acid and H₂O, dried over Na₂SO₄, and concentrated under reduced pressure to afford an acetylated derivative (4.7 mg).^{13,14}

Acetylated derivative: ¹H NMR (CDCl₃, 500 MHz) 5.45 (1H, dd, *J* = 10.5, 5.0 Hz, H-2), 5.23 (1H, s, H-12a), 5.09 (1H, s, H-12b), 4.55 (1H, dd, *J* = 6.5, 4.5 Hz, H-6), 3.67 (1H, dd, *J* = 11.5, 8.0 Hz, H-10), 2.56 (1H, m, H-4a), 2.34 (1H, m, H-4b), 2.34 (1H, m, H-5a), 2.14 (1H, dd, *J* = 20.0, 5.5 Hz, H-1a), 2.03 (3H, s, acetoxy CH₃), 1.97 (3H, s, acetoxy CH₃), 1.80 (1H, m, H-8a), 1.69 (1H, dd, *J* = 19.5, 6.5 Hz, H-1b), 1.64 (1H, m, H-9a), 1.64 (1H, m, H-9b), 1.55 (1H, m, H-5b), 1.53 (1H, m, H-8b), 1.21 (3H, s, H₃-13), 1.10 (3H, s, H₃-15), 0.75 (3H, s, H₃-14); HRESIMS *m/z* 316.1980 [M + Na]⁺ (calcd. for C₁₉H₃₀NaO₅, 316.1985).

X-ray Diffraction Analysis. Single crystal X-ray data for **A1** were collected on a Bruker D8 Venture diffractometer using graphite-monochromated Mo K α radiation (λ = 0.71073 Å) at 298 K. Data collection, cell refinement, and data reduction were performed with Bruker Instrument Service v4.2.2, APEX2¹⁵ and SAINT V8.34A,¹⁶ respectively. Absorption correction using equivalent reflections was performed with the SADABS program.¹⁷ The structure solutions and full-matrix least-squares refinements based on F^2 were performed with the SHELX package^{18,19} and were refined with fixed individual displacement parameters [$U_{iso}(H) = 1.2 U_{eq}(C_{sp^2} \text{ and } C_{ar})$ or $1.5 U_{eq}(C_{sp^3})$] using a riding model. All non-hydrogen atoms were refined anisotropically and occupational disorder was observed in C4 and C4' atoms. Crystallographic tables were constructed using Olex2.²⁰ X-ray crystallographic data in cif format is available at CCDC 2193776 and can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif. Additional crystallographic information is available in the Supporting Information.

Crystallographic data of A1: C₁₅H₂₆O₃ (M = 254.37 g/mol): trigonal, space group P3₂, a = 14.1989(5) Å, b = 14.1989(5) Å, c = 6.3380(3) Å, α = 90°, β = 90°, γ = 120°, V = 1106.60(8) Å³, Z = 3, T = 273.15 K, μ (Mo K α) = 0.078 mm⁻¹, F(000) =

420.2, crystal size = $0.424 \times 0.33 \times 0.194 \text{ mm}^3$, $\rho_{\text{calc}} = 1.1450 \text{ g/cm}^3$; of the 18081 reflections measured ($5.74^\circ \leq 2\theta \leq 50.7^\circ$), 2700 were unique ($R_{\text{int}} = 0.0704$, $R_{\text{sigma}} = 0.0348$) and were used in all calculations. The final R_1 was 0.0421 ($I > 2\sigma(I)$), and wR_2 was 0.0937 (all data).

ECD Calculation. The ECD spectra were calculated as described previously.¹⁰

ASSOCIATED CONTENT

Supporting Information. HRESIMS, IR, NMR and X-ray crystallography data of **A1** and the acetylated derivative are available in the Supporting Information.

AUTHOR INFORMATION

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Author Contributions. The manuscript was written through contributions from all authors. All authors approved the final version of the manuscript. All authors contributed equally.

Notes. The authors declare no competing financial interest.

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Conflict of Interest.

The authors declare that they have no conflict of interest.

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SUPPORTING INFORMATION**Structure Revision of the Sesquiterpene Nordine based on NMR-spectroscopic Analysis and X-ray crystallography**

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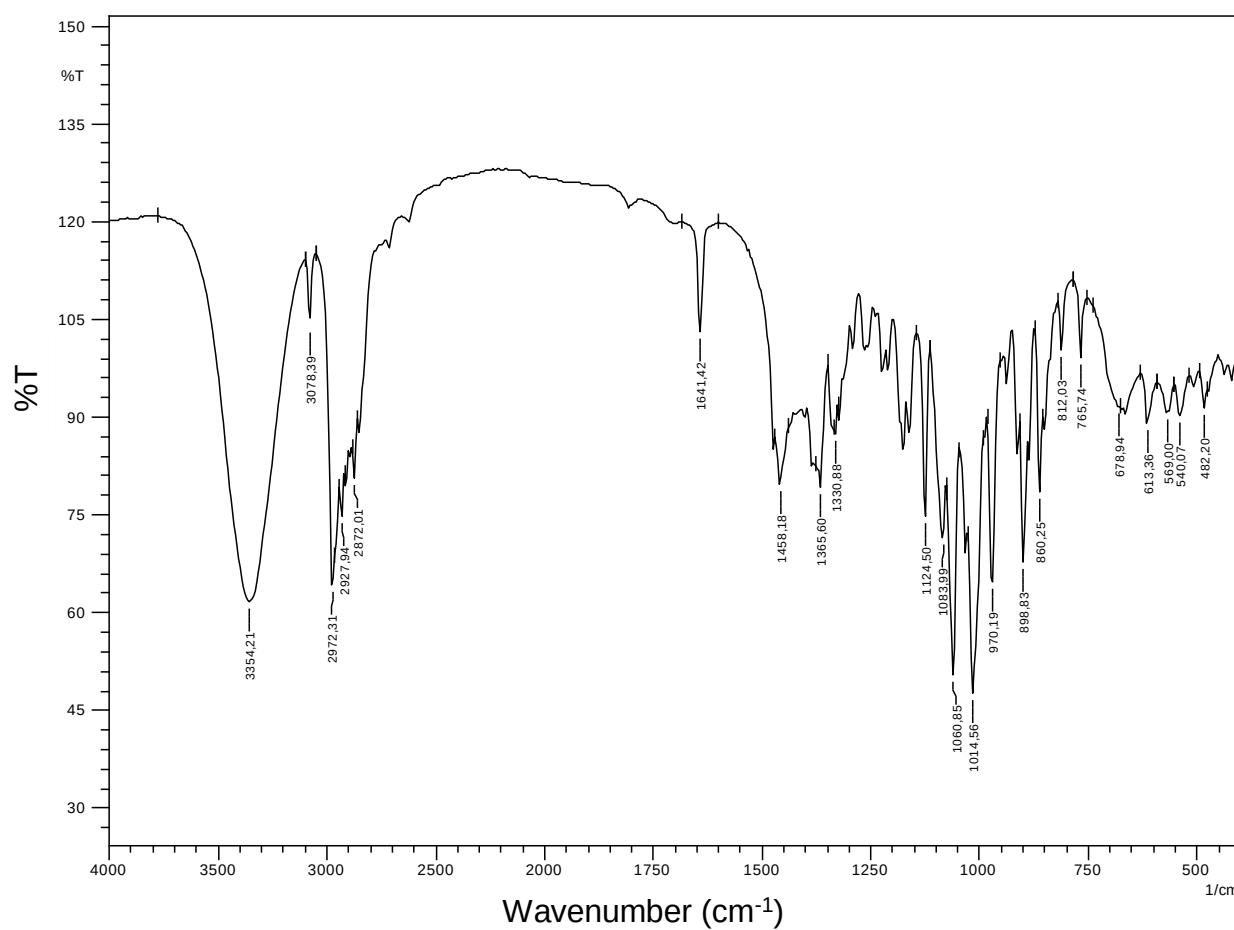
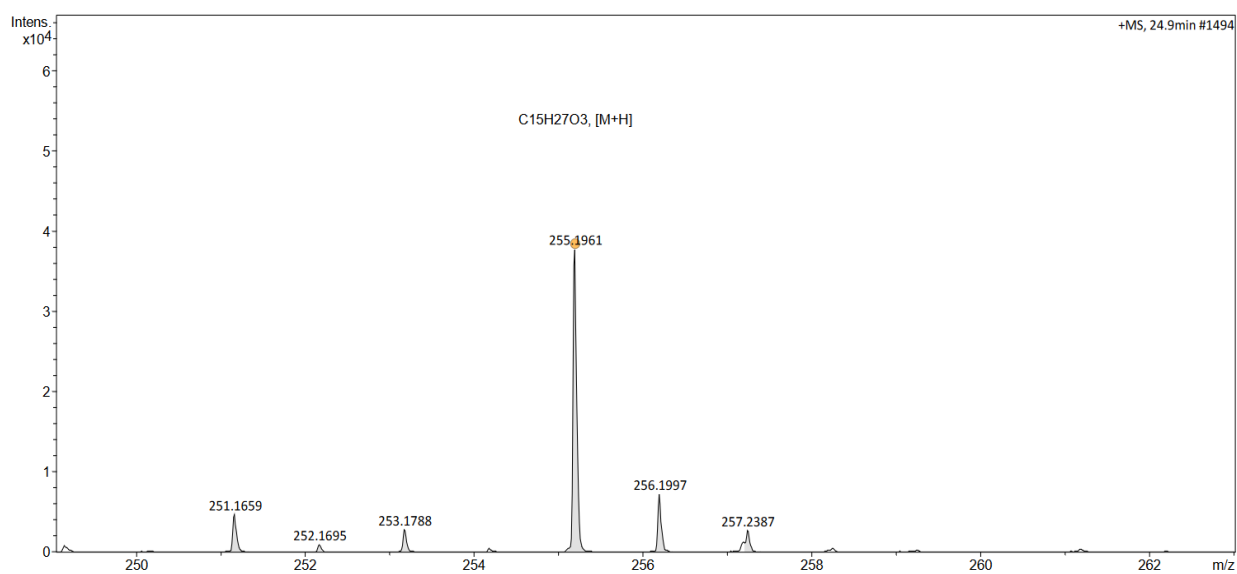
Figure S1. IR spectrum of compound **A1**.**Figure S2.** HRESIMS [M + H]⁺ spectrum of compound **A1**.

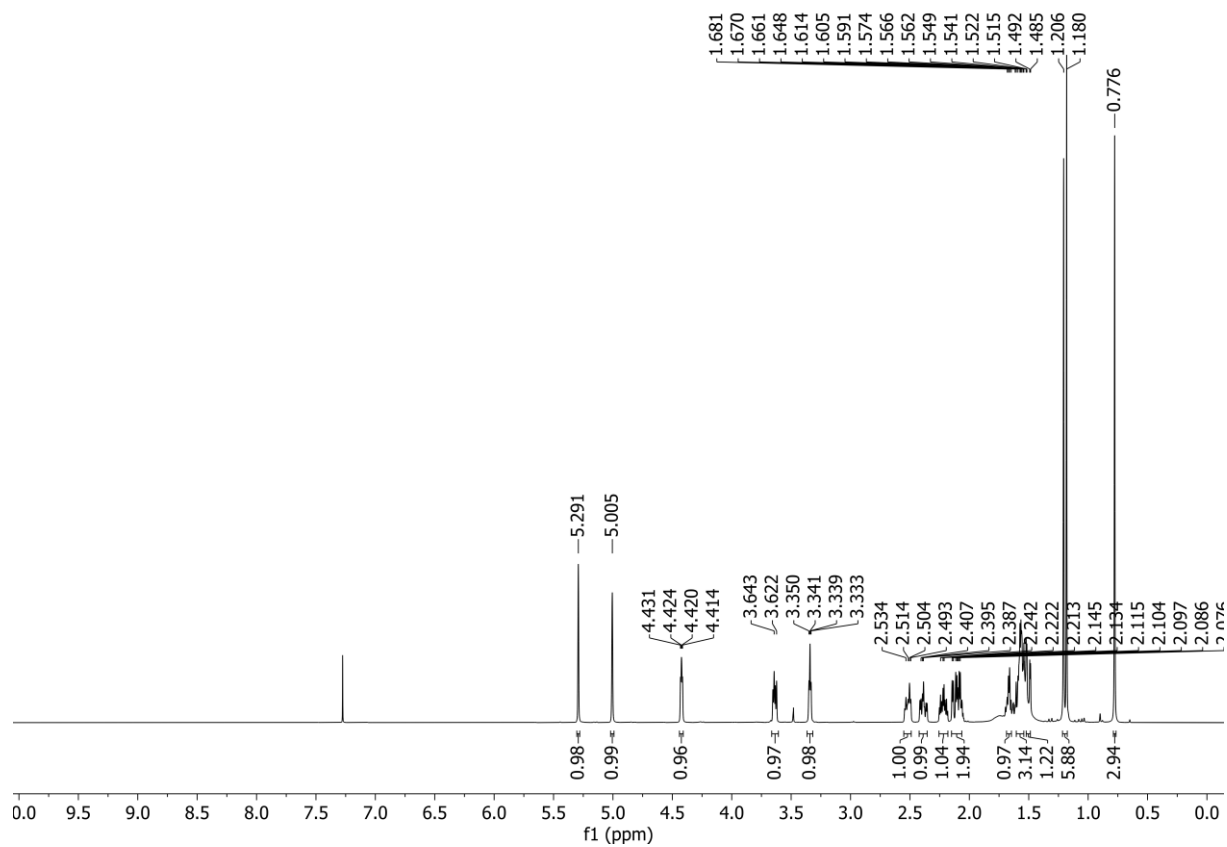
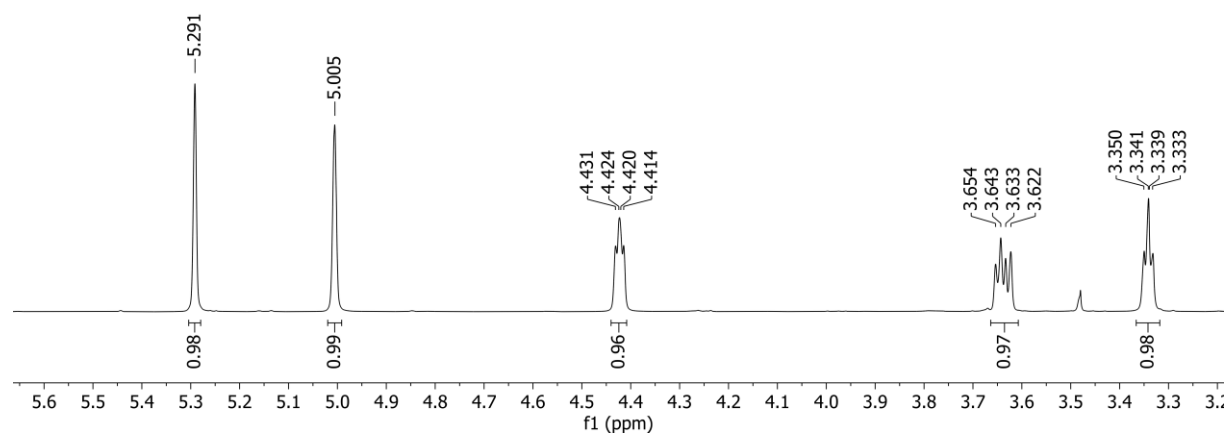
Figure S3. ^1H NMR (500 MHz, CDCl_3) spectrum of **A1**.**Figure S4.** ^1H NMR (500 MHz, CDCl_3) spectrum of **A1** (expansion).

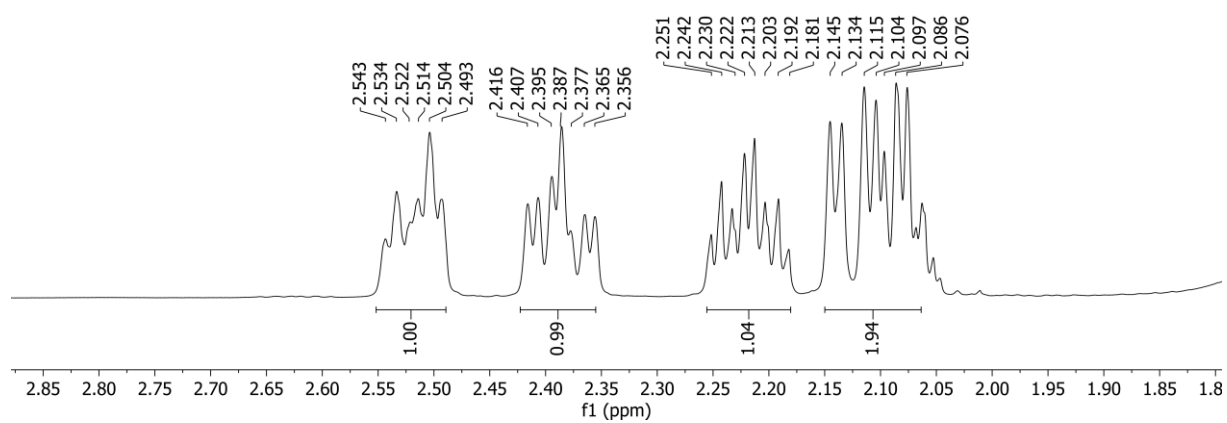
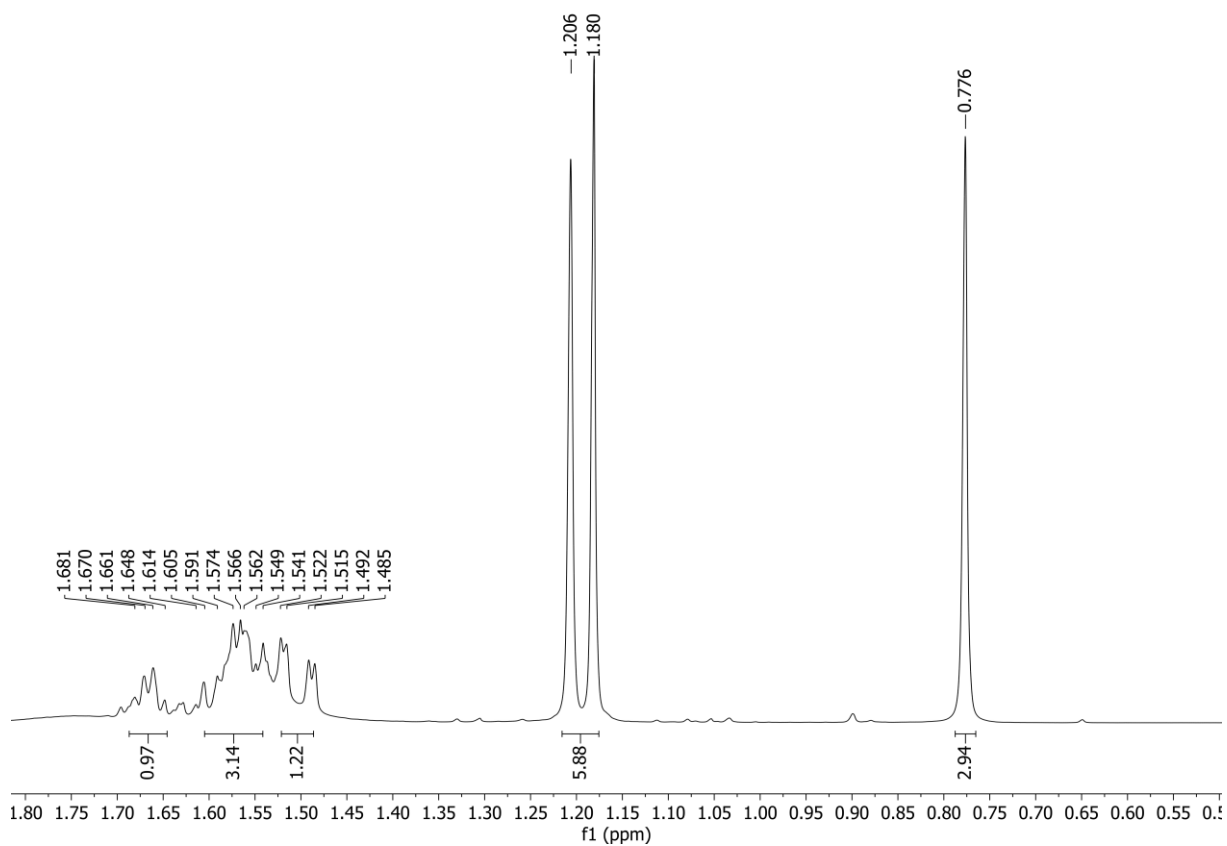
Figure S5. ^1H NMR (500 MHz, CDCl_3) spectrum of **A1** (expansion).**Figure S6.** ^1H NMR (500 MHz, CDCl_3) spectrum of **A1** (expansion).

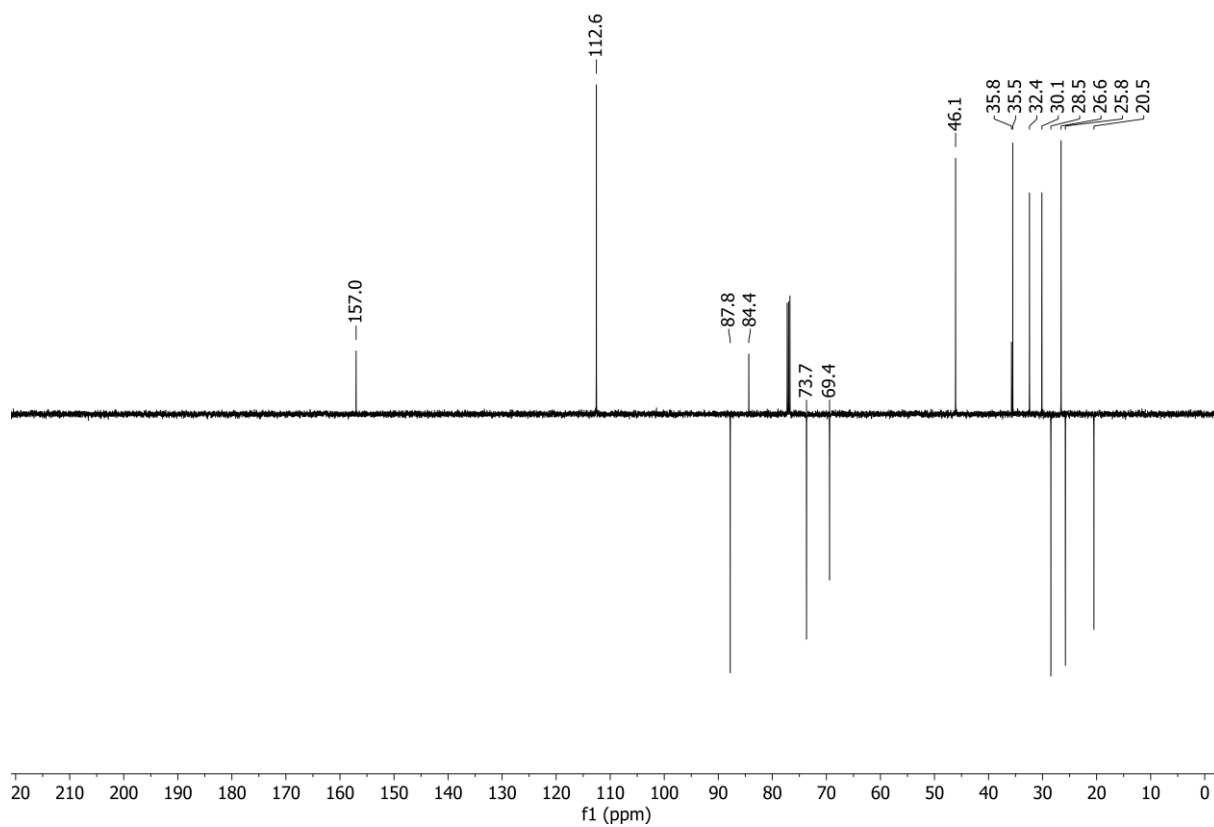
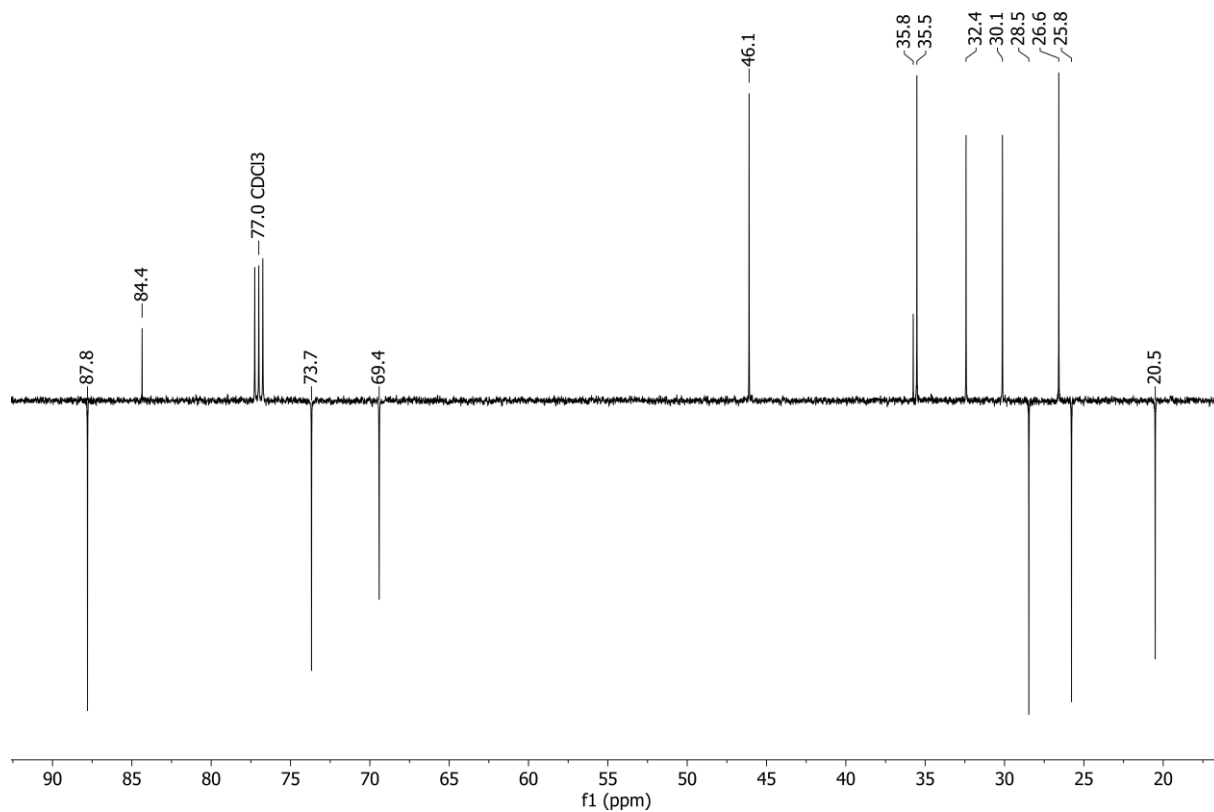
Figure S7. ^{13}C -APT NMR (125 MHz, CDCl_3) spectrum of **A1**.**Figure S8.** ^{13}C -APT NMR (125 MHz, CDCl_3) spectrum of **A1** (expansion).

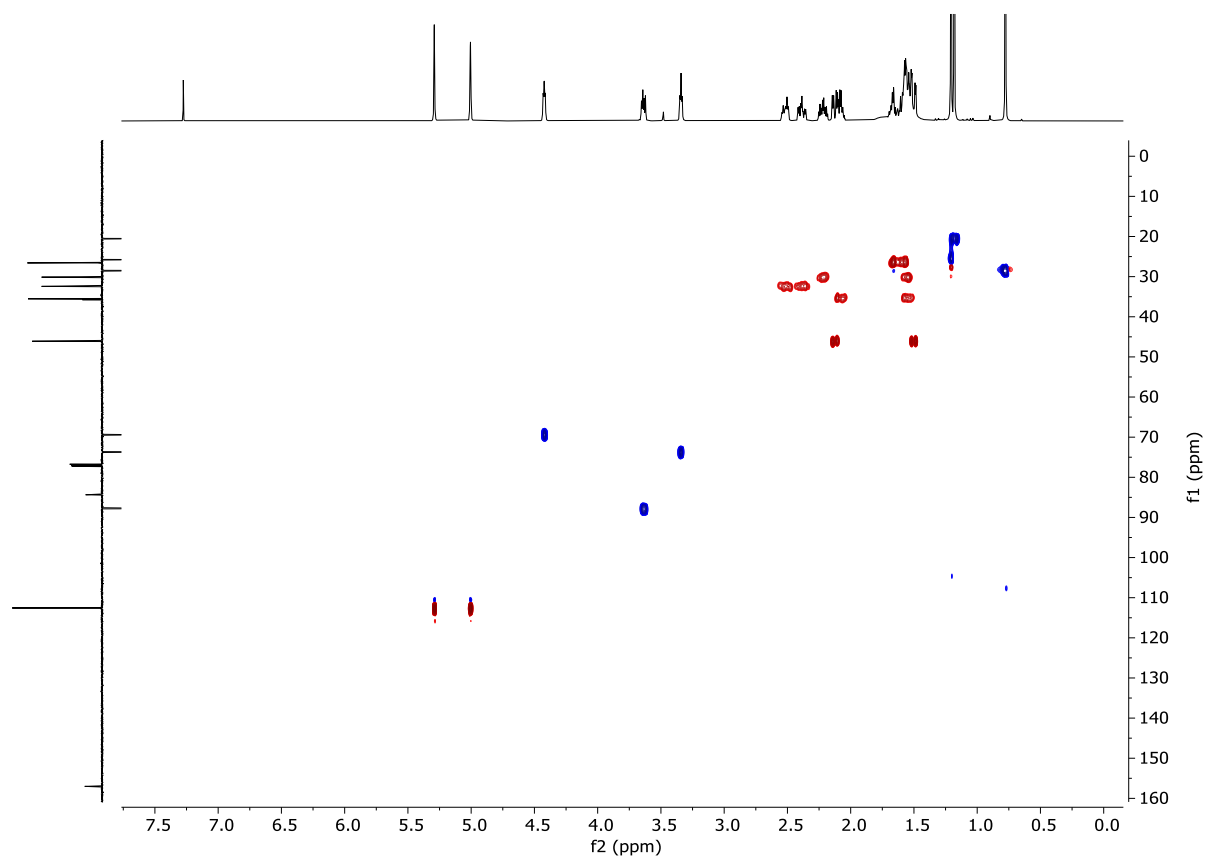
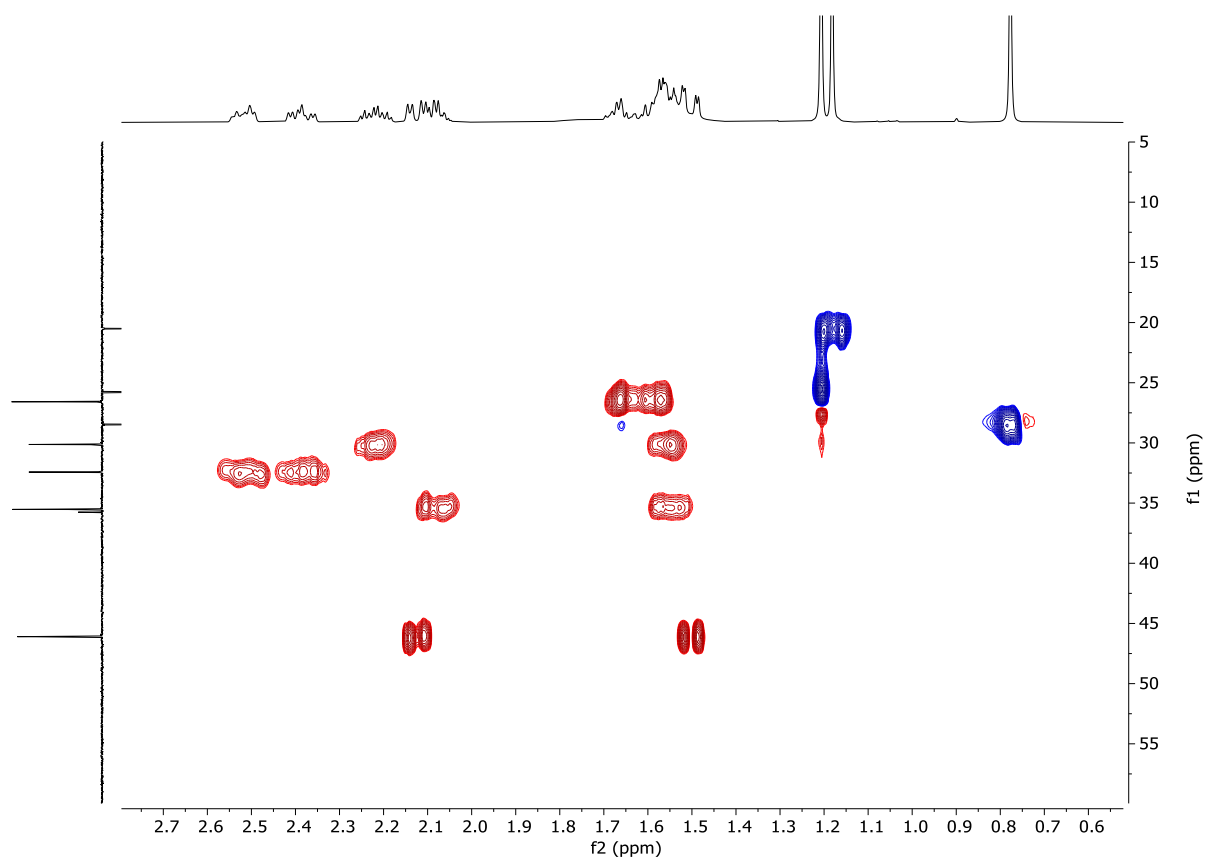
Figure S9. HSQC NMR (500MHz, CDCl₃) spectrum of **A1**.**Figure S10.** HSQC NMR (500MHz, CDCl₃) spectrum of **A1** (expansion).

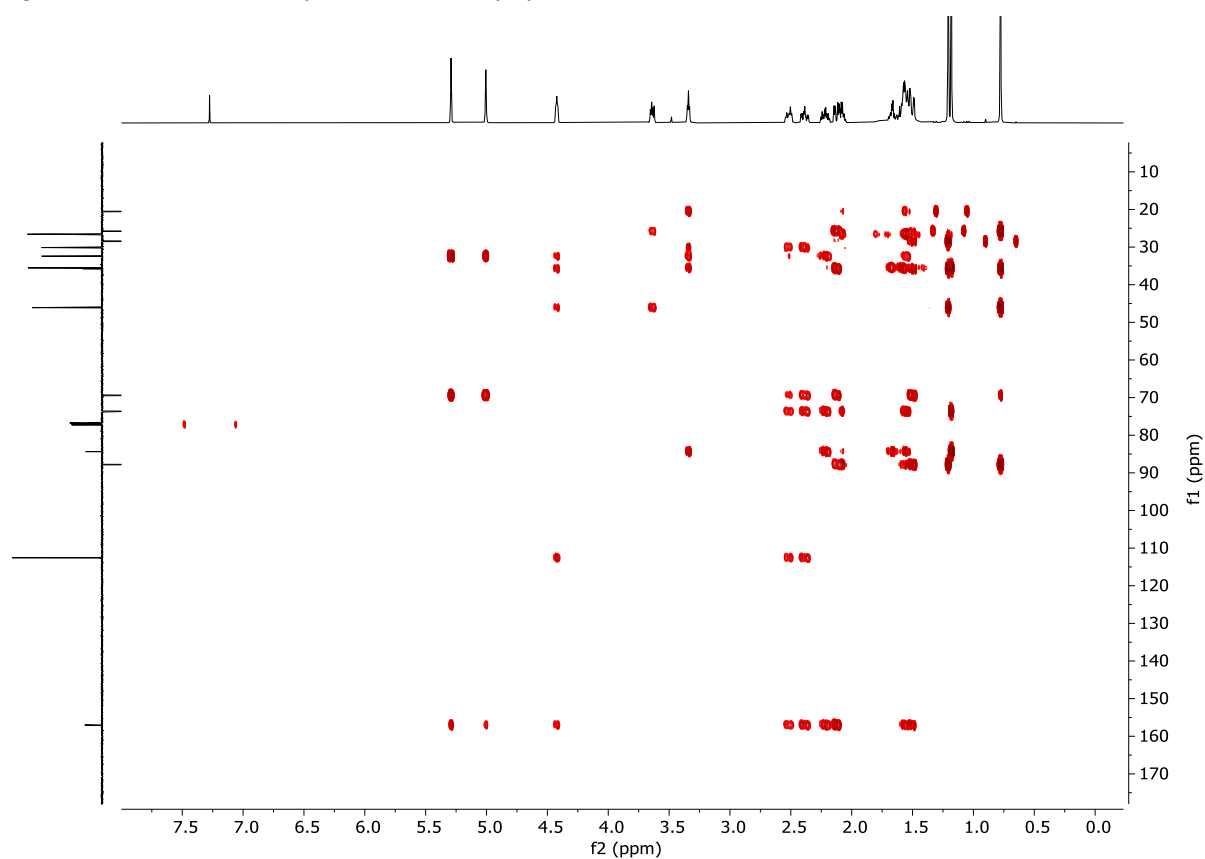
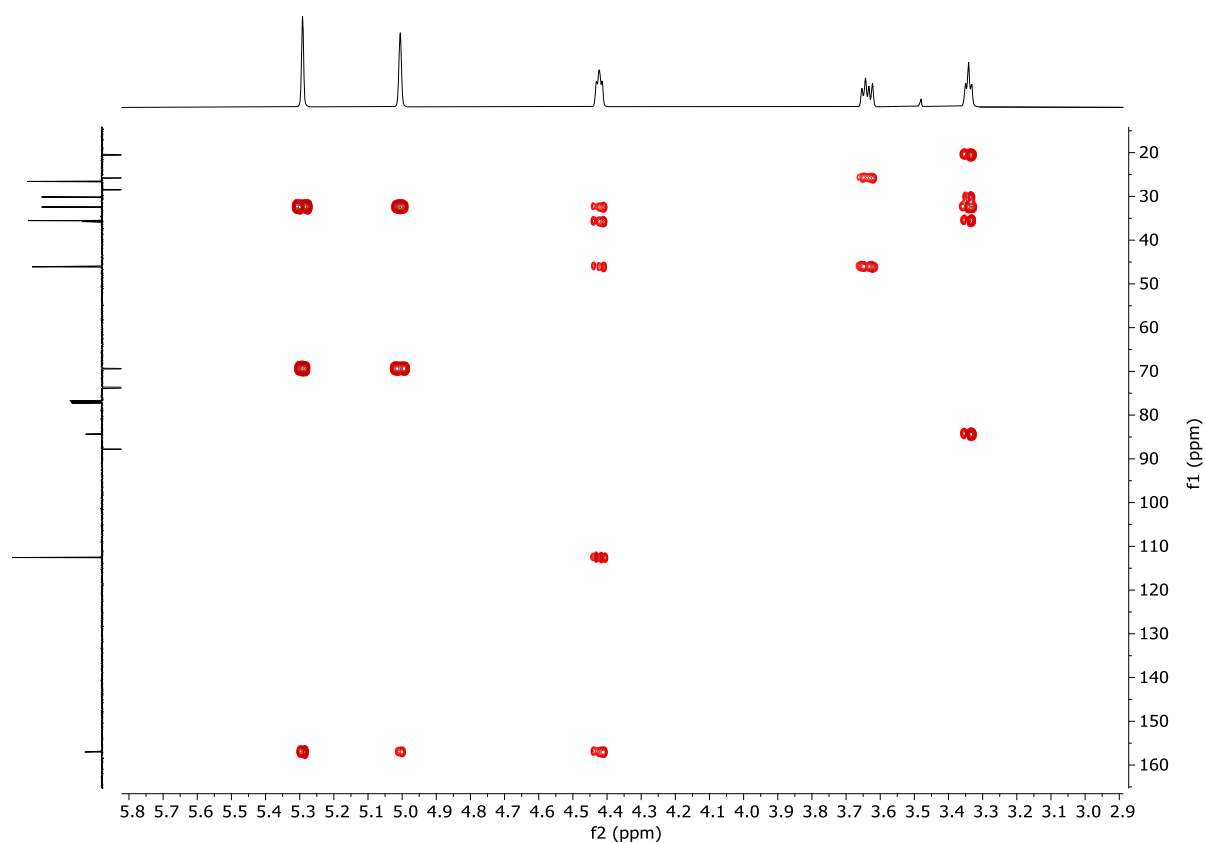
Figure S11. HMBC NMR (500MHz, CDCl_3) spectrum of **A1**.**Figure S12.** HMBC NMR (500MHz, CDCl_3) spectrum of **A1** (expansion).

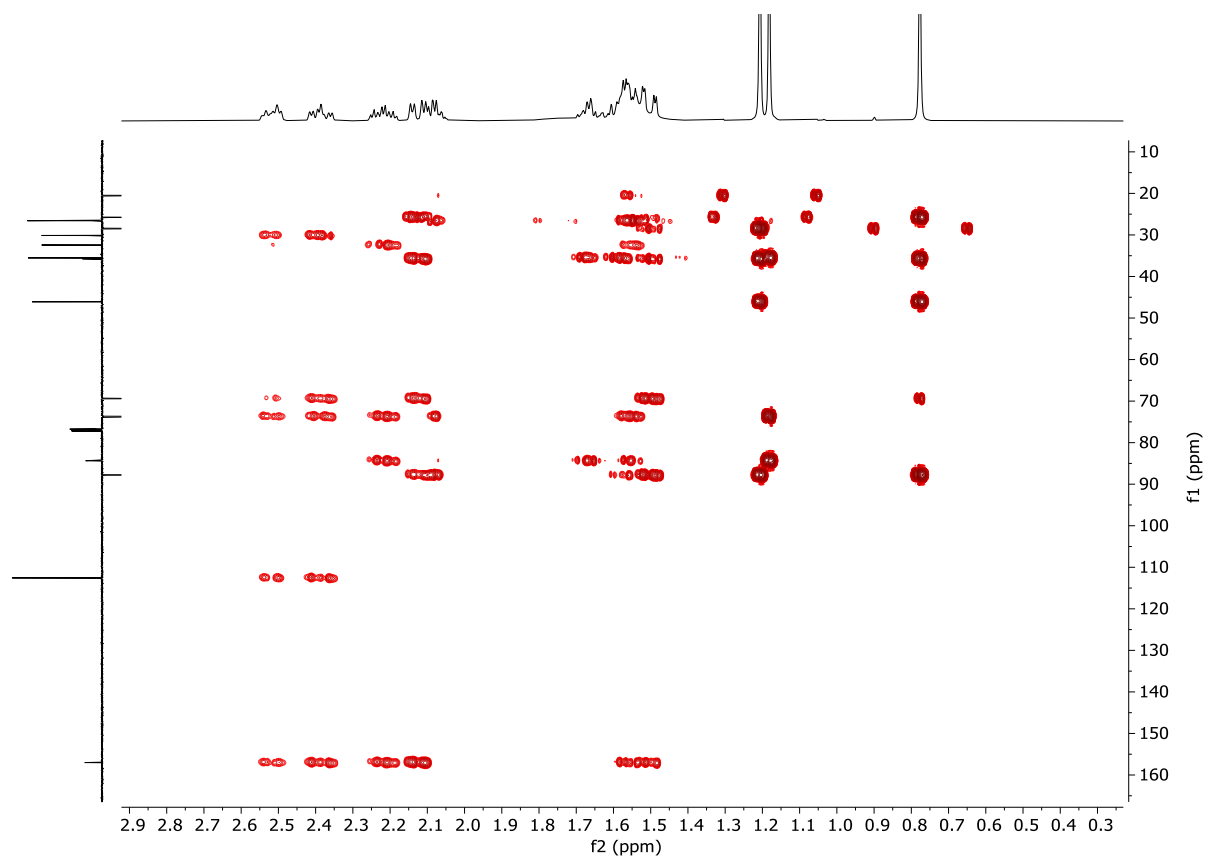
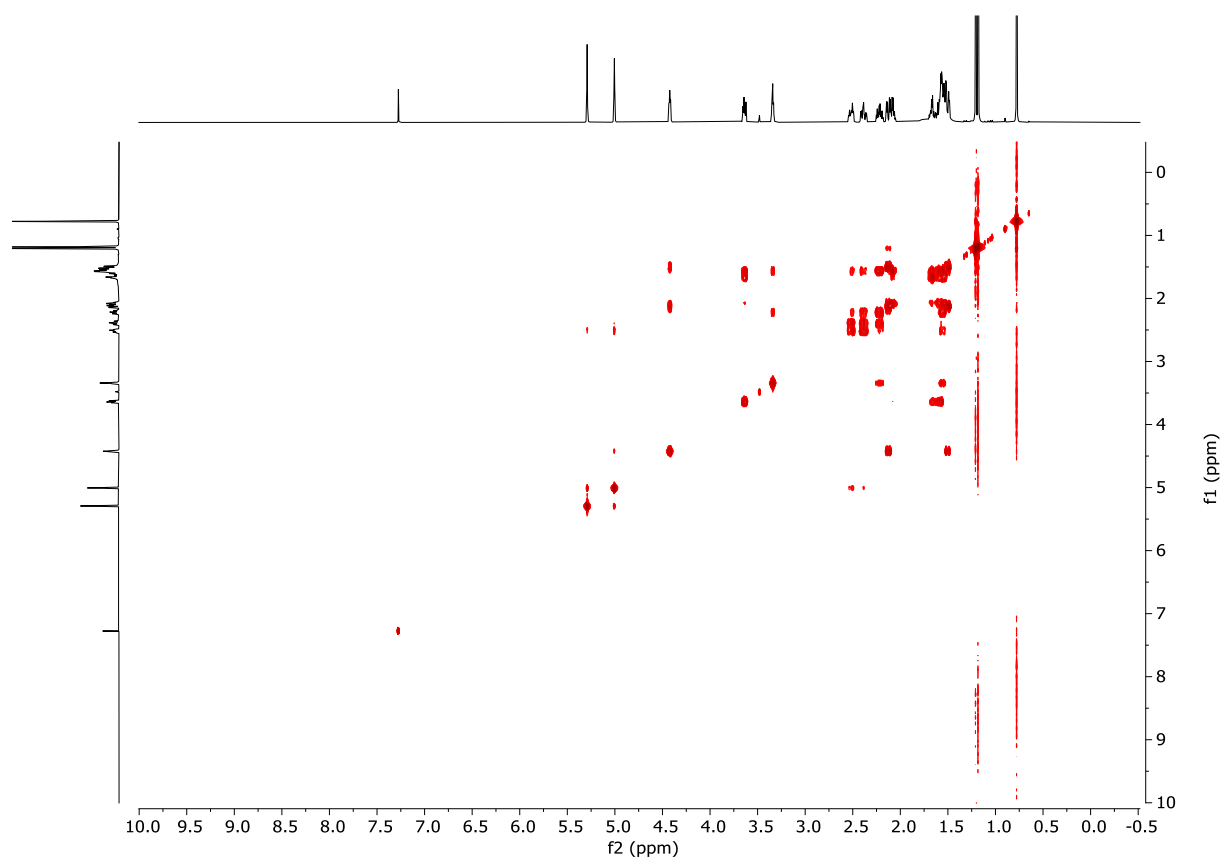
Figure S13. HMBC NMR (500MHz, CDCl_3) spectrum of **A1** (expansion).**Figure S14.** COSY NMR (500 MHz, CDCl_3) spectrum of **A1**.

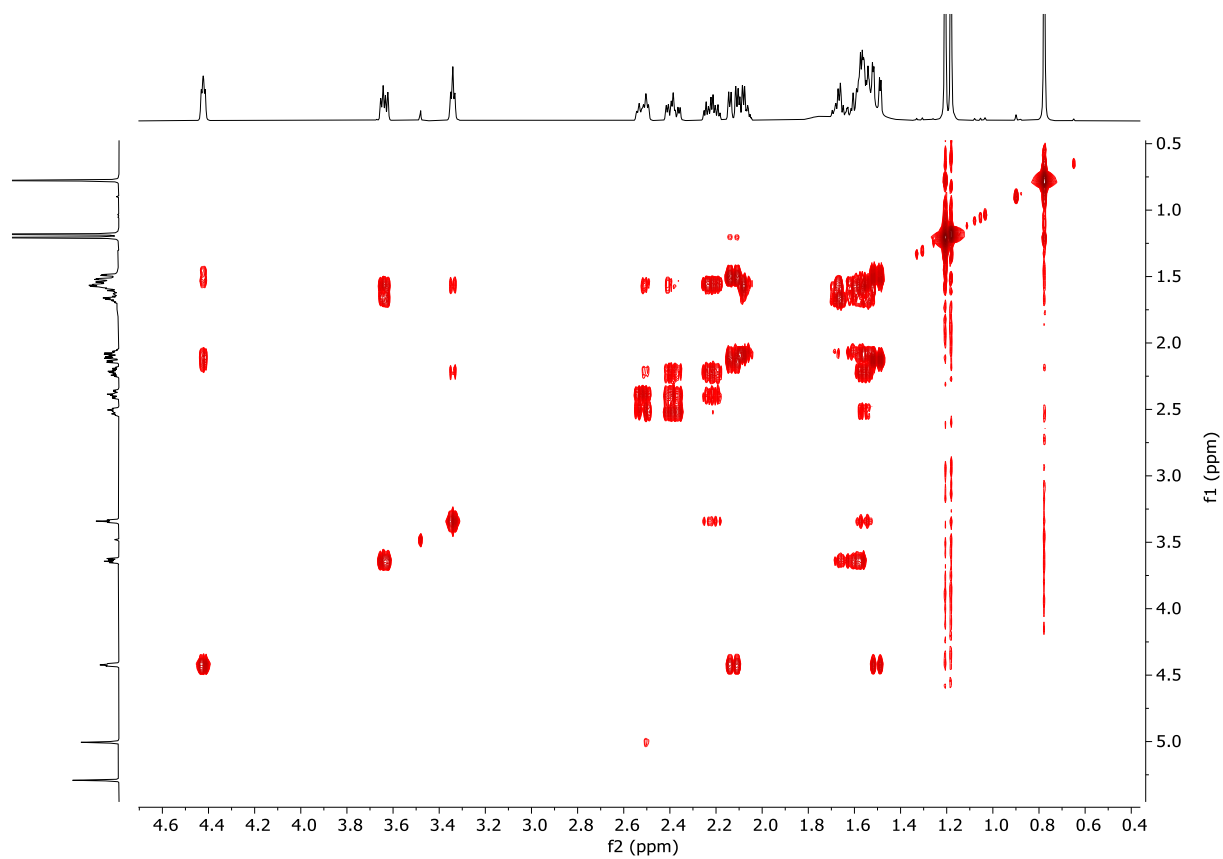
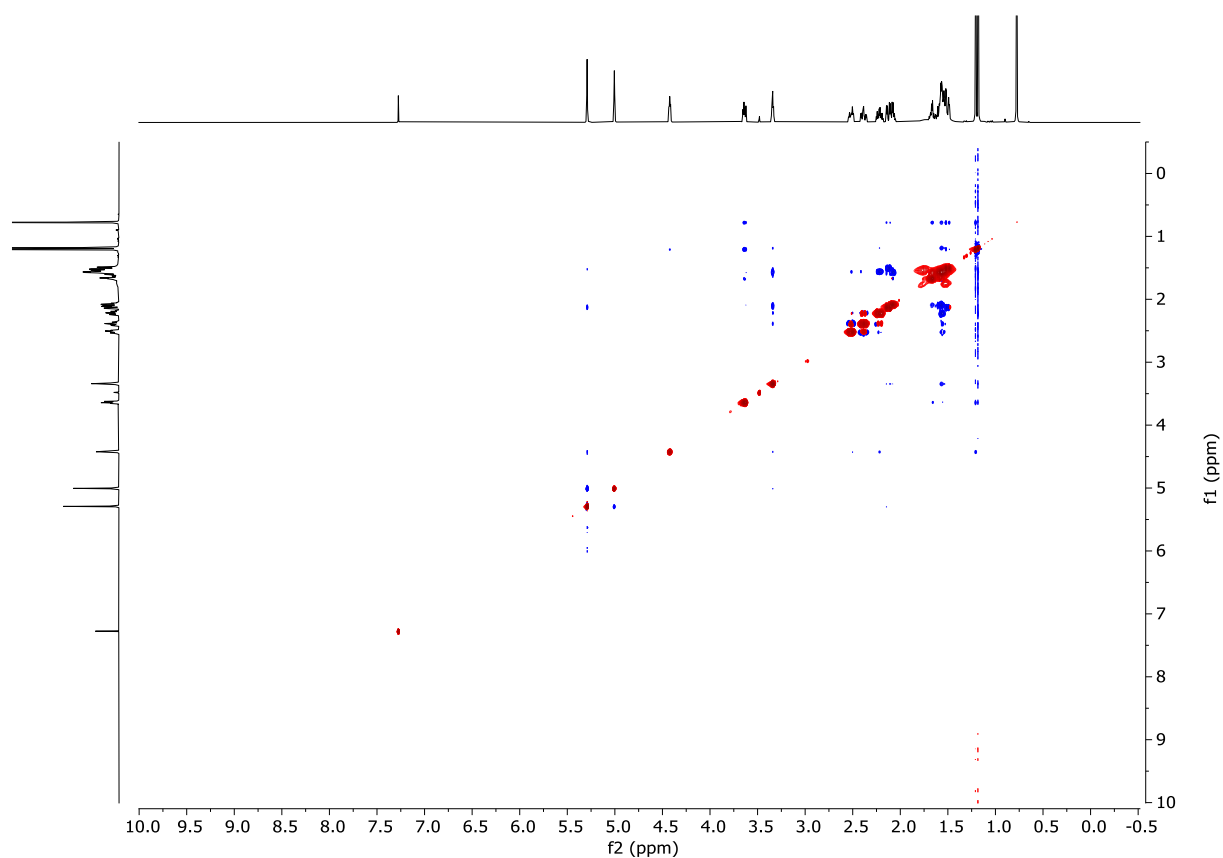
Figure S15. COSY NMR (500 MHz, CDCl_3) spectrum of **A1** (expansion).**Figure S16.** NOESY NMR (500 MHz, CDCl_3) spectrum of **A1**.

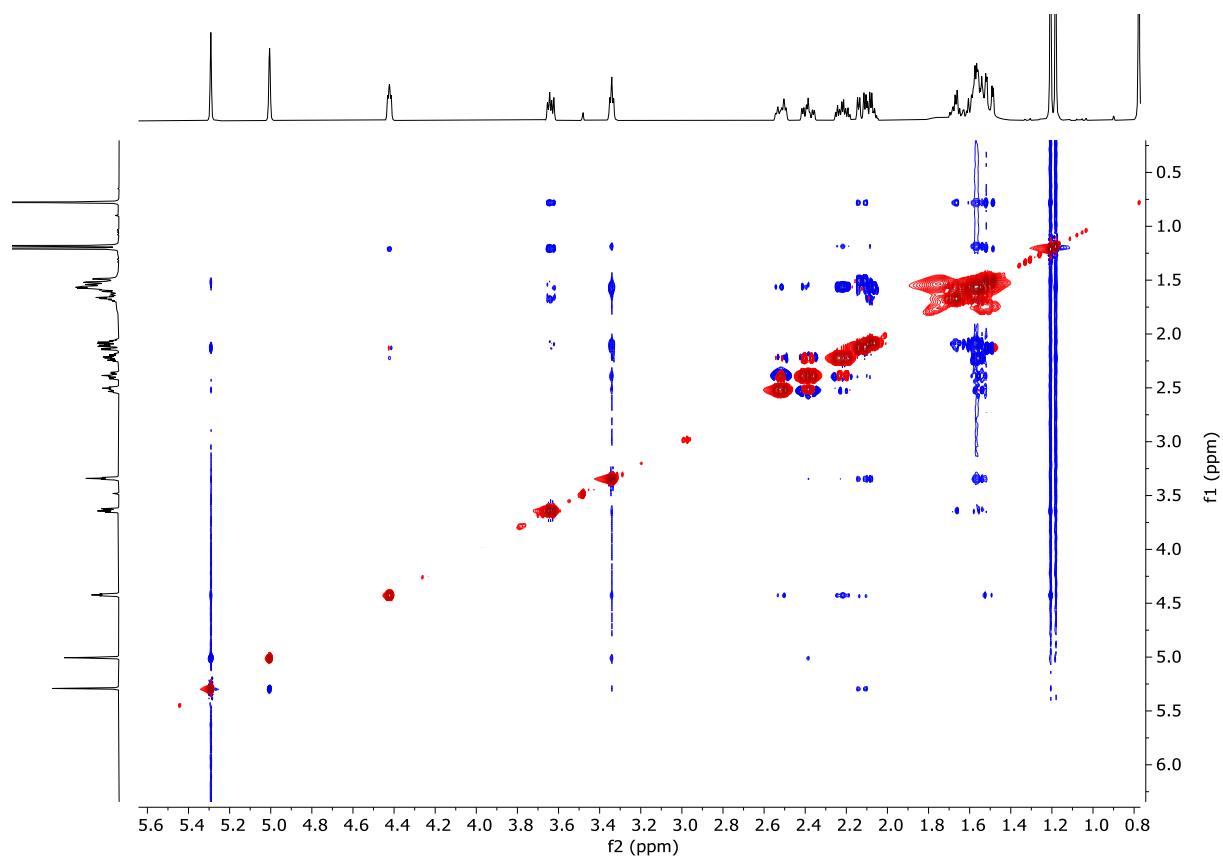
Figure S17. NOESY NMR (500 MHz, CDCl_3) spectrum of **A1** (expansion).

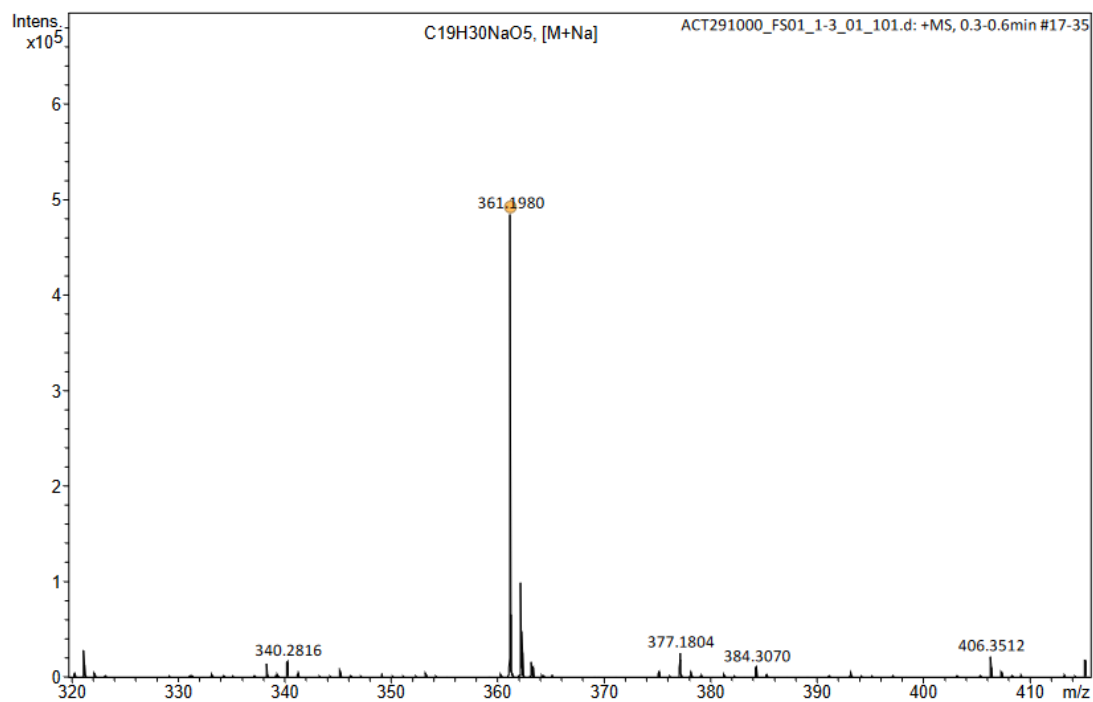
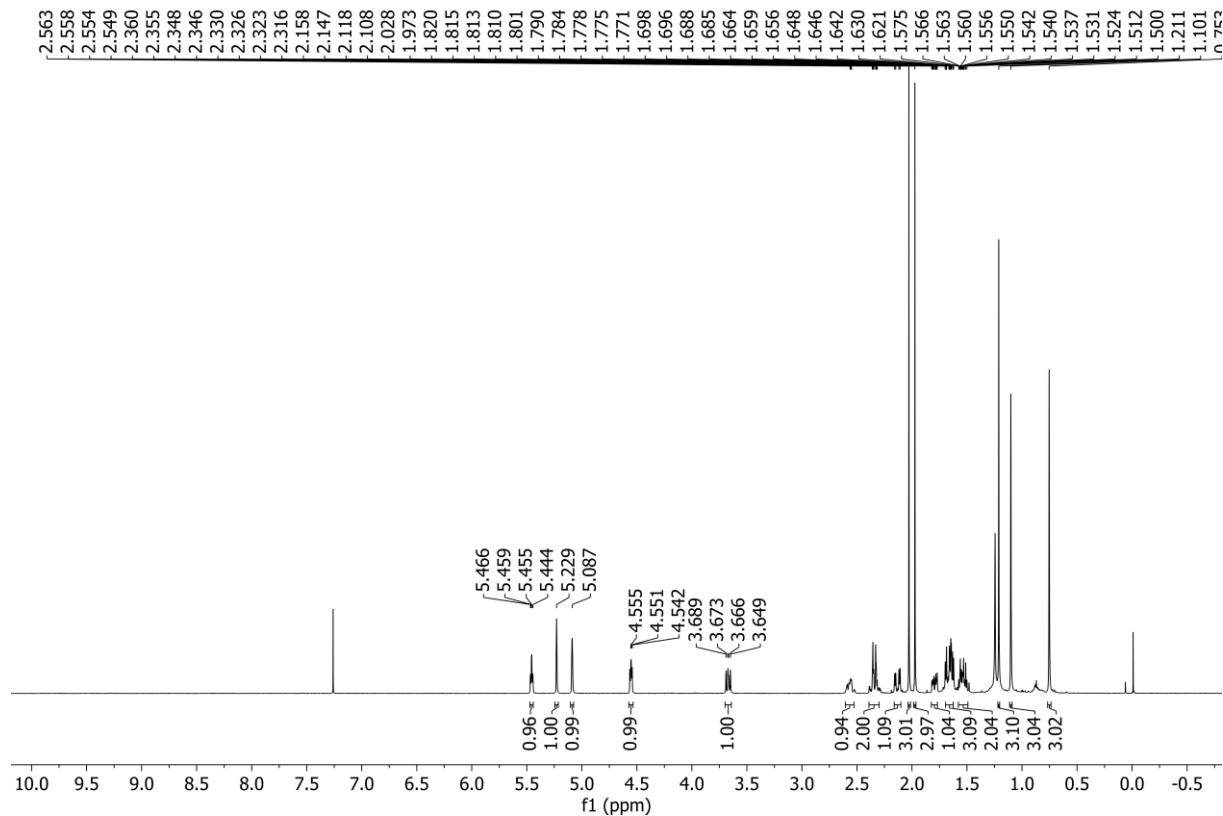
Figure S18. HRESIMS $[M + Na]^+$ spectrum of acetylated **A1**.**Figure S19.** 1H NMR (500 MHz, $CDCl_3$) spectrum of acetylated **A1**.

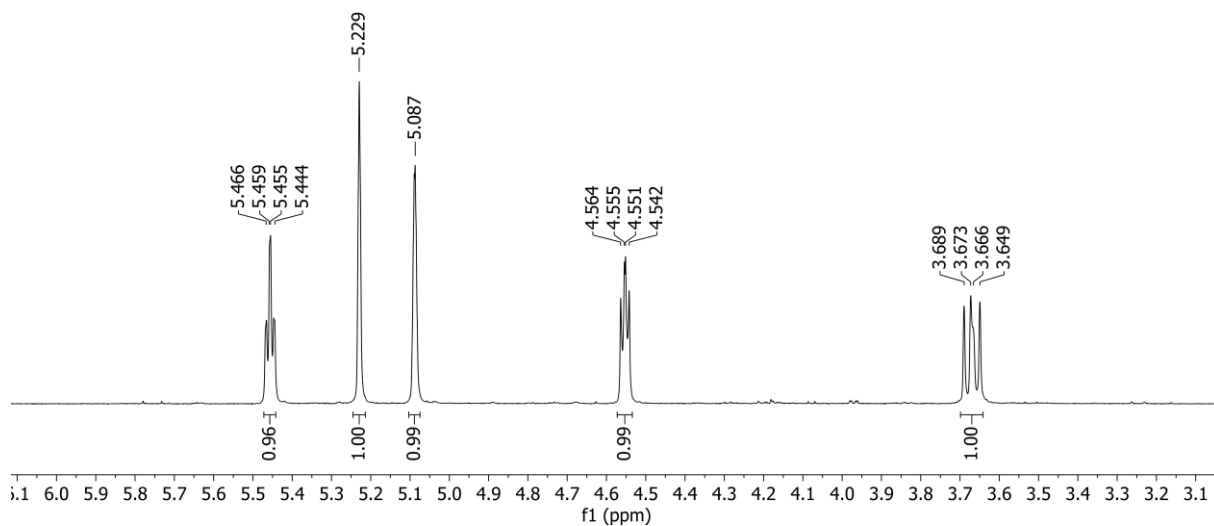
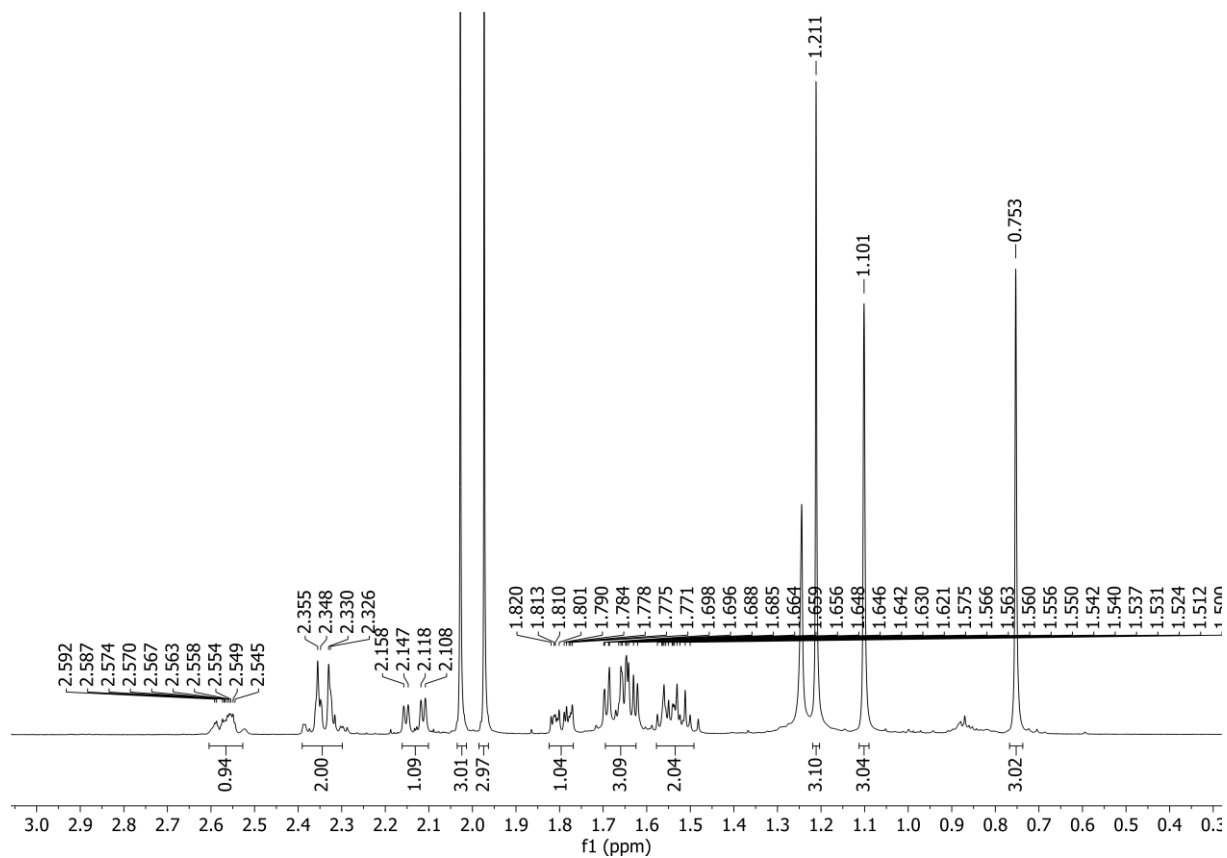
Figure S20. ^1H NMR (500 MHz, CDCl_3) spectrum of acetylated **A1** (expansion).**Figure S21.** ^1H NMR (500 MHz, CDCl_3) spectrum of acetylated **A1** (expansion).

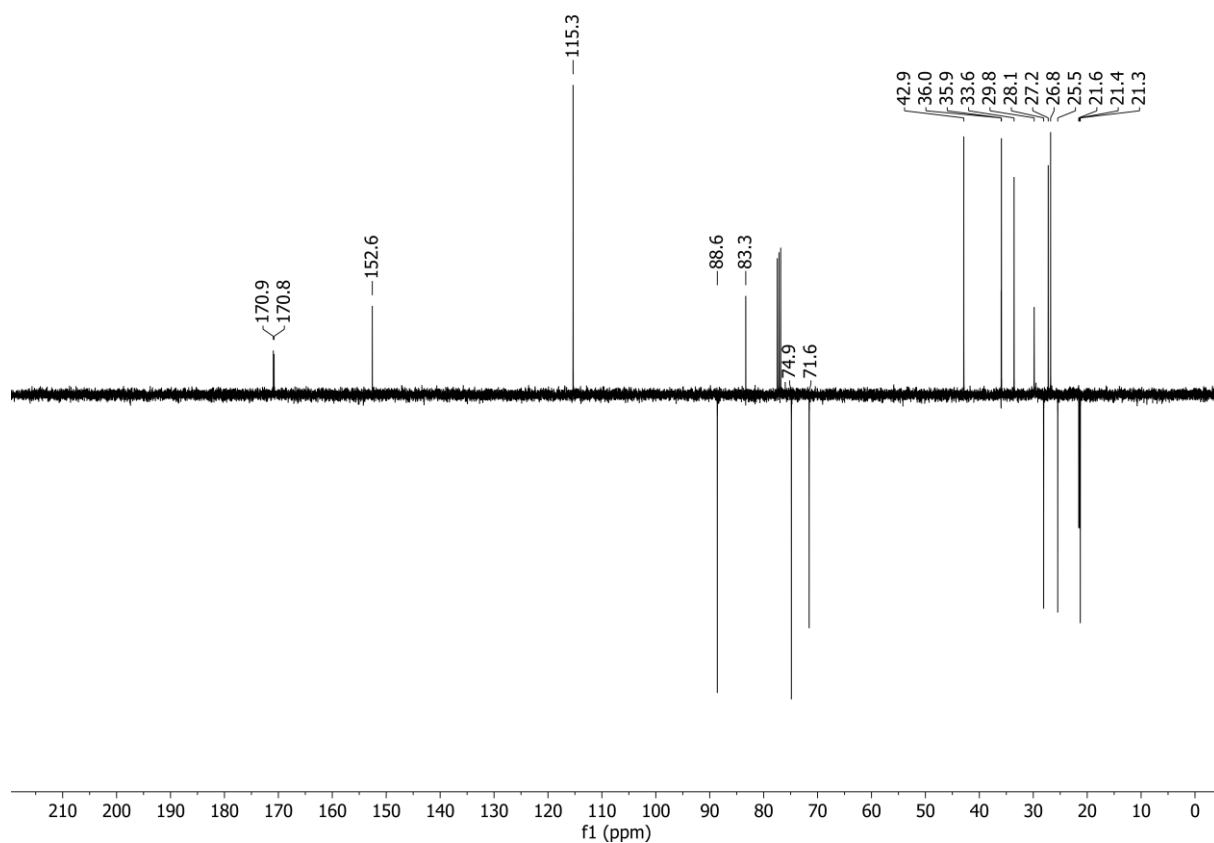
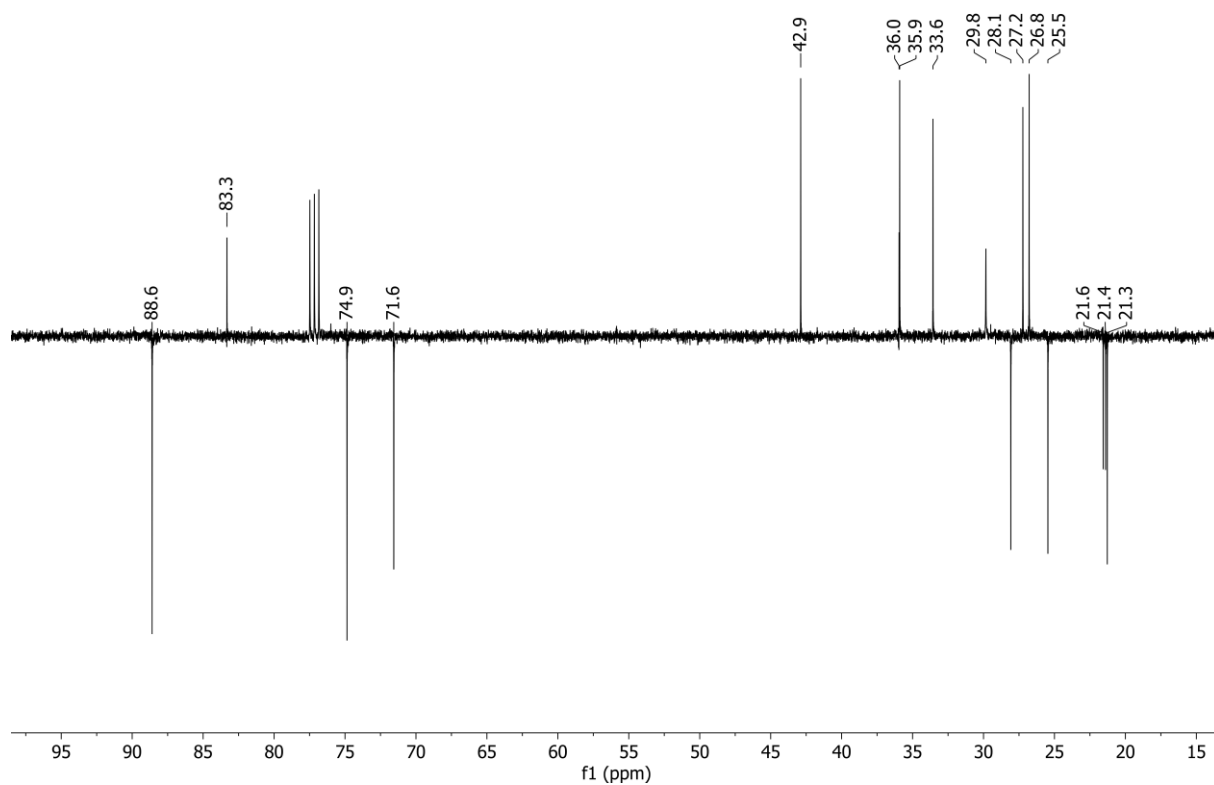
Figure S22. ^{13}C -APT NMR (500 MHz, CDCl_3) spectrum of acetylated **A1**.**Figure S23.** ^{13}C APT NMR (CDCl_3 , 125 MHz) spectrum of acetylated **A1**.

Figure S24. HSQC NMR (500MHz, CDCl₃) spectrum of acetylated **A1**.

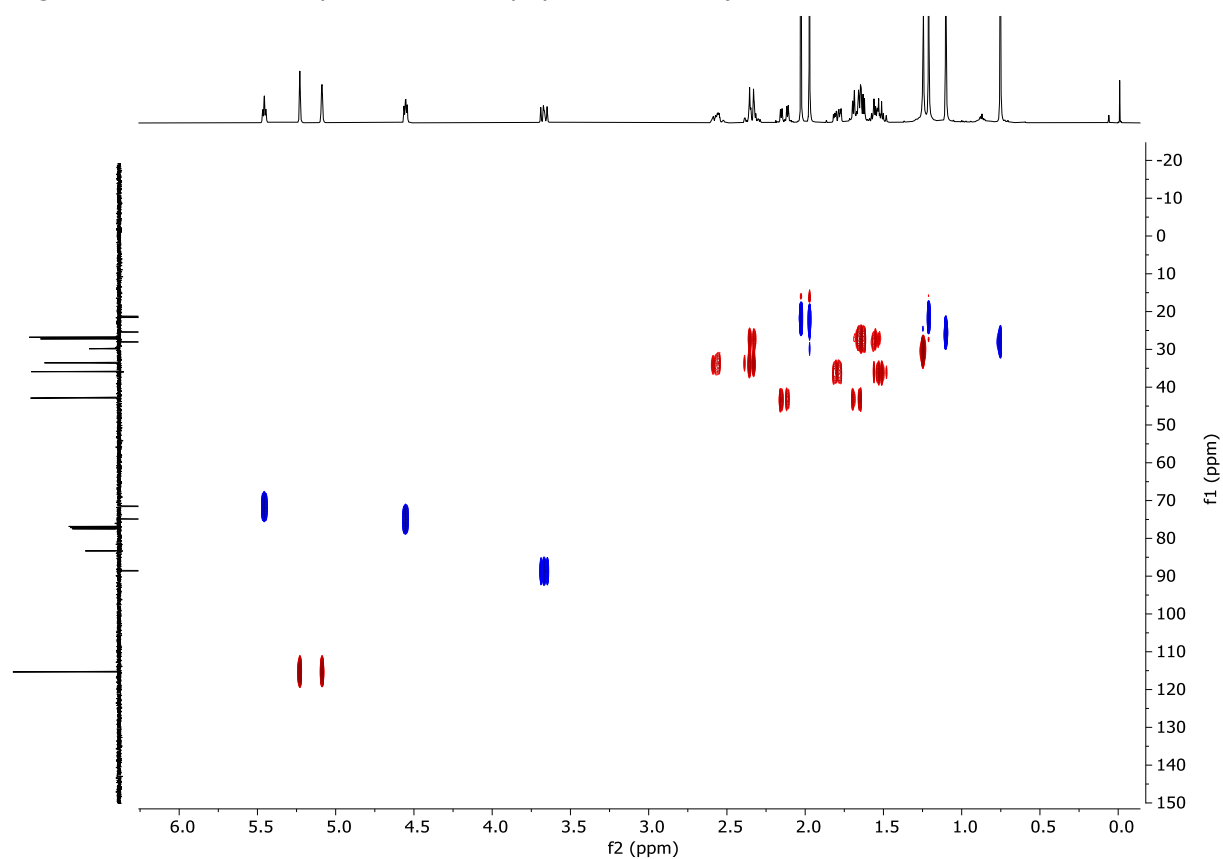


Figure S25. HSQC NMR (500MHz, CDCl₃) spectrum of acetylated **A1** (expansion).

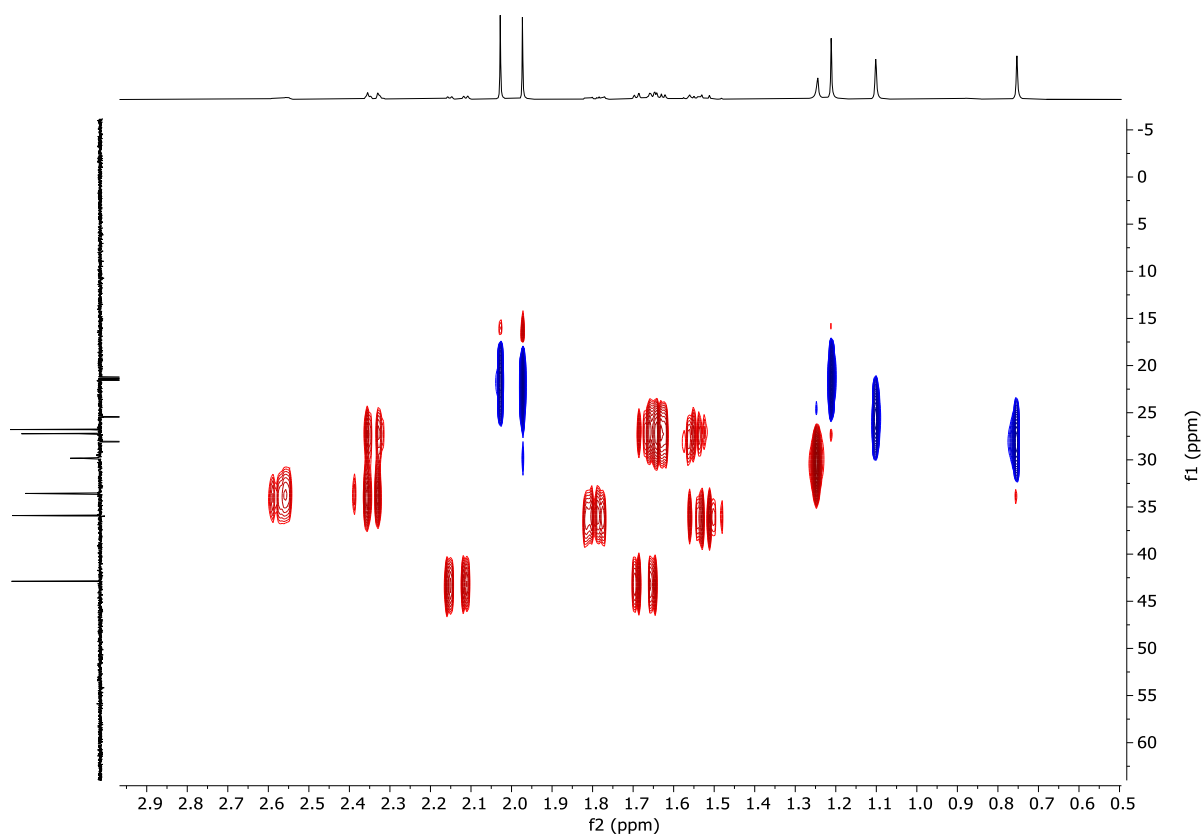


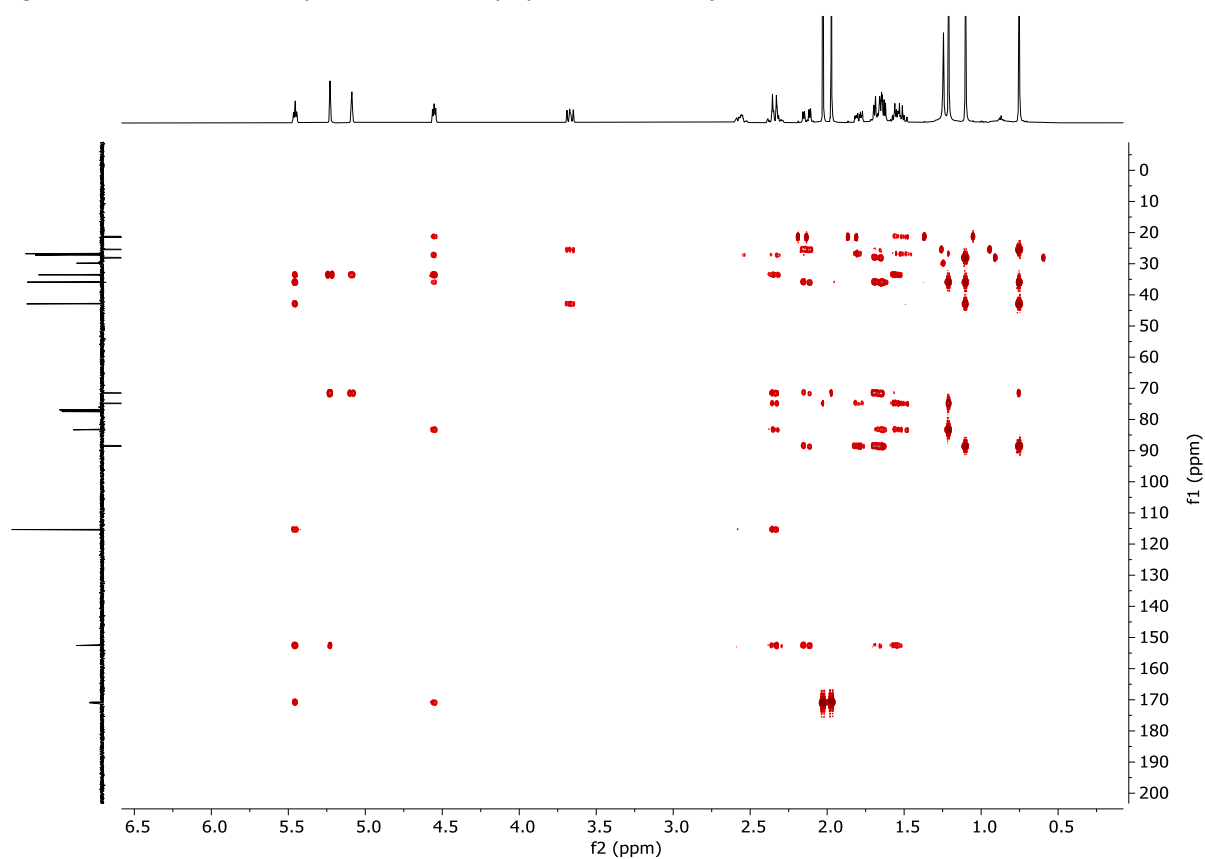
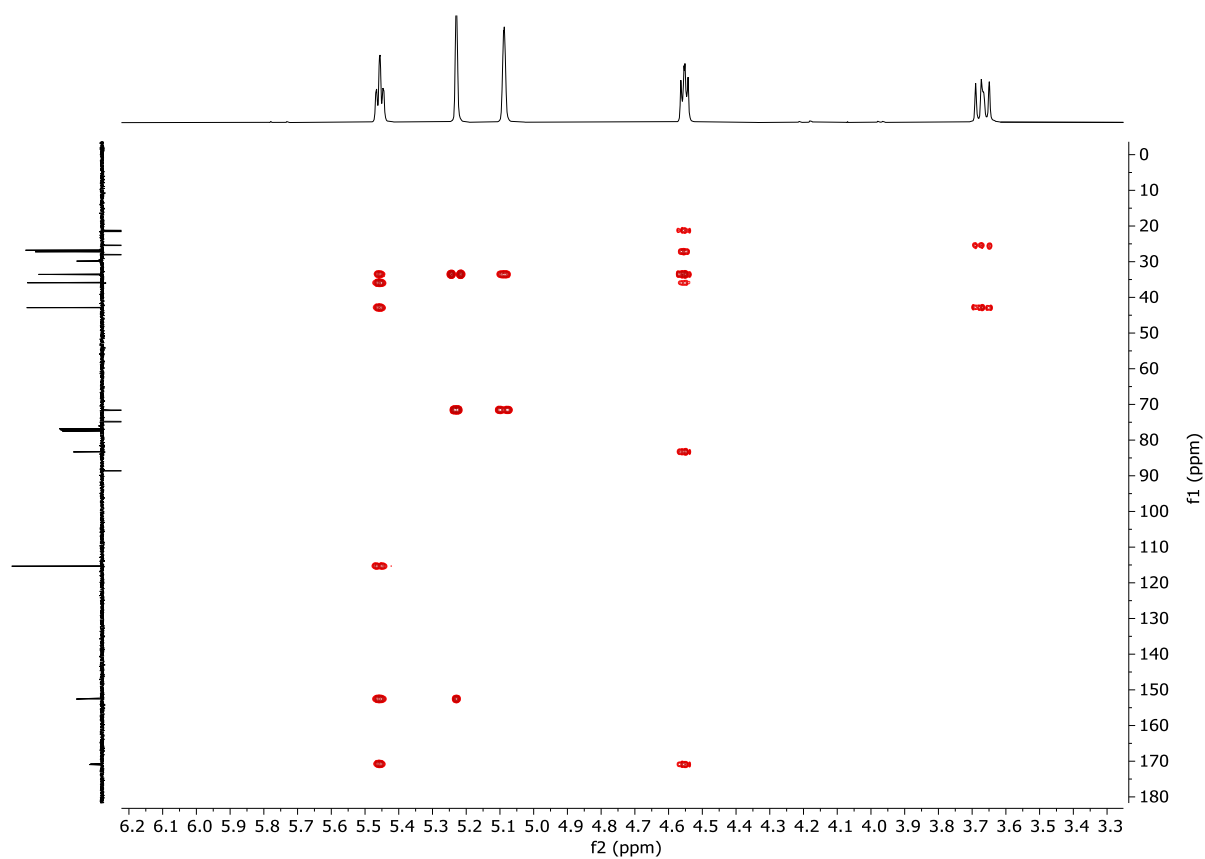
Figure S26. HMBC NMR (500MHz, CDCl_3) spectrum of acetylated **A1**.**Figure S27.** HMBC NMR (500MHz, CDCl_3) spectrum of acetylated **A1** (expansion).

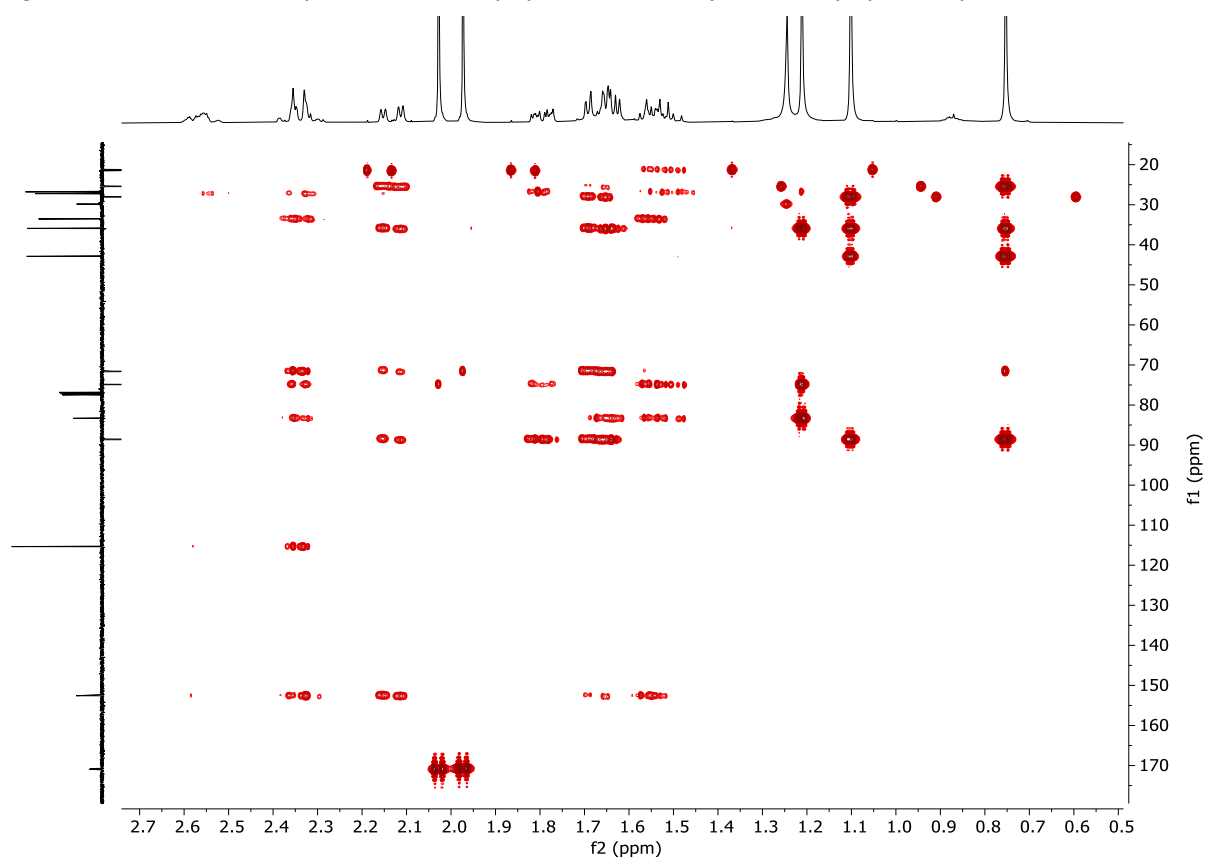
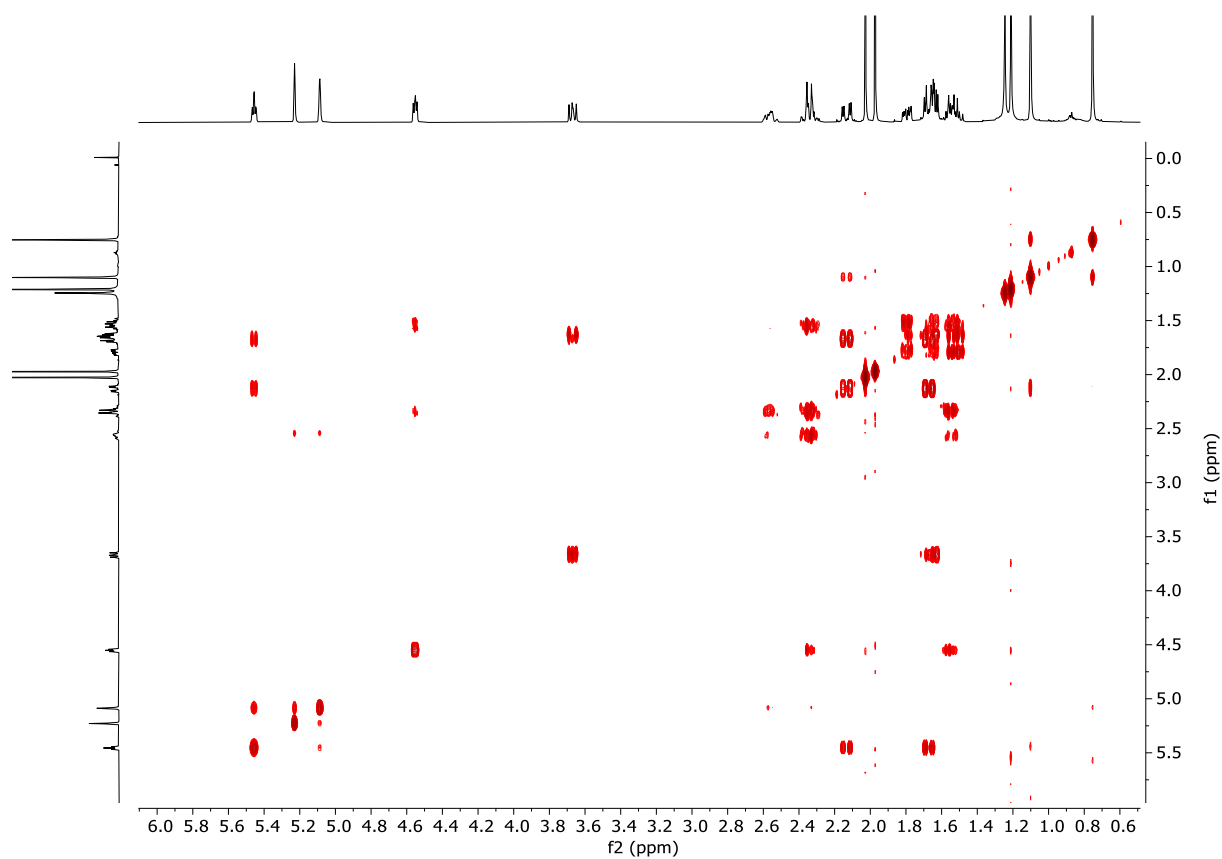
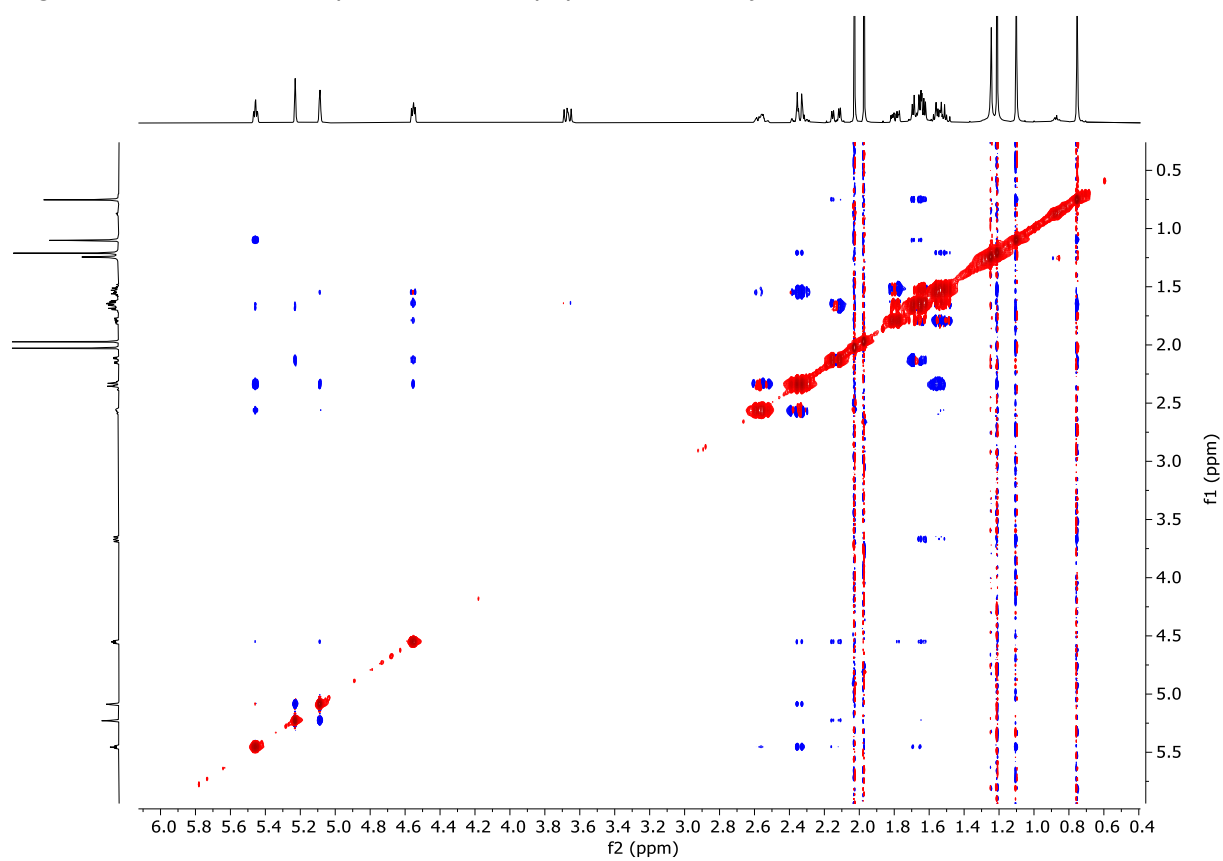
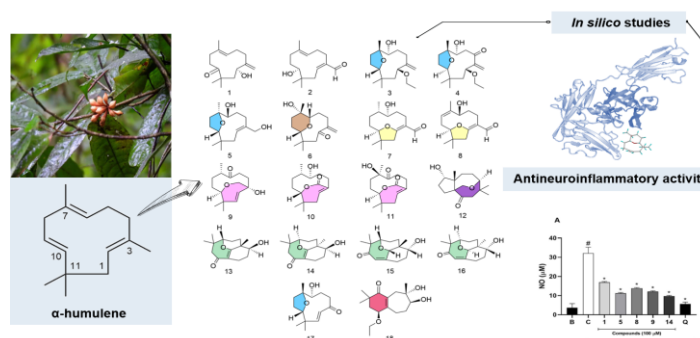
Figure S28. HMBC NMR (500MHz, CDCl_3) spectrum of acetylated **A1** (expansion).**Figure S29.** COSY NMR (500 MHz, CDCl_3) spectrum of acetylated **A1**.

Figure S30. NOESY NMR (500 MHz, CDCl_3) spectrum of acetylated **A1**.

5.2 Artigo 2: Dolichocarpols G-X: Unveiling Novel Macrocyclic Humulene Sesquiterpenoids from *Anaxagorea dolichocarpa*



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ABSTRACT

In our ongoing study of natural products, 11 novel macrocyclic humulene sesquiterpenoids, namely, dolichocarpols G–Q (**1–11**), 5 bicyclic, dolichocarpols R–V (**12–16**), and 2 norsesquiterpenes, dolichocarpols W–X (**17–18**) were isolated from the roots of *Anaxagorea dolichocarpa*. Their structures were unequivocally determined by the analysis of NMR, HRESIMS, and IR data, along with NMR and ECD quantum-mechanical calculations, followed by the application of the DP4+ method. Most compounds possess an ether bridge between different carbons, whereas compounds **13/14** and **15/16** are diastereomers isomerized at C-7/C-10 and at C-7/C-10/C-12, respectively. The antineuroinflammatory activity of compounds **1–18** was tested in an LPS/IFN- γ -stimulated BV2 microglial cell line. Compounds **1**, **5**, **8**, **9** and **14** significantly reduced nitric oxide (NO) levels in a concentration-dependent manner (25–200 μ M). Moreover, possible interactions between these bioactive compounds and inducible nitric oxide synthase (iNOS) were predicted via *in silico studies*. NO is known as an inflammatory mediator in neurodegenerative diseases; therefore, the effects of these compounds indicate their potential antineuroinflammatory activity.

Keywords: *Anaxagorea dolichocarpa*; Annonaceae; Antineuroinflammatory; Humulene.

1. Introduction

Anaxagorea (Annonaceae) is the only genus in the subfamily Anaxagoreoideae, comprising approximately 30 species with a disjunct distribution in the Americas and Southeast Asia (Chatrou et al., 2012; Li and Xu, 2020). Among its species, *Anaxagorea dolichocarpa* Sprague & Sandwith stands out for being widely distributed in South America, where it can be found in Colombia, Venezuela, Costa Rica, the Guianas, and Brazil, primarily in the Amazon rainforest and Atlantic forest regions (Gottsberger, 2016; Maas and Westra, 1984a, 1984b; Pontes et al., 2004).

This species has garnered interest due to the presence of a wide range of bioactive compounds, particularly alkaloids and terpenoids, which are distributed in different parts of the plant, such as the bark, leaves, and roots (Cascaes et al., 2023; Hocquemiller et al., 1981; Lucio et al., 2011; Sales et al., 2020a, 2020b). Due to its rich phytochemical composition, this species has demonstrated a range of pharmacological activities, including antiviral, antinociceptive, antitumor, anti-inflammatory and cytotoxic properties. Additionally, it is notable for its use in traditional medicine, where it is employed in the treatment of colds, flu, and headaches. Moreover, its bark is used as an ingredient in curare and for making fishing lines, dental floss, ropes, and baskets, highlighting its economic importance (Almeida et al., 2011; Brandão et al., 2011; Gripp et al., 2020; Li and Xu, 2020; Lucio et al., 2011; Pinheiro et al., 2016; Schults and Raffauf, 1990).

In our continuing study of humulene-type sesquiterpenoids, we isolated six unprecedented macrocyclic humulenes from *Anaxagorea dolichocarpa*, named dolichocarpols A–F (Sales et al., 2020a), and nordine, which was first obtained from the roots of *Anaxagorea javanica* (Husain et al., 2012). The reanalysis of the ^{13}C spectral data of nordine revealed that it has an ether cycle between carbons C-10 and C-7 instead of between C-10 and C-6 (Andrade et al., 2022). In this work, we report the isolation and structural elucidation of 18 new macrocyclic humulene sesquiterpenoids (Fig. 1) together with the *in vitro* evaluation of their antineuroinflammatory effects using BV2 cells. Additionally, the affinity and stability of the interactions between these compounds and inducible nitric oxide synthase (iNOS) were assessed through molecular docking and molecular dynamics simulations.

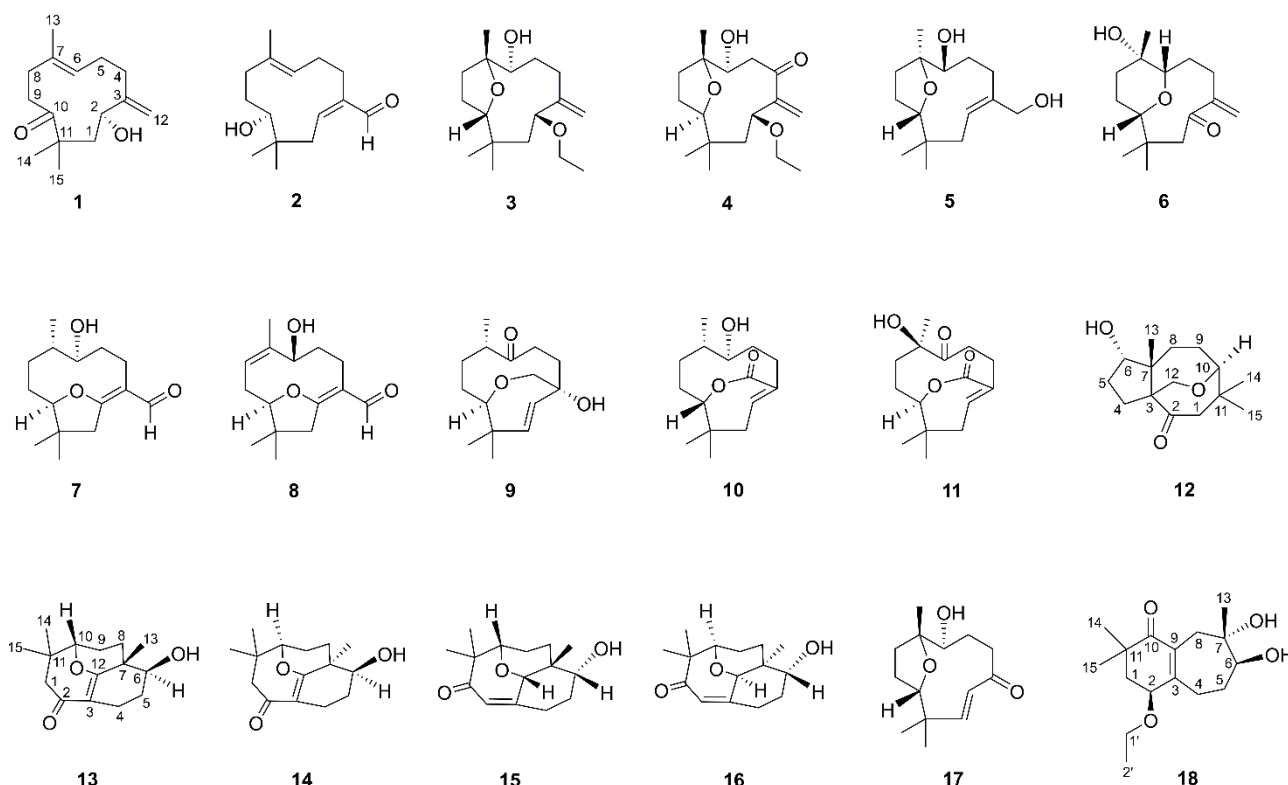


Fig. 1. Chemical Structures of Compounds 1–18.

2. Results and discussion

2.1. Isolation and structure elucidation

The roots of *Anaxagorea dolichocarpa* were subjected to dehydration followed by extraction with ethanol three times at room temperature. The resulting ethanolic extract was suspended in a MeOH-H₂O solution (7:3) and subjected to sequential partitioning utilizing *n*-hexane, chloroform, and ethyl acetate (EtOAc). The fractions soluble in *n*-hexane and chloroform were further separated via silica gel chromatography and medium-pressure liquid chromatography, respectively. The fractions obtained were subsequently purified through preparative high-performance liquid chromatography (HPLC), resulting in the isolation of 11 new macrocyclic humulene-type sesquiterpenoids: dolichocarpols G–J (**1–4**), L (**6**), Q–V (**11–16**) and 2 new nor-sesquiterpenes, dolichocarpols W and X (**17–18**), all from the *n*-hexane phase. Additionally, 5 more were isolated from the chloroform phase: dolichocarpols K (**5**) and M–P (**7–10**).

Compound **1** was obtained as a colorless oil with a positive optical rotation of $[\alpha]^{20}_{\text{D}} +35.1$ (c. 0.1, CHCl_3). The molecular formula was determined to be $\text{C}_{15}\text{H}_{24}\text{O}_2$ on the basis of the HRESIMS ion at m/z 237.1859 $[\text{M} + \text{H}]^+$ (calcd. for $\text{C}_{15}\text{H}_{25}\text{O}_2$, 237.1849, $\Delta = -4.3$ ppm), with four indexes of hydrogen deficiency. The IR spectrum (Fig. S32) displayed characteristic absorptions for hydroxy (3433 cm^{-1}) and ketone carbonyl (1718 cm^{-1}) groups. The 1D NMR data (Tables 1 and 2) were characteristic of macrocyclic humulene-type sesquiterpenes. The ^{13}C NMR spectra confirmed the presence of 15 carbons ascribable to three methyls, six methylenes (five aliphatics and one olefinic at δ_{C} 112.1), two methines (one oxymethine at δ_{C} 71.5 and one olefinic at δ_{C} 126.0), and four quaternary carbons (one aliphatic at δ_{C} 48.2, one carbonyl at δ_{C} 215.7 and two olefinic at δ_{C} 134.8 and δ_{C} 156.6). The ^1H NMR data (Table 2) revealed three singlet methyl groups (δ_{H} 1.59, δ_{H} 1.19, δ_{H} 1.20), an olefinic proton [δ_{H} 5.12 (dd, $J = 10.5, 5.5$ Hz)], an oxymethine [δ_{H} 3.60 (d, $J = 9.0$ Hz)], and two olefinic protons (δ_{H} 5.04 s, δ_{H} 4.93 s). In the HMBC spectrum (Fig. 2), the correlations from $\text{H}_2\text{-1}$ to C-3, C-10, C-14, and C-15, along with the correlations from $\text{H}_2\text{-12}$ to C-2, C-3, and C-4, established the carbonyl at C-10, as well as a double bond between C-3 and C-12. Additionally, the correlations from $\text{H}_3\text{-13}$ to C-6 and C-8 located the second double bond between C-6 and C-7. In the COSY spectrum (Fig. 2), a sequence of correlations between $\text{H}_2\text{-8}/\text{H}_2\text{-9}$ and $\text{H-6}/\text{H}_2\text{-5}/\text{H}_2\text{-4}$ was observed along with the correlation between H-2 and $\text{H}_2\text{-1}$, which was consistent with the proposed structure. The relative configuration was deduced from the NOESY data (Fig. 3) through correlations from H-2 to $\text{H}_3\text{-15}$, $\text{H-4}\beta$ and $\text{H-1}\beta$, which suggested that these protons were cofacial and β -oriented. To establish the absolute configuration of compound **1**, ECD analyses were conducted. The results demonstrated that the calculated ECD spectrum for 2S matched the experimental ECD spectrum, allowing assignment of the absolute configuration of compound **1** as 2S (Fig. S31). Therefore, the structure of **1** was established, and it was named dolichocarpol G.

Compound **2** was isolated as a colorless oil. The molecular formula was established as $\text{C}_{15}\text{H}_{24}\text{O}_2$, implying four indexes of hydrogen deficiency, on the basis of HRESIMS $[\text{M} + \text{H}]^+$ at m/z 237.1855 (calcd. for $\text{C}_{15}\text{H}_{25}\text{O}_2$, 237.1849, $\Delta = -2.5$ ppm). The IR spectrum (Fig. S40) showed absorption bands at 3443 cm^{-1} (hydroxy), 1714 cm^{-1} (carbonyl) and 1685 cm^{-1} (double bond). The ^1H NMR data (Table 2) resembled those of **1**, comprising characteristic signals for olefinic protons at δ_{H} 5.11 (m) and δ_{H} 6.56 (dd, $J = 12.0, 3.0$ Hz), oxymethine at δ_{H} 3.18 (d, $J = 6.5$ Hz) and

aldehydic protons [δ_{H} 9.34 (d, $J = 1.0$ Hz)]. The ^{13}C NMR spectrum (Table 1) exhibited 15 carbon signals, including two double bonds (δ_{C} 152.9/142.9 and δ_{C} 136.8/124.9), one oxymethine (δ_{C} 76.2) and one aldehyde carbonyl (δ_{C} 195.6). As with compound **1**, the long-range HMBC correlations (Fig. 2) from H-10 to C-1, C-14, C-15 and from H₃-13 to C-6, C-7, and C-8 established a hydroxy group at C-10 and a double bond between C-6/C-7, respectively. Additionally, the cross peaks from H-2 to C-4 and C-12 were attributed to the aldehyde carbonyl at C-12. In a manner similar to that of **1**, the COSY spectra (Fig. 2) revealed correlations between protons H-10/H₂-9/H₂-8, H-6/H₂-5/H₂-4 and H-2/H₂-1. The NOESY spectrum showed correlations between H-2 and H-12 and from H-10 to H-2, H-6 and H₃-15, indicated that they are cofacial. Finally, the comparison of the ECD experimental spectra with the calculated one led us to assign the absolute configuration of 10*S* for **2** (Fig. 4). Hence, the structure of **2** was determined, and it was designated as dolichocarpol H.

Compound **3** was isolated as a colorless oil. The HRESIMS spectrum showed the $[\text{M} + \text{H}]^+$ ion at m/z 283.2259 (calcd. for $\text{C}_{17}\text{H}_{31}\text{O}_3$, 283.2268, $\Delta = 3.0$ ppm), which is compatible with the molecular formula of $\text{C}_{17}\text{H}_{30}\text{O}_3$ and corresponds to three indexes of hydrogen deficiency. The ^{13}C NMR data (Table 1) were similar to those of nordine (Andrade et al., 2022), but with two additional signals at δ_{C} 63.3 and δ_{C} 15.6 (ethoxy group) and the deshielding of C-2 ($\Delta\delta_{\text{C}} +7.5$). In the ^1H NMR spectrum (Table 2), the two double quartets at δ_{H} 3.37 and 3.26 (dq, $J = 14.0, 7.0$ Hz) corresponding to the oxymethylene hydrogens and the triplet at δ_{H} 1.12 (t, $J = 7.0$ Hz) attributed to the methyl CH_3 -2', along with the HMBC correlations from H-2 to C-1, C-1', C-3, C-11 and C-12, confirmed the ethoxy group at C-2. The remaining correlations were identical to those observed in nordine, confirming the ether bridge between C-10 and C-7. To establish the relative configurations of C-2, C-6, C-7 and C-10, GIAO-DFT calculations of the ^{13}C and ^1H nuclear magnetic shielding constants (σ) were conducted for all 8 possible diastereomers. The DP4+ methodology was applied, resulting in a probability of 100% for the candidate with the 2*R**,6*S**,7*R**,10*S** configuration (Table S3). Thus, the absolute configuration was determined by comparing the calculated ECD data with the experimental ECD spectra, which closely matched, and consequently, the absolute configuration was established as (2*R*,6*S*,7*R*,10*S*) (Fig. 4). Therefore, the structure of **3** was determined, and it was named dolichocarpol I.

Table 1
¹³C NMR spectroscopic data for compounds **1–18** in CDCl₃ (δ_{H} in ppm, *J* in Hz)

No.	1 ^a	2 ^a	3 ^a	4 ^a	5 ^b	6 ^a	7 ^a	8 ^a	9 ^b	10 ^a	11 ^a	12 ^a	13 ^a	14 ^b	15 ^a	16 ^a	17 ^a	18 ^a
1	44.0	39.5	44.1	47.4	36.7	51.2	41.1	41.1	137.9	38.4	38.7	54.5	56.2	55.8	206.3	205.8	140.9	41.2
2	71.5	152.9	76.8	73.3	131.1	199.9	176.7	176.0	132.8	135.2	136.7	212.5	202.4	203.3	127.1	126.8	134.0	74.6
3	156.3	142.9	153.6	156.4	133.4	153.5	115.3	116.2	75.2	139.2	136.9	65.1	126.8	124.2	157.4	159.8	205.5	159.4
4	36.6	24.2	33.9	203.3	29.6	29.2	19.0	19.2	34.9	33.9	31.4	31.7	21.3	19.2	32.8	31.3	40.2	23.9
5	30.1	26.1	30.3	40.0	32.7	24.6	33.5	32.3	40.3	34.4	32.6	30.8	26.9	26.7	32.5	30.3	27.7	28.9
6	126.0	124.9	73.8	77.2	76.0	83.4	67.6	67.2	211.5	75.4	215.9	84.1	72.2	74.6	76.5	73.3	75.7	77.2
7	134.8	136.8	84.4	84.7	83.6	70.2	31.8	141.3	47.0	39.1	80.5	49.7	44.1	42.6	42.1	42.1	85.2	71.6
8	34.8	33.6	35.3	35.0	36.7	40.5	32.0	122.4	30.6	24.0	30.3	30.4	33.1	31.3	21.5	28.3	36.0	31.9
9	34.5	34.8	26.5	25.5	26.6	24.0	26.3	29.6	28.5	23.9	25.8	26.9	21.1	22.3	19.8	20.0	27.4	132.0
10	215.7	76.2	88.2	86.4	88.0	83.4	92.7	87.3	84.0	86.0	85.7	81.1	87.6	87.4	76.6	76.9	87.3	202.9
11	48.2	39.1	35.8	35.8	37.4	41.1	40.2	39.0	39.2	42.3	42.0	37.0	39.5	39.6	53.6	53.5	39.3	41.1
12	112.1	195.6	113.1	118.2	62.7	114.5	189.4	189.8	70.5	171.1	170.6	70.2	168.4	169.0	80.6	79.9	-	-
13	16.4	19.7	20.4	21.8	27.8	20.5	14.2	17.0	30.5	16.6	24.8	25.5	17.1	22.0	24.2	21.2	22.3	25.1
14	26.4	24.5	28.4	29.7	27.8	26.8	29.1	29.8	35.4	31.3	25.6	27.0	25.2	31.4	22.0	27.1	28.7	25.1
15	22.2	21.5	25.4	26.2	27.4	21.4	21.8	21.6	30.5	25.4	31.3	29.7	31.4	24.9	27.3	22.0	30.0	26.5
1'	-	-	63.3	63.2	-	-	-	-	-	-	-	-	-	-	-	-	-	65.1
2'	-	-	15.6	15.4	-	-	-	-	-	-	-	-	-	-	-	-	-	15.6

^a Recorded at 125 MHz.

^b Recorded at 100 MHz.

Table 2¹H NMR spectroscopic data for compounds **1–9** in CDCl₃ (δ_{H} in ppm, *J* in Hz)

No.	1 ^a	2 ^a	3 ^a	4 ^a	5 ^b	6 ^a	7 ^a	8 ^a	9 ^b
1a	1.94 dd (15.0, 1.0)	2.48 dd (16.0, 12.0)	1.91 dd (15.0, 4.5)	1.89 dd (14.0, 10.0)	2.36 m	2.52 d (11.5)	2.94 d (18.0)	2.94 d (18.0)	5.55 dd (12.0, 0.8)
1b	1.57 dd (15.0, 9.0)	1.90 dd (16.0, 3.0)	1.51 dd (14.5, 4.5)	1.69 dl (14.0)	1.94 m	2.27 d (11.5)	2.77 d (18.0)	2.77 d (18.0)	-
2	3.60 d (9.0)	6.56 dd (12.0, 3.0)	3.98 t (4.0)	4.30 dl (11.0)	5.65 dd (10.8, 4.0)	-	-	-	5.24 dd (12.0, 2.4)
3	-	-	-	-	-	-	-	-	-
4a	2.34 m	2.57 m	2.40 m	-	2.35 m	2.67 m	2.53 m	2.41 dt (13.5, 4.5)	2.49 td (12.0, 1.2)
4b	2.10 m	1.97 m	2.40 m	-	2.16 m	2.33 m	2.23 td (13.5, 5.0)	2.26 dd (13.5, 4.5)	1.55 m
5a	2.19 m	2.19 m	2.23 m	3.24 dd (13.5, 3.5)	2.20 m	1.72 m	1.67 m	1.91 m	2.87 dd (12.0, 17.2)
5b	2.08 m	2.19 m	1.53 m	2.55 dd (13.5, 7.5)	1.51 m	1.72 m	1.49 m	1.76 m	2.06 dd (8.0, 17.2)
6	5.12 dd (10.5, 5.5)	5.11 m	3.31 t (4.5)	3.95 dd (7.5, 3.5)	3.51 sl	2.88 dd (4.0, 2.0)	3.47 dd (11.5, 3.5)	4.22 dd (11.0, 4.0)	-
7	-	-	-	-	-	-	2.56 m	-	2.41 m
8a	2.67 td (12.5, 2.0)	2.25 m	2.09 m	1.85 m	1.94 m	1.86 m	1.60 m	5.37 ddd (11.5, 5.5, 1.0)	1.77 m
8b	1.97 m	1.80 ddd (15.5, 13.5, 2.5)	1.53 m	1.61 m	1.55 m	1.54 m	1.60 m	-	1.58 m
9a	3.00 td (13.0, 2.0)	1.70 ddd (15.5, 13.5, 2.5)	1.64 m	1.65 m	1.76 m	1.56 m	1.60 m	2.22 dd (12.0, 4.0)	1.71 m
9b	2.00 m	1.40 m	1.60 m	1.59 m	1.55 m	1.47 m	1.50 m	2.04 m	1.71 m
10	-	3.18 d (6.5)	3.65 dd (10.5, 5.5)	3.42 dd (11.0, 5.0)	3.67 dd (9.2, 6.0)	2.86 dd (3.5, 2.0)	4.19 dd (11.5, 2.5)	3.95 dd (12.0, 3.5)	3.68 dd (8.8, 3.6)
11	-	-	-	-	-	-	-	-	-
12a	5.04 s	9.34 d (1.0)	5.21 s	5.81 s	4.23 d (11.2)	5.22 s	9.57 s	9.61 s	4.02 dd (12.4, 2.4)
12b	4.93 s	-	5.03 s	5.68 s	3.98 d (11.2)	5.20 s	-	-	3.62 dd (12.4, 2.4)
13	1.59 s	1.50 s	1.18 s	1.23 s	1.15 s	1.14 s	0.85 d (6.5)	1.79 s	1.02 d (6.8)
14	1.19 s	0.93 s	0.74 s	0.82 s	0.87 s	0.97 s	1.17 s	1.22 s	1.36 s
15	1.20 s	1.20 s	1.15 s	1.10 s	1.15 s	1.00 s	1.14 s	1.17 s	1.00 s
1'a	-	-	3.37 dq (9.5, 7.0)	3.34 dq (9.5, 2.5)	-	-	-	-	-
1'b	-	-	3.26 dq (9.0, 7.0)	3.31 dq (9.5, 2.5)	-	-	-	-	-
2'	-	-	1.13 t (7.0)	1.13 t (7.0)	-	-	-	-	-

^a Recorded at 500 MHz.^b Recorded at 400 MHz.

Compound **4** was obtained as a colorless oil, and its HRESIMS data revealed the $[M + Na]^+$ ion peak at m/z 319.1871 (calcd. for $C_{17}H_{28}NaO_4$, 319.1880, $\Delta = 2.8$ ppm), which is consistent with the molecular formula of $C_{17}H_{28}O_4$, implying an index of hydrogen deficiency of four. The analysis of the 1D NMR data (Tables 1 and 2) indicated that **4** highly resembled **3**, except for the presence of a ketone carbonyl signal (δ_C 203.3) and the deshielding of C-5 ($\Delta\delta_C +9.7$) and C-12 ($\Delta\delta_C +5.3$), which suggested its location at C-4. In the HMBC spectrum (Fig. 2), the correlations of H-2, H-5, H-6 and H₂-12 with C-4 confirmed the position of the carbonyl, whereas the remaining correlations were the same as those observed in **3**. The NOESY correlations (Fig. 3) from H₃-13 to H-8 β and H-6 suggested that these protons adopted the β -orientation, whereas the correlations from H₃-14 to H-10 and H-1 α suggested that they were cofacial and adopted the α -orientation. Thus, the relative configurations of C-2, C-6, C-7 and C-10 were assigned through calculations of the ^{13}C and 1H σ values of all 8 possible diastereomers. The DP4+ method indicates a probability of 97.63% for the candidate 2*S**,6*R**,7*S**,10*S** (Table S3). Finally, the absolute configuration was found to be 2*R*,6*S*,7*R*,10*R* on the basis of a comparison of the experimental and calculated ECD spectra (Fig. S31). Therefore, the structure of **4** was determined and named dolichocarpol J.

Compound **5** was obtained as a colorless oil with a molecular formula of $C_{15}H_{26}O_3$ on the basis of its HRESIMS peak at m/z 277.1783 $[M + Na]^+$ (calcd. to $C_{15}H_{26}NaO_3$, 277.1774 $\Delta = -3.2$ ppm), indicating that the hydrogen deficiency index is equal to three. Unlike that of compound **4**, the IR spectrum did not show carbonyl group absorption, but the absorptions of the hydroxy group (3450 cm^{-1}) and double bond (1641 cm^{-1}) remained. The 1D NMR data (Tables 1 and 2) of **5** were similar to those of **3**, except for the double bond between C-3/C-12 which was replaced by an oxymethylene group (δ_C 62.8) and a double endo bond (δ_C 131.2/133.6). The HMBC cross-peaks (Fig. 2) from H-2 to C-1, C-3, C-4, C-11 and C-12 located the double bond between C-2/C-3 and the oxymethylene group at C-12. As in compounds **3** and **4**, the values of C-10 (δ_C 88.0) and C-7 (δ_C 83.6) were compatible with the ether bridge between these carbons. To confirm this proposal, an acetylated derivative was obtained, and its HRESIMS data revealed a sodium adduct ion peak $[M + Na]^+$ at m/z 361.1995 (calcd. for $C_{19}H_{30}NaO_5$, 361.1985, $\Delta = -2.5$ ppm), which was compatible with the molecular formula $C_{19}H_{30}O_5$, confirming the double acetylation. In the ^{13}C and 1H NMR data of acetylated **5** (Figs. S74–75), the presence of two carbonyls (δ_C 171.6, δ_C 170.9) and two methyls (δ_C 21.4, δ_C 21.2) and the deshielding of H-6 ($\Delta\delta_H +0.96$) and H₂-12 ($\Delta\delta_H +0.55$, $+0.58$) corroborated the acetylation at these positions and confirmed the proposed structure. The relative configuration was determined through ^{13}C

and ^1H NMR calculations of the 4 possible diastereomers, along with the DP4+ method, which indicated a probability of 100% for the candidate $6S^*,7R^*,10R^*$ (Table S3). Lastly, the similarity of the experimental and calculated ECD spectra established the absolute configuration of $6R,7S,10S$. Thus, it was possible to determine the structure of **5**, which was named dolichocarpol K.

Compound **6** was isolated as a colorless oil. Its molecular formula was established as $\text{C}_{15}\text{H}_{24}\text{O}_3$ on the basis of its HRESIMS peak at m/z 275.1616 $[\text{M} + \text{Na}]^+$ (calcd. for $\text{C}_{15}\text{H}_{24}\text{NaO}_3$, 275.1618 $\Delta = 0.6$ ppm), corresponding to four indexes of hydrogen deficiency. Further analyses of the 1D NMR data (Tables 1 and 2) revealed that they resembled those of **3–5**, suggesting a similar humulene backbone. However, key differences were observed for the values of C-6 ($\Delta\delta_{\text{C}} +7.7$) and the shielding for C-10 ($\Delta\delta_{\text{C}} -4.13$), C-7 ($\Delta\delta_{\text{C}} -14.0$) and C-5 ($\Delta\delta_{\text{C}} -9.70$), which suggested an ether fusion between C-10 and C-6. In the HMBC spectrum (Fig. 2), the mutual correlations from H_2 -12 to C-2, C-3 and C-4 associated with those of H-10 to C-1, C-6, C-8, C-11 and from H-6 to C-4, C-8, C-10 and C-13 placed the carbonyl at C-2, an exocyclic double bond between C-3/C-12 and confirmed the ether bridge between C-10/C-6. The NOESY correlations (Fig. 3) from H-10 to H_3 -15 and from H-6 to H_3 -13/H-4 β indicated that these protons were cofacial and β -oriented. NMR calculations of all 4 possible diastereomers confirmed the relative configuration, and the DP4+ methodology indicated a probability of 100% for the candidate $6R^*,7R^*,10R^*$ (Table S3). To conclude, the ECD calculated spectrum for $6S,7S,10S$ closely matched the experimental spectrum, thereby establishing the absolute configuration as $6S,7S,10S$ (Fig. 4). Therefore, the structure of **6** was determined, and it was named dolichocarpol L.

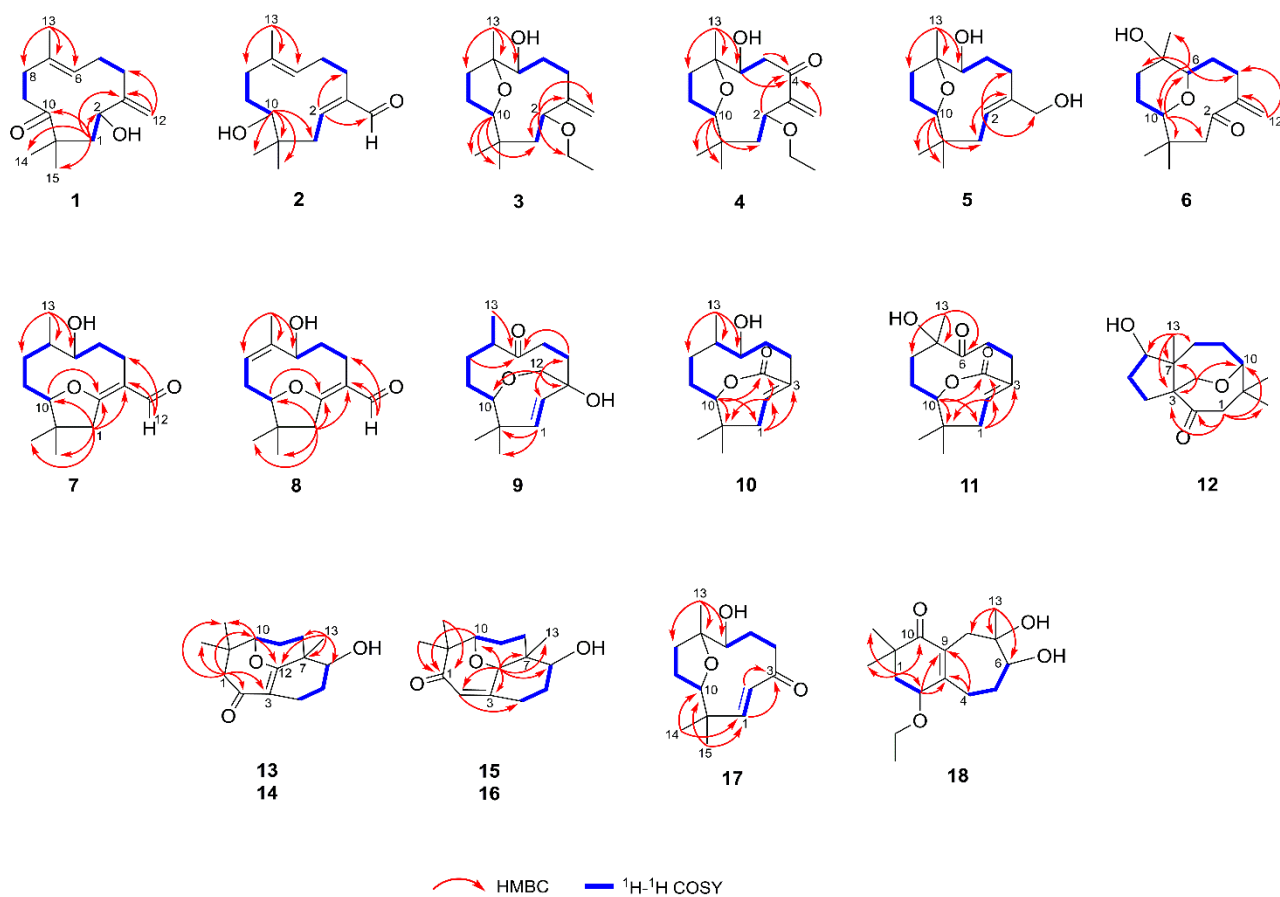


Fig. 2. Key HMBC and ^1H - ^1H COSY correlations of compounds 1-18.

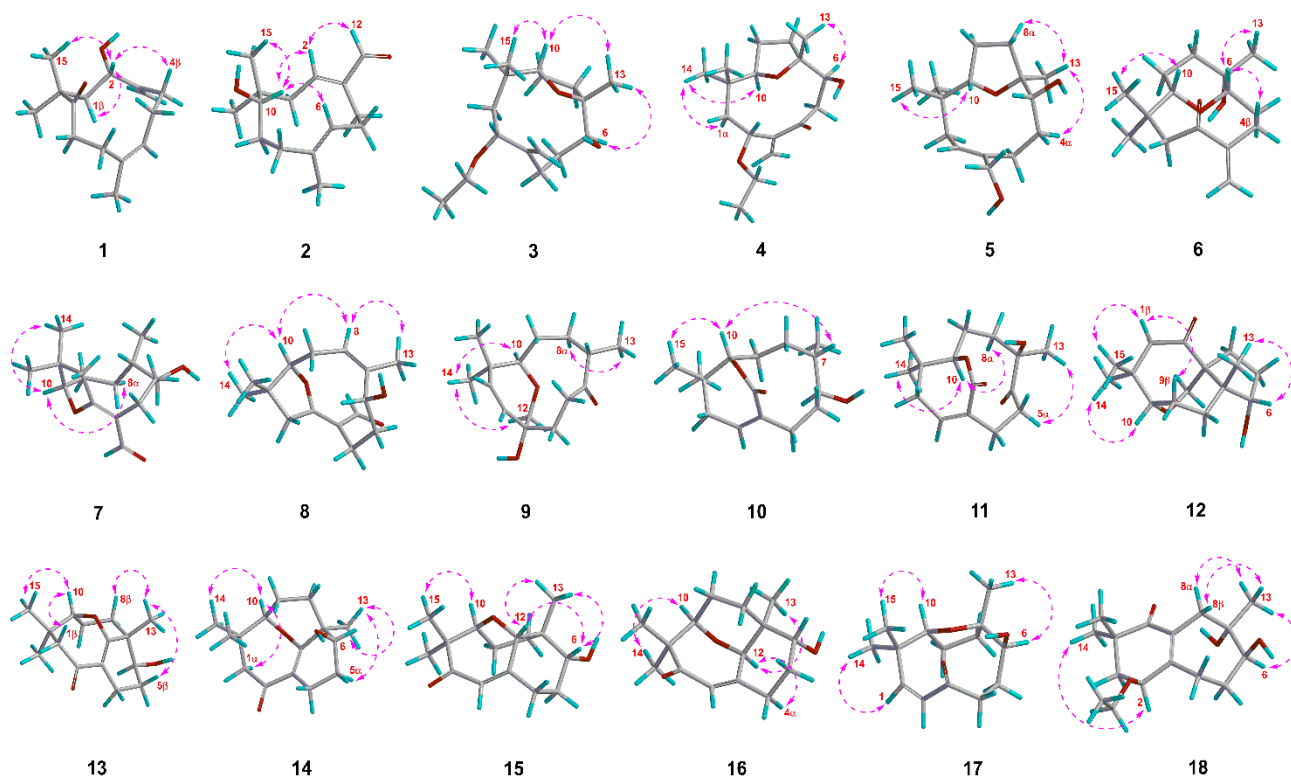


Fig. 3. Key NOESY correlations of compounds 1-18.

Compounds **7** and **8** were obtained as colorless oils. For **7**, the HRESIMS data established the molecular formula of $C_{15}H_{24}O_3$ on the basis of the $[M + H]^+$ ion at m/z 253.1793 (calc. for $C_{15}H_{25}O_3$, 253.1798 Δ = 2.2 ppm), indicating four indexes of hydrogen deficiency. For **8**, the molecular formula was determined to be $C_{15}H_{22}O_3$ $[M + Na]^+$ at m/z 273.1469 (calcd. for $C_{15}H_{22}NaO_3$, 273.1461 Δ = -3.0 ppm), corresponding to five indexes of hydrogen deficiency. Their 1D and 2D NMR data (Tables 1, 2 and Fig. 2) resembled those of dolichocarpol B (Sales et al., 2020a), suggesting that they are macrocyclic humulene sesquiterpenes with an ether bridge between C-10 and C-2. However, the HSQC spectra (Figs. S93 and S101) of **7** and **8** showed absorptions at δ_H 9.57 s/ δ_C 189.4 and δ_H 9.61 s/ δ_C 189.8, respectively, which was consistent with an aldehyde carbonyl rather than an acid. Additionally, the 1H spectrum of **8** showed a singlet at δ_H 1.79, characteristic of a methyl group above a double bond (δ_C 141.3/122.4), which was not observed in **7**. The HMBC correlations of compound **8** from H_3 -13 to C-6, C-7, C-8, and from H-12 to C-3 and C-4 defined the position of the double olefin bond between carbons C-7/C-8 and the aldehyde group at C-12, respectively. To determine the relative configuration, ^{13}C and 1H NMR calculations were performed on all 4 possible diastereomers of compound **7** and the 2 possible diastereomers of compound **8**. The DP4+ methodology indicates relative configurations of 6*S**,7*S**,10*R** for **7** and 6*R**,10*R** for **8**, with probabilities of 99.94% and 100%, respectively (Table S3). Finally, the absolute configurations were determined by comparing the experimental and theoretical ECD spectra, establishing the configurations as 6*S*,7*S*,10*R* for **7** and 6*R*,10*R* for **8**. Consequently, the structures of compounds **7** and **8** were assigned and named dolichocarpols M and N, respectively.

Compound **9** was isolated as a colorless oil. The molecular formula was established as $C_{15}H_{24}O_3$ based on the ion at m/z 253.1793 $[M + H]^+$ (calcd for $C_{15}H_{25}O_3$, 253.1798, Δ = 1.9 ppm) from its HRESIMS data, indicating an index of hydrogen deficiency equal to four. The ^{13}C NMR data (Table 1) revealed 15 signals, including signals for two olefinic carbons (δ_C 137.9/132.8), one oxymethylenic carbon (δ_C 84.0), one nonhydrogenated carbon (δ_C 75.2), one oxymethylenic carbon (δ_C 70.5) and one carbonyl (δ_C 211.5). The 1H NMR data (Table 2) revealed a doublet at δ_H 1.02 (d, J = 6.8 Hz, 3H) attributed to CH_3 -13, suggesting that an ether bridge did not form between C-10 and C-7, as in **3–5**. In the HSQC spectrum (Fig. S109), two doublets (δ_H 5.55, dd, J = 12.0, 0.8 Hz; δ_H 5.24 dd, J = 12.0, 2.4 Hz) were associated with the protons of the C-1 (δ_C 137.9) and C-2 (δ_C 132.8) carbons, which agreed with the correlations from H-1 to H-2/H-10 and from H-2 to H-1/H-12 observed in the COSY spectra (Fig. 2). The mutual HMBC correlations from H_3 -13, H-8, H-5 and H-4 to

the carbon at δ_c 211.5 placed the carbonyl at C-6. Similarly, the correlations from H-1 to C-10, C-11, C-15 and from H-2 to C-11/C-12 confirmed the presence of a double bond between C-1 and C-2. Additionally, the cross-peak correlation from H₂-12 to C-2, C-3, C-4 and C-10 established a new ether fusion between C-10/C-12/C-3. Moreover, the NOESY correlations of H-10/H₃-14, H-12 α /H₃-14 and H₃-13/H-8 α suggested that all these protons adopted the α -orientation. Additionally, the DP4+ methodology, which is based on the calculated values of ^{13}C and ^1H σ in the 4 possible diastereomers, indicated a probability of 100% for the relative $3R^*,7S^*,10R^*$ configuration (Table S3). Thus, comparison of the experimental ECD spectrum with the calculated one established that the absolute configuration of **9** was $3R,7S,10R$ (Fig. 4). Therefore, the structure was determined, and **9** was named dolichocarpol O.

Compounds **10** and **11** were obtained as colorless oils. Their IR spectra showed bands at 3481/3437 cm^{-1} and 1709/1707 cm^{-1} characteristic of hydroxy and carbonyl groups, respectively. For **10**, a molecular formula of $\text{C}_{15}\text{H}_{24}\text{O}_3$ was established from the HRESIMS data at m/z 275.1622 $[\text{M} + \text{Na}]^+$ (calcd. for $\text{C}_{15}\text{H}_{24}\text{NaO}_3$, 275.1618 $\Delta = -1.7$ ppm), corresponding to four indexes of hydrogen deficiency. In **11**, HRESIMS showed the $[\text{M} + \text{H}]^+$ ion peak at m/z 267.1593 (calcd. for $\text{C}_{15}\text{H}_{23}\text{O}_4$, 267.1591, $\Delta = -0.6$ ppm), which was compatible with the molecular formula $\text{C}_{15}\text{H}_{22}\text{O}_4$. Like that of compound **9**, the ^1H NMR data (Table 3) of **10** revealed a doublet for the CH_3 -13 (δ_{H} 1.01, d, $J = 6.8$ Hz, 3H), which was absent in **11**. The ^{13}C NMR data (Table 1) of both compounds showed the presence of 15 carbons. The common shifts at δ_c 171.1/170.6, δ_c 139.2/136.9 and δ_c 135.2/136.7 were characteristic of an ester carbonyl and a double bond. Additionally, **11** contained a nonhydrogenated carbinolic carbon (δ_c 80.5) and a carbonyl (δ_c 215.9), whereas **10** contained an oxymethine (δ_c 75.4). In the HMBC spectra of both compounds (Fig. 2), the cross-peaks from H-10 and H-2 to C-12, C-11 and C-1 defined the fusion via C-10/C-12/C-3 through a lactone instead of an ether, as in the previous compounds. Additionally, in **10**, the mutual correlation from H₃-13 to C-6, C-7 and C-8 defined the position of the OH group at C-6, whereas the correlation from H₂-1 to C-2/C-3 established a double bond between these carbons. On the other hand, in **11**, the cross-peaks from H₃-13 to C-6, C-7 and C-8 established the position of the ketone carbonyl at C-6. In the COSY spectrum (Fig. 2), the correlations between H-10/H₂-9 in both compounds, together with the sequence of correlations between H-6/H-7/H₂-5 present only in **10**, were consistent with the proposed structures. ^{13}C and ^1H NMR data were calculated for 4 possible diastereomers of compound **10** and 2 possible diastereomers of compound **11**. The DP4+ methodology confirmed the relative configurations of $6R^*,7R^*,10R^*$ for **10** and $7S^*,10S^*$ for

11, with probabilities of 100% and 99.53%, respectively (Table S3). Lastly, through an careful comparison of both experimental and calculated ECD spectra, it was determined that the absolute configurations of compounds **10** and **11** are 6*S*,7*S*,10*S* and 7*R*,10*R*, respectively. As a result of this detailed analysis, the structures of **10** and **11** were conclusively established and have been named dolichocarpol P and dolichocarpol Q, respectively.

Compound **12** was obtained as a colorless oil, and its IR spectrum revealed hydroxy (3437 cm^{-1}) and carbonyl (1688 cm^{-1}) groups. The molecular formula was determined to be $\text{C}_{15}\text{H}_{24}\text{O}_3$ by HRESIMS on the basis of analysis of the ion at m/z 275.1627 $[\text{M} + \text{Na}]^+$ (calcd. for $\text{C}_{15}\text{H}_{24}\text{NaO}_3$, 275.1618 $\Delta = -3.5\text{ ppm}$), corresponding to four indexes of hydrogen deficiency. The ^{13}C NMR data (Table 1) disclosed a total of 15 carbon resonances, including those of one carbonyl group (δ_{C} 212.5), two oxymethines (δ_{C} 84.1, 81.1), three nonhydrogenated carbons, six methylenic carbons (one oxygenated at δ_{C} 70.2) and three methyls (CH_3 -13: δ_{C} 25.5; CH_3 -14: δ_{C} 27.0; CH_3 -15: δ_{C} 29.7). The ^1H NMR data (Table 3) revealed three single signals corresponding to the methyl groups, with the two doublets at δ_{H} 4.63 (d, $J = 12.0\text{ Hz}$) and δ_{H} 3.59 (d, $J = 12.0\text{ Hz}$) corresponding to H_2 -12 (oxymethylenic), and signals at δ_{H} 3.87 (dd, $J = 7.0, 2.0\text{ Hz}$) and δ_{H} 3.73 (td, $J = 5.0, 1.0\text{ Hz}$), corresponding to the two oxymethines. The HMBC cross peaks (Fig. 2) from H_2 -12 to C-2, C-3, C-4, and C-7 combined with those from H_3 -13 to C-3, C-6, C-7 and C-8 established a new C-C fusion between C-3/C-7 not found in compounds **1–11** and placed the OH group at C-6. Moreover, the correlations from the same H_2 -12 to C-10 and from H_2 -1 to C-2, C-3, C-10, C-11, C-14 and C-15 placed the carbonyl at C-2 and established the same ether bridge between C-10/C-12/C-3 as in compound **9**. The sequence of correlations of homonuclear couplings in the COSY spectrum (Fig. 2) between H_2 -4/ H_2 -5/ H -6 and H_2 -8/ H_2 -9/ H -10 corroborated the proposed structure. The NOESY spectra (Fig. 3) revealed correlations between H -6/ H_3 -13 and from H -1 β to H_3 -15/ H -9 β , indicating that they were cofacial with the β -orientation. Additionally, NMR calculations for the 8 possible diastereomers of compound **12** confirmed the relative configuration, and the DP4+ methodology indicated a probability of 100% for the candidate 3*R**,6*R**,7*S**,10*R** (Table S4). To conclude, the absolute configuration of **12** was proposed to be 3*S*,6*S*,7*R*,10*S* on the basis of a comparison of the calculated and experimental ECD spectra (Fig. 4). Thus, the structure of **12** was characterized, and it was named dolichocarpol R.

Table 3¹H NMR spectroscopic data for compounds **10–18** in CDCl₃ (δ_{H} in ppm, *J* in Hz)

No.	10^b	11^a	12^a	13^a	14^b	15^a	16^a	17^b	18^a
1a	2.28 dddd (14.8, 8.4, 5.2, 2.8)	2.25 dddd (15.5, 8.5, 5.5, 3.0)	2.80 d (14.0)	2.92 d (14.5)	2.94 d (15.2)	-	-	5.52 d (13.6)	1.86 dd (13.0, 8.0)
1b	1.94 dd (14.8, 8.4)	1.93 dd (15.0, 8.5)	2.35 d (14.0)	2.29 d (14.5)	2.28 d (15.2)	-	-	-	2.11 dd (13.5, 5.0)
2	6.25 ddd (8.0, 5.6, 2.4)	6.20 ddd (8.5, 5.5, 2.0)	-	-	-	5.91 s	5.89 s	5.97 d (13.6)	3.99 dd (8.0, 5.0)
3	-	-	-	-	-	-	-	-	-
4a	2.92 m	2.96 m	1.58 m	2.41 ddd (16.0, 6.0, 2.0)	2.24 m	2.28 dt (12.5, 4.5)	2.62 m	2.61 ddd (13.6, 10.8, 2.8)	2.75 m
4b	2.19 m	2.61 td (13.5, 3.5)	1.58 m	1.99 m	2.24 m	2.19 td (12.5, 4.5)	2.22 m	2.45 m	2.19 m
5a	2.05 m	3.09 ddd (14.5, 13.0, 3.0)	2.15 m	1.83 m	1.79 m	2.00 m	1.86 m	2.12 m	1.80 m
5b	1.66 m	2.17 ddd (14.5, 5.0, 3.5)	2.15 m	1.68 m	1.79 m	1.57 m	1.86 m	1.90 m	1.80 m
6	3.36 dd (10.0, 4.8)	-	3.87 dd (7.0, 2.0)	3.58 dd (11.5, 3.0)	3.84 dd (7.6, 3.2)	3.58 dd (11.0, 4.5)	3.60 dd (7.0, 3.5)	3.62 dd (8.0, 1.2)	3.61 t (4.5)
7	1.60 m	-	-	-	-	-	-	-	-
8a	1.58 m	1.88 m	2.35 m	1.93 m	2.12 m	1.80 m	1.75 m	1.91 m	2.93 d (13.5)
8b	1.45 m	1.88 m	1.71 m	1.67 m	1.65 m	1.27 m	1.75 m	1.67 m	2.53 d (13.5)
9a	2.13 m	1.75 m	2.31 m	1.73 m	2.00 m	1.94 m	1.88 m	1.84 m	-
9b	1.58 m	1.59 m	1.98 m	1.56 m	1.86 m	1.94 m	1.88 m	1.58 m	-
10	4.12 dd (12.4, 5.6)	4.11 dd (13.5, 6.0)	3.73 td (5.0, 1.0)	4.16 td (7.5, 0.5)	4.15 dd (7.2, 5.2)	3.76 dd (8.0, 5.5)	3.73 t (8.0)	3.70 dd (10.4, 5.6)	-
11	-	-	-	-	-	-	-	-	-
12a	-	-	4.63 d (12.0)	-	-	4.00 s	4.28 s	-	-
12b	-	-	3.59 d (12.0)	-	-	-	-	-	-
13	1.01 d (6.8)	1.32 s	0.84 s	1.18 s	1.27 s	1.34 s	1.25 s	1.24 s	1.19 s
14	1.19 s	0.93 s	1.00 s	0.88 s	1.05 s	1.04 s	1.32 s	1.01 s	1.09 s
15	0.99 s	1.17 s	1.11 s	1.11 s	0.97 s	1.33 s	1.03 s	1.38 s	1.17 s
1'a	-	-	-	-	-	-	-	-	3.70 dq (14.0, 7.0)
1'b	-	-	-	-	-	-	-	-	3.48 dq (14.0, 7.0)
2'	-	-	-	-	-	-	-	-	1.23 t (7.0)

^a Recorded at 500 MHz.^b Recorded at 400 MHz.

Compounds **13** and **14** were isolated as colorless oils with molecular formulas of $C_{15}H_{22}O_3$, as determined by analysis of their HRESIMS ions at m/z 273.1458 and 273.1456 $[M + Na]^+$ (calc. for $C_{15}H_{22}NaO_3$, 273.1461 $\Delta = 1.1$ and 2.1 ppm), respectively. Both of their IR spectra showed hydroxy groups (3437 cm^{-1}), double bonds (1640 cm^{-1}) and carbonyl groups (1708 cm^{-1}). The 1D NMR spectral data of **13** and **14** (Tables 1 and 3) were similar to those of compounds **1–12** including three methyl groups (δ_C 17.1/22.0, δ_C 31.4/31.4, δ_C 25.2/24.9), two oxymethine carbons (δ_C 87.6/87.4, δ_C 72.2/74.6), one conjugated carbonyl (δ_C 202.5/203.3), one double bond (δ_C 126.8/124.2, δ_C 168.4/169.0) and five methylenic carbons. In the HMBC (Fig. 2), the mutual correlations from H-1 to C-2, C-3, C-10, C-14, C-15 and from H-10 to C-1, C-12 and C-14 established an ether fusion via C-10/C-12/C-3, whereas the correlations from H₃-13 to C-6, C-7, C-8 and C-12 defined the OH group at C-6 and a new C–C fusion between C-3/C-12/C-7. Thus, the double bond was inserted between C-12/C-3, and the carbonyl was positioned at C-2. Similar to **12**, the sequence of vicinal correlations in the COSY spectrum (Fig. 2) between H₂-4/H₂-5/H-6 and H₂-8/H₂-9/H-10 corroborated the structural proposals. The only significant differences between compounds **13** and **14** were observed in the chemical shift values of the CH₃-13 methyl group (**13**: δ_H 1.18, δ_C 17.1; **14**: δ_H 1.27, δ_C 22.0) and the coupling constants of H-6. Compound **13** exhibited a larger coupling ($J = 11.5$, 3.0 Hz), whereas compound **14** exhibited a smaller coupling ($J = 7.6$, 3.2 Hz) (Table 3 and Fig. S189). These data suggest that the compounds are diastereomers. To confirm this proposal, the relative configurations of **13** and **14** were established via ^{13}C and 1H NMR calculations. The DP4+ methodology, calculated between the 4 possible diastereomers, indicates probabilities of 100% for the relative configuration of 6*R**,7*S**,10*R** for **13** and 6*S**,7*S**,10*R** for **14** (Table S4). Lastly, the absolute configurations of compounds **13** (6*S*,7*R*,10*S*) and **14** (6*S*,7*S*,10*R*), established by superimposing the experimental ECD spectra with the theoretical ones, confirmed these proposals (Fig. 4). As a result, the structures of **13** and **14** were determined to be new bicyclic humelenes with a new C–C fusion not present in the previous compounds and were named dolichocarpols S and T, respectively.

Compounds **15** and **16** were obtained as colorless oils. The IR spectra of both compounds revealed olefinic double bonds (1640 cm^{-1}), hydroxy groups (3426 cm^{-1}) and carbonyl groups (1708 cm^{-1}). The molecular formulas of these compounds were identical to those of **13** and **14** ($C_{15}H_{22}O_3$) on the basis of their HRESIMS data (m/z

251.1642 and m/z 251.1647 $[M + H]^+$ (calcd. for $C_{15}H_{23}O_3$, 251.1642 $\Delta = -0.3$ and -2.2 ppm), corresponding to five indexes of hydrogen deficiency. The similarity of the 1D NMR spectral data (Tables 1 and 3) with those of **13** and **14** was altered only by the presence of an olefinic proton (δ_H 5.91/5.89), a further oxymethine proton (δ_H 4.00/4.28) and the carbons at δ_C 80.6/79.9, δ_C 127.1/126.8 and δ_C 157.4/159.8. The HMBC cross-peaks (Fig. 2) from the olefinic proton H-2 to C-4, C-11 and C-12 and from H-10, H₃-14, and H₃-15 to C-1 combined with those from H-12 to C-2 and C-3 defined that the carbonyl was located at C-1 and that the double bond was between C-2/C-3. In addition, the correlations from H-12 to C-6, C-7, C-8, C-10 and C-13 confirmed the same C–C fusion as that in **13** and **14**. The COSY spectra (Fig. 2) maintained the same sequence of vicinal correlations between H₂-4/H₂-5/H-6 and H₂-8/H₂-9/H-10, which is compatible with the respective structural proposals. Compounds **15** and **16** exhibited differences in their chemical shifts at C-6 (**15**: δ_C 76.5; **16**: δ_C 73.3), C-13 (**15**: δ_C 24.2; **16**: δ_C 21.2), and H-12 (**15**: δ_H 4.00 s; **16**: δ_H 4.28 s). Additionally, differences in the coupling constants of H-6 (**15**: $J = 11.0, 4.5$ Hz; **16**: $J = 7.0, 3.5$ Hz) were observed. These differences suggest that the compounds are pairs of diastereomers, and these changes are linked to the spatial arrangement of CH₃-13, H-10 and H-12 (Fig. S189). To confirm this hypothesis, the relative configuration was determined by ^{13}C and 1H NMR calculations. The DP4+ method, used to performed calculations on the 8 possible diastereomers, indicates a probability of 99.75% for the $6S^*,7S^*,10R^*,12R^*$ configuration for **15** and a probability of 100% for the $6R^*,7S^*,10R^*,12R^*$ configuration for **16** (Table S4). Additionally, comparison of the experimental and calculated ECD spectra confirmed this proposal and determined the absolute configurations of **15** as $6R,7R,10S,12S$ and **16** as $6R,7S,10R,12R$ (Fig. 4). Therefore, the structures of compounds **15** and **16** were established and named dolichocarpols U and V, respectively.

The molecular formula of compound **17** was deduced by HRESIMS as $C_{14}H_{22}O_3$ based on the ion at m/z 261.1452 $[M + Na]^+$ (calcd for $C_{14}H_{22}NaO_3$, 261.1461), suggesting that it might be a norsesquiterpene. The 1D NMR data (Tables 1 and 3) were similar to those of **3**, with the same ether bridge existing between C-10 (δ_C 87.3) and C-7 (δ_C 85.2). However, the 1H NMR spectrum showed two doublets at δ_H 5.50 (d, $J = 13.6$ Hz) and 5.97 (d, $J = 13.6$ Hz) instead of two singlets. Additionally, in the ^{13}C NMR/DEPT spectrum, a carbonyl signal was observed at δ_C 205.5, along with signals corresponding to only four methylene

carbons (δ_c 40.2, δ_c 27.7, δ_c 36.0, and δ_c 27.4) instead of five. The COSY spectrum (Fig. 2) showed the sequence of correlations among the protons H₂-4/H₂-5/H-6 and H₂-8/H₂-9/H-10, as well as between the two olefinic protons. The HMBC cross-peaks (Fig. 2) from H₃-14 and H₃-15 to C-1, C-10 and C-11 defined that one oxymethine carbon and the double bond were located at C-10 and C-1/C-2, respectively. Similarly, the correlation of the olefinic protons H-1 and H-2 with C-3, along with the mutual correlation of the methyl protons H₃-13 with C-6, C-7 and C-8, indicated that the carbonyl was located at C-3 and that the second oxymethine carbon was located at C-6, respectively. The relative configuration of **17** was determined via NMR calculations of the 4 possible diastereomers. The DP4+ methodology established a probability of 100% for the 6*S**,7*R**,10*S** configuration (Table S4). Consequently, the ECD spectrum calculated for 6*S*,7*R*,10*S* matched the experimental spectrum, confirming its absolute configuration (Fig. S31). Hence, compound **17** was characterized and identified as a new norsesquiterpene named dolichocarpol W.

Obtained as a colorless oil, compound **18** exhibited a molecular formula of C₁₆H₂₆O₄, established from its HRESIMS data at *m/z* 305.1718 [M + Na]⁺ (calcd for C₁₆H₂₆NaO₄, 305.1723), corresponding to four indexes of hydrogen deficiency. Its 1D NMR data (Tables 1 and 3) resembled those of dolichocarpol F (Sales et al., 2020a), suggesting that **18** possesses a similar backbone. Despite this, one methine proton was absent, a carbonyl group remained (δ_c 203.0), and an oxymethine carbon (δ_c 77.3) besides a double bond (δ_c 159.5/132.2) were added. However, unlike that of dolichocarpol F, the DEPT spectrum of **18** (Table 1) contained only four methylene carbons (δ_c 41.3, δ_c 24.0, δ_c 29.1, δ_c 32.0), and the COSY correlations (Fig. 2) revealed only the H₂-1/H-2 and H₂-4/H₂-5/H-6 moieties. Furthermore, the HMBC cross-peaks (Fig. 2) from H₂-1 to C-2, C-3, C-10, C-14, and C-15, combined with those from H-2 and H-4 to C-3 and C-9, established a new C–C fusion between C-3/C-9. Moreover, the HMBC correlations of H₂-1 and H₂-8 to C-10 inferred that a carbonyl was located at this position, whereas the correlations of H₃-13 to C-6, C-7, and C-8 indicated that the OH group was located at C-6 and C-7. Additionally, as in **3** and **4**, the ethoxy group at C-2 was identified at δ_c 65.2 and δ_c 15.7, as confirmed by the correlations from H-2 to C-1, C-1', and C-3. Therefore, the presence of the ethoxy group in compounds **3**, **4**, and **18** is likely to be an artifact of isolation generated during the extraction procedure using EtOH. According to the literature (Gong et al., 2012; Liu et al., 2014; Wu et al., 2015), several terpenoids with ethoxy and methoxy

groups have been reported as artifacts due to the extraction process. Finally, NMR calculations of the 4 possible diastereomers confirmed the relative configuration of compound **18**. When the DP4+ methodology was applied, a probability of 100% was obtained for the 2S*,6S*,7S* configuration (Table S4). Thus, the absolute configuration of 2S,6S,7S was determined on the basis of the similarity of the experimental and calculated ECD spectra (Fig. 4). Therefore, **18** was proposed as a new nor-sesquiterpene named dolichocarpol X.

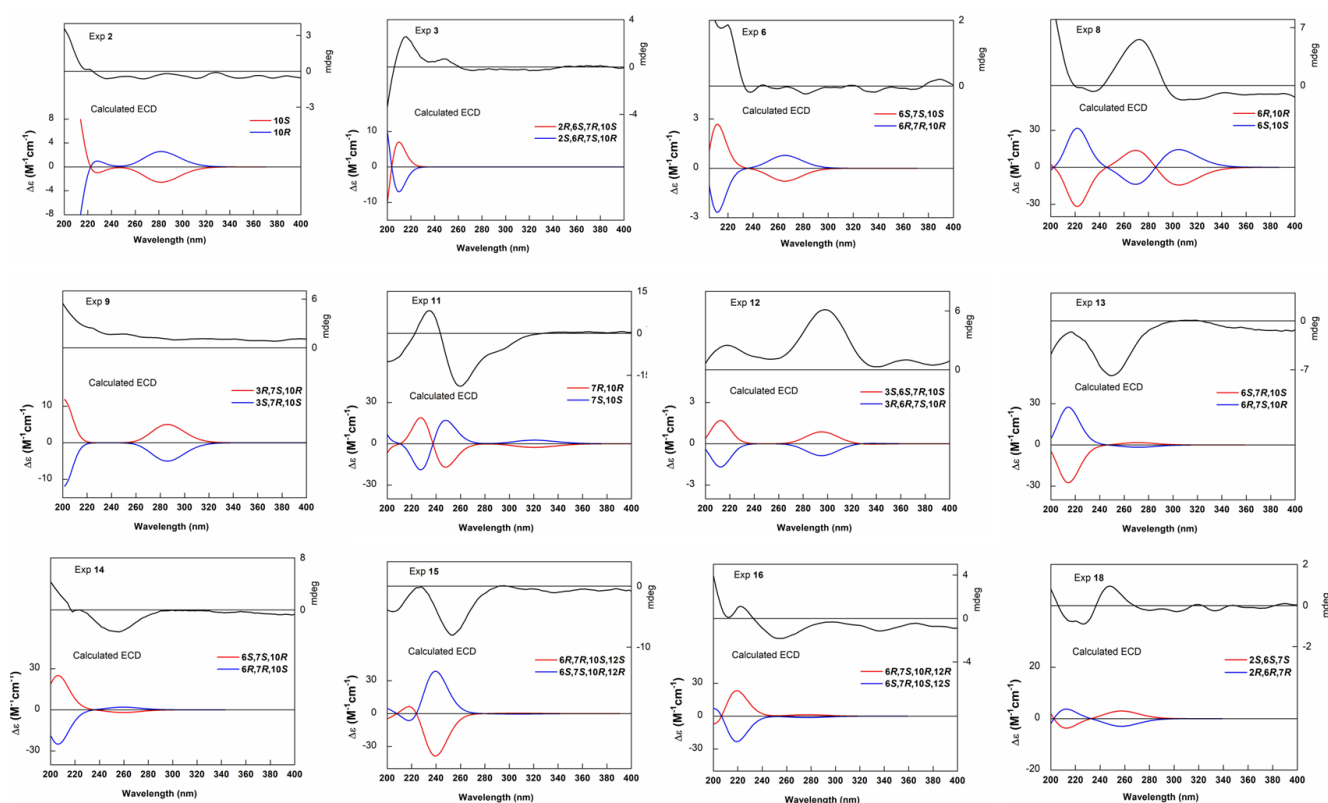


Fig. 4. Experimental and calculated ECD spectra of compounds **2**, **3**, **6**, **8**, **9**, **11**, **12**, **13–16**, **18**.

2.2. Evaluation of antineuroinflammatory activity

The antineuroinflammatory effects of all the isolated compounds were assayed in the LPS/IFN- γ -stimulated BV2 cell line. First, noncytotoxic concentrations of compounds **1–18** in BV2 microglial cells were determined by the MTT assay, a colorimetric test that evaluates metabolic cell activity. A reduction in cell viability above 20% was observed only for compound **18** at 200 μ M (cell viability: 74.7 $\pm$ 0.3%). However, the viability of the cells remained unaffected by the other compounds, with reductions not exceeding 20% at all the tested concentrations (Table S33).

Therefore, in the first set of experiments, we used 100 μM as a safe concentration to investigate the effects of compounds **1–18** on NO levels in BV2 cells. Compounds **1**, **5**, **8**, **9** and **14** significantly reduced NO levels in BV2 cells, demonstrating their antineuroinflammatory activity (Fig. 5). Considering that these are **novel** compounds, we conducted an additional evaluation using concentrations ranging from **25** to **200** μM to better understand their effects. The majority of the compounds reduced NO levels in a concentration-dependent manner, reaching a maximum effect similar to that of quercetin (Fig. 5). The literature indicates that NO serves as a neurotransmitter, activating inflammatory pathways implicated in neuroinflammation related to neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease, amyotrophic lateral sclerosis, ischemia, and multiple sclerosis. Recently, phytochemicals have demonstrated significant efficacy in combating the increased production of NO in central nervous system disorders (Subedi et al., 2015). Overall, the levels of NO can be reduced by the direct inhibition of iNOS (inducible NO synthase), by the action of immunomodulatory cytokines, as well as by the action of NO-sequestering compounds (Fricker et al., 1997; Green et al., 1994; Kou et al., 2021; Liu et al., 2019).

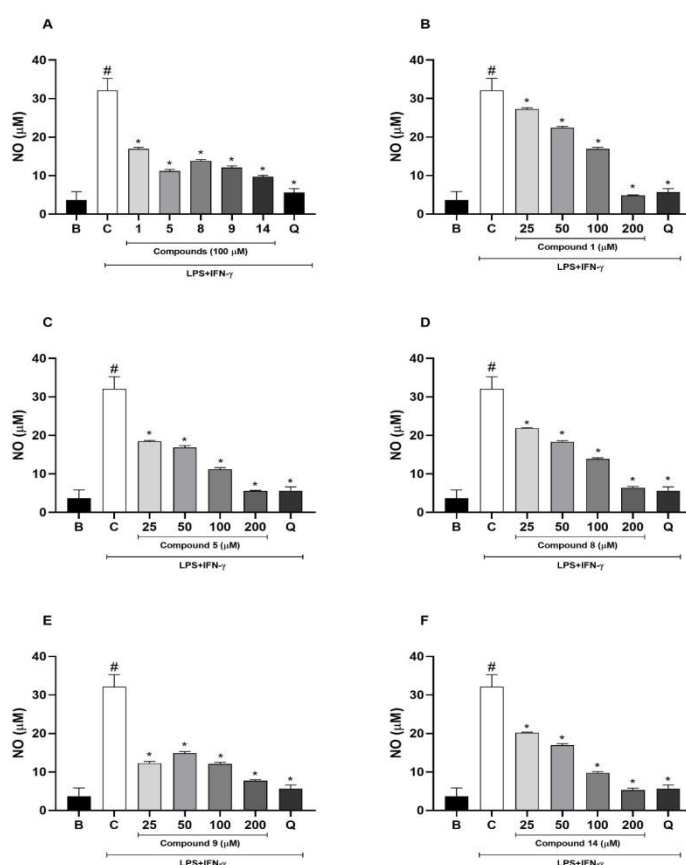


Fig. 5. Inhibitory effect of compounds **1**, **5**, **8**, **9** and **14** on NO Production in stimulated BV2 microglia cells. Results are expressed as means $\pm$ SEMs ($n = 5$). B: basal. C: control. Q: quercetin (positive control, 20 μ M). # $p < 0.05$ versus basal group; * $p < 0.05$ versus control group.

2.3. *In silico studies*

To assess the binding energies and interaction affinities with the i-Nos target (PDB: 1NSI) of compounds **1**, **5**, **8**, **9** and **14**, molecular docking and molecular dynamics simulations were carried out, as this enzyme plays a crucial role in the inflammatory process by catalyzing the production of nitric oxide (NO) (Cinelli et al., 2020). iNOS is highly relevant in inflammation because it is induced in response to inflammatory cytokines, such as TNF- α and IL-1 β , leading to the production of large amounts of NO. Depending on the context, NO can exert pro-inflammatory or anti-inflammatory effects (Godfrey; Kornberg, 2020; Inkanuwat et al., 2018). The investigation of the potential antineuroinflammatory activity of rare sesquiterpenes through iNOS modulation is particularly important, as similar studies have been conducted by Liu et al. (2018) using compounds derived from the extract of *Inula japonica* flowers. The molecular docking revealed that the compounds presented negative binding energy values and activity probability values greater than 0.5, which indicated their affinity for the target. The control (quercetin) ($p = 1$) was the compound with the highest affinity, but **9** ($p = 0.6431$) and **1** ($p = 0.6805$) presented activity probability values very close to the values demonstrated by the positive control (1400W) ($p = 0.6842$). Compounds **5** ($p = 0.6988$) and **8** ($p = 0.7475$) showed greater affinity than the control (1400W) and interactions for the inhibition of the enzyme, such as residue Glu 377 (Fig. S190), a residue necessary for the binding and coordination of the heme group and essential for the catalytic activity of iNOS (Li et al., 1999). Furthermore, similar interactions were observed between the compounds under study and the control compounds quercetin and 1400W, which refer to hydrophobic interactions between residues Phe369 and Trp194. With respect to the molecular dynamics simulations, compounds **1**, **5**, **8**, **9** and **14** (**9** being the most stable) presented greater stability with respect to the target than did the control compounds due to the lower root mean square deviation (RMSD) values in certain stages of the simulation (Fig. S191), and the complexes under study were consistent with respect to the root mean square fluctuation (RMSF) values (Fig. S192) and protein packaging through radius of gyration values (Fig. S193), revealing similar

patterns between the isolated protein and the complex protein–ligand throughout the simulation. This consistency indicates the stability of the molecule–protein interaction and demonstrates that significant variations that could hinder the interaction did not occur in the protein and that the molecules, when coupled to proteins, do not present significant variations in relation to the protein alone. This finding indicates that the complexes are stable, with low fluctuations in the tertiary structure of the enzymes.

3. Conclusions

In summary, 18 novel macrocyclic humulene sesquiterpenoids, namely dolichocarpols G–X (**1–18**), were isolated from the roots of *A. dolichocarpa*. Their structures were determined unambiguously by extensive spectroscopic analysis along with NMR and ECD quantum-mechanical calculations. Dolichocarpols S–V (**13–16**) are diastereomers featuring an uncommon C–C fusion between carbons C-3/C-12/C-7, whereas dolichocarpols W–X (**17–18**) are nor-sesquiterpenes, which are relatively rare in naturally occurring macrocyclic humulene type. Furthermore, the tested compounds **1**, **5**, **8**, **9** and **14** didn't presented any cytotoxicity and effectively reduced NO levels in a concentration-dependent manner, reaching a maximum effect similar to the positive control quercetin. These findings enriches the structural types and biological activities of the macrocyclic humulene sesquiterpenoids in the genus *Anaxagorea* and suggests that these compounds may potentially exhibit characteristics as antineuroinflammatory agents and have further prospects for exploration.

4. Experimental

4.1. General experimental procedures

Optical rotations were measured on a Jasco P-2000 polarimeter at 20 °C in CHCl₃. IR spectra were acquired on a Shimadzu IRPrestige-21 spectrometer as well as an FTIR spectrophotometer (Shimadzu IRSpirit-T) with a QATR-S accessory. 1D and 2D NMR spectra were recorded on a Bruker AVANCE III HD spectrometer (500/400 and 125/100 MHz for ¹H and ¹³C, respectively) using CHCl₃ residual solvent (δ_{H} 7.26 and δ_{C} 77.0) as an internal standard. HRESIMS analysis was carried out using a Bruker spectrometer (micrOTOFII) operating in positive mode. Medium-

pressure liquid chromatography (MPLC) was performed on silica gel 60 (40–63 μm , 230–400 mesh, SiliCycle). Thin-layer chromatography (TLC) was carried out using precoated silica gel F-254 aluminum sheets (SiliCycle), and the compound spots were observed under UV light at 254 and 366 nm and stained with iodine vapor. For preparative high-performance liquid chromatography (HPLC), a Shimadzu apparatus with an SPD-M10A VP diode array detector and a YMC-Triart C₁₈ preparative column (250 mm $\times$ 10 mm and 5 μm particles) were used with a flow rate of 8.0 mL/min. The experimental ECD spectra were recorded with a Jasco J-1100 spectrometer (Jasco, Tokyo, Japan) in the 200–400 nm region using the following parameters: bandwidth, 1 nm; response, 1 s; scanning speed, 100 nm/min; number of accumulations, 3; room temperature (25 °C); sample in acetonitrile solution; cell path length, 0.1 cm; and concentration, 0.1 mg/ml.

4.2. Plant material

The *Anaxagorea dolichocarpa* roots were collected in May 2021 at Cruz do Espírito Santo, Paraíba, Brazil (7°05'26.3"S, 35°04'32.6"W) and identified by Dr. M. F. Agra. A voucher specimen (AGRA & GOES 5543) was deposited at the Herbarium Prof. Lauro Pires Xavier, Federal University of Paraíba (UFPB), Brazil. A registration with the number **AE4B71A** was secured in the National Management System of Genetic Patrimony and Associated Traditional Knowledge (SISGEN).

4.3. Extraction and isolation

The roots (2.20 kg) were air-dried and powdered and subsequently extracted three times with 95% EtOH (4 L, 72 h each) at room temperature. After evaporation of the solvent under reduced pressure, a crude residue (65 g) was obtained. 50 g of the residue was suspended in a 7:3 mixture of MeOH-H₂O and subjected to a partitioning process using *n*-hexane, chloroform, EtOAc and *n*-butanol to yield the corresponding fractions. Part of the hexane-soluble fraction (8.0 g) was processed through column chromatography (CC) using silica gel, employing a gradient of hexane, CHCl₃, EtOAc and MeOH for elution. Upon TLC analysis, the resulting products were grouped to afford fractions **1–6**. Fraction **3** (2.8 g) was separated by MPLC with silica gel and eluted with a gradient mixture of hexane, CHCl₃, EtOAc, and MeOH. The fractions obtained were combined according to the TLC profile to

afford subfractions **1–12**. Subfraction **5** (400 mg) was subjected to preparative HPLC with the following gradient elution: solvent A = H₂O; solvent B = CH₃CN; elution system = 0–60 min (25–70% B); and 60–75 min (70–100% B). This process yielded compounds **1** (3.5 mg), **3** (18.0 mg), **4** (5.0 mg), **11** (4.0 mg) and **18** (5.1 mg). Subfraction **6** (400 mg) was also subjected to preparative HPLC via gradient elution: solvent A = H₂O; solvent B = CH₃CN; elution system = 0–90 min (10–54% B); 90–100 min (54–100% B). This process yielded compounds **2** (4.5 mg), **6** (10.2 mg), **13** (4.0 mg), **14** (6.5 mg), **15** (4.0 mg) and **16** (4.2 mg). Subfraction **7** (120 mg) was purified through HPLC with subsequent gradient elution: solvent A = H₂O; solvent B = CH₃CN; elution system = 0–60 min (20–55% B); and 60–70 min (55–100% B). This process yielded compounds **12** (3.9 mg) and **17** (1.4 mg). The CHCl₃ soluble fraction (6.0 g) was separated via MPLC using silica gel and eluted employing a gradient mixture of hexane, CHCl₃, EtOAc, and MeOH. After this, the obtained fractions were combined on the basis of TLC profiles, fractions **1–8** were obtained. Fraction **3** (125 mg) was purified through preparative HPLC utilizing a solvent gradient of H₂O (solvent A) and CH₃CN (solvent B): elution profile = 0–75 min (15–60% B); 75–85 min (60–100% B). This process yielded compound **10** (12.0 mg). Fraction **4** (425 mg) was subjected to preparative HPLC with a solvent gradient of H₂O (solvent A) and CH₃CN (solvent B): elution profile = 0–80 min (10–45% B) and 80–90 min (45–100% B). This process resulted in compounds **5** (8.0 mg), **7** (5.0 mg), **8** (6.0 mg) and **9** (6.2 mg).

4.4. Spectroscopic data

Dolichocarpol G (1): colorless oil; $[\alpha]_D^{20} +35.1$ (c. 0.1, CHCl₃); IR (KBr) $\nu_{\max}$ 3433, 2966, 2929, 2872, 1718, 1450, 1369, 1246, 1080, 1024, 900 cm⁻¹; ¹H and ¹³C NMR data, see Tables 1 and 2; HRESIMS m/z 237.1859 [M + H]⁺ (calcd for C₁₅H₂₅O₂, 237.1849).

Dolichocarpol H (2): colorless oil; $[\alpha]_D^{20} +4.5$ (c. 0.1, CHCl₃); IR (film) $\nu_{\max}$ 3443, 2958, 2869, 1714, 1685, 1456, 1370, 1186, 1065, 1039 cm⁻¹; ¹H and ¹³C NMR data, see Tables 1 and 2; HRESIMS m/z 237.1855 [M + H]⁺ (calcd for C₁₅H₂₅O₂, 237.1849).

Dolichocarpol I (3): colorless oil; $[\alpha]_D^{20} -9.5$ (c. 0.1, CHCl₃); IR (KBr) $\nu_{\max}$ 3374, 2958, 2929, 2872, 1640, 1450, 1370, 1123, 1079, 1059, 1013, 898, 855 cm⁻¹; ¹H and

^{13}C NMR data, see Tables 1 and 2; HRESIMS m/z 283.2259 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{17}\text{H}_{31}\text{O}_3$, 283.2268).

Dolichocarpol J (4): colorless oil; $[\alpha]^{20}_{\text{D}} +18.7$ (c. 0.1, CHCl_3); IR (KBr) ν_{max} 3446, 2966, 2929, 2872, 1712, 1676, 1450, 1267, 1217, 1066, 1016 cm^{-1} ; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS m/z 319.1871 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{17}\text{H}_{28}\text{NaO}_4$, 319.1880).

Dolichocarpol K (5): colorless oil; $[\alpha]^{20}_{\text{D}} +18.0$ (c. 0.1, CHCl_3); IR (KBr) ν_{max} 3450, 2958, 2929, 2870, 1641, 1456, 1367, 1215, 1056, 1004 cm^{-1} ; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS m/z 277.1783 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{15}\text{H}_{26}\text{NaO}_3$, 277.1774).

Dolichocarpol L (6): colorless oil; $[\alpha]^{20}_{\text{D}} -6.6$ (c. 0.1, CHCl_3); IR (film) ν_{max} 3423, 2955, 2866, 1711, 1665, 1456, 1372, 1105, 1071, 1033, 910 cm^{-1} ; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS m/z 275.1616 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{15}\text{H}_{24}\text{NaO}_3$, 275.1618).

Dolichocarpol M (7): colorless oil; $[\alpha]^{20}_{\text{D}} +28.5$ (c. 0.1, CHCl_3); IR (KBr) ν_{max} 3483, 2960, 2933, 1735, 1629, 1446, 1371, 1246, 1070, 1022 cm^{-1} ; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS m/z 253.1793 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{15}\text{H}_{25}\text{O}_3$, 253.1798).

Dolichocarpol N (8): colorless oil; $[\alpha]^{20}_{\text{D}} +76.1$ (c. 0.1, CHCl_3); IR (KBr) ν_{max} 3412, 2962, 2933, 1718, 1627, 1444, 1361, 1220, 1062, 1002, 920, 869 cm^{-1} ; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS m/z 273.1469 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{15}\text{H}_{22}\text{NaO}_3$, 273.1461).

Dolichocarpol O (9): colorless oil; $[\alpha]^{20}_{\text{D}} +32.8$ (c. 0.1, CHCl_3); IR (KBr) ν_{max} 3398, 2960, 2927, 1708, 1448, 1375, 1251, 1120, 1056, 1033 cm^{-1} ; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS m/z 253.1793 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{15}\text{H}_{25}\text{O}_3$, 253.1798).

Dolichocarpol P (10): colorless oil; $[\alpha]^{20}_{\text{D}} -6.0$ (c. 0.1, CHCl_3); IR (KBr) ν_{max} 3437, 2955, 2926, 2872, 1708, 1467, 1393, 1255, 1117, 1094, 1022 cm^{-1} ; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS m/z 275.1622 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{15}\text{H}_{24}\text{NaO}_3$, 275.1618).

Dolichocarpol Q (11): colorless oil; $[\alpha]^{20}_{\text{D}} -21.6$ (c. 0.1, CHCl_3); IR (KBr) ν_{max} 3481, 2964, 2927, 2872, 1707, 1462, 1390, 1205, 1170, 1082, 1047, 941 cm^{-1} ; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS m/z 267.1593 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{15}\text{H}_{23}\text{O}_4$, 267.1591).

Dolichocarpol R (12): colorless oil; $[\alpha]^{20}_D +6.2$ (c. 0.1, CHCl_3); IR (film) ν_{max} 3437, 2958, 2923, 1688, 1461, 1378, 1258, 1183, 1120, 1051, 1022 cm^{-1} ; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS m/z 275.1627 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{15}\text{H}_{24}\text{NaO}_3$, 275.1618).

Dolichocarpol S (13): colorless oil; $[\alpha]^{20}_D -22.5$ (c. 0.1, CHCl_3); IR (film) ν_{max} 3432, 2955, 2923, 2869, 1726, 1637, 1459, 1375, 1252, 1140, 1045 cm^{-1} ; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS m/z 273.1458 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{15}\text{H}_{23}\text{O}_3$, 273.1461).

Dolichocarpol T (14): colorless oil; $[\alpha]^{20}_D -7.3$ (c. 0.1, CHCl_3); IR (film) ν_{max} 3437, 2955, 2926, 2869, 1708, 1640, 1459, 1375, 1252, 1125, 1056 cm^{-1} ; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS m/z 273.1456 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{15}\text{H}_{22}\text{NaO}_3$, 273.1461).

Dolichocarpol U (15): colorless oil; $[\alpha]^{20}_D -4.5$ (c. 0.1, CHCl_3); IR (film) ν_{max} 3426, 2955, 2920, 2852, 1708, 1640, 1459, 1378, 1091, 1051, 1016 cm^{-1} ; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS m/z 251.1642 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{15}\text{H}_{23}\text{O}_3$, 251.1642).

Dolichocarpol V (16): colorless oil; $[\alpha]^{20}_D -2.6$ (c. 0.1, CHCl_3); IR (film) ν_{max} 3446, 2958, 2926, 1708, 1640, 1459, 1441, 1381, 1252, 1160, 1077, 1048 cm^{-1} ; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS m/z 251.1647 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{15}\text{H}_{23}\text{O}_3$, 251.1642).

Dolichocarpol W (17): colorless oil; $[\alpha]^{20}_D +2.4$ (c. 0.1, CHCl_3); IR (KBr) ν_{max} 3417, 2955, 2869, 1717, 1459, 1375, 1243, 1160, 1082, 1025 cm^{-1} ; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS m/z 261.1452 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{14}\text{H}_{22}\text{NaO}_3$, 261.1461).

Dolichocarpol X (18): colorless oil; $[\alpha]^{20}_D +8.7$ (c. 0.1, CHCl_3); IR (KBr) ν_{max} 3450, 2966, 2926, 2870, 1716, 1662, 1452, 1377, 1288, 1267, 1163, 1089 cm^{-1} ; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS m/z 305.1718 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{16}\text{H}_{26}\text{NaO}_4$, 305.1723).

4.5. NMR and ECD calculations

Randomized conformational searches were performed for all the possible stereoisomers of compounds **1–18** using the Monte Carlo algorithm with an MMFF force field in SPARTAN `14 software. All the conformers within a relative free energy

window of 10 kcal/mol were selected for geometry optimization calculations in the gas phase, employing the B3LYP/6-31(d) level of theory. Vibrational frequency calculations were performed at the same level of theory to confirm that stationary points correspond to minima on the potential energy surface. To determine the relative configuration of the compounds (Albuquerque et al., 2024; Costa et al., 2021), conformers within a relative energy window of 3 kcal/mol were subsequently selected for calculations of ^{13}C and ^1H nuclear magnetic shielding constants (σ) using the GIAO-B3LYP/6-31G (d) level of theory, along with the polarizable continuous model with the integral equation formalism (IEF-PCM) method to simulate solvation by acetonitrile (Miertus et al., 1981). The mean shielding constants of the population were obtained by assuming Boltzmann statistics at a temperature of 298 K. The DP4+ method, which relies on Bayesian analysis, was used to establish a statistical correlation between the calculated and experimental ^{13}C and ^1H chemical shifts. Each structure was ranked on the basis of probability, and probabilities greater than 90% indicated a high level of confidence in which candidate exhibited the best agreement with the experimental data (Grimblat et al., 2015). Whenever the DP4+ methodology displayed a probability lower than 90%, ^{13}C and ^1H σ were recalculated at a higher level of theory: GIAO-B3LYP/6-311+G (d,p). Once the relative configurations were established, ECD simulations were conducted to determine the absolute configurations. For the ECD calculations, a TD-DFT level of theory was applied at CAM-B3LYP/TZVP to calculate the excitation energy (in nm) and rotatory strength R in the dipole velocity form for the first 30 singlet $\rightarrow$ singlet electronic transitions. IEF-PCM was applied to implicitly simulate acetonitrile as the solvent. The final ECD spectra were generated on the basis of Boltzmann statistics of the selected conformers and plotted using Origin 8 software. All quantum-mechanical calculations were performed using the Gaussian 16 software package (Frisch et al., 2016).

4.6. *In silico studies*

4.6.1. *Molecular docking*

Molecular docking simulations were performed with Molegro Virtual Docker 6.0 software and the X-ray diffraction data for human inducible nitric oxide synthase (i-Nos) (PDB: 1NSI) with a length of 2.55 Å were obtained from the Protein Data Bank library (PDB) (<https://www.rcsb.org/structure/1NSI>) (Accessed on January 17, 2024).

Initially, all water and cofactor molecules were deleted from the software workspace. The following standard settings were applied to perform the simulation: score function: MolDock score; ligand evaluation: internal ES, internal H-bond, Sp^2 – Sp^2 torsion; number of runs: 30; algorithm: MolDock SE; and number of poses: 5. The simulation was performed with a GRID radius of 15 Å and a resolution of 0.30 Å. The interactions between the ligands and amino acid residues of i-Nos (PDB: 1NSI) were analyzed using the BIOVIA Discovery Studio, 2021. The compounds under study were designed in the software Marvin Sketch v.23.10, ChemAxon, and subsequently, the standardization of chemical structures in 3D and minimization of the energy of the compounds were carried out via molecular mechanics methods and the semiempirical Austin Model 1 (AM1) method using the SPARTAN `14 software. The 1400W compound (Garvey et al., 1997; Jafarian et al., 2005) was used as a control for comparing binding energy values, and the active site was identified by visualizing the residues corresponding to the active site in the articles referring to each of the proteins in the Protein Data Bank (PDB), as well as by using molecular pocket predictions using the Bite Net platform - Skolteck I Molecule, 2022 (Rajiv et al., 2023).

4.6.2. Molecular dynamics

Molecular dynamics (MD) simulations were performed with GROMACS 5.0 software (Abraham et al., 2015; Berendsen et al., 1995) to estimate the flexibility of interactions between proteins and ligands. The topology of the ligands was prepared with the Automated Topology Builder (Malde et al., 2011) (ATB) using the GROMOS43a1 force field. The simulations used the SPC water model in a cubic box (Bondi, 1964), with neutralization by ions (Cl^- and Na^+) and minimization performed to remove bad contacts. The system was equilibrated at 300 K using the 100 ps V-rescale algorithm, represented by NVT (constant number of particles, volume and temperature), followed by equilibration at 1 atm pressure, and the Parrinello–Rahman algorithm was used at NPT (constant number of particles, pressure and temperature) for up to 100 ps. The simulations lasted 100 ns, with 50,000,000 steps. The stability and flexibility of the complex were evaluated by the RMSD and RMSF values, and the corresponding graphs were generated via Grace software (Pettersen et al., 2020; Nachbagauer et al., 2020).

4.7. Biological assay

4.7.1. Cytotoxicity assay

The cytotoxicity of compounds **1–18** was evaluated via an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (Mosmann et al., 1983). The microglial BV2 cell line was obtained from the Rio de Janeiro Cell Bank (BCRJ), Brazil, and cultured in Roswell Park Memorial Institute medium (RPMI; Sigma Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) and 1% penicillin–streptomycin (Sigma Aldrich, St. Louis, MO, USA) at 37 °C and 5% CO₂. The cells (1×10^5 cells/mL) were plated in 96-well plates and incubated overnight. Then, compounds **1–18** were added at four concentrations (25, 50, 100 or 200 µM) in five replicates, and the plates were incubated for 24 h. After treatment, the supernatant (110 µL) was removed, and 10 µL of MTT solution was added (5 mg/mL) (Sigma Aldrich). The plates were incubated for an additional four hours, and sodium dodecyl sulfate (SDS) (100 µL/well) was added to dissolve the formazan. Optical densities were measured on a spectrophotometer (BioTek Instruments microplate reader, Synergy HT, Winooski, VT, USA) at a wavelength of 570 nm.

4.7.2. NO production assay

For NO determination, BV2 cells (1×10^6 cells/mL) were seeded in 96-well culture plates in RPMI medium supplemented with 10% FBS and 1% penicillin–streptomycin in a 5% CO₂ incubator at 37 °C. After a four-hour period, the cells were stimulated with LPS (500 ng/mL, Sigma Aldrich) and IFN-γ (5 ng/mL, Thermo Fisher) in the presence of compounds **1–18**. In the first set of experiments, all the compounds were tested at a concentration of 100 µM in five replicates. Posteriorly, compounds **1**, **5**, **8**, **9** and **14** were tested at concentrations ranging from 25 to 200 µM. Quercetin (20 µM) was used as the positive control. Additionally, a resting group (unstimulated) and an LPS/IFN-γ-stimulated control group (treated with the vehicle) were included. After 24 hours, the cell-free supernatants were collected, and NO was quantified via the Griess method (Griess 1987).

4.7.3. Statistical analysis

The values are presented as the means $\pm$ standard errors of the means (SEMs). $n = 5$. Group comparisons were conducted using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test ($p < 0.05$). Statistical analysis was performed using GraphPad Prism 8.0.2 (GraphPad Software Inc., San Diego, CA, USA).

CRediT authorship contribution statement

Rodrigo Silva de Andrade: Writing – original draft, Conceptualization, Methodology, Investigation, Data curation. **Kaio Aragão Sales:** Methodology, Investigation. **Edileuza Bezerra de Assis:** Investigation, Visualization, Data curation. **Lucas Silva Abreu:** Formal analysis, Investigation. **Ana Carolina Ferreira de Albuquerque:** Methodology, Formal analysis, Investigation, Visualization. **Fernando Martins dos Santos Junior:** Writing – review & editing, Validation, Supervision. **Natália Ferreira de Sousa:** Methodology, Investigation, Formal analysis. **Paulo Bruno Araújo Loureiro:** Methodology, Investigation. **Geraldo Moisés Wanderley Amorim:** Methodology, Investigation. **Marianna Vieira Sobral:** Writing – review & editing, Validation, Supervision. **Marcus Tullius Scotti:** Validation, Supervision. **Josean Fachine Tavares:** Writing – review & editing, Conceptualization, Validation, Supervision. **Marcelo Sobral da Silva:** Writing – review & editing, Conceptualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The NMR data for all compounds (**1–18**) have been deposited in the Natural Products Magnetic Resonance Database (NP-MRD; www.np-mrd.org) and can be found at NP0333624 (**1**), NP0333625 (**2**), NP0333626 (**3**), NP0333627 (**4**), NP0333628 (**5**), NP0333629 (**6**), NP0333630 (**7**), NP0333631 (**8**), NP0333632 (**9**),

NP0333633 (**10**), NP0333634 (**11**), NP0333635 (**12**), NP0333637 (**13**), NP0333638 (**14**), NP0333639 (**15**), NP0333640 (**16**), NP0333641 (**17**), NP0333642 (**18**).

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Appendix. A Supplementary data

ECD, HRESIMS, IR, NMR, Docking and Molecular dynamics data of the compounds are available in the Supporting Information.

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SUPPORTING INFORMATION

Dolichocarpols G-X: Unveiling Novel Macrocyclic Humulene Sesquiterpenoids from *Anaxagorea dolichocarpa*

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Figure S31. Experimental and calculated ECD spectra of compounds 1–18

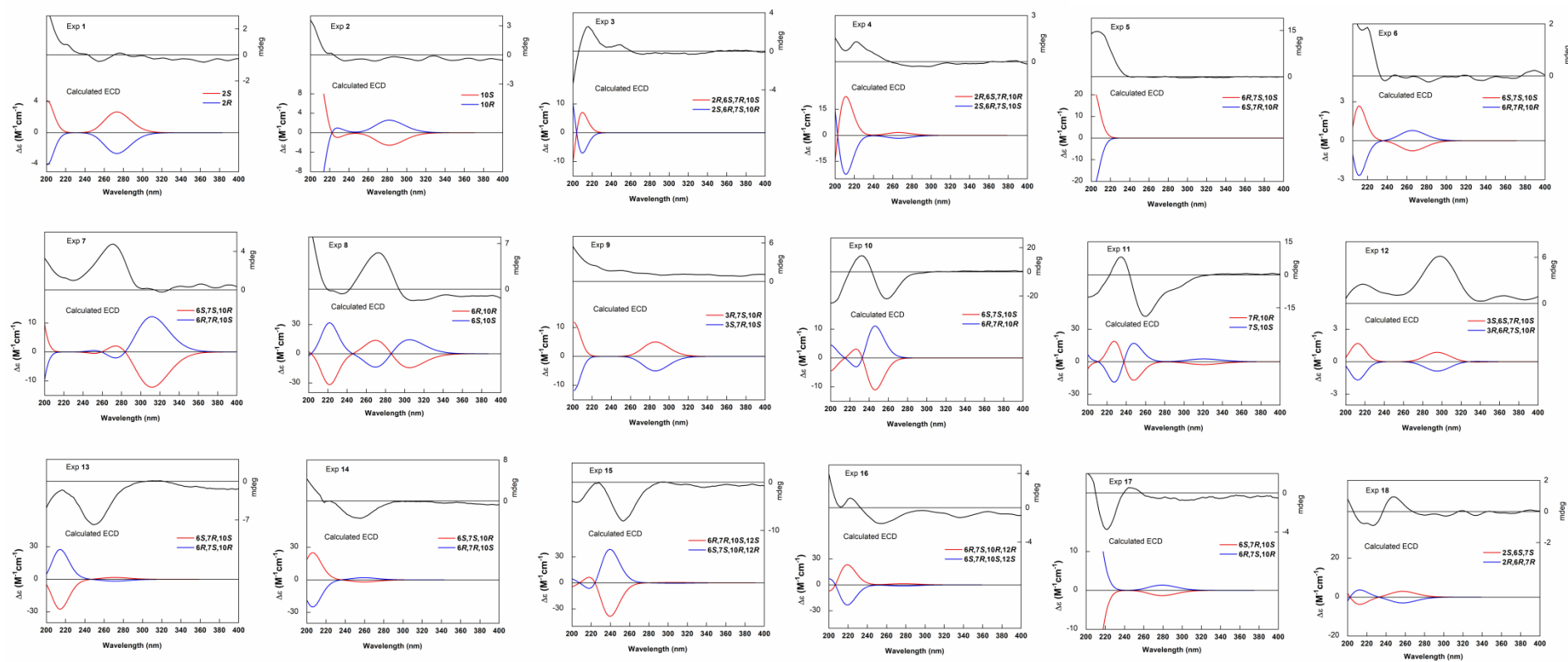


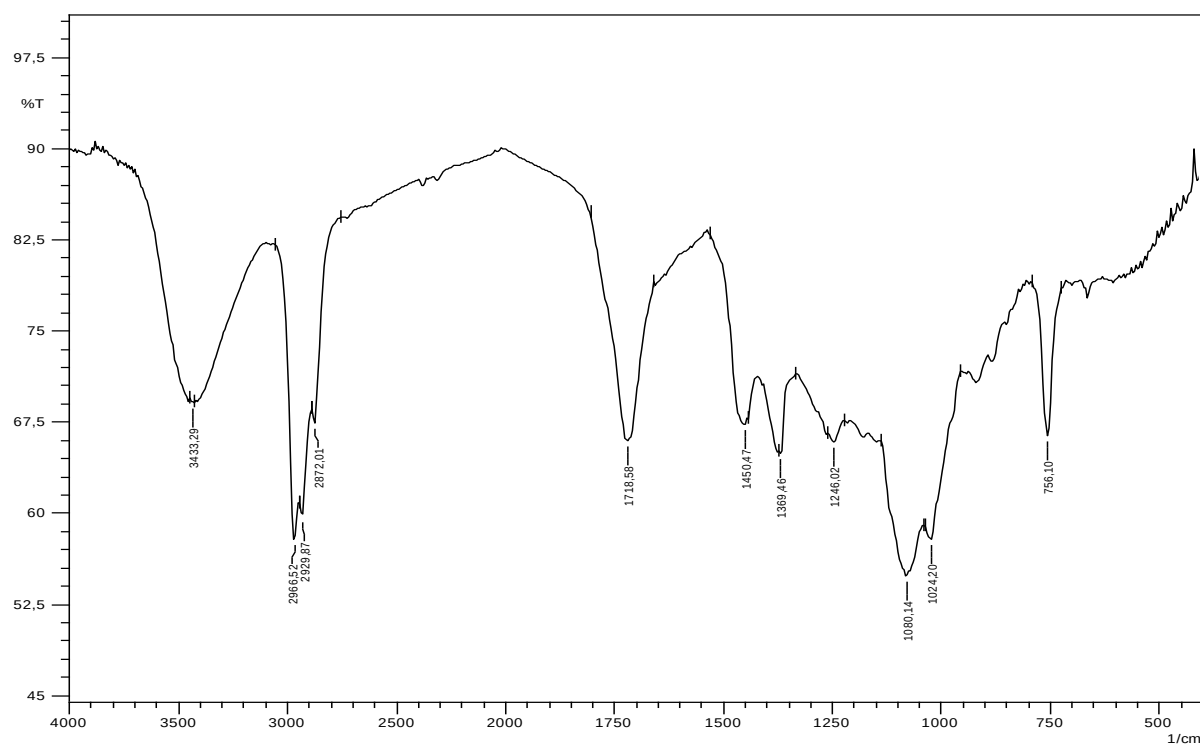
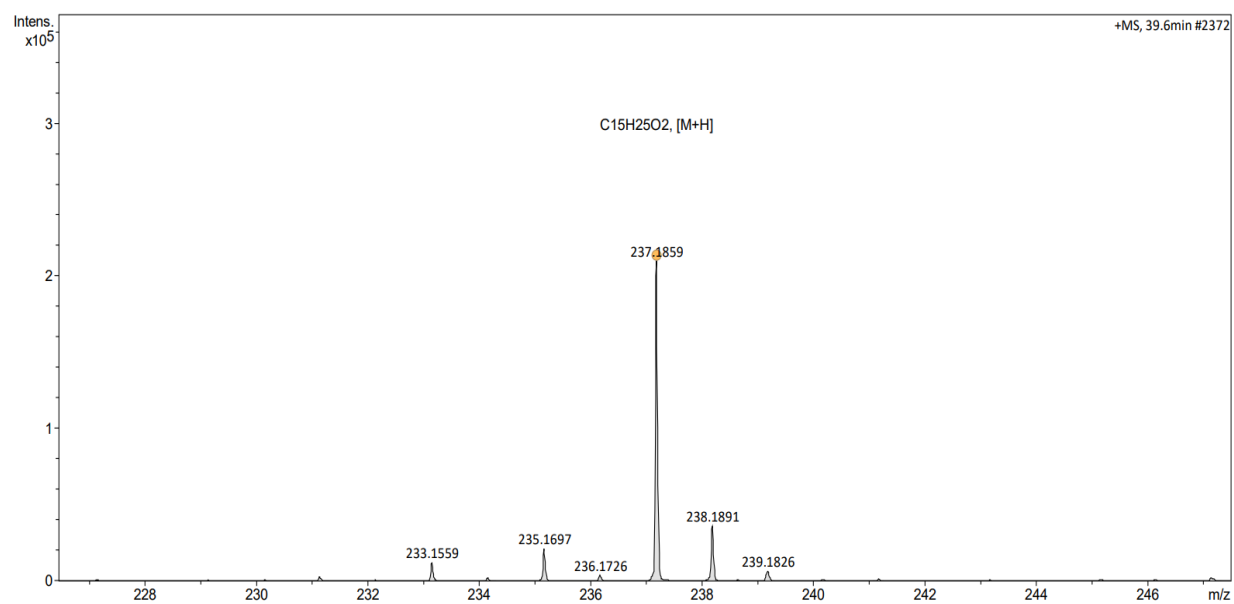
Figure S32. IR spectrum of dolichocarpol G (1)**Figure S33.** HRESIMS $[\text{M} + \text{H}]^+$ spectrum of dolichocarpol G (1)

Figure S34. ^1H NMR spectrum of dolichocarpol G (**1**) (CDCl_3 , 500 MHz)

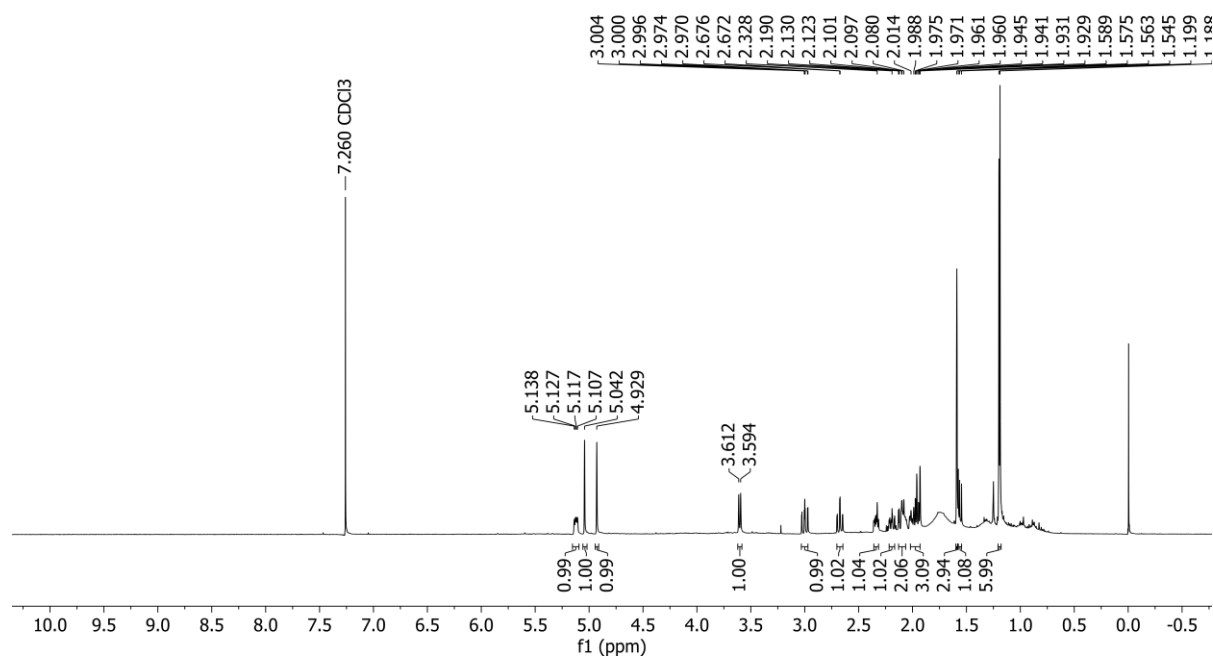


Figure S35. ^{13}C NMR spectrum of dolichocarpol G (**1**) (CDCl_3 , 125 MHz)

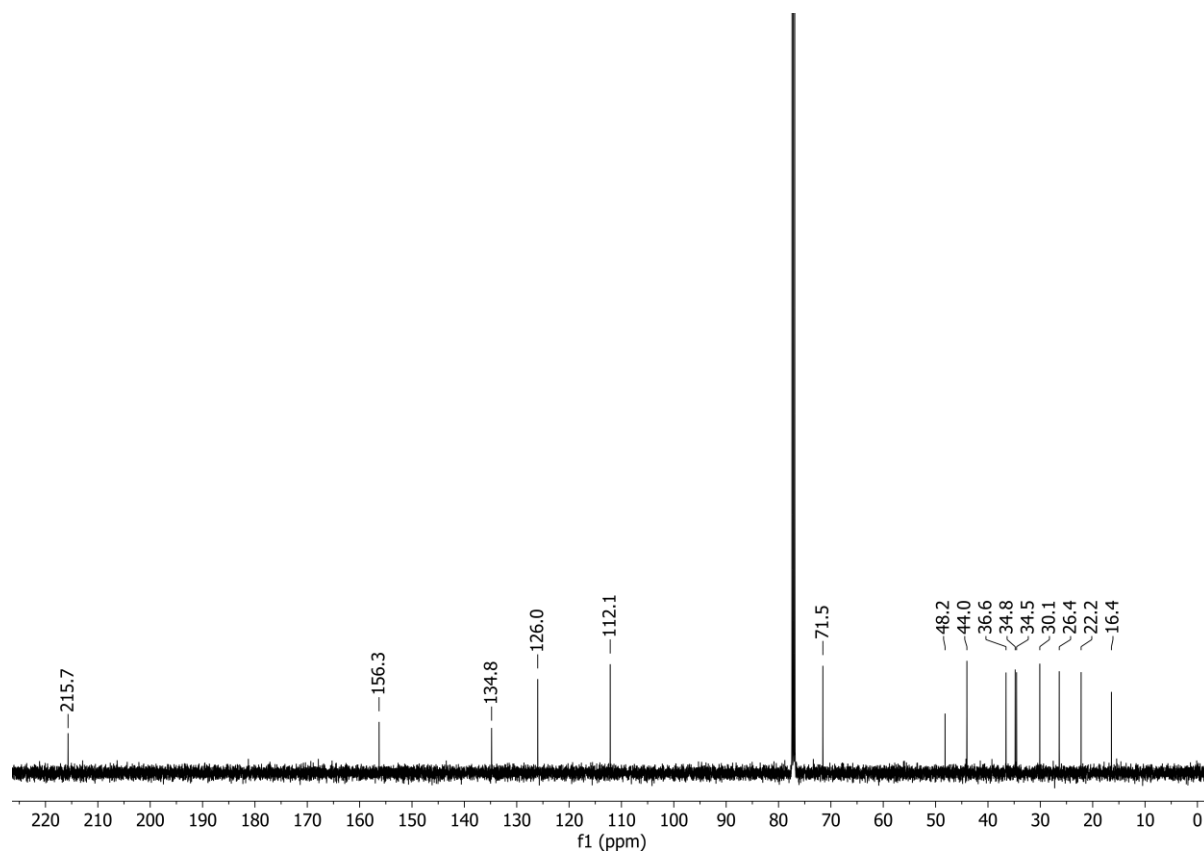


Figure S36. HSQC spectrum of dolichocarpol G (**1**) (CDCl₃, 500 MHz)

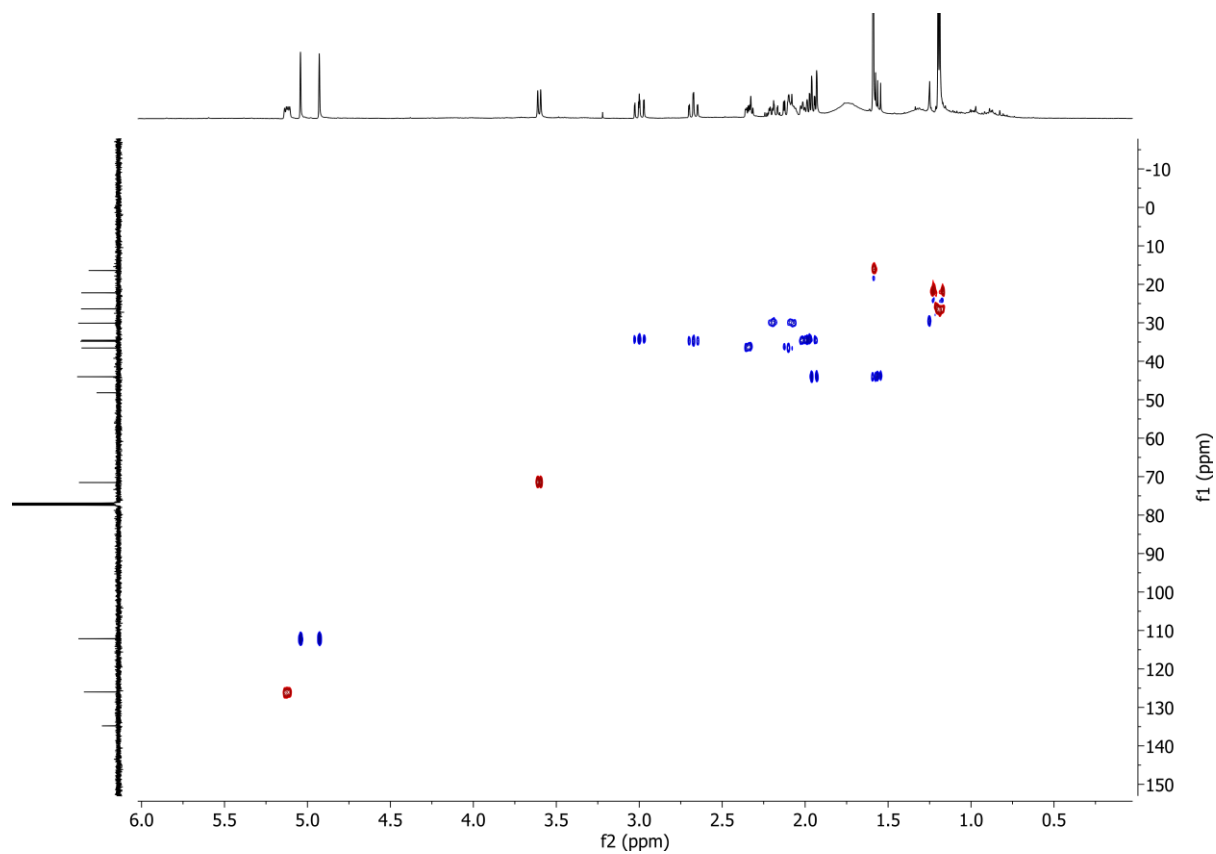


Figure S37. HMBC spectrum of dolichocarpol G (**1**) (CDCl₃, 500 MHz)

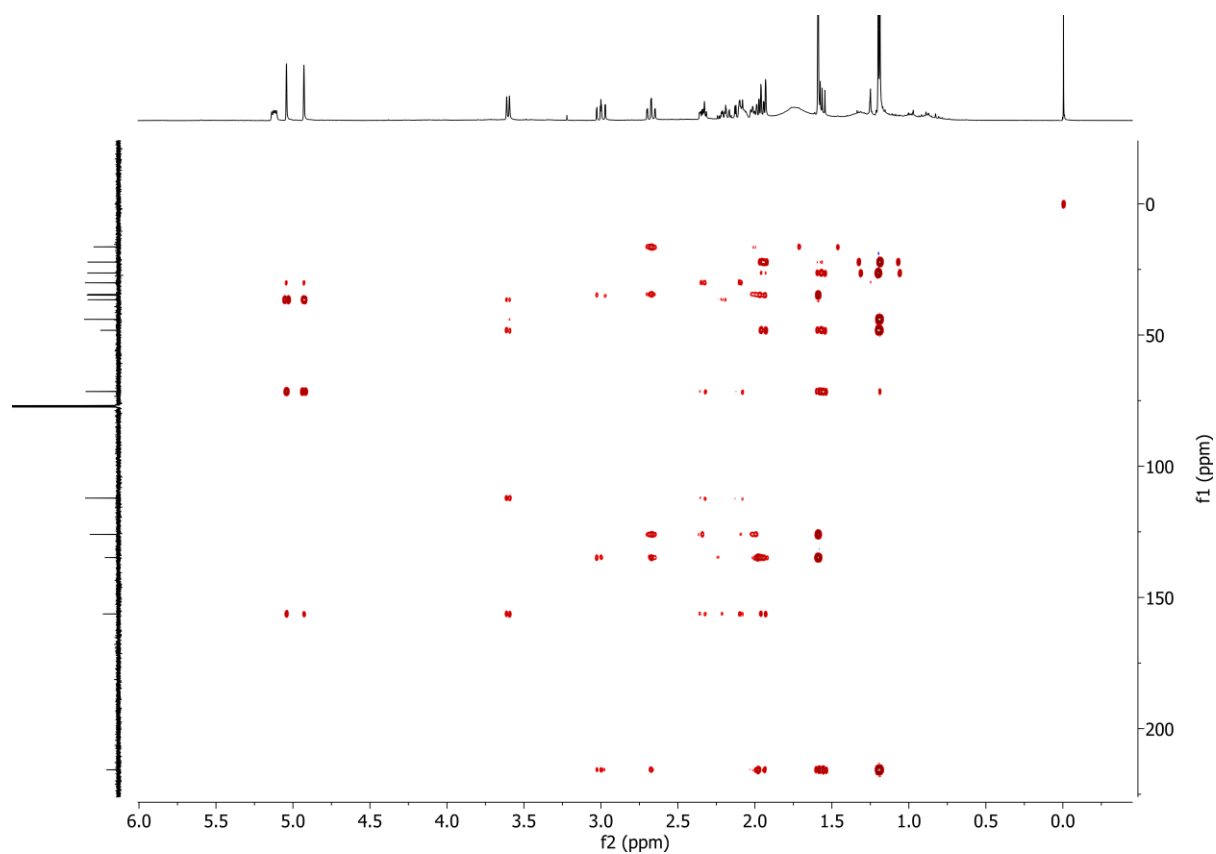


Figure S38. COSY spectrum of dolichocarpol G (**1**) (CDCl₃, 500 MHz)

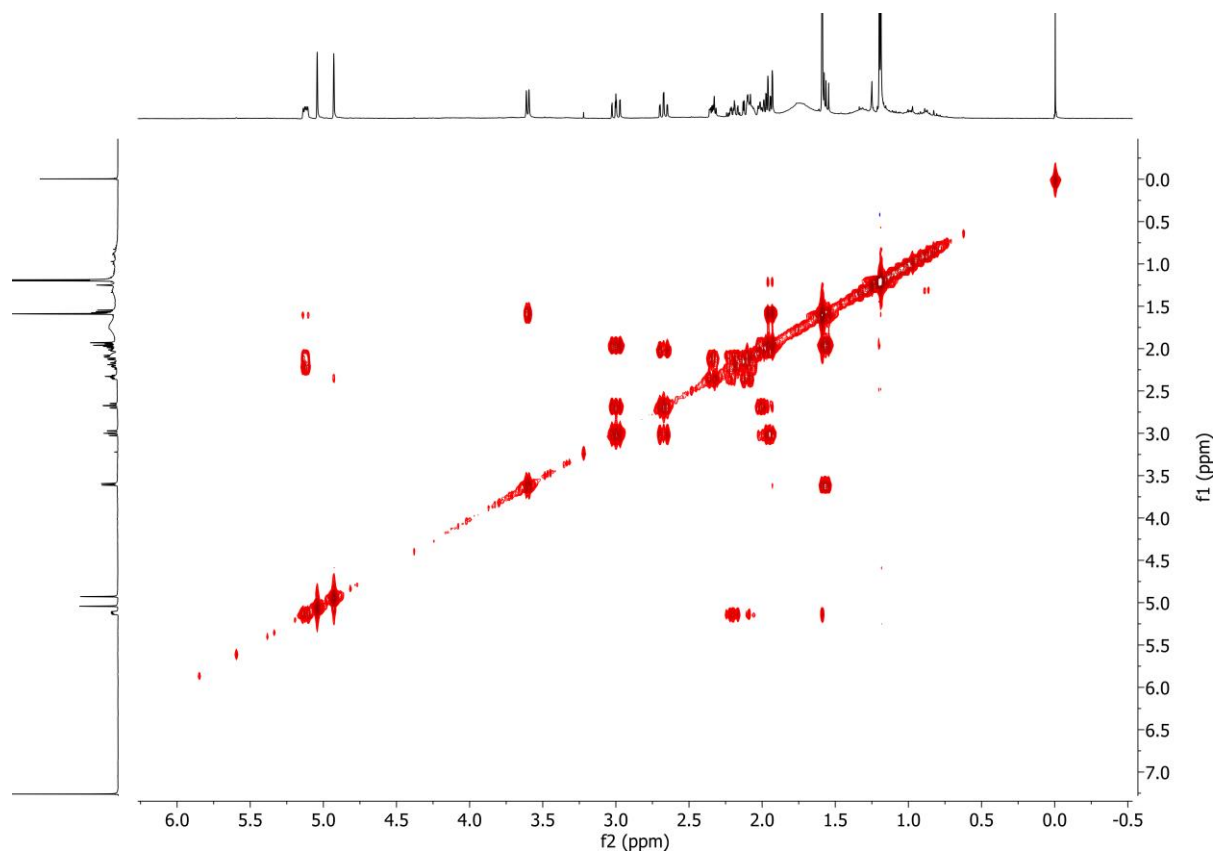


Figure S39. NOESY spectrum of dolichocarpol G (**1**) (CDCl₃, 500 MHz)

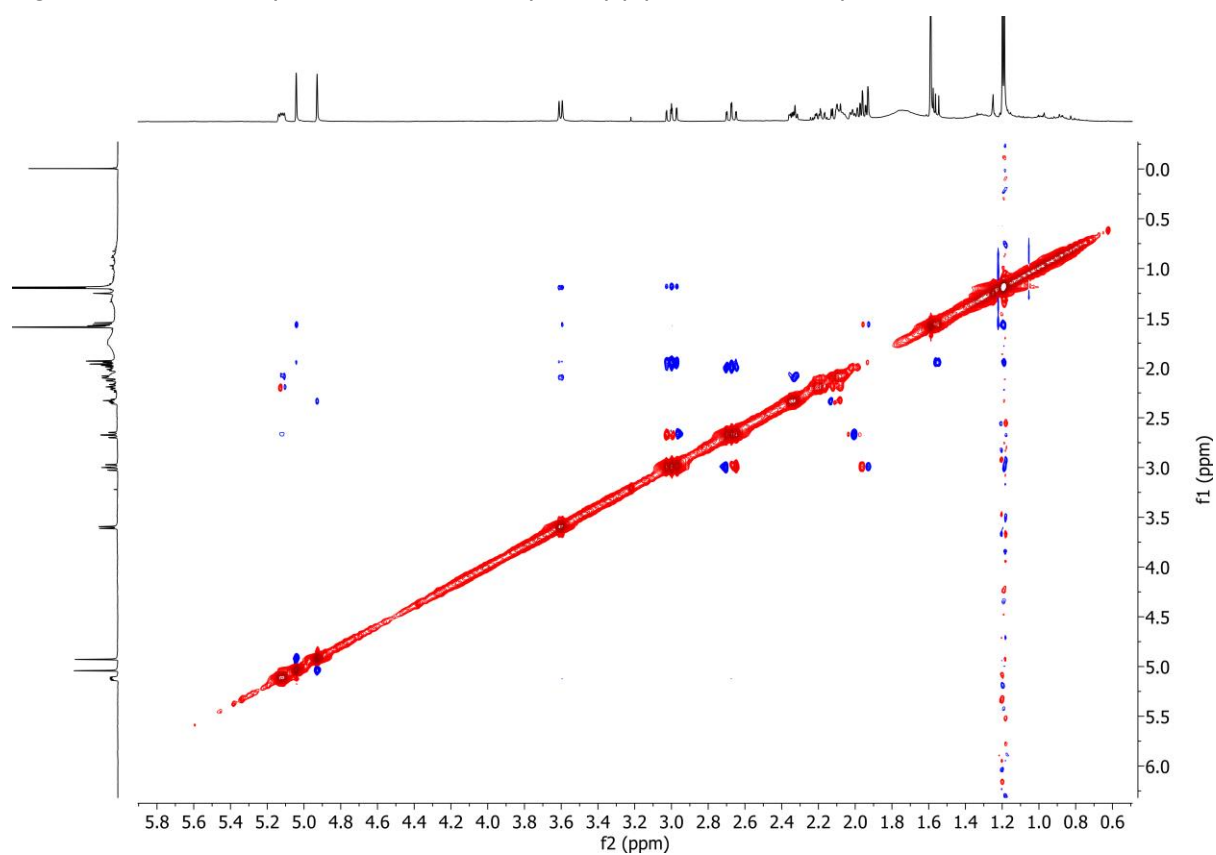


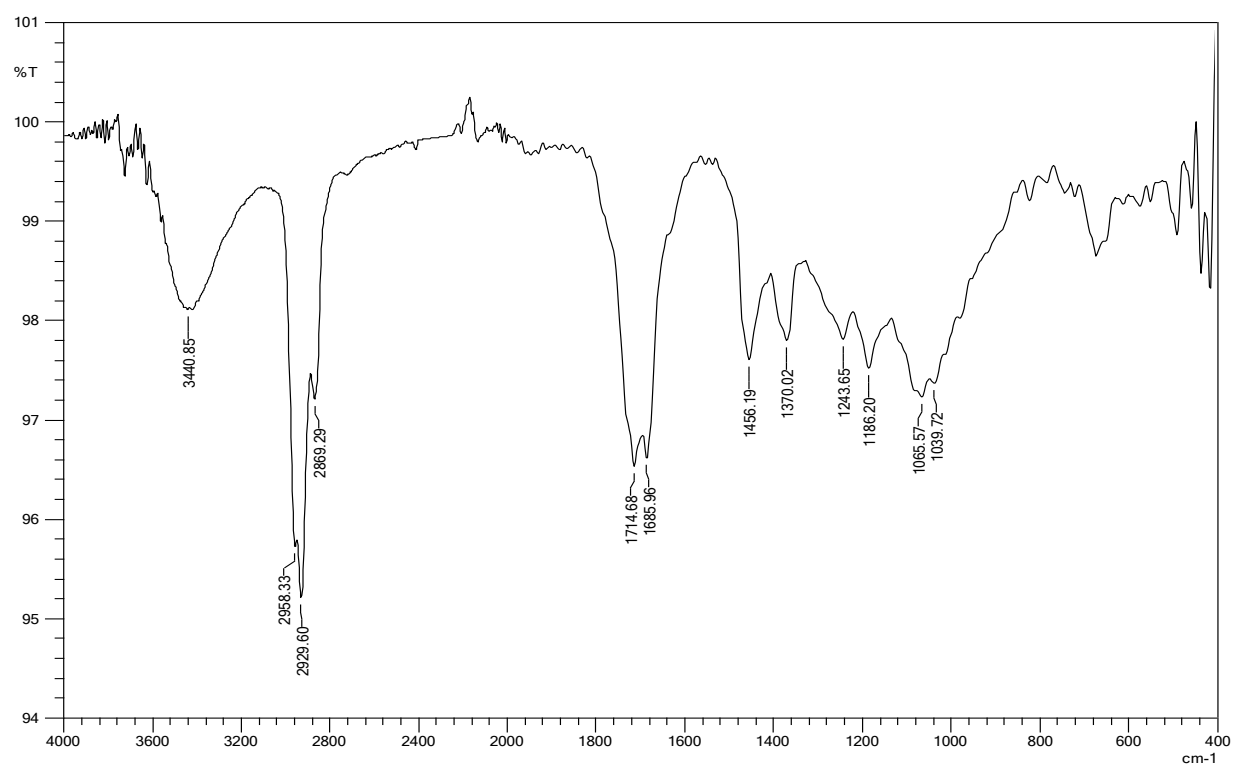
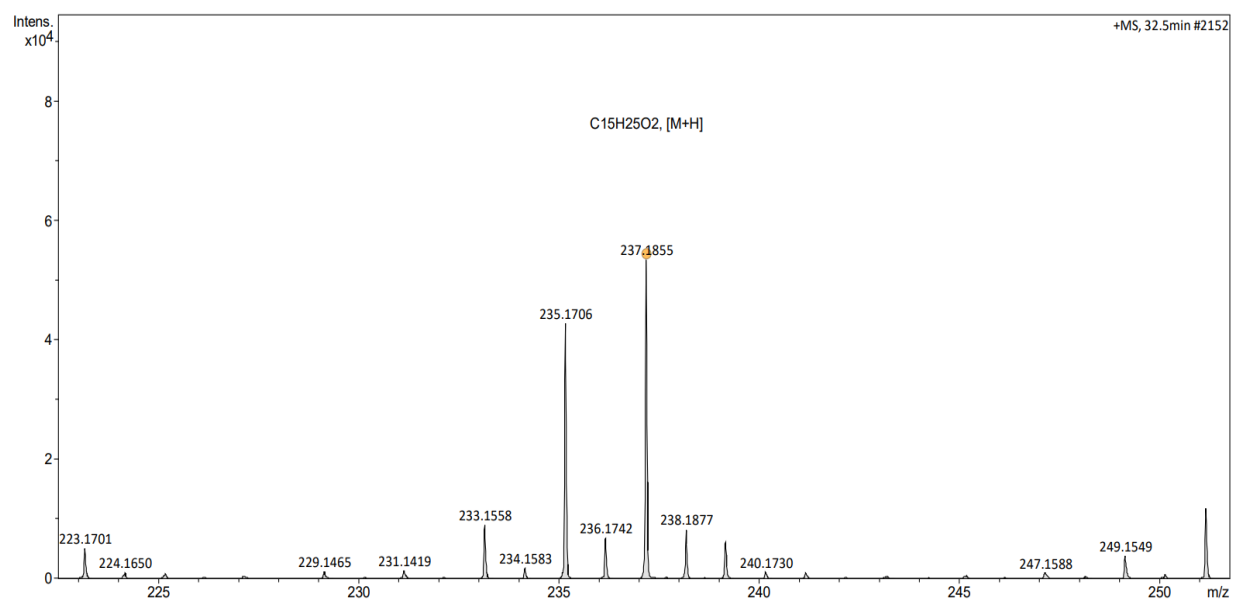
Figure S40. IR spectrum of dolichocarpol H (**2**)**Figure S41.** HRESIMS $[\text{M} + \text{H}]^+$ spectrum of dolichocarpol H (**2**)

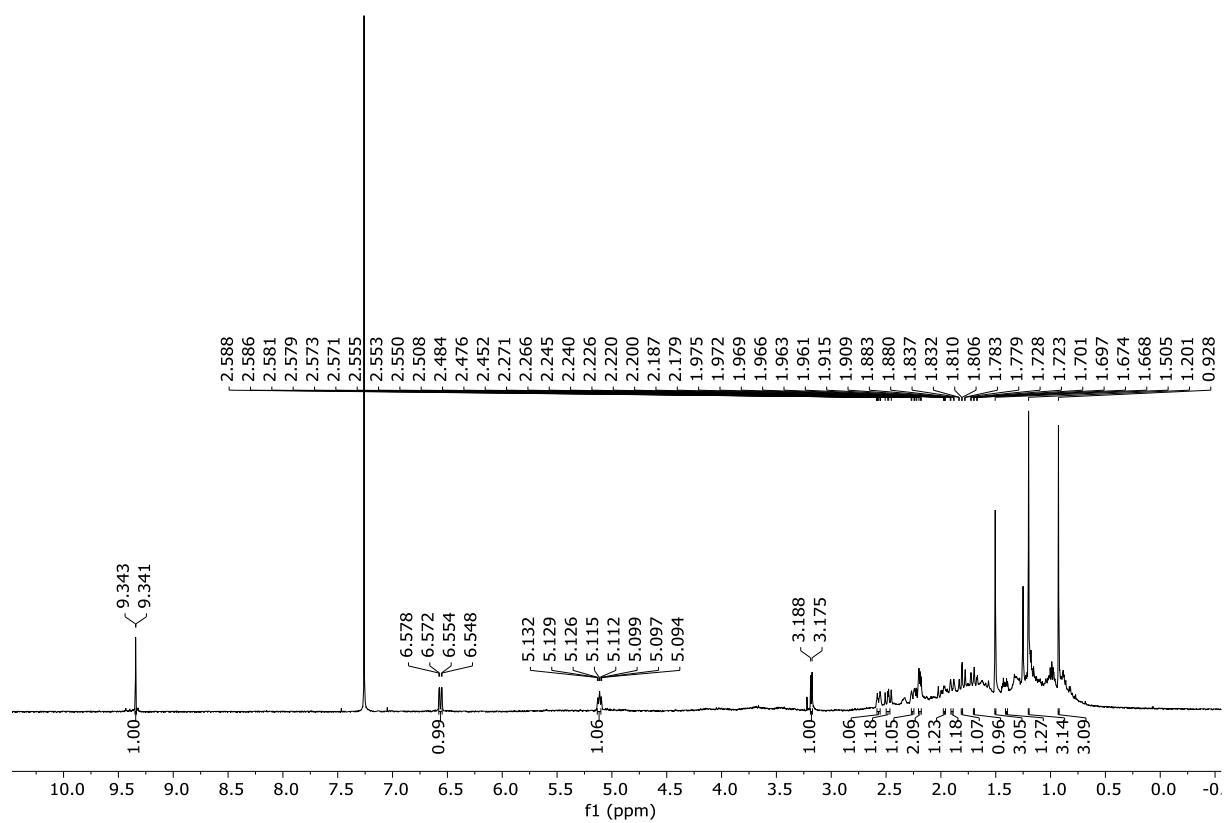
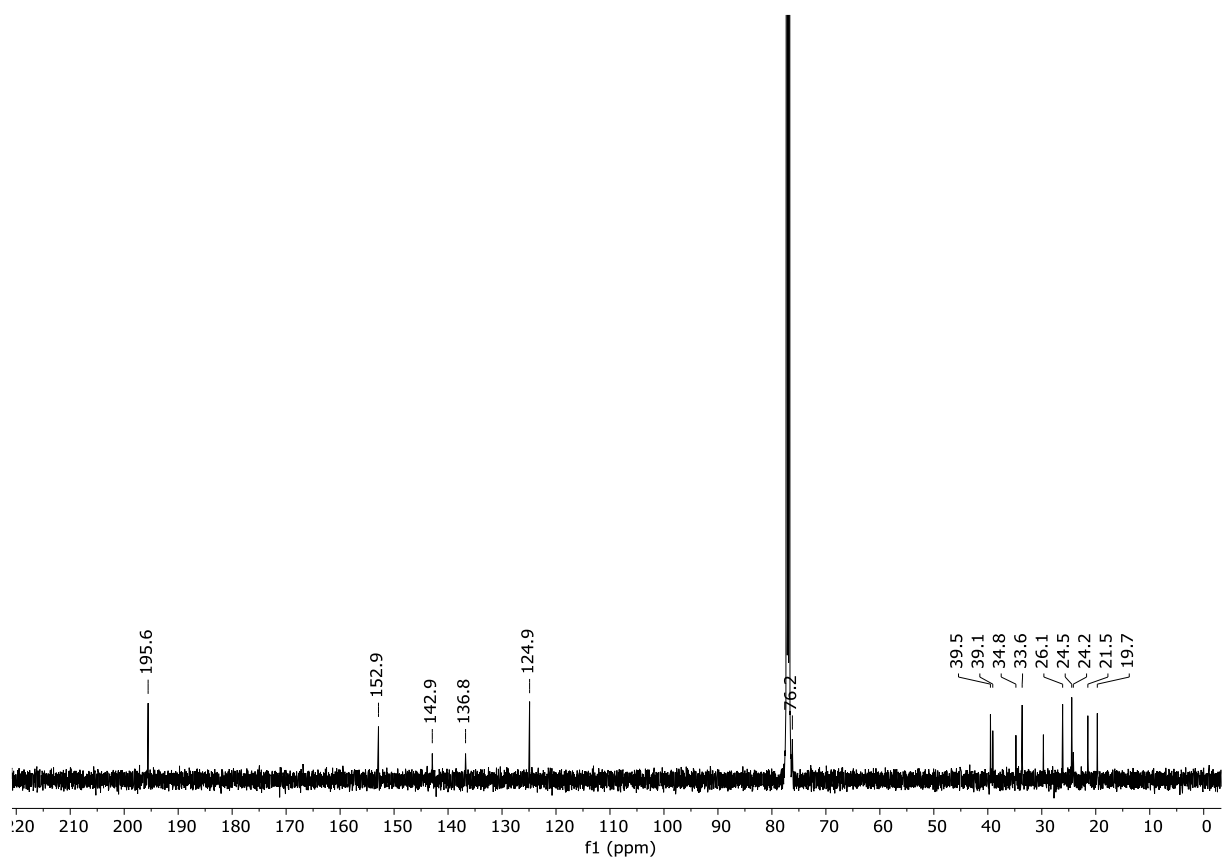
Figure S42. ^1H NMR spectrum of dolichocarpol H (**2**) (CDCl_3 , 500 MHz)**Figure S43.** ^{13}C NMR spectrum of dolichocarpol H (**2**) (CDCl_3 , 125 MHz)

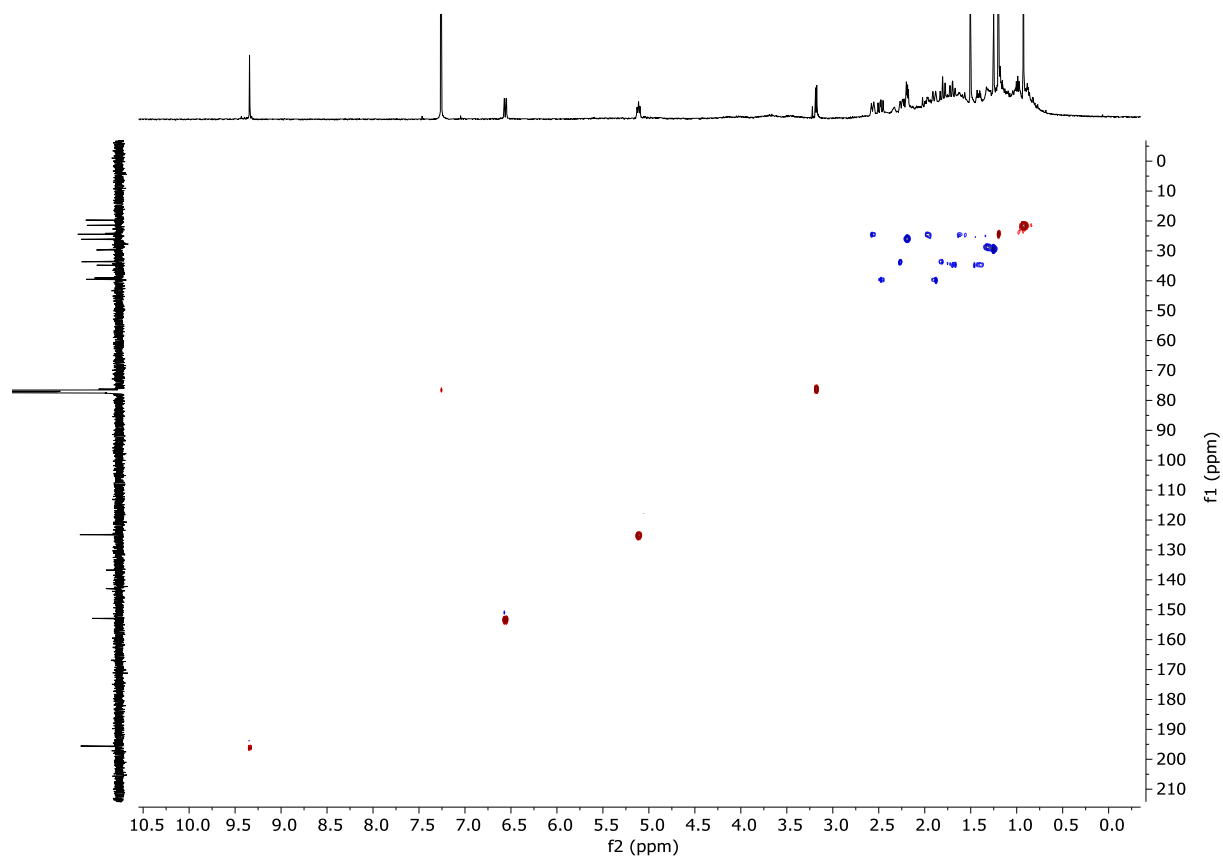
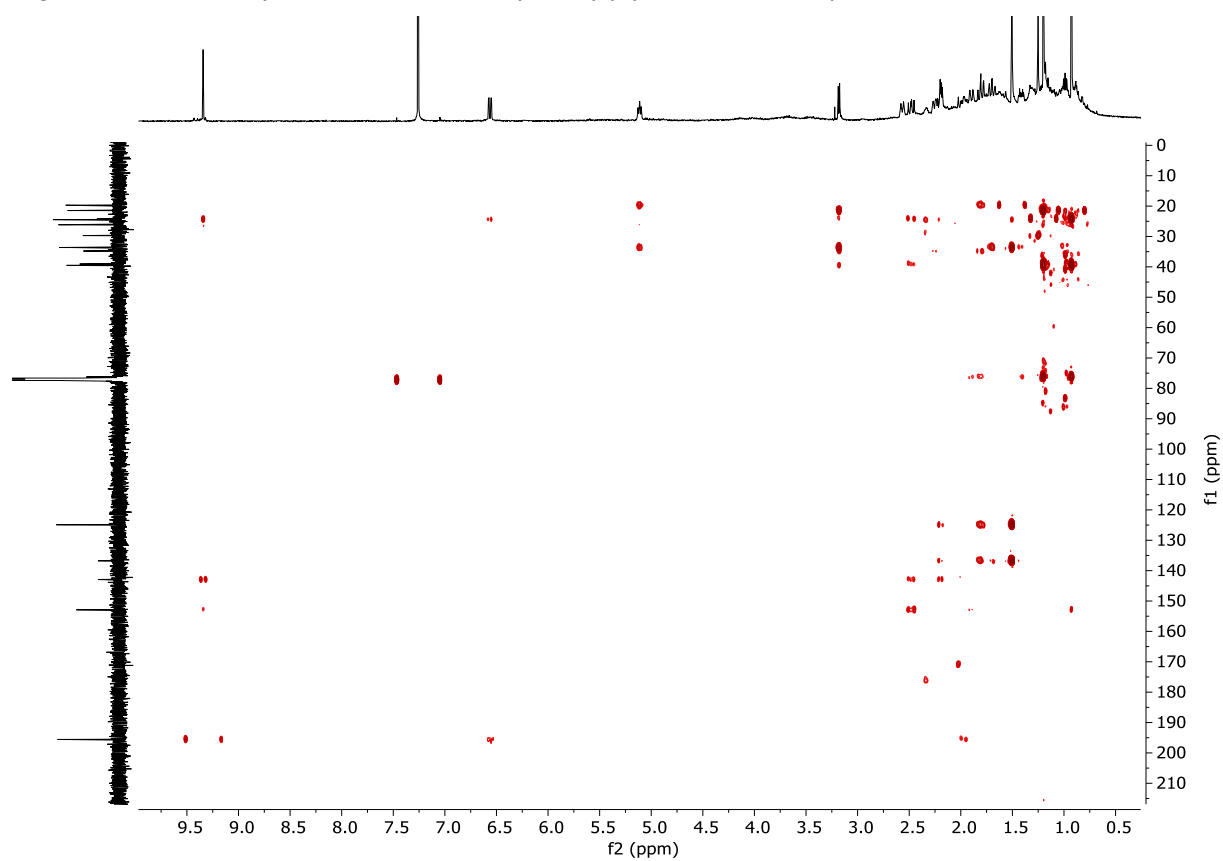
Figure S44. HSQC spectrum of dolichocarpol H (**2**) (CDCl₃, 500 MHz)**Figure S45.** HMBC spectrum of dolichocarpol H (**2**) (CDCl₃, 500 MHz)

Figure S46. COSY spectrum of dolichocarpol H (**2**) (CDCl₃, 500 MHz)

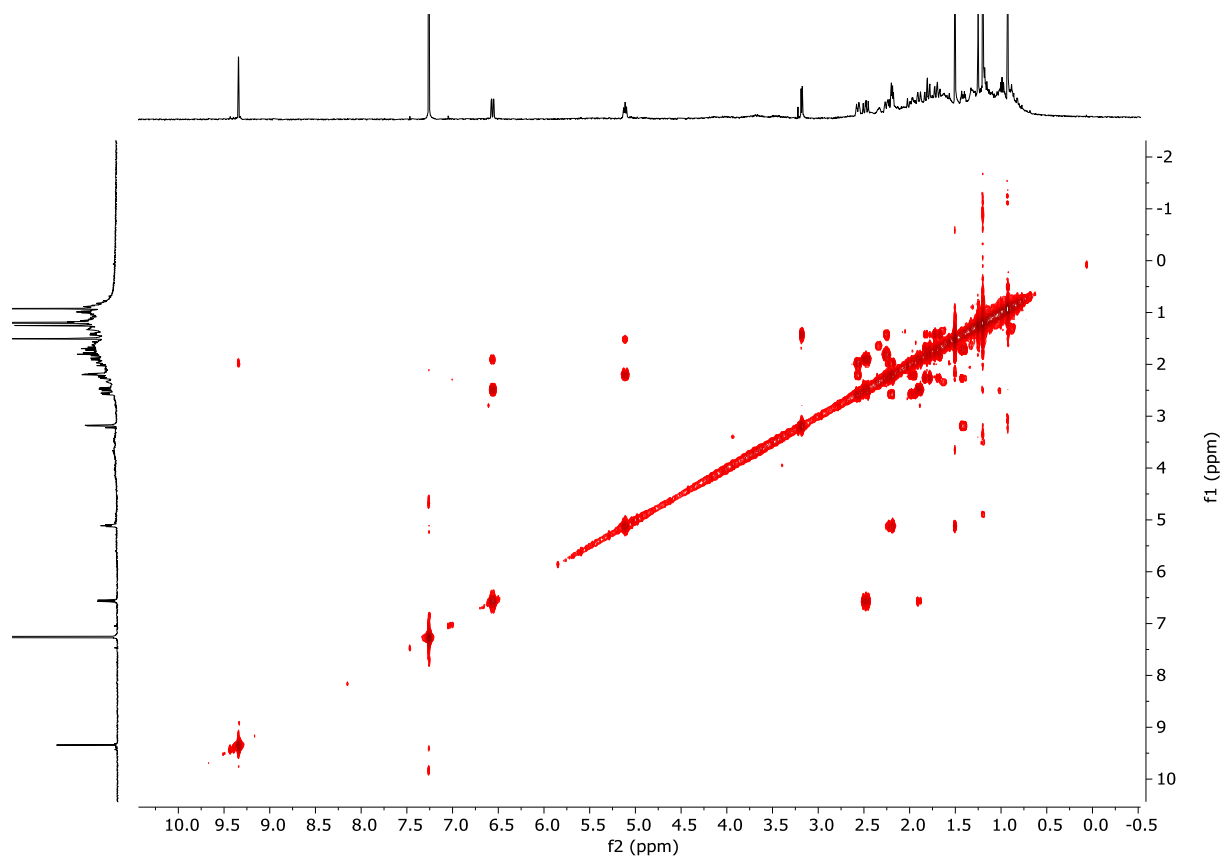


Figure S47. NOESY spectrum of dolichocarpol H (**2**) (CDCl₃, 500 MHz)

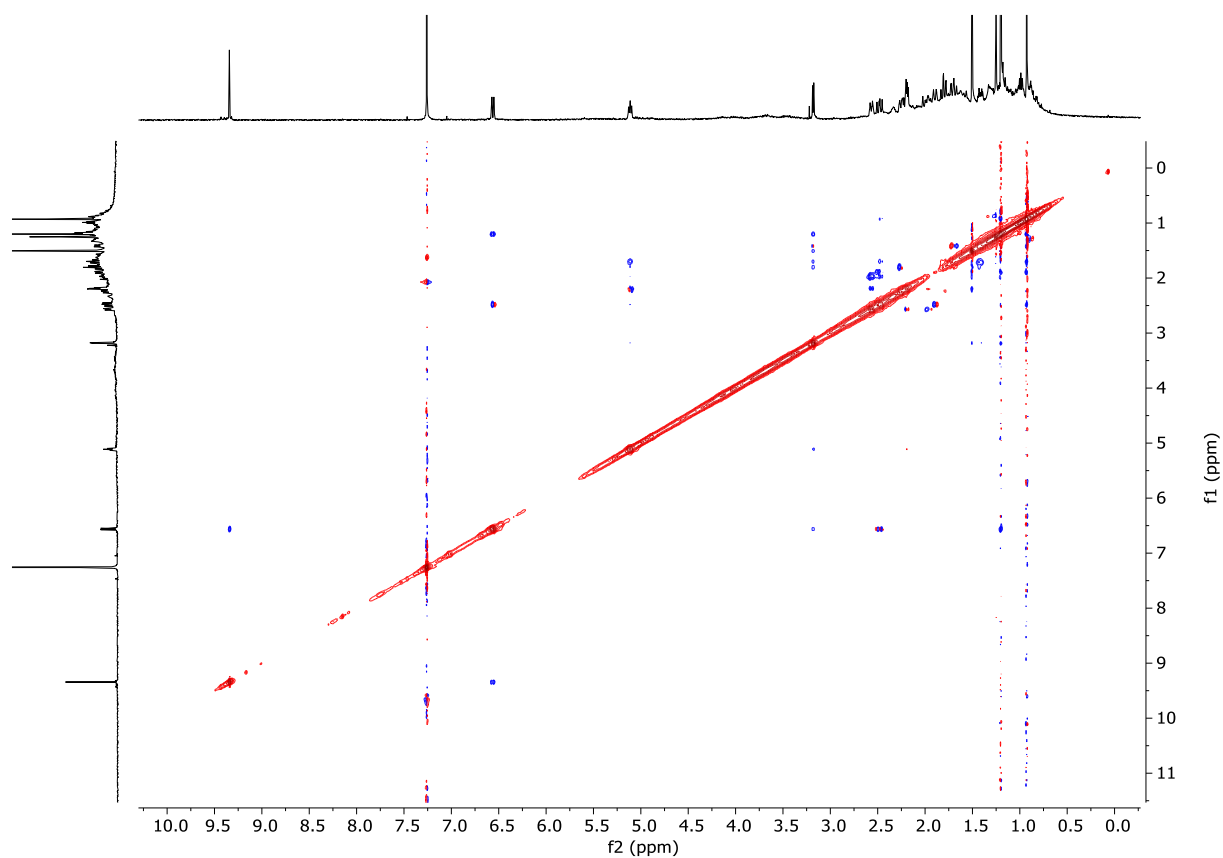


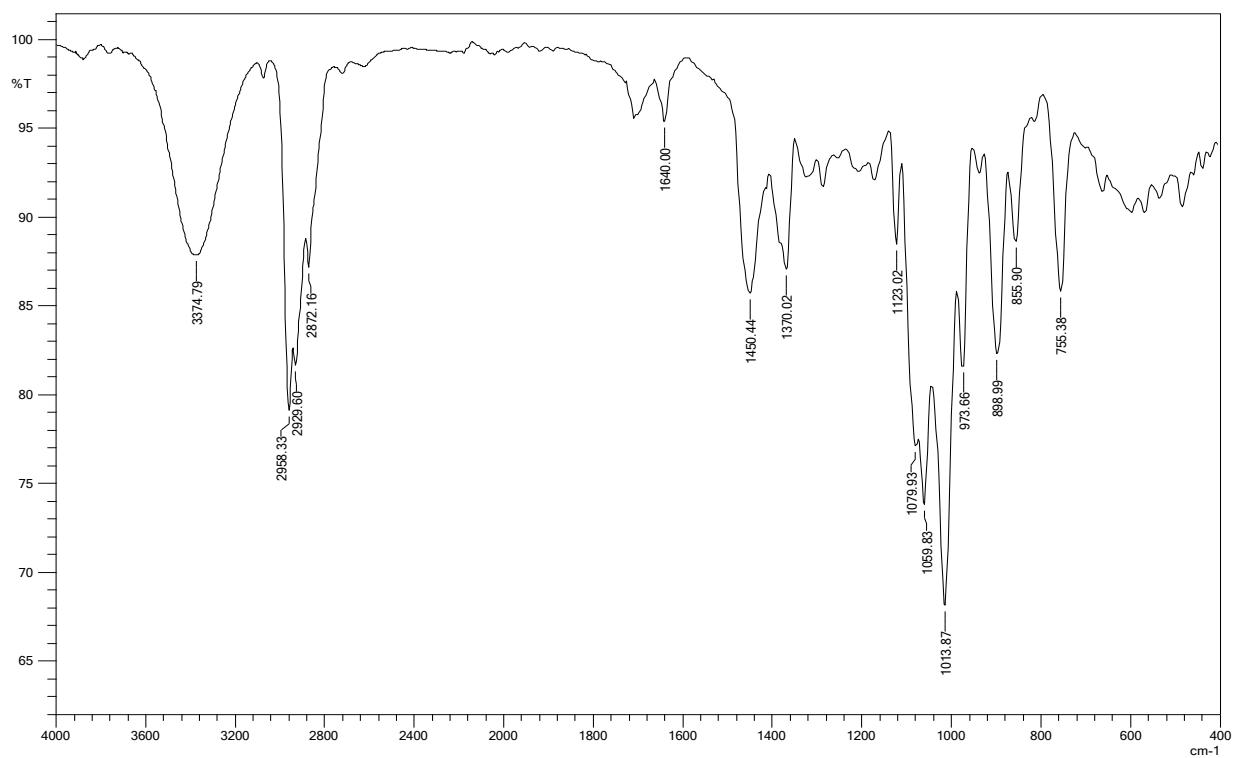
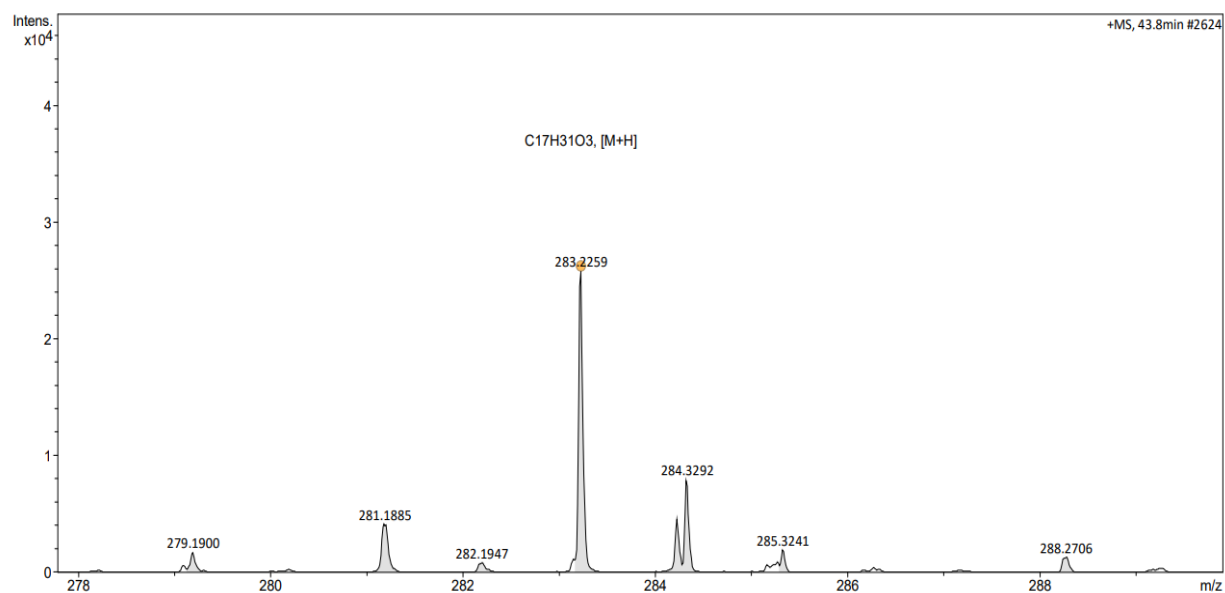
Figure S48. IR spectrum of dolichocarpol I (**3**)**Figure S49.** HRESIMS [M + H]⁺ spectrum of dolichocarpol I (**3**)

Figure S50. ^1H NMR spectrum of dolichocarpol I (**3**) (CDCl_3 , 500 MHz)

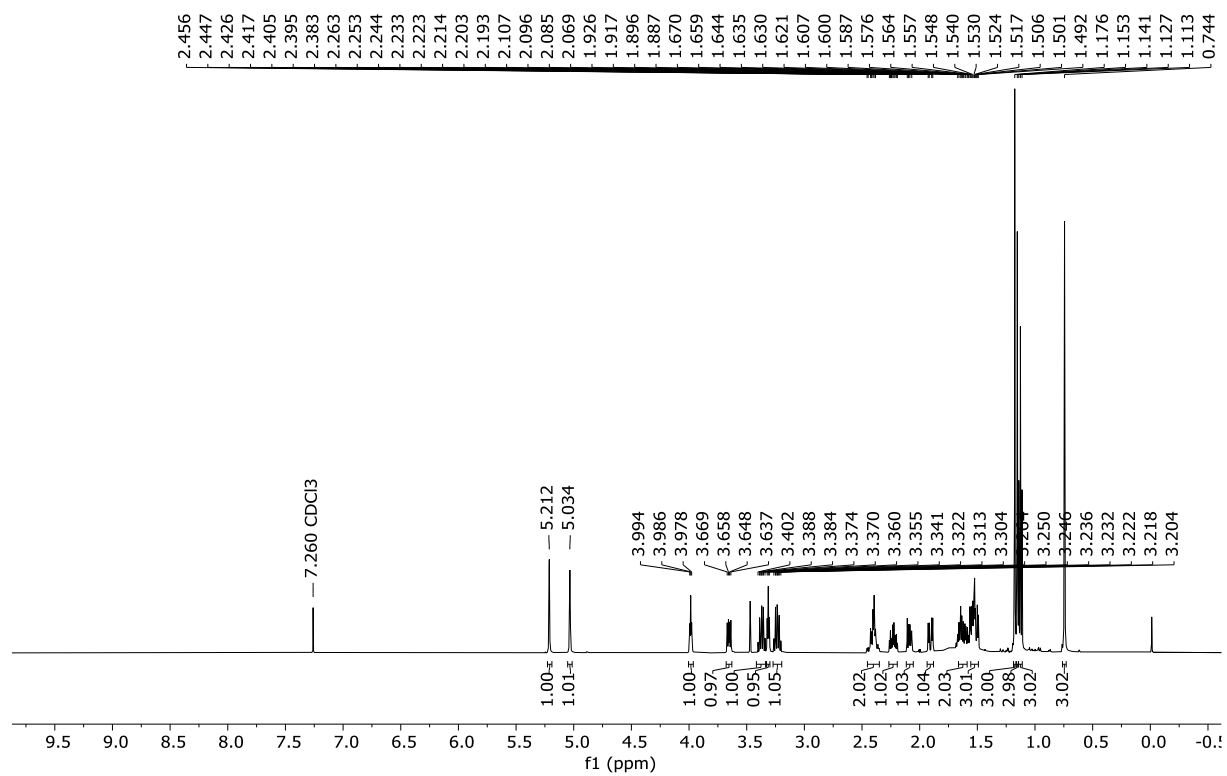


Figure S51. ^{13}C DEPTQ NMR spectrum of dolichocarpol I (**3**) (CDCl_3 , 125 MHz)

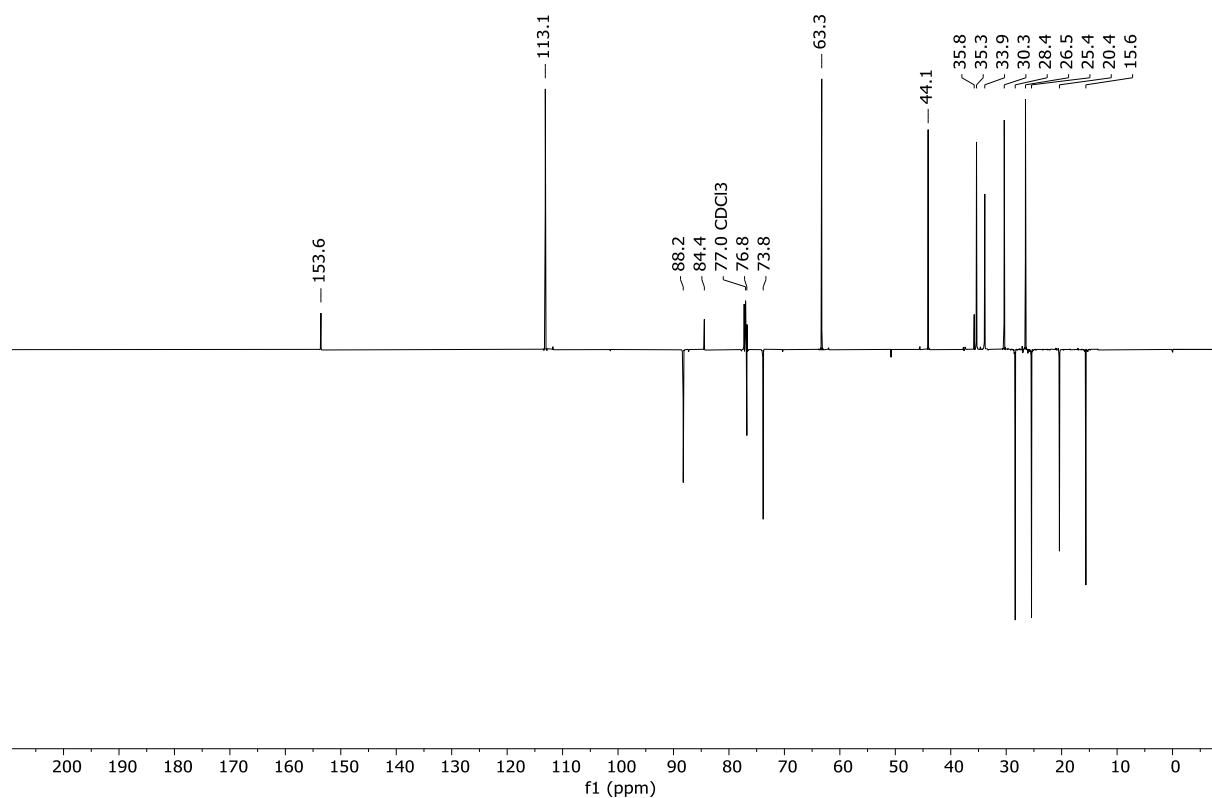


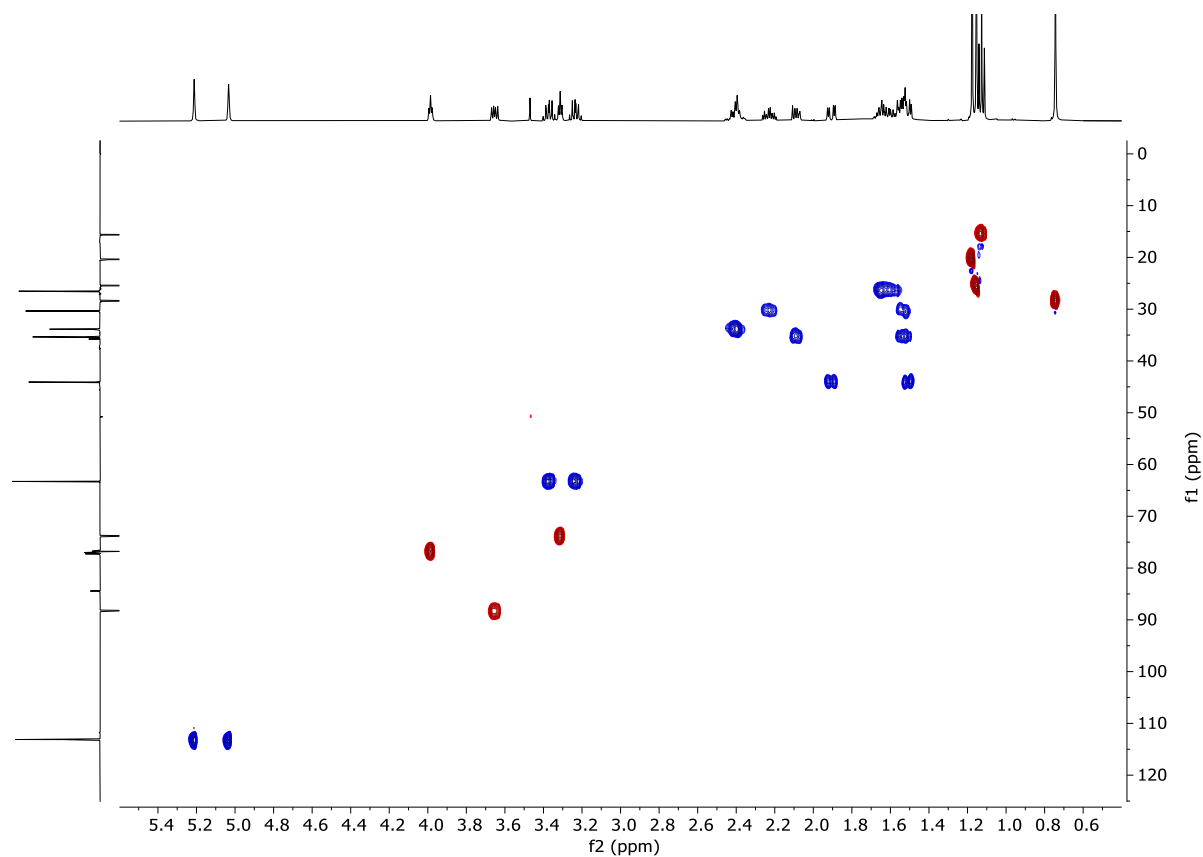
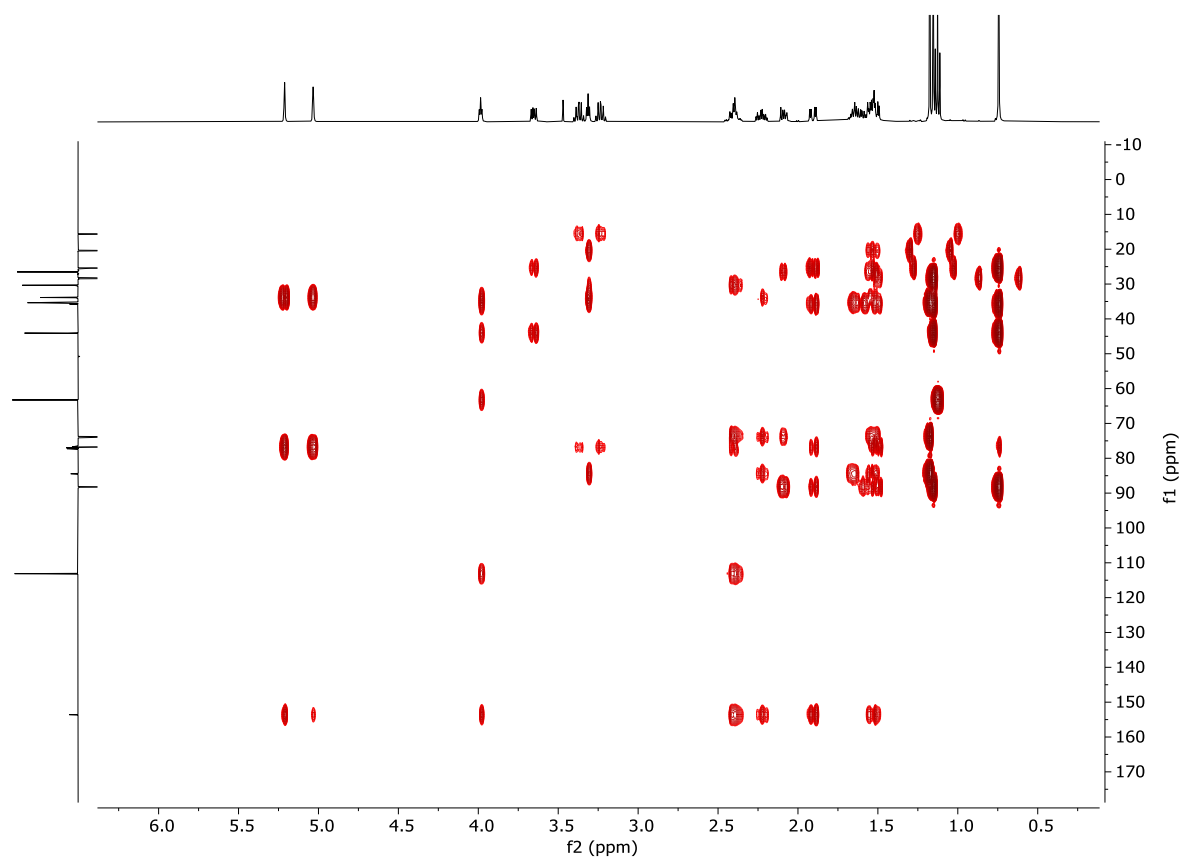
Figure S52. HSQC spectrum of dolichocarpol I (**3**) (CDCl₃, 500 MHz)**Figure S53.** HMBC spectrum of dolichocarpol I (**3**) (CDCl₃, 500 MHz)

Figure S54. COSY spectrum of dolichocarpol I (**3**) (CDCl₃, 500 MHz)

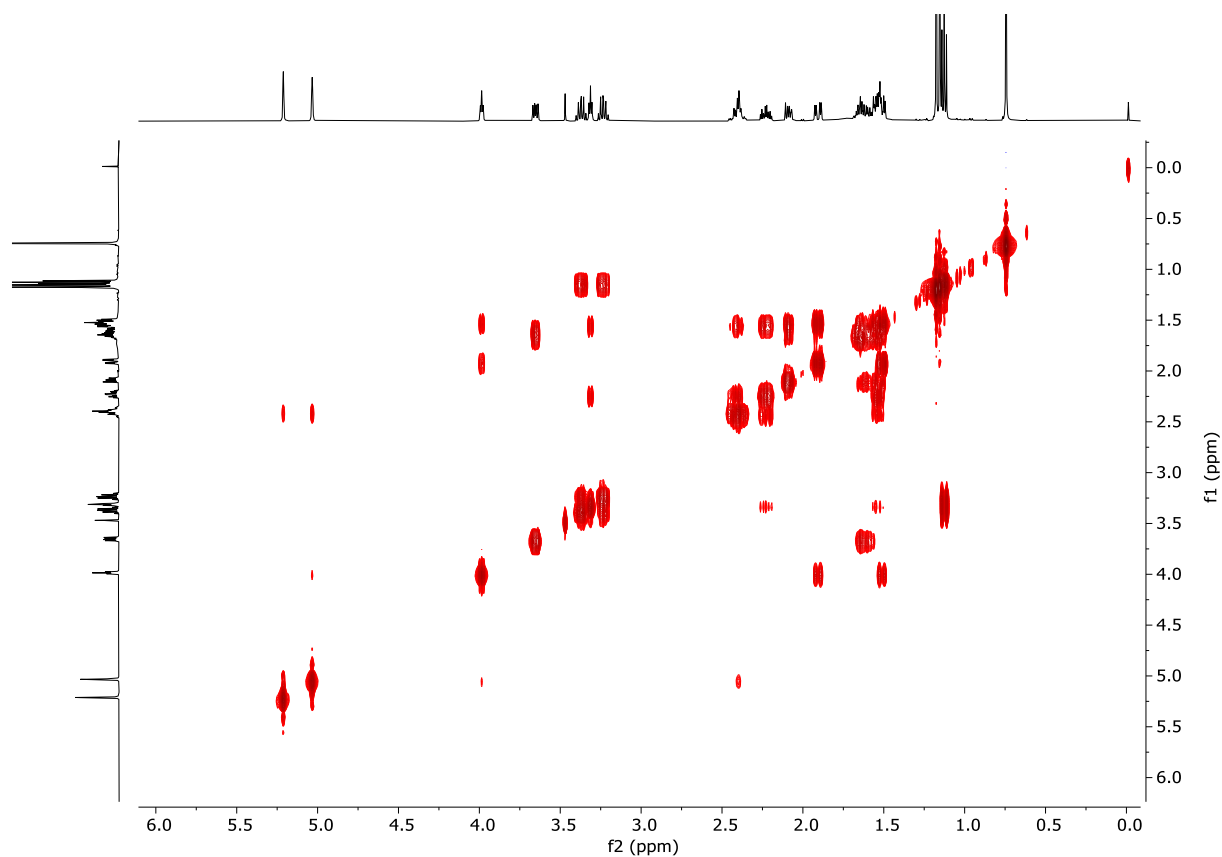


Figure S55. NOESY spectrum of dolichocarpol I (**3**) (CDCl₃, 500 MHz)

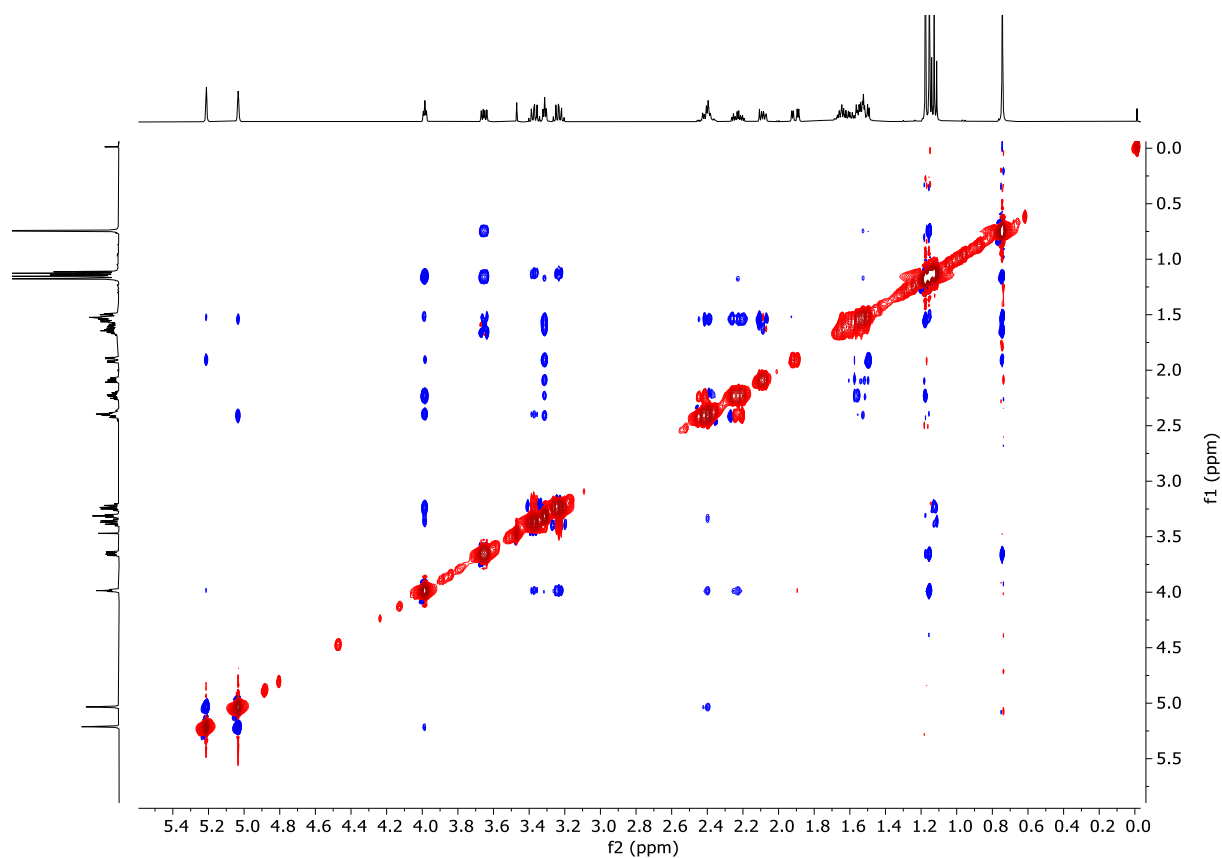


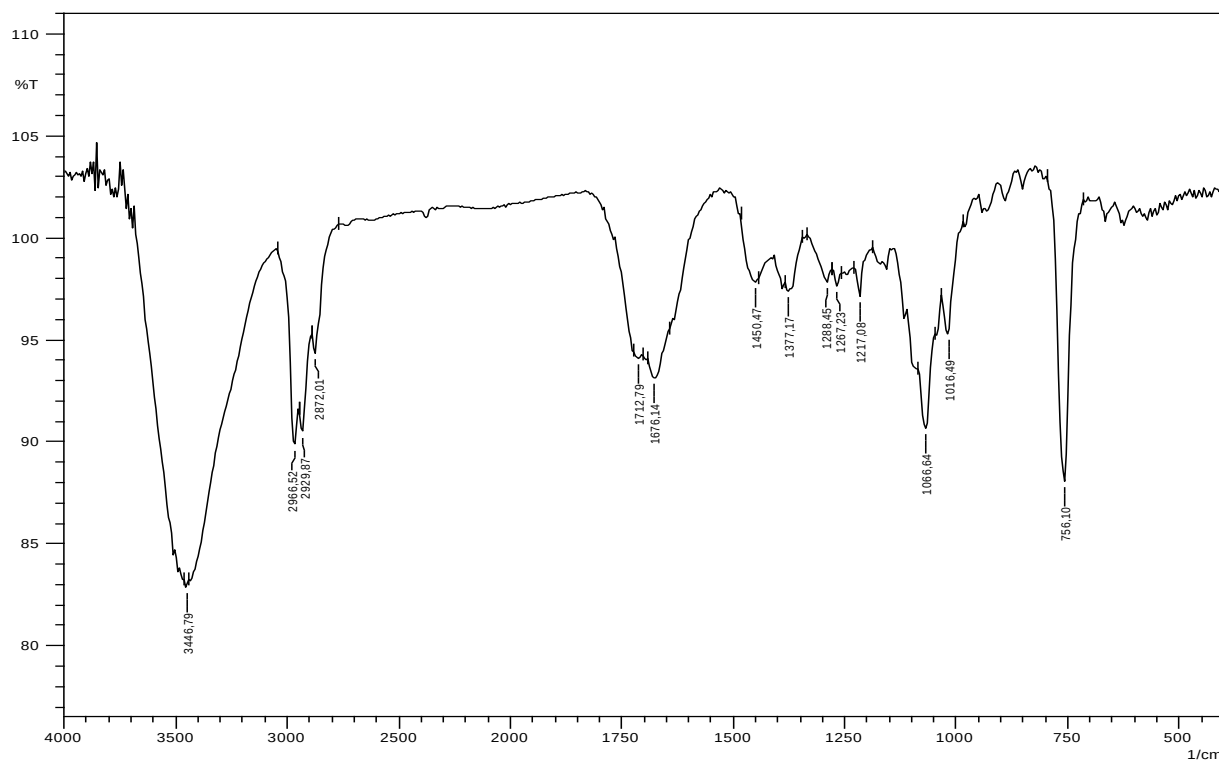
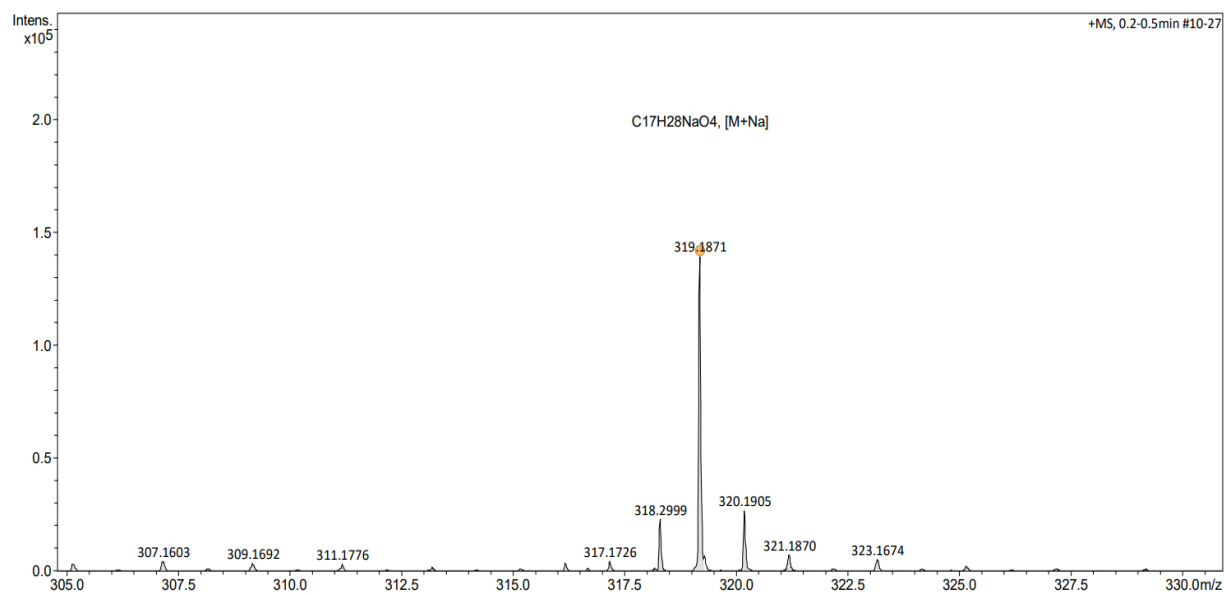
Figure S56. IR spectrum of dolichocarpol J (**4**)**Figure S57.** HRESIMS $[\text{M} + \text{Na}]^+$ spectrum of dolichocarpol J (**4**)

Figure S58. ^1H NMR spectrum of dolichocarpol J (**4**) (CDCl_3 , 500 MHz)

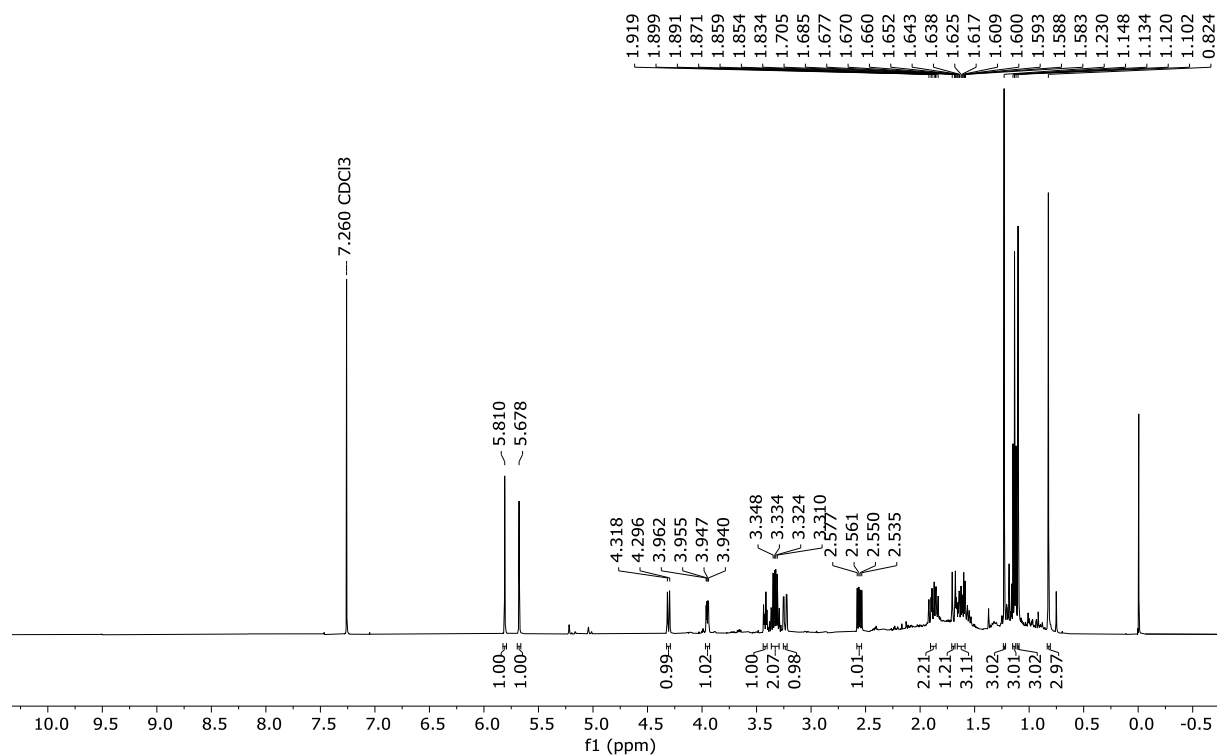


Figure S59. ^{13}C NMR spectrum of dolichocarpol J (**4**) (CDCl_3 , 125 MHz)

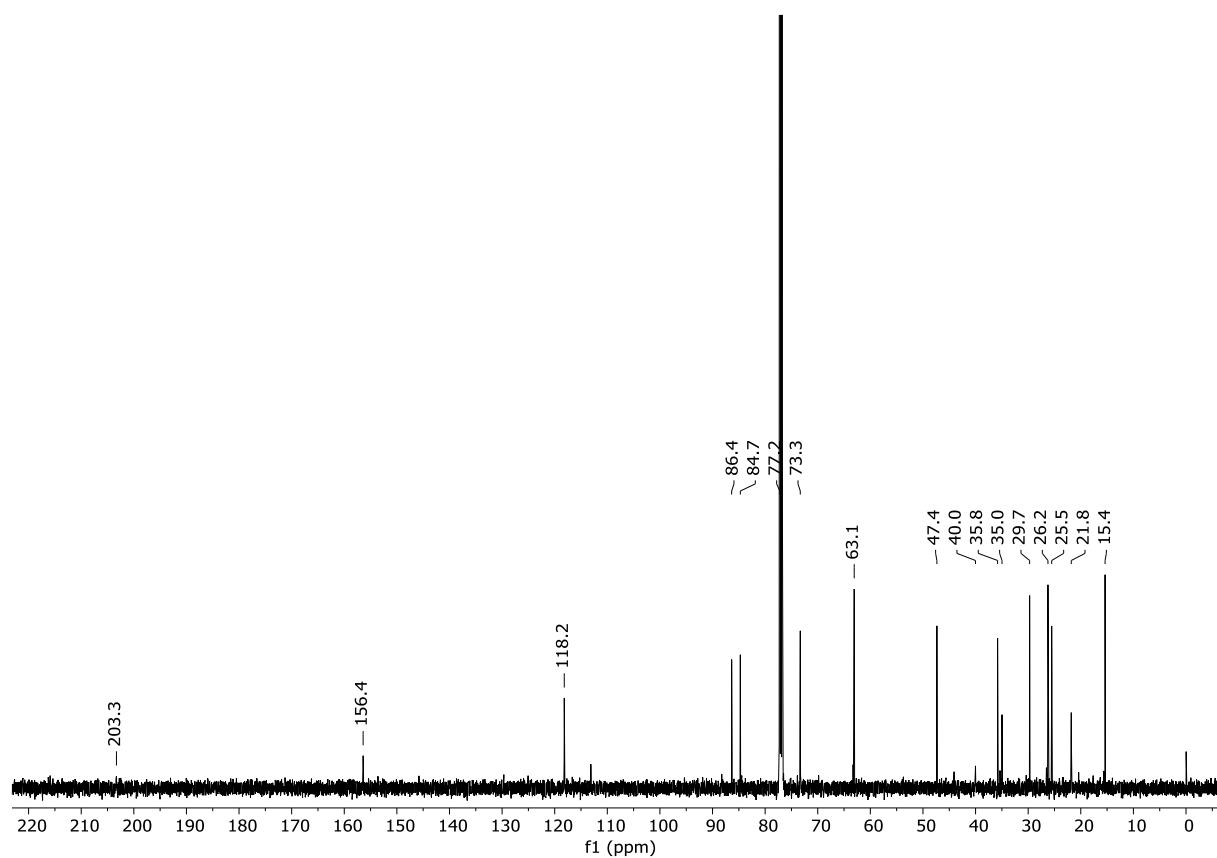


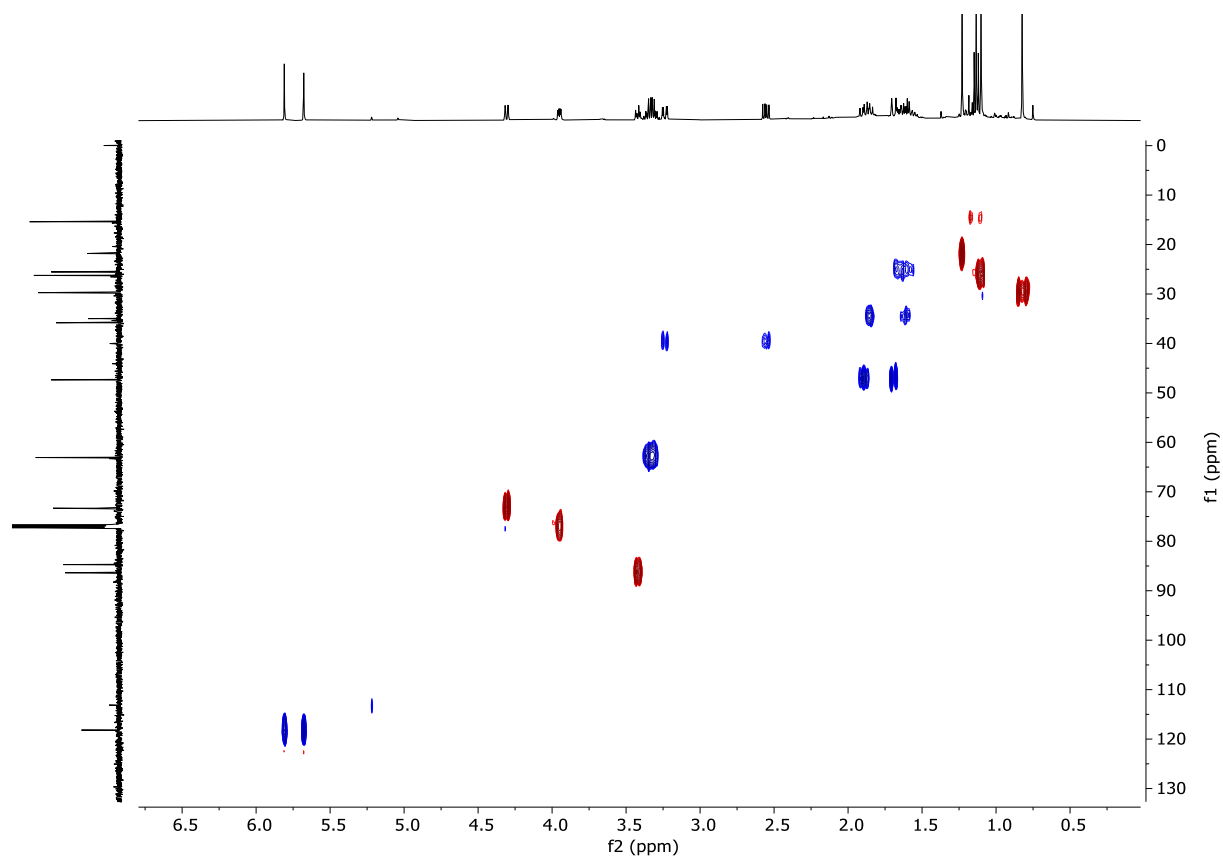
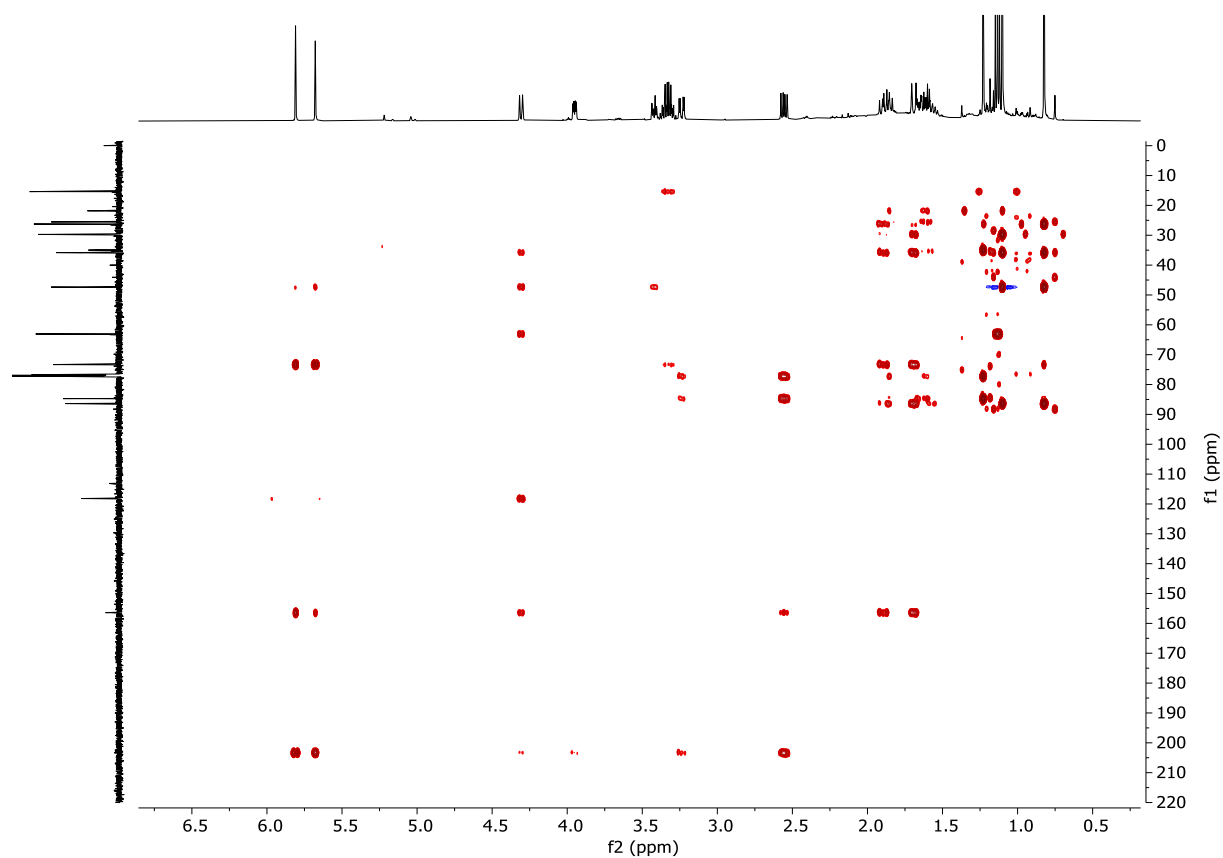
Figure S60. HSQC spectrum of dolichocarpol J (**4**) (CDCl₃, 500 MHz)**Figure S61.** HMBC spectrum of dolichocarpol J (**4**) (CDCl₃, 500 MHz)

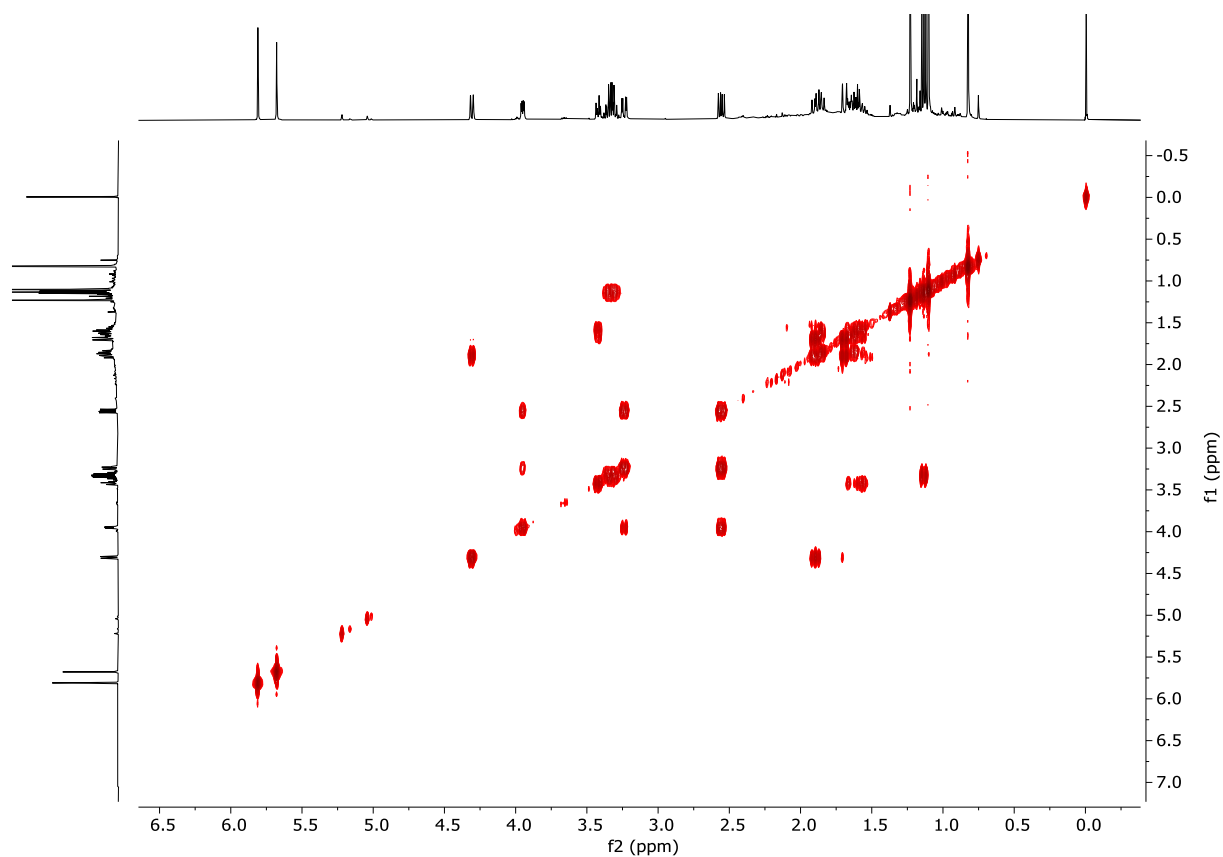
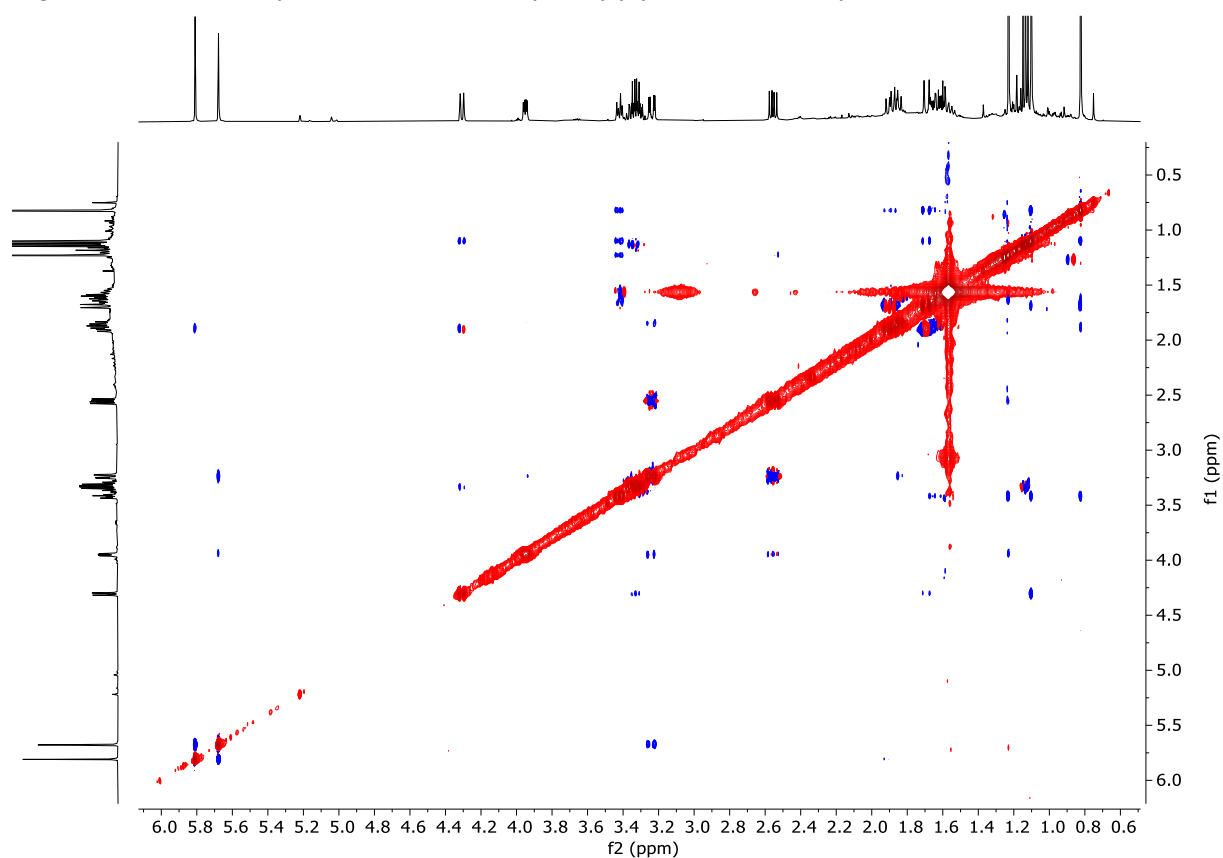
Figure S62. COSY spectrum of dolichocarpol J (**4**) (CDCl₃, 500 MHz)**Figure S63.** NOESY spectrum of dolichocarpol J (**4**) (CDCl₃, 500 MHz)

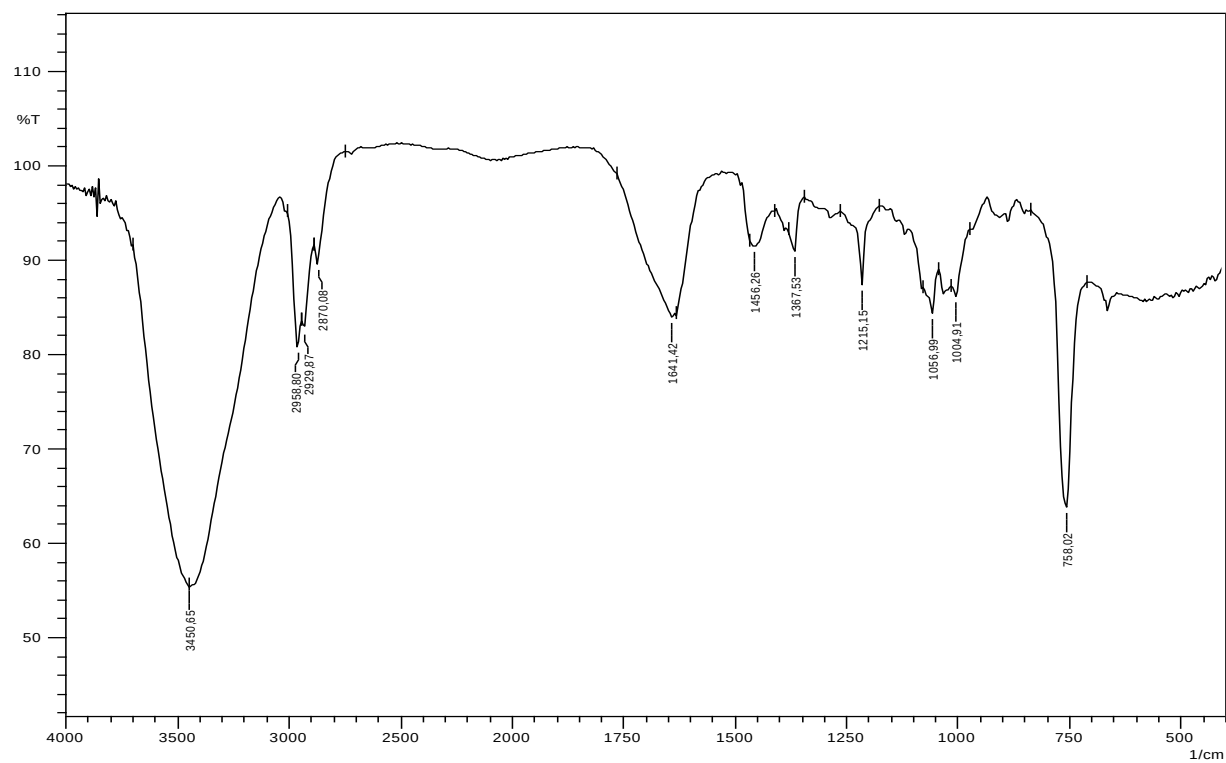
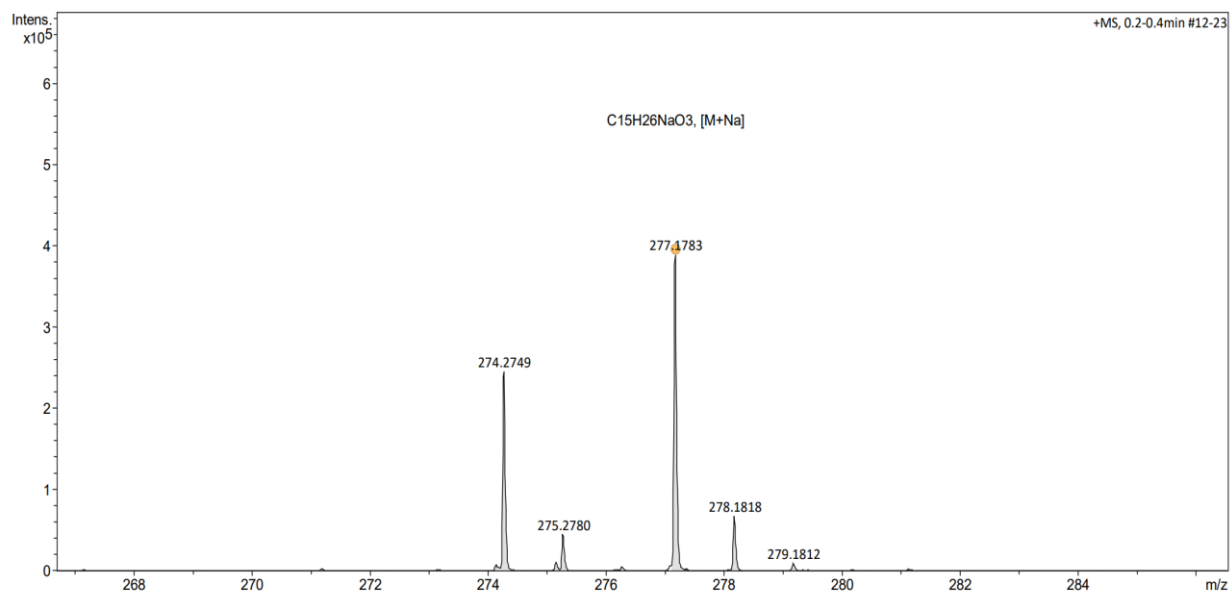
Figure S64. IR spectrum of dolichocarpol K (5)**Figure S65.** HRESIMS $[\text{M} + \text{Na}]^+$ spectrum of dolichocarpol K (5)

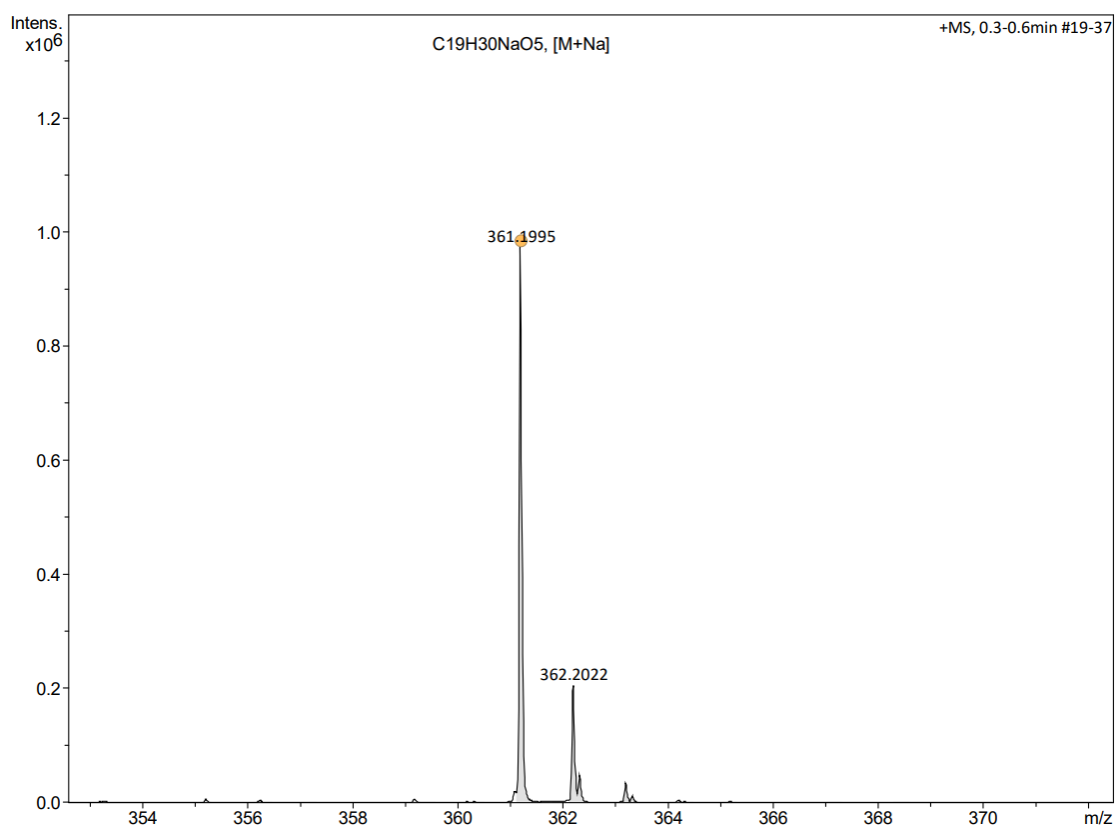
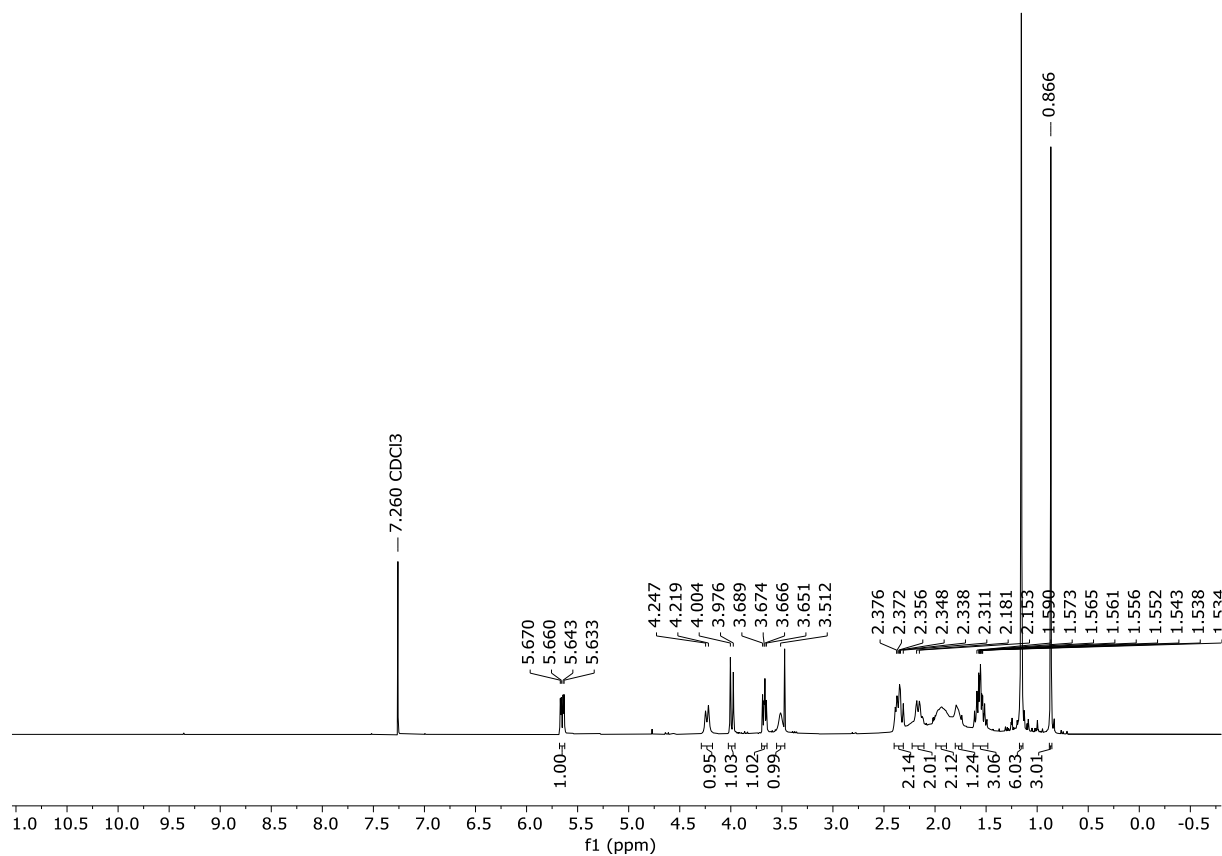
Figure S66. HRESIMS $[M + Na]^+$ spectrum of acetylated **5****Figure S67.** 1H NMR spectrum of dolichocarpol K (**5**) ($CDCl_3$, 400 MHz)

Figure S68. ^{13}C NMR spectrum of dolichocarpol K (**5**) (CDCl_3 , 100 MHz)

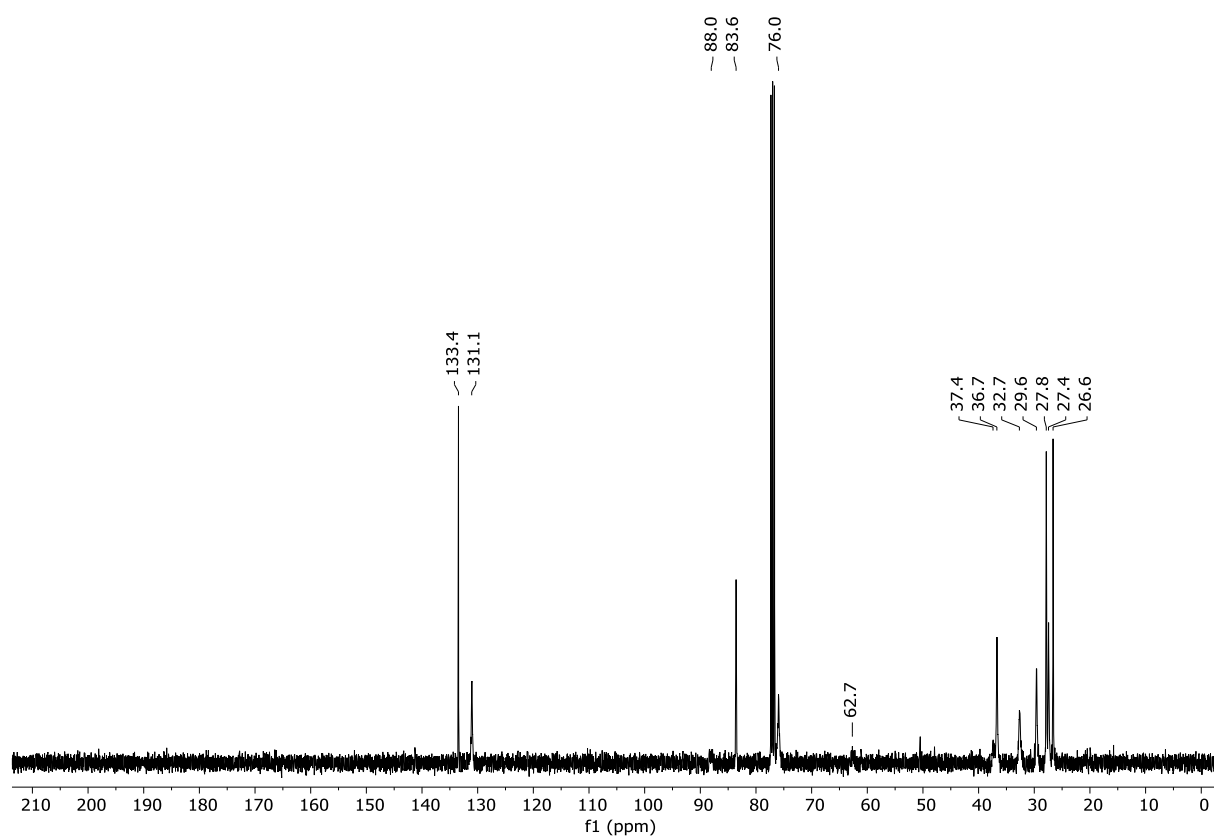


Figure S69. ^{13}C DEPT135 spectrum of dolichocarpol K (**5**) (CDCl_3 , 100 MHz)

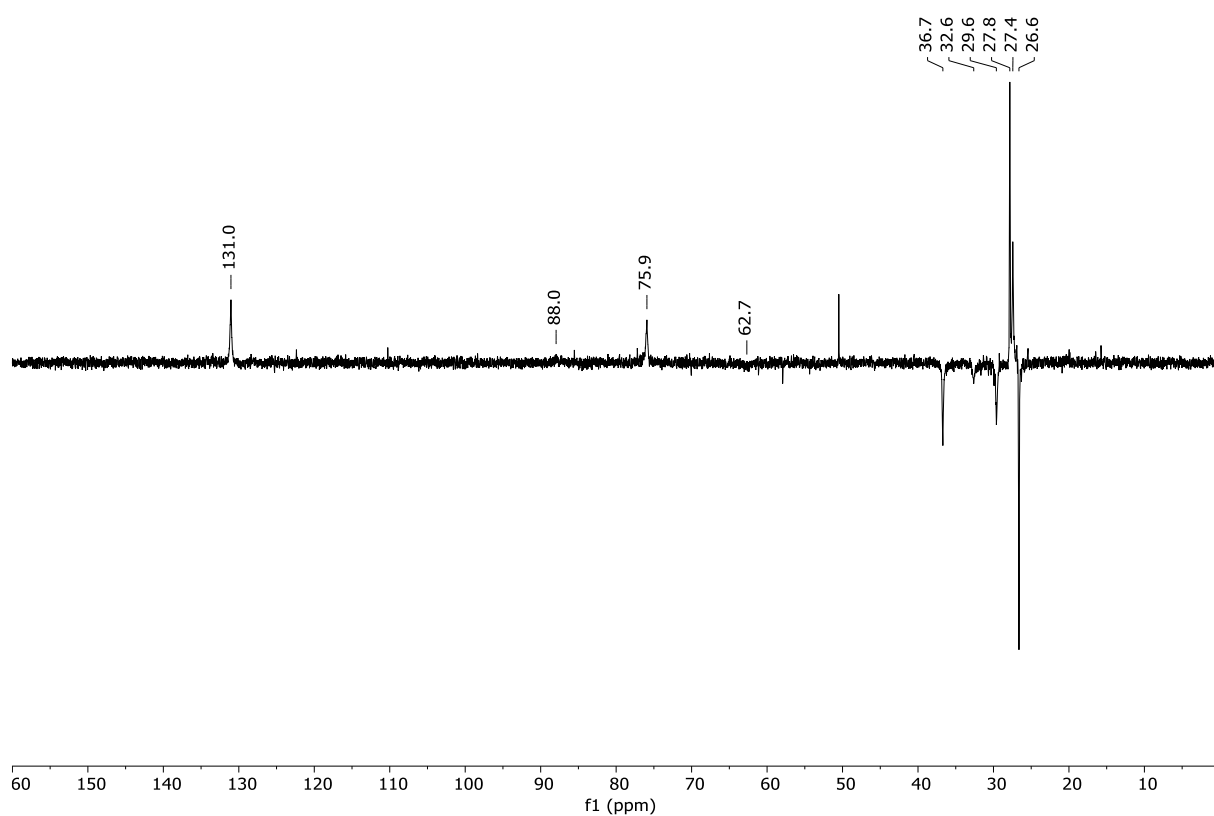


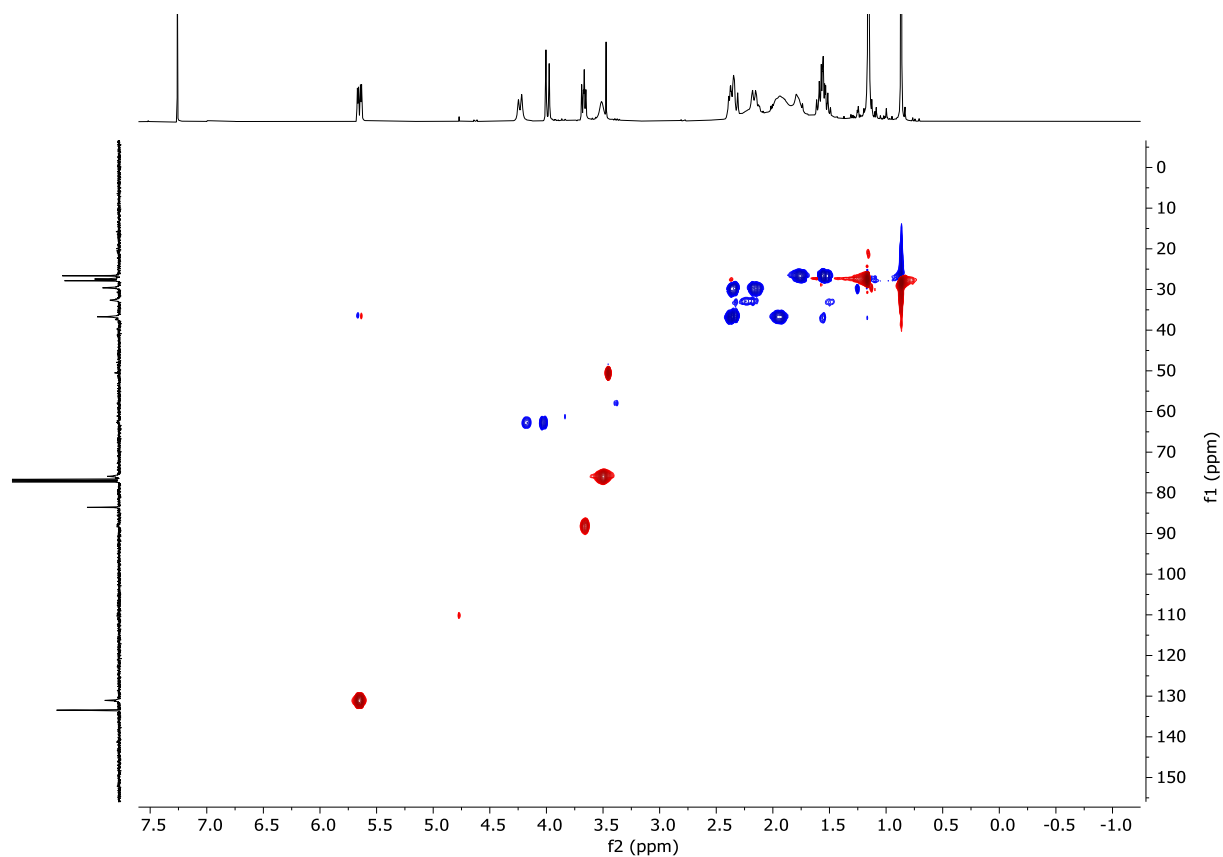
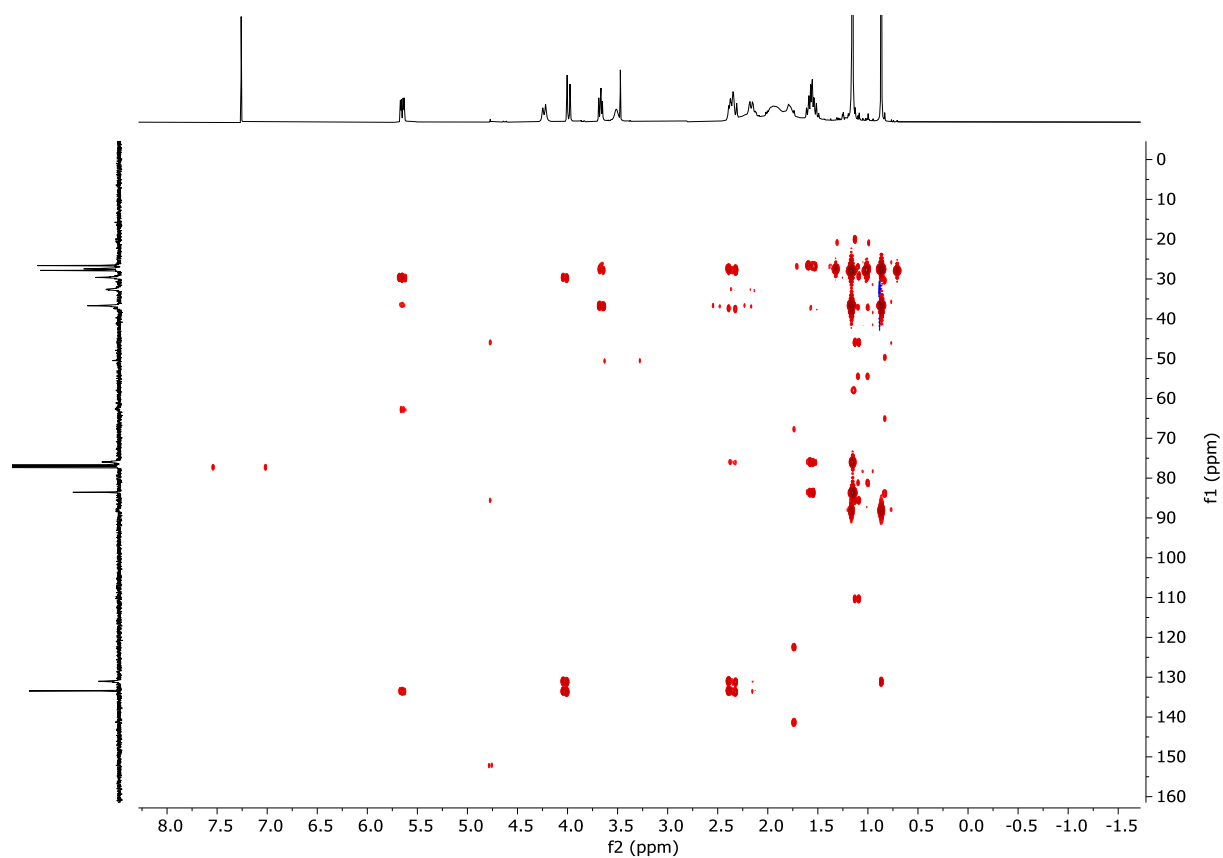
Figure S70. HSQC spectrum of dolichocarpol K (**5**) (CDCl₃, 400 MHz)**Figure S71.** HMBC spectrum of dolichocarpol K (**5**) (CDCl₃, 400 MHz)

Figure S72. COSY spectrum of dolichocarpol K (**5**) (CDCl₃, 400 MHz)

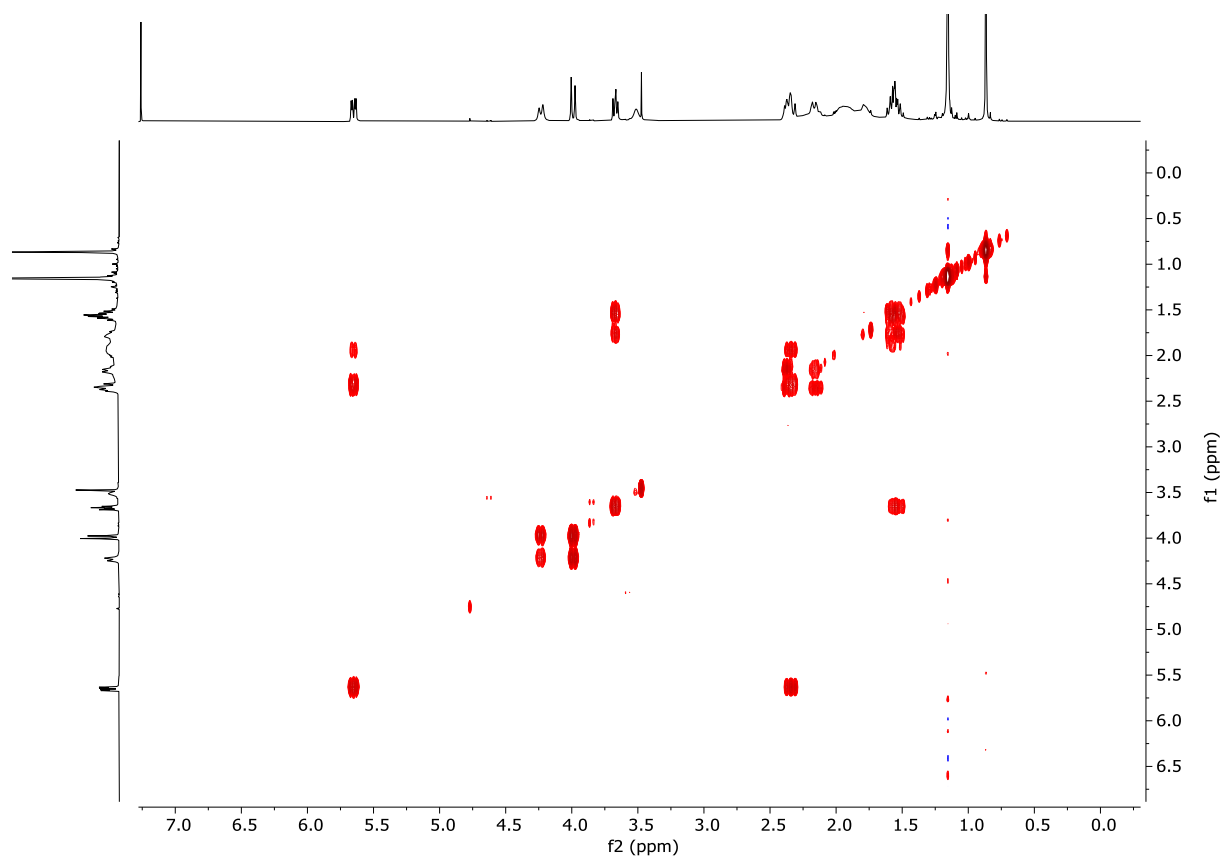


Figure S73. NOESY spectrum of dolichocarpol K (**5**) (CDCl₃, 400 MHz)

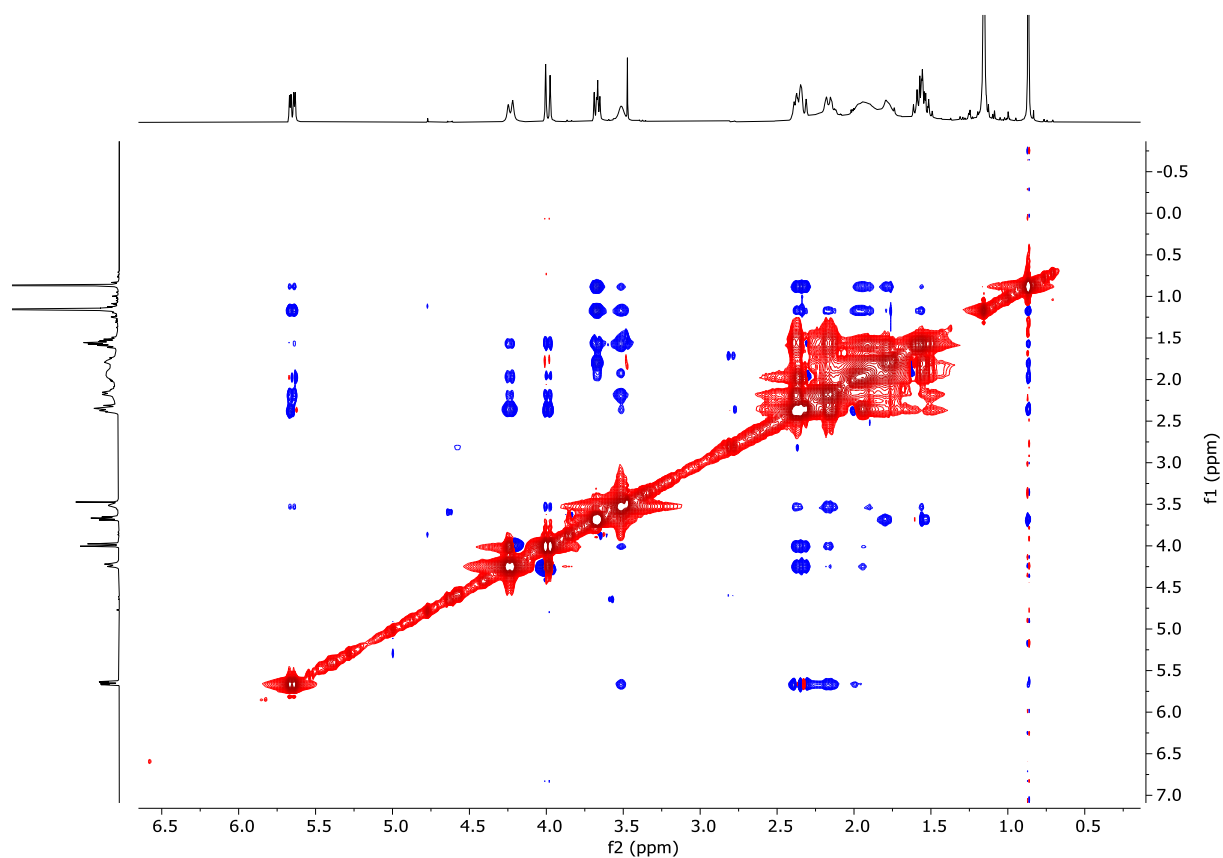


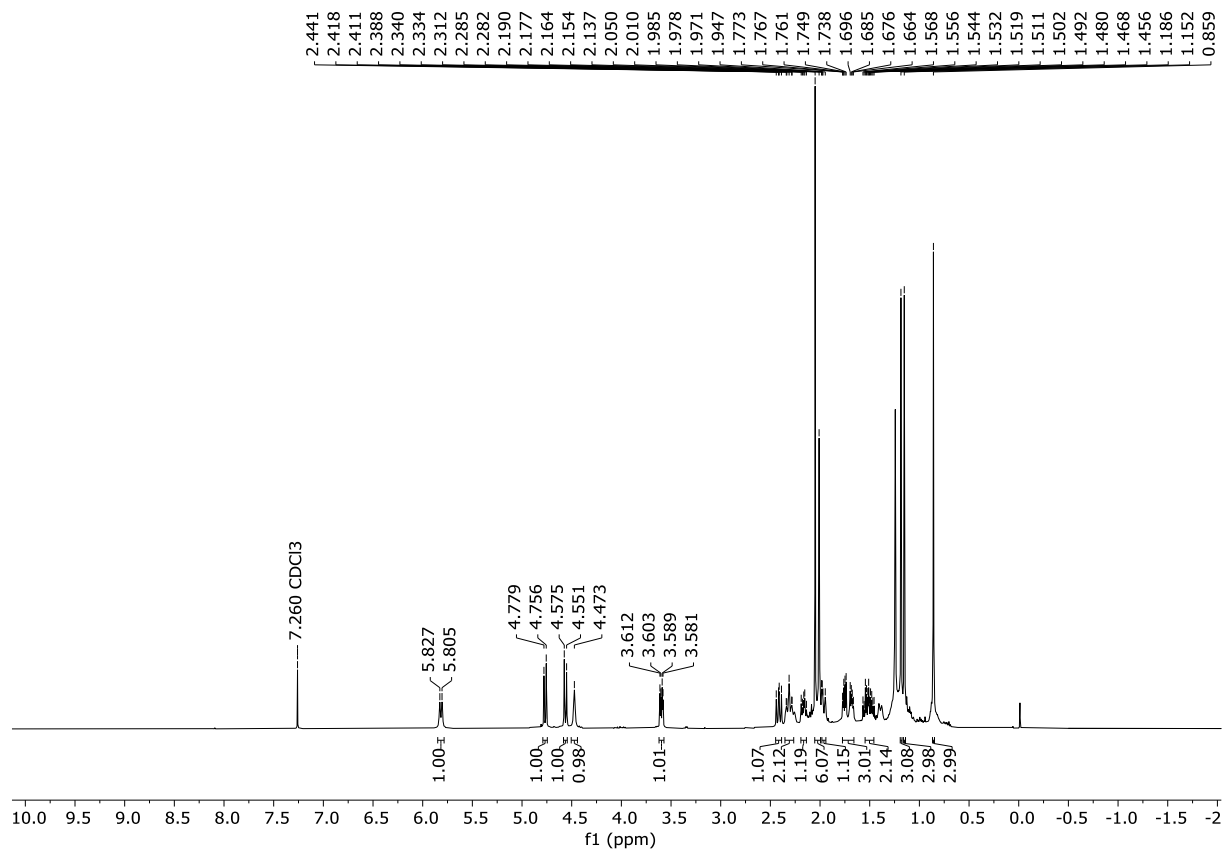
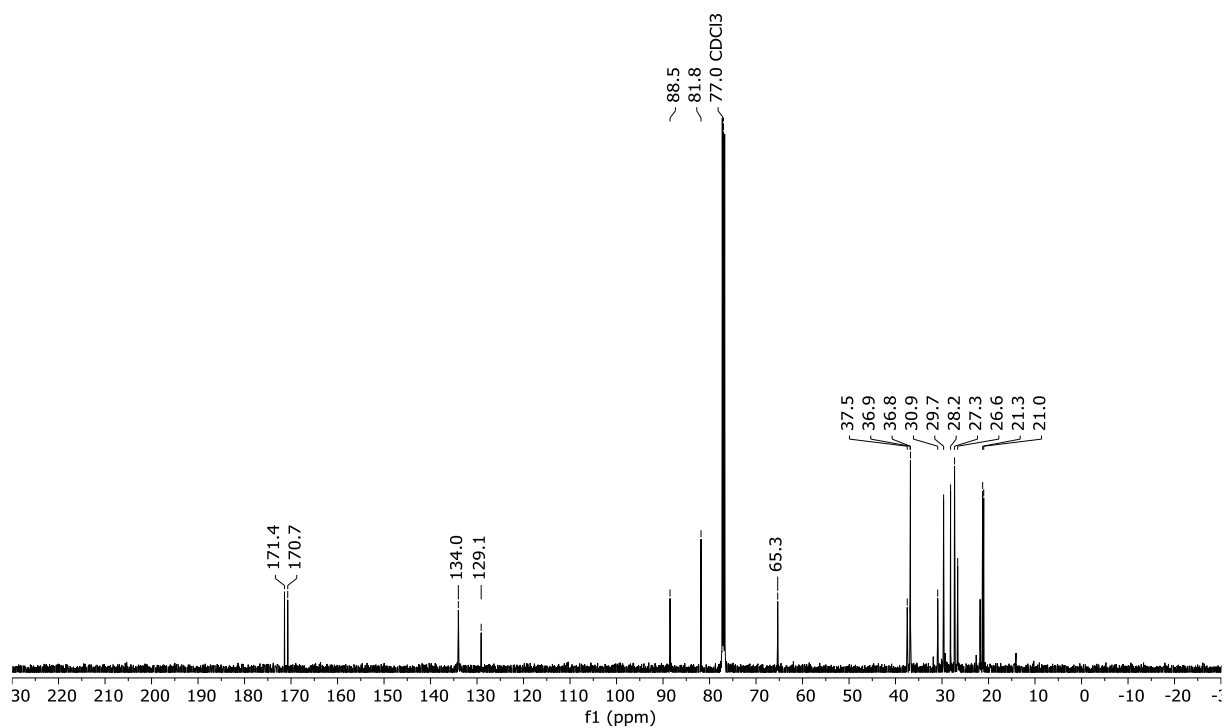
Figure S74. ^1H NMR spectrum of acetylated **5** (CDCl_3 , 500 MHz)**Figure S75.** ^{13}C NMR spectrum of acetylated **5** (CDCl_3 , 125 MHz)

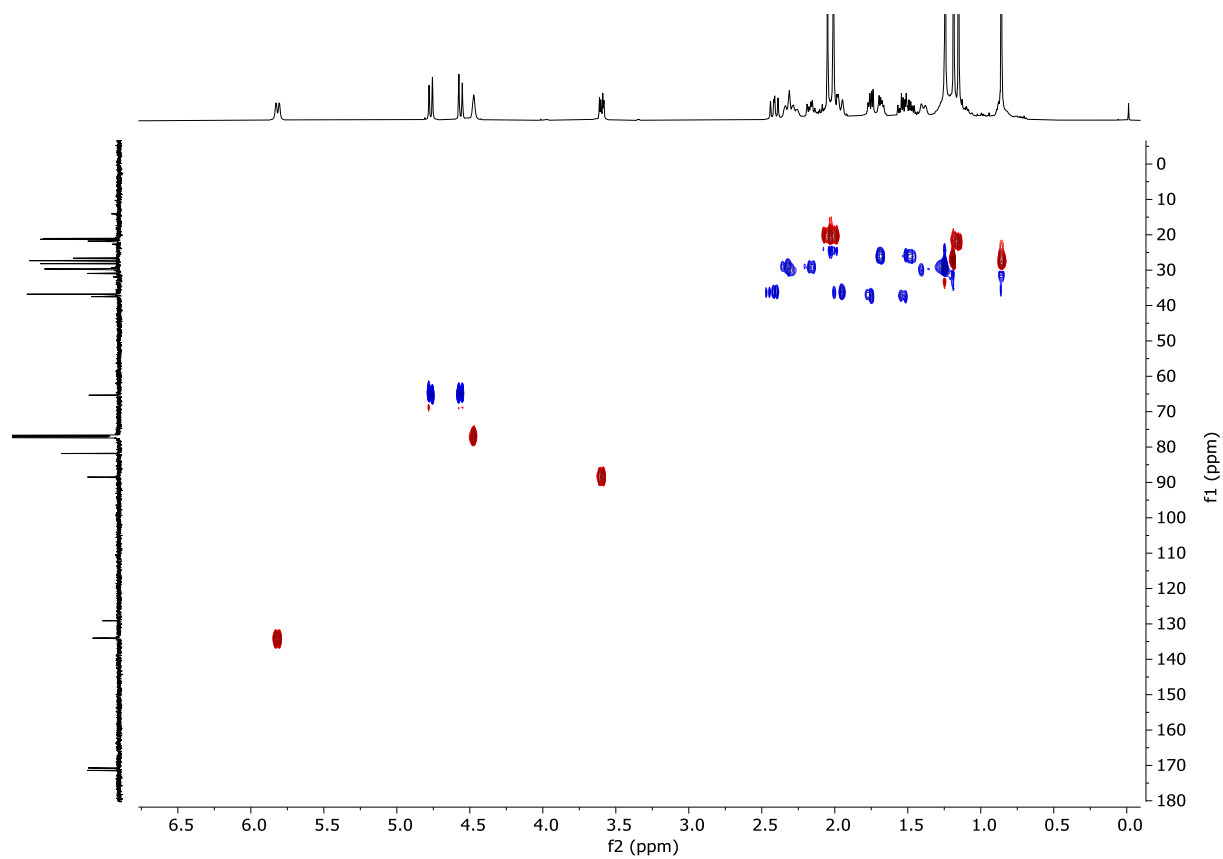
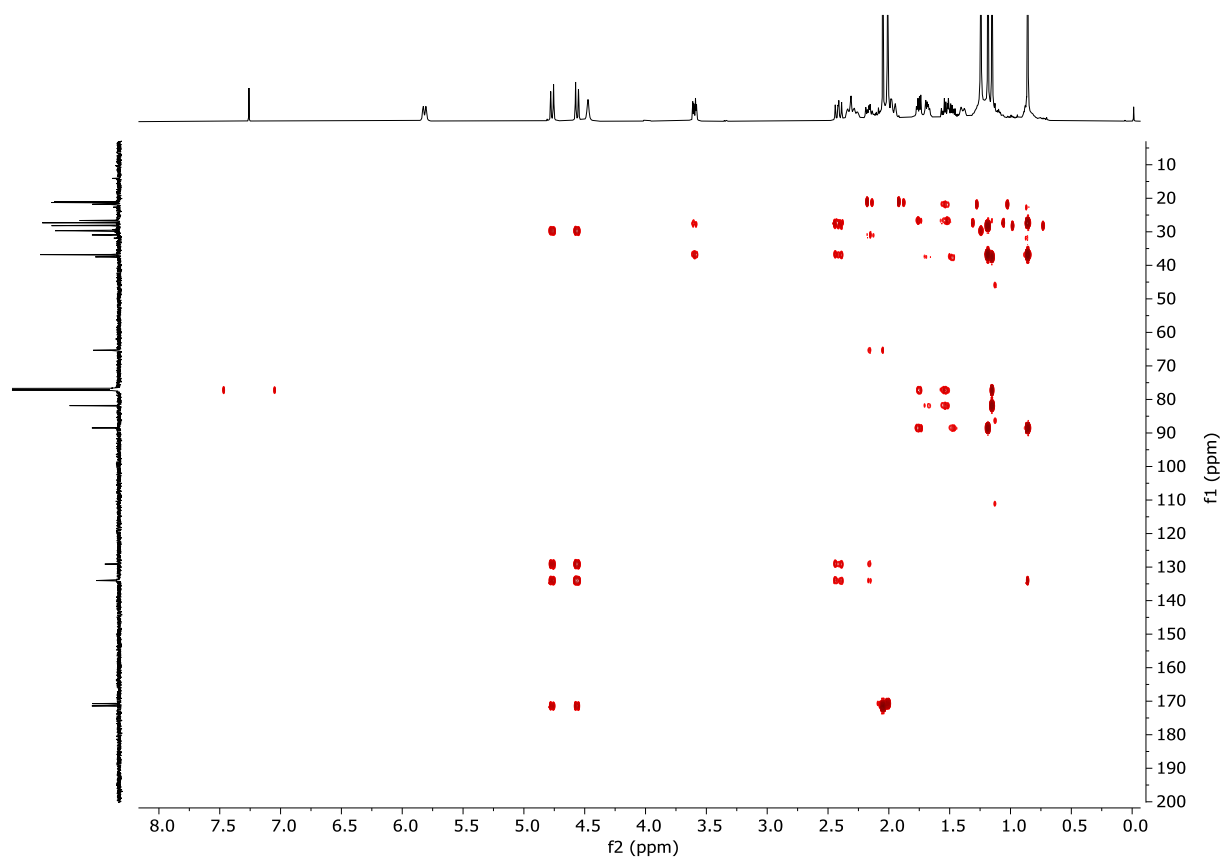
Figure S76. HSQC spectrum of acetylated **5** (CDCl₃, 500 MHz)**Figure S77.** HMBC spectrum of acetylated **5** (CDCl₃, 500 MHz)

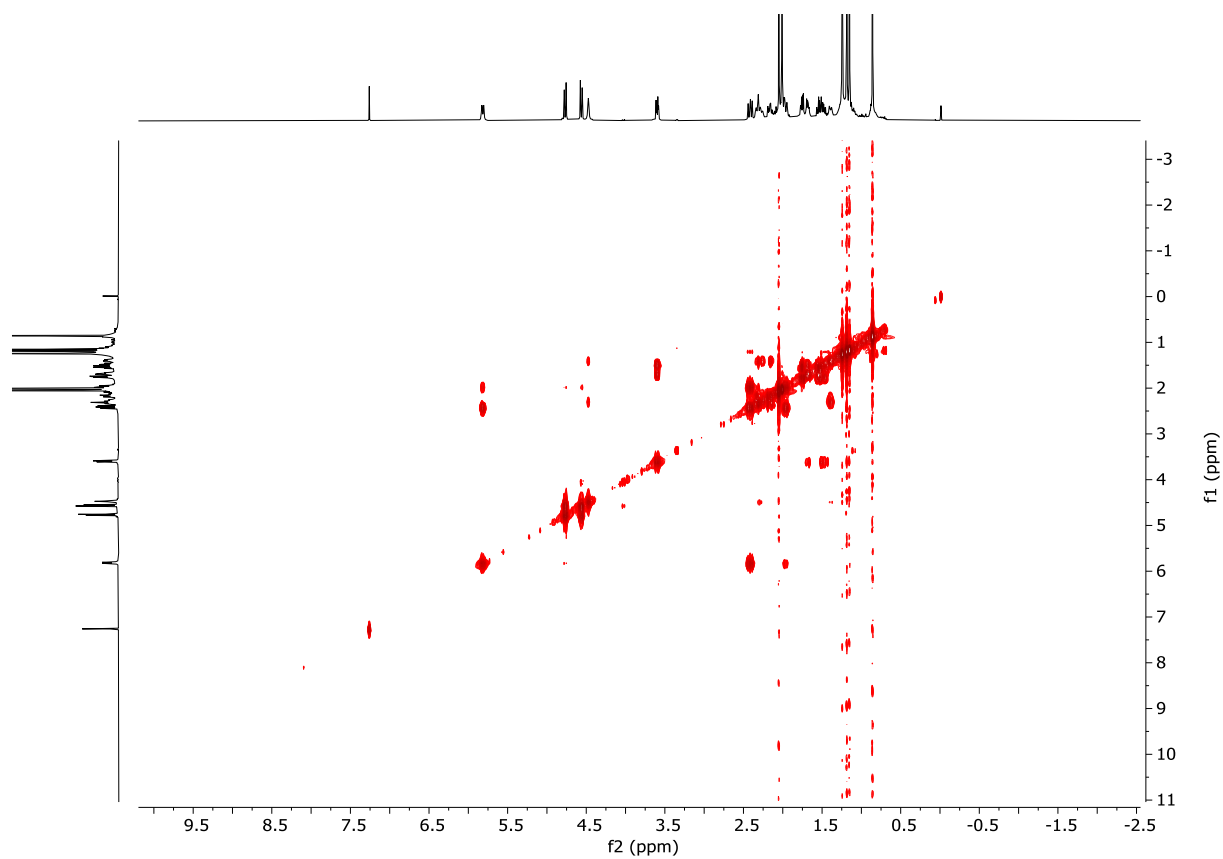
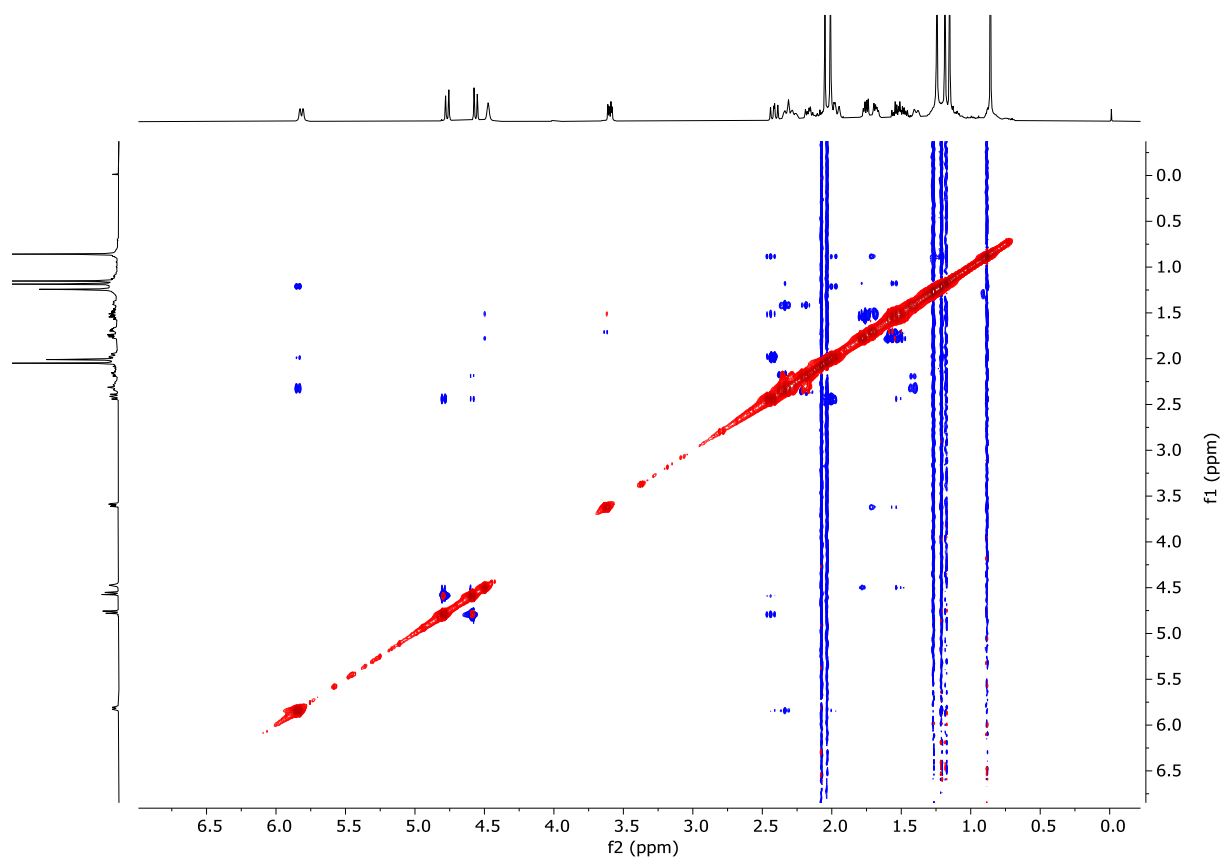
Figure S78. COSY spectrum of acetylated **5** (CDCl₃, 500 MHz)**Figure S79.** NOESY spectrum of acetylated **5** (CDCl₃, 500 MHz)

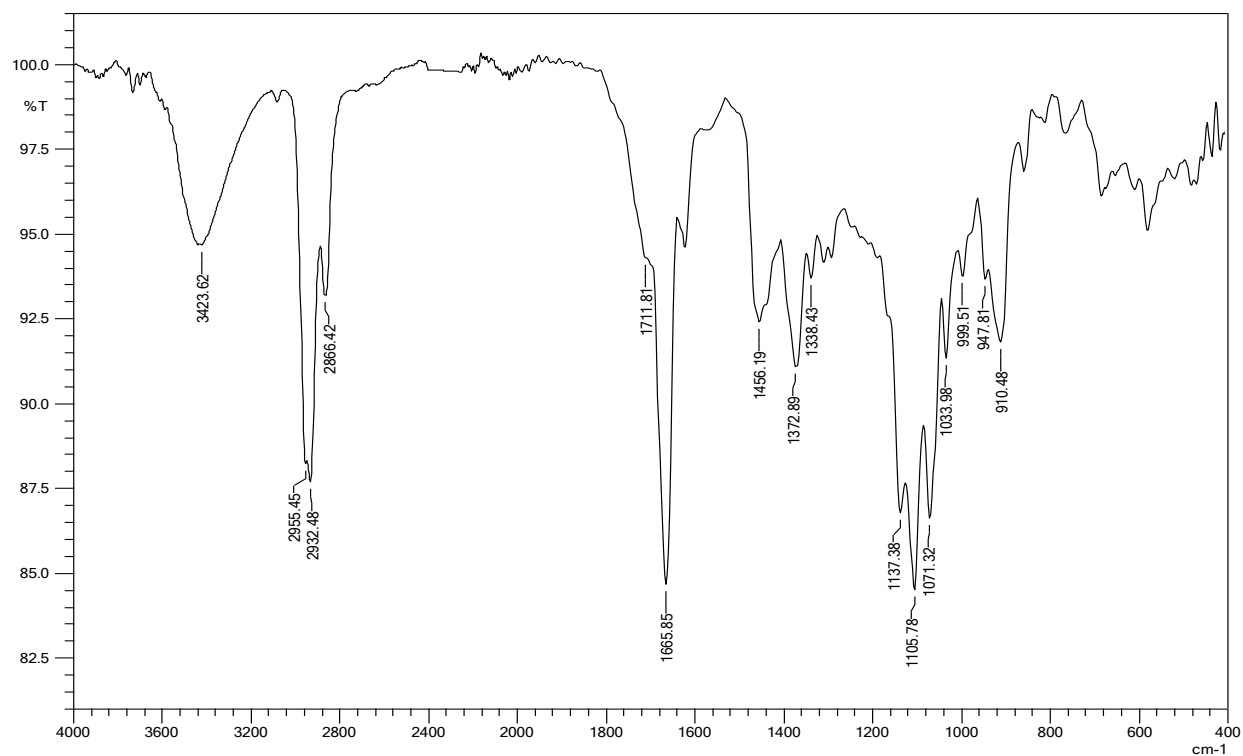
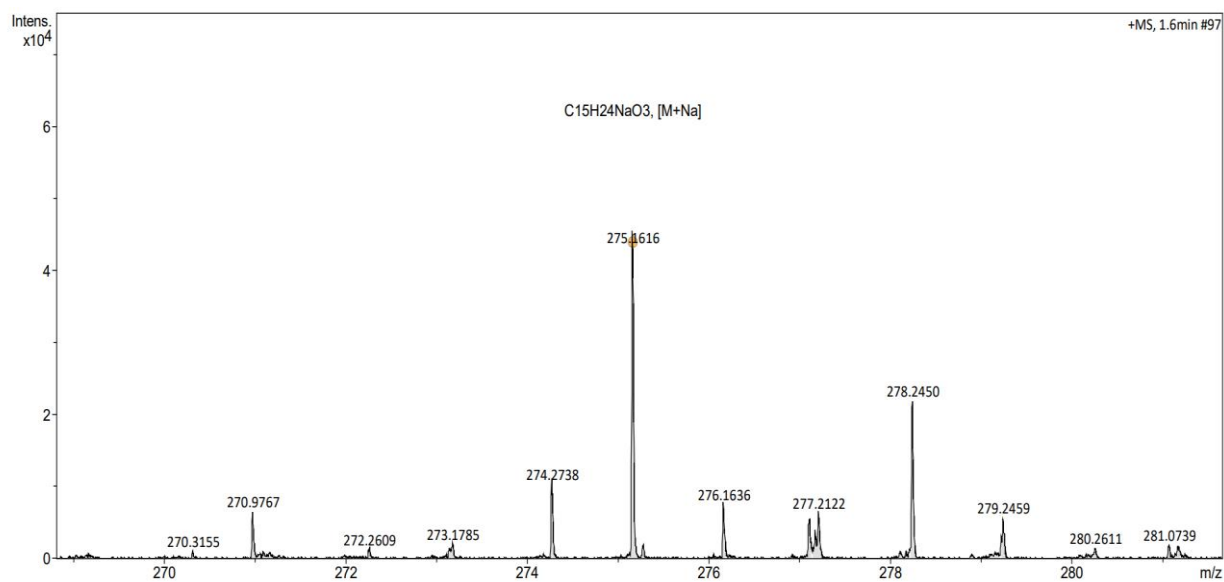
Figure S80. IR spectrum of dolichocarpol L (**6**)**Figure S81.** HRESIMS [M + H]⁺ spectrum of dolichocarpol L (**6**)

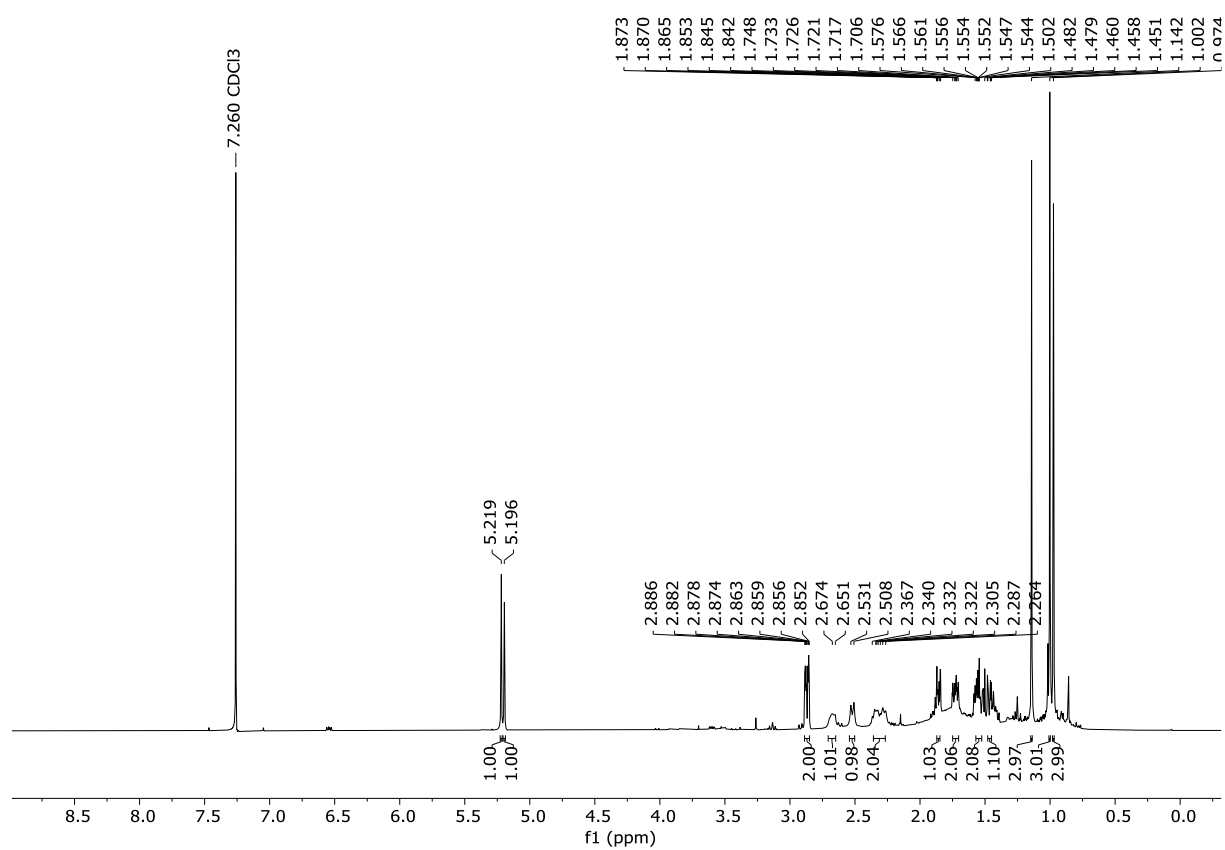
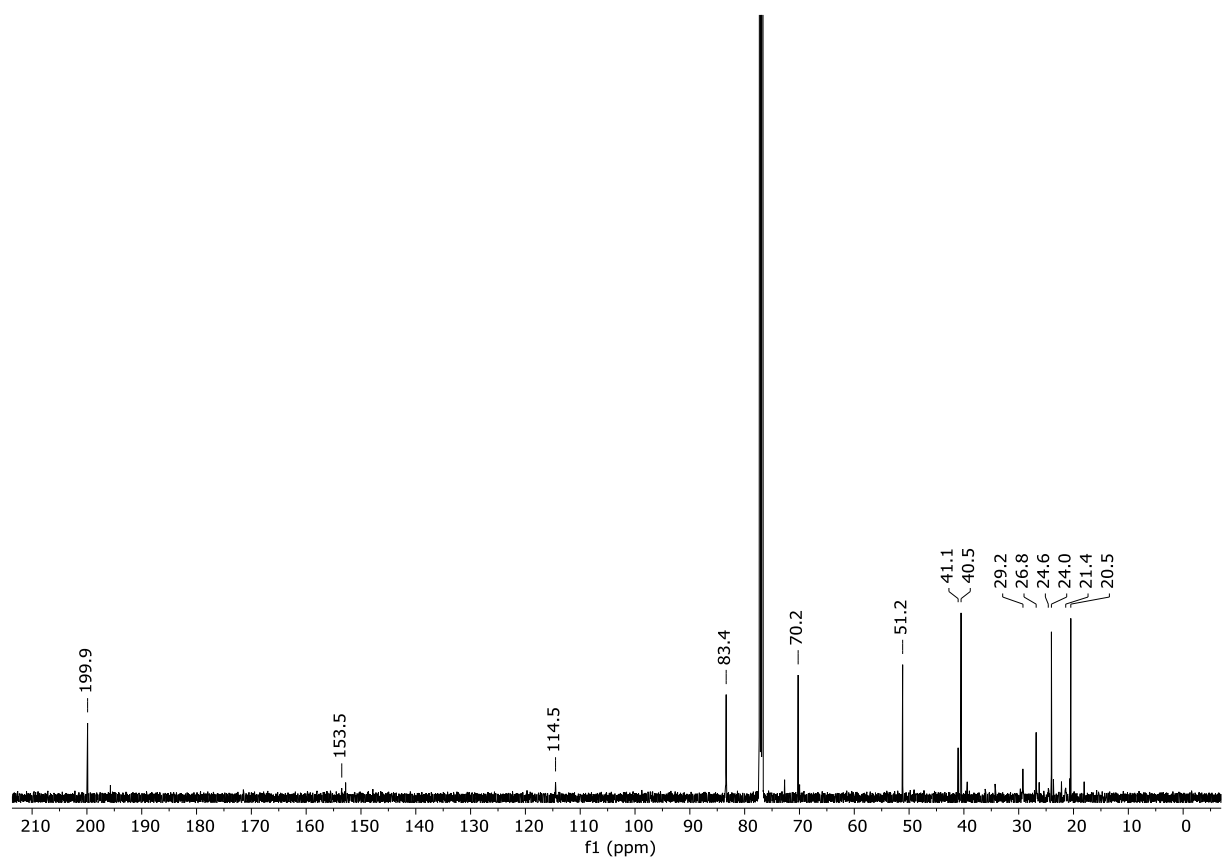
Figure S82. ^1H NMR spectrum of dolichocarpol L (**6**) (CDCl_3 , 500 MHz)**Figure S83.** ^{13}C NMR spectrum of dolichocarpol L (**6**) (CDCl_3 , 125 MHz)

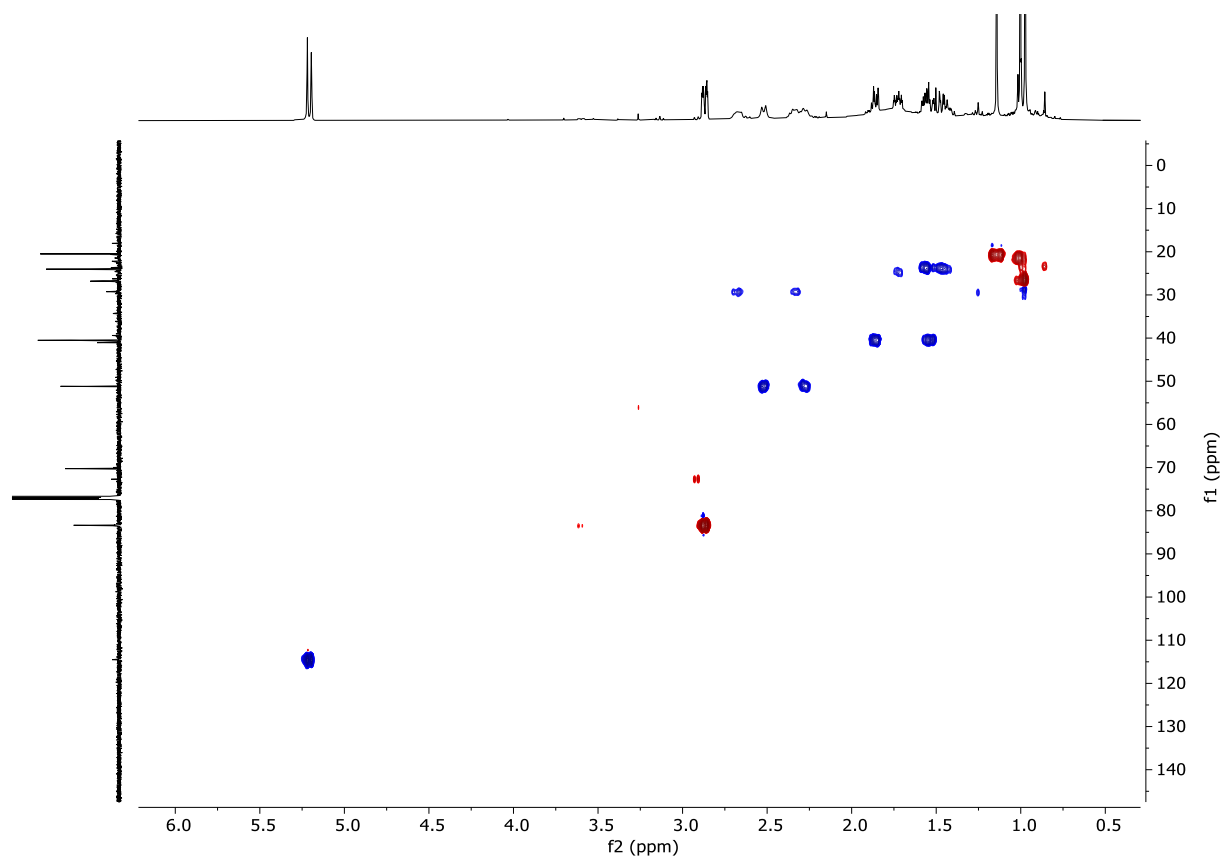
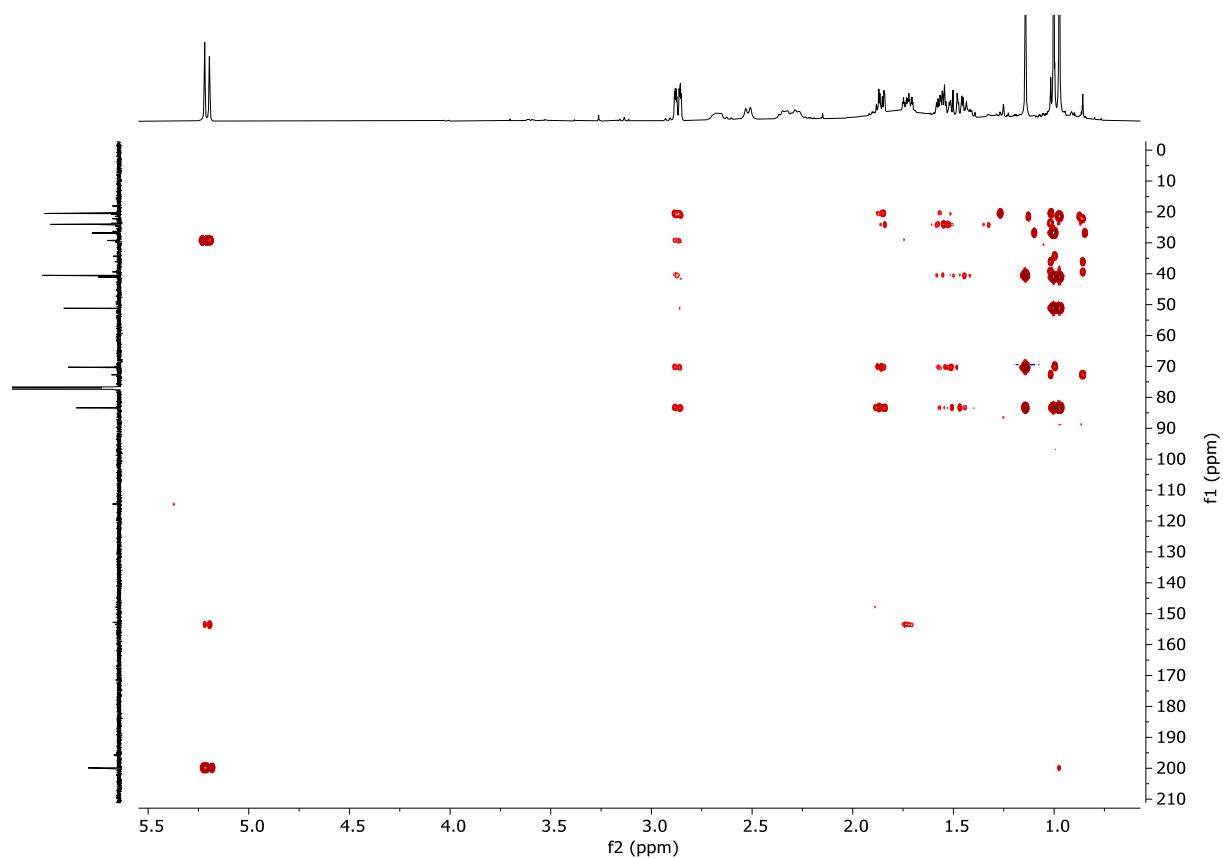
Figure S84. HSQC spectrum of dolichocarpol L (**6**) (CDCl₃, 500 MHz)**Figure S85.** HMBC spectrum of dolichocarpol L (**6**) (CDCl₃, 500 MHz)

Figure S86. COSY spectrum of dolichocarpol L (**6**) (CDCl₃, 500 MHz)

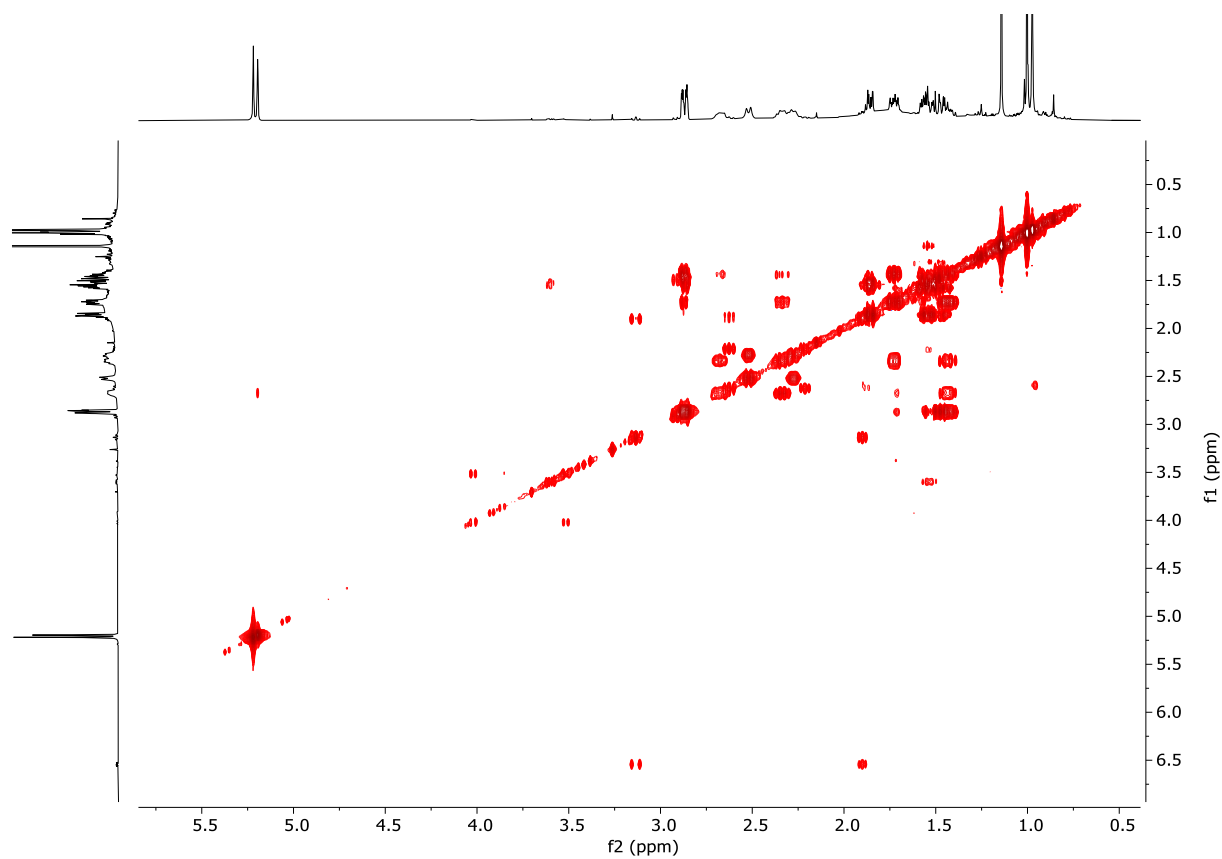


Figure S87. NOESY spectrum of dolichocarpol L (**6**) (CDCl₃, 500 MHz)

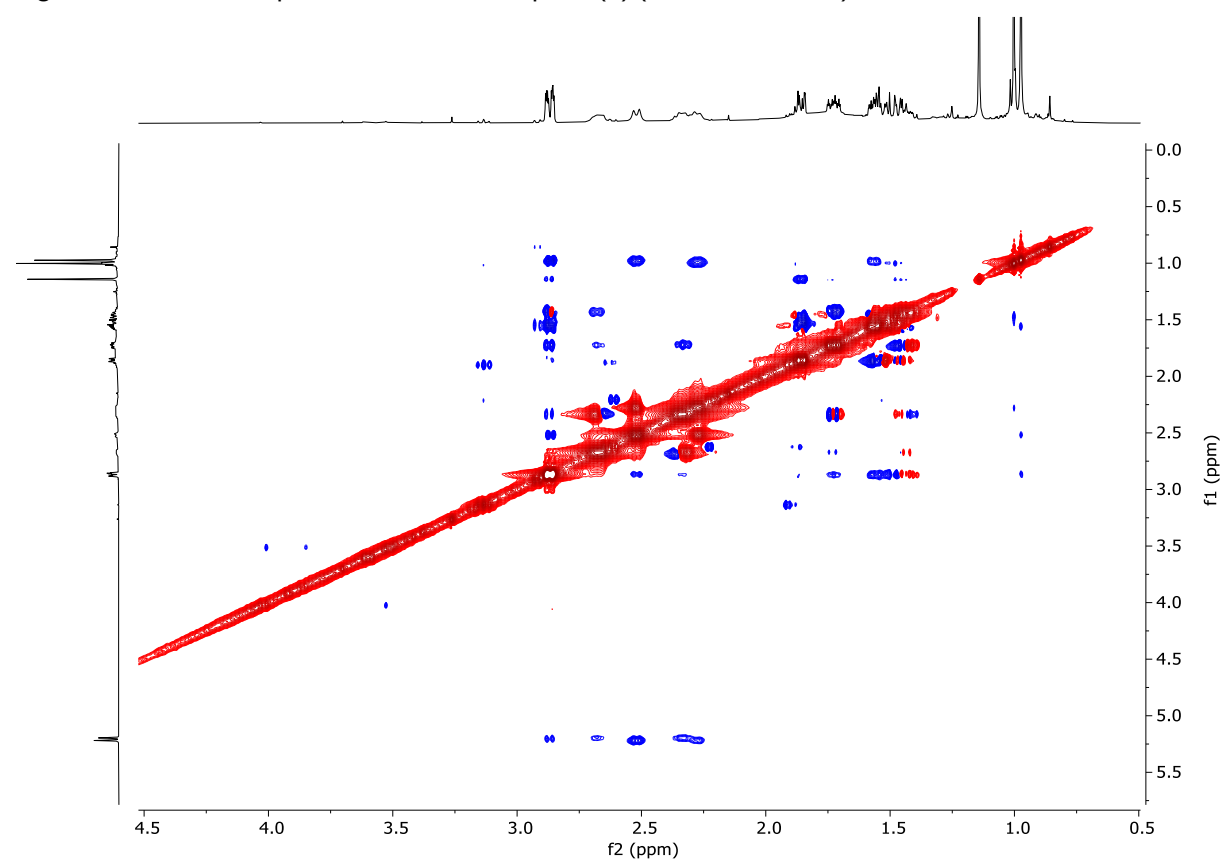


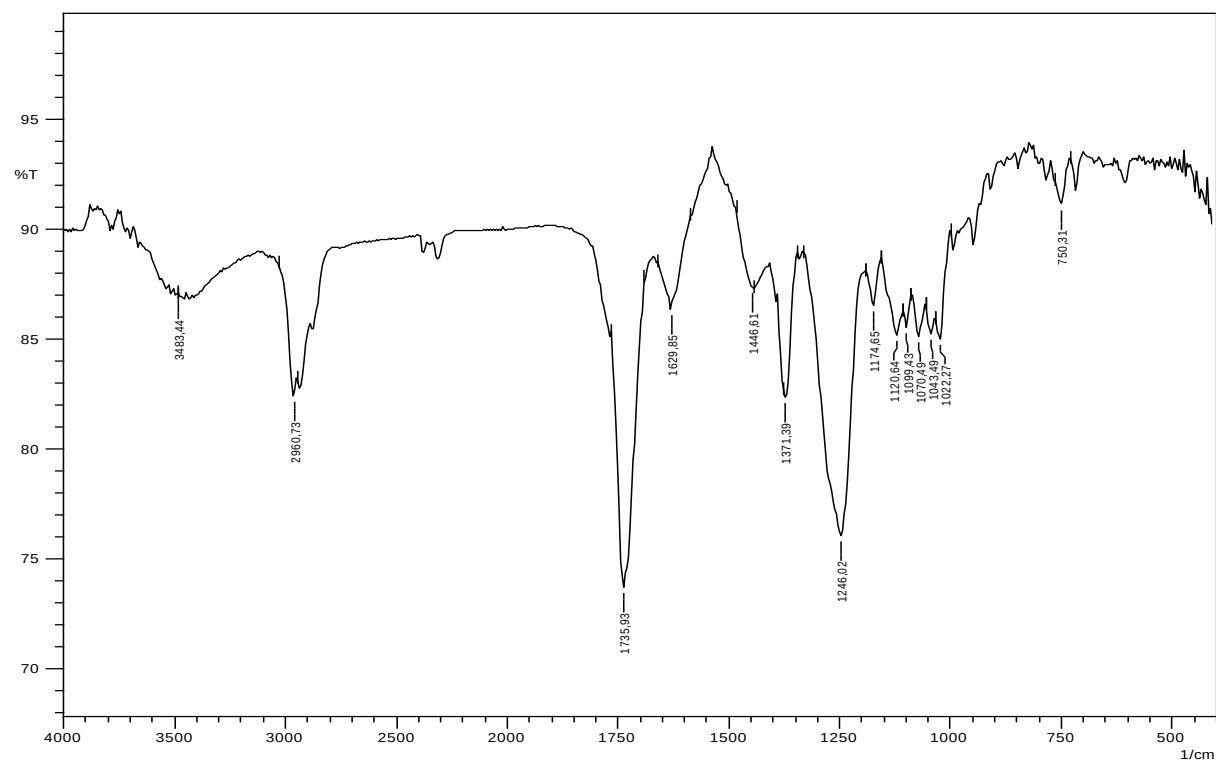
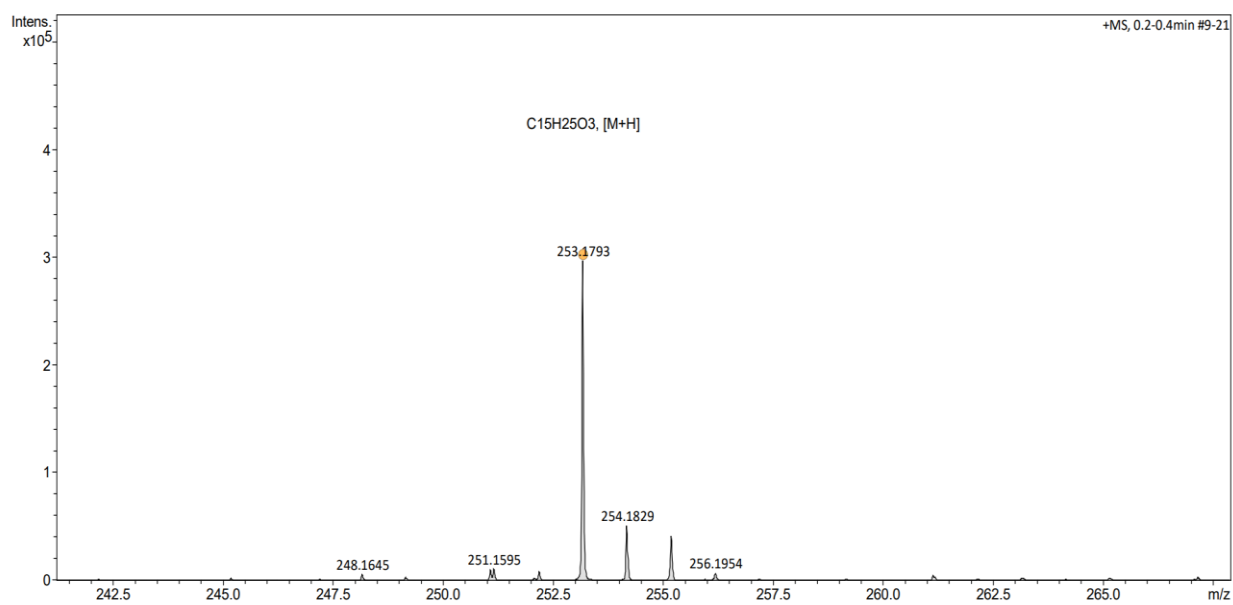
Figure S88. IR spectrum of dolichocarpol M (**7**)**Figure S89.** HRESIMS $[\text{M} + \text{H}]^+$ spectrum of dolichocarpol M (**7**)

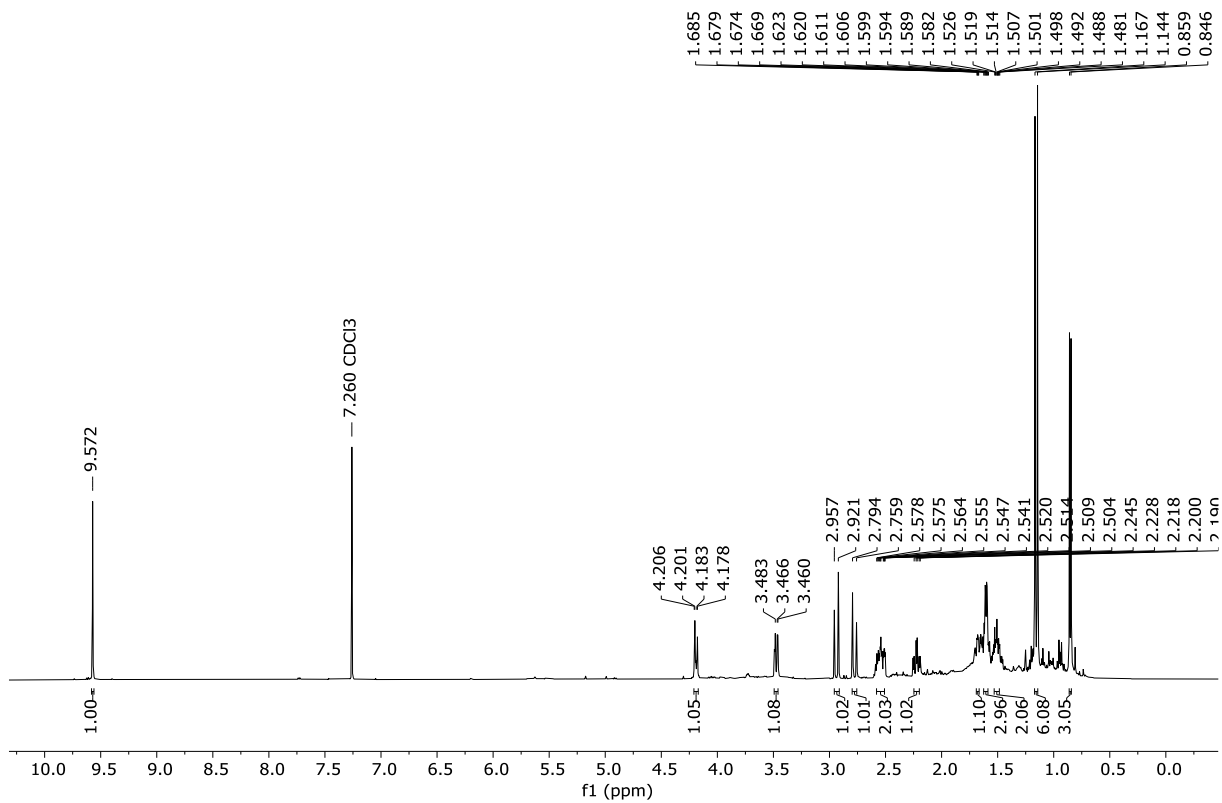
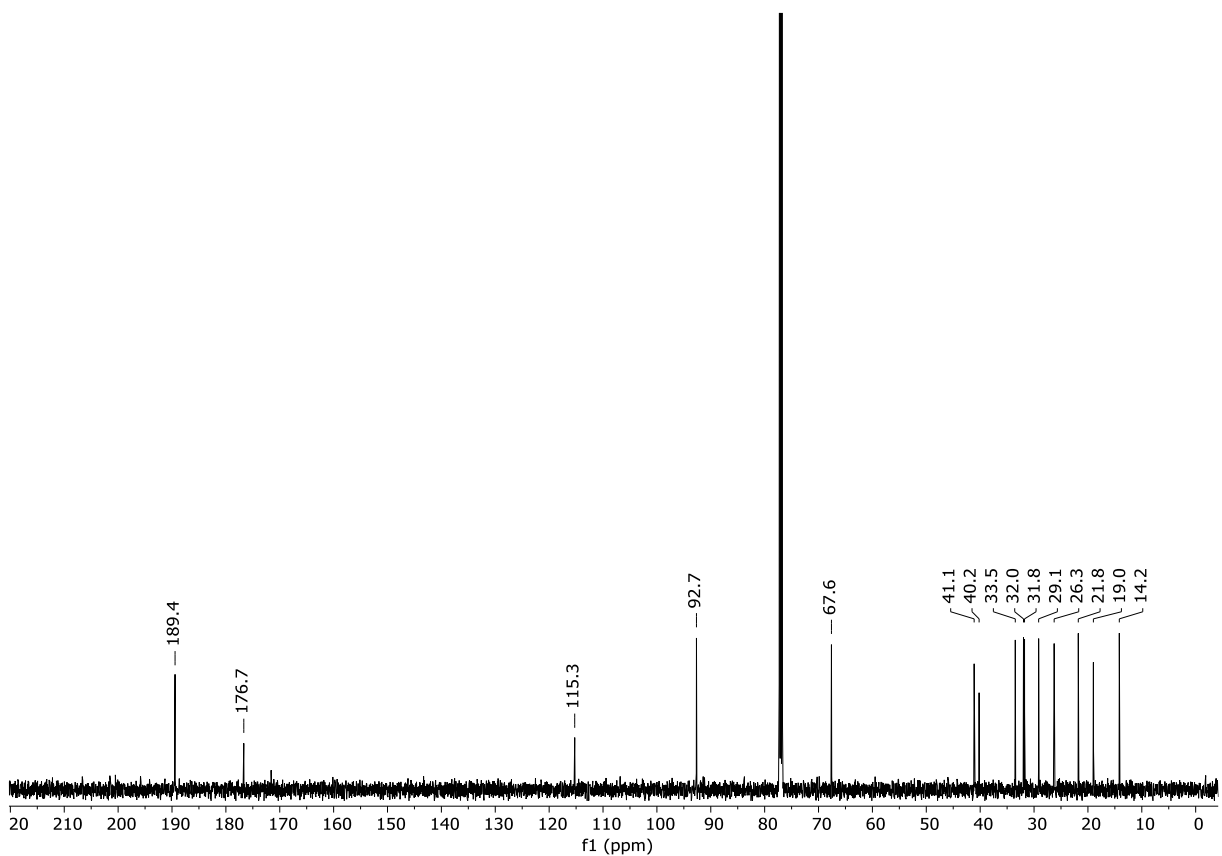
Figure S90. ^1H NMR spectrum of dolichocarpol M (**7**) (CDCl_3 , 500 MHz)**Figure S91.** ^{13}C NMR spectrum of dolichocarpol M (**7**) (CDCl_3 , 125 MHz)

Figure S92. ^{13}C DEPT NMR spectrum of dolichocarpol M (**7**) (CDCl_3 , 125 MHz)

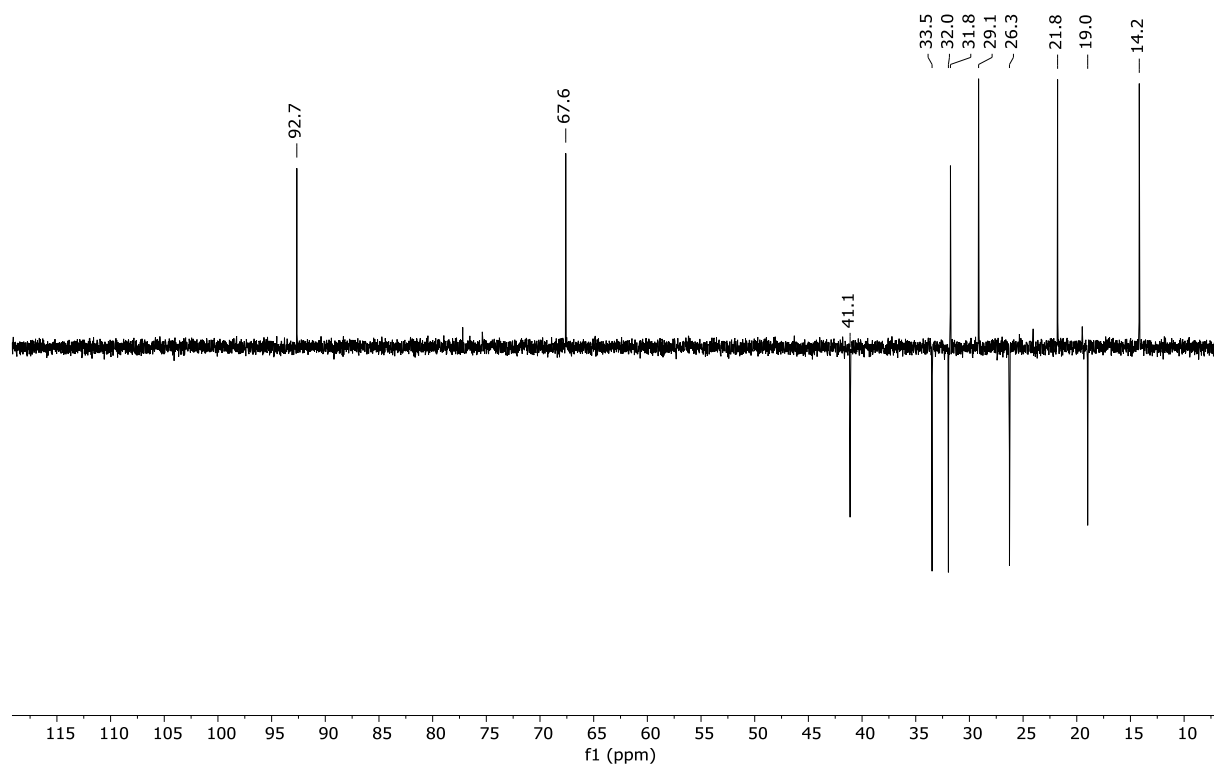


Figure S93. HSQC spectrum of dolichocarpol M (**7**) (CDCl_3 , 500 MHz)

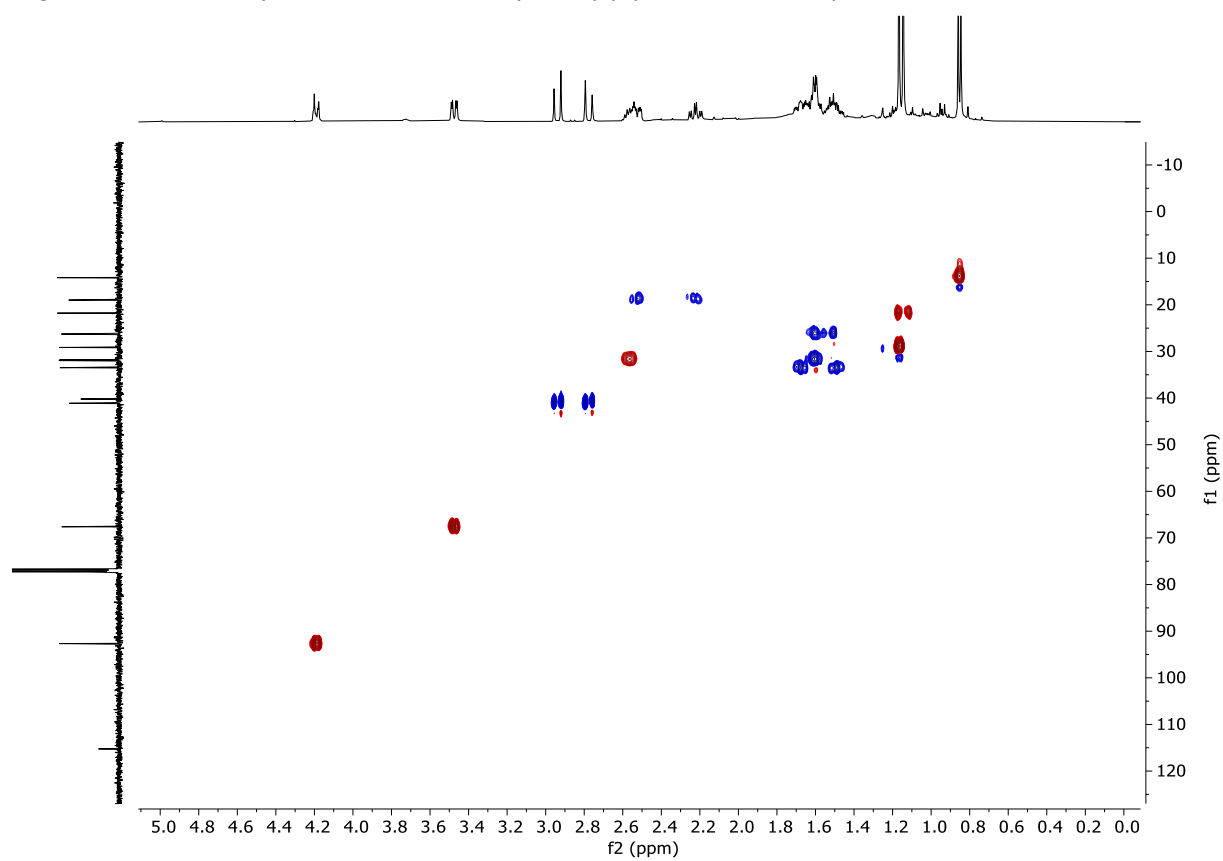


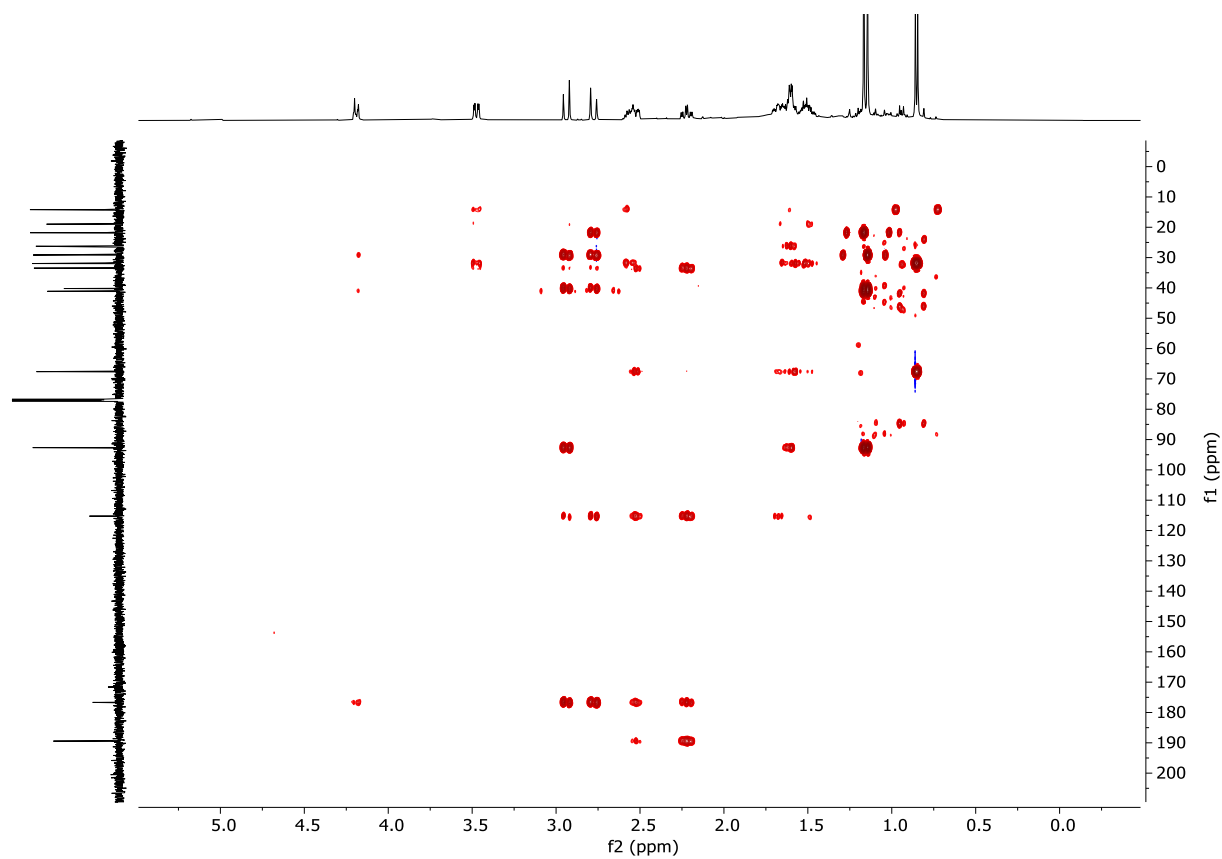
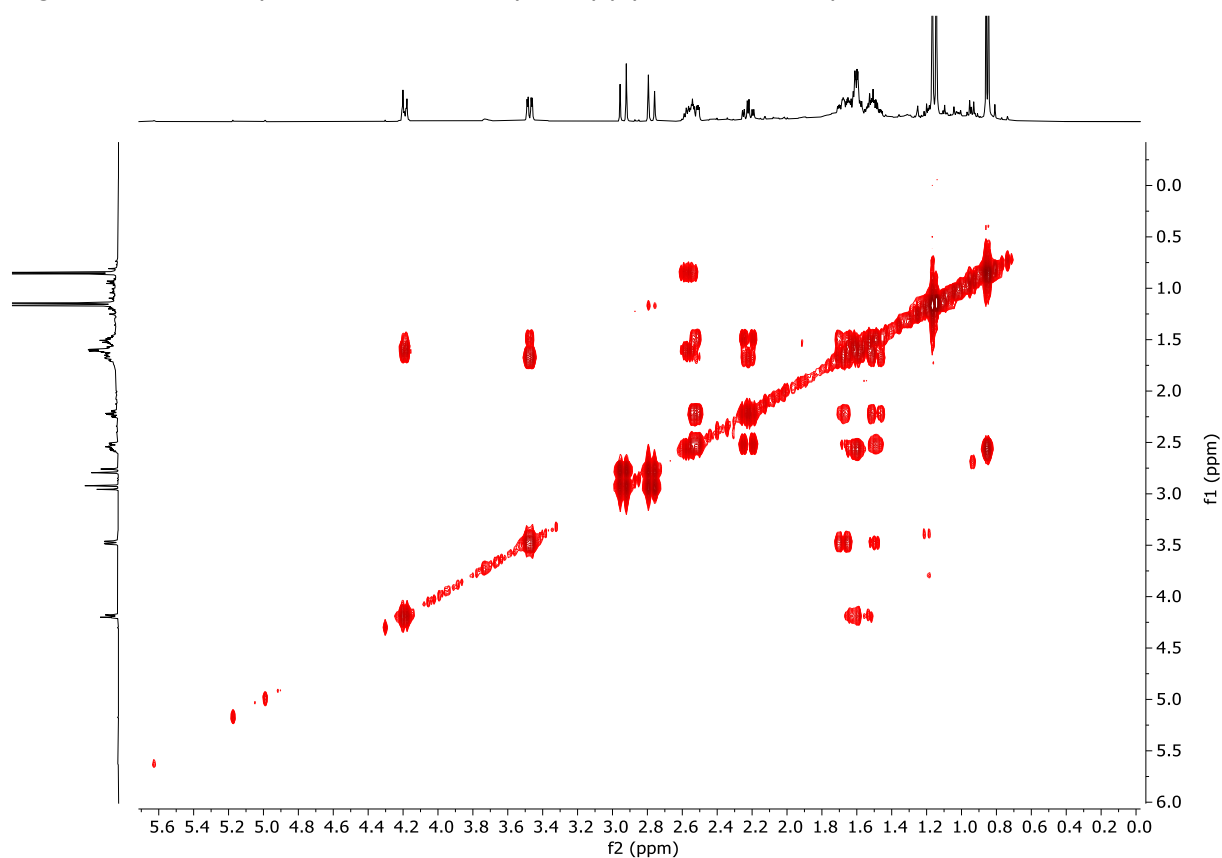
Figure S94. HMBC spectrum of dolichocarpol M (**7**) (CDCl₃, 500 MHz)**Figure S95.** COSY spectrum of dolichocarpol M (**7**) (CDCl₃, 500 MHz)

Figure S96. NOESY spectrum of dolichocarpol M (**7**) (CDCl₃, 500 MHz)

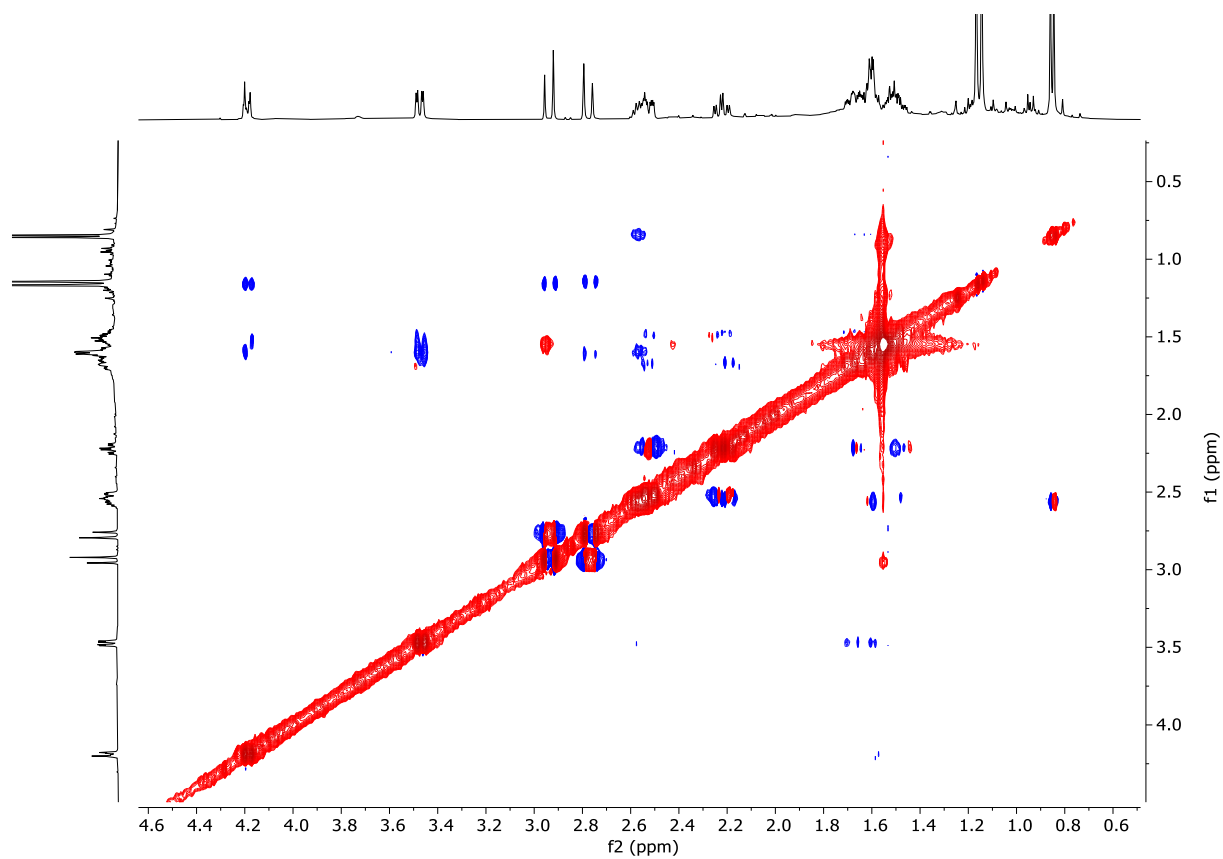


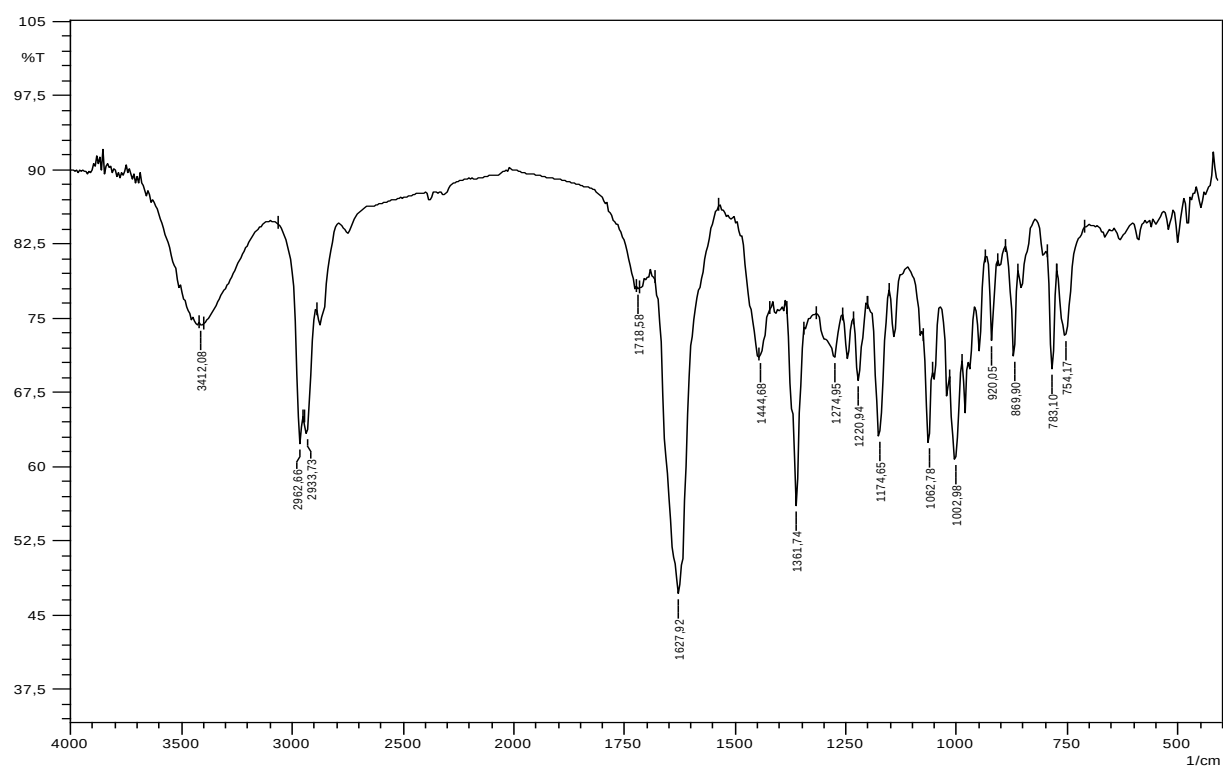
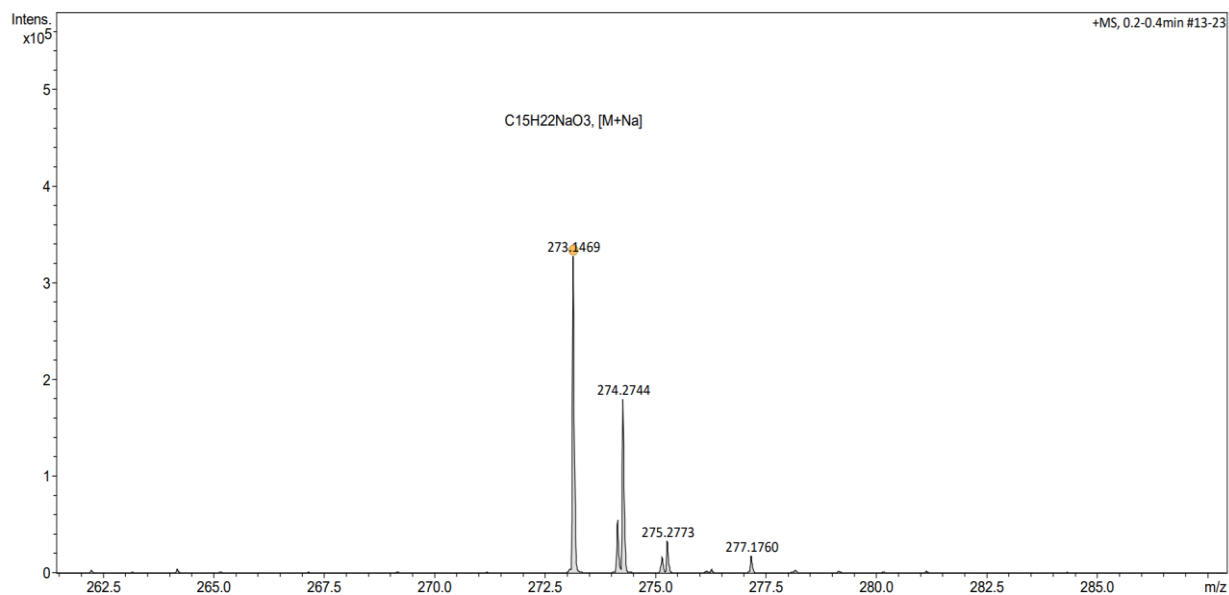
Figure S97. IR spectrum of dolichocarpol N (**8**)**Figure S98.** HRESIMS $[\text{M} + \text{Na}]^+$ spectrum of dolichocarpol N (**8**)

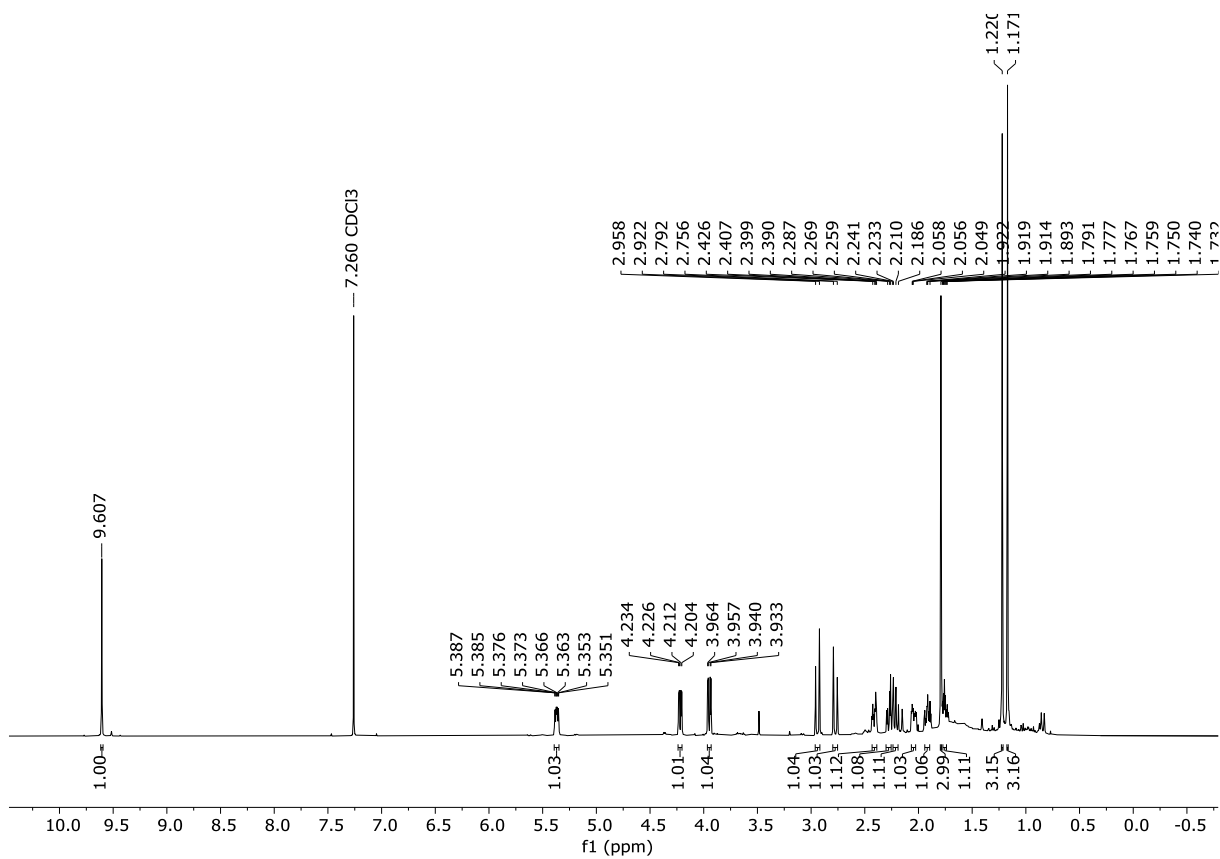
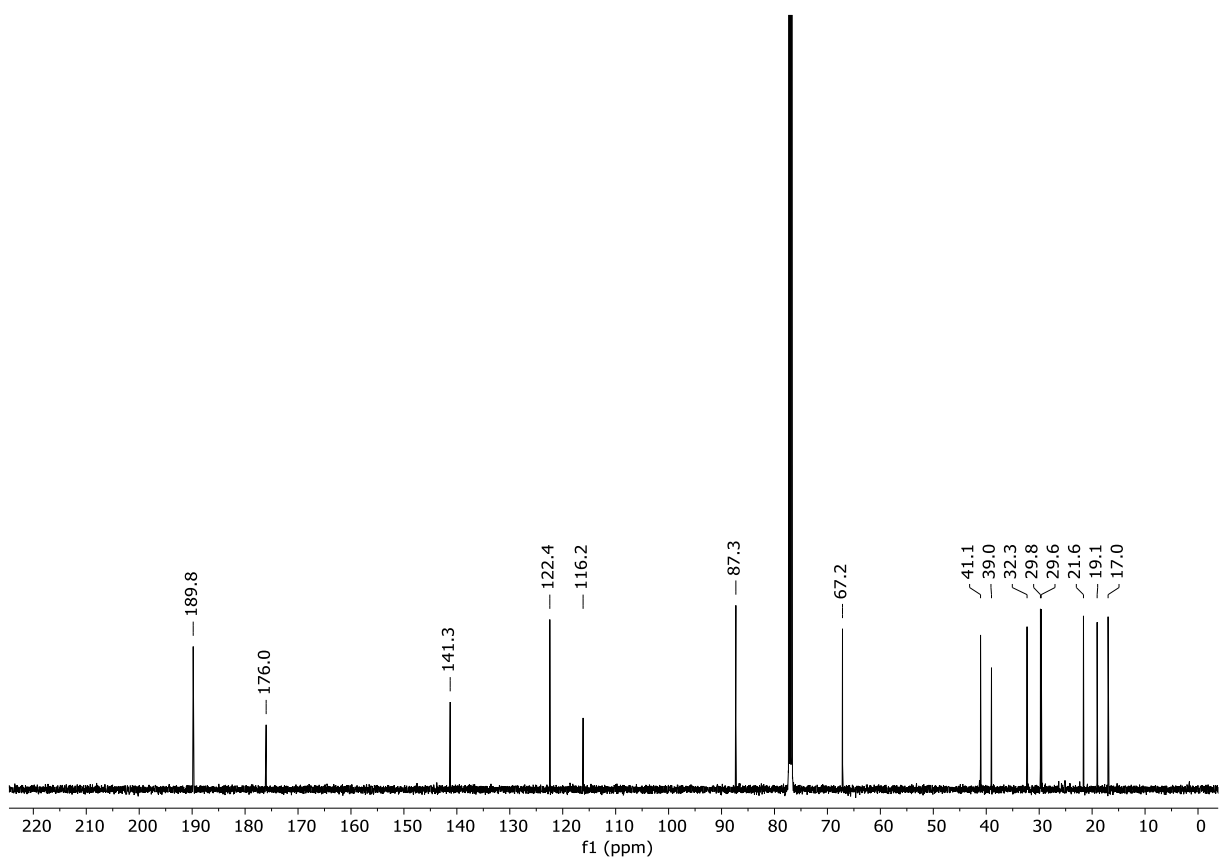
Figure S99. ^1H NMR spectrum of dolichocarpol N (**8**) (CDCl_3 , 500 MHz)**Figure S100.** ^{13}C NMR spectrum of dolichocarpol N (**8**) (CDCl_3 , 125 MHz)

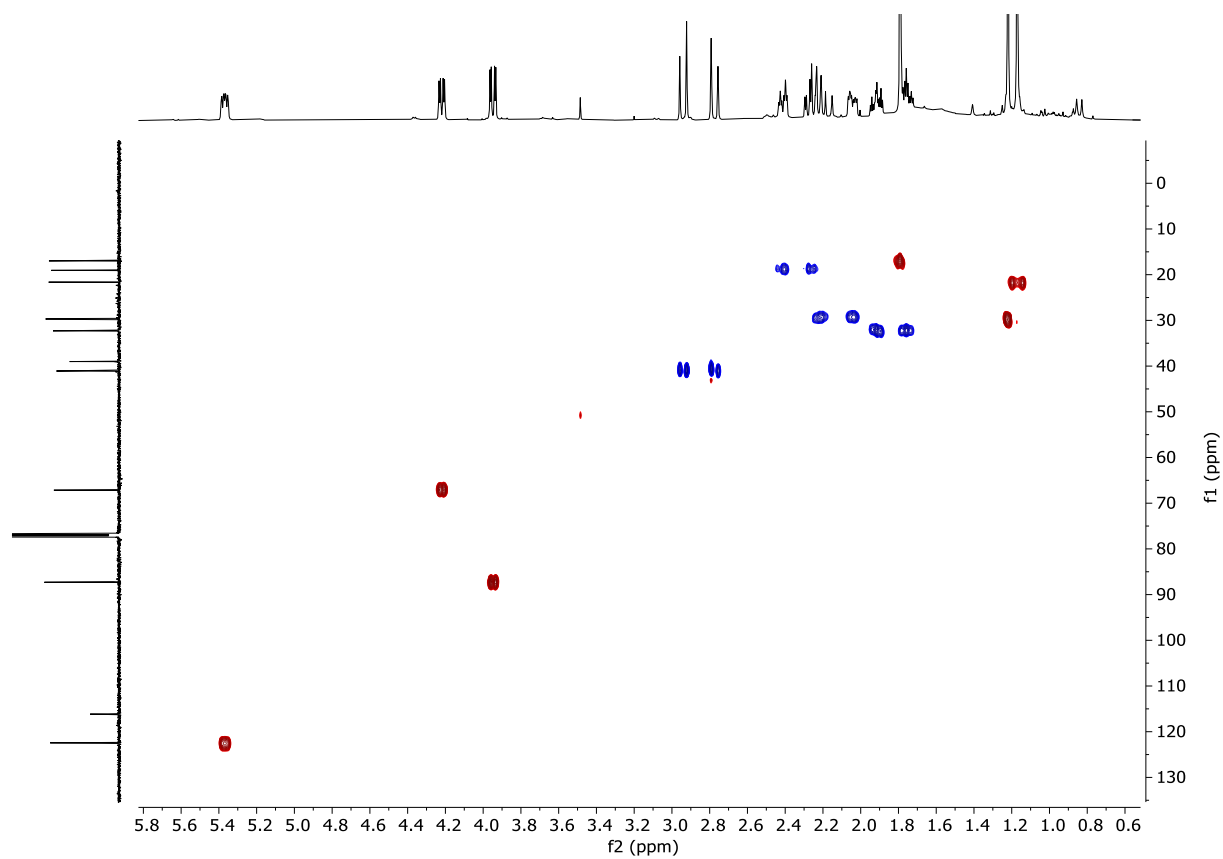
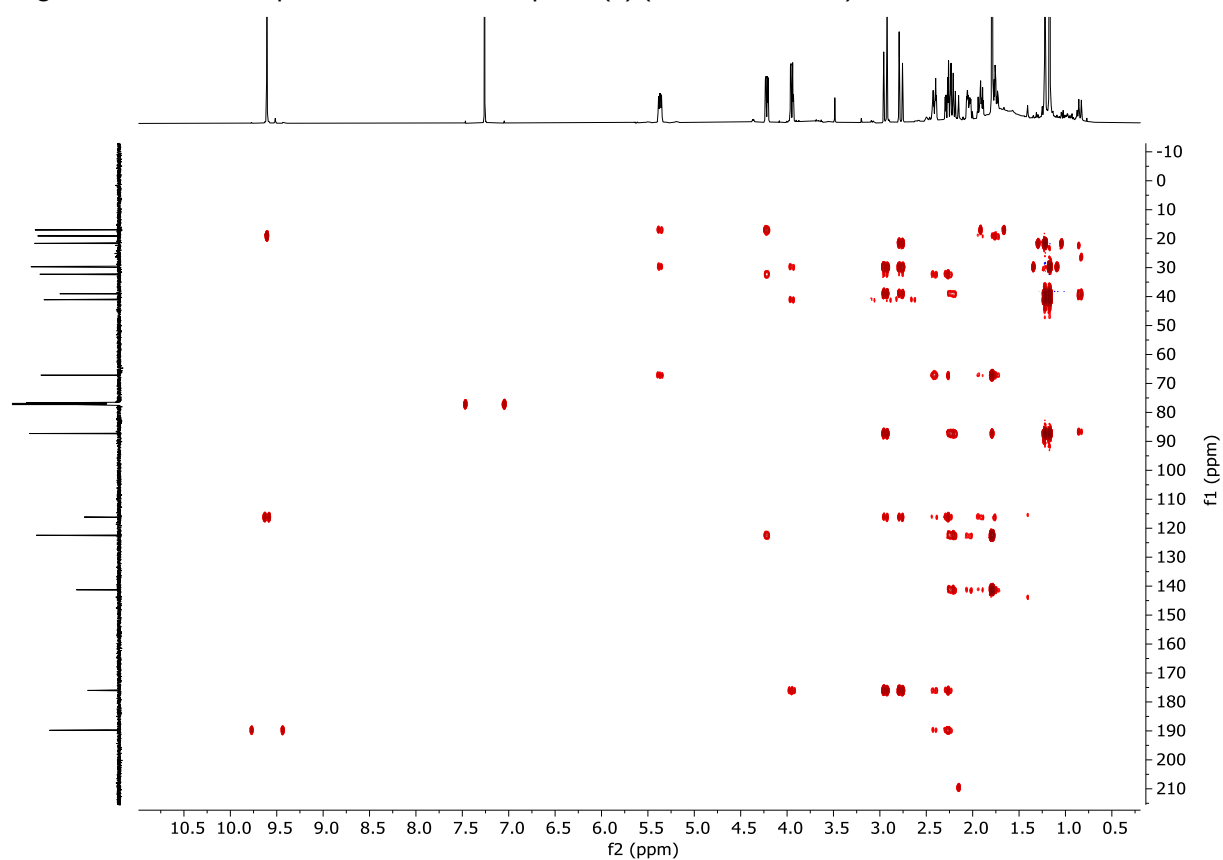
Figure S101. HSQC spectrum of dolichocarpol N (**8**) (CDCl₃, 500 MHz)**Figure S102.** HMBC spectrum of dolichocarpol N (**8**) (CDCl₃, 500 MHz)

Figure S103. COSY spectrum of dolichocarpol N (**8**) (CDCl₃, 500 MHz)

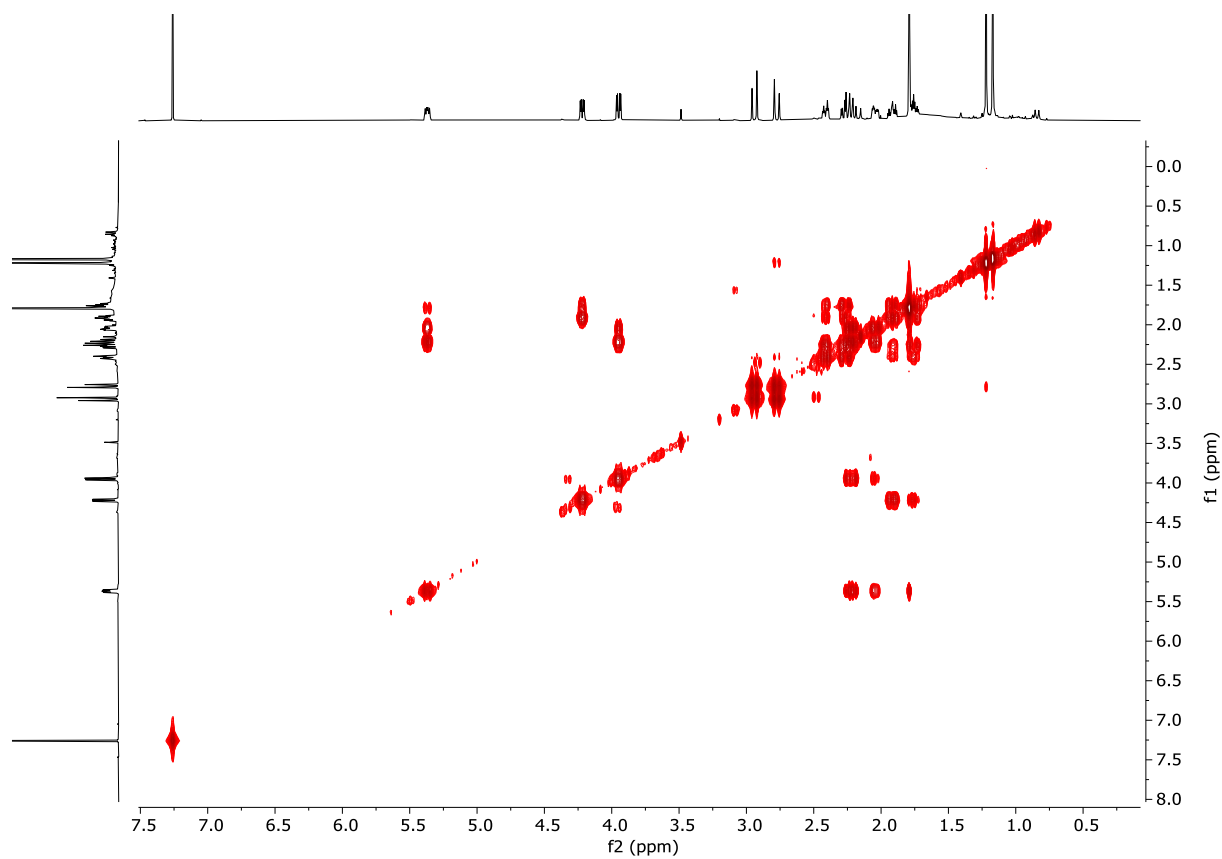


Figure S104. NOESY spectrum of dolichocarpol N (**8**) (CDCl₃, 500 MHz)

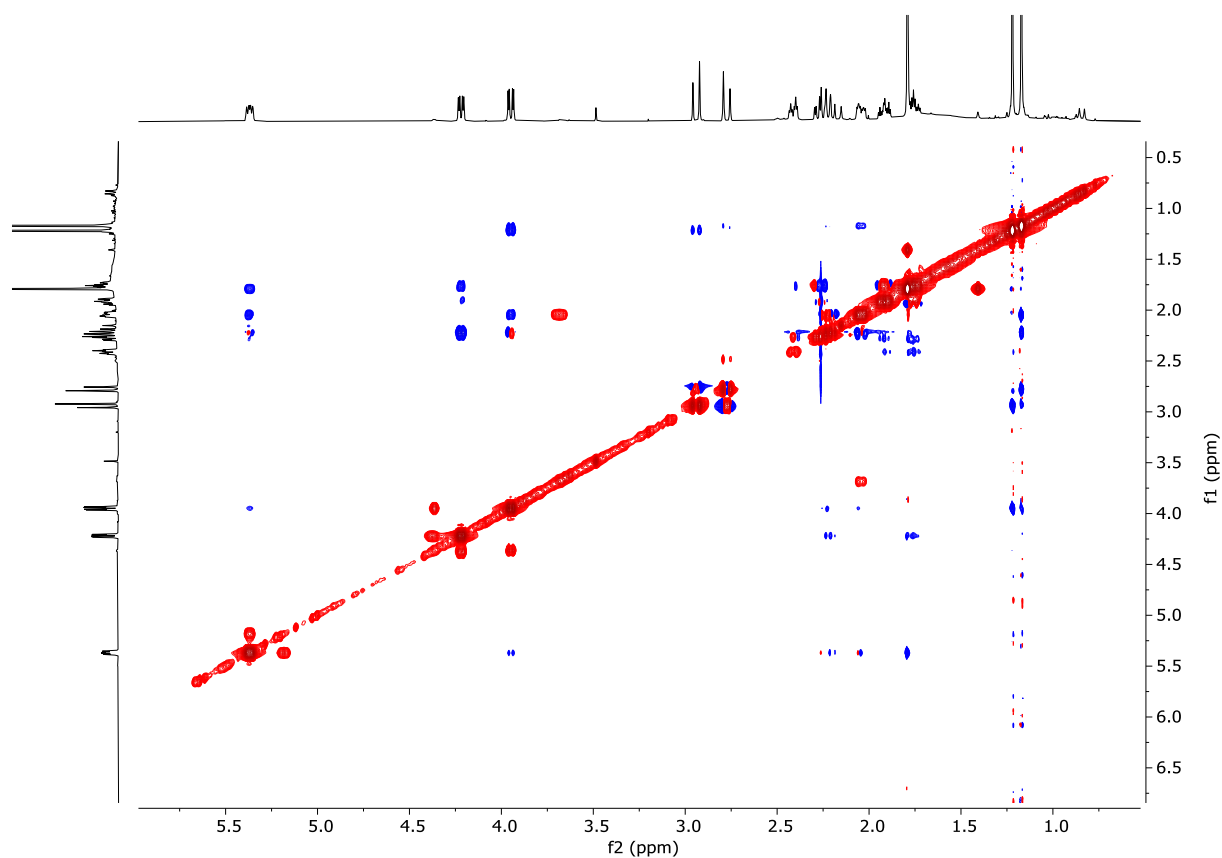


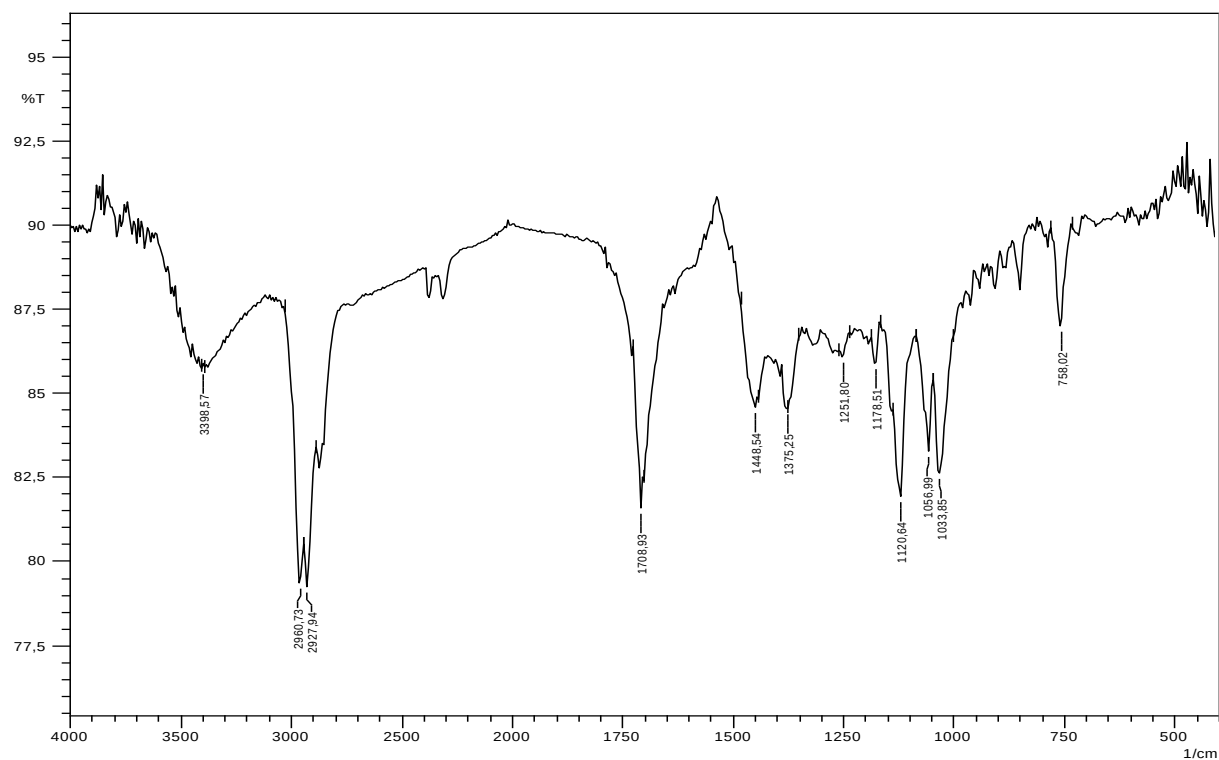
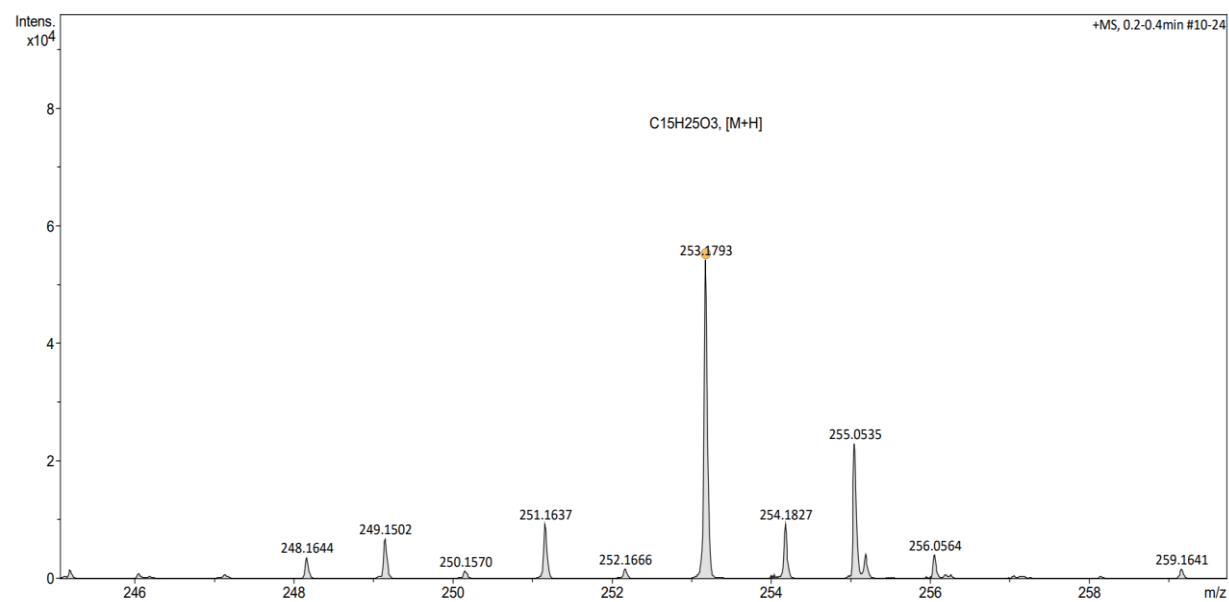
Figure S105. IR spectrum of dolichocarpol O (**9**)**Figure S106.** HRESIMS $[M + H]^+$ spectrum of dolichocarpol O (**9**)

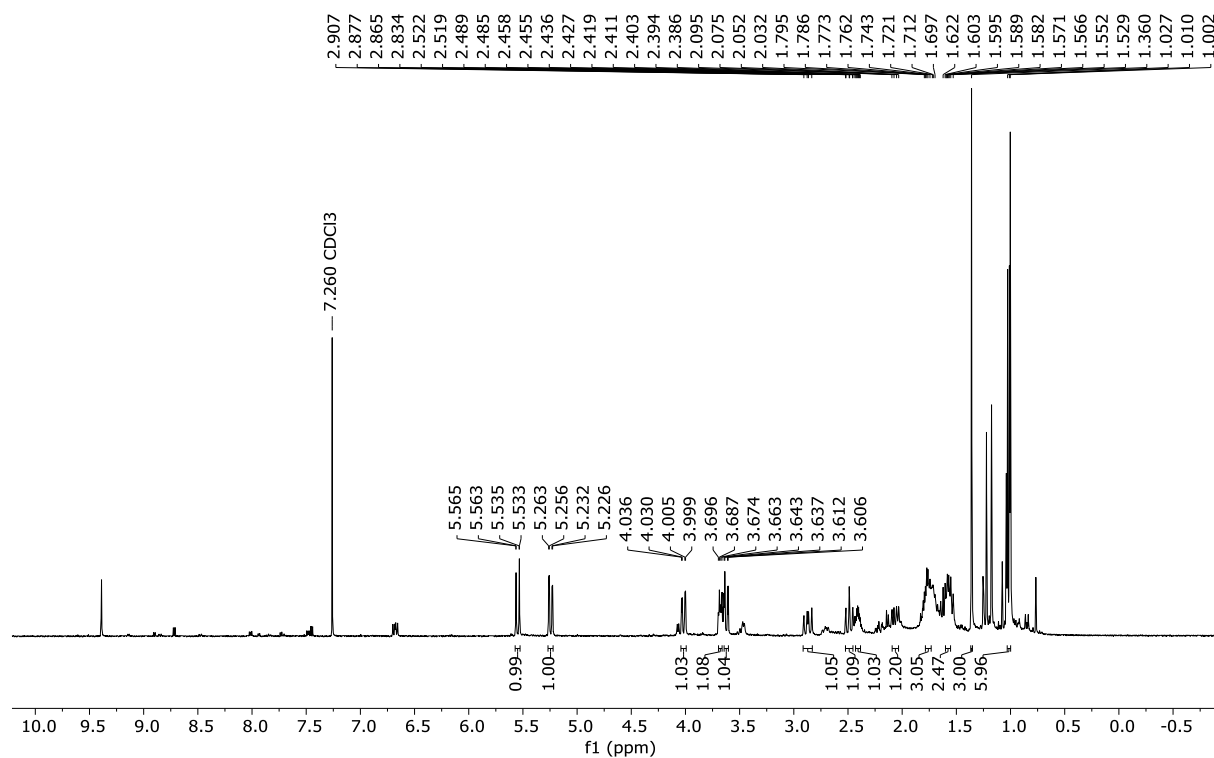
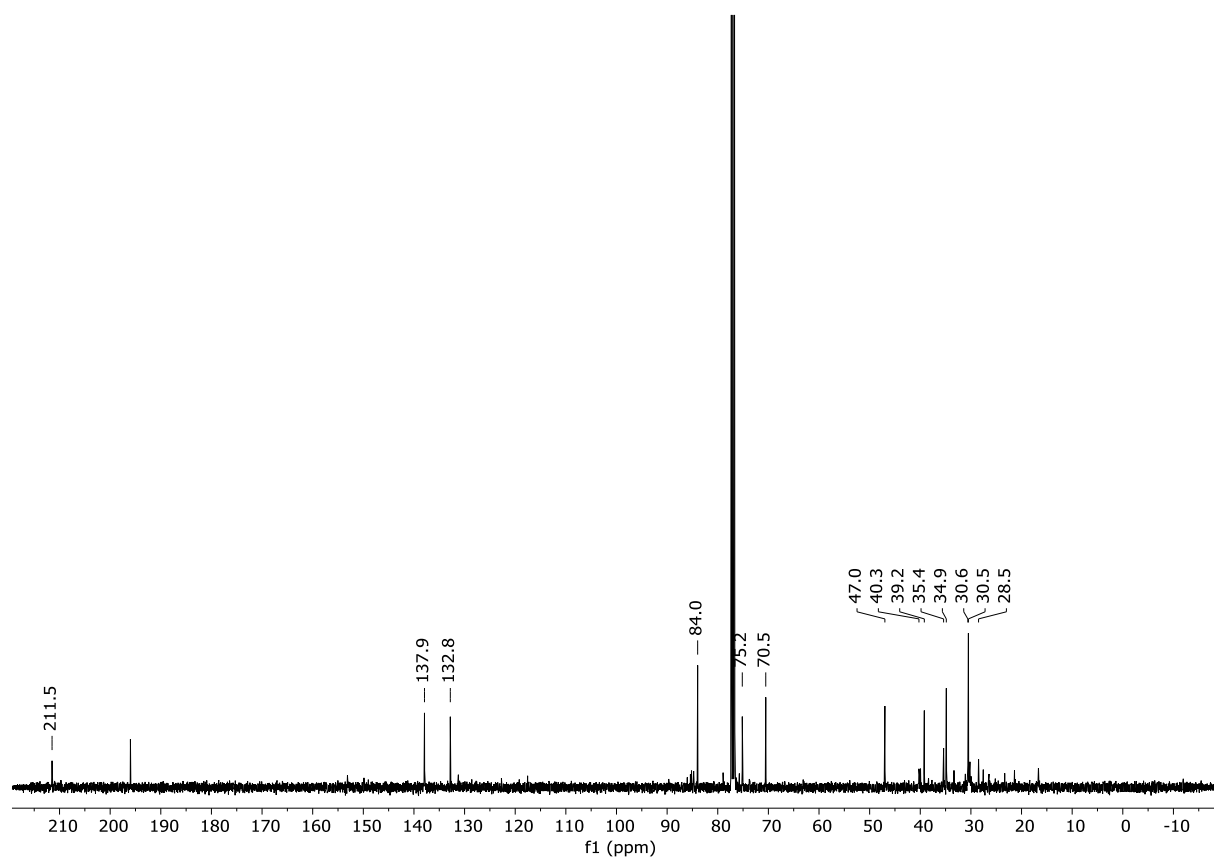
Figure S107. ^1H NMR spectrum of dolichocarpol O (**9**) (CDCl_3 , 400 MHz)**Figure S108.** ^{13}C NMR spectrum of dolichocarpol O (**9**) (CDCl_3 , 100 MHz)

Figure S109. HSQC spectrum of dolichocarpol O (**9**) (CDCl₃, 400 MHz)

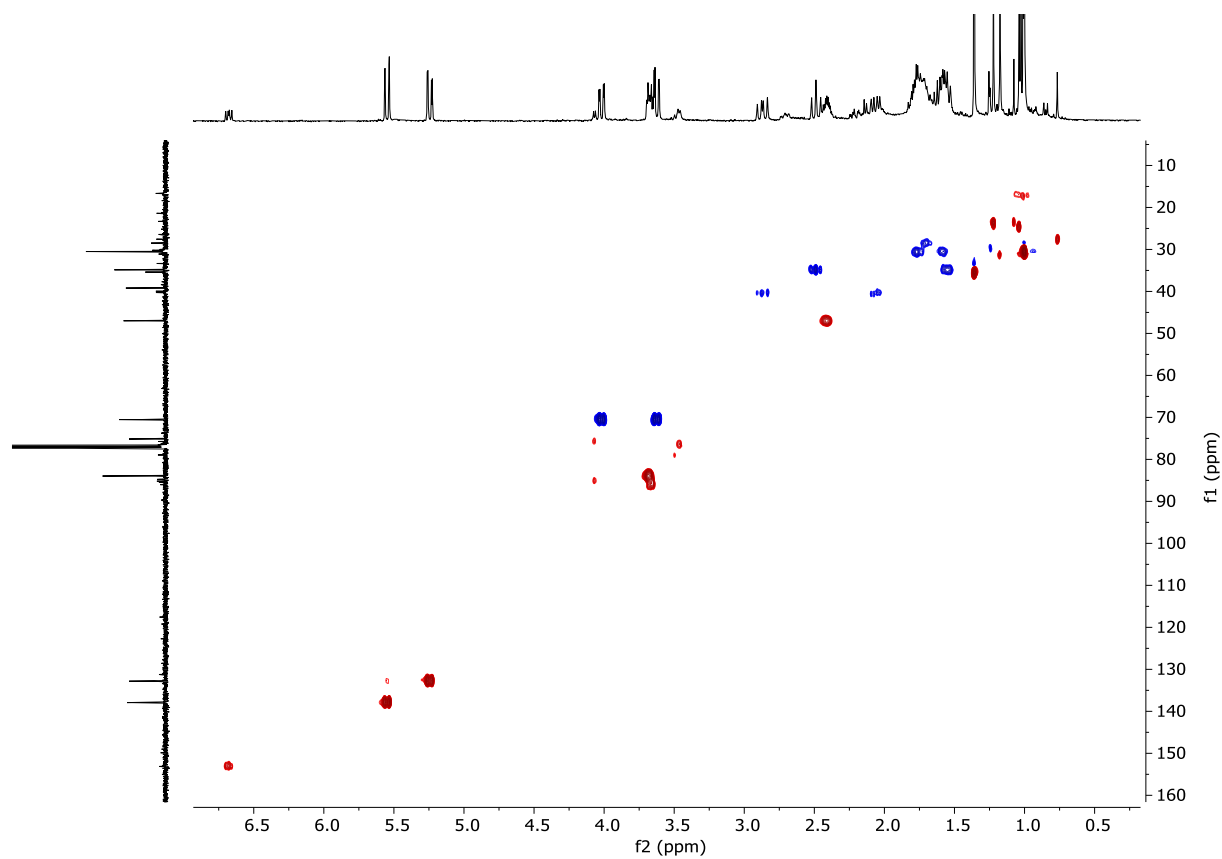


Figure S110. HMBC spectrum of dolichocarpol O (**9**) (CDCl₃, 400 MHz)

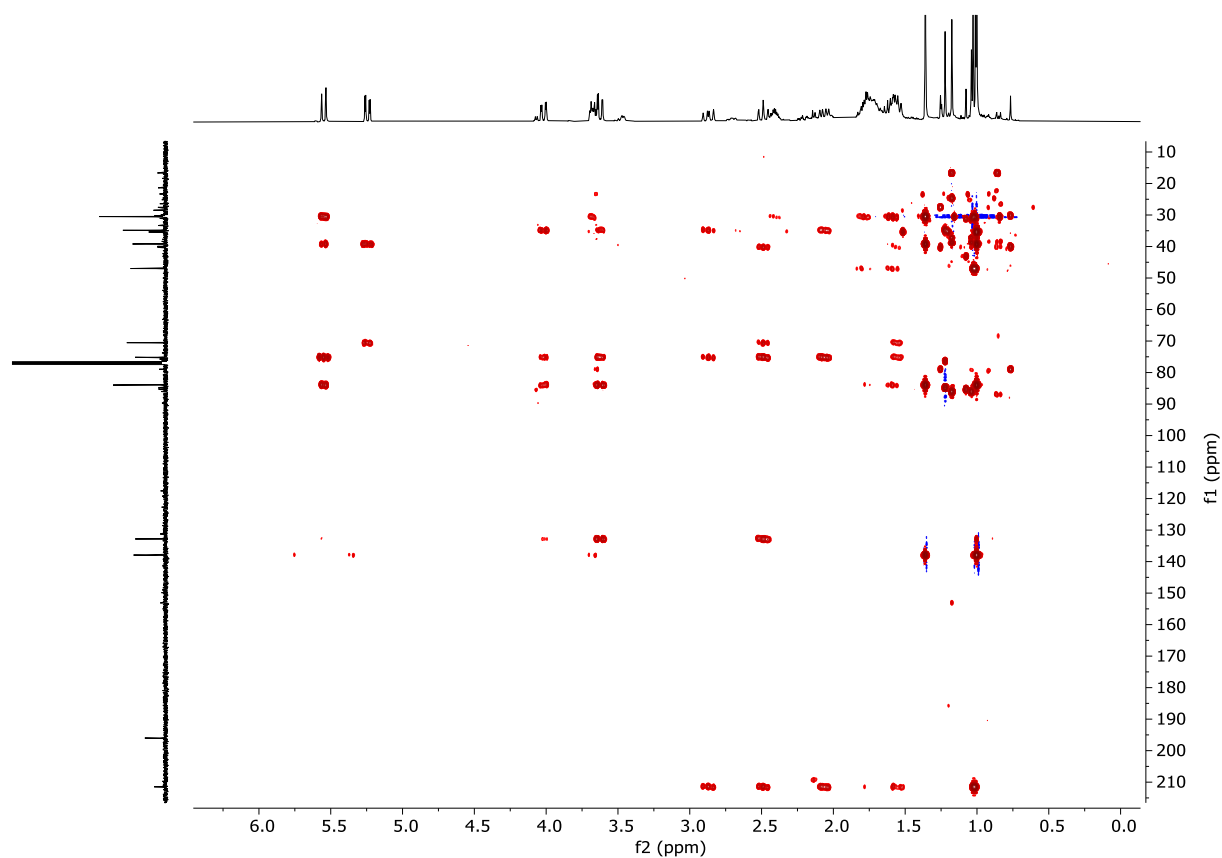


Figure S111. COSY spectrum of dolichocarpol O (**9**) (CDCl₃, 400 MHz)

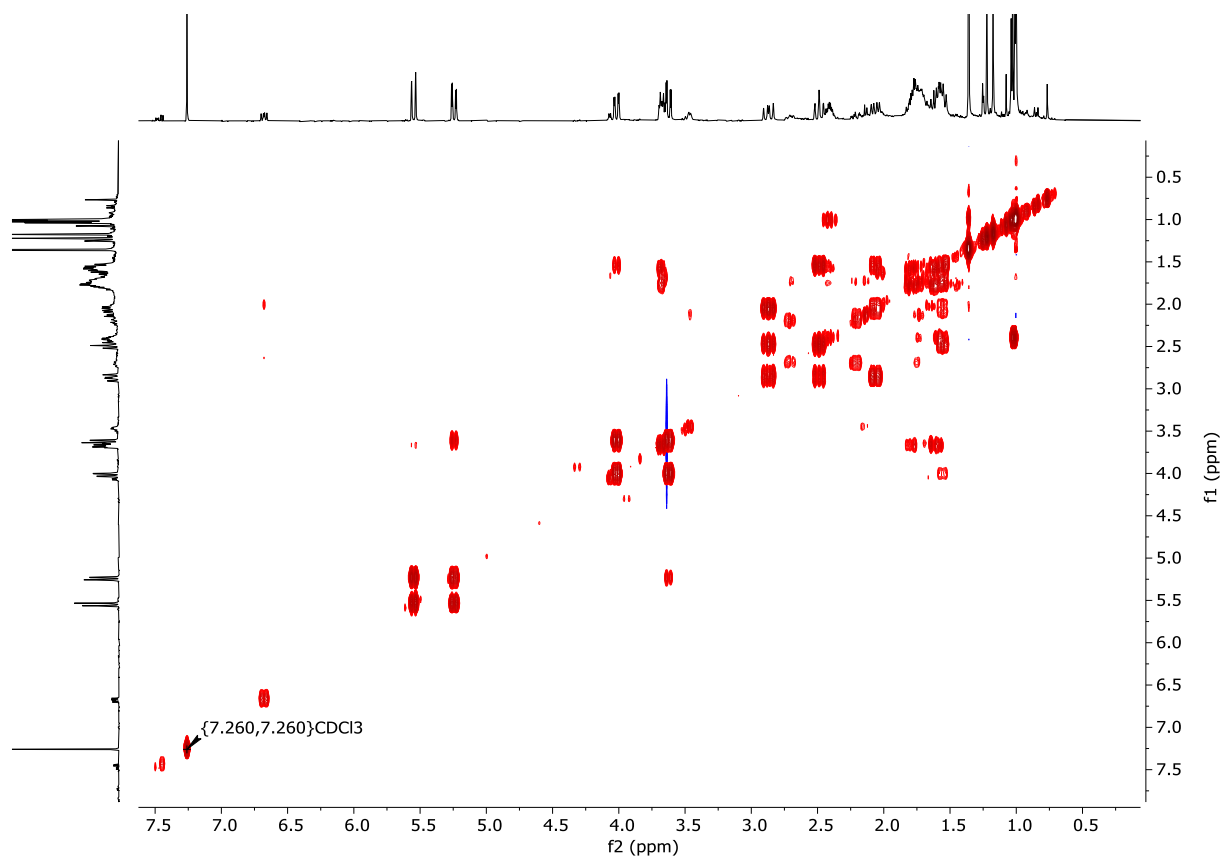


Figure S112. NOESY spectrum of dolichocarpol O (**9**) (CDCl₃, 400 MHz)

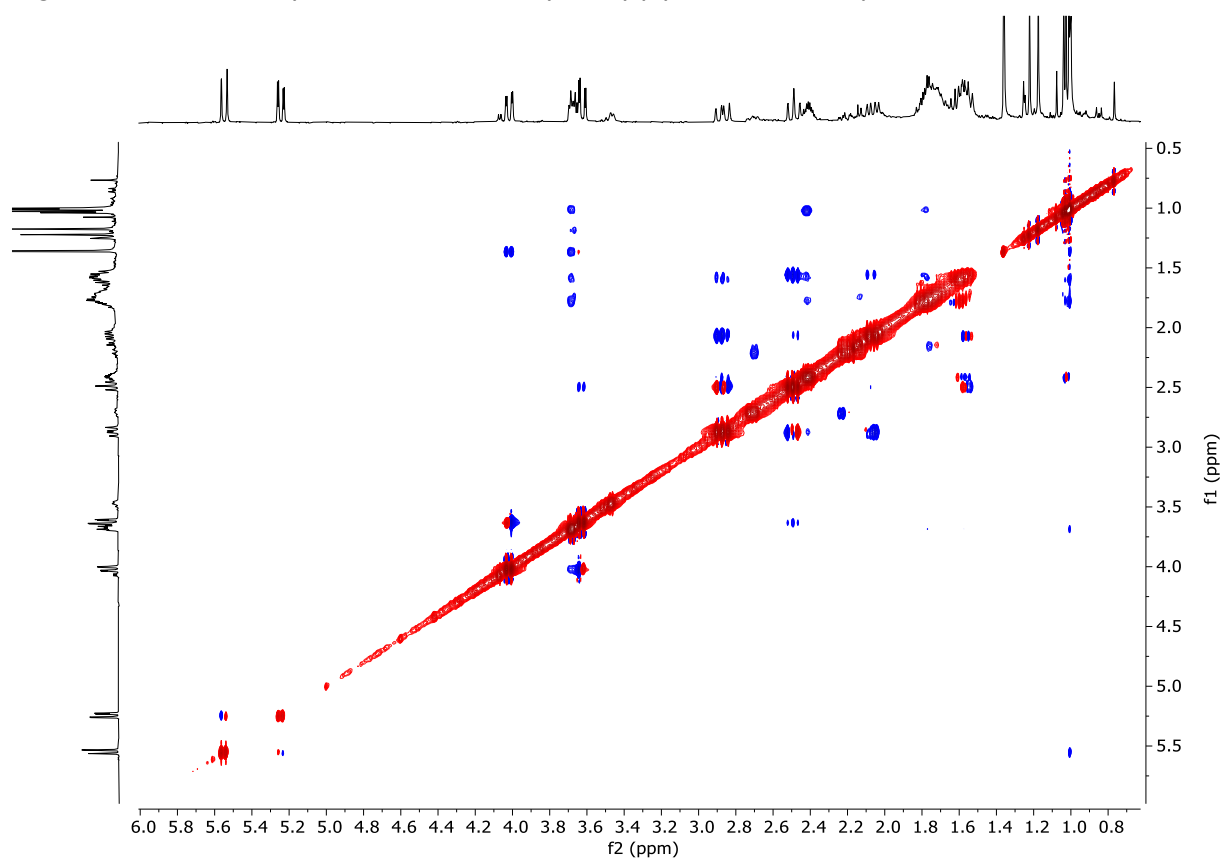


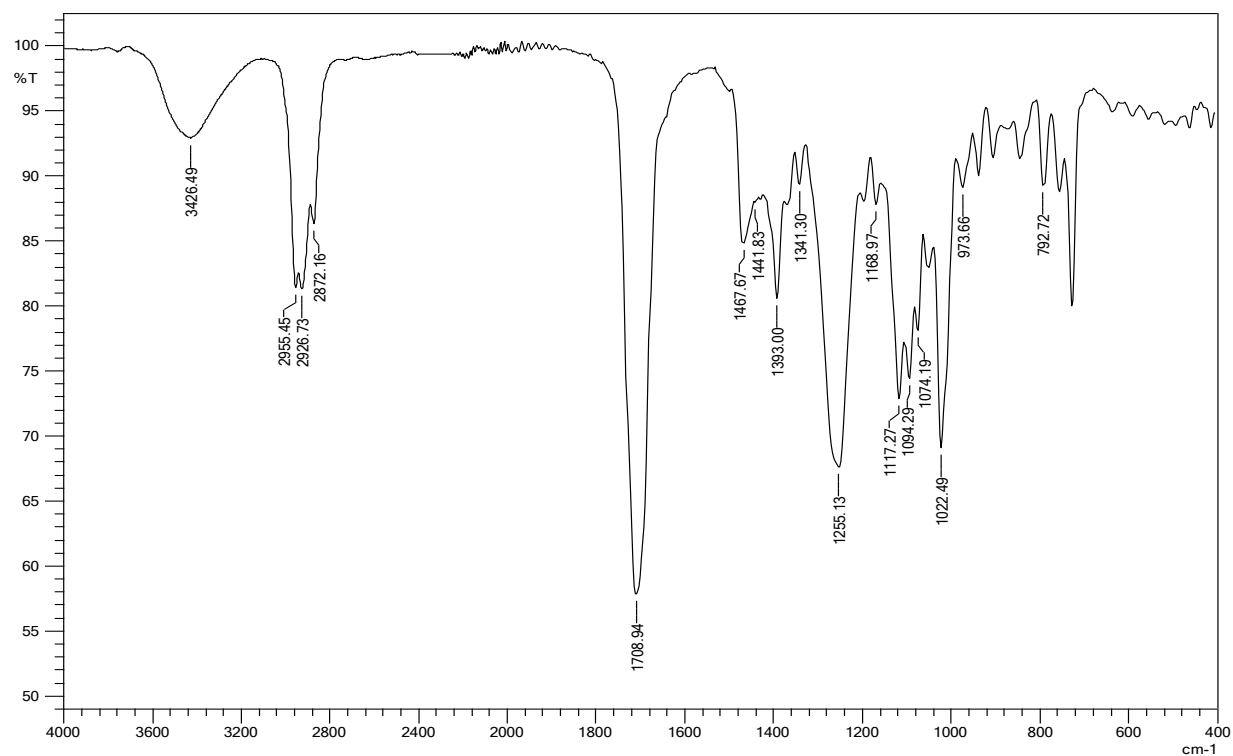
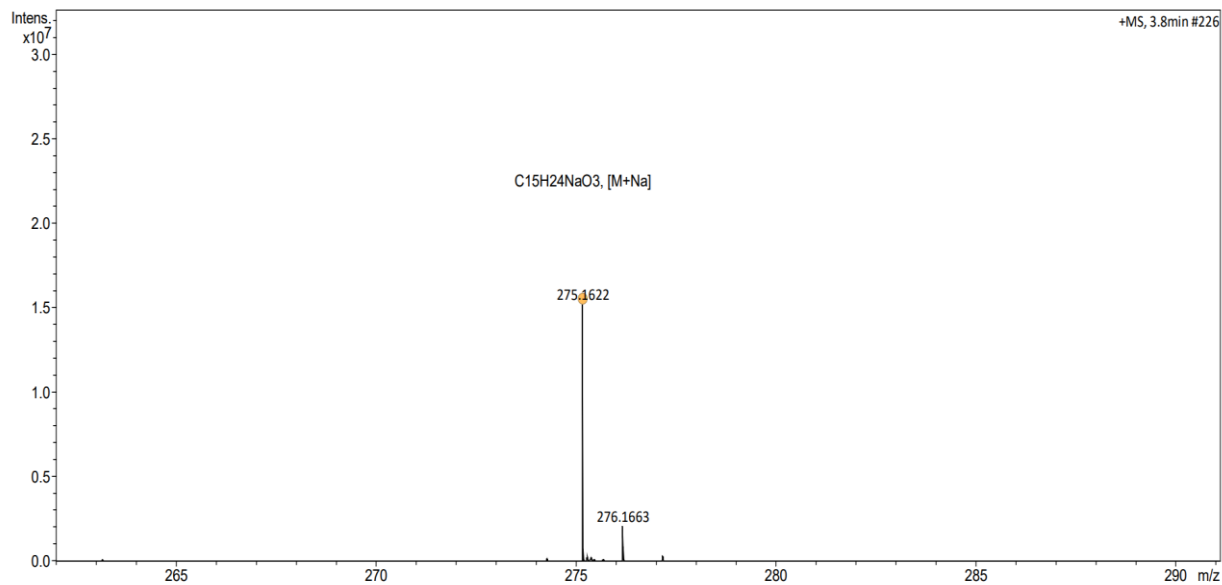
Figure S113. IR spectrum of dolichocarpol P (**10**)**Figure S114.** HRESIMS $[\text{M} + \text{Na}]^+$ spectrum of dolichocarpol P (**10**)

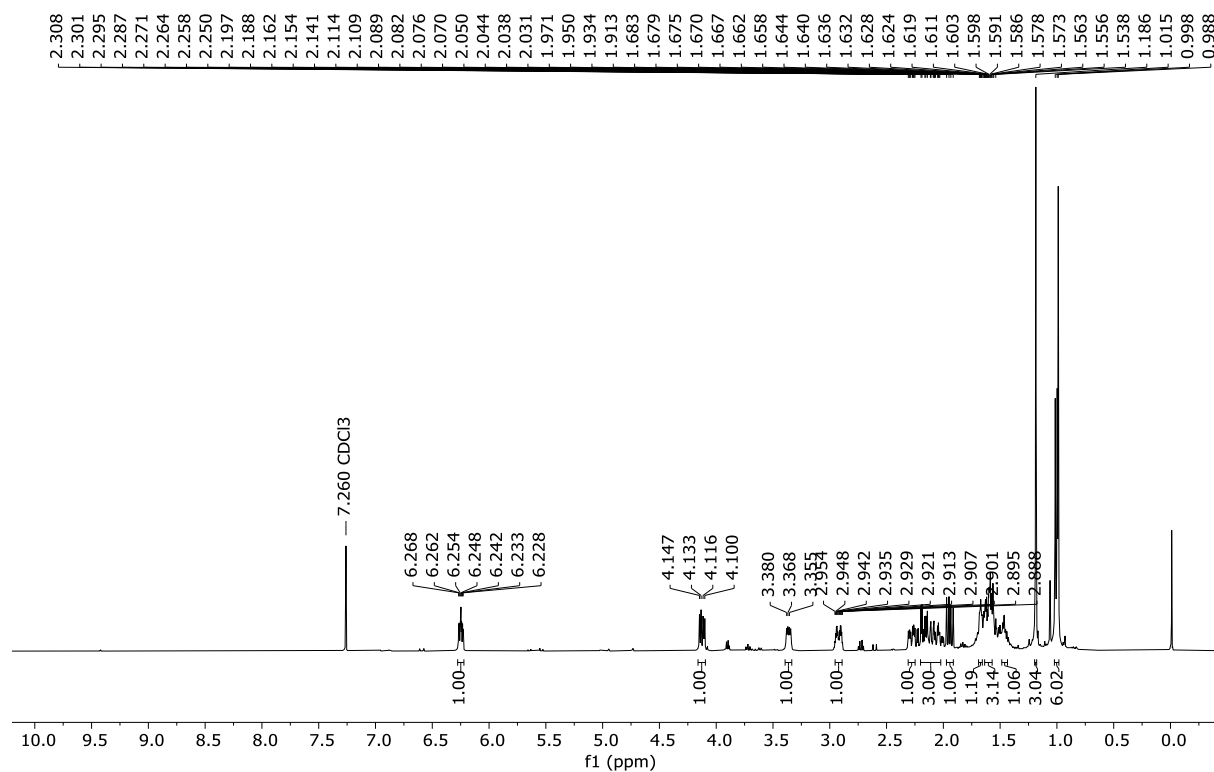
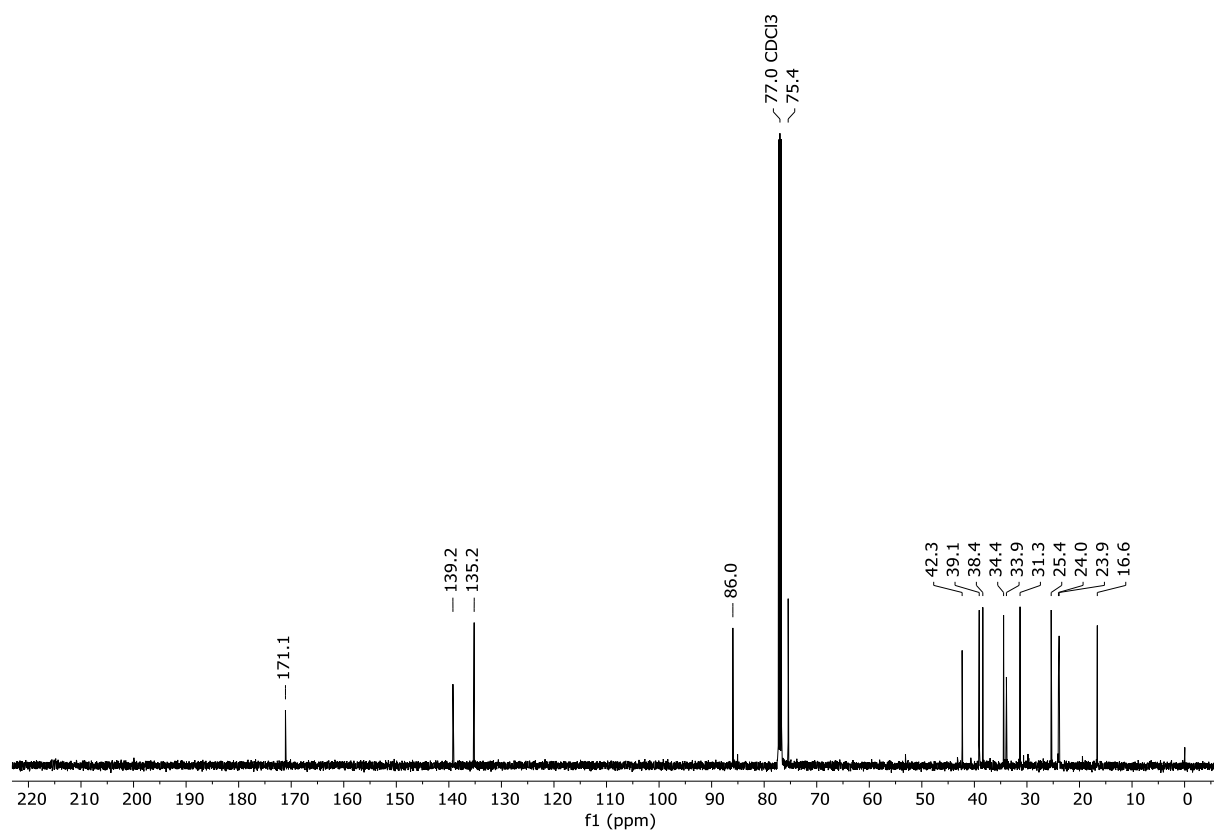
Figure S115. ^1H NMR spectrum of dolichocarpol P (**10**) (CDCl_3 , 400 MHz)**Figure S116.** ^{13}C NMR spectrum of dolichocarpol P (**10**) (CDCl_3 , 125 MHz)

Figure S117. HSQC spectrum of dolichocarpol P (**10**) (CDCl₃, 500 MHz)

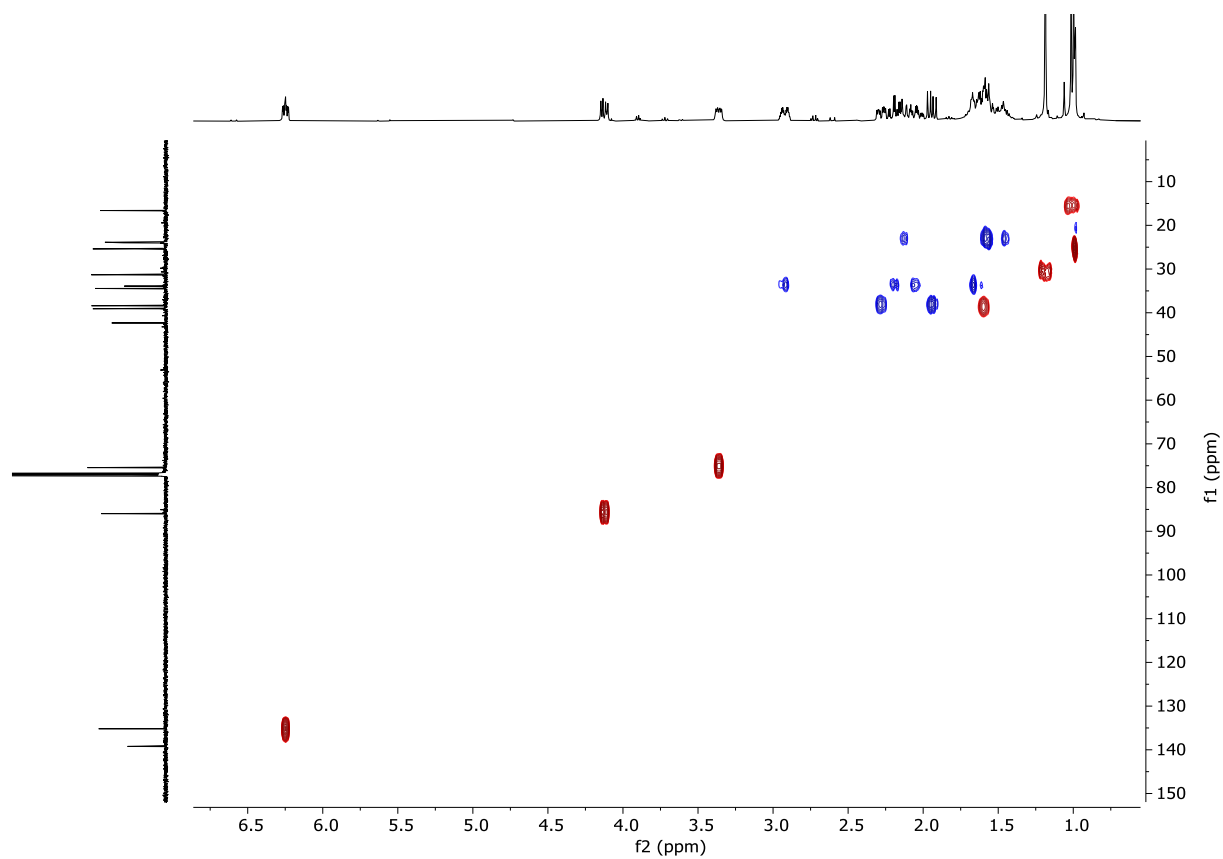


Figure S118. HMBC spectrum of dolichocarpol P (**10**) (CDCl₃, 500 MHz)

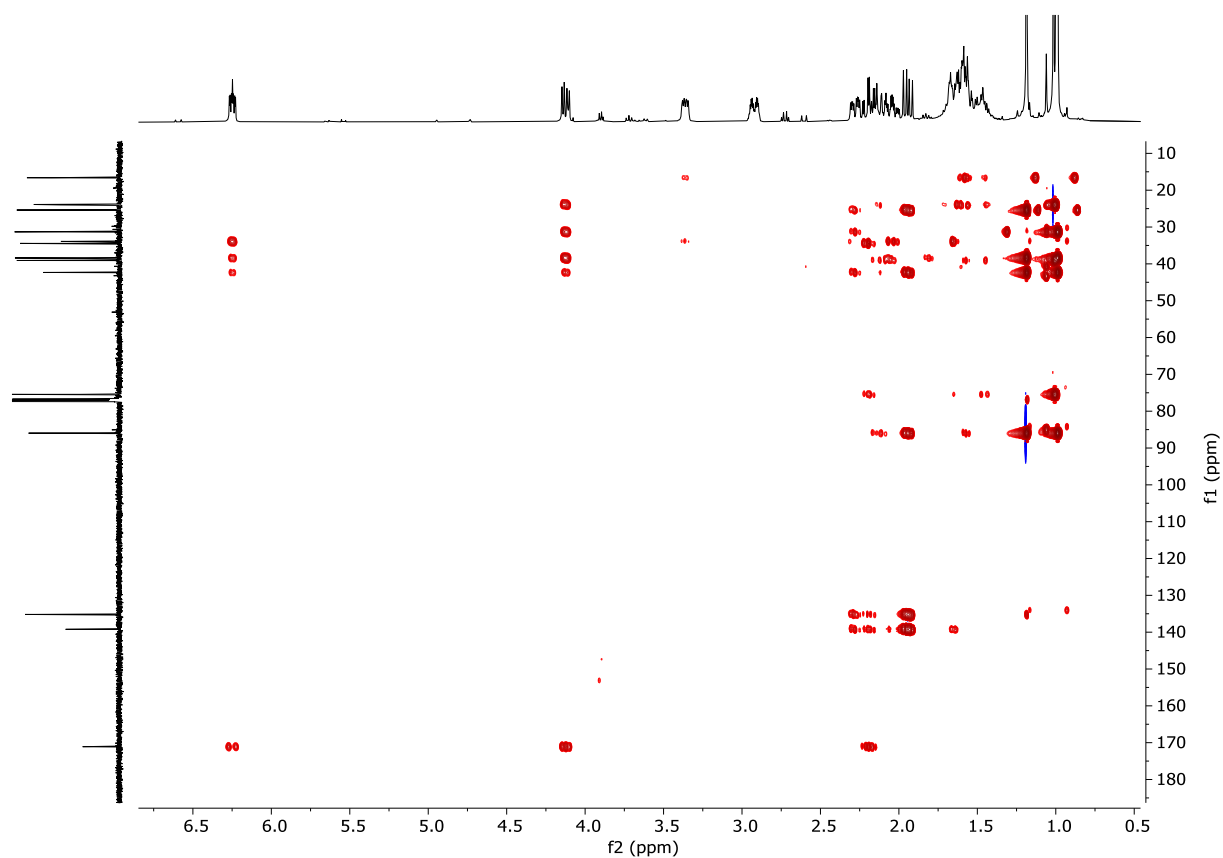


Figure S119. COSY spectrum of dolichocarpol P (**10**) (CDCl₃, 500 MHz)

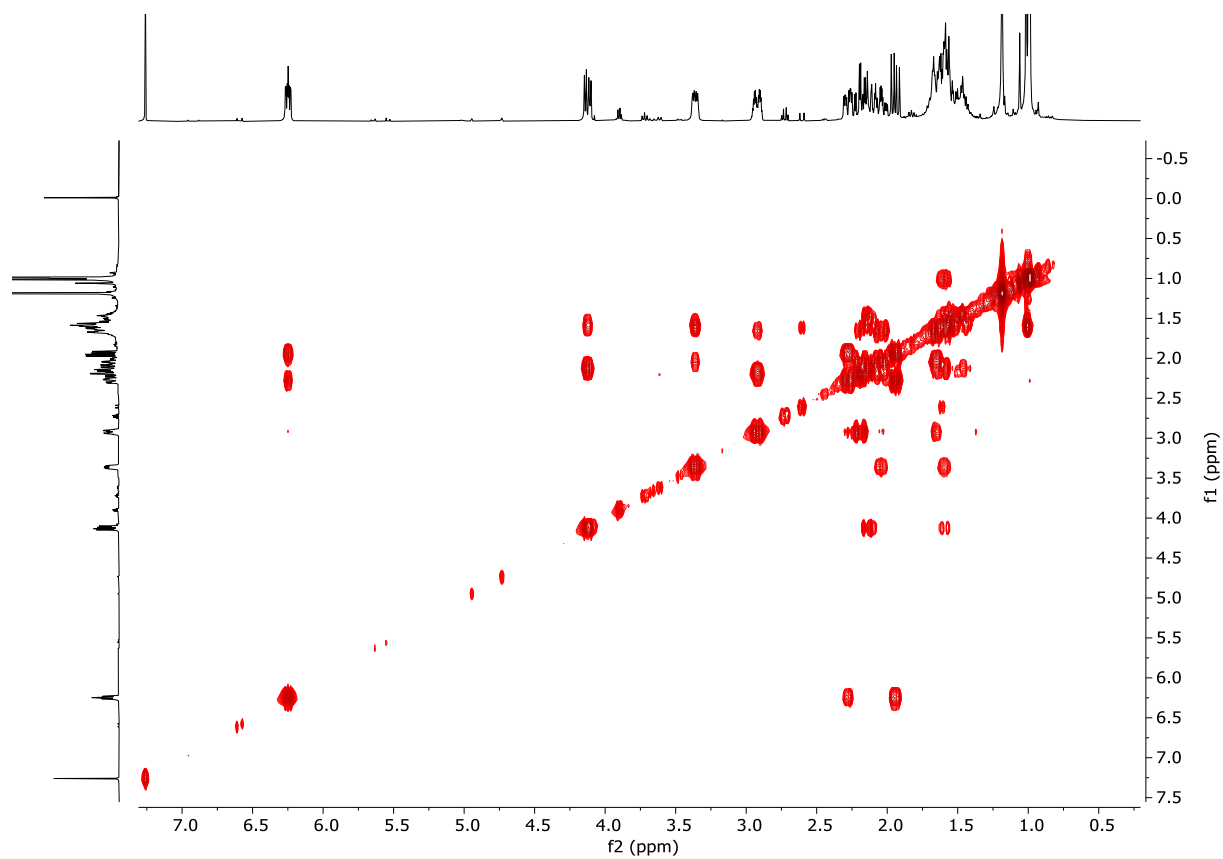


Figure S120. NOESY spectrum of dolichocarpol P (**10**) (CDCl₃, 500 MHz)

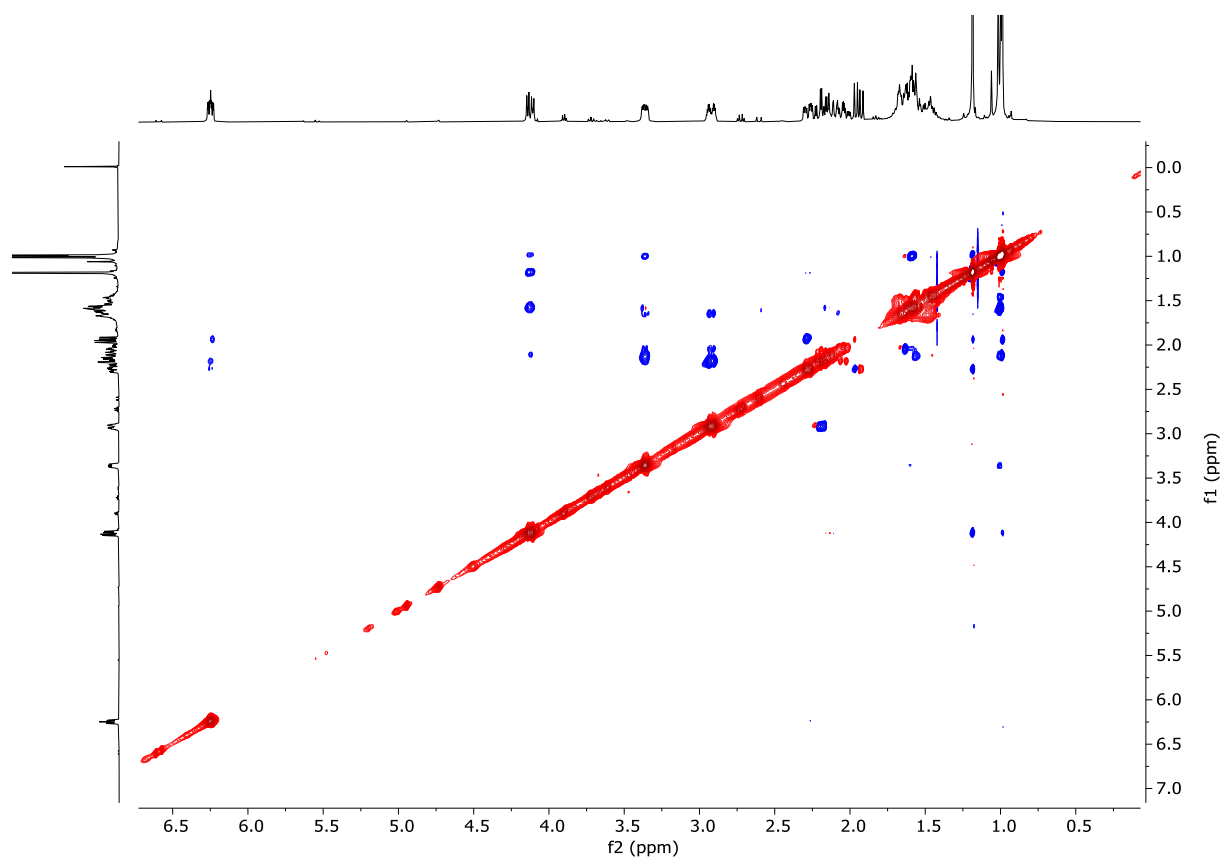


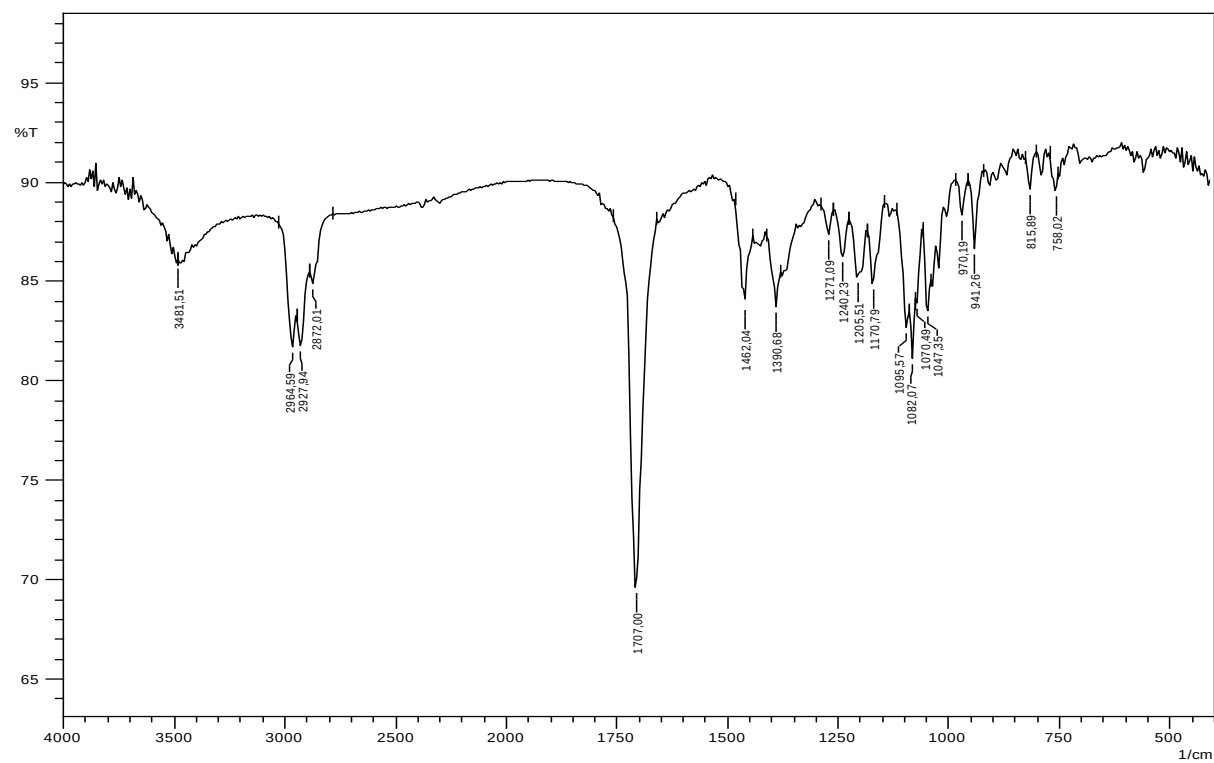
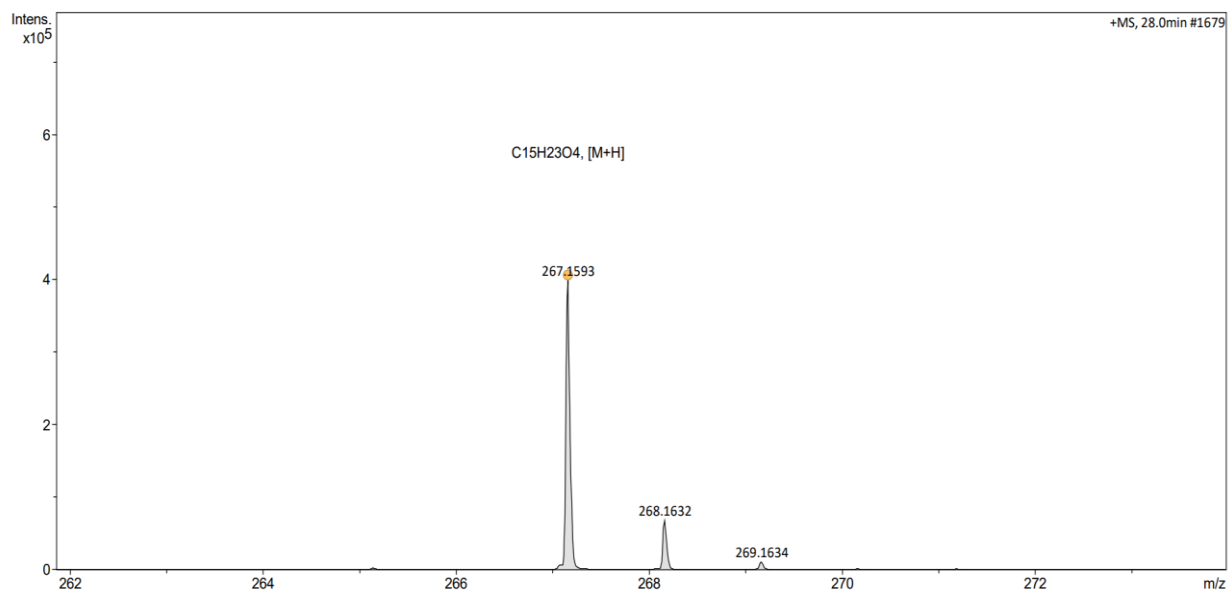
Figure S121. IR spectrum of dolichocarpol Q (**11**)**Figure S122.** HRESIMS $[\text{M} + \text{H}]^+$ spectrum of dolichocarpol Q (**11**)

Figure S123. ^1H NMR spectrum of dolichocarpol Q (**11**) (CDCl_3 , 500 MHz)

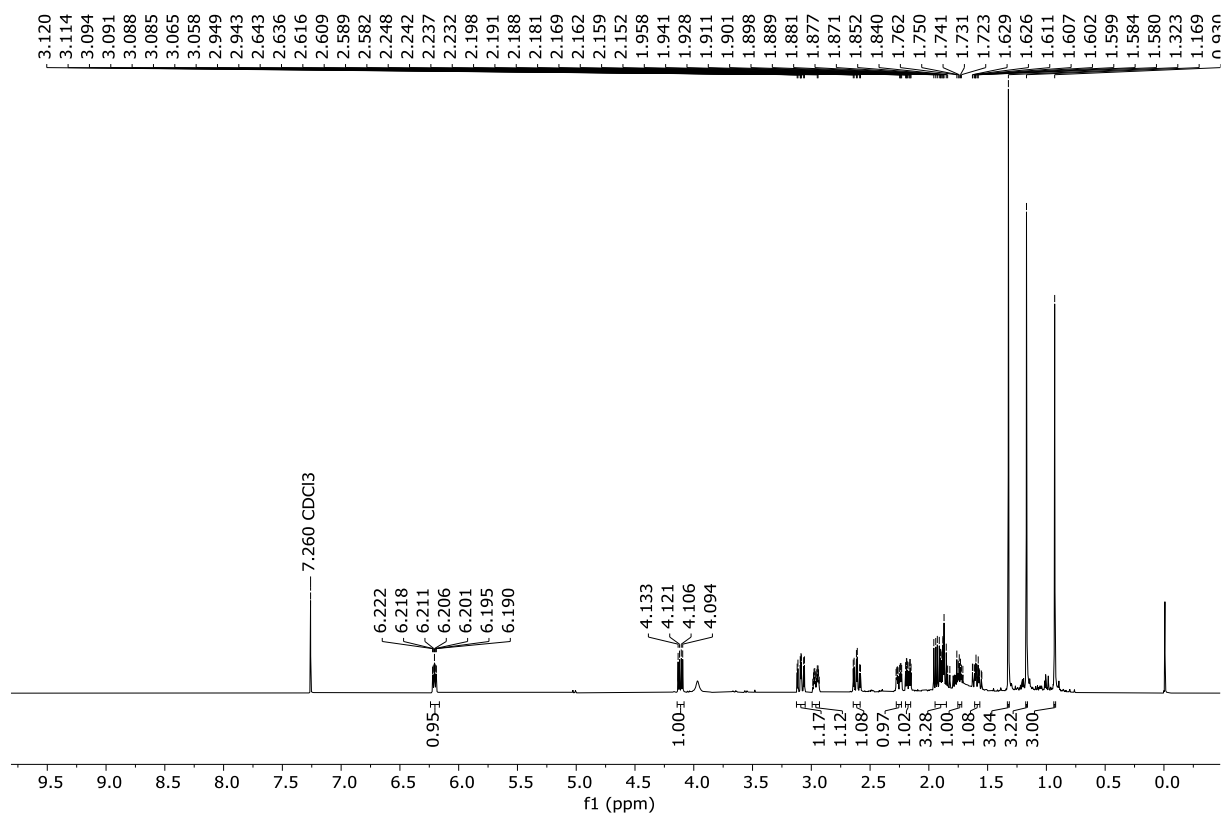


Figure S124. ^{13}C NMR spectrum of dolichocarpol Q (**11**) (CDCl_3 , 125 MHz)

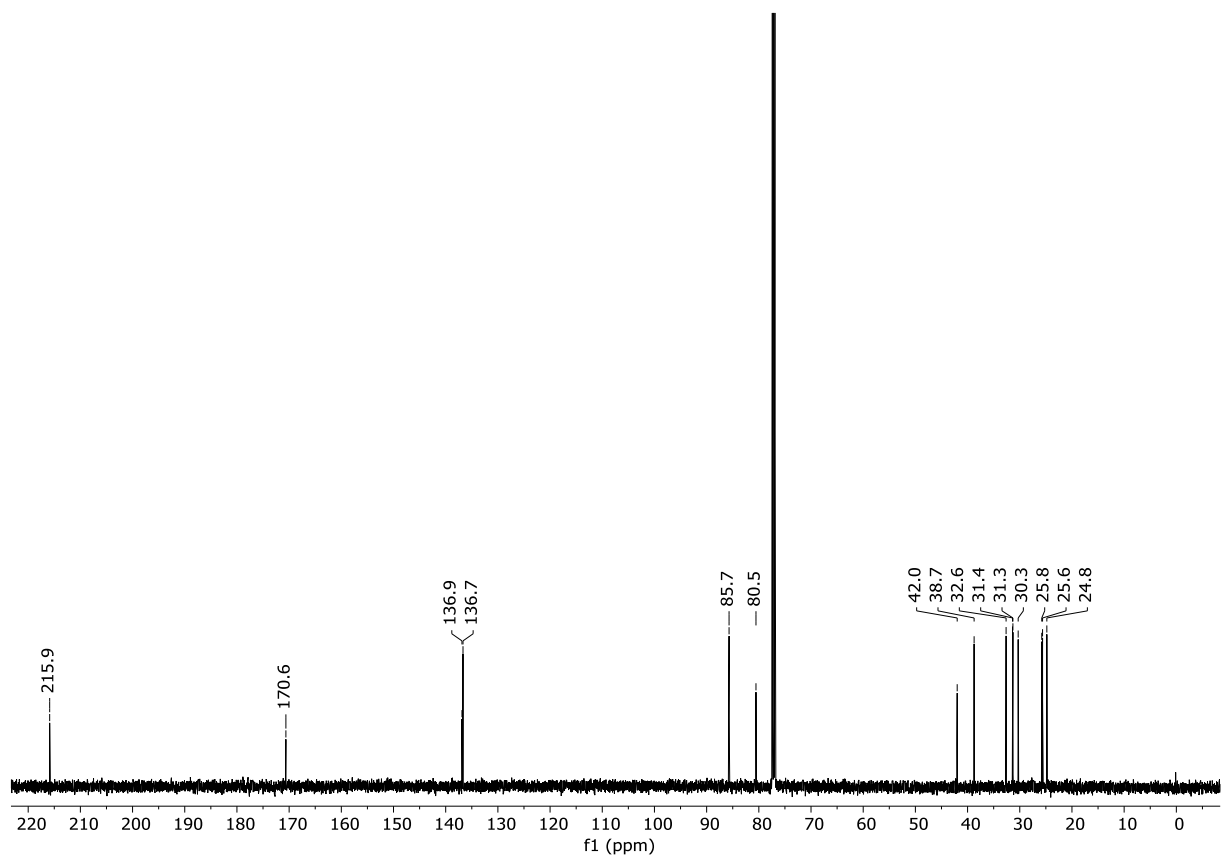


Figure S125. HSQC spectrum of dolichocarpol Q (**11**) (CDCl₃, 500 MHz)

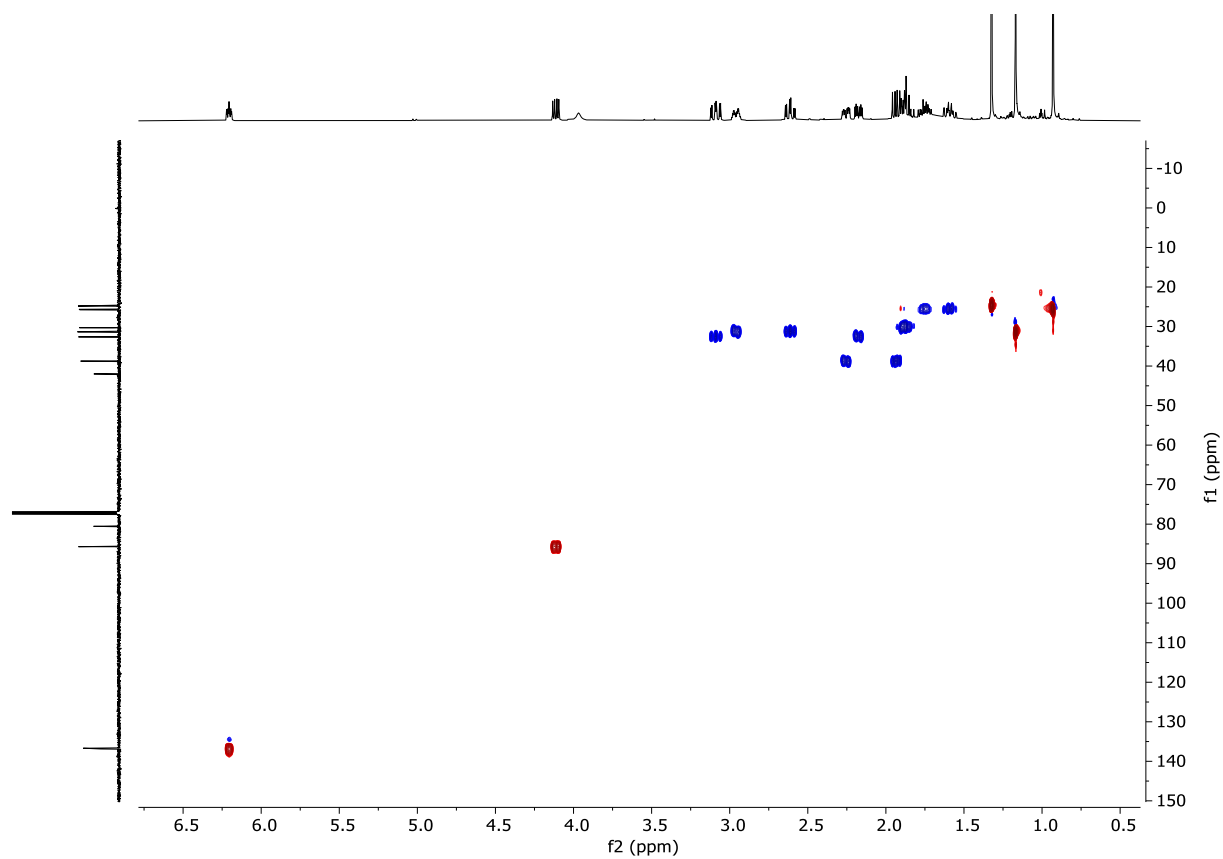


Figure S126. HMBC spectrum of dolichocarpol Q (**11**) (CDCl₃, 500 MHz)

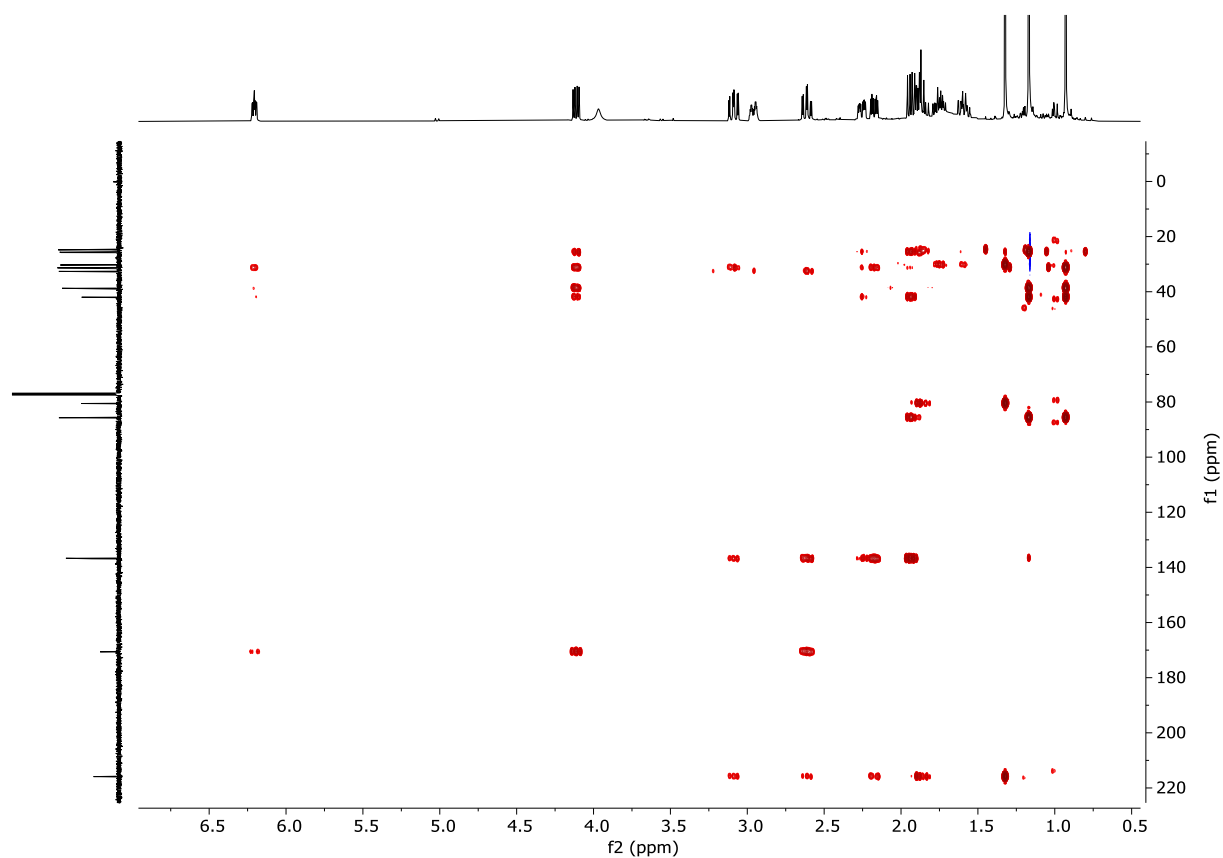


Figure S127. COSY spectrum of dolichocarpol Q (**11**) (CDCl₃, 500 MHz)

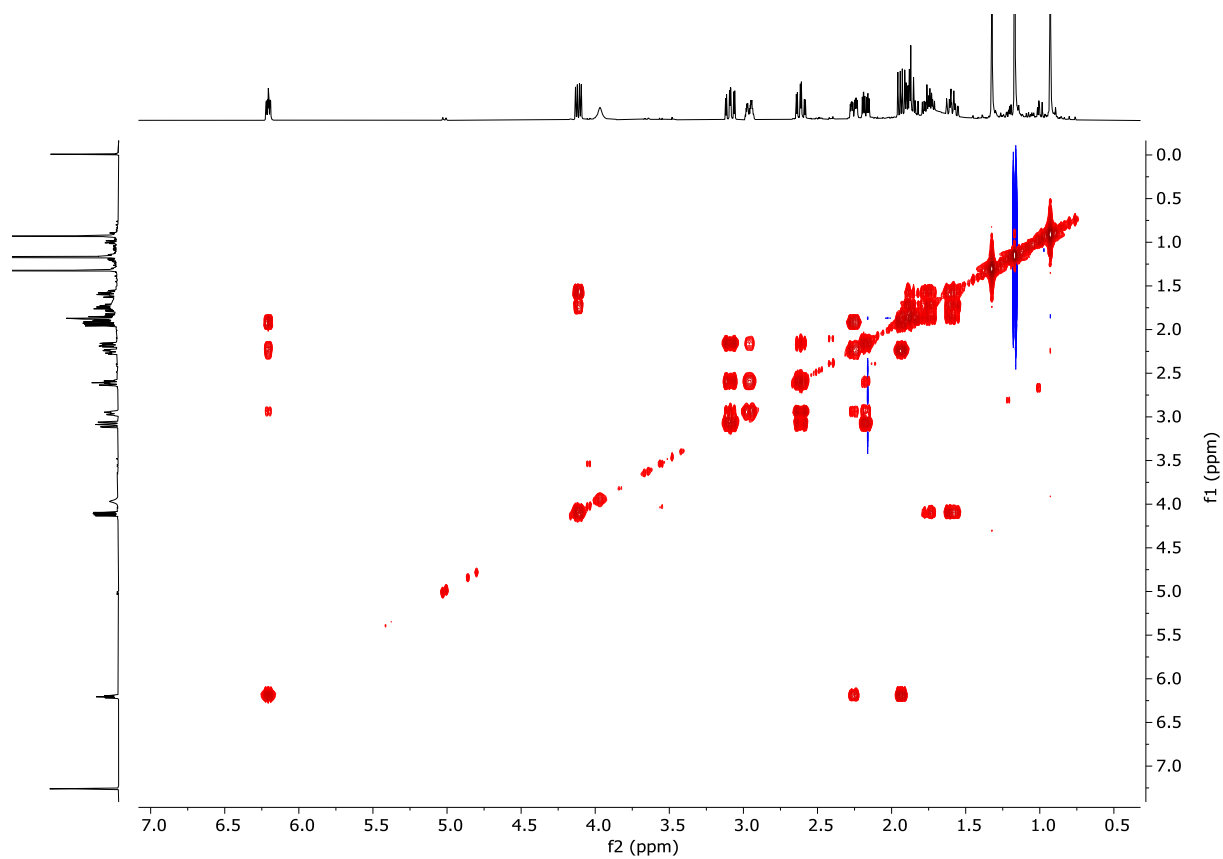


Figure S128. NOESY spectrum of dolichocarpol Q (**11**) (CDCl₃, 500 MHz)

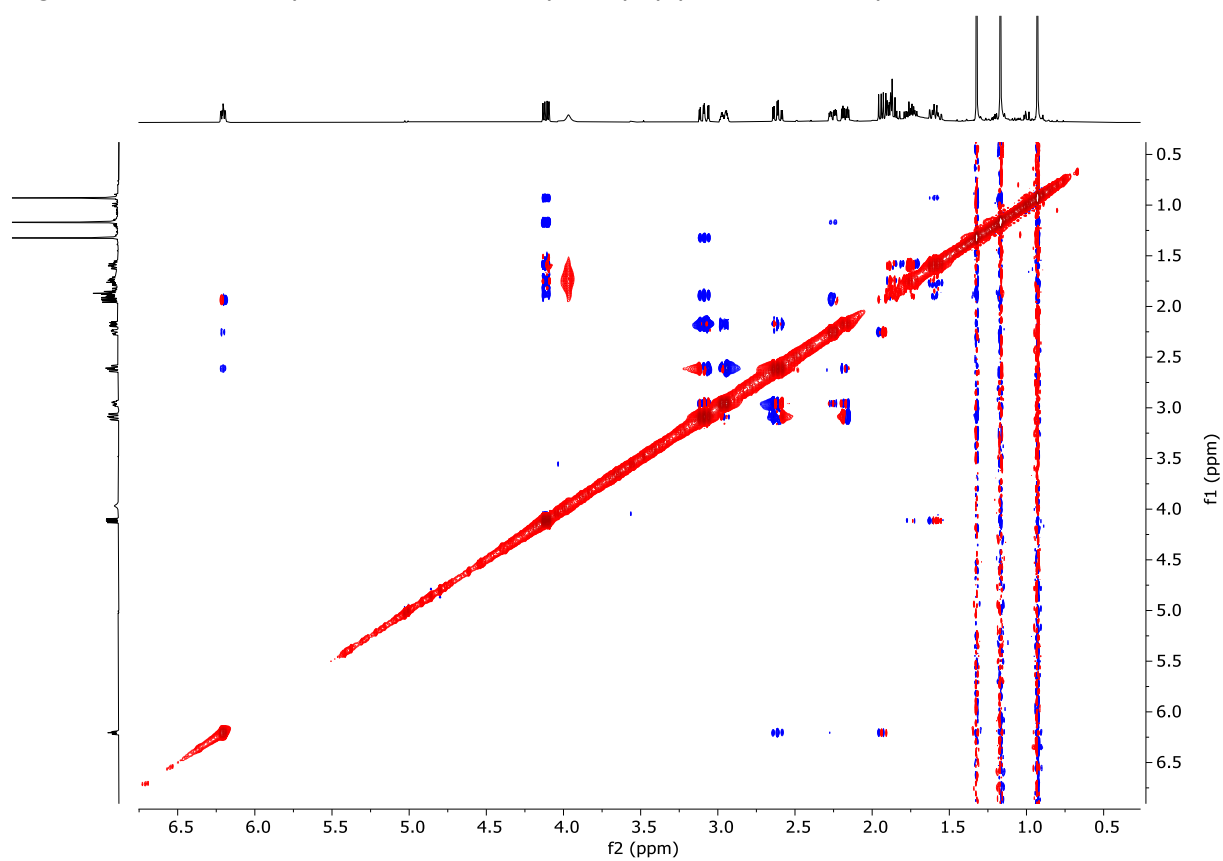


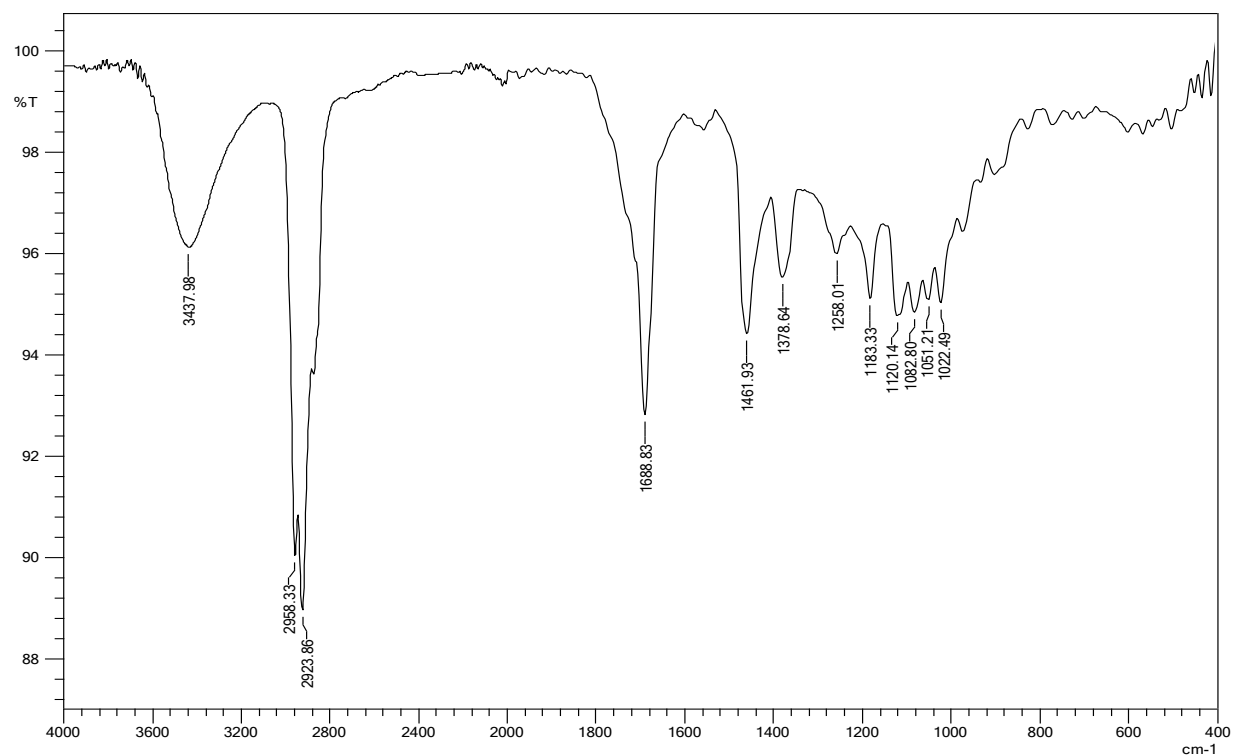
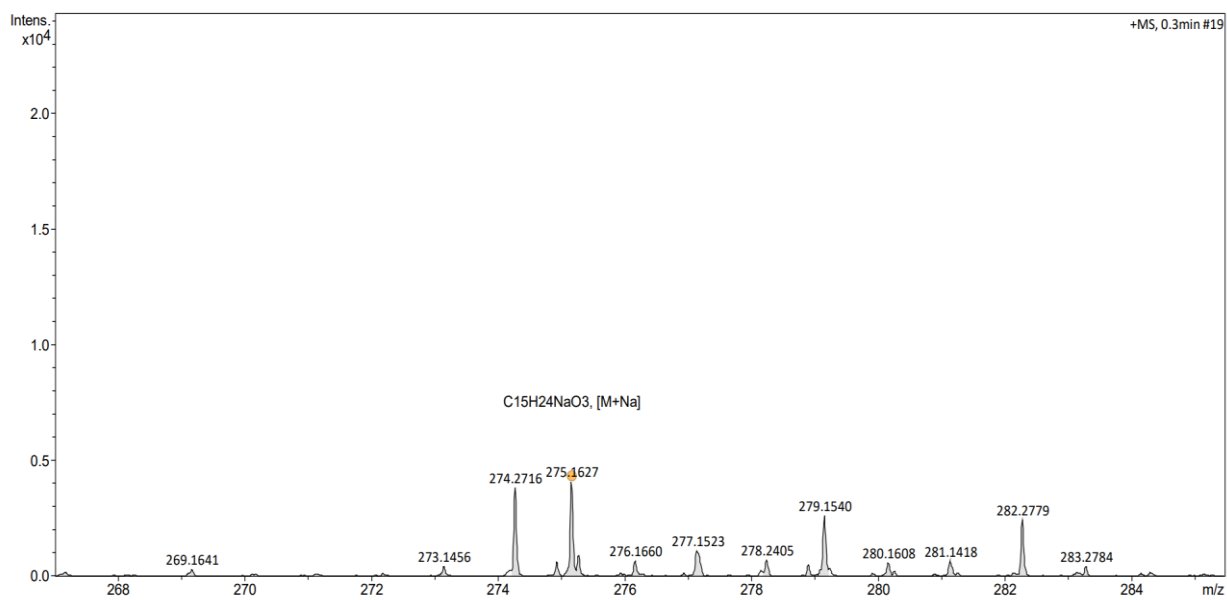
Figure S129. IR spectrum of dolichocarpol R (**12**)**Figure S130.** HRESIMS [M + Na]⁺ spectrum of dolichocarpol R (**12**)

Figure S131. ^1H NMR spectrum of dolichocarpol R (**12**) (CDCl_3 , 500 MHz)

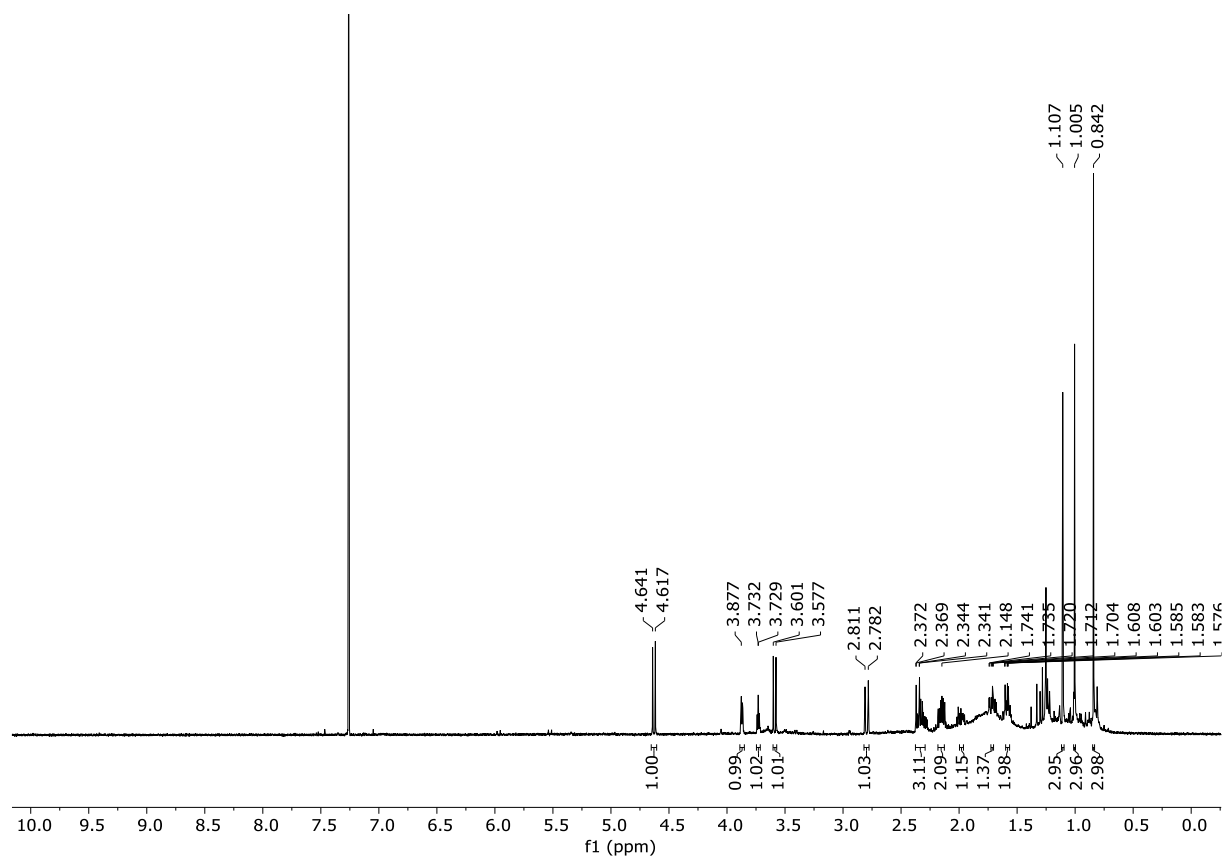


Figure S132. ^{13}C NMR spectrum of dolichocarpol R (**12**) (CDCl_3 , 125 MHz)

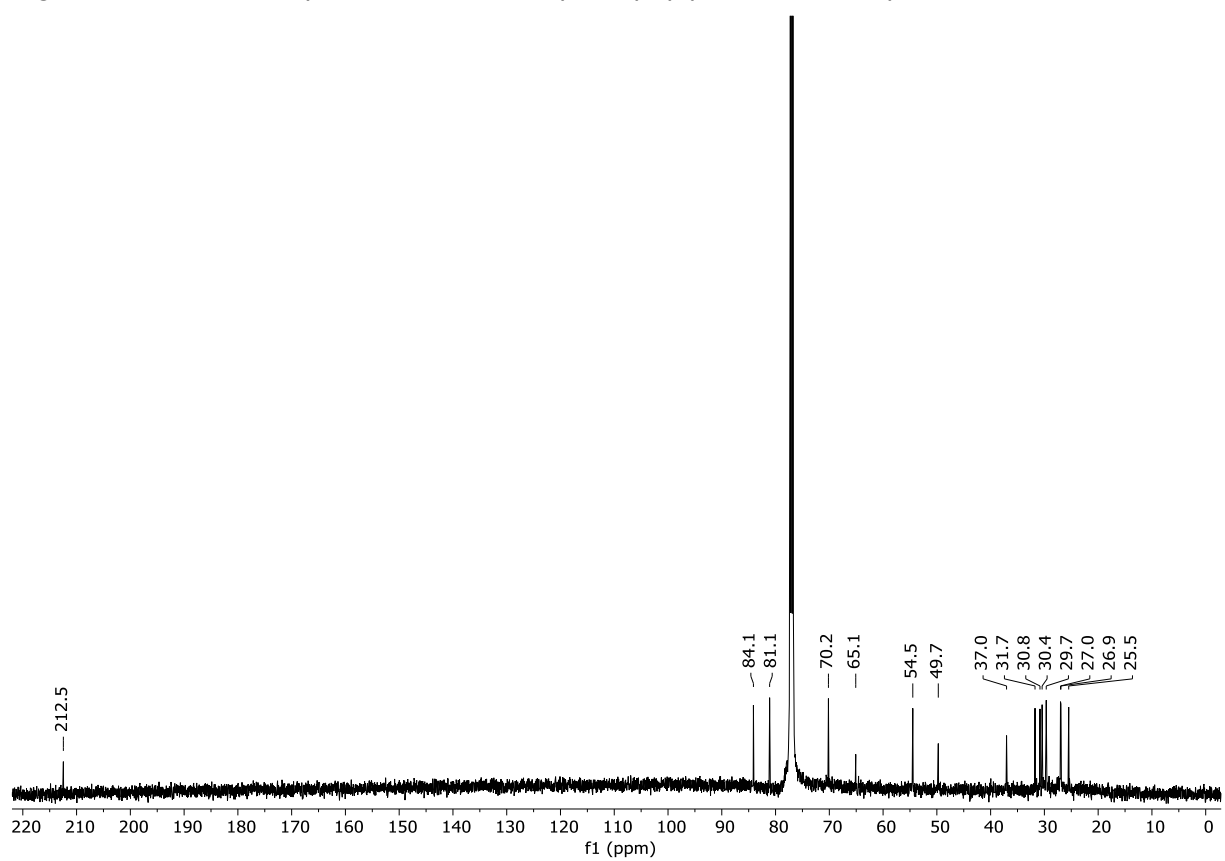


Figure S133. ^{13}C DEPT135 spectrum of dolichocarpol R (**12**) (CDCl_3 , 125 MHz)

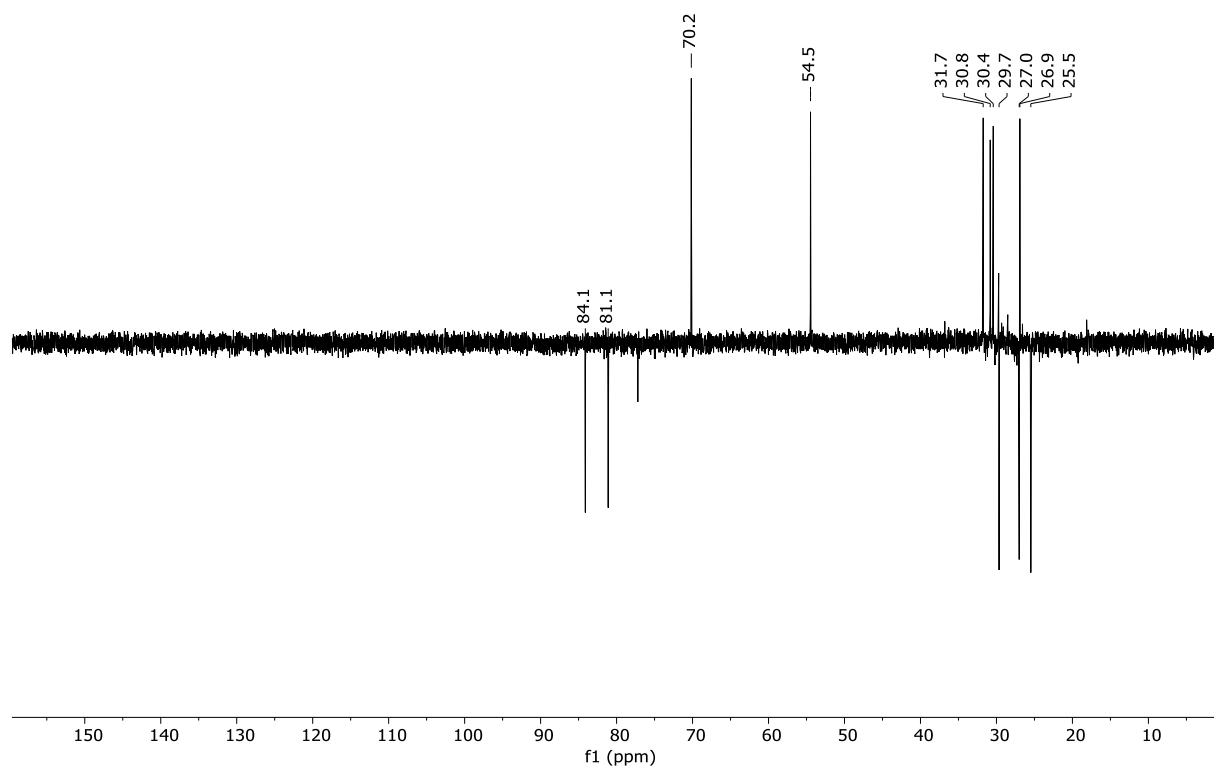


Figure S134. HSQC spectrum of dolichocarpol R (**12**) (CDCl_3 , 500 MHz)

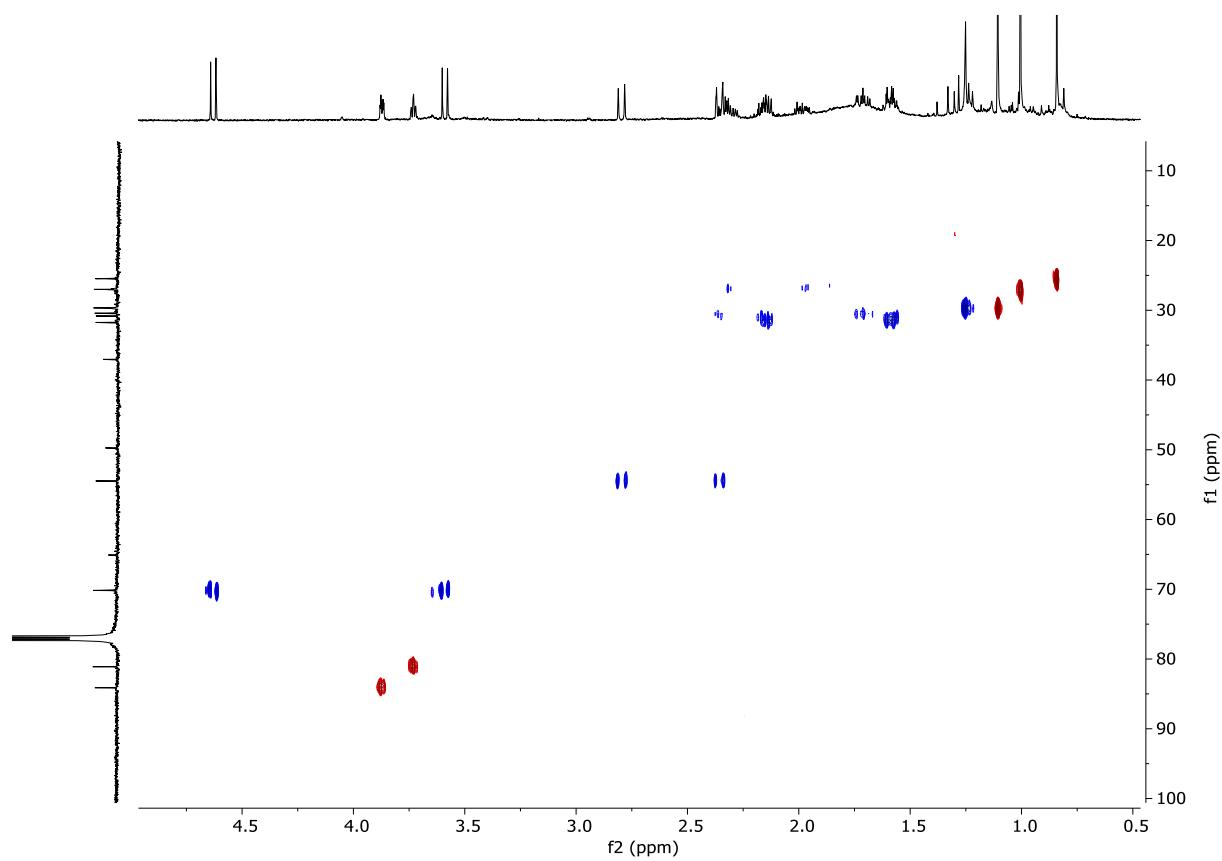


Figure S135. HMBC spectrum of dolichocarpol R (**12**) (CDCl₃, 500 MHz)

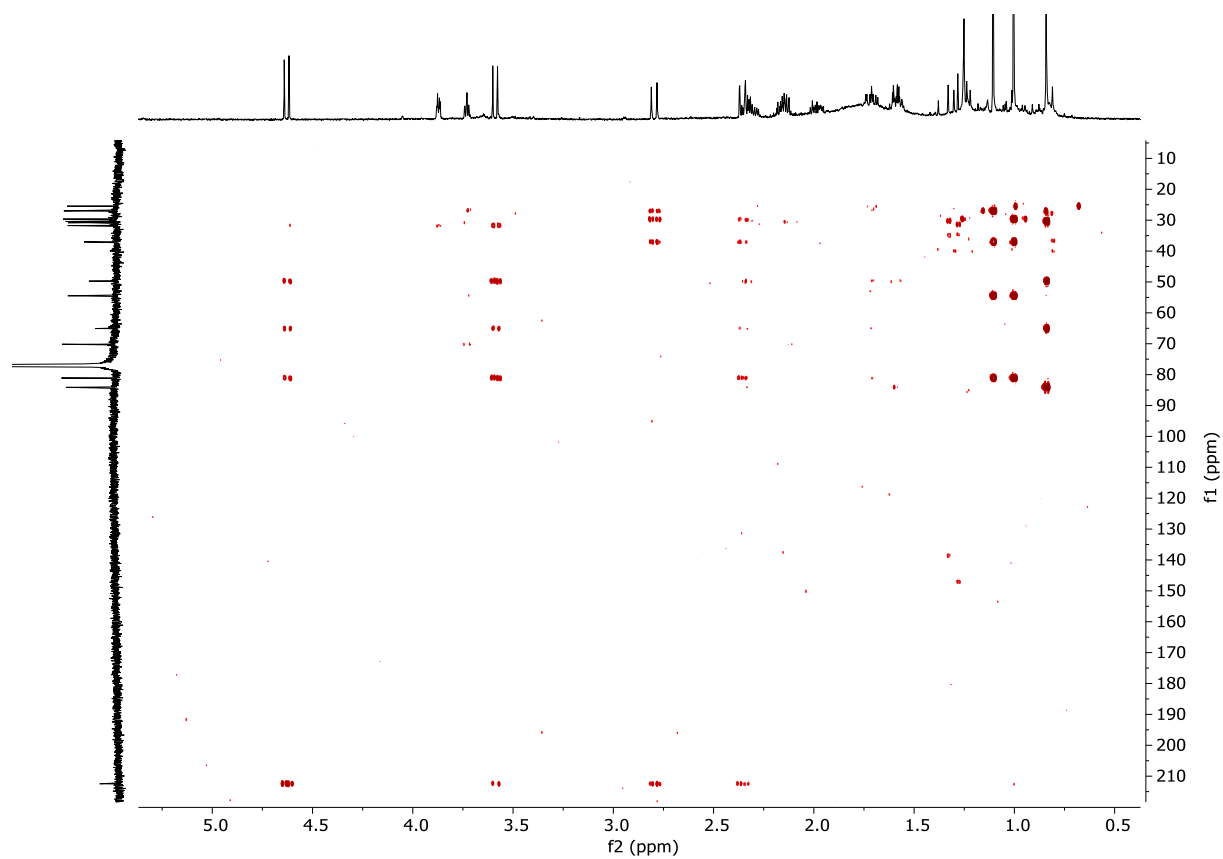


Figure S136. COSY spectrum of dolichocarpol R (**12**) (CDCl₃, 500 MHz)

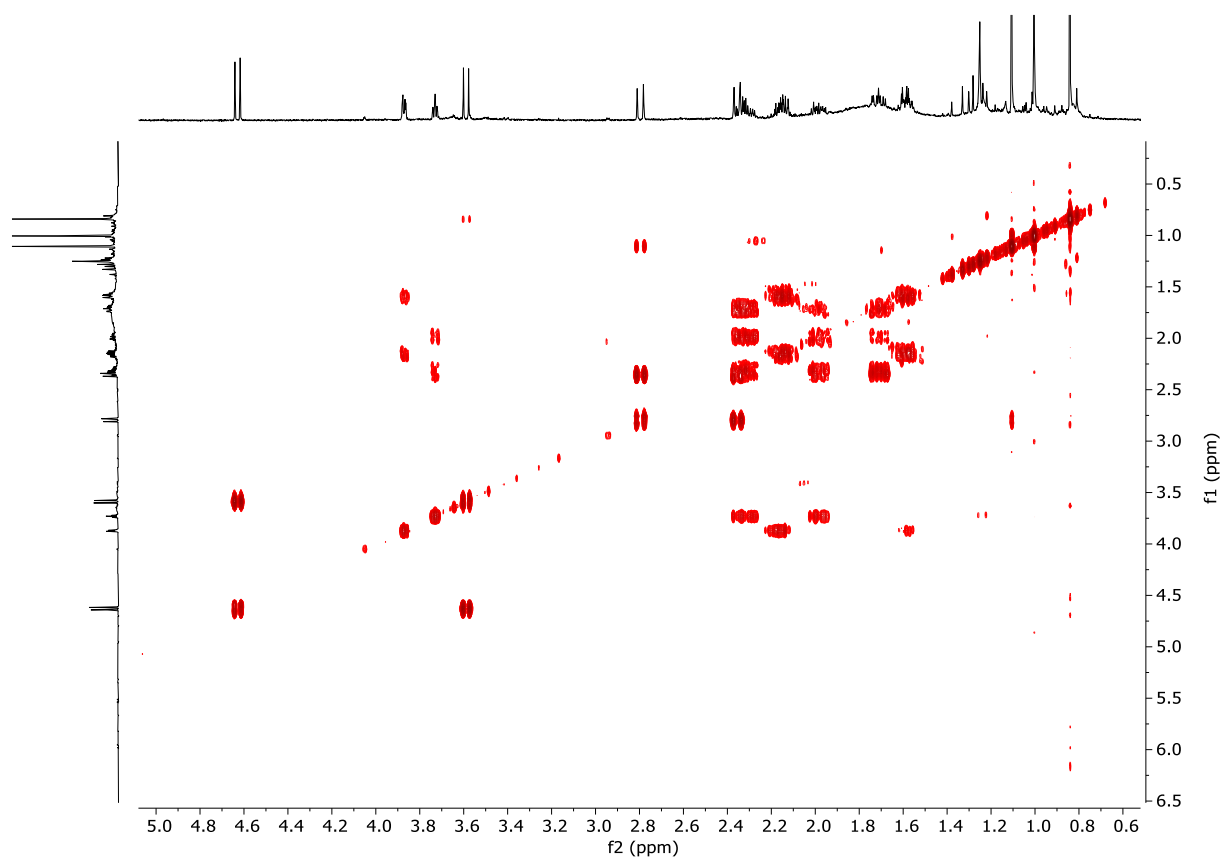


Figure S137. NOESY spectrum of dolichocarpol R (**12**) (CDCl₃, 400 MHz)

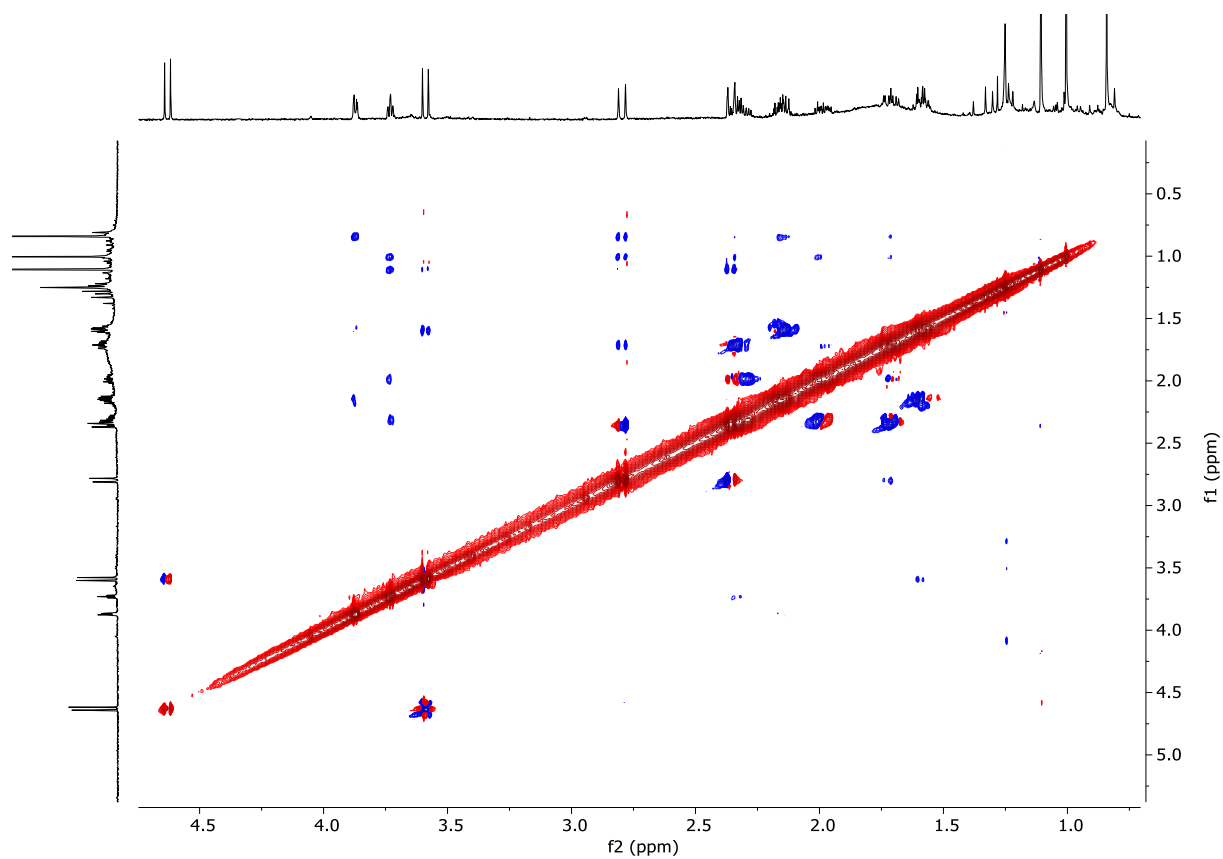


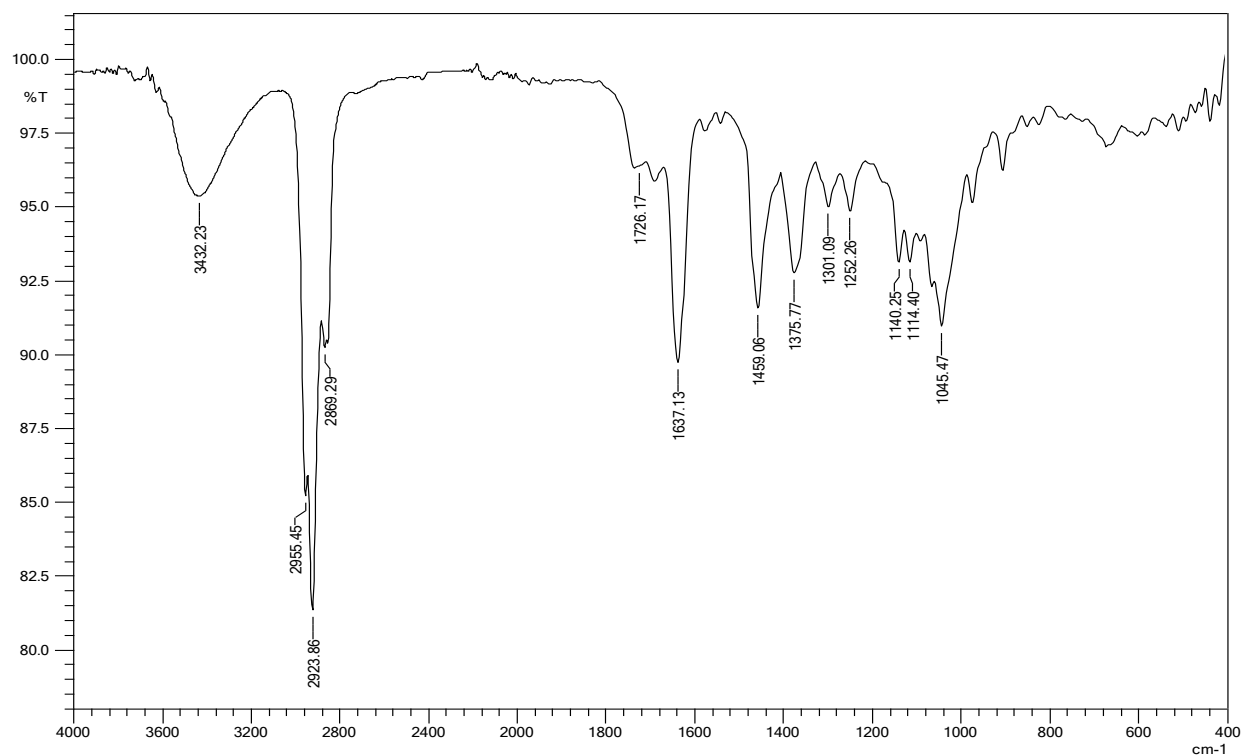
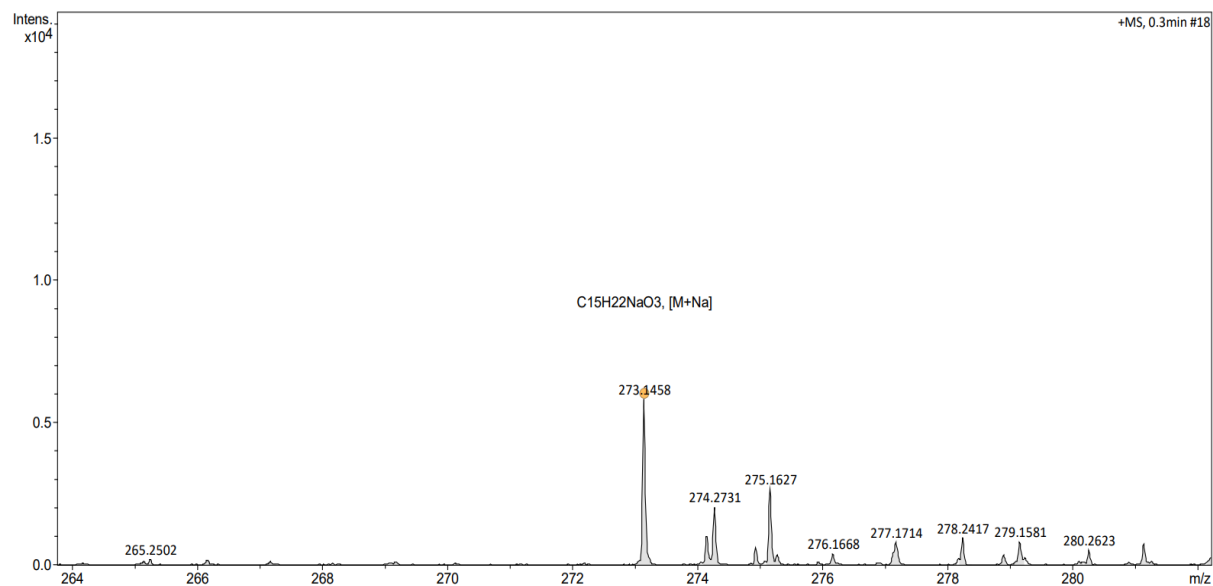
Figure S138. IR spectrum of dolichocarpol S (**13**)**Figure S139.** HRESIMS [M + H]⁺ spectrum of dolichocarpol S (**13**)

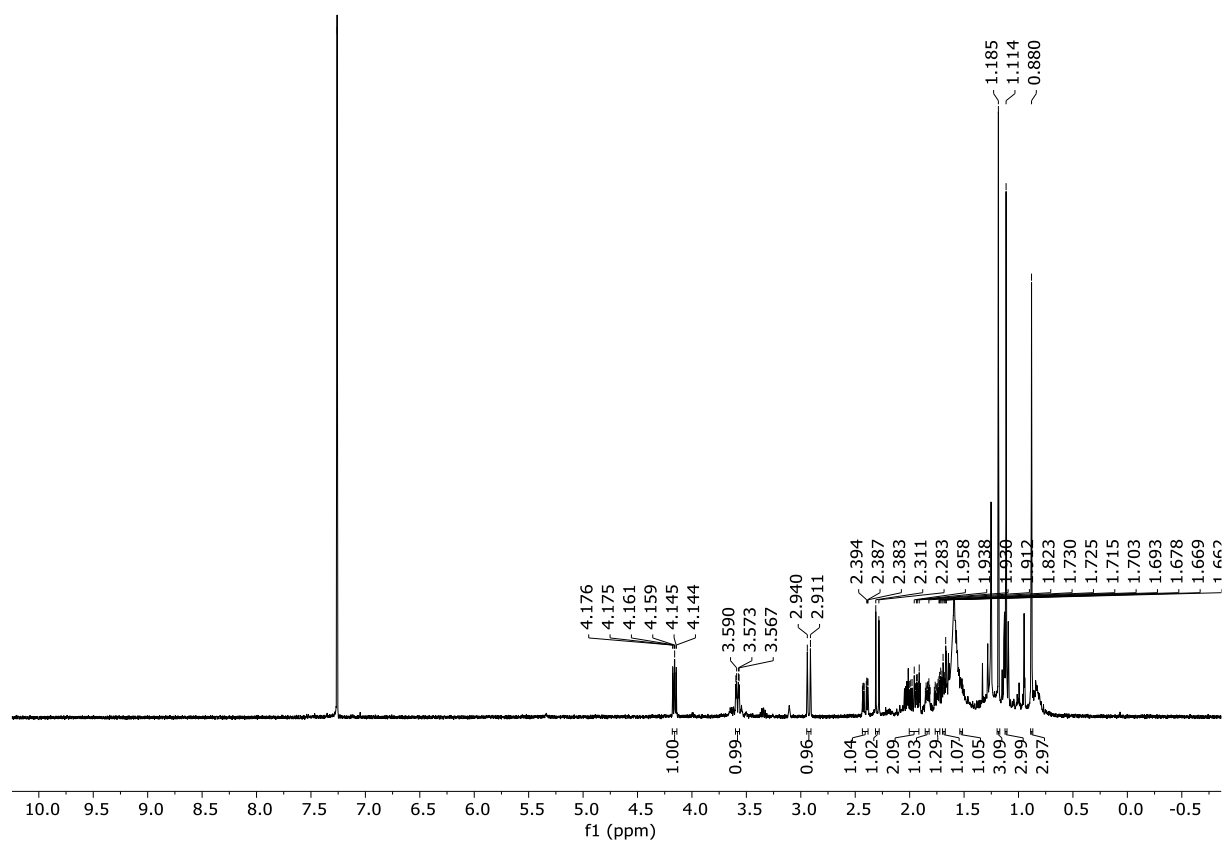
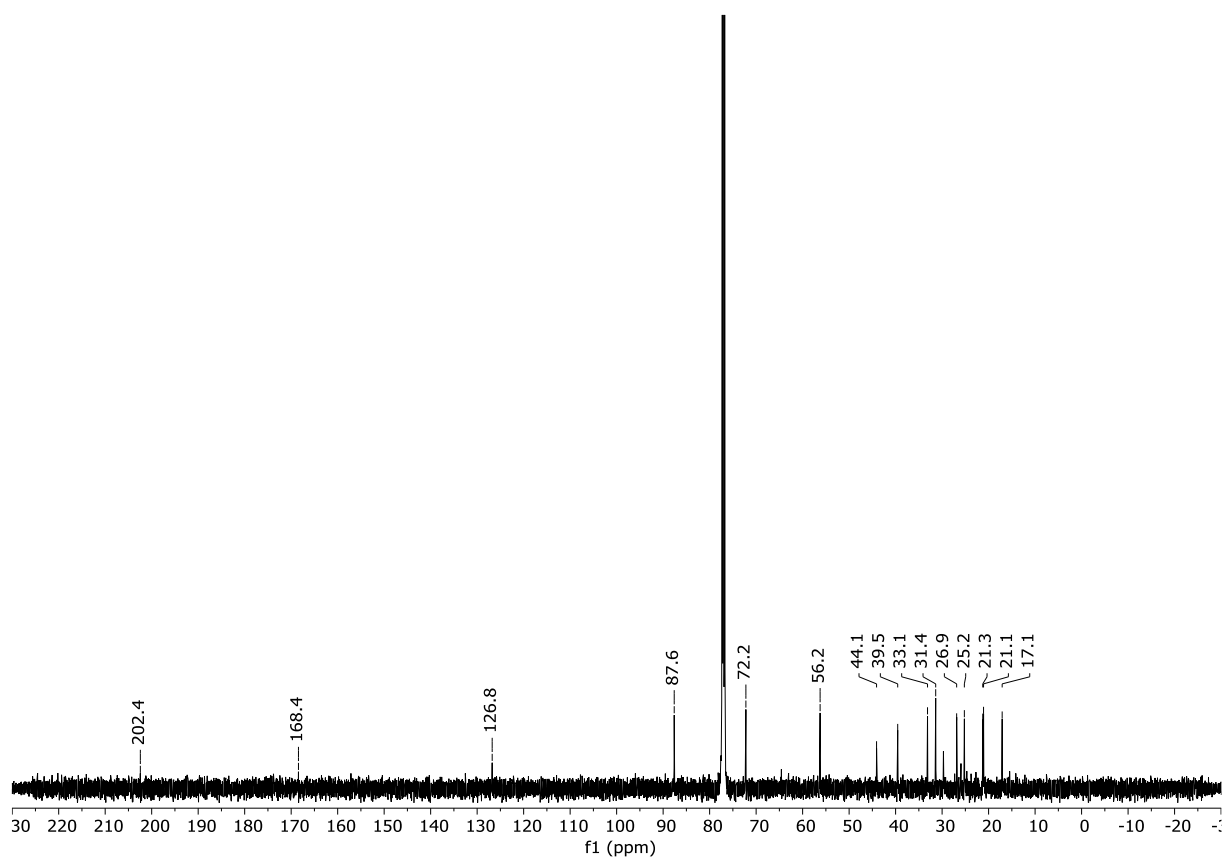
Figure S140. ^1H NMR spectrum of dolichocarpol S (**13**) (CDCl_3 , 500 MHz)**Figure S141.** ^{13}C NMR spectrum of dolichocarpol S (**13**) (CDCl_3 , 125 MHz)

Figure S142. HSQC spectrum of dolichocarpol S (**13**) (CDCl₃, 500 MHz)

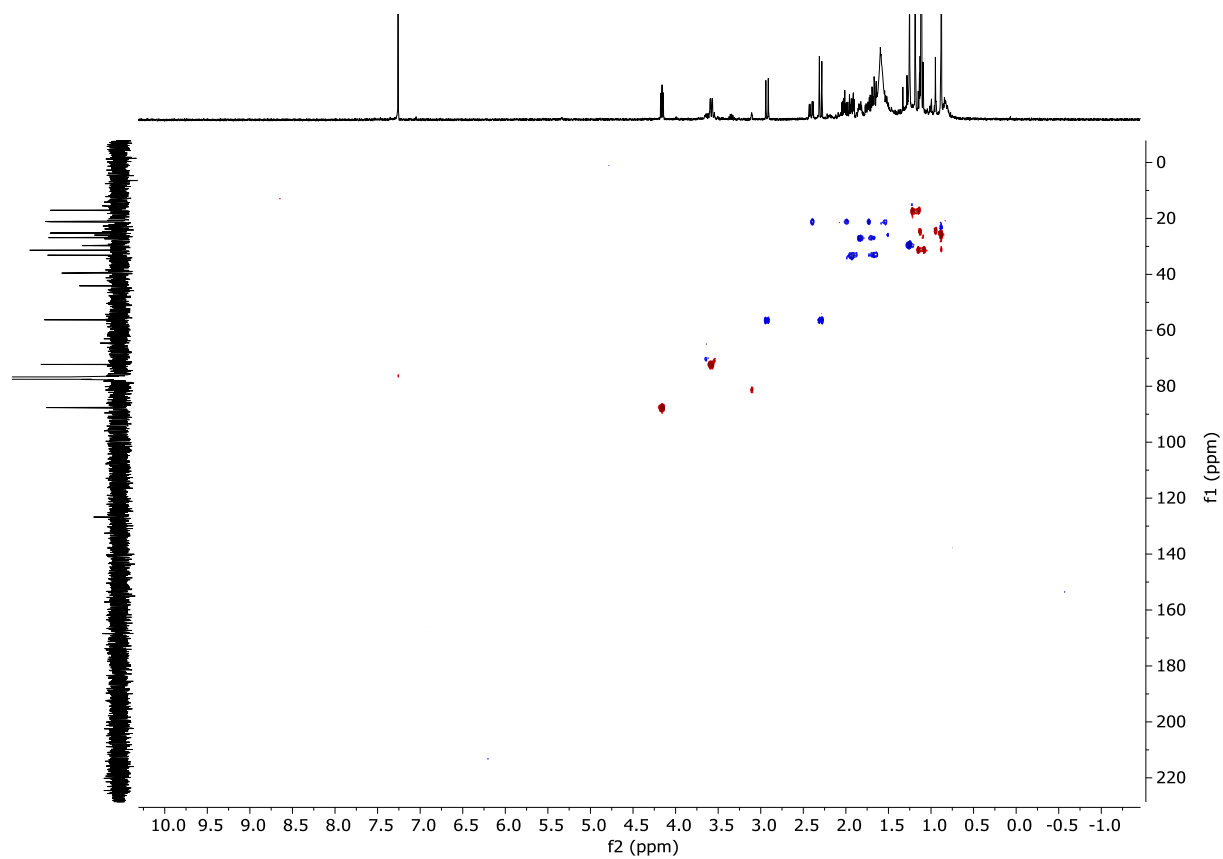


Figure S143. HMBC spectrum of dolichocarpol S (**13**) (CDCl₃, 500 MHz)

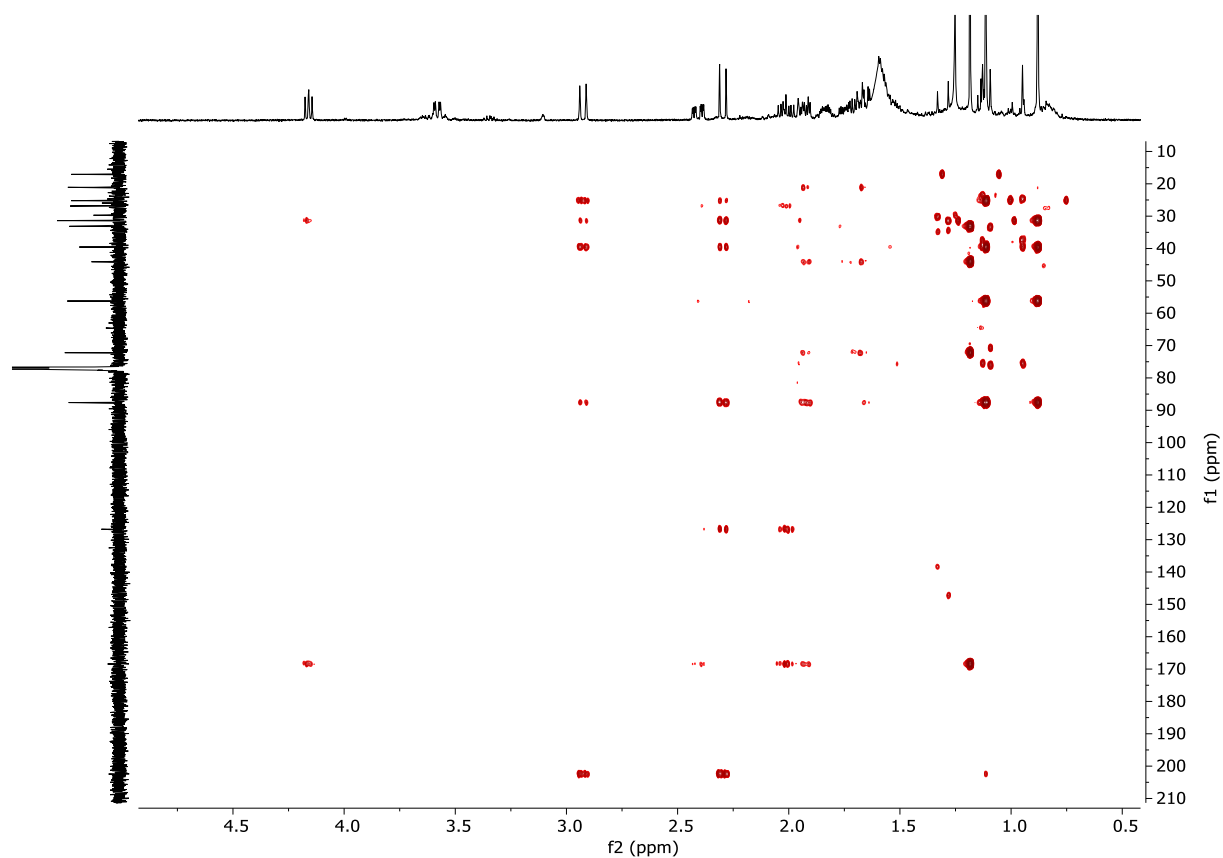


Figure S144. COSY spectrum of dolichocarpol S (**13**) (CDCl₃, 500 MHz)

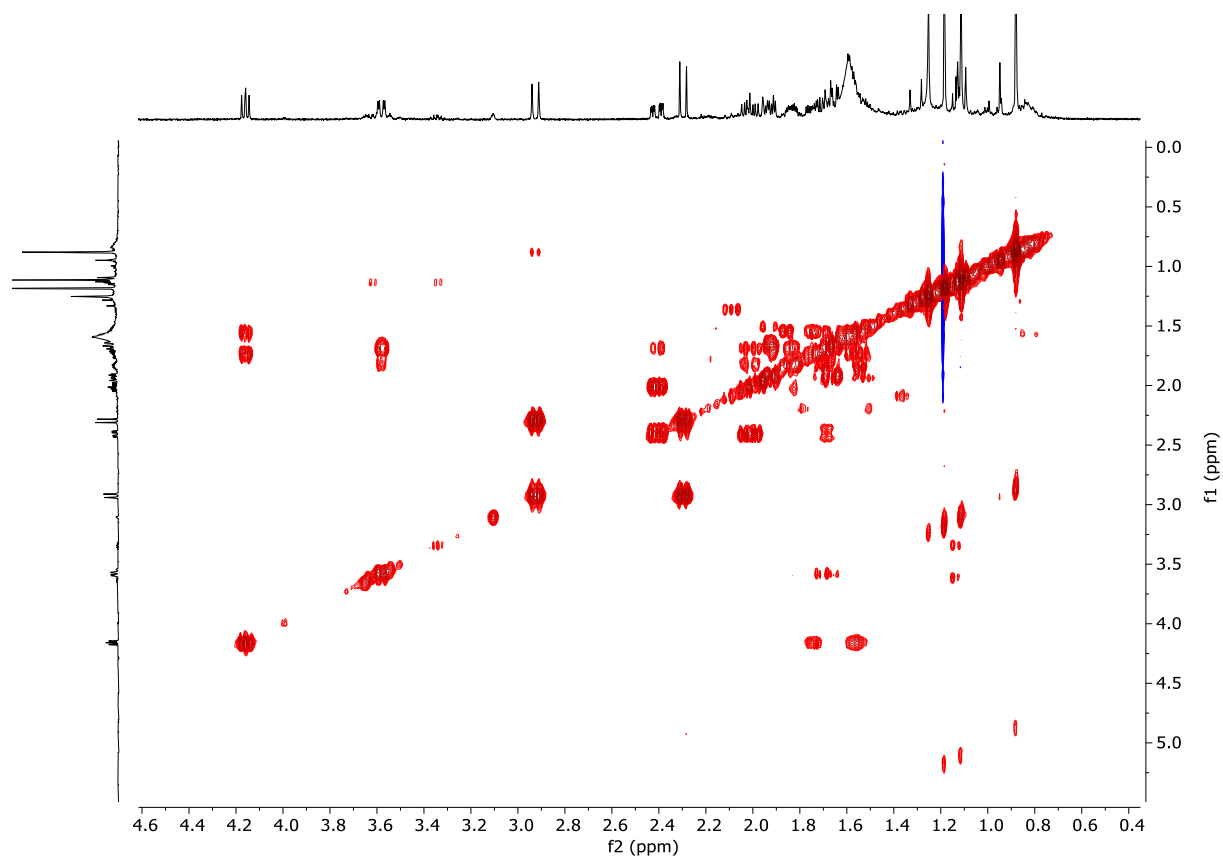


Figure S145. NOESY spectrum of dolichocarpol S (**13**) (CDCl₃, 500 MHz)

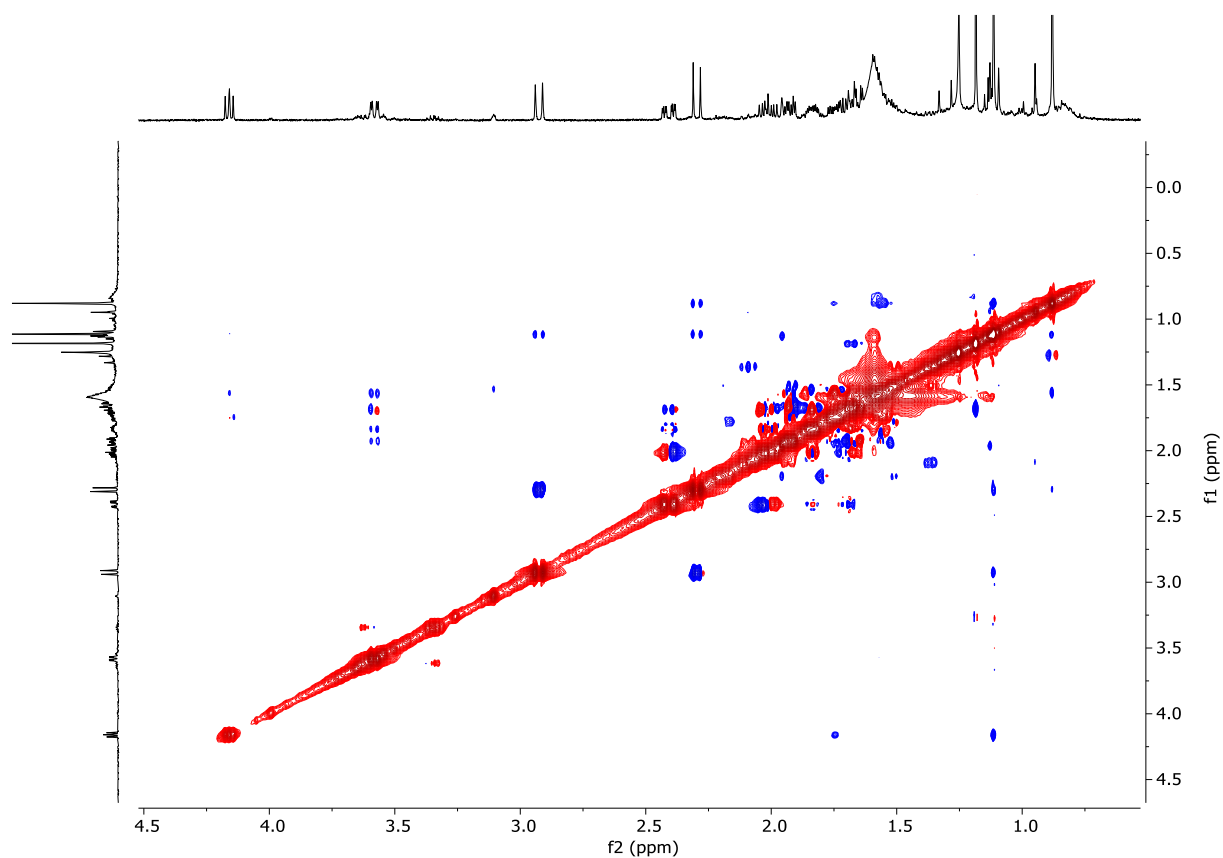


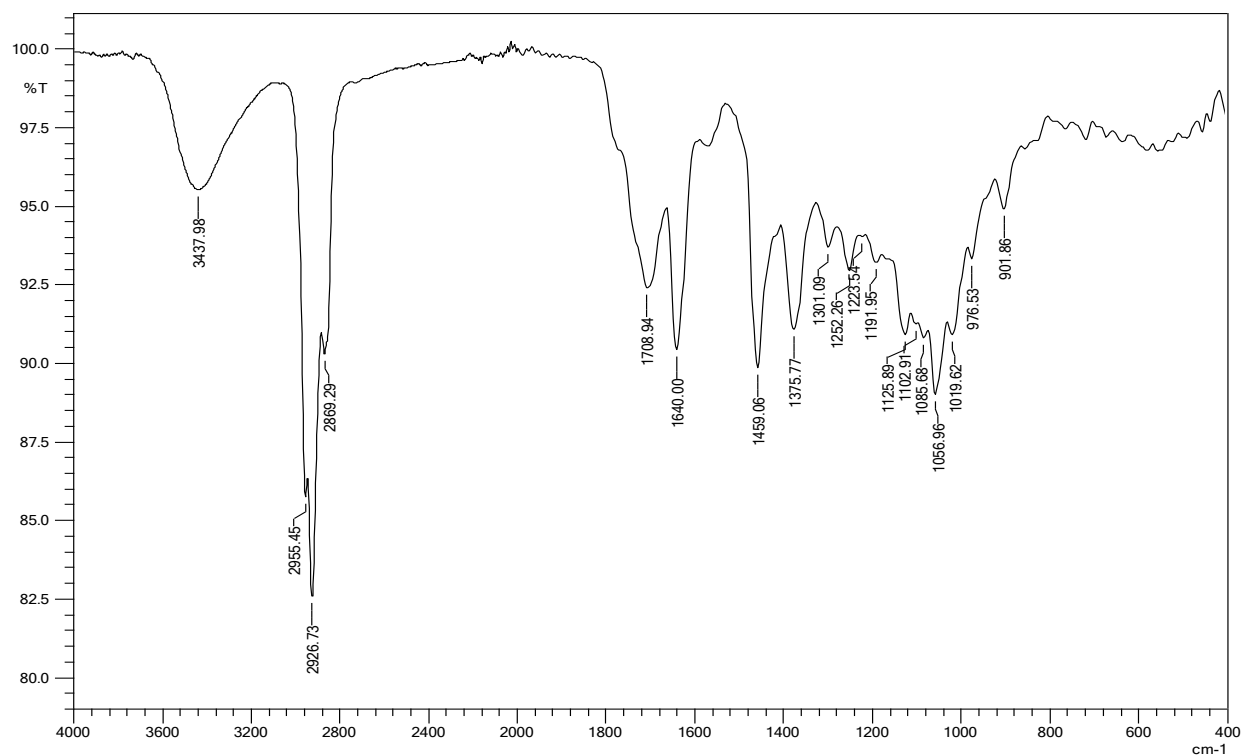
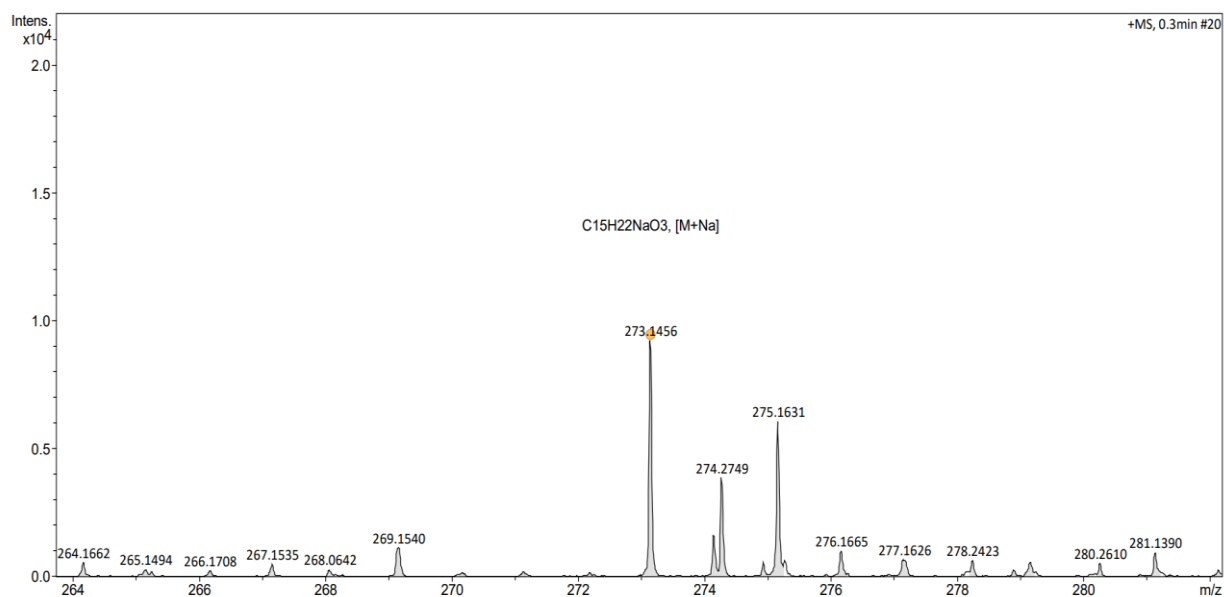
Figure S146. IR spectrum of dolichocarpol T (**14**)**Figure S147.** HRESIMS $[\text{M} + \text{Na}]^+$ spectrum of dolichocarpol T (**14**)

Figure S148. ^1H NMR spectrum of dolichocarpol T (**14**) (CDCl_3 , 400 MHz)

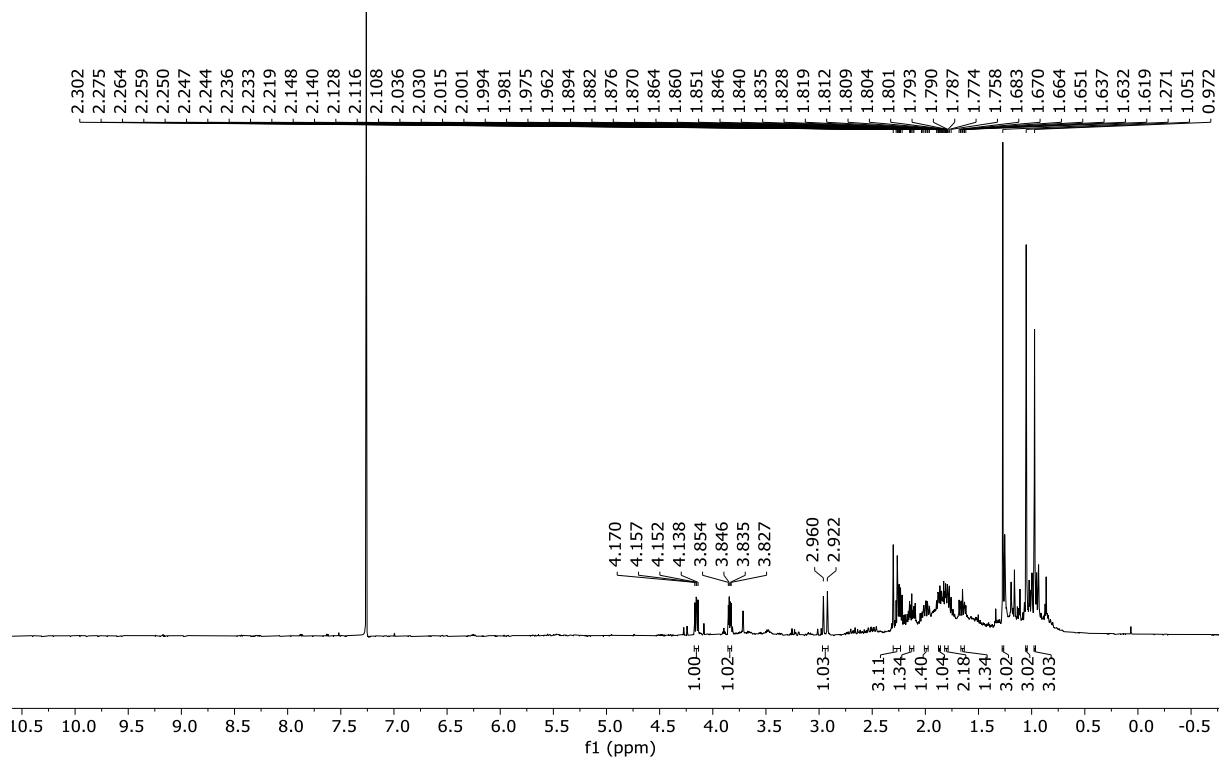


Figure S149. ^{13}C NMR spectrum of dolichocarpol T (**14**) (CDCl_3 , 100 MHz)

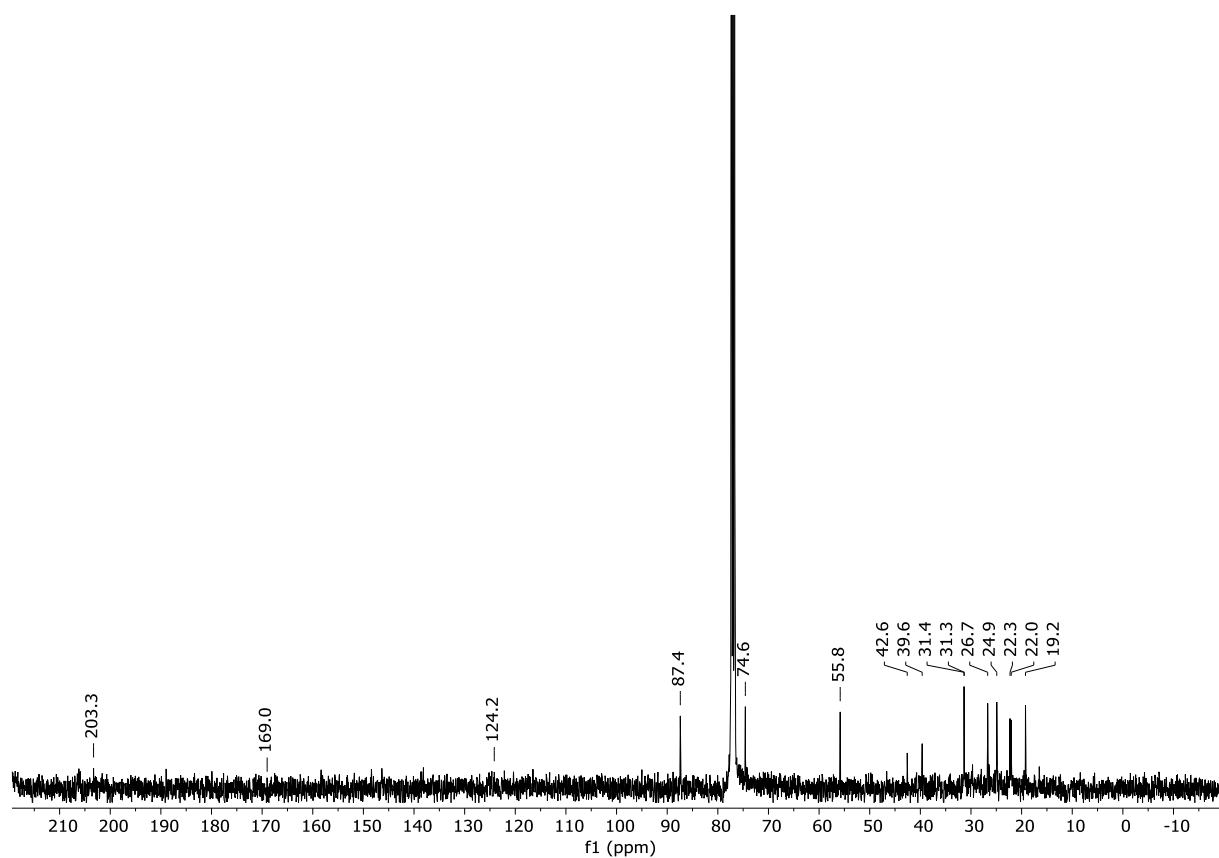


Figure S150. HSQC spectrum of dolichocarpol T (**14**) (CDCl₃, 400 MHz)

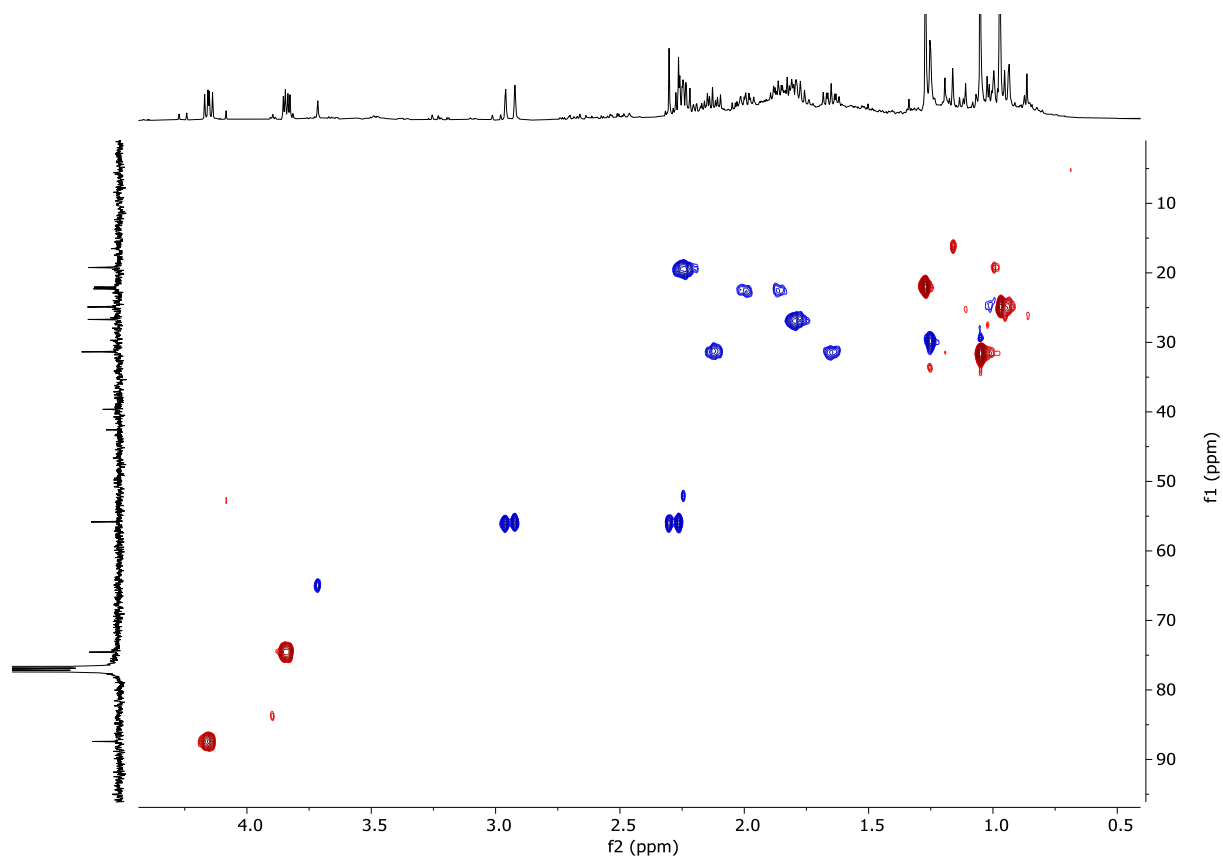


Figure S151. HMBC spectrum of dolichocarpol T (**14**) (CDCl₃, 400 MHz)

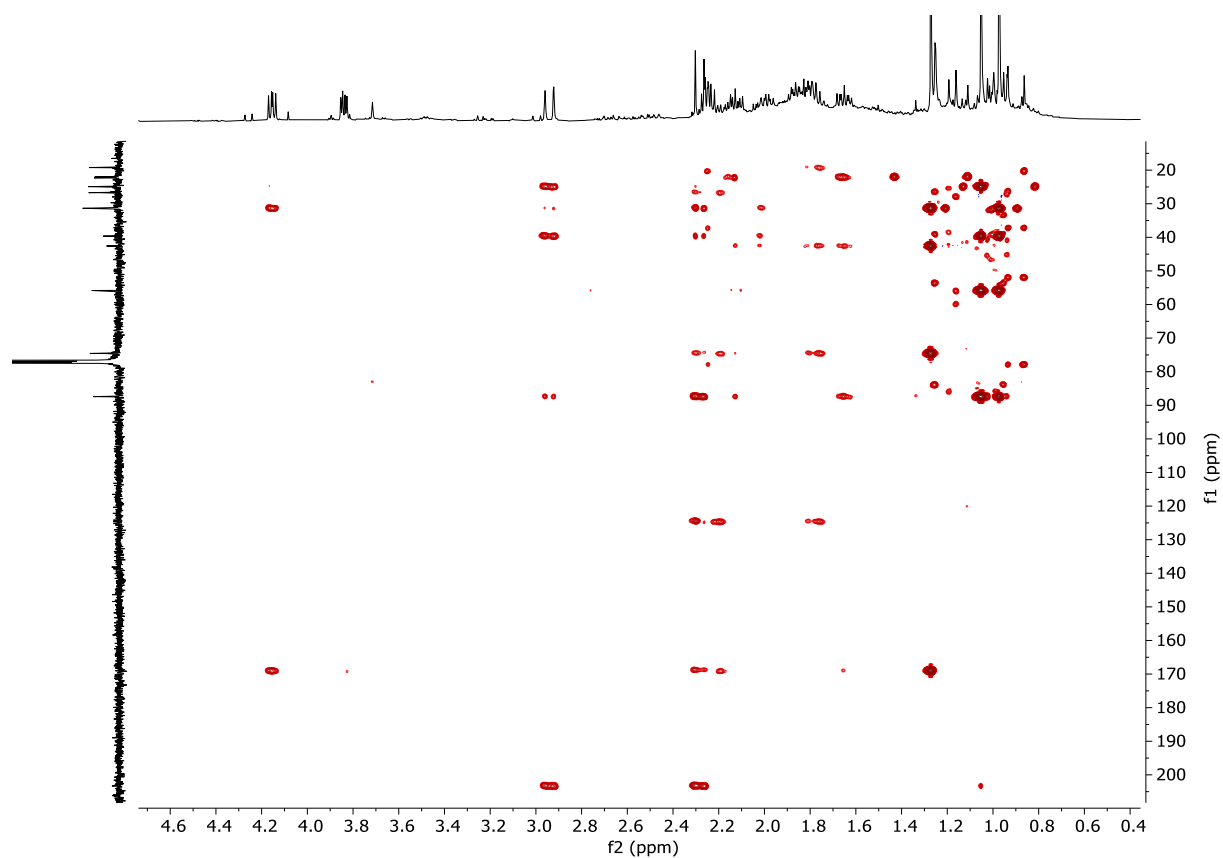


Figure S152. COSY spectrum of dolichocarpol T (**14**) (CDCl₃, 400 MHz)

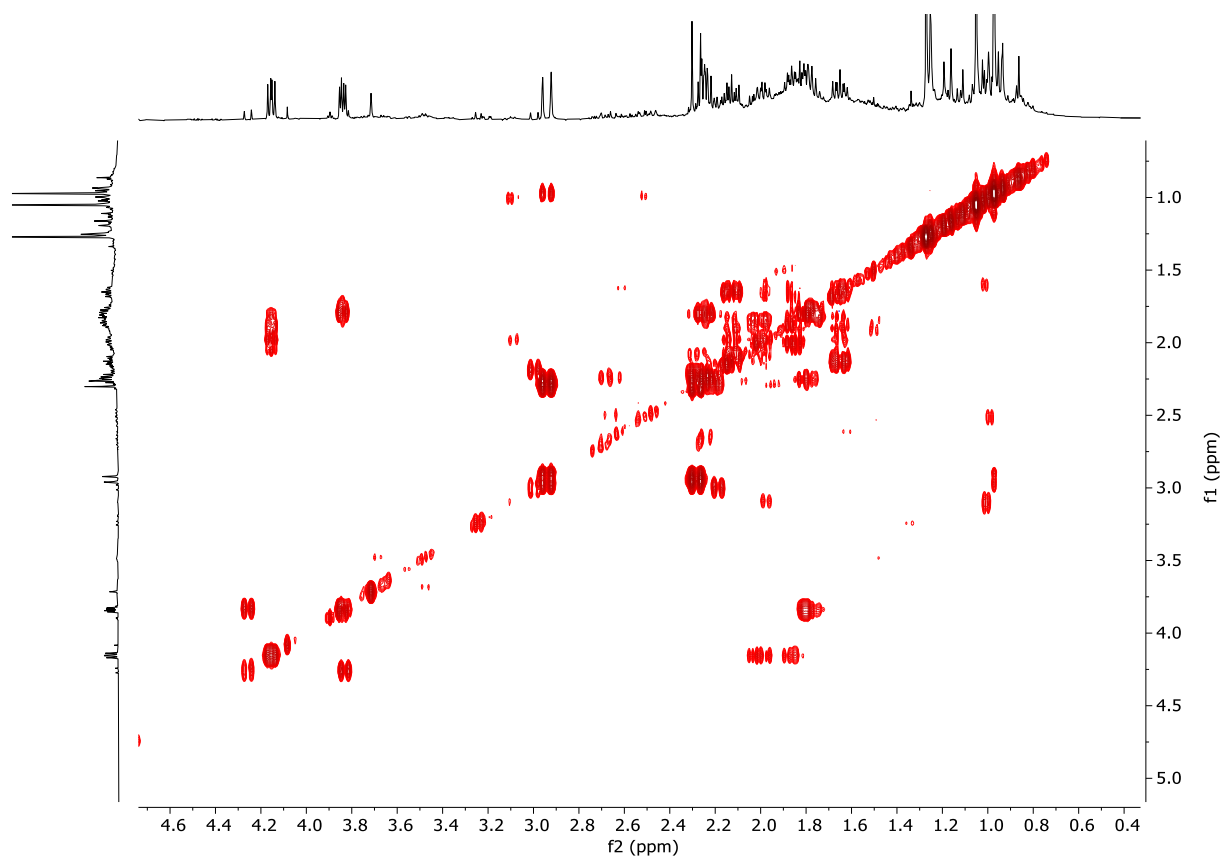


Figure S153. NOESY spectrum of dolichocarpol T (**14**) (CDCl₃, 400 MHz)

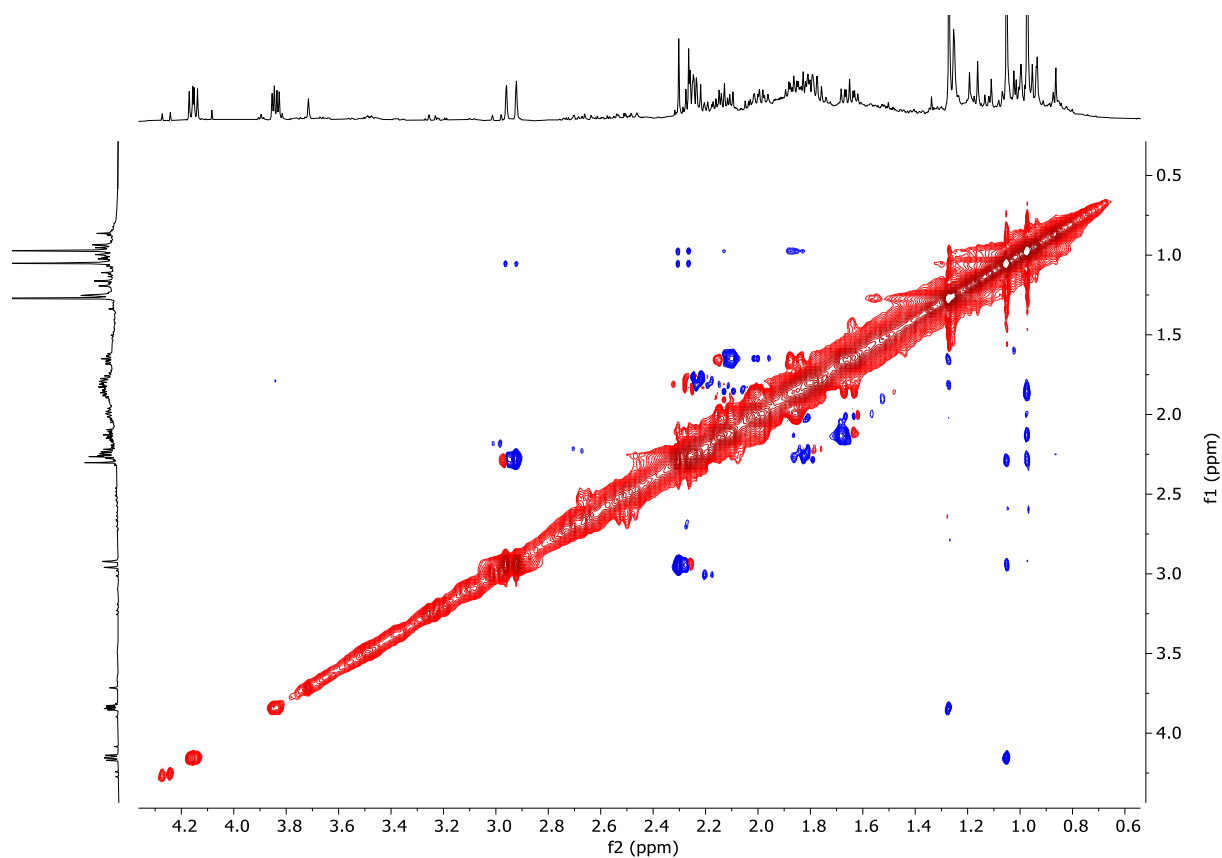


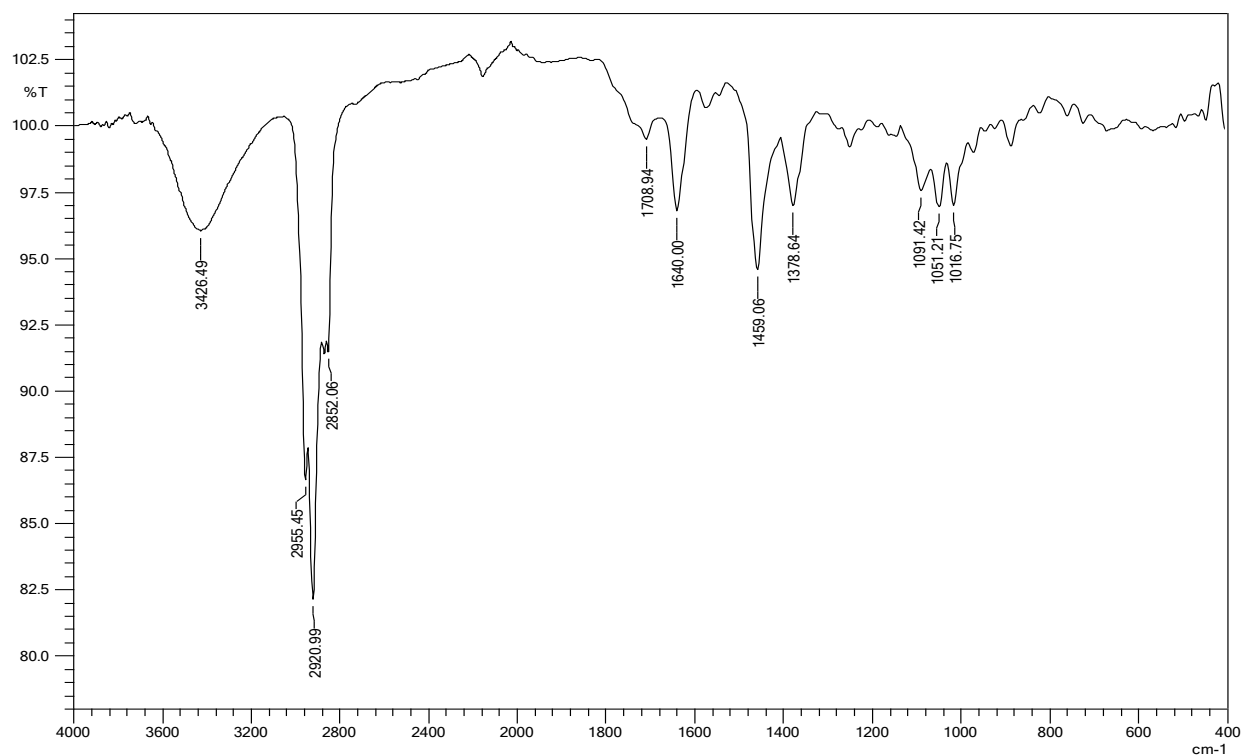
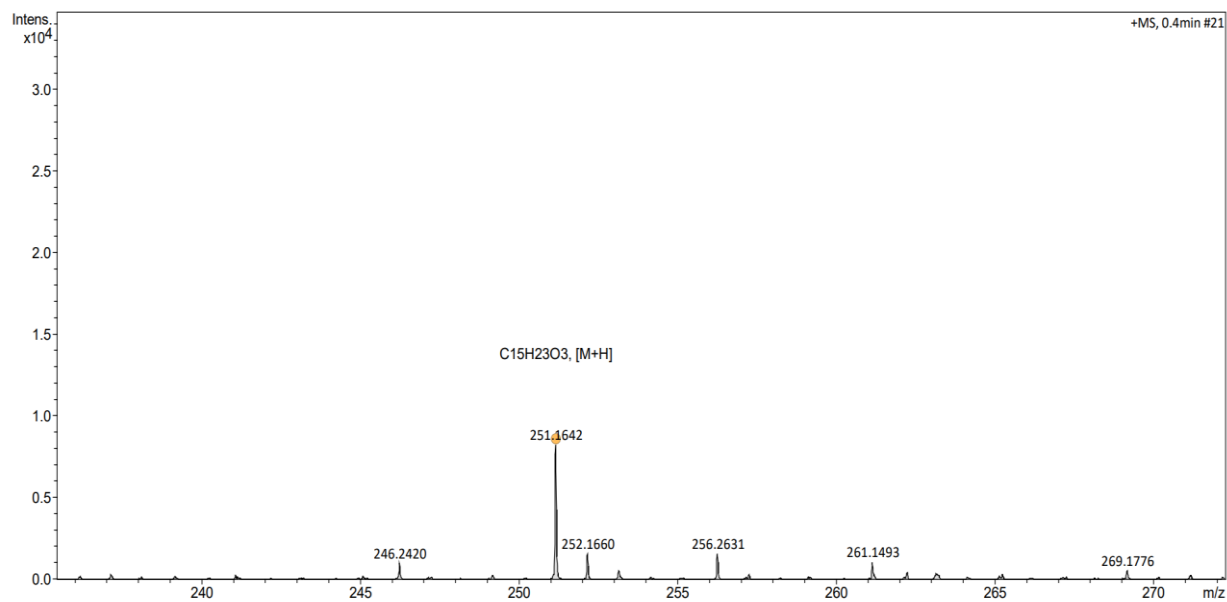
Figure S154. IR spectrum of dolichocarpol U (**15**)**Figure S155.** HRESIMS [M + H]⁺ spectrum of dolichocarpol U (**15**)

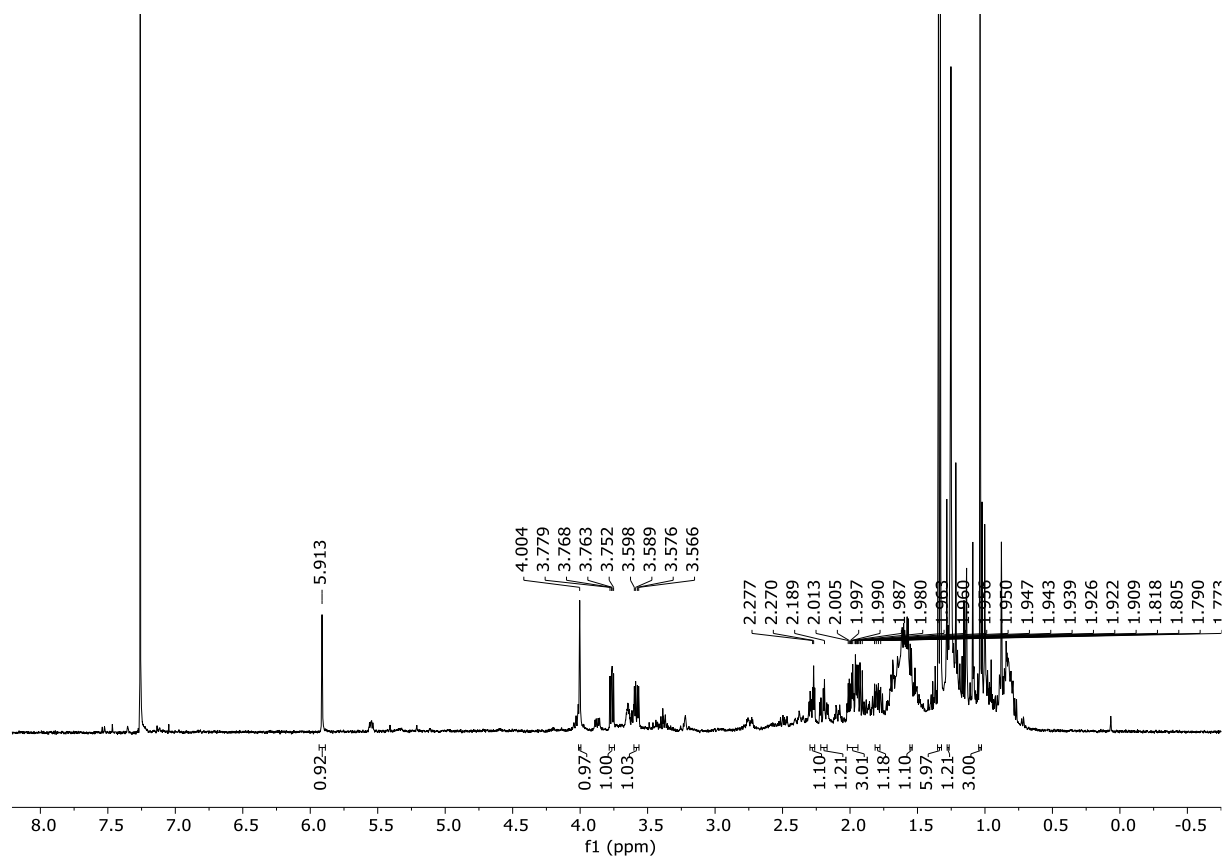
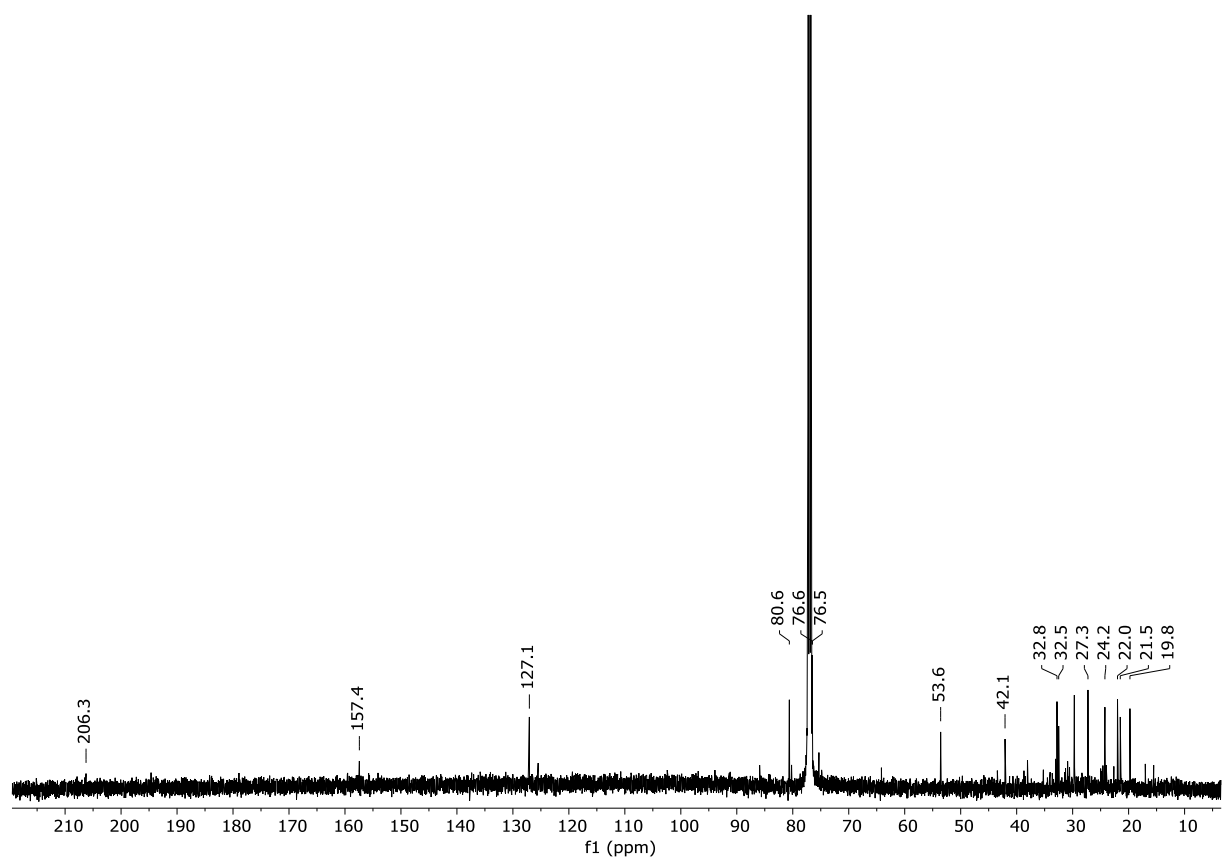
Figure S156. ^1H NMR spectrum of dolichocarpol U (**15**) (CDCl_3 , 500 MHz)**Figure S157.** ^{13}C NMR spectrum of dolichocarpol U (**15**) (CDCl_3 , 125 MHz)

Figure S158. ^{13}C DEPT135 spectrum of dolichocarpol U (**15**) (CDCl_3 , 125 MHz)

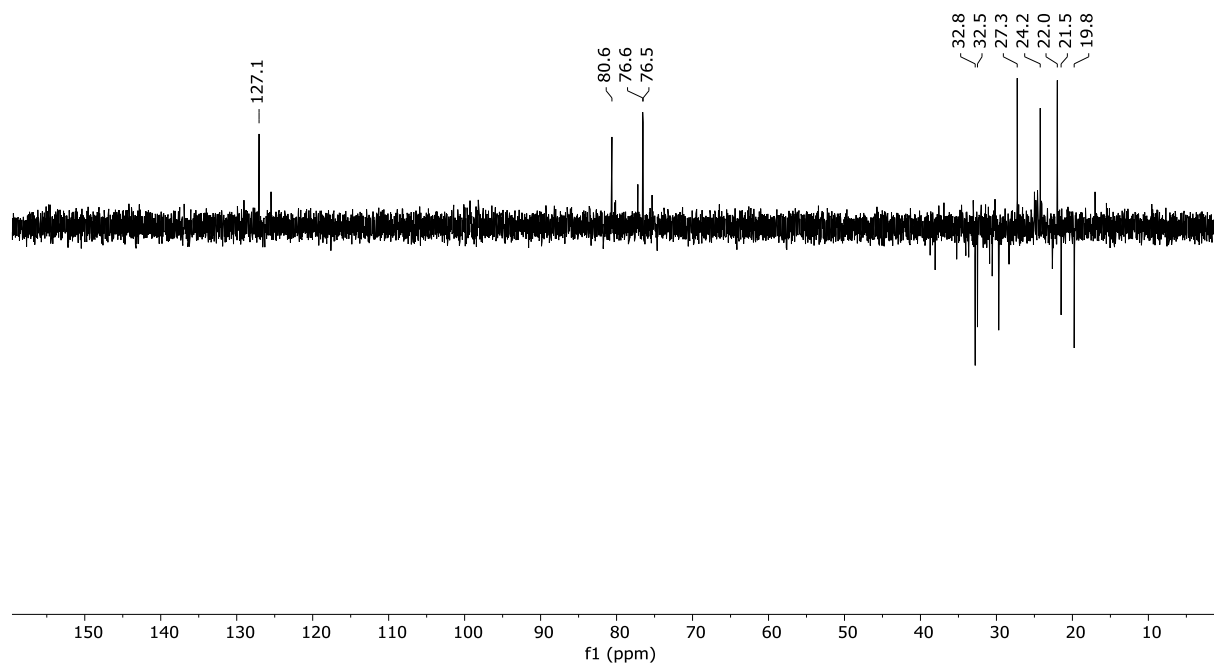


Figure S159. HSQC spectrum of dolichocarpol U (**15**) (CDCl_3 , 500 MHz)

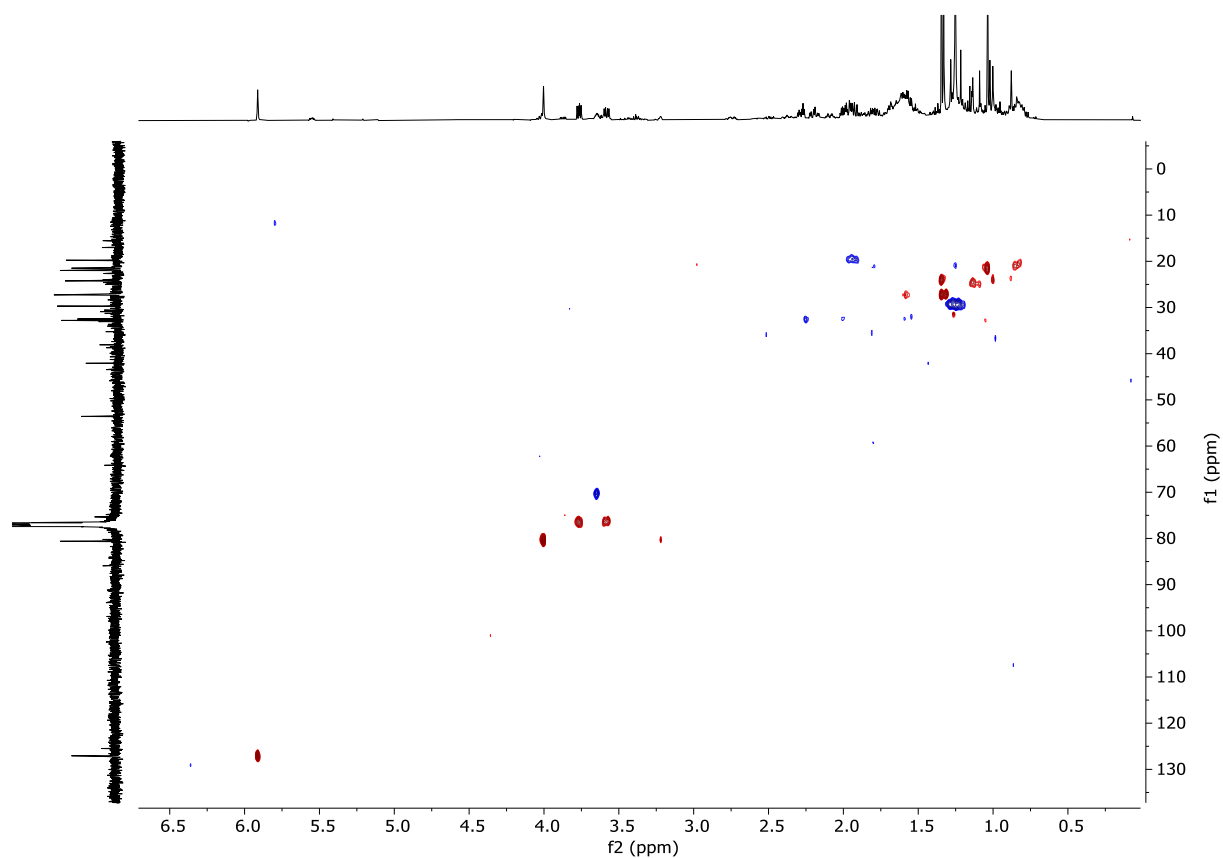


Figure S160. HMBC spectrum of dolichocarpol U (**15**) (CDCl₃, 500 MHz)

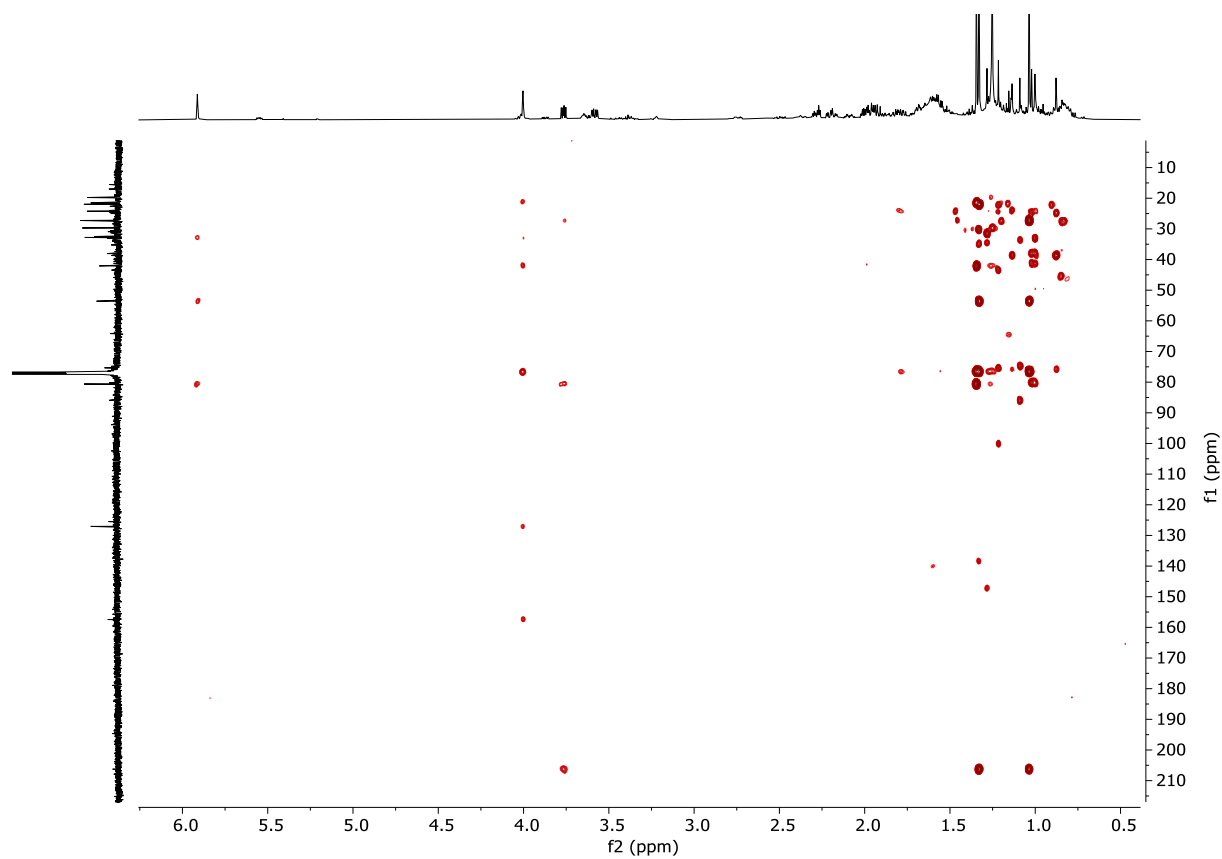


Figure S161. COSY spectrum of dolichocarpol U (**15**) (CDCl₃, 500 MHz)

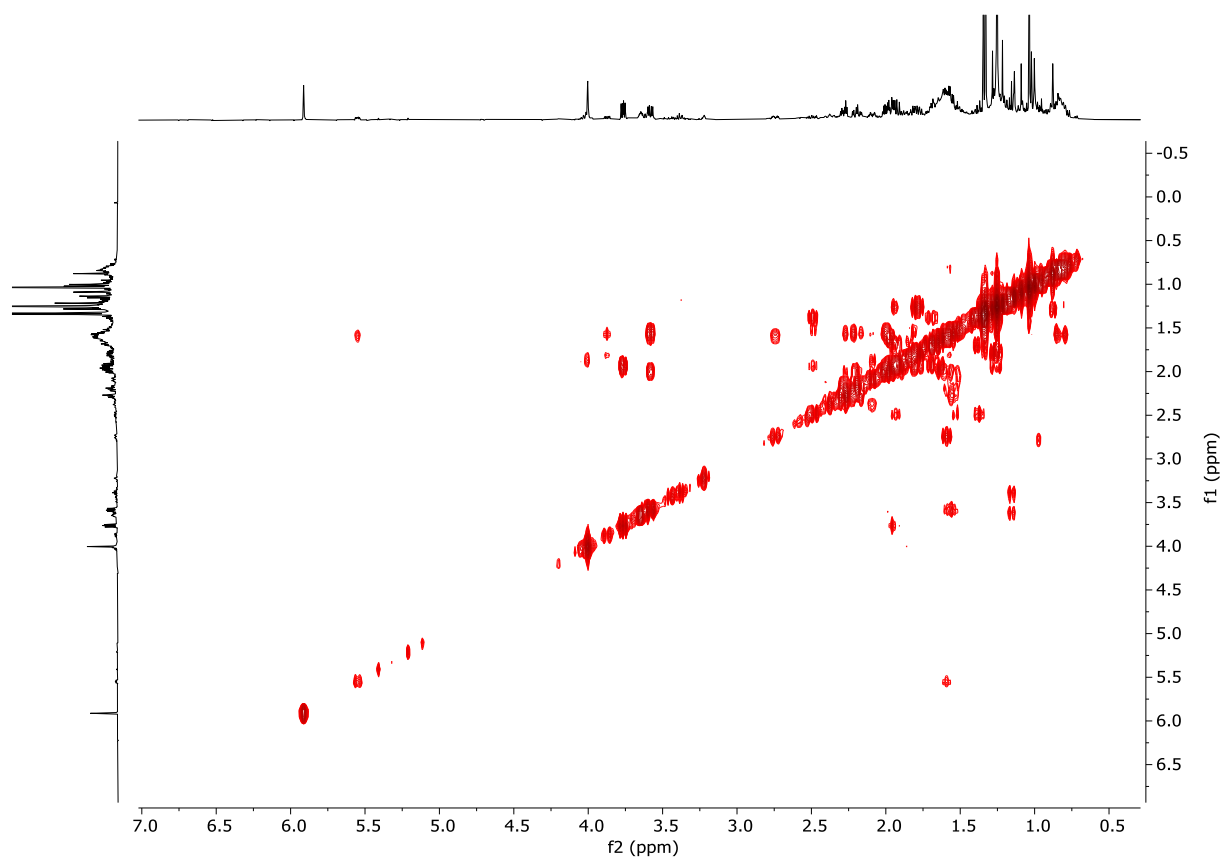


Figure S162. NOESY spectrum of dolichocarpol U (**15**) (CDCl₃, 500 MHz)

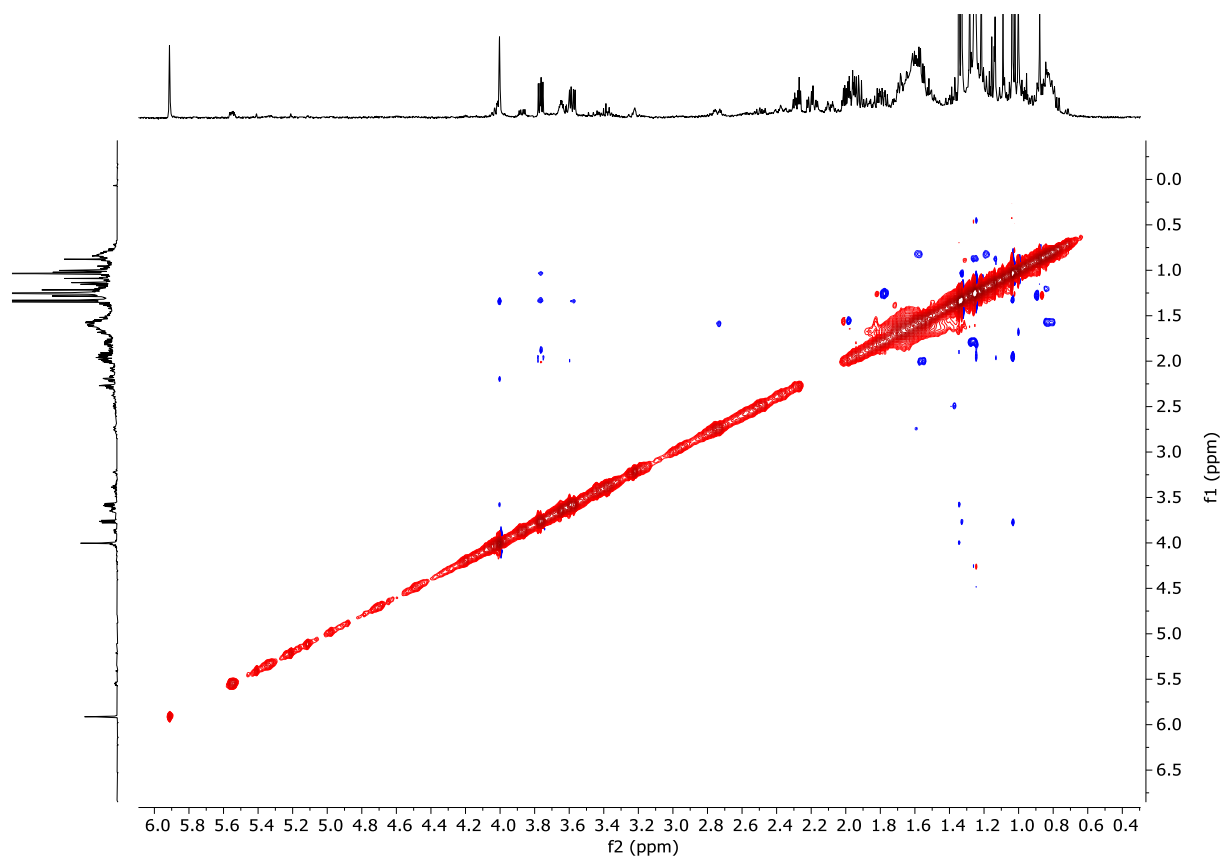


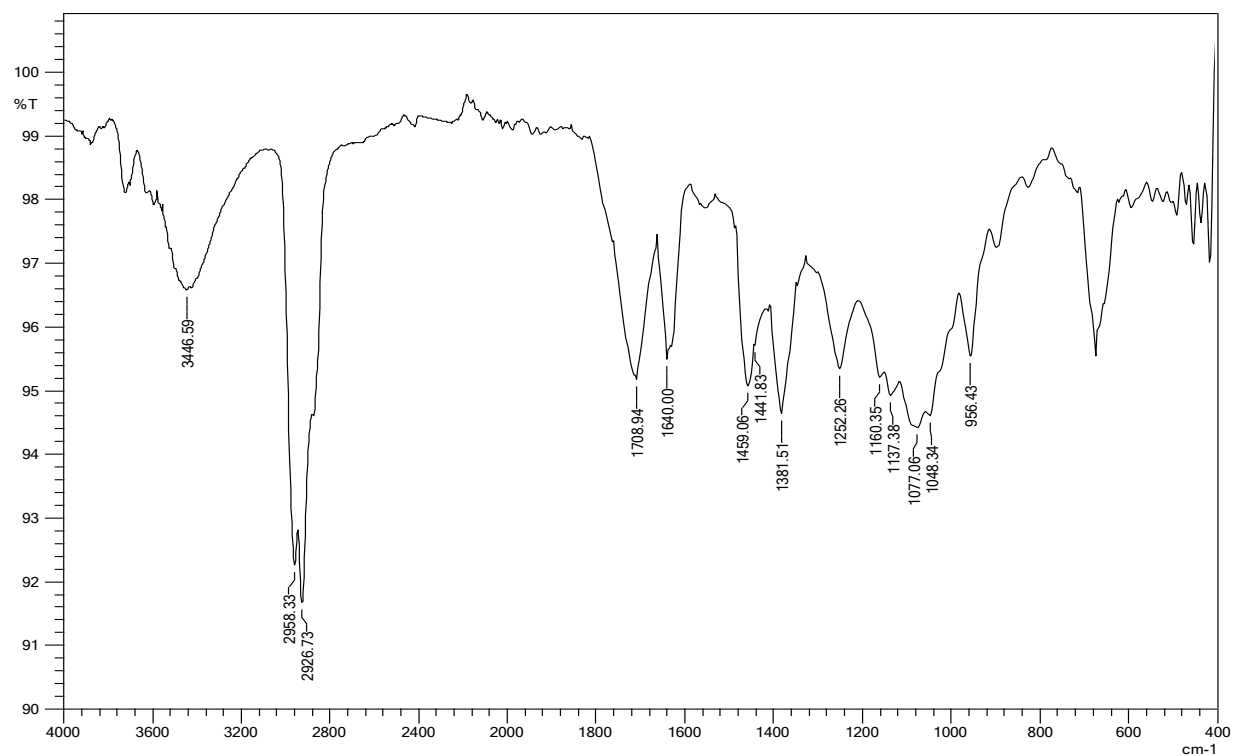
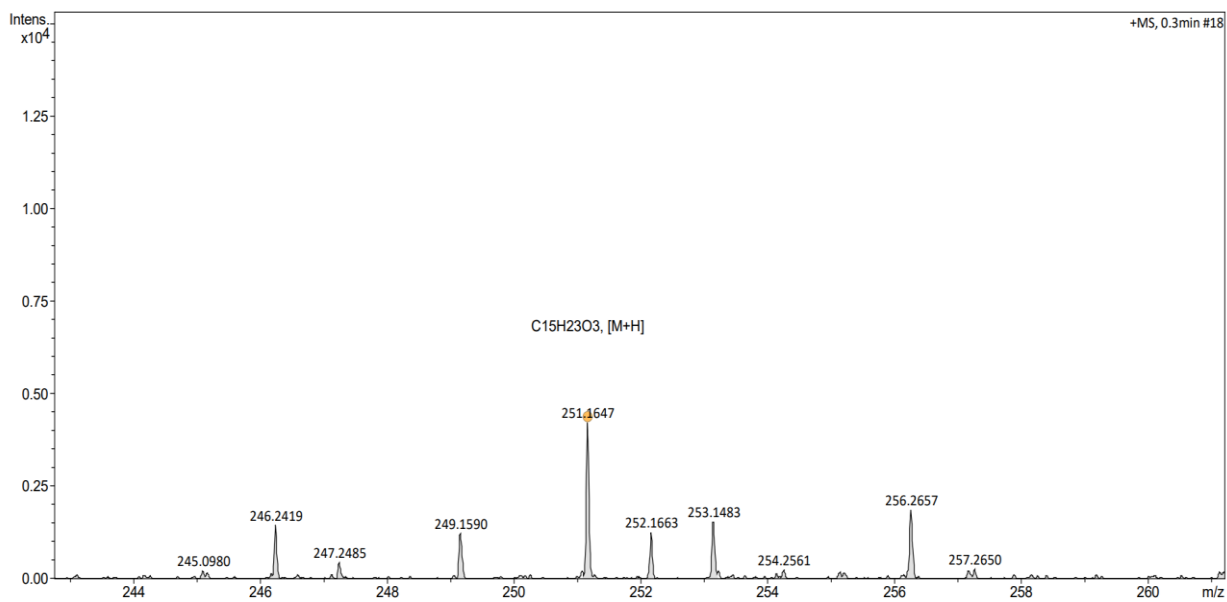
Figure S163. IR spectrum of dolichocarpol V (**16**)**Figure S164.** HRESIMS [M + H]⁺ spectrum of dolichocarpol V (**16**)

Figure S165. ^1H NMR spectrum of dolichocarpol V (**16**) (CDCl_3 , 500 MHz)

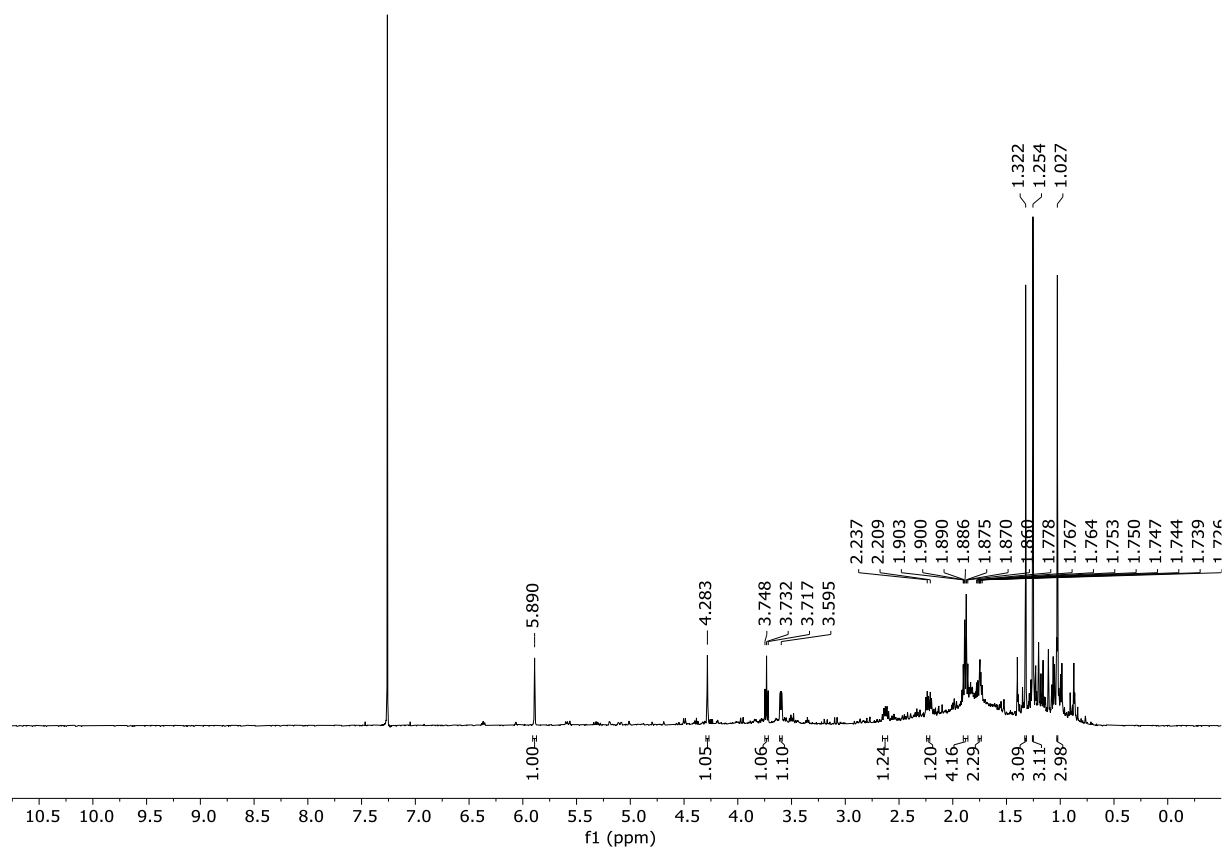


Figure S166. ^{13}C NMR spectrum of dolichocarpol V (**16**) (CDCl_3 , 125 MHz)

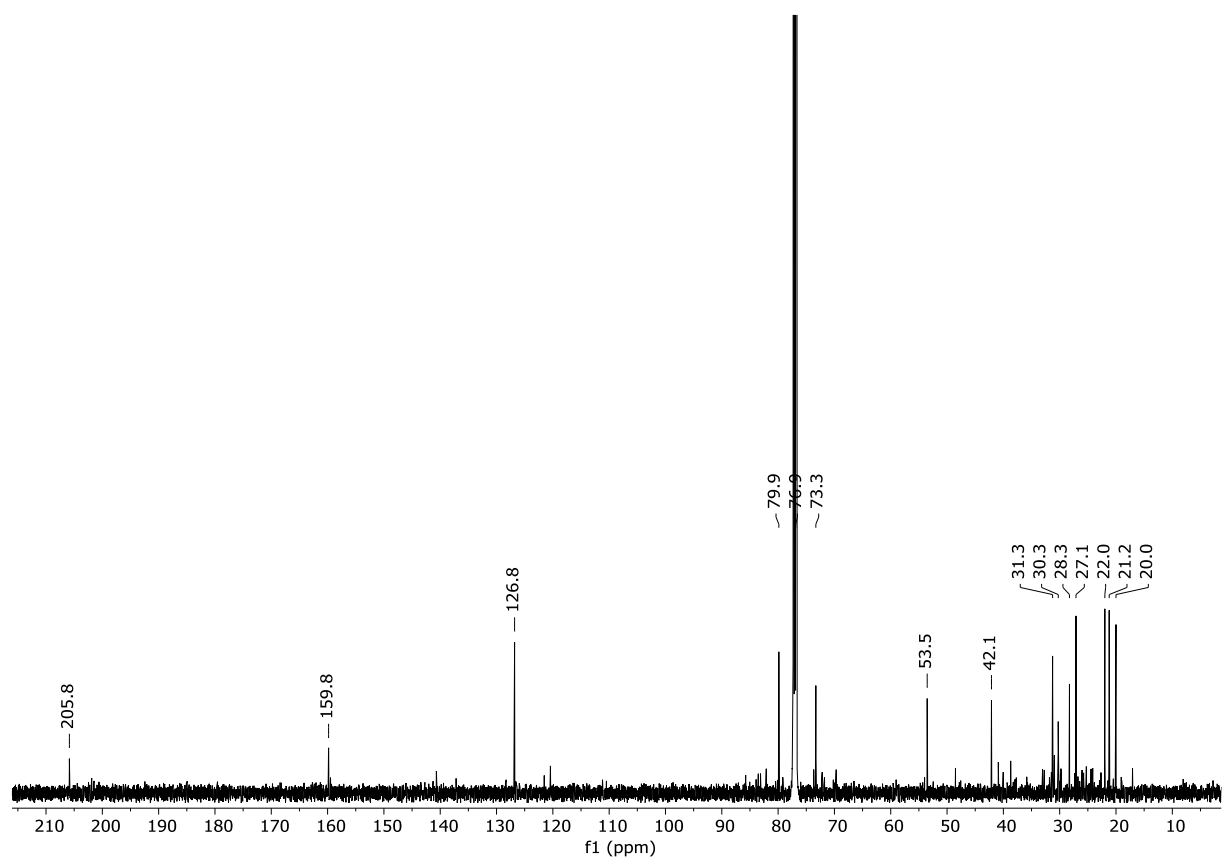


Figure S167. HSQC spectrum of dolichocarpol V (**16**) (CDCl₃, 500 MHz)

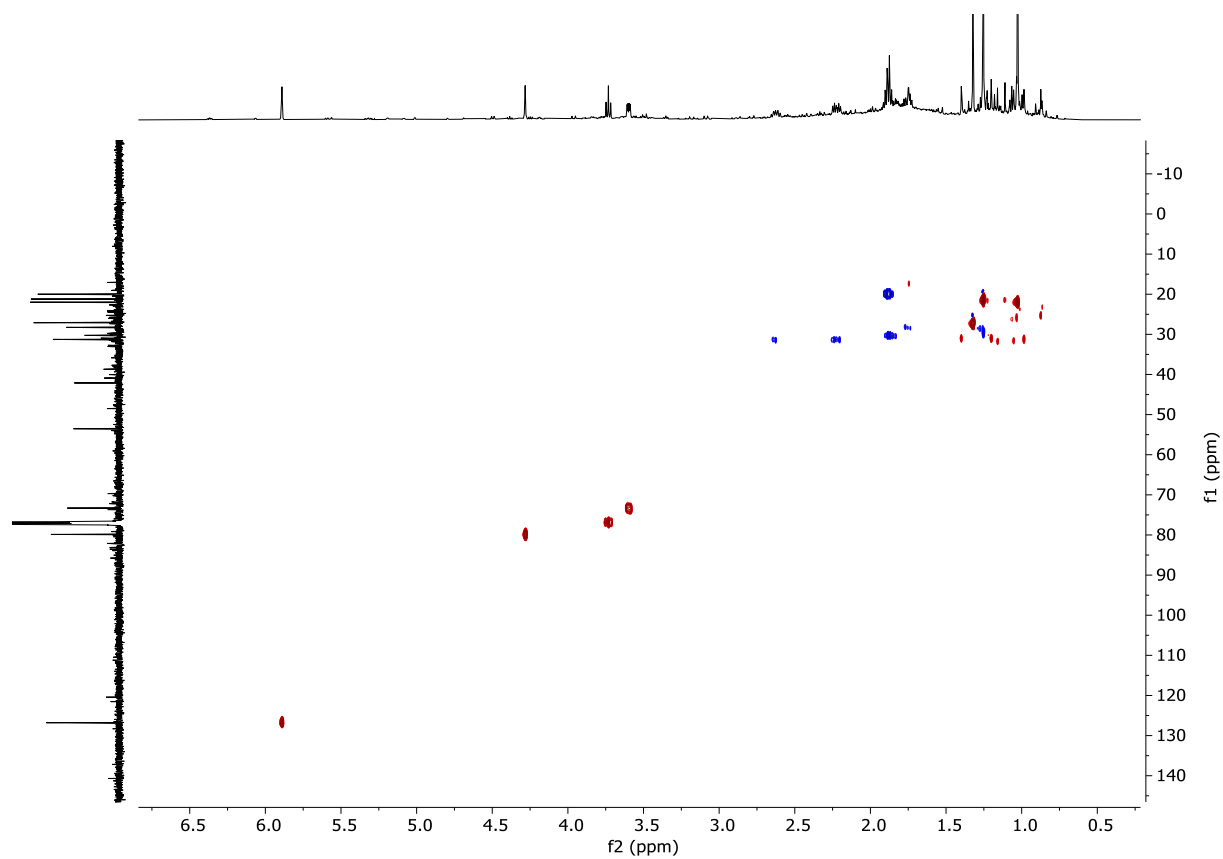


Figure S168. HMBC spectrum of dolichocarpol V (**16**) (CDCl₃, 500 MHz)

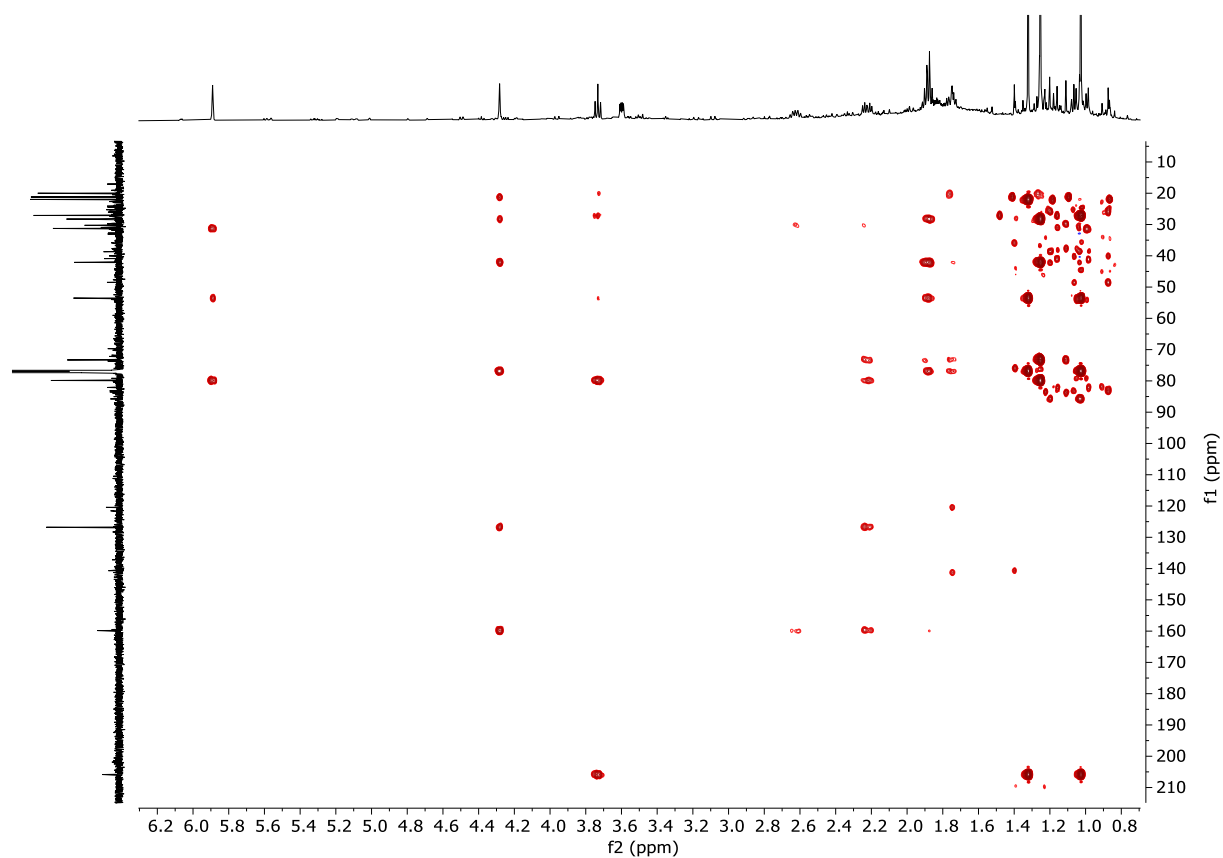


Figure S169. COSY spectrum of dolichocarpol V (**16**) (CDCl₃, 500 MHz)

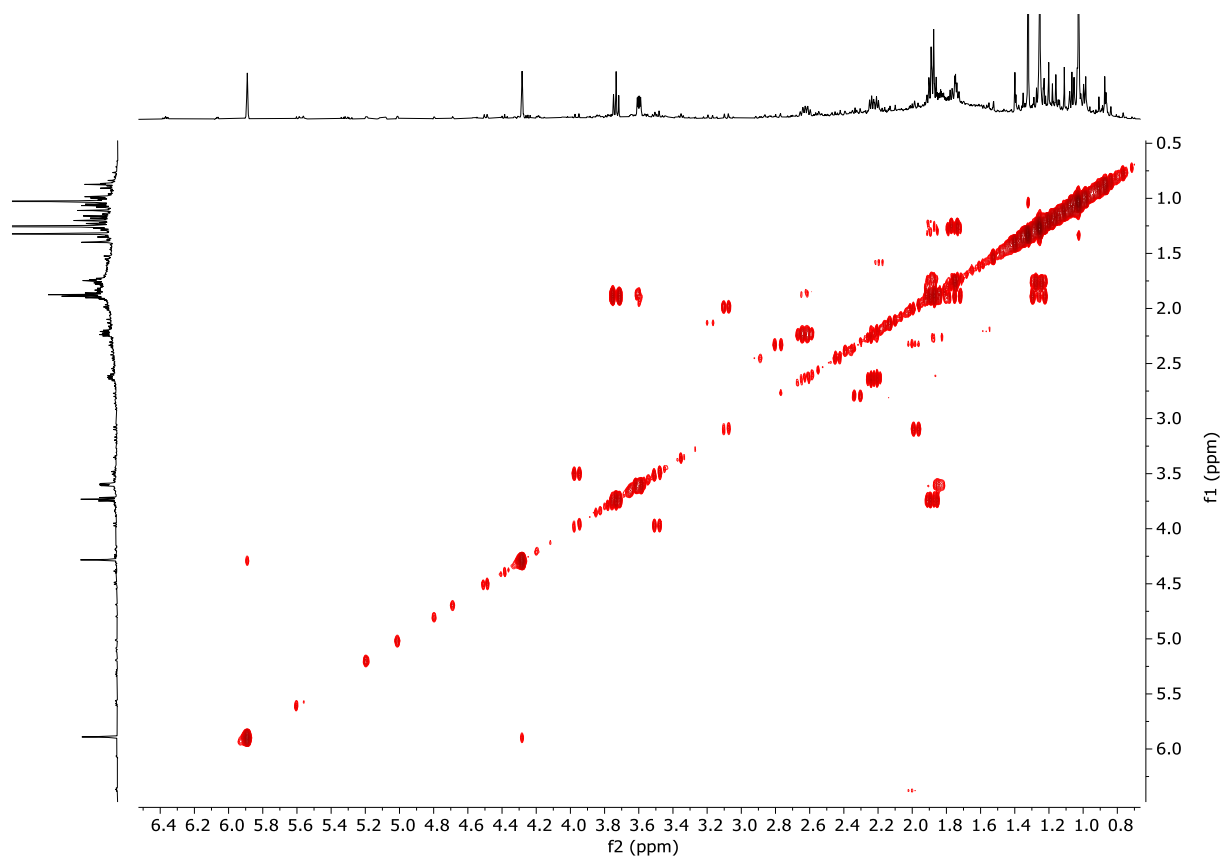


Figure S170. NOESY spectrum of dolichocarpol V (**16**) (CDCl₃, 500 MHz)

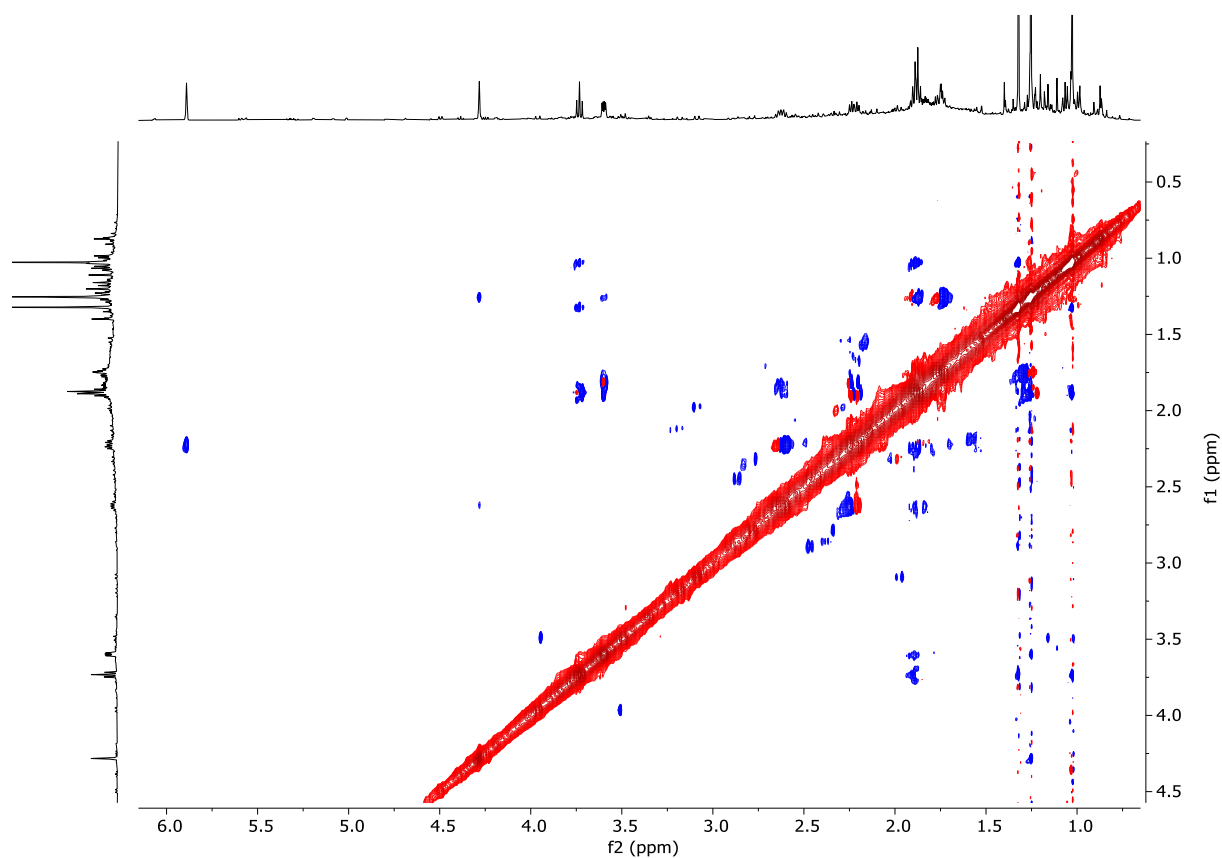


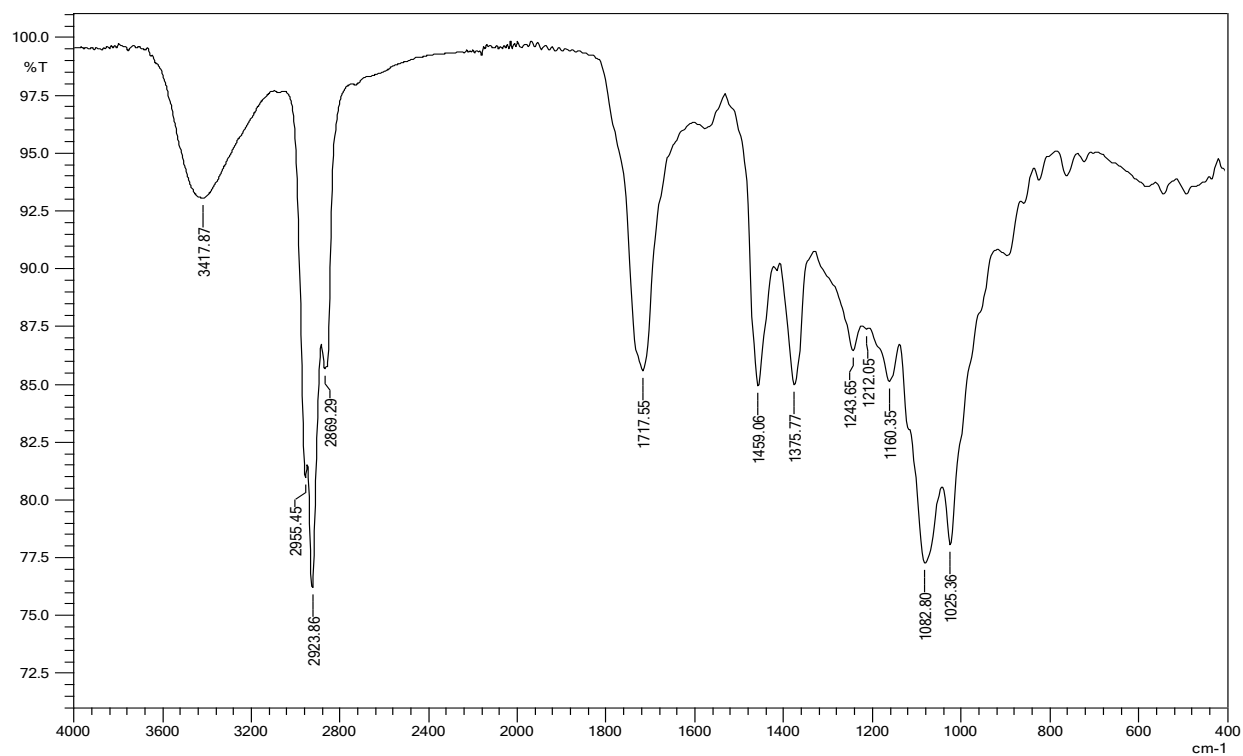
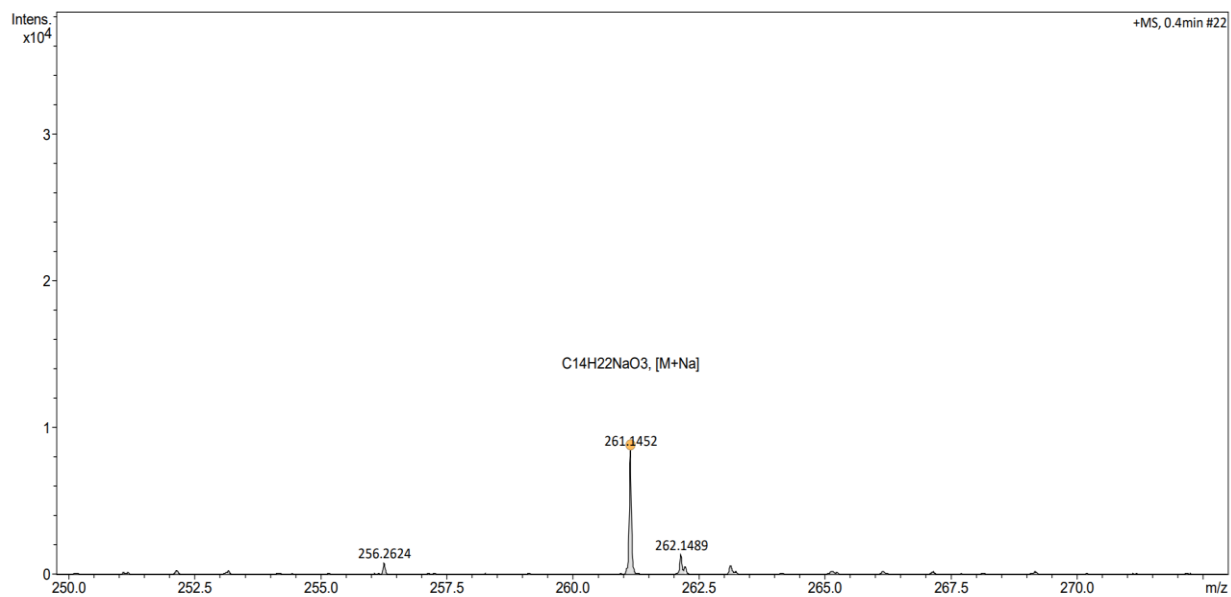
Figure S171. IR spectrum of dolichocarpol W (**17**)**Figure S172.** HRESIMS $[\text{M} + \text{Na}]^+$ spectrum of dolichocarpol W (**17**)

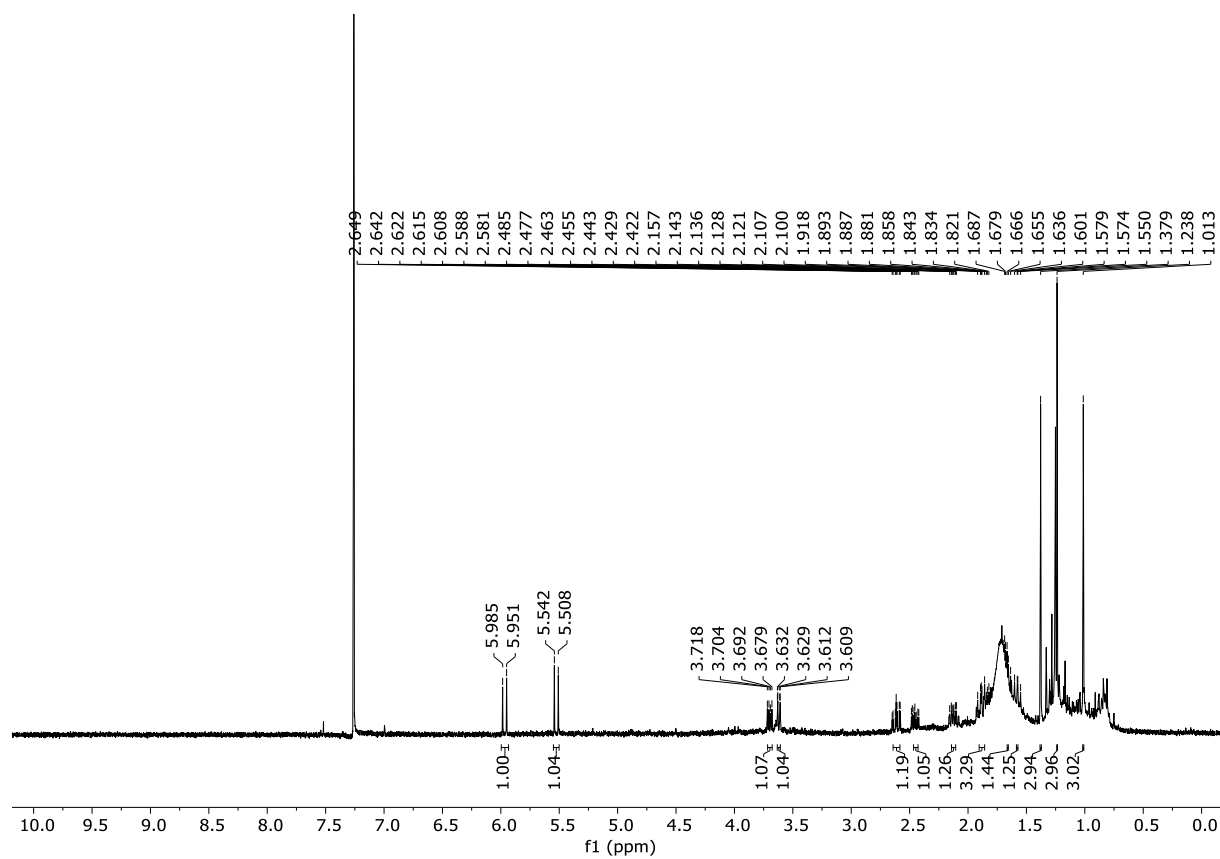
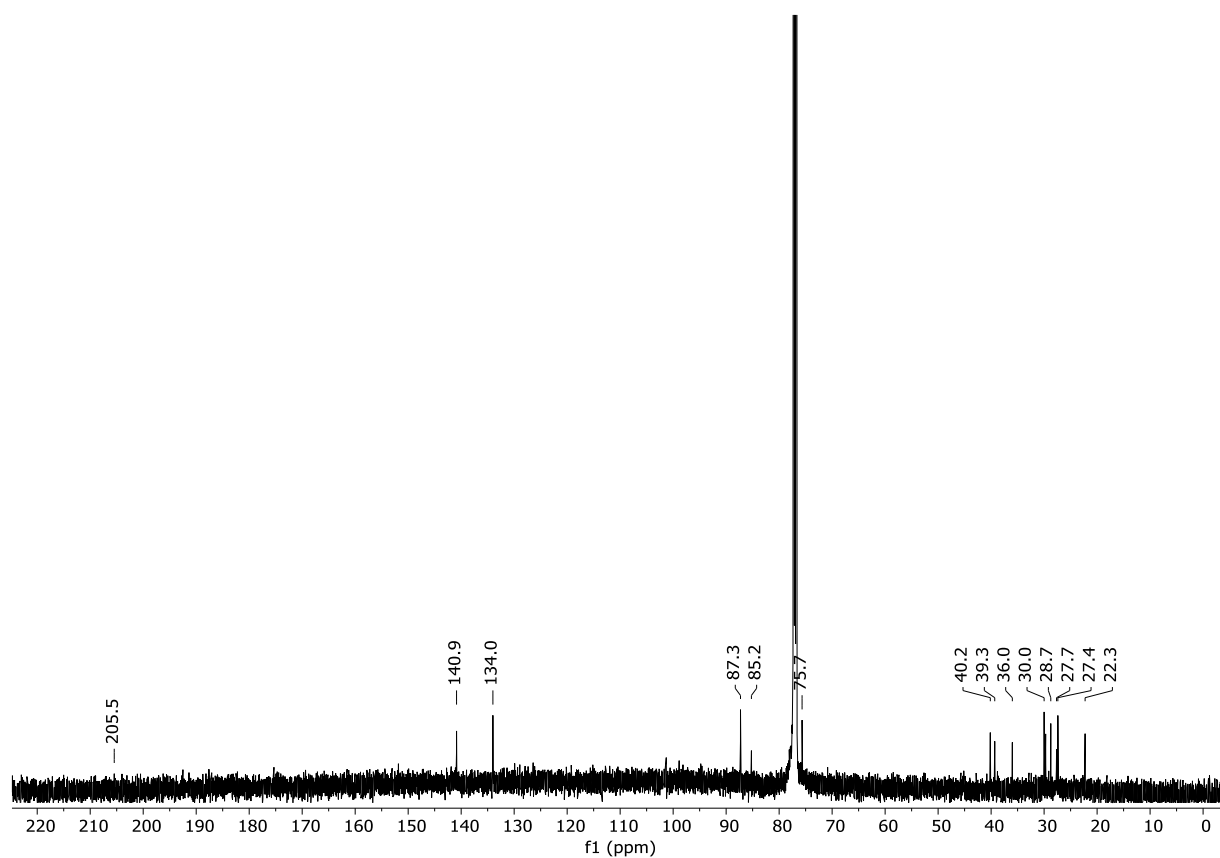
Figure S173. ^1H NMR spectrum of dolichocarpol W (**17**) (CDCl_3 , 400 MHz)**Figure S174.** ^{13}C NMR spectrum of dolichocarpol W (**17**) (CDCl_3 , 125 MHz)

Figure S175. ^{13}C DEPT135 spectrum of dolichocarpol W (**17**) (CDCl_3 , 125 MHz)

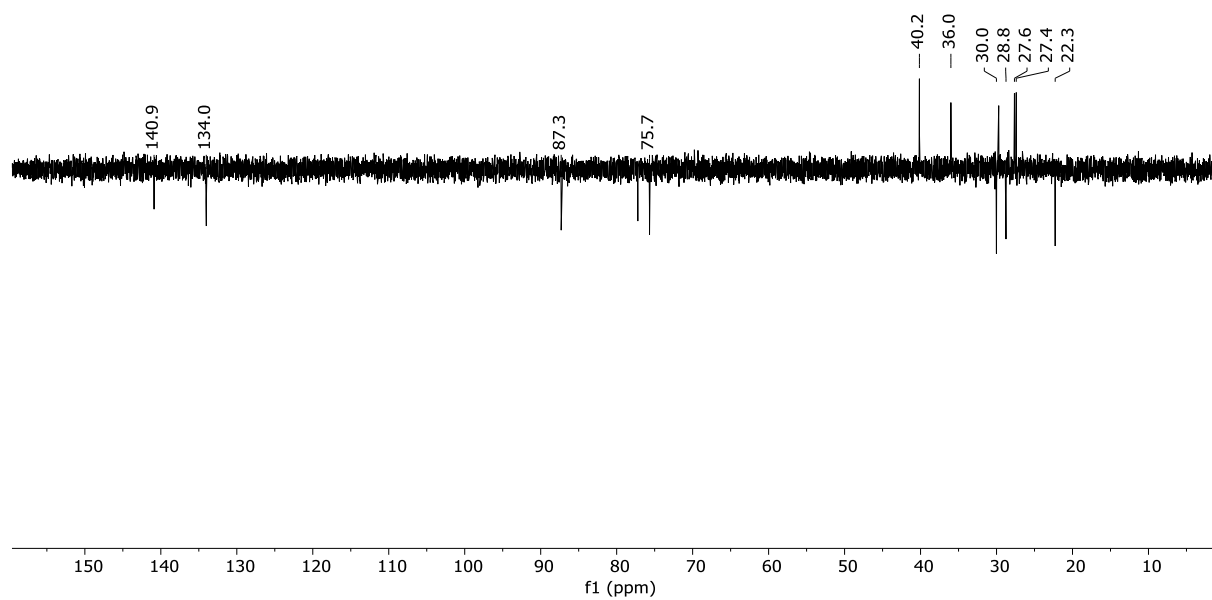


Figure S176. HSQC spectrum of dolichocarpol W (**17**) (CDCl_3 , 400 MHz)

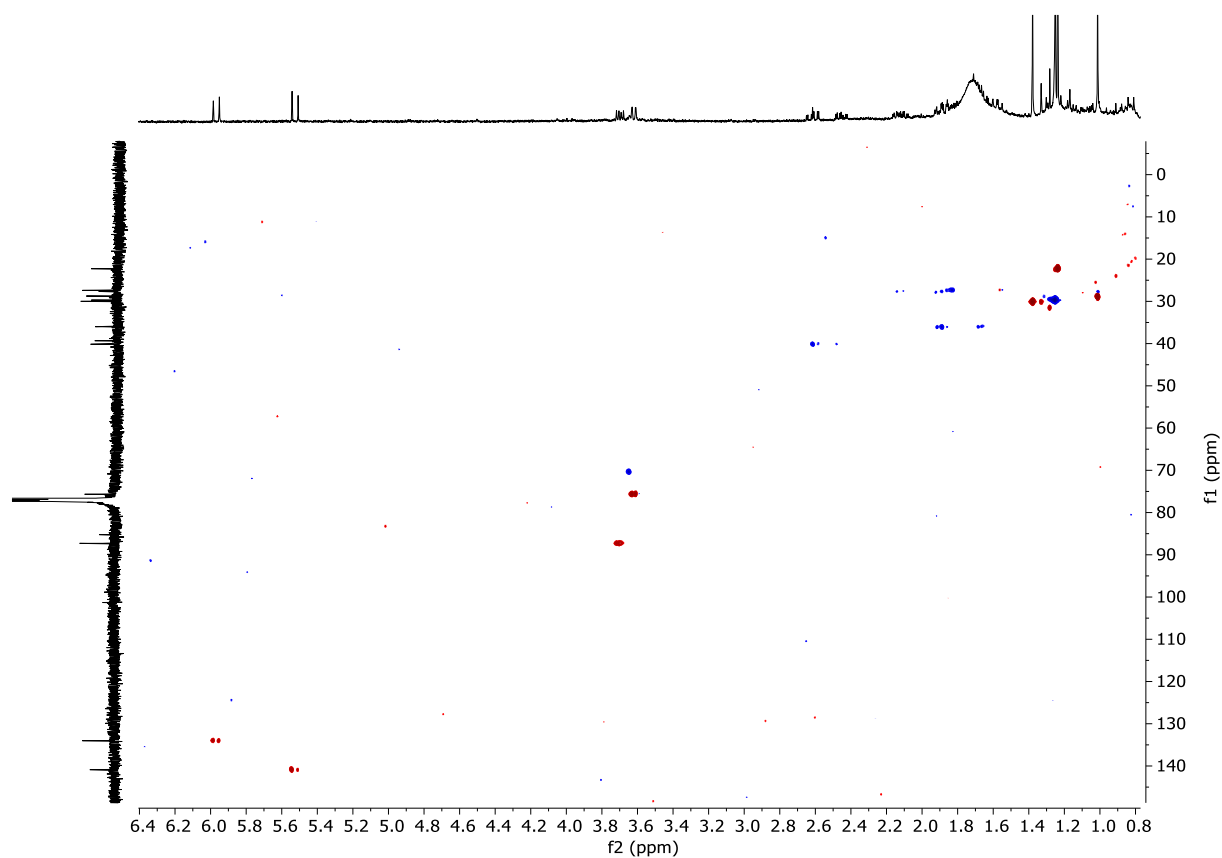


Figure S177. HMBC spectrum of dolichocarpol W (**17**) (CDCl₃, 400 MHz)

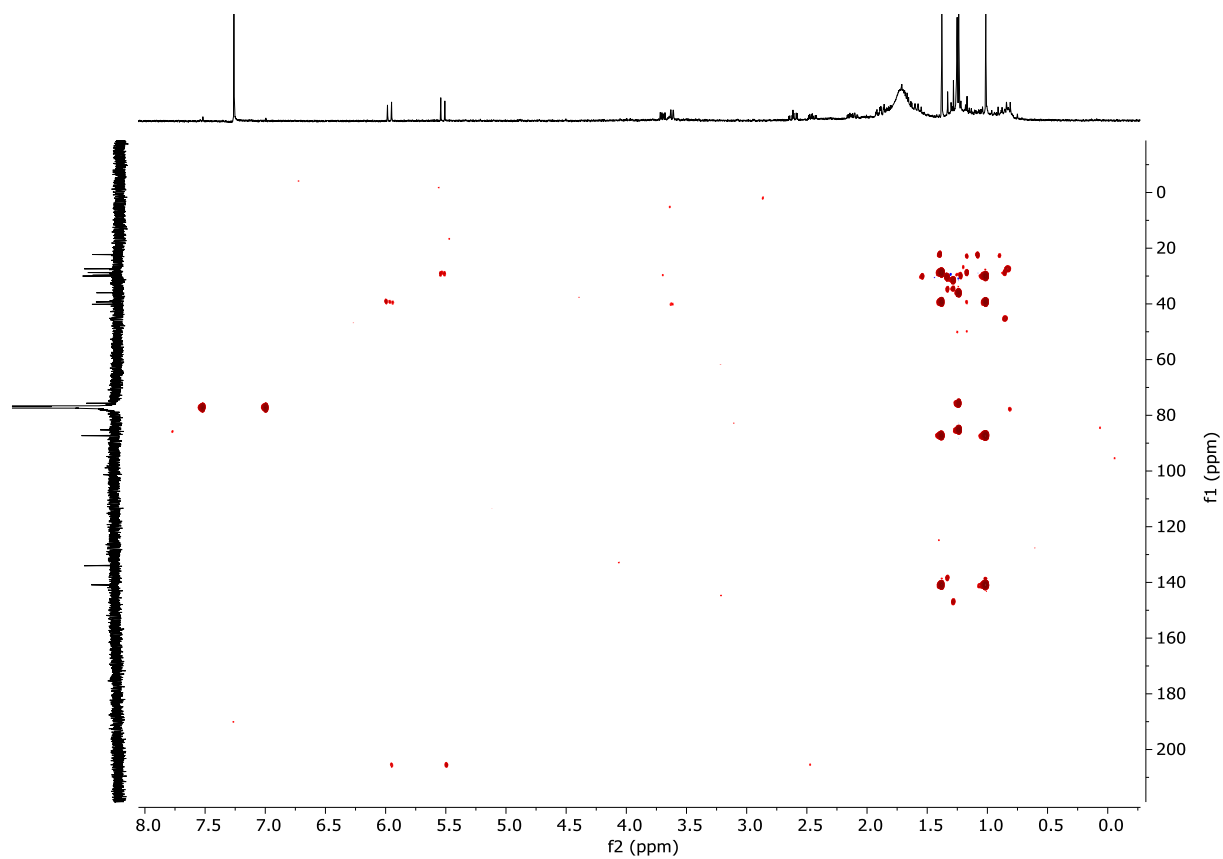


Figure S178. COSY spectrum of dolichocarpol W (**17**) (CDCl₃, 400 MHz)

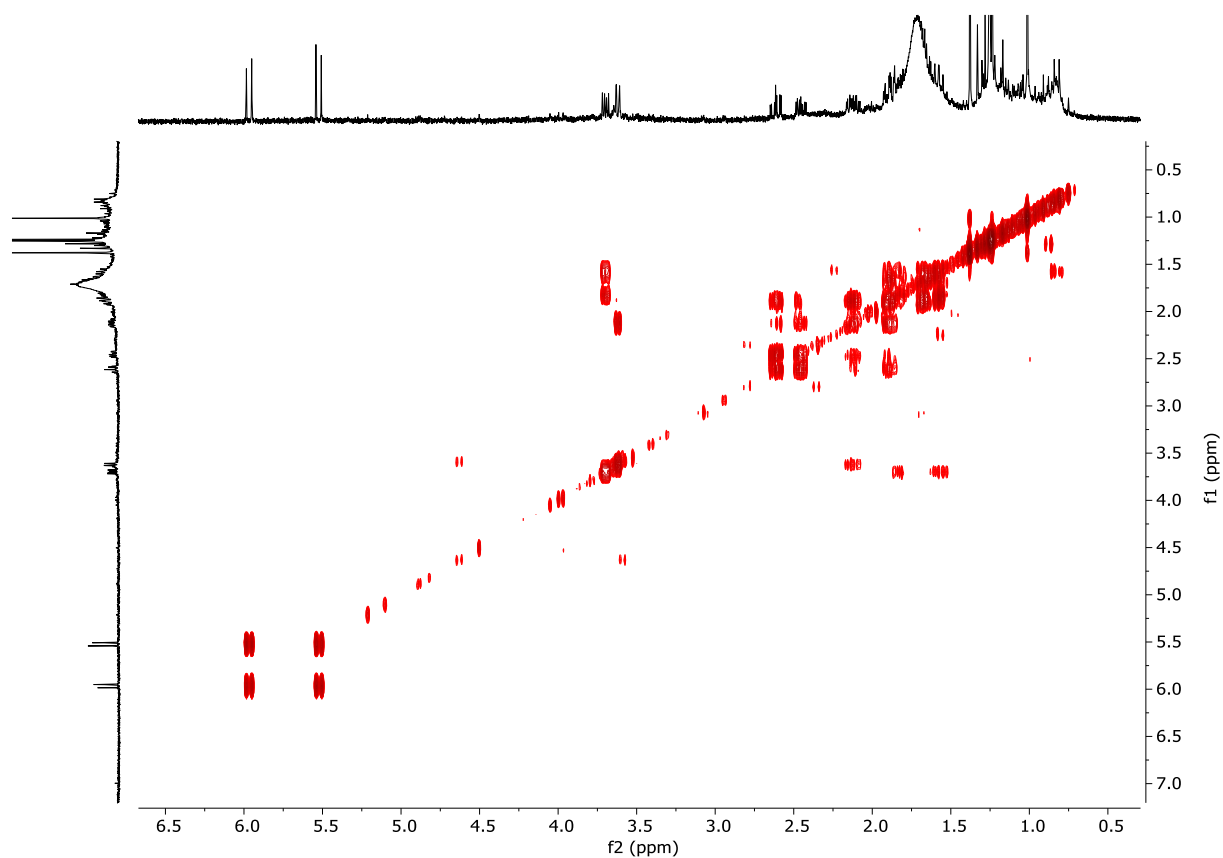


Figure S179. NOESY spectrum of dolichocarpol W (**17**) (CDCl₃, 400 MHz)

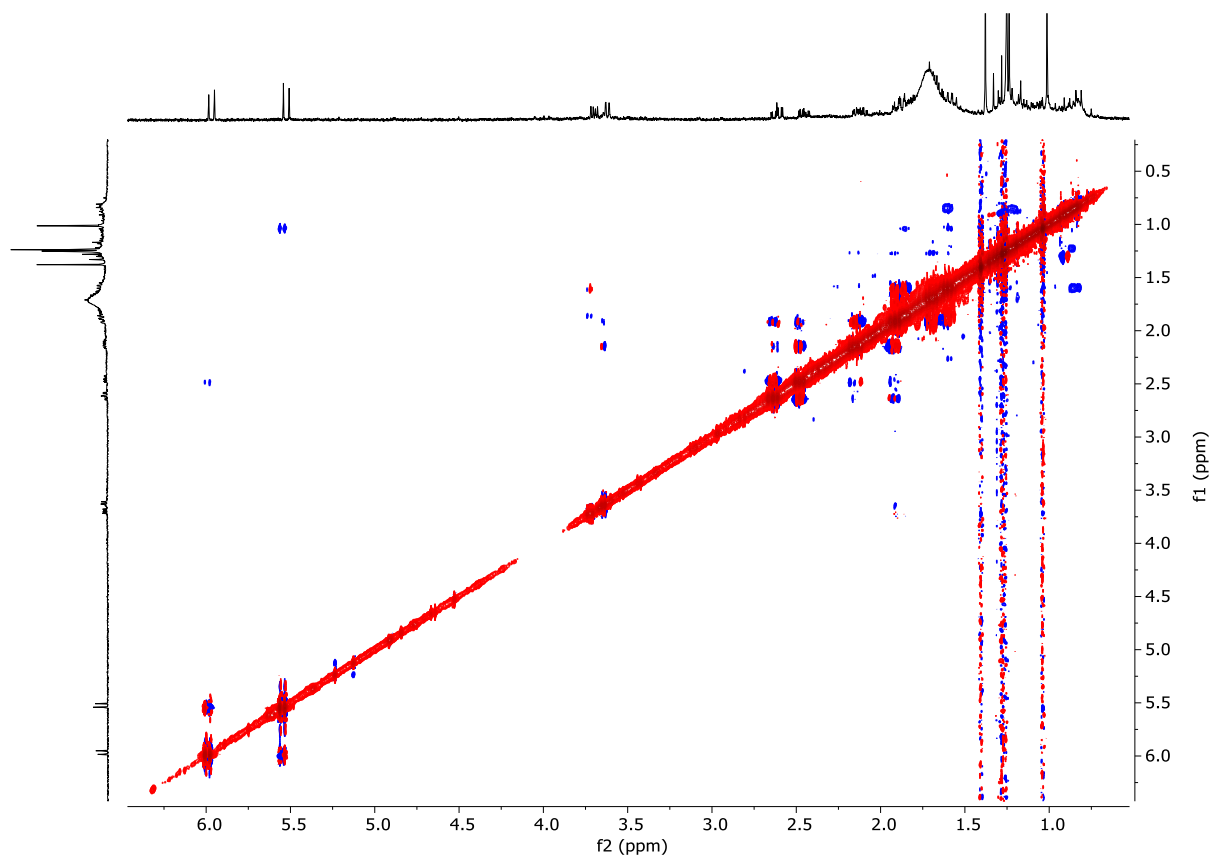


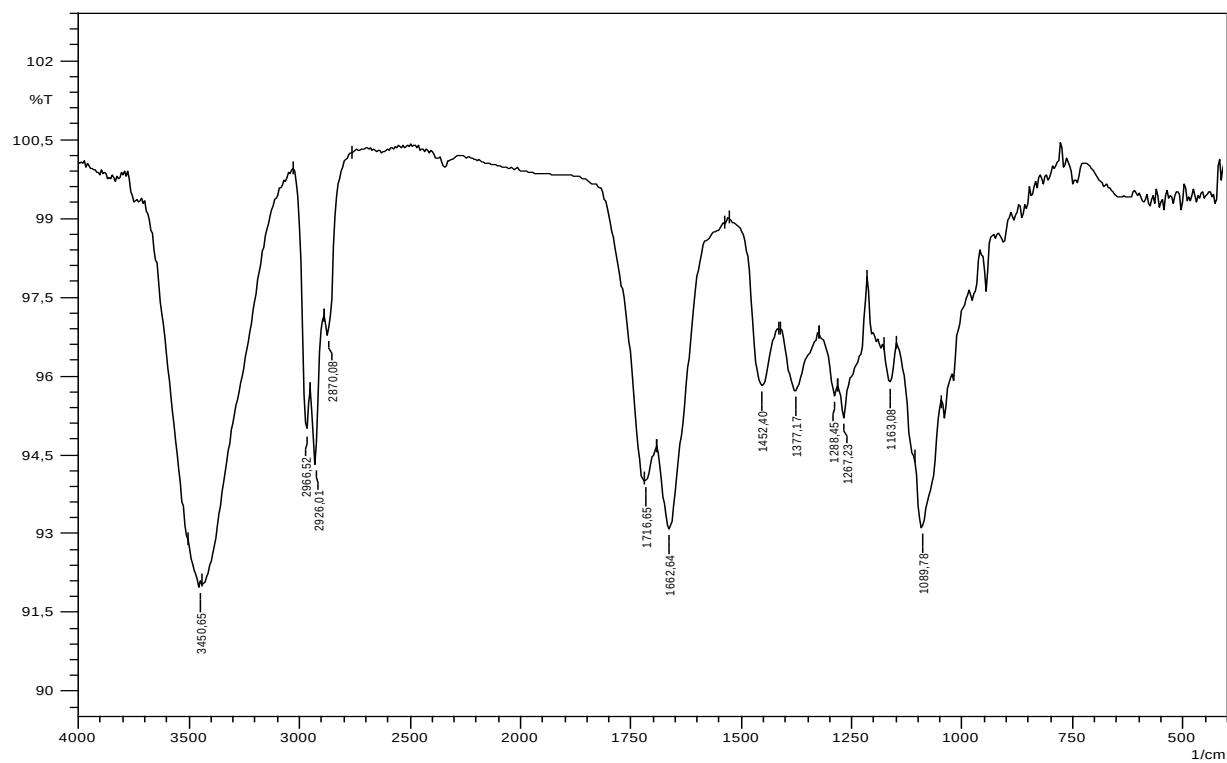
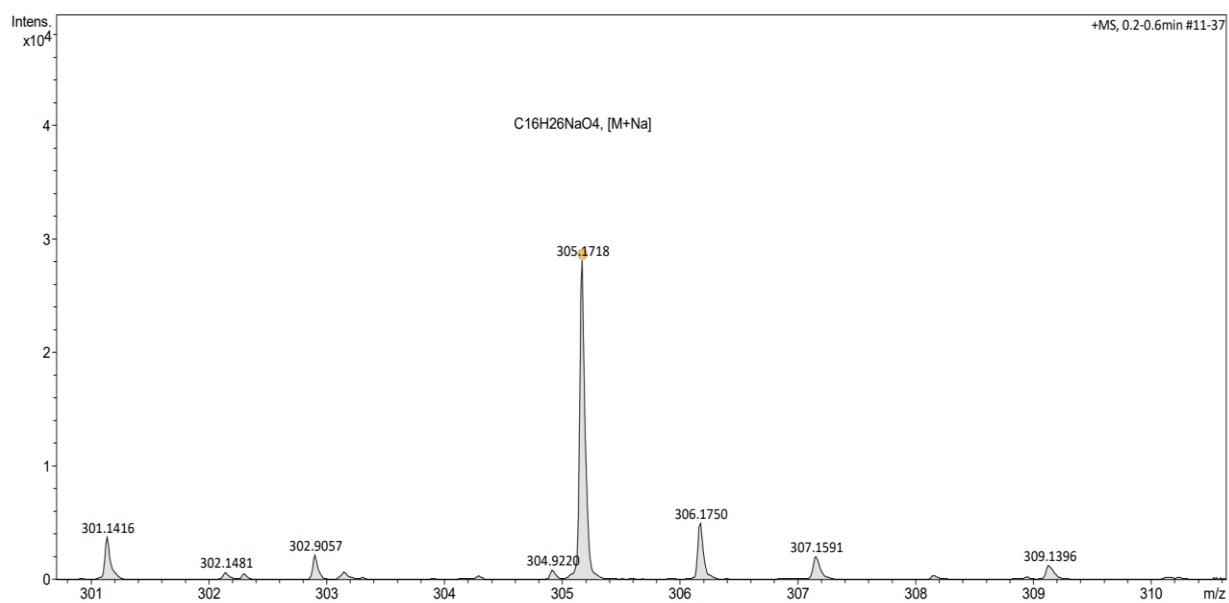
Figure S180. IR spectrum of dolichocarpol X (**18**)**Figure S181.** HRESIMS $[\text{M} + \text{Na}]^+$ spectrum of dolichocarpol X (**18**)

Figure S182. ^1H NMR spectrum of dolichocarpol X (**18**) (CDCl_3 , 500 MHz)

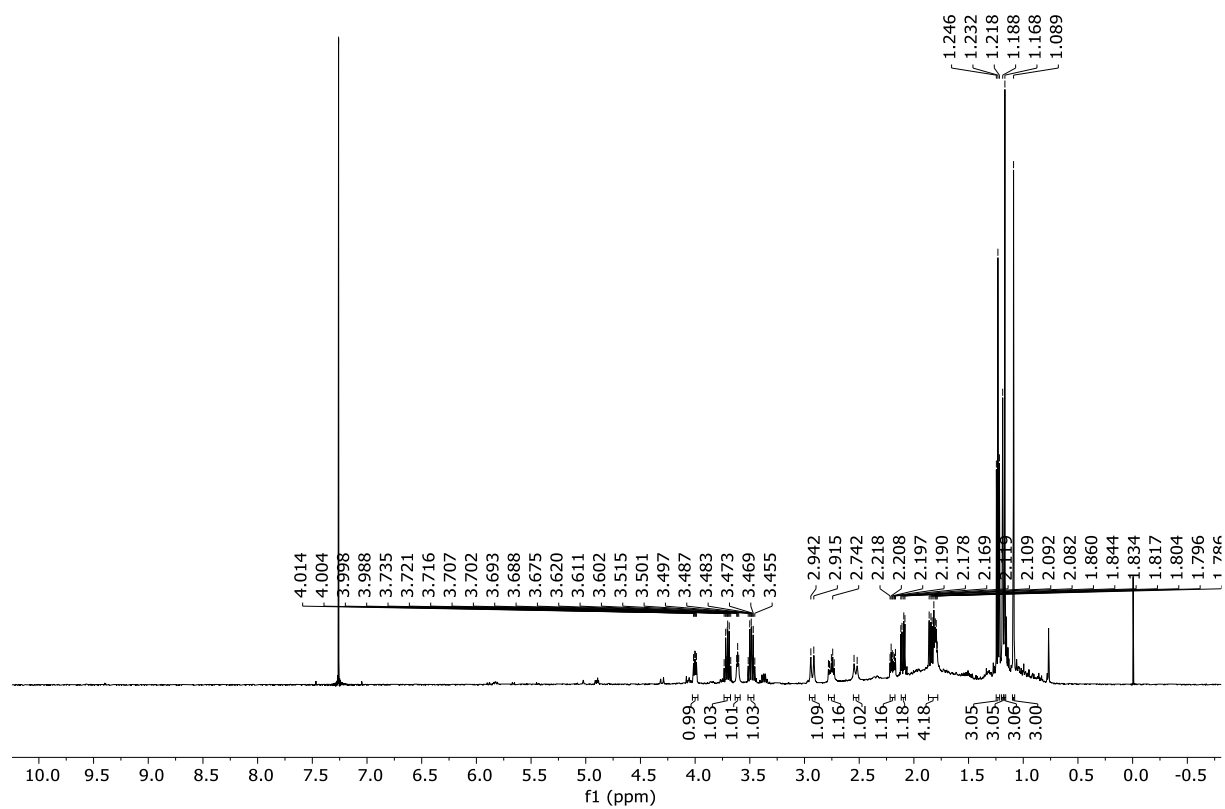


Figure S183. ^{13}C NMR spectrum of dolichocarpol X (**18**) (CDCl_3 , 125 MHz)

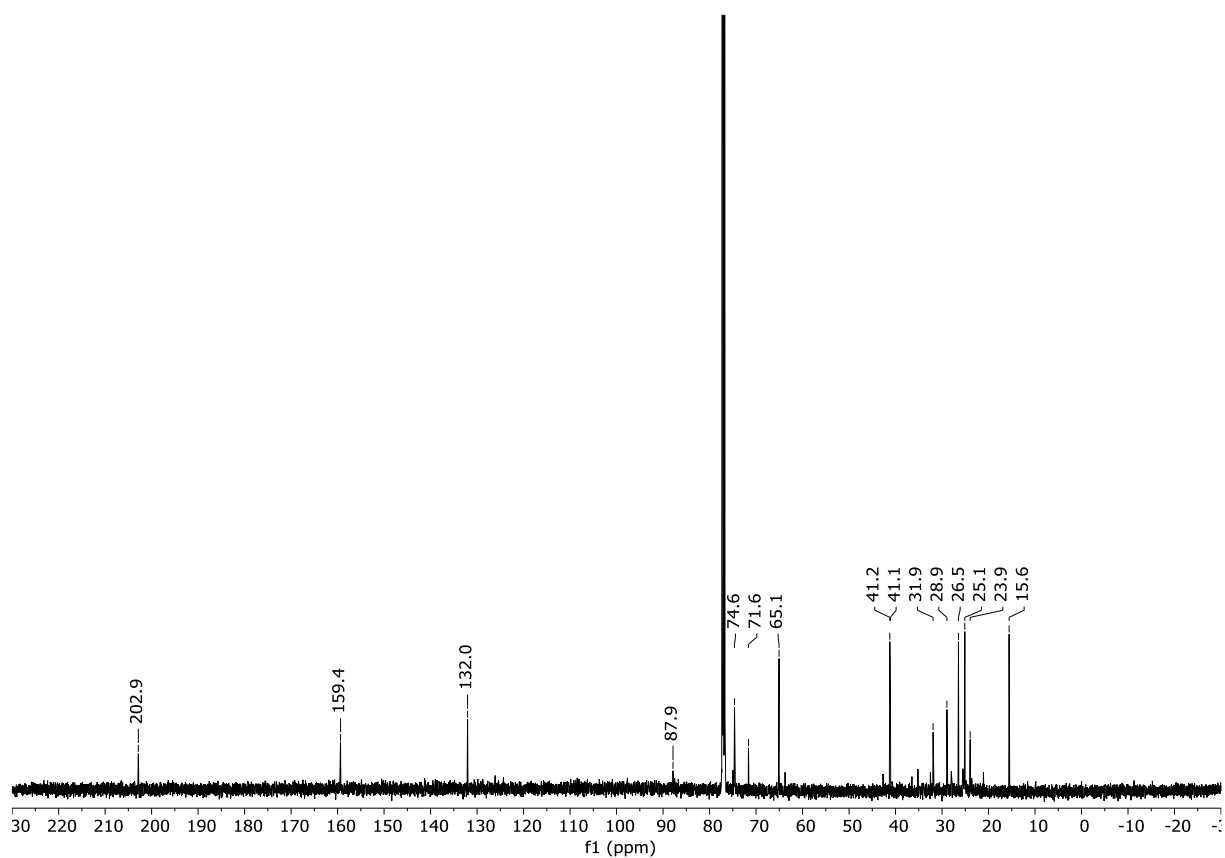


Figure S184. ^{13}C DEPT135 spectrum of dolichocarpol X (**18**) (CDCl_3 , 125 MHz)

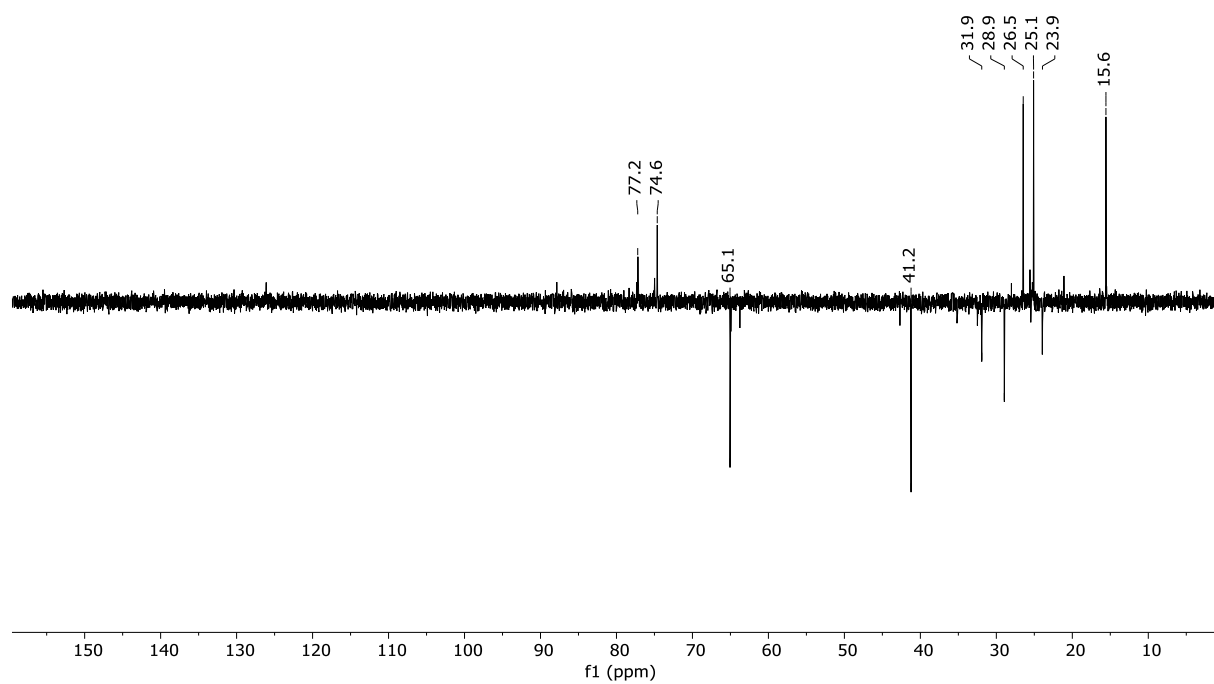


Figure S185. HSQC spectrum of dolichocarpol X (**18**) (CDCl_3 , 500 MHz)

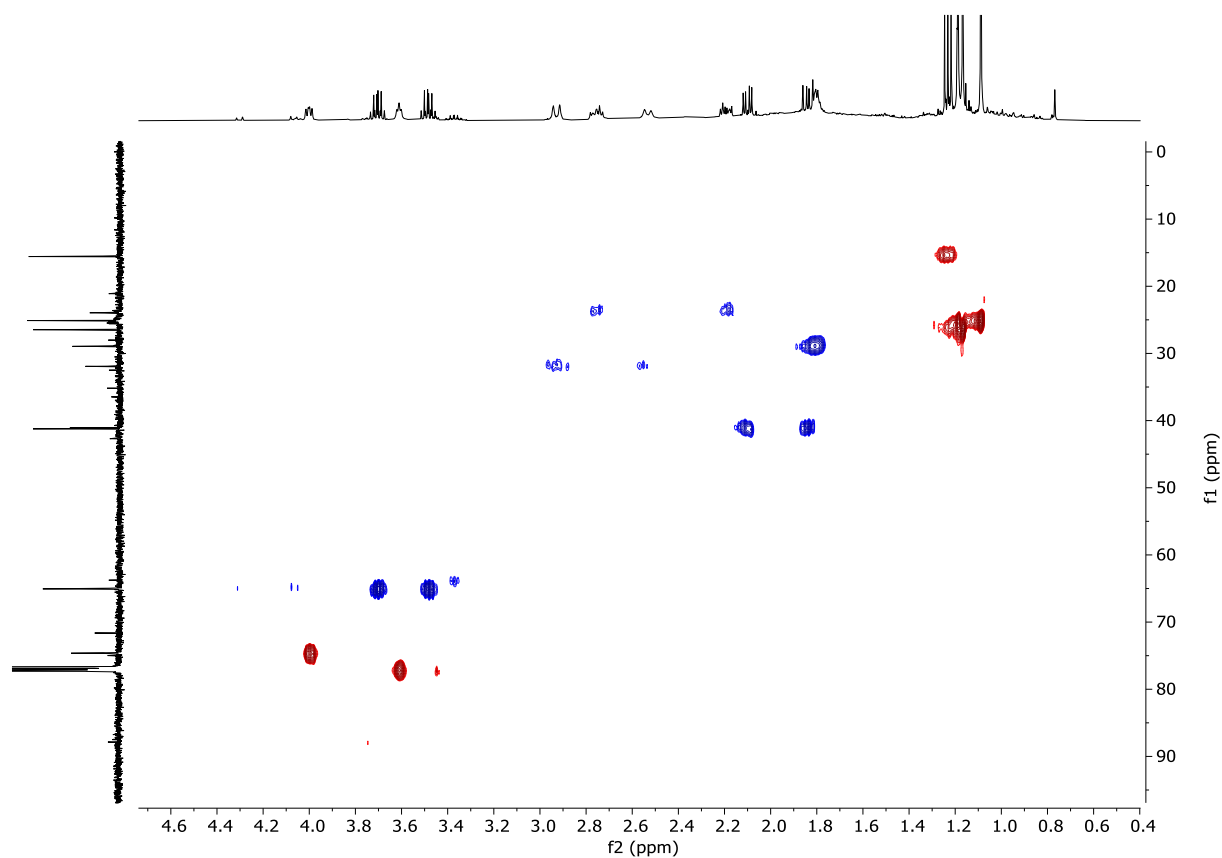


Figure S186. HMBC spectrum of dolichocarpol X (**18**) (CDCl₃, 500 MHz)

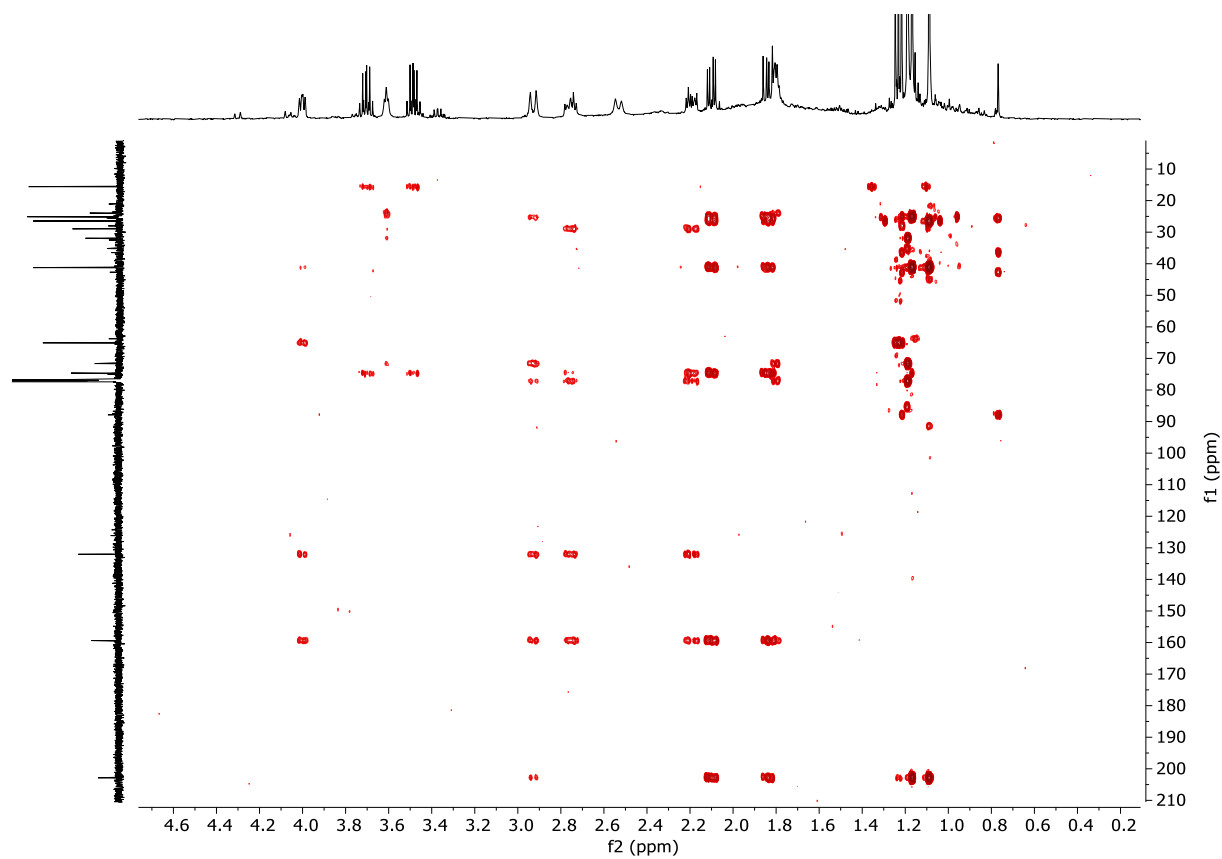


Figure S187. COSY spectrum of dolichocarpol X (**18**) (CDCl₃, 500 MHz)

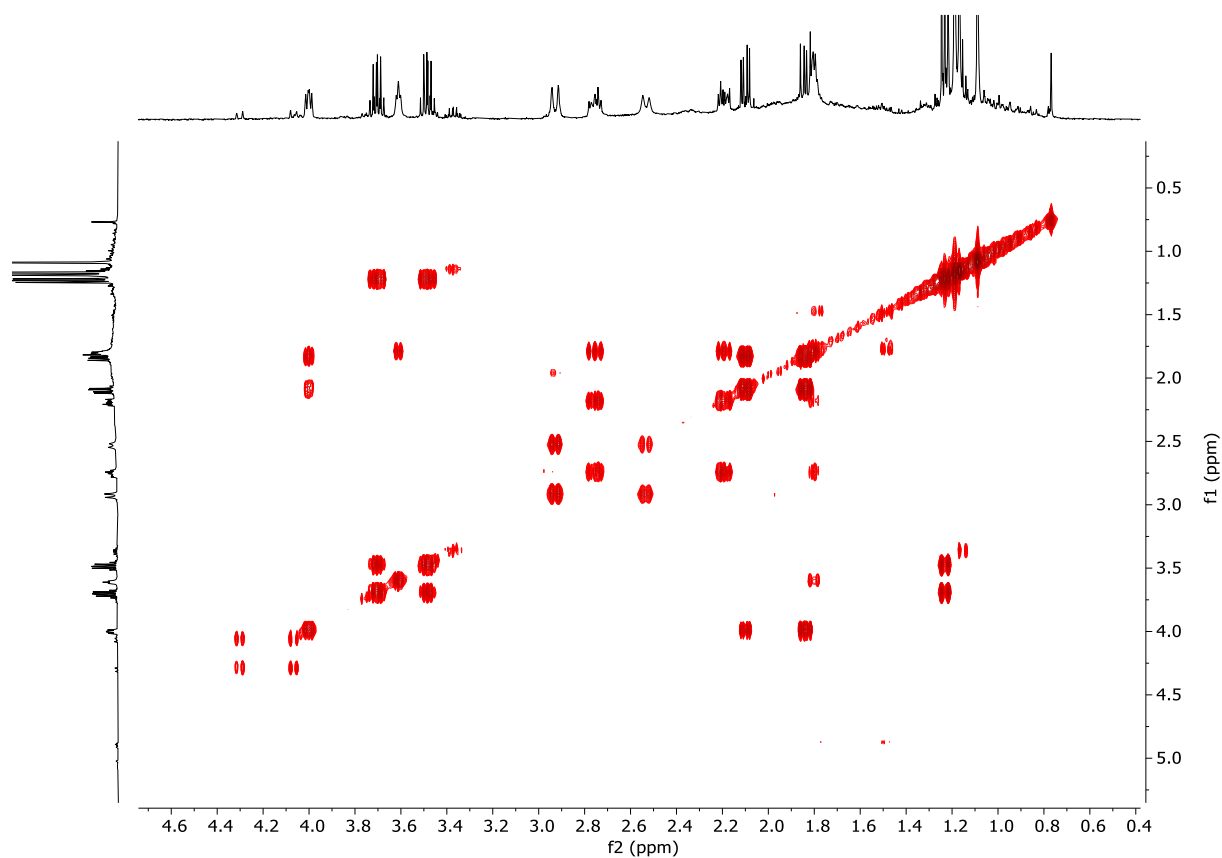


Figure S188. NOESY spectrum of dolichocarpol X (**18**) (CDCl_3 , 500 MHz)

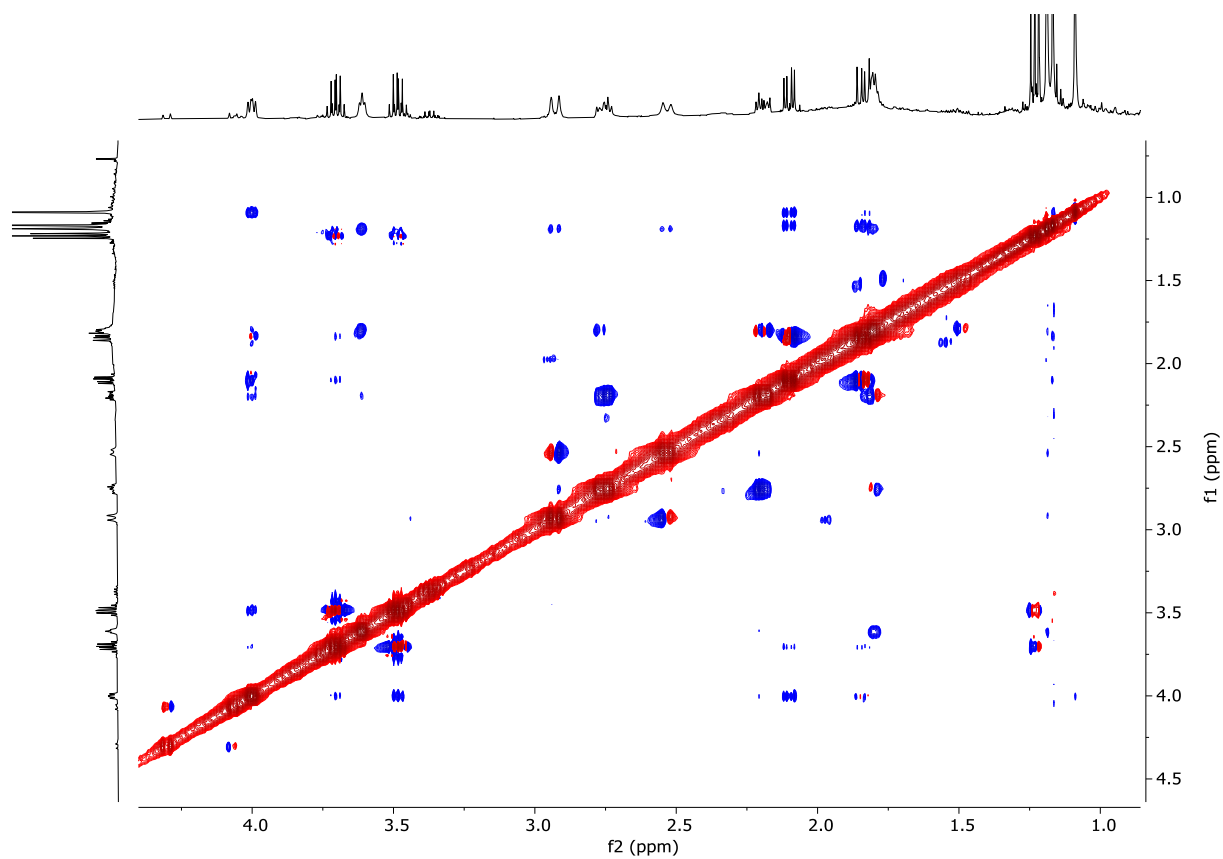


Figure S189. Comparison between the optimized lowest energy conformers for compounds **13–14** and **15–16**

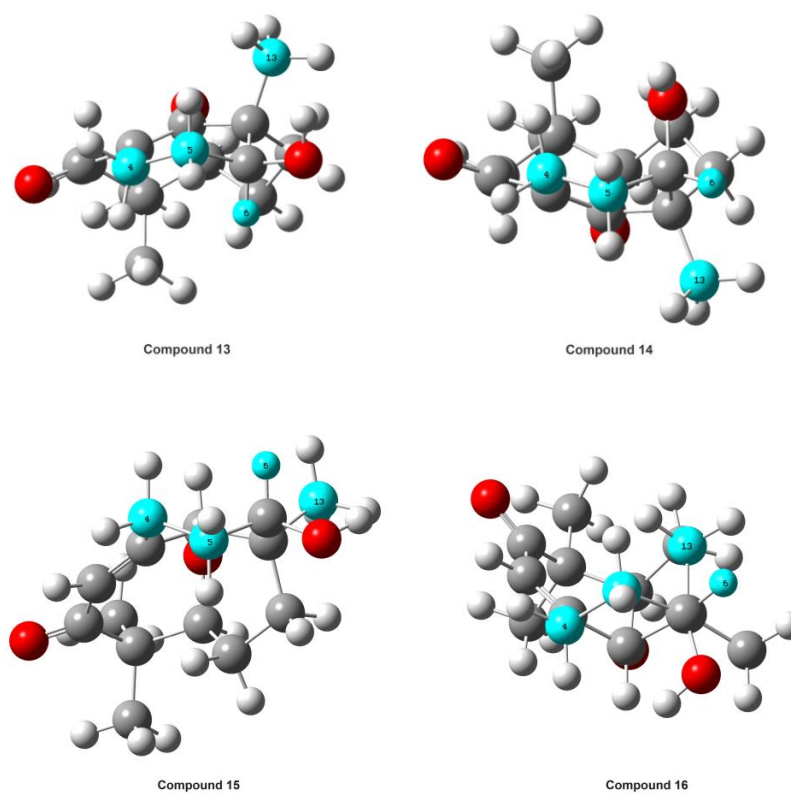
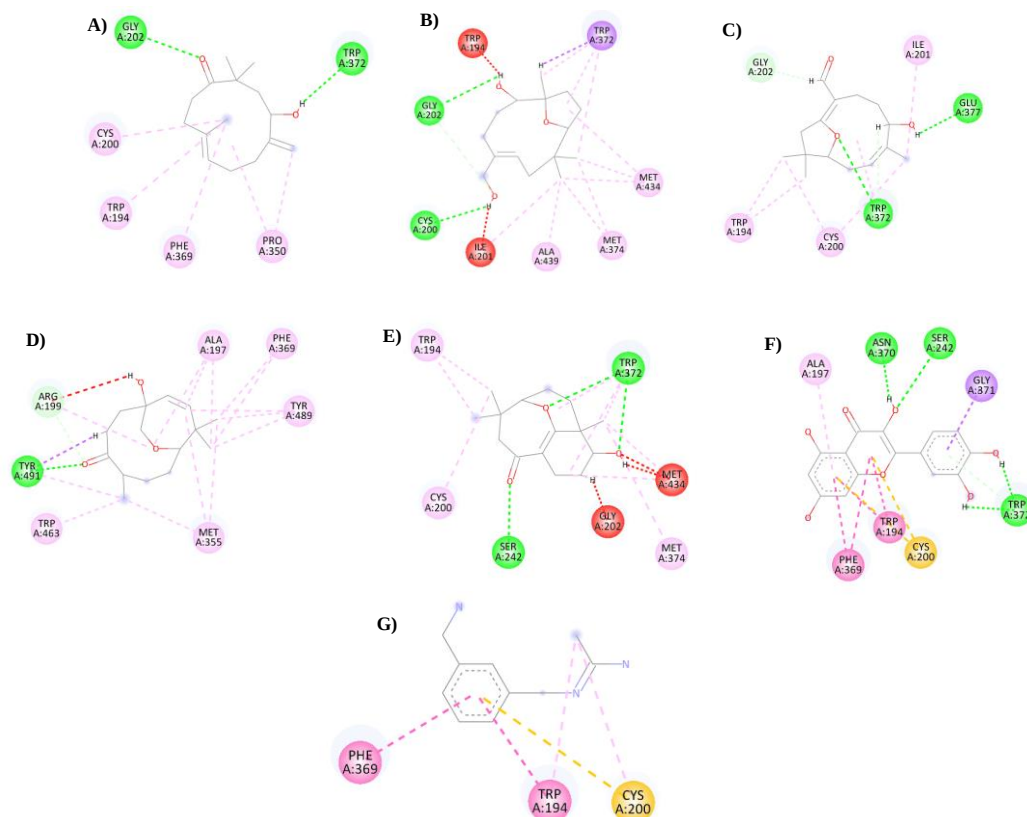
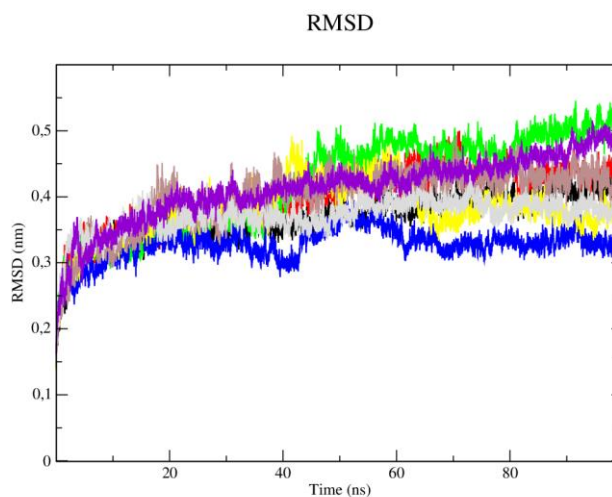
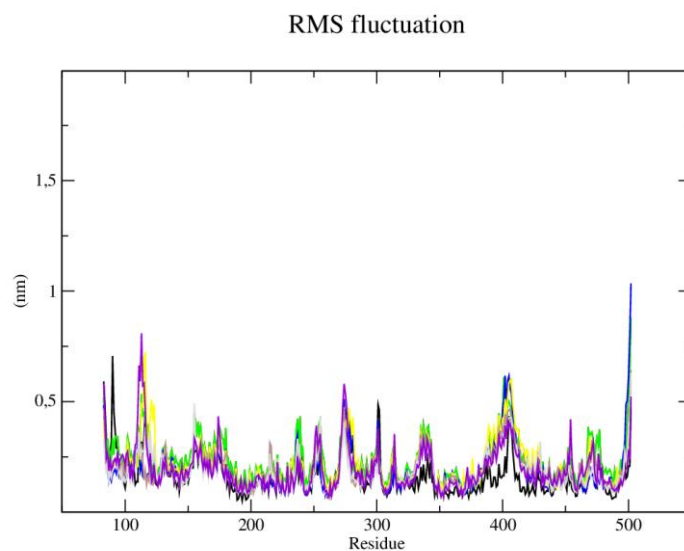


Figure S190. 2D molecular interaction map of compounds **1**, **5**, **8**, **9**, **14**

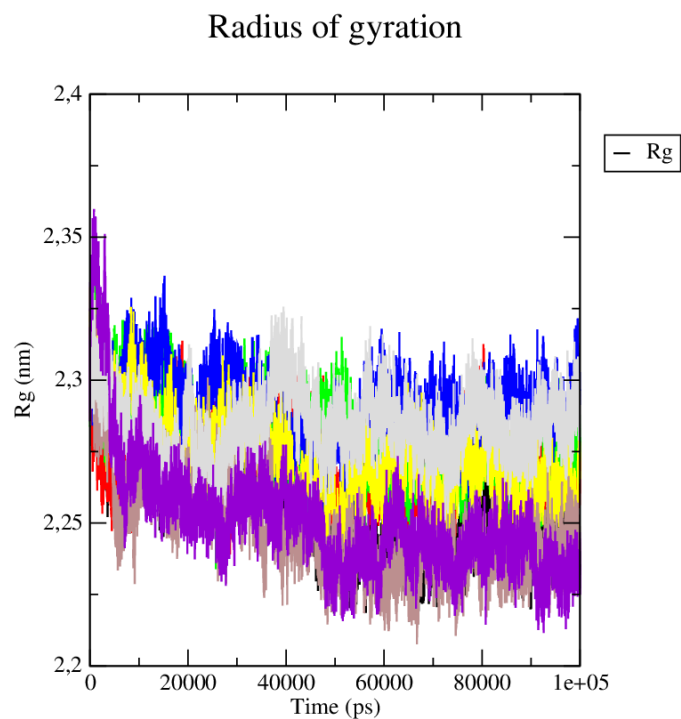
A) Compound **1**, B) Compound **5**, C) Compound **8**, D) Compound **9**, E) Compound **14**, F) **Quercetin**, G) **1400W** positive control with the i-Nos enzyme (PDB: 1NSI). **Interactions:** Orange (Pi-Sulfur), Dark pink (Pi-pi stacked and Pi-pi T-shaped), Light pink (Alkyl and Pi-alkyl), Lilac (Pi-sigma), Dark green (conventional hydrogen bonding), Light green (Carbon Hydrogen bond), Red (unfavorable interaction). Residues: Trp (Tryptophan), Cys (Cysteine), Ser (Serine), Met (Methionine), Gly (Glycine), Ile (Isoleucine), Ala (Alanine), Arg (Arginine), Phe (Phenylalanine), Tyr (Tyrosine), Glu (Glutamic Acid), Pro (Proline), Asn (Asparagine).

Figure S191. Root-mean-square deviation (RMSD) of compounds **1**, **5**, **8**, **9**, **14**

I-Nos (PDB: 1NSI) (black line), **1** (brown line), **5** (red line), **8** (green line), **9** (blue line), **14** (yellow line), **Quercetin** (gray line) and Compound **1400-W** (lilac line).

Figure S192. Root-mean-square fluctuation (RMSF) of compounds **1**, **5**, **8**, **9**, **14**

I-Nos (PDB: 1NSI) (black line), **1** (brown line), **5** (red line), **8** (green line), **9** (blue line), **14** (yellow line), **Quercetin** (gray line) and Compound **1400-W** (lilac line).

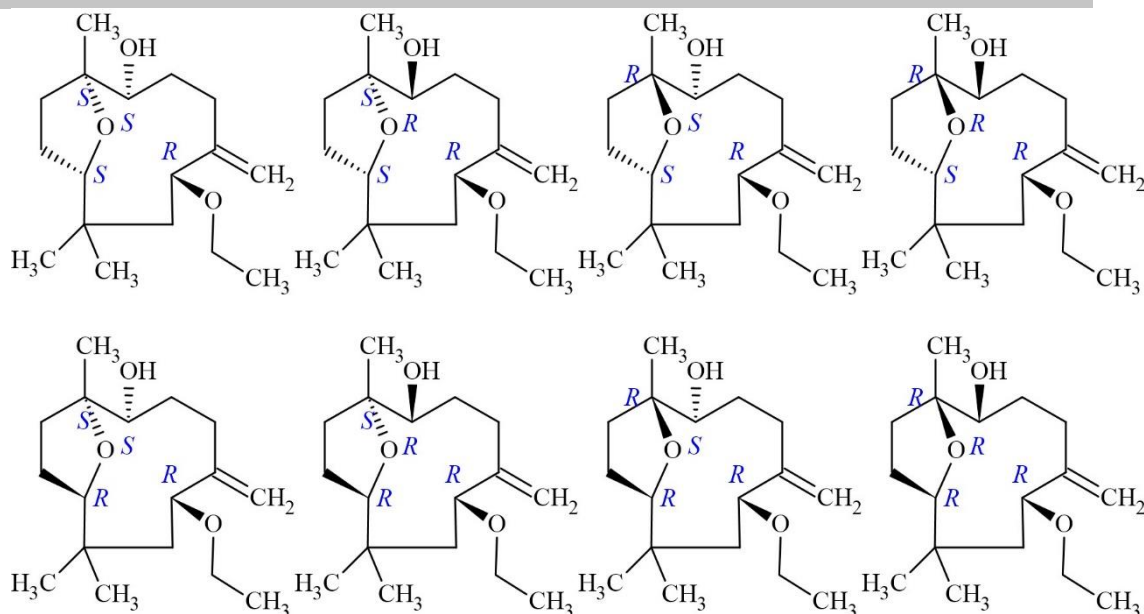
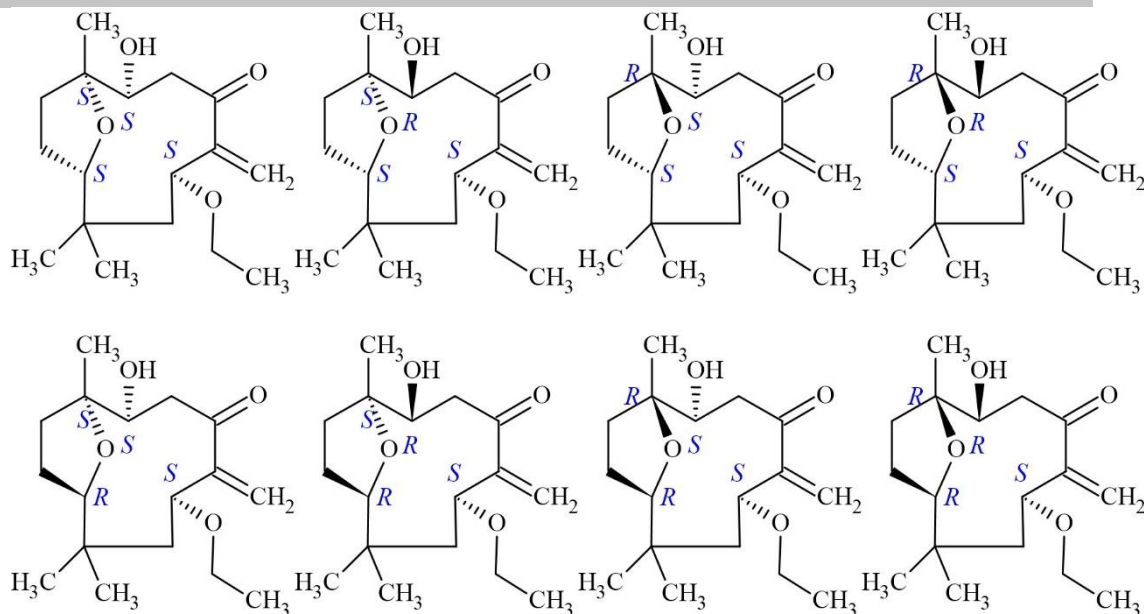
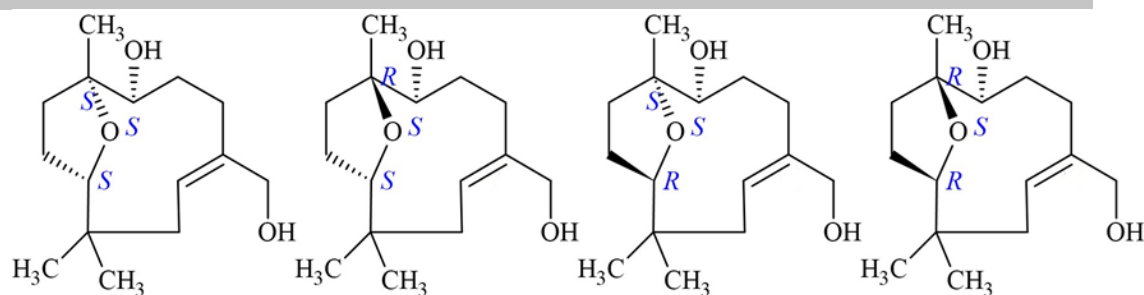
Figure S193. Radius of gyration (Rg) of compounds **1**, **5**, **8**, **9**, **14**

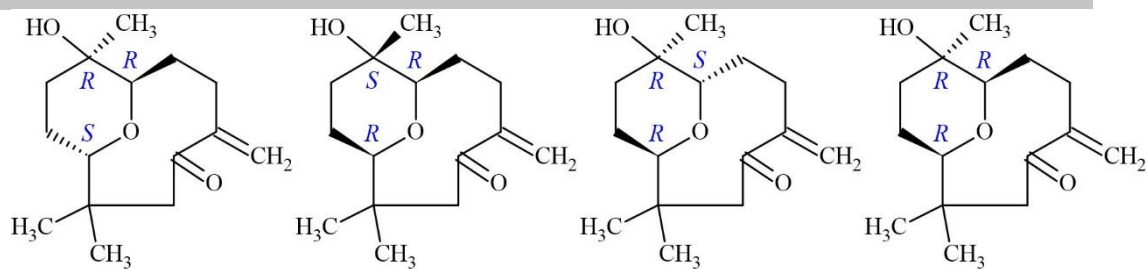
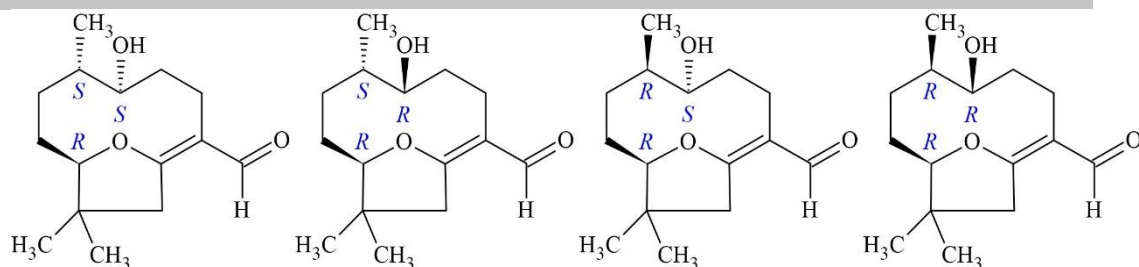
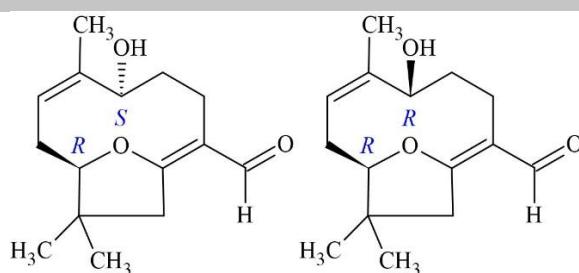
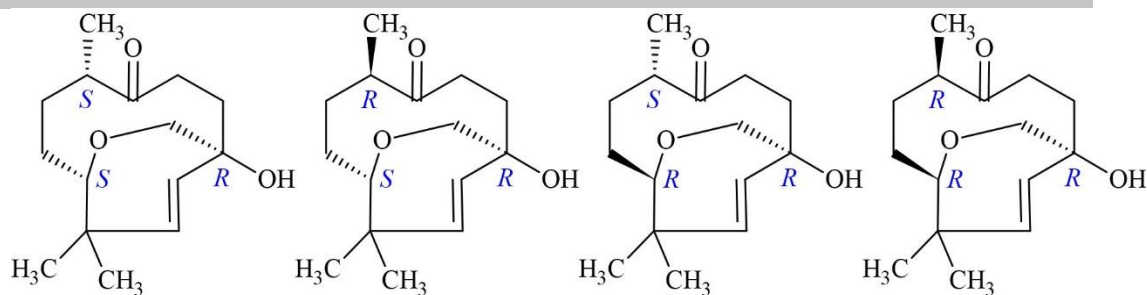
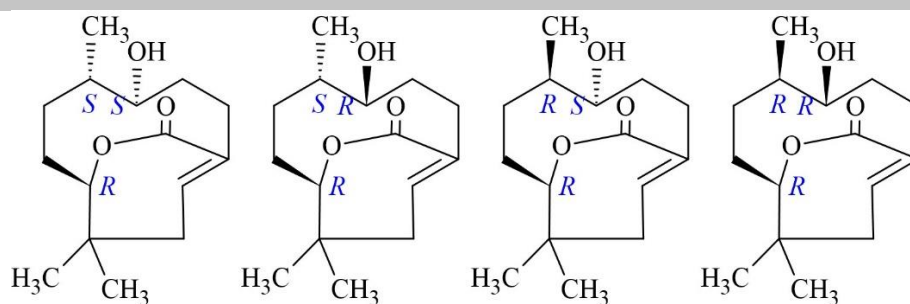
I-Nos (PDB: 1NSI) (black line), **1** (brown line), **5** (red line), **8** (green line), **9** (blue line), **14** (yellow line), **Quercetin** (gray line) and Compound **1400-W** (lilac line).

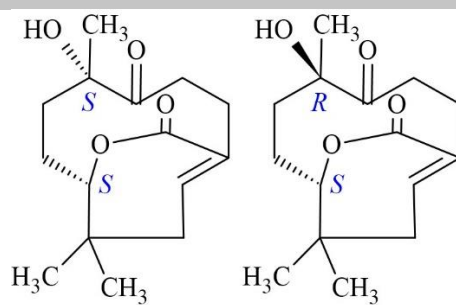
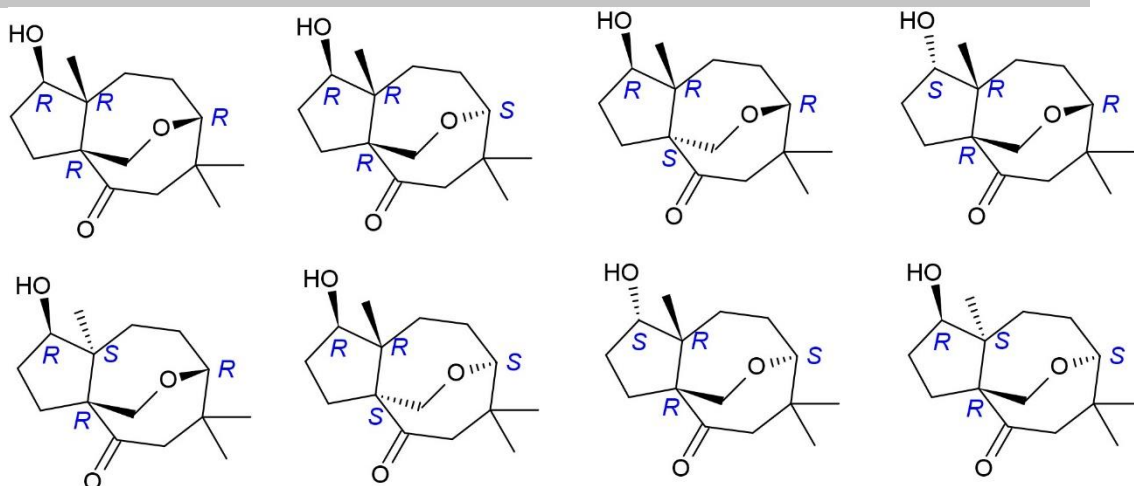
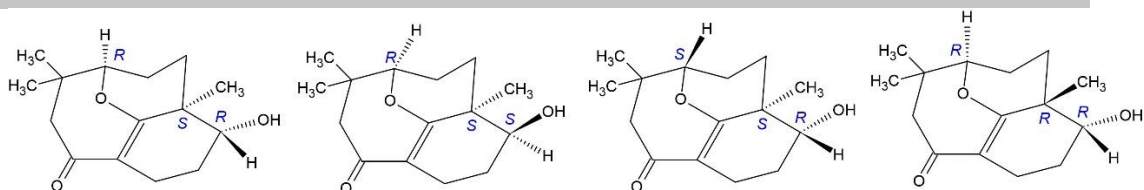
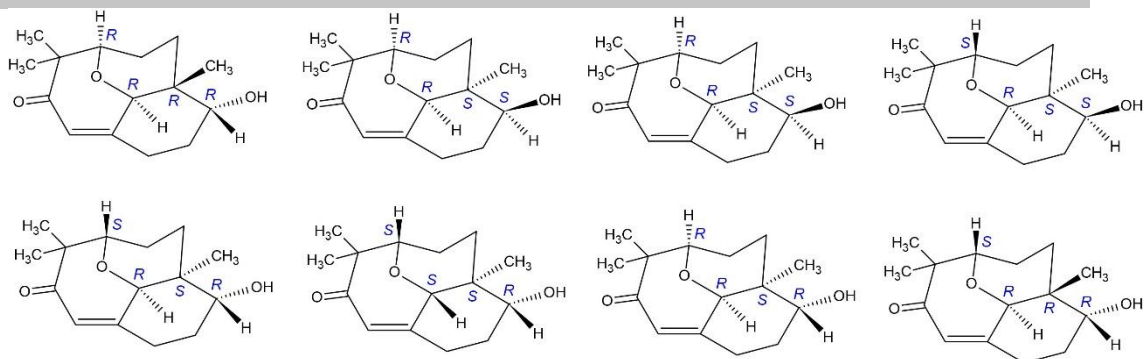
Table S1. Binding energy and activity probability values of the compounds under study on the i-Nos protein according to the MolDock Score (kJ/mol) scoring function

Compounds	i-Nos (PDB: 1NSI)	
	Binding energy (kJ.mol ⁻¹)	Probability value (p)
1	-94.9808	0.6805
5	-97.5387*	0.6988*
8	-104.326*	0.7475*
9	-89.7609	0.6431
14	-77.3582	0.5542
Quercetin	-139.566	1
Positive control (1400W)	-95.5013	0.6842

Caption: In bold, the lowest energy is highlighted and with (*) the target where the test compounds presented lower energy values when compared to at least one of the control compounds under study is highlighted.

Table S2. Structural representation of all possible diastereomers of compounds **3–18****Compound 3****Compound 4****Compound 5**

Compound 6**Compound 7****Compound 8****Compound 9****Compound 10**

Compound 11**Compound 12****Compounds 13 and 14****Compounds 15 and 16**

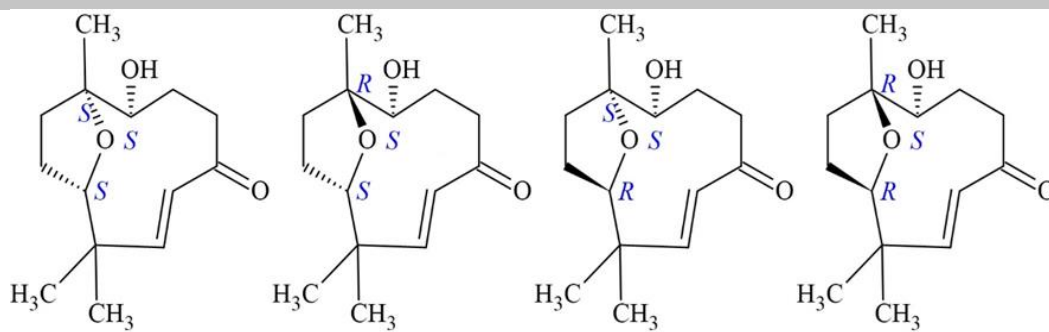
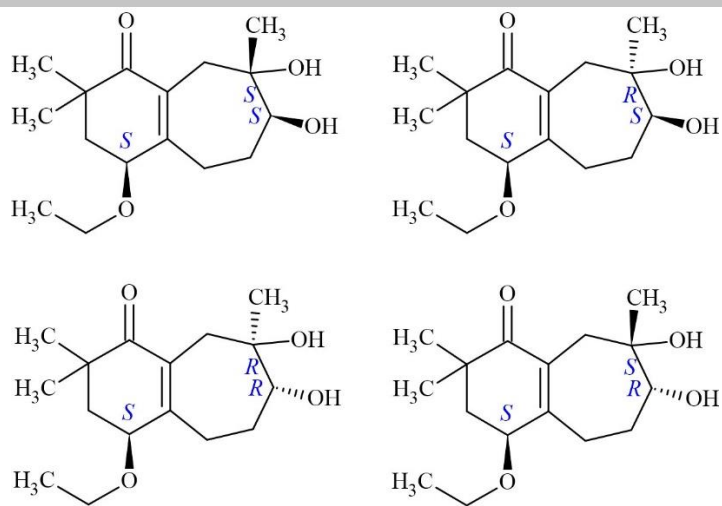
Compound 17**Compound 18**

Table S3. DP4+ probabilities obtained by correlating the calculated ^{13}C and ^1H NMR nuclear magnetic shielding tensors of all possible diastereomers of compounds **3–11** with the experimental NMR data

Compound 3								
	2 <i>R</i> *,6 <i>R</i> *, 7 <i>R</i> *,10 <i>R</i> *	2 <i>R</i> *,6 <i>S</i> *, 7 <i>R</i> *,10 <i>R</i> *	2 <i>R</i> *,6 <i>R</i> *, 7 <i>S</i> *,10 <i>R</i> *	2 <i>R</i> *,6 <i>S</i> *, 7 <i>S</i> *,10 <i>R</i> *	2 <i>R</i> *,6 <i>R</i> *, 7 <i>R</i> *,10 <i>S</i> *	2 <i>R</i> *,6 <i>S</i> *, 7 <i>R</i> *,10 <i>S</i> *	2 <i>R</i> *,6 <i>R</i> *, 7 <i>S</i> *,10 <i>S</i> *	2 <i>R</i> *,6 <i>S</i> *, 7 <i>S</i> *,10 <i>S</i> *
DP4+ (%)	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00
Compound 4								
	2 <i>S</i> *,6 <i>R</i> *, 7 <i>R</i> *,10 <i>R</i> *	2 <i>S</i> *,6 <i>S</i> *, 7 <i>R</i> *,10 <i>R</i> *	2 <i>S</i> *,6 <i>R</i> *, 7 <i>S</i> *,10 <i>R</i> *	2 <i>S</i> *,6 <i>S</i> *, 7 <i>S</i> *,10 <i>R</i> *	2 <i>S</i> *,6 <i>R</i> *, 7 <i>R</i> *,10 <i>S</i> *	2 <i>S</i> *,6 <i>S</i> *, 7 <i>R</i> *,10 <i>S</i> *	2 <i>S</i> *,6 <i>R</i> *, 7 <i>S</i> *,10 <i>S</i> *	2 <i>S</i> *,6 <i>S</i> *, 7 <i>S</i> *,10 <i>S</i> *
DP4+ (%)	0.00	0.00	97.63	0.00	0.01	0.00	2.73	0.00
Compound 5								
	6 <i>S</i> *,7 <i>S</i> *,10 <i>S</i> *		6 <i>S</i> *,7 <i>S</i> *,10 <i>R</i> *		6 <i>S</i> *,7 <i>R</i> *,10 <i>S</i> *		6 <i>S</i> *,7 <i>R</i> *,10 <i>R</i> *	
DP4+ (%)	0.00		0.00		0.00		100.00	
Compound 6								
	6 <i>R</i> *,7 <i>R</i> *,10 <i>R</i> *		6 <i>R</i> *,7 <i>R</i> *,10 <i>S</i> *		6 <i>S</i> *,7 <i>R</i> *,10 <i>R</i> *		6 <i>R</i> *,7 <i>S</i> *,10 <i>R</i> *	
DP4+ (%)	100.00		0.00		0.00		0.00	
Compound 7								
	6 <i>R</i> *,7 <i>R</i> *,10 <i>R</i> *		6 <i>S</i> *,7 <i>R</i> *,10 <i>R</i> *		6 <i>R</i> *,7 <i>S</i> *,10 <i>R</i> *		6 <i>S</i> *,7 <i>S</i> *,10 <i>R</i> *	
DP4+ (%)	0.04		0.02		0.01		99.94	
Compound 8								
	6 <i>R</i> *,10 <i>R</i> *				6 <i>S</i> *,10 <i>R</i> *			
DP4+ (%)	100.00				0.00			
Compound 9								
	3 <i>R</i> *,7 <i>R</i> *,10 <i>R</i> *		3 <i>R</i> *,7 <i>S</i> *,10 <i>R</i> *		3 <i>R</i> *,7 <i>R</i> *,10 <i>S</i> *		3 <i>R</i> *,7 <i>S</i> *,10 <i>S</i> *	
DP4+ (%)	0.00		100.00		0.00		0.00	
Compound 10								
	6 <i>R</i> *,7 <i>R</i> *,10 <i>R</i> *		6 <i>S</i> *,7 <i>R</i> *,10 <i>R</i> *		6 <i>R</i> *,7 <i>S</i> *,10 <i>R</i> *		6 <i>S</i> *,7 <i>S</i> *,10 <i>R</i> *	
DP4+ (%)	100.00		0.00		0.00		0.00	
Compound 11								
	7 <i>S</i> *,10 <i>S</i> *				7 <i>R</i> *,10 <i>S</i> *			
DP4+ (%)	99.53				0.47			

Table S4. DP4+ probabilities obtained by correlating the calculated ^{13}C and ^1H NMR nuclear magnetic shielding tensors of all possible diastereomers of compounds **12–18** with the experimental NMR data

Compound 12								
	3 <i>R</i> *,6 <i>R</i> *, 7 <i>R</i> *,10 <i>R</i> *	3 <i>R</i> *,6 <i>R</i> *, 7 <i>R</i> *,10 <i>S</i> *	3 <i>S</i> *,6 <i>R</i> *, 7 <i>R</i> *,10 <i>R</i> *	3 <i>R</i> *,6 <i>S</i> *, 7 <i>R</i> *,10 <i>R</i> *	3 <i>R</i> *,6 <i>R</i> *, 7 <i>S</i> *,10 <i>R</i> *	3 <i>S</i> *,6 <i>R</i> *, 7 <i>R</i> *,10 <i>S</i> *	3 <i>R</i> *,6 <i>S</i> *, 7 <i>R</i> *,10 <i>S</i> *	3 <i>R</i> *,6 <i>R</i> *, 7 <i>S</i> *,10 <i>S</i> *
DP4+ (%)	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00
Compound 13								
	6 <i>R</i> *,7 <i>S</i> *,10 <i>R</i> *		6 <i>R</i> *,7 <i>R</i> *,10 <i>R</i> *		6 <i>R</i> *,7 <i>S</i> *,10 <i>S</i> *		6 <i>S</i> *,7 <i>S</i> *,10 <i>R</i> *	
DP4+ (%)	100.00		0.00		0.00		0.00	
Compound 14								
	6 <i>R</i> *,7 <i>S</i> *,10 <i>R</i> *		6 <i>R</i> *,7 <i>R</i> *,10 <i>R</i> *		6 <i>R</i> *,7 <i>S</i> *,10 <i>S</i> *		6 <i>S</i> *,7 <i>S</i> *,10 <i>R</i> *	
DP4+ (%)	0.00		0.00		0.00		100.00	
Compound 15								
	6 <i>R</i> *,7 <i>R</i> *, 10 <i>R</i> *,12 <i>S</i> *	6 <i>R</i> *,7 <i>R</i> *, 10 <i>R</i> *,12 <i>R</i> *	6 <i>R</i> *,7 <i>S</i> *, 10 <i>R</i> *,12 <i>S</i> *	6 <i>S</i> *,7 <i>R</i> *, 10 <i>R</i> *,12 <i>S</i> *	6 <i>R</i> *,7 <i>S</i> *, 10 <i>R</i> *,12 <i>R</i> *	6 <i>S</i> *,7 <i>S</i> *, 10 <i>R</i> *,12 <i>S</i> *	6 <i>R</i> *,7 <i>S</i> *, 10 <i>S</i> *,12 <i>S</i> *	6 <i>S</i> *,7 <i>S</i> *, 10 <i>R</i> *,12 <i>R</i> *
DP4+ (%)	0.00	0.00	0.00	0.00	0.25	0.00	0.00	99.75
Compound 16								
	6 <i>R</i> *,7 <i>R</i> *, 10 <i>R</i> *,12 <i>S</i> *	6 <i>R</i> *,7 <i>R</i> *, 10 <i>R</i> *,12 <i>R</i> *	6 <i>R</i> *,7 <i>S</i> *, 10 <i>R</i> *,12 <i>S</i> *	6 <i>S</i> *,7 <i>R</i> *, 10 <i>R</i> *,12 <i>S</i> *	6 <i>R</i> *,7 <i>S</i> *, 10 <i>R</i> *,12 <i>R</i> *	6 <i>S</i> *,7 <i>S</i> *, 10 <i>R</i> *,12 <i>S</i> *	6 <i>R</i> *,7 <i>S</i> *, 10 <i>S</i> *,12 <i>S</i> *	6 <i>S</i> *,7 <i>S</i> *, 10 <i>R</i> *,12 <i>R</i> *
DP4+ (%)	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00
Compound 17								
	6 <i>S</i> *,7 <i>S</i> *,10 <i>R</i> *		6 <i>S</i> *,7 <i>S</i> *,10 <i>S</i> *		6 <i>S</i> *,7 <i>R</i> *,10 <i>R</i> *		6 <i>S</i> *,7 <i>R</i> *,10 <i>S</i> *	
DP4+ (%)	0.00		0.00		0.00		100.00	
Compound 18								
	2 <i>S</i> *,6 <i>S</i> *,7 <i>R</i> *		2 <i>S</i> *,6 <i>R</i> *,7 <i>S</i> *		2 <i>S</i> *,6 <i>R</i> *,7 <i>R</i> *		2 <i>S</i> *,6 <i>S</i> *,7 <i>S</i> *	
DP4+ (%)	0.00		0.00		0.00		100.00	

Table S5. Calculated ^{13}C NMR nuclear magnetic shielding tensors (σ) and chemical shifts (δ), in ppm, of all possible diastereomers of **3**

Carbons	$2R^*,6R^*,7R^*,10R^*$		$2R^*,6S^*,7R^*,10R^*$		$2R^*,6R^*,7S^*,10R^*$		$2R^*,6S^*,7S^*,10R^*$		$2R^*,6R^*,7R^*,10S^*$		$2R^*,6S^*,7R^*,10S^*$		$2R^*,6R^*,7S^*,10S^*$		$2R^*,6S^*,7S^*,10S^*$	
	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ
1	135.1	55.1	137.8	52.4	144.4	45.8	144.2	46.1	139.2	51.1	143.6	46.7	144.7	45.5	144.8	45.4
2	107.0	83.3	107.3	82.9	109.2	81.0	112.1	78.2	107.2	83.0	107.6	82.6	106.7	83.5	106.5	83.7
3	32.9	157.3	40.8	149.5	43.4	146.8	44.1	146.1	34.4	155.8	40.5	149.8	46.2	144.0	40.7	149.5
4	152.6	37.7	154.1	36.2	161.5	28.7	158.5	31.8	154.4	35.8	158.2	32.0	163.6	26.6	163.4	26.8
5	158.0	32.2	155.6	34.7	155.9	34.4	155.8	34.5	156.4	33.8	155.3	34.9	156.7	33.5	156.4	33.9
6	112.3	77.9	119.9	70.4	115.2	75.1	112.0	78.2	111.7	78.6	116.0	74.2	119.8	70.4	111.7	78.5
7	106.3	83.9	106.4	83.8	103.9	86.4	103.4	86.8	102.6	87.7	104.1	86.1	107.2	83.0	106.6	83.6
8	153.2	37.0	150.2	40.1	153.4	36.8	156.5	33.8	151.0	39.3	152.0	38.2	150.0	40.2	155.1	35.1
9	160.8	29.4	160.4	29.9	158.5	31.7	156.7	33.5	157.5	32.7	160.9	29.4	161.5	28.7	161.7	28.6
10	104.5	85.7	107.6	82.6	105.1	85.1	105.0	85.3	104.1	86.2	104.3	85.9	108.7	81.5	107.0	83.2
11	152.6	37.7	152.5	37.7	151.6	38.6	151.9	38.3	150.7	39.5	151.9	38.4	153.2	37.0	153.4	36.9
12	85.5	104.8	84.6	105.6	81.8	108.4	84.7	105.5	82.0	108.3	83.6	106.6	79.3	110.9	79.2	111.0
13	163.9	26.3	168.9	21.4	167.1	23.2	162.0	28.2	162.9	27.3	168.3	21.9	169.8	20.4	162.6	27.7
14	161.3	28.9	168.9	21.4	165.8	24.4	166.0	24.2	160.6	29.7	160.3	30.0	165.3	24.9	165.5	24.7
15	170.2	20.0	161.3	28.9	155.6	34.7	155.7	34.5	161.9	28.3	162.7	27.6	165.3	24.9	165.8	24.5
1'	127.4	62.9	127.1	63.1	127.3	62.9	127.0	63.2	126.8	63.4	127.8	62.4	128.7	61.6	128.6	61.7
2'	173.0	17.3	172.6	17.6	173.3	16.9	173.2	17.0	172.4	17.9	173.2	17.0	173.6	16.6	173.6	16.7

Chemical shifts were obtained as $\delta_{\text{calc}} = \sigma_{\text{TMS}} - \sigma$, where σ_{TMS} represents the nuclear magnetic shielding tensor of the reference compound (tetramethylsilane, TMS).

Table S6. Calculated ^1H NMR nuclear magnetic shielding tensors (σ) and chemical shifts (δ), in ppm, of all possible diastereomers of **3**

Hydrogens	$2R^*,6R^*,7R^*,10R^*$		$2R^*,6S^*,7R^*,10R^*$		$2R^*,6R^*,7S^*,10R^*$		$2R^*,6S^*,7S^*,10R^*$		$2R^*,6R^*,7R^*,10S^*$		$2R^*,6S^*,7R^*,10S^*$		$2R^*,6R^*,7S^*,10S^*$		$2R^*,6S^*,7S^*,10S^*$	
	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ
1a	30.19	1.98	30.22	1.96	29.92	2.25	29.95	2.23	29.86	2.31	29.97	2.21	29.82	2.35	29.72	2.45
1b	30.32	1.85	30.26	1.91	30.85	1.33	30.85	1.32	30.25	1.93	30.50	1.68	30.79	1.38	30.74	1.43
2	28.05	4.12	28.19	3.98	27.97	4.20	27.76	4.41	27.96	4.21	28.27	3.90	28.18	4.00	28.24	3.94
4a	29.55	2.62	29.64	2.53	29.48	2.70	29.57	2.60	29.57	2.60	29.86	2.31	29.26	2.91	29.27	2.91
4b	29.52	2.66	29.53	2.64	30.09	2.08	29.88	2.29	29.52	2.65	29.72	2.45	30.12	2.06	30.00	2.17
5a	29.68	2.49	29.92	2.26	30.15	2.02	30.48	1.70	29.81	2.36	30.26	1.92	30.31	1.87	30.03	2.14
5b	30.11	2.07	30.43	1.74	30.22	1.95	30.27	1.90	30.08	2.09	30.14	2.04	30.16	2.01	29.85	2.32
6	28.47	3.70	27.96	4.22	28.35	3.82	28.39	3.79	28.38	3.80	28.33	3.84	27.78	4.40	28.45	3.72
8a	29.51	2.67	29.97	2.20	30.47	1.70	30.53	1.65	29.79	2.39	29.93	2.25	30.40	1.77	30.57	1.61
8b	30.48	1.69	30.35	1.82	30.04	2.14	29.94	2.23	30.34	1.83	30.45	1.73	30.03	2.14	29.60	2.57
9a	30.46	1.72	30.49	1.68	30.09	2.09	29.88	2.30	30.45	1.72	30.53	1.65	30.59	1.58	30.58	1.59
9b	30.49	1.68	30.50	1.68	30.31	1.87	30.36	1.82	30.15	2.03	30.37	1.80	30.60	1.58	30.55	1.62
10	28.30	3.88	28.26	3.92	28.09	4.08	28.03	4.15	27.98	4.19	28.26	3.91	28.06	4.11	28.07	4.11
12a	26.67	5.50	26.83	5.35	27.13	5.04	27.29	4.88	26.64	5.53	26.96	5.21	26.94	5.24	26.80	5.37
12b	26.83	5.34	26.74	5.44	27.13	5.05	27.25	4.92	26.60	5.58	26.89	5.29	26.98	5.20	27.04	5.13
13	30.95	1.22	30.94	1.24	30.94	1.23	30.85	1.33	30.79	1.38	30.95	1.22	31.01	1.17	30.96	1.21
14	31.25	0.92	31.07	1.11	31.09	1.08	31.03	1.14	31.14	1.03	31.31	0.87	31.26	0.92	31.24	0.94
15	31.02	1.15	31.18	0.99	31.09	1.09	31.07	1.11	30.70	1.48	30.91	1.27	31.15	1.03	31.18	0.99
1'a	28.62	3.55	28.58	3.59	28.56	3.61	28.53	3.64	28.57	3.60	28.62	3.55	28.59	3.58	28.62	3.55
1'b	28.85	3.32	28.73	3.45	28.85	3.33	28.75	3.42	28.80	3.38	28.96	3.22	28.99	3.18	29.05	3.12
2'	31.02	1.16	30.96	1.21	31.02	1.16	30.99	1.18	30.89	1.29	31.02	1.16	31.07	1.11	31.06	1.11

Chemical shifts were obtained as $\delta_{\text{calc}} = \sigma_{\text{TMS}} - \sigma$, where σ_{TMS} represents the nuclear magnetic shielding tensor of the reference compound (tetramethylsilane, TMS).

Table S7. Calculated ^{13}C NMR nuclear magnetic shielding tensors (σ) and chemical shifts (δ), in ppm, of all possible diastereomers of **4**

Carbons	$2S^*,6R^*,7R^*,10R^*$		$2S^*,6S^*,7R^*,10R^*$		$2S^*,6R^*,7S^*,10R^*$		$2S^*,6S^*,7S^*,10R^*$		$2S^*,6R^*,7R^*,10S^*$		$2S^*,6S^*,7R^*,10S^*$		$2S^*,6R^*,7S^*,10S^*$		$2S^*,6S^*,7S^*,10S^*$	
	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ
1	135.6	54.6	136.2	54.0	135.8	54.4	135.5	54.7	144.5	45.7	143.5	46.7	136.1	54.1	135.8	54.4
2	116.8	73.5	114.1	76.2	114.7	75.6	113.5	76.7	112.9	77.3	114.5	75.7	114.9	75.3	114.1	76.1
3	38.3	151.9	39.0	151.3	37.1	153.2	33.8	156.5	41.6	148.7	41.8	148.5	36.9	153.3	34.0	156.2
4	-10.4	200.6	-3.4	193.6	-11.9	202.1	-8.4	198.7	-12.0	202.2	-8.9	199.1	-12.7	202.9	-8.9	199.1
5	151.1	39.1	144.6	45.6	149.4	40.9	145.0	45.2	152.0	38.2	151.9	38.4	150.3	40.0	145.5	44.7
6	106.7	83.5	104.9	85.3	108.1	82.1	109.7	80.6	109.0	81.2	110.0	80.3	107.7	82.5	109.8	80.4
7	104.3	85.9	105.6	84.7	103.9	86.3	105.2	85.0	103.6	86.7	103.5	86.8	104.0	86.2	105.6	84.6
8	157.3	32.9	148.0	42.2	151.7	38.5	155.2	35.0	154.9	35.3	153.9	36.3	152.1	38.1	155.8	34.4
9	159.9	30.3	159.2	31.0	160.5	29.8	159.4	30.8	159.4	30.8	162.3	28.0	160.9	29.3	160.0	30.2
10	107.7	82.6	107.4	82.8	106.9	83.4	104.4	85.8	104.0	86.3	105.0	85.3	107.0	83.2	104.8	85.4
11	153.6	36.6	152.5	37.7	152.7	37.5	152.5	37.8	151.6	38.6	151.6	38.6	153.1	37.2	153.1	37.1
12	77.4	112.9	75.8	114.4	74.6	115.6	78.1	112.2	75.4	114.8	70.2	120.1	74.7	115.5	79.1	111.1
13	161.7	28.5	166.8	23.5	164.8	25.4	163.3	27.0	164.4	25.8	168.2	22.0	164.7	25.5	163.6	26.6
14	164.5	25.7	163.6	26.6	161.6	28.7	161.3	28.9	160.9	29.4	160.9	29.3	162.0	28.3	161.8	28.4
15	164.6	25.6	163.8	26.4	168.9	21.3	170.0	20.2	164.3	25.9	160.1	30.1	169.2	21.0	170.6	19.6
1'	127.2	63.1	126.4	63.8	127.2	63.0	126.7	63.5	126.3	64.0	127.4	62.8	127.5	62.7	127.1	63.2
2'	173.8	16.5	172.4	17.9	173.4	16.9	173.0	17.3	173.4	16.9	173.4	16.8	173.8	16.4	173.6	16.7

Chemical shifts were obtained as $\delta_{\text{calc}} = \sigma_{\text{TMS}} - \sigma$, where σ_{TMS} represents the nuclear magnetic shielding tensor of the reference compound (tetramethylsilane, TMS)]

Table S8. Calculated ^1H NMR nuclear magnetic shielding tensors (σ) and chemical shifts (δ), in ppm, of all possible diastereomers of **4**

Hydrogens	2S*,6R*, 7R*,10R*		2S*,6S*, 7R*,10R*		2S*,6R*, 7S*,10R*		2S*,6S*, 7S*,10R*		2S*,6R*, 7R*,10S*		2S*,6S*, 7R*,10S*		2S*,6R*, 7S*,10S*		2S*,6S*, 7S*,10S*	
	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ
1a	30.15	2.03	29.90	2.28	30.25	1.93	30.27	1.91	30.29	1.89	30.54	1.63	30.38	1.79	30.31	1.86
1b	30.44	1.74	30.29	1.88	30.31	1.87	30.20	1.97	30.11	2.07	29.96	2.21	30.32	1.86	30.37	1.80
2	27.76	4.42	27.63	4.54	27.71	4.46	27.59	4.58	27.63	4.55	27.77	4.41	27.78	4.40	27.68	4.49
5a	29.05	3.12	28.92	3.25	29.15	3.03	29.39	2.79	28.94	3.23	29.84	2.33	29.26	2.92	29.12	3.06
5b	29.69	2.49	29.61	2.56	29.75	2.42	29.01	3.17	29.97	2.20	29.19	2.99	29.81	2.37	29.52	2.66
6	28.22	3.96	27.53	4.64	28.01	4.16	28.14	4.03	28.23	3.95	28.05	4.12	28.09	4.08	28.20	3.97
8a	29.27	2.91	29.94	2.24	30.30	1.87	29.47	2.70	29.66	2.51	30.21	1.96	30.43	1.75	30.59	1.59
8b	30.70	1.47	30.01	2.17	30.36	1.82	30.49	1.69	30.65	1.52	30.33	1.85	30.39	1.78	29.60	2.58
9a	30.25	1.92	30.13	2.04	30.36	1.82	30.30	1.87	30.50	1.68	30.21	1.97	30.58	1.60	30.46	1.71
9b	30.42	1.75	30.23	1.95	30.50	1.68	30.36	1.82	30.60	1.57	30.22	1.96	30.43	1.75	30.39	1.78
10	28.82	3.35	28.53	3.64	28.18	4.00	28.36	3.81	28.42	3.76	28.17	4.01	28.23	3.94	28.47	3.71
12a	26.46	5.71	26.31	5.86	26.31	5.87	26.43	5.74	26.32	5.85	26.39	5.79	26.37	5.80	26.55	5.62
12b	26.47	5.70	26.30	5.88	26.37	5.80	26.44	5.73	26.34	5.83	26.13	6.05	26.45	5.73	26.60	5.58
13	31.01	1.16	30.80	1.37	30.96	1.22	30.92	1.26	31.05	1.13	30.91	1.26	31.03	1.15	31.02	1.16
14	31.27	0.90	31.06	1.11	31.24	0.94	31.21	0.96	31.12	1.05	31.16	1.01	31.32	0.86	31.32	0.85
15	31.22	0.96	30.99	1.19	31.11	1.07	31.07	1.10	31.24	0.94	31.27	0.90	31.18	0.99	31.16	1.01
1'a	28.72	3.45	28.50	3.67	28.63	3.54	28.63	3.54	28.64	3.54	28.56	3.62	28.69	3.49	28.72	3.45
1'b	28.84	3.34	28.64	3.54	28.62	3.55	28.62	3.56	28.67	3.50	28.64	3.54	28.70	3.47	28.70	3.48
2'	31.05	1.13	30.82	1.36	30.98	1.19	30.99	1.19	30.98	1.20	30.99	1.19	31.05	1.12	31.08	1.09

Chemical shifts were obtained as $\delta_{\text{calc}} = \sigma_{\text{TMS}} - \sigma$, where σ_{TMS} represents the nuclear magnetic shielding tensor of the reference compound (tetramethylsilane, TMS).

Table S9. Calculated ^{13}C NMR nuclear magnetic shielding tensors (σ) and chemical shifts (δ), in ppm, of all possible diastereomers of **5**

Carbons	6S*,7S*,10S*		6S*,7S*,10R*		6S*,7R*,10S*		6S*,7R*,10R*	
	σ	δ	σ	δ	σ	δ	σ	δ
1	148.0	42.2	149.2	41.0	148.4	41.8	148.0	42.2
2	63.7	126.5	67.6	122.6	66.1	124.2	66.9	123.4
3	52.0	138.2	48.6	141.7	50.7	139.5	50.8	139.4
4	159.2	31.0	161.4	28.9	163.1	27.1	161.3	28.9
5	154.5	35.7	153.3	36.9	155.2	35.0	153.6	36.6
6	113.3	77.0	110.5	79.8	112.4	77.8	117.0	73.3
7	107.0	83.2	102.6	87.7	101.3	89.0	107.1	83.2
8	153.5	36.7	152.9	37.3	149.7	40.5	148.7	41.5
9	161.0	29.3	155.0	35.2	155.0	35.2	160.3	30.0
10	106.5	83.8	103.7	86.6	102.1	88.2	107.6	82.6
11	149.4	40.8	148.8	41.5	146.6	43.7	150.5	39.8
12	117.8	72.5	118.9	71.4	6.3	183.9	120.7	69.6
13	163.0	27.2	160.3	29.9	160.6	29.6	168.3	21.9
14	166.5	23.8	154.1	36.2	153.1	37.1	164.2	26.0
15	164.8	25.5	118.9	71.4	162.1	28.1	165.8	24.5

Chemical shifts were obtained as $\delta_{\text{calc}} = \sigma_{\text{TMS}} - \sigma$, where σ_{TMS} represents the nuclear magnetic shielding tensor of the reference compound (tetramethylsilane, TMS).

Table S10. Calculated ^1H NMR nuclear magnetic shielding tensors (σ) and chemical shifts (δ), in ppm, of all possible diastereomers of **5**

Hydrogens	6S*,7S*,10S*		6S*,7S*,10R*		6S*,7R*,10S*		6S*,7R*,10R*	
	σ	δ	σ	δ	σ	δ	σ	δ
1a	30.39	1.79	30.27	1.91	28.69	3.49	29.35	2.82
1b	29.41	2.76	28.99	3.19	29.81	2.37	30.27	1.90
2	26.23	5.95	26.16	6.02	25.68	6.50	26.24	5.93
4a	28.57	3.61	29.68	2.49	28.70	3.48	28.84	3.33
4b	30.18	1.99	28.93	3.24	29.49	2.69	29.81	2.37
5a	29.93	2.24	29.79	2.38	29.43	2.75	30.10	2.07
5b	29.68	2.49	29.99	2.19	29.59	2.59	29.91	2.26
6	28.16	4.01	28.08	4.09	27.86	4.31	27.88	4.30
8a	29.30	2.87	29.63	2.54	29.74	2.44	30.12	2.06
8b	30.43	1.74	30.28	1.90	29.53	2.64	29.76	2.42
9a	30.45	1.72	29.79	2.39	29.87	2.30	30.36	1.82
9b	30.49	1.69	30.26	1.92	29.18	2.99	30.35	1.82
10	27.40	4.77	27.84	4.34	27.48	4.70	28.13	4.04
12a	27.81	4.37	27.71	4.46	27.37	4.80	27.84	4.33
12b	27.69	4.48	27.71	4.47	27.29	4.88	27.56	4.61
13	30.87	1.31	30.50	1.68	30.13	2.05	30.74	1.43
14	30.70	1.47	30.69	1.49	30.35	1.82	30.99	1.18
15	31.10	1.07	30.76	1.42	30.34	1.84	30.97	1.20

Chemical shifts were obtained as $\delta_{\text{calc}} = \sigma_{\text{TMS}} - \sigma$, where σ_{TMS} represents the nuclear magnetic shielding tensor of the reference compound (tetramethylsilane, TMS).

Table S11. Calculated ^{13}C NMR nuclear magnetic shielding tensors (σ) and chemical shifts (δ), in ppm, of all possible diastereomers of **6**

Carbons	6R*,7R*,10R*		6R*,7R*,10S*		6S*,7R*,10R*		6R*,7S*,10R*	
	σ	δ	σ	δ	σ	δ	σ	δ
1	139.4	50.9	139.4	50.8	139.7	50.5	139.1	51.1
2	-3.9	194.1	-10.0	200.2	-9.7	199.9	-3.6	193.9
3	40.7	149.5	40.4	149.8	40.2	150.1	39.5	150.8
4	158.5	31.7	157.2	33.1	156.7	33.5	156.5	33.8
5	162.1	28.1	158.8	31.4	158.3	31.9	160.4	29.8
6	106.5	83.7	112.5	77.8	112.6	77.6	105.4	84.8
7	120.6	69.7	120.6	69.6	119.4	70.8	119.7	70.5
8	150.8	39.5	152.4	37.8	150.7	39.5	147.9	42.3
9	165.6	24.6	166.8	23.4	165.3	24.9	163.5	26.7
10	107.9	82.3	111.3	78.9	111.3	78.9	107.7	82.5
11	144.8	45.4	139.6	50.6	139.3	50.9	145.2	45.0
12	78.7	111.5	81.7	108.5	82.1	108.2	79.9	110.3
13	166.3	23.9	164.8	25.5	167.5	22.7	168.1	22.1
14	162.8	27.4	165.0	25.2	164.5	25.8	165.7	24.5
15	167.6	22.7	158.0	32.2	157.9	32.3	162.0	28.2

Chemical shifts were obtained as $\delta_{\text{calc}} = \sigma_{\text{TMS}} - \sigma$, where σ_{TMS} represents the nuclear magnetic shielding tensor of the reference compound (tetramethylsilane, TMS).

Table S12. Calculated ^1H NMR nuclear magnetic shielding tensors (σ) and chemical shifts (δ), in ppm, of all possible diastereomers of **6**

Hydrogens	6<i>R</i>*,7<i>R</i>*,10<i>R</i>*		6<i>R</i>*,7<i>R</i>*,10<i>S</i>*		6<i>S</i>*,7<i>R</i>*,10<i>R</i>*		6<i>R</i>*,7<i>S</i>*,10<i>R</i>*	
	σ	δ	σ	δ	σ	δ	σ	δ
1a	29.67	2.51	29.94	2.23	29.06	3.11	29.77	2.40
1b	29.90	2.27	29.03	3.14	29.94	2.24	29.69	2.48
4a	29.63	2.54	30.02	2.15	29.24	2.94	29.77	2.40
4b	29.80	2.38	29.19	2.99	30.01	2.16	29.67	2.51
5a	30.53	1.64	30.63	1.54	30.26	1.92	30.63	1.55
5b	30.50	1.67	30.35	1.82	30.69	1.48	30.35	1.82
6	28.83	3.34	28.44	3.73	28.61	3.56	29.02	3.16
8a	30.47	1.71	30.59	1.58	30.44	1.73	30.33	1.85
8b	30.53	1.65	30.33	1.84	30.45	1.72	30.60	1.58
9a	30.67	1.51	30.38	1.79	30.26	1.91	30.57	1.61
9b	30.37	1.80	30.08	2.10	30.21	1.97	30.42	1.75
10	28.91	3.27	28.32	3.86	28.40	3.77	28.95	3.22
12a	26.86	5.32	26.79	5.38	26.81	5.36	26.91	5.26
12b	26.85	5.32	26.84	5.33	26.86	5.31	26.88	5.29
13	31.15	1.03	31.04	1.13	30.96	1.22	30.97	1.21
14	31.13	1.04	30.78	1.39	30.75	1.42	31.10	1.07
15	31.09	1.09	31.08	1.09	31.08	1.10	31.04	1.13

Chemical shifts were obtained as $\delta_{\text{calc}} = \sigma_{\text{TMS}} - \sigma$, where σ_{TMS} represents the nuclear magnetic shielding tensor of the reference compound (tetramethylsilane, TMS).

Table S13. Calculated ^{13}C NMR nuclear magnetic shielding tensors (σ) and chemical shifts (δ), in ppm, of all possible diastereomers of **7**

Carbons	6R*,7R*,10R*		6S*,7R*,10R*		6R*,7S*,10R*		6S*,7S*,10R*	
	σ	δ	σ	δ	σ	δ	σ	δ
1	144.2	46.0	145.5	44.7	144.8	45.4	144.9	45.3
2	12.7	177.6	14.1	176.2	13.3	177.0	14.2	176.0
3	76.4	113.8	77.9	112.3	75.0	115.2	73.9	116.3
4	170.2	20.1	168.0	22.2	169.5	20.7	168.3	21.9
5	161.6	28.6	155.4	34.8	154.5	35.7	151.9	38.3
6	115.1	75.2	115.7	74.5	114.7	75.6	113.5	76.7
7	146.0	44.2	150.4	39.8	149.9	40.3	151.6	38.6
8	161.0	29.2	163.5	26.8	160.8	29.5	158.8	31.4
9	156.8	33.4	156.3	33.9	159.4	30.8	158.6	31.6
10	96.0	94.2	97.7	92.6	100.0	90.2	99.2	91.0
11	148.1	42.2	148.0	42.2	147.8	42.4	148.3	41.9
12	5.9	184.3	11.9	178.3	6.7	183.6	10.7	179.5
13	168.7	21.5	167.5	22.8	166.7	23.6	169.9	20.3
14	159.2	31.1	159.4	30.9	158.3	31.9	157.4	32.8
15	167.7	22.5	168.4	21.8	166.5	23.7	166.7	23.6

Chemical shifts were obtained as $\delta_{\text{calc}} = \sigma_{\text{TMS}} - \sigma$, where σ_{TMS} represents the nuclear magnetic shielding tensor of the reference compound (tetramethylsilane, TMS).

Table S14. Calculated ^1H NMR nuclear magnetic shielding tensors (σ) and chemical shifts (δ), in ppm, of all possible diastereomers of **7**

Hydrogens	6<i>R</i>*,7<i>R</i>*,10<i>R</i>*		6<i>S</i>*,7<i>R</i>*,10<i>R</i>*		6<i>R</i>*,7<i>S</i>*,10<i>R</i>*		6<i>S</i>*,7<i>S</i>*,10<i>R</i>*	
	σ	δ	σ	δ	σ	δ	σ	δ
1a	29.21	2.97	28.50	3.68	29.27	2.90	28.96	3.21
1b	29.45	2.72	29.66	2.51	29.53	2.64	29.61	2.56
4a	29.60	2.58	29.68	2.49	29.77	2.40	29.82	2.35
4b	30.20	1.98	30.14	2.04	30.21	1.97	30.06	2.11
5a	30.00	2.18	30.44	1.74	30.30	1.88	30.47	1.70
5b	30.57	1.61	30.45	1.73	30.17	2.01	30.37	1.81
6	28.86	3.31	28.52	3.65	28.42	3.76	28.55	3.63
7	30.63	1.54	30.18	2.00	30.62	1.55	30.42	1.76
8a	31.12	1.05	31.16	1.01	30.28	1.90	30.13	2.05
8b	30.76	1.42	30.00	2.18	31.20	0.97	31.09	1.09
9a	30.27	1.90	30.18	2.00	30.07	2.11	30.10	2.08
9b	30.34	1.84	30.30	1.88	30.24	1.94	30.19	1.99
10	27.67	4.51	27.76	4.42	27.75	4.42	27.79	4.38
12a	22.17	10.00	22.21	9.97	21.89	10.29	21.99	10.18
13	31.21	0.97	31.29	0.89	31.22	0.96	31.12	1.06
14	30.91	1.26	30.95	1.23	30.89	1.28	30.90	1.28
15	30.96	1.21	30.90	1.27	30.96	1.22	30.93	1.25

Chemical shifts were obtained as $\delta_{\text{calc}} = \sigma_{\text{TMS}} - \sigma$, where σ_{TMS} represents the nuclear magnetic shielding tensor of the reference compound (tetramethylsilane, TMS)

Table S15. Calculated ^{13}C NMR nuclear magnetic shielding tensors (σ) and chemical shifts (δ), in ppm, of all possible diastereomers of **8**

Carbons	6 <i>R</i> *,10 <i>R</i> *		6 <i>S</i> *,10 <i>R</i> *	
	σ	δ	σ	δ
1	146.9	43.4	145.4	44.8
2	18.7	171.5	16.1	174.1
3	75.4	114.8	78.4	111.9
4	164.0	26.2	165.2	25.1
5	155.7	34.6	158.2	32.1
6	121.6	68.6	123.2	67.0
7	50.0	140.3	52.0	138.2
8	70.8	119.5	67.1	123.1
9	159.9	30.4	155.4	34.9
10	106.6	83.6	97.1	93.1
11	145.0	45.2	146.3	43.9
12	10.7	179.6	11.4	178.8
13	169.9	20.3	169.4	20.8
14	162.3	27.9	159.7	30.6
15	168.7	21.6	168.5	21.7

Chemical shifts were obtained as $\delta_{\text{calc}} = \sigma_{\text{TMS}} - \sigma$, where σ_{TMS} represents the nuclear magnetic shielding tensor of the reference compound (tetramethylsilane, TMS).

Table S16. Calculated ^1H NMR nuclear magnetic shielding tensors (σ) and chemical shifts (δ), in ppm, of all possible diastereomers of **8**

Hydrogens	6<i>R</i>*,10<i>R</i>*		6<i>S</i>*,10<i>R</i>*	
	σ	δ	σ	δ
1a	29.19	2.98	29.18	3.00
1b	29.70	2.48	29.90	2.28
4a	29.71	2.46	29.71	2.46
4b	30.03	2.15	30.44	1.74
5a	29.70	2.47	30.15	2.03
5b	30.59	1.58	30.56	1.62
6	27.53	4.65	27.77	4.41
8a	26.54	5.63	26.36	5.81
9a	29.41	2.76	29.76	2.42
9b	30.12	2.06	29.64	2.53
10	27.99	4.18	27.80	4.38
12a	22.29	9.88	22.11	10.06
13	30.61	1.56	30.43	1.74
14	31.02	1.15	31.00	1.18
15	31.01	1.16	30.84	1.33

Chemical shifts were obtained as $\delta_{\text{calc}} = \sigma_{\text{TMS}} - \sigma$, where σ_{TMS} represents the nuclear magnetic shielding tensor of the reference compound (tetramethylsilane, TMS).

Table S17. Calculated ^{13}C NMR nuclear magnetic shielding tensors (σ) and chemical shifts (δ), in ppm, of all possible diastereomers of **9**

Carbons	3R*,7R*,10R*		3R*,7S*,10R*		3R*,7R*,10S*		3R*,7S*,10S*	
	σ	δ	σ	δ	σ	δ	σ	δ
1	56.5	133.7	57.0	133.2	51.6	138.6	32.5	157.8
2	60.8	129.4	60.7	129.5	57.8	132.4	62.6	127.7
3	115.2	75.1	114.9	75.4	117.5	72.7	109.5	80.7
4	151.8	38.4	153.5	36.8	151.9	38.4	149.8	40.4
5	153.9	36.3	147.7	42.5	152.6	37.7	150.0	40.3
6	-17.1	207.4	-15.9	206.1	-24.2	214.5	-20.4	210.6
7	137.1	53.1	140.9	49.3	142.5	47.7	142.4	47.8
8	157.5	32.8	155.8	34.4	154.5	35.7	153.6	36.6
9	155.4	34.8	155.8	34.4	160.7	29.5	159.3	30.9
10	106.4	83.8	107.8	82.4	110.0	80.2	93.7	96.6
11	147.2	43.0	147.4	42.9	147.1	43.1	133.6	56.6
12	118.5	71.8	119.4	70.8	116.5	73.7	106.4	83.8
13	168.9	21.3	170.1	20.2	171.6	18.7	167.8	22.4
14	152.9	37.3	155.1	35.1	161.0	29.2	170.5	19.7
15	159.3	30.9	160.0	30.3	168.8	21.4	168.2	22.0

Chemical shifts were obtained as $\delta_{\text{calc}} = \sigma_{\text{TMS}} - \sigma$, where σ_{TMS} represents the nuclear magnetic shielding tensor of the reference compound (tetramethylsilane, TMS).

Table S18. Calculated ^1H NMR nuclear magnetic shielding tensors (σ) and chemical shifts (δ), in ppm, of all possible diastereomers of **9**

Hydrogens	3<i>R</i>*,7<i>R</i>*,10<i>R</i>*		3<i>R</i>*,7<i>S</i>*,10<i>R</i>*		3<i>R</i>*,7<i>R</i>*,10<i>S</i>*		3<i>R</i>*,7<i>S</i>*,10<i>S</i>*	
	σ	δ	σ	δ	σ	δ	σ	δ
1a	26.32	5.85	26.35	5.82	26.49	5.69	25.35	6.82
2	26.64	5.54	26.64	5.53	26.42	5.76	26.19	5.99
4a	30.04	2.14	29.74	2.43	29.88	2.29	30.27	1.91
4b	30.30	1.87	30.72	1.45	29.98	2.20	30.29	1.89
5a	29.33	2.84	29.21	2.97	29.48	2.70	29.22	2.96
5b	29.78	2.39	29.98	2.20	29.46	2.72	29.77	2.41
7	29.66	2.52	29.61	2.57	29.10	3.07	29.57	2.60
8a	30.59	1.59	30.41	1.76	30.37	1.81	30.08	2.09
8b	30.14	2.04	30.54	1.63	30.20	1.97	30.79	1.38
9a	30.23	1.95	30.24	1.94	30.48	1.69	30.65	1.52
9b	30.36	1.81	30.36	1.81	30.69	1.48	30.95	1.23
10	28.22	3.95	28.14	4.03	28.35	3.83	28.35	3.83
12a	28.12	4.06	28.26	3.92	28.52	3.66	29.22	2.95
12b	28.43	3.74	28.54	3.64	28.34	3.84	28.45	3.73
13	31.06	1.11	31.17	1.01	31.07	1.10	31.18	0.99
14	30.84	1.34	30.82	1.35	31.24	0.93	30.83	1.35
15	31.06	1.11	31.17	1.00	31.09	1.08	31.02	1.16

Chemical shifts were obtained as $\delta_{\text{calc}} = \sigma_{\text{TMS}} - \sigma$, where σ_{TMS} represents the nuclear magnetic shielding tensor of the reference compound (tetramethylsilane, TMS)

Table S19. Calculated ^{13}C NMR nuclear magnetic shielding tensors (σ) and chemical shifts (δ), in ppm, of all possible diastereomers of **10**

Carbons	6R*,7R*,10R*		6S*,7R*,10R*		6R*,7S*,10R*		6S*,7S*,10R*	
	σ	δ	σ	δ	σ	δ	σ	δ
1	151.7	38.6	151.5	38.7	151.6	38.7	151.5	38.7
2	55.6	134.7	56.7	133.6	54.4	135.8	55.8	134.5
3	54.2	136.1	53.2	137.0	54.0	136.2	53.5	136.7
4	154.3	35.9	158.3	31.9	154.7	35.6	157.3	32.9
5	153.8	36.4	155.2	35.0	158.8	31.5	158.5	31.8
6	116.0	74.3	115.0	75.2	118.2	72.0	114.5	75.8
7	148.1	42.2	151.6	38.6	150.1	40.2	148.2	42.1
8	163.9	26.3	162.7	27.5	162.8	27.4	161.5	28.7
9	163.6	26.7	161.5	28.7	161.8	28.5	160.6	29.6
10	105.2	85.0	103.3	86.9	105.3	84.9	104.7	85.5
11	143.2	47.0	143.9	46.3	143.4	46.8	143.7	46.5
12	25.8	164.5	24.5	165.7	25.5	164.7	25.2	165.1
13	171.5	18.7	170.8	19.5	173.5	16.8	168.6	21.7
14	158.7	31.6	158.6	31.6	158.4	31.8	158.6	31.7
15	165.1	25.1	164.9	25.3	165.2	25.0	165.2	25.0

Chemical shifts were obtained as $\delta_{\text{calc}} = \sigma_{\text{TMS}} - \sigma$, where σ_{TMS} represents the nuclear magnetic shielding tensor of the reference compound (tetramethylsilane, TMS).

Table S20. Calculated ^1H NMR nuclear magnetic shielding tensors (σ) and chemical shifts (δ), in ppm, of all possible diastereomers of **10**

Hydrogens	6<i>R</i>*,7<i>R</i>*,10<i>R</i>*		6<i>S</i>*,7<i>R</i>*,10<i>R</i>*		6<i>R</i>*,7<i>S</i>*,10<i>R</i>*		6<i>S</i>*,7<i>S</i>*,10<i>R</i>*	
	σ	δ	σ	δ	σ	δ	σ	δ
1a	29.84	2.33	29.84	2.34	29.82	2.35	29.82	2.35
1b	30.25	1.93	30.25	1.92	30.24	1.94	30.28	1.90
2	25.65	6.52	25.69	6.48	25.60	6.58	25.65	6.52
4a	29.35	2.82	29.56	2.61	29.36	2.81	29.50	2.68
4b	29.96	2.21	29.79	2.38	30.00	2.18	29.77	2.40
5a	30.12	2.06	30.27	1.90	30.12	2.05	30.27	1.91
5b	30.64	1.54	30.14	2.03	30.84	1.33	30.28	1.89
6	28.58	3.60	28.15	4.02	28.11	4.07	28.20	3.97
7	30.61	1.57	30.39	1.78	30.37	1.80	30.39	1.79
8a	30.65	1.52	30.61	1.56	30.98	1.19	30.78	1.40
8b	30.68	1.50	30.47	1.70	30.70	1.48	30.70	1.47
9a	29.86	2.31	29.22	2.95	29.72	2.46	28.99	3.18
9b	30.55	1.63	30.56	1.61	30.56	1.61	30.57	1.60
10	27.96	4.22	27.95	4.22	27.94	4.24	28.00	4.18
13	31.14	1.04	31.16	1.02	31.33	0.84	31.36	0.81
14	31.03	1.14	31.05	1.13	31.03	1.14	31.05	1.13
15	31.15	1.02	31.15	1.02	31.17	1.01	31.18	0.99

Chemical shifts were obtained as $\delta_{\text{calc}} = \sigma_{\text{TMS}} - \sigma$, where σ_{TMS} represents the nuclear magnetic shielding tensor of the reference compound (tetramethylsilane, TMS).

Table S21. Calculated ^{13}C NMR nuclear magnetic shielding tensors (σ) and chemical shifts (δ), in ppm, of all possible diastereomers of **11**

Carbons	7S*,10S*		7R*,10S*	
	σ	δ	σ	δ
1	140.2	50.0	139.7	50.6
2	41.2	149.0	42.6	147.7
3	38.5	151.7	38.5	151.7
4	148.4	41.8	148.7	41.5
5	147.9	42.3	144.3	45.9
6	-55.8	246.0	-48.9	239.1
7	94.5	95.7	97.4	92.8
8	139.5	50.8	141.7	48.5
9	156.1	34.1	158.1	32.1
10	92.1	98.1	94.2	96.0
11	135.3	54.9	135.2	55.0
12	1.3	189.0	0.9	189.3
13	153.1	37.1	153.1	37.1
14	158.4	31.8	158.2	32.1
15	161.4	28.8	160.5	29.7

Chemical shifts were obtained as $\delta_{\text{calc}} = \sigma_{\text{TMS}} - \sigma$, where σ_{TMS} represents the nuclear magnetic shielding tensor of the reference compound (tetramethylsilane, TMS).

Table S22. Calculated ^1H NMR nuclear magnetic shielding tensors (σ) and chemical shifts (δ), in ppm, of all possible diastereomers of **11**

Hydrogens	7S*,10S*		7R*,10S*	
	σ	δ	σ	δ
1a	30.00	2.17	29.96	2.21
1b	29.93	2.24	29.96	2.21
2	25.42	6.76	25.51	6.66
4a	29.06	3.11	29.05	3.13
4b	29.04	3.14	28.96	3.21
5a	27.96	4.21	28.96	3.21
5b	29.80	2.38	29.44	2.73
8a	29.24	2.93	29.94	2.23
8b	30.24	1.94	29.49	2.69
9a	30.16	2.01	30.39	1.79
9b	30.16	2.02	29.97	2.20
10	28.20	3.98	28.96	3.21
13	30.68	1.50	30.61	1.57
14	30.95	1.23	30.90	1.28
15	31.02	1.15	31.11	1.07

Chemical shifts were obtained as $\delta_{\text{calc}} = \sigma_{\text{TMS}} - \sigma$, where σ_{TMS} represents the nuclear magnetic shielding tensor of the reference compound (tetramethylsilane, TMS).

Table S23. Calculated ^{13}C NMR nuclear magnetic shielding tensors (σ) and chemical shifts (δ), in ppm, of all possible diastereomers of **12**

Carbons	$3R^*,6R^*,7R^*,10R^*$		$3R^*,6R^*,7R^*,10S^*$		$3S^*,6R^*,7R^*,10R^*$		$3R^*,6S^*,7R^*,10R^*$		$3R^*,6R^*,7S^*,10R^*$		$3S^*,6R^*,7R^*,10S^*$		$3R^*,6S^*,7R^*,10S^*$		$3R^*,6R^*,7S^*,10S^*$	
	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ
1	135.7	54.6	129.9	60.3	128.3	62.0	136.0	54.2	135.9	54.3	135.9	54.3	130.2	60.0	128.4	61.8
2	-24.6	214.9	-20.0	210.2	-20.6	210.9	-30.9	221.1	-19.0	209.2	-17.5	207.7	-25.8	216.0	-21.1	211.4
3	125.2	65.0	124.5	65.7	124.6	65.6	123.4	66.8	124.4	65.9	125.1	65.1	122.5	67.7	123.2	67.0
4	158.4	31.8	163.8	26.5	163.3	26.9	153.8	36.4	155.0	35.2	159.6	30.6	162.7	27.5	159.7	30.6
5	157.1	33.1	157.6	32.6	157.5	32.7	153.3	36.9	157.3	33.0	159.3	30.9	154.7	35.5	155.5	34.7
6	110.5	79.7	110.5	79.8	111.1	79.1	106.8	83.4	105.9	84.3	109.8	80.4	109.0	81.2	106.5	83.8
7	135.5	54.8	127.0	63.2	130.3	59.9	133.0	57.3	134.5	55.8	138.3	51.9	124.2	66.0	127.7	62.5
8	156.6	33.6	144.6	45.7	149.6	40.7	159.0	31.3	160.2	30.1	156.5	33.7	149.8	40.5	155.2	35.1
9	158.9	31.4	158.9	31.3	161.8	28.4	159.3	31.0	161.0	29.2	162.4	27.8	159.3	30.9	161.6	28.7
10	107.3	83.0	110.9	79.3	111.8	78.5	107.5	82.8	110.5	79.7	110.0	80.2	110.9	79.3	112.7	77.6
11	149.0	41.2	137.6	52.6	138.2	52.0	148.0	42.2	149.2	41.0	150.0	40.2	135.9	54.4	138.3	52.0
12	120.2	70.0	123.3	66.9	118.9	71.4	120.2	70.0	120.1	70.1	120.8	69.4	123.1	67.1	117.9	72.3
13	175.0	15.2	172.6	17.7	174.6	15.6	167.5	22.7	165.1	25.2	172.4	17.8	164.9	25.3	167.2	23.0
14	157.3	32.9	165.8	24.4	165.4	24.8	157.2	33.1	160.1	30.1	160.7	29.5	165.9	24.3	165.4	24.9
15	162.3	27.9	167.6	22.6	167.4	22.9	163.3	26.9	162.3	27.9	162.1	28.2	167.6	22.7	167.4	22.9

Chemical shifts were obtained as $\delta_{\text{calc}} = \sigma_{\text{TMS}} - \sigma$, where σ_{TMS} represents the nuclear magnetic shielding tensor of the reference compound (tetramethylsilane, TMS).

Table S24. Calculated ^1H NMR nuclear magnetic shielding tensors (σ) and chemical shifts (δ), in ppm, of all possible diastereomers of **12**

Hydrogens	$3R^*,6R^*,7R^*,10R^*$		$3R^*,6R^*,7R^*,10S^*$		$3S^*,6R^*,7R^*,10R^*$		$3R^*,6S^*,7R^*,10R^*$		$3R^*,6R^*,7S^*,10R^*$		$3S^*,6R^*,7R^*,10S^*$		$3R^*,6S^*,7R^*,10S^*$		$3R^*,6R^*,7S^*,10S^*$	
	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ
1a	29.15	3.03	28.90	3.28	30.13	2.04	28.95	3.22	29.66	2.51	29.38	2.79	28.80	3.38	28.85	3.32
1b	30.02	2.16	30.28	1.90	28.91	3.27	29.99	2.19	29.54	2.64	29.78	2.39	30.14	2.03	30.16	2.01
4a	30.67	1.51	30.81	1.37	30.96	1.21	30.33	1.84	30.00	2.17	31.19	0.98	30.57	1.61	30.32	1.86
4b	30.88	1.29	30.51	1.66	30.36	1.82	30.80	1.37	30.75	1.42	29.83	2.35	30.61	1.57	30.55	1.62
5a	30.31	1.86	30.59	1.59	30.54	1.63	29.62	2.55	30.60	1.58	30.75	1.42	29.74	2.43	30.57	1.60
5b	30.31	1.86	30.04	2.13	30.40	1.77	30.47	1.71	30.05	2.12	30.30	1.88	30.38	1.79	30.06	2.11
6	27.79	4.38	27.71	4.47	28.23	3.94	28.39	3.79	28.17	4.01	27.88	4.29	28.46	3.71	28.18	3.99
8a	30.59	1.59	30.29	1.88	30.24	1.94	30.86	1.32	29.67	2.50	30.48	1.70	30.64	1.53	29.50	2.67
8b	30.72	1.46	30.65	1.52	30.11	2.07	30.28	1.89	30.71	1.46	30.02	2.16	29.91	2.27	30.64	1.53
9a	30.11	2.07	30.65	1.52	30.45	1.72	30.09	2.09	30.06	2.12	30.25	1.92	30.59	1.58	30.48	1.70
9b	30.31	1.86	30.13	2.04	30.54	1.63	30.29	1.88	30.27	1.91	30.11	2.07	30.10	2.07	30.39	1.79
10	28.23	3.95	28.23	3.95	28.36	3.81	28.24	3.93	28.34	3.83	28.33	3.84	28.42	3.76	28.35	3.82
12a	27.98	4.20	28.16	4.01	28.06	4.11	27.91	4.27	28.35	3.82	28.09	4.09	28.03	4.14	28.05	4.12
12b	28.37	3.81	28.32	3.86	28.20	3.97	28.46	3.71	27.38	4.79	28.56	3.62	28.35	3.83	27.58	4.59
13	30.97	1.21	31.02	1.16	31.25	0.93	30.98	1.20	31.30	0.87	31.31	0.87	31.04	1.14	31.21	0.96
14	30.97	1.20	31.09	1.09	31.08	1.09	30.96	1.22	31.04	1.14	31.08	1.10	31.05	1.12	31.08	1.10
15	31.17	1.00	31.09	1.09	31.07	1.11	31.15	1.03	31.21	0.97	31.23	0.95	31.05	1.13	31.05	1.12

Chemical shifts were obtained as $\delta_{\text{calc}} = \sigma_{\text{TMS}} - \sigma$, where σ_{TMS} represents the nuclear magnetic shielding tensor of the reference compound (tetramethylsilane, TMS).

Table S25. Calculated ^{13}C NMR nuclear magnetic shielding tensors (σ) and chemical shifts (δ), in ppm, of all possible diastereomers of **13** and **14**

Carbons	6R*,7S*,10R*		6R*,7R*,10R*		6R*,7S*,10S*		6S*,7S*,10R*	
	σ	δ	σ	δ	σ	δ	σ	δ
1	130.9	59.3	132.0	58.2	132.0	58.3	131.5	58.7
2	-2.5	192.8	1.2	189.1	1.3	188.9	-2.7	193.0
3	64.5	125.8	66.5	123.7	67.3	122.9	66.1	124.1
4	163.3	27.0	167.2	23.0	167.2	23.0	166.0	24.3
5	158.8	31.4	158.3	31.9	159.7	30.5	159.9	30.3
6	116.7	73.6	110.7	79.5	114.0	76.2	114.6	75.7
7	139.9	50.3	142.3	48.0	143.1	47.2	141.5	48.7
8	152.0	38.2	162.0	28.2	160.5	29.7	153.6	36.6
9	163.9	26.4	166.3	23.9	166.1	24.1	162.8	27.5
10	100.3	90.0	109.7	80.6	109.8	80.4	100.6	89.6
11	145.4	44.9	145.1	45.1	145.2	45.0	146.0	44.3
12	17.7	172.5	13.2	177.0	13.1	177.1	17.4	172.8
13	168.3	21.9	170.5	19.7	173.7	16.5	163.7	26.5
14	162.1	28.1	165.6	24.6	165.7	24.6	162.9	27.3
15	155.4	34.8	159.2	31.0	159.2	31.0	156.3	33.9

Chemical shifts were obtained as $\delta_{\text{calc}} = \sigma_{\text{TMS}} - \sigma$, where σ_{TMS} represents the nuclear magnetic shielding tensor of the reference compound (tetramethylsilane, TMS).

Table S26. Calculated ^1H NMR nuclear magnetic shielding tensors (σ) and chemical shifts (δ), in ppm, of all possible diastereomers of **13** and **14**

Hydrogens	6<i>R</i>*,7<i>S</i>*,10<i>R</i>*		6<i>R</i>*,7<i>R</i>*,10<i>R</i>*		6<i>R</i>*,7<i>S</i>*,10<i>S</i>*		6<i>S</i>*,7<i>S</i>*,10<i>R</i>*	
	σ	δ	σ	δ	σ	δ	σ	δ
1a	28.53	3.65	29.11	3.07	29.15	3.02	28.65	3.53
1b	29.74	2.44	30.24	1.93	30.30	1.88	29.85	2.33
4a	29.53	2.64	29.66	2.51	29.79	2.39	29.74	2.44
4b	29.66	2.51	29.89	2.28	29.82	2.35	29.66	2.51
5a	30.08	2.10	29.97	2.21	30.10	2.07	30.19	1.99
5b	30.09	2.08	30.54	1.64	30.66	1.52	30.12	2.05
6	27.95	4.22	28.07	4.11	28.20	3.97	27.95	4.22
8a	29.71	2.46	29.88	2.29	30.24	1.93	30.08	2.10
8b	29.93	2.24	30.88	1.29	30.61	1.57	29.63	2.55
9a	30.01	2.16	30.07	2.11	30.08	2.09	30.06	2.11
9b	30.05	2.13	30.11	2.07	30.14	2.03	29.87	2.30
10	27.35	4.82	27.88	4.29	27.92	4.26	27.50	4.68
13	30.56	1.62	30.94	1.23	30.86	1.32	30.67	1.51
14	30.77	1.41	31.20	0.98	31.19	0.98	30.87	1.30
15	30.61	1.57	31.11	1.07	31.10	1.07	30.77	1.40

Chemical shifts were obtained as $\delta_{\text{calc}} = \sigma_{\text{TMS}} - \sigma$, where σ_{TMS} represents the nuclear magnetic shielding tensor of the reference compound (tetramethylsilane, TMS).

Table S27. Calculated ^{13}C NMR nuclear magnetic shielding tensors (σ) and chemical shifts (δ), in ppm, of all possible diastereomers of **15** and **16**

Carbons	6 <i>R</i> *,7 <i>R</i> *, 10 <i>R</i> *,12 <i>S</i> *		6 <i>R</i> *,7 <i>R</i> *, 10 <i>R</i> *,12 <i>R</i> *		6 <i>R</i> *,7 <i>S</i> *, 10 <i>R</i> *,12 <i>S</i> *		6 <i>S</i> *,7 <i>R</i> *, 10 <i>R</i> *,12 <i>S</i> *		6 <i>R</i> *,7 <i>S</i> *, 10 <i>R</i> *,12 <i>R</i> *		6 <i>S</i> *,7 <i>S</i> *, 10 <i>R</i> *,12 <i>S</i> *		6 <i>R</i> *,7 <i>S</i> *, 10 <i>S</i> *,12 <i>S</i> *		6 <i>S</i> *,7 <i>S</i> *, 10 <i>R</i> *,12 <i>R</i> *	
	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ
1	-14.7	204.9	-7.2	197.4	-8.2	198.4	-14.0	204.3	-7.6	197.8	-8.5	198.7	-7.1	197.3	-9.0	199.2
2	63.3	126.9	69.7	120.5	67.7	122.6	62.3	127.9	67.2	123.0	67.9	122.3	69.0	121.3	67.5	122.7
3	37.4	152.8	31.9	158.4	33.2	157.0	34.0	156.3	29.7	160.5	32.0	158.2	33.7	156.5	28.4	161.8
4	167.6	22.7	154.4	35.8	163.5	26.7	162.8	27.4	154.9	35.4	163.5	26.7	156.3	33.9	155.6	34.6
5	159.7	30.5	154.3	36.0	159.3	30.9	159.0	31.2	155.9	34.4	158.6	31.7	154.5	35.7	156.7	33.6
6	117.4	72.9	113.0	77.2	116.1	74.1	116.7	73.5	116.0	74.2	115.3	74.9	112.2	78.0	114.4	75.8
7	151.6	38.7	140.4	49.8	149.3	40.9	150.1	40.1	143.3	47.0	148.9	41.3	142.8	47.4	144.2	46.1
8	159.4	30.8	162.1	28.1	152.4	37.9	155.3	35.0	160.0	30.2	152.6	37.7	161.0	29.2	162.6	27.6
9	163.1	27.2	164.3	25.9	164.0	26.2	163.6	26.7	167.3	23.0	161.5	28.7	165.2	25.0	166.0	24.2
10	103.5	86.7	113.9	76.3	105.5	84.7	105.7	84.5	113.8	76.4	104.9	85.3	113.7	76.5	113.2	77.0
11	125.7	64.5	131.5	58.7	125.8	64.4	124.9	65.4	134.4	55.8	125.1	65.1	131.8	58.4	133.8	56.5
12	110.7	79.5	113.5	76.7	112.1	78.1	111.0	79.2	110.0	80.2	114.6	75.6	110.0	80.2	109.8	80.4
13	168.5	21.7	164.9	25.3	175.1	15.1	175.3	14.9	167.4	22.8	169.0	21.2	170.7	19.5	159.8	30.4
14	167.2	23.0	167.3	23.0	164.8	25.4	163.4	26.8	166.2	24.0	164.2	26.0	167.9	22.4	166.4	23.9
15	157.9	32.3	158.1	32.1	162.3	28.0	157.5	32.7	161.0	29.2	161.6	28.6	158.7	31.5	161.5	28.7

Chemical shifts were obtained as $\delta_{\text{calc}} = \sigma_{\text{TMS}} - \sigma$, where σ_{TMS} represents the nuclear magnetic shielding tensor of the reference compound (tetramethylsilane, TMS).

Table S28. Calculated ^1H NMR nuclear magnetic shielding tensors (σ) and chemical shifts (δ), in ppm, of all possible diastereomers of **15** and **16**

Hydrogens	6<i>R</i>*,7<i>R</i>*, 10<i>R</i>*,12<i>S</i>*		6<i>R</i>*,7<i>R</i>*, 10<i>R</i>*,12<i>R</i>*		6<i>R</i>*,7<i>S</i>*, 10<i>R</i>*,12<i>S</i>*		6<i>S</i>*,7<i>R</i>*, 10<i>R</i>*,12<i>S</i>*		6<i>R</i>*,7<i>S</i>*, 10<i>R</i>*,12<i>R</i>*		6<i>S</i>*,7<i>S</i>*, 10<i>R</i>*,12<i>S</i>*		6<i>R</i>*,7<i>S</i>*, 10<i>S</i>*,12<i>S</i>*		6<i>S</i>*,7<i>S</i>*, 10<i>R</i>*,12<i>R</i>*	
	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ	σ	δ
2	26.53	5.65	26.39	5.78	26.64	5.53	26.62	5.55	26.46	5.72	26.49	5.68	26.43	5.74	26.58	5.59
4a	29.20	2.97	30.00	2.17	29.30	2.88	29.25	2.92	29.33	2.85	29.30	2.88	29.60	2.57	29.70	2.48
4b	29.76	2.42	30.11	2.06	29.71	2.46	29.59	2.59	30.10	2.08	29.39	2.78	30.10	2.08	29.99	2.18
5a	30.24	1.94	29.99	2.19	30.20	1.97	30.41	1.76	30.26	1.91	29.89	2.29	30.11	2.07	30.30	1.88
5b	30.32	1.86	30.33	1.85	30.47	1.70	30.66	1.51	30.29	1.88	30.36	1.82	30.52	1.65	30.50	1.67
6	28.31	3.86	28.14	4.04	28.36	3.82	27.94	4.24	28.40	3.77	27.94	4.23	28.24	3.93	28.43	3.74
8a	29.75	2.43	30.22	1.96	30.16	2.02	30.25	1.92	30.30	1.87	29.81	2.37	30.45	1.72	30.78	1.39
8b	30.88	1.30	30.77	1.40	30.44	1.73	30.69	1.48	30.78	1.39	30.32	1.85	30.50	1.68	29.86	2.32
9a	30.34	1.84	30.17	2.01	30.27	1.91	30.43	1.74	30.18	2.00	30.04	2.14	30.24	1.94	30.42	1.76
9b	30.01	2.17	30.11	2.06	30.25	1.93	30.17	2.00	30.22	1.96	30.05	2.13	30.27	1.90	29.97	2.21
10	27.98	4.20	28.15	4.03	28.13	4.05	28.09	4.09	28.31	3.87	27.96	4.21	28.20	3.97	28.23	3.95
12	27.86	4.32	27.04	5.13	27.74	4.44	28.01	4.16	27.60	4.57	26.90	5.27	28.33	3.85	28.01	4.16
13	31.21	0.96	31.32	0.85	31.10	1.07	31.26	0.92	30.84	1.33	30.96	1.21	31.40	0.77	30.90	1.27
14	30.95	1.23	31.11	1.06	31.11	1.07	30.95	1.23	31.17	1.01	30.94	1.24	31.18	1.00	31.22	0.95
15	30.87	1.30	30.78	1.39	30.90	1.27	30.90	1.27	30.82	1.36	30.70	1.47	30.86	1.31	30.86	1.31

Chemical shifts were obtained as $\delta_{\text{calc}} = \sigma_{\text{TMS}} - \sigma$, where σ_{TMS} represents the nuclear magnetic shielding tensor of the reference compound (tetramethylsilane, TMS).

Table S29. Calculated ^{13}C NMR nuclear magnetic shielding tensors (σ) and chemical shifts (δ), in ppm, of all possible diastereomers of **17**

Carbons	6S*,7S*,10R*		6S*,7S*,10S*		6S*,7R*,10R*		6S*,7R*,10S*	
	σ	δ	σ	δ	σ	δ	σ	δ
1	45.0	145.2	33.3	157.0	54.2	136.0	47.4	142.8
2	61.4	128.9	64.1	126.2	65.2	125.0	62.1	128.2
3	-6.6	196.9	-6.5	196.8	-11.0	201.2	-12.2	202.4
4	147.5	42.7	151.9	38.3	149.8	40.4	148.3	42.0
5	160.8	29.4	158.4	31.9	158.7	31.5	159.8	30.4
6	113.8	76.4	112.9	77.3	118.4	71.8	114.5	75.7
7	103.2	87.0	109.6	80.6	105.5	84.7	102.7	87.5
8	151.7	38.5	154.9	35.3	149.2	41.0	152.7	37.5
9	158.4	31.9	161.3	28.9	160.4	29.8	158.5	31.8
10	102.4	87.9	97.5	92.8	108.6	81.6	102.9	87.4
11	146.3	43.9	147.3	42.9	146.4	43.8	146.7	43.5
13	163.4	26.8	164.1	26.1	168.4	21.8	164.9	25.4
14	164.8	25.5	167.3	22.9	167.4	22.8	159.9	30.4
15	165.3	24.9	168.6	21.6	163.4	26.8	157.5	32.8

Chemical shifts were obtained as $\delta_{\text{calc}} = \sigma_{\text{TMS}} - \sigma$, where σ_{TMS} represents the nuclear magnetic shielding tensor of the reference compound (tetramethylsilane, TMS).

Table S30. Calculated ^1H NMR nuclear magnetic shielding tensors (σ) and chemical shifts (δ), in ppm, of all possible diastereomers of **17**

Hydrogens	6 <i>S</i> *,7 <i>S</i> *,10 <i>R</i> *		6 <i>S</i> *,7 <i>S</i> *,10 <i>S</i> *		6 <i>S</i> *,7 <i>R</i> *,10 <i>R</i> *		6 <i>S</i> *,7 <i>R</i> *,10 <i>S</i> *	
	σ	δ	σ	δ	σ	δ	σ	δ
1a	25.61	6.56	25.28	6.89	26.68	5.49	26.05	6.12
2	25.46	6.71	25.46	6.72	26.21	5.97	25.80	6.37
4a	29.56	2.61	29.47	2.71	28.95	3.22	29.51	2.67
4b	30.14	2.04	30.17	2.00	29.66	2.51	29.54	2.64
5a	29.26	2.92	30.01	2.16	30.02	2.16	29.98	2.19
5b	30.39	1.79	30.10	2.08	30.42	1.75	30.01	2.16
6	28.21	3.96	28.17	4.01	27.85	4.33	28.38	3.79
8a	29.88	2.29	29.51	2.67	29.94	2.24	30.19	1.98
8b	30.43	1.75	30.50	1.68	30.18	2.00	30.25	1.93
9a	30.02	2.16	30.66	1.51	30.31	1.86	30.23	1.95
9b	30.38	1.79	30.43	1.74	30.34	1.83	30.31	1.87
10	28.05	4.13	27.63	4.54	28.12	4.05	28.04	4.13
13	31.05	1.12	31.14	1.04	30.89	1.28	30.82	1.36
14	31.04	1.13	31.03	1.14	31.02	1.16	30.98	1.19
15	30.91	1.27	31.01	1.16	31.05	1.12	30.49	1.68

Chemical shifts were obtained as $\delta_{\text{calc}} = \sigma_{\text{TMS}} - \sigma$, where σ_{TMS} represents the nuclear magnetic shielding tensor of the reference compound (tetramethylsilane, TMS).

Table S31. Calculated ^{13}C NMR nuclear magnetic shielding tensors (σ) and chemical shifts (δ), in ppm, of all possible diastereomers of **18**

Carbons	2S*,6S*,7R*		2S*,6R*,7S*		2S*,6R*,7R*		2S*,6S*,7S*	
	σ	δ	σ	δ	σ	δ	σ	δ
1	147.9	42.3	147.3	42.9	150.0	40.2	150.3	39.9
2	113.8	76.4	113.6	76.6	115.2	75.1	114.7	75.5
3	29.3	160.9	29.6	160.6	31.7	158.5	33.4	156.8
4	161.4	28.8	162.7	27.5	160.5	29.7	161.7	28.6
5	156.9	33.3	158.9	31.3	160.0	30.2	160.4	29.8
6	113.0	77.2	114.3	75.9	110.5	79.7	113.7	76.5
7	117.2	73.0	116.5	73.7	115.8	74.4	119.7	70.5
8	153.8	36.4	155.5	34.8	153.5	36.7	154.2	36.0
9	59.3	130.9	60.1	130.2	59.7	130.6	58.5	131.7
10	-7.6	197.9	-5.4	195.7	-5.5	195.7	-11.9	202.2
11	145.0	45.2	145.6	44.7	146.8	43.4	147.2	43.1
13	162.2	28.0	164.9	25.3	170.3	19.9	162.5	27.7
14	160.4	29.9	161.2	29.0	162.0	28.2	162.5	27.7
15	162.3	28.0	163.2	27.0	163.8	26.4	163.6	26.7
1'	124.3	65.9	124.6	65.6	125.3	64.9	124.5	65.7
2'	171.6	18.6	172.1	18.1	173.2	17.0	173.1	17.2

Chemical shifts were obtained as $\delta_{\text{calc}} = \sigma_{\text{TMS}} - \sigma$, where σ_{TMS} represents the nuclear magnetic shielding tensor of the reference compound (tetramethylsilane, TMS).

Table S32. Calculated ^1H NMR nuclear magnetic shielding tensors (σ) and chemical shifts (δ), in ppm, of all possible diastereomers of **18**

Hydrogens	2S*,6S*,7R*		2S*,6R*,7S*		2S*,6R*,7R*		2S*,6S*,7S*	
	σ	δ	σ	δ	σ	δ	σ	δ
1a	29.72	2.46	29.73	2.44	30.03	2.14	30.18	1.99
1b	29.95	2.22	30.12	2.06	30.22	1.95	29.94	2.23
2	27.53	4.64	27.67	4.50	27.81	4.36	27.95	4.23
4a	29.10	3.07	29.66	2.51	29.69	2.49	29.12	3.05
4b	29.41	2.77	28.92	3.25	29.42	2.76	30.00	2.18
5a	30.12	2.05	30.14	2.03	30.23	1.95	30.55	1.62
5b	30.03	2.15	29.97	2.21	30.69	1.48	30.16	2.02
6	28.22	3.96	28.13	4.05	28.38	3.80	28.50	3.67
8a	29.04	3.14	29.37	2.81	30.12	2.05	29.34	2.83
8b	29.44	2.73	29.11	3.06	29.13	3.05	29.69	2.49
13	30.69	1.48	30.89	1.28	31.19	0.99	30.97	1.20
14	30.67	1.51	30.80	1.38	30.95	1.22	30.79	1.39
15	30.70	1.47	30.82	1.35	31.00	1.17	31.05	1.13
1'a	27.96	4.22	28.04	4.13	28.23	3.94	28.20	3.98
1'b	28.42	3.76	28.48	3.69	28.64	3.53	28.65	3.53
2'	30.64	1.53	30.73	1.45	30.92	1.26	30.89	1.28

Chemical shifts were obtained as $\delta_{\text{calc}} = \sigma_{\text{TMS}} - \sigma$, where σ_{TMS} represents the nuclear magnetic shielding tensor of the reference compound (tetramethylsilane, TMS).

Table S33. Cytotoxicity of compounds **1–18** in stimulated BV2 microglia cell line

Compound	Cell viability (%)			
	25 μ M	50 μ M	100 μ M	200 μ M
Control	100 $\pm$ 5.9			
1	91.8 $\pm$ 1 ^a	91.8 $\pm$ 1.6 ^a	91.07 $\pm$ 1.1 ^a	92.5 $\pm$ 1 ^a
2	82.03 $\pm$ 2 ^a	80.3 $\pm$ 3.9 ^a	82.8 $\pm$ 2.1 ^a	79.9 $\pm$ 1.1 ^a
3	101.0 $\pm$ 3.9	95.9 $\pm$ 4.6	95.1 $\pm$ 2.8	100.5 $\pm$ 3.9
4	107.9 $\pm$ 4.5	103.2 $\pm$ 4.9	106.1 $\pm$ 5	99.5 $\pm$ 4.8
5	100.9 $\pm$ 1.6	100.8 $\pm$ 2.2	101.3 $\pm$ 0.9	101.6 $\pm$ 2.1
6	97.4 $\pm$ 1.6	83.8 $\pm$ 6.9	89.7 $\pm$ 1.3	86.3 $\pm$ 1.4
7	108.6 $\pm$ 3.8	102.9 $\pm$ 5.9	95.7 $\pm$ 1.8	94.8 $\pm$ 3.7
8	108.7 $\pm$ 0.5	104.4 $\pm$ 1.2	97.3 $\pm$ 0.7	90.7 $\pm$ 2.6 ^a
9	102.2 $\pm$ 0.7	100.9 $\pm$ 1.6	96.4 $\pm$ 1.5	90.0 $\pm$ 0.9 ^a
10	103.7 $\pm$ 1.5	96.8 $\pm$ 2.5	95.4 $\pm$ 1.6	91.1 $\pm$ 1.2 ^a
11	96.76 $\pm$ 2.9	99.4 $\pm$ 3.9	98.9 $\pm$ 3.6	103.6 $\pm$ 4.9
12	93.02 $\pm$ 0.9	93.3 $\pm$ 2.2	92.2 $\pm$ 1.8	96.9 $\pm$ 3.4
13	96.1 $\pm$ 1.7	88.4 $\pm$ 0.8 ^a	88.3 $\pm$ 0.7 ^a	91.6 $\pm$ 2.4
14	106.6 $\pm$ 0.8	101.8 $\pm$ 0.2	93.9 $\pm$ 1.9	87.4 $\pm$ 1.5 ^a
15	92.9 $\pm$ 1.8	93.8 $\pm$ 2.5	102.9 $\pm$ 2.9	99.3 $\pm$ 2.7
16	101.3 $\pm$ 1.6	96.8 $\pm$ 1.4	106.6 $\pm$ 5.5	109.6 $\pm$ 6.6
17	103.6 $\pm$ 4.4	92.2 $\pm$ 4.4	90.4 $\pm$ 1.1	89.4 $\pm$ 2.2
18	84.92 $\pm$ 0.2 ^a	78.9 $\pm$ 1 ^a	80.2 $\pm$ 1 ^a	74.7$\pm$0.3^a

Values represent means $\pm$ Standard Error of the Mean (SEM). n = 5. ^aDifferent from the vehicle-treated control group, $p < 0.05$.

6. CONCLUSÕES

O estudo fitoquímico do extrato etanólico das raízes de *Anaxagorea dolichocarpa* resultou no isolamento de dezenove substâncias, as quais tiveram suas estruturas determinadas inequivocamente por meio da análise dos dados de RMN, HRESIMS e IV, juntamente com cálculos quânticos de RMN e ECD. Foram isolados dezoito sesquiterpenos macrocíclicos do tipo humuleno inéditos na literatura, denominados de dolichocarpols G-X (**1-18**) e um que já havia sido isolado previamente (nordine), porém neste trabalho foi feita a correção da sua estrutura bem como a determinação da sua estereoquímica absoluta por Raios-X e ECD.

Os compostos dolichocarpols G-X foram testados a fim de avaliar sua citotoxicidade e atividade antineuroinflamatória em uma linhagem celular BV2 estimulada com LPS/IFN- γ . Apenas o composto dolichocarpol X apresentou redução na viabilidade celular acima de 20% a 200 μ M. No entanto, a viabilidade das células permaneceu inalterada pelos outros compostos, com reduções que não excederam 20% em todas as concentrações testadas. Os compostos dolichocarpols G, K, N, O e T além de não apresentarem citotoxicidade aparente, reduziram significativamente os níveis de NO nas células BV2 em concentrações variando de 25 a 200 μ M, chegando a atingir um efeito máximo semelhante ao do controle (quercetina).

Nos estudos de docking molecular, os compostos dolichocarpols G, K, N, O e T apresentaram valores de energia de ligação negativa e valores de probabilidade de atividade superiores a 0,5, indicando afinidade pelo alvo (i-Nos). Os compostos que apresentaram maior estabilidade foram K e N com valores de $p = 0,6988$ e $0,7475$, respectivamente. Já na dinâmica molecular, o composto dolichocarpol O foi o que apresentou maior estabilidade, indicando que os complexos são estáveis, com baixas flutuações na estrutura terciária da enzima.

Por fim, os resultados indicam que a metodologia utilizada mostrou-se adequada para o isolamento de sesquiterpenos, demonstrando que existe uma considerável presença desses na espécie, corroborando com estudos anteriores. Dessa forma, esses achados ampliam a compreensão sobre a diversidade estrutural e atividade biológica dos sesquiterpenos macrocíclicos humulenos da família Annonaceae e da espécie *Anaxagorea dolichocarpa*, contribuindo para o seu conhecimento fitoquímico e farmacológico.

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