Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Strain and genome sequencing

Streptomyces malaysiensis DSM 4137 was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany). For whole-genome shotgun sequencing, TruSeq shotgun, TruSeq PCR-free shotgun and Nextera mate-pair libraries were constructed from high molecular weight genomic DNA, using the manufacturer's protocols. Sequencing was carried out on an Illumina MiSeq platform using the Illumina V2 500 cycle kit run in 2 x 250 bp mode. Reads were processed using an in-house Illumina adapter trimming tool (fastq_miseq_trimmer).[1] Read pairs were then pre-assembled using FLASH v1.2.11 [https://ccb.jhu.edu/software/FLASH/].[2] Nextera matepair linker sequence was used for splitting of matepair reads (fastq_miseq_trimmer). Sequence assembly was done using the Phrap, Ngen (DNAstar) and Newbler assemblers and editing was done using consed version 16. Repeats were resolved by doing a mini-assembly for the individual sections of the genome, and the resulting consensus was integrated into the main genome assembly. The complete genome sequence will be reported separately (M. Samborskyy *et al*., *ms* in preparation).

Gene cloning by homologous recombination in yeast

Genomic DNA was isolated from *Streptomyces malaysiensis* DSM 4137 grown in Gym 65 liquid culture (glucose: 4.0 g, yeast extract: 4.0 g, malt extract: 4.0 g, water: 1 L, pH 7.2). Genome sequencing reveiled the presence of four terpene cyclase genes whose translated amino acid sequences are shown in Figure S1. The gene of the isoafricanol synthase was amplified from genomic DNA using the short primers listed in Table S1. The obtained PCR product was used as a template in a second PCR with the long primers in Table S1 elongated with homology arms (underlined) for homologous recombination with the HindIII and EcoRI digested vector pYE-Express[3] in *S. cerevisiae* FY834. Transformation of the yeast with the PCR product and the linearised vector was performed using the LiOAc/SS carrier DNA protocol,[4] followed by plating of the cells on SM-URA[4] agar and growth for three days at 28 °C to allow for homologous recombination. Plasmid DNA was isolated from the yeast using the Zymoprep Yeast Plasmid Miniprep II kit (Zymo Research, Irvine, USA) and subsequently shuttled into *E. coli* BL 21 cells by electroporation. The plasmid sequence was confirmed by sequencing.

Table S1 Primers for cloning of the isoafricanol synthase.

Primer	**Sequence**
PR117f_Isoafricanol	ATGCACACGCACGCTTCGCG
PR117r_Isoafricanol	CTACGCGAGCTGAGAGGGC
PR118f_Isoafricanol	GGCAGCCATATGGCTAGCATGACTGGTGGAATGCACACGCACGCTTCGCG
PR118r_Isoafricanol	TCTCAGTGGTGGTGGTGGTGGTGCTCGAGTCTACGCGAGCTGAGAGGGC

Incubation experiment of purified enzyme with FPP and product isolation

E. coli BL 21 transformants were inoculated in a 2YT liquid preculture (tryptone: 16 g, yeast extract: 10 g, NaCl: 5 g, water: 1 L, pH 7.2) containing kanamycin (50 mg/L) over night. The preculture was used to inoculate 2YT expression cultures (6 x 1 L) containing kanamycin (50 mg/L). Cells were grown to an OD_{600} = 0.4 at 37 °C and 160 rpm, followed by cooling of the cultures to 18 °C for 30 minutes. IPTG (0.4 μM) was added and the culture was incubated at 18 °C and 160 rpm overnight. *E. coli* cells were harvested by centrifugation at 4 °C and 3600 rpm for 30 min. The supernatant was discarded and the cell pellets were resuspended in 2 x 10 mL lysis buffer (20 mM TRIS, 20 mM imidazole, 1 mM $MgCl_2$, pH 7.0) for each 1 L culture. Cell disruption was done by ultra-sonication on ice for 6 x 60 sec. The soluble enzyme fractions were harvested at 4 °C and 8000 rpm by repeated centrifugation (2 x 10 min). Protein purification was performed by Ni^{2+}-NTA affinity chromatography with Ni^{2+}-NTA superflow (Novagen) using binding buffer (20 mM TRIS, 20 mM imidazole, 1 mM $MgCl_2$, pH 7.0) and elution buffer (20 mM TRIS, 500 mM imidazole, 1 mM $MgCl_2$, pH 7.0). All wash and elution fraction were checked by SDS-PAGE. Incubation experiments were performed with the pure protein fractions (60 mL, 0.39 mg/mL by Bradford assay) and incubation buffer (40 mL, 50 mM TRIS,1 mM $MgCl_2$, pH 7.0) containing FPP (20 mg, 0.5 mg/mL) at 28 °C over night. The reaction mixture was extracted with hexane (3 x 60 mL). The combined organic layers were dried with $MgSO_4$ and concentrated under reduced pressure. Column chromatography on silica gel of the crude product with pentane/diethyl ether (5:1) yielded the pure sesquiterpene alcohol for structure elucidation by NMR.

For the experiments to study the enzyme mechanism 0.8 mg of the labelled substrates (12-^{13}C)FPP,[5] (13-^{13}C)FPP,[5] (3-^{13}C,2-^{2}H)FPP[6] and (*R*)- or (*S*)-(1-^{13}C,1-^{2}H)FPP[7] dissolved in incubation buffer (3 mL) were incubated with a preparation of isoafricanol synthase (3 mL) under the same conditions as described above. The substrates (2-^{13}C,1,1-$^{2}H_2$)DMAPP[6] (0.5 mg) + IPP (1 mg) and (*R*)- or (*S*)-(1-^{13}C,1-^{2}H)GPP[7] (1 mg) + IPP (1 mg), dissolved in incubation buffer (3 mL), were incubated with preparations of *S. coelicolor* FPP synthase (2 mL, ca. 0.1 mg/mL by Bradford assay)[8] and isoafricanol synthase (2 mL). The reaction mixtures were extracted with ($^{2}H_6$)benzene (0.6 mL), the extracts were dried with $MgSO_4$ and directly analysed by NMR. For the incubation experiments with (*R*)- and (*S*)-(1-^{13}C,1-^{2}H)FPP the reaction mixtures were extracted with 1.5 mL pentane, the solvent was removed and the product was dissolved in (^{2}H)chloroform (0.5 mL) and directly analysed by NMR.

Isoafricanol (1). Yield: 2.8 mg. HRMS (TOF): obs. *m*/*z* 222.1966 (calcd., formula) = (222.1978, $C_{15}H_{26}O^+$, $[M]^+$). IR (diamond ATR): $\tilde{\nu}$ = 3503 (w), 3056 (w), 2987 (m), 2952 (s), 2925 (s), 2866 (s), 1677 (w), 1457 (m), 1381 (m), 1362 (m), 1259 (s), 1163 (m), 1097 (s), 1016 (s), 980 (m), 941 (m), 905 (w), 879 (m), 849 (m), 798 (s) cm^{-1}.

NMR data are presented in Table 1 of main text.

A) Geosmin synthase

VTQPFRLPDFYMPYPARLNPHVQEAREHSTQWAREMEMLEGSGIWEQEDLDAHDYALLCAYTHPDCSG
TELSLVTDWYVWVFFFDDHFLETFKRSQDRAAGKAYLDRLPAFMPMDPADGTPEPTNPVEAGLADLWA
RTVPSMSSDWRARFRESTENLLNESLWELSNINIDRVPNPVEYIEMRRKVGGAPWSAGLVEHAVRAEV
PAVIAGSRPMRVLRDAFADAVHLRNDLFSYQREIEEEGELSNGVLVLETFLDCTTQEAADSVNELLTS
RLQQFEDTALTELGPLFAEHGLDPAACAGVLAYVKGLQDWQSGGHEWHMRSSRYMNGAHEAGGAHGAD
GARGAHGVHEAGGAHGTNGAGGTGRRSERAPWSPFALTGLGVSAASLPLTTGRAEAARARRFSHVPFQ
RTGPSLLPDISMPFTLRLNAHLPTARRHLVDWAHRMGILEPQPGVPGSQVWDERRLLAADLPLCAAGI
HPDGSPDELDVASGWLAWGTYADDYYPAVFGRTHDLAGARACNARLGAFMPLDAGPTPVPANALERSL
ADLWGRTAGPMEDAARRDLRQAIEDMTASWLWELANQTKHRIPDPVDYIEMRRHTFGSDLTMSLCRLA
HGRRVPPEIYRSGPVQSLESAAANYATLLNDVFSYQKEIEFEGEVHNGVLVVQNFFDCDYPTGVAIVN
DLMTSRMRQFQHVAEHELPVLYDDFGLGPEARKAMDGYVEELSHWMTGILNWHREVPRYREEELLRVH
RRPVGARRRPVAAGAGAGARAVAPWHGPTGLGTSAARVPVPVGAGLSARP

B) 2-Methylisoborneol synthase

MPTPAPADAPAPAPEARAAGGALDRILRGPSGLGTVSLRPARFEGASAPVEAPAAPAPPAPPAEGSPV
PGLYHHPVPEPDPVRVAEVGRRIKSWALDEVELYPEDWEDQFDGFSVGRYMVACHPDAPTVDHLMLAT
RLMVAENAVDDCYCEDHGGSPIGLGGRLLLAHTALDPLHTTKEYQPRWAESLHSDAPRRAYRSAMEYF
HGAASPSQADRFRHDMARLHLGYLAEAAWAQTDHVPEVWEYLAMRQFNNFRPCPTITDTVGGYELPAD
LHAGPAMQRVIALAGNATTIVNDLYSYTKELDSPGRHLNLPVVIAEREGIADRDAYMKAIDVHNELMH
DFEAEAAALAAACPVPSVGRFLRGVAVWVDGNHYWHRTNTYRYSLPDFW

C) Cyclooctat-9-en-7-ol synthase

MTTGLSTAGAQDIGRSSVRPYLEECTRRFQEMFDRHVVTRPTKVELTDAELREVIDDCNAAVAPLGKT
VSDERWISYVGVVLWSQSPRHIKDMEAFKAVCVLNCVTFVWDDMDPALHDFGLFLPQLRKICEKYYGP
EDAEVAYEAARAFVTSDHMFRDSPIKAALCTTSPEQYFRFRVTDIGVDFWMKMSYPIYRHPEFTEHAK
TSLAARMTTRGLTIVNDFYSYDREVSLGQITNCFRLCDVSDETAFKEFFQARLDDMIEDIECIKAFDQ
LTQDVFLDLIYGNFVWTTSNKRYKTAVNDVNSRIQ

D) Isoafricanol synthase

MHTHASRPHARQSALPRRAALFDFPASADLSPDTGAARQHTIQWLSRFRVFENHASVEEYDALRFDVL
TGLFYPRATGADLNLGSDLVGWYFVFDDQFDGELGCRPEEVARLVADVIRVTEEDMAPGGTGGGEGPL
LESFRDLWHRINSGRPRVWRDRFRHHWLEYLHSYHREALERTGAAPADGGGDAPRSVEDVLALRRHSI
GVQPCLDLNEPFGGYTLPSALHGGFPLARMREATDDVVVFTNDIASLDKELAVGDVHNSVIVQWKLAG
GGVEDAVRHIAGLANARYGWFEETAARLPELLAEAGADPGTHRAVGRYVDGMRHVMTGNLGWSLRTAR
YDERGTEAVSGGRERPWARLTGAEDLIRAGRGAPPPPGSGPDTRQPMPSEPSQLA

Fig. S1 Amino acid sequences of native terpene cyclases from *S. malaysiensis*. Highly conserved motifs required for enzyme functionality are marked in yellow.

Comment [JSD]: These highlighted passages are not changes. See figure legend!

Headspace sampling by CLSA

Streptomyces malaysiensis DSM 4137 was grown on Gym 65 agar plates (glucose: 4.0 g, yeast extract: 4.0 g, malt extract: 4.0 g, $CaCO_3$ 2.0 g, agar agar 16.0 g, water: 1 L, pH 7.2) for five days. The volatiles released by Streptomyces *malaysiensis* DSM 4137 were trapped by use of the CLSA (closed-loop stripping analysis) technique as described previously.[9] The extract was directly measured by GC/MS.

GC-/MS and GC/Q-TOF analysis

GC-MS analyses were carried out with a 7890B gas chromatograph connected to a 5977A inert mass detector (Agilent) fitted with a HP5-MS fused silica capillary column (30 m, 0.25 mm i. d., 0.50 μm film). Instrumental GC parameters were (1) inlet pressure: 77.1 kPa, He 23.3 mL min^{-1}, (2) injection volume: 1-2 μL, (3) split ratio: 50:1 (60 s valve time), (4) GC program: 5 min at 50 °C increasing at 10 °C min^{-1} to 320 °C, (5) carrier gas: He 1 mL min^{-1}. The MS parameters were (1) transfer line: 250 °C, (2) electron energy: 70 eV. Retention indices (*I*) were determined from a homologous series of n-alkanes (C8-C40).

HRMS analyses were carried out with a 7890B gas chromatograph connected to a 7200 accurate-mass Q-TOF mass detector (Agilent) eqipped with a HP5-MS fused silica capillary column (30 m, 0.25 mm i. d., 0.50 μm film). Instrumental GC parameters were (1) inlet pressure: 83.2 kPa, He 24.6 mL min^{-1}, (2) injection volume: 1 μL, (3) split ratio: 50:1 (60 s valve time), (4) GC program: 5 min at 50 °C increasing at 10 °C min^{-1} to 320 °C, (5) carrier gas: He 1 mL min^{-1}. The MS parameters were (1) transfer line: 250 °C, (2) electron energy: 70 eV.

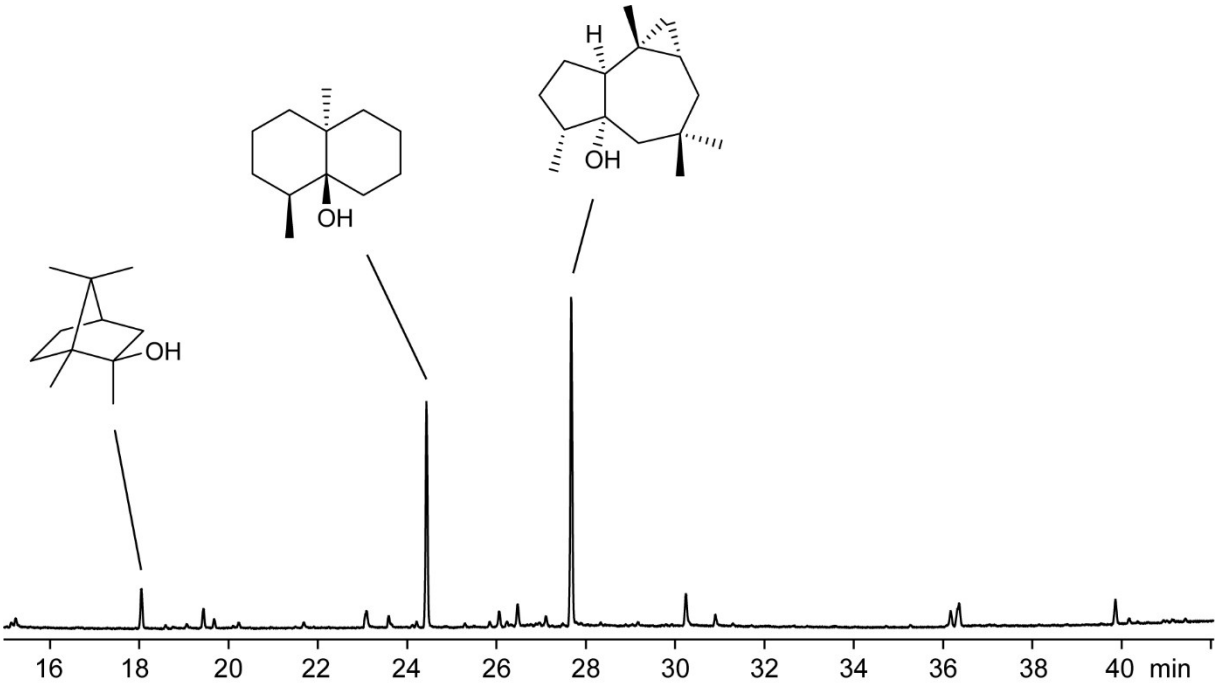

Fig. S2 Total ion chromatogram of a headspace extract from *S. malaysiensis*.

MGSSHHHHHHSSGLVPRGSHMASMTGGMHTHASRPHARQSALPRRAALFDFPASADLSPDTGAARQHT
IQWLSRFRVFENHASVEEYDALRFDVLTGLFYPRATGADLNLGSDLVGWYFVFDDQFDGELGCRPEEV
ARLVADVIRVTEEDMAPGGTGGGEGPLLESFRDLWHRINSGRPRVWRDRFRHHWLEYLHSYHREALER
TGAAPADGGGDAPRSVEDVLALRRHSIGVQPCLDLNEPFGGYTLPSALHGGFPLARMREATDDVVVFT
NDIASLDKELAVGDVHNSVIVQWKLAGGGVEDAVRHIAGLANARYGWFEETAARLPELLAEAGADPGT
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DTRQPMPSEPSQLA

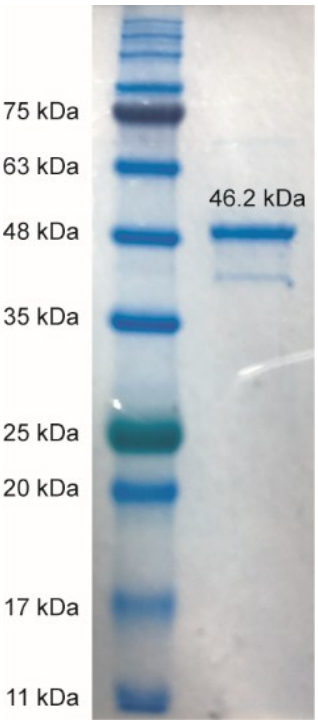


Fig. S3 Amino acid sequence of recombinant isoafricanol synthase ($(His)_6$ tag underlined) and SDS PAGE of the purified enzyme.

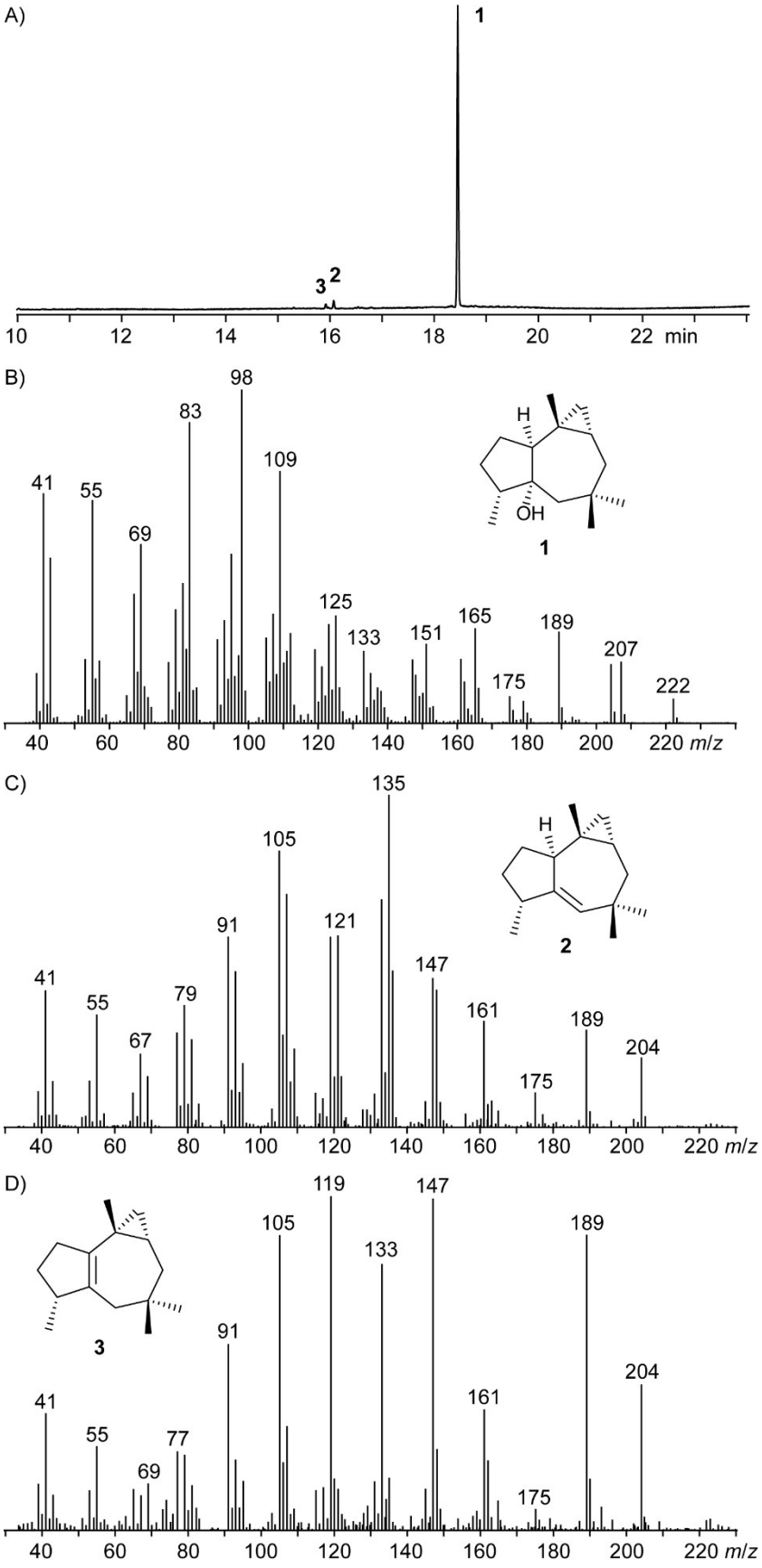


Fig. S4 GC/MS analysis of products obtained from FPP with isoafricanol synthase. A) Total ion chromatogram, B) mass spectrum of isoafricanol (**1**), C) mass spectrum of african-1-ene (**2**), and C) mass spectrum of african-2(6)-ene (**3**).

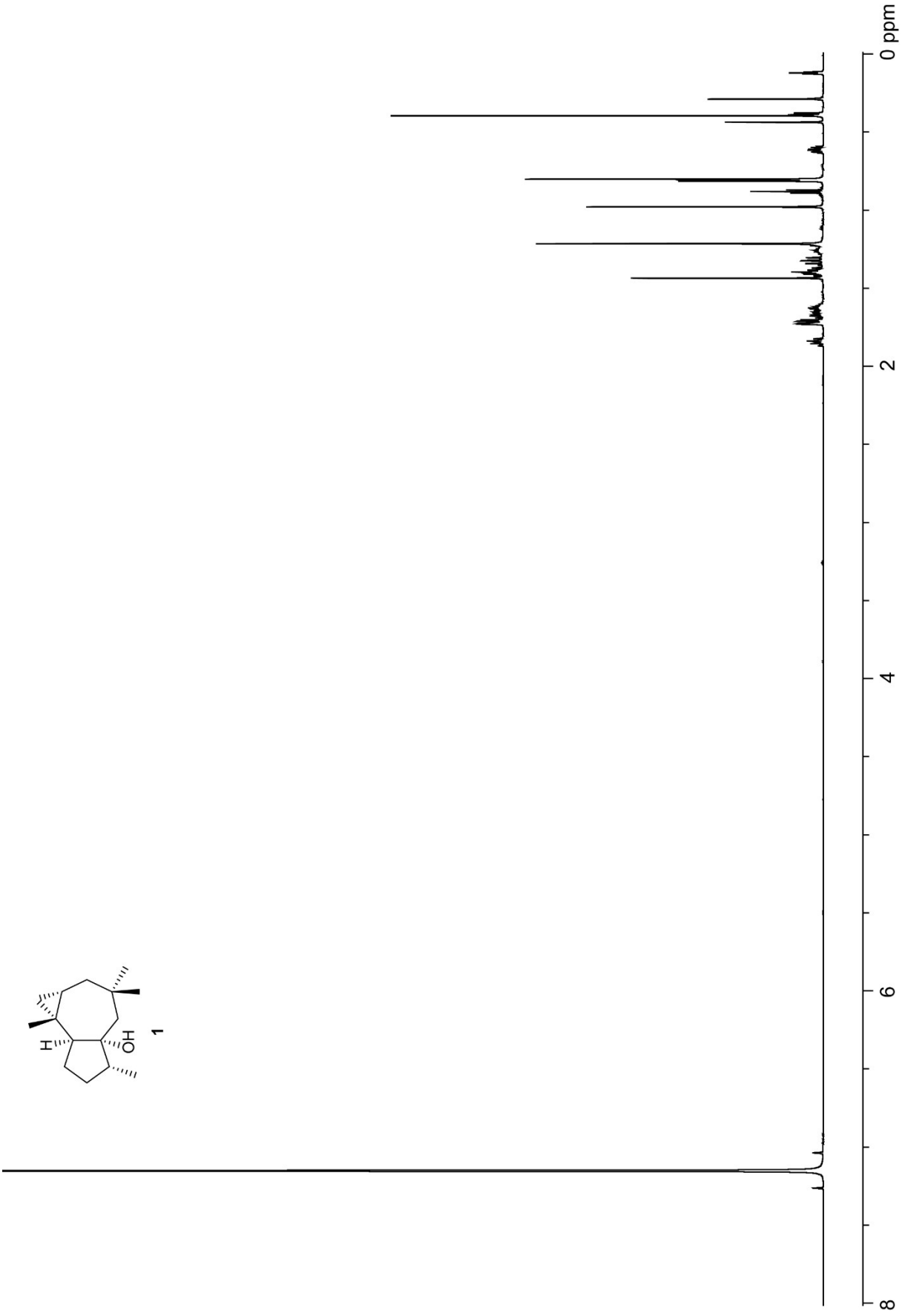


Fig. S5 ^{1}H-NMR spectrum of **1** in ($^{2}H_6$)benzene (700 MHz).

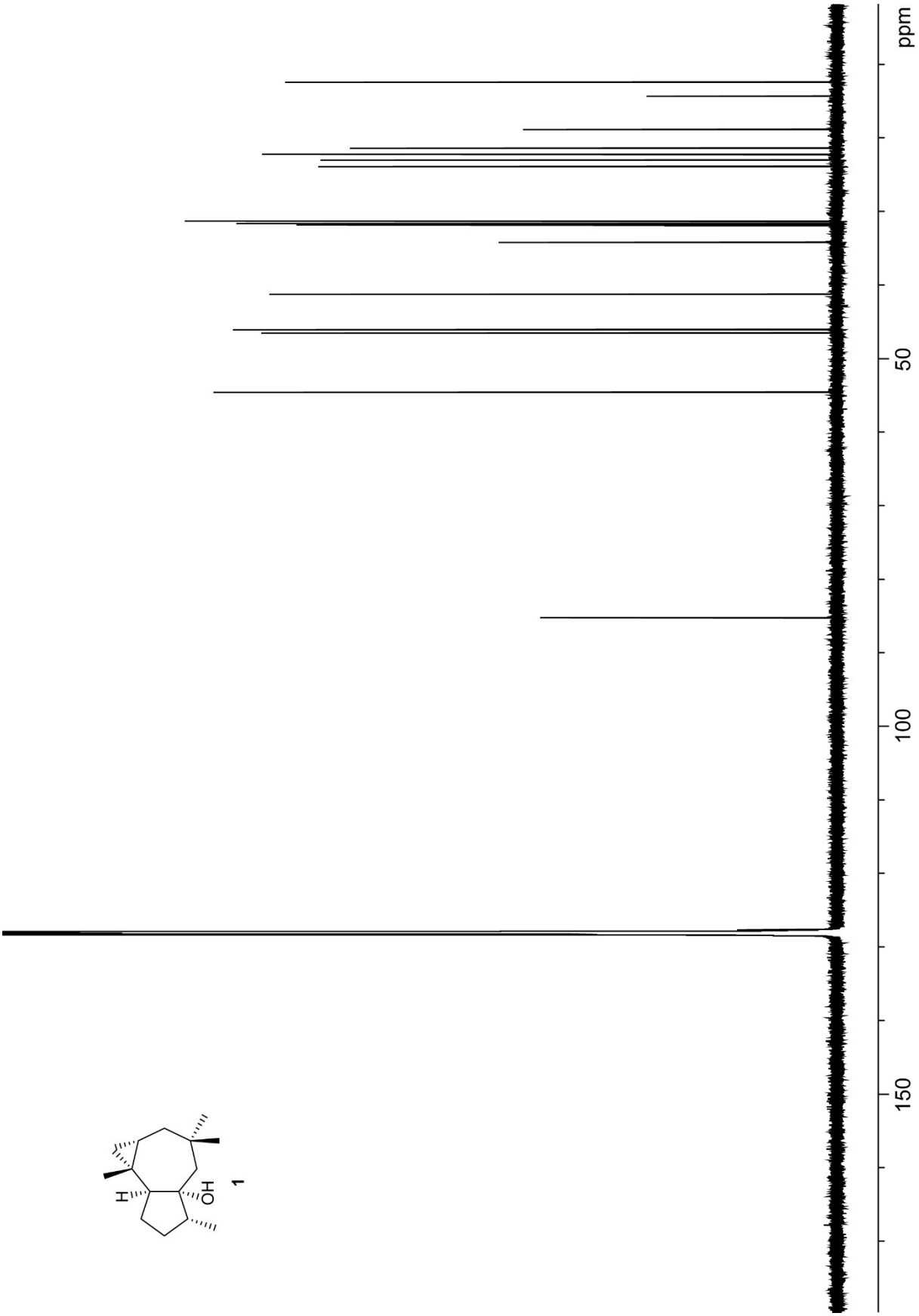


Fig. S6 ^{13}C-NMR spectrum of **1** in ($^{2}H_6$)benzene (175 MHz).

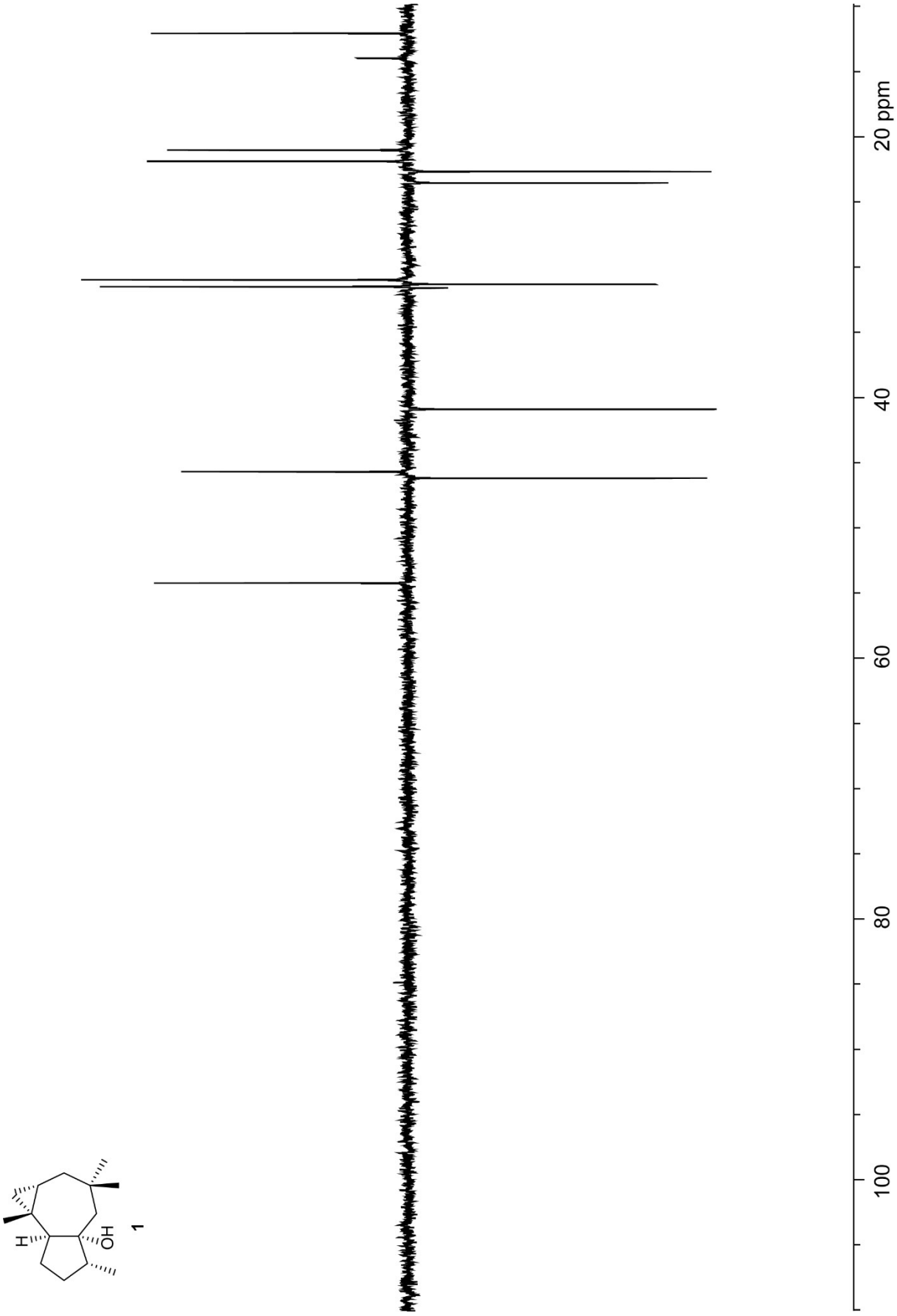


Fig. S7 DEPT spectrum of **1** in ($^2H_6$)benzene (175 MHz).

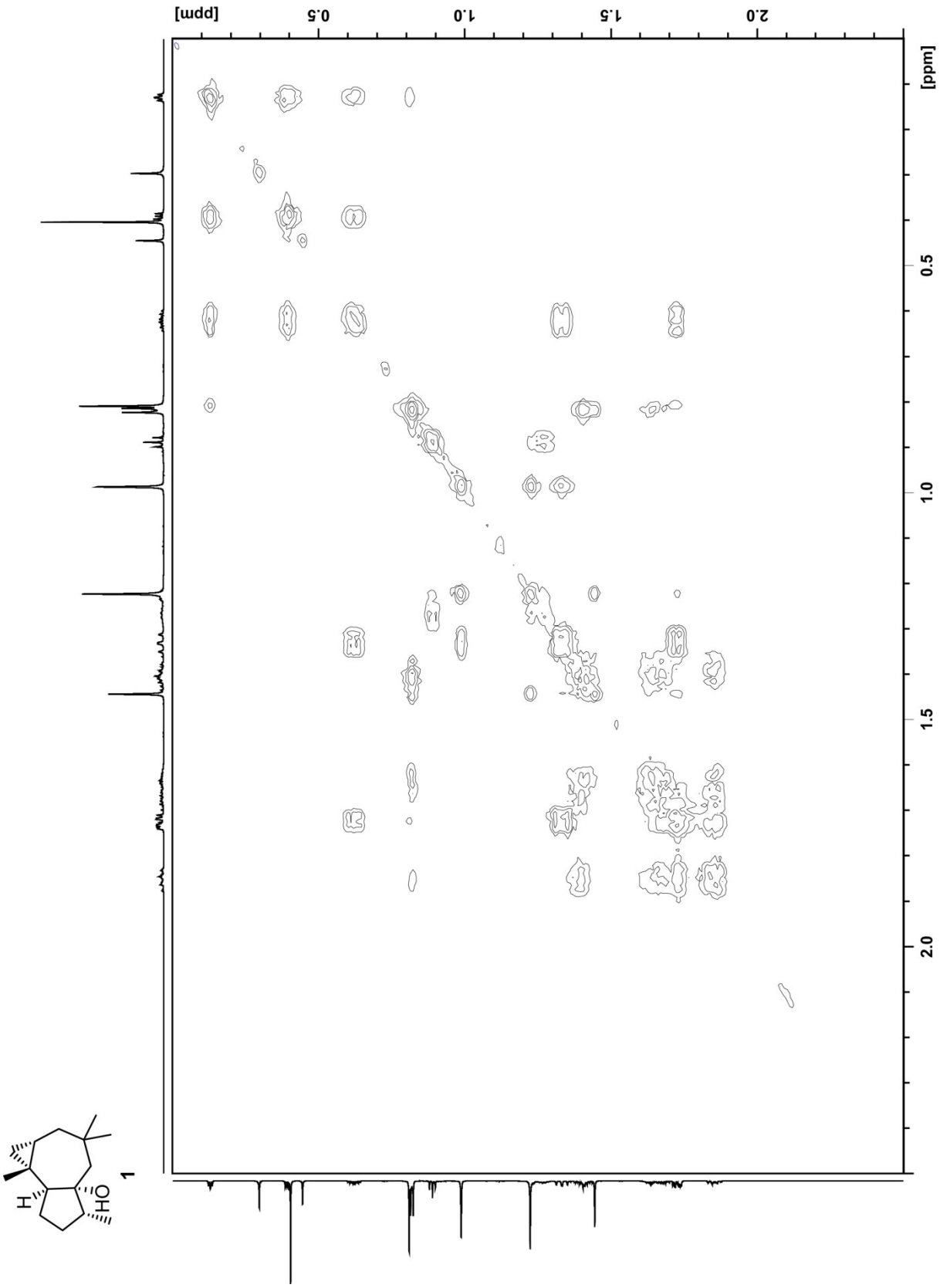


Fig. S8 $^{1}H,^{1}H$-COSY spectrum of **1** in ($^{2}H_{6}$)benzene.

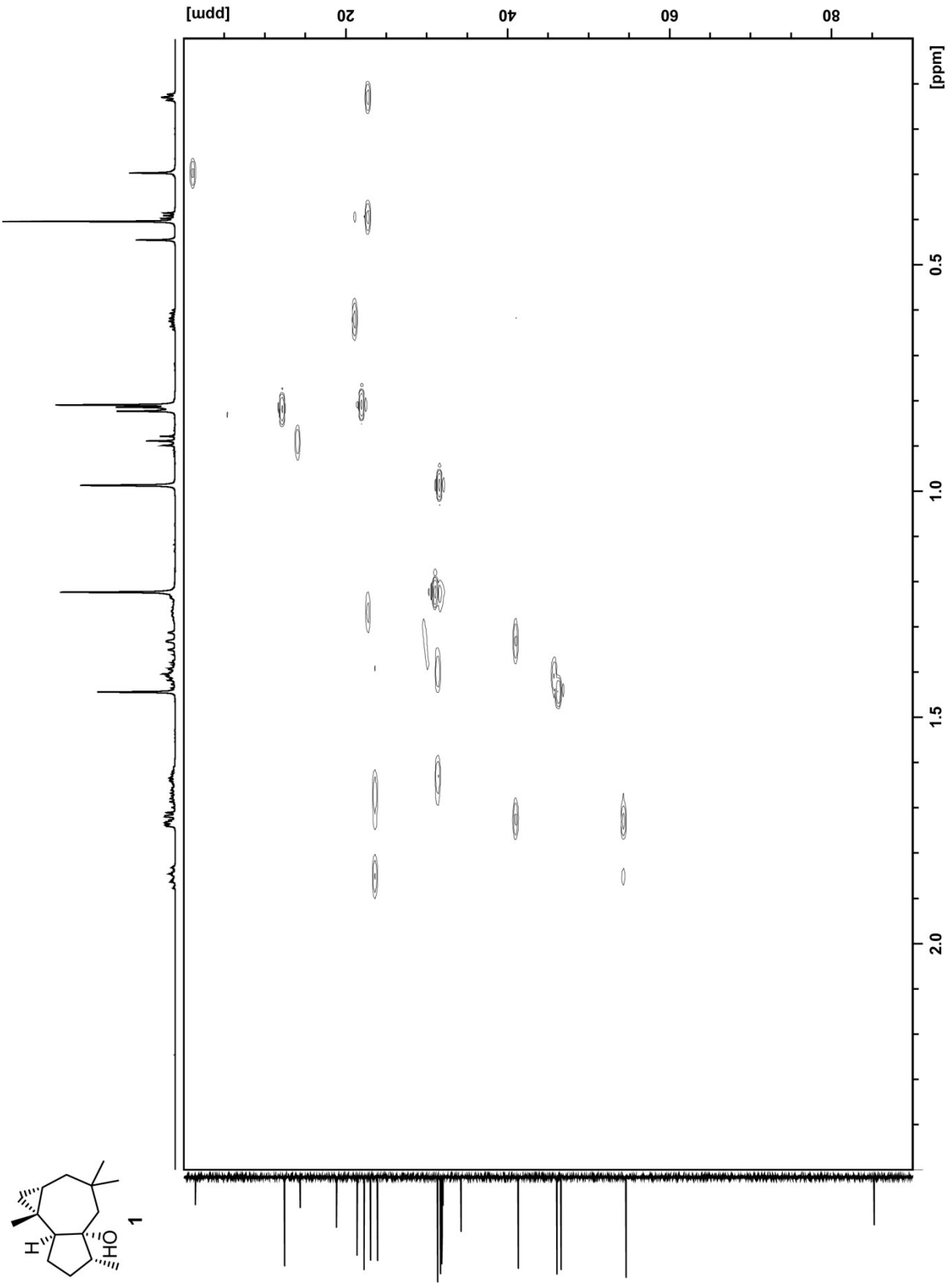


Fig. S9 HSQC spectrum of **1** in ($^2H_6$)benzene.

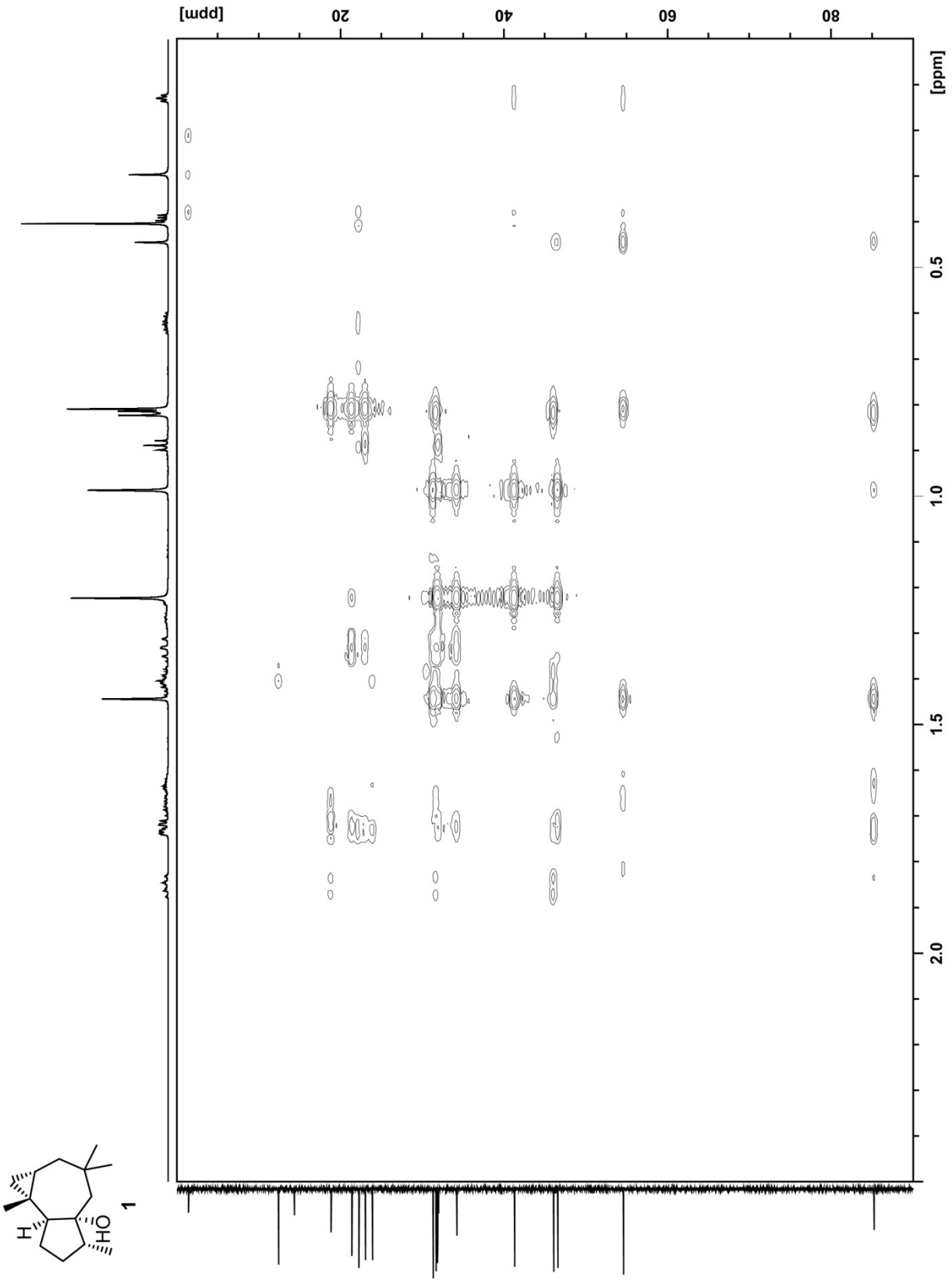


Fig. S10 HMBC spectrum of **1** in ($^2H_6$)benzene.

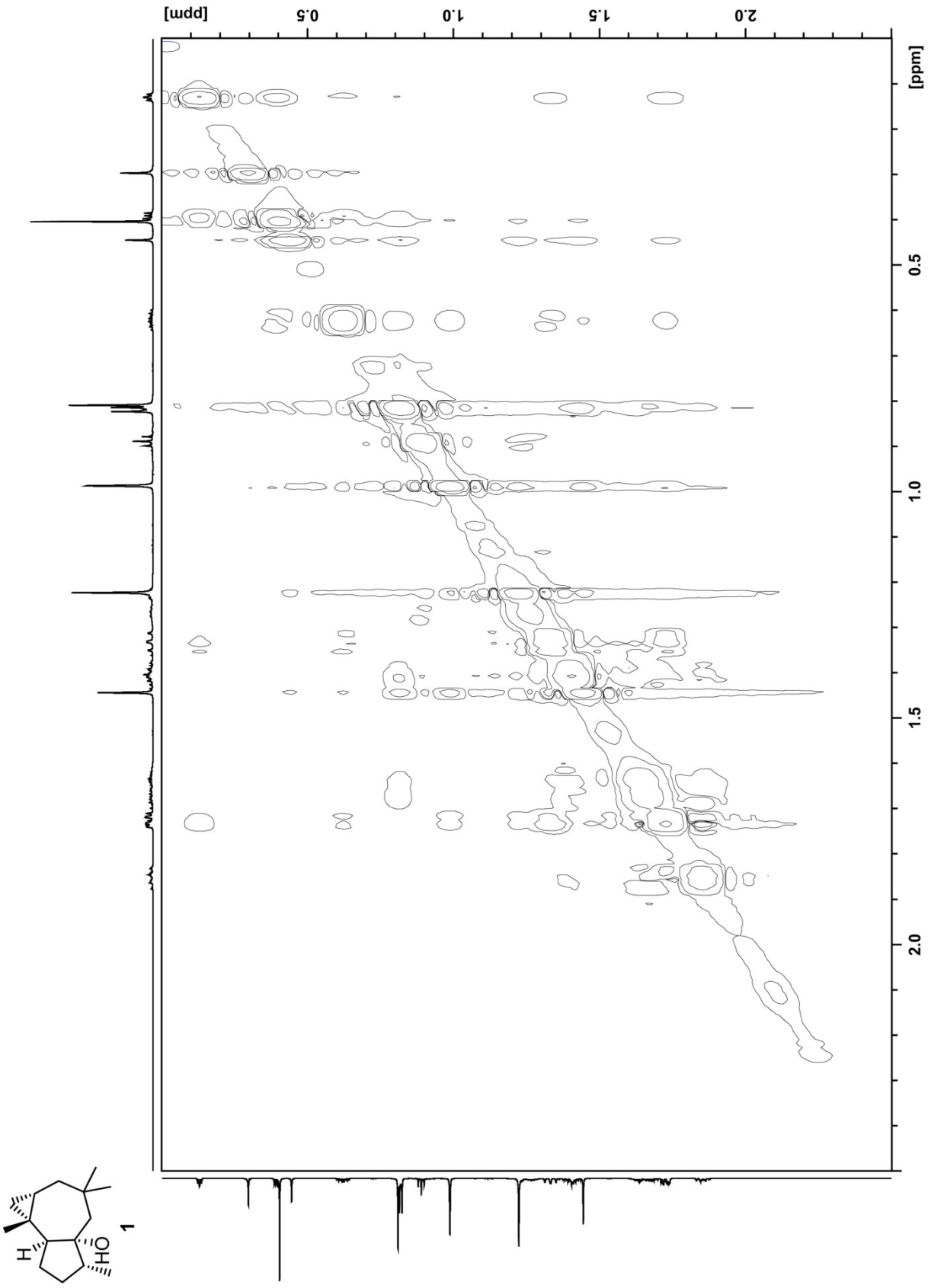
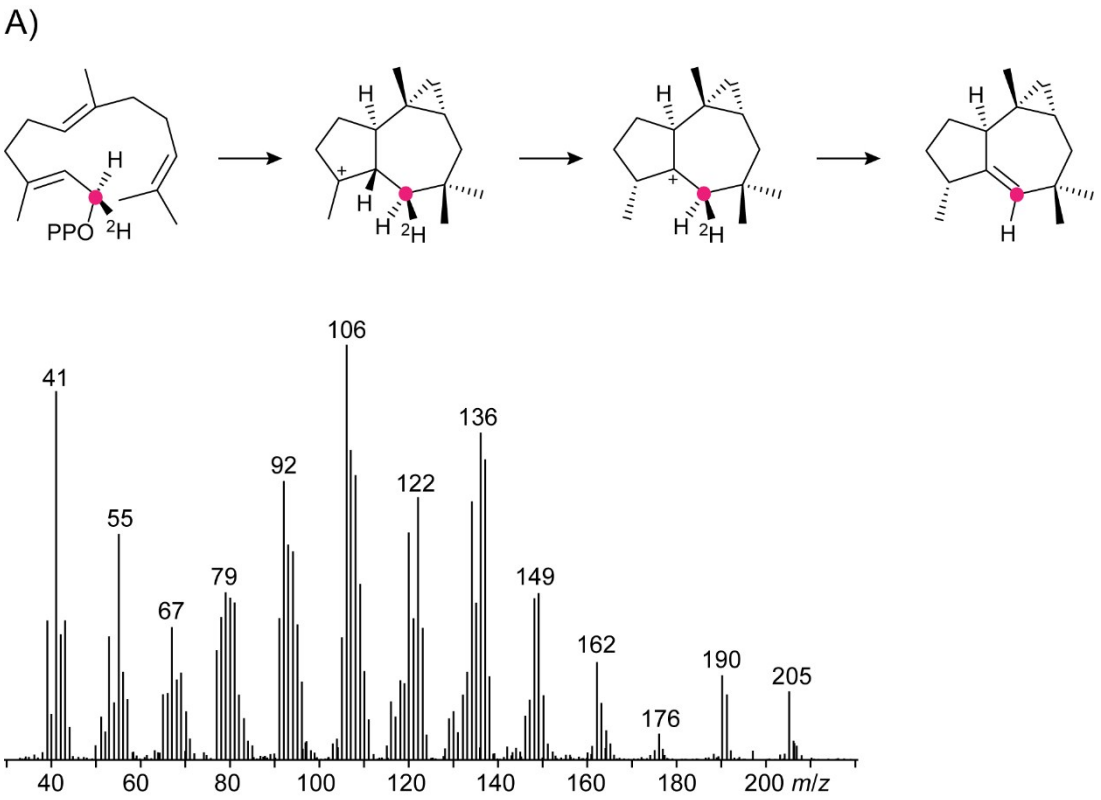


Fig. S11 NOESY spectrum of **1** in ($^2H_6$)benzene.



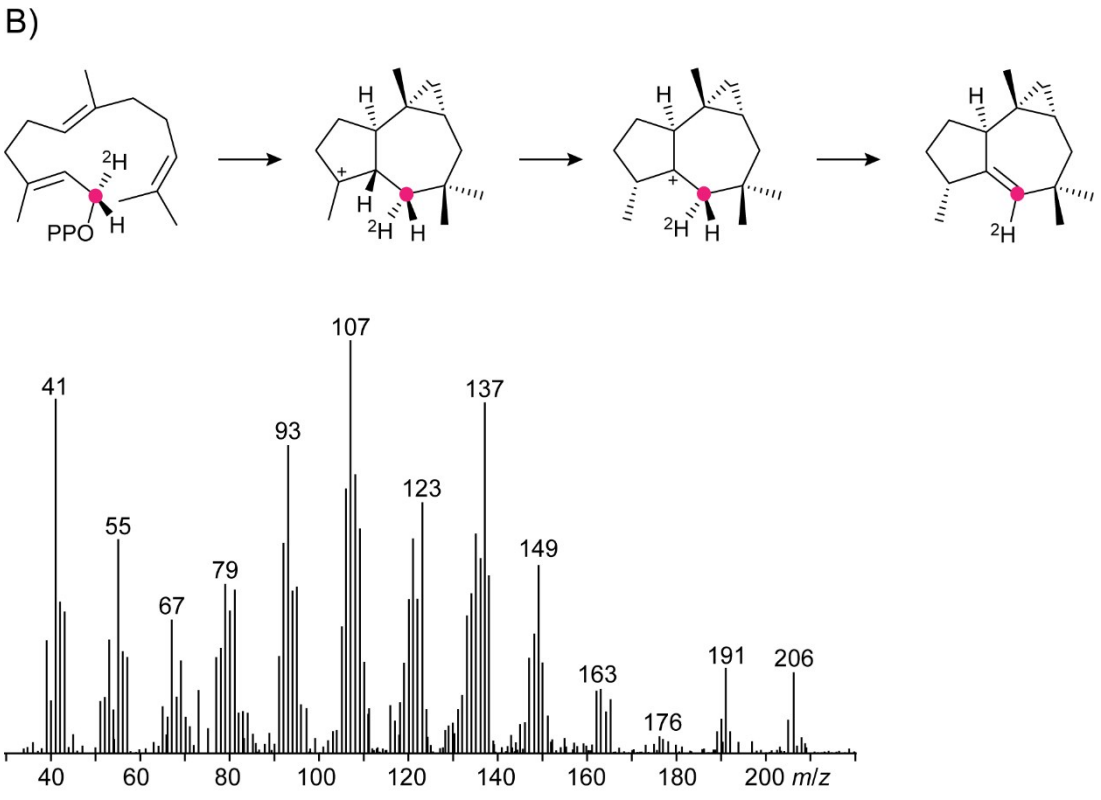


Fig. S12 GC/MS analysis of labelled **2** obtained from A) (*R*)-(1-^{13}C,1-^{2}H)FPP and B) (*S*)-(1-^{13}C,1-^{2}H)FPP by enzymatic conversion with isoafricanol synthase. Coloured dots indicate ^{13}C-labelled carbons.

References

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