Electronic Supporting Information

Biosynthetic characterization of the antifungal fernane-type triterpenoid polytolypin for generation of new analogues via combinatorial biosynthesis

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Supplementary Methods

General materials and experimental procedures

The biochemical reagents and kits were purchased from TaKaRa Bio Inc. (Dalian, China), Thermo Fisher Scientific Inc. (Shenzhen, China), or Sangon Biotech Co., Ltd. (Shanghai, China), unless noted otherwise. The synthesis of biosynthetic genes (*polA-polE* and $EfuA_{TC}$) and oligonucleotide primers were completed by Tsingke Biotech Co., Ltd. (Beijing, China) or Sangon Biotech Co., Ltd. All the chemicals were purchased from Guanghua Sci-Tech Co., Ltd. (Guangdong, China), Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China) or Yuwang Industrial Co., Ltd. (Shandong, China).

HPLC-MS analyses were performed on a Dionex UltiMate 3000 HPLC system (Thermo Scientific, USA) with a COSMOSIL $3C_{18}$-EB Column (4.6 ID × 150 mm) and an amaZon SL ion trap mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) source (Bruker, USA). The semi-preparative HPLC was performed on a Dionex UltiMate 3000 HPLC system using a YMC-Pack ODS-A column (10.0 mm i.d. ×250 mm, 5 μm). Column chromatography was carried out with silica gel (200–300 mesh) (Qingdao Haiyang Chemical Group Corporation, China). The UV data, IR data and optical rotation values were respectively measured on a JASCO V-550 UV/vis spectrometer, a JASCO FT/IR-4600 plus spectrometer, and a JASCO P1020 digital polarimeter from JASCO International CO., Ltd. (Tokyo, Japan). 1D and 2D NMR spectra were recorded on Bruker AV 400/600 spectrometers (Bruker, USA) using the solvent signals (pyridine-d_5: δ_H 7.57/δ_C 135.5; CD_3OD: δ_H 3.30/δ_C 49.0; $CDCl_3$: δ_H 7.26/δ_C 77.0) as the reference.

Strains and media

Humicola fuscoatra NRRL 22980 was provided by the ARS Culture Collection. The quadruple auxotrophic *Aspergillus oryzae* NSAR1 (*niaD*$^-$, *sC*$^-$, Δ*argB*, *adeA*$^-$)[1] was used as the host for heterologous expression. The *A. oryzae* NSAR1 transformant was firstly grown in 10 mL DPY medium (2% dextrin, 1% polypeptone, 0.5% yeast extract, 0.05% $MgSO_4·7H_2O$, 0.5% KH_2PO_4) for 1-2 days at 28 °C and 150 rpm. And then, the mycelia were transferred into the modified Czapek-Dox (CD) medium (0.3% $NaNO_3$, 0.2% KCl, 0.05% $MgSO_4·7H_2O$, 0.1% KH_2PO_4, 0.002% $FeSO_4·7H_2O$, 1% polypeptone, 2% starch, pH 5.5) to induce the expression of exogenous genes under the *amyB* promoter. *Escherichia coli* DH5α (TaKaRa) was used for construction of recombinant plasmids in LB medium with 100 mg/L ampicillin.

Construction of recombinant plasmids

polA-polE and $EfuA_{TC}$ were amplified from the recombinant plasmids containing the chemically synthesized genes using the primers listed in Table S1, and $fsoA_{TC}$ was amplified from the genomic DNA of *H. fuscoatra* NRRL 22980, which were cloned into the linearized pTAex3 vector, respectively (Table S2). And then, the gene cassettes containing the *amyB* promoter and terminator were amplified from the pTAex3 based recombinant plasmids, and inserted into pAdeA or pUSA.

Transformation of *A. oryzae* NSAR1

The *A. oryzae* NSAR1 transformants were obtained via PEG-mediated transformation of protoplast. The spore suspension of *A. oryzae* NASR1 was cultivated in 10 mL DPY medium at 200 rpm and 28 °C for 2 days. Then the culture broth was transferred into 100 mL DPY medium and cultured for 1 day. The mycelia were harvested by filtration, and digested using the Yatalase solution (1% Yatalase, 0.6 M $(NH_4)_2SO_4$, 50 mM maleic acid, pH 5.5) to remove the cell walls. The protoplast pellet was collected through centrifugation at 1500 rpm for 10 min, and then washed twice with TF Solution 2 (1.2 M sorbitol, 35 mM NaCl, 50 mM $CaCl_2{\cdot}2H_2O$, 10 mM Tris-HCl, pH 7.5). After that, TF Solution 2 was added to adjust the protoplast concentration to around 1.0×10^7 cell/mL. 200 μL protoplast suspension and 10 μg plasmids (< 20 μL) were gently mixed and placed on ice for 30 min, followed by addition of 1.35 mL TF Solution 3 (60% PEG4000, 50 mM CaCl2·2H2O, 10 mM Tris-HCl, pH 7.5) in three times. After incubation at room temperature for 20 min, 5 mL TF Solution 2 was added, and the mixture was subjected to centrifugation at 1500 rpm for 10 min. The precipitate was resuspended in 200 μL TF Solution 2 and spread on the selective under-layer medium, which was covered with the selective upper-layer medium. The selective medium was composed of 0.1% $(NH_4)_2SO_4$, 0.2% NH_4Cl, 0.05% NaCl, 0.05% KCl, 0.1% KH_2PO_4, 0.05% $MgSO_4{\cdot}7H_2O$, 0.002% $FeSO_4{\cdot}7H_2O$, 1.2 M sorbitol, 2% glucose and 1.5% agar for under-layer (or 0.8% agar for upper-layer) supplemented with appropriate ingredients for auxotrophic complementation. And the transformants could be obtained after incubation at 30 °C for 3-5 days. All the transformants used in the work are listed in Table S3.

HPLC-MS analysis of metabolites from *A. oryzae* NSAR1 transformants

After growing in the modified CD medium for 6 days, mycelia of the *A. oryzae* NSAR1 transformant were harvested and extracted with ethanol. The crude extract was resuspended in methanol for HPLC-MS analysis. The mobile phase was composed of water with 0.1% formic acid

(A) and acetonitrile with 0.1% formic acid (B). For analysis of metabolites from *A. oryzae* NSAR1 transformants harboring one or two exogenous genes, the samples were subjected to a linear gradient elution of 50–100% B (0–10 min) and 100% B (10–50 min) at the flow rate of 1 mL/min. For analysis of metabolites from *A. oryzae* NSAR1 transformants harboring two to five exogenous genes, the samples were subjected to a linear gradient elution of 10–100% B (0–40 min) and 100% B (40–65 min) at the flow rate of 1 mL/min.

Purification procedure of compounds

Purification process for **1**

Mycelia and culture medium from 9.5 L culture of AO-PolA/B/C/E were extracted with ethanol and ethyl acetate, respectively. The crude extract was subjected to silica gel column chromatography with stepwise elution of cyclohexane and ethyl acetate. The fraction containing **1** was further purified by semi-preparative HPLC (YMC-Pack ODS-A column, 3 mL/min) with isocratic elution of 65% CH_3CN–H_2O containing 0.1% formic acid to yield **1** (7.5 mg).

Purification process for **2**

Mycelia from 5 L culture of AO-PolA were extracted with ethanol. The crude extract was subjected to silica gel column chromatography with stepwise elution of cyclohexane and ethyl acetate. **2** (10.0 mg) was obtained through recrystallization of the fraction containing **2**.

Purification process for **3**

Mycelia from 6 L culture of AO-PolA/C were extracted with ethanol. The crude extract was subjected to silica gel column chromatography with stepwise elution of cyclohexane and ethyl acetate. The fraction containing **3** was further purified by semi-preparative HPLC (YMC-Pack ODS-A column, 3 mL/min) with isocratic elution of 99% CH_3CN–H_2O containing 0.1% formic acid to yield **3** (8.0 mg).

Purification process for **4**

Mycelia from 4 L culture of AO-PolA/B/C were extracted with ethanol. The crude extract was subjected to silica gel column chromatography with stepwise elution of cyclohexane and ethyl acetate. The fraction containing **4** was further purified by semi-preparative HPLC (YMC-Pack ODS-A column, 3 mL/min) with isocratic elution of 85% CH_3CN–H_2O containing 0.1% formic acid to yield **4** (5.8 mg).

Purification process for **5**

Mycelia from 9 L culture of AO-PolA/C/E were extracted with ethanol. The crude extract was subjected to silica gel column chromatography with stepwise elution of cyclohexane and ethyl acetate. The fraction containing **5** was further purified by semi-preparative HPLC (YMC-Pack ODS-A column, 3 mL/min) with isocratic elution of 75% CH_3CN–H_2O containing 0.1% formic acid to yield **5** (26.4 mg).

Purification process for **6**

Mycelia from 1 L culture of AO-EfuA_{TC} were extracted with ethanol. The crude extract was subjected to silica gel column chromatography with stepwise elution of cyclohexane and ethyl acetate to give **6** (14.8 mg).

Purification process for **7**

Mycelia from 1 L culture of AO-FsoA_{TC} were extracted with ethanol. The crude extract was subjected to silica gel column chromatography with stepwise elution of cyclohexane and ethyl acetate to give **7** (16.5 mg).

Purification process for **8**

Mycelia from 5 L culture of AO-EfuA_{TC}/PolC were extracted with ethanol. The crude extract was subjected to silica gel column chromatography with stepwise elution of cyclohexane and ethyl acetate. The fraction containing **8** was further purified by semi-preparative HPLC (YMC-Pack ODS-A column, 3 mL/min) with isocratic elution of 90% CH_3CN–H_2O containing 0.1% formic acid to yield **8** (15.0 mg).

Purification process for **9**

Mycelia from 10 L culture of AO-EfuA_{TC}/PolB/C were extracted with ethanol. The crude extract was subjected to silica gel column chromatography with stepwise elution of cyclohexane and ethyl acetate. The crude fraction containing **9** was further purified by semi-preparative HPLC (YMC-Pack ODS-A column, 3 mL/min) with isocratic elution of 75% CH_3CN–H_2O containing 0.1% formic acid to yield **9** (5.0 mg).

Purification process for **10**

Mycelia from 20 L culture of AO-EfuA_{TC}/PolC/E were extracted with ethanol. The crude extract was subjected to silica gel column chromatography with stepwise elution of cyclohexane and ethyl acetate. The fraction containing **10** was further purified by HPLC (COSMOSIL 3C_{18}-EB Column, 1 mL/min) with isocratic elution of 55% CH_3CN–H_2O containing 0.1% formic acid to

yield **10** (5.6 mg).

Purification process for **11**

Mycelia from 10 L culture of AO-EfuA$_{TC}$/PolB/C/E were extracted with ethanol. The crude extract was subjected to silica gel column chromatography with stepwise elution of cyclohexane and ethyl acetate. The fraction containing **11** was further purified by semi-preparative HPLC (YMC-Pack ODS-A column, 3 mL/min) with isocratic elution of 51% CH_3CN–H_2O containing 0.1% formic acid to yield **11** (6.6 mg).

Purification process for **12**

Mycelia from 9 L culture of AO-FsoA$_{TC}$/PolC were extracted with ethanol. The crude extract was subjected to silica gel column chromatography with stepwise elution of cyclohexane and ethyl acetate. The fraction containing **12** was further purified by semi-preparative HPLC (YMC-Pack ODS-A column, 3 mL/min) with isocratic elution of 90% CH_3CN–H_2O containing 0.1% formic acid to yield **12** (7.0 mg).

Purification process for **13**

Mycelia from 7.5 L culture of AO-FsoA$_{TC}$/PolB/C were extracted with ethanol. The crude extract was subjected to silica gel column chromatography with stepwise elution of cyclohexane and ethyl acetate. The fraction containing **13** was further purified by semi-preparative HPLC (YMC-Pack ODS-A column, 3 mL/min) with isocratic elution of 80% CH_3CN–H_2O containing 0.1% formic acid to yield **13** (5.3 mg).

Structural characterization

Compound **1**: white powder; $[\alpha]_D^{29}$ = +42.2 (*c* 0.5, C_5H_5N); APCI-MS (positive) *m/z* 519 $[M + H]^+$; NMR spectra, see Figs. S22-S27; NMR data, see Table S7. Compound **1** was determined to be the known compound polytolypin (CAS Registry Number: 174158-04-4), and its NMR data in pyridine is firstly reported in this work.

Compound **2**: colorless needle crystal; $[\alpha]_D^{29}$ = -37.4 (*c* 0.5, CH_2Cl_2); APCI-MS (positive) *m/z* 409 $[M + H – H_2O]^+$; NMR spectra, see Fig. S1; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 145.1, 116.1, 79.2, 59.5, 54.1, 50.7, 47.9, 42.8, 41.5, 38.9, 36.8, 36.2, 36.0, 35.3, 32.3, 30.6, 30.3, 28.2, 27.7, 27.5, 24.1, 24.0, 22.9, 22.1, 21.0, 20.0, 16.0, 14.6, 14.0, 12.8. The NMR data are in good agreement with those of motiol (CAS Registry Number: 2566-82-7).[2-5]

Compound **3**: colorless needle crystal; $[\alpha]_D^{30}$ = +10.5 (*c* 0.5, C_5H_5N); UV (CH_3OH) λ_{max} (log ε)

205 (2.48) nm；IR (KBr) ν_{max} 3434, 2945, 2885, 2865, 1631, 1469, 1384, 1076 cm^{-1}; APCI-MS (positive) *m/z* 439 $[M + H - H_2O]^+$; NMR spectra, see Figs. S3-S8; NMR data, see Table S4. Compound **3** was identified as 3β-hydroxyfern-7-en-23-oic acid.

Compound **4**: colorless powder; $[\alpha]_D^{29}$ = +11.1 (*c* 0.5, C_5H_5N); UV (CH_3OH) λ_{max} (log ε) 205 (3.40) nm；IR (KBr) ν_{max} 3367, 2947, 2888, 2868, 1706, 1384, 1043 cm^{-1}; APCI-MS (negative) *m/z* 471 $[M - H]^-$;NMR spectra, see Figs. S10-S15; NMR data, see Table S5. Compound **4** was identified as 2α,3β-dihydroxyfern-7-en-23-oic acid.

Compound **5**: white powder; $[\alpha]_D^{29}$ = +85.7 (*c* 0.2, C_5H_5N); UV (CH_3OH) λ_{max} (log ε) 205 (3.35) nm；IR (KBr) ν_{max} 3485, 2948, 2897, 2871, 1697, 1469, 1401, 1113 cm^{-1}; APCI-MS (positive) *m/z* 503 $[M + H]^+$; NMR spectra, see Figs. S16-S21; NMR data, see Table S6. Compound **5** was identified as 1β,3β-dihydroxyfern-7-en-23,25-dioic acid.

Compound **6**: colorless needle crystal; $[\alpha]_D^{26}$ = -9.5 (*c* 0.63, $CHCl_3$); NMR spectra, see Fig. S29; APCI-MS (positive) *m/z* 409 $[M + H - H_2O]^+$; ^{13}C NMR (150 MHz, $CDCl_3$) δ_C 151.1, 116.2, 79.1, 59.6, 52.0, 44.3, 42.9, 40.0, 39.3, 39.3, 37.8, 37.6, 36.7, 36.7, 36.1, 30.8, 29.3, 28.2, 28.1, 27.4, 25.2, 23.0, 22.1, 20.1, 19.1, 17.9, 15.8, 15.4, 15.0, 14.0. The NMR data are in good agreement with those of fernenol (CAS Registry Number: 4966-00-1).[6, 7]

Compound **7**: colorless needle crystal; $[\alpha]_D^{26}$ = +22.3 (*c* 0.28, $CHCl_3$); NMR spectra, see Fig. S30; APCI-MS (positive) *m/z* 409 $[M + H - H_2O]^+$; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 134.3, 134.1, 79.0, 59.8, 52.7, 50.4, 42.9, 41.0, 38.9, 37.6, 36.7, 35.9, 35.4, 30.7, 30.2, 28.3, 28.0, 28.0, 27.2, 26.9, 22.9, 22.1, 22.0, 20.3, 20.2, 19.1, 18.9, 15.8, 15.5, 14.6. The NMR data are in good agreement with those of isomotiol (CAS Registry Number: 4575-73-9).[8, 9]

Compound **8**: colorless needle crystal; $[\alpha]_D^{29}$ = +59.5 (*c* 0.5, C_5H_5N); UV (CH_3OH) λ_{max} (log ε) 203 (3.46) nm；IR (KBr) ν_{max} 3444, 2946, 2888, 2865, 1713, 1634, 1378, 1079 cm^{-1}; APCI-MS (positive) *m/z* 439 $[M + H – H_2O]^+$; NMR spectra, see Figs. S31-S36; NMR data, see Table S8. Compound **8** was identified as fernenolic acid.

Compound **9**: white powder; $[\alpha]_D^{29}$ = +22.9 (*c* 0.5, C_5H_5N); APCI-MS (negative) *m/z* 471 $[M - H]^-$; NMR spectra, see Figs. S37-S42; NMR data, see Table S9. Compound **9** was identified as the known compound retigeric acid A (CAS Registry Number: 35591-41-4).Compound **10**: white powder; $[\alpha]_D^{29}$ = +25.0 (*c* 0.5, C_5H_5N); UV (CH_3OH) λ_{max} (log ε) 205 (3.39) nm；IR (KBr) ν_{max} 3390, 2948, 2884, 1631, 1384, 1083, 1035 cm^{-1}; APCI-MS (positive) *m/z* 471 $[M + H – H_2O]^+$; NMR

spectra, see Figs. S43-S48; NMR data, see Table S10. Compound **10** was identified as 1β,3β,25-trihydroxyfern-9(11)-en-23-oic acid.

Compound **11**: white powder; $[\alpha]_D^{29}$ = -110.1 (*c* 0.5, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 204 (3.61) nm; IR (KBr) ν_{max} 3371, 2925, 2885, 1728, 1643, 1424, 1385, 1159, 1059 cm^{-1}; APCI-MS (positive) *m/z* 519 $[M + H]^+$; NMR spectra, see Figs. S50-S55; NMR data, see Table S11. Compound **11** was identified as 1β,2α,3β-trihydroxyfern-9(11)-en-23,25-dioic acid.

Compound **12**: colorless needle crystal; $[\alpha]_D^{30}$ = +35.1 (*c* 0.5, C_5H_5N); UV (CH_3OH) λ_{max} (log ε) 205 (3.59) nm; IR (KBr) ν_{max} 3515, 2966, 2940, 2873, 1698, 1386, 1099 cm^{-1}; APCI-MS (positive) *m/z* 439 $[M + H - H_2O]^+$; NMR spectra, see Figs. S56-S61; NMR data, see Table S12. Compound **12** was identified as 3β-hydroxyfern-8-en-23-oic acid.

Compound **13**: white powder; $[\alpha]_D^{29}$ = +10.7 (*c* 0.5, C_5H_5N); UV (CH_3OH) λ_{max} (log ε) 204 (3.37) nm; IR (KBr) ν_{max} 3363, 2934, 2885, 1701, 1456, 1381, 1054 cm^{-1}; APCI-MS (negative) *m/z* 471 $[M - H]^-$; NMR spectra, see Figs. S62-S67; NMR data, see Table S13. Compound **13** was identified as 2α,3β-dihydroxyfern-8-en-23-oic acid.

Antifungal assay

Antifungal activity was measured in 96-well microtiter plates using the 2-fold dilution assay. Two fungi strains *Candida albicans* FIM709 and *Aspergillus niger* R330 were cultivated on sabouraud dextrose agar medium (2% glucose, 1% peptone and 1.8% agar) at 32 °C for 4–7 days. The spores were collected and adjusted to a concentration of 10^7-10^9 /mL with 0.9% saline. Subsequently, 100 μL of mixtures was added to 200 mL sabouraud dextrose broth as spore solution. Compounds were dissolved in DMSO to a concentration of 12.5 mg/mL. 2 μL of sample solution was mixed with 200 μL of spore solution, which was then further half diluted with spore solution until the concentration reaching 0.06 μg/mL. Amphotericin B was used as the positive control and DMSO as the negative control. The 96-well microtiter plates were placed at 32 °C for 48 hours. The minimum inhibition concentration (MIC) was defined based on the minimal concentration in which fungi cannot grow.

Supplementary Notes

MPSYHNTDKTLLGDARQSLQQAVDYSLGCQQPDGHWVAPVMADATFTAQYVFFKHQIP
ELSLDEDGPEIQRWLLGEQTADGSWTLAPDLPGNLSTTVEAYLALRILGVPKSDQAMLRA
RDFVVRNGGVEGVRFFTRFFLATFGLVPWTAIPQMPAELILLPTFMFLNIYVLSSWARSTLI
PILLVRHHEPVYALPNGQSANNNFLDELWCNPGEKNIPFALPLWDLLRRYQWIEFAFTLLD
HILALFGGLRRWPCRHMALKRCTAWLLEHQEESGDWAGFFPPIHGSIWALLLDGFSFQSE
VIRLGMEALERLVVIDPKGKWVQSTVSPCWDTALMANALCDAGMSGDTRLAKATQWLR
DRQLMVSHGDWRNYANTQQAGGWSFQYFNSFYPDVDDTAVVIMTLIKEDPNCTNSDCV
MNGVEWMLGMQSRDGGWGAFDVNNNARWLHKIPFSDMDSLVDPSTSDVTGRILECLGL
LLSQRKSPLSPRWRHRLQASSAKAIAFLAKEQESSGAWWGRWGNNYHYGTANVLRGLA
WFAQTDPSAQMMCMRTLSWIDETQNADGGWGETLASYVDKSLAGLGRSTAAHTAWALE
SLLRFRLPSDQAIERGVRWLIDNQQPNVDGYYYGTKWQAGAGQGASWRFDHAYVGTGF
PSVLYLGYPYYHHLFPIQALSRYIDKASRQGIETLRIPSSSAVILDRPNVLL

Note S1 The amino acid sequence of $EfuA_{TC}$

MDMAPDELDELRGSAQRALEQAIDFSFSCQQDDGHWVAPVSADATFTAQYVMFKHAIPA
LNLDISGAEAAALRHWLLGDQNAAEGSWGLAPGLPGNLSTTVEAYLALRLLGVPSSNPA
LQQARRFVLAHGGISRVRFFTRFFLATFGLFPWSAIPQMPAELILMPKWAPLNIYVLSSWAR
STLIPILVVRHHEPLYPLPNAQSDPNSGFLDELWLDPTNKEVPFAPPLWDMFHGRDRDVVK
LAFTLGDKALAQIGGLKKGPQRRLALRRCIEWLLEHQEETGDWAGFFPPMHGSVWALLL
EGFSLEHDVVKRGLEALERLAVNDESGKWLQSTVSPCWDTALMVKALCDAGLGLGGAE
AAKGNRHARVTTAVDWVRSLQLLGPQGDWRVYSRNQRPGGWSFEYNNTWYPDVDDTA
VVVMMLVTHDPAAVESNAVEMGIEWILGMQNHDGGWGAFDTNNDALWLHKIPFSDMDS
LVDPSTSDVTGRMLECFGMLLTHRKGGLRLRPELSQRLHESAQKALAFLFREQTASGAW
WGRWGCNYNYGTTNVLRGLPAFCGDKEVARAALRAVLWLEKCQNKDGGWGETLLSYG
HPDLAGKGPSTAAHTAWALDALLRFRPASDPALQKGVQWLVSNQVPKTEEKRHWASWPS
DLYVGTGFPNVLYLGYPFYHHHFAISALARFLDRTDEPDQDRDLPLL

Note S2 The amino acid sequence of $FsoA_{TC}$

Supplementary Tables

Table S1 Primers used in the study

Primer	**Sequence (5′ to 3′)**	**Usage**
Inf-*polA*-pTAex3-SmaI-F	TCGAGCTCGGTACCCATGATGCCCACCAAATCTTT	Cloning *polA* from the recombinant plasmid containing the chemically synthesized *polA* to construct pTAex3-*polA*
Inf-*polA*-pTAex3-SmaI-R	CTACTACAGATCCCCTCACACGACAGGTTCAGGAA	
Inf-*polB*-pTAex3-SmaI-F	CGAATTCGAGCTCGGTACCCATGTACAGCTTCTTCCTAGT	Cloning *polB* from the recombinant plasmid containing the chemically synthesized *polB* to construct pTAex3-*polB* and pUSA- *polB*
Inf-*polB*-pTAex3-SmaI-R	ACGAGCTACTACAGATCCCCTCAGCCTTGTTTCCGTCTCC	
Inf-*polC*-pTAex3-SmaI-F	CGAATTCGAGCTCGGTACCCATGGCAGTGTTCAAACTCGA	Cloning *polC* from the recombinant plasmid containing the chemically synthesized *polC* to construct pTAex3-*polC* and pUSA- *polC*
Inf-*polC*-pTAex3-SmaI-R	ACGAGCTACTACAGATCCCCTCAGGGTATCTCTCTCTCCT	
Inf-*polD*-pTAex3-SmaI-F	CGAATTCGAGCTCGGTACCCATGCAACTTACTGTGGTGTT	Cloning *polD* from the recombinant plasmid containing the chemically synthesized *polD* to construct pTAex3-*polD* and pUSA- *polD*
Inf-*polD*-pTAex3-SmaI-R	ACGAGCTACTACAGATCCCCTTAAACAAAGTGGACGTCAT	
Inf-*polE*-pTAex3-SmaI-F	CGAATTCGAGCTCGGTACCCATGTCTGGGGAAACAGCCTT	Cloning *polE* from the recombinant plasmid containing the chemically synthesized *polE* to construct pTAex3-*polE* and pUSA- *polE*
Inf-*polE*-pTAex3-SmaI-R	ACGAGCTACTACAGATCCCCCTAGTGCTTCCTCCGACGAA	
Inf-*efuA*$_{TC}$-pTAex3-SmaI-F	TCGAGCTCGGTACCCATGCCGTCTTACCACAACAC	Cloning *efuA*$_{TC}$ from the recombinant plasmid containing the chemically synthesized *efuA*$_{TC}$ to construct pTAex3-*efuA*$_{TC}$
Inf-*efuA*$_{TC}$-pTAex3-SmaI-R	CTACTACAGATCCCCCTACAAGAGTACGTTCGGAC	
Inf-*fsoA*$_{TC}$-pTAex3-SmaI-F	TCGAGCTCGGTACCCACACACAATGGACATGGCGC	Cloning *fsoA*$_{TC}$ from *Humicola fuscoatra* NRRL 22980 genome to construct pTAex3-*fsoA*$_{TC}$
Inf-*fsoA*$_{TC}$-pTAex3-SmaI-R	CTACTACAGATCCCCTCAAACGTGCCGAGTCATGAG	
Inf-*polD*-pUSA-BamHI-F	GTCTATTATAGGAAAGGATCCCAATCTTCAAGAGCAGAAT	Cloning *polD* from pTAex3-*polD* to construct pUSA-*polC-polD*
Inf-*polD*-pUSA-BamHI-R	CAAGATGACTCTAGAGGATCGTAAGATACATGAGCTTCGG	
Inf-pAdeA-Parm-F	GCAGGTCGACTCTAGACGACTCCAATCTTCAAGAGC	Cloning target genes from pTAex3-based plasmids to construct recombinant pAdeA plasmids
Inf-pTA-Tamy-R1	AACGCGCTCGCGAGCAAGTACCATACAGTACCGCG	
Inf-pTA-Parm-F1	GCTCGCGAGCGCGTTCCACTGCATCATCAGTCTAG	
Inf-pAdeA-Tamy-R	TAGTAGATCCTCTAGAGTAAGATACATGAGCTTCGG	

Table S2 Plasmids used in the study

Plasmid	**Characteristic**	**Source**
pTAex3	Plasmid containing *argB* marker gene cassette for gene expression in *A. oryzae* NSAR1 along with the ampicillin resistance gene cassette	Fujii *et al.*[10]
pUSA	Plasmid containing *sC* marker gene cassette for gene expression in *A. oryzae* NSAR1 along with the ampicillin resistance gene cassette	Yamada *et al.*[11]
pAdeA	Plasmid containing *adeA* marker gene cassette for gene expression in *A. oryzae* NSAR1 along with the ampicillin resistance gene cassette	Jin *et al.*[12]
pTAex3-*polA*	pTAex3 containing *polA* under the *amyB* promoter	This work
pTAex3-*polB*	pTAex3 containing *polB* under the *amyB* promoter	This work
pTAex3-*polC*	pTAex3 containing *polC* under the *amyB* promoter	This work
pTAex3-*polD*	pTAex3 containing *polD* under the *amyB* promoter	This work
pTAex3-*polE*	pTAex3 containing *polE* under the *amyB* promoter	This work
pTAex3-$efuA_{TC}$	pTAex3 containing $efuA_{TC}$ under the *amyB* promoter	This work
pTAex3-$fsoA_{TC}$	pTAex3 containing $fsoA_{TC}$ under the *amyB* promoter	This work
pUSA-*polB*	pUSA containing *polB* under the *amyB* promoter	This work
pUSA-*polC*	pUSA containing *polC* under the *amyB* promoter	This work
pUSA-*polD*	pUSA containing *polD* under the *amyB* promoter	This work
pUSA-*polE*	pUSA containing *polE* under the *amyB* promoter	This work
pUSA-*polC*-*polD*	pUSA containing *polC* and *polD* under the *amyB* promoter	This work
pAdeA-*polB*	pAdeA containing *polB* under the *amyB* promoter	This work
pAdeA-*polD*	pAdeA containing *polD* under the *amyB* promoter	This work
pAdeA-*polE*	pAdeA containing *polE* under the *amyB* promoter	This work
pAdeA-*polB*-*polE*	pAdeA containing *polB* and *polE* under the *amyB* promoter	This work

Table S3 *A. oryzae* NSAR1 transformants used in the study

Strain	**Description**
AO-PolA	The *A. oryzae* NSAR1 transformant harboring pTAex3-*polA*
AO-PolA/B	The *A. oryzae* NSAR1 transformant harboring pTAex3-*polA* and pUSA-*polB*
AO-PolA/C	The *A. oryzae* NSAR1 transformant harboring pTAex3-*polA* and pUSA-*polC*
AO-PolA/D	The *A. oryzae* NSAR1 transformant harboring pTAex3-*polA* and pUSA-*polD*
AO-PolA/E	The *A. oryzae* NSAR1 transformant harboring pTAex3-*polA* and pUSA-*polE*
AO-PolA/B/C	The *A. oryzae* NSAR1 transformant harboring pTAex3-*polA* and pUSA-*polC* and pAdeA-*polB*
AO-PolA/C/D	The *A. oryzae* NSAR1 transformant harboring pTAex3-*polA* and pUSA-*polC* and pAdeA-*polD*
AO-PolA/C/E	The *A. oryzae* NSAR1 transformant harboring pTAex3-*polA* and pUSA-*polC* and pAdeA-*polE*
AO-PolA/B/C/E	The *A. oryzae* NSAR1 transformant harboring pTAex3-*polA* and pUSA-*polC* and pAdeA-*polB-polE*
AO-PolA/B/C/D/E	The *A. oryzae* NSAR1 transformant harboring pTAex3-*polA* and pUSA-*polC-polD* and pAdeA-*polB-polE*
AO-EfuA$_{TC}$	The *A. oryzae* NSAR1 transformant harboring pTAex3-*efuA$_{TC}$*
AO-EfuA$_{TC}$/PolC	The *A. oryzae* NSAR1 transformant harboring pTAex3-*efuA$_{TC}$* and pUSA-*polC*
AO-EfuA$_{TC}$/PolB/C	The *A. oryzae* NSAR1 transformant harboring pTAex3-*efuA$_{TC}$* and pUSA-*polC* and pAdeA-*polB*
AO-EfuA$_{TC}$/PolC/E	The *A. oryzae* NSAR1 transformant harboring pTAex3-*efuA$_{TC}$* and pUSA-*polC* and pAdeA-*polE*
AO-EfuA$_{TC}$/PolB/C/E	The *A. oryzae* NSAR1 transformant harboring pTAex3-*efuA$_{TC}$* and pUSA-*polC* and pAdeA-*polB-polE*
AO-FsoA$_{TC}$	The *A. oryzae* NSAR1 transformant harboring pTAex3-*fsoA$_{TC}$*
AO-FsoA$_{TC}$/PolC	The *A. oryzae* NSAR1 transformant harboring pTAex3-*fsoA$_{TC}$* and pUSA-*polC*
AO-FsoA$_{TC}$/PolB/C	The *A. oryzae* NSAR1 transformant harboring pTAex3-*fsoA$_{TC}$* and pUSA-*polC* and pAdeA-*polB*
AO-FsoA$_{TC}$/PolC/E	The *A. oryzae* NSAR1 transformant harboring pTAex3-*fsoA$_{TC}$* and pUSA-*polC* and pAdeA-*polE*
AO-FsoA$_{TC}$/PolB/C/E	The *A. oryzae* NSAR1 transformant harboring pTAex3-*fsoA$_{TC}$* and pUSA-*polC* and pAdeA-*polB-polE*

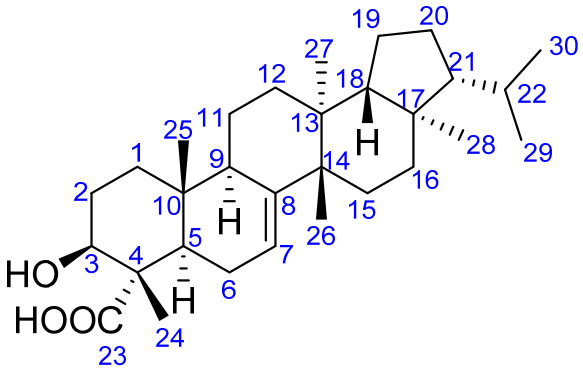

Table S4 NMR data of **3** in pyridine-d_5 (^{1}H at 400 MHz and ^{13}C at 100 MHz)

No.	δ_C, type	δ_H (J in Hz)[a]	^{1}H-^{1}H COSY	HMBC	NOESY
1	37.1, CH_2	a: 1.68	1b, 2	3, 5, 10	25
		b: 1.28	1a, 2	2, 3, 9, 25	3, 5, 9
2	28.5[b], CH_2	2.02	1a, 1b, 3	1, 3, 4, 10	24, 25
3	75.5, CH	4.70	2	1, 2, 4, 23, 24	1b, 5
4	53.7, C				
5	48.0, CH	2.58	6a, 6b	3, 4, 6, 7, 9, 10, 23, 24, 25	1b, 3
6	26.4, CH_2	a: 2.39	5, 6b, 7	5, 7, 8	
		b: 2.32	5, 6a, 7	5, 7, 8	
7	116.4, CH	5.42	6a, 6b, 9	5, 6, 9, 14	15b, 26
8	145.7, C				
9	48.4, CH	2.58	7, 11a, 11b	5, 7, 8, 10, 25	1b, 27
10	35.2, C				
11	16.4, CH_2	a: 1.58	9, 11b, 12	12	
		b: 1.46	9, 11a, 12	9, 12	
12	32.5, CH_2	1.34	11a, 11b	9, 13, 14, 18, 27	
13	36.2, C				
14	41.8, C				
15	30.5, CH_2	a: 1.60	15b, 16a, 16b	8, 26	28
		b: 1.47	15a, 16a, 16b	16, 17	7
16	36.5, CH_2	a: 1.66	15a, 15b, 16b	14, 15, 18	28, 29
		b: 1.43	15a, 15b, 16a	15	29
17	43.0, C				
18	54.3, CH	1.42	19a, 19b	19, 21	21, 26
19	20.2, CH_2	a: 1.36	18, 19b, 20a, 20b	18	
		b: 1.20	18, 19a, 20a, 20b	21	
20	28.4[b], CH_2	a: 1.75	19a, 19b, 20b, 21		30
		b: 1.15	19a, 19b, 20a, 21		
21	59.5, CH	0.87	20a, 20b, 22	22	18
22	30.8, CH	1.37	21, 29, 30	21	28
23	180.1, C				
24	11.3, CH_3	1.74, s		3, 4, 5, 23	2, 25
25	13.5, CH_3	0.92, s		1, 5, 9, 10	1a, 2, 24
26	24.2, CH_3	1.07, s		8, 13, 14, 15	7, 18
27	21.2, CH_3	0.90, s		12, 13, 14, 18	9
28	14.1, CH_3	0.70, s		16, 17, 18, 21	15a, 16a, 22
29	22.2, CH_3	0.89, d (6.4)	22	21, 22, 30	16a, 16b
30	23.1, CH_3	0.83, d (6.4)	22	21, 22, 29	20a

[a] The indiscernible signals from overlap or the complex multiplicity are reported without designating multiplicity.

[b] The assignments could be interchanged.

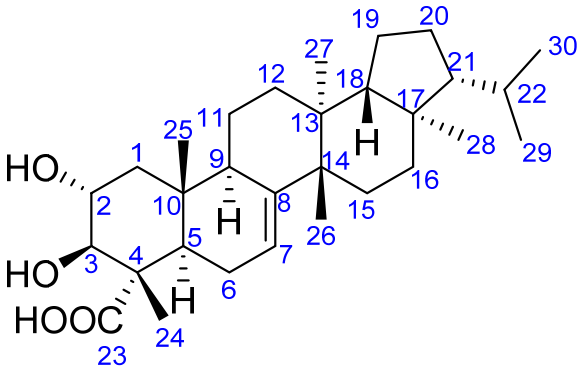

Table S5 NMR data of **4** in pyridine-d_5 (^{1}H at 400 MHz and ^{13}C at 100 MHz)

No.	δ_C, type	δ_H (J in Hz)[a]	^{1}H-^{1}H COSY	HMBC	ROESY
1	46.2, CH_2	a: 2.35	1b, 2	3, 5	25
		b: 1.64	1a, 2	2, 25	3, 5
2	68.7, CH	4.32, td (10.4, 4.0)	1a, 1b, 3		24, 25
3	80.7, CH	4.65, d (9.6)	2	2, 4, 23, 24	1b, 5
4	54.0, C				
5	48.2, CH	2.75	6	4, 6, 10, 24, 25	1b, 3
6	26.1, CH_2	2.35	5, 7	5	
7	116.5, CH	5.43	6, 9	5, 9, 14	15b
8	145.4, C				
9	48.8, CH	2.73	7, 11a, 11b	10, 25	27
10	36.9, C				
11	16.5, CH_2	a: 1.66	9, 11b, 12		27
		b: 1.56	9, 11a, 12		25
12	32.5, CH_2	1.30	11a, 11b		
13	36.2, C				
14	41.8, C				
15	30.5, CH_2	a: 1.60	15b, 16a, 16b		27, 28
		b: 1.45	15a, 16a, 16b	13, 16, 17	7
16	36.4, CH_2	a: 1.65	15a, 15b, 16b	14	28
		b: 1.41	15a, 15b, 16a		
17	42.9, C				
18	54.2, CH	1.40	19a, 19b	19	21, 26
19	20.2, CH_2	a: 1.36	18, 19b, 20a, 20b		
		b: 1.23	18, 19a, 20a, 20b		
20	28.4, CH_2	a: 1.73	19a, 19b, 20b, 21		
		b: 1.15	19a, 19b, 20a, 21		
21	59.5, CH	0.85	20a, 20b, 22		18
22	30.8, CH	1.35	21, 29, 30		28
23	179.7, C				
24	12.5, CH_3	1.80, s		3, 4, 5, 23	2, 25
25	14.6, CH_3	1.02, s		1, 5, 9, 10	1a, 2, 11b, 24
26	24.2, CH_3	1.05, s		8, 13, 14, 15	18
27	21.2, CH_3	0.87, s		12, 13, 14, 18	9, 11a, 15a
28	14.1, CH_3	0.68, s		16, 17, 18, 21	15a, 16a, 22
29	22.2, CH_3	0.88, d (6.4)	22	21, 22, 30	
30	23.1, CH_3	0.83, d (6.4)	22	21, 22, 29	

[a] The indiscernible signals from overlap or the complex multiplicity are reported without designating multiplicity.

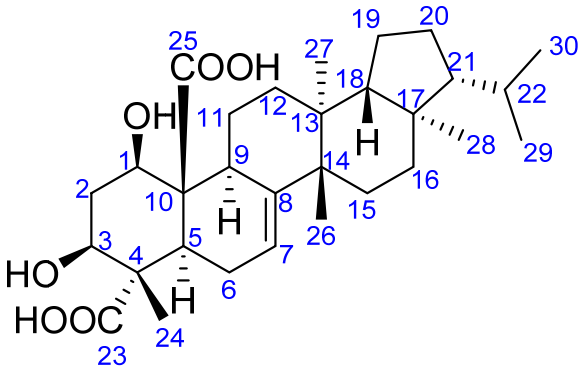

Table S6 NMR data of **5** in pyridine-d_5 (^{1}H at 400 MHz and ^{13}C at 100 MHz)

No.	δ_C, type	δ_H (J in Hz)[a]	^{1}H-^{1}H COSY	HMBC	ROESY
1	78.5, CH	3.98, dd (12.0, 4.0)	2a, 2b	2, 9, 25	3, 5
2	40.9, CH_2	a: 2.84, q (12.0)	1, 2b, 3	1, 3, 4, 10	24
		b: 2.71	1, 2a, 3	1, 3, 4, 10	
3	73.0, CH	4.92, dd (12.0, 4.4)	2a, 2b	23, 24	1, 5
4	54.0, C				
5	44.8, CH	2.66, dd (10.4, 5.6)	6a, 6b	4, 6, 10, 24, 25	1, 3
6	27.2, CH_2	a: 3.21	5, 6b, 7	5, 7, 8	
		b: 2.53, dt (16.8, 4.8)	5, 6a, 7	10	
7	118.6, CH	5.78	6a, 6b	5, 6, 9, 14	26
8	143.4, C				
9	47.2, CH	3.28	11a, 11b	1, 8, 10, 12, 13, 25	27
10	51.1, C				
11	21.1, CH_2	a: 3.27	9, 11b, 12a, 12b	8, 12, 13	
		b: 1.92	9, 11a, 12a, 12b	12	26
12	33.2, CH_2	a: 1.43	11a, 11b, 12b	13, 27	
		b: 1.35	11a, 11b, 12a	9, 27	
13	35.8, C				
14	42.6, C				
15	30.9, CH_2	a: 1.69	15b, 16a, 16b	14, 26	
		b: 1.60	15a, 16a, 16b	13, 16	
16	36.4, CH_2	a: 1.64	15a, 15b, 16b	17, 18, 28	29
		b: 1.42	15a, 15b, 16a	15, 17, 28	
17	42.8, C				
18	54.2, CH	1.40	19a, 19b	13, 17, 19, 27, 28	21
19	20.0, CH_2	a: 1.31	18, 19b, 20a, 20b	18	
		b: 1.21	18, 19a, 20a, 20b		
20	28.3, CH_2	a: 1.70	19a, 19b, 20b, 21		30
		b: 1.11	19a, 19b, 20a, 21	19, 21	
21	59.4, CH	0.81	20a, 20b, 22	16, 17, 20	18
22	30.6, CH	1.32	21, 29, 30		
23	179.3, C				
24	10.2, CH_3	1.91, s		3, 4, 5, 23	2a
25	178.4, C				
26	24.7, CH_3	1.25, s		8, 13, 14, 15	7, 11b
27	21.6, CH_3	1.00, s		12, 13, 14, 18	9, 28
28	14.2, CH_3	0.70, s		16, 17, 18, 21	27
29	22.0, CH_3	0.85, d (6.4)	22	21, 22, 30	16a
30	23.0, CH_3	0.79, d (6.4)	22	21, 22, 29	20a

[a] The indiscernible signals from overlap or the complex multiplicity are reported without designating multiplicity.

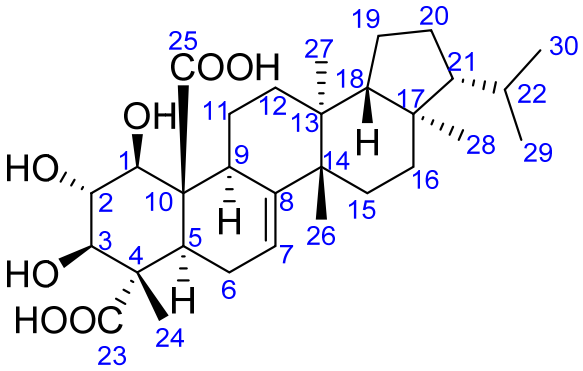

Table S7 NMR data of **1** in pyridine-d_5 (^{1}H at 600 MHz and ^{13}C at 150 MHz)

No.	δ_C, type	δ_H (J in Hz)[a]	^{1}H-^{1}H COSY	HMBC	ROESY
1	83.8, CH	3.97, d (8.4)	2	2, 9, 10, 25	3, 5,
2	75.4, CH	4.66, t (9.0)	1, 3	1, 3	24
3	77.9, CH	4.84, d (9.0)	2	2, 4, 23, 24	1, 5
4	53.5, C				
5	44.6, CH	2.84	6a, 6b	3, 4, 6, 9, 10, 24	1, 3, 9
6	27.1, CH_2	a: 3.31	5, 6b, 7		24
		b: 2.55, br d (17.4)	5, 6a, 7	7, 8, 10	
7	118.7, CH	5.80	6a, 6b	5, 9	26
8	143.4, C				
9	48.0, CH	3.35	11a, 11b		5, 27
10	51.7, C				
11	21.1, CH_2	a: 3.33	9, 11b, 12a, 12b		
		b: 2.01	9, 11a, 12a, 12b		26
12	33.3, CH_2	a: 1.45	11a, 11b, 12b	14	27
		b: 1.37	11a, 11b, 12a		
13	35.9, C				
14	42.6, C				
15	31.0, CH_2	a: 1.66	15b, 16a, 16b	14, 17	27, 28
		b: 1.58	15a, 16a, 16b		26
16	36.5, CH_2	a: 1.65	15a, 15b, 16b	14, 17, 18	29
		b: 1.43	15a, 15b, 16a	14, 17, 21, 28	
17	42.8, C				
18	54.3, CH	1.42	19a, 19b	13, 17, 21, 27, 28	21, 26
19	20.1, CH_2	a: 1.31	18, 19b, 20a, 20b		
		b: 1.21	18, 19a, 20a, 20b		27, 28
20	28.4, CH_2	a: 1.71	19a, 19b, 20b, 21		30
		b: 1.13	19a, 19b, 20a, 21		22, 28, 30
21	59.4, CH	0.81	20a, 20b, 22	16, 17	18
22	30.7, CH	1.34	21, 29, 30	21, 29, 30	20b, 28
23	178.8, C				
24	11.7, CH_3	1.98, s		3, 4, 5, 23	2, 6a
25	178.4, C				
26	24.7, CH_3	1.27, s		8, 13, 14, 15	7, 11b, 15b, 18
27	21.7, CH_3	0.99, s		12, 13, 14, 18	9, 12a, 15a, 19b, 28
28	14.2, CH_3	0.70, s		16, 17, 18, 21	15a, 19b, 20b, 22, 27
29	22.1, CH_3	0.86, d (6.0)	22	21, 22, 30	16a
30	23.0, CH_3	0.80, d (6.0)	22	21, 22, 29	20a, 20b

[a] The indiscernible signals from overlap or the complex multiplicity are reported without designating multiplicity.

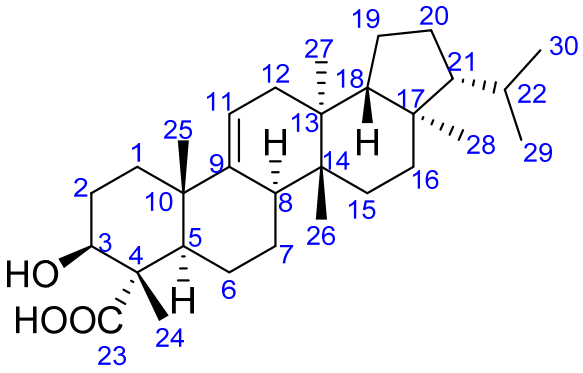

Table S8 NMR data of **8** in pyridine-d_5 (^{1}H at 600 MHz and ^{13}C at 150 MHz)

No.	δ_C, type	δ_H (J in Hz)[a]	^{1}H-^{1}H COSY	HMBC	ROESY
1	40.0, CH_2	a: 2.05	1b, 2a, 2b	3, 5, 10, 25	11
		b: 1.62	1a, 2a, 2b	2, 3, 10, 25	3, 5
2	28.8, CH_2	a: 2.04	1a, 1b, 2b, 3	1, 3, 4, 10	
		b: 2.00	1a, 1b, 2a, 3	1, 3, 4, 10	
3	75.7, CH	4.67, dd (10.2, 6.0)	2a, 2b	4, 23, 24	1b, 5
4	53.7, C				
5	41.9, CH	2.56, dd (12.6, 7.2)	6a, 6b	3, 4, 6, 9, 10, 23, 24, 25	1b, 3, 8
6	20.9, CH_2	a: 2.02	5, 6b, 7a, 7b	4, 5, 7, 8, 10	
		b: 1.92	5, 6a, 7a, 7b	5, 7, 10	
7	18.1, CH_2	a: 1.63	6a, 6b, 7b, 8	6	
		b: 1.40	6a, 6b, 7a, 8	6, 8, 9	
8	40.1, CH	2.21, br d (13.2)	7a, 7b, 11		5, 27
9	151.7, C				
10	37.7, C				
11	116.7, CH	5.42	8, 12a, 12b	8, 9, 12, 13	1a
12	36.9, CH_2	a: 1.65	11, 12b	9, 11, 18, 27	26
		b: 1.56, dd (17.4, 4.2)	11, 12a	9, 11, 13, 14, 27	
13	36.9, C				
14	37.9, C				
15	29.3, CH_2	a: 1.25	15b, 16a, 16b		
		b: 1.19	15a, 16a, 16b	16, 26	
16	36.4, CH_2	a: 1.60	15a, 15b, 16b	14, 17, 28	28, 29
		b: 1.34	15a, 15b, 16a	17, 28	
17	43.1, C				
18	52.1, CH	1.50, dd (12.6, 7.2)	19a, 19b	12, 17, 19, 20, 21, 27, 28	21, 26
19	20.3, CH_2	a: 1.32	18, 19b, 20a, 20b		
		b: 1.26	18, 19a, 20a, 20b	18	
20	28.4, CH_2	a: 1.74	19a, 19b, 20b, 21	17, 19, 21	30
		b: 1.14	19a, 19b, 20a, 21	19	22
21	59.6, CH	0.88	20a, 20b, 22	16, 17, 20, 28	18
22	30.9, CH	1.37	21, 29, 30	21, 30	20b, 28
23	180.6, C				
24	11.9, CH_3	1.76, s		3, 4, 5, 23	25
25	25.8, CH_3	1.23, s		1, 5, 9, 10	24
26	15.7, CH_3	0.80, s		8, 13, 14, 15	12a, 18
27	16.0, CH_3	0.78, s		12, 13, 14, 18	8
28	14.0, CH_3	0.72, s		16, 17, 18, 21	16a, 22
29	22.2, CH_3	0.88, d (6.6)	22	21, 22, 30	16a
30	23.1, CH_3	0.84, d (6.6)	22	21, 22, 29	20a

[a] The indiscernible signals from overlap or the complex multiplicity are reported without designating multiplicity.

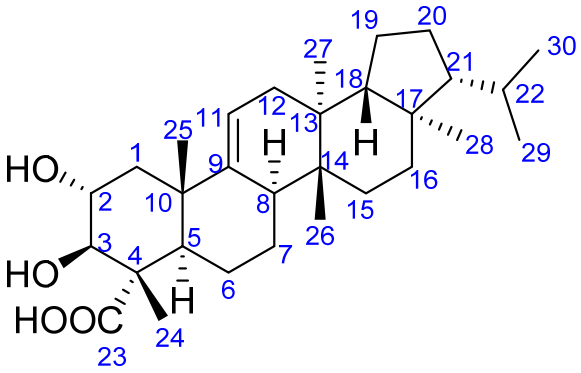

Table S9 NMR data of **9** in pyridine-d_5 (^{1}H at 600 MHz and ^{13}C at 150 MHz)

No.	δ_C, type	δ_H (J in Hz)[a]	^{1}H-^{1}H COSY	HMBC	ROESY
1	48.8, CH_2	a: 2.72, dd (12.6, 4.2)	1b, 2	2, 3, 5, 10, 25	11, 25
		b: 1.93	1a, 2	2, 3, 9, 10, 25	3, 5
2	68.8, CH	4.29, td (10.2, 4.2)	1a, 1b, 3	1, 3	24, 25
3	80.8, CH	4.60, d (9.6)	2	1, 2, 4, 5, 23, 24	1b, 5
4	53.6, C				
5	42.1, CH	2.70, dd (11.4, 7.8)	6a, 6b	1, 3, 4, 6, 9, 10, 24, 25	1b, 3, 8
6	20.6, CH_2	a: 2.02, br q (12.0)	5, 6b, 7a, 7b	4, 5, 7, 8, 10	
		b: 1.89	5, 6a, 7a, 7b		
7	18.0, CH_2	a: 1.61	6a, 6b, 8	9	
		b: 1.39	6a, 6b, 8		
8	39.9, CH	2.21, br d (13.8)	7a, 7b, 11		5, 27
9	151.2, C				
10	39.5, C				
11	116.9, CH	5.49	8, 12a, 12b	8, 10, 12	1a, 25
12	36.8, CH_2	a: 1.61, br d (17.4)	11, 12b	9, 11, 13, 18	
		b: 1.49	11, 12a	9, 11, 13, 27	
13	36.9, C				
14	37.8, C				
15	29.2, CH_2	1.17	16a, 16b		27, 28
16	36.3, CH_2	a: 1.57	15, 16b	15, 17, 28	28, 29
		b: 1.31	15, 16a		26
17	43.0, C				
18	52.0, CH	1.46	19a, 19b	12, 13, 14, 17, 19, 21, 27, 28	21, 26
19	20.2, CH_2	a: 1.27	18, 19b, 20a, 20b		
		b: 1.21	18, 19a, 20a, 20b	13	
20	28.3, CH_2	a: 1.72	19a, 19b, 20b, 21	17, 19, 21	30
		b: 1.12	19a, 19b, 20a, 21	19	
21	59.6, CH	0.84	20a, 20b, 22		18
22	30.8, CH	1.35	21, 29, 30	21, 29, 30	28
23	179.6, C				
24	13.0, CH_3	1.80, s		3, 4, 5, 23	2
25	26.7, CH_3	1.30, s		1, 5, 9, 10	1a, 2, 11
26	15.6, CH_3	0.77, s		8, 13, 14, 15	16b, 18
27	15.8, CH_3	0.73, s		12, 13, 14, 18	8, 15
28	13.9, CH_3	0.68, s		16, 17, 18, 21	15, 16a, 22
29	22.2, CH_3	0.86, d (6.6)	22	21, 22, 30	16a
30	23.1, CH_3	0.82, d (6.6)	22	21, 22, 29	20a

[a] The indiscernible signals from overlap or the complex multiplicity are reported without designating multiplicity.

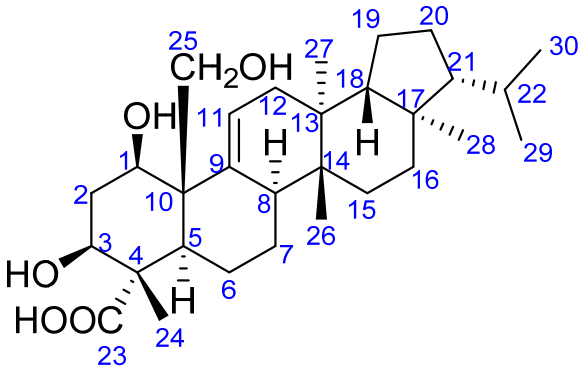

Table S10 NMR data of **10** in pyridine-d_5 (^{1}H at 600 MHz and ^{13}C at 150 MHz)

No.	δ_C, type	δ_H (J in Hz)[a]	^{1}H-^{1}H COSY	HMBC	NOESY
1	78.7, CH	4.51	2	2, 3, 9, 10, 25	5
2	39.5, CH_2	2.61	1, 3	1, 3, 4, 10	24, 25a
3	72.8, CH	4.83	2	1, 2, 4, 5, 23, 24	5
4	53.5, C				
5	40.9, CH	2.75, dd (12.6, 7.2)	6a, 6b	1, 3, 4, 6, 9, 10, 23, 24, 25	1, 3, 8
6	21.8, CH_2	a: 2.18	5, 6b, 7a, 7b	4, 5, 7, 8	24, 25b
		b: 2.01	5, 6a, 7a, 7b	5, 7, 10	
7	17.8, CH_2	a: 1.67	6a, 6b, 7b, 8		
		b: 1.63	6a, 6b, 7a, 8		
8	40.9, CH	2.35, br d (12.6)	7a, 7b, 11	9	5, 27
9	143.9, C				
10	48.6, C				
11	122.7, CH	6.98	8, 12a, 12b	8, 10, 13	
12	37.6, CH_2	a: 1.89, br d (17.4)	11, 12b	9, 11, 13, 18, 27	18, 26
		b: 1.67	11, 12a	9, 11, 13, 14, 27	27
13	36.8, C				
14	38.4, C				
15	29.4, CH_2	1.25	16a, 16b	13, 14, 16, 17	27, 28
16	36.4, CH_2	a: 1.59	15, 16b	14, 15, 17, 28	28, 29
		b: 1.35	15, 16a	15, 28	26
17	43.1, C				
18	52.0, CH	1.52, dd (13.2, 7.2)	19a, 19b	13, 14, 17, 19, 20, 21, 27, 28	12a, 21, 26
19	20.3, CH_2	a: 1.27	18, 19b, 20a, 20b	17, 18, 21	
		b: 1.22	18, 19a, 20a, 20b	17, 18, 21	
20	28.4, CH_2	a: 1.71	19a, 19b, 20b, 21	17, 19	30
		b: 1.12	19a, 19b, 20a, 21	19, 21	22, 28, 30
21	59.6, CH	0.85	20a, 20b, 22	17, 20, 28	18
22	30.9, CH	1.36	21, 29, 30	17, 21, 29, 30	20b, 28
23	180.0, C				
24	12.0, CH_3	1.83, s		3, 4, 5, 23	2, 6a
25	63.3, CH_2	a: 4.67, d (10.8)	25b	1, 5, 9, 10	2
		b: 3.98, d (10.8)	25a	1, 5, 9, 10	6a
26	15.8, CH_3	1.02, s		8, 13, 14, 15	12a, 16b, 18
27	16.4, CH_3	0.88, s		12, 13, 14, 18	8, 12b, 15, 28
28	14.0, CH_3	0.71, s		16, 17, 18, 21	15, 16a, 20b, 22, 27
29	22.2, CH_3	0.87, d (6.6)	22	21, 22, 30	16a
30	23.1, CH_3	0.82, d (6.6)	22	21, 22, 29	20a, 20b

[a] The indiscernible signals from overlap or the complex multiplicity are reported without designating multiplicity.

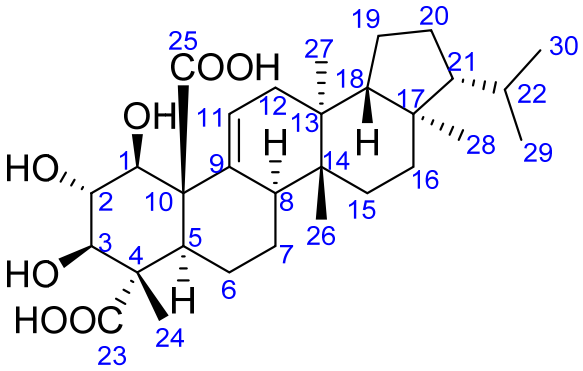

Table S11 NMR data of **11** in CD_3OD (^1H at 600 MHz and ^{13}C at 150 MHz)

No.	δ_C, type	δ_H (J in Hz)[a]	^1H-^1H COSY	HMBC	NOESY
1	82.7, CH	3.11, d (9.0)	2	2, 9, 10, 25	3, 5, 11
2	76.3, CH	3.68	1, 3	1, 3	24
3	78.7, CH	3.77, d (8.4)	2	2, 4, 23, 24	1, 5
4	53.8, C				
5	41.4, CH	1.98, dd (11.4, 7.2)	6a, 6b	1, 3, 4, 6, 9, 10, 23, 24, 25	1, 3
6	22.3, CH_2	a: 2.18	5, 6b, 7a, 7b	4, 5, 7, 8	24
		b: 1.44	5, 6a, 7a, 7b	7	
7	19.9, CH_2	a: 1.93	6a, 6b, 7b, 8	8	26
		b: 1.54	6a, 6b, 7a, 8	6	
8	40.9, CH	2.17	7a, 7b, 11	15	27
9	143.1, C				
10	52.9, C				
11	122.5, CH	6.46	8, 12a, 12b	8, 9, 10, 13	1
12	37.9, CH_2	a: 1.69	11, 12b, 27	9, 11, 13, 14, 27	26
		b: 1.56	11, 12a	9, 11, 13, 14, 27	27
13	37.5, C				
14	39.1, C				
15	30.7, CH_2	a: 1.48	15b, 16a, 16b	16	
		b: 1.34	15a, 16a, 16b	13, 17	26
16	37.3, CH_2	a: 1.66	15a, 15b, 16b	15, 17, 18, 28	28, 29
		b: 1.42	15a, 15b, 16a	17, 28	
17	44.1, C				
18	53.4, CH	1.62	19a, 19b	16, 17, 19, 20, 21, 27, 28	21, 26
19	21.1, CH_2	a: 1.39	18, 19b, 20a, 20b		
		b: 1.34	18, 19a, 20a, 20b	13, 17, 18, 20	27, 28
20	29.3, CH_2	a: 1.85	19a, 19b, 20b, 21	17, 19, 21	30
		b: 1.24	19a, 19b, 20a, 21		22, 28, 30
21	61.0, CH	0.97	20a, 20b, 22	20, 22, 28	18
22	32.1, CH	1.45	21, 29, 30	21, 29, 30	20b, 28
23	181.4, C				
24	11.8, CH_3	1.12, s		3, 4, 5, 23	2, 6a
25	181.6, C				
26	16.4, CH_3	0.76, s		8, 13, 14, 15	7a, 12a, 15b, 18
27	16.0, CH_3	0.89, s	12a	12, 13, 14, 18	8, 12b, 19b
28	14.5, CH_3	0.80, s		16, 17, 18, 21	16a, 19b, 20b, 22
29	22.6, CH_3	0.90, d (6.6)	22	21, 22, 30	16a
30	23.4, CH_3	0.84, d (6.6)	22	21, 22, 29	20a, 20b

[a] The indiscernible signals from overlap or the complex multiplicity are reported without designating multiplicity.

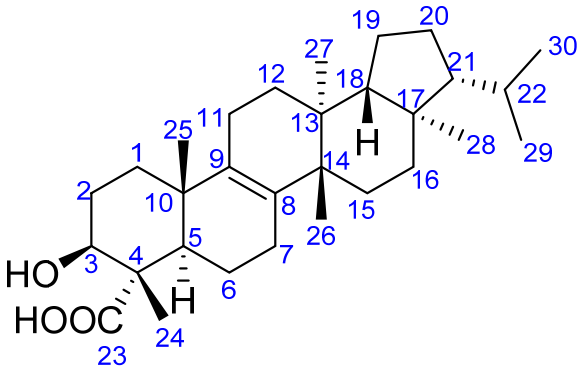

Table S12 NMR data of **12** in pyridine-d_5 (^{1}H at 600 MHz and ^{13}C at 150 MHz)

No.	δ_C, type	δ_H (J in Hz)[a]	^{1}H-^{1}H COSY	HMBC	ROESY
1	36.0, CH_2	a: 1.85, dt (13.2, 3.0)	1b, 2a, 2b	2, 3, 5, 9, 10, 25	11a, 25
		b: 1.41	1a, 2a, 2b	2, 3, 5, 9, 10, 25	3, 5
2	28.6, CH_2	a: 2.04	1a, 1b, 2b, 3	1, 3, 4, 10	
		b: 2.00	1a, 1b, 2a, 3	1, 3, 4, 10	24, 25
3	75.3, CH	4.71, dd (11.4, 4.8)	2a, 2b	1, 2, 4, 5, 23, 24	1b, 5
4	54.6, C				
5	47.2, CH	2.38, dd (10.2, 4.2)	6a, 6b	1, 3, 4, 6, 7, 9, 10, 24, 25	1b, 3
6	22.1, CH_2	a: 1.79	5, 6b, 7a, 7b	4, 5, 7, 8, 10	
		b: 1.77	5, 6a, 7a, 7b	4, 5, 7, 8, 10	
7	27.1, CH_2	a: 2.14	6a, 6b, 7b	8, 9	26
		b: 2.08	6a, 6b, 7a	8, 9	
8	134.4, C				
9	134.6, C				
10	37.2, C				
11	19.2, CH_2	a: 2.13	11b, 12a, 12b	8, 9, 12	1a, 27
		b: 1.91	11a, 12a, 12b	8, 9, 12, 13	25
12	30.5, CH_2	a: 1.44	11a, 11b, 12b	9, 11, 13, 18, 27	
		b: 1.27	11a, 11b, 12a	9, 11, 13, 14, 18, 27	
13	36.9, C				
14	41.3, C				
15	27.1, CH_2	a: 1.61	15b, 16a, 16b	16, 26	
		b: 1.25	15a, 16a, 16b	13, 17	
16	36.1, CH_2	a: 1.59	15a, 15b, 16b	14, 15, 17, 18, 28	29
		b: 1.41	15a, 15b, 16a	15, 17, 21, 28	26
17	43.0, C				
18	52.9, CH	1.47	19a, 19b	13, 14, 17, 19, 21, 27, 28	21, 26
19	20.6, CH_2	a: 1.38	18, 19b, 20a, 20b	17, 18, 21	
		b: 1.26	18, 19a, 20a, 20b	13, 17, 18	
20	28.6, CH_2	a: 1.76	19a, 19b, 20b, 21	17, 18, 19, 21	30
		b: 1.17	19a, 19b, 20a, 21	18, 19, 21, 22	
21	59.8, CH	0.89	20a, 20b, 22	16, 17, 20, 22, 28, 29, 30	18
22	30.9, CH	1.38	21, 29, 30	17, 21, 30	
23	180.6, C				
24	12.2, CH_3	1.69, s		3, 4, 5, 23	2b, 25
25	20.6, CH_3	1.11, s		1, 5, 9, 10	1a, 2b, 11b, 24
26	22.3, CH_3	1.00, s		8, 13, 14, 15	7a, 16b, 18
27	16.0, CH_3	0.77, s		12, 13, 14, 18	11a
28	14.7, CH_3	0.74, s		16, 17, 18, 21	
29	22.2, CH_3	0.88, d (6.0)	22	21, 22, 30	16a
30	23.1, CH_3	0.84, d (6.0)	22	21, 22, 29	20a

[a] The indiscernible signals from overlap or the complex multiplicity are reported without designating multiplicity.

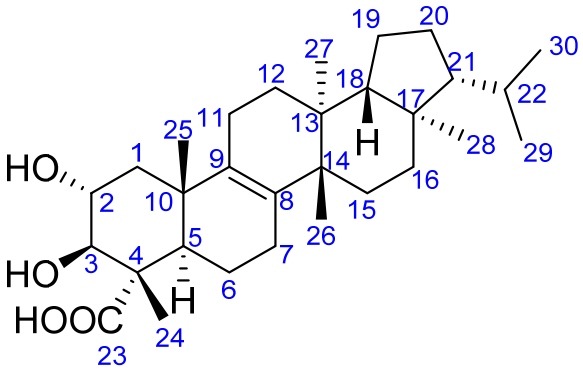

Table S13 NMR data of **13** in pyridine-d_5 (^{1}H at 600 MHz and ^{13}C at 150 MHz)

No.	δ_C, type	δ_H (J in Hz)[a]	^{1}H-^{1}H COSY	HMBC	ROESY
1	44.9, CH_2	a: 2.52	1b, 2	2, 3, 5	11a, 11b, 25
		b: 1.75	1a, 2	2, 3, 9, 10, 25	3
2	69.3, CH	4.33, br t (9.0)	1a, 1b, 3	3	24, 25
3	80.7, CH	4.67, d (9.0)	2	1, 2, 4, 23, 24	1b, 5
4	54.6, C				
5	47.3, CH	2.55	6a, 6b	4, 7, 9, 10, 23, 24, 25	3
6	21.5, CH_2	a: 1.83	5, 6b, 7a, 7b	8, 10	
		b: 1.77	5, 6a, 7a, 7b		
7	27.0, CH_2	a: 2.12	6a, 6b, 7b	5, 8, 9	15b, 26
		b: 2.06	6a, 6b, 7a	8,	
8	134.2, C				
9	134.5, C				
10	38.8, C				
11	19.3, CH_2	a: 2.19	11b, 12a, 12b	8, 9, 12	1a, 27
		b: 1.93	11a, 12a, 12b	8, 9, 12, 13	1a, 25
12	30.3, CH_2	a: 1.39	11a, 11b, 12b	11, 13, 18, 27	
		b: 1.21	11a, 11b, 12a	9, 11, 13, 14, 18, 27	
13	36.8, C				
14	41.2, C				
15	27.0, CH_2	a: 1.58	15b, 16a, 16b		27, 28
		b: 1.22	15a, 16a, 16b	13, 14	7a
16	36.1, CH_2	a: 1.56	15a, 15b, 16b	14, 17, 18, 28	
		b: 1.39	15a, 15b, 16a		
17	42.9, C				
18	52.8, CH	1.43	19a, 19b	12, 13, 14, 16, 17, 19, 21, 27, 28	21, 26
19	20.5, CH_2	a: 1.33	18, 19b, 20a, 20b	17	
		b: 1.21	18, 19a, 20a, 20b	20	
20	28.5, CH_2	a: 1.74	19a, 19b, 20b, 21	17, 21	30
		b: 1.13	19a, 19b, 20a, 21		22
21	59.7, CH	0.86	20a, 20b, 22	16, 17, 28	18
22	30.8, CH	1.36	21, 29, 30	21, 29, 30	20b, 28
23	180.2, C				
24	13.3, CH_3	1.75, s		3, 4, 5, 23	2
25	21.8, CH_3	1.18, s		1, 5, 9, 10	1a, 2, 11b
26	22.2, CH_3	0.97, s		8, 13, 14, 15	7a, 18
27	15.8, CH_3	0.71, s		12, 13, 14, 18	11a, 15a
28	14.6, CH_3	0.71, s		16, 17, 18, 21	15a, 22
29	22.1, CH_3	0.86, d (6.0)	22	21, 22, 30	
30	23.0, CH_3	0.82, d (6.0)	22	21, 22, 29	20a

[a] The indiscernible signals from overlap or the complex multiplicity are reported without designating multiplicity.

Supplementary Figures

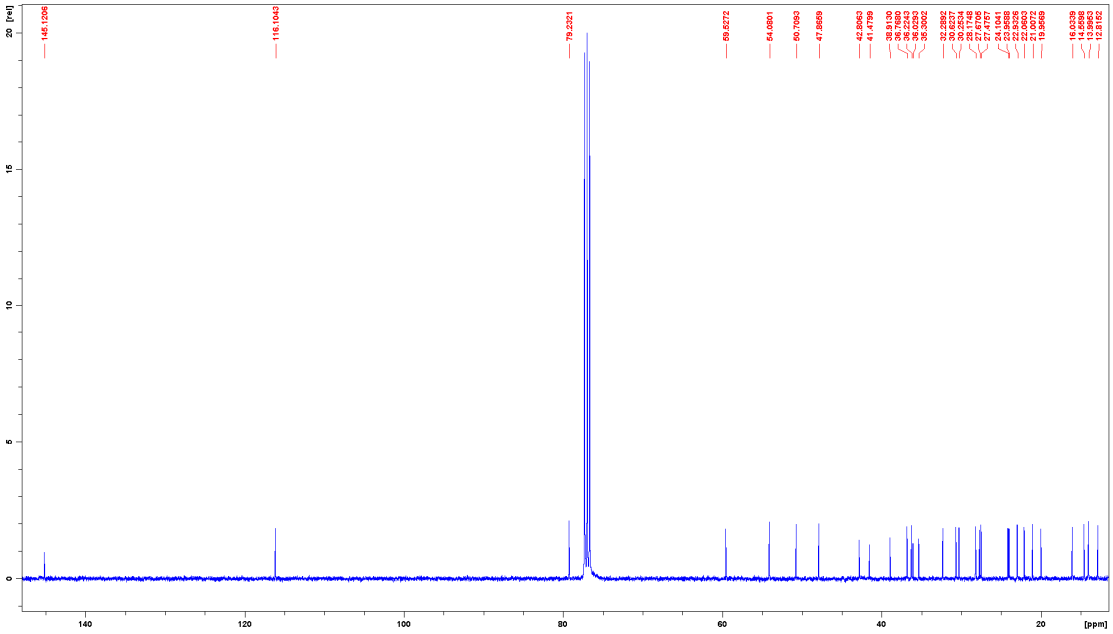

Figure S1 ^{13}C NMR spectrum of **2** in $CDCl_3$ at 100 MHz

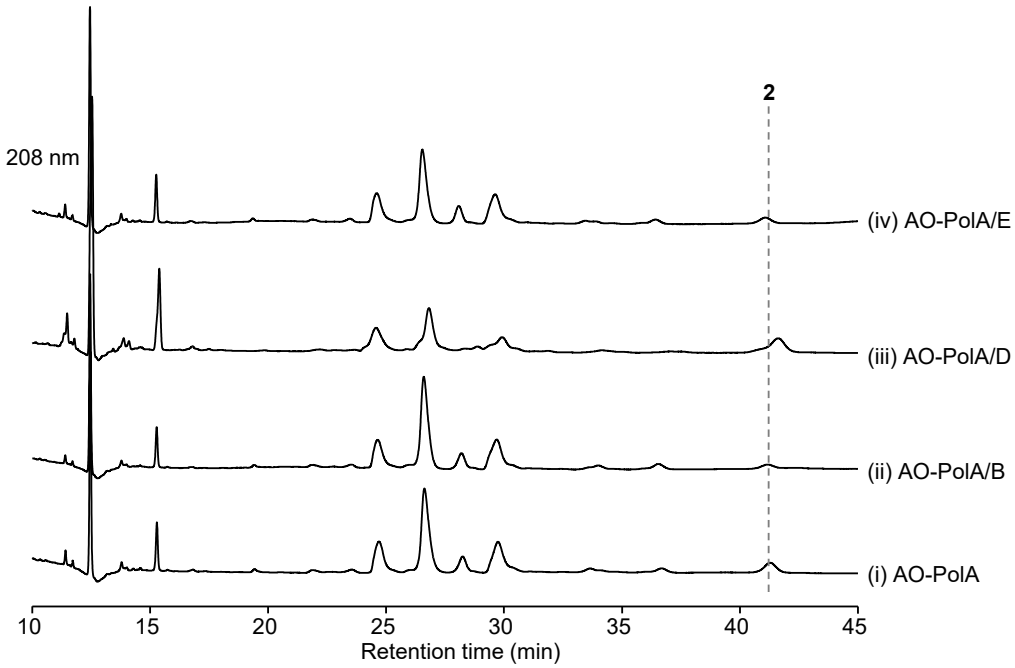

Figure S2 HPLC analysis of *A. oryzae* NSAR1 transformants expressing two genes in the *pol* cluster

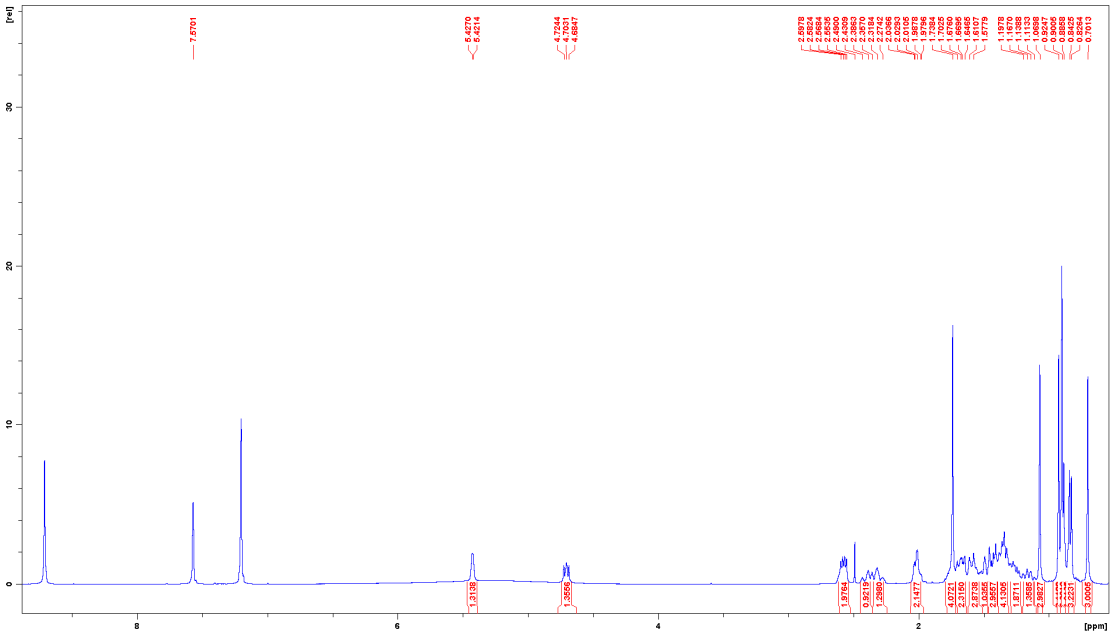

Figure S3 ^{1}H NMR spectrum of **3** in pyridine-d_5 at 400 MHz

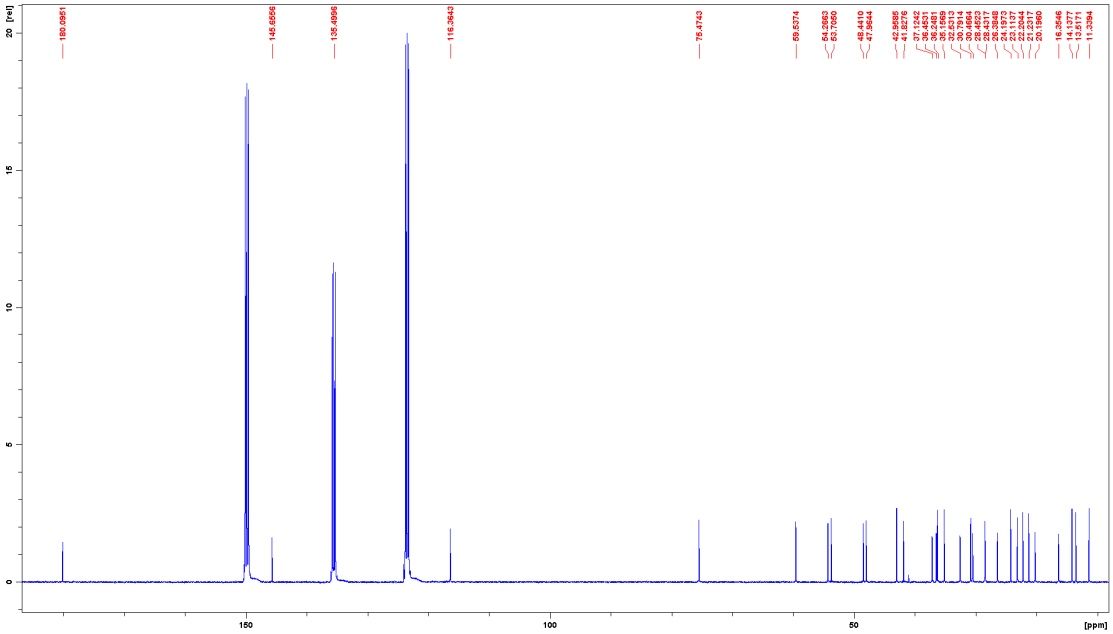

Figure S4 ^{13}C NMR spectrum of **3** in pyridine-d_5 at 100 MHz

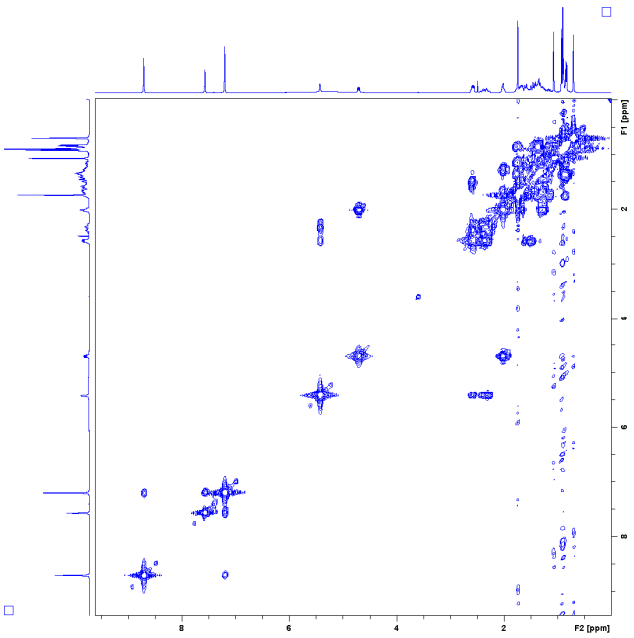

Figure S5 ^{1}H-^{1}H COSY spectrum of **3** in pyridine-d_5 at 400 MHz

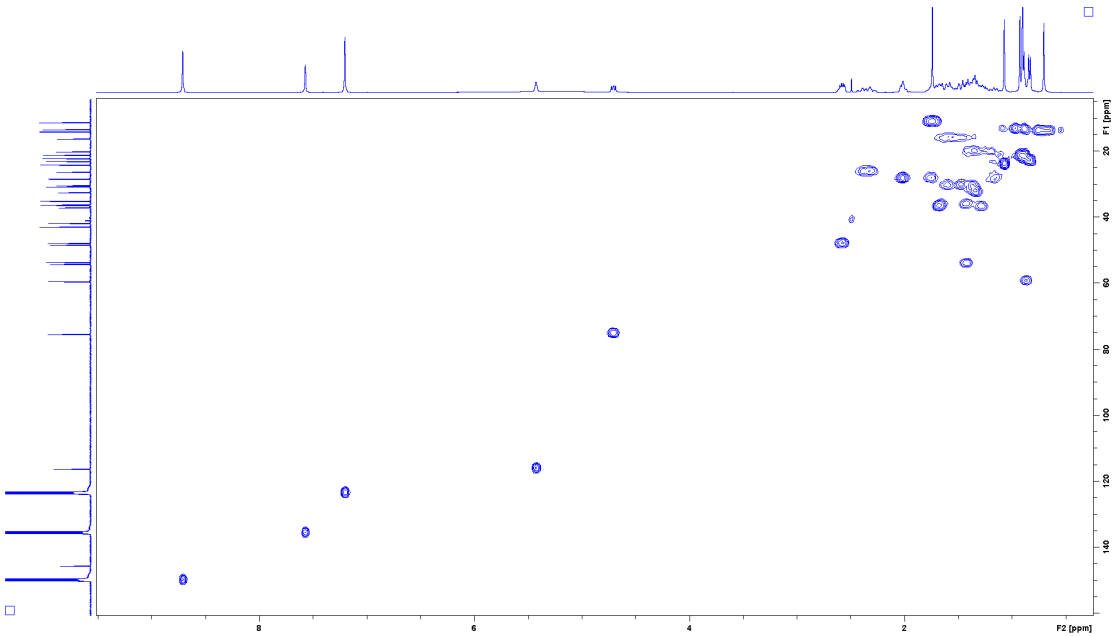

Figure S6 HSQC spectrum of **3** in pyridine-d_5 at 400 MHz

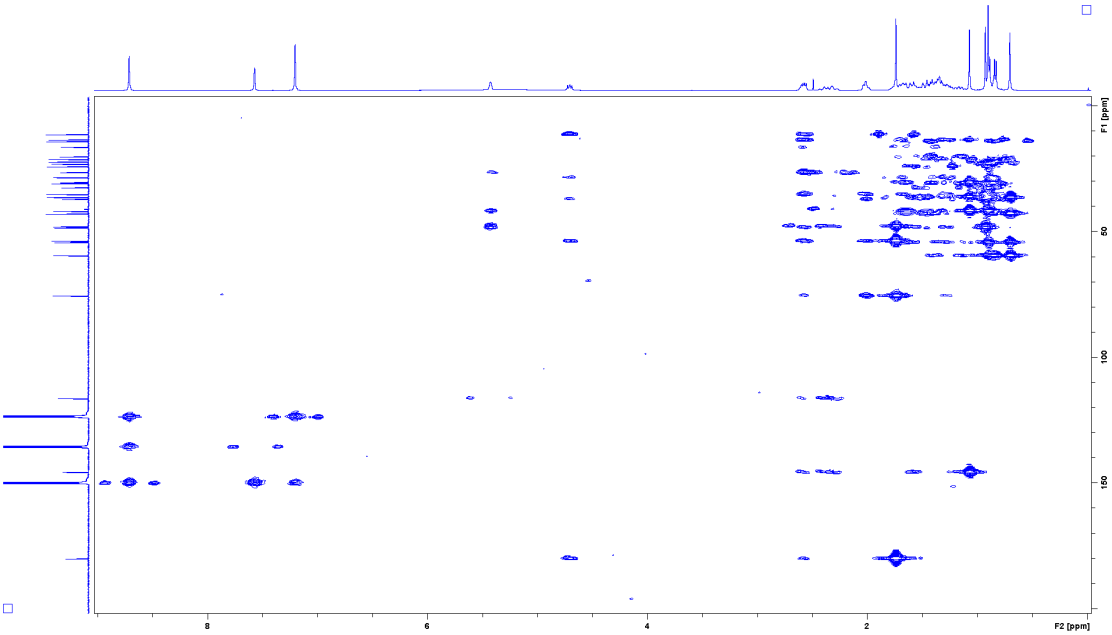

Figure S7 HMBC spectrum of **3** in pyridine-d_5 at 400 MHz

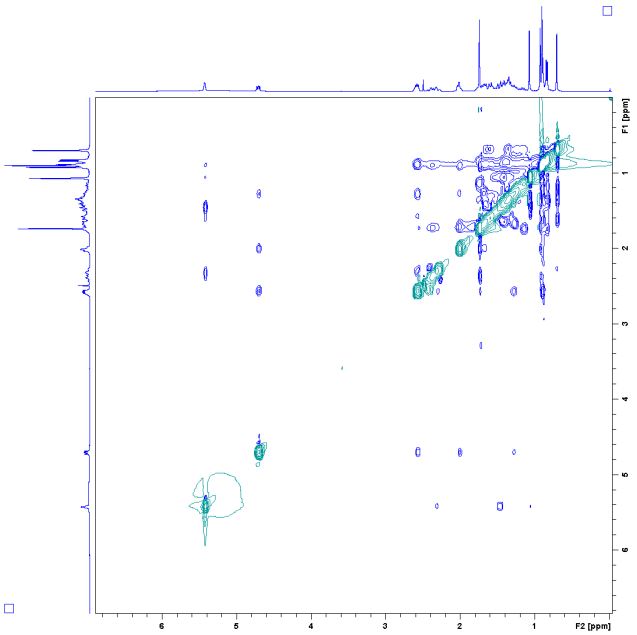

Figure S8 NOESY spectrum of **3** in pyridine-d_5 at 400 MHz

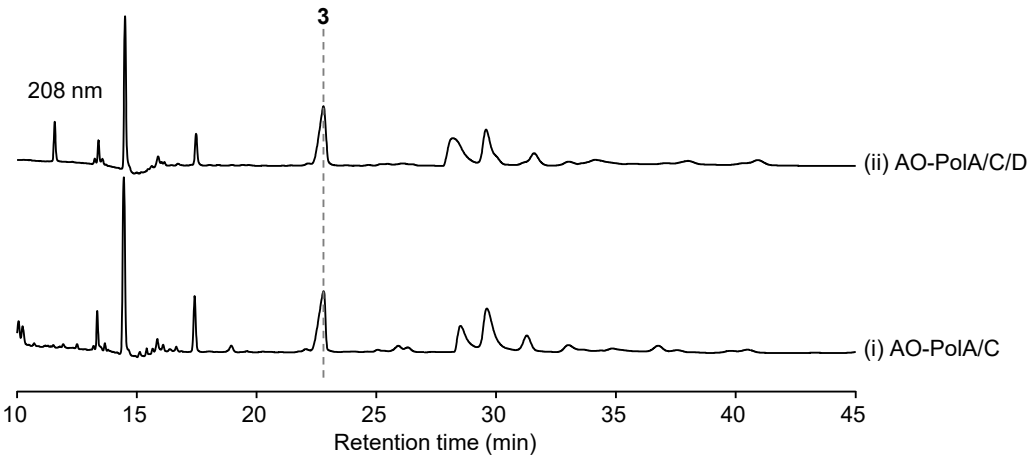

Figure S9 HPLC analysis of *A. oryzae* NSAR1 transformants expressing three genes in the *pol* cluster

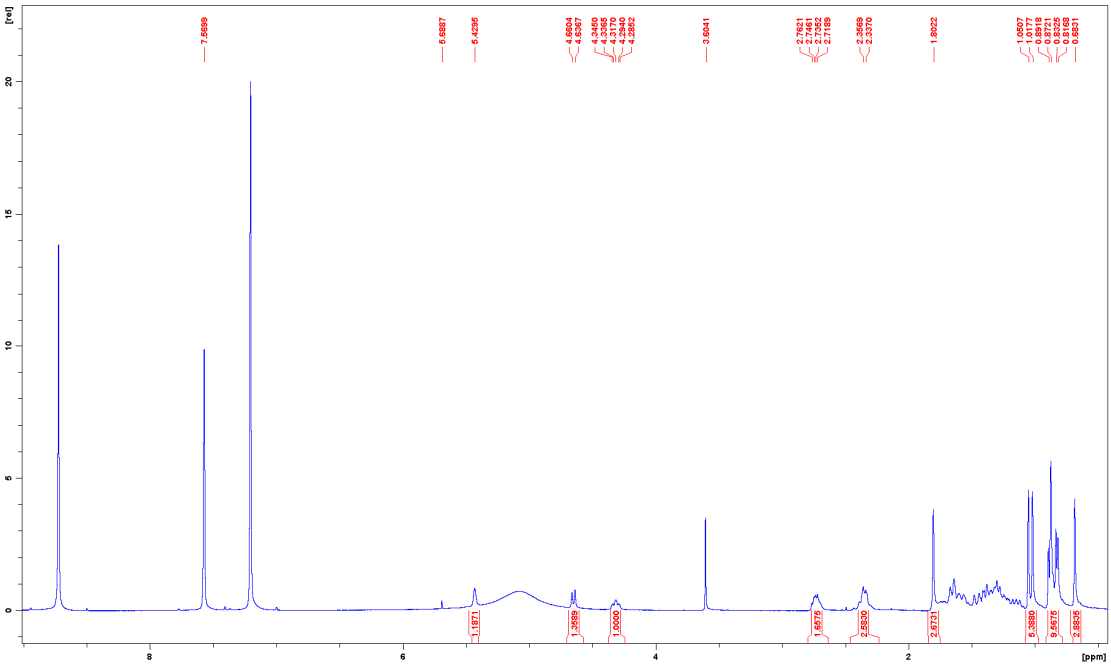

Figure S10 ^{1}H NMR spectrum of **4** in pyridine-d_5 at 400 MHz

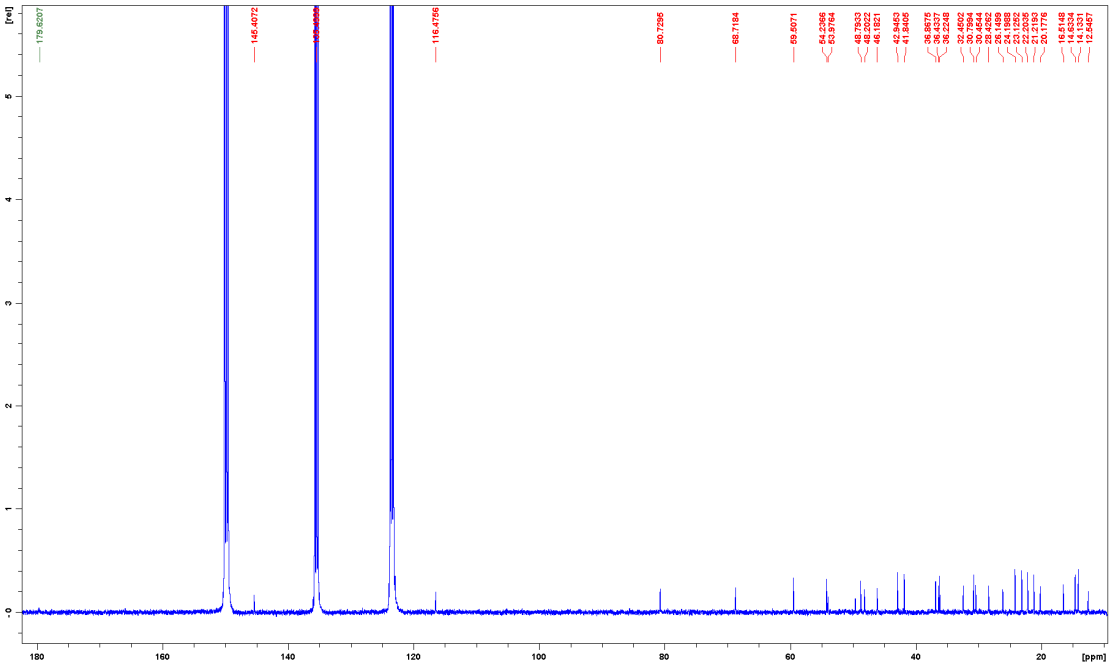

Figure S11 ^{13}C NMR spectrum of **4** in pyridine-d_5 at 100 MHz

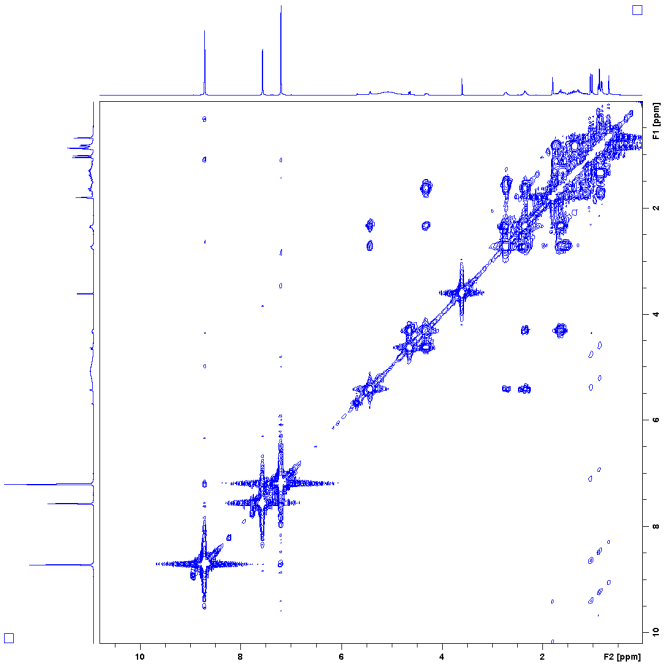

Figure S12 ^{1}H-^{1}H COSY spectrum of **4** in pyridine-d_5 at 400 MHz

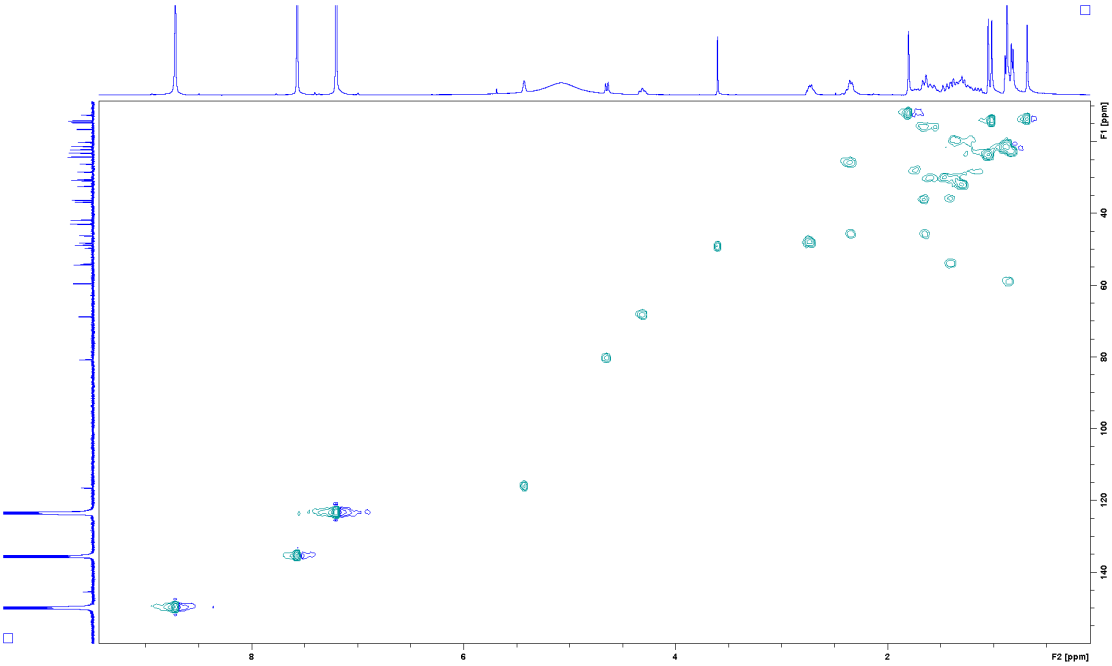

Figure S13 HSQC spectrum of **4** in pyridine-d_5 at 400 MHz

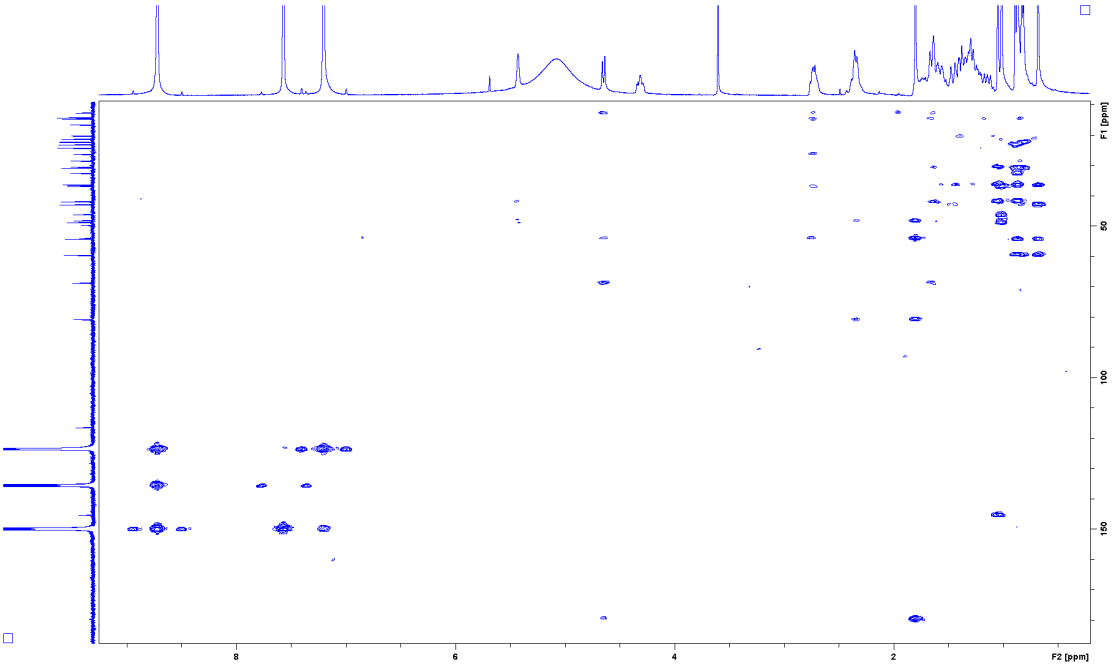

Figure S14 HMBC spectrum of **4** in pyridine-d_5 at 400 MHz

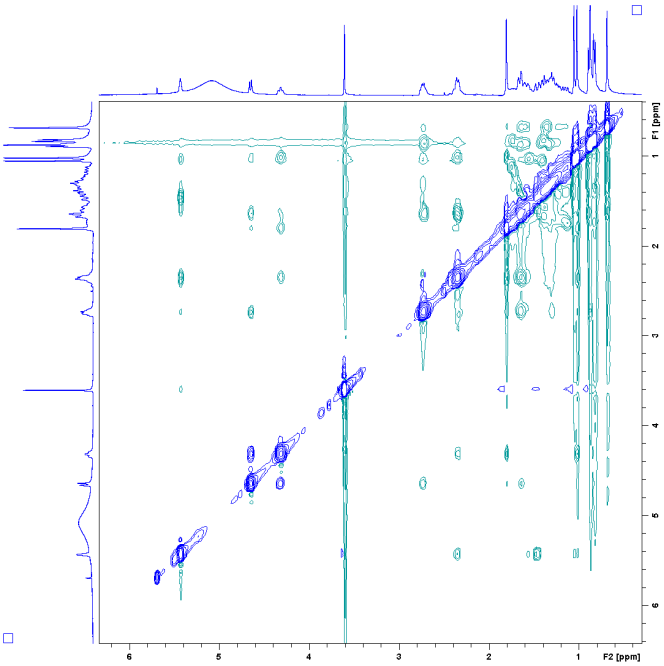

Figure S15 ROESY spectrum of **4** in pyridine-d_5 at 400 MHz

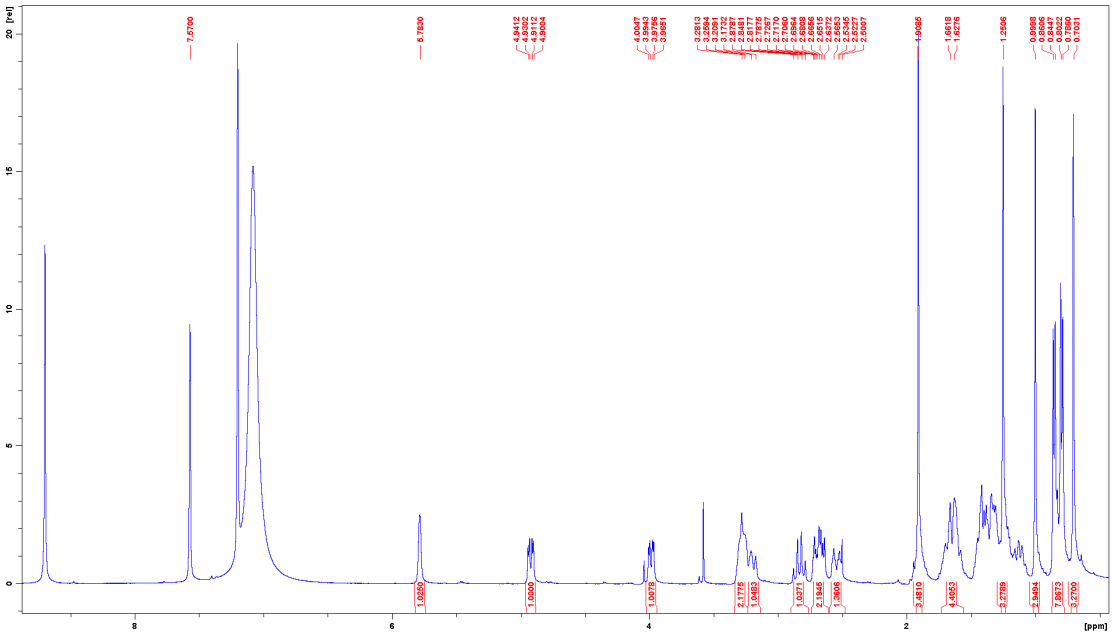

Figure S16 ^{1}H NMR spectrum of **5** in pyridine-d_5 at 400 MHz

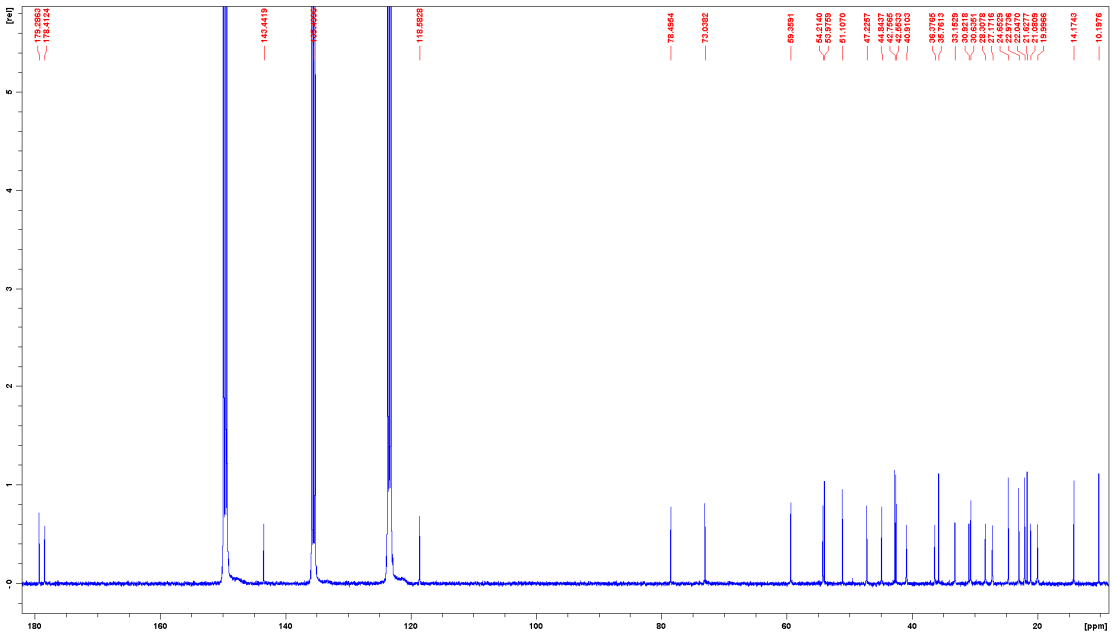

Figure S17 ^{13}C NMR spectrum of **5** in pyridine-d_5 at 100 MHz

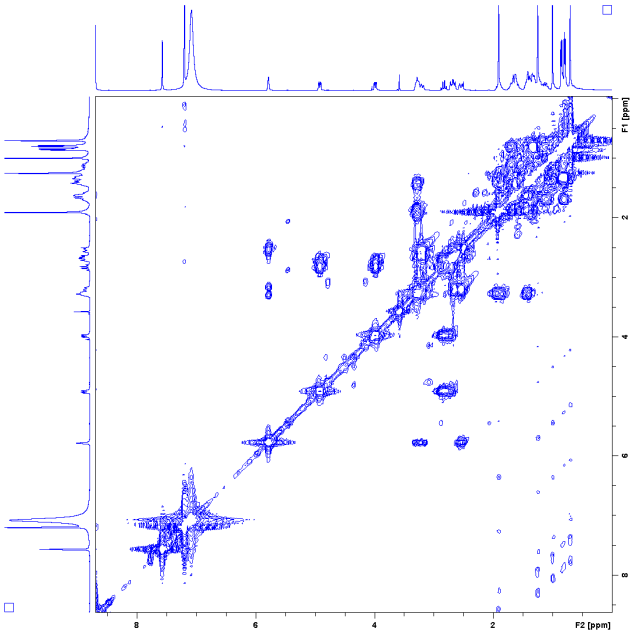

Figure S18 ^{1}H-^{1}H COSY spectrum of **5** in pyridine-d_5 at 400 MHz

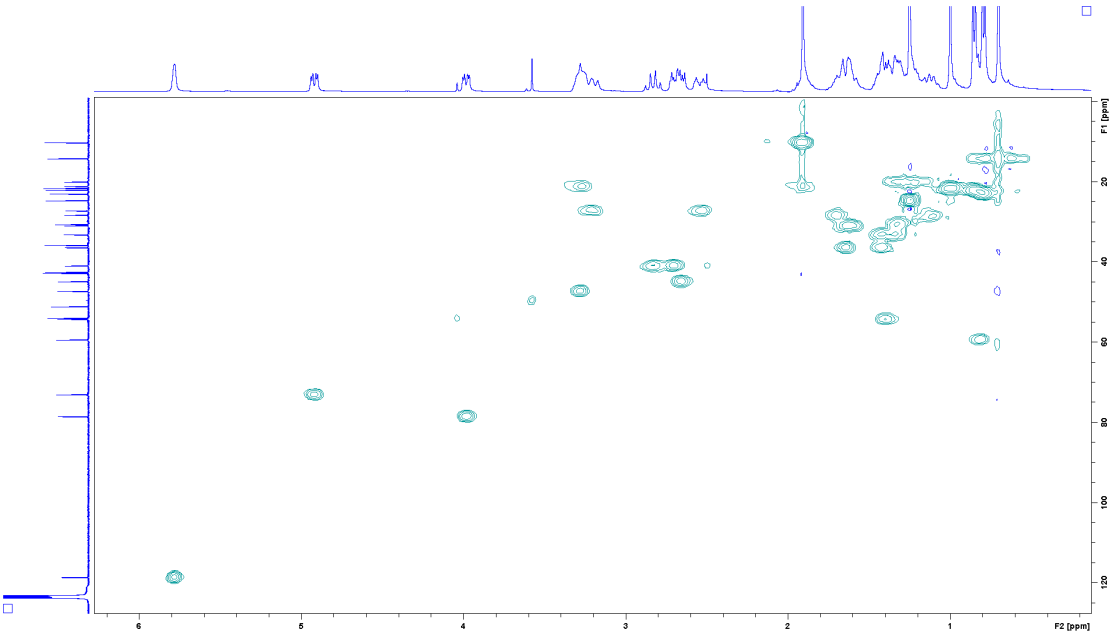

Figure S19 HSQC spectrum of **5** in pyridine-d_5 at 400 MHz

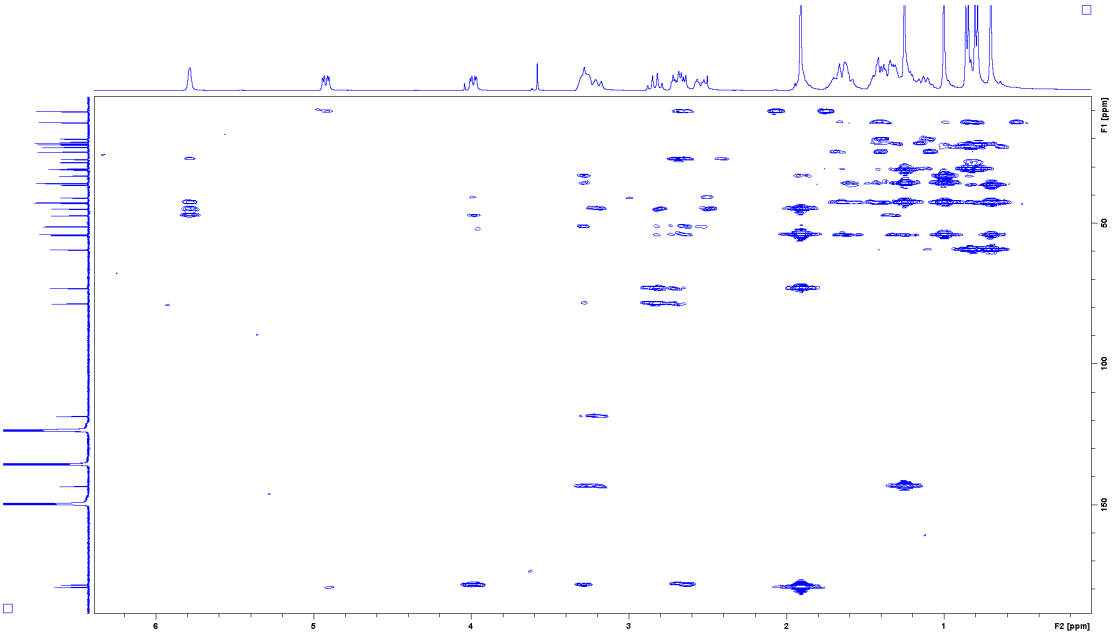

Figure S20 HMBC spectrum of **5** in pyridine-d_5 at 400 MHz

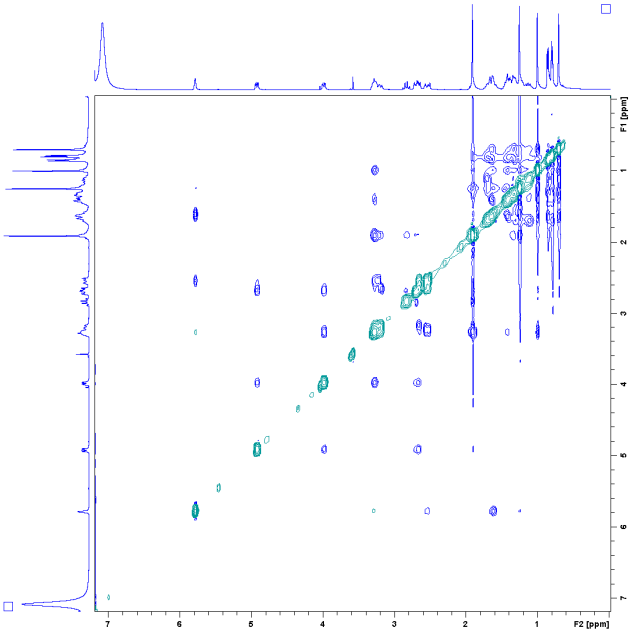

Figure S21 ROESY spectrum of **5** in pyridine-d_5 at 400 MHz

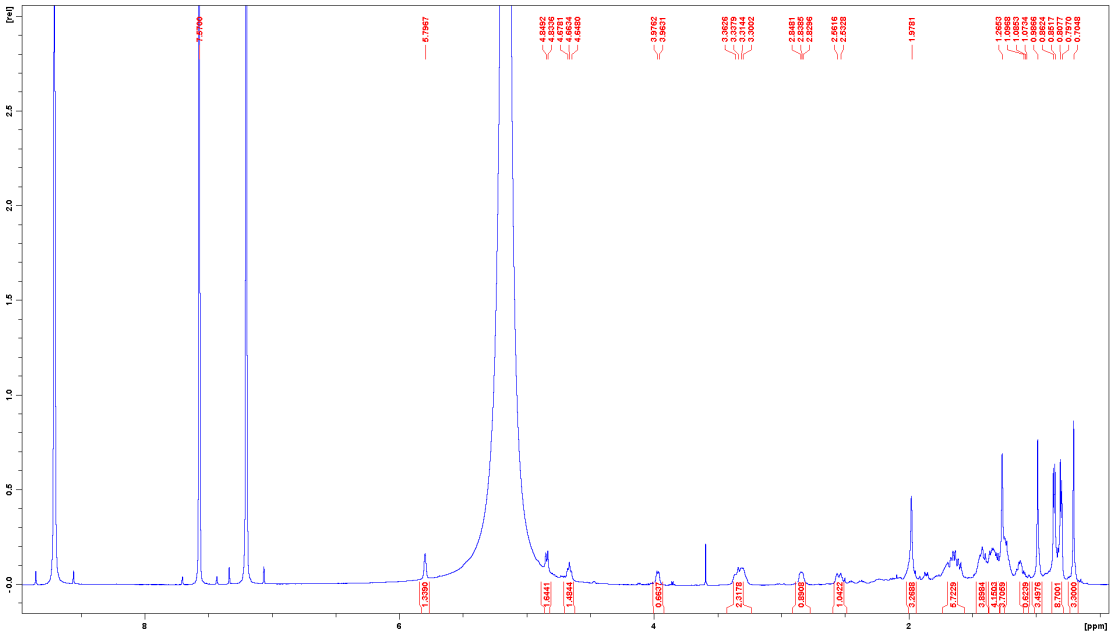

Figure S22 ^{1}H NMR spectrum of **1** in pyridine-d_5 at 600 MHz

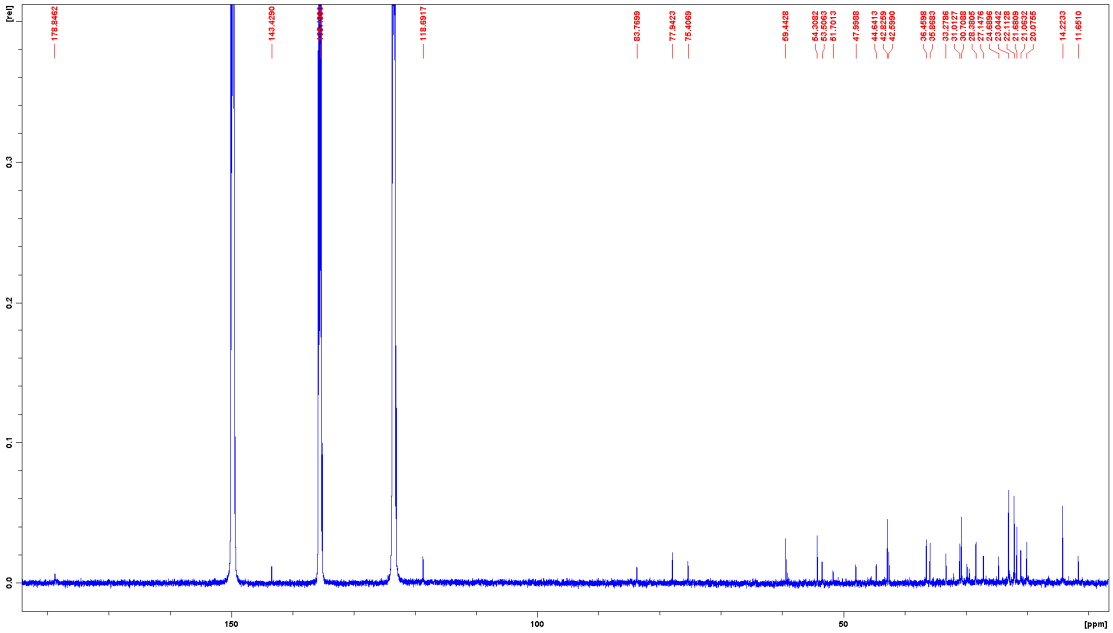

Figure S23 ^{13}C NMR spectrum of **1** in pyridine-d_5 at 150 MHz

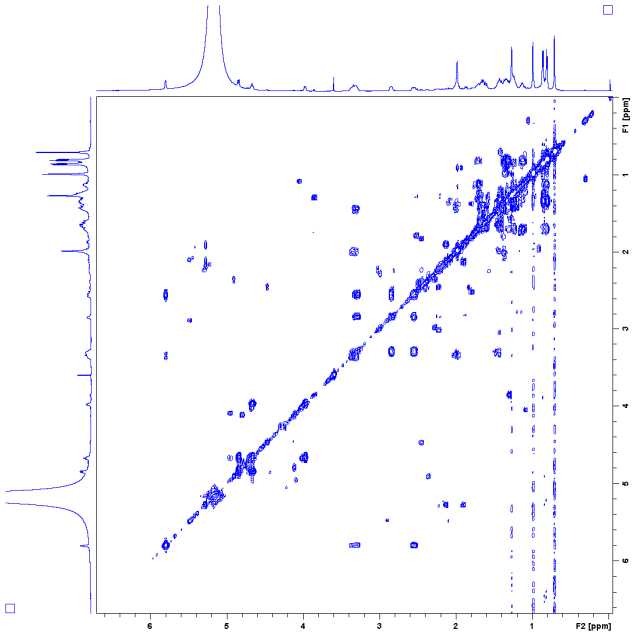

Figure S24 ^{1}H-^{1}H COSY spectrum of **1** in pyridine-d_5 at 600 MHz

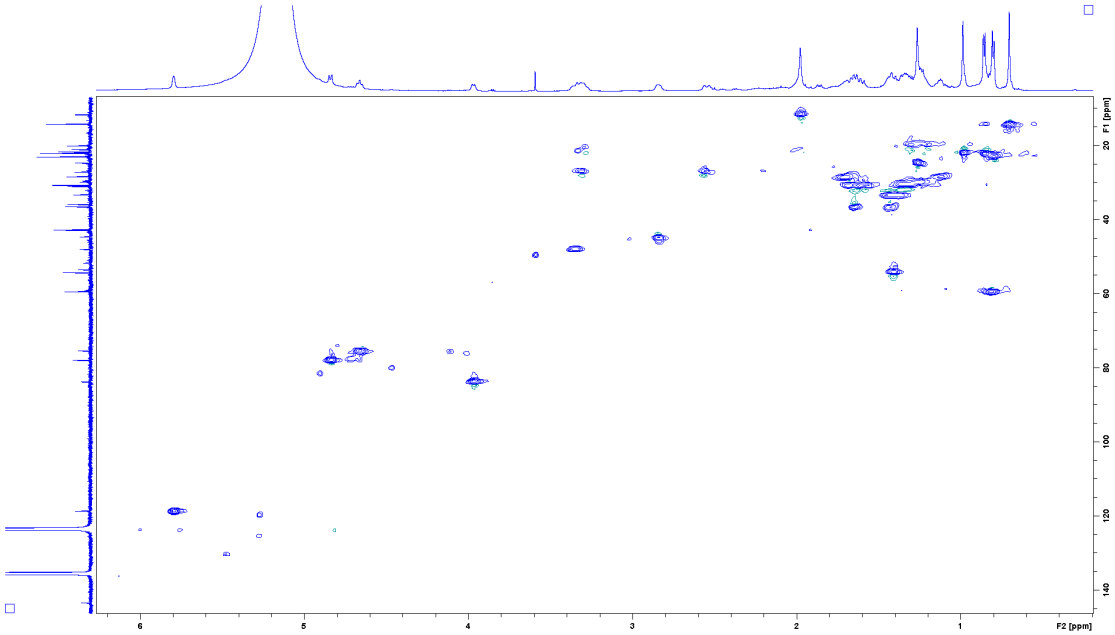

Figure S25 HSQC spectrum of **1** in pyridine-d_5 at 600 MHz

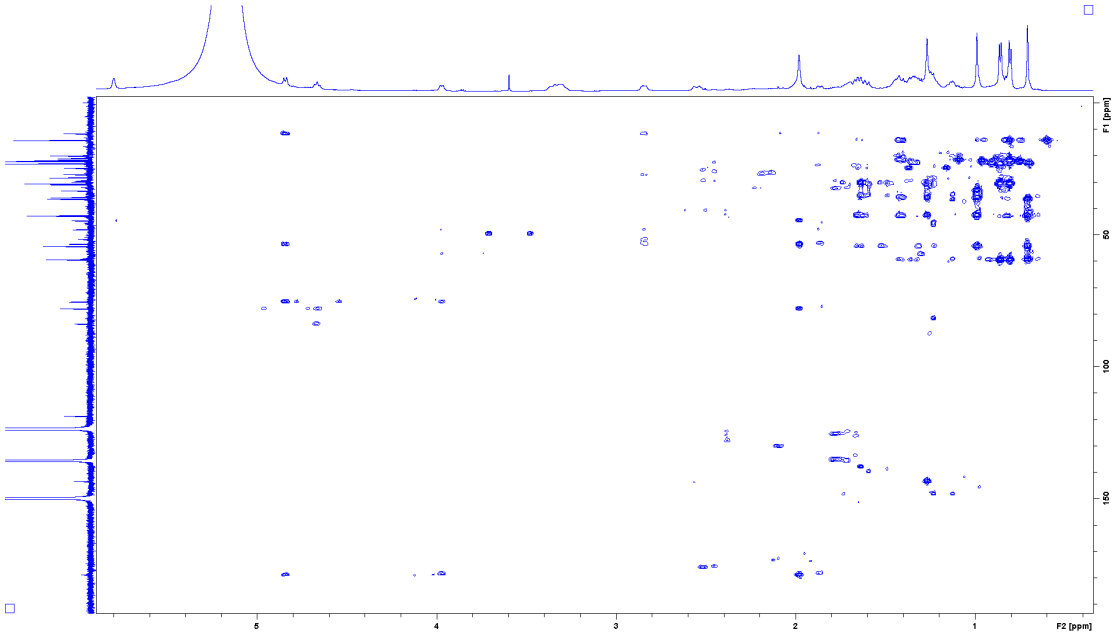

Figure S26 HMBC spectrum of **1** in pyridine-d_5 at 600 MHz

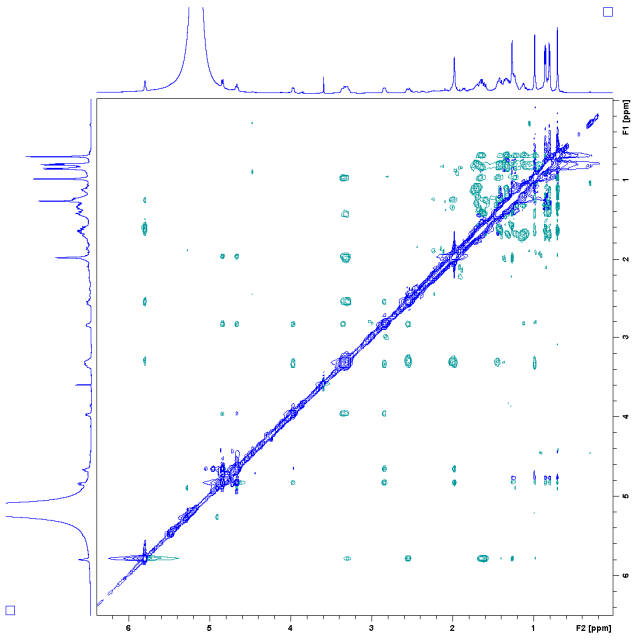

Figure S27 ROESY spectrum of **1** in pyridine-d_5 at 600 MHz

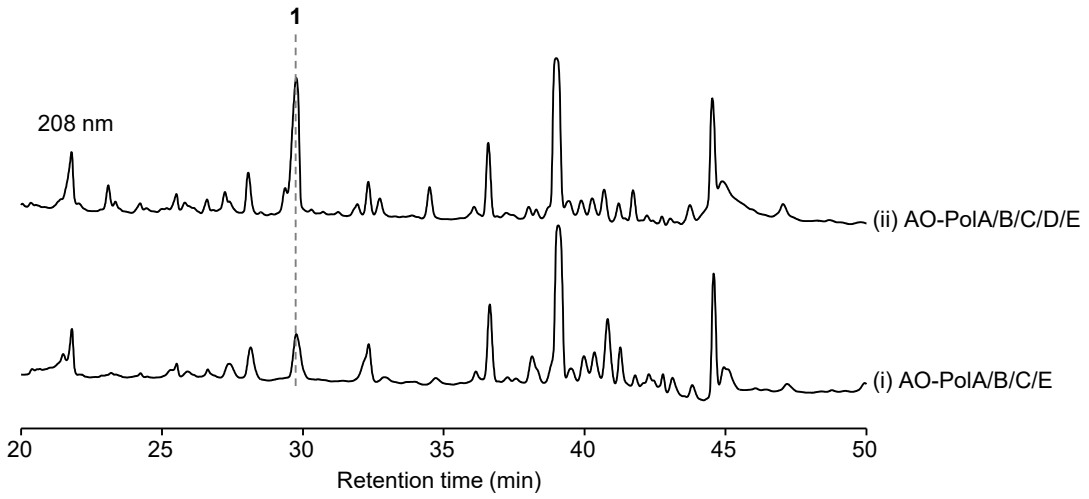

Figure S28 HPLC analysis of the *A. oryzae* NSAR1 transformant expressing all the five genes in the *pol* gene cluster

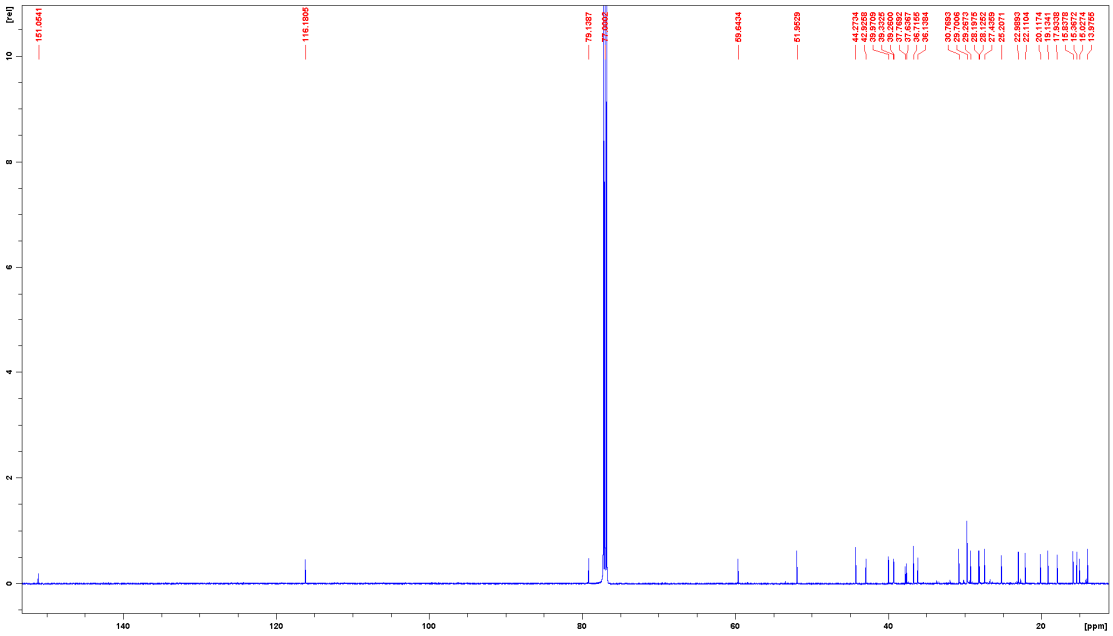

Figure S29 ^{13}C NMR spectrum of **6** in $CDCl_3$ at 150 MHz

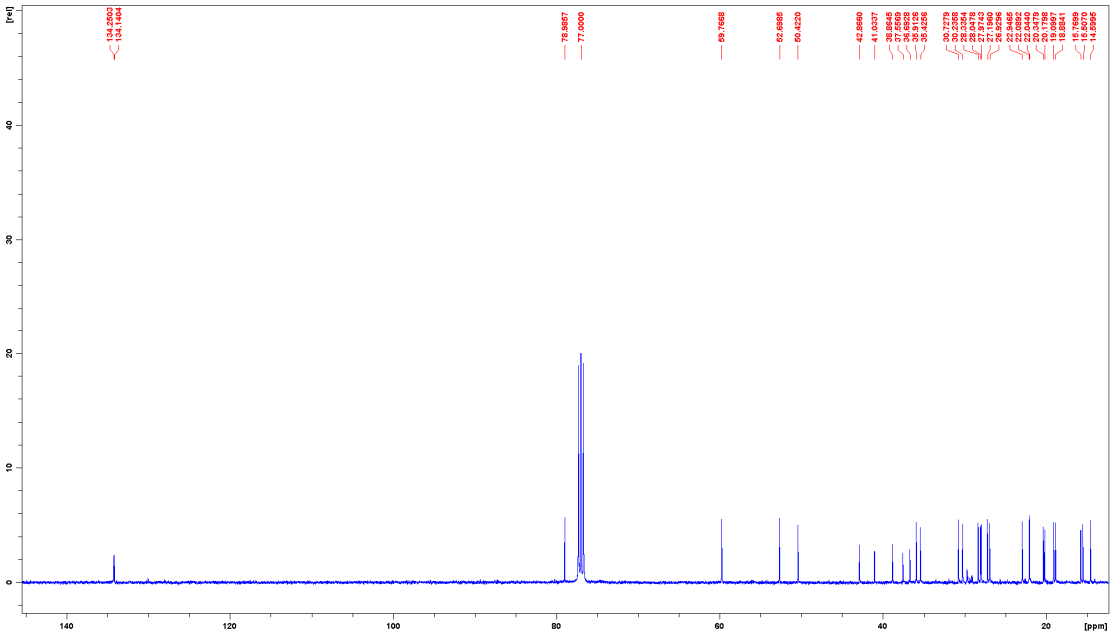

Figure S30 ^{13}C NMR spectrum of **7** in $CDCl_3$ at 100 MHz

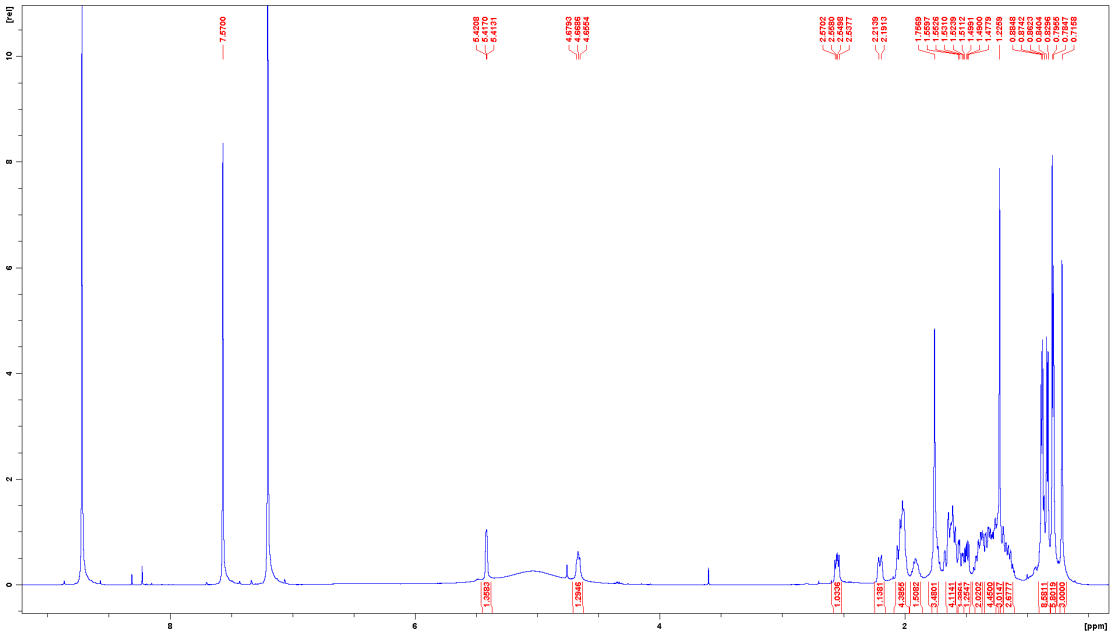

Figure S31 ^{1}H NMR spectrum of **8** in pyridine-d_5 at 600 MHz

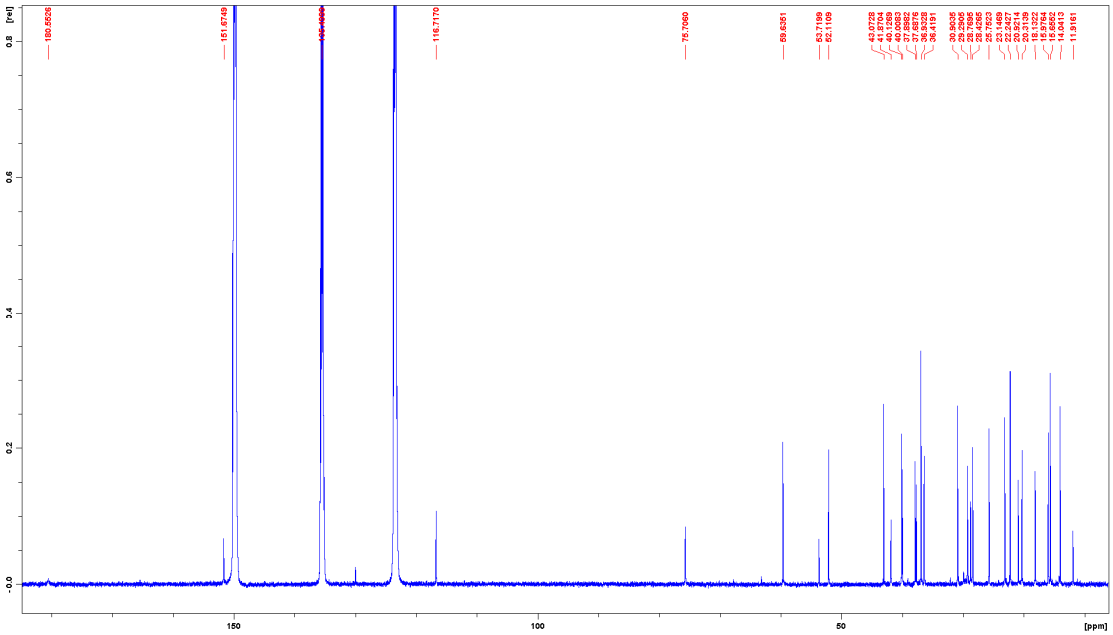

Figure S32 ^{13}C NMR spectrum of **8** in pyridine-d_5 at 150 MHz

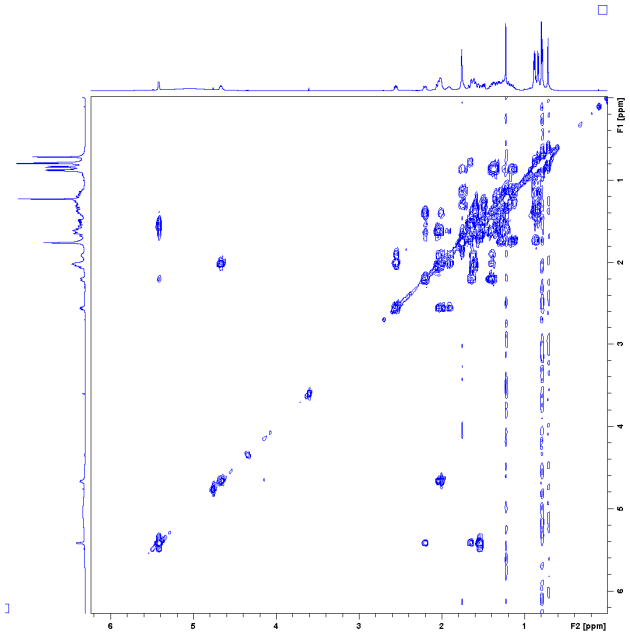

Figure S33 ^{1}H-^{1}H COSY spectrum of **8** in pyridine-d_5 at 600 MHz

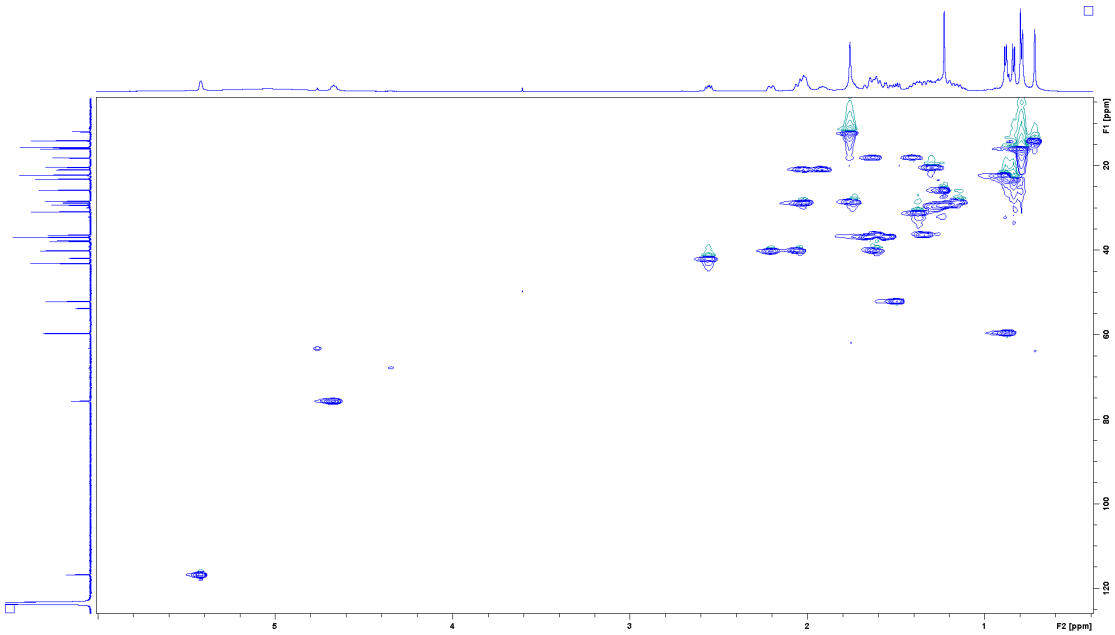

Figure S34 HSQC spectrum of **8** in pyridine-d_5 at 600 MHz

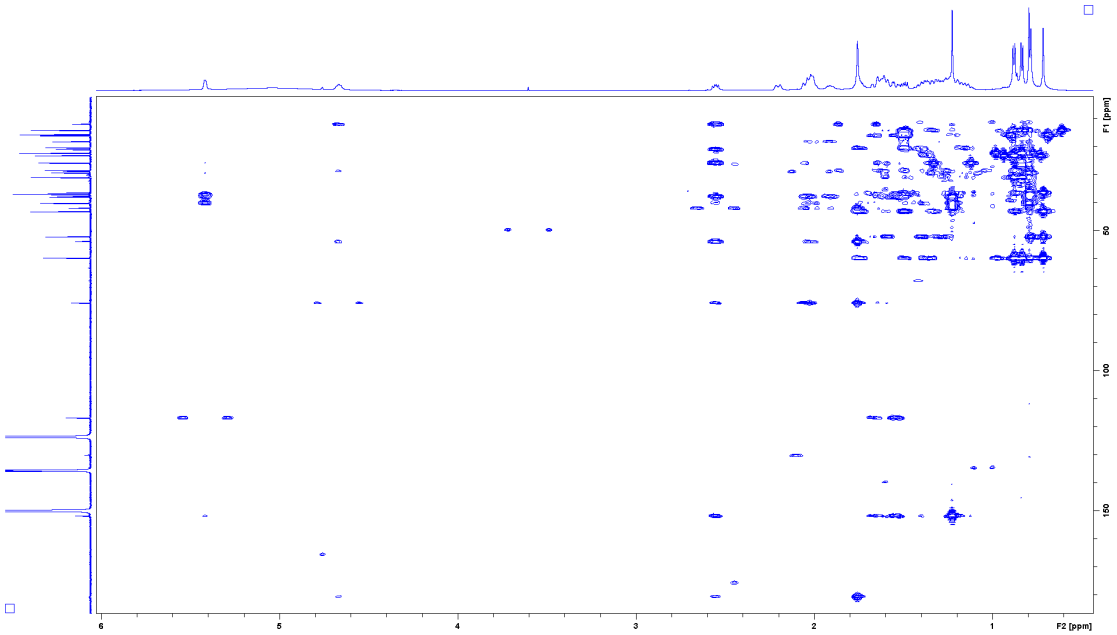

Figure S35 HMBC spectrum of **8** in pyridine-d_5 at 600 MHz

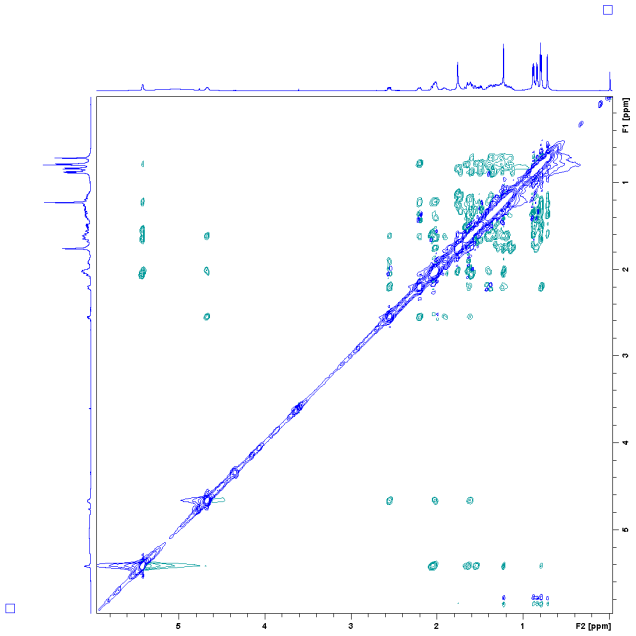

Figure S36 ROESY spectrum of **8** in pyridine-d_5 at 600 MHz

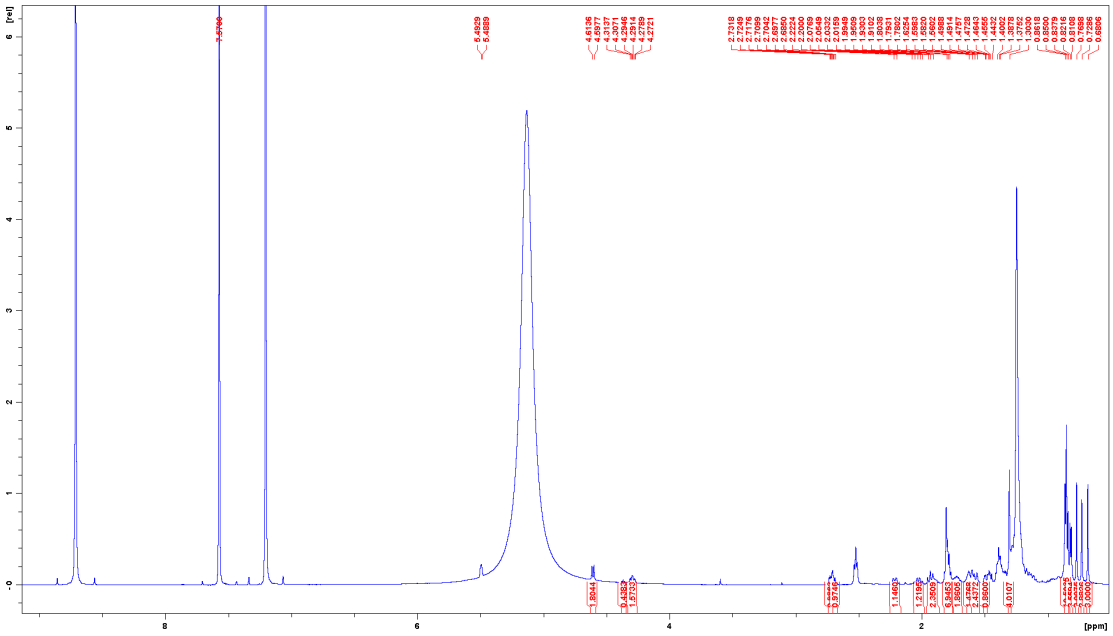

Figure S37 ^{1}H NMR spectrum of **9** in pyridine-d_5 at 600 MHz

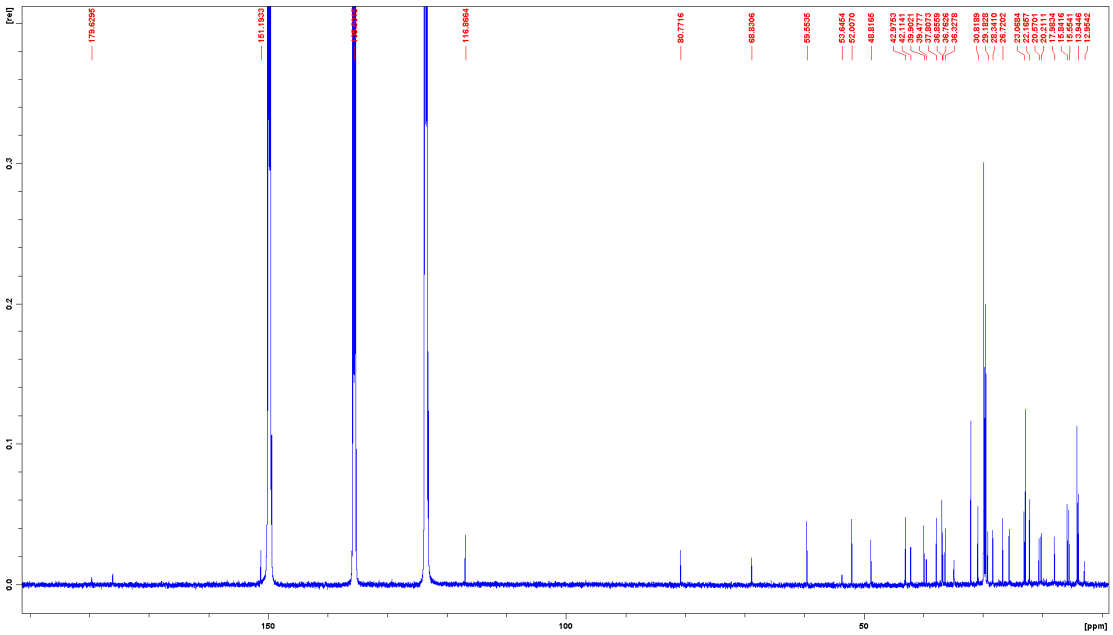

Figure S38 ^{13}C NMR spectrum of **9** in pyridine-d_5 at 150 MHz

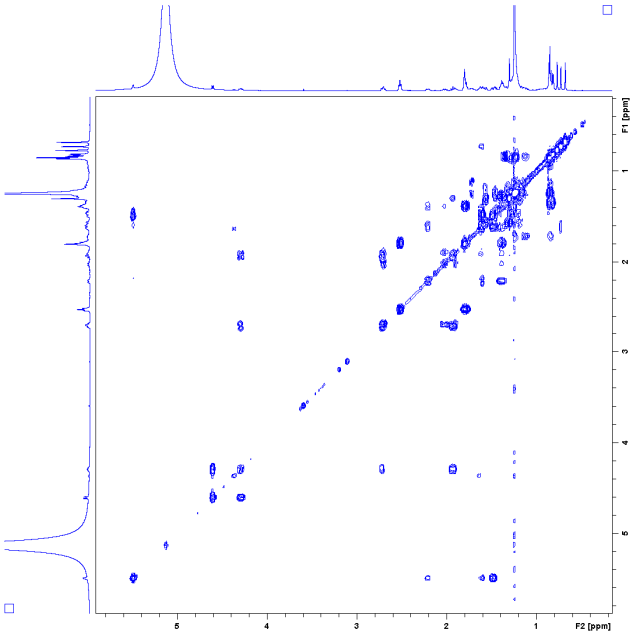

Figure S39 ^{1}H-^{1}H COSY spectrum of **9** in pyridine-d_5 at 600 MHz

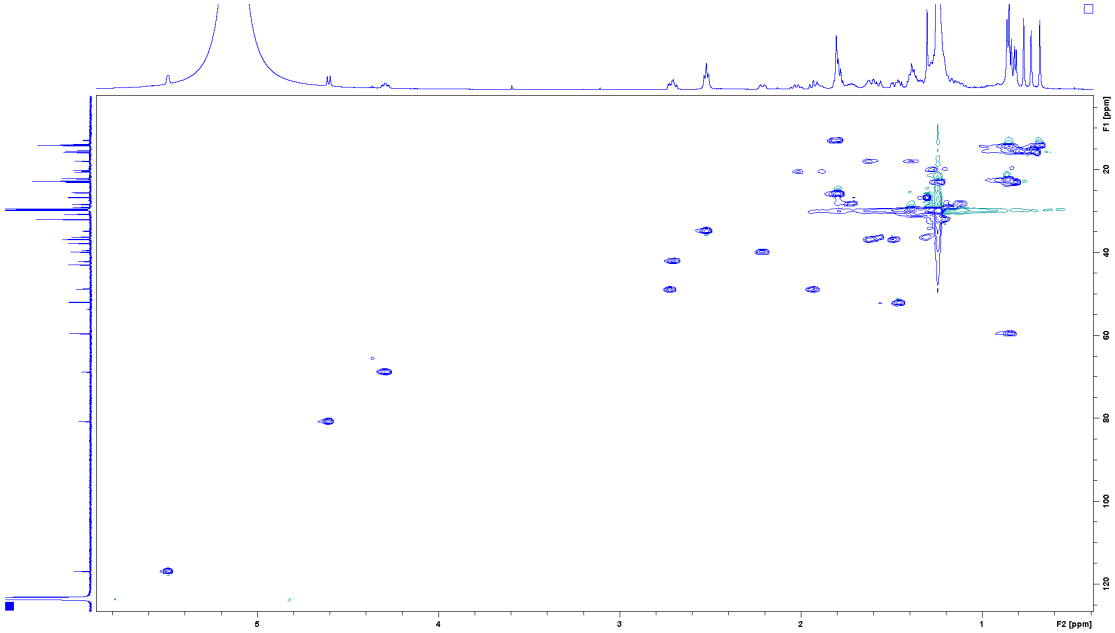

Figure S40 HSQC spectrum of **9** in pyridine-d_5 at 600 MHz

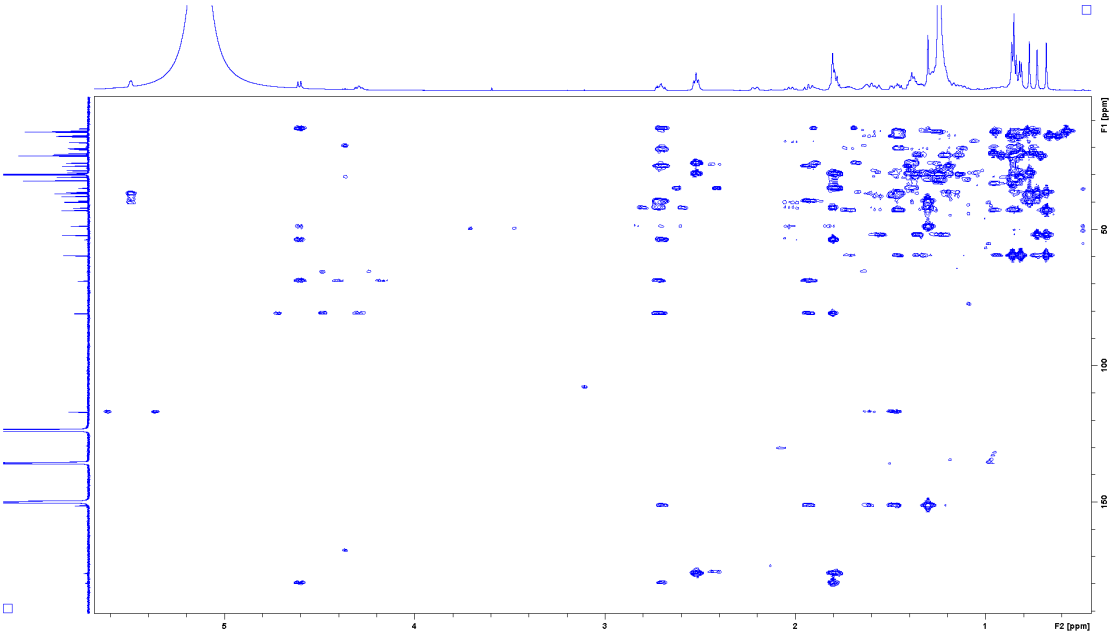

Figure S41 HMBC spectrum of **9** in pyridine-d_5 at 600 MHz

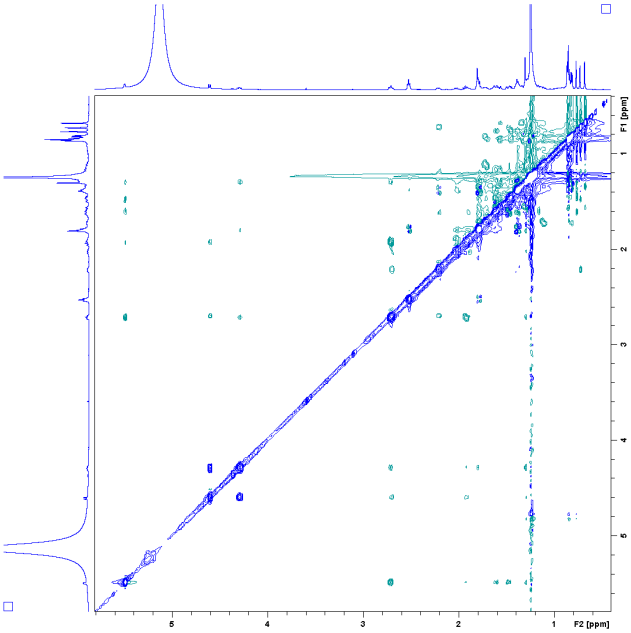

Figure S42 ROESY spectrum of **9** in pyridine-d_5 at 600 MHz

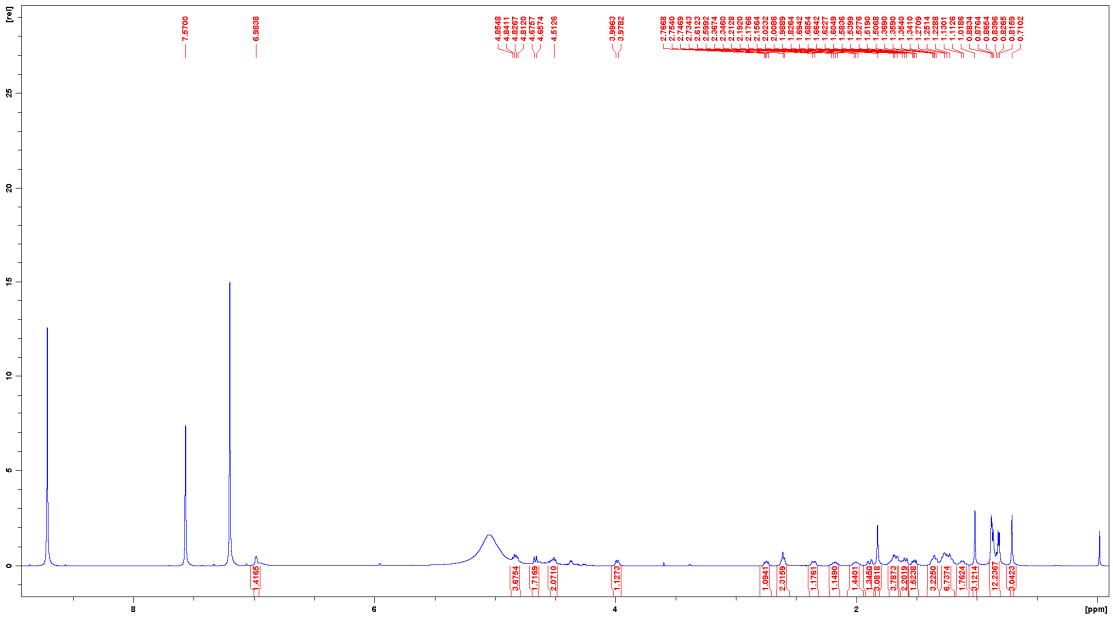

Figure S43 ^{1}H NMR spectrum of **10** in pyridine-d_5 at 600 MHz

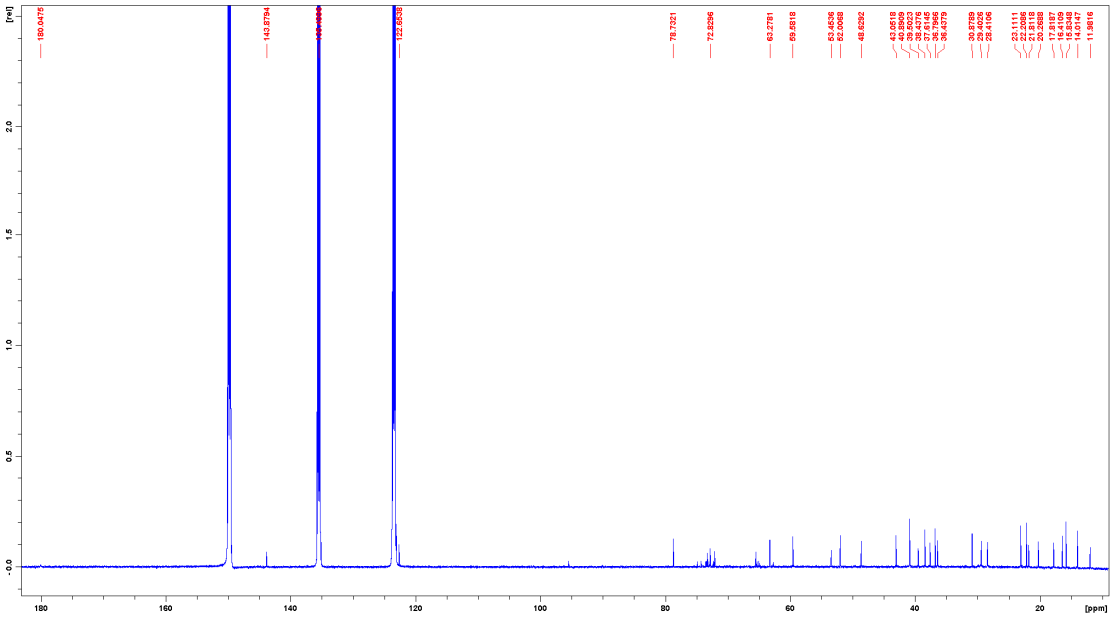

Figure S44 ^{13}C NMR spectrum of **10** in pyridine-d_5 at 150 MHz

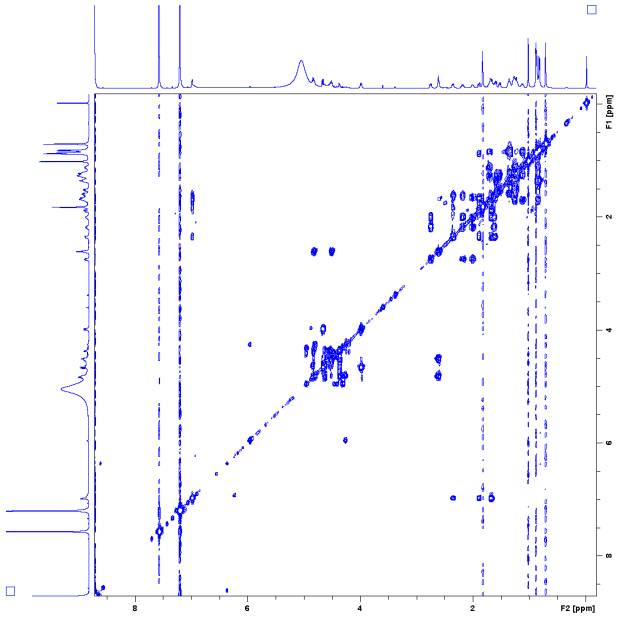

Figure S45 ^{1}H-^{1}H COSY spectrum of **10** in pyridine-d_5 at 600 MHz

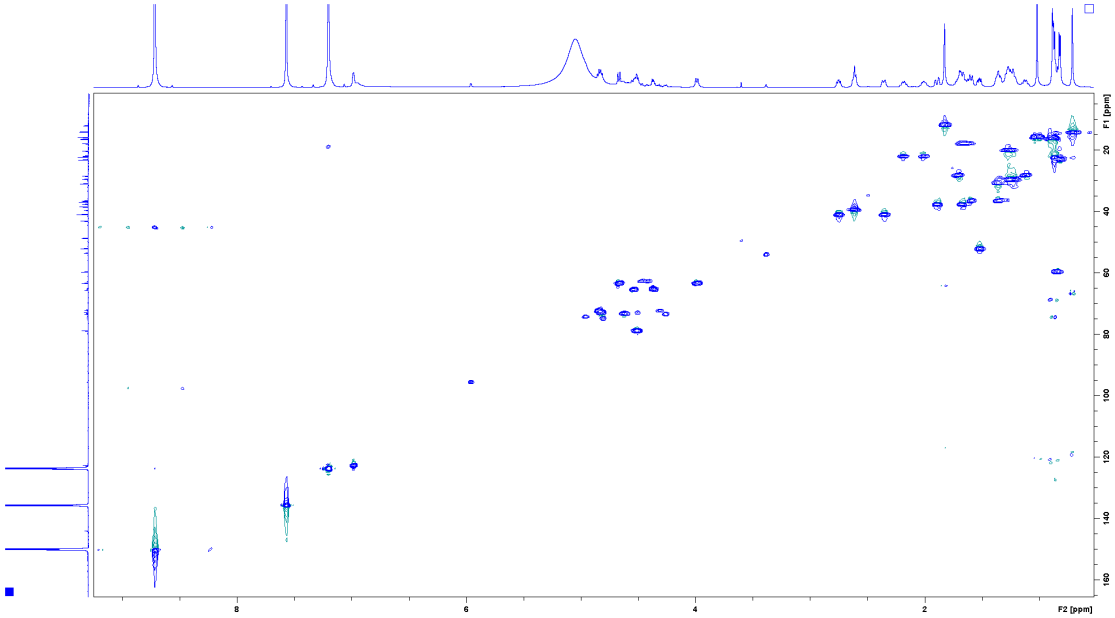

Figure S46 HSQC spectrum of **10** in pyridine-d_5 at 600 MHz

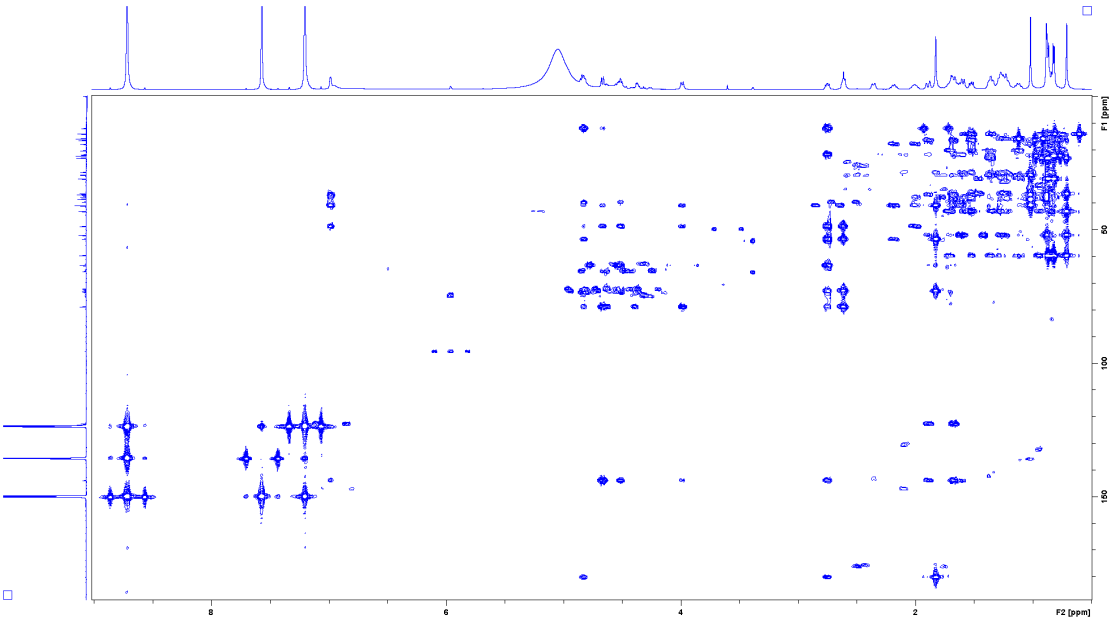

Figure S47 HMBC spectrum of **10** in pyridine-d_5 at 600 MHz

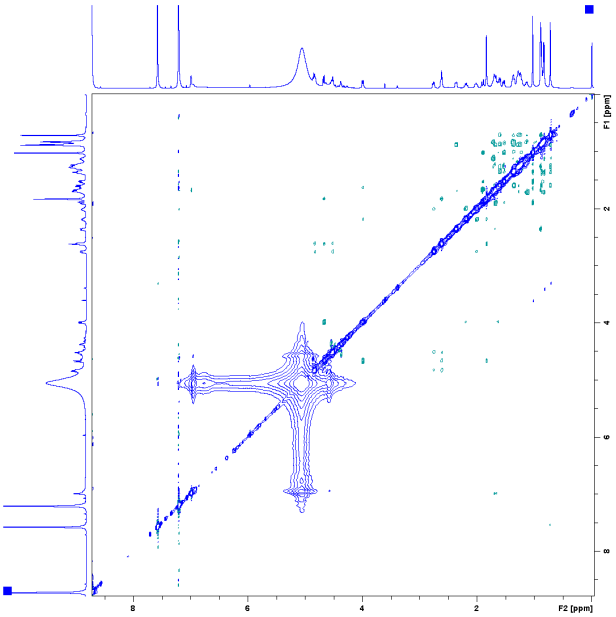

Figure S48 ROESY spectrum of **10** in pyridine-d_5 at 600 MHz

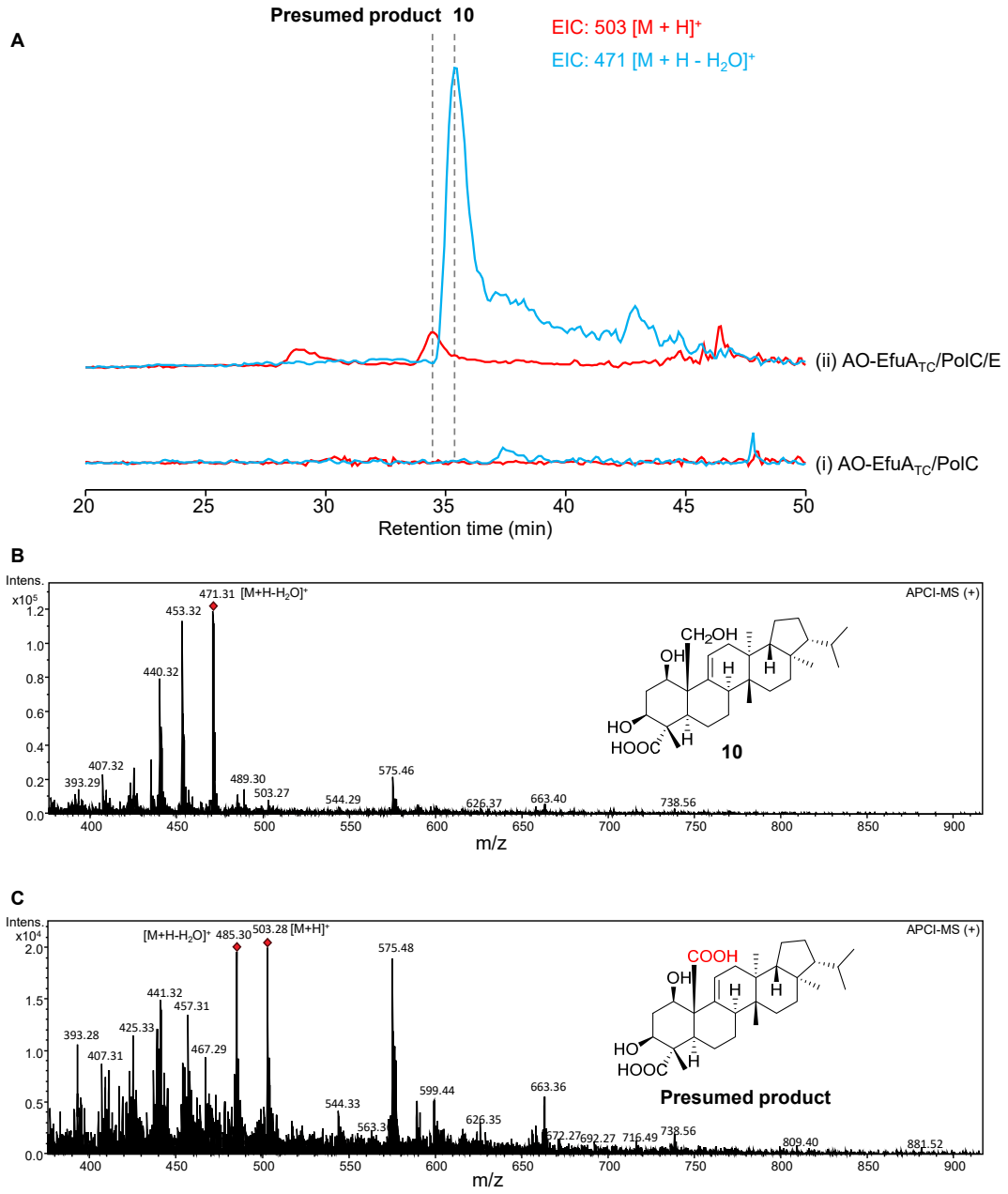

Figure S49 MS analysis of *A. oryzae* NSAR1 transformants co-expressing *efuA*$_{TC}$ and *polC/E* (A) Extracted ion chromatogram of **10** and the presumed product; (B) APCI-MS of **10**; (C) APCI-MS of the presumed product

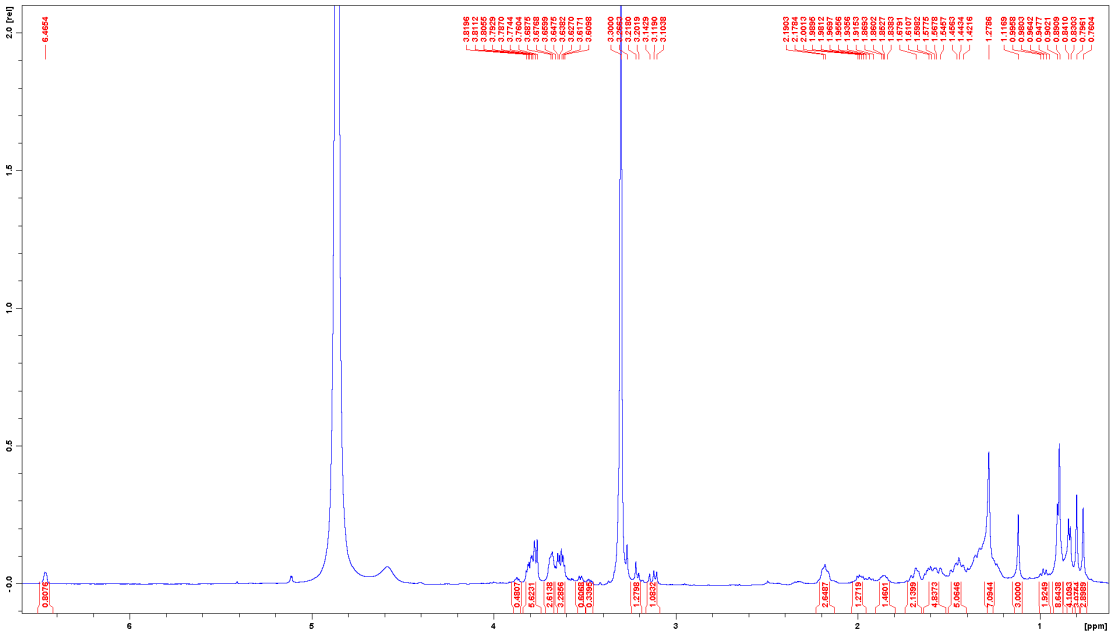

Figure S50 ^{1}H NMR spectrum of **11** in CD_3OD at 600 MHz

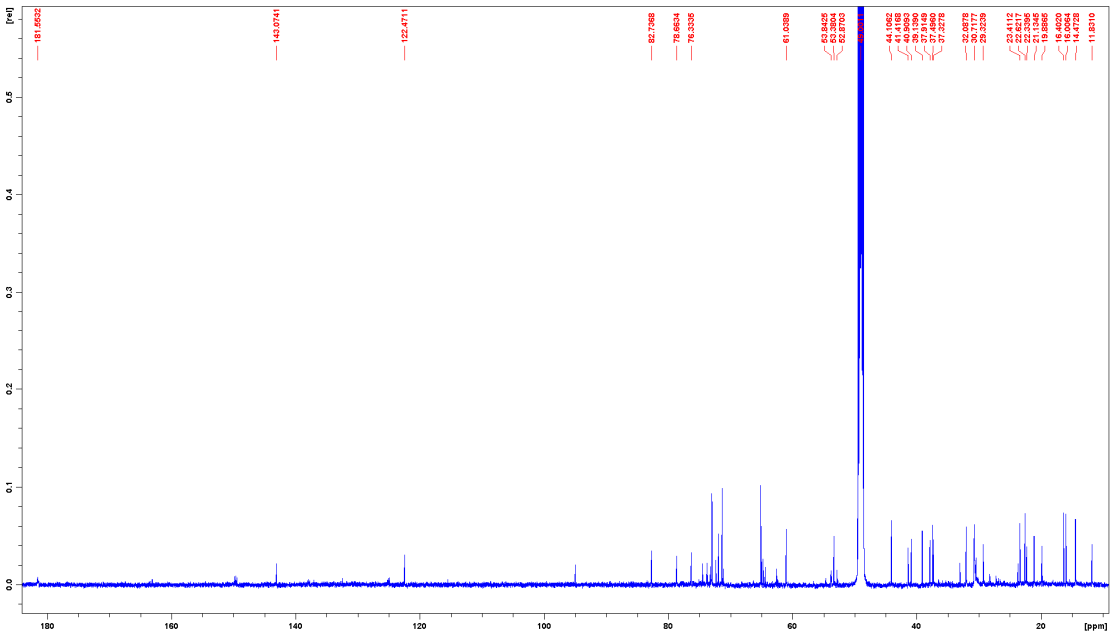

Figure S51 ^{13}C NMR spectrum of **11** in CD_3OD at 150 MHz

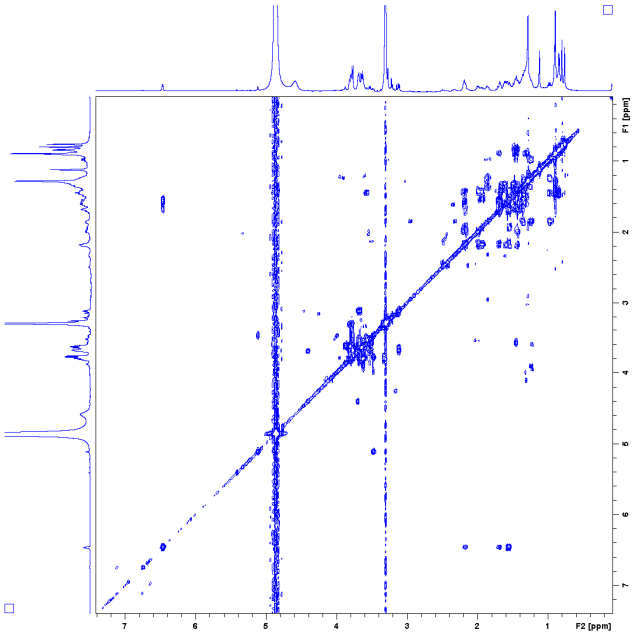

Figure S52 ^{1}H-^{1}H COSY spectrum of **11** in CD_3OD at 600 MHz

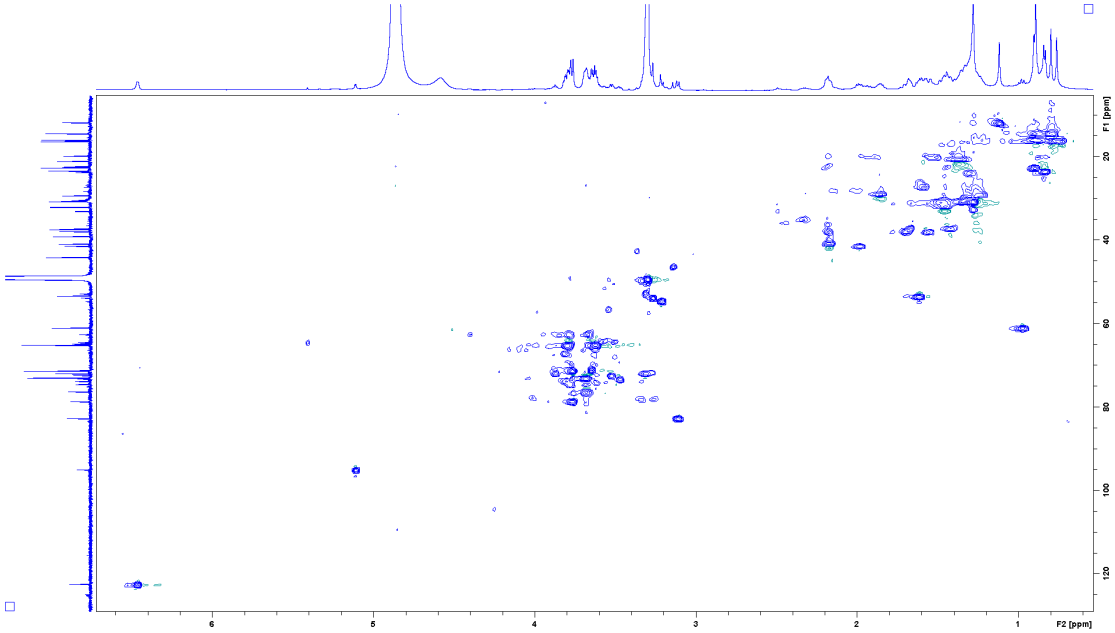

Figure S53 HSQC spectrum of **11** in CD_3OD at 600 MHz

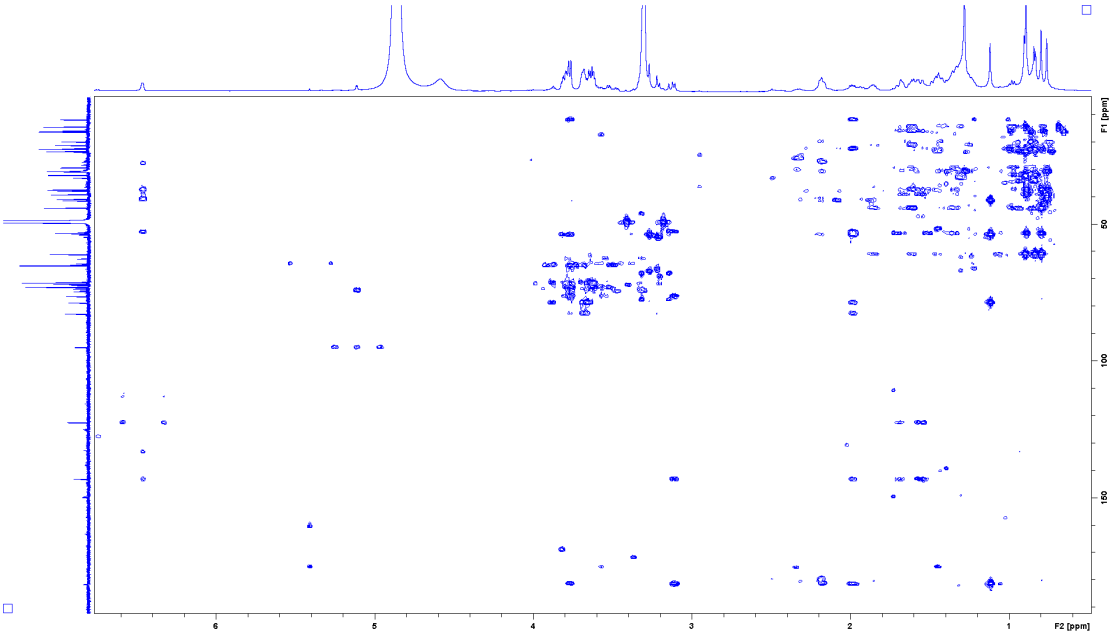

Figure S54 HMBC spectrum of **11**in CD_3OD at 600 MHz

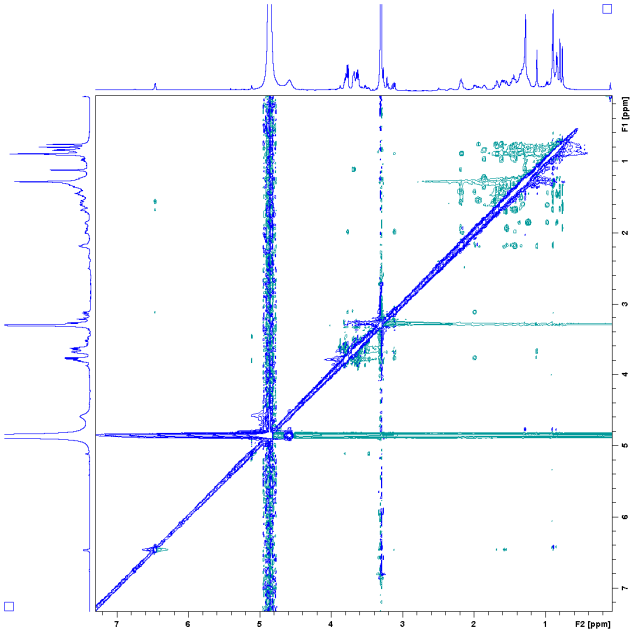

Figure S55 NOESY spectrum of **11** in CD_3OD at 600 MHz

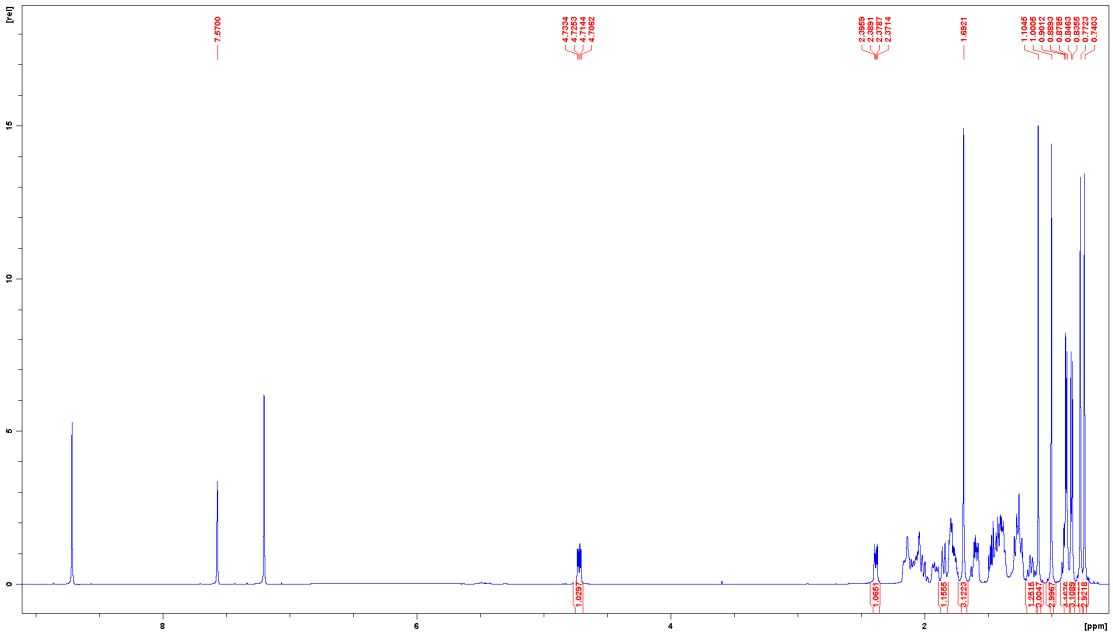

Figure S56 ^{1}H NMR spectrum of **12** in pyridine-d_5 at 600 MHz

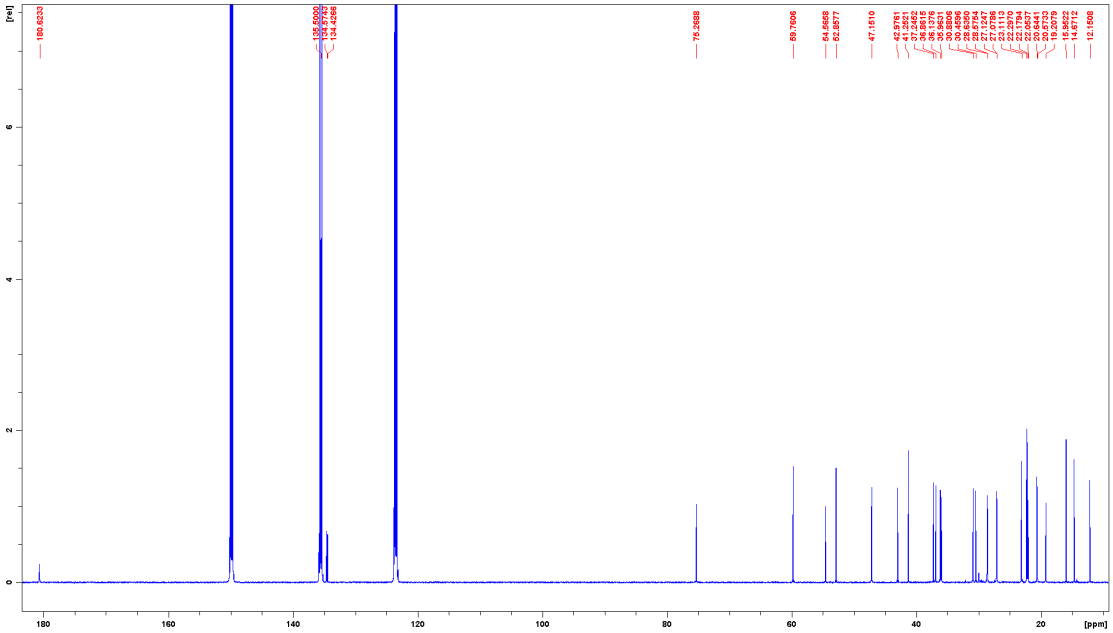

Figure S57 ^{13}C NMR spectrum of **12** in pyridine-d_5 at 150 MHz

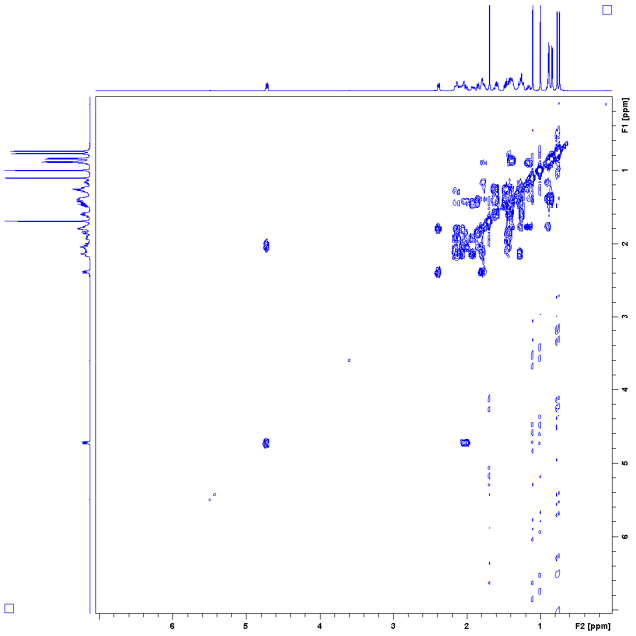

Figure S58 ^{1}H-^{1}H COSY spectrum of **12** in pyridine-d_5 at 600 MHz

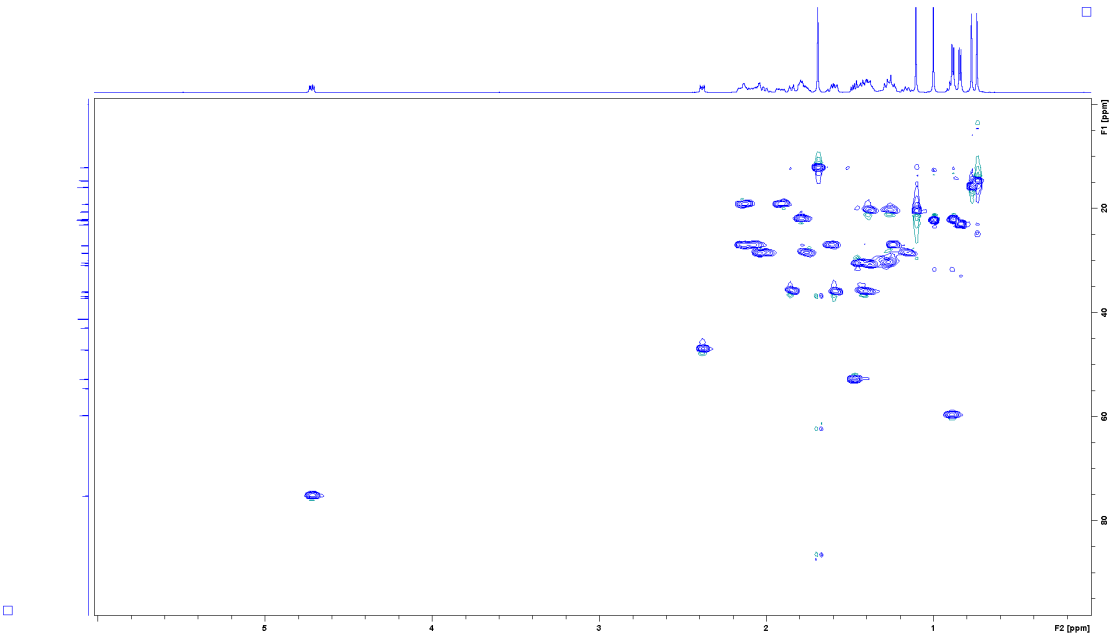

Figure S59 HSQC spectrum of **12** in pyridine-d_5 at 600 MHz

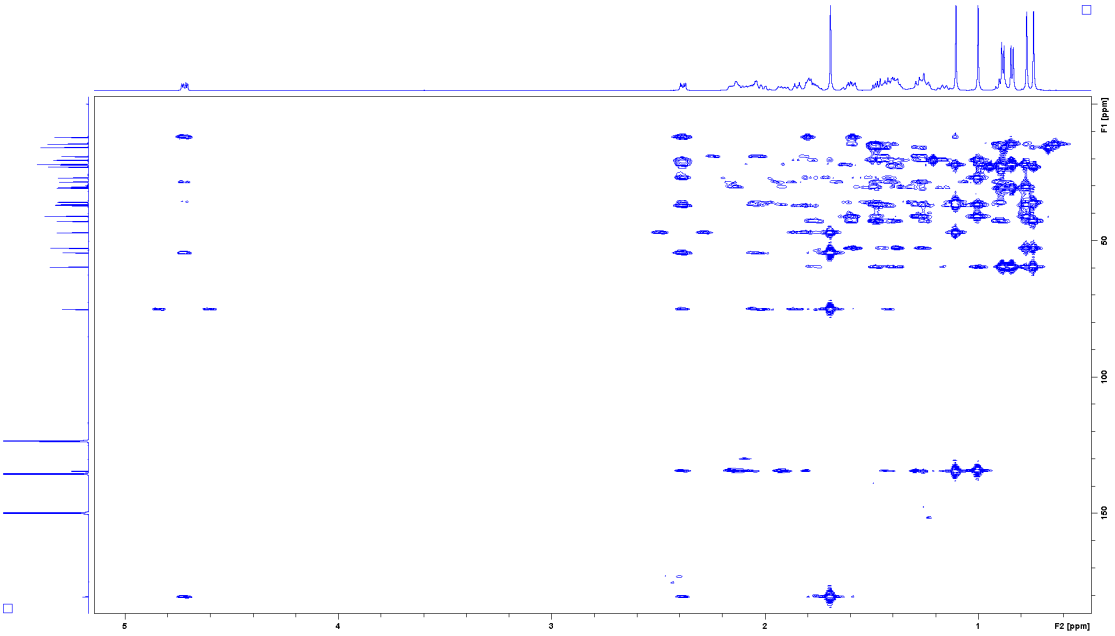

Figure S60 HMBC spectrum of **12** in pyridine-d_5 at 600 MHz

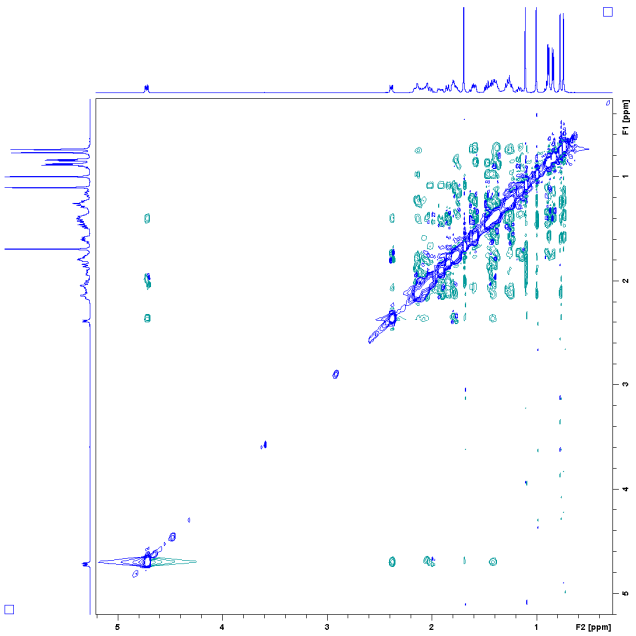

Figure S61 ROESY spectrum of **12** in pyridine-d_5 at 600 MHz

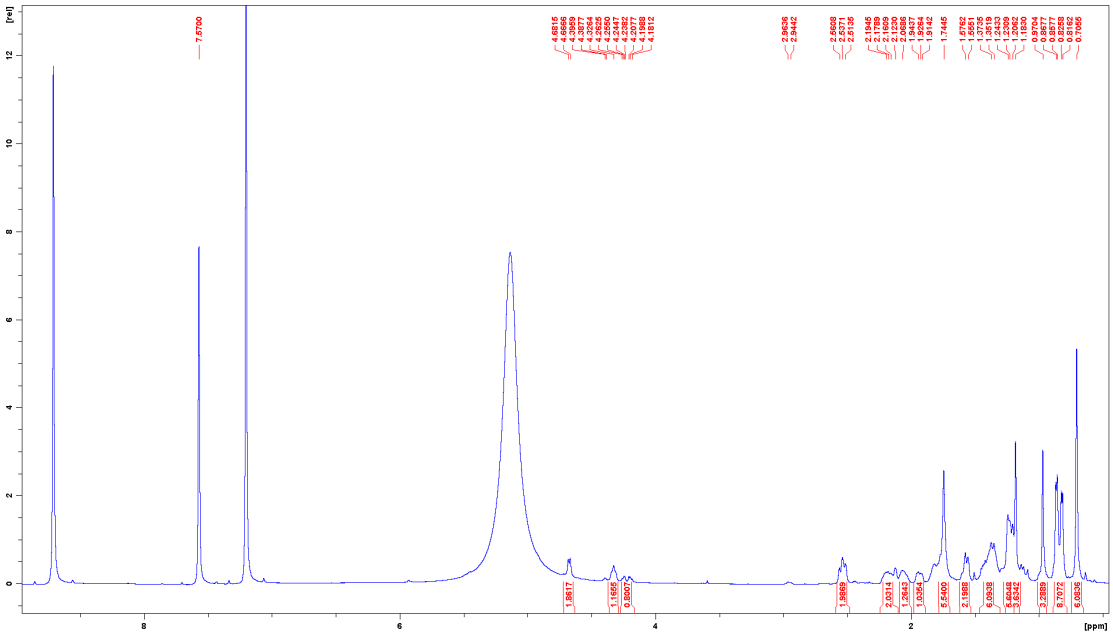

Figure S62 ^{1}H NMR spectrum of **13** in pyridine-d_5 at 600 MHz

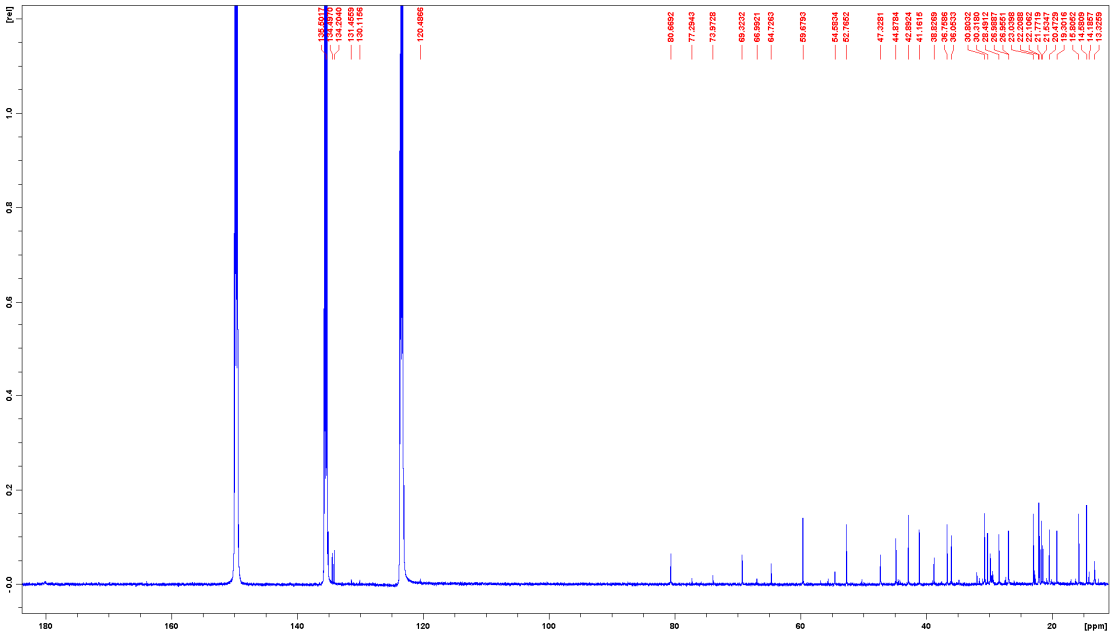

Figure S63 ^{13}C NMR spectrum of **13** in pyridine-d_5 at 150 MHz

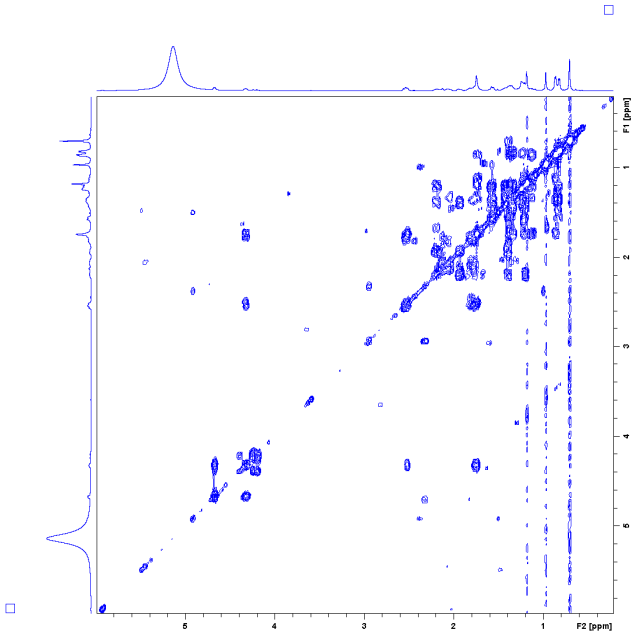

Figure S64 ^{1}H-^{1}H COSY spectrum of **13** in pyridine-d_5 at 600 MHz

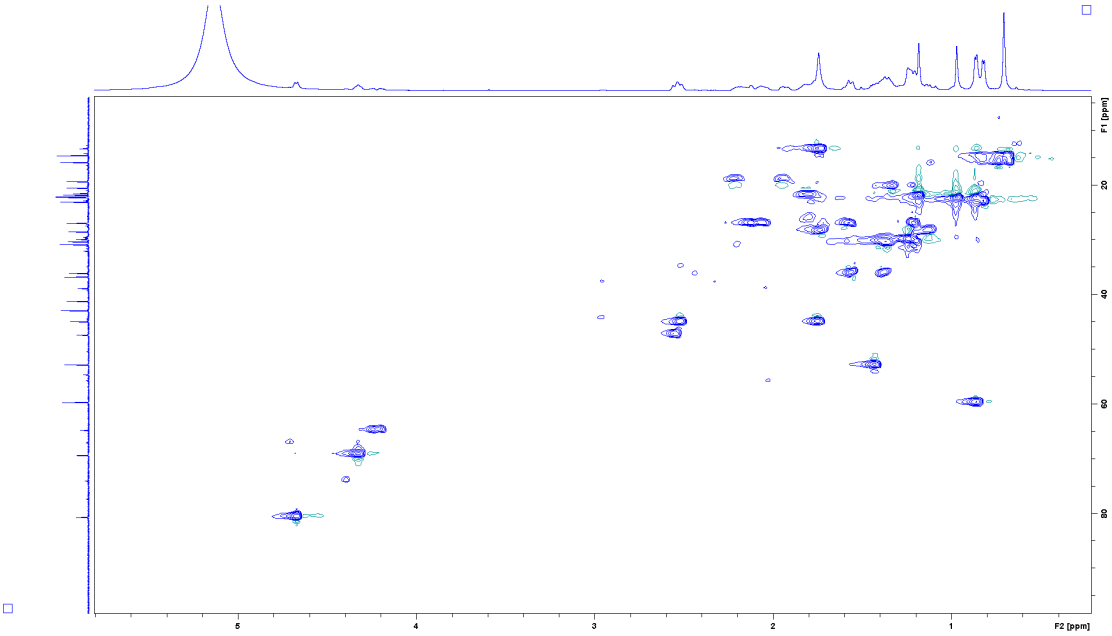

Figure S65 HSQC spectrum of **13** in pyridine-d_5 at 600 MHz

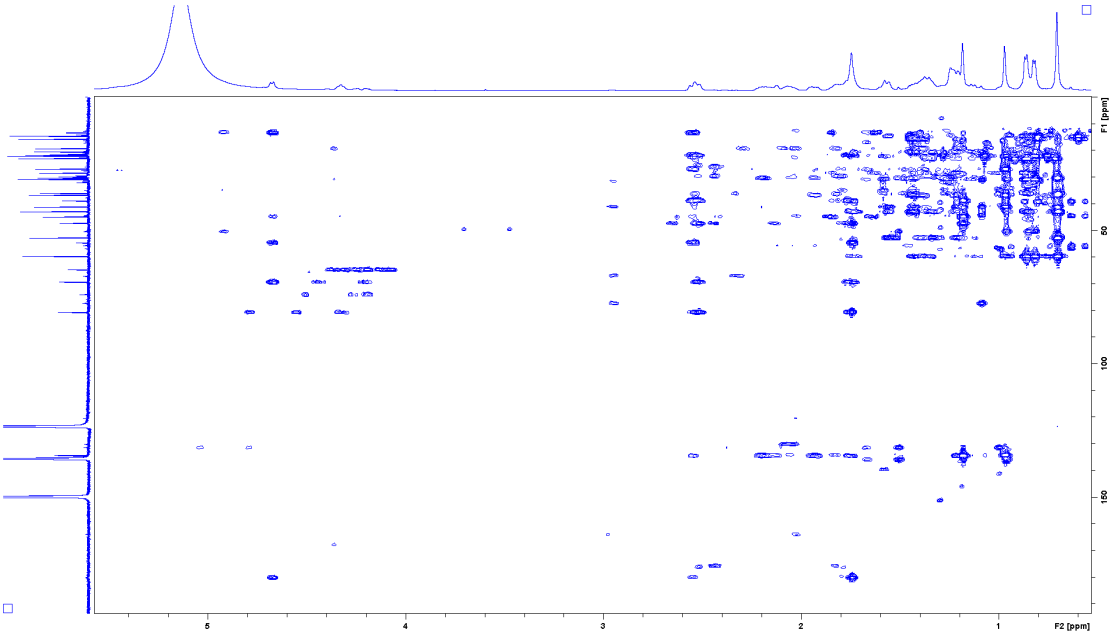

Figure S66 HMBC spectrum of **13** in pyridine-d_5 at 600 MHz

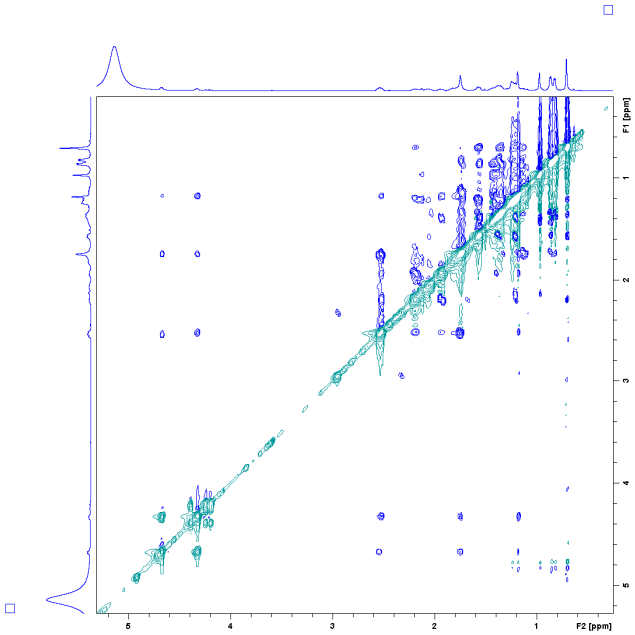

Figure S67 ROESY spectrum of **13** in pyridine-d_5 at 600 MHz

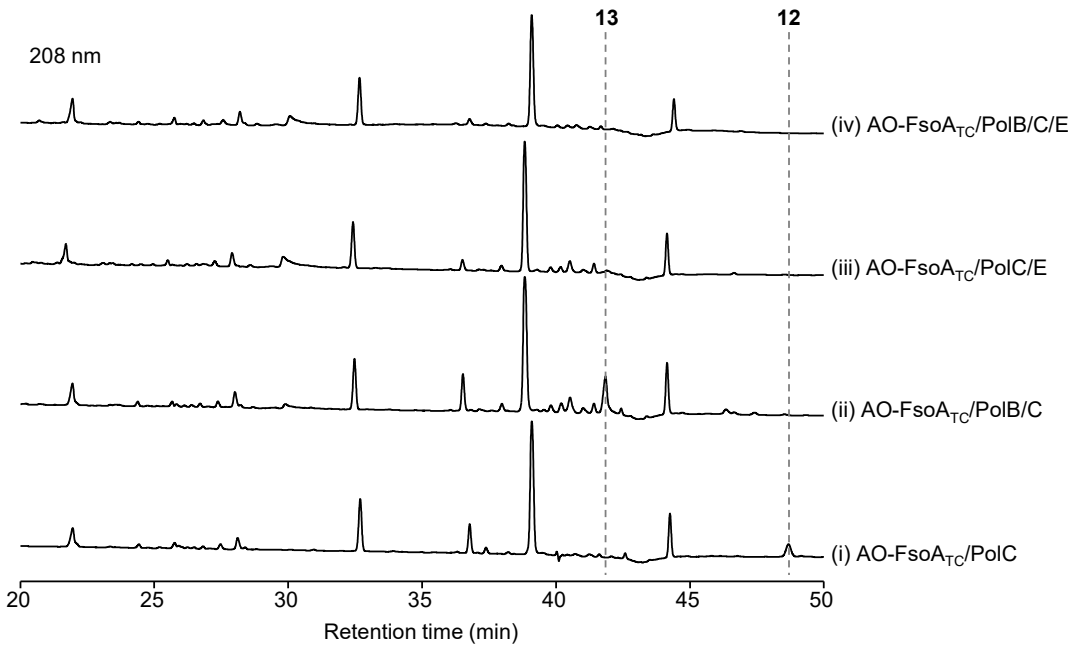

Figure S68 HPLC analysis of *A. oryzae* NSAR1 transformants co-expressing *fsoA$_{TC}$* and *polB/C/E*

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