Supplemental Synthetic Methods and Characterization, and Supplemental Figures and Tables

for

Rational Design of Mechanism-Based Inhibitors and Activity-Based Probes for the Identification of Retaining α-L-Arabinofuranosidases

Nicholas G.S. McGregor[a] Marta Artola,[b] Alba Nin-Hill,[c] Daniël Linzel,[b] Mireille Haon,[d] Jos Reijngoud,[e] Arthur Ram,[e] Marie-Noëlle Rosso,[d] Gijsbert A. van der Marel,[b] Jeroen D.C. Codée,[b] Gilles P. van Wezel[e] Jean-Guy Berrin,[d] Carme Rovira,[c,f,*] Herman S. Overkleeft*[,b] Gideon J. Davies*[,a]

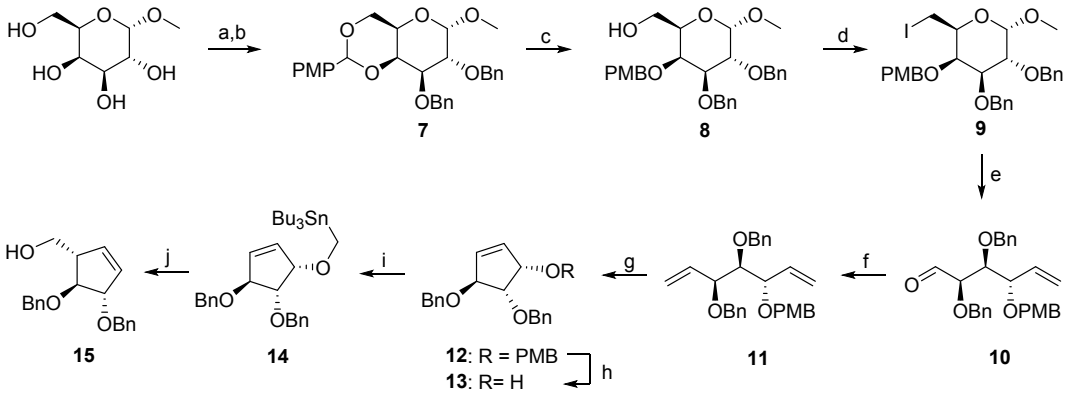
[a]York Structural Biology Laboratory, Department of Chemistry, The University of York, Heslington, York, YO10 5DD [b]Leiden Institute of Chemistry, Leiden University, Einsteinweg 55, 2300 RA Leiden, The Netherlands, [c]Departament de Química Inorgànica i Orgànica (Secció de Química Orgànica) & Institut de Química Teòrica i Computacional (IQTCUB), Universitat de Barcelona, Martí i Franquès 1, 08028 Barcelona, Spain, [d]INRA, Aix Marseille University, Biodiversité et Biotechnologie Fongiques (BBF), UMR1163, F-13009 Marseille, France. [e]Molecular Microbiology and Biotechnology, Institute of Biology Leiden, Leiden University, Sylviusweg 72, 2333 BE Leiden, The Netherlands, [f]Institució Catalana de Recerca i Estudis Avançats (ICREA), 08020 Barcelona, Spain.

Supplemental Methods

General Synthetic Experimental Details

All synthetic reagents were of a commercial grade and were used as received unless stated otherwise. Acetonitrile (ACN), dichloromethane (DCM), tetrahydrofuran (THF) and *N*,*N*-dimethylformamide (DMF) were stored over 4 Å molecular sieves, which were dried *in vacuo* before use. All reactions were performed under a nitrogen atmosphere unless stated otherwise. Solvents used for flash column chromatography were of pro analysis quality. Reactions were monitored by analytical thin-layer chromatography (TLC) using Merck aluminum sheets pre-coated with silica gel 60 with detection by UV absorption (254 nm) and by spraying with a solution of $(NH_4)_6Mo_7O_{24}{\cdot}H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4{\cdot}H_2O$ (10 g/L) in 10% sulfuric acid followed by charring at ±150 ˚C or by spraying with an aqueous solution of $KMnO_4$ (7%) and K_2CO_3 (2%) followed by charring at ±150 °C. Column chromatography was performed manually using either Baker or Screening Device silica gel 60 (0.04 - 0.063 mm) or a Biotage Isolera™ flash purification system using silica gel cartridges (Screening devices SiliaSep HP, particle size 15-40 µm, 60A) in the indicated solvents. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AV-400 (400/101 MHz), Bruker AV-500 (500/126 MHz), AV-600 (600/152 MHz) and AV-850 (850/214 MH) spectrometer in the given solvent. Chemical shifts are given in ppm relative to the chloroform residual solvent peak or tetramethylsilane (TMS) as internal standard. The following abbreviations are used to describe peak patterns when appropriate: s (singlet), d (doublet), t (triplet), qt (quintet), m (multiplet), br (broad), ar (aromatic), app (apparent). 2D NMR experiments (HSQC, COSY and NOESY) were carried out to assign protons and carbons of the new structures and assignation follows the general numbering shown in protected galactose **6** and cyclopentene **15**. High-resolution mass spectra (HRMS) of compounds were recorded with a LTQ Orbitrap (Thermo Finnigan). Optical rotations were measured on an Anton Paar MCP automatic polarimeter (Sodium D-line, λ = 589 nm). LC/MS analysis was performed on an LCQ Advantage Max (Thermo Finnigan) ion-trap spectrometer (ESI+) coupled to a Surveyor HPLC system (Thermo Finnigan) equipped with a C18 column (Gemini, 4.6 mm x 50 mm, 3 µm particle size, Phenomenex) equipped with buffers A: H_2O, B: acetonitrile (MeCN) and C: 1% aqueous TFA or 50 mM NH_4HCO_3 in H_2O. For reversed-phase HPLC-MS purifications of ABPs **4** and **5** an Agilent Technologies 1200 series prep-LCMS with a 6130 Quadrupole MS system was used equipped with buffers A: 50 mM NH_4HCO_3 in H_2O and B: MeCN.

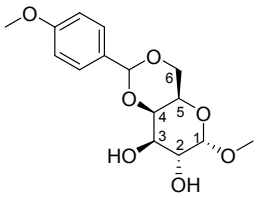
Synthesis of Cyclopentene 15



Scheme 1. Synthesis of α-L-arabinofuranose-configured cyclopentene **12**. Reagents and conditions: a) (1*S*)-(+)-10-Camphorsulfonic acid, CH_3CN, 50 °C, 300 mbar, 2.5 h; b) BnBr, NaH, TBAI, DMF, 0 °C, rt, 18 h, 74% over two steps; c) BH_3·THF, Bu_2BOTf, DMF, 0 °C, 15 min, 90%; d) I_2, TPP, THF, reflux, 3 h, 79%; e) Activated Zn powder, THF, 35 °C, 2 h, 84%; f) $Ph_3P^+CH_3Br^-$, *n*-BuLi, THF, –78 °C to –20 °C for 1 h, then rt, 18 h, 73%; g) Grubb's II cat., DCM, reflux, 18 h, 90%; h) DDQ, DCM, 0 °C, rt, 2 h, 86%; i) Bu_3SnMeI, KH, dibenzo-18-crown-6, THF, 0 °C, rt, 18 h, 91%; j) *n*-BuLi, THF, –78 °C, rt, 18 h, 68%.

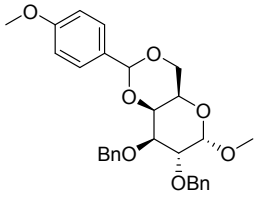
Cyclopentene **15** was synthesized in nine steps from commercial methyl α-D-galactopyranoside with n 15% overall total yield. First, *p*-methoxybenzylidene acetal was installed at C4 and C6 (carbohydrate numbering) by treatment with anisaldehyde dimethylacetal and was subsequently benzylated at C2 and C3 to afford intermediate **7** in 74% over 2 steps (Scheme 1). Selective opening of the PMP-group in compound **7** was achieved with Bu_2BOTf and BH_3·THF, and subsequent nucleophilic substitution of the primary alcohol with iodine, followed by Vasella fragmentation with activated zinc powder afforded intermediate **10** which could be scaled up to 56 mmol with moderate yields. Wittig olefination of aldehyde **10** and subsequent ring-closing metathesis (RCM) with second generation Grubb's catalyst afforded **11**. PMB group was then selectively removed with DDQ and intermediate **14** was obtained by subsequent alkylation with freshly synthesized Bu_3SnMeI. Wittig-Still rearrangement of intermediate **14** with *n*-BuLi at –78 °C afforded key cyclopentene **15**.

(2S,4aR,7R,8R,8aR)-6-Methoxy-2-(4-methoxyphenyl)hexahydropyrano[3,2-d][1,3]dioxine-7,8-diol



α-D-galactopyranoside-1-*O*-methyl (25.0 g, 128 mmol), camphorsulphonic acid (2.99 g, 12.9 mmol), and 4-methoxybenzaldehyde dimethyl acetal (26.0 mL, 154 mmol) were suspended in ACN (250 mL) and stirred on a rotary evaporator at 300 mbar and 50 °C until around 25% of the solvent was left (2.5 h). The resulting pink reaction mixture was quenched with Et_3N until neutral pH and a colour change was observed (pink to green). The reaction mixture was concentrated *in vacuo* and the resulting yellow crude oil was used in the following reaction without further purification.

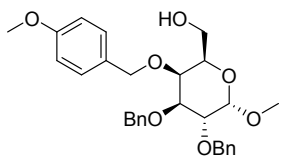
*(2S,4aR,6S,7R,8S,8aS)-7,8-Bis(benzyloxy)-6-ethoxy-2-(4-methoxyphenyl)hexahydropyrano[3,2-d][1,3]dioxine (**7**)*



NaH (13.5 g, 563 mmol) was added slowly (over 15 min) to a stirring solution of crude **7** and TBAI (2.39 g, 6.46 mmol) in DMF (500 mL) at 0 °C. After 10 min, BnBr (38.0 mL, 320 mmol) was added dropwise to the resulting reaction mixture and was stirring at rt overnight. The resulting orange mixture was quenched by slow addition of MeOH (120 mL), diluted with EtOAc (400 mL) and washed with brine (300 mL) and water (1.20 L). The aqueous phase was extracted with EtOAc (3 × 300 mL). The combined organic phases were dried ($MgSO_4$), filtered and concentrated under reduced vacuum, after which silica gel column chromatography (0% → 50% EtOAc in pentane) yielded the desired compound in 74% over 2 steps (46.6 g, 94.6 mmol). $[\alpha]_D^{20}$ = +61.3 (*c* = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.44 (d, *J* = 8.6 Hz, 2H, CH_{PMP} Ar), 7.42 – 7.26 (m, 10H, CH Bn), 6.88 (d, *J* = 8.8 Hz, 2H, CH_{PMP} Ar), 5.43 (s, 1H, C*H*PhOCH$_3$), 4.88 (d, *J* = 12.0 Hz, 1H, C*H*HPh), 4.82 (d, *J* = 12.4 Hz, 1H, C*H*HPh), 4.75 (d, *J* = 2.7 Hz, 1H, CH-1), 4.74 (d, *J* = 18.6 Hz, 1H, C*H*HPh), 4.68 (d, *J* = 12.0 Hz, 1H, C*H*HPh), 4.18 (dd, *J* = 12.4, 1.5 Hz, 1H, CH-6a), 4.16 – 4.14 (dd, *J* = 4.06, 1H, CH-4), 4.06 (dd, *J* = 10.1, 3.5 Hz, 1H, CH-2), 3.98 (dd, *J* = 4.6, 2.6 Hz, 1H, CH-3), 3.95 (d, *J* = 3.4 Hz, 1H, CH-6b), 3.79 (s, 3H, PhOCH_3), 3.55 (s, 1H, CH-5), 3.38 (s, 3H, OCH_3 Anom). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 160.1, 138.9, 138.7, 130.5 (4C_q Ar), 128.4, 128.2, 127.8, 127.7, 127.6 (12CH Ar), 113.5 (2CH_{PMP}), 101.1 (*C*HPhOCH$_3$), 99.6 (CH-1),

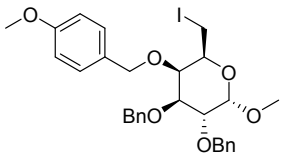
76.1 (CH-3), 75.5 (CH-2), 74.8 (CH-4), 73.9, 72.3 ($2CH_2Ph$), 69.4 (CH_2-6), 62.5 (CH-5), 55.6 (OCH_3), 55.4 (OCH_3). HRMS: calcd. for $[C_{29}H_{33}O_7]^+$ 493.5755; found 493.2225. HRMS: calcd. for $[C_{29}H_{32}NaO_7]^+$ 515.5572; found 515.2043.

*((2R,3S,4S,5R,6S)-4,5-Bis(benzyloxy)-6-methoxy-3-((4-methoxybenzyl)oxy)tetrahydro-2H-pyran-2-yl)methanol (**8**)*



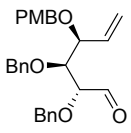
BH_3·THF (167 mL, 1 M in THF) was slowly added to a yellow solution of **7** (8.62 g, 17.5 mmol) in 75 mL of dry DCM at 0 °C. After subsequent slow addition of Bu_2BOTf (10 mL, 1 M in DCM), the reaction was stirred for 30 minutes and quenched with Et_3N (12 mL) until neutral pH. The reaction mixture was concentrated and the crude was purified by silica gel column chromatography (10% → 60% EtOAc in pentane) to afford the title compound as a colorless oil (9.62 g, 15.8 mmol, 90%). $[\alpha]_D^{20}$ = –2.6 (*c* = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.49 – 7.25 (m, 10H, CH Ar), 7.22 (d, *J* = 8.7 Hz, 2H, CH Ar), 6.83 (d, *J* = 8.7 Hz, 2H, CH Ar), 4.89 (d, *J* = 3.7 Hz, 1H, C*H*HPh), 4.86 (d, *J* = 4.1 Hz, 1H, C*H*HPh), 4.83 (d, *J* = 12.1 Hz, 1H, C*H*HPh), 4.73 (d, *J* = 11.8 Hz, 1H, C*H*HPh), 4.70 (s, 1H, CH-1), 4.68 (d, *J* = 7.8 Hz, 1H, C*H*HPh), 4.57 (d, *J* = 11.4 Hz, 1H, C*H*HPh), 4.04 (dd, *J* = 10.1, 3.6 Hz, 1H, CH-2), 3.92 (dd, *J* = 10.1, 2.8 Hz, 1H, CH-3), 3.85 (d, *J* = 2.6 Hz, 1H, CH-4), 3.74 (s, 3H, $PhOCH_3$), 3.67 (d, *J* = 9.1 Hz, 2H, CH_2OH), 3.44 (d, *J* = 6.2 Hz, 1H, CH-5), 3.33 (s, 3H, OCH_3 Anom). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 159.4, 138.8, 138.5, 130.4 ($4C_q$ Ar), 130.2, 128.5, 128.2, 127.8, 127.6 (12CH Ar), 113.9 (2CH Ar), 98.8 (CH-1), 79.2 (CH-4), 76.6 (CH-2), 74.7 (CH-3), 74.1 (CH_2 PMB), 73.6, 73.6 ($2CH_2Ph$), 70.4 (CH-5), 62.4 (CH_2-6), 55.4 (OCH_3), 55.3 (OCH_3). HRMS: calcd. for $[C_{29}H_{34}NaO_7]^+$ 517.5732; found 517.2205.

*(2S,3R,4S,5R,6S)-3,4-Bis(benzyloxy)-6-(iodomethyl)-2-methoxy-5-((4-methoxybenzyl)oxy)tetrahydro-2H-pyran (**9**)*



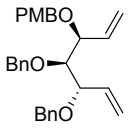
Triphenylphosphine (7.50 g, 28.6 mmol) and imidazole (2.60 g, 38.2 mmol) were added to a solution of **8** (9.35 g, 18.9 mmol) in THF (230 mL). The resulting solution was heated to reflux (90 °C) and an iodine solution (7.22 g, 28.4 mmol) in THF (115 mL) was added slowly through a canula. After 2 h of refluxing the mixture was allowed to cool to rt and was subsequently diluted with EtOAc (200 mL). The reaction mixture was washed with water (250 mL), brine (50 mL) and sat. aq. $Na_2S_2O_3$ (200 mL). The aqueous phase was then extracted with EtOAc (2 x 100 mL). The combined organic phases were dried with Na_2SO_4, filtered and concentrated *in vacuo*. Purification by silica column chromatography (10% → 20% EtOAc in pentane) afforded compound **9** as a brown solid (15.0 mmol, 79%). $[\alpha]_D^{20}$ = +12.7 (*c* = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.44 – 7.24 (m, 10H, CH Ar), 7.22 (d, *J* = 8.6 Hz, 2H, CH Ar), 6.83 (d, *J* = 8.7 Hz, 2H, CH Ar), 4.94 (d, *J* = 11.0 Hz, 1H, C*H*HPh), 4.88 (d, *J* = 11.8 Hz, 1H, C*H*HPh), 4.82 (d, *J* = 12.1 Hz, 1H, C*H*HPh), 4.74 (d, *J* = 11.8 Hz, 1H, C*H*HPh), 4.68 (4.66 (d, = 17.1 Hz, 1H, C*H*HPh 4.64 (d, *J* = 8.6 Hz, 1H, CH-1), 4.57 (d, *J* = 11.1 Hz, 1H, C*H*HPh), 4.01 (d, *J* = 3.6 Hz, 1H, CH-3), 3.98 (t, *J* = 3.2 Hz, 1H, CH-2), 3.91 (dd, *J* = 10.1, 2.7 Hz, 1H, CH-4), 3.81 ((t, *J* = 7.0 Hz, 1H, CH-5), 3.75 (s, 3H, $PhOCH_3$), 3.40 (s, 3H, OCH_3 Anom.), 3.20 (dd, *J* = 10.0, 7.8 Hz, 1H, C*H*HI), 3.01 (dd, *J* = 10.1, 6.0 Hz, 1H, C*H*HI). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 159.3, 138.6, 138.3, 130.3 ($4C_q$ Ar), 130.0, 128.3, 128.0, 127.7, 127.5 (12CH Ar), 113.7 (2CH Ar), 98.7 (CH-1), 79.0 (CH-4), 76.0 (CH-3), 75.3 (CH-2), 74.5 (CH_2 PMB), 73.5 (CH_2 Bn), 71.2 (CH-5), 55.7 (OCH_3), 55.2 (OCH_3). HRMS: calcd. for $[C_{29}H_{33}INaO_6]^+$ 627.4707; found 627.1236.

*(2R,3S,4S)-2,3-Bis(benzyloxy)-4-((4-methoxybenzyl)oxy)hex-5-enal (**10**)*



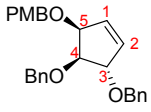
A solution of iodine **9** (1.98 g, 3.27 mmol) in a 9:1 THF:H_2O mixture (30 mL) was degassed using N_2 while sonicating for 15 min. Activated zinc powder (4.95 g, 75.7 mmol) was added to the solution and the resulting mixture was sonicated under a N_2-atmosphere at 35 °C for 2 h. Then, a new spot was observed by TLC (R_f 0.4–0.7 pentane–EtOAc 8:2) and the mixture was filtered through Celite®, washed with THF and diethyl ether, and subsequently concentrated. The resulting yellow emulsion was diluted with diethyl ether (60 mL) and washed with water (40 mL) and brine (20 mL). The aqueous phase was extracted again with diethyl ether (20 mL). The combined organic phases were dried (Na_2SO_4), filtered and concentrated. Purification by silica column chromatography (0% → 10% EtOAc in pentane) afforded the desired product **10** as an oil (2.77 mmol, 84%). $[\alpha]_D^{20}$ = +39.7 (*c* = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.62 (d, *J* = 1.7 Hz, 1H, CH=O), 7.43 – 7.24 (m, 10H, CH Ar), 7.20 (d, *J* = 8.7 Hz, 2H, CH Ar), 6.87 (d, *J* = 8.7 Hz, 1H, CH Ar), 5.97 – 5.84 (m, 1H, C*H*=CH_2), 5.53 – 5.39 (m, 2H, CH=C*H*$_2$), 4.67 (d, *J* = 11.8 Hz, 1H, C*H*HPh), 4.61 (d, *J* = 11.2 Hz, 1H, C*H*HPh), 4.56 (d, *J* = 11.8 Hz, 1H, C*H*HPh), 4.50 (dd, *J* = 11.1, 2.6 Hz, 2H, CH_2Ph), 4.19 – 4.06 (m, 3H, C*H*HPh, CH-2, CH-4), 3.88 (dd, *J* = 7.8, 3.8 Hz, 1H, CH-3), 3.81 (s, 3H, $PhOCH_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 202.2 (CH=O), 158.9, 137.3, 137.0 (3C_q Ar), 135.4 (CH-5), 129.6 (C_q Ar), 129.3, 128.1, 127.9, 127.5 (12CH Ar), 119.9 (CH_2OPMB), 113.4 (2CH Ar), 83.7 (CH-2/CH-4), 80.9 (CH-3), 78.5 (CH-2/CH-4), 74.0, 73.1, 69.4 (3CH_2Ph), 54.9 (OCH_3). HRMS: calcd. for $[C_{28}H_{30}NaO_5]^+$ 469.5322; found 469.2000.

*((((3S,4R,5S)-5-((4-Methoxybenzyl)oxy)hepta-1,6-diene-3,4-diyl)bis(oxy))bis(methylene))dibenzene (**11**)*



$MePPh_3Br$ (6.75 g, 18.9 mmol) was added to a flame-dried flask and dissolved in dry THF (100 mL). The resulting suspension was cooled to –74 °C, after which *n*-BuLi (8.50 mL, 19.6 mmol) was added dropwise. The resulting yellow suspension was allowed to warm to –20 °C and was stirred for 1 h. Aldehyde **10** (2.80 g, 6.28 mmol) was dissolved in dry THF (3 mL), added slowly *via* the cold wall of the flask and the resulting mixture was allowed to warm to rt and stirred overnight. The suspension became from yellow to orange to dark red. After 20 h, TLC revealed a higher running spot (R_f 0.7, pentane–EtOAc 9:1) and the mixture was quenched using a sat. aq. NH_4Cl solution (400 mL) and extracted with diethyl ether (400 mL). The aqueous phase was extracted again with diethyl ether (200 mL). The combined organic phases were dried ($MgSO_4$), filtered and concentrated. The resulting crude was purified by silica column chromatography (0% → 10% EtOAc in pentane), which afforded diene **11** in 73% yield (2.03 g, 4.56 mmol). $[\alpha]_D^{20}$ = +30.7 (*c* = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.43 – 7.19 (m, 10H, CH Ar), 7.14 (d, *J* = 8.6 Hz, 2H, CH Ar), 6.81 (d, *J* = 8.6 Hz, 2H, CH Ar), 6.03 – 5.68 (m, 2H, 2C*H*=CH_2), 5.41 – 5.18 (m, 4H, 2CH=C*H*$_2$), 4.71 (d, *J* = 11.3 Hz, 1H, C*H*HPh), 4.68 – 4.56 (m, 2H, CH_2Ph), 4.40 (dd, *J* = 48.3, 11.5 Hz, 2H, CH_2Ph), 4.14 (d, *J* = 11.1 Hz, 1H, C*H*HPh), 4.09 – 3.98 (m, 2H, CH-3, CH-5), 3.75 (s, 3H, $PhOCH_3$), 3.65 – 3.51 (m, 1H, CH-4). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 159.1, 138.7, 138.7 (3C_q Ar), 136.0, (2C*H*=CH_2), 130.7 (C_q Ar), 129.4, 128.3, 128.1, 127.5, (12CH Ar), 119.2, 118.7 (2C*H*$_2$=CH), 113.8 (2CH Ar), 83.9 (CH-4), 80.7, 80.0 (CH-3, CH-5), 75.0 (CH_2 PMB), 70.7, 69.9 (2CH_2 Bn), 55.3 (OCH_3). HRMS: calcd. for $[C_{29}H_{32}NaO_4]^+$ 467.5602; found 467.2200.

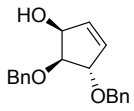
*((((1R,2S,5S)-5-((4-Methoxybenzyl)oxy)cyclopent-3-ene-1,2-diyl)bis(oxy))bis(methylene))dibenzene (**12**)*



Diene **11** (3.09 g, 6.96 mmol) was dissolved in DCM (200 mL) and added to a flame-dried flask, which was subsequently degassed for 15 min. To the resulting solution, second generation Grubb's catalyst (365 mg, 0.429 mmol) was added. The mixture was carefully degassed while sonicating for 30 min and covered from light. After refluxing for 68 h, the mixture was concentrated *in vacuo*, and silica gel column chromatography (0% → 10% EtOAc in pentane) afforded the desired cyclopentene **12** as an oil in 90% yield (2.69 g, 6.47 mmol). $[\alpha]_D^{20}$ = +168.4 (*c* = 1, $CHCl_3$). ^{1}H

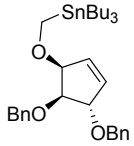
NMR (400 MHz, $CDCl_3$) δ = 7.46 – 7.26 (m, 12H, CH Ar), 6.85 (d, *J* = 8.6 Hz, 2H, CH Ar), 6.05 (d, *J* = 7.8 Hz, 1H, CH-2), 5.99 (d, *J* = 6.3 Hz, 1H, CH-1), 4.81 (d, *J* = 4.8 Hz, 1H, CH-3), 4.74 (d, *J* = 11.8 Hz, 1H, C*H*HPh), 4.67 (d, *J* = 11.7 Hz, 1H, C*H*HPh), 4.61 (d, *J* = 11.5 Hz, 1H, C*H*HPh), 4.58 (d, *J* = 11.7 Hz, 1H, C*H*HPh), 4.53 (d, *J* = 5.8 Hz, 1H, CH-5), 4.51 (s, 2H, CH_2 PMB), 3.95 (t, *J* = 5.3 Hz, 1H, CH-4), 3.80 (s, 3H, $PhOCH_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 159.3, 138.5, 138.4 ($3C_q$ Ar), 136.1 (CH-2), 132.0 (CH-1), 130.8 (C_q Ar), 129.7, 128.5, 128.1, 128.0, 127.8, 113.9 (14CH Ar), 86.6 (CH-5), 84.3 (CH-4), 78.4 (CH-3), 72.2 ($2CH_2Ph$), 70.7 (CH_2Ph PMB), 55.4 (OCH_3). HRMS: calcd. for $[C_{27}H_{28}NaO_4]^+$ 439.5062; found 439.1880.

*(1S,4S,5S)-4,5-Bis(benzyloxy)cyclopent-2-en-1-ol (**13**)*



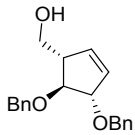
A solution of protected cyclopentene **12** (1.85 g, 4.45 mmol) in DCM (3.6 mL) and H_2O (2.2 mL) was cooled to 0 °C. DDQ (2.01 g, 8.89 mmol) was added and the resulting black mixture was allowed to warm to rt, stirred for 2 h, and filtered over Celite®. The residue was washed with DCM (150 mL) and was washed with water (150 mL) and $NaHCO_3$ (50 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Column chromatography (0% → 15% EtOAc in pentane) afforded the title compound **13** as an off-white solid in 86% yield (1.13 g, 3.80 mmol). $[\alpha]_D^{20}$ = +127.4 (*c* = 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ = 7.44 – 7.28 (m, 10H, CH Ar), 6.06 – 6.01 (m, 2H, CH-1, CH-2), 4.71 (d, *J* = 6.8 Hz, 1H, CH-3), 4.67 (d, *J* = 4.8 Hz, 2H, CH_2Ph), 4.65 (dd, *J* = 3.5, 1.5 Hz, 1H, CH-5), 4.58 (s, 2H, CH_2Ph), 3.97 (dd, *J* = 5.7, 3.6 Hz, 1H, CH-4). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 135.6, 134.0 (CH-1, CH-2), 128.7, 128.6, 128.2, 128.2, 127.9 (10CH Ar), 86.5 (CH-3), 83.2 (CH-4), 73.0 (CH-5), 72.8, 72.0 ($2CH_2Ph$).HRMS: calcd. for $[C_{19}H_{20}NaO_3]^+$ 319.3552; found 319.1314.

*((((1S,4S,5R)-4,5-Bis(benzyloxy)cyclopent-2-en-1-yl)oxy)methyl)tributylstannane (**14**)*



To a cold solution of cyclopentenol **13** (652 mg, 2.20 mmol) in THF (6 mL) at 0 °C, KH (747 mg 30% dispersed in mineral oil, 5.59 mmol) and dibenzo-18-crown-6 (41 mg, 0.11 mmol) were added. To this mixture, freshly prepared Bu_3SnMeI (0.8 mL, 2.6 mmol) was added slowly. The resulting brown suspension was allowed to warm to rt and after 1h all starting material was consumed. After quenching with a few milliliters of water dropwise at 0 °C, water (30 mL), EtOAc (15 mL) and brine (10 mL) was added to dissolve the emulsion and the organic phase dried ($MgSO_4$), filtrated, and concentrated *in vacuo*. Flash purification by silica gel column chromatography (0% → 5% EtOAc in pentane) afforded the desired product as a colorless oil (2.0 mmol, 91%). $[\alpha]_D^{20}$ = +133.8 (*c* = 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ = 7.44 – 7.28 (m, 10H, CH Ar), 6.06 (s, 2H, CH-1, CH-2), 4.78 (d, *J* = 11.8 Hz, 1H, C*H*HPh), 4.74 (d, *J* = 4.7 Hz, 1H, CH-3/CH-5), 4.67 (d, *J* = 11.7 Hz, 1H, C*H*HPh), 4.63 – 4.52 (m, 2H, CH_2Ph), 4.38 (dd, *J* = 5.7, 2.0 Hz, 1H, CH-3/CH-5), 3.92 (dd, *J* = 5.6, 4.8 Hz, 1H, CH-4), 3.79 – 3.66 (m, 2H, CH_2SnBu_3), 1.56 – 1.37 (m, 6H, $3SnCH_2CH_2CH_2CH_3$), 1.28 (dq, *J* = 14.4, 7.3 Hz, 6H, 3 $SnCH_2CH_2CH_2CH_3$), 0.88 (m, 15H, $3SnCH_2CH_2CH_2CH_3$, $3SnCH_2CH_2CH_2CH_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 138.7, 128.6 ($2C_q$ Ar), 136.1, 132.1 (CH-1, CH-2), 128.5, 127.9, 127.7, 127.6 (10CH Ar), 86.7 (CH-3/CH-5), 84.2 (CH-4), 82.8 (CH-3/CH-5), 72.0, 71.9 ($2CH_2Ph$), 59.3 (CH_2SnBu_3), 29.3 ($3SnCH_2CH_2CH_2CH_3$), 27.5 ($3SnCH_2CH_2CH_2CH_3$), 13.9 ($3SnCH_2CH_2CH_2CH_3$), 9.1 ($3SnCH_2CH_2CH_2CH_3$). HRMS: calcd. for $[C_{32}H_{48}NaO_3Sn]^+$ 622.4322; found 623.2538.

*((1S,4S,5S)-4,5-Bis(benzyloxy)cyclopent-2-en-1-yl)methanol (**15**)*

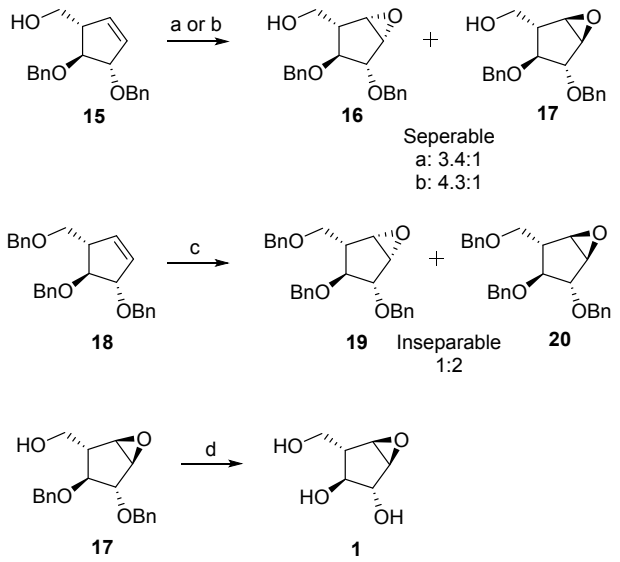


Stanylated cyclopentenol **14** (634 mg, 1.06 mmol) in THF (15 mL) was cooled down to –75 °C, and *n*-BuLi (2.3 M in hexanes, 0.7 mL, 1.59 mmol) was added dropwise along the cold wall of the flask. The resulting light green solution was allowed to heat up to rt and stirred overnight. The mixture had become dark brown and was poured into an ice-

cooled solution of saturated NH_4Cl (40 mL), washed with water and extracted with EtOAc (2x). The organic phase was dried ($MgSO_4$), filtrated, concentrated under reduced pressure and purified by silica gel column chromatography (0% → 40% EtOAc in pentane) to afford the title compound **15** (0.70 mmol, 68%). $[\alpha]_D^{20}$ = +11.6 (*c* = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.45 – 7.27 (m, 10H, CH Ar), 5.98 – 5.91 (m, 1H, CH-2), 5.87 (dd, *J* = 6.0, 2.0 Hz, 1H, CH-1), 4.61 (dd, *J* = 20.4, 1.5 Hz, 5H, $2CH_2Ph$, CH-3), 3.99 (t, *J* = 3.1 Hz, 1H, CH-4), 3.76 – 3.58 (m, 2H, CH_2OH), 2.87 (dd, *J* = 2.7, 0.7 Hz, 1H, CH-5). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 138.3, 138.2 ($2C_q$ Ar), 134.4 (CH-1), 131.0 (CH-2), 128.6, 128.0, 127.9, 127.8 (10CH Ar), 88.7 (CH-3), 86.8 (CH-4), 71.8, 71.4 ($2CH_2Ph$), 64.2 (CH_2OH), 53.3 (CH-5). HRMS: calcd. for $[C_{20}H_{22}NaO_3]^+$ 333.3822; found 333.1472.

Synthesis of α-L-arabino-cyclophellitol epoxide 1

The first step towards the designed covalent inhibitors —α-L-epoxide and α-L-aziridine— was stereoselective epoxidation of cyclopentene **15**. We rationalized that treatment of cyclopentene **15** with *m*-CPBA would lead to predominant β-L-epoxidation where the neighbouring primary alcohol would play a directing role by hydrogen bonding with *m*-CPBA. Indeed, m-CPBA epoxidation at 50 °C overnight—resulted indeed in a 3.4:1 separable mixture of β-L- and α-L-epoxide in 62%. Cooling the mixture to 4 °C and slowing the reaction for 4 days increased the β-L and α-L ratio to 4.3:1, with a higher reaction yield (91%). In order to synthesize the α-L-epoxide selectively, cyclopentene **15** was benzylated and subjected to epoxidation with *m*-CPBA. However, although the β-L and α-L ratio was improved to 1:2 due to steric hindrance and the absence of the H-bonding of the primary alcohol, a non-separable mixture by column chromatography was obtained. Final arabinofuranosidase-configured α-L-epoxide **1** was obtained by hydrogenation of partially benzylated **17** with Pearson's catalyst.



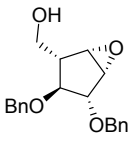
Scheme 2. Synthesis of epoxides. Reagents and conditions: a) *m*-CPBA, DCM, 50 °C, 18 h, 62%, 3.4:1 of **16**:**17**. b) *m*-CPBA, DCM, 0 °C, 4 days, 91%, 4.3:1 of **16**:**17**. c) *m*-CPBA, DCM, 50 °C, 18 h, 62%. d) H_2, $Pd(OH)_2$, MeOH, 18 h, 50%.

*Synthesis of β- and α-epoxide **16** and **17***

A solution of primary alcohol **15** (200 mg, 0.64 mmol) in DCM (6 mL) was added to a buffer (pH 6.9) of Na_2HPO_4 (6.5 mL) and NaH_2PO_4 (6.5 mL) at 0 °C under vigorous stirring. Then, *m*-CPBA (305 mg, 1.77

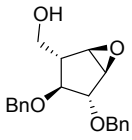
mmol) was added to the vigorously stirring solution and the mixture was stirred at 4 °C for 4 days. The aqueous phase was extracted with EtOAc (2 × 25 mL) and the combined organic phases were dried ($MgSO_4$), filtered and the solvent removed under reduced pressure. The reaction mixture was purified by silica gel column chromatography (0% → 70% EtOAc in pentane) to afford the β-epoxide **17** as a white solid (155 mg, 0.47 mmol, 74%). α-Epoxide **16** was isolated as well as minor product (36 mg, 0.11 mmol, 17%).

*((1S,2S,3S,4R,5S)-3,4-Bis(benzyloxy)-6-oxabicyclo[3.1.0]hexan-2-yl)methanol; β-epoxide (**16**)*



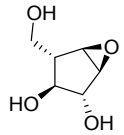
$[\alpha]_D^{20}$ = –12.6 (*c* = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.45 – 7.27 (m, 10H, CH Ar), 4.79 (d, *J* = 11.9 Hz, 1H, C*H*HPh), 4.69 (d, *J* = 11.3 Hz, 2H, CH_2Ph), 4.55 (d, *J* = 11.6 Hz, 1H, C*H*HPh), 4.11 (dd, *J* = 5.3, 1.3 Hz, 1H, CH-3), 3.91 (dd, *J* = 10.6, 5.4 Hz, 1H, C*H*HOH), 3.84 (dd, *J* = 10.6, 7.5 Hz, 1H, C*H*HOH), 3.56 (m, 3H, CH-1, CH-2, CH-4), 2.23 (tdd, *J* = 7.2, 5.4, 1.4 Hz, 1H, CH-5). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 138.2, 136.9 (2C_q Ar), 128.7, 128.6, 128.1, 128.0, 127.9 (10CH Ar), 85.7 (CH-3), 81.0 (CH-4), 72.7, 71.9 (2CH_2Ph), 62.2 (CH_2OH), 55.1, 54.0 (CH-1, CH-2), 46.8 (CH-5). HRMS: calcd. for $[C_{20}H_{22}NaO_4]^+$ 349.3812; found 349.1420.

*((1R,2S,3S,4R,5R)-3,4-Bis(benzyloxy)-6-oxabicyclo[3.1.0]hexan-2-yl)methanol; α-epoxide (**17**)*



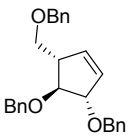
^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.44 – 7.18 (m, 10H, CH Ar), 4.60 – 4.40 (m, 4H, 2CH_2Ph), 4.00 (s, 1H, CH-3), 3.74 (s, 1H, CH-4), 3.69 (d, *J* = 5.7 Hz, 2H, CH_2OH), 3.57 (s, 2H, CH-1, CH-2), 2.61 – 2.40 (m, 1H, CH-5). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 137.9, 137.2 (2C_q Ar), 130.2, 129.9, 128.7, 128.6, 128.3, 128.0, 127.9 (10CH Ar), 86.4 (CH-4), 82.1 (CH-3), 72.2, 71.7 (2CH_2Ph), 62.4 (CH_2OH), 59.1, 57.8 (CH-1, CH-2), 48.8 (CH-5). HRMS: calcd. for $[C_{20}H_{22}NaO_4]^+$ 349.3812; found 349.1416. HRMS: calcd. for $[C_{20}H_{22}KO_4]^+$ 365.4898; found 365.1147.

*(1S,2R,3S,4S,5R)-4-(Hydroxymethyl)-6-oxabicyclo[3.1.0]hexane-2,3-diol (**1**)*



Epoxide **17** (26.4 mg, 0.081 mmol) was dissolved in MeOH (5 mL) and thoroughly degassed with argon, after which 20% $Pd(OH)_2$ (11 mg, 0.02 mmol) was added H_2-gas was bubbled through the mixture (5 min) and the reaction mixture was stirred overnight under a H_2-atmosphere. After removal of H_2 with argon, the mixture was filtered over Celite®, washed with MeOH (40 mL) and concentrated under reduced pressure. The crude was purified by silica gel column chromatography (0% → 20% MeOH in DCM) to afford the title compound **1** (5.0 mg, 42%). $[\alpha]_D^{20}$ = –13.6 (*c* = 1, MeOH). ^{1}H NMR (500 MHz, MeOD) δ = 3.94 (s, 1H, CH-3), 3.73 (s, 1H, CH-4), 3.68 (dd, *J* = 10.8, 5.6 Hz, 1H, C*H*HOH), 3.62 (dd, *J* = 10.8, 6.8 Hz, 1H, C*H*HOH), 3.57 (d, *J* = 2.2 Hz, 1H, CH-2), 3.50 – 3.45 (m, 1H, CH-1), 2.26 (t, *J* = 6.2 Hz, 1H, CH-5). ^{13}C NMR (126 MHz, MeOD) δ = 81.9 (CH-4), 78.0 (CH-3), 61.9 (CH_2OH), 60.2 (CH-1), 59.99 (CH-2), 52.3 (CH-5). HRMS: calcd. for $[C_6H_{10}NaO_4]^+$ 146.1420; the exact mass cannot be detected.

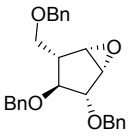
*((((1S,2S,5S)-5-((Benzyloxy)methyl)cyclopent-3-ene-1,2-diyl)bis(oxy))bis(methylene))dibenzene (**18**)*



Primary alcohol **15** (189 mg, 0.61 mmol) and TBAI (12 mg, 0.03 mmol) were dissolved in 4 mL DMF. NaH (60% dispersed in mineral oil, 53 mg, 1.33 mmol) was slowly added at 0 °C. Then, BnBr (0.12 mL, 1.01 mmol) was added dropwise to the cold solution and the mixture was stirred overnight. The resulting yellow solution was quenched with MeOH (1 mL, dropwise), extracted with EtOAc (2x) , and washed with brine and water. The combined organic phases were dried, filtered and concentrated *in vacuo*, and successive silica gel column chromatography (0% → 5% EtOAc in pentane) yielded pure product **16** (0.50 mmol, 82%). $[\alpha]_D^{20}$ = +0.20 (*c* = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.41 – 7.27 (m, 15H, CH Ar), 5.88 (dd, *J* = 4.7, 3.1 Hz, 2H,

CH-1, CH-2), 4.64 (d, *J* = 4.7 Hz, 2H, CH_2Ph), 4.63 (d, *J* = 2.7 Hz, 1H, CH-3), 4.56 (s, 2H, CH_2Ph), 4.54 (s, 2H, CH_2Ph), 3.99 (t, *J* = 3.4 Hz, 1H, CH-4), 3.58 – 3.40 (m, 2H, CH_2OBn), 2.98 – 2.91 (m, 1H, CH-5). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 138.6, 138.5, 138.4 ($3C_q$ Ar), 134.5, 130.3 (CH-1, CH-2), 128.5, 128.0, 127.8, 127.7 (15CH Ar), 89.7 (CH-3), 87.1 (CH-4), 73.3 (CH_2Ph), 72.2 (CH_2OBn), 71.7, 71.3 ($2CH_2Ph$), 51.5 (CH-5). HRMS: calcd. for $[C_{27}H_{28}NaO_3]^+$ 423.5072; found 423.1937.

*(1S,2R,3S,4S,5S)-2,3-Bis(benzyloxy)-4-((benzyloxy)methyl)-6-oxabicyclo[3.1.0]hexane (**19**)*

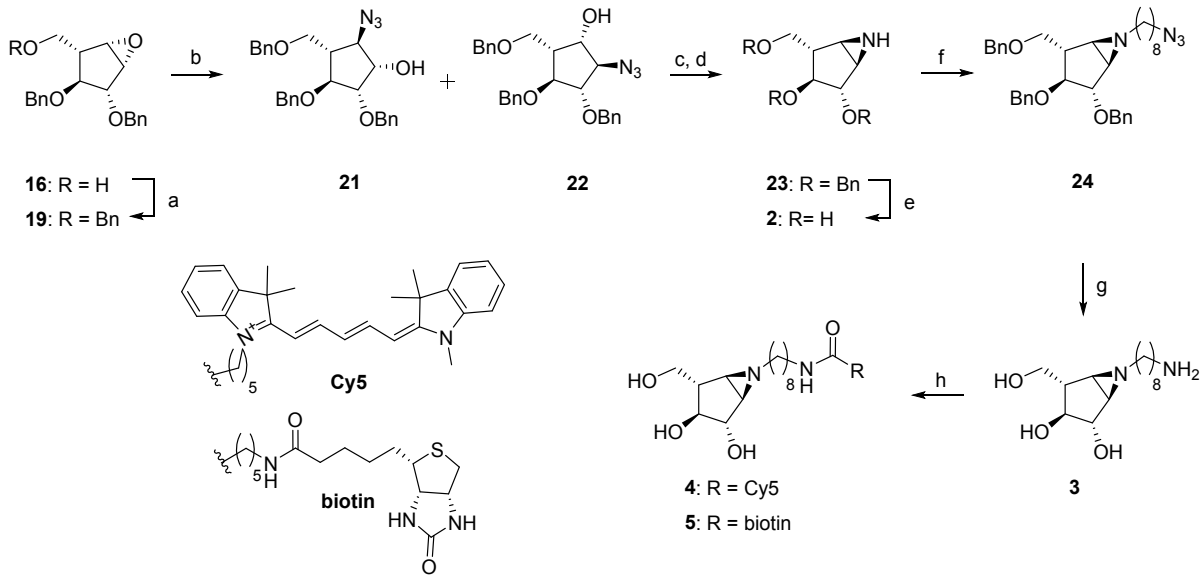


Epoxide **16** (80 mg, 0.245 mmol) was dissolved in DMF (1.6 mL) and cooled to 0 °C. TBAI (14.2 mg, 0.038 mmol) and 60% NaH (25 mg, 0.625 mmol) was added. Then, BnBr (0.05 mL, 0.421 mmol) was added dropwise and the resulting yellow solution was stirred overnight at rt. The reaction mixture was quenched with MeOH, extracted with EtOAc, washed with brine and water, dried ($MgSO_4$), filtered and concentrated under reduced pressure. Finally, silica gel column chromatography (0% → 30% EtOAc in pentane) afforded the desired product (80 mg, 78%). $[\alpha]_D^{20}$ = –27.7 (*c* = 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ = 7.48 – 7.20 (m, 15H, CH Ar), 4.78 (d, *J* = 11.9 Hz, 1H, C*H*HPh), 4.73 – 4.60 (m, 2H, CH_2Ph), 4.55 (s, 2H, CH_2Ph), 4.51 (d, *J* = 11.6 Hz, 1H, C*H*HPh), 4.10 (dd, *J* = 5.3, 1.3 Hz, 1H, CH-3), 3.66 (d, *J* = 1.8 Hz, 1H, C*H*HOBn), 3.64 (d, *J* = 4.9 Hz, 1H, C*H*HOBn), 3.63 (dd, *J* = 3.1, 1.4 Hz, 1H, CH-1/CH-2), 3.56 (dd, *J* = 3.1, 1.3 Hz, 1H, CH-1/CH-2), 3.40 (dd, *J* = 7.1, 5.3 Hz, 1H, CH-4), 2.35 (dddd, *J* = 8.6, 7.2, 5.8, 1.4 Hz, 1H, CH-5). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 138.4, 138.3, 138.1, ($3C_q$ Ar), 128.6, 128.5, 128.0, 127.8 (15CH Ar), 85.8 (CH-3), 81.5 (CH-4), 73.5, 72.7, 71.8 ($3CH_2Ph$), 69.1 (CH_2OBn), 55.3, 54.5 (CH-1, CH-2), 45.2 (CH-5). HRMS: calcd. for $[C_{27}H_{28}NaO_4]^+$ 439.5062; found 439.1878.

Synthesis of α-L-*arabino*-cyclophellitol aziridines 2-5

With cyclopentene **18** in hand, considering that the stereochemistry of vicinal hydroxyls in α-L-arabinofuranoside are in opposite stereochemistry than the desired α-L-aziridine, direct aziridination was first contemplated to form the α-L-aziridine aided by steric hindrance of the vicinal protecting groups. Unfortunately, no aziridination was observed with 3-amino-2-(trifluoromethyl)quinazolin-4(3*H*)-one (Q-CF_3) as nitrogen donor and PIDA to form the reactive acetylated quinazolinone nor with *O*-(2,4-dinitrophenyl)hydroxylamine (DPH) and a ruthenium catalyst. Probably the alkene is not accessible enough due to the benzyl groups and the conformation adopted by the cyclopentene.

The synthesis towards the aziridine **2** inhibitor was pursued by first benzylation of the primary hydroxyl of epoxide **16** and subsequent S_N1 ring opening with sodium azide. This afforded two separable **21** and **22** regio-isomers in 1:2 ratio in 77% (Scheme 3). Hydroxyls of **21** and **22** were first tosylated and subsequently treated with triphenylphosphine (TPP) and diisopropylethylamine (DIPEA) at 60 °C to obtain the benzylated aziridine **23**. Final aziridine **2** was obtained after benzyls deprotection under Birch conditions in a reasonably good yield. For the synthesis of activity based probes aziridine **23** was alkylated with 8-azidooctyl triflate and after subsequent deprotection under Birch reduction conditions with sodium and *t*-butanol, deprotected amino-octylaziridine **3** was obtained. Aziridine **3** was ultimately coupled with either Cy5-OSu or biotin-OSu esters in the presence of DIPEA as a base, and the α-L-arabinofuranosidase configured aziridine ABPs **4** and **5** were finally purified by HPLC-MS purification.

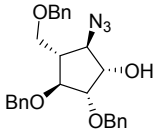


Scheme 3. Synthesis of α-L-aziridines **2-5**. Reagents and conditions: a) BnBr, NaH, TBAI, DMF, rt, 18 h, 78%. b) NaN_3, $LiClO_4$, DMF, 100 °C, 18 h 77%. c) TsCl, DMAP, TEA, DCM, 0 °C, 18 h, 50%. d) TPP, DIPEA, THF:H_2O, reflux, 1.5 h, 56%. e) Li, NH_3, –60 °C, 1 h, 66%. f) 8-azidooctyl triflate, DIPEA, DCM, 0 °C to rt, 18 h, 57%. g) Na, NH_3, *t*-BuOH, –60 °C, 1 h, 95%. h) Cy5-Osu or biotin-OSu, DIPEA, DMF, 18 h, **4**: 56% and **5**: 19%.

*Synthesis of azido alcohols **21** and **22***

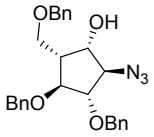
NaN_3 (132 mg, 2.03 mmol) and $LiClO_4$ (409 mg, 3.84 mmol) were added to a stirring solution of perbenzylated epoxide **19** (80 mg, 0.192 mmol) in DMF (2 mL). The reaction mixture was stirred at 100 °C overnight and then quenched with water (2 mL). The mixture was extracted with EtOAc (3 × 5 mL) and the combined organic phases were washed with water (10 mL) and brine (10 mL), and the combined aqueous phases were extracted again with EtOAc (10 mL). The combined organic phases were dried ($MgSO_4$), filtered, concentrated under reduced pressure and purified by silica gel column chromatography to afford the desired products **21** (22 mg, 25%) and **22** (46 mg, 52%).

*(1S,2R,3S,4S,5S)-2-Azido-4,5-bis(benzyloxy)-3-((benzyloxy)methyl)cyclopentan-1-ol (**21**)*



$[\alpha]_D^{20}$ = +9.6 (*c* = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.49 – 7.21 (m, 15H, CH Ar), 4.74 (d, *J* = 11.6 Hz, 1H, C*H*HPh), 4.64 (d, *J* = 11.6 Hz, 1H, C*H*HPh), 4.58 (d, *J* = 11.7 Hz, 1H, C*H*HPh), 4.55 – 4.47 (m, 2H, CH_2Ph), 4.44 (d, *J* = 11.8 Hz, 1H, C*H*HPh), 4.15 (ddd, *J* = 7.8, 5.4, 1.4 Hz, 1H, CH-4), 4.02 (ddd, *J* = 6.8, 5.0, 1.3 Hz, 1H, CH-1), 3.80 – 3.69 (m, 3H, CH-2, CH-3, C*H*HOBn), 3.64 (dd, *J* = 9.5, 5.1 Hz, 1H, C*H*HOBn), 2.31 (tdd, *J* = 7.8, 5.0, 4.0 Hz, 1H, CH-5). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 138.1, 137.8, 137.3 (3C_q Ar), 128.7, 128.6, 128.2, 128.1, 128.0 (15CH Ar), 86.2 (CH-2), 82.5 (CH-1), 74.6 (CH-4), 73.8, 72.8, 72.6 (3CH_2Ph), 71.0 (CH-3), 68.1 (CH_2OBn), 45.2 (CH-5). HRMS: calcd. for $[C_{27}H_{29}N_3NaO_4]^+$ 482.5352; found 482.2047. HRMS: calcd. for $[C_{27}H_{29}N_3NaO_4]^+$ 498.6438; found 498.1784.

*(1S,2R,3S,4S,5R)-2-Azido-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)cyclopentan-1-ol (**22**)*

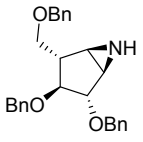


$[\alpha]_D^{20}$ = –29.1 (*c* = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.31 (m, 15H, CH Ar), 4.59 (d, *J* = 11.5 Hz, 1H, C*H*HPh), 4.56 – 4.46 (m, 5H, 2CH_2Ph, C*H*HPh), 4.04 (dd, *J* = 7.4, 5.5 Hz, 1H), 3.87 (dd, *J* = 5.5, 2.7 Hz, 1H, CH-3), 3.79 (dd, *J* = 5.6, 2.7 Hz, 1H, CH-2), 3.67 (dd, *J* =

8.4, 7.4 Hz, 1H, CH-1), 3.53 (d, *J* = 5.4 Hz, 2H, CH_2OBn), 2.03 (dq, *J* = 8.4, 5.4 Hz, 1H, CH-5). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 138.1, 138.0, 137.4 ($3C_q$ Ar), 128.8, 128.6, 128.5, 128.3, 128.1, 128.0, 127.9, 127.8 (15CH Ar), 82.3 (CH-2), 80.8 (CH-1), 75.8 (CH-3), 73.3, 72.2, 72.0 ($3CH_2Ph$), 68.8 (CH_2OBn), 66.8 (CH-4), 48.0 (CH-5). HRMS: calcd. for $[C_{27}H_{29}N_3NaO_4]^+$ 482.5352; found 482.2047. HRMS: calcd. for $[C_{27}H_{29}N_3NaO_4]^+$ 498.6438; found 498.1784.

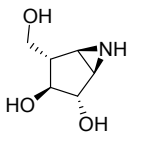
*(1R,2S,3S,4S,5R)-2,3-Bis(benzyloxy)-4-((benzyloxy)methyl)-6-azabicyclo[3.1.0]hexane (**23**)*

First, Et_3N (0.06 mL, 0.446 mmol)) and DMAP (4 mg, 0.036 mmol) were added to a solution of azido alcohols **21** and **22** (82 mg, 0.178 mmol) in DCM (2 mL) at 0 °C and stirred for 15 min. TsCl (45 mg, 0.236 mmol) was added subsequently and the mixture was allowed to warm to rt and stirred overnight. Then H_2O (1 mL) was added and the mixture was extracted with DCM and washed with water. The organic phase was dried ($MgSO_4$), filtered and the solvents were removed under reduced pressure. Flash purification by silica gel column chromatography (0 → 15% EtOAc in pentane) afforded an inseparable mixture of both isomers (55 mg, 0.089mmol, 50%). HRMS: calcd. for $[C_{34}H_{35}N_3NaO_6S]^+$ 636.7182; found 636.2137.



DIPEA (0.04 mL) was added to a suspension of the previous mixture of azido tosylates (55 mg, 0.089 mmol) and triphenylphosphine (82 mg, 0.312 mmol) in 2.5 mL of a mixture of 4:1 THF:H_2O. This was stirred at 60 °C for 1.5 h and then allowed to cool to rt. After removal of THF under reduced pressure, the reaction mixture was diluted with EtOAc (8 mL) and washed with additional H_2O (6 mL). The aqueous phase was extracted with EtOAc (2 × 10 mL) and the combined organic phases were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (0% → 5% MeOH in DCM, then 20% → 100% EtOAc in pentane) afforded the desired aziridine **23** (21 mg, 0.050 mmol, 56%). $[\alpha]_D^{20}$ = +1.6 (*c* = 1, MeOH). ^1H NMR (400 MHz, MeOD) δ = 7.42 – 7.25 (m, 13H, CH Ar), 7.21 (d, *J* = 6.3 Hz, 2H, CH Ar), 4.51 (d, *J* = 11.9 Hz, 1H, C*H*HPh), 4.48 – 4.35 (m, 5H, $2CH_2Ph$, C*H*HPh), 3.92 (s, 1H, CH-3), 3.83 (s, 1H, CH-4), 3.54 (dd, *J* = 9.2, 6.9 Hz, 1H, C*H*HOBn), 3.39 (t, *J* = 9.1 Hz, 1H, C*H*HOBn), 2.58 – 2.50 (m, 2H, CH-1, CH-5), 2.47 (d, *J* = 3.2 Hz, 1H, CH-2). ^{13}C NMR (101 MHz, MeOD) δ = 139.6, 139.3, 139.0 ($3C_q$ Ar), 129.5, 129.4, 129.2, 128.9, 128.8, 128.7 (15CH Ar), 84.6 (CH-4), 84.2 (CH-3), 74.1, 72.5, 72.1 ($3CH_2Ph$), 70.7 (CH_2OBn), 48.1 (CH-5), 39.1 (CH-1), 38.8 (CH-2). HRMS: calcd. for $[C_{27}H_{30}NO_3]^+$ 416.5405; found 416.2217. HRMS: calcd. for $[C_{27}H_{29}NNaO_3]^+$ 438.5222; found 438.2035.

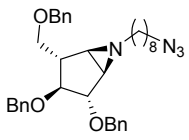
*(1R,2S,3S,4S,5R)-4-(Hydroxymethyl)-6-azabicyclo[3.1.0]hexane-2,3-diol (**2**)*



Lithium (13 mg, 1.87 mmol) was added to a solution of condensed ammonia (4 mL) at –60 °C. Then, a solution of protected aziridine **23** (21 mg, 0.050 mmol) in THF (1 mL) was added dropwise to the resulting dark blue ammonia solution. The mixture was stirred at –60 °C for 1 h, after which several drops of MeOH were added until the dark blue color had disappeared. After adding a few additional drops of H_2O, the ammonia was evaporated by stirring at rt under normal atmosphere in the fume hood. The mixture was then concentrated, dissolved in water and brought to pH 7 with Amberlite 120 H^+. The resin bound to the aziridine was then washed with water (2×) and subsequently eluted with NH_4OH (3x). After filtration, the final aziridine **2** was obtained in 66% yield (4.83 mg, 0.033 mmol). $[\alpha]_D^{20}$ = –41.0 (*c* = 0.2, MeOH). ^1H NMR (500 MHz, D_2O) δ = 4.04 (s, 1H, CH-4), 3.88 (s, 1H, CH-3), 3.66 (d, *J* = 6.7 Hz, 2H, CH_2OH), 2.60 (s, 2H, CH-1, CH-2), 2.22

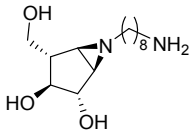
(t, *J* = 6.5 Hz, 1H, CH-5). ^{13}C NMR (126 MHz, D_2O) δ = 80.2 (CH-3), 77.1 (CH-4), 61.6 (CH_2OH), 50.3 (CH-5), 39.3, 37.4 (CH-1, CH-2). HRMS: calcd. for $[C_6H_{12}NO_3]^+$ 146.1655; found 146.0813.

*(1R,2S,3S,4S,5R)-6-(Azidomethyl)-2,3-bis(benzyloxy)-4-((benzyloxy)methyl)-6-azabicyclo[3.1.0]hexane (**24**)*



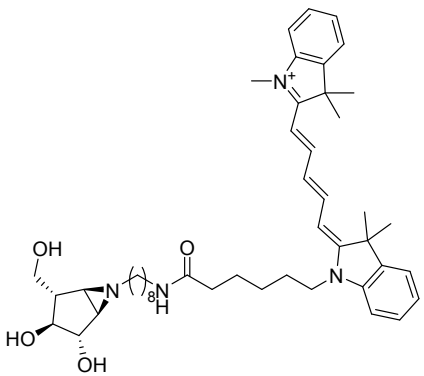
Perbenzylated aziridine **23** (359 mg, 0.864 mmol) was co-evaporated with toluene (3×), subsequently dissolved in DCM (8 mL), and cooled to 0 °C. DIPEA (0.17 mL, 0.950 mmol) and 8-azidooctyl triflate (0.3 M, 5.7 mL, 1.71 mmol) was added dropwise and the resulting mixture was stirred at rt overnight. The reaction mixture was then quenched with MeOH (25 mL) and a saturated $NaHCO_3$ solution (100 mL) was added and extracted with EtOAc (100 mL). The aqueous phase was further extracted with EtOAc (3 × 60 mL) and the organic phase was then washed with brine (100 mL). Subsequently, the combined organic phases were dried over $MgSO_4$, filtered, concentrated, and purified by Silica gel column chromatography (0% → 80% EtOAc in pentane) to afford the desired product **24** in 57% yield as an oil (278 mg, 0.490 mmol). $[\alpha]_D^{20}$ = –9.265 (*c* = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.37 – 7.24 (m, 13H, CH Ar), 7.24 – 7.19 (m, 2H, CH Ar), 4.58 – 4.50 (m, 2H, CH_2Ph), 4.47 (d, *J* = 12.1 Hz, 2H, CH_2Ph), 4.42 – 4.27 (m, 2H, CH_2Ph), 3.86 (s, 1H, CH-3), 3.68 (s, 1H, CH-4), 3.50 – 3.38 (m, 1H, C*H*HOBn), 3.38 – 3.29 (m, 1H, C*H*HOBn), 3.25 (t, *J* = 7.0 Hz, 2H, CH_2N_3), 2.45 (t, *J* = 7.8 Hz, 1H, CH-5), 2.34 – 2.11 (m, 2H, CH_2N), 2.04 (s, 2H, CH-1, CH-2), 1.65 – 1.49 (m, 4H, $2CH_2$), 1.39 – 1.25 (m, 8H, $4CH_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 128.5, 128.4, 128.2, 127.8, 127.7, 127.6 (15CH Ar), 88.1 (CH-4), 85.2 (CH-3), 73.2, 71.4, 71.2 ($3CH_2Ph$) 70.6 (CH_2OBn), 59.2 (CH_2N), 51.6 (CH_2N_3), 47.3 (CH-5), 47.0 (CH-1, CH-2), 29.8, 29.6, 29.2, 29.0, 27.5, 26.8 ($6CH_2$).

*(1R,2S,3S,4S,5R)-6-(Aminomethyl)-4-(hydroxymethyl)-6-azabicyclo[3.1.0]hexane-2,3-diol (**3**)*



Sodium (303 mg, 13.2 mmol) was dissolved in condensed ammonia (9 mL) at –60 °C and a solution of protected aziridine **24** (250 mg, 0.440 mmol) and *t*-BuOH (0.42 mL, 4.40 mmol) in THF (2.2 mL) was added dropwise to the resulting dark blue ammonia solution. The mixture was stirred at –60 °C for 1 h, after which drops of MeOH were added until the dark blue color had disappeared. After adding a few drops of H_2O, the ammonia was removed by stirring at rt under normal atmosphere in the fume hood. The mixture was then concentrated, dissolved in water and brought to pH 7 with Amberlite 120 H^+. The aziridine bound to the resin was then washed with water (2×) through a filter and subsequently the aziridine was eluted with NH_4OH (8×). The filtrate was then concentrated to afford the title compound **3** in 95% yield (114 mg, 0.43 mmol). $[\alpha]_D^{20}$ = 2.20 (*c* = 1, MeOH). ^{1}H NMR (500 MHz, MeOD) δ = 3.84 (s, 1H, CH-4), 3.63 – 3.58 (m, 2H, C*H*$_2$OH), 3.57 (s, 1H, CH-3), 2.70 – 2.57 (m, 2H, CH_2NH_2), 2.33 – 2.25 (m, 3H, CH_2N, CH-1/2), 2.23 (d, *J* = 5.4 Hz, 1H, CH-1/2), 2.12 (t, *J* = 6.4 Hz, 1H, CH-5), 1.58 – 1.50 (m, 2H, CH_2), 1.49 – 1.42 (m, 2H, CH_2), 1.44 – 1.30 (m, 8H, $4CH_2$). ^{13}C NMR (126 MHz, MeOD) δ = 81.6 (CH-3), 77.6 (CH-4), 62.4 (CH_2OH), 58.5 (CH_2N), 52.5 (CH-5), 48.4 (CH-1/2), 46.3 (CH-1/2), 42.6 (CH_2NH_2), 33.9, 33.6, 30.7, 30.5, 28.3, 28.0 ($6CH_2$). LCMS: calcd. for $[C_{14}H_{29}N_2O_3]^+$ 273.3965; found 273.1071.

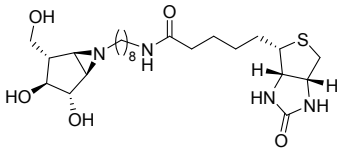
*2-((1E,3E)-5-((E)-1-(6-((((1R,2S,3S,4S,5R)-2,3-Dihydroxy-4-(hydroxymethyl)-6-azabicyclo[3.1.0]hexan-6-yl)methyl)amino)-6-oxohexyl)-3,3-dimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-1,3,3-trimethyl-3H-indol-1-ium (**4**)*



A solution of aziridine **3** (8.0 mg, 0.029 mmol), DIPEA (6.2 µL, 0.035 mmol) and Cy5-OSu ester (18.7 mg, 0.032 mmol) in DMF

(2mL) was stirred for 24 h under an argon atmosphere. Then, the starting material was consumed (checked by LC/MS), and the reaction mixture was concentrated under reduced pressure and purified by semi-preparative reversed HPLC (linear gradient: 58%→61% B in A, 12min; solutions used A: 50mM NH_4HCO_3 in H_2O; B: acetonitrile), the fractions were concentrated and lyophilized to afford final Cy5 probe **4** in 56% yield as a blue solid (12.7 mg, 0.016 mmol). ^{1}H NMR (600 MHz, MeOD) δ = 8.25 (t, *J* = 13.1 Hz, 2H, 2CH Ar), 7.50 (d, *J* = 7.4 Hz, 2H, 2CH Ar), 7.45 – 7.37 (m, 2H, 2CH Ar), 7.33 – 7.26 (m, 4H, 2CH Ar, 2CH=C*H*), 6.63 (t, *J* = 12.4 Hz, 1H, CH=C*H*), 6.28 (dd, *J* = 13.7, 7.5 Hz, 2H, 2CH=C*H*), 5.50 (s, 1H, CH), 4.11 (t, *J* = 7.4 Hz, 2H, CH_2C=O), 3.83 (s, 1H, CH-4), 3.63 (s, 3H, CH_3), 3.59 (dd, *J* = 8.5, 6.5 Hz, 2H, CH_2OH), 3.56 (s, 1H, CH-3), 3.12 (t, *J* = 7.2 Hz, 2H, CH_2), 2.28 (d, *J* = 4.0 Hz, 1H, CH-1), 2.25 (t, *J* = 6.9 Hz, 2H, CH_2), 2.23 – 2.21 (m, 1H, CH-2), 2.21 – 2.18 (m, 2H, CH_2), 2.11 (t, *J* = 6.5 Hz, 1H, CH-5), 1.94 (s, 6H, 2CH_3), 1.85 – 1.80 (m, 2H, CH_2), 1.75 (s, 6H, 2CH_3), 1.73 – 1.68 (m, 2H, CH_2), 1.54 – 1.51 (m, 2H, 2CH_2), 1.50 – 1.45 (m, 4H, 2CH_2), 1.38 – 1.26 (m, 6H, 3CH_2). ^{13}C NMR (214 MHz, MeOD) δ = 177.3 (C=O), 174.3, 173.3 (2C_q), 154.1 (CH Ar), 142.8, 142.2, 141.2, 141.1 (C_q Ar), 129.5, 128.4, 125.2, 124.9, 124.8, 122.0, 121.9, 110.6, 110.4, 103.0, 102.9 (12CH Ar/CH=CH), 80.1 (CH-3), 76.2 (CH-4), 61.0 (CH_2OH), 57.0 (CH_2), 51.1 (CH-5), 46.9 (CH-2), 44.9 (CH-1), 43.4, 39.0, 35.3 (3CH_2), 30.0 (CH_3N^+), 29.3, 29.2, 29.0, 28.9, 26.9, 26.8 (6CH_2), 26.6 (CH_3), 26.5 (CH_3), 26.4, 26.0, 25.2 (3CH_2), 20.7 (2CH_3). LCMS: calcd. for $[C_{46}H_{65}N_4O_4]^+$ 737.5000; found 737.53.

*N-(((1R,2S,3S,4S,5R)-2,3-Dihydroxy-4-(hydroxymethyl)-6-azabicyclo[3.1.0]hexan-6-yl)methyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (**5**)*

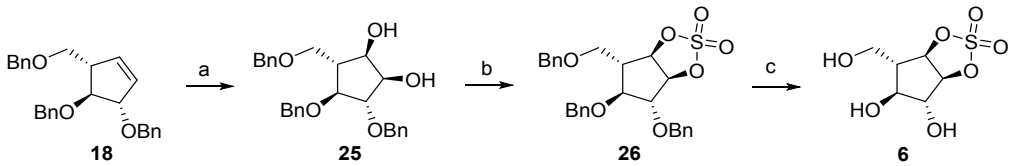


Aziridine **3** (7.5 mg, 0.028 mmol), biotin-Osu ester (13 mg, 0.038 mmol), and DIPEA (5.8 µL, 0.033 mmol) were dissolved in DMF (1.4 mL), and the resulting solution was protected from light and stirred overnight at rt. The reaction mixture was then concentrated and purified by HPLC-MS. After semi-preparative reversed HPLC (linear gradient: 22%→34% B in A, 12min, solutions used A: 50 mM NH_4HCO_3 in H_2O, B: acetonitrile), the fractions were concentrated and lyophilized to afford the desired biotinylated probe **5** in 19% yield as a white solid product (2.66 mg, 0.005 mmol). ^{1}H NMR (850 MHz, MeOD) δ = 4.53 – 4.41 (m, 1H, CH biotin), 4.30 (dd, *J* = 7.9, 4.4 Hz, 1H, CH biotin), 3.84 (s, 1H, CH-3/4), 3.64 – 3.53 (m, 2H, CH_2OH), 3.57 (s, 1H, CH-3/4), 3.23 – 3.18 (m, 1H, CH biotin), 3.16 (ddd, *J* = 13.3, 10.8, 6.3 Hz, 2H, CH_2C=O), 2.93 (dd, *J* = 12.8, 5.0 Hz, 1H, C*H*HS), 2.73 – 2.68 (m, 1H, C*H*HS), 2.29 (d, *J* = 3.3 Hz, 1H, CH-1/2), 2.28 (td, *J* = 7.0, 2.0 Hz, 2H, CH_2), 2.24 (dd, *J* = 4.0, 1.5 Hz, 1H, CH-1/2), 2.19 (td, *J* = 7.4, 2.1 Hz, 2H, CH_2), 2.13 – 2.11 (m, 1H, CH-5), 1.74 (ddt, *J* = 13.6, 9.1, 6.1 Hz, 1H, C*H*H), 1.70 – 1.64 (m, 2H, CH_2), 1.64 – 1.55 (m, 1H, C*H*H), 1.52 (dd, *J* = 16.3, 9.2 Hz, 2H, CH_2), 1.50 – 1.46 (m, 2H, CH_2), 1.44 (dt, *J* = 16.0, 8.0 Hz, 2H, CH_2), 1.38 – 1.26 (m, 8H, 4CH_2). ^{13}C NMR (214 MHz, MeOD) δ = 176.0 (C=O biotin), 166.1 (C=O), 81.5 (CH-3/4), 77.6 (CH-3/4), 63.4 (CH), 62.4 (CH_2OH), 61.6 (CH), 58.5 (CH_2), 57.0 (SCH), 52.5 (CH-5), 48.3 (CH-1/2), 46.3 (CH-1/2), 41.1, 40.4, 36.8, 30.7, 30.6, 30.4, 30.4, 29.8, 29.5, 28.4, 27.9, 27.0 (12CH_2). LCMS: calcd. for $[C_{24}H_{43}N_4O_5S]^+$ 499.2949; found 499.27.

Synthesis of α-L-*arabino*-cyclosulfate 6

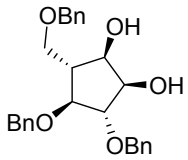
The synthesis of irreversible α-L-arabinofuranose configured cyclic sulfate **6** (Scheme 4) started from benzylated cyclopentene **18** which was oxidized with a mixture of $NaIO_4$ and $RuCl_3 \cdot 3H_2O$ affording

exclusively cis-α-L-diol **25** in 48% yield. Diol **25** was then treated with thionyl chloride and triethylamine and the sulfite mixture were further oxidized to the cyclic sulfate **26**. Benzyls were finally removed by hydrogenation using Pearson's catalyst to afford final cyclosulfate **6** in 24% yield.



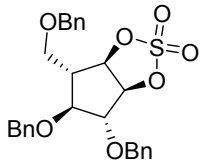
Scheme 4. Reagents and conditions towards cyclosulfate **3**. a) $NaIO_4$, $RuCl_3 \cdot 3\ H_2O$, EtOAc/CH_3CN/H_2O, 0 °C, 3 h, 48%. b) i) $SOCl_2$, Et_3N, DCM, 0 °C, 30 min. ii) $NaIO_4$, $RuCl_3 \cdot 3H_2O$, EtOAc/CH_3CN/H_2O, 0 °C, 3 h, 51% c) H_2, $Pd(OH)_2$, MeOH, 18 h, 24%.

*(1R,2R,3S,4S,5R)-3,4-Bis(benzyloxy)-5-((benzyloxy)methyl)cyclopentane-1,2-diol (**25**)*



A solution of $NaIO_4$ (247 mg, 1.16 mmol) and $RuCl_3 \cdot 3H_2O$ (24.7 mg, 0.09 mmol) in 6 mL water was added slowly to a vigorously stirring solution of benzylated cyclopentene **18** (306 mg, 0.76 mmol) in a mixture of EtOAc and acetonitrile (1:1, 23 mL) at 0 °C. After 3 h, $Na_2S_2O_3$ (0.1 M, 30 mL) was added and the mixture was extracted with EtOAc (3x). The combined organic phases were washed with brine and water, dried ($MgSO_4$), filtered, concentrated and purified by silica gel column chromatography (0% → 60% EtOAc in pentane) to afford diol **25** in 48% yield (161 mg, 0.37 mmol). $[\alpha]_D^{20}$ = –6.00 (*c* = 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.38 – 7.03 (m, 15H, CH Ar), 4.65 (d, *J* = 11.7 Hz, 1H, C*H*HPh), 4.58 – 4.51 (m, 2H, CH_2Ph), 4.50 – 4.42 (m, 3H, CH_2Ph, C*H*HPh), 3.94 (dd, *J* = 6.6, 2.6 Hz, 3H, CH-1, CH-2, CH-3), 3.69 (dd, *J* = 7.6, 4.7 Hz, 1H, CH-4), 3.59 (dd, *J* = 9.2, 4.9 Hz, 1H, C*H*HOBn), 3.47 (dd, *J* = 9.2, 6.5 Hz, 1H, C*H*HOBn), 3.41 (d, *J* = 7.5 Hz, 1H, OH), 3.33 (s, 1H, OH), 2.32 (qd, *J* = 6.8, 4.7 Hz, 1H, CH-5). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 138.2, 138.1, 138.0 ($3C_q$ Ar), 128.4, 128.3, 127.9, 127.7, 127.6 (15CH Ar), 88.7 (CH-1/2), 82.1 (CH-4), 74.8 (CH-1/2), 73.3 (CH_2Ph), 72.8 (CH-3), 72.0, 71.8 ($2CH_2$Ph), 70.0 (CH_2OBn), 48.7 (CH-5). HRMS: calcd. for $[C_{27}H_{30}NaO_5]^+$ 457.5212; found 457.1979. HRMS: calcd. for $[C_{27}H_{30}KO_5]^+$ 473.6298; found 473.1908.

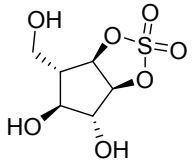
*(3aS,4R,5S,6S,6aR)-4,5-Bis(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-4H-cyclopenta[d][1,3,2] dioxathiole 2,2-dioxide (**26**)*



$SOCl_2$ (0.05 mL) was added dropwise to a solution of diol **25** (77 mg, 0.177 mmol) and Et_3N (0.11 mL) in DCM (9 mL) at 0 °C. After 40 min, the mixture was diluted with Et_2O (9 mL) and washed with H_2O (20 mL) and brine (18 mL). The organic phase was washed, dried ($MgSO_4$) and concentrated. The resulting crude was co-evaporated with toluene twice and re-dissolved in a mixture of ACN (7.5 mL) and CCl_4 (7.5 mL), and cooled to 0 °C. A solution of $RuCl_3 \cdot 3H_2O$ (5 mg, 0.018 mmol) and $NaIO_4$ (79 mg, 0.368 mmol) in water (14 mL) was added slowly and the reaction mixture was stirred for 3 h. The reaction mixture was extracted with Et_2O and washed with brine (2x). The aqueous phases were extracted again with Et_2O, and the combined organic fractions were dried ($MgSO_4$), concentrated under reduced pressure and purified by silica gel flash column chromatography (0% → 20% EtOAc in pentane) to afford the pure perbenzylated cyclic sulfate **26** in 51% yield (50,7 mg, 0.102 mmol).$[\alpha]_D^{20}$ = –19.0 (*c* = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.41 – 7.27 (m, 11H, CH Ar), 7.26 – 7.20 (m, 4H, CH Ar), 5.24 – 5.13 (m, 1H, CH-1), 4.96 (dd, *J* = 7.9, 4.9 Hz, 1H, CH-2), 4.73 (dd, *J* = 11.5, 10.0 Hz, 2H, CH_2Ph), 4.58 (d, *J* = 11.6 Hz, 1H, C*H*HPh), 4.53 (d, *J* = 11.6 Hz, 1H, C*H*HPh), 4.45 (d, *J* = 11.8 Hz, 1H, C*H*HPh), 4.36 (d, *J* = 11.8 Hz, 1H,

C*H*HPh), 4.32 (dd, *J* = 7.8, 4.9 Hz, 1H, CH-3), 3.94 (dd, *J* = 10.2, 7.9 Hz, 1H, CH-4), 3.60 (dd, *J* = 9.8, 3.0 Hz, 1H, C*H*HOBn), 3.52 (dd, *J* = 9.8, 3.4 Hz, 1H, C*H*HOBn), 2.55 (ddt, *J* = 10.0, 6.5, 3.1 Hz, 1H, CH-5). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 137.8, 137.6, 136.7 ($3C_q$ Ar), 128.7, 128.6, 128.5, 128.4, 128.2, 128.1, 127.9 (15CH Ar), 87.1 (CH-3), 85.3 (CH-2), 80.4 (CH-1), 78.0 (CH-4), 73.4, 73.2, 72.8 ($3CH_2$Ph), 65.2 (CH_2OBn), 47.6 (CH-5). HRMS: calcd. for $[C_{27}H_{28}NaO_7S]^+$ 519.5632; found 519.1447. HRMS: calcd. for $[C_{27}H_{28}KO_7S]^+$ 535.6718; found 535.1184 .

(3a*S*,4*R*,5*S*,6*S*,6a*R*)-4,5-Dihydroxy-6-(hydroxymethyl)tetrahydro-4*H*-cyclopenta[*d*][1,3,2]dioxathiole 2,2-dioxide (6)

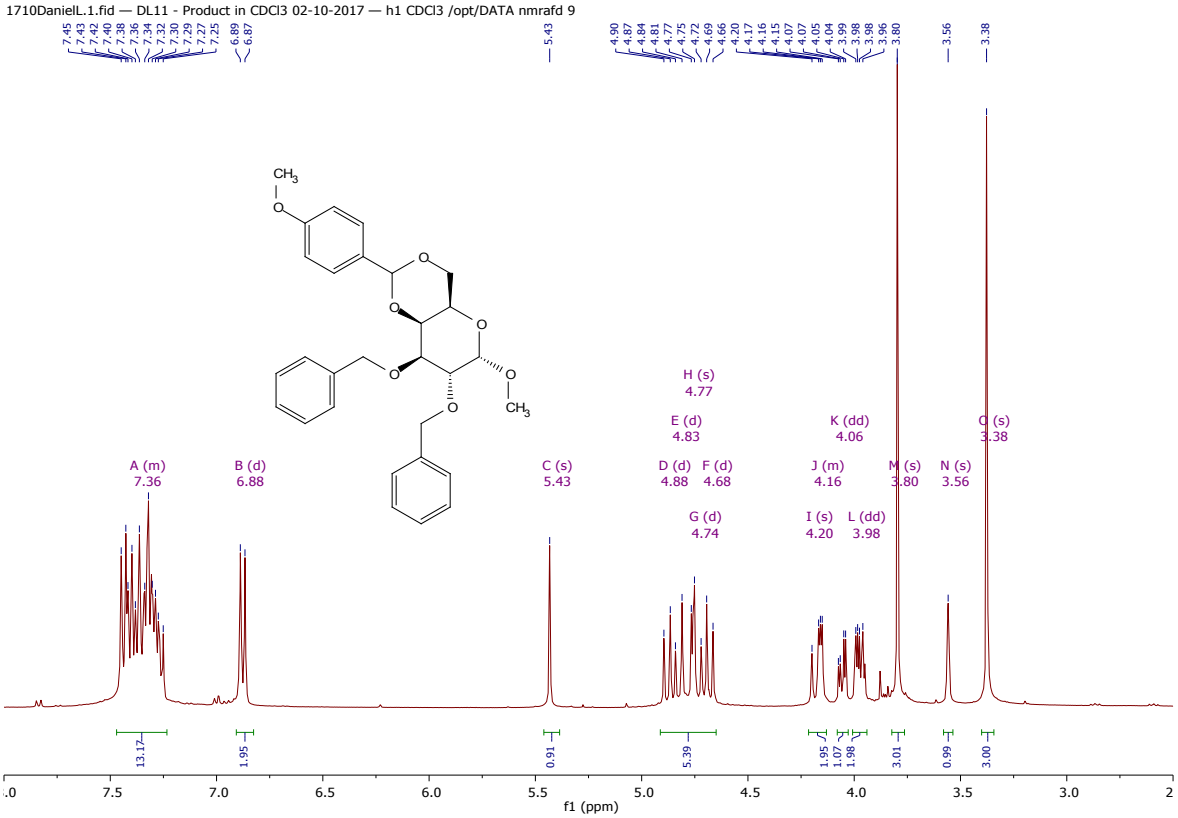


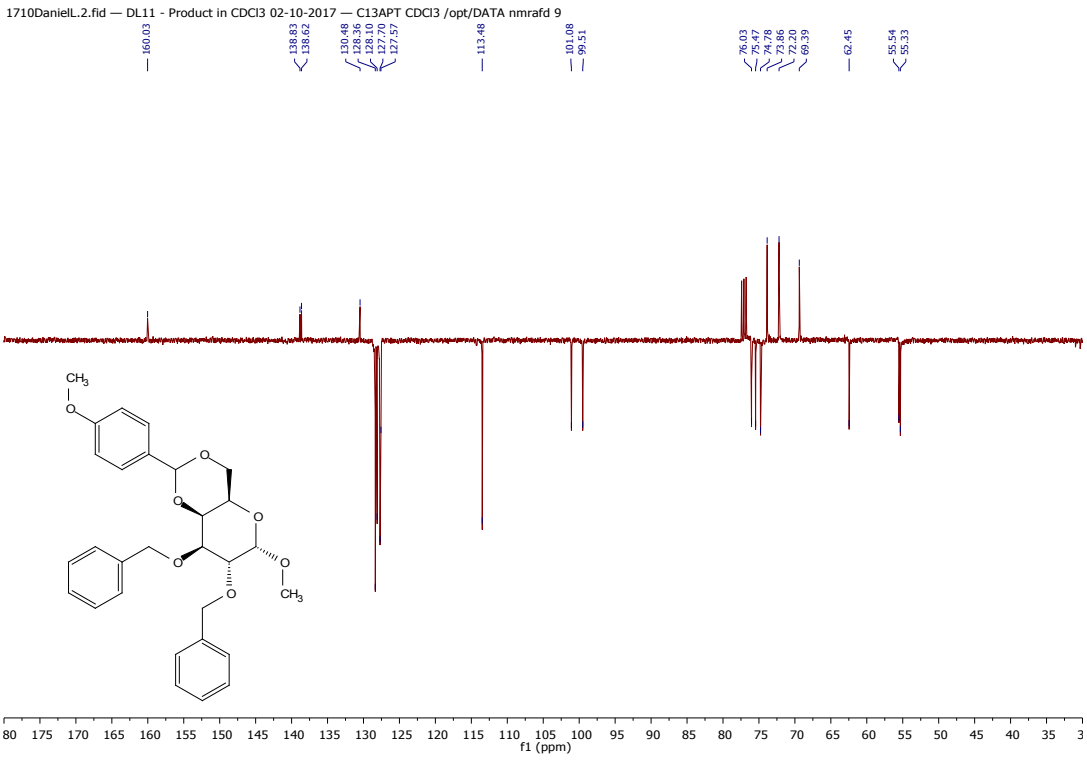
3

Benzylated cyclosulfate **26** (45 mg, 0.090 mmol) was dissolved in MeOH (6.0 mL) and thoroughly degassed with argon. $Pd(OH)_2$ (20% on carbon, 19 mg, 0.027 mmol) was added to the solution and hydrogenated with a H_2 balloon at rt overnight. The reaction mixture was bubbled with argon and filtered over Celite®, and the celite was washed several times with MeOH. The filtrate was concentrated under reduced pressure and purified by silica gel column chromatography (0% → 15% MeOH in DCM) to afford final arabinofuranose-configured cyclosulfate **6** in 24% yield (4.98 mg, 0.022 mmol). $[\alpha]_D^{20}$ = –14.0 (*c* = MeOH). ^{1}H NMR (500 MHz, MeOD) δ = 5.17 (dd, *J* = 8.0, 6.3 Hz, 1H, CH-1), 4.92 (dd, *J* = 8.0, 5.9 Hz, 1H, CH-2), 4.17 (dd, *J* = 8.6, 5.8 Hz, 1H, CH-3), 3.81 (dd, *J* = 11.3, 3.7 Hz, 1H, C*H*HOH), 3.71 – 3.68 (m, 1H, C*H*HOH), 3.68 – 3.65 (m, 1H, CH-4), 2.29 (dtd, *J* = 9.8, 6.0, 3.7 Hz, 1H, CH-5). ^{13}C NMR (126 MHz, MeOD) δ = 87.8 (CH-2), 82.6 (CH-1), 81.4 (CH-3), 73.6 (CH-4), 59.8 (CH_2), 51.8 (CH-5). HRMS: calcd. for $[C_6H_{10}NaO_7S]^+$ 249.1882; the exact mass cannot be detected.

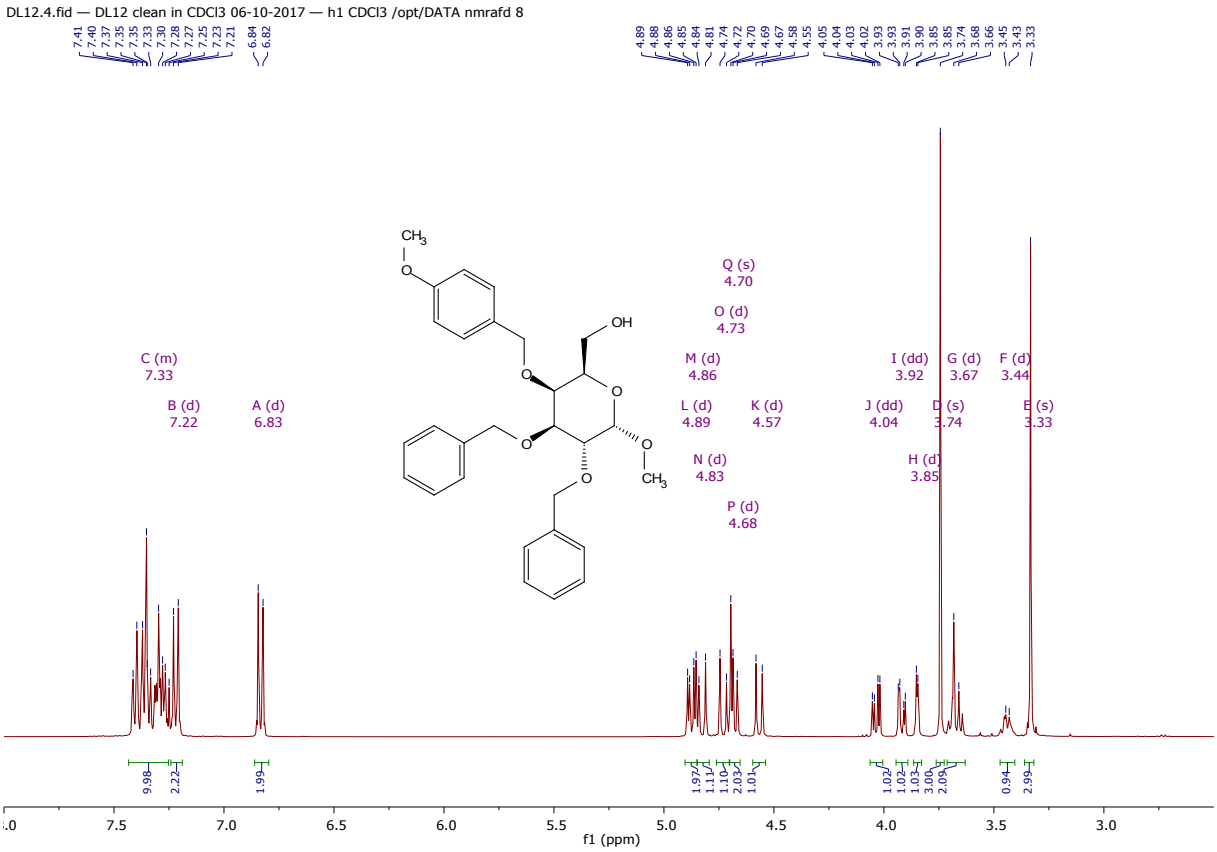
NMR Spectra

^{1}H-NMR and ^{13}C-NMR spectra of **7** in $CDCl_3$

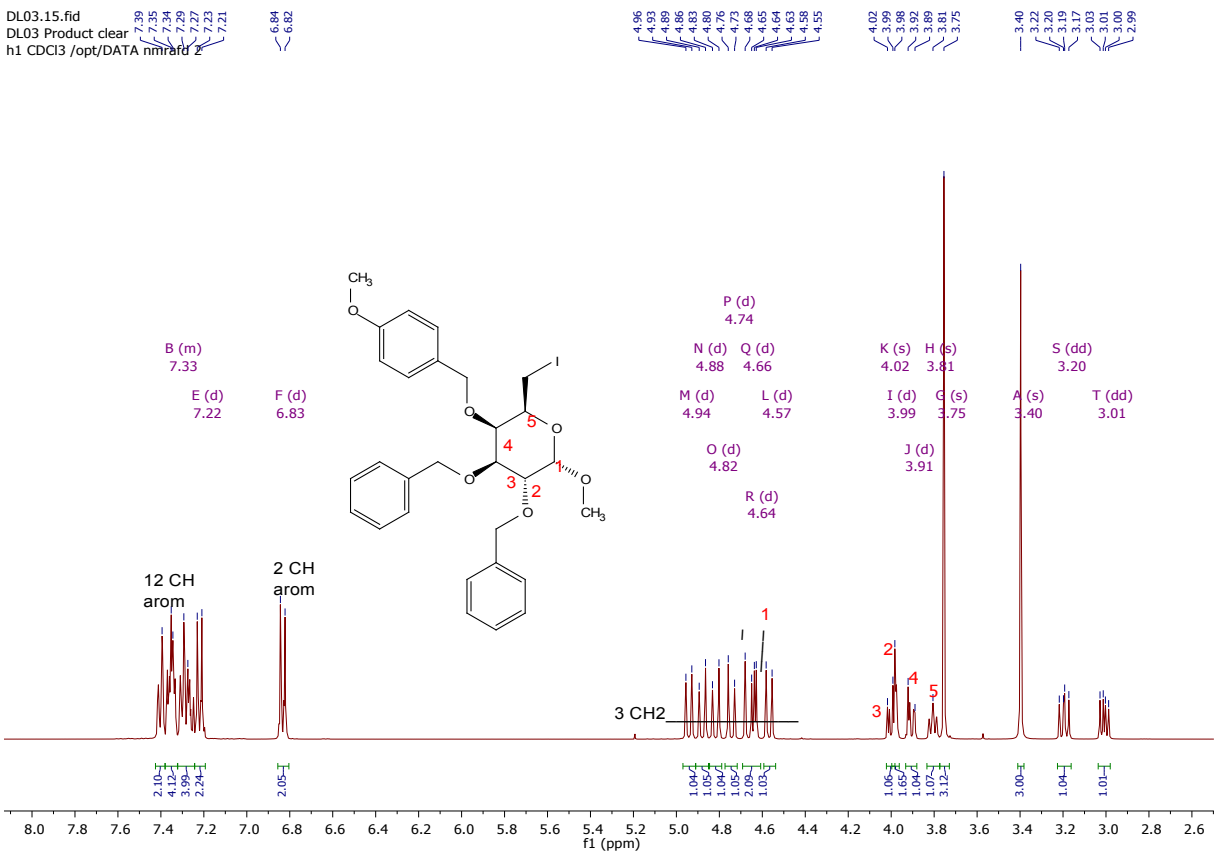


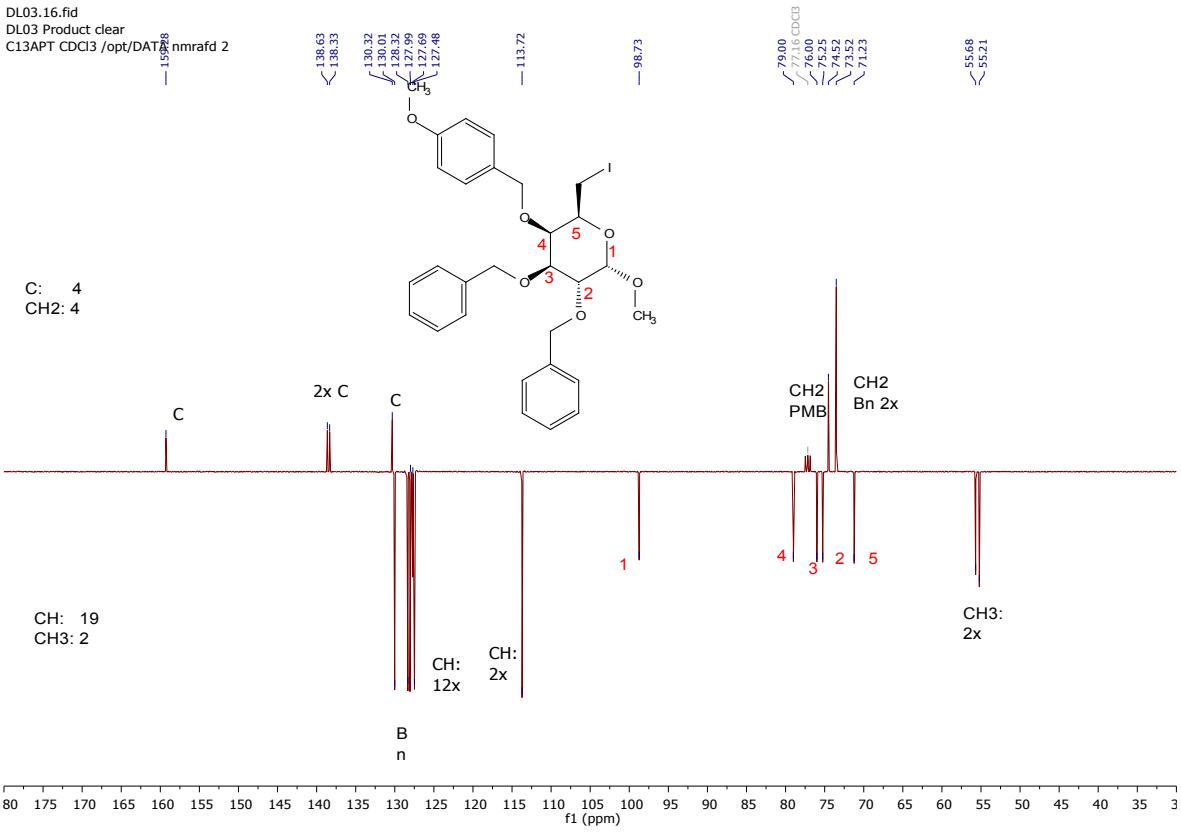


^{1}H-NMR and ^{13}C-NMR spectra of **8** in $CDCl_3$

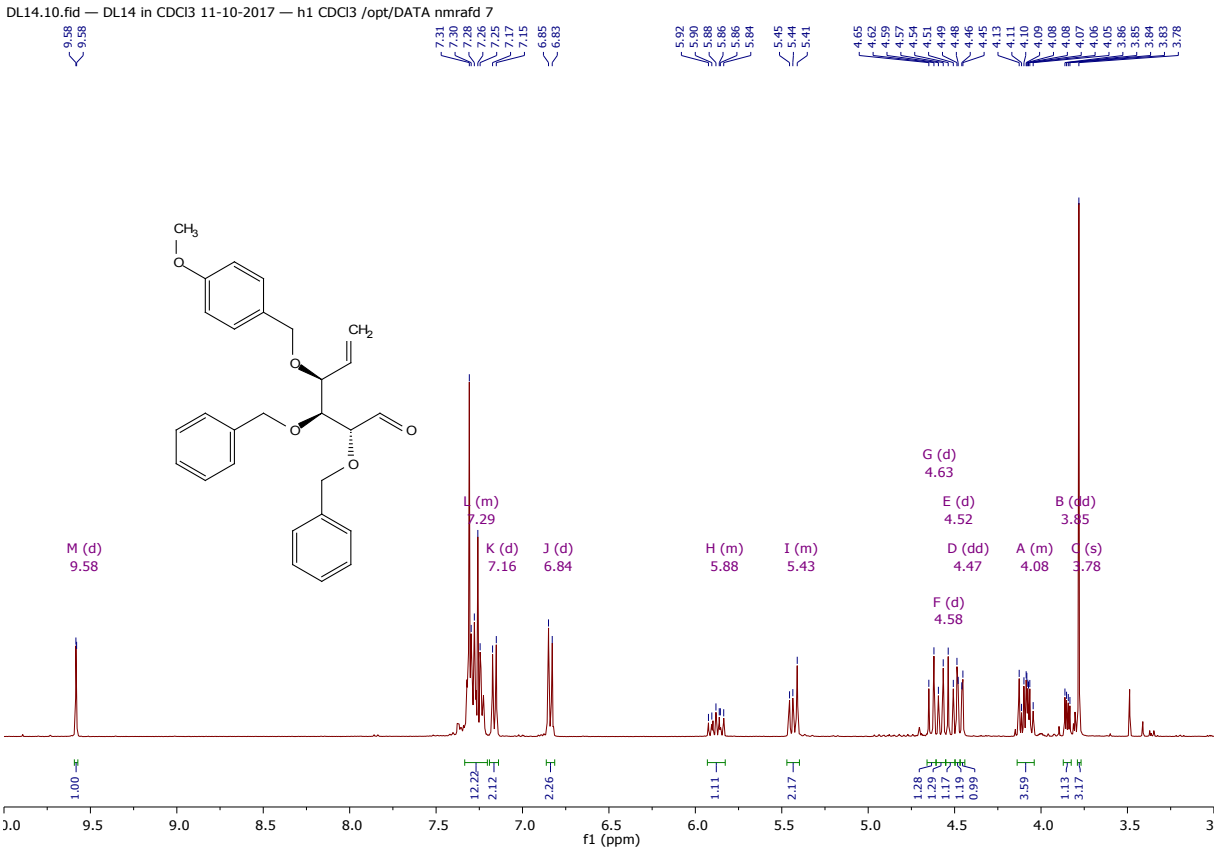


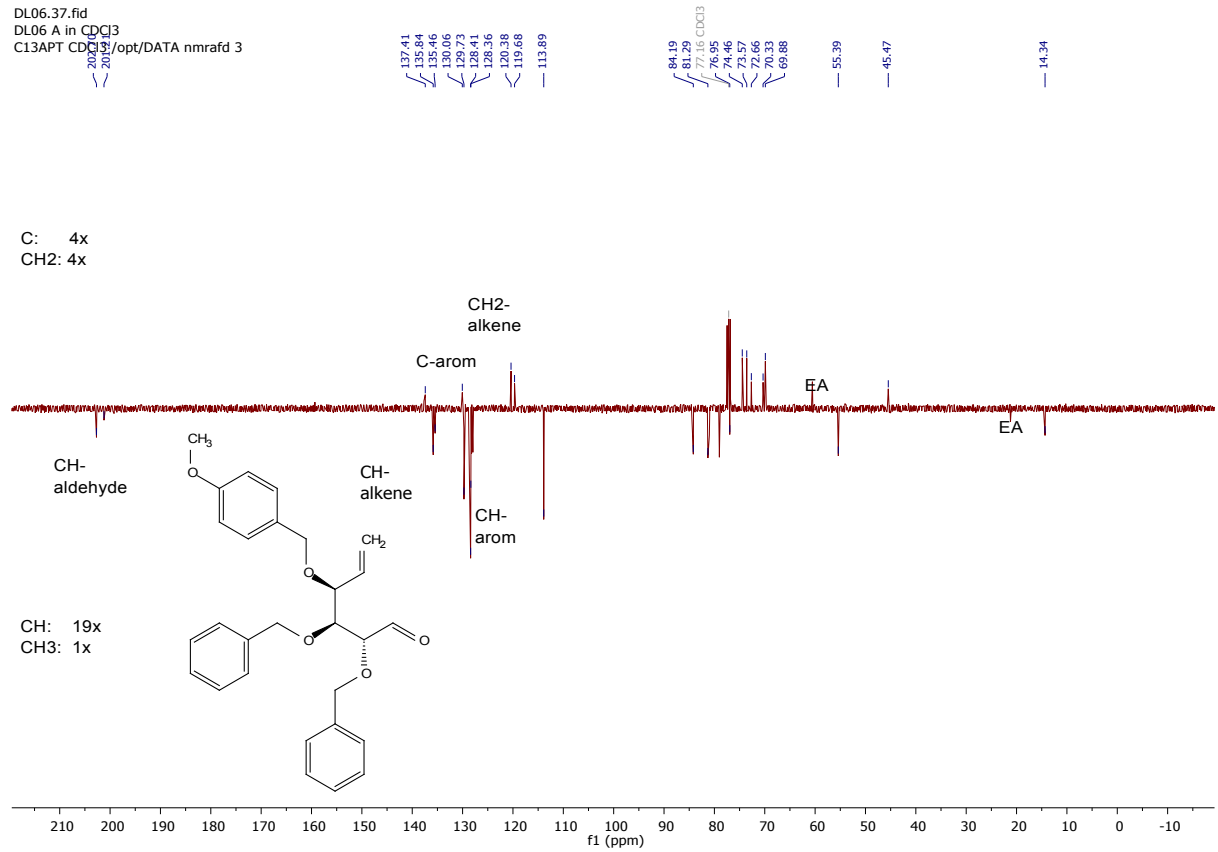
^{1}H-NMR and ^{13}C-NMR spectra of **9** in $CDCl_3$



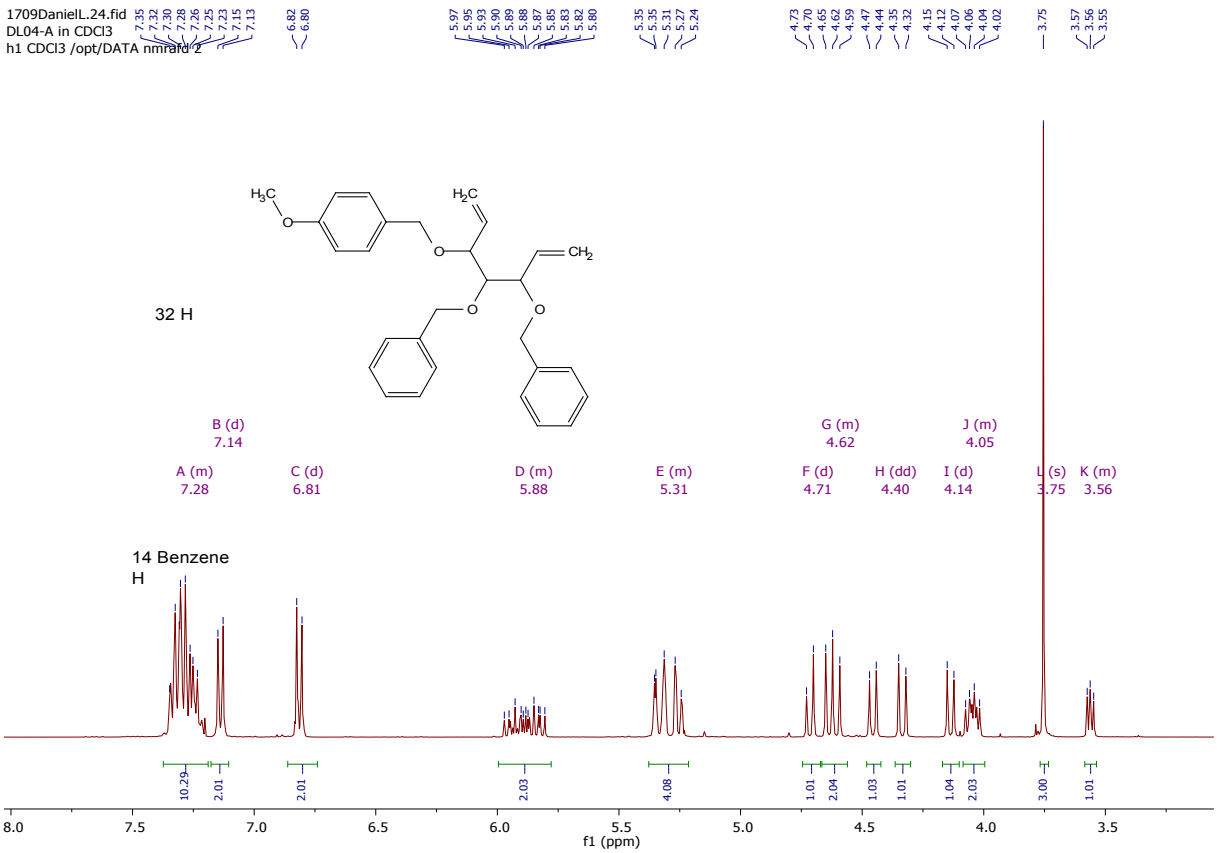


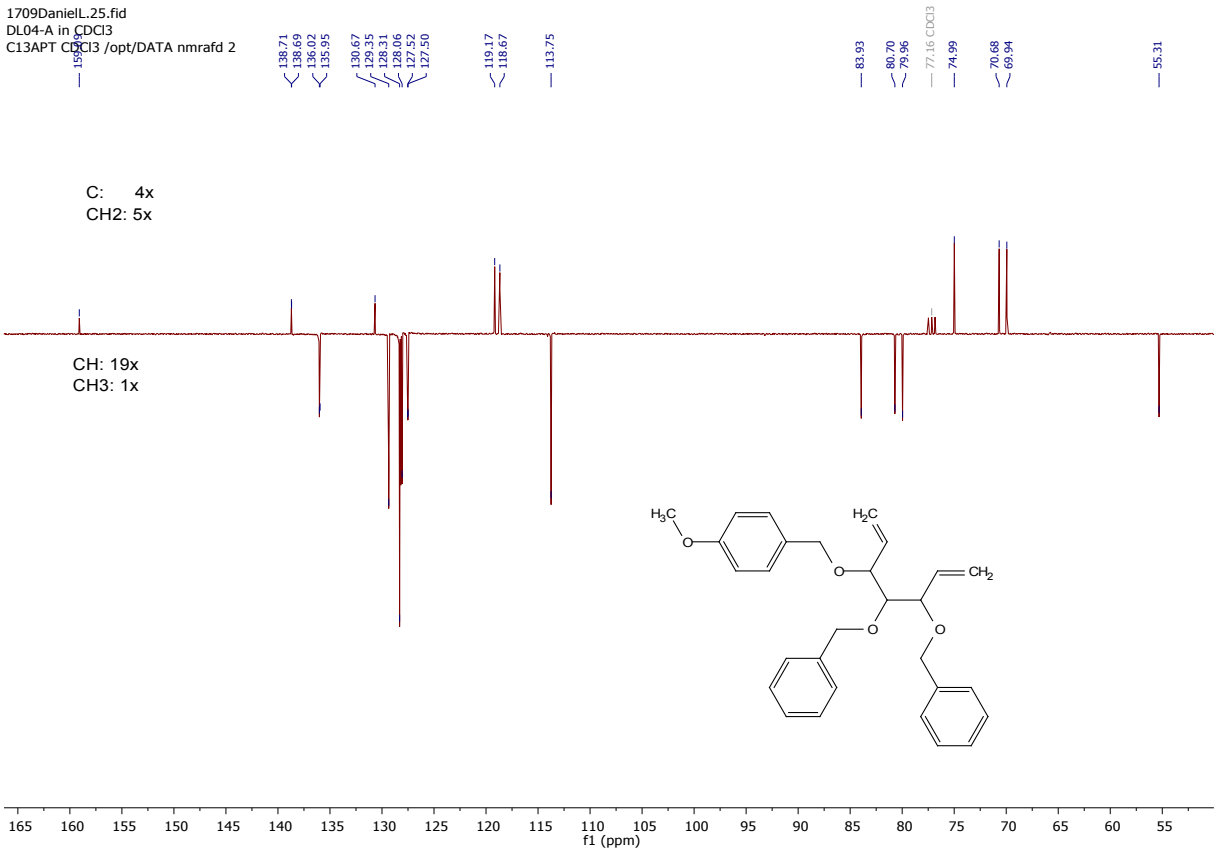
^{1}H-NMR and ^{13}C-NMR spectra of **10** in $CDCl_3$



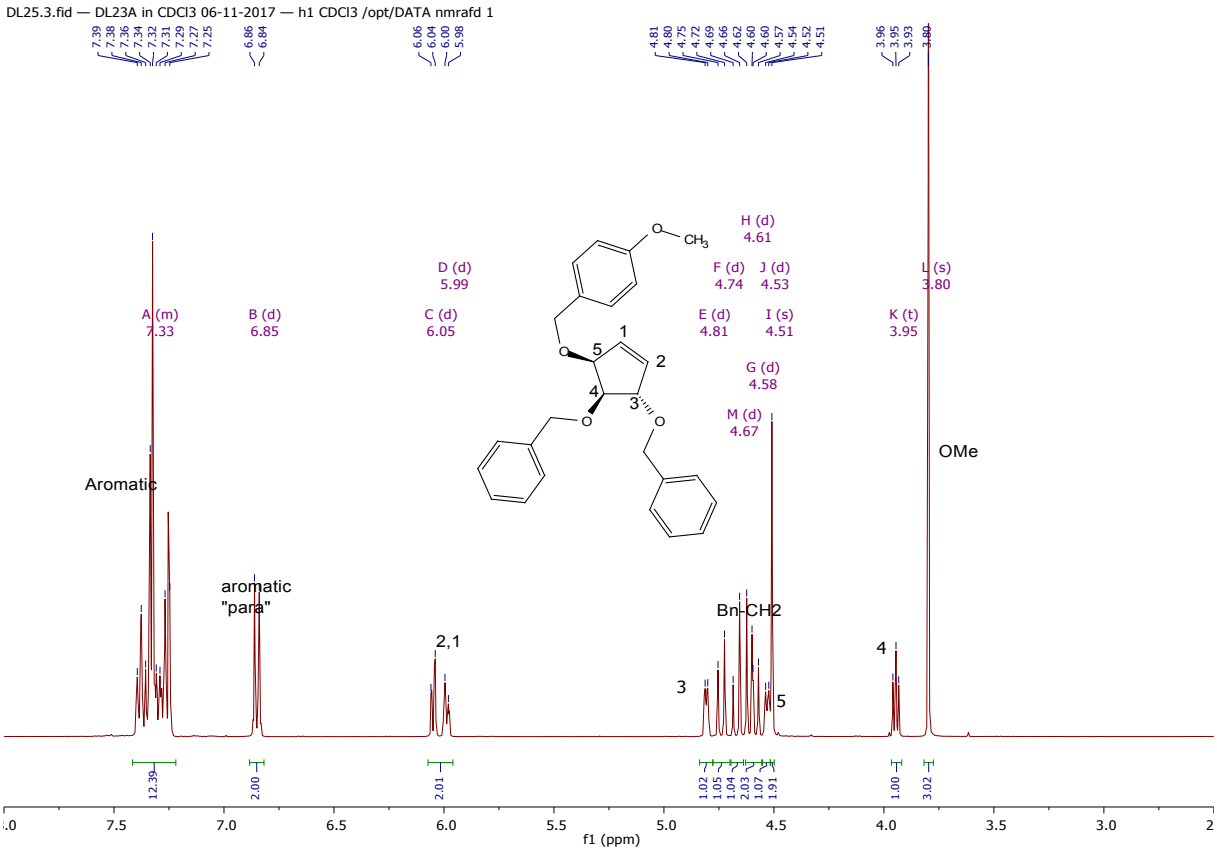


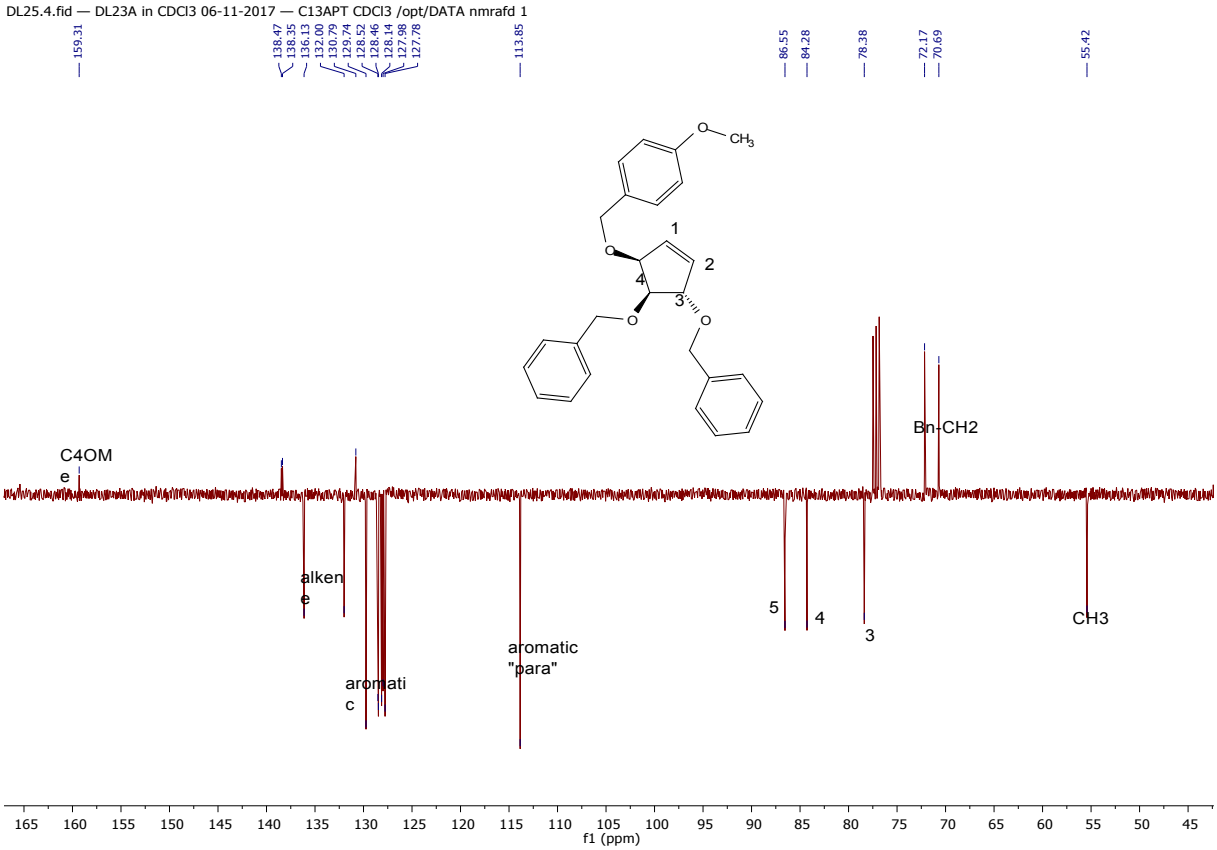
^{1}H-NMR and ^{13}C-NMR spectra of **11** in $CDCl_3$



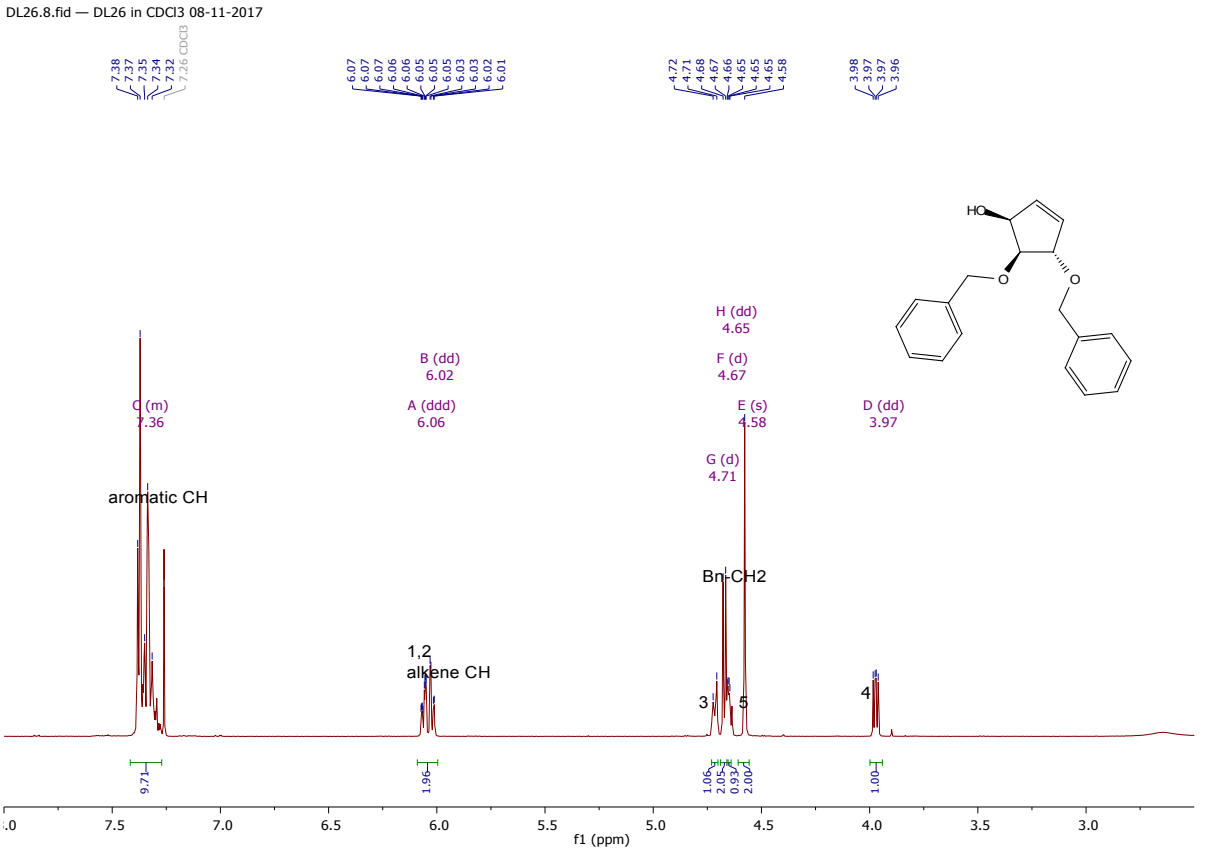


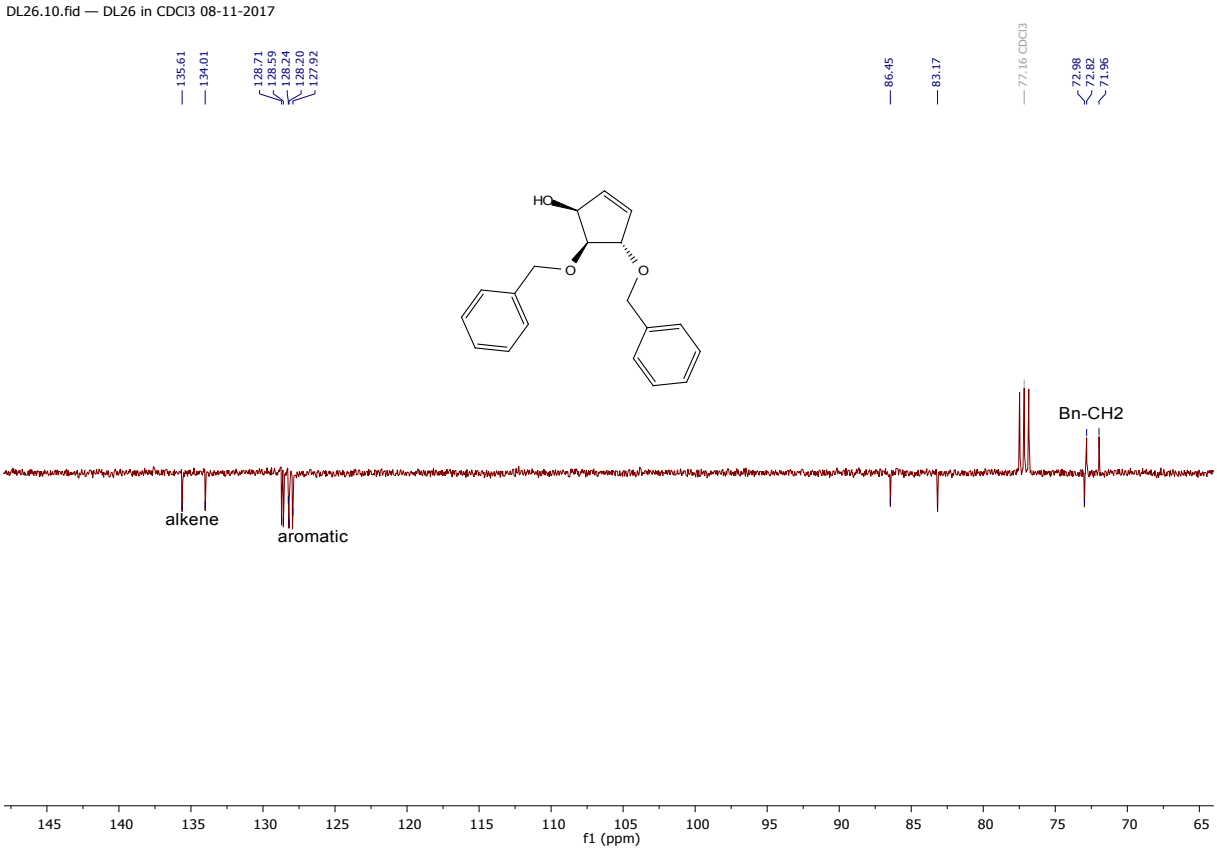
^{1}H-NMR and ^{13}C-NMR spectra of **12** in $CDCl_3$



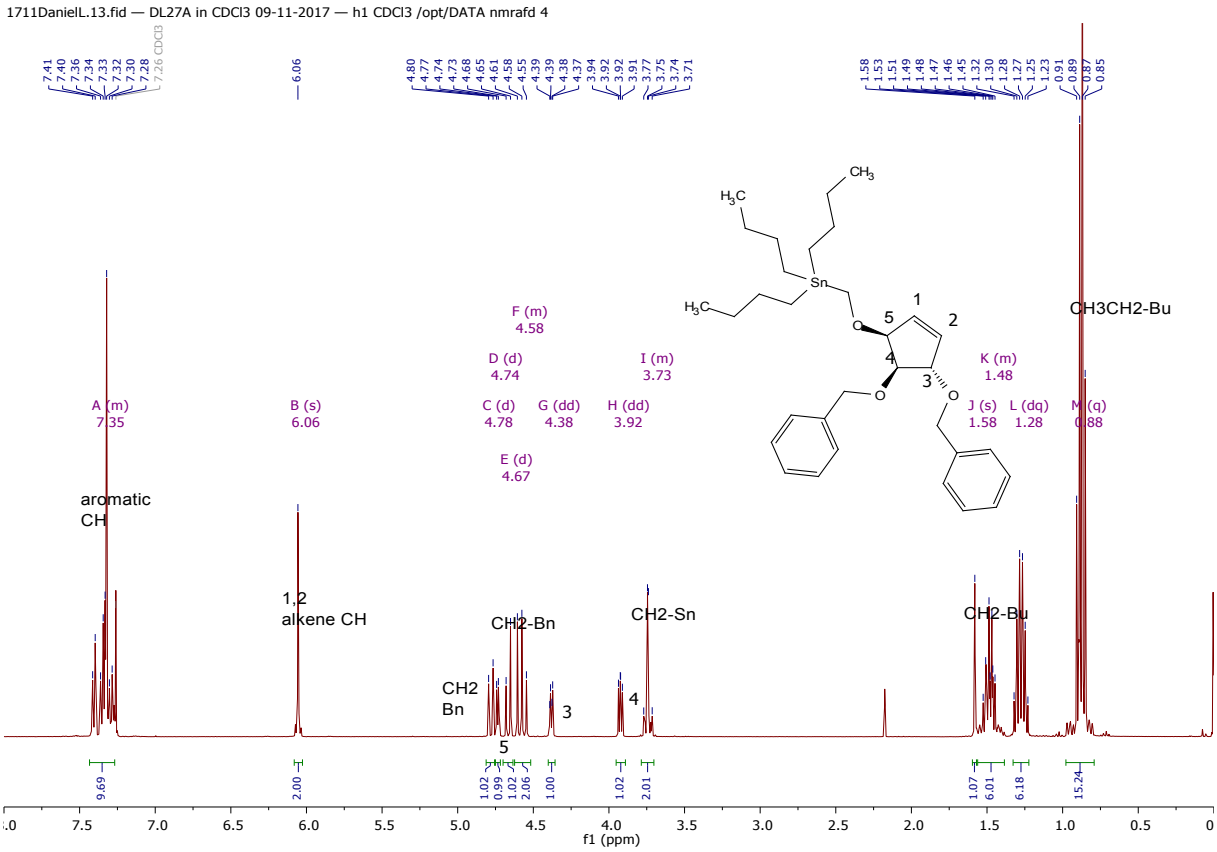


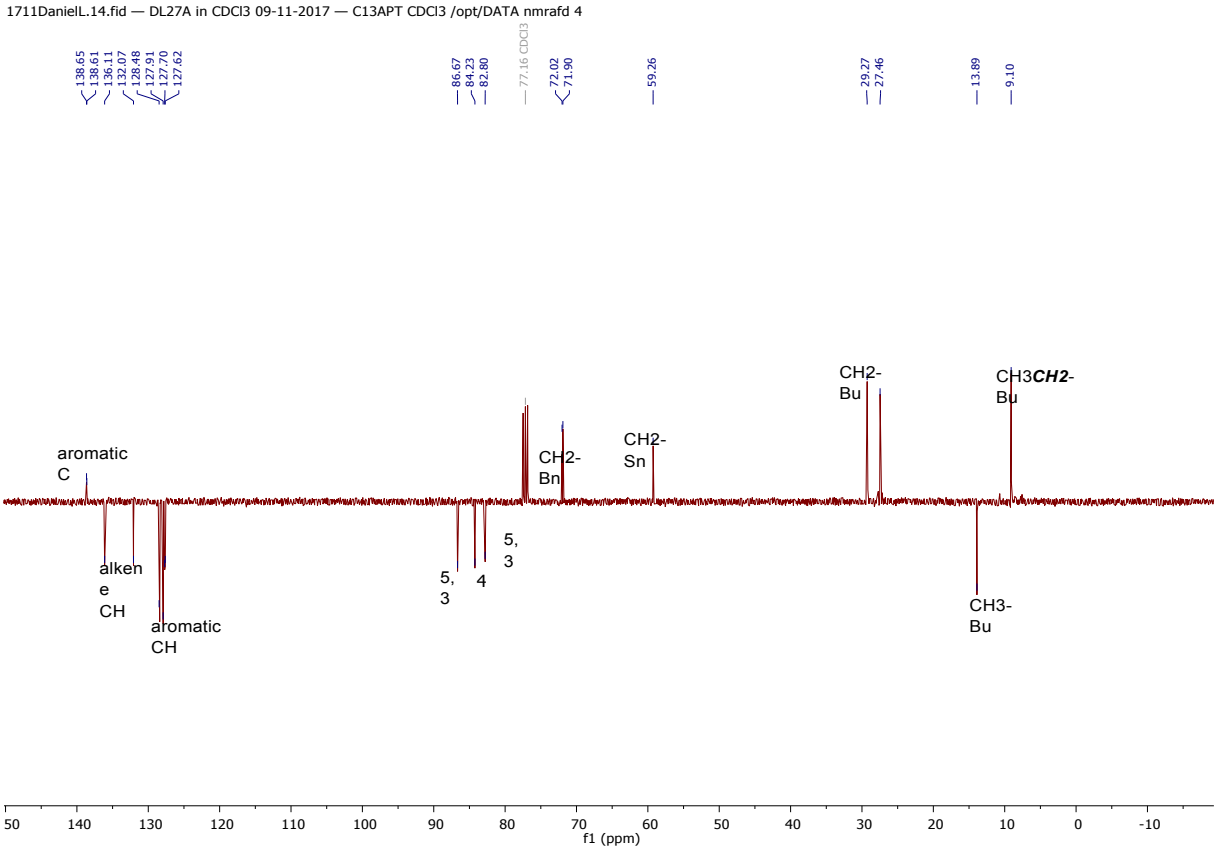
^{1}H-NMR and ^{13}C-NMR spectra of **13** in $CDCl_3$



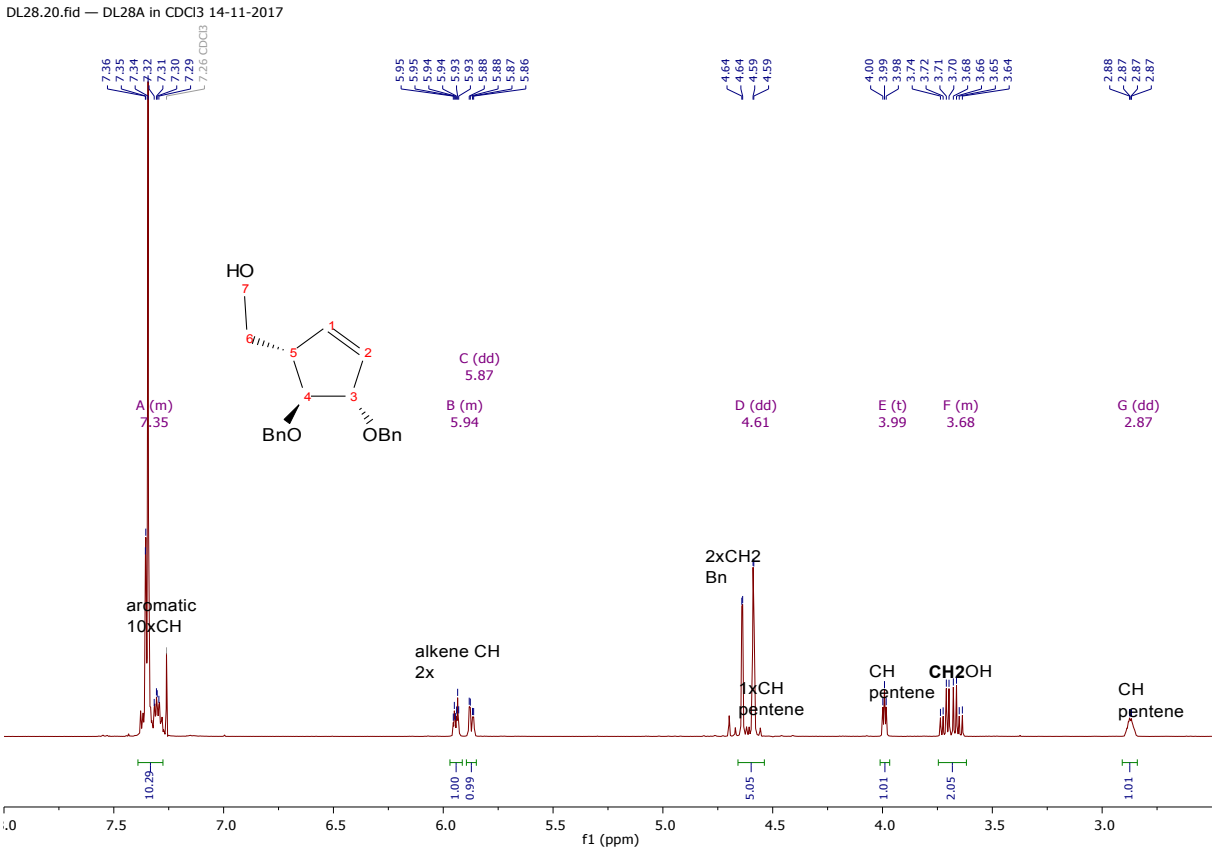


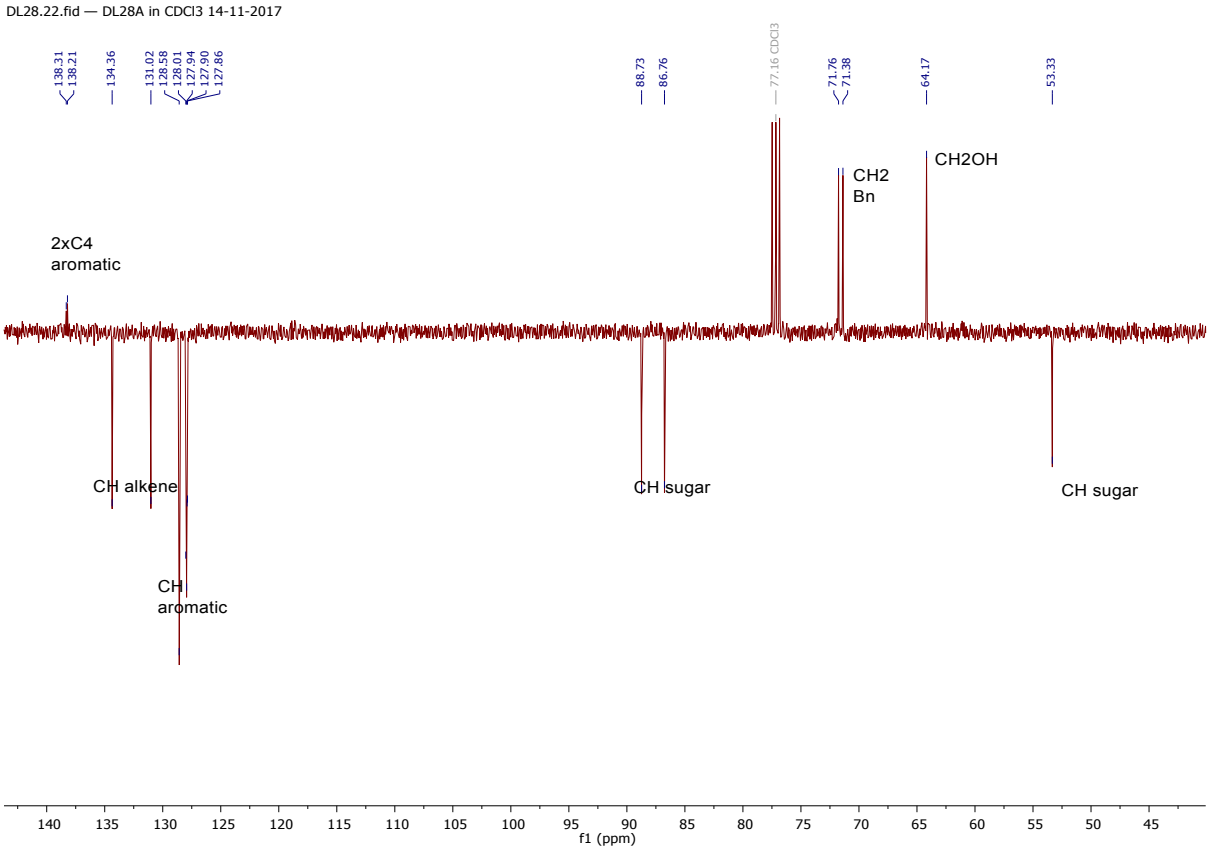
^{1}H-NMR and ^{13}C-NMR spectra of **14** in $CDCl_3$



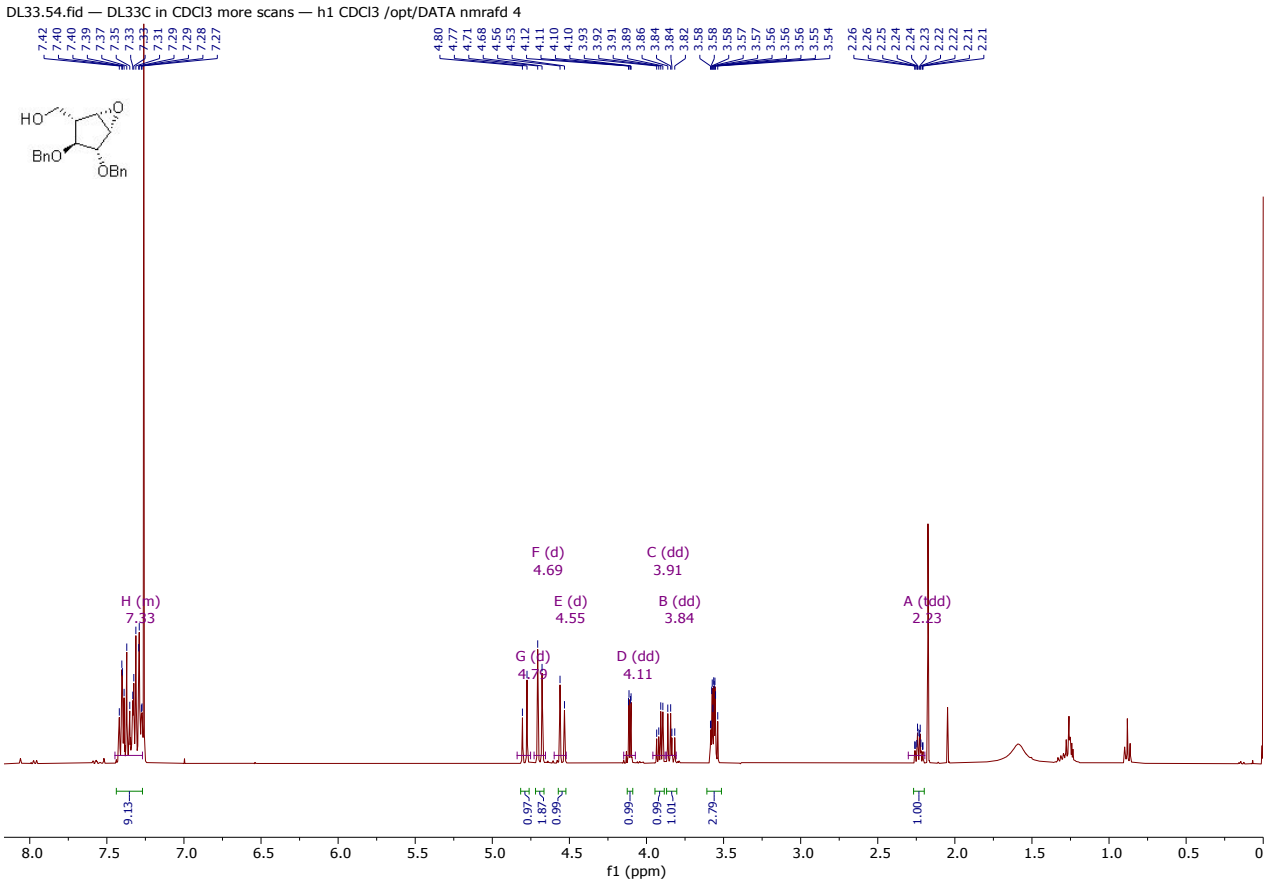


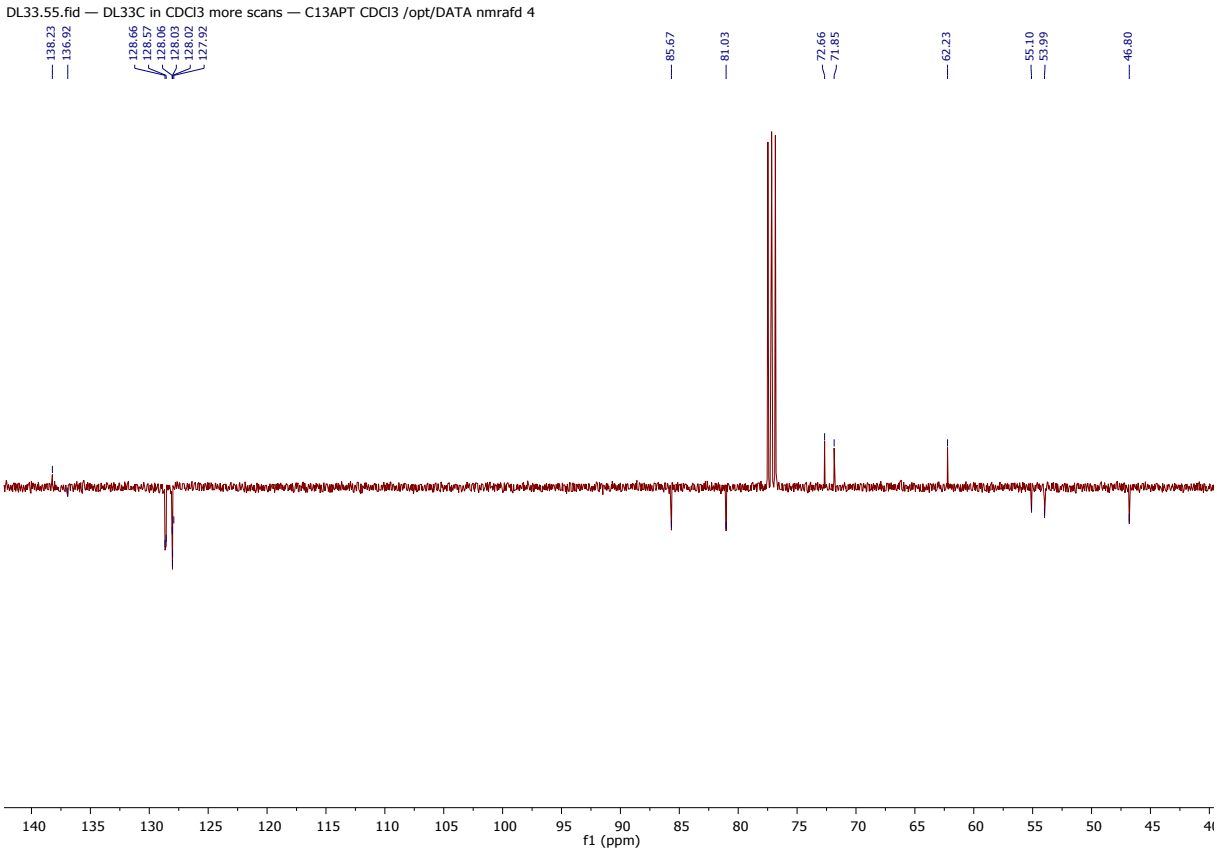
^{1}H-NMR and ^{13}C-NMR spectra of **15** in $CDCl_3$



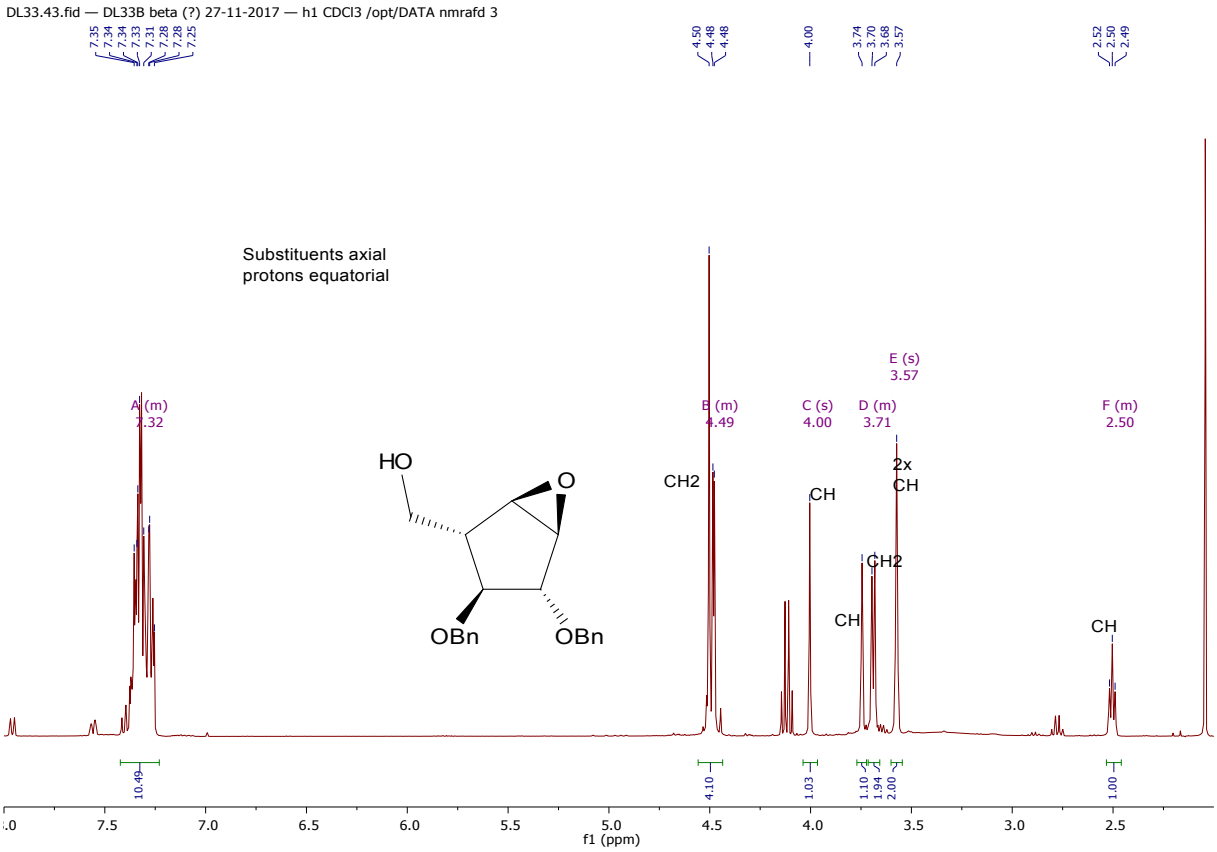


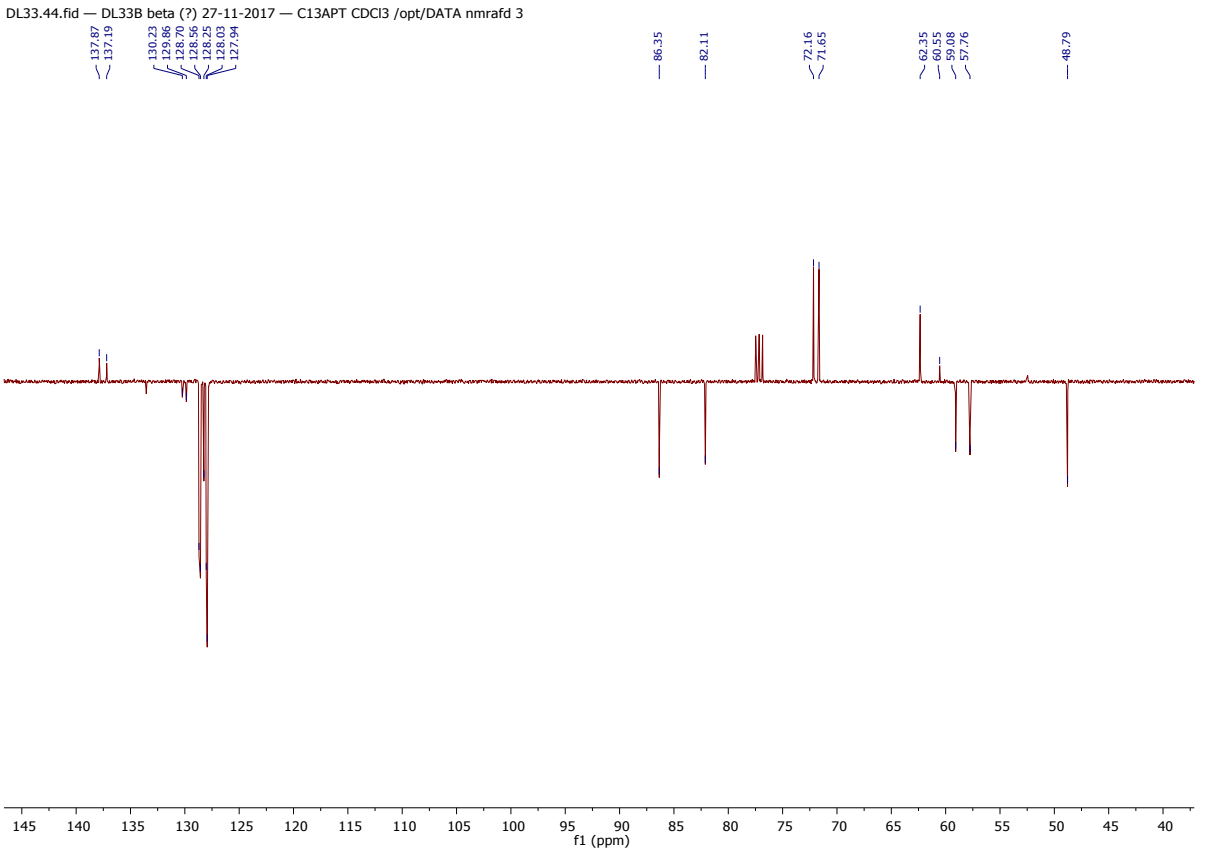
^{1}H-NMR and ^{13}C-NMR spectra of **16** in $CDCl_3$



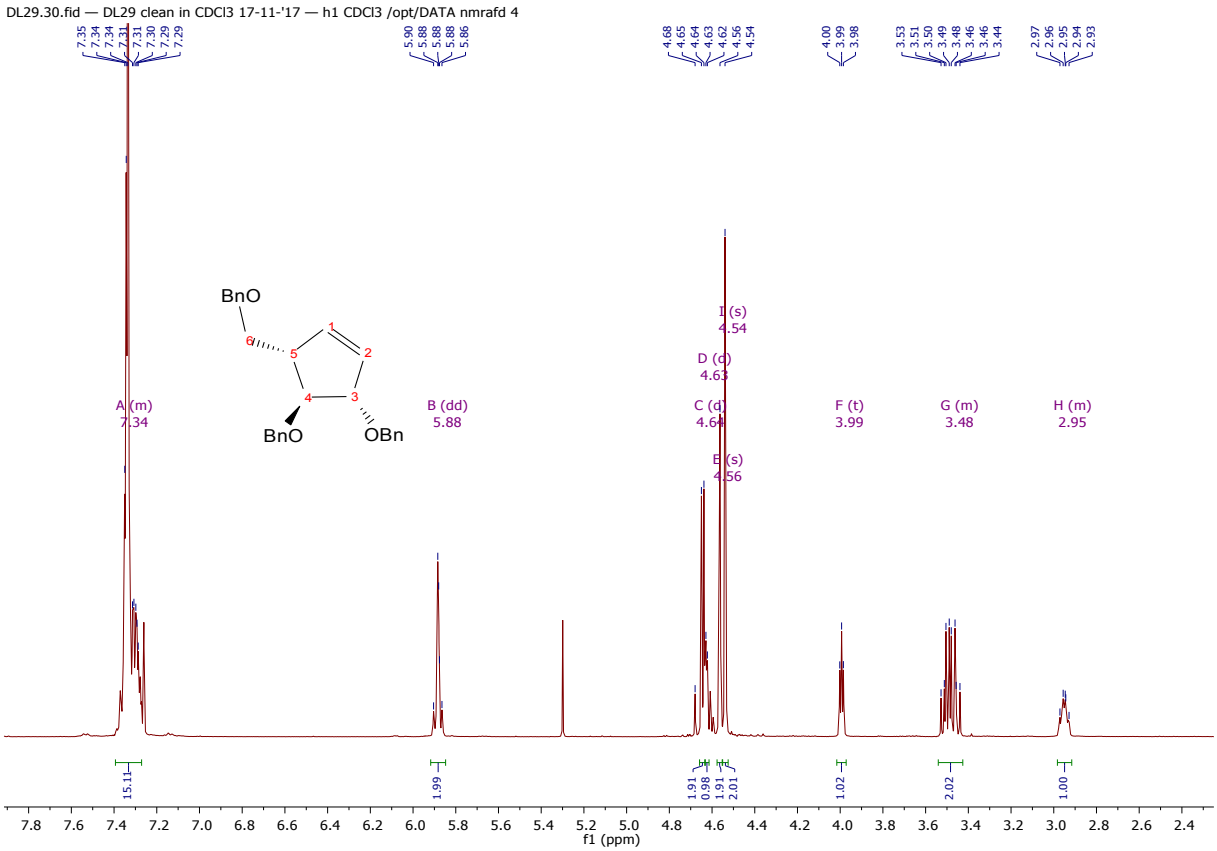


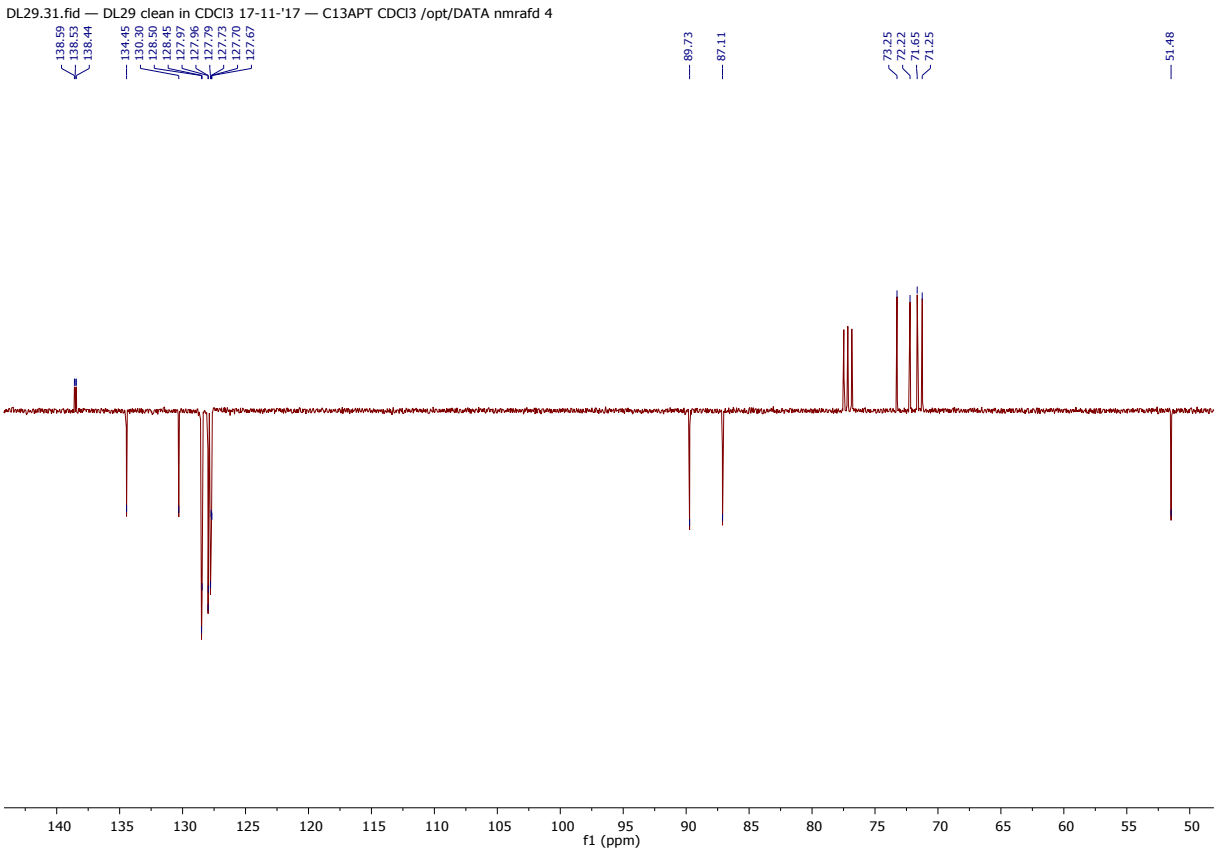
^{1}H-NMR and ^{13}C-NMR spectra of **17** in $CDCl_3$



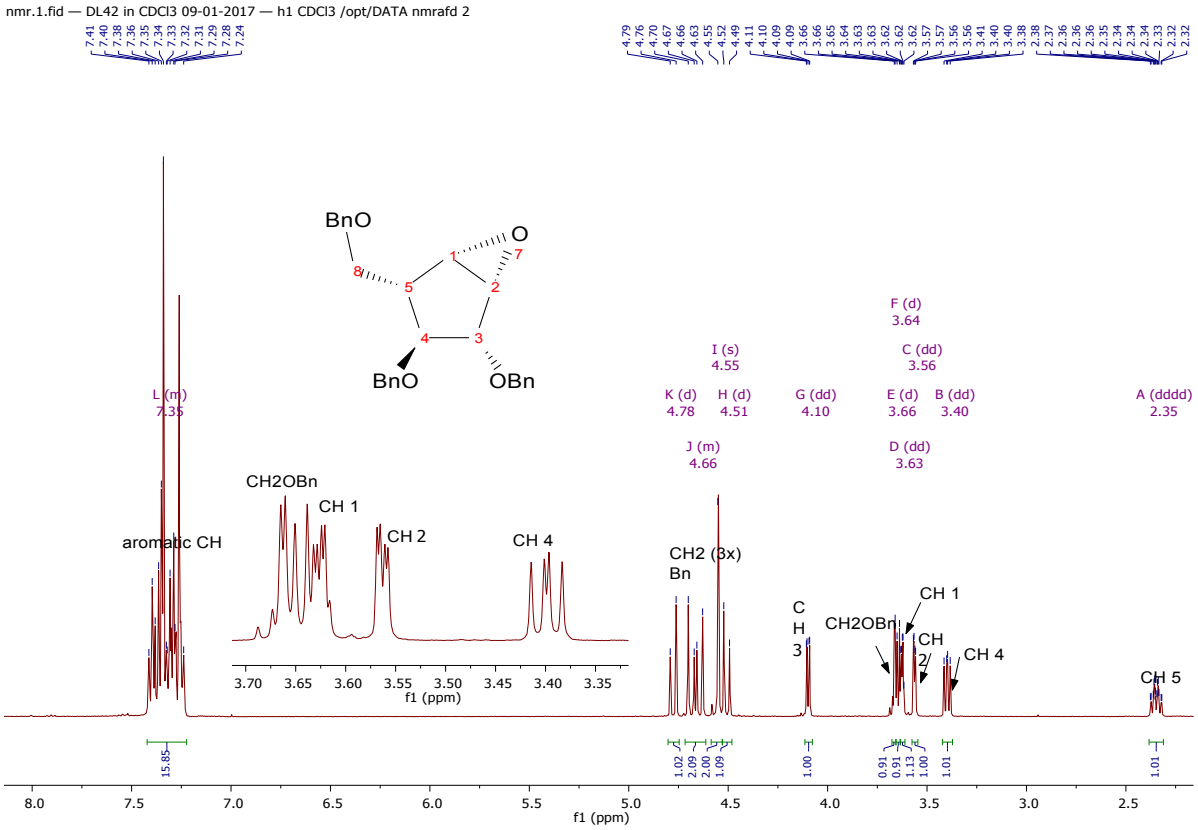


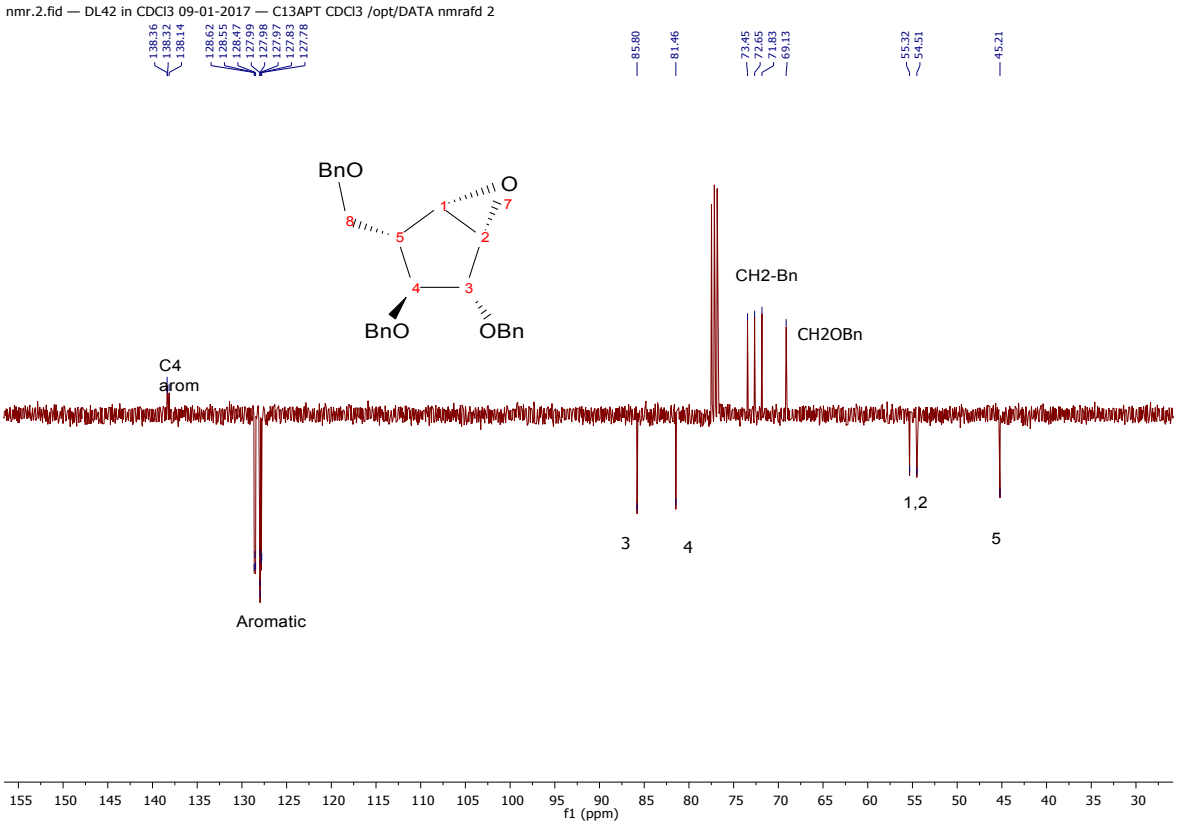
^{1}H-NMR and ^{13}C-NMR spectra of **18** in $CDCl_3$



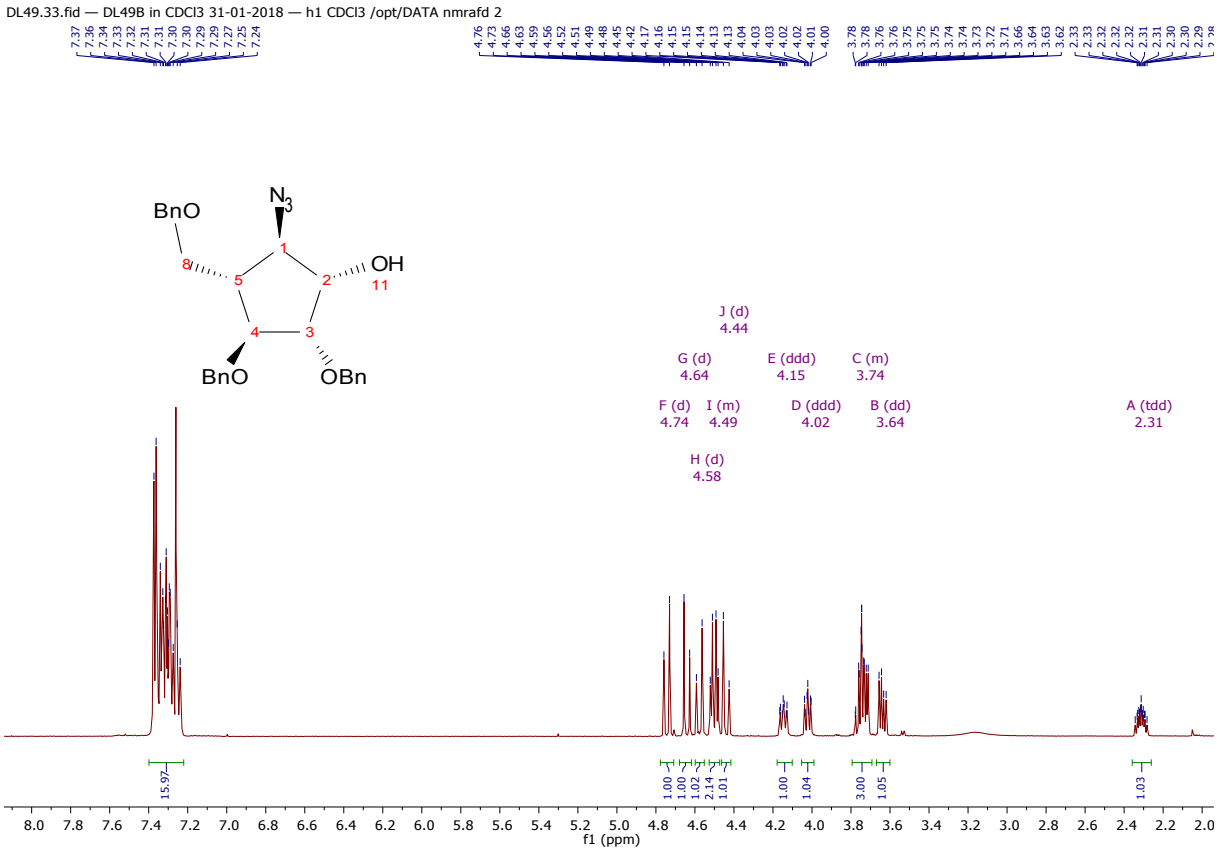


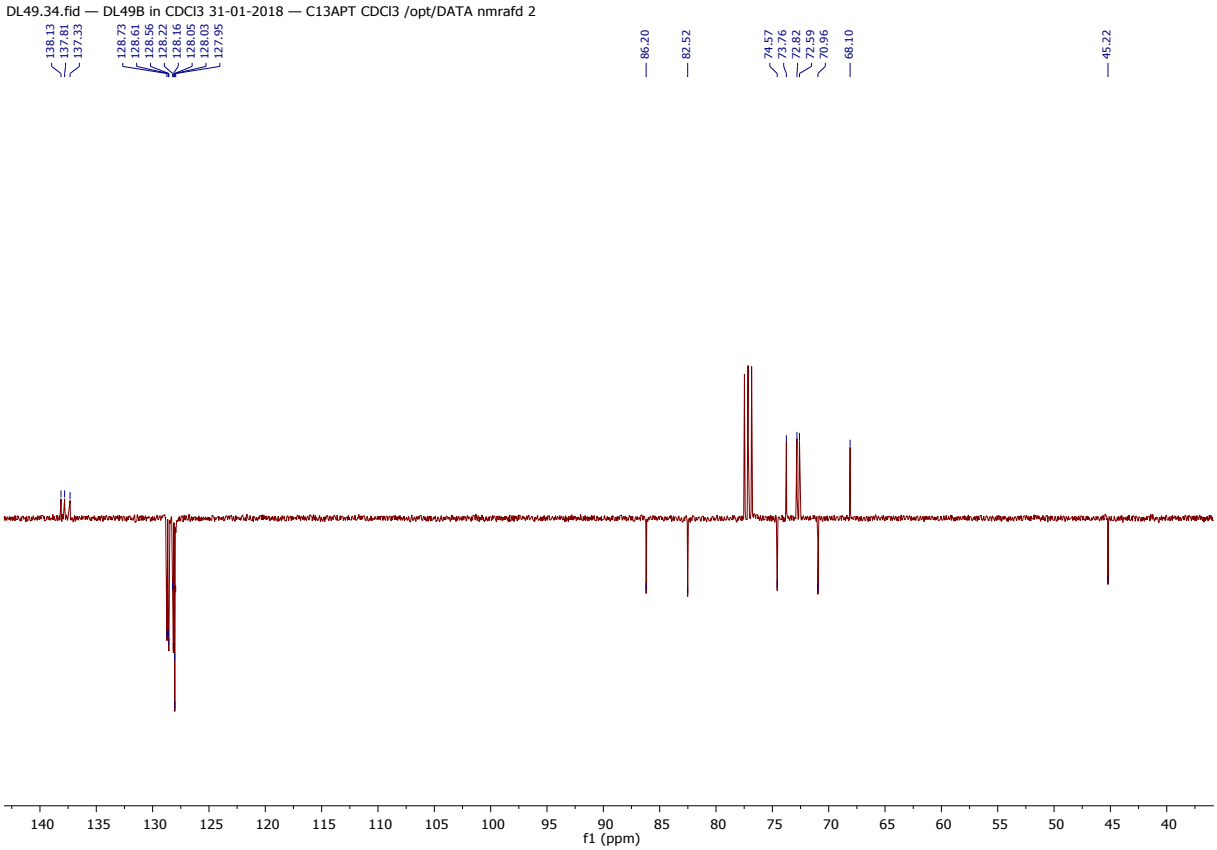
^{1}H-NMR and ^{13}C-NMR spectra of **19** in $CDCl_3$





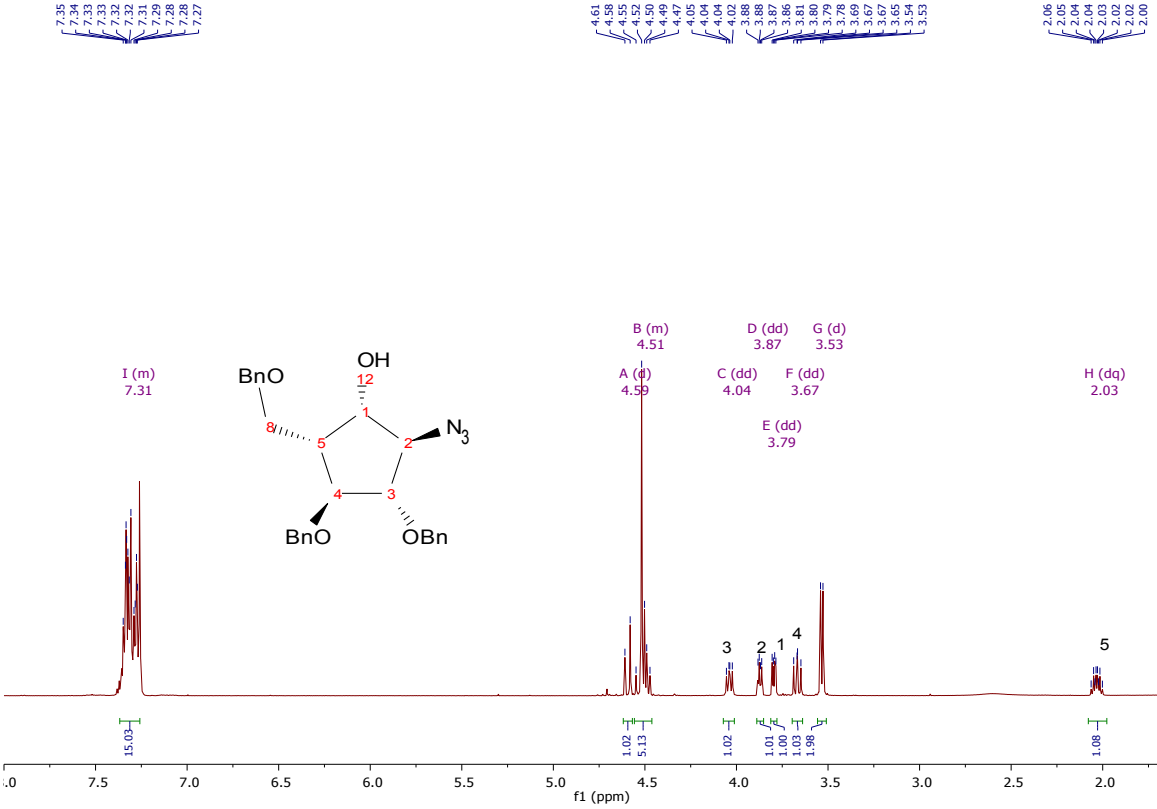
^{1}H-NMR and ^{13}C-NMR spectra of **21** in $CDCl_3$



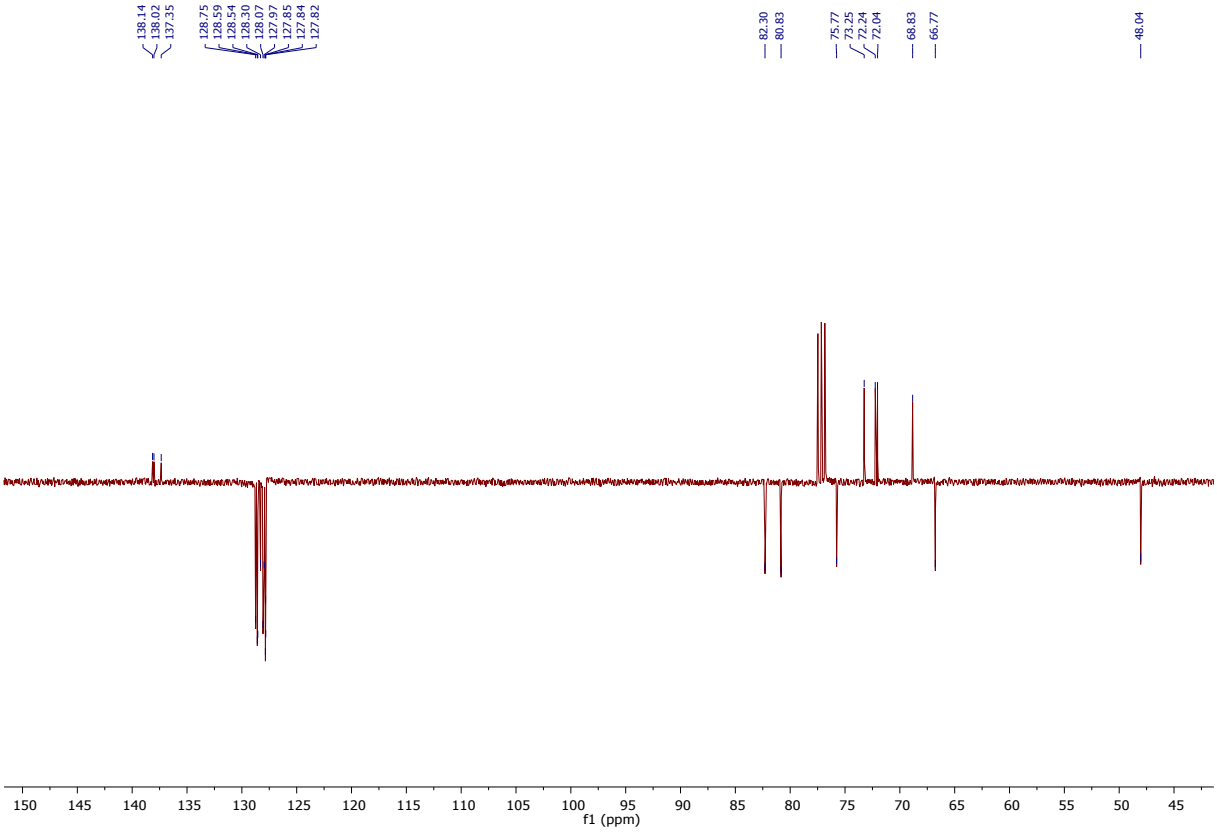


^{1}H-NMR and ^{13}C-NMR spectra of **22** in $CDCl_3$

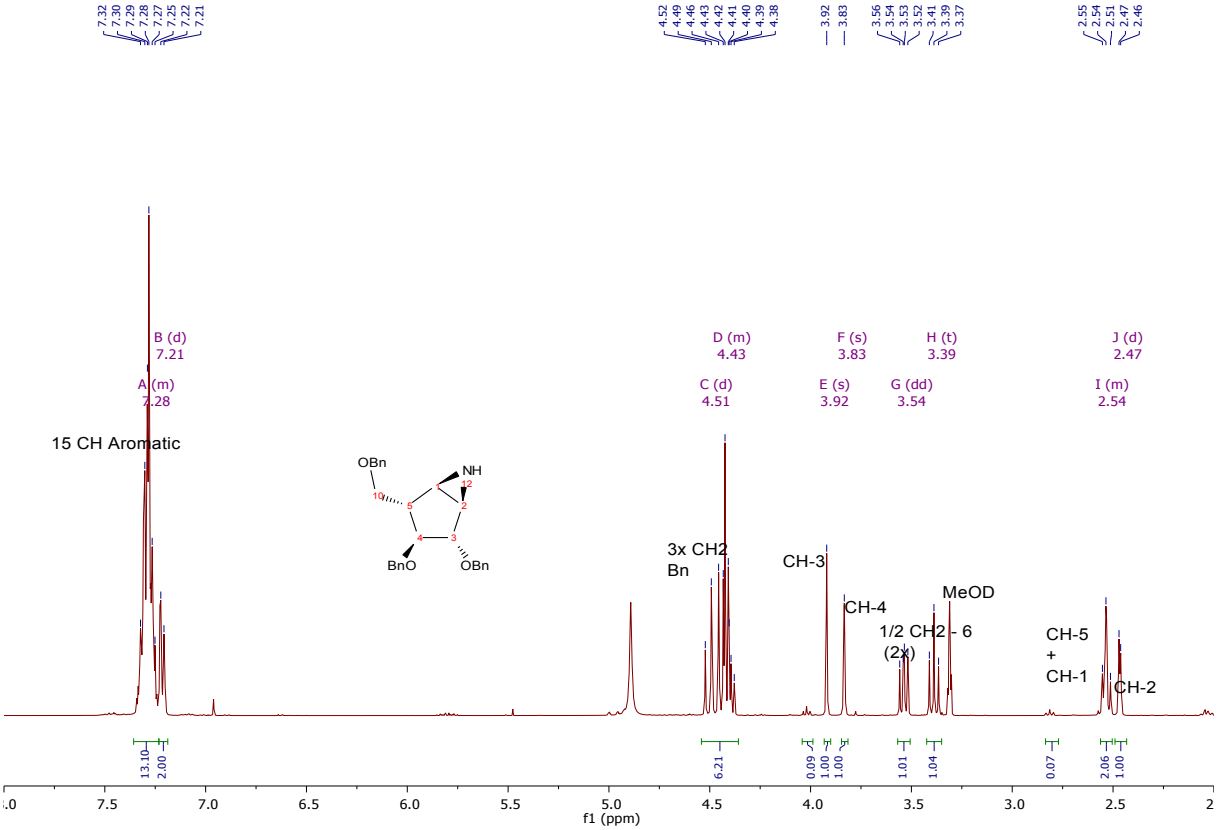
DL49.29.fid — DL49A in CDCl3 31-01-2018 — h1 CDCl3 /opt/DATA nmrafd 1

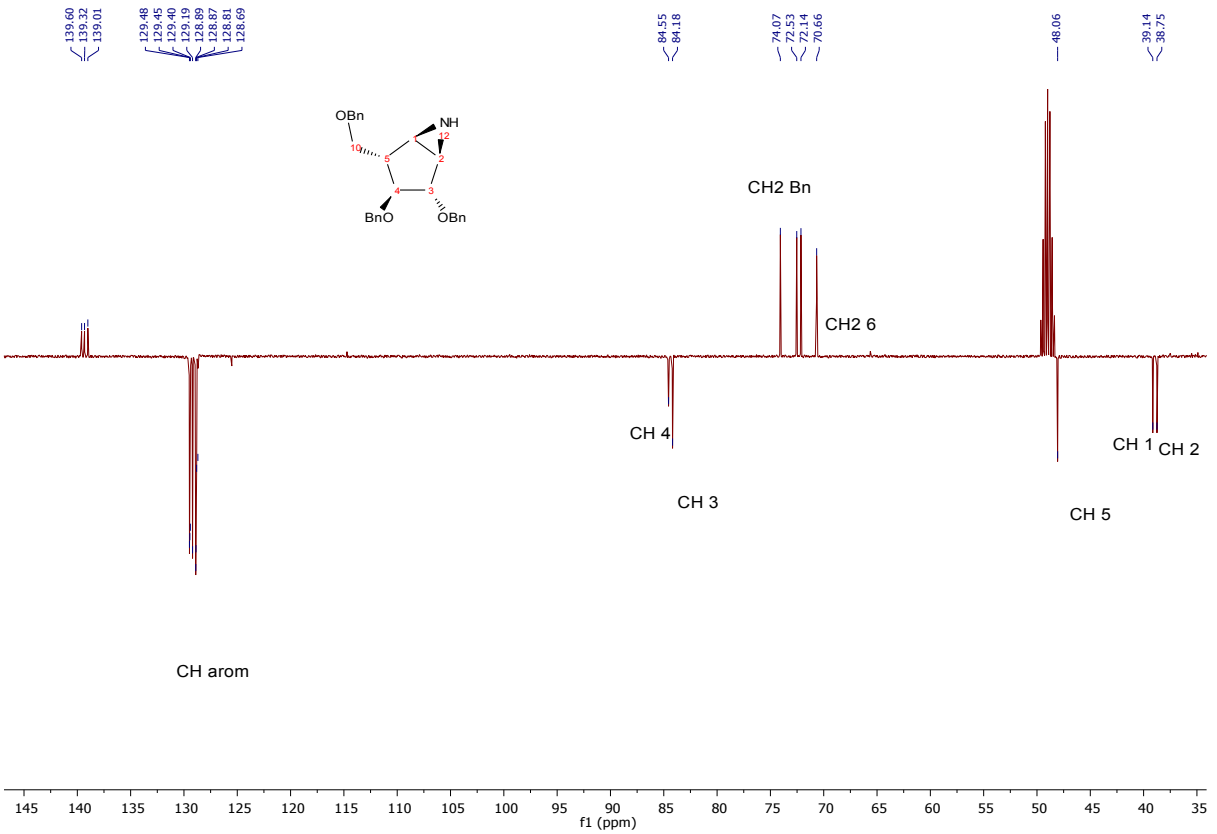


DL49.30.fid — DL49A in CDCl3 31-01-2018 — C13APT CDCl3 /opt/DATA nmrafd 1

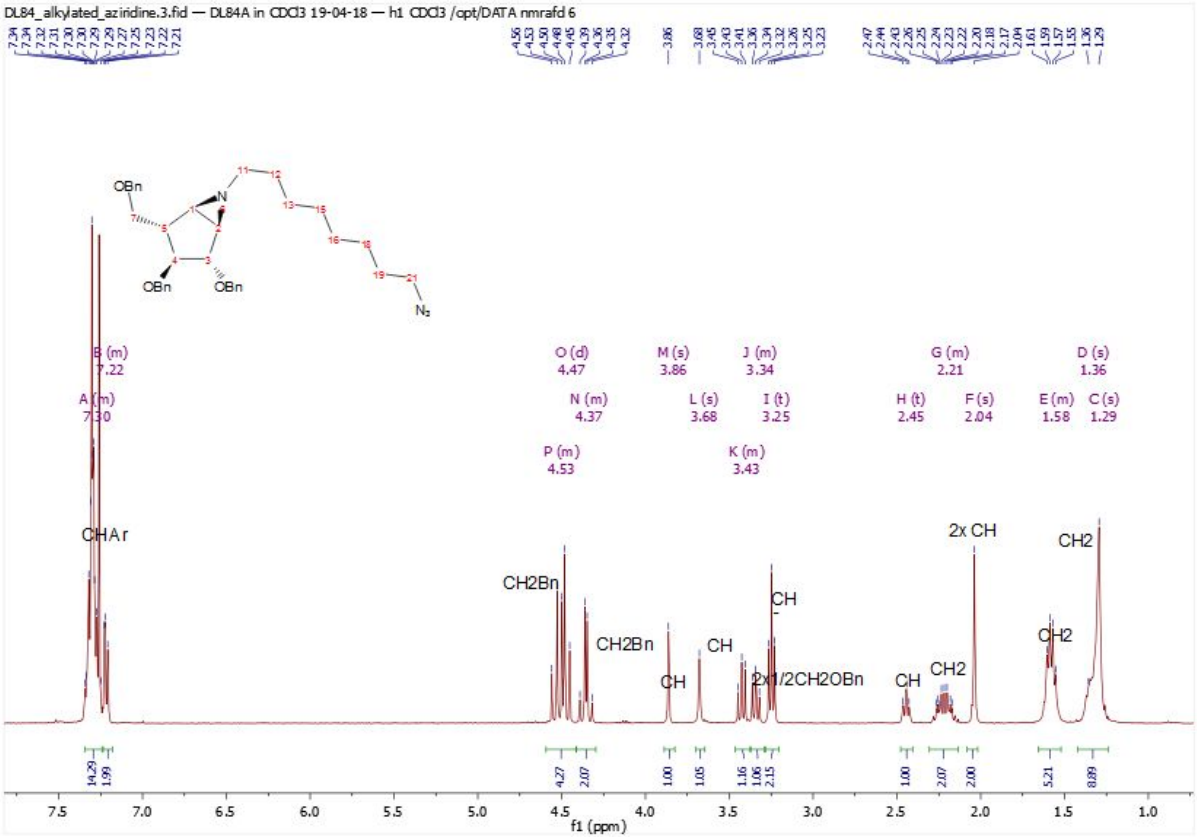


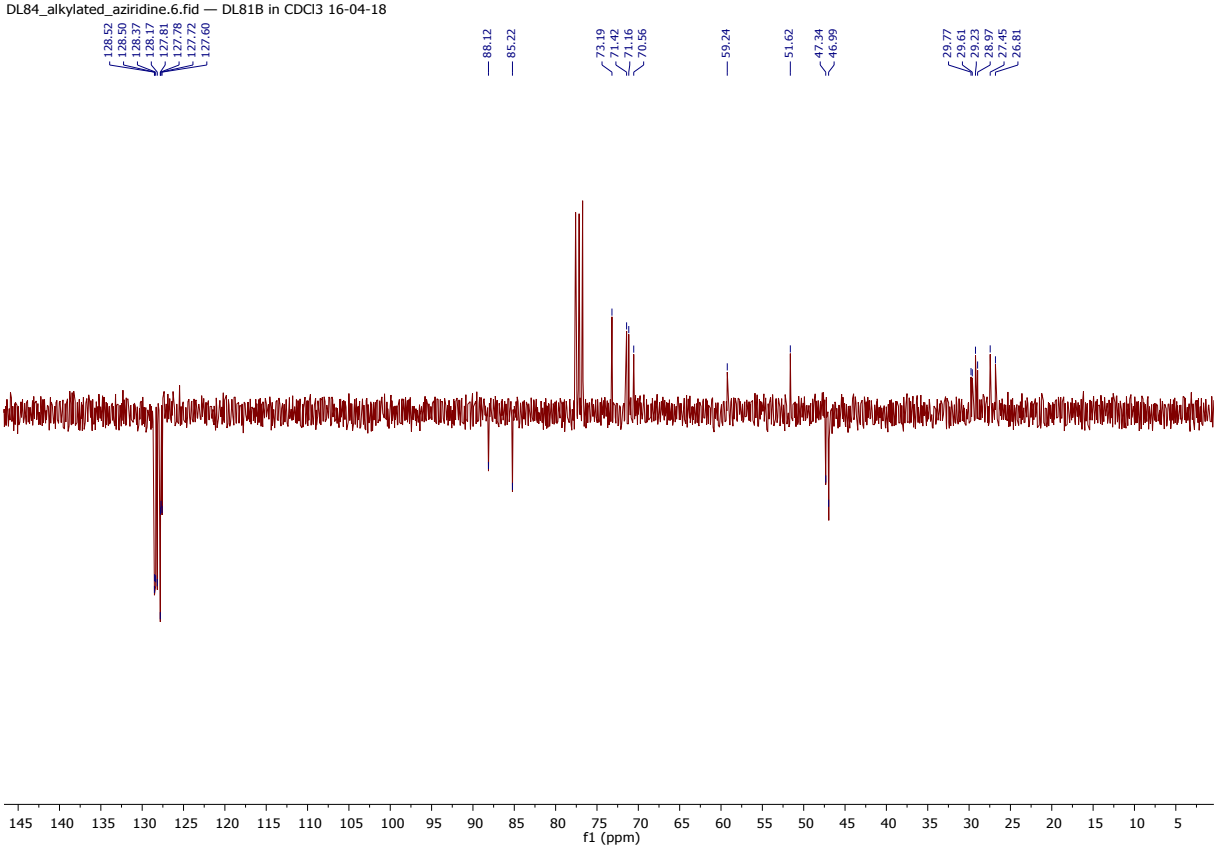
^{1}H-NMR and ^{13}C-NMR spectra of **23** in MeOD



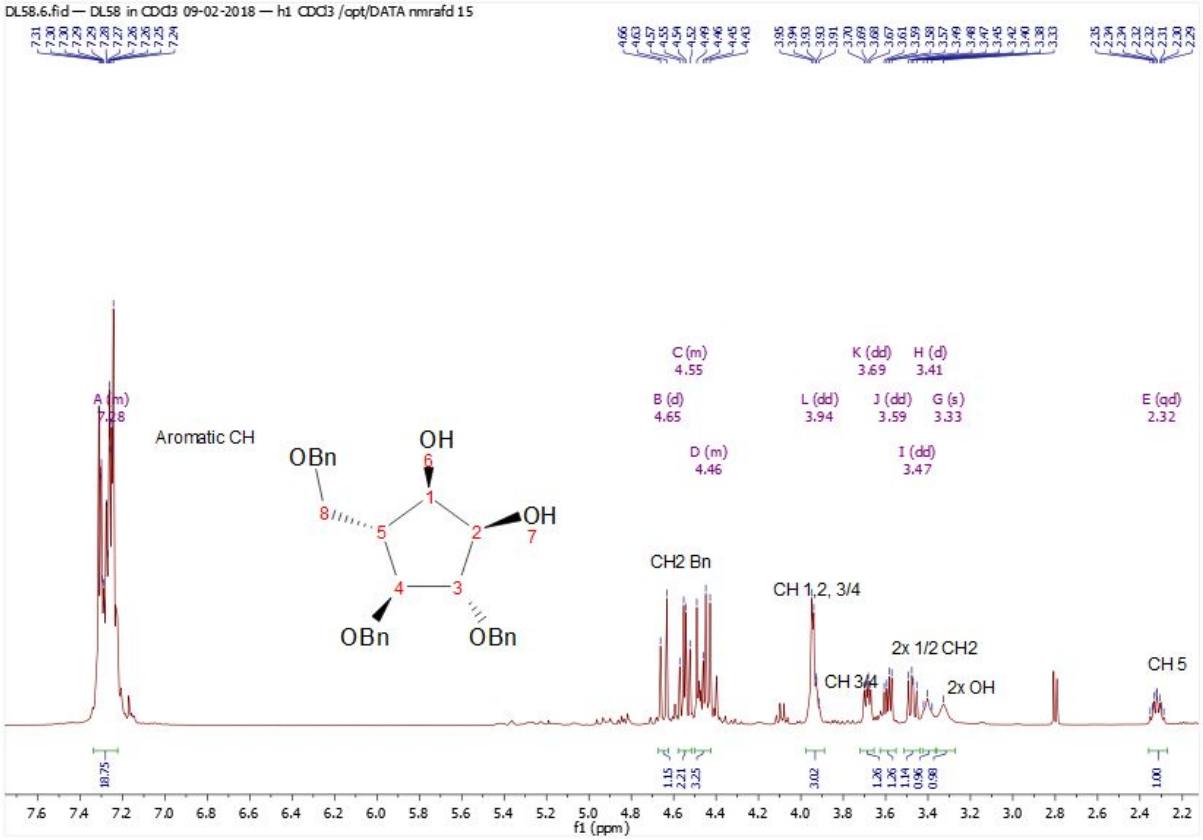


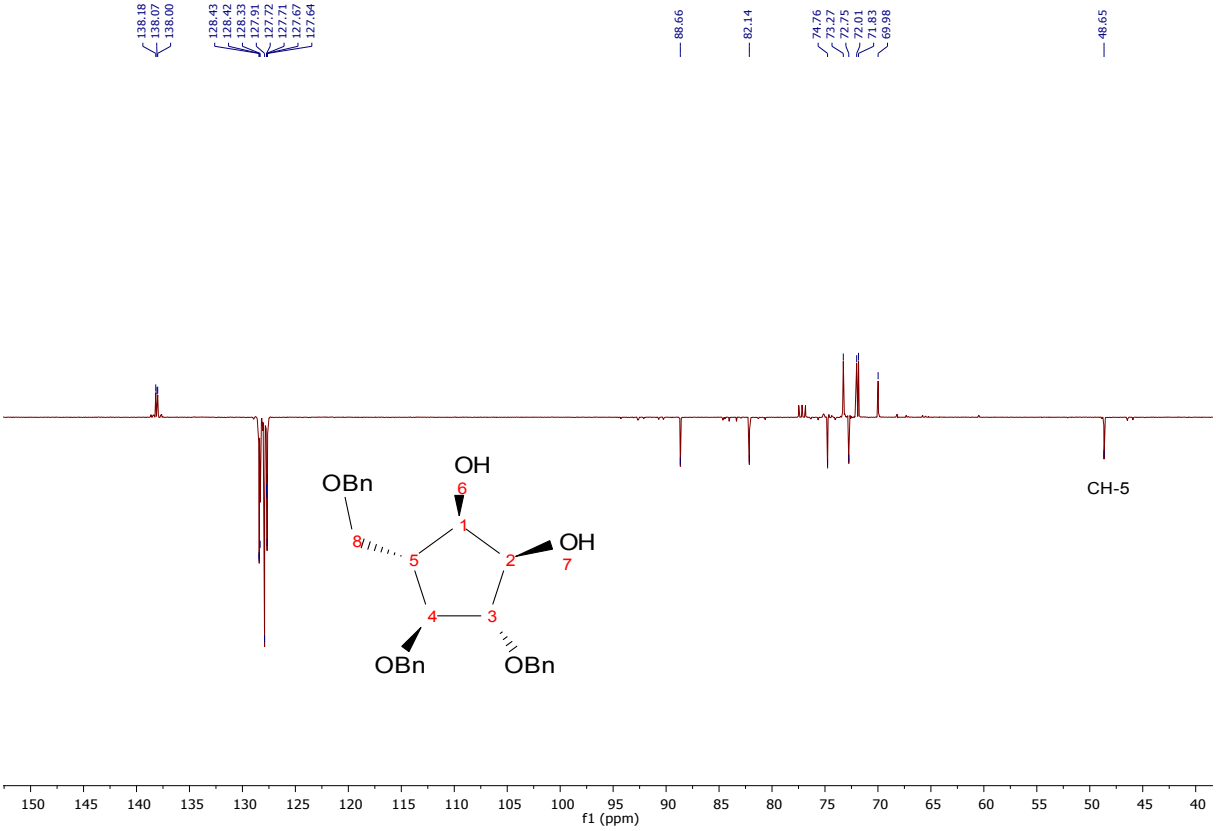
^{1}H-NMR and ^{13}C-NMR spectra of **24** in MeOD



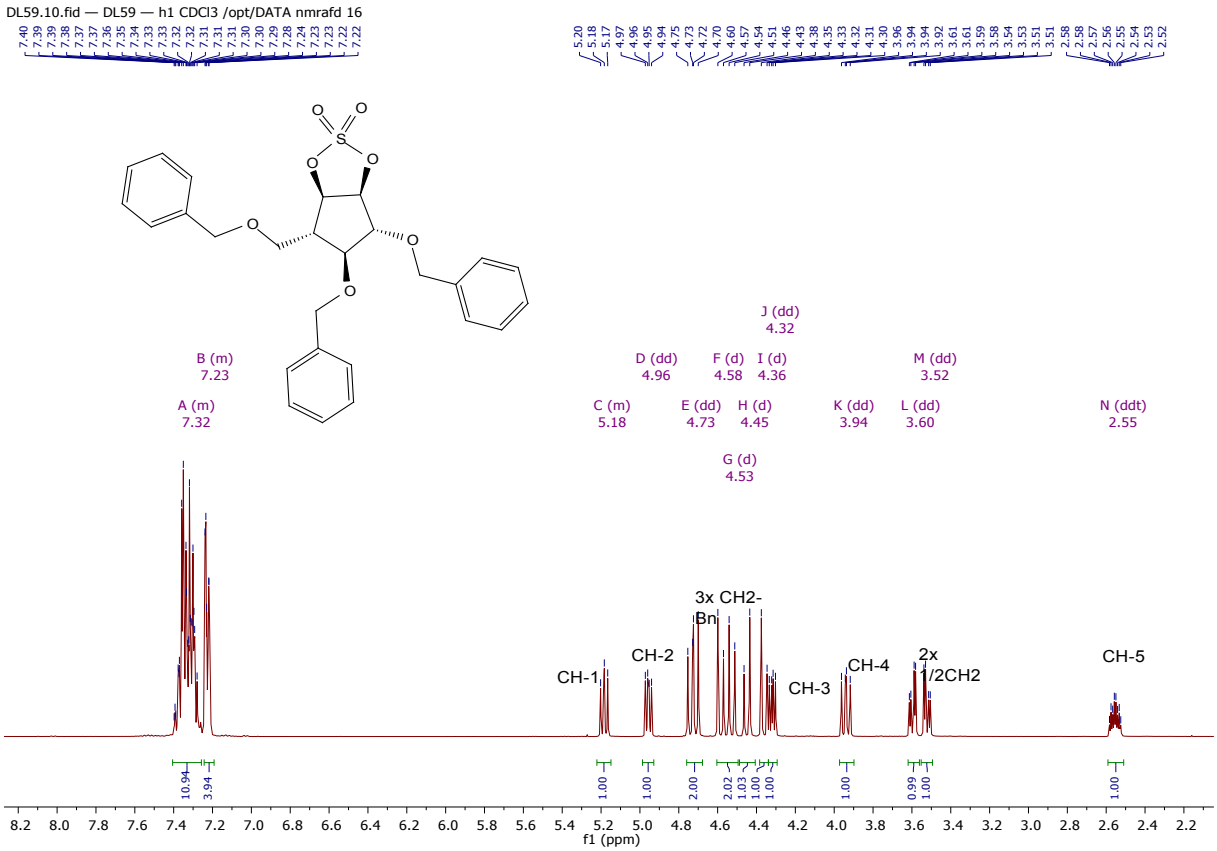


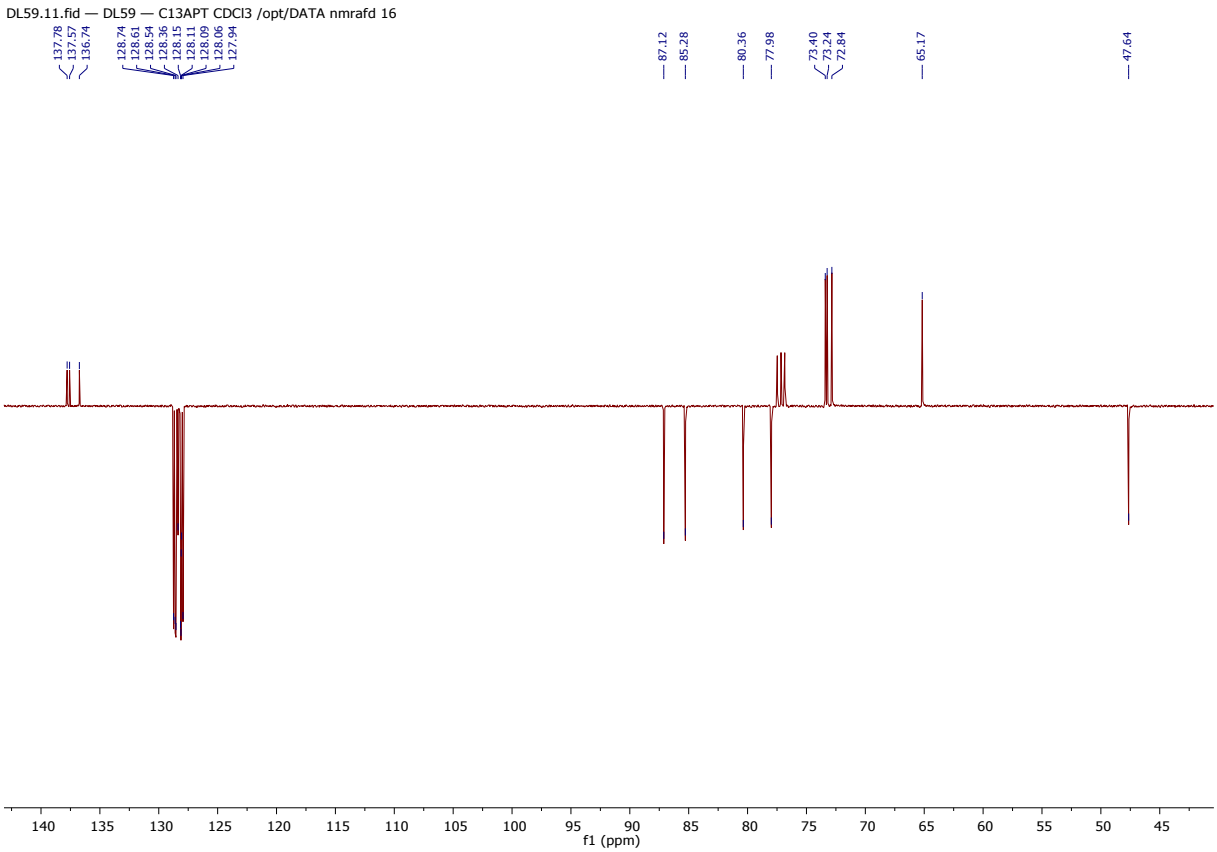
^{1}H-NMR and ^{13}C-NMR spectra of **25** in $CDCl_3$



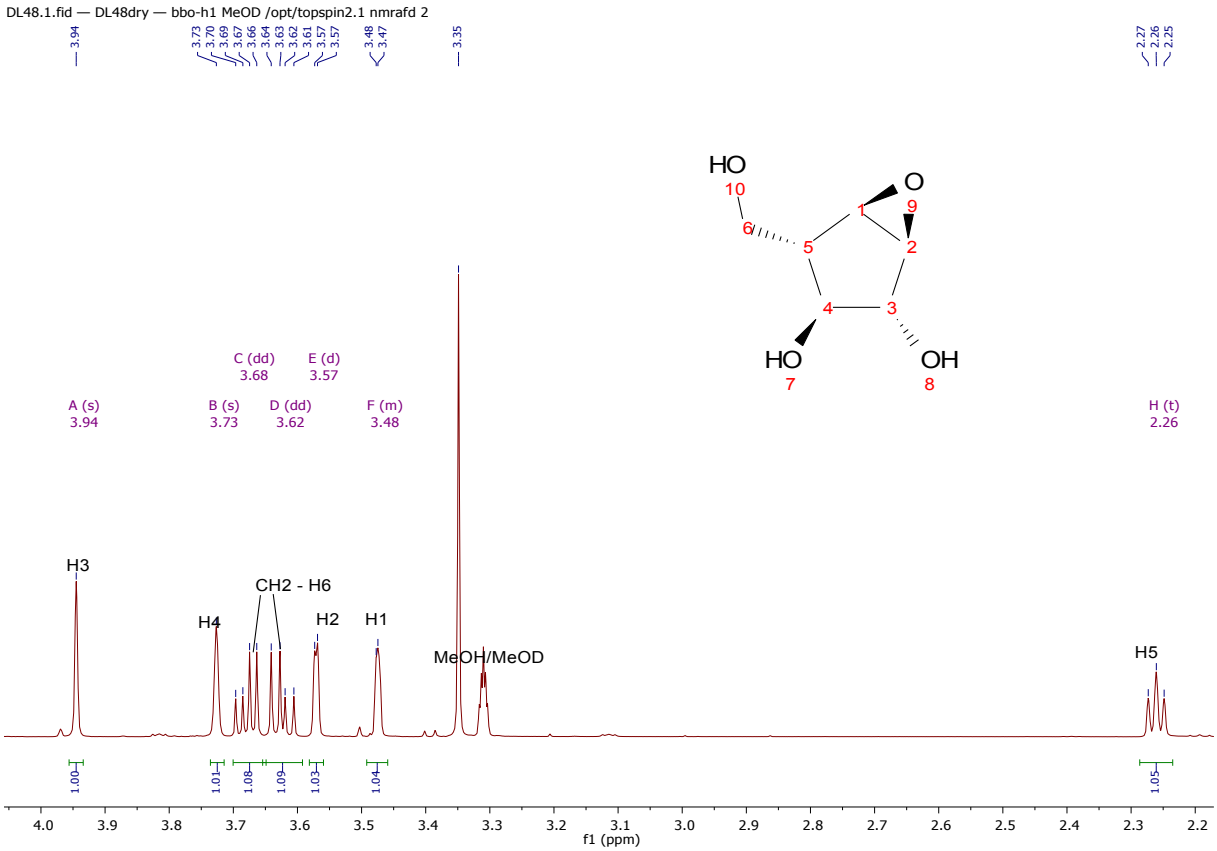


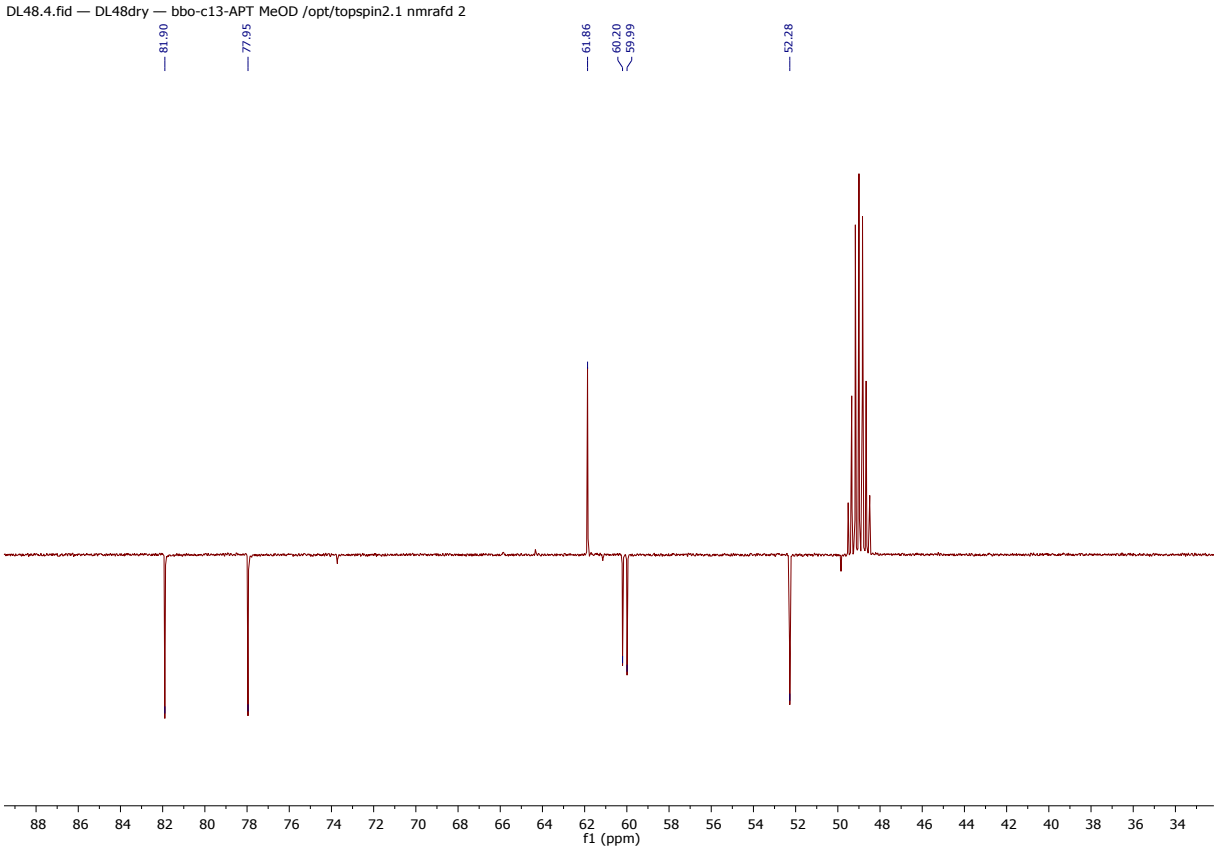
^{1}H-NMR and ^{13}C-NMR spectra of **26** in $CDCl_3$



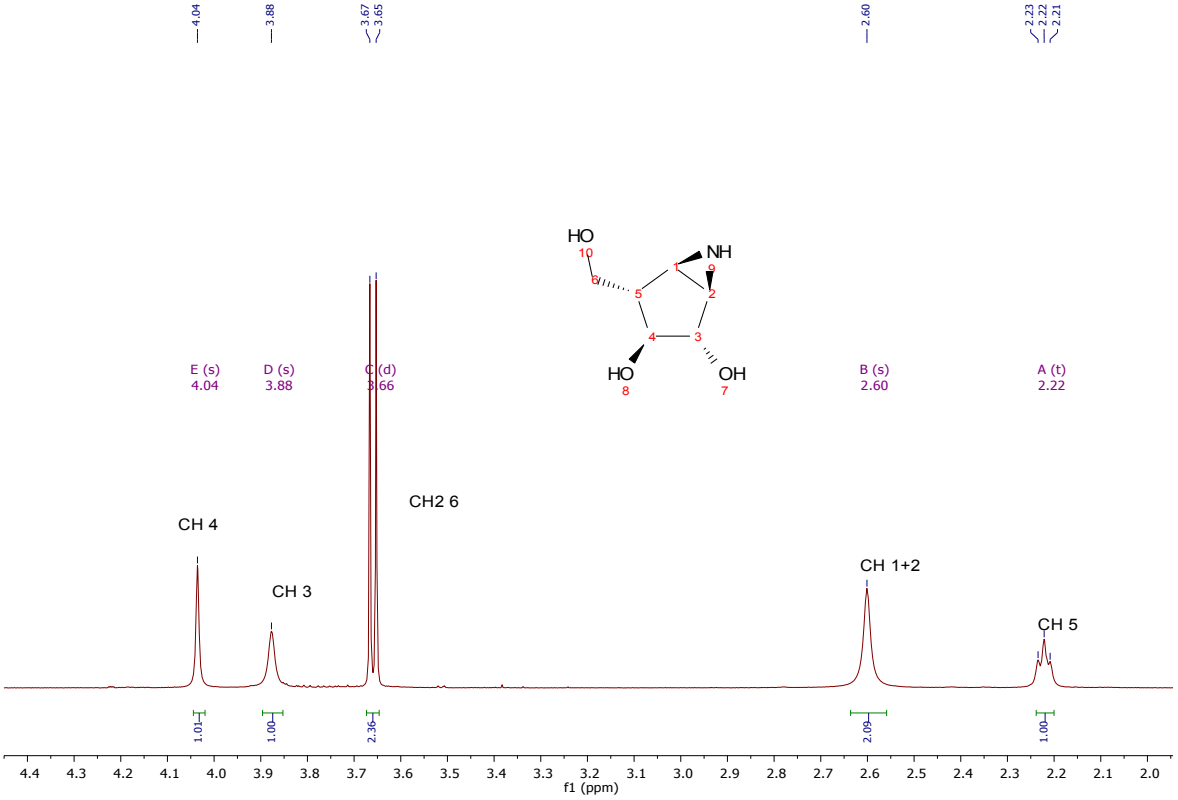


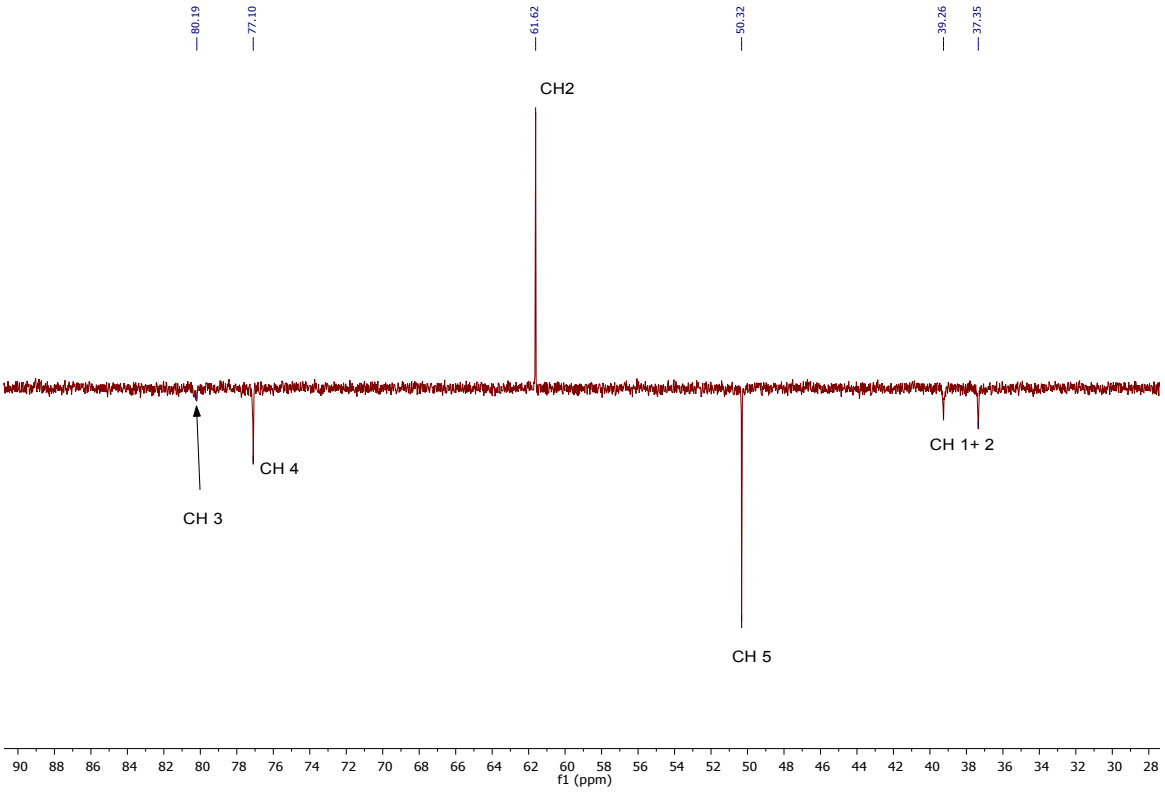
^{1}H-NMR and ^{13}C-NMR spectra of **1** in MeOD





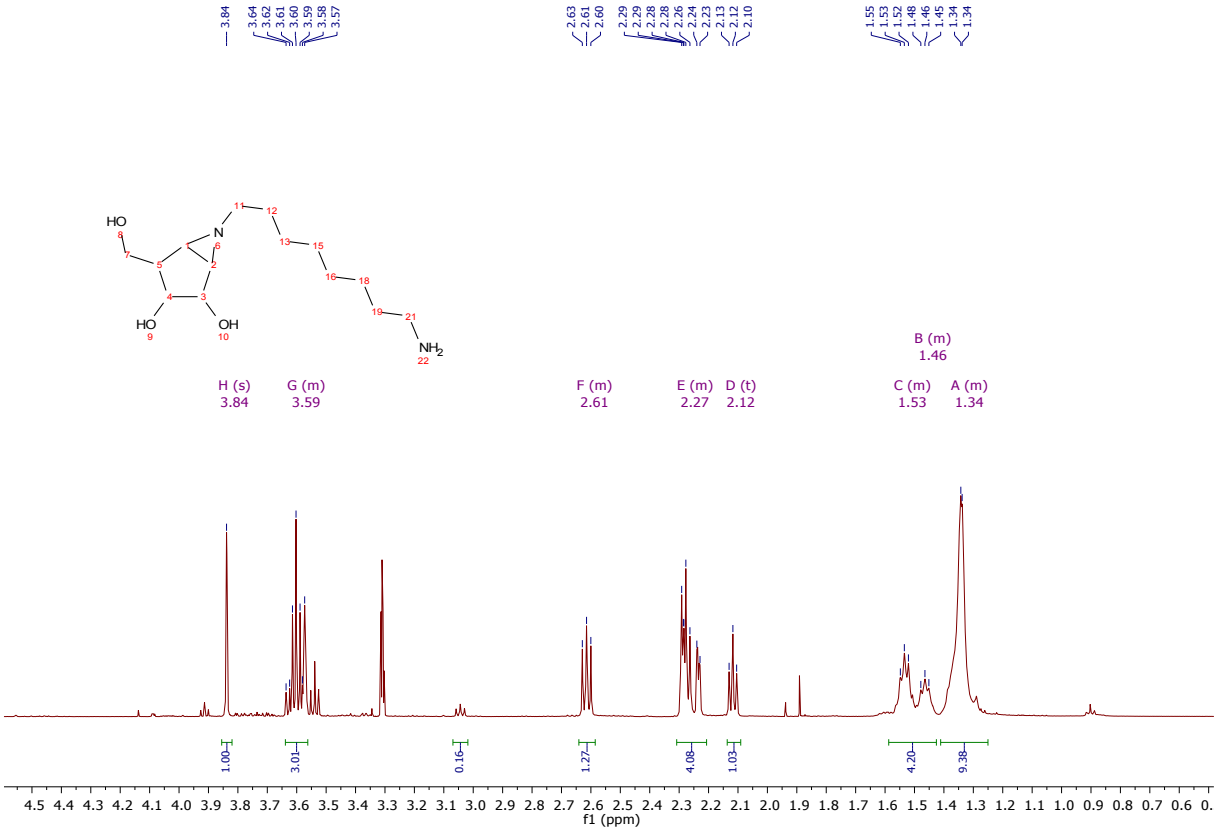
^{1}H-NMR and ^{13}C-NMR spectra of **2** in D_2O



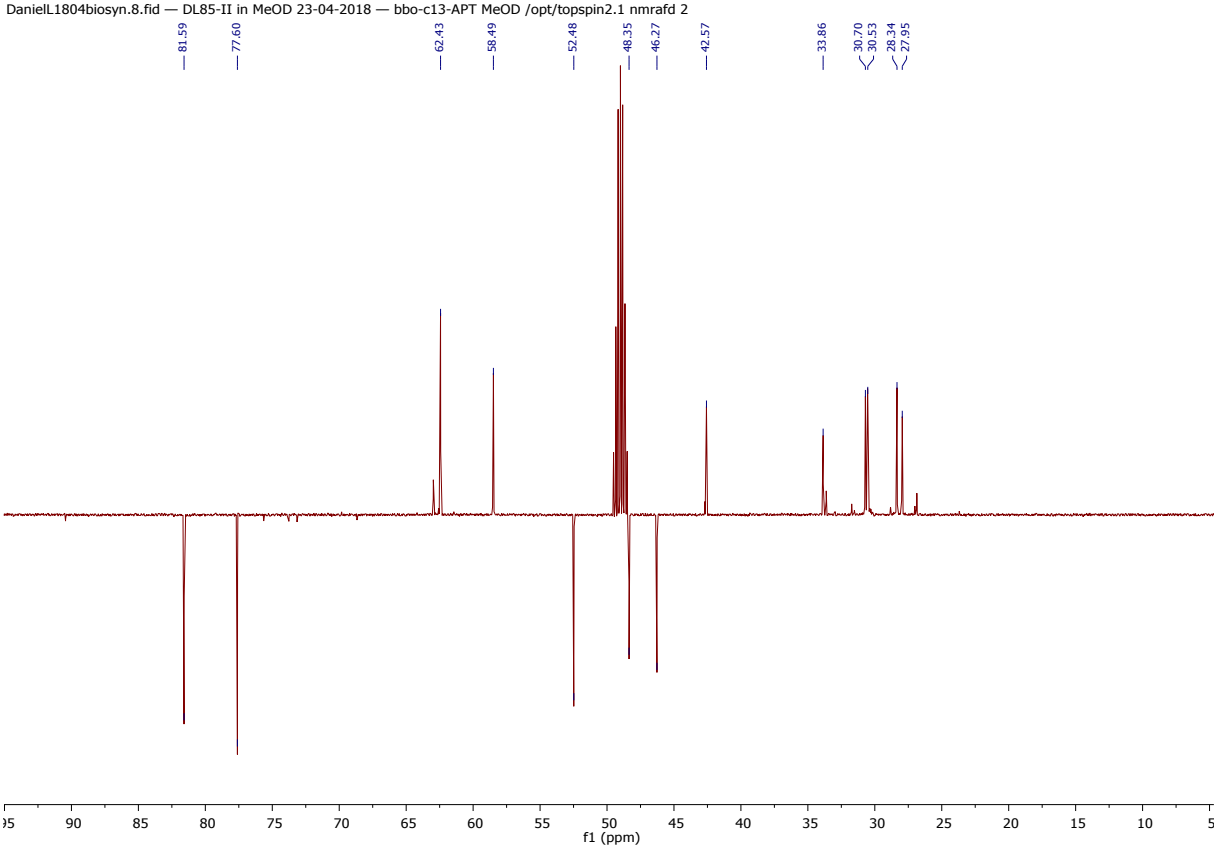


^{1}H-NMR and ^{13}C-NMR spectra of **3** in MeOD

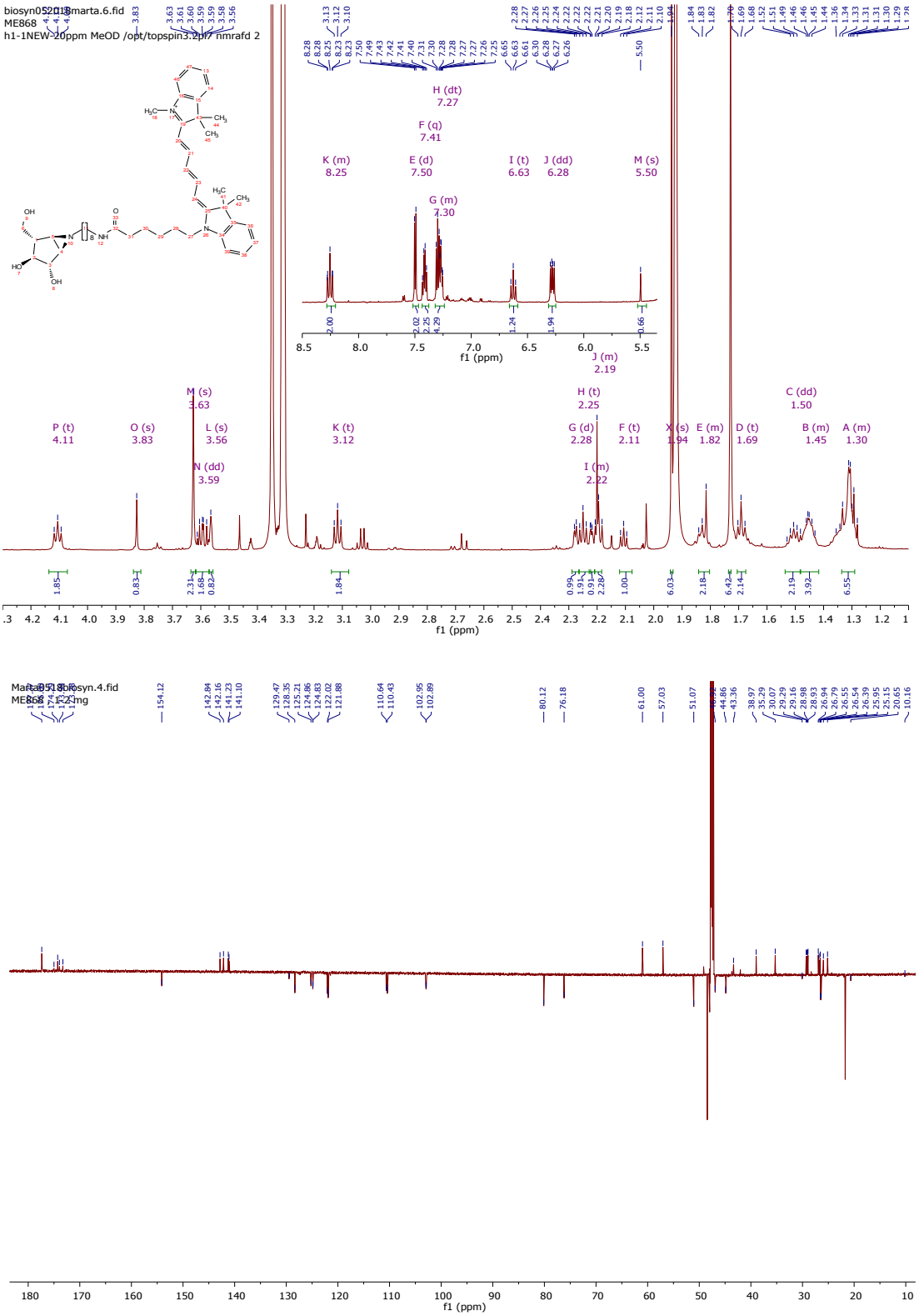
DanielL1804biosyn.5.fid — DL85-II in MeOD 23-04-2018 — bbo-h1 MeOD /opt/topspin2.1 nmrafd 2



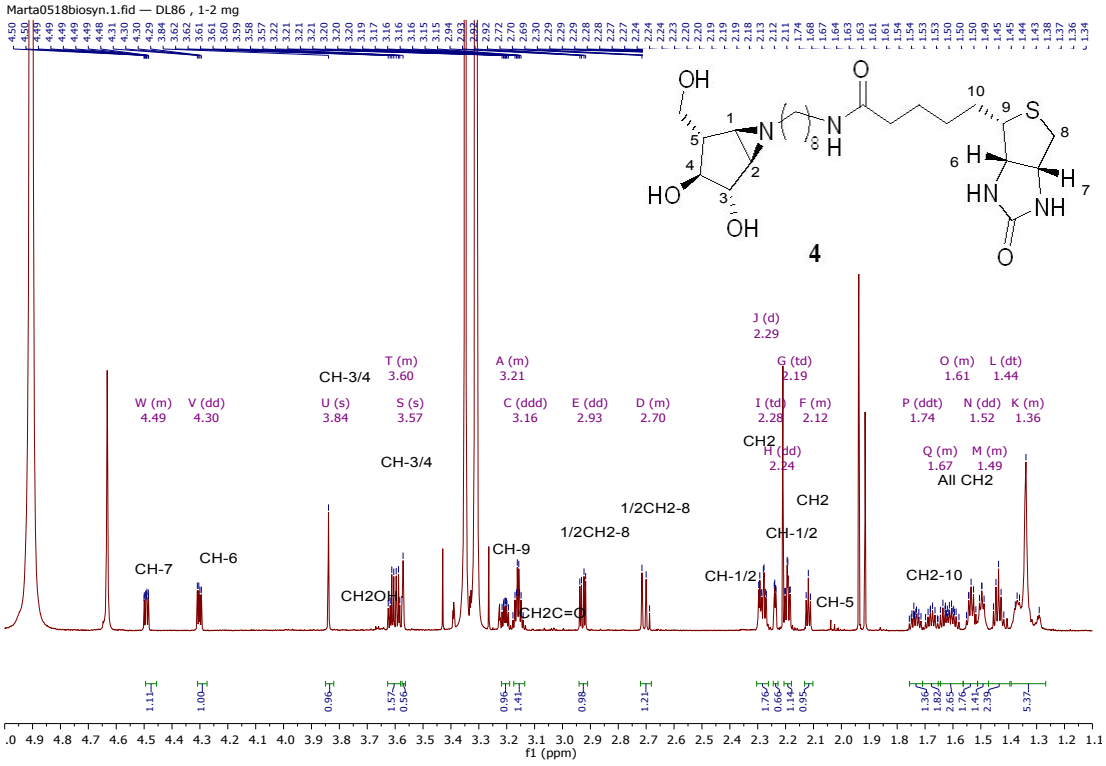
DanielL1804biosyn.8.fid — DL85-II in MeOD 23-04-2018 — bbo-c13-APT MeOD /opt/topspin2.1 nmrafd 2

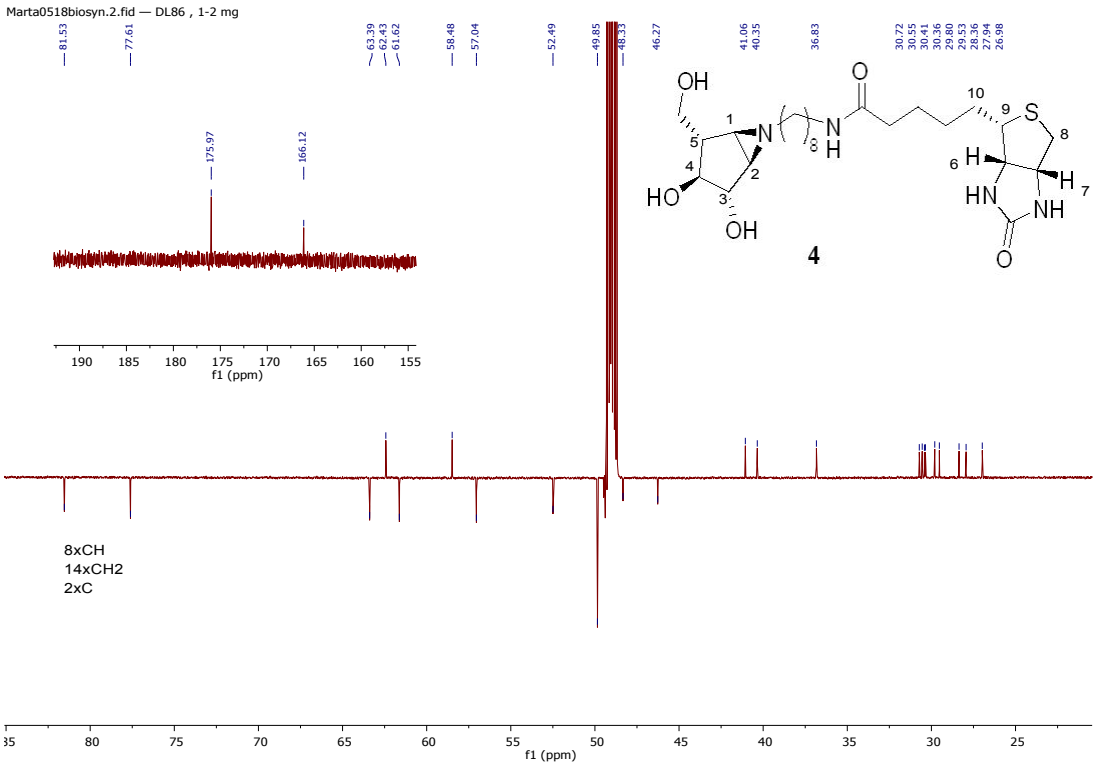


^{1}H-NMR and ^{13}C-NMR spectra of **4** in $MeOD_4$

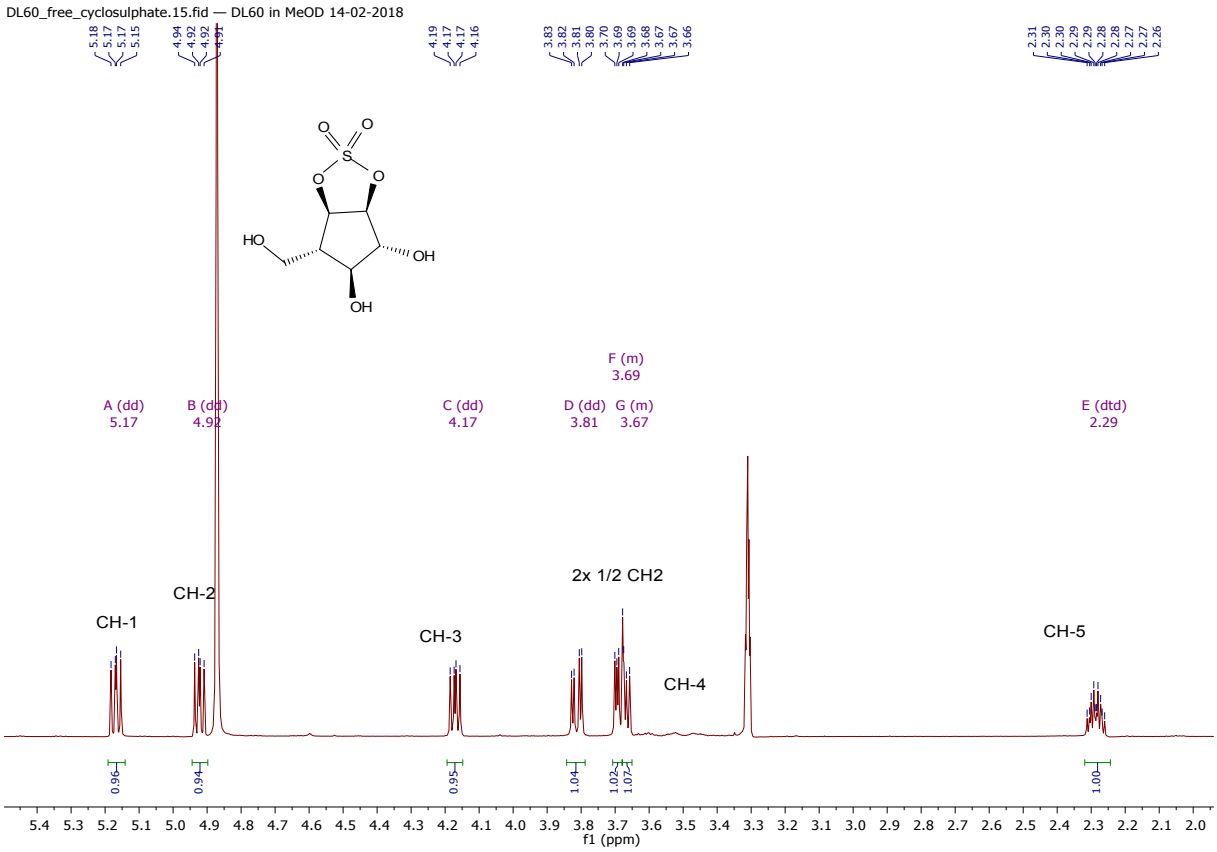


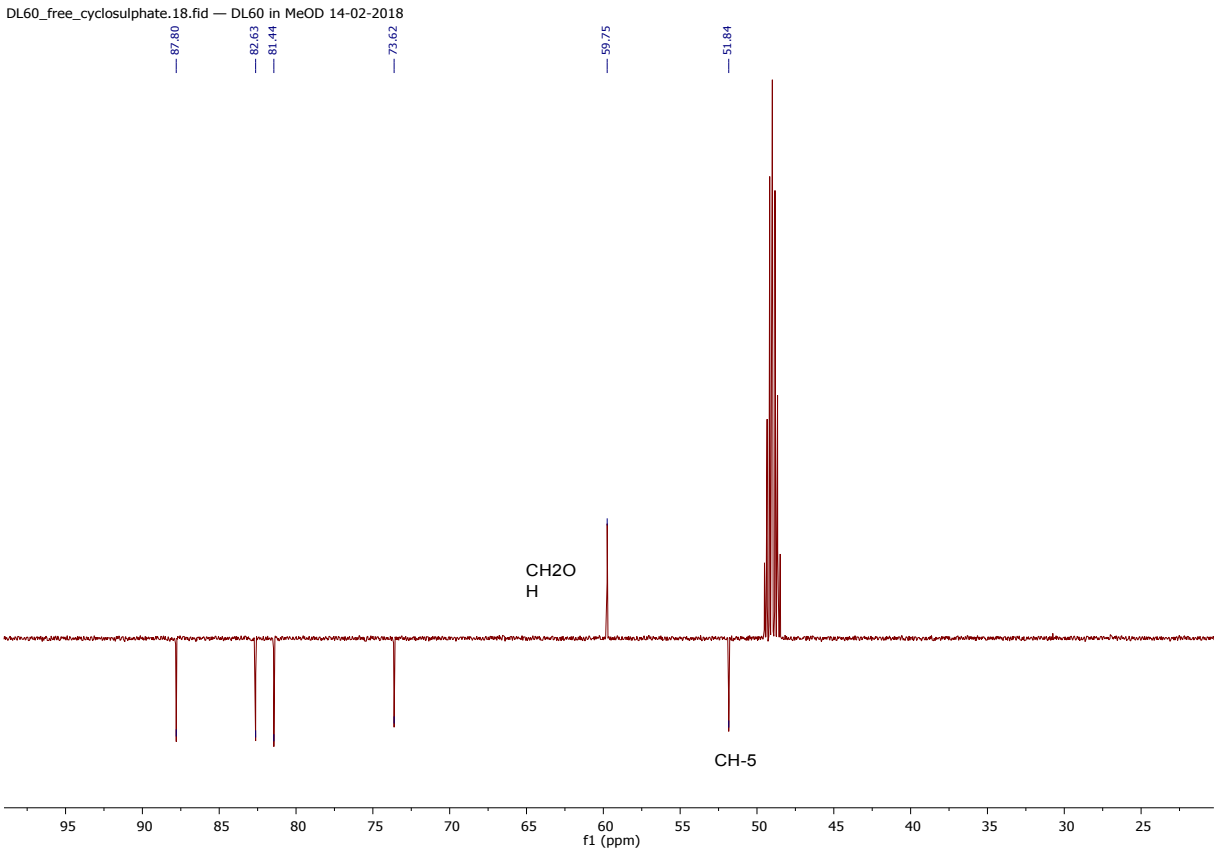
^{1}H-NMR and ^{13}C-NMR spectra of **5** in $MeOD_4$

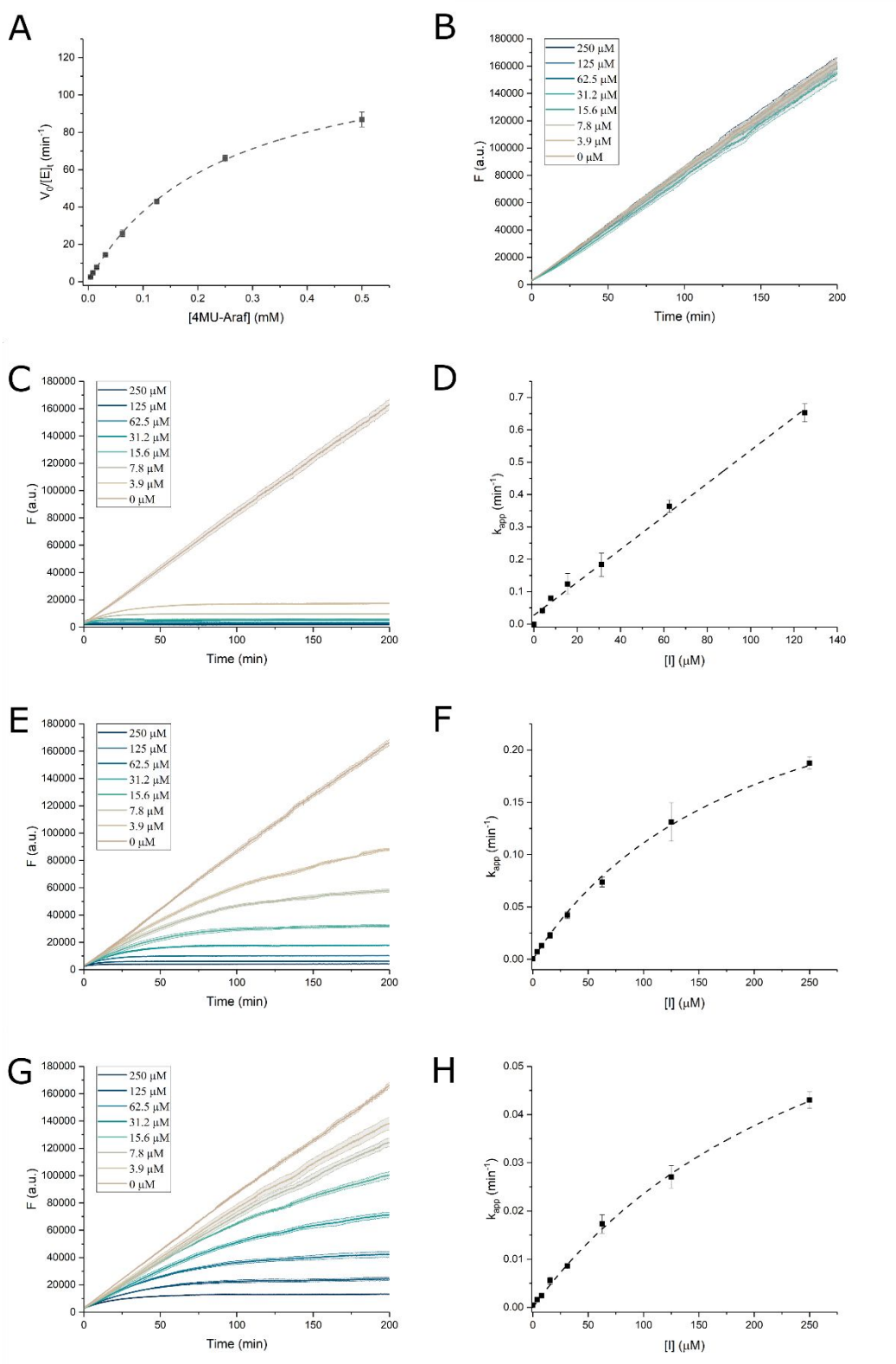




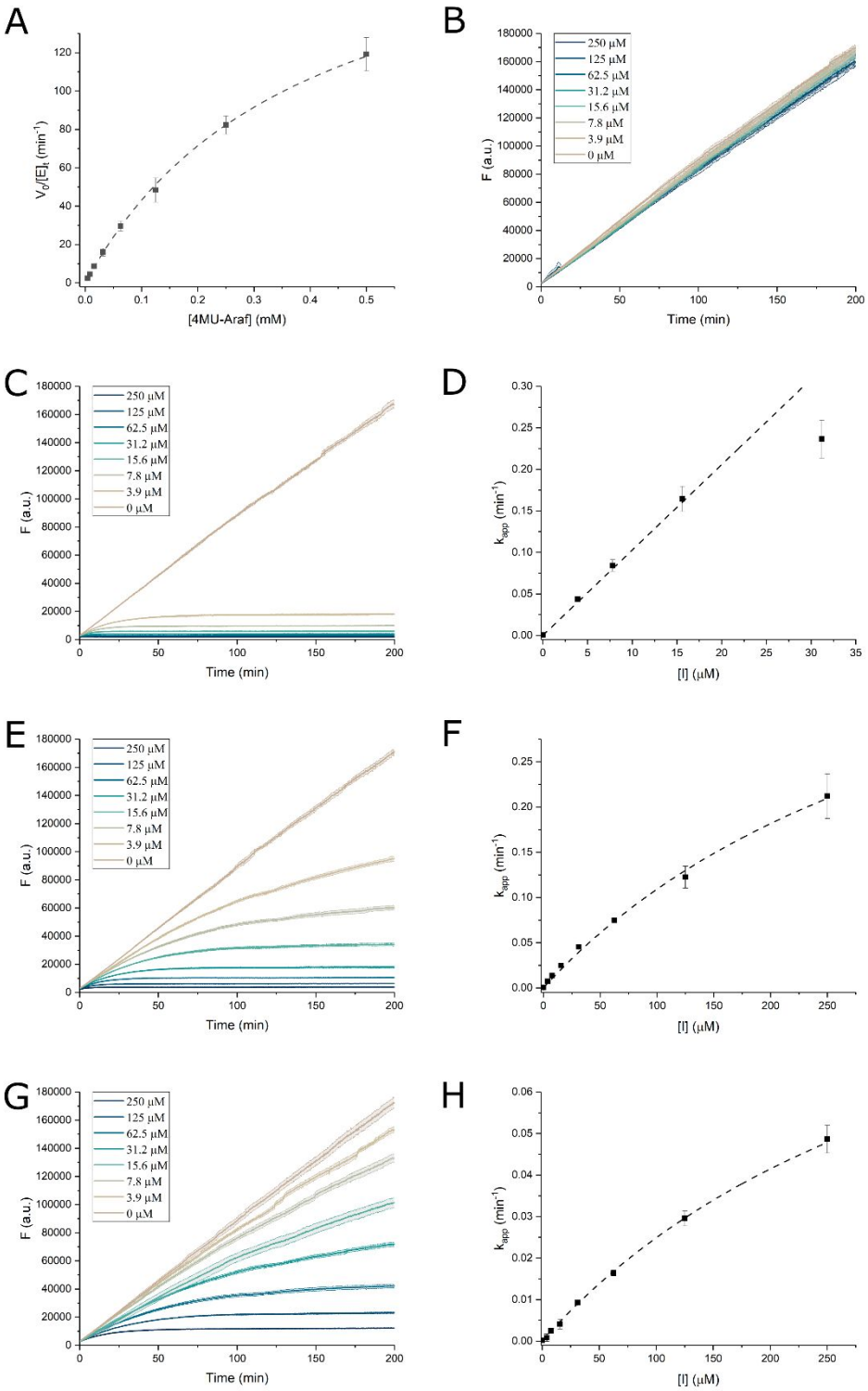
^{1}H-NMR and ^{13}C-NMR spectra of **6** in MeOD



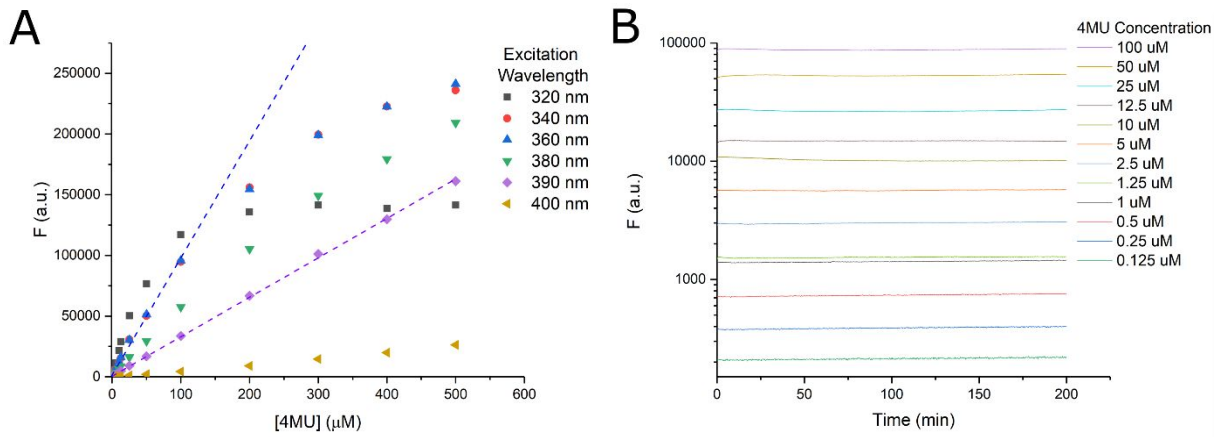




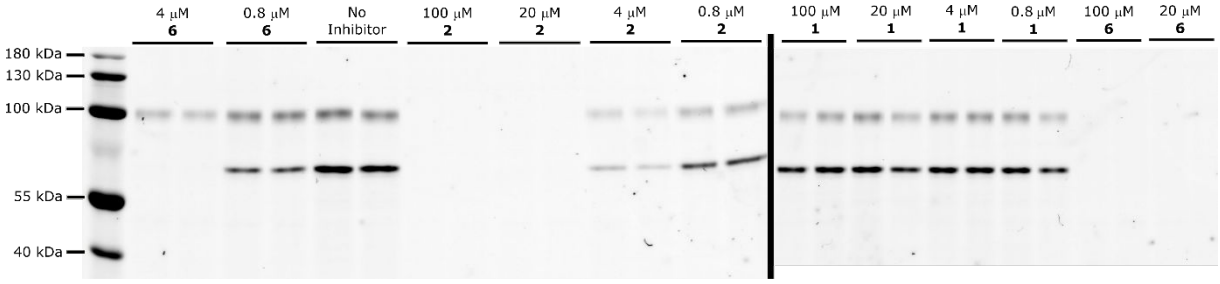
Supplemental Figure 1: Inhibition kinetics of AnAbfA. A) Plot of rate vs. substrate concentration measured for AnAbfA hydrolyzing 4MU-Araf with the hyperbolic fit shown as a dotted line. B) Plot of fluorescence vs. time for AnAbfA in the presence of difference concentrations of inhibitor **1**. Each line represents the average of four measurements with the standard deviation represented as thinner lines above and below the average. C) Plot of fluorescence vs. time for AnAbfA in the presence of difference concentrations of inhibitor **6** as shown in panel B. D) Plot of apparent decay constant (k_{app}) extracted from an exponential decay fit of the curves shown in panel C vs. inhibitor concentration with the hyperbolic fit shown as a dotted line. Error bars are the standard deviation of the k_{app} values extracted from each of the four measured curves. Panels E and F are the same measurements as panels C and D, but made with inhibitor **2**. Panels G and H are the same measurements as panels C and D, but made with inhibitor **3**.



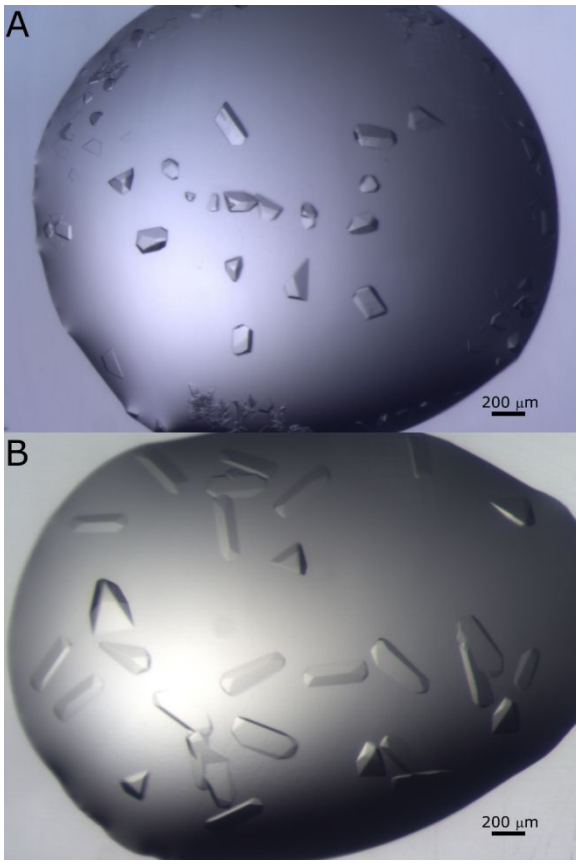
Supplemental Figure 2: Inhibition kinetics of AkAbfB. A) Plot of rate vs. substrate concentration measured for AkAbfB hydrolyzing 4MU-Araf with the hyperbolic fit shown as a dotted line. B) Plot of fluorescence vs. time for AkAbfB in the presence of difference concentrations of inhibitor **1**. Each line represents the average of four measurements with the standard deviation represented as thinner lines above and below the average. C) Plot of fluorescence vs. time for AkAbfB in the presence of difference concentrations of inhibitor **6** as shown in panel B. D) Plot of apparent decay constant (k_{app}) extracted from an exponential decay fit of the curves shown in panel C vs. inhibitor concentration with the hyperbolic fit shown as a dotted line. Error bars are the standard deviation of the kapp values extracted from each of the four measured curves. Panels E and F are the same measurements as panels C and D, but made with inhibitor **2**. Panels G and H are the same measurements as panels C and D, but made with inhibitor **3**.



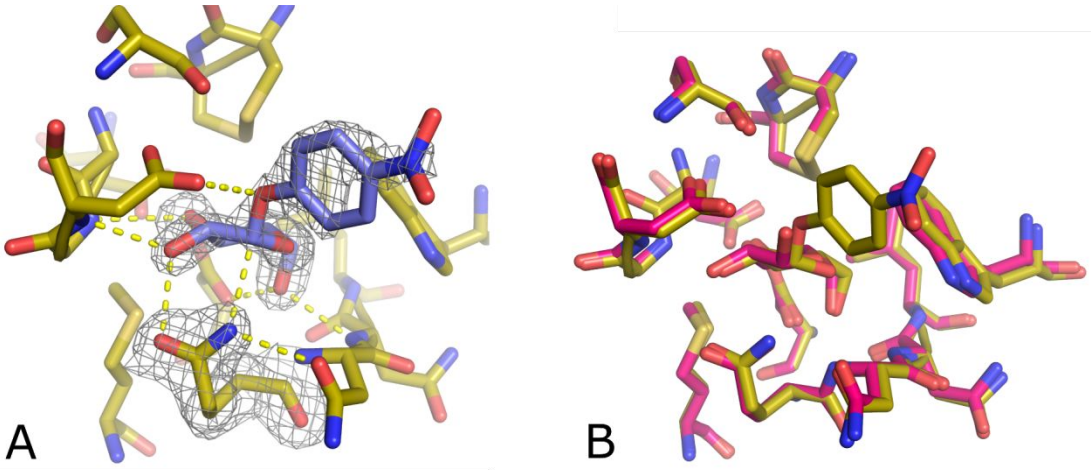
Supplemental Figure 3: Determination of the impact of inner filter effects and stability of fluorescence over the course of the inhibition measurement. A) Epifluorescence intensity vs. [4MU] measured in 40 µL total volume of pH 7 phosphate buffer in a black 384-well plate using different excitation wavelengths. A linear best fit for F vs. [4MU] from 0.125 to 100 µM at 360 nm excitation is shown as a dashed blue line. A linear best fit for F vs. [4MU] from 0.125 to 500 µM at 390 nm excitation is shown as a dashed purple line. B) 4MU epifluorescence intensity measured over 200 minutes with a 15 second cycle time using an excitation wavelength of 360 nm at 25°C for concentrations ranging from 0.125-100 µM in 40 µL of pH 7 phosphate buffer in a black 384-well plate, plotted on a log scale



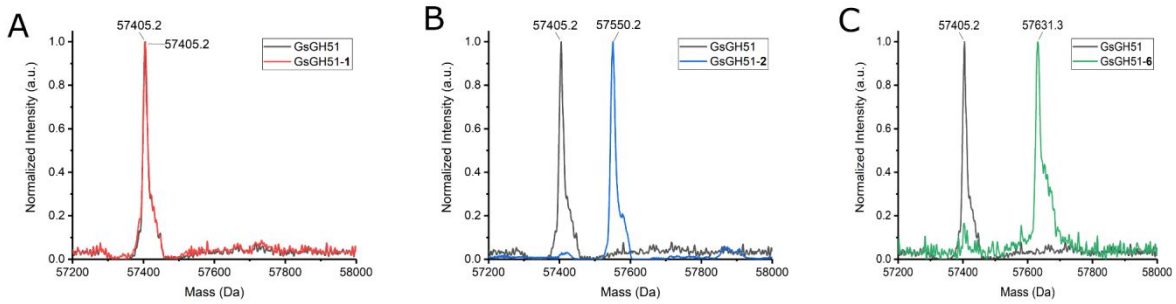
Supplemental Figure 4: Comparison of inhibitor pre-treatment efficiency for blocking α-L-arabinofuranosidase active sites. To identify conditions suitable for blocking α-L-arabinofuranosidase active sites prior to probe labelling, pretreatments with 0.8-100 µM of inhibitors **1**, **2**, or **6** were performed followed by staining with probe **4**. Each pretreatment and staining was run in duplicate and were loaded into the gel in replicate pairs. The compound number and concentration are given above each pair of lanes.



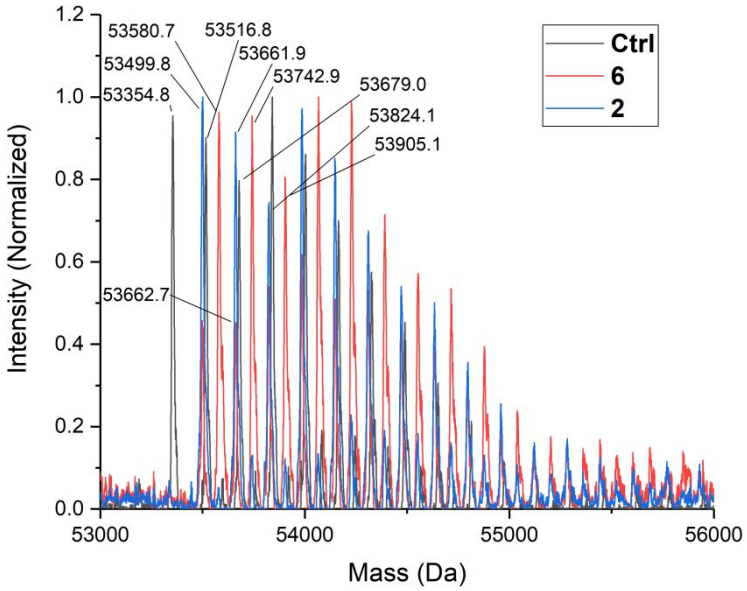
Supplemental Figure 5: A) Crystals of GsGH51 grown at 293 K from a mixture of 1200 nL of 8 mg/mL GsGH51 in 5 mM Tris, 1 mM EDTA, pH 8 mixed with 600 nL of 0.1 M Tris, pH 7.5, 5% 2-propanol, 0.7 M NH_4F, 20% PEG3350. B) Crystals of EndoH-deglycosylated AkAbfB grown at 293 K from a mixture of 1200 nL of 10 mg/mL AkAbfB in 20 mM pH 5.0 sodium acetate mixed with 600 nL of 50% PEG400, 0.4 M Li_2SO_4, 100 mM sodium acetate, pH 4.5.



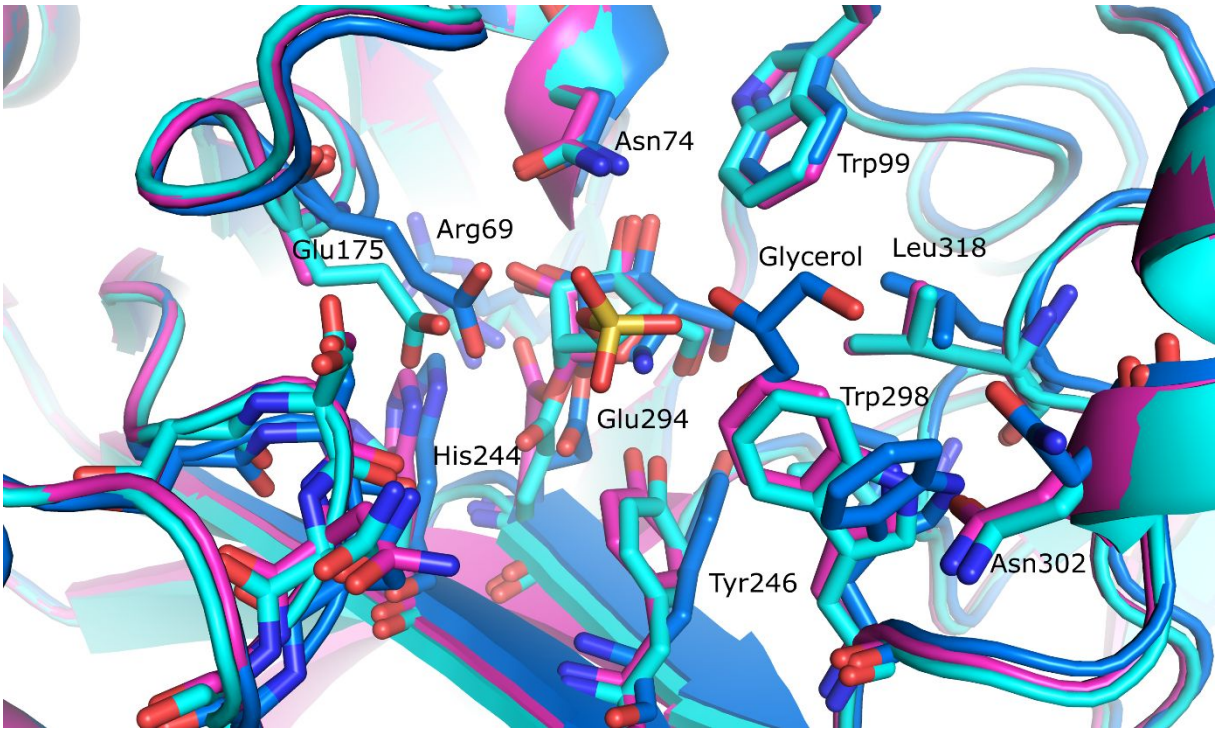
Supplemental Figure 6: Structure of the AkAbfB (E221Q) PNP-Araf Michaelis complex. A) Electron density of the PNP-Araf molecule (blue) within the AkAbfB (E221Q) active site (yellow) contoured to 1σ. Apparent hydrogen bonding interactions are shown as yellow dotted lines. B) Superposition of the AkAbfB (E221Q) Michaelis complex (yellow) with the AkAbfB product complex (fuchsia, PDBID: 1WD4).



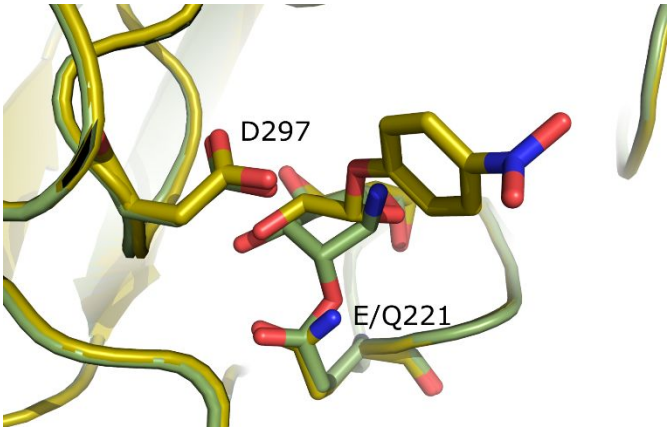
Supplemental Figure 7: Deconvoluted intact MS measurements of GsGH51 before and after treatment with inhibitors **1** (A), **2** (B) and **6** (C). Predicted mass = 57406.3, expected Δm (**1**) = 146, expected Δm (**2**) = 145, expected Δm (**6**) = 226



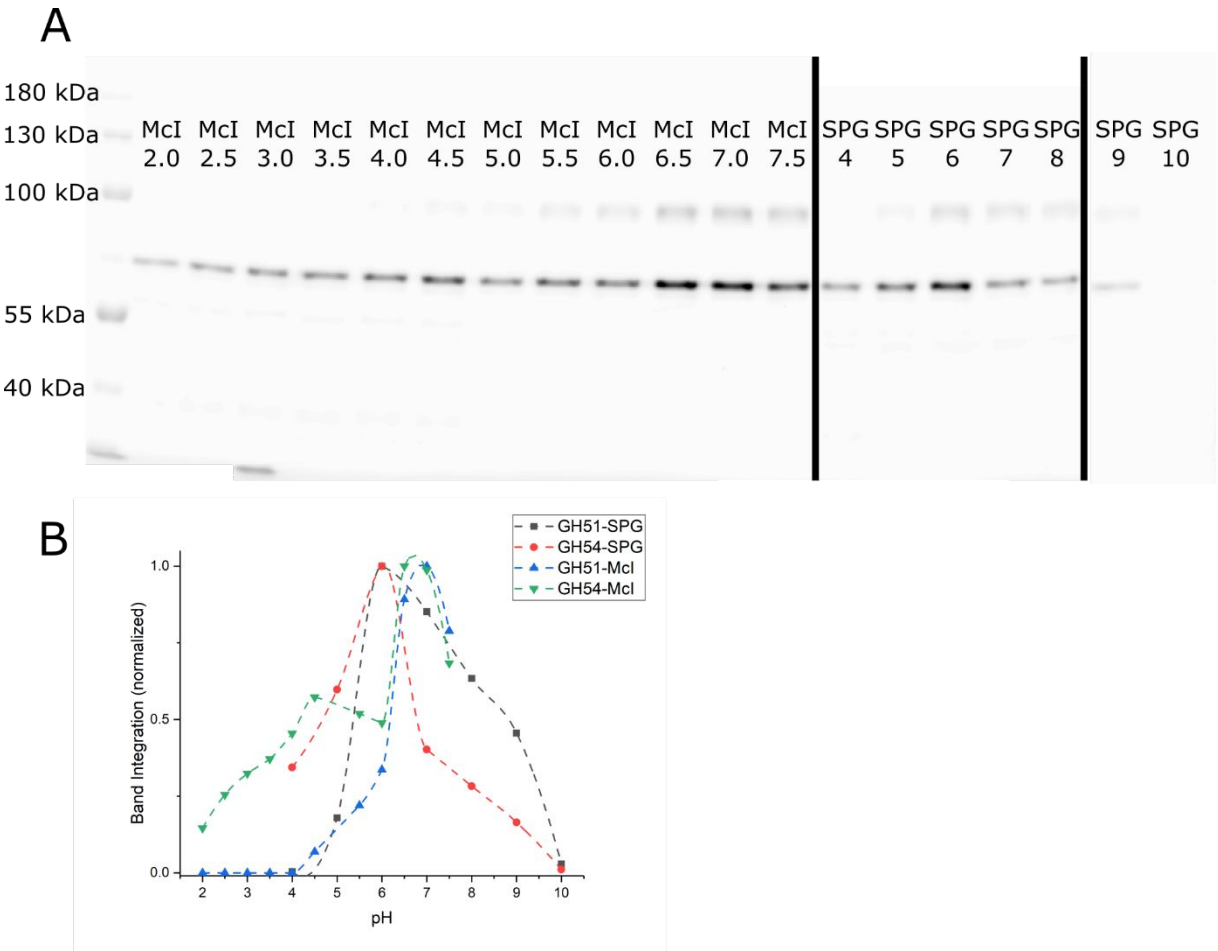
Supplemental Figure 8: Deconvoluted intact MS measurements of AkAbfB before and after treatment with inhibitors **2** and **6**. The predicted mass is unknown due to unknown glycosylation remaining after EndoH treatment. Expected Δm (**2**) = 145, expected Δm (**6**) = 226. Note the additional peak series in the **6**-treated sample, which corresponds to a loss of 80 Da, the mass of SO_3. We attribute this to in-source fragmentation.



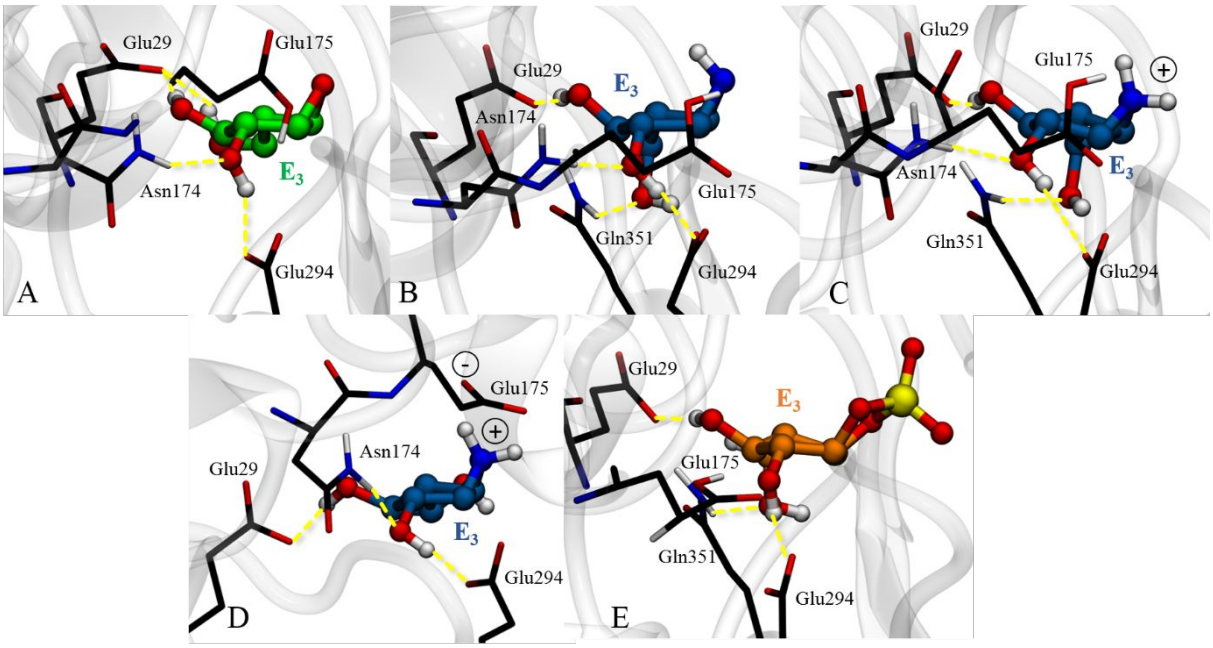
Supplemental Figure 9: Perturbation of the GsGH51 active site following reaction with **2**. The complex with **2** is shown in blue, the complex with **6** is shown in cyan, and the complex with arabinofuranose (PDBID: 1PZ2) is shown in fuchsia. With the exception of Asn74 and Trp99 (shown for reference), the ligand and the active site residues with significantly different positions in complex with **2** are shown as sticks.



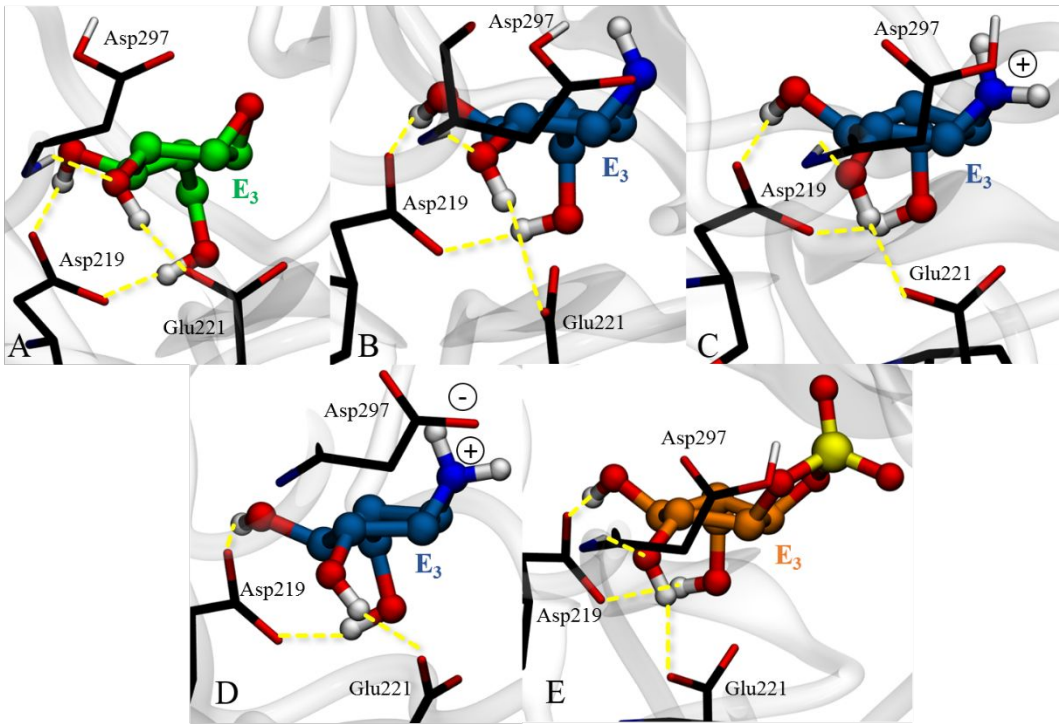
Supplemental Figure 10: Overlay of PNP-Araf and **2** in the active site of AkAbfB (E221Q) (gold) and AkAbfB (green), respectively, showing the displacement of the C1, C2, and O (or C6) positions between initial binding and the formation of the covalent intermediate.



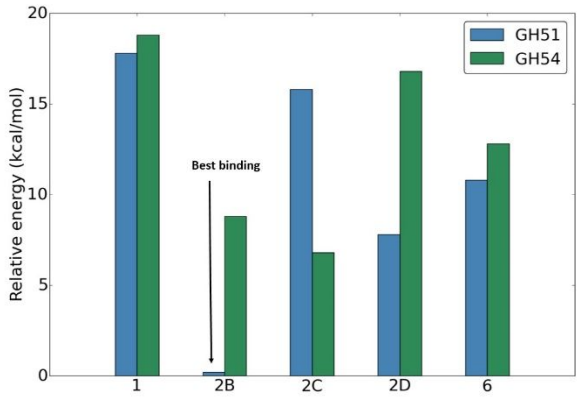
Supplemental Figure 11: Fluorescence image (Cy5) following SDS-PAGE separation of *A. niger* arabinan secretome treated with ABP **4** in buffers at different pH values. A) Three separate gels, which were imaged in parallel, are shown, separated by black lines. The buffer used during treatment with ABP **4** is labelled above each lane (McI for McIlvane buffer and SPG for SPG buffer). B) Plot of normalized integrated band intensity vs. pH for both the upper (GH51) and lower (GH54) bands in the McI and SPG buffer systems.



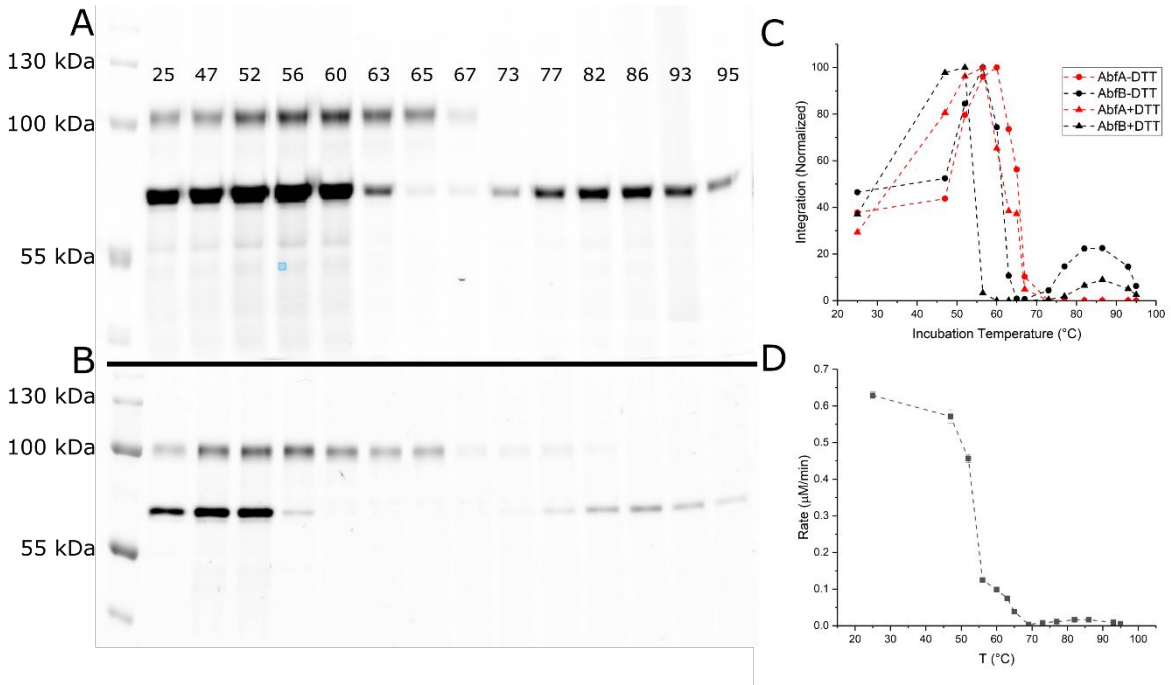
Supplemental Figure 12: Simulated optimized Michaelis complex configurations of inhibitors **1** (A), **2** (B, C, D), and **6** (E) used for the calculation of binding energy within the active site of GsGH51. B shows the protonated general acid/base with deprotonated aziridine nitrogen, C shows the protonated general acid/base with protonated aziridine nitrogen, D shows the deprotonated general acid/base with protonated aziridine nitrogen.



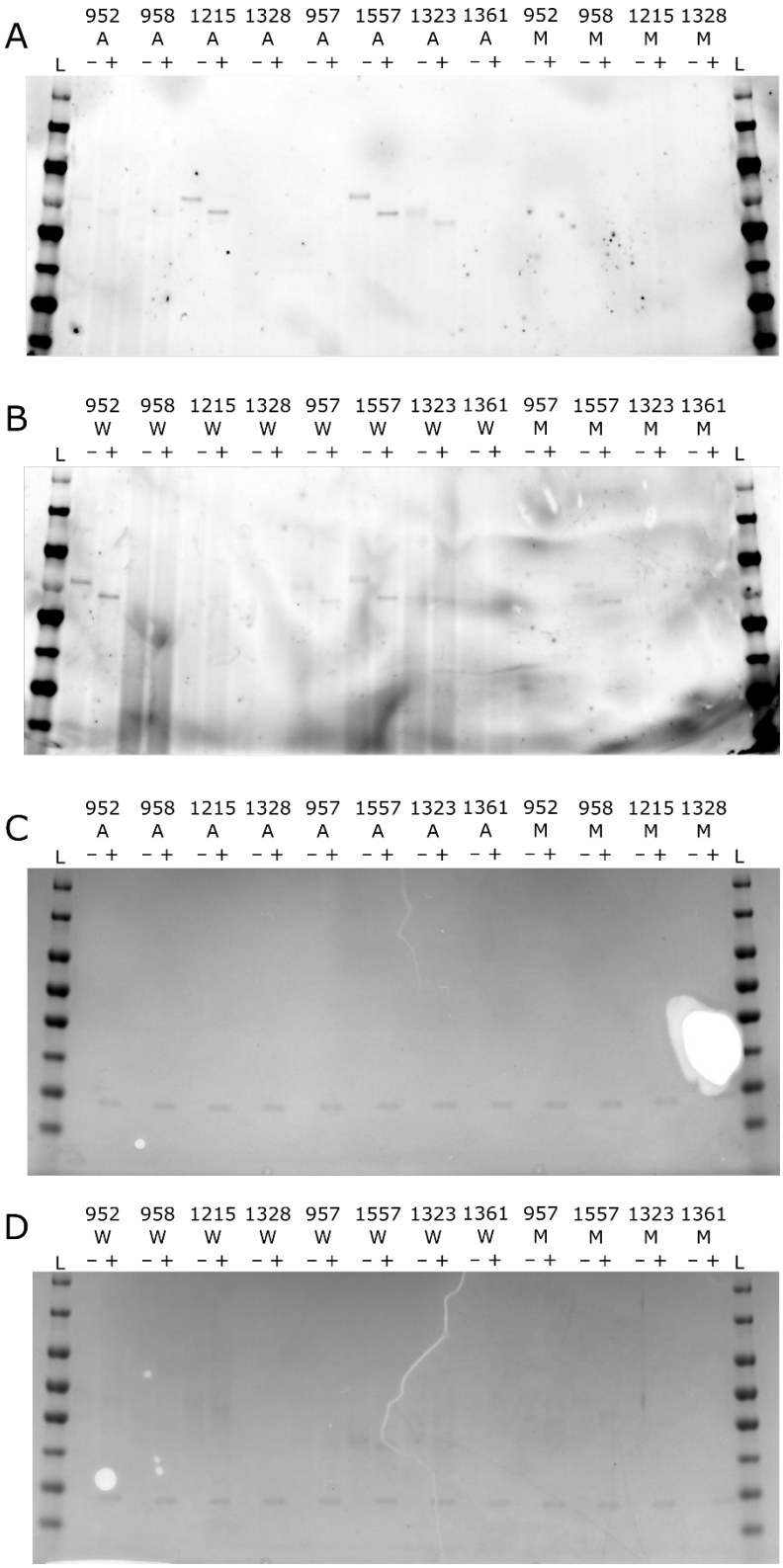
Supplemental Figure 13: Simulated optimized Michaelis complex configurations of inhibitors **1** (A), **2** (B, C, D), and **6** (E) used for the calculation of binding energy within the active site of AkAbfB. B shows the protonated general acid/base with deprotonated aziridine nitrogen, C shows the protonated general acid/base with protonated aziridine nitrogen, D shows the deprotonated general acid/base with protonated aziridine nitrogen,



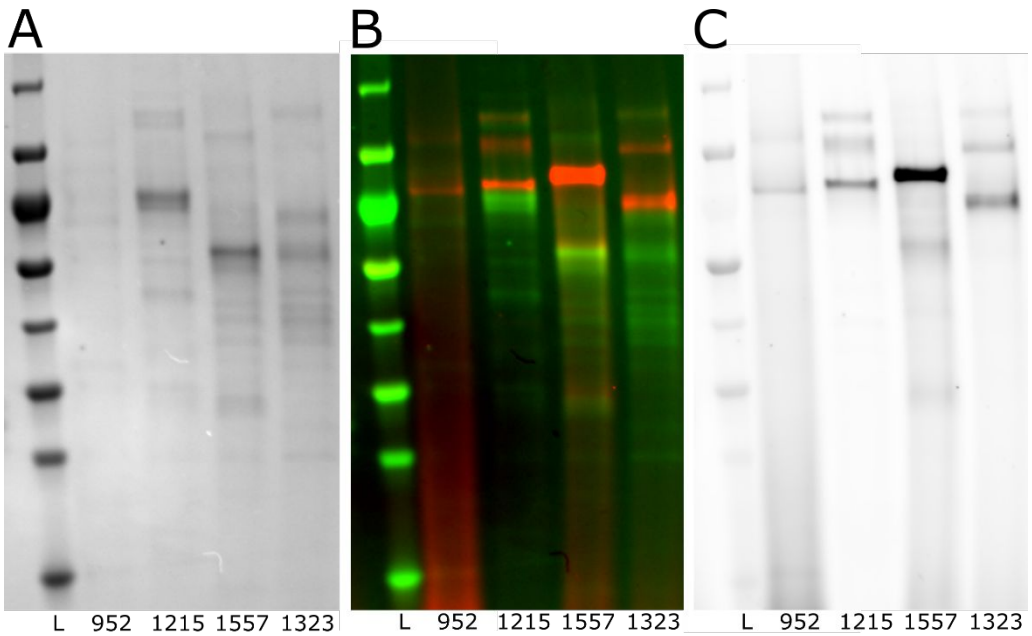
Supplemental Figure 14: Relative energies of the inhibitors binding to the active sites of GsGH51 and AkAbfB. Compounds **2**B, **2**C and **2**D corresponds to the different tested protonation states, following the labels used in Supplemental Figure 10 and 11. **2**B corresponds to protonated general acid/base with deprotonated aziridine nitrogen of **2**; **2**C corresponds to protonated general acid/base with protonated aziridine nitrogen of **2** and **2**D corresponds to deprotonated general acid/base with protonated aziridine nitrogen of **2**.



Supplemental Figure 15: A) Fluorescence image (Cy5) following SDS-PAGE separation of *A. niger* arabinan secretome treated with ABP **4** following incubation at different temperatures. The incubation temperature prior to labelling with ABP **4** is written above each lane in °C. B) Replication of the experiment shown in A, but with the addition of 5 mM DTT prior to incubation at temperature. C) Plot of normalized integrated fluorescence intensity for the AbfA (red) and AbfB (black) bands vs. pre-treatment temperature with (triangles) and without (circles) DTT. D) Plot of initial 4MU-Araf hydrolysis rate vs. pre-incubation temperature for 5 µL of arabinan secretome treated with DTT diluted into 45 µL of 50 µM 4MU-Araf in pH 6.5 phosphate buffer. Each point represents the average of 4 technical replicates with error bars representing the standard deviation.



Supplemental Figure 16: SDS-PAGE of basidiomycete secretomes. A, B) Cy5 fluorescence scans of basidiomycete secretomes treated with ABP **4** and separated on SDS-PAGE. Labels are shown above each lane. L indicates ladder, A indicates secretome grown on aspen pulp, W indicates secretome grown on wheat straw, M indicates secretome grown on maltose, + indicates treatment with PNGaseF, - indicates no treatment with PNGaseF, the number above each lane is the BRFM number for the strain from which the secretome was isolated. C, D) Coomassie staining of the same gels shown in A and B; labelling is identical.



Supplemental Figure 17: Basidiomycete secretomes stained with ABP **4**. Panel A is white light imaging following Coomassie staining, panel C is Cy5 fluorescence, and panel B is Cy5 fluorescence (red) superimposed on the Coomassie image (image inverted, contrast optimized and false coloured green). Lane 1 contains 5 µL of Pageruler 10-180 kDa prestained ladder. Lanes 2-5 are *T. gibbosa, A. biennis, L. menziesii,* and *F. fomentarius,* respectively.

Supplemental Tables

Supplemental Table 1: Kinetic parameters determined for the hydrolysis of 4MU-α-L-arabinofuranoside by AnAbfA and AkAbfB

	K_M (μM)	k_{cat} (min^{-1})	k_{cat}/K_M ($s^{-1}M^{-1}$)
AnAbfA (GH51)	240±20	130±10	9000
AkAbfB (GH54)	390±30	210±10	9000

Supplemental Table 2: Data collection and refinement statistics (molecular replacement)

	GsGH51		AkGH54		AkGH54 (E221Q)
	Complex with **2** (PDB 6SXV)	Complex with **6** (PDB 6SXU)	Complex with **2** (PDB 6SXT)	Complex with **6** (PDB 6SXS)	PNP-Araf Complex (PDB 6SXR)
Data collection					
Space group	H3	H3	$H3_2$	$H3_2$	$H3_2$
a, *b*, *c* (Å)	178.994 178.994 100.893	178.470 178.470 100.411	112.232 112.232 342.730	111.136 111.136 342.742	111.970 111.970 341.390
α, β, γ (°)	90.000 90.000 120.000	90.000 90.000 120.000	90.000 90.000 120.000	90.000 90.000 120.000	90.000 90.000 120.000
Resolution (Å)	29.83-1.40 (1.44-1.40)*	29.75-1.40 (1.42-1.40)	84.54-1.47 (1.49-1.47)	83.92-1.86 (1.89-1.86)	93.28-1.64 (1.67-1.64)
R_{meas}	0.092 (0.748)	0.058 (0.765)	0.109 (3.128)	0.133 (5.537)	0.129 (2.319)
$I / \sigma I$	10.8 (1.5)	18.7 (2.3)	13.0 (0.7)	10.2 (0.5)	12.5 (1.0)
Completeness (%)	99.5 (94.5)	99.8 (97.4)	99.3 (98.5)	100 (100)	98.2 (97.0)
Redundancy	6.2 (5.8)	6.2 (6.1)	12.4 (10.7)	12.1 (12.8)	12.6 (12.2)
Refinement					
Resolution (Å)	29.83-1.40	29.75-1.40	84.69-1.47	84.06-1.86	93.45-1.64
No. reflections	235420	235394	141273	68786	99210
R_{work} / R_{free}	0.124/0.154	0.115/0.143	0.157/0.179	0.181/0.215	0.159/0.181
No. atoms					
Protein	7951	8960	3628	3568	3572
Ligand/ion	110	77	202	131	260
Water	869	913	456	236	352
B-factors					
Protein	20.7	17.1	20.9	43.7	26.8
Ligand/ion	33.1	28.0	42.1	58.5	50.4
Water	31.9	30.1	34.3	49.4	37.6
R.m.s. deviations					
Bond lengths (Å)	0.019	0.017	0.016	0.012	0.014
Bond angles (°)	2.17	2.00	1.94	1.77	1.87

*Values in parentheses are for highest-resolution shell.

Supplemental Table 3: Basidiomycete strains screened for α-L-arabinofuranosidase production

Strain	BRFM ID	Classification	Origin	Ref
Trametes gibbosa	952	Basidiomycota, Polyporaceae	France, Ariège, Moulis, Beech Forest	1
Polyporus brumalis	958	Basidiomycota, Polyporaceae	France, Hautes Pyrénées, Puydarieux	2
Abortiporus biennis	1215	Basidiomycota, Meruliaceae	France, Orne	3
Hexagonia nitida	1328	Basidiomycota, Polyporaceae	France, Vaucluse	3
Trametes ljublarskyi	957	Basidiomycota, Polyporaceae	France	3,4
Leiotrametes menziesii	1557	Basidiomycota, Polyporaceae	Martinique (Island)	3
Fomes fomentarius	1323	Basidiomycota, Polyporaceae	France, Corsica	3
Trametes Meyenii	1361	Basidiomycota, Polyporaceae	India	3

Supplemental References

(1) Berrin, J. G.; Navarro, D.; Couturier, M.; Olivé, C.; Grisel, S.; Haon, M.; Taussac, S.; Lechat, C.; Courtecuisse, R.; Favel, A.; Coutinho, P. M.; Lesage-Meessen, L. Exploring the Natural Fungal Biodiversity of Tropical and Temperate Forests toward Improvement of Biomass Conversion. *Appl. Environ. Microbiol.* **2012**, *78* (18), 6483–6490. https://doi.org/10.1128/AEM.01651-12.

(2) Miyauchi, S.; Rancon, A.; Drula, E.; Hage, H.; Chaduli, D.; Favel, A.; Grisel, S.; Henrissat, B.; Herpoël-Gimbert, I.; Ruiz-Dueñas, F. J.; Chevret, D.; Hainaut, M.; Lin, J.; Wang, M.; Pangilinan, J.; Lipzen, A.; Lesage-Meessen, L.; Navarro, D.; Riley, R.; Grigoriev, I. V.; Zhou, S.; Raouche, S.; Rosso, M.-N. Integrative Visual Omics of the White-Rot Fungus Polyporus Brumalis Exposes the Biotechnological Potential of Its Oxidative Enzymes for Delignifying Raw Plant Biomass. *Biotechnol. Biofuels* **2018**, *11* (1), 201. https://doi.org/10.1186/s13068-018-1198-5.

(3) Zhou, S.; Raouche, S.; Grisel, S.; Navarro, D.; Sigoillot, J. C.; Herpoël-Gimbert, I. Solid-State Fermentation in Multi-Well Plates to Assess Pretreatment Efficiency of Rot Fungi on Lignocellulose Biomass. *Microb. Biotechnol.* **2015**, *8* (6), 940–949. https://doi.org/10.1111/1751-7915.12307.

(4) Paës, G.; Navarro, D.; Benoit, Y.; Blanquet, S.; Chabbert, B.; Chaussepied, B.; Coutinho, P. M.; Durand, S.; Grigoriev, I. V.; Haon, M.; Heux, L.; Launay, C.; Margeot, A.; Nishiyama, Y.; Raouche, S.; Rosso, M.-N.; Bonnin, E.; Berrin, J.-G. Tracking of Enzymatic Biomass Deconstruction by Fungal Secretomes Highlights Markers of Lignocellulose Recalcitrance. *Biotechnol. Biofuels* **2019**, *12* (1), 76. https://doi.org/10.1186/s13068-019-1417-8.