Supporting Information for:

Probing the Aglycon Specificity of an Engineered Glycosyltransferase

Richard W. Gantt, Randal D. Goff, Gavin J. Williams, and Jon S. Thorson*

University of Wisconsin National Cooperative Drug Discovery Group, Laboratory for Biosynthetic Chemistry, School of Pharmacy, 777 Highland Avenue, Madison, WI 53705, USA
E-mail: jsthorson@pharmacy.wisc.edu

Table of Contents

1. Full citations of references [13], [22], [30], and [35].

[13] M. Veyhl, K. Wagner, C. Volk, V. Gorboulev, K. Baumgarten, W. Weber, M. Schaper, B. Bertram, M. Wiessler, H. Koepsell, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 2914-2919.

[22] J. P. Holland, F. I. Aigbirhio, H. M. Betts, P. D. Bonnitcha, P. Burke, M. Christlieb, G. C. Churchill, A. R. Cowley, J. R. Dilworth, P. S. Donnelly, J. C. Green, J. M. Peach, S. R. Vasudevan, J. E. Warren, *Inorg. Chem.* **2007**, *46*, 465-485.

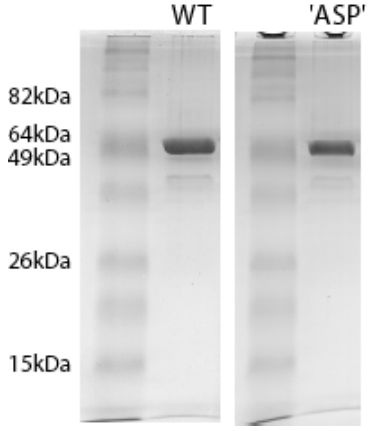
[30] C. Zhang, C. Albermann, X. Fu, N. R. Peters, J. D. Chisholm, G. Zhang, E. J. Gilbert, P. G. Wang, D. L. Van Vranken, J. S. Thorson, *ChemBioChem* **2006**, *7*, 795-804.

[35] D. N. Bolam, S. Roberts, M. R. Proctor, J. P. Turkenburg, E. J. Dodson, C. Martinez-Fleites, M. Yany, B. G. Davis, G. J. Davies, H. J. Gilbert, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 5336-5341.

2. General methods. All chemicals and reagents were purchased from Sigma-Aldrich, Fluka, or New England Biolabs unless otherwise stated. In addition to the previously reported putative aglycons [(**11**, **118**-**119**)[S1] (**14**, **32**, and **38**)[S2] (**45**, **70** and **139**)[S3] **55**,[S4] **73**[S5] and **132**-**136**[S6]], **29**, **50**, and **56** were a gift from Prof. Joe Langenhan, Seattle Univ.; **39** was a gift from Prof. Ben Shen, Univ. of Wisconsin-Madison; **74** and **127** were a gift from Prof. Dr. Sarah O'Connor, Massachusetts Inst. of Tech., USA; **85** was a gift from Prof. Dr. Michael Thomas, Univ. of Wisconsin-Madison; **105-112** were a gift from Prof. Dr. David L. Jakeman, Dalhousie University, Nova Scotia; **41** and **64** were isolated from fermentation; and the synthesis of **16** and **136** (Timmons, S. unpublished) will be reported elsewhere. Plasmid pET28/OleD was obtained from Prof. Hung-Wen Liu (Univ. of Texas-Austin, Austin, USA). LC/ESI-MS mass spectra were obtained using electrospray ionization on an Agilent 1100 HPLC-MSD SL quadrupole mass spectrometer connected to a UV/Vis diode array detector. High resolution mass spectra were determined utilizing electrospray ionization on a Waters (Micromass) LCT instrument (Beverly, MA, USA) with a time-of-flight analyzer and all HRMS samples contained an aliquot of a known compound (lock mass). Routine TLC analyses were accomplished on aluminum TLC plates coated with 0.2 mm silica gel from Sigma-Aldrich and monitoring at 254 nm. Flash column chromatography was achieved on 40 – 63 μm, 60 Å silica gel (Silicycle, Quebec, Canada). Unless otherwise noted, compounds were characterized by NMR with a ^{UNITY}INOVA 400 MHz instrument (Varian, Palo Alto, CA, USA) in conjunction with a QN Switchable BB probe (Varian, Palo Alto, CA, USA). ^{1}H and ^{13}C chemical shifts were referenced to internal solvent resonances and reported relative to TMS. Multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), qn (quintet), m (multiplet) and br (broad). Italicized elements or groups are those that are responsible for the shifts. Chemical shifts are reported in parts per million (ppm) and coupling constants *J* are given in Hz. NMR assignments were performed with the aid of COSY, TOCSY, HSQC, and HMBC experiments.

3. Protein expression and purification. Single colonies of *E. coli* BL21(DE3)pLysS (Stratagene, La Jolla, CA, USA) transformed with either pET28a/OleD or pET28a/OleD[A242V/S132F/P67T] vector[S7] were used to inoculate 3 mL LB medium supplemented with 50 μg mL^{-1} kanamycin and cultured overnight at 37°C. The entire starter culture was then transferred to 1 L LB medium supplemented with 50 μg mL^{-1} kanamycin and grown at 37°C until the OD600 reached ~0.7. Isopropyl β-D-thiogalactoside (IPTG) was subsequently added to a final concentration of 0.4 mM and the culture was incubated at 28ºC for approximately 18 hours. Cell pellets were collected by centrifugation at 10,000 *g* at 4°C for 20 min and the supernatant discarded. Pellets were resuspended in 10 mL lysis buffer (20 mM phosphate buffer, pH 7.4, 0.5 M NaCl, 10 mM imidazole) and were lysed by sonication. Cell debris was removed by centrifugation at 10,000 *g* at 4°C for 20 min and the cleared supernatant immediately applied to 2 mL of nickel nitrilotriacetic acid (Ni-NTA) resin (QIAgen

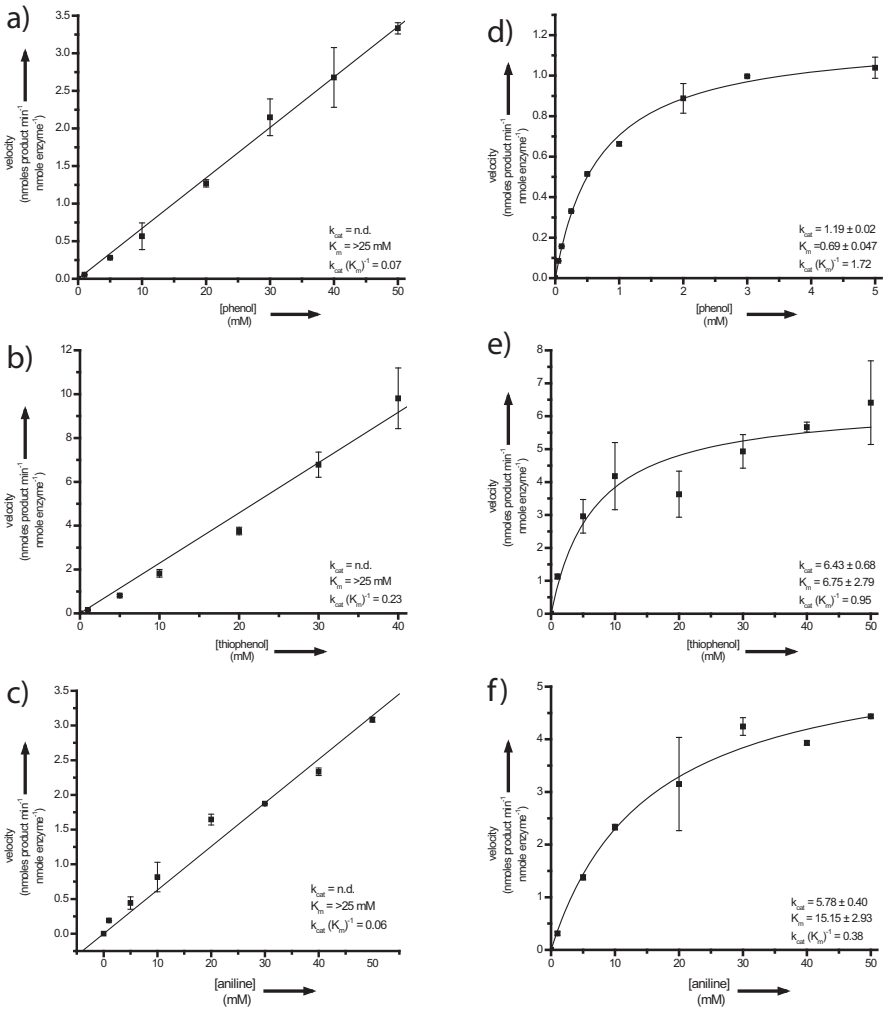
Valencia, CA, USA) pre-equilibrated with lysis buffer. Protein was allowed to bind for 30 min at 4°C with gentle agitation, and the resin washed with 50 mL lysis buffer (x 4). Finally, the enzyme was eluted by incubation of the resin with 2 mL lysis buffer containing 250 mM imidazole for 10 min at 4°C with gentle agitation. The purified protein was applied to a PD-10 desalting column (Amersham Biosciences, Piscataway, NJ, USA), equilibrated with 50 mM Tris-HCl (pH 8.0), and eluted as described by the manufacturer. Protein aliquots were immediately flash frozen in liquid nitrogen and stored at -80°C. Protein purity was confirmed by SDS-PAGE to be >95% and protein concentration for all studies was determined using the Bradford Protein Assay Kit from Bio-Rad (Hercules, CA, USA).



Supplementary Figure 1. SDS-PAGE gel of purified WT and 'ASP' OleD. Molecular weights for the standard ladder are labeled at left.

4. Acceptor library screening. Reactions were conducted in a final volume of 100 µL and contained 50 µg of purified enzyme, 2.5 mM UDP-glucose, 50 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, and 1 mM of aglycon unless otherwise noted. Two separate control reactions for each aglycon that withheld either enzyme or UDP-glucose were performed in parallel. Reactions were allowed to proceed at 25 ^{o}C for ~16 hr, quenched with an equal volume of MeOH, centrifuged at 10,000 *g* for 10 min and the supernatant removed for analysis. The clarified reaction mixtures were analyzed by analytical reverse-phase HPLC with a 250 mm x 4.6 mm Gemini 5µ C18 column (Phenomenex, Torrance, CA, USA) using various methods (See **Supp. Info. 8.2**.). HPLC peak areas were integrated with Star Chromatography Workstation Software (Varian, Palo Alto, CA, USA) and the total percent conversion calculated as a percent of the total peak area of substrate and product(s). Reactions which displayed potential new product(s) via HPLC were further analyzed by tandem LC/ESI-MS using a 250 mm x 4.6 mm Gemini 5µ C18 analytical column. If both WT and 'ASP' reactions resulted in chromatographically identical product(s), only the reaction with the highest percent conversion was analyzed via LC-MS. Chromatographic methods, reactant and product retention times, and MS determinations for all positive substrates are summarized in **Supp. Info. 8.1**-**8.2**.

5. Determination of Kinetic Parameters. Assays were performed in a final volume of 200 µL 50 mM Tris-HCl (pH 8.0), and contained constant concentrations of enzyme (40 µg for 'ASP' reactions or 120 µg for WT reactions) and saturating UDP-glucose (2.5 mM) while varying the phenol (**8**), thiophenol (**6**), or aniline (**34**) concentration (0-50 mM). Aliquots (50 µL) were removed at 10 min (where the rate of product formation was determined to be linear), mixed with an equal volume of ice cold MeOH, and centrifuged at 10,000 *g* for 10 min. Supernatants were analyzed by analytical reverse-phase HPLC as described in the preceding section. HPLC peak areas were integrated with Star Chromatography Workstation Software (Varian, Palo Atlo, CA, USA) and the total percent conversion calculated as a percent of the total peak area of substrate and product(s). All experiments were performed in triplicate. Initial velocities were fitted to the Michaelis-Menten equation using Origin Pro 7.0 software. OleD wild-type enzyme could not be saturated with acceptors **8**, **6**, and **34.** Consequentially, k_{cat}/K_m for wild-type was determined by linear regression.



Supplementary Figure 2. Determination of kinetic parameters for WT and 'ASP' OleD; experiments were performed in triplicate with standard deviation noted. WT with varied acceptors (a) **8**, (b) **6**, and (c) **34**. 'ASP' with varied acceptor (d) **8**, (e) **6**, and (f) **34**.

5.1. Supplementary Table 1. Kinetic parameters determined for WT and 'ASP' OleD with phenol (**8**), thiophenol (**6**), or aniline (**34**) and saturating [UDP-Glc].

Substrate	Enzyme	k_{cat} (min^{-1})	K_m (mM)	k_{cat} / K_m (mM^{-1}min^{-1})	$(k_{cat} / K_m)_{'ASP'}/(k_{cat} / K_m)_{WT}$
phenol	WT	n.a.[a]	n.a.	0.07	---
(**8**)	'ASP'	1.19 ± 0.02	0.69 ± 0.05	1.72	24.6
thiophenol	WT	n.a.	n.a.	0.23	---
(**6**)	'ASP'	6.43 ± 0.68	6.75 ± 2.79	0.95	4.1
aniline	WT	n.a.	n.a.	0.08	---
(**34**)	'ASP'	5.78 ± 0.40	15.15 ± 2.93	0.38	4.8

[a] n.a., not available

6. Scale-Up and Characterization of Representative Glucosides.

6.1. General Reaction Procedure. Reactions were conducted in 10 mL Tris-HCl (50 mM, pH 8.0) containing substrate **6**, **8**, or **24** (20 mM), UDP-glucose (100 mM), and $MgCl_2$ (5 mM) at 25 ^{o}C with agitation. Aliquots of 'ASP' enzyme (4.4 mg) were added to each reaction at 0, 7,

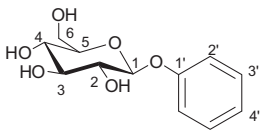
and 19 hours. The reactions allowed to proceed for a total of 42 hours and were subsequently frozen (-80ºC), lyophilized, resuspended in 2 mL of ice cold MeOH, and filtered. Glucosides were isolated by collecting fractions from analytical reverse-phase HPLC with a 250 mm x 4.6 mm Gemini 5µ C18 column (Phenomenex, Torrance, CA, USA) using the appropriate method (**Supp. Info. 8.2**). The product-containing fractions were subsequently collected and lyophilized. Products were confirmed by HRMS as previously described and via ^{1}H and ^{13}C NMR using a Varian ^{UNITY}INOVA 500 MHz instrument (Palo Alto, CA, USA) with a Protasis/MRM CapNMR capillary probe (Savoy, IL, USA). ^{13}C assignments were attained through gHSQC and gHMBC methods.

6.2. Phenyl β-D-glucopyranoside (140). ^{1}H NMR (CD_3OD, 500 MHz) δ 7.32 (m, 2 H, $H_{3'}$), 7.14 (d, J = 8.2 Hz, 2 H, $H_{2'}$), 7.05 (m, 1 H, $H_{4'}$), 4.96 (d, J = 6.7 Hz, 1 H, H_1), 3.94 (d, J = 12.0 Hz, 1 H, H_{6A}), 3.75 (dd, J = 12.0, 5.0 Hz, 1 H, H_{6B}), 3.55-3.45 (m, 4 H, $H_{2\text{-}5}$); ^{13}C NMR (CD_3OD, 500 MHz) δ 158.0 ($C_{1'}$), 129.3, ($C_{3'}$), 122.3 ($C_{4'}$), 116.6 ($C_{2'}$), 101.2 (C_1), 76.9 (C_5), 73.8 (C_2), 70.3 (C_4), 63.4 (C_3), 61.4 (C_6); HRMS-ESI (*m/z*): $[M+Na]^+$ calcd for $C_{12}H_{16}NaO_6$, 279.0840; found 279.0833.

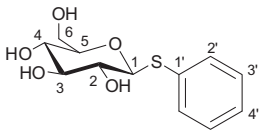
6.3. Phenyl 1-thio-β-D-glucopyranoside (141). ^{1}H NMR (CD_3OD, 500 MHz) δ 7.64 (d, J = 7.3 Hz, 2 H, $H_{2'}$), 7.38 (m, 2 H, $H_{3'}$), 7.35-7.30 (m, 1 H, $H_{4'}$), 4.68 (d, J = 9.6 Hz, 1 H, H_1), 3.95 (d, J = 12.0 Hz, 1 H, H_{6A}), 3.74 (dd, J = 12.0, 5.2 Hz, 1 H, H_{6B}), 3.46 (m, 1 H, H_3), 3.43-3.24 (m, 2 H, $H_{4,5}$), 3.29 (m, 1 H, H_2); ^{13}C NMR (CD_3OD, 500 MHz) δ 134.2 ($C_{1'}$), 131.5 ($C_{2'}$), 128.8 ($C_{3'}$), 127.2 ($C_{4'}$), 88.2 (C_1), 80.9 (C_4), 78.6 (C_3), 72.6 (C_2), 70.2 (C_5), 61.7 (C_6); HRMS-ESI (*m/z*): $[M+Na]^+$ calcd for $C_{12}H_{16}NaO_5S$, 295.0611; found 295.0624.

6.4. Phenyl 1-amino-β-D-glucopyranoside (142). ^{1}H NMR (CD_3OD, 500 MHz) δ 7.16 (m, 2 H, $H_{3'}$), 6.81 (d, J = 7.6 Hz, 2 H, $H_{2'}$), 6.73 (m, 1 H, $H_{4'}$), 4.57 (d, J = 7.8 Hz, 1 H, H_1), 3.87 (d, J = 11.7 Hz, 1 H, H_{6A}), 3.73-3.66 (m, 1 H, H_{6B}), 3.49 (t, J = 7.9 Hz, 1 H, H_3), 3.44-3.33 (m, 3 H, $H_{2,4,5}$); ^{13}C NMR (CD_3OD, 500 MHz) δ 147.0 ($C_{1'}$), 128.8, ($C_{3'}$), 118.4 ($C_{4'}$), 113.9 ($C_{2'}$), 85.8 (C_1), 77.9 (C_3), 77.2 (C_5), 73.5 (C_4), 70.7 (C_2), 61.6 (C_6); HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{12}H_{16}NNaO_5$, 278.0999; found 278.1006.

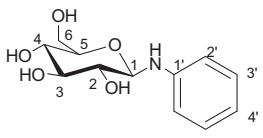
6.5. Phenyl β-D-glucopyranose-(1→2)-1-thio-β-D-glucopyranoside (143). ^{1}H NMR (acetone-d_6, 500 MHz) δ 7.61 (m, 2 H, $H_{2''}$), 7.33 (m, 2 H, $H_{3''}$), 7.28 (m, 1 H, $H_{4''}$), 4.77 (d, J = 9.8 Hz, 1 H, $H_{1'}$), 4.65 (d, J = 7.8 Hz, 1 H, H_1), 3.91-3.85 (m, 2 H, $H_{6A,6A'}$), 3.73-3.66 (m, 3 H, $H_{3',6B,6B'}$), 3.51-3.47 (m, 1 H, $H_{2'}$), 3.46-3.37 (m, 5 H, $H_{3\text{-}5,4',5'}$), 3.31-3.27 (m, 1 H, H_2); ^{13}C NMR (acetone-d_6, 500 MHz) δ 131.5 ($C_{2''}$), 128.9 ($C_{3''}$), 127.1 ($C_{4''}$), 104.6 (C_1), 85.7 ($C_{1'}$), 81.8 ($C_{2'}$), 80.7, 78.5 ($C_{3'}$), 77.3, 76.9, 75.3 (C_2), 71.0, 70.3, 62.4 ($C_6/C_{6'}$), 62.3 ($C_6/C_{6'}$); MS-ESI (*m/z*): $[M+Cl]^-$ calcd for $C_{18}H_{26}ClO_{10}S$, 469.1; 469.0 observed.[1]



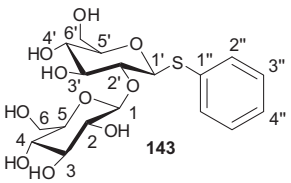
140



141



142



143

[1] The disaccharide linkage of **143** was determined to be 1→2 through comparison of COSY and TOCSY NMR experiments of the peracetylated analogs of **143** and its monosaccharide, **141**. The H2 proton of peracetylated **141** appears at 4.97 ppm ($CDCl_3$, 500 MHz), while the H2 proton of the proximal sugar of peracetylated **143** is at 3.86 ppm, indicating the position of glycosylation.

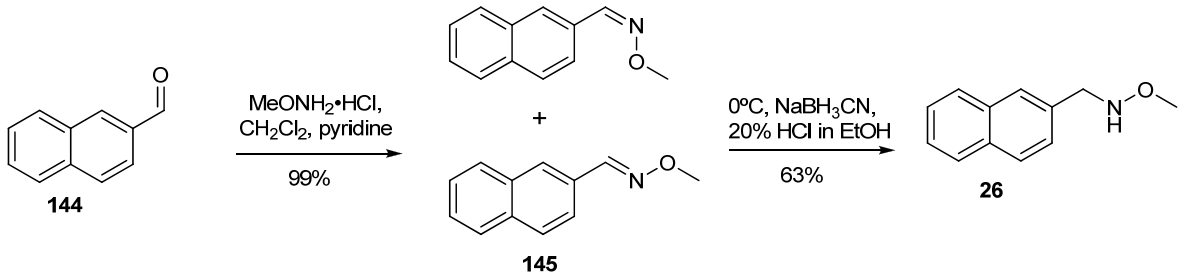
7. Aglycon syntheses and spectral data.

7.1. General reductive amination procedure. Aldehyde (12.5 mmol) was dissolved in CH_2Cl_2 to a final concentration of 0.45 M. To this was added 1.4 equivalents of $MeONH_2$•HCl and 2.2 equivalents of pyridine and the mixture was stirred for 2 hours at RT. TLC analysis at this stage revealed the substrate to be completely consumed with two products being formed. The reaction mixture was subsequently washed with 5% aqueous HCl (3 x 50 mL) and saturated NaCl (2 x 50 mL). The resulting organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to provide the crude oxime (>80%) which was used in subsequent reactions without further purification.

Crude oxime was dissolved in EtOH to a final concentration of 1.5 M. The reaction mixture was cooled to 0 ºC, 3 equivalents of $NaBH_3CN$ were added, and the solution was stirred for 15 min. An equal volume of 20% HCl in EtOH chilled to 0 °C was subsequently added in a drop-wise fashion over 10 min. The reaction was then allowed to warm to RT and stirred overnight. TLC analysis revealed complete consumption of substrate and a single new product. The reaction was neutralized with the addition of Na_2CO_3 until the evolution of gas halted, concentrated under reduced pressure, and CH_2Cl_2 (20 mL) was added. The resulting mixture was washed with saturated $NaHCO_3$ (2 x 50 mL), dried over Na_2SO_4, and the collected organics concentrated under reduced pressure. The concentrate was purified by flash chromatography (1:1 hexanes:CH_2Cl_3) to yield the desired methoxyamine product in > 50% yield.

7.2. *N*-methoxy-2-naphthalenemethanamine (26). 2-Naphthaldehyde (**144**; 2.0 g, 12.5 mmol) provided of the desired oxime **145** (2.3 g, 99% crude yield) as a white solid. TLC R_f = 0.48, 0.60 (1:8 EtOAc:hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (s, 1 H, NCH), 7.92-7.80 (m, 5 H, Ph), 7.56-7.48 (m, 2 H, Ph), 4.058 (s, 3 H, OCH_3),[2] 4.055 (s, 3 H, OCH_3)[2]; ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.0, 134.4, 133.5, 130.2, 128.9, 128.6, 128.6, 128.2, 127.2, 126.9, 62.4; HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{12}H_{12}NO$, 186.0841; found 186.0920.

Oxime **145** (2.3 gm, 12.4 mmol) yielded **26** (1.5 g, 63% yield) as an orange oil. TLC R_f = 0.36 (1:4 EtOAc:hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84-7.74 (m, 4 H, Ph), 7.52-7.39 (m, 3 H, Ph), 5.80 (br s, 1 H, NH), 4.18 (s, 2 H, NHCH_2), 3.50 (t, J = 0.4 Hz, 3 H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.5, 133.7, 133.1, 128.4, 128.1, 128.0, 127.9, 127.2, 126.3, 126.1, 62.2, 56.6; HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{12}H_{14}NO$, 188.1070; found 188.1069.

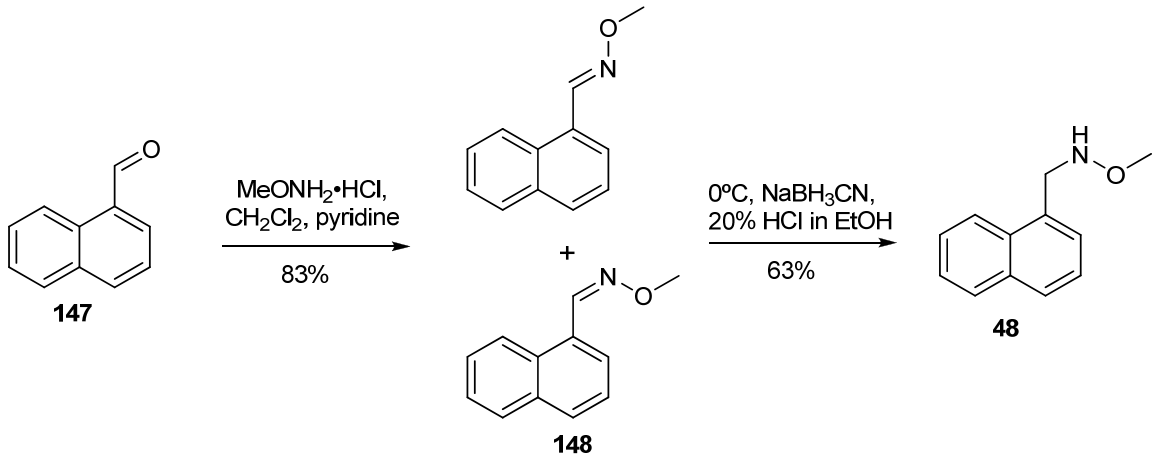


7.3. *N*-methoxy-1-naphthalenemethanamine (48). 1-Naphthaldehyde (**147**; 2.0 g, 12.7 mmol) gave oxime **148** (2.0 g, 83% crude yield) as a yellow oil. TLC R_f = 0.56, 0.65 (1:4 EtOAc:hexanes,); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (s, 1 H, NCH), 8.52 (m, 1 H, Ph), 7.84

[2] Signals observed in ^{1}H spectrum at 4.058 and 4.055 for OCH_3 are from the presence of (*E*) and (*Z*)-isomers.

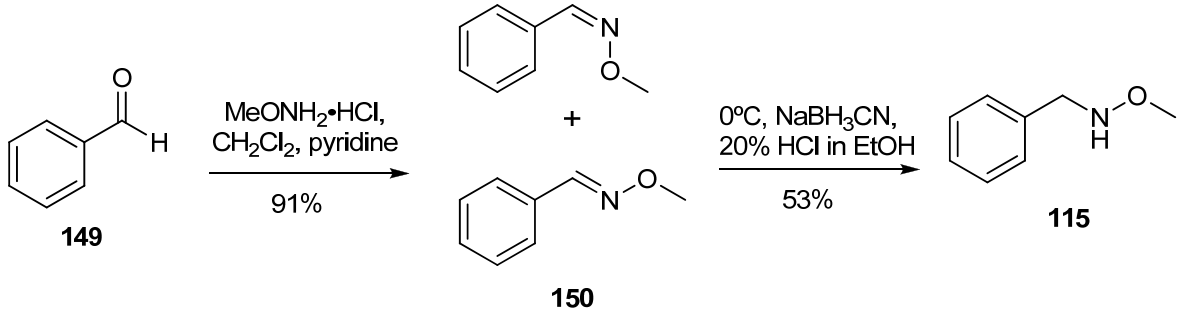
(m, 2 H, Ph), 7.74 (m, 1 H, Ph), 7.58-7.42 (m, 3 H, Ph), 4.06 (s, 3 H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.5, 133.9, 130.8, 130.5, 128.8, 128.1, 127.4, 127.1, 126.2, 125.4, 124.6, 62.2; HRMS-ESI (*m/z*): $[M]^{+\bullet}$ calcd for $C_{12}H_{11}NO$, 185.0836; found 185.0842.

Oxime **148** (2.0 gm, 10.5 mmol) yielded **48** (1.2 g, 63% yield) as a yellow oil. TLC R_f = 0.41 (1:4::EtOAc:hexanes); ^1H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, *J* = 8.8 Hz, 1 H, Ph), 7.84 (d, *J* = 8.4 Hz, 1 H, Ph), 7.77 (d, *J* = 8.4 Hz, 1 H, Ph), 7.56-7.36 (m, 4 H, Ph), 5.77 (br s, 1 H, NH), 4.51 (s, 2 H, NHC*H*$_2$), 3.53 (d, *J* = 0.4 Hz, 3 H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 134.1, 133.0, 132.3, 129.0, 128.7, 127.8, 126.5, 126.0, 125.7, 124.0, 62.1, 54.1; HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{12}H_{14}NO$, 188.1070; found 188.1070.



7.4. *N*-methoxybenzylamine (115). Benzaldehyde (**149**; 4.8 g, 49.2 mmol) afforded oxime **150** (6.1 g, 91% crude yield) as a colorless oil. TLC R_f = 0.82 (1:8 EtOAc:hexanes); ^1H NMR (400 MHz, $CDCl_3$) δ 8.03 (s, 1 H, NCH), 7.58-7.51 (m, 2 H, Ph), 7.37-7.32 (m, 3 H, Ph), 3.95 (s, 3 H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.8, 132.5, 130.0, 129.0, 127.3, 62.2[3]; HRMS-ESI (*m/z*): $[M]^{+\bullet}$ calcd for C_8H_9NO, 135.0679; found 135.0684.

Oxime **150** (6.1 g, 44.9 mmol) provided desired methoxyamine **115** (3.3 g, 53% yield) as a colorless oil. TLC R_f = 0.31 (1:8 EtOAc:hexanes); ^1H NMR (400 MHz, $CDCl_3$) δ 7.38-7.22 (m, 5 H, Ph), 5.71 (br s, 1 H, NH), 4.04 (s, 2 H, C*H*$_2$NH), 3.50 (d, *J* = 0.4 Hz, 3 H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.9, 129.1, 128.7, 127.7, 62.1, 56.5[4]; HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_8H_{12}NO$, 138.0914; found 138.0916.



[3] Not all phenyl ^{13}C resonances were observed, presumably due to spectral overlap in the 132.5-127.3 ppm region.

[4] Not all phenyl ^{13}C resonances were observed, presumably due to spectral overlap in the 129.1-127.1 ppm region.

8. Acceptor library screening summary and methods.

8.1. Data summary.

Compound Number	Compound Name	WT % conversion[a]	'ASP' % conversion[a]	substrate retention time	calculated substrate mass (*m/z*)	observed substrate mass (*m/z*)		product retention time	expected product mass (*m/z*)	observed product mass (*m/z*)	product designation[b]	HPLC method[c]	comments
3	benzhydrol	>99.9	>99.9	19.7	184.2	n.f.		12.9	508.2	531.0 $[M+Na]^+$	DI	A	
								13.3	508.2	531.0 $[M+Na]^+$	DI		
								14.0	346.4	381.2 $[M+Cl]^-$	MONO		
4	β-zearalenol	91.8	>99.9	18.1	320.2	321.2 $[M+H]^+$		8.1	482.2	483.2 $[M+H]^+$	MONO	A	
								9.6	482.2	483.2 $[M+H]^+$	MONO		
								9.7	644.3	679.2 $[M+Cl]^-$	DI		
								10.5	644.3	667.7 $[M+Na]^+$	DI		
								11.7	482.2	483.2 $[M+H]^+$	MONO		
								12.7	644.3	643.2 $[M-H]^-$	DI		
								12.8	644.3	643.2 $[M-H]^-$	DI		
								13.9	482.2	483.2 $[M+H]^+$	MONO		
5	2-iodophenol	70	99.7	18.6	219.9	243.8 $[M+H]^+$		11.7	382.0	404.4 $[M+Na]^+$	MONO	A	
6	thiophenol	61.3	93.7	20.8	110.2	n.f.		8.4	434.1	469.0 $[M+Cl]^-$	DI	A	
								9.4	272.1	307.2 $[M+Cl]^-$	MONO		
7	6-hydroxy-4-methylcoumarin	21	92	13.0	176.2	175.2 $[M-H]^-$		8.7	338.3	373.2 $[M+Cl]^-$, 339.0 $[M+H]^+$	MONO	A	0.2 mM final concentration
8	phenol	17	89	12.8	94.0	129.5 $[M+Cl]^-$		7.9	256.3	291.2 $[M+Cl]^-$	MONO	A	
9	kaempferol	71.3	88.0	17.4	286.0	285.0 $[M-H]^-$		7.1	448.1	483.0 $[M+Cl]^-$	MONO	A	
								8.2	610.2	609.0 $[M-H]^-$	DI		
								8.6	610.2	609.0 $[M-H]^-$	DI		
								8.8	610.2	609.0 $[M-H]^-$	DI		
								9.5	610.2	609.0 $[M-H]^-$; 645.0 $[M+Cl]^-$	DI		
								10.0	610.2	609.0 $[M-H]^-$; 645.0 $[M+Cl]^-$	DI		
								10.9	610.2	609.0 $[M-H]^-$	DI		
								11.2	610.2	645.0 $[M+Cl]^-$	DI		
								11.8	610.2	645.0 $[M+Cl]^-$	DI		
								12.3	448.1	447.0 $[M-H]^-$	MONO		
								12.4	448.1	447.0 $[M-H]^-$	MONO		
								12.8	448.1	447.0 $[M-H]^-$	MONO		
10	1,8-dihydroxyanthraquinone	81	78	24.2	240.0	240.8 $[M+H]^+$		13.3	402.1	403.0 $[M+H]^+$	MONO	A	
11	digitoxin neoaglycon	79	77	14.8	403.3	404.0 $[M+H]^+$		12.8	565.3	566.0 $[M+H]^+$	MONO	A	0.25 mM final concentration
12	4-nitrophenol	33	74	15.4	139.0	138.4 $[M-H]^-$		9.6	301.3	336.4 $[M+Cl]^-$	MONO	A	
13	phenylhydrazine	5	73	13.3	108.1	109.2 $[M+H]^+$		11.3	270.1	270.8 $[M+H]^+$; 292.8 $[M+Na]^+$; 268.8 $[M-H]^-$; 304.8 $[M+Cl]^-$	MONO	C	
14	$^{-}T_0$- calicheamicin fragment	0	69	12.3	584.1	582.8 [M-H]-		11.89	746.1	781.0 $[M+Cl]^-$	MONO	B	0.1 final concentration
15	*N*-hydroxybenzamide	25	66	11.8	137.1	138.2 $[M+H]^+$		13.8	299.1	300.2 $[M+H]^+$	MONO	J	
16	warfarin neoaglycon	6.2	64.9	21.4	339.2	340.6 $[M+H]^+$		13.7	501.2	524.2 $[M+Na]^+$	MONO	D	
								13.9	501.2	524.0 $[M+Na]^+$	MONO		
								14.2	501.2	524.0 $[M+Na]+$	MONO		
17	mitoxantrone	11	63	16.1	444.2	445.2 $[M+H]^+$		15.6	606.3	607.0 $[M+H]^+$	MONO	E	0.25 mM final concentration
18	β-estradiol	0	59.7	20.2	272.2	273.2 $[M+H]^+$		11.8	596.3	619.2 $[M+Na]^+$	DI	A	
								13.1	596.3	619.4 $[M+Na]^+$	DI		
								14.7	434.2	435.4 $[M+H]^+$	MONO		
19	diphenylamine	51.0	48.9	24.1	169.1	170.0 $[M+H]^+$		13.3	493.2	494.2 $[M+H]^+$; 516.0 $[M+Na]^+$; 491.8 $[M-H]^-$; 528.2 $[M+Cl]^-$	DI	G	
								14.0	331.1	331.6 $[M+H]^+$; 353.6 $[M+Na]^+$;	MONO		
20	phenoxyethanethiol	32.0	48.9	21.9	154.0	n.f.		8.2	478.2	513.0 $[M+Cl]^-$	DI	A	
								11.7	316.1	351.0 $[M+Cl]^-$	MONO		
21	2-naphthalenemethanol	80.3	46.1	21.5	158.1	n.f.		16.2	482.2	505.0 $[M+Na]^+$	DI	E	
								17.1	482.2	504.8 $[M+Na]^+$	DI		
								17.5	320.1	342.6 $[M+Na]^+$	MONO		
22	benzylmercaptan	37	45	21.4	286.1	n.f.		10.3	286.3	321.2 $[M+Cl]^-$	MONO	A	
23	*N*-ethylaniline	10.2	41.2	20.1	121.1	122.2 $[M-H]^-$		9.4	445.2	446.2 $[M+H]^+$; 468.2 $[M+Na]^+$	DI	G	
								10.7	283.1	283.8 $[M+H]^+$; 305.8 $[M+Na]^+$	MONO		
24	digitoxigenin	8.9	39.9	17.9	374.2	375.0 $[M+H]^+$		12.2	536.3	537.0 $[M+H]^+$	MONO	A	
								13.5	536.3	537.0 $[M+H]^+$	MONO		
25	3,4-dichloroaniline	9	39	15.3	161.0	162.2 $[M+H]^+$		13.0	323.0	328.8 $[M+H]^+$	MONO	D	0.2 mM final concentration
26	*N*-methoxy-2-naphthalenemethanamine	64.2	37.9	19.5	187.1	210.2 $[M+Na]^+$		11.6	511.2	510.2 $[M+H]^+$	DI	G	
								12.1	511.2	534.2 $[M+Na]^+$	DI		
								13.6	349.1	372.2 $[M+Na]^+$	MONO		
27	plumbagin	11	35	20.9	188.1	189.0 $[M+H]^+$		11.1	350.1	348.6 $[M-H]^-$; 384.8 $[M+Cl]^-$	MONO	A	
28	acetaminophen	3	35	13.4	151.1	152.1 $[M+H]^+$		11.5	313.2	314.1 $[M+H]^+$	MONO	E	
29	*N*-benzoxybenzhydrylamine	87	32	26.2	289.2	290.0 $[M+H]+$		15.9	613.3	613.8 $[M+H]^+$;635.6 $[M+Na]^+$	DI	D	
								17.2	451.1	452.2 $[M+H]^+$; 474.0 $[M+Na]^+$	MONO		

Compound Number	Compound Name	WT % Conversion	'ASP' % Conversion	substrate retention time	calculated substrate mass (*m/z*)	observed substrate mass (*m/z*)		product retention time	expected product mass (*m/z*)	observed product mass (*m/z*)	product designation	HPLC method	comments
30	alazarin	27	31	19.6	240.0	238.6 $[M-H]^-$		13.4	402.1	425.0 $[M+Na]^+$; 400.8 $[M-H]^-$	MONO	A	
31	cannabidiol	49	29	24.8	314.2	415.2 $[M+H]^+$		24.5	476.3	477.3 $[M+H]^+$	MONO	E	0.1 mM final concentration
32	pseudo-aglycon - calicheamicin fragment	43	27	19.4	1050.1	1049.1 $[M-H]^-$		9.9	1212.1	1247.1 $[M+Cl]^-$	MONO	B	0.1 mM final concentration
33	*N*-methylaniline	4.4	27.2	18.1	107.1	n.f.		8.1	431.2	432.2 $[M+H]^+$; 454.0 $[M+Na]^+$	DI	G	0.2 mM final concentration
								9.1	269.1	269.8 $[M+H]^+$; 291.8 $[M+Na]^+$	MONO		
34	aniline	3	27	12.8	93.1	94.2 $[M+H]^+$		8.0	255.1	256.0 $[M+H]^+$	MONO	G	
35	1-pyrenemethanol	2.6	26.7	22.5	232.1	255.0 $[M+Na]^+$		14.0	556.2	591.2 $[M+Cl]^-$	DI	A	
								15.2	556.2	591.2 $[M+Cl]^-$	DI		
								16.0	394.1	429.0 $[M+Cl]^-$	MONO		
36	ampicillin	3	27	9.8	349.1	350.2 $[M+H]^+$		15.4	511.2	511.8 $[M+H]^+$; 533.8 $[M+Na]^+$	MONO	A	
								16.1	511.2	511.8 $[M+H]^+$; 533.8 $[M+Na]^+$	MONO		
37	benzhydrylamine	34	27	19.1	183.1	206.0 $[M+Na]^+$		18.1	345.2	345.8 $[M+H]^+$; 367.6 $[M+Na]^+$	MONO	E	
38	α3I - calicheamicin fragment	21	25	17.4	1210.2	1245.1 $[M+Cl]^-$		15.2	1372.2	1407.1 $[M+Cl]^-$	MONO	B	0.1 mM final concentration
39	fredericamycin A	17	25	23.6		540.0 $[M+H]^+$		10.0	701.2	700.8 $[M-H]^-$	MONO	A	0.25 mM final concentration
40	dynemicin A	45	25	24.9	537.1	537.8 $[M+H]^+$		14.8	699.6	722.2 $[M+Na]^+$	MONO	M	0.1 mM final concentration
41	hydramycin	7	24	19.1	408.1	407.0 [M-H]-		15.7	570.1	569.0 [M-H]	MONO	L	
42	2-phenylethanethiol	31	23	22.8	138.2	172.2 $[M+Cl]^-$		11.8	300.1	335.0 $[M+Cl]^-$	MONO	A	
43	pyrimethamine	36	21	20.2	248.1	249.2 $[M+H]^+$		17.8	410.1	411.2 $[M+H]^+$	MONO	J	
44	7-amino-4-methylcoumarin	1	18	12.3	175.1	176.2 $[M+H]^+$		9.2	337.3	338.0 $[M+H]^+$	MONO	A	0.2 mM final concentration
45	staurosporine aglycon	5	15	19.5	311.1	312.2 $[M+H]^+$		14.6	473.2	474.8 $[M+H]^+$	MONO	A	0.05 mM final concentration
46	benzylalcohol	8	14	11.4	108.1	107.0 $[M-H]^-$		8.75	270.1	305.0 $[M+Cl]^-$	MONO	A	
47	phenylacetic hydrazide	0	13	12.8	150.1	151.2 $[M+H]^+$		15.2	312.1	313.2 $[M+H]^+$	MONO	J	
48	*N*-methoxy-1-naphthalenemethanamine	18	11	19.8	187.1	188.2 $[M+H]^+$		13.5	349.1	350.2 $[M+H]^+$	MONO	H	
49	brefeldin A	1	10	16.0	280.2	281.2 $[M+H]^+$			442.2	477.2 $[M+Cl]^-$	MONO	A	
50	*N*-ethoxybenzhydrylamine	41	10	24.8	227.1	250.0 $[M+Na]^+$		14.2	389.2	412.2 $[M+Na]^+$	MONO	D	
51	benzaldehyde oxime	3	9	14.8	121.1	122.2 $[M+H]^+$		9.9	283.1	284.2 $[M+H]^+$	MONO	A	
52	benzoic hydrazide	3	7	5.7	136.1	137.0 $[M+H]^+$		7.2	298.1	299.2 $[M+H]^+$	MONO	A	
53	sulfanilamide	0	6.7	10.6	172.0	173.1 $[M+H]^+$		3.7	334.1	335.1 $[M+H]^+$	MONO	E	
								5.9	334.1	335.1 $[M+H]^+$; 369.0 $[M+Cl]^-$	MONO		
54	4-biphenylacetic acid	0.8	5.9	20.3	212.1	213.2 $[M+H]^+$		15.9	374.1	397.0 $[M+Na]^+$	MONO	A	
								16.0	374.1	397.0 $[M+Na]^+$	MONO		
								16.2	374.1	397.0 $[M+Na]^+$	MONO		
55	deacetyl-colchicine	21	3	11.0	357.2	358.0 $[M+H]^+$		11.4	519.0	520.0 $[M+H]^+$	MONO		0.5 mM final concentration
56	*N*-methoxybenzhydrylamine	9	2	23.6	213.2	248.6 $[M+Cl]$		13.4	375.2	398.0 $[M+Na]^+$	MONO	D	
57	nystatin A1	41.6	0.0	15.2	925.5	926.0 $[M+H]^+$		12.7	1087.6	1088.4 $[M+H]^+$	MONO	F	0.25 mM final concentration
								13.9	1087.6	1087.8 $[M+H]^+$	MONO		
								14.1	1087.6	1088.2 $[M+H]^+$	MONO		
58	2-naphthoic acid	0.2	4.6	17.4	172.1	173.2 $[M+H]^+$		12.0	334.1	357.2 $[M+Na]^+$	MONO	A	
								12.3	334.1	357.2 $[M+Na]^+$	MONO		
								12.6	334.1	357.2 $[M+Na]^+$	MONO		
59	1-hydroxyanthraquinone	3	4	24.1	224.1	224.8 $[M+H]^+$		13.1	386.1	386.8 $[M+H]^+$	MONO	A	
60	3-iodobenzoic acid	0.6	3.2	18.7	248.0	249.0 $[M+H]^+$		13.4	410.0	445.0 $[M+Cl]^-$	MONO	A	
								13.6	410.0	445.0 $[M+Cl]^-$	MONO		
								13.9	410.0	445.0 $[M+Cl]^-$	MONO		
								15.0	410.0	445.0 $[M+Cl]^-$	MONO		
61	sulfapyridine	1	3	15.4	249.1	250.1 $[M+H]^+$		12.6	411.1	412.2 $[M+H]^+$	MONO	E	
62	daunorubicin	0.4	2.8	14.5	527.2	528.2 [M+H]+		11.1	689.2	690.2 $[M+H]^+$	MONO	A	
								13.3	689.2	690.2 $[M+H]^+$	MONO		
63	trimethoprim	2	2	15.4	290.1	291.3 $[M+H]^+$		12.0	452.2	453.2 $[M+H]^+$	MONO	E	
64	calicheamicin γ	3	1	15.6	1367.3	1402.2 $[M+Cl]^-$		9.9	1529.3	1564.3 $[M+Cl]^-$	MONO	B	0.1 mM final concentration
65	3-chlorobenzoic acid	0.1	1.8	17.2	156.0	157.0 $[M+H]^+$		11.9	318.1	317.0 $[M-H]^-$	MONO	A	
								12.4	318.1	317.0 $[M-H]^-$	MONO		
								12.8	318.1	317.0 $[M-H]$	MONO		

Compound Number	Compound Name	WT % Conversion	'ASP' % Conversion	substrate retention time	calculated substrate mass (*m/z*)	observed substrate mass (*m/z*)		product retention time	expected product mass (*m/z*)	observed product mass (*m/z*)	product designation	HPLC method	comments
66	ivermectin	0	2	33.2	874.5	897.2 $[M+Na]^+$		23.4	1036.6	1059.2 $[M+Na]^+$	MONO	K	0.1 mM final concentration
67	phenylacetic acid	0	2	14.1	136.1	137.2 $[M+H]^+$		9.9	298.1	333.2 $[M+Cl]^-$	MONO	A	
68	2-benzoylbenzoic acid	1	1	18.2	226.1	227.2 $[M+H]^+$		13.2	388.1	411.2 $[M+Na]^+$	MONO	A	
								13.4	388.1	411.0 $[M+Na]^+$	MONO		
								13.6	388.1	411.0 $[M+Na]^+$	MONO		
								13.7	388.1	411.2 $[M+Na]^+$	MONO		
								13.9	388.1	411.2 $[M+Na]^+$	MONO		
69	chloramphenicol	1	1	14.4	322.0	321.0 $[M-H]$-		12.4	484.1	483.0 $[M-H]^-$	MONO	A	
70	staurosporine glucoside	0	1	14.6	473.2	472.2 $[M-H]^-$		7.6	635.2	670.0 $[M+Cl]^-$	MONO	A	0.05 mM final concentration
71	avermectin	0	1	26.3	872.5	895.2 $[M+Na]^+$		18.2	1034.5	1058.0 $[M+Na]^+$	MONO	K	0.1 mM final concentration
72	indole	0	0.4	18.4	117.1	118.2 $[M+H]^+$		10.6	279.1	280.0 $[M+H]^+$	MONO	A	
73	ivermectin psuedo-aglycon	0	0.2	27.8	730.4	753.0 $[M+Na]^+$		18.65	892.5	915.6 $[M+Na]^+$	MONO	K	0.01 mM final concentration

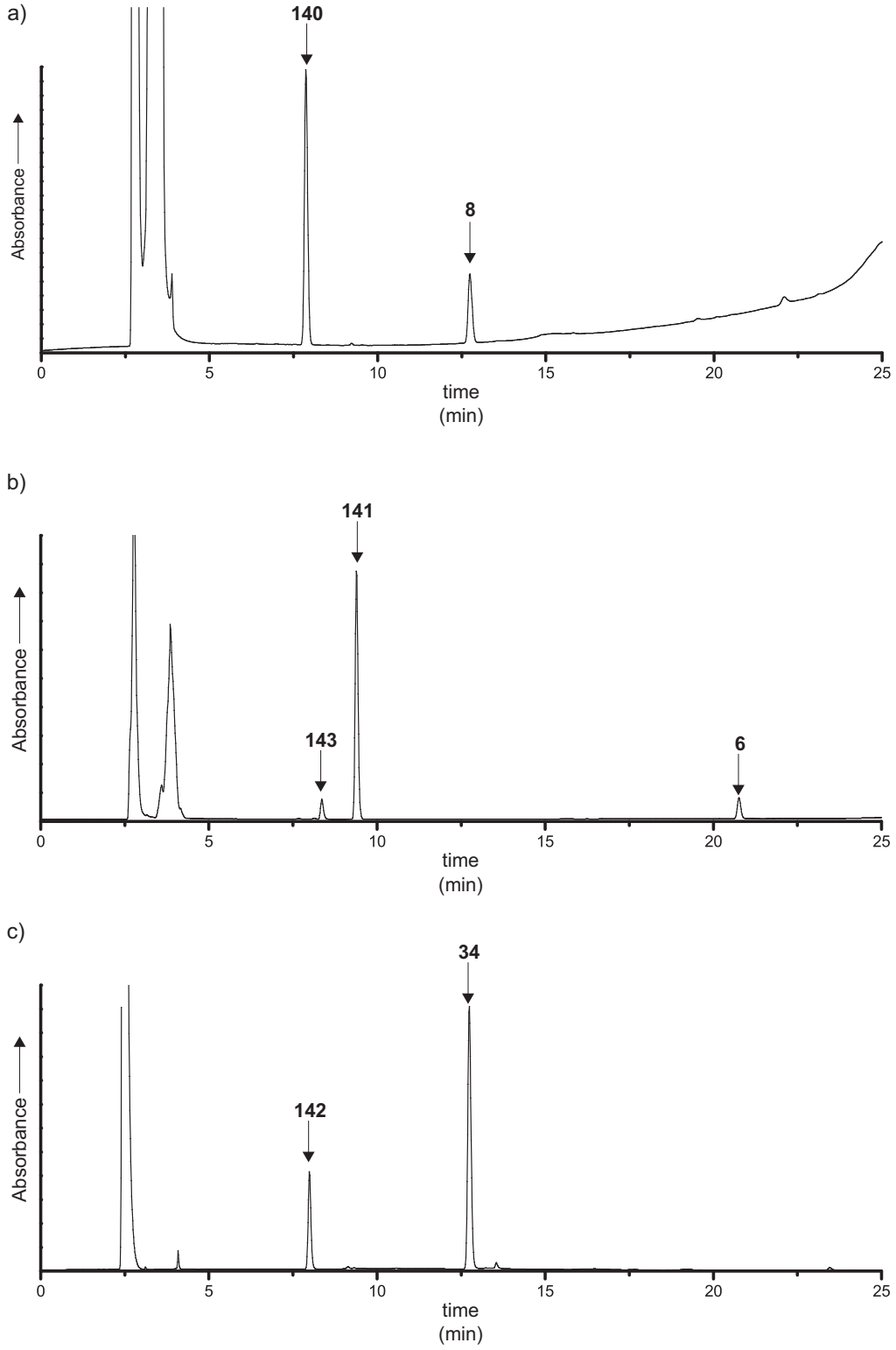
[a] % conversion is calculated as the sum of all observed products; [b] MONO – mass corresponds to monosaccharide product, DI – mass corresponds to disaccharide product; [c] refer to Supp. Info. 8.2 for HPLC methods; [d] n.f. – not found

8.2. HPLC methods.

Method	Solvent A[a]	Solvent B	Gradient	Detection (nm)
A	0.01% TFA	CH_3CN	10-75% B, 20 min; 75-100% B, 1min; 100% B, 5 min	254
B	0.01% TFA	CH_3CN	10-100% B, 20 min	280
C	50 mM Tris, pH 8.0	CH_3CN	5% B, 2.5 min; 5-60% B, 18.5 min; 60-100% B, 1 min; 100% B, 6 min	254
D	ddH_2O	CH_3CN	10-75% B, 20 min; 75-100% B, 1min; 100% B, 14 min	254
E	ddH_2O	CH_3CN	2% B, 2.5 min; 2-60% B, 17.5 min; 60-100% B, 1 min; 100% B, 4 min	254
F	0.01% TFA	CH_3CN	10-75% B, 20 min; 75-100% B, 1min; 100% B, 5 min	305
G	10 mM NH_4CO_3, pH 10.0	CH_3CN	10-75% B, 20 min; 75-100% B, 1min; 100% B, 5 min	254
H	ddH_2O	CH_3CN	10-75% B, 20 min; 75-100% B, 1min; 100% B, 5 min	254
J	ddH_2O	CH_3CN	5% B, 5 min; 5-75% B, 15 min; 75-100% B, 1 min; 100% B, 5 min	254
K	ddH_2O	CH_3CN	30-70% B, 5 min; 70-100% B, 30 min; 100% B, 3 min	243
L	0.01% TFA	CH_3CN	10-100% B, 15 min; 100% B, 20 min	254
M	0.01% TFA	CH_3CN	10-75% B, 20 min; 75-100% B, 1min; 100% B, 5 min	320

[a] 0.1% formic acid in ddH_2O was utilized for LC/ESI-MS methods when solvent A was ddH_2O or 0.1% trifluoroacetic acid in ddH_2O. 5 mM ammonium bicarbonate (pH 8.0) was utilized for LC/ESI-MS methods when solvent A was 50 mM Tris-HCl [pH 8.0] in ddH_2O or 10 mM ammonium bicarbonate (pH 10.0) in ddH_2O.

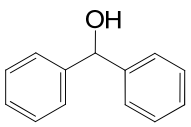
8.3 Representative HPLC traces.



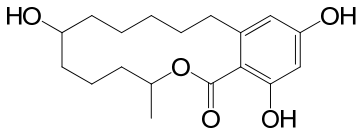
Supplementary Figure 3. Representative HPLC traces for OleD reactions with **a**) phenol (**8**), **b**) thiophenol (**6**), and **c**) aniline (**34**).

9. Structures of aglycon library members.

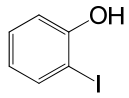
Supplementary Figure 4. Compounds that resulted in observable glucosylation (**3**-**73**) are listed in descending order of observed 'ASP' conversion. Compounds with no observable glucosylation (**74**-**139**) are listed in alphabetical order. Italic numbers noted under compound number and name denote observed percent conversion for *WT/'ASP'*.



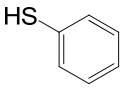
3: benzhydrol
>99.9/>99.9



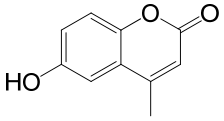
4: β-zearalenol
>99.9/92



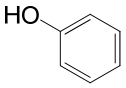
5: 2-iodophenol
70/>99



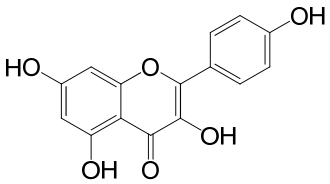
6: thiophenol
61/94



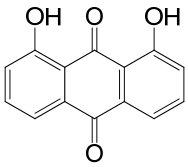
7: 6-hydroxy-4-methylcoumarin
21/92



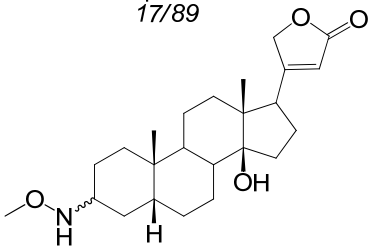
8: phenol
17/89



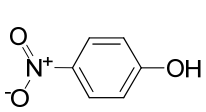
9: kaempferol
71/88



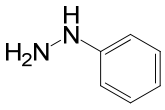
10: 1,8-dihydroxyanthraquinone
81/78



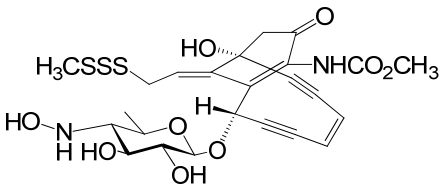
11: digitoxin neoalgycon
77/79



12: 4-nitrophenol
33/74

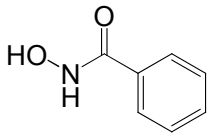


13: phenylhydrazine
5/73

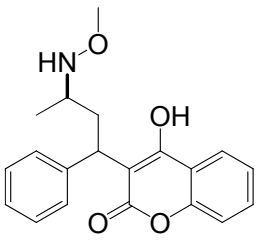


14: calicheamicin fragment T_0
0/69

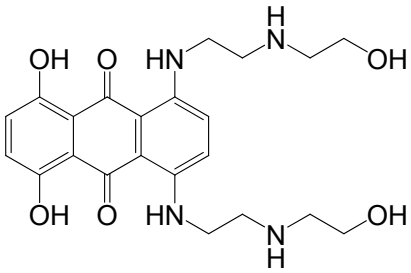
Structures of All Library members (con't)



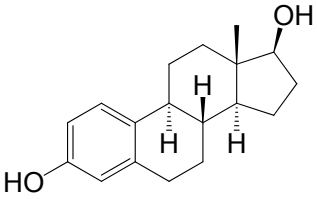
15: *N*-hydroxybenzamide (benzohydroxamic acid)
25/66



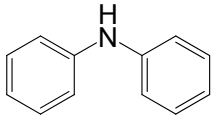
16: warfarin neoaglycon
6/65



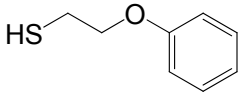
17: mitoxantrone
11/63



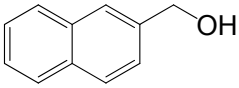
18: β-estradiol
0/60



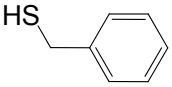
19: diphenylamine
51/49



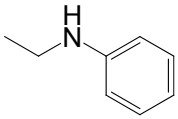
20: phenoxyethanethiol
32/49



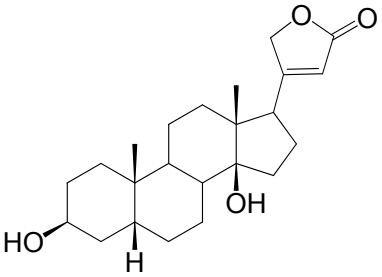
21: 2-naphthalenemethanol
80/46



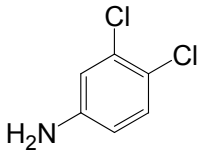
22: benzylmercaptan
37/45



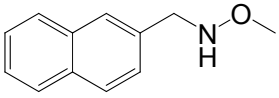
23: *N*-ethylaniline
10/41



24: digitoxigenin
9/40

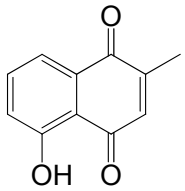


25: 3,4-dichloroaniline
9/39

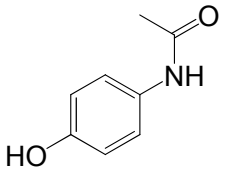


26: *N*-methoxy-2-naphthalenemethanamine
64/38

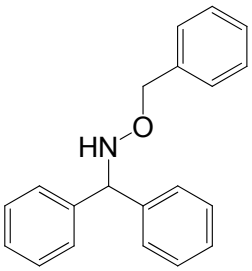
Structures of All Library members (con't)



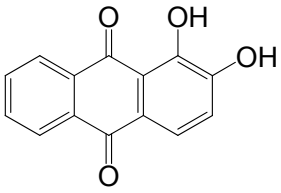
27: plumbagin
11/35



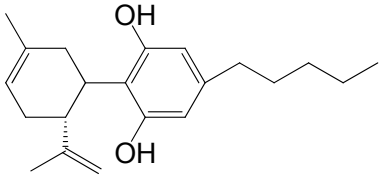
28: acetaminophen
3/35



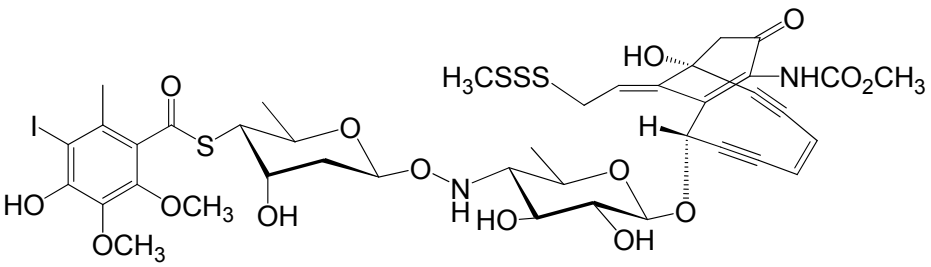
29: *N*-benzoxybenzhydrylamine
87/32



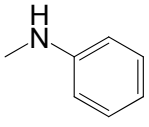
30: alizarin
27/31



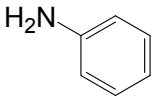
31: cannabidiol
49/29



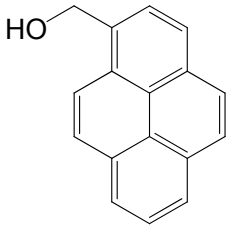
32: calicheamicin fragment psuedo-aglycon
43/27



33: *N*-methylaniline
4/27

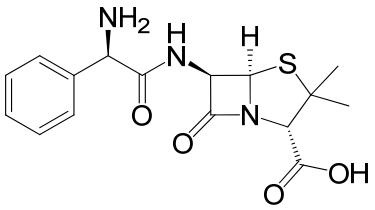


34:aniline
3/27

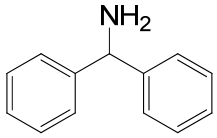


35: 1-pyrenemethanol
3/27

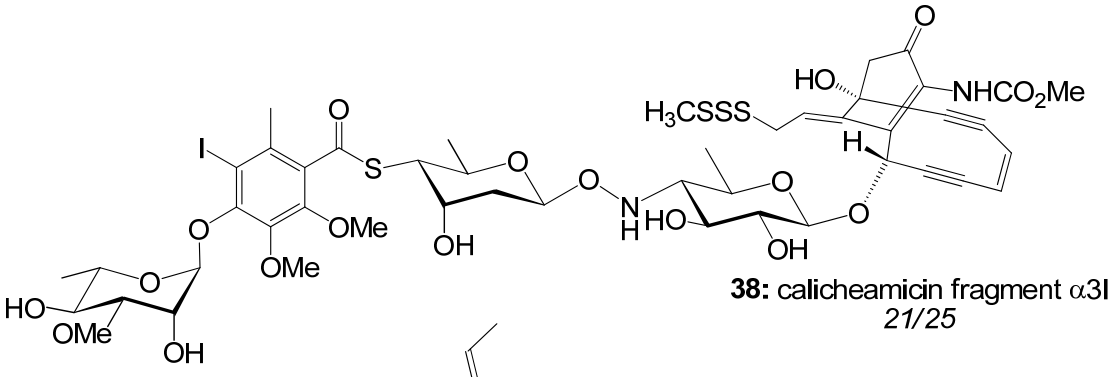
Structures of All Library members (con't)



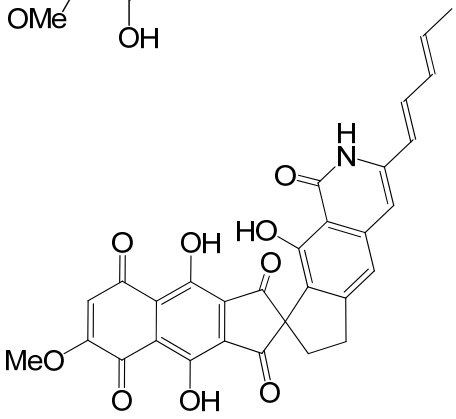
36: ampicillin
3/27



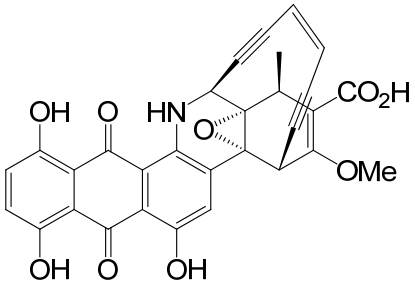
37: benzhydrylamine
(aminodiphenylmethane)
34/27



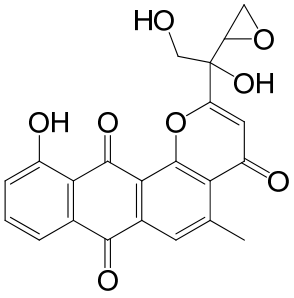
38: calicheamicin fragment α3I
21/25



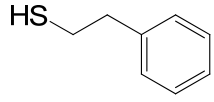
39: fredericamycin A
17/25



40: dynemicin A
24/25

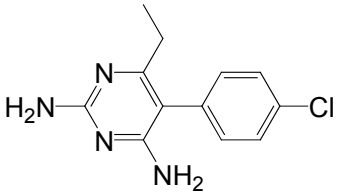


41: hydramycin
7/24

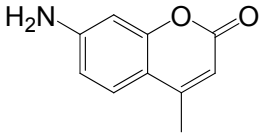


42: 2-phenylethanethiol
31/23

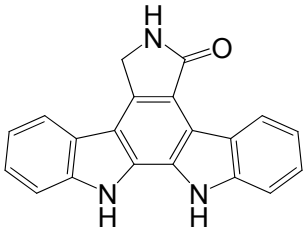
Structures of All Library members (con't)



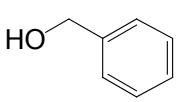
43: pyrimethamine
36/21



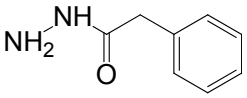
44: 7-amino-4-methylcoumarin
1/18



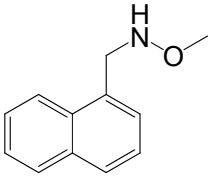
45: staurosporine aglycon
5/15



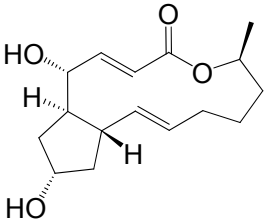
46: benzylalcohol
8/14



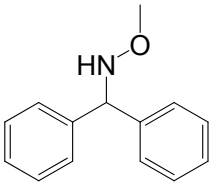
47: phenylacetic hydrazide
0/13



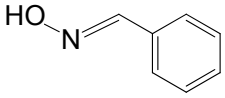
48: *N*-methoxy-1-naphthalenemethanamine
18/11



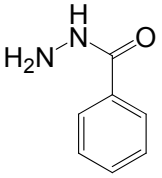
49: brefeldin A
1/10



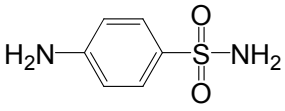
50: *N*-methoxybenzhydrylamine
9/2



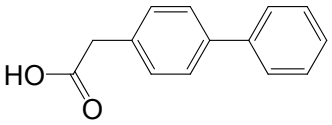
51: benzaldehyde oxime
3/9



52: benzoic hydrazide
3/7

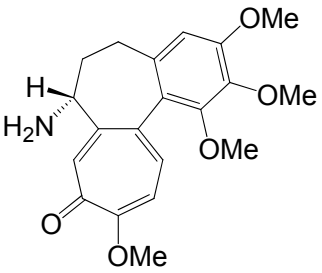


53: sulfanilamide
0/7

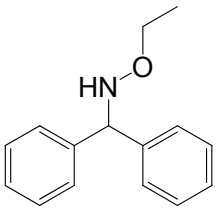


54: 4-biphenylacetic acid
1/6

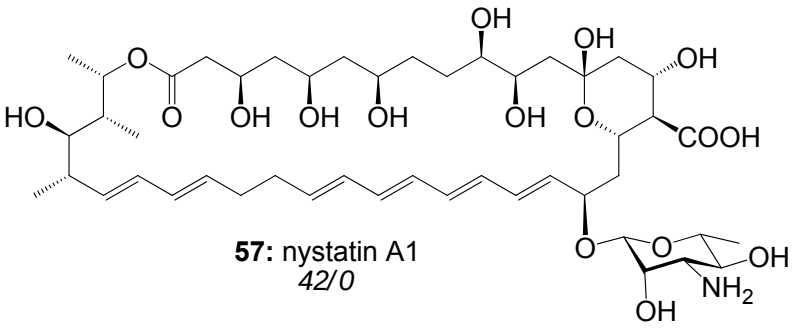
Structures of All Library members (con't)



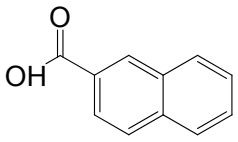
55: deacetyl-colchicine
21/3



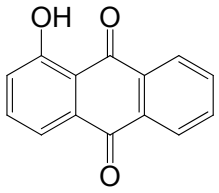
56: *N*-ethoxydiphenylmethylamine
41/10



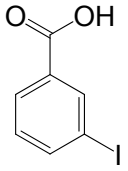
57: nystatin A1
42/0



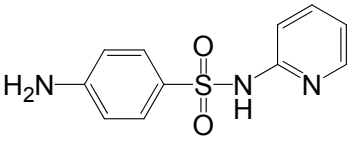
58: 2-naphthoic acid
0.2/5



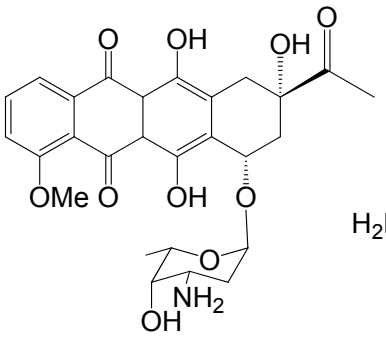
59: 1-hydroxyanthraquinone
3/4



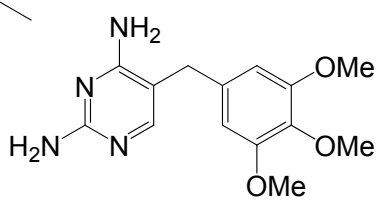
60: 3-iodobenzoic acid
1/3



61: sulfapyridine
1/3

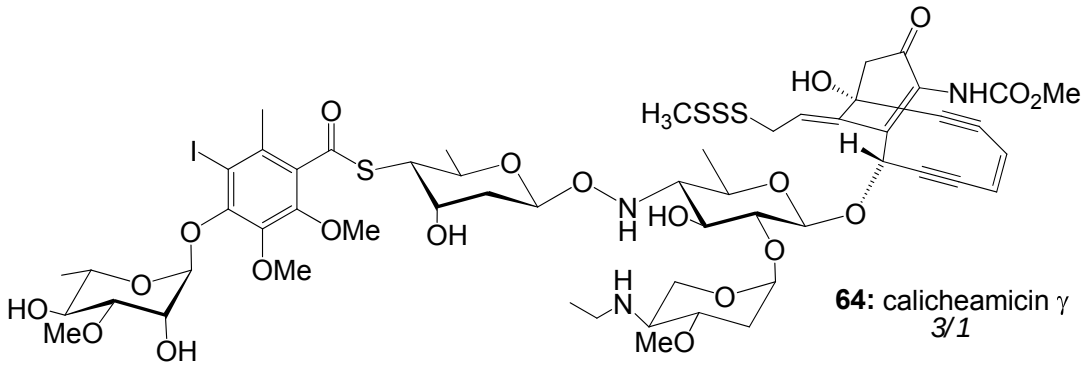


62: daunorubicin
0/3

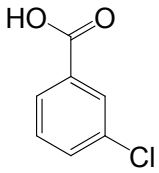


63: trimethoprim
2/2

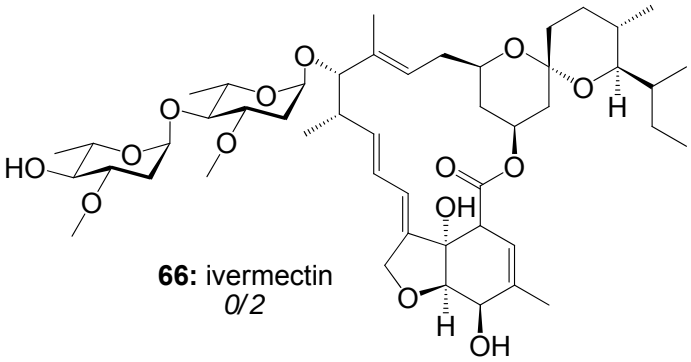
Structures of All Library members (con't)



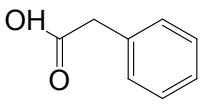
64: calicheamicin γ
3/1



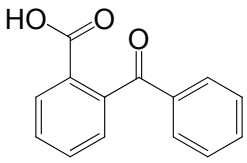
65: 3-chlorobenzoic acid
0/2



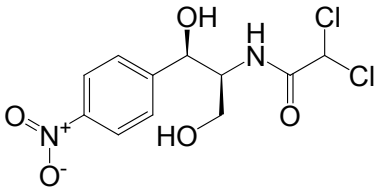
66: ivermectin
0/2



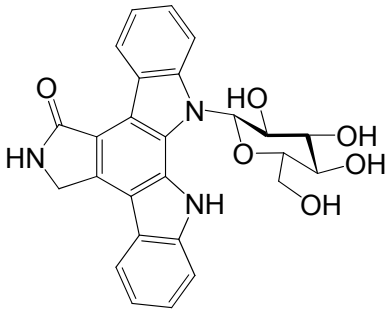
67: phenylacetic acid
0/2



68: 2-benzoylbenzoic acid
1/1

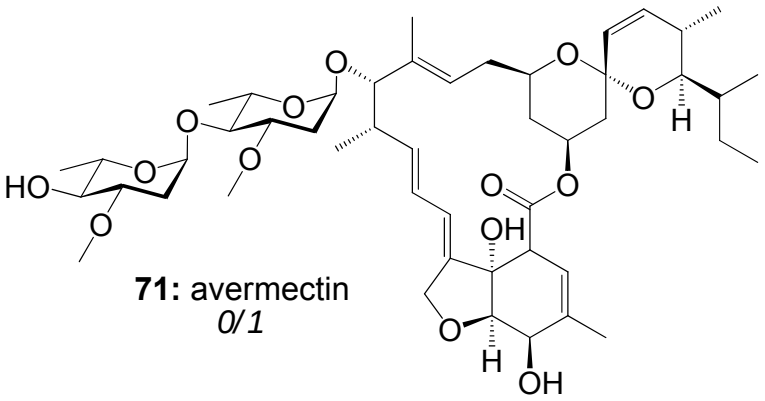


69: chloramphenicol
1/1

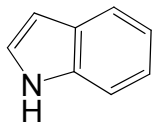


70: staurosporine glucoside
0.1/1

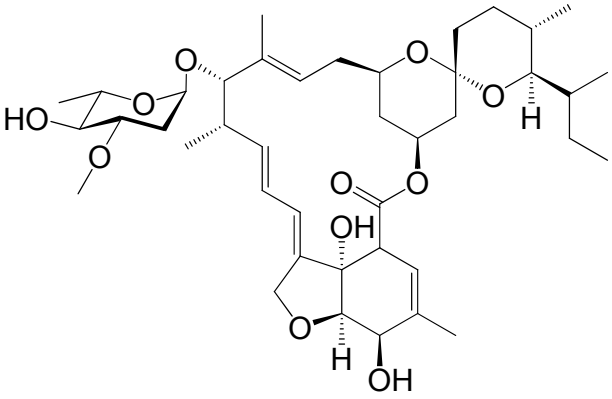
Structures of All Library members (con't)



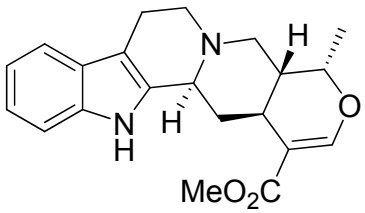
71: avermectin
0/1



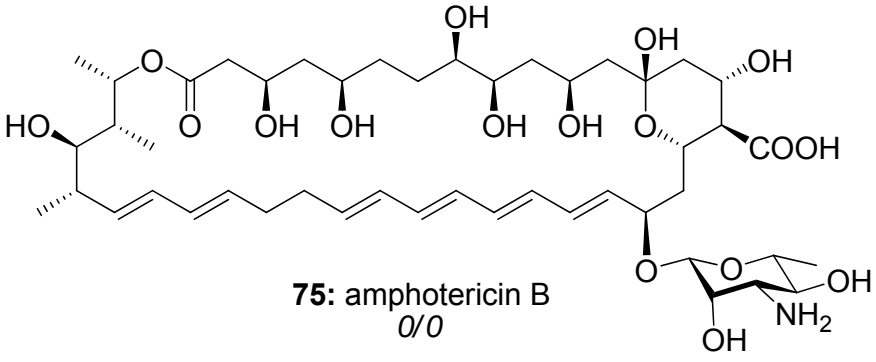
72: indole
0/0.4



73: ivermectin psuedo-aglycon
0/0.2

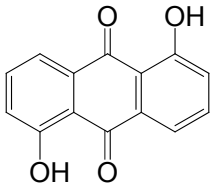


74: ajmalicine
0/0

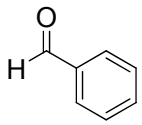


75: amphotericin B
0/0

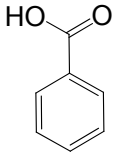
Structures of All Library members (con't)



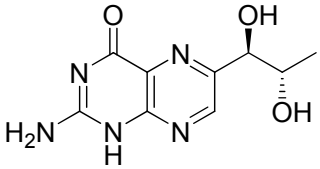
76: anthrarufin
0/0



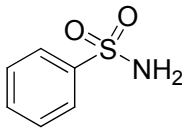
77: benzaldehyde
0/0



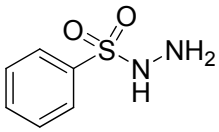
78: benzoic acid
0/0



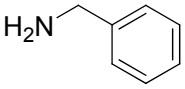
79: 6-biopterin
0/0



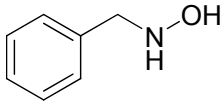
80: benzenesulfonamide
0/0



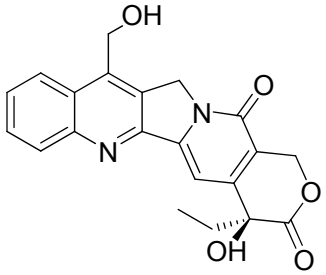
81: benzenesulfonyl hydrazide
0/0



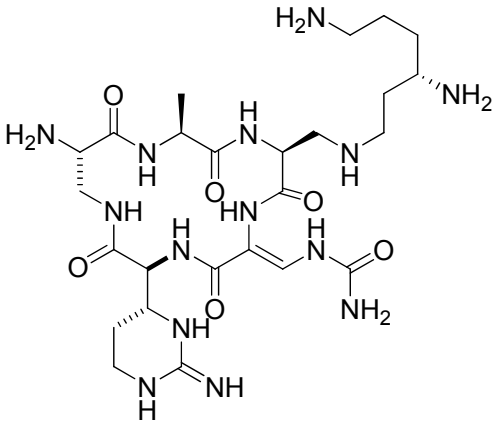
82: benzylamine
0/0



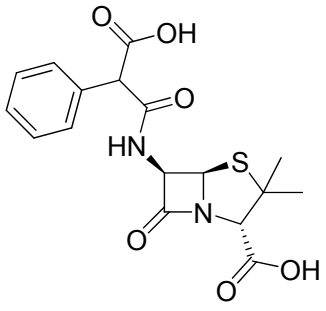
83: *O*-benzylhydroxylamine
0/0



84: camptothecin
0/0

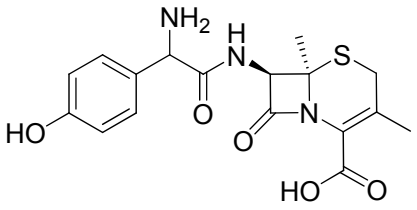


85: capreomycin 1B
0/0

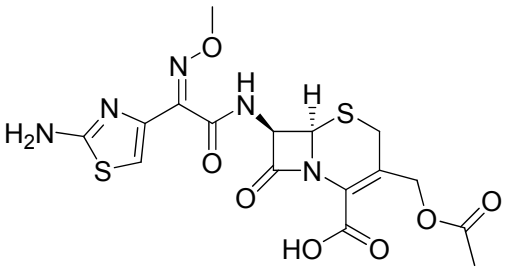


86: carbenicillin
0/0

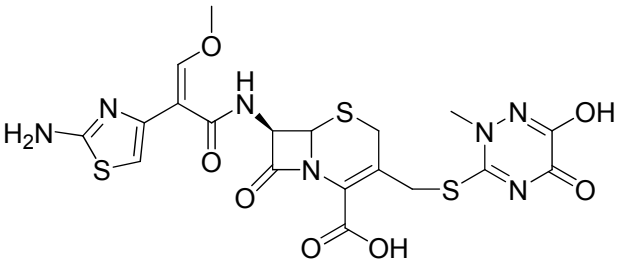
Structures of All Library members (con't)



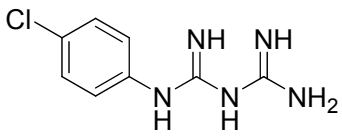
87: cefadroxil
0/0



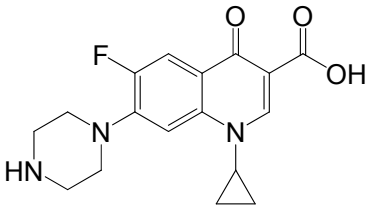
88: cefotaxime
0/0



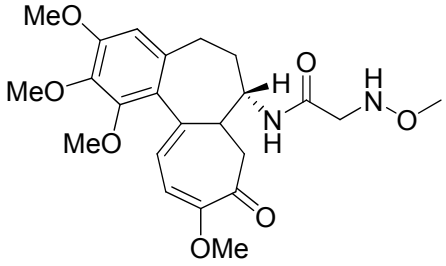
89: ceftriaxone
0/0



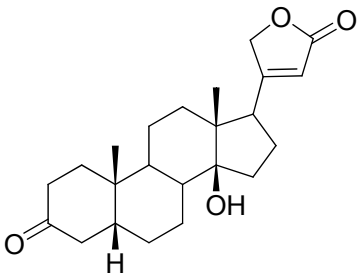
90: 1-(4-chlorophenyl)biguanide
0/0



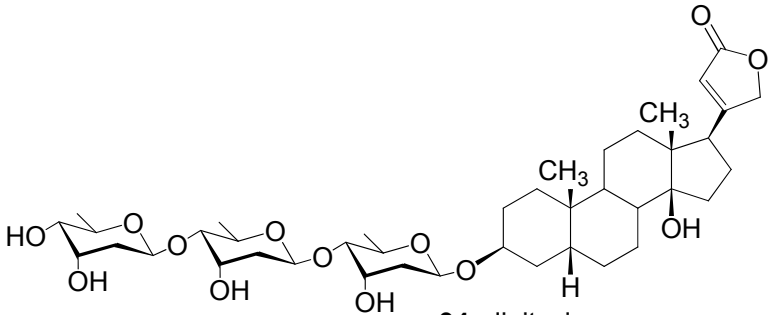
91: ciprofloxacin
0/0



92: colchicine neoaglycon
0/0

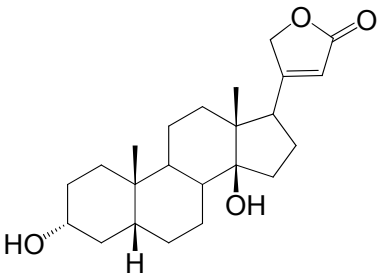


93: digitoxone
0/0

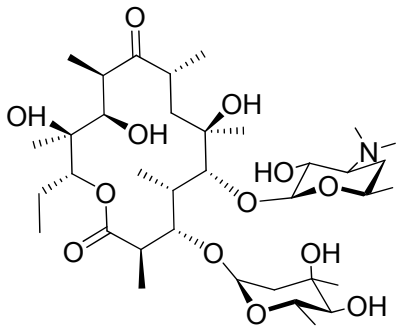


94: digitoxin
(digoxin)
0/0

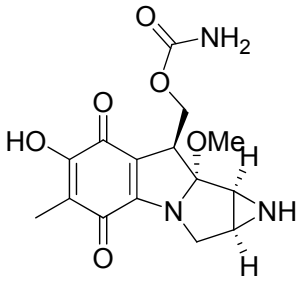
Structures of All Library members (con't)



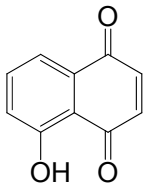
95: epi-digitoxigenin
0/0



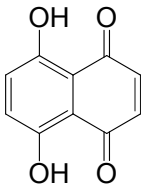
96: erythromycin
0/0



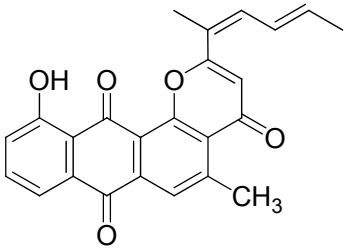
97: hydroxymitomycin C
0/0



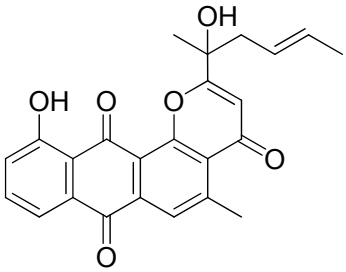
98: 5-hydroxy-1,4-naphthaquinone
0/0



99: 5,8-hydroxy-
1,4-naphthaquinone
0/0

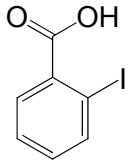


100: α-indomycinone
0/0

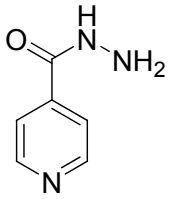


101: β-indomycinone
0/0

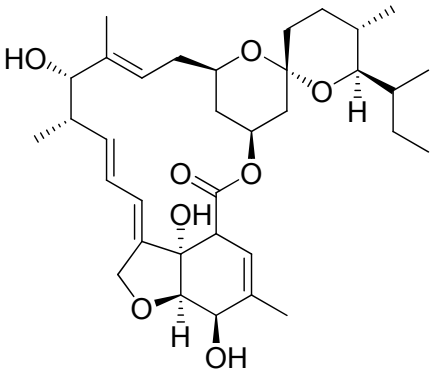
Structures of All Library members (con't)



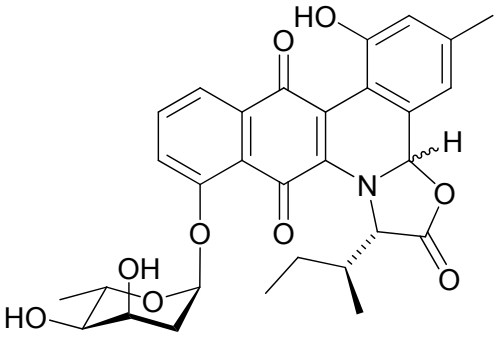
102: 2-iodobenzoic acid
0/0



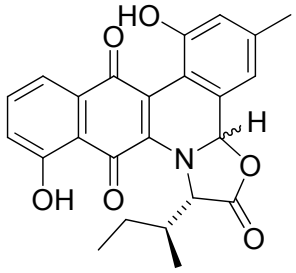
103: isoniazid
0/0



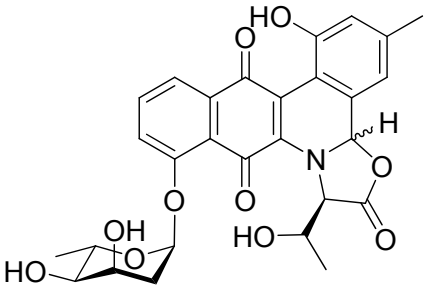
104: ivermectin aglycon
0/0



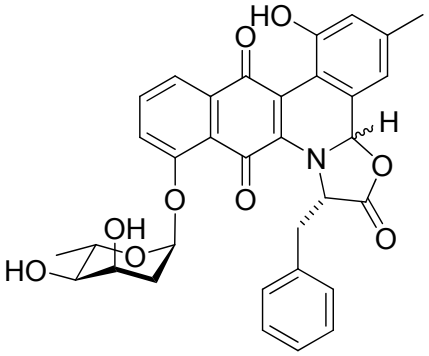
105: jadomycin B
0/0



106: jadomycin B aglycone
0/0

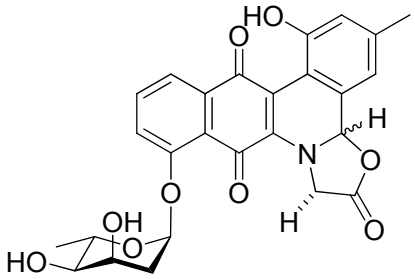


107: jadomycin DT

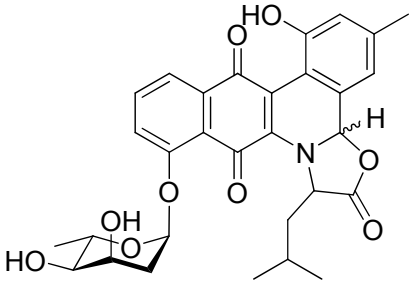


108: jadomycin F

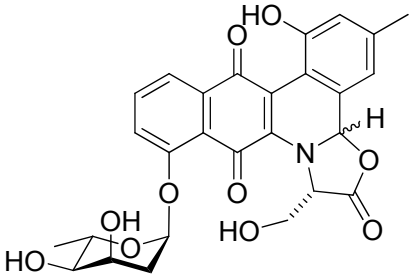
Structures of All Library members (con't)



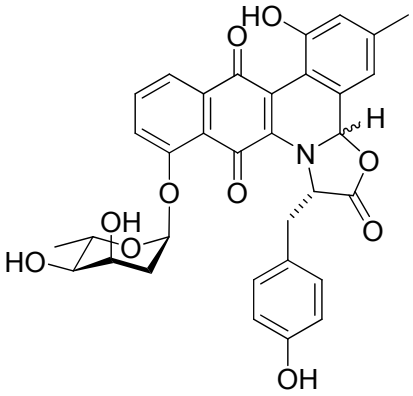
109: jadomycin G



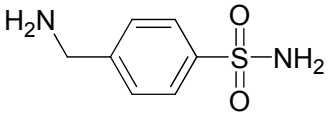
110: jadomycin L



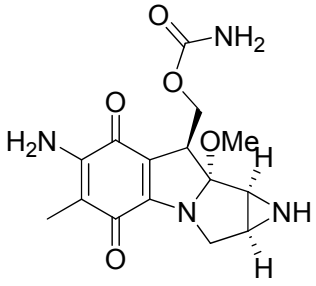
111: jadomycin S



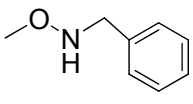
112: jadomycin Y



113: mafenide
0/0

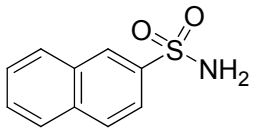


114: mitomycin C
0/0

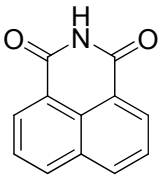


115: *N*-methoxybenzylamine
0/0

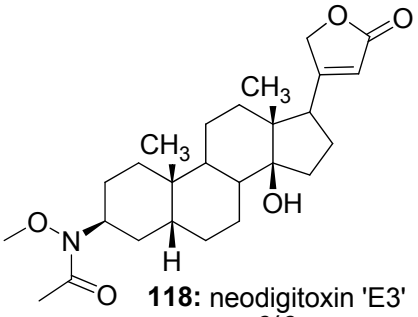
Structures of All Library members (con't)



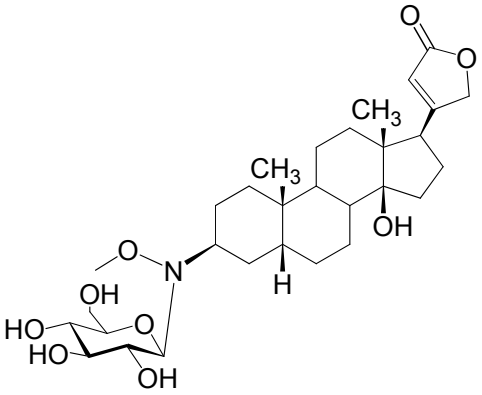
116: naphthalene-2-sulfonamide
0/0



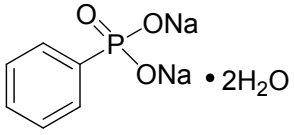
117: 1,8-naphthalimide
0/0



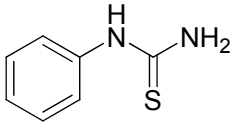
118: neodigitoxin 'E3'
0/0



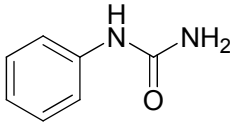
119: neodigitoxin glucoside
0/0



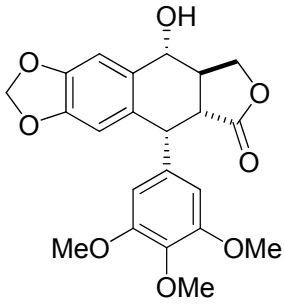
120: sodium phenyl phosphate dibasic dihydrate
0/0



121: *N*-phenylthiourea
0/0

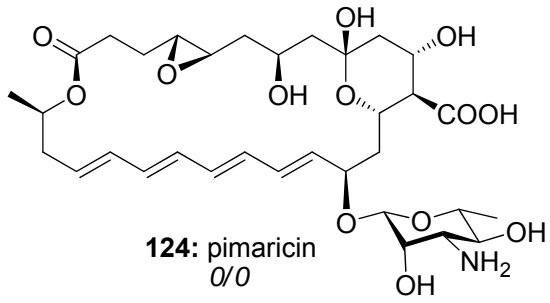


122: *N*-phenylurea
0/0

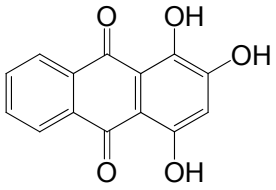


123: podophyllotoxin
0/0

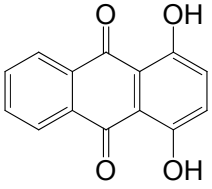
Structures of All Library members (con't)



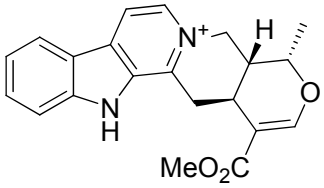
124: pimaricin
0/0



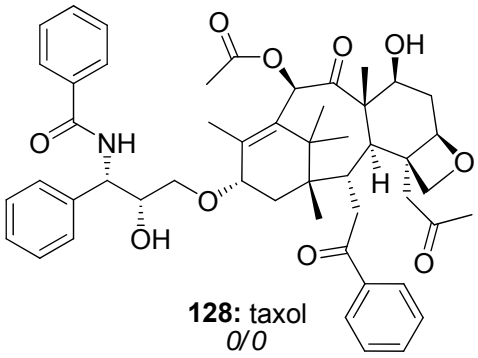
125: purpurin
0/0



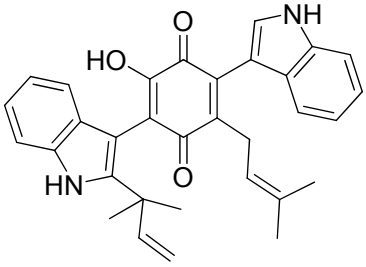
126: quinizarin
0/0



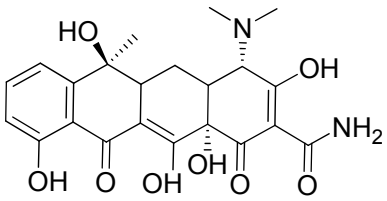
127: serpentine
0/0



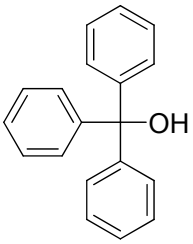
128: taxol
0/0



129: terrequinone A
0/0

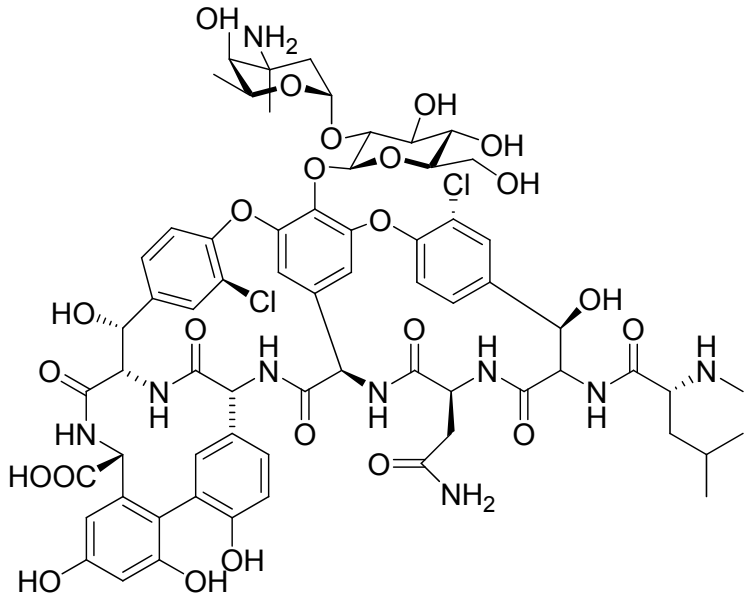


130: tetracycline
0/0

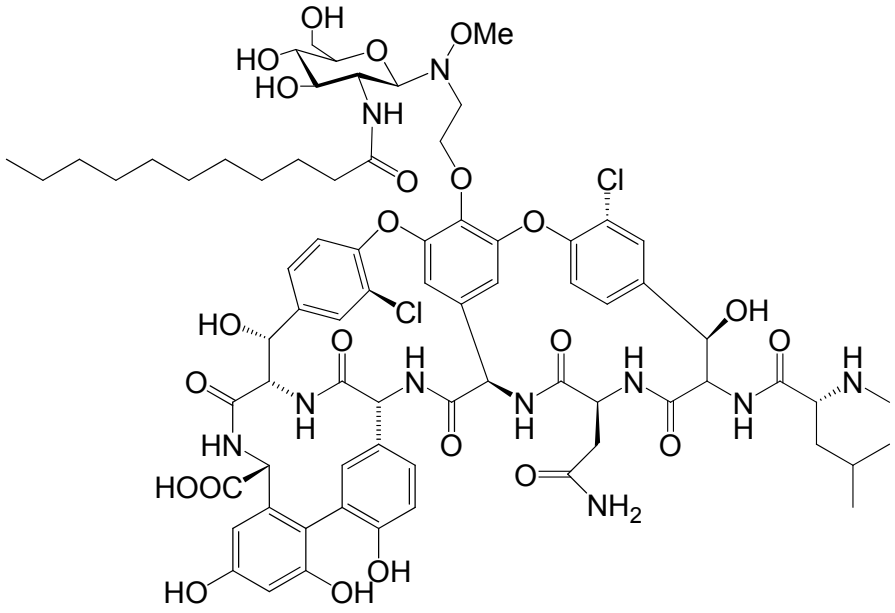


131: triphenolmethanol
0/0

Structures of All Library members (con't)

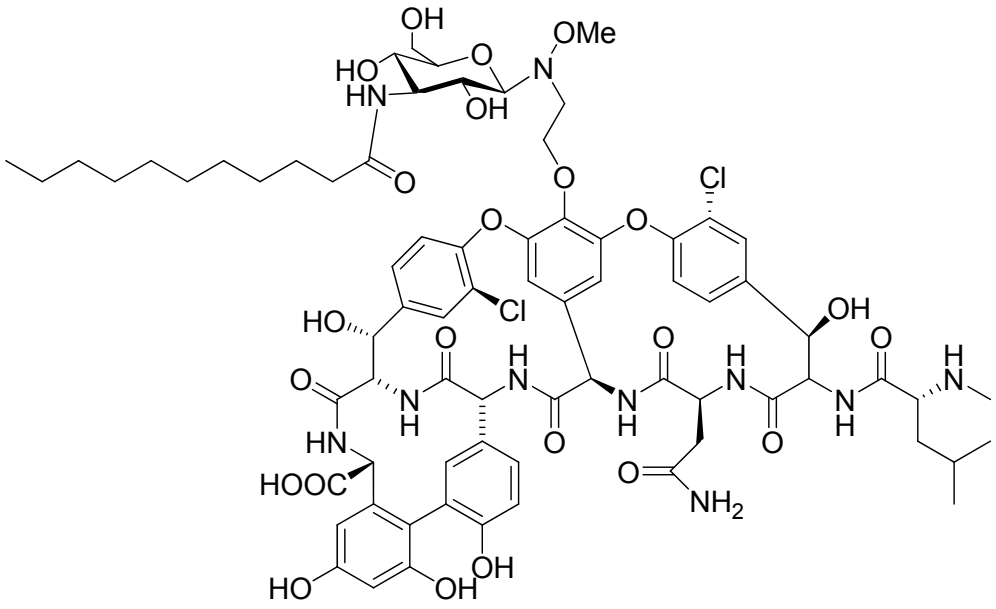


132: vancomycin
0/0

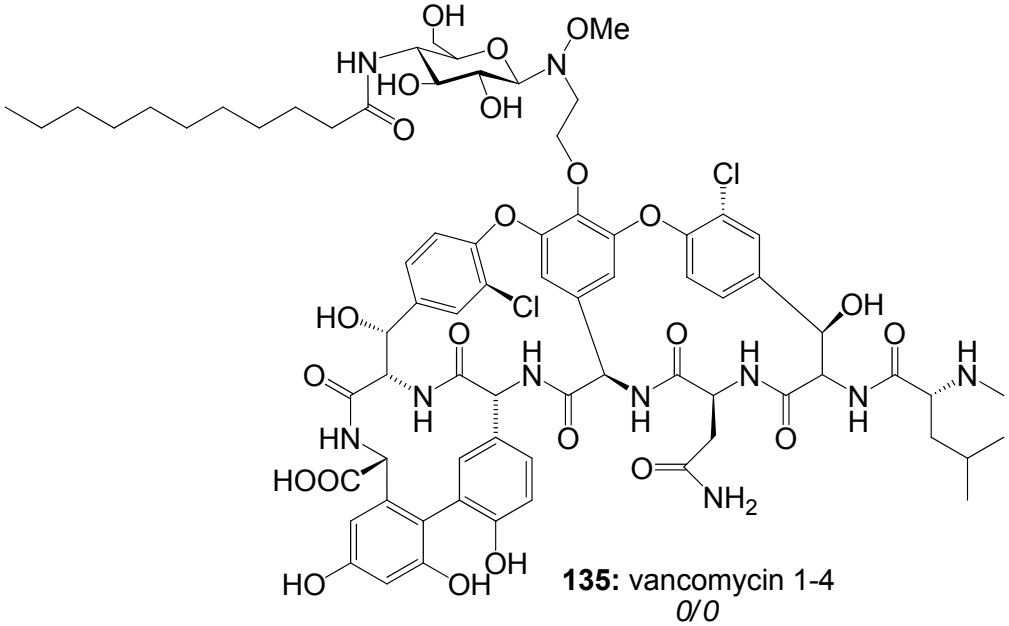


133: vancomycin 1-1
0/0

Structures of All Library members (con't)

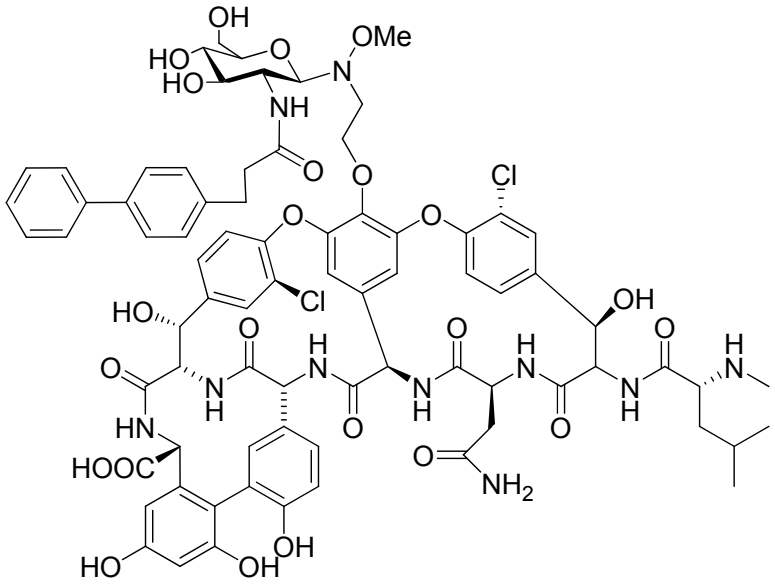


134: vancomycin 1-2
0/0

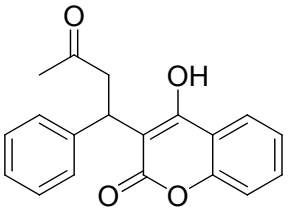


135: vancomycin 1-4
0/0

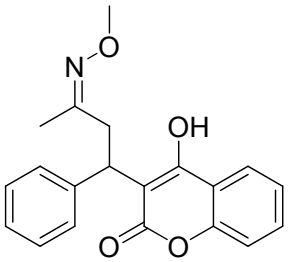
Structures of All Library members (con't)



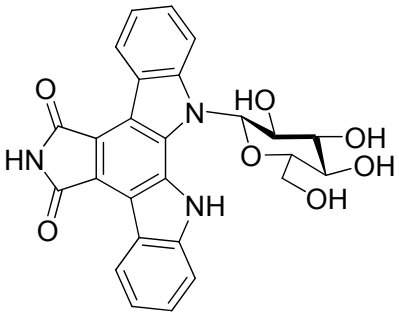
134: vancomycin 1-5
0/0



135: warfarin
0/0



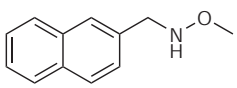
136: warfarin oxime
0/0



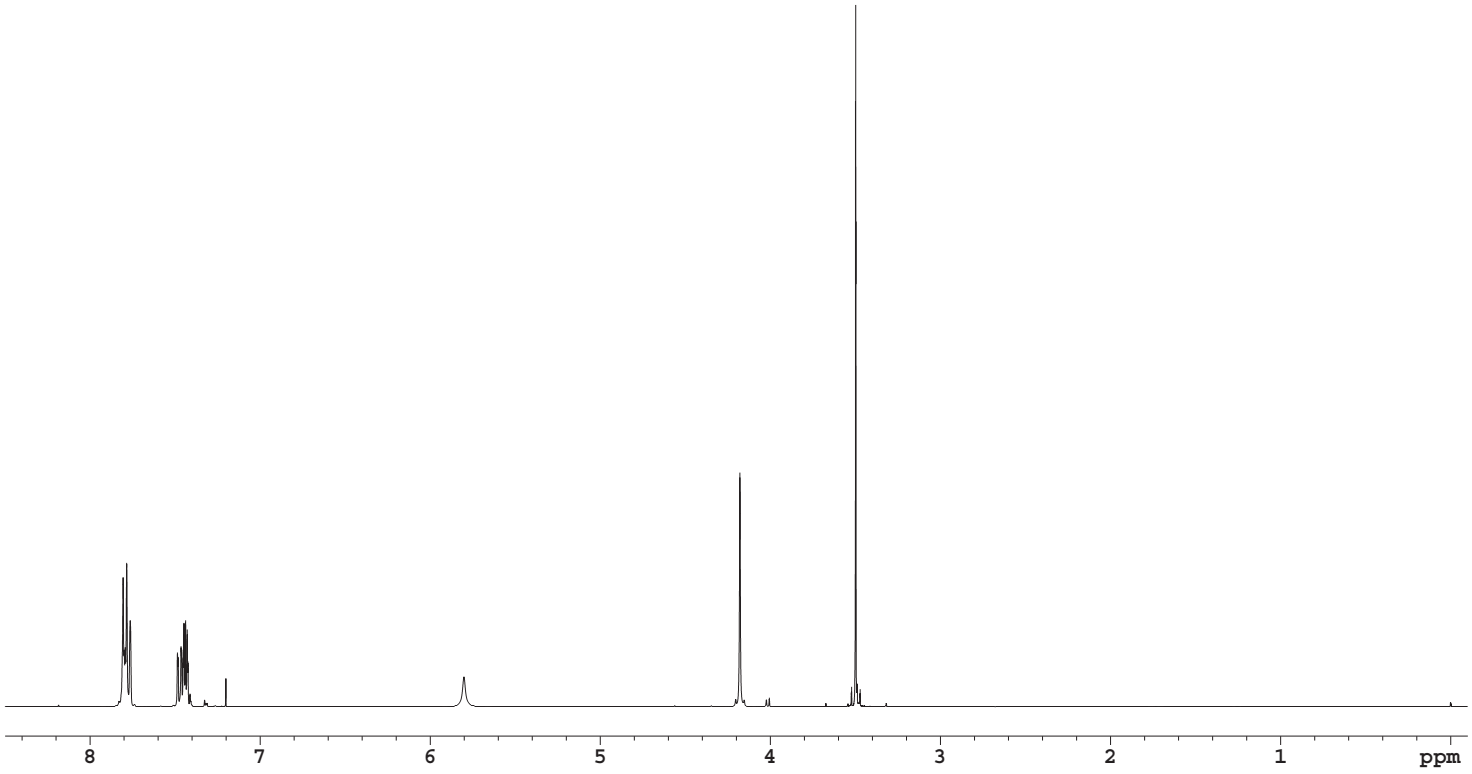
137: '131'
0/0

10. ^{1}H and ^{13}C NMR Spectra.

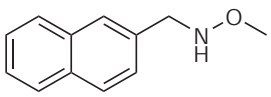
^{1}H, 400 MHz, $CDCl_3$



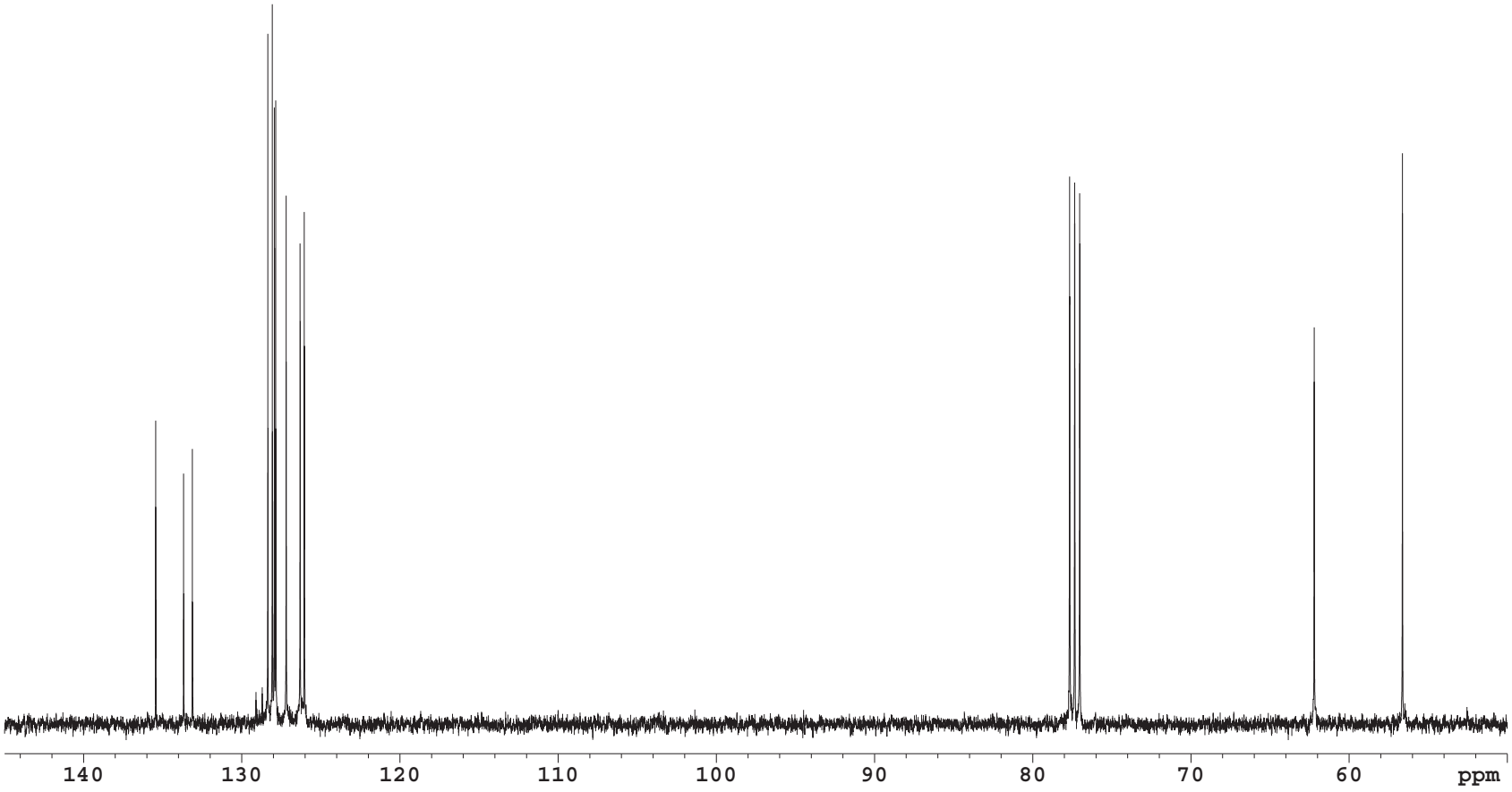
26



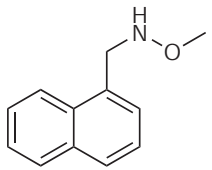
^{13}C, 100 MHz, $CDCl_3$



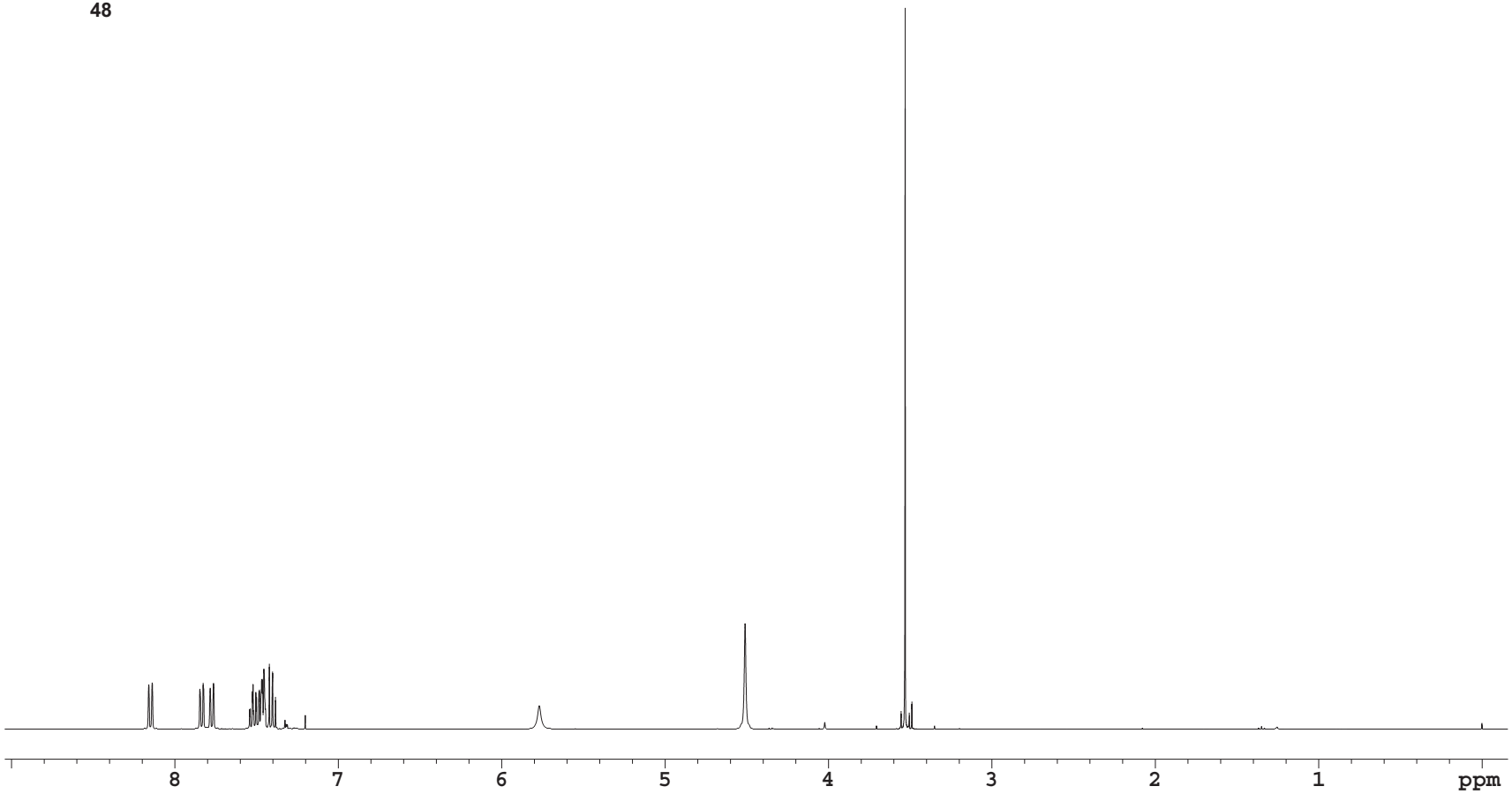
26



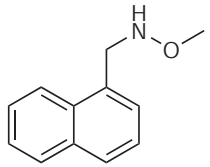
^{1}H, 400 MHz, $CDCl_3$



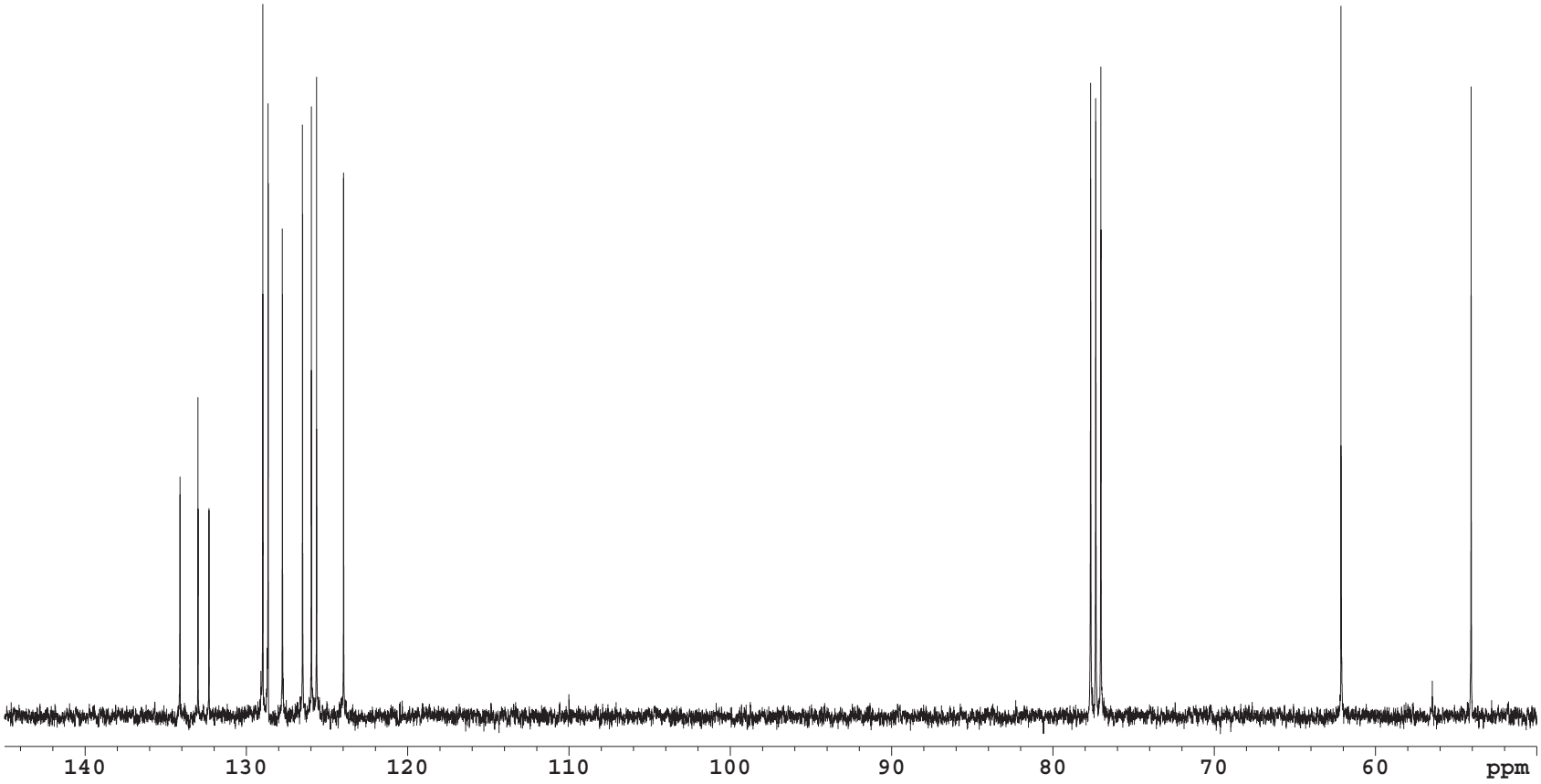
48



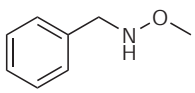
^{13}C, 100 MHz, $CDCl_3$



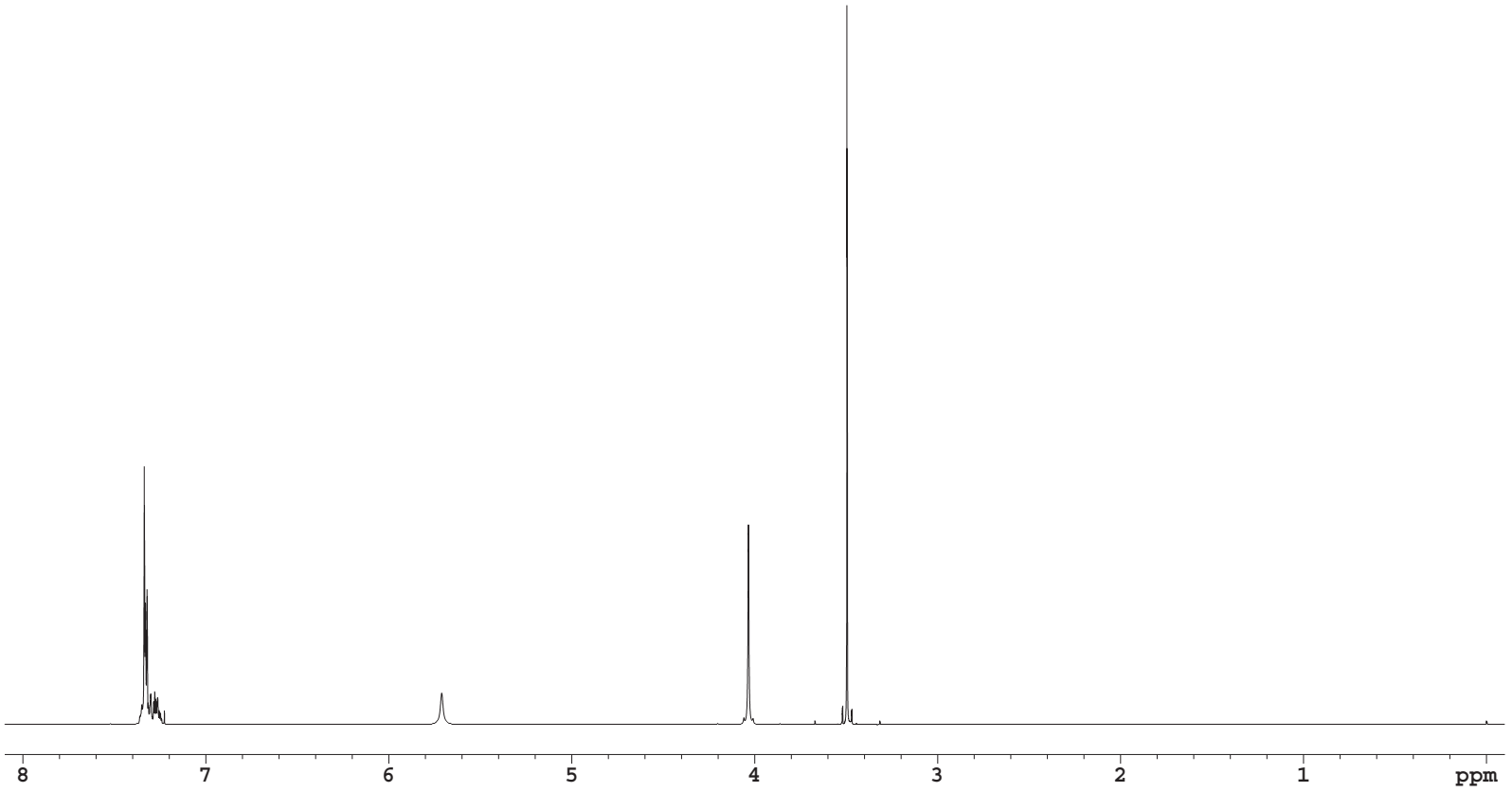
48



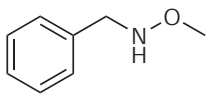
^{1}H, 400 MHz, $CDCl_3$



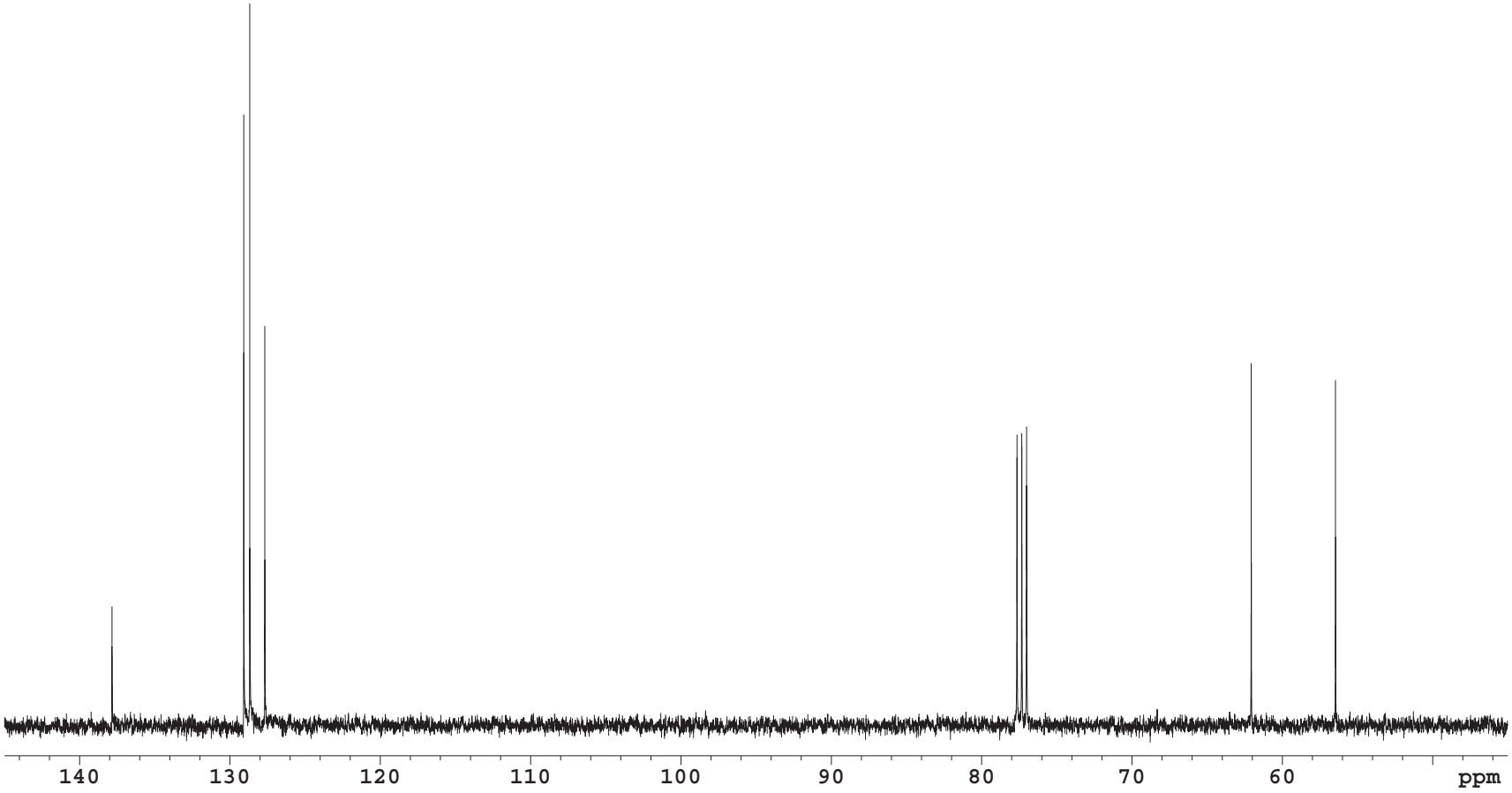
115



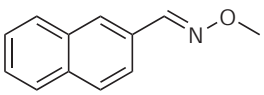
^{13}C, 100 MHz, $CDCl_3$



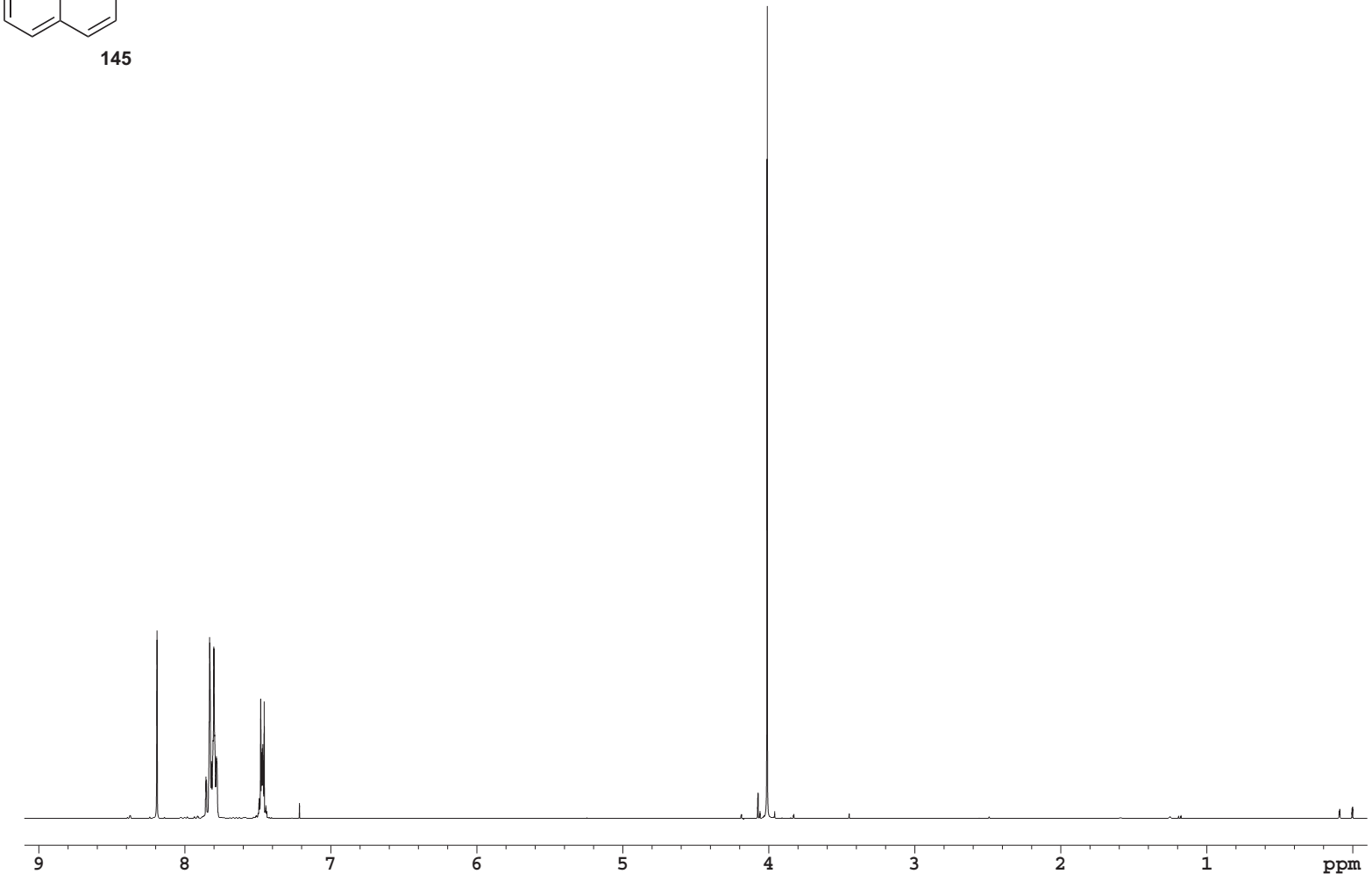
115



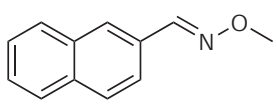
^{1}H, 400 MHz, $CDCl_3$



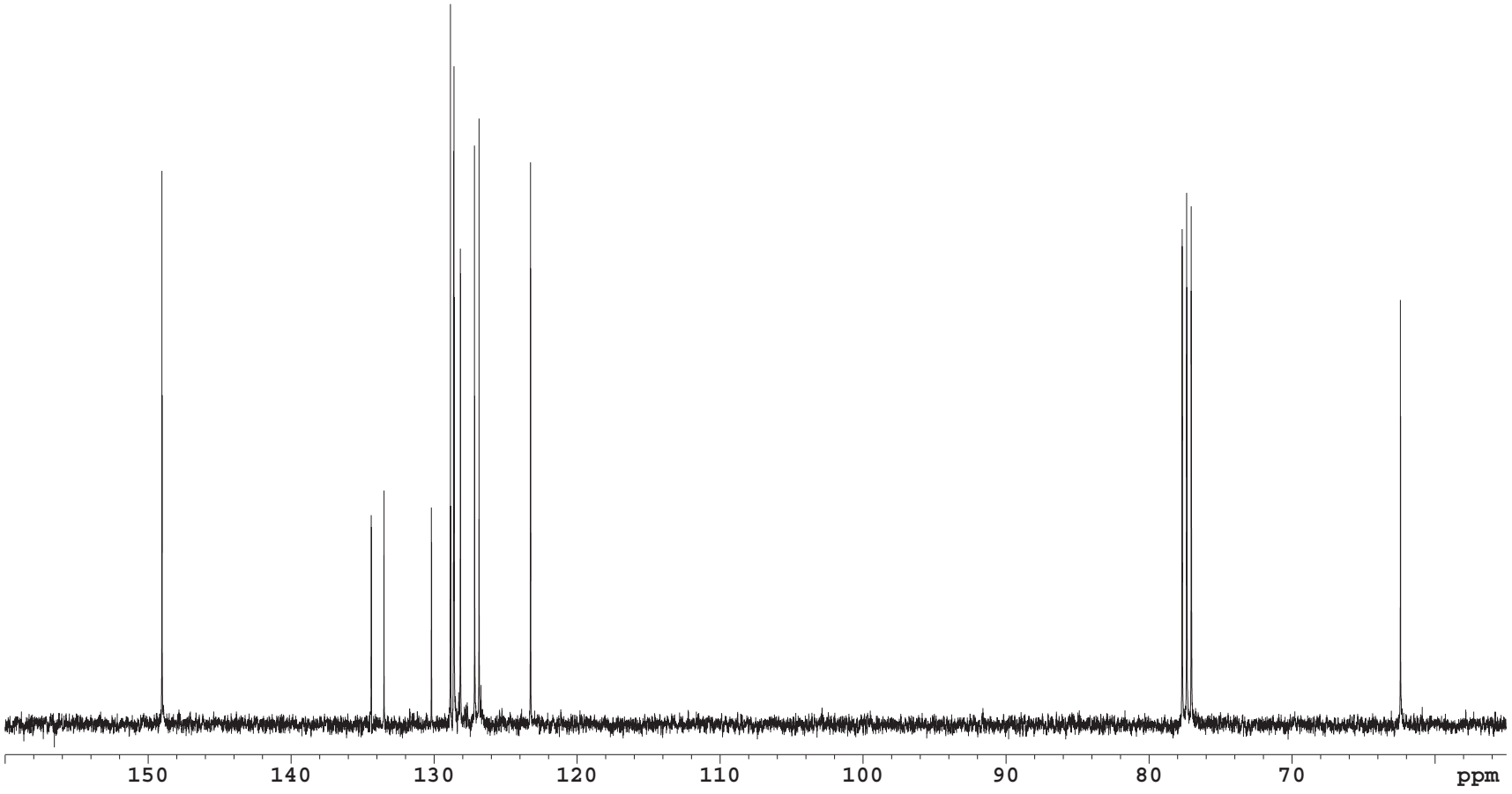
145



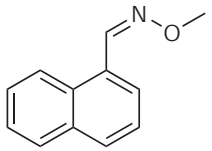
^{13}C, 100 MHz, $CDCl_3$



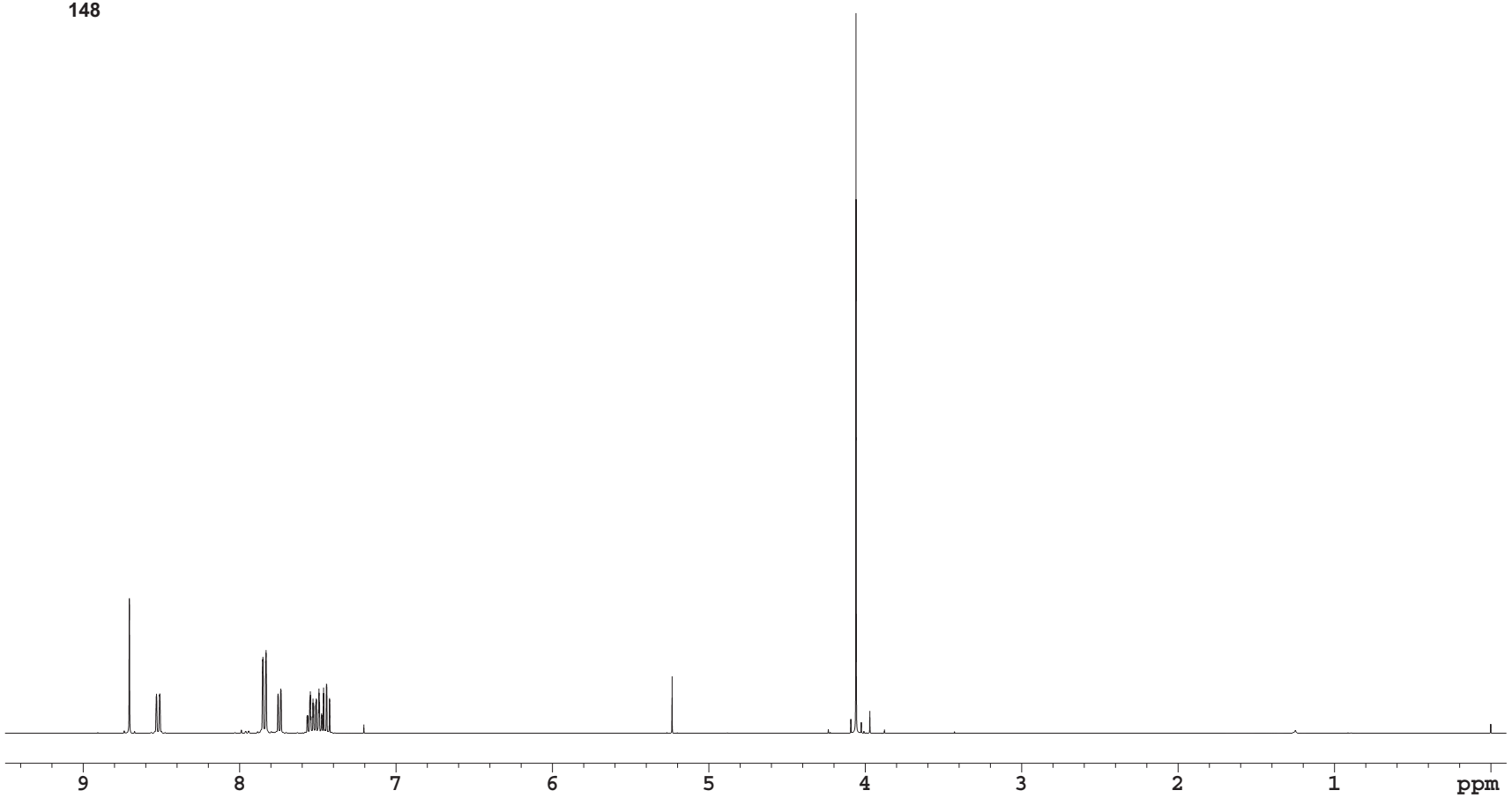
145



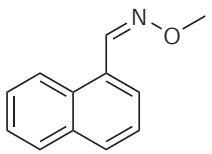
^{1}H, 400 MHz, $CDCl_3$



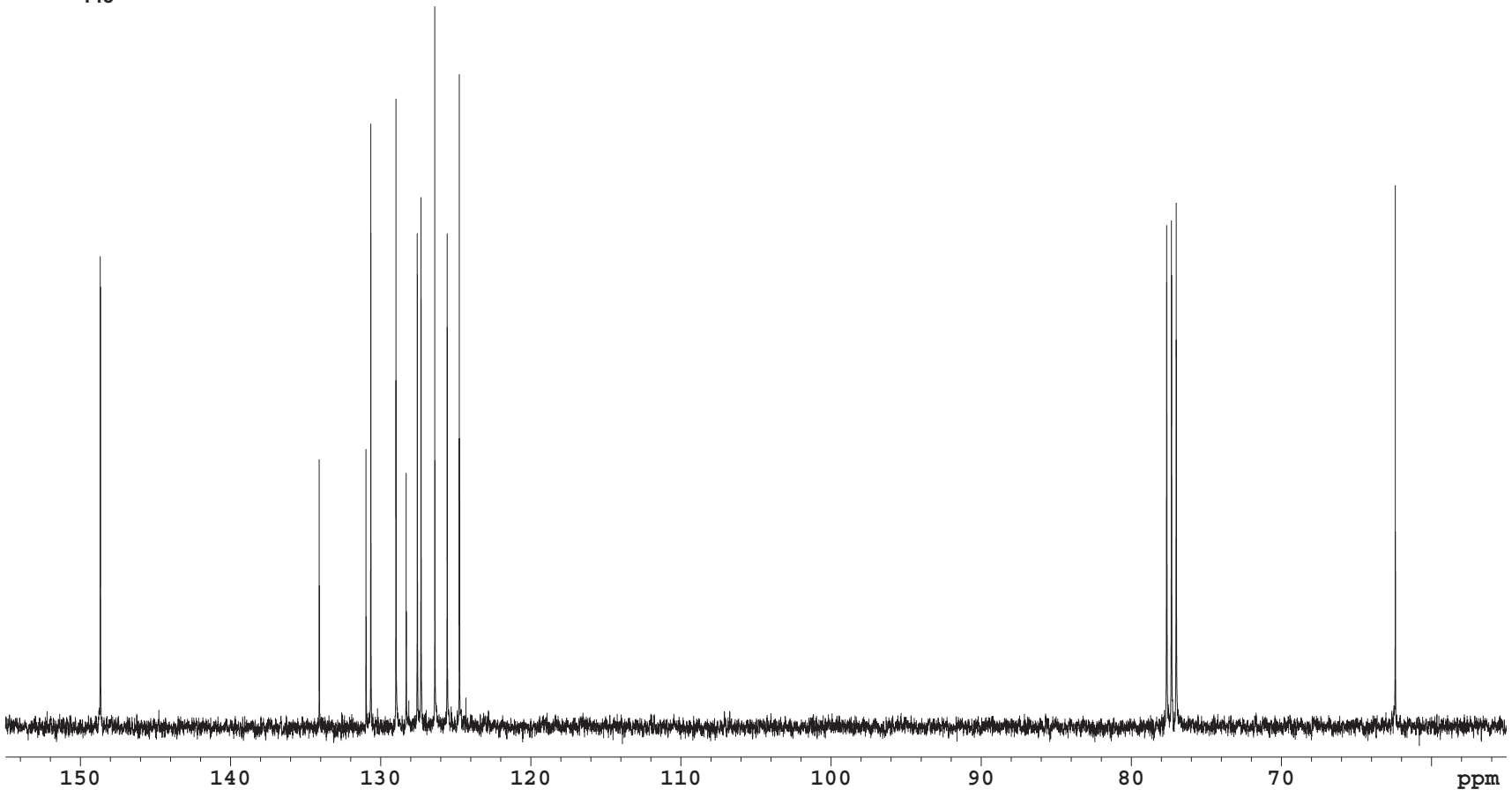
148



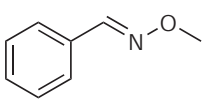
^{13}C, 100 MHz, $CDCl_3$



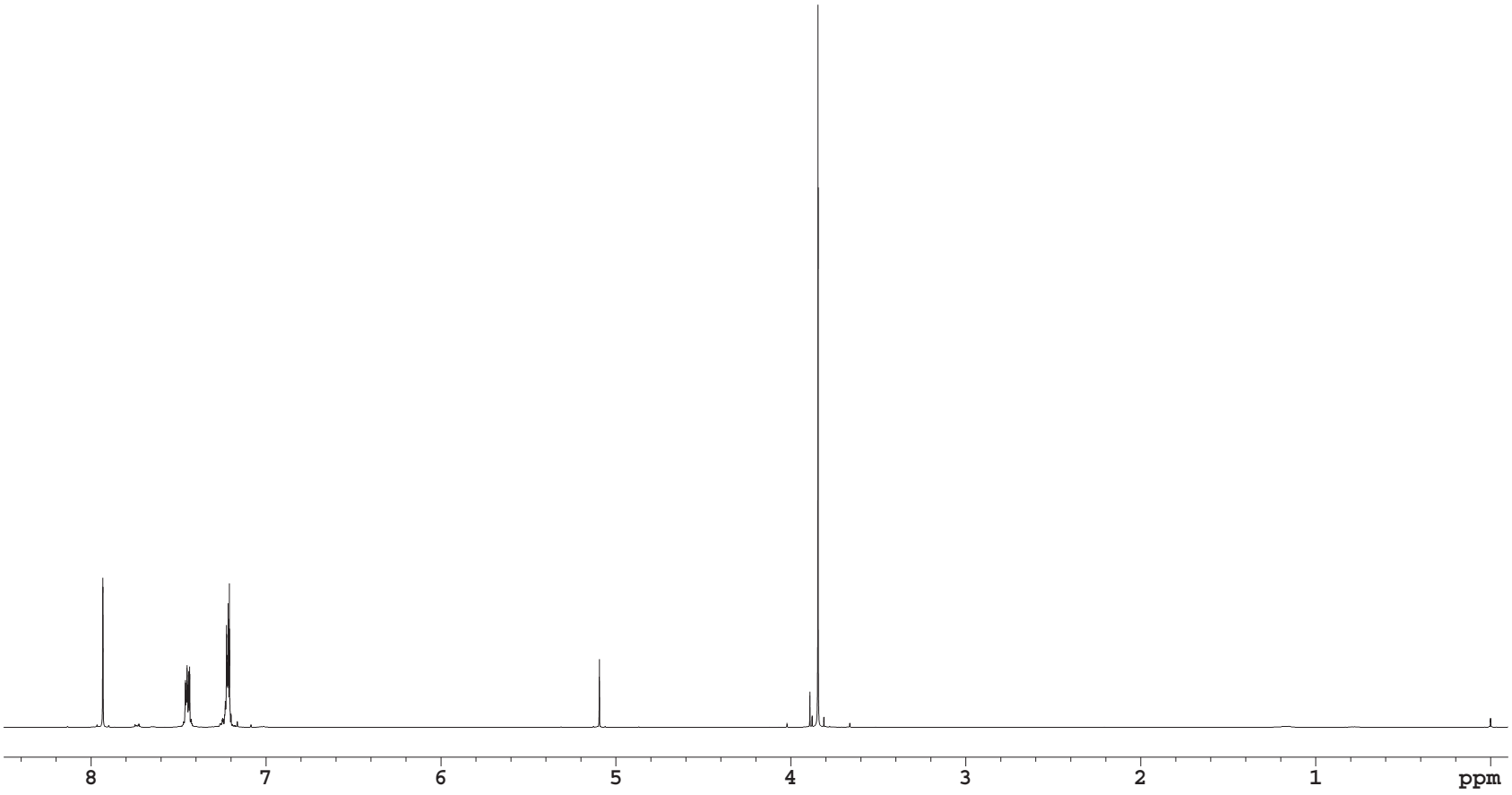
148



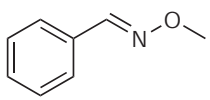
^{1}H, 400 MHz, $CDCl_3$



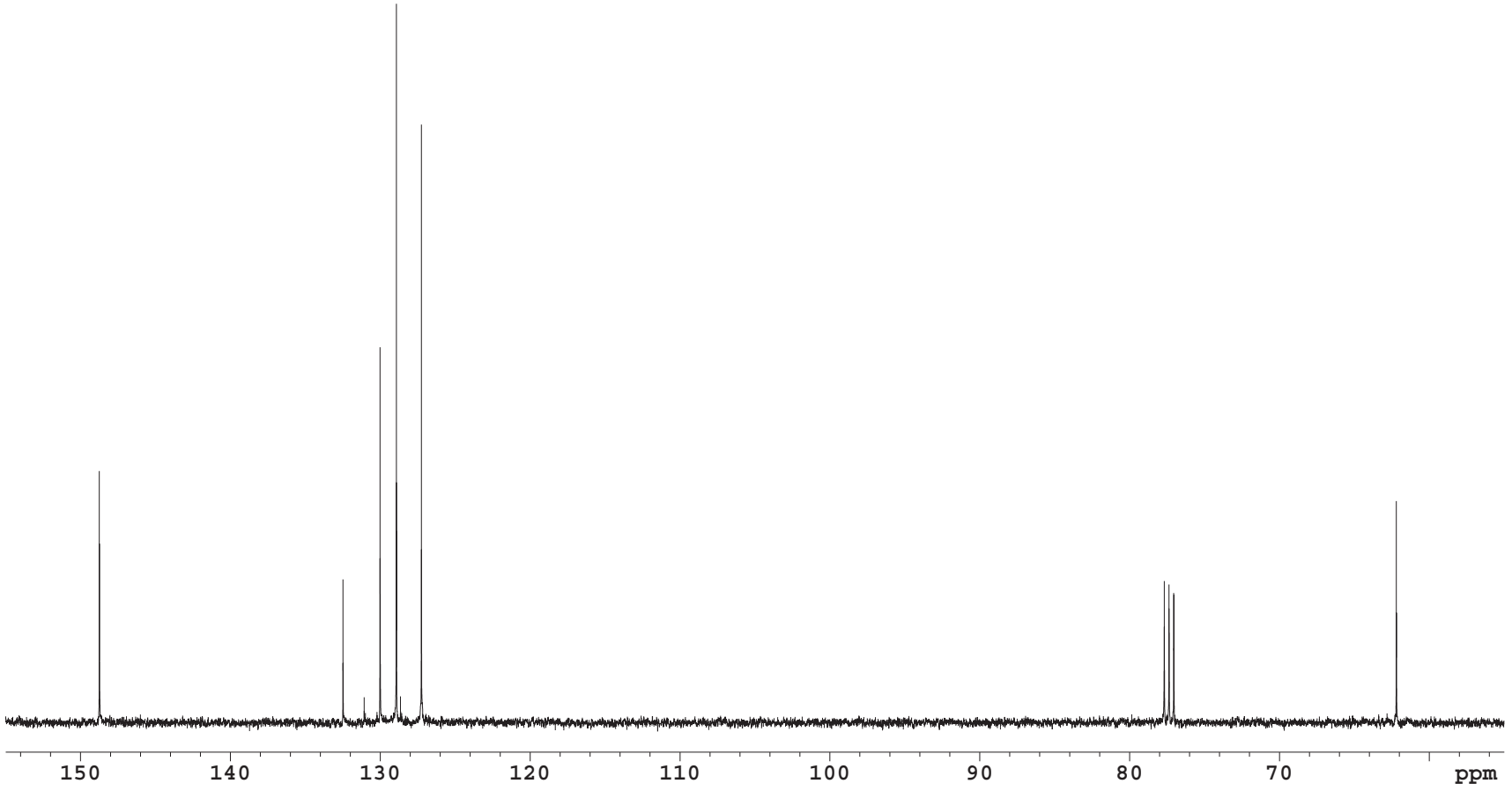
150



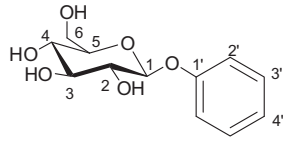
^{13}C, 100 MHz, $CDCl_3$



150

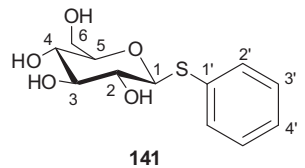


^{1}H, 500 MHz, CD_3OD



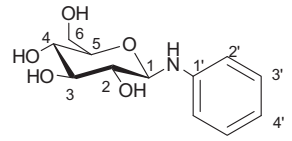
140

^{1}H, 500 MHz, CD_3OD



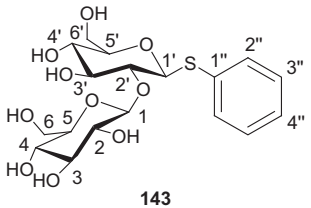
141

^{1}H, 500 MHz, CD_3OD



142

^{1}H, 500 MHz, acetone-d_6



143

11. Supplementary references.

[S1] J. M. Langenhan, N. R. Peters, I. A. Guzei, F. M. Hoffmann, J. S. Thorson, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 12305-12310.

[S2] C. Zhang, E. Bitto, R. D. Goff, S. Singh, C. A. Bingman, B. R. Griffith, C. Albermann, G. N. Phillips Jr., J. S. Thorson, *Chem. Biol.* **2008**, In press.

[S3] C. Zhang, C. Albermann, X. Fu, N. R. Peters, J. D. Chisholm, G. Zhang, E. J. Gilbert, P. G. Wang, D. L. Van Vranken, J. S. Thorson, *ChemBioChem* **2006**, *7*, 795-804.

[S4] A. Ahmed, N. R. Peters, M. K. Fitzgerald, J. A. Watson Jr., F. M. Hoffmann, J. S. Thorson, *J. Am. Chem. Soc.* **2006**, *128*, 14224-14225.

[S5] C. Zhang, C. Albermann, X. Fu, J. S. Thorson, *J. Am. Chem. Soc.* **2006**, *128*, 16420-16421.

[S6] B. R. Griffith, C. Krepel, X. Fu, S. Blanchard, A. Ahmed, C. E. Edmiston, J. S. Thorson, *J. Am. Chem. Soc.* **2007**, *129*, 8150-8155.

[S7] G. J. Williams, C. Zhang, J. S. Thorson, *Nat. Chem. Biol.* **2007**, *3*, 657-662.