Supporting Information

A Novel Ascaroside Controls the Parasitic Life Cycle of the Entomopathogenic Nematode *Heterorhabditis bacteriophora*

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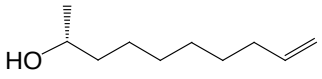
Supplementary Methods

General synthetic methods

All non-aqueous reactions were carried out under inert atmosphere (N_2) using ACS Certified ≥ 99% reagents and ACS Certified ≥ 98.5% or HPLC grade solvents, which were used as received. Tetrahydrofuran (THF) was dried via passage over a column of activated alumina. High-performance liquid chromatography (HPLC) was performed using only HPLC grade water and acetonitrile on a traditional reverse-phase column. Flash column chromatography was performed using 60Å silica gel. ^{1}H NMR and ^{13}C NMR spectroscopy were performed on a Bruker AV-400, Varian VS-700 or Varian INOVA 600. Mass spectra were obtained using an Agilent 6210 electrospray time-of-flight mass spectrometer. Infrared spectroscopy was performed on a Bruker Tensor 27 FT-IR spectrometer. Optical rotation measurements were obtained using a JASCO P-2000 polarimeter.

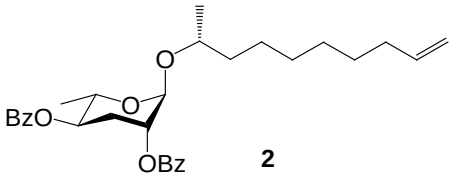
Synthesis of 1:



1

To a suspension of 82.4 mg (3.39 mmol) of magnesium turnings in 0.44 mL dry THF was added 0.52 mL (3.4 mmol) 7-bromo-1-heptene in 2.9 mL dry THF dropwise over the course of 4 minutes. The resulting suspension was brought to reflux and stirred for 12 h until all magnesium had been consumed. The resulting cloudy, pale-yellow mixture was allowed to cool to 25°C. To a separate flask was added 34.2 mg (0.238 mmol) CuBr in 5 mL dry THF. This suspension was cooled to -78°C. To this chilled suspension was added the previously synthesized 7-heptenylmagnesium bromide dropwise over 3 minutes via syringe. This resulting suspension was allowed to stir for 10 minutes, upon which it turned dark gray with black solids. To this suspension was added 0.17 mL (2.4 mmol) (R)-(+)-propylene oxide at once, upon which it turned from dark gray to black. The suspension was allowed to warm to 25°C and was stirred for 3 h. To the black suspension was added 4 mL sat. NH_4Cl at once. The aqueous layer was separated from the organic layer and then extracted with 3 x 10 mL THF. The resulting organic extracts were dried over $MgSO_4$ and filtered to obtain 509.3 mg of dark yellow oil after rotary evaporation. Silica gel column chromatography (54.9 g silica gel, 20% ethyl acetate in hexanes) afforded 225.2 mg (43%) of a colorless oil. $[\alpha]_D^{25}$ = -19.8, *c* 1.87 (CH_2Cl_2); IR (cm^{-1}): 3334; ^1H NMR (400 MHz, $CDCl_3$): δ 5.79 (ddt, 1H, J=17.1 Hz, J=10.2 Hz, J=6.7 Hz); 4.97 (d, 1H, J=17.2 Hz); 4.91 (d, 1H, J=10.2 Hz); 3.76 (m, 1H); 2.02 (q, 2H, J=6.9 Hz); 1.36 (m, 10H); 1.16 (d, 3H, J=6.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 139.1; 114.2; 68.2; 39.3; 33.8; 29.5; 29.1; 28.8; 25.7; 23.5.

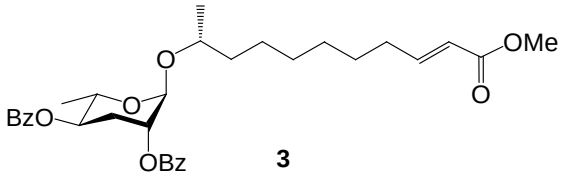
Synthesis of 2:



2

A suspension of 500.3 mg (1.404 mmol) dibenzoyl ascarylose, 350.4 mg (2.242 mmol) alcohol **1** and 166.0 mg 4Å molecular sieves in 16 mL CH_2Cl_2 was cooled to 0°C. To this suspension was added 0.77 mL (6.1 mmol) $BF_3{\cdot}OEt_2$ at once. The resulting suspension was stirred at 0°C for 2.5 h. 20 mL saturated $NaHCO_3$ solution was then added. Upon cessation of effervescence, the aqueous layer was separated from the organic layer and then extracted with 3 x 10 mL 20% *i*PrOH in CH_2Cl_2. The resulting organic extracts were dried over $MgSO_4$ and filtered to obtain 847.3 mg of oil. Silica gel column chromatography (53 g silica gel, gradient run from 50% hexanes in CH_2Cl_2 to 100% CH_2Cl_2) afforded 487.6 mg (70%) of a colorless syrup. $[\alpha]_D^{25}$ = -14.0, *c* 2.33 (CH_2Cl_2); IR (cm^{-1}): 1722; HRMS (*m/z*): $[M+Na]^+$ calcd. for $C_{30}H_{38}O_6Na$ 517.2561, found 517.2564; ^1H NMR (400 MHz, $CDCl_3$): δ 8.12 (d, 2H, J=7.2 Hz); 8.05 (d, 2H, J=7.2 Hz); 7.58 (t, 2H, J=6.8 Hz); 7.46 (m, 4H); 5.82 (ddt, 1H, J=17.1, J=10.2 Hz, J=6.7 Hz); 5.19 (td, 1H, J=10.5 Hz, J=4.1 Hz); 5.15 (m, 1H); 5.00 (d, 1H, J=17.2 Hz); 4.96 (s, 1H); 4.94 (d, 1H, J=11.1 Hz); 4.13 (dq, 1H, J=9.7 Hz, J=6.2 Hz); 3.85 (m, 1H); 2.42 (dt, 1H, J=13.3 Hz, J=3.4 Hz); 2.22 (td, 1H, J=11.0 Hz, J=12.4 Hz, J=2.9 Hz); 2.07 (q, 2H, J=6.8 Hz); 1.65 (m, 1H); 1.45 (m, 9H); 1.29 (d, 3H, J=6.2 Hz), 1.19 (d, 3H, J=6.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.8; 165.6; 139.1; 133.2; 133.1; 130.0; 129.8; 129.6; 128.4; 114.2; 93.8; 72.6; 71.2; 70.7; 66.9; 37.1; 33.8; 29.7; 29.4; 29.1; 28.9; 25.7; 19.2; 17.9.

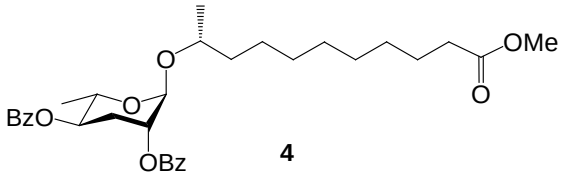
Synthesis of 3:



3

To a solution of 480.2 mg (0.9367 mmol) alkene **2** and 0.42 mL (4.7 mmol) methyl acrylate in 29.8 mL CH_2Cl_2 at reflux was added 79.5 mg (0.0937 mmol) Grubbs 2nd generation ruthenium catalyst at once. The resulting solution was allowed to stir at reflux for 6 h. The reaction mixture was concentrated to 404.0 mg of brown oil. Silica gel column chromatography (41 g silica gel, gradient run from 2% diethyl ether in hexanes to 10% diethyl ether in hexanes) afforded 485.1 mg (91%) of a yellow oil. $[\alpha]_D^{25}$ = -1.3, *c* 2.75 (CH_2Cl_2); IR (cm^{-1}): 1721; HRMS (*m/z*): $[M+Na]^+$ calcd. for $C_{32}H_{40}O_8Na$ 575.2615, found 575.2606; ^1H NMR (400 MHz, $CDCl_3$): δ 8.11 (d, 2H, J=7.1 Hz); 8.04 (d, 2H, J=7.2 Hz); 7.58 (t, 2H, J=7.2 Hz); 7.46 (m, 4H); 6.98 (dt, 1H, J=15.6 Hz, J=6.9 Hz); 5.82 (d, 1H, J=15.6 Hz); 5.18 (td, 1H, J=10.5 Hz, J=4.0 Hz); 5.15 (m, 1H); 4.95 (s, 1H); 4.11 (dq, 1H, J=9.7 Hz, J=6.1 Hz); 3.84 (m, 1H); 3.70 (s, 3H); 2.42 (dt, 1H, J=13.3 Hz, J=3.7 Hz); 2.22 (m, 3H); 1.67 (m, 1H); 1.43 (m, 9H); 1.28 (d, 3H, J=6.2 Hz); 1.19 (d, 3H, J=6.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.1; 165.7; 165.6; 149.6; 133.2; 133.1; 130.0; 129.8; 129.5; 128.4; 120.9; 93.8; 72.6; 71.2; 70.6; 66.9; 51.3; 37.0; 32.1; 29.7; 29.3; 29.1; 28.0; 25.6; 19.1; 17.8.

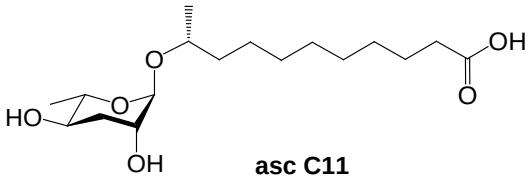
Synthesis of 4:



4

A suspension of 422.8 mg (0.7650 mmol) unsaturated ester **3** and 50.4 mg 10 % palladium on activated carbon in 46.0 mL EtOAc was vacuum purged and back filled with H_2 from a balloon. The resulting suspension was allowed to stir at 23^{o}C for 22 h. The reaction mixture was filtered through Celite® and concentrated to obtain 411.5 mg (97%) of colorless oil that required no further purification. $[\alpha]_D^{25}$ = -2.1, *c* 4.04 (CH_2Cl_2); IR (cm^{-1}): 1722; HRMS (*m/*z): $[M+Na]^+$ calcd. for $C_{32}H_{42}O_8Na$ 577.2772, found 577.2779; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 (d, 2H, J=7.4 Hz); 8.04 (d, 2H, J=7.4 Hz); 7.57 (t, 2H, J=6.8 Hz); 7.45 (m, 4H); 5.18 (td, 1H, J=10.4 Hz, J=4.2 Hz); 5.15 (m, 1H); 4.95 (s, 1H); 4.12 (dq, 1H, J=9.7 Hz, J=6.1 Hz); 3.84 (m, 1H); 3.64 (s, 3H); 2.42 (dt, 1H, J=13.3 Hz, J=4.1 Hz); 2.29 (t, 2H, J=7.5 Hz); 2.21 (td, 1H, J=12.4 Hz, J=2.8 Hz); 1.70 (m, 3H); 1.42 (m, 11H); 1.28 (d, 3H, J=6.2 Hz); 1.18 (d, 3H, J=6.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.1; 165.6; 165.5; 133.1; 133.0; 129.9; 129.7; 129.5; 128.3; 93.7; 72.5; 71.1; 70.6; 66.8; 51.3; 37.0; 33.9; 29.6; 29.4; 29.3; 29.1; 29.0; 25.6; 24.8; 19.1; 17.8.

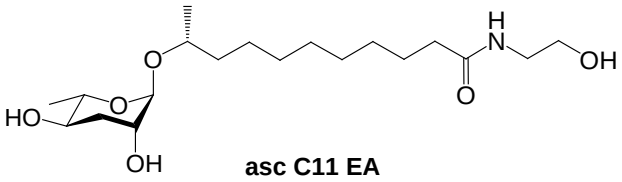
Synthesis of asc C11 (free acid):



asc C11

To a suspension of 55.8 mg (0.101 mmol) saturated ester **4** in 7.9 mL *t*BuOH was added 7.9 mL sat. 1M LiOH at once. The resulting solution was allowed to stir at 23°C for 19 h. 1N HCl was then added dropwise until the reaction mixture reached a pH=3. 5.0 mL 20% *i*PrOH in CH_2Cl_2 was added to the acidified reaction mixture, and the layers were separated. The aqueous layer was extracted with an additional 8 x 5 mL 20% *i*PrOH in CH_2Cl_2. Solid NaCl was then added until saturation was obtained, and the aqueous layer was further extracted with 3 x 5 mL 20% *i*PrOH in CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and filtered. Evaporation of solvent afforded 128.3 mg of an oil. HPLC purification afforded 19.4 mg (58%) of a colorless oil. $[\alpha]_D^{25}$ = -54.5, *c* 0.99 (CH_3OH); IR (cm^{-1}): 3390; 1737; HRMS (*m/z*): $[M+Na]^+$ calcd. for $C_{17}H_{32}O_6Na$ 355.2091, found 355.2074; ^1H NMR (400 MHz, CD_3OD): δ 4.68 (s, 1H); 3.77 (m, 2H); 3.68 (m, 1H); 3.58 (td, 1H, J=10.3 Hz, J=4.4 Hz); 2.29 (t, 2H, J=7.6 Hz); 2.06 (dt, 1H, J=13.0 Hz, J=3.8 Hz); 1.83 (td, 1H, J=12.3 Hz, J=2.9 Hz); 1.58 (m, 3H); 1.34 (m, 11H); 1.20 (d, 3H, J=6.1 Hz); 1.11 (d, 3H, J=6.1 Hz); ^{13}C NMR (400 MHz, $CDCl_3$): δ 174.5; 96.0; 71.6; 69.8; 69.3; 68.1; 37.1; 35.1; 34.1; 29.4; 29.3; 29.1; 29.0; 25.6; 24.9; 18.9; 17.6.

Synthesis of asc C11 EA:

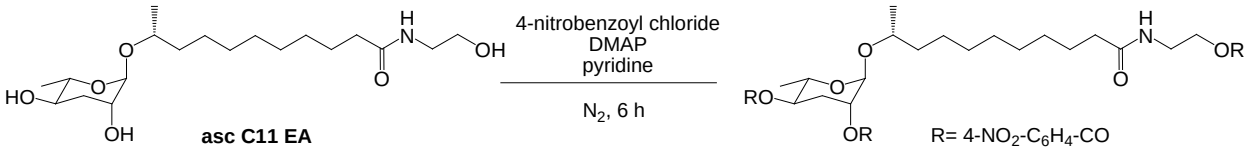


asc C11 EA

To a solution of 57.8 mg (0.104 mmol) saturated ester **4** in 4.3 mL pyridine at reflux was added 0.1 mL (2 mmol) ethanolamine at once. The resulting solution was allowed to stir at reflux for 48 h, in which time formation of the amide bond and monodebenzoylation occurred (as judged with ^{1}H NMR). The reaction mixture was concentrated to a yellow syrup. This syrup was dissolved in 7.3 mL *t*BuOH. 7.3 mL 1M LiOH was then added at once. This solution was allowed to stir for 24 h at 23^{o}C. 1N HCl was then added dropwise until the reaction mixture reached a pH=5. 5.0 mL 20% *i*PrOH in CH_2Cl_2 was then added to the acidified reaction mixture and the layers were separated. The aqueous layer was extracted with an additional 6 x 5 mL 20% *i*PrOH in CH_2Cl_2. The aqueous layer was then saturated with solid NaCl and further extracted with 3 x 5 mL 20% *i*PrOH in CH_2Cl_2. The combined organic layers were dried over Na_2SO_4, filtered and concentrated to a pale yellow oil. HPLC purification afforded 31.5 mg (81%, 2 steps) of a colorless oil. $[\alpha]_D^{25}$ = -49.4, *c* 1.56 (CH_3OH); IR (cm^{-1}): 3314; 1651; HRMS (*m/*z): $[M+Na]^+$ calcd. for $C_{19}H_{37}NO_6Na$ 398.2513, found 398.2496; see Supplemental Table 1.

Establishing absolute stereochemistry

Derivatization of asc C11 EA with 4-nitrobenzoyl chloride.



Natural or synthetic asc C11 EA (0.5mg) and a catalytic amount of 4-dimethylaminopyridine were added to 300 µL of a stirred solution of 4-nitrobenzoyl chloride in pyridine (10 mg/mL). The reaction mixture was allowed to stir at room temperature under N_2 for 6 h and was subsequently quenched with 100 µL of water. The crude reaction mixture was dried *in vacuo* and the resulting residue was chromatographed on silica gel eluted with 2:1 ethyl acetate/hexanes to afford the 2', 4', 2"-tri-4-nitrobenzoate derivative. ^{1}H NMR (600 MHz, CD_3OD): δ 8.39 (d, 2H); 8.34 (d, 2H); 8.33 (d, 2H); 8.31 (d, 2H); 8.24 (d, 2H); 8.23 (d, 2H); 5.13 (m, 1H); 5.13 (m, 1H); 4.98 (s, 1H); 4.40 (t, 2H); 4.18 (dq, 1H); 3.85 (m, 1H); 3.58 (t, 2H); 2.46 (dt, 1H); 2.24 (ddd, 1H); 2.18 (t, 2H); 1.60 (m, 2H); 1.45 (m, 1H); 1.38 (m, 1H); 1.27 (br s, 11H); 1.26 (d, 3H); 1.18 (d, 3H).

Circular Dichroism. The absolute stereochemistry of natural asc C11 EA was determined to be 10*R*, 2'*R*, and 4'*R* by comparing the circular dichroism (CD) spectra of the 2', 4', 2"-tri-4-nitrobenzoate derivatives of the natural and synthetic asc C11 EA. Both CD spectra displayed a negative Cotton Effect at 220 nm and a positive Cotton Effect at 268 nm. Circular dichroism spectra were acquired on an AVIV Circular Dichroism Model 202 spectrometer in a 350 µL Hellma quartz cuvette. The data were obtained in methanol using two scans in the range of 200-340 nm and obtaining data points every 1 nm with an averaging time of 3 s and a 1 nm bandwidth.

Secretion of asc C11 EA by *H. bacteriophora* adults. Approximately 100 μL of settled *H. bacteriophora* adults were washed and incubated in 1 mL of Ringer's solution for 3 h with gentle shaking. After 3 h, the adults were still moving and alive. The worms were allowed to settle, and the supernatant was collected and dried prior to extraction with MeOH. The secretions were re-dissolved in 100 μL MeOH and 5 μL was injected onto an Agilent 6130 single quadrupole LCMS. The concentration of asc C11 EA in the 1 mL of Ringer's solution was estimated to be 5 nM, using an LCMS-based standard curve generated with synthetic asc C11 EA.

Figure S1. Comparison of the activity of crude pheromone extract (at 1X) and synthetic asc C11 EA (at 1 μM) in the IJ recovery assay. Data represent the average of two independent assays (± 1 SD).

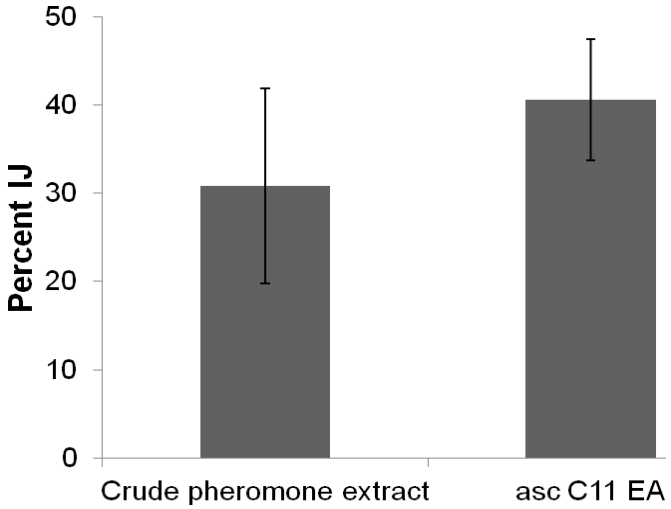


Figure S2. NMR spectra of natural asc C11 EA in methanol-d_4. (A) ^{1}H NMR spectrum of natural asc C11 EA in methanol-d_4. (B) dqf-COSY spectrum of natural asc C11 EA in methanol-d_4. (C) gHSQC spectrum of natural asc C11 EA in methanol-d_4. (D) gHMBC spectrum of natural asc C11 EA in methanol-d_4. (E) ROESY spectrum of natural asc C11 EA in methanol-d_4.

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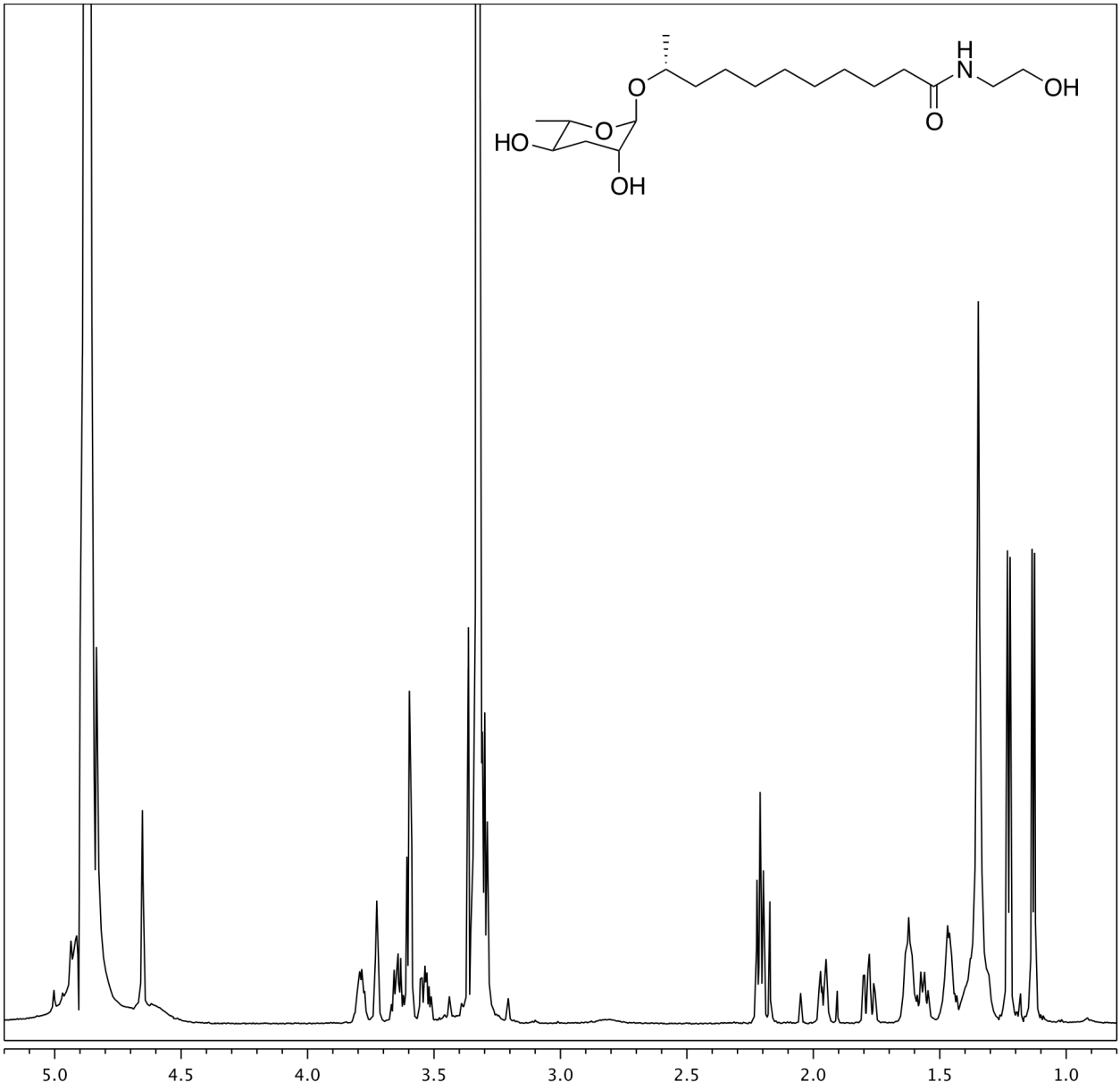

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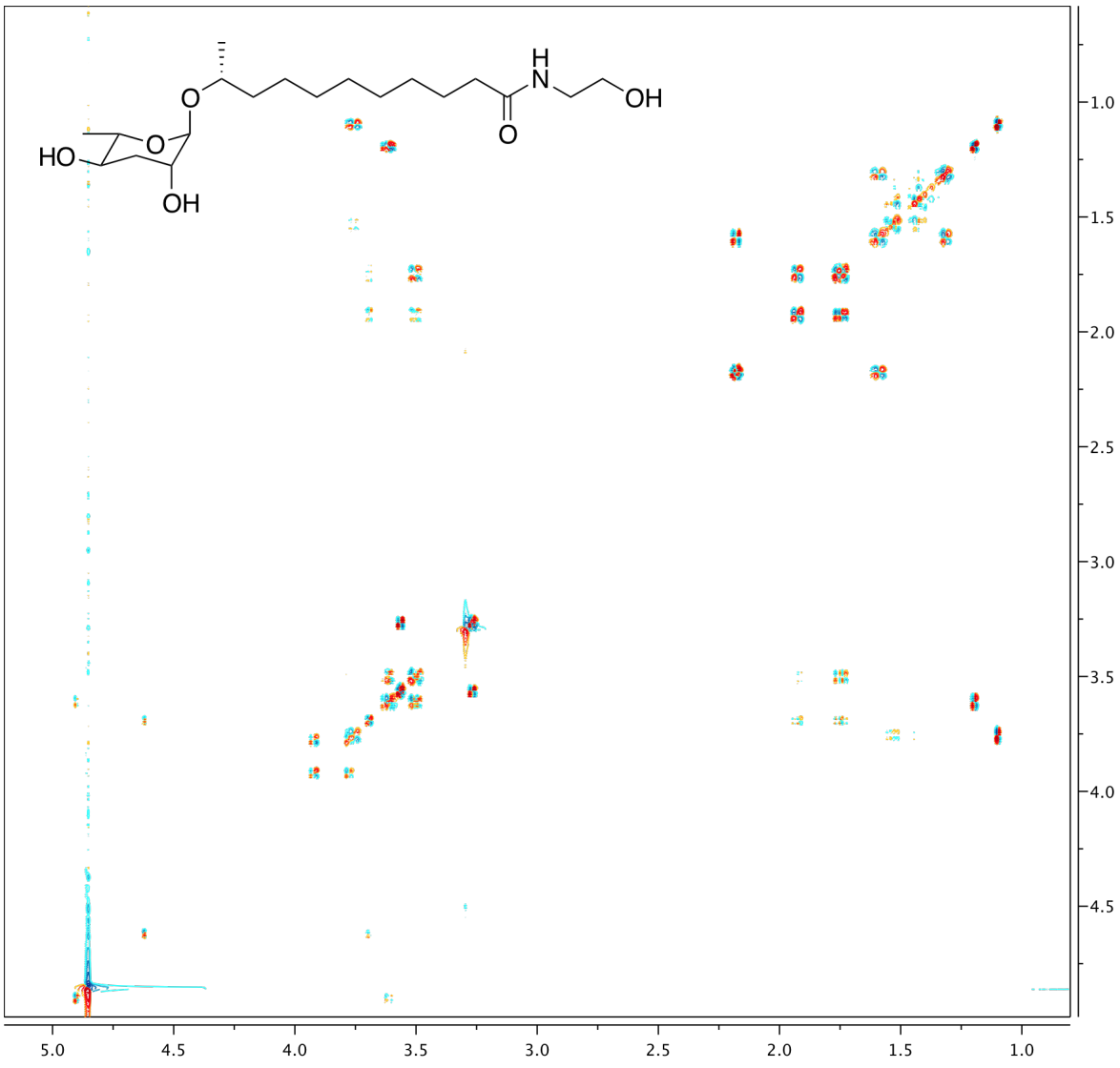

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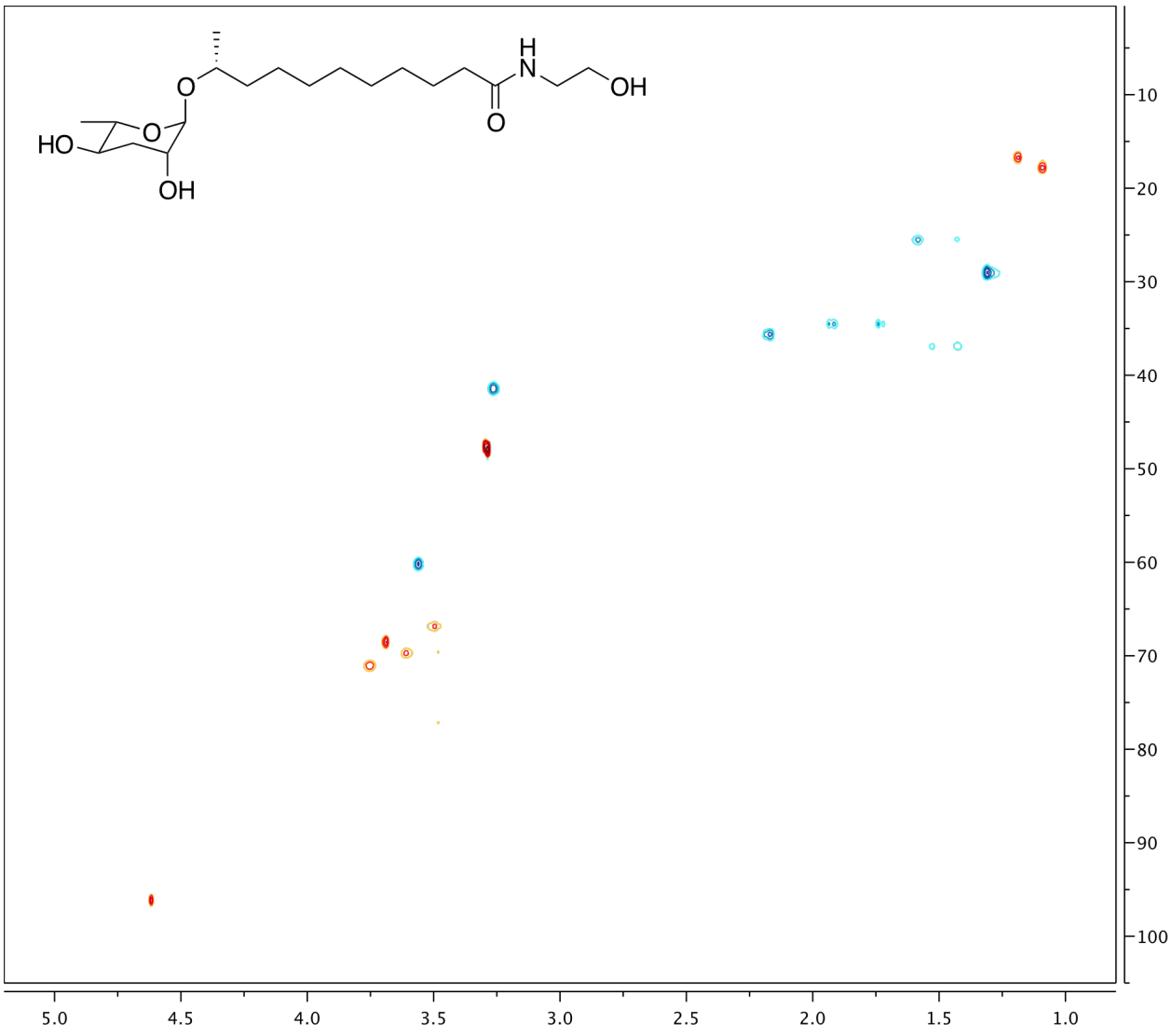
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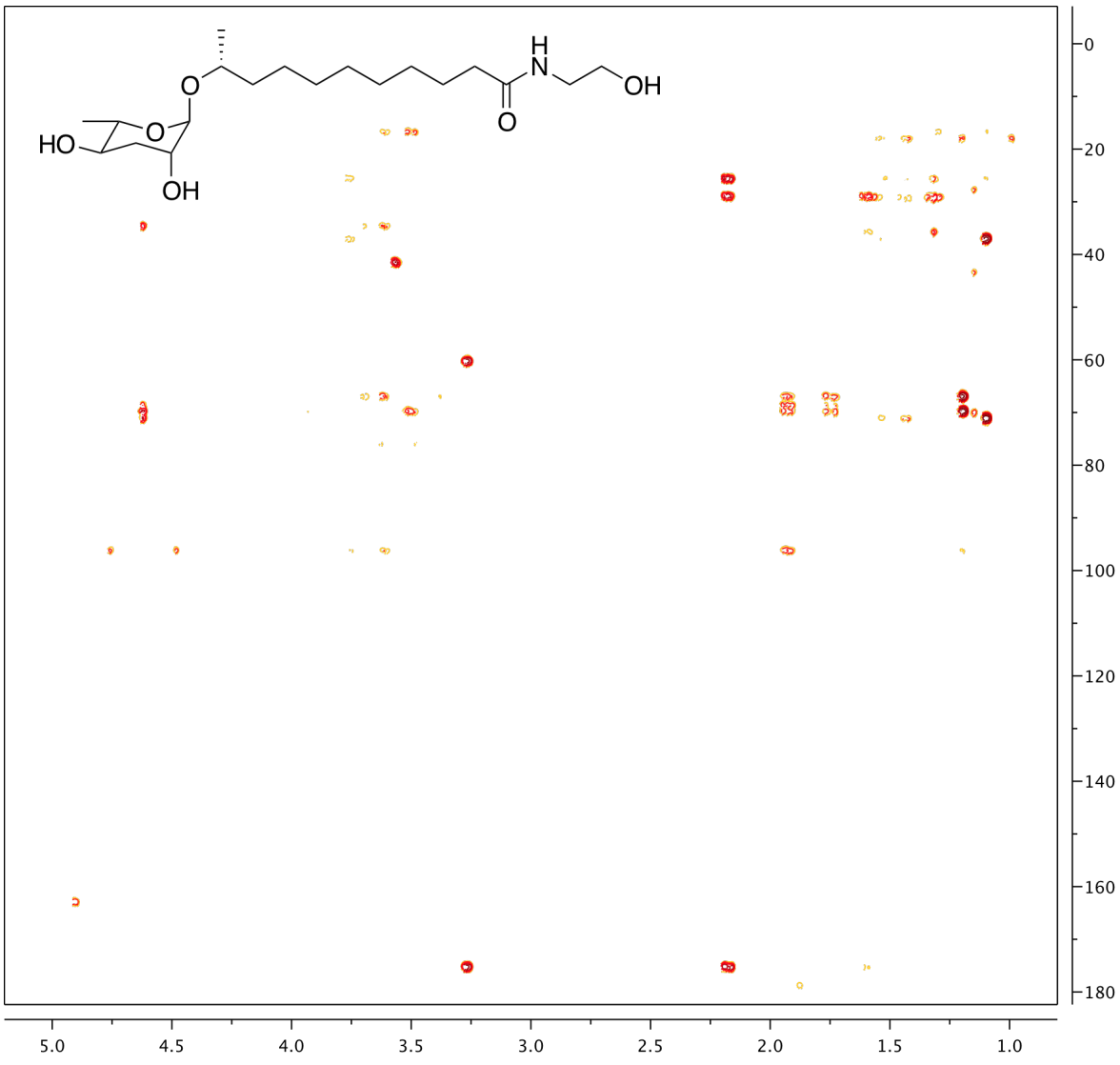
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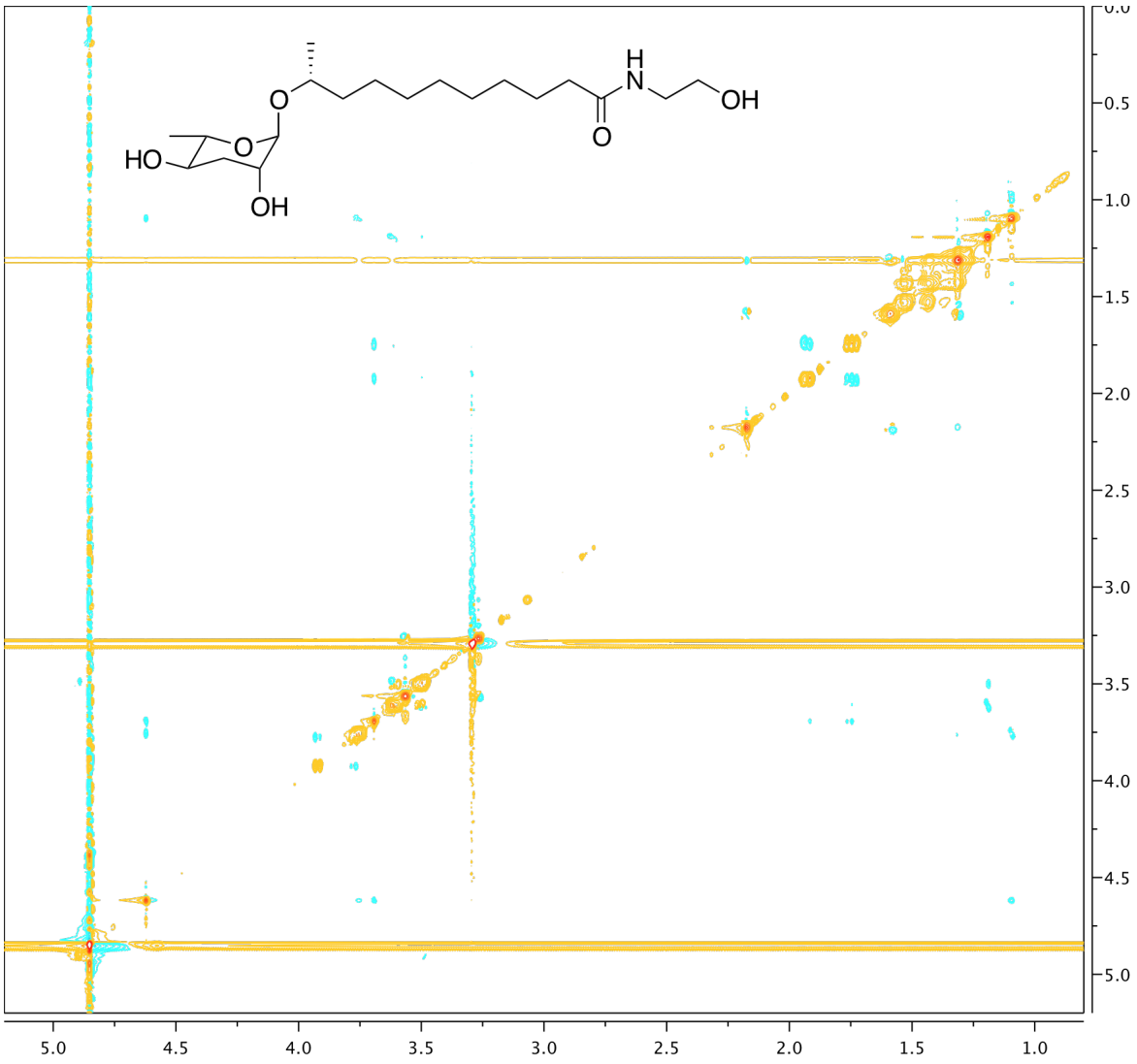
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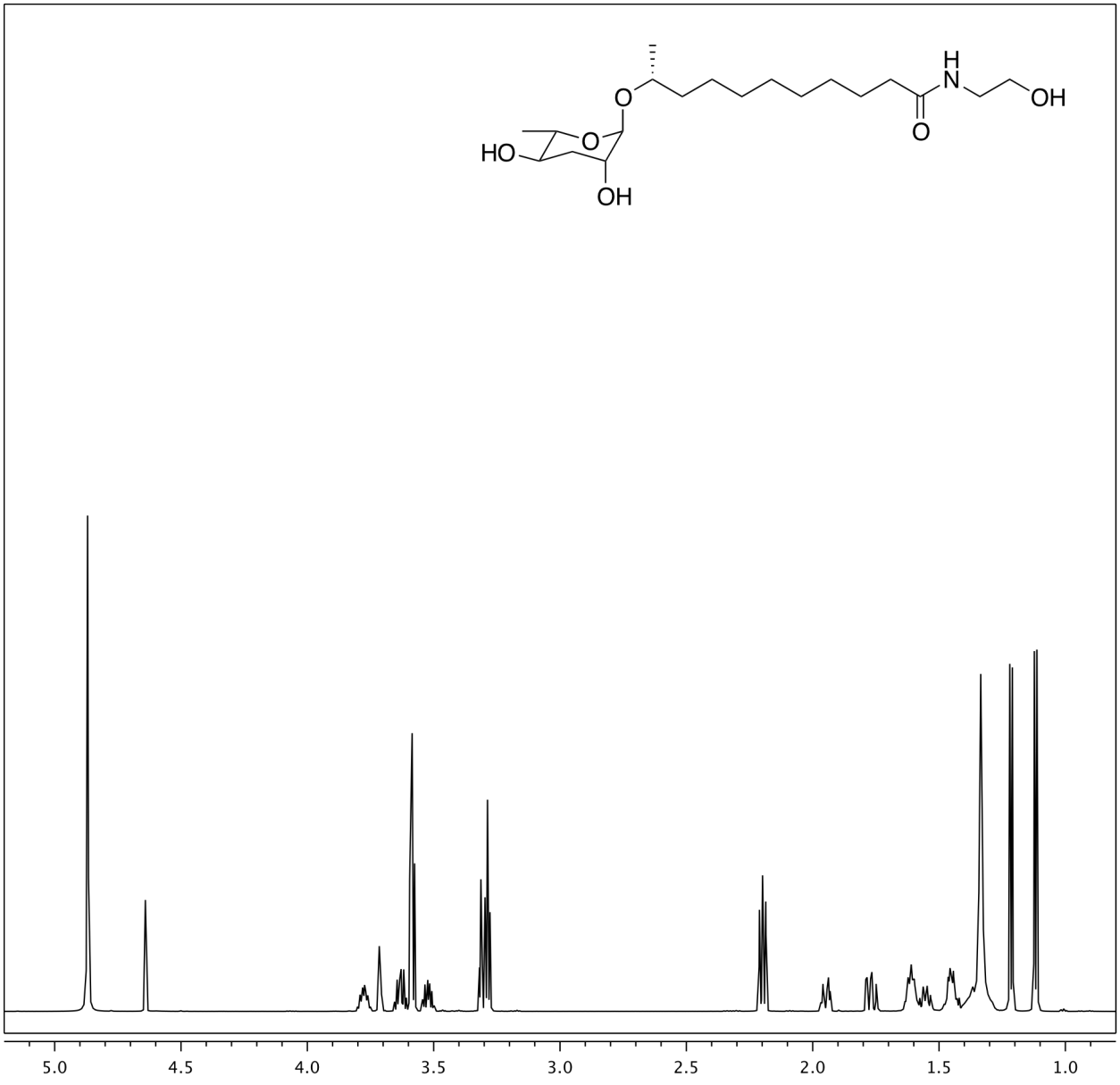
Figure S3. NMR spectra of synthetic asc C11 EA in methanol-d_4. (A) ^{1}H NMR spectrum of synthetic asc C11 EA in methanol-d_4. (B) dqf-COSY spectrum of synthetic asc C11 EA in methanol-d_4. (C) gHSQC spectrum of synthetic asc C11 EA in methanol-d_4. (D) gHMBC spectrum of synthetic asc C11 EA in methanol-d_4. (E) ROESY spectrum of synthetic asc C11 EA in methanol-d_4.

A



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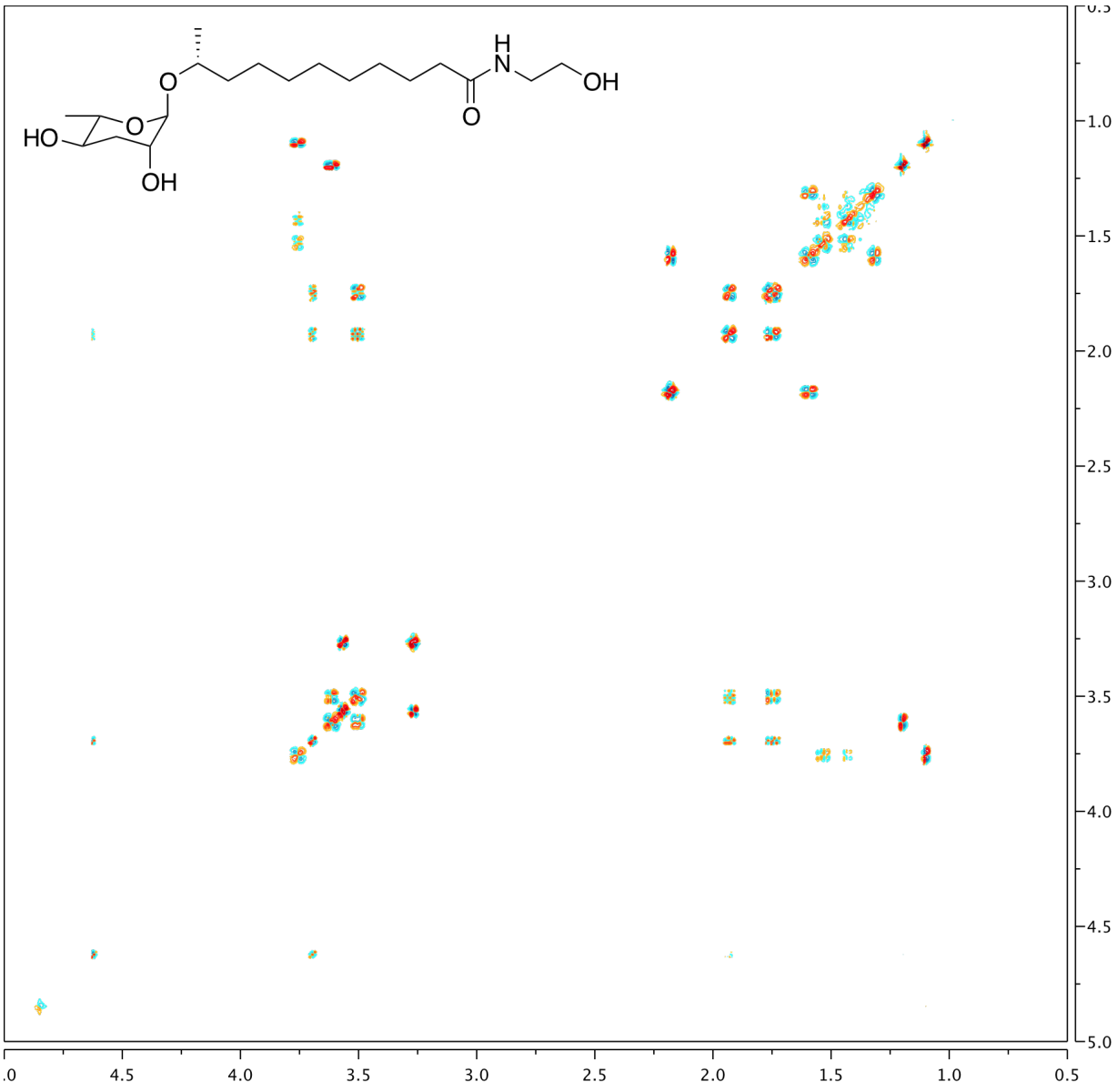
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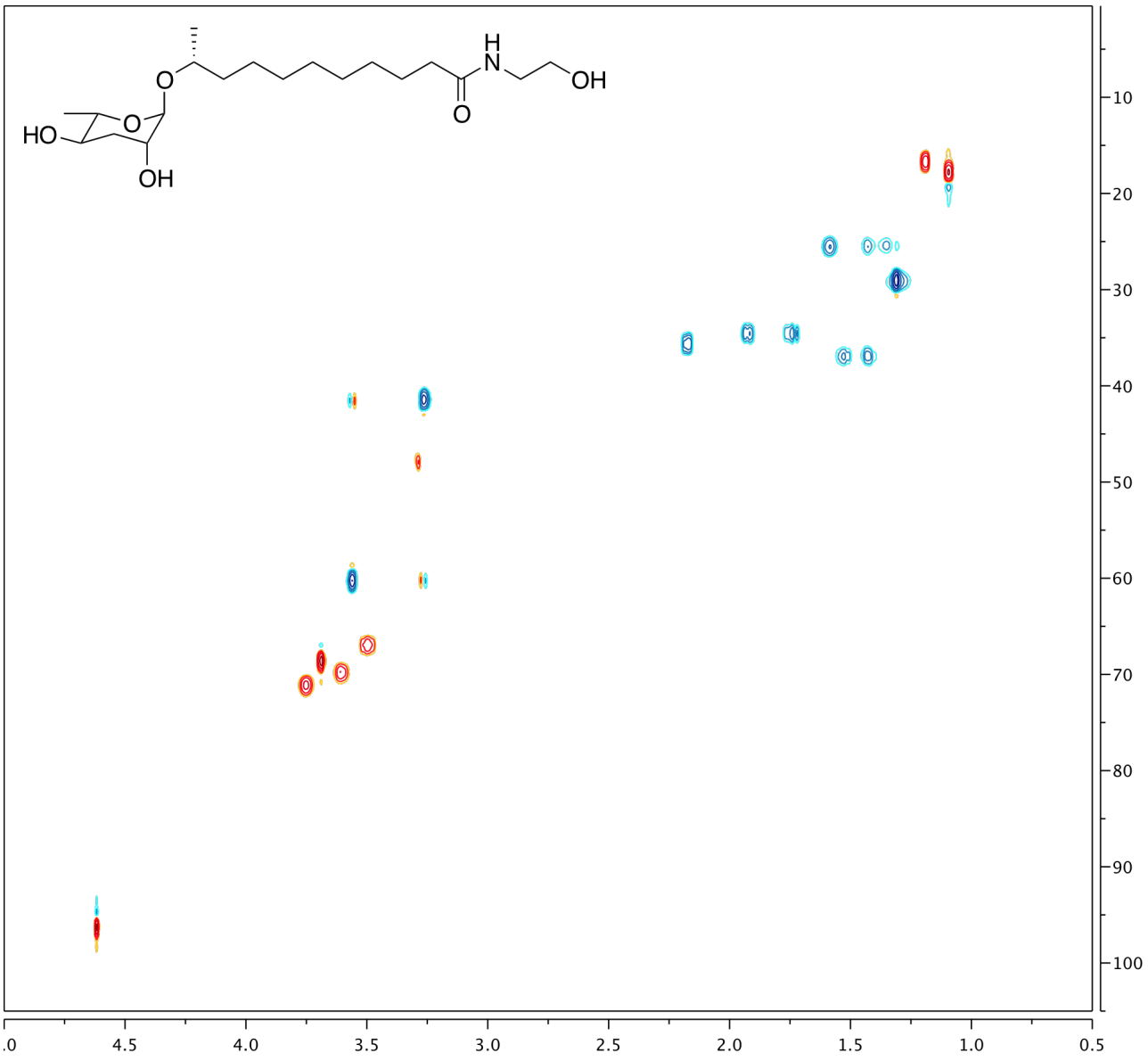
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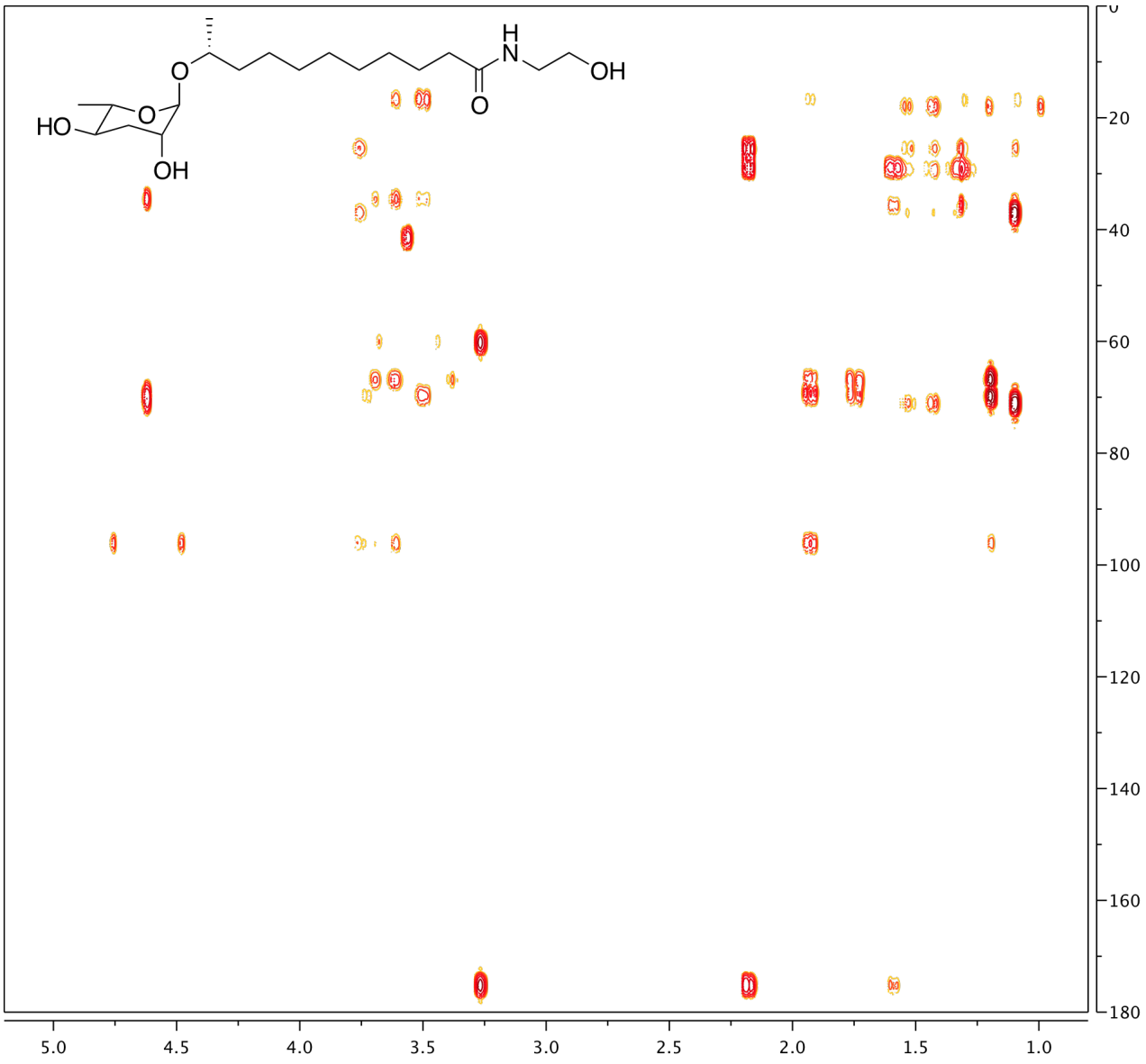
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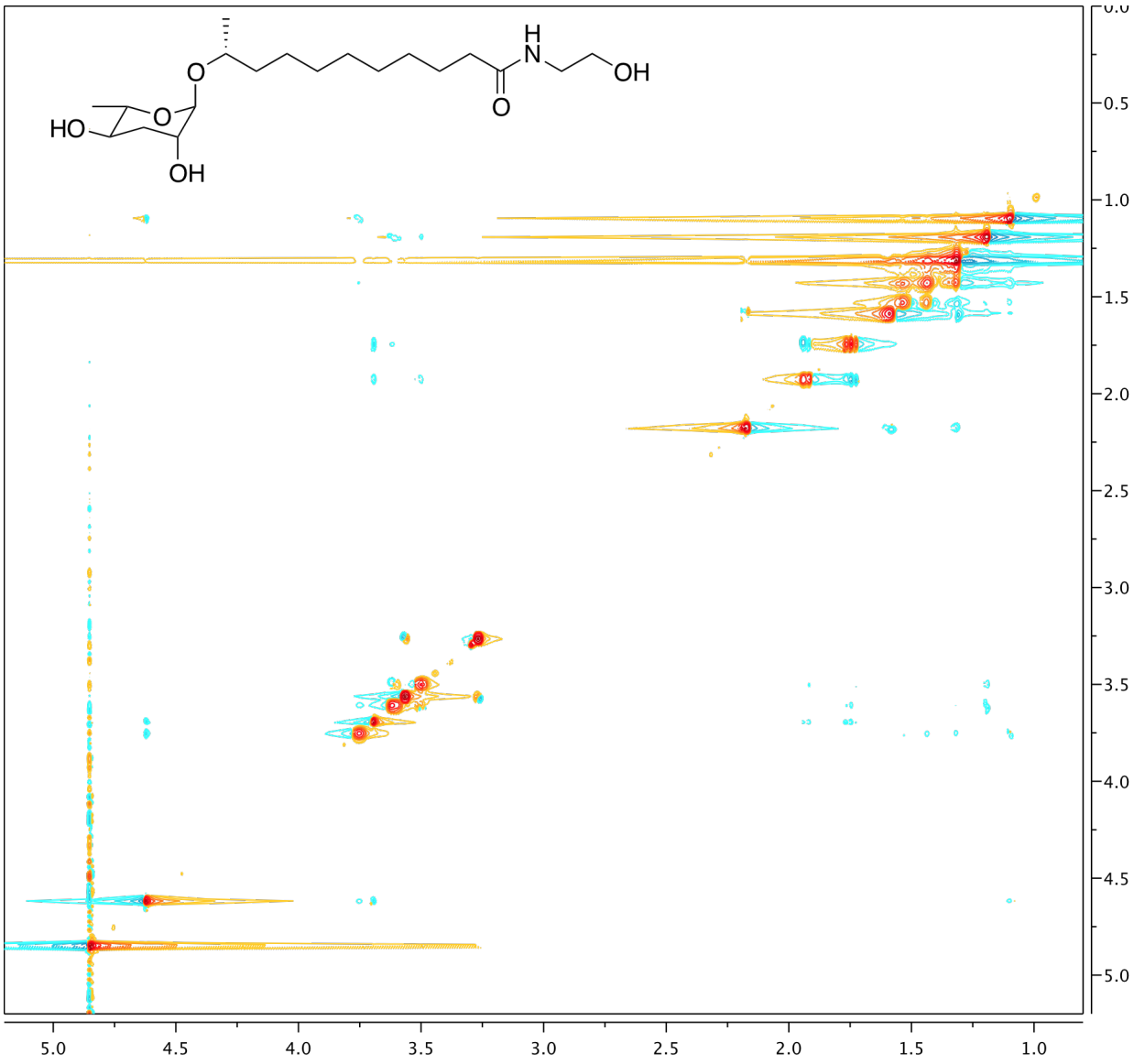
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Table S1. NMR shifts derived from ^{1}H, dqf-COSY, gHSQC, and gHMBC spectra of natural and synthetic asc C11 EA in methanol-d_4.

Natural asc C11 EA				**Synthetic asc C11 EA**		
No.	δ_H mult. [*J* (Hz)]	δ_C	HMBC	δ_H mult. [*J* (Hz)]	δ_C	HMBC
1		175.2			175.3	
2	2.19, t ($J_{2,3}$=7.2)	35.6	C-1,3,4	2.19, t ($J_{2,3}$=7.2)	35.7	C-1,3,4,1”,2”
3	1.61, m	25.5	C-1,2,4	1.61, m	25.5	C-1,2,4
4-7	1.33, br s	29.0	C-2,3,8	1.33, br s	29.0	C-2,3,8
8a	1.45, m	25.5	C-7,9	1.45, m	25.5	C-7,9
8b	1.35, m		C-7,9	1.35, m		C-7,9
9a	1.55, m	36.9	C-10,11	1.55, m	36.9	C-8,10,11
9b	1.45, m		C-10,11	1.45, m		C-8,10,11
10	3.77, m	71.1	C-1’,8,9	3.77, m	71.2	C-1’,8,9
11	1.11, d ($J_{10,11}$=6.1)	17.8	C-9,10	1.11, d ($J_{10,11}$=6.1)	17.8	C-8,9,10
1”	3.28, t ($J_{1”,2”}$=5.9)	41.2	C-1, 2”	3.29, t ($J_{1”,2”}$=5.9)	41.4	C-1, 2”
2”	3.58, t	60.2	C-1”	3.58, t	60.3	C-1”
1’	4.64, s	96.2	C-3’,5’,10	4.64, s	96.3	C-3’,5’,10
2’	3.71, dt ($J_{1’,2’}$=1.7)	68.5	C-3’,4’	3.71, dt ($J_{1’,2’}$=1.7)	68.7	C-1’,3’,4’
3’ ax	1.75, ddd ($J_{2’,3’ax}$=2.9)	34.5	C-2’,4’,5’	1.75, ddd ($J_{2’,3’ax}$=2.9)	34.5	C-1’,2’,4’,5’
3’ eq	1.94, dt ($J_{2’,3’eq}$=2.9, $J_{3’ax,3’eq}$=13.1)		C-1’,2’,4’,5’	1.95, dt ($J_{2’,3’eq}$=2.9, $J_{3’ax,3’eq}$=13.1)		C-1’,2’,4’,5’
4’	3.52, ddd ($J_{3’ax,4’}$=11.3, $J_{3’eq,4’}$=4.6)	66.9	C-3’,5’,6’	3.52, ddd ($J_{3’ax,4’}$=11.3, $J_{3’eq,4’}$=4.6)	67.0	C-3’,5’,6’
5’	3.63, dq ($J_{4’,5’}$ =9.4)	69.7	C-1’,3’,4’,6’	3.63, dq ($J_{4’,5’}$ =9.4)	69.8	C-1’,3’,4’,6’
6’	1.21, d ($J_{5’,6’}$=6.2)	16.7	C-1’,4’,5’	1.21, d ($J_{5’,6’}$=6.2)	16.8	C-1’,4’,5’