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Supplementary Figures and Supporting Information File

Structural hybridization of three aminoglycoside antibiotics yields a potent broad-spectrum bactericide that eludes bacterial resistance enzymes

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Index of content:

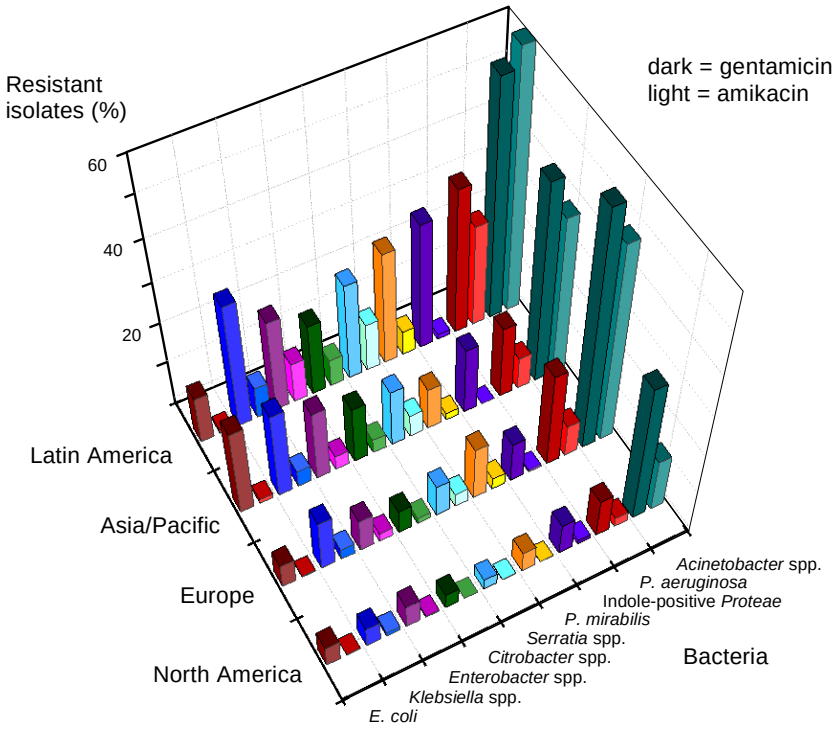


Figure S1. Prevalence of clinical isolates resistant to gentamicin (left darker bars) and amikacin (right lighter bars) identified by the SENTRY Program between 1998-2007. This plot was constructed with raw data from reference 1.

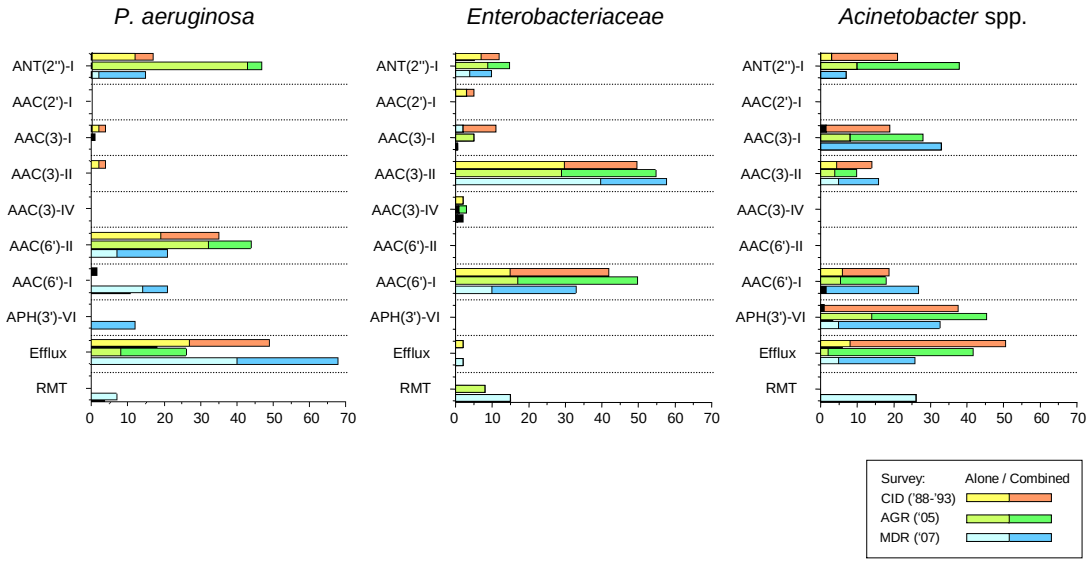
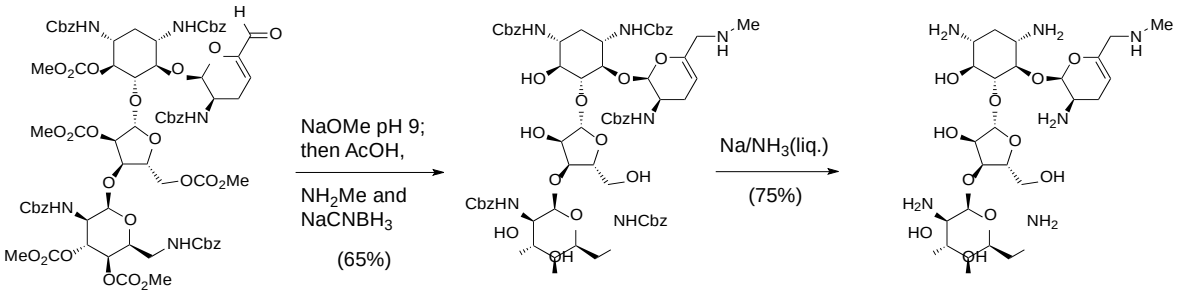


Figure S2. Distribution of aminoglycoside resistance mechanisms in isolates from the CID and SENTRY program collections from three time periods. Bars indicate the overall prevalence of each resistance mechanism, and the darker section indicates the sub-proportion expressed in combination with other mechanisms. Plot adapted from reference 2. RMT = rRNA methyltransferase.



Scheme S1. Synthesis of N6'-substituted G52-neomycin hybrid **26**.

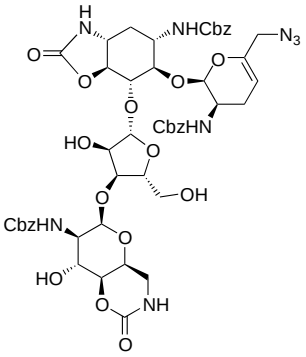
General Procedures

All reactions were carried out under an inert atmosphere of argon with dry solvents, using anhydrous conditions unless otherwise stated. Dry dichloromethane (DCM) and tetrahydrofuran (THF) were obtained from a solvent delivery system with activated alumina columns. Methanol (MeOH) was distilled from CaH_2 under argon. Reagents were purchased at the highest commercial quality and used without further purification. Flash column chromatography was performed with silica gel from SilicaFlash P60, particle size 40-63 μm, 230-400 mesh and distilled hexanes, ethyl acetate (EtOAc) or DCM. Free amines were purified with DCM applying gradients of 'ammoniacal MeOH' (referring to a 1:9 solution which was freshly prepared with 28% ammonia liquor before use). Deprotected free-base aminoglycosides were purified with homogeneous solvent systems consisting of $CHCl_3$/MeOH/$NH_4OH_{(aq)}$ in ratios ranging from 2:3:0.5 to 2:3:2. Yields refer to chromatographically and spectroscopically homogeneous material. Low temperature experiments conducted for longer than 3 h were conducted with a Cryocool apparatus with an acetone bath. Reactions were monitored by direct-injection low resolution mass spectrometry (LRMS) and thin layer chromatography (TLC) carried out on 0.25 mm E. Merck silica precoated plates (60F-254), visualized under UV light developed with acidified ammonium molybdate/cerium sulfate and heat. NMR spectra were recorded on Bruker ARX-400, AV-400 or AV-700 instruments and are calibrated using residual undeuterated solvent as an internal reference. The following abbreviations were used to explain multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Low resolution mass spectra (LRMS) were recorded on a Thermo Finnigan Surveyor MSQ and high resolution mass spectra (HRMS) were recorded on an Agilent Technologies LC-MSD TOF mass spectrometer by electrospray ionization in positive mode. Either protonated

molecular ions $[M+H]^+$ or sodium adducts $[M+Na]^+$ were used for empirical formula confirmation. IR spectra were recorded on a Perkin-Elmer Spectrum One FT-IR using NaCl tablets. Optical rotations were recorded in a 1 dm cell at ambient temperature, on a Perkin-Elmer 343 polarimeter. Analytical HPLC was performed in Achaogen Inc. using mobile phases with 0.1% HFBA, column Sunfire C18, 3x50mm, 2.5 µm, flow of 0.5 mL/min at 40 ºC and Chemiluminescent Nitrogen Detection (CLND), water/MeOH gradient A: 25 to 95% in 20 min or gradient B: 30 to 75% in 30 min.

General procedure for Birch reduction

Approx. 5 to 7 mL of ammonia was condensed into a two-neck flask equipped with a cold finger condenser at -78 °C. A solution of protected aminoglycoside (25 to 50 µmol) in anhydrous THF (1 mL) was added to the ammonia solution, followed by a drop of *t*BuOH. Approx. 20 to 30 mg (~1 mmol) of sodium metal were added to the mixture, which was stirred vigorously at -78 °C until the reaction turned deep blue. After 5 min, LRMS analysis indicated complete removal of the protecting groups, and the reaction was quenched with excess AcOH (100 µL). The ammonia was slowly evaporated by bubbling argon at RT, to give a white residue of salts, which was dissolved in $CHCl_3$/MeOH/NH_4OH (2:3:0.5) and purified by column chromatography using the same solvent system, increasing the proportion of ammonia as required. The fractions containing aminoglycoside were identified by TLC, collected and evaporated under vacuum to furnish a wet residue, which was dissolved in a minimum volume of water and freeze-dried. The dry residue obtained was redissolved in a minimum of water, at which point insoluble traces of silica were generally observed, and were removed by filtration of the solution through a 0.45 µm syringe filter. Finally, freeze-drying the filtrate yielded the aminoglycoside analog as the free-base fluffy powder. For characterization purposes, the aminoglycoside was redissolved in a minimum volume of water, treated with AcOH (50 µL) and freeze-dried to provide the aminoglycoside acetate salts, which were obtained as yellow solids.



14

6'-Azido-1,6-4''',6'''-*bis*-carbamate-3,2',2'''-*N*-Cbz-4',5'-dehydro-3',4'-dideoxy-neomycin (14).

Compound **13** (100 mg, 78 μmol), was dried by evaporation three times from toluene, dissolved in anhydrous DMF (2 mL), cooled to 0 °C, and treated dropwise with KHMDS (470 μL, 0.235 mmol, 0.5 M in toluene) and stirred overnight at 0 °C, when LRMS indicated complete consumption of the starting material and the major ions corresponded to the *bis*-carbamate **14**. The reaction was quenched with AcOH (30 μL), evaporated under high-vacuum at 50 °C to a residue, which was diluted with THF and was filtered through a 0.45 μm syringe filter. The volatiles were evaporated to a residue, which was purified by column chromatography (5 → 7 → 8% ammoniacal MeOH in DCM), to yield 48.9 mg of the title compound **14** (59%, 46 μmol), as an off-white amorphous solid.

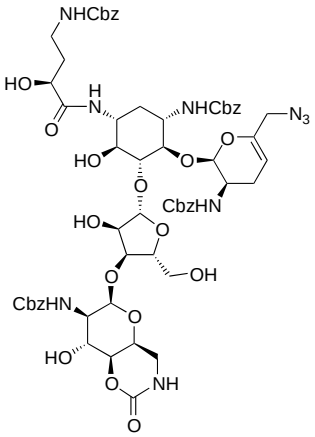
R_f = 0.3, 20:5:3 $CHCl_3$/EtOAc/MeOH

HRMS (ESI) calcd. for $C_{49}H_{56}N_8O_{19}$, M + H^+ = 1061.3734, found 1061.3729 (-0.56 ppm).

^{1}H NMR (CD_3OD, 400 MHz) δ 7.47-7.08 (m, 15H), 5.59 (s, 1H), 5.21-4.90 (m, 8H), 4.57 (s, 1H), 4.33-4.18 (m, 3H), 4.13 (dd, *J* = 7.80, 5.54 Hz, 1H), 4.07 (s, 1H), 4.02-3.94 (m, 1H), 3.92 (s, 1H), 3.86-3.74 (m, 2H), 3.74-3.58 (m, 4H), 3.56-3.35 (m, 5H), 3.27 (s, 1H), 2.21-1.89 (m, 3H), 1.51 (dd, *J* = 22.72, 11.37 Hz, 1H).

^{13}C NMR (CD_3OD, 100 MHz) δ 160.6, 157.1, 156.8, 156.3, 153.2, 144.3, 136.4 - 136.2 (3C), 127.8 - 127.1 (15C), 107.7, 98.9, 97.2, 95.6, 83.0, 81.7, 78.3, 77.9, 76.2, 74.4, 72.8, 67.6, 66.3, 66.1, 65.9, 63.0, 62.0, 53.5, 51.9, 51.8, 50.9, 46.7, 43.1, 31.4, 21.5.

FTIR (NaCl): 3381, 2102, 1698, 1429, 1348, 1038 cm^{-1}.



15

6'-Azido-*N*-1-((*S*)-4-(benzyloxycarbonylamino)-2-hydroxybutanoyl)-3''',6'''-carbamate-3, 2',2'''-*N*-Cbz-4',5'-dehydro-3',4'-dideoxy-neomycin (15).

Bis-carbamate **14** (44 mg, 42 µmol) was dissolved in DMF (2 mL), treated with 0.5 M LiOH (530 µL, 0.266 mmol) and stirred overnight, when LRMS indicated deprotection of the C1-amino group, and starting material was not detected on TLC (20:5:3, $CHCl_3$, EtOAc, MeOH, Rf ~ 0). The reaction was diluted with THF (10 mL) and filtered through a 0.45 µm syringe filter, and the filtrate was evaporated under high-vacuum at 60 °C, to give a residue which was redissolved in THF and refiltered. The filtrate was evaporated under vacuum to a residue, which was used without further purification.

(*S*)-4-(Benzyloxycarbonylamino)-2-hydroxybutanoic acid (22.3 mg, 88 µmol) was dissolved in THF (1 mL), treated with *N*-hydroxysuccinamide (10.1 mg, 88 µmol), DIPEA (46 µL, 0.155 mmol) and EDC (18.6 mg, 97 µmol), and stirred for 2 h. The C1 free-amine intermediate was dissolved in dry THF (1 mL), and treated with the active-ester solution, which was stirred overnight, when LRMS indicated complete consumption of the free-amine intermediate, and the major ions corresponded to the N1-amide product **15**. The reaction was quenched with sat. NH_4Cl, evaporated under vacuum, diluted with DCM, washed successively with 2 N HCl and sat. $NaHCO_3$, dried over Na_2SO_4 and filtered. The organic layer was evaporated to a residue, which was purified by column chromatography (9 → 10% ammoniacal MeOH in DCM), to yield 21.1 mg of the title compound **15** (40% for 2 steps, 16.6 µmol), as an off-white amorphous solid.

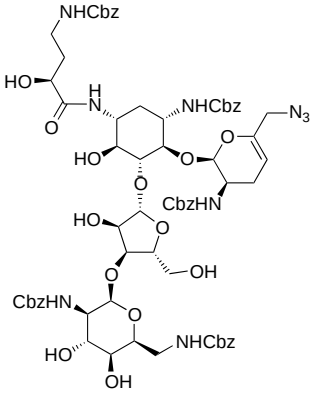
R_f = 0.2, 20:5:3 $CHCl_3$/EtOAc/MeOH

HRMS (ESI) calcd. for $C_{60}H_{71}N_9O_{22}$, M + H^+ = 1270.4785 (-0.14 ppm).

^{1}H NMR (CD_3OD, 400 MHz) δ 7.44-7.09 (m, 20H), 5.58 (s, 1H), 5.16-4.90 (m, 10H), 4.61-4.52 (m, 2H), 4.27-4.15 (m, 3H), 4.11 (s, 1H), 4.07-4.03 (m, 1H), 4.01 (dd, *J* = 7.43, 3.89 Hz, 1H), 3.95-3.86 (m, 2H), 3.76 (dd, *J* = 9.94, 6.41 Hz, 1H), 3.71-3.53 (m, 5H), 3.53-3.38 (m, 4H), 3.31 (d, *J* = 13.21 Hz, 1H), 3.24-3.12 (m, 2H), 2.18-2.05 (m, 1H), 2.05-1.96 (m, 1H), 1.96-1.83 (m, 2H), 1.75 (dt, *J* = 13.39, 13.35, 6.47 Hz, 1H), 1.41 (dd, *J* = 24.28, 12.06 Hz, 1H).

^{13}C NMR (CD_3OD, 100 MHz) δ 175.12, 157.17 - 156.37 (4C), 153.17, 144.23, 136.59 - 136.31 (4C), 127.80 - 126.94 (20C), 108.51, 98.94, 97.79, 95.10, 85.24, 81.60, 77.70, 76.84, 76.37, 74.33, 73.56, 69.04, 67.50, 66.08, 66.04, 65.75, 65.66, 62.85, 61.53, 51.95, 51.77, 49.46, 49.15, 46.82, 43.09, 36.27, 33.79, 32.89, 21.50.

FTIR (NaCl): 3369, 2102, 1698, 1429, 1040 cm^{-1}.



16

6'-Azido-*N*-1-((*S*)-4''''-(benzyloxycarbonylamino)-2''''-hydroxybutanoyl)-3,2',2''',6'''-*N*-Cbz-4',5'-dehydro-3',4'-dideoxy-neomycin (16).

Compound **15** (21 mg, 16.6 μmol) was dried by evaporation three times from toluene, dissolved in anhydrous benzyl alcohol (2 mL), treated with sodium benzyloxide (100 μL, 1 M in benzyl alcohol, 0.10 mmol), and stirred for 16 h, when LRMS monitoring indicated that an equilibrium was reached, favouring the 6'''-*N*-Cbz product **4.33**. The reaction was quenched with AcOH (10 μL, 0.17 mmol), diluted with DCM (10 mL) and the solution was loaded onto silica gel for purification by column chromatography (2 → 4 → 8% MeOH/DCM), to yield 19.5 mg of the title compound **16** (85%, 14 μmol), as an off-white amorphous solid.

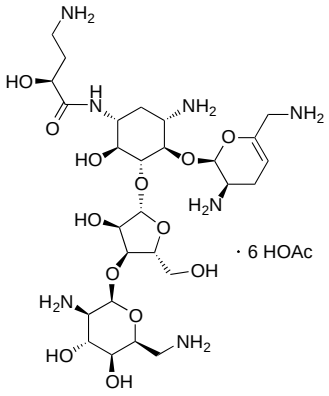
R_f = 0.4, 20:5:3 $CHCl_3$/EtOAc/MeOH

HRMS (ESI) calcd. for $C_{67}H_{79}N_9O_{23}$, M + Na^+ = 1400.5181, found 1400.5180 (-0.1 ppm).

^{1}H NMR (CD_3OD, 700 MHz) δ 7.37-7.11 (m, 25H), 5.55 (s, 1H), 5.11-4.89 (m, 11H), 4.57 (s, 1H), 4.55-4.51 (m, 1H), 4.15-4.10 (m, 2H), 3.98 (dd, *J* = 7.72, 3.88 Hz, 1H), 3.91 (dd, *J* = 7.95, 4.51 Hz, 1H), 3.87-3.82 (m, 2H), 3.77 (s, 1H), 3.74 (ddd, *J* = 11.36, 6.36, 2.13 Hz, 1H), 3.67-3.55 (m, 3H), 3.55-3.48 (m, 3H), 3.45 (d, *J* = 13.68 Hz, 1H), 3.43-3.34 (m, 3H), 3.34-3.26 (m, 2H), 3.22-3.18 (m, 1H), 3.14 (td, *J* = 13.28, 6.26, 6.26 Hz, 1H), 2.08 (dd, *J* = 15.57, 12.20 Hz, 1H), 2.02-1.94 (m, 1H), 1.92-1.87 (m, 1H), 1.85 (ddd, *J* = 14.01, 7.13, 4.28 Hz, 1H), 1.72 (dt, *J* = 13.66, 13.38, 6.88 Hz, 1H), 1.39-1.29 (m, 1H).

^{13}C NMR (CD_3OD, 100 MHz) δ 175.5, 157.8 - 156.7 (5C), 144.6, 137.0 - 136.7 (5C), 128.1 - 127.1 (25C), 109.2, 99.3, 98.8, 95.6, 85.7, 82.3, 77.5, 77.0, 74.1, 73.9, 73.2, 70.2, 69.4, 67.8, 66.5, 66.2, 66.2, 66.1, 66.0, 61.9, 52.7, 52.3, 49.8, 49.5, 48.1, 41.2, 36.6, 34.2, 33.3, 21.9.

FTIR (NaCl): 3380, 2102, 1693, 1431, 1527, 1031 cm^{-1}.



17

***N*-1-((*S*)-4-Amino-2-hydroxybutanoyl)-4',5'-dehydro-3',4'-dideoxy-neomycin (17).**

Compound **17** (31 mg, 22 μmol) was submitted to the general Birch reduction procedure. The column chromatography solvents were $CHCl_3$/MeOH/NH_4OH, 2:3:1 followed by 2:3:2. The procedure yielded 14.2 mg of the acetate salt of the title compound **17** (63%, 13.6 μmol), as a yellow amorphous solid.

R_f = 0.1, 2:3:2 $CHCl_3$/MeOH/NH_4OH.

$[\alpha]^{22}_{D}$ 27.5º (*c* 0.71, H_2O).

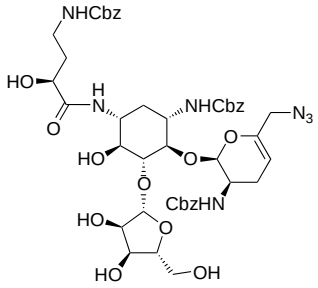
HRMS (ESI) calcd. for $C_{27}H_{51}N_7O_{13}$, M + H^+ = 682.3618, found 682.3620 (-0.29 ppm).

^{1}H NMR (D_2O, 700 MHz) δ 5.66 (d, *J* = 1.20 Hz, 1H), 5.25-5.24 (m, 1H), 5.21 (d, *J* = 2.45 Hz, 1H), 5.16 (t, *J* = 3.76, 3.76 Hz, 1H), 4.47 (dd, *J* = 5.92, 5.26 Hz, 1H), 4.32 (dd, *J* =

4.90, 2.53 Hz, 1H), 4.27 (dd, *J* = 8.16, 3.89 Hz, 2H), 4.19-4.17 (m, 1H), 4.12 (dt, *J* = 5.69, 5.65, 3.42 Hz, 1H), 4.06 (dd, *J* = 10.13, 9.41 Hz, 1H), 3.93 (dt, *J* = 4.96, 4.85, 1.33 Hz, 1H), 3.89 (ddd, *J* = 12.31, 10.60, 4.29 Hz, 1H), 3.82 (dd, *J* = 12.05, 3.29 Hz, 1H), 3.78-3.77 (m, 1H), 3.75 (t, *J* = 9.26, 9.26 Hz, 1H), 3.68 (dd, *J* = 12.14, 5.40 Hz, 1H), 3.65-3.58 (m, 3H), 3.55-3.52 (m, 1H), 3.46 (ddd, *J* = 12.69, 10.58, 4.27 Hz, 1H), 3.38 (dd, *J* = 13.64, 6.67 Hz, 1H), 3.32 (dd, *J* = 13.61, 3.91 Hz, 1H), 3.14-3.05 (m, 2H), 2.64 (ddd, *J* = 18.35, 4.68, 3.63 Hz, 1H), 2.34 (ddd, *J* = 18.52, 4.29, 3.58 Hz, 1H), 2.21 (td, *J* = 12.79, 4.31, 4.31 Hz, 1H), 2.12 (dddd, *J* = 14.22, 8.11, 6.62, 3.87 Hz, 1H), 1.93 (s, 18H AcOD), 1.98-1.94 (m, 1H), 1.75 (dd, *J* = 12.64, 12.63 Hz, 1H).

^{13}C NMR (D2O, 175 MHz) δ 179.3 (AcOD), 175.5, 143.3, 110.2, 100.4, 96.7, 95.4, 84.4, 81.2, 78.1, 75.9, 73.2, 72.9, 70.0, 69.4, 67.6, 67.2, 61.1, 50.7, 48.7, 48.5, 45.7, 40.5, 40.4, 36.5, 30.8, 29.5, 23.0 (AcOD), 22.0.

LC/CLND gradient A, R_t = 11.3 min, 96% purity.



20

6'-Azido-4',5'-dehydro-3',4'-dideoxy-*per*-*N*-Cbz-butirosin (20).

Compound **13** was treated with sodium periodate,[3] followed by treatment with DBU to cleave ring D as previously reported (58% yield).[4] Compound **18** (53 mg, 63 μmol) was dried by evaporation three times from toluene, dissolved in anhydrous DMF (2 mL), cooled to 0 °C, was treated dropwise with KHMDS (380 μL, 0.190 mmol, 0.5 M in toluene) and stirred with cooling for 5 h, when LRMS indicated complete consumption of the starting material and the major ions corresponded to the N1,O6-oxazilidinone: HRMS (ESI) calcd. for $C_{34}H_{39}N_6O_{13}$, M + H^+ = 741.2726, found 741.2712 (-1.85 ppm). The reaction was quenched with AcOH (20 μL) and the volume of DMF was reduced under high-vacuum at 50 °C to a residue, which was triturated with 10% Et_2O/Hex. The collected crude oxazilidinone intermediate was treated with 6 mL of 2:1 THF:sat. $Ba(OH)_2$, at 60 °C overnight, when starting material was not detected on TLC (20:5:3, $CHCl_3$, EtOAc, MeOH,

Rf ~ 0) and LRMS indicated complete deprotection of the C1-amino group: HRMS (ESI) calcd. for $C_{33}H_{42}N_6O_{12}$, M + H^+ = 715.2933, found 715.2938 (0.4 ppm). The suspension of salts were filtered out through a 0.45 µm syringe filter washing with THF, the filtrate was evaporated under high-vacuum at 60 °C, giving a residue that was diluted in THF and re-filtered. The filtrate was evaporated to a residue and used for amide coupling without further purification.

(*S*)-4-(Benzyloxycarbonylamino)-2-hydroxybutanoic acid (32 mg, 0.126 mmol) was dissolved in THF (1 mL), treated with *N*-hydroxysuccinamide (14.5 mg, 0.126 mmol), DIPEA (70 µL, 0.4 mmol) and EDC (27 mg, 0.141 mmol), and stirred for 2 h. A solution of free-amine intermediate dissolved in THF (1 mL) was treated with the active-ester mixture, which was stirred overnight, when LRMS indicated complete consumption of the starting material C1-amine, and the major ions corresponded to the N1-amide product. The reaction was quenched with sat. NH_4Cl, diluted with DCM, washed successively with 2 N HCl and sat. $NaHCO_3$, dried over Na_2SO_4 and filtered. The organic fraction was evaporated to a residue, which was purified by column chromatography (5 → 8% ammoniacal MeOH in DCM), to yield 22.4 mg of the title compound **20** (38% for 3 steps, 23.6 µmol), as an off-white amorphous solid.

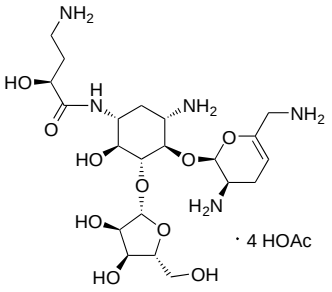
R_f = 0.4, 20:5:3 $CHCl_3$/EtOAc/MeOH

HRMS (ESI) calcd. for $C_{45}H_{56}N_7O_{16}$, M + Na^+ = 972.3598, found 972.3591 (-0.71 ppm).

^{1}H NMR (CD_3OD, 400 MHz) δ 7.39-7.18 (m, 15H), 5.62 (d, *J* = 1.42 Hz, 1H), 5.13 (s, 1H), 5.10-4.90 (m, 6H), 4.55 (d, *J* = 3.62 Hz, 1H), 4.10 (d, *J* = 3.69 Hz, 1H), 4.00 (ddd, *J* = 8.89, 7.12, 4.42 Hz, 2H), 3.84 (dt, *J* = 6.34, 5.97, 3.25 Hz, 1H), 3.77 (ddd, *J* = 10.97, 6.68, 2.29 Hz, 1H), 3.74-3.65 (m, 1H), 3.65-3.54 (m, 4H), 3.54-3.45 (m, 3H), 3.41 (dd, *J* = 9.92, 9.18 Hz, 1H), 3.25-3.14 (m, 2H), 2.10 (dd, *J* = 15.34, 11.95 Hz, 1H), 2.01 (dd, *J* = 6.32, 5.08 Hz, 1H), 1.97-1.84 (m, 3H), 1.76 (dt, *J* = 13.23, 13.20, 6.28 Hz, 1H), 1.42 (dd, *J* = 24.37, 12.03 Hz, 1H).

^{13}C NMR (CD_3OD, 100 MHz) δ 175.1, 157.2, 156.9, 156.4, 144.2, 136.6, 136.4, 136.4, 127.7 - 127.0 (15C), 109.0, 98.9, 95.0, 85.4, 83.1, 76.4, 74.8, 73.5, 69.9, 69.0, 66.1, 65.7, 65.6, 62.3, 52.0, 49.5, 49.2, 46.2, 36.3, 33.8, 32.9, 21.5.

FTIR (NaCl): 3366, 2102, 1693, 1433, 1041 cm^{-1}.



21

4',5'-Dehydro-3',4'-dideoxy-butirosin (21).

Compound **20** (22.4 mg, 23 µmol) was submitted to the general Birch reduction procedure. The column chromatography solvents were $CHCl_3$/MeOH/NH_4OH, 2:3:1 followed by 2:3:2. The procedure yielded 9.2 mg of the acetate salt of the title compound **21** (52%, 12.1 µmol), as a yellow amorphous solid.

R_f = 0.2, 2:3:2 $CHCl_3$/MeOH/NH_4OH.

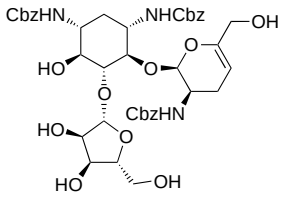
$[\alpha]^{22}_D$ 12.6º (*c* 0.46, H_2O).

HRMS (ESI) calcd. for $C_{21}H_{40}N_5O_{10}$, M + H^+ = 522.2770, found 522.2766 (-0.67 ppm).

^{1}H NMR (D_2O, 400 MHz) δ 5.74 (d, *J* = 1.20 Hz, 1H), 5.21 (d, *J* = 1.26 Hz, 1H), 5.19 (dd, *J* = 4.00, 3.26 Hz, 1H), 4.29 (dd, *J* = 8.15, 3.97 Hz, 1H), 4.16 (dd, *J* = 4.64, 1.00 Hz, 1H), 4.12 (dd, *J* = 6.74, 4.82 Hz, 1H), 3.98 (dt, *J* = 6.64, 6.53, 2.68 Hz, 1H), 3.93-3.86 (m, 2H), 3.86-3.80 (m, 2H), 3.75 (dd, *J* = 9.50, 8.85 Hz, 1H), 3.66-3.60 (m, 3H), 3.56 (dd, *J* = 10.23, 9.25 Hz, 1H), 3.31 (dt, *J* = 10.89, 10.74, 3.60 Hz, 1H), 3.18-3.05 (m, 2H), 2.58 (ddd, *J* = 18.04, 5.34, 4.21 Hz, 1H), 2.40-2.29 (m, 1H), 2.18-2.09 (m, 2H), 1.97 (dt, *J* = 14.58, 14.49, 7.74 Hz, 1H), 1.88 (s, 7H AcOD), 1.62 (q, *J* = 12.50, 12.50, 12.46 Hz, 1H).

^{13}C NMR (D_2O, 100 MHz) δ 180.0 (AcOD), 175.2, 142.7, 110.0, 100.2, 96.0, 84.2, 81.8, 77.6, 74.6, 72.7, 69.1, 69.1, 61.4, 48.3, 48.2, 45.5, 40.1, 36.1, 30.5, 29.2, 22.2 (AcOD), 22.1.

LC/CLND gradient A, R_t = 9.7 min, 97.7% purity.



S1

6'-Deamino-4',5'-dehydro-3',4'-dideoxy-6'-hydroxy-*per*-*N*-Cbz-ribostamycin (S2).

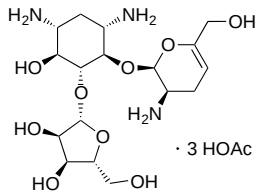
Compound **12** was treated with $NaCNBH_3$ and 20 drops of AcOH in THF, followed by treatment with 5 mL anhydrous MeOH to approx. pH 8 to 9 to afford the reported intermediate 4',5'-Dehydro-3',4'-dideoxy-6,3',2'',5'',3''',4'''-*per*-*N*-Cbz-paromomycin.[4] This diol intermediate (106 mg, 85 µmol) was dissolved in MeOH (5 mL), treated with 10 equiv. $NaIO_4$ and stirred vigorously overnight,[3] when LRMS indicated complete consumption of starting material. The major ion observed corresponded to the sodium adduct of the MeOH *bis*-hemiacetal of the 3''',4'''-*bis*-aldehyde intermediates. The suspension was filtered through a 0.45 µm syringe filter, and the filtrate was reduced under vacuum, diluted with DCM and washed with sat. NaCl. The combined organic layers were evaporated to a residue, which was dissolved in MeOH (3 mL), treated with 1 equiv. DBU and stirred for 4 h, when LRMS indicated complete elimination of the ring D system, leaving the 3'' alcohol free. The reaction was neutralized with AcOH, the volume was reduced under vacuum to a residue, which was diluted with DCM and passed through a silica pad washing with 10% MeOH/DCM. The filtrate was evaporated to a residue, which was purified by column chromatography solvents (3 → 5% MeOH/DCM). The procedure yielded 28 mg of the title compound **S2** (40%, 34 µmol), as a white amorphous solid.

R_f = 0.6, 20:5:3 $CHCl_3$/EtOAc/MeOH

HRMS (ESI) calcd. for $C_{41}H_{49}N_3O_{15}$, M + H^+ = 824.3236, found 824.3216 (-2.5 ppm).

^{1}H NMR (CD_3OD, 400 MHz) δ 7.39-7.13 (m, 15H), 5.58 (s, 1H), 5.13 (s, 1H), 5.07-4.90 (m, 6H), 4.46 (d, *J* = 3.13 Hz, 1H), 4.06 (d, *J* = 3.83 Hz, 1H), 3.96 (dd, *J* = 5.84, 4.85 Hz, 1H), 3.86-3.68 (m, 4H), 3.68-3.57 (m, 2H), 3.56-3.45 (m, 3H), 3.45-3.33 (m, 1H), 3.34-3.24 (m, 1H), 2.06-1.81 (m, 3H), 1.29 (dd, *J* = 25.12, 12.63 Hz, 1H).

^{13}C NMR (CD_3OD, 100 MHz) δ 156.9, 156.9, 156.8, 147.7, 136.5, 136.4, 136.3, 127.7 - 127.1 (15C), 108.9, 97.1, 94.5, 85.2, 83.0, 77.7, 75.1, 74.8, 74.1, 69.9, 66.1, 65.7, 65.7, 62.3, 61.2, 51.2, 49.5, 33.8, 21.6.



23

6'-Deamino-4',5'-dehydro-3',4'-dideoxy-6'-hydroxy-ribostamycin (23).

Compound **S1** (27.4 mg, 33 μmol) was submitted to the general Birch reduction procedure. The column chromatography solvent was $CHCl_3$/MeOH/NH_4OH, 2:3:0.5. The procedure yielded 15.8 mg of the acetate salt of the title compound **23** (79%, 26 μmol), as a yellow amorphous solid.

R_f = 0.2, 2:3:1 $CHCl_3$/MeOH/NH_4OH.

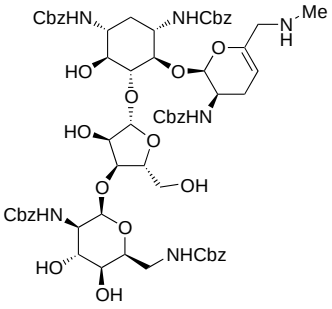
$[\alpha]^{22}_D$ 16.0º (*c* 0.79, H_2O).

HRMS (ESI) calcd. for $C_{17}H_{31}N_3O_9$, M + H^+ = 422.2133, found 422.2134 (0.24 ppm).

^{1}H NMR (D_2O, 700 MHz) δ 5.59 (d, *J* = 1.38 Hz, 1H), 5.17 (d, *J* = 1.36 Hz, 1H), 5.01 (t, *J* = 3.67, 3.67 Hz, 1H), 4.13 (dd, *J* = 4.76, 1.58 Hz, 1H), 4.09 (dd, *J* = 6.70, 4.81 Hz, 1H), 4.02 (dd, *J* = 10.41, 9.25 Hz, 1H), 3.98 (s, 1H), 3.97 (s, 1H), 3.94 (dt, *J* = 6.65, 6.51, 2.61 Hz, 1H), 3.86 (dt, *J* = 5.77, 5.73, 1.37 Hz, 1H), 3.81 (dd, *J* = 12.17, 2.89 Hz, 1H), 3.73 (t, *J* = 9.20, 9.20 Hz, 1H), 3.63-3.57 (m, 2H), 3.44 (ddd, *J* = 12.80, 10.64, 3.97 Hz, 1H), 3.28 (ddd, *J* = 12.37, 10.95, 4.05 Hz, 1H), 2.57 (ddd, *J* = 17.88, 5.47, 3.90 Hz, 1H), 2.42 (ddd, *J* = 12.63, 4.46, 3.90 Hz, 1H), 2.27 (ddd, *J* = 18.34, 4.79, 4.36 Hz, 1H), 1.87 (s, 13H AcOD), 1.79 (q, *J* = 12.64, 12.64, 12.60 Hz, 1H).

^{13}C NMR (D_2O, 175 MHz) δ 180.8 (AcOD), 148.8, 110.4, 97.3, 96.1, 83.8, 82.3, 77.7, 74.9, 71.8, 69.6, 62.0, 60.7, 49.7, 48.3, 46.0, 27.8, 22.9 (AcOD), 22.4.

LC/CLND gradient B, R_t = 9.3 min, 97% purity.



S2

1,3,2',2''', 6'''-*N*-Cbz-6'-*N*-methyl-4',5'-dehydro-3',4'-dideoxy-neomycin (S2).

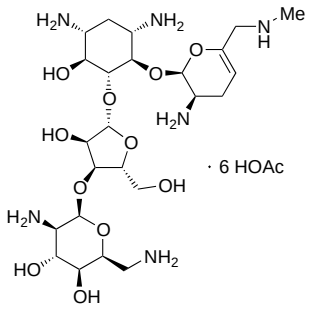
Sodium methoxide in MeOH was prepared by addition of a piece of sodium (~20 mg) to anhydrous MeOH (20 mL). The resulting alkaline solution was diluted with anhydrous MeOH to approx. pH 8 to 9 (Accutint pH paper roll). Aldehyde **12** (94 mg, 61 µmol) was dissolved with the dilute NaOMe solution (5 to 15 mL) and stirred at RT for 3 h. TLC and LRMS was used to monitoring until complete deprotection of the methylcarbonate groups was observed: HRMS (ESI) calcd. for $C_{63}H_{71}N_5O_{22}$, M + Na^+ = 1272.4483, found 1272.4472 (-1 ppm). The solution was acidified with 100 µL of AcOH, cooled to 0 °C, then treated with methylamine (91 µL, 2 M in THF, 0.18 mmol) and $NaCNBH_3$ (120 µL, 1 M in THF, 0.12 mmol), stirred and allowed to warm to RT overnight. The reaction was diluted with DCM, washed with sat. $NaHCO_3$, dried over Na_2SO_4 and filtered. The organic fraction was evaporated to a residue, which was purified by column chromatography (5 → 8% ammoniacal MeOH in DCM) yielding 44.5 mg (58%, 35 µmol) of the title compound **S2**, as an off-white amorphous solid.

R_f = 0.3, 15% of ammoniacal MeOH/$CHCl_3$

HRMS (ESI) calcd. for $C_{64}H_{76}N_6O_{21}$, M + H^+ = 1265.5136, found 1265.5137 (0.05 ppm).

^{1}H NMR (CD_3OD, 400 MHz) δ 7.44-7.12 (m, 25H), 5.52 (s, 1H), 5.17-4.94 (m, 11H), 4.90 (s, 1H), 4.50 (d, *J* = 2.97 Hz, 1H), 4.25-4.10 (m, 2H), 4.02-3.94 (m, 1H), 3.94-3.87 (m, 2H), 3.84 (s, 1H), 3.82-3.76 (m, 1H), 3.68-3.61 (m, 1H), 3.61-3.51 (m, 3H), 3.50-3.46 (m, 1H), 3.46-3.30 (m, 5H), 3.00 (d, *J* = 13.44 Hz, 1H), 2.81 (d, *J* = 13.56 Hz, 1H), 2.18 (s, 3H), 2.14-2.06 (m, 1H), 2.05-1.91 (m, 2H), 1.37 (dd, *J* = 24.33, 12.21 Hz, 1H).

^{13}C NMR (CD_3OD, 100 MHz) δ 159.3 - 158.5 (5C), 147.9, 138.3 - 138.2 (5C), 129.7 - 128.7 (25C), 110.3, 100.4, 99.1, 97.2, 87.1, 83.7, 79.0, 78.6, 75.7, 75.7, 74.8, 71.7, 69.3, 68.0, 67.7 - 67.7 (5C), 63.3, 53.8, 52.9, 51.6, 47.0, 42.7, 35.5, 35.2, 23.6.



26

6'-*N*-Methyl-4',5'-dehydro-3',4'-dideoxy-neomycin (26).

Compound **S2** (44.5 mg, 35 μmol) was submitted to the general Birch reduction procedure. The column chromatography solvents were $CHCl_3$/MeOH/NH_4OH, 2:3:1 followed by 2:3:2. The procedure yielded 25.1 mg of the acetate salt of title compound **26** (75%, 26 μmol), as a yellow amorphous solid.

R_f = 0.2, 2:3:2 $CHCl_3$/MeOH/NH_4OH.

$[\alpha]^{22}_D$ 29.0º (*c* 0.61, H_2O).

HRMS (ESI) calcd. for $C_{24}H_{46}N_6O_{11}$, M + H^+ = 595.3297, found 595.3286 (-1.91 ppm).

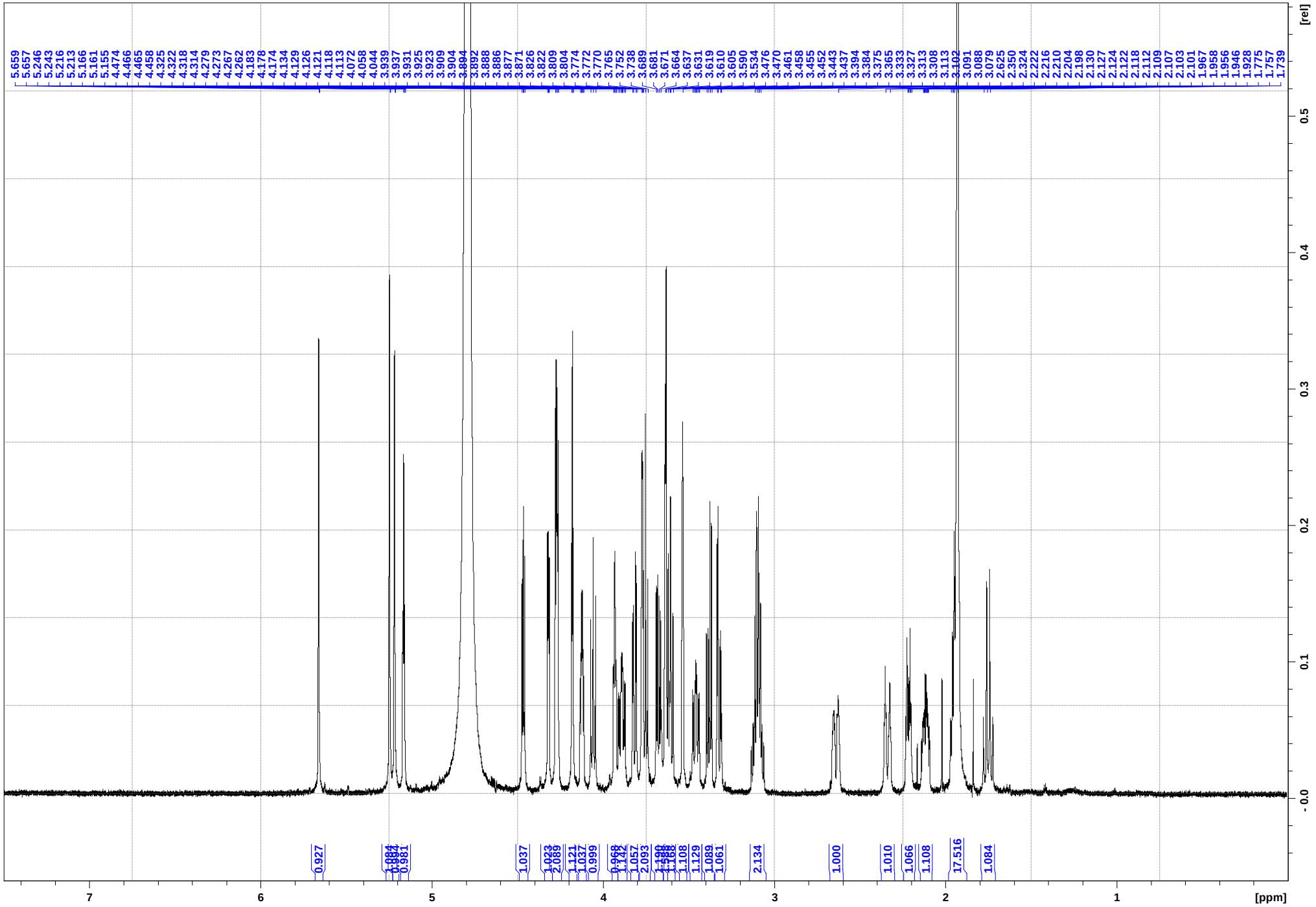
^{1}H NMR (D_2O, 700 MHz) δ 5.63 (d, *J* = 1.14 Hz, 1H), 5.22 (d, *J* = 1.42 Hz, 1H), 5.21 (t, *J* = 3.76, 3.76 Hz, 1H), 5.19 (d, *J* = 2.50 Hz, 1H), 4.42 (t, *J* = 5.48, 5.48 Hz, 1H), 4.29 (dd, *J* = 4.68, 2.82 Hz, 1H), 4.24 (dd, *J* = 5.92, 3.97 Hz, 1H), 4.16 (t, *J* = 3.04, 3.04 Hz, 1H), 4.11 (dt, *J* = 5.75, 5.63, 3.54 Hz, 1H), 3.96 (t, *J* = 9.73, 9.73 Hz, 1H), 3.88 (dt, *J* = 5.46, 5.19, 1.51 Hz, 1H), 3.80 (dd, *J* = 12.13, 3.14 Hz, 1H), 3.75 (d, *J* = 2.98 Hz, 1H), 3.65-3.60 (m, 1H), 3.65 (dd, *J* = 12.14, 5.56 Hz, 1H), 3.52-3.47 (m, 2H), 3.43-3.41 (m, 1H), 3.29-3.18 (m, 3H), 3.24 (dt, *J* = 11.86, 11.53, 4.19 Hz, 1H), 2.64 (s, 3H), 2.61 (ddd, *J* = 8.78, 5.76, 3.52 Hz, 1H), 2.36 (ddd, *J* = 13.35, 5.02, 3.90 Hz, 1H), 2.32 (ddd, *J* = 18.38, 4.71, 3.13 Hz, 1H), 1.85 (s, 18H AcOD), 1.73 (dd, *J* = 25.04, 12.83 Hz, 1H).

^{13}C NMR (D_2O, 175 MHz) δ 181.2 (AcOD), 141.7 110.3, 102.5, 96.5, 95.5, 83.9, 81.4, 78.4, 76.0, 73.2, 72.0, 70.1, 67.6, 67.2, 61.1, 50.8, 49.8, 49.3, 48.2, 45.6, 40.3, 31.9, 28.5, 23.1 (AcOD), 22.9.

LC/CLND gradient A, R_t = 11.7 min, 94% purity.

Experimental references

1. Biedenbach, D. J.; Jones, R. N.; Miller, G. H.; Armstrong, E. S., Ten year trend in aminoglycoside resistance from a worldwide collection of Gram-negative pathogens (1998-2007). In *19th European Congress of Clinical Microbiology and Infectious Diseases*, Poster #636, Helsinki, Finland, 2009.
2. Armstrong, E. S.; Biedenbach, D. J.; Jones, R. N.; Miller, G. H., Surveying aminoglycoside-resistance mechanisms: a tool for the development of neoglycosides. In *19th European Congress of Clinical Microbiology and Infectious Diseases*, Poster #643, Helsinki, Finland, 2009. http://www.achaogen.com/s/ECCMID_P-643.pdf (accessed 09/2015).
3. Hanessian, S.; Takamoto, T.; Masse, R., Aminoglycoside antibiotics: oxidative degradations leading to novel biochemical probes and synthetic intermediates. *The Journal of antibiotics* **1975,** *28* (10), 835-7.
4. Hanessian, S.; Maianti, J. P.; Matias, R. D.; Feeney, L. A.; Armstrong, E. S., Hybrid aminoglycoside antibiotics via Tsuji palladium-catalyzed allylic deoxygenation. *Organic letters* **2011,** *13* (24), 6476-9.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0.927 1.037 1.023 2.089 1.121 1.037 0.999 0.968 1.142 1.057 2.093 1.108 1.129 1.089 1.061 2.134 1.000 1.010 1.066 1.108 17.516 1.084
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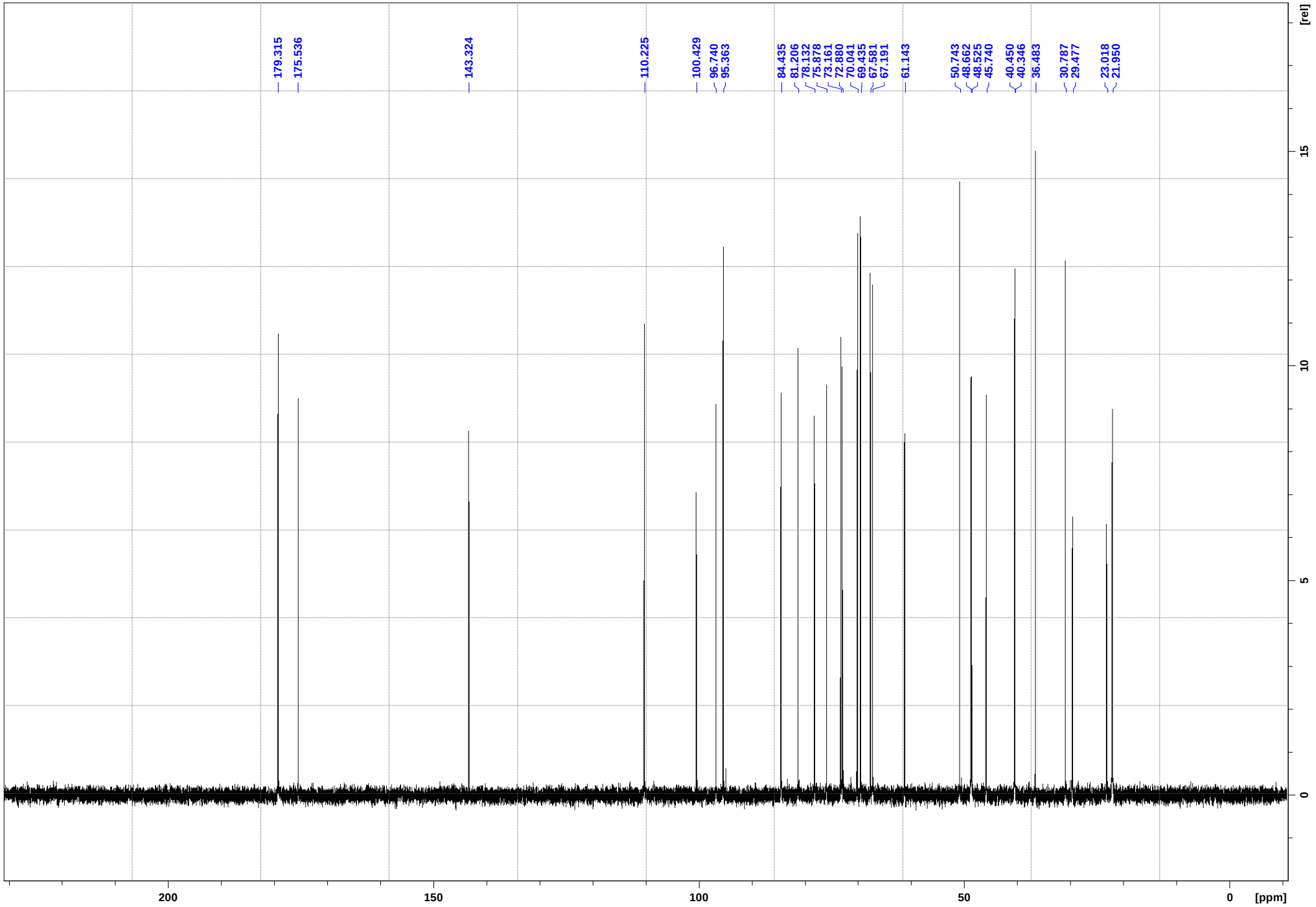

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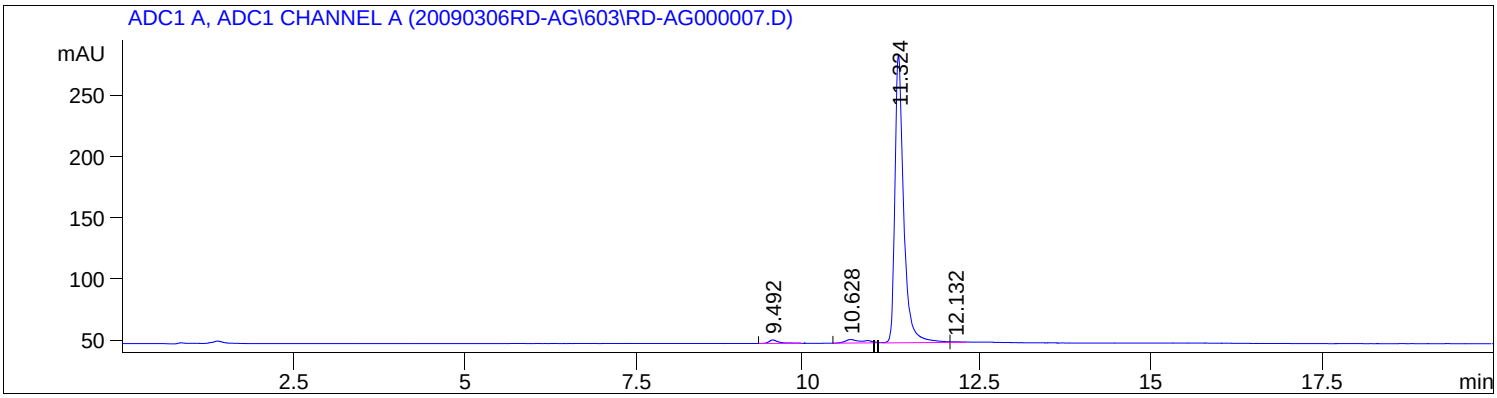


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Use Multiplier & Dilution Factor with ISTDs


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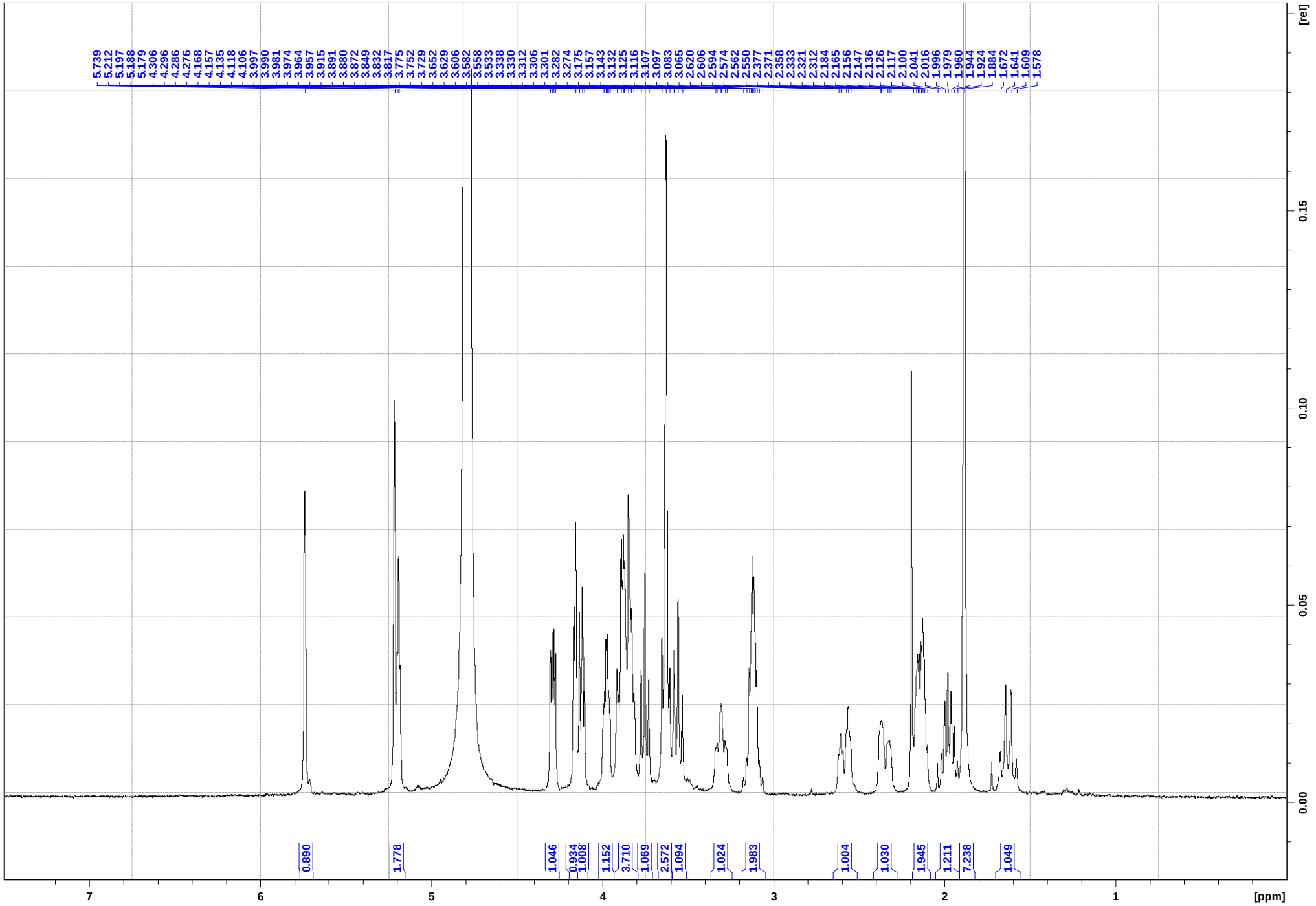
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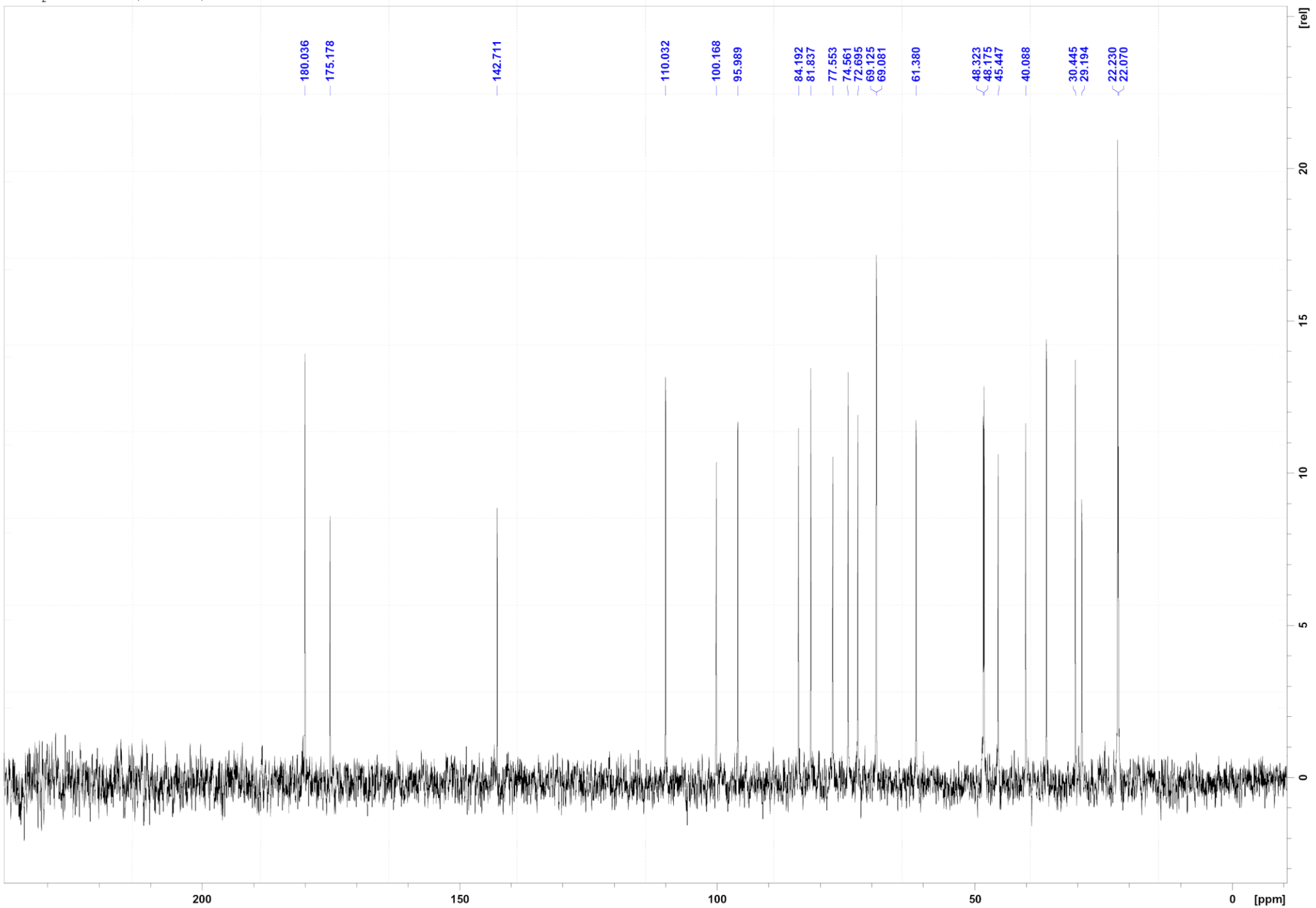
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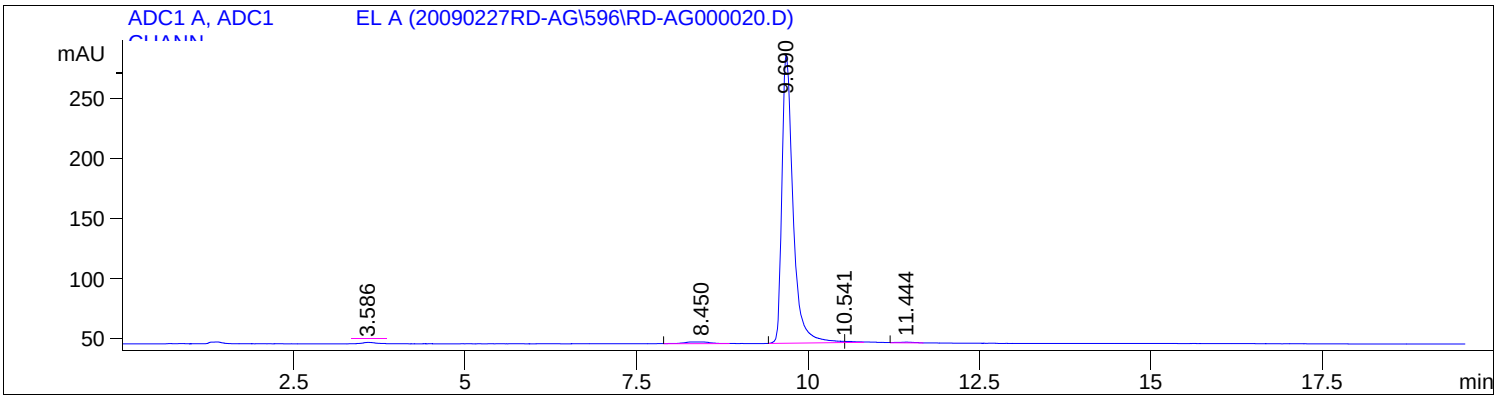


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Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


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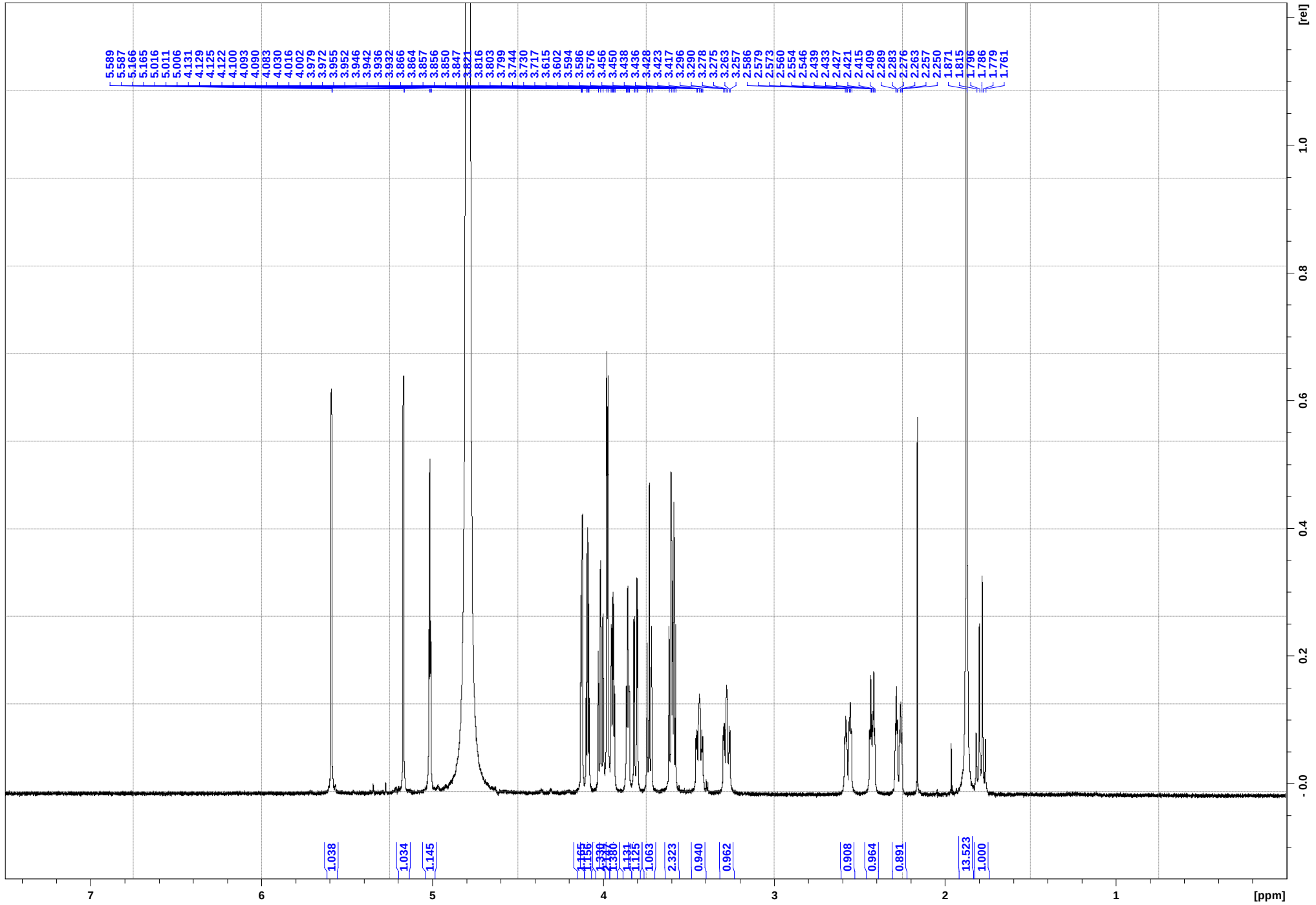
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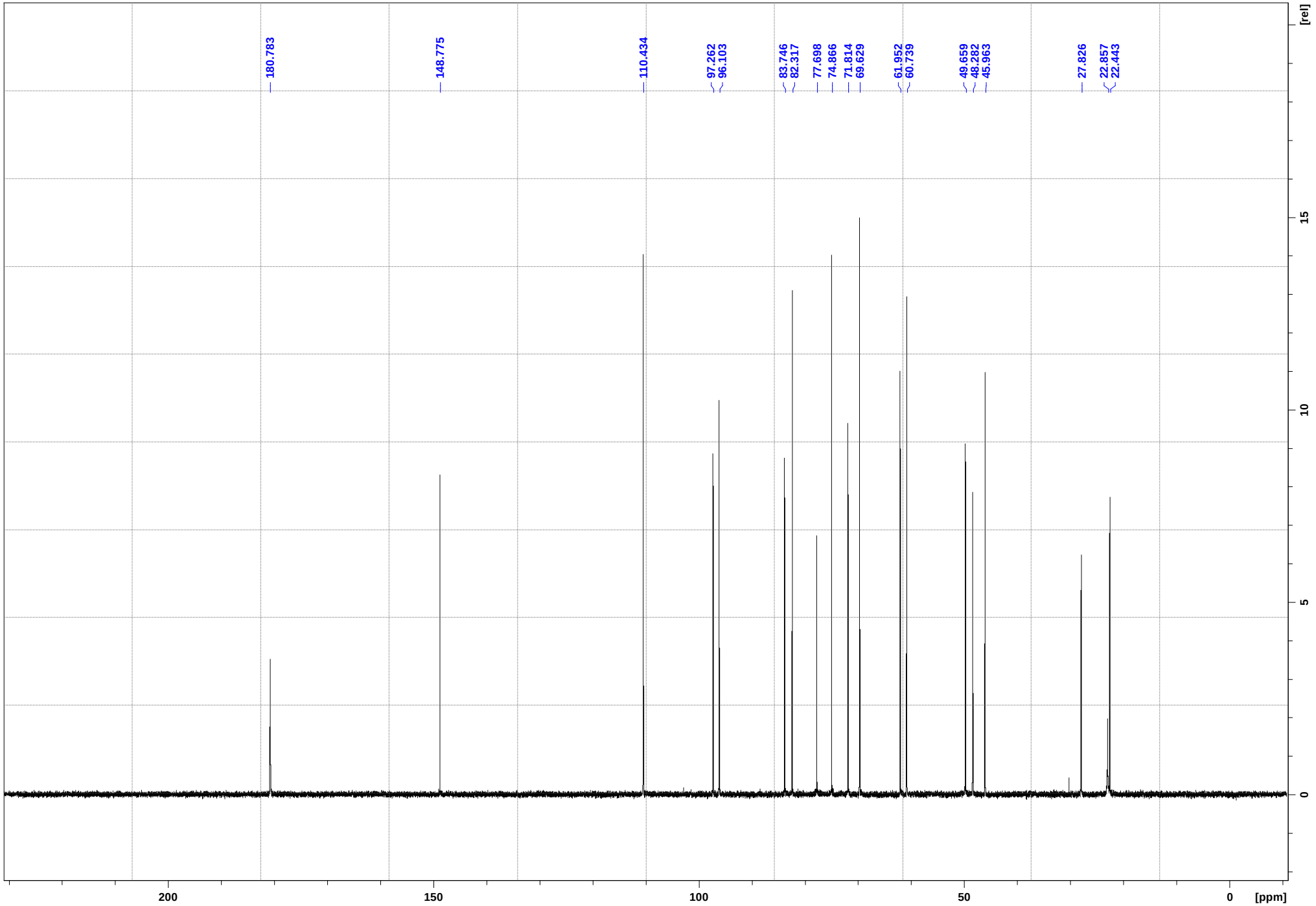
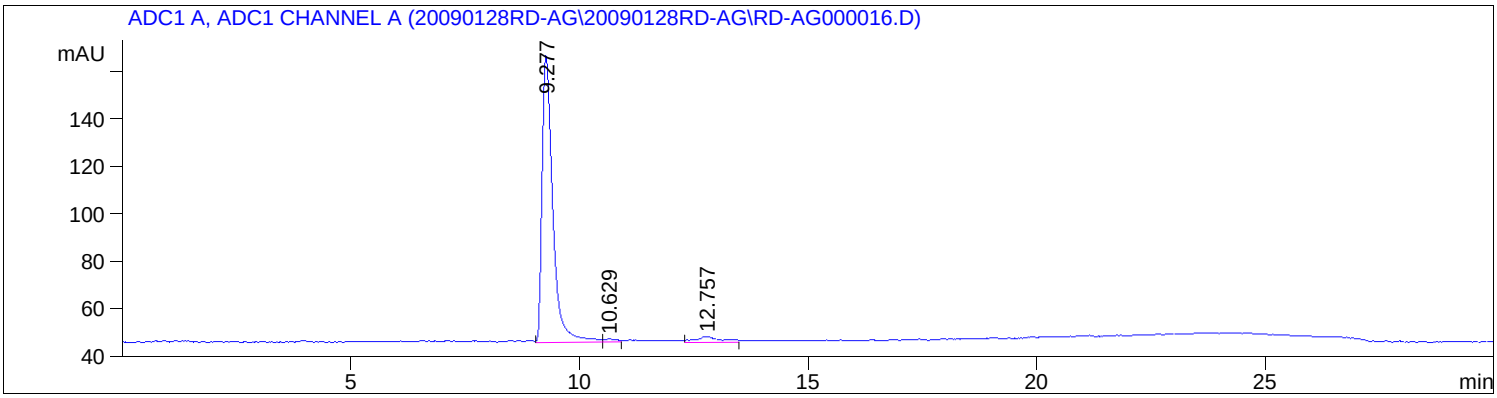
5.011
5.006
4.131
4.129
4.125
4.122
4.100
4.093
4.090
4.083
4.030
4.016
4.002
3.979
3.972
3.955
3.952
3.946
3.942
3.936
3.932
3.866
3.864
3.857
3.856
3.850
3.847
3.821
3.816
3.803
3.799
3.744
3.730
3.717
3.615
3.602
3.594
3.586
3.576
3.456
3.450
3.438
3.436
3.428
3.423
3.417
3.296
3.290
3.278
3.275
3.263
3.257
2.586
2.579
2.573
2.560
2.554
2.546
2.439
2.433
2.427
2.421
2.415
2.409
2.289
2.283
2.276
2.263
2.257
2.250
1.871
1.815
1.796
1.786
1.779
1.761
1.038
1.034
1.145
1.063
2.323
0.940
0.962
0.908
0.964
0.891
13.523
1.000
[rel]
1.0
0.8
0.6
0.4
0.2
- 0.0
7
6
5
4
3
2
1
[ppm]

"Compound 4.25 (JPM5-17)" 3 1 D: Hanessian

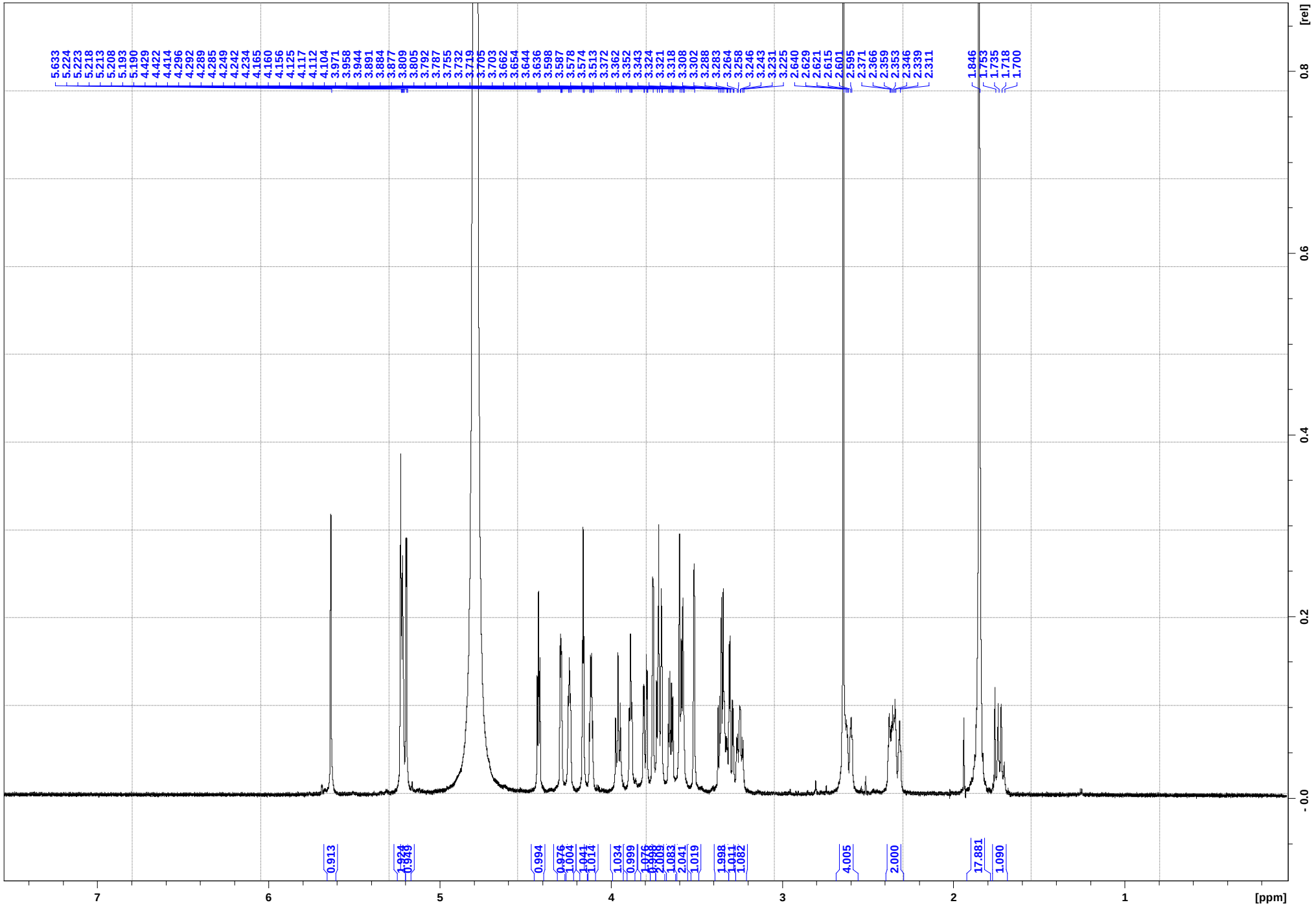


Data File C:\CHEM32\1\DATA\20090128RD-AG\20090128RD-AG\RD-AG000016.D
Sample Name: JPM-5-17

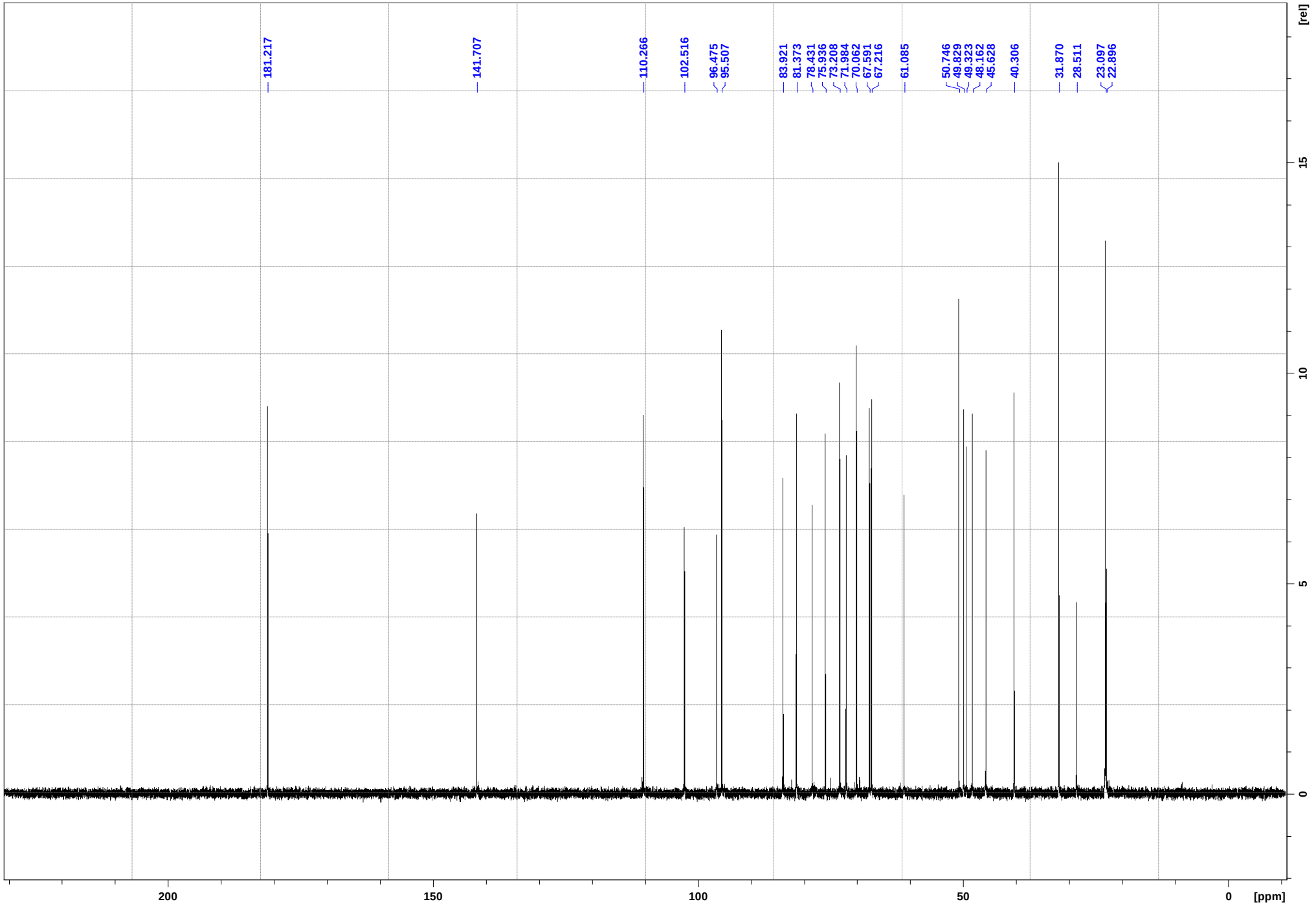
```
==========================================================================
Acq. Operator   : rd                                Seq. Line :  16
Acq. Instrument : Chemstation 4                      Location : Vial 71
Injection Date  : 1/28/2009 11:48:42 PM                   Inj :   1
                                                  Inj Volume : 2 µl
Acq. Method     : C:\Chem32\1\DATA\20090128RD-AG\20090128RD-AG\05-30-75B_210_30M.M
Last changed    : 1/23/2009 12:23:25 PM by rc
Analysis Method : C:\CHEM32\1\METHODS\INT_30MIN.M
Last changed    : 1/28/2009 8:43:05 AM by rc
                  (modified after loading)
Method Info     : Integration method.

Sample Info     : 20x dilution
```




```
==========================================================================
                        Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: ADC1 A, ADC1 CHANNEL A

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   9.277 MF    0.2662 1937.68665  121.29505  96.9886
   2  10.629 FM    0.2323    9.60944 6.89434e-1   0.4810
   3  12.757 MM    0.4517   50.55310    1.86522   2.5304

Totals :                  1997.84919  123.84971

==========================================================================
                        *** End of Report ***
```

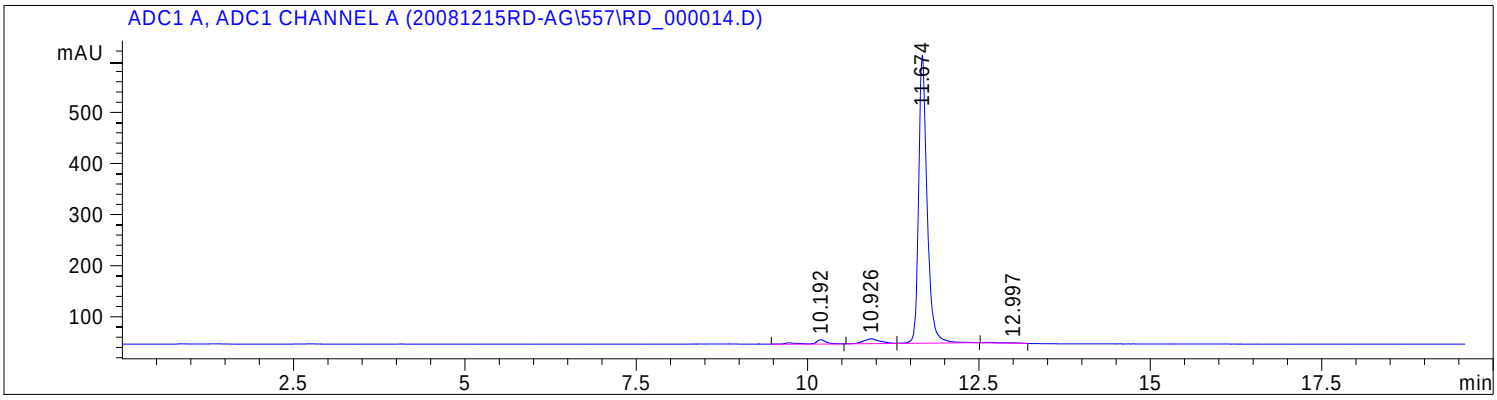

"Compound 4.21 (JPM4-169)" 1 1 D: Hanessian
5.633 5.224 5.223 5.218 5.213 5.208 5.193 5.190 4.429 4.422 4.414 4.296 4.292 4.289 4.285 4.249 4.242 4.234 4.165 4.160 4.156 4.125 4.117 4.112 4.104 3.971 3.958 3.944 3.891 3.884 3.877 3.809 3.805 3.792 3.787 3.755 3.732 3.719 3.705 3.703 3.662 3.654 3.644 3.636 3.598 3.587 3.578 3.574 3.513 3.372 3.362 3.352 3.343 3.324 3.321 3.318 3.308 3.302 3.288 3.283 3.264 3.258 3.246 3.243 3.231 3.225 2.640 2.629 2.621 2.615 2.601 2.595 2.371 2.366 2.359 2.353 2.346 2.339 2.311 1.846 1.753 1.735 1.718 1.700
0.913 0.994 0.976 1.004 1.041 1.014 1.034 0.999 1.083 2.041 1.019 1.998 1.011 1.082 4.005 2.000 17.881 1.090
7 6 5 4 3 2 1 [ppm]
[rel] 0.8 0.6 0.4 0.2 - 0.0

"Compound 4.21 (JPM4-169)" 2 1 D: Hanessian



Data File C:\CHEM32\1\DATA\20081215RD-AG\557\RD_000014.D
Sample Name: JPM4-169

```
==========================================================================
Acq. Operator   : rd                                Seq. Line :  14
Acq. Instrument : Chemstation 8                      Location : Vial 9
Injection Date  : 12/15/2008 9:52:48 PM                  Inj :   1
                                                   Inj Volume : 20 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 2 µl
Acq. Method     : C:\Chem32\1\DATA\20081215RD-AG\557\05-25-95B_20M.M
Last changed    : 10/24/2008 3:35:56 PM by rd
Analysis Method : C:\CHEM32\1\METHODS\INT_30MIN.M
Last changed    : 12/16/2008 8:35:51 AM by rd
                  (modified after loading)
Method Info     : Integration method.

Sample Info     : 20x dilution
```




```
==========================================================================
                          Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: ADC1 A, ADC1 CHANNEL A

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  10.192 BB    0.1923  111.17039    8.09142   2.2071
   2  10.926 BV    0.2445  150.17834    9.29495   2.9815
   3  11.674 VB    0.1313 4749.79053  558.00244  94.2971
   4  12.997 MM    0.4246   25.90739    1.01685   0.5143

Totals :                  5037.04665  576.40566


==========================================================================
                          *** End of Report ***
```