Supporting Information

for

Effects of the 1-*N*-(4-Amino-2*S*-hydroxybutyryl) and 6′-*N*-(2-Hydroxyethyl) Substituents on Ribosomal Selectivity, Cochleotoxicity and Antibacterial Activity in the Sisomicin Class of Aminoglycoside Antibiotics

Amr Sonousi,[a] Vikram A. Sarpe,[a] Margarita Brilkova,[b] Jochen Schacht,[c] Andrea Vasella,[d,] Erik C. Böttger,[b,*] and David Crich[a,*]*

a: Department of Chemistry, Wayne State University, Detroit, MI 48202, USA

b: Institut für Medizinische Mikrobiologie, Universität Zürich, 8006 Zürich, Switzerland

c: Kresge Hearing Research Institute, Department of Otolaryngology, University of Michigan, Ann Arbor, MI 48109, USA

d: Laboratorium für Organische Chemie, ETH Zürich, 8093 Zürich, Switzerland

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General Experimental. All reagents and solvents were purchased from commercial suppliers and were used without further purification unless otherwise specified. All organic extracts were dried over sodium sulfate and concentrated under vacuum. Chromatographic purifications were carried out over silica gel or CM Sephadex C-25 as stated. Analytical thin-layer silica gel chromatography was performed with pre-coated glass backed plates and visualized by UV irradiation (254 nm) or by staining with 25% H_2SO_4 in EtOH or ceric ammonium molybdate solution. Specific rotations were obtained using a digital polarimeter in the solvent specified. High resolution mass spectra were recorded with an electrospray source coupled to a time-of-flight mass analyzer (Waters). ^{1}H, ^{13}C and 2D NMR spectra were recorded on 600 MHz, 500 MHz and 400 MHz instruments. Ammonical methanol was prepared from ammonium hydroxide solution (28% in water) and methanol in 1:9 ratio.

1,3,2′-Trideamino-1,3,2′-triazido-3′′-*N*-(phenylazo)sisomicin-6′-carboxaldehyde (8). To a solution of 1,3,2′,6′-tetra-deamino-1,3,2′,6′-tetraazido-3′′-*N*-(phenylazo)sisomicin **7**[1] (90 mg, 0.14 mmol) in CH_2Cl_2 (2 mL) and pyridine (234 μL, 2.75 mmol), was added at room temperature SeO_2 (152 mg, 1.37 mmol). The reaction mixture was stirred at room temperature for 48 h then was diluted with CH_2Cl_2, and washed with saturated aqueous $NaHCO_3$ followed by brine. The organic layer was dried on Na_2SO_4, concentrated and purified by column chromatography on silica gel basified with Et_3N (49:1 CH_2Cl_2/ methanol) to give the title compound as a yellow gum (66 mg, 77%). $[\alpha]^{21}_D$ +105.70 (*c* 0.67, MeOH). ^{1}H NMR (600 MHz, CD_3OD) δ 9.20 (s, 1H, H-6′), 7.41 – 7.37 (m, 2H, Arom.), 7.28 (t, *J* = 7.8 Hz, 2H, Arom.), 7.09 (t, *J* = 7.3 Hz, 1H, Arom.), 6.20 (dd, *J* = 5.2, 3.1 Hz, 1H, H-4′), 5.97 (d, *J* = 2.4 Hz, 1H, H-1′), 5.47 (d, *J* = 3.8 Hz, 1H, H-1′′), 4.39 (dd, *J* = 11.4, 3.8 Hz, 1H, H-2′′), 4.31 – 4.27 (m, 2H, H-5a′′, H-3′′), 3.73 – 3.65 (m, 2H, H-5, H-4), 3.63 (ddd, *J* = 12.4, 9.9, 4.5 Hz, 1H, H-1), 3.53 – 3.49 (m, 1H, H-2′), 3.46 – 3.36 (m, 2H, H-3, H-6), 3.32 (s, 3H, NC*H*$_3$), 3.31 – 3.28 (m, 1H, H-5a′′), 2.70 – 2.57 (m, 2H, H-3′), 2.24 (dt, *J* = 12.4, 4.5 Hz, 1H, H-2a), 1.42 (q, *J* = 12.4 Hz, 1H, H-2b), 1.10 (s, 3H, 4′′-C*H*$_3$). ^{13}C NMR (151 MHz, CD_3OD) δ 186.6 (C-6′), 151.1, 148.7 (C-5′), 128.3, 124.8 (s, arom.), 121.2 (C-4′), 120.2 (s, arom), 98.9 (C-1′′), 96.6 (C-1′), 79.9 (C-6), 79.7 (C-4), 74.5 (C-5), 73.2 (C-4′′), 68.7

(C-5″), 68.4 (C-3″), 65.2 (C-2″), 60.6 (C-1), 60.0 (C-3), 54.1 (C-2′), 32.3 (C-2), 21.8 (C-3′), 21.1 (N*C*H$_3$). ESIHRMS calculated for $C_{25}H_{32}N_{12}O_8$ $[M+Na]^+$, 651.2364; found, 651.2361.

6′-*N*-(2-Hydroxyethyl)-1,3,2′-trideamino-1,3,2′-triazido-3″-*N*-(phenylazo)sisomicin (9). To a stirred solution of **8** (66 mg, 105.0 μmol) in MeOH (1.5 mL), was added ethanolamine (19 μL, 315.0 μmol) followed by AcOH (1.5 μL, 26.3 μmol) at room temperature. After stirring for 15 min, $NaCNBH_3$ (66 mg, 1.05 mmol) was added in one portion and the reaction mixture was stirred at room temperature for 14 h. The reaction mixture was diluted with CH_2Cl_2, and washed with saturated aqueous $NaHCO_3$ followed by brine. The organic layer was dried on Na_2SO_4, concentrated and purified by column chromatography on silica gel (9:1 CH_2Cl_2/ methanol) to give the title compound as a pale yellow solid (45 mg, 63%). $[\alpha]^{21}_D$ +65 (*c* 0.5, MeOH). ^{1}H NMR (600 MHz, CD_3OD) δ 7.43 – 7.36 (m, 2H, Arom.), 7.28 (t, *J* = 7.9 Hz, 2H, Arom.), 7.10 (t, *J* = 7.3 Hz, 1H, Arom.), 5.89 (d, *J* = 2.2 Hz, 1H, H-1′), 5.48 (d, *J* = 3.8 Hz, 1H, H-1″), 5.08 (dd, *J* = 5.0, 2.2 Hz, 1H, H-4′), 4.39 (dd, *J* = 11.4, 3.8 Hz, 1H, H-2″), 4.29 – 4.26 (m, 2H, H-5a″, H-3″), 3.75 – 3.69 (m, 4H, NHCH$_2$C*H*$_2$O, H-4, H-5), 3.69 – 3.62 (m, 1H, H-1), 3.54 – 3.43 (m, 4H, H-3, H-6a′, H-6b′, H-2′), 3.37 (t, *J* = 6.5 Hz, 1H, H-6), 3.32 (s, 3H, NC*H*$_3$), 3.26 – 3.27 (m, 1H, H-5b″), 3.01 – 2.93 (m, 2H, NHC*H*$_2$CH$_2$O), 2.49 – 2.39 (m, 1H, H-3a′), 2.36 – 2.25 (m, 2H, H-3b′, H-2a), 1.44 (q, *J* = 12.5 Hz, 1H, H-2b), 1.09 (s, 3H, 4″-C*H*$_3$). ^{13}C NMR (151 MHz, CD_3OD) δ 151.1 (s, arom.), 143.8 (C-5′), 128.3, 124.9, 120.2 (s, arom.), 99.8 (C-4′), 98.9 (C-1″), 96.9 (C-1′), 79.9 (C-6), 79.3 (C-4), 74.5 (C-5), 73.1 (C-4″), 68.7 (C-5″), 68.4 (C-3″), 65.2 (C-2″), 60.7 (C-1), 60.3 (C-2′), 57.9 (C-NHCH$_2$*C*H$_2$OH), 54.6 (C-3), 49.2 (C-6′), 49.0 (NH*C*H$_2$CH$_2$OH), 32.5 (C-2), 21.1 (N*C*H$_3$), 20.9 (C-3′). ESIHRMS calculated for $C_{27}H_{39}N_{13}O_8$ $[M+H]^+$, 674.3123; found, 674.3121.

6′-*N*-(2-Hydroxyethyl)sisomicin Pentaacetate Salt (10). A solution of **9** (44 mg, 65.3 μmol) in THF (0.8 mL) was treated with solution of PMe_3 in THF (1M; 463.5 μL, 463.5 μmol) for 2 h, at room temperature. After complete consumption of the starting material, 1 N NaOH (0.5 mL) was added to the reaction mixture and stirring continued for 5 h, before the solvents were evaporated on and the residue was absorbed on silica gel and purified by column chromatography on silica gel (15% to 60% ammonical MeOH in CH_2Cl_2). The product-containing fractions were concentrated, dissolved in ethanol (1.5 mL), and treated with sodium hypophosphite (13.6 mg, 154.5 μmol) and trifluoroacetic acid (71 μL, 927 μmol). After stirring for 5.5 h, the reaction mixture was neutralized using Amberlite® IRA400 hydroxide form, filtered, and dried. The

crude product was dissolved in deionized water (0.6 mL), acidified with glacial acetic acid to pH 3-4 and loaded on a Sephadex C25 column. Elution with water followed by a gradient of 0.1% to 1% NH_4OH in deionized water gave a series of product-containing fractions, which were combined, evaporated, taken up in 10% AcOH and lyophilized to give 6′-*N*-(2-hydroxyethyl)sisomicin as its pentaacetate salt in the form of a white solid (19.3 mg, 37%). $[\alpha]^{21}_D$ +70.4 (*c* 0.23, H_2O). ^{1}H NMR (600 MHz, D_2O) δ 5.47 (d, *J* = 1.6 Hz, 1H, H-1′), 5.09 (t, *J* = 3.6 Hz, 1H, H-4′), 4.92 (d, *J* = 3.6 Hz, 1H, H-1″), 4.00 (dd, *J* = 10.9, 3.6 Hz, 1H, H-2″), 3.80 (d, *J* = 12.9 Hz, 1H, H-5b″), 3.73 (t, *J* = 9.8 Hz, 1H, H-4), 3.69 – 3.62 (m, 3H, H-2′, $NHCH_2C\mathit{H}_2$-OH), 3.56 – 3.53 (m, 3H, H-6a′, H-5, H-6b′), 3.49 (t, *J* = 9.7 Hz, 1H, H-6), 3.27 – 3.26 (m, 3H, H-1′, H-5b″, H-3), 3.23 – 3.17 (m, 1H, H-3), 3.05 – 2.95 (m, 2H, $NC\mathit{H}_2$-CH_2O), 2.71 (s, 3H, $NC\mathit{H}_3$), 2.52 – 2.43 (m, 1H, H-3b′), 2.27 – 2.22 (m, 1H, H-3a′), 2.22-2.16 (m, 1H, H-2a), 1.62 (q, *J* = 12.4 Hz, 1H, H-2b), 1.14 (s, 3H, 4″-$C\mathit{H}_3$). ^{13}C NMR (151 MHz, D_2O) δ 181.2 ($CH_3\mathit{C}OOH$), 141.6 (C-5′), 102.6 (C-4′), 101.0 (C-1″), 96.6 (C-1′), 83.7 (C-6), 79.7 (C-4), 73.6 (C-5), 69.8 (C-4″), 67.5 (C-5″), 66.2 (C-2″), 63.5 (C-3″), 56.4 ($NHCH_2\mathit{C}H_2O$), 49.9 (C-1), 48.3 ($NH\mathit{C}H_2CH_2OH$), 48.1 (C-3), 47.9 (C-6′), 45.9 (C-2′), 34.4 (NCH_3), 28.6 (C-2), 23.1 ($\mathit{C}H_3COOH$), 22.7 (C-3′), 20.8 (C-4″-$\mathit{C}H_3$). ESIHRMS calculated for $C_{21}H_{41}N_5O_8$ $[M+Na]^+$, 514.2853; found, 514.2838.

6′-*N*-(*p*-Nitrobenzyloxycarbonyl)sisomicin (12). Sisomicin sulfate (2.00 g, 2.88 mmol) was added to a stirred suspension of Amberlite IRA-400 (OH form) (20 g) in methanol (15 mL). after stirring overnight, the suspension was filtered, and the filtrate was concentrated to give sisomicin free base (1.54 g). A solution of sisomicin free base (1.29 g) in methanol (30 mL) was treated with zinc acetate dihydrate (1.90 g, 8.67 mmol) and stirred for 1 h before a solution of *N*-(*p*-nitrobenzyloxycarbonyloxy)succinimide[2-3] (0.85 g, 2.88 mmol) in dichloromethane (10 mL) was by syringe pump over 3 h. After stirring overnight the reaction mixture was concentrated to dryness then dissolved in 10% NH_4OH solution (8 mL). The aqueous layer was washed with dichloromethane (3 mL) and extracted with 30% isopropanol in dichloromethane. The extracts were washed with a 10% NH_4OH: brine (7:3) mixture, dried over Na_2SO_4, filtered and concentrated to give **12** (1.35 g, 75%) as colorless foam. *Rf* = 0.55 (CH_2Cl_2:MeOH:NH_4OH = 3:2:1); $[\alpha]_D^{25}$ = +101.1 (*c* = 0.01, MeOH); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.19 (d, *J* = 8.4 Hz, 2H, PNZ-*m*Hs), 7.49 (d, *J* = 8.4 Hz, 2H, PNZ-*o*Hs), 5.44 (s, 1H, H-1′), 5.21 – 5.15 (m, 2H, $C\mathit{H}_2$-4-$NO_2C_6H_4$), 4.90 (d, *J* = 2.6 Hz, 1H, H-1″), 4.80 (d, *J* = 2.9 Hz, 1H, H-4′), 3.89 (dd, *J* = 13.9,

5.9 Hz, 1H, H-6′), 3.79 (d, *J* = 12.1 Hz, 1H, H-5″), 3.57 – 3.44 (m, 5H, H-2″, H-4, H-5, H-5″, H-6′), 3.07 – 3.04 (m, 1H, H-2′), 3.01 (t, *J* = 9.4 Hz, 1H, H-6), 2.82 – 2.77 (m, 2H, H-1, H-3), 2.57 (s, 3H, NC*H*$_3$), 2.45 (d, *J* = 9.9 Hz, 1H, H-3″), 2.16 – 2.12 (m, 1H, H-3′), 2.00 – 1.91 (m, 2H, H-2, H-3′), 1.23 – 1.15 (m, 1H, H-2), 1.13 (s, 3H, 4″-C*H*$_3$); ^{13}C NMR (151 MHz, $CDCl_3$): δ 156.2 (s, C=O), 147.4 (s, C-5′), 145.8 , 144.5, 128.0, 123.7 (s, arom.), 101.2 (s, C-1″), 99.1 (s, C-1′), 98.3 (s, C-4′), 89.5 (s, C-6), 82.6 (s, C-4), 75.6 (s, C-5), 71.1 (s, C-4″), 69.4 (s, C-2″), 67.3 (s, C-5″), 65.2 (s, C-3″), 64.8 (s, *C*H_2-4-$NO_2C_6H_4$), 50.6 (s, C-1), 49.7 (s, C-3), 47.0 (s, C-2′), 43.5 (s, C-6′), 39.3 (s, C-2), 38.7 (s, C- N*C*H_3), 25.9 (s, C-3′), 24.2 (s, C-4″-*C*H_3). ESI-HRMS: *m/z* calcd. for $C_{27}H_{43}N_6O_{11}$ $[M+H]^+$ 627.2990, found: 627.2971.

6′-*N*-(*p*-Nitrobenzyloxycarbonyl)-2′,3-di-*N*-(*tert*-butyloxycarbonyl)sisomicin (13). A solution of **12** (1.00 g, 1.60 mmol) and zinc acetate dihydrate (1.05 g, 8.67 mmol) in methanol (20 mL) was stirred for 1 h before a solution of *N*-(*tert*-butoxycarbonyloxy)succinimide (0.65 g, 3.02 mmol) in THF (10 mL) was added by syringe pump over 4 h. After stirring overnight (triethylamine 0.17 mL) was added, followed by *N*-(*tert*-butoxycarbonyloxy)succinimide (0.18 g, 0.81 mmol) in THF (2 mL). The resulting solution was stirred for 24 h then quenched by addition of glycine (0.48 g, 6.4 mmol) and evaporated to dryness. The crude product was dissolved in dichloromethane (50 mL) and washed with 30% aqueous NH_4OH solution, dried over Na_2SO_4 and concentrated. The residue was purified by chromatography over silica gel (6% to 8% of ammonical methanol in dichloromethane) to give **13** (0.69 g, 52%) as a white solid *Rf*= 0.5 (25% ammonical MeOH in CH_2Cl_2); $[\alpha]_D^{25}$= +131.7 (*c* = 0.01, MeOH); ^{1}H NMR (600 MHz, CD_3OD): δ 8.21 (d, *J* = 8.4 Hz, 2H, PNZ-*m*Hs), 7.58 (d, *J* = 8.4 Hz, 2H, PNZ-*o*Hs), 5.40 (s, 1H, H-1′), 5.26 – 5.17 (m, 2H, C*H*$_2$-4-$NO_2C_6H_4$), 4.95 (d, *J* = 3.7 Hz, 1H, H-1″), 4.73 (br s, 1H, H-4′), 3.97 (d, *J* = 12.1 Hz, 1H, H-5″), 3.87 (d, *J* =15.4 Hz, 1H, H-6′), 3.75 (dd, *J* = 10.5 Hz, 3.5 Hz, 1H, H-2″), 3.69 (t, *J* = 7.9 Hz, 1H, H-5), 3.65 (d, *J* = 15.4 Hz, 1H, H-6′), 3.50 (t, *J* = 8.1 Hz, 1H, H-5), 3.45 (m, 2H, H-3, H-4), 3.32 (d, *J* = 12.1 Hz, 1H, H-5″), 3.08 (t, *J* = 9.4 Hz, 1H, H-6), 2.82 (t, *J* = 8.4 Hz, 1H, H-1), 2.57 (s, 3H, NC*H*$_3$), 2.53 (d, *J* = 10.3 Hz, 1H, H-3″), 2.08 – 2.03 (m, 2H, H-3′), 1.99 (br d, *J* =11.4 Hz, 1H, H-2), 1.42 (s, 9H, C(C*H*$_3$)$_3$), 1.40 (s, 9H, C(C*H*$_3$)$_3$), 1.26 (m, 1H, H-2), 1.16 (s, 3H, 4″-C*H*$_3$); ^{13}C NMR (151 MHz, CD_3OD): δ 157.1, 156.5, 156.3 (s, C=O), 147.4 (s, arom.), 146.4 (s, C-5′), 144.7 (s, arom.), 127.6, 123.2 (s, arom.), 101.0 (s, C-1″), 97.0 (s, C-1′), 95.9 (s, C-4′), 88.6 (s, C-6), 80.2 (s, C-4), 78.8 (s, *C*$(CH_3)_3$), 75.6 (s, C-5), 71.4 (s, C-4″), 69.2 (s, C-2″), 68.0 (s, C-5″), 64.7 (s, *C*H_2-4-$NO_2C_6H_4$), 64.0 (s, C-3″), 50.3 (s, C-1), 49.3

(s, C-3), 46.9 (s, C-2′), 42.6 (s, C-6′), 36.9 (s, N*C*H$_3$), 35.4 (s, C-2), 27.4 (s, C(*C*H$_3$)$_3$), 27.3 (s, C(*C*H$_3$)$_3$), 22.1 (s, C-3′), 22.0 (s, C-4″-*C*H$_3$). ESI-HRMS: *m/z* calcd. for $C_{37}H_{59}N_6O_{15}$ $[M+H]^+$ 827.4038, found: 827.4023.

6′-*N*-(*p*-Nitrobenzyloxycarbonyl)-3″-*N*-(phenylazo)-2′,3-di-*N*-(*tert*-butyloxycarbonyl)sisomicin (14). A stirred solution of **13** (675 mg, 0.82 mmol) in acetonitrile:H_2O (1:1, 14 mL) was treated with K_2CO_3 (1.13 g, 8.17 mmol) and cooled to 0 ^oC in an ice bath. A solution of phenyldiazonium tetrafluoroborate (173 mg, 0.90 mmol) in acetonitrile (7 mL) was added by syringe pump over 1 h at 0 ^oC, after which the reaction was complete. The reaction mixture was diluted with ethyl acetate and washed with water, and brine. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by chromatography over silica gel (6% to 10% of methanol in dichloromethane) to give **14** (0.67 g, 88%) as a buff gum. *Rf* = 0.3 (10% MeOH in CH_2Cl_2); $[\alpha]_D^{25}$ = +127.6 (*c* = 0.01, MeOH); ^{1}H NMR (600 MHz, CD_3OD): δ 8.22 (d, *J* = 8.4 Hz, 2H, PNZ-*m*Hs), 7.58 (d, *J* = 8.1 Hz, 2H, PNZ-*o*Hs), 7.38 (d, *J* = 7.7 Hz, 2H, Ph-*o*Hs), 7.28 (t, *J* = 7.7 Hz, 2H, Ph-*m*Hs), 7.10 (t, *J* = 7.2 Hz, 1H, Ph-*p*H), 5.41 (s, 1H, H-1′), 5.26 – 5.17 (m, 2H, C*H*$_2$-4-$NO_2C_6H_4$), 5.16 (d, *J* = 3.7 Hz, 1H, H-1″), 4.73 (br s, 1H, H-4′), 4.44 (dd, *J* = 11.0 Hz, 3.7 Hz, 1H, H-2″), 4.21 (d, *J* = 11.0 Hz, 1H, H-3″), 4.13 (d, *J* =11.7 Hz, 1H, H-5″), 3.88 (d, *J* = 15.4 Hz, 1H, H-6′), 3.71 (t, *J* = 8.1 Hz, 1H, H-2′), 3.65 (d, *J* = 15.4 Hz, 1H, H-6′), 3.58 – 3.56 (m, 1H, H-5), 3.50 – 3.41 (m, 2H, H-3, H-4), 3.38 (d, *J* =11.7 Hz, 1H, H-5″), 3.31 (s, 3H, NC*H*$_3$), 3.17 (t, *J* = 9.4 Hz, 1H, H-6), 2.88 (t, *J* = 8.4 Hz, 1H, H-1), 2.10 – 2.06 (m, 2H, H-3′), 1.99 (br d, *J* =13.2, 1H, H-2), 1.42 (s, 9H, C(C*H*$_3$)$_3$), 1.41 (s, 9H, C(C*H*$_3$)$_3$), 1.31 – 1.25(m, 1H, H-2), 1.09 (s, 3H, 4″-C*H*$_3$); ^{13}C NMR (151 MHz, CD_3OD): δ 157.1, 156.5, 156.4 (s, C=O), 151.0, 147.4 (s, arom.), 146.5 (s, C-5′), 144.7, 128.5, 128.3, 127.7, 127.6, 124.9, 123.2, 120.2 (s, arom.), 101.5 (s, C-1″), 97.1 (s, C-1′), 95.9 (s, C-4′), 88.4 (s, C-6), 80.2 (s, C-4), 78.9 (s, *C*(CH$_3$)$_3$), 75.6 (s, C-5), 72.9 (s, C-4″), 69.1 (s, C-5″), 68.0 (s, C-3″), 65.6 (s, C-2″), 64.7 (s, *C*H$_2$-4-$NO_2C_6H_4$), 50.4 (s, C-1), 49.3 (s, C-3), 46.9 (s, C-2′), 42.6 (s, C-6′), 35.4 (s, C-2), 32.6 (s, N*C*H$_3$), 27.4 (s, C(*C*H$_3$)$_3$), 27.2 (s, C(*C*H$_3$)$_3$), 22.1 (s, C-3′), 21.2 (s, C-4″-*C*H$_3$). ESI-HRMS: *m/z* calcd. for $C_{43}H_{63}N_8O_{15}$ $[M+H]^+$ 931.4413, found: 931.4388.

1-*N*-(*tert*-Butyloxycarbonylamino-2(*S*)-hydroxybutyryl)-6′-*N*-(*p*-nitrobenzyloxycarbonyl)-3″-*N*-(phenylazo)-2′,3-di-*N*-(*tert*-butyloxycarbonyl)sisomicin (15). A solution of *N*-Boc-4-amino-2(*S*)-hydroxy-butyric acid[4] (15.3 mg, 0.069 mmol), *N*-hydroxy-5-norbornene-2,3-dicarboximide[5] (12.5 mg, 0.069 mmol), ethyldiisopropylamine (18.0 mg, 0.14 mmol) and EDCI

(13.4 mg, 0.069 mmol) in DMF (0.2 mL) was stirred for 0.5 h before **14** (50 mg, 0.053 mmol) in DMF (2 mL) was added. Stirring was continued for 24 h at room temperature before the reaction mixture was cooled to 0 °C, quenched with saturated aqueous $NaHCO_3$ (0.5 mL), and extracted with ethyl acetate. The organic layer was washed with saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated to dryness. The residue was purified via silica gel chromatography eluting with 25% to 40% acetone in hexanes to give **15** (54 mg, 89%) as a white foam. *Rf* = 0.3 (50% acetone in hexanes); $[\alpha]_D^{25}$ = +83.0 (*c* = 0.01, MeOH); ^{1}H NMR (600 MHz, CD_3OD): δ 8.23 (d, *J* = 8.4 Hz, 2H, PNZ-*m*Hs), 7.59 (d, *J* = 8.4 Hz, 2H, PNZ-*o*Hs), 7.38 (d, *J* = 7.7 Hz, 2H, Ph-*o*Hs), 7.28 (t, *J* = 7.9 Hz, 2H, Ph-*m*Hs), 7.09 (t, *J* = 7.2 Hz, 1H, Ph-*p*H), 5.40 (s, 1H, H-1′), 5.27 – 5.18 (m, 3H, C*H*$_2$-4-$NO_2C_6H_4$, H-1″), 4.74 (br s, 1H, H-4′), 4.34 (dd, *J* = 11.2 Hz, 3.5 Hz, 1H, H-2″), 4.19 (m, 2H, H-3″, H-5″), 3.96 (dd, *J* = 8.4 Hz, 3.7 Hz, 1H, C*H*(OH)CH_2CH_2), 3.88 – 3.84 (m, 2H, H-1, H-6′), 3.71 (t, *J* = 8.1 Hz, 1H, H-2′), 3.68 – 3.60 (m, 3H, , H-5, H-6, H-6′), 3.51 – 3.47 (m, 2H, H-3, H-4), 3.33 (d, *J* = 12.1 Hz, 1H, H-5″), 3.29 (s, 3H, NC*H*$_3$), 3.13-3.00 (m, 2H, CH(OH)CH_2C*H*$_2$), 2.11 – 2.00 (m, 3H, H-3′, H-2), 1.85 – 1.82 (m, 1H, CH(OH)C*H*$_2$$CH_2$), 1.73 – 1.67 (m, 1H, CH(OH)C*H*$_2$$CH_2$), 1.54 – 1.51 (m, 1H, H-2), 1.42 – 1.40 (m, 27H, C(C*H*$_3$)$_3$), 1.08 (s, 3H, 4″-C*H*$_3$); ^{13}C NMR (151 MHz, CD_3OD): δ 175.7, 157.2, 157.1, 156.4 (s, C=O), 151.0, 147.5 (s, arom.), 146.5 (s, C-5′), 144.7, 128.3, 127.6, 124.9, 123.2, 120.2 (s, arom.), 95.6 (s, C-1″), 97.2 (s, C-1′), 96.0 (s, C-4′), 81.5 (s, C-6), 79.8 (s, C-4), 79.0, 78.6 (s, *C*$(CH_3)_3$), 75.9 (s, C-5), 73.0 (s, C-4″), 69.4 (s, *C*H(OH)CH_2CH_2), 69.1 (s, C-5″), 68.6 (s, C-3″), 65.4 (s, C-2″), 64.7 (s, *C*H_2-4-$NO_2C_6H_4$), 49.2 (s, C-3), 49.0 (s, C-1), 47.0 (s, C-2′), 42.6 (s, C-6′), 36.3 (s, CH(OH)CH_2*C*H_2), 33.9 (s, CH(OH)*C*H_2CH_2), 33.7 (s, C-2), 32.8 (s, N*C*H_3), 27.4 (s, C(*C*H_3)$_3$), 27.2 (s, C(*C*H_3)$_3$), 22.1 (s, C-3′), 21.2 (s, C-4″-*C*H_3). ESI-HRMS: *m/z* calcd. for $C_{52}H_{77}N_9NaO_{19}$ $[M+Na]^+$ 1154.5233, found: 1154.5212.

1-*N*-(*tert*-Butyloxycarbonylamino-2(*S*)-hydroxybutyryl)-3″-*N*-(phenylazo)-2′,3-di-*N*-(*tert*-butyloxycarbonyl)sisomicin (16). A stirred solution of **15** (50 mg, 0.08 mmol) in 1,4-dioxane (1 mL) was treated with 1 N aqueous NaOH (1 mL) and heated to 40 ^oC for 14 h. The reaction mixture was diluted with H_2O (2 mL) and extracted with ethyl acetate. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography over silica gel (6% to 15% of ammonical methanol in dichloromethane) to give **16** (38 mg, 70%) as a white solid. *Rf* = 0.5 (20% ammonical MeOH in CH_2Cl_2); $[\alpha]_D^{25}$ = +116.5 (*c* = 0.01, MeOH); ^{1}H NMR (600 MHz, CD_3OD):

δ 7.38 (d, *J* = 7.7 Hz, 2H, Ph-*o*Hs), 7.28 (t, *J* = 7.9 Hz, 2H, Ph-*m*Hs), 7.10 (t, *J* = 7.2 Hz, 1H, Ph-*p*H), 5.44 (s, 1H, H-1′), 5.20 (d, *J* = 3.3 Hz, H-1″), 4.72 (br s, 1H, H-4′), 4.35 (dd, *J* = 11.4 Hz, 3.7 Hz, 1H, H-2″), 4.23 – 4.19 (m, 2H, H-3″, H-5″), 3.96 (dd, *J* = 8.4 Hz, 3.7 Hz, 1H, C*H*(OH)CH_2CH_2), 3.94 – 3.86 (m, 1H, H-1), 3.75 – 3.69 (m, 2H, H-6, H-2′), 3.65 – 3.60 (m, 1H, H-5), 3.55 (t, 1H, *J* = 9.4 Hz, H-4), 3.51 – 3.45 (m, 1H, H-3), 3.33 (d, *J* =12.1 Hz, 1H, H-5″), 3.29 (s, 3H, NCH_3), 3.23 – 3.15 (m, 2H, H-6′), 3.13 – 3.02 (m, 2H, CH(OH)CH_2CH_2), 2.14 – 2.00 (m, 3H, H-3′, H-2), 1.88 – 1.82 (m, 1H, CH(OH)CH_2CH_2), 1.73 – 1.67 (m, 1H, CH(OH)CH_2CH_2), 1.57 – 1.51 (m, 1H, H-2), 1.42 – 1.40 (m, 27H, C(CH_3)$_3$), 1.08 (s, 3H, 4″-CH_3); ^{13}C NMR (151 MHz, CD_3OD): δ 175.7, 157.1, 156.4, 156.3 (s, C=O), 151.0 (s, arom.), 149.4 (s, C-5′), 128.3, 124.9, 120.2 (s, arom.), 95.6 (s, C-1″), 97.3 (s, C-1′), 95.2 (s, C-4′), 81.5 (s, C-6), 80.1 (s, C-4), 78.9, 78.6 (s, *C*(CH_3)$_3$), 75.9 (s, C-5), 73.0 (s, C-4″), 69.4 (s, *C*H(OH)CH_2CH_2), 69.1 (s, C-5″), 68.6 (s, C-3″), 65.4 (s, C-2″), 49.2 (s, C-3), 49.0 (s, C-1), 47.0 (s, C-2′), 43.4 (s, C-6′), 36.4 (s, CH(OH)CH_2*C*H_2), 33.9 (s, CH(OH)*C*H_2CH_2), 33.7 (s, C-2), 32.7 (s, N*C*H_3), 27.43 (s, C(*C*H_3)$_3$), 27.42 (s, C(*C*H_3)$_3$), 22.1 (s, C-3′), 21.2 (s, C-4″-*C*H_3). ESI-HRMS: *m/z* calcd. for $C_{44}H_{72}N_8NaO_{15}$ $[M+Na]^+$ 975.5015, found: 975.4996.

1-*N*-(4-Amino-2(*S*)-hydroxybutyryl)sisomicin (11). A solution of **16** (50 mg, 0.052 mmol) in dichloromethane (1 mL) and the solution was cooled to 0 °C, stirred and treated with trifluoroacetic acid (1 mL). After stirring for 45 min the reaction mixture the reaction mixture was diluted with dry dichloromethane (10 mL) and toluene (10 mL) and concentrated. The crude mixture was diluted with dry dichloromethane (10 mL) and concentrated twice, after which the residue was purified by Sephadex C-25 chromatography eluting with a gradient of 0.1% to 3% ammonia in deionized water. The product-containing fractions were combined, acidified with glacial acetic acid and lyophilized to give **11** as white foam (24 mg, 54%). $[\alpha]_D^{25}$ = +34.5 (*c* = 0.01, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 5.43 (s, 1H, H-1′), 5.02 (t, *J* = 3.5 Hz, H, H-4′), 4.96 (d, *J* = 4.0 Hz, 1H, H-1″), 4.08 (dd , *J* = 9.2 Hz, 3.7 Hz, 1H, C*H*(OH)CH_2CH_2), 3.98 (d , *J* = 12.8 Hz, 1H, H-5″), 3.94 – 3.90 (m, 1H, H-1), 3.84 (dd, *J* = 11.0 Hz, 3.9 Hz, 1H, H-2″), 3.74 (t, *J* = 9.7 Hz, 1H, H-4), 3.70 (t, *J* = 5.1 Hz, 1H, H-2′), 3.64 (t, *J* = 9.7 Hz, 1H, H-6), 3.57 (t, *J* = 9.7 Hz, 1H, H-5), 3.49 (s, 2H, H-6′), 3.28 – 3.23 (m , 1H, H-3), 3.21 (d, *J* = 12.8 Hz, 1H, H-5″), 3.11 (d, *J* = 11.0 Hz, 1H, H-3″), 2.98 (t, *J* =7.3 Hz, 2H, CH(OH)CH_2CH_2), 2.71 (s, 3H, NCH_3), 2.52 – 2.46 (m, 1H, H-3′), 2.23 – 2.17 (m, 1H, H-3′), 2.03 – 1.95 (m, 2H, H-2, CH(OH)CH_2CH_2), 1.82 – 1.75 (m, 1H, CH(OH)CH_2CH_2), 1.63-1.57 (m, 1H, H-2), 1.14 (s, 3H, 4″-CH_3); ^{13}C NMR (151

MHz, D_2O): δ 181.2 (s, CH_3*C*OOH), 175.4 (s, NHCO), 143.3 (s, C-5′), 100.3 (s, C-4′), 98.1 (s, C-1″), 97.0 (s, C-1′), 80.0 (s, C-4), 78.8 (s, C-6), 73.8 (s, C-5), 69.8 (s, C-4″), 69.5 (s, *C*H(OH)CH_2CH_2), 66.8 (s, C-5″), 66.0 (s, C-2″), 64.2 (s, C-3″), 48.8 (s, C-1), 48.4 (s, C-3), 46.0 (s, C-2′), 40.5 (s, C-6′), 36.8 (s, CH(OH)CH_2*C*H_2), 34.9 (s, N*C*H_3), 30.7 (s, CH(OH)*C*H_2CH_2), 30.4 (s, C-2), 23.2 (s, *C*H_3COOH), 23.0 (s, C-3′), 20.8 (s, 4″-*C*H_3). ESI-HRMS: *m/z* calcd. for $C_{23}H_{45}N_6O_9$ $[M+H]^+$ 549.3248, found: 549.3234.

6′-*N*-(2-*tert*-Butyldimethylsiloxyethyl)-1-*N*-(*tert*-butyloxycarbonylamino-2(*S*)-hydroxybutyryl)-3″-*N*-(phenylazo)-2′,3-di-*N*-(*tert*-butyloxycarbonyl)sisomicin (17). *tert*-Butyldimethylsilyloxyacetaldehyde (20 μL, 0.11 mmol) and ethyldiisopropylamine (27.1 mg, 0.21 mmol) were added to a stirred solution of **16** (100 mg, 0.11 mmol) in dry THF (2.5 mL). The reaction mixture was stirred for 10 min before sodium triacetoxyborohydride (26.7 mg, 0.13 mmol) was added, after which stirring was continued for 24 h at room temperature. The solvent was evaporated under vacuum and the residue was purified by column chromatography on silica gel eluting with 4% to 15% ammonical methanol in dichloromethane to give **17** (59mg, 51%) as a white foam). *Rf* = 0.5 (10% ammonical MeOH in CH_2Cl_2); $[\alpha]_D^{25}$ = +95.3 (*c* = 0.01, MeOH); ^{1}H NMR (600 MHz, CD_3OD): δ 7.38 (d, *J* = 7.7 Hz, 2H, Ph-*o*Hs), 7.28 (t, *J* = 7.7 Hz, 2H, Ph-*m*Hs), 7.10 (t, *J* = 7.3 Hz, 1H, Ph-*p*H), 5.43 (s, 1H, H-1′), 5.20 (d, *J* = 3.3 Hz, H-1″), 4.76 (br s, 1H, H-4′), 4.34 (dd, *J* = 11.4 Hz, 3.3 Hz, 1H, H-2″), 4.22 – 4.19 (m, 2H, H-3″, H-5″), 3.96 (dd, *J* = 8.4 Hz, 3.7 Hz, 1H, C*H*(OH)CH_2CH_2), 3.88 – 3.83 (m, 1H, H-1), 3.77 – 3.67 (m, 4H, $NHCH_2CH_2O$, H-6, H-2′), 3.65 – 3.60 (m, 1H, H-5), 3.57 (t, 1H, *J* = 8.4, H-4), 3.51 – 3.46 (m, 1H, H-3), 3.33 (d, *J* =12.1 Hz, 1H, H-5″), 3.31 – 3.30 (m, 1H, H-6′), 3.29 (s, 3H, N*C*H_3), 3.16 (d, *J* =13.6 Hz, 1H, H-6′), 3.12 – 3.02 (m, 2H, CH(OH)CH_2CH_2), 2.80 – 2.64 (m, 2H, $NHCH_2CH_2O$), 2.14 – 2.00 (m, 3H, H-3′, H-2), 1.88 – 1.82 (m, 1H, CH(OH)CH_2CH_2), 1.73 – 1.67 (m, 1H, CH(OH)CH_2CH_2), 1.59 – 1.51 (m, 1H, H-2), 1.42 – 1.40 (m, 27H, C(CH_3)$_3$), 1.08 (s, 3H, 4″-CH_3), 0.91 (s, 9H, SiC(CH_3)$_3$), 0.09 (s, 6H, Si(CH_3)$_2$); ^{13}C NMR (151 MHz, CD_3OD): δ 175.7, 157.1, 156.5, 156.4 (s, C=O), 151.0 (s, arom.), 146.5 (s, C-5′), 128.3, 124.9, 120.2 (s, arom.), 99.6 (s, C-1″), 97.6 (s, C-4′), 97.2 (s, C-1′), 81.5 (s, C-6), 79.8 (s, C-4), 78.9, 78.8, 78.6 (s, *C*(CH_3)$_3$), 75.9 (s, C-5), 73.0 (s, C-4″), 69.4 (s, *C*H(OH)CH_2CH_2), 69.2 (s, C-5″), 68.6 (s, C-3″), 65.4 (s, C-2″), 61.6 (s, $NHCH_2CH_2O$), 50.7 (s, C-6′), 49.9 (s, $NHCH_2CH_2O$), 49.3 (s, C-3), 49.0 (s, C-1), 47.1 (s, C-2′), 36.4 (s, CH(OH)CH_2CH_2), 33.93 (s, CH(OH)CH_2CH_2), 33.87 (s, C-2), 32.7 (s, N*C*H_3), 27.5 (s, C(*C*H_3)$_3$), 27.4 (s, C(*C*H_3)$_3$), 25.0(s, SiC(*C*H_3)$_3$), 22.2 (s, C-3′), 21.2

(s, C-4″-*C*H$_3$), 17.8 (s, Si*C*(CH$_3$)$_3$), -6.6(s, Si(*C*H$_3$)$_2$). ESI-HRMS: *m/z* calcd. for $C_{52}H_{91}N_8O_{16}Si$ $[M+H]^+$ 1111.6322, found: 1111.6307.

6′-*N*-(2-Hydroxyethyl)-1-*N*-(4-amino-2(*S*)-hydroxybutyryl)sisomicin Pentaacetate Salt [Plazomicin.5AcOH] (4). A solution of **17** (59 mg, 0.054 mmol) in dichloromethane (1 mL) was cooled to 0 °C with stirring before trifluoroacetic acid (1 mL) was added. The reaction mixture was stirred for 6 h then was diluted with dry dichloromethane (10 mL) and toluene (10 mL) and concentrated. The crude product was diluted with dry dichloromethane (10 mL) and concentrated twice before the residue was purified by Sephadex C-25 chromatography eluting with a gradient of 0.1% to 3% ammonia in deionized water. The product-containing fractions were combined, acidified with glacial acetic acid and lyophilized to give **4** (19 mg, 40%). $[\alpha]_D^{25}$ = +46.5 (*c* = 0.01, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 5.51 (s, 1H, H-1′), 5.16 (t, *J* = 3.5 Hz, H, H-4′), 4.99 (d , *J* = 4.0 Hz, 1H, H-1″), 4.11 (dd , *J* =9.4 Hz, 3.9 Hz, 1H, C*H*(OH)CH$_2$CH$_2$), 4.00 (d , *J* = 12.8 Hz, 1H, H-5″), 3.99-3.93 (m, 1H, H-1), 3.84 (dd, *J* = 11.0 Hz, 4.0 Hz, 1H, H-2″), 3.81 (t, *J* = 9.9 Hz, 1H, H-4), 3.77 (t, *J* = 5.3 Hz, 1H, H-2′), 3.71 (t, *J* = 5.1 Hz, 2H, NHCH$_2$C*H*$_2$O), 3.69 – 3.65 (m, 2H, H-6, H-6′), 3.64 – 3.44 (m , 2H, H-5, H-6′), 3.35 – 3.26 (m , 1H, H-3), 3.24 (d, *J* = 12.8 Hz, 1H, H-5″), 3.15 (d, *J* = 11.0 Hz, 1H, H-3″), 3.09 – 3.06 (m, 2H, NHC*H*$_2$CH$_2$O), 3.01 (t, *J* = 7.2 Hz, 2H, CH(OH)CH$_2$C*H*$_2$), 2.74 (s, 3H, NC*H*$_3$), 2.58 – 2.52 (m, 1H, H-3′), 2.29 – 2.24 (m, 1H, H-3′), 2.07 (dt, *J* = 13.2 Hz, 4.4 Hz, 1H, H-2), 2.04 – 1.98 (m, 1H, CH(OH)C*H*$_2$CH$_2$), 1.84 – 1.79 (m, 1H, CH(OH)C*H*$_2$CH$_2$), 1.64 (q, 1H, *J* = 12.5 Hz, H-2), 1.17 (s, 3H, 4″-C*H*$_3$); ^{13}C NMR (151 MHz, D_2O): δ 181.2 (s, CH$_3$*C*OOH), 175.4 (s, NHCO), 141.7 (s, C-5′), 102.5 (s, C-4′), 98.0 (s, C-1″), 96.9 (s, C-1′), 79.8 (s, C-4), 78.8 (s, C-6), 73.8 (s, C-5), 69.8 (s, C-4″), 69.4 (s, *C*H(OH)CH$_2$CH$_2$), 66.8 (s, C-5″), 65.9 (s, C-2″), 64.2 (s, C-3″), 56.4 (s, NHCH$_2$*C*H$_2$O), 48.8 (s, C-1), 48.31 (s, NH*C*H$_2$CH$_2$O), 48.26 (s, C-3), 47.9 (s, C-6′), 45.9 (s, C-2′), 36.8 (s, CH(OH)CH$_2$*C*H$_2$), 34.9 (s, N*C*H$_3$), 30.7 (s, CH(OH)*C*H$_2$CH$_2$), 30.4 (s, C-2), 23.1 (s, *C*H$_3$COOH), 23.0 (s, C-3′), 20.8 (s, 4″-*C*H$_3$). ESI-HRMS: *m/z* calcd. for $C_{25}H_{49}N_6O_{10}$ $[M+H]^+$ 593.3510, found: 593.3481.

Bacterial strains. Clinical isolates of *E. coli* and *S. aureus* were obtained from the Diagnostic Department, Institute of Medical Microbiology, University of Zurich. MIC values were determined by broth microdilution assays as described.[6]

Recombinant microorganisms. The construction of these strains derived from single rRNA allelic *M. smegmatis* ΔrrnB, has been described previously.[7-8]

Cell-free translation assays. S-30 extracts and purified ribosomes were used for cell-free translation assays as described previously.[6] Firefly luciferase mRNA was used as reporter to monitor translation activity. Luminescence was measured using a luminometer Flx800 (Bio-Tek Instruments).

Cochlear Explants. Drugs were screened for toxicity to hair cells in cochlear explants from CBA/J mice on postnatal day 2 to 3.[9] The dissected tissue was placed on a collagen-coated incubation dish in 1 mL of serum-free Basal Medium Eagle plus serum-free supplement (Invitrogen), 1% BSA, 2 mM glutamine, and 5 mg/ mL glucose. The explants were incubated (37 °C, 5% CO_2) for 4 h, and an additional 1 mL of culture medium was added to submerse the explants. After 24 h the medium was exchanged for new medium containing the drugs, and the explants were incubated for an additional 24 or 72 h. Cultures were fixed overnight in 4% (vol/vol) paraformaldehyde at 4 °C, and then permeabilized for 30 min with 3% (vol/vol) Triton X-100 in PBS, and washed three times with PBS. Following incubation at room temperature for 40 min with rhodamine-phalloidin (Molecular Probes), hair cell presence was determined by light microscopy of the phalloidin-stained stereociliary bundles and circumferential F-actin rings on the cuticular plate.

References

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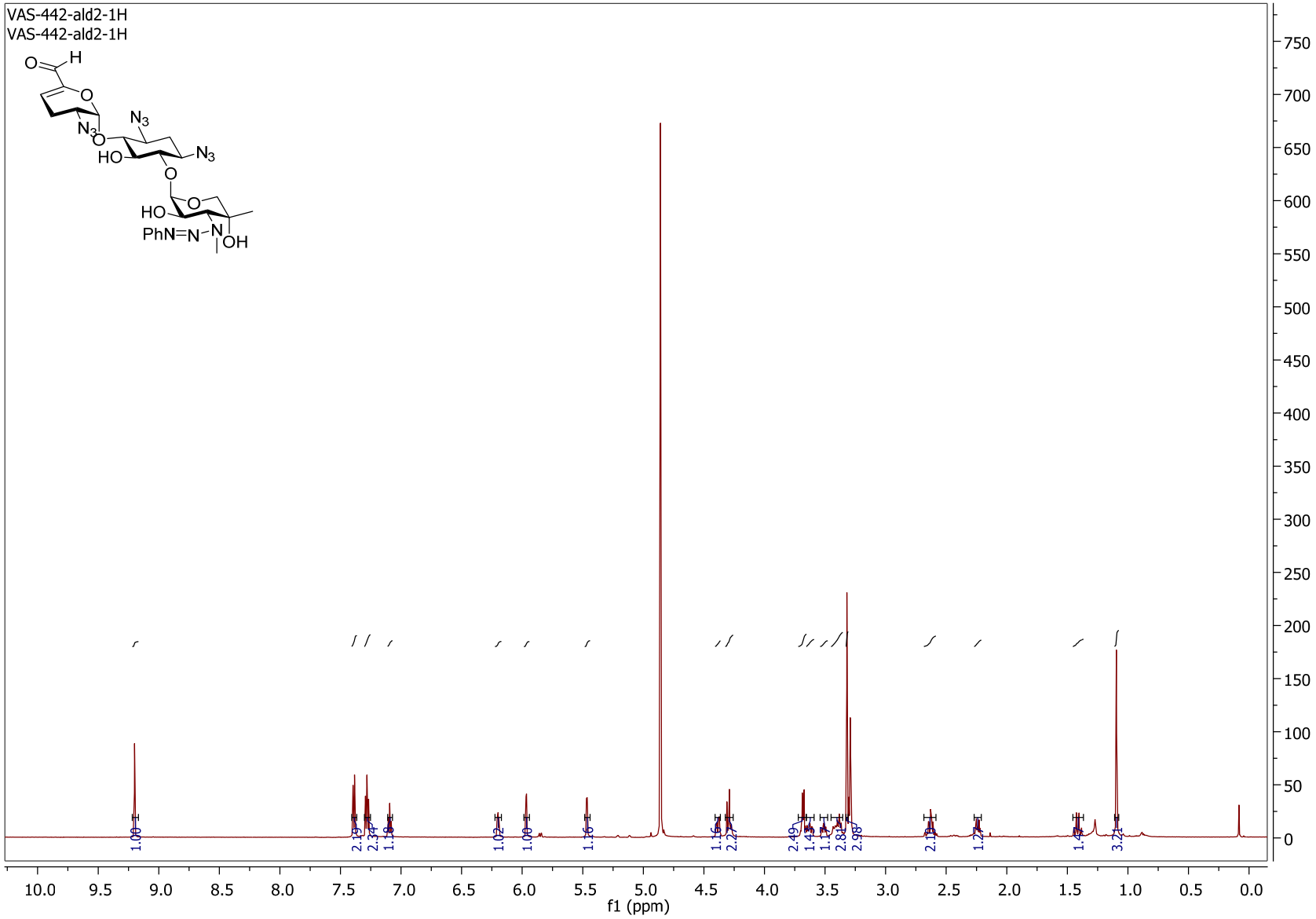
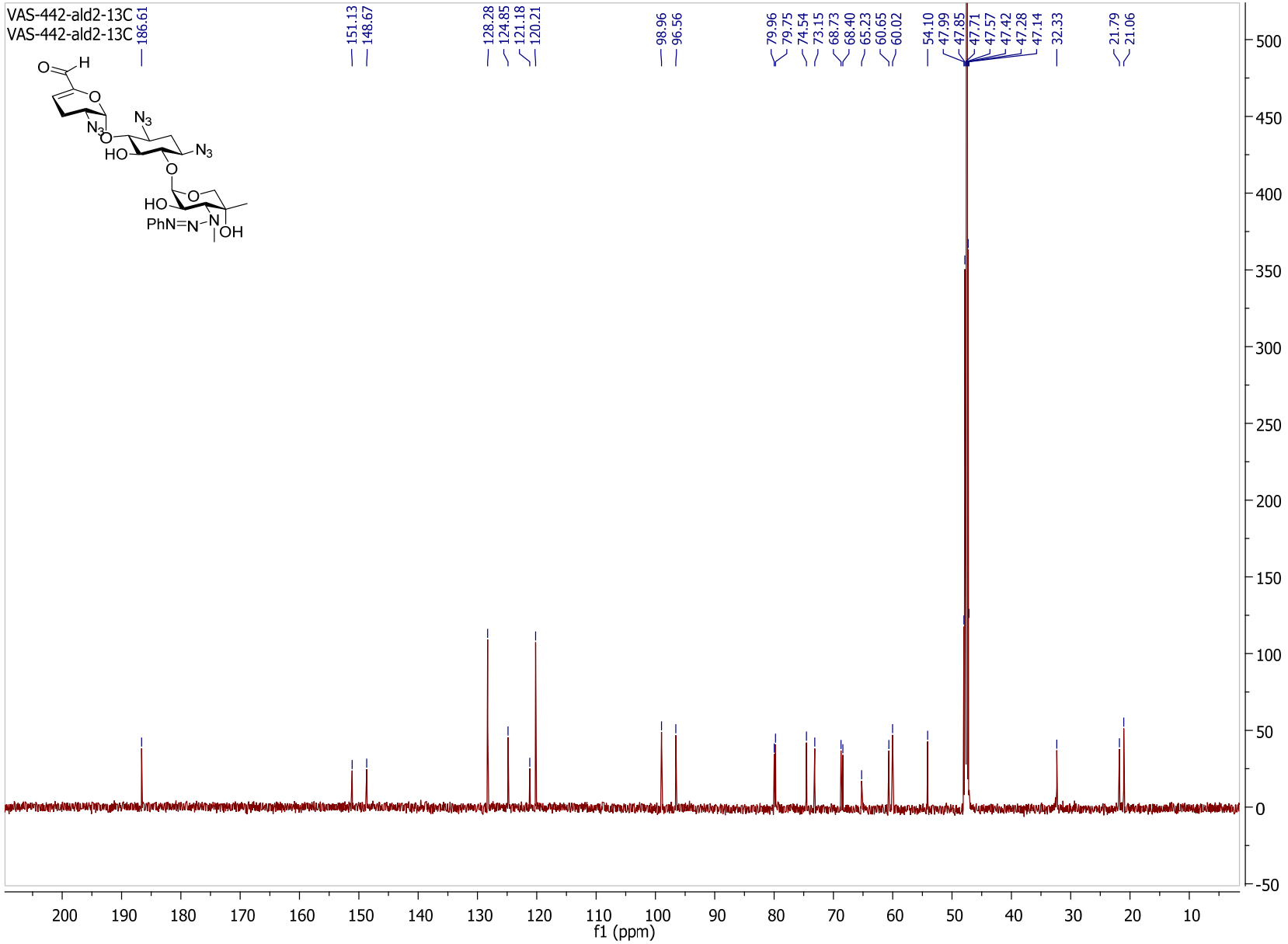
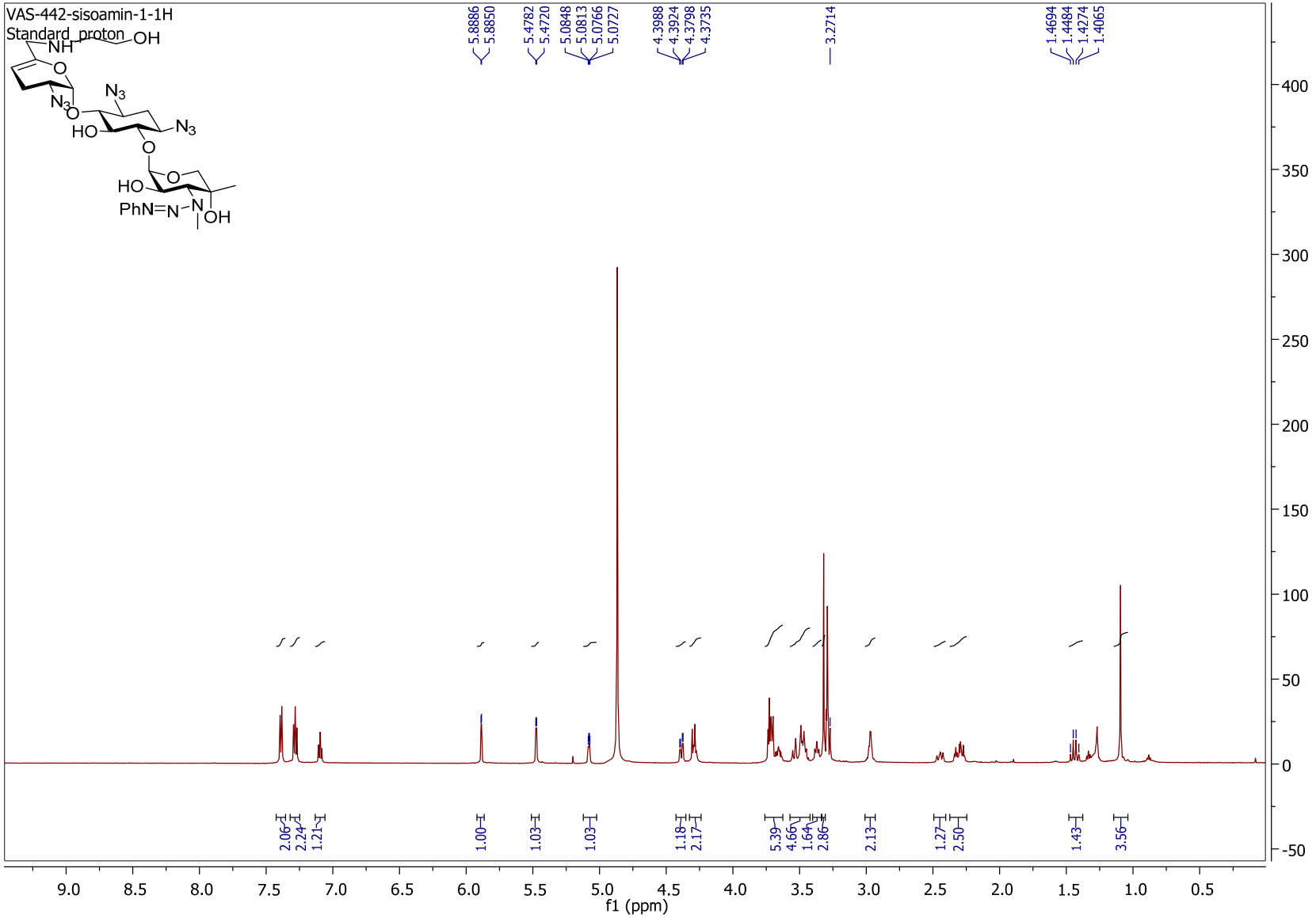
^{1}H NMR (600 MHz,CD_3OD) Spectrum of 1,3,2′-Trideamino-1,3,2′-triazido-3′′-*N*-(phenylazo)sisomicin-6′-carboxaldehyde (8)



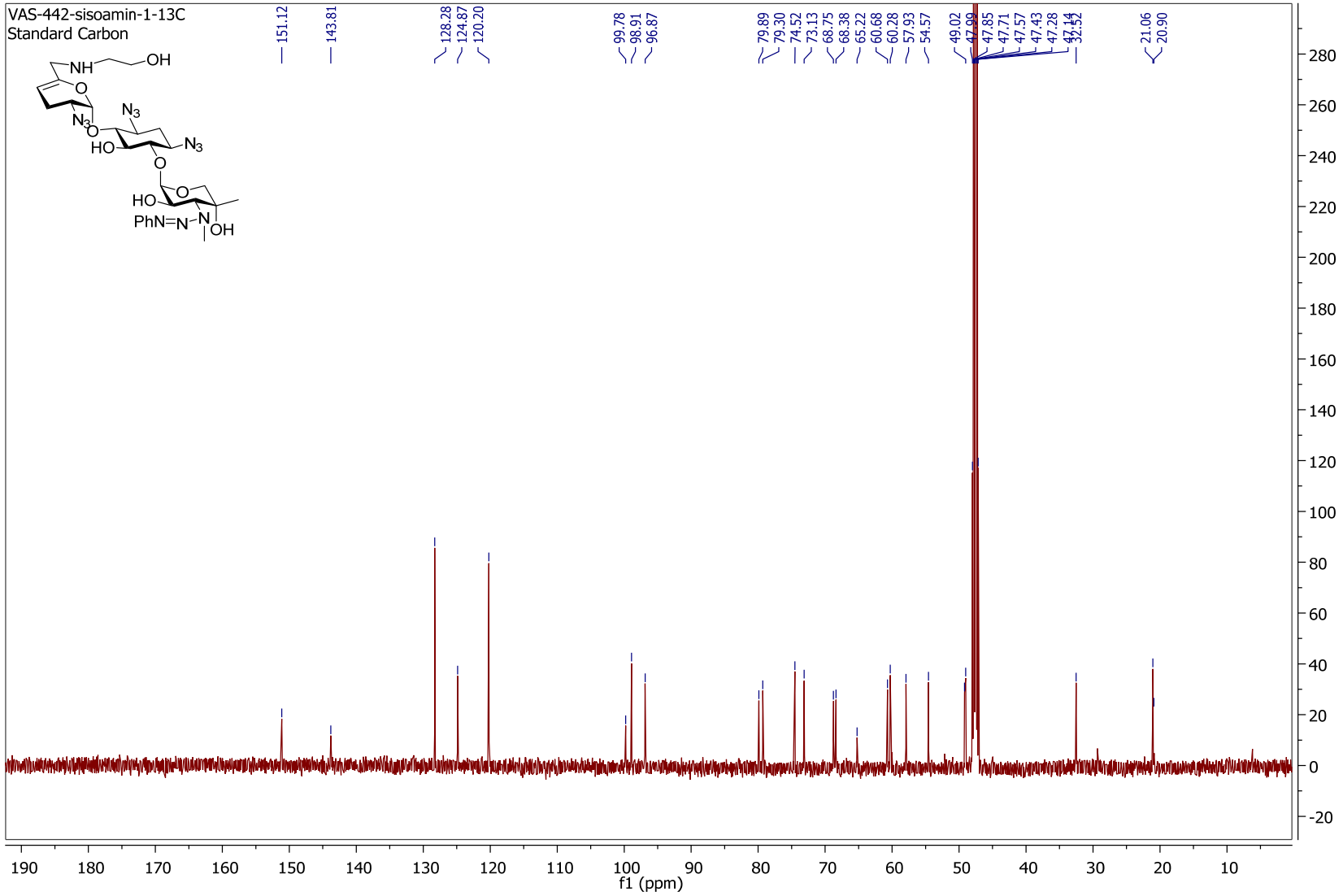
^{13}C NMR (151 MHz,CD_3OD) Spectrum of 1,3,2′-Trideamino-1,3,2′-triazido-3′′-*N*-(phenylazo)sisomicin-6′-carboxaldehyde (8)



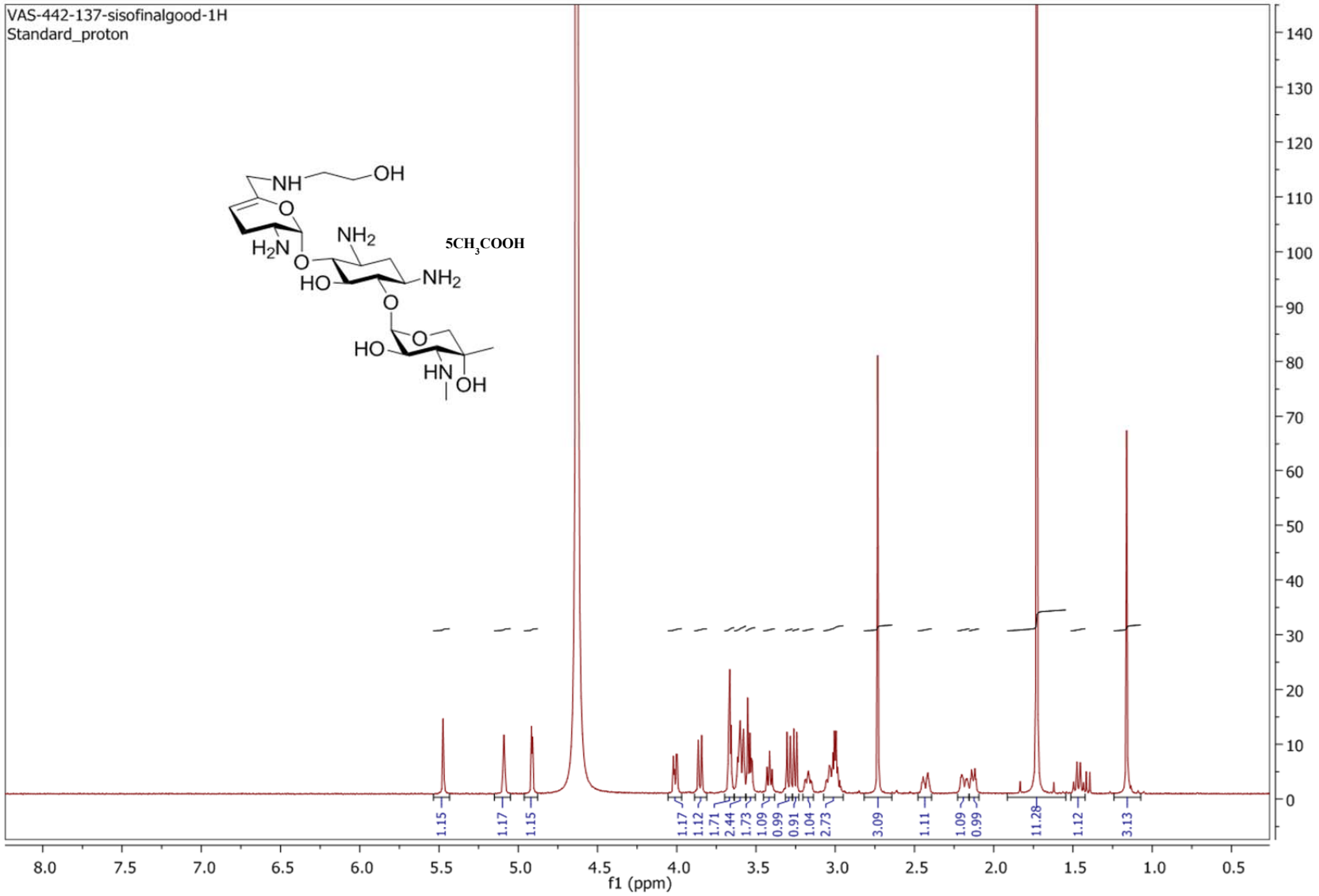
^{1}H NMR (600 MHz, CD_3OD) Spectrum of 6′-*N*-(2-Hydroxyethyl)-1,3,2′-trideamino-1,3,2′-triazido-3″-*N*-(phenylazo)sisomicin (9)



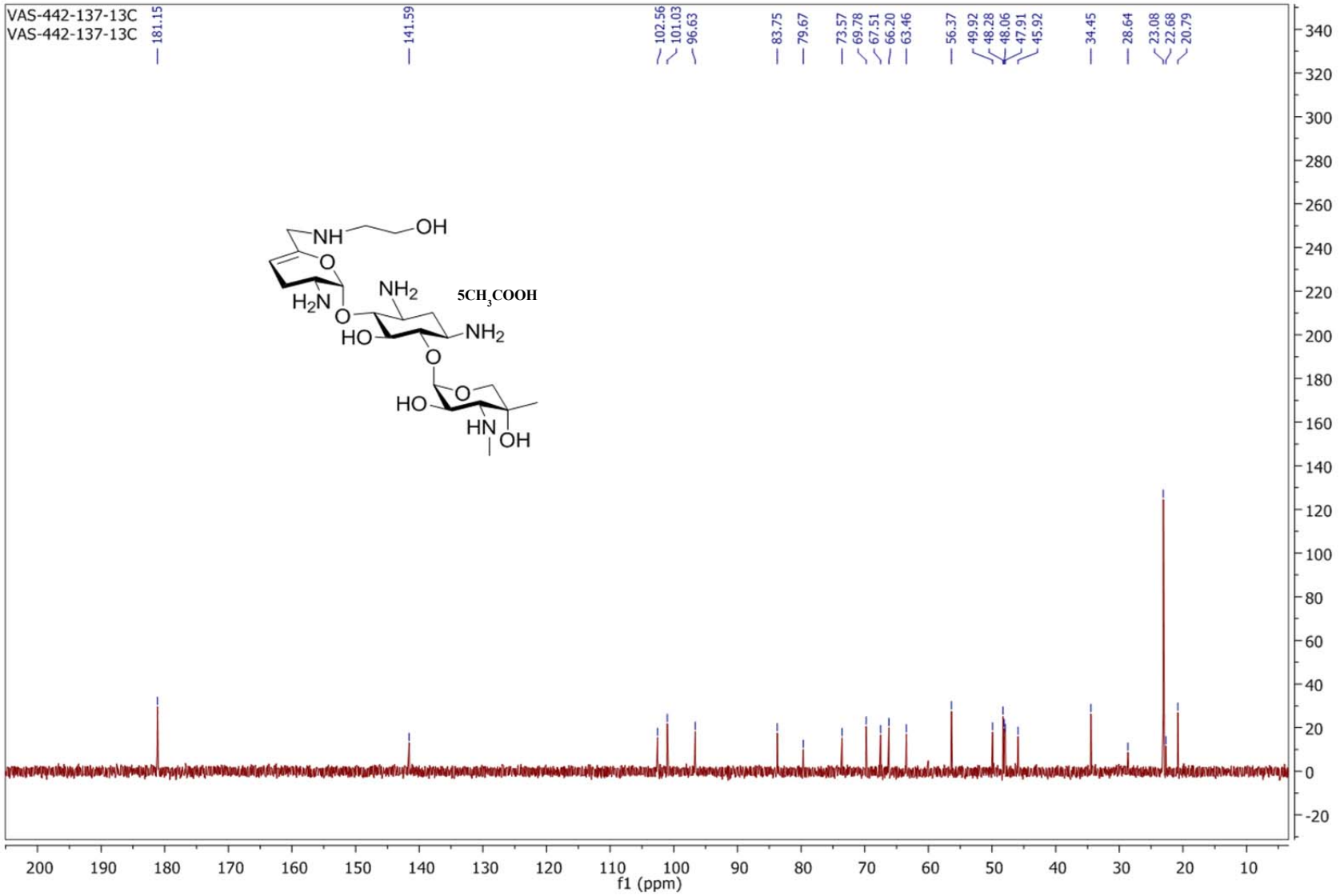
^{13}C NMR (600 MHz,CD_3OD) Spectrum of 6′-*N*-(2-Hydroxyethyl)-1,3,2′-trideamino-1,3,2′-triazido-3′′-*N*-(phenylazo)sisomicin (9)



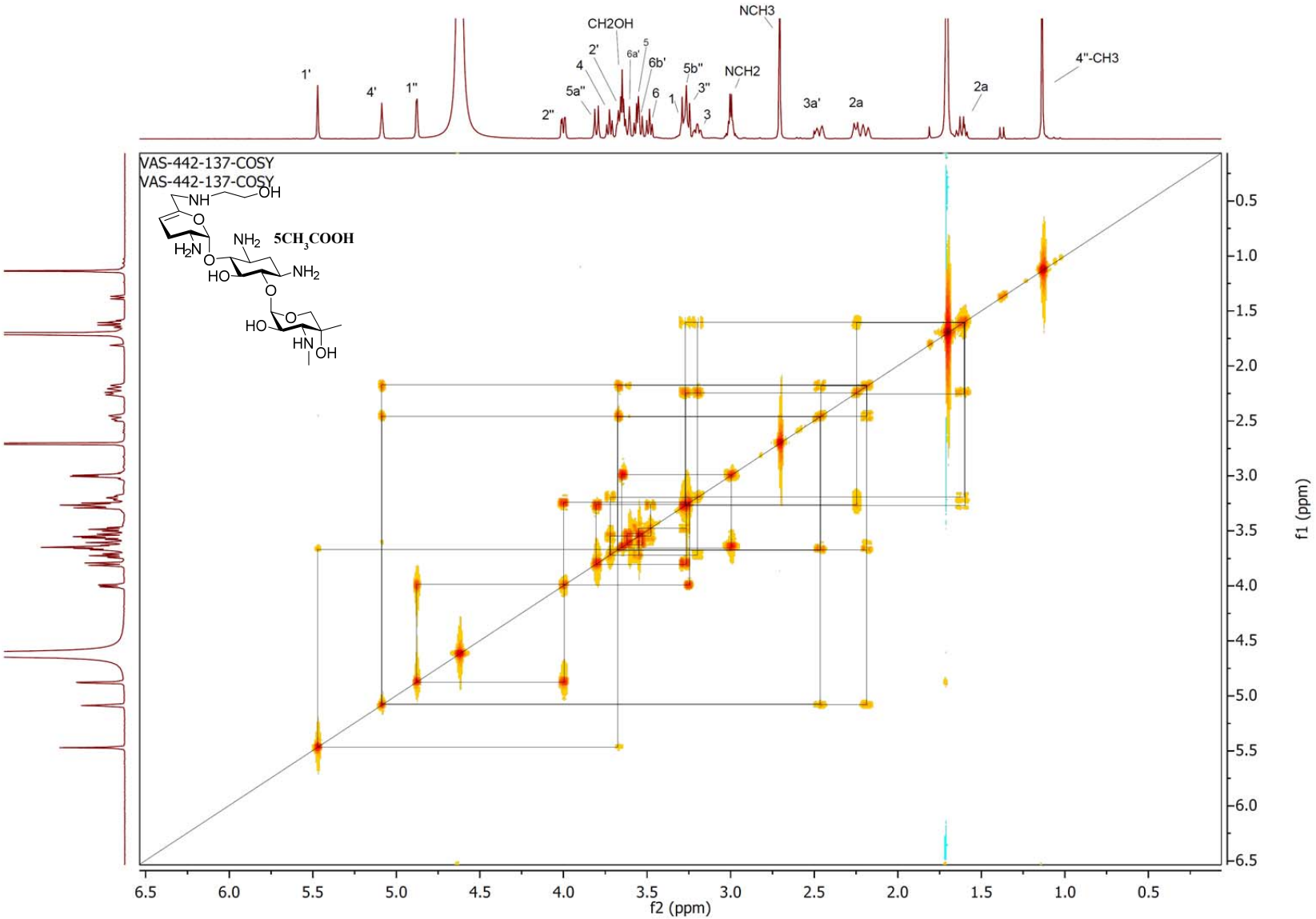
^{1}H NMR (600 MHz, D_2O) Spectrum of 6′-*N*-(2-Hydroxyethyl)sisomicin Pentaacetate Salt (10)



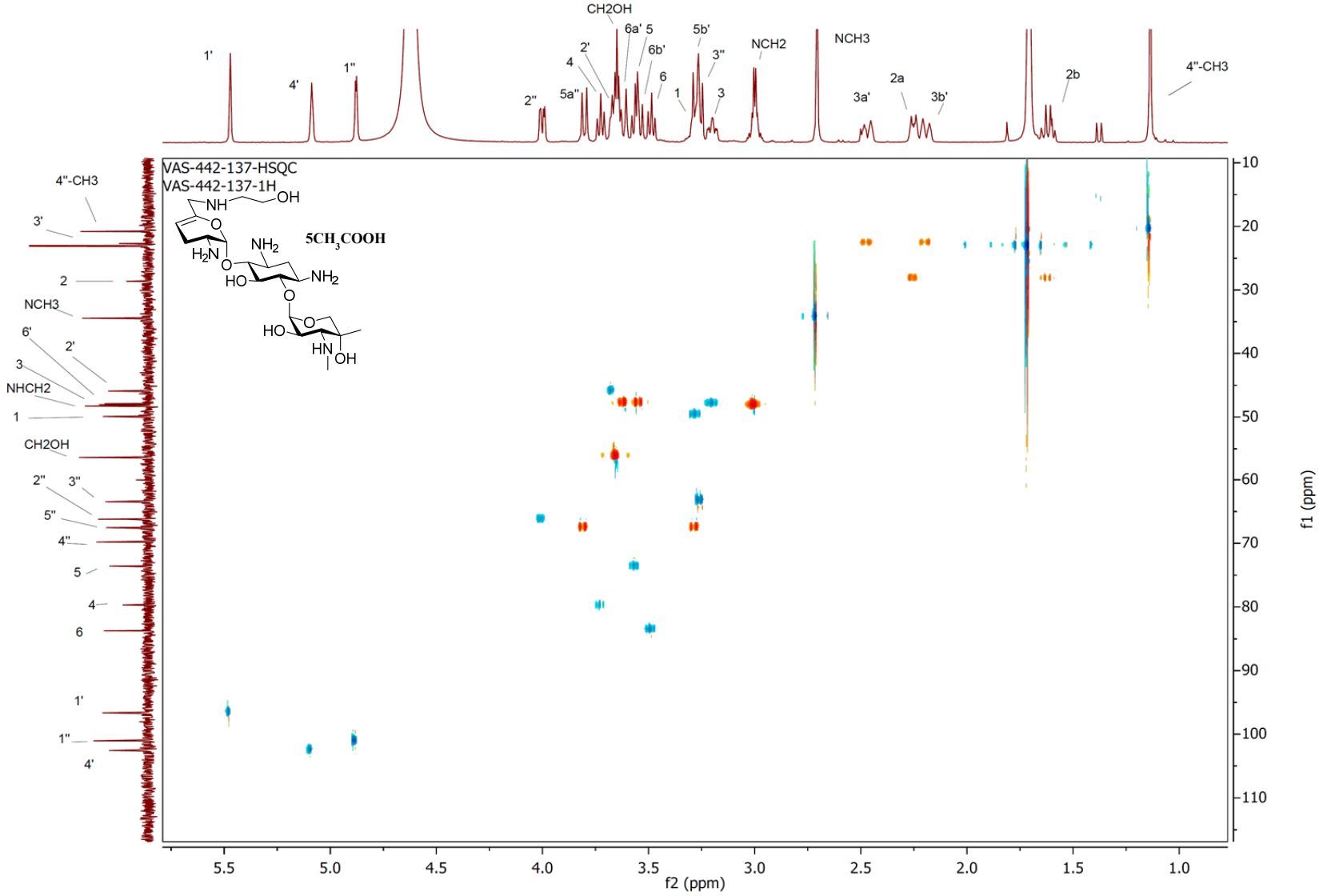
^{13}C NMR (151 MHz, D_2O) Spectrum of 6′-*N*-(2-Hydroxyethyl)sisomicin Pentaacetate Salt (10)



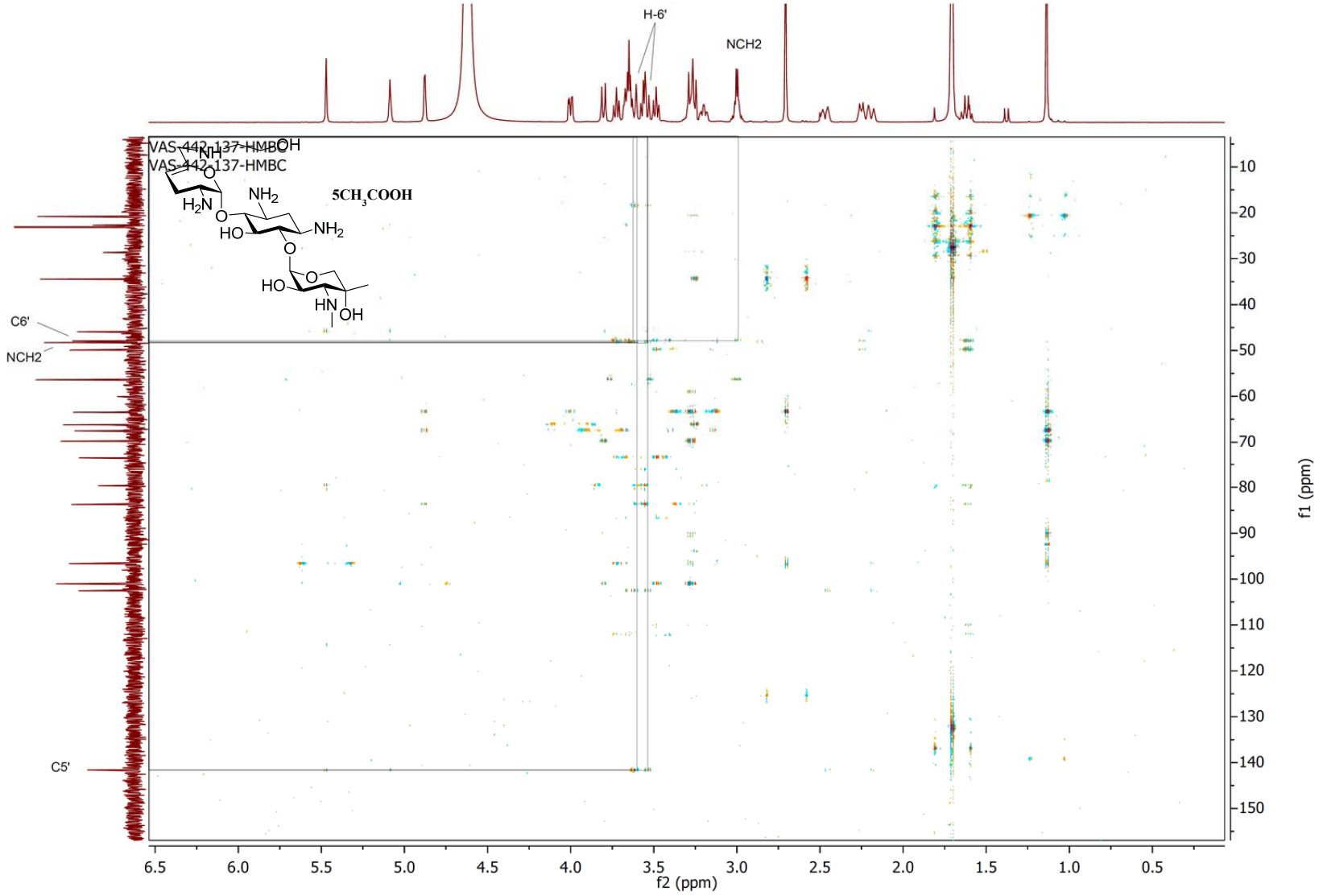
COSY NMR (600 MHz, D_2O) Spectrum of 6′-*N*-(2-Hydroxyethyl)sisomicin Pentaacetate Salt (10)



HSQC NMR (600 MHz, D_2O) Spectrum of 6′-*N*-(2-Hydroxyethyl)sisomicin Pentaacetate Salt (10)



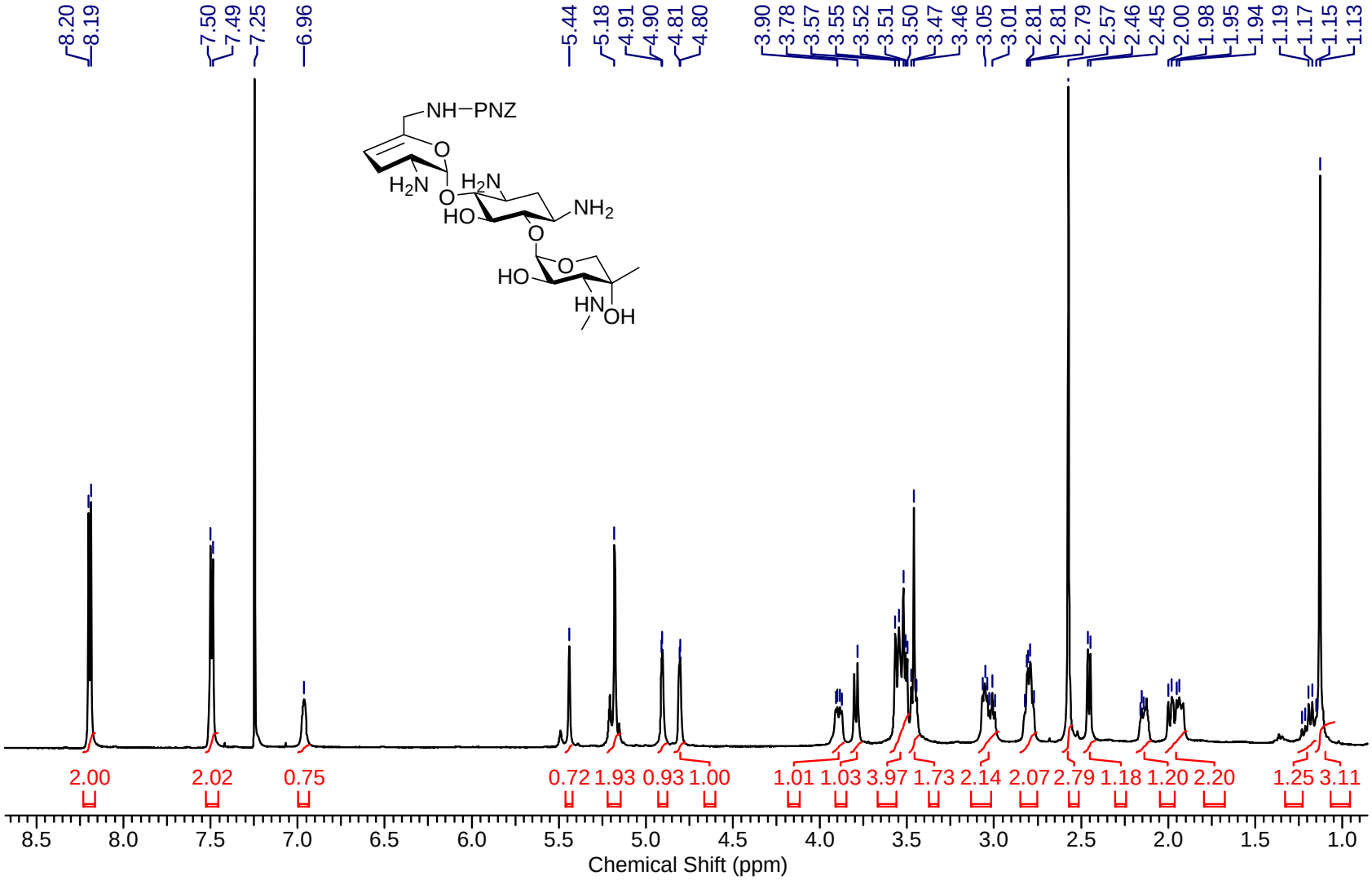
HMBC NMR (600 MHz, D_2O) Spectrum of 6′-*N*-(2-Hydroxyethyl)sisomicin Pentaacetate Salt (10)



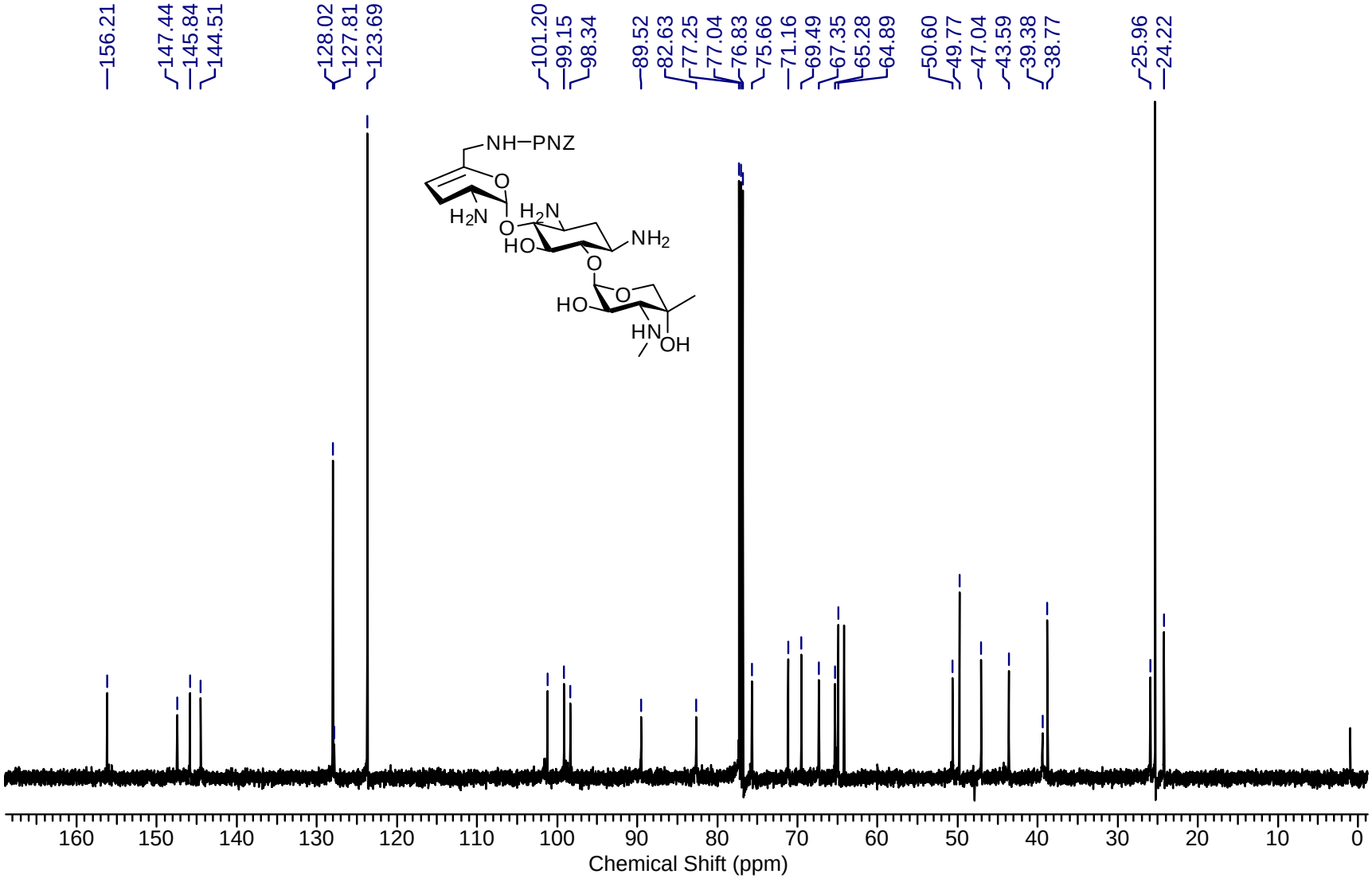
Important 2D NMR correlations of 6′-*N*-(2-Hydroxyethyl)sisomicin Pentaacetate Salt (10)

Signal 1 (δ)	Assignment	Signal 2 (δ)	Assignment	Correlation
5.47 (d)	H-1′	96.6	C-1′	HSQC
5.09 (t)	H-4′	102.6	C-4′	HSQC
4.88 (d)	H-1″	101.0	C-1″	HSQC
3.73 (t)	H-4	96.6	C-1′	HMBC
3.73 (t)	H-4	3.23-3.17 (m)	H-3	COSY
3.62 – 3.52	H-6′	48.3	NH***C***H_2CH_2OH	HMBC
3.05-2.95	NC***H***$_2CH_2OH$	47.9	C-6′	HMBC

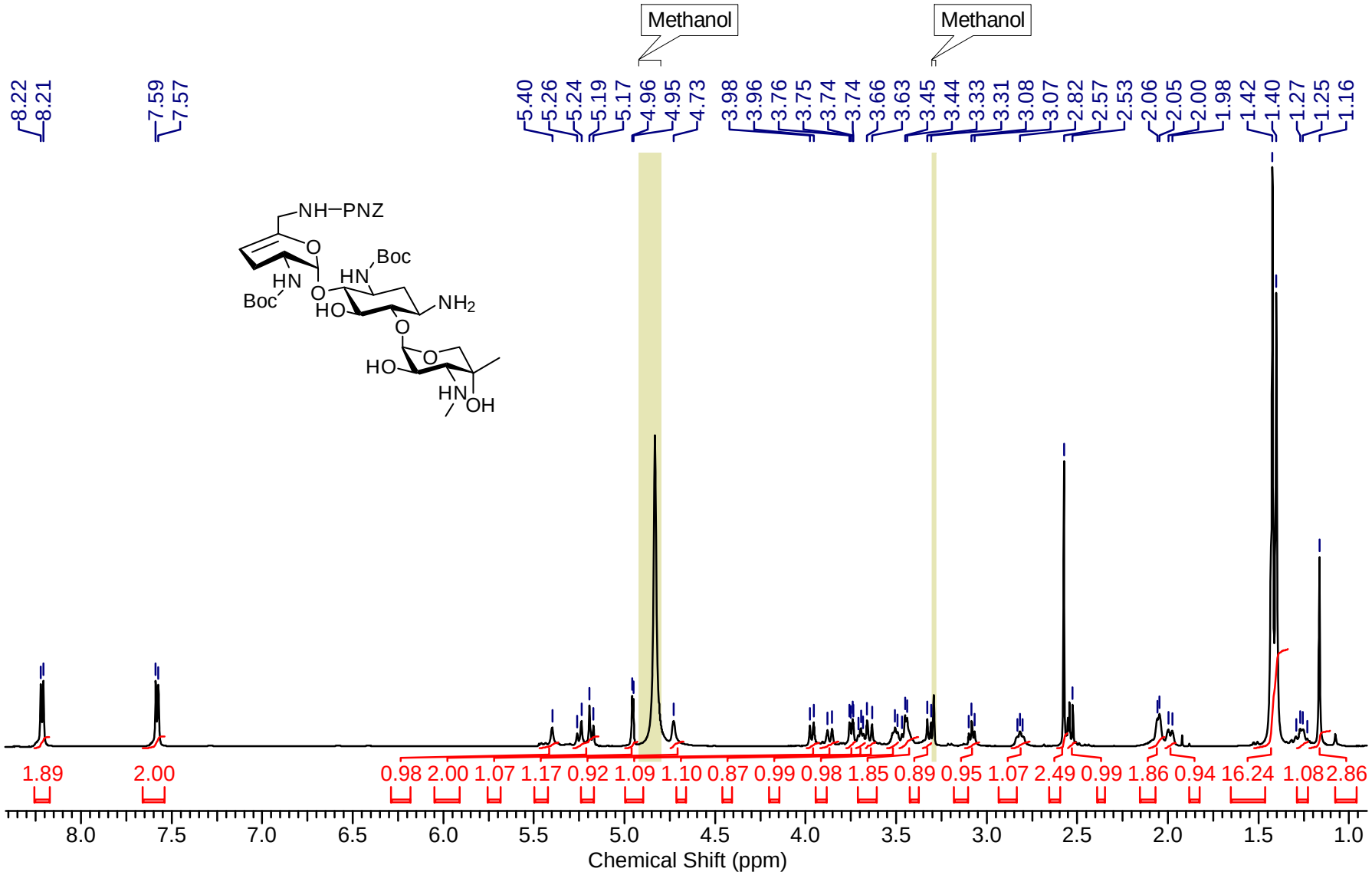
^{1}H NMR (600 MHz,$CDCl_3$) Spectrum of 6′-*N*-(*p*-Nitrobenzyloxycarbonyl)sisomicin (12)



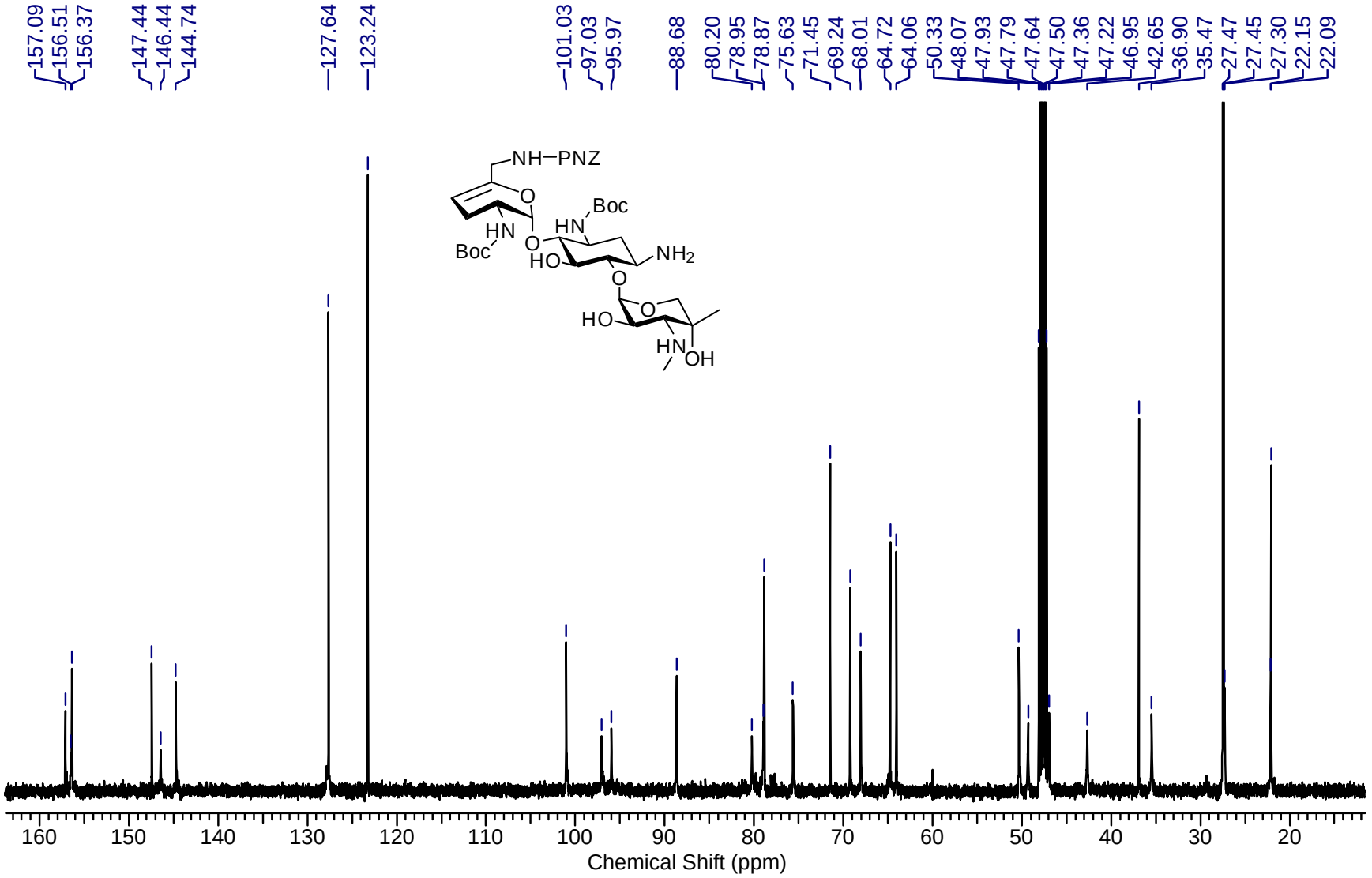
^{13}C NMR (151 MHz, $CDCl_3$) Spectrum of 6′-*N*-(*p*-Nitrobenzyloxycarbonyl)sisomicin (12)



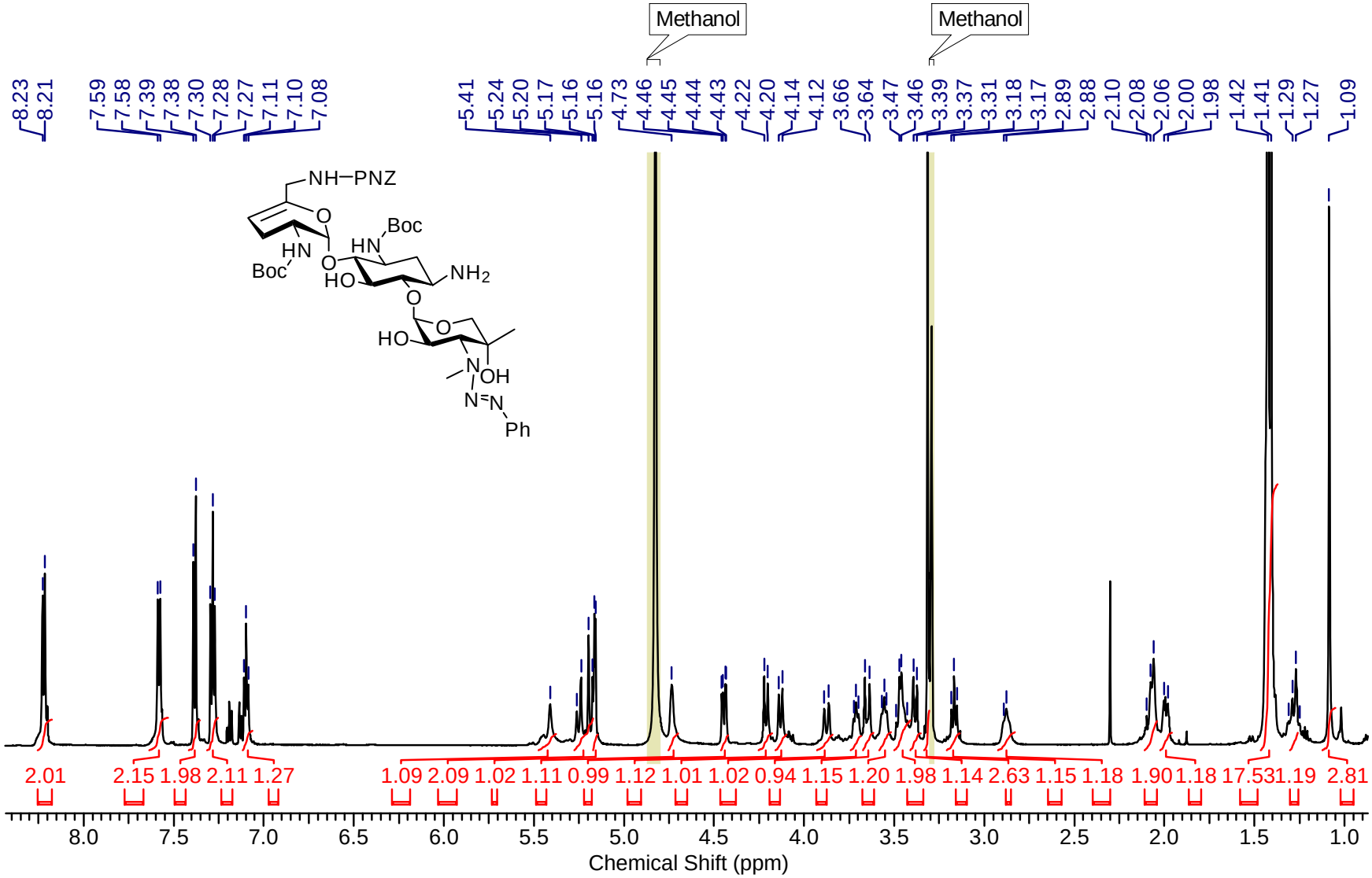
^{1}H NMR (600 MHz, CD_3OD) Spectrum of 6′-*N*-(*p*-Nitrobenzyloxycarbonyl)-2′,3-di-*N*-(*tert*-butyloxycarbonyl)sisomicin (13)



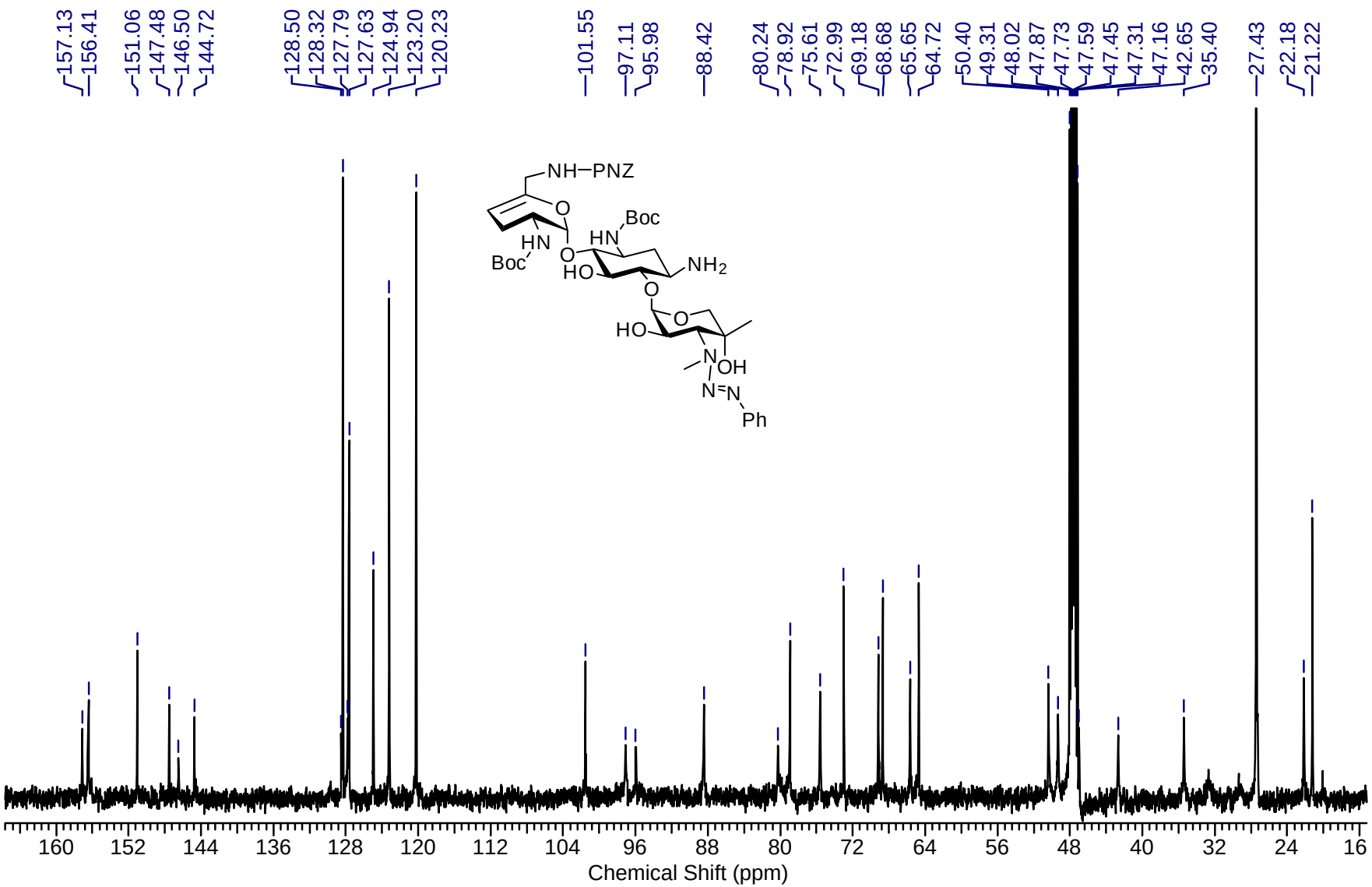
^{13}C NMR (151 MHz, CD_3OD) Spectrum of 6′-*N*-(*p*-Nitrobenzyloxycarbonyl)-2′,3-di-*N*-(*tert*-butyloxycarbonyl)sisomicin (13)



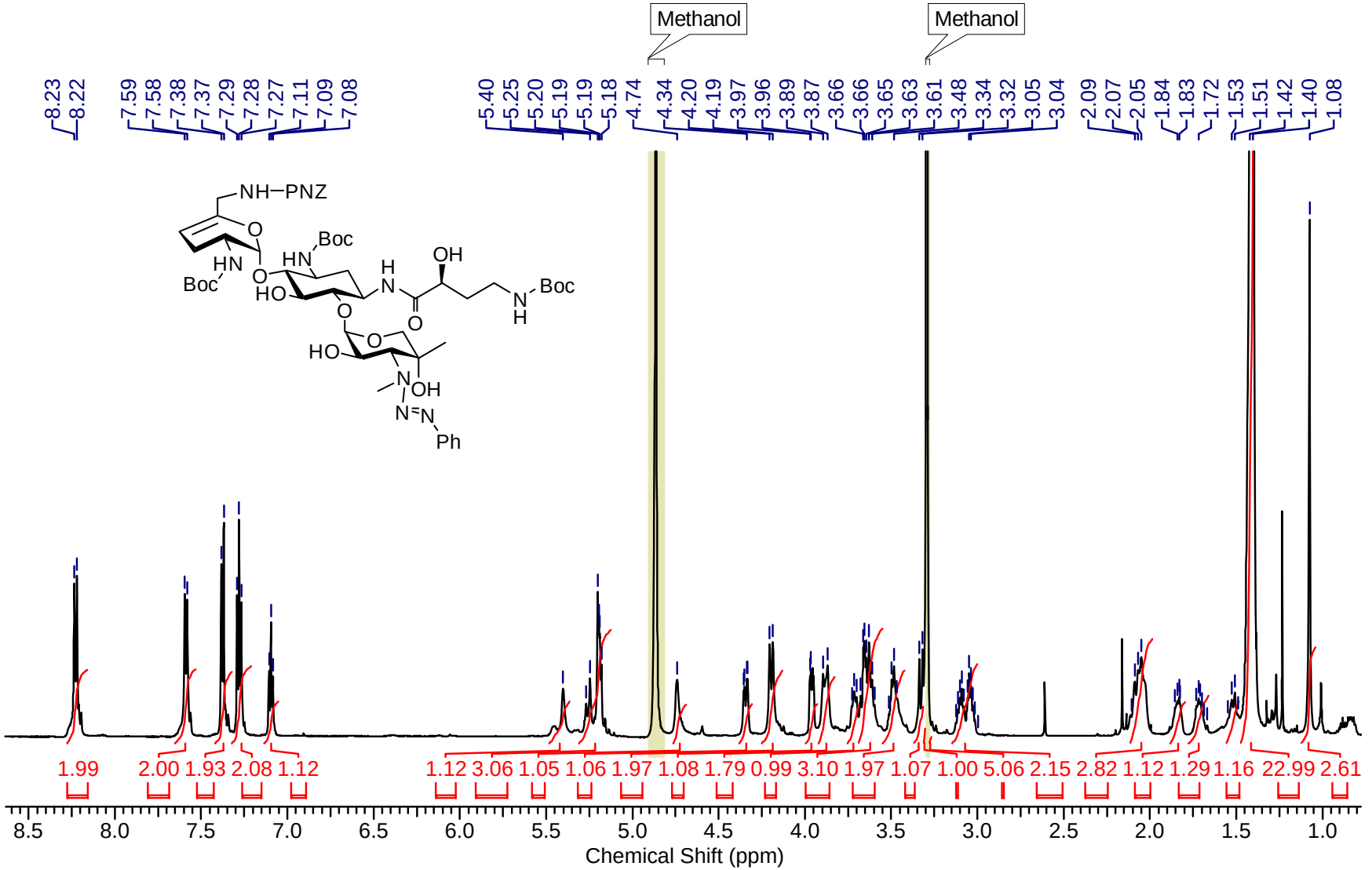
[1]HNMR (600 MHz, CD_3OD) Spectrum of 6′-*N*-(*p*-Nitrobenzyloxycarbonyl)-3′′-*N*-(phenylazo)-2′,3-di-*N*-(*t*-butyloxycarbonyl)sisomicin (14)



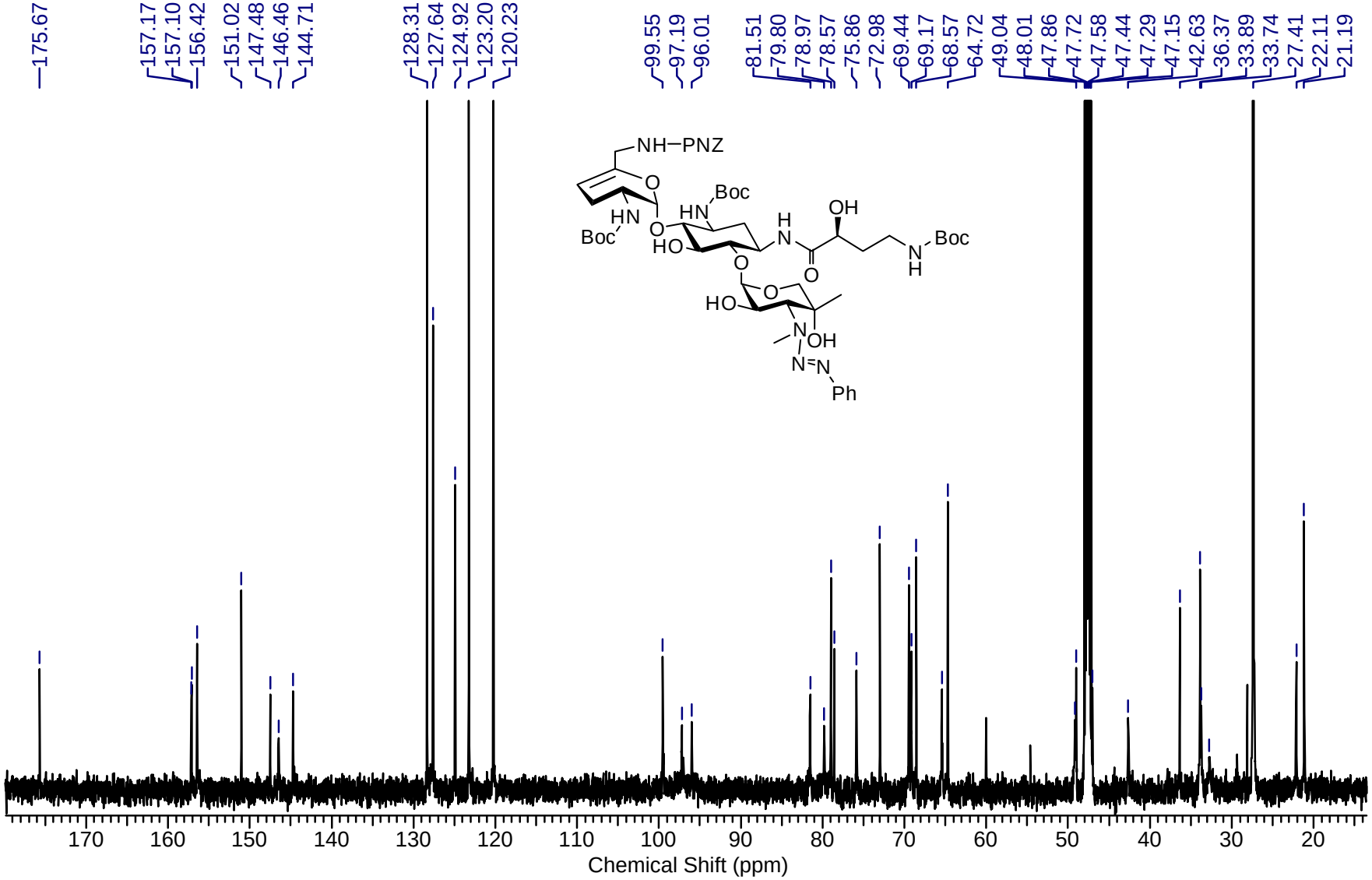
^{13}C NMR (151 MHz, CD_3OD) Spectrum of 6′-*N*-(*p*-Nitrobenzyloxycarbonyl)-3′′-*N*-(phenylazo)-2′,3-di-*N*-(*t*-butyloxycarbonyl)sisomicin (14)



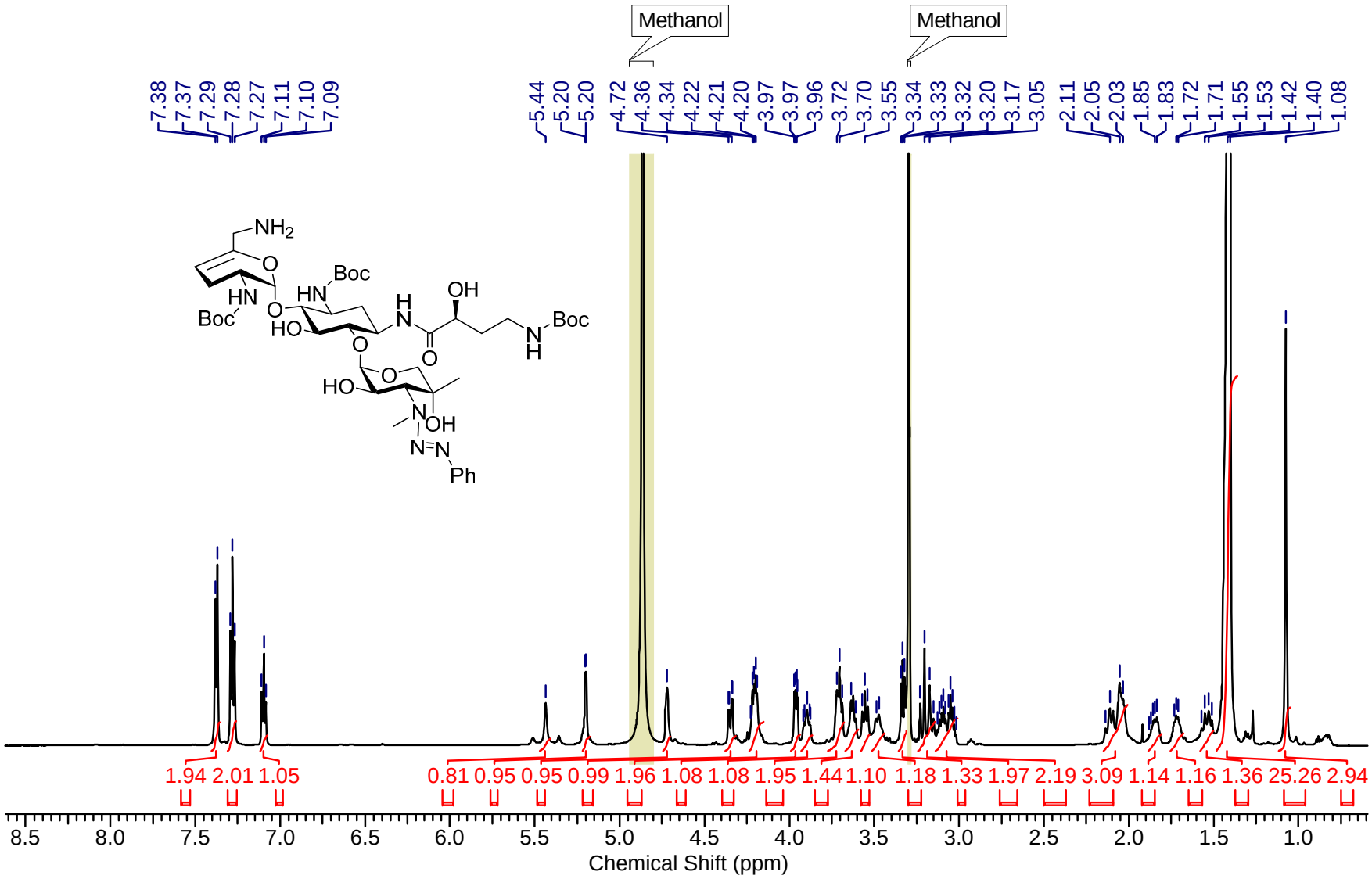
^{1}H NMR (600 MHz, CD_3OD) Spectrum of 1-*N*-(*tert*-Butyloxycarbonylamino-2(*S*)-hydroxybutyryl)-6′-*N*-(*p*-nitrobenzyloxycarbonyl)-3′′-*N*-(phenylazo)-2′,3-di-*N*-(*tert*-butyloxycarbonyl)sisomicin (15)



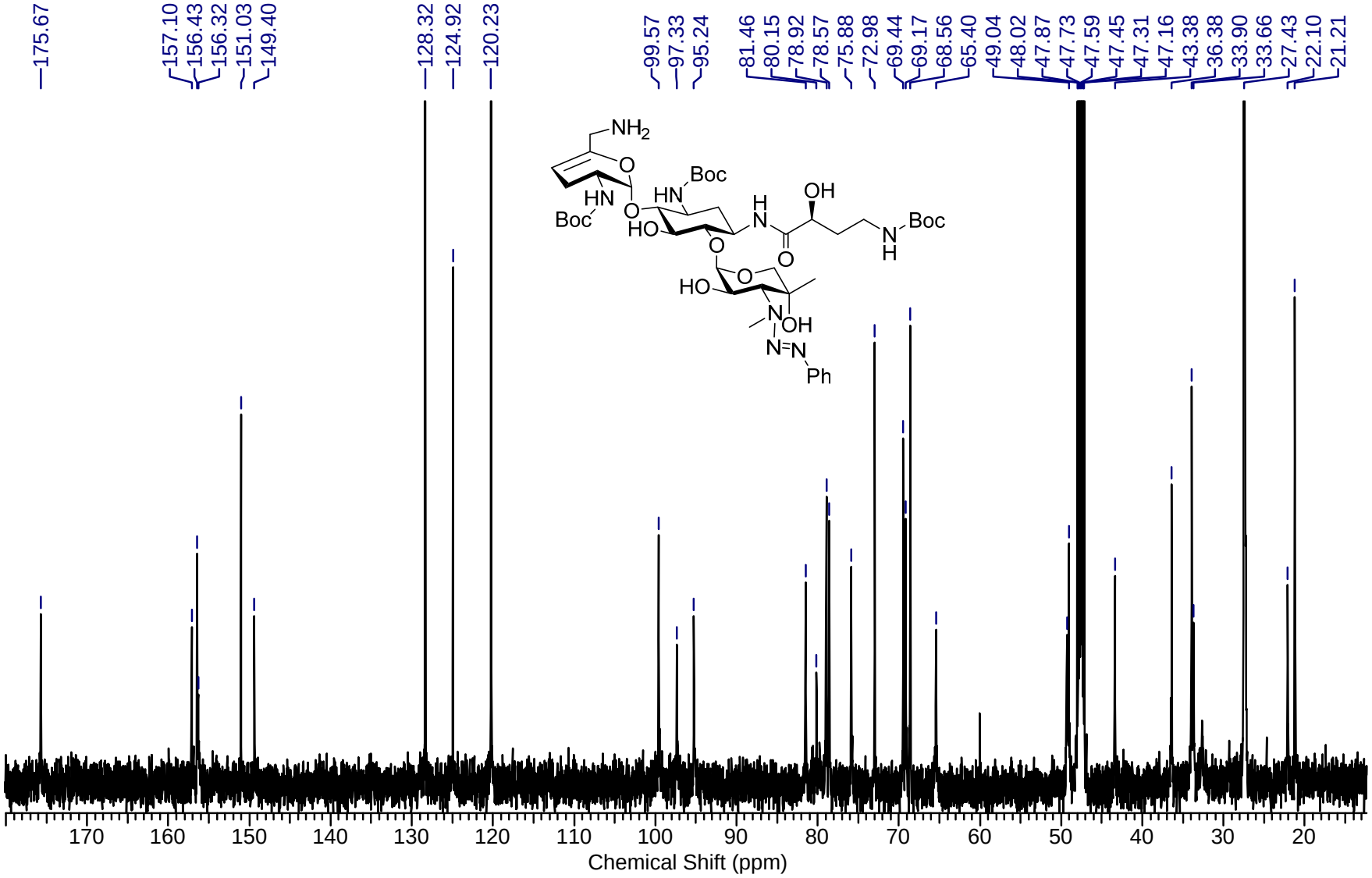
^{13}C NMR (151 MHz, CD_3OD) Spectrum of 1-*N*-(*tert*-Butyloxycarbonylamino-2(*S*)-hydroxybutyryl)-6′-*N*-(*p*-nitrobenzyloxycarbonyl)-3′′-*N*-(phenylazo)-2′,3-di-*N*-(*tert*-butyloxycarbonyl)sisomicin (15)



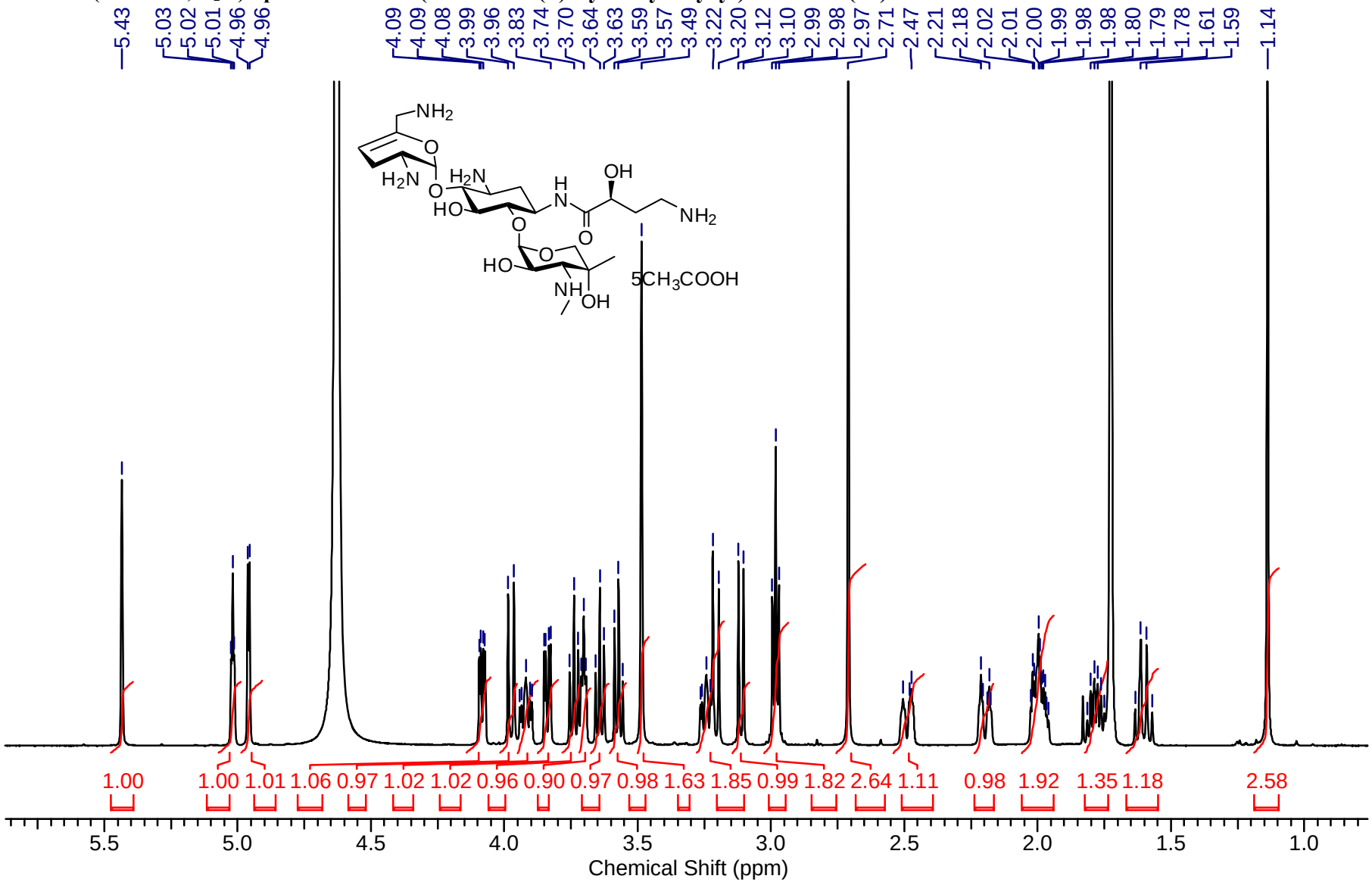
^{1}H NMR (600 MHz, CD_3OD) Spectrum of 1-*N*-(*tert*-Butyloxycarbonylamino-2(*S*)-hydroxybutyryl)-3′′-*N*-(phenylazo)-2′,3-di-*N*-(*tert*-butyloxycarbonyl)sisomicin (16)



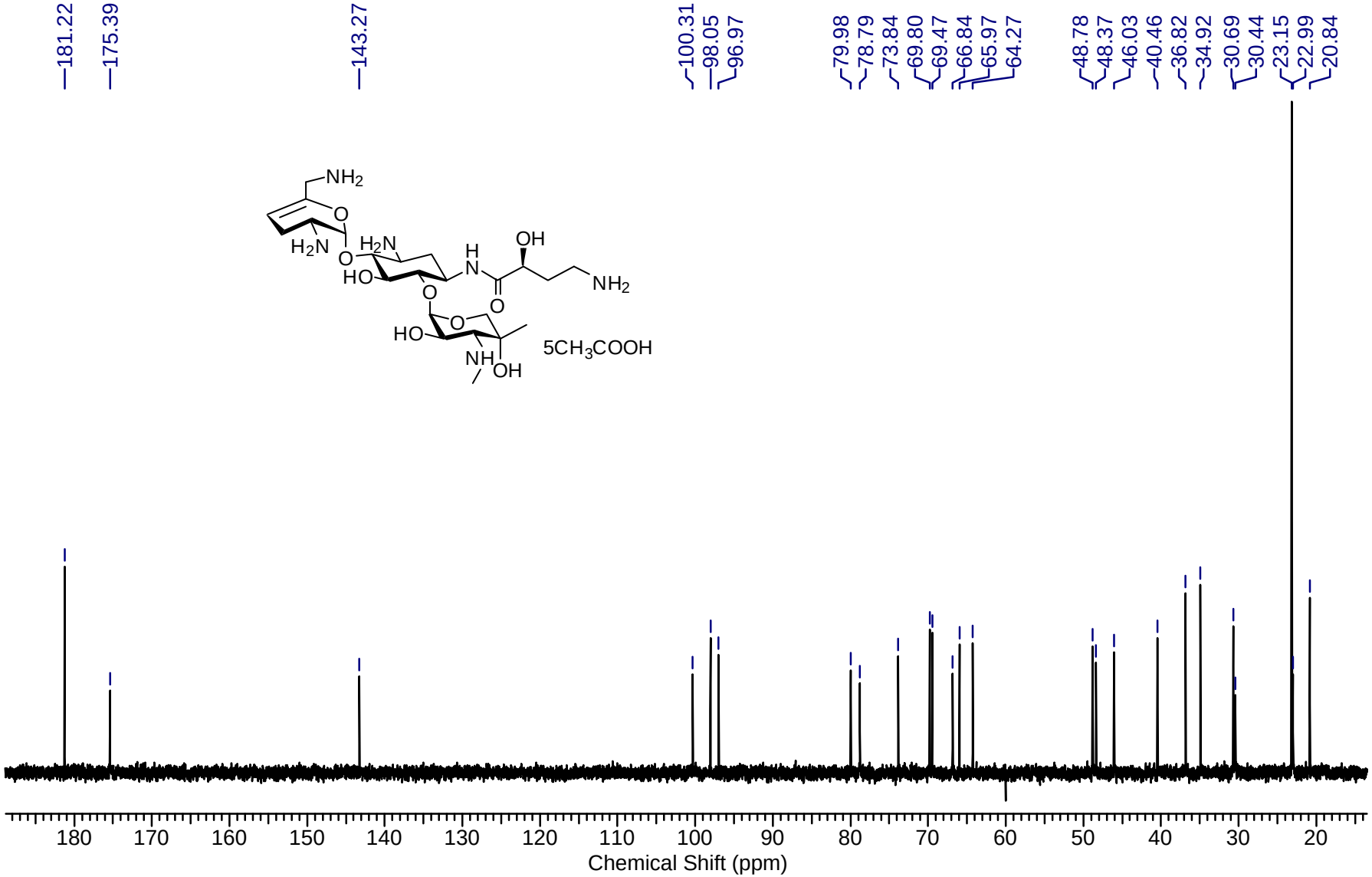
^{13}C NMR (151 MHz, CD_3OD) Spectrum of 1-*N*-(*tert*-Butyloxycarbonylamino-2(*S*)-hydroxybutyryl)-3′′-*N*-(phenylazo)-2′,3-di-*N*-(*tert*-butyloxycarbonyl)sisomicin (16)



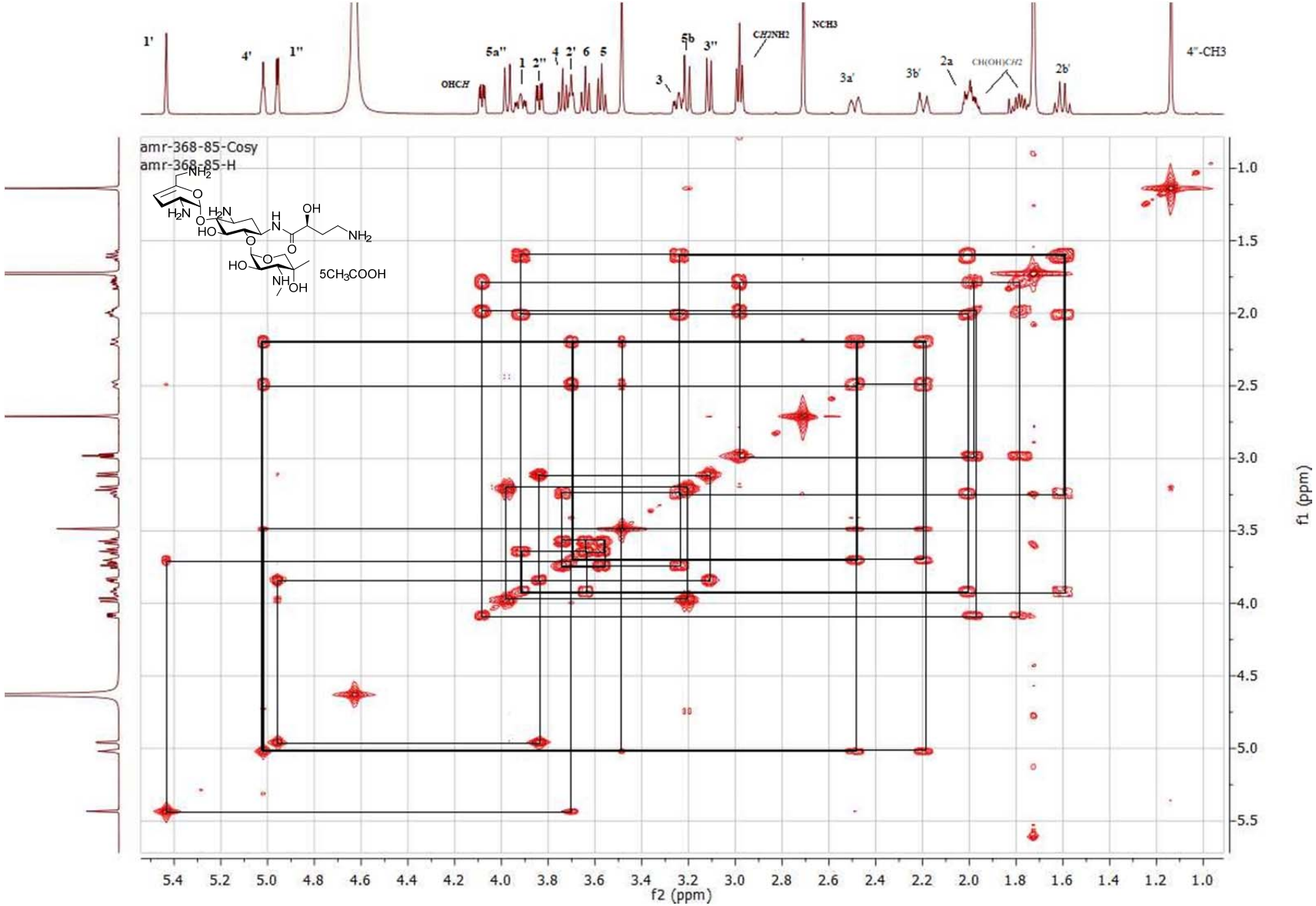
^{1}H NMR (600 MHz, D_2O) Spectrum of 1-*N*-(4-Amino-2(*S*)-hydroxybutyryl)sisomicin (11)



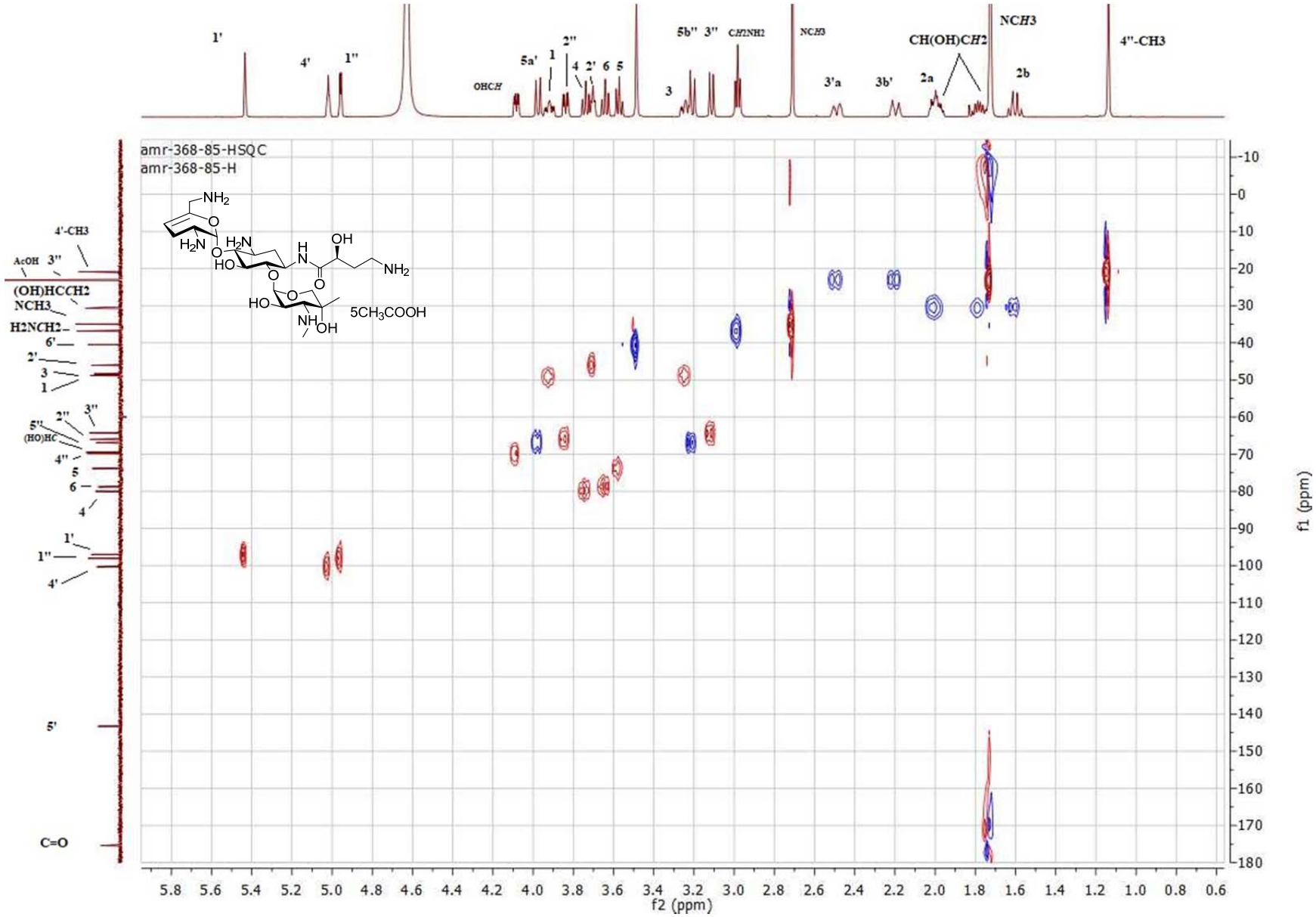
^{13}C NMR (151 MHz, D_2O) Spectrum of 1-*N*-(4-Amino-2(*S*)-hydroxybutyryl)sisomicin (11)



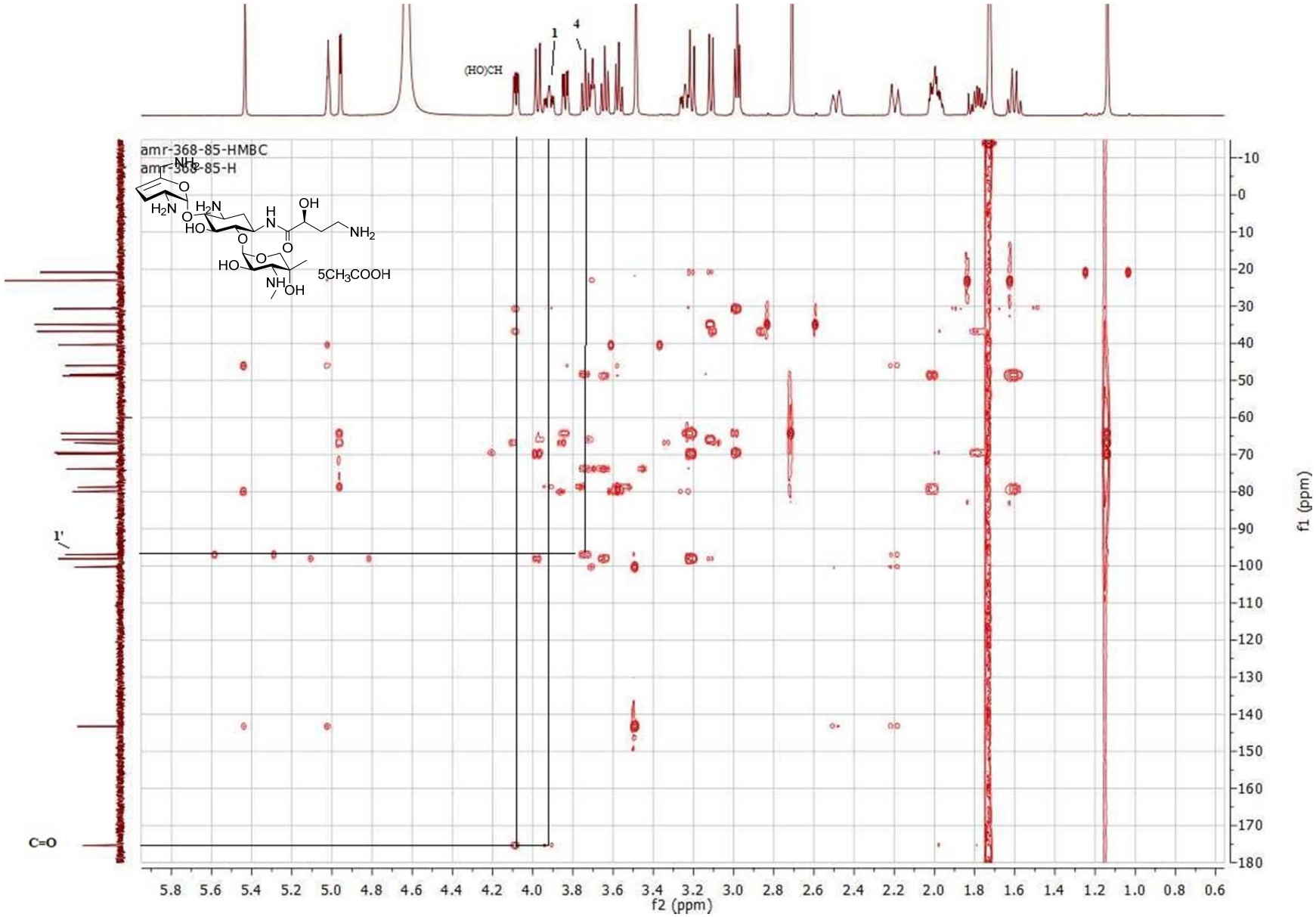
COSY NMR (600 MHz, D_2O) Spectrum of 1-*N*-(4-Amino-2(*S*)-hydroxybutyryl)sisomicin (11)



HSQC (600 MHz, D_2O) Spectrum of 1-*N*-(4-Amino-2(*S*)-hydroxybutyryl)sisomicin (11)



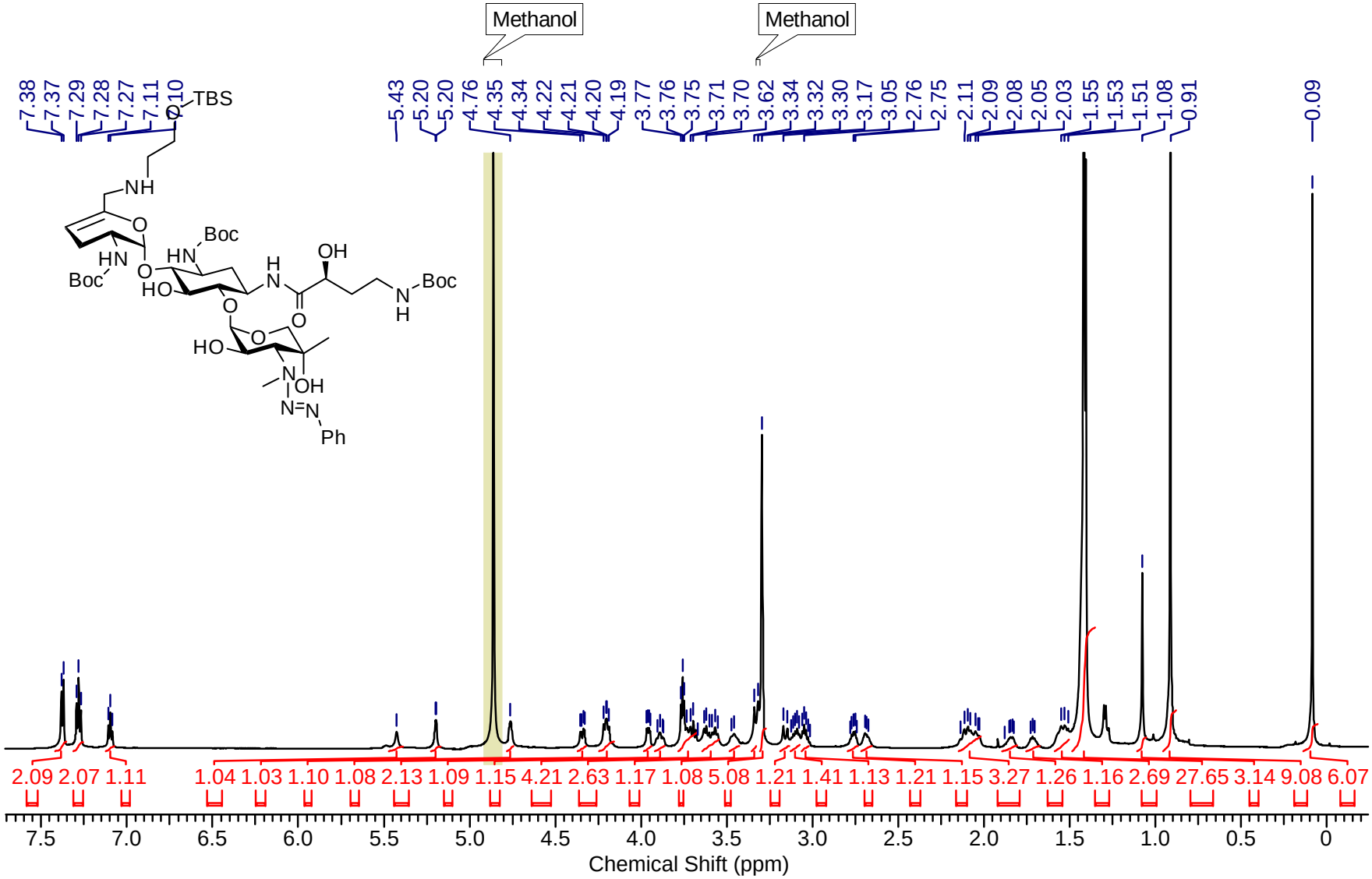
HMBC (600 MHz, D_2O) Spectrum of 1-*N*-(4-Amino-2(*S*)-hydroxybutyryl)sisomicin (11)



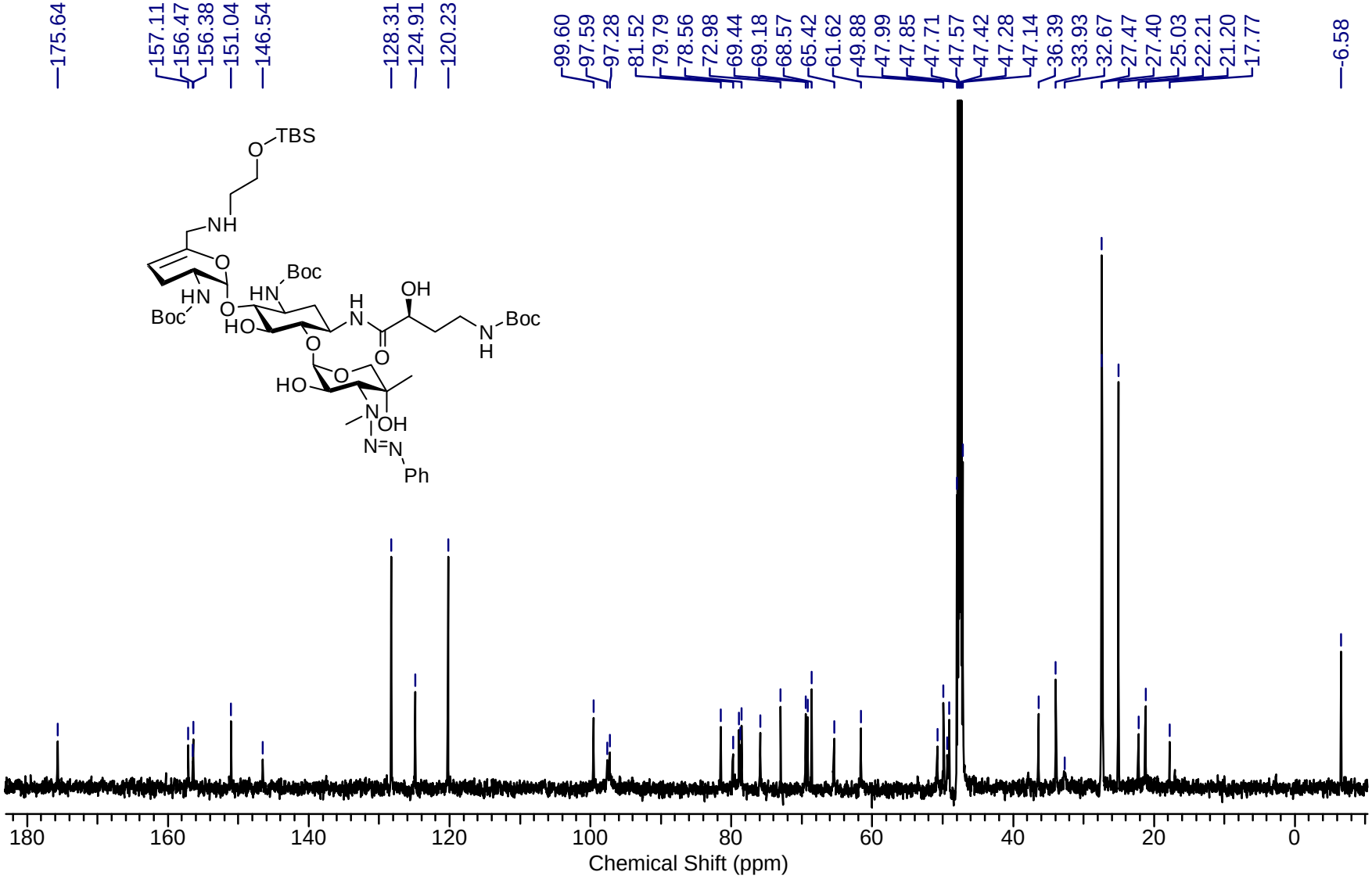
Important 2D NMR correlations of 1-*N*-(4-Amino-2(*S*)-hydroxybutyryl)sisomicin (11)

Signal 1 (δ)	Assignment	Signal 2 (δ)	Assignment	Correlation
5.43 (d)	H-1′	97.0	C-1′	HSQC
5.02 (t)	H-4′	100.3	C-4′	HSQC
4.96 (d)	H-1″	98.1	C-1″	HSQC
4.08 (dd)	O=CC***H***(OH)	2.03-1.95	CH(OH)C***H***$_2$CH$_2$	COSY
3.92 (m)	H-1	175.4	NH***C***=O	HMBC
3.74 (t)	H-4	97.0	C-1′	HMBC
3.74 (t)	H-4	3.25 (m)	H-3	COSY

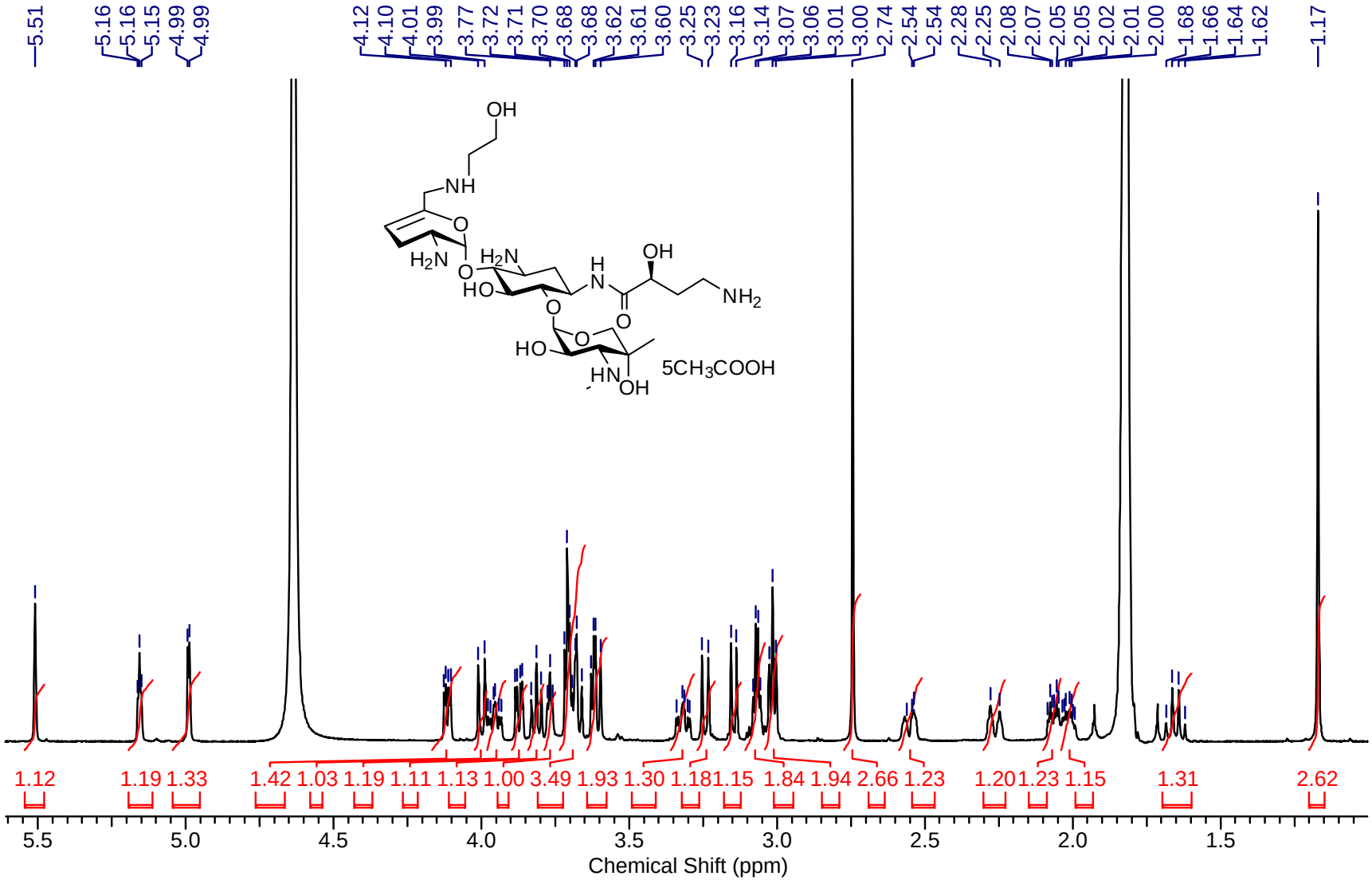
^{1}H NMR (600 MHz, CD_3OD) Spectrum of 6′-*N*-(2-*tert*-Butyldimethylsiloxyethyl)-1-*N*-(*tert*-butyloxycarbonylamino-2(*S*)-hydroxybutyryl)-3′′-*N*-(phenylazo)-2′,3-di-*N*-(*tert*-butyloxycarbonyl)sisomicin (17)



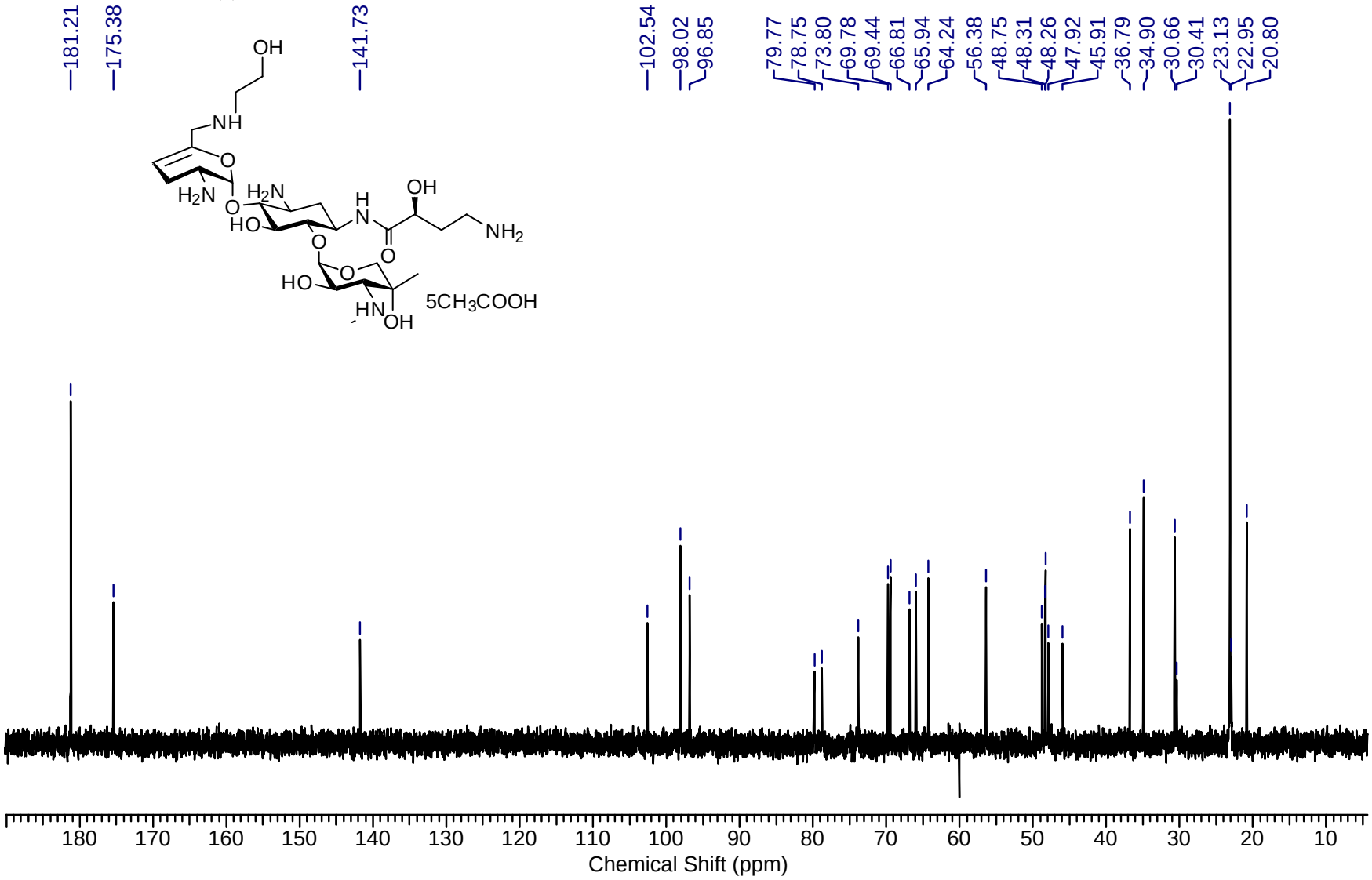
^{13}C NMR (151 MHz, CD_3OD) Spectrum of 6′-*N*-(2-*tert*-Butyldimethylsiloxyethyl)-1-*N*-(*tert*-butyloxycarbonylamino-2(*S*)-hydroxybutyryl)-3′′-*N*-(phenylazo)-2′,3-di-*N*-(*tert*-butyloxycarbonyl)sisomicin (17)



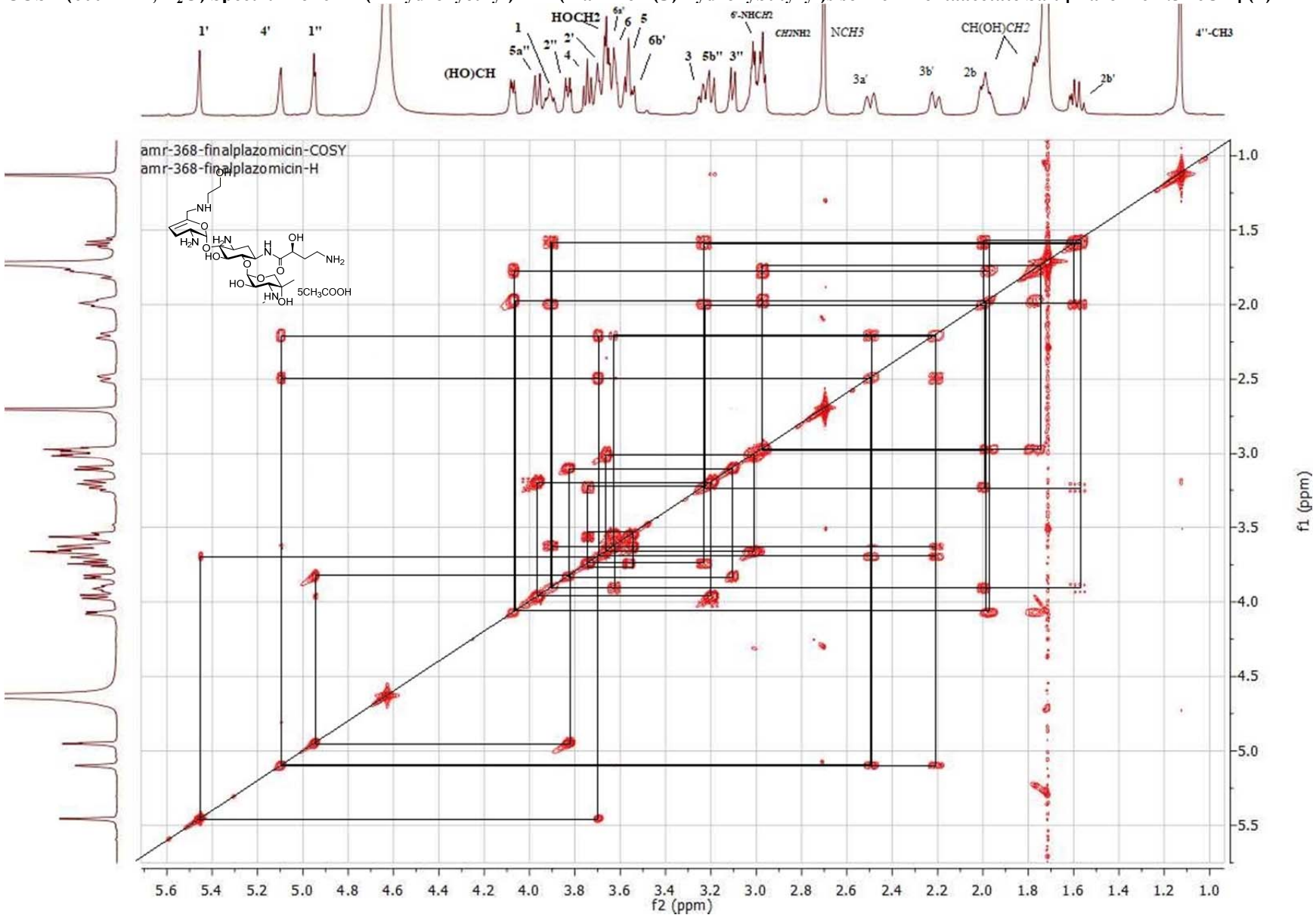
^{1}H NMR (600 MHz, D_2O) Spectrum of 6′-*N*-(2-Hydroxyethyl)-1-*N*-(4-amino-2(*S*)-hydroxybutyryl)sisomicin Pentaacetate Salt [Plazomicin.5AcOH] (4)



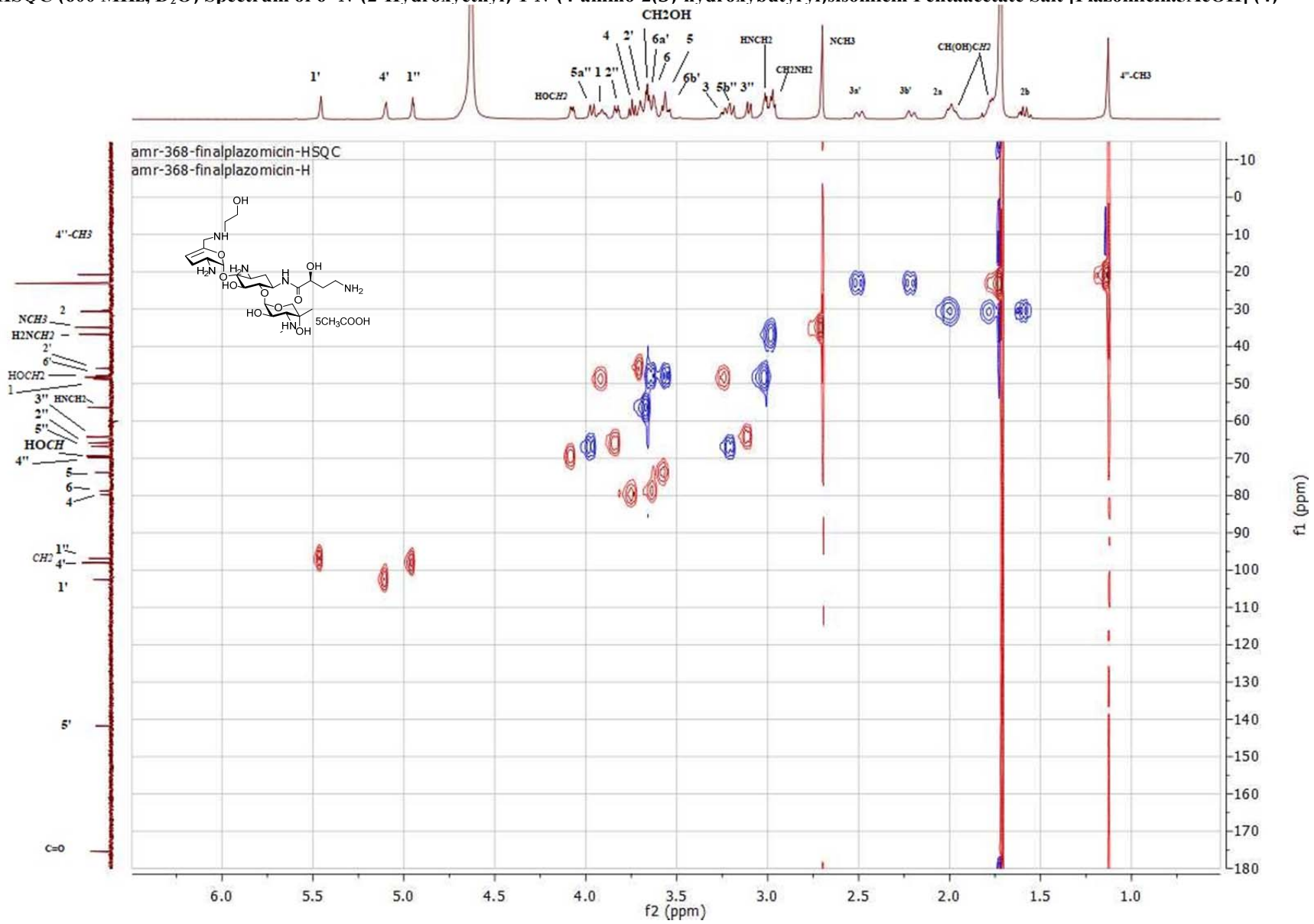
^{13}C NMR (151 MHz, D_2O) Spectrum of 6′-*N*-(2-Hydroxyethyl)-1-*N*-(4-amino-2(*S*)-hydroxybutyryl)sisomicin Pentaacetate Salt [Plazomicin.5AcOH] (4)



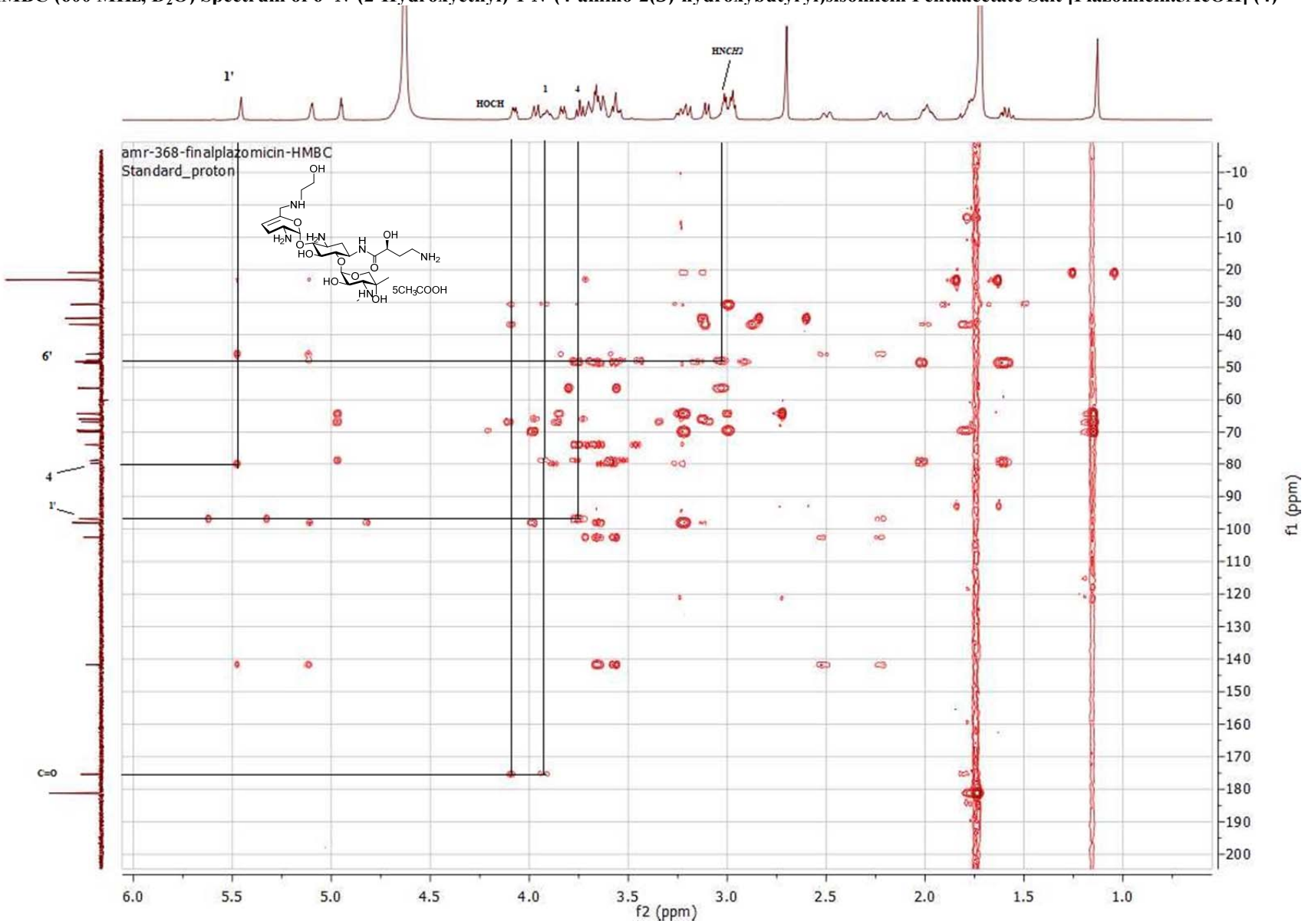
COSY (600 MHz, D_2O) Spectrum of 6′-*N*-(2-Hydroxyethyl)-1-*N*-(4-amino-2(*S*)-hydroxybutyryl)sisomicin Pentaacetate Salt [Plazomicin.5AcOH] (4)



HSQC (600 MHz, D_2O) Spectrum of 6′-*N*-(2-Hydroxyethyl)-1-*N*-(4-amino-2(*S*)-hydroxybutyryl)sisomicin Pentaacetate Salt [Plazomicin.5AcOH] (4)



HMBC (600 MHz, D_2O) Spectrum of 6′-*N*-(2-Hydroxyethyl)-1-*N*-(4-amino-2(*S*)-hydroxybutyryl)sisomicin Pentaacetate Salt [Plazomicin.5AcOH] (4)



Important 2D NMR correlations of 6′-*N*-(2-Hydroxyethyl)-1-*N*-(4-amino-2(*S*)-hydroxybutyryl)sisomicin Pentaacetate Salt [Plazomicin.5AcOH] (4)

Signal 1 (δ)	Assignment	Signal 2 (δ)	Assignment	Correlation
5.51 (s)	H-1′	96.9	C-1′	HSQC
5.51 (s)	H-1′	79.8	C-4	HMBC
5.16 (t)	H-4′	102.5	C-4′	HSQC
4.99 (d)	H-1″	98.0	C-1″	HSQC
3.95 (m)	H-1	175.4	NH***C***=O	HMBC
3.81 (t)	H-4	96.9	C-1′	HMBC
3.81 (t)	H-4	3.31 (m	H-3	COSY
3.07 (q)	NHC$H_2$$CH_2$O	47.9	C-6′	HMBC
3.65-3.89 (m)	H-6′	48.31	NH***C***$H_2$$CH_2$OH	HMBC