The Asymmetric Synthesis of (2*S*,3*R*)-Capreomycidine and the Total Synthesis of Capreomycin IB.

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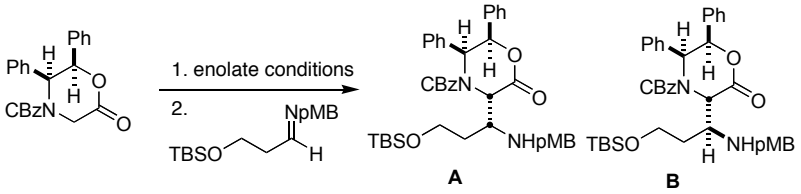
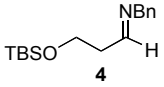
SUPPORTING INFORMATION



Effect of enolate on diastereoselectivity.

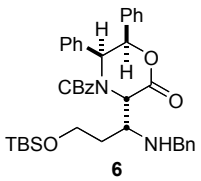
CONDITIONS	YIELD	dr (A:B)[a]
1. LHMDS, 2. Me_2AlCl	57%	3.2:1
1. LHMDS, 2. Et_2AlCl	~50%	3.5:1
LDA	N.R.	N/A
1. $TiCl_4$, 2. Et_3N	<20%	nd[b]
1. LDA, 2. Cp_2ZrCl_3	N.R.	N/A

[a]determined by ^{1}H-NMR.[b]dr not determined



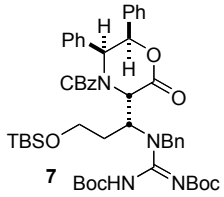
3-(*tert*-butyldimethylsiloxy)-*N*-benzyl-propanaldimine (4). To benzylamine (663 mg, 6.19 mmol, 1 eq.) and alumina (3.8 g), was added a solution of 3-(*tert*-butyldimethylsiloxy)-propanal (1.17 g, 6.19 mmol, 1 eq.) in CH_2Cl_2 (23 mL) at 0°C. The heterogeneous mixture was stirred for 25 min. at 0°C then filtered and evaporated (in vacuo). The product (**4**), isolated as a pale oil, was used crude for next step.

^{1}H NMR (300 MHz) ($CDCl_3$) δ $CHCl_3$: 0.11 (6H, s); 0.95 (9H, s); 2.59 (2H, q, J=6.2 Hz); 3.94 (2H, t, J=6.2Hz); 4.63 (2H, s); 7.26-7.40 (5H, m); 7.90 (1H, t, J=4.8Hz). ^{13}C NMR (75 MHz) ($CDCl_3$) δ $CDCl_3$: -5.0, 18.5, 26.1, 39.4, 60.7, 65.4, 127.0, 128.0, 128.5, 139.2, 164.5. IR (NaCl, neat) 3087, 3064, 3029, 2955, 2928, 2885, 2856, 1669, 1495, 1472, 1463, 1387, 1255, 1100, 837 cm^{-1}.



Mannich product (6). Under argon atmosphere, compound (-) **5** (755 mg, 1.95 mmol, 1 eq.) was dissolved in dry tetrahydrofuran (42 mL). The resulting solution was cooled to -78°C. LHMDS (3.3 mL, 2.0 mmol, 1.03 eq., 0.61 M in THF) was added, and the reaction stirred 15 min. Dimethylaluminum chloride (2.0 mL, 2.0 mmol., 1.03 eq., 1M in hexanes) was added dropwise, and the mixture stirred 15 min. In a separate flask, compound **4** was dissolved in 4 mL dry THF under argon atmosphere, and added *via* canula to the awaiting aluminum enolate. The reaction was stirred 2 h at -78°C. Saturated aqueous sodium bicarbonate was added and the quenched reaction was warmed to room temp. After filtering the resulting suspension through celite, the mother liquor was extracted 3x with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and evaporated (in vacuo). This resulted in a pale orange oil that was purified by silica gel chromatography (eluted with 9:1 hexanes:ethyl acetate) to yield 704 mg (54%) of **6** as an inseparable 3.3:1 mixture of diastereomers (^{1}H-NMR). Both diastereomers were taken on to the next step.

^{1}H NMR (300 MHz) (DMSO-d_6, 393K) (major diastereomer) δ DMSO: 0.07 (6H, s); 0.90 (9H, s); 1.70 (1H, dddd, J=14.3, 9.2, 5.9, 5.9 Hz); 2.09 (1H, dddd, J=11.0, 6.6, 4.4, 4.4 Hz); 3.50(1H, sym. m); 3.83 (1H, 1/2 ABq, J=5.5 Hz); 3.85 (1H, 1/2 ABq, J=5.5 Hz); 3.93 (2H, s); 4.88 (1H, 1/2 ABq, J=12.5 Hz); 4.96 (1H, 1/2 ABq, J=12.5 Hz); 5.07 (1H, d, J=5.9 Hz); 5.25 (1H, d, J=3.3 Hz); 6.35 (1H, d, J=3.3 Hz); 6.60 (2H, d, J=7.0 Hz); 6.93-7.35 (18H, m). ^{13}C NMR (100 MHz) ($CDCl_3$) (major diastereomer) δ -5.1, 18.5, 26.2, 36.2, 52.5, 58.9, 60.2, 60.3, 61.4, 68.0, 79.1, 126.6, 126.7, 127.2, 127.5, 127.6, 127.8, 127.9, 127.9, 128.1, 128.1, 128.4, 128.6, 128.6, 128.8, 129.0, 134.6, 135.6, 136.5, 140.9, 156.0, 169.3. IR (NaCl, deposited from CH_2Cl_2): 3032, 2928, 2855, 1752, 1708, 1497, 1454, 1398, 1250, 1102, 836, 775 cm^{-1}. HRMS (FAB+) calc. for $C_{40}H_{49}N_2O_5Si$ (MH^+) 665.3411; found 665.3417.

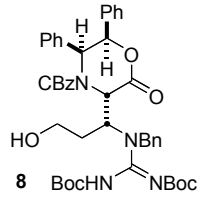


7

Guanidine 7. To a solution of **6** (510 mg, 0.77 mmol., 1 eq.) and *N,N'*-bis-*tert*-butoxycarbonyl-*S*-methylisothiourea (290 mg, 1.00 mmol., 1.3 eq.) under an argon atmosphere in DMF (3.9 Ml), is added triethylamine (322 _l, 2.31 mmol., 3.0 eq.). After dissolution, silver triflate (277 mg, 1.08 mmol., 1.4 eq.) was added and the heterogeneous reaction stirred 3h. Dilution of the reaction with ethyl acetate was followed by filtration through celite to remove any solids. The resulting organic layer was washed twice with brine and dried over anhydrous sodium sulfate. Filtration, followed by removal of the solvent under reduced pressure resulted in an orange oil that was subjected to silica gel chromatography (eluted with 7:1 hexanes:ethyl acetate), providing 518 mg (74%) of **7** as a white foam.

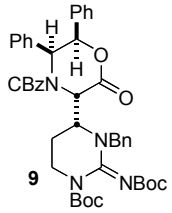
^{1}H NMR (300 MHz) ($CDCl_3$) (unresolvable rotamers) spectrum appears as follows: δ $CHCl_3$: -0.04 and 0.01 (6H, s); 0.83 (9H, s); 1.40 (9H, s); 1.58 (9H, s); 1.81-1.98 (1H, broad m); 2.04-2.22 (1H, broad m); 3.41-3.55 (1H, m); 3.55-3.68 (1H, m); 4.59 (1H, broad d, J=18.7 Hz); 4.77 (1H, broad d, J=12.1 Hz); 4.83-4.99 (2H, m); 5.31 (1H, broad d, J=11.0 Hz); 5.38 (1H, broad s); 5.74-5.91 (1H, broad m); 6.58 (2H, broad d, J=7.3 Hz); 6.67 (2H, broad d, J=7.0 Hz); 6.87 (1H, broad s); 7.02-7.48 (16H, m). ^{13}C NMR (75 MHz) ($CDCl_3$) δ -5.2, 18.3, 26.1, 28.3, 28.6, 32.1,

49.0, 54.8, 59.0, 59.7, 62.3, 68.0, 78.7, 79.2, 81.7, 125.9, 127.1, 127.2, 127.5, 127.7, 128.0, 128.1, 129.2, 134.6, 135.8, 137.3, 149.8, 153.4, 155.5, 160.7, 168.1. IR (NaCl, depos. from CH_2Cl_2): 3403, 3064, 3033, 2955, 2930, 1760, 1701, 1600, 1489, 1455, 1393, 1367, 1297, 1252, 1146, 1123, 837 cm^{-1}. HRMS (FAB) calc. for $C_{51}H_{67}N_4O_9Si$ (MH^+) 907.4677; found 907.4671. $[\alpha]_D^{25}$=+15.6 (*c*=0.55, CH_2Cl_2).



8

Alcohol 8. Compound **7** (198 mg, 0.22 mmol., 1 eq.) was dissolved in 14.5 mL acetonitrile. Upon addition of 5% aqueous HF in acetonitrile (7.3 mL) the mixture was stirred until the starting material was consumed (2.5 h). At that point, solid sodium bicarbonate was added and the mixture stirred until the bubbling ceased (30 min.). After filtration to remove the remaining bicarbonate and evaporation of the solvent under reduced pressure, the crude product was subjected to flash silica gel chromatography (Whatman brand silica gel (230-400 mesh), eluted with 4:1 dichloromethane:ethyl acetate). This resulted in the isolation of 141 mg (81%) of **8** as a pale foam. It is necessary to use **8** immediately for the next step as it is unstable.
^{1}H NMR (unresolvable rotamers). ^{13}C NMR (unresolvable rotamers). IR (NaCl, neat) 3393, 3064, 3032, 2978, 1757, 1700, 1653, 1635, 1601, 1497, 1394, 1287, 1147 cm^{-1}. HRMS (FAB) calc. for $C_{45}H_{53}N_4O_9$ (MH^+) 793.3812; found 793.3807.

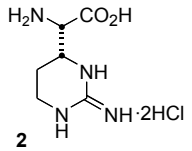


9

Cyclic guanidine 9. Diisopropylazodicarboxylate (45 mL, 0.23 mmol., 1.5 eq.) was added to a solution of **8** (122 mg, 0.15 mmol., 1 eq.) and triphenylphosphine (60 mg, 0.23 mmol., 1.5 eq.) in THF (5.3 mL) at 0°C under argon atmosphere. After stirring 10 minutes at 0°C, the reaction was allowed to warm to room temperature and stir 30 min. The THF was removed *in vacuo*, and the crude oil subjected to silica gel chromatography (eluted with 88:12 dichloromethane:ether). This purification provided 102 mg (88%) of **9** as a white amorphous solid. M.p. 199°C (recryst. *i*-PrOH/water).

^{1}H NMR (300 MHz) (DMSO-d_6, 393K) δ DMSO: 1.42 (9H, s); 1.47 (9H, s); 2.08-2.34 (2H, m); 3.40 (1H, ddd, J=12.8, 7.7, 5.1 Hz); 4.08 (1H, ddd, J=12.8, 8.4, 8.4 Hz); 4.40 (1H, m); 4.41 (1H, 1/2 ABq, J=14.7 Hz); 4.92-5.09 (2H, sym. m); 5.17 (1H, d, J=3.3 Hz); 5.18 (1H, 1/2 ABq, J=14.7 Hz); 5.36 (1H, d, J=8.1 Hz); 6.17 (1H, d, J=3.3 Hz); 6.52 (2H, d, J=7.3 Hz); 6.96-7.45 (18H, m). ^{13}C NMR (75 MHz) ($CDCl_3$) (unresolvable rotamers, resonances are reported as observed at 298 K, * denotes minor rotamer) δ $CDCl_3$:21.9, 22.2, 26.2, 27.2, 28.4, 28.8, 42.4, 53.5, *53.9, *55.7, 57.4, *58.7, 59.7, 61.6, *61.9, 68.7, 78.8, 79.0, 82.8, 126.5, 127.2, 127.8, 128.2, 128.7, 129.0, 129.3, *133.7, 134.1, 134.7, 136.0, *136.6, *151.2, 151.8, *152.8, 154.3, 156.3, 159.6, *159.9, *167.3, 168.3. IR (NaCl, deposited from CH_2Cl_2): 2976, 1751, 1734, 1717,

1701, 1684, 1676, 1616, 1576, 1456, 1394, 1314, 1247, 1139 cm^{-1}. HRMS (FAB) calc. for $C_{45}H_{51}N_4O_8$ (MH^+) 775.3707; found 775.3707. $[\alpha]_D^{25}$=+16.7 (*c*=0.55, CH_2Cl_2).



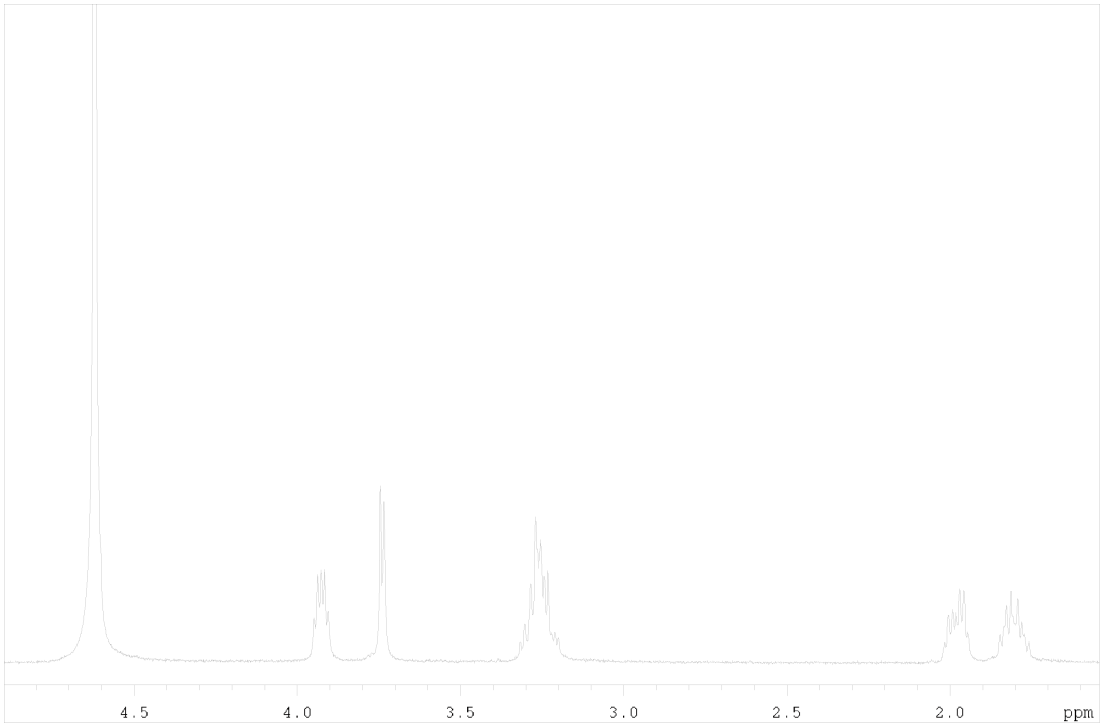
2

Capreomycidine·HCl (2). A solution of **9** (215 mg, 0.28 mmol., 1 eq) in 3:1 THF:EtOH (32.4 mL) was purged with argon for 5 min. Palladium chloride (146 mg, 0.83 mmol., 3 eq.) was added to the solution in a pressure tube. The tube was then pressurized and evacuated five times with hydrogen gas. Pressurization of the tube to 100 psi with hydrogen gas was followed by stirring of the reaction for 4 days at room temperature. Release of the hydrogen pressure was followed by purging with argon. The solution was filtered through Celite and evaporated *in vacuo*. The off-white residue was triturated with ether and dried under vacuum. This residue was then taken up in 2.5 mL 0.5 M HCl (prepared with dd H_2O) and refluxed for 1.5 h. Lyophilization of this reaction mixture provided crude (2*S*,3*R*)-capreomycidine **2** as its dihydrochloride salt. Dissolution of the crude product in MeOH was followed by addition of pyridine to ~pH 5. Addition of absolute EtOH resulted in the precipitation of 28 mg (48%) capreomycidine monohydrochloride as a white amorphous solid. The synthetic **2** agreed with the natural product by spectroscopic methods and molar optical rotation (synthetic: $[M]_D^{20}$=+28.2, natural: $[M]_D^{20}$=+32.5). Molar optical rotation is defined as $[M]_D^{20}=[\alpha]_D^{20}$ x MW / 100.

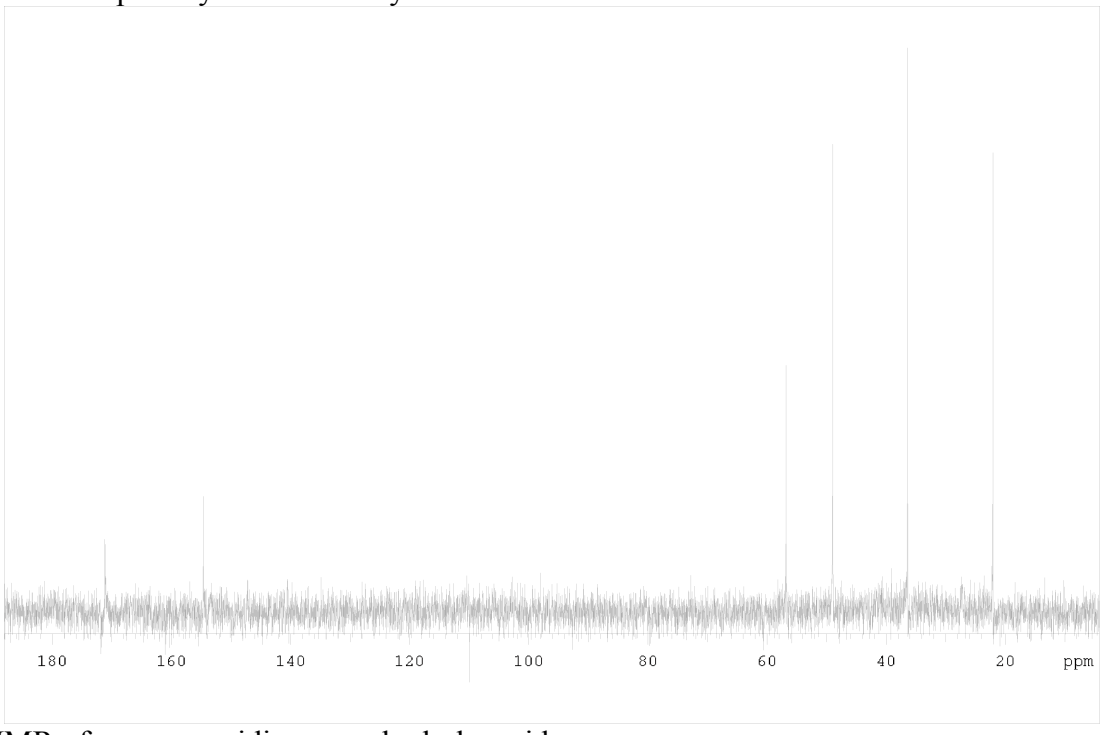
For analytical purposes, the monohydrobromide salt was formed in the following manner: The dihydrochloride salt was taken up in deionized water, treated with 2 drops of 28% aqueous HBr, and lyophilized overnight. The resulting off-white residue was dissolved in a minimal amount of methanol, and the solution neutralized with pyridine. Absolute ethanol was added until capreomycidine mono-HBr (**2·HBr**) began to precipitate as a white solid which was recovered by filtration and dried.

To compare to natural **2**, synthetic **2** was passed down a column of Dowex 50WX2-100 (H^+ form), washed with dd H_2O and eluted with 1.5% NH_4OH. The eluent was evaporated, dissolved in a minimal amount of dd H_2O containing several equivalents of NH_4Oac, and lyophilized overnight. The resulting residue (capreomycidine·2HOAc) was found to be identical to natural **2** by ^1H and ^{13}C-NMR.

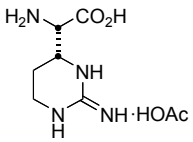
^1H NMR (monohydrobromide) (400 MHz) (D_2O) δ 1.94-2.02 (1H, m); 2.12-2.20 (1H, m); 3.38-3.49 (2H, m); 3.91 (1H, d, J=4.7 Hz); 4.11 (1H, ddd, J=8.6, 4.3, 4.3 Hz). ^{13}C NMR (100 MHz) (D_2O): δ 22.0, 36.4, 48.9, 56.8, 154.7, 171.1. IR (1% KBr): 3566, 3397, 3066, 2927, 1669, 1663, 1646, 1617, 1576, 1569, 1533, 1448, 1418, 1374, 1339, 1113.



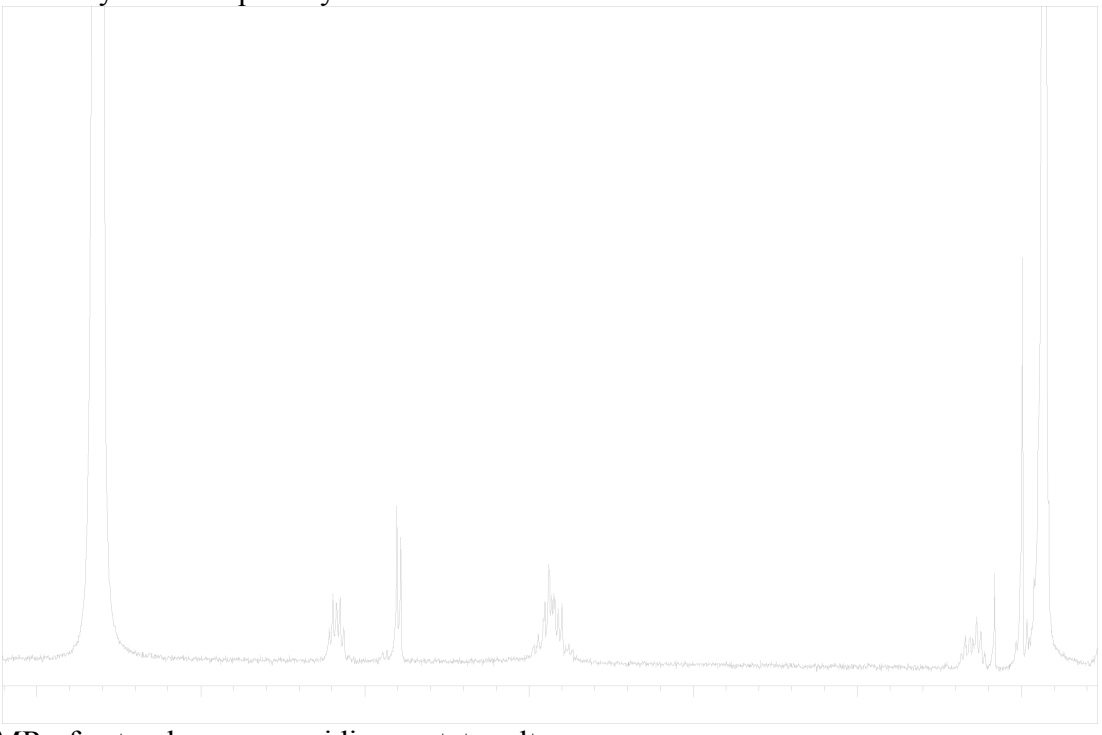
^{1}H-NMR of capreomycidine monohydrobromide.



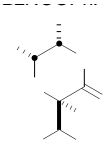
^{13}C-NMR of capreomycidine monohydrobromide.



^{1}H-NMR of synthetic capreomycidine acetate salt.

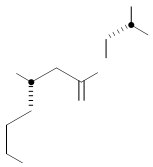


^{1}H-NMR of natural capreomycidine acetate salt.



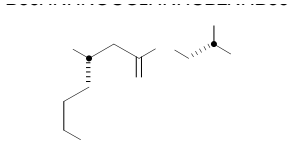
Diethylacetal 10. A solution of **(+) 5** (500 mg, 1.29 mmol, 1 eq.) was dissolved in CH_2Cl_2 (20 mL) and cooled to -78°C. While stirring, $TiCl_4$ (280 μL, 2.58 mmol., 2 eq.) was added, followed by triethylamine (360 μL, 2.58 mmol., 2 eq.) to provide a dark blue enolate solution. After stirring for 15 min., triethyl orthoformate (1.3 mL, 7.74 mmol, 6 eq.) was added, and the solution warmed slowly to 0°C. After stirring 45 min. at 0°C, 0.025 M pH 7 phosphate buffer was added, and the mixture stirred 15 min. The quenched reaction was partitioned between sat. aq. $NaHCO_3$ and CH_2Cl_2. The organic layer was removed and washed twice with water. Upon drying the organic layer over anhydrous sodium sulfate, the solution was filtered and evaporated to provide an off white solid. Silica gel chromatography (eluted with 6:4:1 CH_2Cl_2:hexanes:EtOAc) provided 539 mg (85%) of pure **10** as a white solid. Recrstalization from CH_2Cl_2 / hexanes provided heavy white needles (m.p. 151°C).
^{1}H NMR (300MHz) (DMSO-d_6, 373K) δ DMSO: 1.21 (3H, t, J=7.0 Hz); 3.70 (2H, m); 3.81 (2H, m); 5.00 (2H, m); 5.11 (1H, d, J=2.8 Hz); 5.26 (1H, d, J=3.1 Hz); 6.39 (1H, d, J=3.1 Hz); 6.61 (2H, d, J=7.5 Hz); 7.00 (1H, d, J=2.6 Hz); 7.01-7.27 (13H, m). ^{13}C NMR (75 MHz) ($CDCl_3$) (unresolvable rotamers, resonances reported as observed at 298K, *denotes minor rotamer) δ $CDCl_3$: 15.3, 60.0, 60.7, 60.9, *64.5 , 64.6, 65.1, 65.2, 67.8, *68.4, 79.0, *79.3, 102.8, *103.5, 126.4, 126.5, 127.4, 127.4, 127.5, 127.6, 127.8, 128.0, 128.2, 128.3, 128.6, 128.7, 134.7, 135.4, 135.5, 136.1, *153.7, 155.0, *166.0, 166.3. IR (NaCl, deposited from CH_2Cl_2): 3031, 2975, 2894, 1754, 1705, 1497, 1454, 1402, 1372, 1344, 1319, 1302, 1268, 1245, 1107, 1070, 1031, 980. HRMS (FAB^+) calc. for $C_{29}H_{32}NO_6$ (MH^+) 490.2229; found 490.2213. $[\alpha]_D^{25}$ = -20.6 (*c*=0.5, CH_2Cl_2).

***R*-α-formylglycine diethylacetal (11).** A solution of **10** (370 mg, 0.76 mmol., 1 eq.) and 20% $Pd(OH)_2$ on activated carbon (106 mg, 0.15 mmol., 0.2 eq.) in 3:1 THF:EtOH (23.5 mL) was purged with argon for 10 min. The tube was then filled with hydrogen gas to 85 psi. The pressure was released, and the tube refilled. This was repeated 3 times more. The pressurized tube was then stirred for 2 days at room temperature. After the 2 days, the pressure was released, the solution purged with argon, and the solution diluted with 15 mL MeOH. The 20% $Pd(OH)_2$ on activated carbon was removed by filtration through celite. Evaporation of the filtrate and trituration of the residue with ether provided 134 mg (99%) of **11** as a white solid (m.p. 160-165°C (decomp), recryst. MeOH/CH_2Cl_2).
^{1}H NMR (400MHz) (CD_3OD) δ CD_2HOD: 1.20 (3H, t, J=7.0 Hz); 1.25 (3H, t, J=7.0 Hz); 3.59-3.83 (5H, m); 5.00 (1H, broad s). ^{13}C NMR (100 MHz) (CD_3OD) δ CD_3OD: 15.6, 15.6, 58.8, 65.0, 66.7, 102.0, 171.1. IR (NaCl, depos. from MeOH): 3233, 2974, 2908, 1671, 1652, 1567, 1498, 1392, 1338, 1296, 1266, 1157, 1114, 1091, 1057, 1027 cm^{-1}. HRMS (FAB^+) calc. for $C_7H_{16}NO_4$ (MH^+) 178.1079; found 178.1082. $[\alpha]_D^{25}$ = +14.4 (*c*=0.55, MeOH).



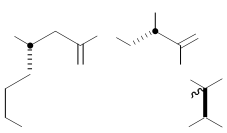
***N*-CBz-DAPA-*N*-(*N,N'*-di-Boc-L-Lys)-OMe (15):** To a suspension of **13** (130 mg, 0.45 mmol., 1 eq.) and **14** (200 mg, 0.45 mmol., 1 eq.) in methylene chloride (8 mL) was added *N*-methylmorpholine (99 μL, 0.90 mmol, 2 eq.) at 0°C under argon. This mixture was stirred 1h. at 0°C and overnight at room temperature. The reaction mixture was then evaporated and taken up in EtOAc with a small amount of methylene chloride and methanol. The organic solution was washed with 0.5 M citric acid, saturated aqueous sodium bicarbonate, and twice with brine. The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated to provide 240 mg (92%) of **15** as a white amorphous solid.

^{1}H NMR (400MHz) (DMSO-d_6, D_2O exchange) δ CD2HSOCD$_3$: 1.16-1.40 (4H, m); 1.34 (18H, s); 2.10 (1H, dd, J=7.5, 14.5 Hz); 2.19 (1H, dd, J=6.0, 14.5 Hz); 2.83 (2H, broad t.); 3.32 (1H, dd, J=7.0, 13.5 Hz); 3.38 (1H, dd, J=5.5, 13.5); 3.60 (3H, s); 3.65 (1H, m); 4.15 (1H, dd, J=6.0, 6.0 Hz); 5.02 (2H, s); 7.22-7.40 (5H, m). ^{13}C NMR (75MHz) ($CDCl_3$) δ $CDCl_3$: 26.8, 28.5, 28.6, 31.9, 40.3, 41.1, 48.0, 52.8, 54.6, 67.1, 79.1, 79.4, 128.2, 128.5, 136.1, 155.8, 156.2, 171.0, 172.0. IR (NaCl, depos. from $CHCl_3$): 3345, 3064, 3035, 2981, 2939, 1748, 1685, 1651, 1530, 1449, 1391, 1366, 1329, 1276, 1169, 1109, 1062, 1028, 1014, 970. HRMS (FAB$^+$) calc. for $C_{28}H_{45}N_4O_9$ (MH^+) 581.3187, found 581.3179. $[\alpha]_D^{25}$=+14.5 (*c*=0.55, $CHCl_3$).



***N*-CBz-DAPA-*N*-(*N,N'*-di-Boc-L-Lys)-OH (16):** To a solution of **15** (99 mg, 0.17 mmol, 1 eq) in THF (1.8 mL) and MeOH (1.8 mL) was added 2N NaOH (217 mL, 0.43 mmol, 2.6 eq) dropwise. After the starting material was consumed by TLC (1h), the reaction was diluted with water and acidified to pH 3 with aq. HCl. The solution was then extracted three times with EtOAc, and then the combined extracts were washed twice with brine. Drying of the organic layer over anhydrous Na_2SO_4 was followed by filtration and evaporation to provide 96 mg (quant) of **16** as a clear oil.

^{1}H NMR (400MHz) (DMSO-d_6) δ DMSO: 1.20-1.40 (4H, m); 1.36 (18H, s); 2.12 (1H, dd, J=7.9, 14.3 Hz); 2.21 (1H, dd, J=5.5, 13.9 Hz); 2.84 (2H, broad m); 3.27 (1H, m); 3.40-3.50 (1H, m); 3.67 (1H, broad s); 4.07 (1H, m); 5.02 (1H, 1/2 ABq, J=13.2 Hz); 5.04 (1H, 1/2 Abq, J=13.2 Hz); 6.62 (1H, d, J=8.7 Hz); 6.73 (1H, broad t); 7.30-7.36 (5H, m); 7.41 (1H, d, J=7.9 Hz); 7.93 (1H, broad t). ^{13}C NMR (100MHz) (DMSO-d_6) δ DMSO: 26.3, 28.3, 28.3, 31.3, 41.2, 47.5, 54.0, 65.5, 77.3, 77.4, 127.8, 127.8, 128.3, 136.9, 155.0, 155.5, 155.9, 170.7, 172.0. IR (NaCl, depos. from $CHCl_3$): 3334, 3066, 3035, 2977, 2934, 1693, 1526, 1454, 1411, 1393, 1367, 1342, 1291, 1251, 1170, 1064, 1028. HRMS (FAB$^+$) calc. for $C_{27}H_{43}N_4O_9$ (MH^+) 567.3030, found 567.3033. $[\alpha]_D^{25}$=-3.3 (*c*=1.0, $CHCl_3$).

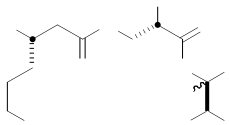


***N*-CBz-DAPA-*N*-(*N,N'*-di-Boc-L-Lys)-DEA-OEt (17):**A stirred solution of EtOH (10 mL) at 0°C was treated with acetyl chloride (2 mL, 30 mmol.). This mixture was warmed to room

temperature and stirred for 30 min. The resulting ethanolic HCl solution was added to a round bottomed flask containing **11** (109 mg, 0.62 mmol., 1 eq.). After stirring the reaction at reflux for 2.5 h, the solvent was removed *in vacuo* to provide 144 mg (96%) of **12** as a yellow solid which was used crude in the next step.

A solution of **16** (50 mg, 0.088 mmol, 1 eq) and **12** (21 mg, 0.088 mmol, 1 eq) in CH_2Cl_2 (1.8 mL) was cooled to 0°C. Dropwise addition of *N*-methylmorpholine (20 μL, 0.18 mmol, 2.05 eq) to the solution was followed by the addition of HOBt (18 mg, 0.13 mmol, 1.5 eq) and EDCI (18 mg, 0.092 mmol, 1.05 eq). The reaction was stirred for 1h at 0°C, diluted with EtOAc and washed with sat. aq. $NaHCO_3$, dilute aq. HCl, and brine twice. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to provide a crude yellow solid which was purified by silica gel chromatography (gradient elution from 25:1 MeOH:CH_2Cl_2 to 20:1 MeOH:CH_2Cl_2) to provide 60 mg (91%) of **17** as an amorphous white solid that is an ~2.6:1 mixture of inseparable epimers (^{1}H NMR).

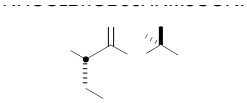
^{1}H NMR (400MHz) (DMSO-d_6) (mixture of epimers, * denotes minor epimer) δ DMSO: 1.07 (3H, t, J=7.0 Hz); 1.07 (3H, t, J=7.0 Hz); 1.18 (3H, t, J=7.0 Hz); *1.17 (3H, t, J=7.0 Hz); 2.12 (1H, dd, J=7.5, 13.9 Hz); 2.21 (1H, dd, J=5.8, 14.1 Hz); 2.84 (2H, symm. M); 3.15-3.40 (2H, m); 3.42-3.52 (2H, m); 3.53-3.73 (3H, m); 4.03-4.16 (2H, symm. m); 4.22 (1H, q, J=7.9 Hz); 4.44-4.49 (1H, m); 4.70 (1H, d, J=5.8 Hz); *4.71 (1H, d, J=4.9 Hz); 5.00 (1H, 1/2 ABq, J=12.6 Hz); *5.01 (1H, 1/2 ABq, J=12.6 Hz); 5.04 (1H, 1/2 ABq, J=12.6 Hz); *5.04 (1H, 1/2 ABq, J=12.6 Hz); 6.61 (1H, d, J=8.7 Hz); 6.72 (1H, t, 5.7 Hz); 7.28-7.39 (6H, m); 7.74 (1H, m); *8.18 (1H, d, J=8.1 Hz); 8.23 (1H, d, J=8.3 Hz). ^{13}C NMR (100 MHz) (5:1 CD_3OD:$CDCl_3$) δ CD_3OD 14.6, 15.6, 15.6, 27.6, 29.0,29.0, 32.9, 41.1, 42.3, 42.8, 56.1, 56.3, 56.4, 62.6, 64.4, 64.6, 64.7, 67.9, 79.8, 80.1, *102.1, 102.2, 129.0, 129.1, 129.5, 137.9, 157.7, 158.2, 158.4, 170.3, 172.5, 174.1. IR (NaCl, depos. from CH_2Cl_2): 3304, 2977, 2933, 2481, 2422, 1743, 1680, 1662, 1534, 1421, 1393, 1367, 1327, 1299, 1253, 1176, 1116, 1071, 1029, 1002 cm-1. HRMS (FAB^+) calc. for $C_{36}H_{60}N_5O_{12}$ (MH^+) 754.4238; found 754.4251.



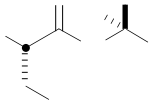
Compound 18. A solution of **17** (50 mg, 0.066 mmol, 1 eq) and $Pd(OH)_2$ (23 mg, 0.033 mmol, 0.5 eq) in absolute ethanol (2 mL) was purged with argon for 10 min. Hydrogen was then bubbled through the solution and a balloon of hydrogen fixed to the top of the flask. After stirring for 1h, the starting material was shown to be consumed by TLC. The reaction was then purged with argon, filtered through celite and evaporated to provide 41 mg (quant) of **18** as a clear oil and an ~2:1 mixture of insepaprable epimers. The deprotected compound was used immediately in the subsequent coupling reaction in order to avoid intramolecular *N,N'*-acyl migration.

^{1}H NMR (400 MHz) (DMSO-d_6, * denotes minor diastereomer) δ: 1.09 (3H, t, J=7.0 Hz); 1.09 (3H, t, J=7.0 Hz); 1.19 (3H, t, J=7.0 Hz); 1.21-1.38 (4H, m); 1.36 (18H, broad s); 1.97 (2H, broad s); 2.16 (1H, dd, J=7.2, 14.1 Hz); 2.21 (1H, dd, J=6.8, 14.3 Hz); 2.85 (2H, m); 3.02 (2H, m); 3.30 (1H, m,); 3.49 (2H, m); 3.62 (2H, m); 3.69 (1H, broad s); 4.11 (2H, m); 4.48 (1H, m); *4.74 (1H, d, J=4.3 Hz); 4.75 (1H, d, J=3.0 Hz); 6.63 (1H, d, J=9.0 Hz); 6.75 (1H, t, J=5.1 Hz); 7.74 (1H, m); 8.23 (1H, m). ^{13}C NMR (75 MHz) (CD_3OD) δ CD_3OD: 14.7, 15.7, 27.8, 29.0, 29.0, 33.5, 41.2, 43.3, 44.8, 55.8, 56.3, 62.7, 64.9, 79.9, 80.1, 102.5, 157.8, 158.4, 170.4, 174.0,

175.7. IR (NaCl, deposited from CH_2Cl_2): 3330, 2977, 2933, 1734, 1690, 1520, 1451, 1415, 1392, 1366, 1344, 1271, 1251, 1171, 1112, 1068, 1025 cm^{-1}. HRMS (FAB^+) calc. for $C_{28}H_{54}N_5O_{10}$ (MH^+) 620.3871; found 620.3853.

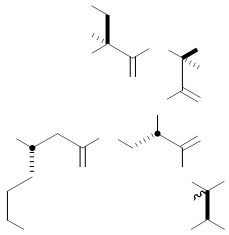


Boc-Asn-Ala-OBn: A round bottomed flask was charged with *N*-Boc-Asn-OH (464mg, 2 mmol, 1 eq.) and alanine benzyl ester hydrochloride (431 mg, 2 mmol., 1 eq.) under argon. Methylene chloride (40 mL) was added and the resulting suspension was cooled to 0°C. Triethylamine (558 μL, 4 mmol, 2 eq.) was added and the suspension became clear. EDCI (403 mg, 2.1 mmol, 1.05 eq.) and HOBt (405 mg, 3 mmol., 1.5 eq.) were added at 0°C, and the reaction was allowed to warm to room temperature with stirring overnight. The reaction mixture was diluted with ethyl acetate, washed with sat. aq. sodium bicarbonate, dilute aqueous HCl, and brine three times. The organic layer was dried over sodium sulfate, filtered, and evaporated to provide 646 mg (82 %) of Boc-Asn-Ala-OBn as a white solid (m.p. 144°C, recryst. EtOAc).
^1H NMR (300MHz) (DMSO-d_6) δ DMSO: 1.29 (3H, d, J=7.3 Hz); 1.36 (9H, s); 2.32 (1H, dd, J=15.0, 8.8 Hz); 2.38 (1H, dd, J=15.0, 4.8 Hz); 4.23-4.36 (2H, m); 5.1 (2H, s); 6.86 (1H, d, J=8.1Hz); 6.89 (1H, s); 7.19 (1H, s); 7.30-7.40 (5H, m); 8.25 (1H, d, J=7.0 Hz). ^{13}C NMR (75MHz) ($CDCl_3$) δ $CDCl_3$: 18.2, 28.6, 37.3, 48.6, 51.1, 67.3, 80.5, 128.3, 128.5, 128.7, 135.4, 155.8, 171.1, 172.4, 173.6. IR (NaCl, depos. from CH_2Cl_2): 3400, 3329, 3206, 2982, 1740, 1692, 1654, 1526, 1456, 1410, 1391, 1368, 1325, 1254, 1169, 1053 cm-1. HRMS (FAB^+) calc. for $C_{19}H_{28}N_3O_6$ (MH^+) 394.1978; found 394.1967. $[\alpha]_D^{25}$: +5.8 (*c*=0.55, CH_2Cl_2).



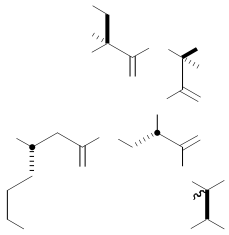
Boc-Asn-Ala-OH (19). A solution of 2*S*-(2*S*-tert-Butoxycarbonylamino-3-carbamoyl-propionylamino)-propionic acid, benzyl ester (386 mg, 0.98 mmol, 1 eq) in MeOH (18 mL) was purged with argon. To this solution was added 10% palladium on carbon (103 mg, 0.097 mmol, 0.1 eq). Hydrogen gas was bubbled through the mixture and a balloon of hydrogen attached to the flask. After stirring for 3h, the starting material was shown to be consumed by TLC (75:20:5 CH_2Cl_2:EtOAc:*i*-PrOH). Argon was bubbled through the reaction and the palladium on carbon removed by filtration through celite. Evaporation of the solvent provided **19** (291 mg, 98% yield) as a white crystalline solid (m.p.=195-197°C, recryst. MeOH / Et_2O).

^1H NMR (400 MHz) (DMSO-d_6, D_2O exchange) δ: 1.24 (3H, d, J=7.5 Hz); 1.35 (9H, s); 2.32 (1H, dd, J=9.0, 15.0 Hz); 2.42 (1H, dd, J=4.5, 15.0 Hz); 4.16 (1H, q, J=7.5 Hz); 4.23 (1H, dd, J=4.5, 9.0 Hz). ^{13}C NMR (100 MHz) (DMSO-d_6, D_2O exchange) δ: 17.5, 28.5, 37.5, 47.9, 51.4, 78.9, 155.6, 171.9, 172.1, 174.3. IR (NaCl, depos. from MeOH): 3584, 3320, 3210, 2980, 2936, 1717, 1662, 1615, 1558, 1539, 1507, 1456, 1393, 1367, 1318, 1296, 1238, 1162, 1054, 1022. HRMS (FAB^+) calc. for $C_{12}H_{22}N_3O_6$ (MH^+) 304.1509; found 304.1502. $[\alpha]_D^{25}$=-14.7 (*c*=0.6, MeOH).



***N*$^{\alpha}$-(Boc-Asn-Ala-)-DAPA-*N*$^{\alpha}$-(*N,N'*-di-Boc-L-Lys)-DEA-OEt (20):** A solution of **19** (22 mg, 0.072 mmol, 1 eq), **18** (45 mg, 0.072 mmol, 1 eq), and HOBt (14 mg, 0.11 mmol, 1.5 eq) in THF (2.3 mL) and DMF (1.3 mL) was cooled to 0°C. Diisopropylcarbodiimide (17 μL, 0.11 mmol, 1.5 eq) was added dropwise and the reaction stirred 5h, allowing to warm to room temp. The reaction mixture was then treated with two drops of water, and the solvent removed *in vacuo*. The crude residue was purified by silica gel chromatography (gradient elution from 5% to 15% MeOH in CH_2Cl_2) to provide the desired dipeptide with some HOBt contamination. A second silica gel purification using the same solvent conditions provided 57 mg (88%) of **20** (2.5:1 mixture of inseparable epimers) as a white amorphous solid.

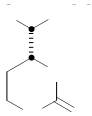
^{1}H NMR (400 MHz) (DMSO-d_6, *denotes minor epimer) δ: 1.07 (3H, t, J=6.8 Hz); 1.08 (3H, t, J=7.0 Hz); 1.17 (3H, t, J=7.0 Hz); 1.21 (3H, d, J=7.0 Hz); 1.20-1.49 (4H, m, partially buried); 1.36 (18H, s); 1.37 (9H, s); 2.17 (2H, m); 2.41 (1H, m); 2.54 (1H, dd, J=5.8, 14.9 Hz, partially buried); 2.84 (2H, m); *3.15 (1H, m); 3.25 (1H, m); 3.37 (1H, m, partially buried); 3.48 (2H, m); 3.59 (2H, m); 3.67 (1H, broad m, partially buried); 4.09 (2H, m); 4.20 (1H, m, partially buried); 4.24 (1H, q, J=7.3 Hz, partially buried); 4.41 (1H, m, partially buried); 4.44 (1H, dd, J=6.0, 8.1 Hz partially buried); 4.72 (1H, d, J=6.0 Hz); 6.61 (1H, d, J=8.7 Hz); 6.73 (1H, t, J=5.3 Hz); 6.93 (2H, m); *7.36 (1H, broad s); 7.37 (1H, broad s); *7.55 (1H, t, J=5.9 Hz); 7.64 (1H, t, J=5.6 Hz); *7.95 (1H, d, J=6.8 Hz); 8.00 (1H, d, J=6.6 Hz); *8.04 (1H, d, J=8.9 Hz); 8.08 (1H, d, J=8.3 Hz); 8.11 (1H, d, J=8.3 Hz); *8.16 (1H, d, J=8.1 Hz). ^{13}C NMR (100 MHz) (DMSO-d_6, *denotes minor epimer) δ: 14.0, 15.0, 15.0, 17.9, *18.2, 26.4, 28.2, 28.3, 28.3, 31.6, 37.2, 40.3, 41.2, 47.5, 48.7, 51.1, 52.4, 54.8, 60.6, 62.3, 62.5, 62.7, 77.3, 77.4, 78.3, 100.3, 155.1, 155.1, 155.5, 168.9, 169.8, 170.5, 171.4, 171.9, 172.1. IR (NaCl, depos. from 10% MeOH in CH_2Cl_2): 3461, 3285, 3077, 2978, 2932, 1744, 1691, 1665, 1641, 1547, 1529, 1449, 1392, 1367, 1326, 1272, 1253, 1174, 1121, 1068, 1028. HRMS (FAB$^+$) calc. for $C_{40}H_{73}N_8O_{15}$ (MH^+) 905.5195; found 905.5199.



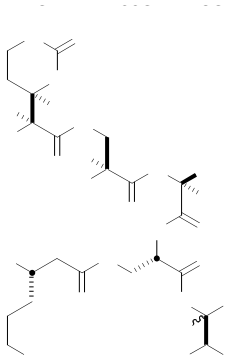
***N*$^{\alpha}$-(*N*$^{\alpha}$-Boc-DAPA-Ala-)-DAPA-*N*$^{\alpha}$-(*N,N'*-di-Boc-L-Lys)-DEA-OEt (21):** To a solution of **20** (57 mg, 0.063 mmol, 1 eq) in acetonitrile (1.15 mL) and water (1.15 mL), bis(trifluoroacetoxy)iodosobenzene (41 mg, 0.094 mmol, 1.5 eq) was added followed by dimethylformamide (1.15 mL). After stirring the reaction for 15 min, pyridine (15 mL, 0.19 mmol, 3 eq) was added, and the resulting solution stirred for an additional 2.5 h. The reaction

was then reduced to ca. 1/2 its volume by rotary evaporation, diluted with EtOAc, and partitioned with brine. Removal of the organic layer was followed by extraction of the aqueous layer with EtOAc. Upon drying of the combined organic extracts over anhydrous Na_2SO4, the solids were removed by filtration and the filtrate concentrated to dryness. Silica gel chromatography (gradient elution from 5% to 15% MeOH in CH_2Cl_2) provided 48 mg (87%) of **21** as a clear glass that was an ~2.6:1 mixture of epimers. The product was used immediately for the subsequent coupling reaction in order to avoid *N,N'*-acyl migration.

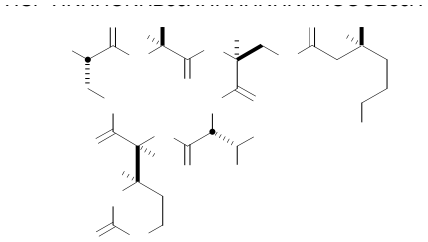
^{1}H NMR (300 MHz) (DMSO-d_6, 353 K, D_2O exchange, * denotes minor diastereomer) δ DMSO: 1.10 (3H, t, J=7.0 Hz); 1.10 (3H, t, J=7.0 Hz); *1.19 (3H, t, J=7.1 Hz); 1.20 (3H, t, J=7.1 Hz); *1.27 (3H, d, J=7.2 Hz); 1.27 (3H, d, J=7.1 Hz); 1.29-1.46 (4H, m, partially buried); 1.37 (18H, s); 1.39 (9H, s); 2.22 (2H, m); 2.86 (2H, m, partially buried); 2.89 (2H, m, partially buried); 3.29 (1H, dd, J=7.9, 13.6); 3.37 (1H, dd, J=5.7, 13.6); 3.52 (2H, m, partially buried); 3.61 (2H, m, partially buried); 3.69 (1H, broad m, partially buried); 4.01 (1H, t, J=6.1 Hz); 4.11 (2H, m); 4.25 (1H, q, J=7.1 Hz); *4.26 (1H, q, J=7.1 Hz); 4.43 (1H, m, partially buried); 4.45 (1H, d, J=5.3 Hz); 4.73 (1H, d, J=5.3 Hz); *4.74 (1H, d, J=5.3 Hz). ^{13}C NMR (100 MHz) (CD_3OD) δ CD_3OD: 14.7, 15.6, 15.6, 15.6, 17.7, 27.8, 28.8, 29.0, 29.0, 33.1, 41.3, 42.1, 43.1, 44.5, 51.3, 51.4, 54.9, 56.6, 62.7, 64.6, 64.8, 80.0, 80.2, 81.0, 102.4, 158.0, 158.1, 158.7, 170.8, 172.0, 173.8, 174.5, 175.3. IR (NaCl, depos. from $CHCl_3$): 3306, 3061, 2978, 2934, 1683, 1668, 1523, 1454, 1392, 1367, 1345, 1250, 1202, 1170, 1113, 1065, 1024. HRMS (FAB^+) calc. for $C_{39}H_{73}N_8O_{14}$ (MH^+) 877.5246, found 877.5236.



***N*δ-CBz-capreomycidine (22).** A solution of **2** (19 mg, 0.08 mmol, 1 eq) in dd H_2O (1.5 mL) and 2N NaOH (50 μL, prepared from dd H_2O) was treated with benzyl chloroformate (23 μL, 0.16 mmol, 2 eq) and stirred at room temp for 1h. At that point, and additional 100 μL of 2N NaOH was added and the reaction stirred an additional 1h. The mixture was diluted with dd H_2O and extracted 2x with CH_2Cl_2. Adjustment of the cloudy aqueous layer to pH 3 with aq. HCl (prepared from dd H_2O), was followed by evaporation to dryness. The residue was then dissolved with dd H_2O and loaded onto a Waters C18 Sep-Pak cartridge (prepared by washing with 3 x 5 mL MeCN followed by 3 x 5 mL dd H_2O). Any water insoluble material was kept in the flask. After washing the loaded Sep-Pak cartridge with dd H_2O (~7 mL) the product was eluted back into the flask containing the water insoluble material with 3 x 5 mL MeCN and 3 x 5 mL MeOH. The solvent was removed *in vacuo* to provide 15 mg (56%) of crude **22** as a white solid. This material was used crude in the subsequent coupling reaction.



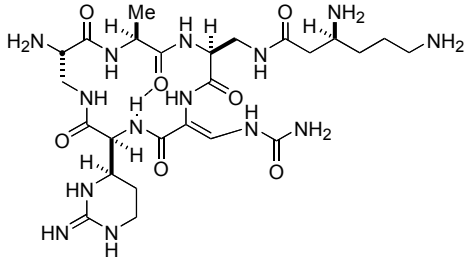
Compound 23. A solution of **21** (20 mg, 0.023 mmol, 1 eq), **22** (8 mg, 0.023 mmol, 1 eq), and HOBt (5 mg, 0.035 mmol, 1.5 eq) in DMF (1 mL) and THF (0.6 mL) was cooled to 0°C and stirred. EDCI (7 mg, 0.035 mmol, 1 eq) was then added, and the reaction stirred for 5h, at which point the starting material appeared to be consumed by TLC. The solvent was then removed *in vacuo* and the resulting residue was subjected to silica gel chromatography (gradient elution from 15% to 25% MeOH in CH_2Cl_2) to provide a partially pure product which was chromatographed again under the same conditions to provide 25 mg (89%) of **23** as a glass. The product was isolated as an inseparable 2.6:1 mixture of epimers.
^{1}H NMR (400 MHz) (DMSO-d_6, 338 K, D_2O exchange, * denotes minor diastereomer) δ DMSO: 1.08 (3H, t, J=6.8 Hz); 1.08 (3H, t, J=7.0 Hz); 1.17 (3H, t, J=7.0 Hz); *1.24 (3H, d, J=7.3 Hz); 1.24 (3H, d, J=7.0 Hz); 1.25-1.42 (4H, m, partially buried); 1.35 (18H, s); 1.38 (9H, s); 1.68 (1H, m); 1.87 (1H, m); 2.20 (2H, m); 2.87 (2H, t, J=5.8 Hz); 3.20 (1H, m); 3.23-3.35 (2H, m, partially buried); 3.40-3.53 (3H, m); 3.59 (2H, m); 3.69 (2H, m); 4.09 (3H, m); 4.16 (1H, d, J=6.8 Hz); 4.26 (1H, q, J=6.8 Hz); 4.44 (1H, m); 4.47 (1H, d, J=5.3 Hz); 4.71 (1H, d, J=5.3 Hz); *4.73 (1H, d, J=5.5 Hz); 5.05 (2H, s); 7.30-7.36 (5H, m). ^{13}C NMR (100 MHz) (CD_3OD, * denotes minor diastereomer) δ CD_3OD: 14.7, 15.6, 15.7, 17.8, 23.9, 27.8, 28.9, 29.0, 29.0, 33.3, 37.9, 41.3, 42.2, 42.6, 43.0, 43.3, 51.3, 51.4, 54.3, 54.9, 55.2, 56.5, 59.4, 62.8, 64.7, 64.8, 64.9, 68.4, 80.0, 80.3, 81.1, *102.3, 102.5, 129.4, 129.5, 129.7, 138.0, 156.0, 157.6, *158.1, 158.7, 158.8, 170.5, 172.3, 172.9, 173.4, 174.3, 175.4. IR (NaCl, deposited from CH_2Cl_2/MeOH): 3302, 3064, 2977, 2934, 1669, 1522, 1455, 1392, 1367, 1249, 1169, 1064. HRMS (FAB$^+$) calc. for $C_{53}H_{89}N_{12}O_{17}$ (MH^+) 1165.6469; found 1165.6459.



Macrocycle 24: A solution of **23** (24 mg, 0.020 mmol, 1 eq)) in ethanol (3 mL) was purged with argon for 10 min. Addition of 10% Pd/C (15 mg, 0.014 mmol, 0.7 eq) was followed by careful bubbling of hydrogen gas through the solution. After fixing a hydrogen balloon to the flask, the reaction was stirred for 2h then purged with argon. The 10% Pd/C was removed by filtration through celite and the filter cake was washed with absolute ethanol. The filtrate was concentrated to dryness. Dissolution of the crude hydrogenation product in absolute ethanol (1.3 mL) was followed by addition of 1N LiOH (80 μL, 0.080 mmol, 4 eq). At 1h, and additional amount of

1N LiOH (40 mL, 0.040 mmol, 2 eq) was added. After 1.5h total reaction time, the reaction mixture was diluted with dd H_2O and adjusted to ~pH 6 with aqueous HCl and evaporated to provide an off-white residue. Dissolution of the residue in dd H_2O was followed by loading onto a Waters C18 sep-pak cartridge (prepared by washing with 3 x 5 mL acetonitrile followed by 3 x 5 mL dd H_2O). The cartridge was washed with 2 x 2 mL dd H_2O which was subsequently discarded. Isolation of the amino acid was accomplished by elution from the Sep-Pak cartridge with 3 x 5 mL acetonitrile and 3 x 5 mL methanol followed by evaporation of the solvent. The crude amino acid was then dissolved in DMF (4 mL) and CH_2Cl_2 (20 mL) followed by the addition of EDCI (30 mg, 0.16 mmol, 7.8 eq) and HOAt (20 mg, 0.15 mmol, 7.3 eq). After stirring the reaction at room temperature for 36h, the solvent was removed *in vacuo* to provide a yellow oily residue. The residue was chromatographed on silica gel (gradient elution from 15% to 30% MeOH in CH_2Cl_2) to provide **24** with some HOAt contaminant present. After dissolution of the residue in 10% MeOH in CH_2Cl_2 and partitioning with brine, the organic layer was removed, dried over Na_2SO_4, filtered, and evaporated. The resulting residue was triturated with 10% MeOH in CH_2Cl_2 and the solvent evaporated to provide 4 mg (20%, opaque glass) of pure **24** as a single diastereomer.

^{1}H NMR (400 MHz) (CD_3OD) □: 1.22 (3H, t, J=7.0 Hz); 1.23 (3H, t, J=6.4 Hz); 1.37 (3H, d, J=7.3 Hz); 1.40-1.61 (4H, m); 1.43 (27H, broad s); 1.81 (1H, m); 2.04 (1H, m); 2.29 (1H, dd, J=6.4, 14.3 Hz); 2.36 (1H, dd, J=7.0, 14.3 Hz); 3.03 (2H, broad t); 3.27-3.41 (2H, m, partially obscured); 3.54-3.86 (9H, m); 4.06 (1H, dd, J=4.9, 9.2 Hz); 4.28 (2H, m); 4.35 (1H, m); 4.59 (1H, d, J=3.6 Hz); 4.79 (1H, d, J=3.2 Hz); 5.06 (1H, d, J=3.8 Hz). ^{13}C NMR (125 MHz) (CD_3OD) □ CD_3OD: 15.7, 15.7, 18.0, 24.2, 27.9, 28.8, 29.0, 29.0, 33.3, 37.7, 40.1, 41.3, 42.3, 43.2, 50.4, 51.2, 54.2, 55.0, 56.4, 58.0, 65.1, 65.3, 65.3, 80.1, 80.3, 81.1, 102.0, 156.2, 157.4, 158.1, 158.7, 171.1, 171.9, 172.2, 172.5, 174.4, 177.1. IR (NaCl, depos. from CH_2Cl_2): 3583, 3287, 3070, 2976, 2931, 1667, 1518, 1454, 1392, 1366, 1303, 1249, 1169, 1107, 1065. HRMS (FAB^+) calc. for $C_{43}H_{77}N_{12}O_{14}$ (MH^+) 985.5682; found 985.5678. [□]$_D^{25}$=-47 (*c*=0.1, MeOH).

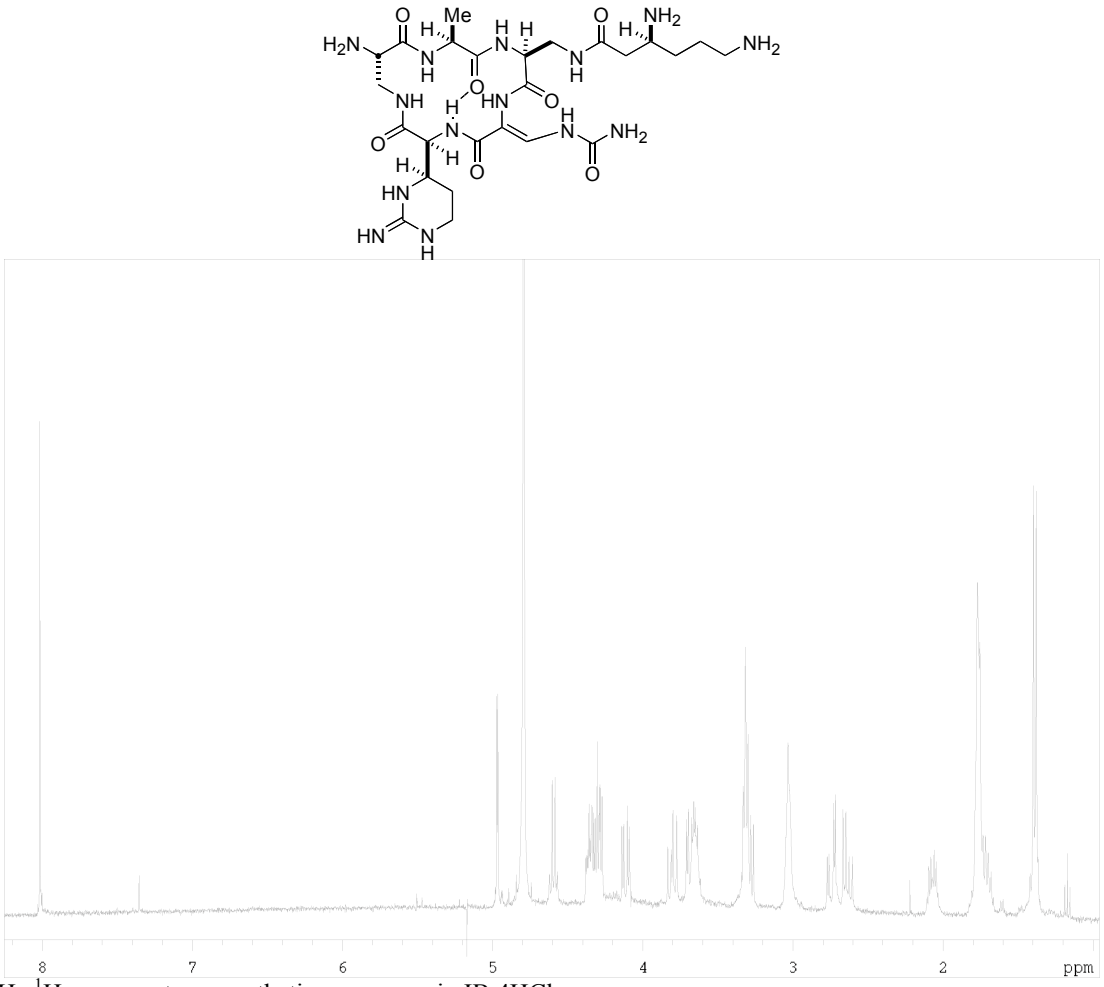


Capreomycin IB·4HCl (1b). Macrocycle **24** (5 mg, 0.0049 mmol, 1 eq) was dissolved in 99% formic acid (500 □L, distilled from phthallic anhydride) and stirred at room temperature for 1.25h. The formic acid was removed *in vacuo*, and the resulting residue dissolved in acetone (400 □L) and 2N HCl (400 □L). After refluxing the resulting solution under argon for 10 min, the solution was cooled to room temperature and urea (35 mg, 0.58 mmol, 119 eq) was added. After stirring for 14h, the solvent was removed *in vacuo*, and the resulting residue was triturated with absolute ethanol to provide 2 mg (50%) of capreomycin IB·4HCl (**1b**) as a white precipitate. The synthetic product matched the natural product by ^{1}H NMR, optical rotation, and TLC (30:10:1 phenol : H_2O : 28% aqueous ammonia, R_f=0.29).

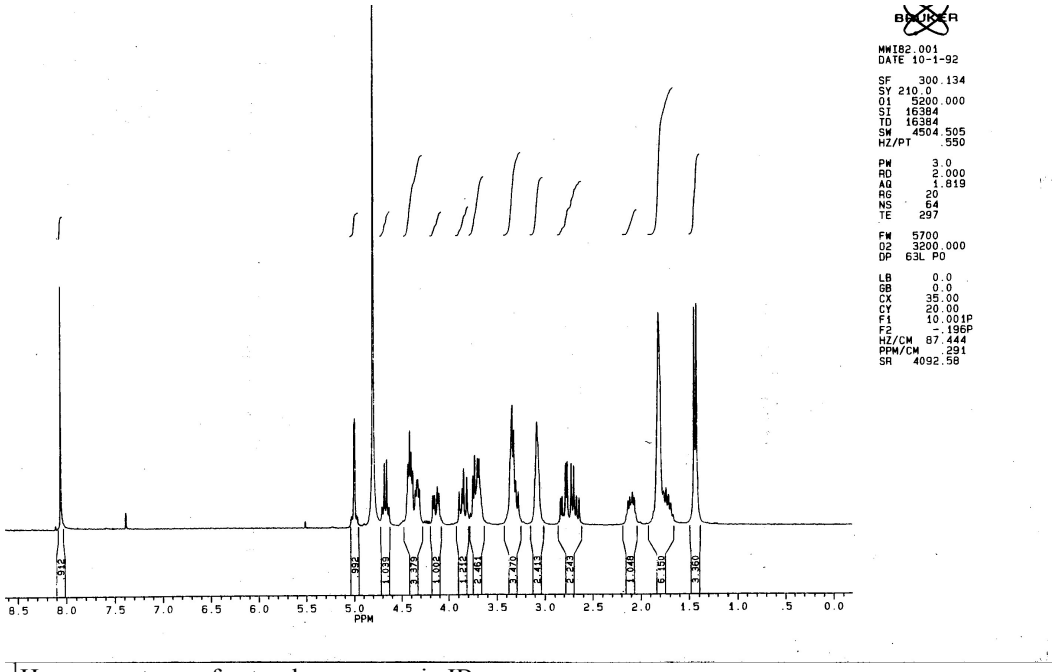
Synthetic **1b** was then combined with a mixture of natural capreomycin IA and IB (**1a,b**) in water, 2 drops of concentrated sulfuric acid were added and the solution evaporated. Absolute

ethanol was added to the residue, and the precipitate collected. Spectral analysis of the mixture of synthetic and natural material (^{1}H NMR) showed that all peaks corresponding to **1b** increased in intensity compared to **1a**.

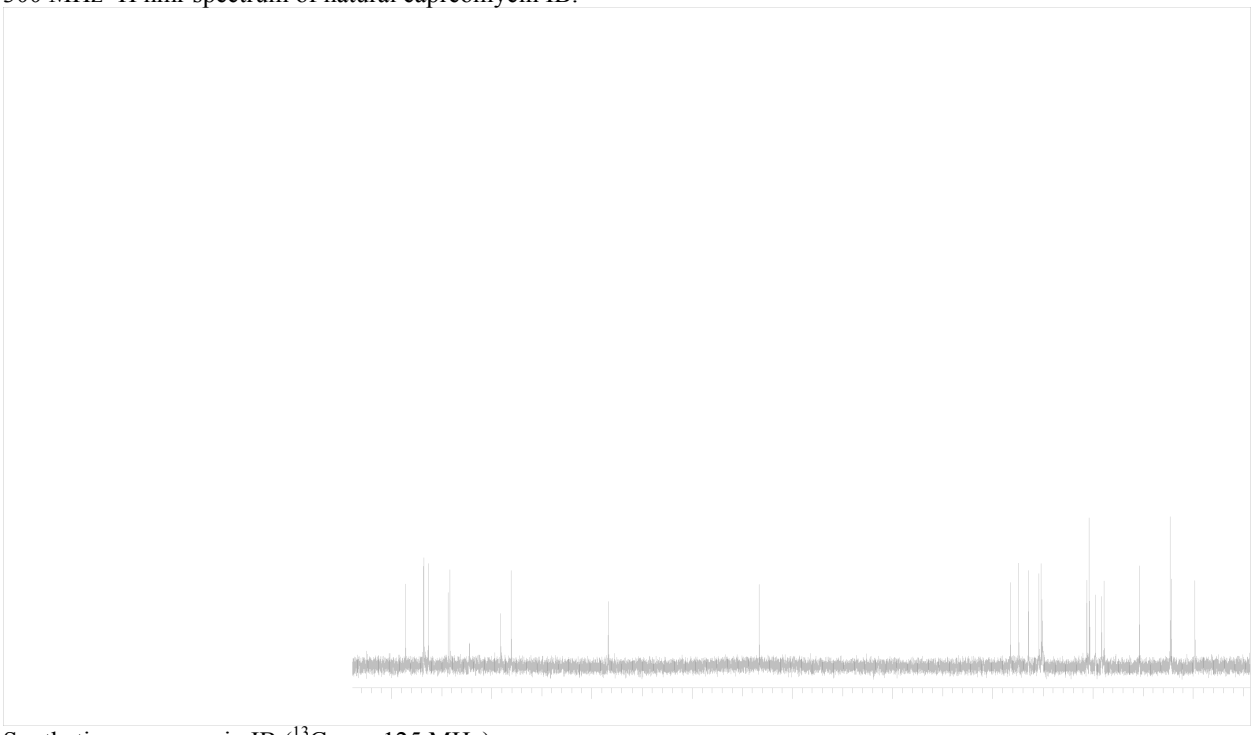
^{1}H NMR (400MHz) (D_2O) □ EtOH: 1.38 (3H, d, J=7.3 Hz); 1.72 (1H, m, partially buried); 1.75 (2H, broad s); 1.77 (2H, broad s); 2.07 (1H, dddd, J=5.1, 5.1, 5.1, 13.6 Hz); 2.64 (1H, dd, J=8.3, 16.4 Hz); 2.74 (1H, dd, J=4.7, 16.2 Hz); 3.03 (2H, broad s); 3.29 (1H, dd, J=7.7, 14.5 Hz, partially buried); 3.31 (2H, t, J=5.8 Hz); 3.65 (1H, m, partially buried); 3.68 (1H, dd, J=5.3, 14.1 Hz, partially buried); 3.80 (1H, dd, J=9.6, 14.1 Hz); 3.68 (1H, dd, J=4.9, 14.1 Hz); 4.27-4.34 (2H, m, partially buried); 4.36 (1H, ddd, J=2.8, 5.3, 8.1 Hz, partially buried); 4.59 (1H, q, J=7.0 Hz); 4.97 (1H, d, J=2.8 Hz); 8.02 (1H, s). ^{13}C NMR (dd H_2O w/ 10% D_2O) (125 MHz) □: 19.6, 24.3, 24.6, 30.7, 37.8, 38.2, 39.5, 40.7, 41.2, 50.1, 50.3, 50.8, 52.8, 54.8, 56.5, 106.5, 136.7, 156.1, 158.2, 168.3, 168.7, 172.7, 173.5, 1735., 177.2. $[\square]_D^{25}$, synthetic: -48 (*c*=0.05, dd H_2O), natural: -44.6 (*c*=0.5, dd H_2O). Electrospray MS (ES^+) ($M+H^+$): calc.: 653.35, found 653.40.



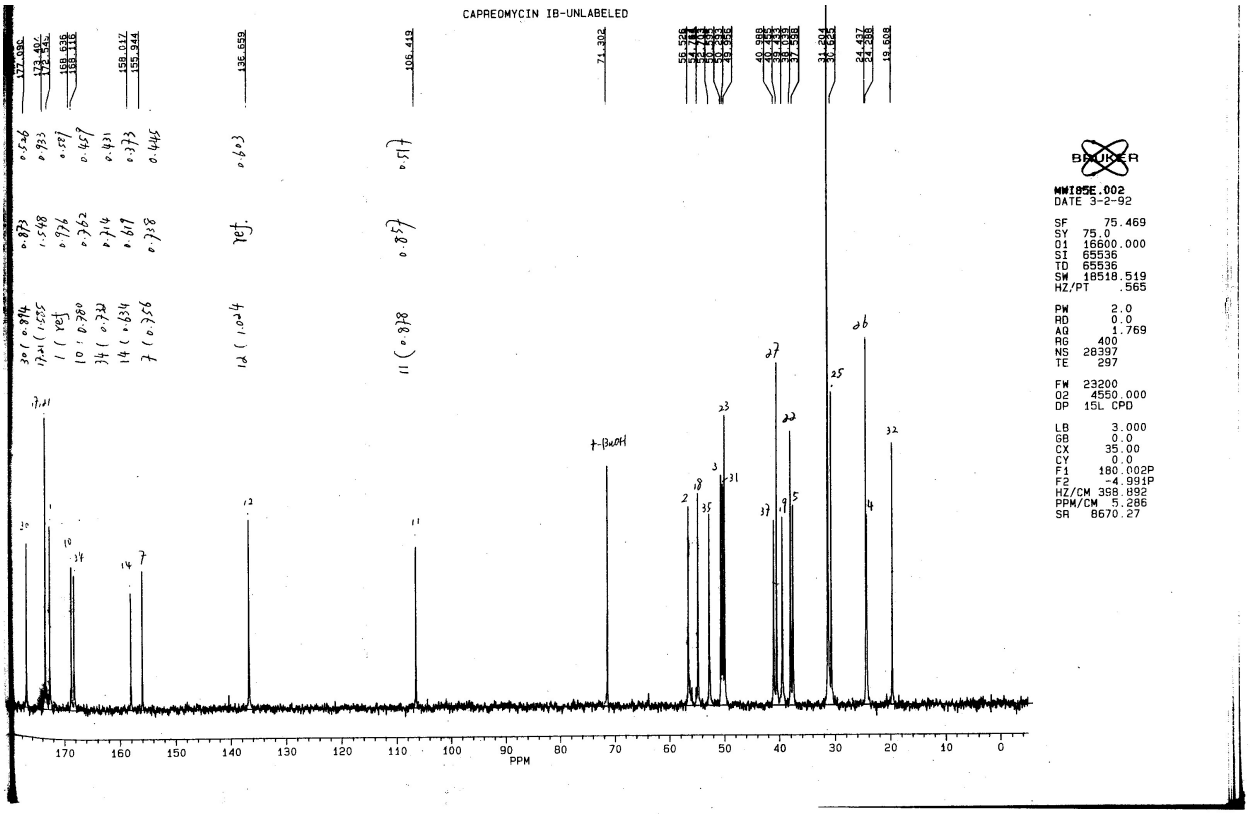
300 MHz ^{1}H nmr spectrum synthetic capreomycin IB·4HCl



300 MHz ^{1}H nmr spectrum of natural capreomycin IB.



Synthetic capreomycin IB (^{13}C nmr 125 MHz)



Natural capreomycin IB (^{13}C nmr 75 MHz).