Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Anthranilic Acid-containing Cyclic Tetrapeptides: At The Crossroads Of Conformational Rigidity And Synthetic Accessibility

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A. General Experimental Information

All reactions were carried out under an inert atmosphere (nitrogen or argon where stated) with dry solvents under anhydrous conditions. Glassware for anhydrous reactions was dried in an oven at 140 ºC for minimum 6 h prior to use. Dry solvents were obtained by passing the previously degassed solvents through activated alumina columns. Yields refer to chromatographically and spectroscopically (^{1}H-NMR) homogeneous materials, unless otherwise stated. Reagents were purchased at a high commercial quality (typically 97 % or higher) and used without further purification, unless otherwise stated. Analytical thin layer chromatography (TLC) was carried out on Merck silica gel plates with QF-254 indicator and visualized by UV and/or phosphomolybdic acid (PMA) stain. Flash column chromatography was performed using silica gel 60 (Silicycle, 230-400 mesh). ^{1}H and ^{13}C spectra were recorded on a 400 MHz spectrometer and were calibrated using residual non-deuterated solvent as an internal reference. The following abbreviations or combinations thereof were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, p = pentet, br = broad singlet, dd = doublet of doublet. Melting points were recorded on an automated melting point apparatus and are uncorrected.

All of the HPLC analyses were carried out with UV detection monitored at 254 nm. Analytical reversed-phase HPLC analyses were performed with a 250 × 4.6 mm C-18 column using gradient conditions (10−90% acetonitrile in water, flow rate = 0.75 mL/min, injection volume = 30 μL).

All the UV spectrums were recorded on a UV spectrometer using a 10 mm quartz cuvette at 20 μM in acetonitrile. Circular Dichroism spectrums were recorded on a CD spectrometer using a 2 mm quartz cuvette at 200 μM in acetonitrile.

B. Synthesis of Cyclic Peptides

General Procedures

Coupling Method I

Boc or Cbz protected amino acid (Pg-AA-OH, 2.0 mmol, 1.0 equiv.) was added to a solution of HOAt (408 mg, 3.0 mmol, 1.5 equiv.) and NMM (220 μL, 2.0 mmol, 1.0 equiv.) in DCM (3.0 mL) at 0 ^{o}C under N_2. The resulting mixture was stirred at 0 ^{o}C for 10 min and then methyl anthranilate (906 mg, 6.0 mmol, 3.0 equiv.) was added, followed by the addition of EDC•HCl (575 mg, 3.0 mmol, 1.5 equiv.) in one portion. The reaction mixture was allowed to warm to room temperature over 1 h and was stirred at room temperature for 6 h. 30 mL DCM was added to dilute the solution and the organic phase was washed with 0.2 M HCl aqueous solution (30 mL × 5). The organic phase was further washed with brine (10 mL), saturated $NaHCO_3$ solution (20 mL × 2) and brine (10 mL), dried over $MgSO_4$ and filtered. The solvent was removed under vacuum to give the crude material. The crude material was used in the next step without further purification. An analytically pure sample was prepared by crystallization from DCM/hexanes.

Coupling Method II

EDC•HCl (287 mg, 1.5 mmol, 1.5 equiv.) was added to a mixture of Boc or Cbz protected amino acid (Pg-AA-OH, 1.0 mmol, 1.0 equiv.), *N*-terminus deprotected linear peptide (1.0 mmol, 1.0 equiv.), HOBt (203 mg, 1.5 mmol, 1.5 equiv.) and DIPEA (175 μL, 1.0 mmol, 1.0 equiv.) in DCM (10.0 mL) at 0 ^{o}C under N_2. The reaction mixture was stirred at 0 ^{o}C for 10 min and was allowed to warm to room temperature. After overnight reaction at room temperature, 40 mL DCM was added to dilute the solution and the organic phase was washed with 0.1 M HCl aqueous solution (30 mL × 2). The organic phase was further washed with brine (10 mL), saturated $NaHCO_3$ solution (30 mL × 2) and brine (10 mL), dried over $MgSO_4$ and filtered. The solvent was removed under vacuum to give the crude material. The crude material was used in the next step without further purification. An analytically pure sample can be prepared by crystallization from DCM/hexanes.

Boc Deprotection Procedure I

Boc protected intermediate (1.0 mmol) was dissolved in DCM (5 mL) at 0 ^{o}C, and the mixture was stirred at 0 ^{o}C for 10 min. TFA (5 mL) was added in one portion and the reaction mixture was stirred for 30 min at room temperature. Toluene (30

mL) was added and the solution was concentrated. Residual TFA was azeotroped 3 times with toluene (3 × 30 mL) to give the crude product. Saturated $NaHCO_3$ (20 mL) was added and the aqueous layer was extracted with DCM (30 mL). The organic layer was further washed with saturated $NaHCO_3$ (20 mL × 2) and brine (10 mL), dried over $MgSO_4$ and filtered. The solvent was removed under vacuum to give the crude material. The crude material was used in the next step without further purification.

Boc Deprotection Procedure II

4 M HCl in dioxane (5 mL) was added to Boc protected intermediate (1.0 mmol) at 0 ^oC, and the mixture was stirred at room temperature for 30 min. Toluene (20 mL) was added and the solution was concentrated. Residual dioxane was azeotroped 3 times with toluene (3 × 20 mL) and then dried under high vacuum for 3 h to give the HCl salt of the Boc deprotected product.

^{t}Bu Deprotection Procedure

^{t}Bu protected cyclic peptides (0.08 mmol) was dissolved in 1:1 TFA/DCM containing 5 % (v/v) triethylsilane (TES) (0.8 mL) at 0 ^oC, and the mixture was stirred at 0 ^oC for 10 min. The reaction mixture was stirred for an additional 60 min at room temperature. Toluene (2 mL) was added and the solution was concentrated. Residual TFA was azeotroped 3 times with toluene (3 × 2 mL) to give the crude product. The crude product was purified with flash chromatography (4 – 8 % MeOH in DCM containing 0.1 % AcOH) to give the pure product.

Cbz Deprotection Procedure

To a solution of Cbz protected substrate in methanol (0.1 M) under nitrogen was added 10 wt % Pd/C (0.05 equiv. Pd). The reaction was placed under an atmosphere of hydrogen (1 atm, balloon) for 12 h. After the reaction finished, the flask was purged with N_2. The reaction mixture was filtered over a Celite pad and concentrated to afford the product. The product was used in the next step without further purification.

Bn Deprotection Procedure

To a solution of Cbz protected substrate in methanol (0.05 M) under nitrogen was added 10 wt % Pd/C (0.15 equiv. Pd). The reaction was placed under an atmosphere of hydrogen (1 atm, balloon) for 24 h. After the reaction finished, the flask was purged with N_2. The reaction mixture was filtered over a Celite pad and

concentrated to afford the product. The product was purified with flash chromatography (4 – 8 % MeOH in CH_2Cl_2) to give the pure product.

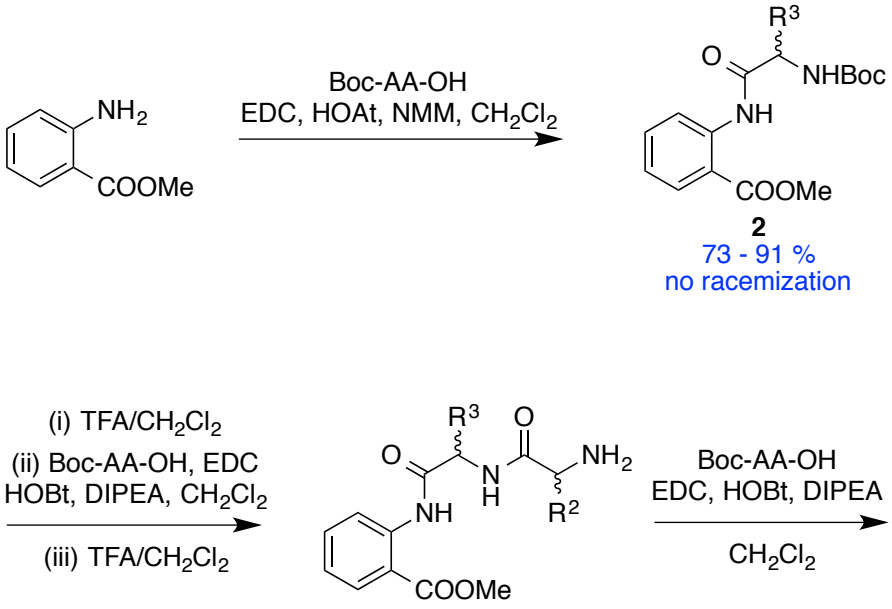
Hydrolysis of Methyl Ester

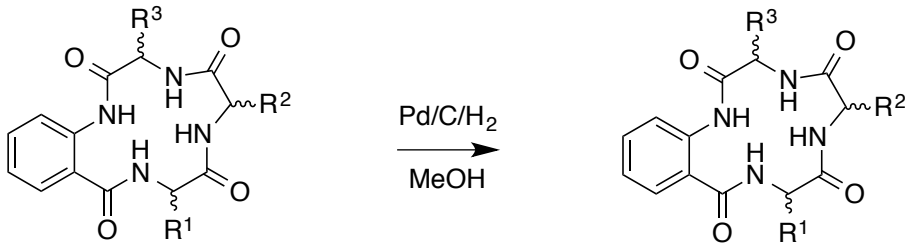
The linear tetrapeptide (0.2 mmol) was added to 2.7 mL THF and the resulting suspension was stirred at room temperature for 10 min. A 0.3 M aqueous solution of LiOH (1.3 mL, 0.4 mmol, 2.0 equiv.) was added and the resulting mixture was stirred at room temperature for 2h. The mixture was concentrated to remove most THF and 0.1 M HCl solution (10 mL) was added. The white solid was filtered, washed with 0.1 M HCl and then water and dried under vacuum to give the product.

Procedure for Cyclization

The deprotected linear peptide (0.2 mmol, 1.0 equiv.) was dissolved in DMF/DCM (1:1 mixture) to give a 2 mM final concentration. HOAt (81.6 mg, 0.6 mmol, 3.0 equiv.) and NMM (88 μL, 0.8 mmol, 4.0 equiv.) were added to the solution, followed by EDC•HCl (114.6 mg, 0.6 mmol, 3.0 equiv.). The resulting mixture was stirred at room temperature under N_2 for 48 h and then concentrated in vacuum to give the crude mixture. The crude product was purified with flash chromatography (3 – 7 % MeOH in DCM) to give the pure product.

***Boc approach* to products 1**





cyclo-LAla-LAla-LPhe-Anth (LLL-**1aaf**), 41 %
cyclo-DAla-LAla-LPhe-Anth (DLL-**1aaf**), 55 %
cyclo-LAla-DAla-LPhe-Anth (LDL-**1aaf**), 48 %
cyclo-DAla-DAla-LPhe-Anth (DDL-**1aaf**), 44 %
cyclo-DGlu'-DAla-LPhe-Anth (DDL-**1e'af**), 35 %
cyclo-LVal-LSer'-LTyr'-Anth (LLL-**1vs'y'**), 45 %
cyclo-DTyr'-DSer'-DVal-Anth (DDD-**1y's'v**), 31 %

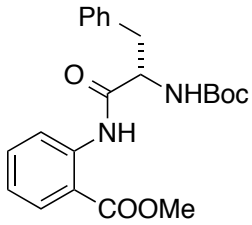
cyclo-LVal-LSer-LTyr-Anth (LLL-**1vsy**), 87 %

Scheme S1. *Boc approach* to products **1**

General Procedures for Boc Approach in Solution

Methyl anthranilate was coupled to the first Boc protected amino acid with "*Coupling Method I*". The Boc protection group was deprotected with procedure described in "*Boc Deprotection Procedure I*" and the resulting material was coupled with the second Boc protected amino acid with "*Coupling Method II*". The tripeptide intermediate was deprotected with "*Boc Deprotection Procedure I*" and coupled with the third Boc amino acid with "*Coupling Method II*" to give the protected tetrapeptide intermediate. The methyl ester of this intermediate was hydrolyzed with the procedure "*Hydrolysis of Methyl Ester*" and the *N*- terminus Boc group was removed with "*Boc Deprotection Procedure II*". The linear tetrapeptide was cyclized with "*Procedure for Cyclization*" to give the cyclic peptide product. If necessary, the cyclic peptide product was deprotected with "*Bn Deprotection Procedure*" or "*^{t}Bu Deprotection Procedure*" to give the final deprotected product.

Methyl (*S*)-2-(2-((*tert*-butoxycarbonyl)amino)-3-phenylpropanamido)benzoate (L-**2f**)

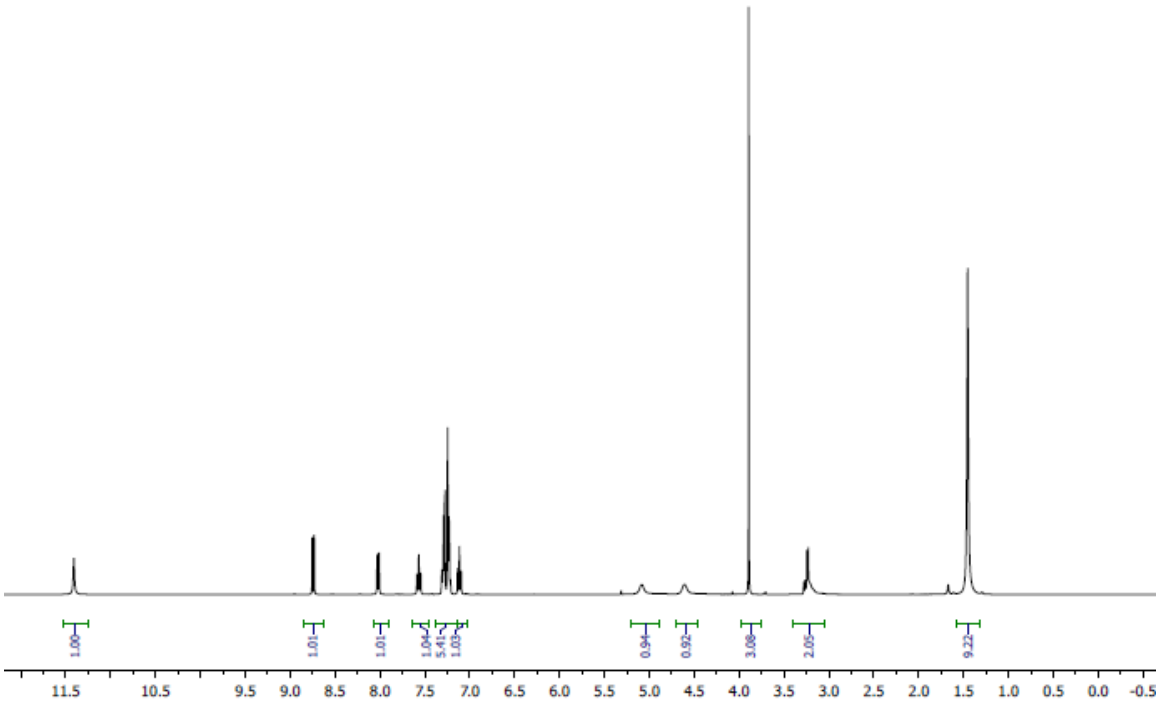


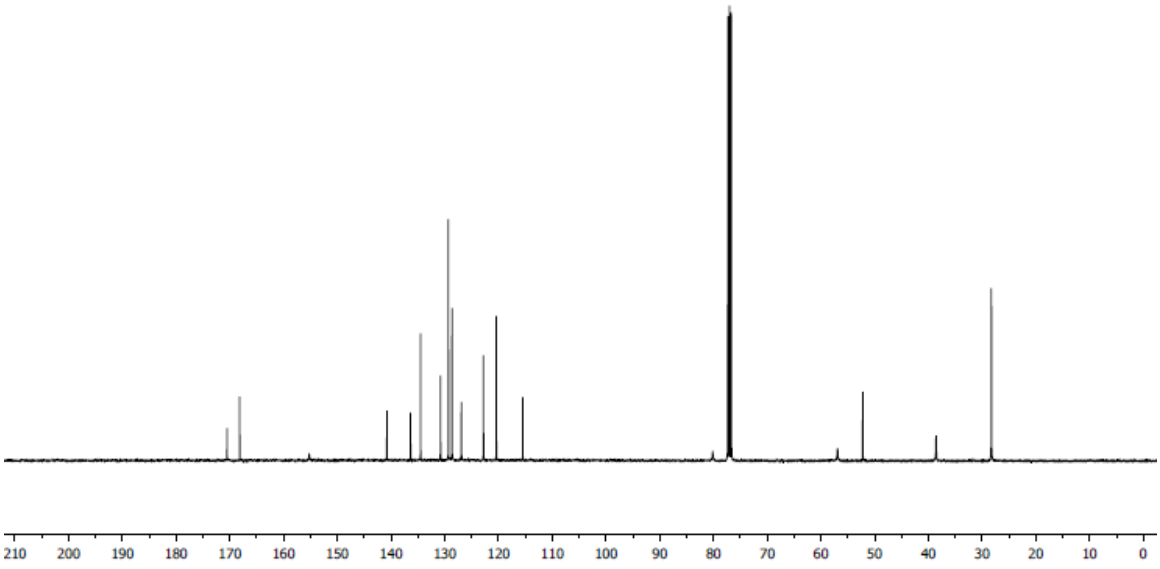
White solid, 83 %; mp = 144.8-145.3 °C;

^{1}H-NMR (400 MHz, $CDCl_3$) δ 11.41 (s, 1H), 8.74 (dd, *J* = 8.5, 0.9 Hz, 1H), 8.02 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.63-7.51 (m, 1H), 7.38-7.15 (m, 5H), 7.15-7.03 (m, 1H), 5.08 (s, 1H), 4.68-4.57 (m, 1H), 3.89 (s, 3H), 3.30-3.10 (m, 2H), 1.46 (s, 9H);

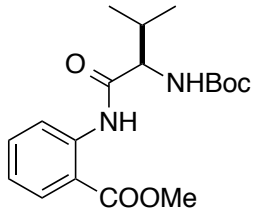
^{13}C-NMR (101 MHz, $CDCl_3$) δ 170.5, 168.1, 155.2, 140.7, 136.3, 134.5, 130.7, 129.3, 128.6, 126.9, 122.8, 120.4, 115.4, 80.1, 56.9, 52.2, 38.5, 28.2;

HRMS (ESI+) m/z calcd for $C_{22}H_{27}N_2O_5$ $(M+H)^+$ 399.1920; found 399.1924.





Methyl (*R*)-2-(2-((*tert*-butoxycarbonyl)amino)-3-methylbutanamido)benzoate (D-**2v**)

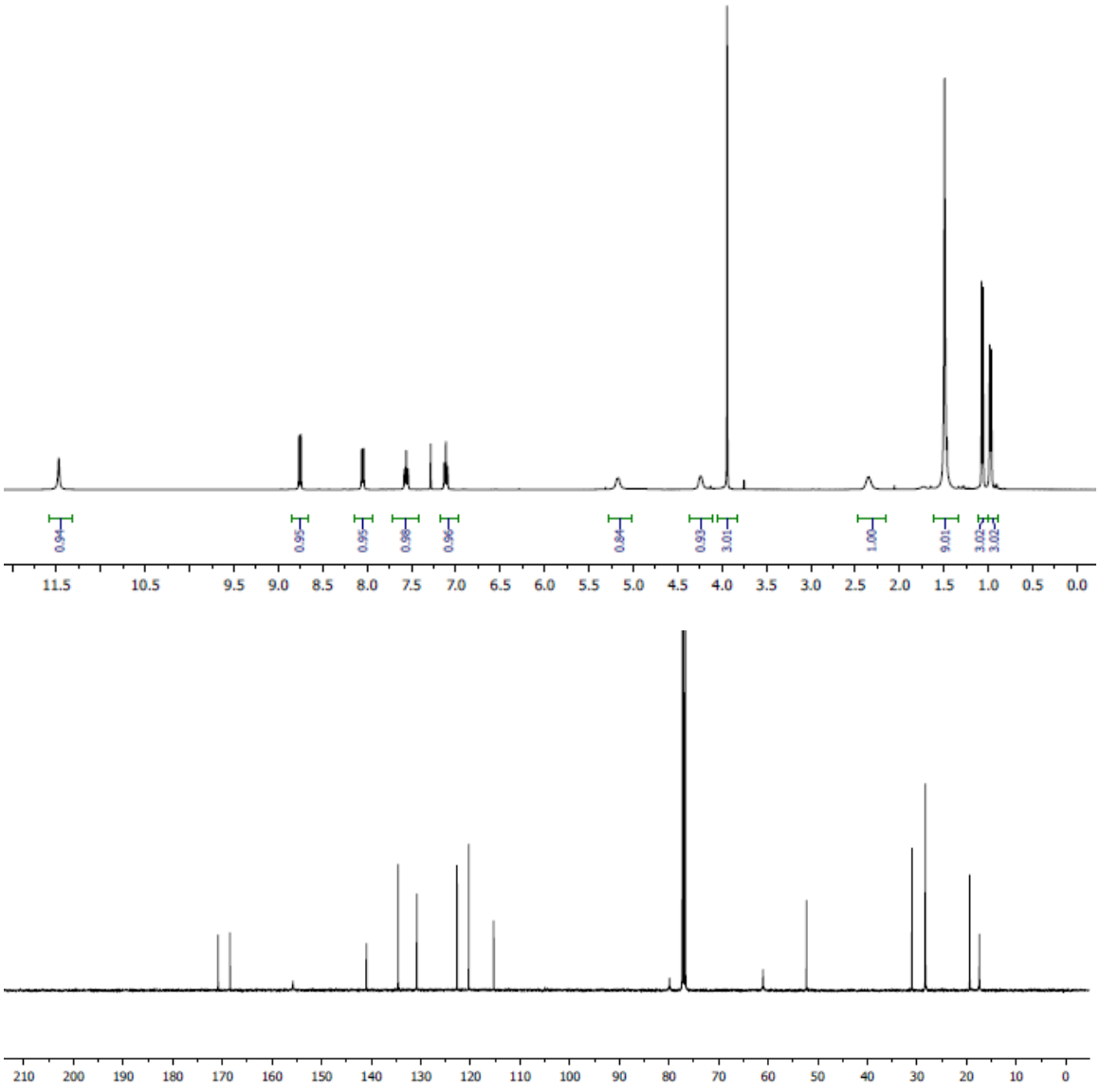


White solid, 73 %; mp = 108.5-109.5 °C;

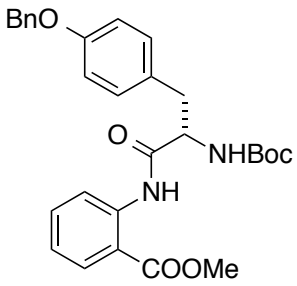
^{1}H-NMR (400 MHz, $CDCl_3$) δ 11.48 (s, 1H), 8.76 (d, *J* = 8.4 Hz, 1H), 8.05 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.71-7.42 (m, 1H), 7.11 (t, *J* = 7.6 Hz, 1H), 5.17 (s, 1H), 4.29-4.17 (m, 1H), 3.94 (s, 3H), 2.45-2.29 (m, 1H), 1.49 (s, 9H), 1.07 (d, *J* = 6.8 Hz, 3H), 0.98 (d, *J* = 6.9 Hz, 3H);

^{13}C-NMR (101 MHz, $CDCl_3$) δ 170.8, 168.4, 155.7, 141.0, 134.5, 130.8, 122.7, 120.3, 115.3, 79.8, 61.0, 52.2, 31.0, 28.3, 19.3, 17.4;

HRMS (ESI+) m/z calcd for $C_{18}H_{27}N_2O_5$ $(M+H)^+$ 351.1920; found 351.1922.



Methyl (*S*)-2-(3-(4-(benzyloxy)phenyl)-2-((*tert*-butoxycarbonyl)amino)propanamido)benzoate (L-**2y'**)

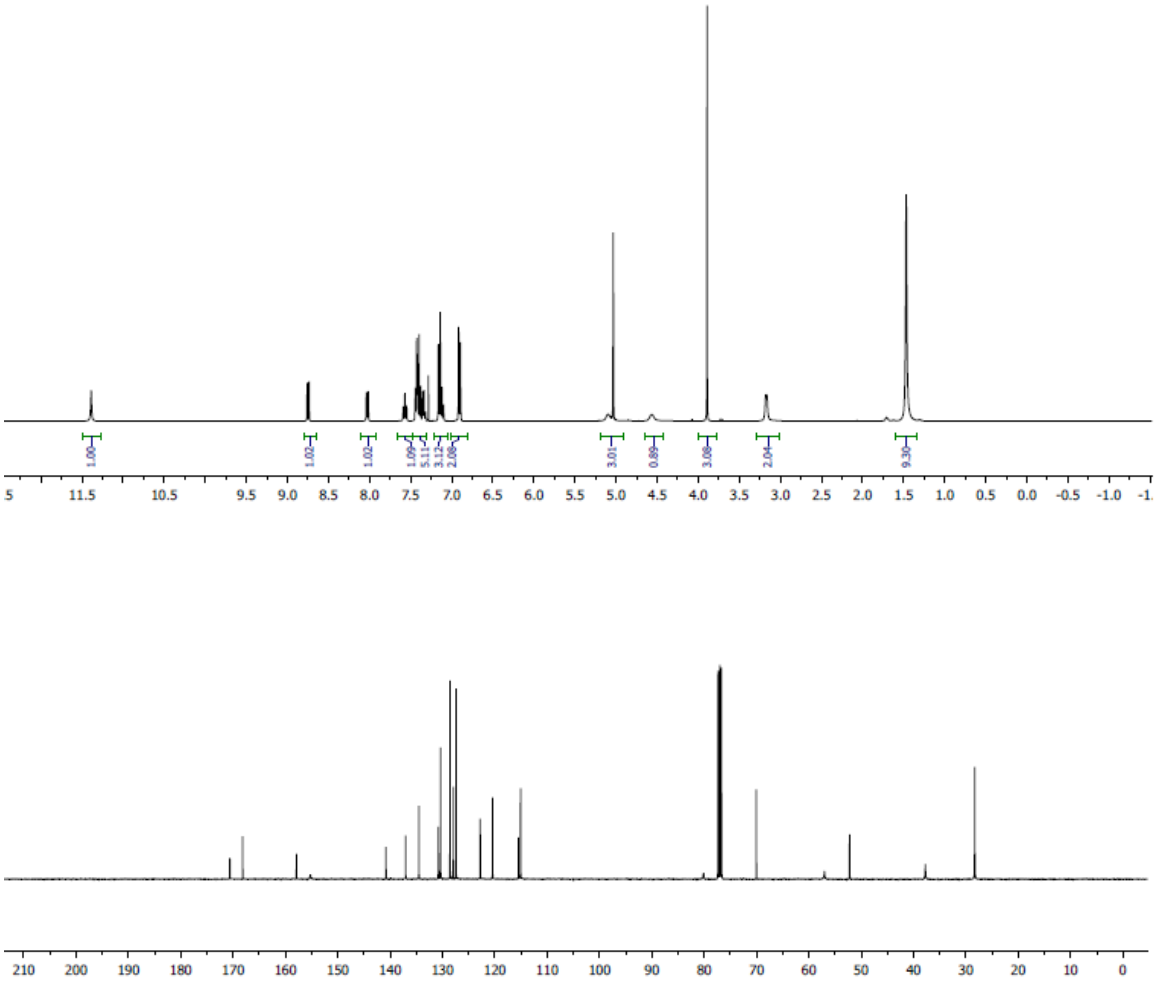


White solid, 91 %; mp = 120.6-121.2 °C;

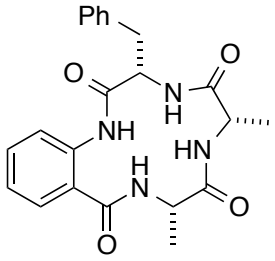
^{1}H NMR (400 MHz, $CDCl_3$) δ 11.39 (s, 1H), 8.75 (d, *J* = 7.9 Hz, 1H), 8.03 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.65-7.47 (m, 1H), 7.47-7.31 (m, 5H), 7.22-7.05 (m, 3H), 7.02-6.81 (m, 2H), 5.10 (s, 1H), 5.03 (s, 2H), 4.61-4.53 (m, 1H), 3.89 (s, 3H), 3.21-3.13 (m, 2H), 1.47 (s, 9H);

^{13}C-NMR (101 MHz, $CDCl_3$) δ 170.6, 168.1, 157.9, 155.2, 140.8, 137.0, 134.5, 130.8, 130.3, 128.6, 128.5, 127.9, 127.4, 122.7, 120.4, 115.4, 115.0, 80.1, 70.0, 57.0, 52.2, 37.7, 28.3;

HRMS (ESI+) m/z calcd for $C_{29}H_{33}N_2O_6$ $(M+H)^+$ 505.2339; found 505.2344.



(3*S*,6*S*,9*S*)-3-Benzyl-6,9-dimethyl-3,4,6,7,9,10-hexahydro-1*H*-benzo[*k*][1,4,7,10]tetraazacyclotridecine-2,5,8,11-tetraone (LLL-**1aaf**)



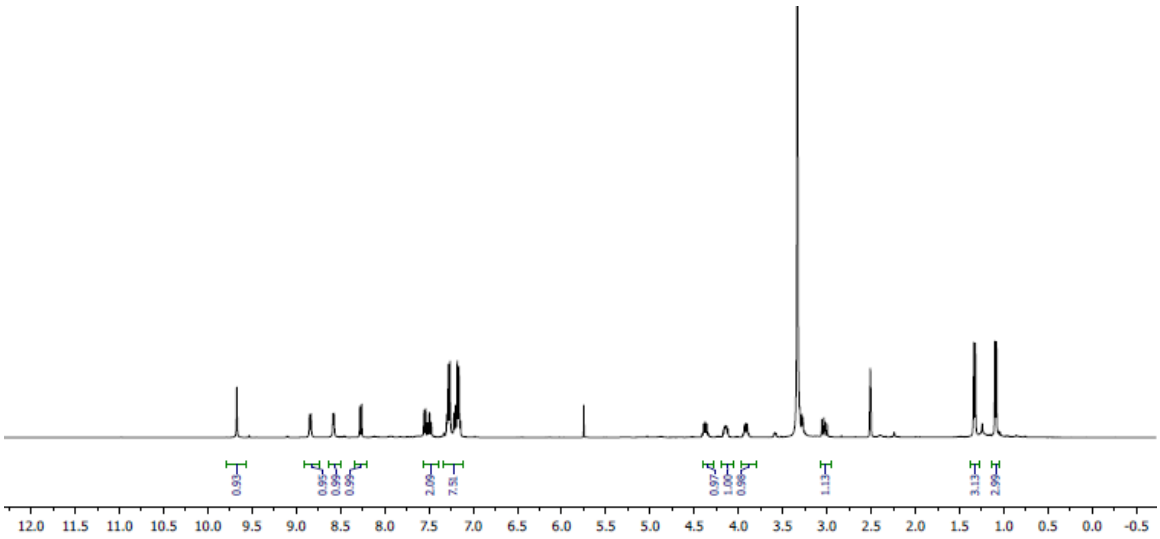
White solid, 41 % over three steps; mp > 270 °C;

^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.67 (s, 1H), 8.84 (d, *J* = 7.0 Hz, 1H), 8.58 (d, *J* = 5.0 Hz, 1H), 8.27 (d, *J* = 7.9 Hz, 1H), 7.57-7.40 (m, 2H), 7.34-7.11 (m, 7H), 4.42-4.33 (m, 1H), 4.19-4.10 (m, 1H), 3.95-3.88 (m, 1H), 3.28-3.35 (m, 1H), 3.03 (dd, *J* = 13.9, 9.7 Hz, 1H), 1.33 (d, *J* = 7.4 Hz, 3H), 1.09 (d, *J* = 6.8 Hz, 3H);

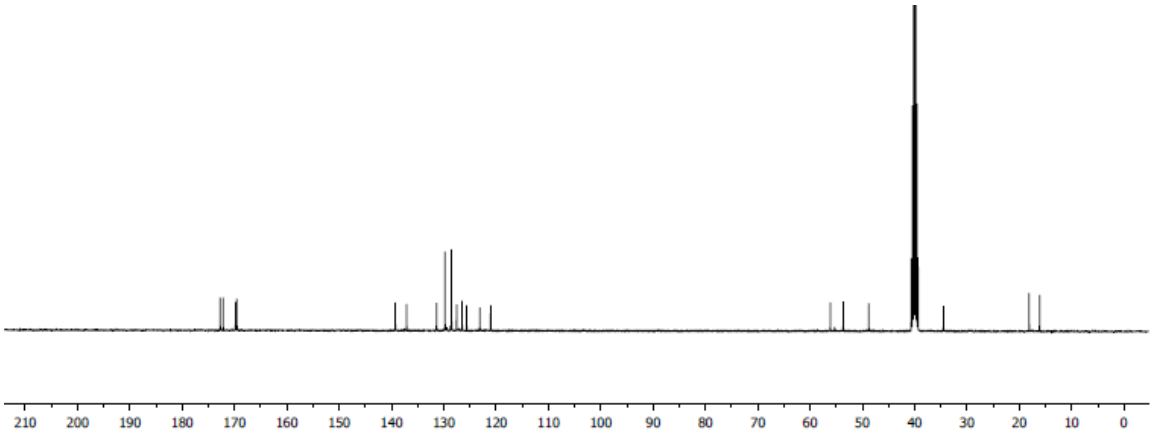
^{13}C-NMR (101 MHz, DMSO-d_6) δ 172.7, 172.1, 169.8, 169.5, 139.2, 137.1, 131.4, 129.7, 128.5, 127.5, 126.5, 125.6, 123.1, 121.0, 56.1, 53.6, 48.7, 34.4, 18.1, 16.1;

HRMS (ESI+) m/z calcd for $C_{22}H_{24}N_4O_4Na$ $(M+Na)^+$ 431.1695; found 431.1679.

^{1}H NMR



^{13}C NMR



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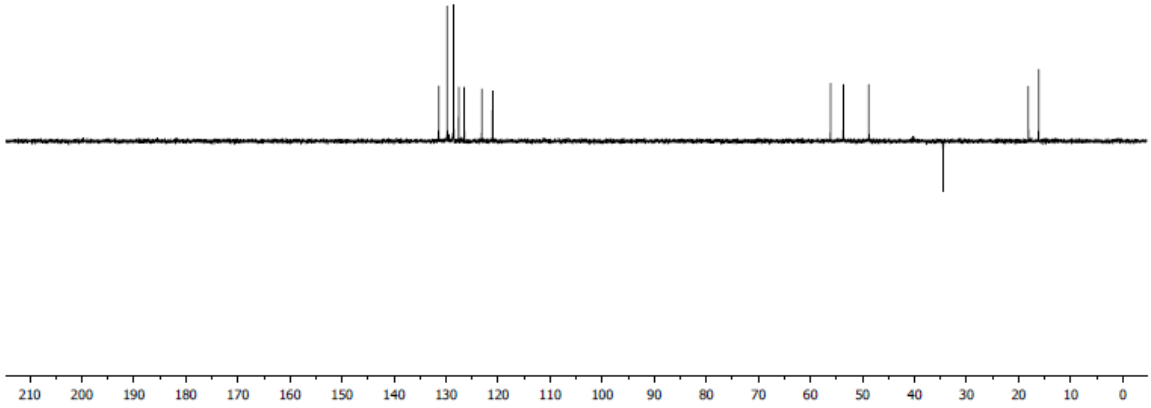
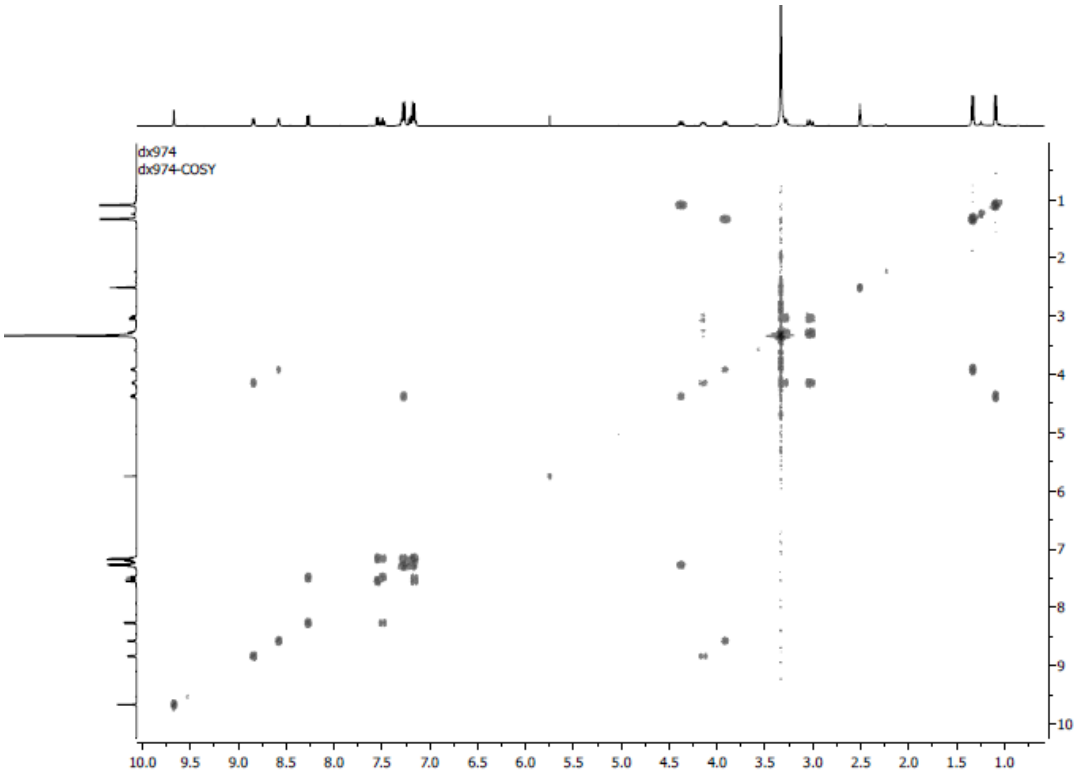


^{1}H-^{1}H COSY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dx974-COSY
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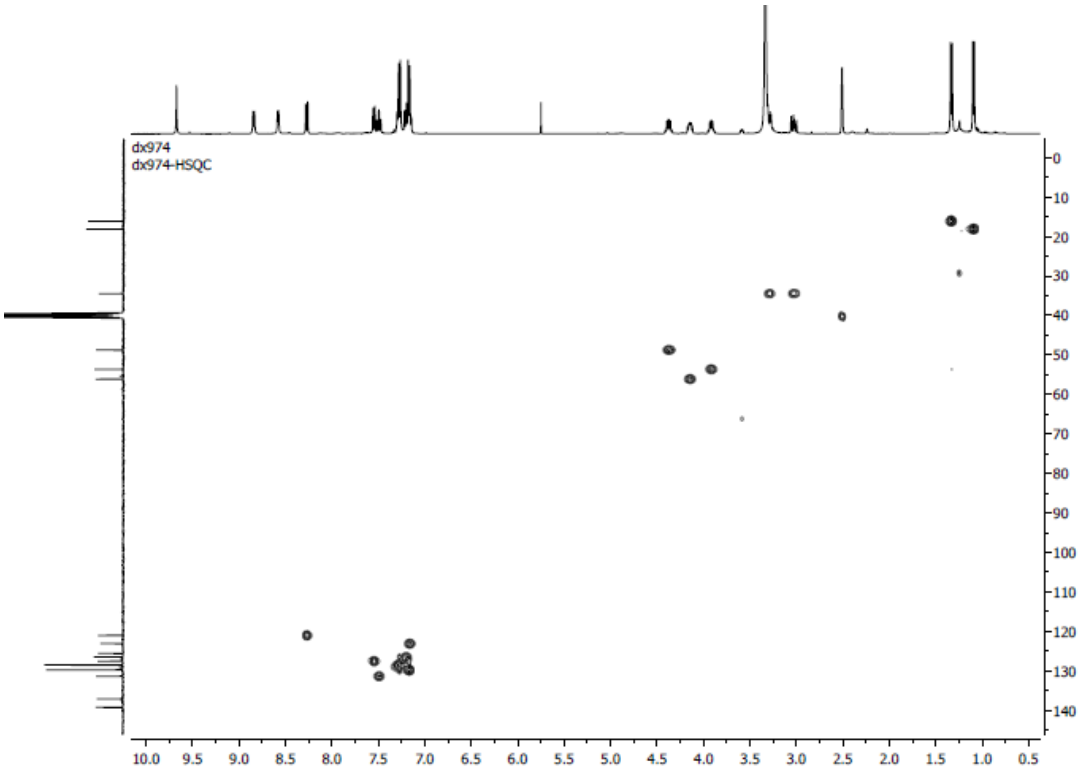
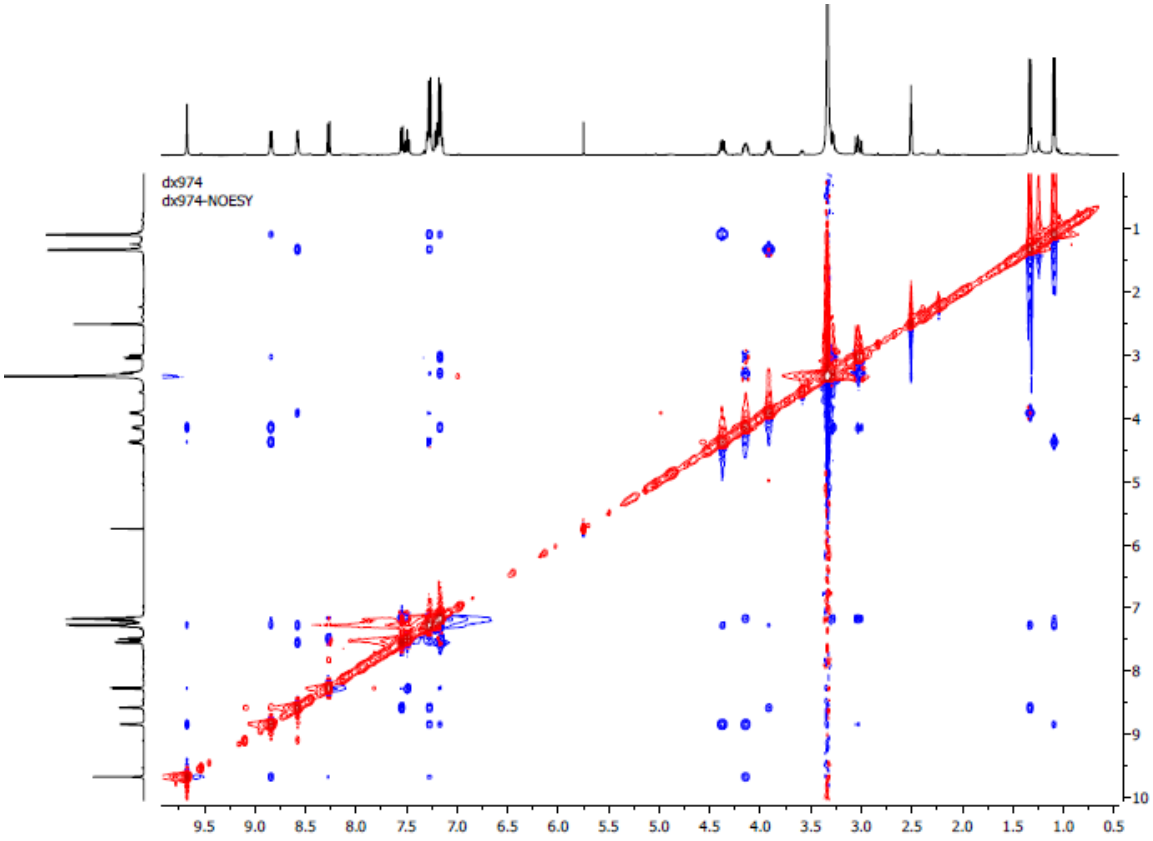
1 2 3 4 5 6 7 8 9 10

HSQC


dx974
dx974-HSQC
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140

NOESY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dx974-NOESY
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4.5
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3.0
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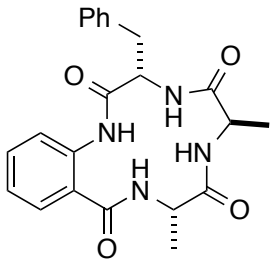
2.0
1.5
1.0
0.5
1
2
3
4
5
6
7
8
9
10

(3*S*,6*R*,9*S*)-3-Benzyl-6,9-dimethyl-3,4,6,7,9,10-hexahydro-1*H*-benzo[*k*][1,4,7,10]tetraazacyclotridecine-2,5,8,11-tetraone (LDL-**1aaf**)



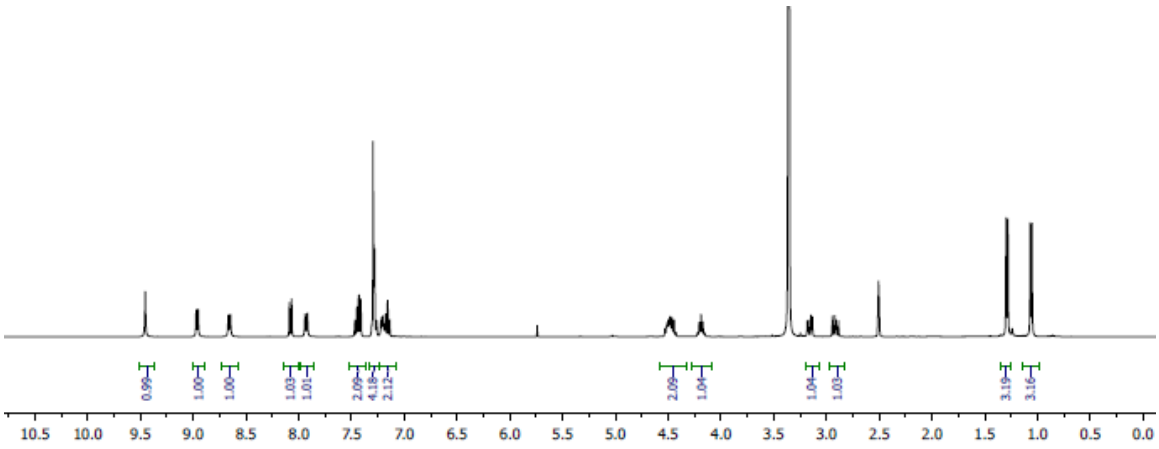
White solid, 48 % over three steps; mp > 270 °C;

^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.45 (s, 1H), 8.96 (d, *J* = 7.2 Hz, 1H), 8.66 (d, *J* = 7.7 Hz, 1H), 8.08 (d, *J* = 8.0 Hz, 1H), 7.93 (d, *J* = 8.8 Hz, 1H), 7.52-7.36 (m, 2H), 7.33-7.23 (m, 4H), 7.23-7.08 (m, 2H), 4.59-4.32 (m, 2H), 4.23-4.15 (m, 1H), 3.16 (dd, *J* = 14.0, 5.1 Hz, 1H), 2.91 (dd, *J* = 14.0, 9.5 Hz, 1H), 1.29 (d, *J* = 6.9 Hz, 3H), 1.06 (d, *J* = 6.8 Hz, 3H);

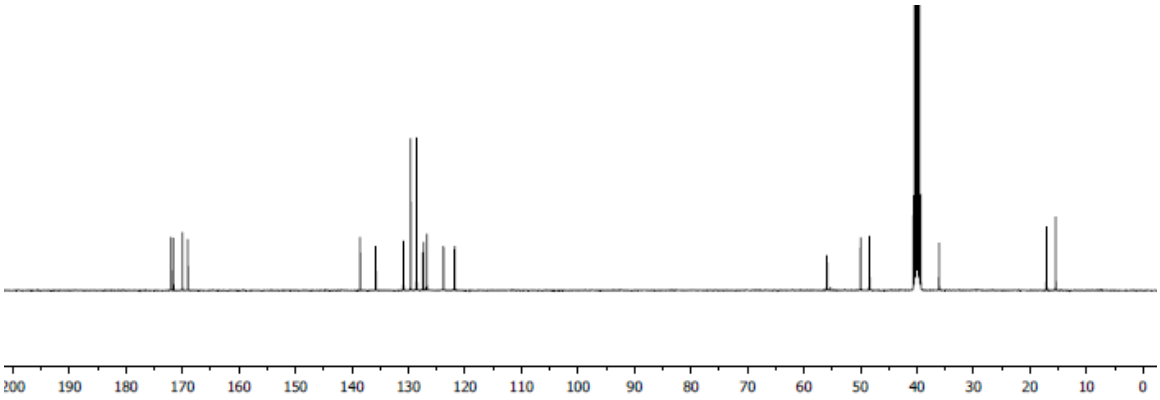
^{13}C-NMR (101 MHz, DMSO-d_6) δ 172.0, 171.5, 170.0, 169.0, 138.5, 135.7, 130.8, 129.6, 128.5, 127.4, 127.3, 126.7, 123.8, 121.8, 55.9, 49.9, 48.4, 36.0, 17.0, 15.4;

HRMS (ESI+) m/z calcd for $C_{22}H_{24}N_4O_4Li$ $(M+Li)^+$ 415.1958; found 415.1948.

^{1}H NMR



^{13}C NMR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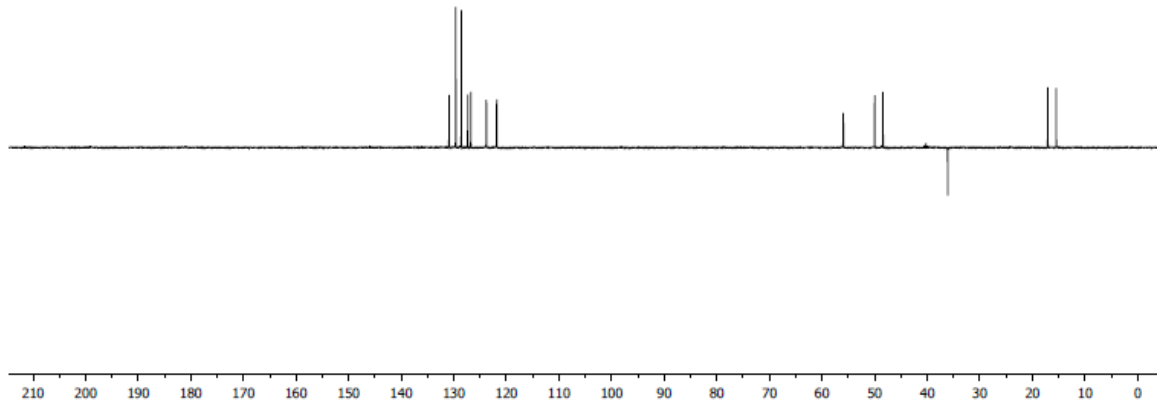
DEPT135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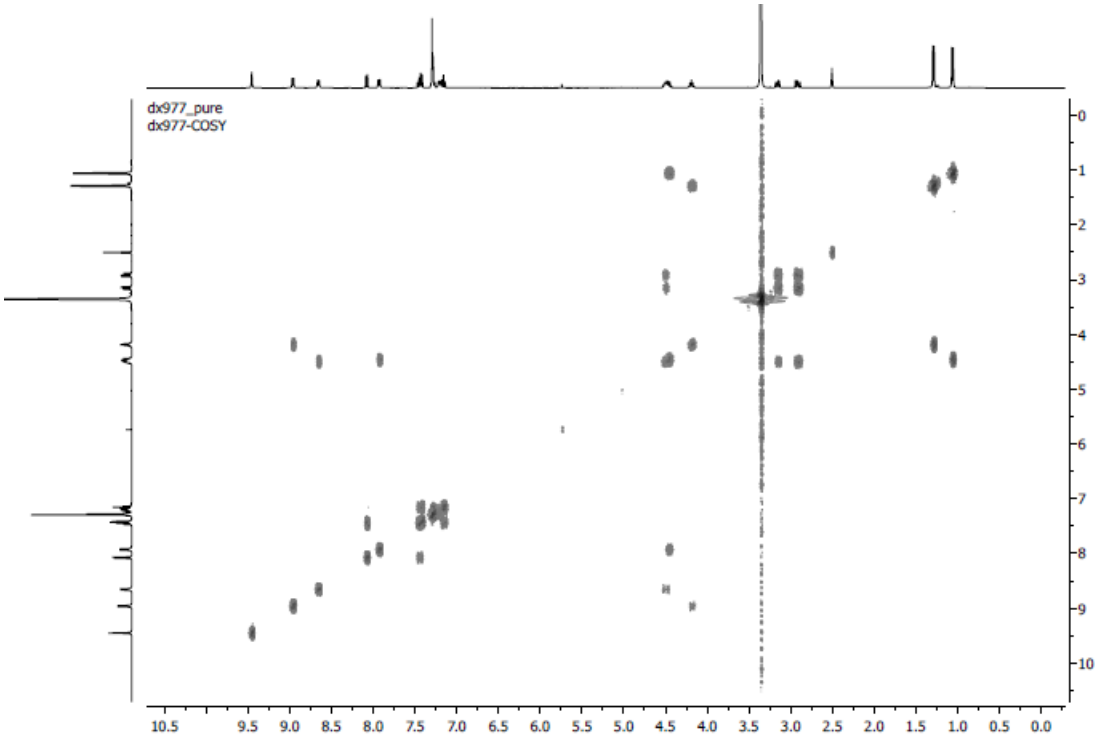
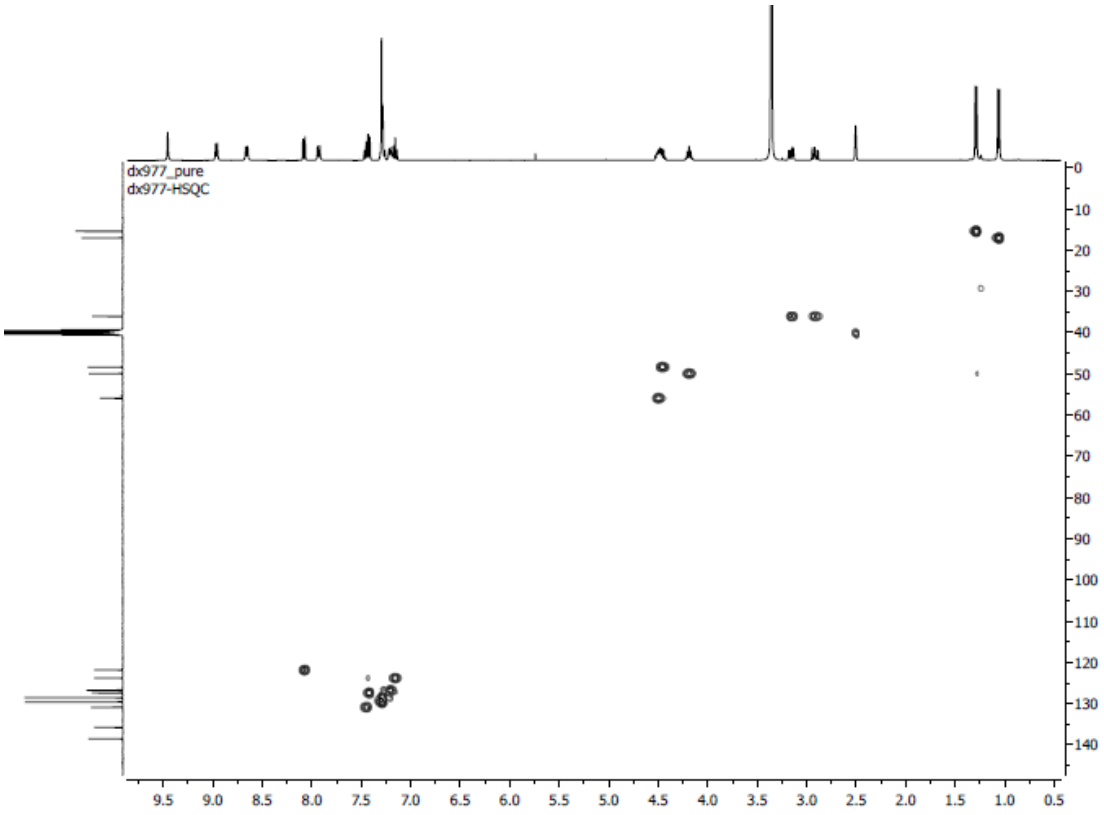
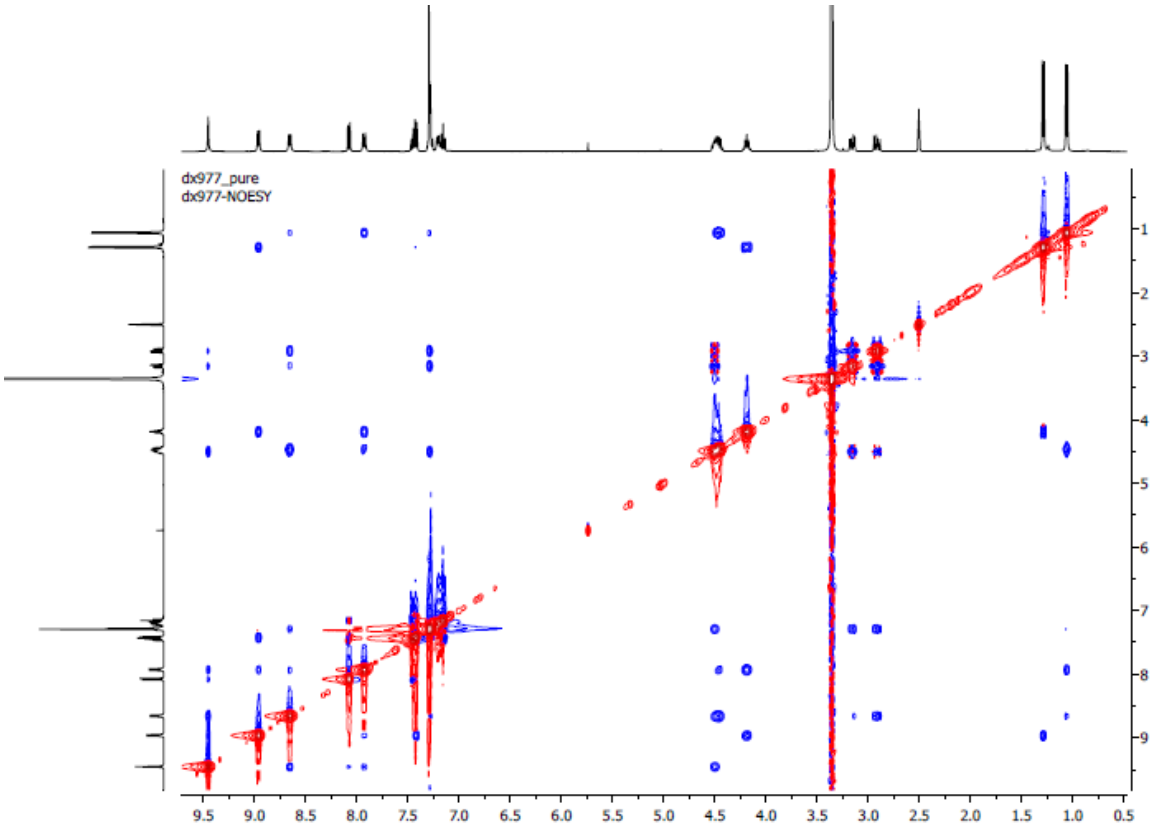
^{1}H-^{1}H COSY



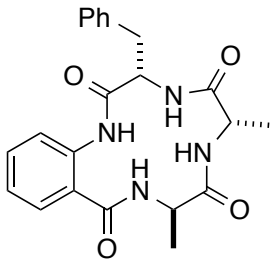
^{1}H-^{13}C HSQC



NOESY


dx977_pure
dx977-NOESY
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
1
2
3
4
5
6
7
8
9

(3*S*,6*S*,9*R*)-3-Benzyl-6,9-dimethyl-3,4,6,7,9,10-hexahydro-1*H*-benzo[*k*][1,4,7,10]tetraazacyclotridecine-2,5,8,11-tetraone (DLL-**1aaf**)



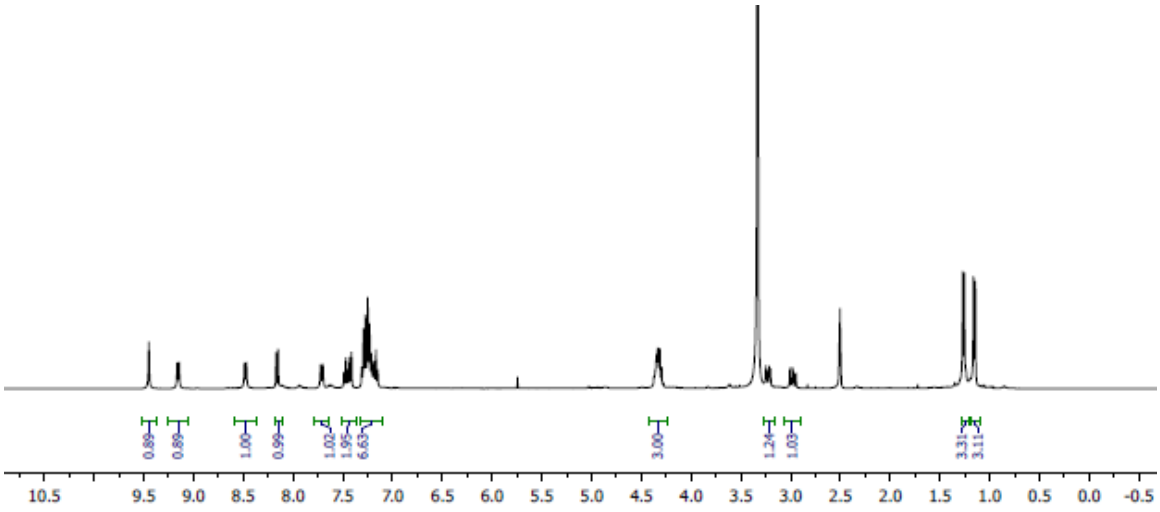
White solid, 55 % over three steps; mp > 270 °C;

^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.45 (s, 1H), 9.15 (d, *J* = 7.7 Hz, 1H), 8.48 (d, *J* = 7.9 Hz, 1H), 8.16 (d, *J* = 8.1 Hz, 1H), 7.71 (d, *J* = 8.9 Hz, 1H), 7.51-7.36 (m, 2H), 7.33-7.11 (m, 6H), 4.43-4.23 (m, 3H), 3.23 (dd, *J* = 13.7, 4.5 Hz, 1H), 2.98 (dd, *J* = 13.8, 10.2 Hz, 1H), 1.26 (d, *J* = 6.9 Hz, 3H), 1.15 (d, *J* = 7.2 Hz, 3H);

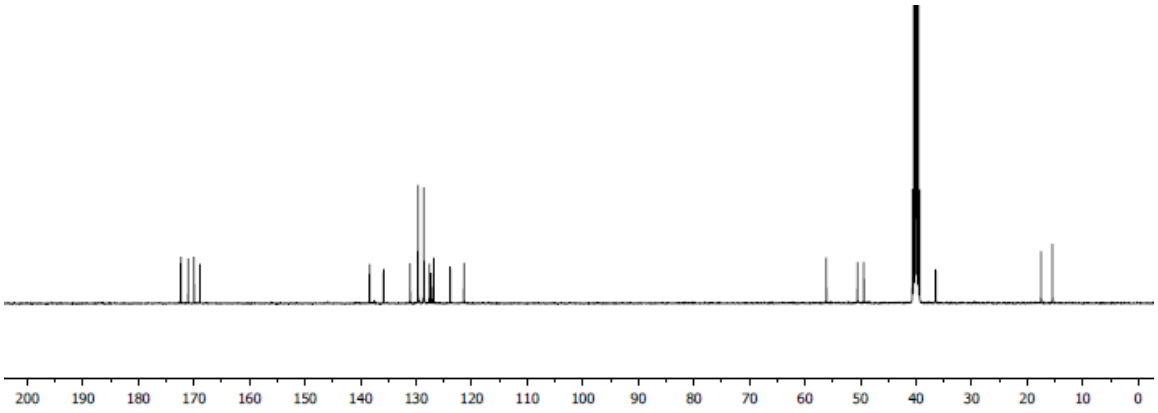
^{13}C-NMR (101 MHz, DMSO-d_6) δ 172.3, 171.0, 169.9, 168.9, 138.3, 135.8, 131.0, 129.6, 128.6, 127.6, 127.3, 126.8, 123.8, 121.3, 56.1, 50.5, 49.4, 36.4, 17.5, 15.4;

HRMS (ESI+) m/z calcd for $C_{22}H_{24}N_4O_4Na$ $(M+Na)^+$ 431.1695; found 431.1682.

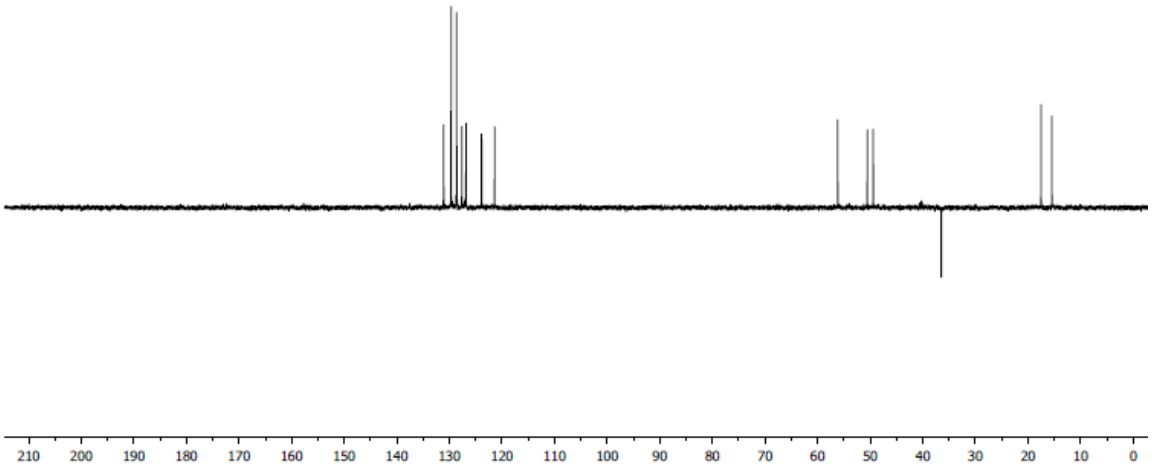
^{1}H NMR



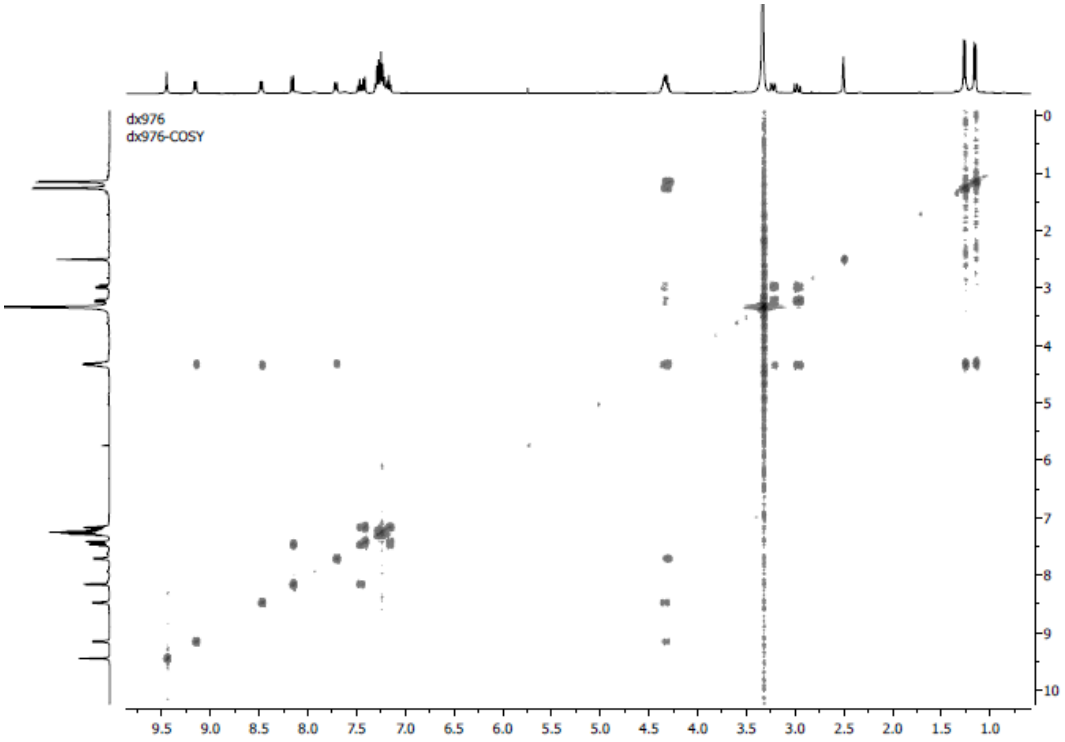
^{13}C NMR


200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

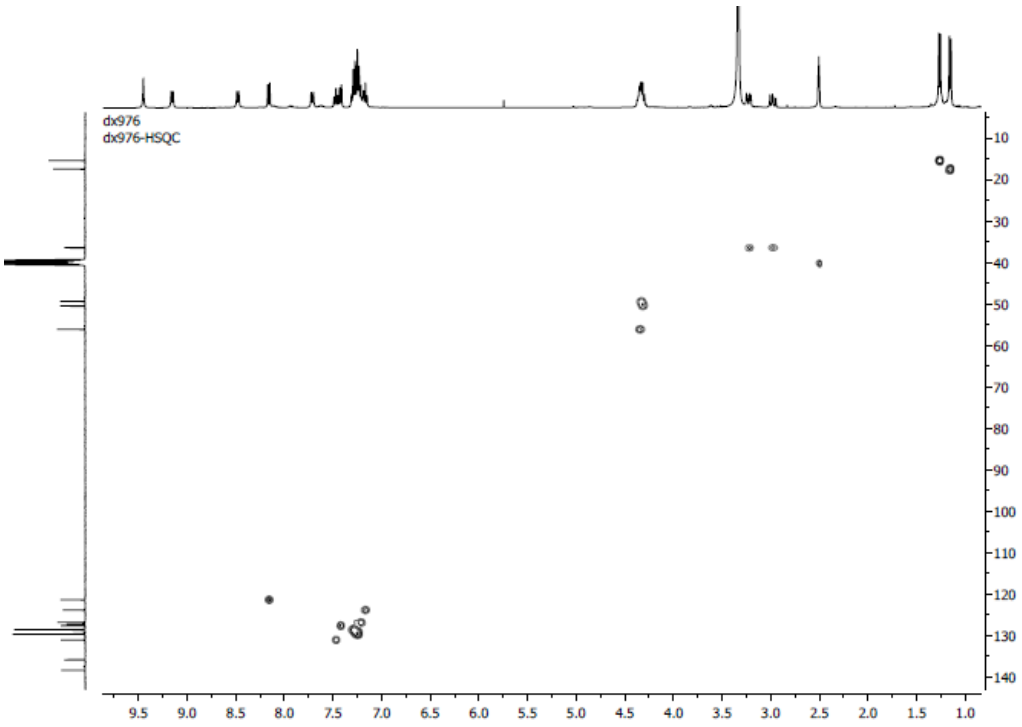
DEPT135


210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

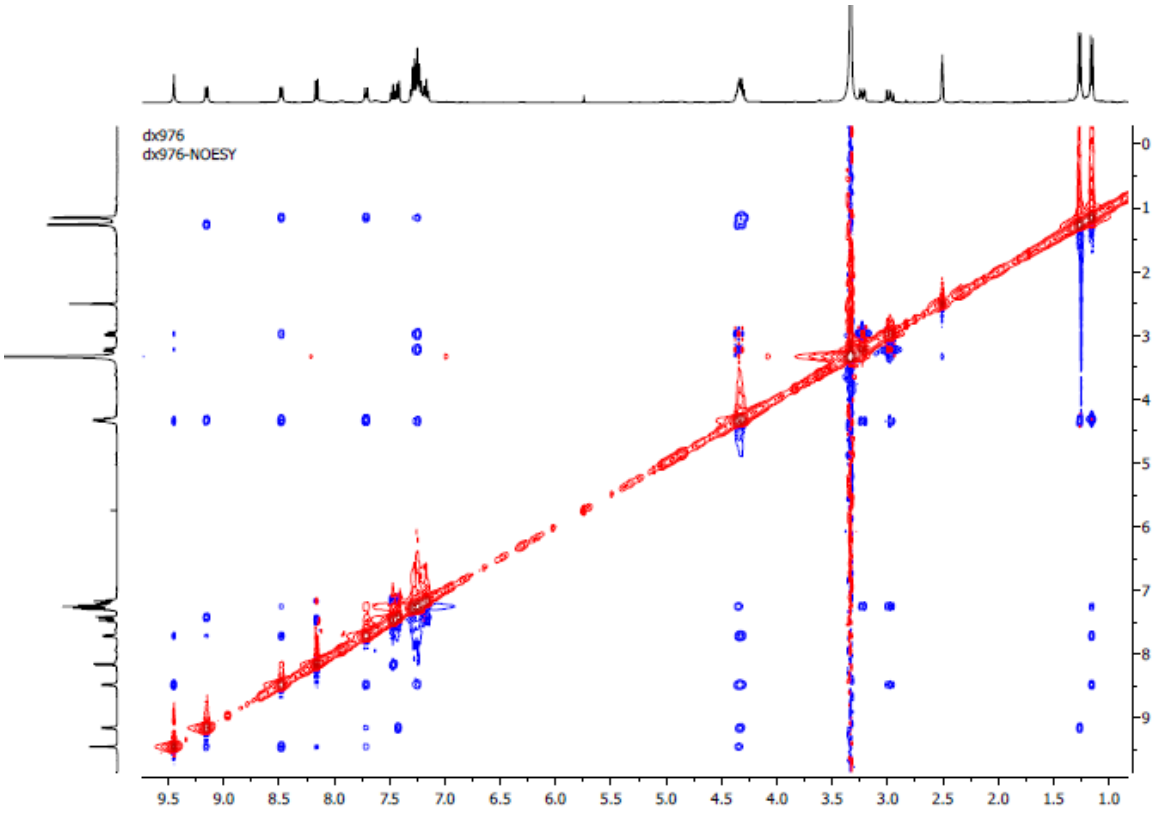
^{1}H-^{1}H COSY



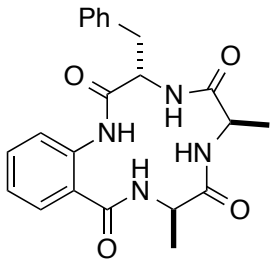
^{1}H-^{13}C HSQC



NOESY


dx976
dx976-NOESY
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0
1
2
3
4
5
6
7
8
9

(3*S*,6*R*,9*R*)-3-Benzyl-6,9-dimethyl-3,4,6,7,9,10-hexahydro-1*H*-benzo[*k*][1,4,7,10]tetraazacyclotridecine-2,5,8,11-tetraone (DDL-**1aaf**)



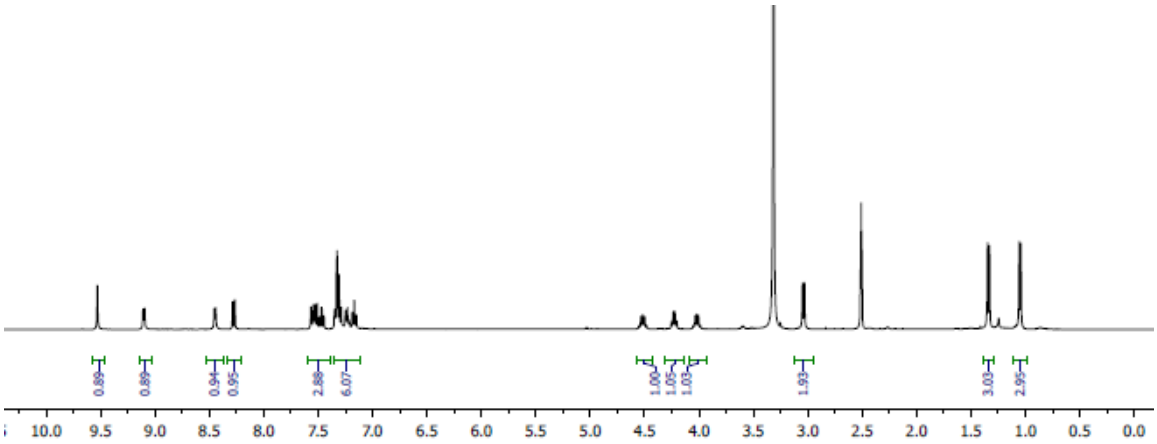
White solid, 44 % over three steps; mp > 270 °C;

^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.53 (s, 1H), 9.10 (d, *J* = 5.6 Hz, 1H), 8.45 (d, *J* = 5.2 Hz, 1H), 8.28 (d, *J* = 8.1 Hz, 1H), 7.60-7.39 (m, 3H), 7.36-7.11 (m, 6H), 4.55-4.48 (m, 1H), 4.26-4.19 (m, 1H), 4.05-3.99 (m, 1H), 3.04 (d, *J* = 7.4 Hz, 2H), 1.34 (d, *J* = 7.4 Hz, 3H), 1.05 (d, *J* = 6.6 Hz, 3H);

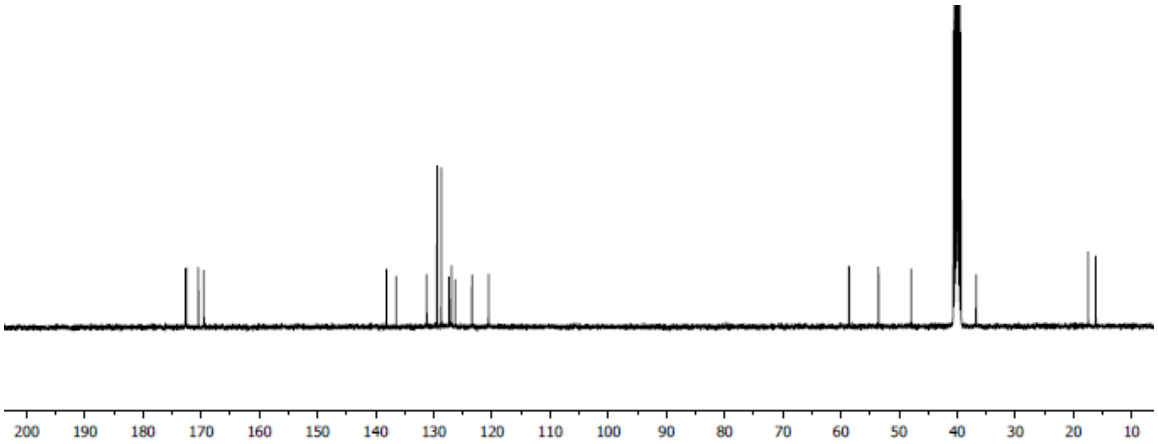
^{13}C-NMR (101 MHz, DMSO-d_6) δ 172.7, 172.5, 170.4, 169.5, 138.1, 136.4, 131.2, 129.4, 128.7, 127.3, 126.9, 126.3, 123.4, 120.5, 58.6, 53.5, 47.9, 36.7, 17.4, 16.1;

HRMS (ESI+) m/z calcd for $C_{22}H_{24}N_4O_4Na$ $(M+Na)^+$ 431.1695; found 431.1716.

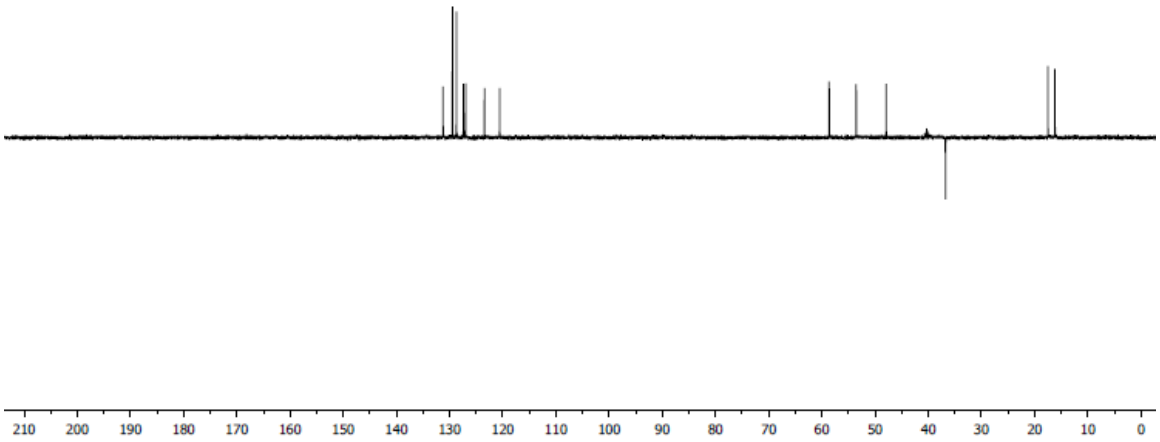
^{1}H NMR



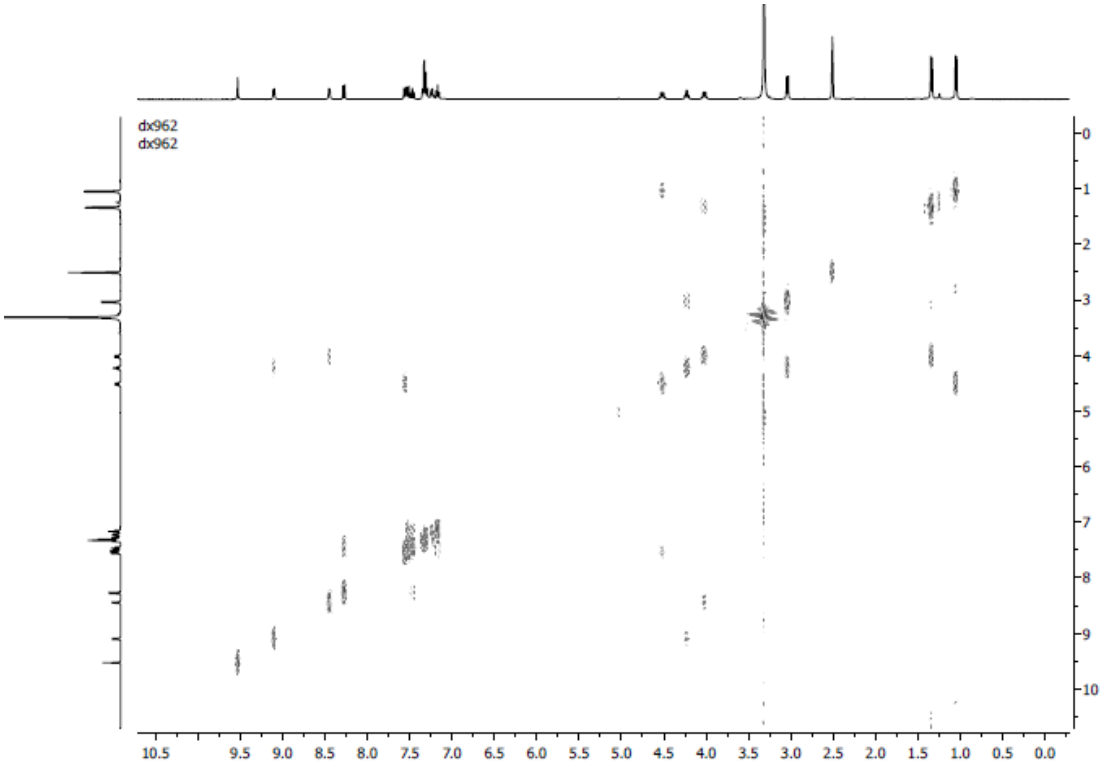
^{13}C NMR


200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10

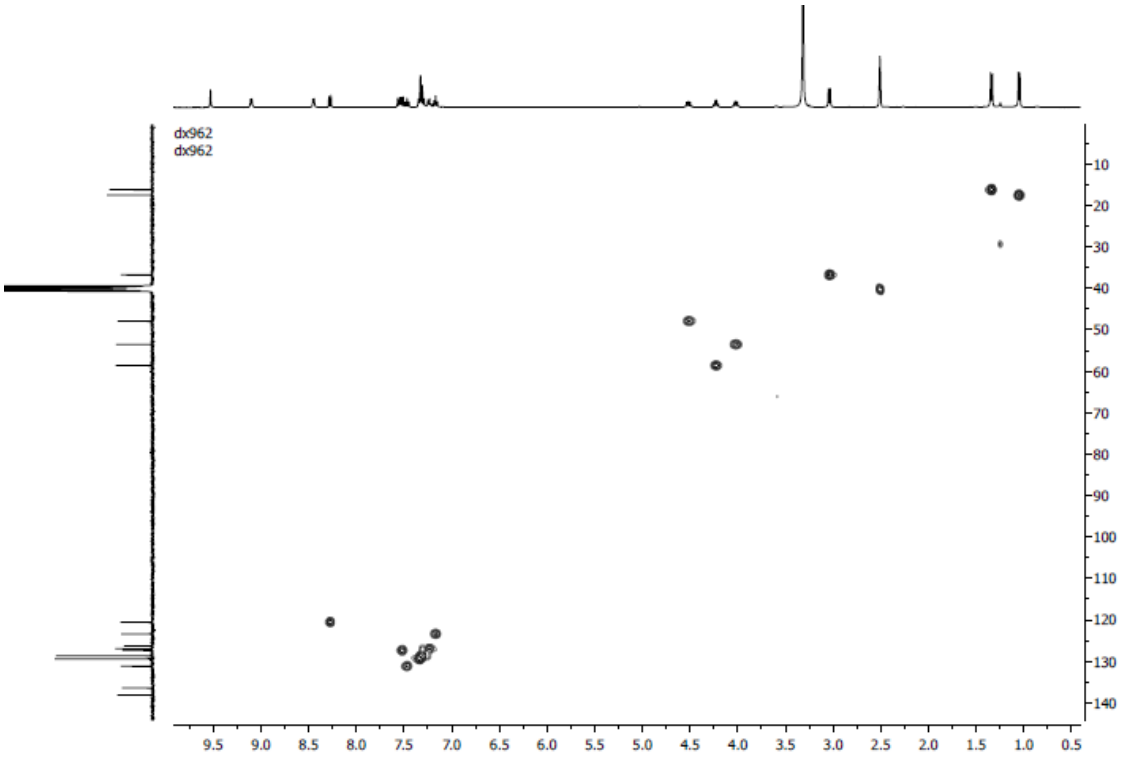
DEPT135


210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

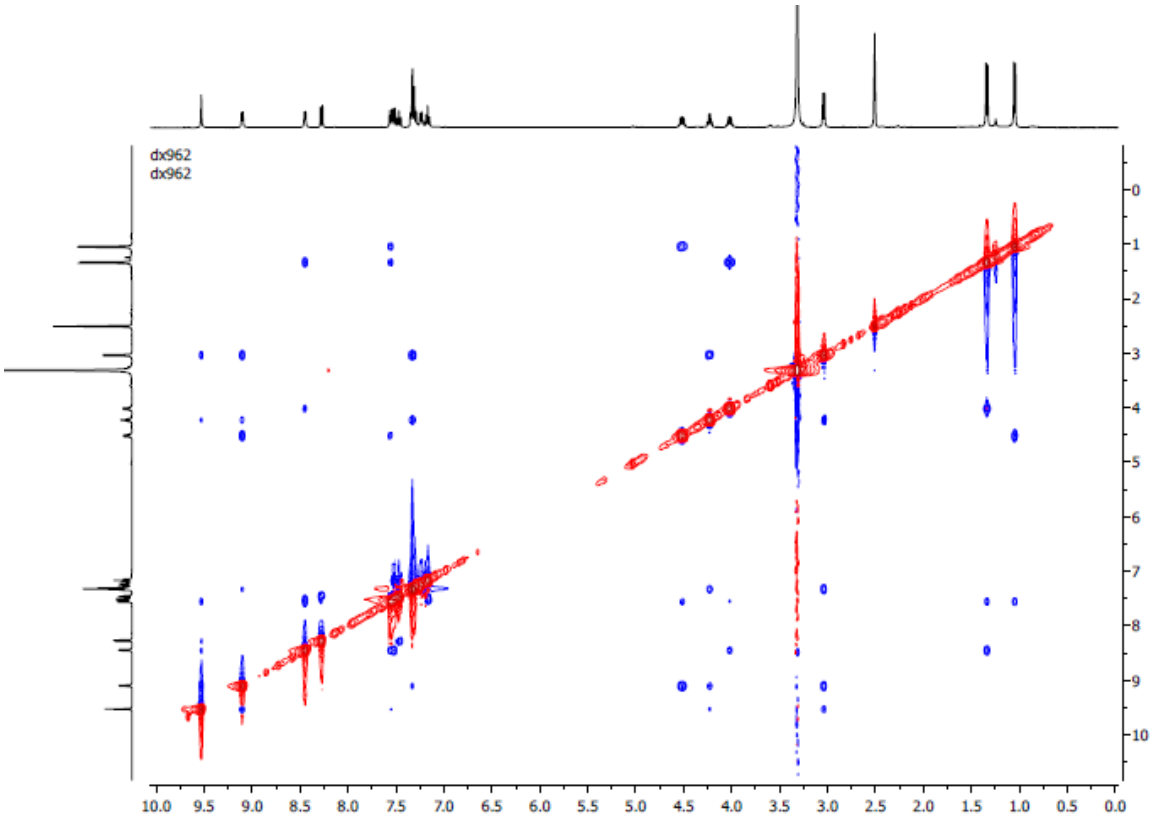
^{1}H-^{1}H COSY



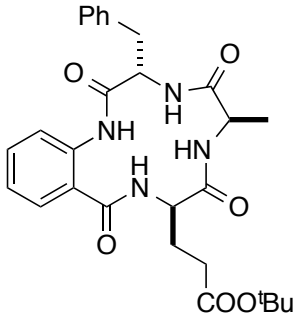
^{1}H-^{13}C HSQC



NOESY


dx962
dx962
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
0 1 2 3 4 5 6 7 8 9 10

tert-Butyl 3-((3*S*,6*R*,9*R*)-3-Benzyl-6-methyl-2,5,8,11-tetraoxo-2,3,4,5,6,7,8,9,10,11-decahydro-1*H*-benzo[*k*][1,4,7,10]tetraazacyclotridecin-9-yl)propanoate (DDL-**1e'af**)



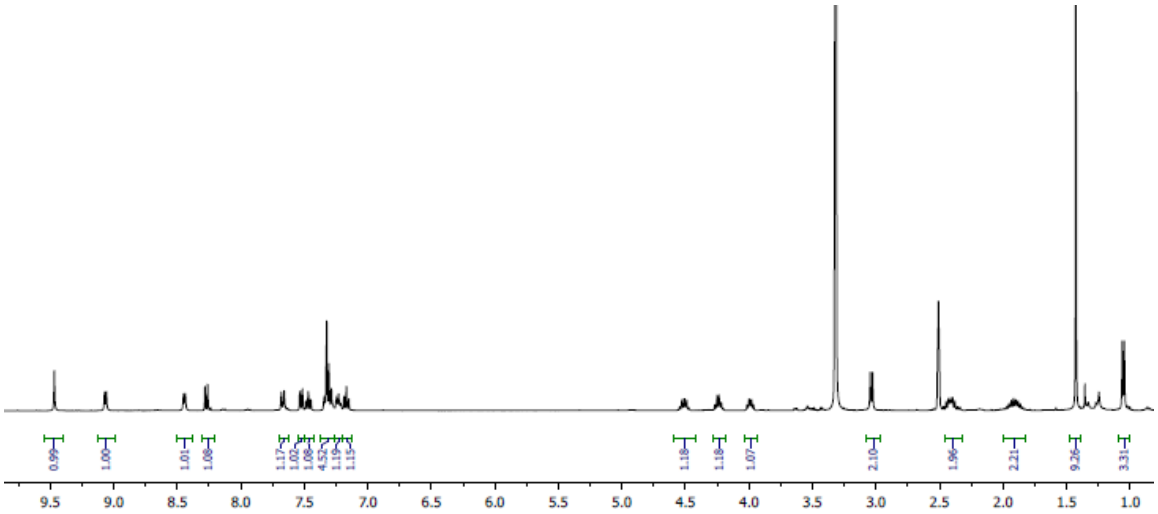
White solid, 35 % over three steps; mp > 270 °C, color change observed at above 230 °C;

^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.47 (s, 1H), 9.06 (d, *J* = 5.8 Hz, 1H), 8.44 (d, *J* = 5.6 Hz, 1H), 8.27 (d, *J* = 8.1 Hz, 1H), 7.67 (d, *J* = 8.8 Hz, 1H), 7.53 (dd, *J* = 7.6, 1.2 Hz, 1H), 7.47 (t, *J* = 7.8 Hz, 1H), 7.35-7.24 (m, 4H), 7.24-7.19 (m, 1H), 7.17 (t, *J* = 7.5 Hz, 1H), 4.60-4.41 (m, 1H), 4.29-4.19 (m, 1H), 4.04-3.95 (m, 1H), 3.04 (d, *J* = 7.4 Hz, 2H), 2.46-2.32 (m, 2H), 1.99-1.83 (m, 2H), 1.43 (s, 9H), 1.05 (d, *J* = 6.7 Hz, 3H);

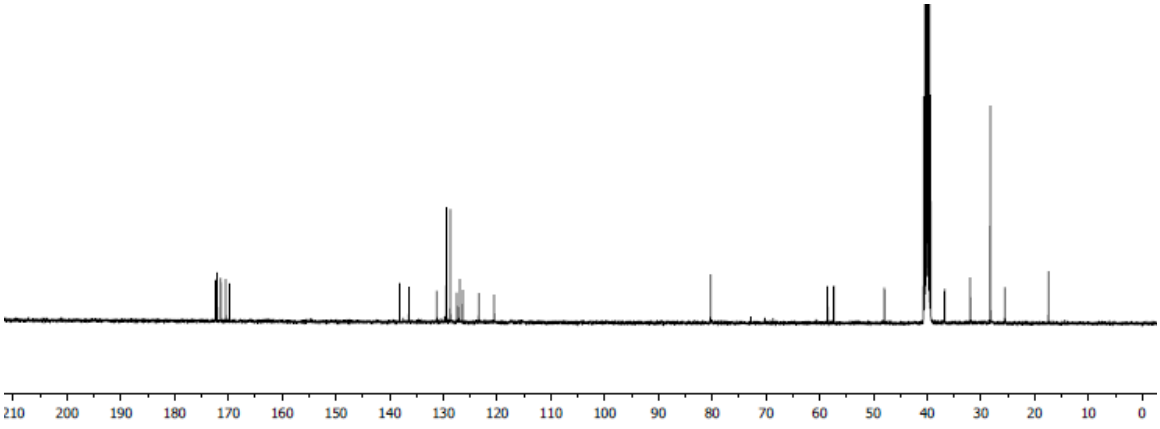
^{13}C-NMR (101 MHz, DMSO-d_6) δ 172.4, 172.0, 171.4, 170.4, 169.7, 138.1, 136.4, 131.2, 129.4, 128.7, 127.5, 126.9, 126.4, 123.4, 120.5, 80.3, 58.5, 57.3, 47.9, 36.7, 31.9, 28.2, 25.5, 17.4;

HRMS (ESI-) m/z calcd for $C_{28}H_{33}N_4O_6$ $(M-H)^-$ 521.2400; found 521.2422.

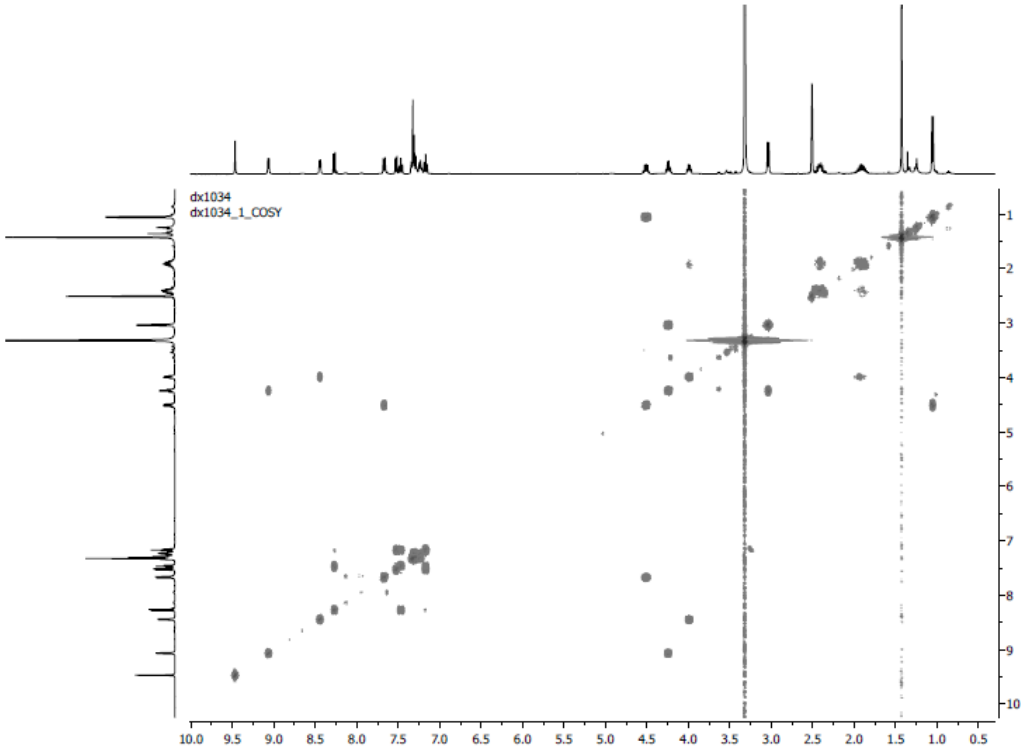
^{1}H NMR


0.99
1.00
1.01
1.08
1.17
1.02
1.08
4.52
1.19
1.15
1.18
1.18
1.07
2.10
1.96
2.21
9.26
3.31
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0

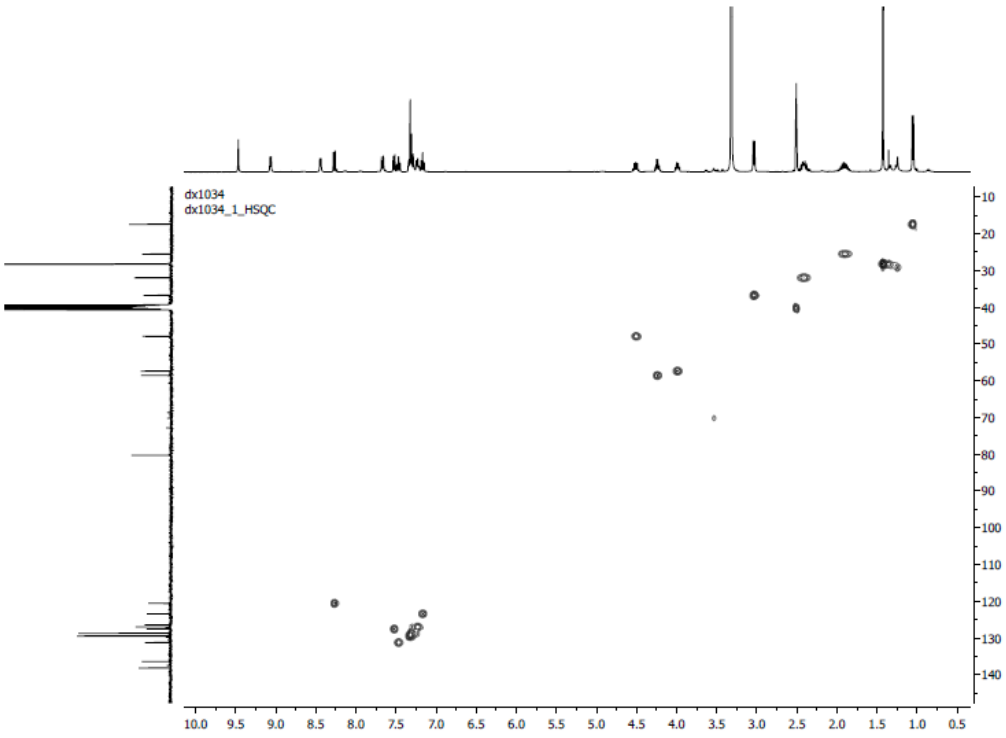
^{13}C NMR


210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

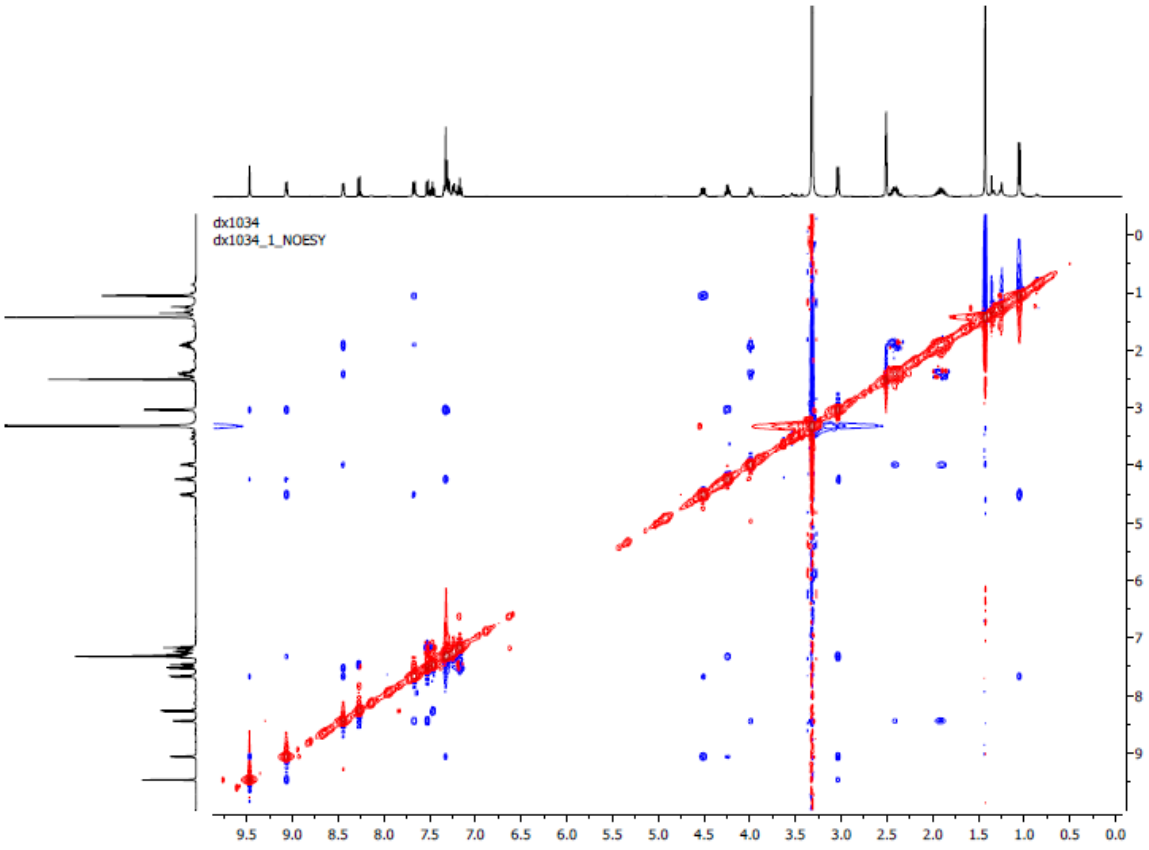
^{1}H-^{1}H COSY



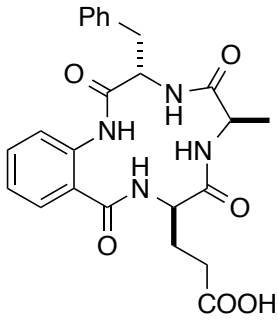
^{1}H-^{13}C HSQC



NOESY


dx1034
dx1034_1_NOESY
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

3-((3*S*,6*R*,9*R*)-3-Benzyl-6-methyl-2,5,8,11-tetraoxo-2,3,4,5,6,7,8,9,10,11-decahydro-1*H*-benzo[*k*][1,4,7,10]tetraazacyclotridecin-9-yl)propanoic acid (DDL-**1eaf**)



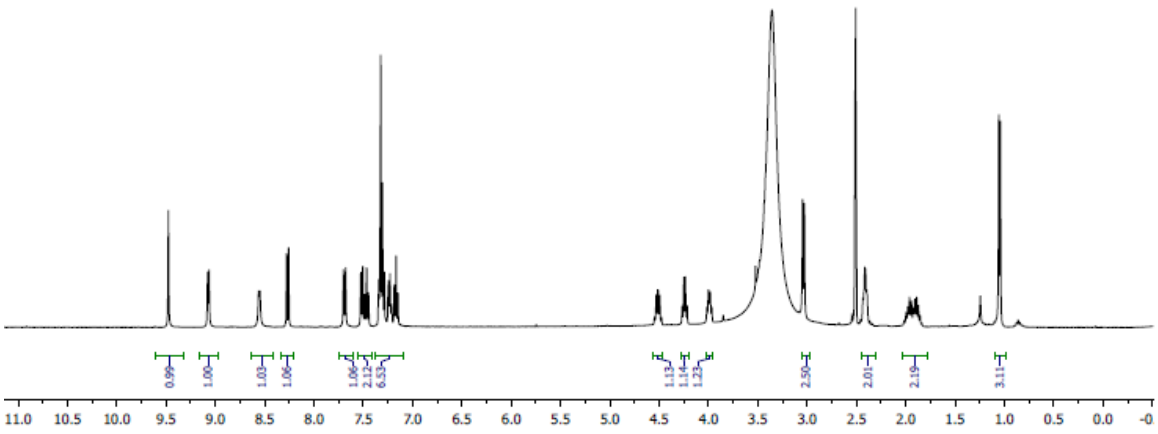
White solid, 87 %; mp > 270 °C;

^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.48 (s, 1H), 9.07 (d, *J* = 5.7 Hz, 1H), 8.55 (d, *J* = 5.1 Hz, 1H), 8.27 (d, *J* = 8.2 Hz, 1H), 7.69 (d, *J* = 8.8 Hz, 1H), 7.56-7.41 (m, 2H), 7.37-7.10 (m, 7H), 4.58-4.48 (m, 1H), 4.29-4.22 (m, 1H), 4.05-3.96 (m, 1H), 3.04 (d, *J* = 7.4 Hz, 2H), 2.46-2.38 (m, 2H), 2.03-1.78 (m, 2H), 1.05 (d, *J* = 6.6 Hz, 3H);

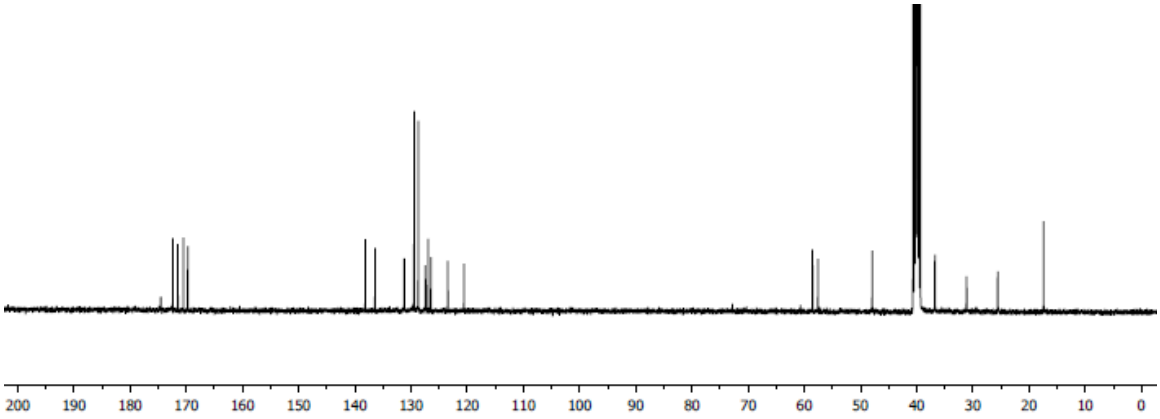
^{13}C-NMR (101 MHz, DMSO-d_6) δ 174.5, 172.4, 171.5, 170.5, 169.7, 138.1, 136.4, 131.2, 129.4, 128.7, 127.4, 126.9, 126.4, 123.4, 120.5, 58.5, 57.5, 47.9, 36.7, 31.1, 25.5, 17.4;

HRMS (ESI-) m/z calcd for $C_{24}H_{25}N_4O_6$ $(M-H)^-$ 465.1774; found 465.1786.

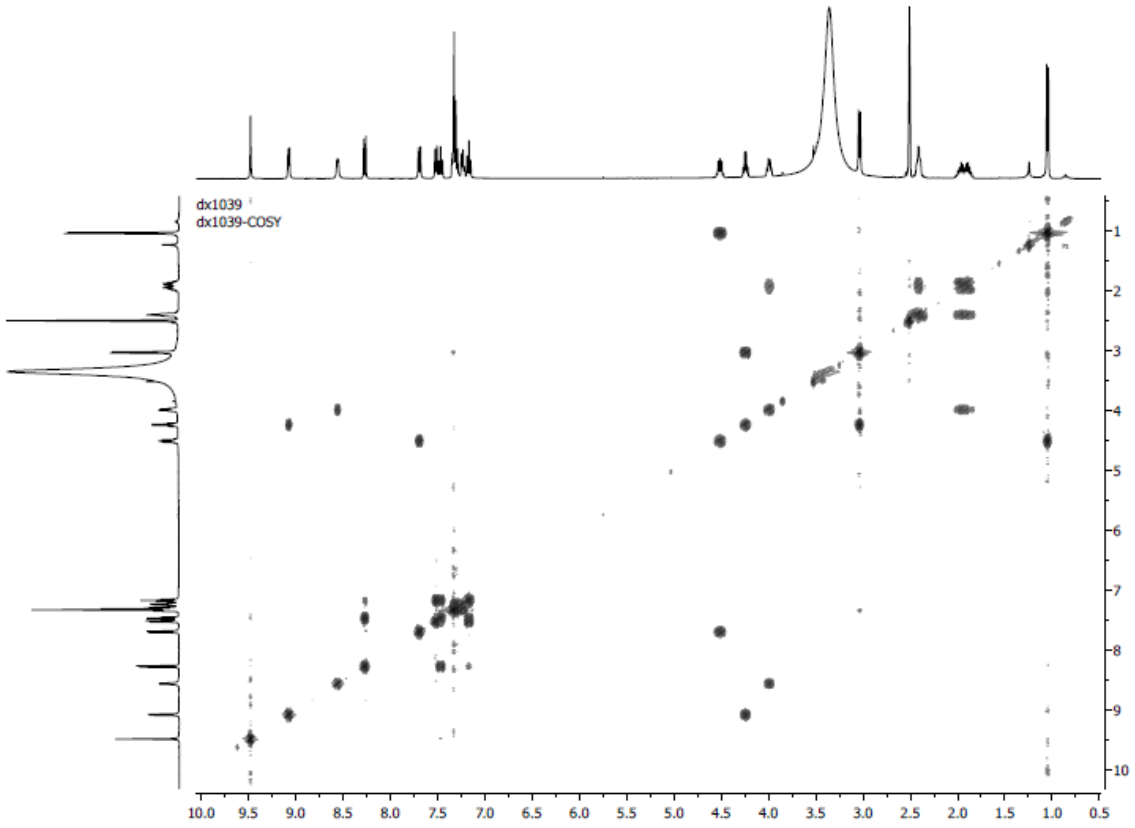
^{1}H NMR



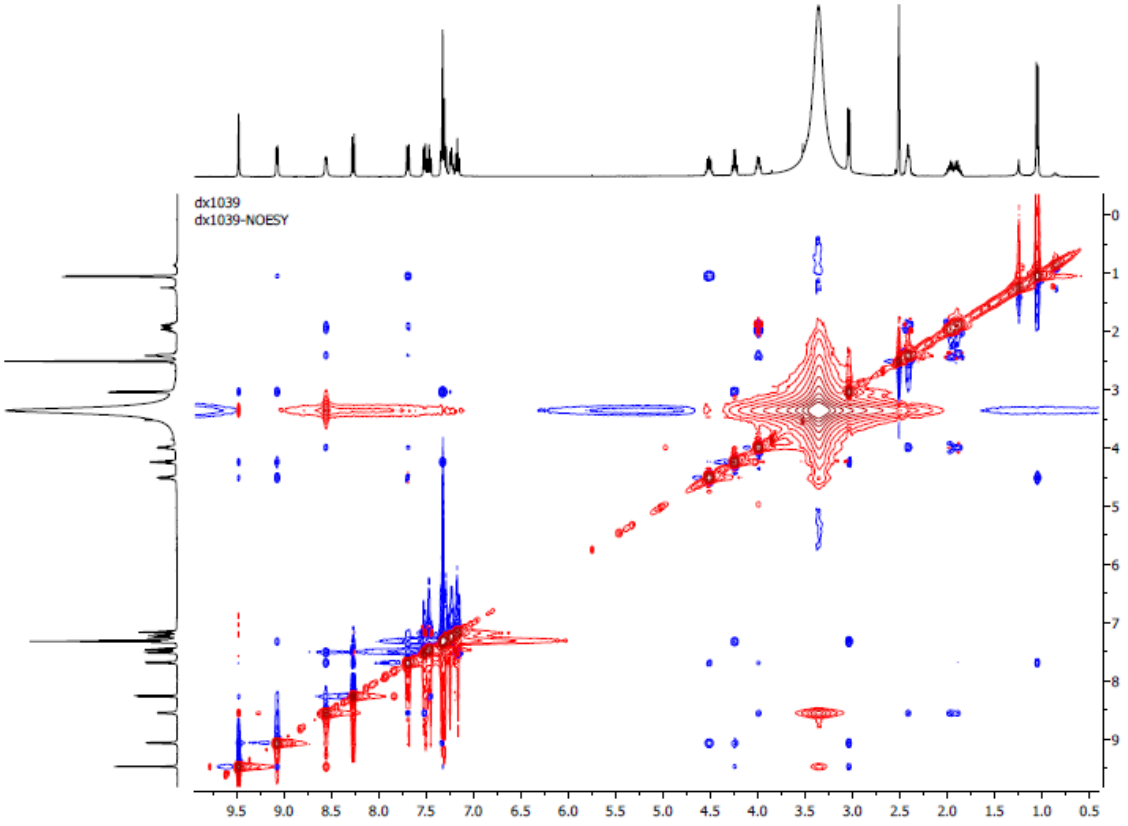
^{13}C NMR


200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

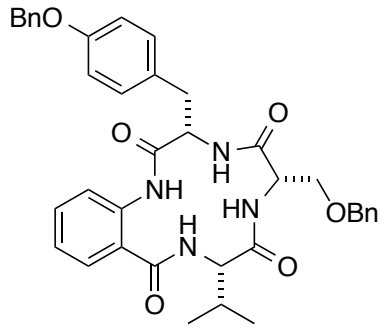
^{1}H-^{1}H COSY


dx1039
dx1039-COSY
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
1 2 3 4 5 6 7 8 9 10

NOESY


dx1039
dx1039-NOESY
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
1
2
3
4
5
6
7
8
9

(3*S*,6*S*,9*S*)-3-(4-(Benzyloxy)benzyl)-6-((benzyloxy)methyl)-9-isopropyl-3,4,6,7,9,10-hexahydro-1*H*-benzo[*k*][1,4,7,10]tetraazacyclotridecine-2,5,8,11-tetraone (LLL-**1vs'y'**)



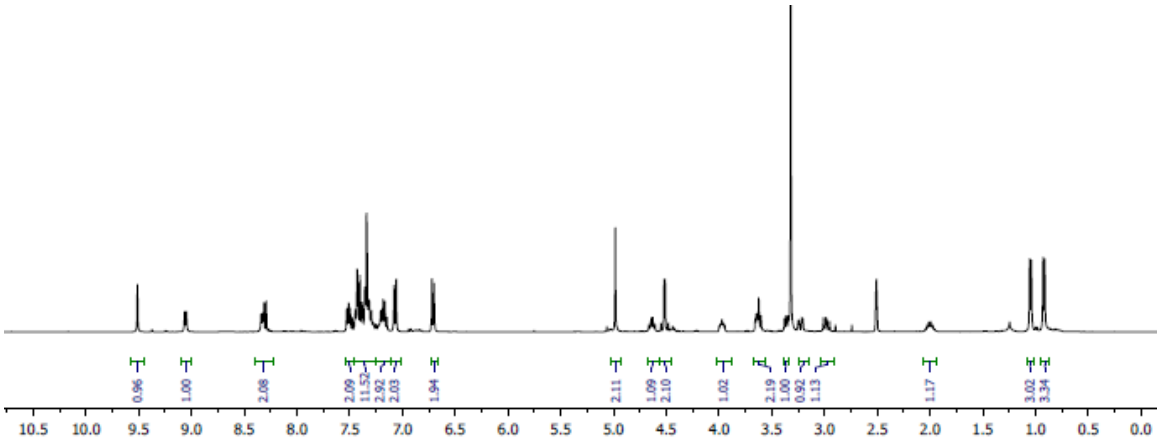
White solid, 45 % over three steps; mp > 270 °C;

^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.51 (s, 1H), 9.06 (d, *J* = 6.8 Hz, 1H), 8.36-8.28 (m, 2H), 7.54-7.47 (m, 2H), 7.47-7.25 (m, 10H), 7.25-7.11 (m, 2H), 7.07 (d, *J* = 8.5 Hz, 2H), 6.71 (d, *J* = 8.5 Hz, 2H), 4.98 (s, 2H), 4.69-4.60 (m, 1H), 4.57-4.46 (m, 2H), 4.03-3.88 (m, 1H), 3.67-3.58 (m, 2H), 3.36 (dd, *J* = 9.2, 5.8 Hz, 1H), 3.24-3.15 (m, 1H), 2.98 (dd, *J* = 14.1, 10.1 Hz, 1H), 2.05-1.97 (m, 1H), 1.05 (d, *J* = 6.6 Hz, 3H), 0.92 (d, *J* = 6.7 Hz, 3H);

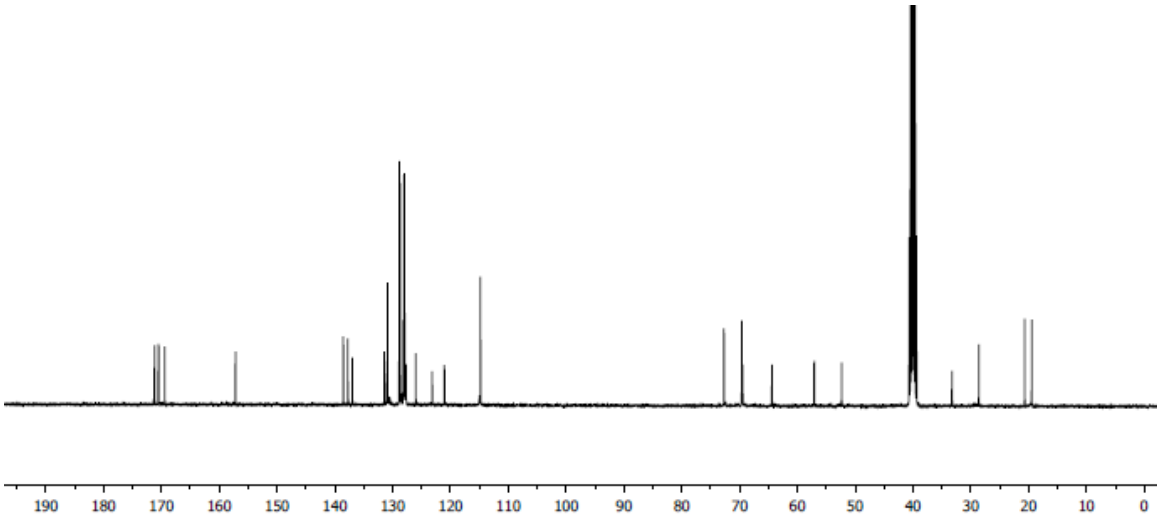
^{13}C-NMR (101 MHz, DMSO-d_6) δ 171.2, 170.5, 170.4, 169.4, 157.1, 138.5, 137.7, 136.9, 131.4, 131.3, 130.9, 128.8, 128.6, 128.2, 128.0, 127.9, 127.8, 127.7, 126.0, 123.1, 121.0, 114.8, 72.7, 69.6, 69.4, 64.4, 57.0, 52.3, 33.2, 28.6, 20.6, 19.4;

HRMS (ESI-) m/z calcd for $C_{38}H_{39}N_4O_6$ $(M-H)^-$ 647.2870; found 647.2850.

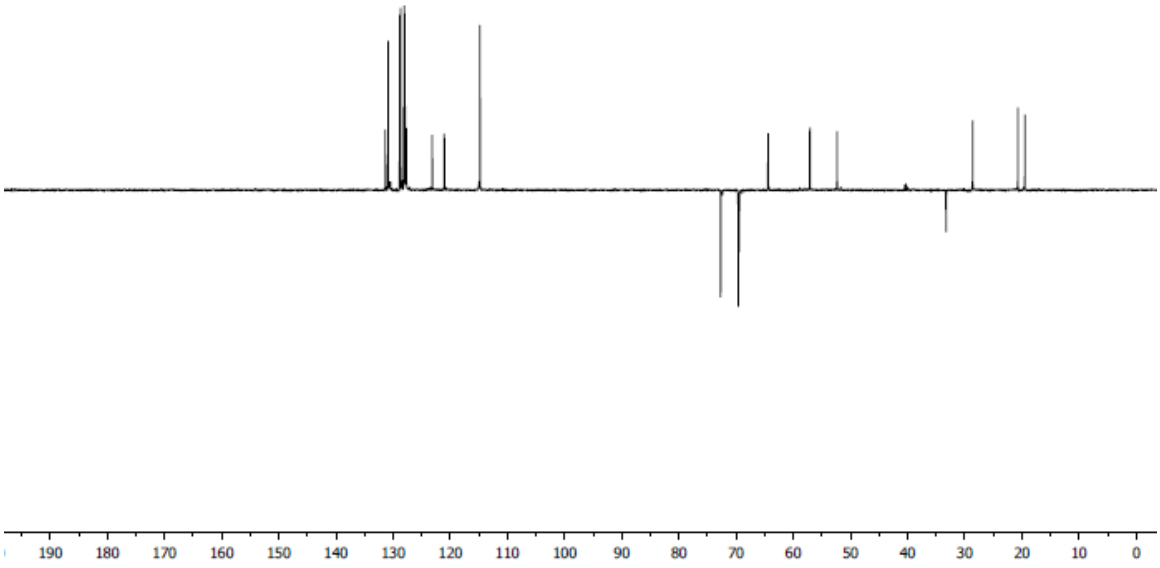
^{1}H NMR



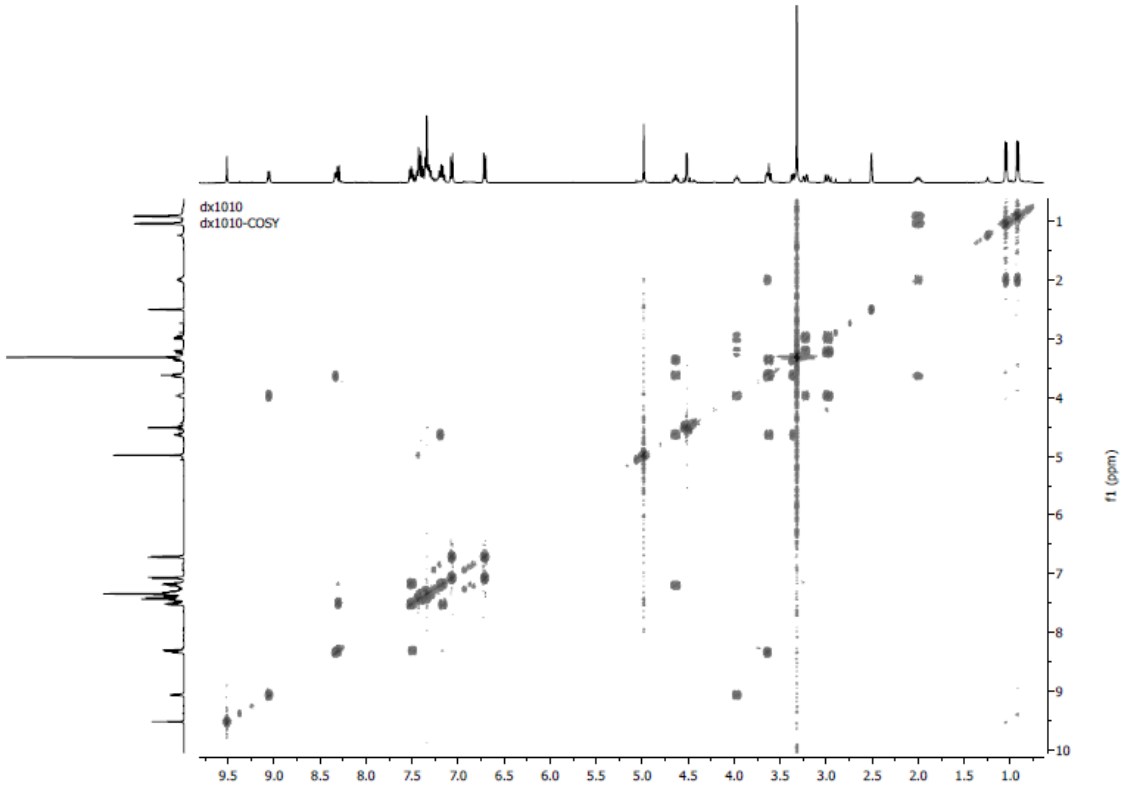
^{13}C NMR



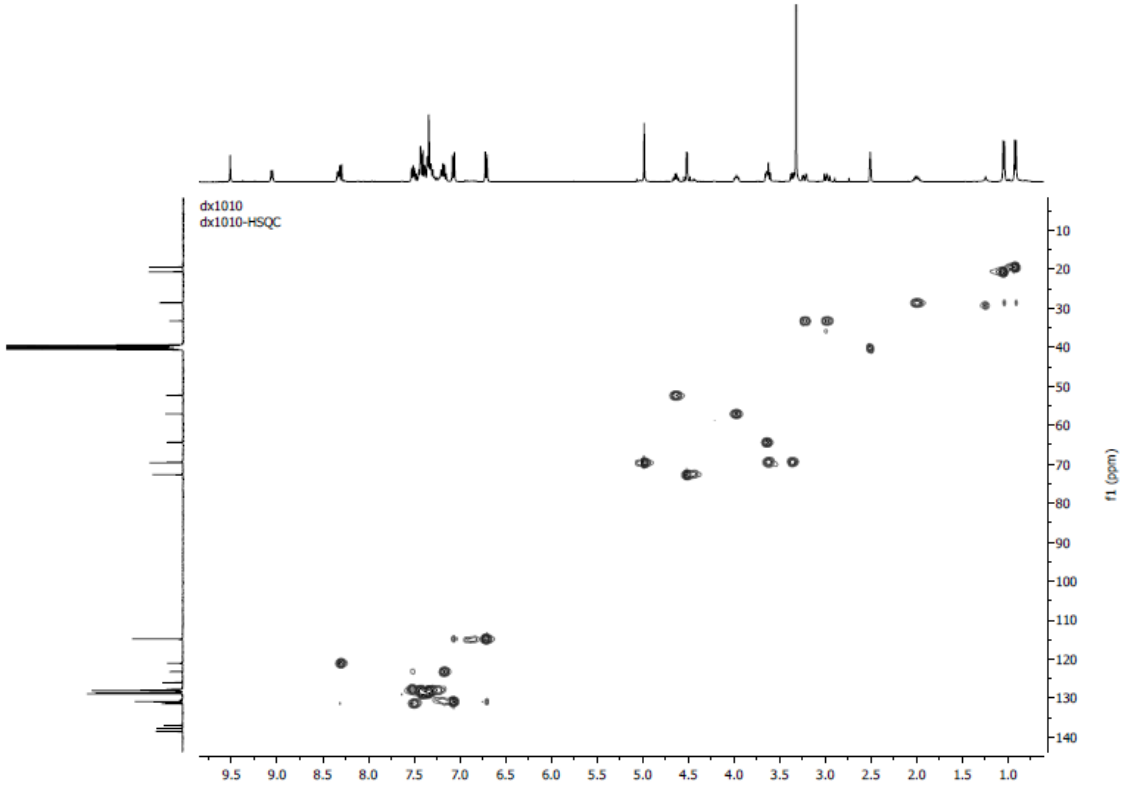
DEPT135


190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

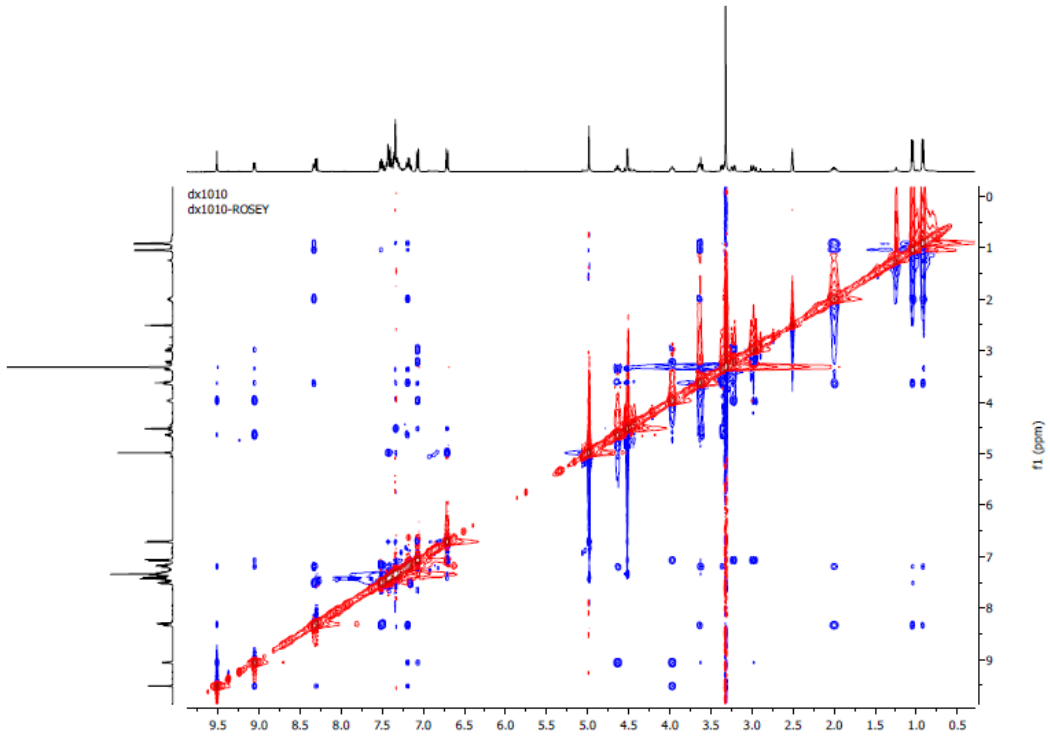
^{1}H-^{1}H COSY


dx1010
dx1010-COSY
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
1 2 3 4 5 6 7 8 9 10
f1 (ppm)

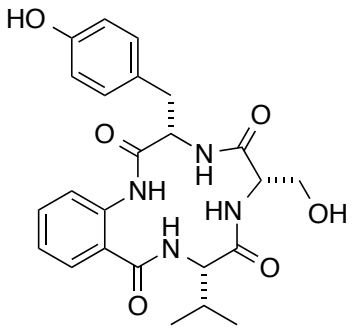
^{1}H-^{13}C HSQC



ROESY



(3*S*,6*S*,9*S*)-3-(4-Hydroxybenzyl)-6-(hydroxymethyl)-9-isopropyl-3,4,6,7,9,10-hexahydro-1*H*-benzo[*k*][1,4,7,10]tetraazacyclotridecine-2,5,8,11-tetraone (LLL-**1vsy**)



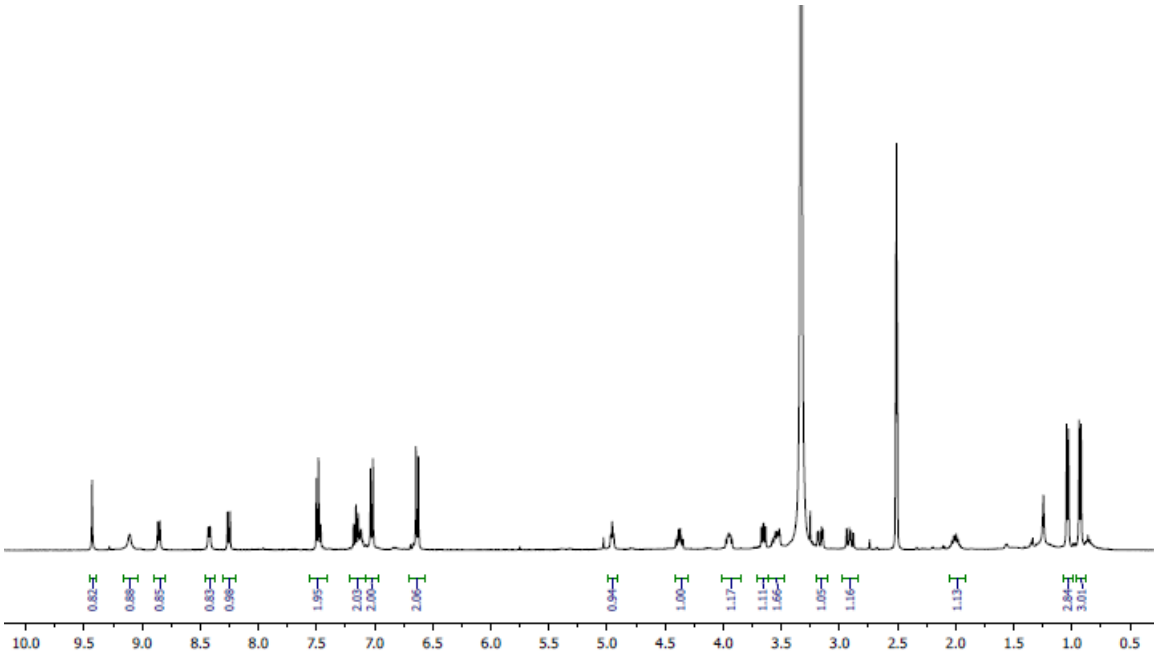
White solid, 87 %; mp > 270 °C, color change observed at above 193 °C;

^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.43 (s, 1H), 9.11 (s, 1H), 8.86 (d, *J* = 7.1 Hz, 1H), 8.42 (d, *J* = 6.0 Hz, 1H), 8.25 (d, *J* = 8.4 Hz, 1H), 7.53-7.45 (m, 2H), 7.21-7.08 (m, 2H), 7.02 (d, *J* = 8.4 Hz, 2H), 6.63 (d, *J* = 8.5 Hz, 2H), 4.96 (t, *J* = 5.3 Hz, 1H), 4.41-4.33 (m, 1H), 3.98-3.91 (m, 1H), 3.65 (dd, *J* = 8.8, 6.3 Hz, 1H), 3.61-3.47 (m, 2H), 3.17 (dd, *J* = 14.0, 4.4 Hz, 1H), 2.91 (dd, *J* = 14.0, 9.5 Hz, 1H), 2.05-1.92 (m, 1H), 1.04 (d, *J* = 6.6 Hz, 3H), 0.93 (d, *J* = 6.7 Hz, 3H);

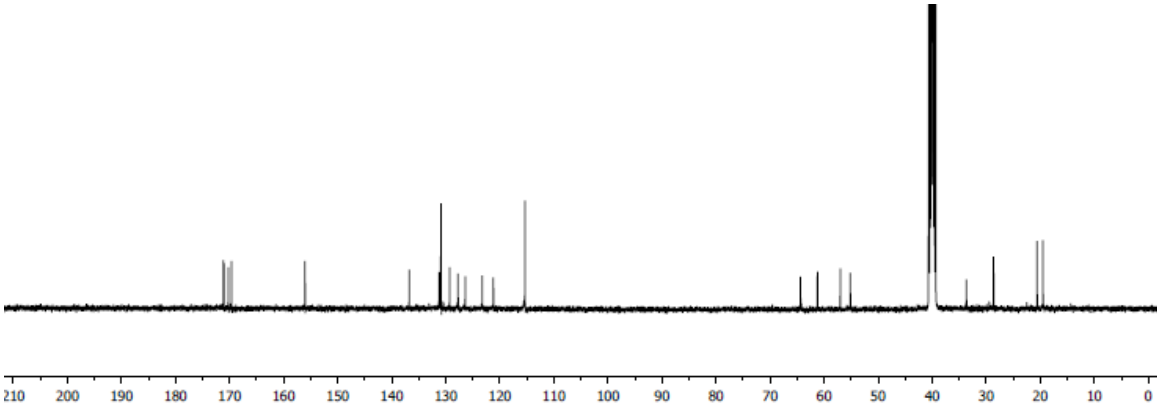
^{13}C-NMR (101 MHz, DMSO-d_6) δ 171.2, 171.1, 170.2, 169.6, 156.0, 136.7, 131.2, 130.9, 129.3, 127.7, 126.4, 123.2, 121.2, 115.3, 64.4, 61.2, 57.0, 55.2, 33.7, 28.6, 20.6, 19.5;

HRMS (ESI-) m/z calcd for $C_{24}H_{27}N_4O_6$ $(M-H)^-$ 467.1931; found 467.1919.

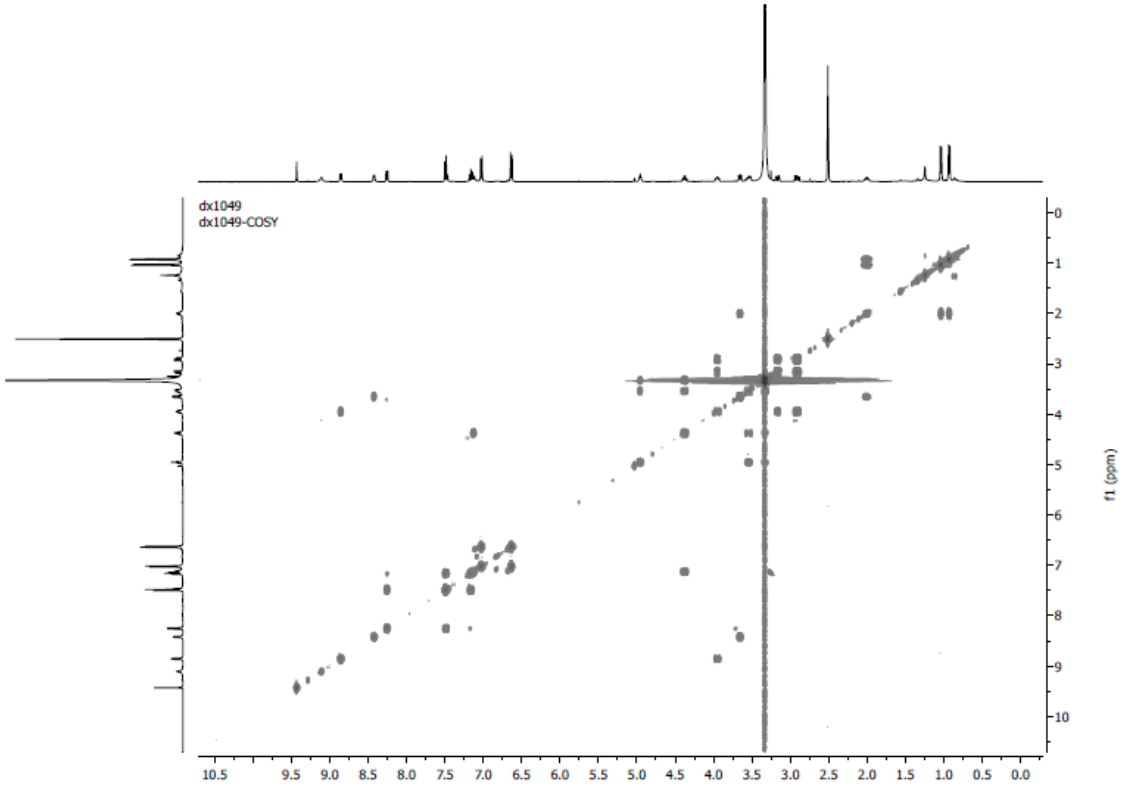
^{1}H NMR


0.82
0.88
0.85
0.83
0.98
1.95
2.03
2.00
2.06
0.94
1.00
1.17
1.11
1.66
1.05
1.16
1.13
2.84
3.01
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5

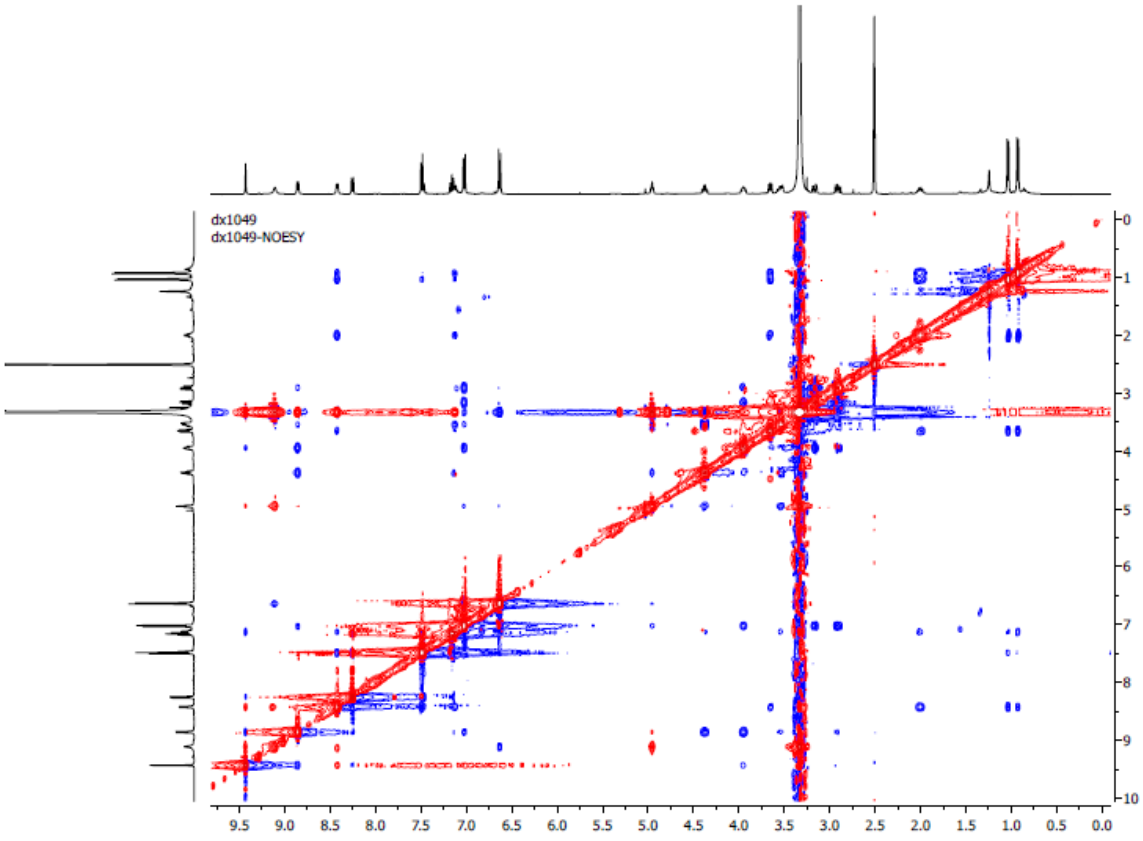
^{13}C NMR


210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

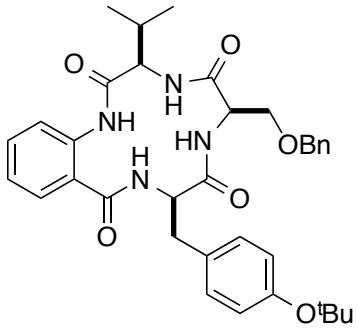
^{1}H-^{1}H COSY


dx1049
dx1049-COSY
f1 (ppm)
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

NOESY


dx1049
dx1049-NOESY
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
0 1 2 3 4 5 6 7 8 9 10

(3*R*,6*R*,9*R*)-6-((Benzyloxy)methyl)-9-(4-(*tert*-butoxy)benzyl)-3-isopropyl-3,4,6,7,9,10-hexahydro-1*H*-benzo[*k*][1,4,7,10]tetraazacyclotridecine-2,5,8,11-tetraone (DDD-**1y's'v**)



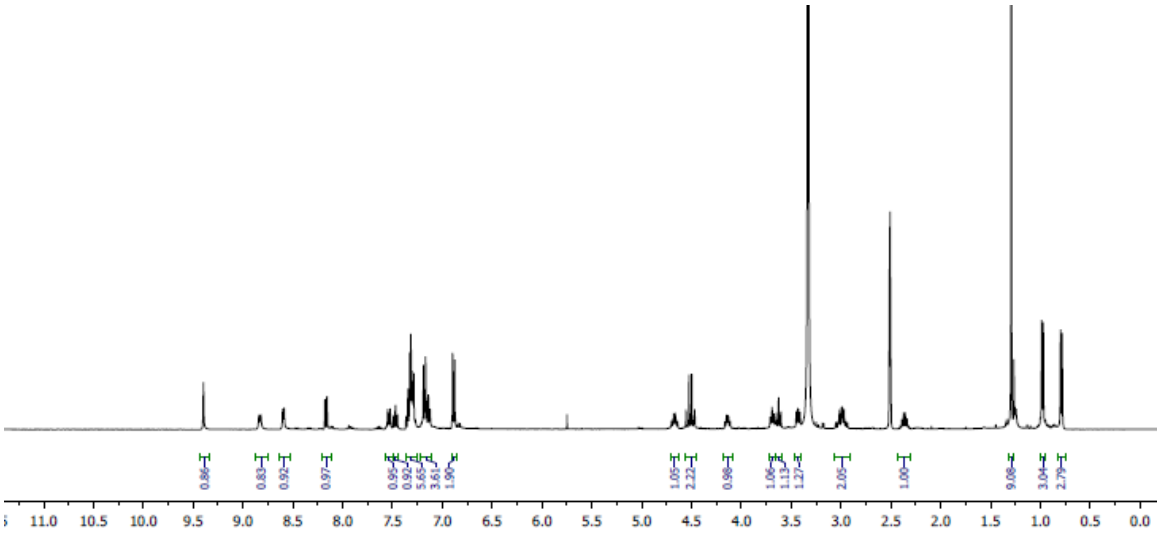
White solid, 31 % over three steps; mp > 270 °C;

^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.40 (s, 1H), 8.83 (d, *J* = 7.3 Hz, 1H), 8.59 (d, *J* = 5.4 Hz, 1H), 8.17 (d, *J* = 8.1 Hz, 1H), 7.54 (d, *J* = 9.5 Hz, 1H), 7.50-7.44 (m, 1H), 7.36-7.26 (m, 6H), 7.21-7.11 (m, 3H), 6.89 (d, *J* = 8.4 Hz, 2H), 4.67 (td, *J* = 9.2, 6.0 Hz, 1H), 4.54 (d, *J* = 12.0 Hz, 1H), 4.48 (d, *J* = 12.0 Hz, 1H), 4.18-4.09 (m, 1H), 3.69 (t, *J* = 7.2 Hz, 1H), 3.63 (t, *J* = 9.3 Hz, 1H), 3.43 (dd, *J* = 9.4, 6.0 Hz, 1H), 3.06-2.93 (m, 2H), 2.68-2.59 (m, 1H), 1.29 (s, 9H), 0.98 (d, *J* = 6.7 Hz, 3H), 0.79 (d, *J* = 6.8 Hz, 3H);

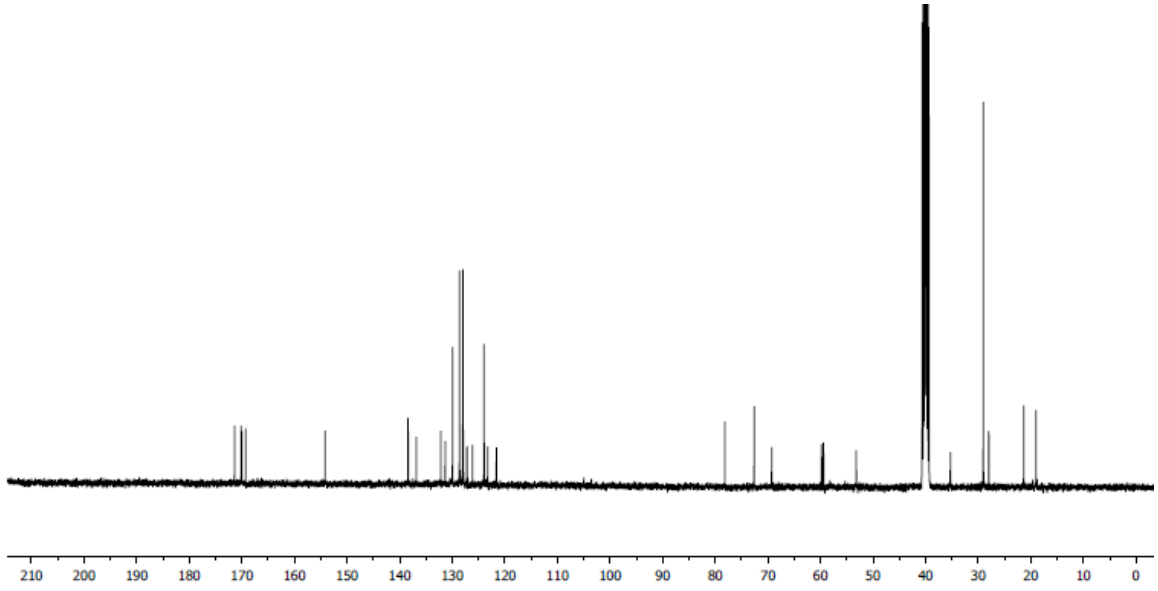
^{13}C-NMR (101 MHz, DMSO-d_6) δ 171.3, 170.1, 170.0, 169.2, 154.1, 138.3, 136.8, 132.1, 131.3, 129.9, 128.6, 128.0, 127.9, 127.2, 126.1, 123.9, 123.2, 121.6, 78.1, 72.6, 69.2, 59.7, 59.4, 53.1, 35.3, 29.0, 27.9, 21.3, 19.0;

HRMS (ESI+) m/z calcd for $C_{35}H_{42}N_4O_6Na$ $(M+Na)^+$ 637.3002; found 637.2988.

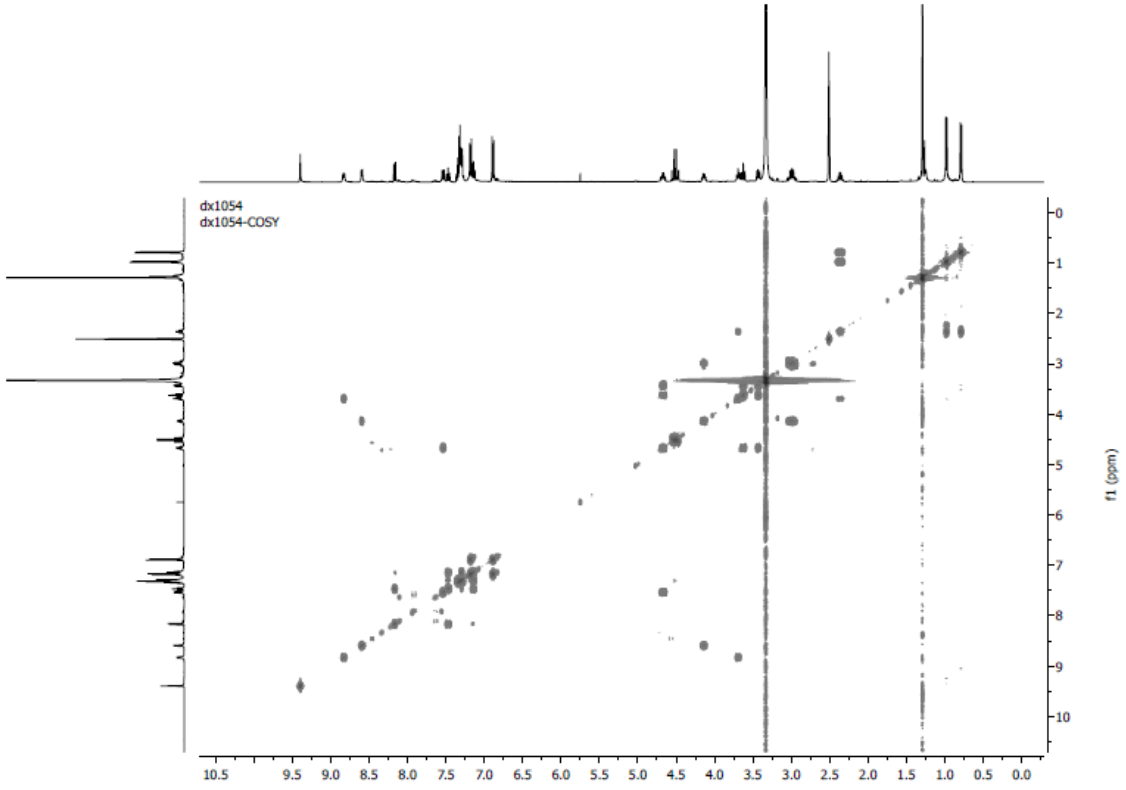
^{1}H NMR


0.86
0.83
0.92
0.97
0.95
0.92
5.65
3.61
1.90
1.05
2.22
0.98
1.06
1.13
1.27
2.05
1.00
9.08
3.04
2.79
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

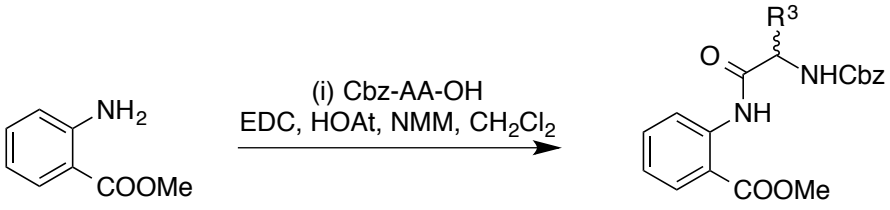
^{13}C NMR


210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

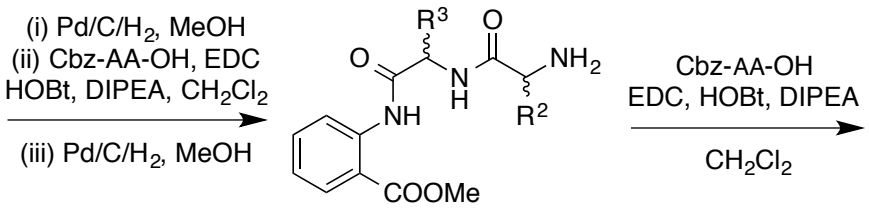
^{1}H-^{1}H NMR

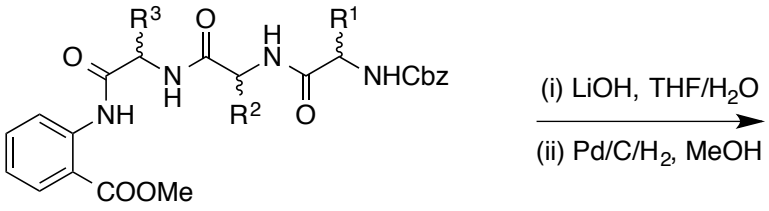

dx1054
dx1054-COSY
f1 (ppm)
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

***Cbz approach* to products 1**

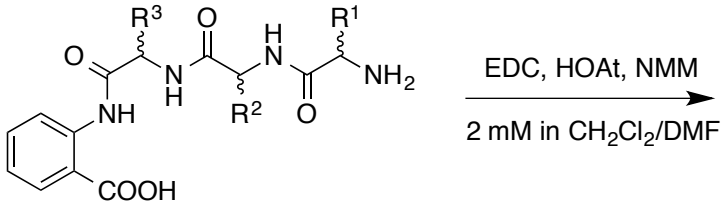


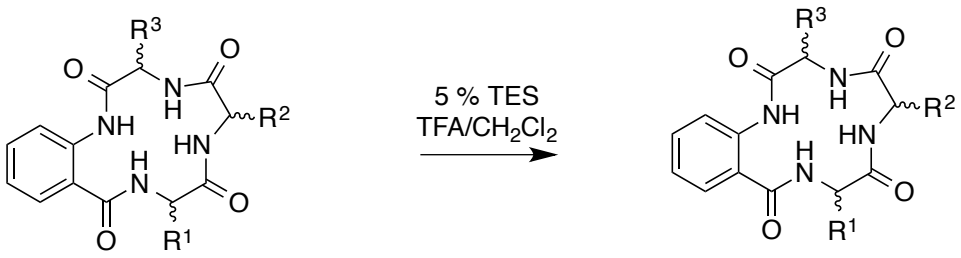
D-**3g'**
82 %





82 - 93 % over four steps





cyclo-LPhe-DAla-LPhe-Anth (LDL-**1faf**), 45 %
cyclo-DPhe-LAla-LGlu'-Anth (DLL-**1fae'**), 42 %

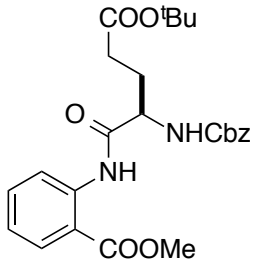
cyclo-DPhe-LAla-LGlu-Anth (DLL-**1fae**), 81 %

Scheme S2. *Cbz approach* to products **1**

General Procedures For Cbz Approach in Solution

Methyl anthranilate was coupled to the first Cbz protected amino acid with "*Coupling Method I*". The Cbz protection group was deprotected with "*Cbz Deprotection Procedure*" and the resulting material was coupled with the second Cbz protected amino acid with "*Coupling Method II*". The tripeptide intermediate was deprotected with "*Cbz Deprotection Procedure*" and coupled with the third Cbz amino acid with "*Coupling Method II*" to give the protected tetrapeptide intermediate. The methyl ester of this intermediate was hydrolyzed with the procedure "*Hydrolysis of Methyl Ester*" and the *N*- terminus Cbz group was removed with "*Cbz Deprotection Procedure*". The linear tetrapeptide was cyclized with "*Procedure for Cyclization*" to give the cyclic peptide product. If necessary, the cyclic peptide product was deprotected with "*^{t}Bu Deprotection Procedure*" to give the final deprotected product.

Methyl (*R*)-2-(2-(((benzyloxy)carbonyl)amino)-5-(*tert*-butoxy)-5-oxopentanamido)benzoate (D-**3g'**)



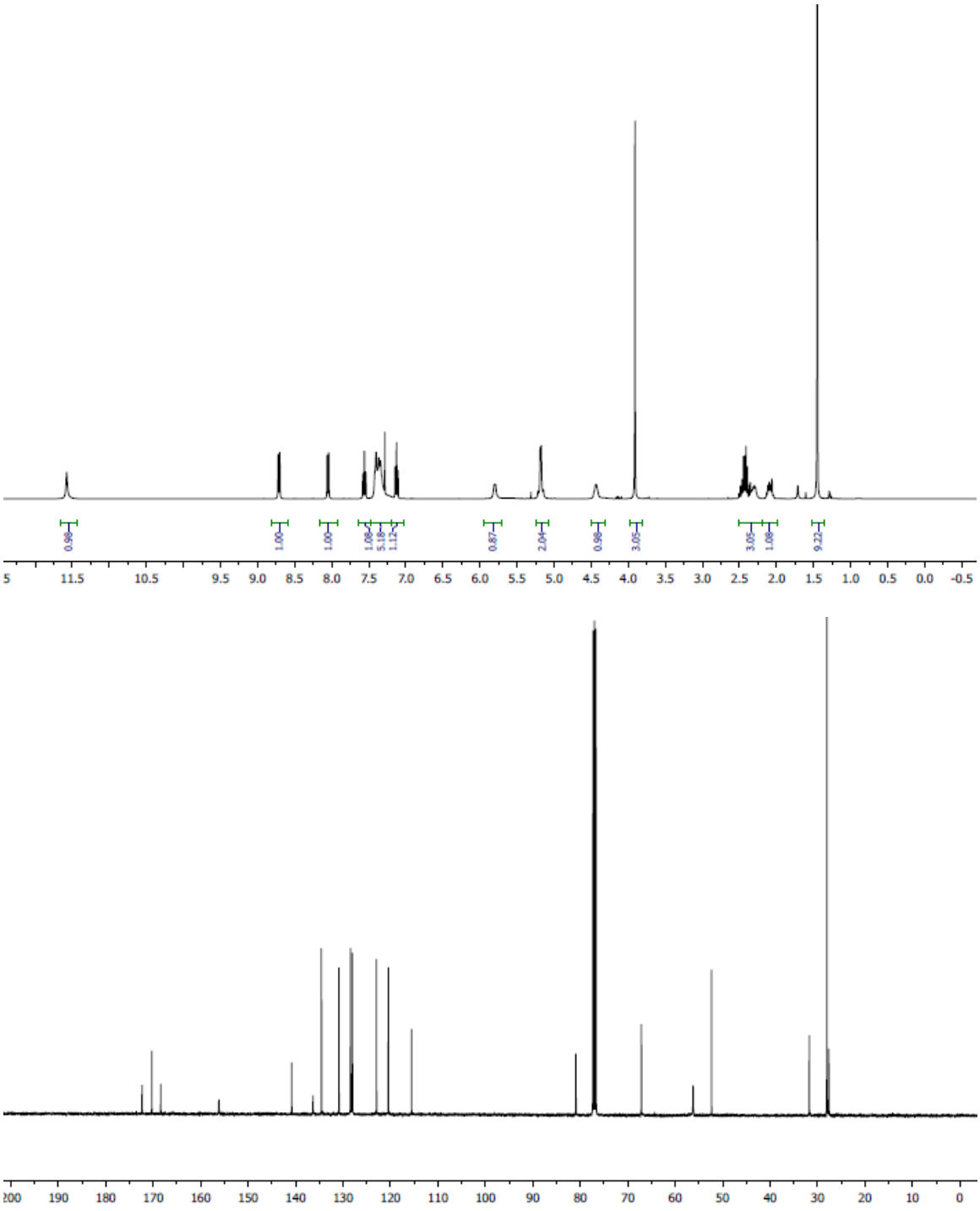
White solid, 82 %; mp = 78.8-79.8 °C;

^{1}H-NMR (400 MHz, $CDCl_3$) δ 11.58 (s, 1H), 8.71 (d, *J* = 8.4 Hz, 1H), 8.05 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.64-7.47 (m, 1H), 7.43-7.30 (m, 5H), 7.20-7.03 (m, 1H), 5.80 (d,

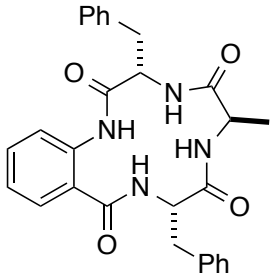
J = 6.2 Hz, 1H), 5.28-5.10 (m, 2H), 4.49-4.38 (m, 1H), 3.91 (s, 3H), 2.50-2.19 (m, 3H), 2.19-1.99 (m, 1H), 1.45 (s, 9H);

^{13}C-NMR (101 MHz, $CDCl_3$) δ 172.3, 170.2, 168.4, 156.1, 140.8, 136.3, 134.5, 130.8, 128.4, 128.0, 128.0, 122.9, 120.4, 115.5, 80.9, 67.1, 56.2, 52.3, 31.7, 28.0, 27.6;

HRMS (ESI+) m/z calcd for $C_{25}H_{31}N_2O_7$ $(M+H)^+$ 471.2131; found 471.2145.



(3*S*,6*R*,9*S*)-3,9-Dibenzyl-6-methyl-3,4,6,7,9,10-hexahydro-1*H*-benzo[*k*][1,4,7,10]tetraazacyclotridecine-2,5,8,11-tetraone (LDL-**1faf**)



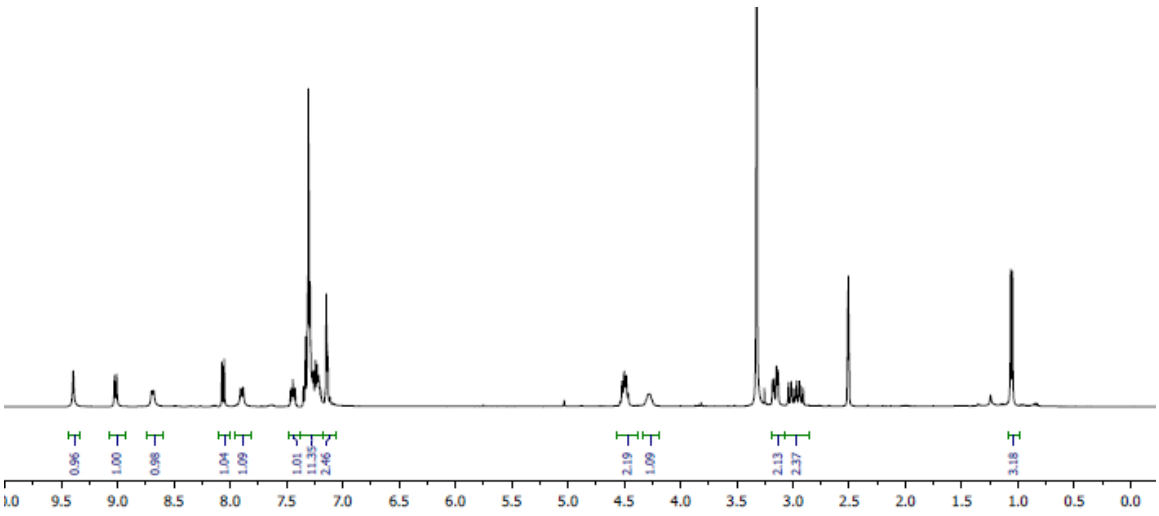
White solid, 45 % over four steps, starting from Boc-deprotected L-**2f**; mp > 270 °C;

^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.39 (s, 1H), 9.02 (d, *J* = 7.5 Hz, 1H), 8.69 (d, *J* = 7.1 Hz, 1H), 8.06 (d, *J* = 8.2 Hz, 1H), 7.90 (d, *J* = 8.8 Hz, 1H), 7.49-7.37 (m, 1H), 7.37-7.17 (m, 10H), 7.17-7.06 (m, 2H), 4.53-4.48 (m, 2H), 4.29-4.25 (m, 1H), 3.19-3.12 (m, 2H), 3.08-2.90 (m, 2H), 1.06 (d, *J* = 6.8 Hz, 3H);

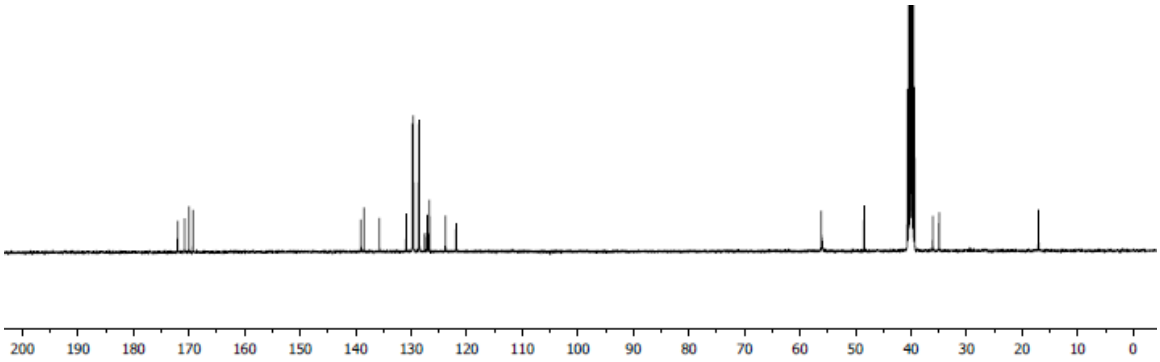
^{13}C-NMR (101 MHz, DMSO-d_6) δ 172.0, 170.8, 170.0, 169.2, 139.0, 138.5, 135.7, 130.9, 129.7, 129.6, 128.6, 128.5, 127.5, 127.1, 126.7, 126.7, 123.8, 121.9, 56.1, 56.0, 48.4, 36.0, 34.9, 17.0;

HRMS (ESI+) m/z calcd for $C_{28}H_{28}N_4O_4Na$ $(M+Na)^+$ 507.2008; found 507.2027.

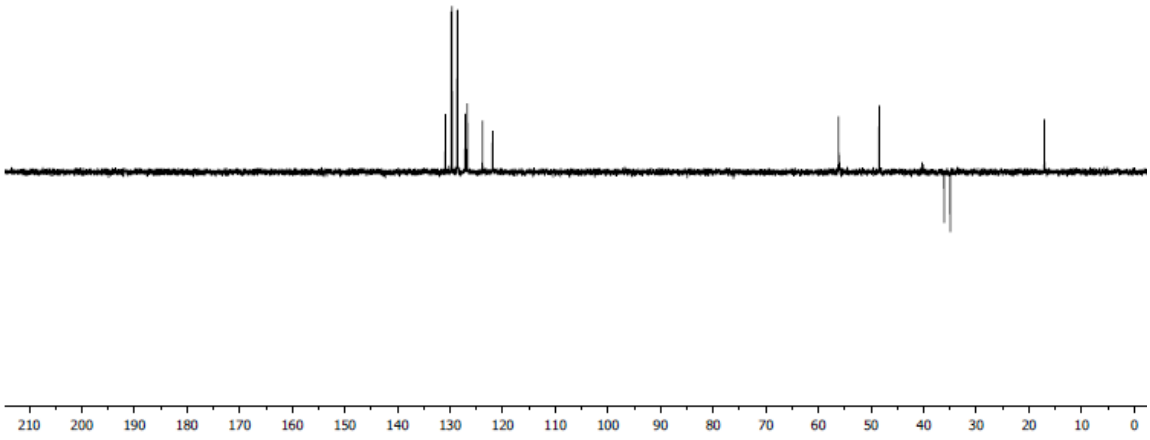
^{1}H NMR



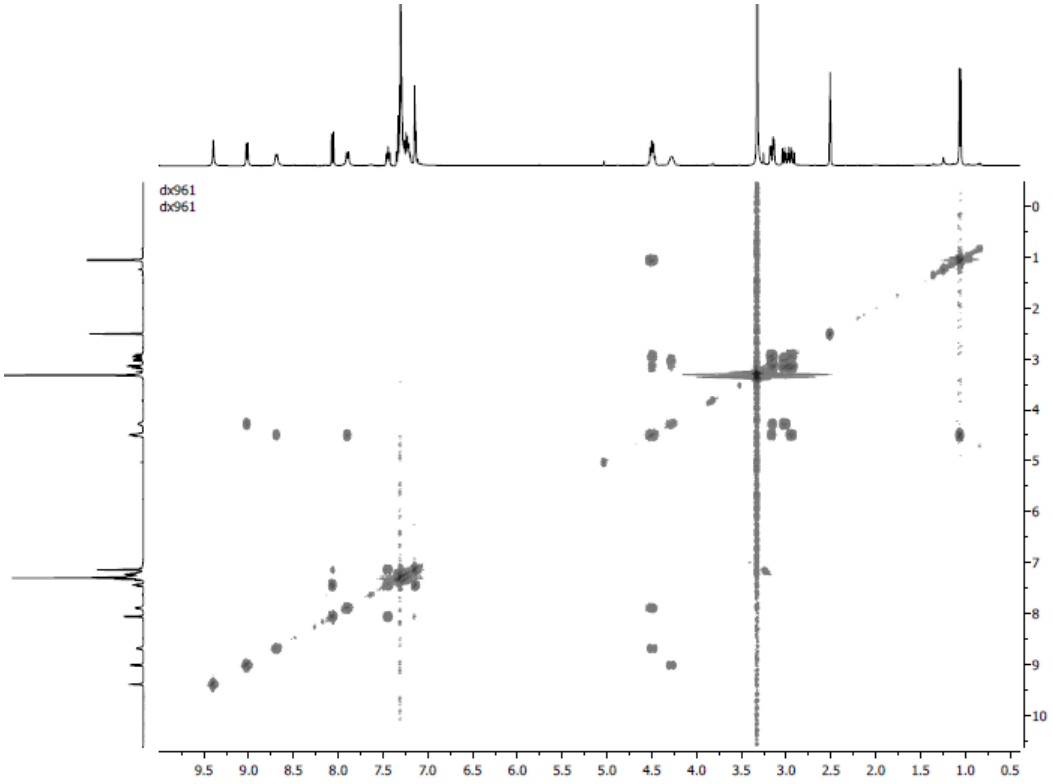
^{13}C NMR


200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

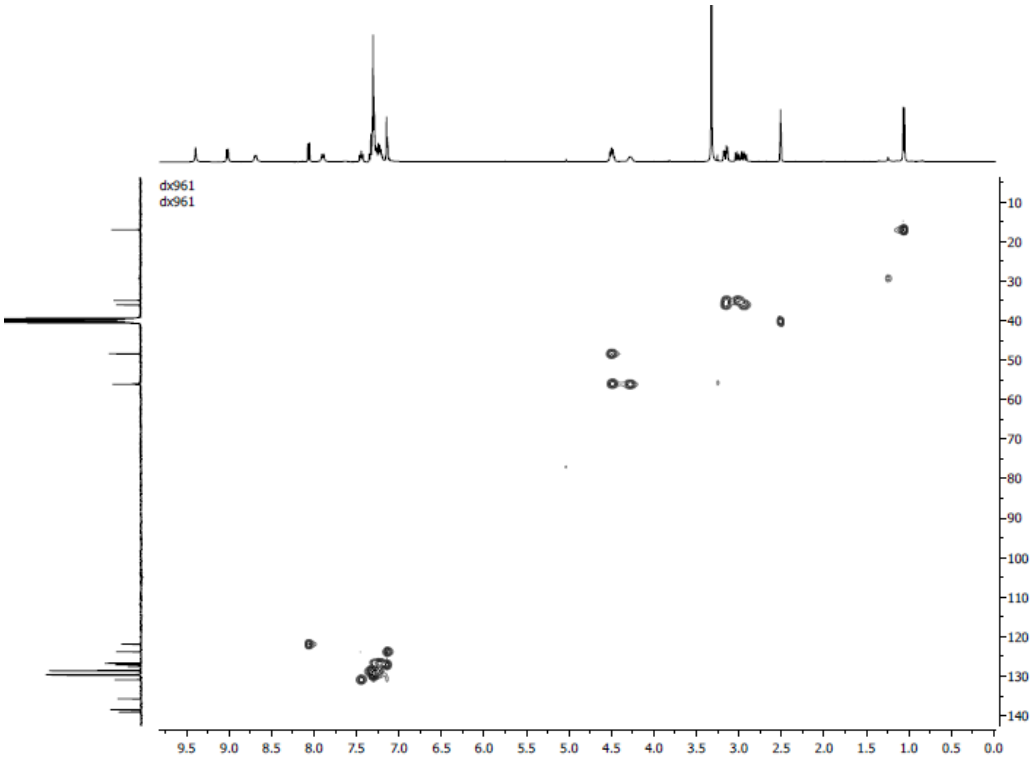
DEPT135


210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

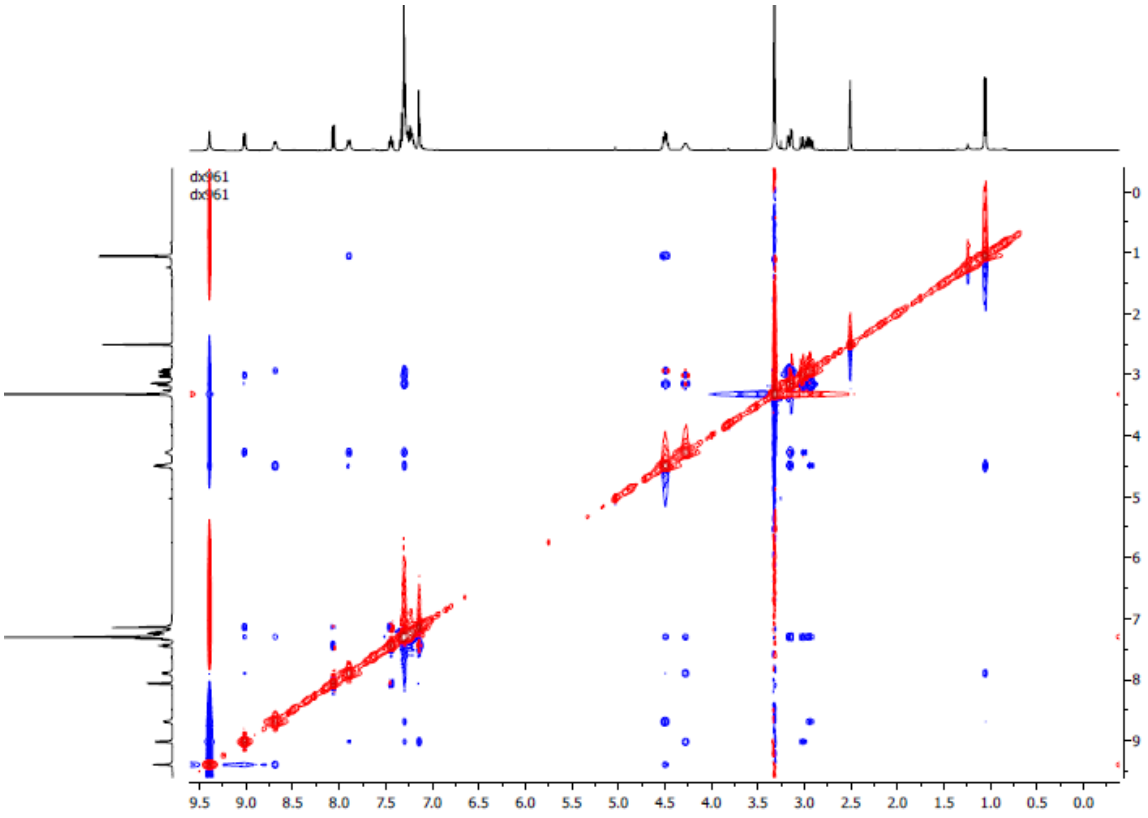
^{1}H-^{1}H COSY



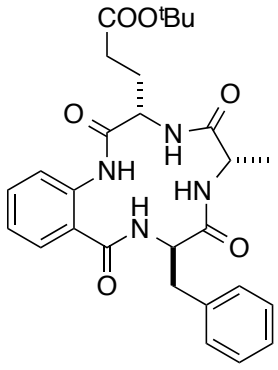
^{1}H-^{13}C HSQC



NOESY


dx961
dx961
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
7
8
9

tert-Butyl 3-((3*S*,6*S*,9*R*)-9-benzyl-6-methyl-2,5,8,11-tetraoxo-2,3,4,5,6,7,8,9,10,11-decahydro-1*H*-benzo[*k*][1,4,7,10]tetraazacyclotridecin-3-yl)propanoate (DLL-**fae'**)



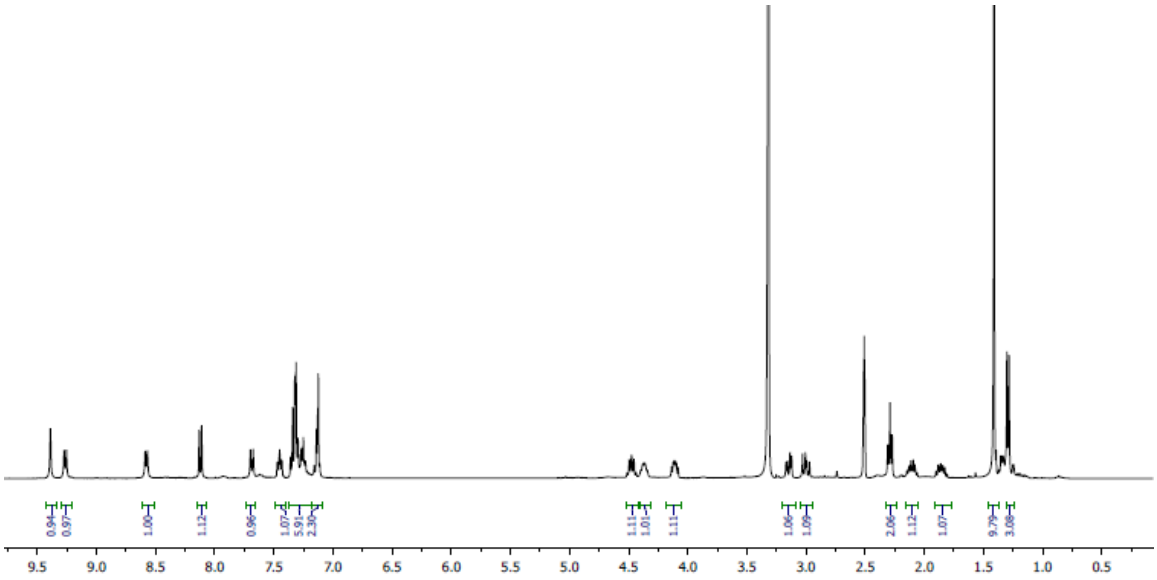
White solid, 42 % over three steps; mp > 270 °C, color change observed at above 215 °C;

^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.39 (s, 1H), 9.26 (d, *J* = 7.9 Hz, 1H), 8.58 (d, *J* = 7.4 Hz, 1H), 8.12 (d, *J* = 8.2 Hz, 1H), 7.69 (d, *J* = 9.2 Hz, 1H), 7.49-7.40 (m, 1H), 7.37-7.18 (m, 5H), 7.18-7.08 (m, 2H), 4.52-4.42 (m, 1H), 4.41-4.32 (m, 1H), 4.16-4.08 (m, 1H), 3.15 (dd, *J* = 13.8, 5.4 Hz, 1H), 3.00 (dd, *J* = 13.8, 9.7 Hz, 1H), 2.29 (t, *J* = 7.5 Hz, 2H), 2.15-2.05 (m, 1H), 1.91-1.77 (m, 1H), 1.41 (s, 9H), 1.29 (d, *J* = 7.2 Hz, 3H);

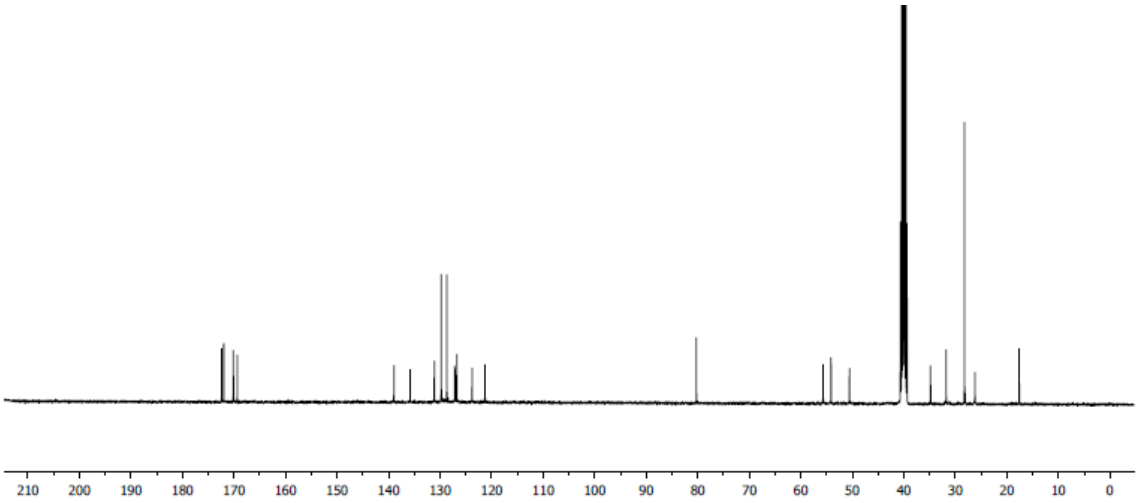
^{13}C-NMR (101 MHz, DMSO-d_6) δ 172.4, 172.0, 170.1, 170.1, 169.3, 138.9, 135.8, 131.1, 129.7, 128.7, 127.1, 127.0, 126.7, 123.7, 121.3, 80.2, 55.6, 54.1, 50.5, 34.8, 31.7, 28.2, 26.2, 17.6;

HRMS (ESI-) m/z calcd for $C_{28}H_{33}N_4O_6$ $(M-H)^-$ 521.2400; found 521.2411.

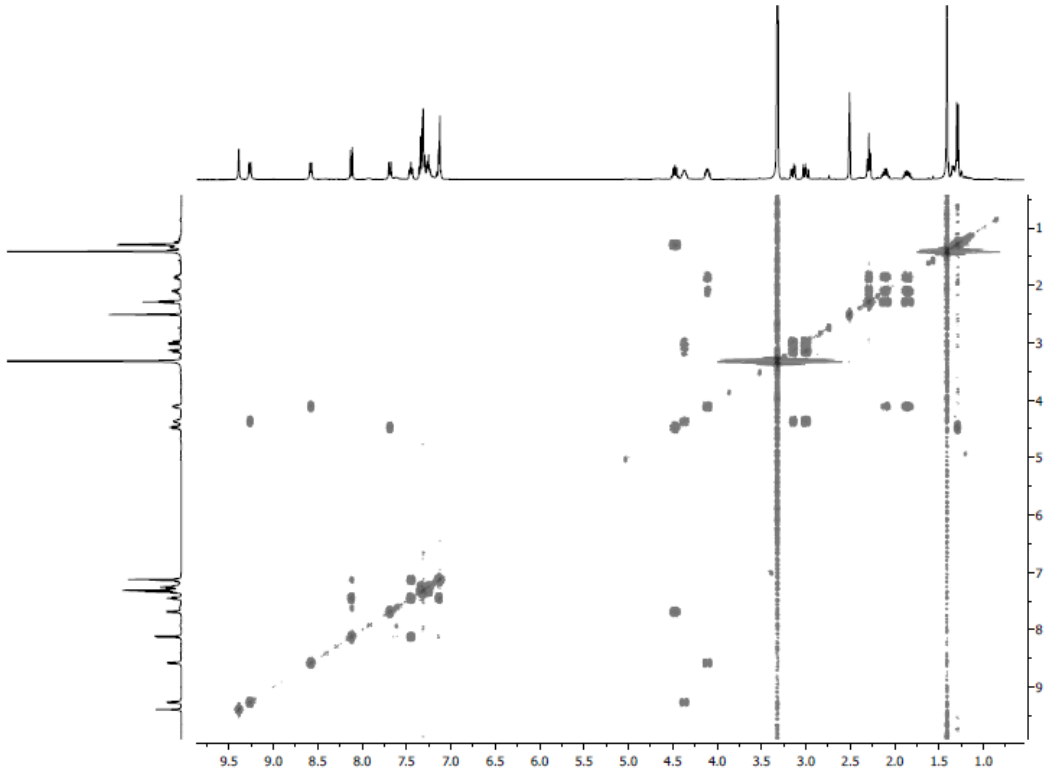
^{1}H NMR


0.94
0.97
1.00
1.12
0.96
1.07
5.91
2.30
1.11
1.01
1.11
1.06
1.09
2.06
1.12
1.07
9.79
3.08
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5

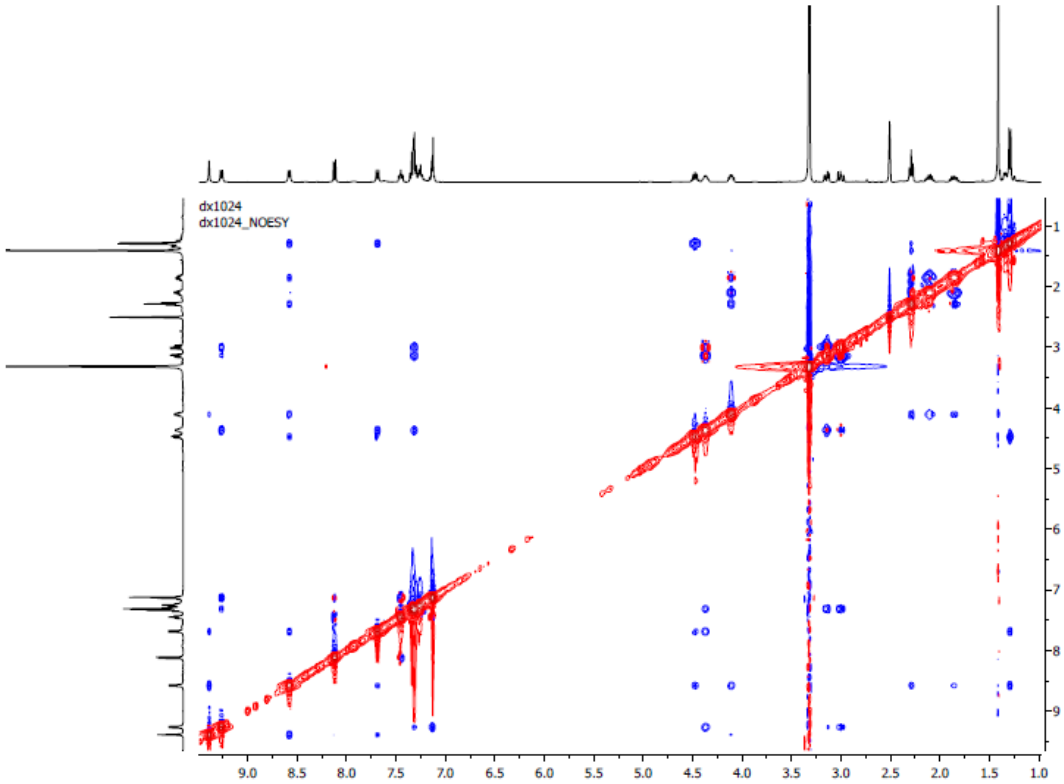
^{13}C NMR


210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

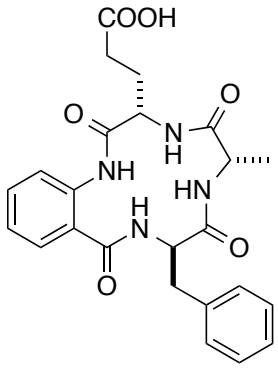
^{1}H-^{1}H COSY



NOESY



3-((3*S*,6*S*,9*R*)-9-Benzyl-6-methyl-2,5,8,11-tetraoxo-2,3,4,5,6,7,8,9,10,11-decahydro-1*H*-benzo[*k*][1,4,7,10]tetraazacyclotridecin-3-yl)propanoic acid (DLL-**fae**)



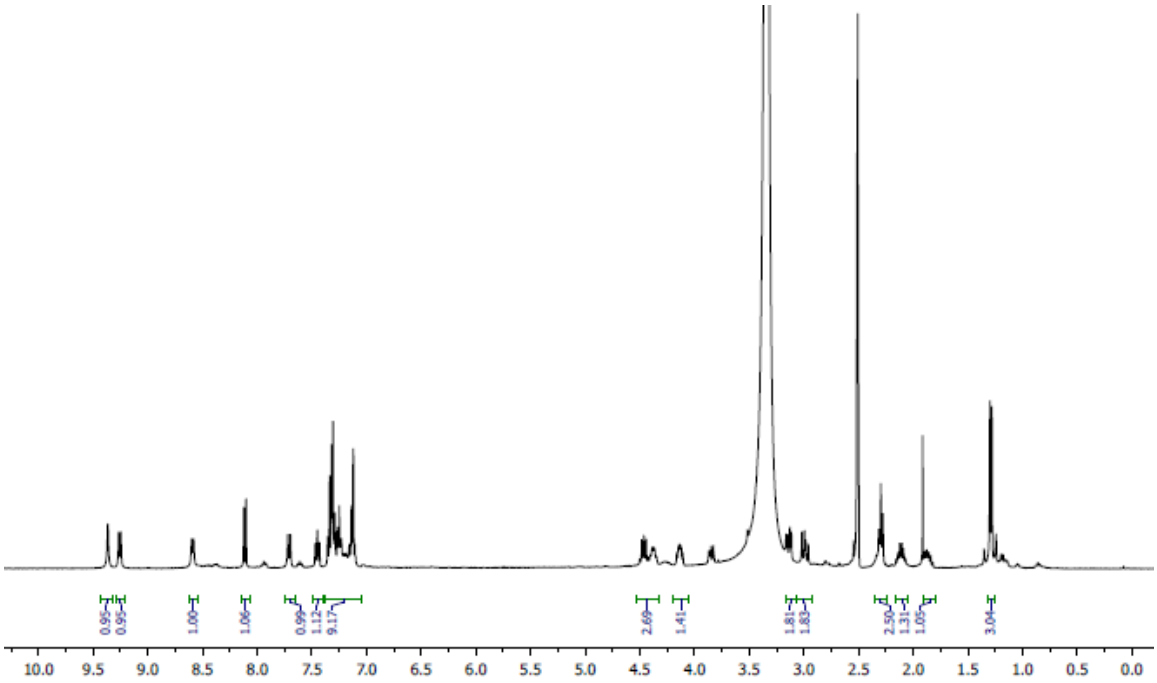
White solid, 81 %; mp > 270 °C;

^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.37 (s, 1H), 9.26 (d, *J* = 8.0 Hz, 1H), 8.59 (d, *J* = 7.3 Hz, 1H), 8.11 (d, *J* = 8.1 Hz, 1H), 7.71 (d, *J* = 9.1 Hz, 1H), 7.50-7.39 (m, 1H), 7.37-7.05 (m, 7H), 4.54-4.32 (m, 2H), 4.19-4.06 (m, 1H), 3.14 (dd, *J* = 13.8, 5.4 Hz, 1H), 2.99 (dd, *J* = 13.8, 9.6 Hz, 1H), 2.30 (t, *J* = 7.6 Hz, 2H), 2.15-2.08 (m, 1H), 1.90-1.79 (m, 1H), 1.29 (d, *J* = 7.3 Hz, 3H);

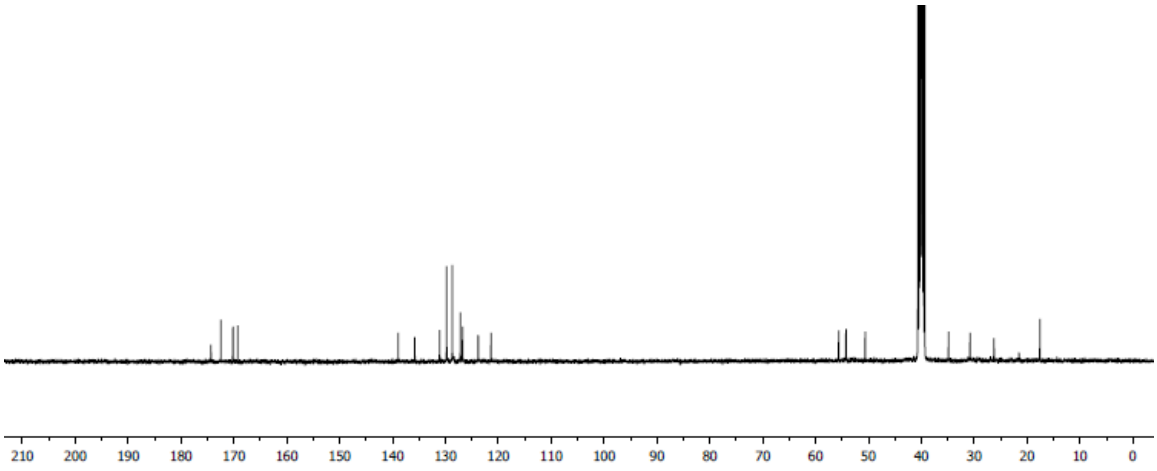
^{13}C-NMR (101 MHz, DMSO-d_6) δ 174.3, 172.4, 170.2, 170.1, 169.2, 138.9, 135.8, 131.1, 129.7, 128.7, 127.1, 127.0, 126.7, 123.8, 121.3, 55.6, 54.2, 50.6, 34.8, 30.8, 26.2, 17.5;

HRMS (ESI-) m/z calcd for $C_{24}H_{25}N_4O_6$ $(M-H)^-$ 465.1774; found 465.1781.

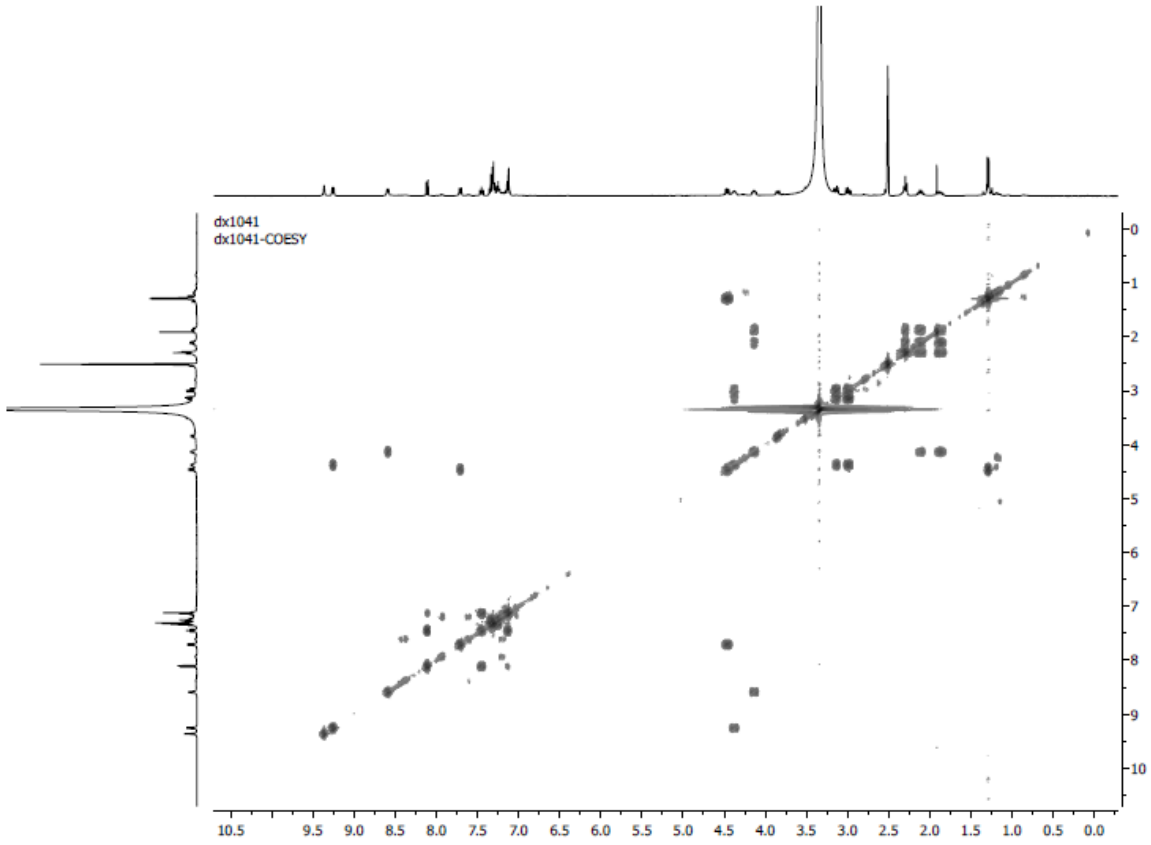
^{1}H NMR


0.95
0.95
1.00
1.06
0.99
1.12
9.17
2.69
1.41
1.81
1.83
2.50
1.31
1.05
3.04
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

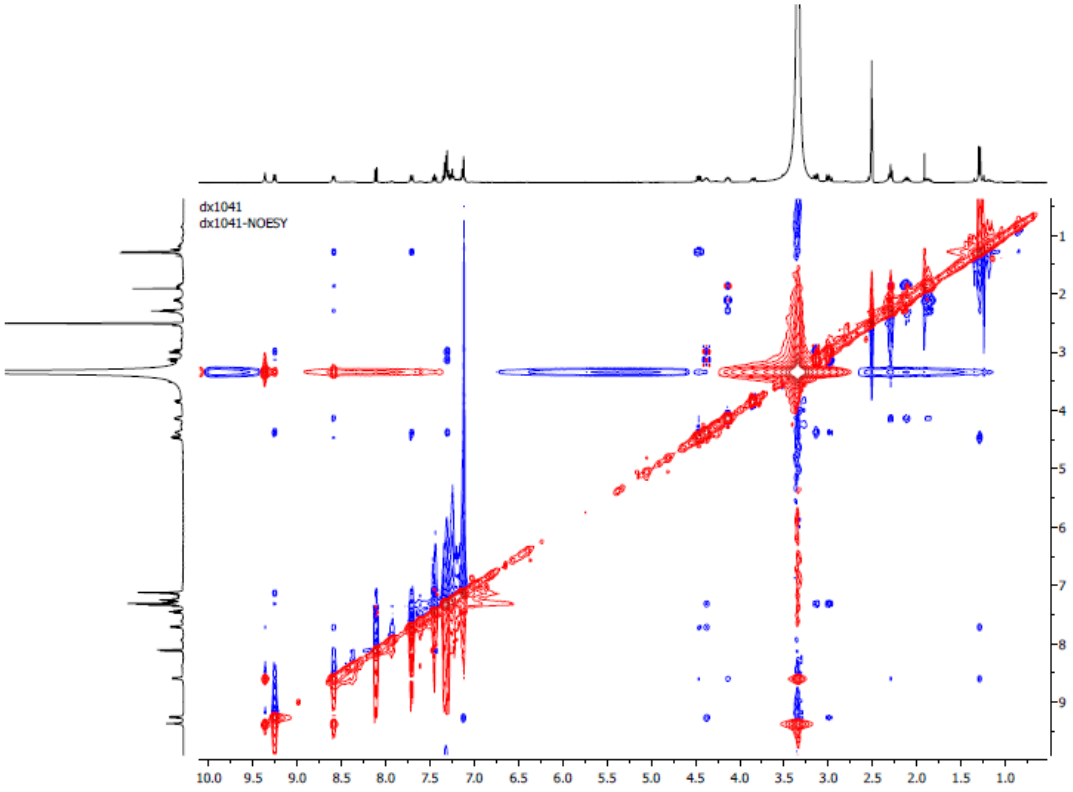
^{13}C NMR


210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

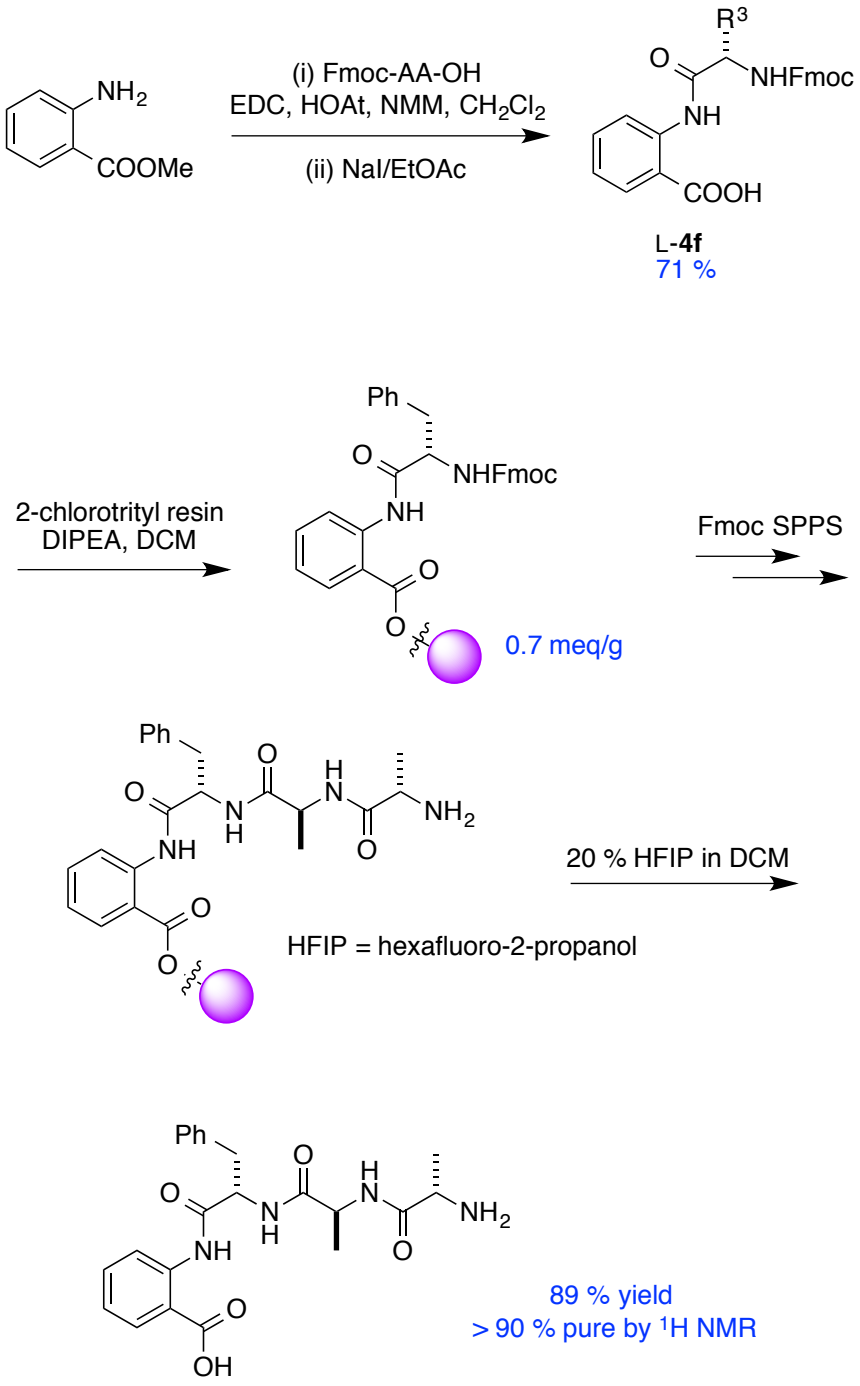
^{1}H-^{1}H COSY


dx1041
dx1041-COESY
0
1
2
3
4
5
6
7
8
9
10
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

NOESY


dx1041
dx1041-NOESY
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
1 2 3 4 5 6 7 8 9

Fmoc solid phase approach to products 1



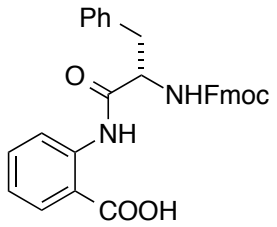
Scheme S3. *Fmoc solid phase approach* to products **1.**

General Procedures for Fmoc Approach on Solid Phase

Synthesis Of Dipeptide Intermediate

Fmoc-Phe-OH (775 mg, 2.0 mmol, 1.0 equiv.) was added to a solution of HOAt (408 mg, 3.0 mmol, 1.5 equiv.) and NMM (220 μL, 2.0 mmol, 1.0 equiv.) in DCM (3.0 mL) at 0 °C under N_2. The resulting mixture was stirred at 0 °C for 10 min and then methyl anthranilate (906 mg, 6.0 mmol, 3.0 equiv.) was added, followed by the addition of EDC•HCl (575 mg, 3.0 mmol, 1.5 equiv.) in one portion. The reaction mixture was allowed to warm to room temperature over 1 h and was stirred at room temperature for 2 h. 30 mL DCM was added to dilute the solution and the organic phase was washed with 0.2 M HCl aqueous solution (30 mL × 5). The organic phase was further washed with brine (10 mL), saturated $NaHCO_3$ solution (20 mL × 2) and brine (10 mL), dried over $MgSO_4$ and filtered. The solvent was removed under vacuum to give the crude material. The crude material was dissolved in EtOAc (10 mL) and LiI (1.07 g, 8.0 mmol, 4.0 equiv.) was added. The mixture was stirred at 80 °C for 18 h and then it was cooled down to room temperature. 0.2 M HCl (20 mL) was added and the mixture was extracted with EtOAc (30 mL × 3). The organic phase was washed with brine (10 mL), dried over $MgSO_4$, filtered and the solvent was removed under vacuum to give the crude product. The crude product was purified by flash chromatography (30% EtOAc in DCM to 100 % EtOAc) to give the desired product L-**4f** as a white solid.

(*S*)-2-(2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-phenylpropanamido)benzoic acid (L-**4f**)



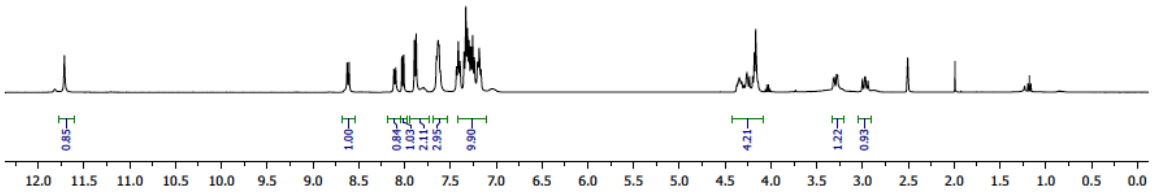
White solid, 71 %; mp = 175.5-176.4 °C;

^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.71 (s, 1H), 8.62 (d, *J* = 8.4 Hz, 1H), 8.11 (d, *J* = 8.0 Hz, 1H), 8.02 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.88-7.83 (m, 2H), 7.68-7.57 (m, 3H), 7.42-7.11 (m, 10H), 4.43-4.08 (m, 4H), 3.30 (dd, *J* = 13.8, 4.2 Hz, 1H), 2.96 (dd, *J* = 13.6 Hz, 10.7 Hz, 1H);

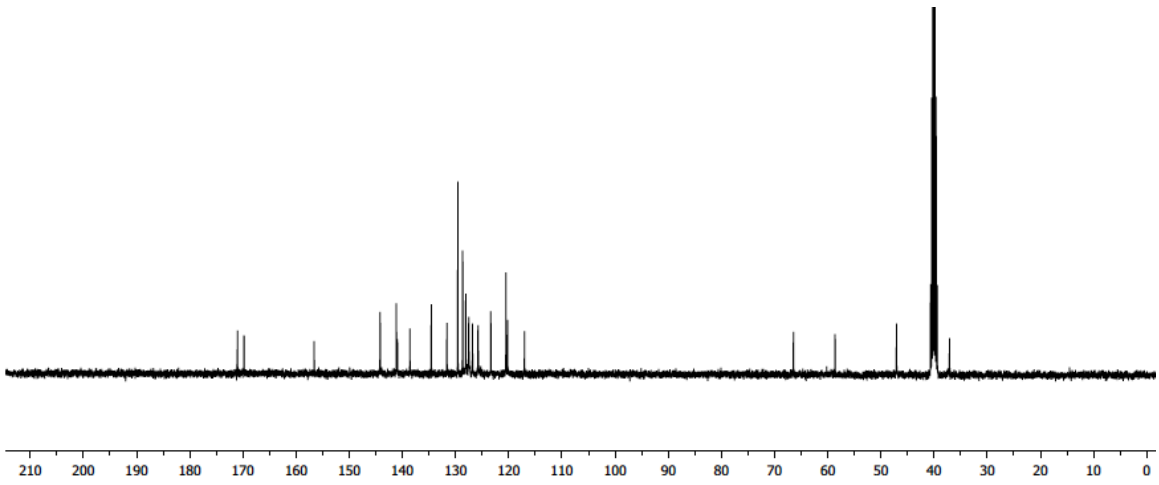
^{13}C-NMR (101 MHz, DMSO-d_6) δ 171.0, 169.8, 156.6, 144.2, 141.1, 140.9, 138.6, 134.6, 131.6, 129.6, 128.7, 128.1, 127.5, 126.8, 125.8, 123.4, 120.5, 120.2, 117.1, 66.5, 58.6, 47.1, 37.1;

HRMS (ESI-) m/z calcd for $C_{31}H_{25}N_2O_5$ $(M-H)^-$ 505.1763; found 505.1781.

^{1}H NMR



^{13}C NMR



Loading of Dipeptide onto 2-Cl-Trityl Resin

Cl-Trt resin (200 mg, 1.4 meq/g) was shaken with anhydrous DCM (4 mL) in a fritted syringe for 30 min. Then the DCM was removed and a mixture of L-**4f** (71 mg, 0.14 mmol) and DIPEA (98 μL, 0.56 mmol) in DCM (2 mL) was added into the syringe and the mixture was shaken at room temperature for 2h. The remaining reactive site was blocked with MeOH/DIPEA (9:1 v/v) for 30 min and the beads were washed with DCM 3 times, MeOH and then DMF 3 times.

Coupling With Amino Acids And Fmoc Deprotection

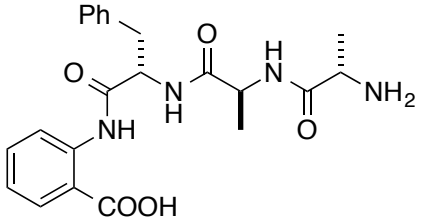
Fmoc protection groups were deprotected by treating the bead with 20 % piperidine in DMF for 1 min, followed by the second treatment with 20 % piperidine in DMF for 15 minutes. The beads were washed with DMF 6 times after the second treatment.

Coupling reactions with amino acids were carried out with 3 equiv. of Fmoc amino acid, 3 equiv. of HBTU, 3 equiv. of HOBt, 6 equiv. of DIPEA in DMF for 1 h at room temperature. The beads were washed with DMF 6 times after the coupling reaction and a few beads were subjected to Kaiser test to confirm the completion of the coupling reaction.

Cleavage From Solid Support

After the last Fmoc deprotection step, the resin was washed with DMF 6 times, MeOH 3 times and DCM 3 times. The linear peptide was cleaved off the bead by treating the beads with HFIP/DCM (1:4 v/v) for 30 min at room temperature. After filteration, the solvents were removed under vacuum and the crude material was dried under high vacuum to give the linear peptide. The crude material was analyzed by HPLC and ^{1}H NMR for its purity.

2-((*S*)-2-((*S*)-2-((*S*)-2-Aminopropanamido)propanamido)-3-phenylpropanamido)benzoic acid (LLL-**aaf** linear peptide, crude material)



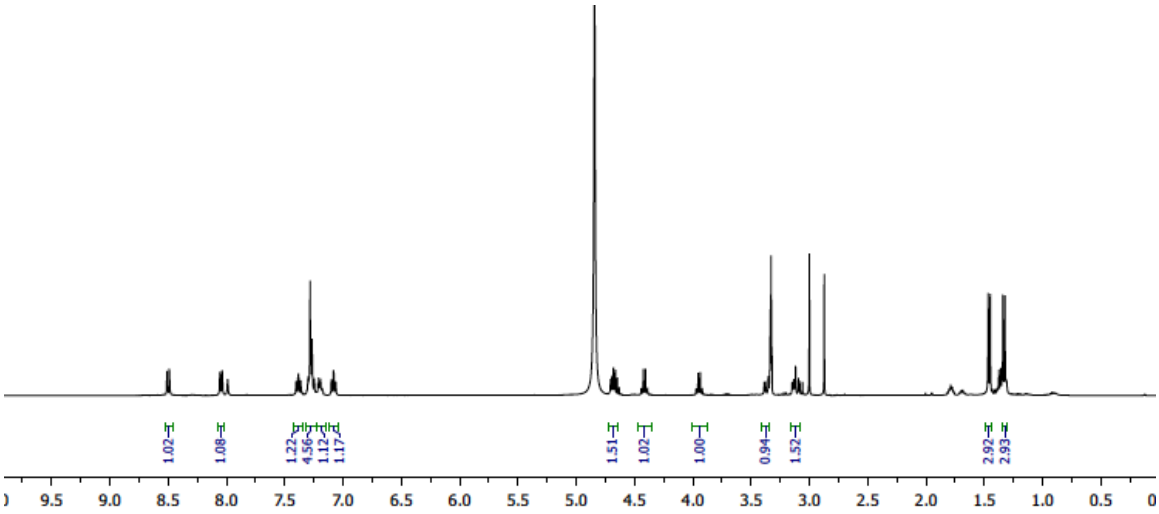
White solid, 89 %;

^{1}H-NMR (400 MHz, MeOH-d_4) δ 8.50 (dd, *J* = 8.3, 0.9 Hz, 1H), 8.05 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.43-7.35 (m, 1H), 7.32-7.23 (m, 4H), 7.23-7.17 (m, 1H), 7.08 (td, *J* = 7.8, 1.1 Hz, 1H), 4.72-4.65 (m, 1H), 4.42 (q, *J* = 7.1 Hz, 1H), 3.95 (q, *J* = 7.0 Hz, 1H), 3.41-3.34 (m, 1H), 3.16-3.08 (m, 1H), 1.46 (d, *J* = 7.0 Hz, 3H), 1.33 (d, *J* = 7.1 Hz, 3H).

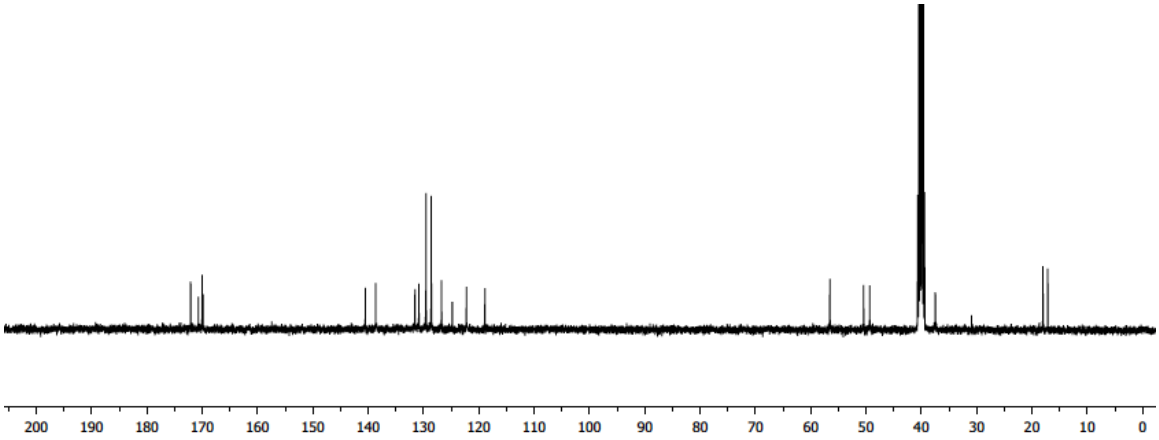
^{13}C-NMR (101 MHz, DMSO-d_6) δ 172.0, 170.6, 170.0, 169.8, 140.5, 138.6, 131.5, 130.8, 129.5, 128.5, 126.7, 124.7, 122.2, 118.8, 56.5, 50.4, 49.3, 37.4, 18.0, 17.1;

HRMS (ESI+) m/z calcd for $C_{22}H_{27}N_4O_5$ $(M+H)^+$ 427.1981; found 427.1999.

^{1}H NMR


1.02
1.08
1.22
4.56
1.12
1.17
1.51
1.02
1.00
0.94
1.52
2.92
2.93
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0

^{13}C NMR


200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

C. QMD and Matching Procedures

Procedures for Matching on Ideal Secondary Structures and NMR Structures

The QMD was performed according to the procedure described previously.[1,2] After the QMD simulation, the conformers were grouped into families base on their Cα - Cβ coordinates. All the conformers within 3.0 kcal/mol were considered to be "preferred". After removing the high-energy conformers, the following number of conformers was used (conformations from simulations in water) for the overlay with secondary structures: LLL-**1aaa**, 1267 conformers, one cluster; LLD-**1aaa**, 977 conformers, one cluster; LDL-**1aaa**, 1322 conformers, one cluster; LDD-**1aaa**, 934 conformers, one cluster; DLL-**1aaa**, 1226 conformers, one cluster; DLD-**1aaa**, 1337 conformers, one cluster; DDL-**1aaa**, 1333 conformers, one cluster; DDD-**1aaa**, 1128 conformers, one cluster.

Standard template for 3_{10}-helix, α-helix, π-helix, β-strand, type I β-turn, type II β-turn, γ-turn, and inverse γ-turn were prepared according to a previous procedure with Discovery Studio 2.5.[1,2] Parallel β-sheet, anti-parallel β-sheet and sheet/turn/sheet templates were obtained by from a previous published procedure.[1,2]

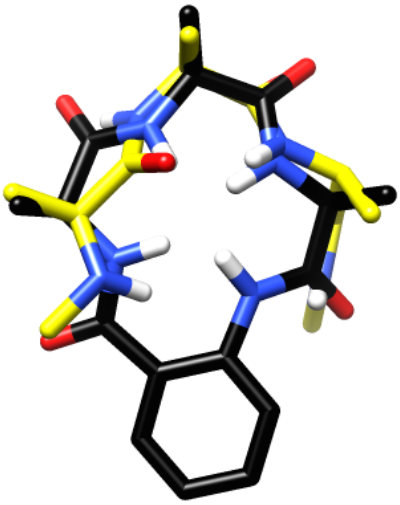
Each of the conformers from QMD was overlaid on ideal secondary structures or the lowest energy NMR conformation using an in-house generated algorithm that compared Cα - Cβ coordinates of the side chains, which generates a list of structures ranked in terms of the root-mean-squared deviation (RMSD) for the overlay process.[1,2]

A comparison between γ-turn and type II β-turn indicated that the side-chain orientations in those two templates were similar in terms of their Cα - Cβ atom coordinates. (Figure S1b)

a

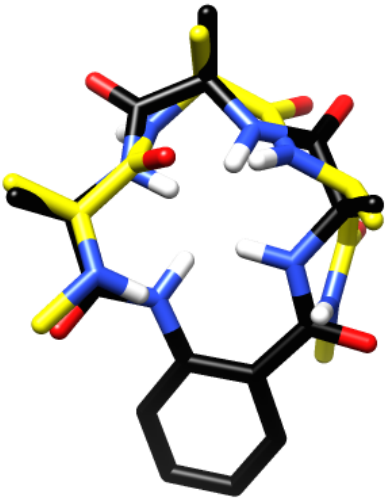
overlays on γ-turn

LLL



RMSD 0.47 Å

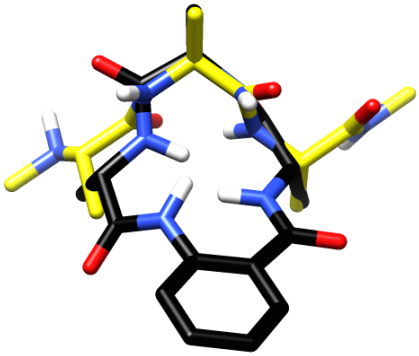
DDD



RMSD 0.38 Å

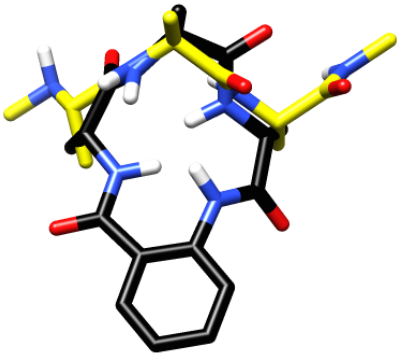
overlays on inverse γ-turn

LDL



RMSD 0.65 Å

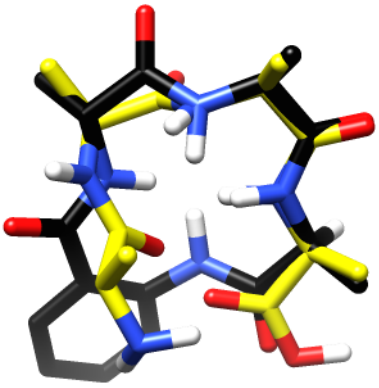
DLD



RMSD 0.61 Å

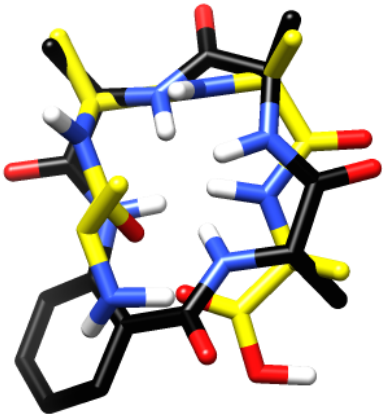
overlays on type I β-turn

DLL



RMSD 0.50 Å

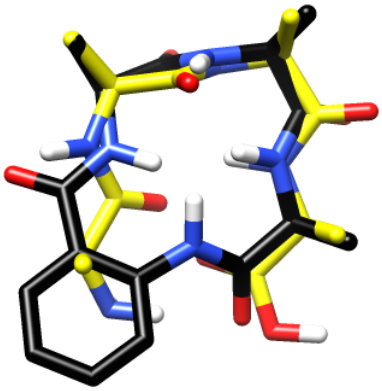
LDD



RMSD 0.67 Å

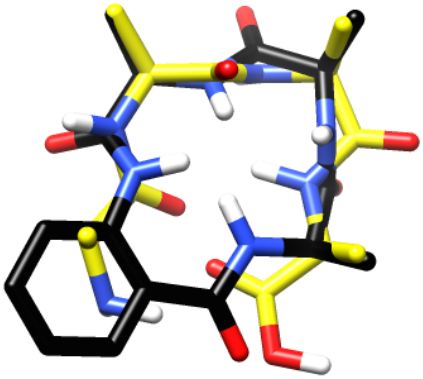
overlays on type II β-turn

LLL



RMSD 0.36 Å

DDD



RMSD 0.31 Å

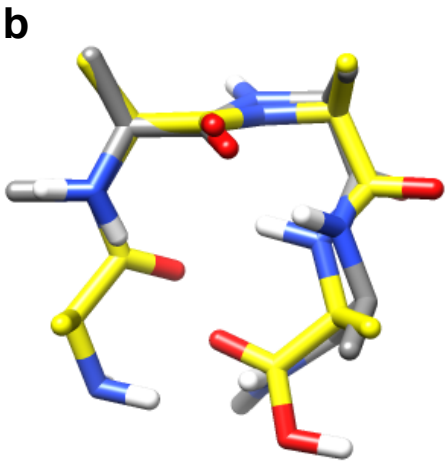


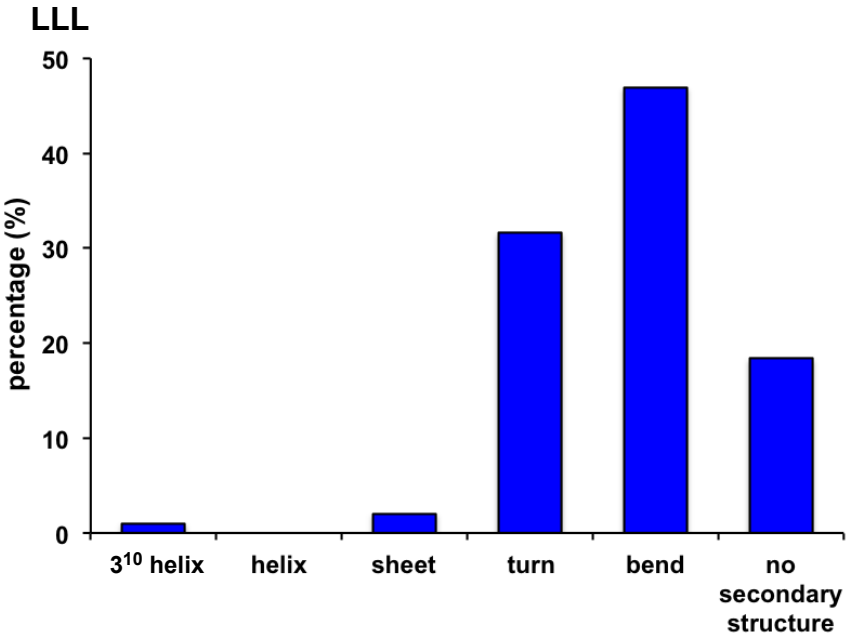
Figure S1. a. Overlays of QMD conformations with type I β-turn, type II β-turn, γ-turn, and inverse γ-turn. **b.** Overlays of γ-turn with type II β-turn.

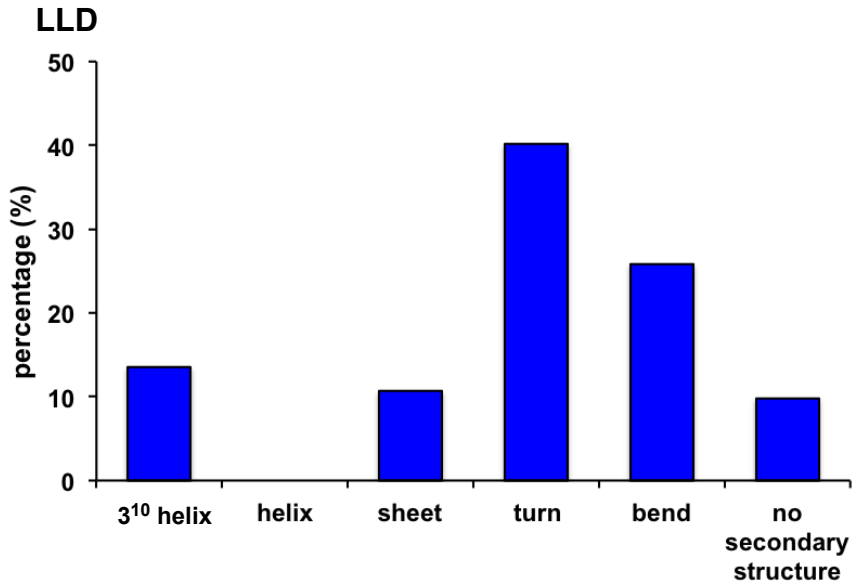
D. Data Mining of 3D Complex Database

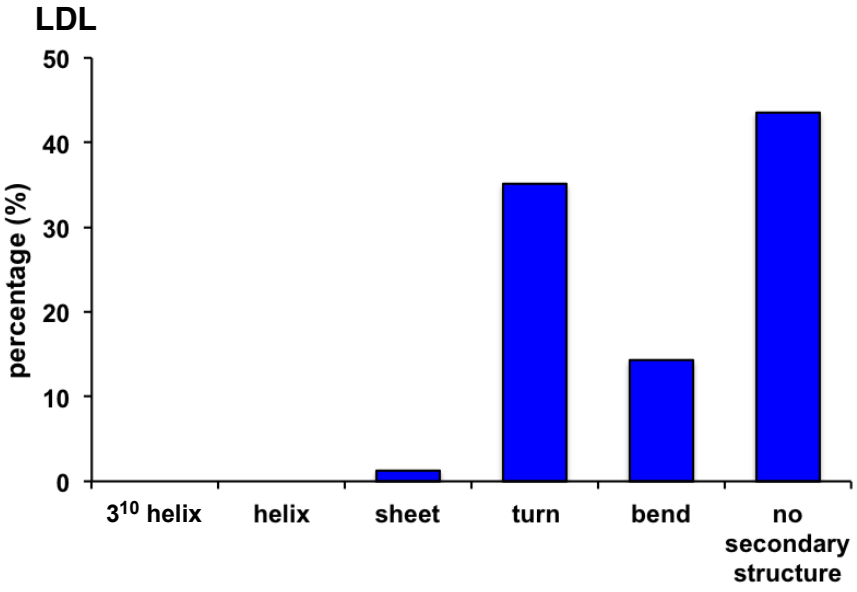
Procedures for Data Mining of 3D Complex Database

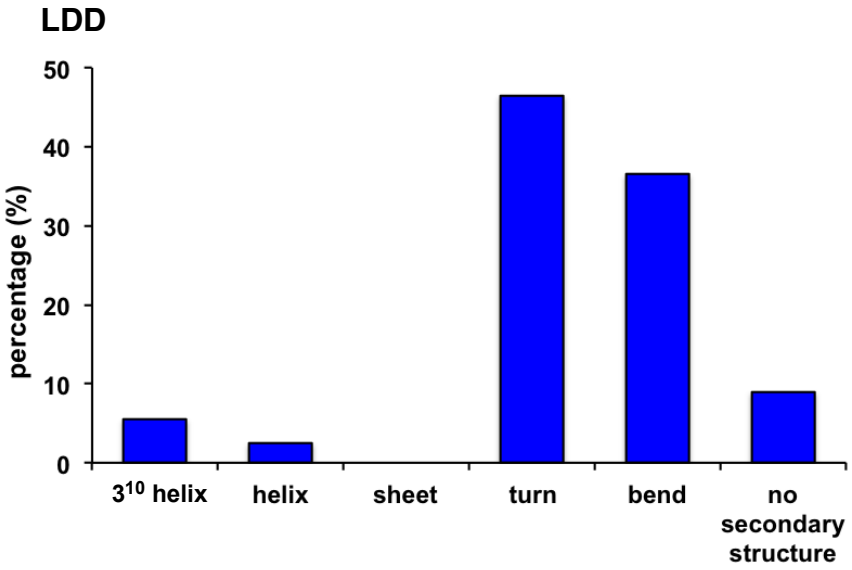
The data mining of 3D complex database was performed for all the diastereomers of **1aaf** according to the procedure described previously.[3]

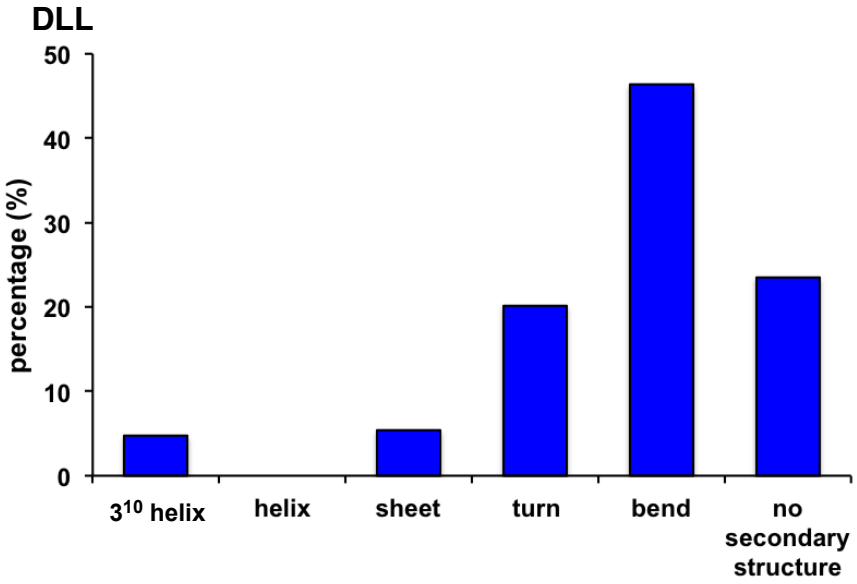
For the top hits based on overlay RMSDs (< 0.3 Å), the overlaid PPI regions were analyzed by DSSP program[4] to assign the secondary structure for each hit.[2] The ϕ, ψ angle of the central residue for all the top hits were measured and plotted in the form of a Ramachandran plot.[5]

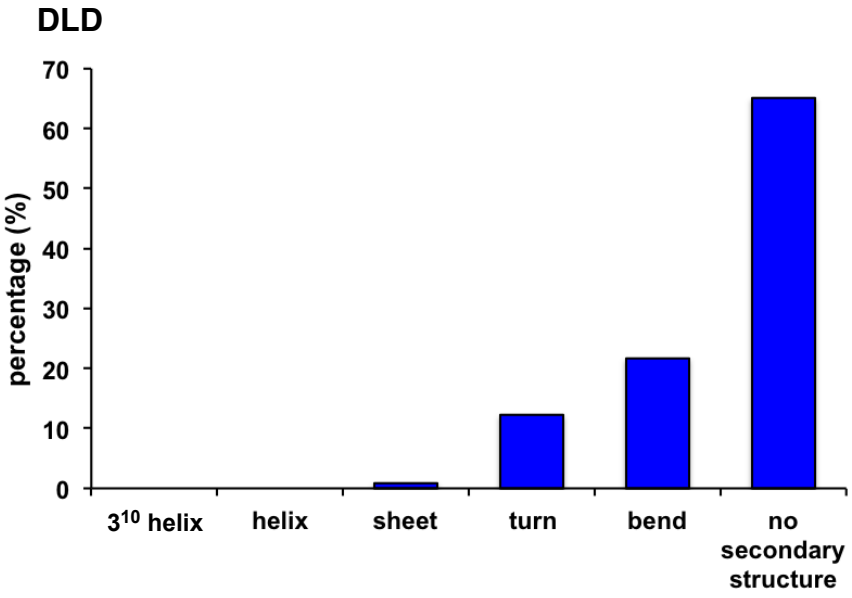


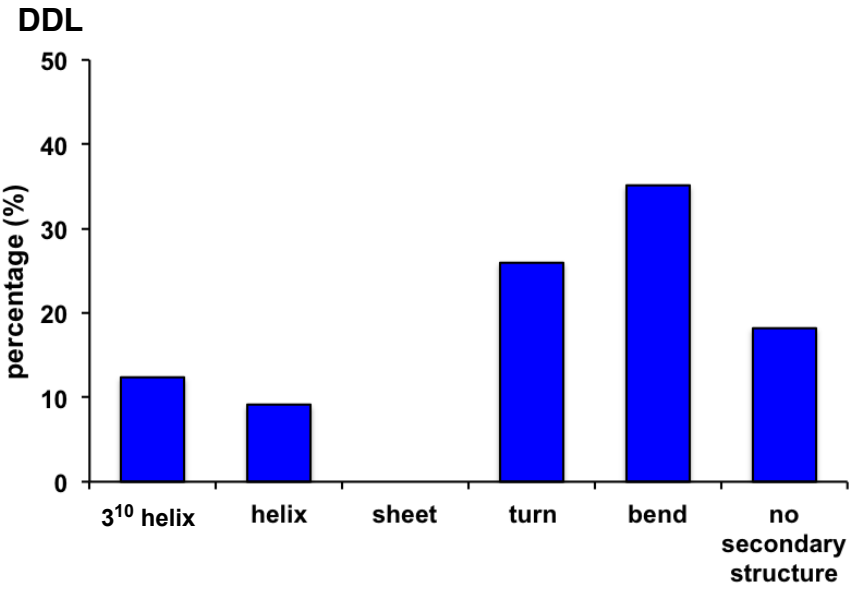

LLD
50
40
30
20
10
0
percentage (%)
3^10 helix
helix
sheet
turn
bend
no secondary structure


LDL
50
40
30
20
10
0
percentage (%)
3^10 helix
helix
sheet
turn
bend
no secondary structure


LDD
percentage (%)
50
40
30
20
10
0
3^10 helix
helix
sheet
turn
bend
no secondary structure


DLL
percentage (%)
50
40
30
20
10
0
3^10 helix
helix
sheet
turn
bend
no secondary structure


DLD
70
60
50
40
30
20
10
0
percentage (%)
3^10 helix
helix
sheet
turn
bend
no secondary structure


DDL
50
40
30
20
10
0
percentage (%)
3^10 helix
helix
sheet
turn
bend
no secondary structure

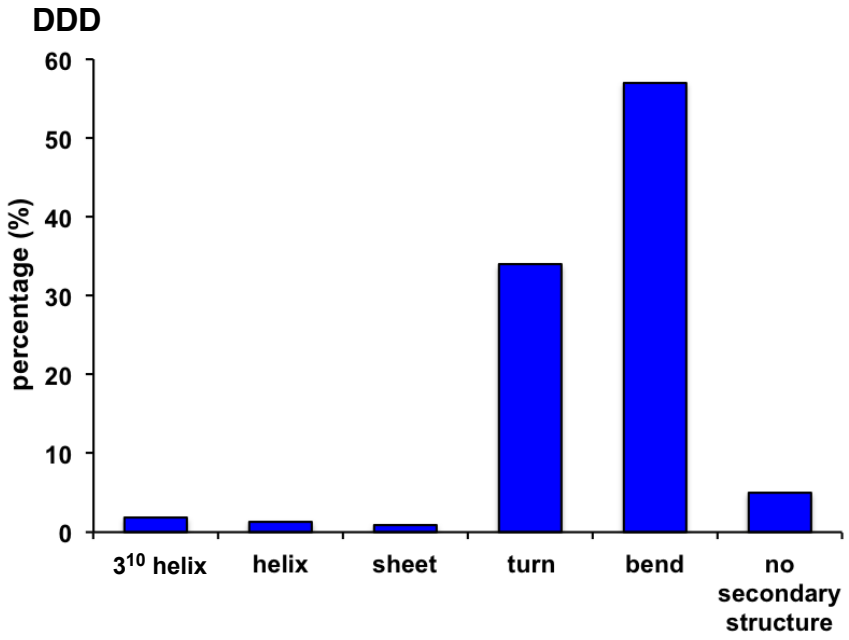
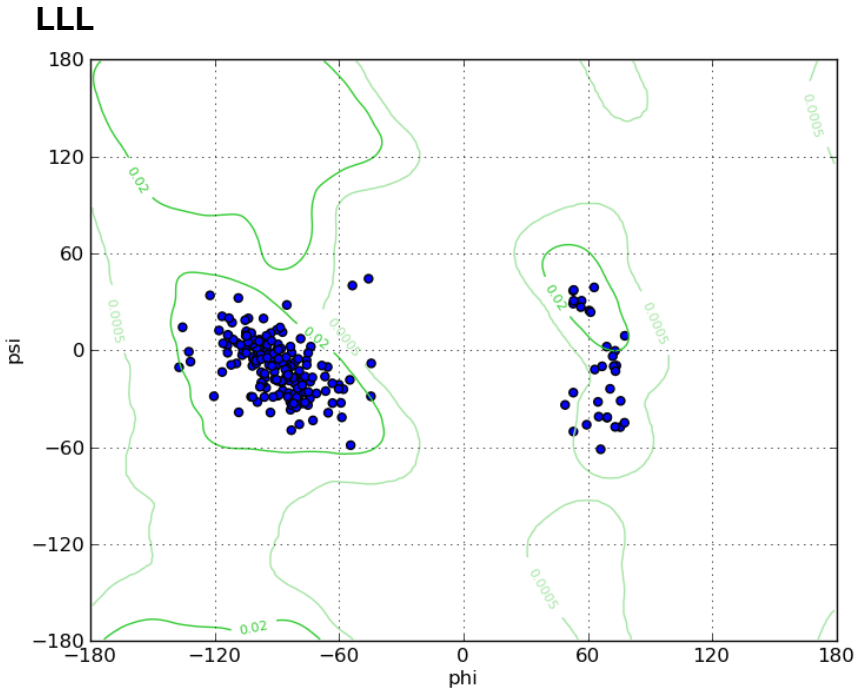
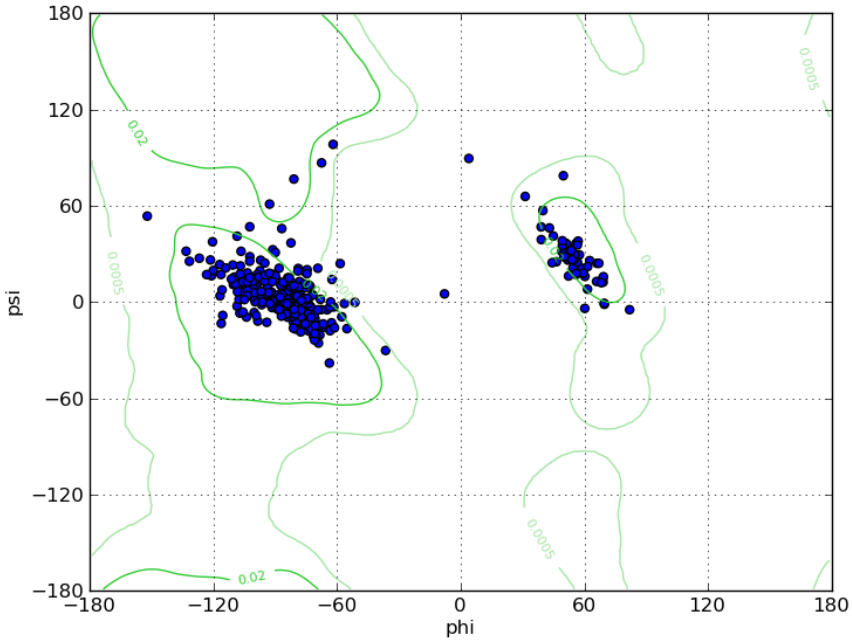


Figure S2. Distribution of best overlays on PPI interface segments with respect to secondary structure.



LLD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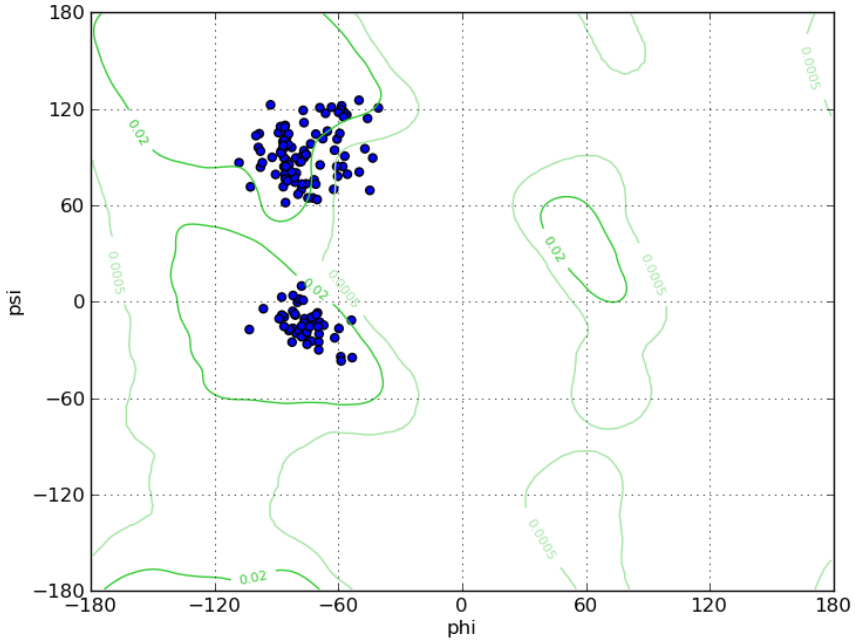
LDL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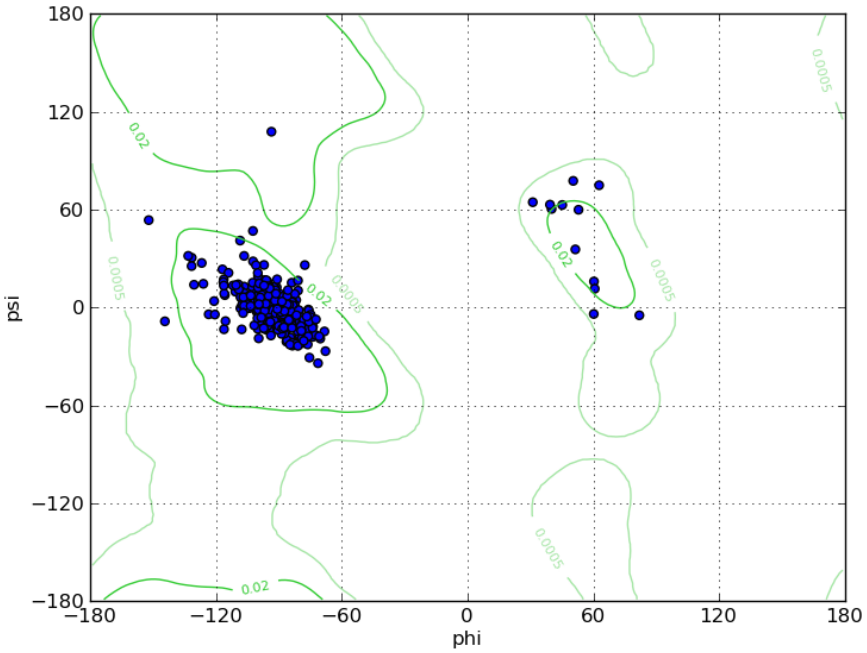
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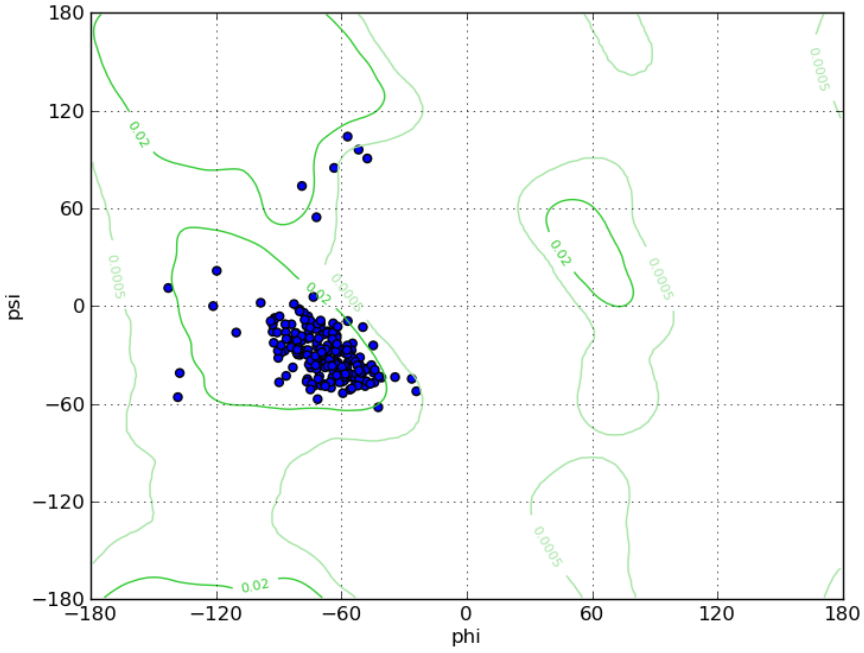
DLL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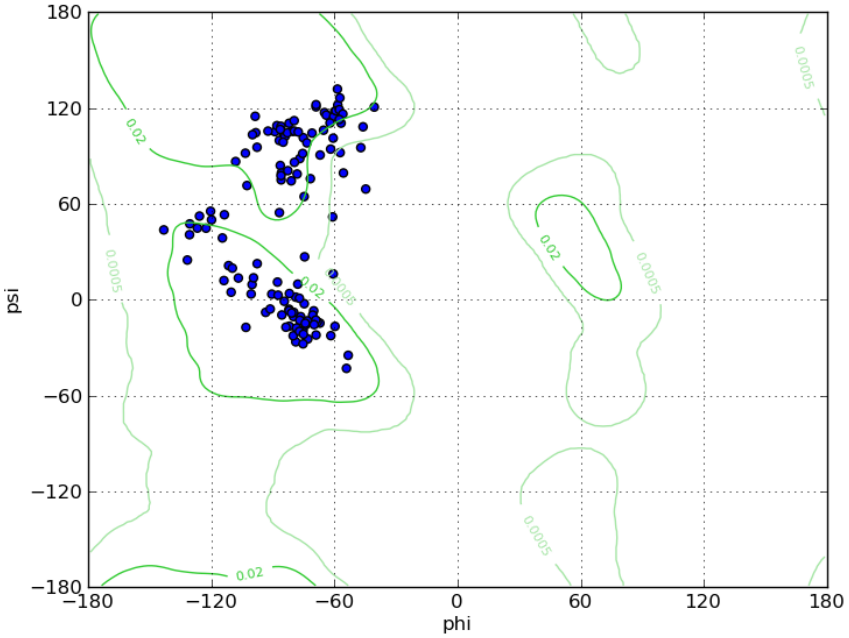
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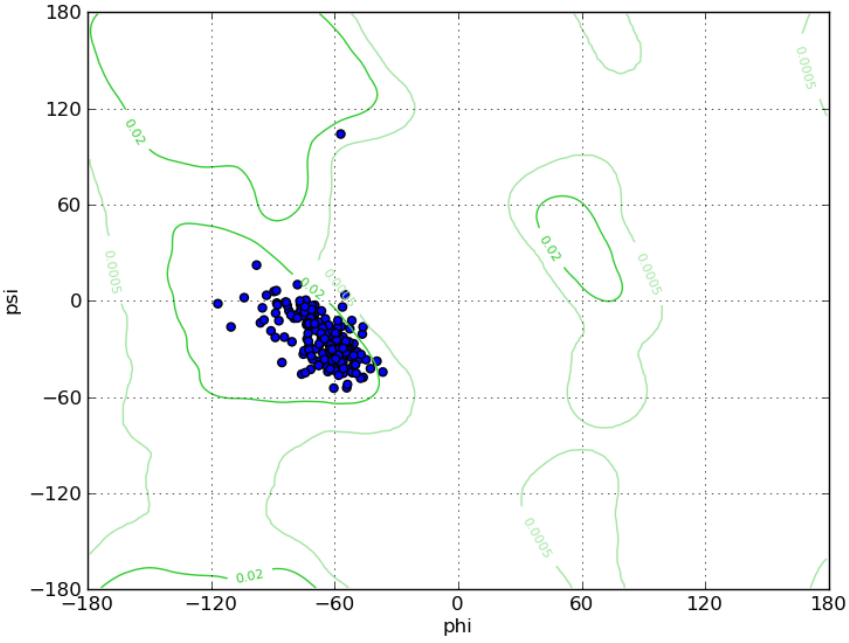
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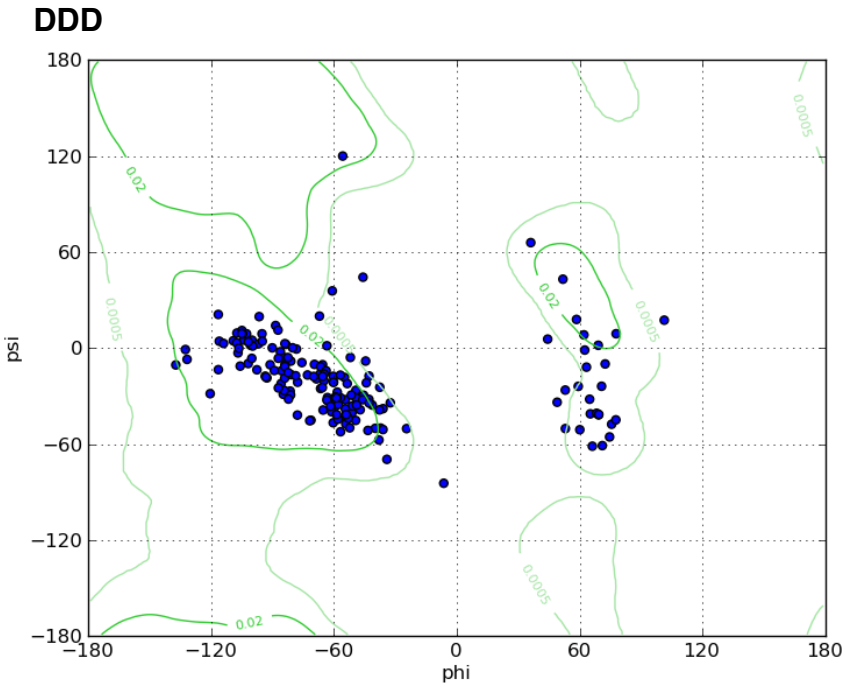


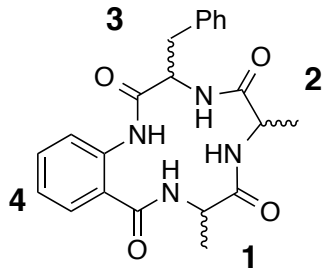
Figure S3. Analysis of the ϕ, ψ angles of the central residue.

E. NMR Structure Determination and Analysis

NMR measurements for cyclic peptides **1** were carried out in DMSO-d_6 with a sample concentration of ~20 mM. NOESY spectra were taken using a mixing time of 400 ms for compounds LLL-**1aaf**, LDL-**1aaf**, DLL-**1aaf**, DDL-**1aaf**, DDL-**1e'af**, DDL-**1eaf**, LLL-**1vsy**, DLL-**1fae'**, DLL-**1fae**, LDL-**1faf**. ROESY spectra were taken using a mixture time of 200 ms for compounds LLL-**1vs'y'** and DDD-**1y's'v**. There was no evidence of cis-amide bonds due to the absence of Cα-Cα or Cα-Cβ couplings across residues.

The observed NOE measurements were summarized in the following tables for **1aaf**. s: strong, 1.8 Å ≤ H-H distance ≤ 2.7 Å; m: medium, 1.8 Å ≤ H-H distance ≤ 3.5 Å; w: weak, 1.8 Å ≤ H-H distance ≤ 5.0 Å. [6,7]

Table S1. Observed NOE measurements.



LLL-**1aaf**

cross peak		intensity	cross peak		intensity
				$H\beta^1$	m
	$H\beta^2$	m		$H\beta^2$	m
	$H\beta^3$	w		$H\alpha^1$	w
NH^3	$H\alpha^2$	m	NH^2	$H\alpha^2$	w
	$H\alpha^2$	m		NH^1	m
	NH^4	m		NH^3	w
				NH^4	w

NH^1	$H\beta^1$	m	NH^4	$H\alpha^3$	w
	$H\alpha^1$	w			

LDL-**1aaf**

cross peak		intensity	cross peak		intensity
	$H\beta^2$	w		$H\beta^2$	m
	$H\beta^3$	m		$H\alpha^1$	m
NH^3	$H\alpha^2/H\alpha^3$	s	NH^2	$H\alpha^2$	w
	NH^2	w		NH^1	w
	NH^4	m		NH^3	w
				NH^4	w

NH^1	$H\beta^1$	m	NH^4	$H\beta^3$	w
	$H\alpha^1$	m		$H\alpha^3$	m

DLL-**1aaf**

cross peak		intensity	cross peak		intensity
	$H\beta^2$	m		$H\beta^2$	m
	$H\beta^3$	m		$H\alpha^1 + H\alpha^2$	s
NH^3	$H\alpha^2 + H\alpha^3$	m	NH^2	NH^1	w
	NH^2	w		NH^4	w
	NH^4	m			
NH^1	$H\beta^1$	m	NH^4	$H\alpha^3$	w
	$H\alpha^1$	m			

DDL-**1aaf**

cross peak		intensity	cross peak		intensity
	$H\beta^3$	m		$H\beta^2$	m
	$H\alpha^3$	w		$H\beta^1$	m
NH^3	$H\alpha^2$	s	NH^2	$H\alpha^1$	w
	NH^4	m		$H\alpha^2$	w
				NH^1	w
NH^1	$H\beta^1$	s	NH^4	$H\beta^3$	w
	$H\alpha^1$	w		$H\alpha^3$	w

NMR structure calculation in DMSO was carried out using the Conformational Searches in MacroModel with distance checks (MacroModel, version 10.0, Schrödinger, LLC, New York, NY). Monte Carlo Multiple Minimum method were used to sample the cyclic peptide conformations. 10000 structures were sampled and minimized with OPLS_2005 force field in dielectric constant 46.7 with a convergence criteria 0.05 kJ/mol over 2000 iterations. Distance constraints from the previous tables were applied during the conformational sampling to eliminate the conformations with distance violations. Duplicate structures base on heavy-atom superposition (RMSD < 0.02 Å) were discarded. The unique conformations within 5 kJ/mol of the global minimum were collected and clustered based on heavy atoms of the macrocyclic scaffold without the Phe side-chain. The clustered conformations were shown in Figure S4.

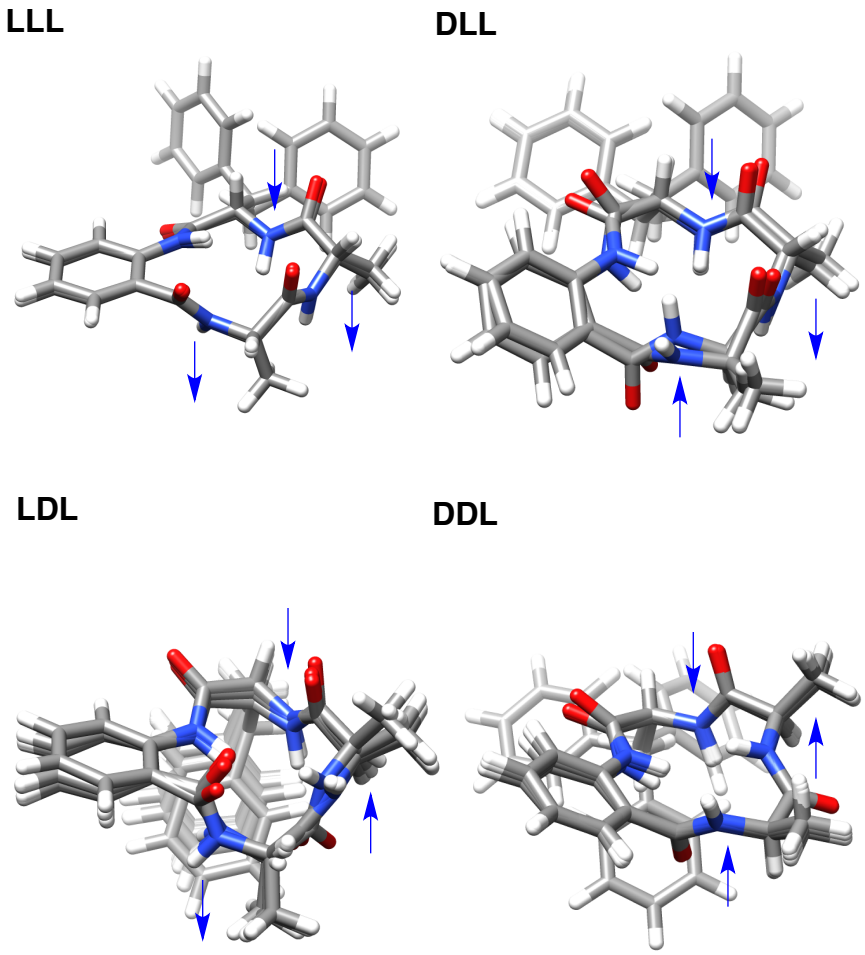


Figure S4. Conformational clusters of **1aaf** with NMR constraints.

F. H/D Exchange of the Amide NH

H/D exchange experiments were explored in $CDCl_3/CD_3OD$. A sample of cyclic peptide **1** was prepared in 450 μL $CDCl_3$ and 50 μL of CD_3OD was added (4 mM final cyclic peptide concentration). The mixture was mixed for 1 min and ^1H NMR of the sample was recorded 3, 5, 7, 10, 15, 20, 25, 30 min after the addition of CD_3OD. The fact that all the amide protons showed significant reduction (> 75 %) in their intensity 30 min after CD_3OD addition indicated that there was no strong intramolecular hydrogen-bond interaction within any diastereomer of the cyclic peptide under the conditions tested.[8,9] The half-lifes of the H/D exchange reactions were summarized in Table S2.

Table S2. H/D exchange half-life $t_{1/2}$ (min).

	NH[1]	**NH[2]**	**NH[3]**	**NH[4]**
LLL-**1aaf**	< 5	15	< 5	< 5
DLL-**1aaf**	< 5	12	< 5	< 5
LDL-**1aaf**	< 5	two peaks overlapped*		< 5
DDL-**1aaf**	< 5	< 5	< 5	< 5

* For the overlapped peak, the peak intensity decreased to 19 % of the original intensity after 30 min.

G. Temperature Dependence of the Amide NH Chemical Shifts

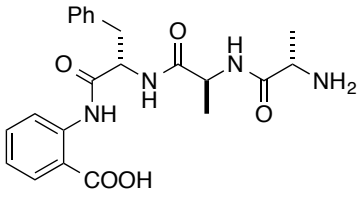
A ~10 mM solution of **1** in DMSO-d_6 was prepared. ^1H NMR measurements were made in the range 303 – 353 K. The first measurement was made at 303 K and the rest of ^1H NMRs were acquired at 10 K intervals. All the spectra obtained were referenced to the solvent peak and the ppm change of the amide NH peaks was monitored. The change in chemical shift was plotted versus the change in temperature and the data was fitted to a linear equation to give the temperature-dependent coefficient ($\Delta\delta/\Delta K$) of the NH proton of interest. The temperature coefficient data in Table S3 indicated that most of the amide protons were shielded from the solvent in the cyclic peptide system.[10,11]

Table S3. Temperature coefficient data of amide *NH*s.

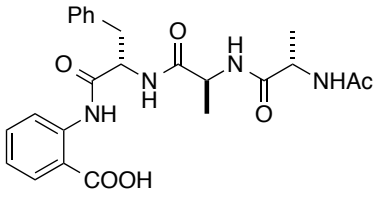
	NH[1]	NH[2]	NH[3]	NH[4]
LLL-**1aaf**	-1.7	-	-4.5	-2.7
DLL-**1aaf**	-4.2	-1.6	-4.9	-2.7
LDL-**1aaf**	-3.7	-3.8	-6.2	-3.7
DDL-**1aaf**	-2.3	-	-6.8	-3.4

H. QikProp Calculation

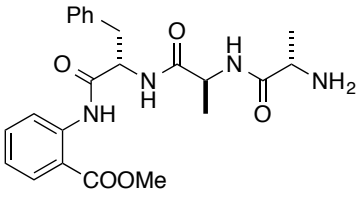
QikProp 3.5 from Schrödinger (2012)[12] was used to evaluate pharmaceutically relevant properties for compounds listed below.



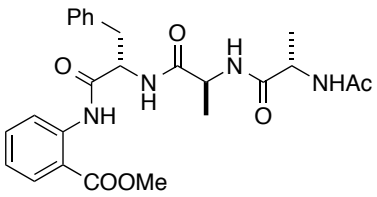
H-**aaf**-OH



Ac-**aaf**-OH



H-**aaf**-OMe



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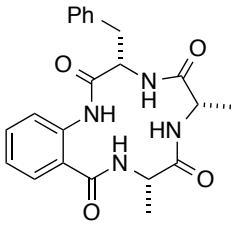

LLL-**1aaf**

I. Stability Analysis of Cyclic Peptide

General Procedure for the pH Stability Assay

A stock solution of cyclic peptide LLL-**1aaf** (66 mM) and triphenylphosphine oxide (TPPO, internal standard, 100 mM) in DMSO was prepared and stored at 25 °C. 9 μL of the DMSO stock solution was dissolved in aqueous solutions with different pH (pH 7.4, PBS buffer; pH 12, 10 mM NaOH; pH 2, 10 mM HCl; all solutions contain 20 % MeOH) to give a 400 μM working solution. The solution was filtered through a 0.2 μm membrane filter and analyzed by reversed phase HPLC (see general methods) at intervals (retention time $t_{\text{LLL-}\mathbf{1aaf}}$ = 15.1 min, t_{TPPO} = 17.9 min). The peak areas of LLL-**1aaf** from rp-HPLC were measured and normalized against peak areas of TPPO and plotted against incubation time. The data points were fitted to first order kinetics to give the rate constant and half-life $t_{1/2}$ of the decomposition reaction.

General Procedure for the Protease Stability Assay

A stock solution of cyclic peptide LLL-**1aaf** (66 mM) and TPPO (100 mM) in DMSO was prepared and stored at 25 °C. A similar stock solution containing 66 mM linear LLL-**aaf** peptide and 100 mM TPPO was prepared in DMSO as a control. A 0.2 unit / μL stock solution of pronase from Streptomyces griseus was prepared in PBS buffer and further diluted to 0.4 unit / mL with PBS buffer containing 20 % MeOH. 4.5 μL of cyclic peptide or linear peptide stock solution was added to 1.5 mL pronase solution and then filtered through a 0.2 μm membrane filter and the resulting solution was analyzed by rp-HPLC (see general methods) at intervals. The peak areas of LLL-**1aaf** or LLL-**aaf** from rp-HPLC were measured and normalized against peak areas of TPPO and plotted against incubation time. Under the experimental condition, no decomposition of LLL-**1aaf** was observed even after 12 h, while for the control linear peptide LLL-**aaf** the half-life of decomposition was about 1.5 h.

J. PAMPA Assay

The PAMPA assay was performed based on a modified procedure.[13]

An artificial membrane (1 % solution of lecithin in dodecane) was carefully applied to a MultiScreen-IP PAMPA filter plate. 150 μL of the compound solution (200 μM containing 5 % DMSO in PBS buffer, pH 7.4) was added to each well of the donor

plate and 400 µL of buffer (5 % DMSO in PBS buffer, pH 7.4) was added to each well of a non-binding acceptor plate. The donor plate was carefully placed on top of the acceptor plate and incubated at room temperature for 16 h. After incubation, samples were taken from the donor and acceptor wells and the compound concentrations were measured by LC-MS. The experiments were performed in triplicates and standard deviations were reported for the results. P_{app} can be calculated from the following equation:

$$P_{app} = -\frac{V_D \bullet V_A}{(V_D+V_A) \bullet A \bullet t} \times \ln\left(1 - \frac{[\text{compound}]_{donor}}{[\text{compound}]_{equilibrium}}\right)$$

The experiments were performed in triplicates and standard deviations were calculated.

Table S4. Calculated cell permeability rates from QikProp, and experimental data from PAMPA assays.

compound	QikProp P_{Caco} (10^6 • cm/s)	PAMPA P_{app} (10^6 • cm/s)
LLL-**1aaf**	14.3	1.53 (± 0.22)
LDL-**1aaf**	21.7	2.04 (± 0.10)
DLL-**1aaf**	33.3	0.87 (± 0.14)
DDL-**1aaf**	15.2	0.57 (± 0.12)
DLL-**1fae'**	17.2	2.01 (± 0.27)
LLL-**1vsy**	3.5	< 0.10
linear control LLL-H-**aaf**-OH	0.15	0.25 (± 0.02)

K. References

(1) Xin, D.; Ko, E.; Perez, L. M.; Ioerger, T. R.; Burgess, K. *Org. Biomol. Chem.* **2013**, *11*, 7789.
(2) Xin, D.; Perez, L. M.; Ioerger, T. R.; Burgess, K. *Angew. Chem. Int. Ed.* **2014**, *53*, 3594.
(3) Ko, E.; Raghuraman, A.; Perez, L. M.; Ioerger, T. R.; Burgess, K. *J. Am. Chem. Soc.* **2013**, *135*, 167.
(4) Kabsch, W.; Sander, C. *Biopolymers* **1983**, *22*, 2577.
(5) Ramachandran, G. N.; Ramakrishnan, C.; Sasisekharan, V. *J. Mol. Biol.* **1963**, *7*, 95.
(6) Glenn, M. P.; Kelso, M. J.; Tyndall, J. D. A.; Fairlie, D. P. *J. Am. Chem. Soc.* **2003**, *125*, 640.
(7) Beierle, J. M.; Horne, W. S.; van Maarseveen, J. H.; Waser, B.; Reubi, J. C.; Ghadiri, M. R. *Angew. Chem. Int. Ed.* **2009**, *48*, 4725.
(8) Steffel, L. R.; Cashman, T. J.; Reutershan, M. H.; Linton, B. R. *J. Am. Chem. Soc.* **2007**, *129*, 12956.
(9) Lingard, H.; Han, J. T.; Thompson, A. L.; Leung, I. K. H.; Scott, R. T. W.; Thompson, S.; Hamilton, A. D. *Angew. Chem. Int. Ed.* **2014**, *53*, 3650.
(10) Ohnishi, M.; Urry, D. W. *Biochem. Biophys. Res. Commun.* **1969**, *36*, 194.
(11) Cierpicki, T.; Otlewski, J. *J. Biomol. NMR* **2001**, *21*, 249.
(12) Duffy, E. M.; Jorgensen, W. L. *J. Am. Chem. Soc.* **2000**, *122*, 2878.
(13) E. Millipore, *Non-cell-based Assays for Drug Transport*, http://www.emdmillipore.com/US/en/product/Non-cell-based-Assays-for-Drug-Transport,MM_NF-C7702 - documentation.