Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry
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Supporting Information for:

A combined solid- and solution-phase approach provides convenient access to analogues of the calcium-dependent lipopeptide antibiotics

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General Procedures

Reagents and General Methods. All reagents employed were of American Chemical Society (ACS) grade or finer and were used without further purification unless otherwise stated. Both L-and D-kynurenine were synthesized according to a previously described procedure[1] and converted into the requisite Fmoc-building blocks based on established protocols.[2] A large scale preparation of Fmoc-(Dmb)Gly-OH was also developed based on existing literature procedures (see supporting information for details). All known compounds prepared had NMR spectra, mass spectra, and optical rotation values consistent with the assigned structures. Orthogonally protected Fmoc-L-Dap(Aloc)-OH and Fmoc-D-Dap(Aloc)-OH were obtained from Iris Biotech GmbH. All reagents employed were of American Chemical Society (ACS) grade or finer and were used without further purification unless otherwise stated. All reactions and fractions from column chromatography were monitored by thin layer chromatography (TLC) using plates with a UV fluorescent indicator (normal SiO_2, Merck 60 F254). One or more of the following methods were used for visualization: UV absorption by fluorescence quenching; iodine staining; phosphomolybdic acid:ceric sulfate:sulfuric acid:H2O (10 g:1.25 g:12 mL:238 mL) spray; and ninhydrin staining. Flash chromatography was performed using Merck type 60, 230-400 mesh silica gel.

Instrumentation for Compound Characterization. NMR spectra were recorded at 300 or 500 MHz with chemical shifts reported in parts per million (ppm) downfield relative to tetramethylsilane (TMS). ^{1}H NMR data are reported in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; qn, quintet and m, multiplet), number of protons, coupling constant (*J*) in Hertz (Hz) and assignment. When appropriate, the multiplicity is preceded by br, indicating that the signal was broad. ^{13}C NMR spectra were recorded at 75.5 MHz with chemical shifts reported relative to $CDCl_3$ δ 77.0. 2-D NMR experiments (TOCSY and HSQC) were performed on a 500 MHz instrument. High-resolution mass spectrometry (HRMS) analysis was performed using an ESI instrument. Circular dichroism spectra were recorded on a Jasco J-810 CD-spectrometer using a 2 mm cuvet.

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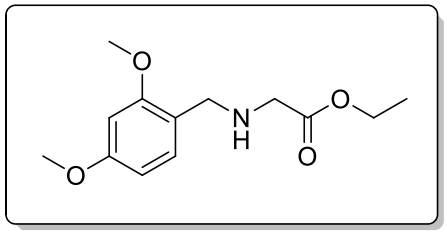
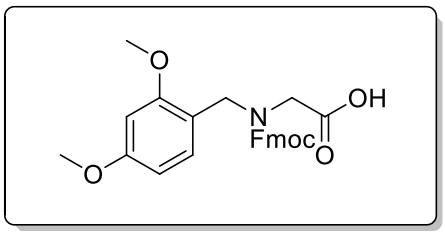
Preparation of Fmoc-(Dmb)Gly-OH

Ethyl 2-((2,4-dimethoxybenzyl)amino)acetate.



2,4-dimethoxybenzaldehyde (3.0 g, 18.0 mmol) was dissolved in dichloroethane (100 ml) and NEt_3 (7.5 ml, 54.0 mmol) was added, followed by glycine ethyl ester hydrochloride (3.77 g, 27.0 mmol). While stirring vigorously $NaB(OAc)_3H$ (7.66 g, 36.2 mmol) was added. After 17 h the reaction was quenched with saturated $NaHCO_3$ (100 ml) and the mixture was extracted with CH_2Cl_2 (3 x 40 ml). The combined organic layers were concentrated under vacuum and applied on a silica column (4:1 EtOAc/CH_2Cl_2). The product was obtained as a white solid (4.57 g, quant.) with analytical data matching that previously reported for the same compound.[3] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.11 (d, *J* = 7.7 Hz, 1H), 6.44-6.40 (m, 2H), 4.22-4.11 (q, *J* = 7.2 Hz, 2H), 3.80 (s, 3H), 3.78 (s, 3H), 3.73 (s, 2H), 3.36 (s, 2H), 1.30-1.22 (m, 3H).

2-((((9H-Fluoren-9-yl)methoxy)carbonyl)(2,4-dimethoxybenzyl)amino)acetic acid.



Ethyl 2-((2,4-dimethoxybenzyl)amino)acetate (4.57 g, 18.0 mmol) was dissolved in dioxane (20 ml) and 1M NaOH (20 ml) was added in 4 ml portions every 5 minutes. After one hour TLC indicated full hydrolysis of the ethyl ester. The mixture was then diluted with saturated $NaHCO_3$ (50 ml) followed by the dropwise addition of Fmoc-OSu (6.41 g, 19.0 mmol) as a solution in dioxane (25 ml) over 30 minutes. The reaction was stirred overnight after which dioxane was removed under vacuum and water was added to a total volume of 100 ml. Solid citric acid was then added to achieve neutral pH. The mixture was then extracted with EtOAc (3 x 100 ml) and the organic layer dried over Na_2SO_4 and concentrated under vacuum. The product was applied to a silica column initially eluting with CH_2Cl_2, moving up to CH_2Cl_2/methanol (9:1). Product containing fraction were combined and after solvent removal Fmoc-(Dmb)Gly-OH was obtained as a nanocrystalline foam (6.0 g, 74% over 2 steps) with analytical data matching that previously reported for the same compound.[4] ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.95 (s, 1H), 7.78-7.72 (m, 2H), 7.60-7.54 (m, 2H), 7.43-7.20 (m, 4H), 6.82 (d, *J* = 8.3 Hz,1H), 6.45 (s, 1H), 6.37 (d, *J* = 8.3 Hz, 1H), 4.57-4.43 (m, 4H), 4.30-4.23 (m, 1H), 4.10 (s, 1H), 3.95 (s, 1H), 3.78 (s, 3H), 3.75 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.6, 160.9, 158.8, 157.17, 156.4, 144.2, 141.6, 131.9, 130.9, 127.9, 127.4, 125.3, 120.2, 117.4, 104.5, 98.6, 68.1, 55.6, 48.6, 48.0, 47.5, 46.4.

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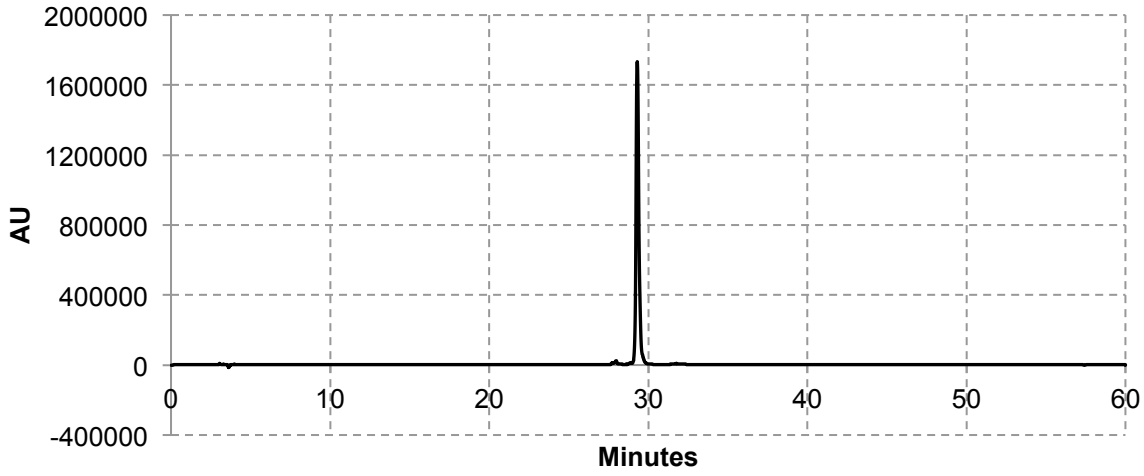
Supplemental Figure S1: HPLC data for compounds **2** and ***ent*-2**

Compound 2:



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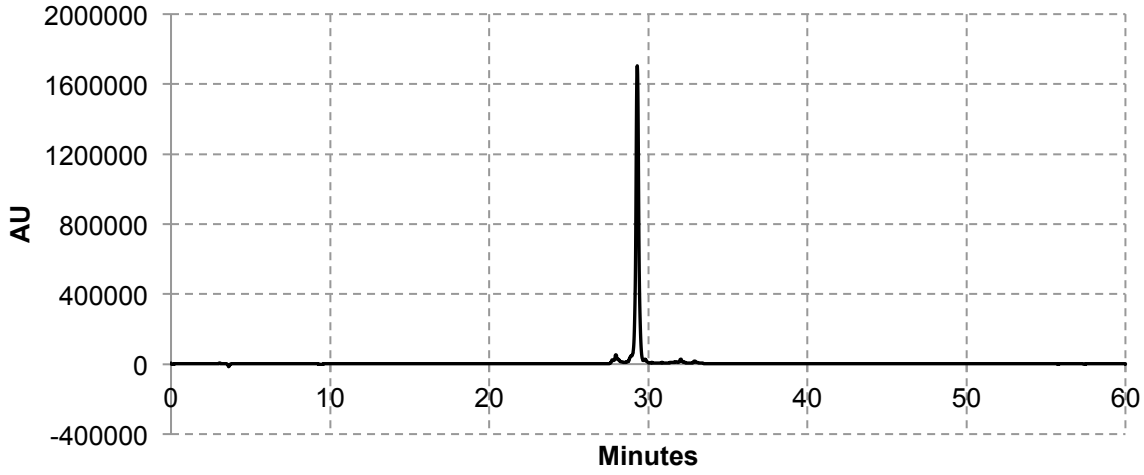
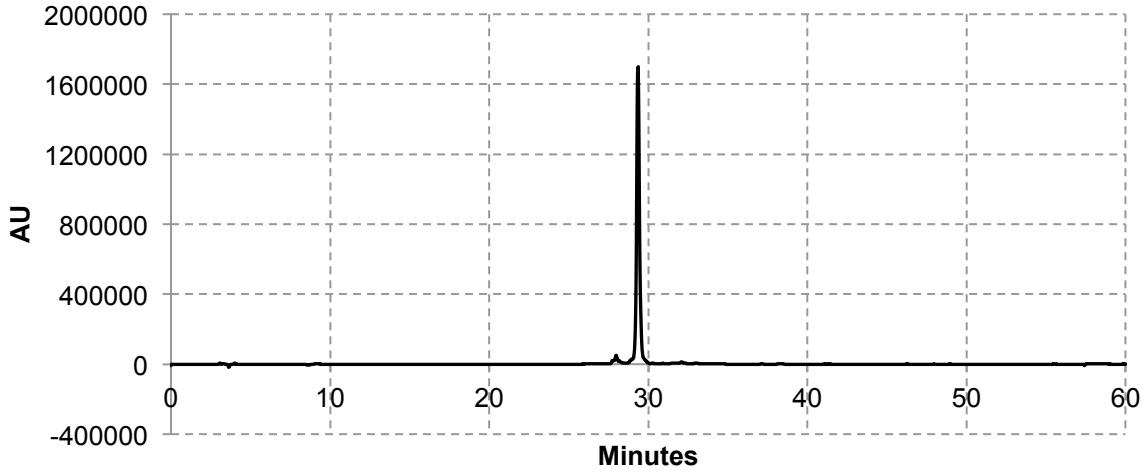


1:1 Mixture of 2 + *ent*-2:



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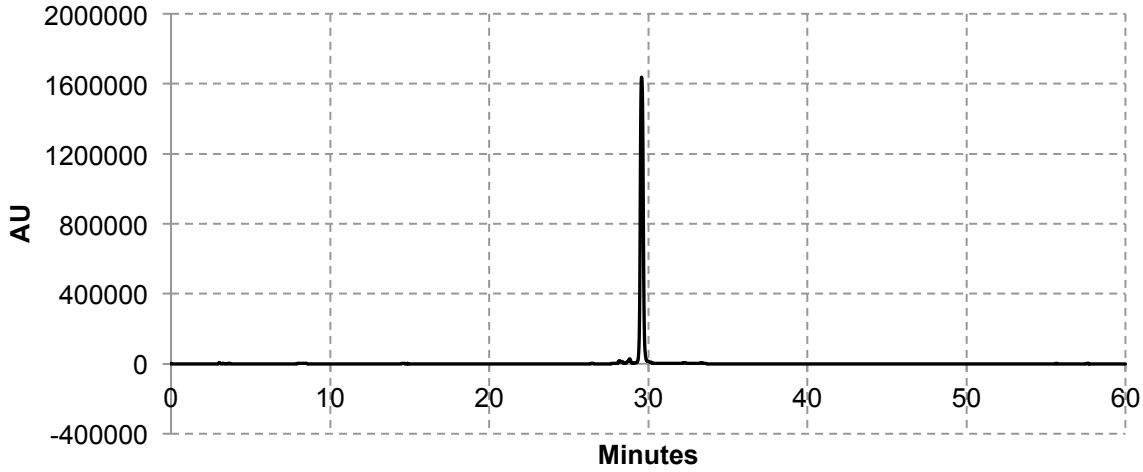
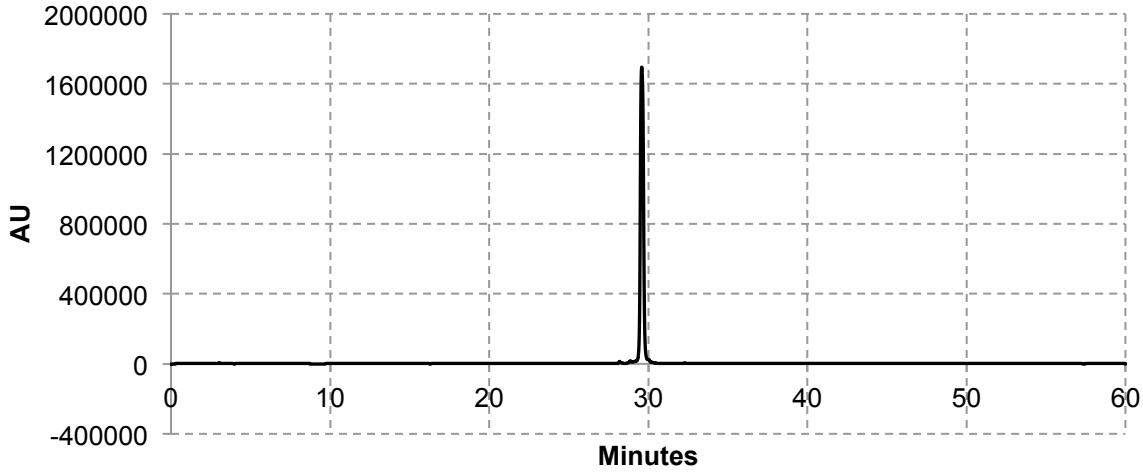
Supplemental Figure S2: HPLC data for compounds **3** and ***ent*-3**

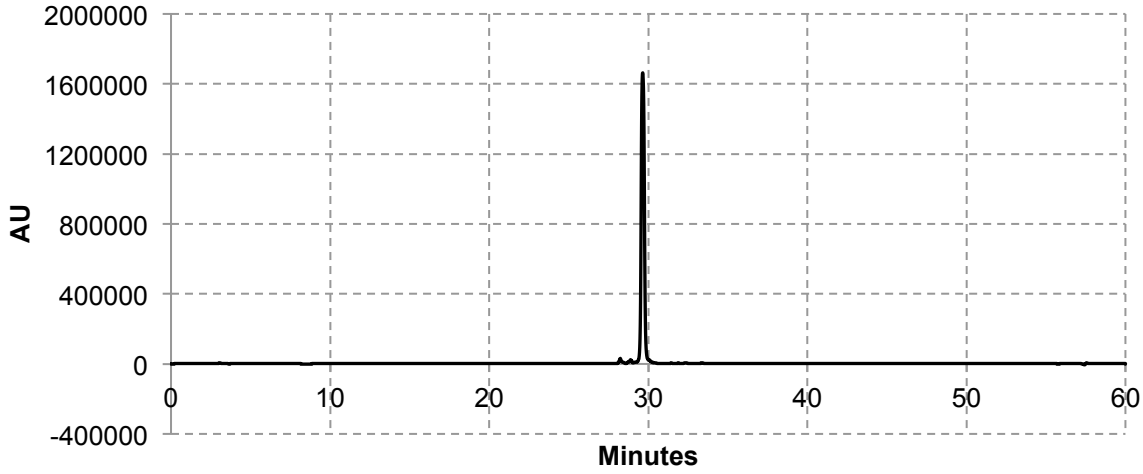
Compound 3:



Compound *ent*-3:



1:1 Mixture of 3 + *ent*-3:



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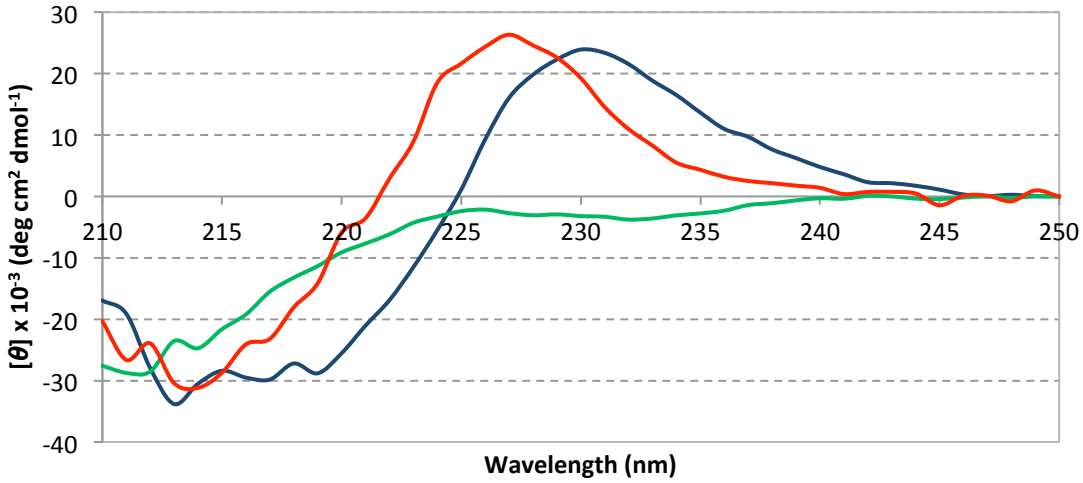
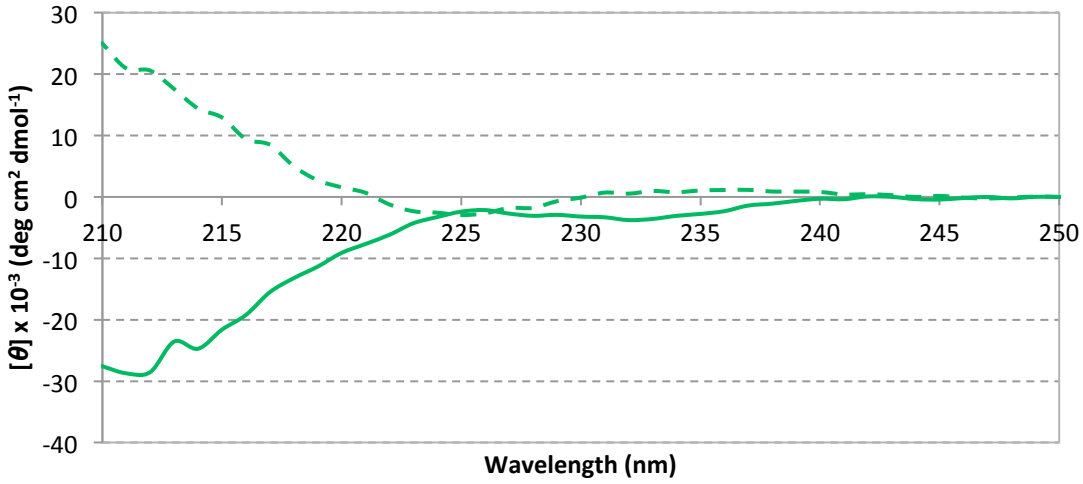
Supplemental Figure S3: Circular Dichroism Spectra

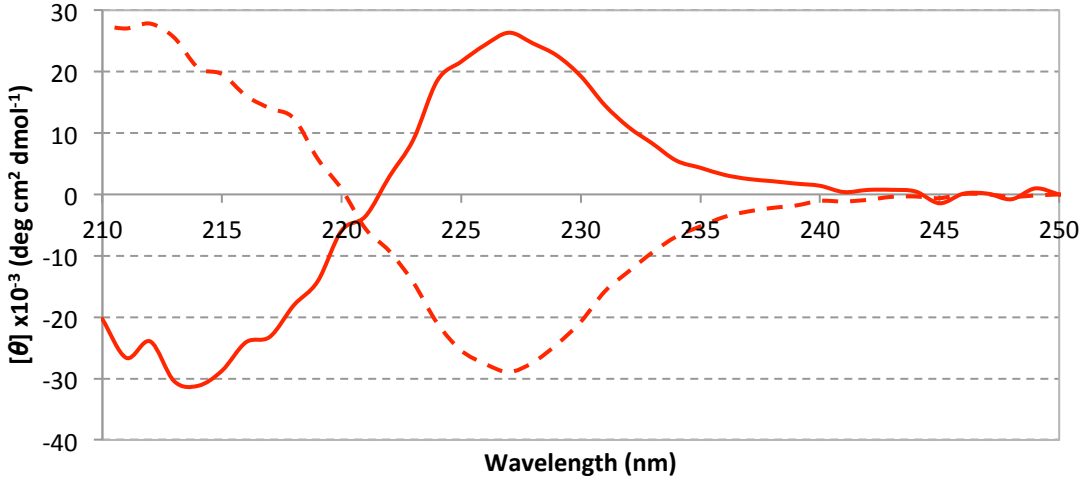
A) Overlay of CD spectra measured for Daptomycin (blue), analogue **2** (green), and analogue **3** (red)



B) CD spectra measured for daptomycin analogue **2** (solid green) and *ent*-**2** (dashed green)



B) CD spectra measured for daptomycin analogue **3** (solid red) and *ent*-**3** (dashed red)



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NMR Chemical shift assignments for compound 2

Residue	$H^{\alpha}(C^{\alpha})$	H^{β} (C^{β})	Sidechain
Tail	---		CH_2 1.07-1.19 (28.5), CH_2 1.20 (31.0), CH_3 0.84 (13.7)
Trp-1	4.43 (53.8)	3.05/2.90 (26.9)	γ2 7.15 (123.4), δ4 7.56 (118.2), δ5 6.95 (117.9), δ6 7.03 (120.5), δ7 7.30 (111.0) H_N 10.77
Asn-2	4.59 (49.5)	2.69/2.53 (35.8)[a]	---
Asp-3	4.53 (49.5)[a]	2.69/2.53 (35.8)[a]	---
Dap-4	4.24-4.27 (51.9)[a]	NA	---
Gly-5	3.72/3.85 (42.0)	---	---
Orn-6	4.20 (52.3)	NA	γ 1.58 (22.9)
Asp-7	4.53 (49.5)[a]	2.69/2.53 (35.8)[a]	---
Ala-8	4.20 (48.2)	1.22 (17.2)	---
Asp-9	4.53 (49.5)[a]	2.69/2.53 (35.8)[a]	---
Gly-10	3.72/3.85 (42.0)	---	---
Ser-11	4.24-4.27 (51.9)[a]	3.60 (61.5)	---
Glu-12	4.45 (49.7)	NA	γ 2.26 (29.9)
Kyn-13	4.24-4.27 (51.9)[a]	3.05/2.90 (26.9)	γ3 6.74 (116.6), γ4 7.22 (133.9), γ5 6.53 (114.2) γ6 7.72 (131.0)

[a] Ambiguous assignments due to overlap of the peaks, NA = not assigned

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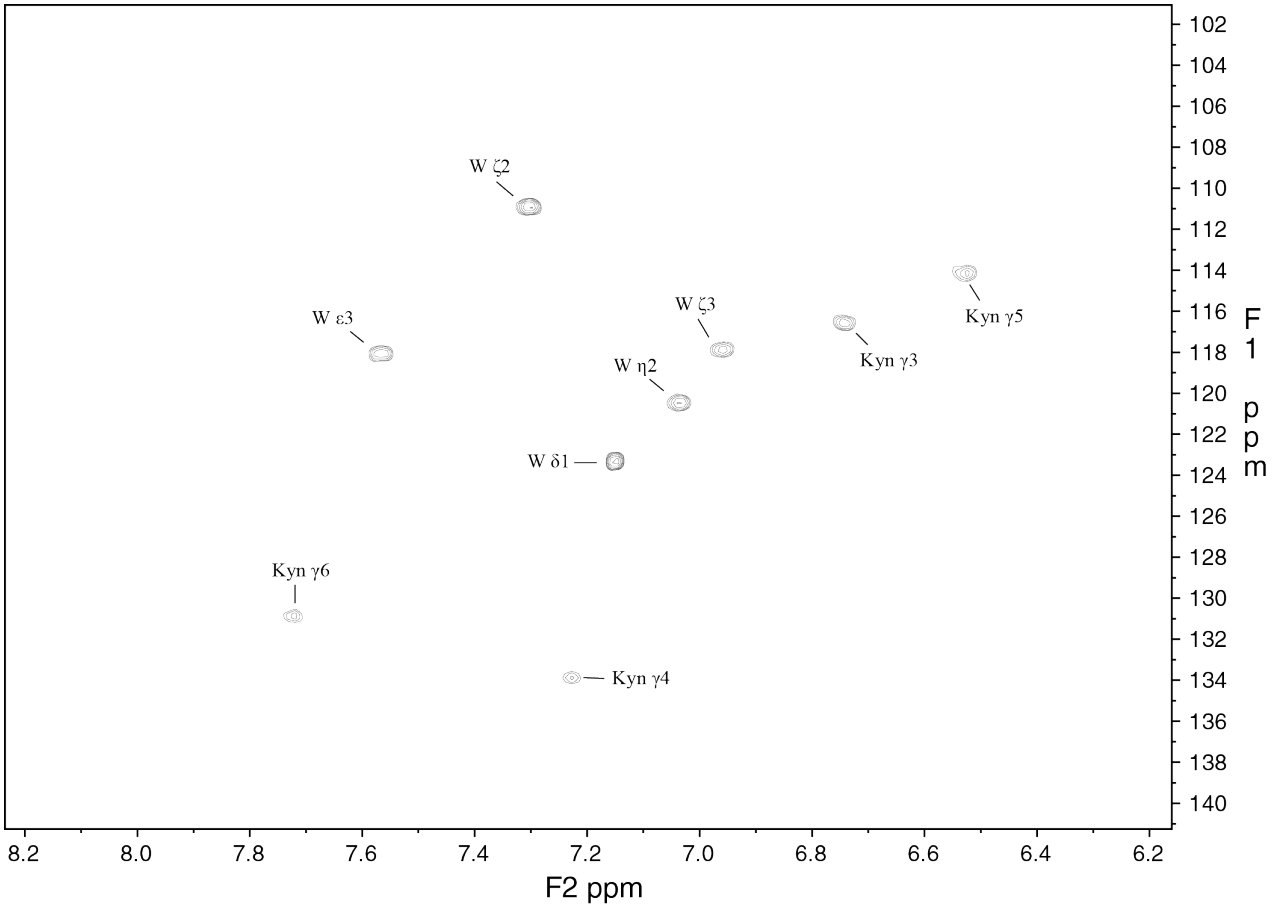
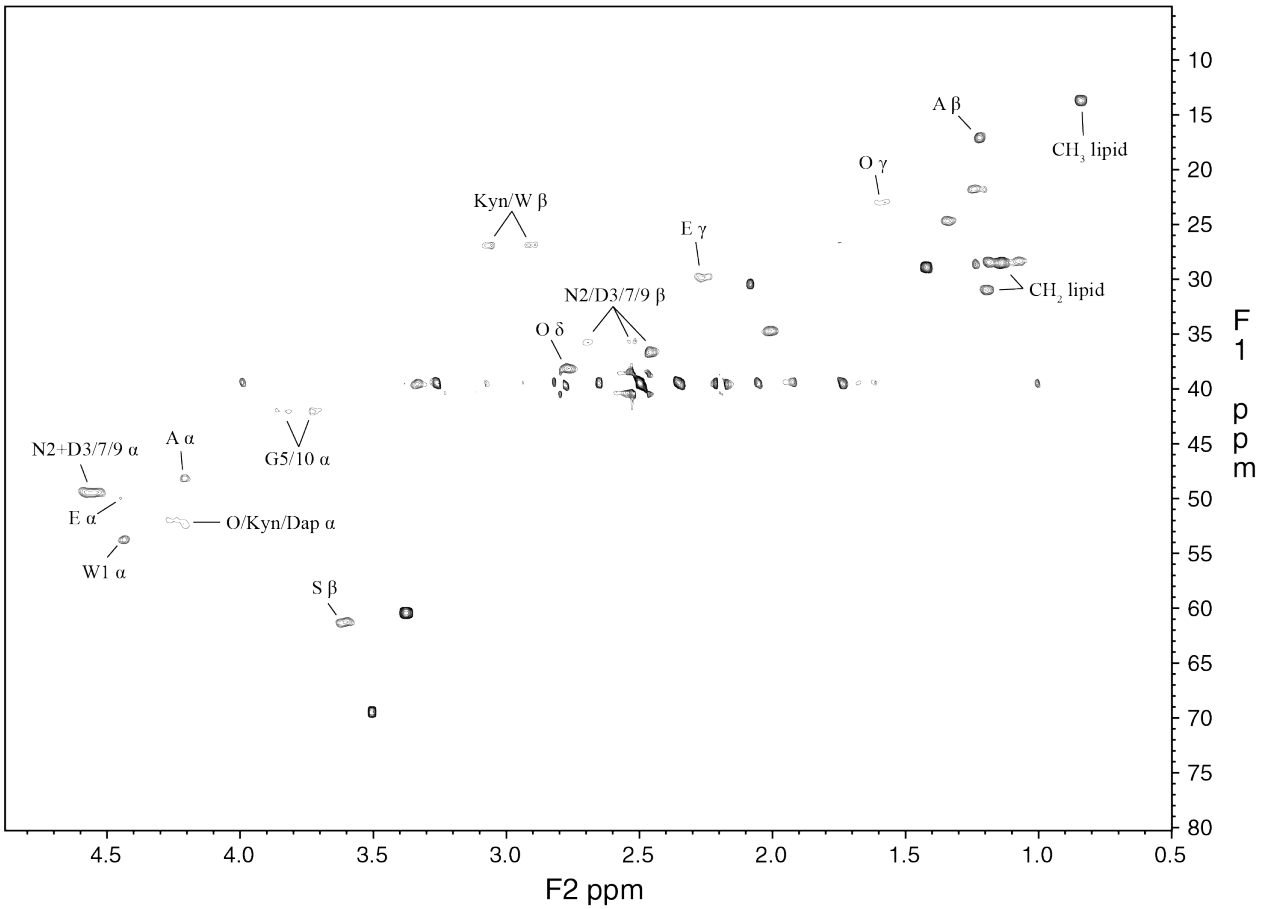
NMR Chemical shift assignments for compound 2





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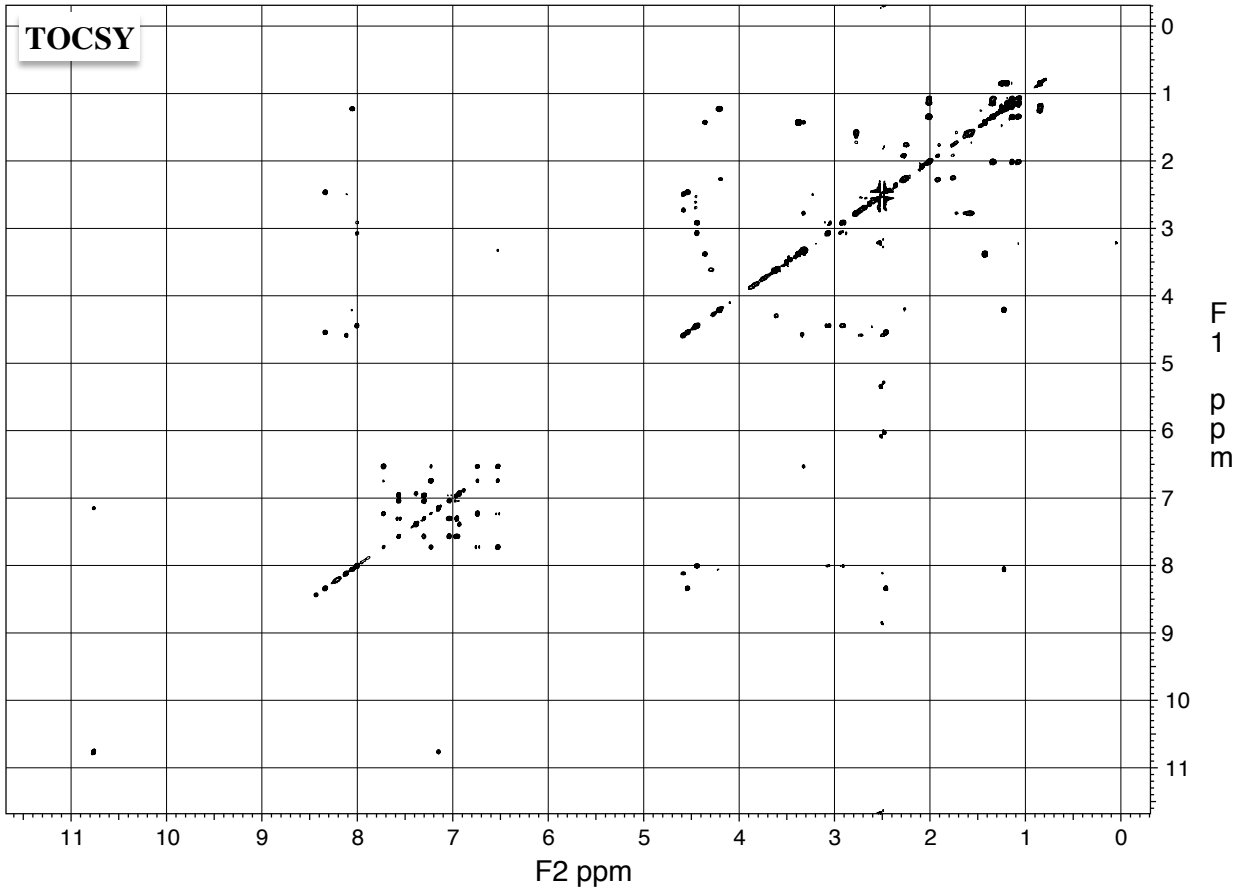
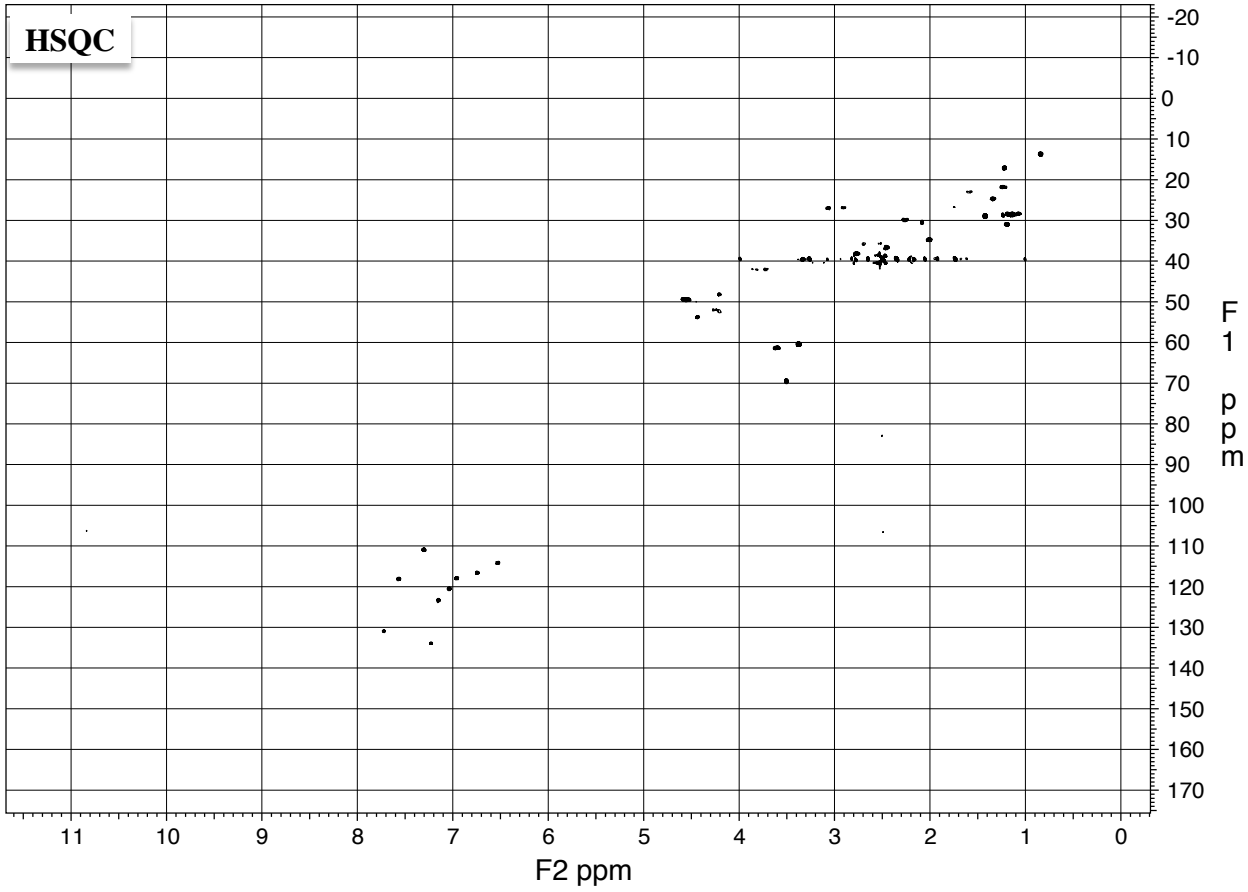
TOCSY and HSQC NMR spectra for compound 2 (measured in DMSO-d_6)





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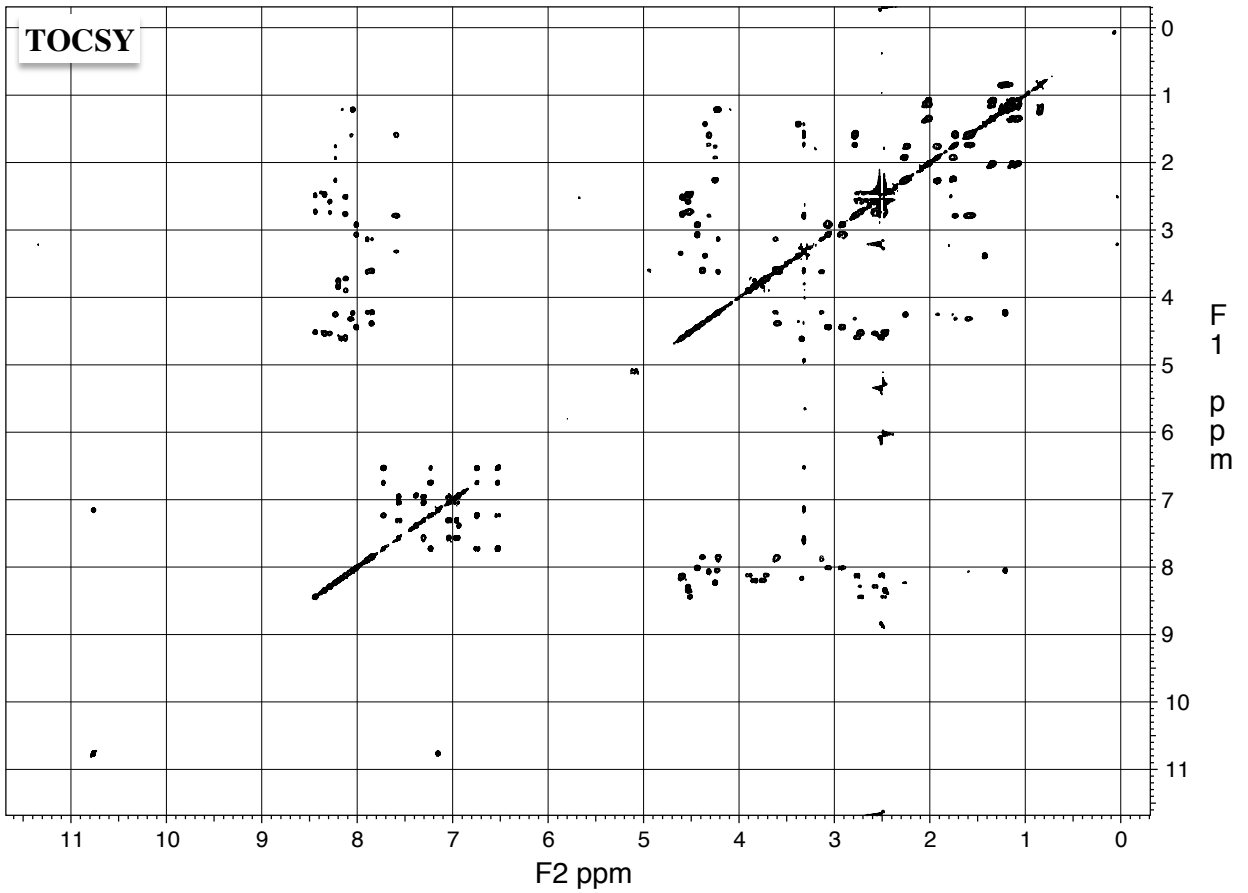
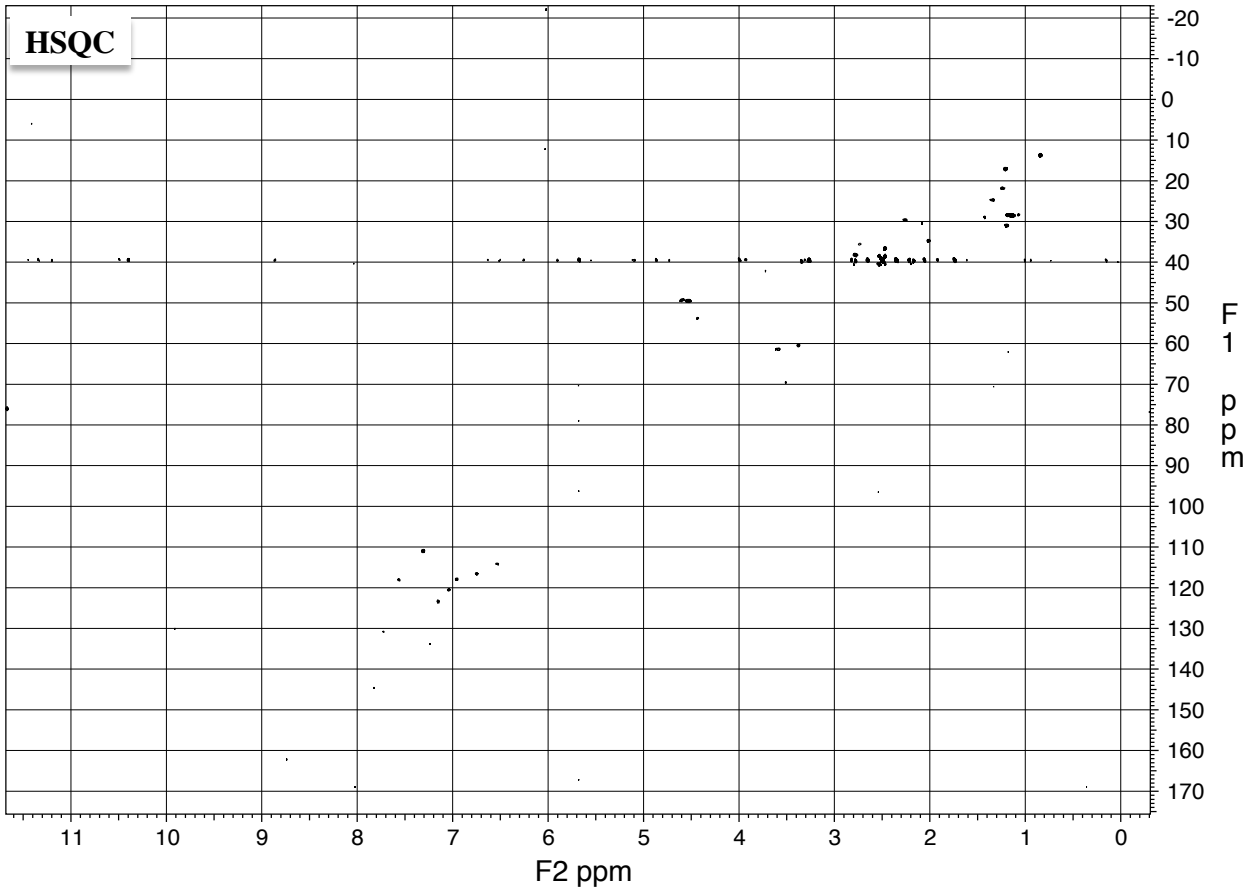
TOCSY and HSQC NMR spectra for compound *ent*-2 (measured in DMSO-d_6)





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NMR Chemical shift assignments for compound 3

Residue	$H^{\alpha}(C^{\alpha})$	H^{β} (C^{β})	Sidechain
Tail	---		CH_2 1.07-1.19 (28.5), CH_2 1.19 (31.0), CH_3 0.84 (13.7)
Trp-1	4.47 (53.8)	3.09/2.92 (26.9)	δ1 7.16 (123.4), ε3 7.58 (118.1), ζ3 6.96 (117.9), η2 7.04 (120.5), ζ2 7.31 (110.9) H_N 10.78
Asn-2	4.60 (49.4)	2.71/2.52 (35.9)[a]	---
Asp-3	4.53 (49.4)[a]	2.71/2.52 (35.9)[a]	---
Dap-4	4.16 (51.9)	3.64/3.03 (39.8)	---
Gly-5	3.87/3.67 (42.2)	---	---
Orn-6	4.33 (51.8)	2.75 (38.2)	γ 1.56 (23.1), δ 2.75 (38.3)
Asp-7	4.53 (49.4 [a]	2.71/2.52 (35.9)[a]	---
Ala-8	4.22 (48.4)	1.18 (17.3)	---
Asp-9	4.53 (49.4)[a]	2.71/2.52 (35.9)[a]	---
Gly-10	3.87/3.67 (42.2)	---	---
Ser-11	4.40 (54.9)	3.60 (61.5)	---
Glu-12	4.35 (51.7)	1.90/1.71 (26.9)	γ 2.21 (29.7)
Trp-13	4.35 (54.0)	3.09/2.92 (26.9)	δ1 7.07 (123.4), ε3 7.47 (118.0), ζ3 6.96 (117.9), η2 7.04 (120.5), ζ2 7.31 (110.9) H_N 10.71

[a] Ambiguous assignments due to overlap of the peaks

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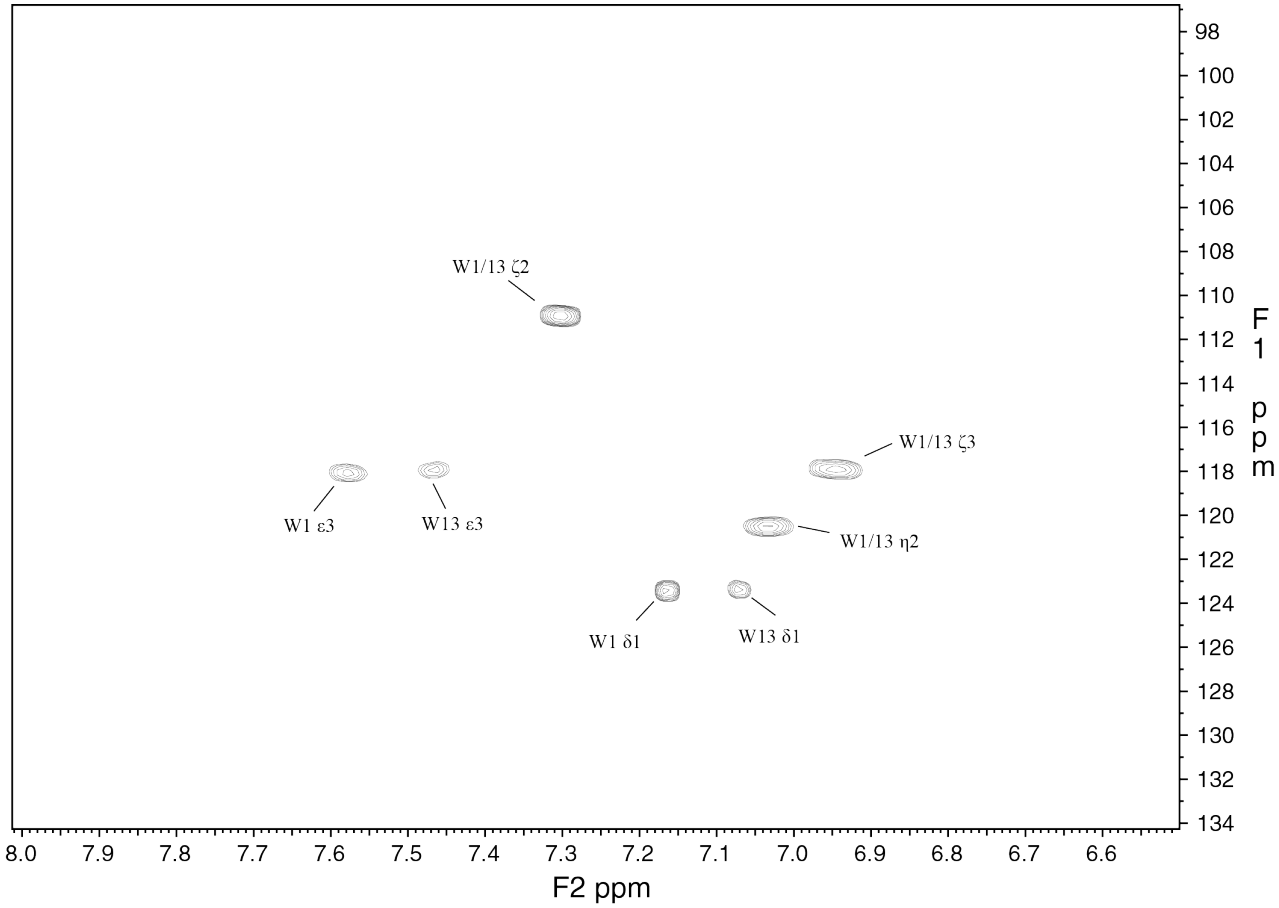
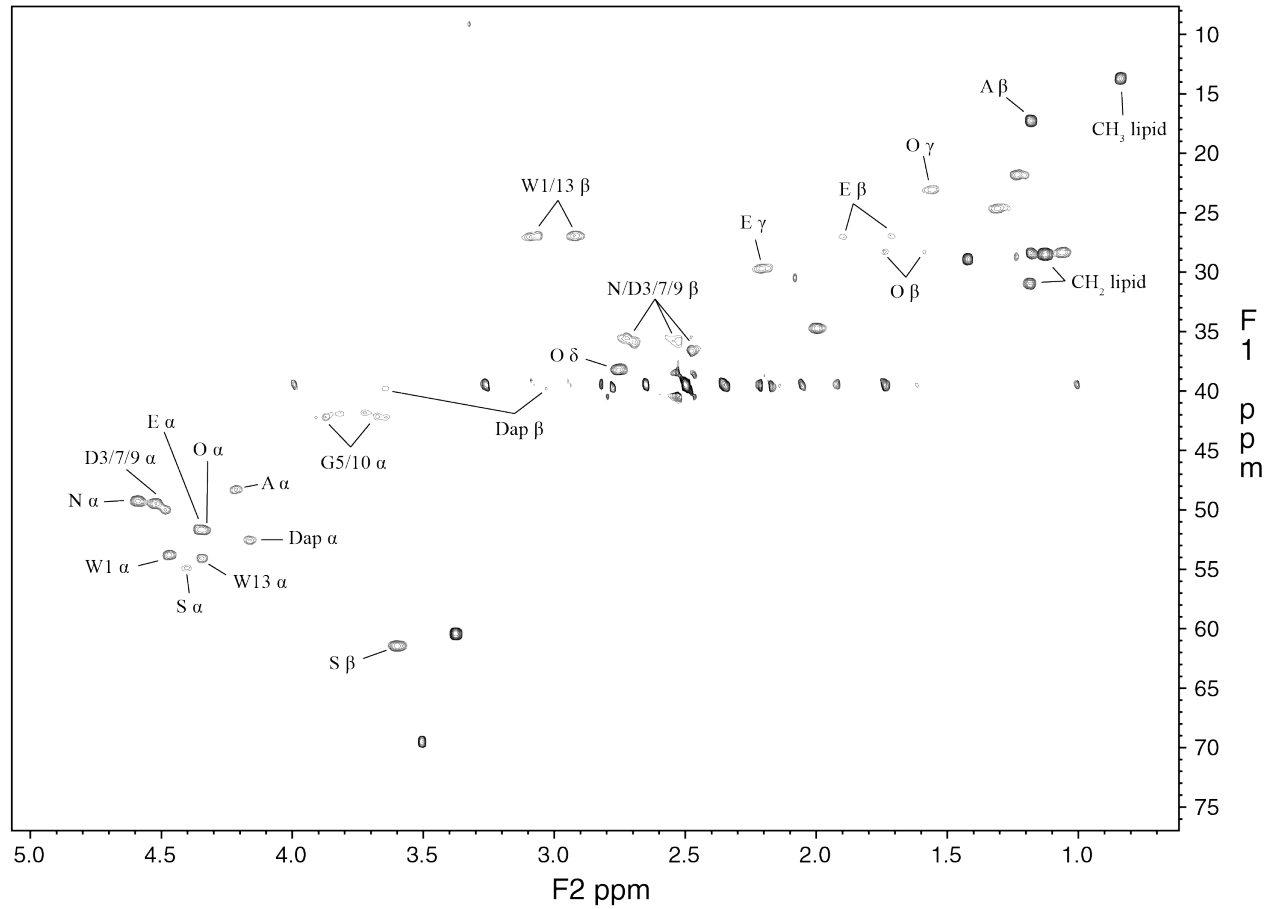
NMR Chemical shift assignments for compound 3





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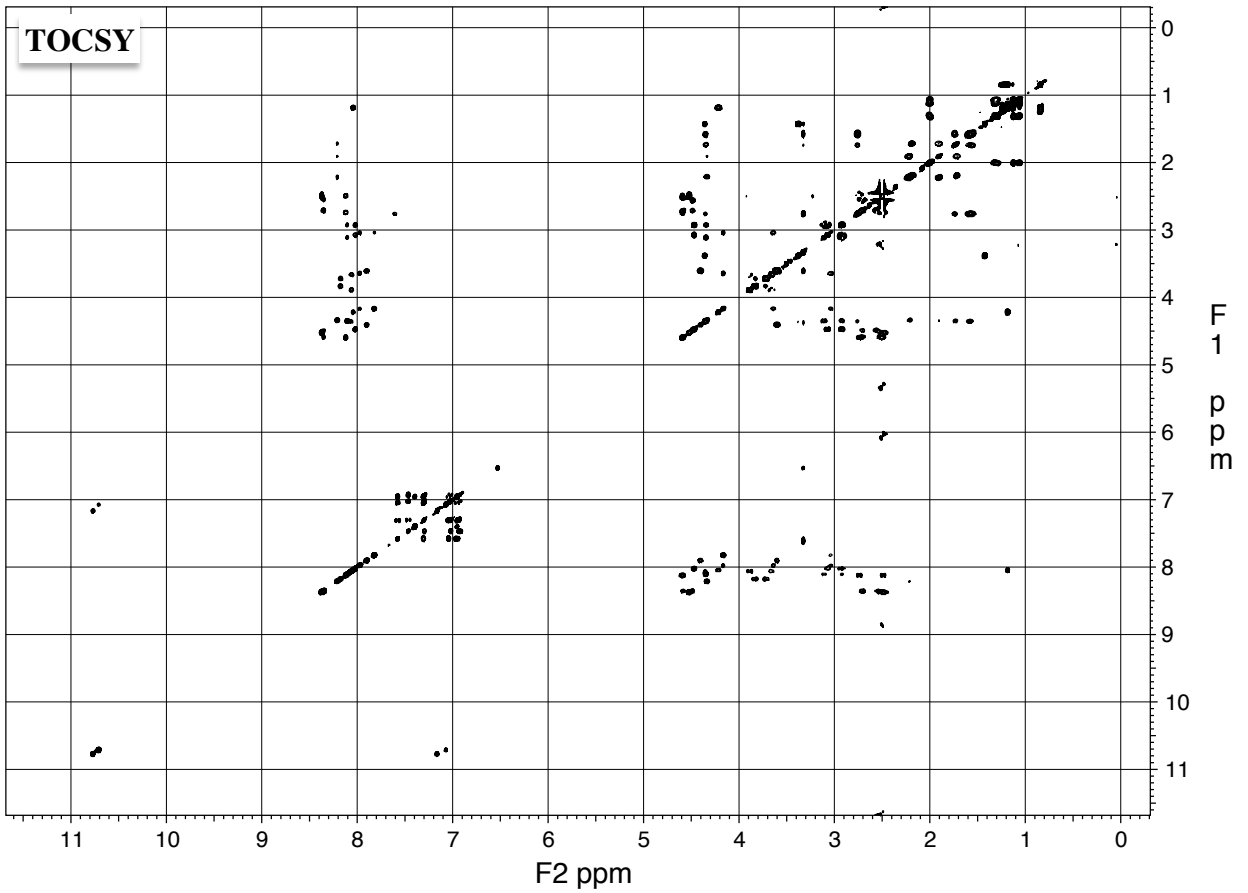
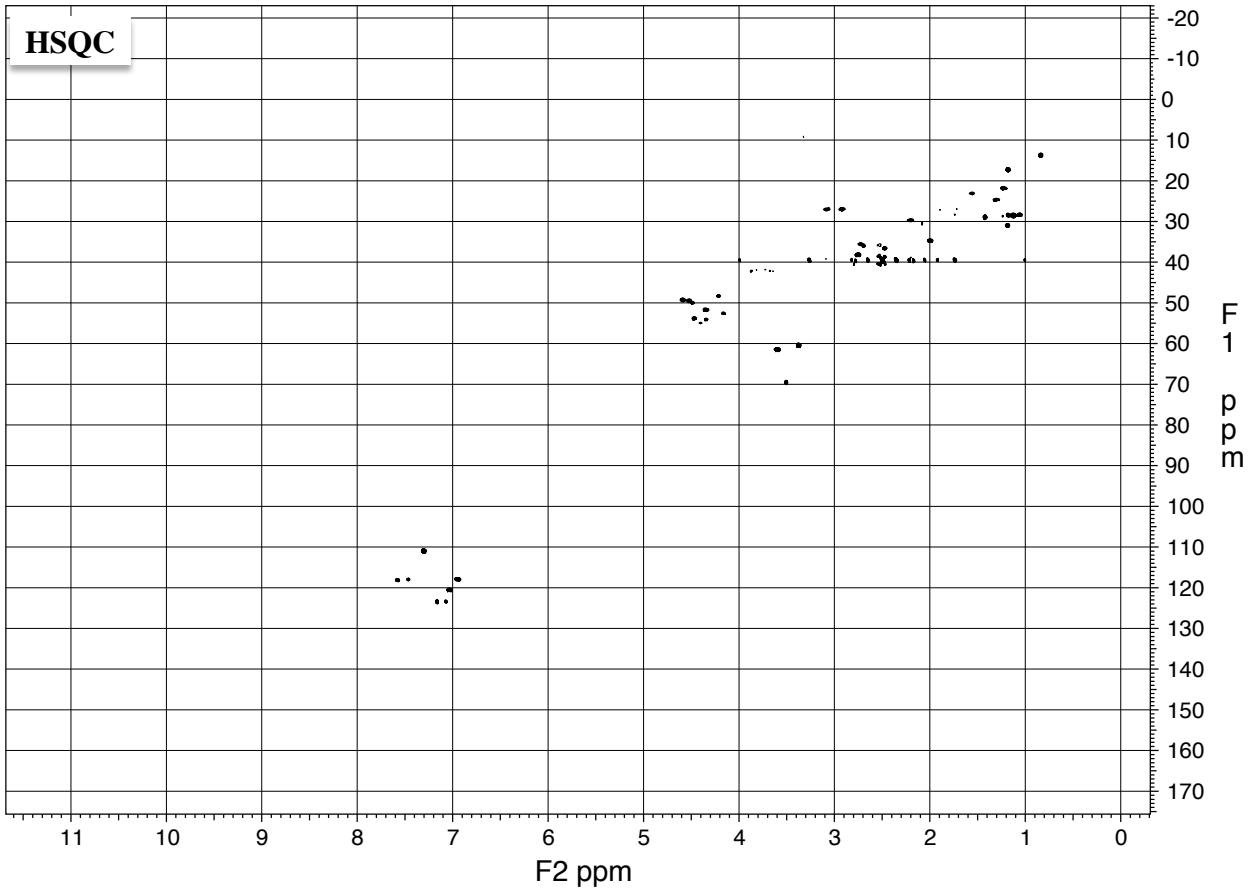
TOCSY and HSQC NMR spectra for compound 3 (measured in DMSO-d_6)





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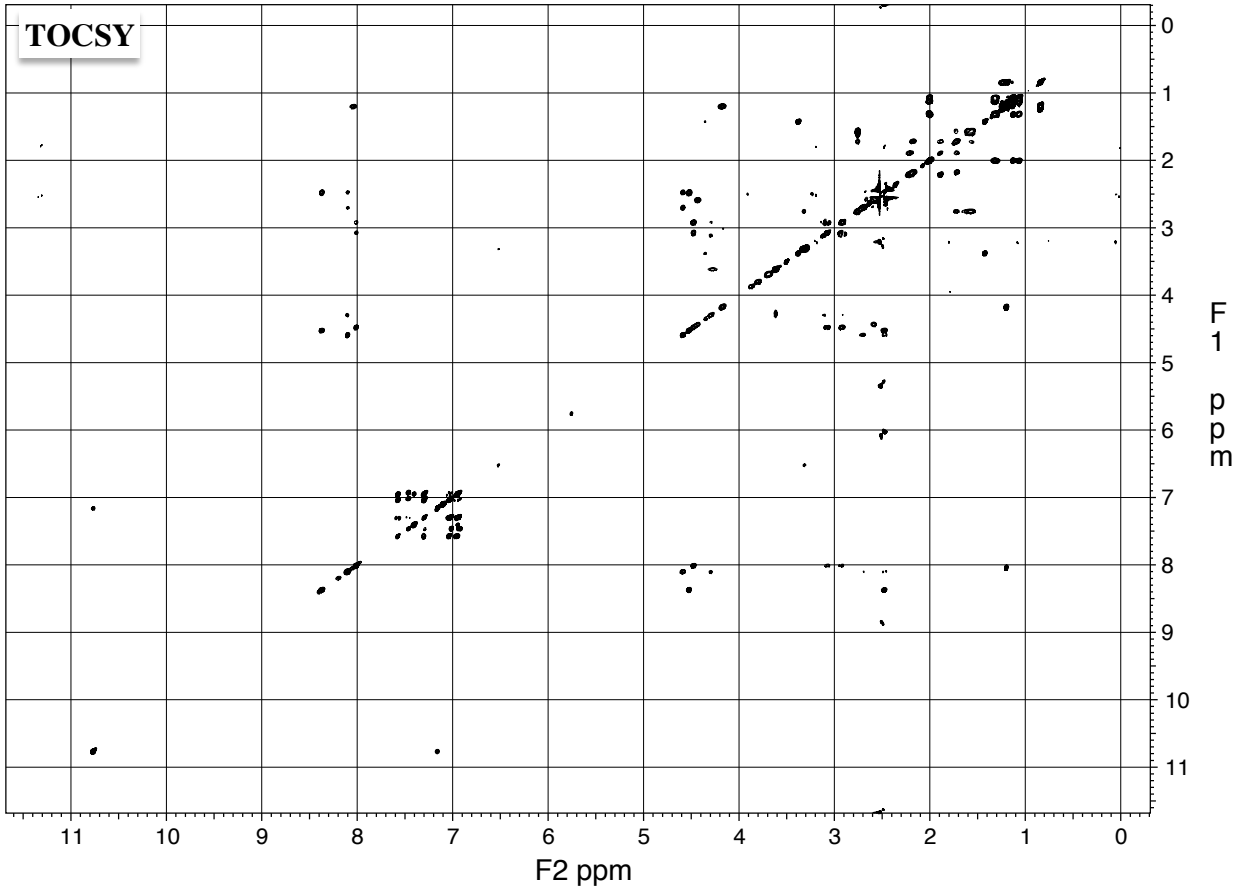
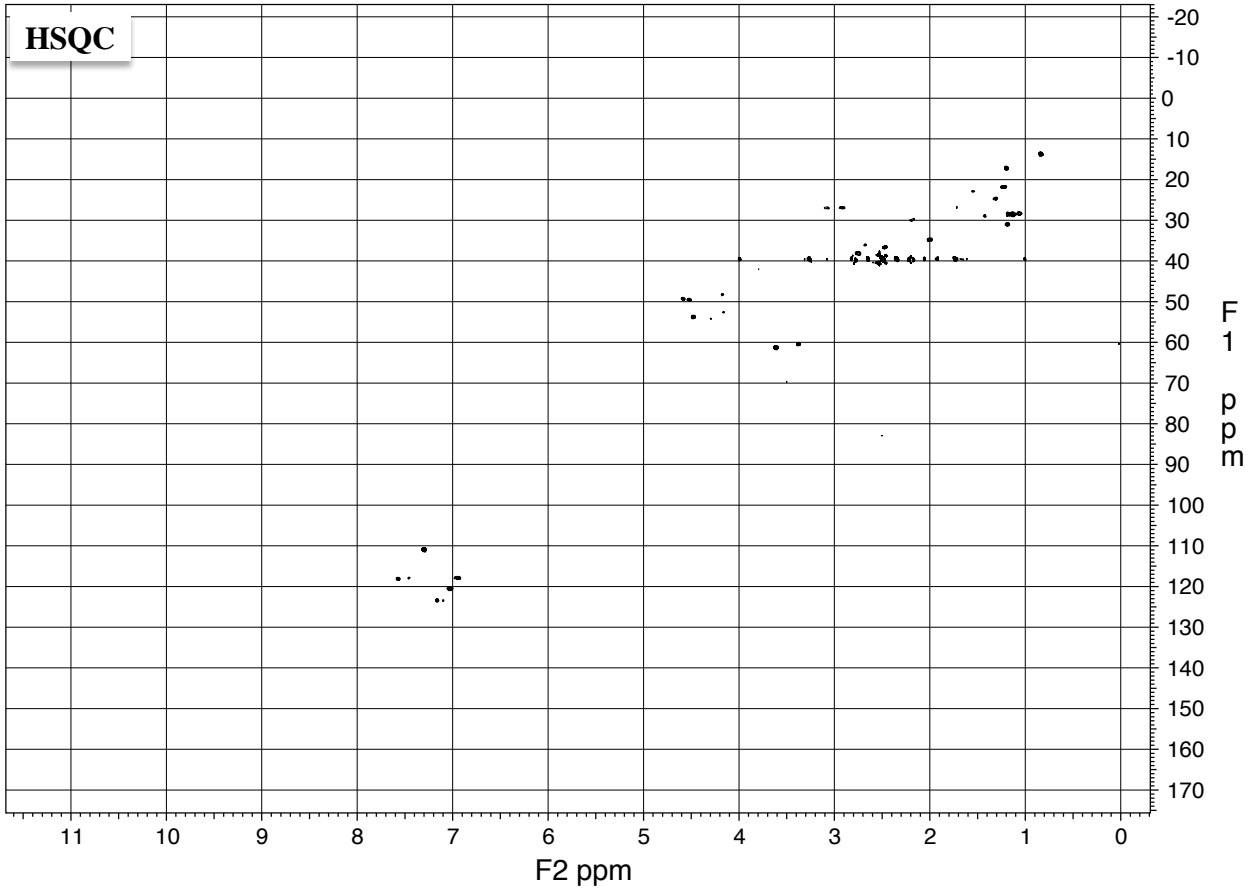
TOCSY and HSQC NMR spectra for compound *ent*-3 (measured in DMSO-d_6)





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