Supporting Information

for

Selective Synthesis of Hydroxy Analogues of Valinomycin using Dioxiranes

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(30 pages, including this cover)

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1. Materials and Methods

The ^{1}H NMR spectra (400 MHz) were referenced to residual isotopic impurity (2.05 ppm) of acetone-d_6 solvent and/or TMS. The ^{13}C NMR spectra (100 MHz) were referenced to the middle peak of acetone-d_6 solvent (30.83 ppm).

The mass spectra were obtained using a Micromass M@LDI™–LR (Waters MS Technologies, time-of-flight mass spectrometer equipped with a nitrogen UV laser (337 nm) reflectron optics, fast dual micro-channel plate (MCP) detector. Previous to MS analysis, the samples (dissolved in acetonitrile) were mixed (1:1, v:v) with a 2,5-dihydroxybenzoic acid (DHB) solution [10 mg/mL, in acetonitrile (saturated with KCl)/water 50:50].

The HPLC analyses were run using a Supelcosil ABZ+plus column (150×4.6 mm, 5 mm) or a silica gel (Ascentis® Si, 250 × 2.1 mm, 5 mm). Preparative HPLC separations were carried out on a silica gel column (Ascentis® Si, 250 × 10 mm, 5 μm).

Acetone and other common solvents were purified by standard methods. Commercial Valinomycin (**2**) of >95% purity (HPLC) was used without further purification. Commercial 1,1,1-trifluoro-2-propanone (TFP) (bp 22 °C), was purified by fractional distillation over granular P_2O_5, stored over 5 Å molecular sieves, and routinely redistilled prior to use. Caroat® triple salt $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$ (a gift from Peroxid-Chemie, Degussa, Germany) was our source of potassium peroxymonosulfate, employed in the synthesis of dioxirane **1b**. Solutions of 0.5-1.0 M methyl(trifluoromethyl)dioxirane (**1b**) in 1,1,1-trifluoropropanone (TFP) were obtained by adopting procedures, equipment, and precautions already reported in detail (Mello, R.; Fiorentino, M.; Fusco, C.; Curci, R. *J. Am. Chem. Soc.* **1989**, *111*, 6749).

The following procedure is representative of valinomycin (**2**) oxidation using TFDO (**1b**):

Monohydroxylation of valinomycin. A standardized cold solution of TFDO (**1b**) in 1,1,1-trifluoropropanone (TFP) (0.9 M, 1 mL, 0.9 mmol) is added in one portion to a stirred solution of VLM (**2**) (100 mg, 0.090 mmol) in acetone (2 mL) kept at 0 °C. The reaction progress is monitored by HPLC (30 minutes, linear gradient of 70-100% acetonitrile in water; flow rate: 1.0 mL/min; UV detector 220

nm). After 6 h reaction time at 0 °C, the solvent is removed under reduced pressure and the product mixture separated from the unreacted starting material by column chromatography (silica gel, ethyl ether/hexane 3:1). This allows the recover of unreacted valinomycin (55 mg, 0.049 mmol). The mixture of products thus separated in turn undergoes treatment with preparative HPLC (hexane/isopropanol 95:5, flow rate 2.0 mL/min, UV detector 220 nm), affording each reaction products as an amorphous solid in > 95% purity (HPLC): **3a** (16.0 mg , 14 μmol), **3b** (10.0 mg , 8.9 μmol), and **3c** (9.1 mg, 8.1 μmol). Based on the amount valinomycin converted (45 mg), the yield of **3a**, **3b**, and **3c** is estimated as 35, 22, and 20%, respectively.

The NMR spectral data of compound **3a-c** are collected in Tables *S1-S6*. Spectra are shown in Figures *S1-S24* .

Structure assignment of compounds **3a**, **3b**, and **3c** was based on their 1D NMR (^{1}H, ^{13}C, DEPT-135) and 2D NMR (COSY, NOESY, HMQC, HMBC) spectra. The procedure relied on sequence-specific assignments based on the residual NH_{i+1}-αH_i correlations provided by the corresponding ^{1}H-^{1}H NOESY NMR spectra. (Cavanagh, J. et al. in *Protein NMR Spectroscopy: Principles and Practice*; Academic, 1996; Chapter 8) This approach was especially useful in discriminating compound **3b** from **3c**, since their structural divergences derive almost entirely from the different residues that are proximal to the hydroxylated valine moiety. For instance, the NMR structure determination of **3b** began by the assignment of the ^{1}H NMR six downfield doublet signals in the 8.06 ÷7.60 ppm range to the resonance of the six amide protons. In turn, by means of the COSY correlations, the signals in the 4.45÷4.20 ppm range could be attributed to the resonance of the α-CH protons of the valine residues. As a result of dioxirane *O*-insertion into the β-CH bond of the D-Val residue, its α-CH proton resonance (4.45 ppm) appears as a clean doublet, due to single coupling with the vicinal NH proton. The latter resonance was eventually identified as a doublet at 7.60 ppm by the corresponding COSY cross-peak.

2. NMR Spectral Data of Compound 3a-c.

Table *S1*. ^{1}H NMR Chemical shift values (ppm) for compound **3a**.[a,b]

	Residue				
	D-β-OH-Hyi	D-Hyi	D-Val	L-Val	L-Lac
N*H*	-	-	7.74 (d, 8.4)[d,e] 7.662 (d, 7.6)[d] 7.657 (d, 7.6)[d]	7.97 (d, 7.6)[d] 7.94 (d, 6.8)[d] 7.92 (d, 7.2)[d]	-
α-C*H*	4.95 (s)	5.01 (d, 4.0) 5.00 (d, 3.6)	4.43 (dd, 8.4, 7.2)[e] 4.39-4.34 (m, 2H)	4.26-4.18 (m, 3H)	5.43-5.35 (m, 3H)
β-C*H*	4.52 (s)[c]	2.39 - 2.14 (m, 8H)			-
β-CH_3	-	-	-	-	1.42 (d, 6.8) 1.409 (d, 6.8) 1.406 (d, 6.4)
γ-CH_3	1.28 (s) 1.24 (s)	1.08 - 0.96 (m, 48H)			-

[a]Data are for spectra in acetone-d_6 at 400 MHz. [b]Signal multiplicity, *J* values (±0.4 Hz), and signal integration values (if >1) are specified in parentheses. [c]Resonance of the β-OH proton. [d]Assigned on the basis of the NOESY spectrum (Fig. *S5*). [e]Relative to the D-Hyi residue sequential to the D-β-OH-Val residue.

Table *S2*. ^{13}C NMR Chemical shift values (ppm) for compound **3a**.[a]

	Residue				
	D-β-OH-Hyi	D-Hyi	D-Val	L-Val	L-Lac
C=O	173.5, 173.4, 173.22, 173.16, 173.0, 172.2, 172.0, 171.4, 171.3, 171.0				
α-*C*H	80.8	80.12 80.08	59.9 59.8 59.6	61.3 61.2 61.1	72.05 71.09 [2]
β-*C*H	72.6[b]	32.17 [2]	31.8, 31.61, 31.58, 31.1, 30.88, 30.86		-
β-*C*H_3	-	-	-	-	18.38 18.35 [2]
γ-*C*H_3	27.8 27.3	20.7 [4], 20.6, 20.5, 20.4 [2], 20.3 [2], 20.2, 19.93, 19.89, 19.7, 18.07, 18.02			-

[a]Data are for spectra in acetone-d_6 at 100 MHz. [b]Resonance of the *C*-OH carbon.

Table *S3*. ^{1}H NMR Chemical shift values (ppm) for compound **3b**.[a,b]

	Residue				
	D-β-OH-Val	D-Hyi	D-Val	L-Val	L-Lac
N*H*	7.60 (d, 8.8)	-	7.72 (d, 8.0)[d] 7.64 (d, 8.4)[d]	8.06 (d, 6.8)[d] 7.89 (d, 7.6)[d] 7.87 (d, 7.6)[d]	-
α-C*H*	4.45 (d, 8.8)	5.04 (d, 3.6)[e] 5.02 (d, 3.2) 5.01(d, 3.6)	4.36 (pseudo-t, 8.0)	4.24-4.16 (m, 2H)	5.39-5.30 (m, 3H)
			4.32-4.28 (m, 2H)		
β-C*H*	4.57 (s)[c]	2.40-2.14 (m, 8H)			-
β-CH_3	-	-	-	-	1.43-1.39 (m, 9H)
γ-CH_3	1.35 (s) 1.31 (s)	1.10-0.97 (m, 48H)			-

[a]Footnote *a*, Table *S1*. [b]Footnote *b*, Table *S1*. [c]Resonance of the β-OH proton. [d]Assigned on the basis of the NOESY spectrum (Fig. *S11*). [e]Relative to the D-Hyi residue sequential to the D-β-OH-Val residue.

Table *S4*. ^{13}C NMR Chemical shift values (ppm) for compound **3b**.[a]

	Residue				
	D-β-OH-Val	D-Hyi	D-Val	L-Val	L-Lac
C=O	173.9, 173.40, 173.38, 173.25, 173.19, 173.0, 172.1, 172.0, 171.5, 171.2, 171.0, 170.7				
α-*C*H	62.9	80.2 [2] 80.1	60.1 59.8	61.3 61.2 61.0	72.3 72.0 71.7
β-*C*H	72.6[b]	32.2 [2] 32.1	31.5, 31.2, 31.11, 31.10, 30.9		-
β-*C*H_3	-	-	-	-	18.6 18.44 18.36
γ-*C*H_3	28.9 28.7	20.64 [2], 20.58 [3], 20.4, 20.32 [2], 20.28, 20.2, 20.0 [2], 19.9, 18.14, 18.09 [2]			-

[a]Footnote *a*, Table *S2*. [b]Resonance of the *C*-OH carbon.

Table *S5*. ^{1}H NMR Chemical shift values (ppm) for compound **3c**.[a,b]

	Residue				
	L-β-OH-Val	D-Hyi	D-Val	L-Val	L-Lac
N*H*	7.70 (d, 7.2)	-	7.81 (d, 7.6)[d] 7.67 (d, 8.0)[d] 7.66 (d, 8.0)[d]	7.95 (d, 7.2)[d] 7.88 (d, 7.2)[d]	-
α-C*H*	4.37 (d, 7.2)	5.04 (d, 3.6, 2H) 5.02 (d, 4.0)	4.37 (pseudo-t, 8.0) 4.31 (pseudo-t, 7.6) 4.30 (pseudo-t, 8.0)	4.28-4.22 (m, 2H)	5.37-5.28 (m, 3H)
β-C*H*	4.52 (s)[c]	2.40-2.19 (m, 8H)			-
β-CH_3	-	-	-	-	1.44-1.40 (m, 9H)
γ-CH_3	1.34 (s) 1.33 (s)	1.08-0.94 (m, 48H)			-

[a]Footnote *a*, Table *S1*. [b]Footnote *b*, Table *S1*. [c]Resonance of the β-OH proton. [d]Assigned on the basis of the NOESY spectrum (Fig. *S19*). [e]Relative to the D-Hyi residue sequential to the L-β-OH-Val.

Table *S6*. ^{13}C NMR Chemical shift values (ppm) for compound **3c**.[a]

	Residue				
	L-β-OH-Val	D-Hyi	D-Val	L-Val	L-Lac
C=O	173.2, 173.0, 172.16, 172.14, 172.12, 171.87, 171.85, 171.82, 171.54, 171.45				
α-*C*H	63.6	80.2 80.1 79.8	60.4 60.2 59.8	61.1 61.0	72.16 72.14 71.03
β-*C*H	72.08[b]	32.3 32.24 32.15	31.5, 31.2, 31.11, 31.10, 30.9		-
CH_3	28.6 (γ-*C*H_3) 28.2 (γ'-*C*H_3)	20.7 [2], 20.5 [2], 20.4 [2], 20.36, 20.2 [2], 20.1, 20.0, 19.9, 18.8, 18.7, 18.6, 18.4, 18.2, 18.03, 18.00			

[a]Footnote *a*, Table *S2*. [b]Resonance of the *C*-OH carbon.

3. 1D-, 2D NMR, and MALDI spectra of compound 3a.

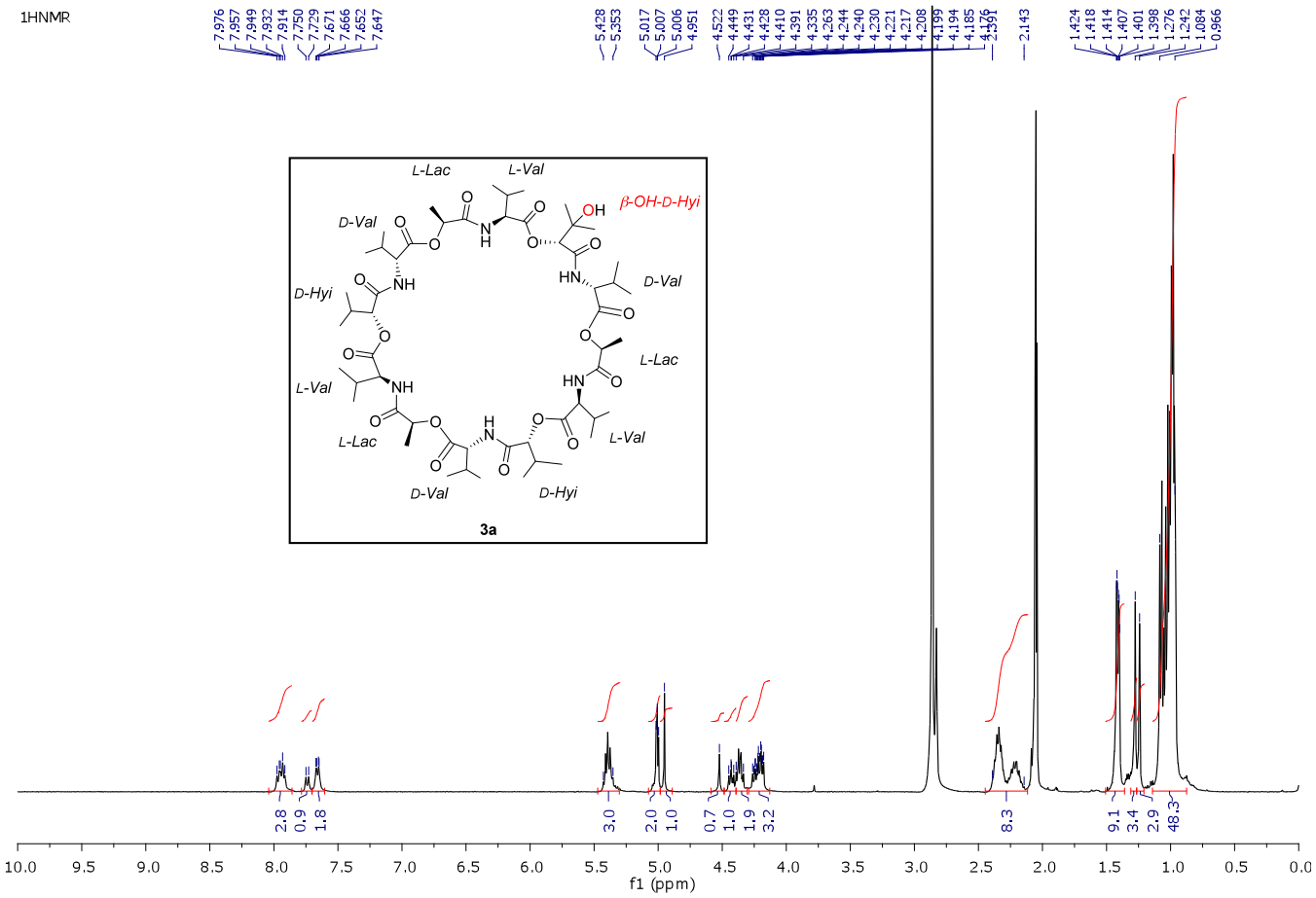

Figure *S1*. ^{1}H NMR spectrum of compound **3a** (acetone-d_6, 400 MHz).

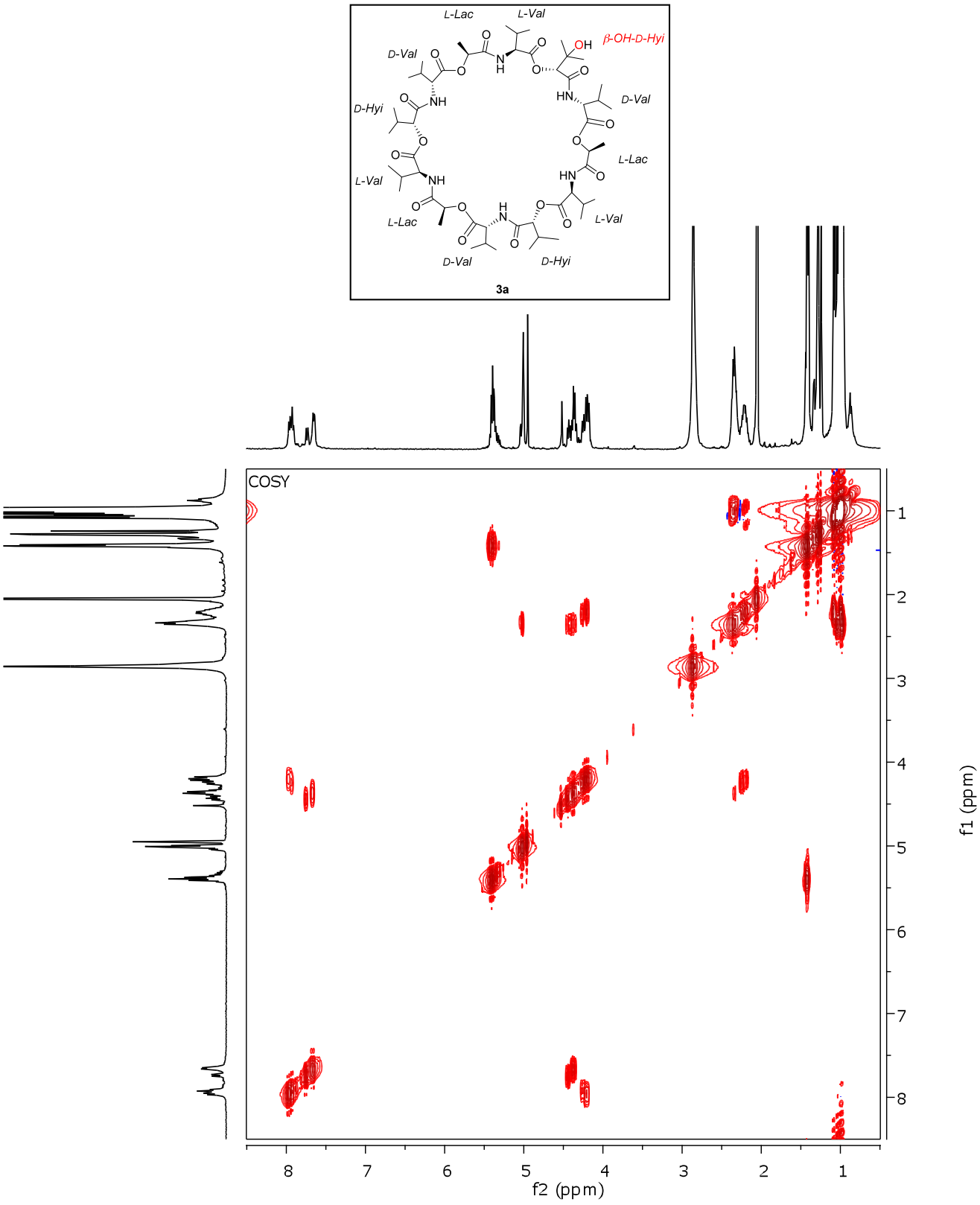

Figure *S2*. COSY NMR spectrum of compound **3a** (acetone-d_6, 400 MHz).

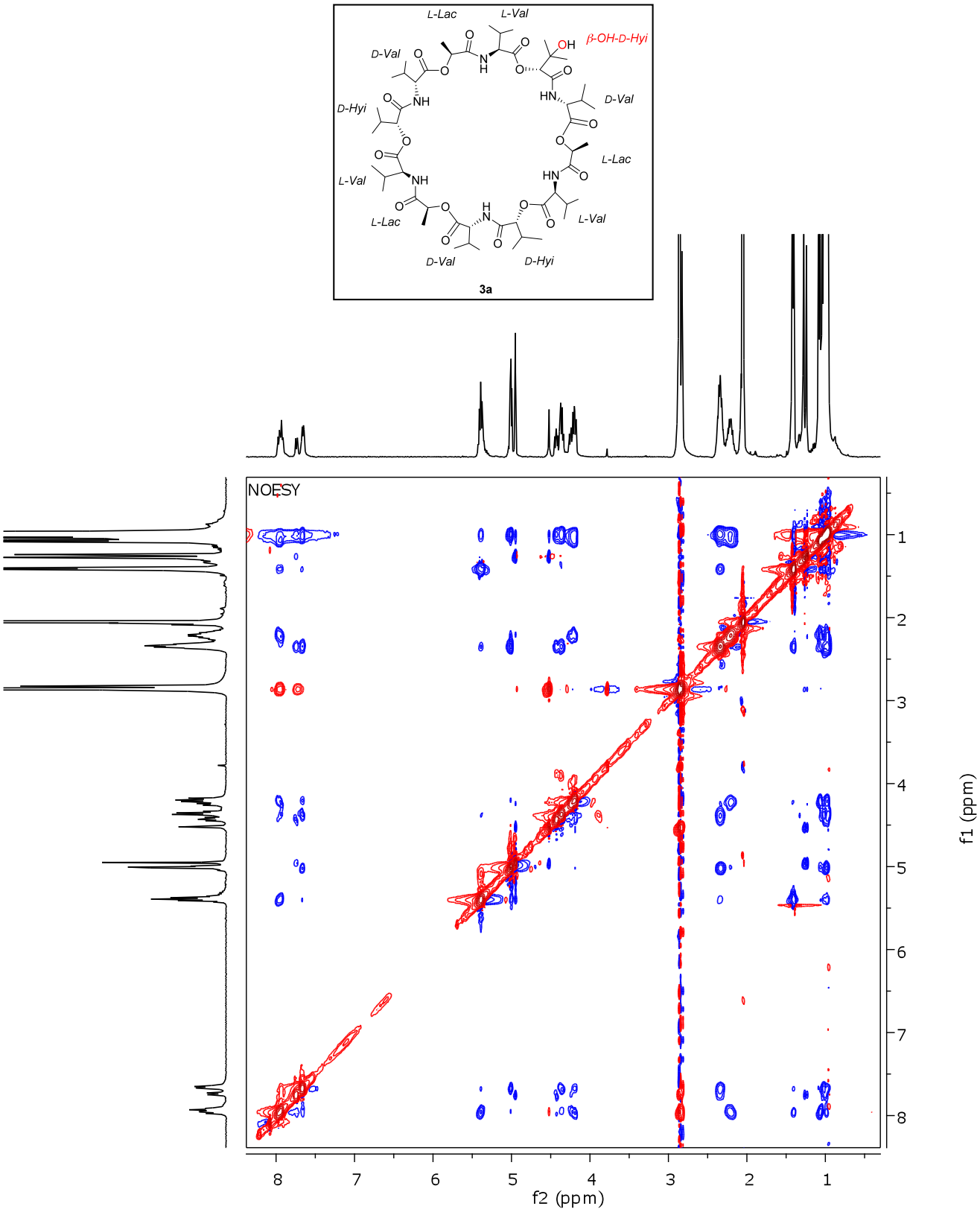

Figure *S3*. NOESY NMR spectrum of compound **3a** (acetone-d_6, 400 MHz, t_{mix} = 0.35 sec).

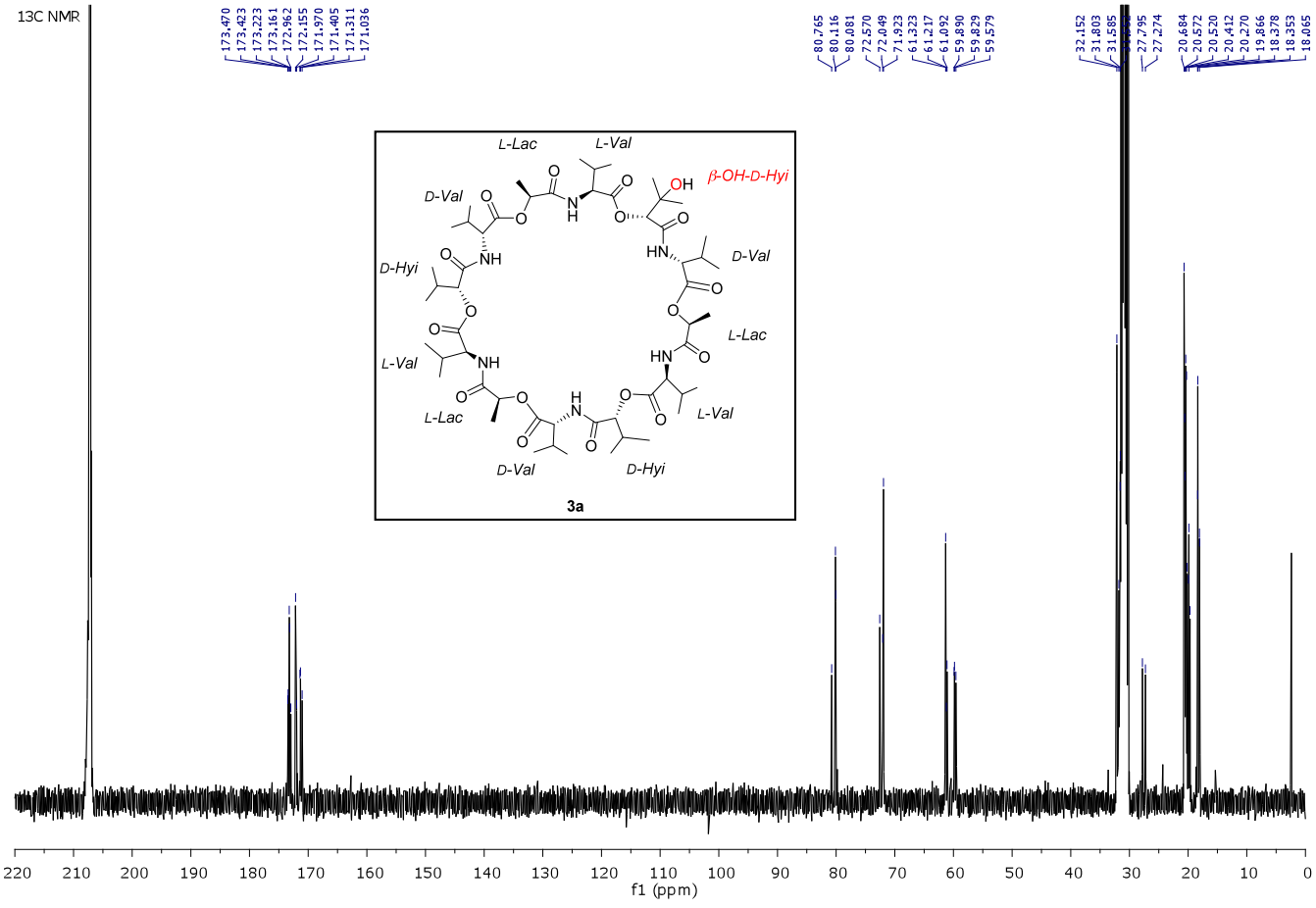

Figure *S4*. ^{13}C NMR spectrum of compound **3a** (acetone-d_6, 100 MHz).

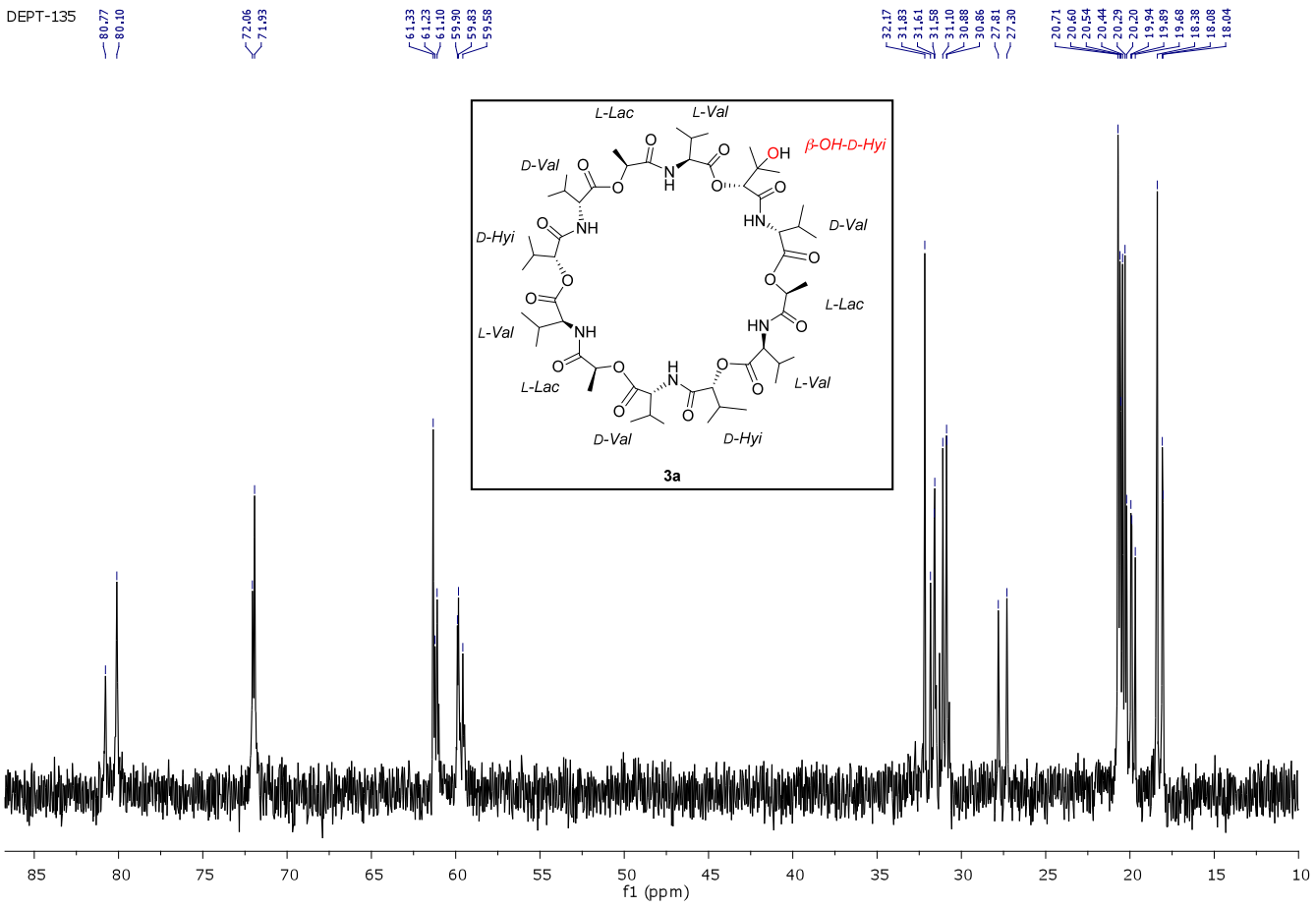

Figure *S5*. ^{13}C DEPT-135 NMR spectrum of compound **3a** (acetone-d_6, 100 MHz).

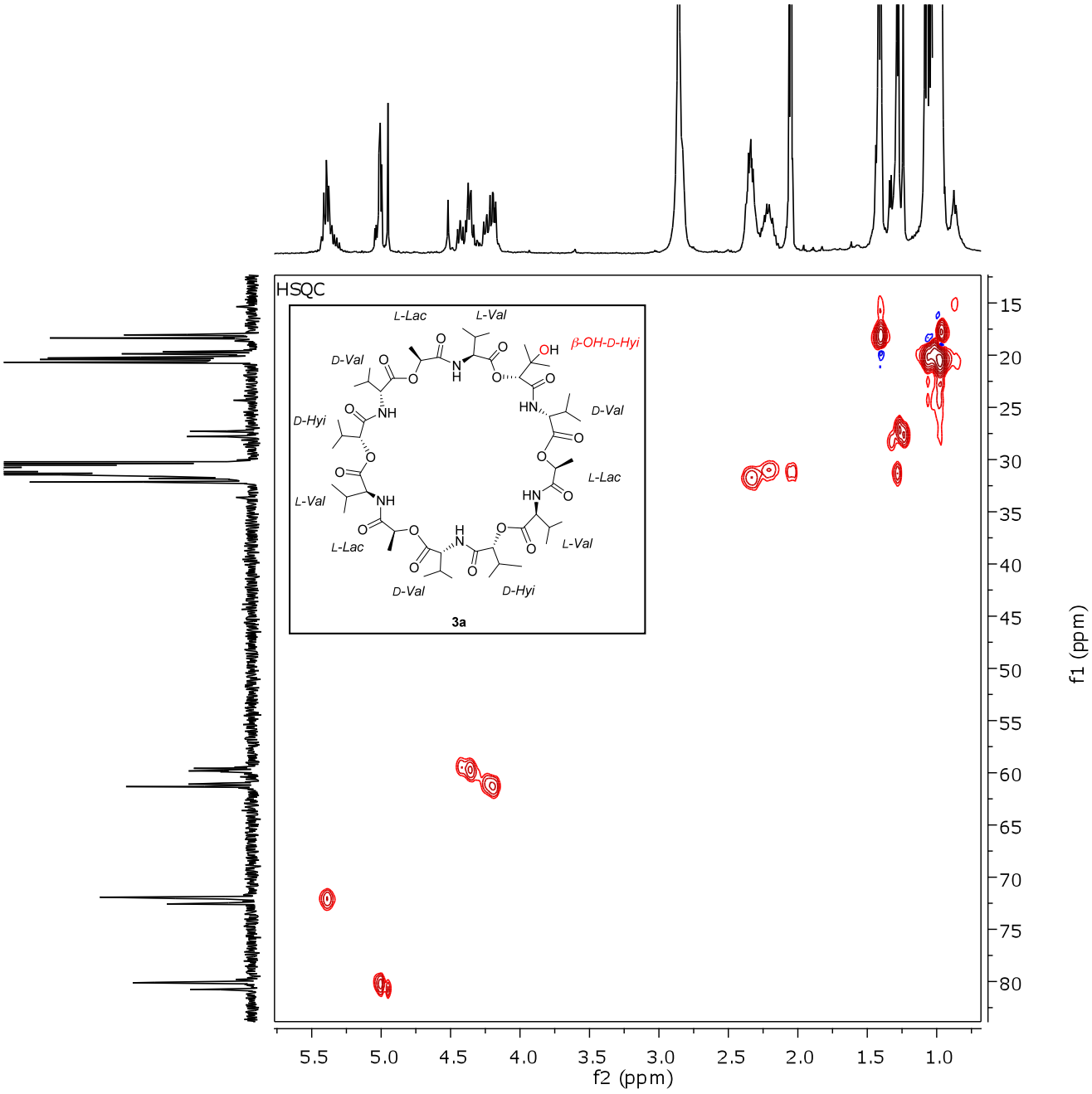

Figure *S6*. HSQC NMR spectrum of compound **3a** (acetone-d_6, 400 MHz).

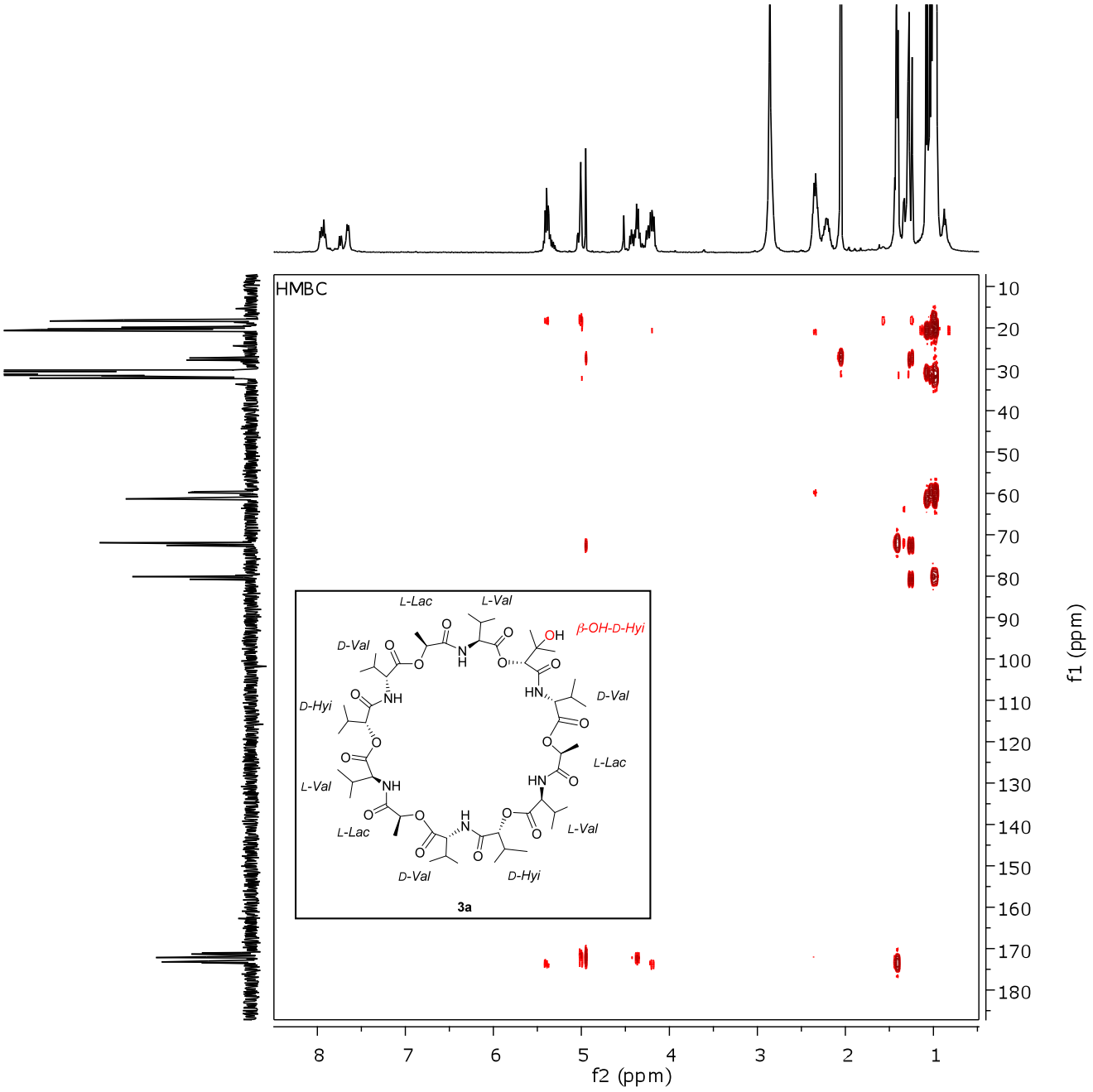

Figure *S7*. HMBC NMR spectrum of compound **3a** (acetone-d_6, 400 MHz).

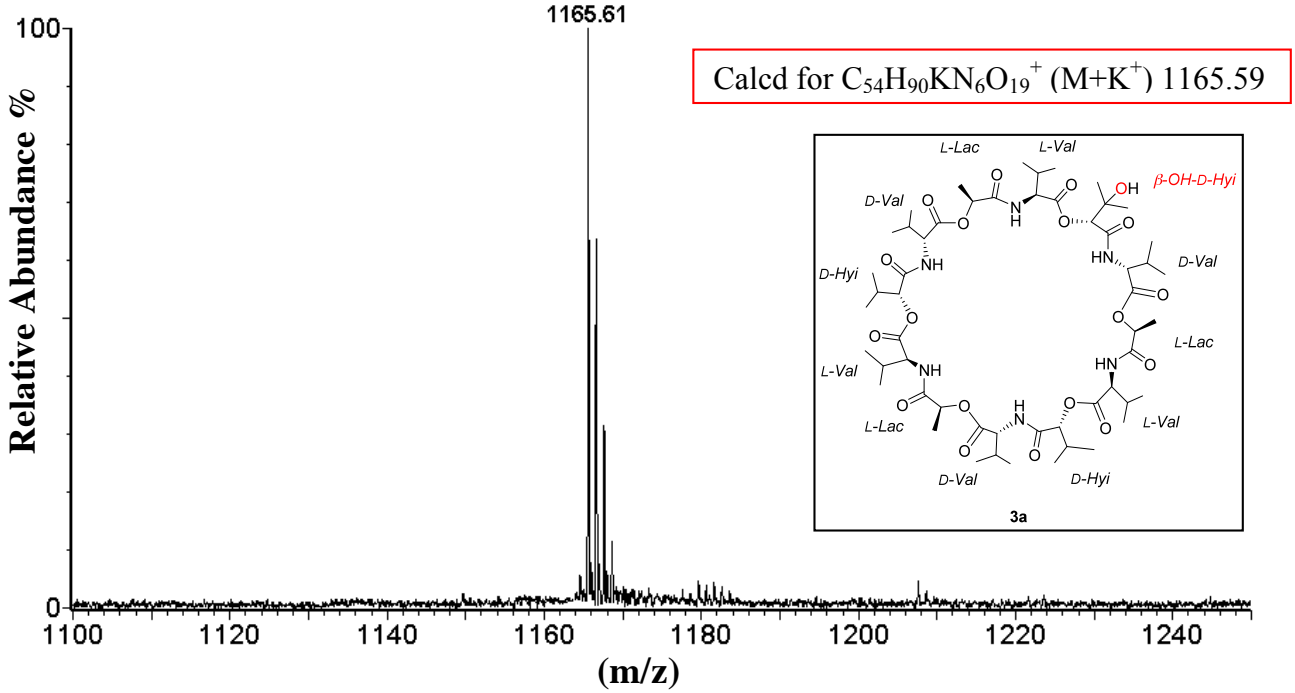

Figure *S8*. MALDI-ToF mass spectrum of compound **3a** in the presence of KCl.

4. 1D-, 2D NMR, and MALDI spectra of compound 3b.

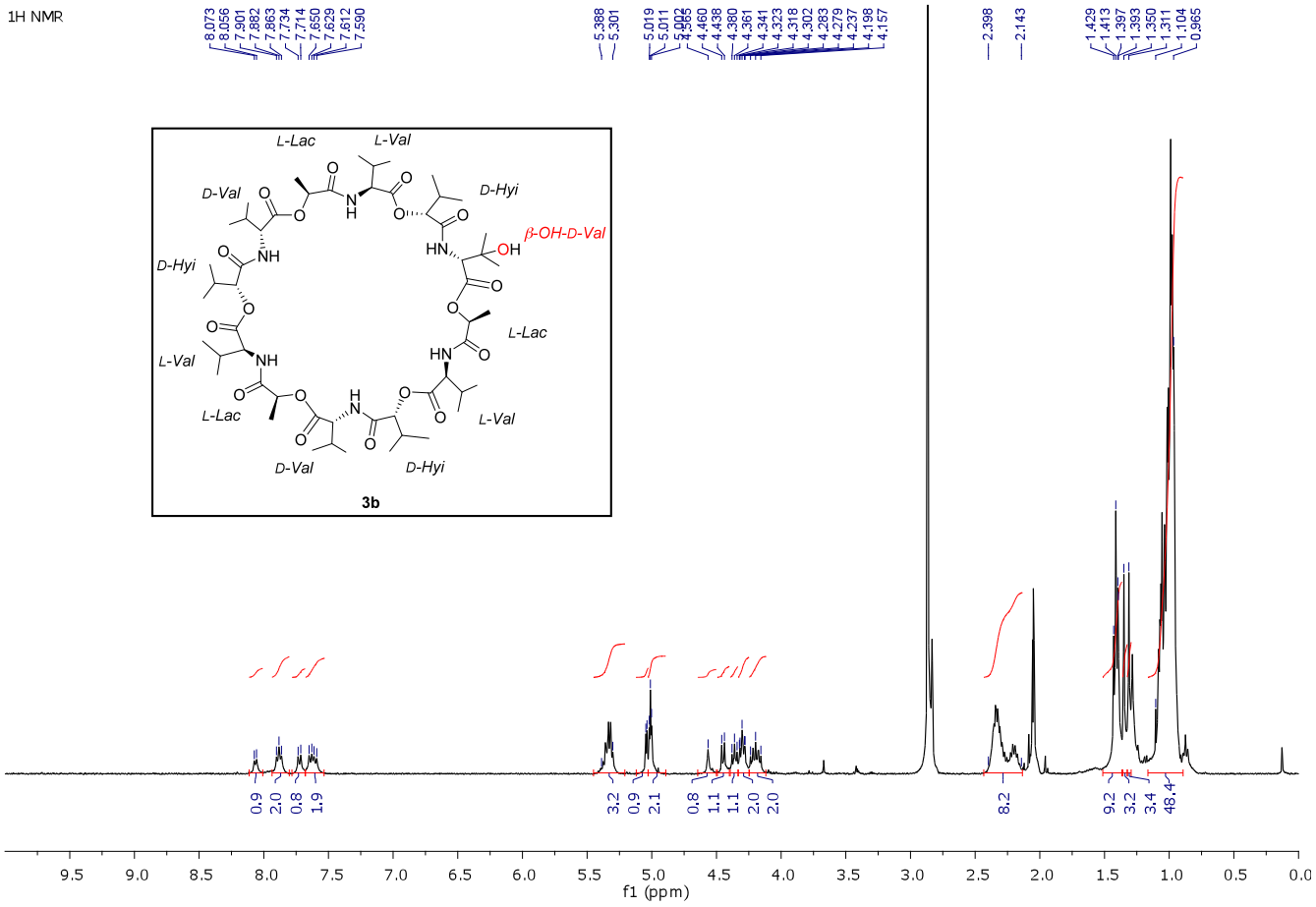

Figure *S9*. ^{1}H NMR spectrum of compound **3b** (acetone-d_6, 400 MHz).

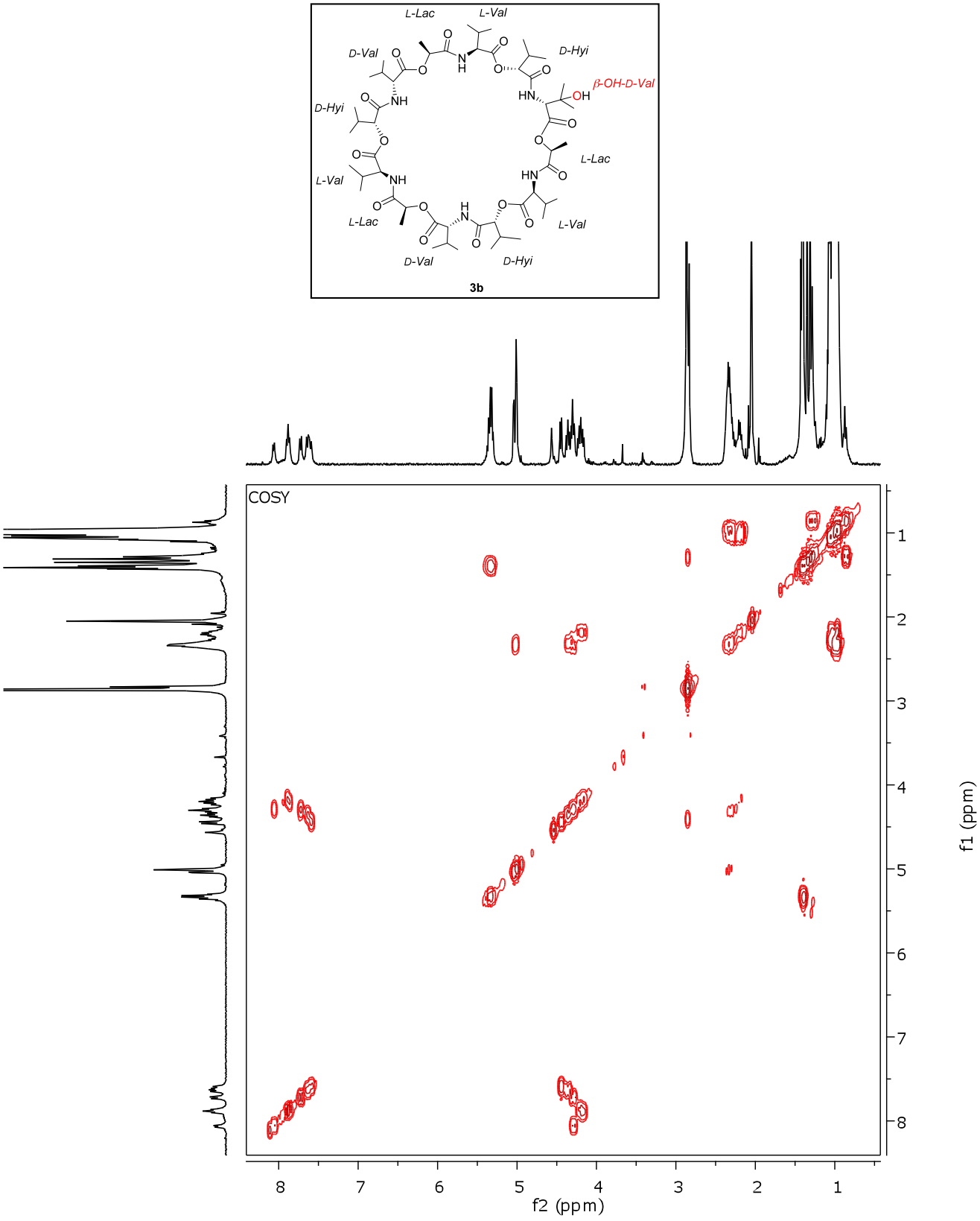

Figure *S10*. COSY NMR spectrum of compound **3b** (acetone-d_6, 400 MHz).

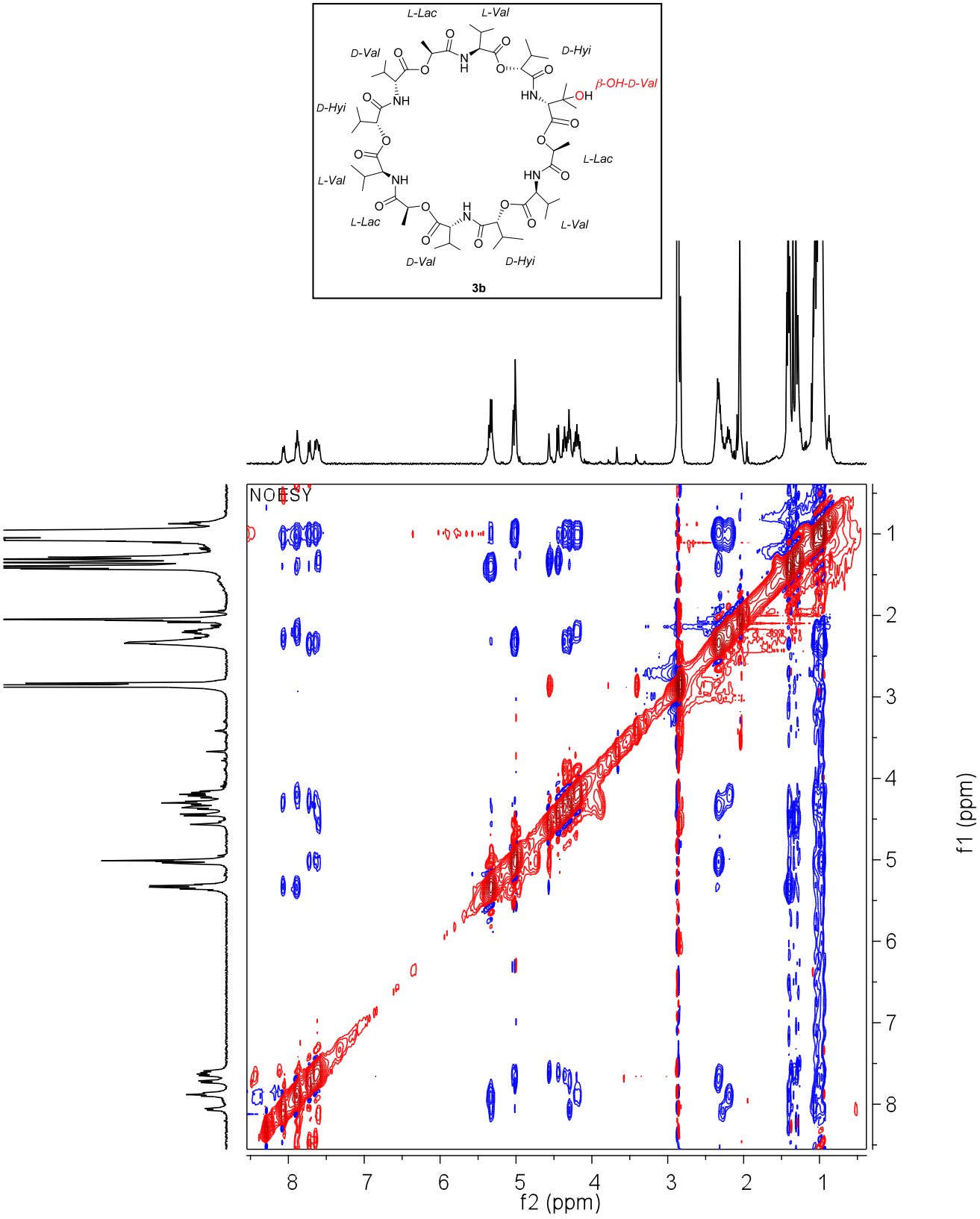

Figure *S11*. NOESY NMR spectrum of compound **3b** (acetone-d_6, 400 MHz, t_{mix} = 0.35 sec).

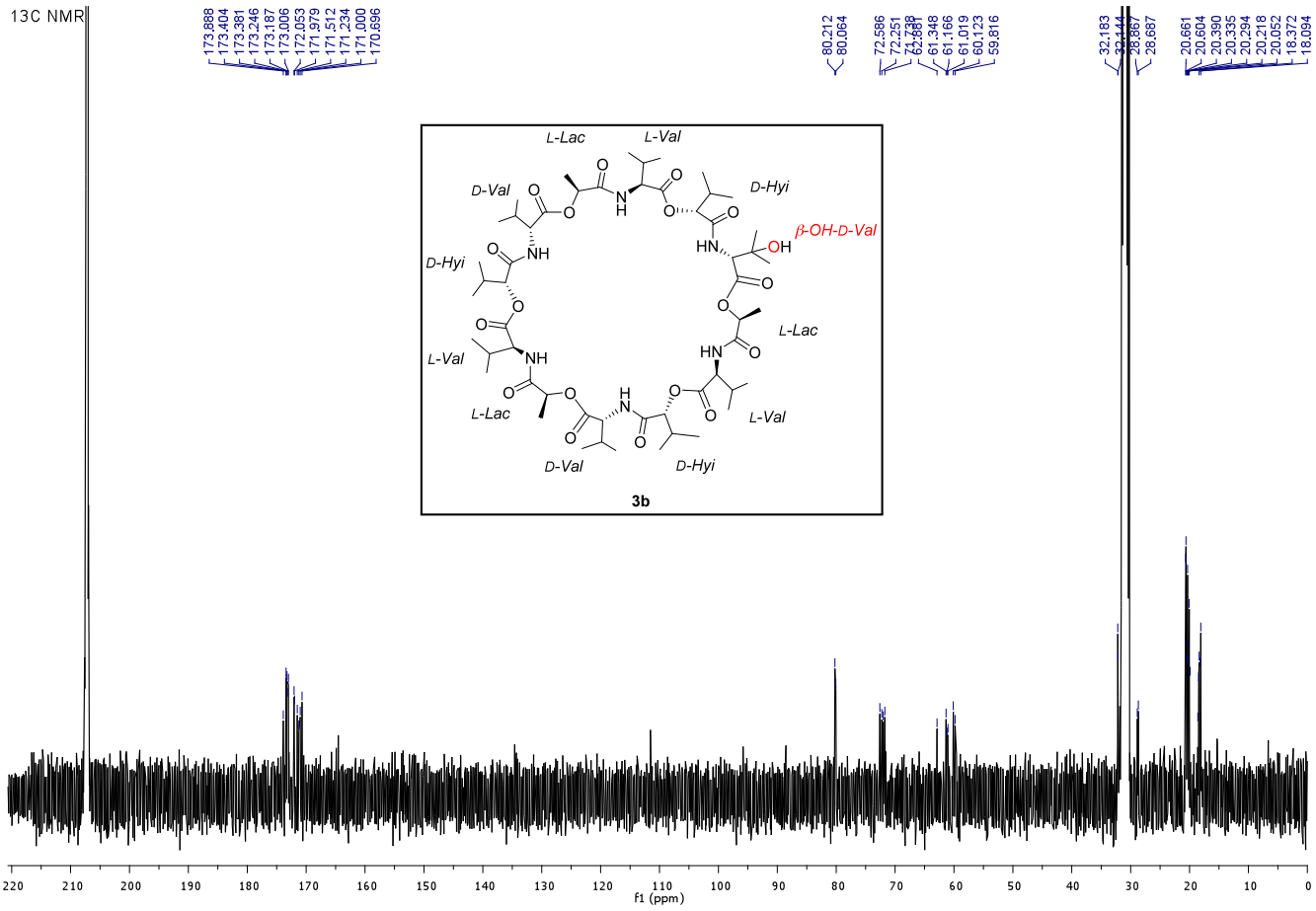

Figure *S12*. ^{13}C NMR spectrum of compound **3b** (acetone-d_6, 100 MHz).

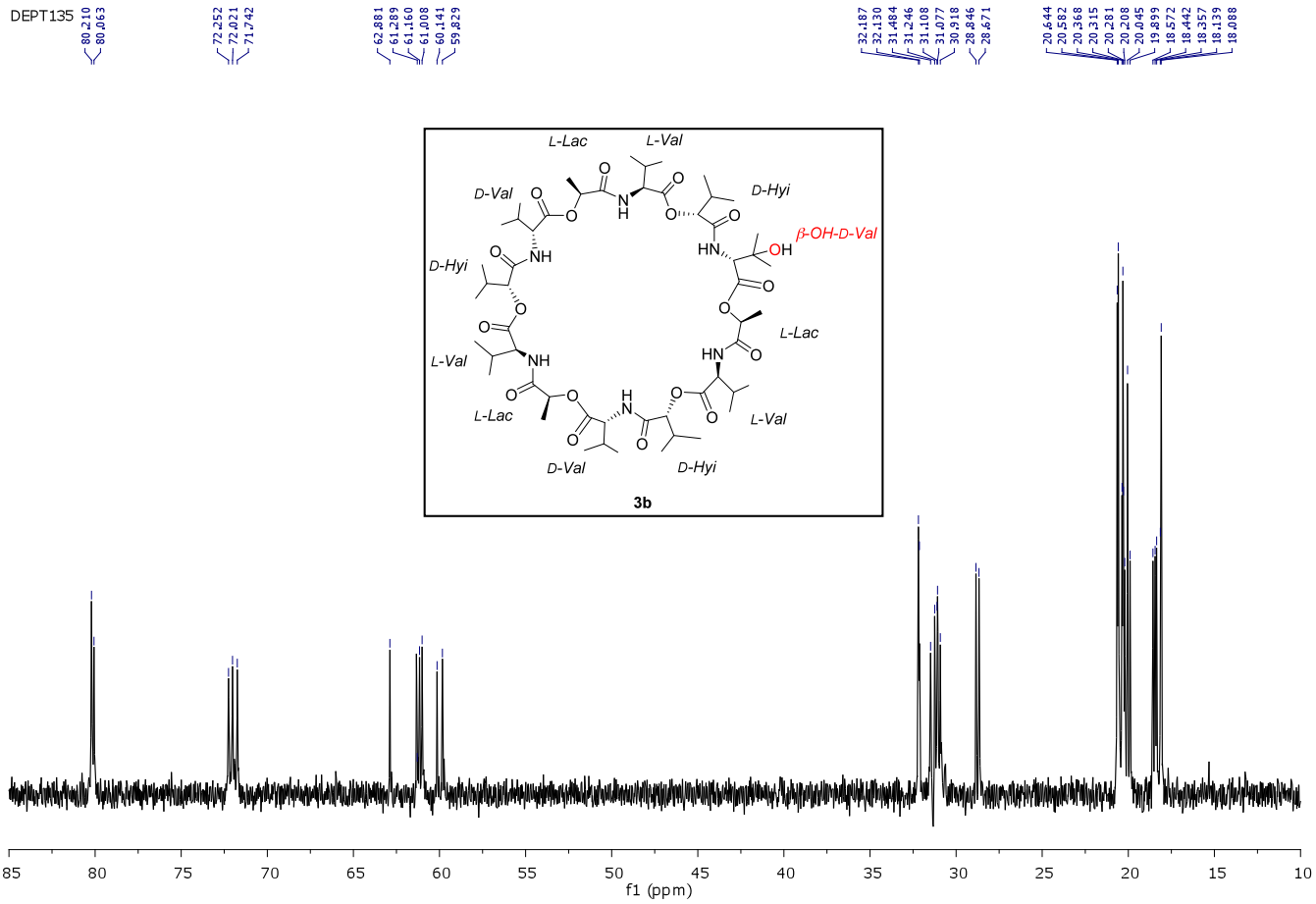

Figure *S13*. ^{13}C DEPT-135 NMR spectrum of compound **3b** (acetone-d_6, 100 MHz).

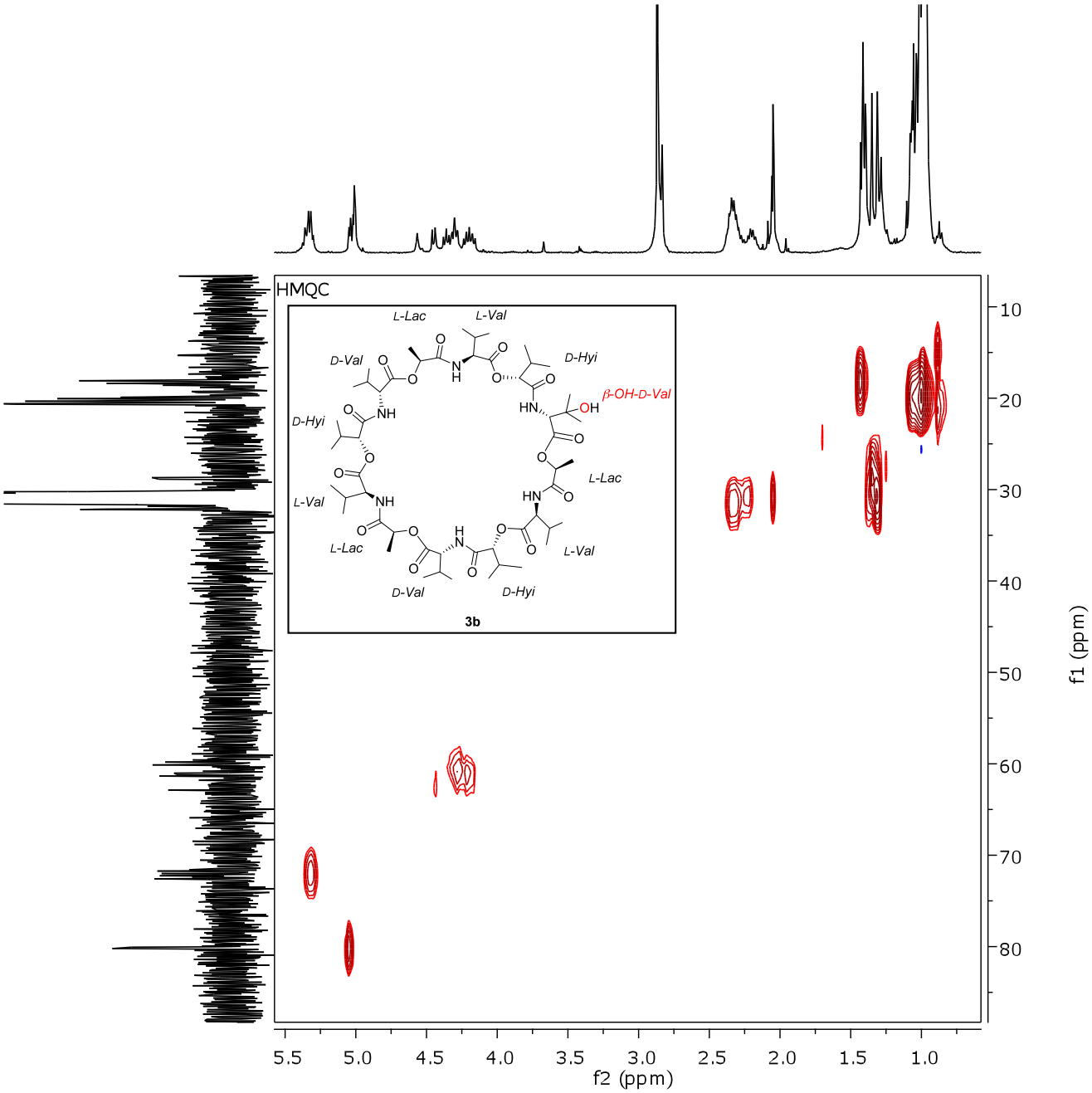

Figure *S14*. HMQC NMR spectrum of compound **3b** (acetone-d_6, 400 MHz).

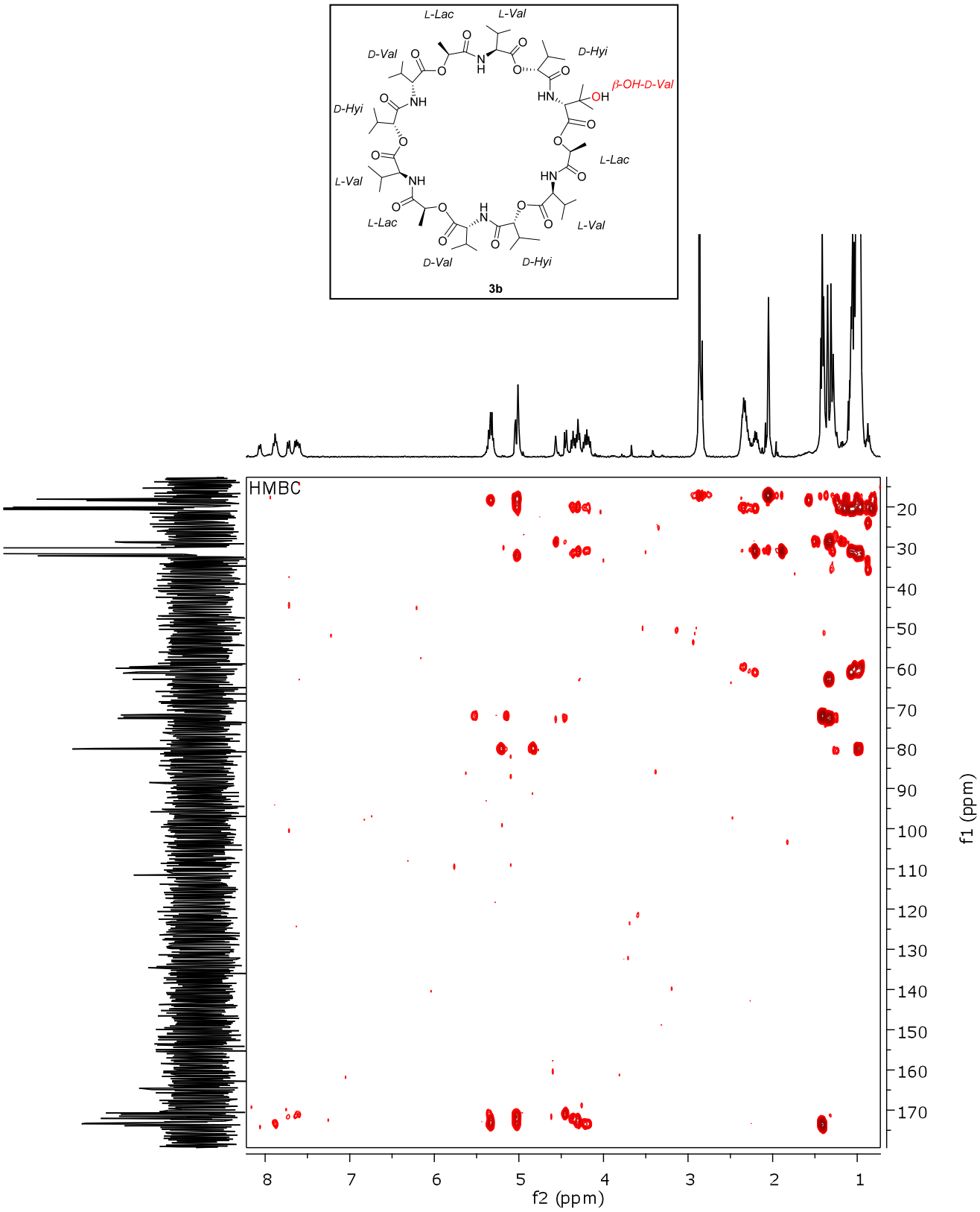

Figure *S15*. HMBC NMR spectrum of compound **3b** (acetone-d_6, 400 MHz).

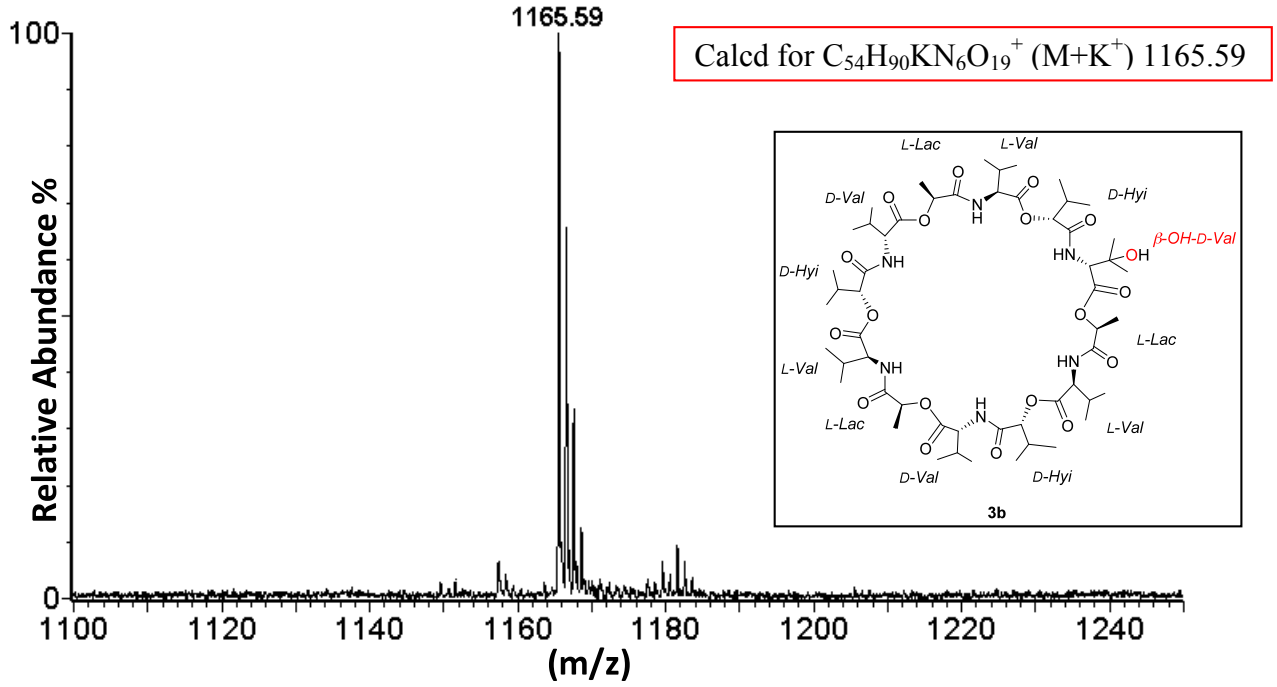

Figure *S16*. MALDI-ToF mass spectrum of compound **3b** in the presence of KCl.

5. 1D-, 2D NMR, and MALDI spectra of compound 3c.

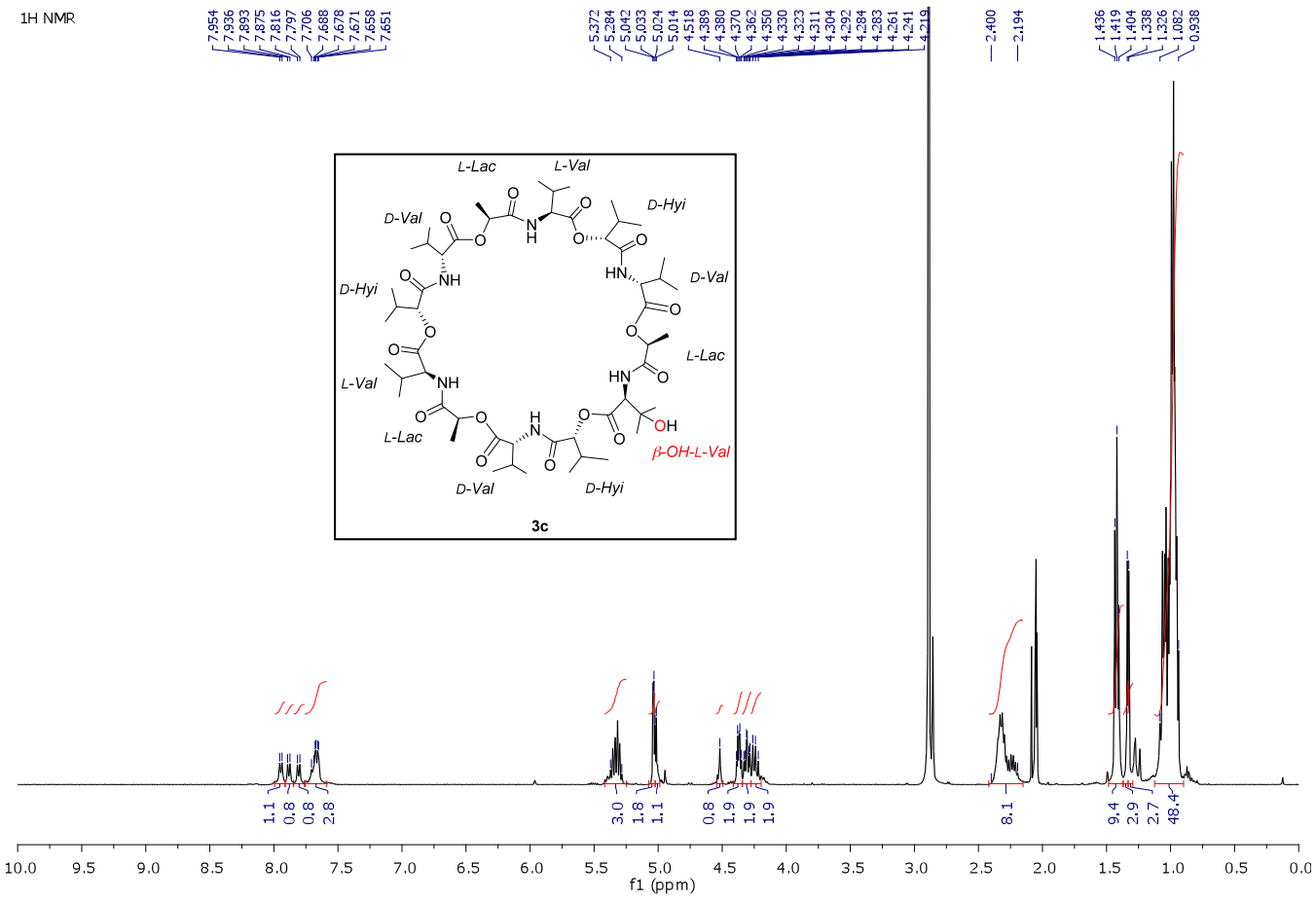

Figure *S17*. ^{1}H NMR spectrum of compound **3c** (acetone-d_6, 400 MHz).

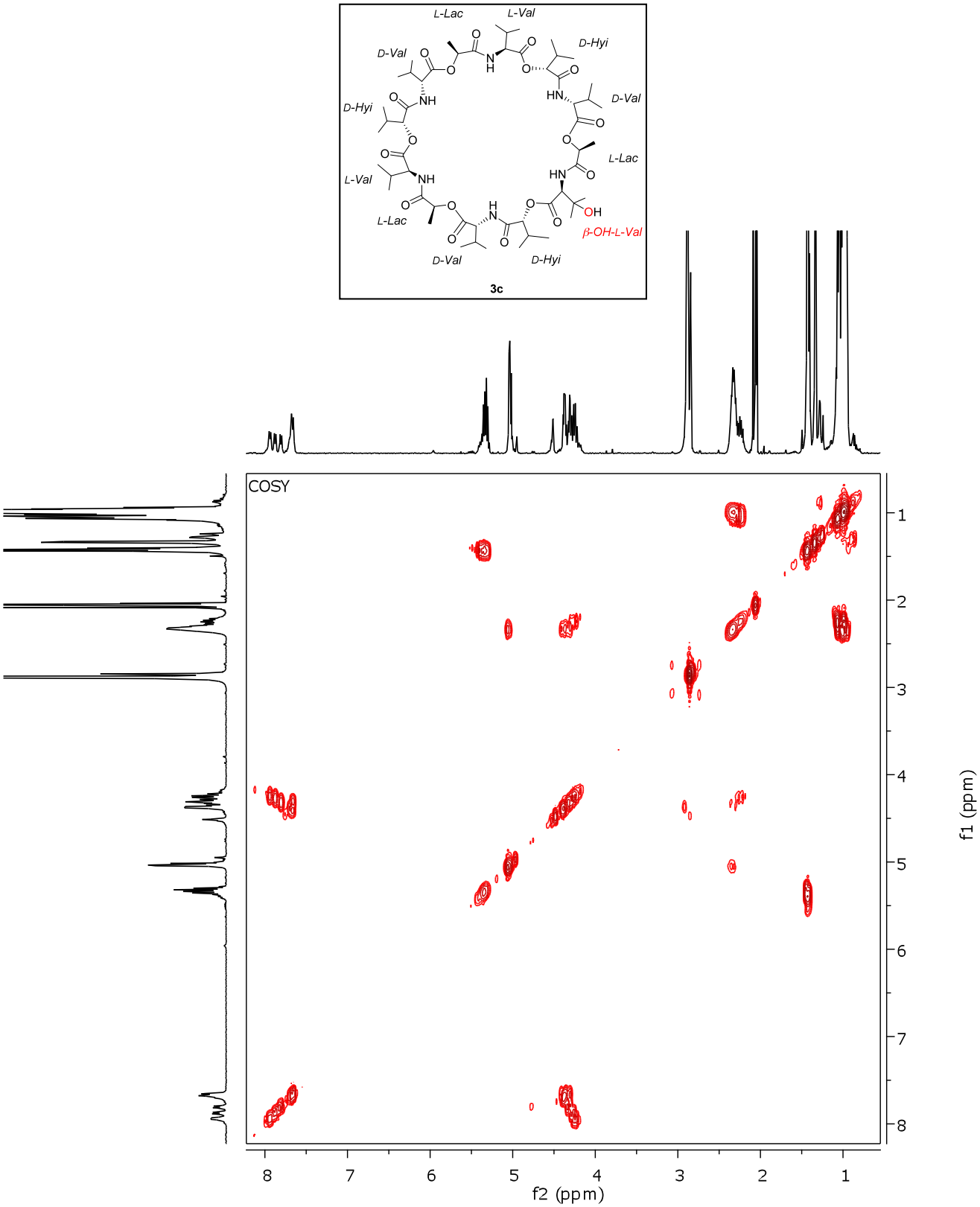

Figure *S18*. COSY NMR spectrum of compound **3c** (acetone-d_6, 400 MHz).

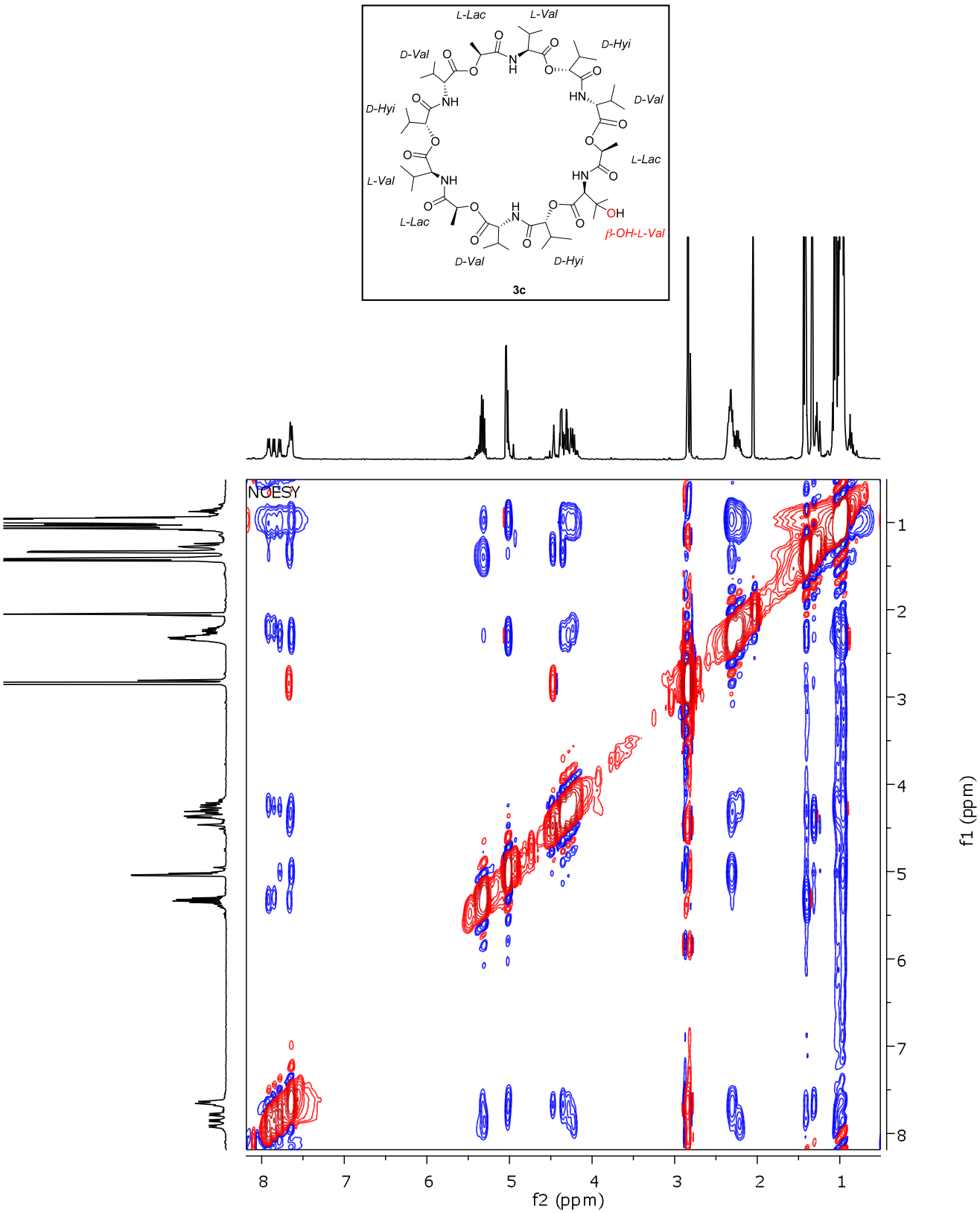

Figure *S19*. NOESY NMR spectrum of compound **3c** (acetone-d_6, 400 MHz, t_{mix} = 0.35 sec).

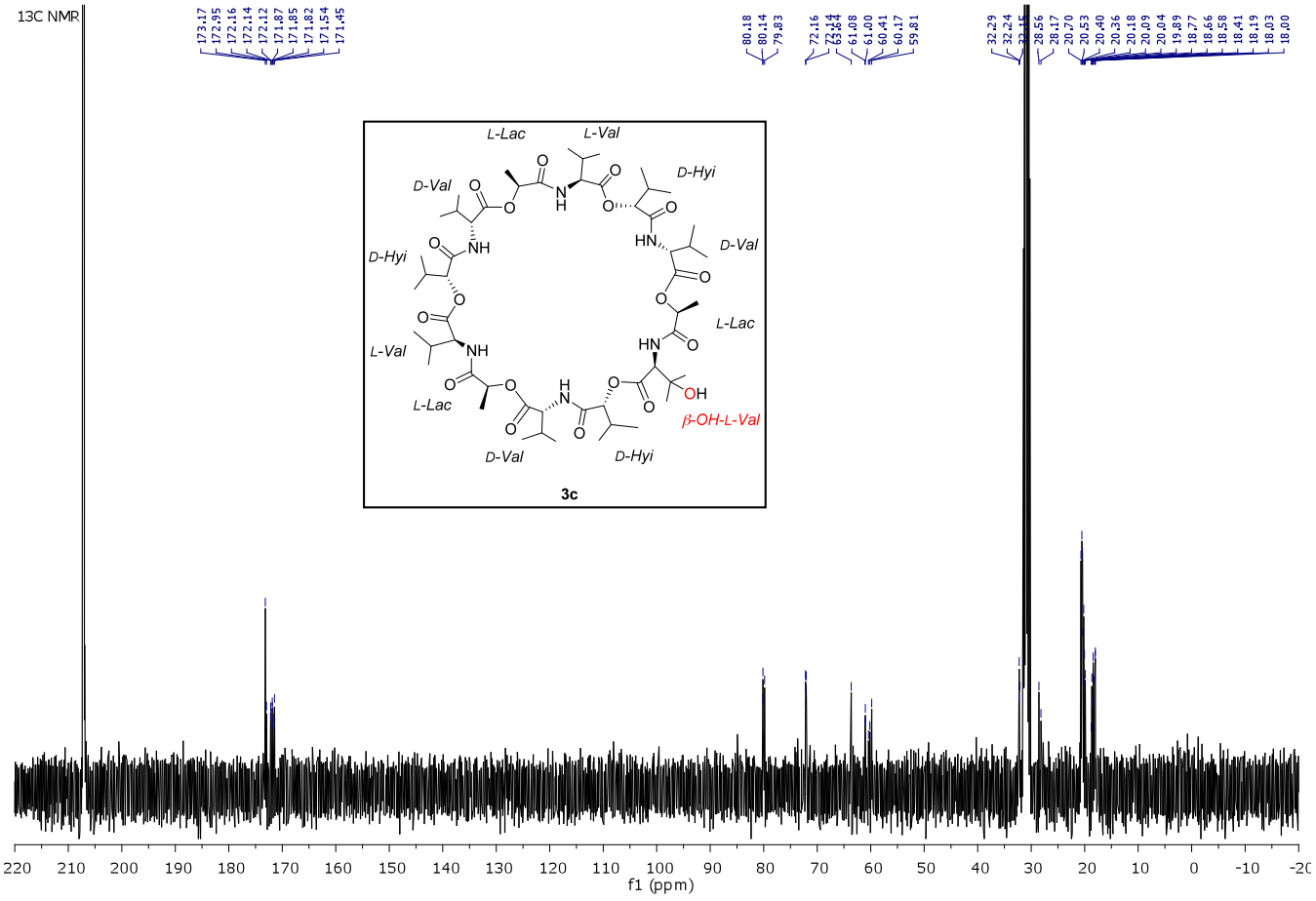

Figure *S20*. ^{13}C NMR spectrum of compound **3c** (acetone-d_6, 100 MHz).

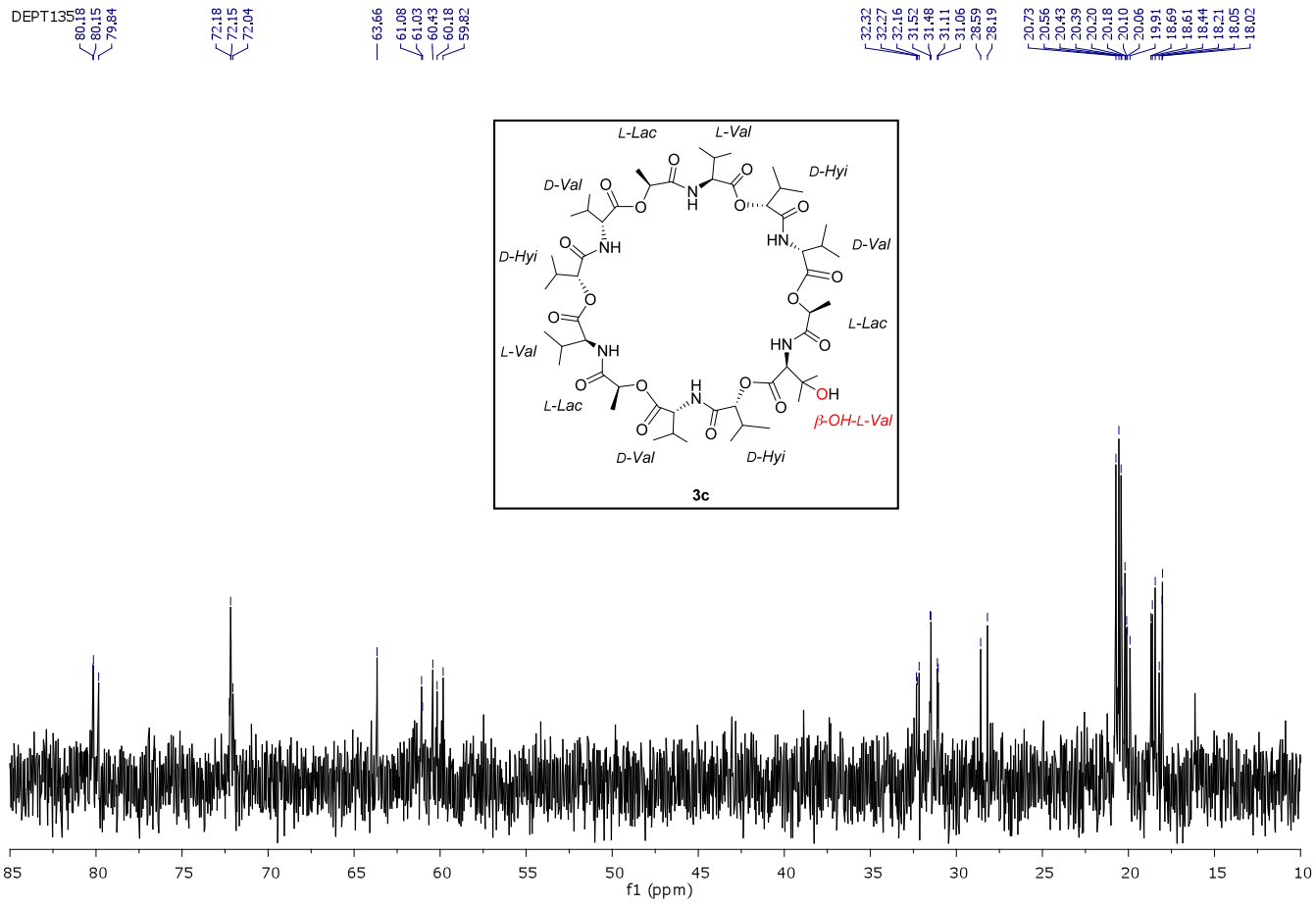

Figure *S21*. ^{13}C DEPT-135 NMR spectrum of compound **3c** (acetone-d_6, 100 MHz).

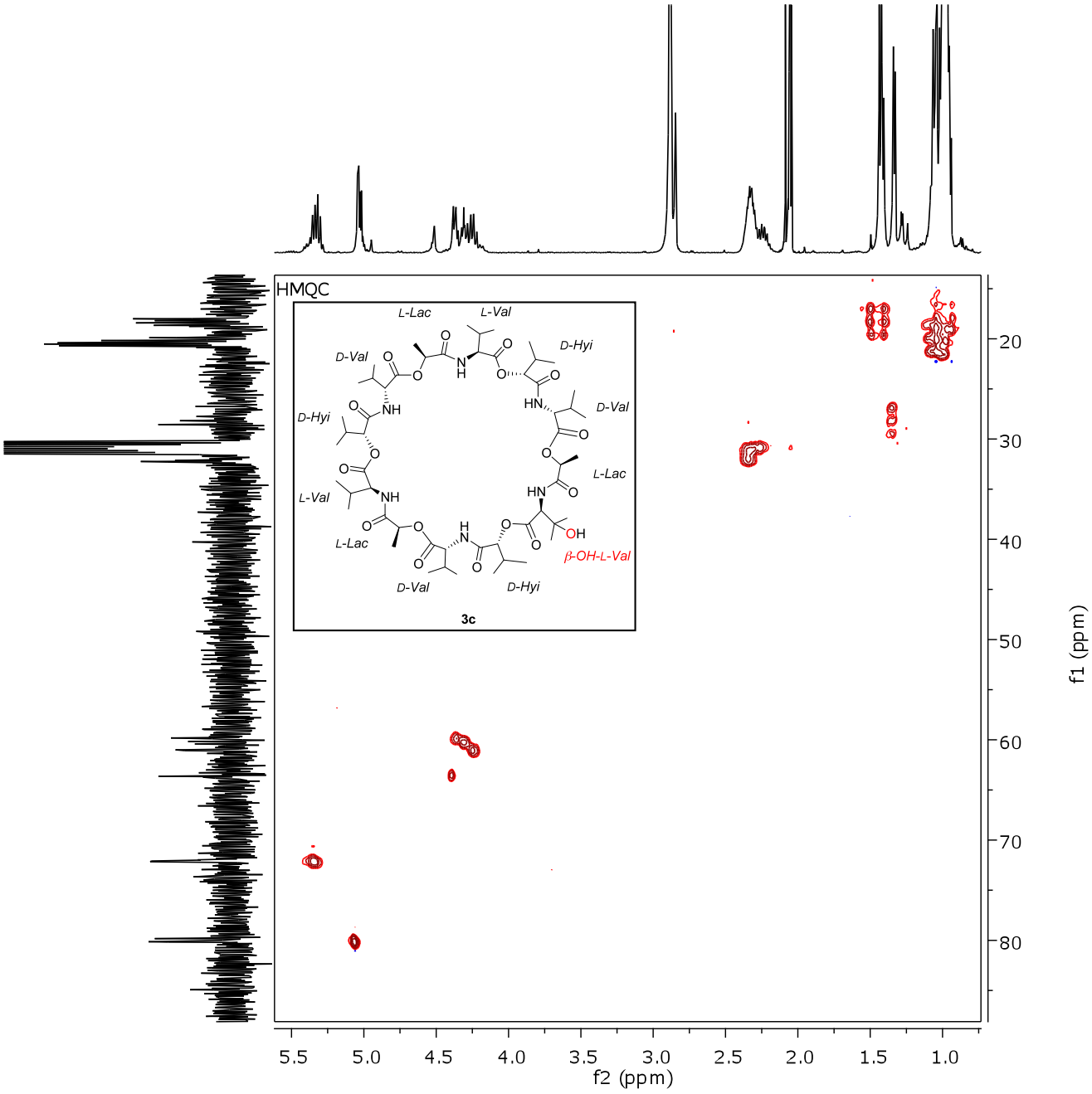

Figure *S22*. HMQC NMR spectrum of compound **3c** (acetone-d_6, 400 MHz).

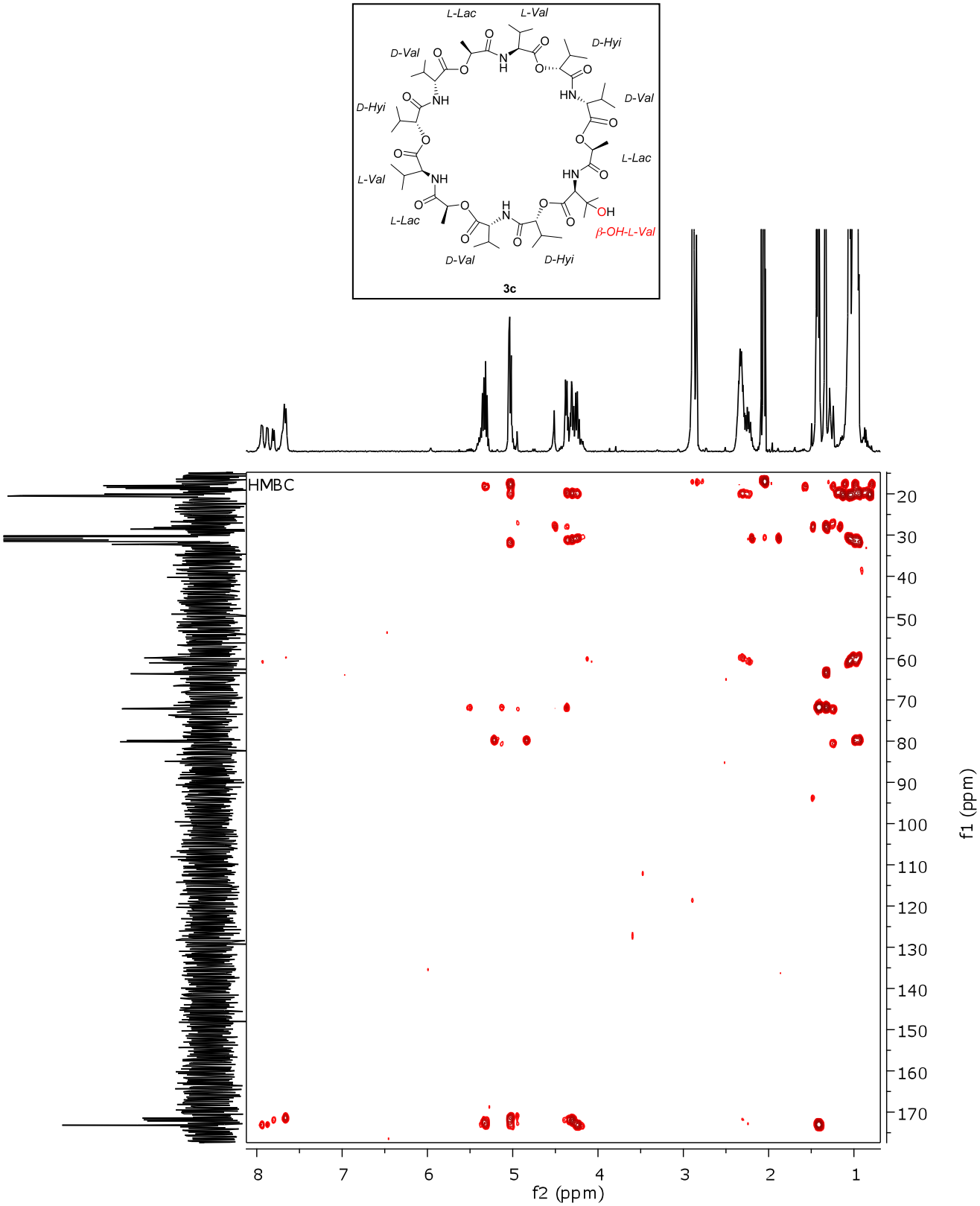

Figure *S23*. HMBC NMR spectrum of compound **3c** (acetone-d_6, 400 MHz).

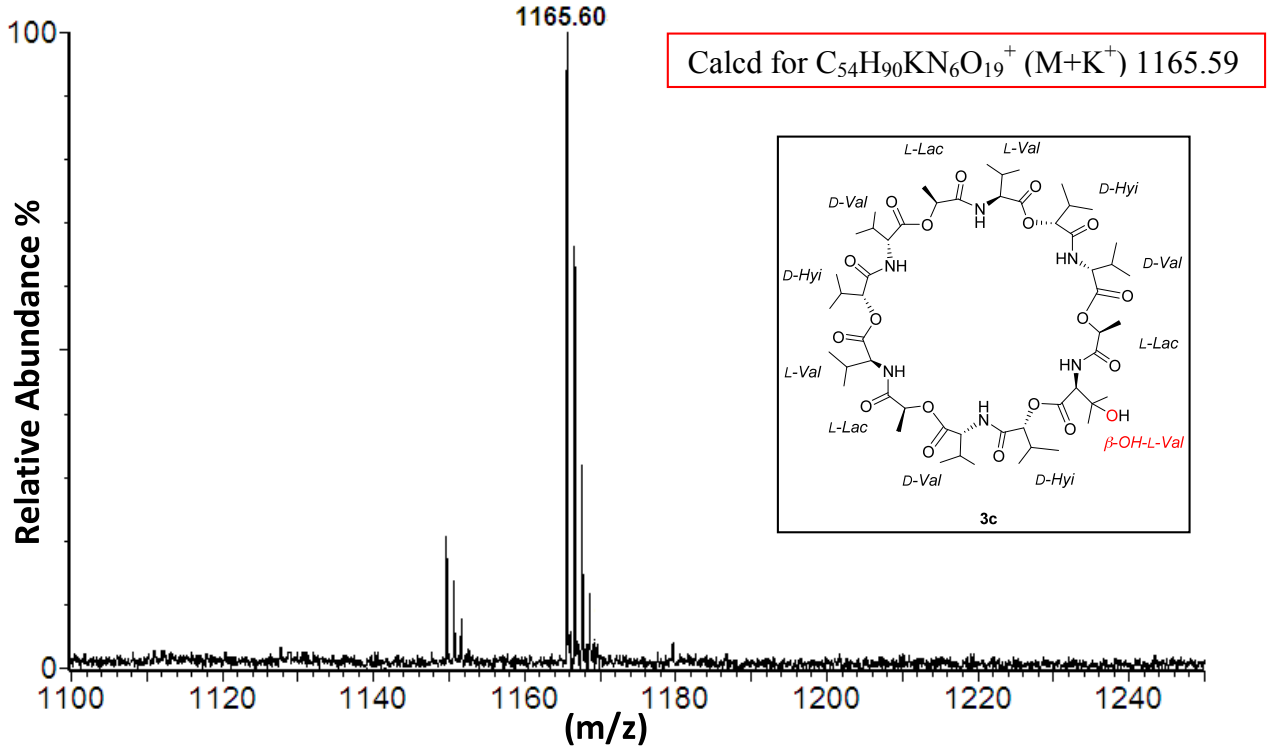

Figure *S24*. MALDI-ToF mass spectrum of compound **3c** in the presence of KCl.