Supporting information

Peripheral cyclic β-amino acids balance stability and edge-protection of β-sandwiches

Gábor Olajos,[a,c] Anasztázia Hetényi,[b] Edit Wéber,[a] Titanilla Szögi,[b] Lívia Fülöp[b] and Tamás A. Martinek[a,c]*

[a] *Institute of Pharmaceutical Analysis, SZTE-MTA Lendület Foldamer Research Group, University of Szeged, Somogyi u. 4., H-6720 Szeged, Hungary. Email: martinek@pharm.u-szeged.hu*

[b] *Department of Medical Chemistry, University of Szeged, Dóm ter 8., H-6720 Szeged, Hungary.*

[c] *MTA-SZTE Biomimetic Systems Research Group, University of Szeged, Dóm ter 8., H-6720 Szeged, Hungary.*

Table of contents

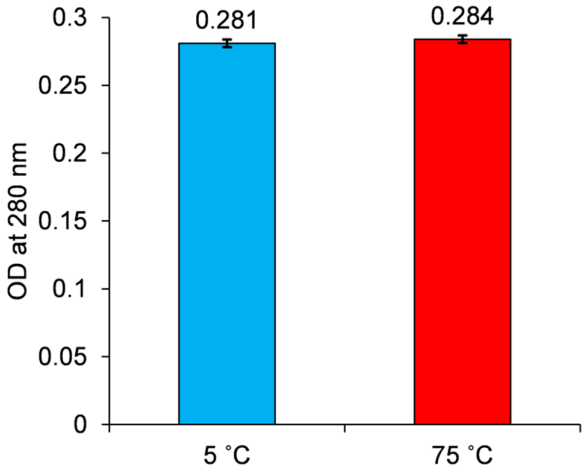


Fig. S1. Optical density at 280 nm measured for **6** at the beginning (blue) and the end (red) of the temperature-dependent CD experiment.

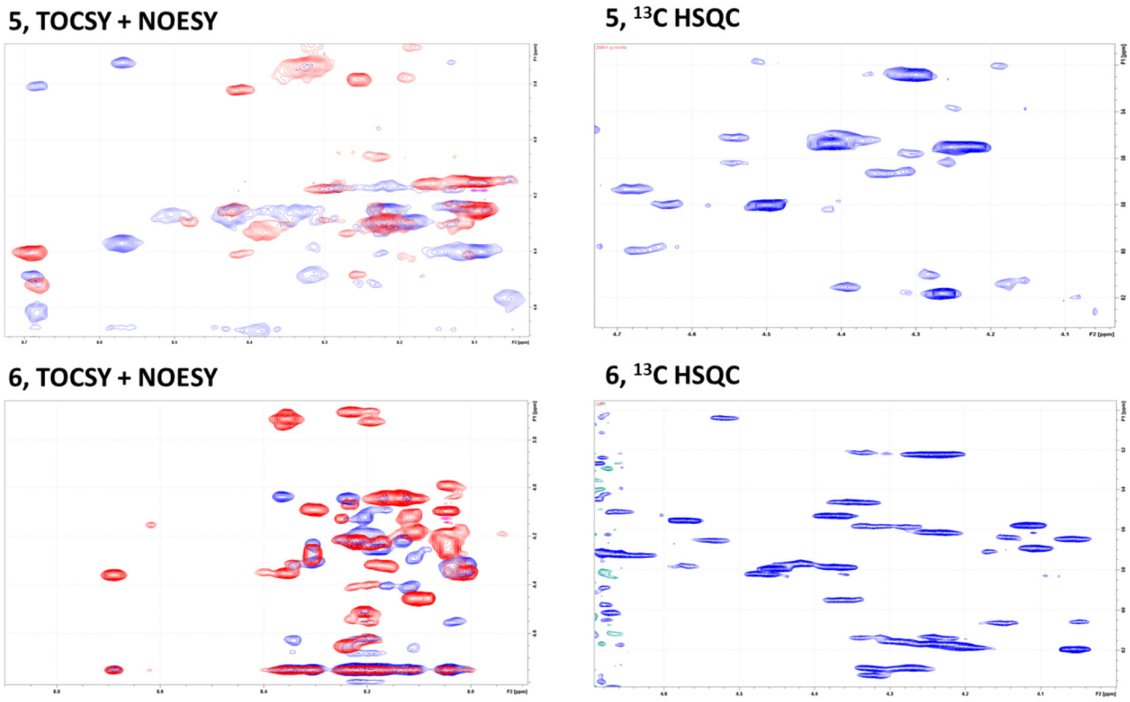


Fig. S2. 2D NMR spectra of the dimeric BB14 and BB15 (compounds **5** and **6**). NH-H_α region of overlaid TOCSY (red) and NOESY (blue) spectra (left), C_α-H_α region of ^{13}C HSQC spectrum (right).

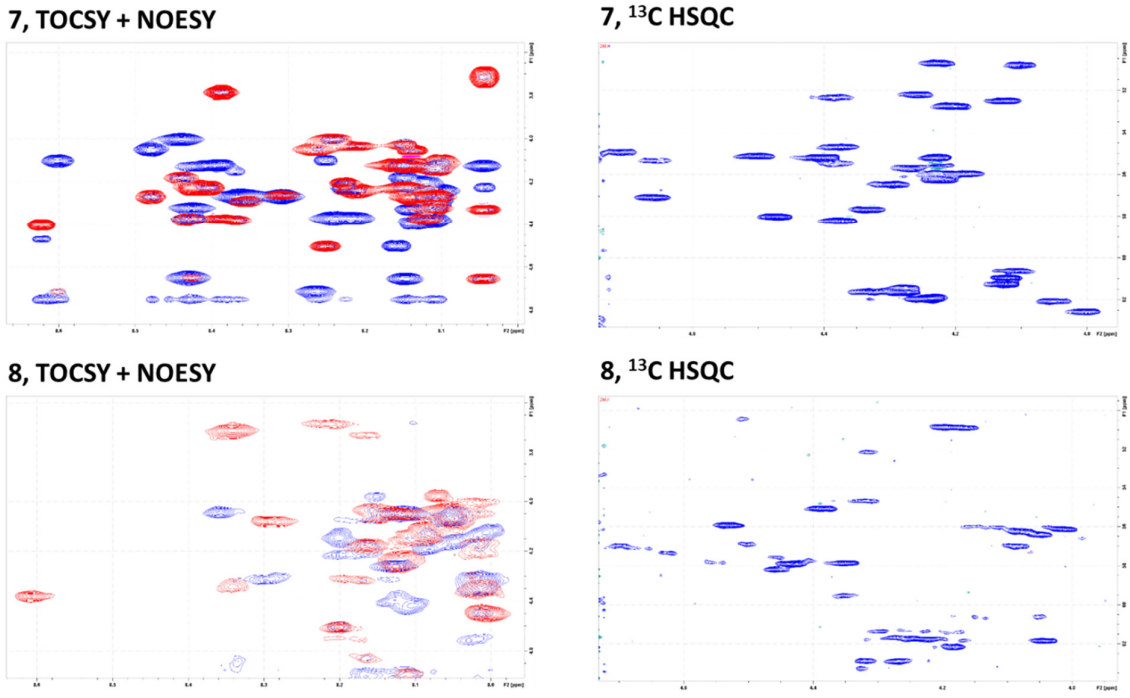


Fig. S3. 2D NMR spectra of the dimeric betabellin analogs (compounds **7** and **8**). NH-H_α region of overlaid TOCSY (red) and NOESY (blue) spectra (left), C_α-H_α region of ^{13}C HSQC spectrum (right).

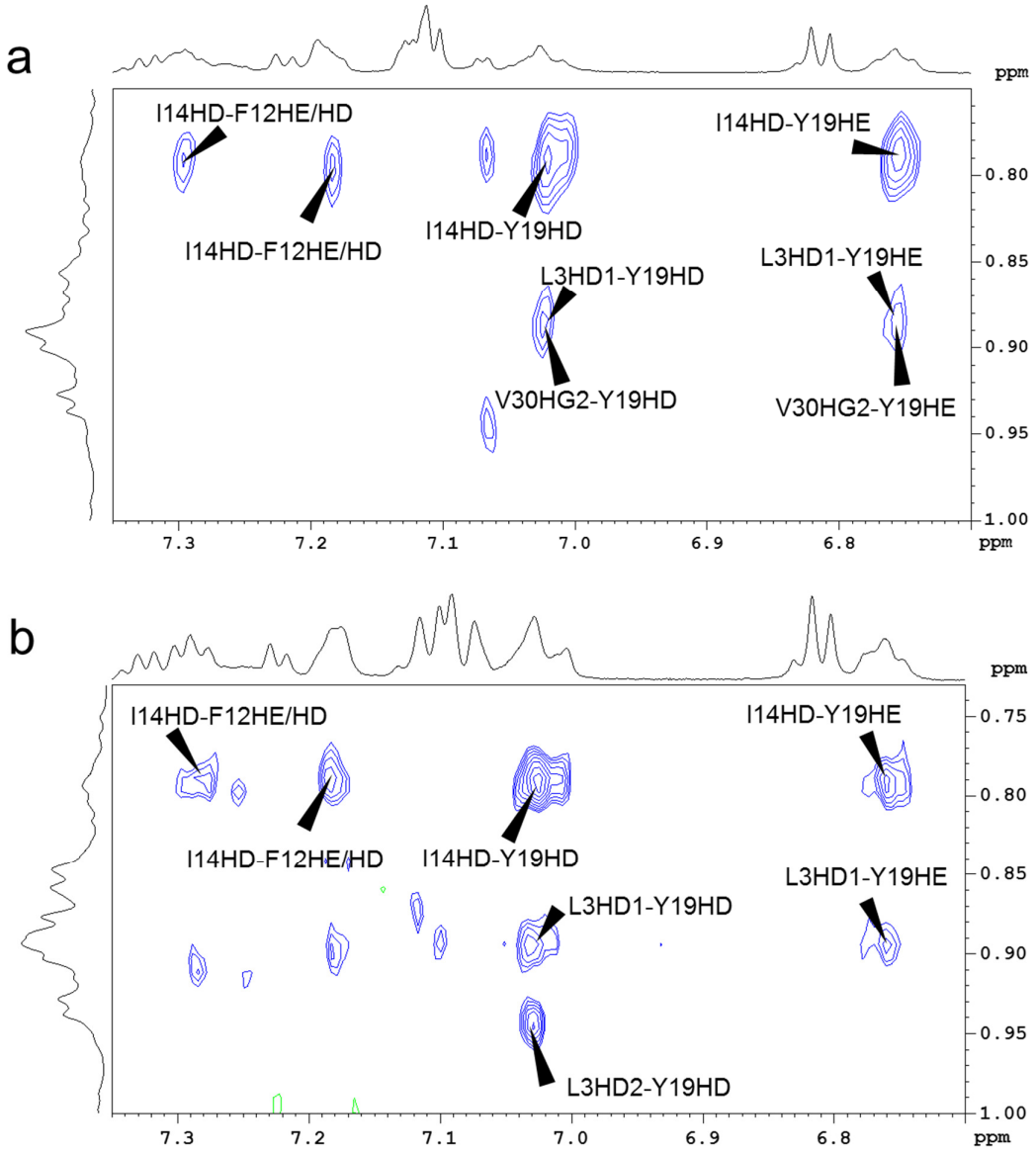


Fig. S4. Detected aliphatic and aromatic side chain contacts showing medium- and long-range interactions in the NOESY spectra of **6** and **8** (**a** and **b**, respectively).

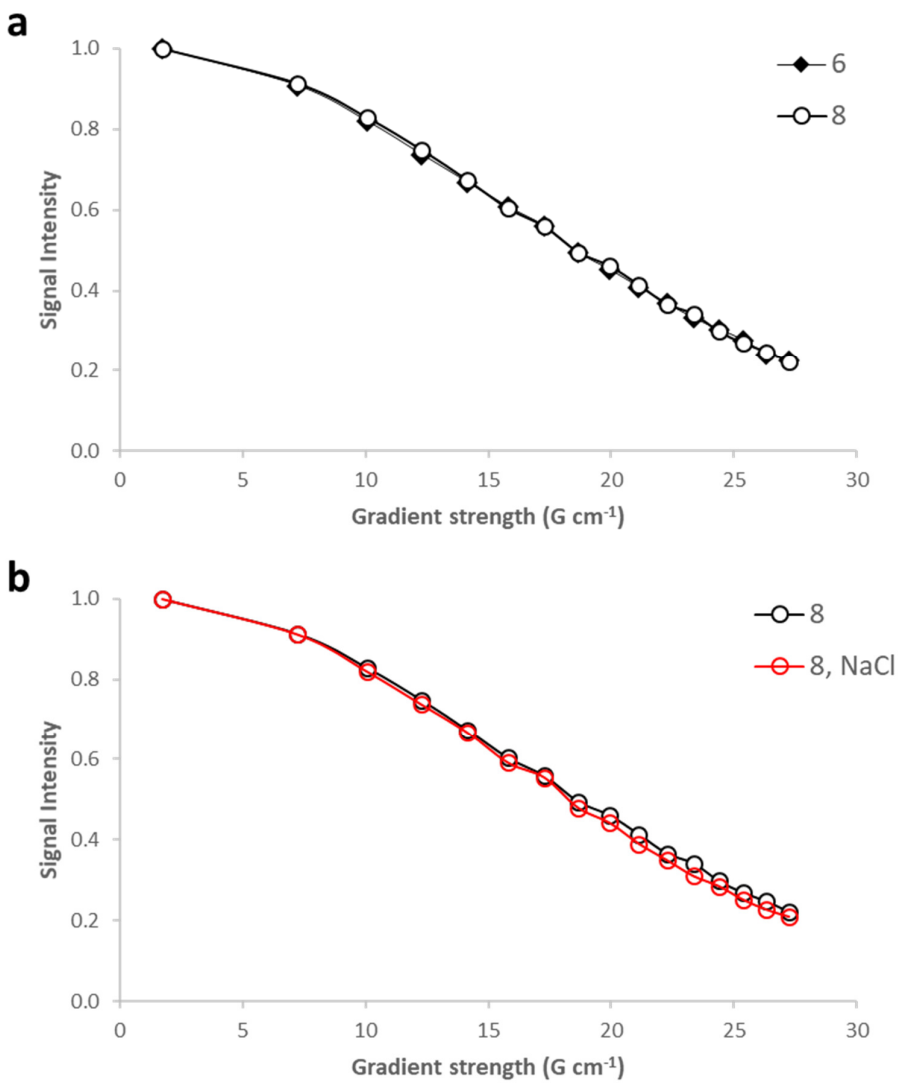


Fig. S5. Diffusion ordered spectroscopy for BB15D and its analog. The obtained signal intensity plots as a function of gradient strength compared for compounds **6** and **8** at low-salt conditions (**a**) and for compound **8** at low- and high-salt conditions (**b**). Pulsed field-gradient spin echo (PFGSE) NMR measurements were performed at 25 °C by using the stimulated echo and longitudinal eddy current delay (LED) pulse sequence with excitation sculpting for water suppression. A time of 3 ms was used for the dephasing/refocusing gradient pulse length, and 300 ms for the diffusion delay. The gradient strength was changed quadratically (from 5% to 80% of the maximum value); the number of steps was 16. Each measurement was run with 32 scans and 8K time domain points. DOSY spectra were processed and evaluated by using the exponential fit implemented in Topspin 3.5.

Table S1. Assigned ^{1}HN, $^{1}H_{\alpha}$, $^{13}C_{\alpha}$, and $^{13}C_{\beta}$ chemical shifts (ppm) for the dimeric peptides. ACHC replacements are highlighted with grey.

	Sequence (BB14 and its analog)	**5**	**7**	**Sequence (BB15 and its analog)**	**6**	**8**
NH	H1	a	a	H1	a	a
H_{α}		a	4.32		a	4.10
C_{α}		a	55.26		a	56.19
C_{β}		a	30.33		31.65	32.35
NH	S2	8.38	a	S2	a	a
H_{α}		a	4.57		4.53	4.53
C_{α}		a	58.06		58.00	57.98
C_{β}		64.69	63.78		63.73	63.77
NH	L3	8.75	8.72	L3	8.79	8.71
H_{α}		4.46	4.50		4.46	4.48
C_{α}		55.11	55.25		55.36	55.14
C_{β}		42.36	42.58		42.32	42.43
NH	T4	8.15	8.24	T4	8.14	8.22
H_{α}		4.31	4.37		4.36	4.34
C_{α}		61.83	61.69		61.65	61.78
C_{β}		69.69	69.91		69.79	69.7
NH	A5	8.30	7.92	A5	8.28	7.82
H_{α}		4.38	2.77		4.33	2.71
C_{α}		52.48	a		52.27	47.54
C_{β}		19.56	50.77		19.41	50.92
NH	S6	8.31	8.14	K6	8.40	8.18
H_{α}		4.55	4.43		4.38	4.21
C_{α}		58.03	57.72		55.89	56.08
C_{β}		63.78	63.86		32.89	33.06
NH	I7	8.21	8.23	I7	8.20	8.11
H_{α}		4.22	4.22		4.55	4.55
C_{α}		aa	61.30		58.26	58.24
C_{β}		38.97	38.93		39.12	39.13
NH	k8	8.48	8.50	p8	a	a
H_{α}		4.32	4.32		4.41	4.41
C_{α}		56.2	56.27		62.96	62.92
C_{β}		32.4	32.91		32.26	32.27
NH	a9	8.29	8.32	k9	8.40	8.40
H_{α}		4.35	4.30		4.19	4.18
C_{α}		52.42	52.81		56.99	57.05
C_{β}		19.45	19.36		33.09	33.02
NH	L10	8.24	8.22	L10	8.27	8.26
H_{α}		4.48	4.47		4.42	4.41
C_{α}		55.39	54.76		54.69	54.74
C_{β}		42.54	42.63		42.33	42.38
NH	T11	8.38	8.31	T11	8.14	a
H_{α}		3.87	4.34		4.31	4.26
C_{α}		64.03	61.99		61.72	61.89

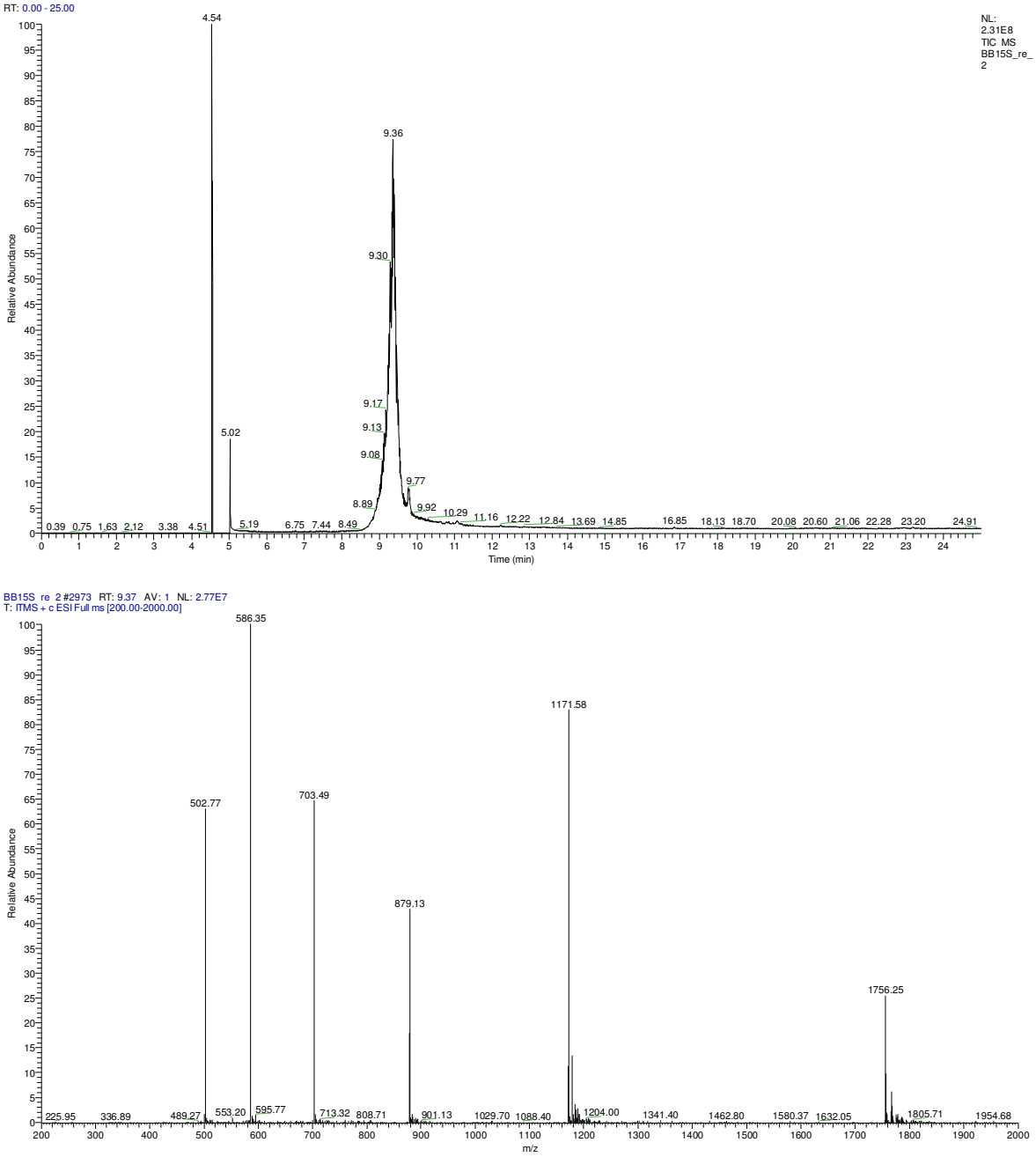
C_β		72.32	69.77		69.95	69.88
NH	I12	8.34	8.20	F12	8.30	8.26
H_α		4.35	4.20		4.72	4.72
C_α		a	60.67		57.33	57.42
C_β		39.76	38.97		40.14	a
NH	H13	a	8.70	S13	8.32	8.31
H_α		a	4.81		4.50	4.51
C_α		a	55.01		57.73	57.82
C_β		30.83	29.51		64.01	64.02
NH	V14	8.35	8.36	I13	8.21	8.196
H_α		4.23	4.15		4.23	4.234
C_α		61.35	62.12		60.69	a
C_β		a	32.98		39.05	39.08
NH	Q15	8.54	8.58	A15	8.30	8.3
H_α		4.36	4.37		4.61	4.6
C_α		55.82	55.78		50.45	50.5
C_β		30.74	29.82		18.33	18.33
NH	a16	8.29	8.41	p16	a	a
H_α		4.36	4.36		4.41	4.41
C_α		52.39	52.27		63.30	63.31
C_β		19.28	19.48		31.91	a
NH	k17	8.40	8.45	h17	8.31	a
H_α		4.36	4.39		4.63	4.59
C_α		55.74	56.52		56.60	56.98
C_β		33.03	33.24		a	a
NH	T18	8.15	8.20	T18	8.11	a
H_α		4.32	4.38		4.30	4.30
C_α		61.84	61.69		61.72	61.8
C_β		69.68	69.81		69.66	69.96
NH	A19	8.42	8.46	Y19	8.30	a
H_α		4.45	4.48		4.65	4.63
C_α		a	52.41		57.82	57.9
C_β		21.41	19.38		a	a
NH	T20	8.17	8.20	T20	8.13	a
H_α		4.35	4.41		4.31	4.32
C_α		61.06	61.50		61.40	61.82
C_β		69.68	70.03		70.07	69.89
NH	C21	6.37	8.52	C21	8.31	a
H_α		4.94	4.75		4.75	a
C_α		54.81	55.39		55.16	a
C_β		a	a		a	a
NH	Q22	8.46	8.53	A22	8.44	8.44
H_α		4.39	4.48		4.42	4.41
C_α		56.68	55.53		52.16	52.18
C_β		31.32	30.03		19.51	19.53
NH	V23	8.22	8.34	V23	8.11	8.11
H_α		4.35	4.10		4.45	4.44

C_α		61.05	62.63		59.55	59.56
C_β		a	32.88		32.99	33.02
NH	k24	8.56	8.54	p24	a	a
H_α		4.26	4.28		4.35	4.36
C_α		55.54	56.02		62.92	62.95
C_β		32.44	32.88		32.15	32.12
NH	a25	8.26	8.25	k25	8.27	8.23
H_α		4.36	4.22		4.15	4.14
C_α		52.4	52.57		56.52	56.46
C_β		19.08	19.32		32.90	32.94
NH	Y26	7.99	8.14	Y26	8.34	8.21
H_α		4.73	4.75		4.75	4.79
C_α		60.01	57.15		57.27	57.04
C_β		41.94	39.13		38.74	a
NH	T27	8.24	8.24	T27	8.21	8.26
H_α		3.84	4.33		4.29	4.27
C_α		64.11	61.97		61.87	62.22
C_β		72.39	70.06		69.98	69.89
NH	V28	8.16	7.85	A28	8.31	8.15
H_α		4.45	2.72		4.32	4.17
C_α		61.55	a		52.27	56.31
C_β		a	50.85		19.41	33.05
NH	H29	8.68	8.35	K29	8.32	8.151
H_α		4.59	4.60		4.32	4.135
C_α		55.13	55.18		56.20	61.9
C_β		29.41	29.08		33.04	33.06
NH	I30	8.28	8.26	V30	8.23	8.15
H_α		4.12	4.22		4.14	4.14
C_α		62.63	61.02		61.99	61.90
C_β		39.02	39.02		32.98	33.06
NH	S31	8.47	8.49	S31	8.46	8.44
H_α		4.47	4.47		4.45	4.44
C_α		57.85	58.27		57.93	57.94
C_β		63.77	63.69		63.78	63.76
NH	E32	8.45	8.52	H32	a	a
H_α		4.30	4.32		4.66	4.62
C_α		55.49	56.00		55.60	55.99
C_β		31.35	30.41		30.28	31.01

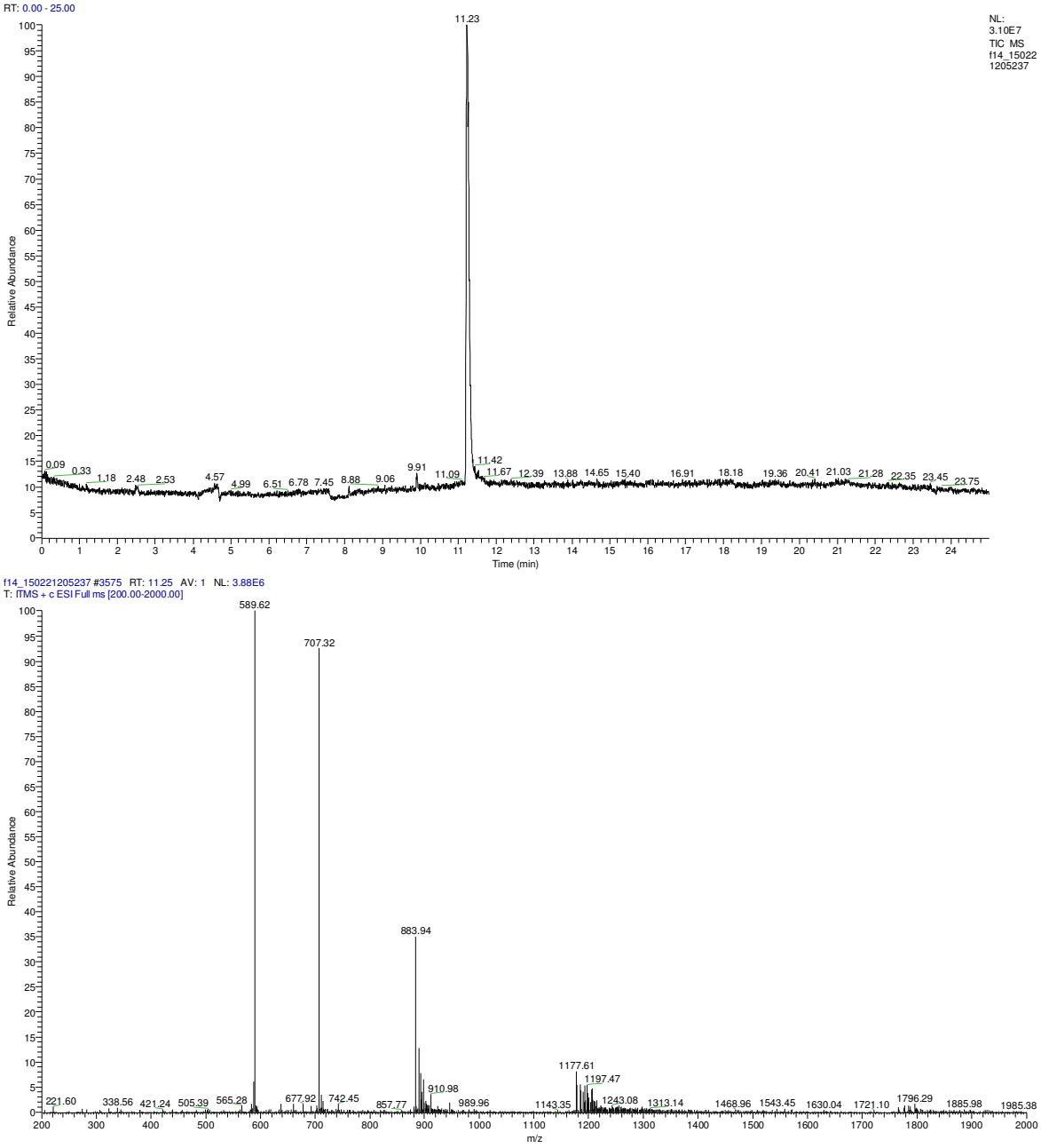
[a] Missing resonance/the resonance could not be assigned due to chemical exchange.

Peptide Characterization

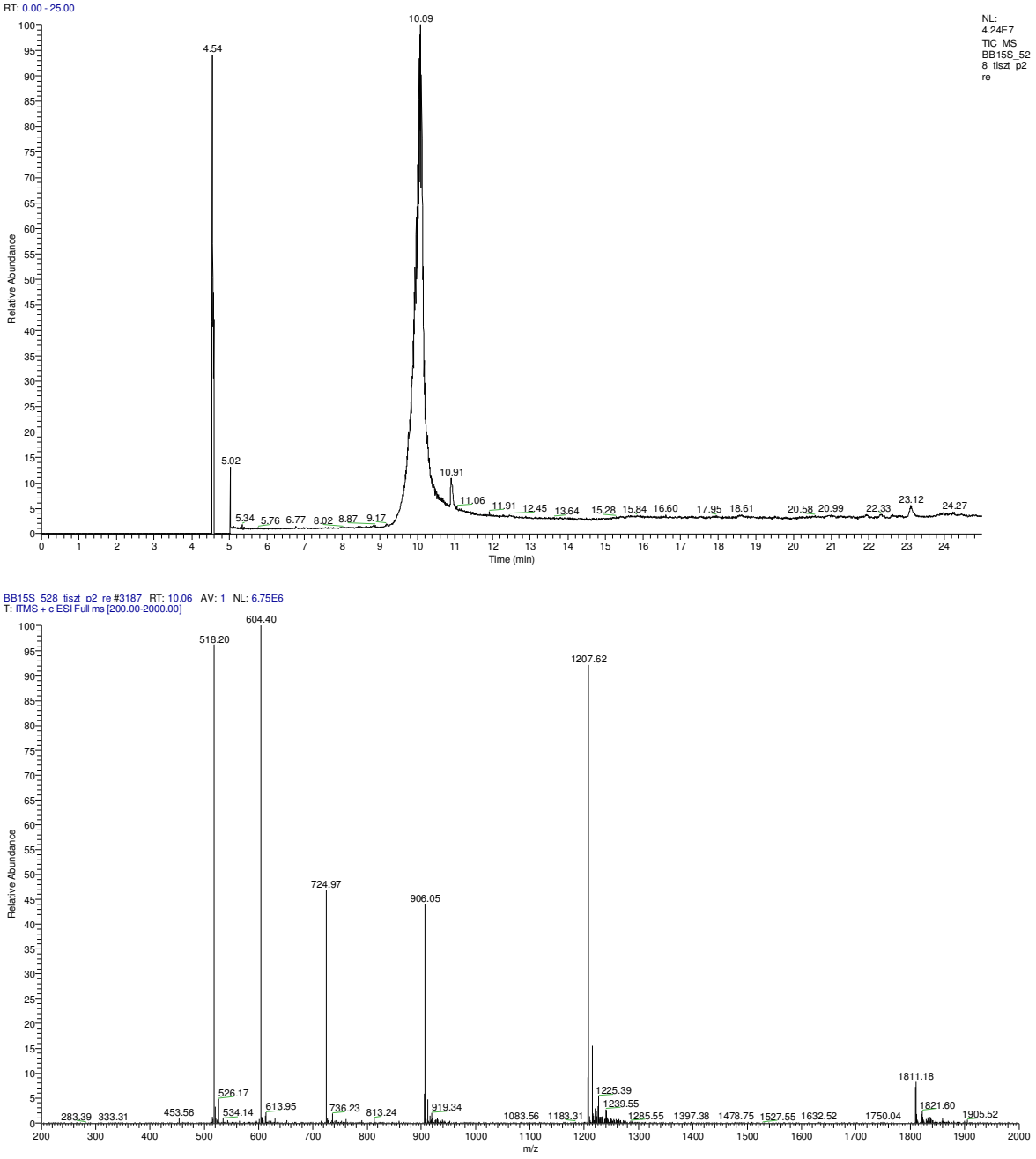
Lyophilized peptides were analyzed by ESI MS and by analytical HPLC measurements on an Aeris Peptide C18 2.5*25 mm column (Phenomenex) with a gradient from 5% A to 80% B over 25 min at a flow rate of 1.2 ml*min^{-1}. Eluents were: A: 0.1% TFA/water; B: 0.1% TFA/20% water/80% ACN. Peptide purity was >95% according to analytical HPLC measurements.



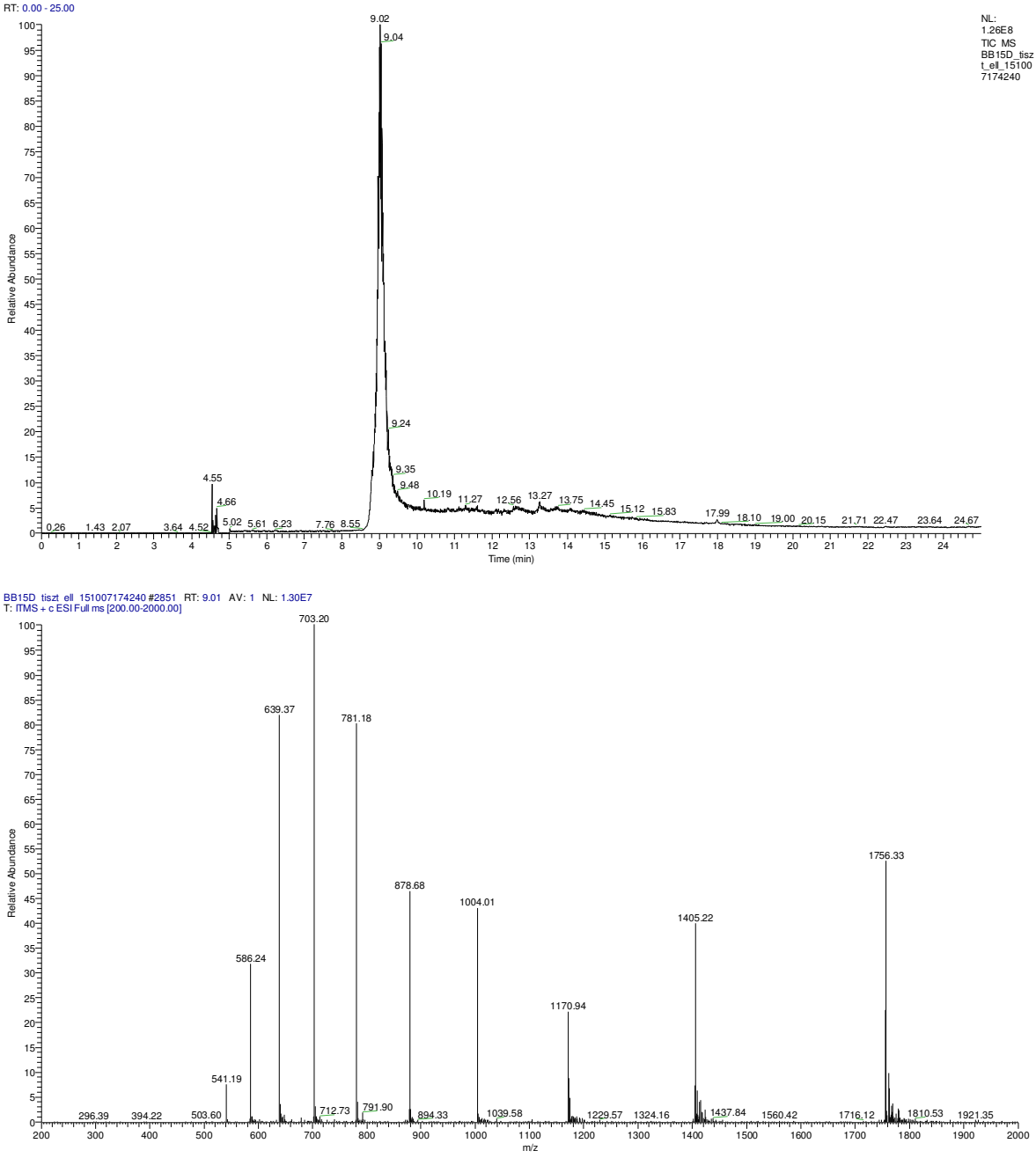
Analytical HPLC chromatogram and ESI-MS spectrum of **2** (monomeric BB15); [MH_2^{2+}]: 1756.25; [MH_3^{3+}]:1171.58 [MH_4^{4+}]: 879.13; [MH_5^{5+}]: 703.49; [MH_6^{6+}]: 586.35; calculated MW: 3511



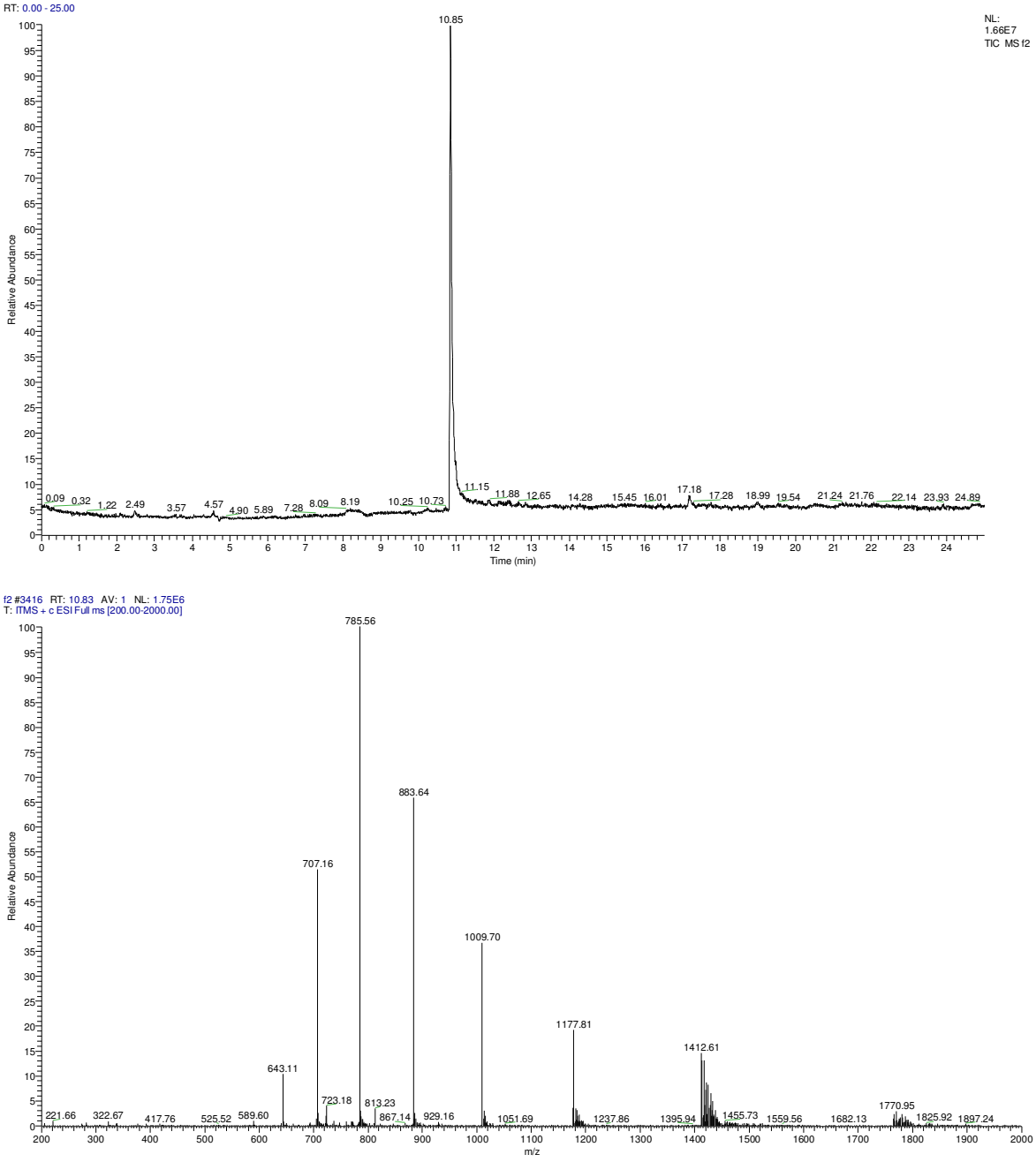
Analytical HPLC chromatogram and ESI-MS spectrum of **3** (monomeric BB14 analog); $[MH_3^{3+}]$:1177.61; $[MH_4^{4+}]$:883.94; $[MH_5^{5+}]$: 707.32; $[MH_6^{6+}]$:589.62; calculated MW: 3531



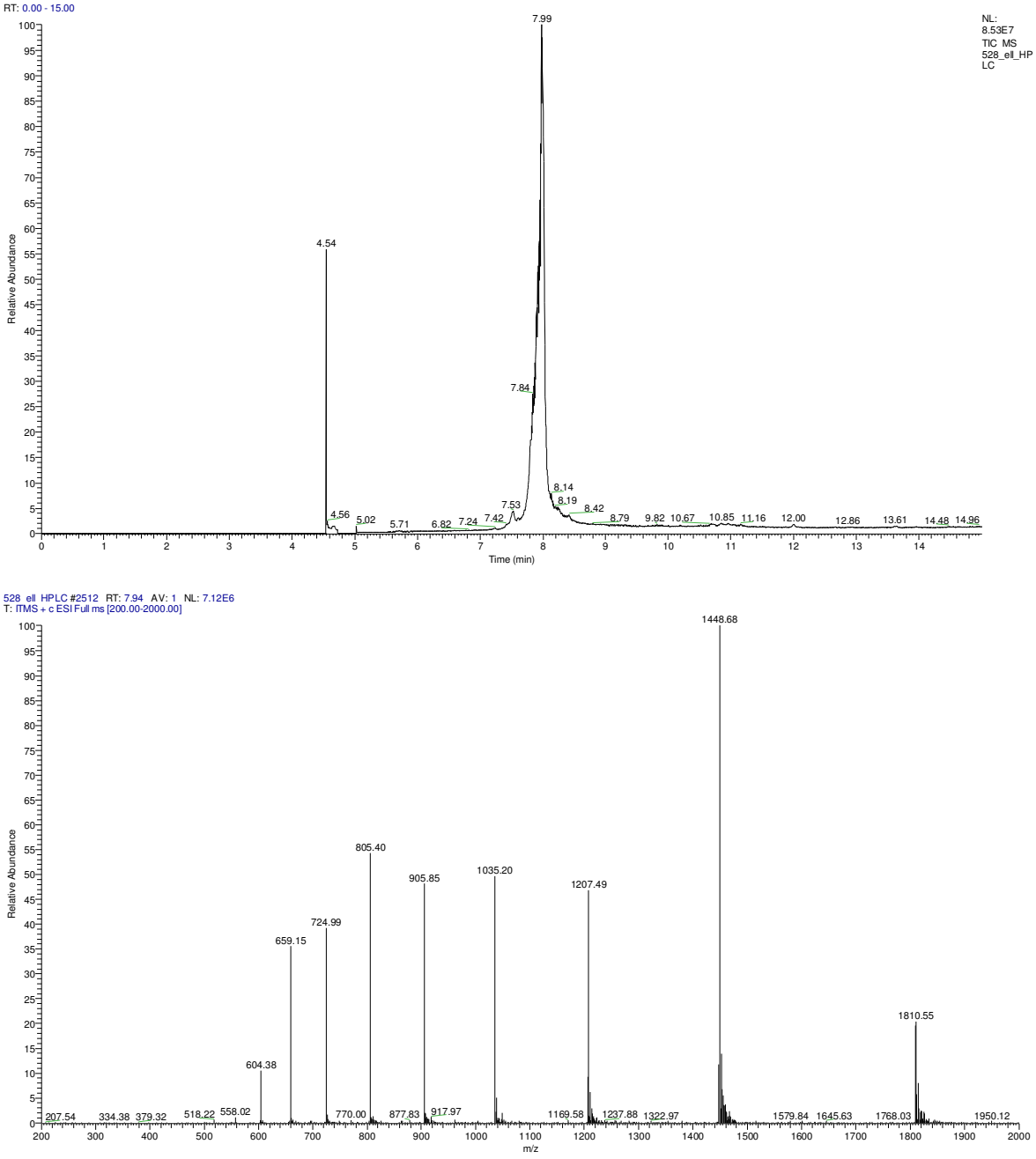
Analytical HPLC chromatogram and ESI-MS spectrum of **4** (monomeric BB15 analog); $[MH_2^{2+}]$: 1811.18; $[MH_3^{3+}]$: 1207.62; $[MH_4^{4+}]$: 906.05; $[MH_5^{5+}]$: 724.97; $[MH_6^{6+}]$: 604.40; calculated MW: 3620



Analytical HPLC chromatogram and ESI-MS spectrum of **6** (dimeric BB15); $[MH_4^{4+}]$: 1756.33; $[MH_5^{5+}]$: 1405.22; $[MH_6^{6+}]$: 1170.94; $[MH_7^{7+}]$: 1004.01; $[MH_8^{8+}]$: 878.68; $[MH_9^{9+}]$: 781.18; calculated MW: 7020



Analytical HPLC chromatogram and ESI-MS spectrum of **7** (dimeric BB14 analog); $[MH_5^{5+}]$: 1412.61; $[MH_6^{6+}]$: 1177.81; $[MH_7^{7+}]$: 1009.70; $[MH_8^{8+}]$: 883.64; $[MH_9^{9+}]$: 785.56; calculated MW: 7060



Analytical HPLC chromatogram and ESI-MS spectrum of **8** (dimeric BB15 analog); [MH_4^{4+}]: 1810.55; [MH_5^{5+}]: 1448.68; [MH_6^{6+}]: 1207.49; [MH_7^{7+}]: 1035.20; [MH_8^{8+}]: 905.85; [MH_9^{9+}]: 805.40; calculated MW: 7238

The characterization of monomeric and dimeric BB14 (compounds **1** and **5)** are described in the supporting material of our previous work[1] (designated as compounds **1** and **7**, respectively).

1. G. Olajos, A. Hetenyi, E. Weber, L. J. Nemeth, Z. Szakonyi, F. Fulop and T. A. Martinek, *Chemistry*, 2015, **21**, 6173-6180.