Org. Biomol. Chem.

Anion binding of a neutral bis(cyclopeptide) in water/methanol mixtures containing up to 95% of water

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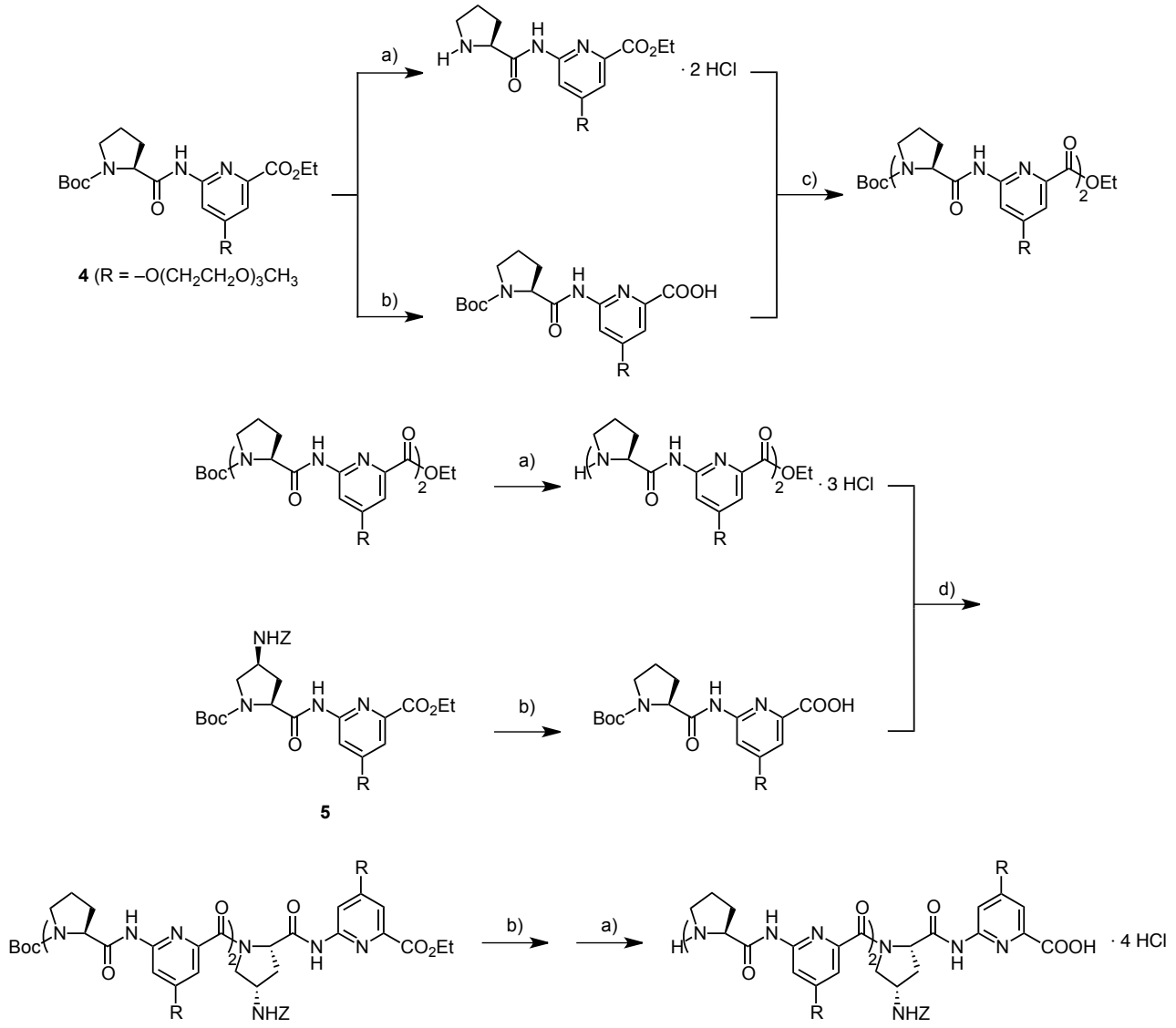
CONTENT

Chain Elongation of Dipeptides **4** and **5**:

Synthetic Strategy. Synthesis of the linear precursor of cyclopeptide **6** involved initial preparation of a tetrapeptide containing two subunits of dipeptide **4**. This tetrapeptide was deprotected at the N-terminus and coupled to C-deprotected dipeptide **5**. The resulting hexapeptide was finally deprotected at both ends.



a) 1.2 equiv 1 N NaOH, 1,4-dioxane, 2 h, 25°C, b) 4 N HCl, 1,4-dioxane, 2 h, 0-5 °C, c) PyCloP, DIEA, CH_2Cl_2, 25 °C, 18 h, d) TBTU, DIEA, DMF, 18 h, 25 °C.

General procedure for the cleavage of ethyl esters. The ester was dissolved in 1,4-dioxane (20 mL/mmol). Addition of aqueous 1 N NaOH (1.2 equiv) was followed by stirring for 2 h at room

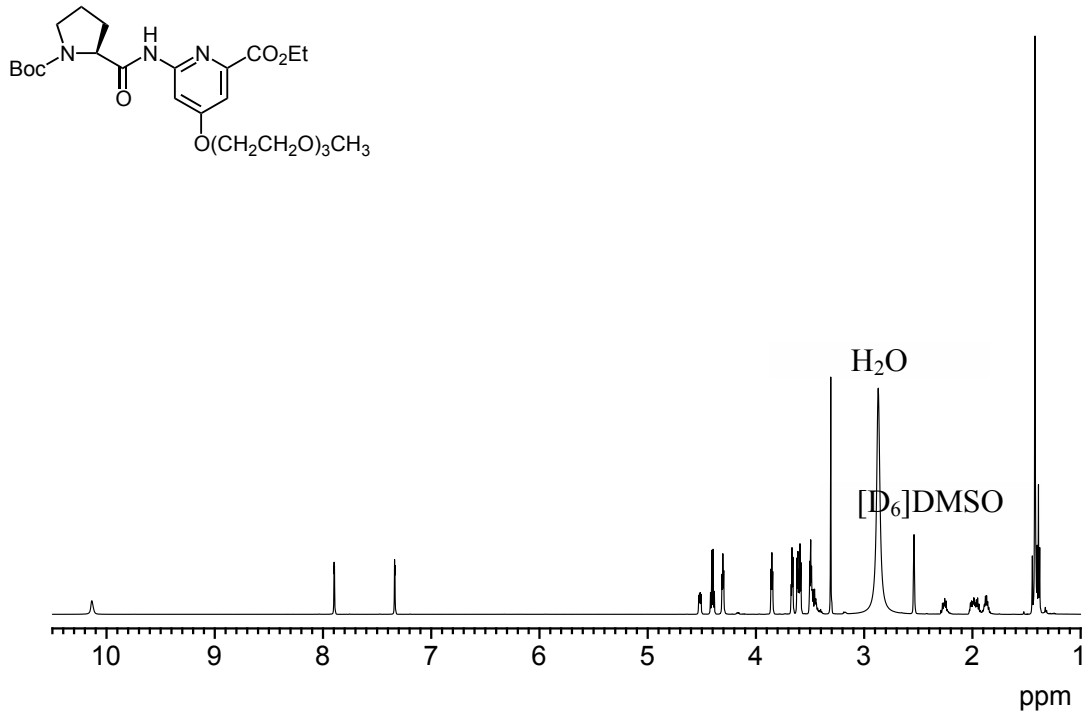
temperature. Afterward, water (20 mL/mmol) was added and the 1,4-dioxane was removed *in vacuo*. The aqueous solution was washed with diethyl ether twice (in the case of the linear hexapeptide this step was omitted) and the organic layers discarded. After acidification of the mixture to pH 2 by addition of 3% aqueous $KHSO_4$ it was extracted with ethyl acetate three times. The combined organic layers were washed three times with water, dried, and evaporated to dryness *in vacuo*. The residue was used for the next step without further purification.

General procedure for the cleavage of *N*-tert-butoxycarbonyl groups. The carbamate was suspended in 1,4-dioxane (20 mL). This suspension was cooled in an ice bath and a 6 N solution of HCl in 1,4-dioxane (40 mL) was added dropwise. The reaction mixture was stirred for 2 h at 0-5 °C and then concentrated to dryness *in vacuo*.

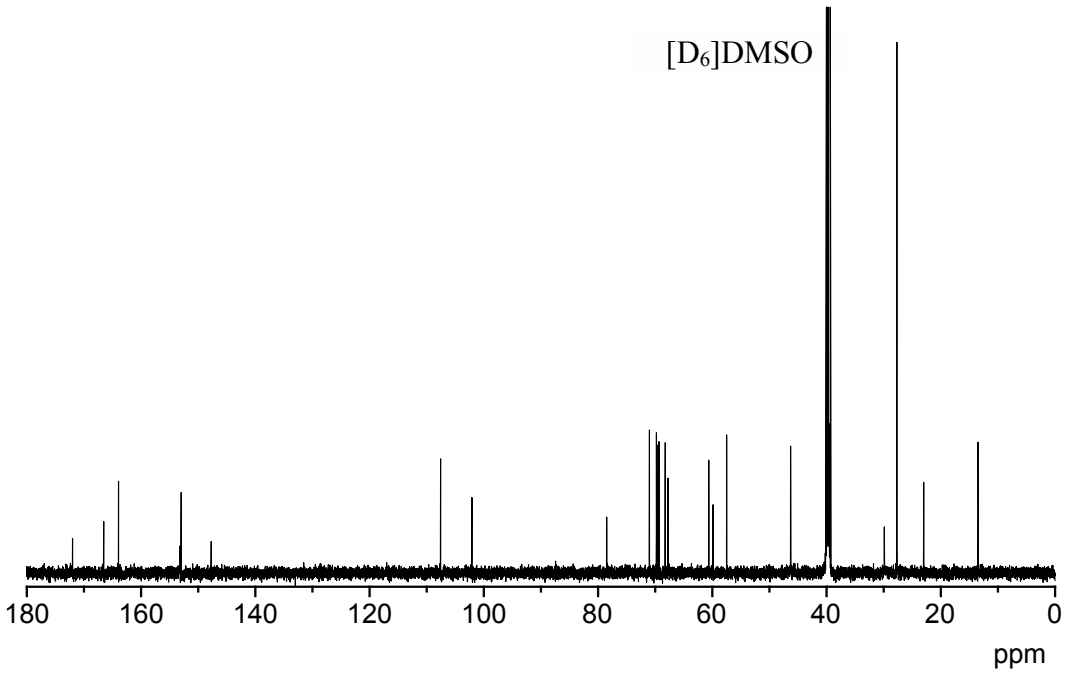
Boc-(Pro-Mapa)$_2$-OEt. Dipeptide **4** (0.82 g, 1.55 mmol) was deprotected at the C-terminus and another portion of **4** (0.74 g, 1.40 mmol) at the N-terminus according to the general procedures. The products were dissolved in dry dichloromethane (30 mL) and PyCloP (0.75 g, 1.86 mmol) and DIEA (1.1 mL, 6.52 mmol) were added. After stirring at 25 °C for 20 h the solvent was removed *in vacuo*, and the residue purified by column chromatography (SiO_2, dichloromethane/methanol, 10:1 (*v/v*)). The product was afforded as an oil, which was used without further purification. Yield 1.18 g (93 %), yellow-orange sticky oil. MS (MALDI-TOF) *m/z* (%) 906 (100 %) $[M+H]^+$, 928 (31 %) $[M+Na]^+$, 944 (9 %) $[M+K]^+$.

Boc-(4Z-Apro)-Mapa-(Pro-Mapa)$_2$-OEt. Tetrapeptide Boc-(Pro-Mapa)$_2$-OEt (1.11 g, 1.23 mmol) was deprotected at the N-terminus and dipeptide **5** (0.82 g, 1.22 mmol) at the C-terminus according to the general procedures. These products were dissolved in DMF (40 mL) and TBTU (0.47 g, 1.46 mmol) and DIEA (1.1 mL, 6.58 mmol) were added. After stirring at 25 °C for 18 h, the solvent was removed *in vacuo* and the residue purified by column chromatography (SiO_2, dichloromethane/methanol, 10:1 (*v/v*)). The resulting oil was used without further purification. Yield 1.48 g (84 %), yellow sticky oil. MS (MALDI-TOF) *m/z* (%) 1434 (100 %) $[M+H]^+$, 1456 (89 %) $[M+Na]^+$, 1472 (41 %) $[M+K]^+$.

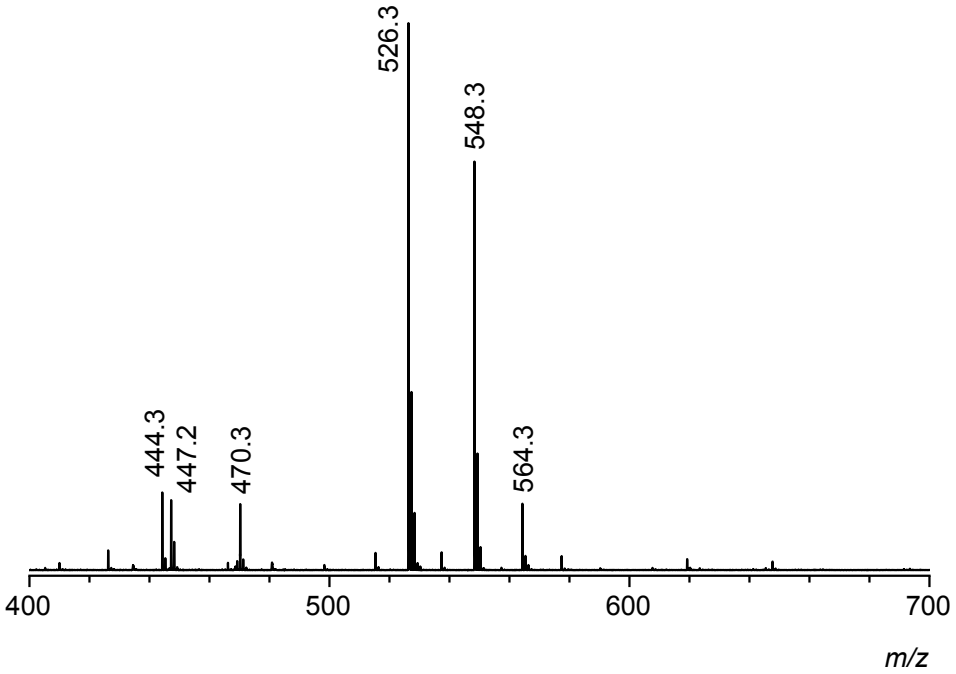
^{1}H NMR Spectrum: Boc-Pro-Mapa-OEt **4** (600 MHz, [D_6]DMSO, 100 °C).



^{13}C NMR Spectrum: Boc-Pro-Mapa-OEt **4** (151 MHz, [D_6]DMSO, 100 °C).

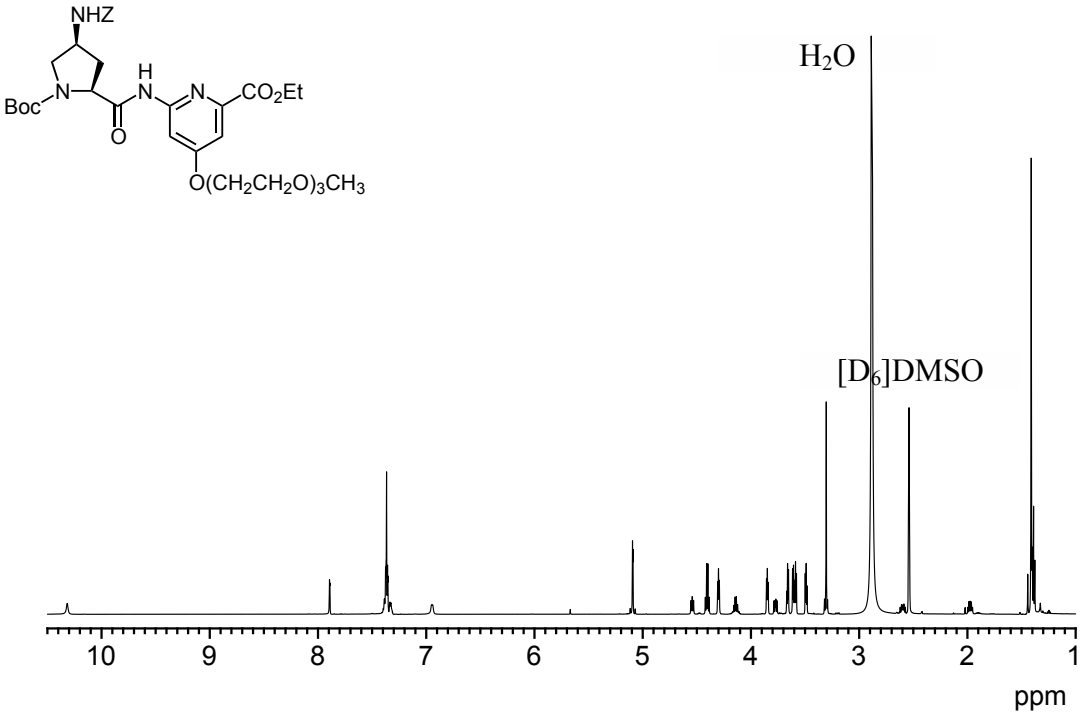


MALDI-TOF MS Spectrum: Boc-Pro-Mapa-OEt **4** (positive mode).

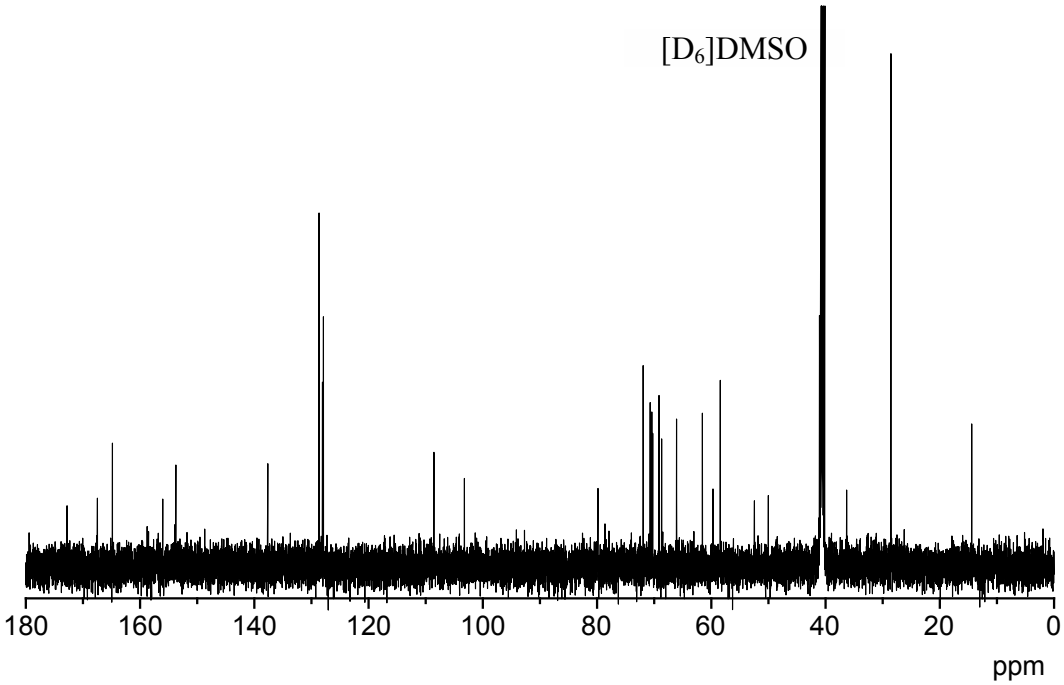


		m/z calcd.	*m/z exp.*
$[M–iso\text{-butene-}C_2H_2+H]^+$	$C_{25}H_{39}N_3O_9 – C_4H_8 – C_2H_2 + H^+$	444.2	444.3
$[M–Boc+Na]^+$	$C_{25}H_{39}N_3O_9 – C_5H_9O_2 + Na^+$	447.2	447.2
$[M–iso\text{-butene}+H]^+$	$C_{25}H_{39}N_3O_9 – C_4H_8 + H^+$	470.2	470.3
$[M+H]^+$	$C_{25}H_{39}N_3O_9 + H^+$	526.3	526.3
$[M+Na]^+$	$C_{25}H_{39}N_3O_9 + Na^+$	548.3	548.3
$[M+K]^+$	$C_{25}H_{39}N_3O_9 + K^+$	564.2	564.3

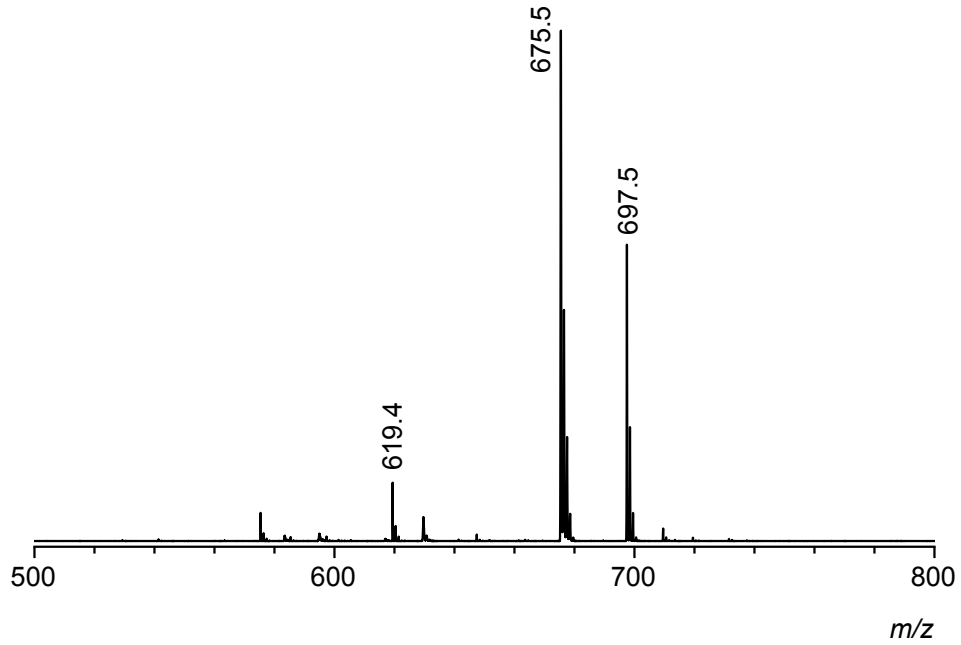
^{1}H NMR Spectrum: Boc-(4Z-Apro)-Mapa-OEt **5** (600 MHz, [D_6]DMSO, 100 °C).



^{13}C NMR Spectrum: Boc-(4Z-Apro)-Mapa-OEt **5** (151 MHz, [D_6]DMSO, 100 °C).

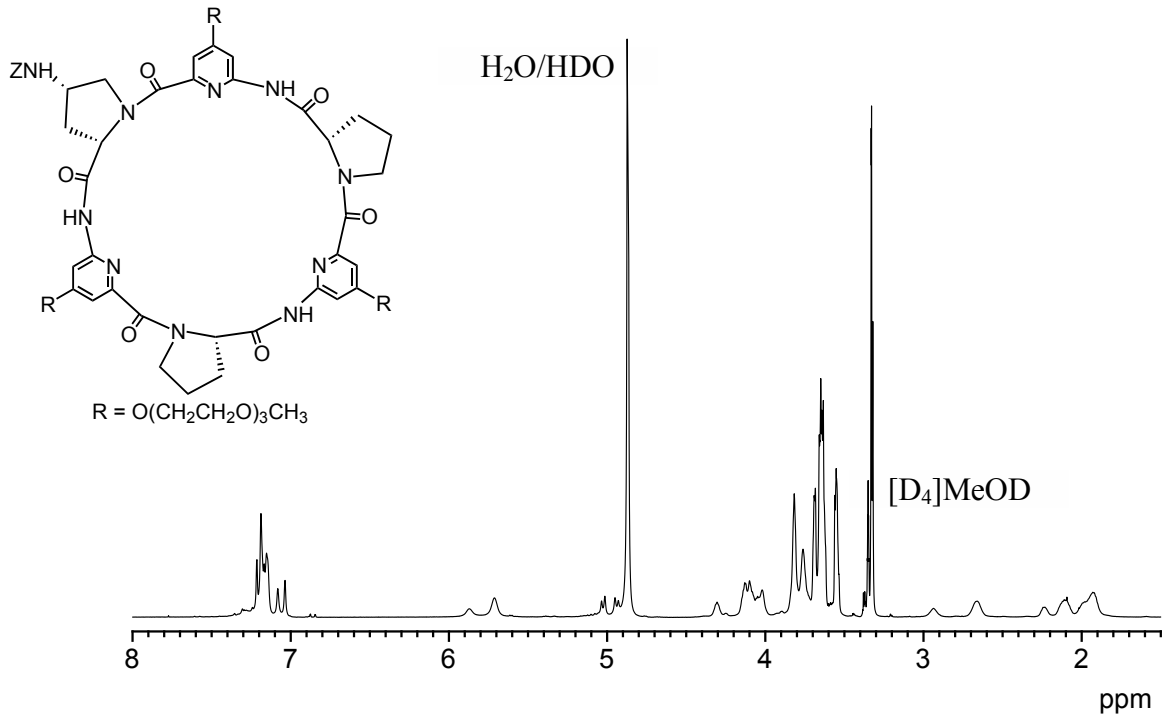


MALDI-TOF MS Spectrum: Boc-(4Z-Apro)-Mapa-OEt **5** (positive mode).

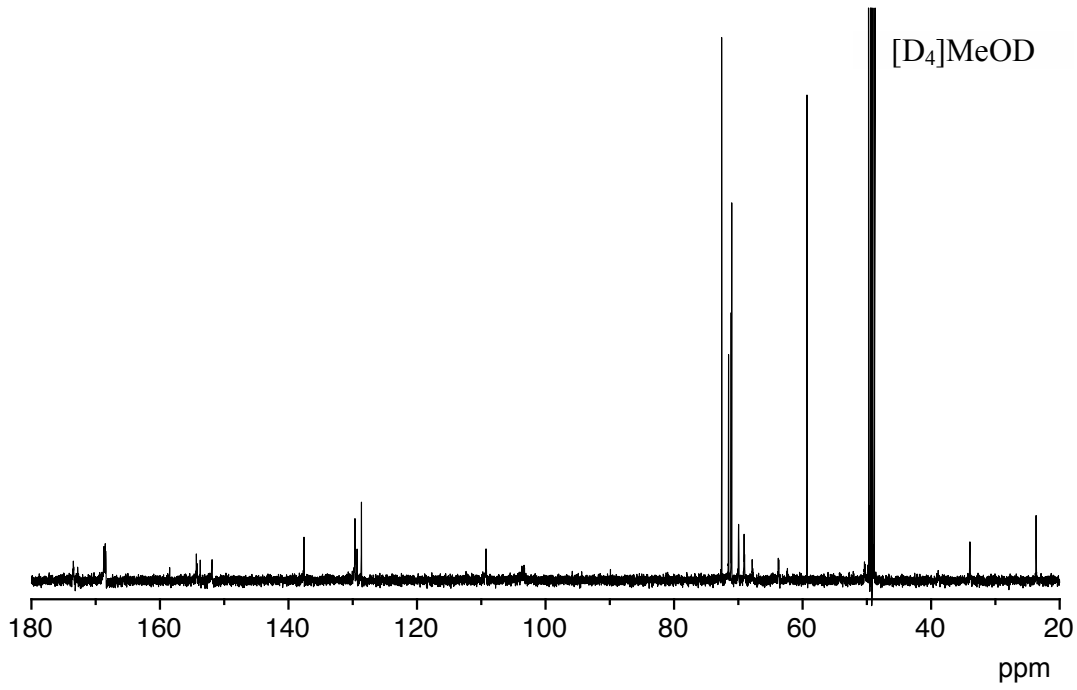


		m/z calcd.	*m/z exp.*
$[M–iso\text{-butene}+H]^+$	$C_{33}H_{46}N_4O_{11} – C_4H_8 + H^+$	619.3	619.4
$[M+H]^+$	$C_{33}H_{46}N_4O_{11} + H^+$	675.3	675.5
$[M+Na]^+$	$C_{33}H_{46}N_4O_{11} + Na^+$	697.3	697.5

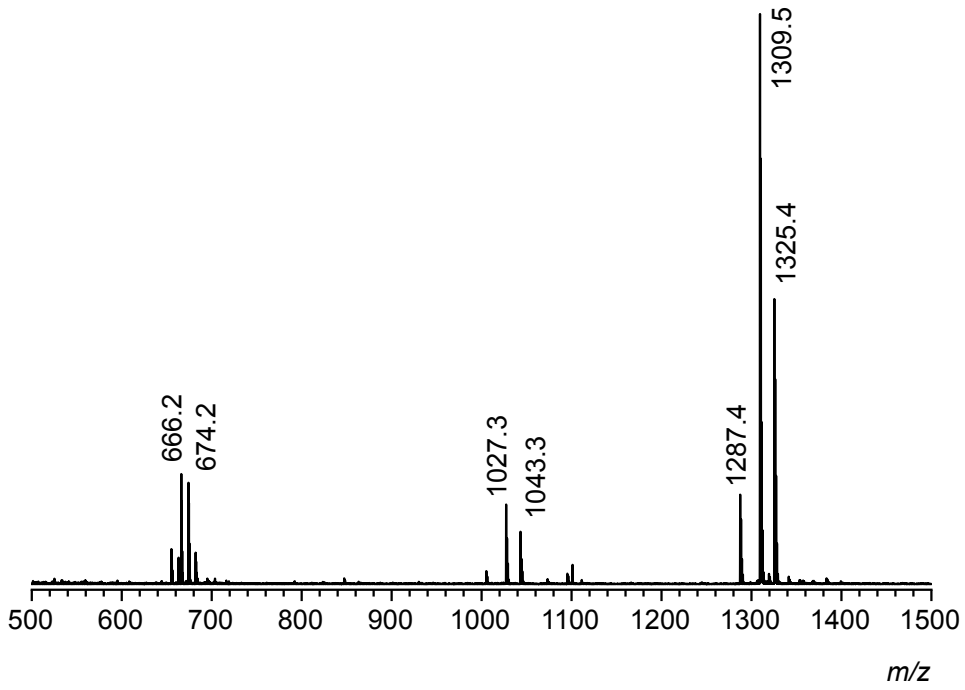
^{1}H NMR Spectrum: *cyclo*[(4Z-Apro)-Mapa-(Pro-Mapa)$_2$] **6** (600 MHz, D_2O/[D_4]MeOD, 1:1 (*v/v*), 25 °C).



^{13}C NMR Spectrum: *cyclo*[(4Z-Apro)-Mapa-(Pro-Mapa)$_2$] **6** (151 MHz, D_2O/[D_4]MeOD, 1:1 (*v/v*), 25 °C).

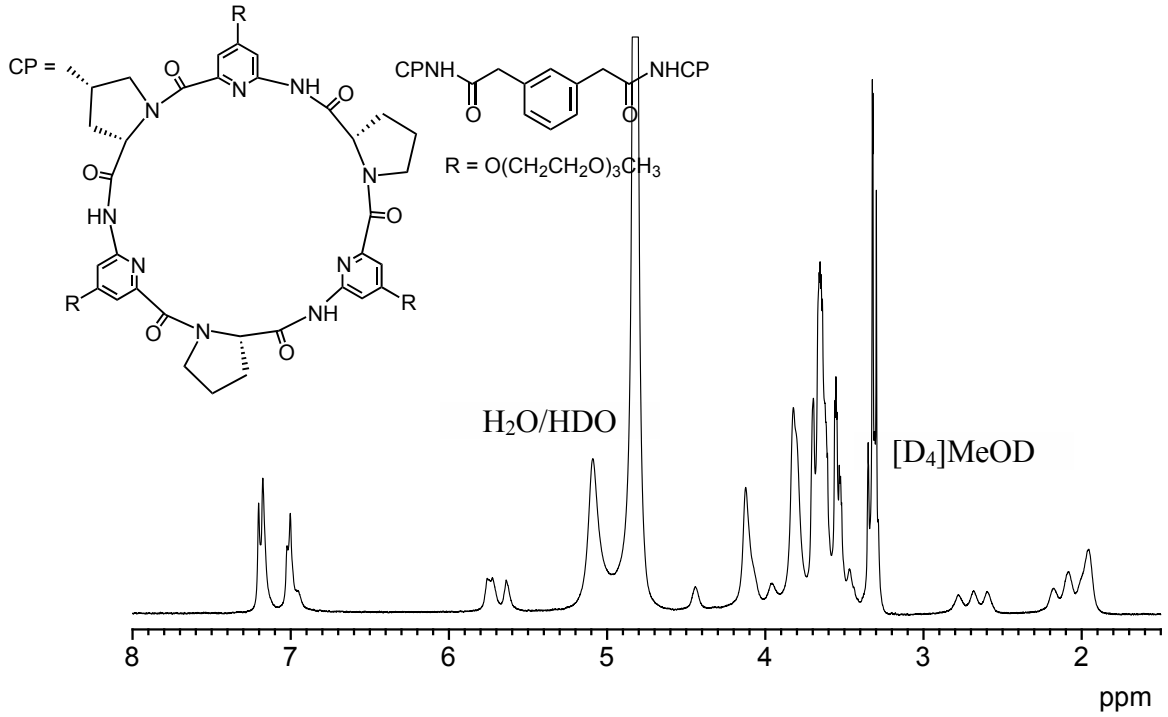


ESI-TOF MS Spectrum: *cyclo*[(4Z-Apro)-Mapa-(Pro-Mapa)$_2$] **6** (positive mode).

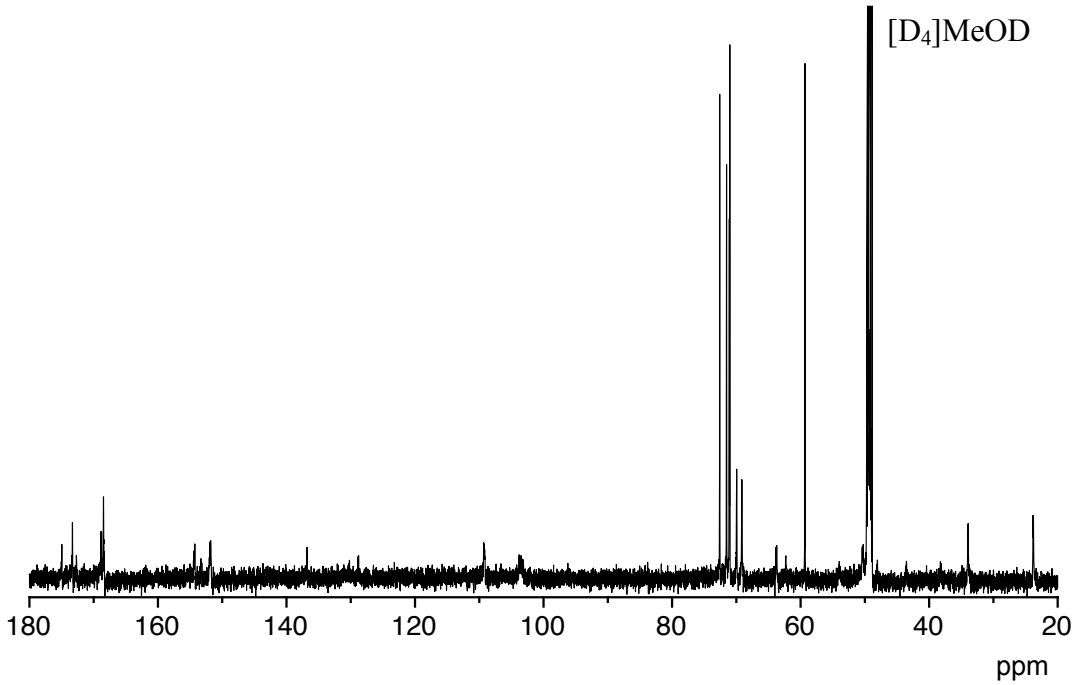


		m/z calcd.	*m/z exp.*
$[M+2Na]^{2+}$	$C_{62}H_{82}N_{10}O_{20} + 2Na^+$	666.3	666.2
$[M+Na+K]^{2+}$	$C_{62}H_{82}N_{10}O_{20} + Na^+ + K^+$	674.3	674.2
$[M–Teg–Z+Na]^+$	$C_{62}H_{82}N_{10}O_{20} – C_7H_{15}O_3 – C_8H_7O_2 + Na^+$	1027.4	1027.3
$[M–Teg–Z+K]^+$	$C_{62}H_{82}N_{10}O_{20} – C_7H_{15}O_3 – C_8H_7O_2 + K^+$	1043.4	1043.3
$[M+H]^+$	$C_{62}H_{82}N_{10}O_{20} + H^+$	1287.6	1287.4
$[M+Na]^+$	$C_{62}H_{82}N_{10}O_{20} + Na^+$	1309.6	1309.5
$[M+K]^+$	$C_{62}H_{82}N_{10}O_{20} + K^+$	1325.5	1325.4

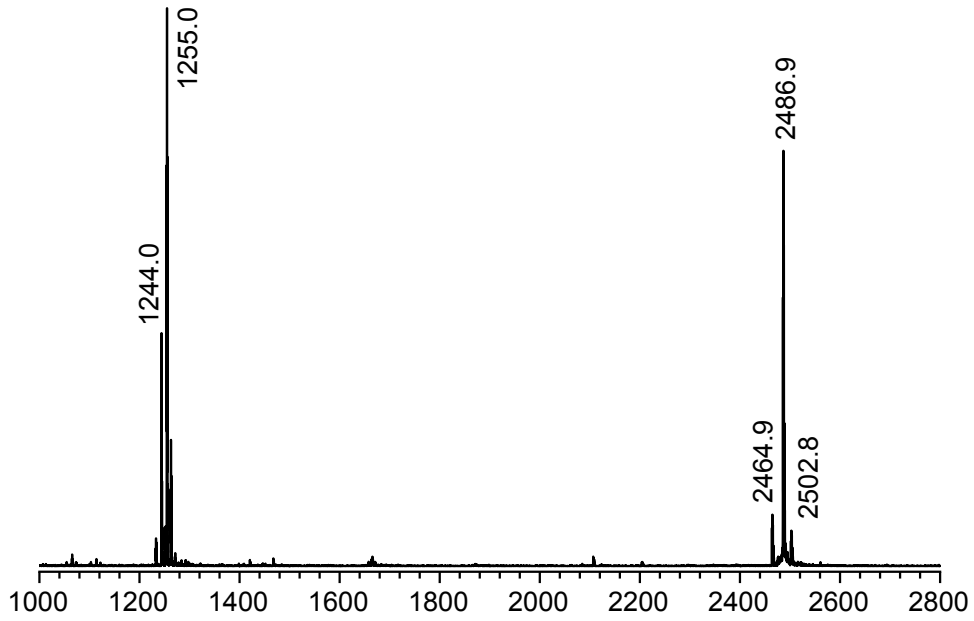
^{1}H NMR Spectrum: {*cyclo*[Apro-Mapa-(Pro-Mapa)$_2$]}$_2$Pda **2b** (600 MHz, D_2O/[D_4]MeOD, 1:1 (*v/v*), 25 °C).



^{13}C NMR Spectrum: {*cyclo*[Apro-Mapa-(Pro-Mapa)$_2$]}$_2$Pda **2b** (151 MHz, D_2O/[D_4]MeOD, 1:1 (*v/v*), 25 °C).



ESI-TOF MS Spectrum: {*cyclo*[Apro-Mapa-(Pro-Mapa)$_2$]}$_2$Pda **2b** (positive mode).

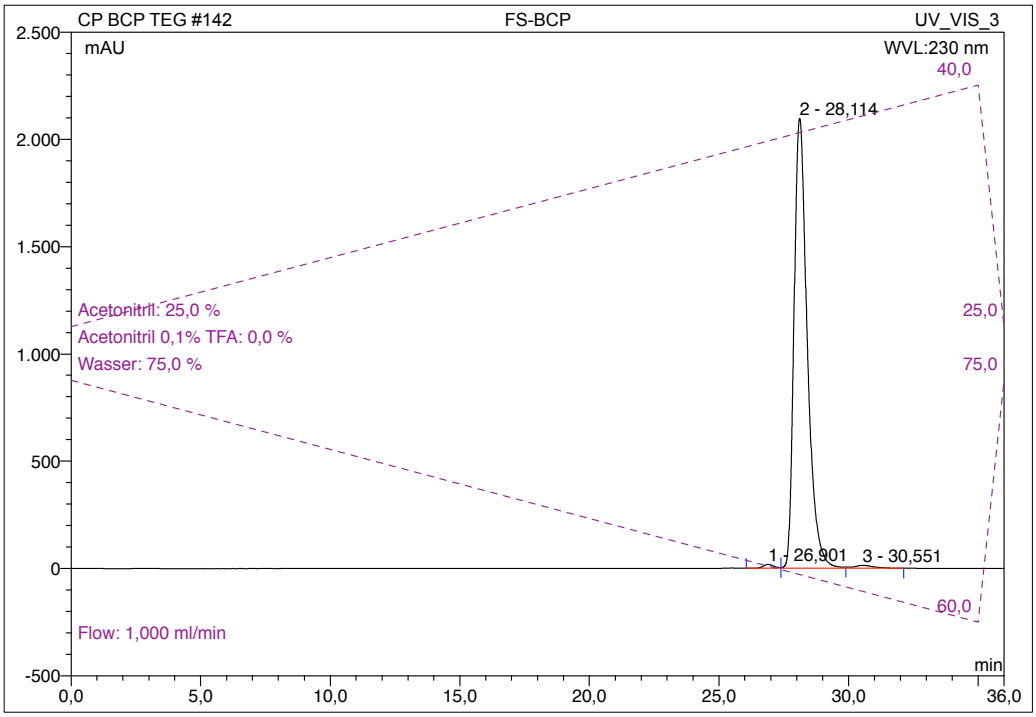


		m/z calcd.	*m/z exp.*
$[M+H+Na]^{2+}$	$C_{118}H_{158}N_{20}O_{38} + H^+ + Na^+$	1243.6	1244.0
$[M+2Na]^{2+}$	$C_{118}H_{158}N_{20}O_{38} + 2\ Na^+$	2554.5	1255.0
$[M+H]^+$	$C_{118}H_{158}N_{20}O_{38} + H^+$	2464.1	2464.9
$[M+Na]^+$	$C_{118}H_{158}N_{20}O_{38} + Na^+$	2486.1	2486.9
$[M+K]^+$	$C_{118}H_{158}N_{20}O_{38} + K^+$	2502.1	2502.8

HPLC: **2b** (Dionex P680 HPLC Pump, ASI-100 Autosampler, TCC-100 Column Oven, UVD 170U UV/Vis Detector, Chromeleon V6.70 Software, Supelco Ascentis® C18 Column, 4.6 × 250 mm, 5 μm)

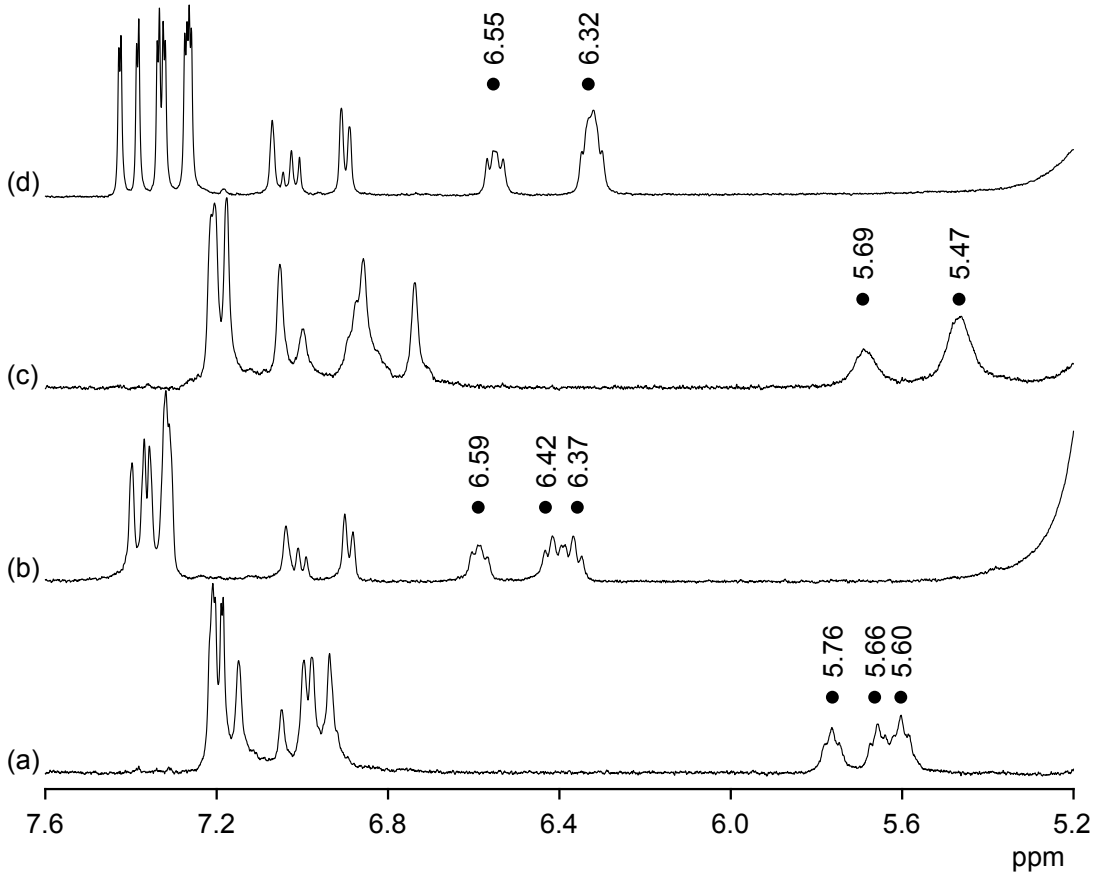
142 FS-BCP

Sample Name:	**FS-BCP**	*Injection Volume [µl]:*	**20,0**
Vial Number:	**GB3**	*Channel:*	**UV_VIS_3**
Sample Type:	**unknown**	*Wavelength [nm]:*	**230**
Control Program:	**AS_Supelco_langsamer AnstiegBCP**	*Bandwidth:*	**1**
Quantif. Method:	**default**	*Dilution Factor:*	**1,0000**
Recording Time:	**21.1.2014 21:29**	*Sample Weight:*	**1,0000**
Run Time (min):	**36,00**	*Sample Amount:*	**1,0000**



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	26,90	n.a.	18,105	8,419	0,67	n.a.	BM
2	28,11	n.a.	2096,508	1243,817	98,38	n.a.	M
3	30,55	n.a.	13,470	12,031	0,95	n.a.	MB
Total:			2128,083	1264,267	100,00	0,000	

Qualitative NMR Spectroscopic Evaluation of Iodide Binding: ^{1}H NMR spectra of bis(cyclopeptide) **2b** (1.8 mM) prior (a) and after (b) the addition of 2 equiv of NaI in in 50% D_2O/CD_3OD and of **2b** (1.0 mM) prior (c) and after (d) the addition of 2 equiv of NaI in in 90% D_2O/CD_3OD. The dots indicate the signals of the H(α) protons on the proline residues of **2b**.



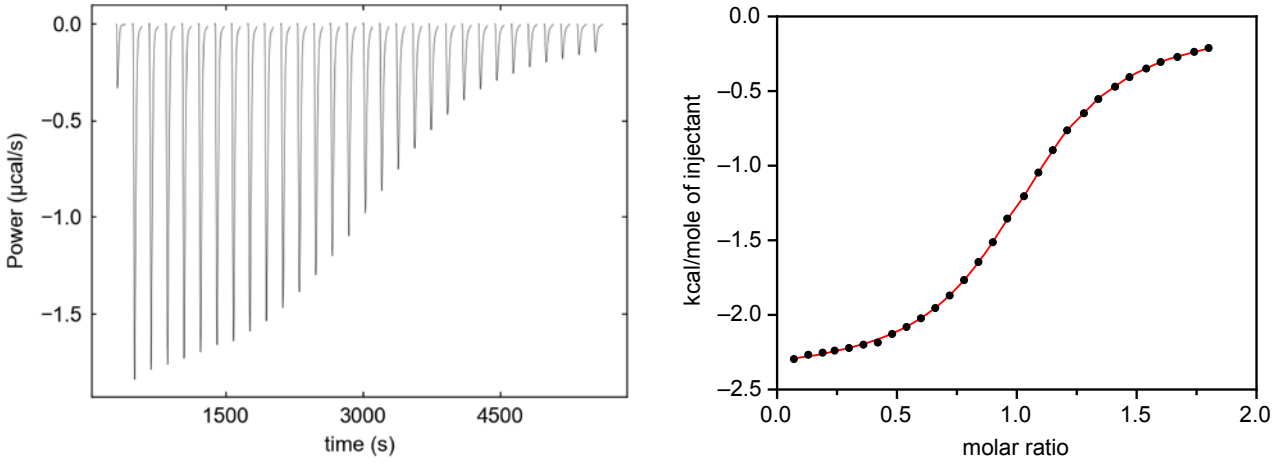
Results of Selected ITC Titrations

Table S1. Concentrations of **2b** and the salts used in the ITC titrations.

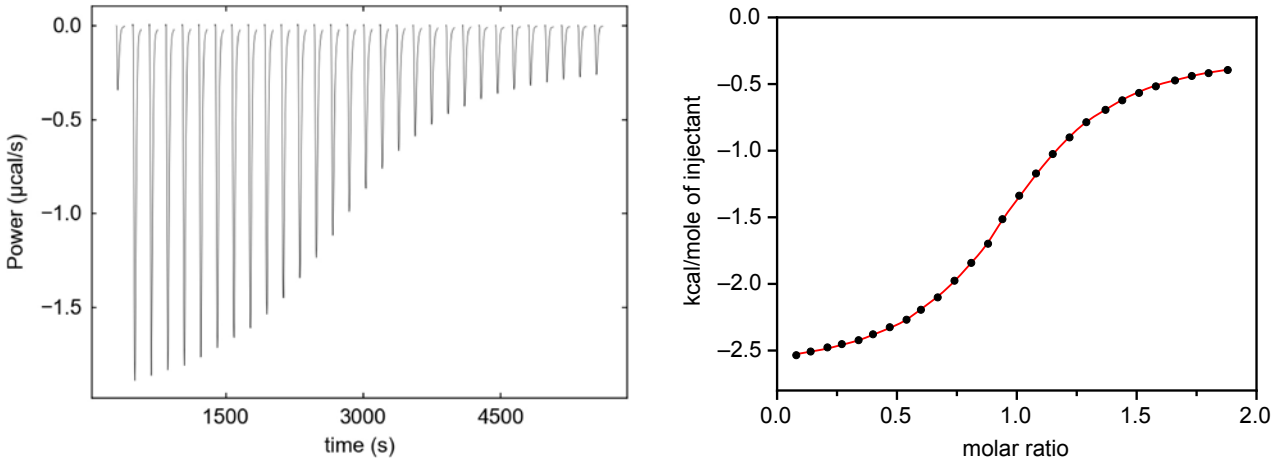
vol% of water in the water/methanol mixture	guest salt	*c*(Receptor) / mM	*c*(Guest) / mM
20%	NaI	0.25	2.5
	NaI + 15-crown-5	0.25 + 2.5 mM 15-crown-5	2.5 + 2.5 mM 15-crown-5
	$(CH_3)_4NI$	0.25	2.5
30%	NaI	0.25	5.0
	Na_2SO_4	0.25	2.5
50%	NaI	0.25	2.5
	Na_2SO_4	0.25	2.5
70%	NaI	0.25	5.0
	Na_2SO_4	0.25	2.5
90%	NaI	0.25	5.0
95%	NaI	0.25	5.0
	Na_2SO_4	0.25	2.5

All of the following thermograms were generated by using NITPIC[1] and the binding isotherms by using Sedphat.[2,3] The circles in the isotherms denote the experimental data and the lines represent the isotherms fitted by using the one-site model.

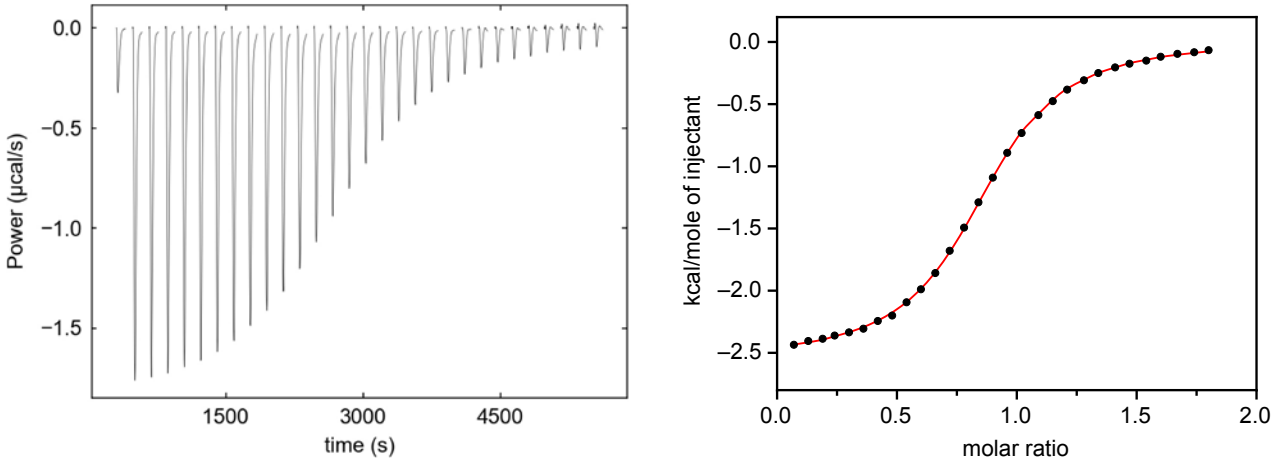
Titration of receptor **2b** with sodium iodide in 20% water/methanol



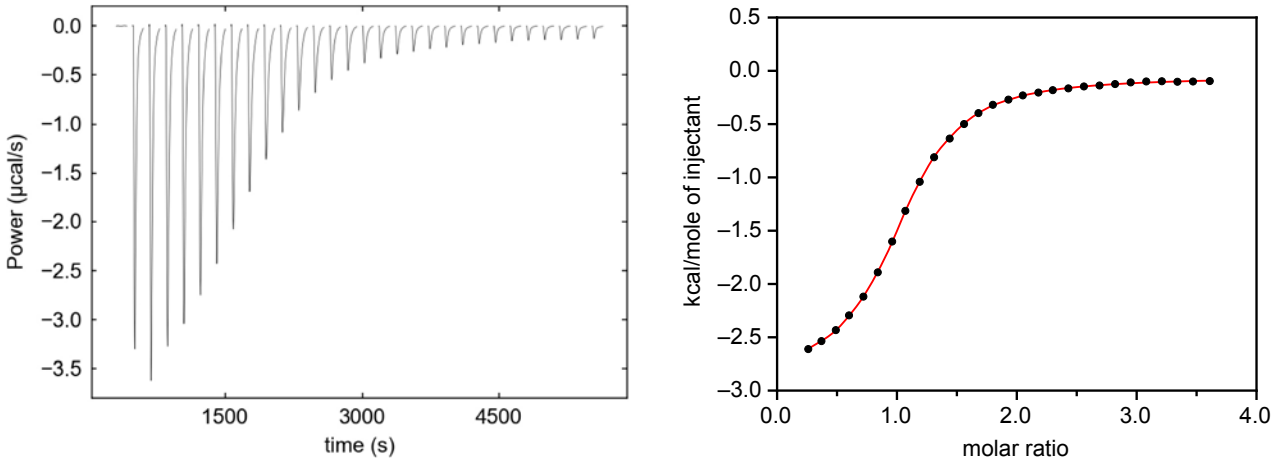
Titration of receptor **2b** with sodium iodide in 20% water/methanol in the presence of 15-crown-5



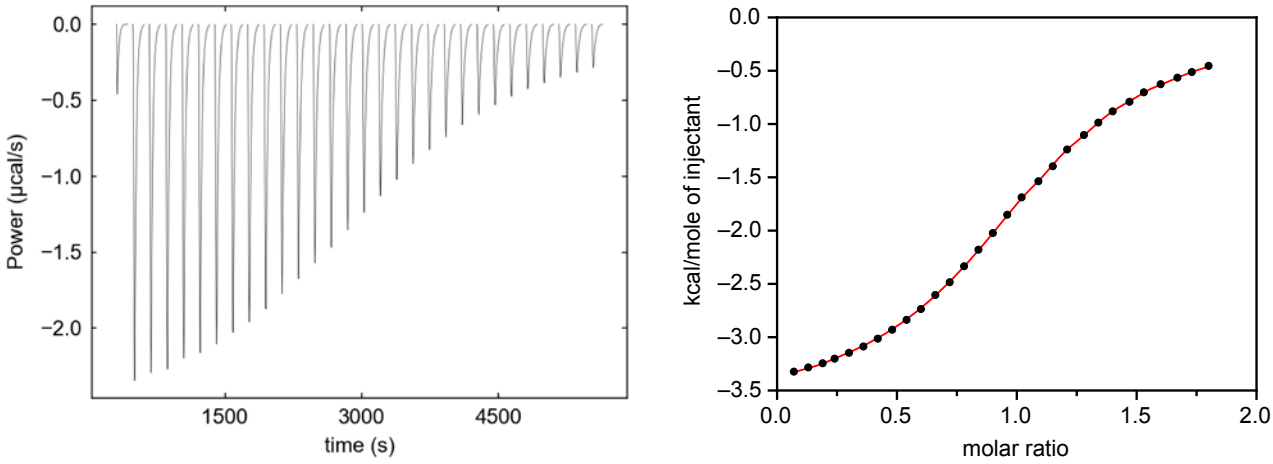
Titration of receptor **2b** with tetramethylammonium iodide in 20% water/methanol



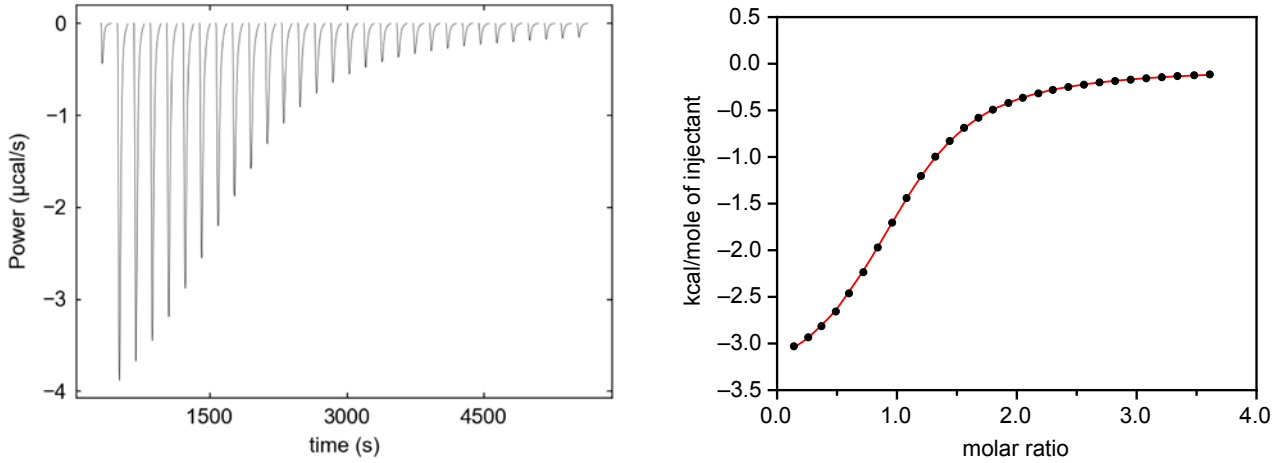
Titration of receptor **2b** with sodium iodide in 30% water/methanol



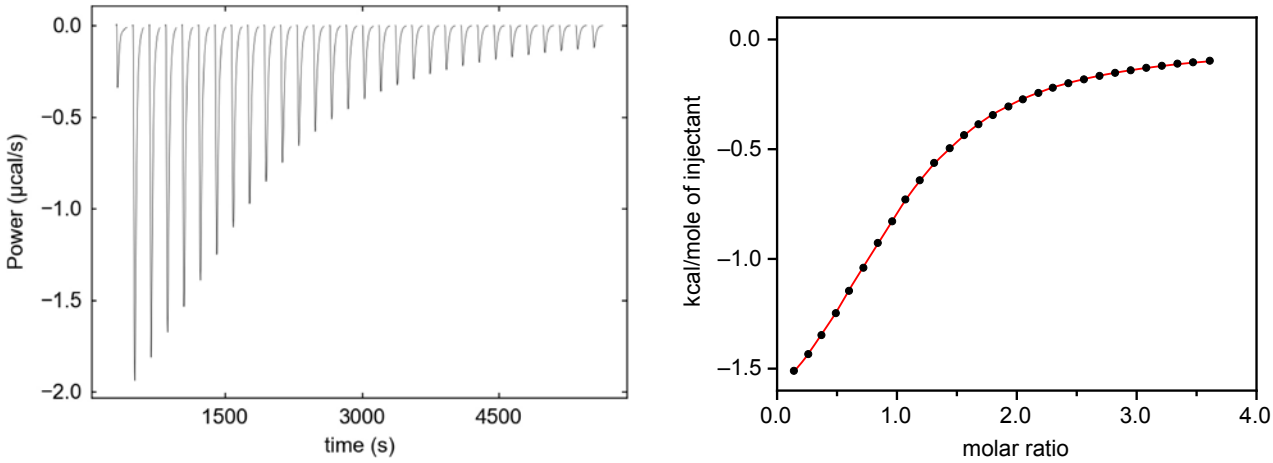
Titration of receptor **2b** with sodium iodide in 50% water/methanol



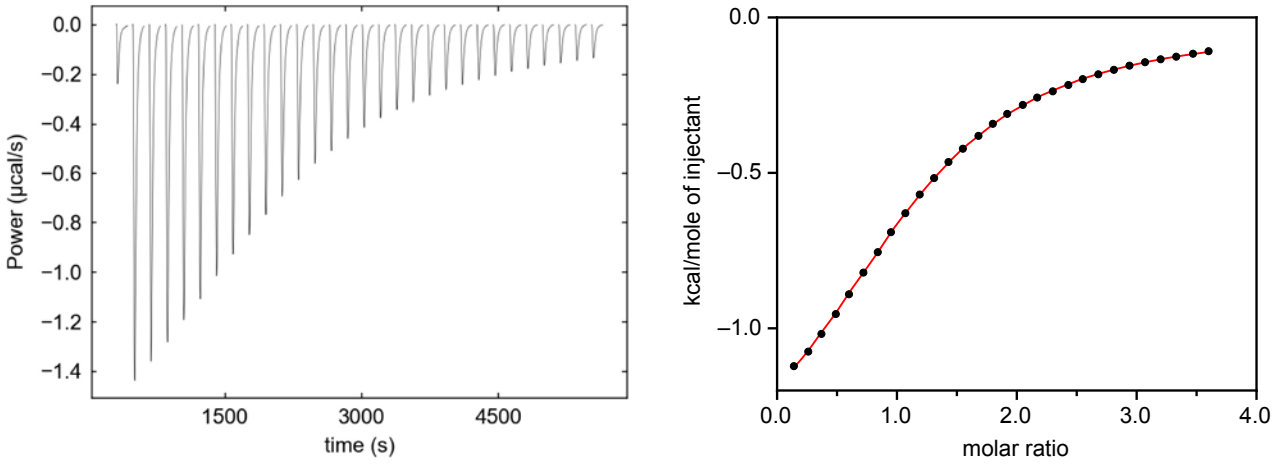
Titration of receptor **2b** with sodium iodide in 70% water/methanol



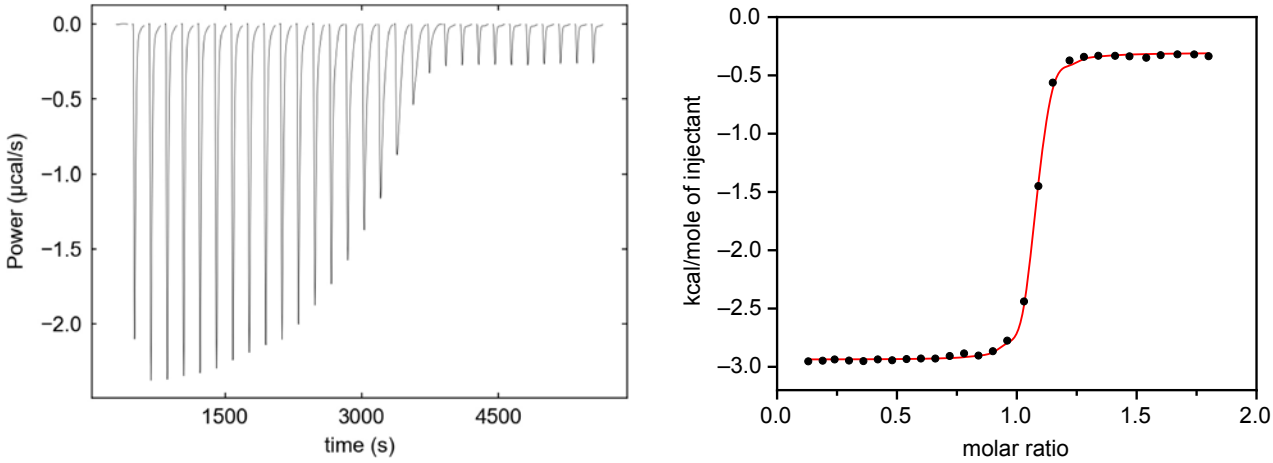
Titration of receptor **2b** with sodium iodide in 90% water/methanol



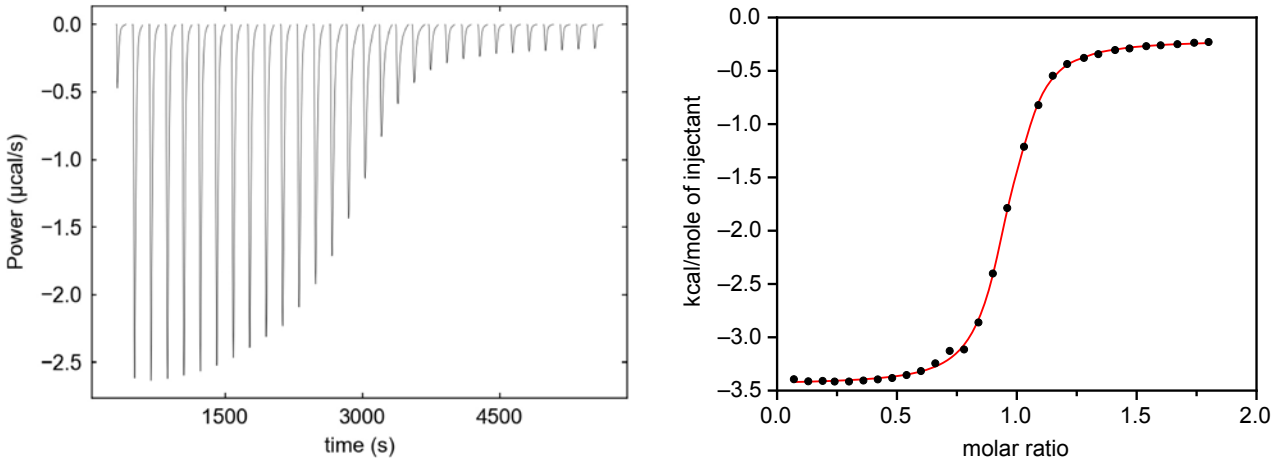
Titration of receptor **2b** with sodium iodide in 95% water/methanol



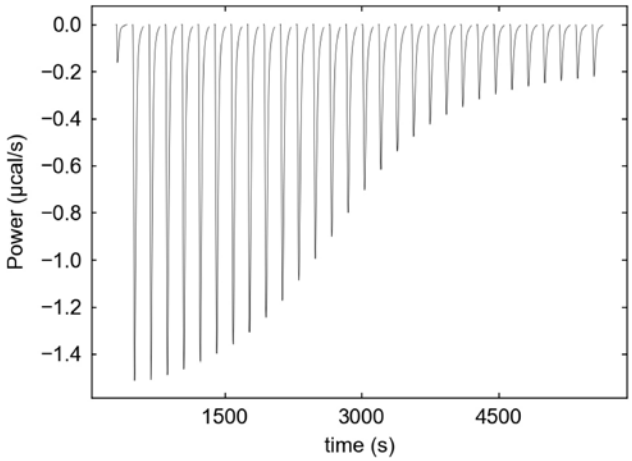
Titration of receptor **2b** with sodium sulfate in 30% water/methanol

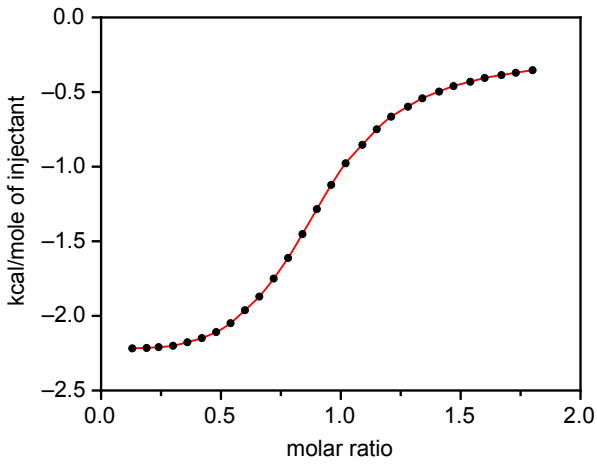


Titration of receptor **2b** with sodium sulfate in 50% water/methanol

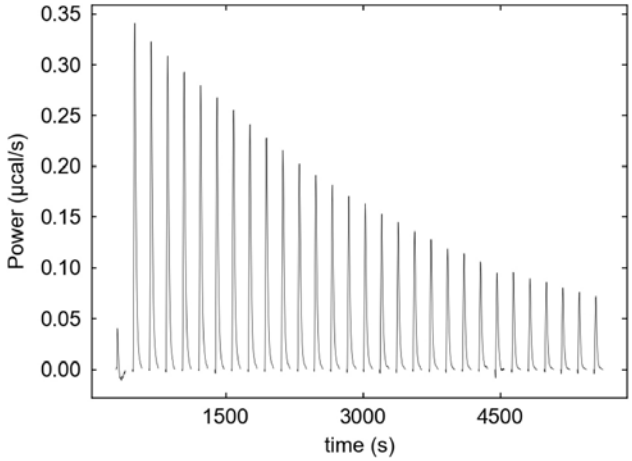


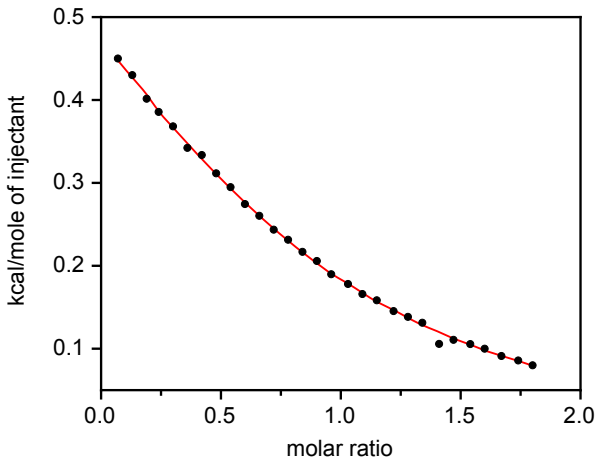
Titration of receptor **2b** with sodium sulfate in 70% water/methanol





Titration of receptor **2b** with sodium sulfate in 95% water/methanol





References

1 S. Keller, C. Vargas, H. Zhao, G. Piszczek, C. A. Brautigam and P. Schuck, *Anal. Chem.*, 2012, **84**, 5066-5073.

2 J. C. D. Houtman, P. H. Brown, B. Bowden, H. Yamaguchi, E. Appella, L. E. Samelson and P. Schuck, *Protein Sci.*, 2007, **16**, 30-42.

3 http://www.analyticalultracentrifugation.com/sedphat/download.htm.