

ELECTRONIC SUPPLEMENTARY INFORMATION

Bilayer thickness determines the alignment of model polyproline helices in lipid membranes

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Details on peptide synthesis

General notes. Fmoc-Oic was prepared from Oic and Fmoc chloride using trimethylsilylchloride assisted protocol, as described.^{S1} *N*-Boc 8-aminooctanoic acid was prepared analogously starting from 8-aminooctanoic acid (0.5 g, 3.14 mmol, 1.05 equiv.) and Boc anhydride (0.65 g, 3.00 mmol, 1 equiv.), the yield was 0.47 g (1.81 mmol, 60% yield). Fmoc-TfmPro and Boc-TfmPro were prepared as described.^{S2} Other chemicals were acquired from commercial sources.

On solid support. Linear peptides were synthesized on 2-chlorotrityl-polystyrene resin. 2-chlorotrityl chloride resin was pre-loaded with Fmoc-Oic using original protocol.^{S3} The peptide sequence was grown conventionally. The coupling steps were performed using *N,N*-dimethylformamide (DMF) as a solvent, 3.0 (or 5.0, or 2.5) equiv. of Fmoc- or Boc-amino acid 2.9 (or 4.9, or 2.4) equiv. HATU, 6 (or 10, or 5) equiv. diisopropylethylamine (DIPEA); coupling time 1.5-3 hours. For the long sequences, the steps after the 10th coupling were performed in DMF – dichloromethane (1:1 vol) mixture. The Fmoc-group was removed with 22% (vol) piperidine in DMF. The peptides were cleaved off from the resin by 15 min shaking with hexafluoro-2-propanol (HFIP) – dichloromethane 1/3 (vol) cocktail.^{S4} Crude peptides were suspended in acetonitrile – water and freeze-dried.

In solution. 1) Peptide coupling. A C-terminally free peptide (1 equiv.) was activated with HATU / DIPEA (1 equiv. / 5 equiv.) in dichloromethane – DMF (1:1 vol) mixture; after 5-10 min corresponding amine (2 equiv.) or peptide (1 equiv.) hydrochloride was added to the mixture. The mixture was shaken for 14 hours, then dichloromethane was added; the organic phase was washed with water, aqueous potassium hydrogen sulphate, aqueous sodium hydrogen carbonate, and brine, dried over magnesium sulphate, filtered and concentrated using nitrogen stream. The residue was suspended in acetonitrile/water and freeze-dried. Note that the washing steps do not eliminate residual unreacted peptide fragments due to negligible solubility of these peptides in water. Final purification was afforded by silica gel chromatography with ethyl acetate/methanol (40:1) mixture used as eluent. The chromatographic purification was repeated until solution ¹⁹F NMR spectra showed absence of the resonance from the remaining coupling reagents. 2) N-terminal deprotection. An aliquot of peptide (25-100 mg) was mixed with 4M hydrogen chloride solution in dioxane (0.3-1.0 ml) for 1.5-2 hours. Bulk solvent was blown off by nitrogen current, the residue was suspended in acetonitrile/water and freeze-dried. 3) Final deprotection. The peptides were stirred in a mixture of conc. hydrochloric acid and methanol (3:8 vol) with palladium on charcoal under hydrogen atmosphere for 2 hours. The solution was filtered, methanol was removed under reduced pressure, the residue was freeze-dried from acetonitrile – water. For synthesis of **7** and **8**: 4) Esterification. 2,2-difluoroethylamine (3 equiv.) and *tert*-butyl nitrite (3.5 equiv.) were mixed in chloroform. After 10 min the mixture was added to the C-terminally free peptide

(1 equiv.) in chloroform. Resulting mixture was shaken for 14 hours. The solvent was blown off by nitrogen current, and the residue was suspended in acetonitrile/water and freeze-dried. The peptide esters were purified by silica gel chromatography using ethyl acetate/methanol (40:1) mixture as eluent. 5) Aminolysis of esters. An aliquot of peptide (50 mg) was mixed with 1,3-diaminopropane (1 ml) for 14 hours (synthesis of **7**). Otherwise, peptide (50 mg) was added to molten 1,8-diaminooctane (mp. 50-54 °C), and the mixture was stirred under heating (~ 65-75 °C) for 2 hours (synthesis of **8**). Water and dichloromethane were added, organic fraction was separated, washed several times with aqueous potassium hydrogen sulphate (the peptide remains in the organic phase!), then washed with brine, dried over magnesium sulphate, filtered and concentrated in vacuum. Final peptides were obtained after removal of the N-terminal Boc-group by treatment with 4M hydrogen chloride in dioxane. All peptides were prepared in 18-83 mg final amounts.

Peptide **1** was prepared from Boc-Oic₄-TfmPro-Oic₄-OH precursor peptide as described.^{S2} Peptide **4** was prepared from the same precursor using conventional N-terminal acetylation and C-terminal methyl esterification in solution, and then purified by silica gel chromatography. Peptides **5** and **6** were prepared analogously.

Final purification. All peptides except peptides **4-6** were additionally purified by reverse phase HPLC on a conventional preparative C18-column using water-acetonitrile gradient with 5 mM hydrochloric acid for ion-pairing.

Analytcs. Identity and purity of final peptides was confirmed by mass-spectra (ESI-Orbitrap), liquid chromatography and ¹H NMR (CD₃OD, 600 MHz) spectra as shown below.

The ¹H NMR spectra were acquired at either 700 or 600 MHz frequency at 298 K in methanol-d₄. The spectra were collected in stimulated echo pulse sequence for the solvent suppression purposes. In few samples (peptides **1** and **12**) ¹H NOESY, ROESY (300 ms mixing) and HOHAHA (dipsi2 60 ms) experiments were run in order to enable resonance assignment. For the rest of the peptides from the series the assignment was assumed from analogy.

The analytical reverse-phase high performance liquid chromatography (RP-HPLC) was performed for all peptide samples. Chromatography was performed on standard C18 analytical column (4.6 mm x 250 mm) thermostated at 40 °C. The settings were as follows: solvent A: 10% aqueous acetonitrile with 0.1% trifluoroacetic acid, solvent B: 90% aqueous acetonitrile with 0.1% trifluoroacetic acid, 1.5 ml min⁻¹ flow rate, linear gradient 5 to 95% solvent B in 15 min, injection volume 10 ml.

Analytical data for peptides

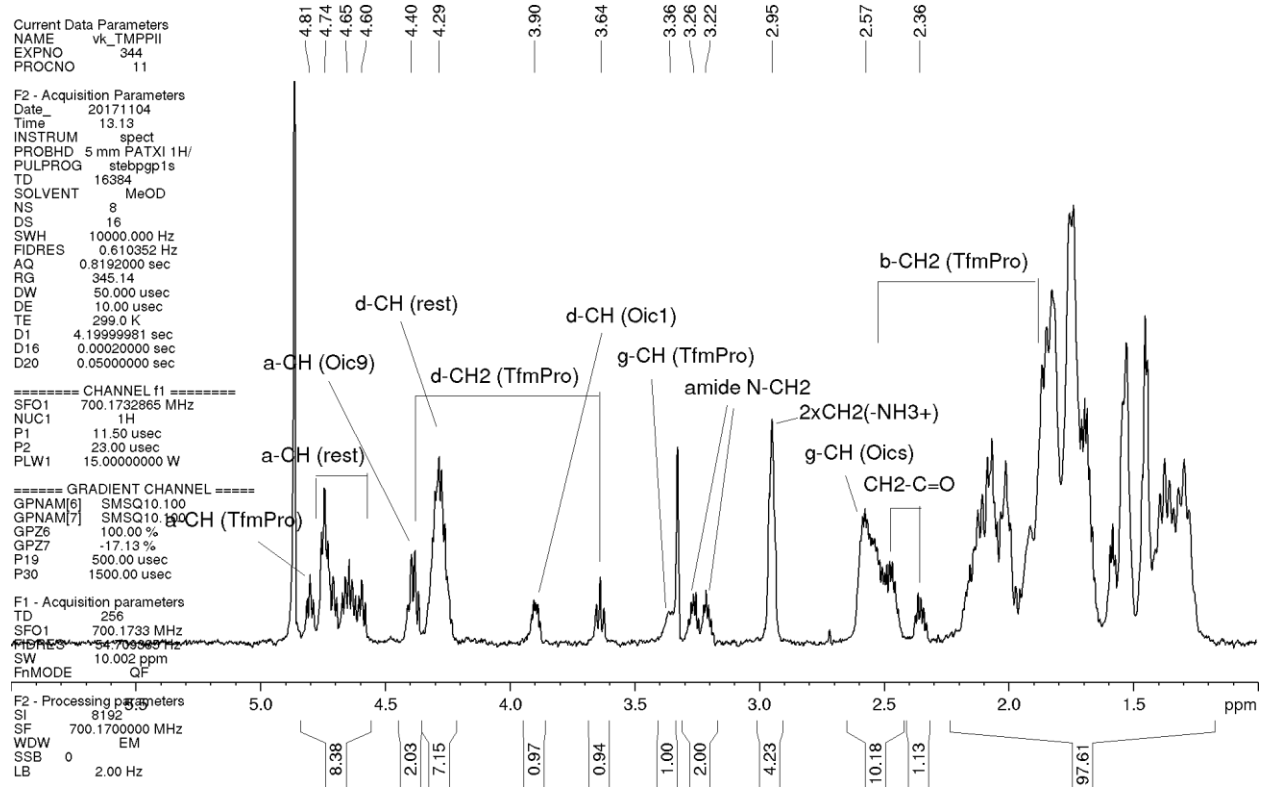
Table S1 Mass-spectrometry data for the peptides

Peptide	Assignment	Mass/charge, Th	
		Expected	Observed (ESI-Orbitrap)
1	[M+H] ⁺	1604.0605	1604.0601
	[M+2H] ²⁺	802.5339	802.5346
2	[M+H] ⁺	1604.0605	1604.0604
	[M+2H] ²⁺	802.5339	802.5350
3	[M+H] ⁺	1604.0605	1604.0614
	[M+2H] ²⁺	802.5339	802.5355
4	[M+H] ⁺	1519.9666	1519.9648
	[M+2H] ²⁺	760.4870	760.4873
5	[M+H] ⁺	1660.1231	1660.1226
	[M+2H] ²⁺	830.5652	830.5663
6	[M+H] ⁺	1448.8819	1448.8824
	[M+2H] ²⁺	724.9446	724.9450
7	[M+H] ⁺	1519.9554	1519.9553
	[M+2H] ²⁺	760.4819	760.4818
8	[M+H] ⁺	1532.9871	1532.9871
	[M+2H] ²⁺	766.9972	766.9979
9	[M+H] ⁺	1755.1603	1755.1594
	[M+2H] ²⁺	878.0838	878.0847
10	[M+H] ⁺	1755.1603	1755.1605
	[M+2H] ²⁺	878.0838	878.0851
11	[M+H] ⁺	1906.2600	1906.2601
	[M+2H] ²⁺	953.6336	953.6349
12	[M+H] ⁺	2057.3597	2057.3601
	[M+2H] ²⁺	1029.1835	1029.1848
13	[M+H] ⁺	2057.3597	2057.3591
	[M+2H] ²⁺	1029.1835	1029.1844

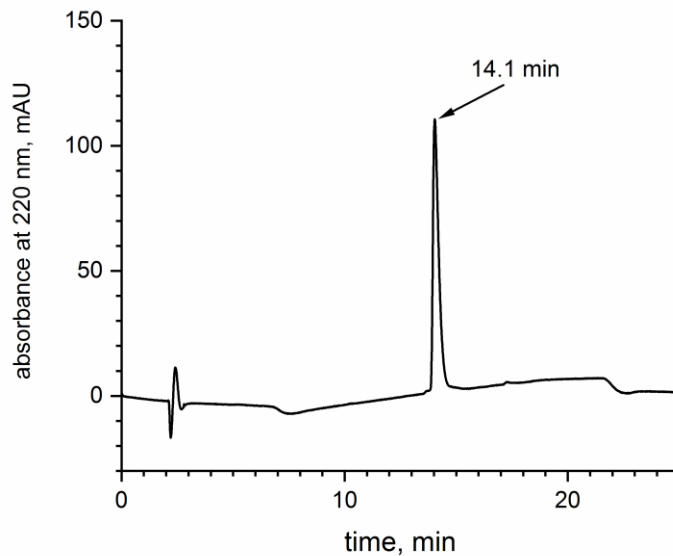
Peptide 1

sequence: $\text{H}_3\text{N}^+(\text{CH}_2)_5\text{CO}-(\text{Oic}_4\text{-TfmPro-Oic}_4)\text{-NH}(\text{CH}_2)_6\text{N}^+\text{H}_3, 2\text{Cl}^-$

^1H NMR spectrum (MeOD, 700 MHz), stimulated echo:



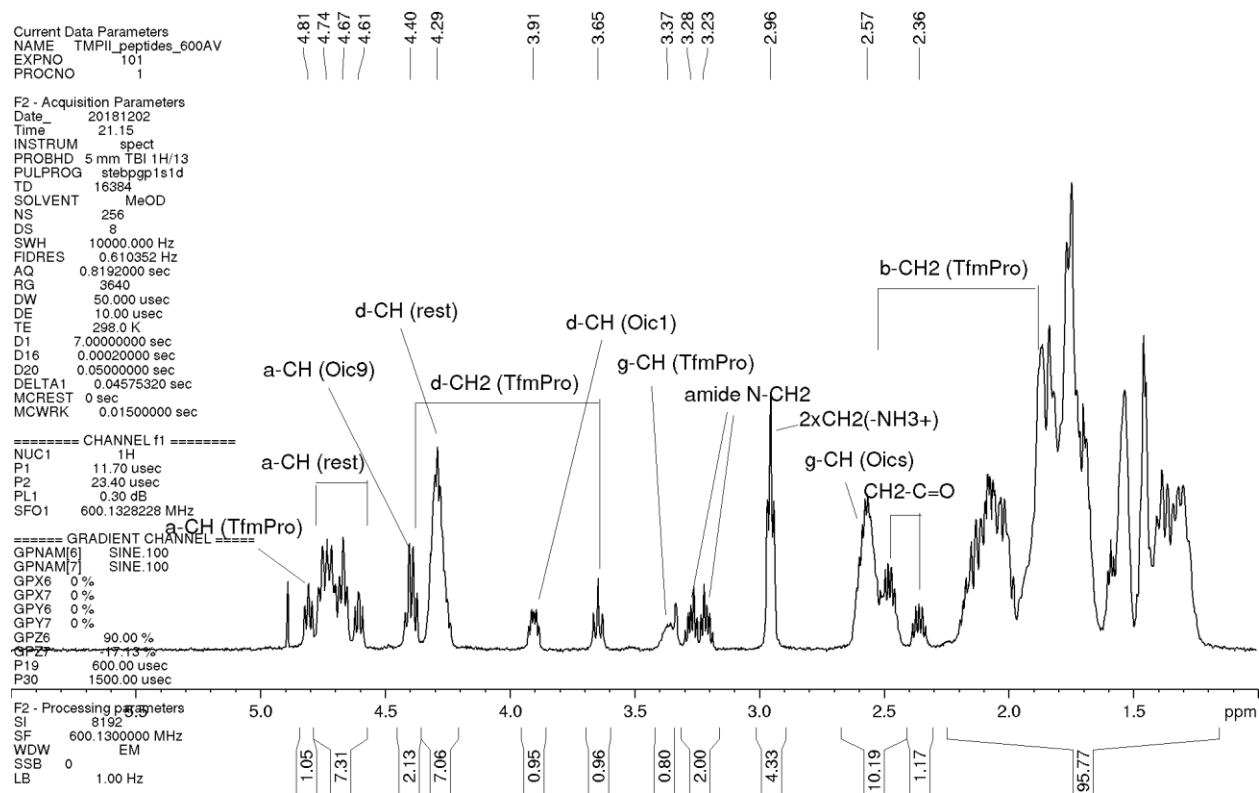
RP-HPLC chromatogram:



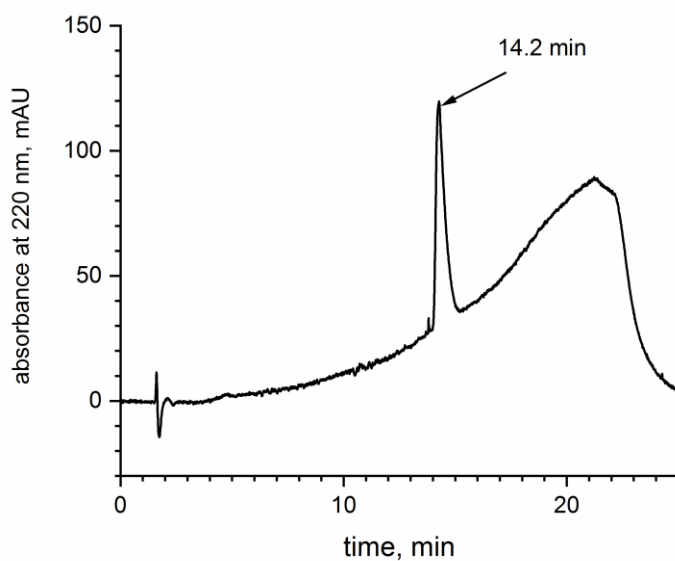
Peptide 2

sequence: $\text{H}_3\text{N}^+(\text{CH}_2)_5\text{CO}-(\text{Oic}_3\text{-TfmPro-Oic}_5)\text{-NH}(\text{CH}_2)_6\text{N}^+\text{H}_3, 2\text{Cl}^-$

^1H NMR spectrum (MeOD, 600 MHz), stimulated echo:



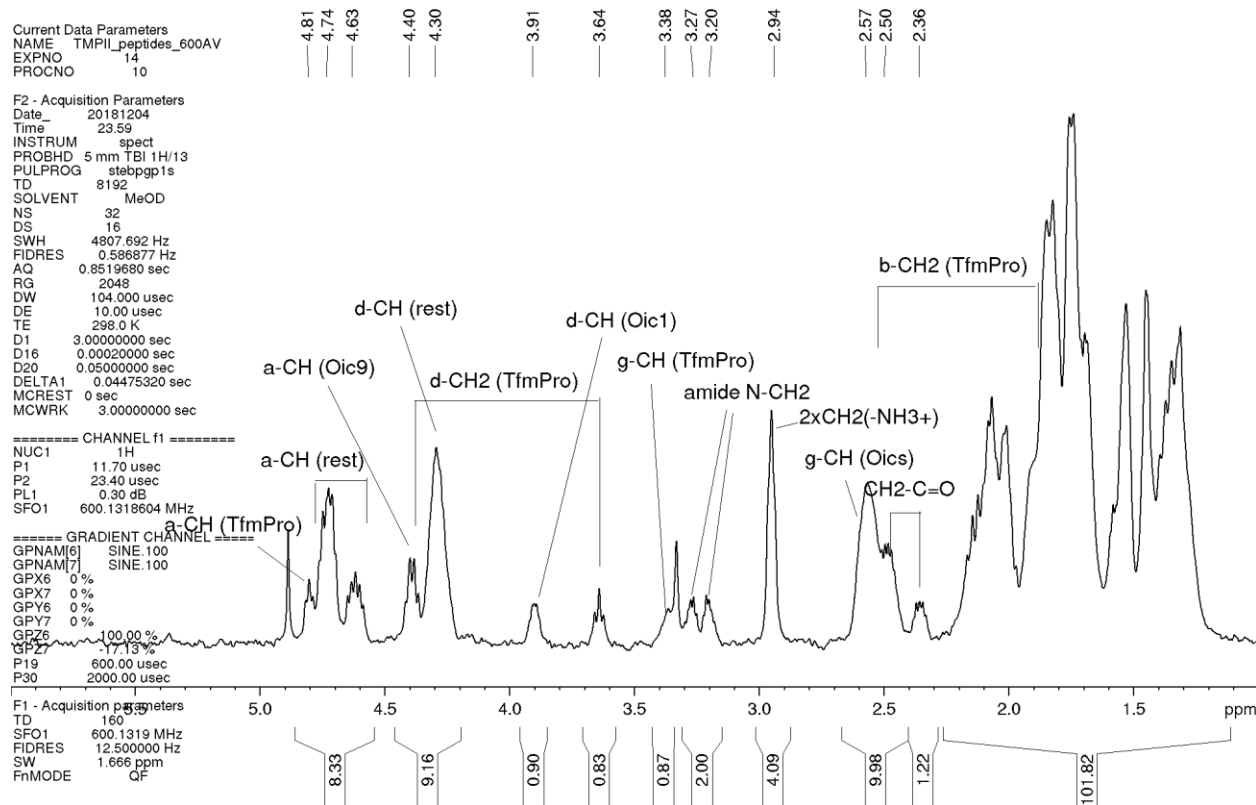
RP-HPLC chromatogram:



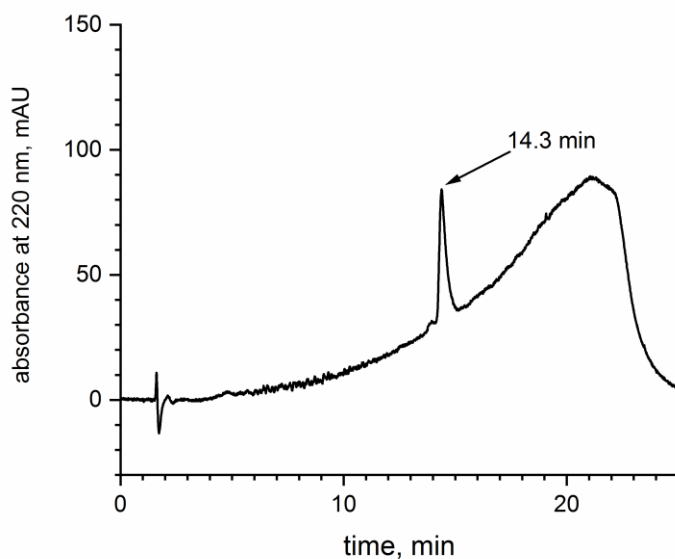
Peptide 3

sequence: $\text{H}_3\text{N}^+(\text{CH}_2)_5\text{CO}-(\text{Oic}_5\text{-TfmPro-Oic}_3)\text{-NH}(\text{CH}_2)_6\text{N}^+\text{H}_3, 2\text{Cl}^-$

^1H NMR spectrum (MeOD, 600 MHz), stimulated echo:



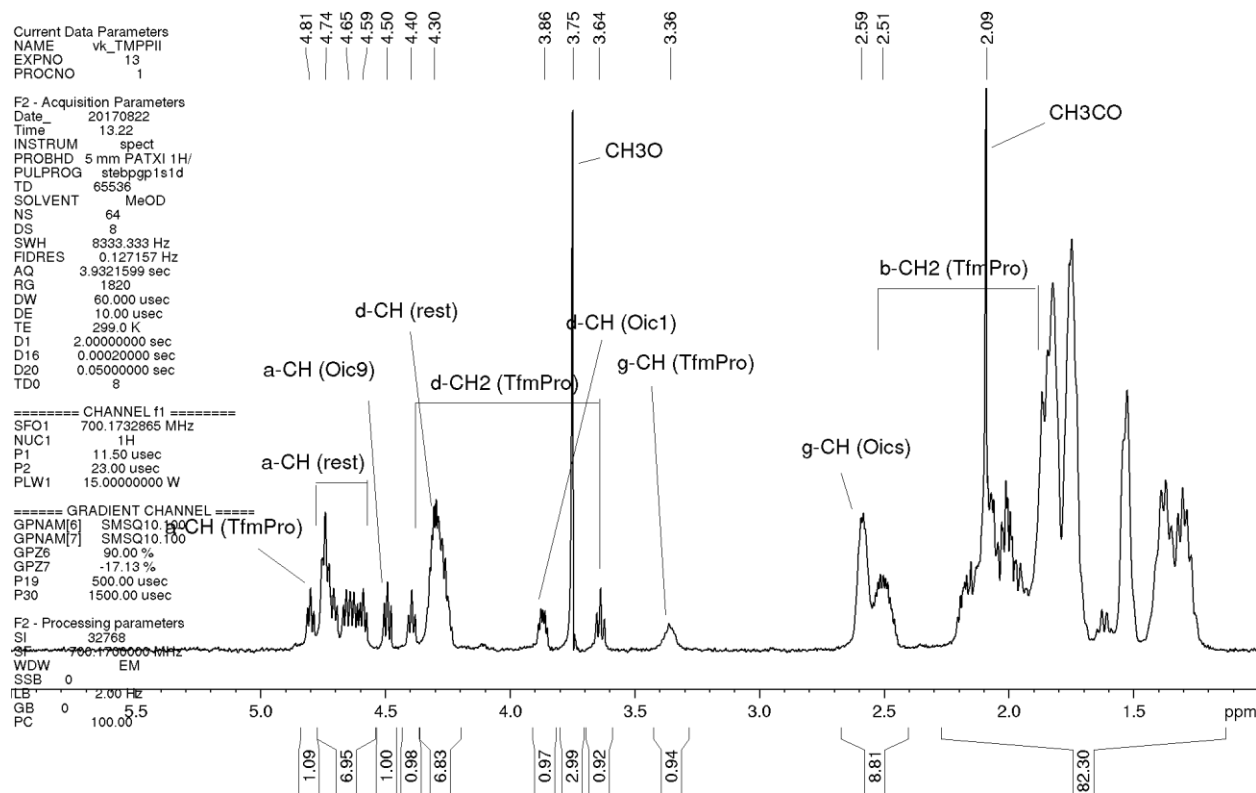
RP-HPLC chromatogram:



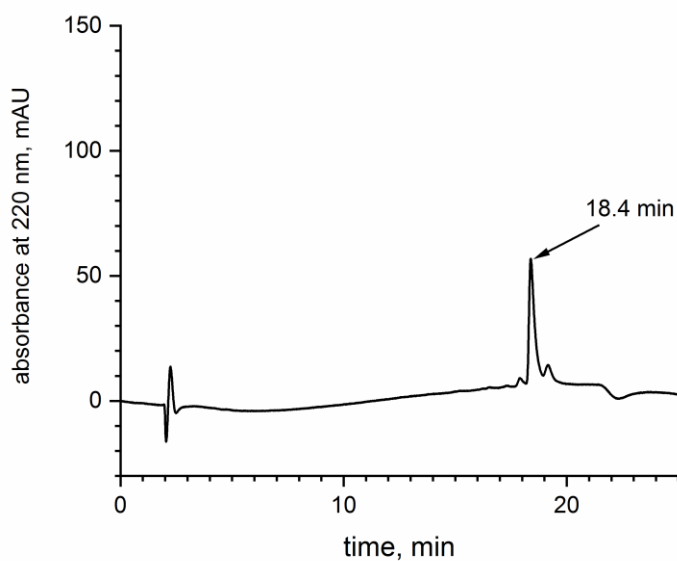
Peptide 4

sequence: H₃CCO-(Oic₄-TfmPro-Oic₄)-OCH₃

¹H NMR spectrum (MeOD, 700 MHz), stimulated echo:



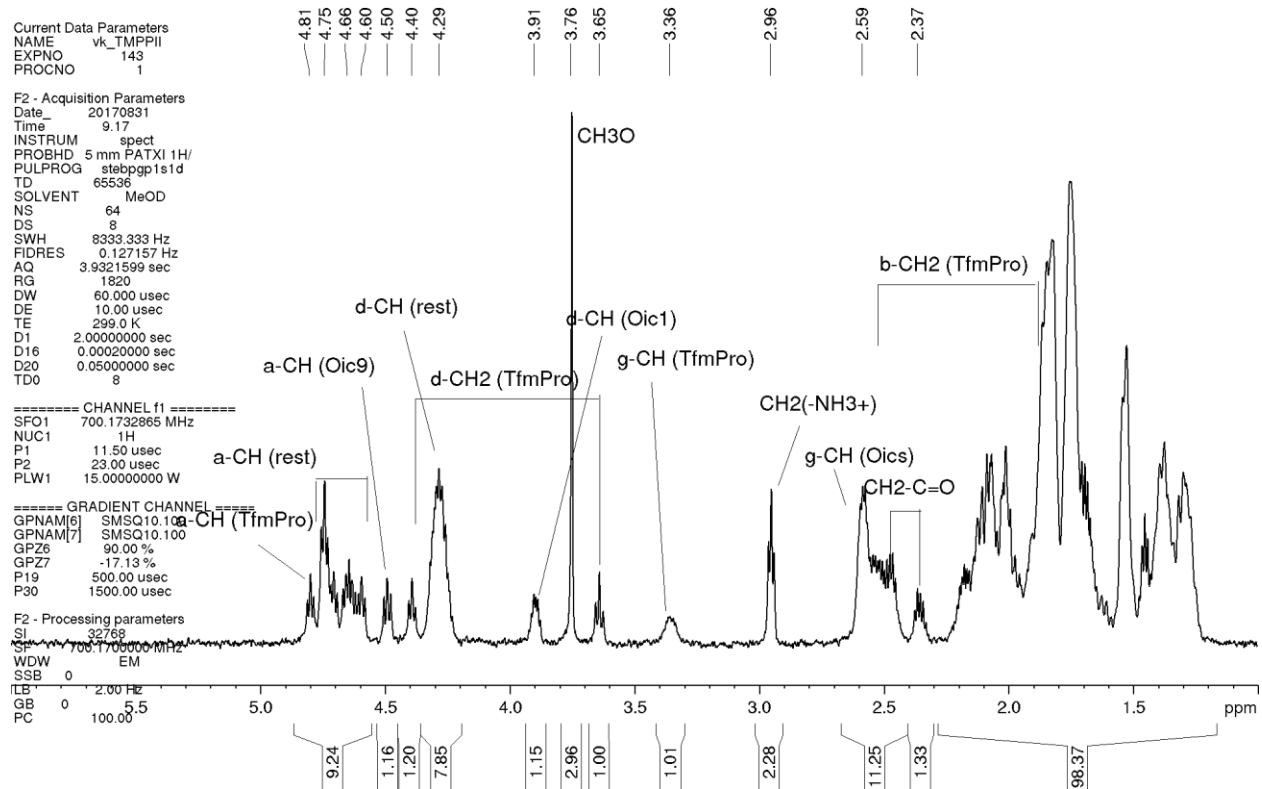
RP-HPLC chromatogram:



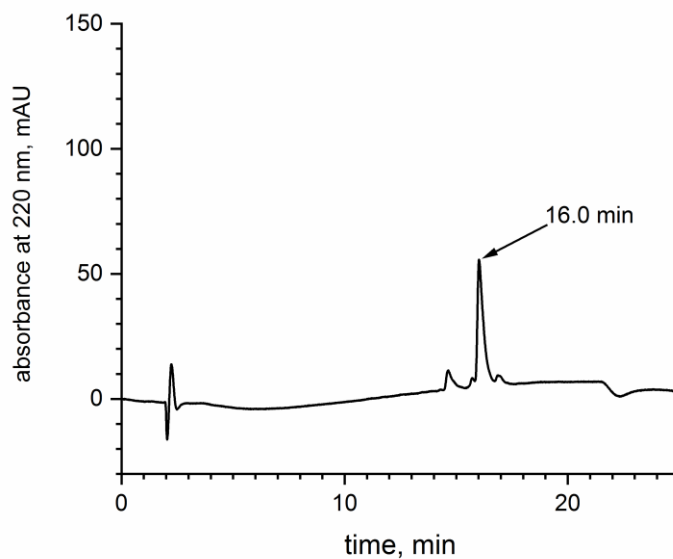
Peptide 5

sequence: $\text{H}_3\text{N}^+(\text{CH}_2)_5\text{CO}-(\text{Oic}_4\text{-TfmPro-Oic}_4)\text{-OCH}_3, \text{Cl}^-$

^1H NMR spectrum (MeOD, 700 MHz), stimulated echo:



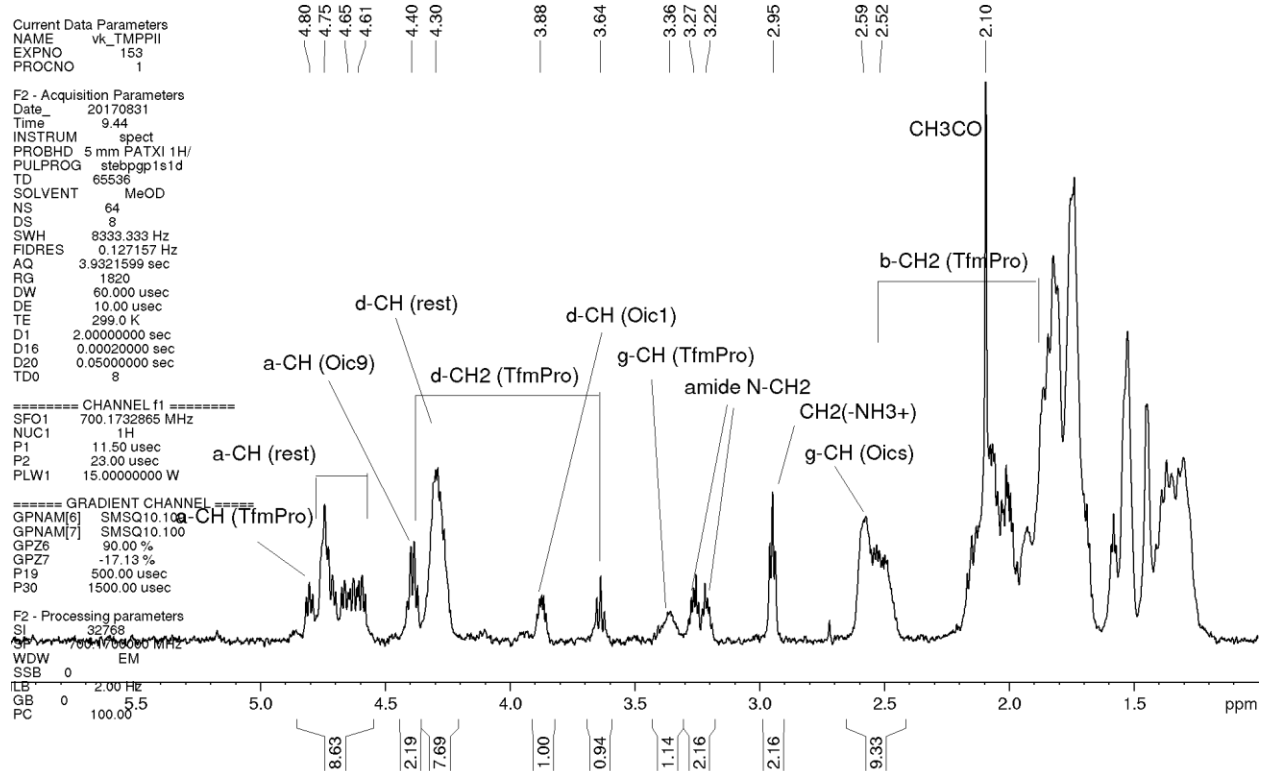
RP-HPLC chromatogram:



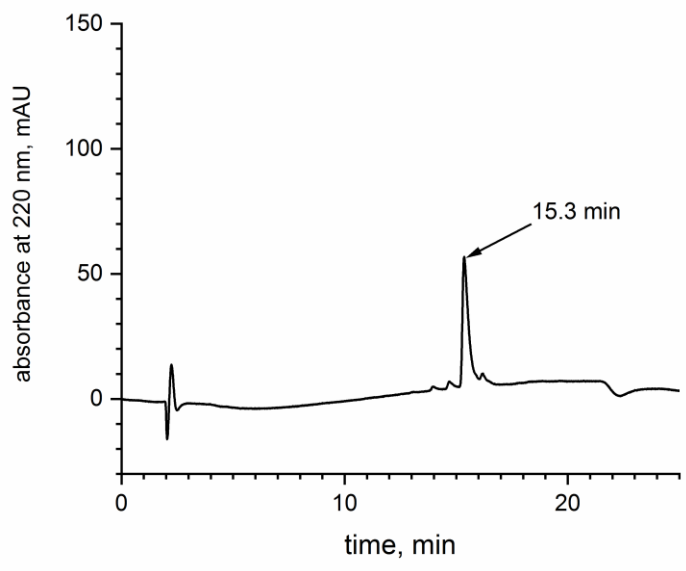
Peptide 6

sequence: $\text{H}_3\text{CCO}-(\text{Oic}_4\text{-TfmPro-Oic}_4)\text{-NH}(\text{CH}_2)_6\text{N}^+\text{H}_3, \text{Cl}^-$

^1H NMR spectrum (MeOD, 700 MHz), stimulated echo:



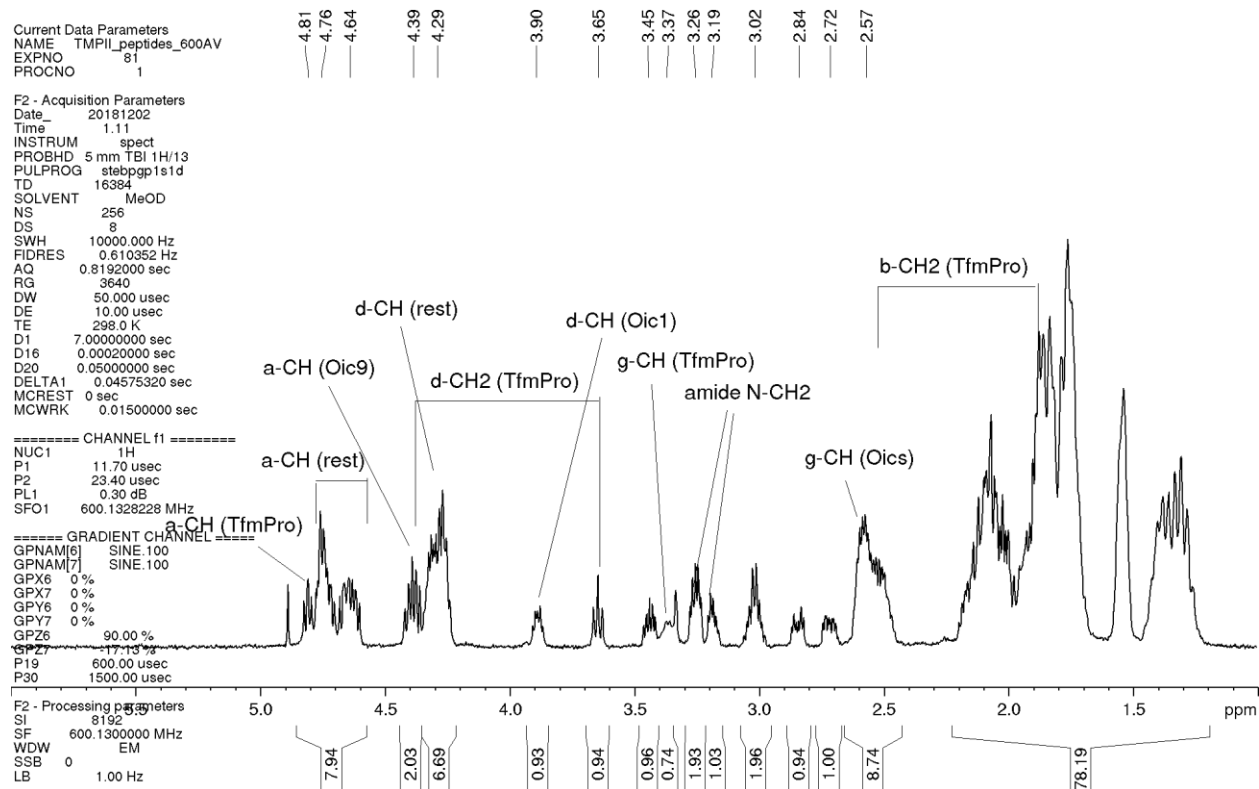
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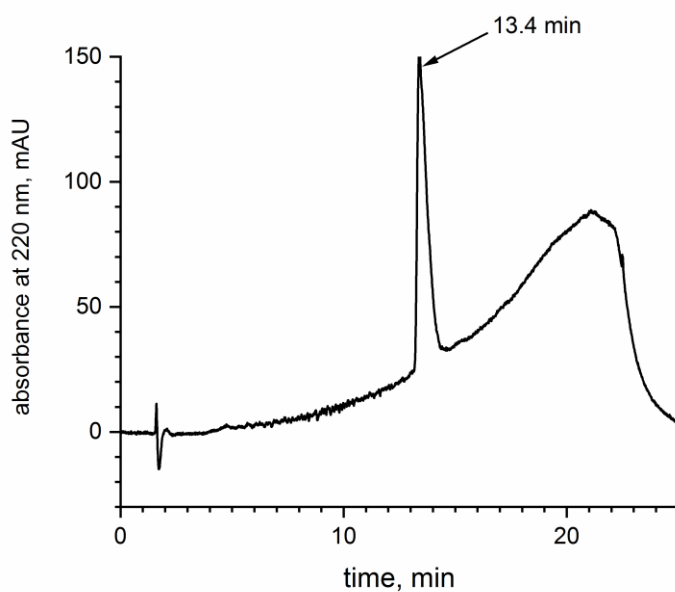
Peptide 7

sequence: $\text{H}_3\text{N}^+(\text{CH}_2)_2\text{CO}-(\text{Oic}_4\text{-TfmPro-Oic}_4)\text{-NH}(\text{CH}_2)_3\text{N}^+\text{H}_3, 2\text{Cl}^-$

^1H NMR spectrum (MeOD, 600 MHz), stimulated echo:



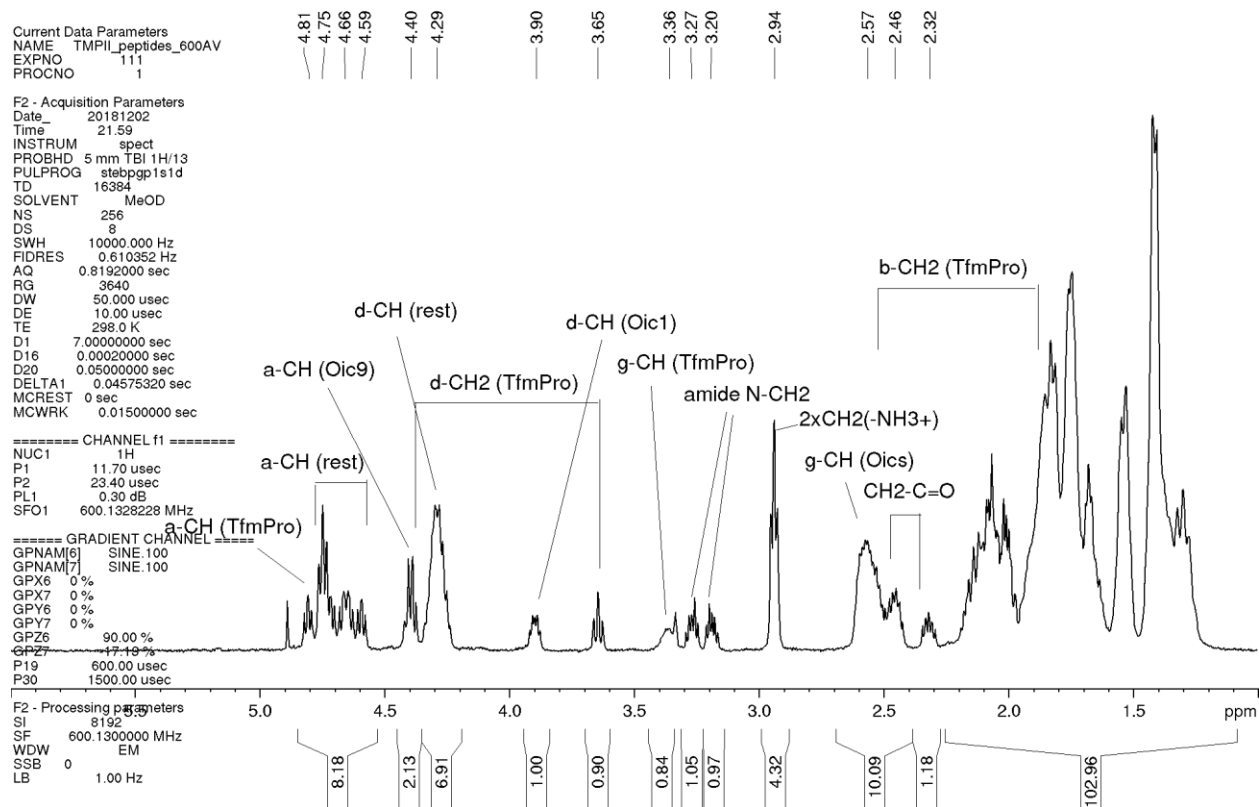
RP-HPLC chromatogram:



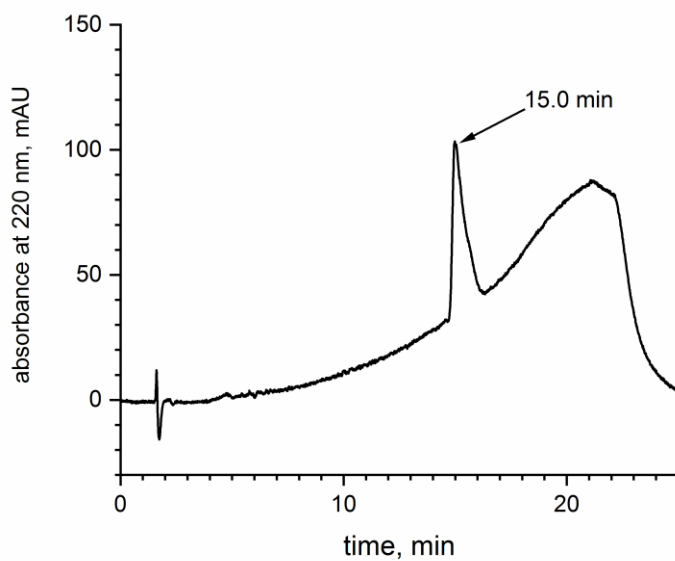
Peptide 8

sequence: $\text{H}_3\text{N}^+(\text{CH}_2)_7\text{CO}-(\text{Oic}_4\text{-TfmPro-Oic}_4)\text{-NH}(\text{CH}_2)_8\text{N}^+\text{H}_3, 2\text{Cl}^-$

^1H NMR spectrum (MeOD, 600 MHz), stimulated echo:



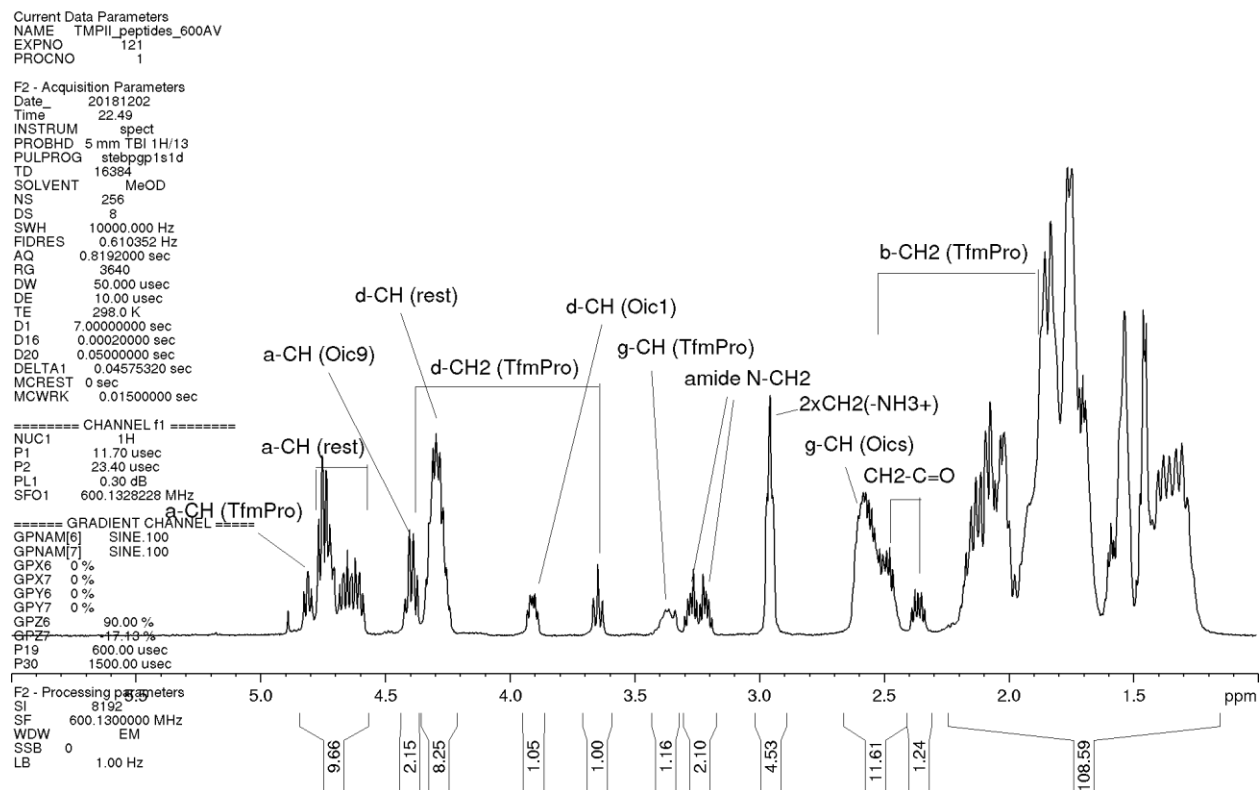
RP-HPLC chromatogram:



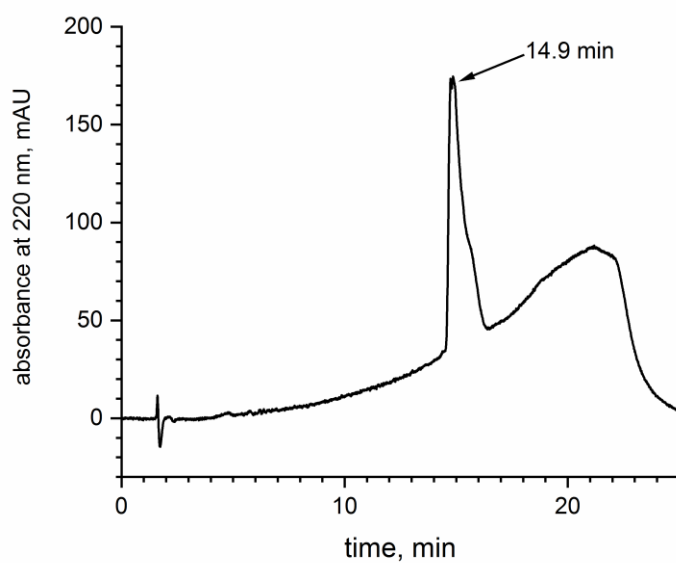
Peptide 9

sequence: $\text{H}_3\text{N}^+(\text{CH}_2)_5\text{CO}-(\text{Oic}_5\text{-TfmPro-Oic}_4)\text{-NH}(\text{CH}_2)_6\text{N}^+\text{H}_3, 2\text{Cl}^-$

^1H NMR spectrum (MeOD, 600 MHz), stimulated echo:



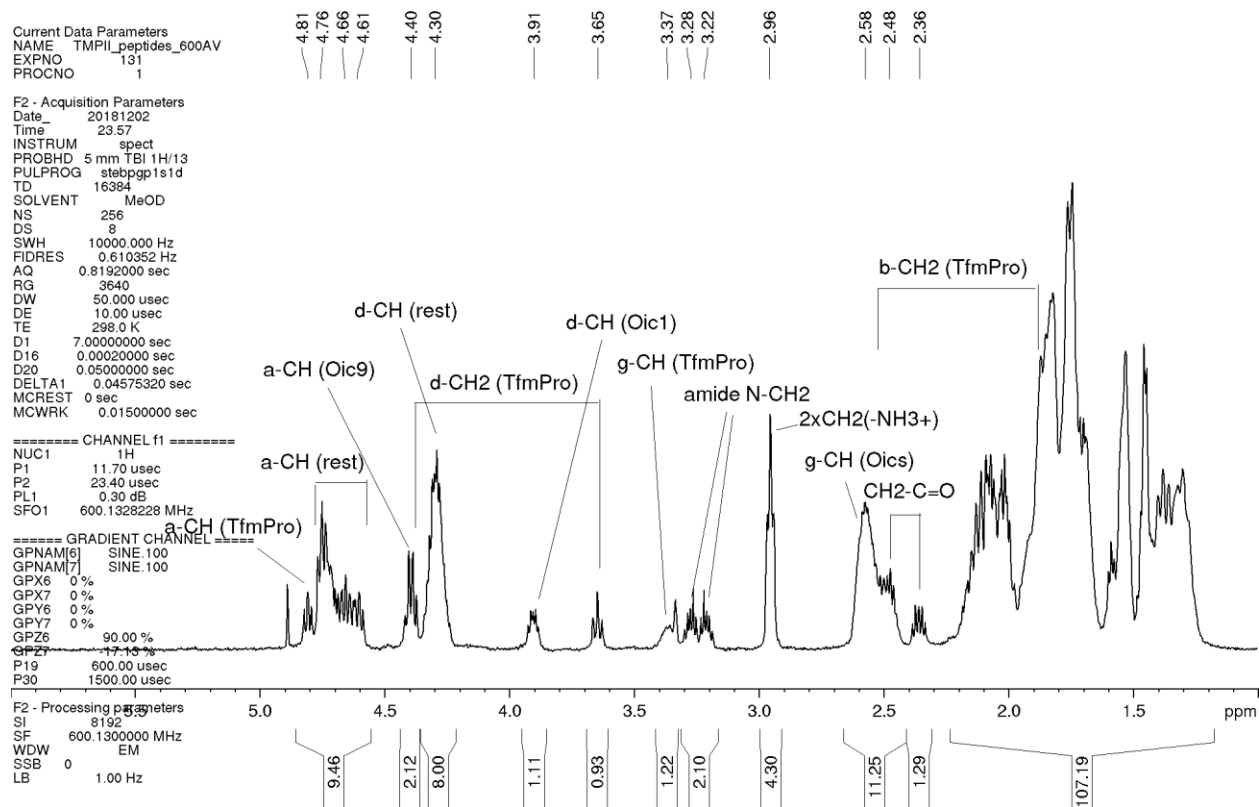
RP-HPLC chromatogram:



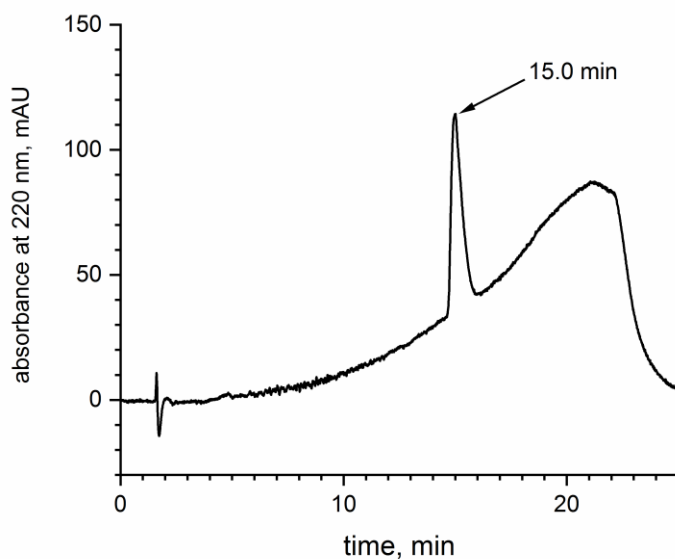
Peptide 10

sequence: $\text{H}_3\text{N}^+(\text{CH}_2)_5\text{CO}-(\text{Oic}_4\text{-TfmPro-Oic}_5)\text{-NH}(\text{CH}_2)_6\text{N}^+\text{H}_3, 2\text{Cl}^-$

^1H NMR spectrum (MeOD, 600 MHz), stimulated echo:



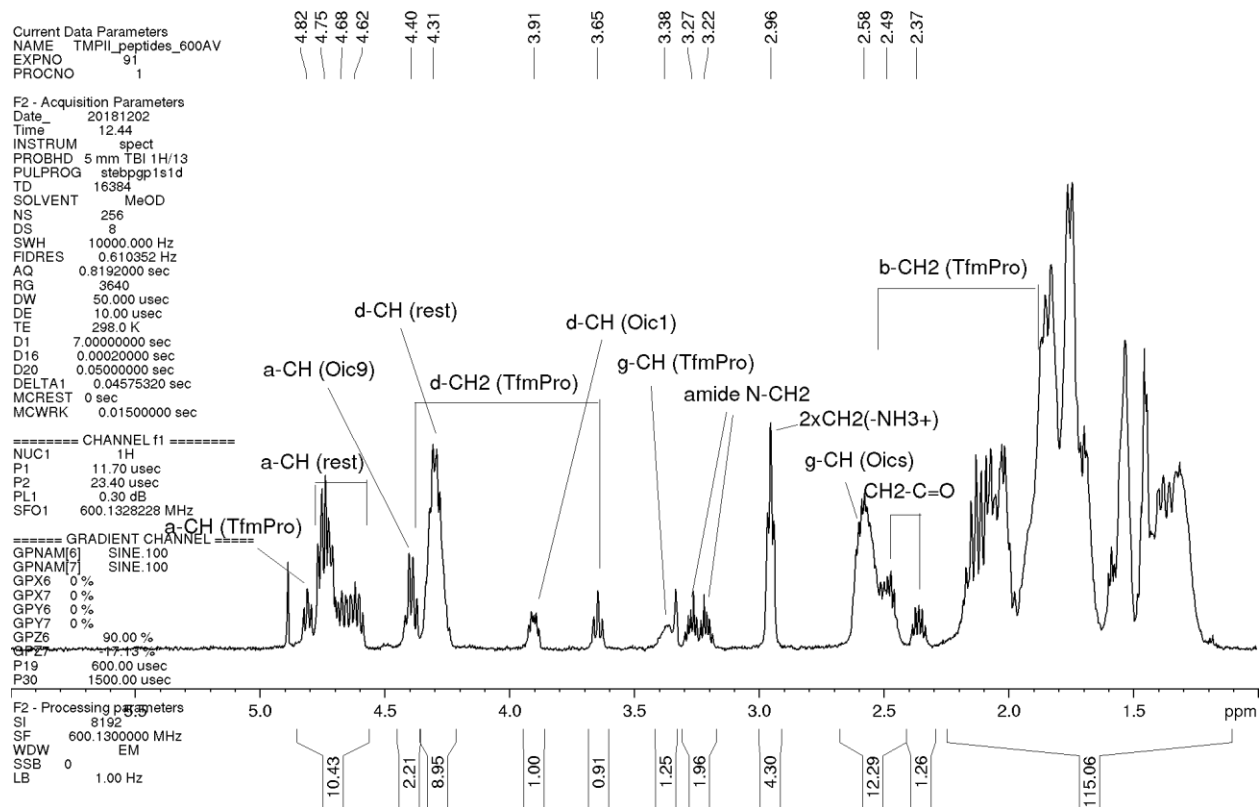
RP-HPLC chromatogram:



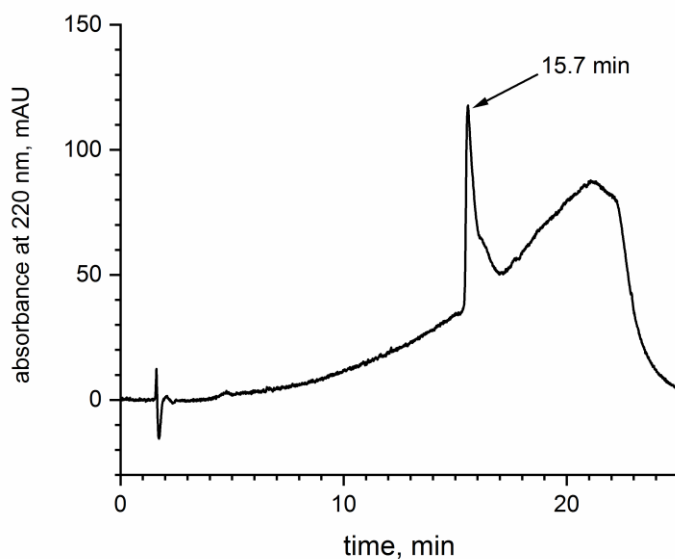
Peptide 11

sequence: $\text{H}_3\text{N}^+(\text{CH}_2)_5\text{CO}-(\text{Oic}_5\text{-TfmPro-Oic}_5)\text{-NH}(\text{CH}_2)_6\text{N}^+\text{H}_3, 2\text{Cl}^-$

^1H NMR spectrum (MeOD, 600 MHz), stimulated echo:



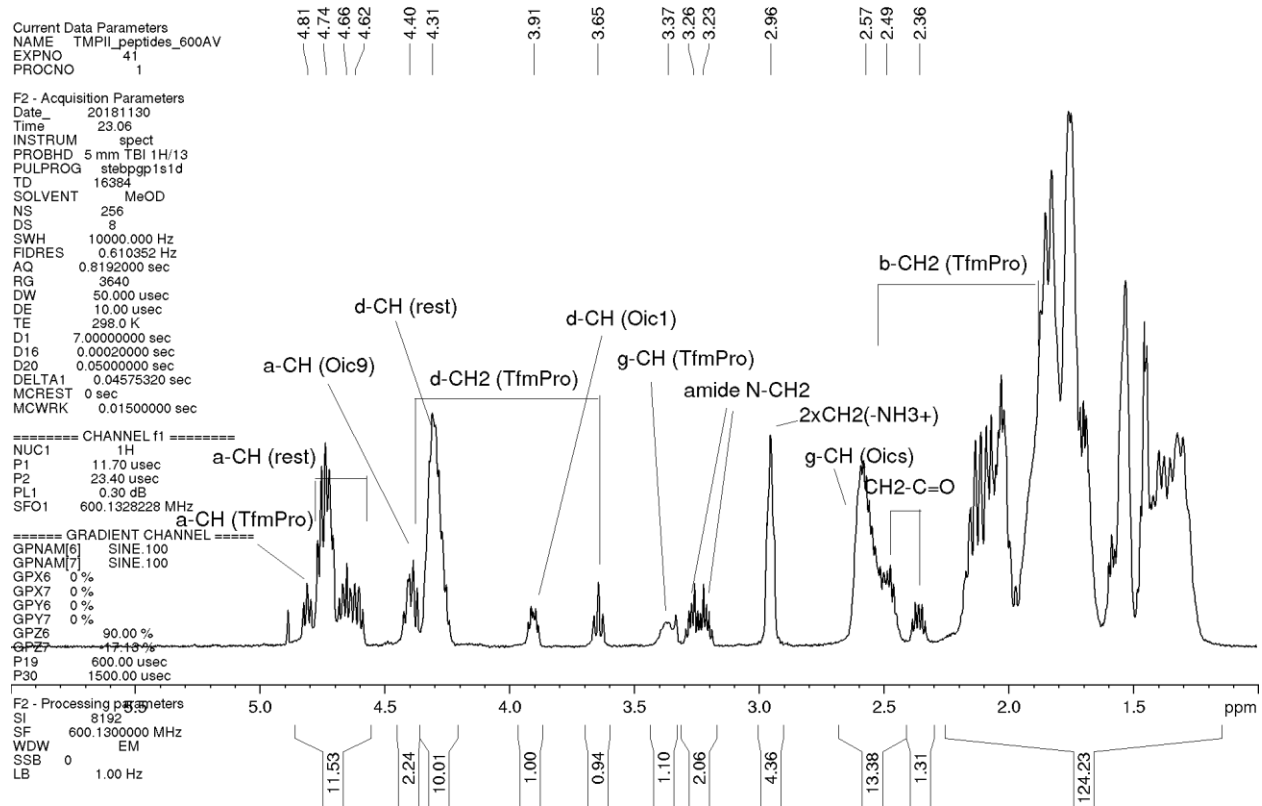
RP-HPLC chromatogram:



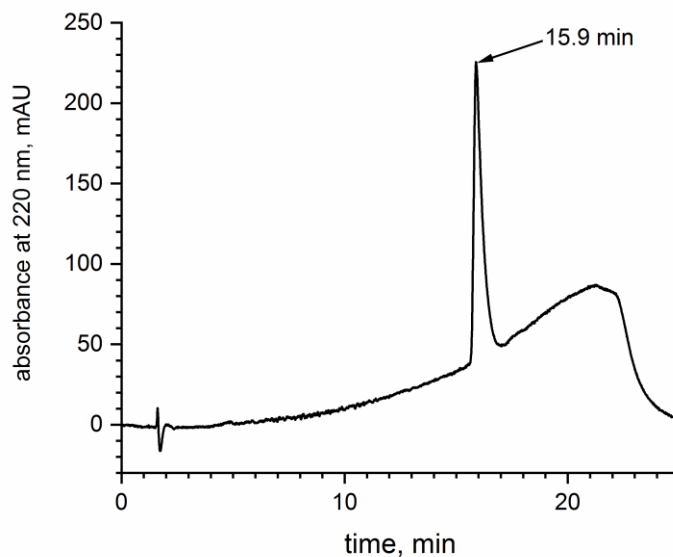
Peptide 12

sequence: $\text{H}_3\text{N}^+(\text{CH}_2)_5\text{CO}-(\text{Oic}_7\text{-TfmPro-Oic}_4)\text{-NH}(\text{CH}_2)_6\text{N}^+\text{H}_3, 2\text{Cl}^-$

^1H NMR spectrum (MeOD, 600 MHz), stimulated echo:



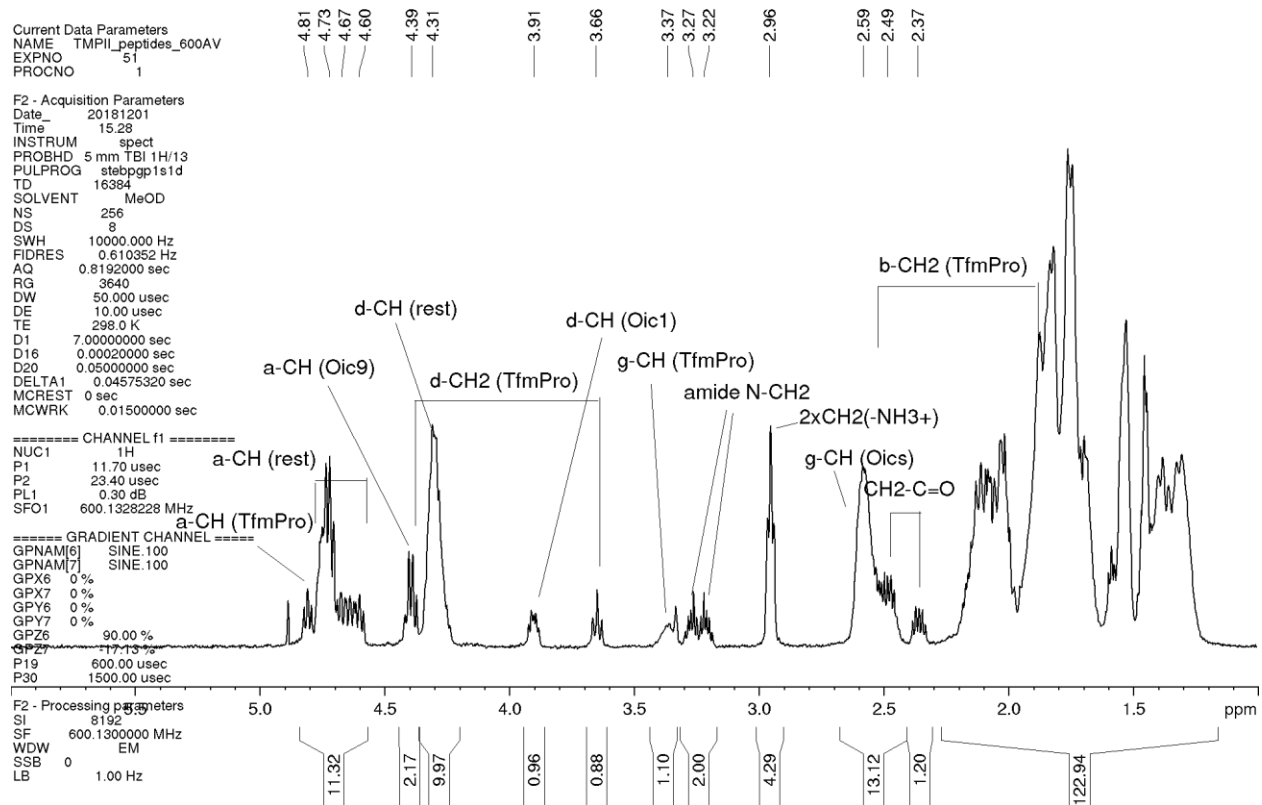
RP-HPLC chromatogram:



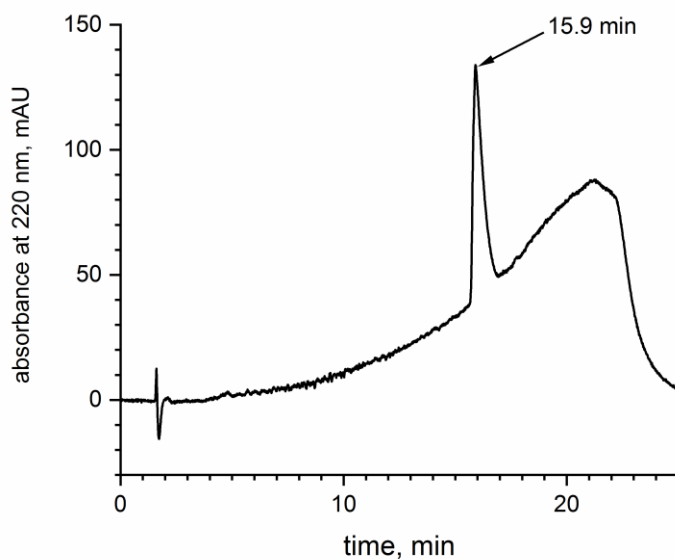
Peptide 13

sequence: $\text{H}_3\text{N}^+(\text{CH}_2)_5\text{CO}-(\text{Oic}_4\text{-TfmPro-Oic}_7)\text{-NH}(\text{CH}_2)_6\text{N}^+\text{H}_3, 2\text{Cl}^-$

^1H NMR spectrum (MeOD, 600 MHz), stimulated echo:



RP-HPLC chromatogram:



Lineshape analysis of the solid-state NMR spectra

The solid-state ^{19}F and ^{31}P NMR spectra of peptide **11** were fitted to determine the different contributions to the spectra. Solid-state ^{19}F NMR spectra were simulated for two triplets, accounting for the two peptide orientation states (TM and SM). Solid-state ^{31}P NMR spectra were calculated assuming a single lipid component. A distribution of local bilayer orientations, e.g., due to mosaic spread, was included in the fitting model. The distribution was formed by two parts: a well-oriented fraction described by a Gaussian distribution of the membrane normal, and a fraction of non-oriented bilayers with a distribution consisting of a powder distribution and a Gaussian distribution with a large width of 20° . For all distributions, the same orientation of the peptide with respect to the local membrane normal, i.e. the same dipolar splitting and chemical shift position corresponding to a perfectly aligned membrane, was used to calculate the resulting lineshape. Rotational diffusion of the peptide around the membrane normal was assumed for all fractions, which is justified as the ^{19}F NMR spectra changed accordingly when changing the sample orientation by 90° . The parameters (width of the mosaic spread, portion of powder spectrum, splitting and chemical shift for perfectly aligned membranes) were adapted until a best fit was obtained. The spectra with the respective fits are displayed in Fig. S1, the best fit parameters are summarized in Table S2. As can be seen, the non-oriented fraction depends on the lipid chain length. As short chain lipids exhibit the largest broadening, it seems plausible that the positive mismatch between peptide and lipid hydrophobic thickness causes the observed perturbation of the membrane alignment. The broadening of ^{19}F and ^{31}P NMR spectra correlate, hence it seems that both peptide orientation and bilayer orientation are affected.

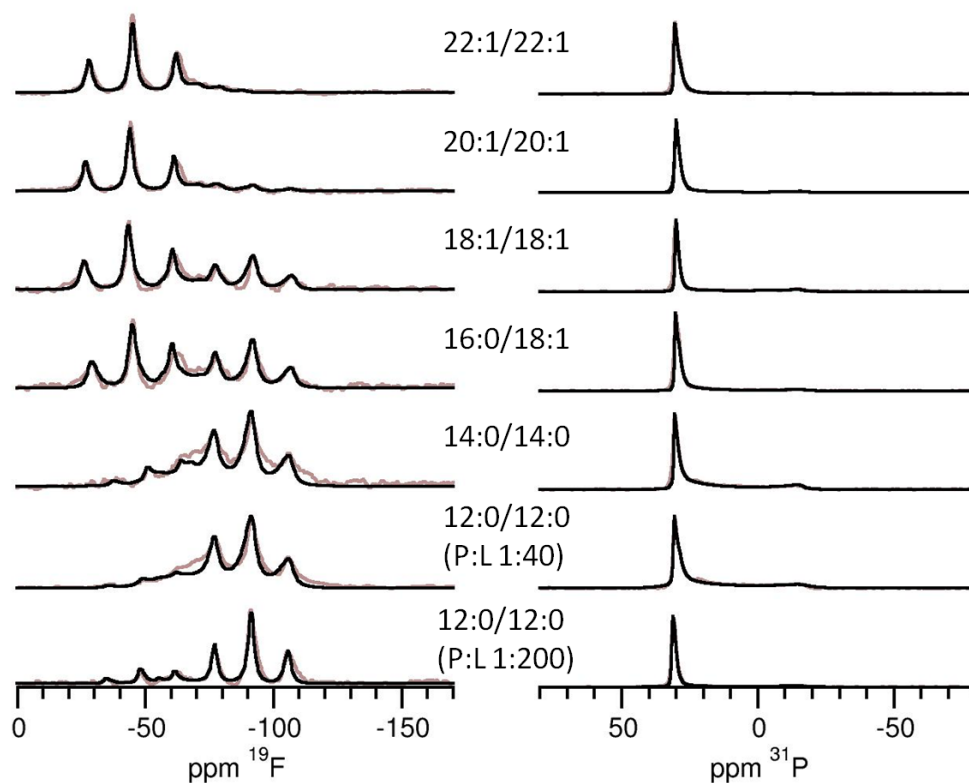


Fig. S1. Lineshape analysis of ^{19}F NMR spectra (left) and ^{31}P NMR spectra (right) of peptide **11** in lipids with different chains (as indicated). All peptides were reconstituted at a lipid:peptide ratio (P/L) as indicated. The fitted spectra are in black, the experimental spectra are in gray.

Table S2. Parameters used for fitting the spectra of peptide **11** in various lipids. The ^{19}F NMR spectra were fitted with two components (TM and SM). A Gaussian distribution with a standard deviation referred to as mosaic spread, and a non-oriented fraction were used to fit the line broadening.

lipid	P:L ratio	TM:SM ratio	^{19}F , peptide TM		^{19}F , peptide SM		^{31}P , lipids	
			mosaic spread	non- oriented fraction	mosaic spread	non- oriented fraction	mosaic spread	non- oriented fraction
22:1/22:1	1:40	0:100	n.a.	n.a.	4°	0.25	9°	0.18
20:1/20:1	1:40	10:90	9°	0	4°	0.22	8°	0.27
18:1/18:1	1:40	42:58	9°	0.22	6°	0.17	7°	0.31
16:0/18:1	1:40	50:50	9°	0.25	8°	0.17	8°	0.30
14:0/14:0	1:40	90:10	10°	0.34	10°	0.71	8°	0.57
12:0/12:0	1:40	97:3	10°	0.43	n.d.	n.d.	9°	0.58
12:0/12:0	1:200	86:14	5°	0.20	10°	0	7°	0.31

References

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- ⁵³ K. Barlos, D. Gatos, J. Kallitsis, G. Papaphotiu, P. Sotiriu, Y. Wenqing and W. Schäfer, *Tetrahedron Lett.*, 1989, **30**, 3943-3946. doi: 10.1016/S0040-4039(00)99290-6
- ⁵⁴ R. Bollhagen, M. Schmiedberger, K. Barlos, E. Grell, *Chem. Commun.*, 1994, 2559-2560. doi: 10.1039/C39940002559