Electronic Supplementary Material (ESI) for RSC Advances.

Supporting information

Selective Homodimerization of Unprotected Peptides Using Hybrid Hydroxydimethylsilane Derivatives

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Abbreviations

ACN, acetonitrile; Alloc, allyloxycarbonyl; Boc, t-butyloxycarbonyl; CM, ChemMatrix; DCM, dichloromethane; DEPT, distortionless enhancement by polarization transfer; DIEA, diisopropylethylamine; DMF, N-N'-dimethylformamide; DMSO, dimethylsylfoxide; DPBS, Dulbelcco's phosphate buffered saline; ESI-MS, electrospray ionization mass spectrometry; Fmoc, fluorenylmethoxycarbonyl; GHRP-6, growth hormone releasing hexapeptide; HBTU, ***N,N,N',N'*-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate; HFIP, hexafluoroisopropanol; HTRF, homogenous time resolved fluorescence;** ICPDMCS, 3-isocyanatopropyldimethylchlorosilane; IP1, inositol monophosphate; LC/MS, tandem liquid chromatography/ mass spectrometry; MW, micro-wave; NMP, N-methyl-2-pyrrolidone; NMR, nuclear magnetic resonance; pip, piperidine; PS, polystyrene; **RP-**HPLC, reversed phase high performance liquid chromatography; RT, room temperature; SPPS, solid phase peptide synthesis; TFA, trifluoroacetic acid; THF, tetrahydrofurane; TIS, triisopropylsilane; Trt, trityl; UV, ultra-violet. Other abbreviations used were those recommended by the IUPAC-IUB Commission (Eur. J. Biochem. 1984, 138, 9-37).

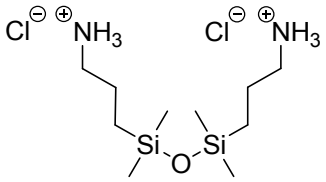
Material and Method

All solvents and reagents were used as supplied. Solvents used for LC/MS were of HPLC grade. Dichloromethane (DCM); N,N-dimethylformamide (DMF) were obtained from Carlo Erba. Fmoc amino acid derivatives, [benzotriazol-1-yloxy(dimethylamino)methylidene]- dimethylazanium hexafluorophosphate (HBTU), Fmoc-Rink amide CM resin, 2-chloro chlorotrityl PS resin, were purchased from Iris Biotech (Marktredwitz, Germany). Diisopropylcarbodiimide (DIC), diisopropylethylamine (DIEA), trifluoroacetic acid (TFA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Hexafluoroisopropanol (HFIP) and triisopropylsilane (TIS) were obtained from Alfa Aeser and Acros respectively. NMR solvents were obtained from Euriso-top.

Samples for LC/MS analyses were prepared in acetonitrile/water (50:50, v/v) mixture, containing 0.1% TFA. The LC/MS system consisted of a Waters Alliance 2695 HPLC, coupled to a Water Micromass ZQ spectrometer (electrospray ionization mode, ESI+). All the analyses were carried out using a Phenomenex Onyx, 25 x 4.6 mm reversed-phase column. A flow rate of 3 mL/min and a gradient of (0-100)% B over 2.5 min were used. Eluent A: water/0.1% HCO2H; eluent B: acetonitrile/0.1% HCO2H. UV detection was performed at 214 nm. Electrospray mass spectra were acquired at a solvent flow rate of 200 µL/min. Nitrogen was used for both the nebulizing and drying gas. The data were obtained in a scan mode ranging from 100 to 1000 m/z or 250 to 1500 m/z to in 0.7 sec intervals.

High Resolution Mass Spectrometric analyses were performed with a Synapt G2-S (Waters) mass spectrometer fitted with an Electrospray Ionisation source. All measurements were performed in the positive ion mode. Capillary voltage: 1000 V; cone voltage: 30 V; source temperature: 120°C; desolvation temperature: 250°C. The data were obtained in a scan mode ranging from 100 to 1500 m/z.

1,3-bis(3-aminopropyl)tetramethyl disiloxane hydrochloride 1a



Commercially available 1,3-bis(3-aminopropyl)tetramethyl disiloxane (500 µL, 1.8 mmol) was poured into 1 M hydrogen chloride ether solution (7.2 mL, 7.2 mmol, 4 eq) cooled in an ice bath. 1,3-bis(3-aminopropyl)tetramethyl disiloxane hydrochloride precipitated immediately. Diethyl ether was removed under reduced pressure to yield compound **1a** as a white powder (100%).

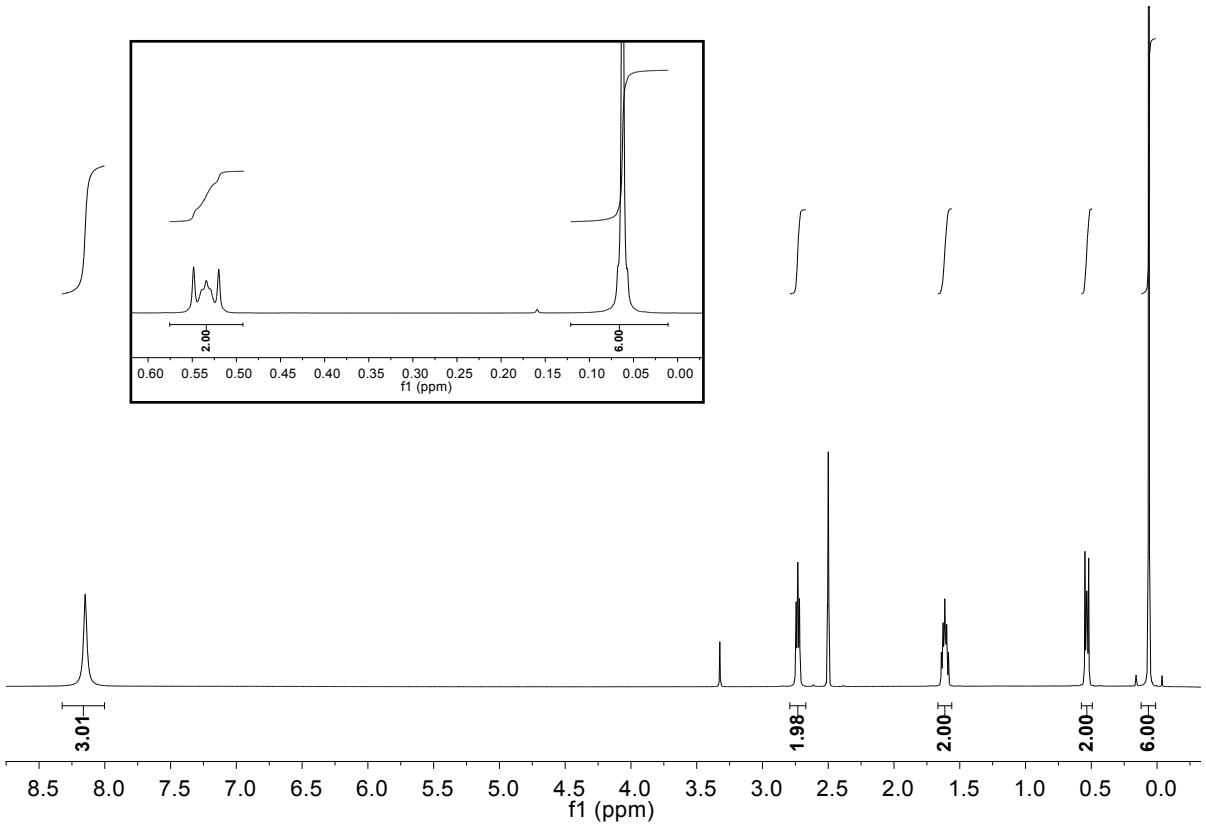


Figure S1. *^{1}H NMR spectrum of **1a** in DMSO-d_6 (600 MHz)*

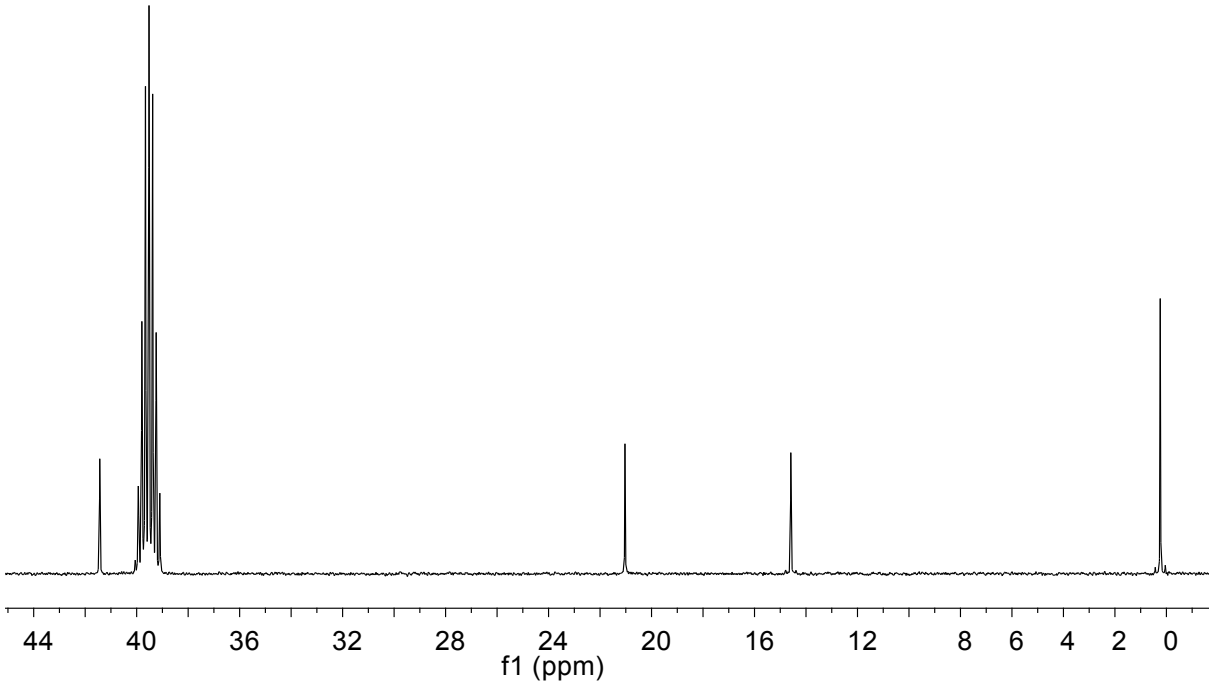


Figure S2. *^{13}C NMR spectrum of **1a** in DMSO-d_6 (151 MHz)*

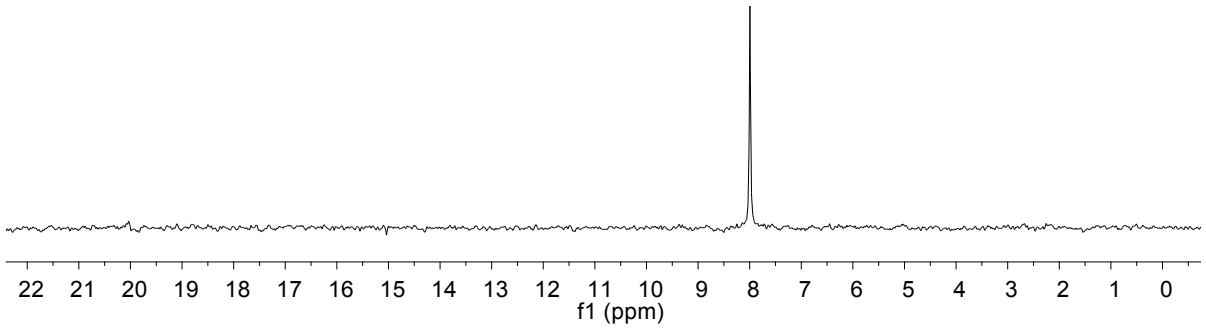


Figure S3. *^{29}Si NMR spectrum of **1a** in DMSO-d_6 (119 MHz)*

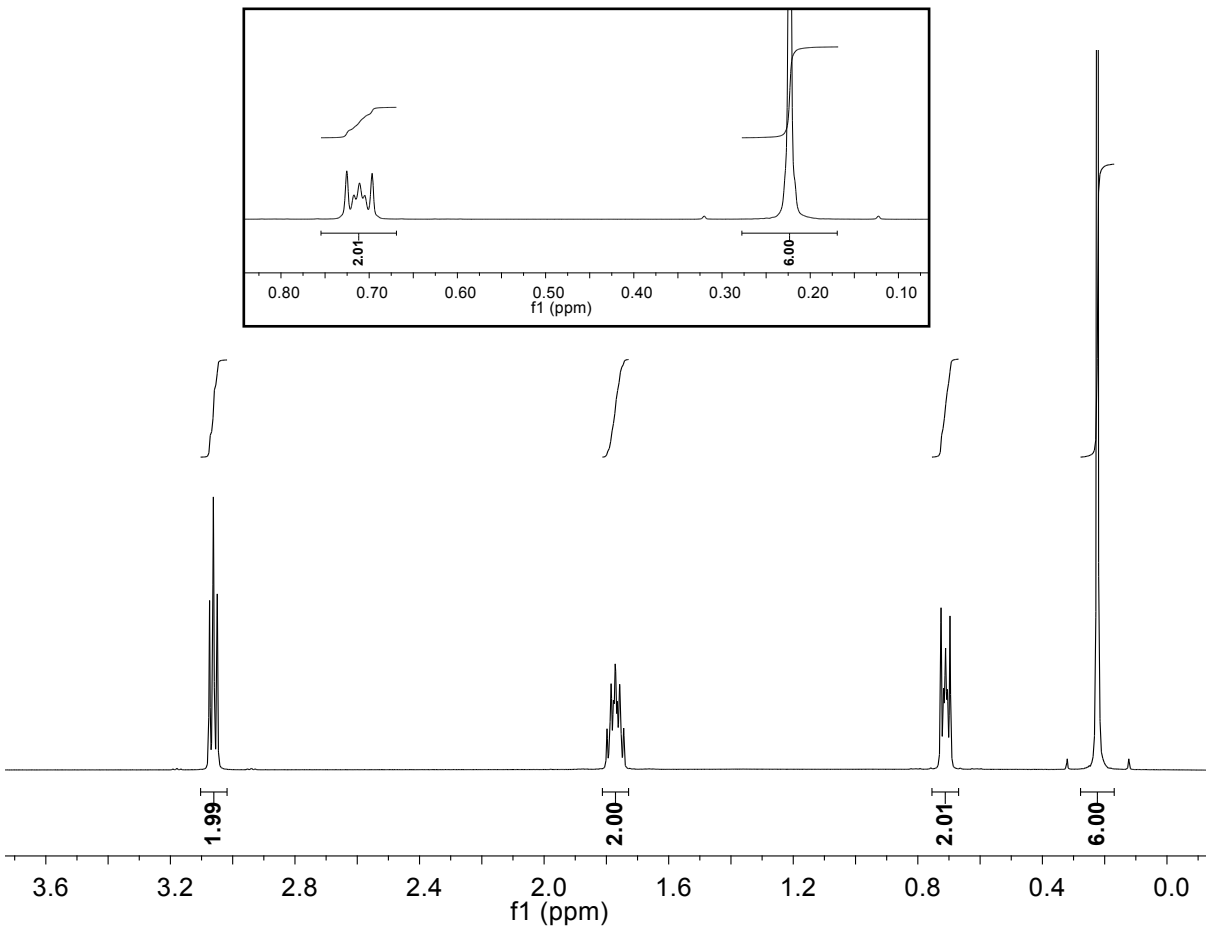


Figure S4. *^{1}H NMR spectrum of **1a** in D_2O (600 MHz)*

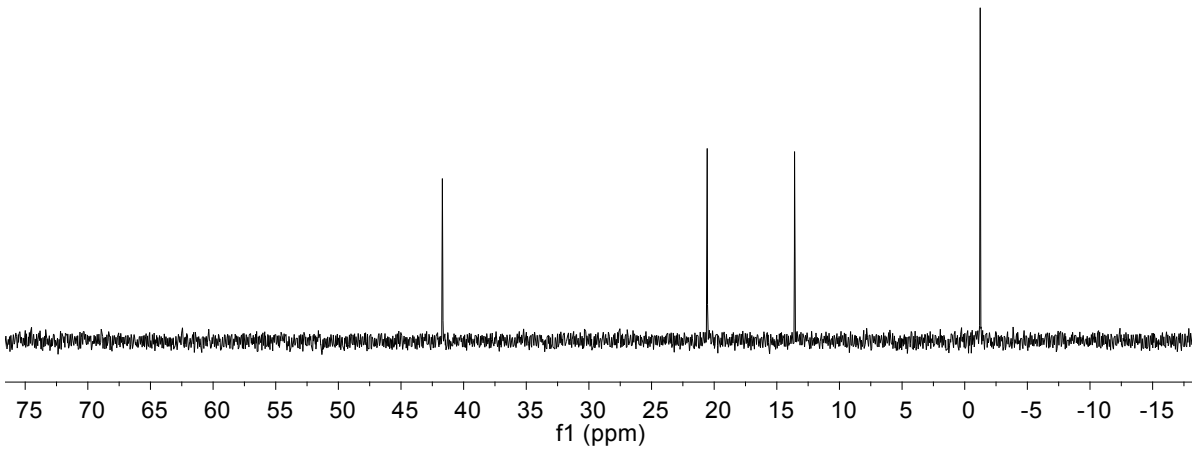


Figure S5. *^{13}C NMR spectrum of **1a** in D_2O (151 MHz)*

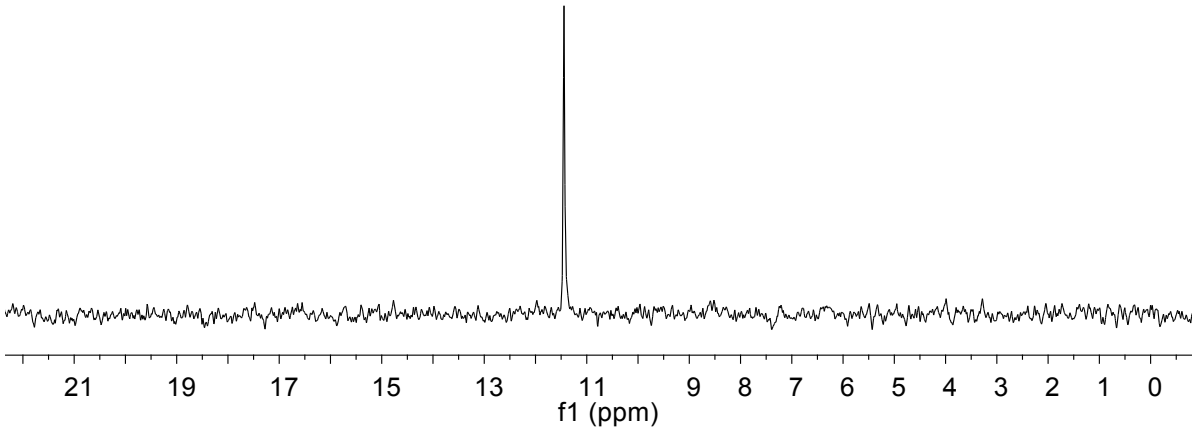
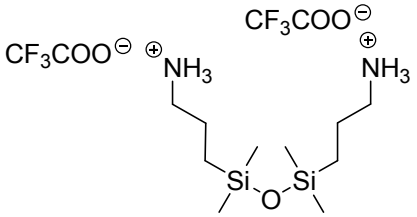


Figure S6. *^{29}Si NMR spectrum of **1a** in D_2O (119 MHz)*

1,3-bis(3-aminopropyl)tetramethyl disiloxane trifluoroacetate 1b



Trifluoroacetic acid (220 µL, 2.89 mmol, 4 eq) was added on 1,3-bis(3-aminopropyl)tetramethyl disiloxane (200 µL, 0.722 mmol) in diethyl ether (2 mL) on an ice bath. Diethyl ether and TFA excess were removed under reduced pressure to yield compound **1b** as a colorless oil (100%).

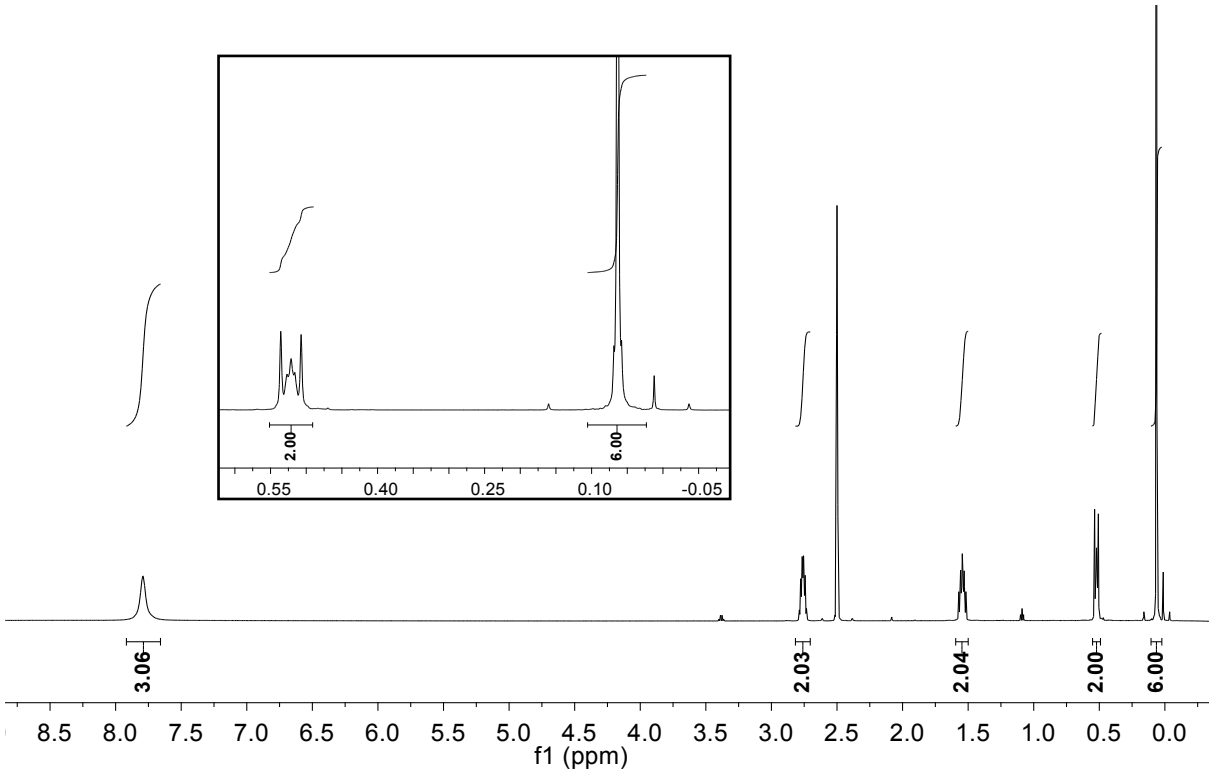


Figure S7. *^{1}H NMR spectrum of **1b** in DMSO-d_6 (600 MHz)*

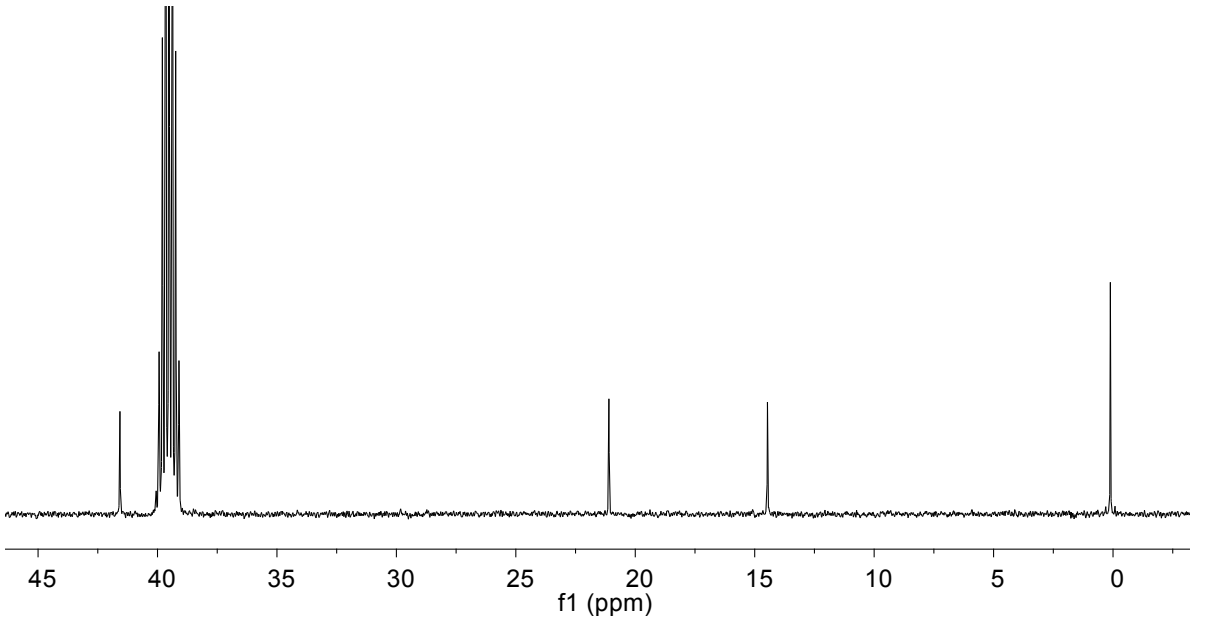


Figure S8. *^{13}C NMR spectrum of **1b** in DMSO-d_6 (151 MHz)*

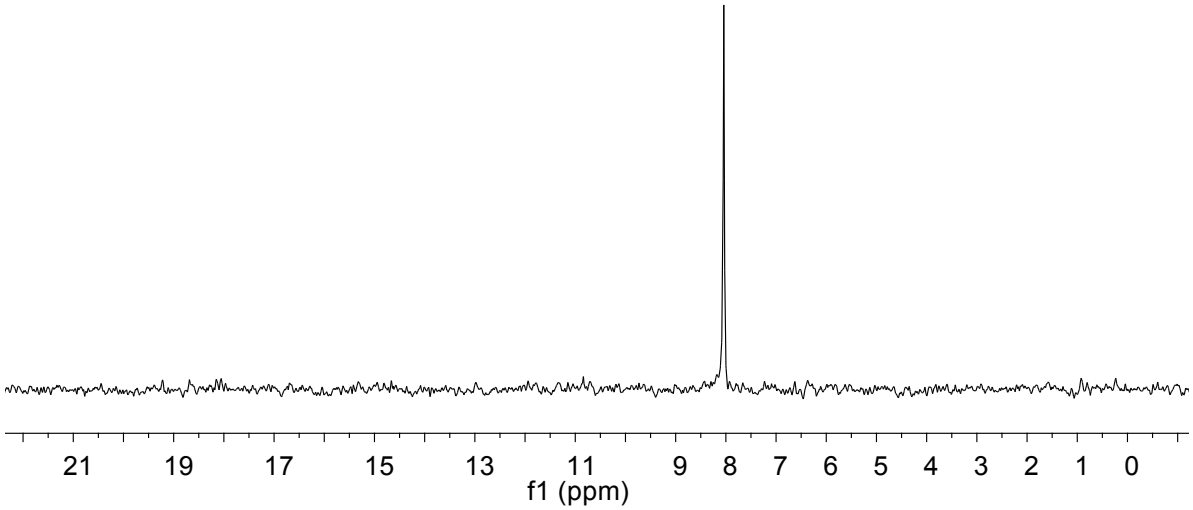
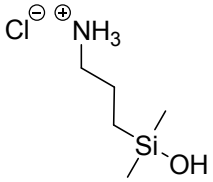


Figure S9. *^{29}Si NMR spectrum of **1b** in DMSO-d_6 (119 MHz)*

(3-aminopropyl)dimethylsilanol hydrochloride 2a



On the one hand, DCl 35% in D_2O (6.00 µL) were added to **1a** (20 mg) in DMSO-d_6 (600 µL) to trigger **1a** hydrolysis into **2a**. The **1a**/**2a** mixture in DMSO-d_6 was analyzed by NMR.

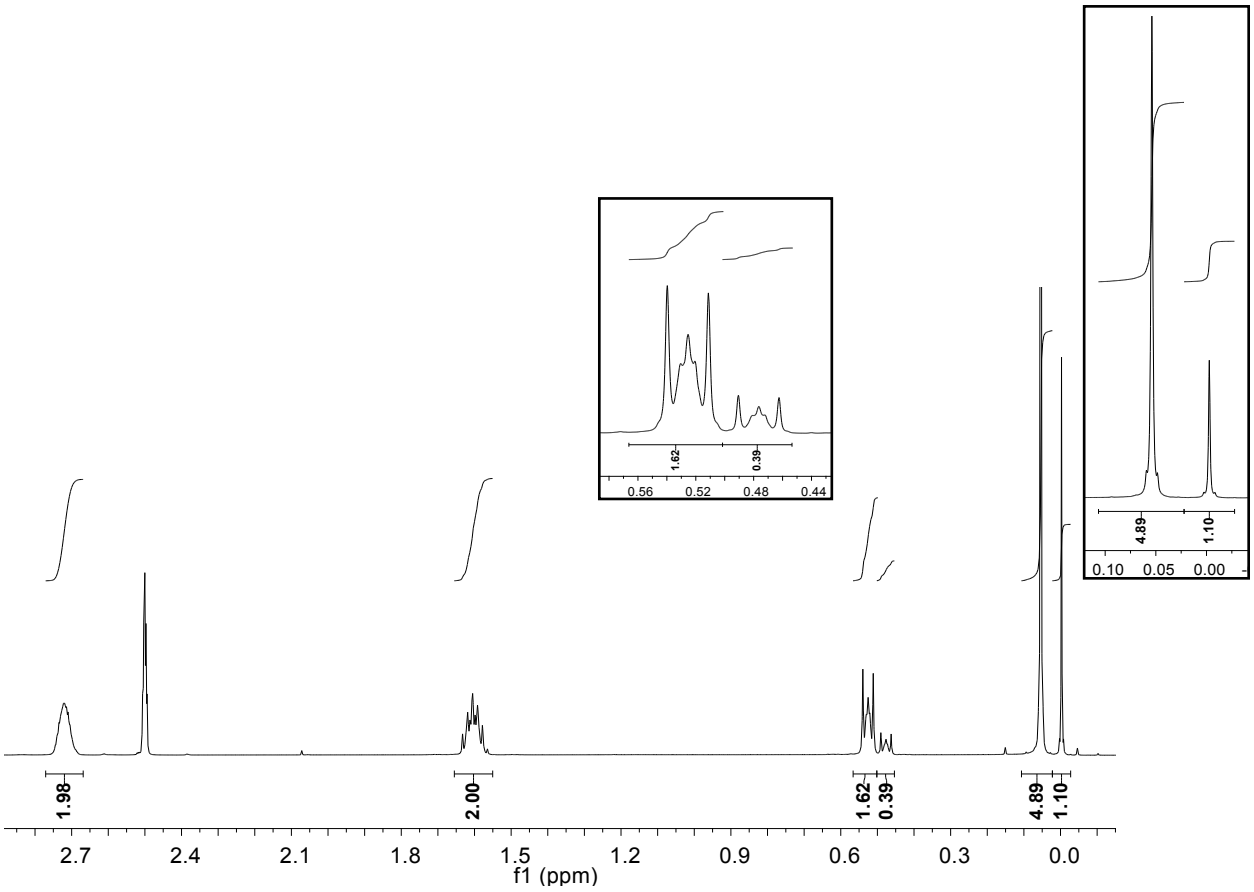


Figure S10. *^{1}H NMR spectrum of **1a/2a** in DMSO-d_6 (600 MHz)*

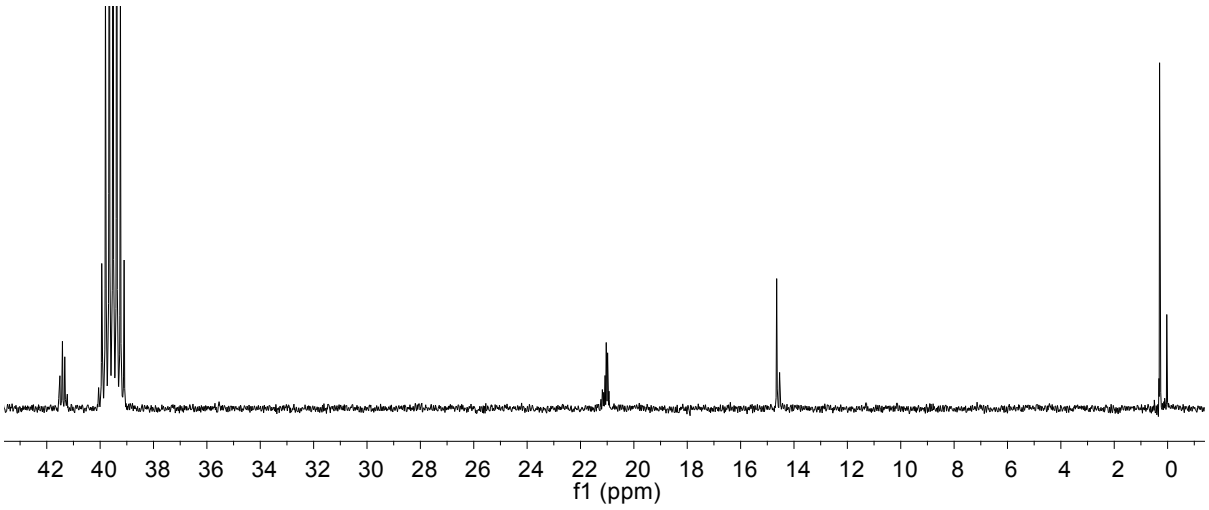


Figure S11. *^{13}C NMR spectrum of **1a/2a** in DMSO-d_6 (151 MHz)*

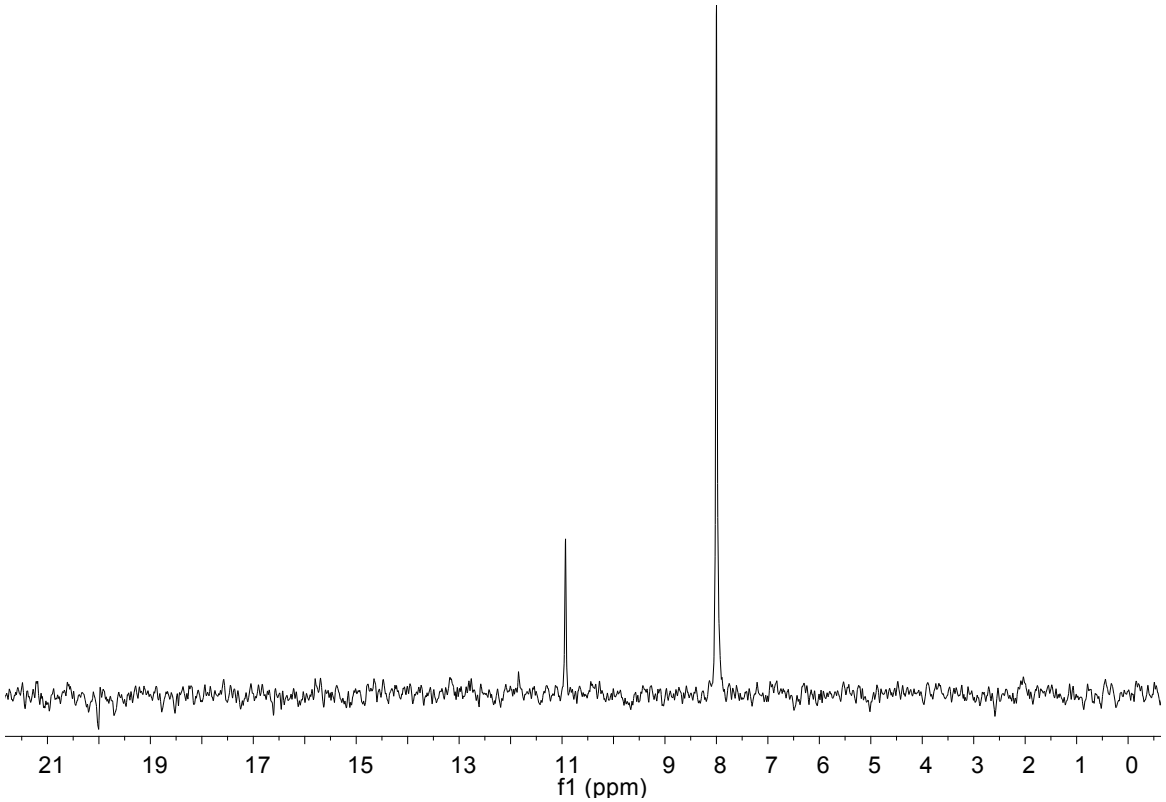


Figure S12. *^{29}Si NMR spectrum of **1a/2a** in DMSO-d_6 (119 MHz)*

On the other hand, 1,3-bis(3-aminopropyl)tetramethyl disiloxane hydrochloride **1a** (20 mg) was solubilized in D_2O (600 µL) and the solution was left aside for 8 weeks to allow **1a** hydrolysis into **2a**. **2a** solution in D_2O was analyzed by NMR. Traces of **1a** are still visible on NMR spectra.

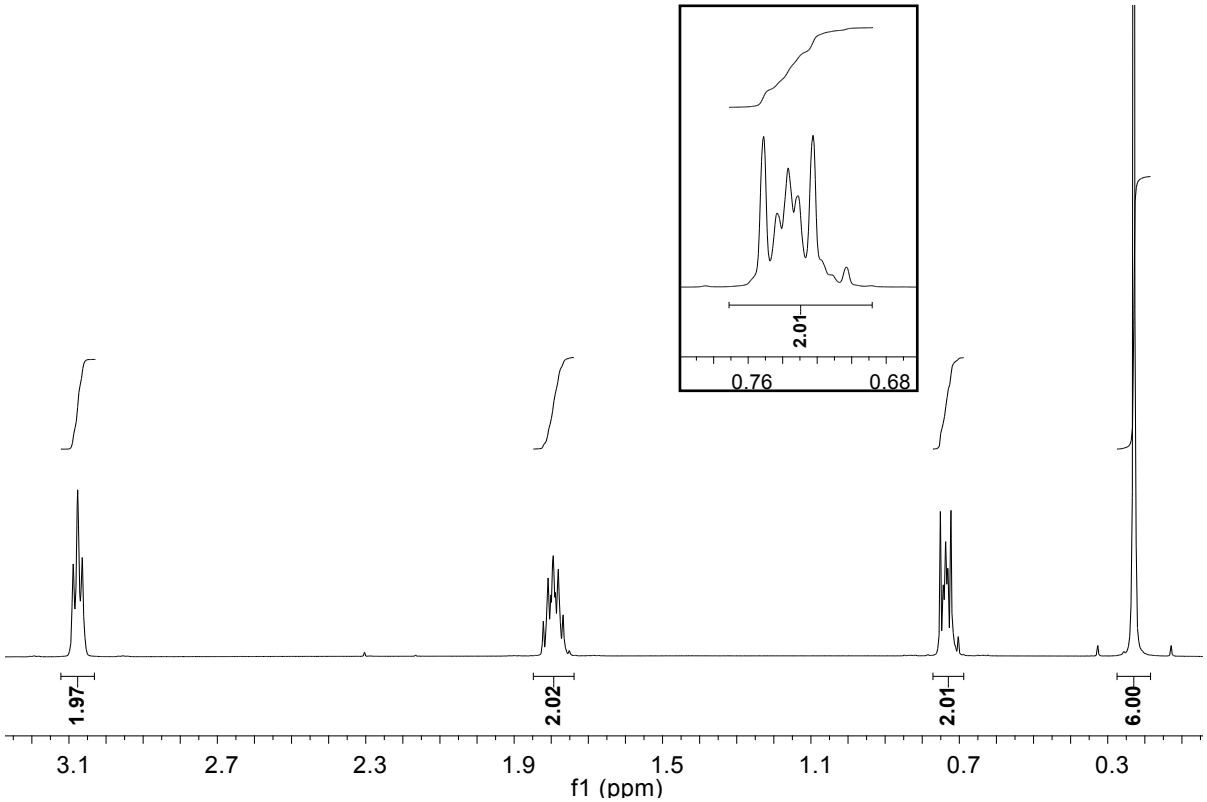


Figure S13. *^{1}H NMR spectrum of **2a** in D_2O (600 MHz)*

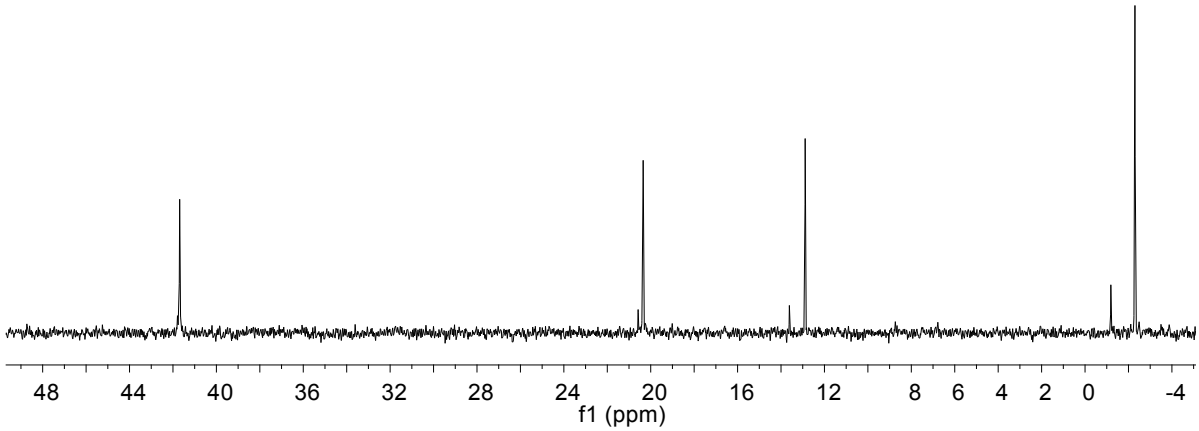


Figure S14. *^{13}C NMR spectrum of **2a** in D_2O (151 MHz)*

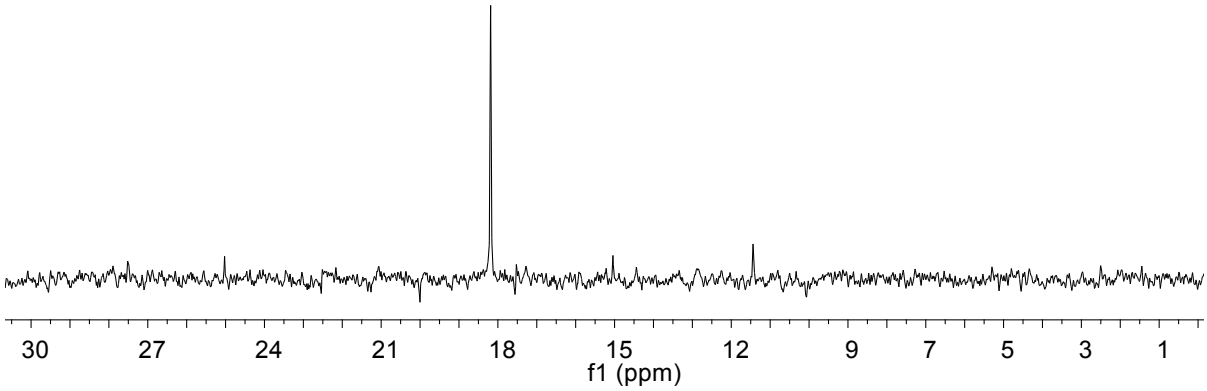
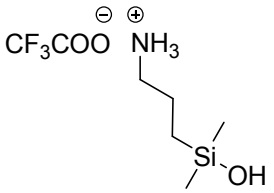


Figure S15. *^{29}Si NMR spectrum of **2a** in D_2O (119 MHz)*

(3-aminopropyl)dimethylsilanol trifluoroacetate 2b



On the one hand, 3-bis(3-aminopropyl)tetramethyl disiloxane trifluoroacetate **1b** (20 mg) was dissolved in DMSO-d_6 (600 µL) and the solution was left aside for 5 weeks to allow **1b** hydrolysis into **2b**. Then the mixture **1b/2b** in DMSO-d_6 was analyzed by NMR.

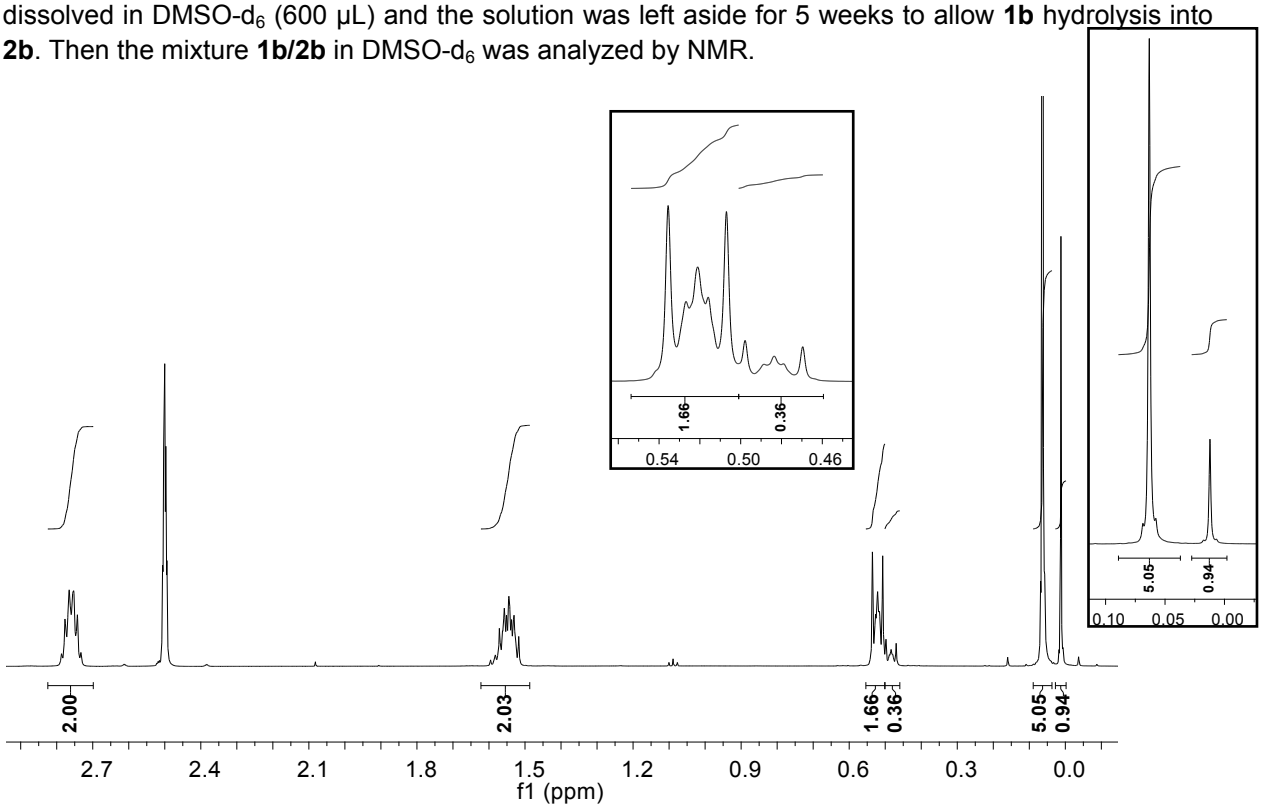


Figure S16. *^{1}H NMR spectrum of **1b/2b** in DMSO-d_6 (600 MHz)*

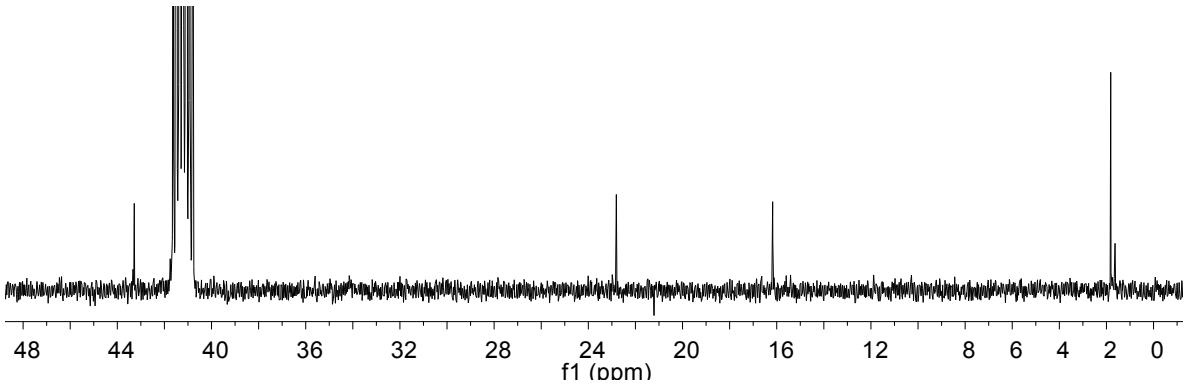


Figure S17. *^{13}C NMR spectrum of **1b/2b** in DMSO-d_6 (151 MHz)*

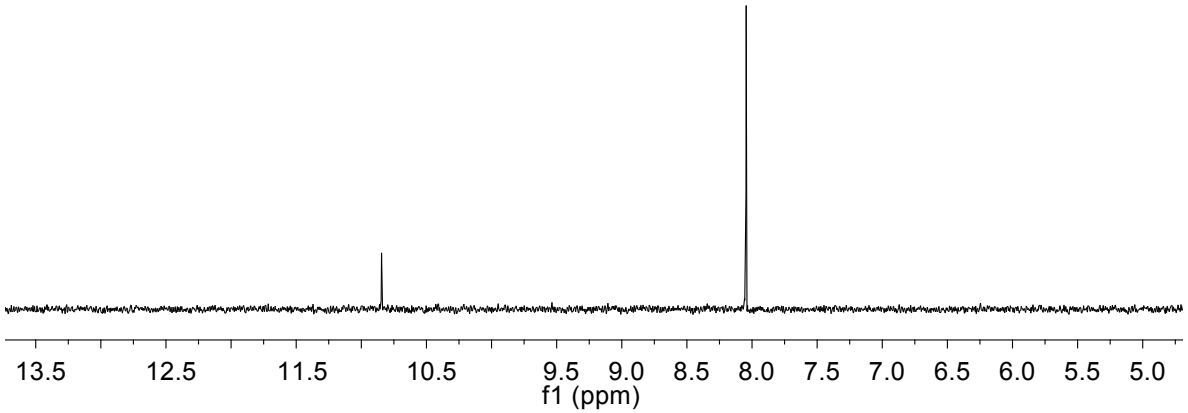


Figure S18.*^{29}Si NMR spectrum of **1b/2b** in DMSO-d_6 (119 MHz)*

On the other hand, 3-bis(3-aminopropyl)tetramethyl disiloxane trifluoroacetate **1b** (20 mg) was dissolved in D_2O (600 µL). **1b** hydrolysis into **2b** occurred. The mixture **1b/2b** in D_2O was analyzed by NMR.

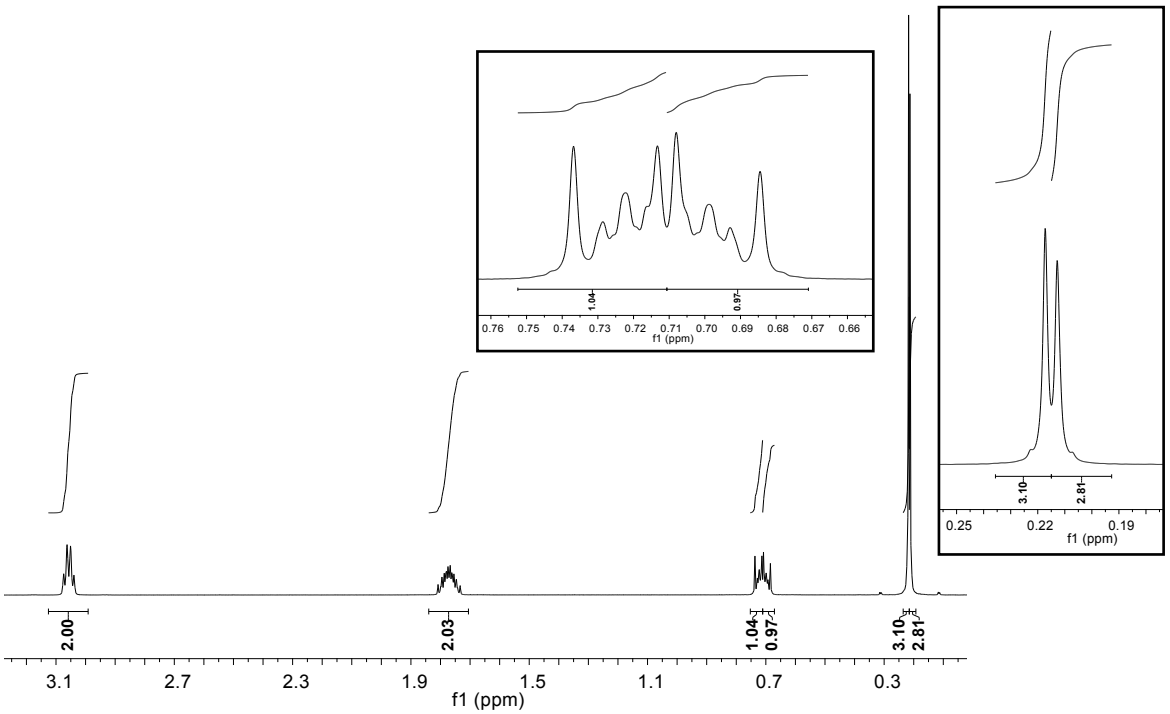


Figure S19. *^{1}H NMR spectrum of **1b**/**2b** in D_2O (600 MHz)*

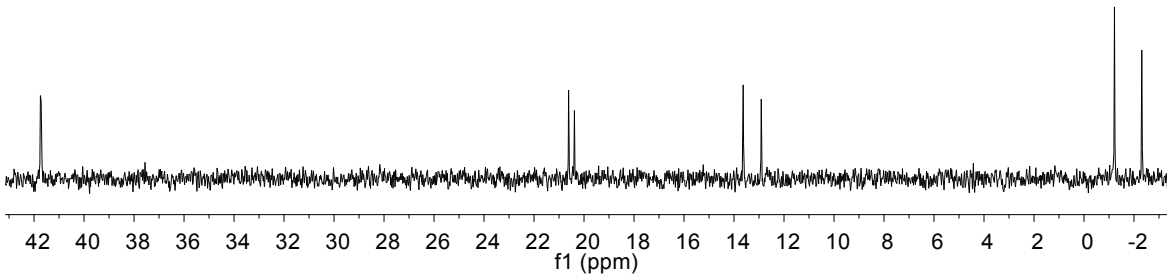


Figure S20. *^{13}C NMR spectrum of **1b**/**2b** in D_2O (151 MHz)*

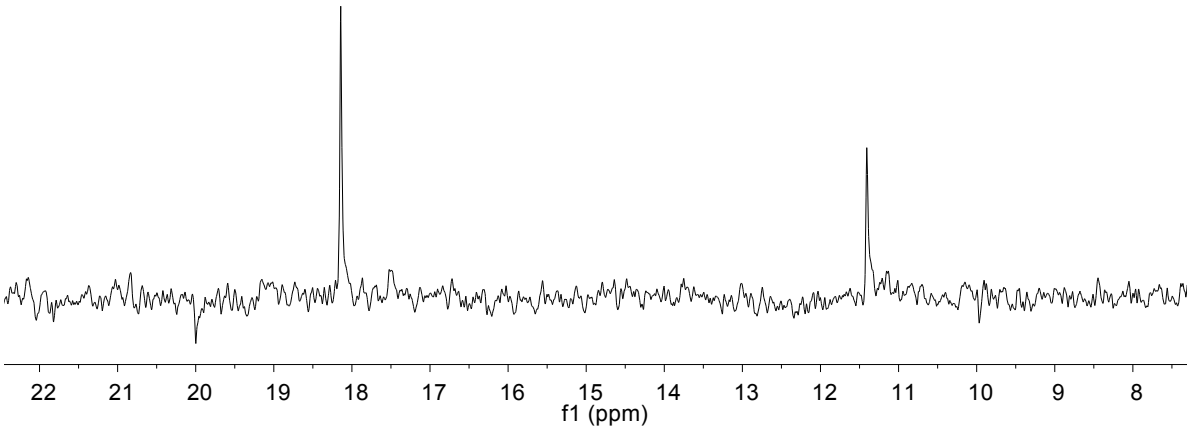


Figure S21. *^{29}Si NMR spectrum of **1b**/**2b** in D_2O (119 MHz)*

Counter-ion effect on NMR signals

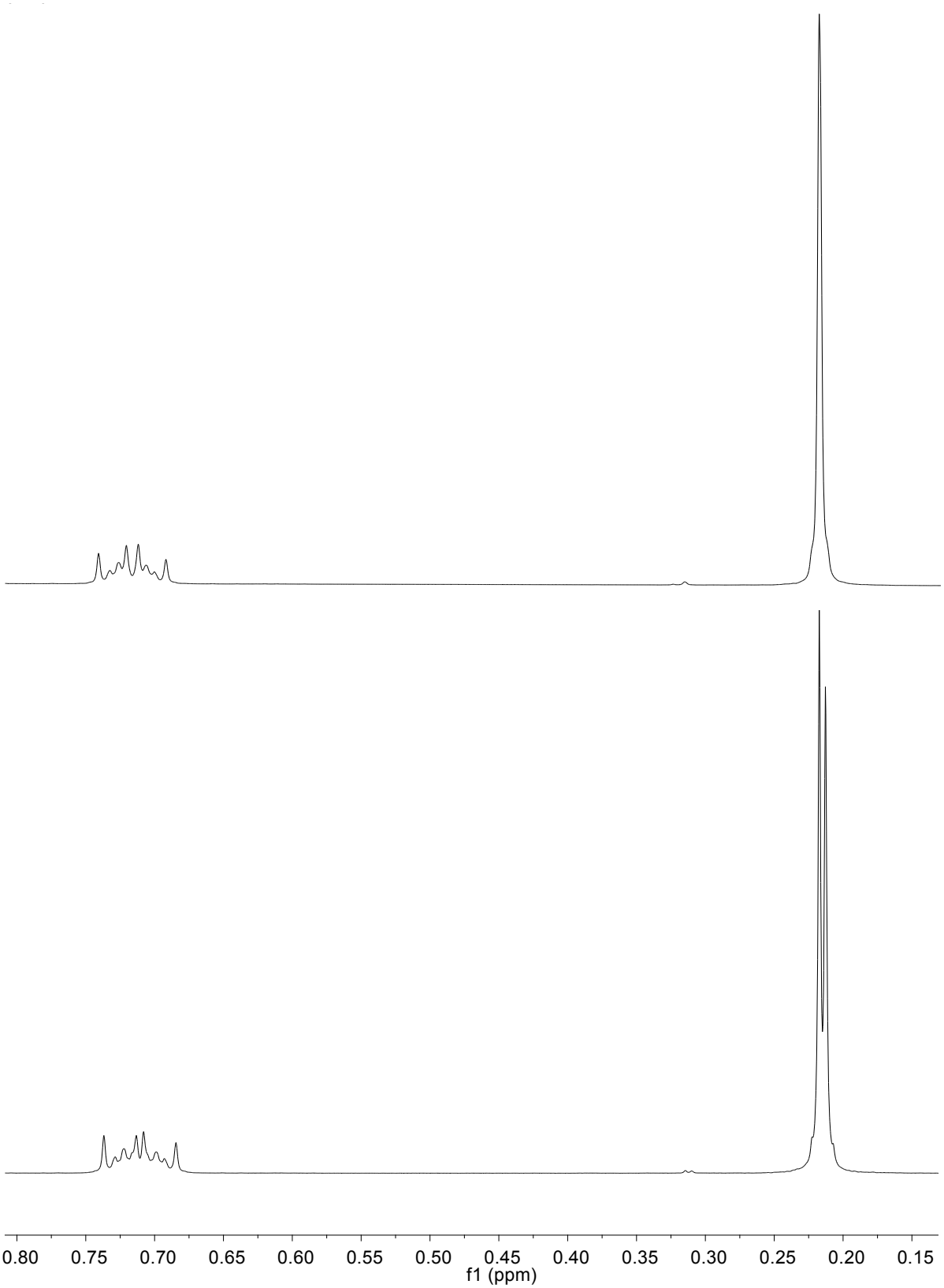


Figure S22. *From hydrochloride to trifluoroacetate salts. Top: ^{1}H NMR spectrum of* ***1a/2a*** *mixture in D_2O. Bottom: ^{1}H NMR spectrum of* ***1b/2b*** *mixture in D_2O.*

DEPT 29Si sequence optimization

In order to decrease the experiment time, we used a DEPT ^{29}Si sequence, with a relaxation delay of 2 seconds, to take advantage of the polarization transfer from hydrogens of methylene in the alpha position to the silicon atom. An optimization was made on this sequence, where the last ^{1}H flip angle was moved from 17° to 45° (Figure S23). The results showed a factor of 2 gain in favor of the angle of 24° compared to the standard DEPT of 45°.

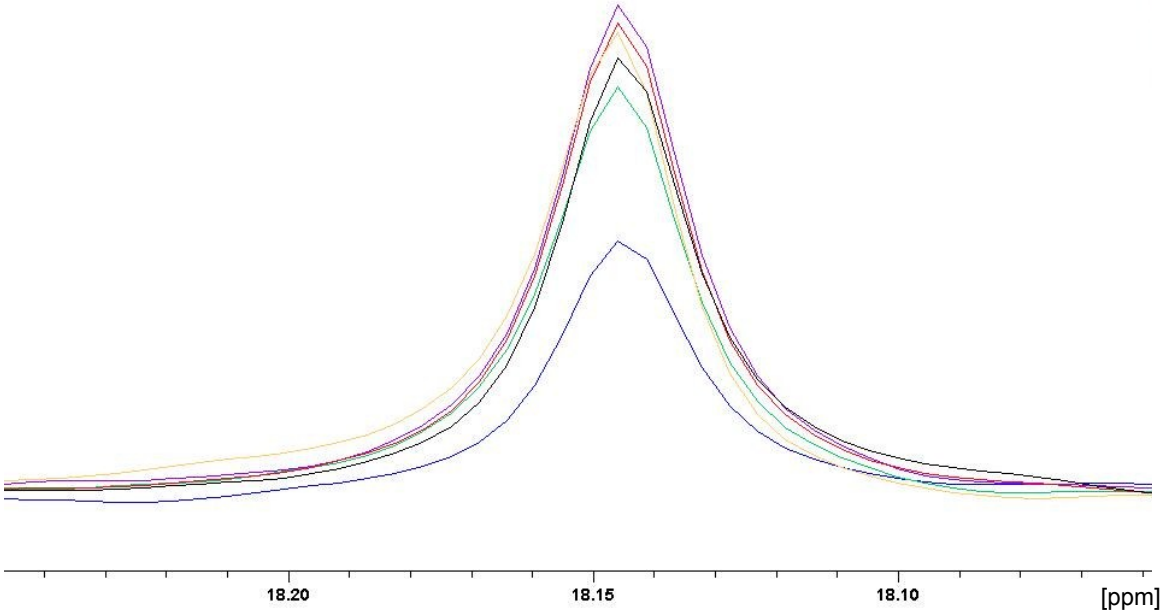


Figure S23. *^{1}H flip angle optimization. 1H flip angle was moved from 17° to 45°: blue 45°, green 35°, purple 24°, red 21°, orange 19.5°, black 17°*

Stability studies of the siloxane bond in different solutions by ^{1}H-NMR

t = 0 min corresponds to the dissolution of 1,3-bis(3-aminopropyl)tetramethyl disiloxane hydrochloride **1a** (20 mg) in DMSO-d_6 or in D_2O-containing buffer that yields a solution at the desired pH. pH were adjusted in advance with Dulbecco's phosphate buffered saline, HCl or NaOH aqueous solutions on blank samples. Dimer percent was reported as a function of time based on the integration of ^{1}H signals from the -CH_2Si group of monomer and dimer.

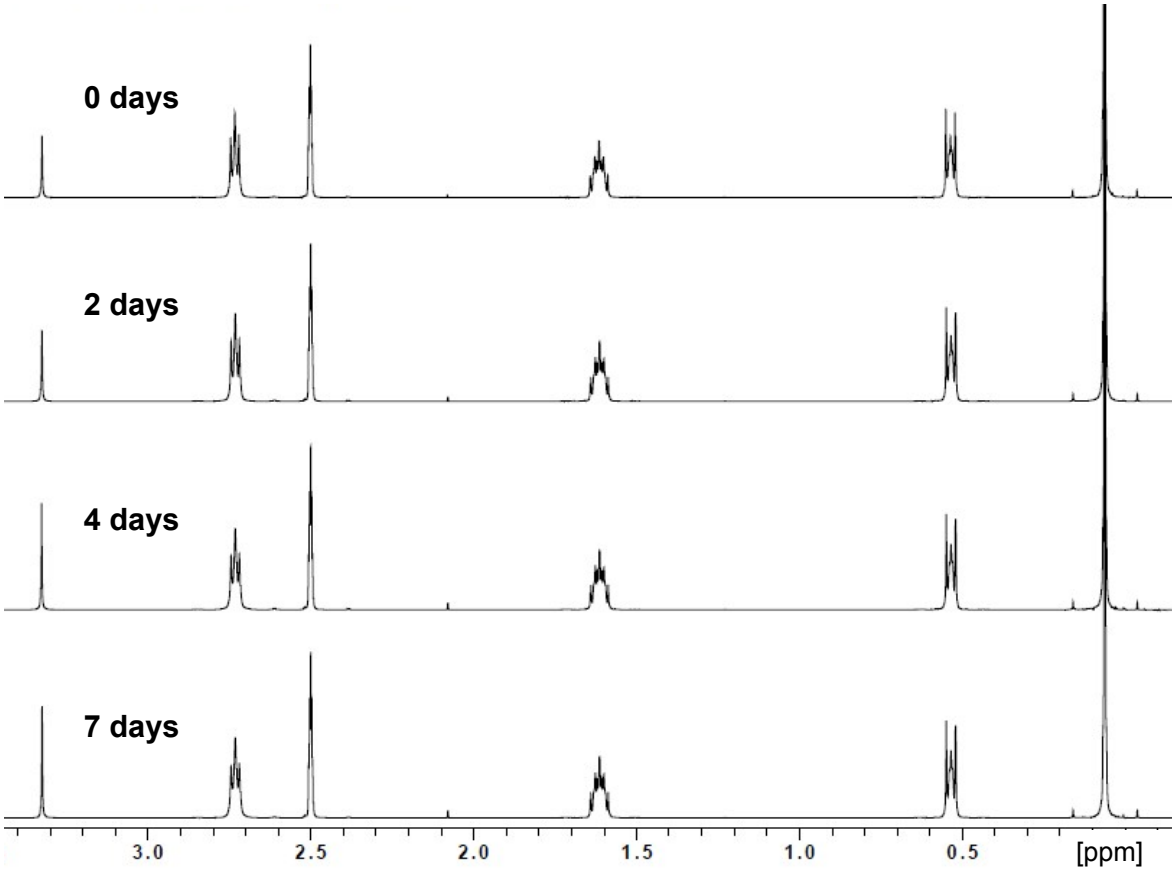


Figure S24. *^{1}H NMR spectra of **1a** in DMSO-d_6*

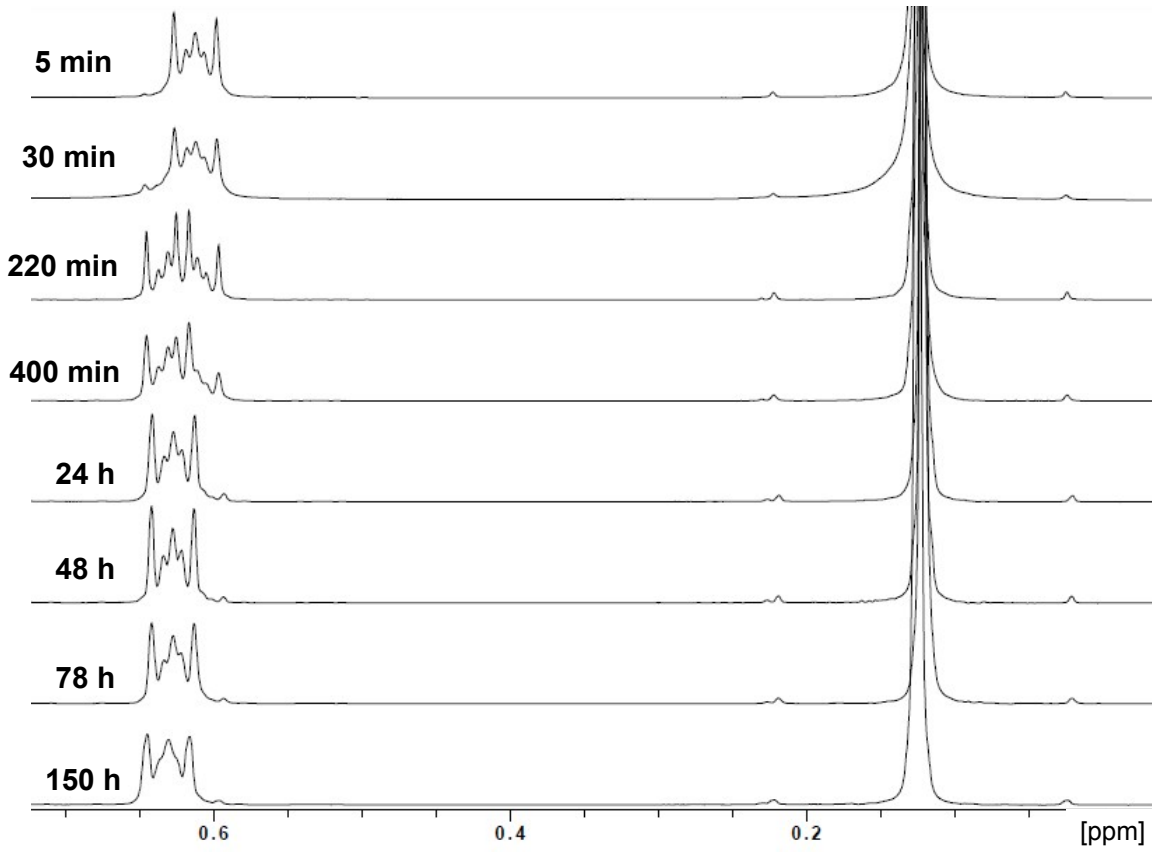


Figure S25. *^{1}H NMR spectra of **1a** at pH 4*

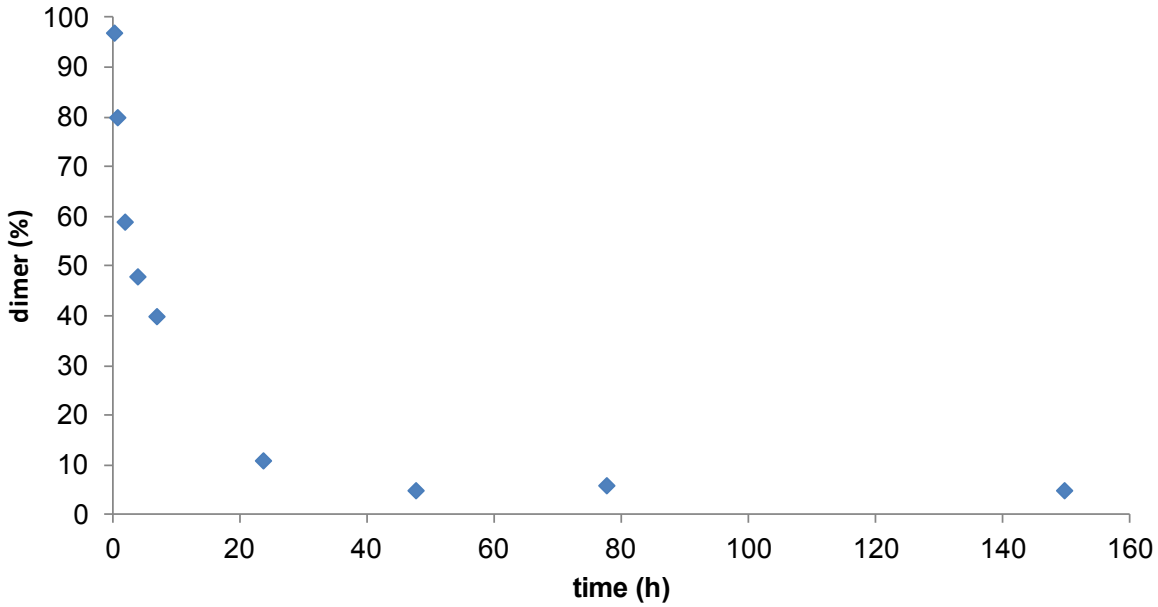


Figure S26. 1a *hydrolysis at pH 4 based on ^{1}H NMR kinetic studies*

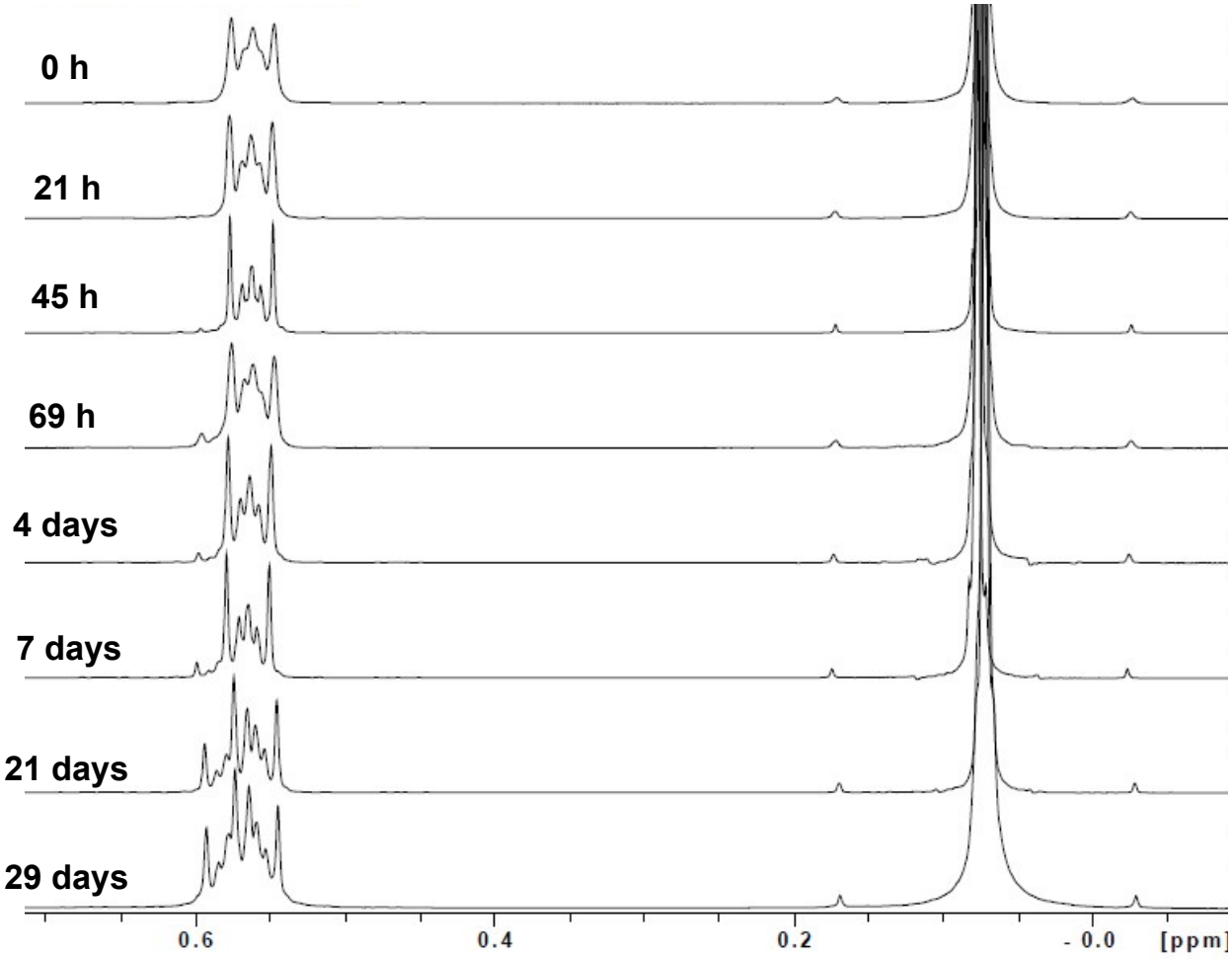


Figure S27. *^{1}H NMR spectra of **1a** at pH 7*

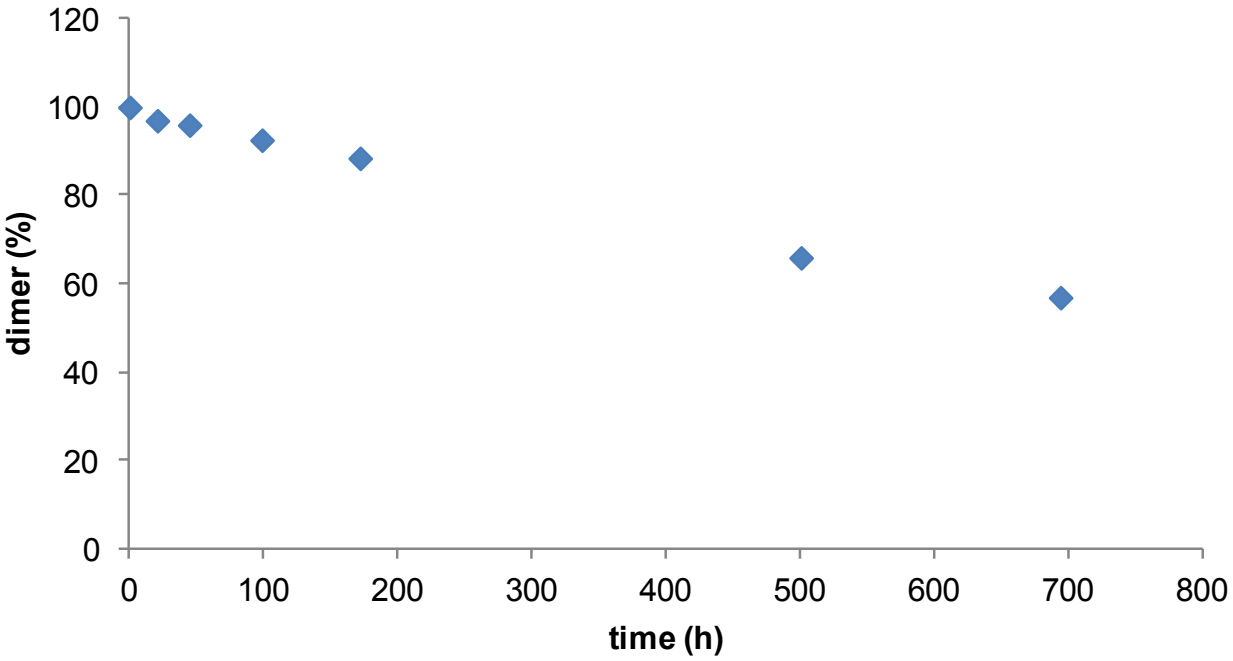


Figure S28. 1a *hydrolysis at pH 7 based on ^{1}H NMR kinetic studies*

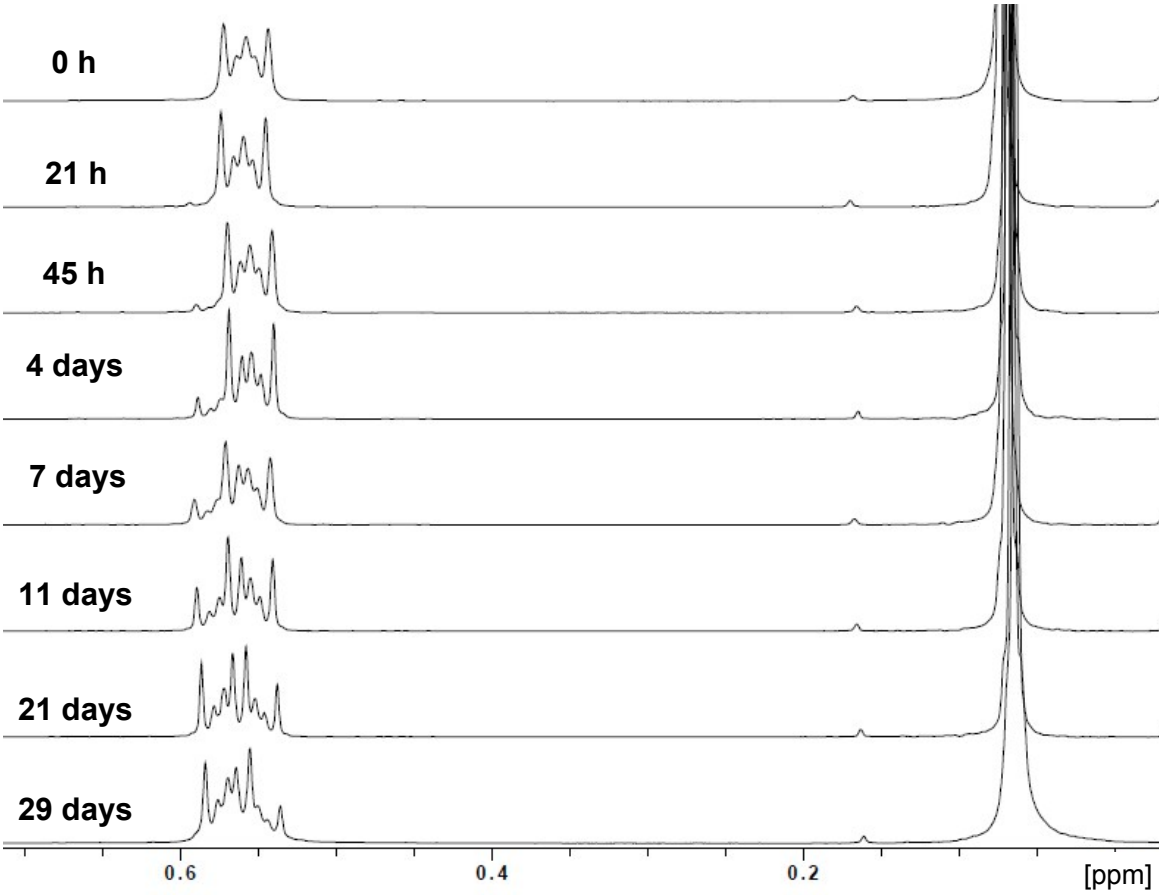


Figure S29. *^{1}H NMR spectra of **1a** at pH 7.4*

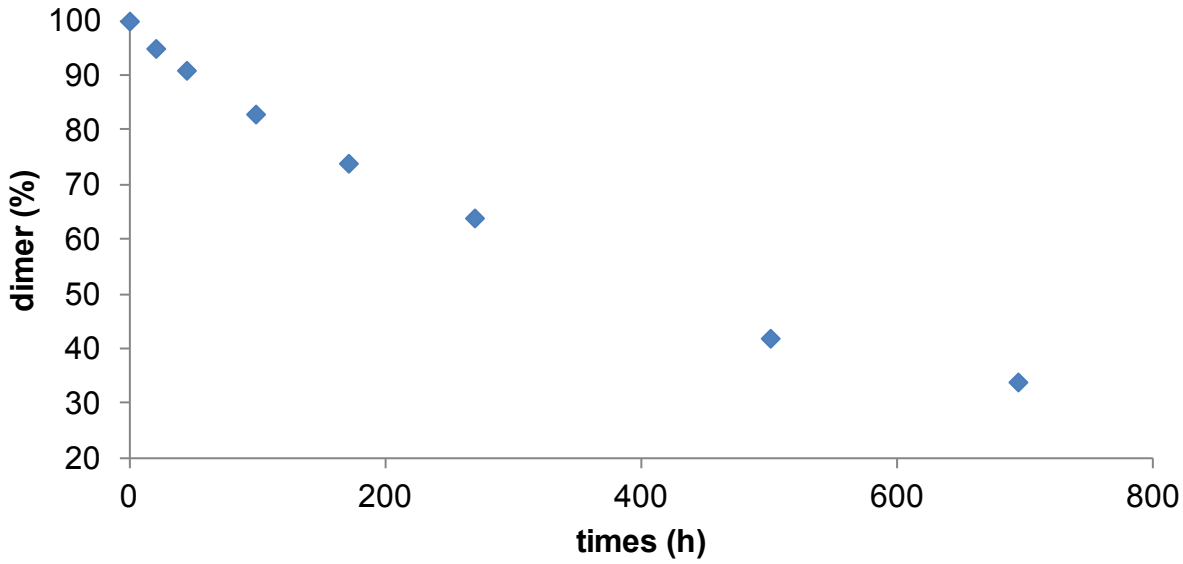


Figure S30. 1a *hydrolysis at pH 7.4 based on ^{1}H NMR kinetic studies*

We focused our attention on ^{1}H signals from -CH_2Si and CH_3Si because they are the farthest signals from the peptide sequence in hybrid peptide displaying the dimethylpropylsiloxane dimerization arm. As a consequence, very few differences in chemical shifts and signal shapes are expected when changing the peptide sequence. Thus, NMR studies on the 1,3-bis(3-aminopropyl)tetramethyl disiloxane model can be generalized to hybrid peptides. In addition, these signals appear in a chemical shift area completely different from peptide signals which makes them easily identifiable and integrable. However, the methylene group in gamma position to the silicon is also a proble for the determination of monomer/dimer ratio (figure S31)

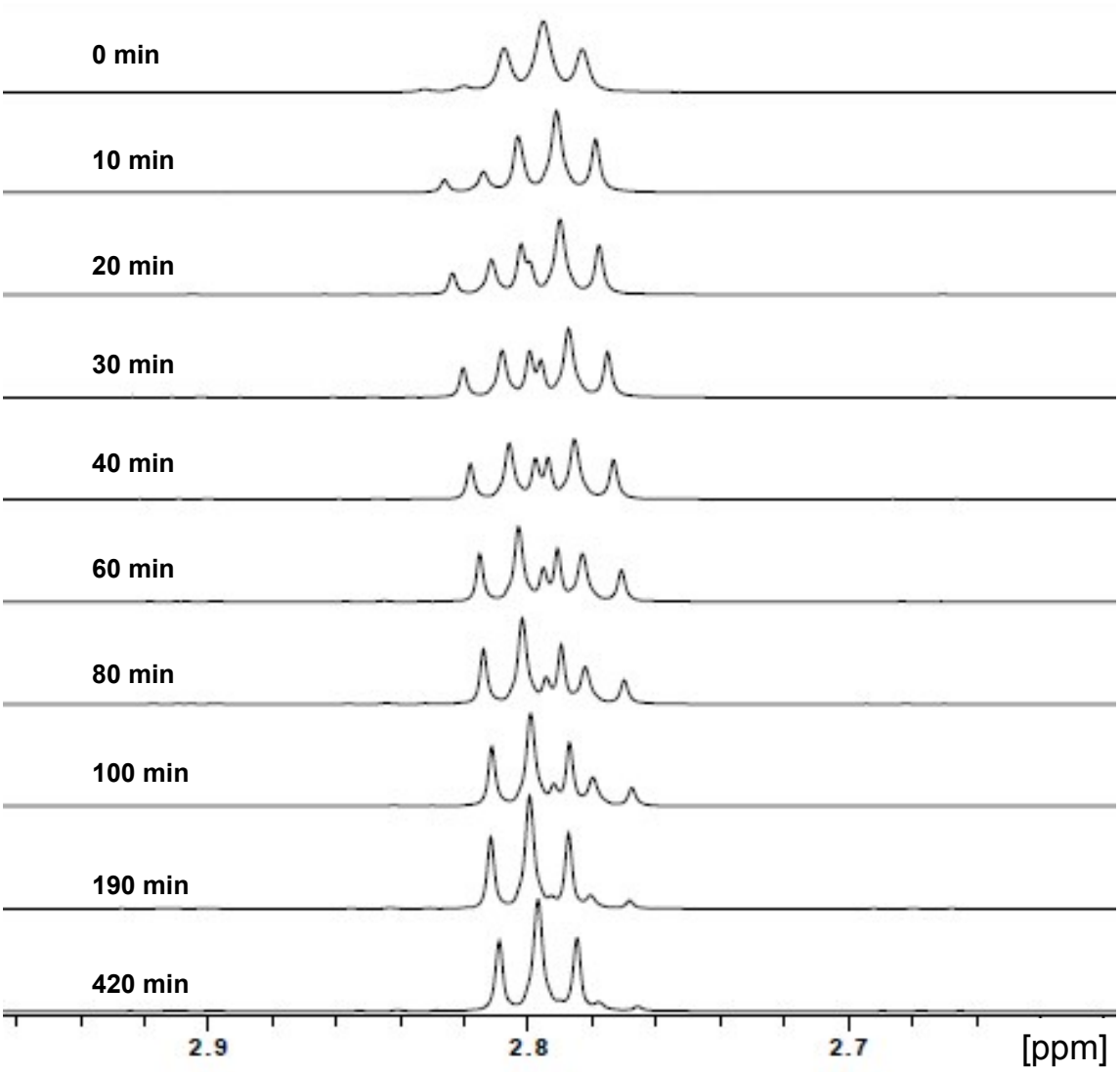


Figure S31. *^{1}H NMR spectra of **1a** at pH 10. Signals from the CH_2 group in gamma position to silicon*

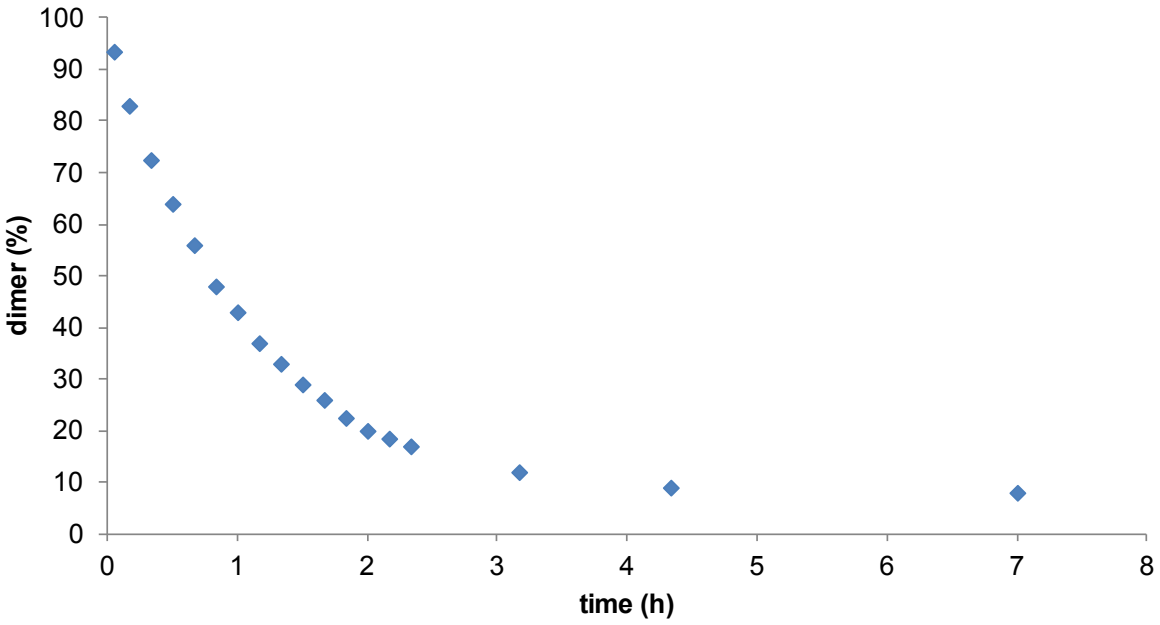


Figure S32. 1a *hydrolysis at pH 10 based on* ^{1}*H NMR kinetic studies*

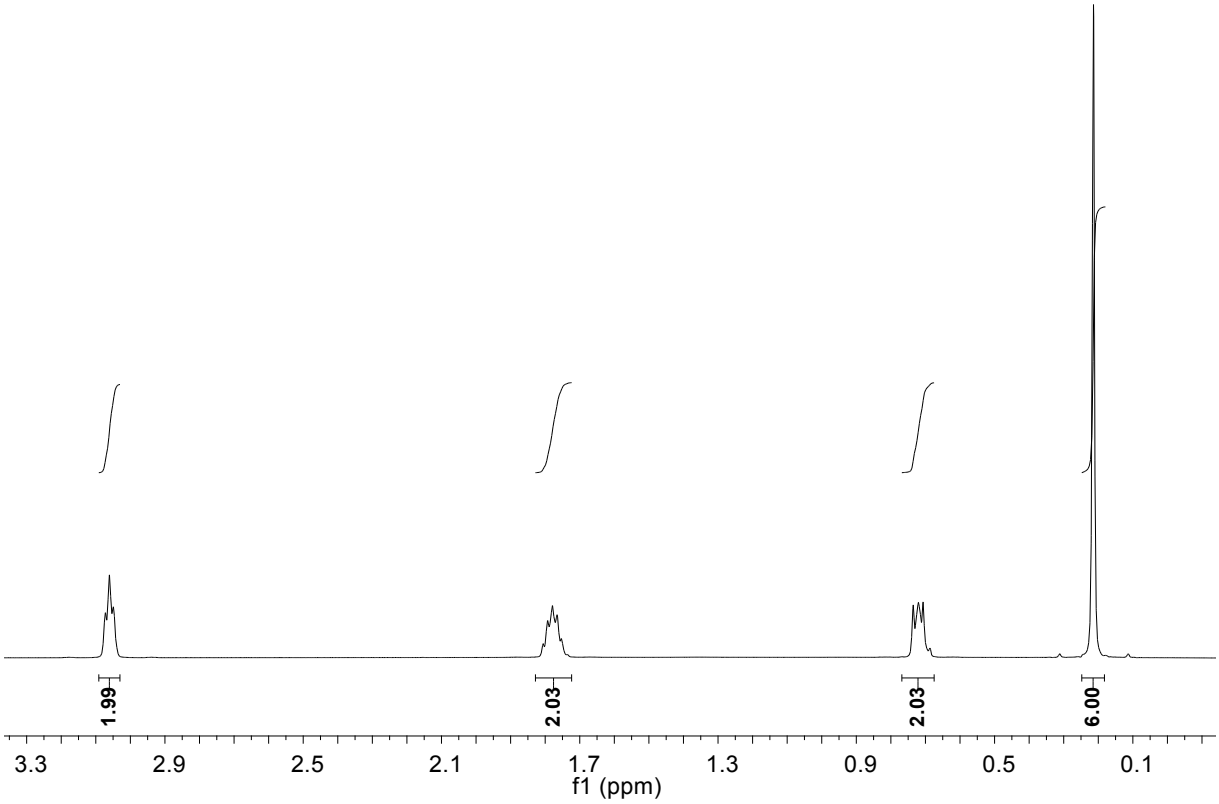


Figure S33. ^{1}*H NMR spectrum of* ***1a*** *in* D_2O *0.1% TFA at t = 0. Hydrolysis occurred immediately. Signals correspond to monomer* ***2a****.*

Silanol condensation upon lyophilization

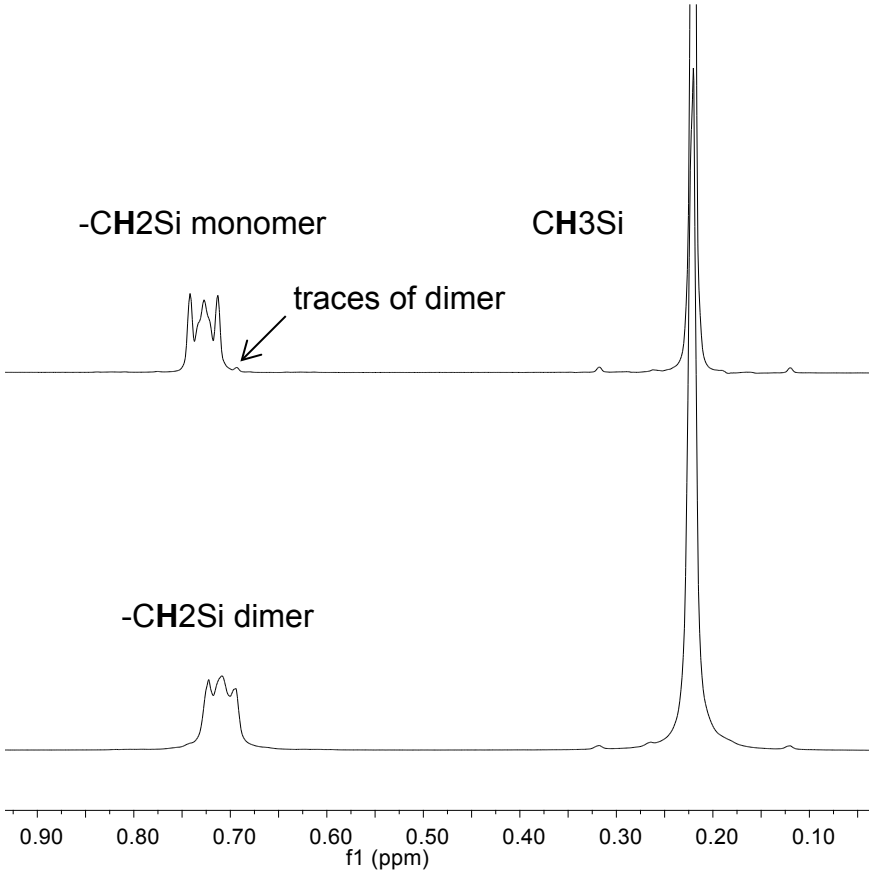
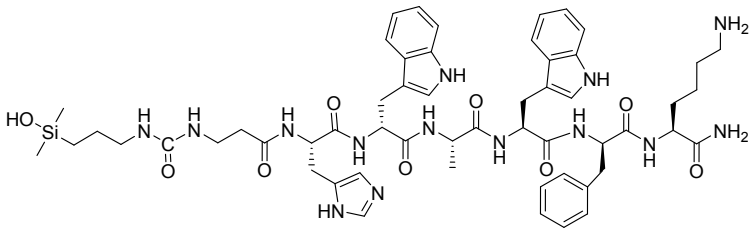


Figure S34. *From monomer to dimer upon lyophilization. Top : ^{1}H NMR spectrum of* ***2a*** *in D_2O/0.1% TFA before lyophilization; bottom : ^{1}H NMR spectrum of the same sample after freeze-drying*

N-ter hybrid monomer 4



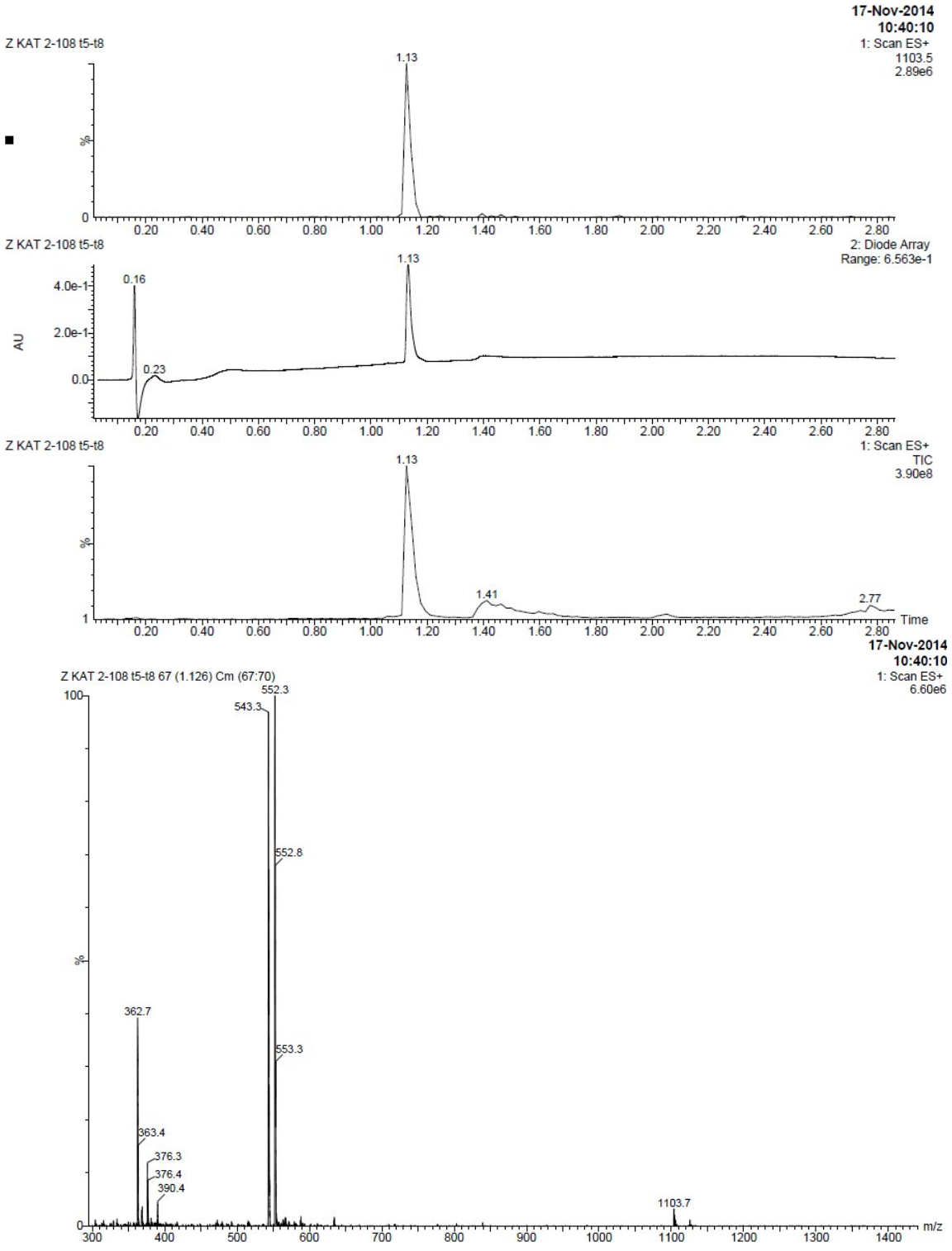
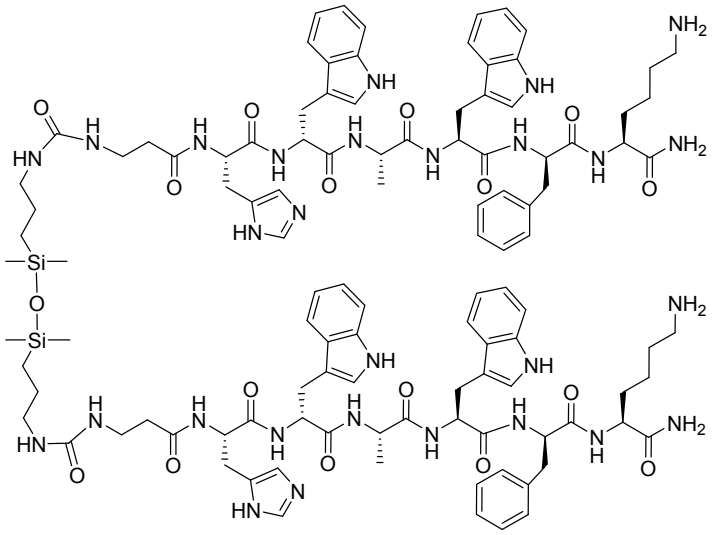


Figure S35. *LC/MS spectrum of* ***4***

N-ter hybrid dimer 5 (JMV 6187)



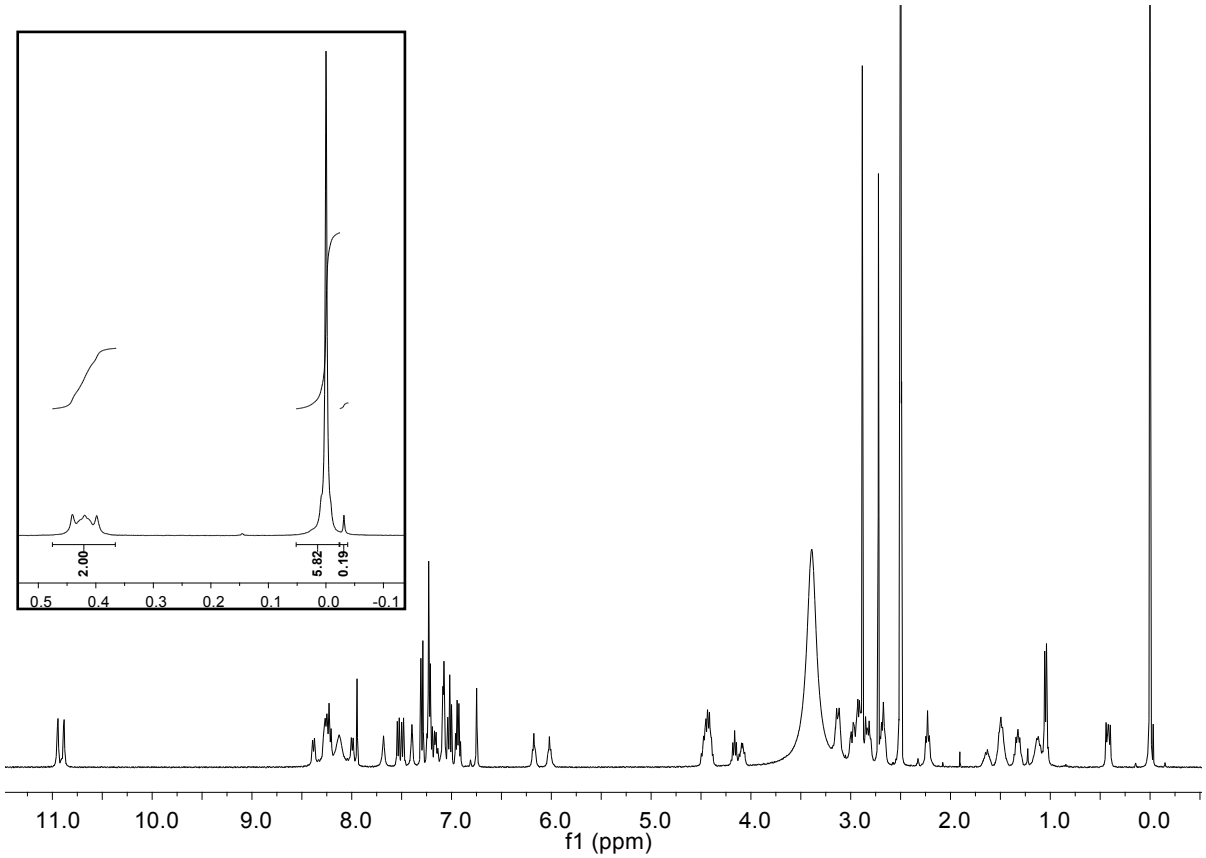


Figure S36. *^{1}H NMR spectrum of 5 in DMSO-d_6 (400 MHz)*

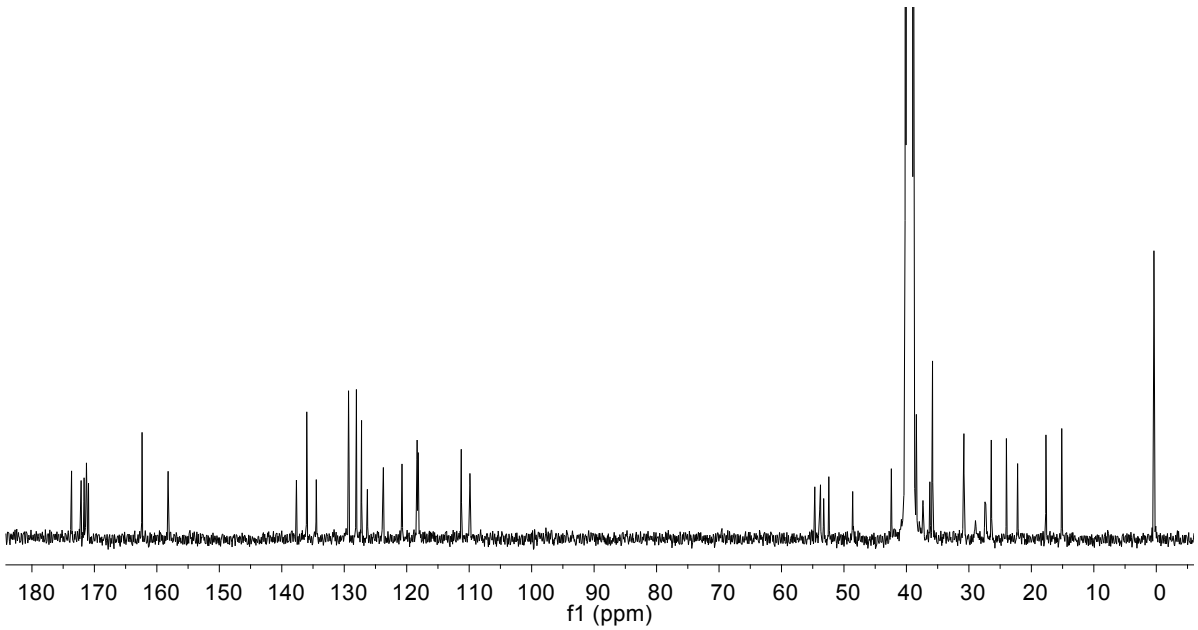
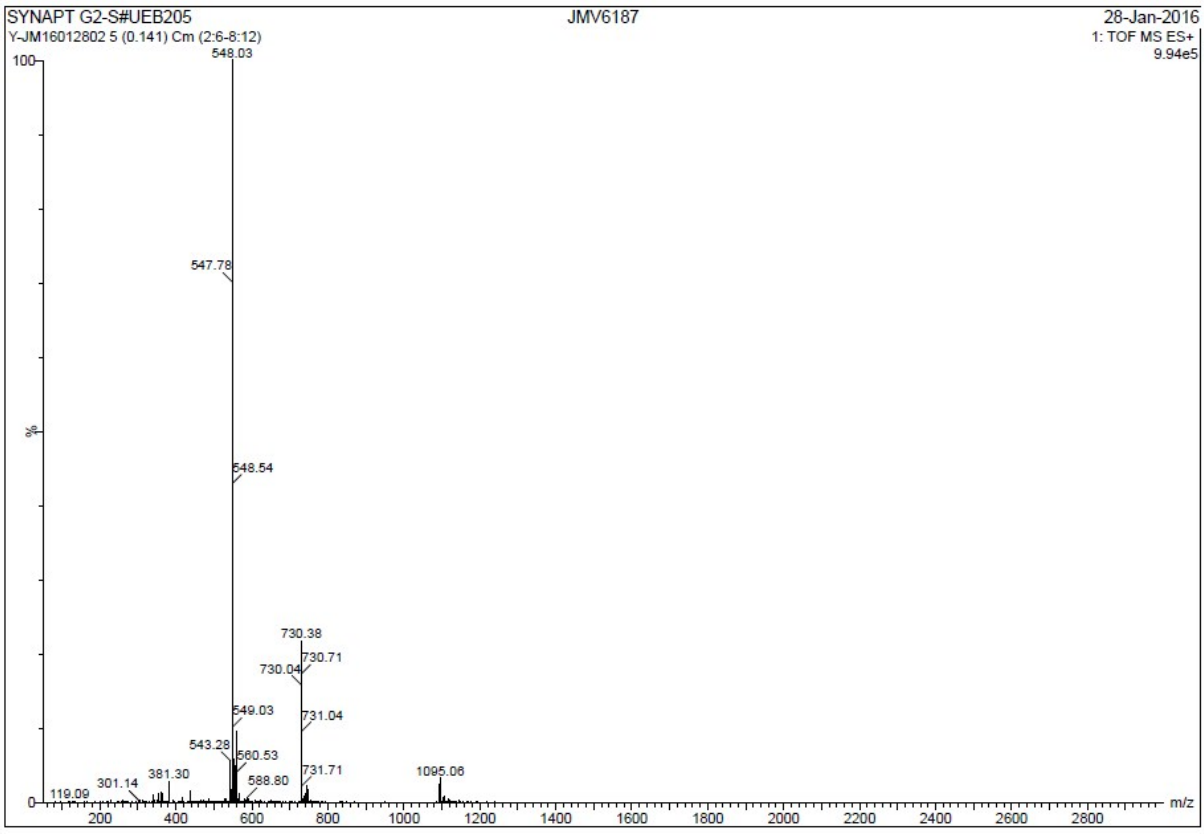


Figure S37. *^{13}C NMR spectrum of **5** in DMSO-d_6 (101 MHz)*



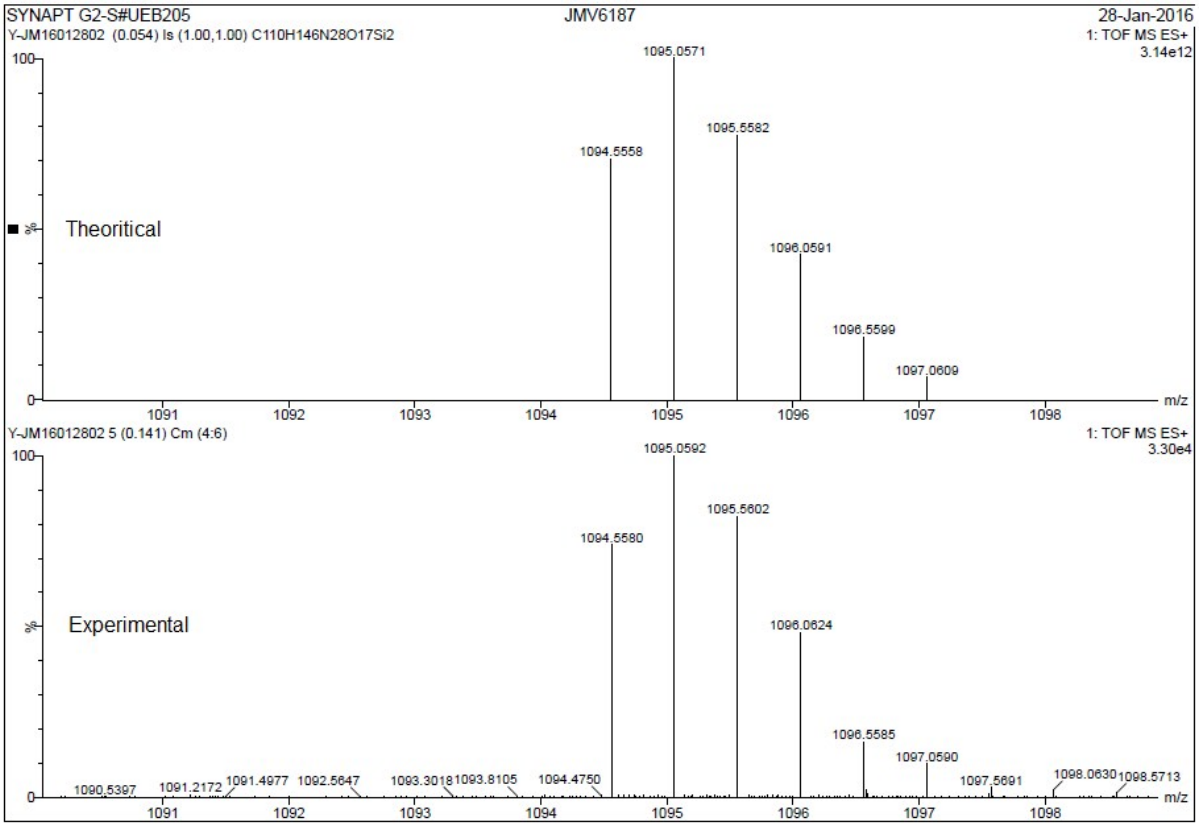
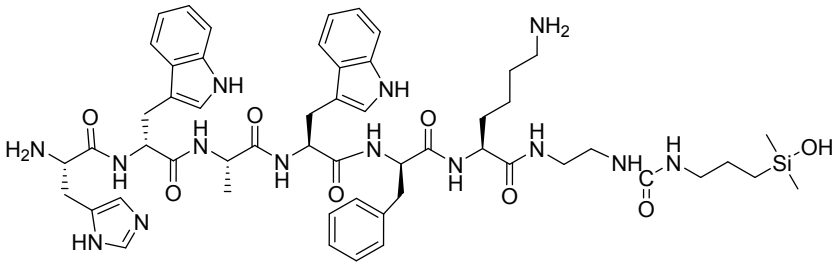


Figure S38**. HR-MS analysis of **5

C-ter hybrid monomer 6



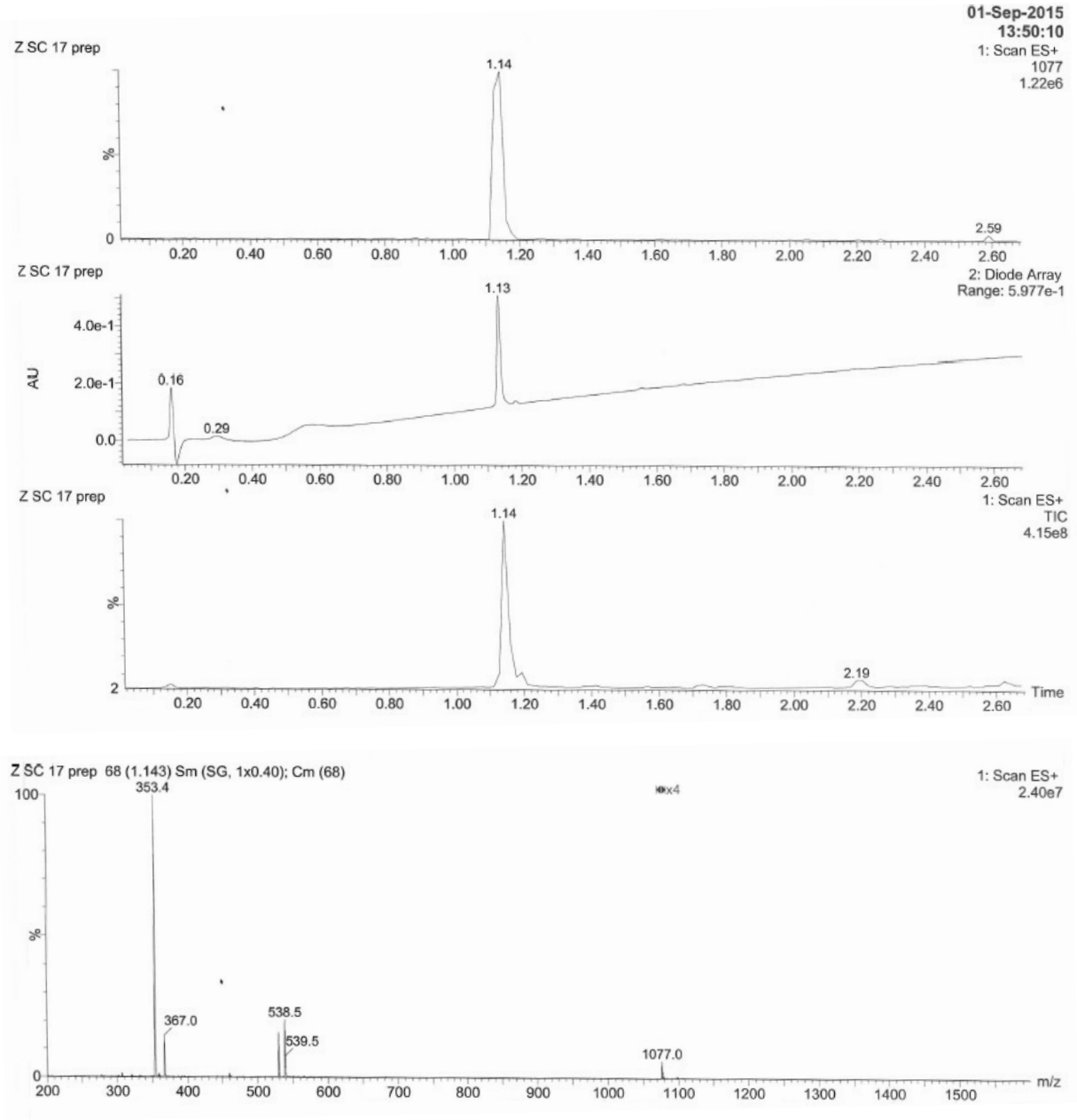
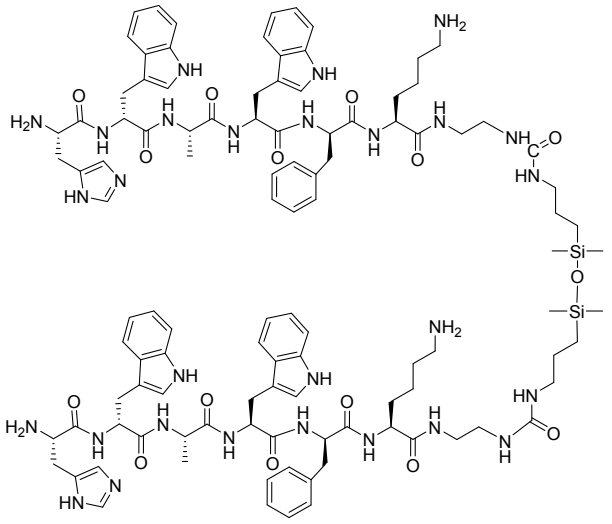
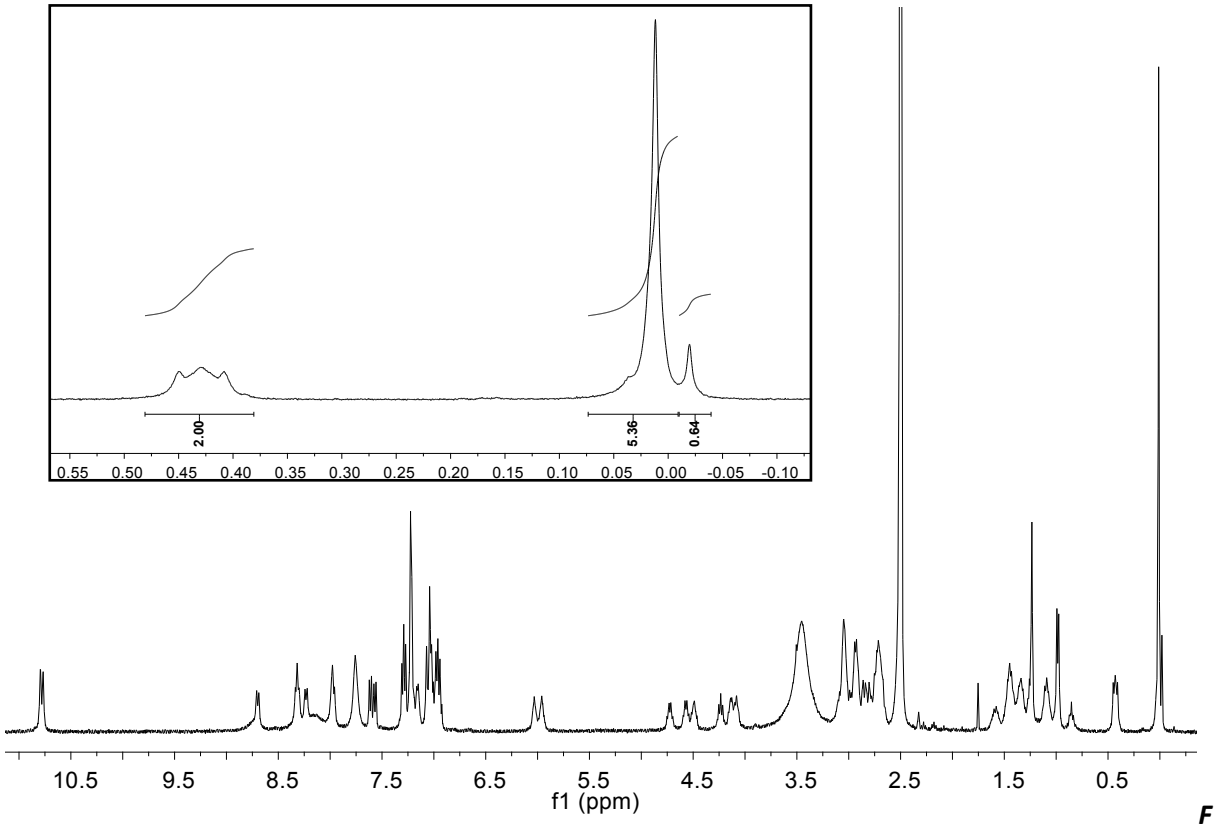


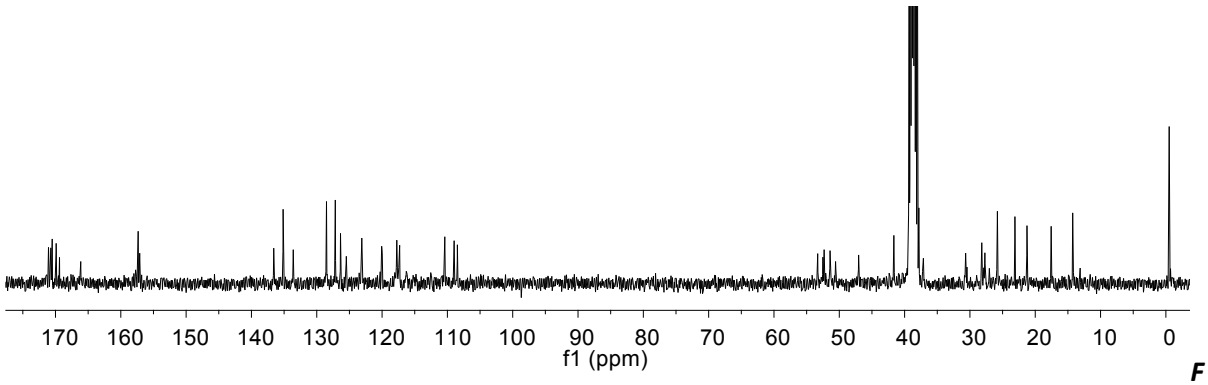
Figure S39. *LC/MS spectrum of* ***6***

C-ter hybrid dimer 7 (JMV 6186)



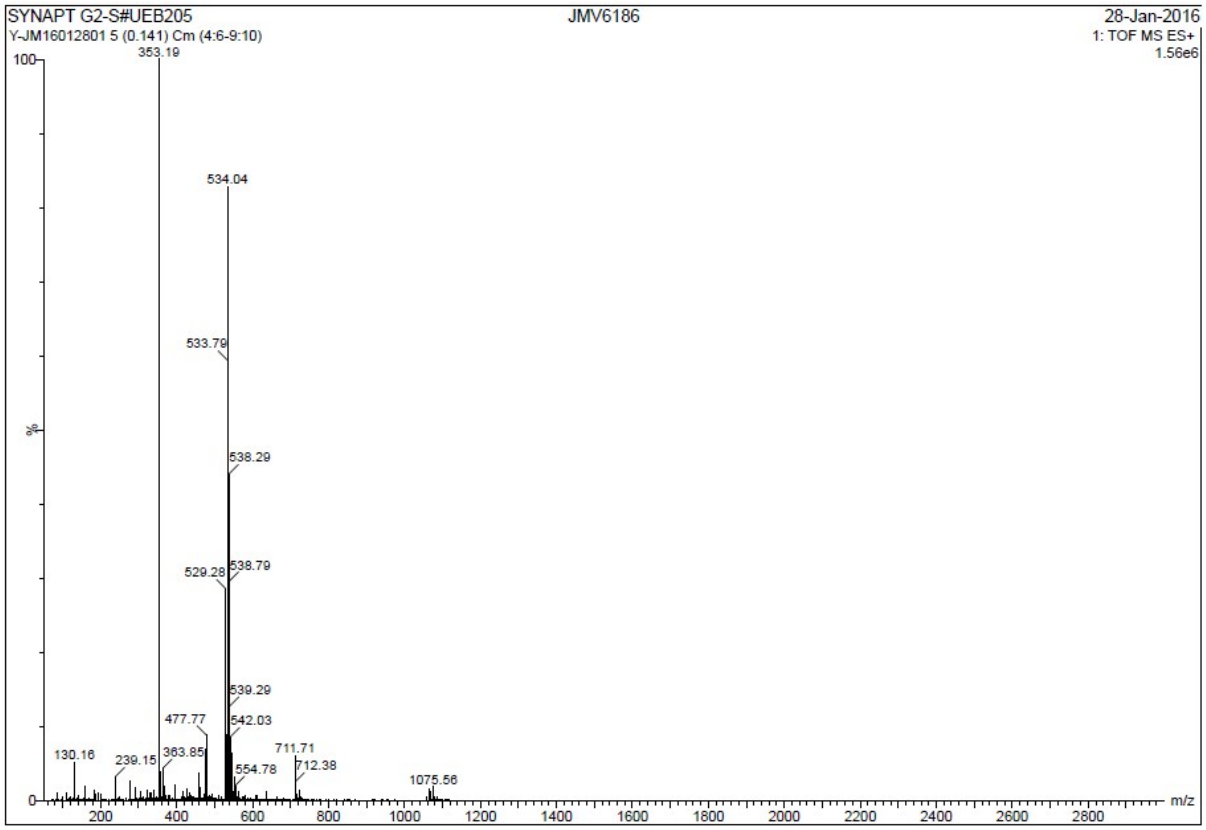


***igure S40**. [1]H NMR spectrum of **7** in DMSO-d_6 (400 MHz)*



F

igure S41. *^{13}C NMR spectrum of **7** in DMSO-d_6 (101 MHz)*



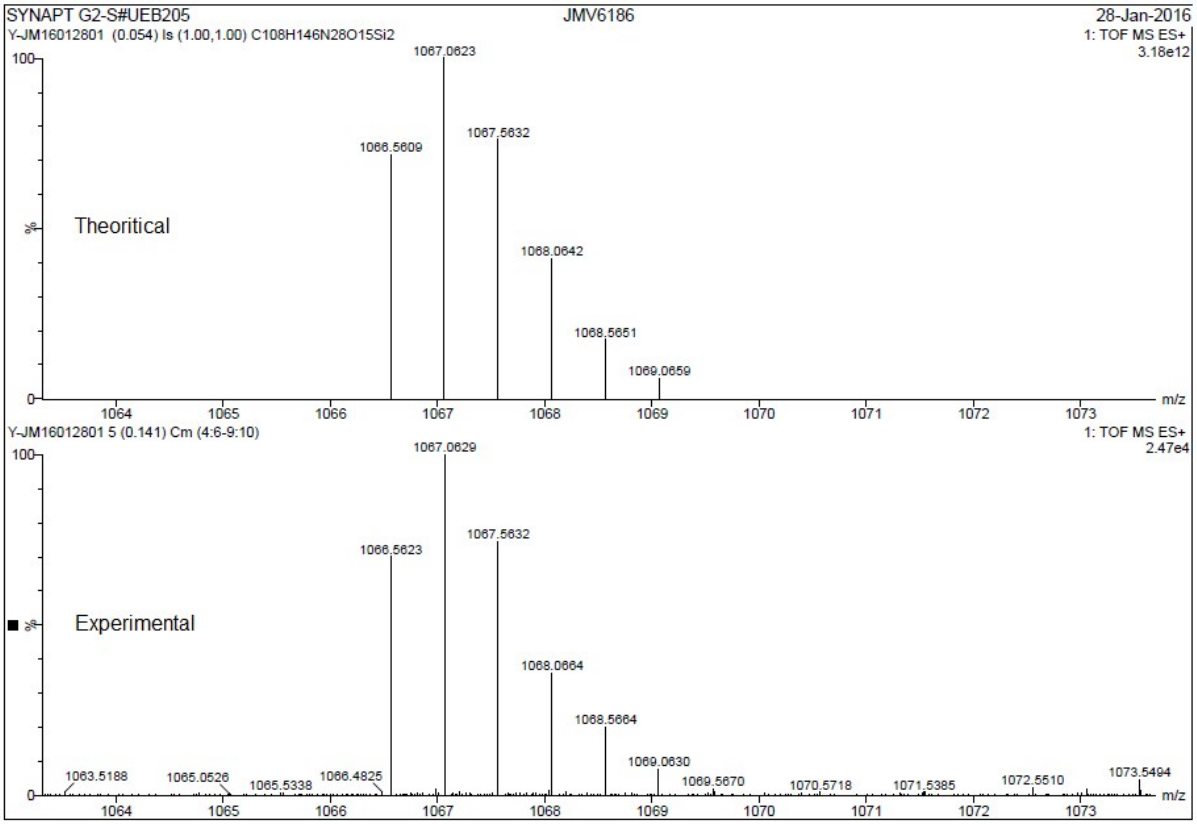
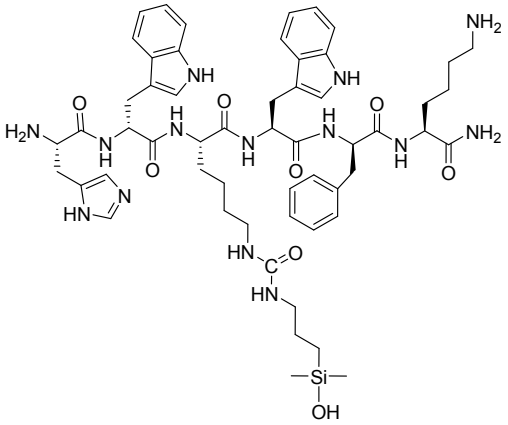
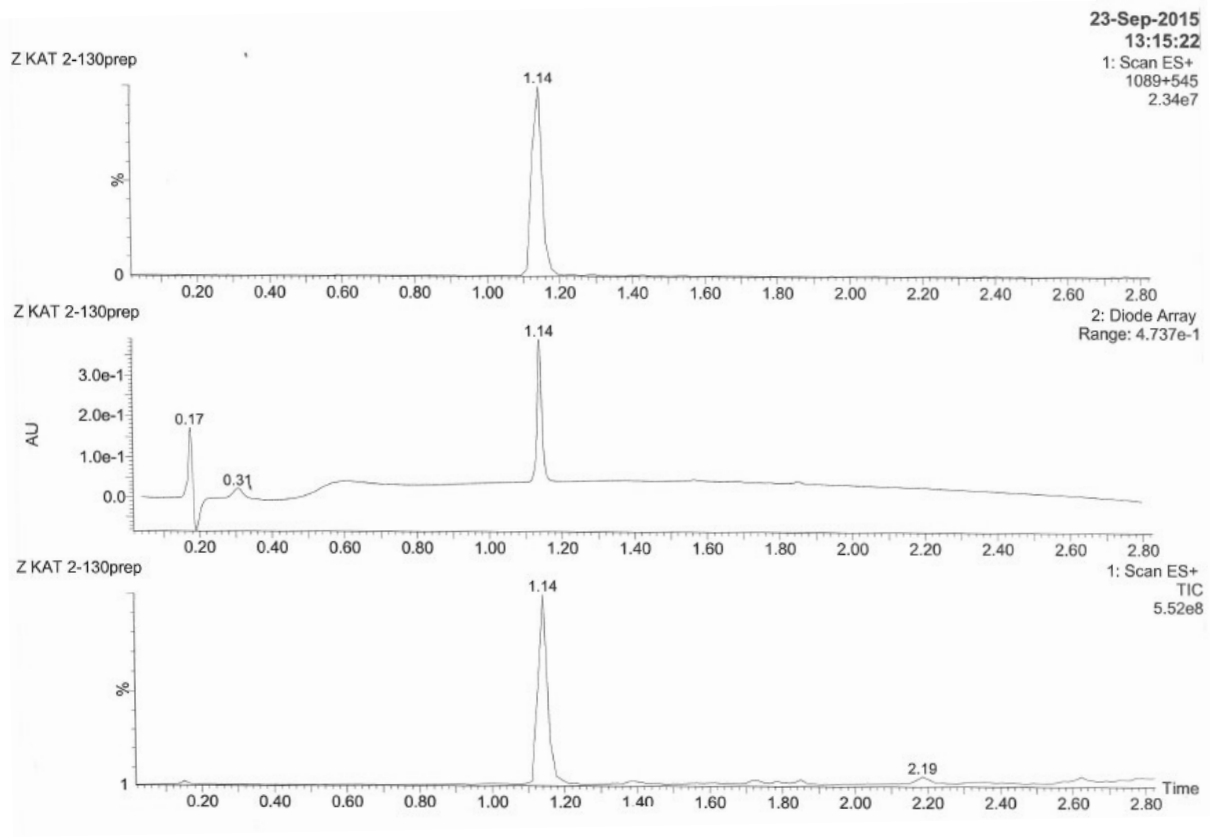


Figure S42. *HR-MS analysis of* ***7***

Lys³ hybrid monomer 8





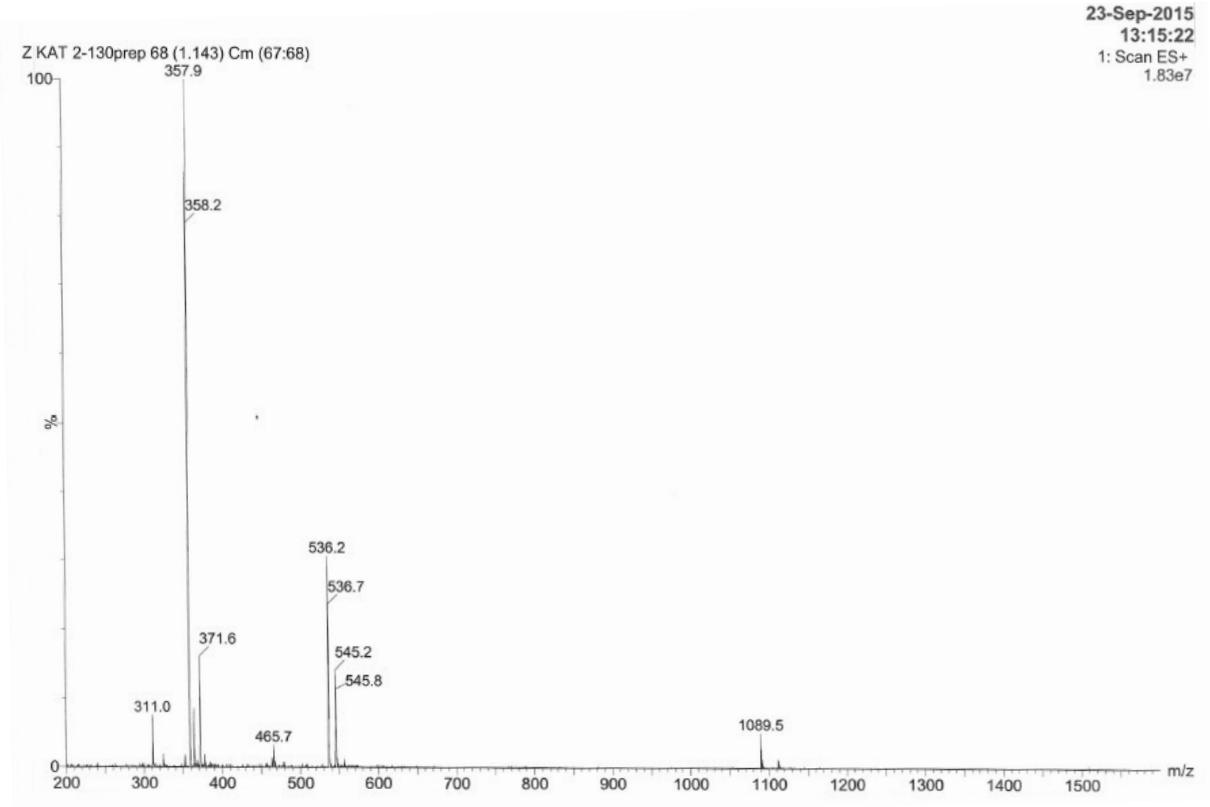


Figure S43. *LC/MS spectrum of* ***8***

Lys³ hybrid dimer 9 (JMV 6185)

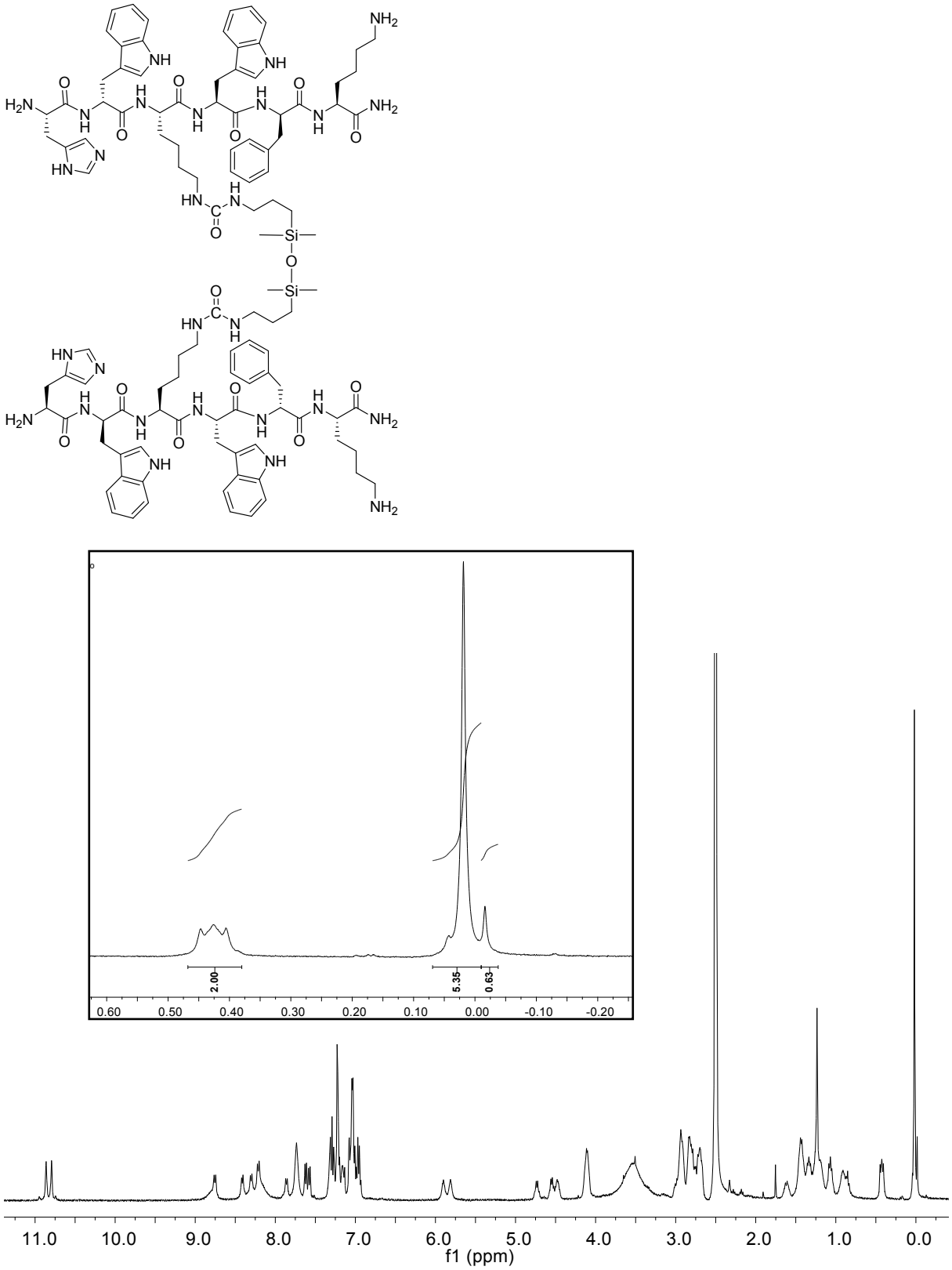


Figure S44. *^{1}H NMR spectrum of **9** in DMSO-d_6 (400 MHz)*

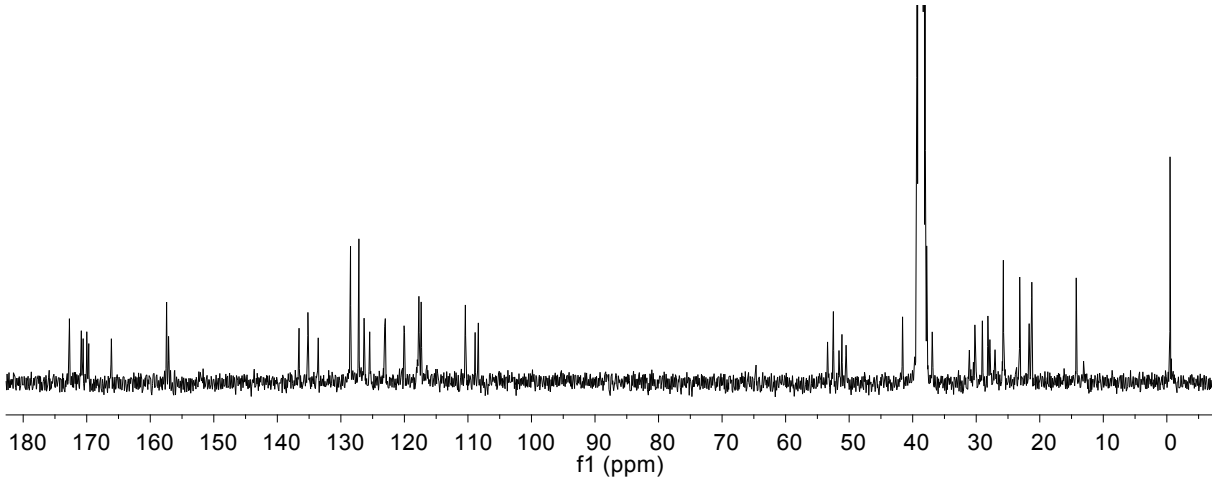
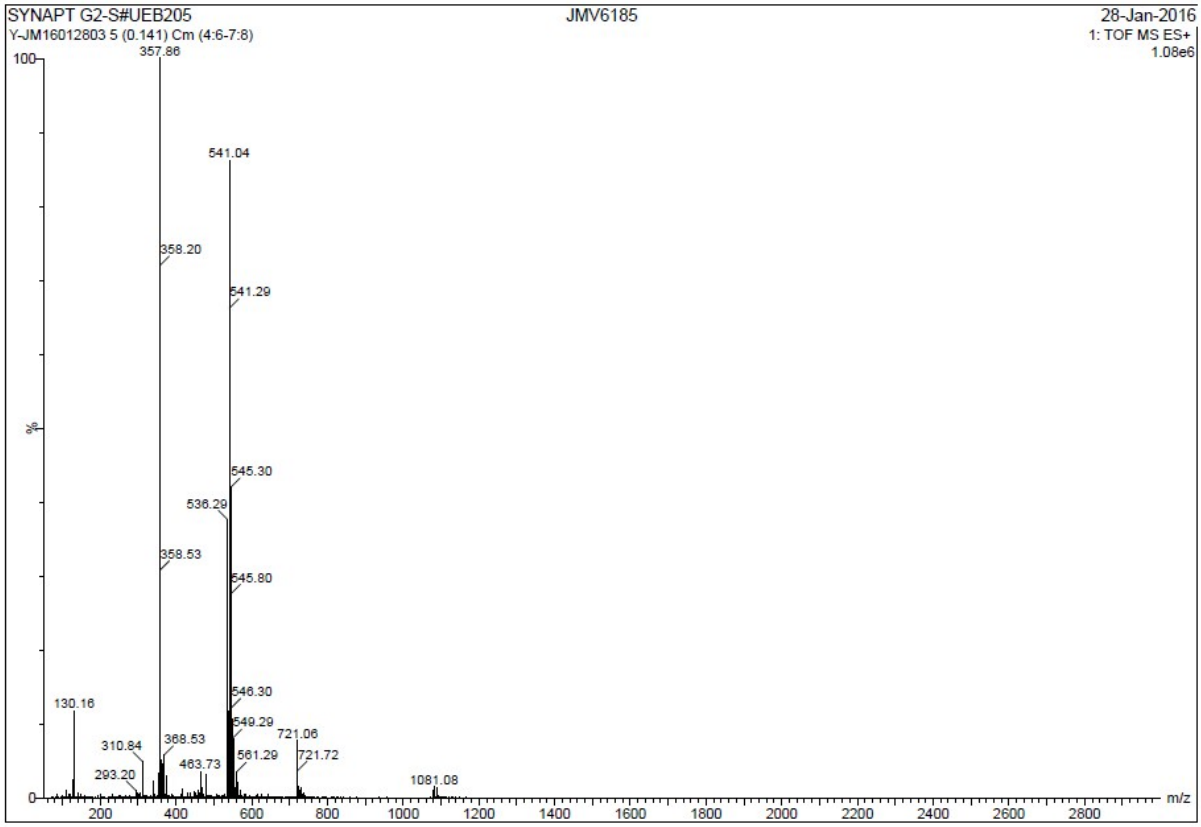


Figure S45. *^{13}C NMR spectrum of **9** in DMSO-d_6 (101 MHz)*



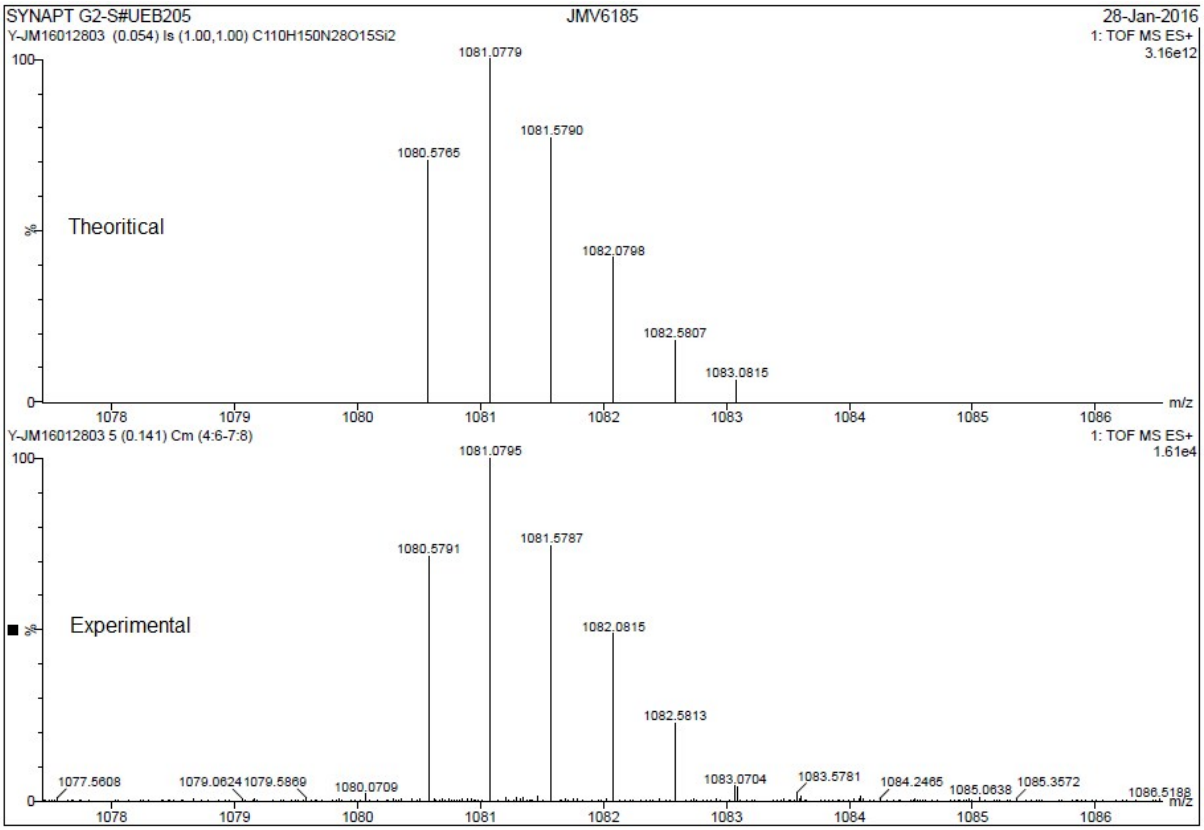
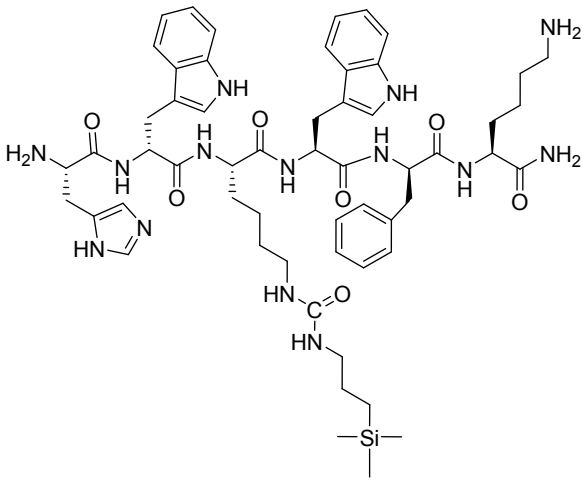


Figure S46. *HR-MS analysis of* ***9***

Lys^3(NH(CH$_2$)$_3$Si(CH$_3$)$_3$) monomer 10 (JMV 6246)



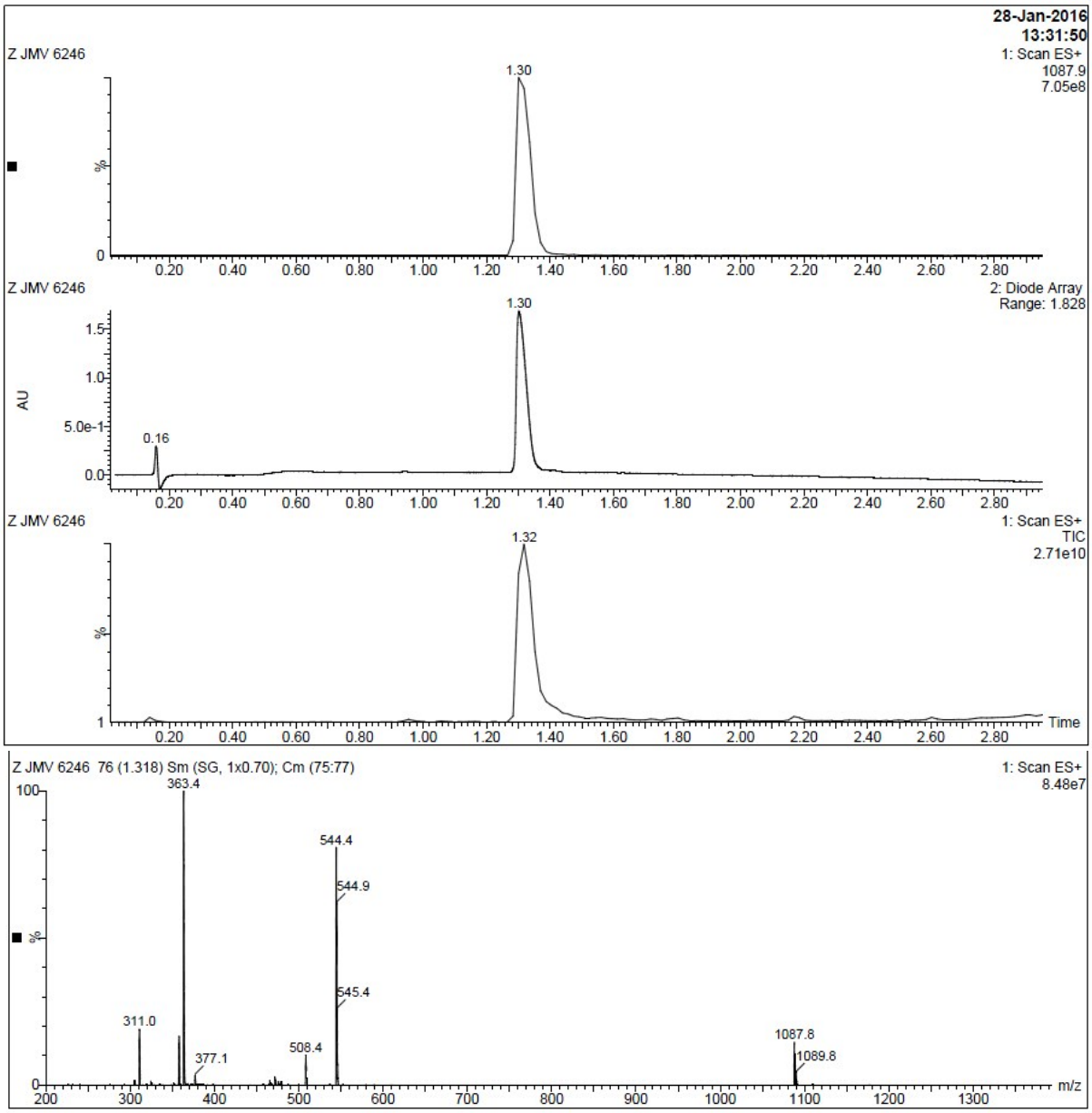


Figure S47. *LC/MS spectrum of* ***10***

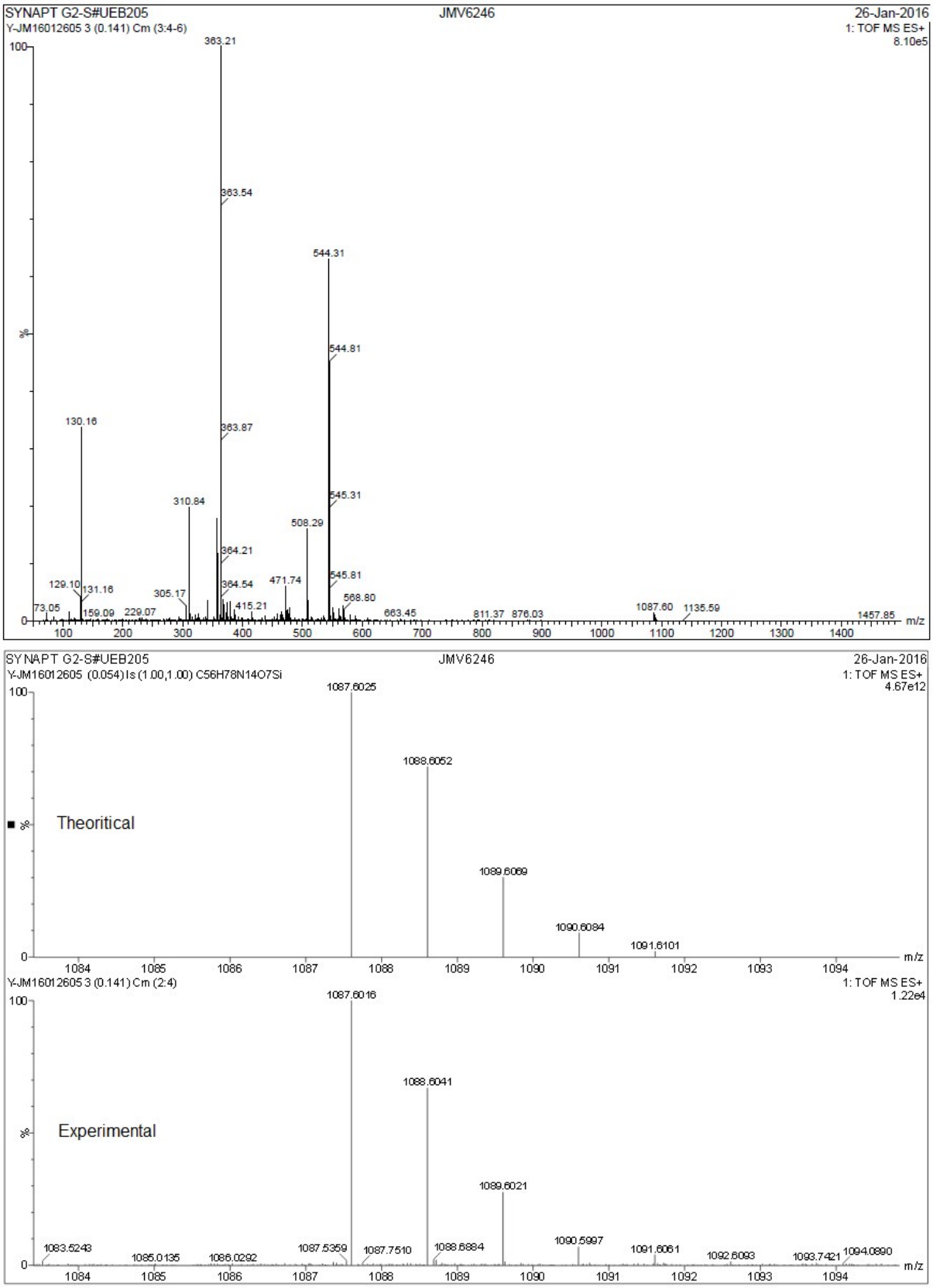
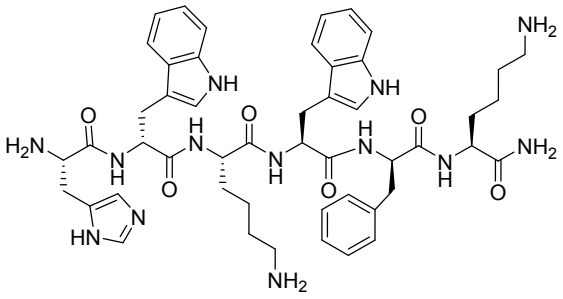
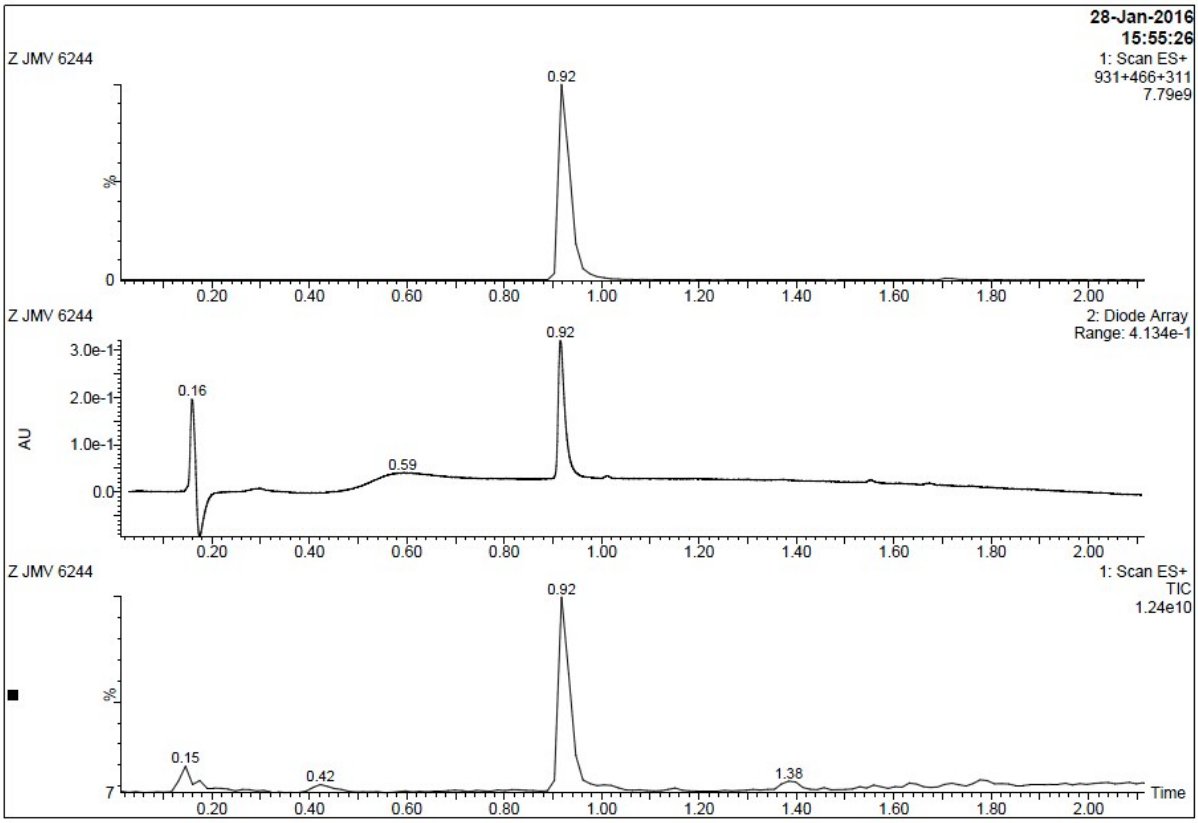


Figure S48. *HR-MS analysis of* ***10***

Lys^3 monomer 11 (JMV 6244)





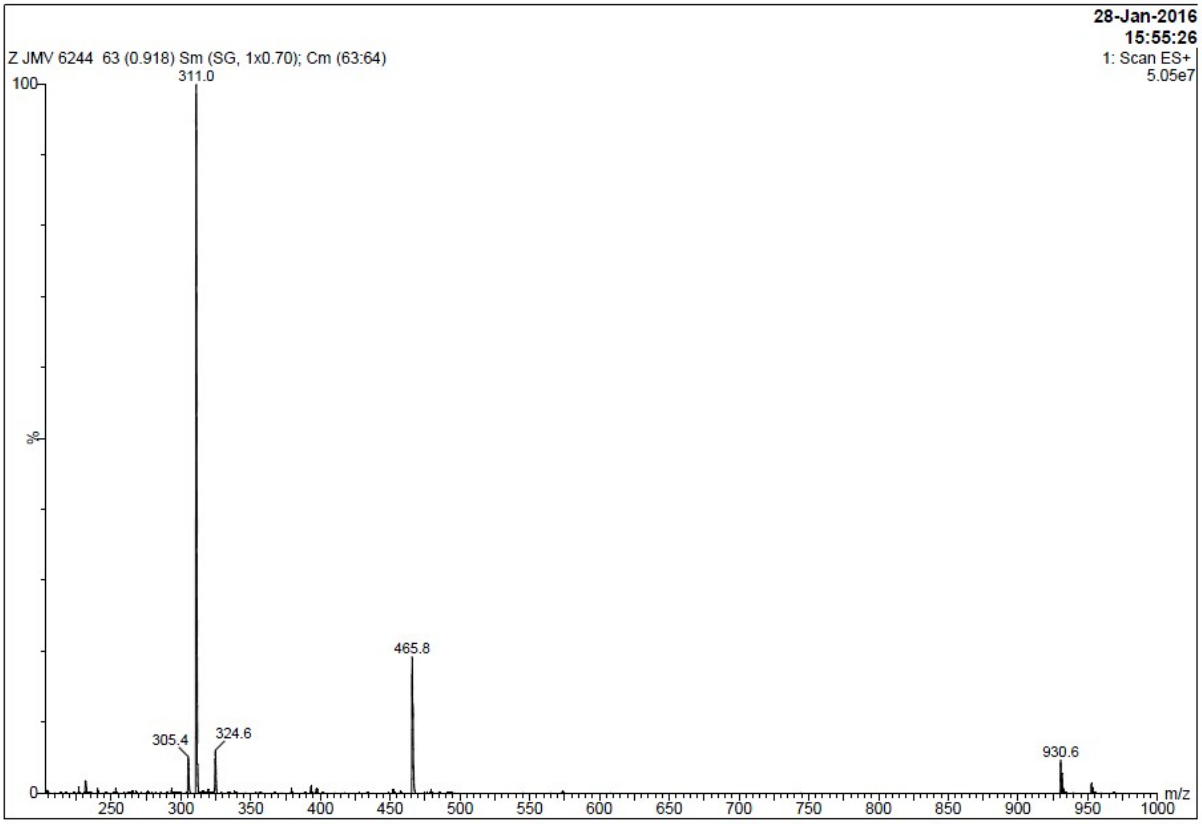


Figure S49. *LC-MS spectrum of* ***11***

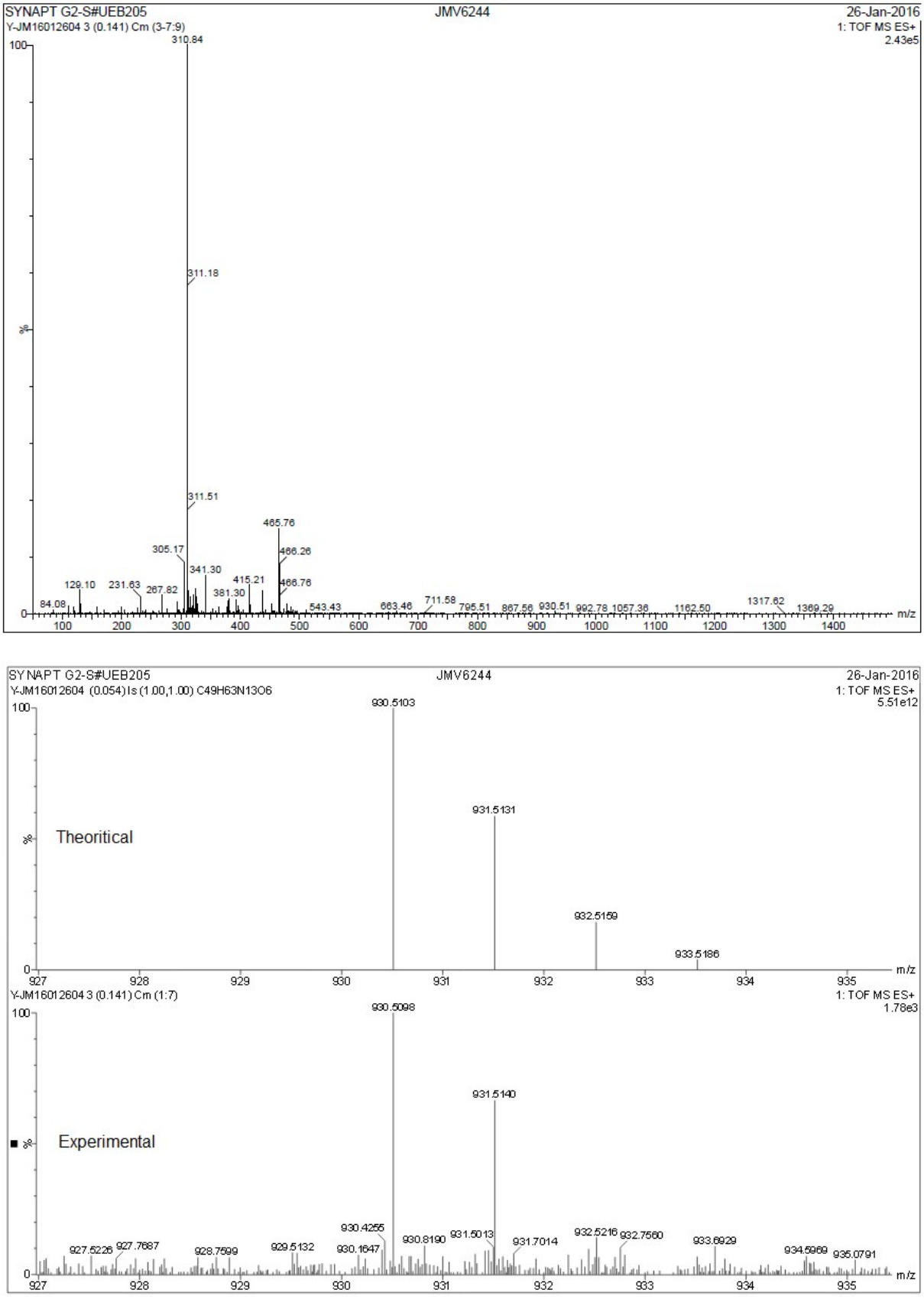
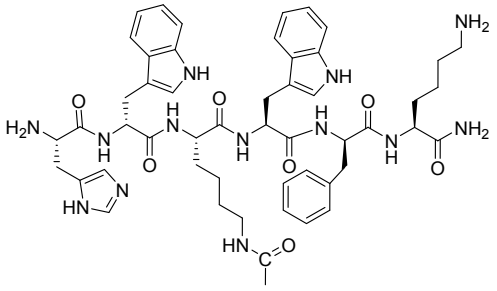
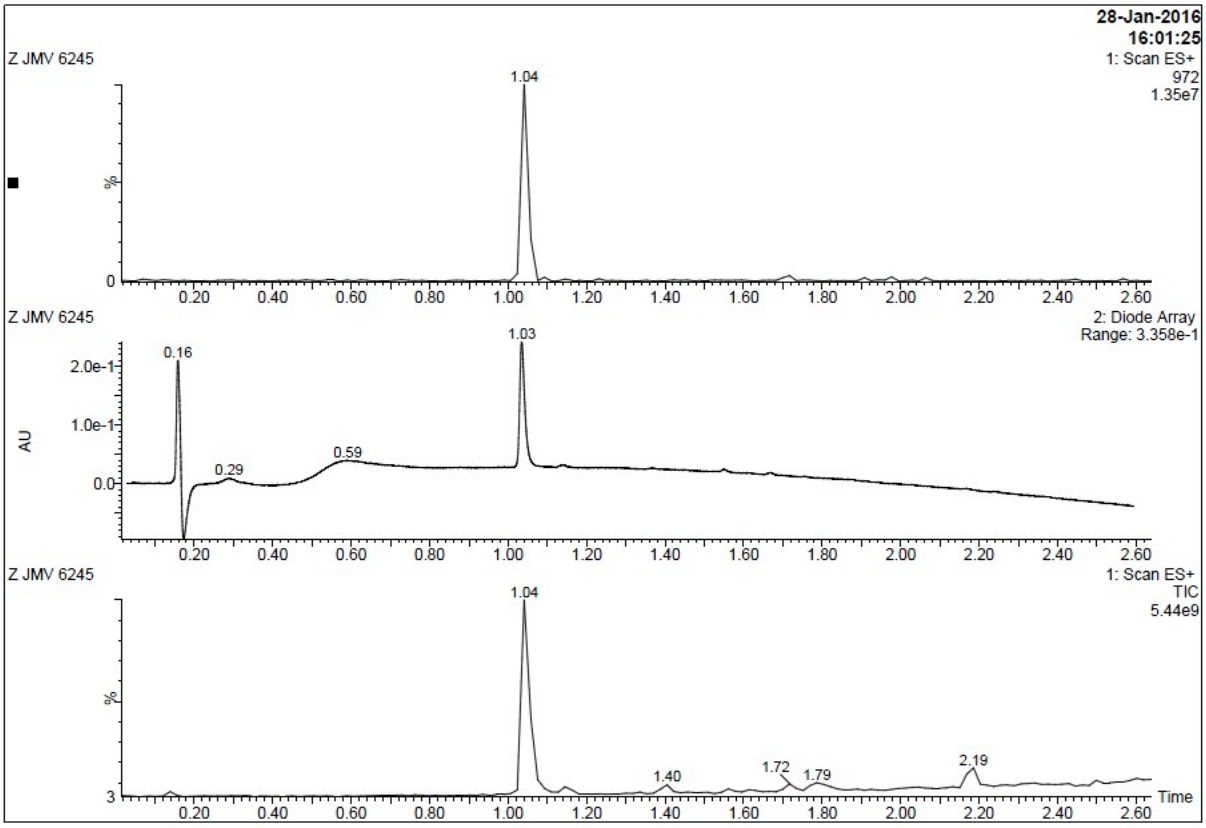


Figure S50. *HR-MS analysis of* ***11***

Lys^3(Ac) monomer 12 (JMV 6245)





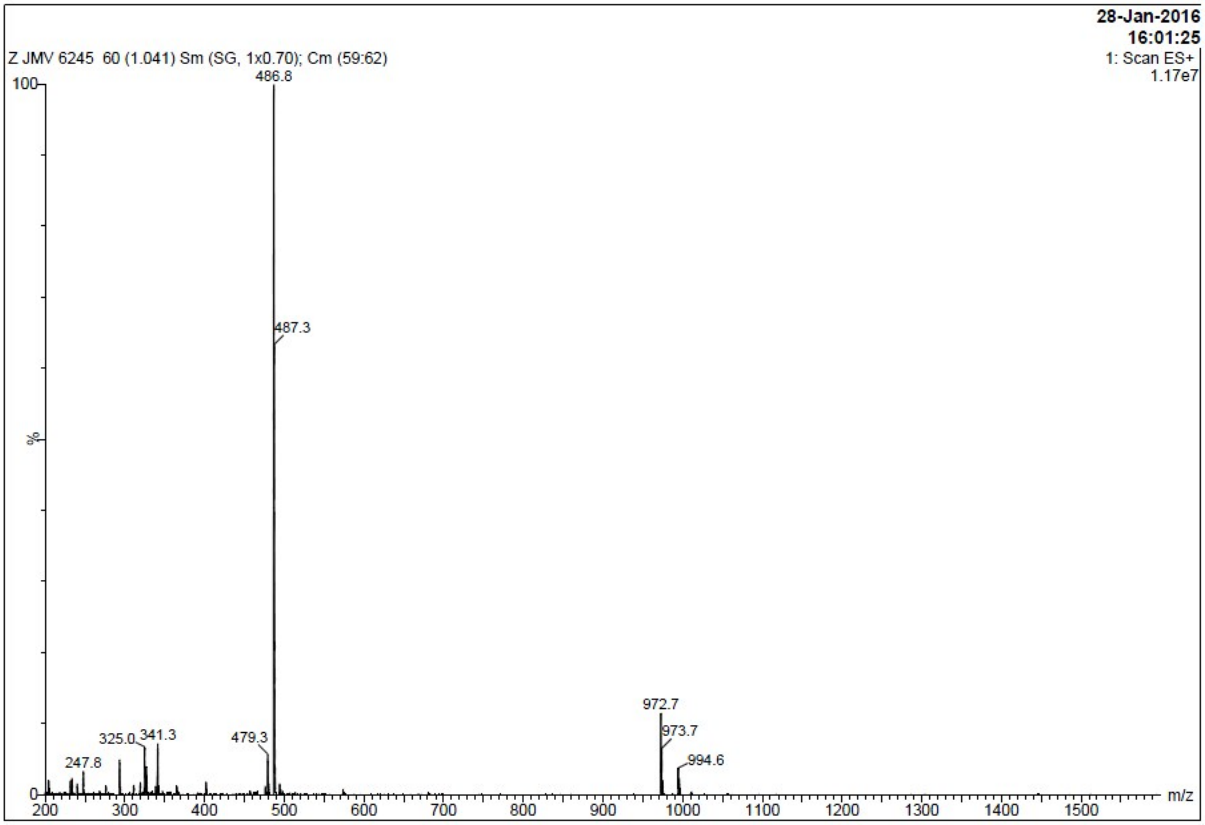


Figure S51. *LC-MS spectrum of* ***12***

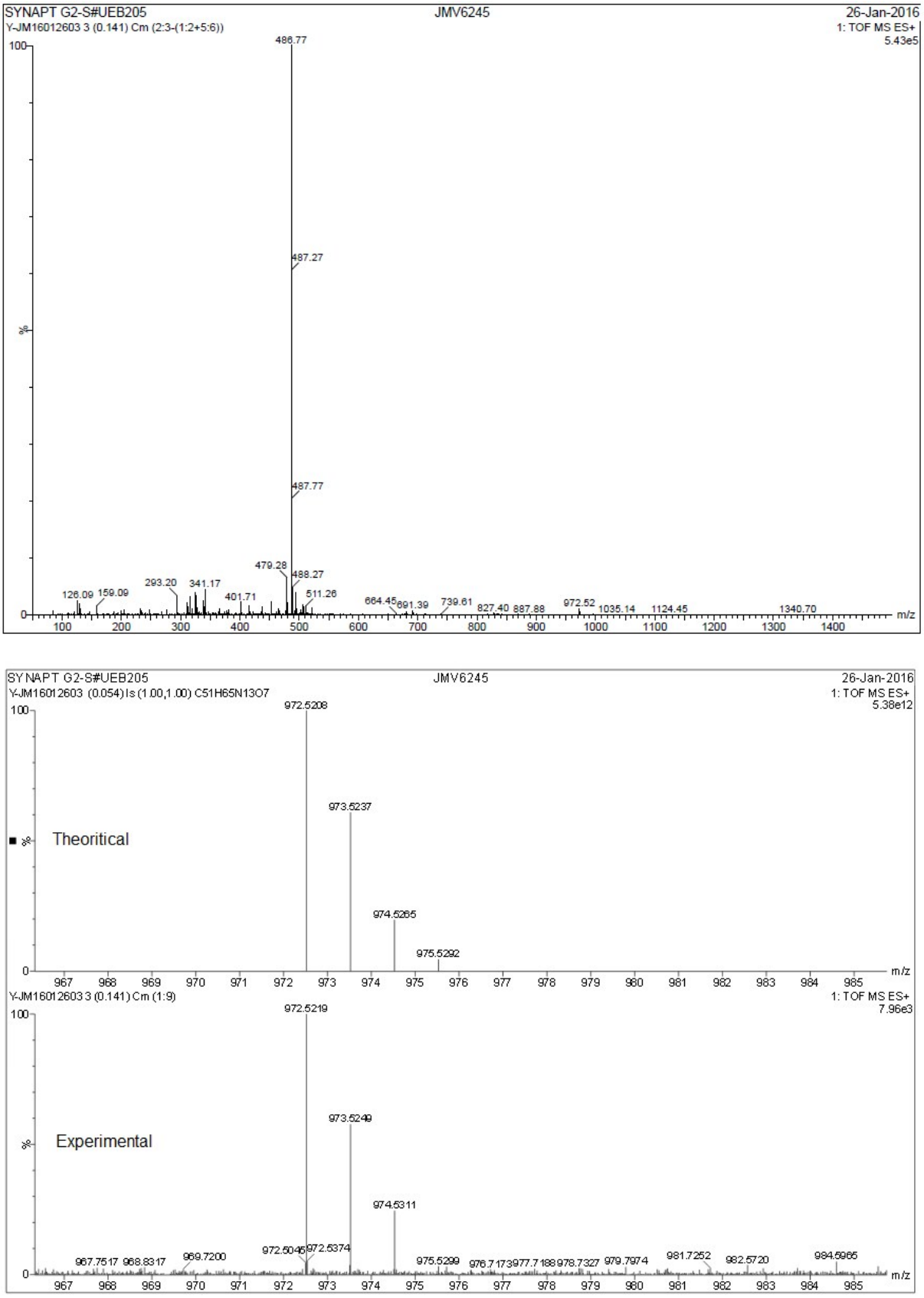


Figure S52. *HR-MS spectrum of* ***12***

Monomeric ligand binding assay

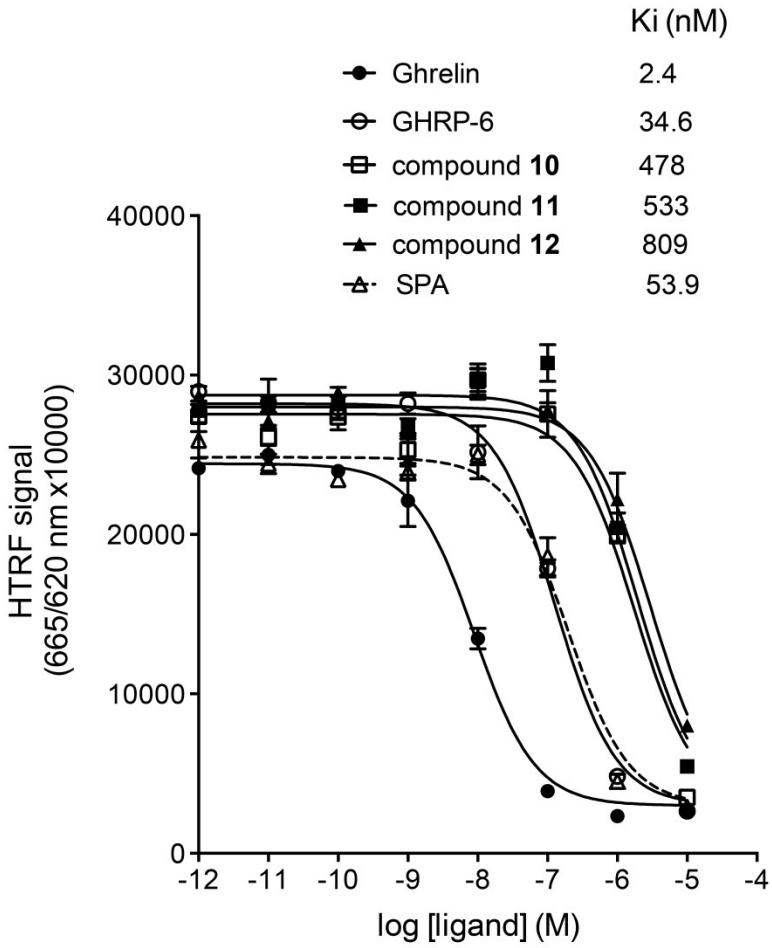


Figure S53. *Binding of compounds **10**, **11** and **12**, monomeric analogues of dimer **9**, to GHS-R1a. Ligands affinities were determined by HTRF-based competition binding assay performed on intact HEK293T cells as described in Materials and Methods. Results are from one representative experiment of two, each performed in triplicate.*

Bioactivity of monomeric ligands

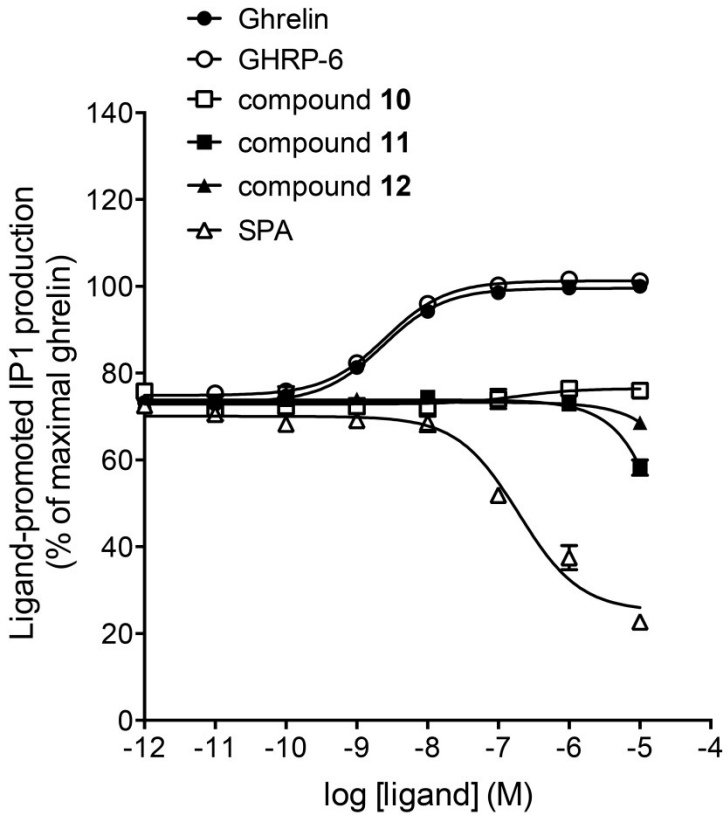


Figure S54. *Signaling property of compounds **10**, **11** and **12**. Efficacy of ligands to stimulate IP1 production was measured on HEK293T cells expressing the GHS-R1a as described in Materials and Methods. Results are one representative experiment of two, each performed in triplicate.*