Electronic supplementary information

Self-mineralization and assembly of a bis-silylated Phe-Phe pseudodipeptide to a structured bioorganic-inorganic material

Saïd Jebors, [a] Laurine Valot, [a,b] Cécile Echalier, [a] Baptiste Legrand, [a] Remi Mikhaleff, [a] Arie Van Der Lee, [c] Raul Arenal,[d] Muriel Amblard, [a] Pascal Dumy, [a] Jean Martinez, [a] Ahmad Mehdi,* [b] and Gilles Subra *[a]

[a] IBMM, Univ Montpellier, CNRS, ENSCM, Montpellier, France

[b] ICGM, Univ Montpellier, CNRS, ENSCM, Montpellier, France

[c] IEM, Univ Montpellier, CNRS, ENSCM, Montpellier, France

[d] Laboratorio de Microscopias Avanzadas (LMA), Instituto de Nanociencia de Aragon (INA), Universidad de Zaragoza, C/ Mariano Esquilor s/n 50018 Zaragoza, Spain

Corresponding Authors

* gilles.subra@umontpellier.fr

* ahmad.mehdi@umontpellier.fr

Table of contents

1. Abbreviations 2

2. Material and Methods 2

3. Synthesis of compound 1 Boc-Phe*gem*Phe-H from Boc-PhePhe-NH_2 4

4. Synthesis of compound 2 H-Phe*gem*Phe-H from 1 Boc-Phe*gem*Phe-H 5

5. Synthesis of compound 3 $(EtO)_3Si$-$(CH_2)_3$-NHCO-Phe*gem*Phe-CONH-$(CH_2)_3$-$Si(EtO)_3$ from compound 2, 2 TFA,H-Phe*gem*Phe-H. 7

6. Synthesis of compound 4 $(Me)_3Si$-$(CH_2)_3$-NHCO-Phe*gem*Phe-CONH-$(CH_2)_3$-$Si(Me)_3$ from compound 2, 2 TFA, H-Phe*gem*Phe-H 11

7. Preparation of M3A and M3B 15

8. X-ray diffraction and crystallography of M3B 15

9. M3A and compound 4 SEM images 17

10. Stability of compound 4 in acidic solution 18

11. Monitoring of M3B formation by ^1H NMR 18

12. Analyses of M3A and M3B materials and compounds 3 and 4 21

13. General scheme of synthesis of compound 5 24

14. Synthesis of compound Boc-Phe*gem*Phe-CONH-$(CH_2)_3$-$Si(Me)_3$ from compound 1 Boc-Phe*gem*Phe-H.....25

15. Synthesis of compound TFA, H-Phe*gem*Phe-CONH-$(CH_2)_3$-$Si(Me)_3$ from compound Boc-Phe*gem*Phe-CONH-$(CH_2)_3$-$Si(Me)_3$.....27

16. Synthesis of compound 5 $(OEt)_3Si$-$(CH_2)_3$-NHCO-Phe*gem*Phe-CONH-$(CH_2)_3$-$Si(Me)_3$ from compound Boc-Phe*gem*Phe-CONH-$(CH_2)_3$-$Si(Me)_3$ 28

17. Preparation of compound M5.....32

18. Analyses of M5 material 32

19. General scheme of synthesis of compound 6 33

20. Synthesis of compound Boc-Leu-Leu-NH_2 34

21. Synthesis of compound Boc-Leu*gem*Leu-H from Boc-Leu-Leu-NH_2 36

22. Synthesis of compound 2TFA, H-Leu*gem*Leu-H from Boc-Leu*gem*Leu-NH_2.....37

23. Synthesis of compound 6 $(OEt)_3Si$-$(CH_2)_3$-NHCO-Phe*gem*Phe-CONH-$(CH_2)_3$-$Si(OEt)_3$ from 2 TFA, H-Leu*gem*Leu-H 39

24. Preparation of compound M6.....42

25. Analyses of M6 material 42

Step by step synthesis of bis-silylated HPhe*gem*Phe 45

1. Abbreviations

ACN, acetonitrile; Boc, t-butyloxycarbonyl; DCM, dichloromethane; DIEA, diisopropylethylamine; DMF, N-N'-dimethylformamide; LC/MS, tandem liquid chromatography/ mass spectrometry; NMR, nuclear magnetic resonance; TFA, trifluoroacetic acid; THF, tetrahydrofurane; TIS, Triisopropylsilane; T3P, Propylphosphonic anhydride; BTIB, Bis[(trifluoroacetoxy)iodo]benzene, NMM, N-methymorpholine; PyBOP, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate; pip, piperidine; IBCF, Isobutyl chloroformate; ICPTES, isocyanatopropyltriethoxysilane; ICPTMS, isocyanatopropyltrimethylsilane; SPPS, solid phase peptide synthesis.

2. Material and Methods

All solvents and reagents were used as supplied. Solvents used for LC/MS were of HPLC grade. DCM and DMF were purchased from Carlo Erba. Fmoc amino acid derivatives were purchased from Iris Biotech (Marktredwitz, Germany). DIEA, TFA were obtained from Sigma-Aldrich (St. Louis, MO, USA). TIS was obtained from Alfa Aesar. NMR solvents were obtained from Euriso-top. ICPTES was obtained from TCI and ICPTMS from Sikémia (Montpellier, France).

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% HCO_2H; eluent B: acetonitrile/0.1% HCO_2H. 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.

NMR spectra were recorded on a Bruker Avance III HD 400 spectrometer equipped with a BBFO probe at the “Laboratoire de Mesure Physique” of the University of Montpellier.

^{13}C and ^{1}H NMR spectra were recorded at 298 K in DMSO-d_6. Chemical shifts (δ) were reported in parts per million (ppm) using residual non-deuterated solvents as internal references (DMSO-d_6, δH = 2.50 ppm). Signals were indicated as s (singlet), d (doublet), t (triplet), q (quartet), dd (double doublet), m (multiplet) … Coupling constants were measured in Hertz.

Spectra were processed, visualized analysed using Topspin 3.5 (Bruker Biospin).

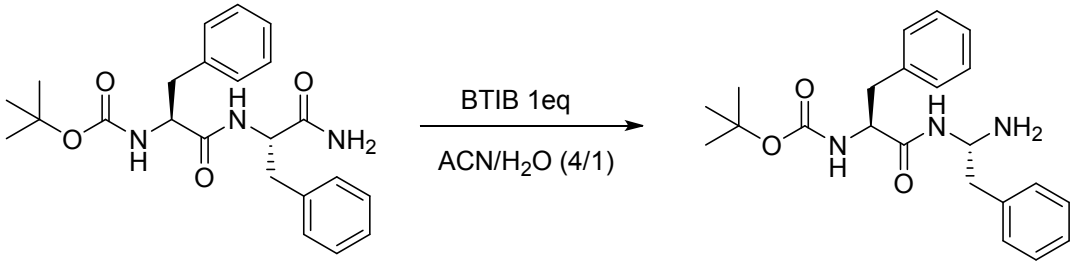
Solid-state NMR spectrum for ^{29}Si was recorded on a Varian VNMRS 300 Solid spectrometer at a magnetic field strength of 7.05 T. A 7.5 mm MAS probe was used with a spinning rate of 5 kHz. Single pulse experiments with a continuous wave ^{1}H decoupling were used for ^{29}Si NMR, with 2 µs π/2 pulse duration and a recycle delay of 60 s. A recycle delay of 200 s was found necessary to allow full ^{29}Si fully relaxation although this did not lead to any change in the relative ratio of the individual components on spectral decomposition. Thus, data with recycle delay of 60 s can be considered as quantitative. ^{13}C solid state NMR analyses were performed on a Varian VNMRS 600 MHz NMR spectrometer (B0 = 14.1 T, $v_0(^{1}H)$ = 599.8 MHz and $v_0(^{13}C)$ = 150.8 MHz). Experiments were performed using a Varian T3 HXY 3.2 mm probe, tuned to ^{1}H and ^{13}C, and spinning at 18 kHz. A ^{1}H-^{13}C CPMAS (Cross Polarization Magic Angle Spinning) pulse sequence was used, with a 1H 90° excitation pulse of 2.5 µs, followed by a ramped contact pulse of 2 ms. Spinal-64 ^{1}H decoupling was applied during acquisition (100 kHz RF). The recycle delay was set to 1-2 s, depending on the sample, and the number of transients acquired ranged from 1430 to 7670. The ^{13}C chemical shifts were referenced to TMS (tetramethylsilane) using the deshielded resonance of adamantane (38.5 ppm) as secondary reference.

Infrared experiments were performed with a Perkin Elmer Spectrum two in ATR mode, with Spectrum software. Samples were analysed in powder, between 450 and 4000cm^{-1}.

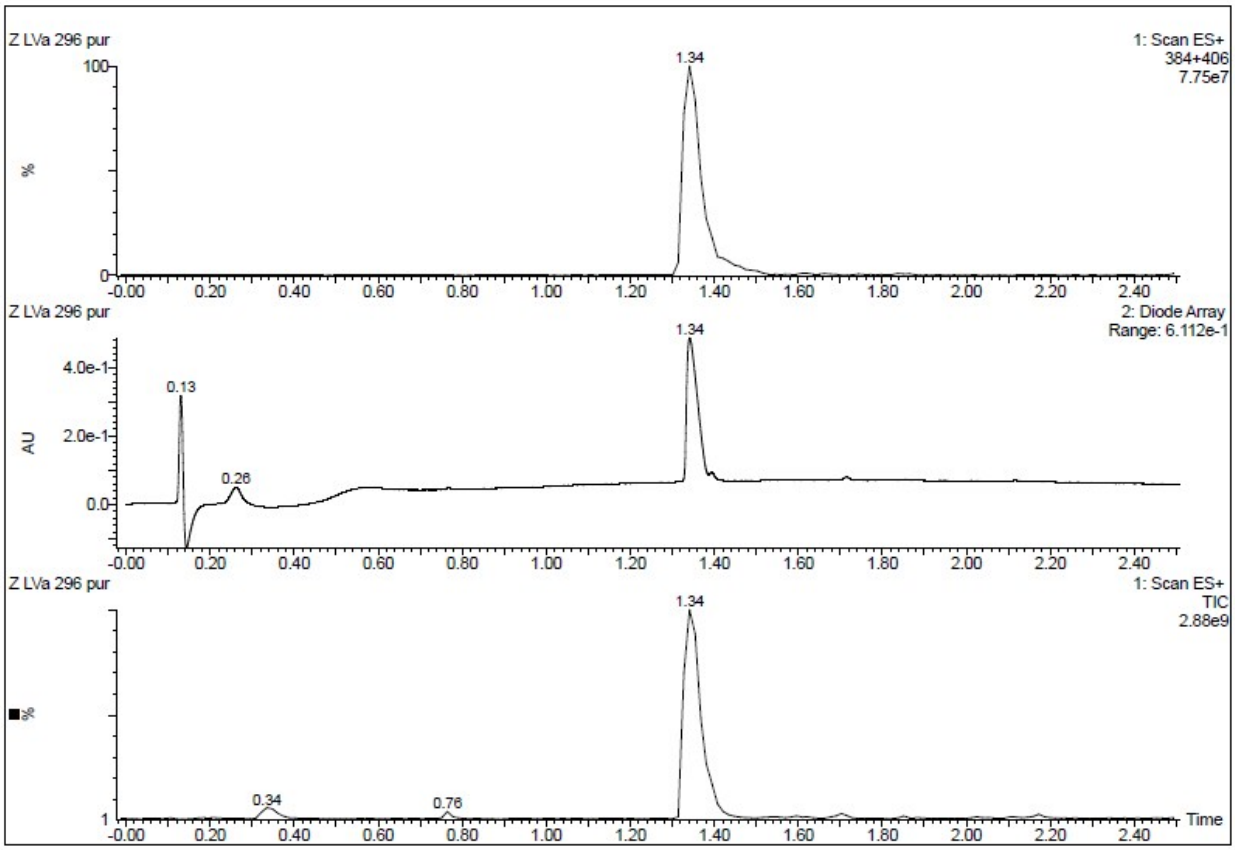
Elemental analysis were performed with a ElementarVario Micro Cube at the “Laboratoire de Mesure Physique” of the University of Montpellier. Carbon, hydrogen and nitrogen analyses were done by burning, and oxygen by pyrolysis. Analyses were done in duplicate.

Powder X-ray diffraction experiments were carried out on a high-resolution Bonse-Hart camera with two germanium channel cuts for very small q values. The wavelength used was 1.542 Å (CuK_α radiation). Saxs and Waxs analyses were performed at the “Laboratoire Charles Coulomb” of the University of Montpellier.

3. Synthesis of compound **1** Boc-Phe*gem*Phe-H from Boc-PhePhe-NH_2



Boc-PhePhe-NH_2 (1 eq, 12.16 mmol, 5 g) and BTIB (1 eq, 12.16 mmol, 5.3 g) were dissolved in an ACN/water (4/1 v/v) solution (243.3 mL). After four hours stirring at room temperature (20°C), the reaction is completed. The solvents are concentrated under reduced pressure, and a white solid is recovered after precipitation in diethyl ether (4.28 g, 92% yield).



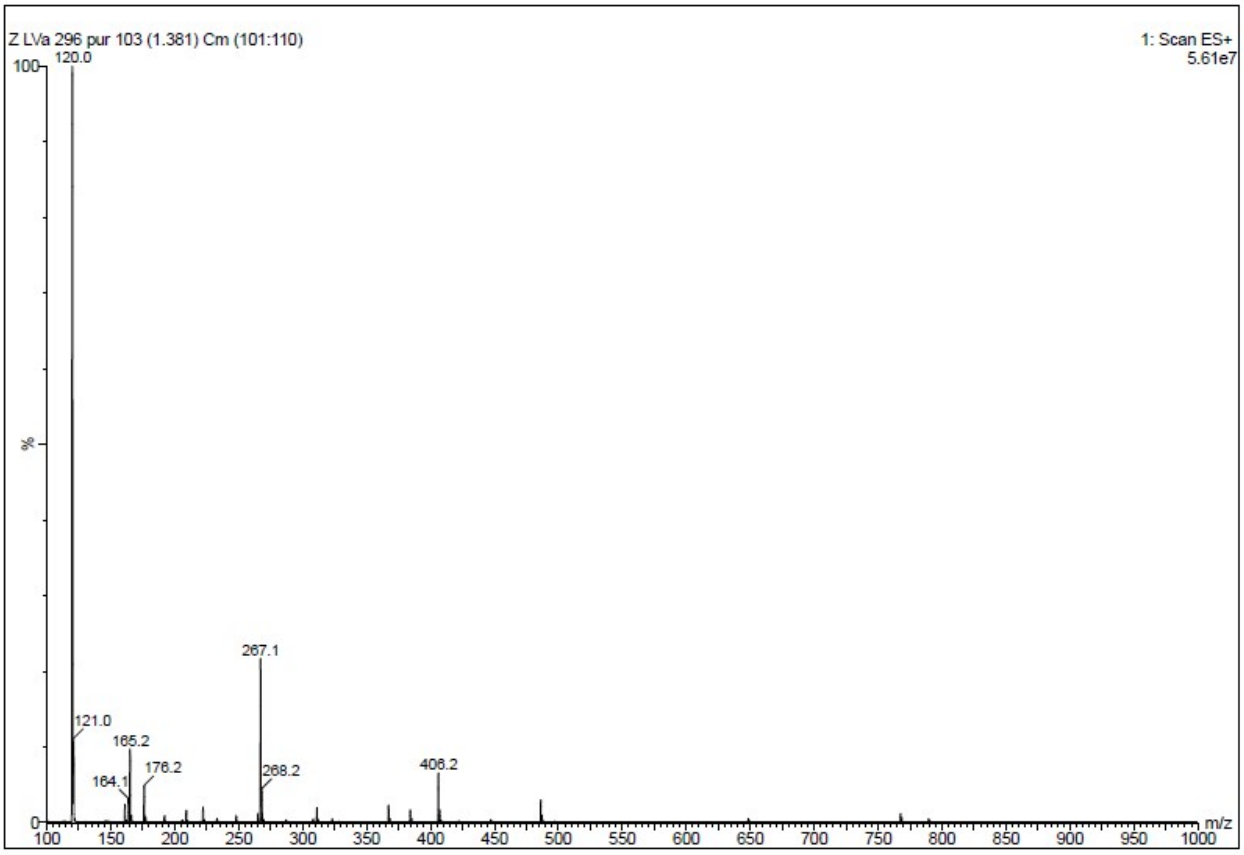
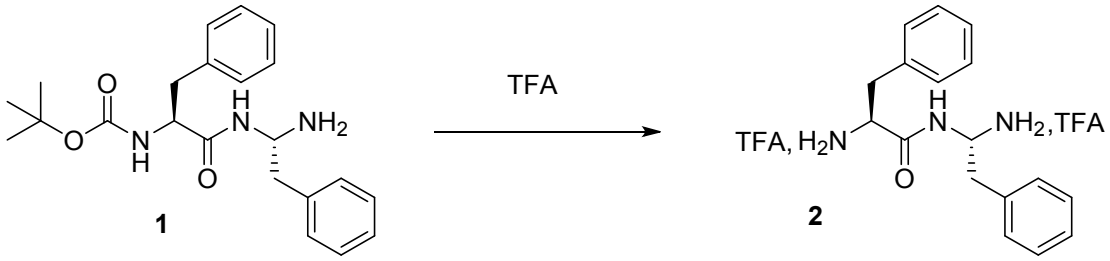
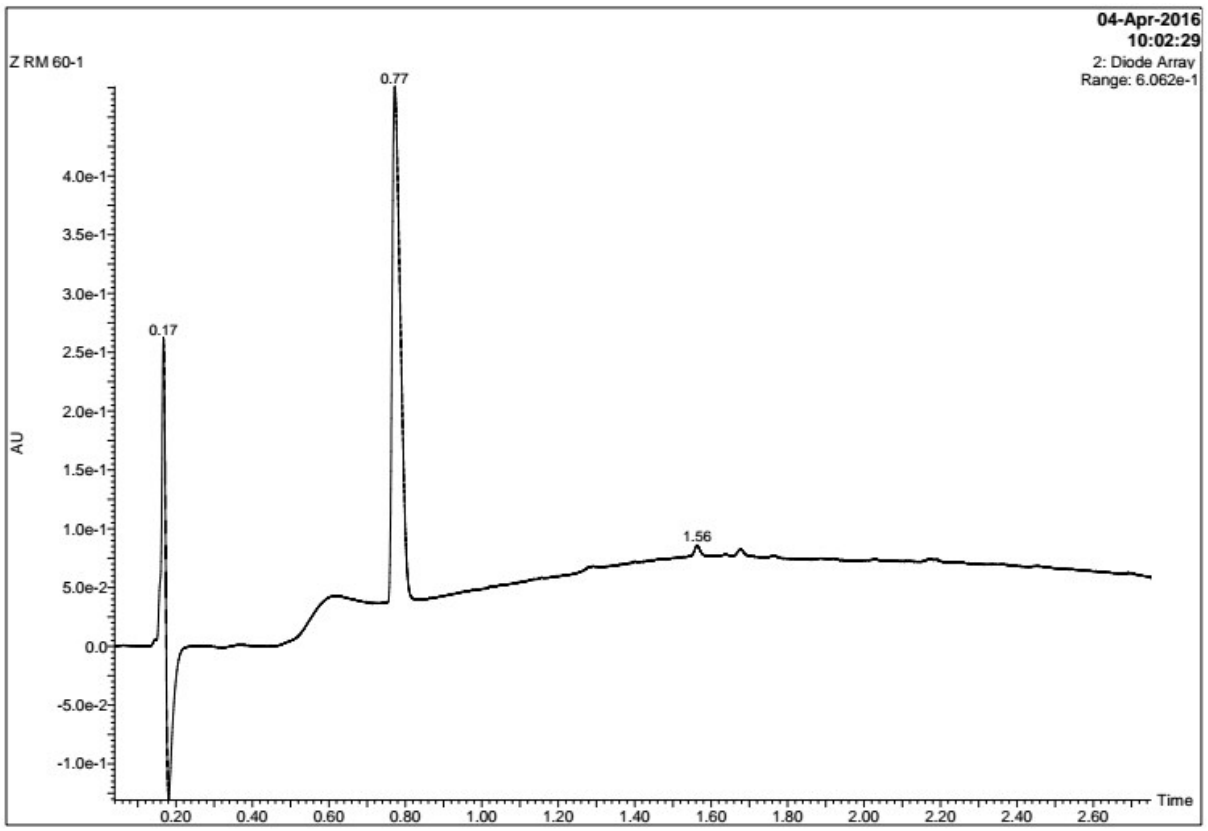


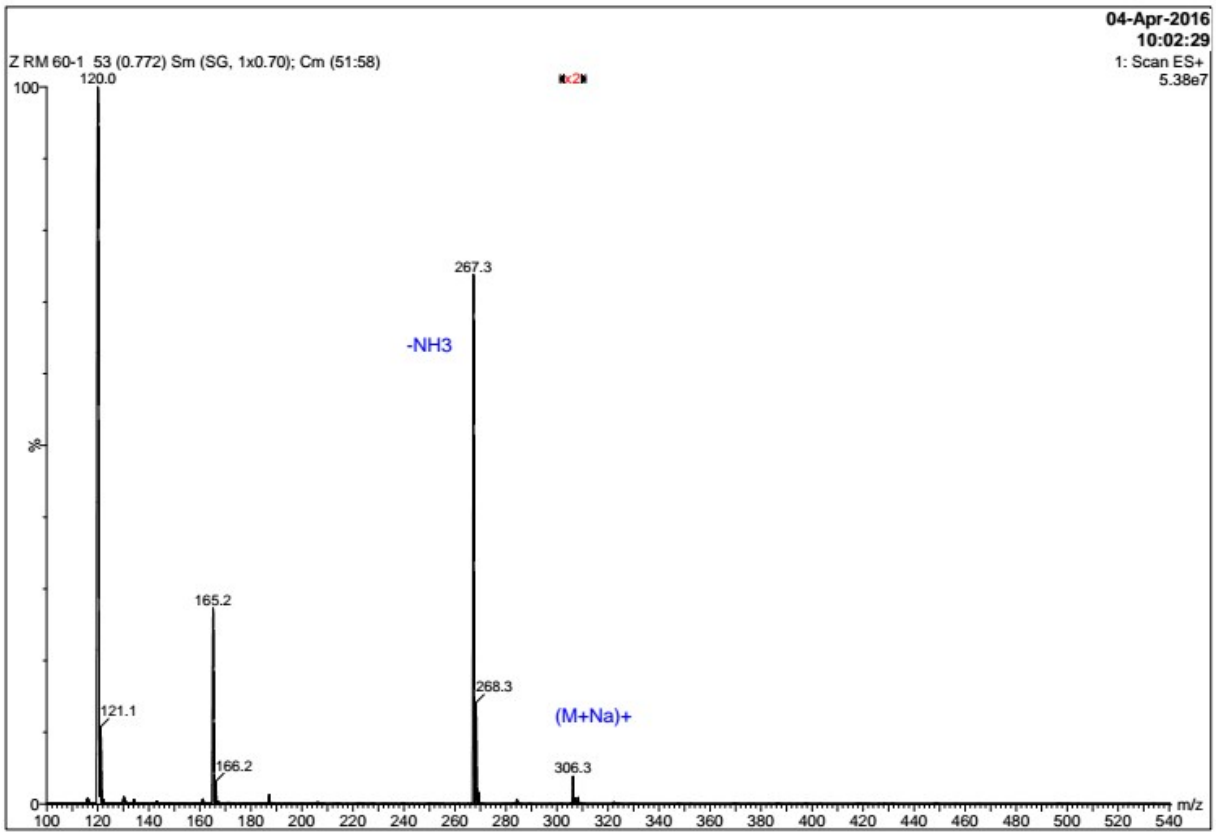
Fig. S1 LC/MS **(ESI$^+$)** of compound **1.** MW 383.49 g/mol
LC/MS (ESI$^+$): t_R = 1.34 min, m/z 406 [M+Na]$^+$.

4. Synthesis of compound **2** H-Phe*gem*Phe-H from **1** Boc-Phe*gem*Phe-H



5.00 g of Boc-Phe*gem*Phe-H (13 mmol) were dissolved in TFA (20 mL). After 10 min at room temperature (20°C), the solution is concentrated under reduced pressure, coevaporated with cyclohexane. An oil is obtained which is dissolved in ACN/water (1/9 v/v) solution (20 mL). The solution was freeze dried. 6.43 g of compound **2** (97% yield) as trifluoracetate salts were obtained.

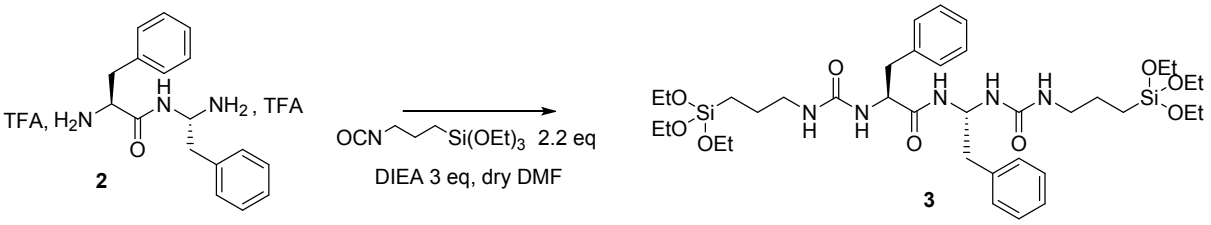




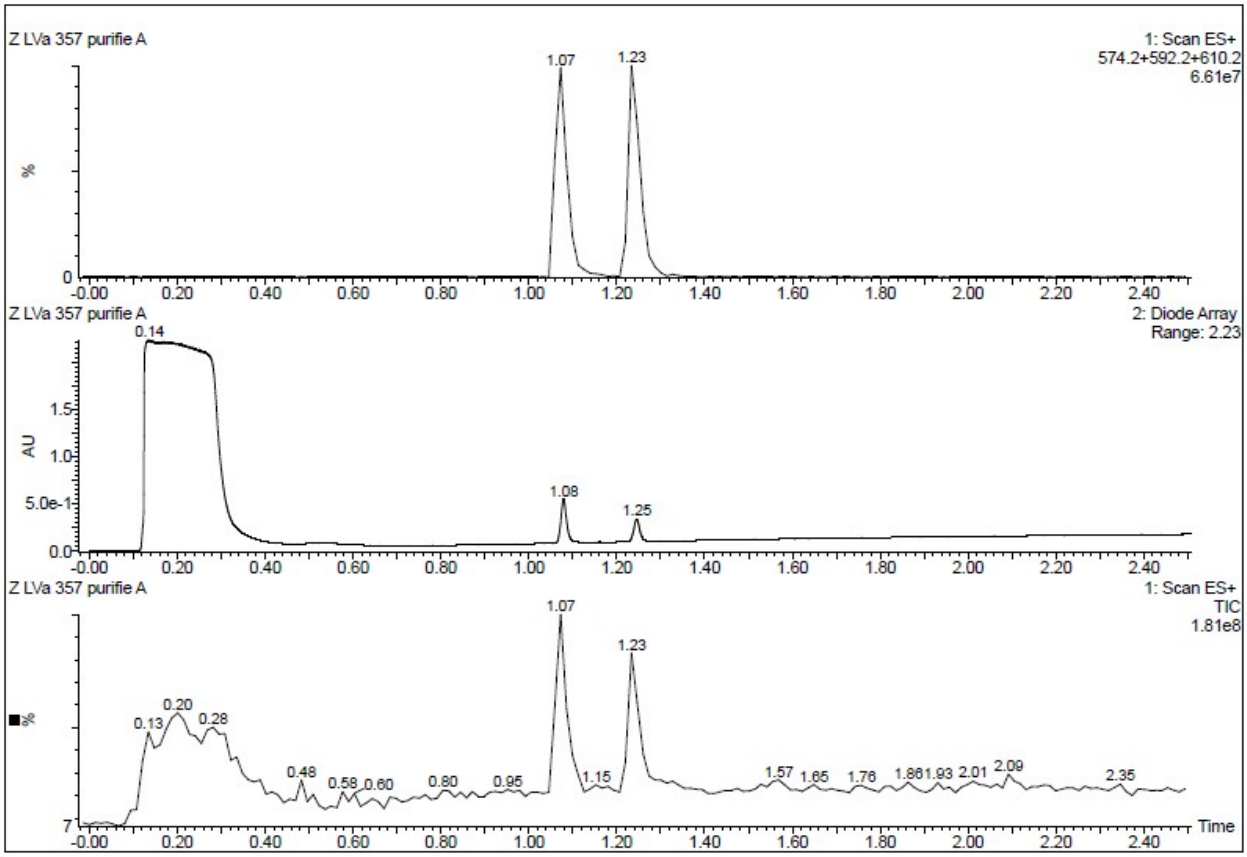
Fig/ S2 LC/MS **(ESI⁺)** of compound **2.** MW 475.4 g/mol (with 2 TFA salts)
LC/MS (ESI⁺): t_R = 0.77 min, m/z 610 $[M\text{-}NH_3]^+$; m/z 306 $[M+Na]^+$.

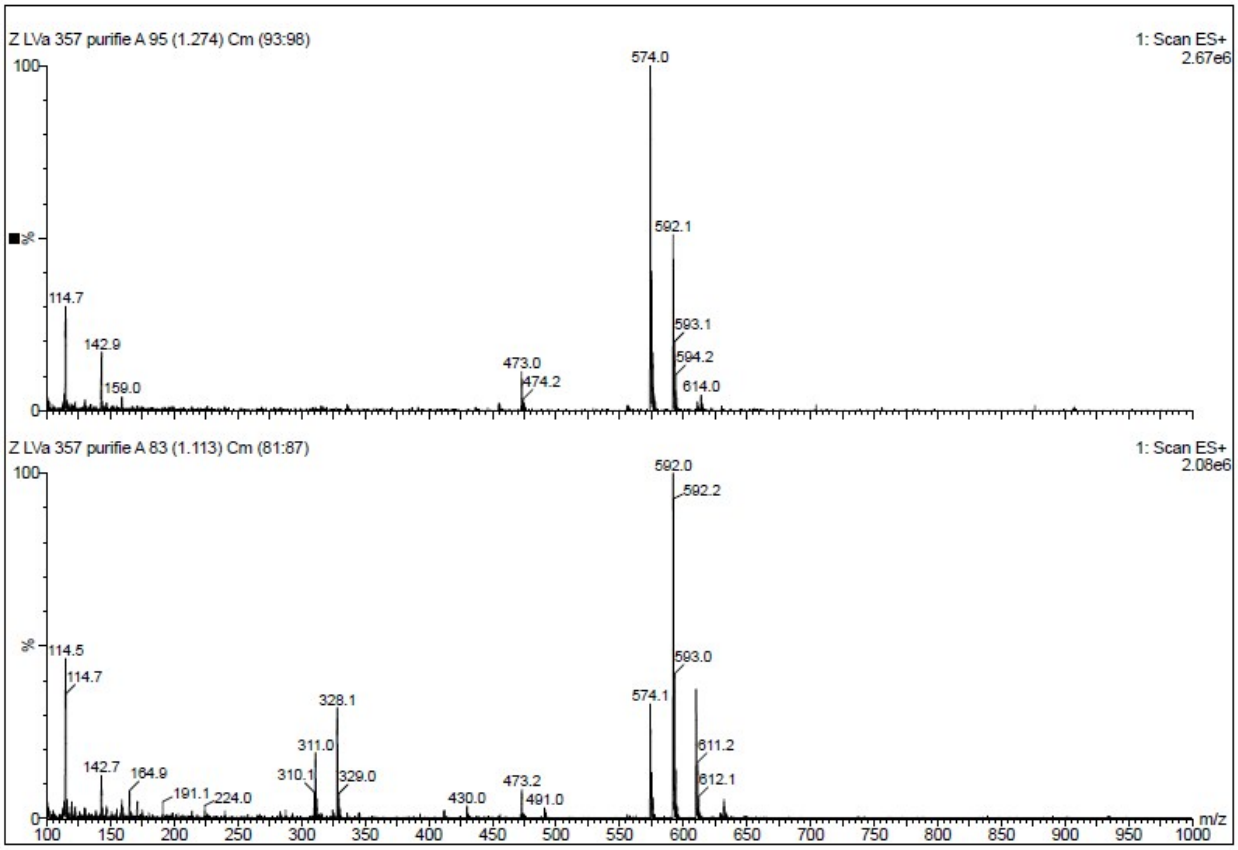
The monoprotonated ion of the desired compound was not observed. This behaviour is typical of the gemdiamino derivatives which undergo a deamination in the spectrometer. However, the loss of ammonia was observed as the sodium adduct. LC/MS (ESI^+): t_R = 0.77 min, m/z 610$[M\text{-}NH_3]^+$; m/z 306 $[M+Na]^+$.

5. Synthesis of compound **3** $(EtO)_3Si\text{-}(CH_2)_3\text{-}NHCO\text{-}Phe\textit{gem}Phe\text{-}CONH\text{-}(CH_2)_3\text{-}Si(EtO)_3$ from compound **2**, 2 TFA,H-Phe*gem*Phe-H.



H-Phe*gem*Phe (1.00 g, 3,53 mmol, 1 eq) was dissolved in a dry DMF solution (20 mL) containing DIEA (1.82 mL, 10.56 mmol, 3 eq) under argon. ICPTES (2.01 mL, 7.74 mmol, 2.2 eq) was added. After 40 minutes at room temperature (20°C), solvent was removed under reduced pressure and the desired compound **3** was precipitated in diethyl ether. After centrifugation and decantation, the solid was washed again with diethyl ether. This washing/centrifugation/decantation procedure was repeated twice. The solid was dried overnight in a vacuum desiccator. 2.28 g of white solid was recovered (83% yield).

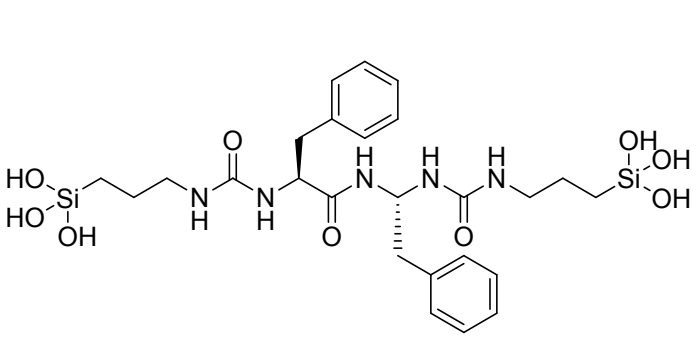




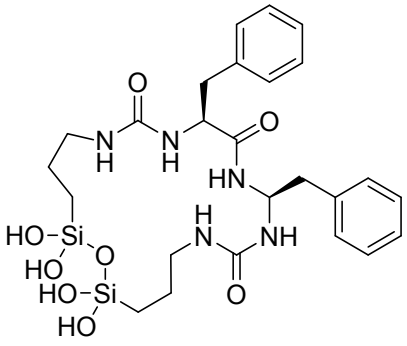
MS spectra t_R =1.08 min and t_R=1.23 min

Fig. S3 LC/MS **(ESI⁺):** of compound **3**, MW 778.10 g/mol
Only the products of hydrolysis of ethoxysilyl groups into silanols are detected. The compound **3** was detected at two retention times due hydrolysis and intramolecular cyclization.

In the spectra description below, M corresponds to molecule **3a** t_R = 1.08 min, MW 609.8 g/mol.
M' corresponds to the molecule **3b** t_R = 1.23 min, MW 591.8 g/mol. This type of behaviour is common for silylated compounds in acidic water/acetonitrile eluents and was already observed.[1]



3a



3b

t_R = 1.08 min, m/z 610 $[M+H]^+$; m/z 632 $[M+Na]^+$; m/z 592 $[M+H-H_2O]^+$; m/z 574 $[M+H-2\ H_2O]^+$
t_R = 1.23 min, m/z 592 $[M'+H]^+$; m/z 614 $[M'+Na]^+$;m/z 574 $[M'+H-H_2O]^+$; m/z 556 $[M'+H-2\ H_2O]^+$

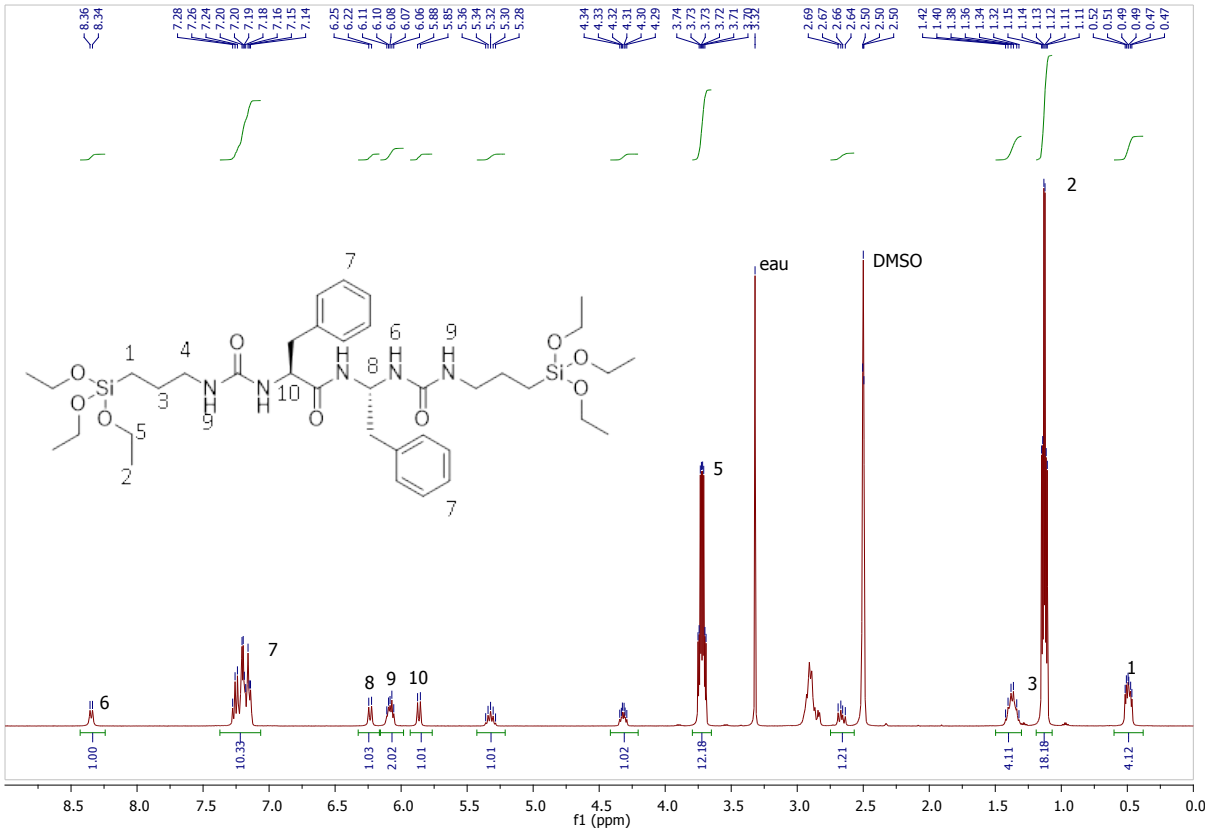


Fig. S4 ^{1}H NMR spectrum of compound **3** in DMSO-d6 (400 MHz).

^{1}H NMR (400 MHz, DMSO) δ 8.35 (d, *J* = 7.5 Hz, 1H) , 7.40 – 7.01 (m, 10H), 6.23 (d, *J* = 8.5 Hz, 1H), 6.08 (dt, *J* = 11.3, 5.6 Hz, 2H), 5.86 (d, *J* = 8.5 Hz, 1H), 5.43 – 5.21 (m, 1H), 4.32 (td, *J* = 8.2, 5.0 Hz, 1H), 3.72 (qd, *J* = 7.0, 3.4 Hz, 12H), 2.88 (dt, *J* = 14.2, 6.5 Hz, 7H), 2.66 (dd, *J* = 13.7, 8.1 Hz, 1H), 1.50 – 1.30 (m, 4H), 1.13 (td, *J* = 7.0, 3.4 Hz, 18H), 0.60 – 0.38 (m, 4H).

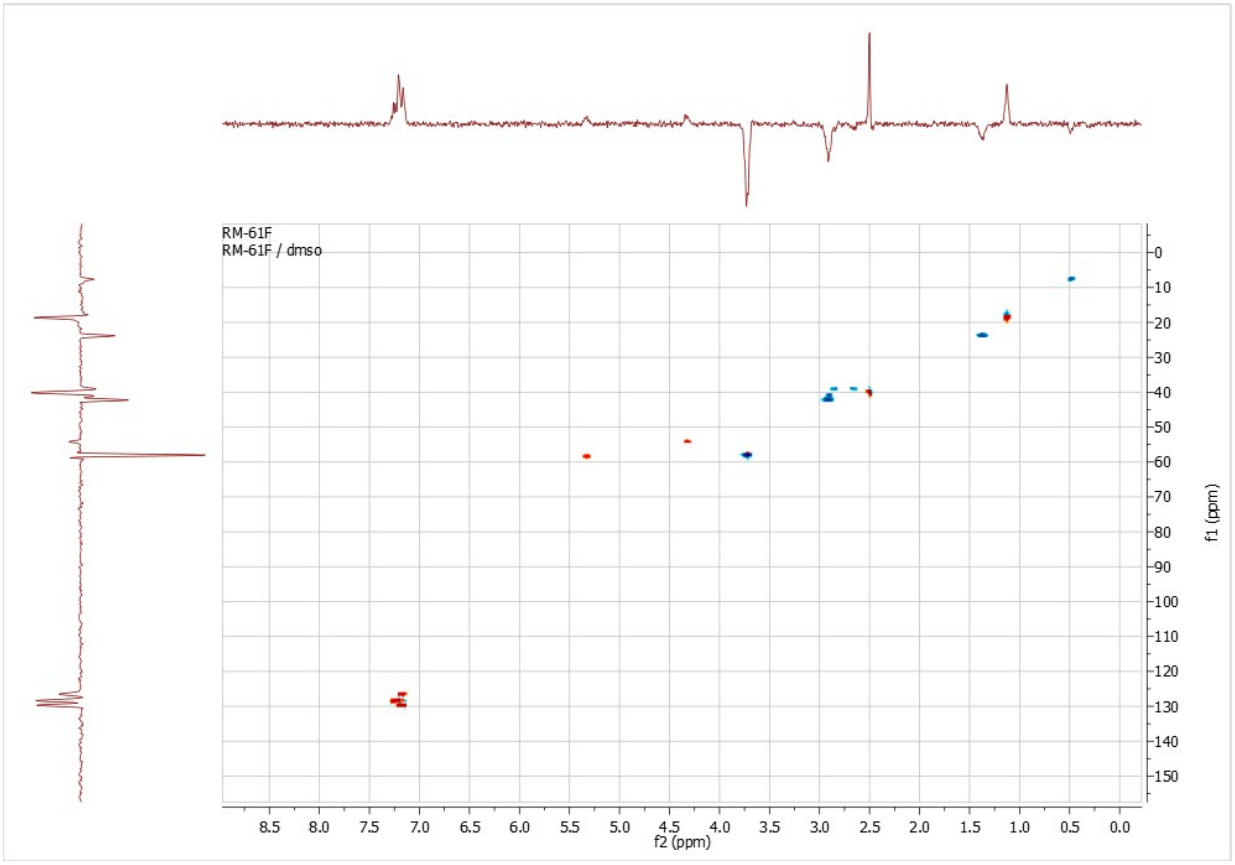


Fig. S5 ^{13}C NMR spectrum of **3** in DMSO-d_6 (500-100.6 MHz).

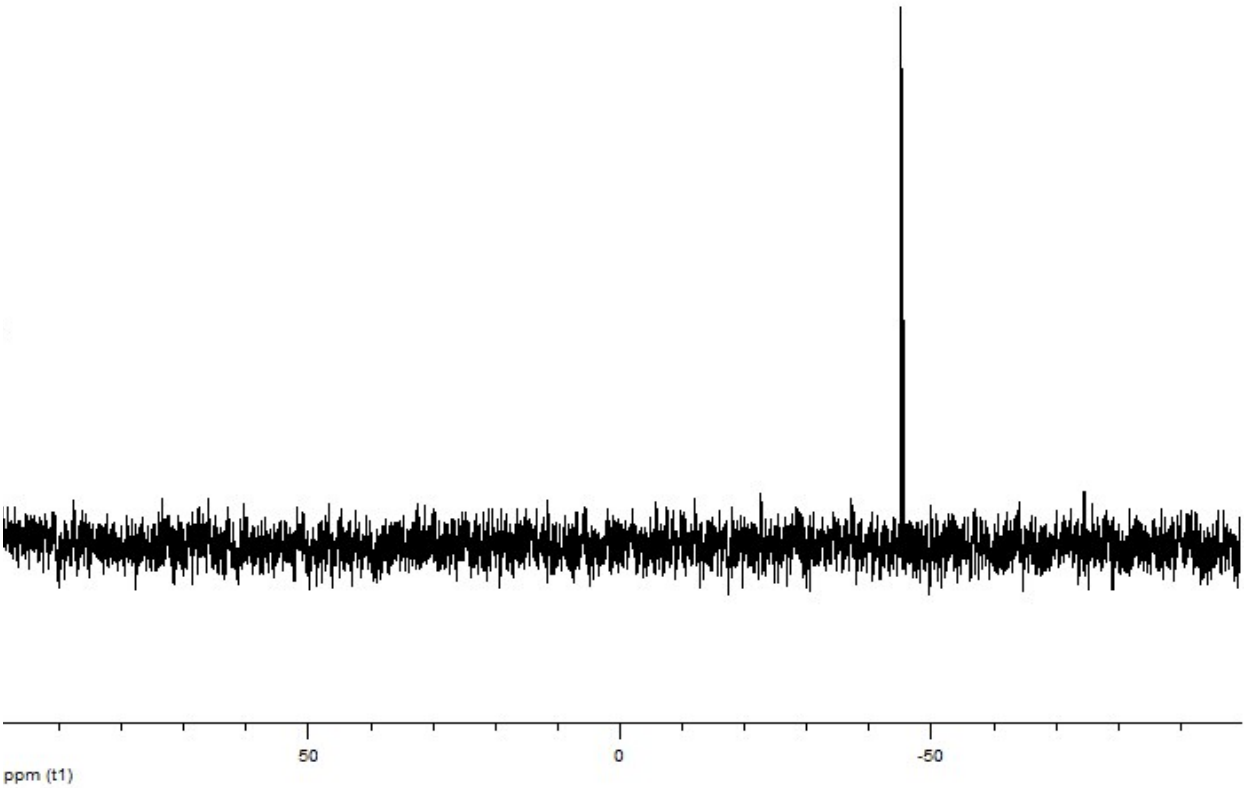
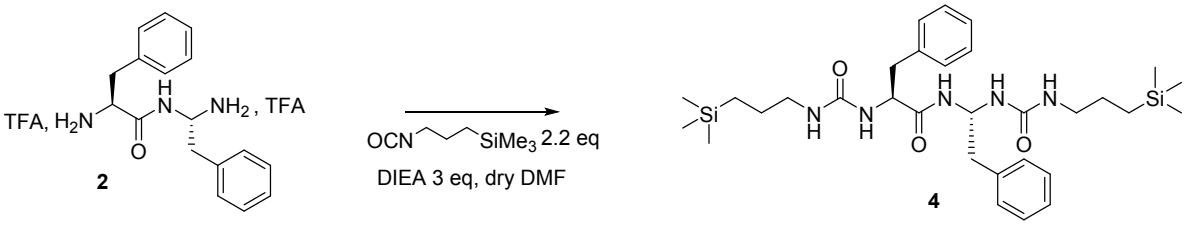
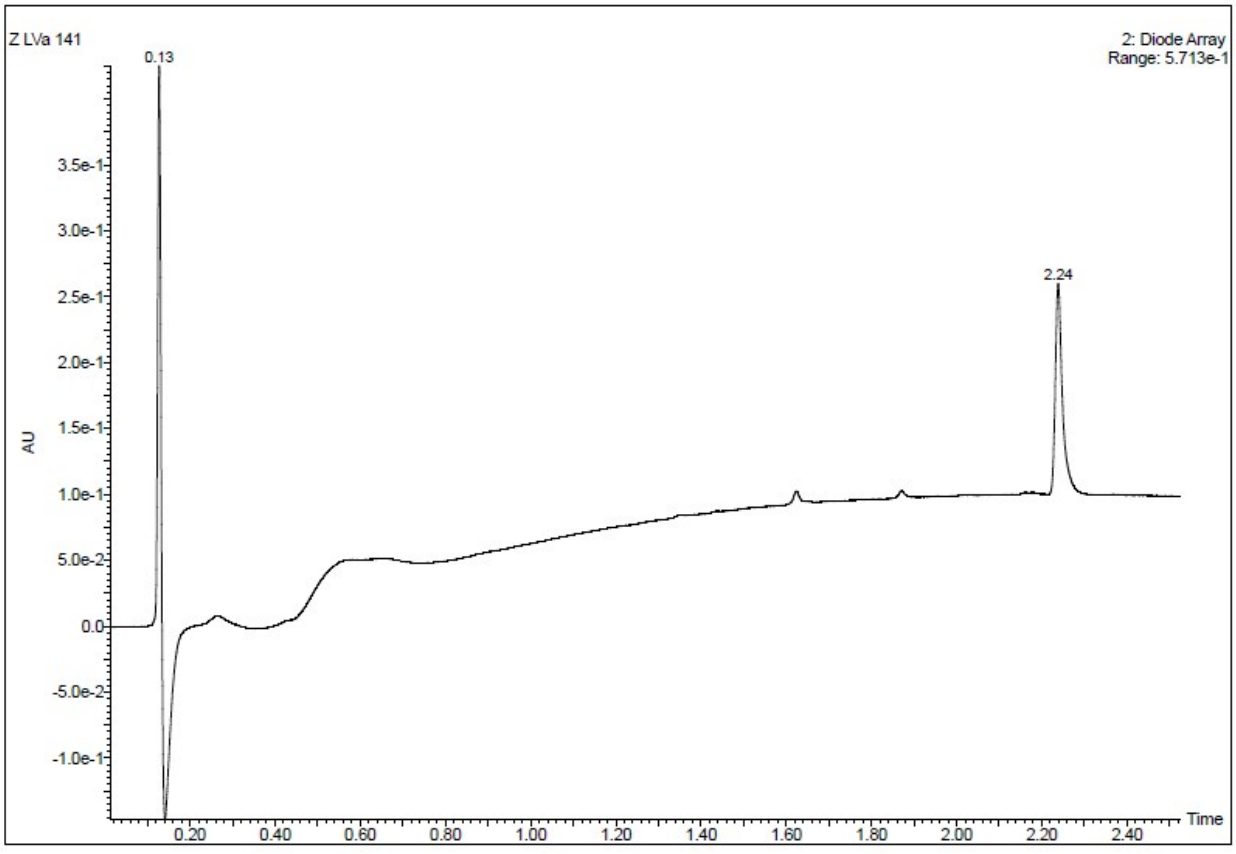


Fig. S6 ^{29}Si NMR spectrum of **3** in DMSO-d_6 (99 MHz).

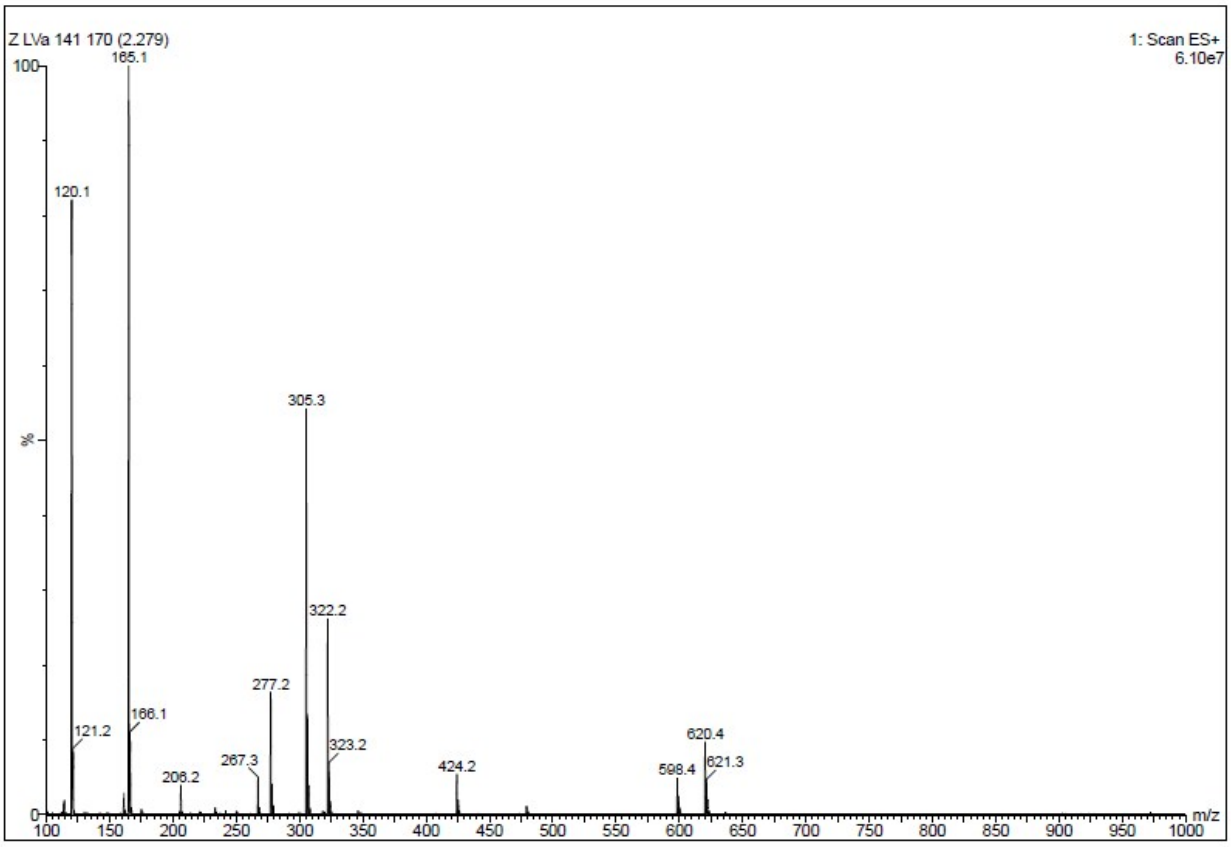
6. Synthesis of compound **4** $(Me)_3Si-(CH_2)_3-NHCO-Phe$*gem*$Phe-CONH-(CH_2)_3-Si(Me)_3$ from compound **2**, 2 TFA, H-Phe*gem*Phe-H



H-Phe*gem*Phe (550 mg, 1.94 mmol, 1 eq) was dissolved in a dry DMF solution (10 mL) containing DIEA (1.0 mL, 5.82 mmol, 3 eq) under argon. ICPTMS (970 µL, 4.27 mmol, 2.2 eq) was added. After 35 minutes at room temperature (20°C), solvent was removed under reduced pressure and the desired compound **4** was precipitated in diethyl ether. After centrifugation and decantation, the solid was washed again with diethyl ether. This washing/centrifugation/decantation procedure was repeated twice. The solid was dried overnight in a vacuum desiccator. 972 mg of white solid was recovered (84% yield).



LC trace at (214 nm)



MS spectra t_R =2.24 min

Fig. S7 LC/MS **(ESI$^+$)** of compound 4, MW 597.94 g/mol

t_R = 2.24 min, m/z 598.4 $[M+H]^+$; m/z 620.4 $[M+Na]^+$; m/z 322.2 $[(M+2H)/2+Na]^+$

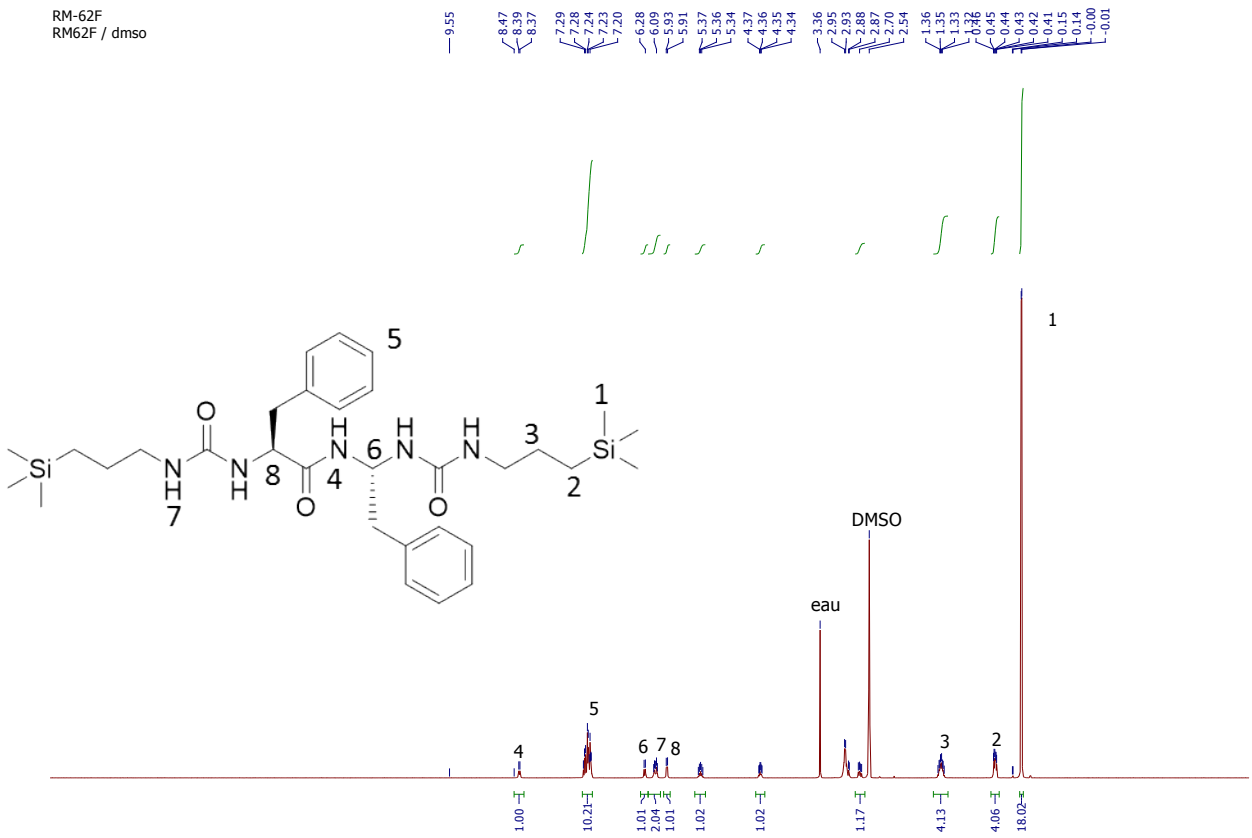


Fig S8 ^{1}H NMR spectrum of **4** in DMSO-d6 (400 MHz).

^{1}H NMR (400 MHz, DMSO) δ 8.41 (t, *J* = 19.3 Hz, 1H), 7.33 – 7.16 (m, 10H), 6.29 (d, *J* = 8.6 Hz, 1H), 6.23 – 6.02 (m, 2H), 5.92 (d, *J* = 8.5 Hz, 1H), 5.35 (p, *J* = 7.2 Hz, 1H), 4.36 (td, *J* = 8.3, 4.9 Hz, 1H), 2.69 (dd, *J* = 13.8, 8.2 Hz, 1H), 1.47 – 1.22 (m, 4H), 0.51 – 0.37 (m, 4H), -0.01 (d, *J* = 4.1 Hz, 18H).

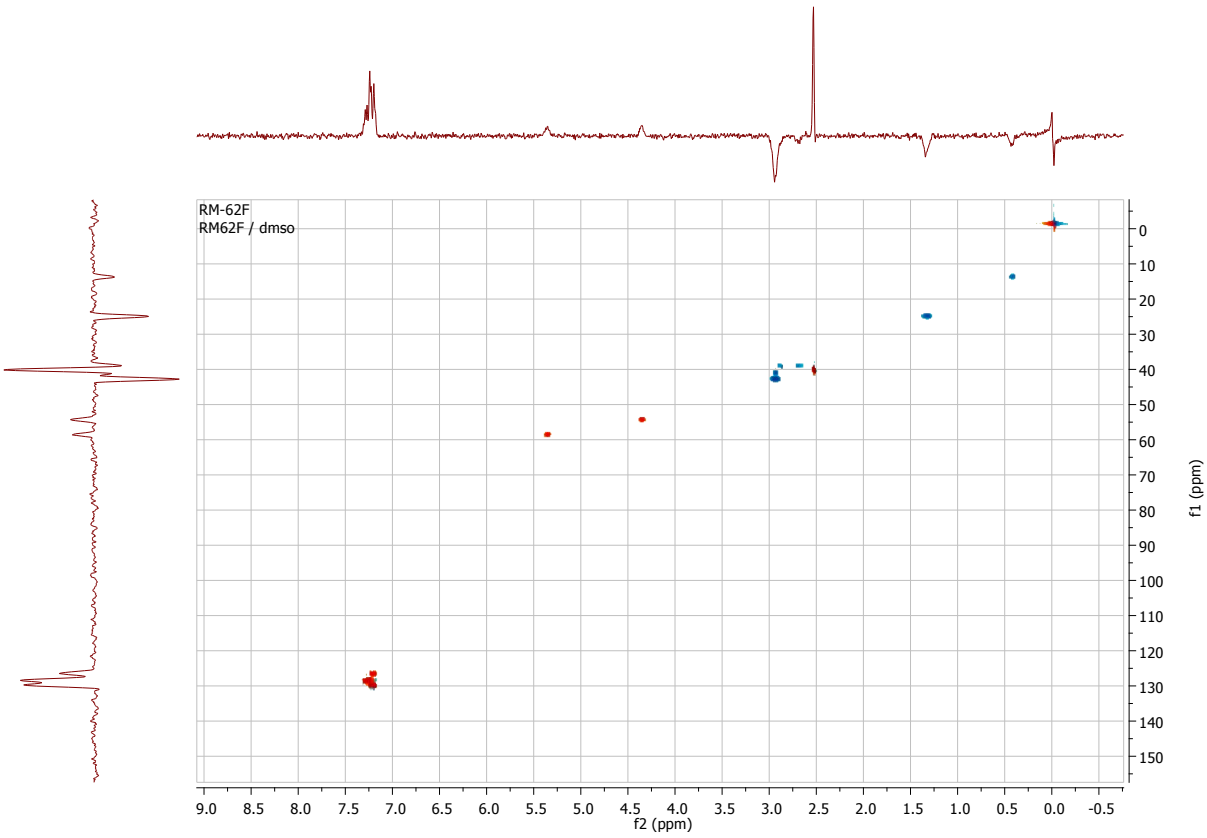


Fig. S9 ^{13}C NMR spectrum of **4** in DMSO-d_6 (500-100.6 MHz).

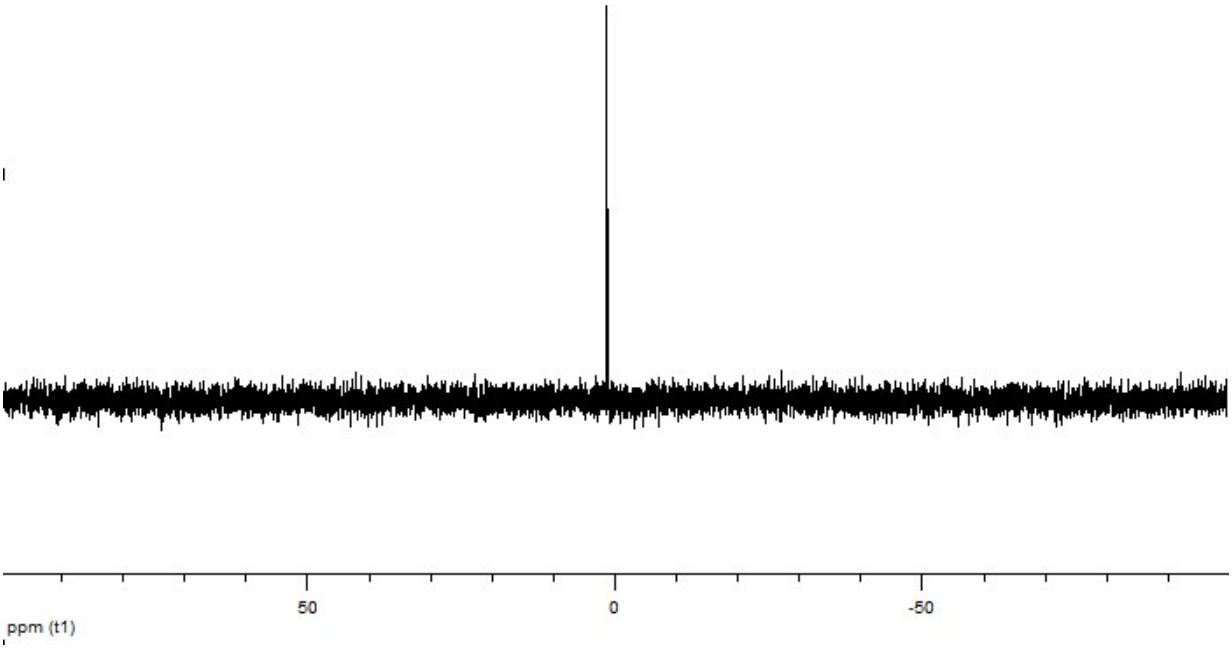
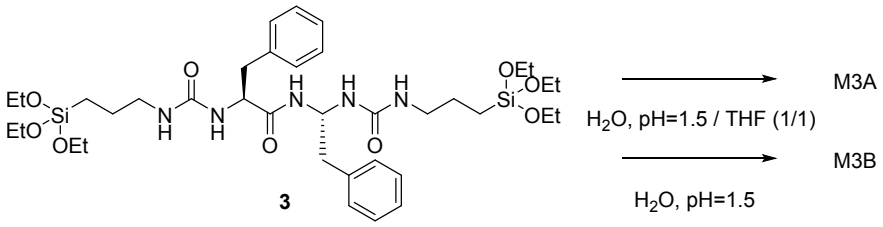


Fig. S10 ^{29}Si NMR spectrum of 4 in DMSO-d_6 (99 MHz).

7. Preparation of **M3A** and **M3B**



In homogeneous conditions, the silylated peptide **3** was solubilized at 38.5 mM (770 mg, 0.99 mmol in 26 mL) in a mixture of water (HCl, pH 1.5)/THF (1/1, v/v) solution. Compound **3** was soluble after sonication. After 4 days under stirring at room temperature (20°C), no precipitate was observable, the solvent was then slowly removed under an extractor hood during one day to induce the polycondensation process. A white product was obtained in the bottom of the beaker, it was washed few times with HCl pH=1.5 solution and dry under vacuum at 50 °C for one night. 565 mg of white solid **M3A** was recovered.

In heterogeneous conditions, the silylated peptide **3** was suspensed at 38.5 mM (676 mg, 0.87 mmol in 22.4 mL) in water-HCl, pH 1.5. Compound **3** was insoluble and stay on the surface even with stirring, for one day, then it became soluble after hydrolysis and precipitate again after condensation. After 4 days under stirring at room temperature (20°C), the white product was filtered and washed few times with HCl pH=1.5 solution and dry under vacuum at 50 °C for one night. 470 mg of white solid **M3B** was recovered.

8. X-ray diffraction and crystallography of **M3B**

Crystal data for compound **4** formula: $C_{31}H_{51}N_5O_3Si_2$, moiety: $C_{31}H_{51}N_5O_3Si_2$, T = 175 K, M_r = 597.95 gmol^{-1}, crystal size = 0.12x0.25x0.30 mm^3, triclinic, spacegroup $P1$, a = 8.9327(3) Å, b = 14.5664(4) Å, c = 21.0900(5) Å, α = 89.048(2)º, β = 82.850(2)º, γ = 75.885(3)º, V = 2640.36(6) Å^3, Z = 3, ρ_{calcd} = 1.128 gcm^{-3}, μ = 0.137 mm^{-1}, θ_{max} = 29.186º, experimental resolution = 0.73 Å, 69359 reflections measured, 24677 unique reflections, 21301 least-squares reflections, 21301 reflections with I>2σ(I), R_{int} = 0.034, <σ(I)/I> = 0.0338, 1154 refined parameters, R_1(I>2σ(I)) = 0.0586, wR_2(I>2σ(I)) = 0.0625, R_1(all data) = 0.0680, wR_2(all data) = 0.0638, GOF = 1.0817, Δρ(min/max) = -0.68/0.93 eÅ^{-3}.

The crystal evaluation and data collection were performed on an Rigaku Oxford Diffraction Gemini-S diffractometer with sealed-tube Mo-$K\alpha$ radiation using the *CrysAlis Pro* program (Rigaku Oxford Diffraction, 2017). The integration of the data frames was done with the same program using default parameters. Lorentz and polarization effects were also corrected, and the empirical absorption correction was done using spherical harmonics employing symmetry-equivalent and redundant data.

The crystal structure was solved using the *ab-initio* iterative charge flipping method with parameters described elsewhere (Van der Lee, 2013) using the *Superflip* program (Palatinus & Chapuis, 2007) and it was refined using full-matrix least-squares procedures as implemented in *CRYSTALS* (Betteridge *et al*., 2003) on all independent reflections with I>2σ(I).

The structure was in an early stage solved in the spacegroup P-1 and refined to an R-value of about 0.10. The resulting structure appeared to be a one-dimensional polymeric structure of compound **4** moieties connected by (unlikely) hydrazine bridges. A reterdermination in the spacegroup P1 gave isolated moieties, but with a pseudo-inversion center present in its 3-dimensional structure (92% fit). The R-factor was, however, much lower (0.0586), and therefore it was decided that the P1 structure was the correct one.

The H atoms were all located in a difference map, but repositioned geometrically. The H atoms were initially refined with soft restraints on the bond lengths and angles to regularise their geometry (C---H in the range 0.93-0.98 Å) and U_{iso}(H) (in the range 1.2-1.5 times U_{eq} of the parent atom), after which the positions were refined with riding constraints (Cooper *et al*, 2010).

There are three independent moieties of **4** in the unit cell (Fig. S1), wich differ in the orientation of the trimethylsilane groups with respect to the molecule's backbones (Fig. S2). The molecules are connected by relatively strong classical hydrogen bonds, (Fig. S1), forming a stacked sequence along the *b*-axis. Fig. S3 gives an ORTEP-style drawing of one of the three molecules.

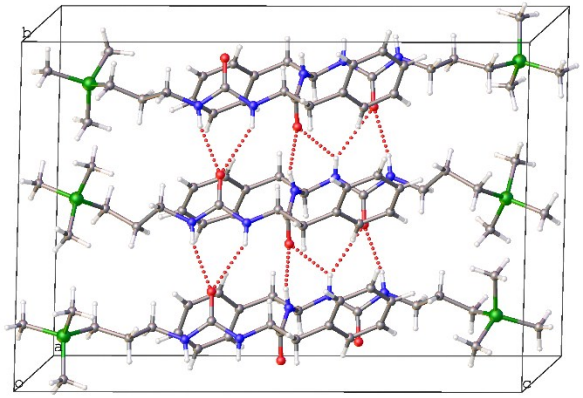


Fig. S11 The three independent moieties of **4** in the unit cell.

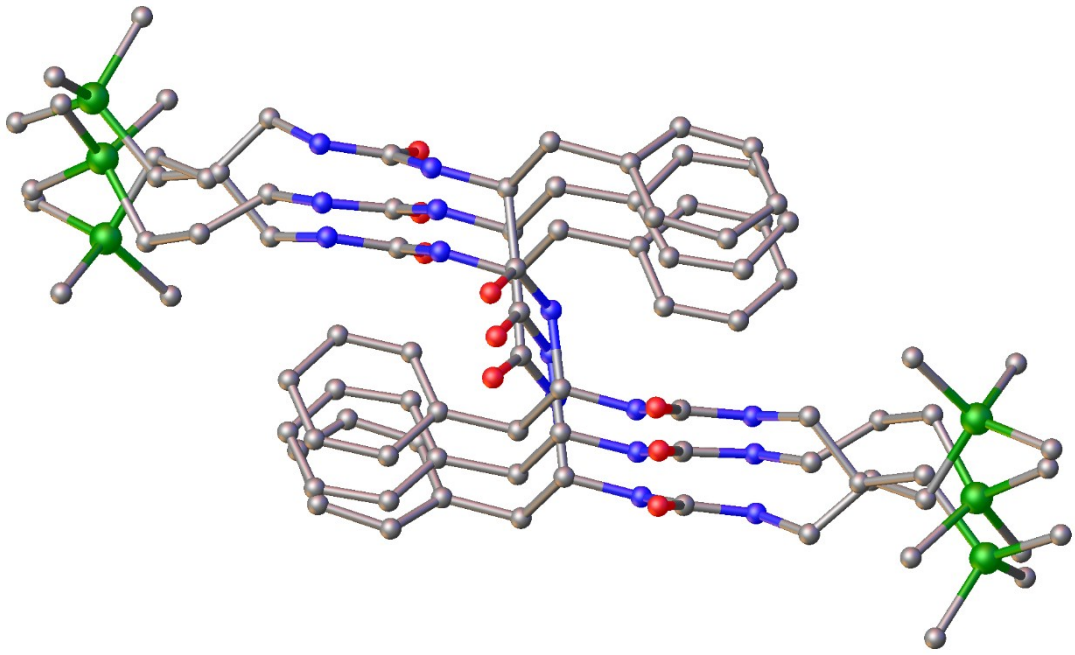


Fig. S12 The three independent moieties of **4** brought close to each other.

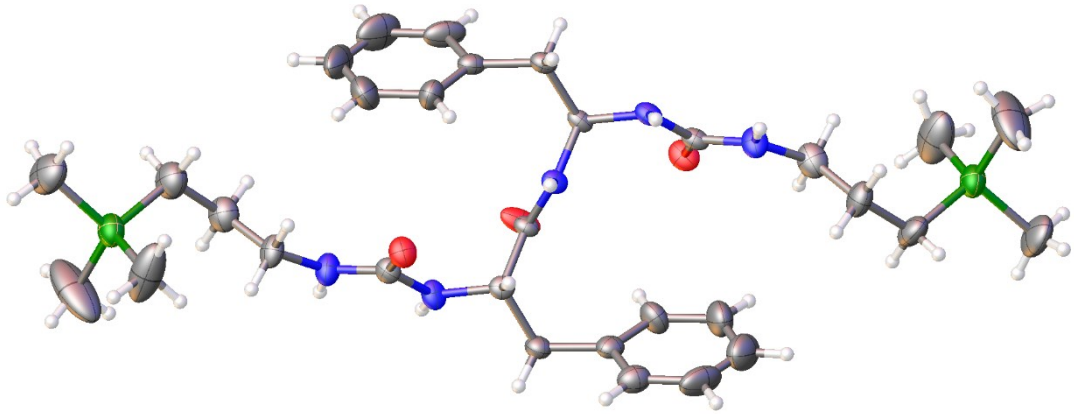


Fig. S13 ORTEP-style drawing of one unit of compound **4** of the three independent molecules with atomic displacement ellipsoids at the 50% probability level.

The structural dawings have been made using *OLEX2* (Dolomanov *et al.*, 2009).

References

CrysAlis^{Pro} Rigaku Oxford Diffraction, England 2017.

A. van der Lee, *J. Appl. Crystallogr.* **2013**, *46*, 1306-1315.

L. Palatinus, G. Chapuis, *J. Appl. Crystallogr.* **2007**, *40*, 786-790.

P. W. Betteridge, J. R. Carruthers, R. I. Cooper, K. Prout, D. J. Watkin, *J. Appl. Crystallogr.* **2003**, *36*, 1487-1487.

R. I. Cooper, A. L. Thompson, & D. J. Watkin. *J. Appl. Crystallogr.* **2010**, 46, 1100-1107.

O.V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard, H. Puschmann, *J. Appl. Crysallogr.* **2009**, *42*, 339-341.

9. **M3A** and compound **4** SEM images

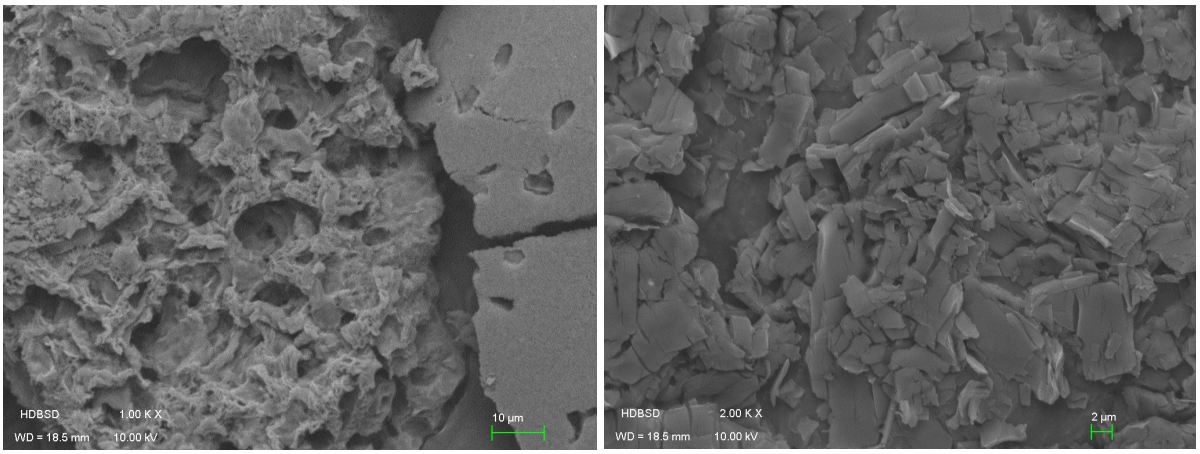


Figure S14. SEM image of amorphous material **M3A** obtained by sol-gel polymerization of **3** during the evaporation of a water (pH 1.5)/ THF (1/1, v/v) solution (left), and SEM image of compound **4** (right).

10. Stability of compound **4** in acidic solution

In heterogeneous conditions, the non-reactive silylated peptide **4** was suspended at 38.5 mM (46 mg, 0.077 mmol in 2 mL) in water-HCl, pH 1.5. 200 µL of this suspension at t=0 was solubilised in 1.5 mL of DMSO-d_6 and analysed by ^{1}H NMR. Compound **4** remained insoluble during the 7 days under stirring. 200 µL of this suspension after 7 days was solubilised in 1.5 mL of DMSO-d_6 and analysed by ^{1}H NMR. Spectra at t=0 and t=7 days were the same, showing no degradation of compound **4**.

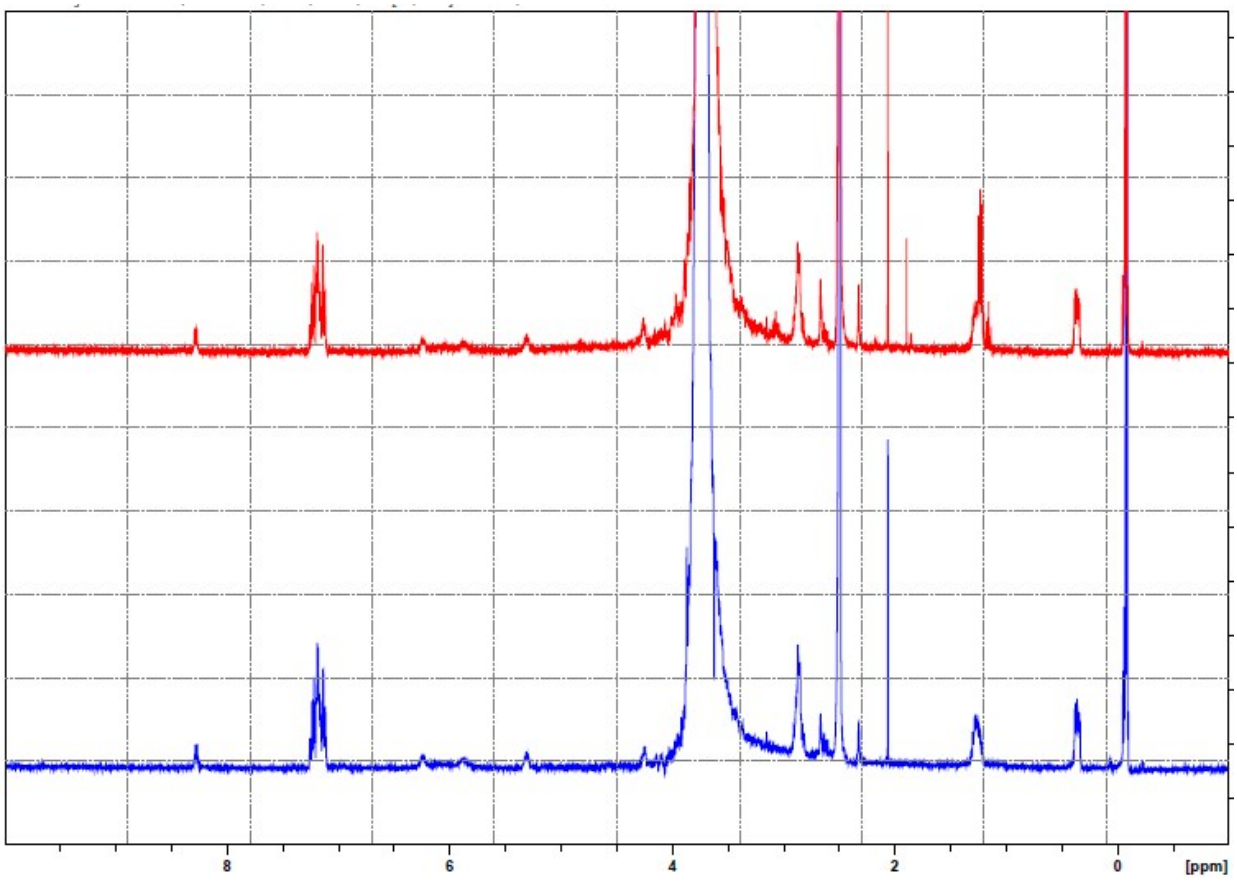
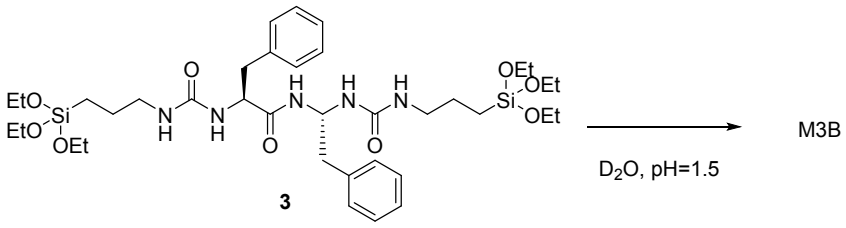


Fig. S15 ^{1}H NMR spectra of compound **4** at t=0 (red) and 7 days (blue) in water-HCl pH=1.5, in DMSO-d6 (400 MHz).

11. Monitoring of **M3B** formation by ^{1}H NMR



Compound **3** (180 mg, 0.23 mmol, 1 eq) was suspended in 7 ml of D_2O at pH 1.5 (using very small amounts of DCl) with magnetic stirring. The sol-gel process, hydrolysis and condensation, was monitored recording ^1H NMR spectra at 298 K at various time intervals during 4 days: typically after 15 minutes, 1, 2, 3, 5, 7, 22, 26, 28, 46, 49, 52, 54, 71, 74 and 78 h. The ethanol release was assessed measuring the intensities of the CH_2 and CH_3 resonances, at 3.6 and 1.1 ppm, respectively. The peptide amount in solution versus time was monitored measuring the aromatics signals centred around 7.3 ppm. We previously showed that the hydrolysis and condensation processes of hybrid silylated peptides can be monitored integrating the methylene protons in alpha position of the silicon versus time. The CH_2SiOH and the CH_2SiO- signals were at 0.5 and 0.6 ppm, respectively.

Fig. S16: Portions of the ^1H NMR spectra of hydrolysed-**3** at various time intervals

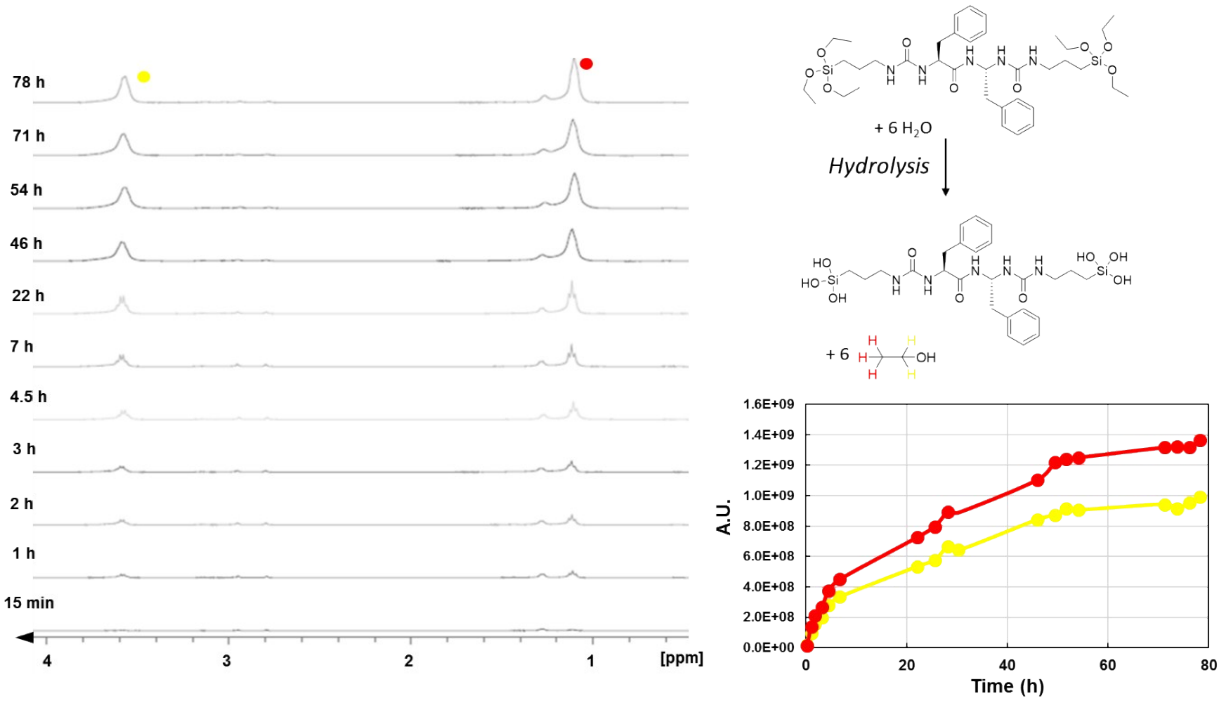


Fig. S16A Variation of the ethanol signals intensities at various time intervals (1.1 and 3.6 ppm).

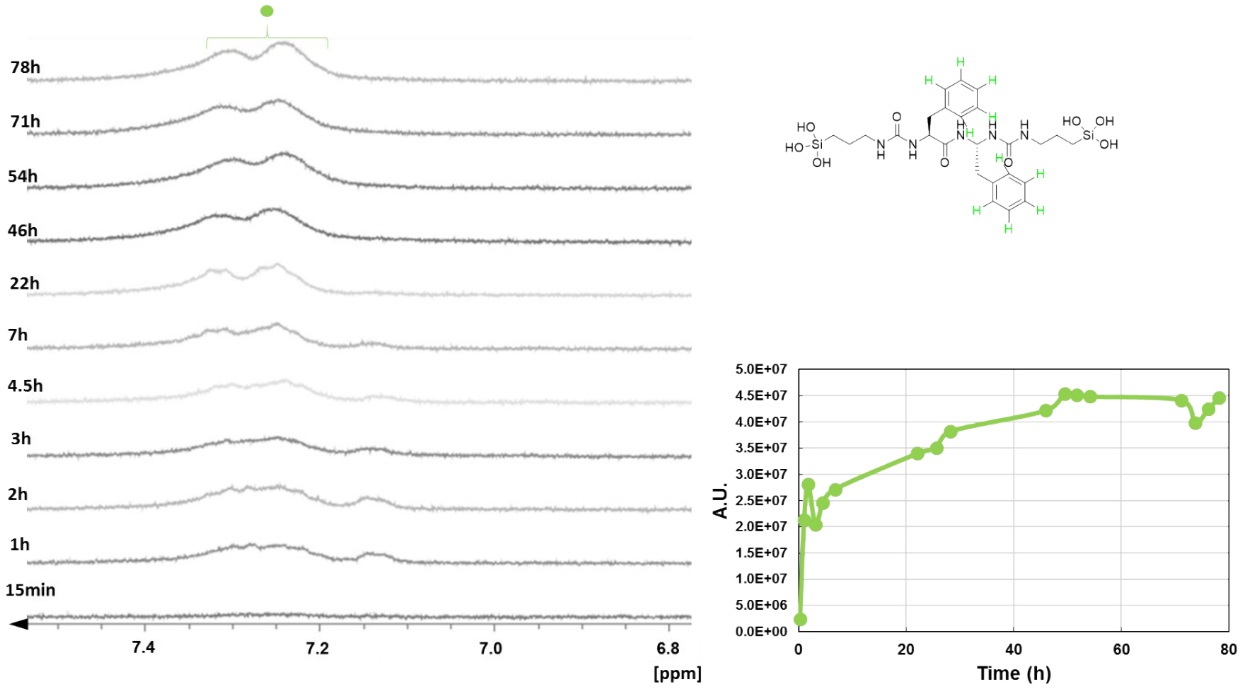


Fig. S16B Variation of the aromatics region intensities of hydrolysed-**3** at various time intervals (7.2-7.35 ppm).

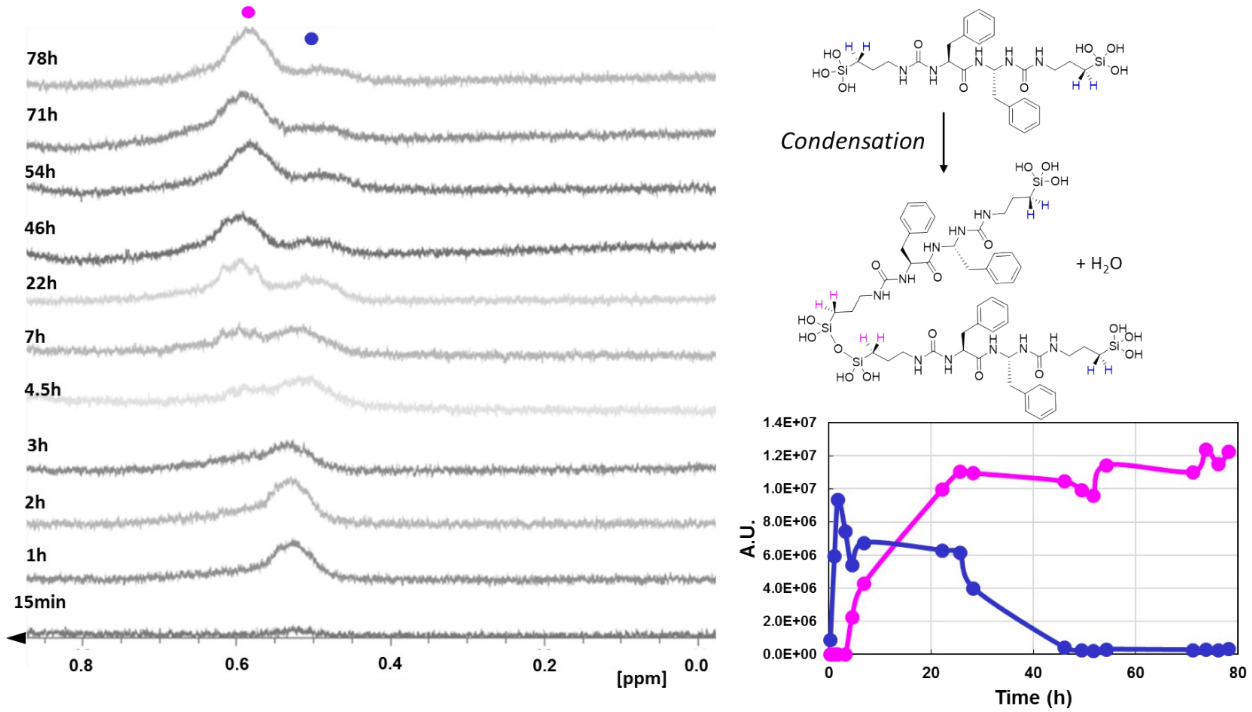


Fig. S16C Variation of the CH_2SiOH (in blue) and CH_2SiO- (in magenta) signals intensities of hydrolysed-**3** at various time intervals (0.5 and 0.6 ppm, respectively).

12. Analyses of **M3A** and **M3B** materials and compounds **3** and **4**

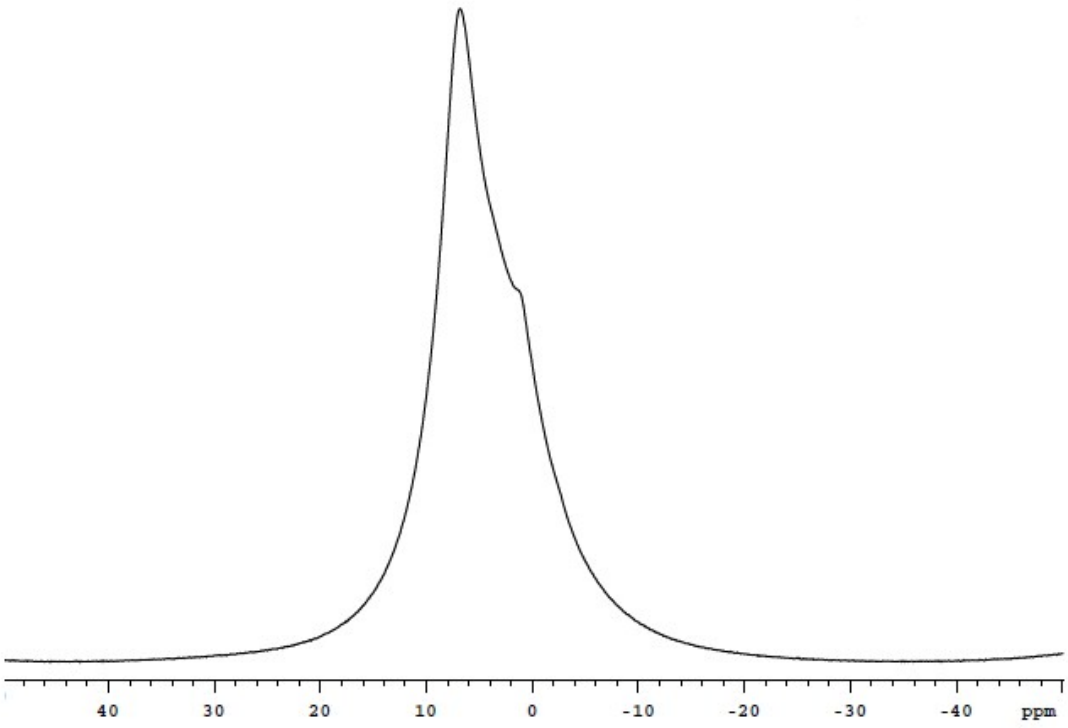


Fig. S17 Solid ^{1}H NMR spectra of material **M3B** (One Pulse, 20 kHz).

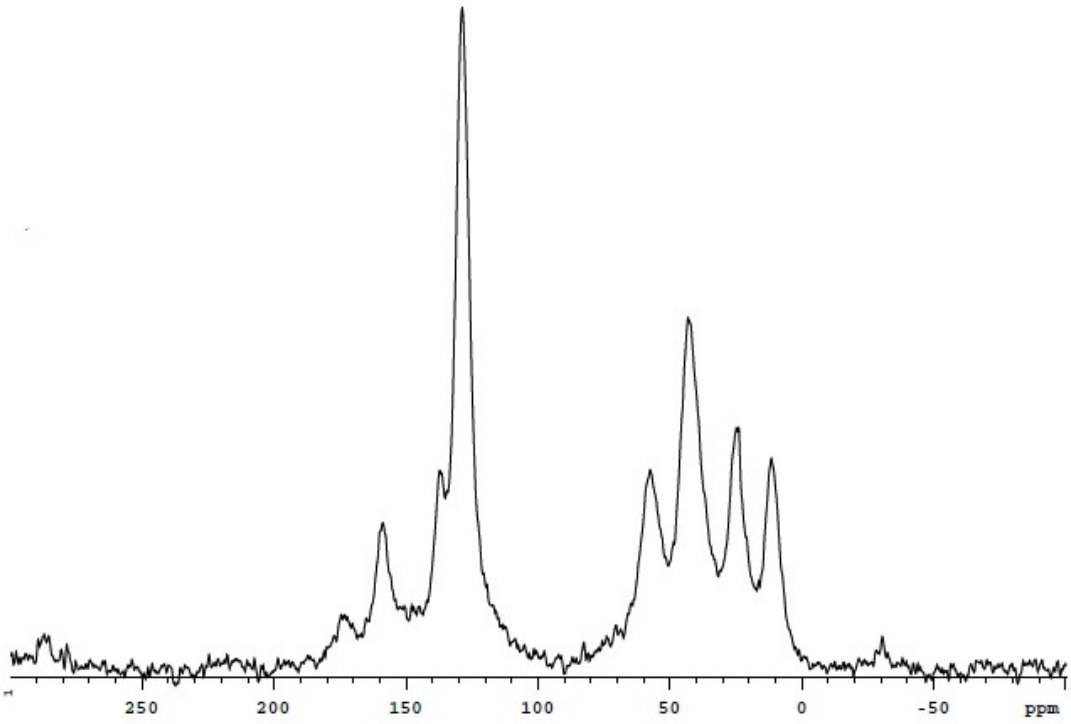


Fig. S18 Solid ^{13}C NMR spectra of material **M3B** (CP-MAS, 12 kHz).

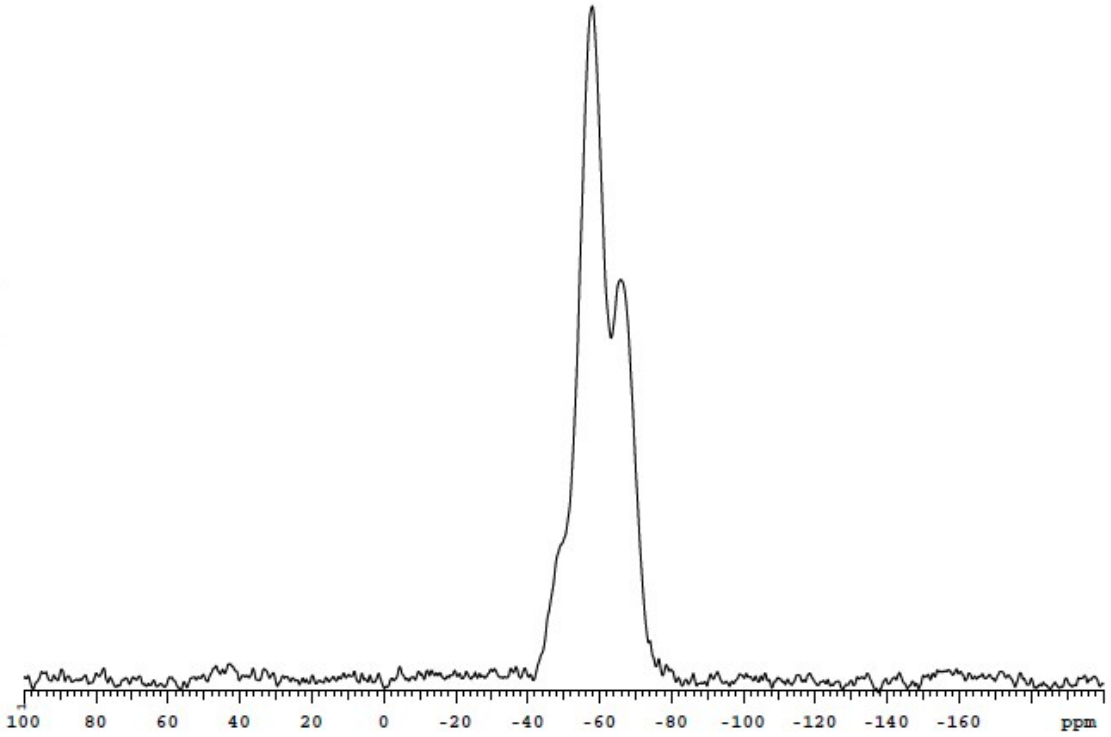


Fig. S19 Solid ^{29}Si NMR spectra of material **M3B** (CP-MAS, 12 kHz).

Material		C (%)	H (%)	N (%)	O (%)
M3A	Mean value	48.068	6.128	11.301	13.077
	Deviation (abs)	0.090	0.091	0.138	0.020
	Deviation (rel %)	0.127	0.128	0.196	0.156
M3B	Mean value	49.481	6.090	11.514	12.685
	Deviation (abs)	0.049	0.124	0.103	0.101
	Deviation (rel %)	0.069	0.176	0.146	0.798

Table S1. Elemental analysis of **M3A** and **M3B** (analysed in duplicate).

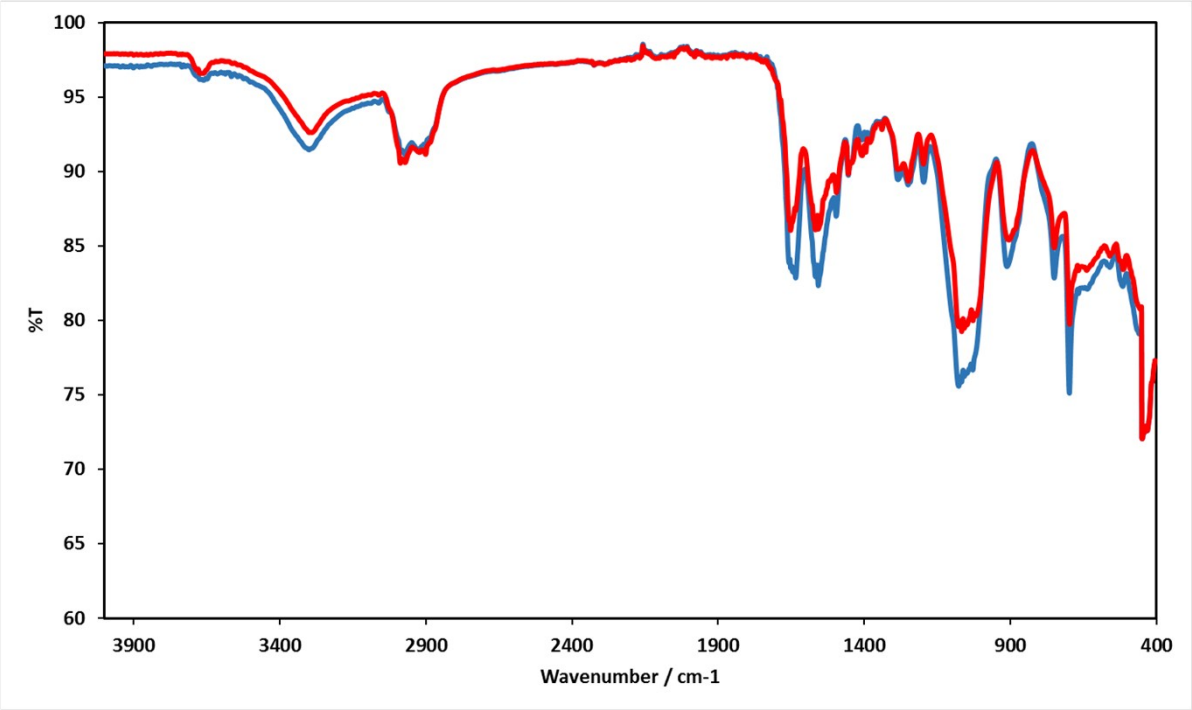


Fig. S20 FTIR spectra of **M3A** (red) and **M3B** (blue).

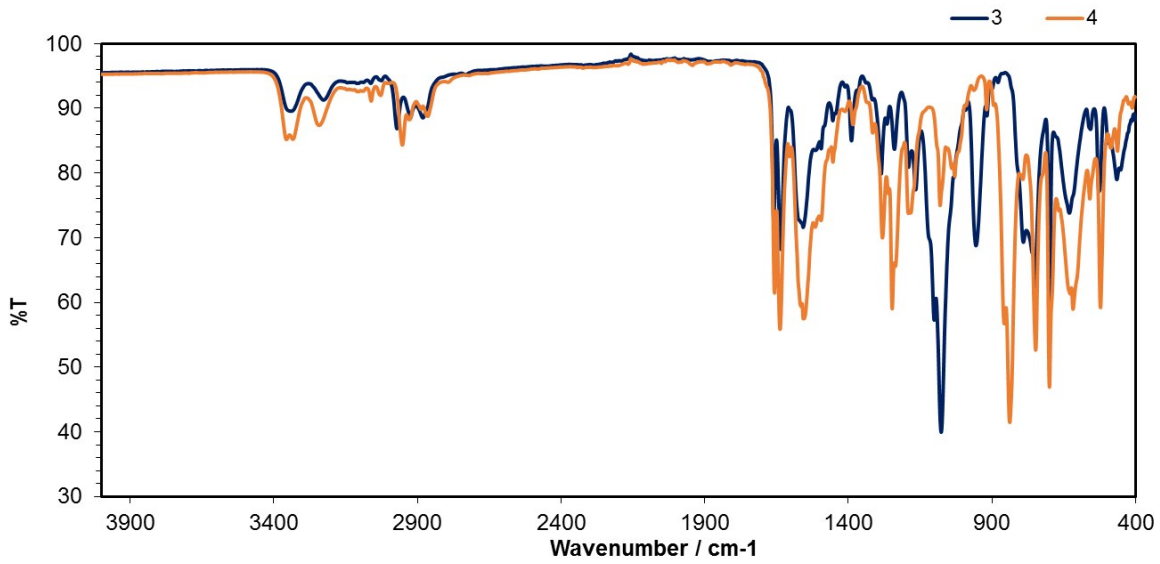


Fig. S21 FTIR spectra of **3** (dark blue) and **4** (orange).

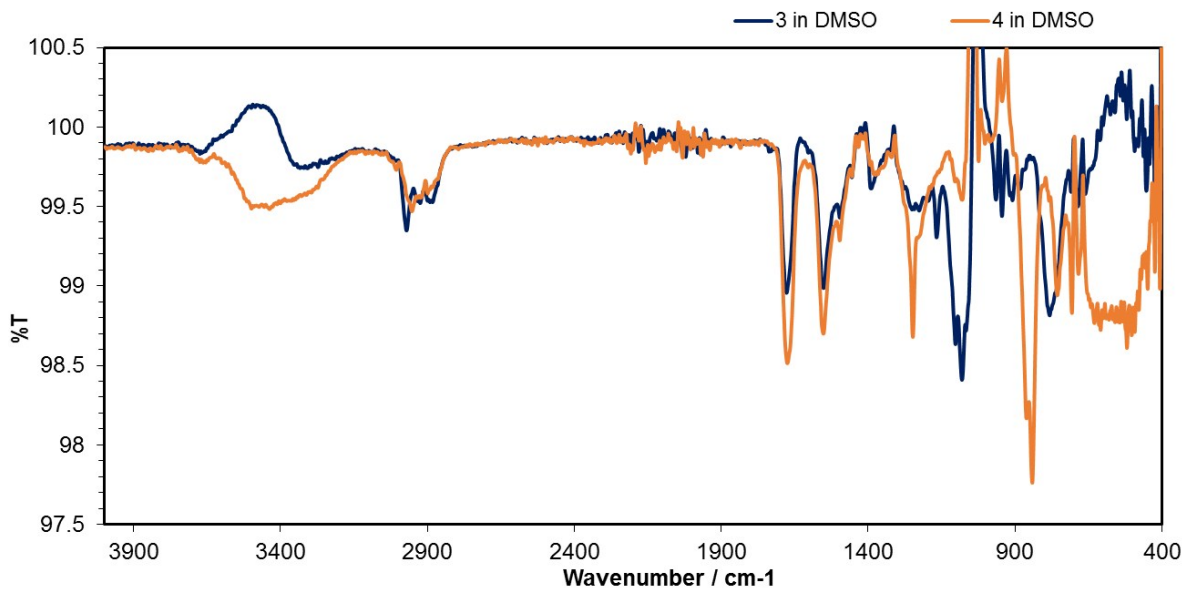


Fig. S22 FTIR spectra of **3** (dark blue) and **4 (**orange) in DMSO at 1.875 %wt.

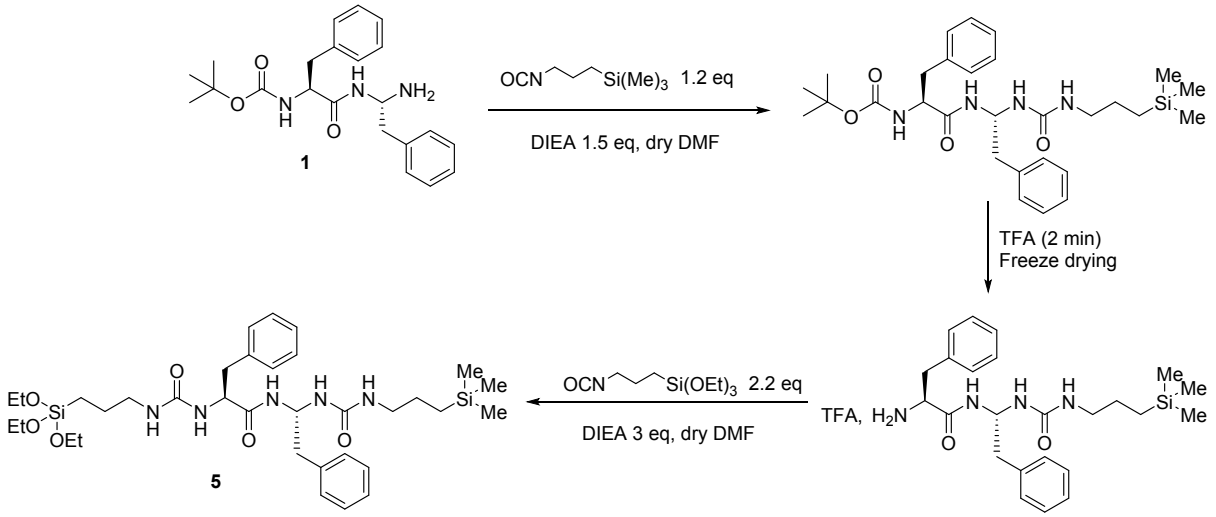
Peak (cm^{-1})	**Assignment**
3299.78	O-H stretch from free Si-OH
2987.95	Aromatic C-H stretch from phenyls
2901.45	Tetragonal C-H stretch from phenylalanines
1651.77	C=O stretch from ureas
1561.79	C=C stretch from phenyls
1494.73	N-H deformation from amides
1453.93	N-H deformation from ureas
1284.82	C-N stretch in α position of amides
1249.15	C-N stretch in α position of ureas
1196.98	C-C stretch from propane arms
1056.87	Si-O-C stretch
903.29	Si-O-Si stretch

Table S2. Peak assignments for FTIR spectrum of **M3A** and **M3B.**

Compound / material	CO amide (amide I) (cm^{-1})	NH amide (amide II) (cm^{-1})	Δν (CO amice - NH amide) (cm^{-1})
3	1638	1565	73
3 in DMSO	1679	1567	112
4	1648	1562	86
4 in DMSO	1672	1549	123
M3A	1649	1567	82
M3B	1652	1559	93
M5	1633	1558	75
M6	1638	1559	79

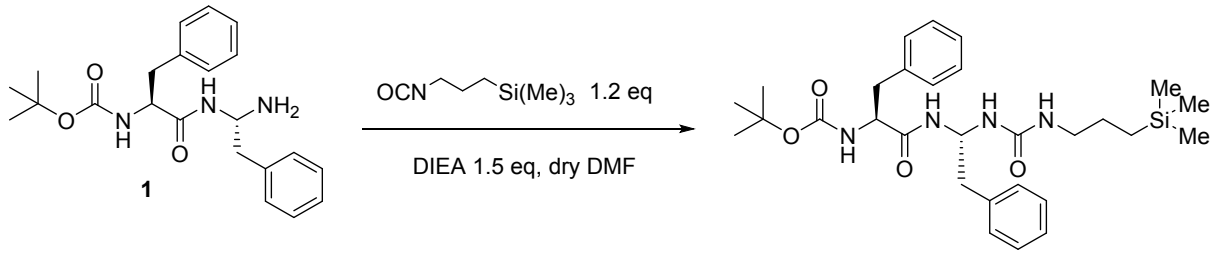
Table S3. Amides bands in FTIR spectrum of **3, 4, M3A** and **M3B.**

13. General scheme of synthesis of compound **5**

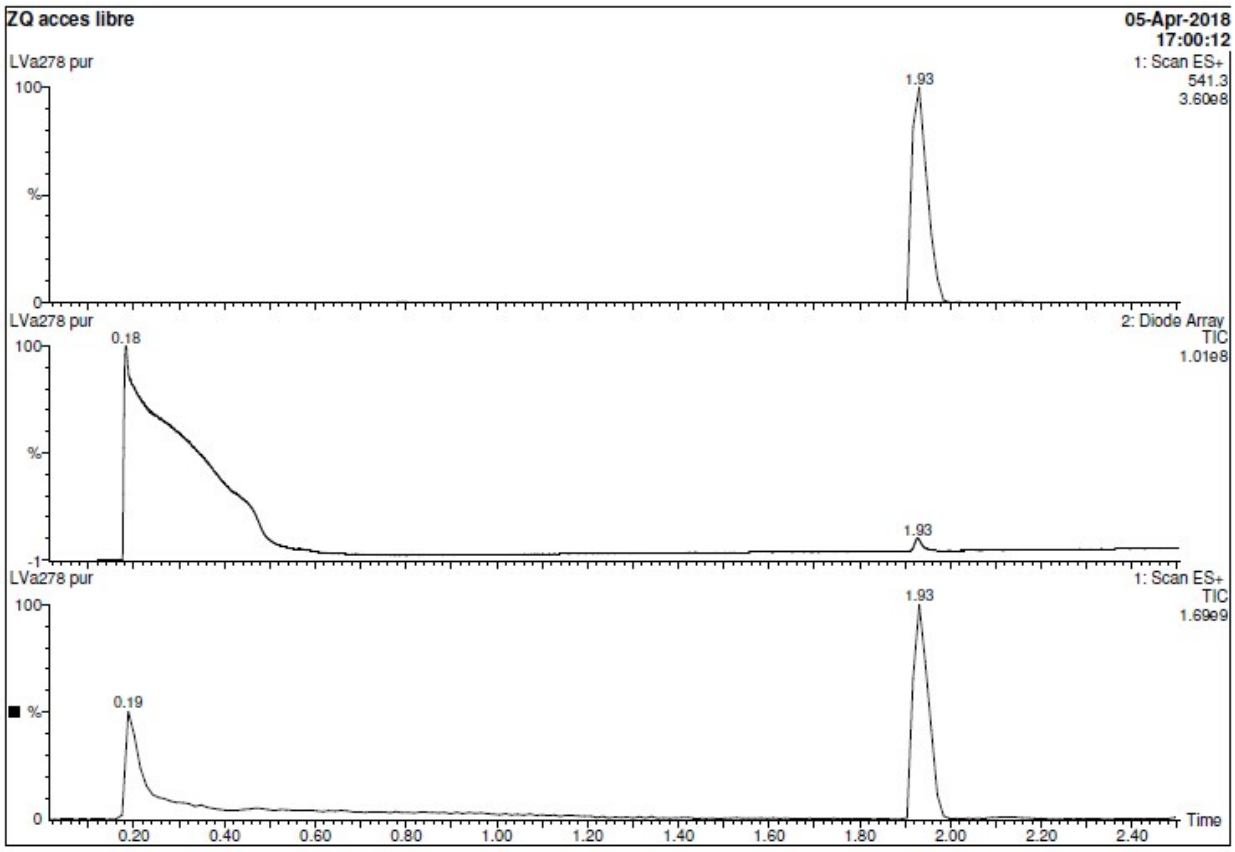


Scheme S1. Synthesis of compound **5.**

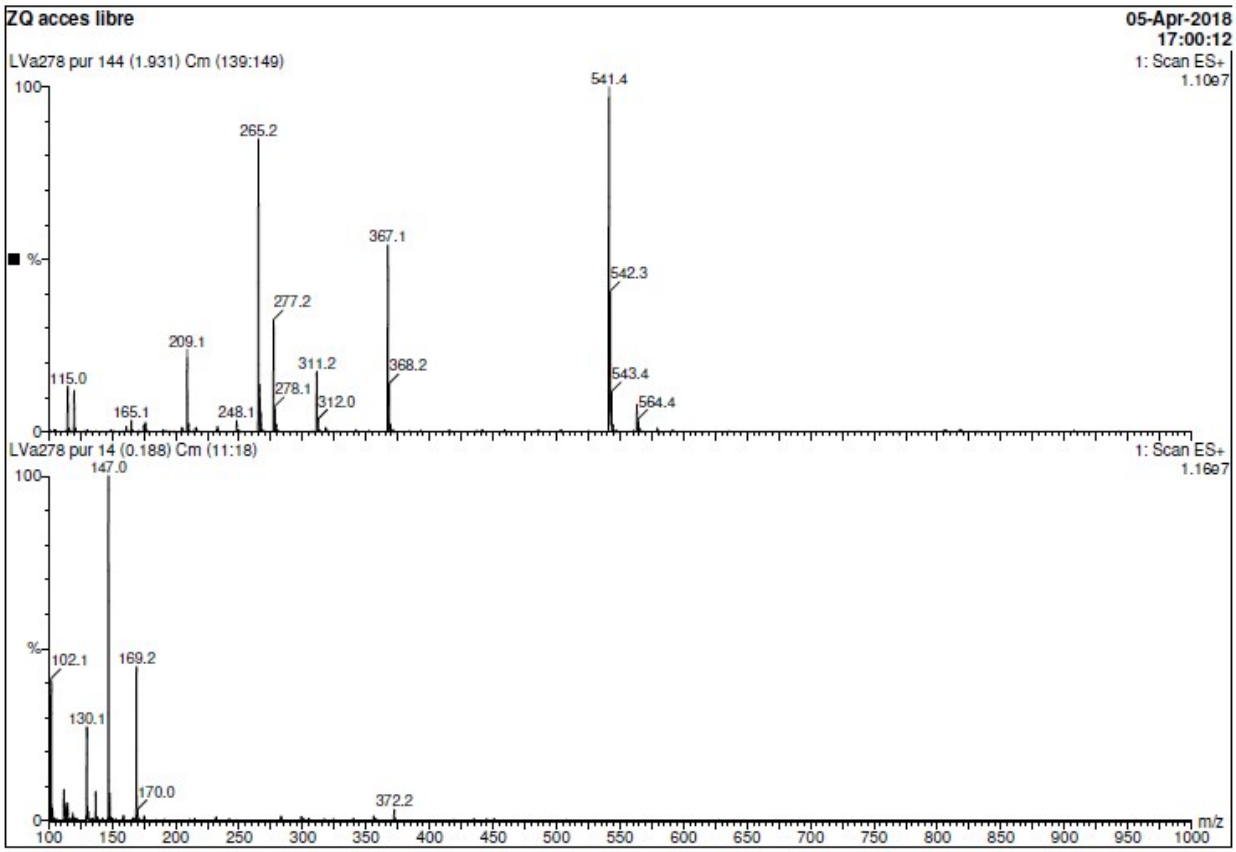
14. Synthesis of compound Boc-Phe*gem*Phe-CONH-$(CH_2)_3$-$Si(Me)_3$ from compound **1** Boc-Phe*gem*Phe-H



Boc-Phe*gem*Phe (500 mg, 1.30 mmol, 1 eq) was dissolved in a dry DMF solution (4.4 mL) containing DIEA (344 µL, 1.96 mmol, 1.5 eq) under argon. ICPTMS (308 µL, 1.56 mmol, 1.2 eq) was added. After 1h30 at room temperature (20°C), solvent was removed under reduced pressure and the desired compound was precipitated in diethyl ether. After centrifugation and decantation, the solid was washed again with diethyl ether. This washing/centrifugation/decantation procedure was repeated twice. The solid was dried overnight in a vacuum desiccator. 364 mg of white solid was recovered (52% yield).



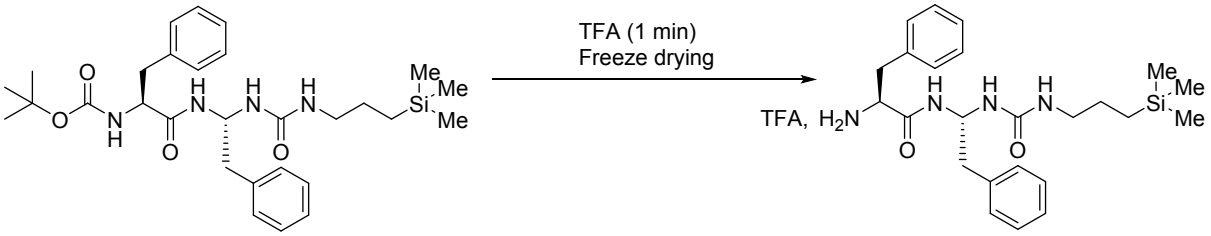
LC trace at (214 nm) and TIC



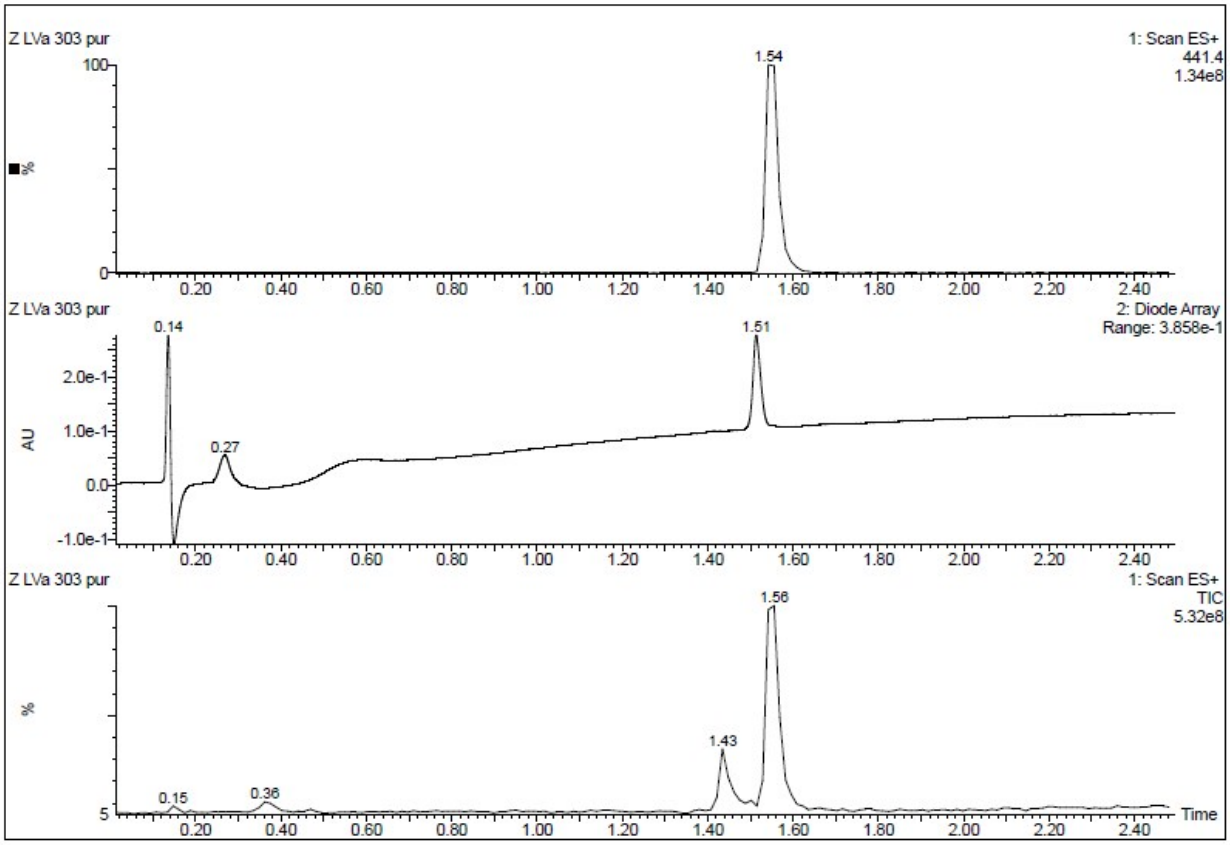
MS spectra t_R = 1.93 min and 0.19 min

Fig. S23 LC/MS (ESI$^+$) of compound Boc-PhegemPhe-CONH-$(CH_2)_3$-Si$(Me)_3$. MW 540.8 g/mol
LC/MS (ESI$^+$): t_R = 1.93 min, m/z 541.3 $[M+H]^+$; m/z 563.3 $[M+Na]^+$.

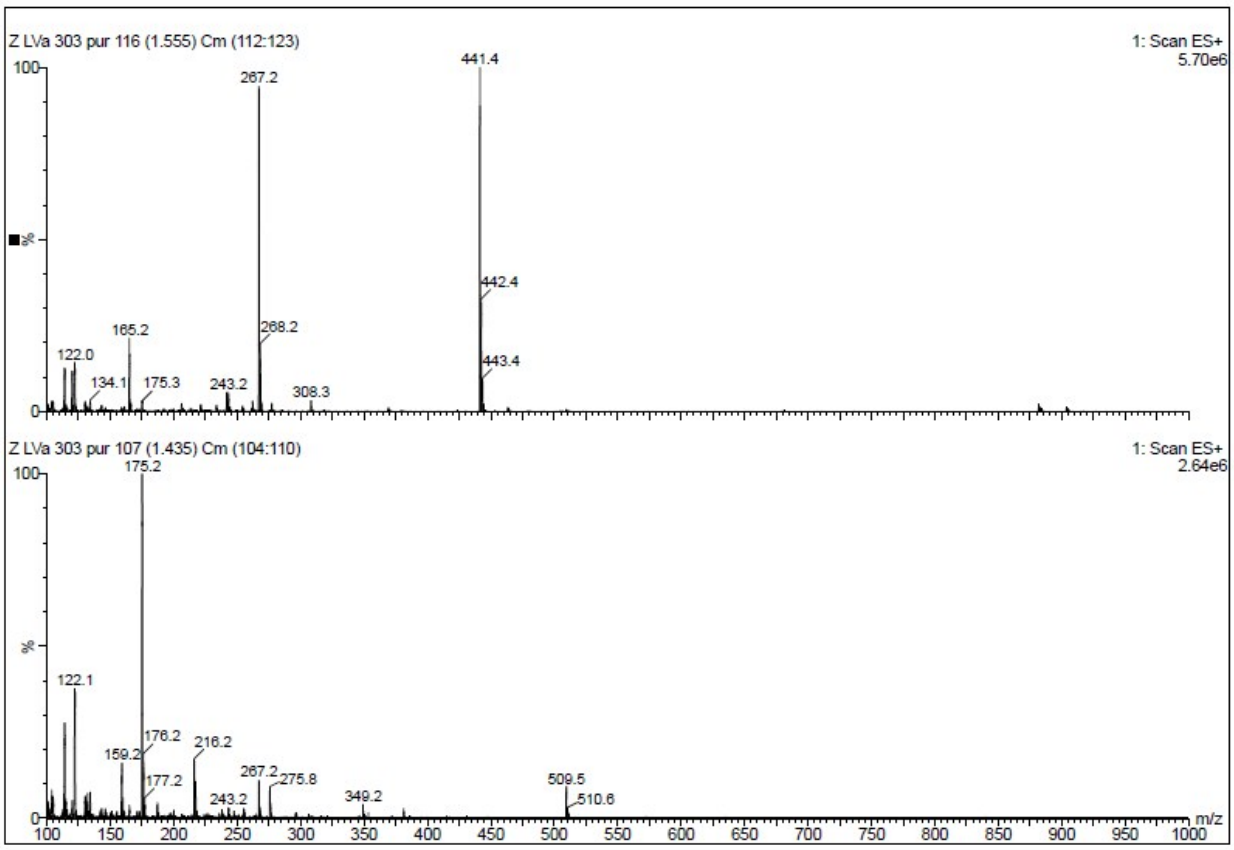
15. Synthesis of compound TFA, H-Phe*gem*Phe-CONH-$(CH_2)_3$-Si$(Me)_3$ from compound Boc-Phe*gem*Phe-CONH-$(CH_2)_3$-Si$(Me)_3$



179 mg of Boc-Phe*gem*Phe- CONH-$(CH_2)_3$-Si$(Me)_3$ (0.331 mmol) were dissolved in TFA (0.9 mL). After 1 min at room temperature (20°C), the solution is diluted in 90 mL of water and freeze dried. 123 mg of compound TFA, H-PhegemPhe-CONH-$(CH_2)_3$-Si$(Me)_3$ (70% yield) as trifluoracetate salts were obtained.



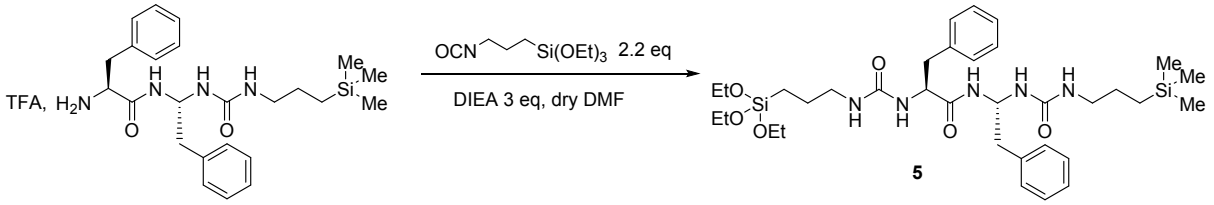
LC trace at (214 nm) and TIC



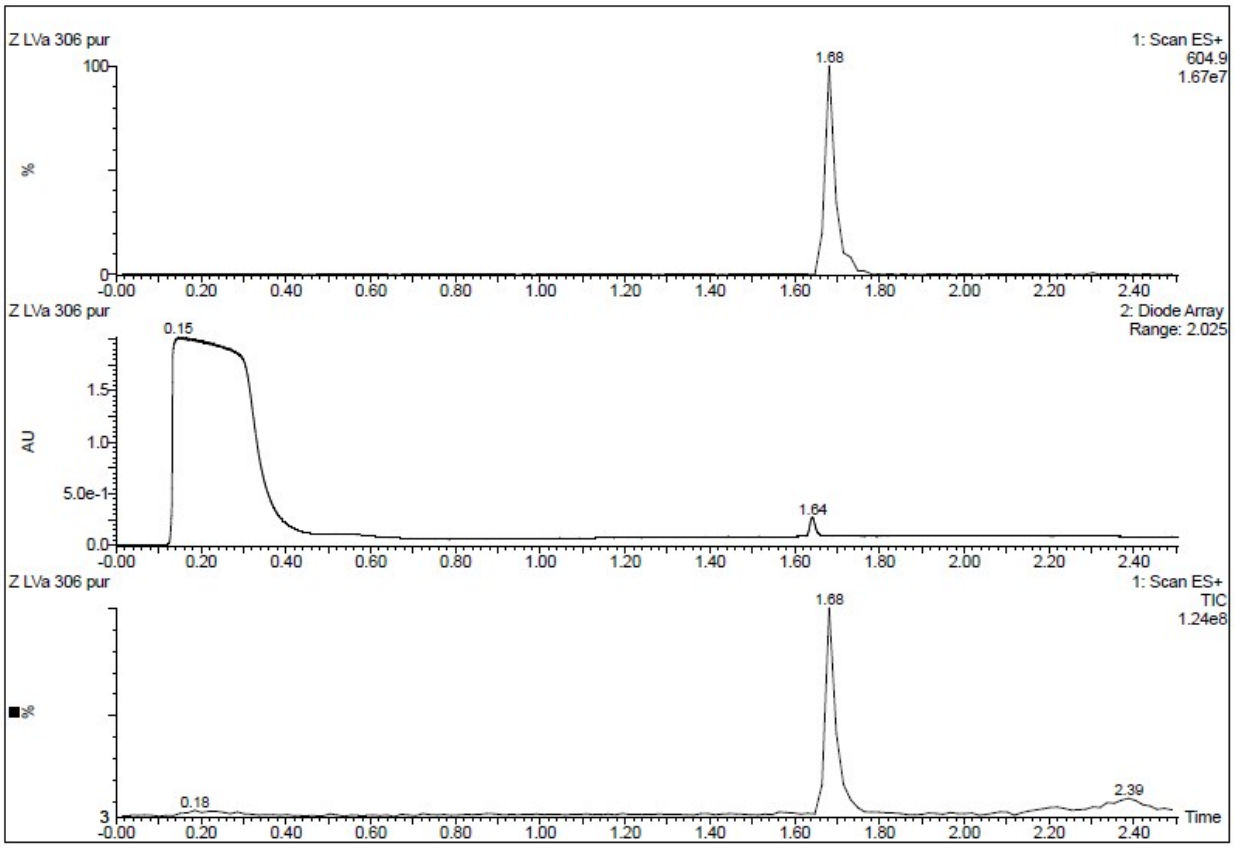
MS spectra t_R = 1.56 min and 1.44 min

Fig. S24 LC/MS **(ESI⁺)** of compound TFA, H-Phe*gem*Phe-CONH-$(CH_2)_3$-$Si(Me)_3$ **.** MW 554.6 g/mol (with 1 TFA salt)
LC/MS (ESI^+): t_R = 1.56 min, m/z 441.4 $[M+H]^+$.

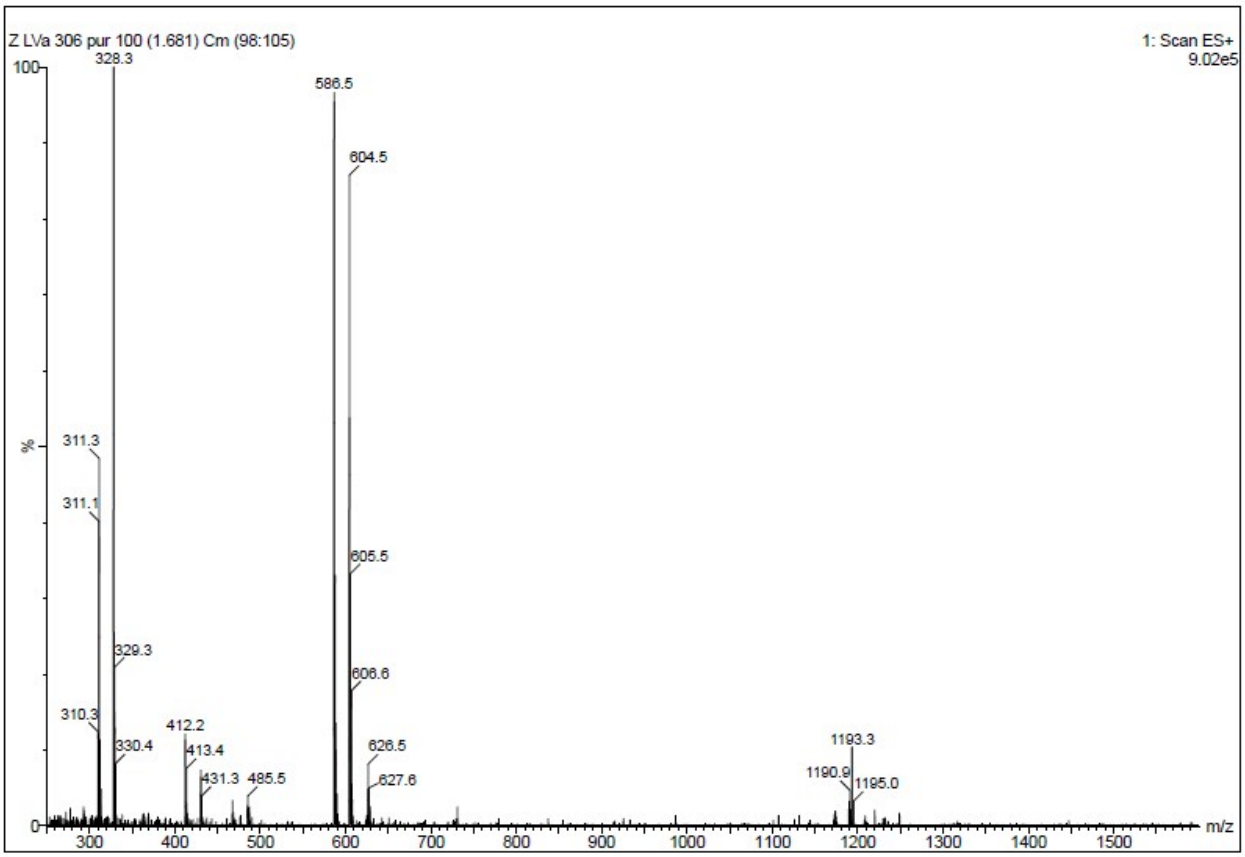
16. Synthesis of compound **5** $(OEt)_3Si$-$(CH_2)_3$-NHCO-Phe*gem*Phe-CONH-$(CH_2)_3$-$Si(Me)_3$ from compound Boc-Phe*gem*Phe-CONH-$(CH_2)_3$-$Si(Me)_3$



H-Phe*gem*Phe-CONH-$(CH_2)_3$-$Si(Me)_3$ (164 mg, 0.296 mmol, 1 eq) was dissolved in a dry DMF solution (1.76 mL) containing DIEA (157 µL, 0.894 mmol, 3 eq) under argon. ICPTES (163 µL, 0.659 mmol, 2.2 eq) was added. After 2 hours at room temperature (20°C), solvent was removed under reduced pressure and the desired compound **5** was precipitated in diethyl ether. After centrifugation and decantation, the solid was washed again with diethyl ether. This washing/centrifugation/decantation procedure was repeated twice. The solid was dried overnight in a vacuum desiccator. 100 mg of white solid was recovered (49% yield).



LC trace at (214 nm) and TIC



MS spectra t_R = 1.68 min

Fig. S25 LC/MS (ESI$^+$) of compound **5.** MW 688.0 g/mol
LC/MS (ESI$^+$): t_R = 1.68 min, m/z 604.5 [M hydrolysed]$^+$; m/z 626.5 [M hydrolysed+Na]$^+$; m/z 1193.3 [M-O-M hydrolysed]$^+$.

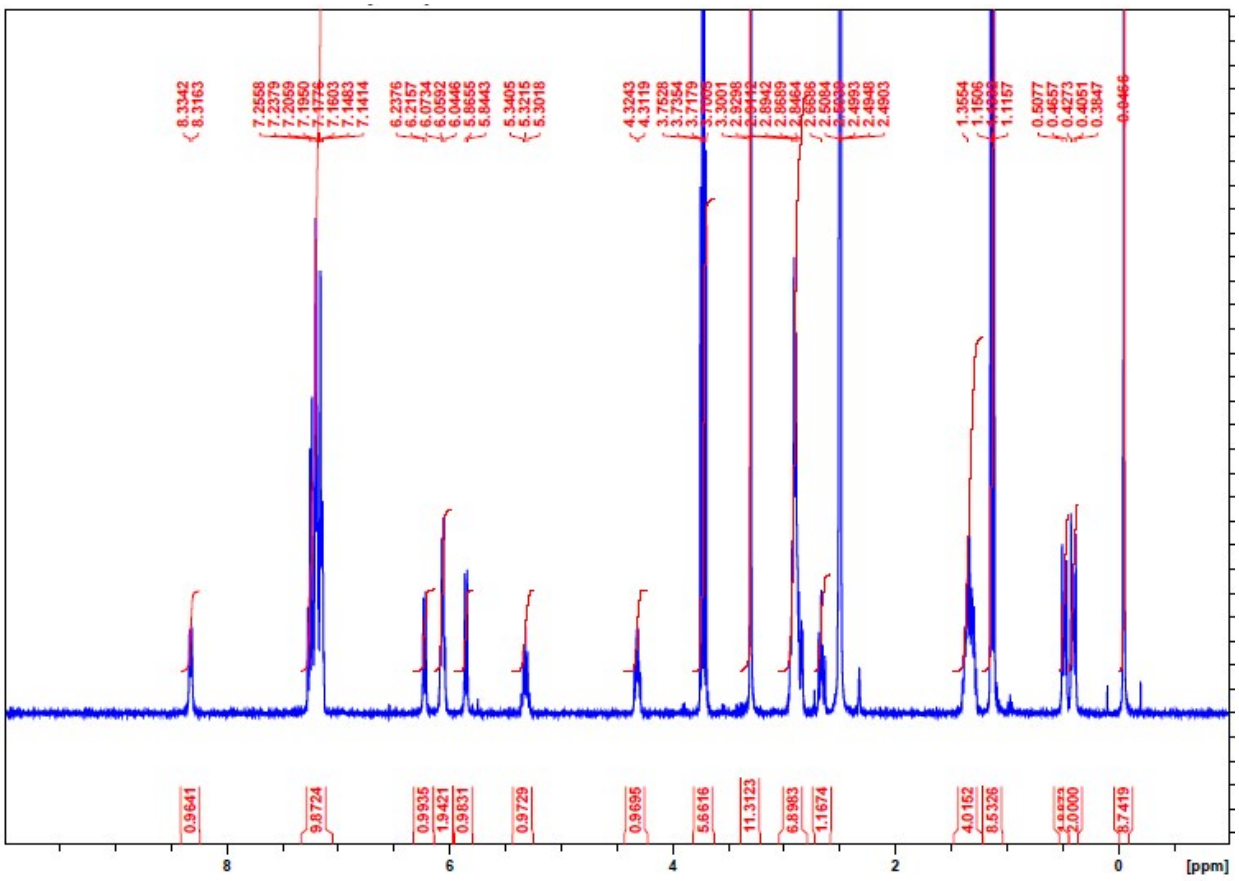


Fig. S26 ^{1}H NMR spectrum of compound **5** in DMSO-d6 (400 MHz).

^{1}H NMR (400 MHz, DMSO) δ 8.32 (d, *J* = 8 Hz, 1H) , 7.25 – 7.14 (m, 10H), 6.22 (d, *J* = 8.7 Hz, 1H), 6.06 (t, *J* = 5.8 Hz, 2H), 5.85 (d, *J* = 8.4 Hz, 1H), 5.34 – 5.30 (m, 1H), 4.32 (t, *J* = 5.0 Hz, 1H), 3.72 (q, *J* = 7.0 Hz, 6H), 2.88 (dt, *J* = 14.2, 6.5 Hz, 7H), 2.66 (dd, *J* = 13.7, 8.1 Hz, 1H), 1.35 – 1.30 (m, 4H), 1.15 (t, *J* = 7.0 Hz, 9H), 0.50 – 0.38 (dd, 4H), 0.01 (s, 9H).

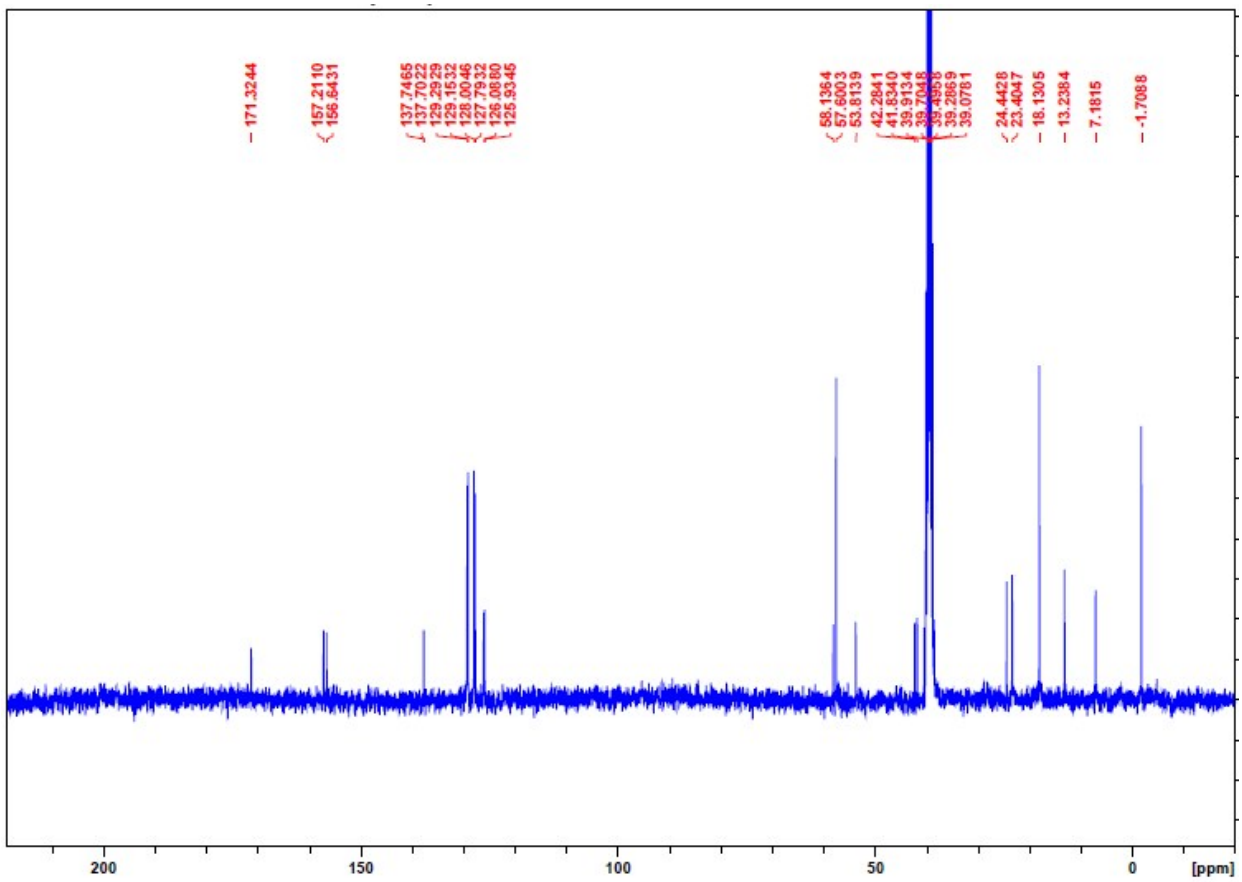


Fig. S27 ^{13}C NMR spectrum of **5** in DMSO-d_6 (100.6 MHz).

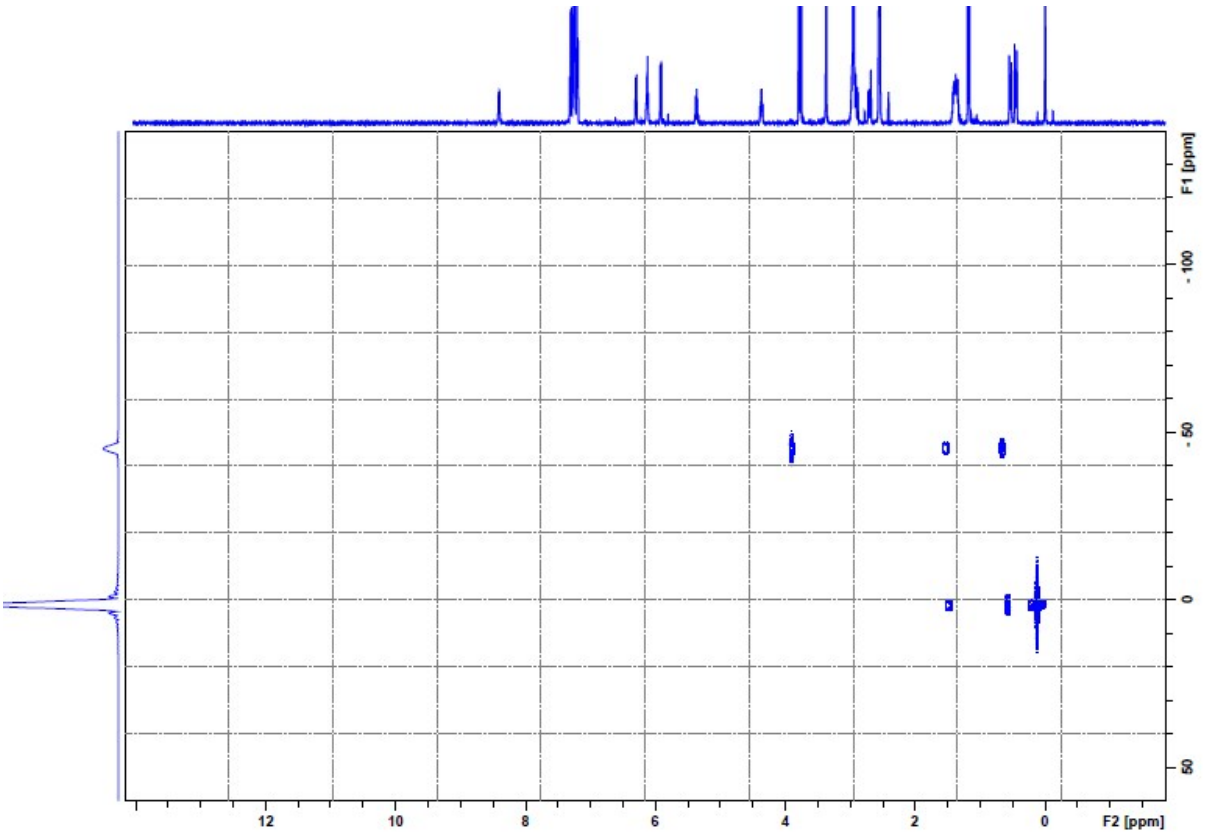
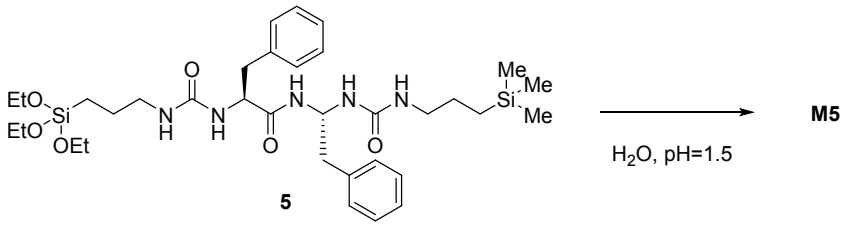


Fig. S28 ^{29}Si-^{1}H NMR spectrum of **5** in DMSO-d_6 (500-99 MHz).

17. Preparation of compound **M5**



In heterogeneous conditions, the silylated peptide **5** was solubilized at 38.5 mM (57.6 mg, 0.084 mmol in 2.2 mL) in water-HCl, pH 1.5. Compound **5** was insoluble and stay on the surface even with stirring, for one day, then it became soluble after hydrolysis and precipitate again after condensation. After 4 days under stirring at room temperature (20°C), the white powder was filtered and washed few times with HCl pH=1.5 solution and dry under vacuum at 50 °C for one night. 43 mg of white solid **M5** was recovered.

18. Analyses of **M5** material

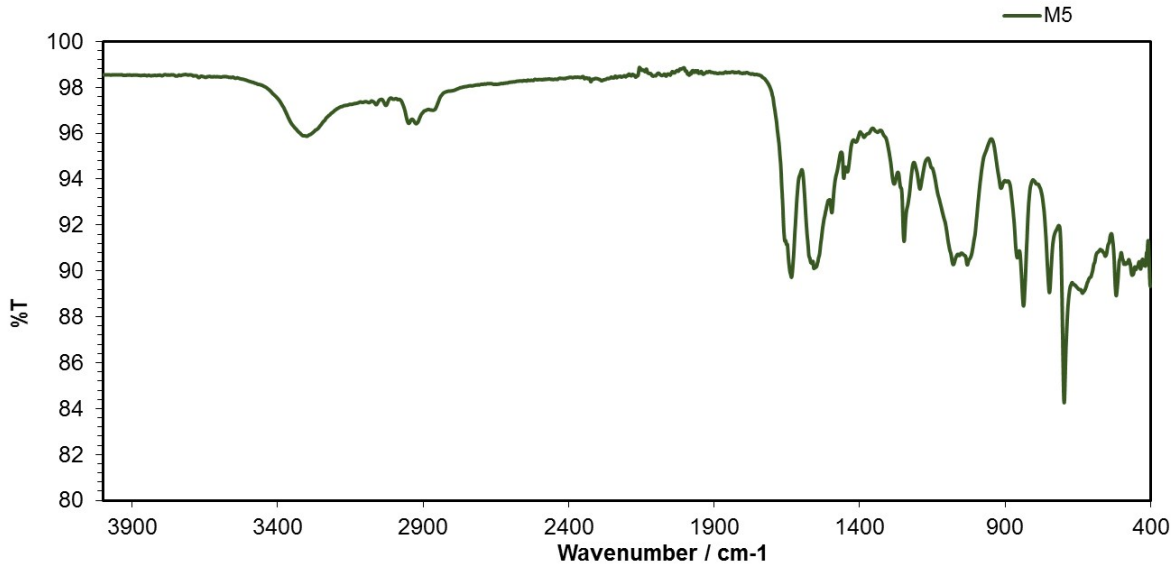


Fig. S29 FTIR spectra of **M5**.

Compound / material	CO amide (amide I) (cm^{-1})	NH amide (amide II) (cm^{-1})	Δν (CO amice - NH amide) (cm^{-1})
M5	1633	1558	75

Table S4. Amides bands in FTIR spectrum of **M5.**

Material		**C (%)**	**H (%)**	**N (%)**	**O (%)**
M5	Mean value	56.768	7.410	11.654	6.703
	Deviation (abs)	0.150	0.133	0.037	0.201

	Deviation (rel %)	0.212	0.188	0.053	3.004

Table S5. Elemental analysis of **M5B** (analysed in duplicate).

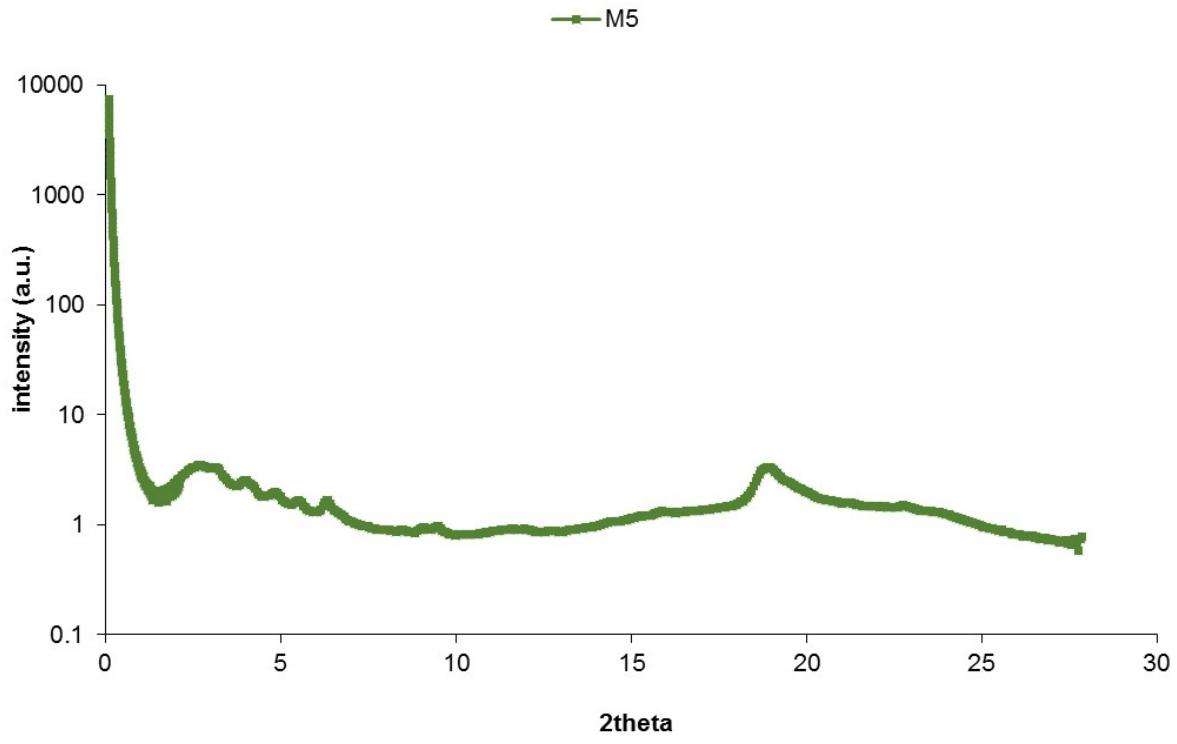
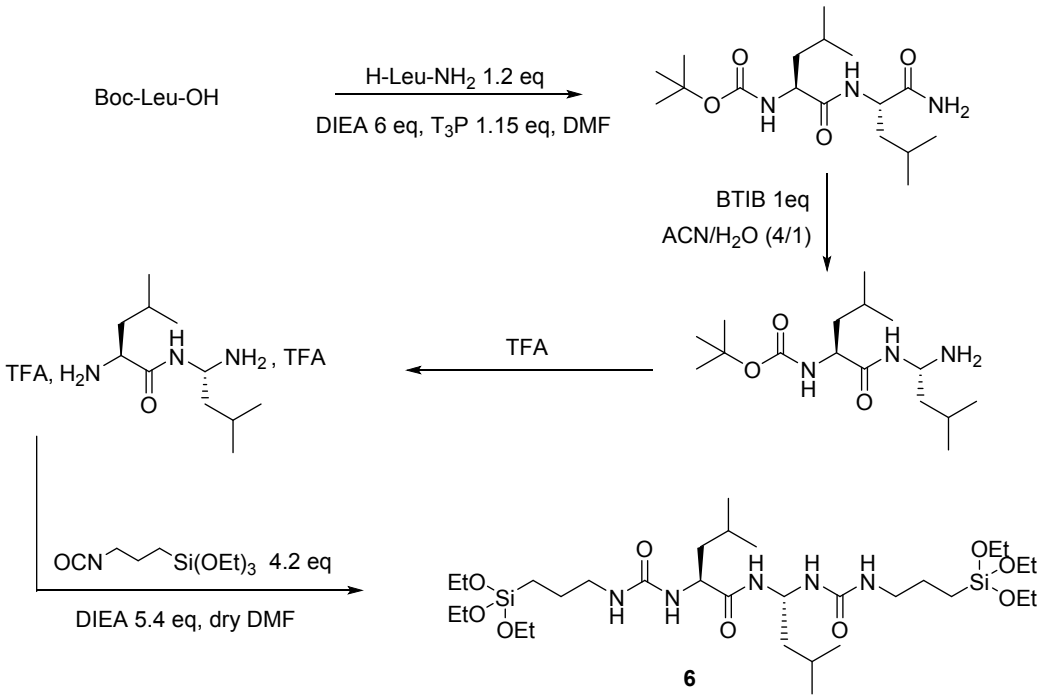


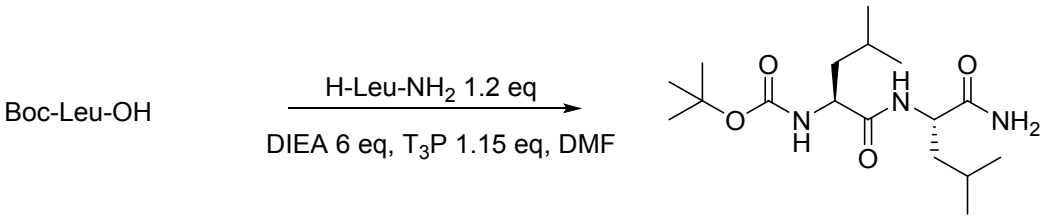
Fig. S30 XRD Saxs and Waxs patterns of **M5.**

19. General scheme of synthesis of compound 6

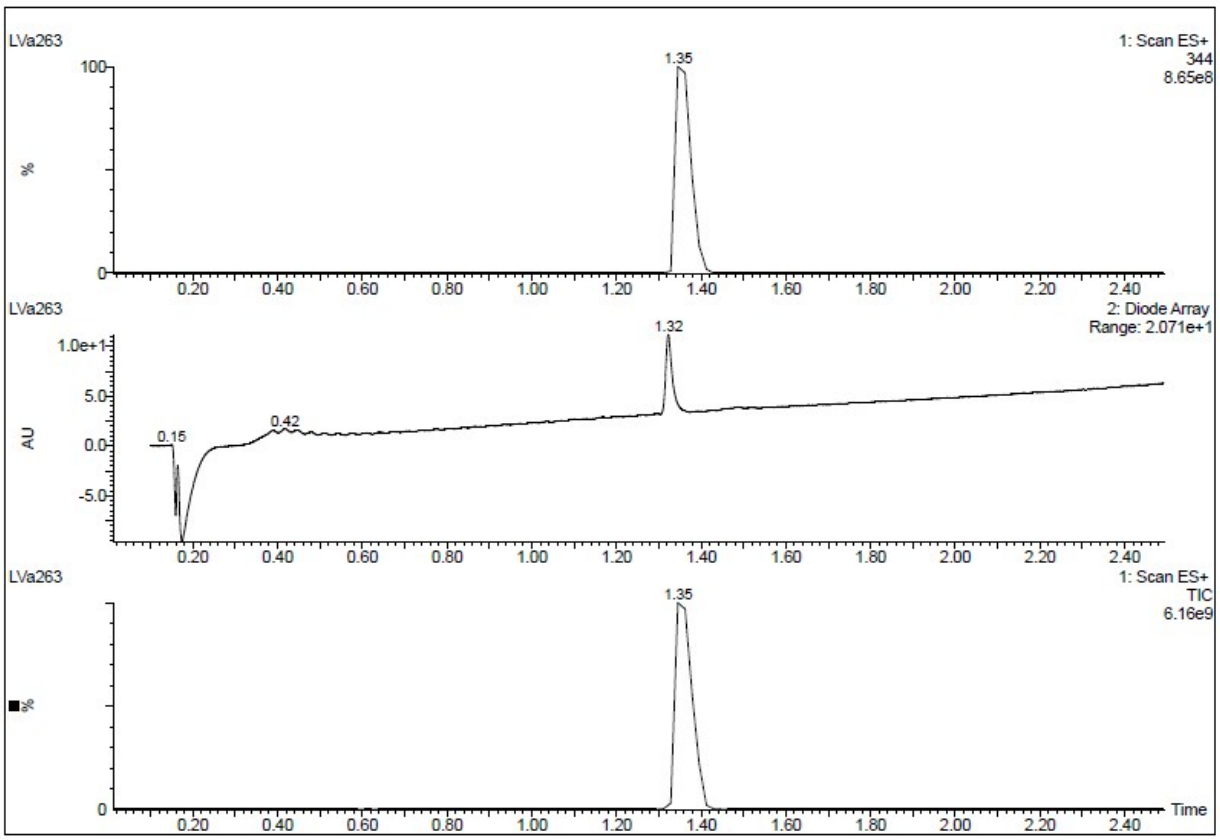


Scheme S2. Synthesis of compound **6**

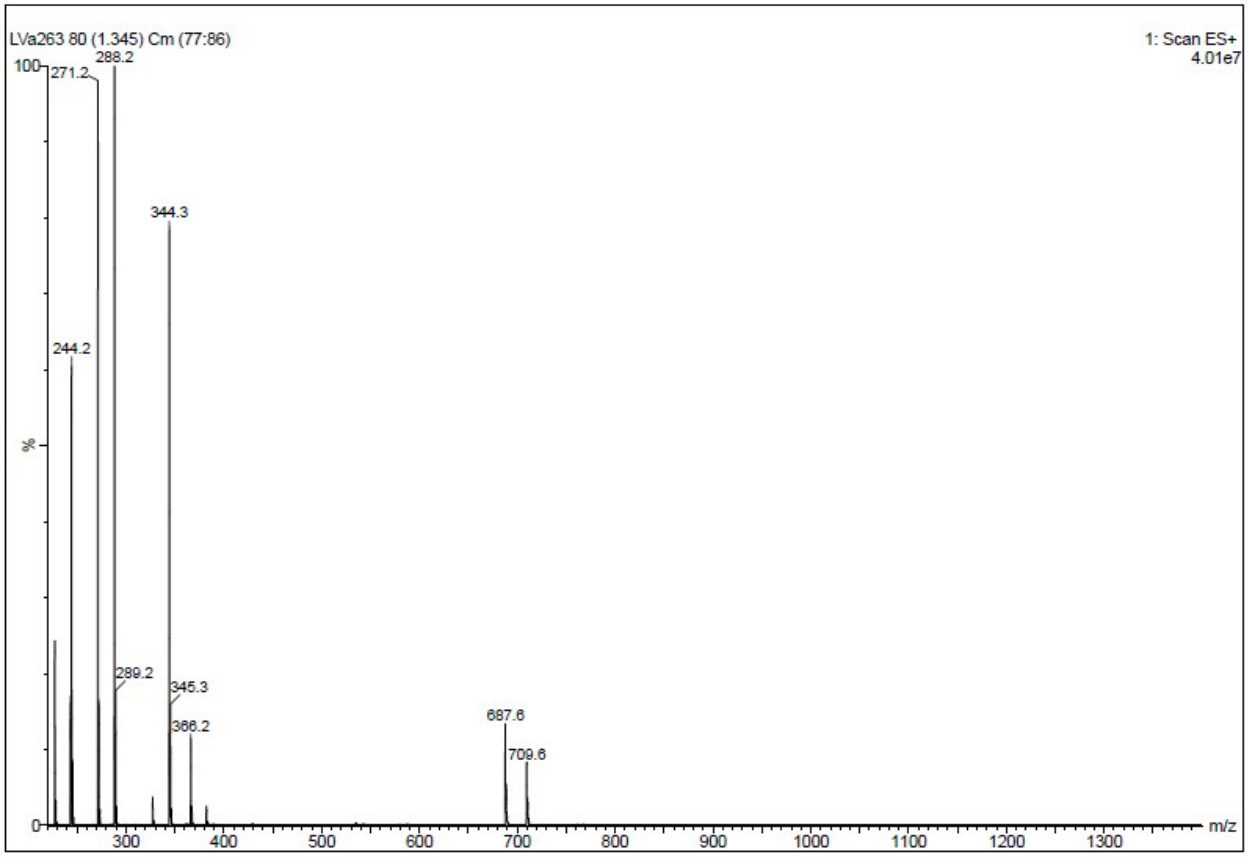
20. Synthesis of compound Boc-Leu-Leu-NH_2



Boc-Leu-OH (1 eq, 12 mmol, 6.00 g) and H-Leu-NH2 (1.2 eq, 14.4 mmol, 2.40 g) were dissolved in 15 mL of DMF. DIEA (6 eq,, 72 mmol, 12.7 mL) is added and then T3P (1.14 eq, 13.7 mmol, 8 mL) is added drop by drop. After five minutes stirring at room temperature (20°C), the reaction is completed. The solvents are concentrated under reduced pressure, and precipitate. It is filtered and wash with diethyl ether. A white solid is recovered after drying under vacuum (1.83 g, 44% yield).



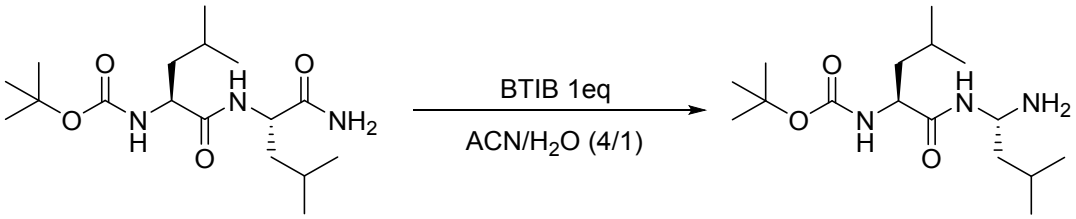
LC trace at (214 nm) and TIC



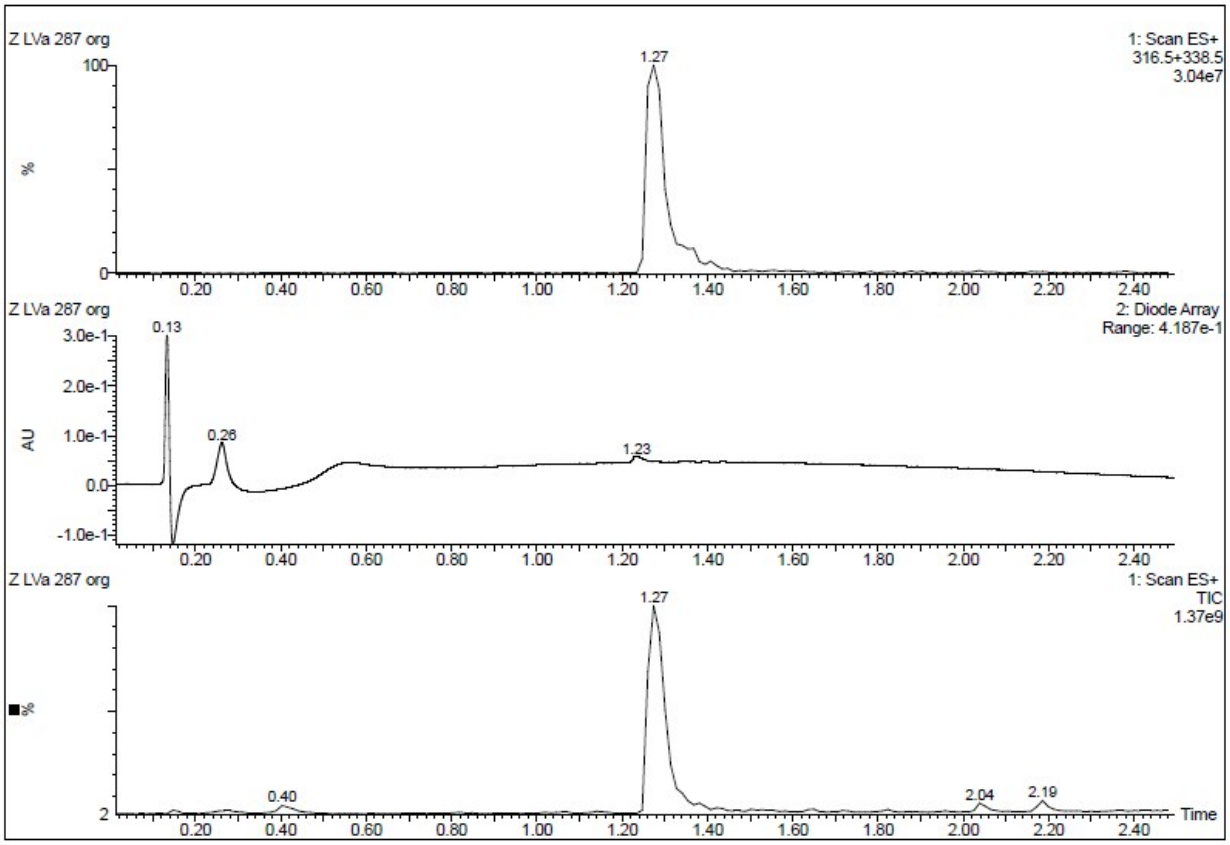
MS spectra t_R = 1.35 min

Fig. S31 LC/MS (ESI$^+$) of compound Boc-Leu-Leu-NH2. MW 343 g/mol
LC/MS (ESI$^+$): t_R = 1.35 min, m/z 344 $[M+H]^+$; m/z 366 $[M+Na]^+$.

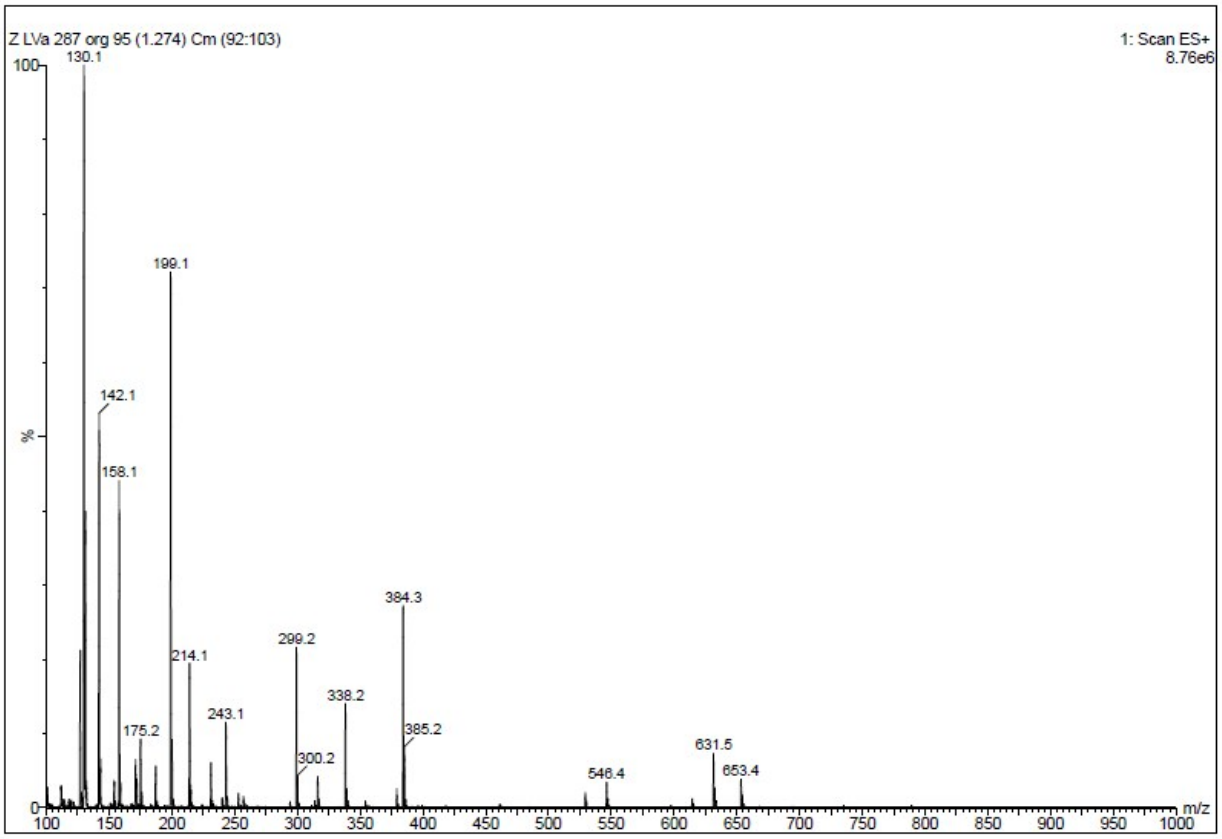
21. Synthesis of compound Boc-Leu*gem*Leu-H from Boc-Leu-Leu-NH_2



Boc-Leu-Leu-NH_2 (1 eq, 4.42 mmol, 1.50 g) and BTIB (1 eq, 4.42 mmol, 1.90 g) were dissolved in an ACN/water (4/1 v/v) solution (100 mL). After four hours stirring at room temperature (20°C), the reaction is completed. The solvents are concentrated under reduced pressure, and a white solid is recovered after precipitation in diethyl ether (554 mg, 40% yield).



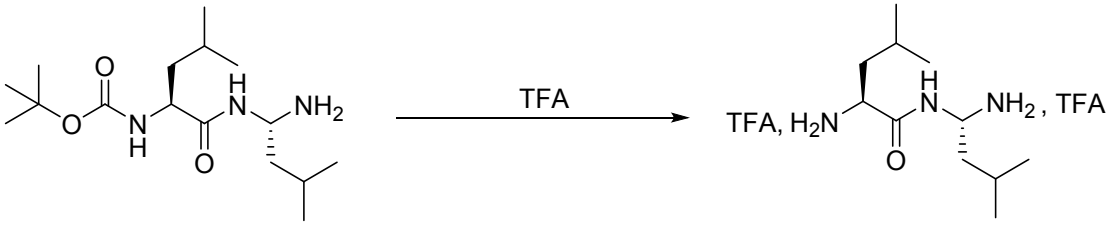
LC trace at (214 nm) and TIC. The compound present a low absorbance at 214 nm.



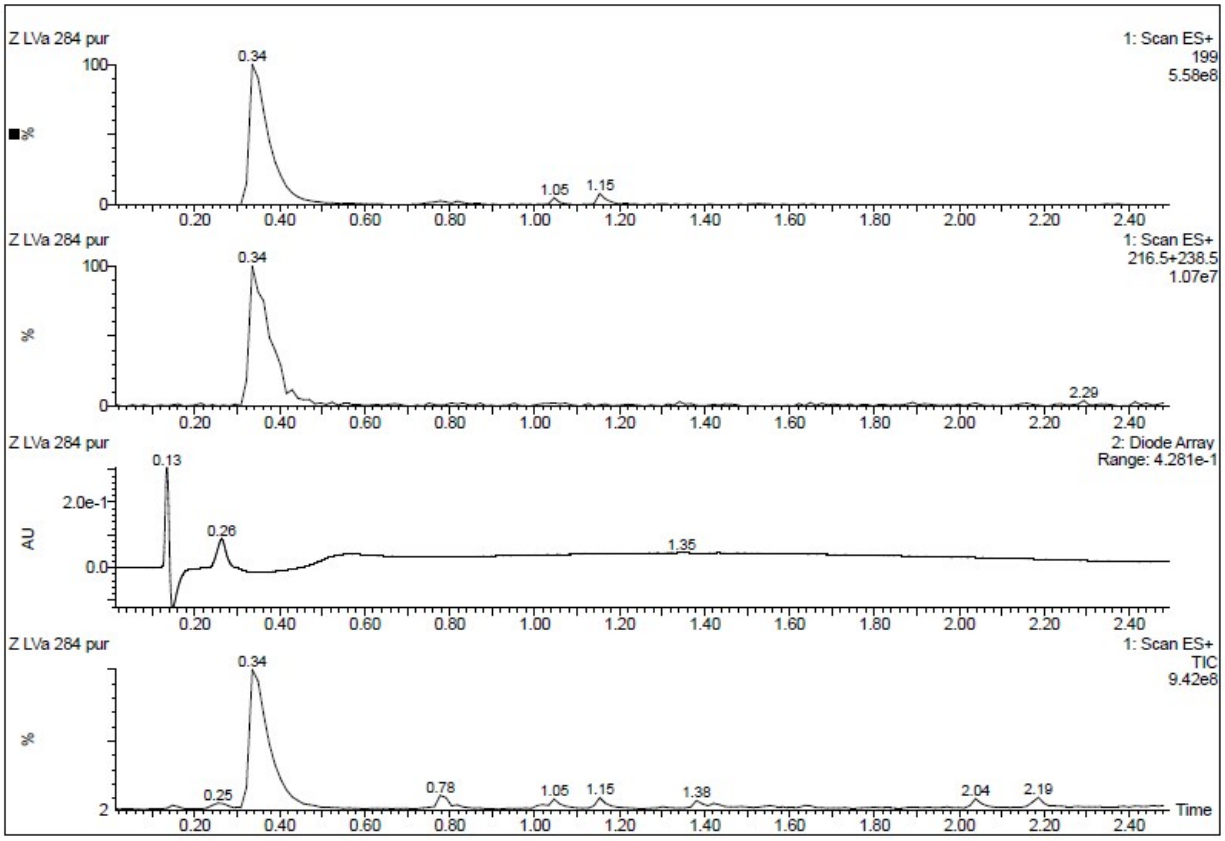
MS spectra t_R = 1.27 min

Fig. S32 LC/MS **(ESI$^+$)** of compound Boc-Leu*gem*Leu-H**.** MW 315.5 g/mol
LC/MS (ESI$^+$): t_R = 1.27 min, m/z 316.2 $[M+H]^+$; m/z 338.2 $[M+Na]^+$.

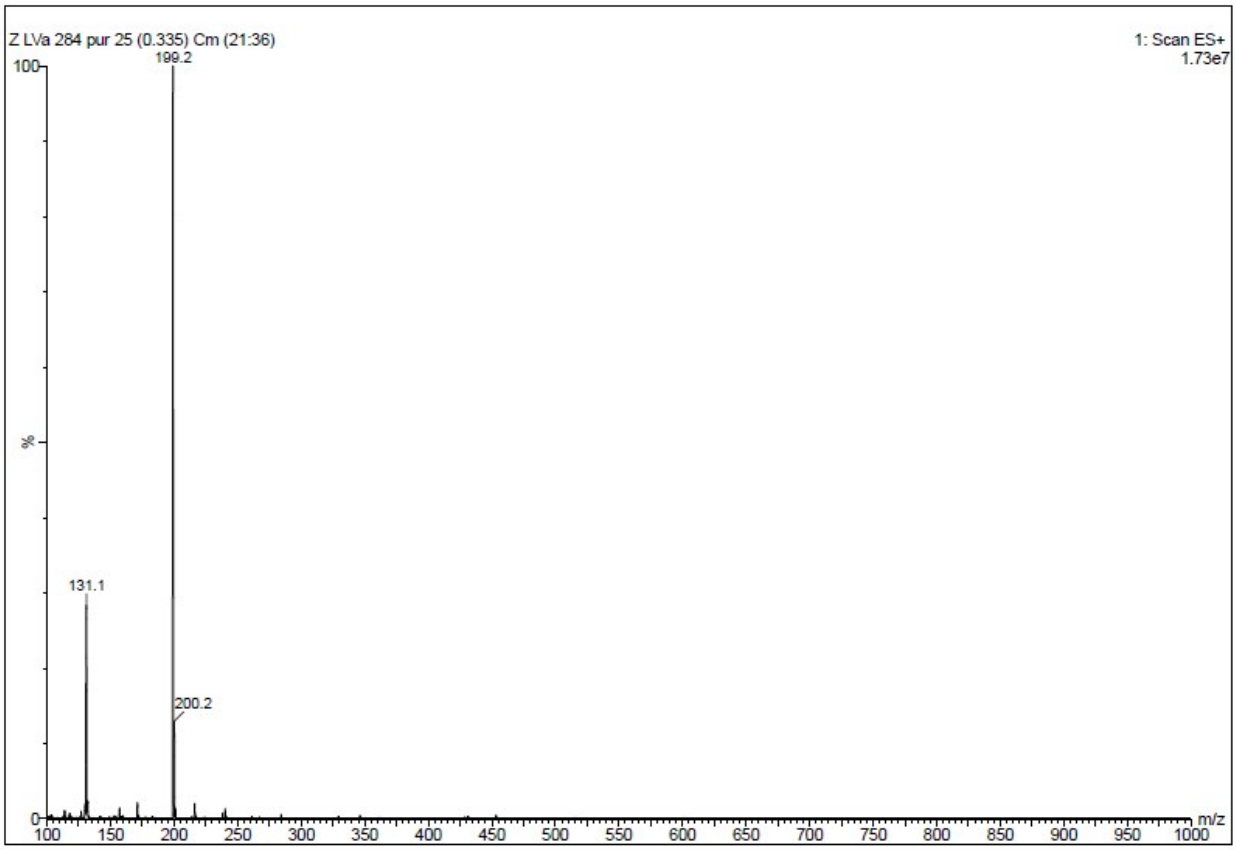
22. Synthesis of compound 2TFA, H-Leu*gem*Leu-H from Boc-Leu*gem*Leu-NH_2



554 mg of Boc-Leu*gem*Leu-H (1.76 mmol) were dissolved in TFA (4 mL). After 10 min at room temperature (20°C), the solution is concentrated under reduced pressure, coevaporated with cyclohexane. An oil is obtained which is dissolved in ACN/water (1/9 v/v) solution (20 mL). The solution was freeze dried. 632 mg of compound H-Leu*gem*Leu-H (81% yield) as trifluoracetate salts were obtained.



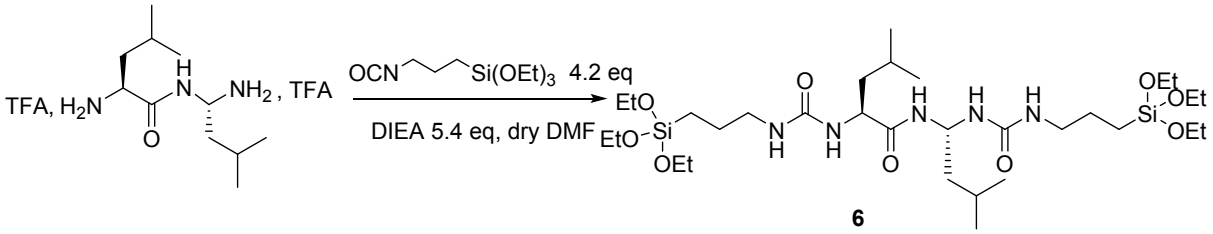
LC trace at (214 nm) and TIC. The compound present a low absorbance at 214 nm.



MS spectra t_R = 0.33 min

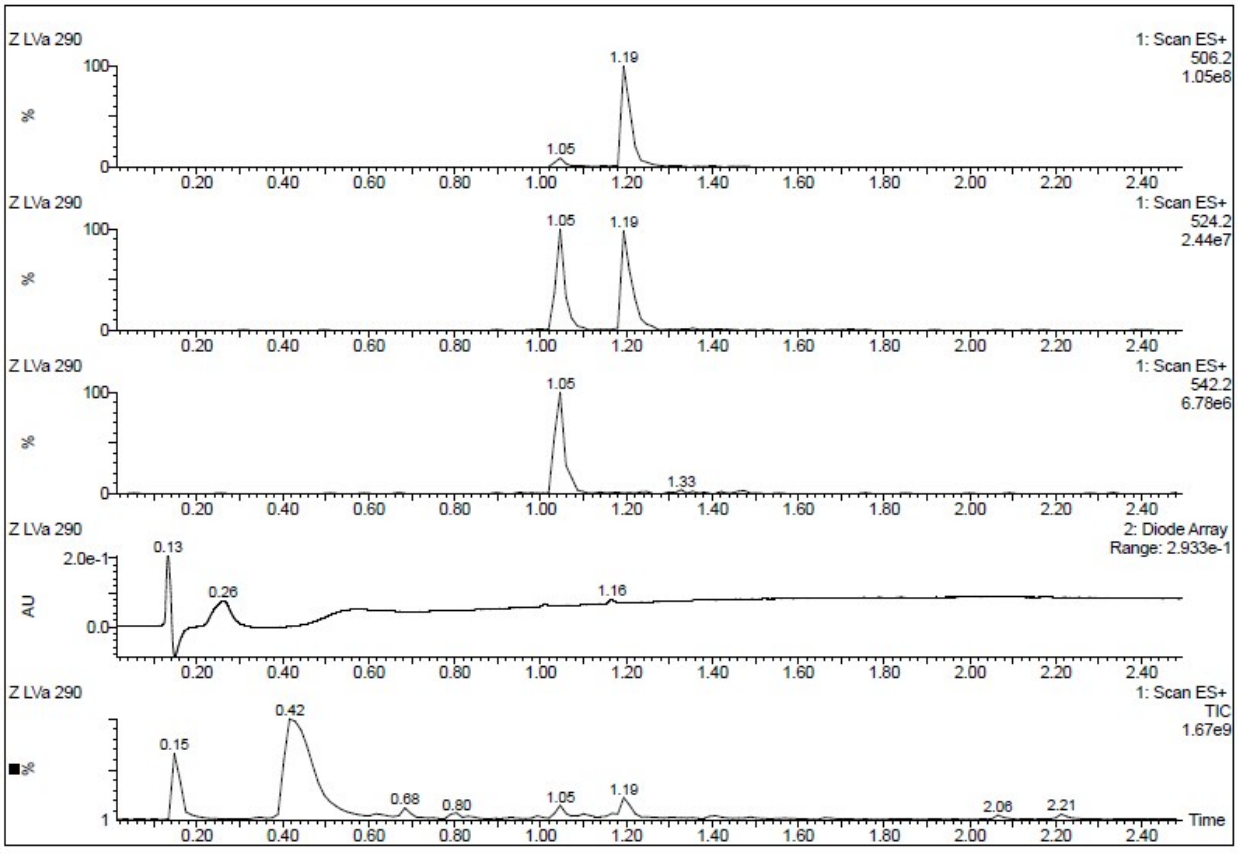
Fig. S33 LC/MS (ESI$^+$) of compound H-Leu*gem*Leu-H. MW 443.4 g/mol (with 2 TFA salts)
LC/MS (ESI$^+$): t_R = 0.33 min, m/z 199.2 $[M\text{-}NH_3]^{+\cdot}$

23. Synthesis of compound **6** $(OEt)_3Si\text{-}(CH_2)_3\text{-}NHCO\text{-}Phe$*gem*$Phe\text{-}CONH\text{-}(CH_2)_3\text{-}Si(OEt)_3$ from 2 TFA, H-Leu*gem*Leu-H

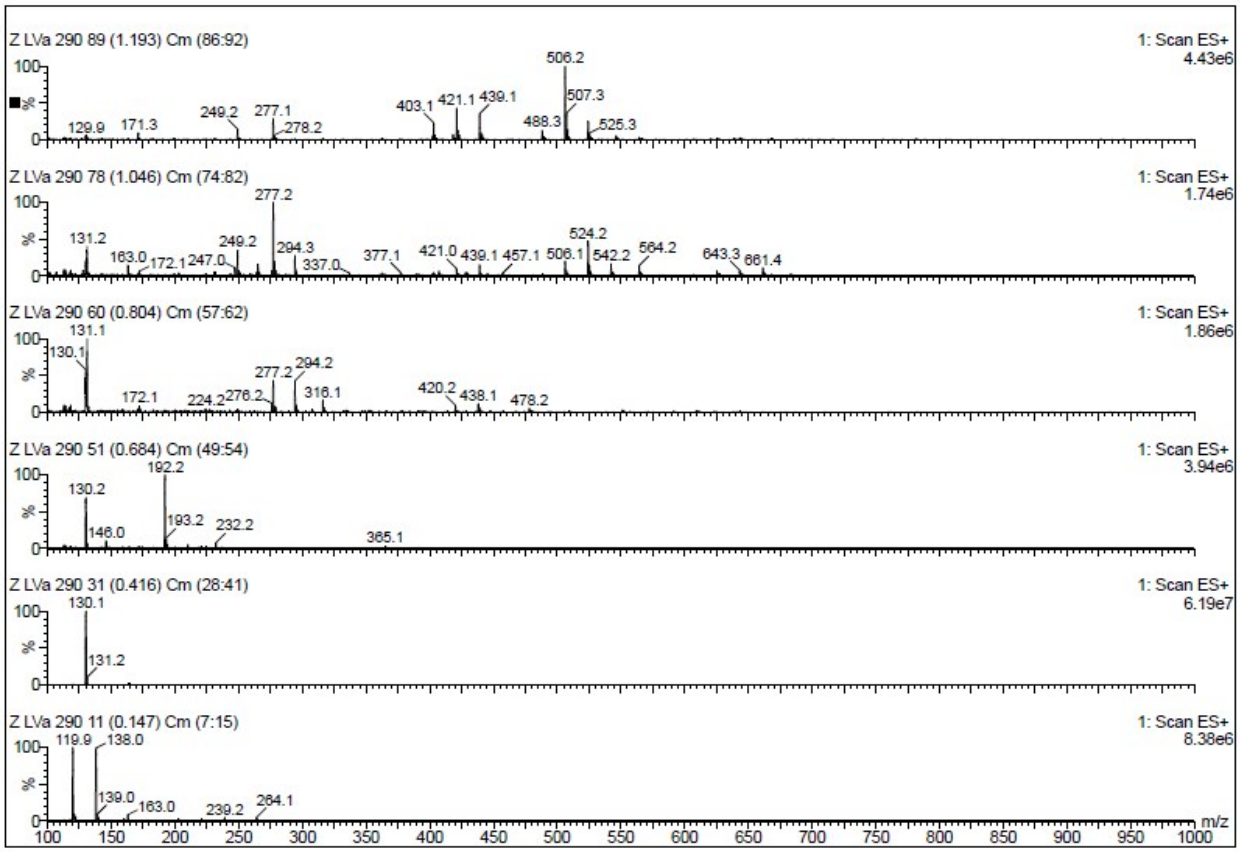


6

H-Leu*gem*Leu-H (560 mg, 1.26 mmol, 1 eq) was dissolved in a dry DMF solution (7.2 mL) containing DIEA (1.2 mL, 6.83 mmol, 5.4 eq) under argon. ICPTES (1.3 mL, 5.26 mmol, 4.2 eq) was added. After 2 hours at room temperature (20°C), solvent was removed under reduced pressure to yield a yellow oil and the desired compound **6** was washed with hexane and evaporated. The product was dried overnight in a vacuum desiccator. 1.1 g of white pasta was recovered (80% yield).



LC trace at (214 nm) and TIC. The compound present a low absorbance at 214 nm.



MS spectra t_R = 1.2 min, 1.05 min, 0.8 min, 0.68 min, 0.42 min, 0.15 min.

Fig. S34 LC/MS (ESI$^+$) of compound **6.** MW 710.1 g/mol
LC/MS (ESI$^+$): t_R = 0.77 min, m/z 542.2 [M hydrolysed]$^+$; m/z 564.2 [M hydrolysed + Na]$^+$, m/z 506 [M hydrolysed – H_2O]$^+$

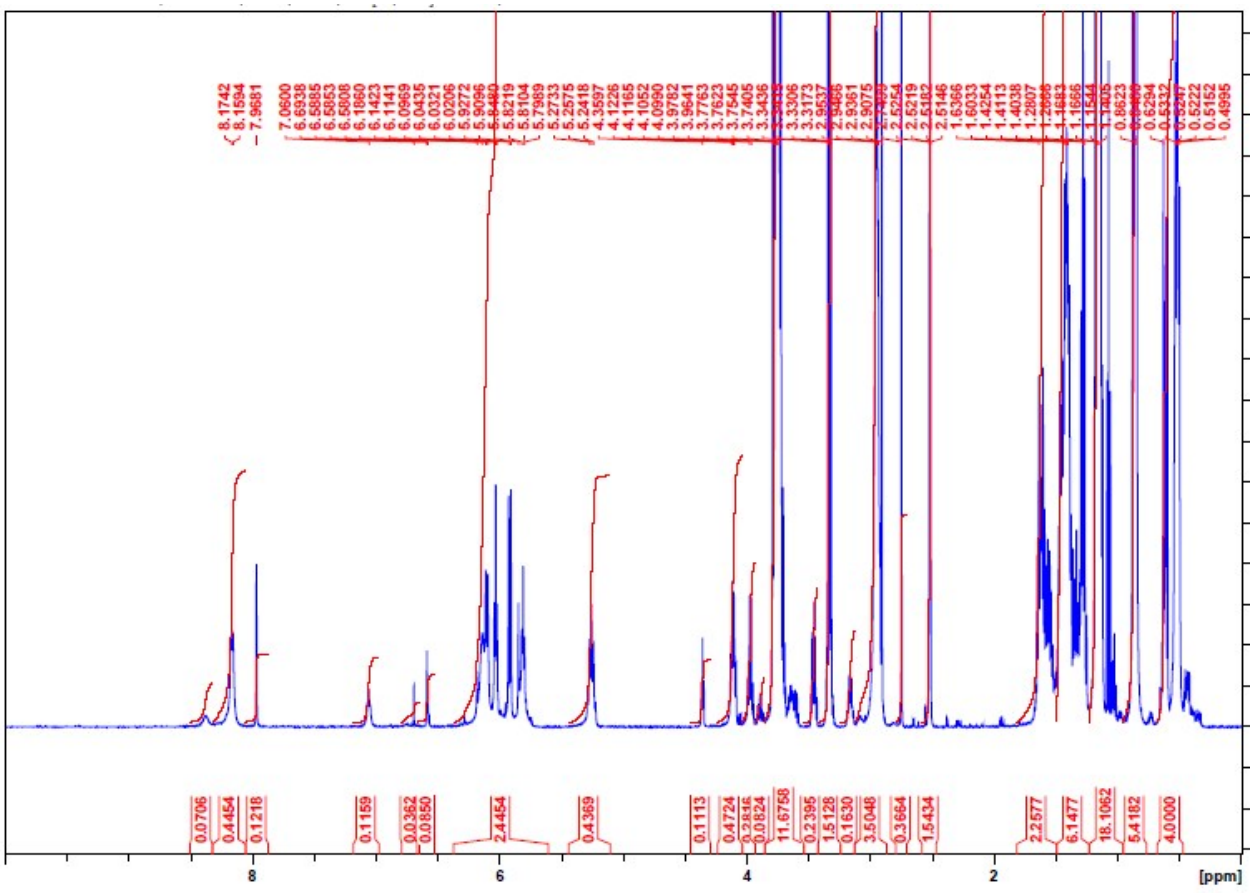


Fig. S35 ^{1}H NMR spectrum of compound **6** in DMSO-d6 (400 MHz).

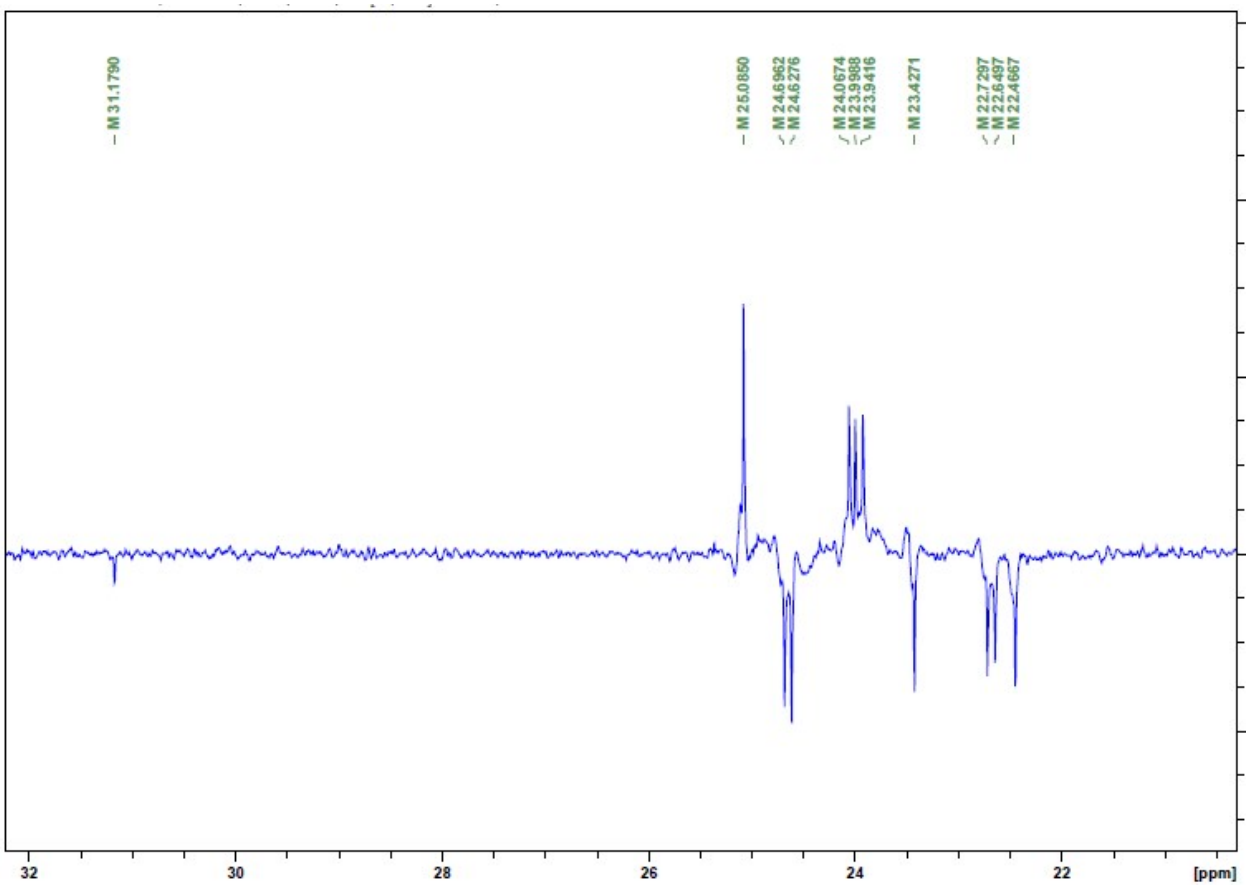


Fig. S36 ^{13}C dept 135 NMR spectrum of **6** in DMSO-d_6 (125.8 MHz).

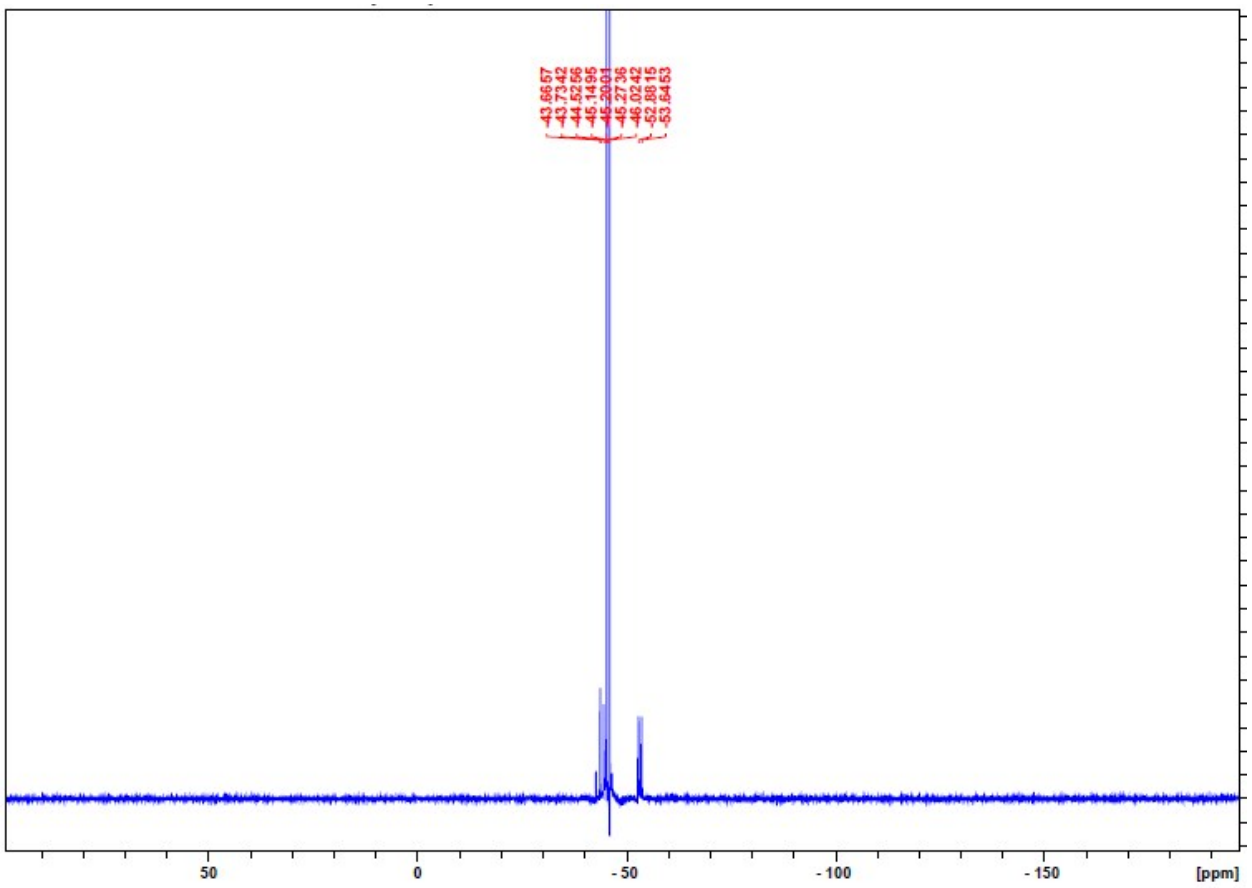
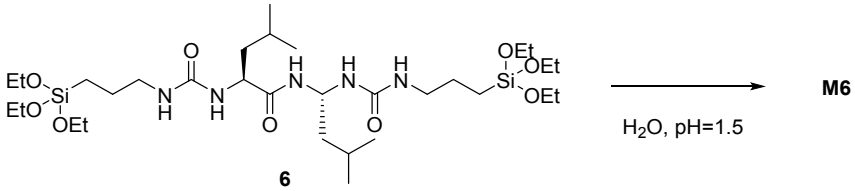


Fig. S37 ^{29}Si NMR spectrum of **6** in DMSO-d_6 (99 MHz).

24. Preparation of compound **M6**



In heterogeneous conditions, the silylated peptide **6** was solubilized at 38.5 mM (960 mg, 1.35 mmol in 35.1 mL) in water-HCl, pH 1.5. Compound **6** was soluble for one hour, then it precipitate after condensation. After 4 days under stirring at room temperature (20°C), the white product was filtered and washed few times with HCl pH=1.5 solution and dry under vacuum at 50 °C for one night. Only 84 mg of white solid **M6** was recovered.

25. Analyses of **M6** material

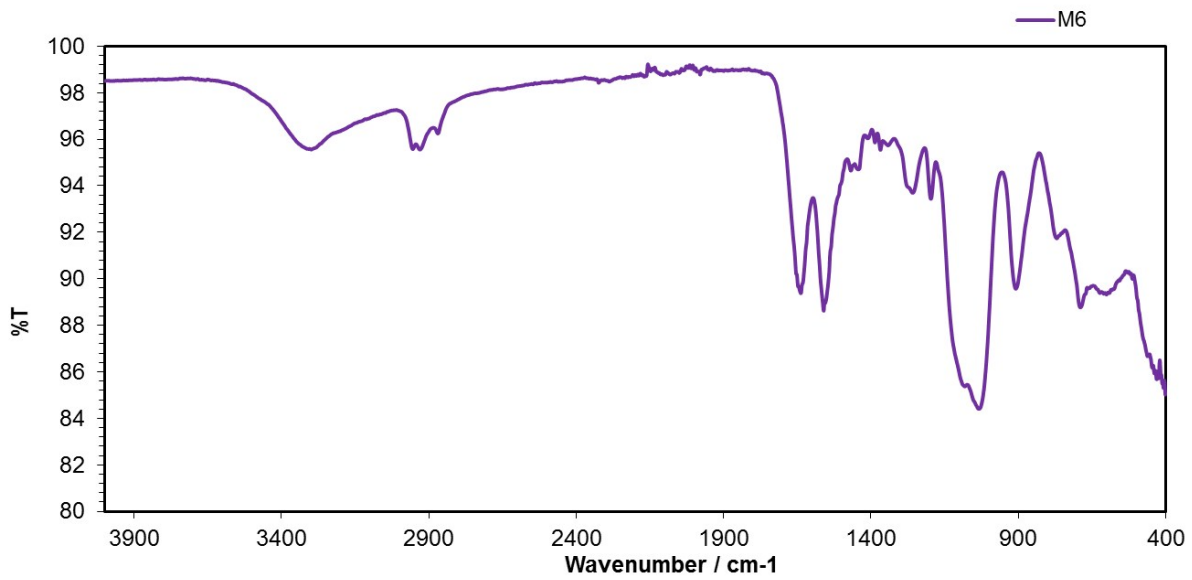


Fig. S38 FTIR spectra of **M6**.

Compound / material	CO amide (amide I) (cm^{-1})	NH amide (amide II) (cm^{-1})	Δν (CO amice - NH amide) (cm^{-1})
M6	1638	1559	79

Table S6. Amides bands in FTIR spectrum of **M6.**

Material		**C (%)**	**H (%)**	**N (%)**	**O (%)**
M6	Mean value	38.222	7.029	13.029	13.259
	Deviation (abs)	0.153	0.104	0.117	0.049
	Deviation (rel %)	0.216	0.147	0.165	0.367

Table S7. Elemental analysis of **M6B** (analysed in duplicate).

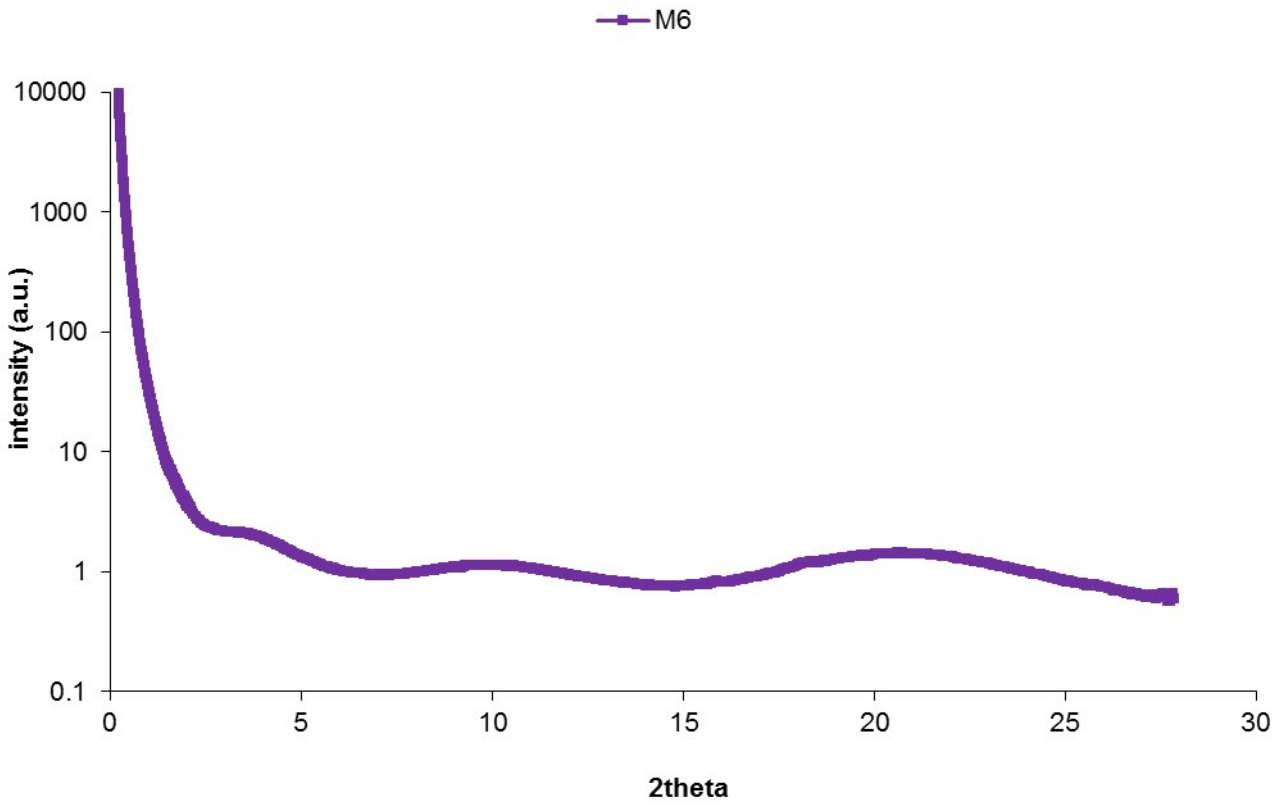
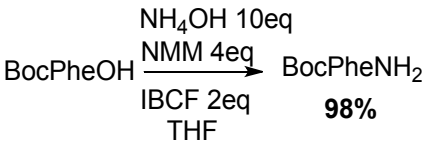


Fig. S39 XRD Saxs and Waxs patterns of **M6**.

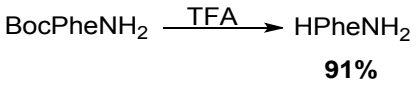
Step by step synthesis of bis-silylated HPhe*gem*Phe

BocPheNH$_2$Synthesis



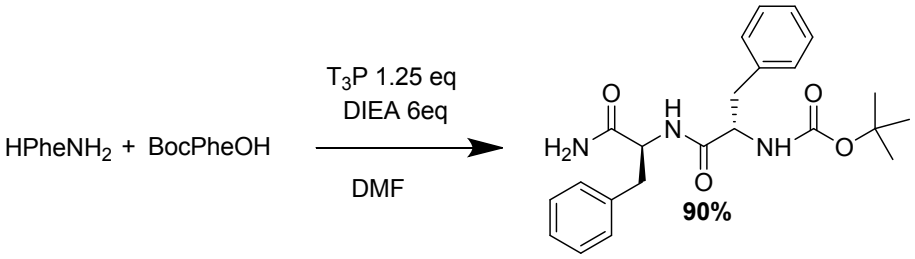
Products	BocPheNH$_2$
Reactants	BocPheOH 1 eq N-methylmorpholine 4 eq Isobutyl chloroformiate 2 eq Ammonia 10eq
Solvents	Tetrahydrofuran
Procedure	1- Add NMM (4 eq, 148 mmol, 28.7 mL) to a solution of BocPheOH (1 eq, 37 mmol, 10 g) in THF (100 mL). 2- Add drop by drop IBCF (2 eq, 74 mmol, 9.6 mL), then again add drop by drop ammonia (10 eq, 370 mmol, 41.24 mL). 3- Allow the reaction to stir during 5 min. The reaction is total. 4- Retake the reaction medium with ethyl acetate and water. 5- Extract the organic phase with $KHSO_4$ and $NaCO_3$ both twice. 6- Dry on $MgSO_4$. 7- Concentrate the obtained product BocPheNH$_2$.
Yield	98%

Deprotection of BocPheNH$_2$



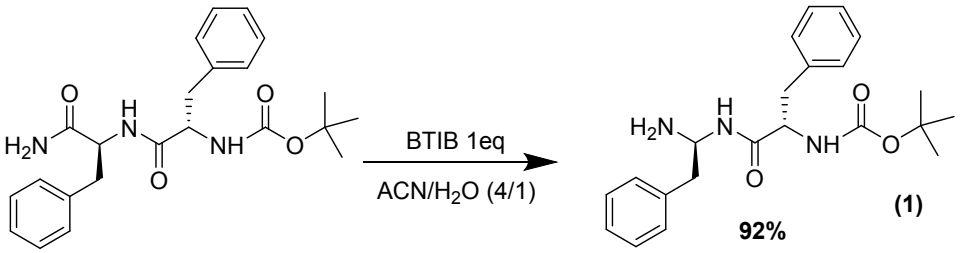
Product	HPheNH$_2$
Reactant/solvent	Trifluoroacetic acid
Procedure	1- Disolve BocPheNH$_2$ (30 mmol, 7.68 g) in TFA (40 mL). 2- After 10 minutes the reaction is over, and then concentrate. 3- Coevaporate the TFA with cyclohexane three times. 4- Pour water and acetonitrile in the obtained oil. 5- Freeze-dry the product.
Yield	91%

Synthesis of BocPhePheNH$_2$



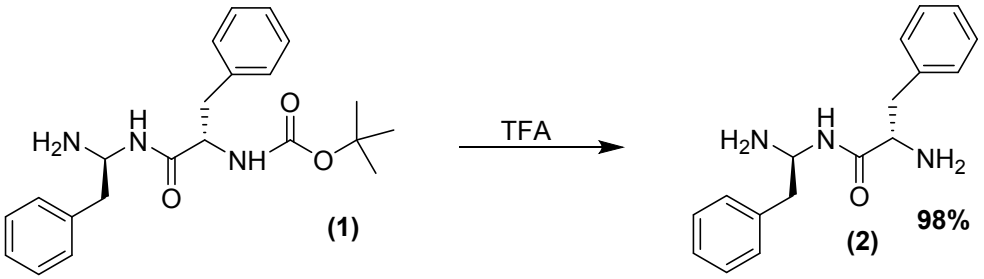
Products	BocPhePheNH$_2$
Reactants	BocPheOH (1 eq), HPheNH$_2$ (1.2 eq) Propylphosphonic anhydride (1 eq) Diisopropylethylamine (6 eq)
solvent	DMF
Procedure	1- Add DIEA (6 eq, 69 mmol, 11.5 mL) to a solution of HPheNH$_2$ (1.2 eq, 14 mmol, 4 g) and BocPheOH (1 eq, 11.6 mmol, 3 g) in DMF. 2- Add drop by drop the T$_3$P (1 eq, 11.6 mmol, 7.3 mL). 3- After 5 min the reaction is completed, add some water to precipitate the dipeptide. 4- Filter the precipitate and wash with ether.
Yield	90%

Synthesis of BocPhe*gem*Phe



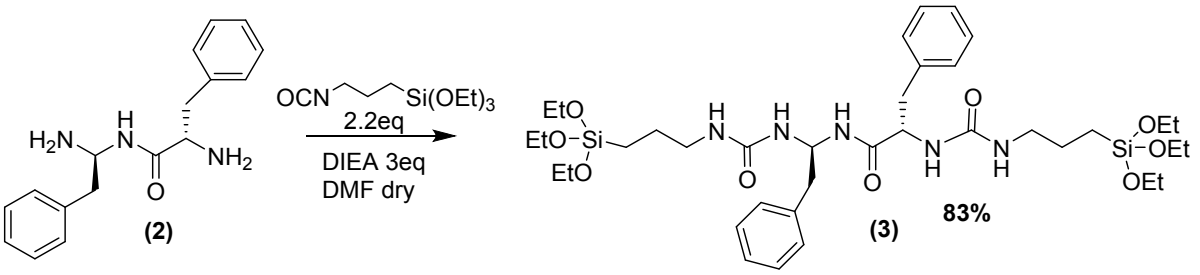
Product	BocPhe*gem*Phe
Reactants	BocPhePheNH$_2$ (1 eq) (Bis(trifluoroacetoxy)iodo)benzene (1 eq)
Solvent	Acetonitrile/water (4/1) (194.56 mL/48.64 mL)
Procedure	1- Dissolve BocPhePheNH$_2$ (1 eq, 12.16 mmol, 5 g) and BTIB (1 eq, 12.16 mmol, 5.3 g) in ACN/H2O (243.2 mL). 2- After 4 hours the reaction is total. 3- Concentrate the product to obtain a crude product. 4- Pour ether on the crude product, the gem dipeptide precipitates. 5- Filter the precipitate.
Yield	92%

Deprotection of BocPhe*gem*Phe



Product	HPhe*gem*Phe
Reactant/solvent	BocPhe*gem*Phe Trifluoroacetic acid
Procedure	1- Disolve BocPhe*gem*Phe (0.287 mmol, 110 mg) in TFA (1.5 mL). 2- After 10 minutes the reaction is over, and then concentrate. 3- Coevaporate the TFA with cyclohexane three times. 4- Pour water and acetonitrile in the obtained oil. 5- Freeze-dry the product.
Yield	98%

Silylation of HPhe*gem*Phe



Product	
Reactants/Solvent	HPhe*gem*Phe 1 eq DIEA 3 eq ICPTES 2.2 eq DMF dry
Procedure	1- Dissolve HPhe*gem*Phe (1 eq, 3.52 mmol, 1 g) in DMF dry under argon 2- Add DIEA (3 eq,10.56 mmol, 1.82 mL) 3- Add isocyanatopropyltrialkoxysilane (2.2 eq, 7.74 mmol, 2.01 mL) 4- After 40 min the reaction is completed. 5- Concentrate the product under vacuo 6- Pour ether, the product precipitates then centrifuge 3 times.
Yield	83%