Electronic Supplementary Information

Bioorganic hybrid OMS by straightforward grafting of trialkoxysilyl-peptides

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1.Materials and Methods

All amino acid derivatives were purchased from IrisBiotech. Other chemicals were purchased from commercial suppliers, Iris Biotech, Senn Chemicals, Merck, Carlo Erba, Fluka, Riedel-de Haën, Sigma Aldrich, Acros Organics, FluoroChem and VWR Prolabo.
The 2-Chloro chlorotrityl chloride resin (100-200 Mesh, loading : 1.6 mmol/g) and the Fmoc-rink amide AM resin (100-200 Mesh, loading 0.7 mmol/g) was purchased from Iris Biotech.

All compounds were analyzed under standard analytical HPLC conditions on a Beckman Gold apparatus composed of the 126 solvent module, the 168 detector, and the 32 Karat software, on a C18 reversed-phase column (VWR chromolith column, 50 mm x 3,9 mm): 100% A to 100% B linear gradient over 3 min at a flow rate of 5 mL/min. Solvent A was H2O + 0.1% TFA. Solvent B was CH_3CN + 0.1% TFA. Detection and purity was performed and determined at 214 nm. The LC/MS analyses were performed on a Quatromicro (Micromass, Manchester, U.K.) triple-quadrupole mass spectrometer fitted with an electrospray interface. Solvents used for HPLC and LC/MS were of HPLC grade.

The nitrogen adsorption isotherms were measured at liquid temperature (77 K) using a Micromeritics Tristar 3000 analyzer. Before the measurements, the samples were out gassed under vacuum for 12 h at 80 °C. The specific surface areas were calculated by the Brunauer–Emmett–Teller (BET) method (using 74 points and starting from 0.01 as the value of the relative pressure) and the pore size distributions were determined by the BJH method applied to the adsorption branch.
Elemental analyses were performed on a FLASH EA 1112 CHNS analyser.
^{1}H-NMR, ^{13}C-NMR and ^{29}Si-NMR spectra were recorded using an AMX 400 Brücker spectrometer operating at 400 MHz, 100 MHz and 75MHz. Deuterated dimethyl sulfoxide was used as solvent. Powder X-ray diffraction patterns were measured on a Bruker D5000 diffractometer equipped with a rotating anode. The wavelength used was 1.542 Å (Cu Kα radiation).
Transmission Electron Microscopy (TEM) observations were carried out at 100 kV on a JEOL 1200 EXII microscope. Samples for TEM measurements were prepared using ultramicrotomy techniques and then deposited on copper grids.
Purification by chromatography was performed on automated flash chromatography system (Biotage) by using pre-loaded silica cartridges.
IR spectra in solid state were realized on a FTS 575C spectrometer Bio Rad.

Chiral HPLC was carried out on a Beckman Gold apparatus composed of the 125 solvent module, the 166 detector, and the 32 Karat software, using chiral pack 250 x 4.6µ, column (isopropanol/hexanes 05:95), UV , 214 nm, 254 nm.

2.Peptide and hybrid peptide synthesis

Hybrid peptides **1** to **4** were prepared on solid-phase (either Rink amide and 2-Chlorotrytil chloride resin respectively for peptide **1,2,3** and **4**) following the general protocol for Fmoc/t-Bu peptide synthesis.

Unprotected peptides: H-Gly-Phe-Glu-NH_2, H-Phe-Ala-Phe-NH_2:
To a pre-swollen suspension of Rink Amide resin in CH_2Cl_2, was added a solution of the Fmoc amino acid (3 eq.), DIEA (6 eq.) and BOP (3 eq.) in DMF. The reaction mixture was agitated at room temperature for 2 h, then washed with DMF (3×), DCM (3×), MeOH(1×) and DCM (1×). Quantitative Fmoc tests were performed as spot checks.

Protected Peptide: Boc-Pro-Pro-Asp(OtBu)-Lys-NH_2
Fmoc-Lys-NH_2 (4 eq.) was anchored on a trityl chloride resin using DIEA (8 eq) and anhydrous DMF. The reaction mixture was agitated at room temperature for 2 h, then washed with DMF/MeOH/DIEA (17/2/1) (3×), DCM (3×), MeOH(1×) and DCM (1×). Quantitative Fmoc tests were performed as spot checks.

Procedure for the solid-phase peptide synthesis
Peptide chains elongation was performed using a Fmoc/tBu strategy with BOP as coupling reagents. The coupling step was carried out on the resin using N^{α}-Fmoc-amino acid (3 eq.) in DMF in the presence of BOP (3 eq.) and DIEA (6 eq.) during 2 hours at room temperature. The N^{α} -Fmoc deprotection step was performed by treatment with a DMF/piperidine solution (2/8 v/v in DMF) for 20 min. After each coupling and deprotection step, the resin was washed with DMF(3×), DCM (3×), MeOH(1×) and DCM (1×). The completion of the reaction was checked by the standard Kaiser test and the TNBS test.

Procedure for Cleavage of Peptides from the solid support
Immobilized peptides were cleaved into solution through agitation of the resin in a mixture of TFA/CH_2Cl_2 (2/1 v/v) or TFE/AcOH/DCM (2/1/7 v/v/v) for 1h, then repeating the cleavage for a further 1h, respectively for unprotected peptide or for protected peptide. After filtration, the solution was concentrated under reduced pressure. The peptide were precipitated with diethyl ether, filtered and dried under high vacuum. All crude compounds were analyzed by analytical HPLC and LC/MS and used without further purification.

Preparation of triethoxysilyl peptides (compounds 1, 3 and 4)
To the solution of peptide (0.1 mmol) in 100 µL of DMF was added DIEA (2.1 eq.) and 3-isocyanatopropyltrimethoxysilane (1.2 eq.). The reaction mixture was allowed to stir for 2 hours at room temperature. After 120 min, reaction was monitored by HPLC. Ether (30 mL) was poured into the reaction mixture to cause precipitation. The precipitate was suspended in ether again and recollected. This procedure was repeated three times to remove TICPS and DIEA. All crude compounds were analyzed by analytical HPLC, LC/MS and NMR and used without further purification.

Preparation of triisopropoxysilyl peptide (example for compound 2):
To a solution of 5-oxo-5-((3-(triisopropoxysilyl)propyl)amino)pentanoic acid in DMF was added DIEA (2.2 eq.) and BOP (1.1 eq) as coupling reagent. After 15 min a solution of

peptide and DIEA was added. The reaction mixture was allowed to stir a room temperature for 2h and monitored by HPLC. Ether (30 mL) was poured into the reaction mixture to induce precipitation of the hybrid peptide compound. The precipitate was suspended in ether again and recollected. This procedure was repeated three times to remove trialkoxysilane and DIEA. Crude compound were analyzed by analytical HPLC, LC/MS and NMR and used without further purification.

3.Preparation of peptide hybrid OMS

Preparation of large pore SBA-15

4.0 g (0.067 mmol) of triblock copolymer EO20PO70EO20 called P123 were dissolved in 160 mL of an acidic aqueous solution (pH=1.5). The obtained solution was then added to 9.40 g (44.9 mmol) of TEOS. The resulting mixture was vigorously stirred for 3 hours at room temperature until a clear solution appears. In order to start the polymerization process, 76 mg (1.8 mmol) of NaF were added and the mixture was heated at 60°C. After aging under regular stirring for 3 days at 60 °C, the resulting material was filtered off and the surfactant (P123) was removed by soxhlet extraction over ethanol 95° for 24 hours. After drying at 100°C under vacuum, 2.75 g of SBA-15 were obtained as white powder.

Preparation of hybrid peptide OMS- by grafting

General procedure:

To a solution of SBA-15 (500 mg) in 20 mL of dry DMF was added hybrid peptide block (50-100 mg). The reaction mixture was allowed to stir for 1 hour at room temperature and 24h at 80°C. The functionalized SBA-15 (SBA-Pep) was filtered off and washed with each 10 mL of DMF (3×), DCM (3×), diethylether (3×), before being dried under vacuum conditions.

Protected hybride peptide block:

The general procedure is followed by treatment of hybrid silica in 5ml of TFA/DCM (2/1 v/v) for 1h. The functionalized deprotected SBA-15 (SBA-Pep) was filtered off and washed with each 10 mL of DMF/DIEA (0.5%) (3×), DMF (3×), DCM (3×), diethylether (3×), before drying *in vacuo*.

4. Hybrid silica-peptide mediated aldolisation

To a suspension of hybrid SBA-peptide catalyst (equivalent to 2 mol%) in desired solvent (6 mL), was added acetone (4 mmol) and NMM (2 mol%). The mixture was stirred for 15 min. Then, 4-nitrobenzaldehyde (1 mmol) was added and stirring was continued until completion of the reaction monitored by HPLC. The reaction mixture was filtered off to remove the catalyst, evaporated *in vacuo*, and the crude product was purified by silica gel column chromatography to afford the pure product.

5. NMR and MS data

Peptide: H-Gly-Phe-Glu-NH_2

^{1}H NMR (400 MHz, DMSO-d_6): 1.76-1.82(m, 1H), 1.91-1.97(m, 1H), 2.23-2.26(m, 2H), 2.77(dd, 1H, J=2.8Hz, 9.6Hz), 3.08(dd, 1H, J=2.8Hz, 9.2Hz), 3.45(d, 1H, J=10.8Hz), 3.58(d, 1H, J=10.8Hz), 4.23 (m, 1H), 4.68(m, 1H), 7.08 (s, 2H), 7.21-7.29(m, 5H), 8.29 (d, 1H, J=5.6Hz), 8.64(d, 2H, J=1H); ^{13}C NMR (100 MHz, DMSO-d_6): 174.4, 173.3, 171.1, 166.2, 137.9, 129.7, 128.6, 126.9, 54.5, 52.4, 40.6, 38.2, 30.7, 27.9.

LC/MS *m/z* 351.2 $[M + H]^+$; HPLC tr, 0.67 min.

Trialkoxysilyl peptide 1: $((EtO)_3Si(CH_2)_3NHCO$-Gly-Phe-Glu-$NH_2)$
^{1}H NMR (400 MHz, DMSO-d_6): 0.47(m, 2H), 1.12(t, 9H, J=6.8), 1.34-1.41(m, 2H), 1.71-1.80(m, 1H), 1.87-1.96(m, 1H), 2.75-2.81(m, 1H,) 2.88-2.93(m, 2H), 2.99-3.04(dd, 1H, J=4.4Hz, 14Hz), 3.49(d, 2H, J=5.6Hz), 3.42-3.46(m, 2H), 3.49(d, 2H, J=5.6Hz), 3.69-3.74(q, 6H, J=6.8Hz, 14Hz), 4.10-4.15 (m, 1H), 4.43-4.49(m, 1H), 6.09(t, 1H, J=5.6Hz), 6.23(t, 1H, J=5.6Hz), 7.04(s, 2H), 7.25-7.40(m, 5H), 8.03(d, 1H, J=8Hz), 8.09(d, 1H, J=8); ^{13}C NMR (100 MHz, DMSO-d_6): 174.5, 173.5, 171.4, 170.9, 158.6, 138.2, 129.6, 128.5, 126.7, 58.1, 54.5, 52.6, 43.5, 42.5, 37.7, 31.1, 27.5, 23.9, 18.7, 7.7. ^{29}Si NMR (75 MHz, DMSO-d_6): -45.1.
HRMS (ESI) *m/z* calcd for C26H44O9N5Si (M+) 598.2908, found 598.2916.

Trialkoxysilyl peptide 2: $((iPrO)_3Si(CH_2)_3NHCO(CH_2)_3CO$-Gly-Phe-Glu-$NH_2)$
^{1}H NMR (400 MHz, DMSO-d_6): 0.45 (m, 2H), 1.10(d, 18H, J=6Hz), 1.40(m, 2H), 1.65(m, 2H), 1.72-1.79(m, 1H), 1.87-1.95(m, 1H), 1.99-2.08(m, 4H), 2.76-2.81(m,1H), 2.94-3.04(m,4H), 3.50-3.57(m,2H), 3.62(m, 2H), 4.06-4.15(m, 3H), 4.47-4.51(m, 1H), 7.07(s, 2H), 7.16-7.21(m, 5H), 7.77(m, 1H), 8.09(m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): 172.2, 171.3, 170.6, 169.7, 168.9, 167.4, 135.8, 127.2, 126.1, 124.3, 64.8, 54.5, 52.6, 42.4, 41.8, 37.6, 35.2, 34.9, 31.2, 27.6, 25.8, 23.4, 21.9, 7.1. ^{29}Si NMR (75 MHz, DMSO-d_6): -48.5.
HRMS (ESI) *m/z* calcd for C33H56O10N5Si (M+) 710.3796, found 710.3781.

Peptide: H-Phe-Ala-Phe-NH_2
^{1}H NMR (400 MHz, DMSO-d_6): 1.21(d, 1H, J=4.8 Hz), 2.85-3.02(m, 2H), 4.04(m, 1H), 4.35 (m, 1H), 4.45 (m, 1H), 7.15-7.21 (m, 10H),8.05 (d, 2H, J=5.6 Hz), 8.1 (s, 2H), 8.6 (d, 2H, J=4.8 Hz) ; ^{13}C NMR (100 MHz, DMSO-d_6): 175.4, 173.8, 171.7, 166.2, 138,1, 129.2, 128.2, 126.3, 55.1, 52.9, 41.8, 38.8, 31.6, 18.5.
LC/MS *m/z* 383.1 $[M + H]^+$; HPLC tr, 0.89 min.

Trialkoxysilyl peptide 3: $((EtO)_3Si(CH_2)_3NHCO$-Phe-Ala-Phe-$NH_2)$
^{1}H NMR (400 MHz, DMSO-d_6): 0.48 (m, 2H), 1.15 (m, 12H), 1.36-1.39 (m, 2H), 3.04 (m, 2H), 3.32 (m, 4H), 3.71-3.75 (q, 6H, J=4.8Hz, 9.2Hz), 4.19 (m, 1H), 4.34-4.39 (m, 2H), 5.95 (m, 1H), 6.14 (m, 1H), 7.18-7.27 (m, 10H), 7.83 (d, 1H, J=4.8), 8.12 (d, 1H, J=5.6). NMR (100 MHz, DMSO-d_6): 206.3, 173.2, 172.8, 172.3, 158.1, 138.4, 129.6, 128.4, 126.6, 58.1, 54.8, 54.2, 49.1, 42.4, 38.6, 37.8, 23.9, 18.7, 18.3, 7.7. ^{29}Si NMR (75 MHz, DMSO-d_6): -45.3.
HRMS (ESI) *m/z* calcd for C31H48O7N5Si (M^+) 630.3323, found 630.3314.

Peptide: Boc-Pro-Pro-Asp(tBu)-Lys-NH_2:
^{1}H NMR (400 MHz, DMSO-d_6): 1.24-1.28 (m, 2H), 1.38 (s, 18H), 1.65-2.21 (m, 12H), 2.66-2.78 (m, 4H), 3.47-3.68 (m, 4H), 4.28-4.48 (m, 4H) , 7.09-7.12 (m, 2H),7.66 (m, 1H) 8.10 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): 173.5, 172.0, 170.4, 169.9, 158.6, 80.9, 79.1, 60.4, 58.2, 53,1, 50.6, 47.1, 39.5, 37.6, 31.8, 28.7, 28.3, 27.1, 24.9, 22.5.
LC/MS *m/z* 611.2 $[M + H]^+$; HPLC tr, 1.11 min.

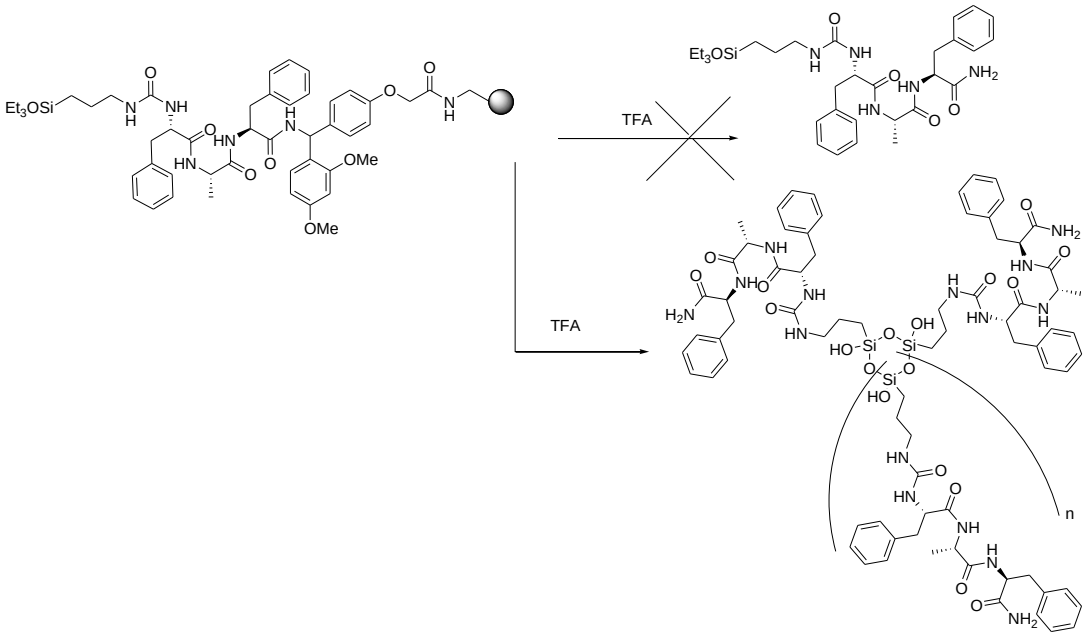
Trialkoxysilyl peptide 4: (Boc-Pro-Pro-Asp(OtBu)-Lys($(EtO)_3Si(CH_2)_3NHCO$)-NH_2)
^{1}H NMR (400 MHz, DMSO-d_6):0.47 (m, 2H), 1.14 (t, 9H, J=7.2 Hz), 1.21 (m,2H), 1.37(s, 18H), 1.59-2.21 (m, 14H), 2.85-3.01 (m,6H), 3.41-3.59 (m, 4H), 3.70-3.76 (q, 6H, J=7.2, J=14), 4.26-4.50 (m, 4H), 5.69-5.80 (m, 2H), 7.05-7.10 (m, 2H) 7.60 (m, 1H), 8.11 (m, 1H).
^{13}C NMR (100 MHz, DMSO-d_6): 173.7, 171.9, 171.8, 170.3, 169.9, 158.4, 153.4, 80.7, 78.7, 59.9, 58.1, 57.8, 52.9, 49.9, 47.1, 46.9, 42.5, 42.3, 37.2, 32.2, 29.3, 29.1, 27.1, 24.9, 24.1, 23.6, 23.1, 18.8, 7.7. ^{29}Si NMR (75 MHz, DMSO-d_6): -45.1.
HRMS (ESI) *m/z* calcd for C39H72O12N7Si (M+) 858.5008, found 858.5001.

Peptide: H-Pro-Pro-Asp-Lys-NH_2:
^{1}H NMR (400 MHz, DMSO-d_6): 1.28 (m, 2H), 1.59 (m,2H), 1.66-2.32 (m, 10H), 2.69-2.75 (m, 2H), 3.25-3.61 (m, 4H), 4.13 (m, 2H), 4.37-4.62 (m, 4H), 7.11-7.21 (m, 2H), 7.72 (m, 1H), 8.13 (m, 1H), 9.62 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): 174.5, 173.3, 172.3, 171.4, 167.9, 60.1, 59.5, 53.3, 50.9, 48.0, 47.1, 37.1, 32.4, 30.3, 29.1, 27.8, 25.6, 24.8, 23.3.
LC/MS *m/z* 456.1 $[M + H]^+$; HPLC tr, 0.91 min.

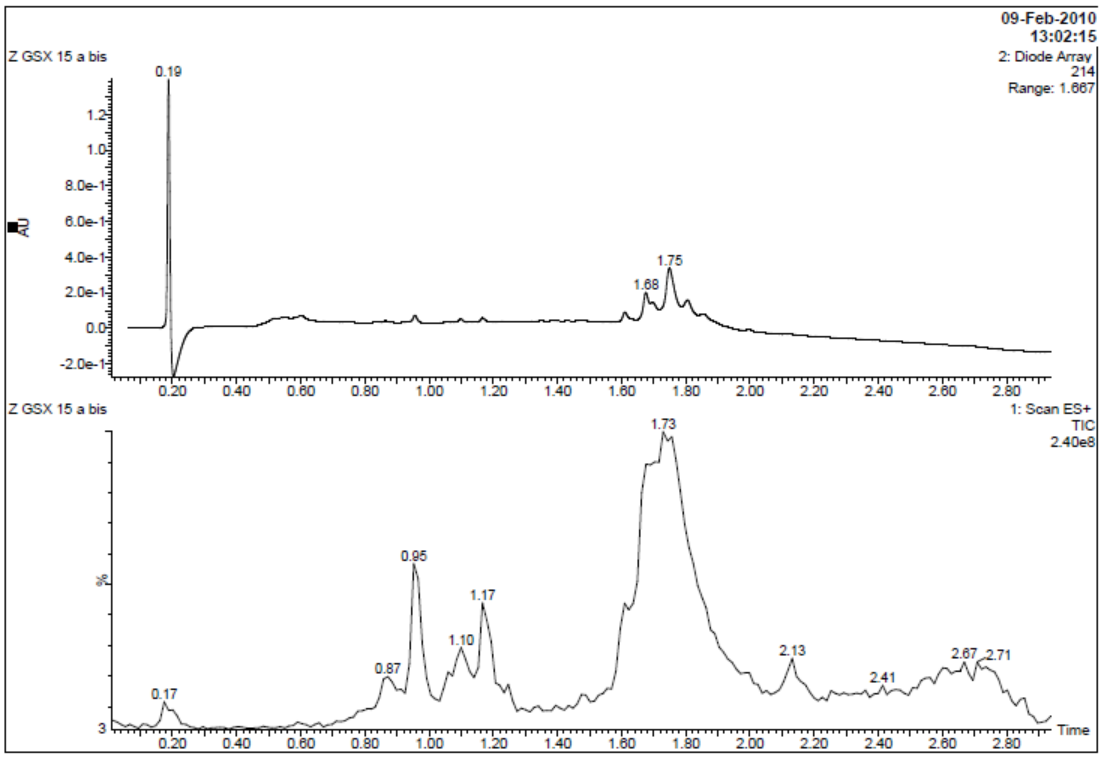
4-hydroxy-4-(4-nitrophenyl)butan-2-one (table 2)
^{1}H NMR (400 MHz, DMSO-d_6): 2.14 (s, 3H), 2.76 (d, 2H, J=6.8Hz), 3.35 (s,1H), 5.18-5.13 (m, 1H), 7.52 (d, 2H, J=8.4), 8.19 (d, 2H, J=8.8Hz). ^{13}C NMR (100 MHz, DMSO-d_6): 206.7, 153.6, 146.9, 127.5, 123.8, 68.3, 62.7, 30.8. LC/MS *m/z* 208 $[M - H]^-$; HPLC tr, 1.00 min.

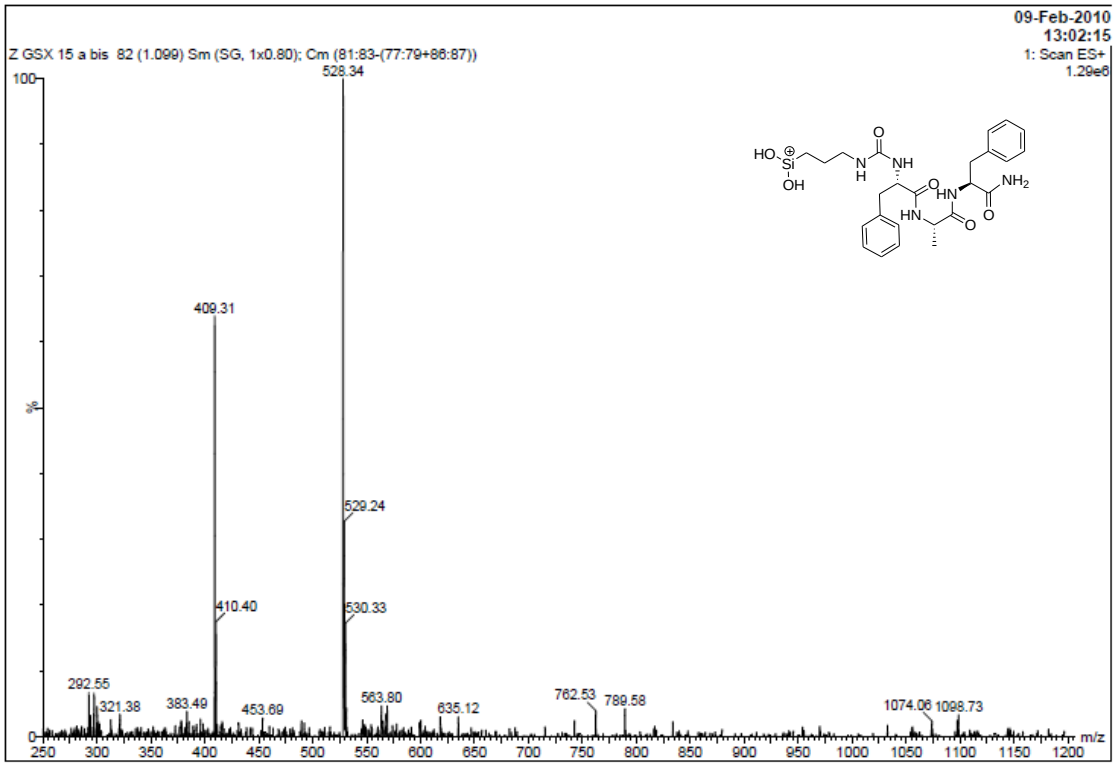
6. Preliminary experiments: direct cleavage of trialkoxysilyl peptides from solid support

Cleavage of resin-supported trialkoxysylil derivatized peptide with TFA

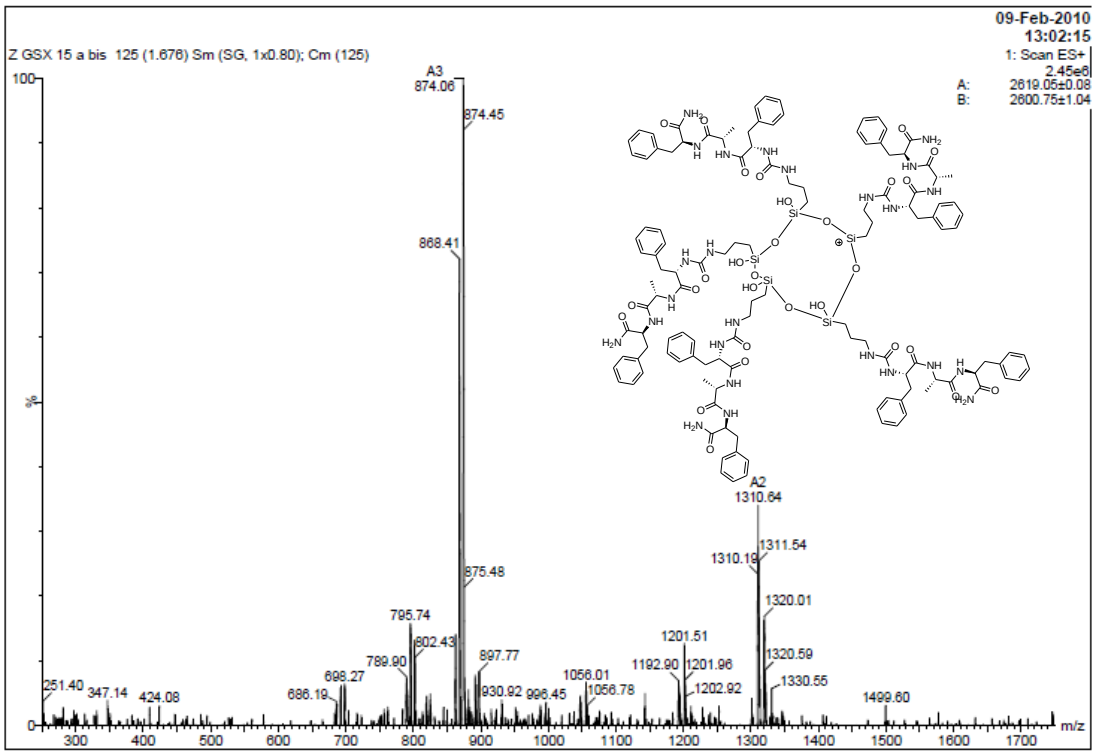


LC/MS trace (up UV 214 nm, down TIC) of crude cleavage mixture after precipitation in diethylether.

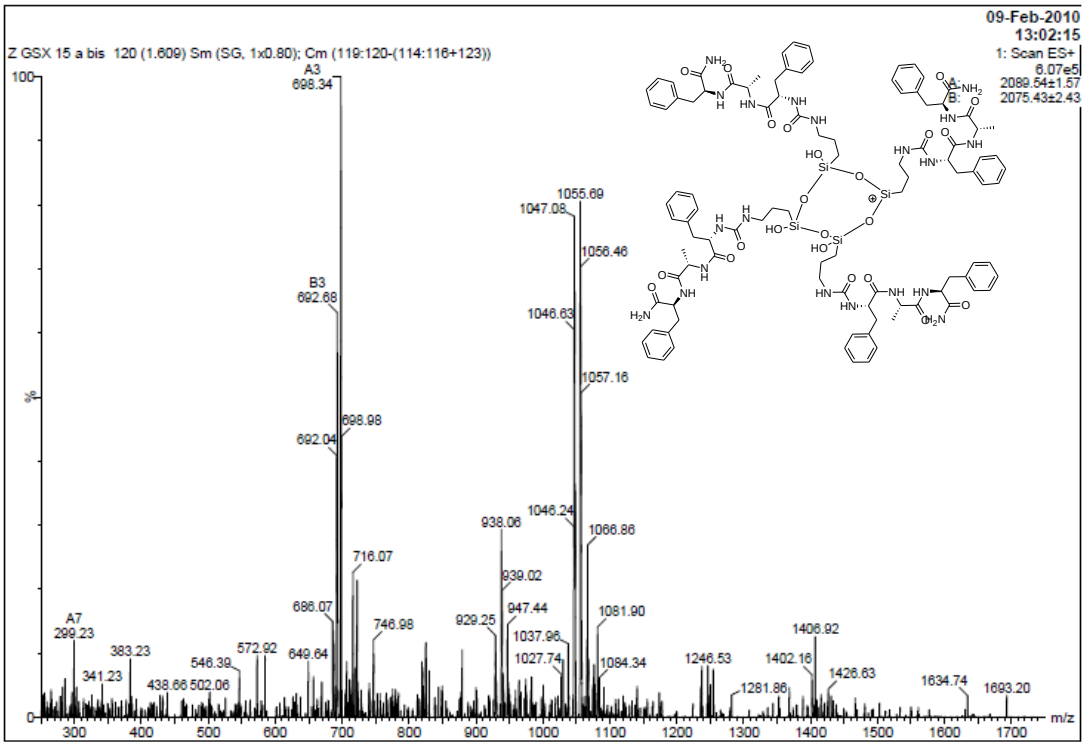




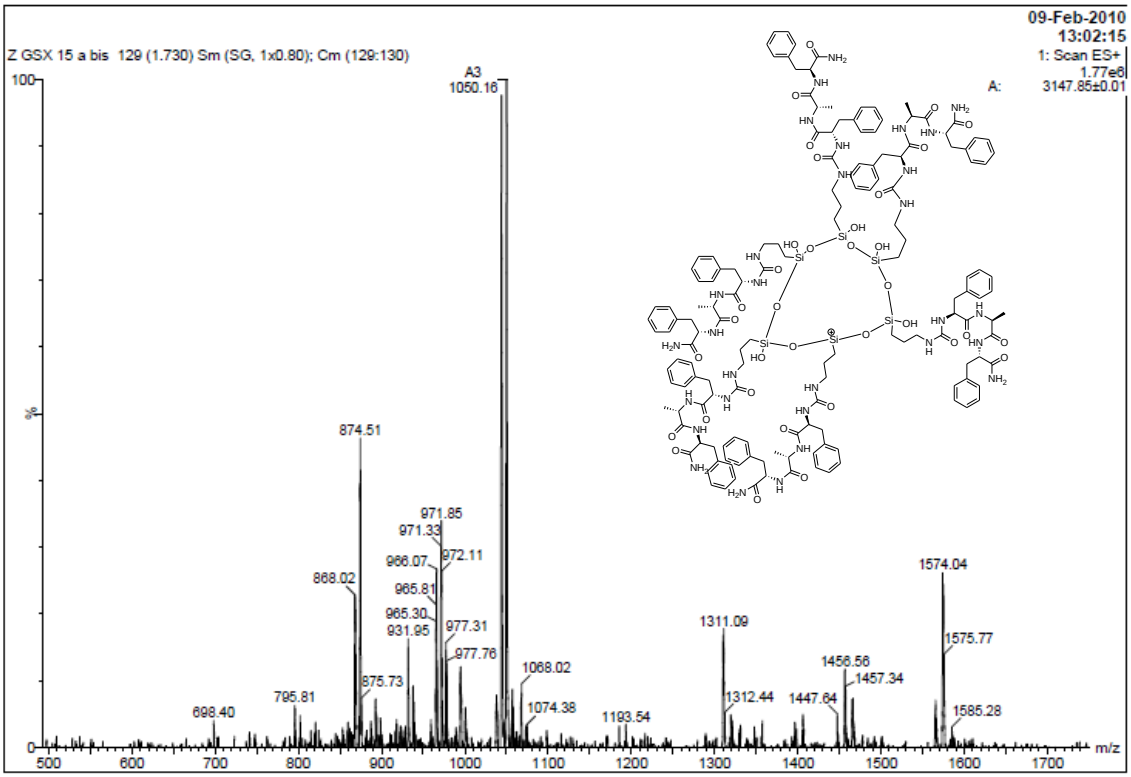
Cyclic pentamer derivative of hybrid peptide **3**



Cyclic tetramer derivative of hybrid peptide **3**

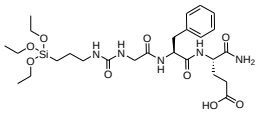


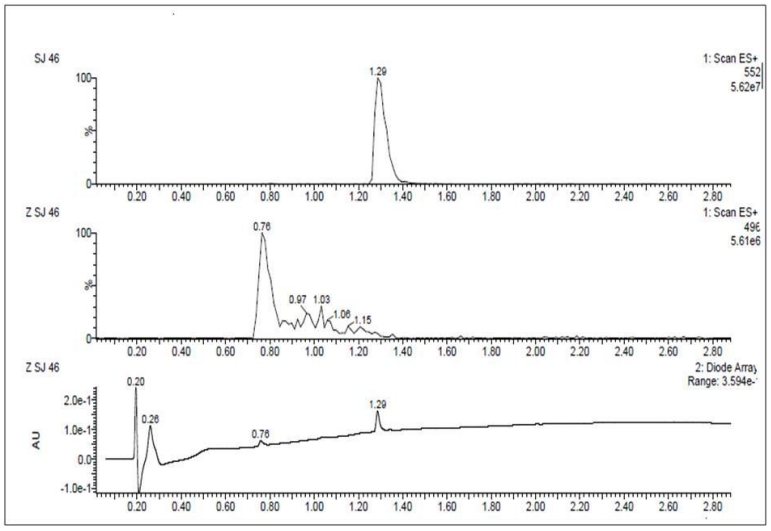
Cyclic hexamer of hybrid peptide **3**

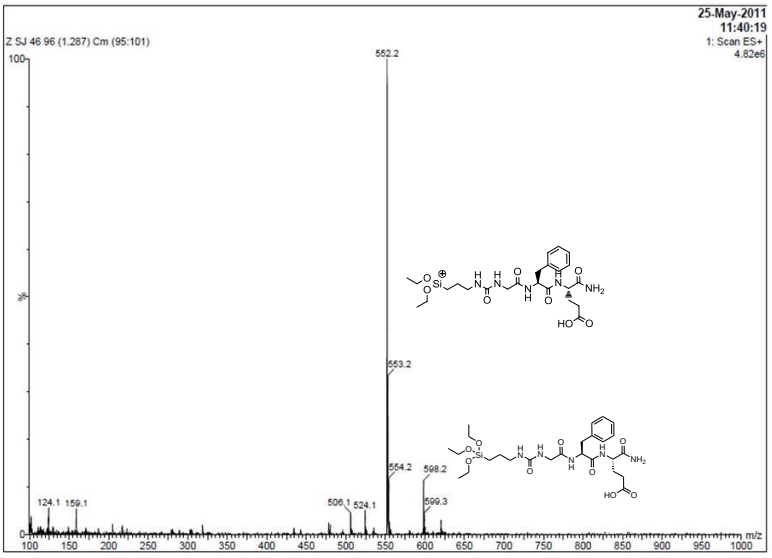


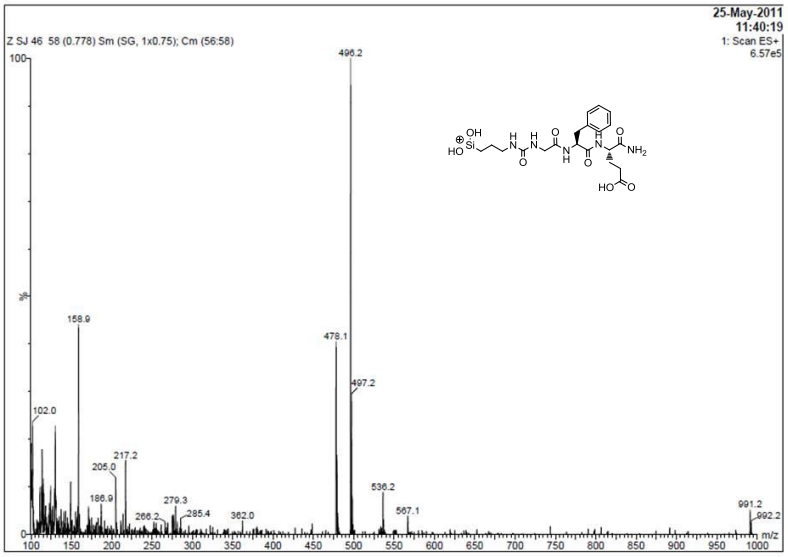
7.LC and MS data of hybrid peptides

compound **1** $(EtO)_3Si(CH_2)_3NHCO$-Gly-Phe-Glu-NH_2

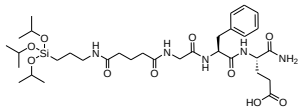


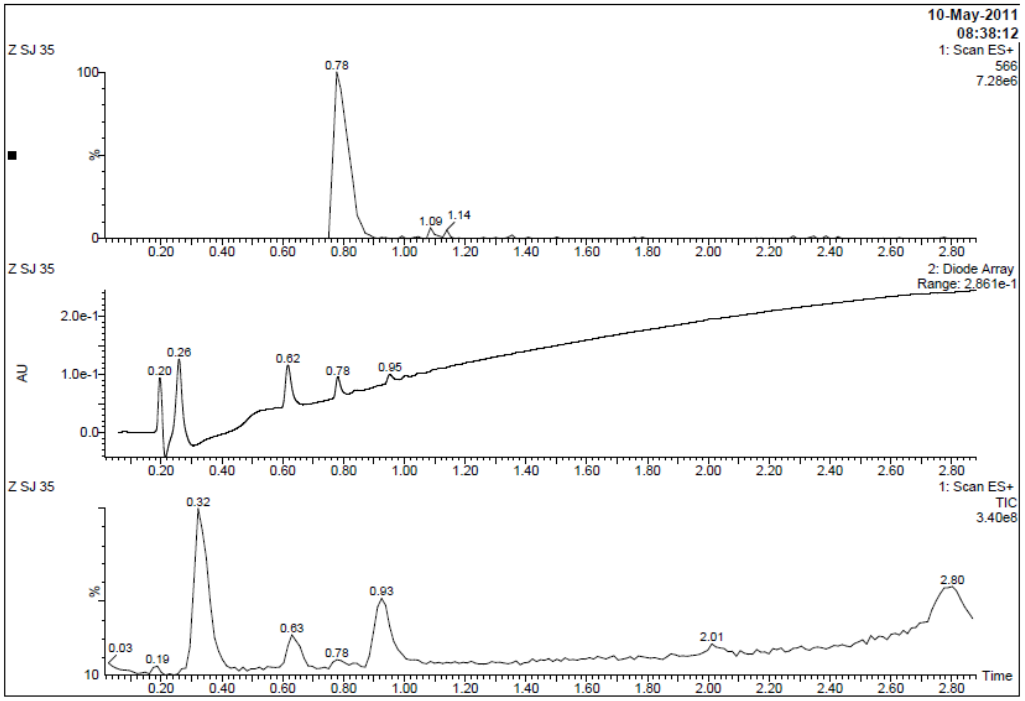


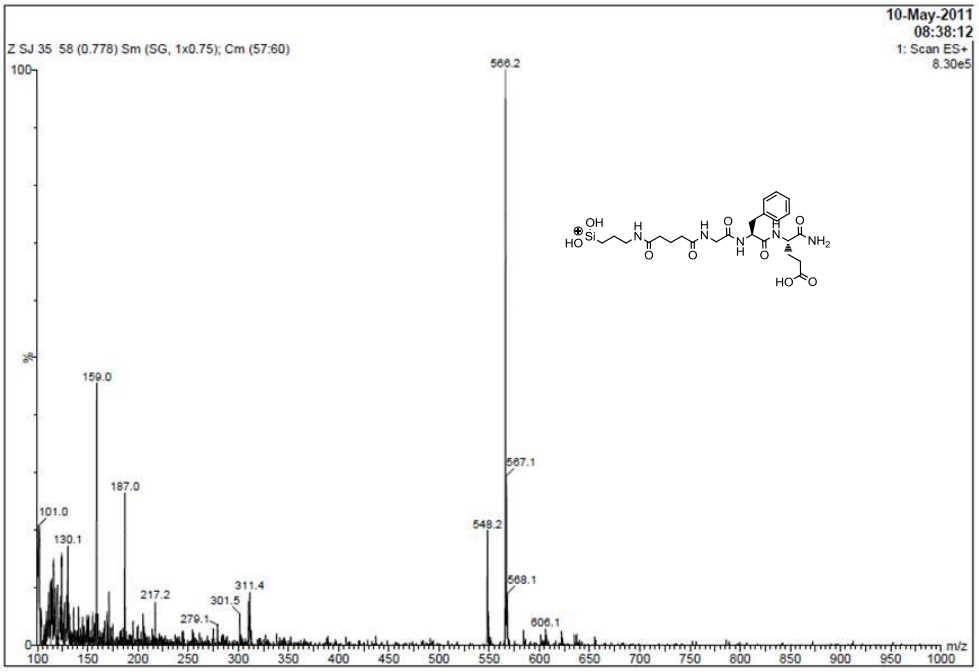




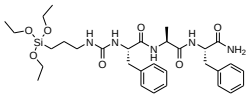
compound **2** $(iPrO)_3Si(CH_2)_3NHCO(CH_2)_3CO$-Gly-Phe-Glu-$NH_2$

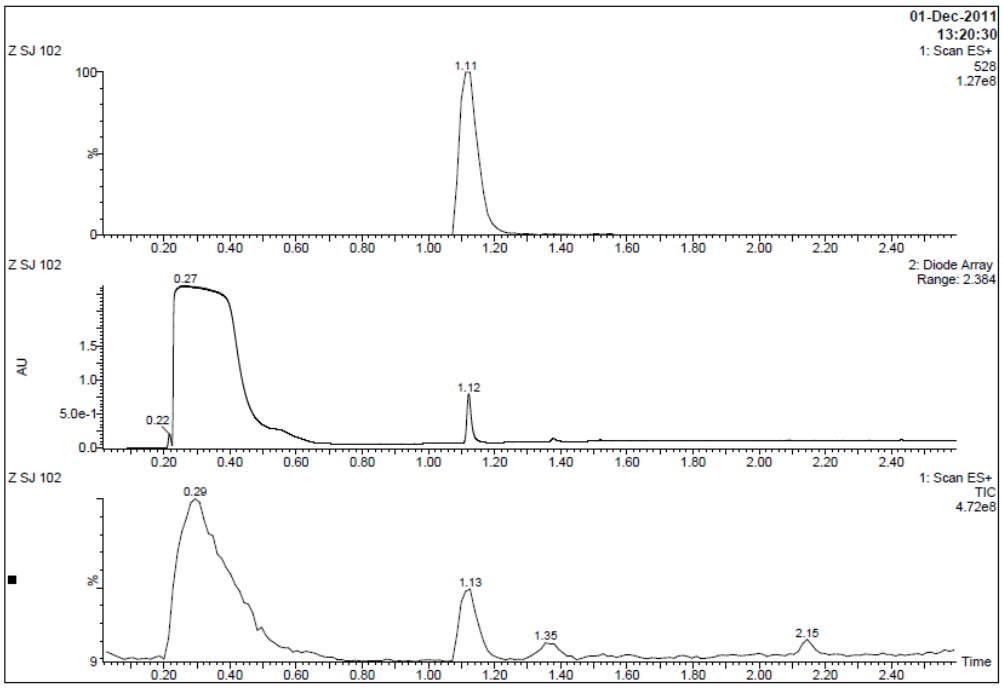


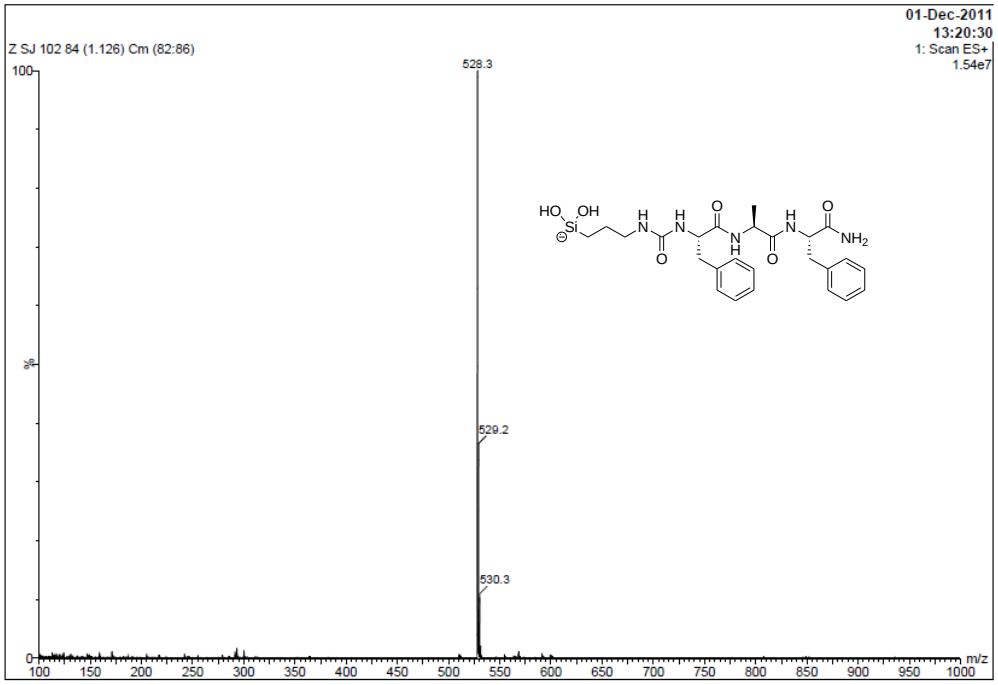




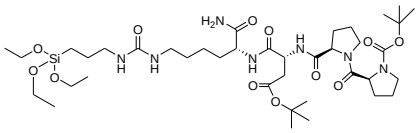
compound **3** $(EtO)_3Si(CH_2)_3NHCO$-Phe-Ala-Phe-NH_2

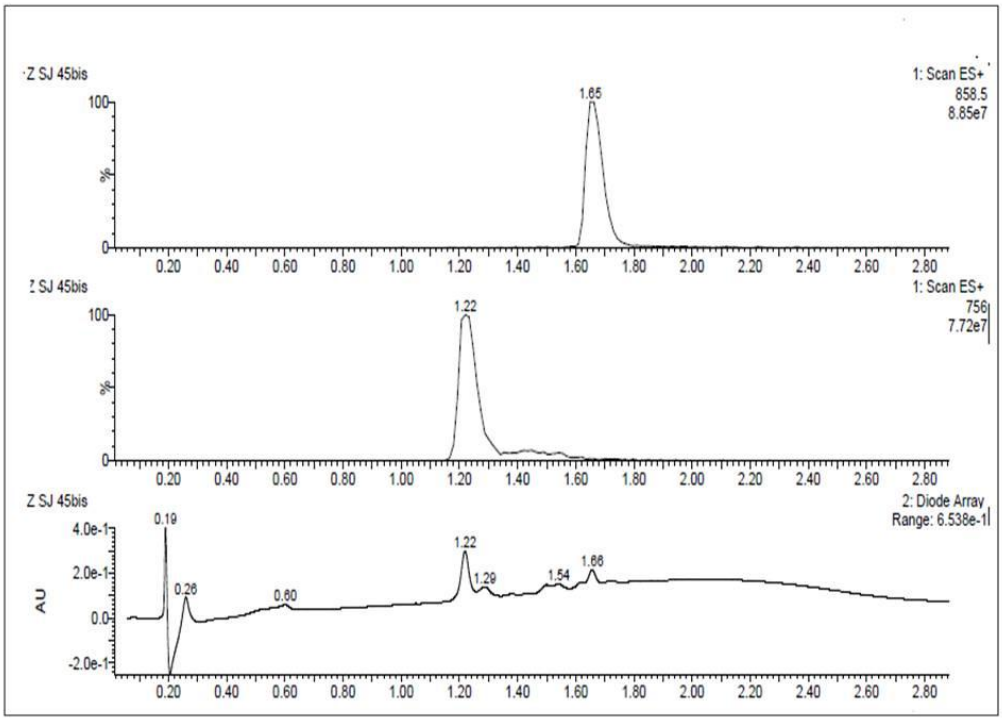


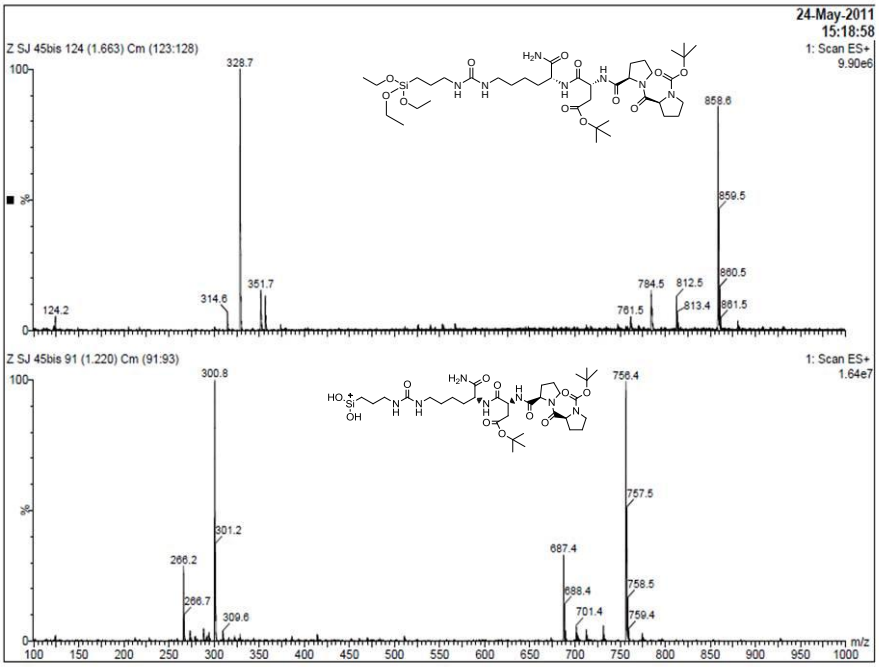




compound **5** Boc-Pro-Pro-Asp(OtBu)-Lys((EtO)$_3$Si(CH$_2$)$_3$NHCO)-NH$_2$



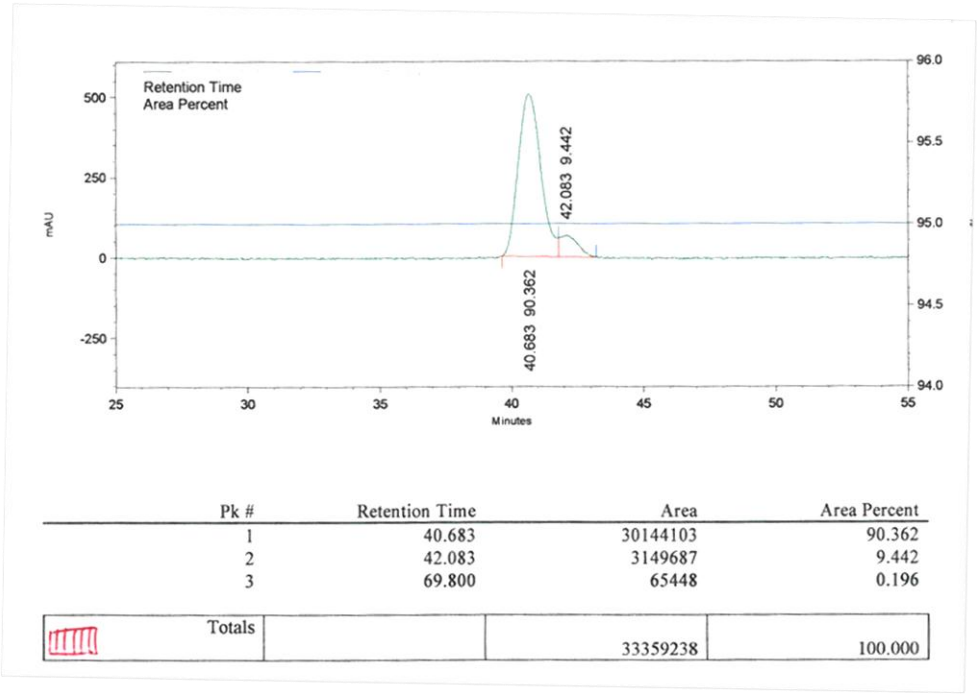




8.Chiral HPLC chromatograms

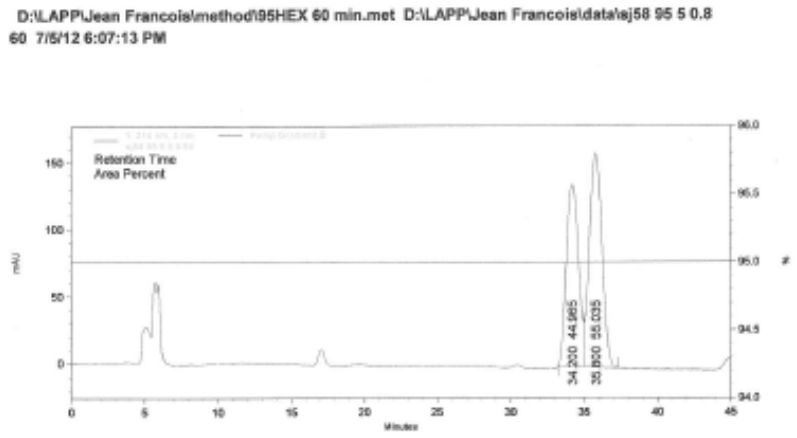
Eniantomeric excess for 4-hydroxy-4-(4-nitrophenyl)butan-2-one (table 2) determined by chiral-phase HPLC analysis:

HPLC: chiral pack (isopropanol/hexane 5/95 30°C) at 1ml/min for soluble catalyst H-PPDK-NH_2



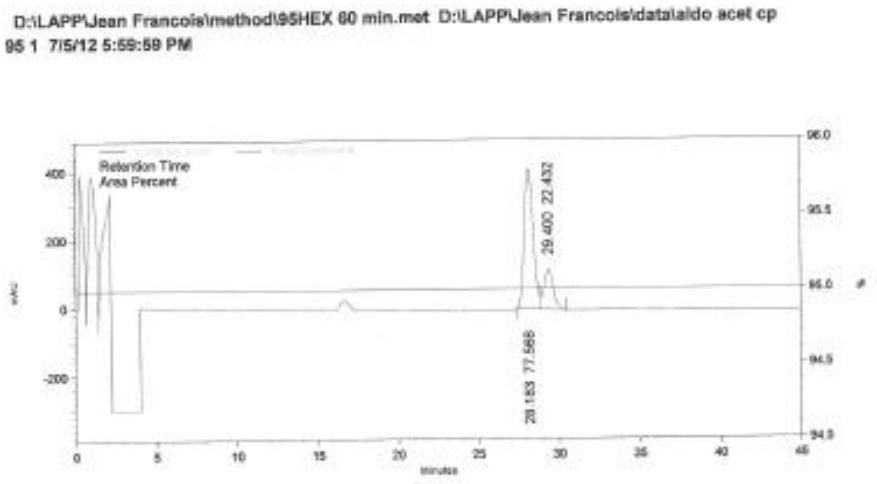
Pk #	Retention Time	Area	Area Percent
1	40.683	30144103	90.362
2	42.083	3149687	9.442
3	69.800	65448	0.196
Totals		33359238	100.000

HPLC: chiral pack (isopropanol/hexane 5/95 30°C) at 1ml/min for material **9** in acetone:



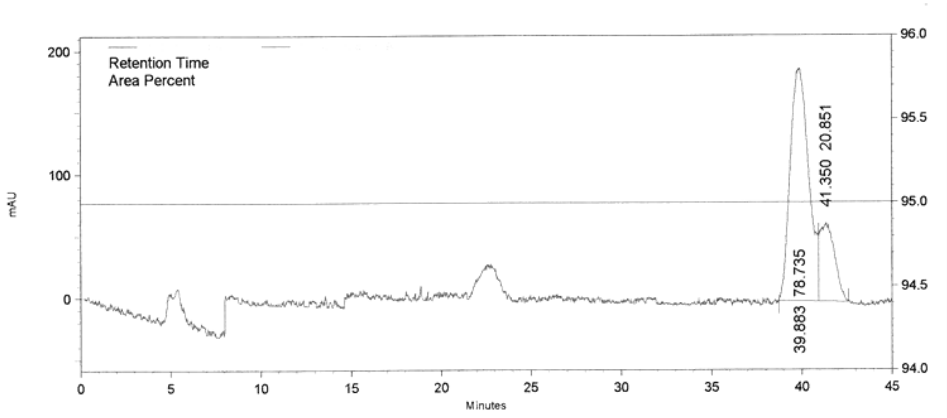
Pk #	Retention Time	Area	Area Percent
1	34.200	7846059	44.965
2	35.800	9603053	55.035
Totals		17449112	100.000

HPLC: chiral pack (isopropanol/hexane 5/95 30°C) at 1ml/min for material **10a** in acetone:



Pk #	Retention Time	Area	Area Percent
1	28.183	15960755	77.568
2	29.400	4615842	22.432
Totals		20576597	100.000

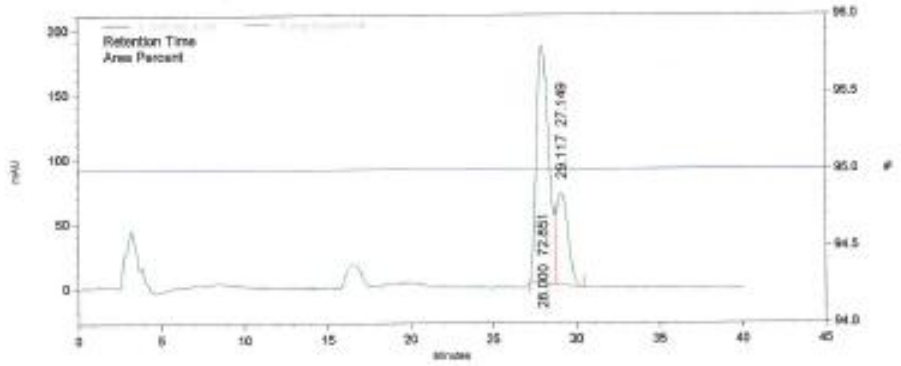
HPLC: chiral pack (isopropanol/hexane 5/95 30°C) at 1ml/min for material **10b** in acetone:



Pk #	Retention Time	Area	Area Percent
1	39.883	13466924	78.735
2	41.350	3566374	20.851
3	66.850	70731	0.414
Totals		17104029	100.000

HPLC: chiral pack (isopropanol/hexane 5/95 30°C) at 1ml/min for material **10a** in $CHCl_3$:

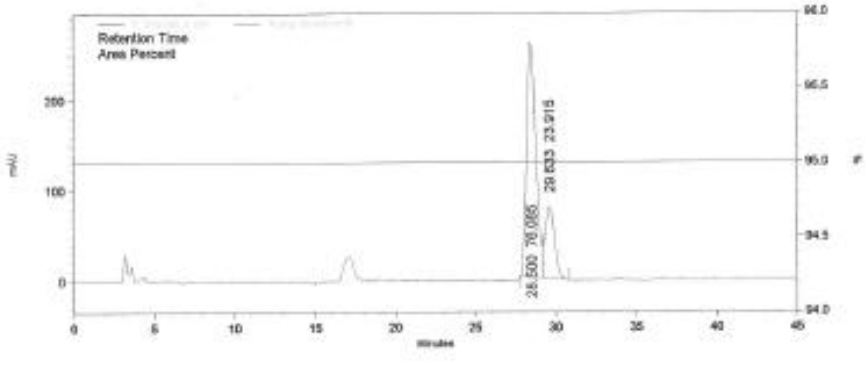
D:\LAPP\Jean Francois\method\95HEX 60 min.met D:\LAPP\Jean Francois\data\aldo chcl3 ap 96 1 7/5/12 6:02:10 PM



Pk #	Retention Time	Area	Area Percent
1	28.000	9360681	72.851
2	29.117	3488342	27.149
Totals		12849023	100.000

HPLC: chiral pack (isopropanol/hexane 5/95 30°C) at 1ml/min for material **10a** in DMF:

D:\LAPP\Jean Francois\method\95HEX 60 min.met D:\LAPP\Jean Francois\data\aldo dmf cp 96 1c 7/5/12 6:03:18 PM

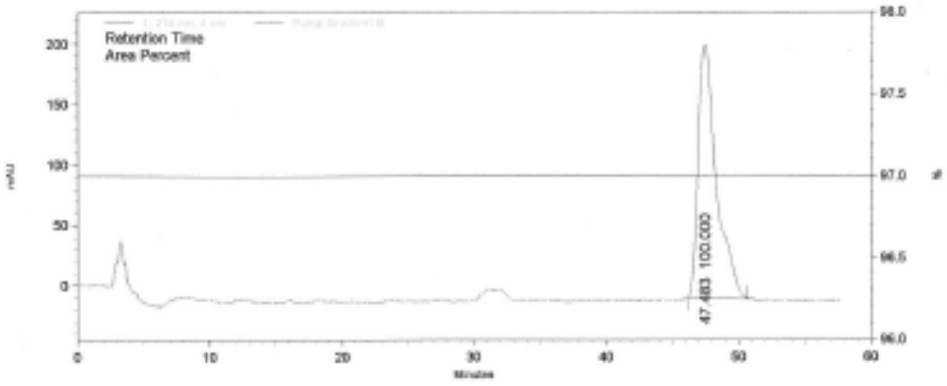


Pk #	Retention Time	Area	Area Percent
1	28.500	10384295	76.085
2	29.633	3263911	23.915
Totals		13648206	100.000

HPLC: chiral pack (isopropanol/hexane 3/97 30°C) at 1ml/min for material **10a** in DMSO:

D:\LAPP\Jean Francois\method\97HEX 100 1.met D:\LAPP\Jean Francois\data\aldo dmso cp 97 1b

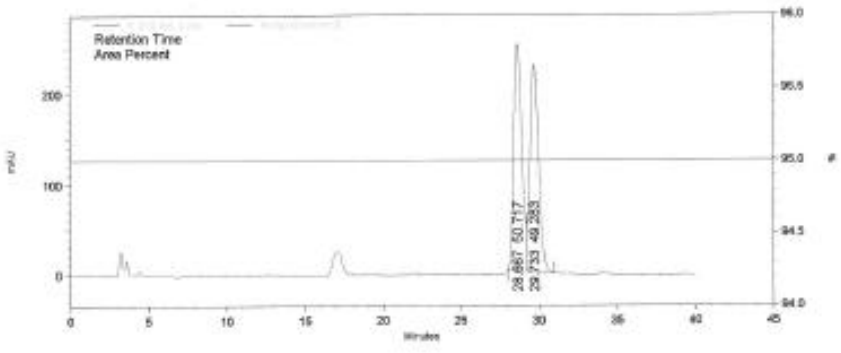
7/5/12 6:24:13 PM



Pk #	Retention Time	Area	Area Percent
1	47.483	19952295	100.000
Totals		19952295	100.000

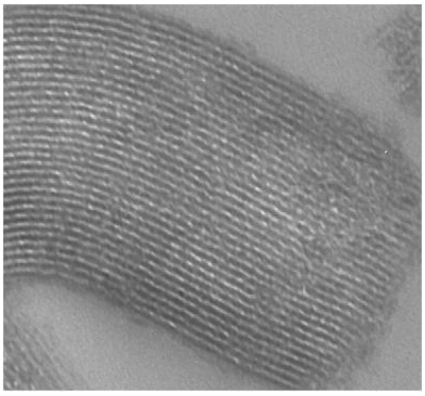
HPLC: chiral pack (isopropanol/hexane 5/95 30°C) at 1ml/min for material **10a** in water:

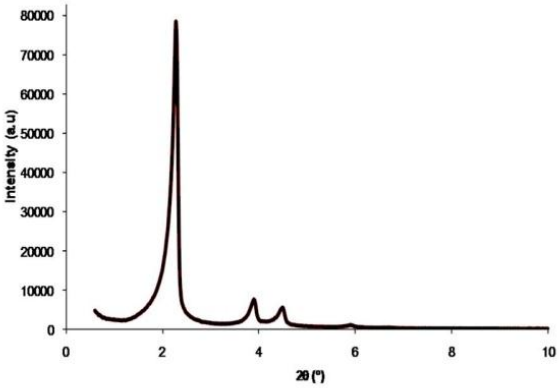
D:\LAPP\Jean Francois\method\95HEX 60 min.met D:\LAPP\Jean Francois\data\aldo H2O cp 95 1 7/5/12 6:04:26 PM



Pk #	Retention Time	Area	Area Percent
1	28.667	9064585	50.717
2	29.733	8808116	49.283
Totals		17872701	100.000

9.TEM and XRD pattern of large pore SBA-15 before peptide grafting





10 Pore distribution curves –Figure 1C and 1D

The pore distributions were determinated from adsorption branch.