Supporting Information

Delivering aminopyridine ligands into cancer cells through conjugation to the cell-penetrating peptide BP16

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Materials and Methods

Unless otherwise stated, common chemicals and solvents (HPLC-grade or reagent-grade quality) were purchased from commercial sources and used without further purification. The 9-fluorenylmethoxycarbonyl (Fmoc) derivatives and Fmoc-Rink-4-methylbenzhydrylamine (MBHA) resin (0.56 mmol/g) were obtained from Senn Chemicals International (Gentilly, France), NovaBiochem (Schwalbach, Germany) or IRIS Biotech GmbH (Marktredwitz, Germany). Ethyl 2-cyano-2-(hydroxyimino)acetate (Oxyma) and 1-[(1-(cyano-2-ethoxy-2-oxoethylideneaminooxy) dimethylaminomorpholino)] uronium hexafluorophosphate (COMU) were purchased from Novabiochem (Nottingham, UK). Trifluoroacetic acid (TFA), triisopropylsilane (TIS), dimethyl sulfoxide (DMSO), *N,N'*-diisopropylcarbodiimide (DIPCDI), *N,N'*-diisoproylethylamine (DIPEA), 5(6)-carboxyfluorescein (CF), LiOH and hydrazine monohydrate were from Sigma–Aldrich Corporation (Madrid, Spain). Cathepsin B (bovine spleen) was purchased from EMD chemicals (San Diego, CA, USA). L-(+) Cysteine was obtained from Fischer Scientific (Waltham, MA USA). Piperidine, tetrabutylammonium fluoride (TBAF), 4-toluenesulfonyl chloride (TsCl), lithium chloride (LiCl) and pyridine were purchased from Fluka (Buchs, Switzerland). Acetic anhydride (Ac_2O) was purchased from Panreac (Barcelona, Spain). Anhydrous $MgSO_4$, NaI, NaOH and acid acetic (AcOH) were purchased from Panreac (Barcelona, Spain). *N*-Methyl-2-pyrrolidinone (NMP), *N,N*-dimethylformamide (DMF), CH_3OH, CH_2Cl_2, CH_3CN, hexane, diethyl ether (Et_2O) and solvents for high performance liquid chromatography (HPLC) were obtained from Scharlau (Sentmenat, Spain). Ethyl acetate (AcOEt) and tetrahydrofuran (THF) were obtained from Carlo Erba (Milan, Italy). StratoSpheres^{TM} PL-HCO_3 MP resins were purchased from Varian (Palo Alto, CA, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and paraformaldehyde were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), phosphate buffered saline (PBS), fetal bovine serum (FBS), penicillin-streptomycin and trypsin were obtained from GIBCO BRL (Grand Island, NY, USA).

Analytical thin layer chromatography (TLC) was performed on precoated TLC plates, silica gel 60 F_{254} (Merck). The spots on the TLC plates were visualized with UV/Vis light (254 nm) and/or stained with a solution of $KMnO_4$ (1.5 g/100 mL H_2O).

Compound purities were determined under standard analytical HPLC conditions with a Dionex liquid chromatography instrument composed of an UV/Vis Dionex UVD170U detector, a P680 Dionex bomb, an ASI-100 Dionex automatic injector, and

CHROMELEON 6.60 software. Detection was performed at 220 nm. Solvent A was 0.1% aq. TFA and solvent B was 0.1% TFA in CH_3CN. Analysis was carried out with a Kromasil 100 C_{18} (4.6 mm × 40 mm, 3.5 μm) column with a 2-100% B linear gradient over 7 min at a flow rate of 1.0 mL/min. Target compounds possess ≥95% purity. All percentages are specified at the end of each synthesis in this section.

Electrospray ionization mass spectrometry (ESI-MS) analyses were performed with an Esquire 6000 ESI ion Trap LC/MS (Bruker Daltonics) instrument equipped with an electrospray ion source (University of Girona). The instrument was operated in the positive ESI(+) ion mode. Samples (5 μL) were introduced into the mass spectrometer ion source directly through an HPLC autosampler. The mobile phase (80:20 CH_3CN/H_2O at a flow rate of 100 μL/min) was delivered by a 1200 Series HPLC pump (Agilent). Nitrogen was employed as both the drying and nebulizing gas.

High-resolution mass spectra (HRMS) were recorded under conditions of ESI with a Bruker MicrOTOF-Q IITM instrument using a hybrid quadrupole time-of-flight mass spectrometer (University of Girona). Samples were introduced into the mass spectrometer ion source by direct infusion through a syringe pump and were externally calibrated using sodium formate. The instrument was operated in the positive ESI(+) ion mode.

HPLC-MS analyses were performed under standard analytical HPLC conditions described above with an Agilent Technologies 1200 HPLC system equipped with a VWD detector and coupled with an Esquire 6000 ESI ion Trap LC/MS (Bruker Daltonics) instrument with an electrospray ion source (University of Girona). The instrument was operated in the positive ESI(+) ion mode.

Microwave-assisted reactions were performed with a single mode Discover S-Class labstation microwave (CEM) (0-300 W). The time, temperature, and power were controlled with the Synergy software. The temperature was monitored through an infrared sensor in the floor of the cavity.

Cell lines

Human breast cancer MCF-7 and pancreas cancer CAPAN-1 cell lines were obtained from the American Tissue Culture Collection (ATCC, Rockville, MD, USA). Human skin fibroblast cells 1BR3G were obtained from EucellBank (University of Barcelona, Barcelona, Spain). Cells were maintained in DMEM supplemented with 10% FBS and 100 UmL^{-1} penicillin-streptomycin at 37 ºC under a humidified atmosphere containing 5% CO_2. Cells were passaged two times per week.

Synthesis of the metal binding peptides BP343, BP344, BP345, BP346, BP349 and BP350

Synthesis of (*S,S'*)-BPBP-Lys-Lys-Leu-Phe-Lys-Lys-Ile-Leu-Lys-Lys-Leu-NH_2 (BPBP-BP16) (BP343). This metal binding peptide was synthesized from the peptidyl resin H-Lys(Boc)-Lys(Boc)-Leu-Phe-Lys(Boc)-Lys(Boc)-Ile-Leu-Lys(Boc)-Lys(Boc)-Leu-Rink-MBHA. This peptidyl resin (50 mg) was subsequently subjected to coupling of 6-[(*tert*-butyldimethylsilyloxy)methyl]nicotinic acid (**1**),[1] TBS removal and chlorination as described for the synthesis of **BP341**. To perform the *N*-alkylation, the peptidyl resin was placed in a 15 mL quartz vial containing a magnetic stir bar and treated with a solution of (2*S*,2*S'*)-1-(pyrid-2-ylmethyl)-2,2'-bipyrrolidine (**3**)[1] (2 equiv), NaI (0.04 equiv) and DIPEA (24 equiv) in NMP. The sealed vial was heated at 125 °C under MW irradiation for 1 h. After the reaction time, upon cooling, the resulting resin was placed in a syringe and the solvent was removed. The resin was washed with NMP (6 × 1 min), CH_3OH (6 × 1 min) and CH_2Cl_2 (1 × 1 min). Acidolytic cleavage, removal of trifluoroacetate counterions and characterization were carried out following the procedure described for **BP341**, leading to the metal binding peptide **BP343** in 94% purity. t_R = 6.68 min. MS (ESI): *m/z* = 1735.1 $[M + H]^+$, 1757.1 $[M + Na]^+$. HRMS (ESI): *m/z* calcd. for $C_{90}H_{157}N_{22}O_{12}$ $[M + 5H]^{5+}$ 347.6465; found 347.6462; calcd. for $C_{90}H_{156}N_{22}O_{12}$ $[M + 4H]^{4+}$ 434.3063; found 434.3061; calcd. for $C_{90}H_{155}N_{22}O_{12}$ $[M + 3H]^{3+}$ 578.7393; found 578.7387.

Synthesis of Ac-Lys-Lys-Leu-Phe-Lys-Lys-Ile-Leu-Lys-Lys-Leu-Lys((*S,S'*)-BPBP)-NH_2 (BP16-BPBP) (BP344). This metal binding peptide was synthesized from the peptidyl resin H-Lys(Boc)-Lys(Boc)-Leu-Phe-Lys(Boc)-Lys(Boc)-Ile-Leu-Lys(Boc)-Lys(Boc)-Leu-Lys(Dde)-Rink-MBHA. This peptidyl resin (50 mg) was acetylated with acetic anhydride/pyridine/CH_2Cl_2 (1:1:1 v/v, 2 × 30 min) under stirring. The resin was then washed with NMP (6 × 1 min) and CH_2Cl_2 (6 × 1 min), and the Kaiser test was used to test the completion of the reaction.[2] The resulting peptidyl resin was treated with hydrazine/NMP (2:98, 5 × 20 min). After these treatments the resin was washed with NMP (6 × 1 min) and CH_2Cl_2 (1 × 1 min). Conjugation of the (S,S')-BPBP ligand at the free amino group was performed as described for metal binding peptide **BP343**. Acidolytic cleavage, removal of trifluoroacetate counterions and characterization were carried out following the procedure described for **BP341**, leading to the metal

binding peptide **BP344** in 90% purity. t_R = 7.39 min. HRMS (ESI): *m/z* calcd. for $C_{98}H_{172}N_{24}O_{14}$ $[M + 6H]^{6+}$ 318.2242; found 318.2241; calcd. for $C_{98}H_{171}N_{24}O_{14}$ $[M + 5H]^{5+}$ 381.6676; found 381.6677; calcd. for $C_{98}H_{170}N_{24}O_{14}$ $[M + 4H]^{4+}$ 476.8327; found 476.8327; calcd. for $C_{98}H_{169}N_{24}O_{14}$ $[M + 3H]^{3+}$ 635.4411; found 635.4416.

Synthesis of ^{Me2}PyTACN-β-Ala-Lys-Lys-Lys-Leu-Phe-Lys-Lys-Ile-Leu-Lys-Lys-Leu-NH_2 (PyTACN-βAK-BP16) (BP345). This metal binding peptide was synthesized from the peptidyl resin H-β-Ala-Lys(Boc)-Lys(Boc)-Lys(Boc)-Leu-Phe-Lys(Boc)-Lys(Boc)-Ile-Leu-Lys(Boc)-Lys(Boc)-Leu-Rink-MBHA. Conjugation of the ^{Me2}PyTACN ligand at the N-terminus amino group, acidolytic cleavage, removal of trifluoroacetate counterions and characterization were carried out following the procedure described for **BP341**. The metal binding peptide **BP345** was obtained in >99% purity. t_R = 6.03 min. MS (ESI): *m/z* = 930.6 $[M + 2H]^{2+}$. HRMS (ESI): *m/z* calcd. for $C_{93}H_{171}N_{25}O_{14}$ $[M + 4H]^{4+}$ 465.5854; found 465.5884; calcd. for $[M + 3H]^{3+}$ $C_{93}H_{170}N_{25}O_{14}$ 620.4448; found 620.4469; calcd. for $C_{93}H_{169}N_{25}O_{14}$ $[M + 2H]^{2+}$ 930.1635; found 930.1637.

Synthesis of (*S,S'*)-BPBP-β-Ala-Lys-Lys-Lys-Leu-Phe-Lys-Lys-Ile-Leu-Lys-Lys-Leu-NH_2 (BPBP-βAK-BP16) (BP346). This metal binding peptide was synthesized from the peptidyl resin H-β-Ala-Lys(Boc)-Lys(Boc)-Lys(Boc)-Leu-Phe-Lys(Boc)-Lys(Boc)-Ile-Leu-Lys(Boc)-Lys(Boc)-Leu-Rink-MBHA. Conjugation of the (*S,S'*)-BPBP ligand at the N-terminus amino group was performed as described for metal binding peptide **BP343**. Acidolytic cleavage, removal of trifluoroacetate counterions and characterization were carried out following the procedure described for **BP341**, leading to the metal binding peptide **BP346** in >99% purity. t_R = 6.02 min. MS (ESI): *m/z* = 645.4 $[M + 3H]^{3+}$, 967.6 $[M + 2H]^{2+}$,1934.2 $[M + H]^{+}$. HRMS (ESI): *m/z* calcd. for $C_{99}H_{173}N_{25}O_{14}$ $[M + 4H]^{4+}$ 484.0893; found 484.0904; calcd. for $C_{99}H_{172}N_{25}O_{14}$ $[M + 3H]^{3+}$ 645.1166; found 645.1181; calcd. for $C_{99}H_{171}N_{25}O_{14}$ $[M + 2H]^{2+}$ 967.1713; found 967.1708.

Synthesis of (*S,S'*)-BPBP-β-Ala-Lys(CF)-Lys-Lys-Leu-Phe-Lys-Lys-Ile-Leu-Lys-Lys-Leu-NH_2 (BPBP-βAK(CF)-BP16) (BP349). This metal binding peptide was synthesized from the peptidyl resin H-β-Ala-Lys(Dde)-Lys(Boc)-Lys(Boc)-Leu-Phe-Lys(Boc)-Lys(Boc)-Ile-Leu-Lys(Boc)-Lys(Boc)-Leu-Rink-MBHA (50 mg). The nicotinic acid derivative **4** was coupled to this peptidyl resin as described for **BP347**. The resulting resin was treated with hydrazine/NMP

(2:98, 5 ×20 min). After these treatments the resin was washed with NMP (6 × 1 min) and CH_2Cl_2 (1 × 1 min). For the derivatization with 5(6)-carboxyfluorescein (CF), this fluorophore (2.5 equiv) was first pre-activated with Oxyma (2.5 equiv) and DIPCDI (2.5 equiv) in CH_2Cl_2/NMP (1:9) for 10 min. The mixture was added to the peptidyl resin and reacted overnight at room temperature protected from light by covering it with aluminium foil due to the light sensitivity of 5(6)-carboxyfluorescein. Completeness of the coupling was confirmed using the Kaiser test.[2] The resin was then washed with NMP (1 × 5 min), piperidine/NMP (1:5, 1 × 15 min), NMP (6 × 1 min), CH_2Cl_2 (6 × 1 min), CH_3OH (6 × 1 min) and CH_2Cl_2 (6 × 1 min), and air dried.[3] The resulting peptidyl resin was cleaved with TFA/H_2O/TIS (95:2.5:2.5) for 3 h at room temperature. Following TFA evaporation and cold Et_2O extraction, the crude was dissolved in H_2O (5 mL) and lyophilized. Characterization was performed as described for **BP341**. The metal binding peptide **BP349** was obtained in >99% purity. t_R = 6.83 and 6.90 min, corresponding to the two isomers of the 5(6)-carboxyfluorescein (CF) labeled peptide. HRMS (ESI): *m/z* calcd. for $C_{120}H_{185}N_{25}O_{20}$ $[M + 6H]^{6+}$ 382.7366; found 382.7375; calcd. for $C_{120}H_{184}N_{25}O_{20}$ $[M + 5H]^{5+}$ 459.0824; found 459.0812; calcd. for $C_{120}H_{183}N_{25}O_{20}$ $[M + 4H]^{4+}$ 573.6012; found 573.6005; calcd. for $C_{120}H_{182}N_{25}O_{20}$ $[M + 3H]^{3+}$ 764.4659; found 764.4664; calcd for $C_{120}H_{181}N_{25}O_{20}$ $[M + 2H]^{2+}$ 1146.1952; found 1146.1963.

Synthesis of (*S*,*S'*)-BPBP-Gly-Phe-Leu-Gly-Lys-Lys-Leu-Phe-Lys-Lys-Ile-Leu-Lys-Lys-Leu-Lys(CF)-NH_2 (BPBP-GFLG-BP16-CF) (BP350). This metal binding peptide was synthesized from the peptidyl resin H-Gly-Phe-Leu-Gly-Lys(Boc)-Lys(Boc)-Leu-Phe-Lys(Boc)-Lys(Boc)-Ile-Leu-Lys(Boc)-Lys(Boc)-Leu-Lys(Dde)-Rink-MBHA (50 mg). The nicotinic acid derivative **4** was coupled to this peptidyl resin as described for **BP347**. The resulting resin was treated with hydrazine/NMP (2:98, 5 × 20 min). After these treatments the resin was washed with NMP (6 × 1 min) and CH_2Cl_2 (1 × 1 min). The derivatization with 5(6)-carboxyfluorescein (CF) was performed as described for **BP349**. The resulting peptidyl resin was cleaved with TFA/H_2O/TIS (95:2.5:2.5) for 3 h at room temperature. Following TFA evaporation and cold Et_2O extraction, the crude was dissolved in H_2O (5 mL) and lyophilized. Characterization was performed as described for **BP341**. The metal binding peptide **BP350** was obtained in 94% purity. t_R = 7.53 and 7.65 min, corresponding to the two isomers of the 5(6)-carboxyfluorescein (CF) labeled peptide. HRMS (ESI): *m/z* calcd. for $C_{136}H_{206}N_{28}O_{23}$ $[M + 6H]^{6+}$ 433.2630; found 433.2635; calcd. for $C_{136}H_{205}N_{28}O_{23}$ [M +

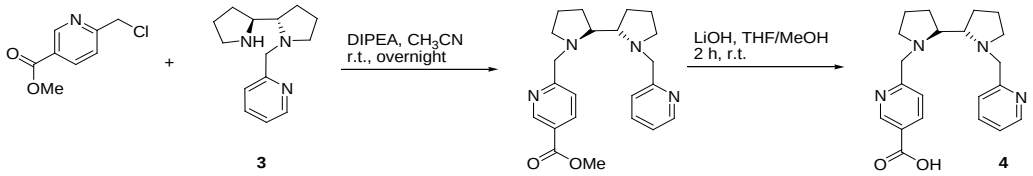
5H]$^{5+}$ 519.7141; found 519.7137; calcd. for $C_{136}H_{204}N_{28}O_{23}$ [M + 4H]$^{4+}$ 649.3908; found 649.3899; calcd. for $C_{136}H_{203}N_{28}O_{23}$ [M + 3H]$^{3+}$ 865.5186; found 865.5197.

1. M. Soler, E. Figueras, J. Serrano-Plana, M. González-Bártulos, A. Massaguer, A. Company, M. Á. Martínez, J. Malina, V. Brabec, L. Feliu, M. Planas, X. Ribas and M. Costas, *Inorg. Chem.*, 2015, **54**, 10542–10558.

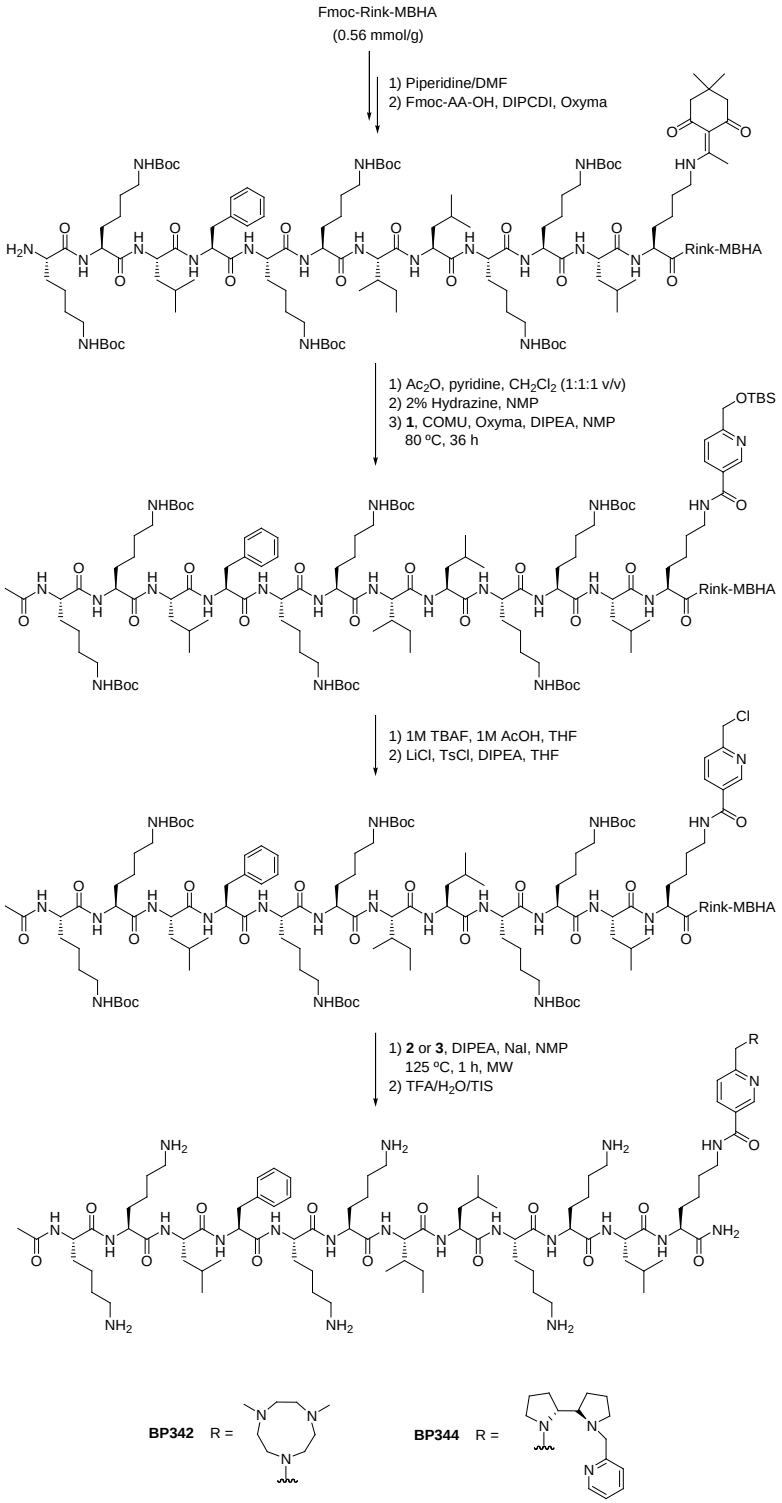
2. E. Kaiser, R. L. Colescott, C. D. Bossinger and P. Cook, *Anal. Biochem.*, 1970, **34**, 595–598.

3. R. Fischer, O. Mader, G. Jung and R. Brock, *Bioconjugate Chem.*, 2003, **14**, 653–660.

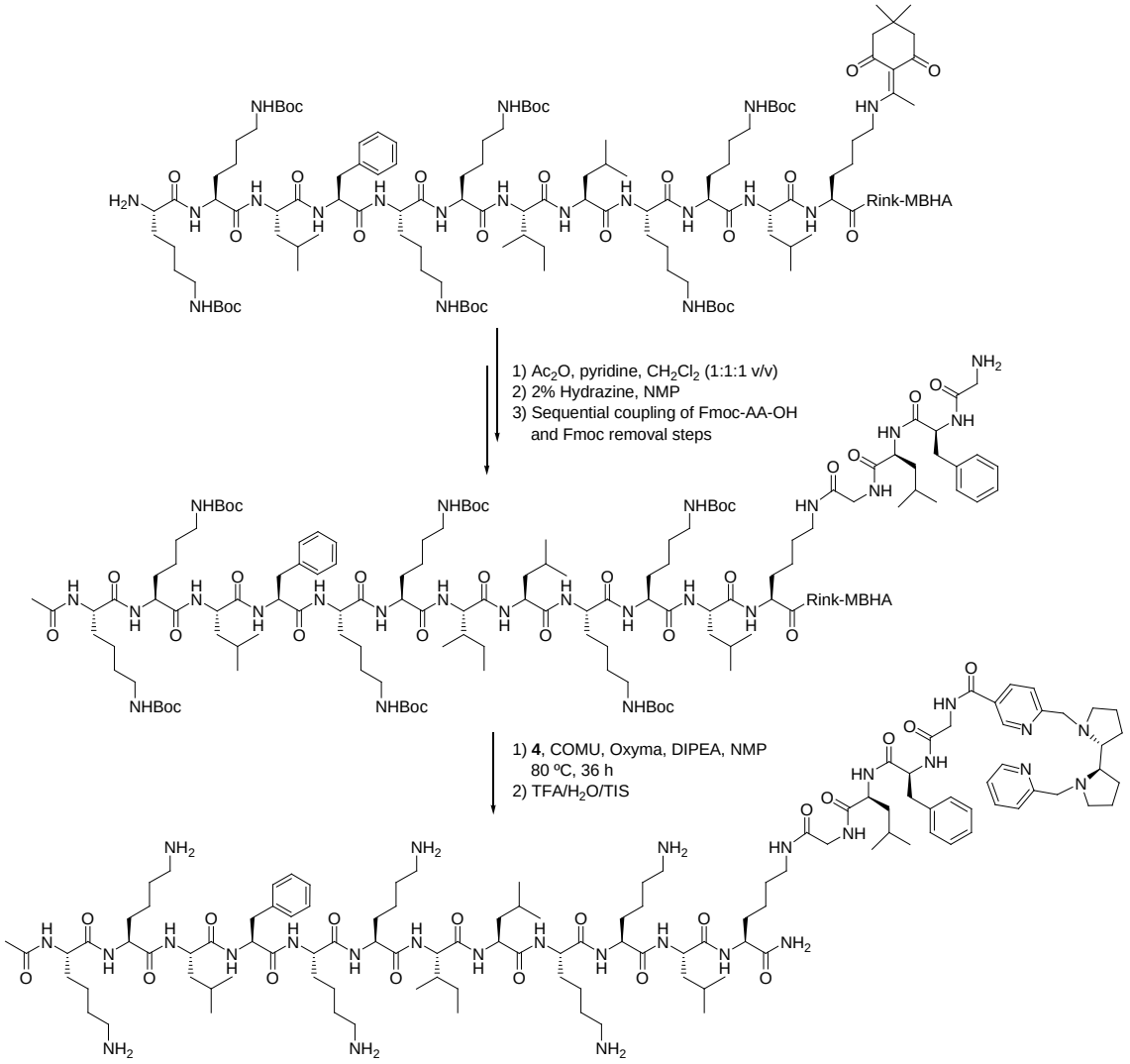
Scheme S1. Synthesis of the (*S,S'*)-BPBP derivative **4**



Scheme S2. Synthesis of the metal binding peptides **BP342** and **BP344**

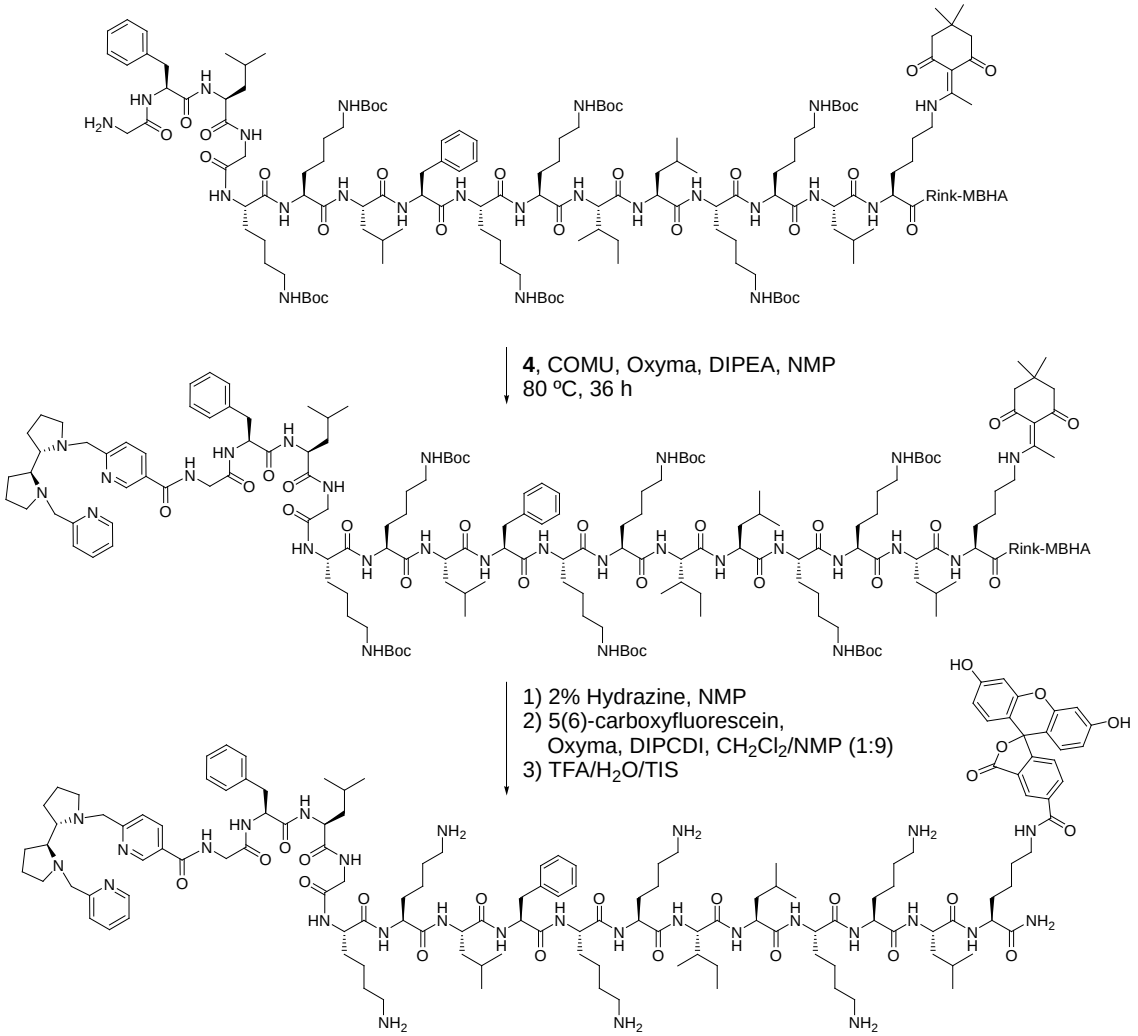


Scheme S3. Synthesis of the metal binding peptide **BP348**



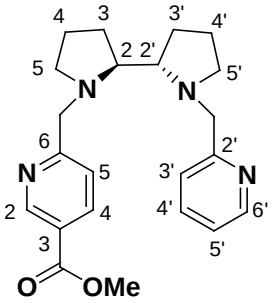
BP348

Scheme S4. Synthesis of the fluorescein-labeled conjugate **BP350**



BP350

Figure S1: a) ^{1}H NMR spectrum (400 MHz, $CDCl_3$), b) ^{13}C NMR spectrum (100 MHz, $CDCl_3$), c) ESI/MS spectrum (*m/z*).



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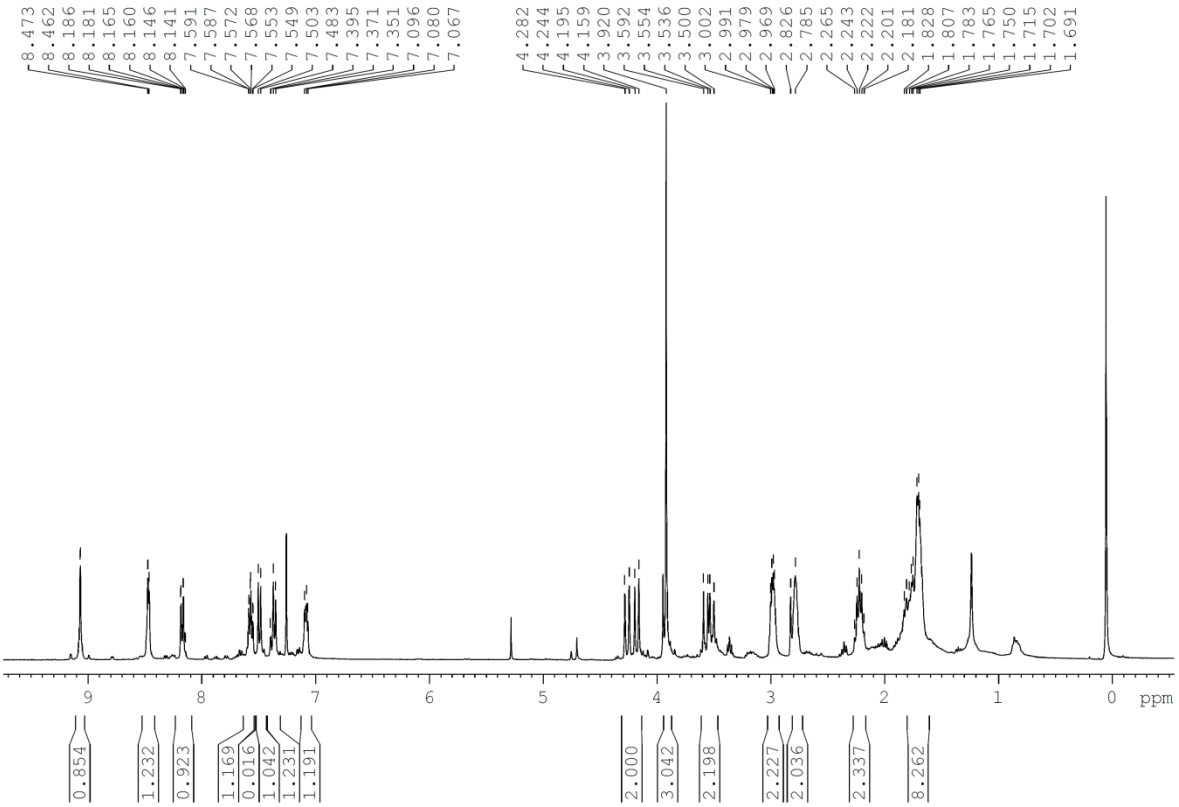
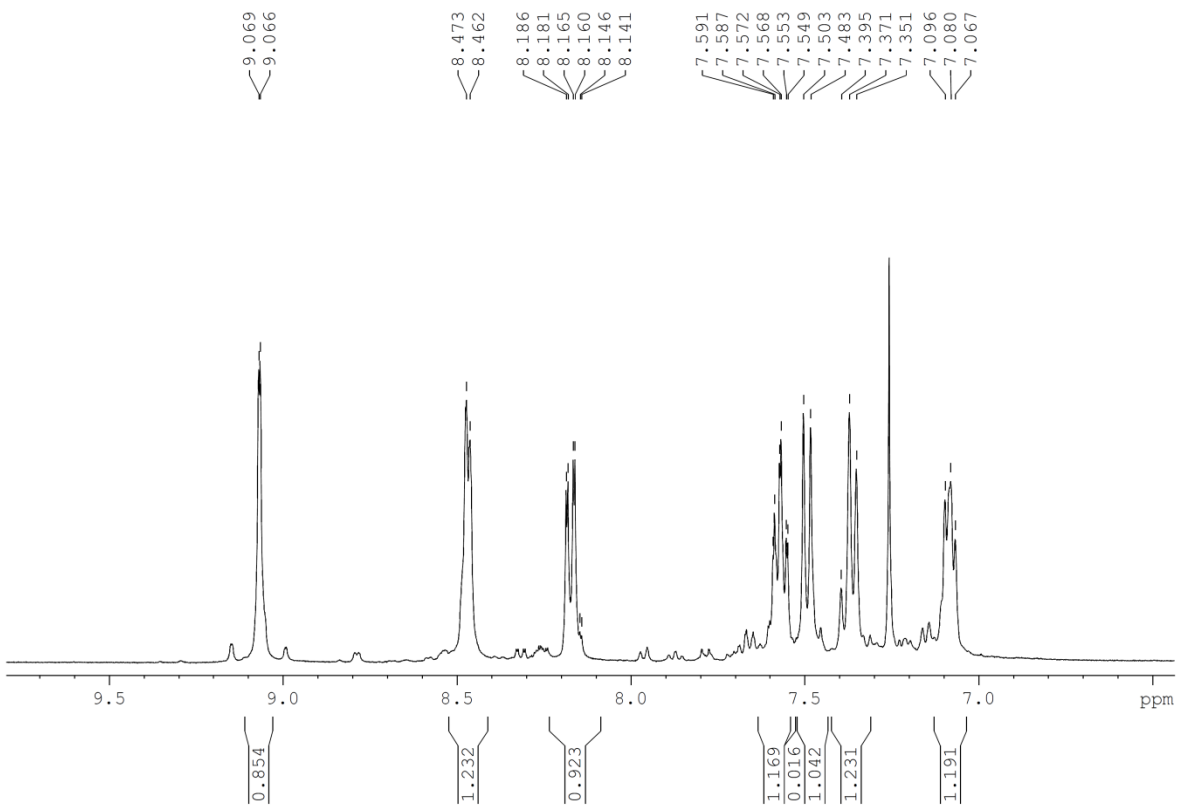
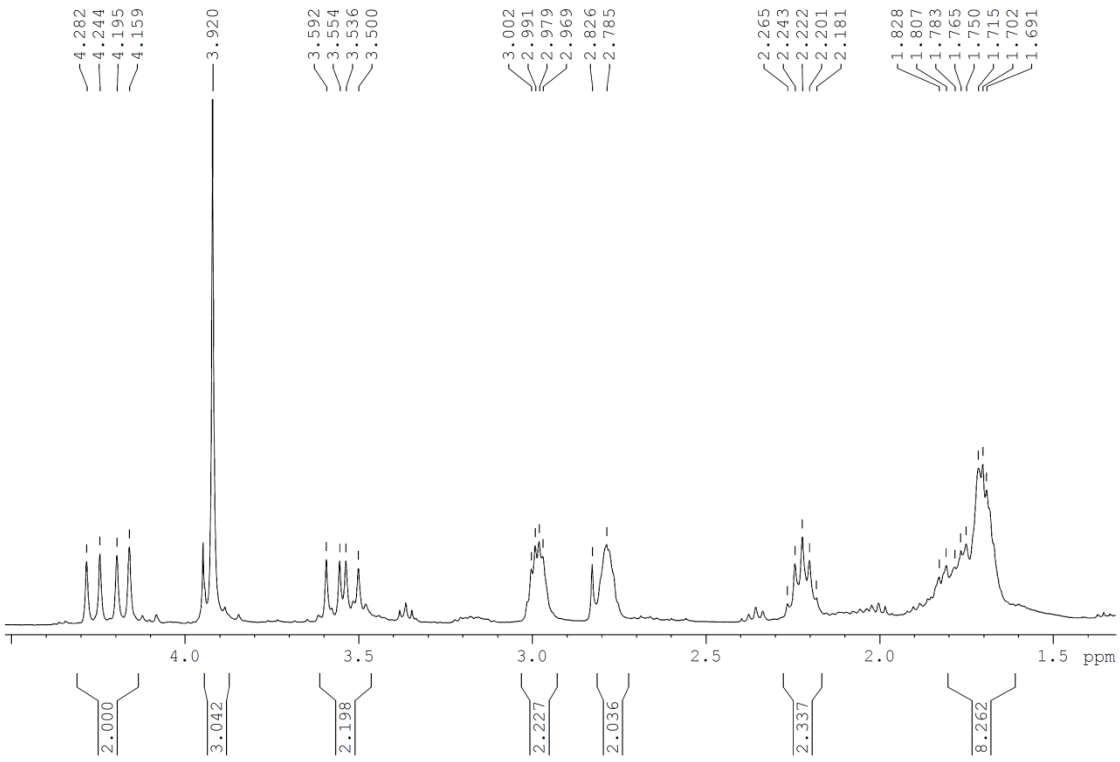


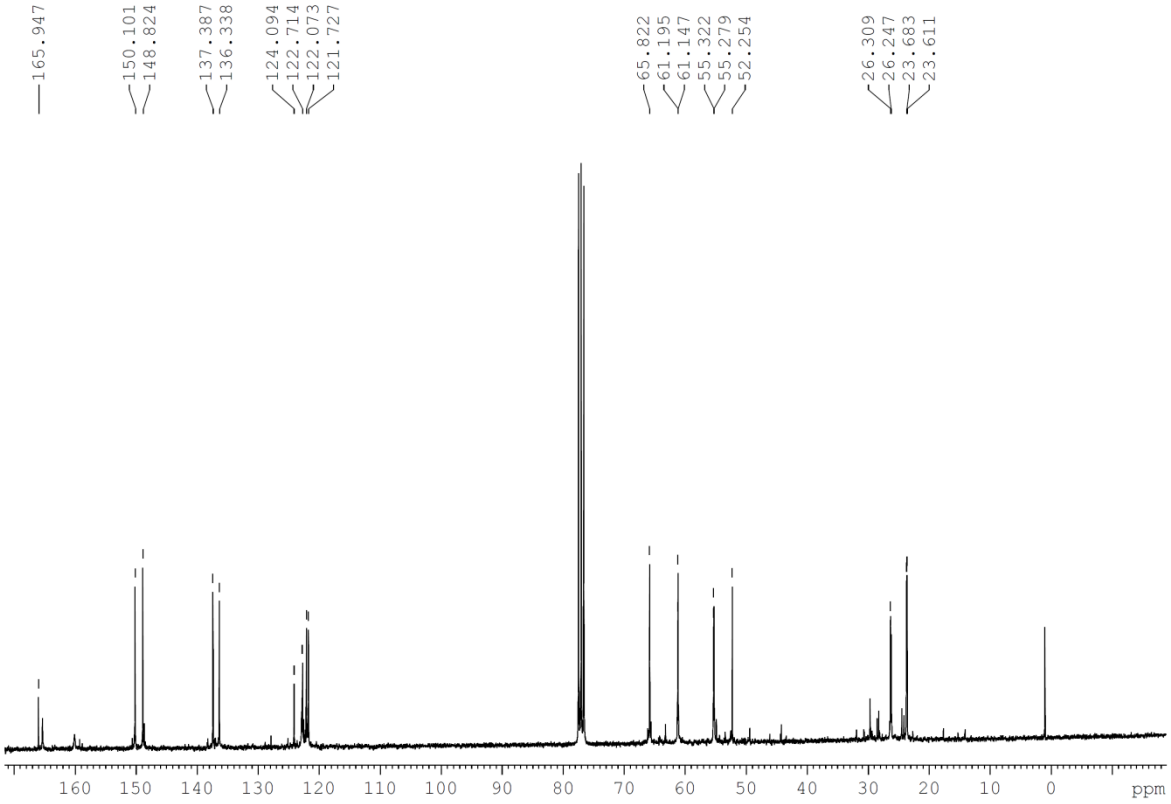


Selected aromatic region.



Selected aliphatic region.

b)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61.147
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23.611
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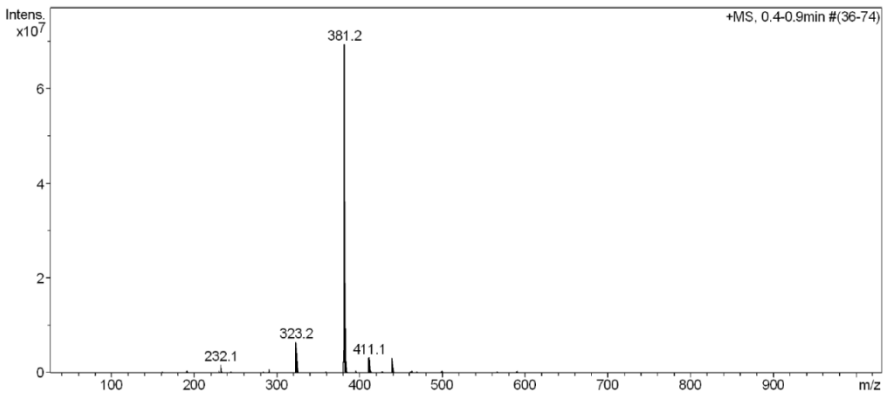
ppm

c)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+MS, 0.4-0.9min #(36-74)
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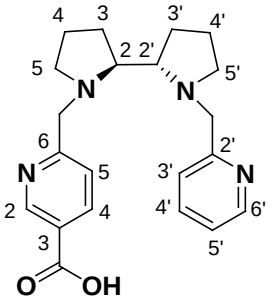
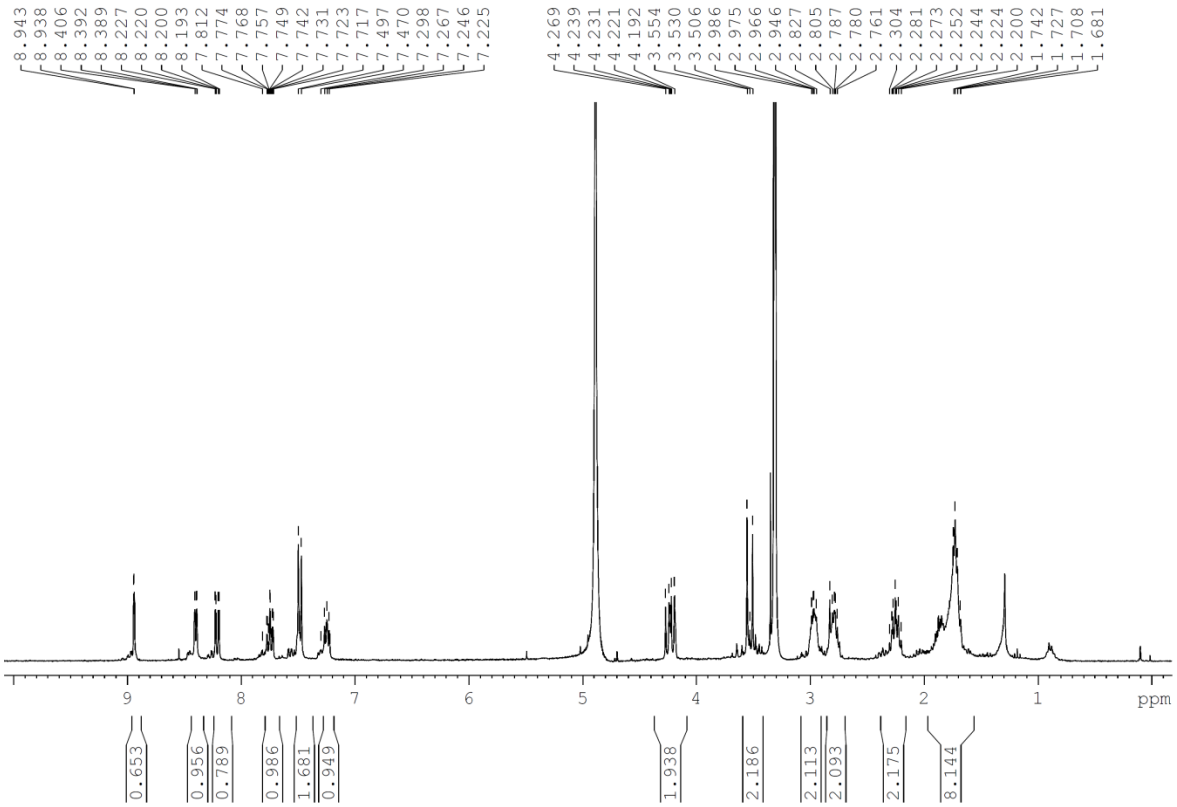
323.2
232.1
411.1
0
2
4
6
100 200 300 400 500 600 700 800 900
m/z

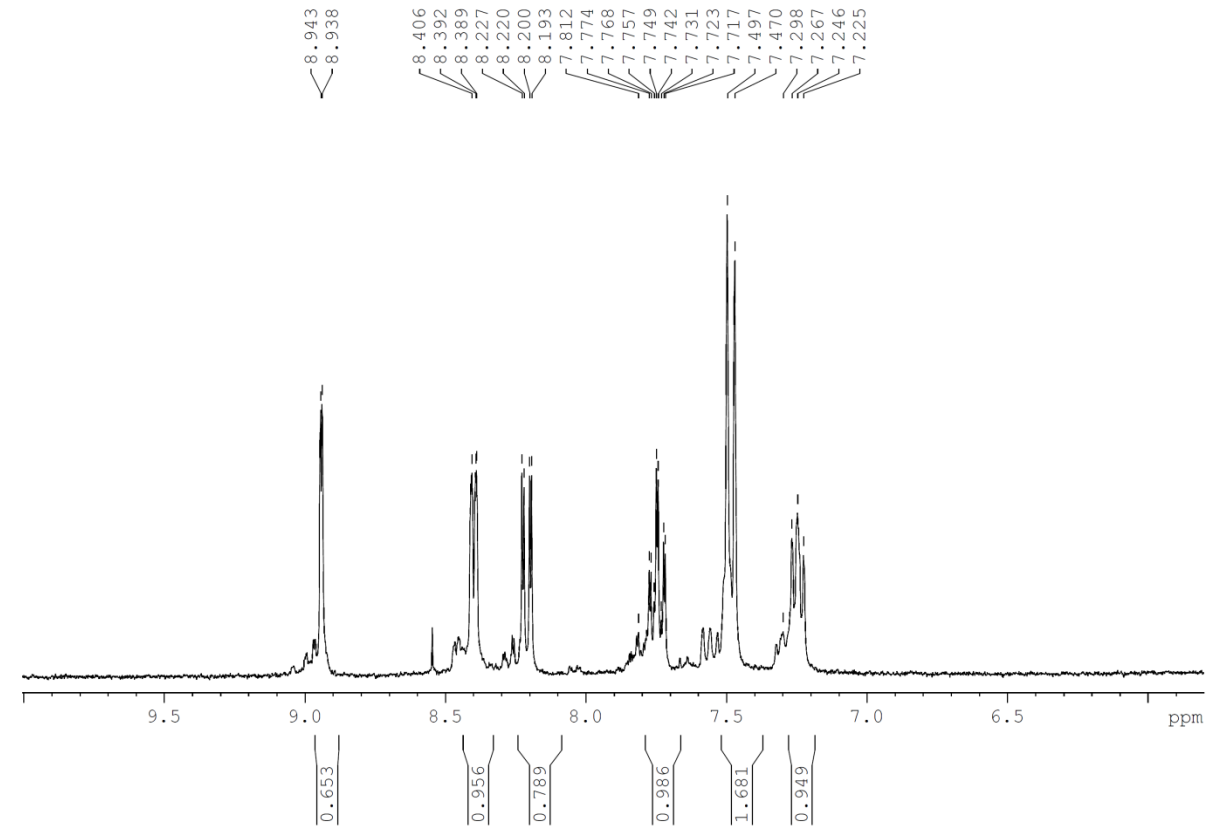
Figure S2: a) ^{1}H NMR spectrum (400 MHz, $CDCl_3$), b) ESI/MS spectrum (*m/z*), c) HRMS spectrum (*m/z*)



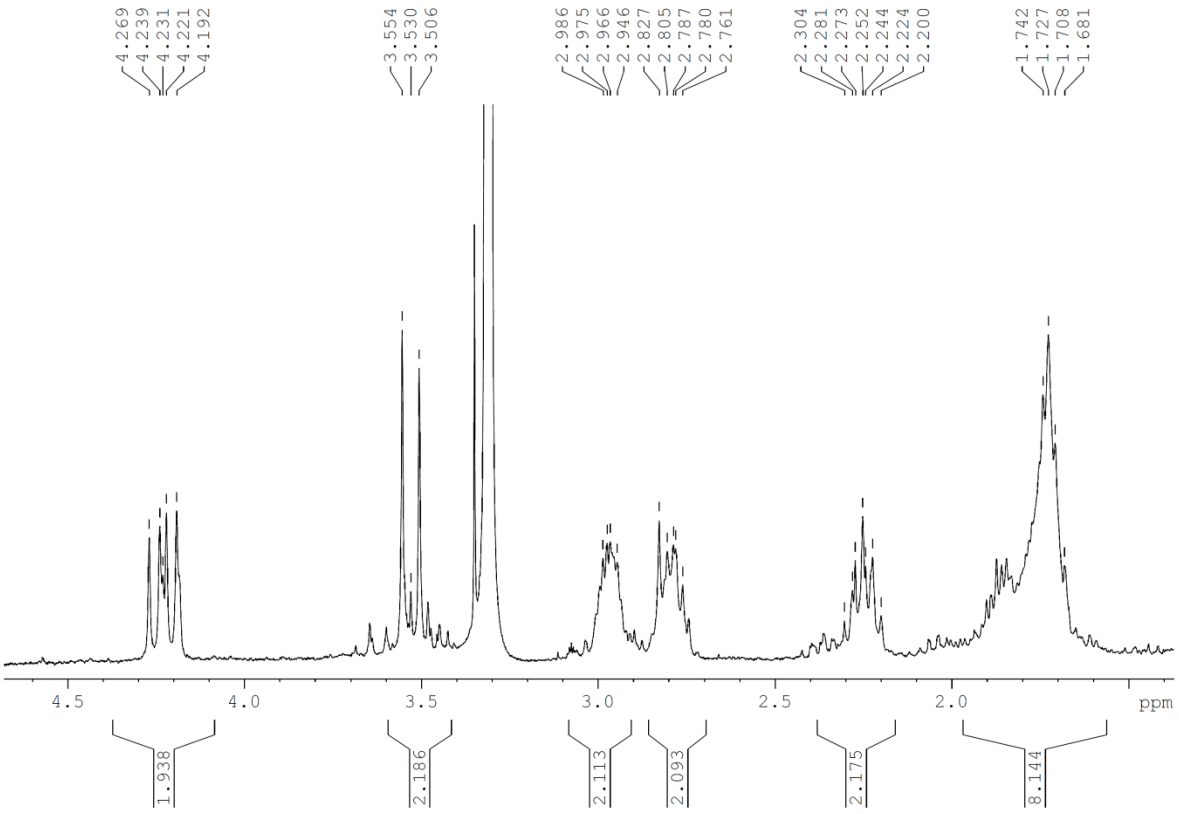
4

a)



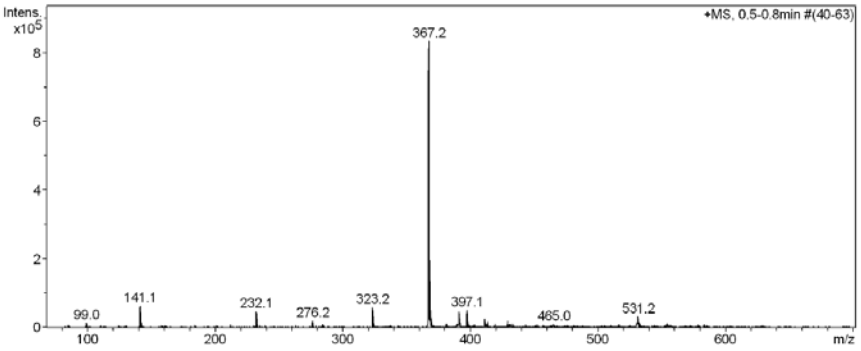


Selected aromatic region.



Selected aliphatic region.

b)



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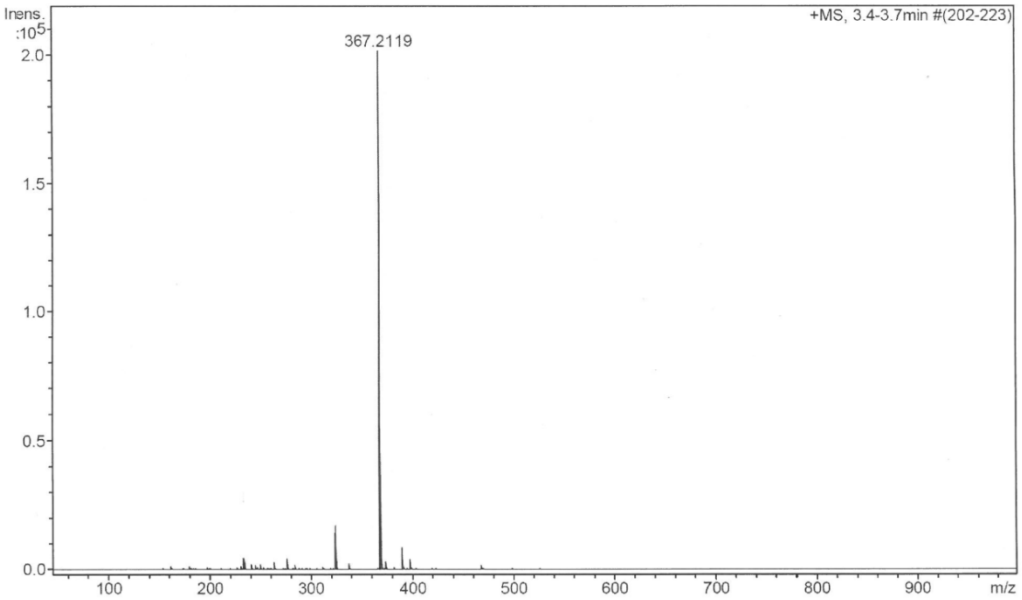
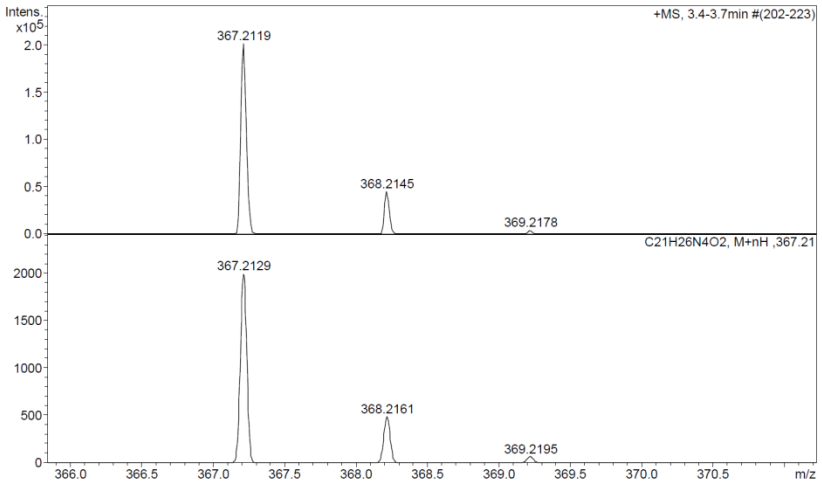
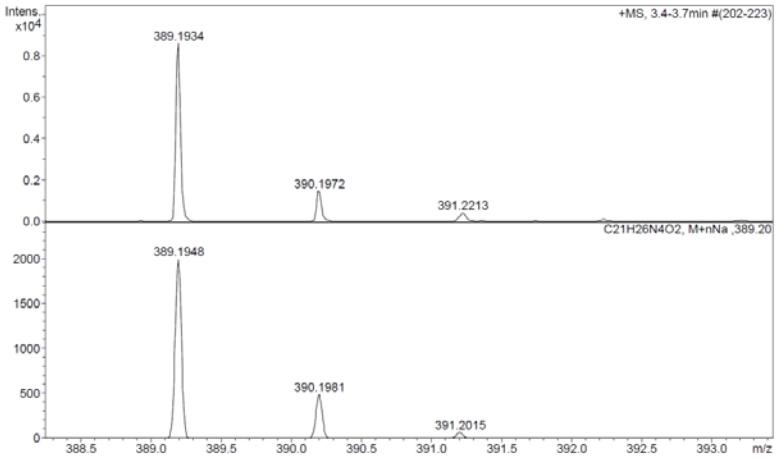




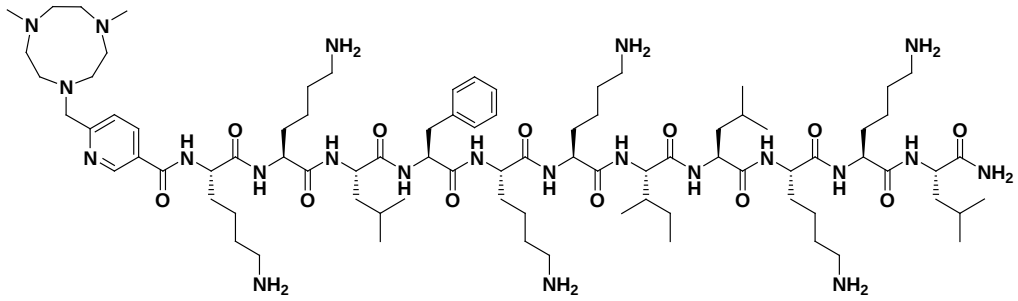
Observed HRMS (top) with the theoretical isotope prediction (bottom).



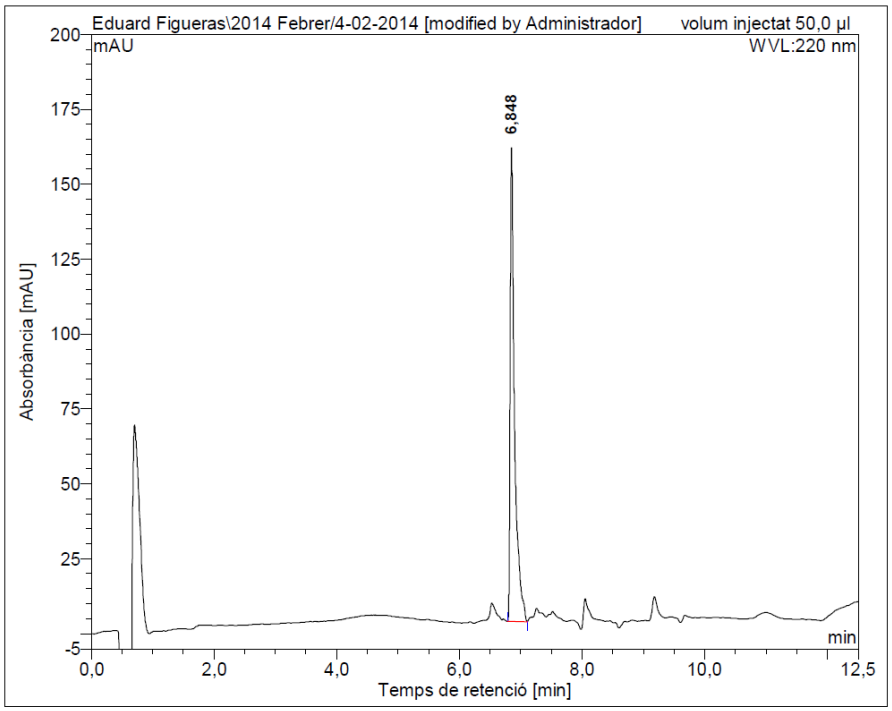
Observed HRMS (top) with the theoretical isotope prediction (bottom).

Figure S3: a) HPLC chromatogram (λ= 220 nm), b) ESI/MS spectrum (*m/z*), c) HRMS spectrum (*m/z*).

PyTACN-BP16 (**BP341**)

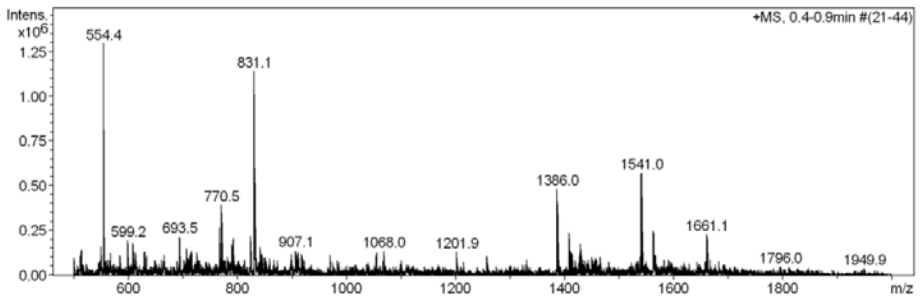


a)

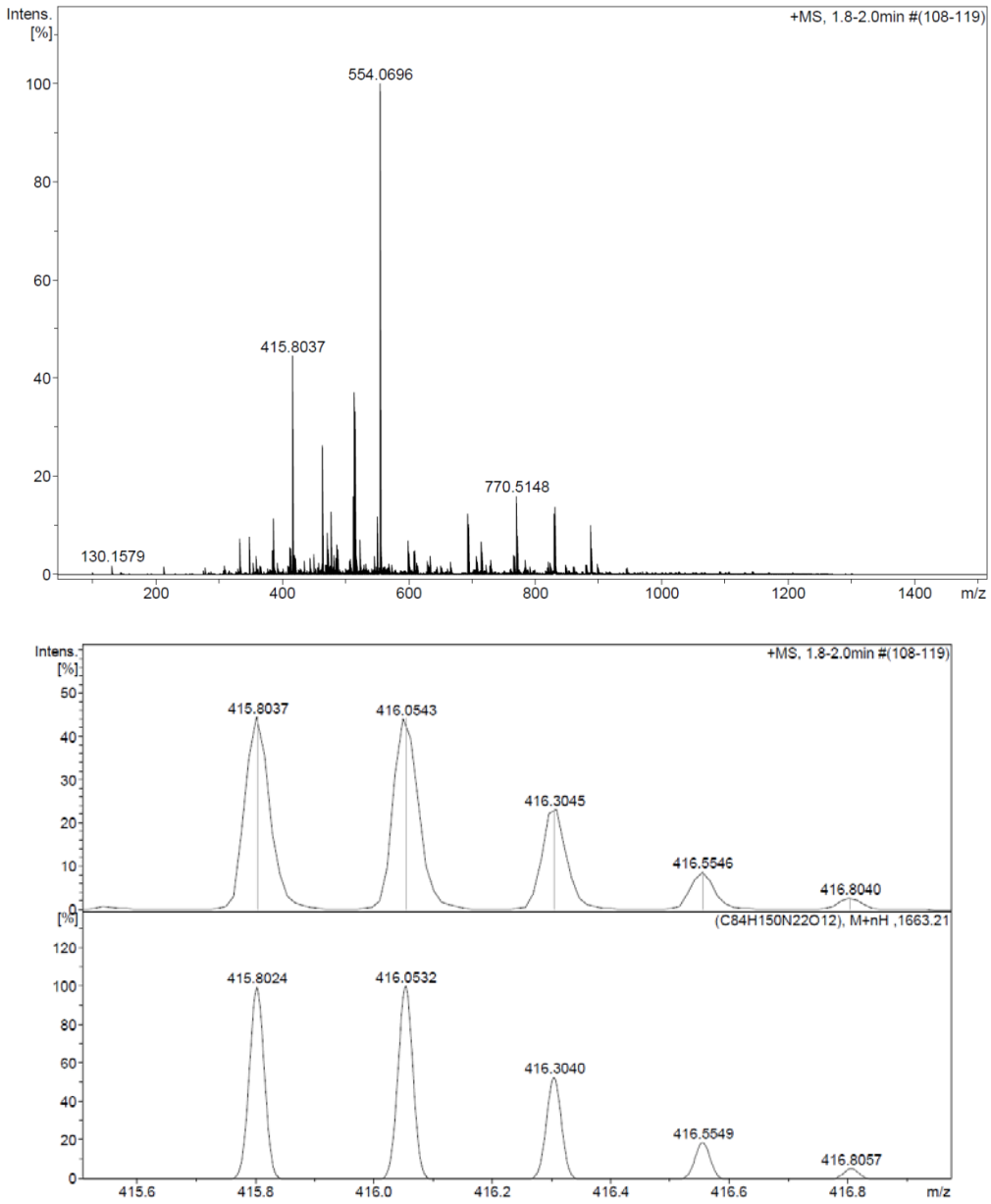


No.	Temps retenció min	alçada mAU	Area mAU*min	Area relativa %
1	6,85	158,135	13,168	100,00
Total:		158,135	13,168	100,00

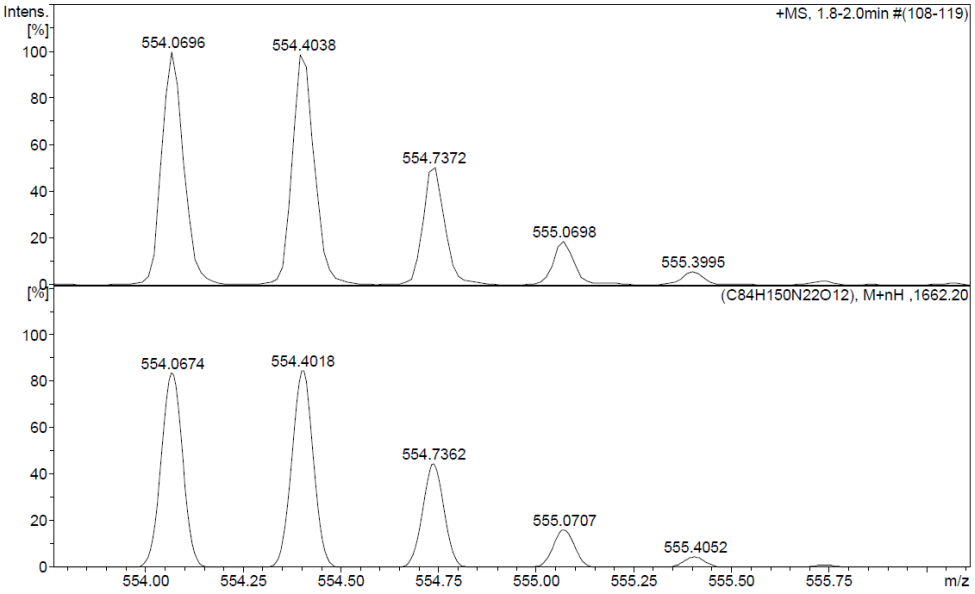
b)



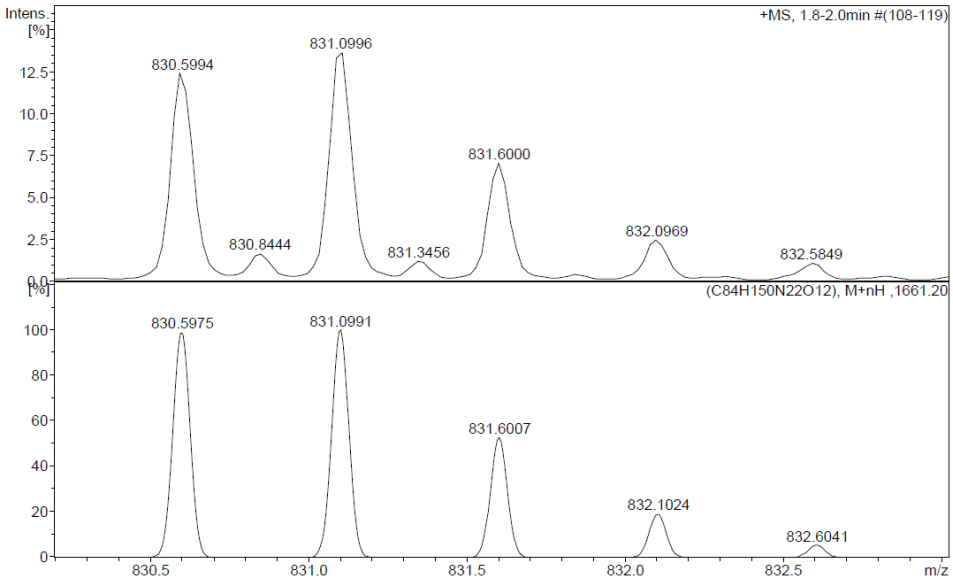
c)



Observed HRMS (top) with the theoretical isotope prediction (bottom).



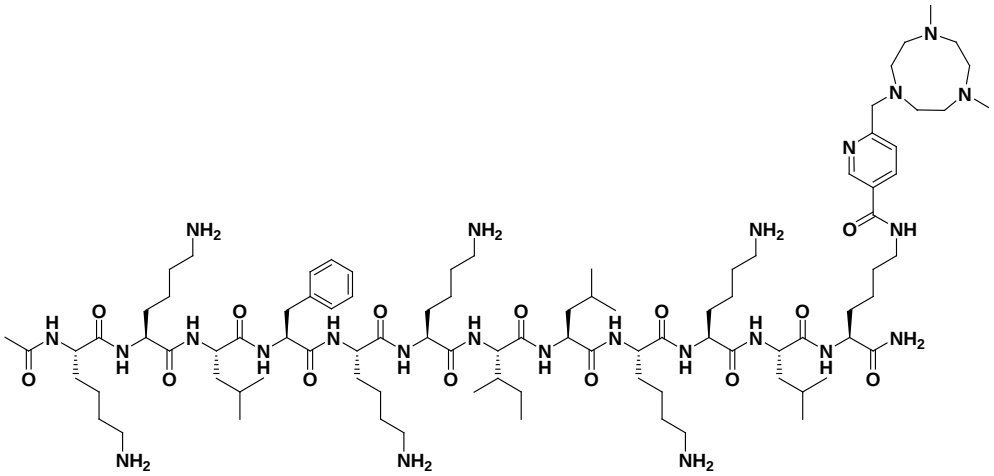
Observed HRMS (top) with the theoretical isotope prediction (bottom).



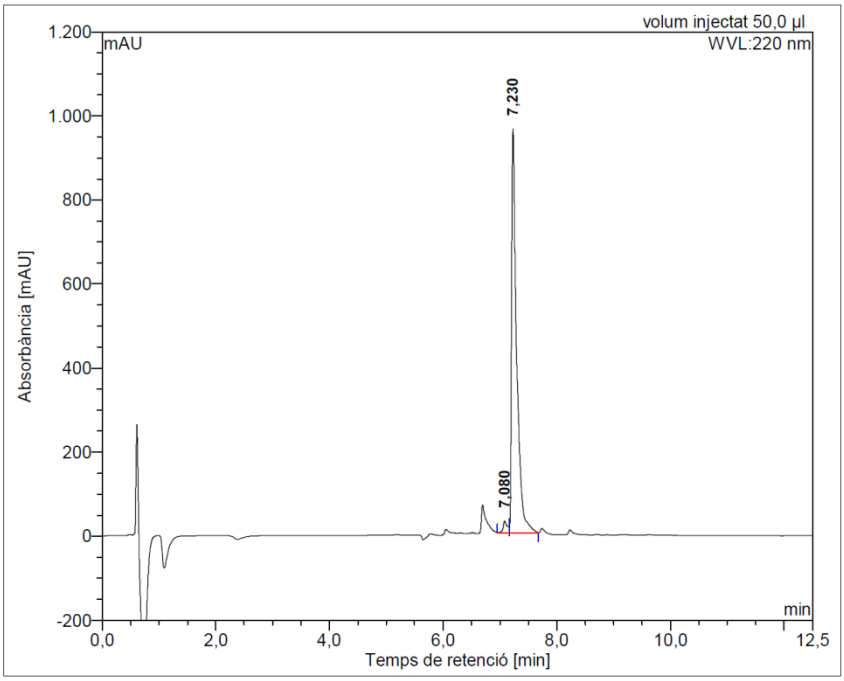
Observed HRMS (top) with the theoretical isotope prediction (bottom).

Figure S4: a) HPLC chromatogram (λ= 220 nm), b) ESI/MS spectrum (*m/z*), c) HRMS spectrum (*m/z*).

BP16-PyTACN (**BP342**)

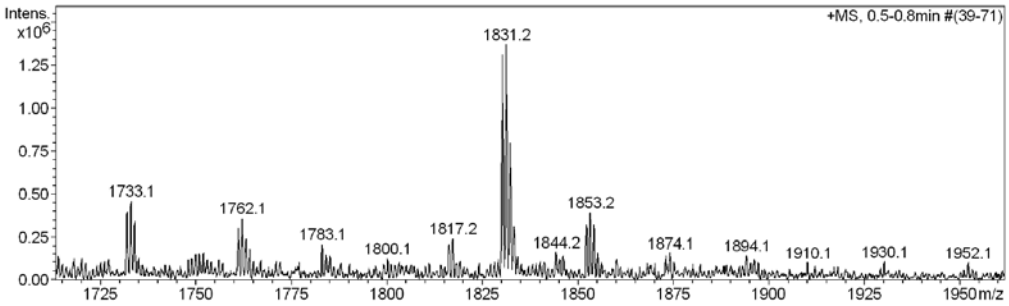


a)

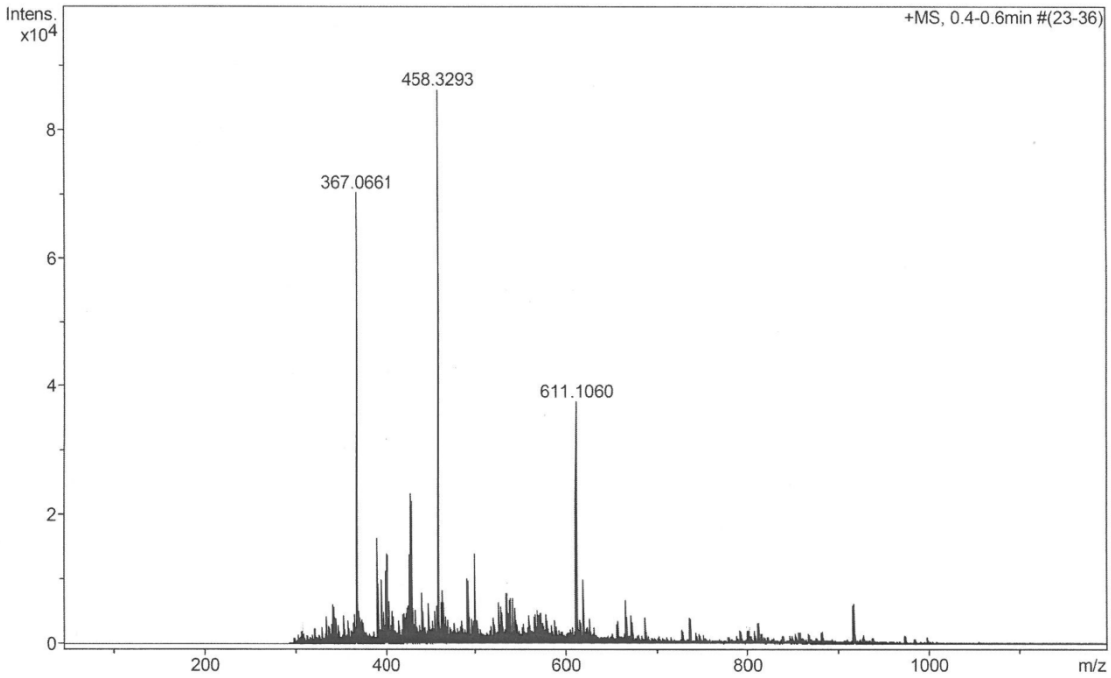


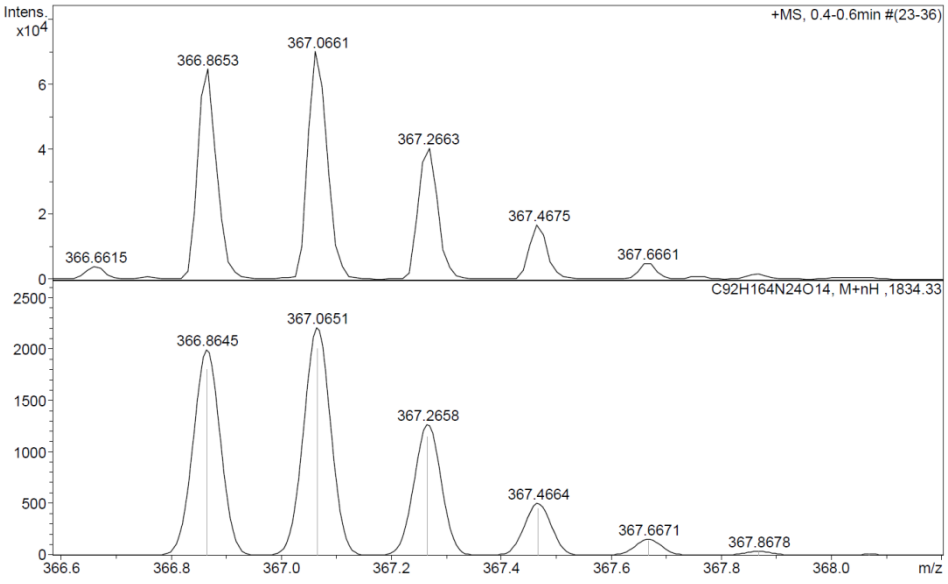
No.	mps retenc min	alçada mAU	Area mAU*min	Area relativa %
1	7,08	27,640	2,269	2,26
2	7,23	960,864	98,165	97,74
Total:		988,504	100,434	100,00

b)

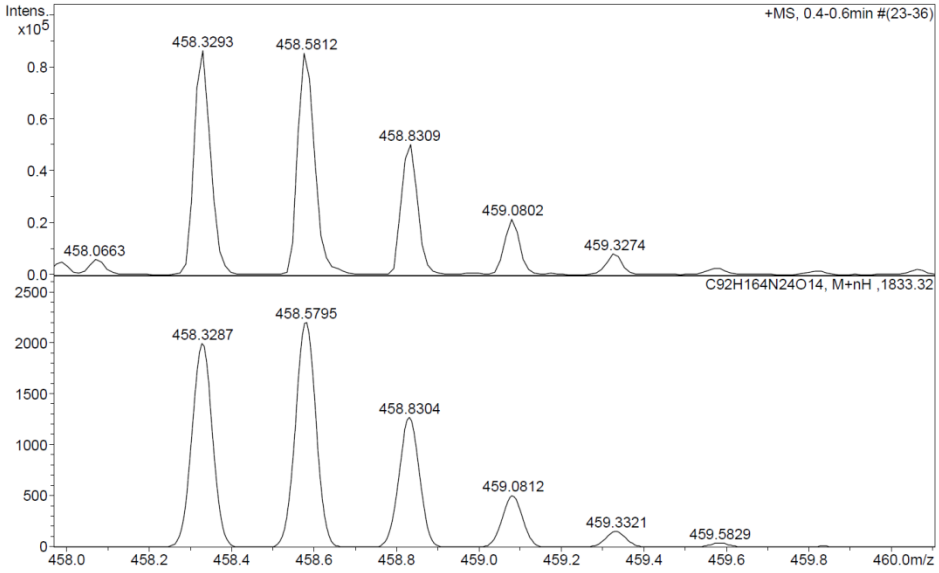


c)

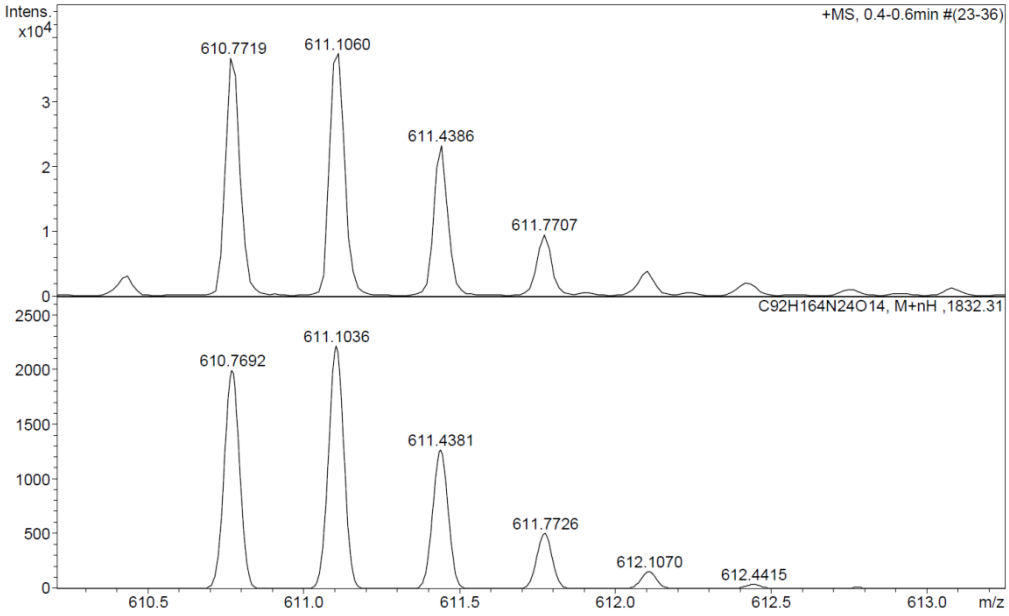




Observed HRMS (top) with the theoretical isotope prediction (bottom).



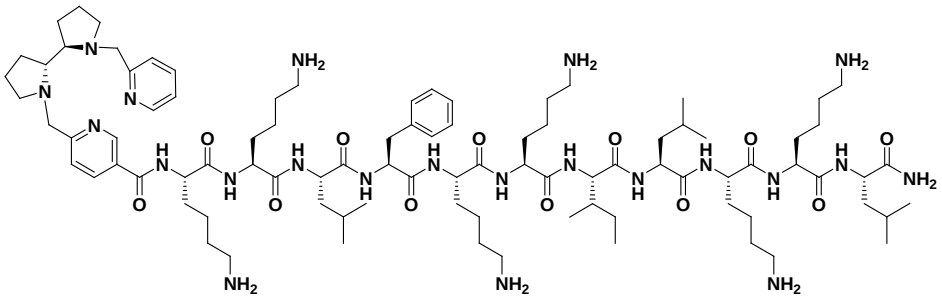
Observed HRMS (top) with the theoretical isotope prediction (bottom).



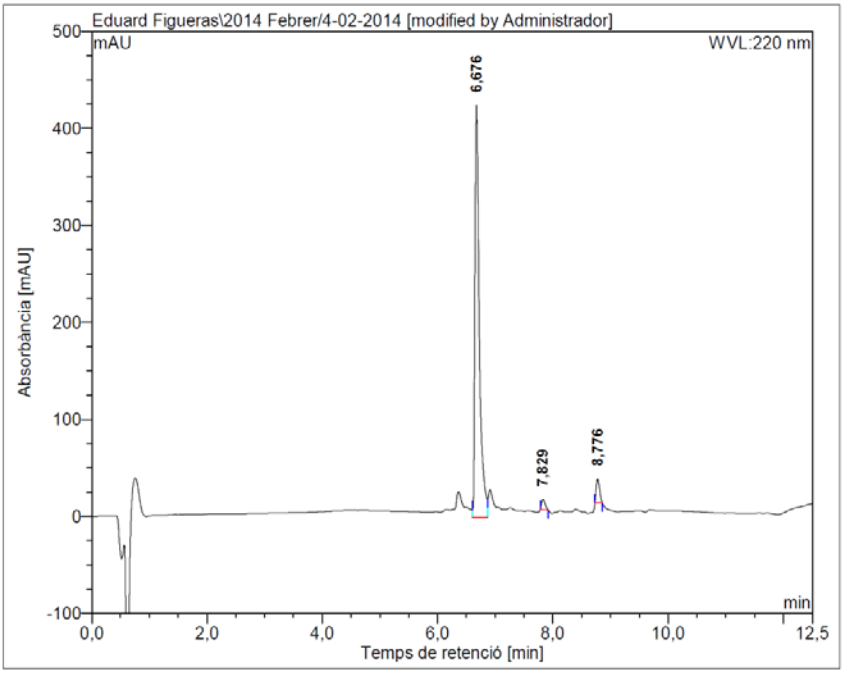
Observed HRMS (top) with the theoretical isotope prediction (bottom).

Figure S5: a) HPLC chromatogram (λ= 220 nm), b) ESI/MS spectrum (*m/z*), c) HRMS spectrum (*m/z*).

BPBP-BP16 (**BP343**)

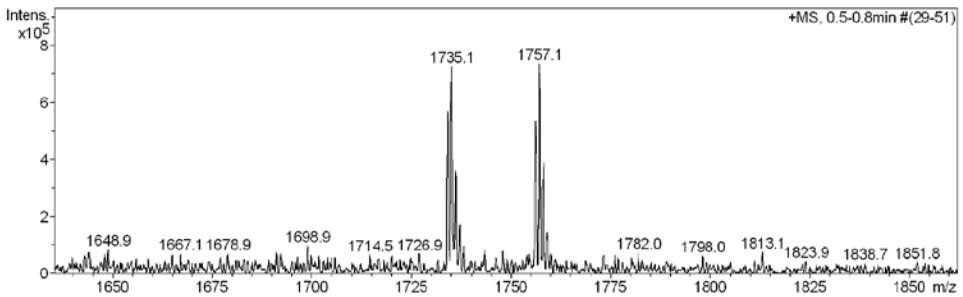


a)

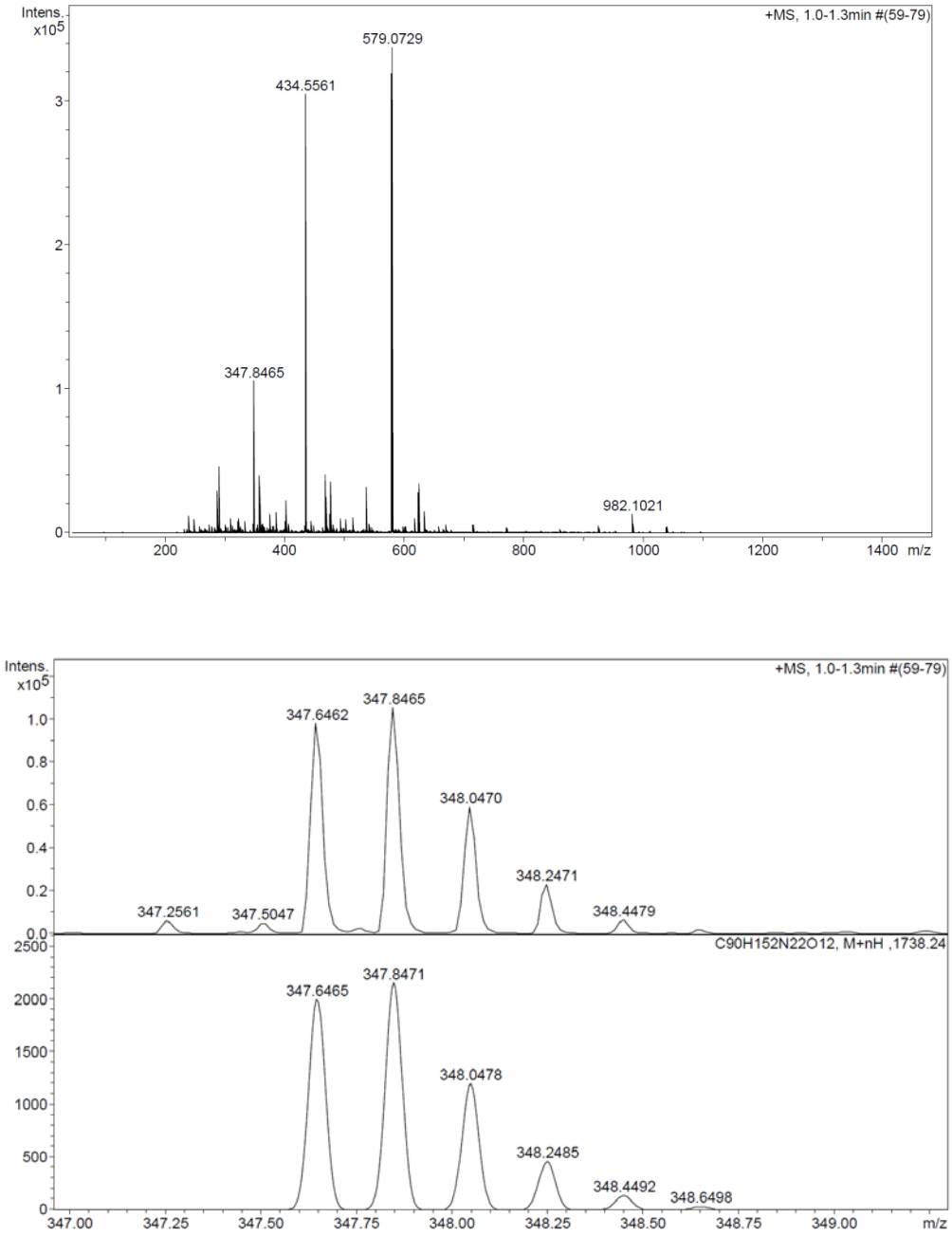


No.	Temps retenció min	alçada mAU	Area mAU*min	Area relativa %
1	6,68	425,084	37,620	93,83
2	7,83	10,533	0,771	1,92
3	8,78	24,513	1,703	4,25
Total:		460,131	40,094	100,00

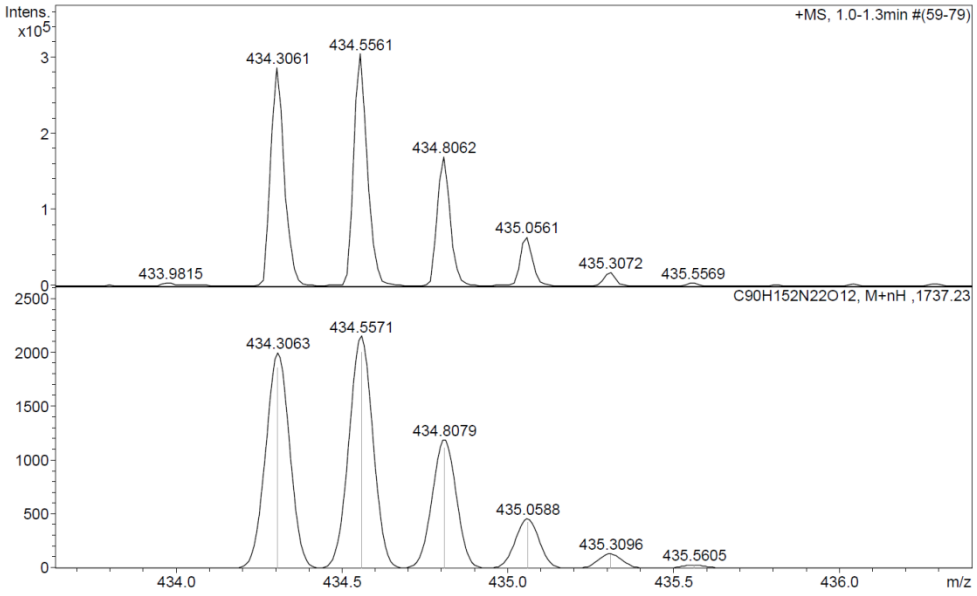
b)



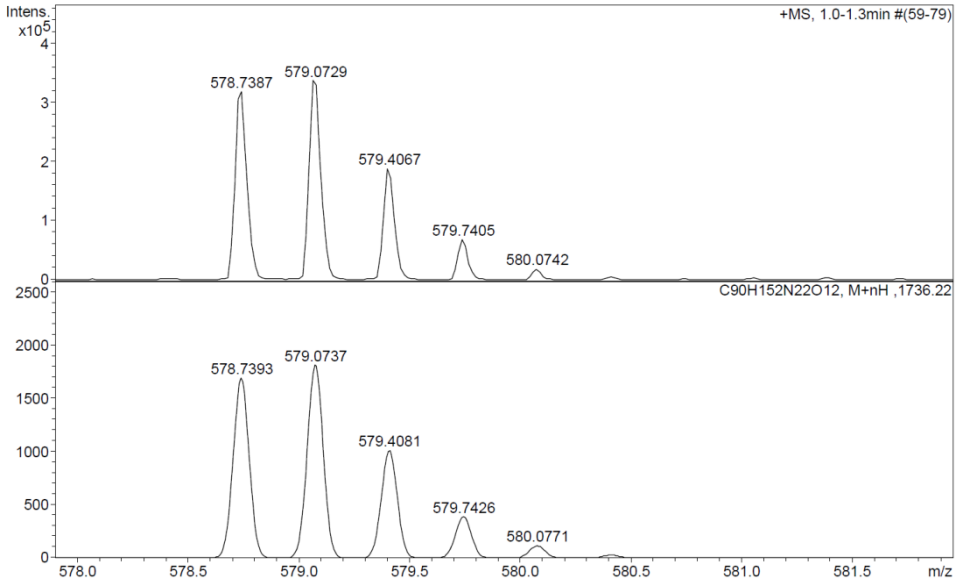
c)



Observed HRMS (top) with the theoretical isotope prediction (bottom).



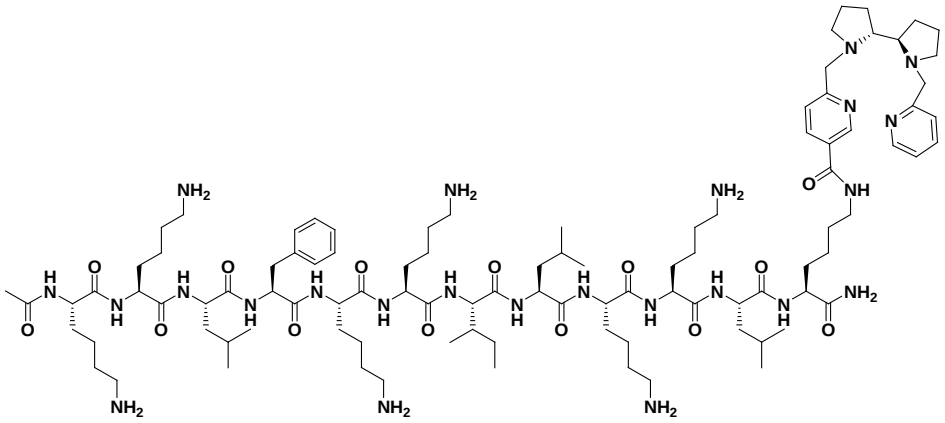
Observed HRMS (top) with the theoretical isotope prediction (bottom).



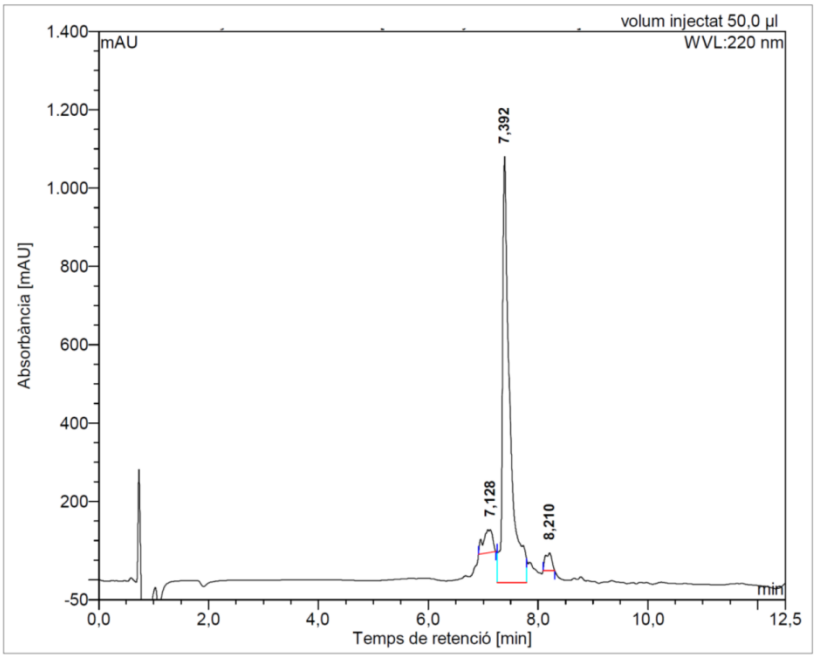
Observed HRMS (top) with the theoretical isotope prediction (bottom).

Figure S6: a) HPLC chromatogram (λ= 220 nm), b) HRMS spectrum (*m/z*).

BP16-BPBP (**BP344**)

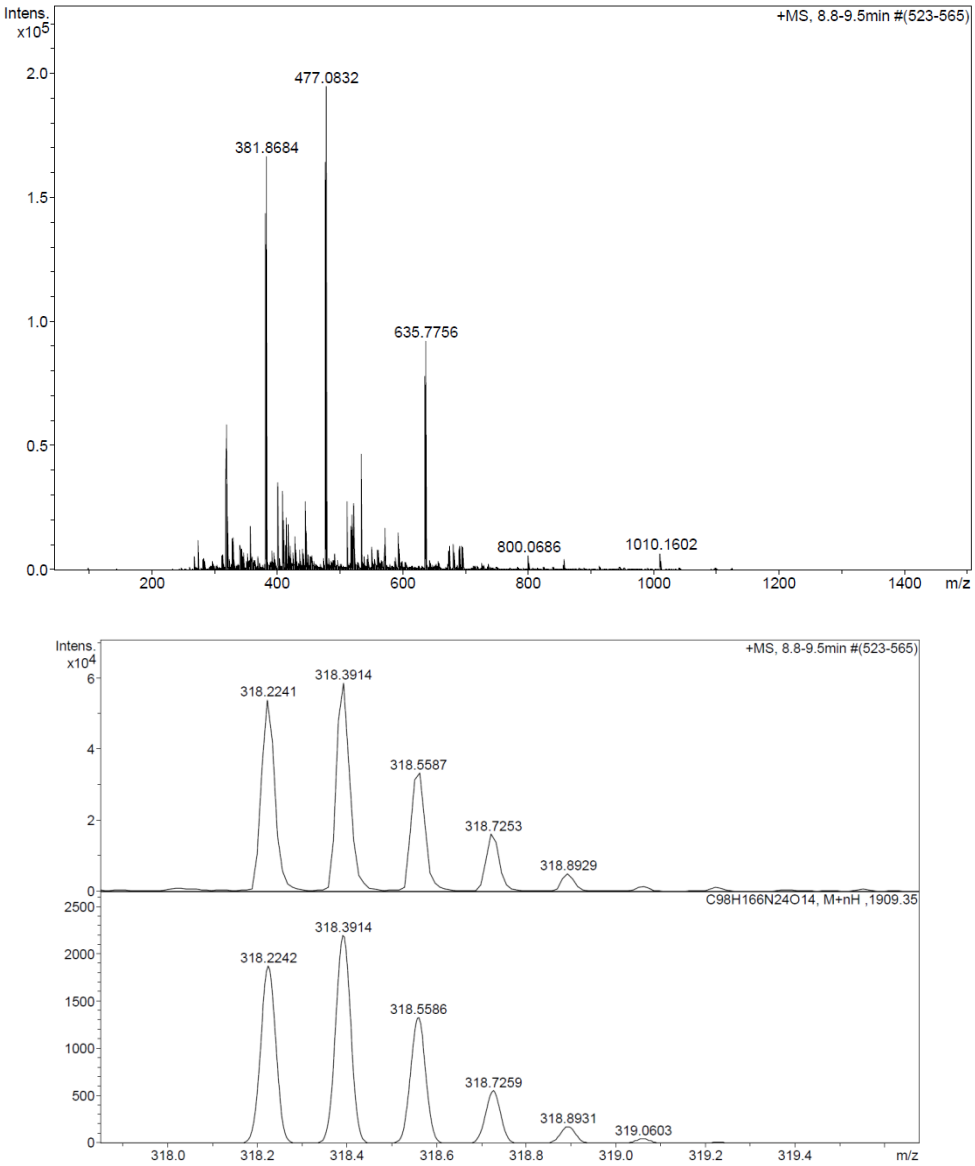


a)

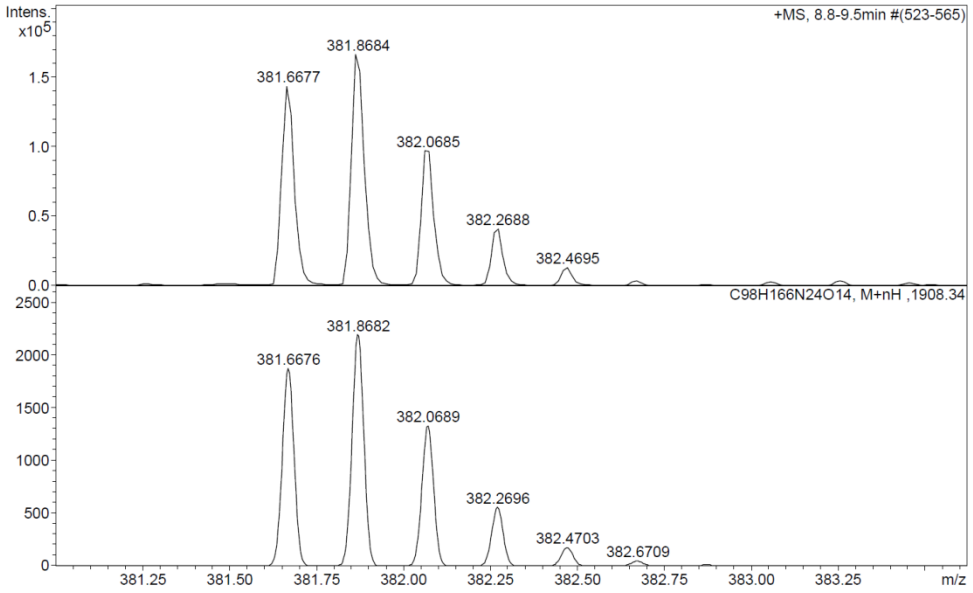


No.	mps retenc min	alçada mAU	Area mAU*min	Area relativa %
1	7,13	58,902	11,867	6,42
2	7,39	1086,835	166,711	90,20
3	8,21	45,621	6,249	3,38
Total:		1191,358	184,827	100,00

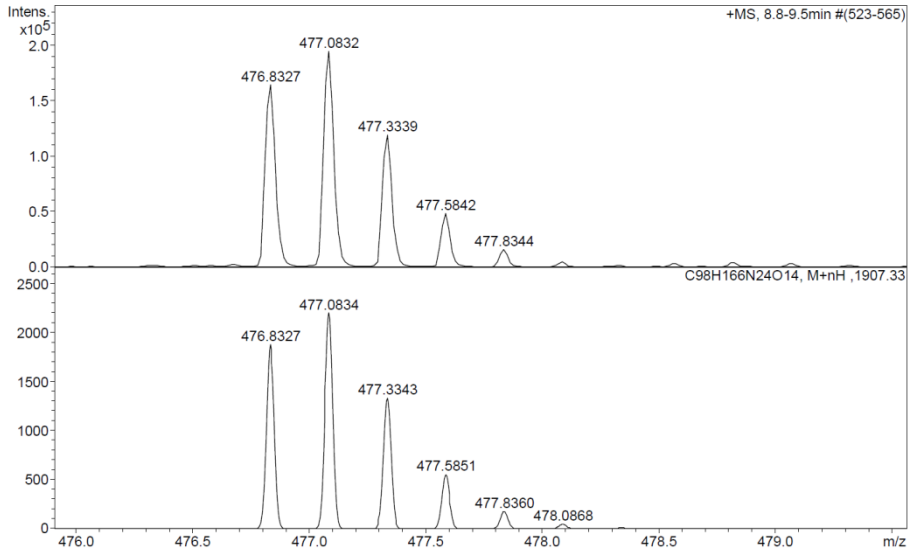
b)



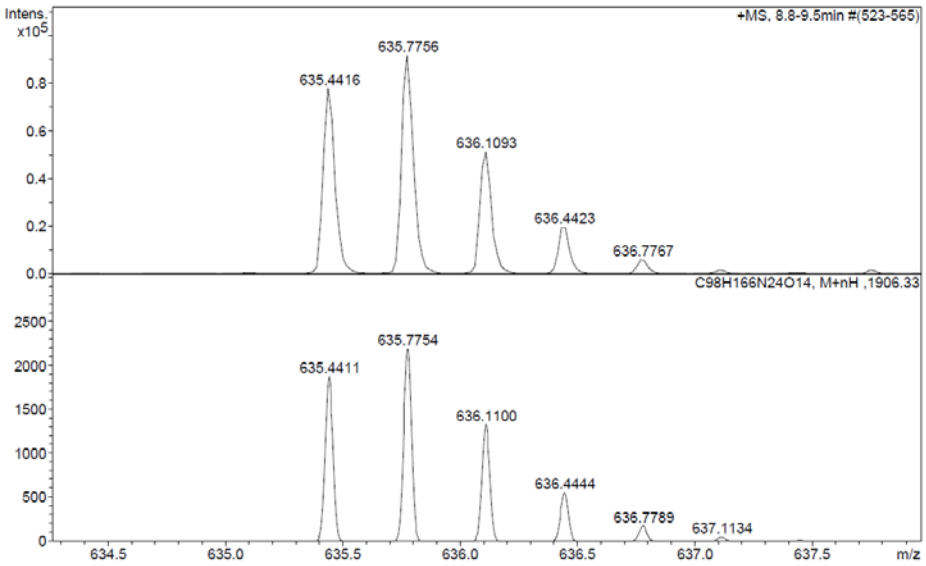
Observed HRMS (top) with the theoretical isotope prediction (bottom).



Observed HRMS (top) with the theoretical isotope prediction (bottom).



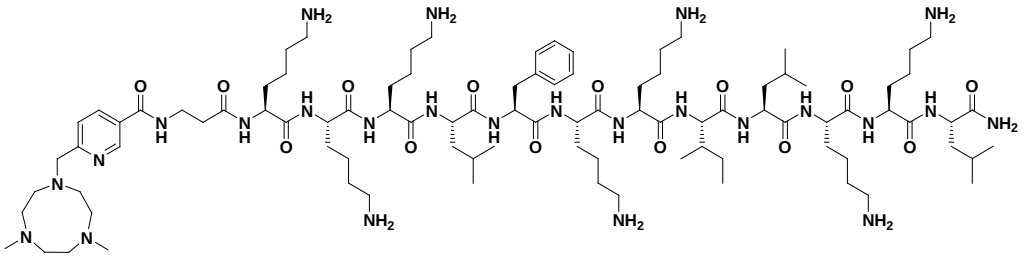
Observed HRMS (top) with the theoretical isotope prediction (bottom).



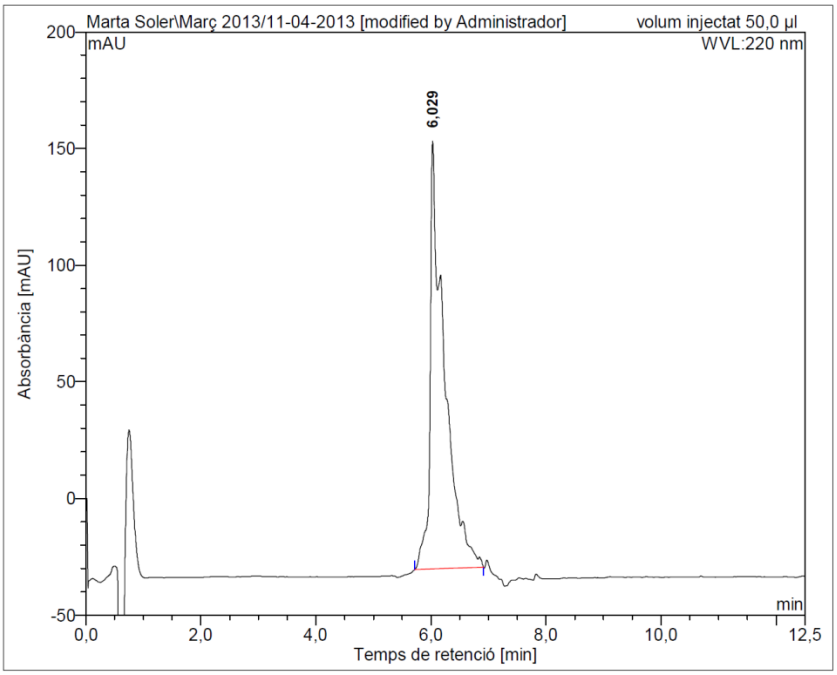
Observed HRMS (top) with the theoretical isotope prediction (bottom).

Figure S7: a) HPLC chromatogram (λ= 220 nm), b) ESI/MS spectrum (*m/z*), c) HRMS spectrum (*m/z*).

PyTACN-βAK-BP16 (**BP345**)

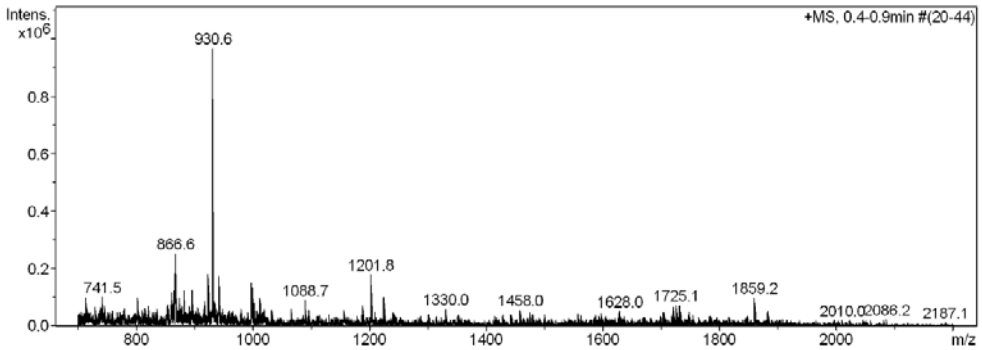


a)

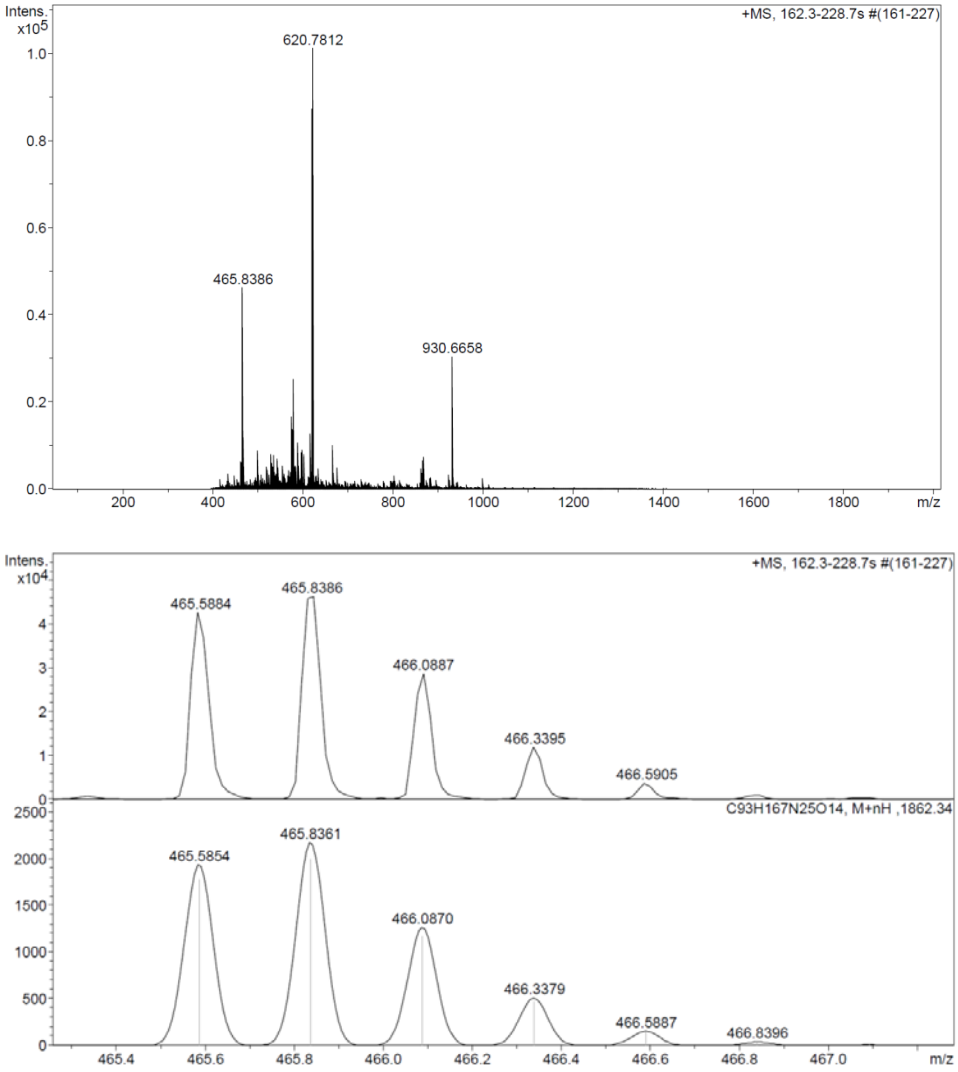


No.	mps retenc min	alçada mAU	Area mAU*min	Area relativa %
1	6,03	183,434	52,770	100,00
Total:		183,434	52,770	100,00

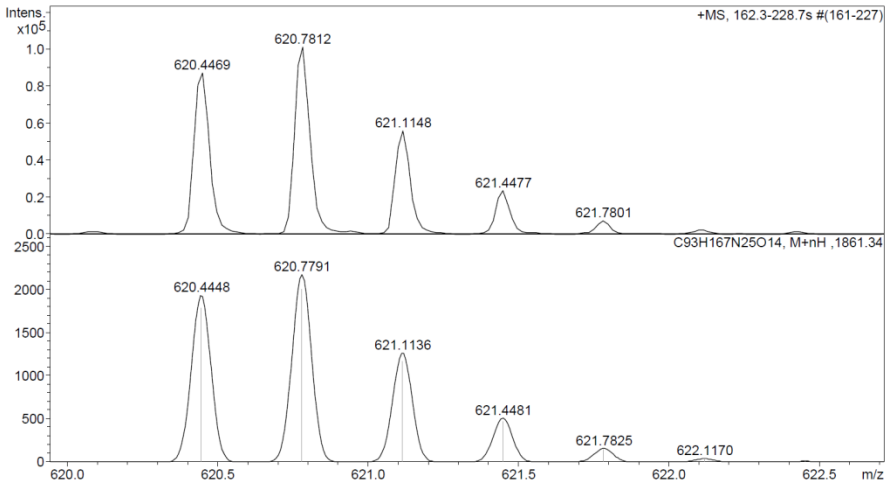
b)



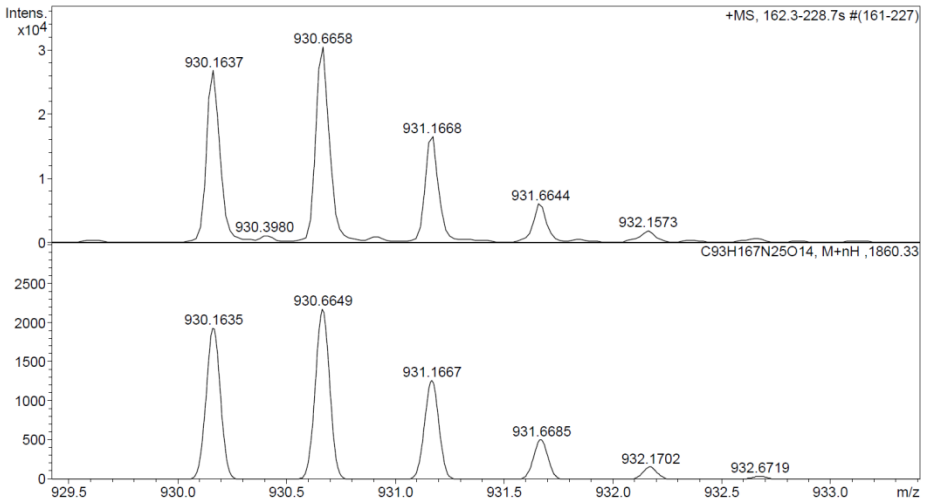
c)



Observed HRMS (top) with the theoretical isotope prediction (bottom).



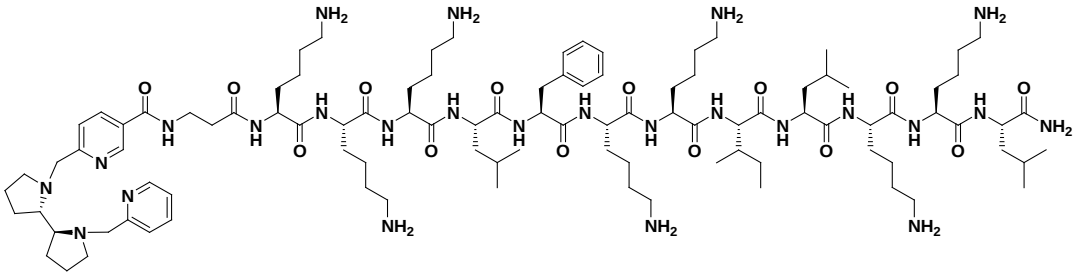
Observed HRMS (top) with the theoretical isotope prediction (bottom).



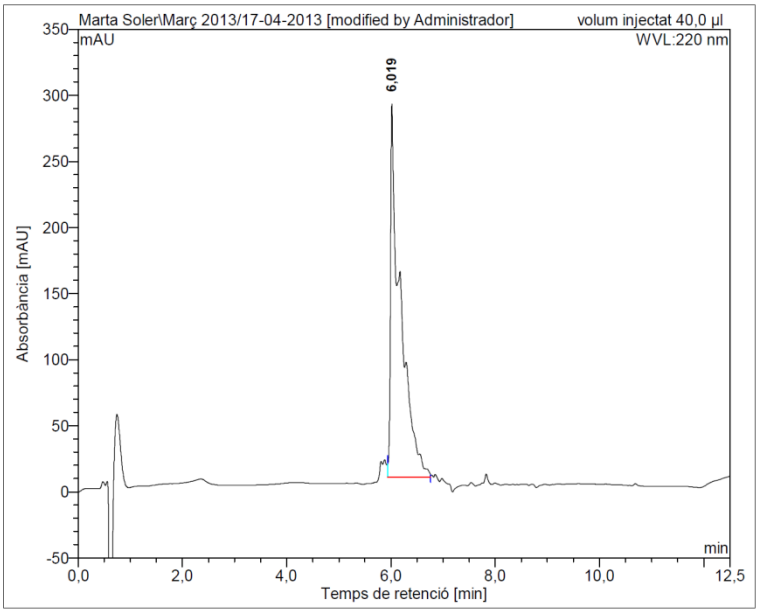
Observed HRMS (top) with the theoretical isotope prediction (bottom).

Figure S8: a) HPLC chromatogram (λ= 220 nm), b) HRMS spectrum (*m/z*).

BPBP-βAK-BP16 (**BP346**)

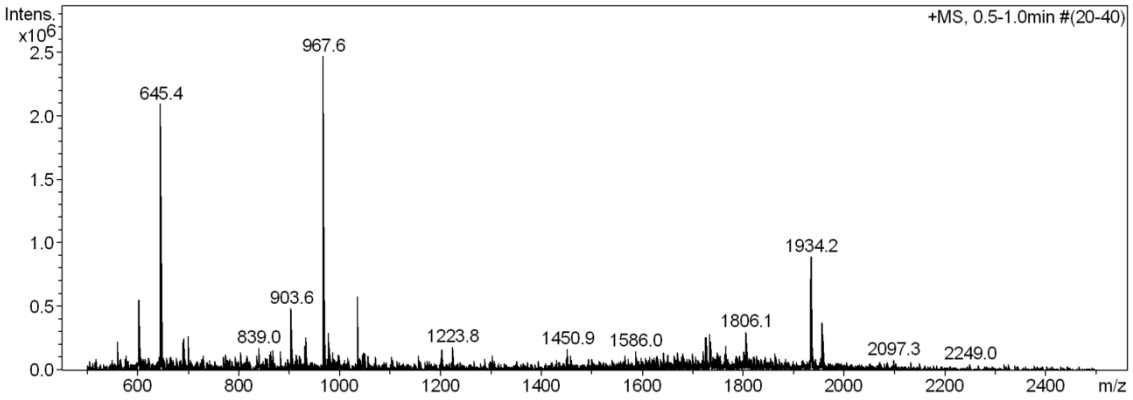


a)

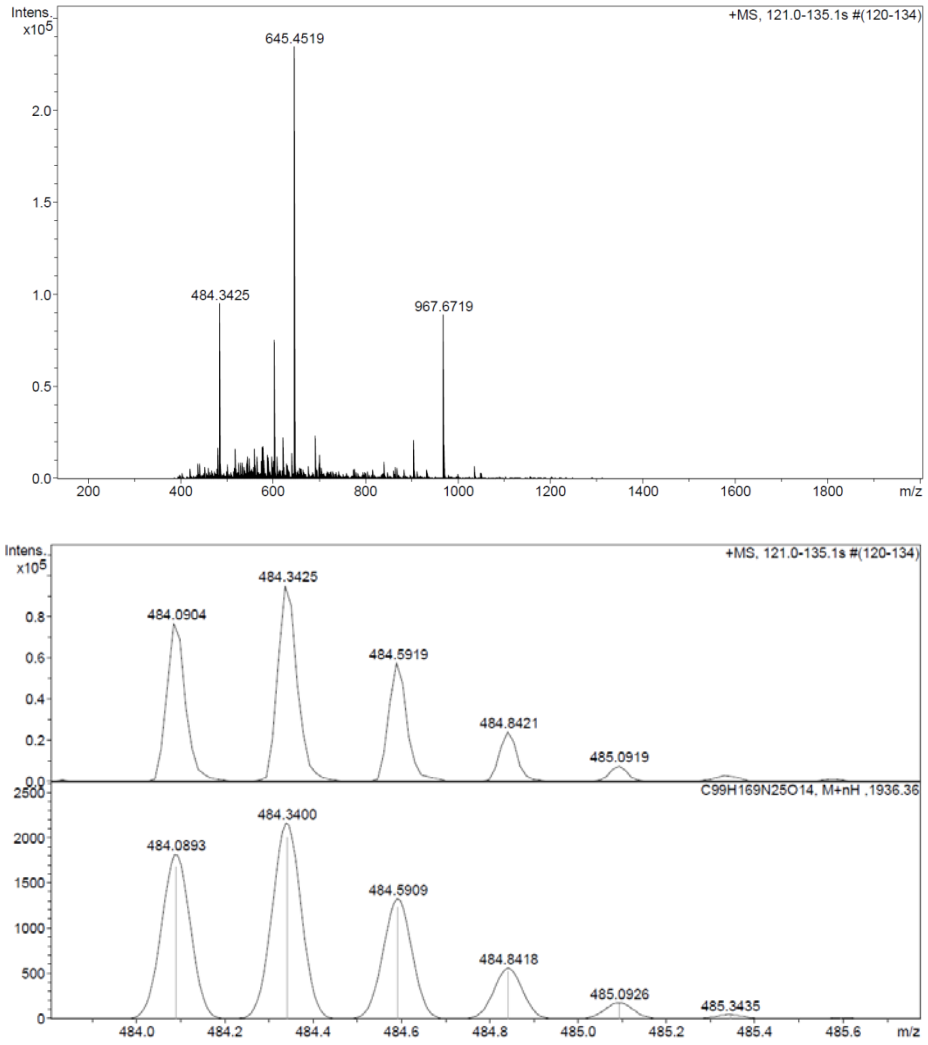


No.	mps retenc min	alçada mAU	Area mAU*min	Area relativa %
1	6,02	282,366	61,202	100,00
Total:		282,366	61,202	100,00

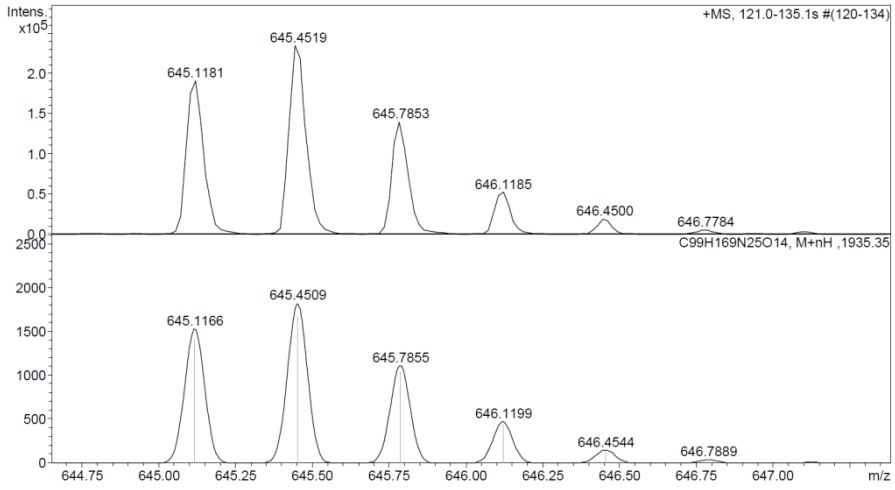
b)



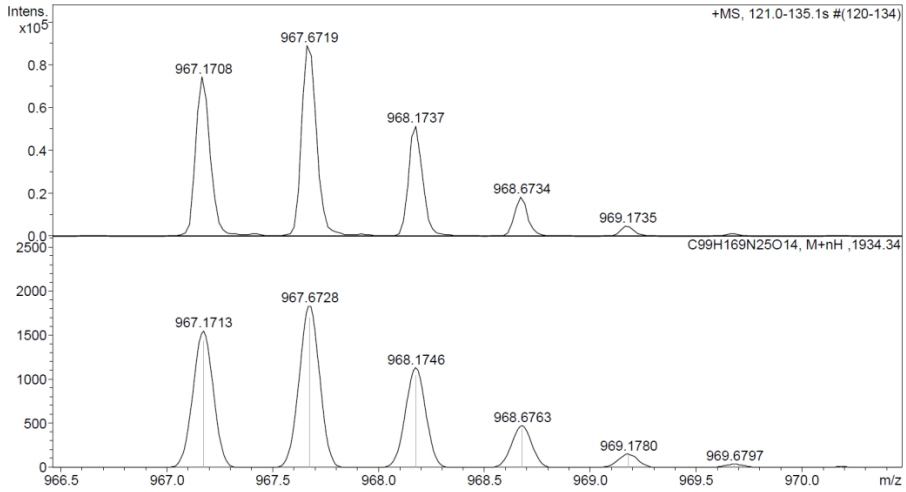
c)



Observed HRMS (top) with the theoretical isotope prediction (bottom).



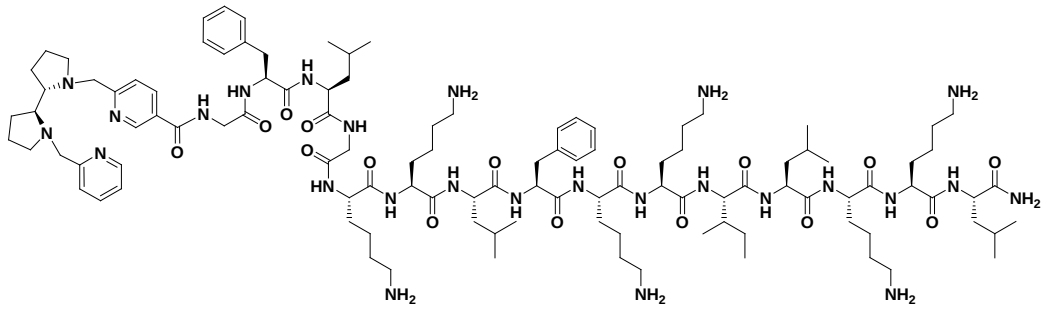
Observed HRMS (top) with the theoretical isotope prediction (bottom).



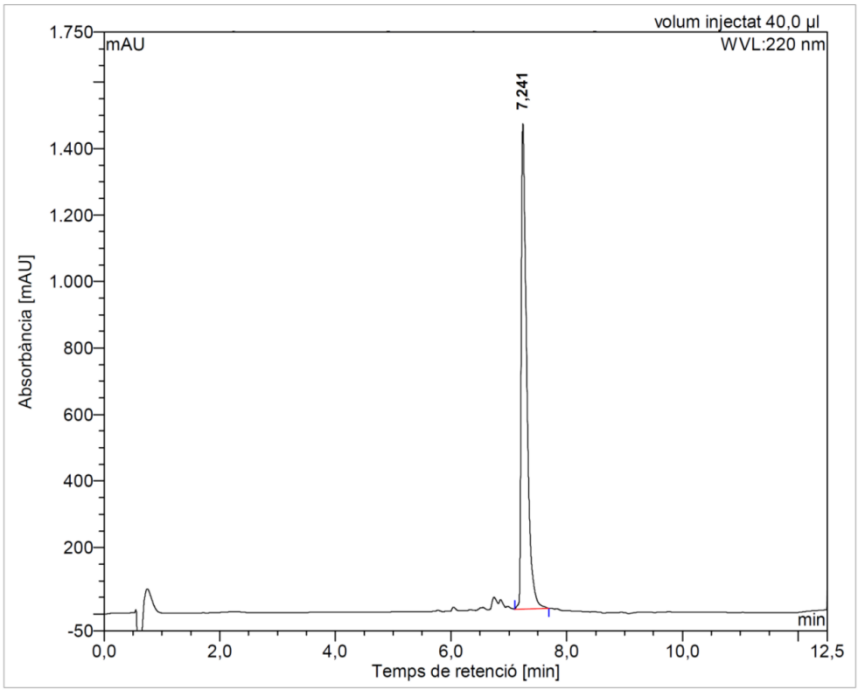
Observed HRMS (top) with the theoretical isotope prediction (bottom).

Figure S9: a) HPLC chromatogram (λ= 220 nm), b) HRMS spectrum (*m/z*).

BPBP-GFLG-BP16 (**BP347**)

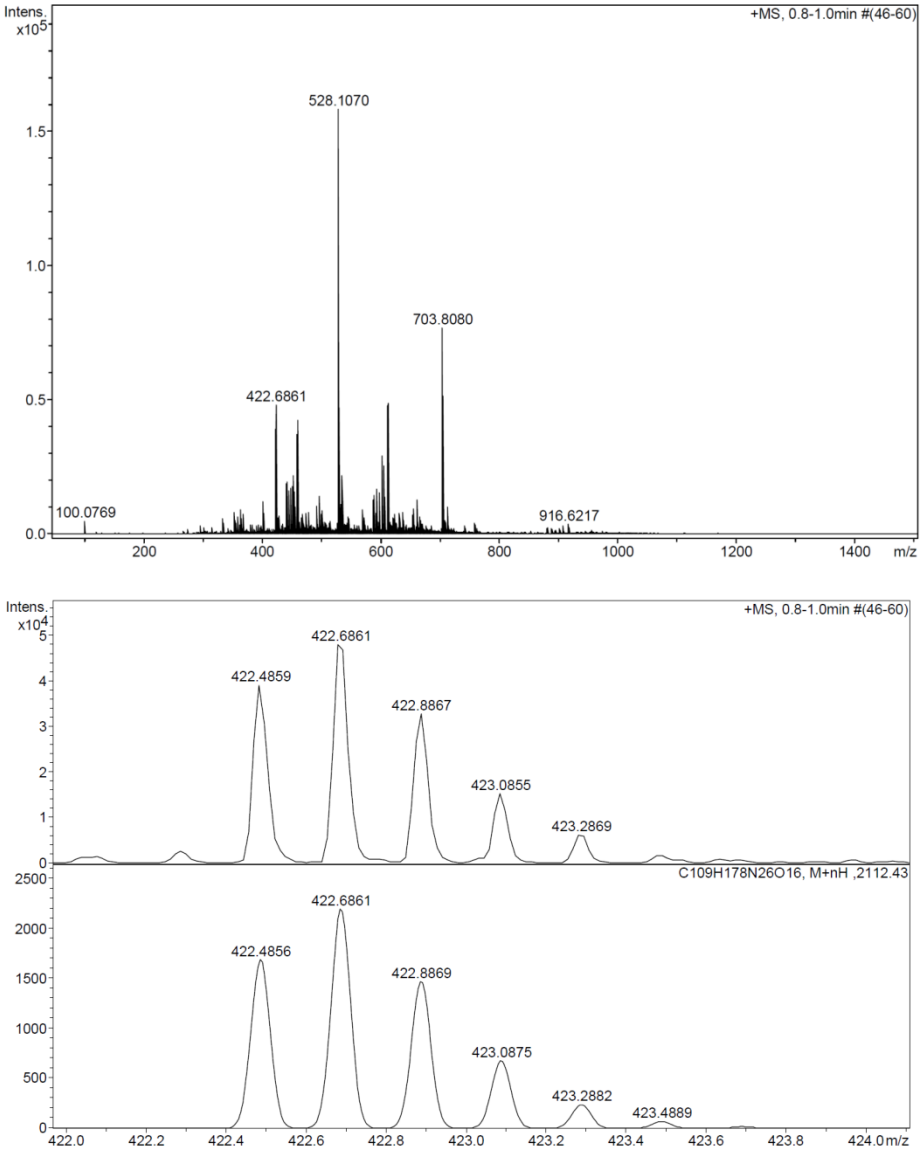


a)

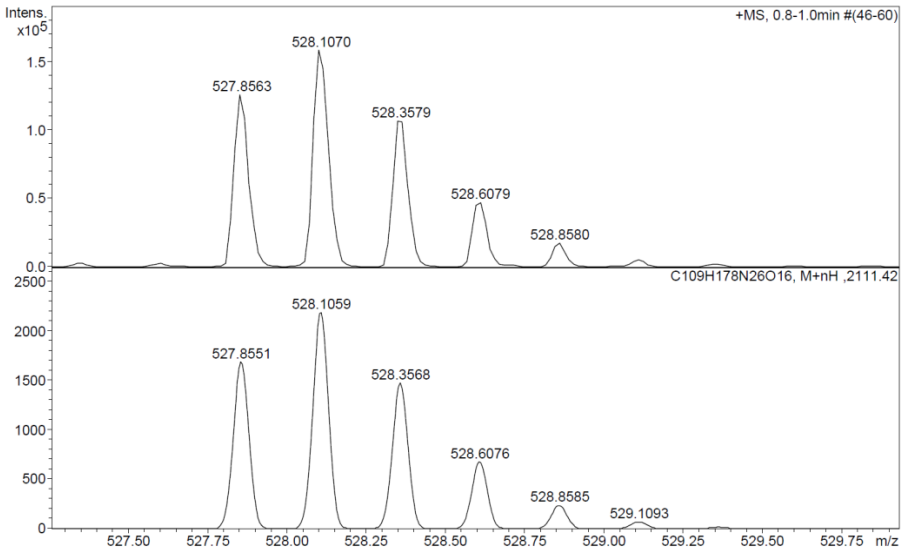


No.	mps retenc min	alçada mAU	Area mAU*min	Area relativa %
1	7,24	1460,388	161,177	100,00
Total:		1460,388	161,177	100,00

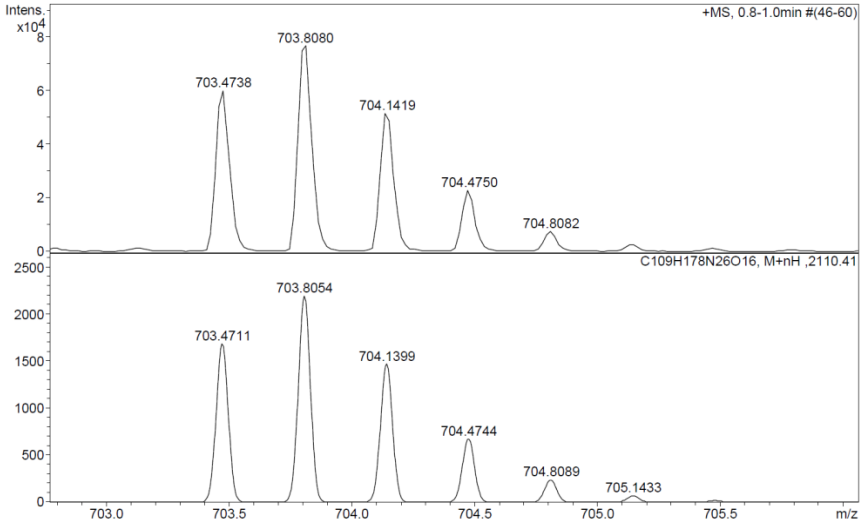
b)



Observed HRMS (top) with the theoretical isotope prediction (bottom).



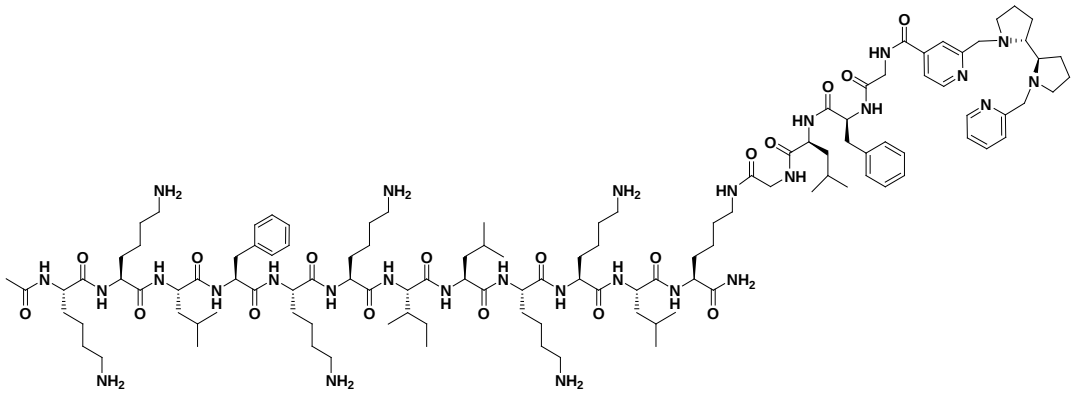
Observed HRMS (top) with the theoretical isotope prediction (bottom).



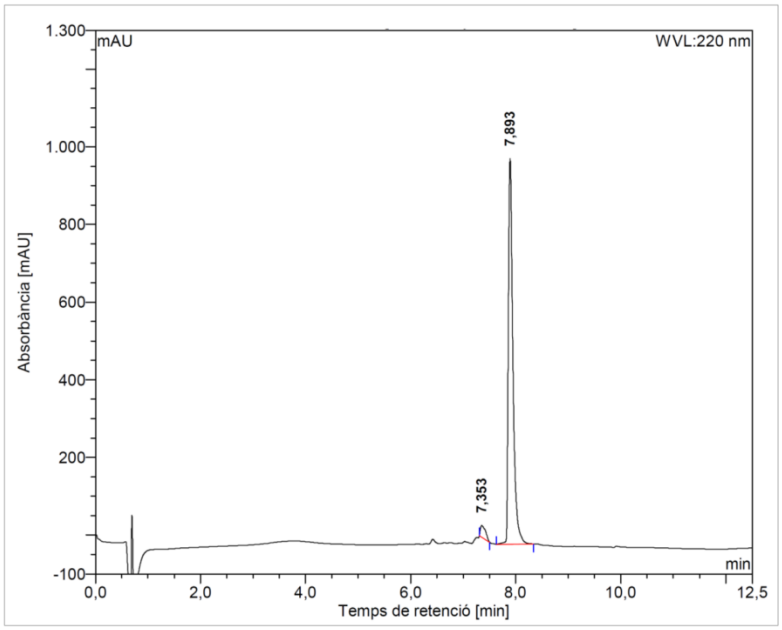
Observed HRMS (top) with the theoretical isotope prediction (bottom).

Figure S10: a) HPLC chromatogram (λ= 220 nm), b) HRMS spectrum (*m/z*).

BP16-GLFG-BPBP (**BP348**)

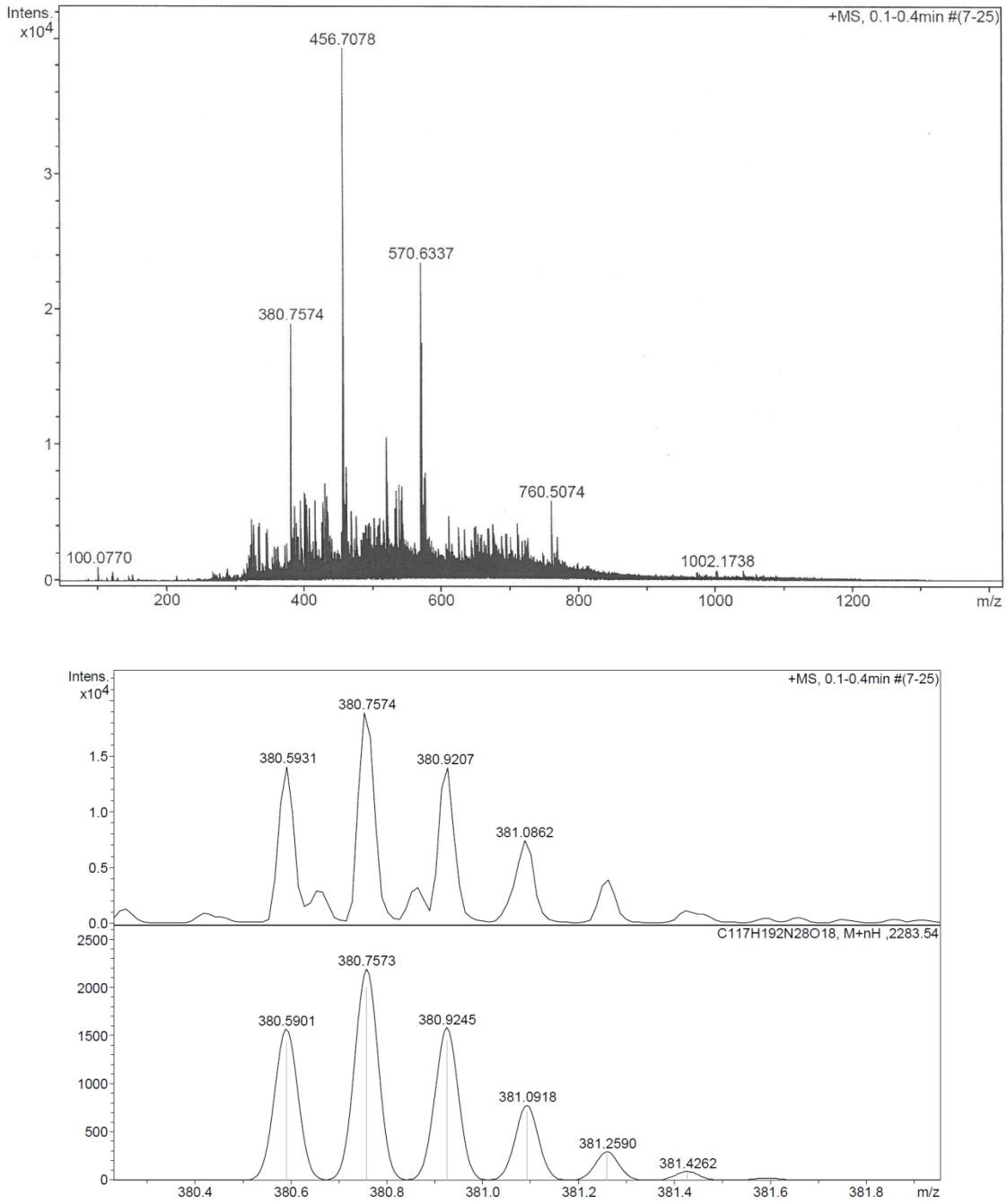


a)

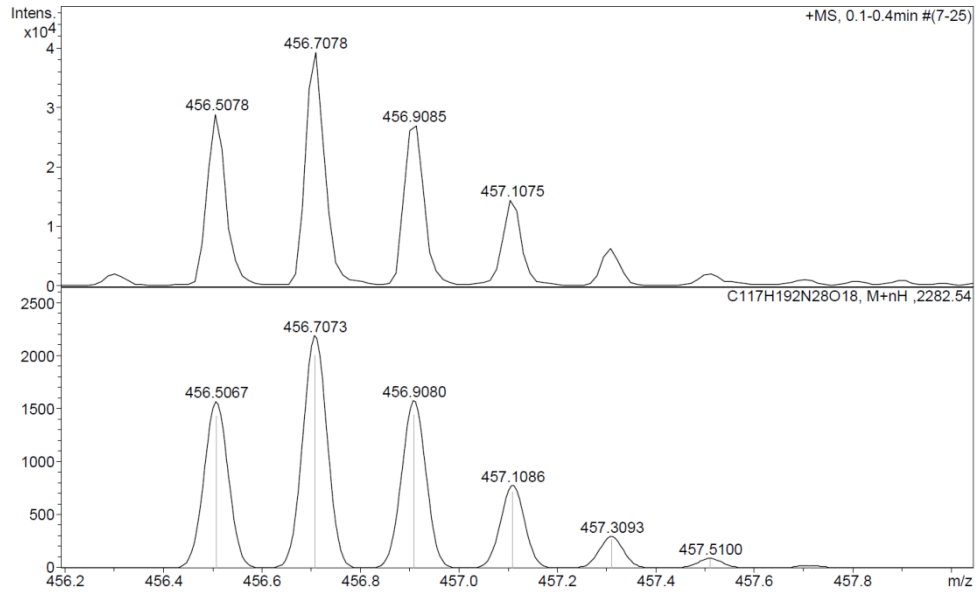


No.	mps retenc min	alçada mAU	Area mAU*min	Area relativa %
1	7,35	30,131	3,543	3,40
2	7,89	993,729	100,753	96,60
Total:		1023,860	104,296	100,00

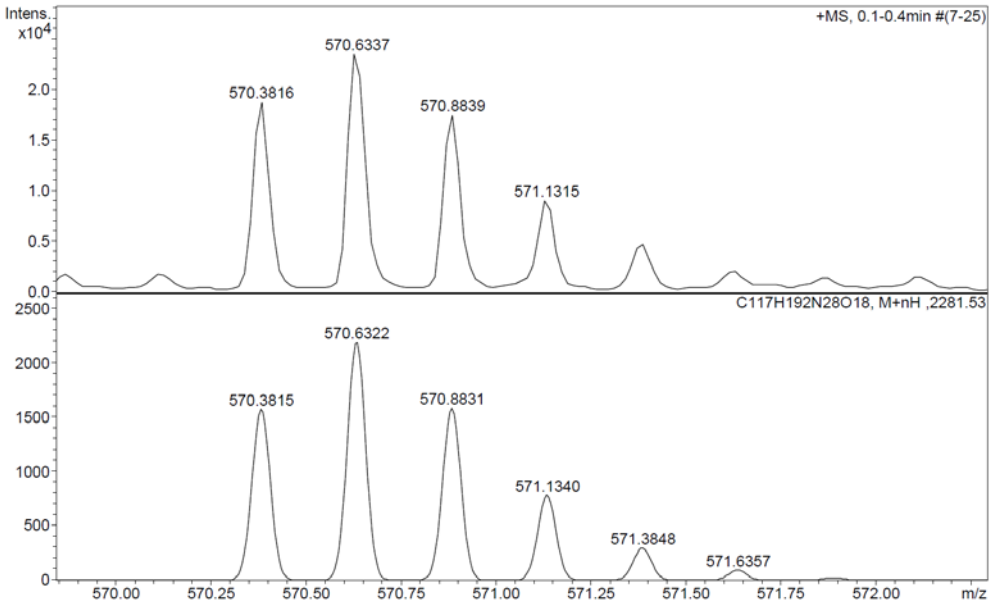
b)



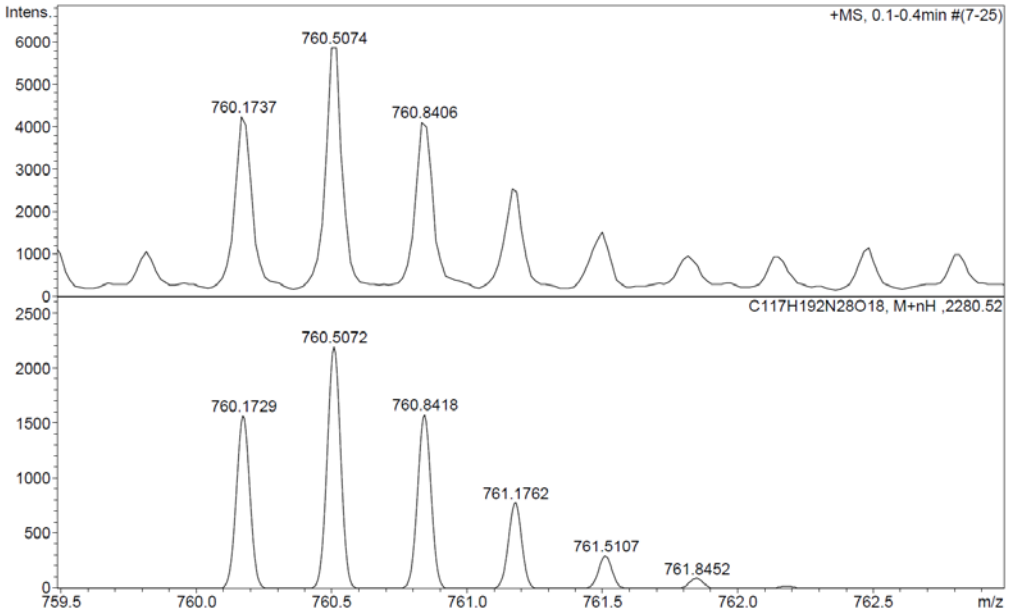
Observed HRMS (top) with the theoretical isotope prediction (bottom).



Observed HRMS (top) with the theoretical isotope prediction (bottom).



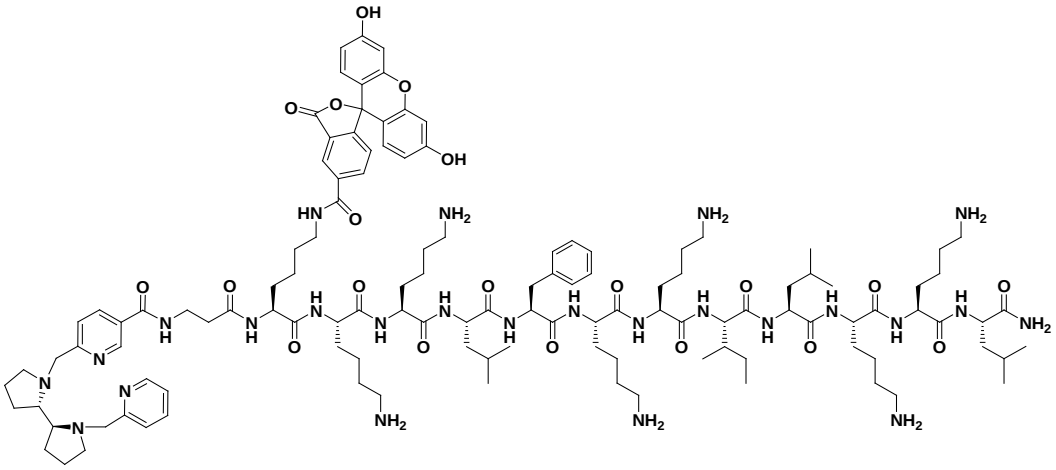
Observed HRMS (top) with the theoretical isotope prediction (bottom).



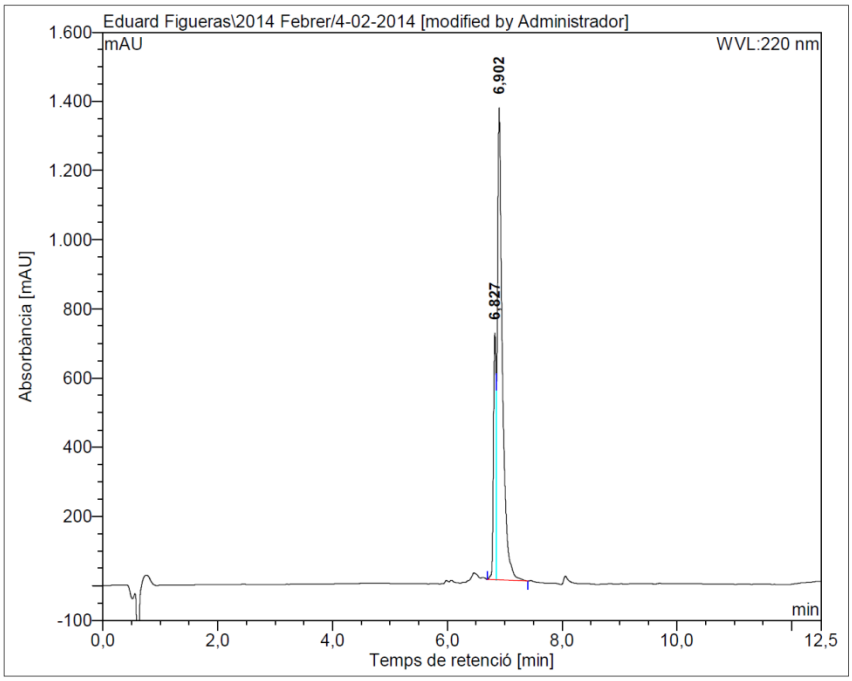
Observed HRMS (top) with the theoretical isotope prediction (bottom).

Figure S11: a) HPLC chromatogram (λ= 220 nm), b) HRMS spectrum (*m/z*).

BPBP-βAK(CF)-BP16 (**BP349)**

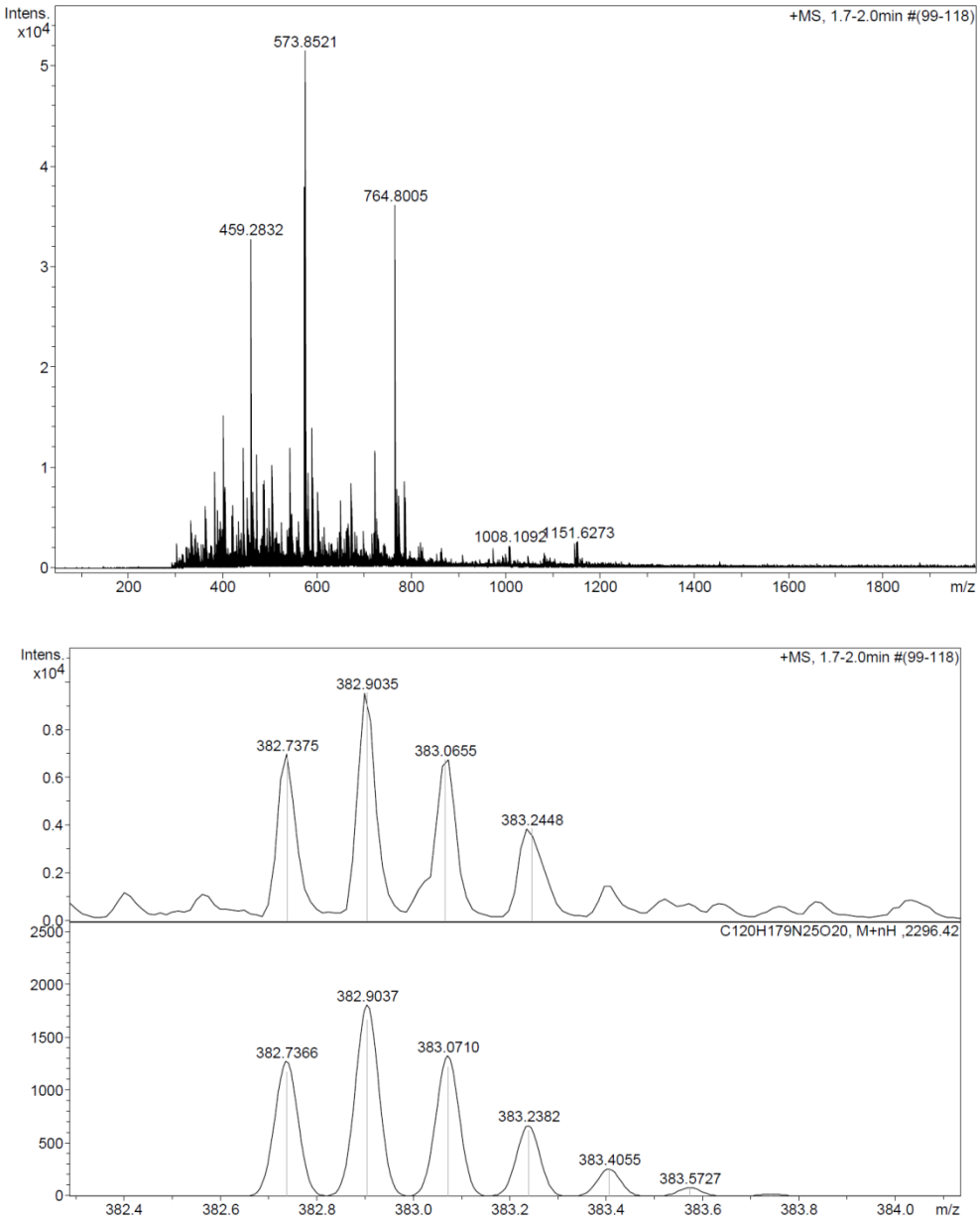


a)

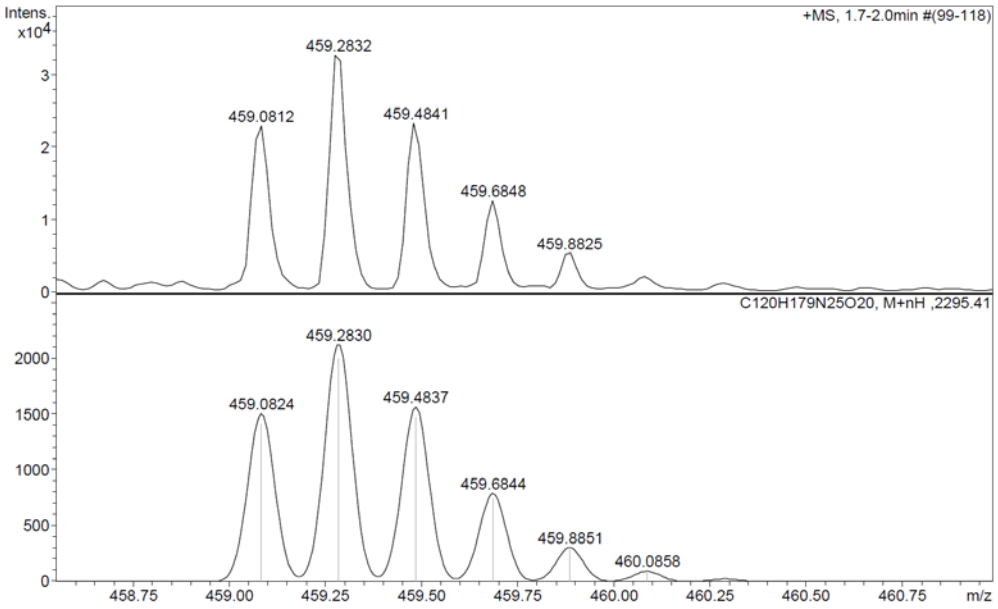


No.	Temps retenció min	alçada mAU	Area mAU*min	Area relativa %
1	6,83	713,518	38,489	21,98
2	6,90	1365,631	136,601	78,02
Total:		2079,149	175,089	100,00

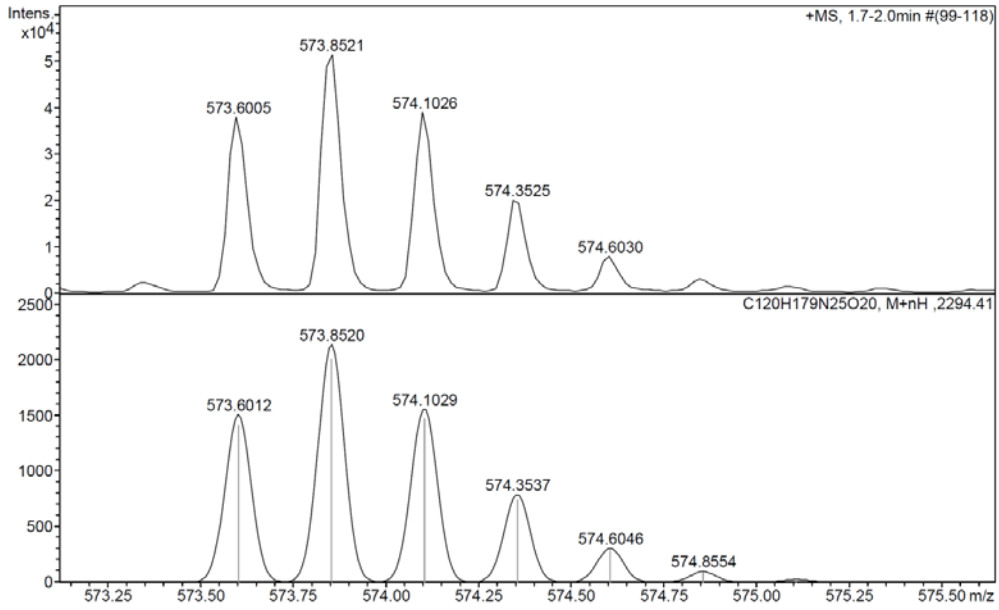
b)



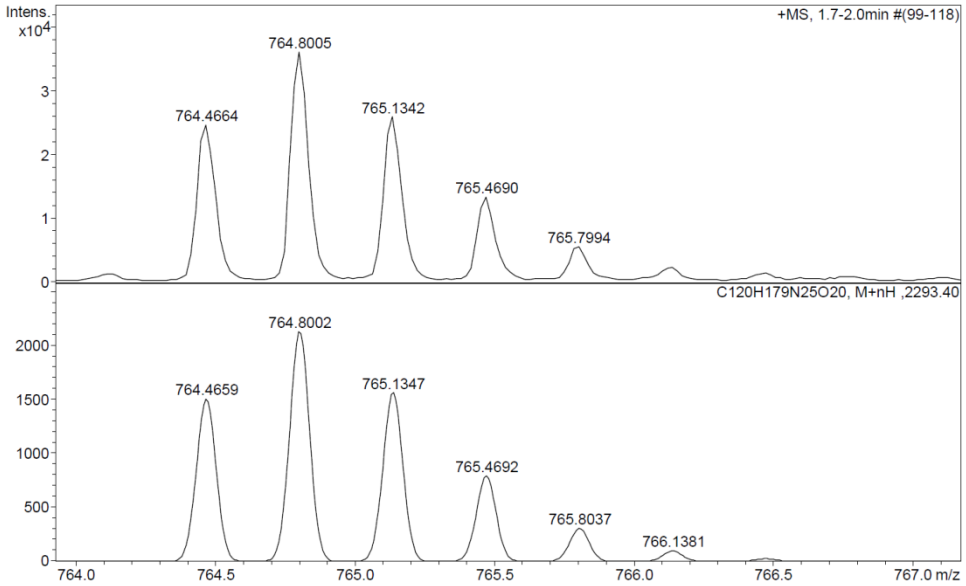
Observed HRMS (top) with the theoretical isotope prediction (bottom).



Observed HRMS (top) with the theoretical isotope prediction (bottom).



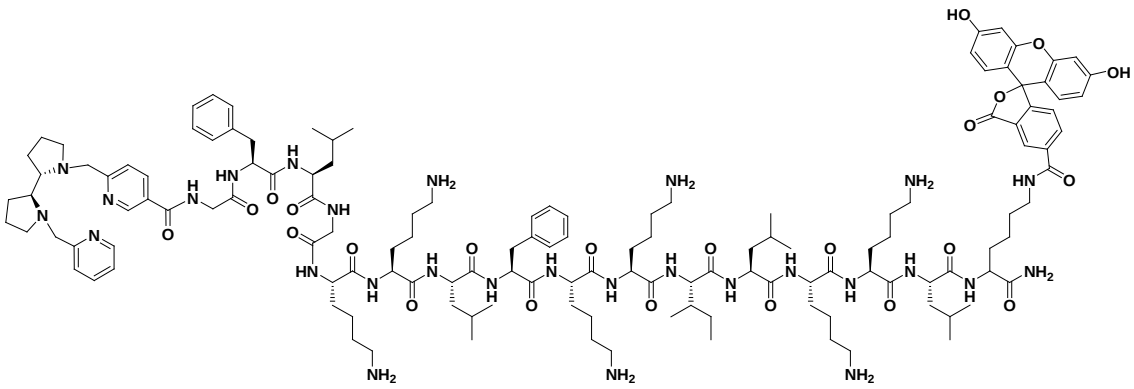
Observed HRMS (top) with the theoretical isotope prediction (bottom).



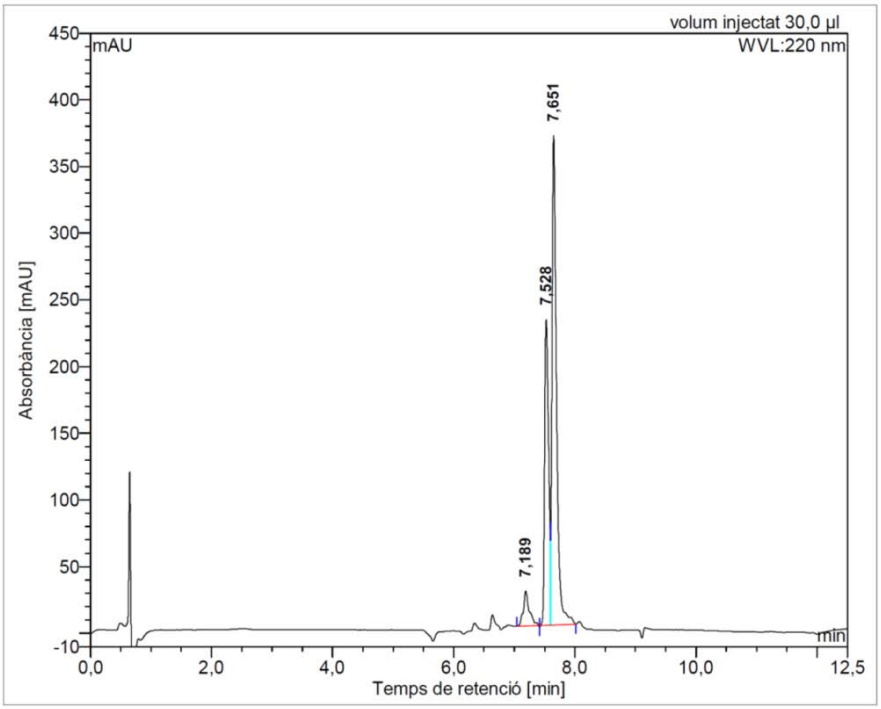
Observed HRMS (top) with the theoretical isotope prediction (bottom).

Figure S12: a) HPLC chromatogram (λ= 220 nm), b) HRMS spectrum (*m/z*).

BPBP-GFLG-BP16-CF (**BP350**)

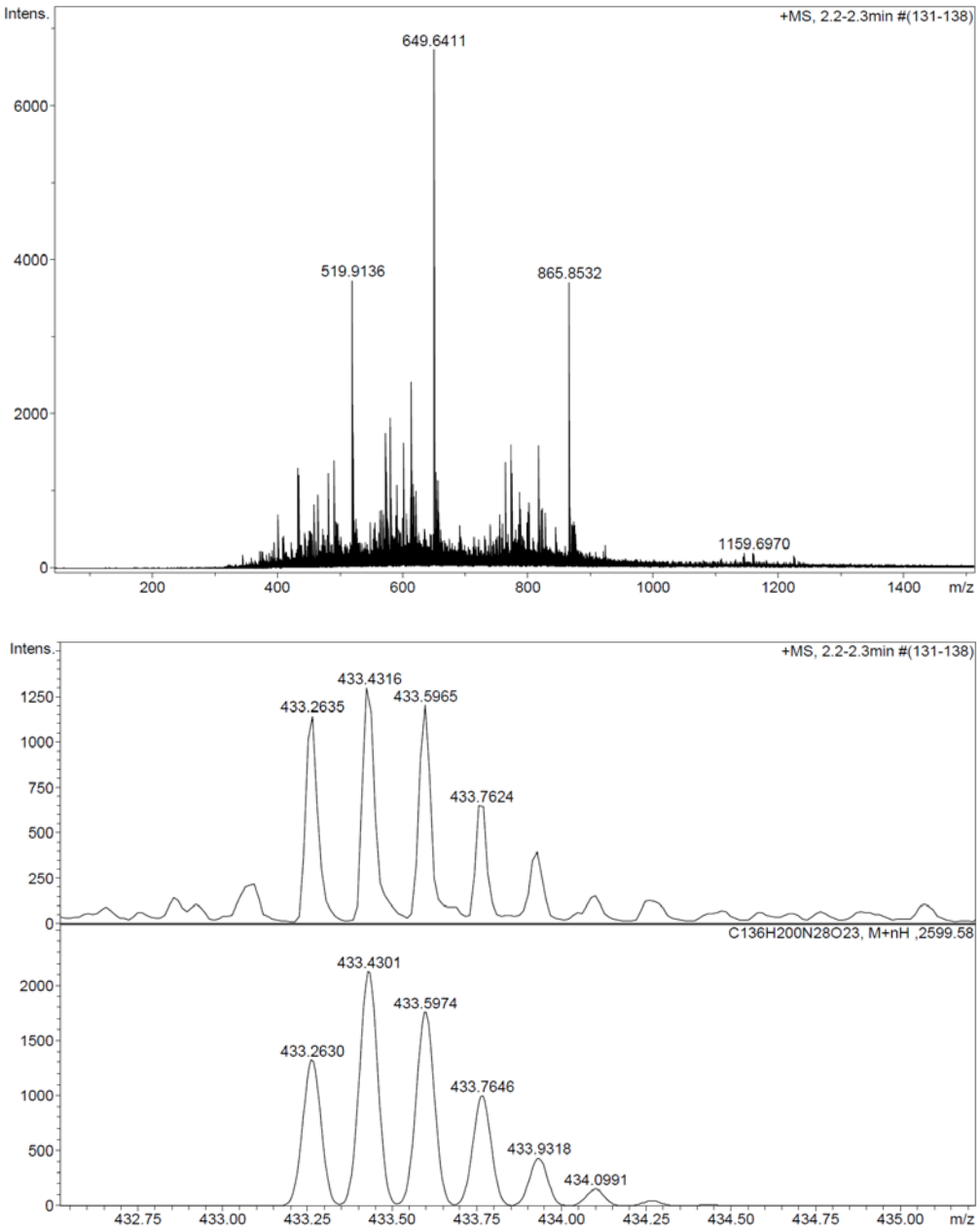


a)

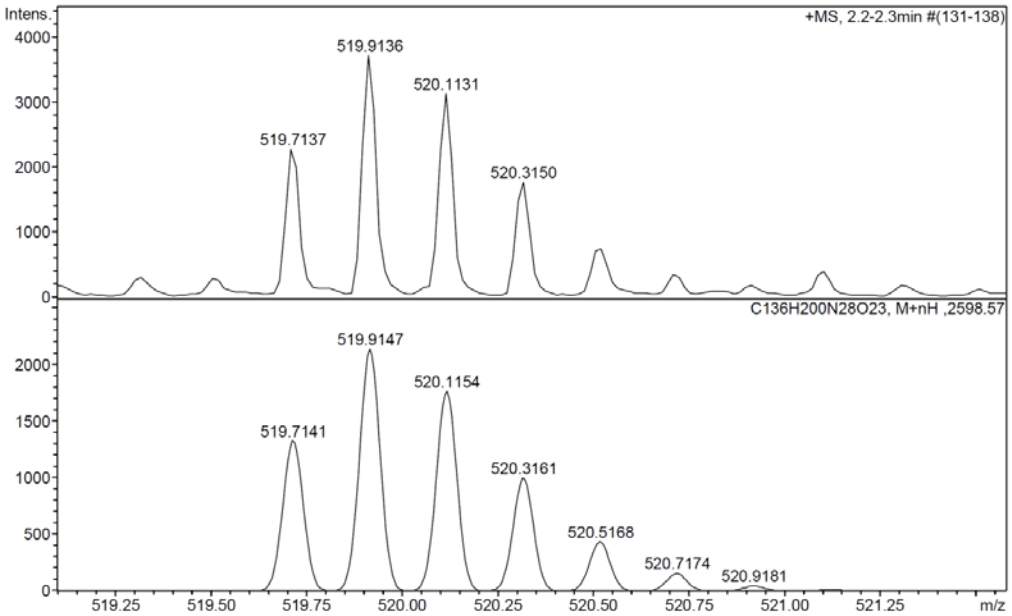


No.	mps retenc min	alçada mAU	Area mAU*min	Area relativa %
1	7,19	26,104	2,979	5,70
2	7,53	229,087	17,335	33,18
3	7,65	366,827	31,932	61,12
Total:		622,017	52,246	100,00

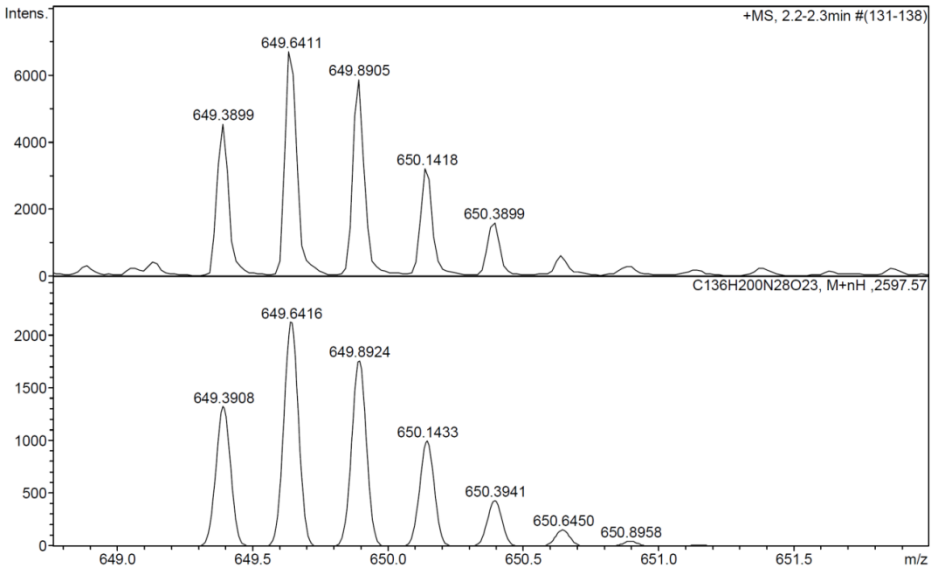
b)



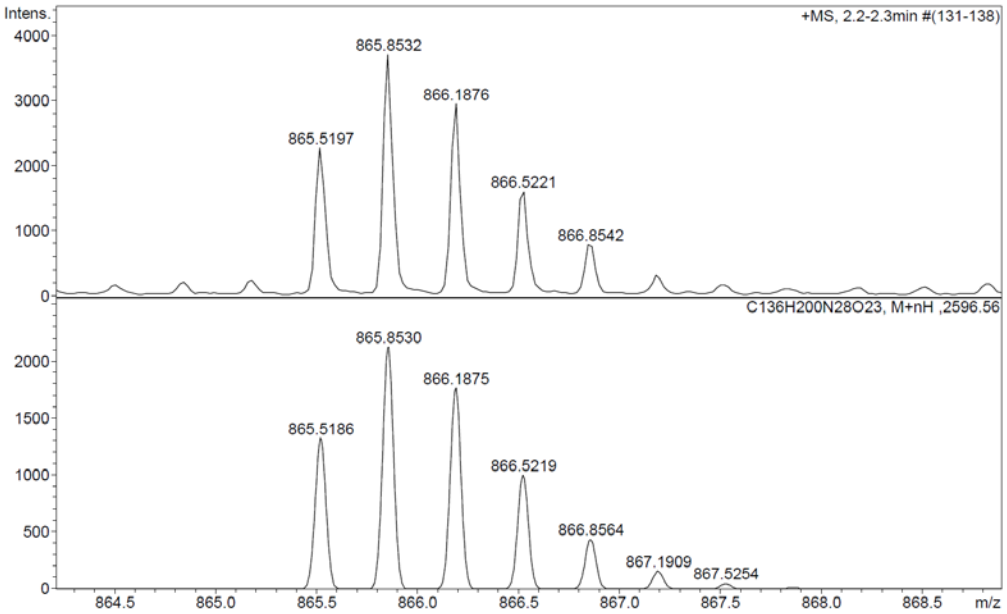
Observed HRMS (top) with the theoretical isotope prediction (bottom).



Observed HRMS (top) with the theoretical isotope prediction (bottom).



Observed HRMS (top) with the theoretical isotope prediction (bottom).



Observed HRMS (top) with the theoretical isotope prediction (bottom).