Modular sub-monomeric cell-penetrating guanidine-rich peptoids – Synthesis, assembly and biological evaluation

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SUPPORTING INFORMATION

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CHEMISTRY PROTOCOLS

MATERIALS AND METHODS (SOLUTION PHASE SYNTHESIS). Starting materials and solvents were purchased from Sigma-Aldrich (Canada), Fisher Scientific (USA), ACP (Canada), Combi-Blocks Inc. (USA), Chem Impex International (USA) or Merck Millipore (USA) and used without any further purification. Flash chromatography was performed using glass columns packed with silica gel (230-400 mesh, Silicycle (Canada)). Compounds purity was measured on an Acquity H-Class UPLC-MS system (PDA UV and SQD2 Mass detectors), equipped with a BEH C18 column (50 × 2.1 mm, 1.7 µm spherical particle size column) (Waters (Canada)) with a 0.8 mL/min flow rate using a gradient of 5–95 % acetonitrile + 0.1 % HCO2H in water + 0.1 % HCO2H over 2.5 min. UPLC-MS system and columns were purchased from Waters (Canada). NMR spectra were obtained on a Bruker (Germany) 300 or 400 MHz. NMR data are reported as follows : chemical shift in parts per million (ppm, δ units) are referenced to the residual protons of the solvent, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, dt = doublet of triplets, m = multiplet, and bs = broad singlet), coupling constant (Hz), and integration.

Allyl(2-bromoethyl)carbamate (2). To a solution of 2-bromoethylamine hydrobromide **1** (25 g, 122 mmol) in THF/H_2O (300 mL/240 mL), cooled to 0 °C, was added $NaHCO_3$ (30.8 g, 367 mmol, 3 eq) followed by allyl chloroformate (15 mL, 141 mmol, 1.15 eq) dropwise. The solution was stirred at rt overnight. THF was evaporated and the aqueous layer was extracted with ethyl acetate (4 x 50 mL). The organic layer was washed with HCl 1M (2 x 50mL) and brine (2 x 50 mL), then dried with MgSO4, filtered and concentrated *in vacuo* to obtain a colourless oil (88 %). This crude was used without any further purification in the next step. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 5.93 (m, 1 H), 5.32 (d, J = 17.4 Hz, 1 H), 5.17 (d, J = 10.5 Hz, 1 H), 5.13 (bs, 1 H), 4.56 (d, J = 5.4 Hz, 2 H), 3.61 (dt, J_1=J_2=5.7 Hz, 2 H), 3.47 (t, J = 5.5 Hz, 2 H). **HRMS** *m/z* 229.9789 $[M + Na]^+$ (229.9787 calcd for $C_6H_{10}BrNNaO_2^+$).

3,5-bis(2-(((allyloxy)carbonyl)amino)ethoxy)benzyl alcohol (4). To 3,5-dihydroxybenzyl alcohol **3** (0.2-2 g, 2 g/L) in acetone, allyl(2-bromoethyl)carbamate (3 eq), K_2CO_3 (3 eq) and TBAI (25% mol) were added and the solution was heated to reflux overnight. After cooling to rt, the crude was concentrated *in vacuo* and purified by flash chromatography with hexanes/ethyl acetate (2:8) (TLC : Rf 0.45, DCM/methanol 9:1). Pure fractions were concentrated to obtain **2** as a white powder (76 % yield, 97 % UPLC-UV purity). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.51 (d, J = 2.1 Hz, 2 H), 6.34 (t, J = 2.0 Hz, 1 H), 5.91 (m, 2 H), 5.35-4.9 overlapped (t, J = 6.2 Hz, 2 H), 5.30 (dd, J_1 = 1.3 Hz, J_2 = 17.3 Hz, 2 H), 5.21 (dd, J_1 = 1.2 Hz, J_2 =10.5 Hz, 2 H), 4.61 (bs, 2 H), 4.56 (d, J = 5.4 Hz, 4 H), 4.00 (t, J = 5.1 Hz, 4 H), 3.57 (dt, J_1 = J_2 = 5.4 Hz, 4 H), 2.04 (bs, 1 H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 159.9, 156.5, 143.9, 132.8, 117.9, 105.5, 100.6, 67.1, 65.8, 65.0, 40.6. **HRMS** *m/z* 417.1631 $[M+Na]^+$ (417.1632 calcd for $C_{19}H_{26}N_2NaO_7^+$).

Methyl 2-(2-nitrophenylsulfonamido)acetate (6). To a solution of glycine methyl ester hydrochloride **5** (4.16 g, 33.1 mmol) in 50 mL DCM was added water (2 mL) and Et_3N (9.4 mL, 67.4 mmol, 2 eq). The solution was cooled to 0 °C, then 2-nitrobenzenesulfonyl chloride (8.2 g, 37.0 mmol, 1.1 eq) was slowly added. The mixture was stirred at rt overnight then concentrated *in vacuo* to remove DCM. The crude mixture was diluted in water/ethyl acetate (70/40 mL) and extracted with ethyl acetate (4 x 40 mL). The combined organic phases were then washed with 1M HCl (50 mL), saturated NaHCO3 (50 mL) and brine (50 mL) before drying with MgSO4, filtered and concentrated *in vacuo*. The resulting solid was washed with toluene in a Büchner funnel to provide a white solid (8.01 g, 88 % yield). The latter was used with no further purification in the next step. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.09 (m, 1 H), 7.94 (m, 1 H), 7.75 (m, 2 H), 6.04 (t, J = 5.7 Hz, 1 H), 4.01 (d, J = 5.7 Hz, 2 H), 3.61 (s, 3 H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 169.1, 147.9, 134.1, 133.8, 133.0, 130.7, 125.8, 52.7, 44.9. **HRMS** *m/z* 297.0156 $[M+Na]^+$ (417.1632 calcd for $C_9H_{10}N_2NaO_6S^+$).

Methyl 2-(N-(3,5-bis(2-(((allyloxy)carbonyl)amino)ethoxy) benzyl)-2-nitrophenylsulfonamido)acetate (7). To a solution of methyl 2-(2-nitrophenylsulfonamido)acetate **6** (927 mg, 3.38 mmol, 1 eq) in 40 mL dry THF cooled to 0°C were added triphenylphosphine (1.33 g, 5.07 mmol, 1.5 eq) and DIAD (1 mL, 5.07 mmol, 1.5 eq). After the reaction medium thickened, a solution of 3,5-bis(2-(((allyloxy)carbonyl)amino)ethoxy)benzyl alcohol (2 g, 5.07 mmol, 1.5 eq) in dry THF was slowly added. The mixture became clearer and was stirred at rt for 20 min before the addition of another solution composed of DIAD (1.33 mL, 2 eq) and triphenylphosphine (1.77 g, 2 eq) in THF. After 20 minutes, the reaction was stopped and THF removed *in vacuo*. The crude mixture was purified via flash chromatography with a gradient of 40-60 % hexanes in ethyl acetate (TLC : Rf 0.31, hexanes/ethyl acetate 3:7). After purification, fractions containing the product were pooled and concentrated *in vacuo* to yield the product as a yellow oil (1.5 g, 68 %, 95 % UPLC-UV purity). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.12-8.04 (m, 1 H), 7.76-7.62 (m, 3 H), 6.38 (bs, 2 H), 6.34 (bs, 1 H), 5.91 (m, 2 H), 5.31 (d, J = 17 Hz, 2 H), 5.22 (d, J = 10 Hz, 2 H), 5.14 (bs, 2 H), 4.59 (s, 2 H), 4.57 (s, 4 H), 4.06 (s, 2 H), 3.94 (t, J = 5.1 Hz, 4 H), 3.36 (s, 3 H), 3.56 (dt, J_1 = J_2 = 5.2 Hz, 4 H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 169.0, 160.0, 156.3, 147.7, 137.0, 133.8, 133.1, 132.8, 131.8, 130.8, 124.2, 117.7, 107.0, 100.9, 66.9, 65.6, 52.2, 51.7, 46.9, 40.3. **HRMS** *m/z* 673.1796 $[M+Na]^+$ (673.1786 calcd for $C_{28}H_{34}N_4NaO_{12}S^+$).

2-(N-(3,5-bis(2-(((allyloxy)carbonyl)amino)ethoxy)benzyl)-2-nitrophenylsulfonamido)acetic acid (S1). **7** (2 g, 3.07 mmol, 1 eq) was diluted in a mixture of methanol (7.7 mL) and 2M NaOH (7.7 mL, 15.4 mmol, 5 eq). The solution was stirred at rt for 2.5 h. Methanol was then removed *in vacuo* and the mixture acidified with 3M HCl until it became cloudy. This aqueous phase was extracted thrice with ethyl acetate and the combined organic phases were dried with MgSO4, filtered and concentrated *in vacuo*. After concentration, a yellow foamy oil was obtained (1.74 g, 89 % yield, 94 % UPLC-UV purity). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 10.75 (bs, 1 H), 7.96 (d, J = 6.8 Hz, 1 H), 7.68-7.44

(m, 3 H), 6.25 (bs, 3 H), 5.77 (m, 2 H), 5.31 (dd, J_1 = 1 Hz, J_2 = 17 Hz, 2 H), 5.22 (dd, J_1 = 1 Hz, J_2 = 10 Hz, 2 H), 5.14 (bs, 2 H), 4.59 (s, 2 H), 4.57 (s, 4 H), 4.06 (s, 2 H), 3.94 (t, J = 5 Hz, 4 H), 3.36 (s, 3 H), 3.56 (t, J = 5 Hz, 4 H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 172.0, 160.0, 156.6, 147.8, 137.0, 133.9, 133.3, 132.7, 132.0, 131.0, 124.3, 118.0, 107.2, 101.3, 67.0, 65.9, 51.8, 47.1, 40.4. **HRMS** *m/z* 659.1627 $[M+Na]^+$ (659.1630 calcd for $C_{27}H_{32}N_4NaO_{12}S^+$).

MATERIALS AND METHODS (SOLID PHASE SYNTHESIS). Protected amino acids were purchased from ChemImpex International (USA). TentaGel S RAM resin (Rink amide resin, 0.22 meq/mg) from Rapp Polymere (Germany) was used. All reactions were carried out at 0.013 M concentration in 6 mL polypropylene cartridges with 20 μm PE frit from Applied Separations (USA). Each step was followed with a sequence of washings with DMF (3 x 4 mL), then isopropanol and DCM alternately (5 x 4 mL each) then DCM (2 x 4 mL). Fmoc deprotections were achieved with a 20% v/v solution of piperidine in DMF during two 15 min cycles. Fluorescein was introduced on the terminal amine by adding a solution of FITC (2 eq) and DIPEA (4 eq) in DCM/pyridine (7:3) to the resin, and letting it react for 30 min. After cleavage from the resin, the cleavage solution was filtered over glass wool and poured into ice-cold ether to precipitate the product. This mixture was centrifuged at 1500 rpm for 15 min and the supernatant removed. The pellet was then dissolved in water (polyarginine, polyamine and polyguanidine compounds) or a mixture of water and acetonitrile (1:1) (polybenzyl compounds) and filtered before purification. Polybenzyl, polyamine and polyguanidine compounds were purified by preparative HPLC (Waters Autosampler 2707, Quaternary gradient module 2535, UV detector 2489, fraction collector WFCIII) equipped with an ACE5 C18 column (250 × 21.2 mm, 5 μm spherical particle size) and water + 0.1% TFA and acetonitrile as eluents. Polyarginines were purified by preparative HPLC (Waters Sample Manager 2767, Binary gradient module 2545, SQ Detector 2) equipped with an XSelect Peptide CSH C18 OBD Prep Column (100 x 19 mm, 5 μm spherical particle size) and water + 0.1 % HCO_2H and acetonitrile + 0.1 % HCO_2H as eluents. Fractions were combined and lyophilized to recover pure compounds, the purity of which was measured on an Acquity H-Class UPLC-MS system (PDA UV and SQD2 Mass detectors, Waters) equipped with a BEH C18 column (50 × 2.1 mm, 1.7 μm spherical particle size column) for polybenzyl (with a 0.8 mL/min flow rate using a gradient of 30–95% (50-95 % for **PBn_5**) acetonitrile + 0.1% HCO_2H in water + 0.1% HCO_2H over 2.5 min) compounds and an HSS T3 column (30 x 2.1 mm, 1.8 μm spherical particle size column) for polyamine, polyguanidine and polyarginine compounds (with a 0.8 mL/min flow rate using a gradient of 1–30 % acetonitrile + 0.1 % HCO_2H in water + 0.1 % HCO_2H over 2.5 min). All compounds, with two exceptions (**PBn_4** and **PBn_5**) displayed a UV purity > 95 % (UV Range 210-400 nm).

Polyarginine transporters (Fluo-βA-R_{8-12}-NH_2 abbreviated "R_{8-12}"). Fmoc-L-Arg(Pbf)-OH or Fmoc-βAla-OH (5 eq) and HATU (5 eq) were solubilized in DMF, before introducing DIPEA (10 eq). The mixture was then added to the resin for 30-min reaction. Peptides were cleaved from the resin by exposing it to a mixture of TFA/water/TIPS (95:2.5:2.5) during 5 h.

Polybenzyl peptoids (Fluo-βA-(*N*Phe)$_{2-6}$-NH_2 abbreviated "PBn_{2-6}"). The bromoacetic acid strategy was applied. Bromoacetic acid (2.5 eq) and DIC (5 eq) were pre-mixed in DCM and left 15 min to allow the symmetric anhydride to form and the urea to precipitate. The solution was then added to the resin with DIPEA (6 eq) for 30-min reaction. Benzylamine (20 eq) diluted in DMF, was then added and the mixture shaken for for 1 h. Fmoc-βAla-OH (5 eq) was coupled using HATU (5 eq) during 2 h. The peptoids were then cleaved from the resin using TFA during 1 h.

Polyamine peptoids (Fluo-βA-(*N*{3,5-bis(2-(amino)ethoxy)) benzyl})$_{2-6}$-NH_2 (PAm_{2-6})) and polyguanidine peptoids (Fluo-βA-(*N*{3,5-bis(2-(guanidino)ethoxy))benzyl})$_{2-6}$-NH_2 ($PGua_{2-6}$)). A nosyl strategy was applied. Compound **S1** (3 eq) was coupled with HATU (3 eq) and DIPEA (5 eq) in DMF for 16 h. The nosyl group was removed using mercaptoethanol (10 eq) and DBU (5 eq) in DMF 2 x 30 min. The Alloc group was removed by using DMBA (2 eq/Alloc) and $Pd(PPh_3)_4$ (0.5 eq/Alloc) in dry DCM under argon 2 x 1 h. After Alloc deprotection, a specific series of washings was performed before the regular ones, using 0.02 M sodium diethyldithiocarbamate in DMF until the washing solution came out colourless. Guanidinylation was performed with Goodman's reagent (1,3-Bis(*tert*-butoxycarbonyl)-2-(trifluoromethanesulfonyl)guanidine) (2 eq/amine) and DIPEA (2 eq/amine) in DCM for 4 h. Cleavage of these peptoids was performed using TFA/water/TIPS (95:2.5:2.5) during 2 h.

BIOLOGICAL ASSAYS PROTOCOLS

Cell culture. HeLa cells were purchased from the American Type Culture Collection (Manassas, VA, USA) and CHO cells K1 and pgsA-745 were kindly provided by Pr Steve Bourgault (Université du Québec à Montréal), HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) high glucose and CHO cells were grown in Ham's F-12 Nutrient Mixture (F-12) L-Glutamine (Invitrogen, Carlsbad, CA, USA). Both culture media were supplemented with 10 % fetal bovine serum (FBS) (Hyclone Laboratories, Logan, UT, USA), and 1 % Penicillin–Streptomycin–Glutamine solution (Invitrogen, Carlsbad, CA, USA). Unless otherwise mentioned, all incubation sequences were done at 37 °C in the presence of 5 % CO_2.

Fluorescence Activated Cell-sorter Scan (FACScan). Approximately 1.2 × 10^5 cells were plated on coverslips in 35 mm culture dishes (Corning Inc., Corning, NY, USA). The cells were grown in their respective media for 24 h. The day of the experiment, cells were treated by adding 5 μM FITC-labeled peptide or peptoids to the culture medium and incubated for 30 min. Cells were washed once with PBS and taken

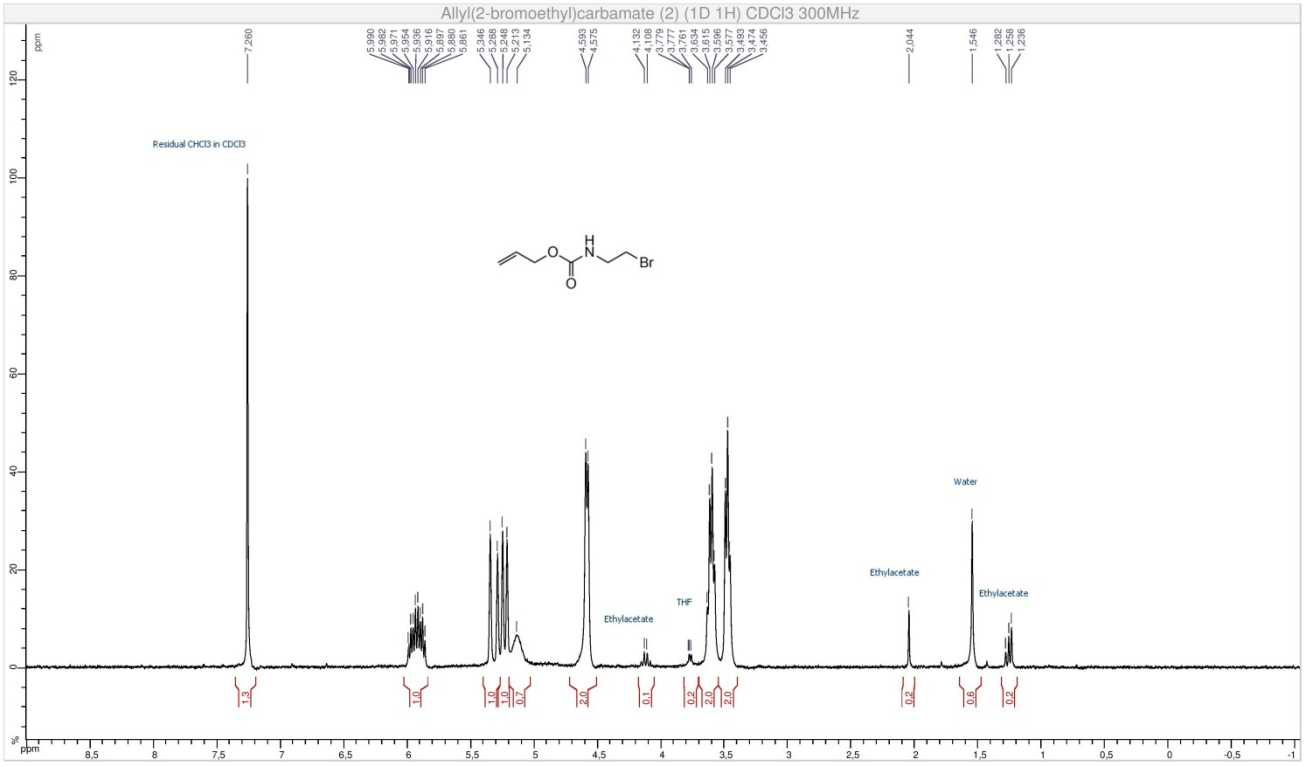
off by treating with Trypsin-EDTA 0.05 % (GIBCO, Invitrogen, Carlsbad, CA, USA) during 7 min. Finally, cells were resuspended in PBS buffer containing 10 µg/mL propidium iodide to establish the live gate to exclude debris and dead cells and 0.05 % w/v Trypan Blue to ensure extracellular fluorescence quenching. A minimum of 10 000 gated events by sample were acquired and analyzed by a FACScan cytometer (Becton Dickinson, Mountain View, CA) equipped with a 15 mW argon ion laser tuned at 488 nm. The emitted fluorescences were split and collected as follows: FITC 530 ± 15 nm (green), PI 585 ± 21 nm (orange).

Cytotoxicity assay (LDH assay). Approximately 4×10^4 cells were seeded into a 24-well plate (Corning Inc., Corning, NY, USA) in 500 μL culture medium. After 24 h, the culture medium was discarded and replaced with 250 μL of medium containing the different compounds (5 or 50 µM). After 24 h, supernatant was collected in 600 μL Eppendorf tubes and wells were gently washed with PBS. Four hundred μL of lysis buffer [1 % Triton X-100 in water] was added to each well and shaken at room temperature during 10 min. Lysates were collected in Eppendorf tubes and both supernatants and lysates were submitted to centrifugation at 1000 rpm for 5 min. Eighty μL of each supernatant and lysate were transferred in a 96-well plate (Becton Dickinson and Co., Franklin Lakes, NJ, USA) and .120 μL of a NAD^+ buffer [10mM in a pH 8.5, 85 mM TAPS and 70 mM lactic acid solution in water] were next added to these wells. The spectrophotometric absorbance of the samples was measured during 2 min at 340 nm using a microplate reader (NanoQuant, Infinite M200, Tecan Trading AG, Männedorf, Switzerland). Untreated cells were used as negative controls and cells treated with 1% Triton X-100 as positive (100% death) controls. Wells containing 80 μL of medium or Triton in absence of cells were used as blanks.

CHARACTERISATIONS OF CHEMICAL COMPOUNDS

Content description. Here are provided the NMR spectra reported above, as well as the MS ionisation profiles of the compounds (complementary to the HRMS measurements), as proof of identity. UV profiles of UPLC injections (using the methods described above) are provided here as proof of purity. For compound **2**, z well known and documented intermediate, only ^{1}H NMR was performed to validate the structure. For solid phase syntheses, as explained above, NMR were non relevant so only MS and UV profiles are provided here

Allyl(2-bromoethyl)carbamate (**2**)



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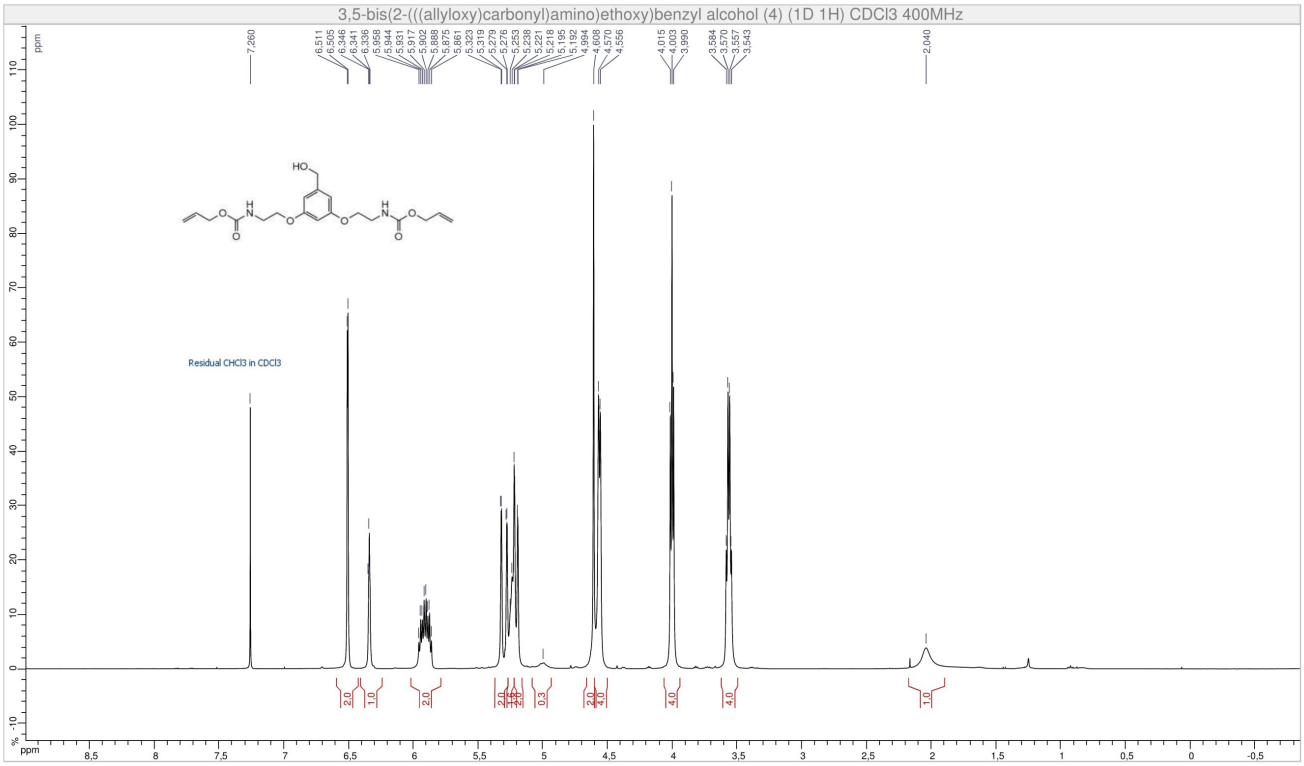

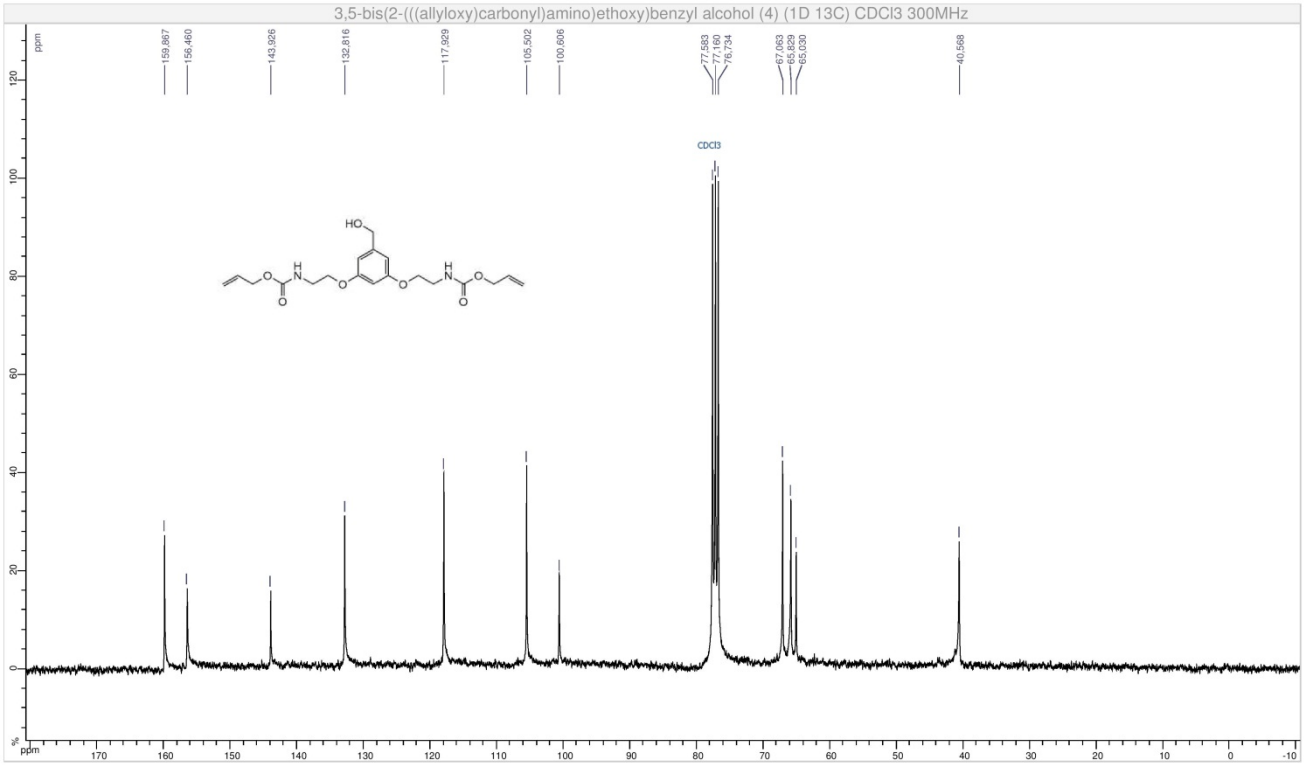

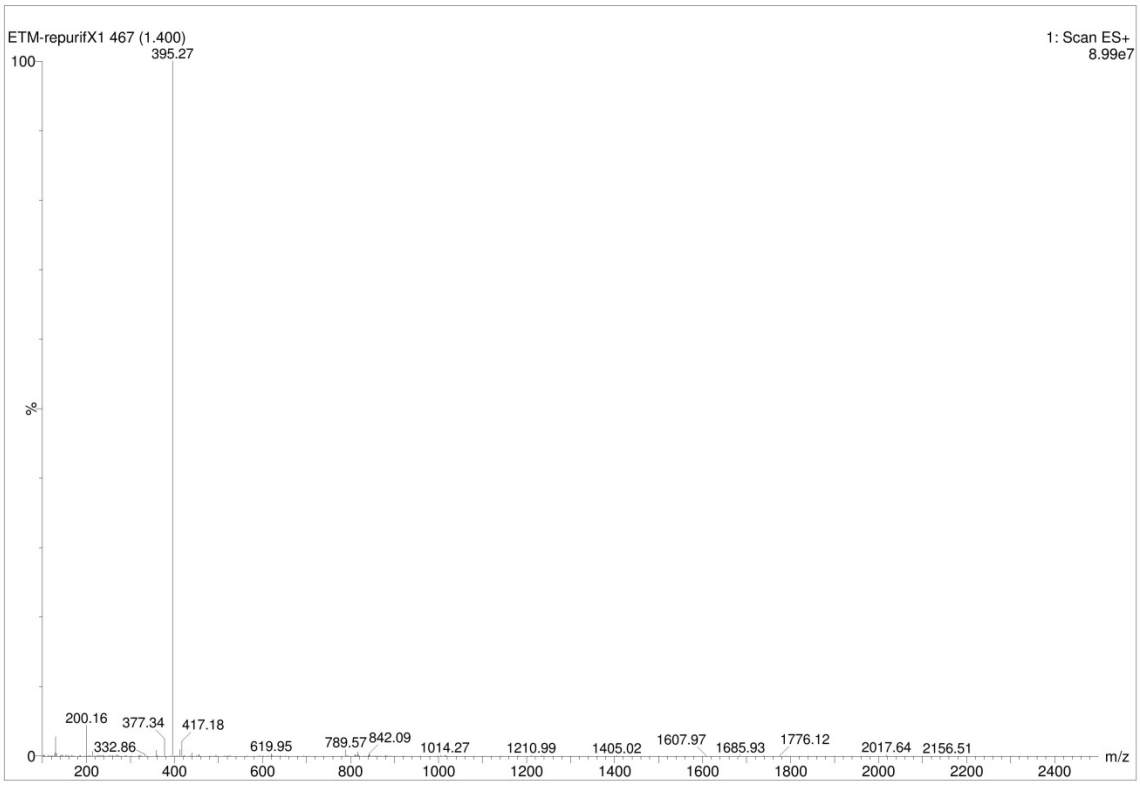
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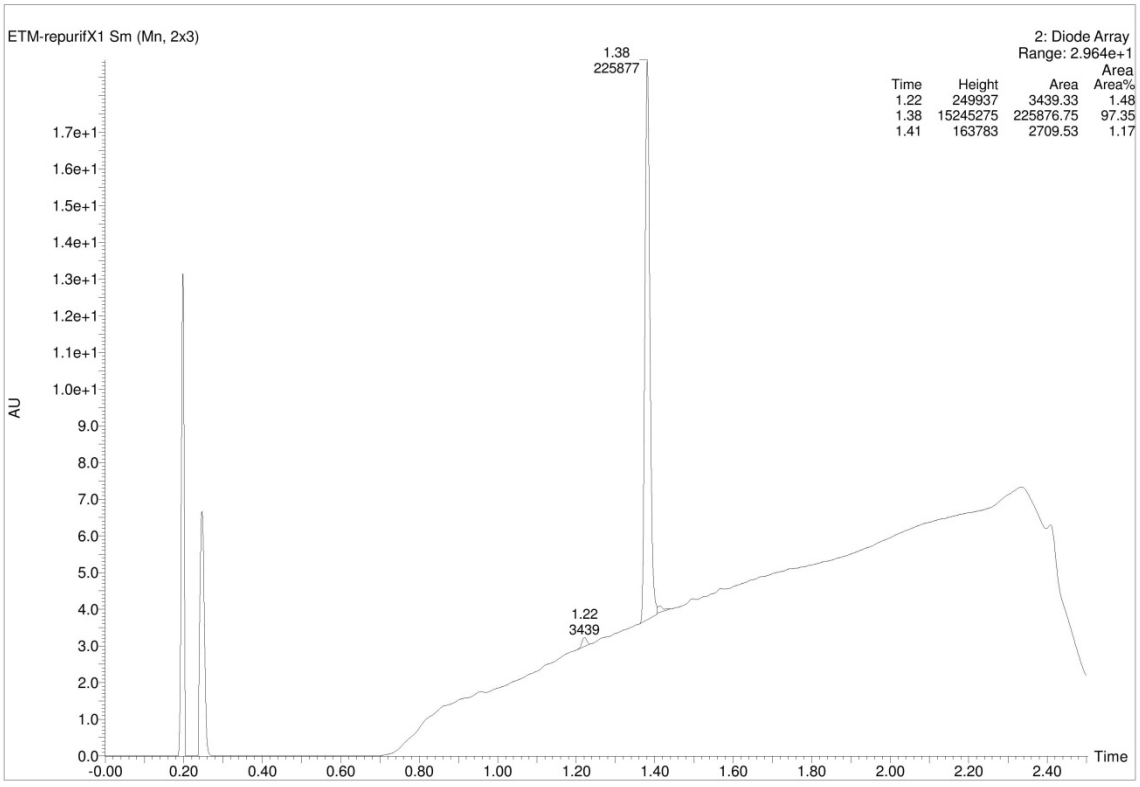
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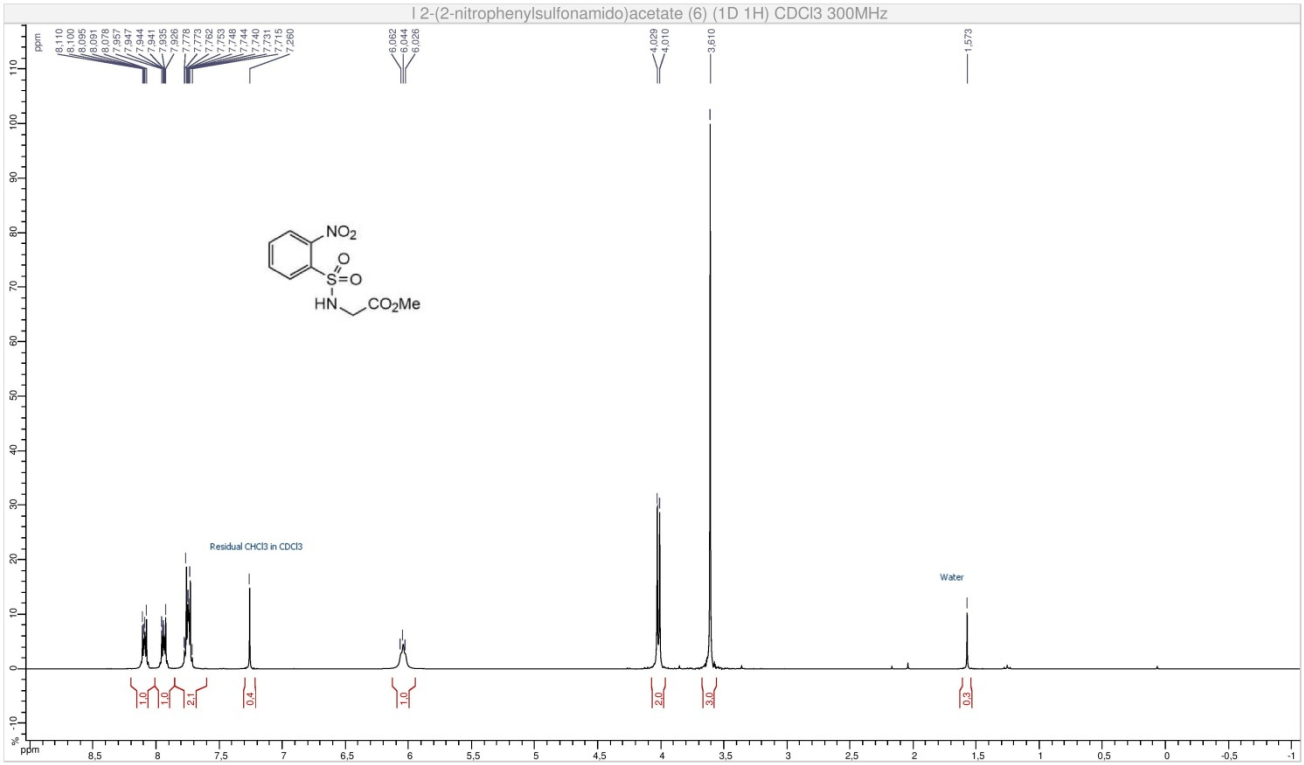
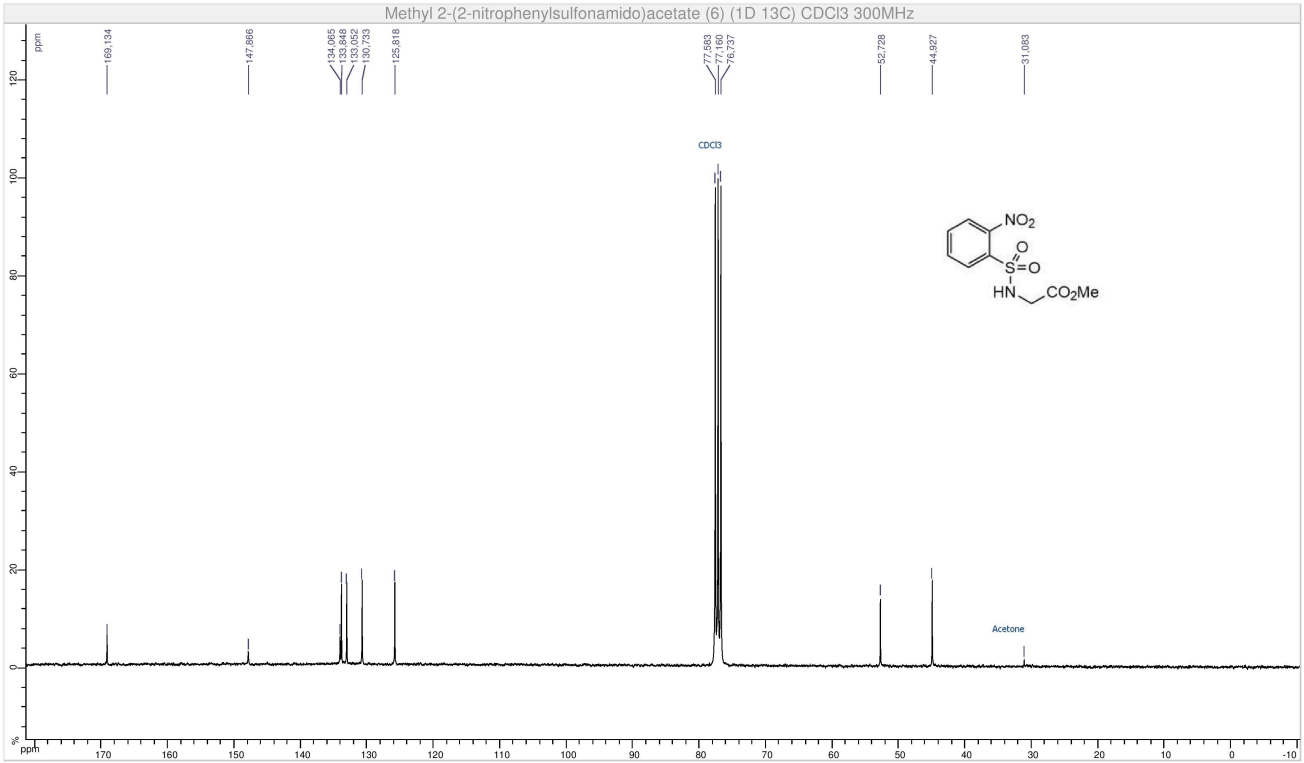
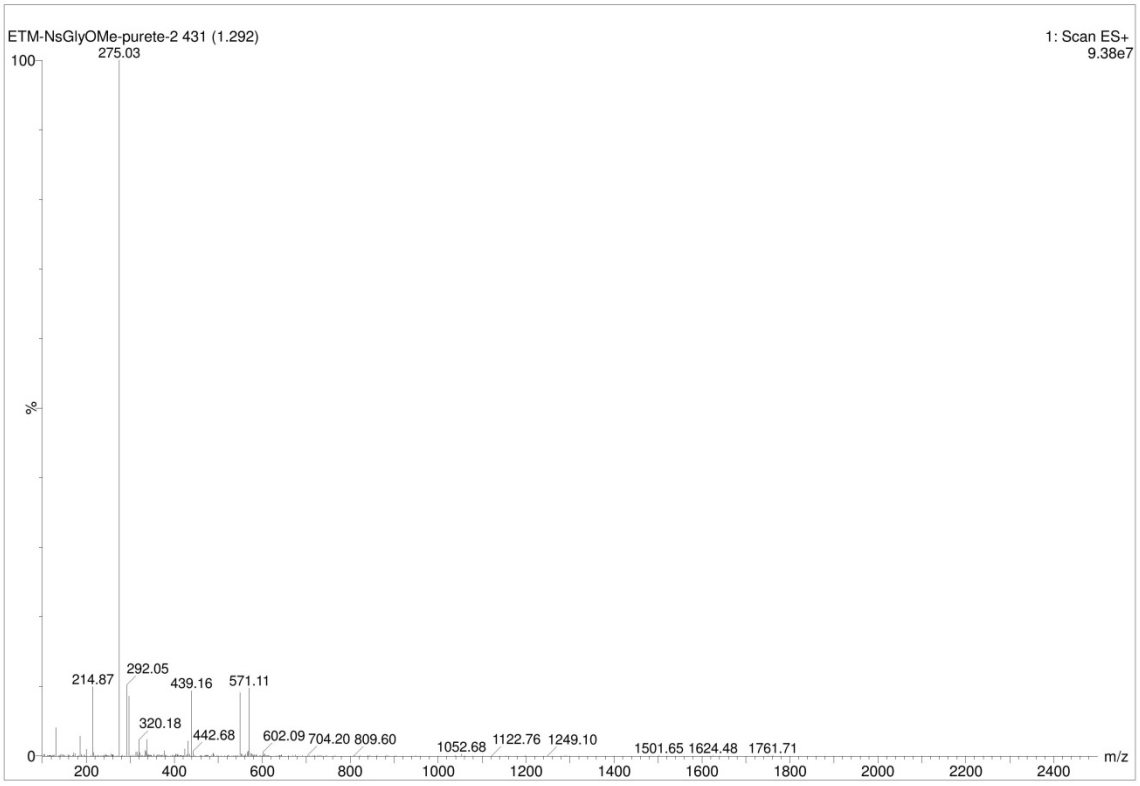
Methyl 2-(2-nitrophenylsulfonamido)acetate (**6**)



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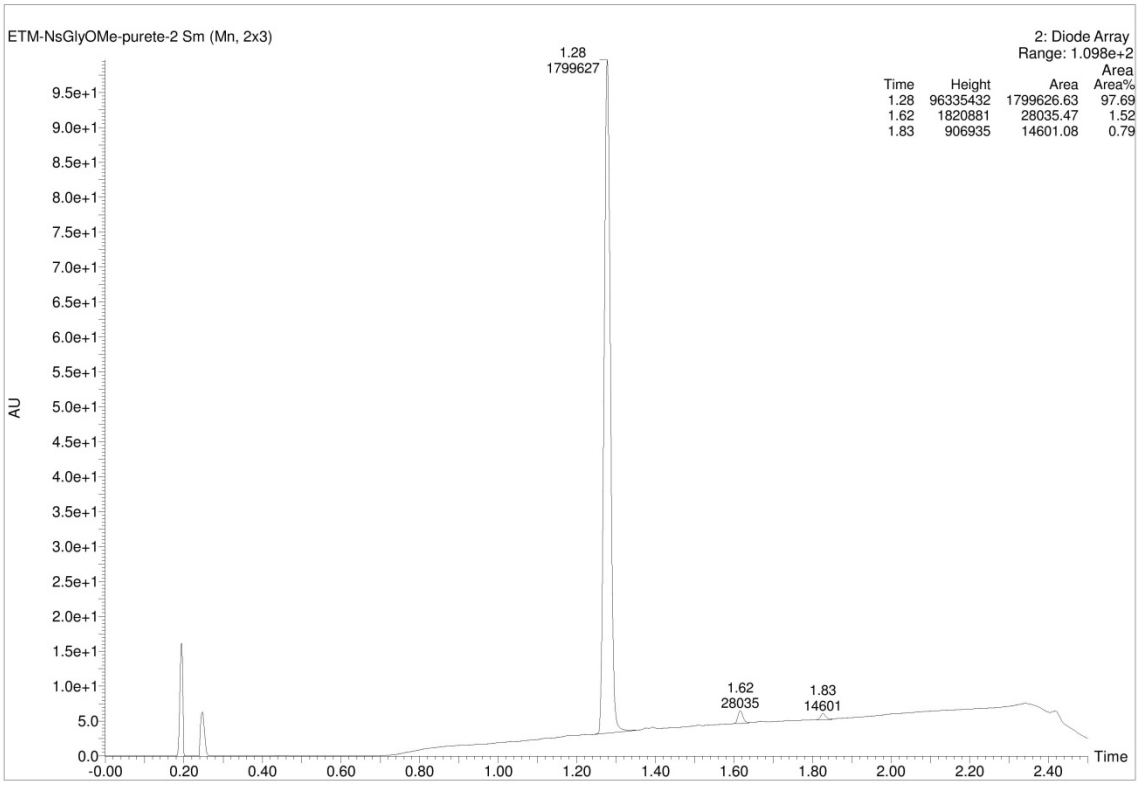
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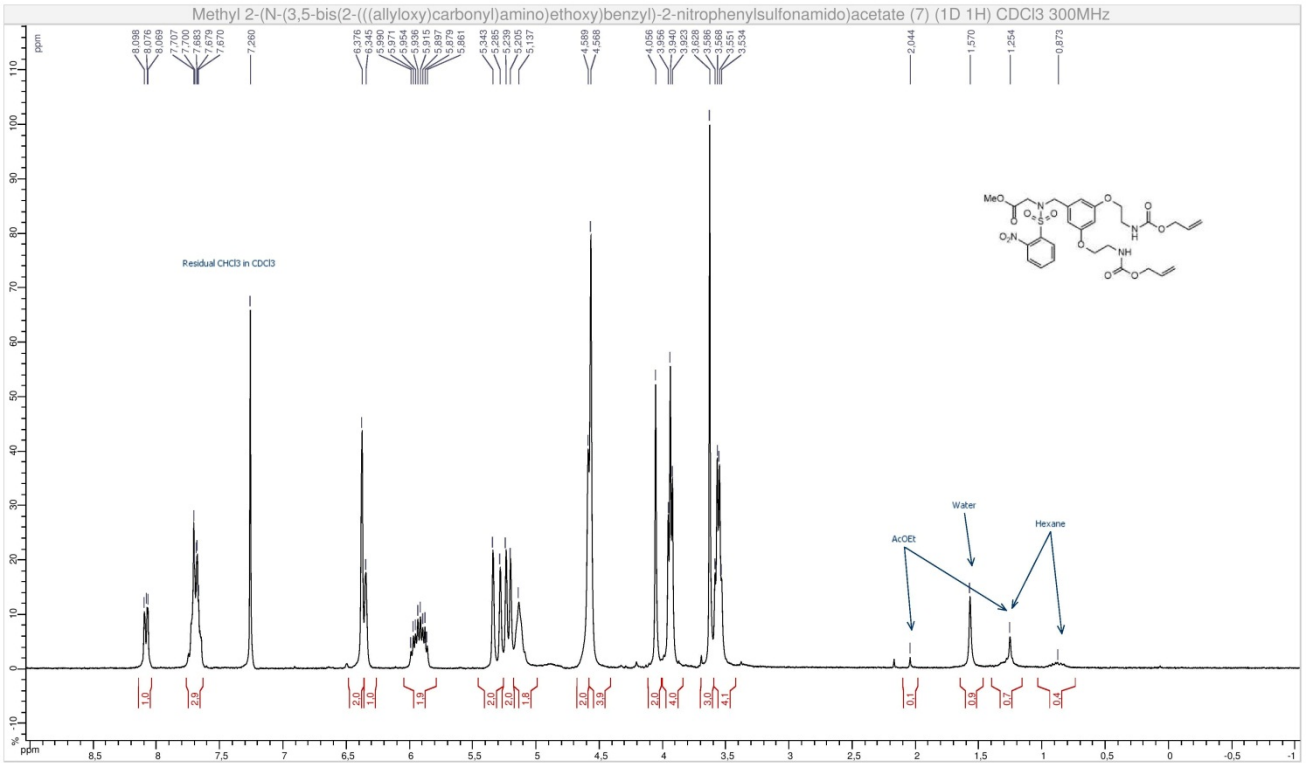
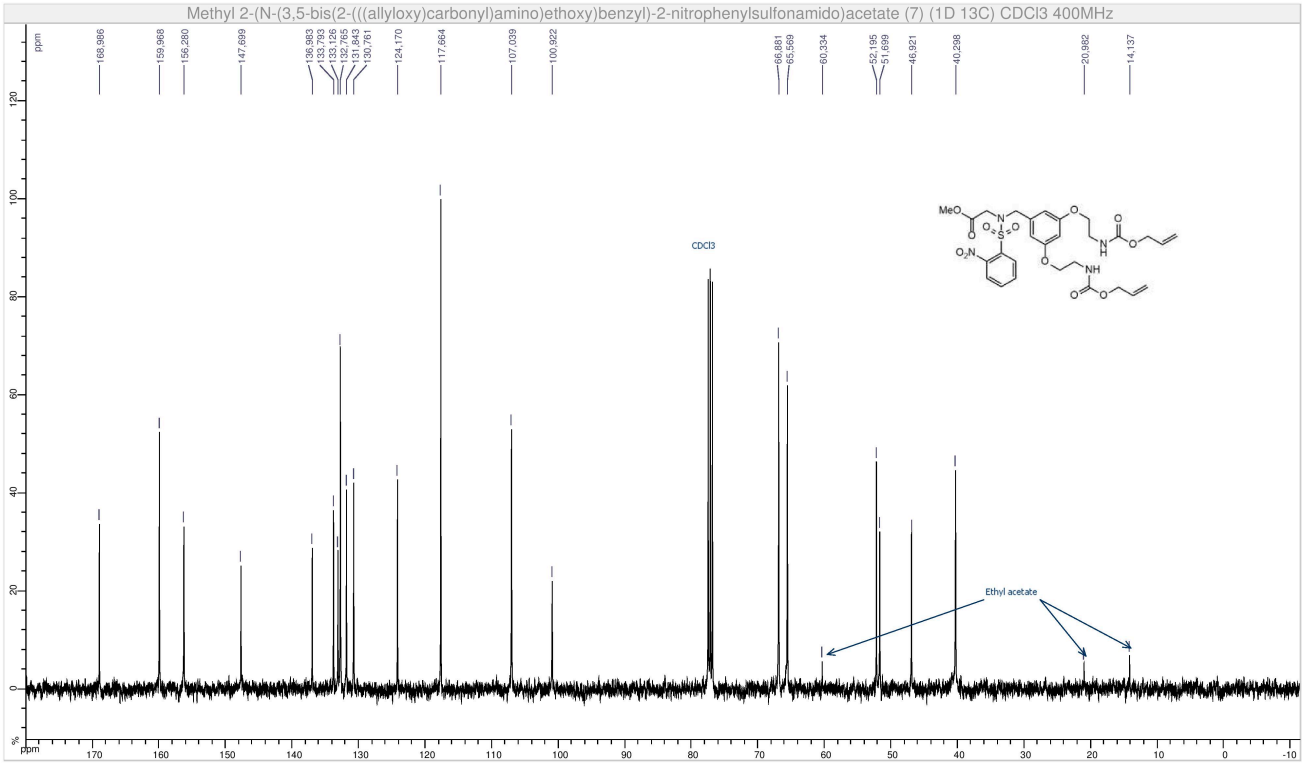
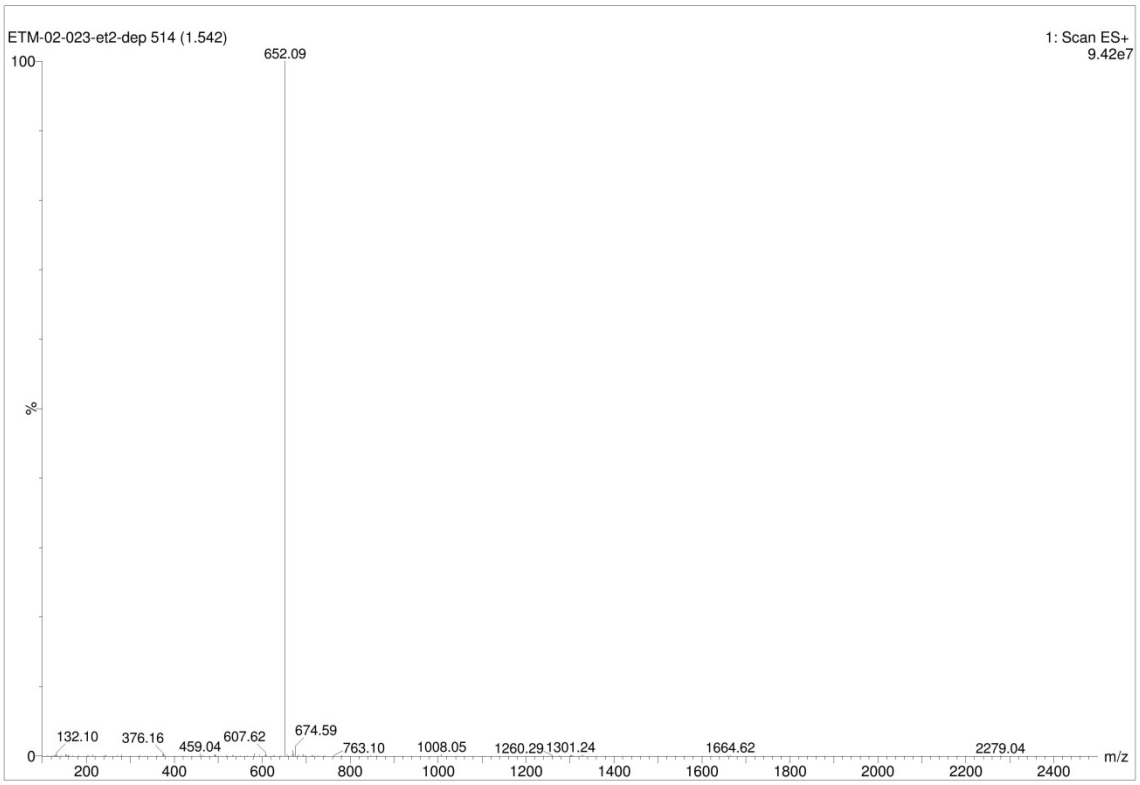
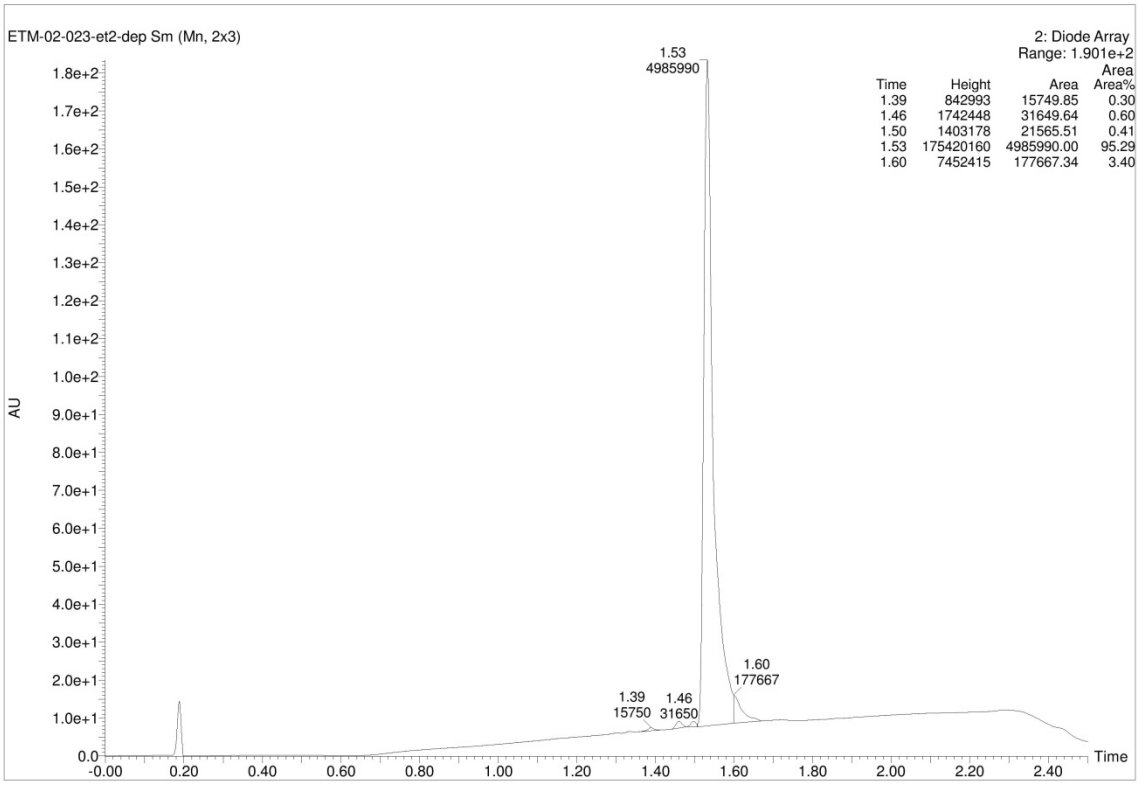
AU
Time
9.5e+1
9.0e+1
8.5e+1
8.0e+1
7.5e+1
7.0e+1
6.5e+1
6.0e+1
5.5e+1
5.0e+1
4.5e+1
4.0e+1
3.5e+1
3.0e+1
2.5e+1
2.0e+1
1.5e+1
1.0e+1
5.0
0.0
-0.00
0.20
0.40
0.60
0.80
1.00
1.20
1.40
1.60
1.80
2.00
2.20
2.40

Methyl 2-(N-(3,5-bis(2-(((allyloxy)carbonyl)amino)ethoxy) benzyl)-2-nitrophenylsulfonamido)acetate (**7**)

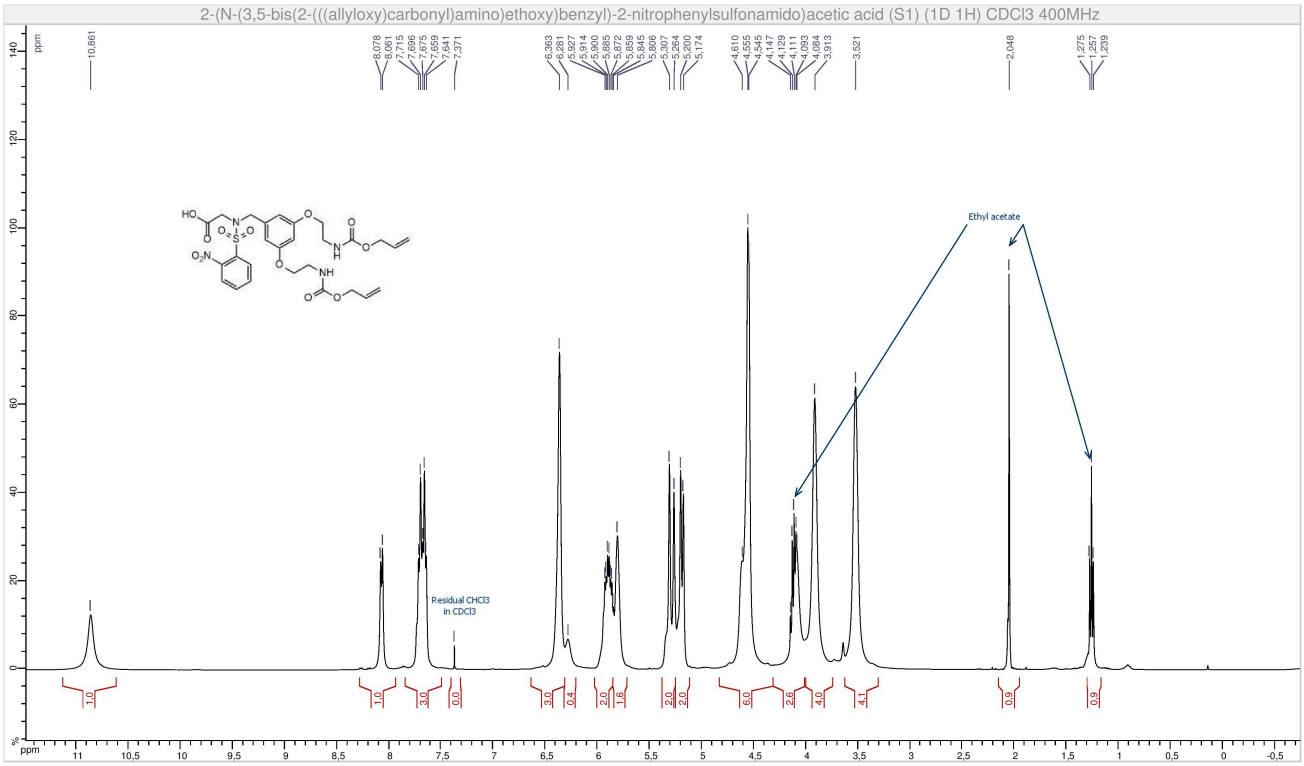


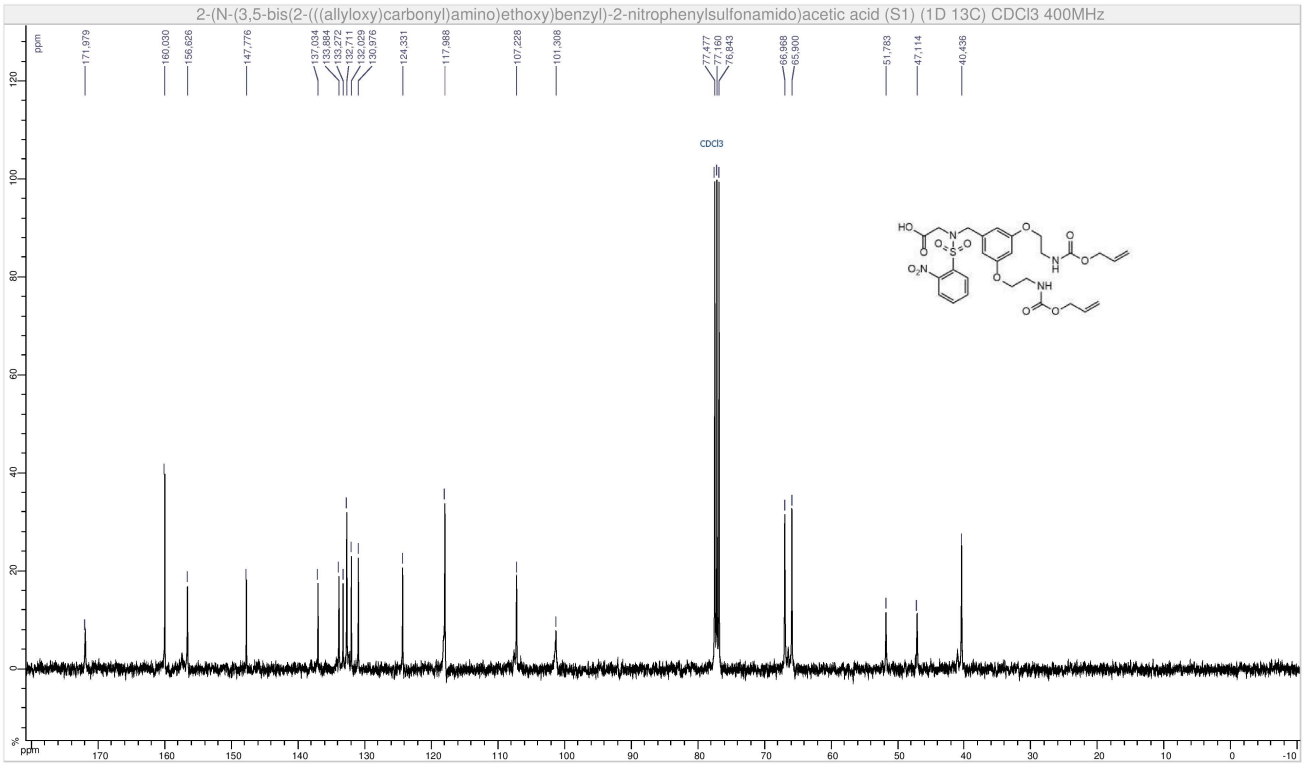


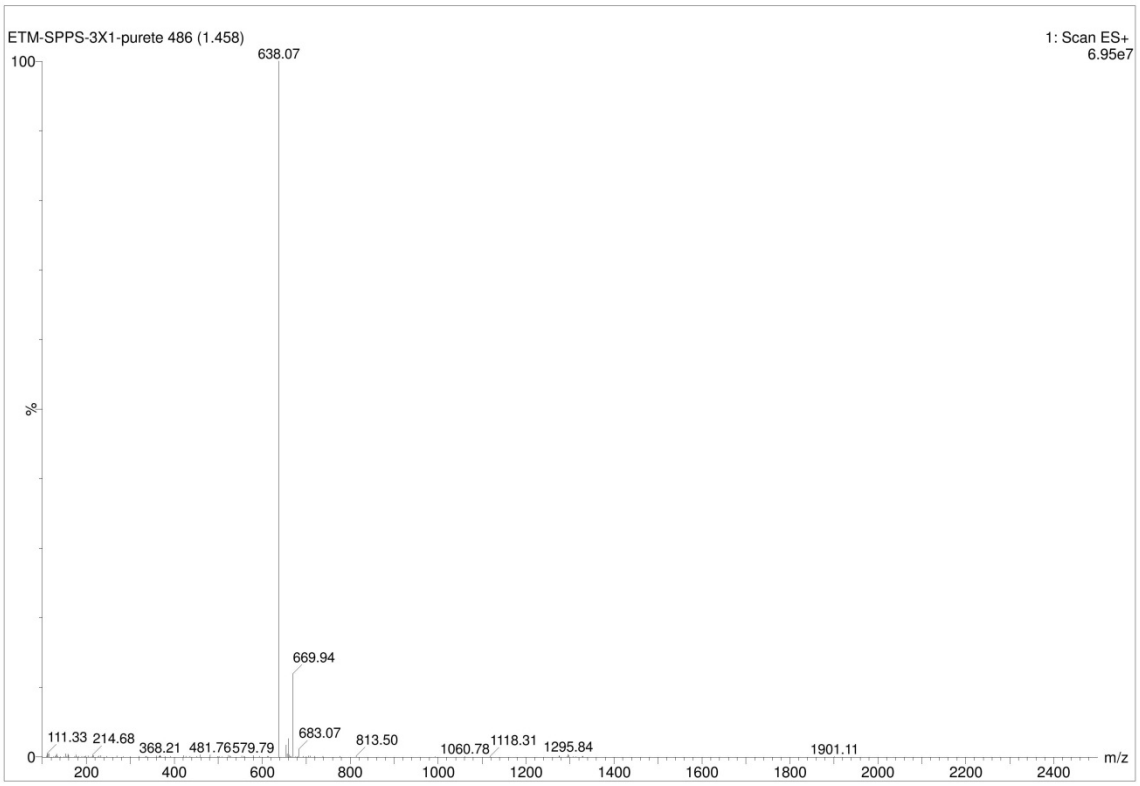

ETM-02-023-et2-dep 514 (1.542)
1: Scan ES+
9.42e7
100
%
0
652.09
132.10
376.16
459.04
607.62
674.59
763.10
1008.05
1260.29
1301.24
1664.62
2279.04
200
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
m/z

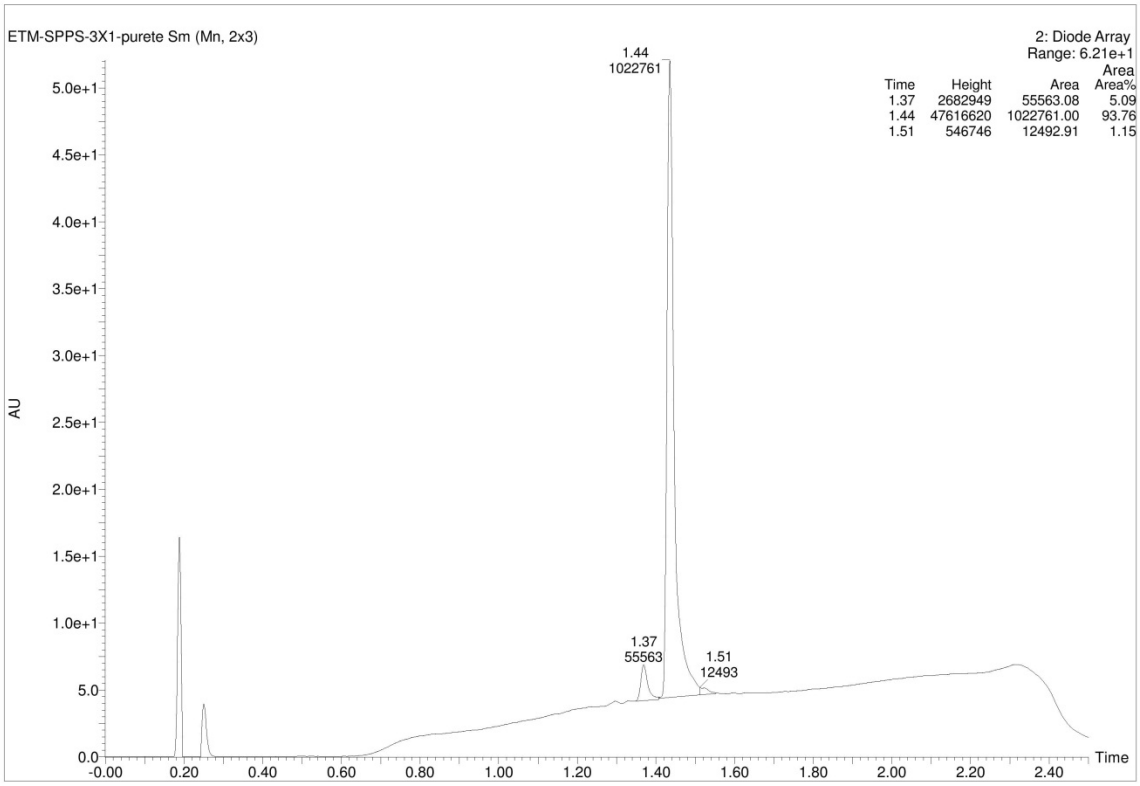

ETM-02-023-et2-dep Sm (Mn, 2x3)
2: Diode Array
Range: 1.901e+2
Area
Time Height Area Area%
1.39 842993 15749.85 0.30
1.46 1742448 31649.64 0.60
1.50 1403178 21565.51 0.41
1.53 175420160 4985990.00 95.29
1.60 7452415 177667.34 3.40
1.53
4985990
1.60
177667
1.39
15750
1.46
31650
AU
Time

2-(N-(3,5-bis(2-(((allyloxy)carbonyl)amino)ethoxy)benzyl)-2-nitrophenylsulfonamido)acetic acid (**S1**)

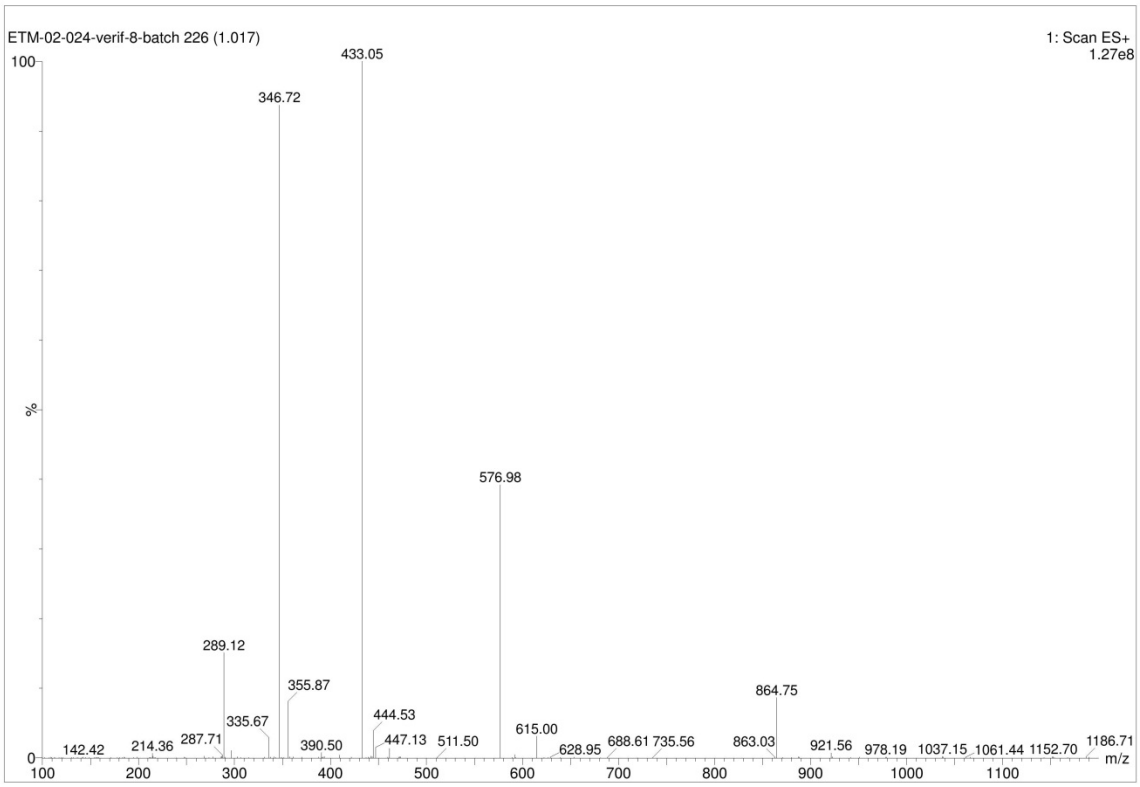


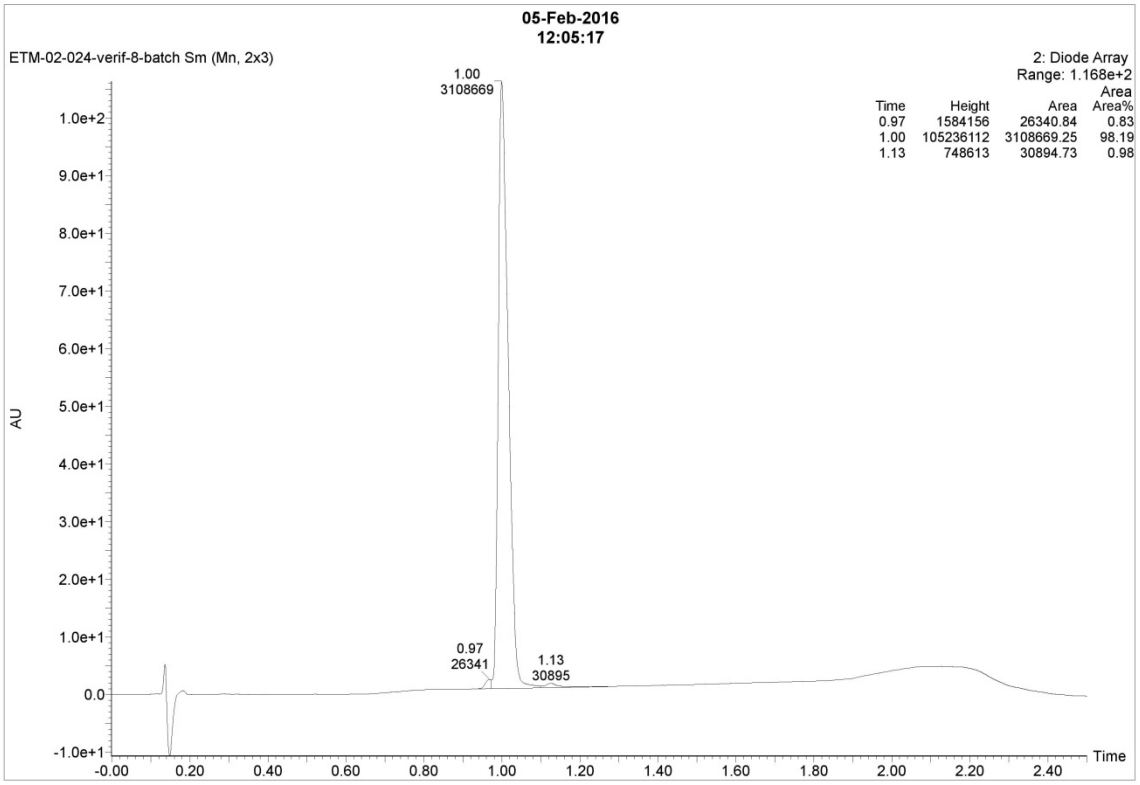



ETM-SPPS-3X1-purete 486 (1.458)
1: Scan ES+
6.95e7
100
%
0
638.07
669.94
683.07
111.33
214.68
368.21
481.76579.79
813.50
1060.78
1118.31
1295.84
1901.11
200
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
m/z

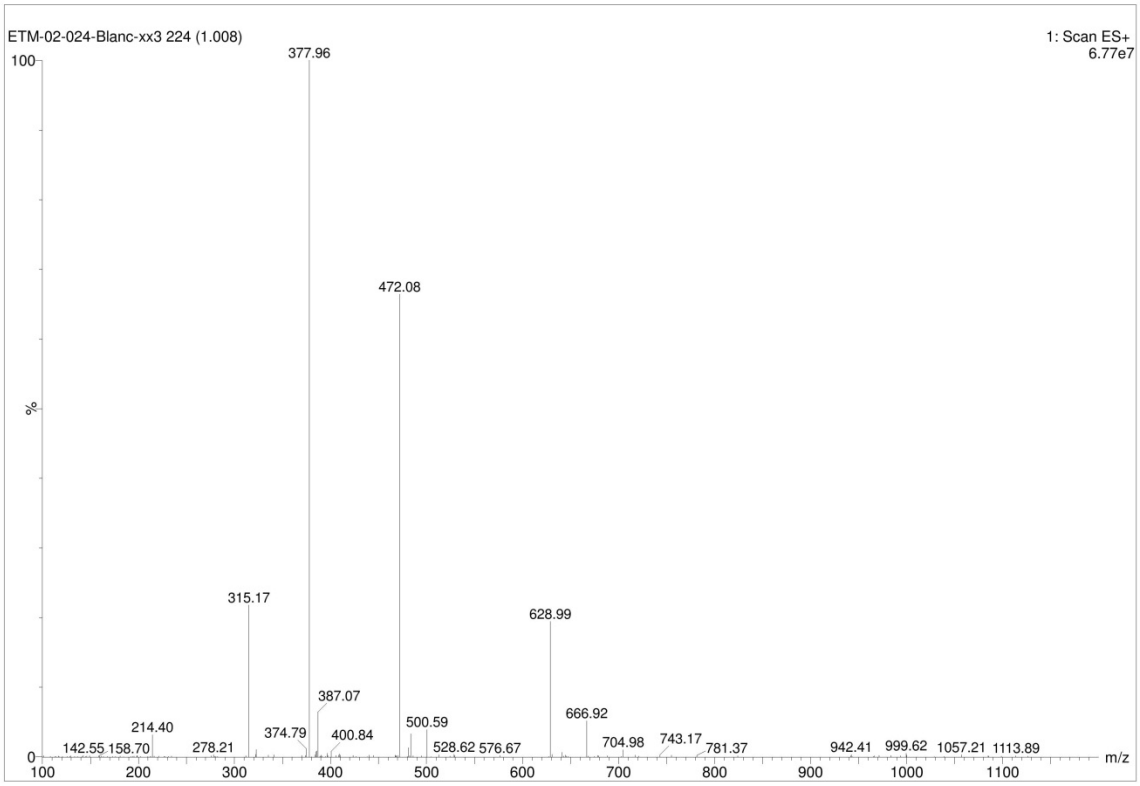

ETM-SPPS-3X1-purete Sm (Mn, 2x3)
2: Diode Array
Range: 6.21e+1
Time Height Area Area%
1.37 2682949 55563.08 5.09
1.44 47616620 1022761.00 93.76
1.51 546746 12492.91 1.15
1.44
1022761
1.37
55563
1.51
12493
AU
5.0e+1
4.5e+1
4.0e+1
3.5e+1
3.0e+1
2.5e+1
2.0e+1
1.5e+1
1.0e+1
5.0
0.0
-0.00
0.20
0.40
0.60
0.80
1.00
1.20
1.40
1.60
1.80
2.00
2.20
2.40
Time

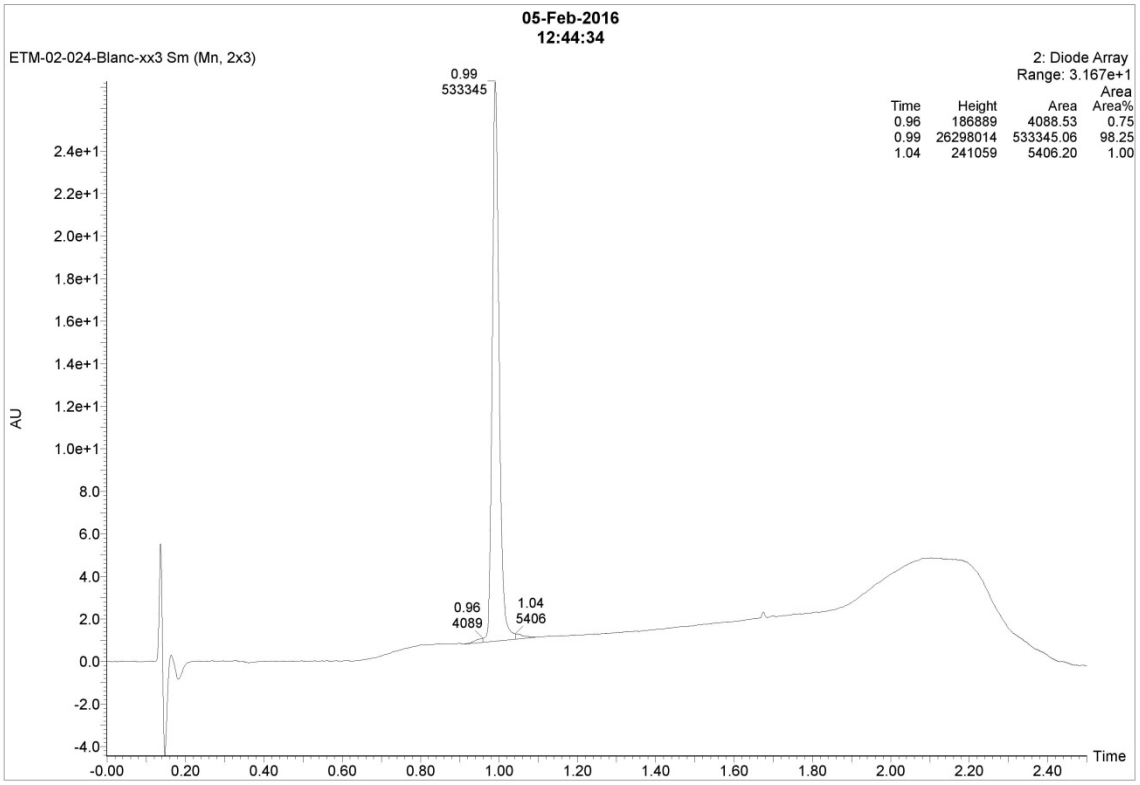
Fluo-βAla-Alarg$_8$-NH$_2$ (**R$_8$**)



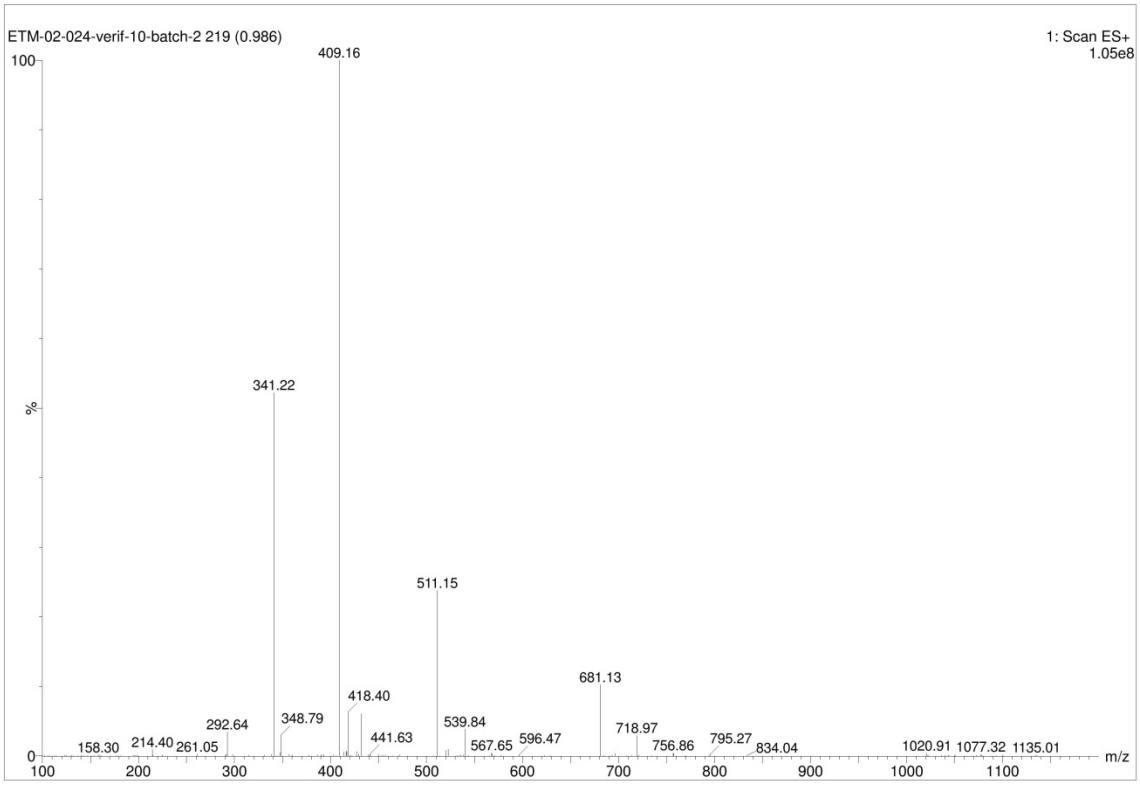


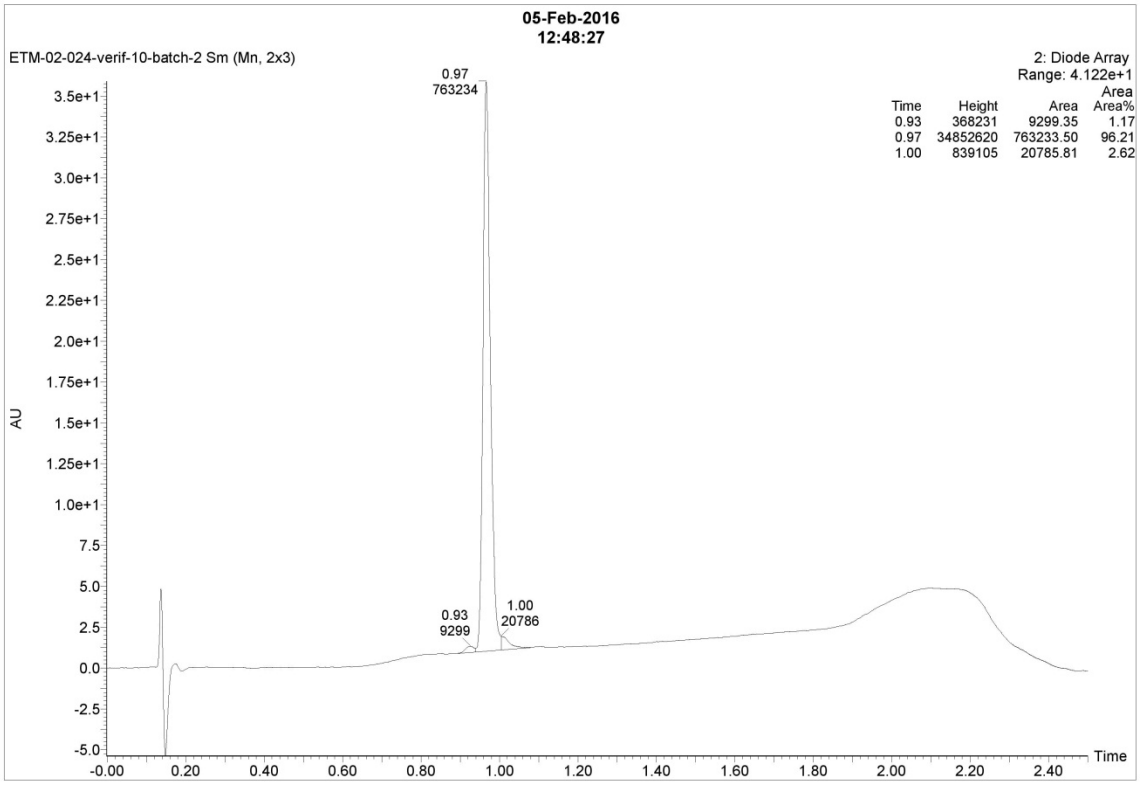
Fluo-βAla-Arg_9-NH_2 (**R_9**)



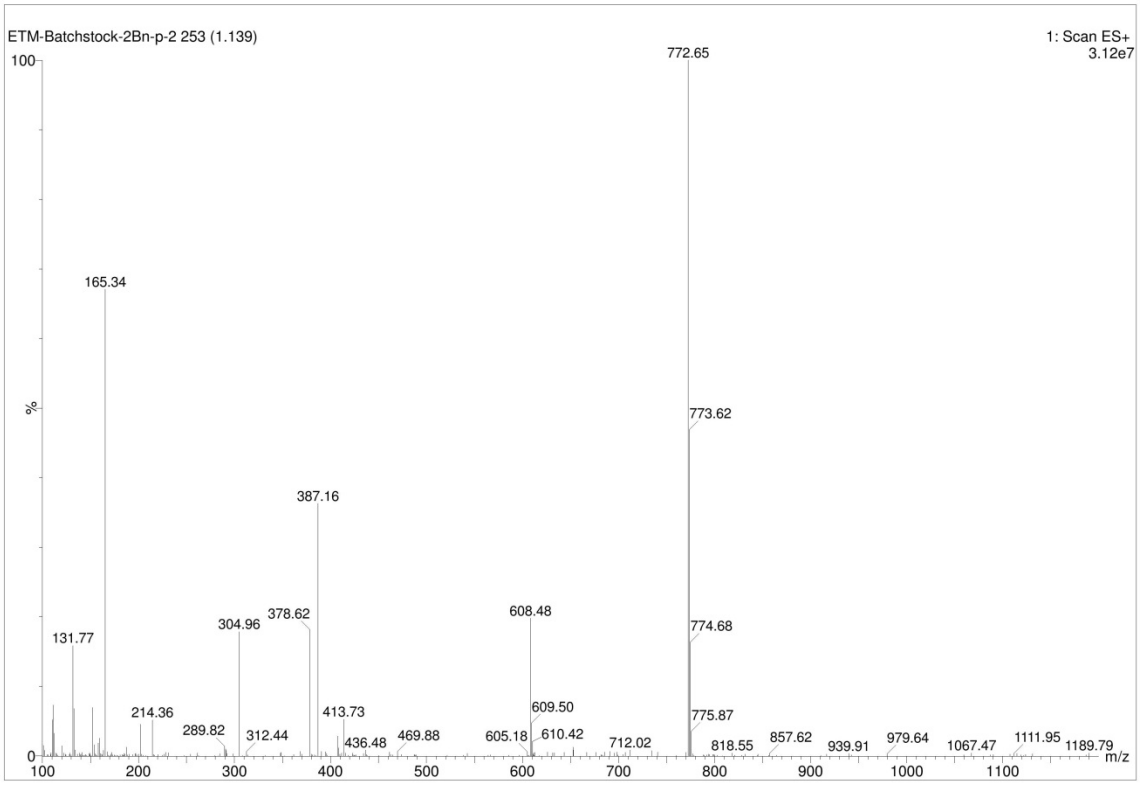


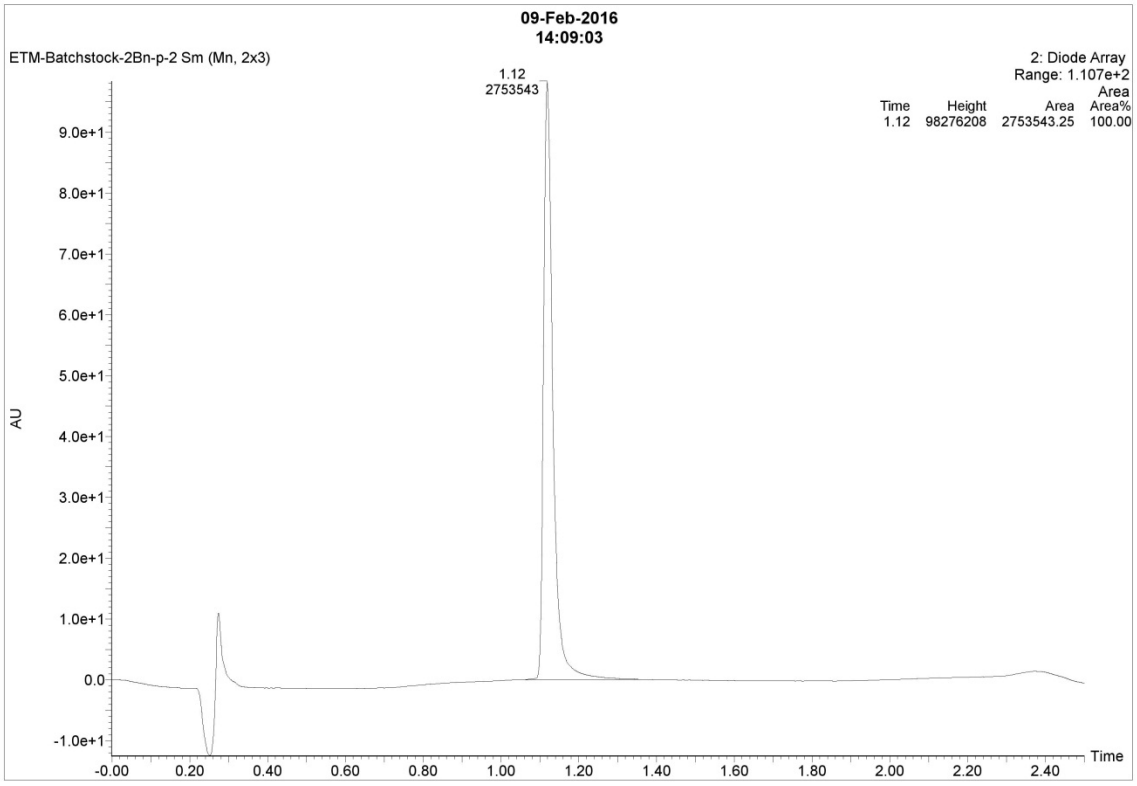
Fluo-βAla-Arg_{10}-NH_2 (**R_{10}**)



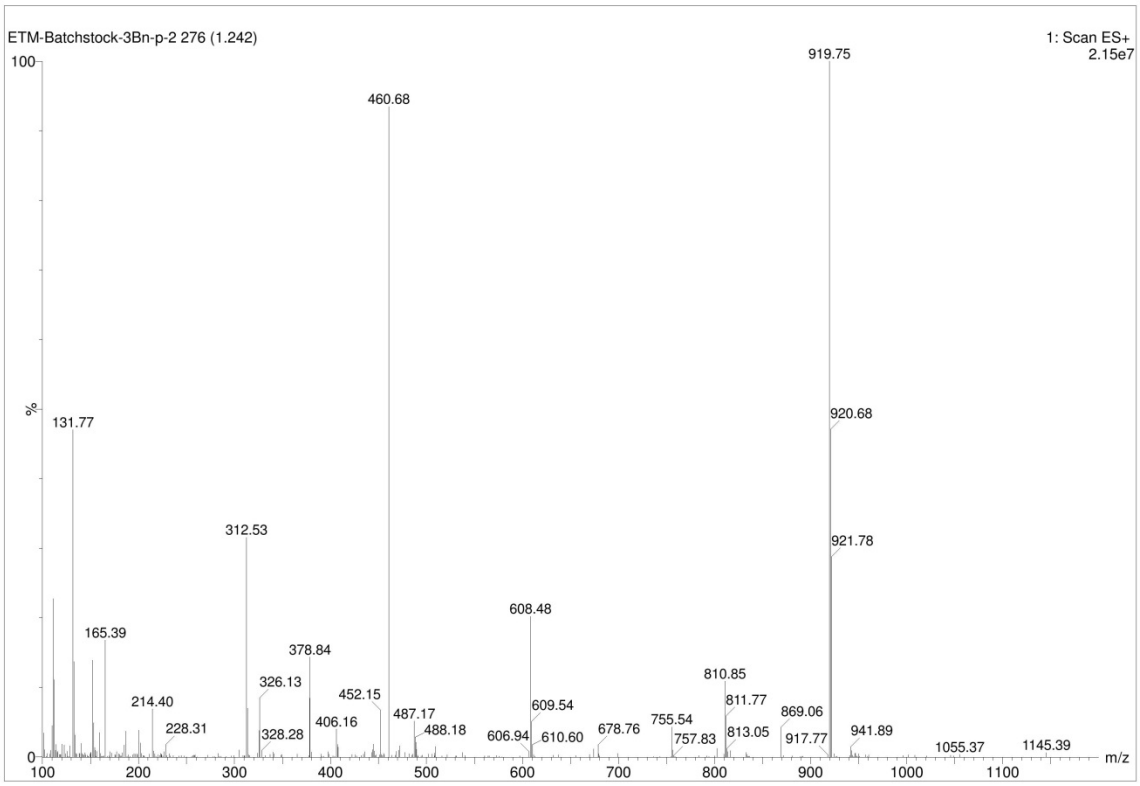


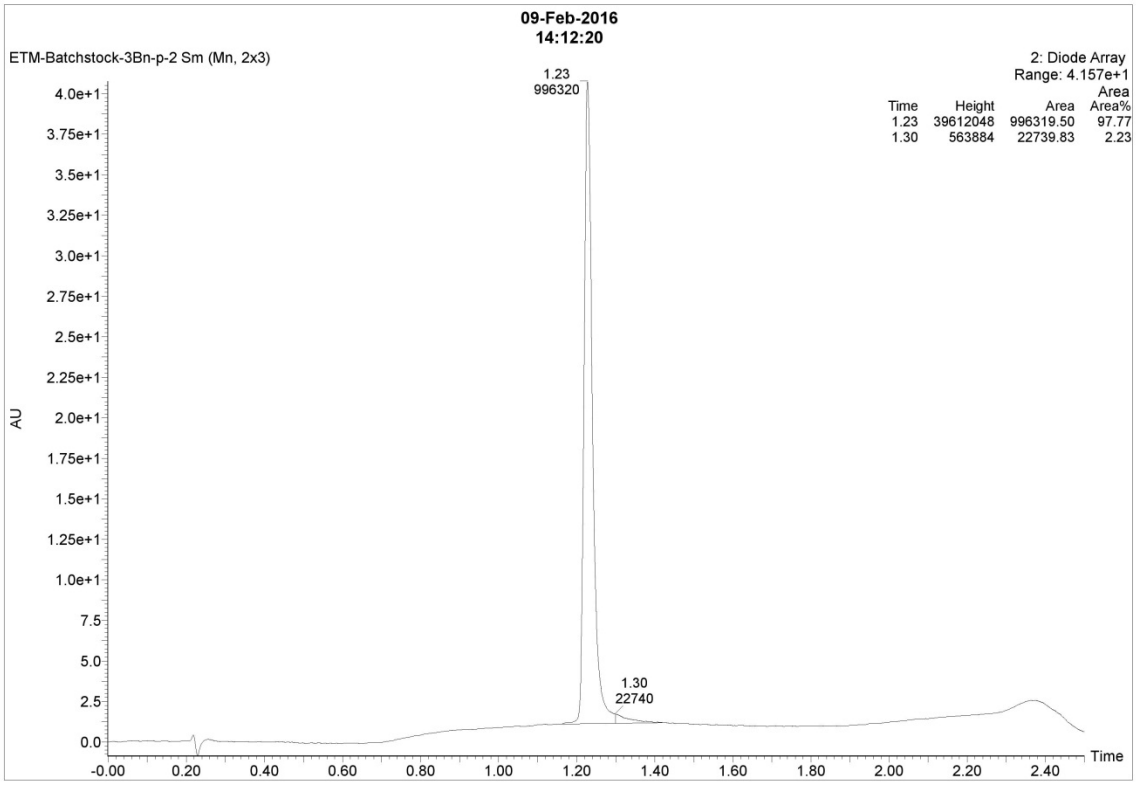
Fluo-βAla-(*N*Phe)$_2$-NH$_2$ (**PBn$_2$**)



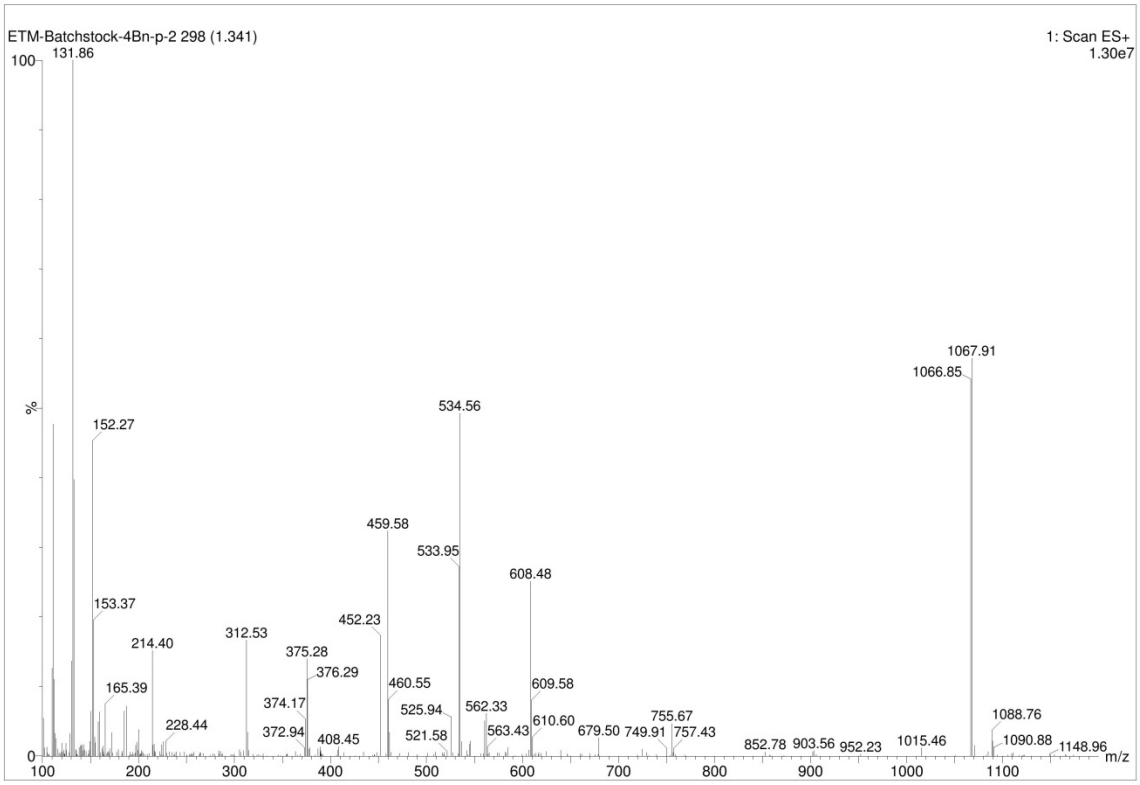


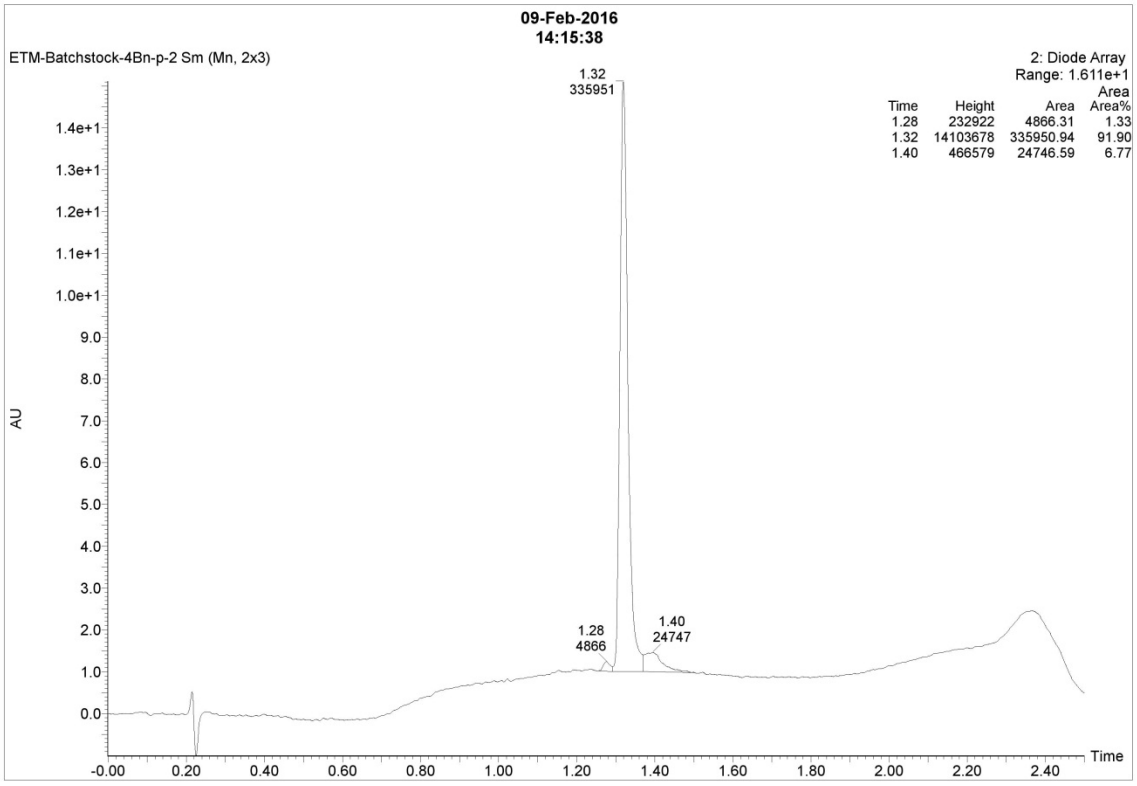
Fluo-βAla-(*N*Phe)$_3$-NH$_2$ (**PBn$_3$**)



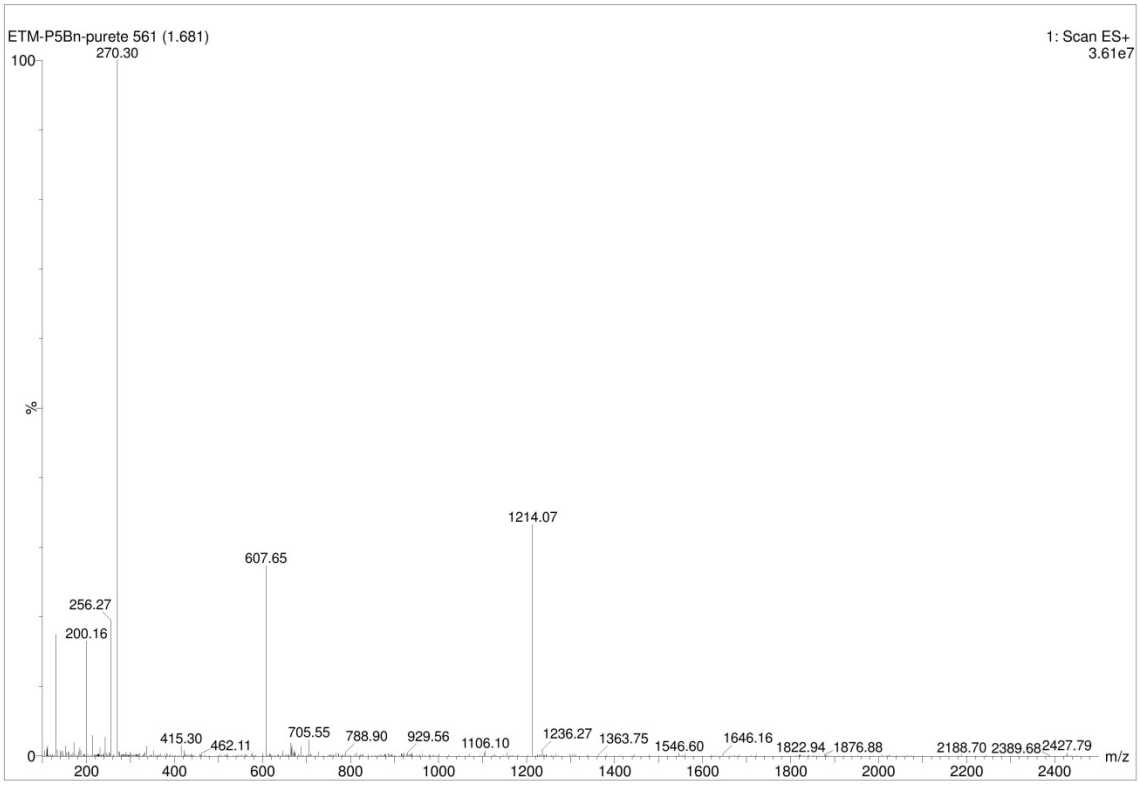


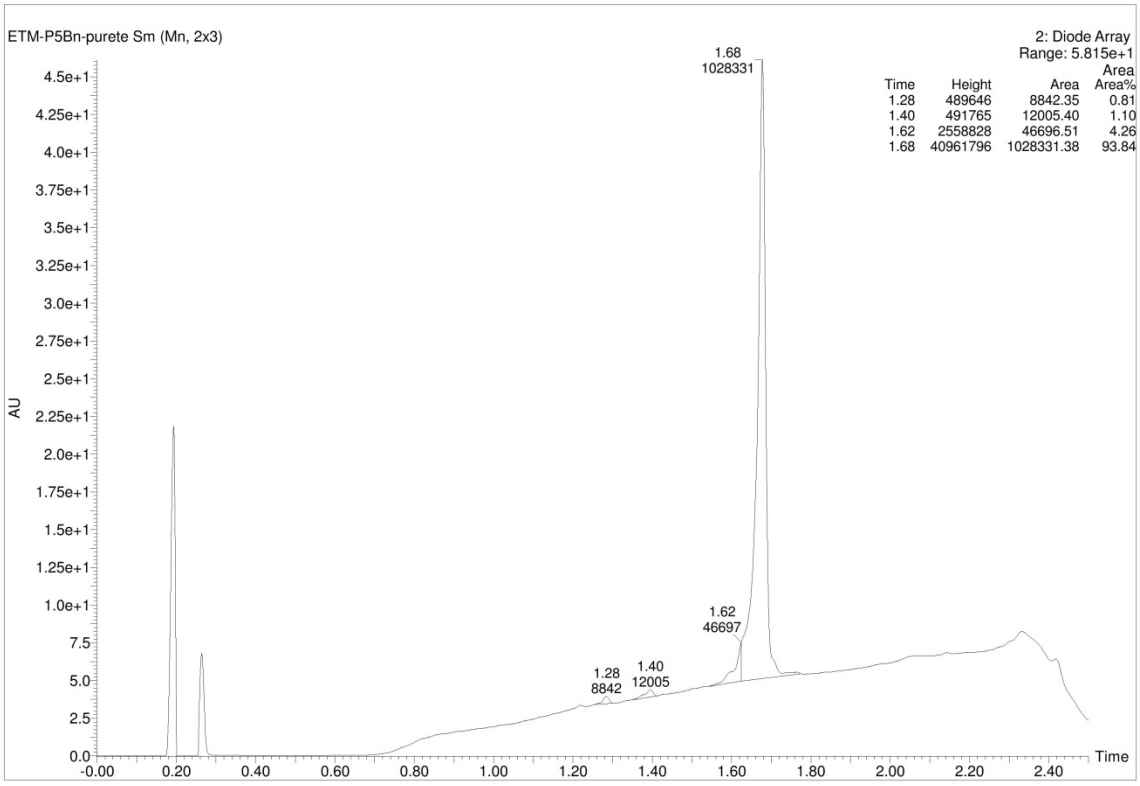
Fluo-βAla-(*N*Phe)$_4$-NH$_2$ (**PBn$_4$**)



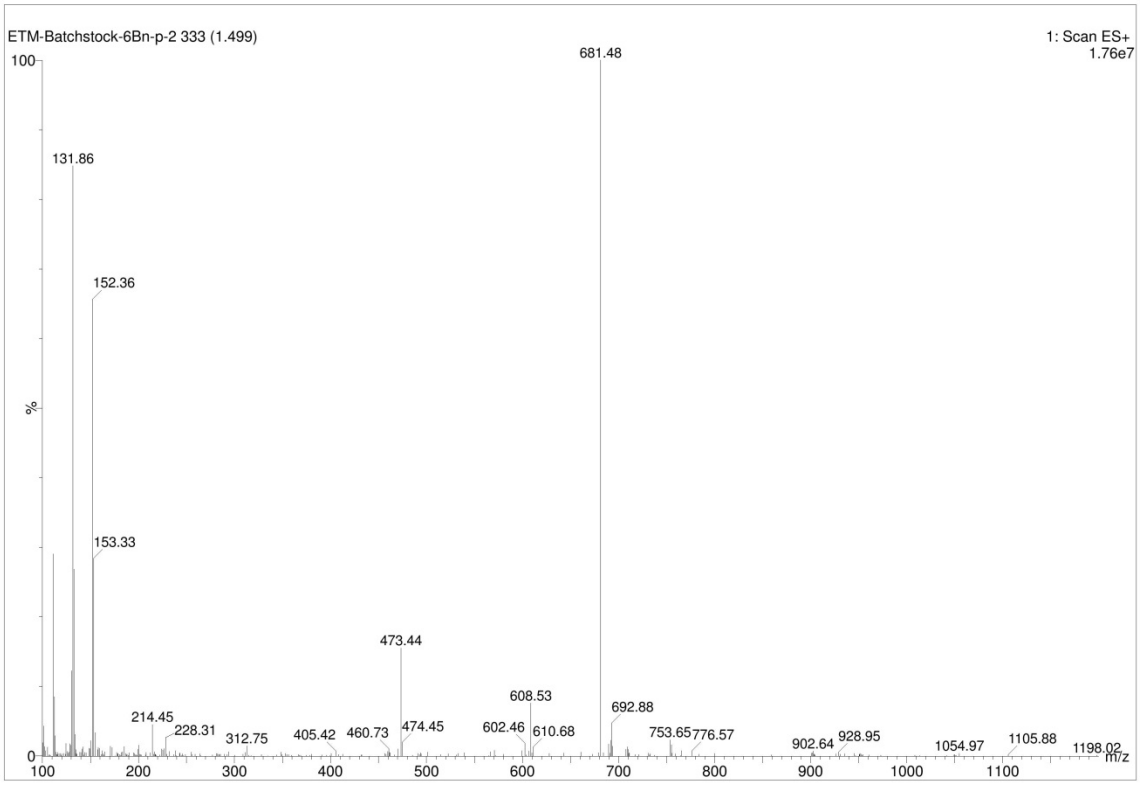


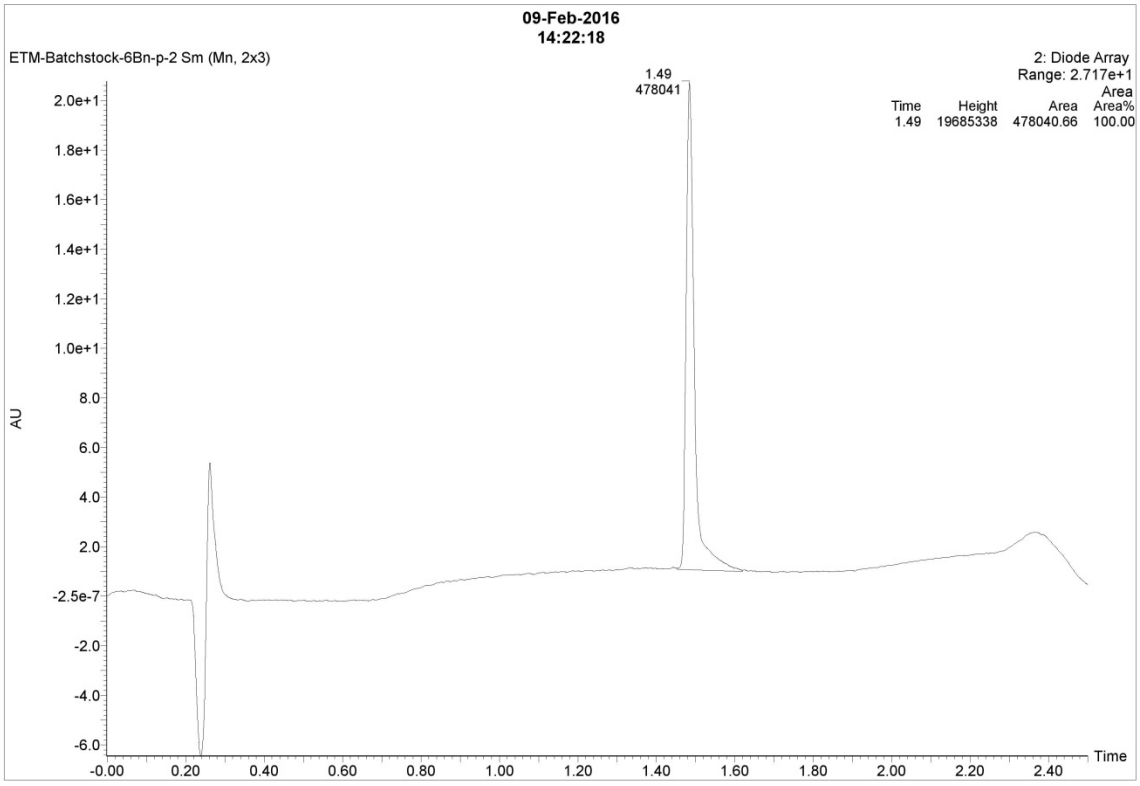
Fluo-βAla-(*N*Phe)$_5$-NH$_2$ (**PBn$_5$**)



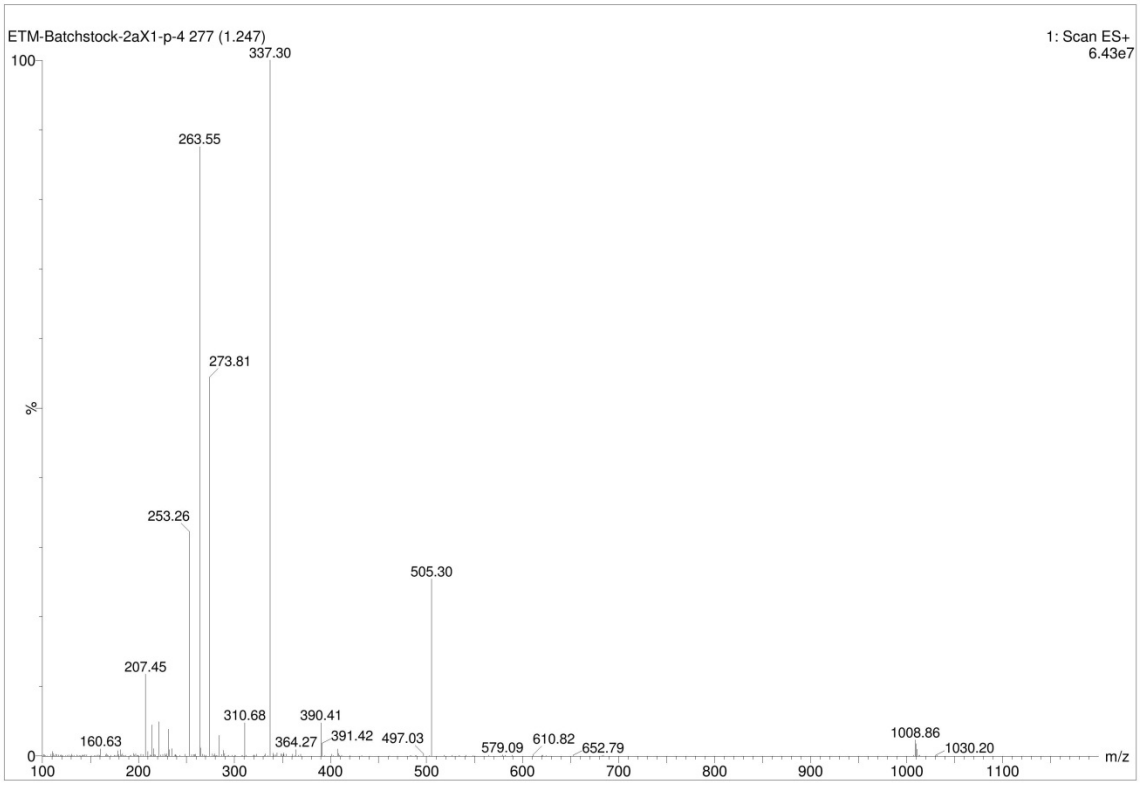


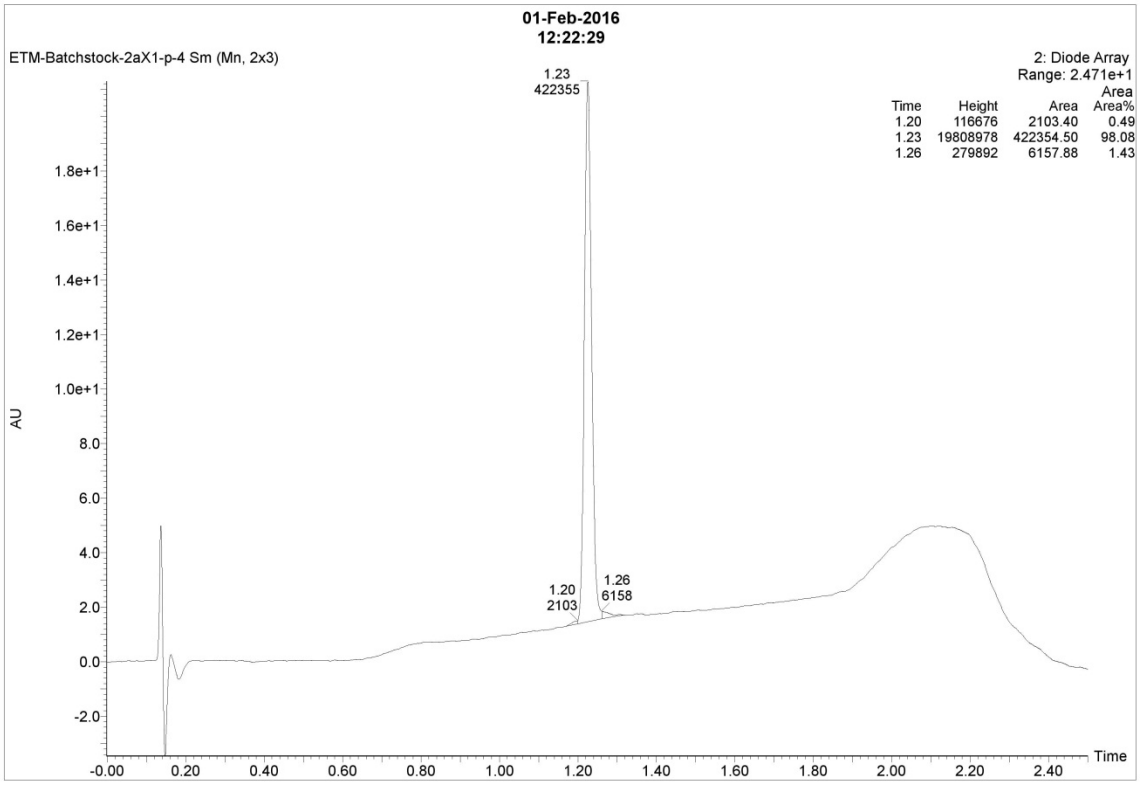
Fluo-βAla-(*N*Phe)$_6$-NH$_2$ (**PBn$_6$**)



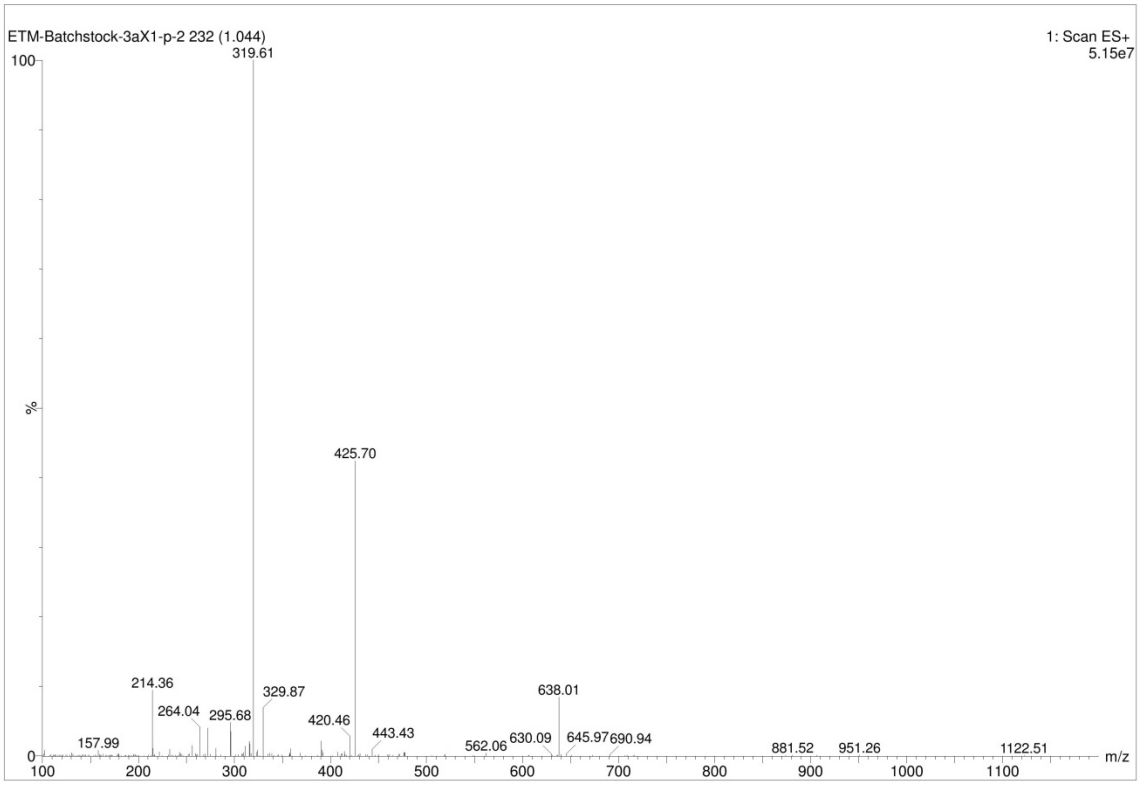


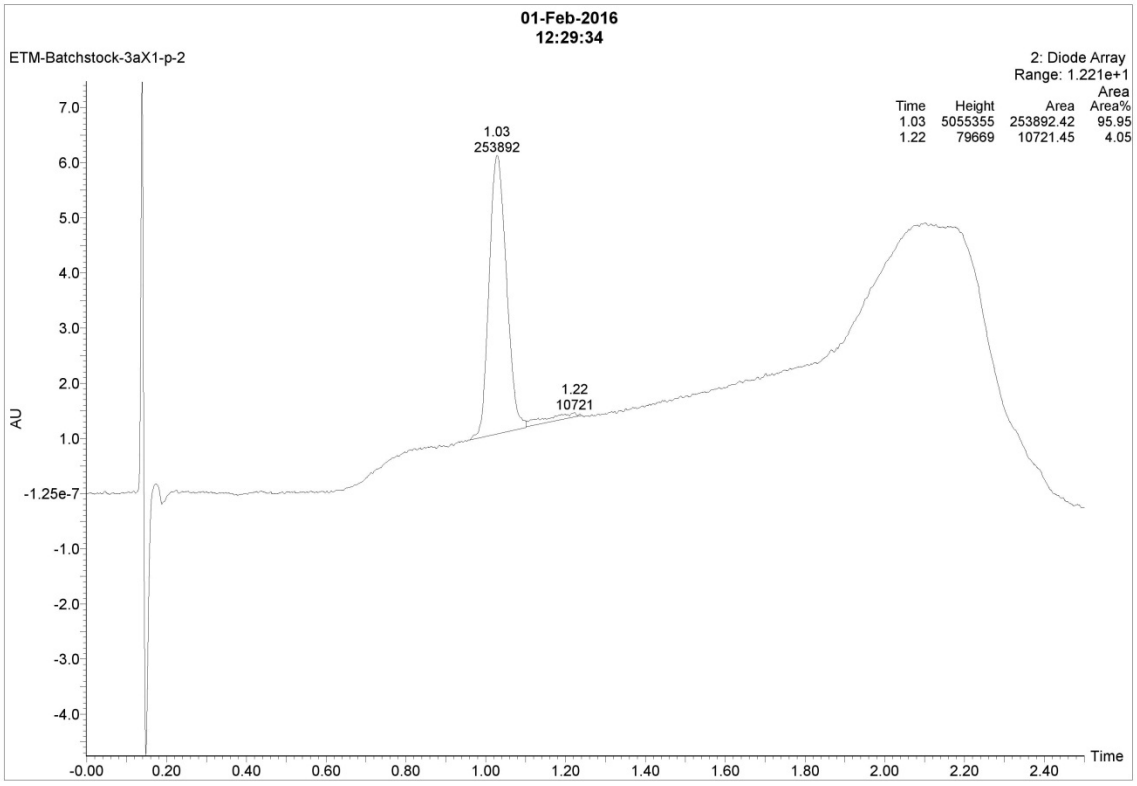
Fluo-βAla-(*N*{3,5-bis(2-(amino)ethoxy)) benzyl})$_2$-NH_2 (**PAm$_2$**)



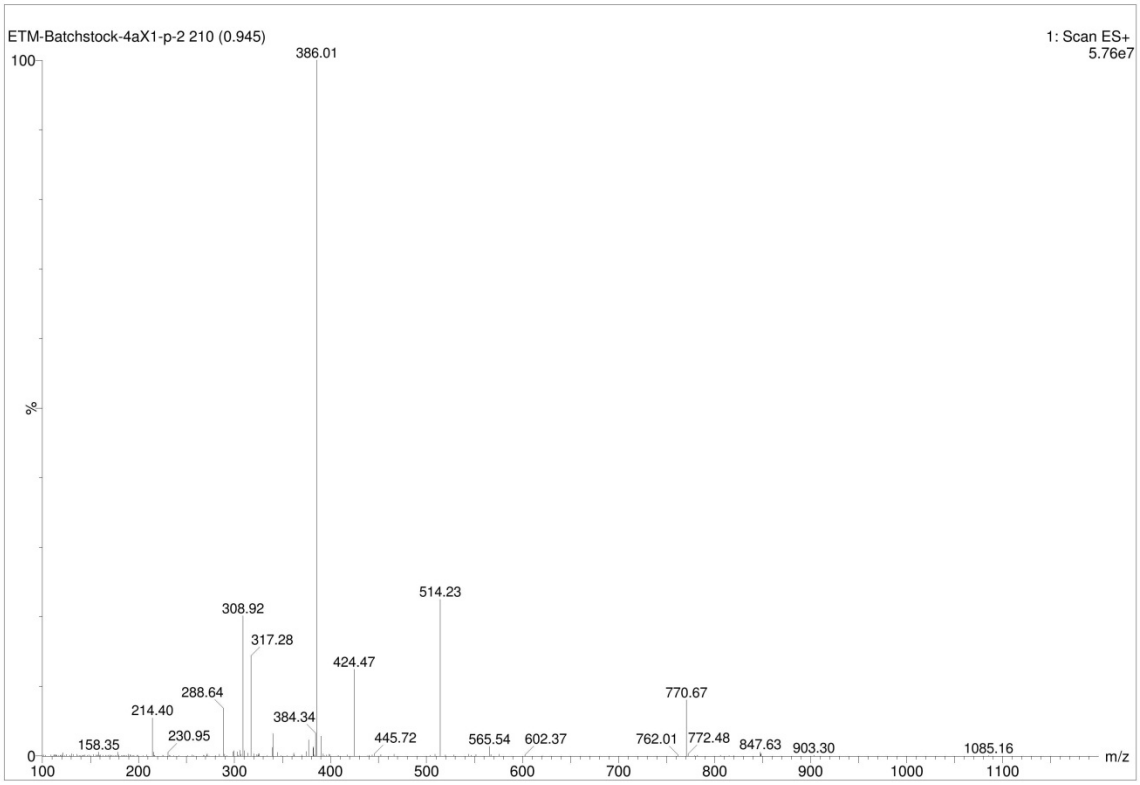


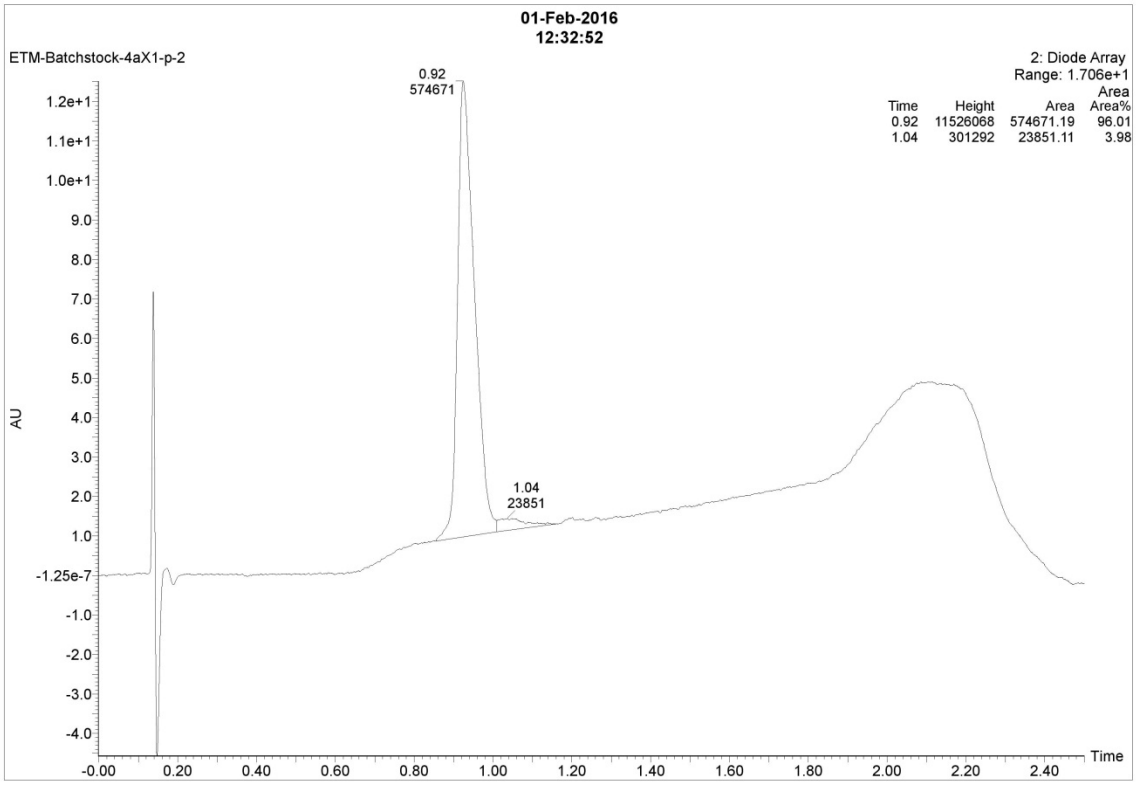
Fluo-βAla-(*N*{3,5-bis(2-(amino)ethoxy)) benzyl})$_3$-NH_2 (**PAm_3**)



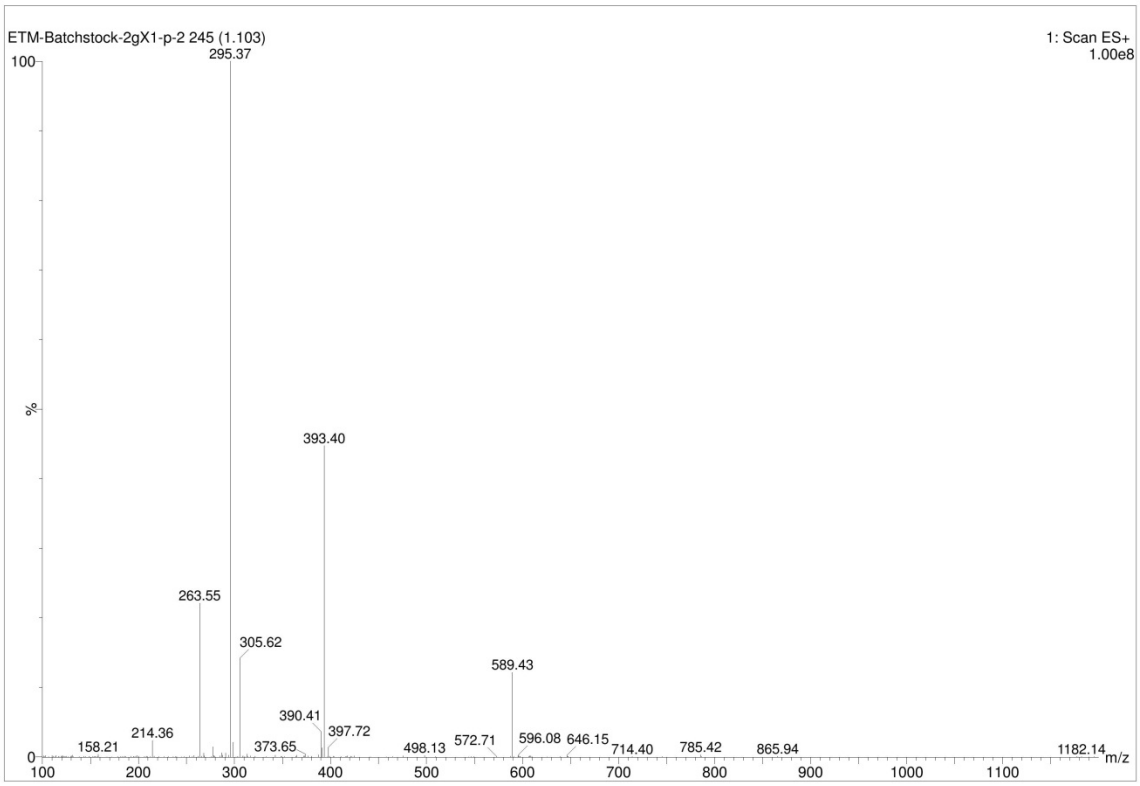


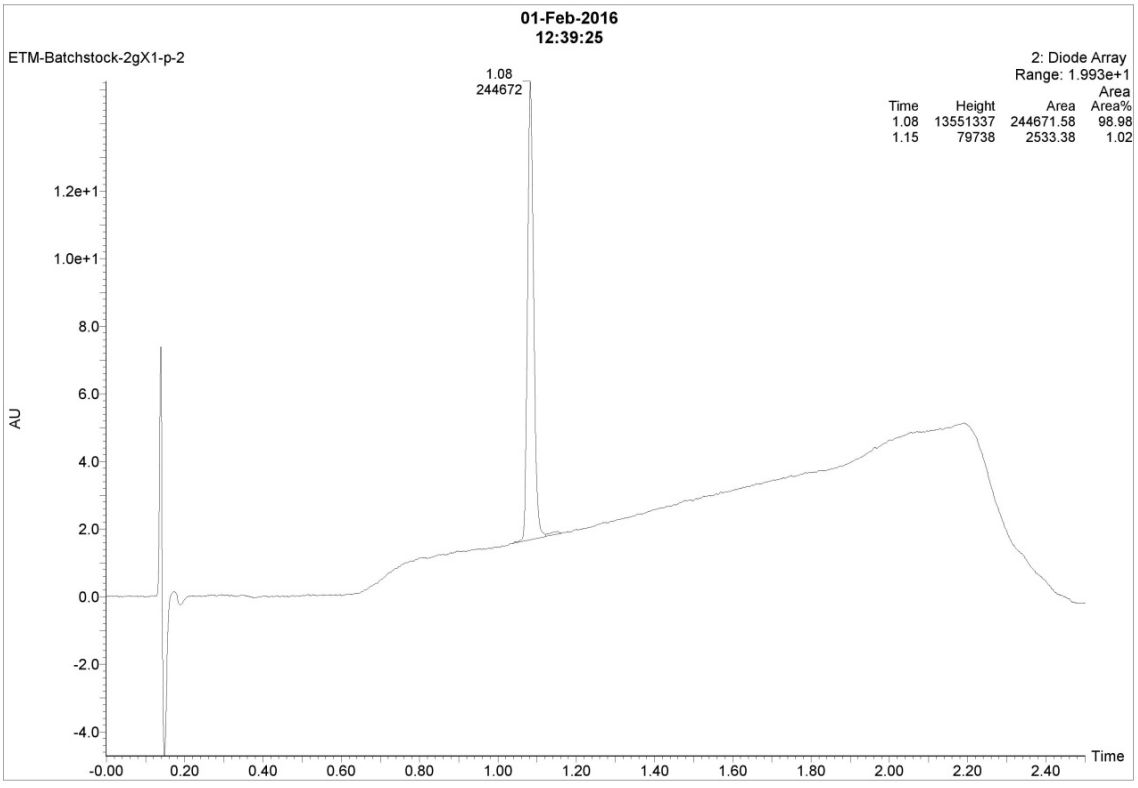
Fluo-βAla-(*N*{3,5-bis(2-(amino)ethoxy)) benzyl})$_4$-NH_2 (**PAm$_4$**)



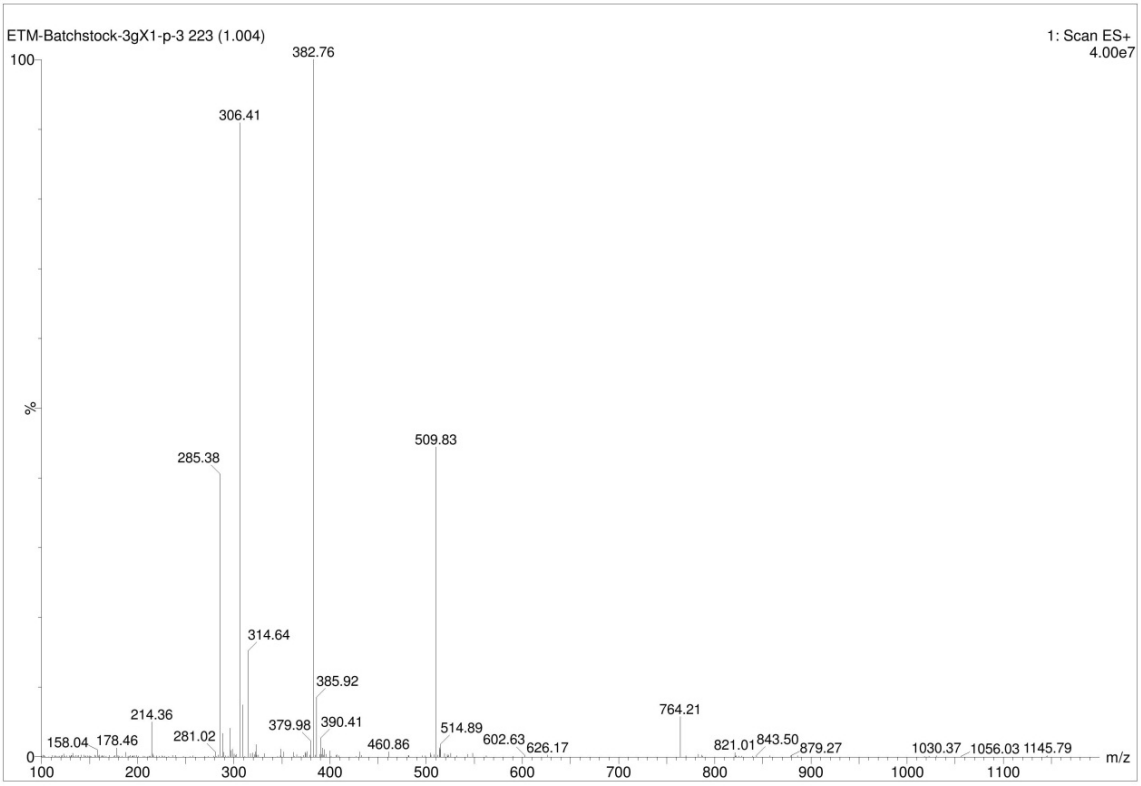


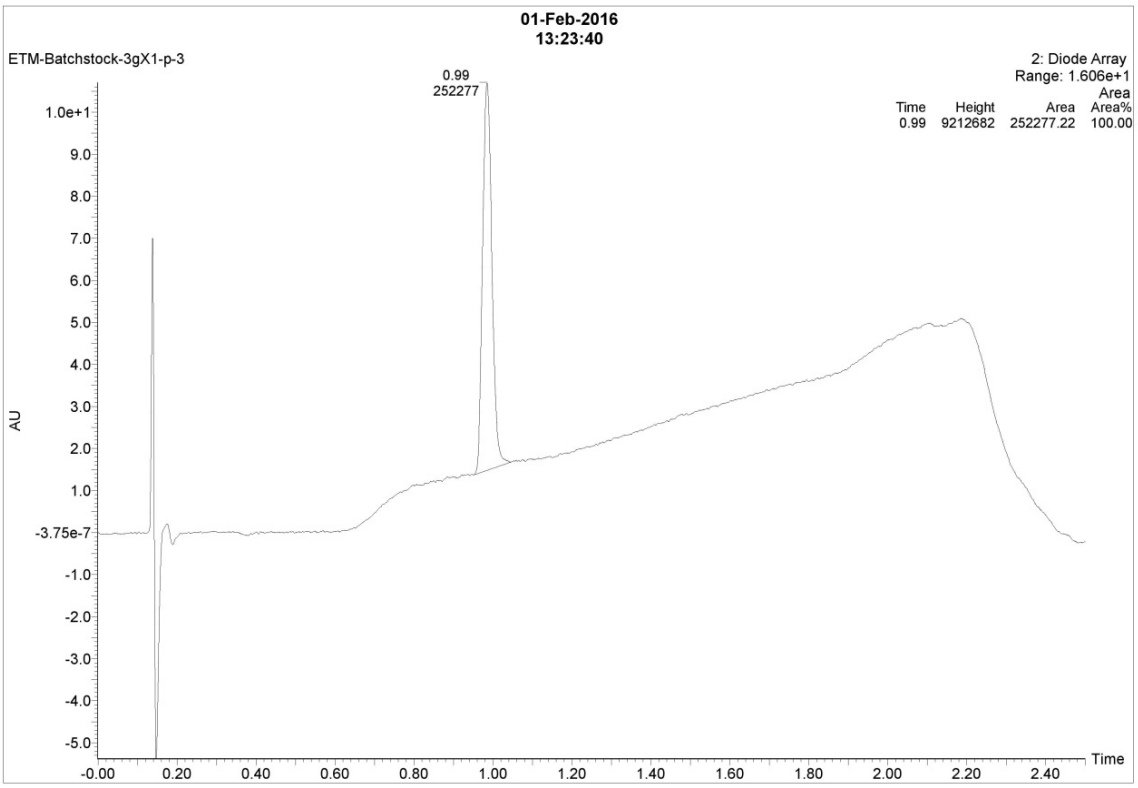
Fluo-βAla-(*N*{3,5-bis(2-(guanidino)ethoxy)) benzyl})$_2$-NH_2 (**PGua$_2$**)



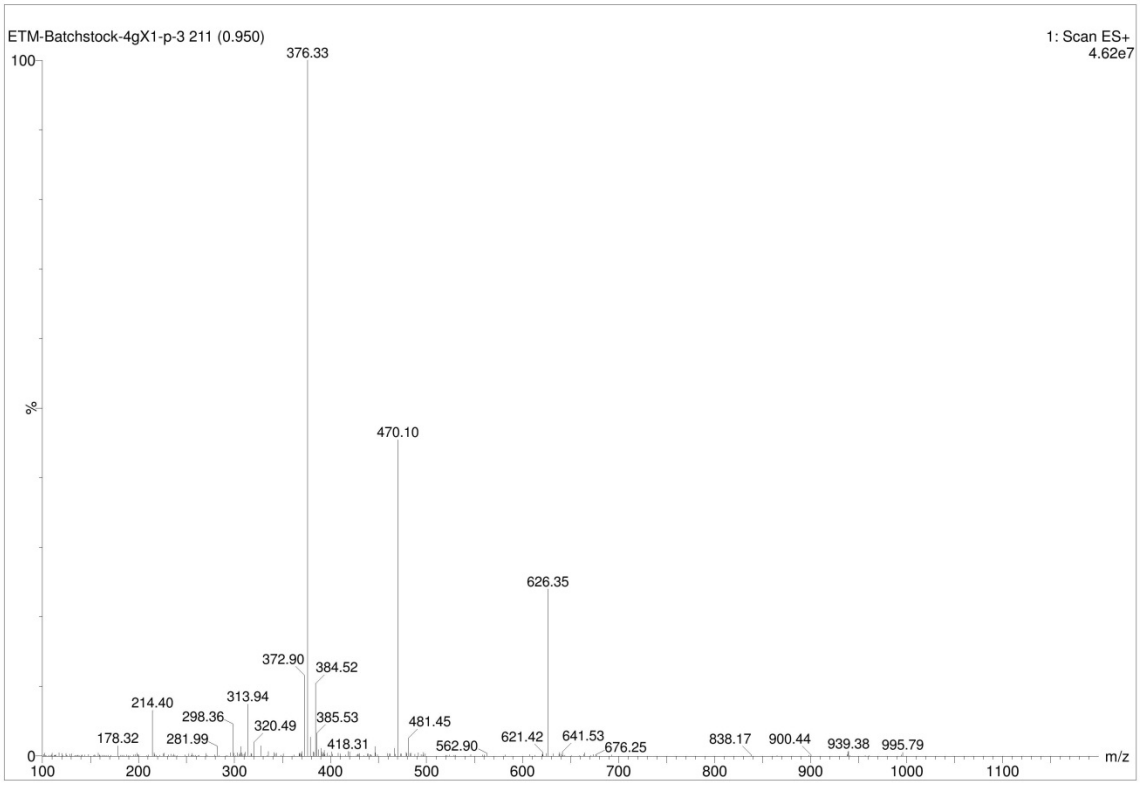


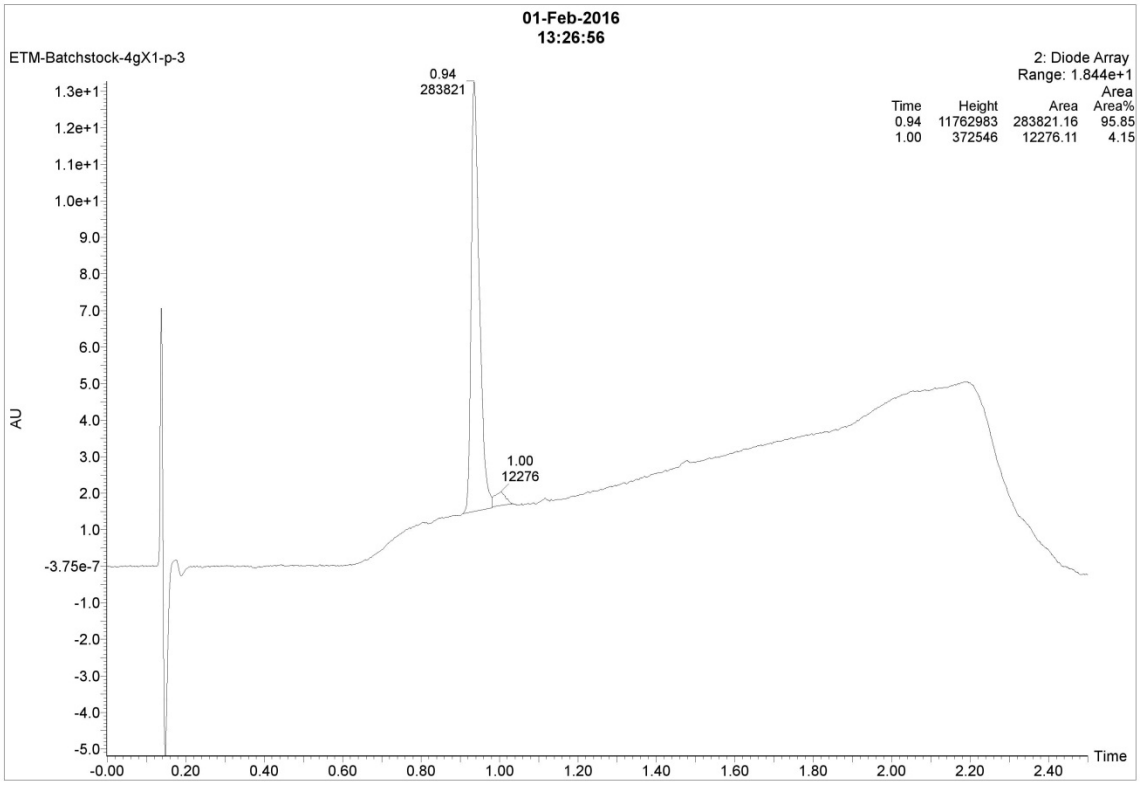
Fluo-βAla-(*N*{3,5-bis(2-(guanidino)ethoxy)) benzyl})$_3$-NH_2 (**PGua$_3$**)



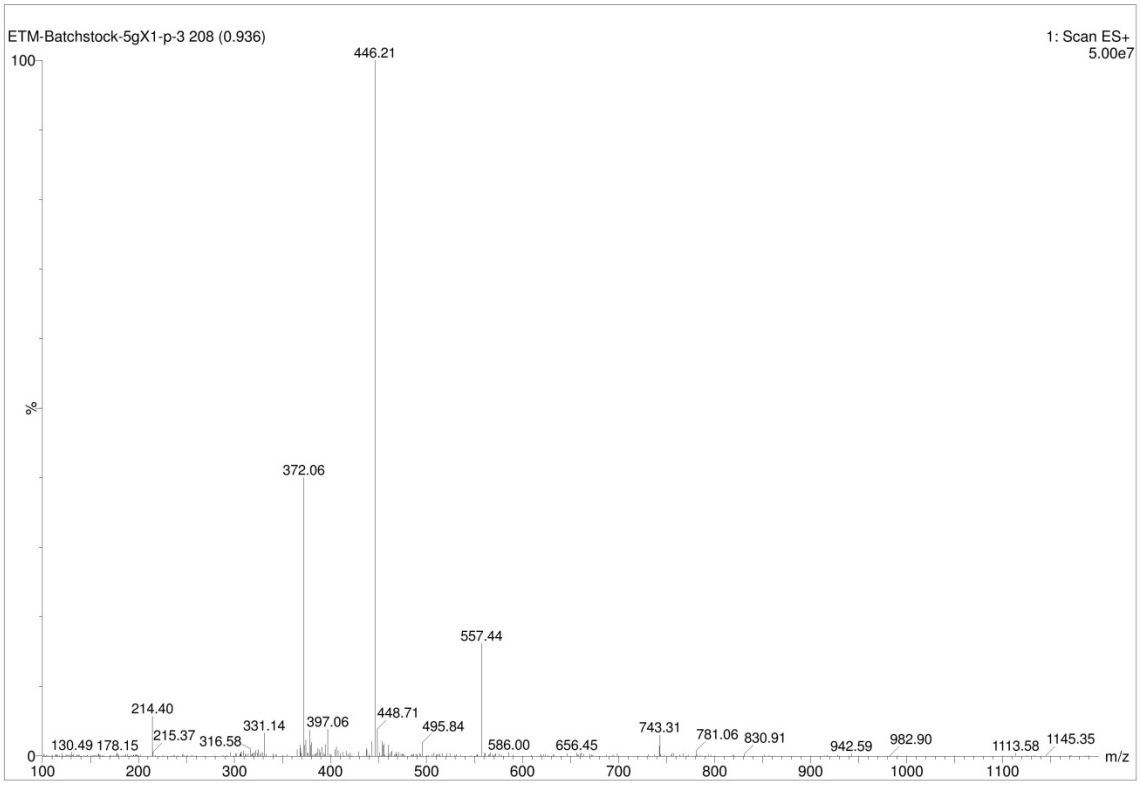


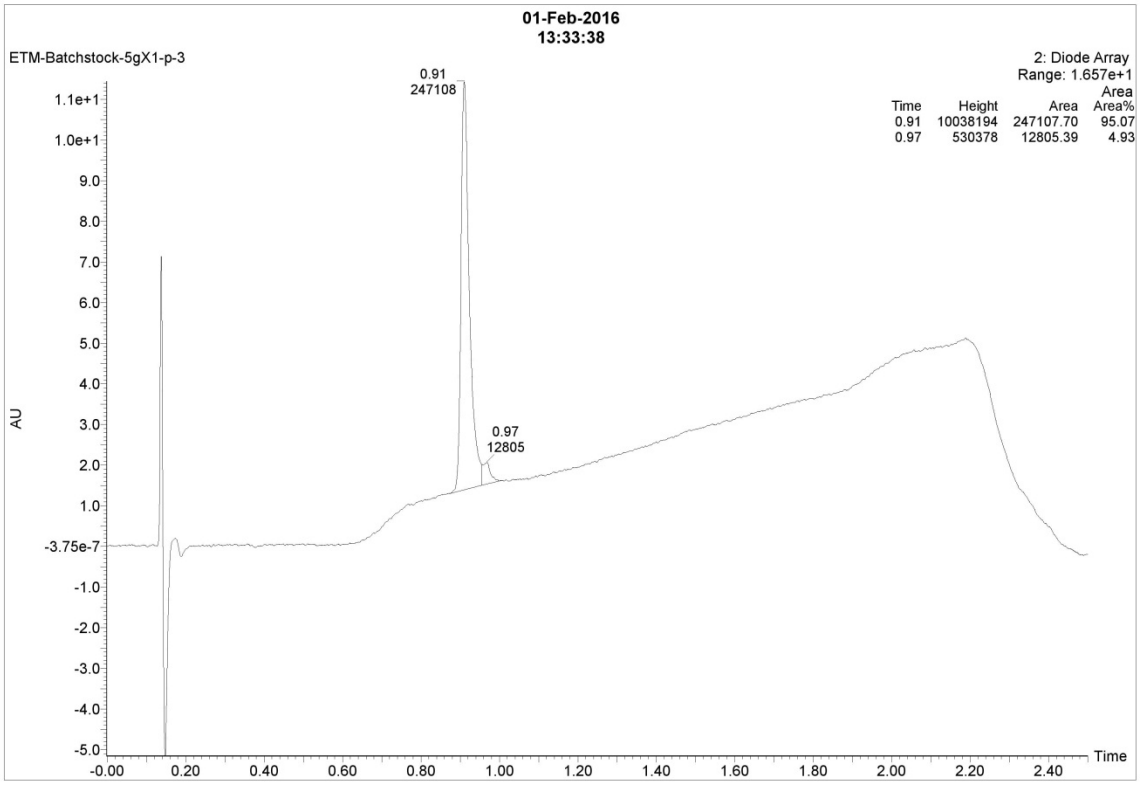
Fluo-βAla-(*N*{3,5-bis(2-(guanidino)ethoxy)) benzyl})$_4$-NH_2 (**PGua$_4$**)





Fluo-βAla-(*N*{3,5-bis(2-(guanidino)ethoxy)) benzyl})$_5$-NH_2 (**PGua$_5$**)





Fluo-βAla-(*N*{3,5-bis(2-(guanidino)ethoxy)) benzyl})$_6$-NH_2 (**PGua$_6$**)