Electronic Supplementary Material (ESI) for MedChemComm.

Supplementary Information

GLYCODENDROPEPTIDES STIMULATE DENDRITIC CELL MATURATION AND T CELL PROLIFERATION. A POTENTIAL INFLUENZA A VIRUS IMMUNOTHERAPY.

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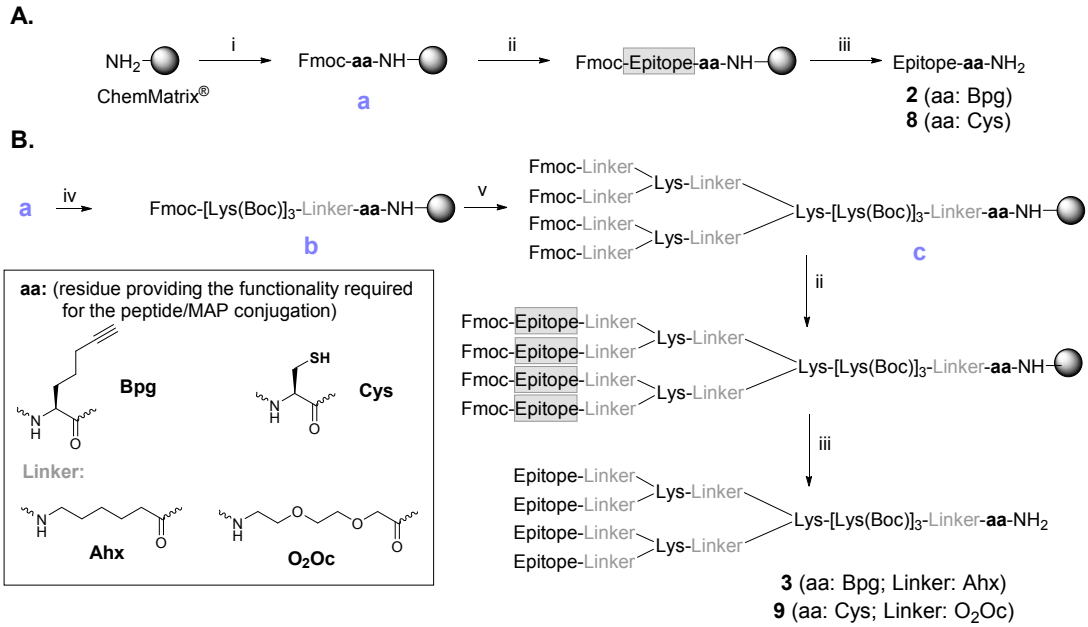
1. Table S1:

Method A						
Compound	**Name**	***n***	***Man***	**Composition**	**MW(mo/av)**	**MW(found)**
Glycodendron (Man_9N_3)						
1	Man_9N_3	-	9	$C_{152}H_{235}N_{39}O_{82}$	3918.5418/3920.7430	3920.8220
Peptides and MAPs ($nNP_{366-374}$-Bpg)						
2	$NP_{366-374}$-Bpg	1	-	$C_{45}H_{73}N_{13}O_{18}S_2$	1147.4638/1148.2720	1148.4704
3	$4NP_{366-374}$-Bpg	4	-	$C_{237}H_{405}N_{65}O_{82}S_8$	5729.7/5733.7	5735.7
Glycodendropeptides ($nNP_{366-374}$-Man_9)						
4 (2+1)	$NP_{366-374}$-Man_9	**1***	**9****	$C_{197}H_{308}N_{52}O_{100}S_2$	5066.0056/5069.0150	5066.0263
5 (3+1)	$4NP_{366-374}$-Man_9	**4***	**9****	$C_{389}H_{640}N_{104}O_{164}S_8$	9648.2703/9654.4430	9648.3698

Method B								
Compound	**Name**	***n***	***p***	***nxp***	***Man***	**Composition**	**MW(mo/av)**	**MW(found)**
Maleimide Dendrons (MD_pAlk)								
6	MD_2Alk	-	2	-	-	$C_{22}H_{22}N_2O_{10}$	474.1274/474.4220	475.0747
7	MD_4Alk	-	4	-	-	$C_{46}H_{48}N_4O_{22}$	1008.2760/1008.8960	1009.2805
Peptides and MAPs ($nNP_{366-374}$-Cys)								
8	$NP_{366-374}$-Cys	1	-	-	-	$C_{41}H_{69}N_{13}O_{18}S_3$	1127.4046/1128.2560	1128.4105
9	$4NP_{366-374}$-Cys	4	-	-	-	$C_{221}H_{378}N_{62}O_{92}S_9$	5660.4/5664.3	5660.2
Dendropeptide Intermediates ($(nNP_{366-374})_p$-Alk)								
10 (6+8)	$(NP_{366-374})_2$-Alk	1	2	2	-	$C_{104}H_{160}N_{28}O_{46}S_6$	2728.9/2730.9	2729.9
11 (7+8)	$(NP_{366-374})_4$-Alk	1	4	4	-	$C_{210}H_{324}N_{56}O_{94}S_{12}$	5517.9/5521.9	5521.9
12 (6+9)	$(4NP_{366-374})_2$-Alk	4	2	8	-	$C_{464}H_{778}N_{126}O_{194}S_{18}$	11795.0/11803.1	11802.0
13 (7+9)	$(4NP_{366-374})_4$-Alk	4	4	16	-	$C_{930}H_{1560}N_{252}O_{390}S_{36}$	23650.0/23666.2	23666.0
Glycodendropeptides ($(nNP_{366-374})_p$-Man_9)								
14 (10+1)	$(NP_{366-374})_2$-Man_9	1	2	**2***	**9****	$C_{256}H_{395}N_{67}O_{128}S_6$	6647.5/6651.7	6647.5
15 (11+1)	$(NP_{366-374})_4$-Man_9	1	4	**4***	**9****	$C_{362}H_{559}N_{95}O_{176}S_{12}$	9436.4360/9442.6630	9436.4658
16 (12+1)	$(4NP_{366-374})_2$-Man_9	4	2	**8***	**9****	$C_{616}H_{1013}N_{165}O_{276}S_{18}$	15713.5/15723.8	15746.0
17 (13+1)	$(4NP_{366-374})_4$-Man_9	4	4	**16***	**9****	$C_{1082}H_{1795}N_{291}O_{472}S_{36}$	27568.5/27587.0	27601.0

MS Characterization of Compounds (*: final number of peptides; **: final number of mannoses)

2. Experimental details for Peptide and MAP synthesis:



Scheme S1. Synthetic routes to peptides **2** and **8** and MAPs **3** and **9**. (i) Fmoc-aa; (ii) Fmoc-SPPS of $NP_{366-374}$ epitope (ASNENMETM); boxes denote the side chain-protected version of the sequence; (iii) TFA acidolysis; (iv) Fmoc-Linker, then 3× Fmoc-Lys(Boc); (v) Fmoc-Lys(Fmoc), then Fmoc-Linker.

Peptides 2 and 8: Residues Fmoc-Bpg (for peptide **2** synthesis) or Fmoc-Cys (for **8**) (1.25 mmol) were loaded on Rink-amide ChemMatrix resin (0.05 mmol) by a manual procedure with HBTU/HOBt (1.25 mmol) and DIEA (2.5 mmol) activation in DMF, in the case of the Fmoc-Bpg, or by an automatic method, when using Fmoc-Cys. To this resin (**a**) the residues of the ASNENMETM ($NP_{366-374}$) sequence were incorporated in automated mode in an ABI433 synthesizer (Applied Biosystems) running standard Fmoc chemistry protocols. Side chains were protected with TFA-labile tert-butyl (Ser, Thr) and trityl (Asn) groups. Couplings were done with 5 mmol each of Fmoc-*L*-amino acid, HBTU and HOBt, and 10 mmol of DIEA, with DMF as solvent. Fmoc groups were removed by 20% piperidine in DMF. Once the protected sequence was assembled, *N*-deprotection followed and acidolysis with TFA/H_2O/TIS (95:2.5:2.5, *v*/*v*, 60 min) gave the final peptides (**2** and **8**), which were isolated by precipitation with cold Et_2O and centrifugation for 3 × 10 min at 4 ºC. The crude material was dissolved in 10% (*v*/*v*) aqueous AcOH, lyophilized and purified by semipreparative RP-HPLC using a 10-35% linear gradient of solvent B into A. Yield of purified **2**, based on initial resin load, was 40%. In contrast, yield of purified **8**, was 46%.

MAPs 3 and 9: Fmoc-Bpg or Fmoc-Cys (0.05 mmol) were loaded on Rink-amide ChemMatrix resin (0.02 mmol) as above, followed by one O_2Oc/Ahx and three Lys(Boc) residues to give **b**. Elongation and branching with Fmoc-Lys(Fmoc) and Fmoc-O_2Oc/Fmoc-Ahx, as outlined in the scheme, led to tetravalent resin **c**, which was elongated with four copies of the ASNENMETM ($NP_{366-374}$) sequence. Aside from the Bpg residue, coupled manually, all other synthetic cycles were done in the automated mode using 2 mmol of Fmoc-amino acid and HBTU/HOBt, and 4 mmol of DIEA, in DMF, as described above. From the first branching point (Fmoc-Lys(Fmoc) onwards), double couplings were systematically performed. Once the protected sequence was assembled, the same work up described above was used to isolate the peptide material. Semipreparative RP-HPLC using a 15-30% linear gradient of B into A furnished **3** and **8** in a global 15% and 20% yield, respectively.

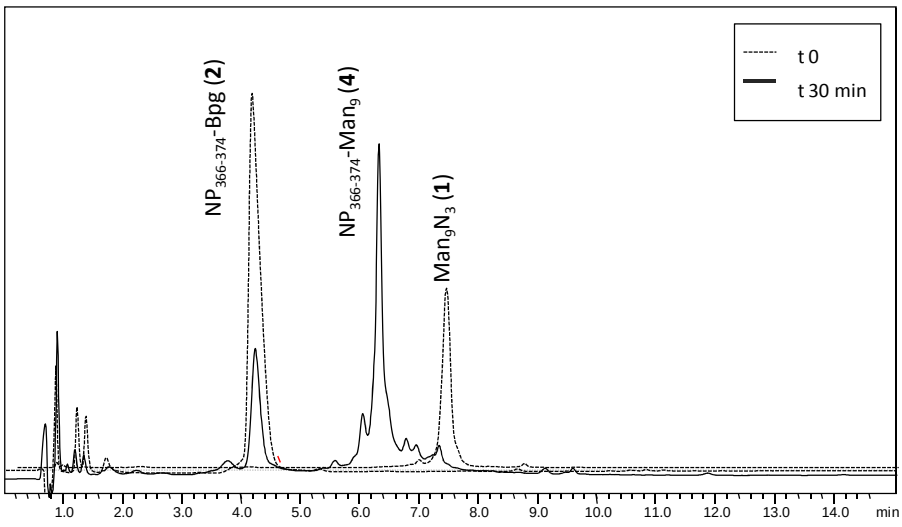
3. Experimental details for GDPs synthesis:

a) Method A:

General Protocol for Glycodendropeptide Synthesis: 0.26 μmol of glycodendron **1** and 0.13 μmol of peptide **2** or MAP **3** were dissolved in 100 μL of 3:2 (*v/v*) THF/phosphate buffer (100 mM, pH 7.4). In a separate vial, $CuSO_4 \cdot 5H_2O$ (0.13 μmol) was dissolved in H_2O (15 μL) and mixed with a solution of TBTA (0.26 μmol) in THF (33 μL). This solution was next added to the original mixture followed by 52 μL of sodium ascorbate (0.52 μmol) in H_2O. After stirring for about 30 min at room temperature (the progression of the reaction was monitorized by analytical RP-HPLC), the reaction mixture was lyophilized, then purified by semipreparative RP-HPLC to give target compounds **4**–**5**.

– **GDP 4 ($NP_{366\text{-}374}$-Man_9):**

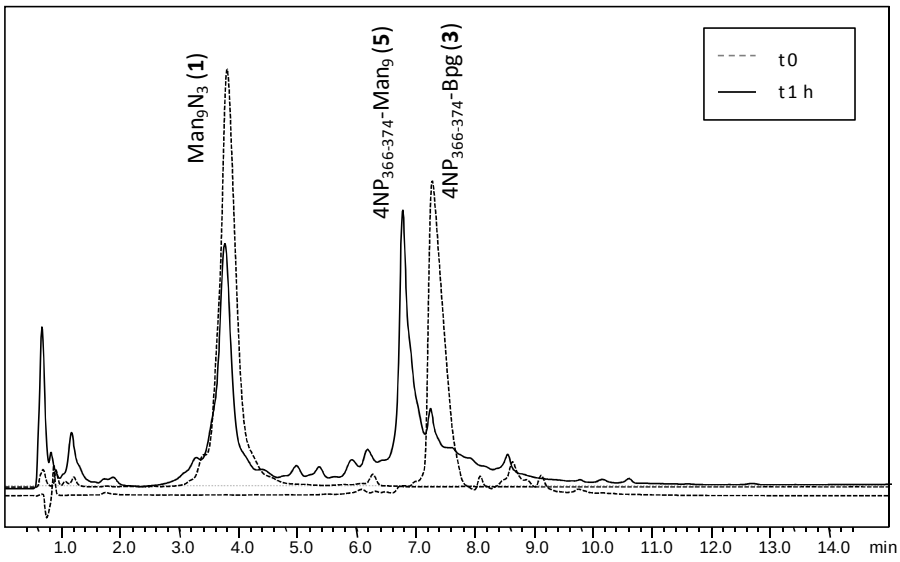
Purification: Semipreparative RP-HPLC eluting with a 15 to 30% linear gradient of B into A over 30min (further HPLC details in Experimental). Yield: **45%**.



HPLC monitoring of the **1** + **2** CuAAC reaction leading to **4** at 0 and 30 minutes. Elution by 15 to 30% linear gradient of B into A over 15min (further HPLC details in Experimental). Column: 4.6×50 mm, 3 μm C8 column (Phenomenex, Torrance, CA).

– **GDP 5 ($4NP_{366\text{-}374}$-Man_9):**

Purification: Semipreparative RP-HPLC eluting with a 17 to 30% linear gradient of B into A over 30min (further HPLC details in Experimental). Yield: **50%**.



HPLC monitoring of the **1** + **3** CuAAC reaction leading to **5** at 0 and 1 hour. Elution by 17 to 30% linear gradient of B into A over 15min (further HPLC details in Experimental). Column: 4.6×50 mm, 3 μm C8 column (Phenomenex, Torrance, CA).

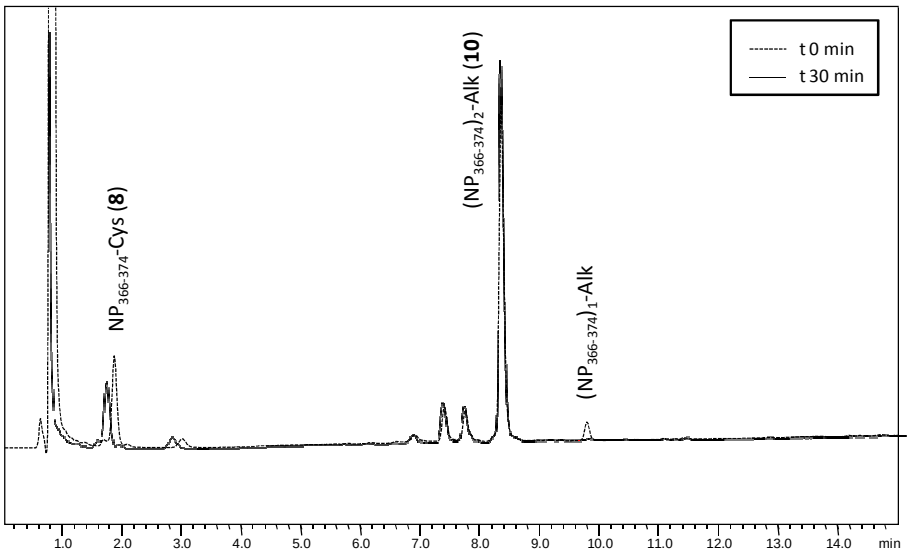
b) Method B:

b.1 First Step: Synthesis of Dendropeptide Intermediates:

General Synthetic Protocol for Dendropeptide Intermediates: To a solution of 0.5 μmol (1 equiv.) of maleimide dendron (0.24 mg of **6** or 0.5 mg of **7**) in CH_3CN (400 μL), a solution of 1.5 equiv./maleimide group of peptide **8** or MAP **9** in 100 mM phosphate buffer, pH 7.4 (800 μL), was added. To monitor the reaction progress, aliquots of the mixture were taken for analytical HPLC and MALDI-TOF MS analysis, and when no further changes in the HPLC profile were observed (reaction times range from few minutes to 24 h, depending on the steric hindrance), the reaction was stopped by addition of glacial AcOH. The product was then isolated by preparative RP-HPLC. The target compounds (**10-13**) were obtained in homogeneous form (> 95%) by analytical HPLC and were satisfactorily characterized by MS (see Table S1, Supporting Information).

– **Dendropeptide Intermediate 10 ($(NP_{365-374})_2$-Alk):**

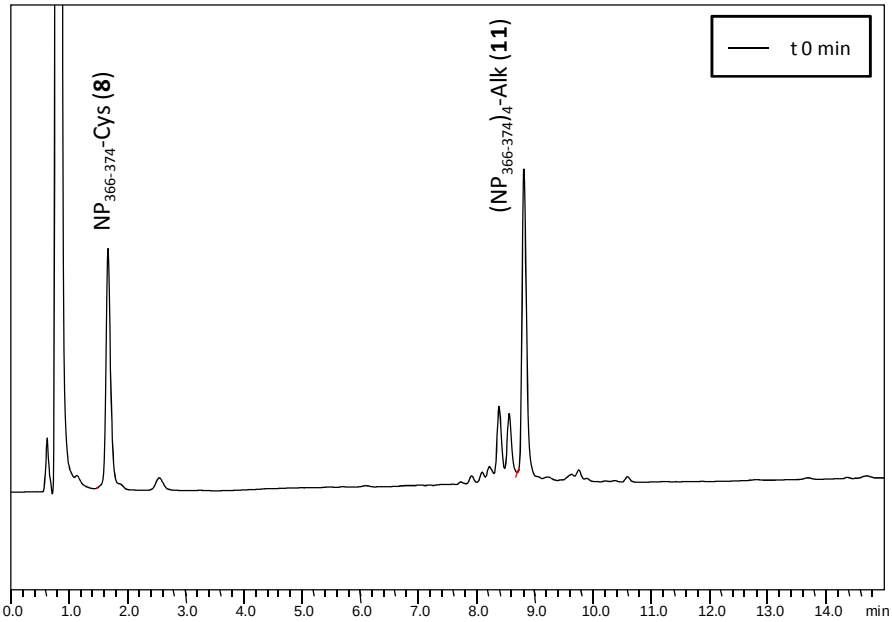
Purification: Semipreparative RP-HPLC eluting with a 15 to 30% linear gradient of B into A over 30min (further HPLC details in Experimental). Yield: **83%**.



HPLC monitoring of the **6** + **8** CuAAC reaction leading to **10** at 0 and 30 min. Elution by 15 to 30% linear gradient of B into A over 15min (further HPLC details in Experimental). Column: 4.6×50 mm, 3 µm C8 column (Phenomenex, Torrance, CA).

– **Dendropeptide Intermediate 11 ($(NP_{365-374})_4$-Alk):**

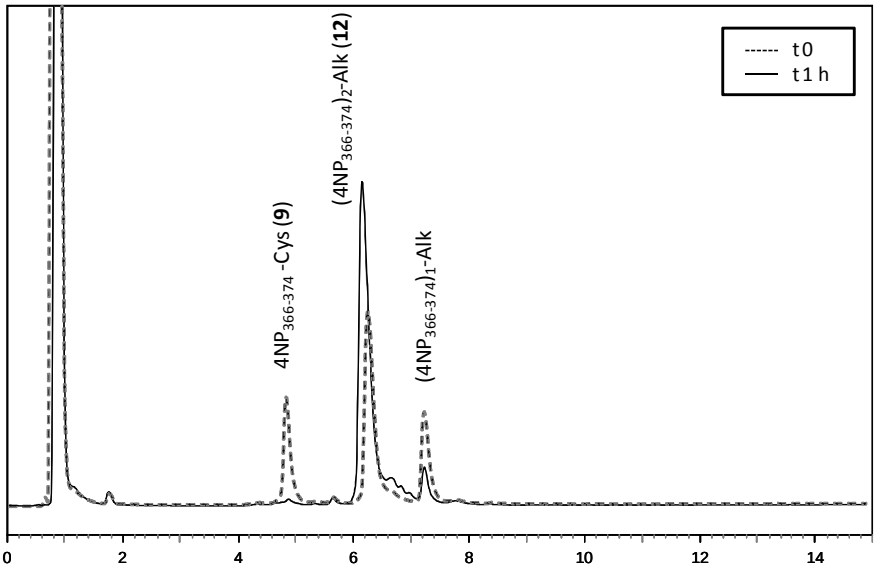
Purification: Semipreparative RP-HPLC eluting with a 15 to 50% linear gradient of B into A over 30min (further HPLC details in Experimental). Yield: **89%**.



HPLC monitoring of the **7** + **8** CuAAC reaction leading to **11** at time 0. Elution by 15 to 50% linear gradient of B into A over 15min (further HPLC details in Experimental). Column: 4.6×50 mm, 3 µm C8 column (Phenomenex, Torrance, CA).

– **Dendropeptide Intermediate 12 ($(4NP_{365-374})_2$-Alk):**

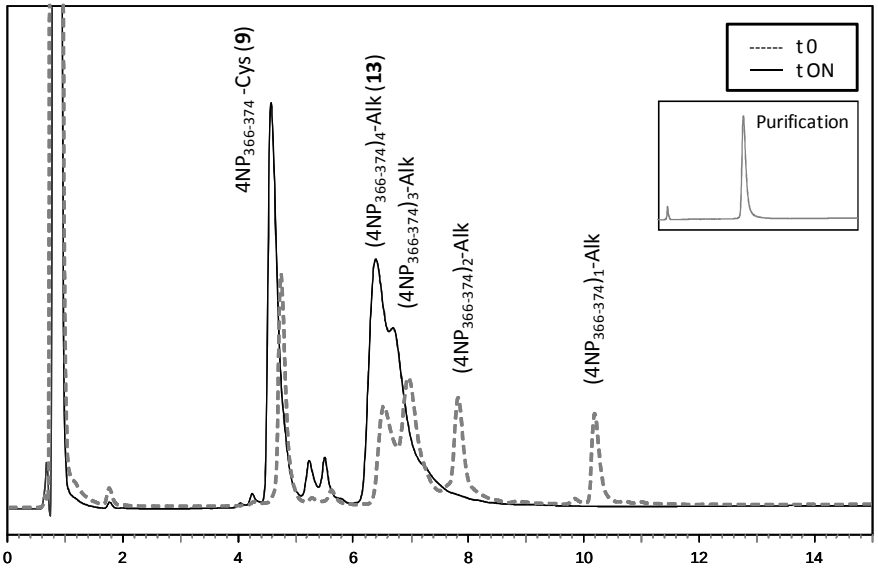
Purification: Semipreparative RP-HPLC eluting with a 15 to 50% linear gradient of B into A over 30min (further HPLC details in Experimental). Yield: **64%**.



HPLC monitoring of the **6** + **9** CuAAC reaction leading to **12** at 0 and 1 hour. Elution by 15 to 50% linear gradient of B into A over 15min (further HPLC details in Experimental). Column: 4.6×50 mm, 3 μm C18 column (Phenomenex, Torrance, CA). Peaks corresponding to the ligation of MAP **9** to one (tr = 7.31 min, $(4NP_{366-374})_1$-Alk) and two (tr = 6.26 min, compound **12**) maleimido groups of compound **6** are shown.

– **Dendropeptide Intermediate 13 ($(4NP_{365-374})_4$-Alk):**

Purification: Semipreparative RP-HPLC eluting with a 15 to 50% linear gradient of B into A over 30min (further HPLC details in Experimental). Yield: **48%**.



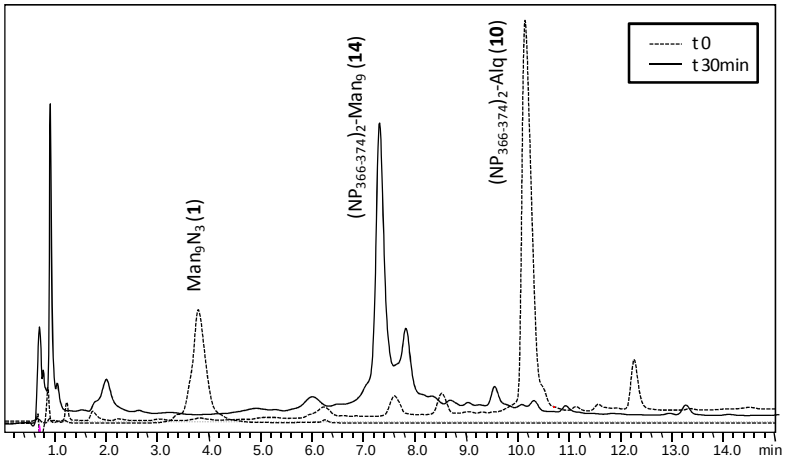
HPLC monitoring of the **7** + **9** CuAAC reaction leading to **13** at 0 and 24 hours. Elution by 15 to 50% linear gradient of B into A over 15min (further HPLC details in Experimental). Column: 4.6×50 mm, 3 μm C18 column (Phenomenex, Torrance, CA). Peaks corresponding to the ligation of MAP **9** to one (tr = 10.22 min, $(4NP_{366-374})_1$-Alk), two (tr = 7.89 min, $(4NP_{366-374})_2$-Alk), three (tr = 6.91 min, $(4NP_{366-374})_3$-Alk) and four (tr = 6.35 min, compound **13**) maleimido groups of compound **7** are shown. Inset: analytical HPLC of purified **13**.

b.2 Second Step: Synthesis of GDPs:

General Protocol for Glycodendropeptide Synthesis: 0.26 μmol of glycodendron **1** and 0.13 μmol of dendropeptide intermediates **10-13** were dissolved in 100 μL of 3:2 (*v/v*) THF/phosphate buffer (100 mM, pH 7.4). In a separate vial, $CuSO_4 \cdot 5H_2O$ (0.13 μmol) was dissolved in H_2O (15 μL) and mixed with a solution of TBTA (0.26 μmol) in THF (33 μL). This solution was next added to the original mixture followed by 52 μL of sodium ascorbate (0.52 μmol) in H_2O. After stirring for about 30 min at room temperature (the progression of the reaction was monitorized by analytical RP-HPLC), the reaction mixture was lyophilized, then purified by semipreparative RP-HPLC to give target compounds **14**–**17**.

– **GDP 14 ($(NP_{365-374})_2$-Man_9):**

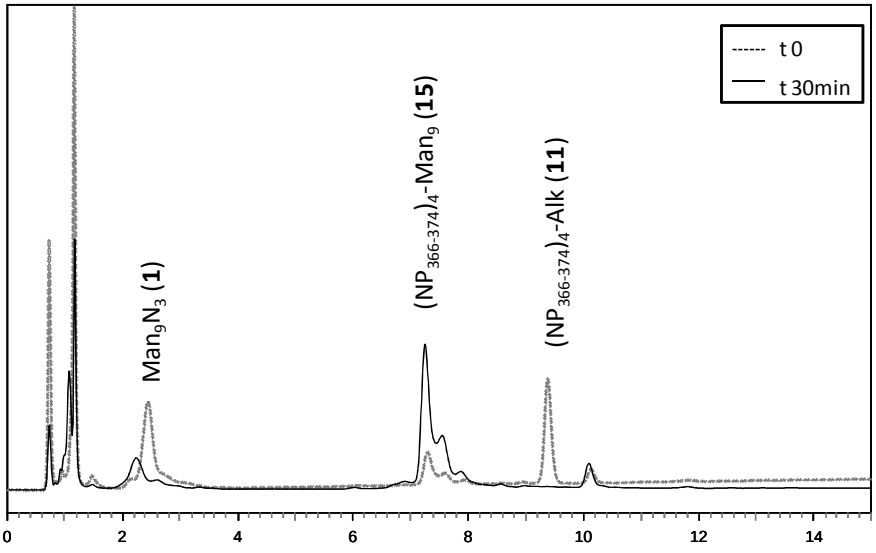
Purification: Semipreparative RP-HPLC eluting with a 17 to 30% linear gradient of B into A over 30min (further HPLC details in Experimental). Yield: **45%**.



HPLC monitoring of the **1** + **10** CuAAC reaction leading to **14** at 0 and 30 minutes. Elution by 17 to 30% linear gradient of B into A over 15min (further HPLC details in Experimental). Column: 4.6×50 mm, 3 µm C8 column (Phenomenex, Torrance, CA).

– **GDP 15 ($(NP_{365-374})_4$-Man_9):**

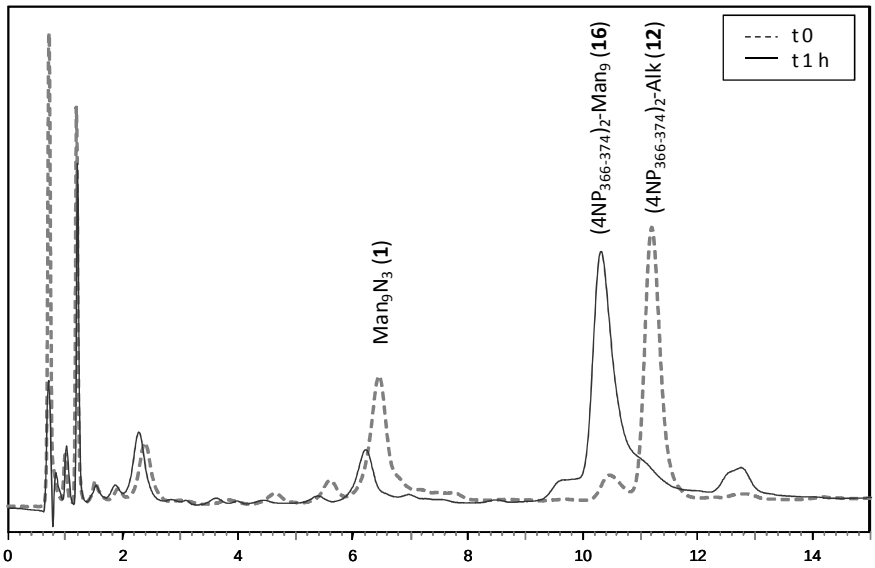
Purification: Semipreparative RP-HPLC eluting with a 20 to 40% linear gradient of B into A over 30min (further HPLC details in Experimental). Yield: **41%**.



HPLC monitoring of the **1** + **11** CuAAC reaction leading to **15** at 0 and 30 minutes. Elution by 20 to 40% linear gradient of B into A over 15min (further HPLC details in Experimental). Column: Column: 4.6×50 mm, 3 µm C8 column (Phenomenex, Torrance, CA).

– **GDP16 ($(4NP_{365-374})_2$-Man_9):**

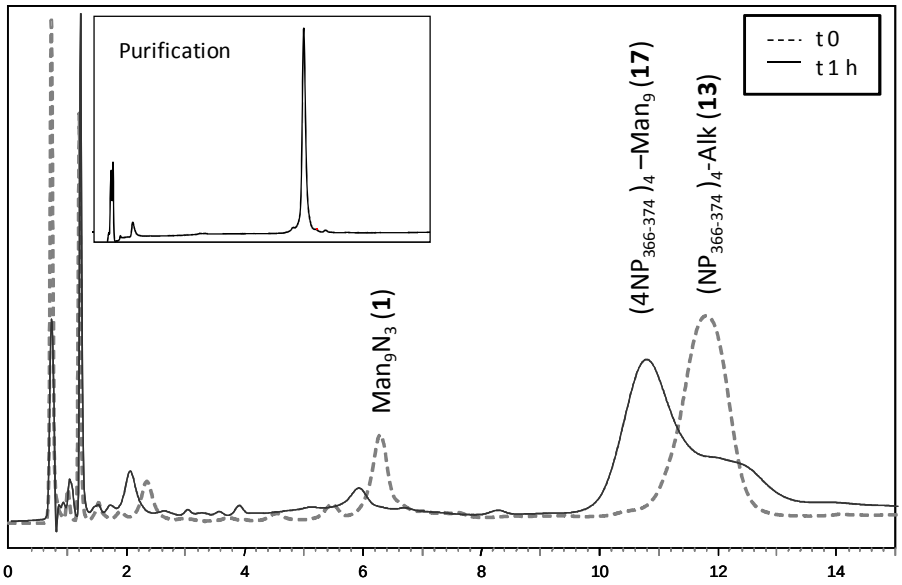
Purification: Semipreparative RP-HPLC eluting with a 17 to 30% linear gradient of B into A over 30min (further HPLC details in Experimental). Yield: **35%**.



HPLC monitoring of the **1** + **12** CuAAC reaction leading to **16** at 0 and 1 hour. Elution by 17 to 30% linear gradient of B into A over 15min (further HPLC details in Experimental). Column: 4.6×50 mm, 3 µm C18 column (Phenomenex, Torrance, CA).

– **GDP 17 ($(4NP_{365-374})_4$-Man_9):**

Purification: Semipreparative RP-HPLC eluting with a 18 to 25% linear gradient of B into A over 30min (further HPLC details in Experimental). Yield: **37%**.

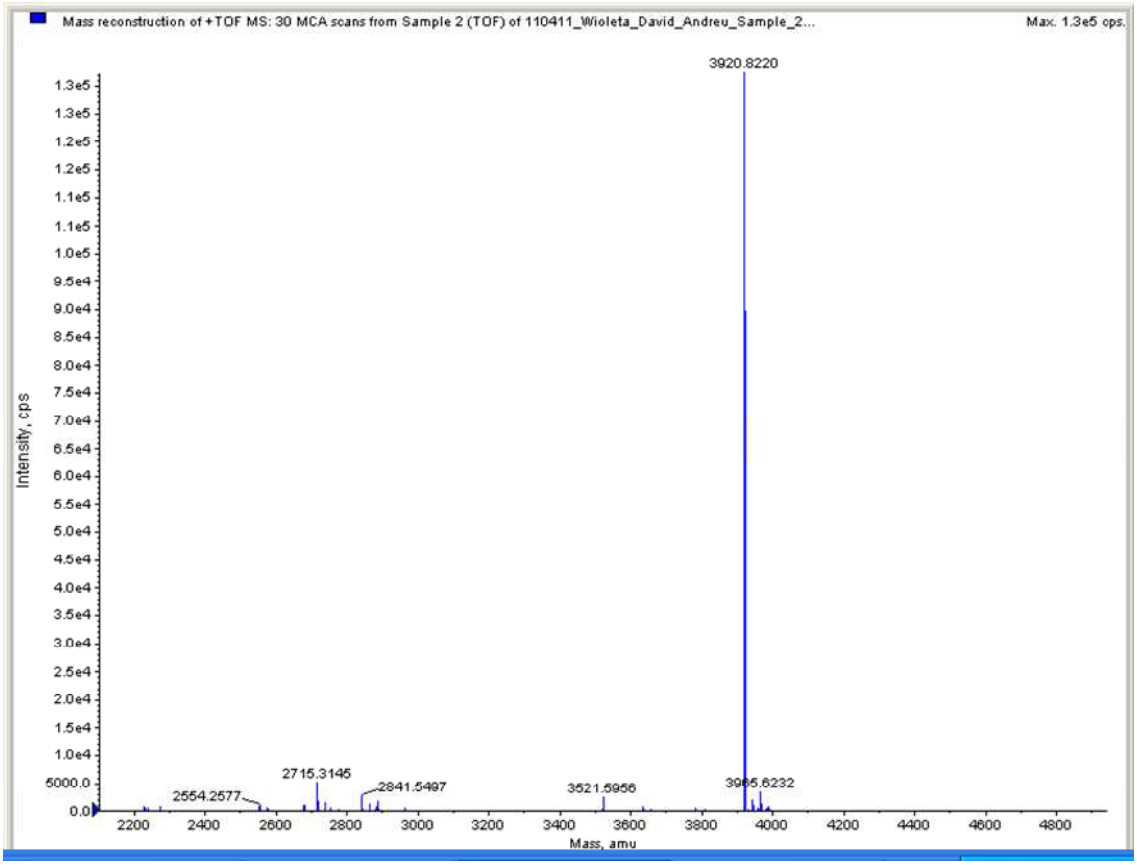


HPLC monitoring of the **1** + **13** CuAAC reaction leading to **17** at 0 and 1 hour. Elution by 18 to 25% linear gradient of B into A over 15min (further HPLC details in Experimental). Column: 4.6×50 mm, 3 µm C18 column (Phenomenex, Torrance, CA). Inset: analytical HPLC of purified **17**.

4. Mass Spectra:

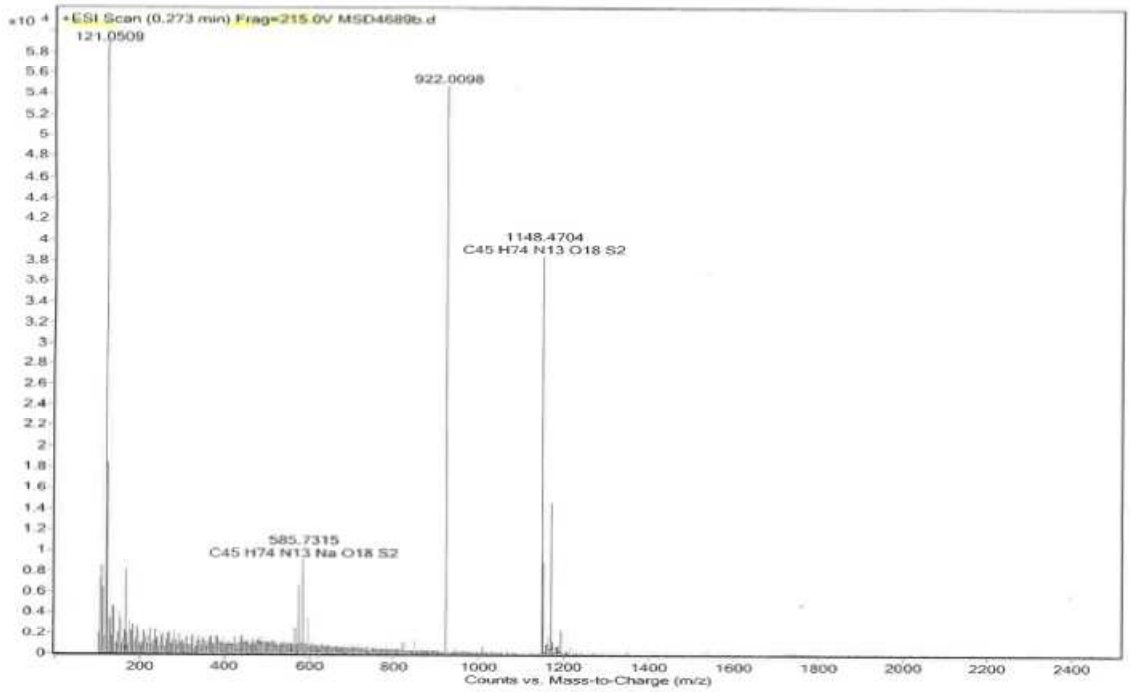
– **Compound 1 (Man_9N_3):** MW (mo/av) = 3918.5418/3920.7430

High Resolution Mass Spectrum.



– **Compound 2 ($NP_{366-374}$-Bpg):** MW (mo/av) = 1147.4638/1148.2720

High Resolution Mass Spectrum.



In this spectrum we can observe *m/z*: 121.0509 (purine) and 922.0098 (HP-0921), corresponding with the internal standard products.

z=1

m/z 1148.4704 consistent with $C_{45}H_{74}N_{13}O_{18}S_2$, thus $[M+H]^+$ (error 0.7ppm)

m/z 1170.4543 consistent with $C_{45}H_{73}N_{13}O_{18}S_2Na$, thus $[M+Na]^+$ (error 0.64ppm)

m/z 1192.4383 consistent with $C_{45}H_{72}N_{13}O_{18}S_2Na_2$, thus $[M-H+2Na]^+$ (error 2.35ppm)

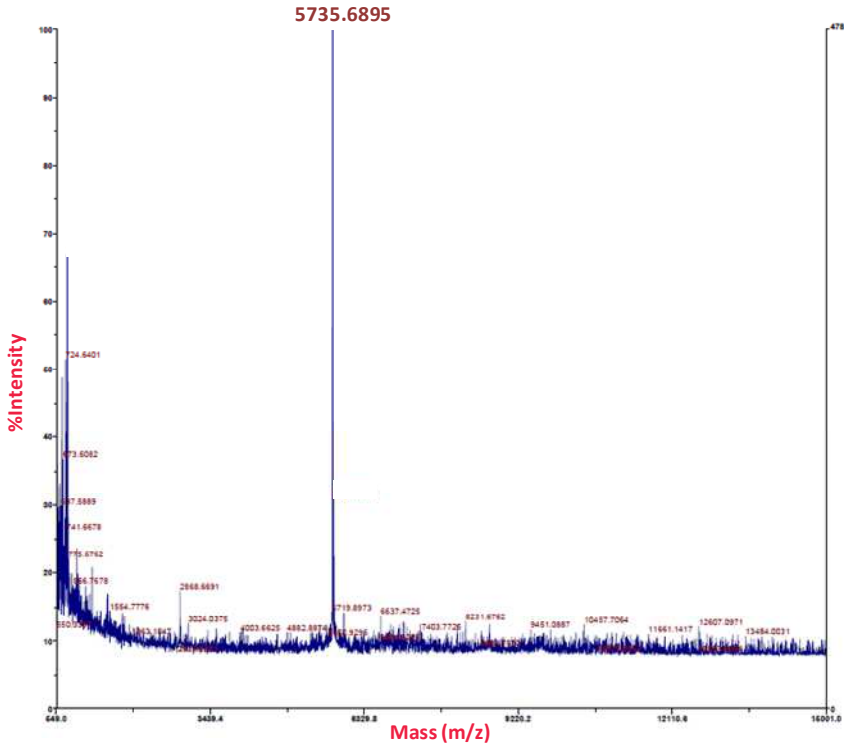
z=2

m/z 574.7392 consistent with $C_{45}H_{75}N_{13}O_{18}S_2$, thus $[M+2H]^{2+}$ (error 1.2ppm)

m/z 585.7315 consistent with $C_{45}H_{74}N_{13}O_{18}S_2Na$, thus $[M+H+Na]^{2+}$ (error 1.47ppm)

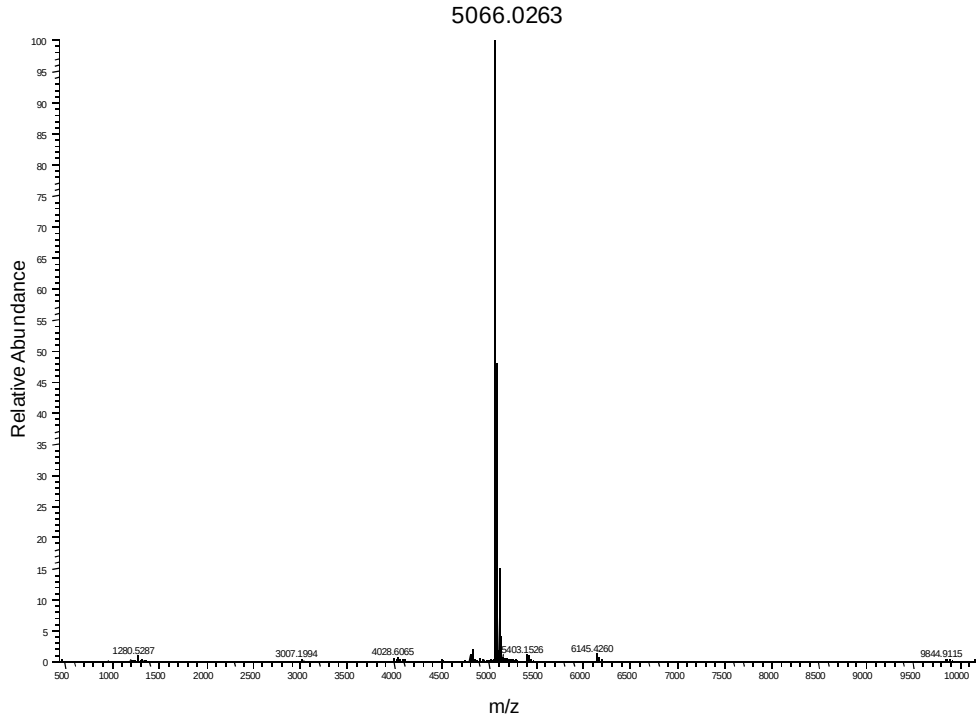
– **Compound 3 (4NP$_{366-374}$-Bpg):** MW (mo/av) = 5729.7/5733.7

MALDI Spectrum



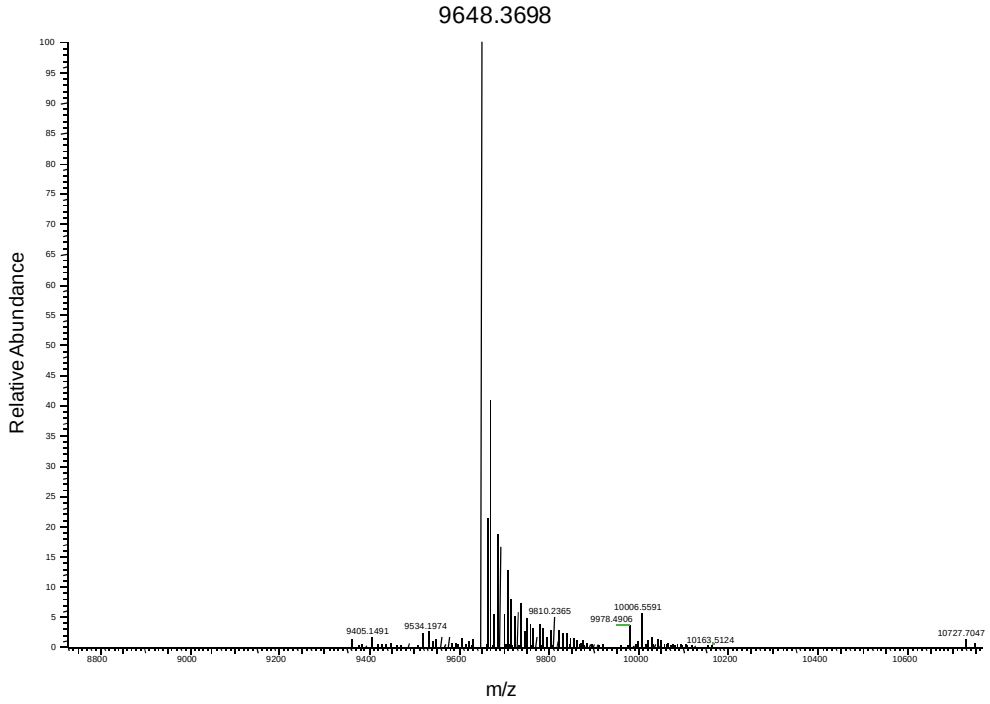
– **Compound 4 (NP$_{366-374}$-Man$_9$):** MW (mo/av) = 5066.0056/5069.0150

High Resolution Mass Spectrum. Deconvoluted and deisotoped data.



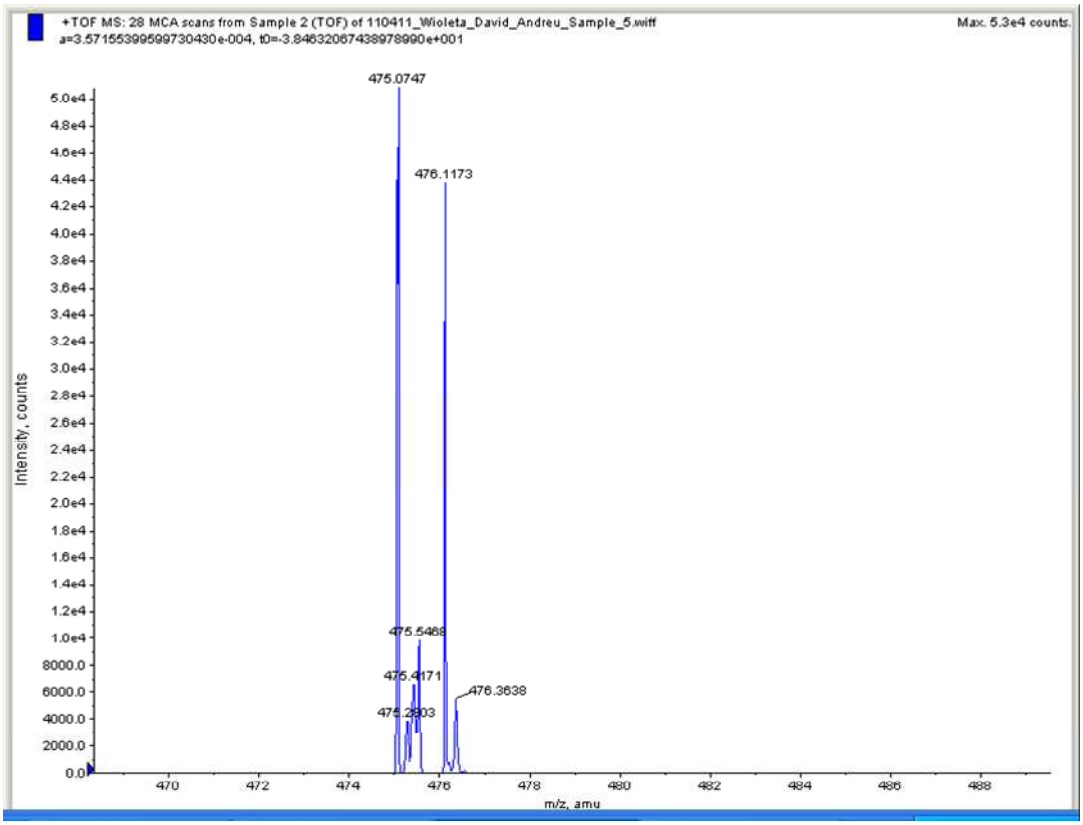
– **Compound 5 ($4NP_{366-374}$-Man_9):** MW (mo/av) = 9648.2703/9654.4430

High Resolution Mass Spectrum. Deconvoluted spectrum. Masses as Charge 0. Deisotoped.



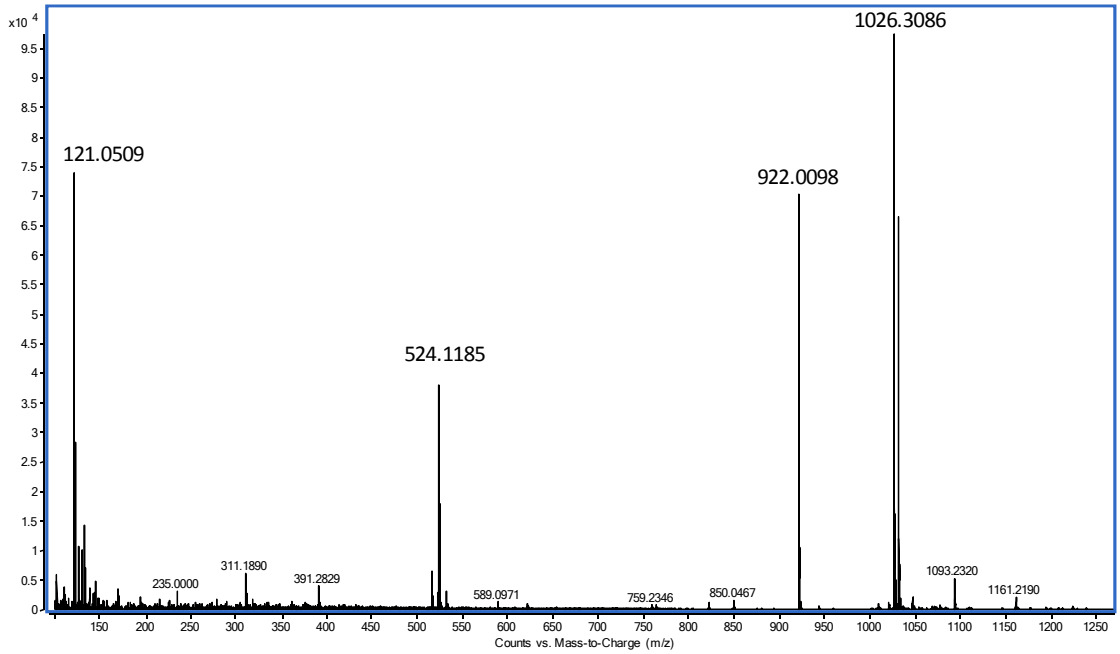
– **Compound 6 (MD_2Alk):** MW(mo/av) = 474.1274/474.4220

High Resolution Mass Spectrum.



– **Compound 7 (MD$_4$Alk):** MW(mo/av) = 1008.2760/1008.8960

High Resolution Mass Spectrum.



In this spectrum we can observe *m/z*: 121.0509 (purine) and 922.0098 (HP-0921), corresponding with the internal standard products.

z=1

m/z 1009.2805 consistent with $C_{46}H_{49}N_4O_{22}$, thus $[M+H]^+$ (error 2.85ppm)

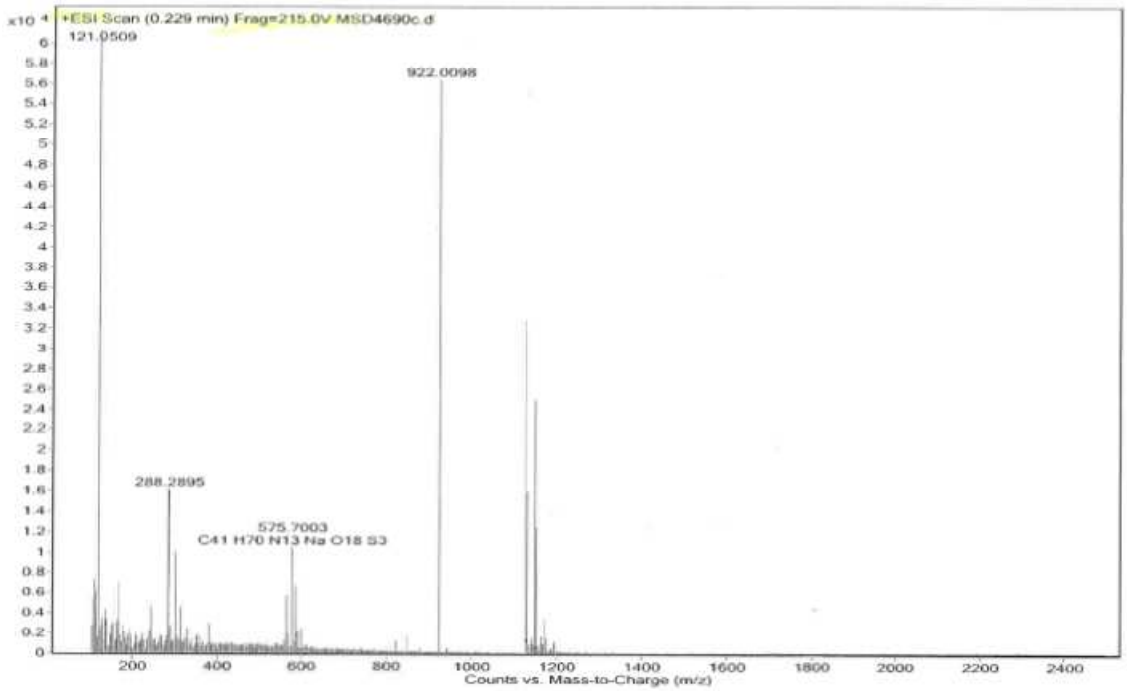
m/z 1026.3086 consistent with $C_{46}H_{52}N_5O_{22}$, thus $[M+NH_4]^+$ (error 1.31ppm)

m/z 1031.2637 consistent with $C_{46}H_{48}N_4O_{22}Na$, thus $[M+Na]^+$ (error 1.57ppm)

m/z 1047.2377 consistent with $C_{46}H_{48}N_4O_{22}K$, thus $[M+Na]^+$ (error 1.37ppm)

– **Compound 8 ($NP_{366-374}$-Cys):** MW (mo/av) = 1127.4046/1128.2560

High Resolution Mass Spectrum.



In this spectrum we can observe *m/z*: 121.0509 (purine) and 922.0098 (HP-0921), corresponding with the internal standard products.

z=1

m/z 1128.4105 consistent with $C_{41}H_{70}N_{13}O_{18}S_3$, thus $[M+H]^+$ (error 1.06ppm)

m/z 1150.3924 consistent with $C_{41}H_{69}N_{13}O_{18}S_3Na$, thus $[M+Na]^+$ (error 1.21ppm)

m/z 1172.3695 consistent with $C_{41}H_{68}N_{13}O_{18}S_3Na_2$, thus $[M-H+2Na]^+$ (error 4.69ppm)

z=2

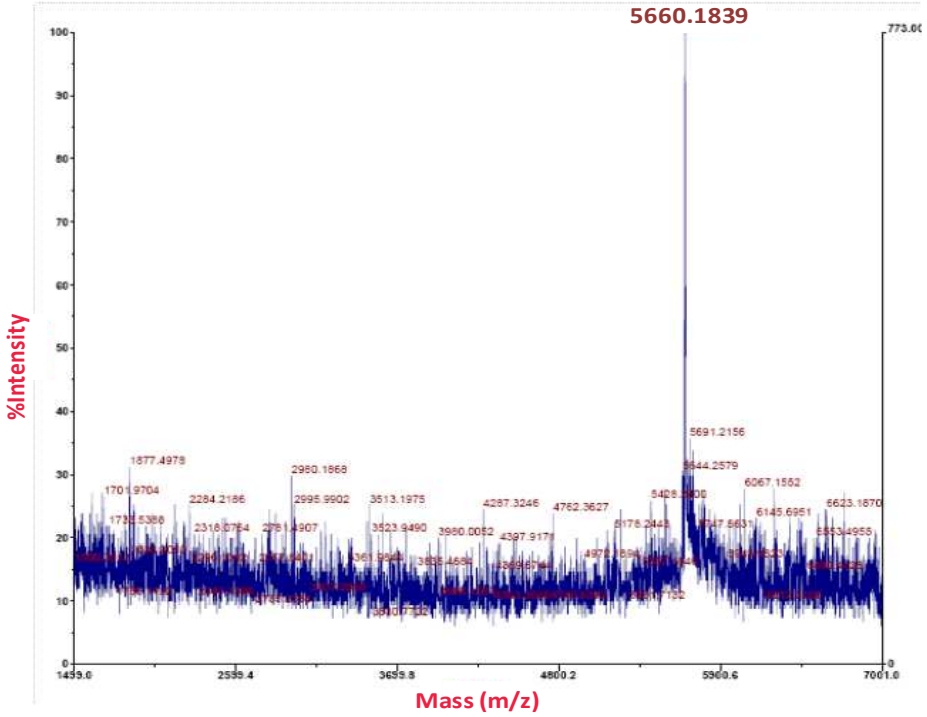
m/z 564.7119 consistent with $C_{41}H_{71}N_{13}O_{18}S_3$, thus $[M+2H]^{2+}$ (error 2.08ppm)

m/z 575.7003 consistent with $C_{41}H_{70}N_{13}O_{18}S_3Na$, thus $[M+H+Na]^{2+}$ (error 0.04ppm)

m/z 586.6928 consistent with $C_{41}H_{69}N_{13}O_{18}S_3Na_2$, thus $[M+2Na]^{2+}$ (error 1.38ppm)

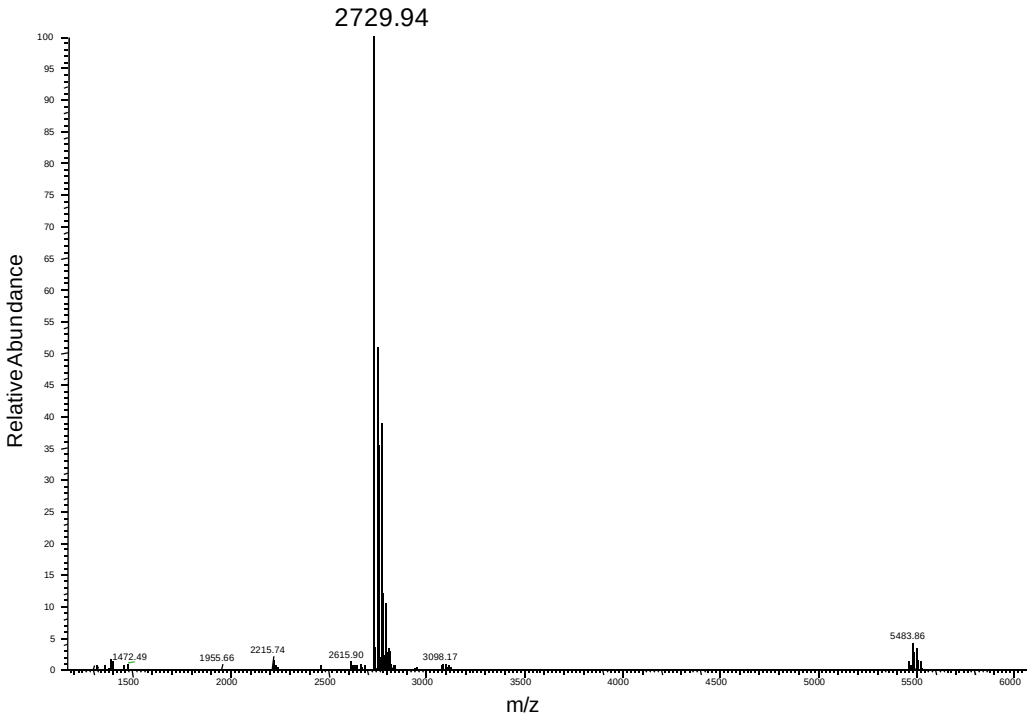
– **Compound 9 (4NP$_{365-374}$-Cys):** MW (mo/av) = 5660.4/5664.3

MALDI spectrum.



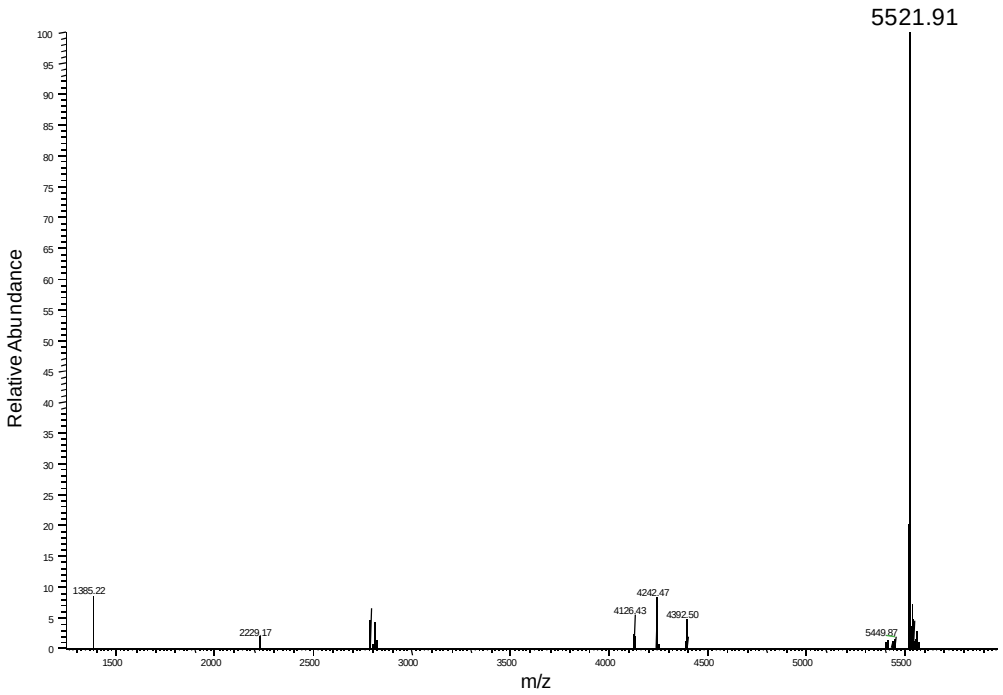
– **Compound 10 ((NP$_{365-374}$)$_2$-Alk):** MW (mo/av) = 2728.9/2730.9

ESI spectrum. Deconvoluted data (masses as charge 0).



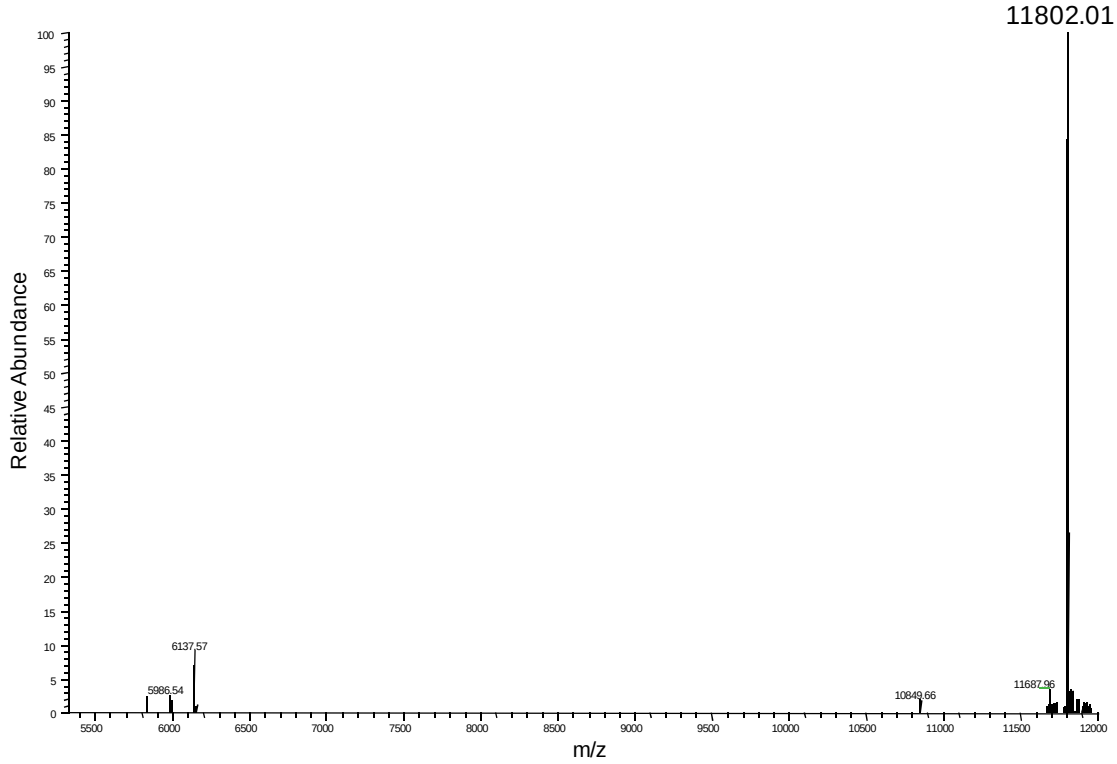
– **Compound 11 ($(NP_{366-374})_4$-Alk):** MW (mo/av) = 5517.9/5521.9

ESI spectrum. Deconvoluted data (masses as charge 0).



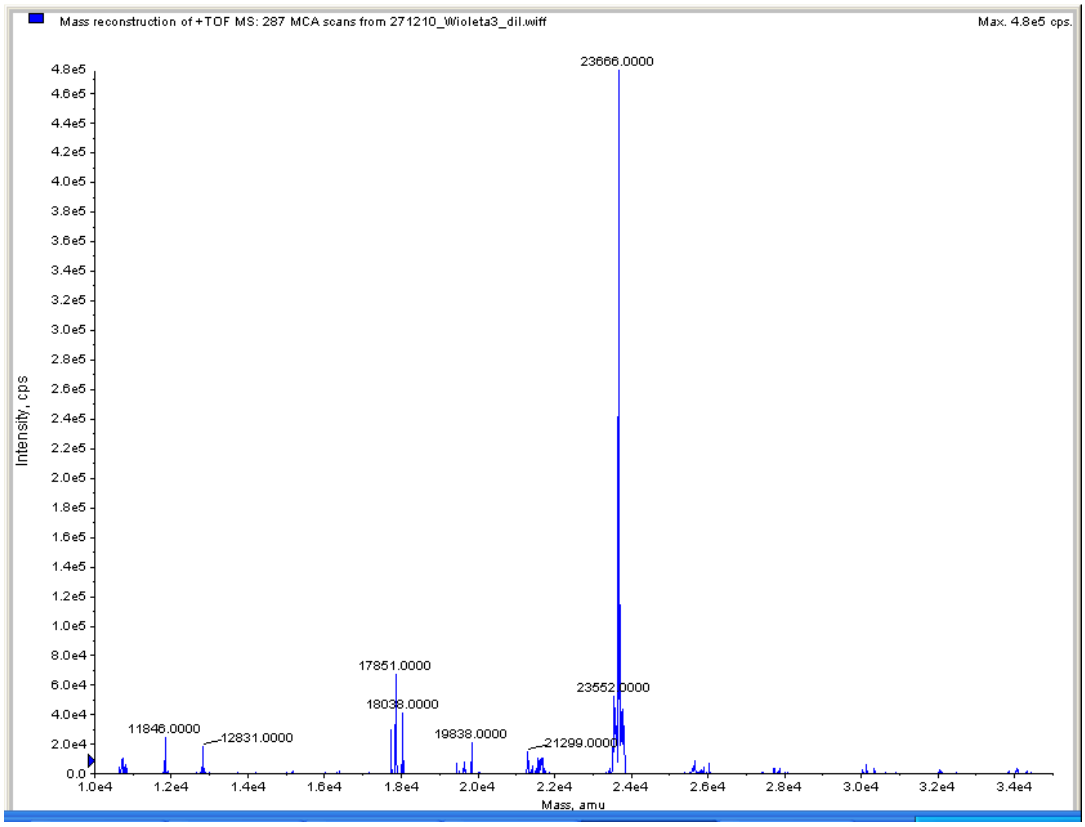
– **Compound 12 ($(4NP_{366-374})_2$-Alk):** MW (mo/av) = 11795.0/11803.1

ESI Spectrum. Deconvoluted data (masses as charge 0).



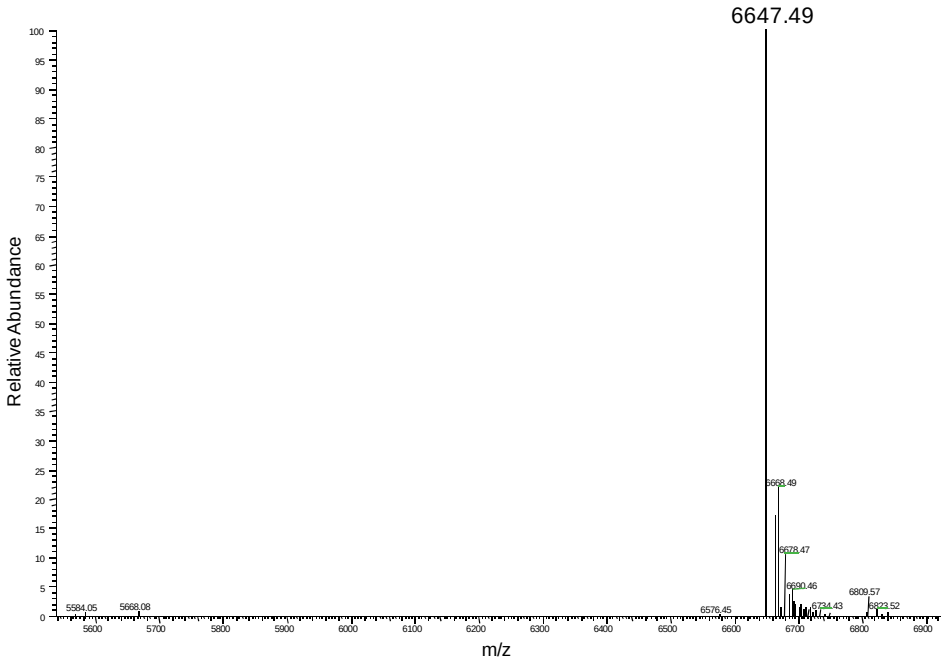
– **Compound 13 ((4NP$_{366-374}$)$_4$-Alk):** MW (mo/av) = 23650.0/23666.2

ESI Spectrum.



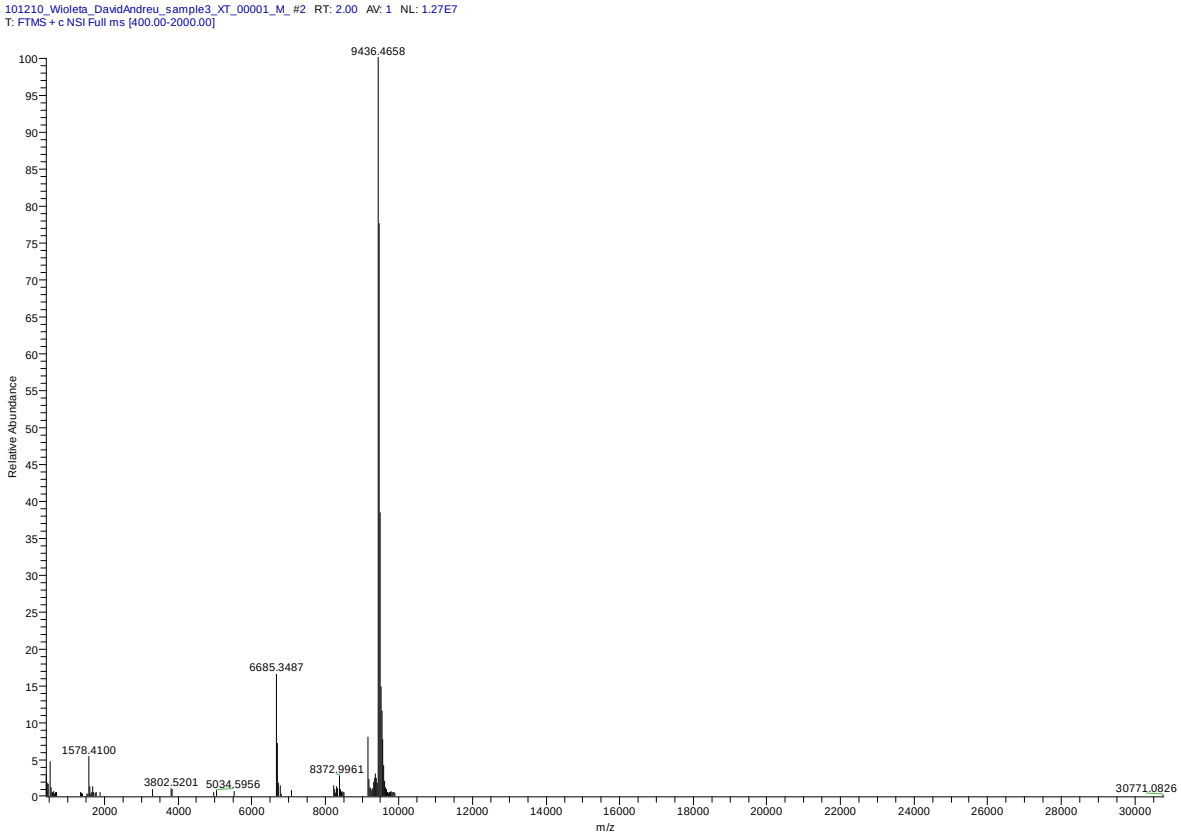
– **Compound 14 ((NP$_{366-374}$)$_2$-Man$_9$):** MW (mo/av) = 6647.5/6651.7

ESI Spectrum. Data deconvoluted.



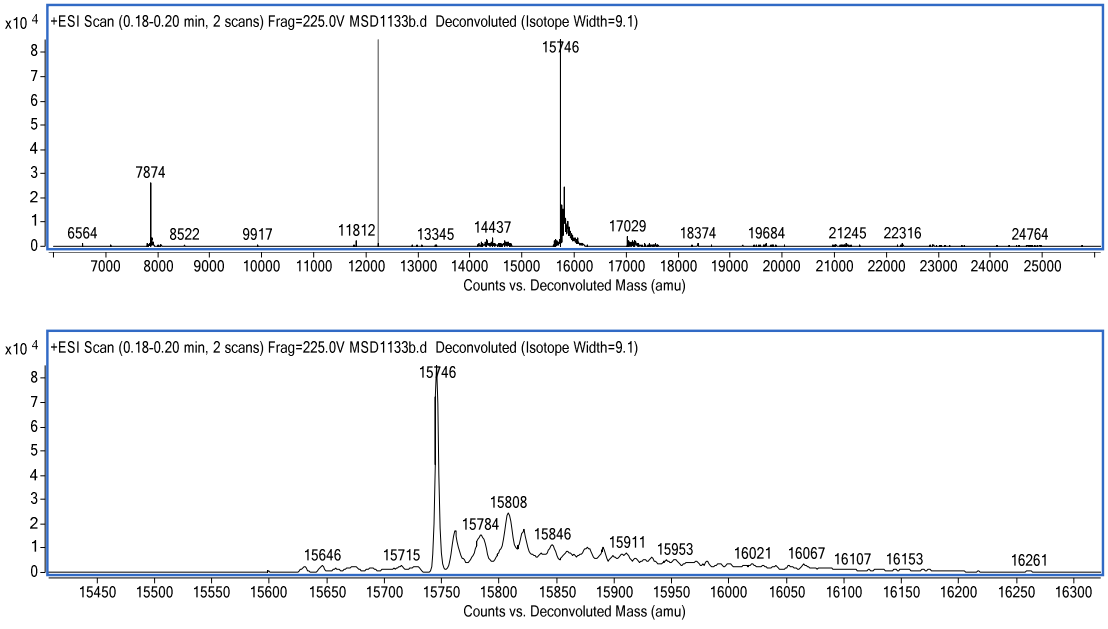
– **Compound 15 ($(NP_{366-374})_4$-Man_9):** MW (mo/av) = 9436.4360/9442.6630

High Resolution Mass Spectrum. Data deconvoluted and deisotoped.



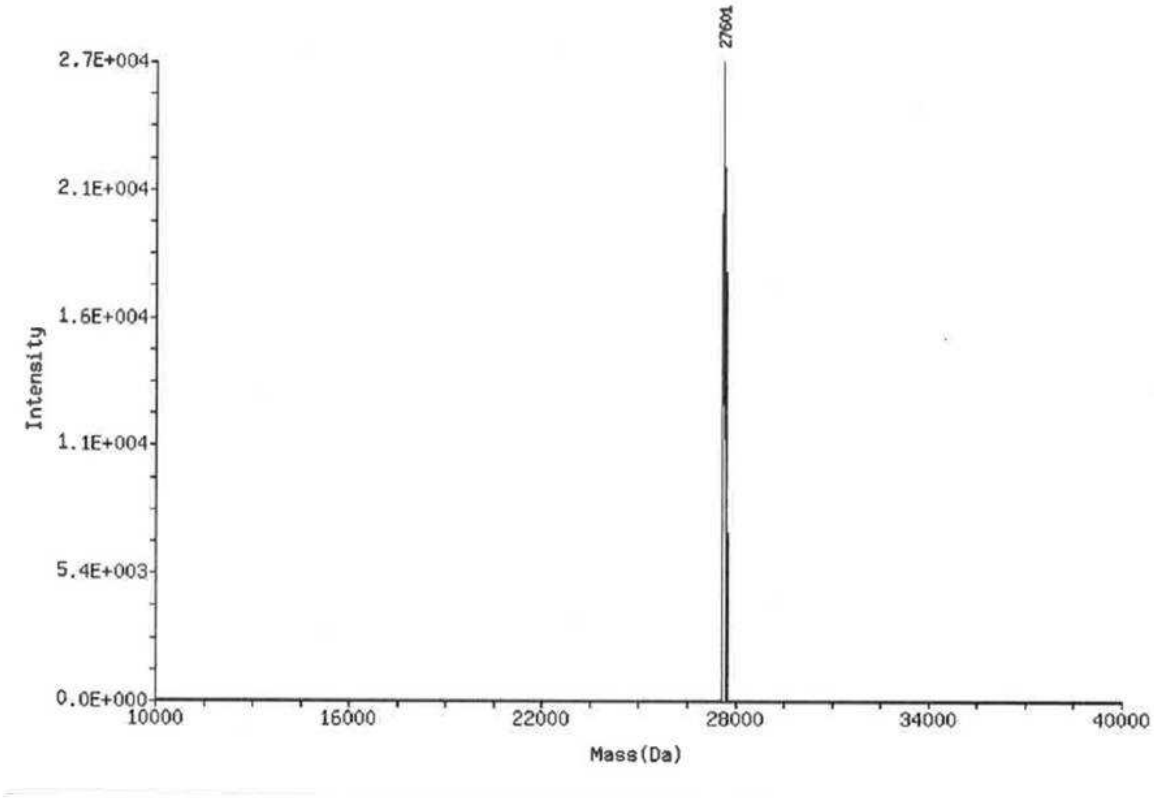
– **Compound 16 ($(4NP_{366-374})_2$-Man_9):** MW (mo/av) = 15713.5/15723.8

ESI Spectrum



– **Compound 17 ($(4NP_{366-374})_4$-Man_9):** MW (mo/av) = 27568.5/27587.0

ESI Spectrum. Deconvoluted spectrum.



5. Toxicity Assays: