Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Scaffold optimization in discontinuous epitope containing protein mimics of gp120 using smart libraries

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General procedure for scaffold conjugation of cyclic peptides by optimized CuAAc, described here for CTV 9.[1-4]

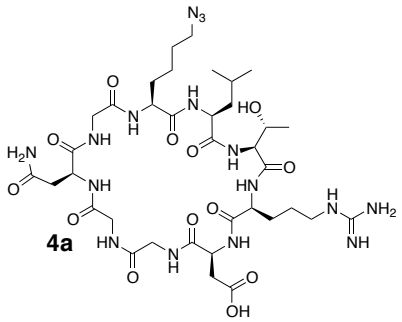
10-fold stock solutions of $CuSO_4$ (26.5 µmol, 6.6 mg in 1 mL H_2O) and sodium ascorbate (53.0 µmol, 10.5 mg in 1 mL H_2O) were prepared. TBTA[3,4] was dissolved in 100 µL DMF. Peptides were dissolved individually in the smallest possible amount (100-500 µL) of DMF (4Å).

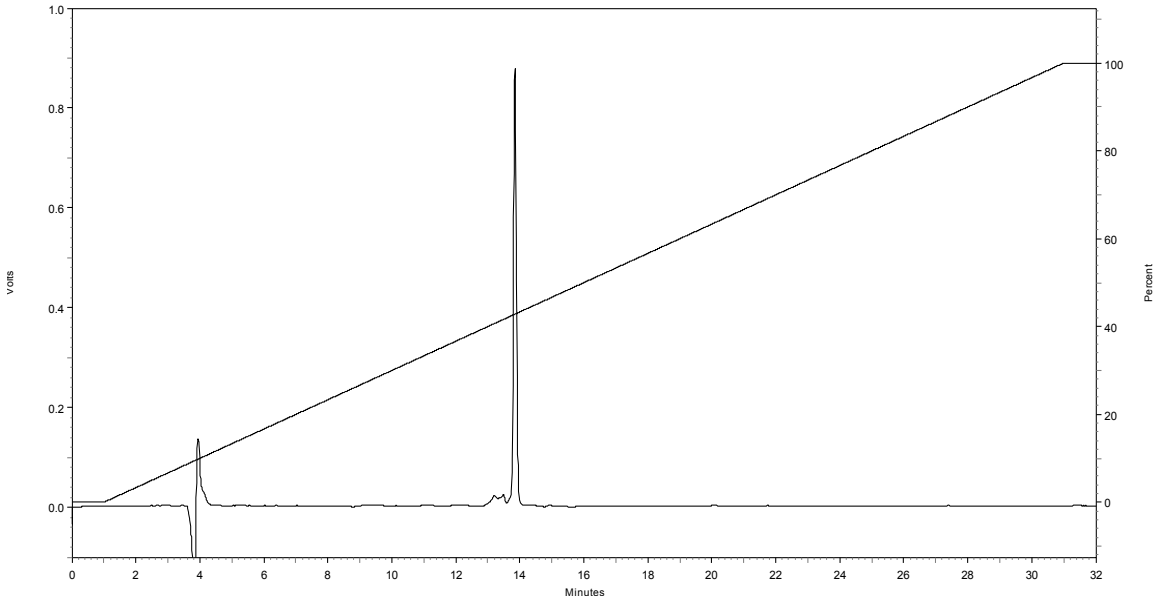
Solutions of three peptides in DMF were prepared: loop 1 (compound **4a**, 4.4 µmol, 4.08 mg), loop 2 (compound **4b**, 4.4 µmol, 6.04 mg) and loop 3 (compound **4c**, 4.4 µmol, 4.71 mg). CTV scaffold **9** (4.4 µmol, 2.30 mg) was added to the microwave vessel and dissolved in 100 µL DMF (4Å). 100 µL of the $CuSO_4$ stock solution (2.65 µmol, 0.66 mg) and 100 µL of the sodium ascorbate stock solution (5.30 µmol, 1.05 mg) were added to the solution, followed by addition the TBTA solution (0.66 µmol, 0.35 mg)). The peptide solutions were combined and added to the reaction mixture. Depending on the amount of DMF that was used to dissolve the peptides, H_2O was added to obtain a 3:2 DMF/H_2O ratio and 1-1.5 mL total volume. The microwave vessel was sealed and allowed to react in the microwave at 80°C during 25 minutes.
After reaction, a sample was taken from the reaction mixture for LC-MS analysis. Based on this analysis, the individual components were purified with preparative HPLC.

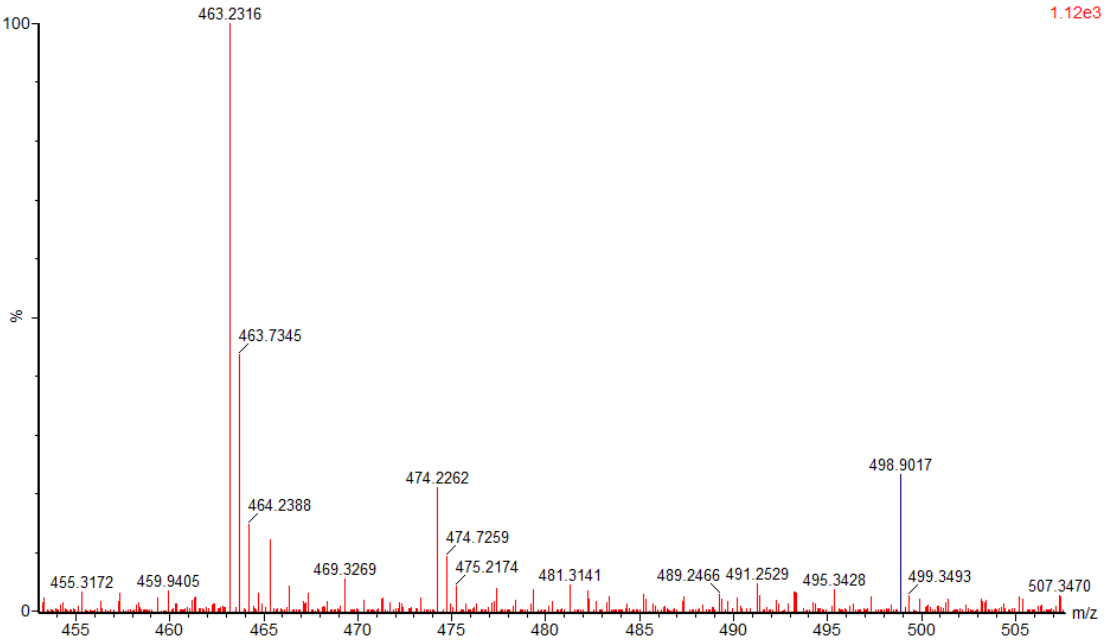
References

1. V. V. Rostovtsev, L. G. Green, V. V. Fokin, and K. B. Sharpless, *Angew. Chem. Int. Ed.*, 2002, 2596.
2. C. W. Tornøe, C. Christensen, and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057–3064.
3. Q. Wang, T. R. Chan, R. Hilgraf, V. V. Fokin, K. B. Sharpless, and M. G. Finn, *J. Am. Chem. Soc.*, 2003, **125**, 3192–3193.
4. T. R. Chan, R. Hilgraf, K. B. Sharpless, and V. V. Fokin, *Org. Lett.*, 2004, **6**, 2853–2855.

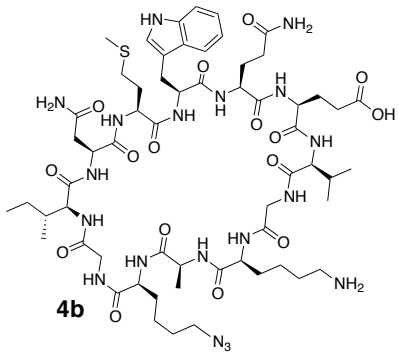
Compound 4a:
Loop 1: K(N_3)LTRDGGNG
$C_{36}H_{60}N_{16}O_{13}$
$[M+H]^+$ monoisotopic calculated: 925.4599
$[M+2H]^{2+}$ calculated: 463.2336
HRMS $[M+2H]^{2+}$ found: 463.2316
Rt= 13.85 min
Purity= 94.5%

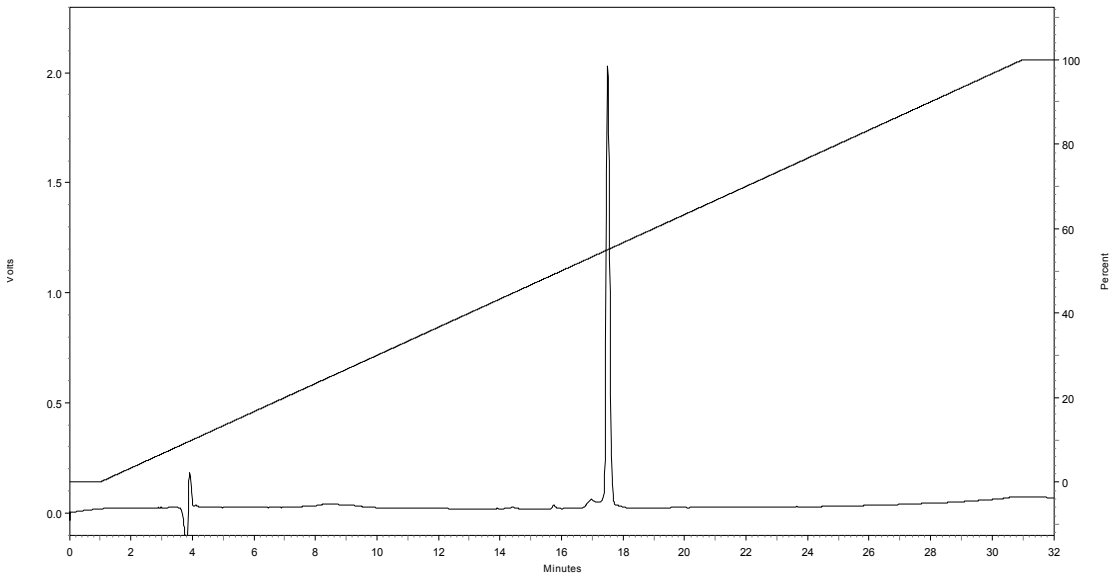


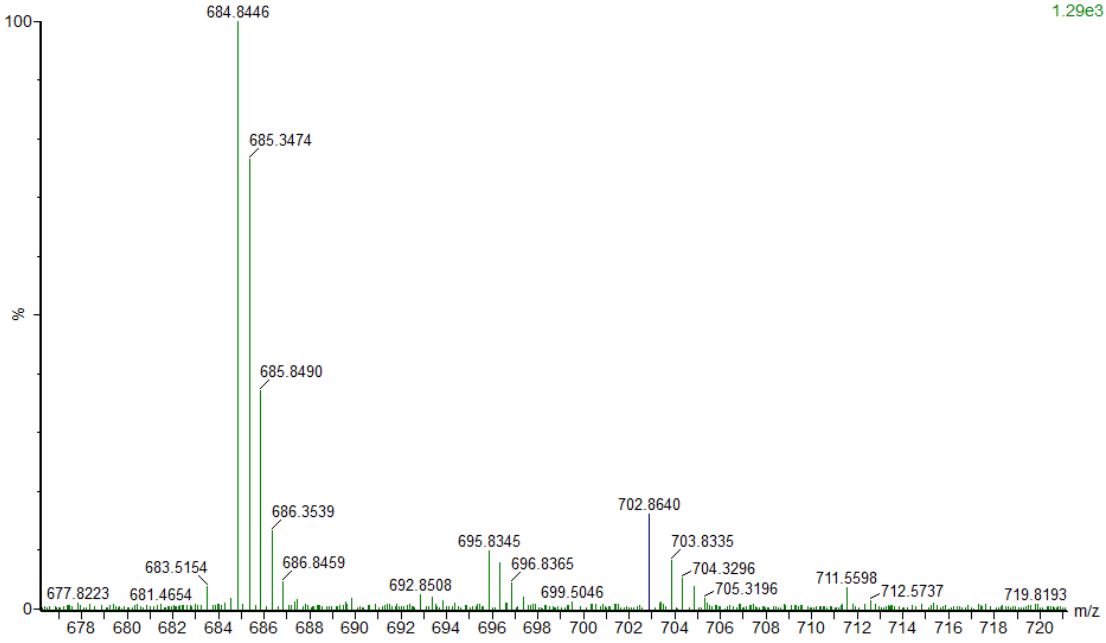




Compound 4b:
Loop 2: INMWQEVGKAK($_3$)G
$C_{60}H_{93}N_{19}O_{16}S$
$[M+H]^+$ monoisotopic calculated: 1368.6841
$[M+2H]^{2+}$ calculated: 684.8457
HRMS $[M+2H]^{2+}$ found: 684.8446
Rt= 17.51 min
Purity= 95.3 %







Compound 4c:

Loop 3: SGGDPEIVTK(N_3)G

$C_{44}H_{70}N_{14}O_{17}$

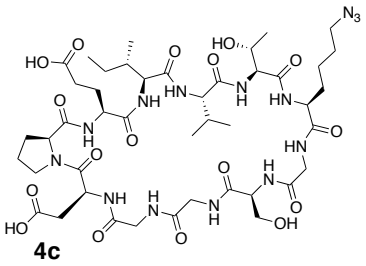
$[M+H]^+$ monoisotopic calculated: 1067.5116

$[M+2H]^{2+}$ calculated: 534.2594

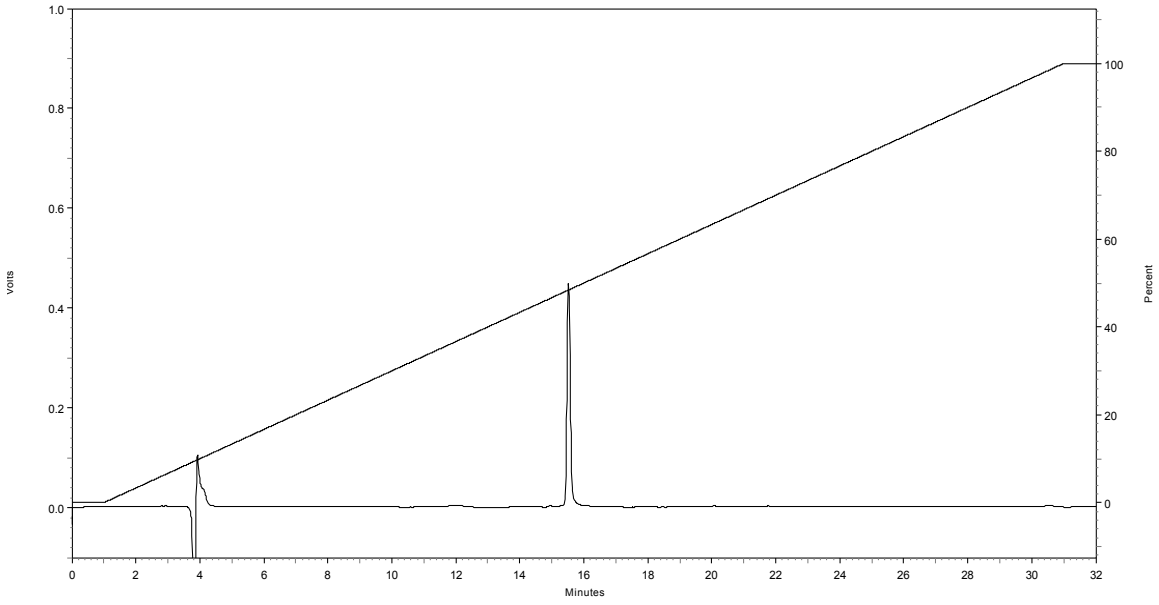
HRMS $[M+2H]^{2+}$ found: 534.2652

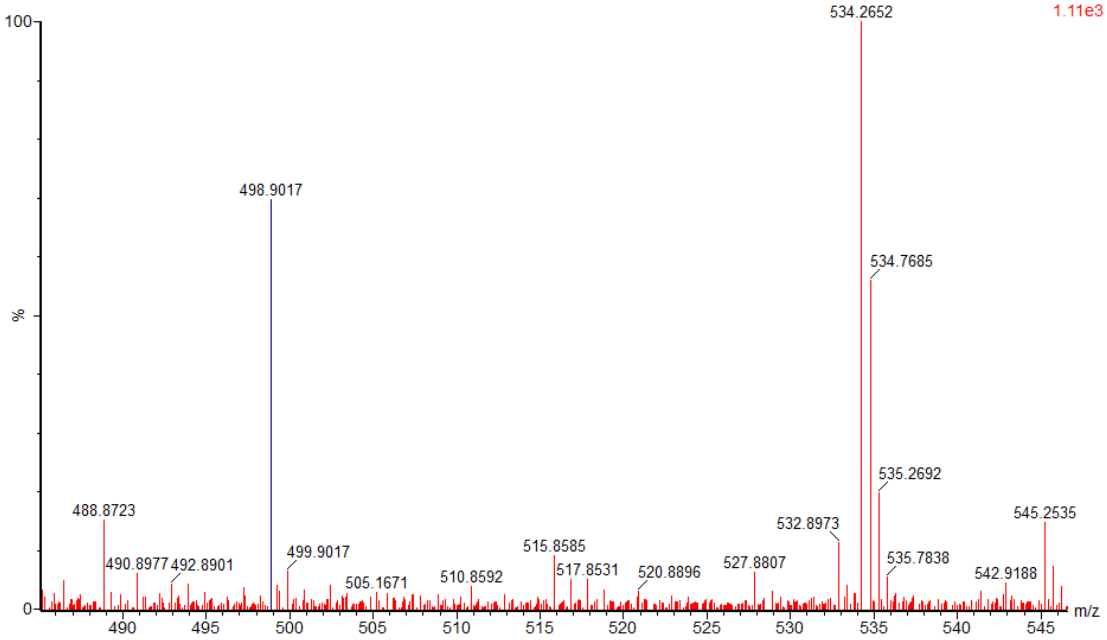
Rt= 15.52 min

Purity= 100%



4c





Loop 3*

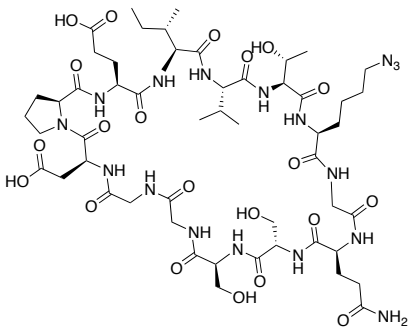
$C_{52}H_{83}N_{17}O_{21}$

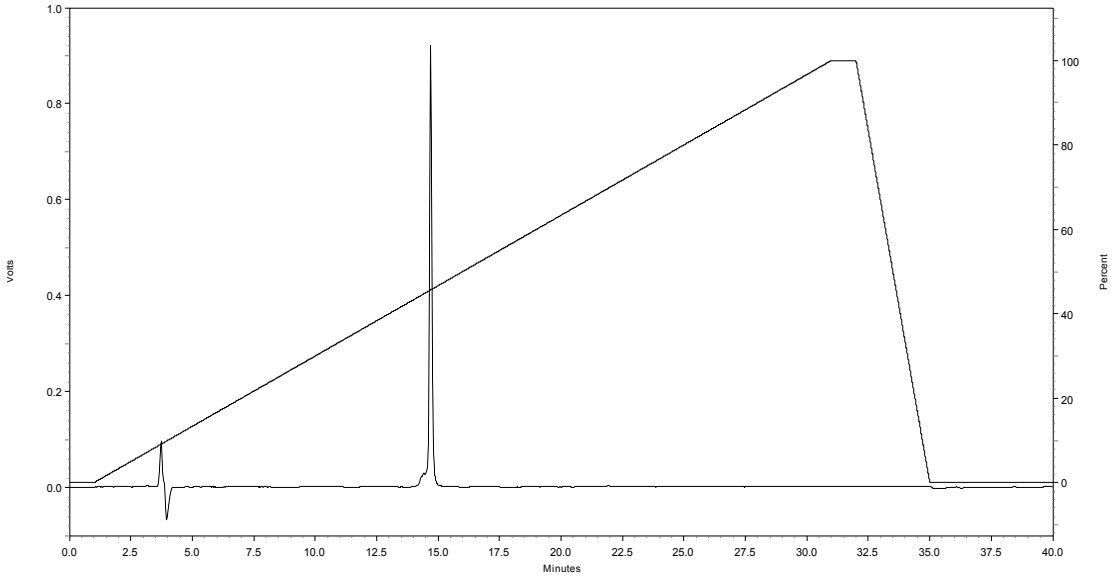
$[M+H]^+$ monoisotopic calculated: 1282.59

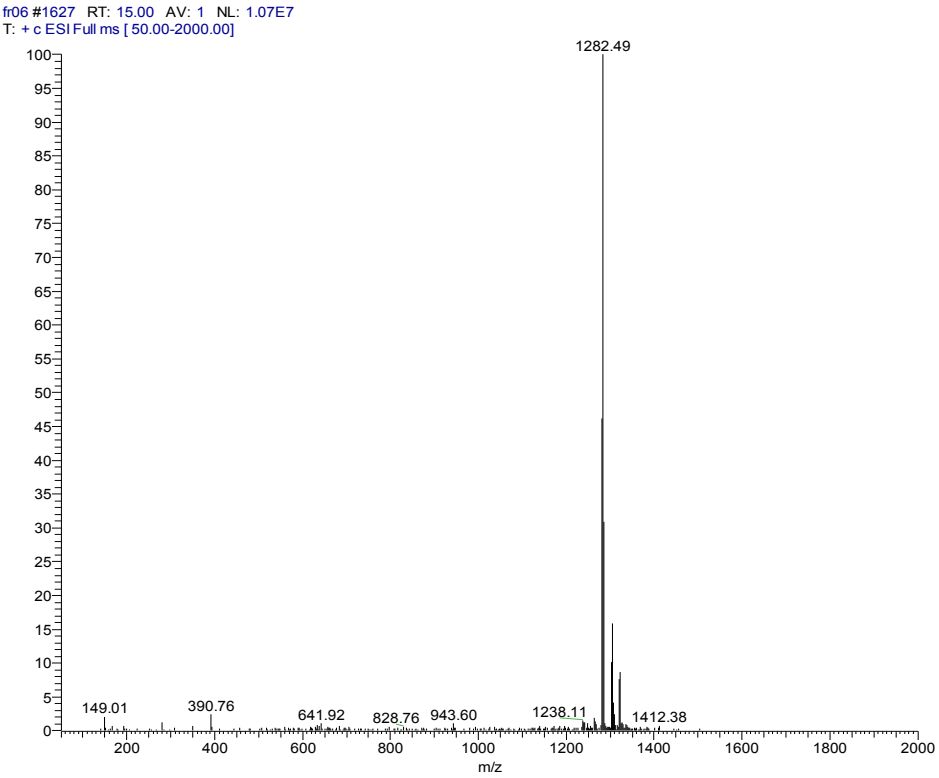
$[M+H]^+$ found: 1282.49

Rt= 14.68 min

Purity= 98.2 %







Dendrimer scaffolded compounds **6**

Loop combination	**Bruto formula**	**[M+H]+ calculated**	**[M+H]+ found**	**Rt (min)**
1-1-2	$C_{164}H_{254}N_{54}O_{50}S$	3812.88	3813.62	15.12
1-2-2	$C_{188}H_{287}N_{57}O_{53}S_2$	4255.10	4256.01	15.92
1-1-3	$C_{148}H_{231}N_{49}O_{51}$	3511.70	3511.14	14.68
1-3-3	$C_{156}H_{241}N_{47}O_{55}$	3653.76	3654.66	15.30
1-2-3	$C_{172}H_{264}N_{52}O_{54}S$	3954.93	3955.97	15.67
2-2-3	$C_{196}H_{297}N_{55}O_{57}S_2$	4398.15	4397.44	16.48
2-3-3	$C_{180}H_{274}N_{50}O_{58}S$	4096.98	4097.66	16.29

Triamine scaffolded compounds **8**

Loop combination	**Bruto formula**	**[M+H]+ calculated**	**[M+H]+ found**	**Rt (min)**
1-1-2	$C_{153}H_{242}N_{54}O_{45}S$	3588.80	3587.57	14.66
1-2-2	$C_{177}H_{275}N_{57}O_{48}S_2$	4032.03	4030.21	15.69
1-1-3	$C_{137}H_{219}N_{49}O_{46}$	3287.63	3286.89	13.97
1-3-3	$C_{145}H_{229}N_{47}O_{50}$	3429.68	3428.99	14.41
1-2-3	$C_{161}H_{252}N_{52}O_{49}S$	3730.85	3729.11	15.29
2-2-3	$C_{185}H_{285}N_{55}O_{52}S_2$	4174.08	4173.43	16.26
2-3-3	$C_{169}H_{262}N_{50}O_{53}S$	3872.91	3872.12	15.89

TAC-scaffolded compounds

Loop combination	**Bruto formula**	**[M+H]+ calculated**	**[M+H]+ found**	**Rt (min)**
1-1-2	$C_{162}H_{249}N_{55}O_{46}S$	3733.86	3732.15	14.87
1-2-2	$C_{186}H_{282}N_{58}O_{49}S_2$	4177.08	4176.55	15.59
1-1-3	$C_{146}H_{226}N_{50}O_{47}$	3432.69	3432.11	14.42
1-3-3	$C_{154}H_{236}N_{48}O_{51}$	3574.74	3574.59	14.94
1-2-3	$C_{170}H_{259}N_{53}O_{50}S$	3875.91	3874.51	15.32
2-2-3	$C_{194}H_{292}N_{56}O_{53}S_2$	4319.13	4318.95	16.17
2-3-3	$C_{178}H_{269}N_{51}O_{54}S$	4017.96	4017.14	15.97

CTV scaffolded compounds **10**

Loop combination	**Bruto formula**	**[M+H]+ calculated**	**[M+H]+ found**	**Rt (min)**
1-1-2	$C_{165}H_{243}N_{51}O_{48}S$	3738.79	3739.16	16.21
1-2-2	$C_{189}H_{276}N_{54}O_{51}S_2$	4183.01	4181.57	16.85
1-1-3	$C_{149}H_{220}N_{46}O_{49}$	3438.61	3438.39	16.10
1-3-3	$C_{157}H_{230}N_{44}O_{53}$	3580.67	3580.33	16.74
1-2-3	$C_{173}H_{253}N_{49}O_{52}S$	3881.84	3881.01	16.77
2-2-3	$C_{197}H_{286}N_{52}O_{55}S_2$	4325.06	4323.22	17.63
2-3-3	$C_{181}H_{263}N_{47}O_{56}S$	4023.89	4022.43	17.41
1-1-3*	$C_{157}H_{233}N_{49}O_{53}$	3653.71	3654.15	16.06
1-3*-3*	$C_{173}H_{256}N_{50}O_{61}$	4010.85	4010.87	16.44