Supporting Information **Lebedev *et al.***

Clickable bifunctional radiometal chelates for peptide labeling

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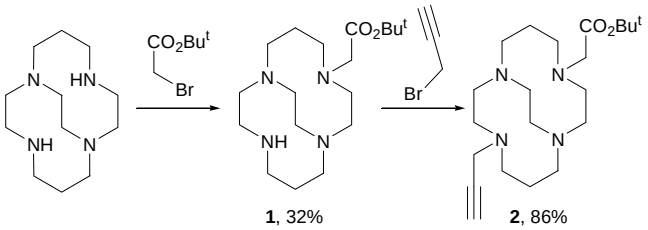
1. Materials and methods

All solvents and reagents were obtained from commercial sources and used as received. Column chromatography was performed on Selecto™ silica gel (Fisher). ^{1}H and ^{13}C NMR spectra were recorded on a Brucker DPX-500 spectrometer. Mass-spectra were obtained on a Waters LC-MS workstation, using 1% TFA in MeCN/ 1% TFA in water as mobile phase.

Analytical and Radio HPLC was performed using a Schimadzu Epic C-18 5 µm column (4.6x250 mm) with two-solvent gradient elution (0.1% TFA in water; 0.1% TFA in acetonitrile). Semi-preparative HPLC was performed using a Waters XBridge Prep BEH130 C18 5 µm column (10x250 mm) and the same solvent system.

2. Synthesis and radiolabeling.

***tert*-butyl (11-prop-2-yn-1-yl-1,4,8,11-tetraazabicyclo[6.6.2]hexadec-4-yl)acetate (2).**



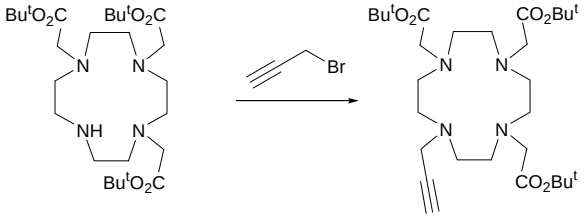
Compound 1 was obtained according to the literature procedure.[1]

To the solution of 1 (20 mg, 0.058 mmol) in acetonitrile (2 ml), K_2CO_3 (14 mg, 0.1 mmol) was added followed by propargyl bromide (12 mg of 80% solution in toluene, 0.1 mmol). Mixture was stirred overnight at room temperature. Solvent was evaporated, and the residue purified on short silica gel column (1 x 3 cm, CH_2Cl_2:MeOH 5:1). Solvent was evaporated and the residual oil was dried in vacuum. Yield: 19 mg, 86%.

^{1}H NMR ($CDCl_3$) δ 3.05-3.65 (m, 14H); 2.75-3.02 (m, 8H); 2.24 (t, J=2.3 Hz, 1H); 1.75-1.90 (m, 2H); 1.61-1.71 (m, 2H); 1.47 (9H, s).

^{13}C NMR ($CDCl_3$) δ 170.2, 82.0, 78.4, 73.5, 58.4, 57.1, 56.0, 55.5, 55.0, 53.7, 52.9, 52.1, 50.9, 50.8, 49.2, 43.0, 28.2, 24.5, 24.4.

tri-*tert*-butyl-2,2',2''-(10-prop-2-yn-1-yl-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (3)



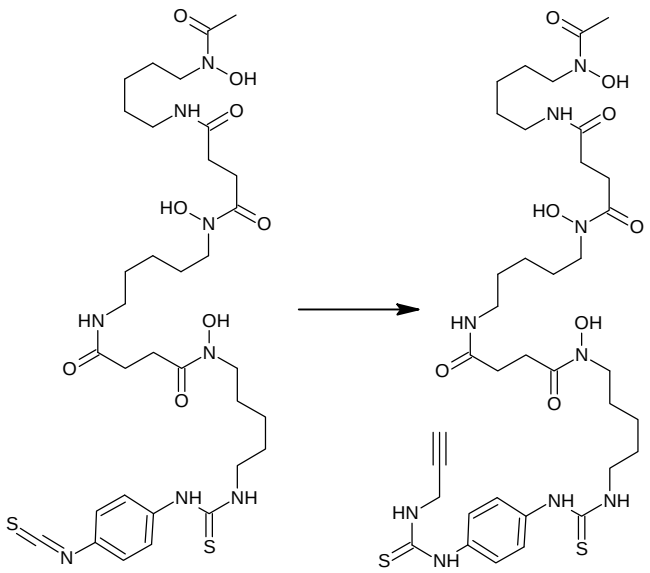
3, 74%

To the solution of **tri-*tert*-butyl 2,2',2''-(1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate** (20 mg, 0.039 mmol) in acetonitrile (2 ml), K_2CO_3 (14 mg, 0.1 mmol) was added followed by propargyl bromide (12 mg of 80% solution in toluene, 0.1 mmol). Mixture was stirred overnight at room temperature. Solvent was evaporated, and the residue purified on short silica gel column (1 x 3 cm, CH_2Cl_2 : MeOH 5:1). Solvent was evaporated and the residual oil was dried in vacuum. Yield: 16 mg, 74%.

^{1}H NMR ($CDCl_3$) δ 2.15-3.3 (m, 24 H); 2.06 (t, J = 2.3 Hz, 1H); 1.38 (s, 18H); 1.33 (s, 9H).

^{13}C NMR ($CDCl_3$) δ 173.4, 172.8, 82.6, 82.4, 78.9, 72.6, 56.7, 56.0, 50.9, 50.7, 49.7, 49.6, 42.9, 27.9, 27.8.

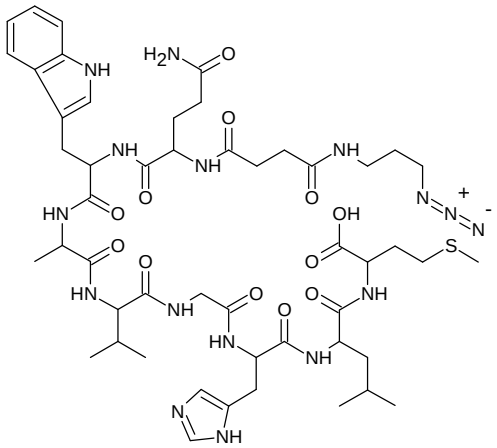
***N*'-{[acetyl(hydroxy)amino]methyl}-*N*-hydroxy-*N*-{[(4-{hydroxy[5-({[(4-{[(prop-2-yn-1-ylamino)carbonothioyl]amino}phenyl)amino]carbonothioyl}amino)pentyl]amino}-4-oxobutanoyl)amino]methyl}succinamide (4)**



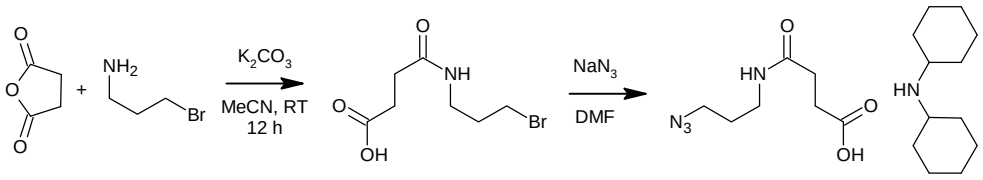
The starting material (10 mg, 0.013 mmol) was dissolved in 1 ml of DMSO. Propargyl amine (5.5 mg, 0.1 mmol) was added and the reaction was left overnight at RT. Water (10 ml) was added to the reaction mixture and the resulting solution was applied onto C-18 cartridge (preconditioned with MeCN [10 ml] and water [20 ml]). Cartridge was washed twice with water (10 ml), then with acetonitrile (10 ml). Organic fraction was collected, dried with Na_2SO_4 and dried in vacuum. Yield: 3.5 mg, 33%.

^{1}H NMR ($CDCl_3$) δ 9.63 (br m, 4H), 7.78 (br m, 3H), 7.48 (d, *J* = 7.3 Hz, 2H), 7.37 (d, *J* = 7.3 Hz, 2H), 5.44 (broad s, 2H), 4.74 (t, *J* = 4.7 Hz, 2H), 3.77 (br s, ~20H, including water), 3.40-3.52 (m, 8H), 2.99 (dd, , *J* = 6.6 Hz, , *J* = 12.6 Hz, 4H), 2.58 (dd, , *J* = 6.7 Hz, , *J* = 11.4 Hz, 4H), 2.54 (br s, 1H), 2.24-2.30 (m, 4H), 1.96 (s, 3H), 1.15-1.60 (m, 18H).

$N_3(CH_2)_3NHCO(CH_2)_2CO$-*QWAVGHLM* (5)



Peptide was synthesized according to Fmoc-protocol (vide infra), azido group was incorporated in a form of non-natural amino acid. synthesized according to the following procedure:



Acetonitrile (50 ml) was added to the mixture of succinic anhydride (1.31 g, 13 mmol), γ-bromopropyl amine hydrobromide (3.51 g, 16 mmol) and potassium carbonate (5.52 g, 40 mmol) and the resulting suspension was stirred vigorously for 12 hours. Water (400 ml) was added, pH was adjusted to 1 with concentrated HCl and the product was extracted with CH_2Cl_2 (3x150 ml). Organic phase was dried over Na_2SO_4 and evaporated. The product was obtained as clear viscous oil. Yield 1.73 g, 56%.

Bromide derivative was dissolved in DMF (6 ml) and NaN_3 (1.35 g, 20 mmol) was added. The mixture was vigorously stirred overnight. The solution was filtered, and the precipitate was washed with DMF (4 ml). Combined DMF solution was diluted with ether (50 ml), the precipitate was collected by centrifugation and washed with ether again. It was dissolved in TFA (3ml), TFA was evaporated in vacuum. The residue was taken up into acetonitrile (20 ml), acetonitrile solution was filtered through a cotton clot, evaporated in vacuum and the residue was redissolved in acetonitrile again (it is important to eliminate even traces of TFA, for it forms an insoluble salt with dicyclohexylamine). Dicyclohexylamine (1 ml) was added to the solution

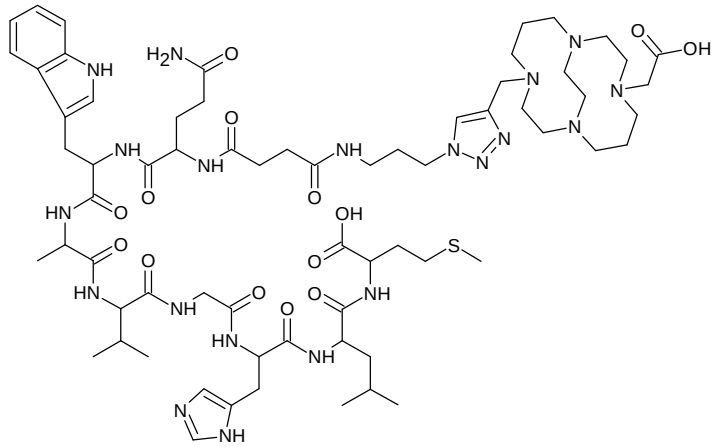
and the resulting solid was filtered, washed with cold acetonitrile and dried in vacuum. Yield 2.13 g, 77%.

^{1}H NMR ($CDCl_3$) δ 7.98 (t, J = 4.8 Hz, 1H), 7.68 (broad s, 2H), 3.32 (t, J = 6.7 Hz, 2H), 3.07 (dd, J_1 = 6.7 Hz, J_2 = 12.6 Hz, 2H), 2.71 (tt, , J = 3.5 Hz , J = 10.6 Hz, 2H), 2.19-2.30 (m, 4H), 1.84 (dd, , J = 2.4 Hz, , J = 12.6 Hz, 4H), 1.51-1.73 (m, 8H), 1.02-1.28 (m, 10H).

Peptide synthesis was then performed on the PS-3 bench-top peptide synthesizer (ProteinTechnologies), employing Fmoc-chemistry. Azide containing acid synthesized as described above was used for the last coupling reaction in the sequence. Wang resin (100 mg, 0.075 mmol of amino acid) preloaded with methionine was treated with DMF for 1 h, before loading into the reaction vial. The following program was used for coupling and deprotection:

Number of repeats	Time, min	Operation
3	0.5	Wash the resin with DMF
2	10	Treat the resin with 20% solution of piperidine in DMF
6	0.5	Wash the resin with DMF
1	1	Dissolve mixture of 0.7 mmol of the amino acid and 0.75 mmol of HBTU in the 0.4 *N*,*N*-methylmorpholine in DMF
1	60	Treat the resin with the solution of the activated amino acid
3	0.5	Wash the resin with DMF
1	1	Dissolve mixture of 0.7 mmol of the amino acid and 0.75 mmol of HBTU in the 0.4 *N*,*N*-methylmorpholine in DMF
1	60	Treat the resin with the solution of the activated amino acid
3	0.5	Wash the resin with DMF

CB-TE2A- *QWAVGHLM* (6)



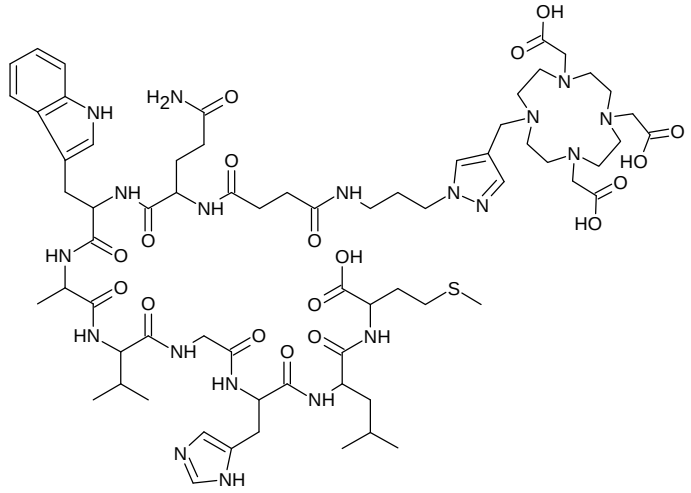
0.3 ml of the peptide **5** solution (10 mg in 1 ml of 50% ^{t}BuOH) was mixed with 0.3 ml of the clickable CB-TE2A (**2**) solution (10 mg in 1 ml of 50% ^{t}BuOH) and $CuCl_2$ (0.1 ml of 0.1 N solution in water). Ascorbic acid (0.3 ml of 0.5 N solution in water) was added. White precipitate appeared immediately. The mixture was vortexed for 2 min, and Na_2S (0.2 ml of 0.1 N solution in water) was added. Black precipitate appeared immediately, the mixture was centrifuged, supernatant was collected and the precipitate was washed with 1 ml of water via suspension/centrifugation. All washings were combined, diluted to 10 ml with water and applied on C-18 cartridge (primed with ethanol [10 ml], then and water [10 ml]). The cartridge was washed with additional 10 ml of water and the product was eluted with acetonitrile (10 ml). Acetonitrile was evaporated in vacuum. To obtain analytically pure material HPLC purification can be performed at this point, otherwise the peptide was deprotected without additional purification. Deprotection was performed by treating the residue with 3 ml of the cleavage cocktail (2.5 *i*Pr_3SiH, 1% of water and 1% of $HSCH_2CH_2SH$ in trifluoroacetic acid) for 7 h. TFA was evaporated in vacuum and the product was isolated by semi-preparative HPLC, 5 ml of the solution ca. 0.2 mg/ml were collected. ($Concentration, mg/ml = \frac{Absorbance_{280nm} \times FW}{5560, AU/mmol/ml}$)

^{64}Cu-CB-TE2A- QWAVGHLM (^{64}Cu-6)

Peptide solution (10 µl, 0.2 mg/ml, in MeCN/H_2O) were mixed with ammonium acetate buffer (10 µl, 0.5 N, pH 6.04) and 0.82 mCi of $^{64}CuCl_2$ (1.5 µl, 6 N HCl) were added. The vial was

sealed and placed in the heating block (70 ^{o}C) for 5 min. The mixture was cooled to room temperature and quenched with EDTA (10 µl of 1 N solution). The product was isolated by preparative radio HPLC (analytical column).

DOTA-QWAVGHLM (7)



The procedure described for **CB-TE2A- QWAVGHLM (6)** was employed. 5 ml of the solution ca. 0.2 mg/ml were collected.

^{64}Cu- DOTA-QWAVGHLM (^{64}Cu-7) was obtained following the procedure for **^{64}Cu-6**, but the reaction was carried out at room temperature.

3. Density Functional Theory (DFT) Calculations

All calculations were conducted using density functional theory (DFT) as implemented in the *Gaussian03* suite of *ab initio* quantum chemistry programs.[2] Geometry optimizations and vibrational frequency calculations were performed by using the unrestricted B3LYP exchange and correlation functionals and the double-ζ 6-31+G(d,p) basis set for all atoms.[3-6] Normal self-consistent field (SCF) and geometry convergence criteria were employed and the geometry of complex **1** was optimized in the gas phase without the use of symmetry constraints. Harmonic frequency analysis based on analytical second derivates was used to characterize the optimized geometries as local minima.

Figure S1. Molecular orbital isosurface generated at 95% of the electron density of the αHOMO-1 orbital of complex 1. The βHOMO is the β-spin orbital spatial counterpart. The picture shows the metal-ligand σ*-antibonding interaction between the Cu^{2+} dz^2 orbital and the pσ-orbitals of the N3 and N7 donor nitrogen atoms.

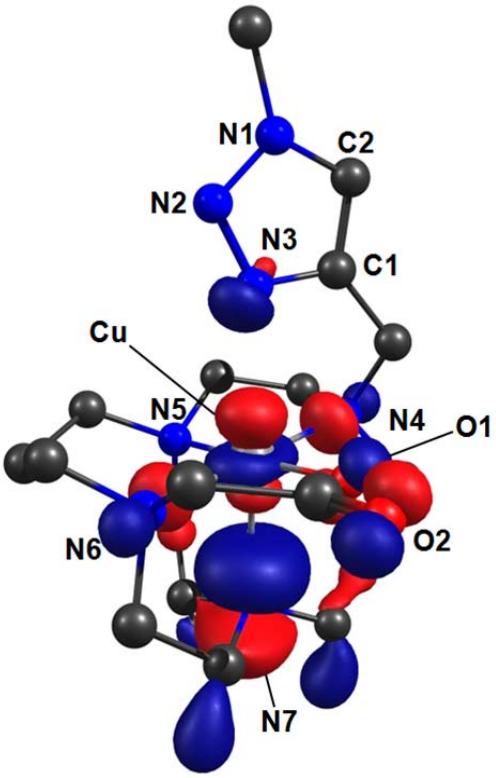


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Table S1. Charge and spin-density analysis for complex **1**.

Atom	Mulliken charge	NPA charge (Total α +β)	Mulliken spin-density	NPA spin-density (Total β - α)
Cu	-0.272	1.328	0.594	0.597
N1	0.194	-0.178	0.000	0.000
N2	0.338	-0.068	-0.001	0.000
N3	-0.401	-0.338	-0.004	0.000
N4	-0.489	-0.604	0.082	0.086
N5	-0.440	-0.619	0.077	0.079
N6	-0.150	-0.616	0.099	0.097
N7	-0.345	-0.627	-0.001	0.000
O1	-0.316	-0.863	0.119	0.121
O2	-0.426	-0.613	0.016	0.017
C1	0.727	-0.069	-0.004	0.000
C2	-0.885	0.048	0.000	0.000
C3	0.638	0.773	-0.004	-0.004

Table 2. NBO analysis of orbital interactions contributing to the stability of complex **1**.[a]

Interaction	NBO interaction energies / kcal mol^{-1}			Percentage contribution / %		
	α-spin	β-spin	Total (α + β)	α-spin	β-spin	Total (α + β)
Ligand-to-metal donation						
N3-Cu	17.26	17.63	34.89	1.96	1.84	1.90
N4-Cu	14.59	36.59	51.18	1.66	3.81	2.78
N5-Cu	19.56	34.74	54.30	2.22	3.62	2.95
N6-Cu	20.91	35.95	56.86	2.38	3.74	3.09
N7-Cu	13.69	15.57	29.26	1.56	1.62	1.59
O1-Cu	29.83	54.57	84.40	3.39	5.68	4.59
Metal-to-ligand donation						
Cu-N3	6.20	6.57	12.77	0.70	0.68	0.69
Cu-N4	5.34	5.56	10.90	0.61	0.58	0.59
Cu-N5	3.66	4.06	7.72	0.42	0.42	0.42
Cu-N6	6.10	3.10	9.20	0.69	0.32	0.50
Cu-N7	2.76	3.07	5.83	0.31	0.32	0.32
Cu-O1	6.96	6.68	13.64	0.79	0.70	0.74
Sum(Ligand-to-Cu)	115.84	195.05	310.89	13.17	20.31	16.90
Sum(Cu-to-ligand)	31.02	29.04	60.06	3.53	3.02	3.26
Sum (all data)	146.86	224.09	370.95	16.70	23.34	20.16
Sum of synergic bonding interactions						
N3-Cu	23.46	24.20	47.66	2.67	2.52	2.59
N4-Cu	19.93	42.15	62.08	2.27	4.39	3.37
N5-Cu	23.22	38.80	62.02	2.64	4.04	3.37
N6-Cu	27.01	39.05	66.06	3.07	4.07	3.59
N7-Cu	16.45	18.64	35.09	1.87	1.94	1.91
O1-Cu	36.79	61.25	98.04	4.18	6.38	5.33
% contribution of metal-to-ligand back-bonding						
Cu-N3	26.43	27.15	26.79	26.43	27.15	26.79
Cu-N4	26.79	13.19	17.56	26.79	13.19	17.56
Cu-N5	15.76	10.46	12.45	15.76	10.46	12.45
Cu-N6	22.58	7.94	13.93	22.58	7.94	13.93
Cu-N7	16.78	16.47	16.61	16.78	16.47	16.61
Cu-O1	18.92	10.91	13.91	18.92	10.91	13.91

[a] Total α-spin NBO interaction energy = 879.48 kcal mol^{-1} (47.8%); total β-spin NBO interaction energy = 960.16 kcal mol^{-1} (52.2%); total energy (α + β-spin NBO interactions) = 1839.64 kcal mol^{-1}.

Table S3. uB3LYP/6-31+G(d,p) optimised Cartesian coordinates of complex **1**.

Atom	*x*	*y*	*z*
6	0.168657	2.74536	-0.88095
6	0.133584	1.902268	-2.17124
6	0.438753	2.541249	1.55949
6	-1.59398	-1.73431	-1.36035
6	0.860552	1.668762	2.750547
6	0.172118	0.302487	2.845889
6	-0.44817	-1.8281	1.861298
6	2.372076	2.19092	0.077473
6	-0.4111	-2.70022	0.599212
6	0.680672	-2.51532	-1.62274
6	3.050019	1.05442	-0.70215
6	1.933714	-1.25227	1.994939
6	3.036341	-0.58363	1.142056
6	2.986874	-1.33149	-1.22804
6	2.150853	-2.62052	-1.1836
1	-0.87138	2.890544	-0.57146
1	0.602802	3.731631	-1.08061
1	-0.65376	2.592305	1.530572
1	0.810171	3.566331	1.70981
1	-2.02594	-2.72248	-1.57685
1	-1.37087	-1.2428	-2.31156
1	-0.90673	0.456702	2.755925
1	0.567757	2.218145	3.654641
1	-1.43261	-1.3739	1.964248
1	-1.31944	-3.31568	0.570595
1	2.599911	3.138135	-0.42796
1	0.361195	-0.12863	3.840922
1	-0.27587	-2.4557	2.748515
1	1.948972	1.570341	2.820624
1	2.805634	2.268993	1.075793
1	0.629412	-1.91115	-2.53246
1	2.788146	1.129451	-1.75923
1	0.314223	-3.52406	-1.86944
1	0.425966	-3.39869	0.642841
1	2.867822	-0.88849	-2.22111
1	2.213932	-1.18786	3.055473
1	4.141621	1.193953	-0.62388
1	3.351688	0.344558	1.621271
1	1.900707	-2.31496	1.753813
1	2.255671	-3.12796	-0.21815
1	2.619622	-3.30342	-1.90443
1	3.917266	-1.24459	1.163069
1	4.051435	-1.6021	-1.12129
7	0.888534	2.046716	0.226779
7	-0.28608	-1.89551	-0.65421
7	0.546482	-0.71094	1.811105
7	2.628896	-0.28724	-0.24321
8	0.032523	2.44681	-3.2584
8	0.174664	0.609094	-1.96728
29	0.356614	0.045079	-0.13007
6	-2.58004	-0.88732	-0.60837
6	-3.96105	-0.90121	-0.61084
7	-2.18282	0.167048	0.159001
7	-3.23324	0.794671	0.628406
7	-4.31772	0.1586	0.159247
1	-4.68701	-1.54207	-1.08969
6	-5.65648	0.647466	0.481997
1	-6.23966	-0.14655	0.954764
1	-5.54027	1.483068	1.172397
1	-6.15859	0.987722	-0.42731

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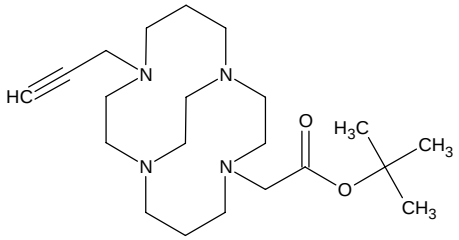
Clickable Cb-TE2A (2): ^{1}H NMR



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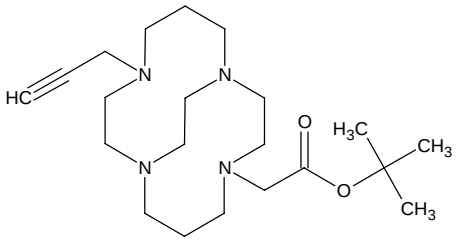
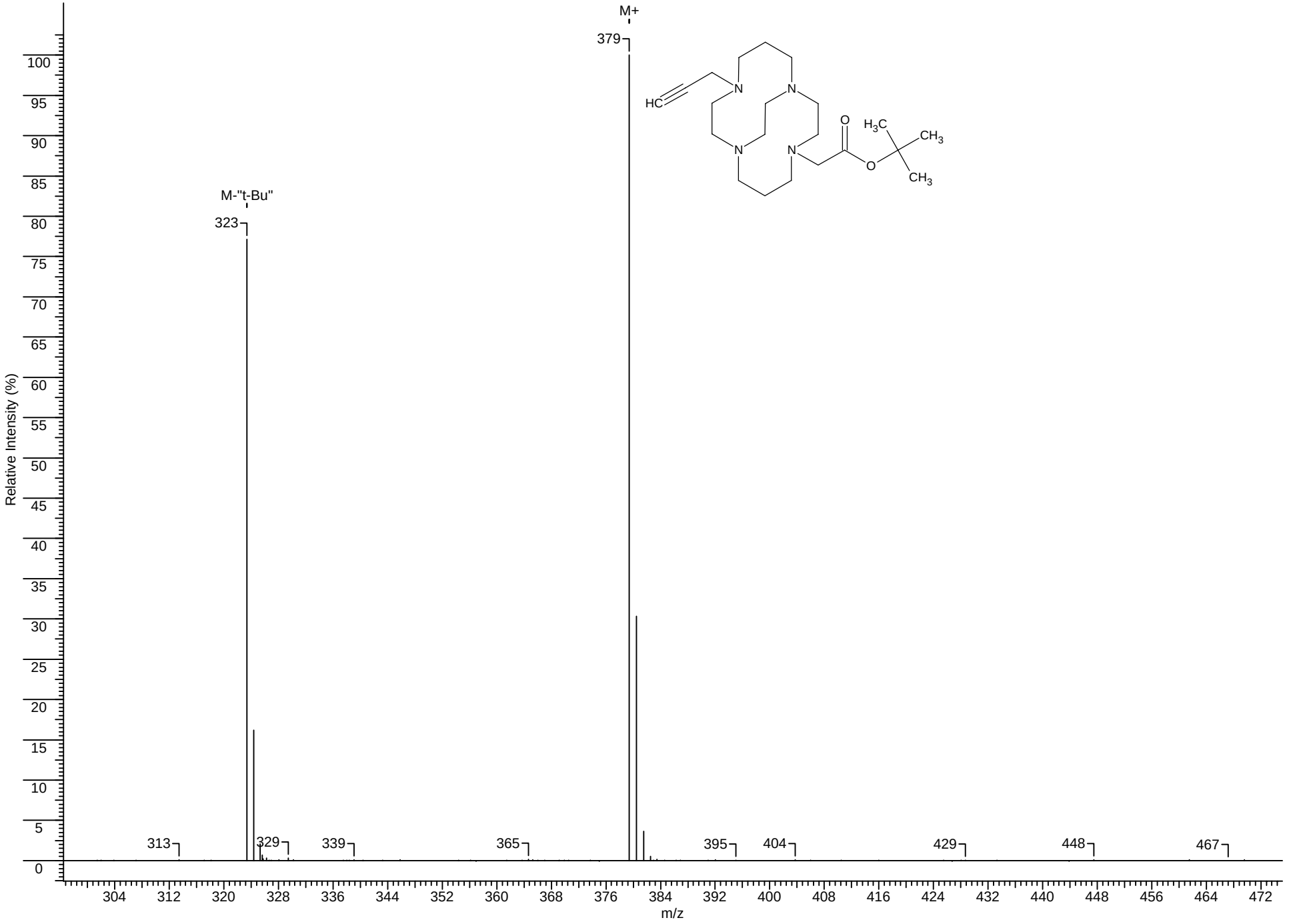
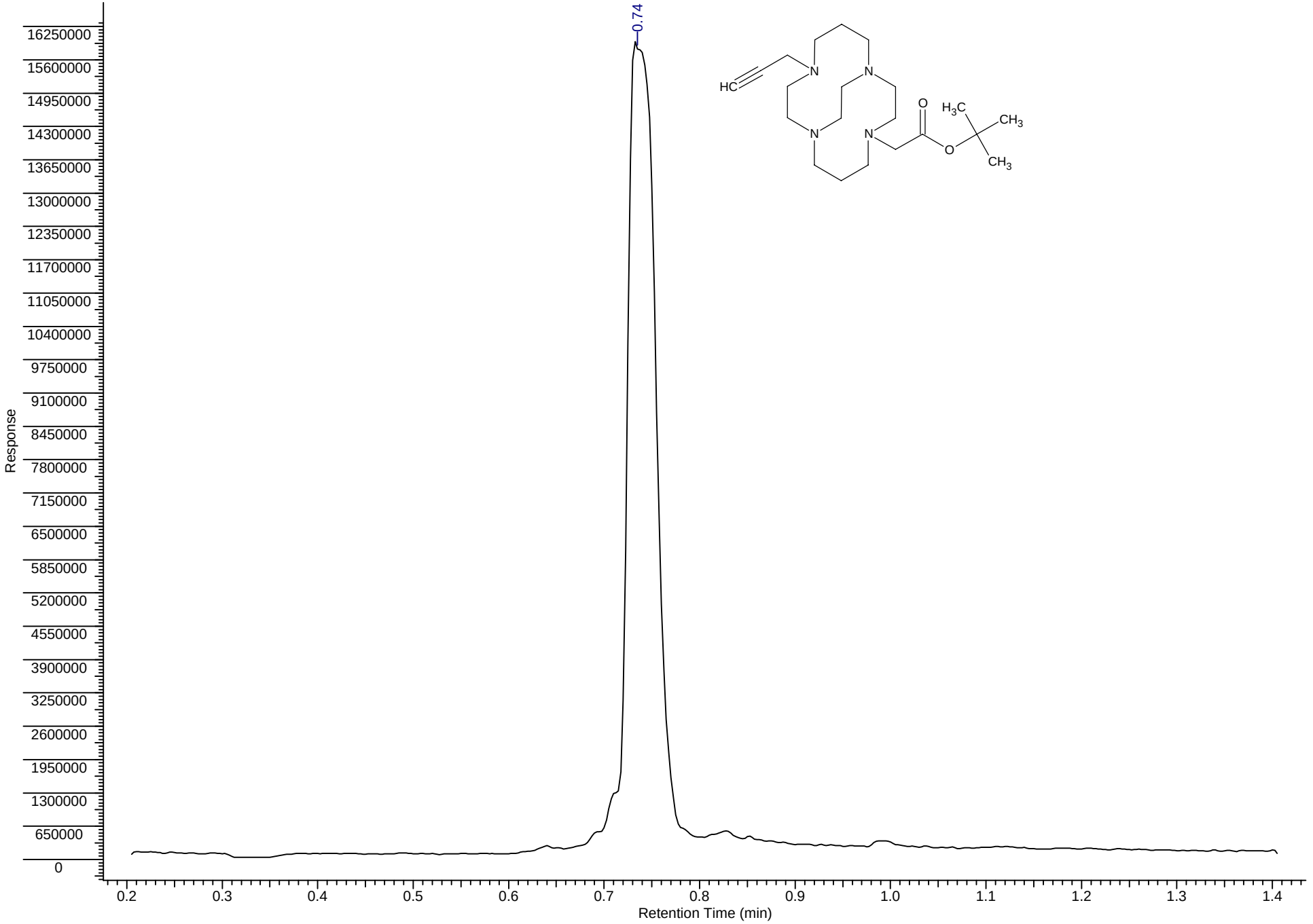


Clickable Cb-TE2A (2): ESI+



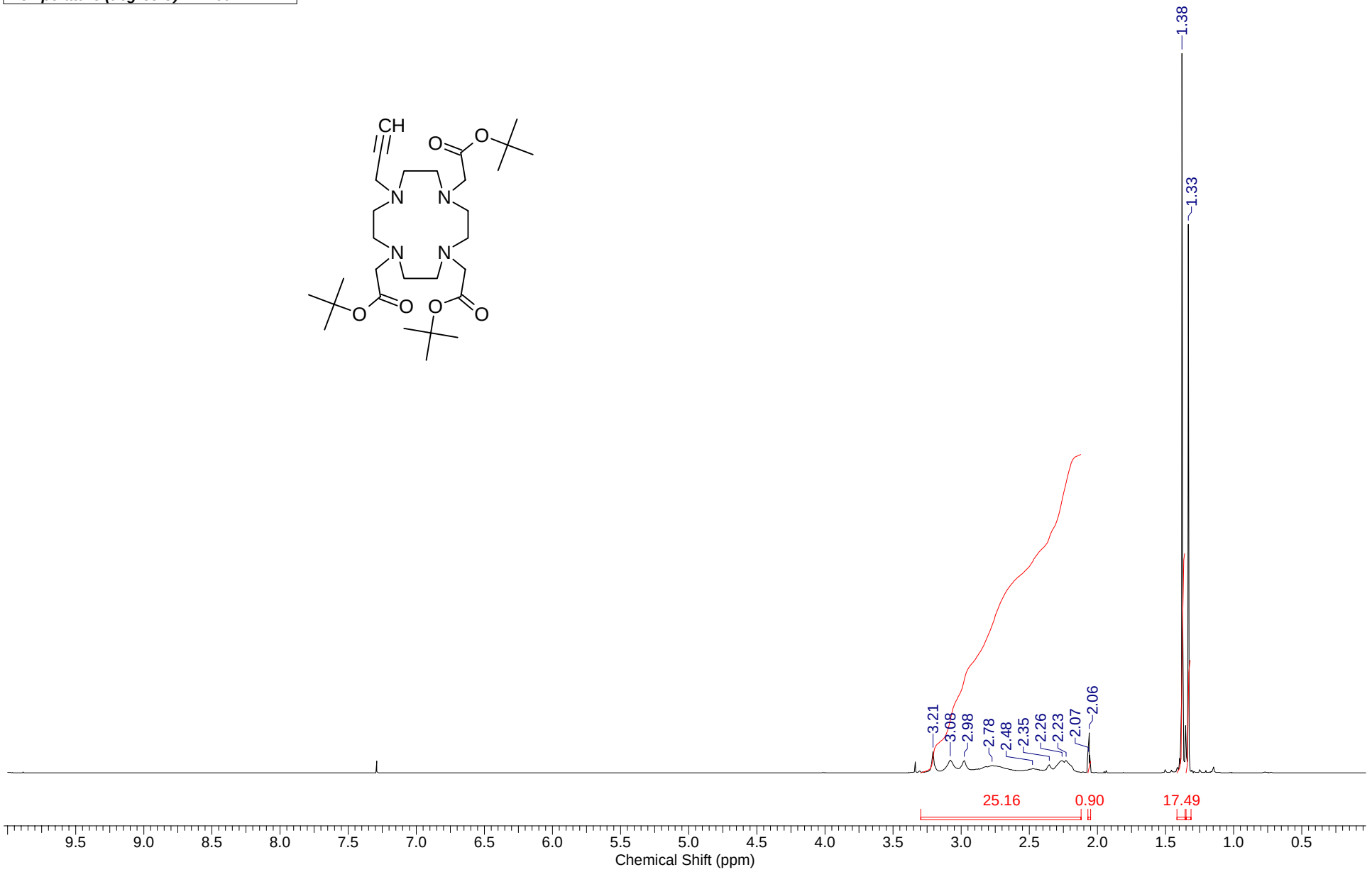
Clickable Cb-TE2A (2): UPLC-MS (TIC)



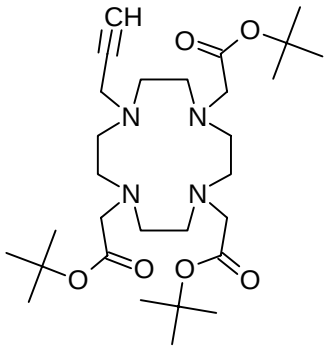
Supplementary Material (ESI) for Chemical Communications

Clickable DOTA (3): ^{1}H NMR

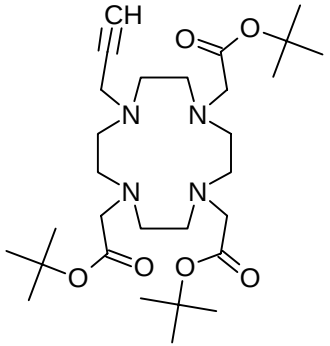
Acquisition Time (sec)	1.6384	***Date***	16 Jul 2009 15:51:28				
File Name	C:\[Artem]\Science\NMR\New Folder\A019101\A019101_001000fid					***Frequency (MHz)***	500.13
Nucleus	1H	***Number of Transients***	18	***Original Points Count***	8192	***Points Count***	65536
Pulse Sequence	zg30	***Solvent***	CHLOROFORM-D			***Sweep Width (Hz)***	5000.00
Temperature (degree C)	24.160						



Clickable DOTA (3): ^{13}C NMR



Clickable DOTA (3): ESI+



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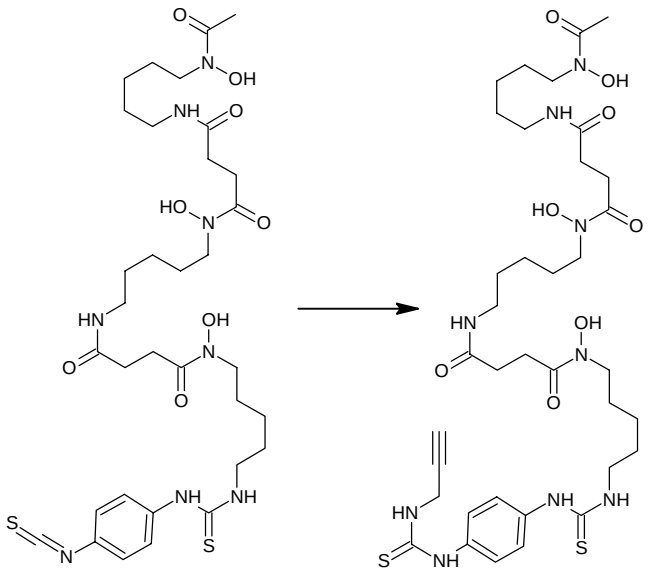
Clickable DFO (4): ^{1}H NMR

Acquisition Time (sec)	3.1719				
Comment	Proton .* lebedeva A014801 (1 1) DMSO 24.0C May_04,2009_12:37 Bruker AVIII 500.13 MHz Respice zg30 1H *.				
Date	04/05/2009 12:47:18	***File Name***	C:\[ARTEM]\SCIENCE\NMR\0148\A014801.ESP		
Frequency (MHz)	500.13	***Nucleus***	1H	***Original Points Count***	32768
Points Count	32768	***Solvent***	DMSO-D6	***Sweep Width (Hz)***	10330.58
Temperature (degree C)	0.000				



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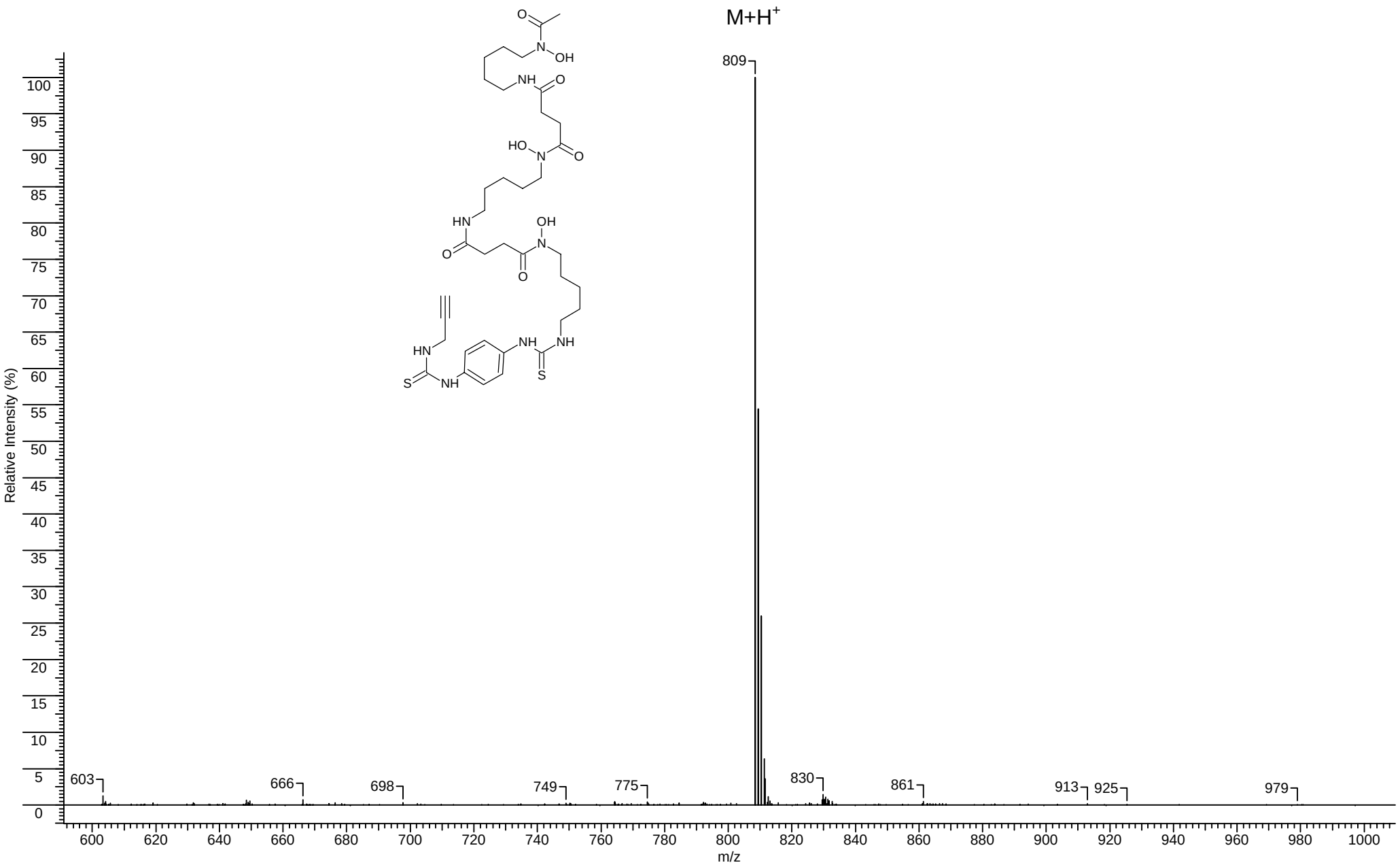
Clickable DFO (4): ESI+



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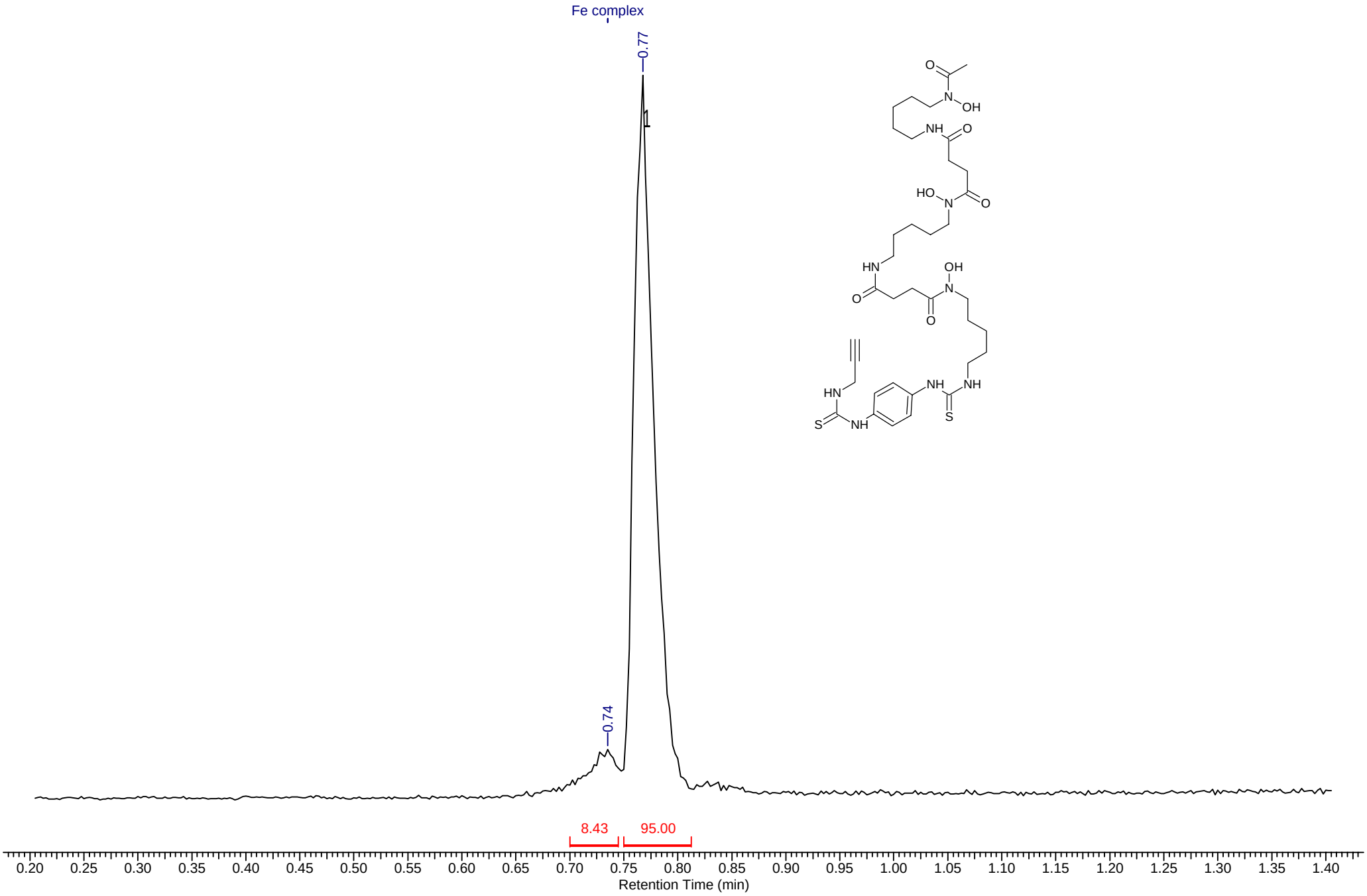
Clickable DFO (4): UPLC-MS (TIC)



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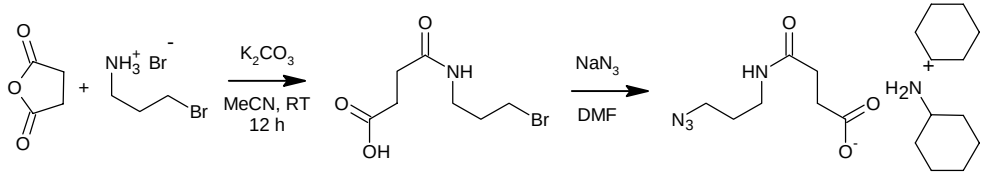
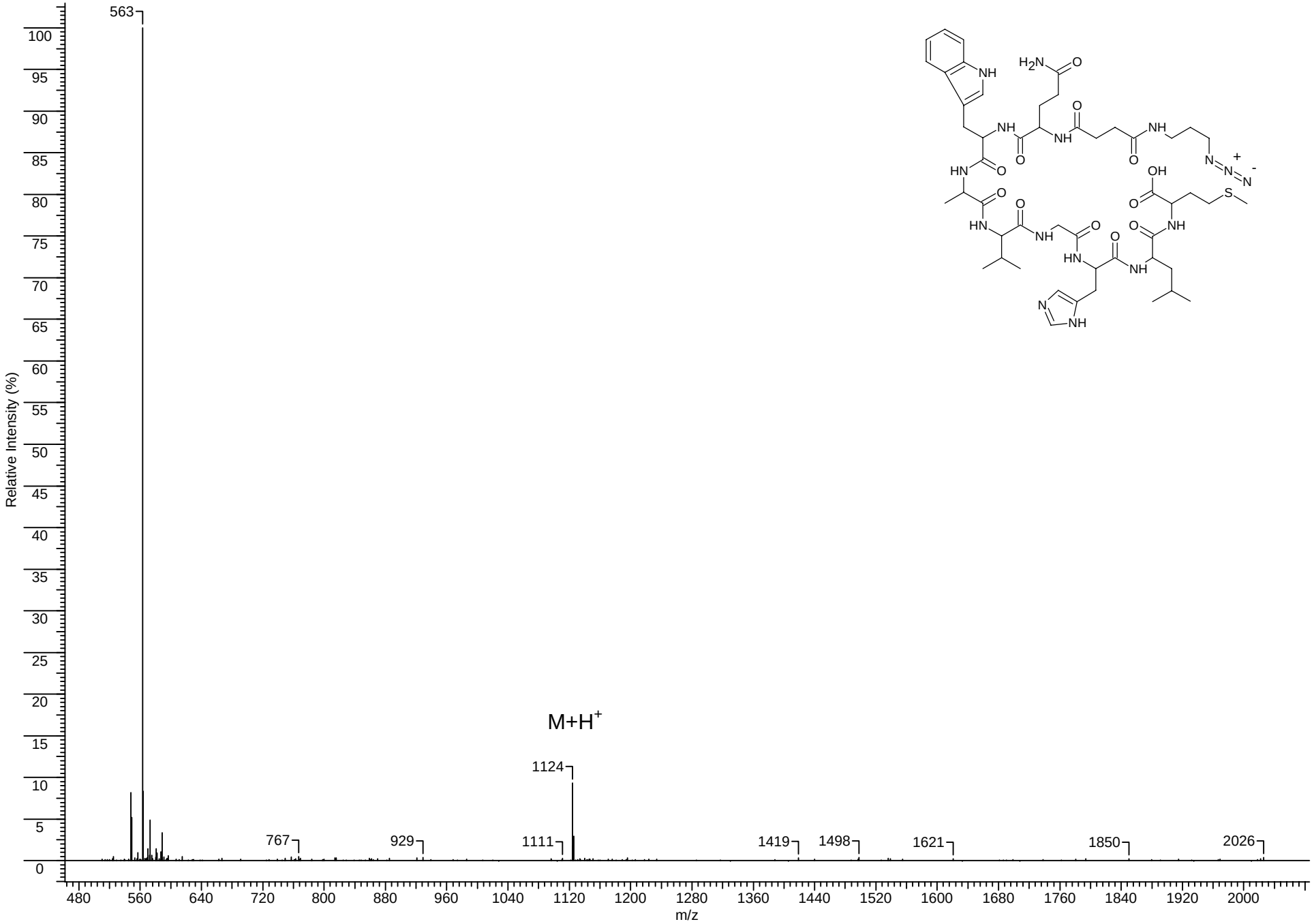
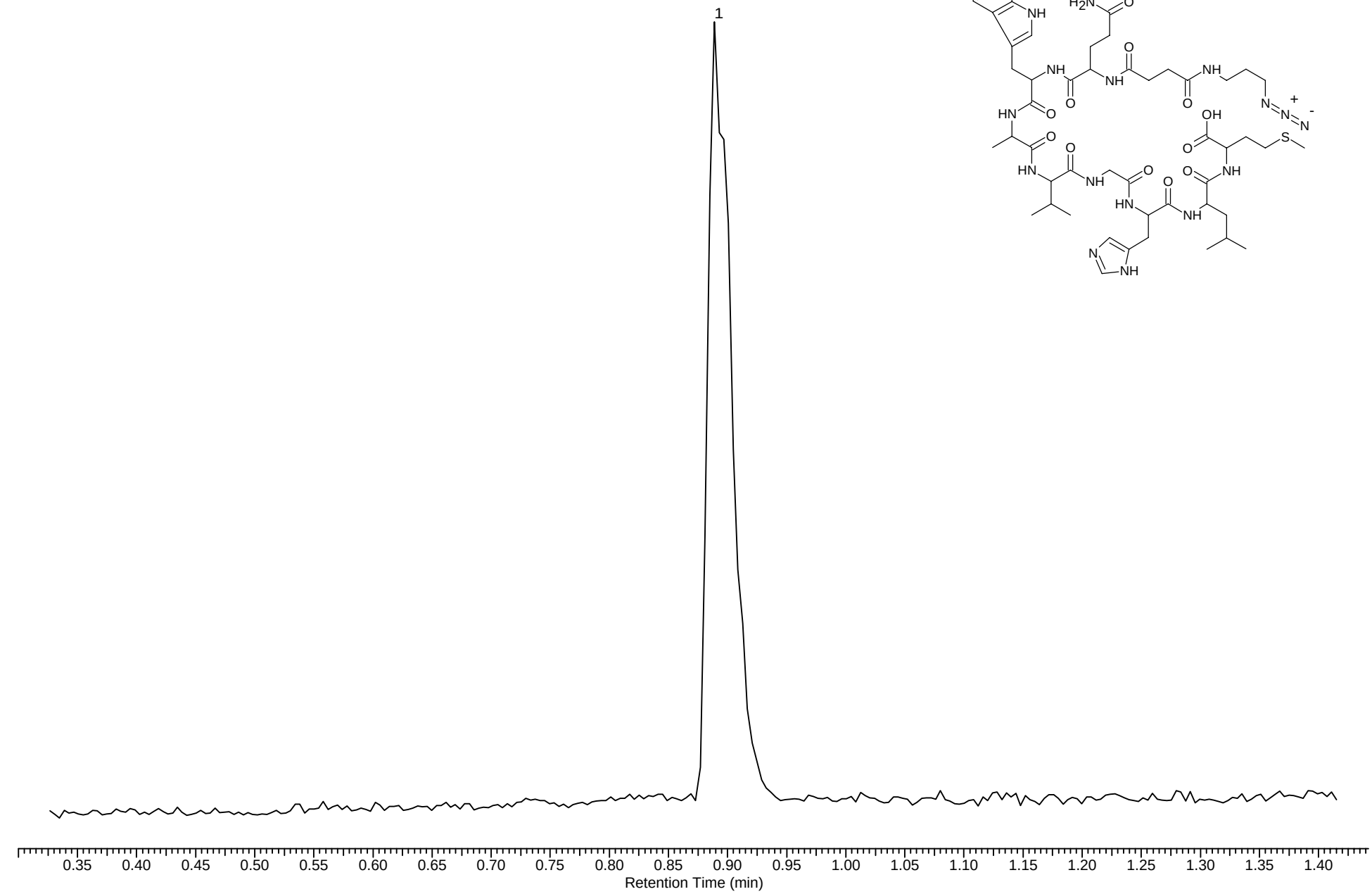


$N_3(CH_2)_2NHCO(CH_2)_2CO$-*QWAVGHLM* (5): ESI+

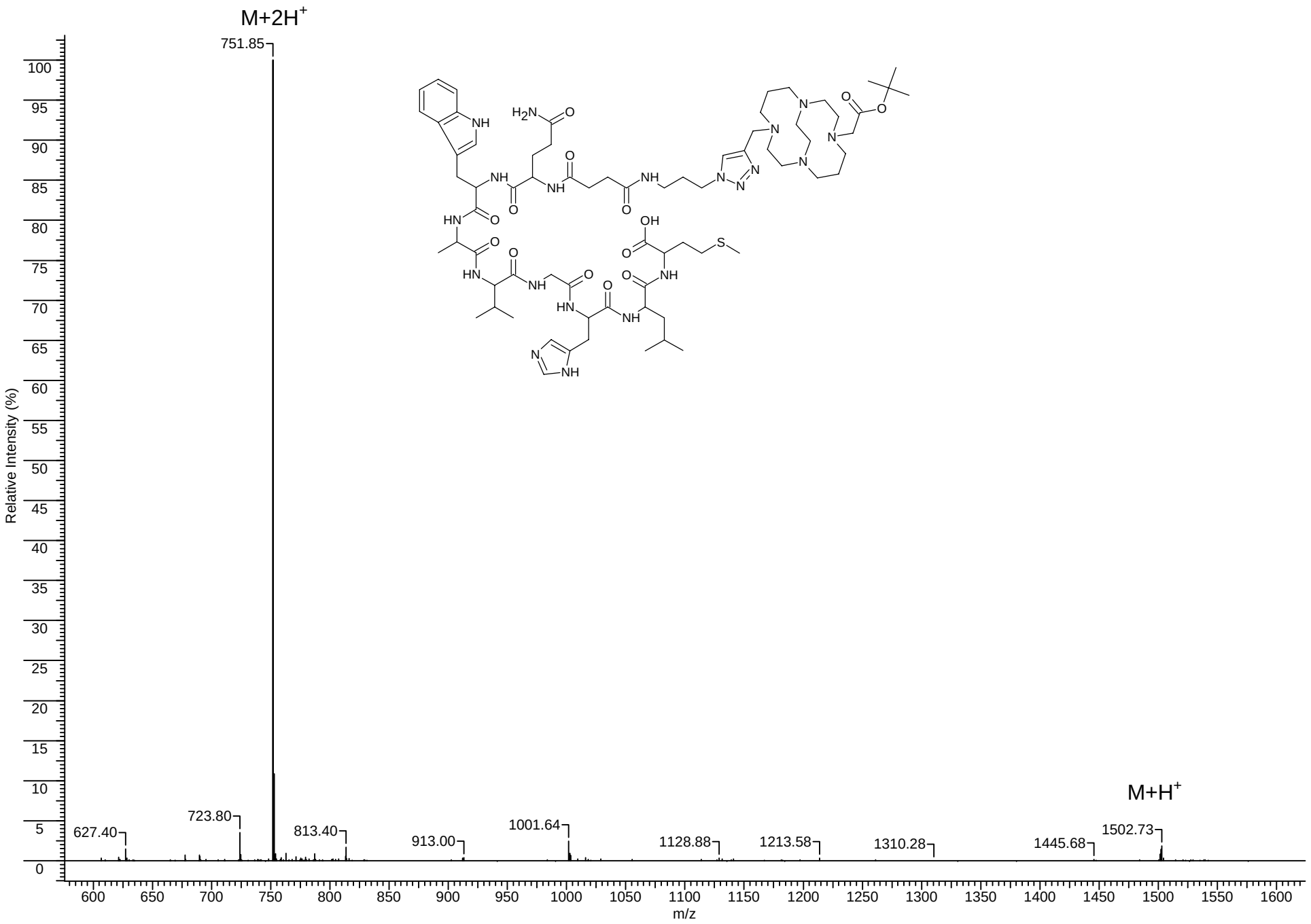


Supplementary Material (ESI) for Chemical Communications

$N_3(CH_2)_2NHCO(CH_2)_2CO$-QWAVGHLM (5): UPLC-MS (TIC)



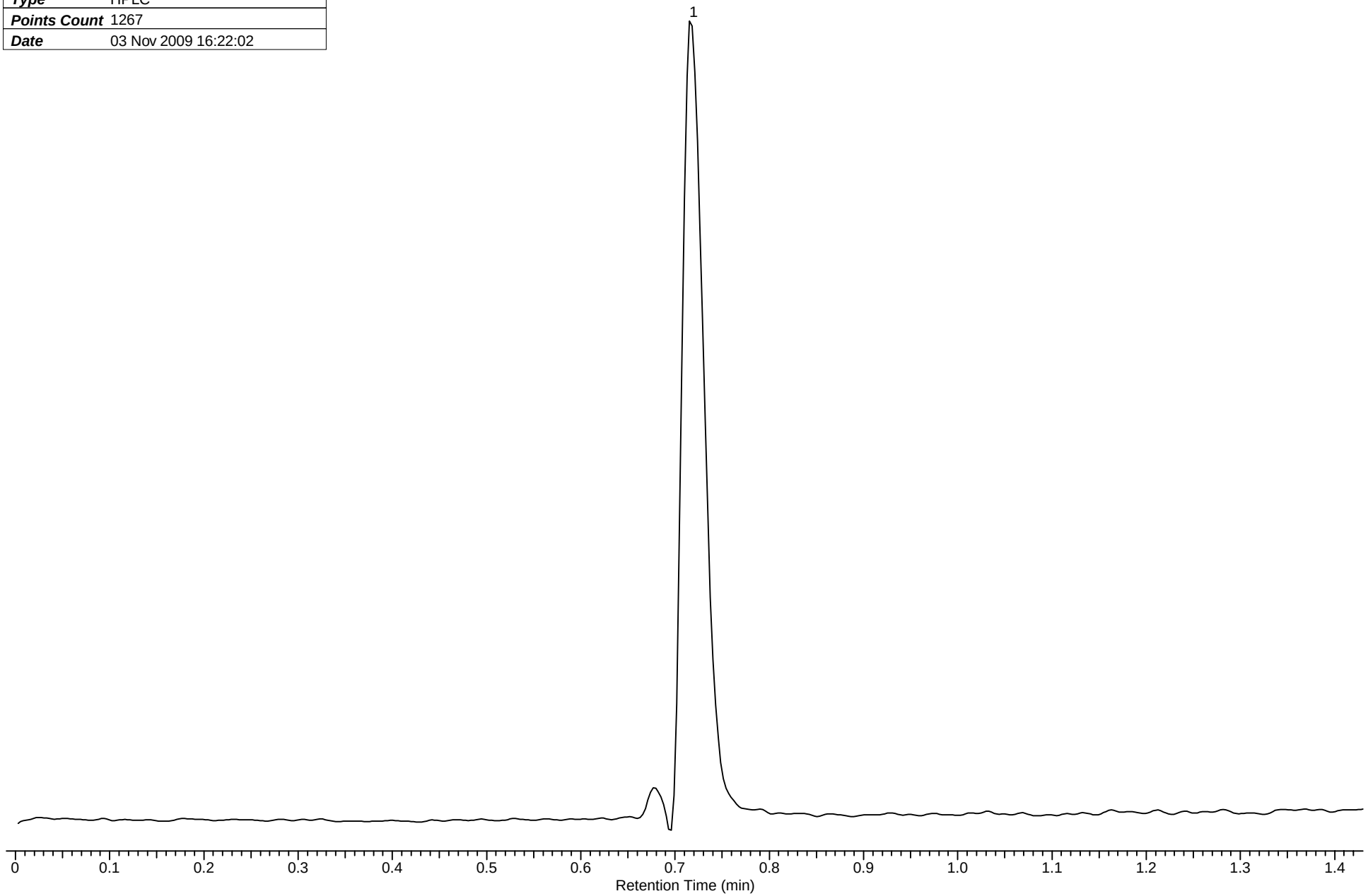
Protected CB-TE2A-GRP (tBu-6): ESI+



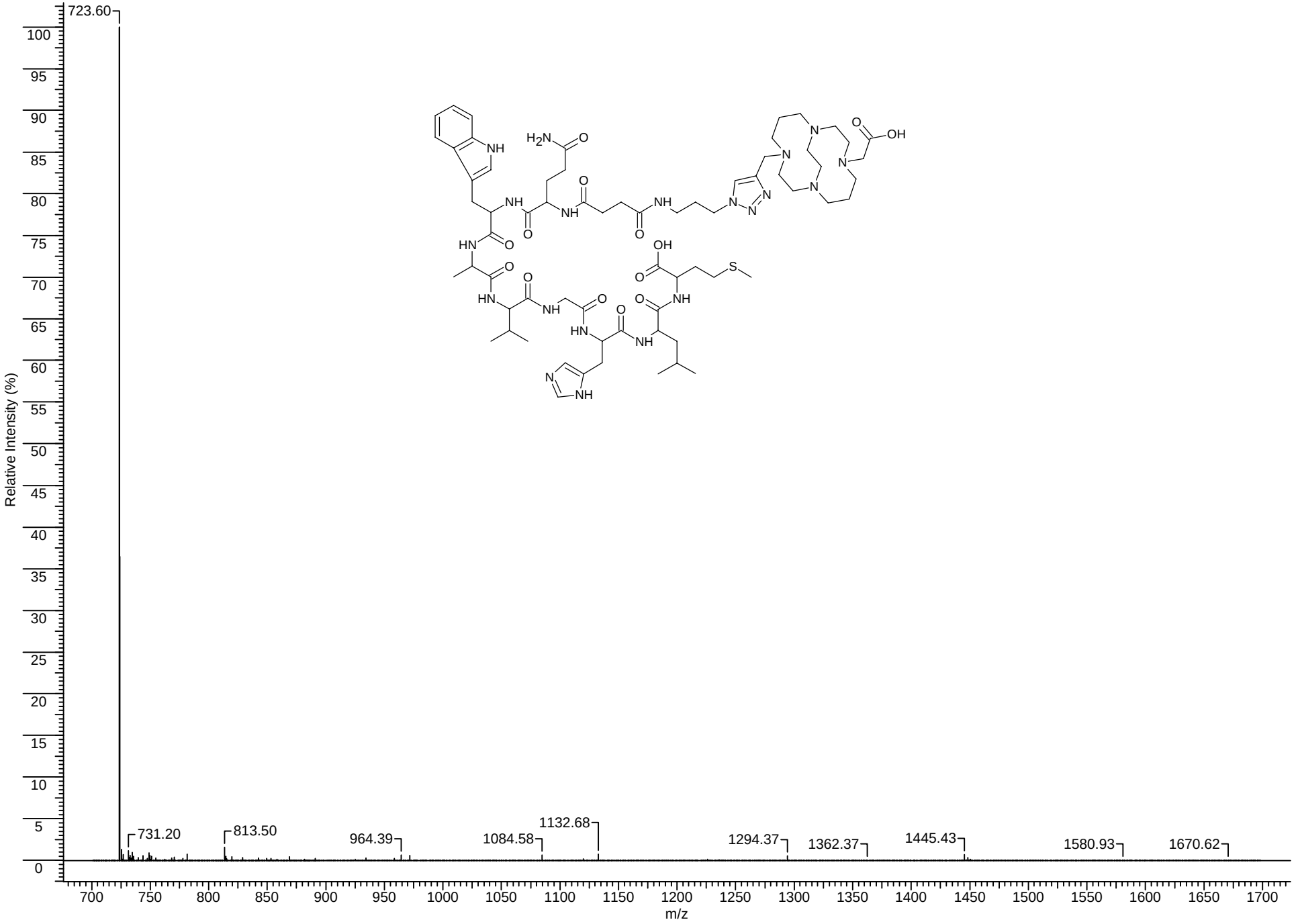
Supplementary Material (ESI) for Chemical Communications

Protected CB-TE2A-GRP (tBu-6): UPLC-MS (TIC)

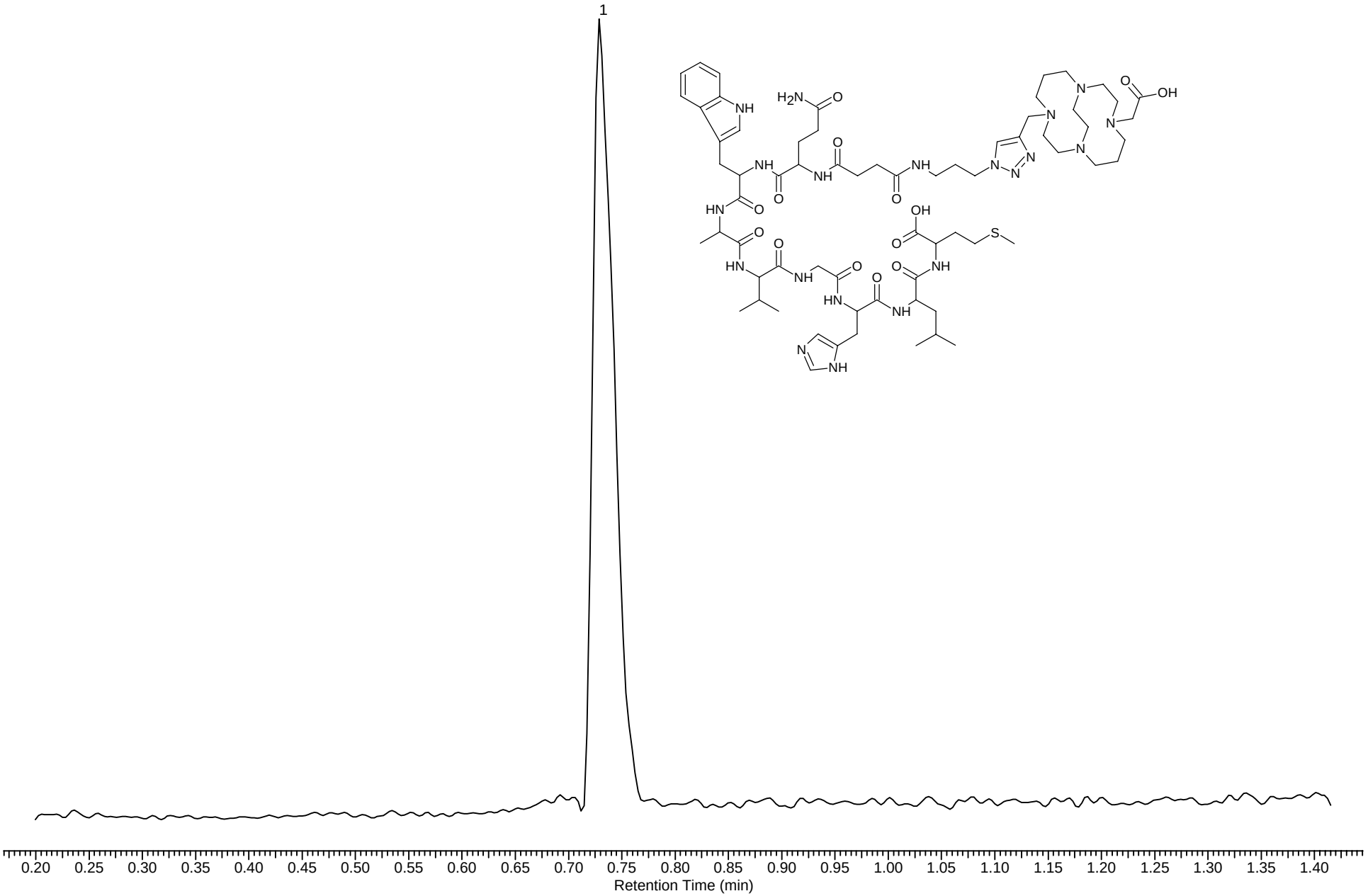
File Name	C:\[Artem]\Science\Publishing\Clickable chelates\Spectra\LC0213TC.TXT
Type	HPLC
Points Count	1267
Date	03 Nov 2009 16:22:02



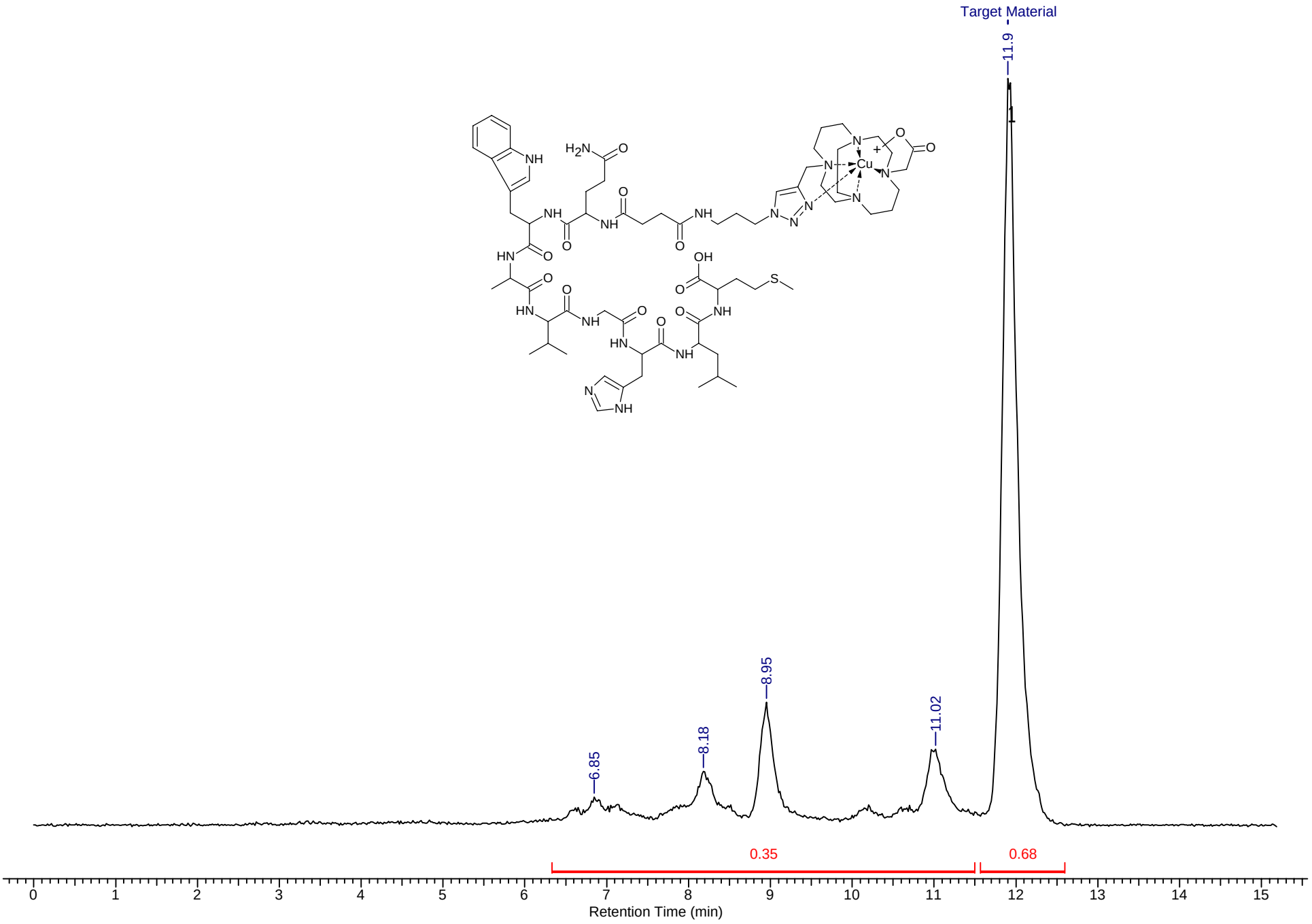
CB-TE2A-GRP (6): ESI+



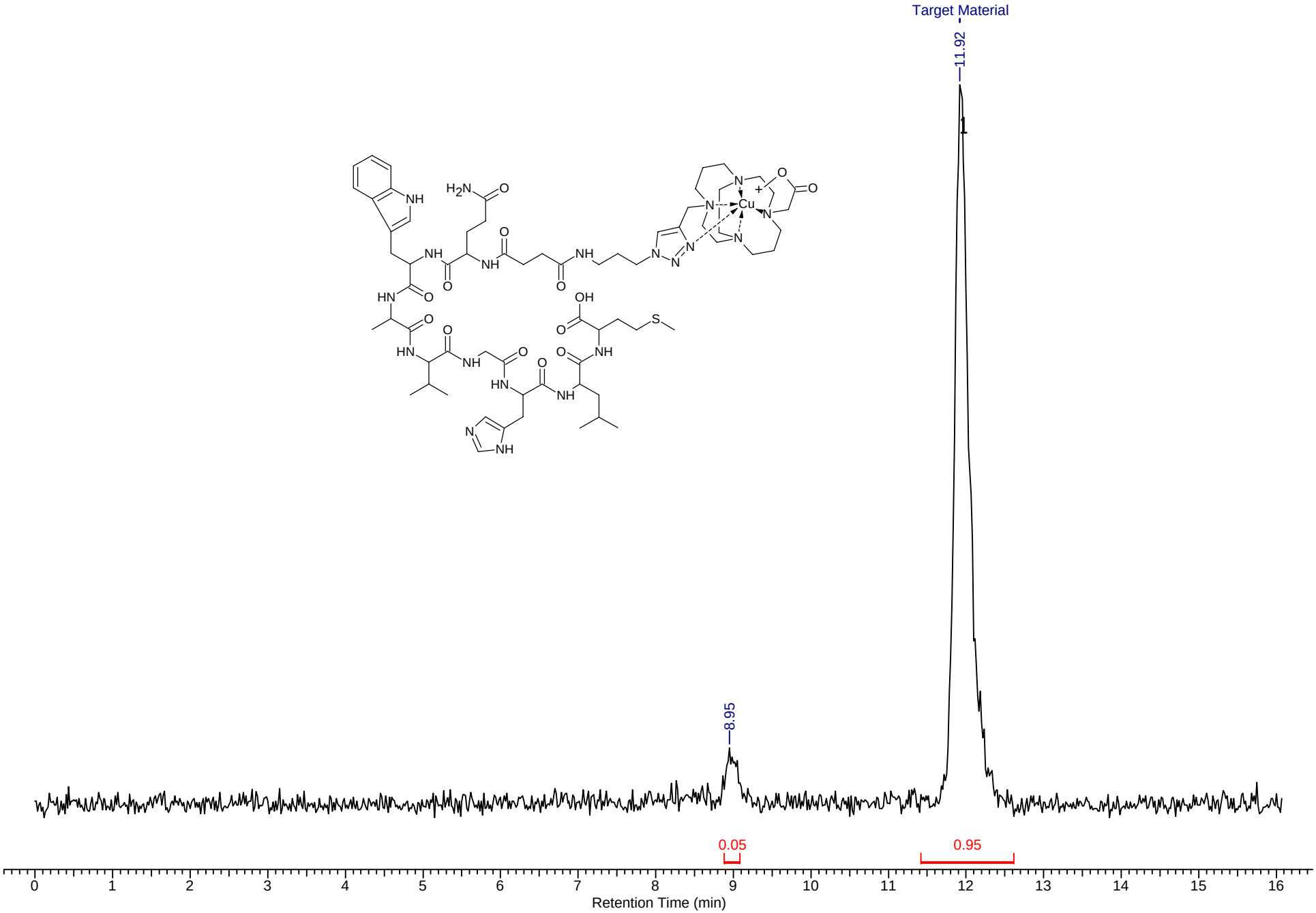
CB-TE2A-GRP (6): UPLC-MS (TIC)



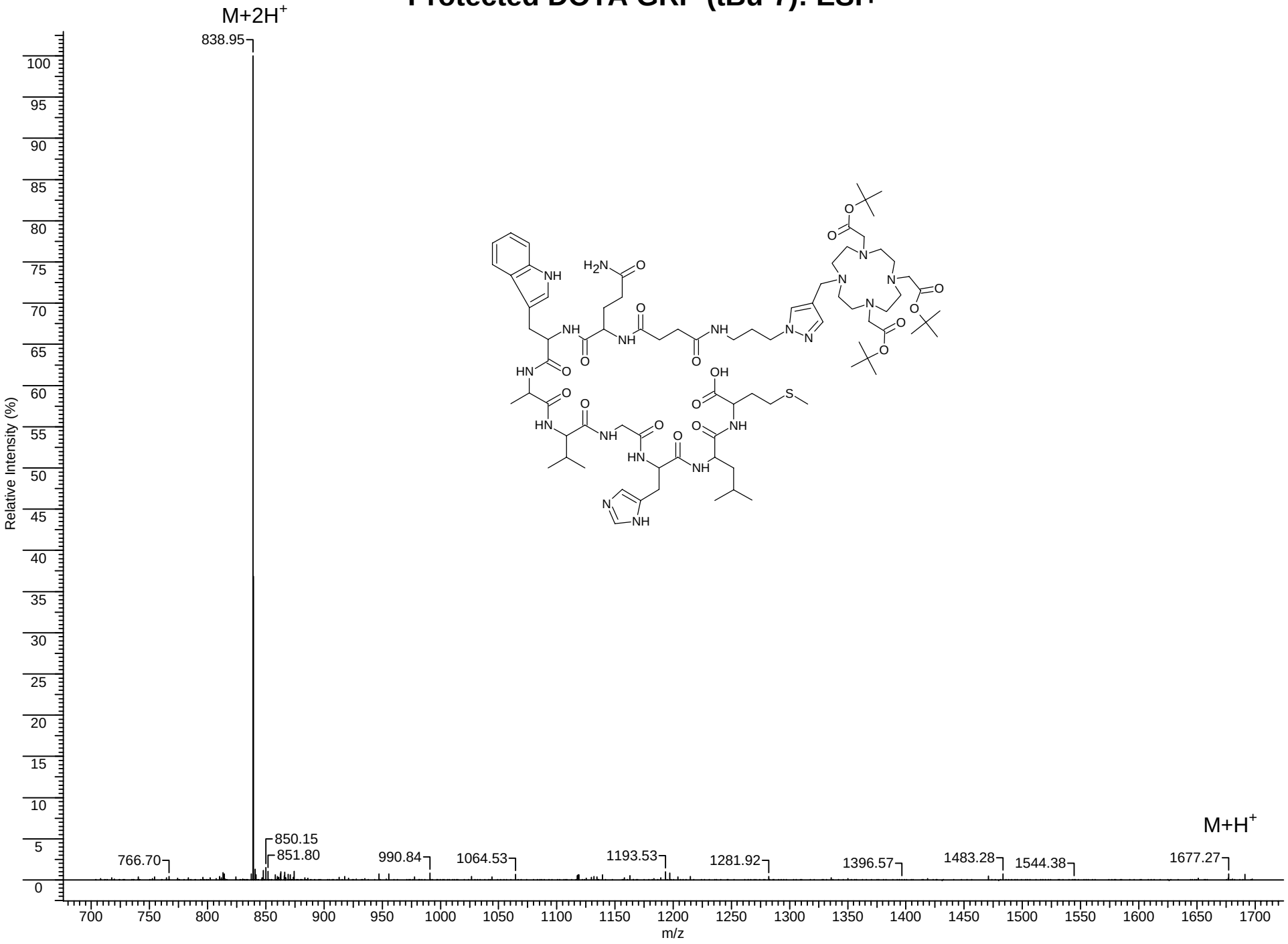
^{64}Cu-CB-TE2A-GRP (^{64}Cu-6): Radio HPLC; Reaction mixture



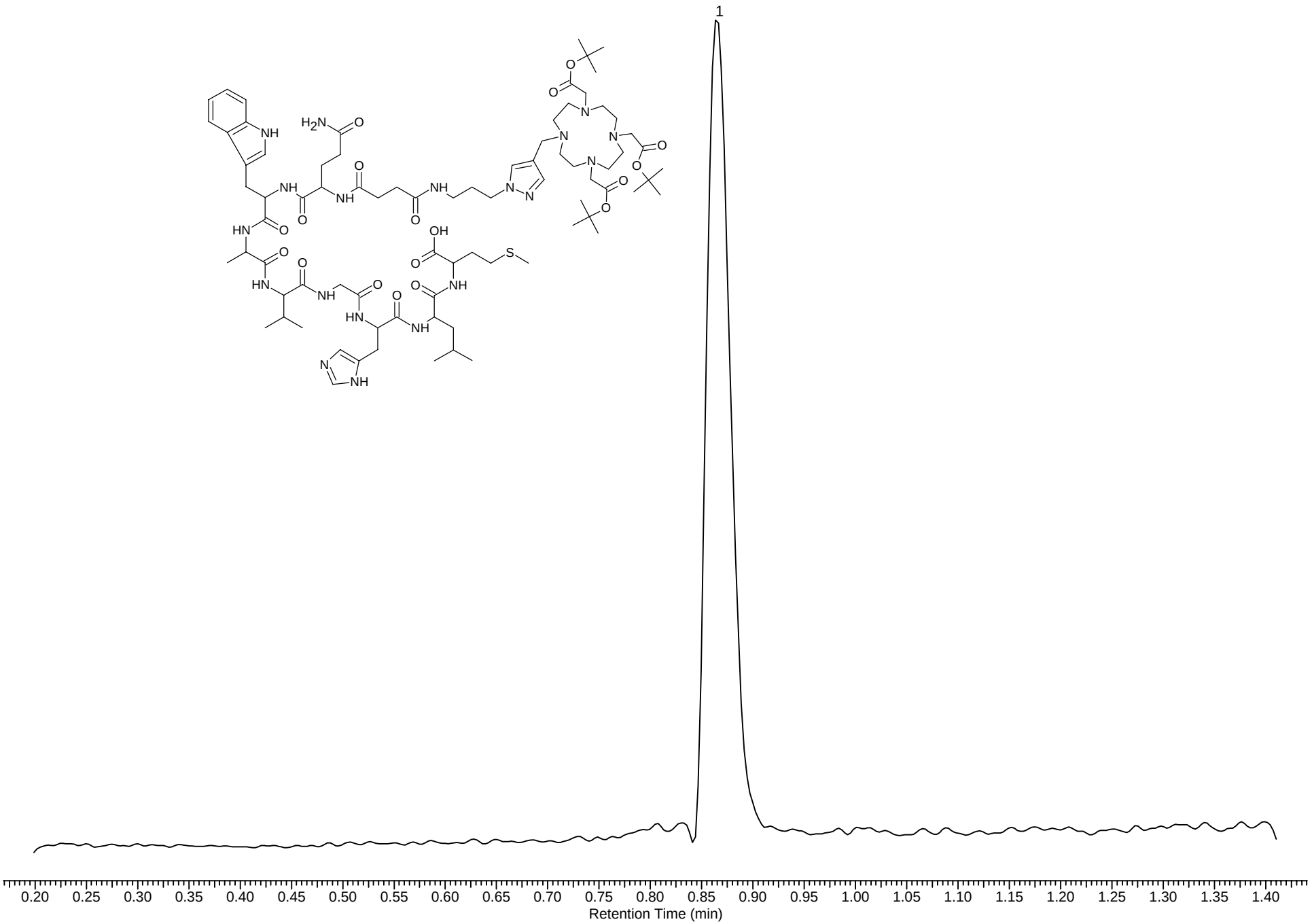
^{64}Cu-CB-TE2A-GRP (^{64}Cu-6): Radio HPLC; After purification



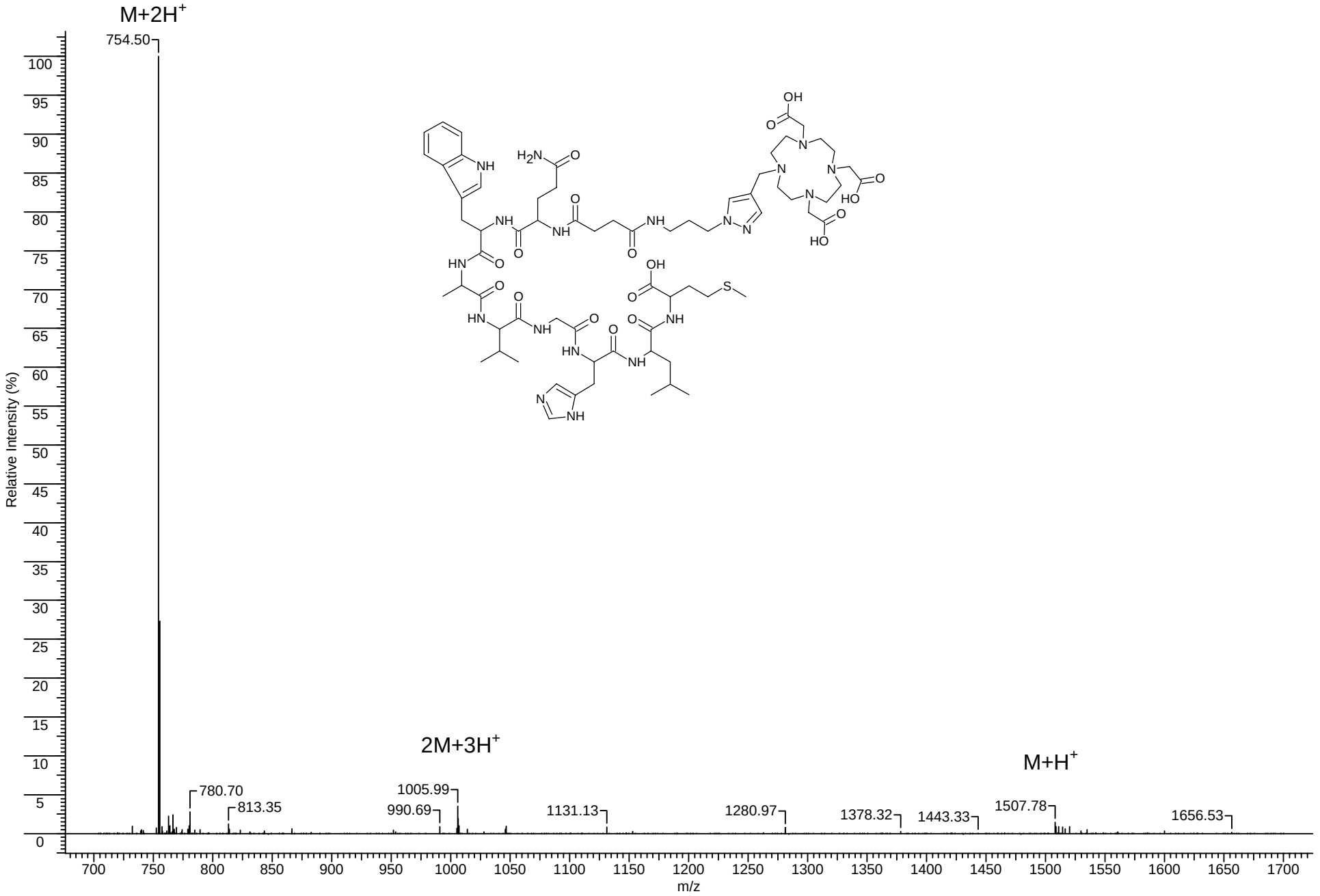
Protected DOTA-GRP (tBu-7): ESI+



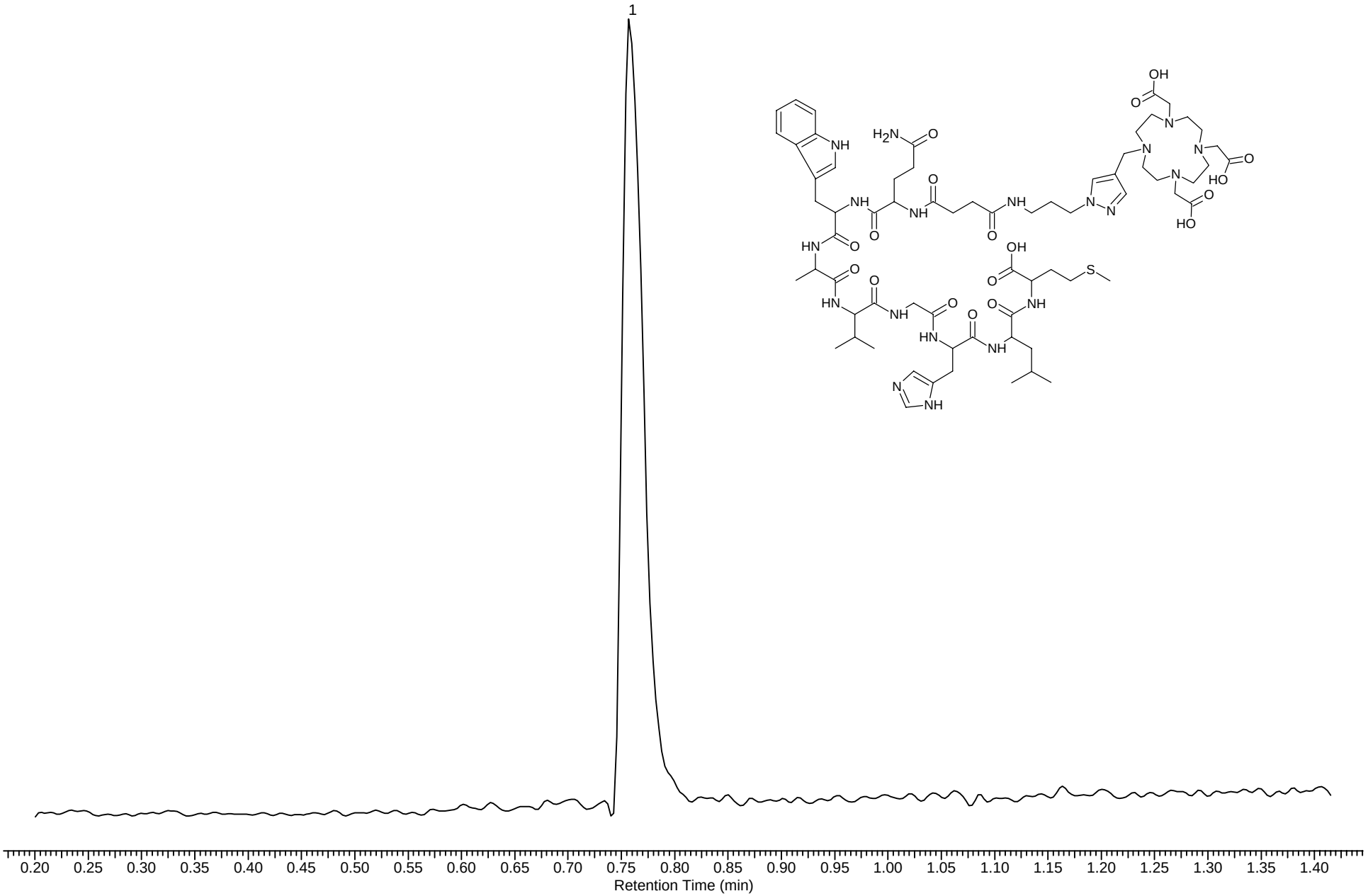
Protected DOTA-GRP (tBu-7): UPLC-MS (TIC)



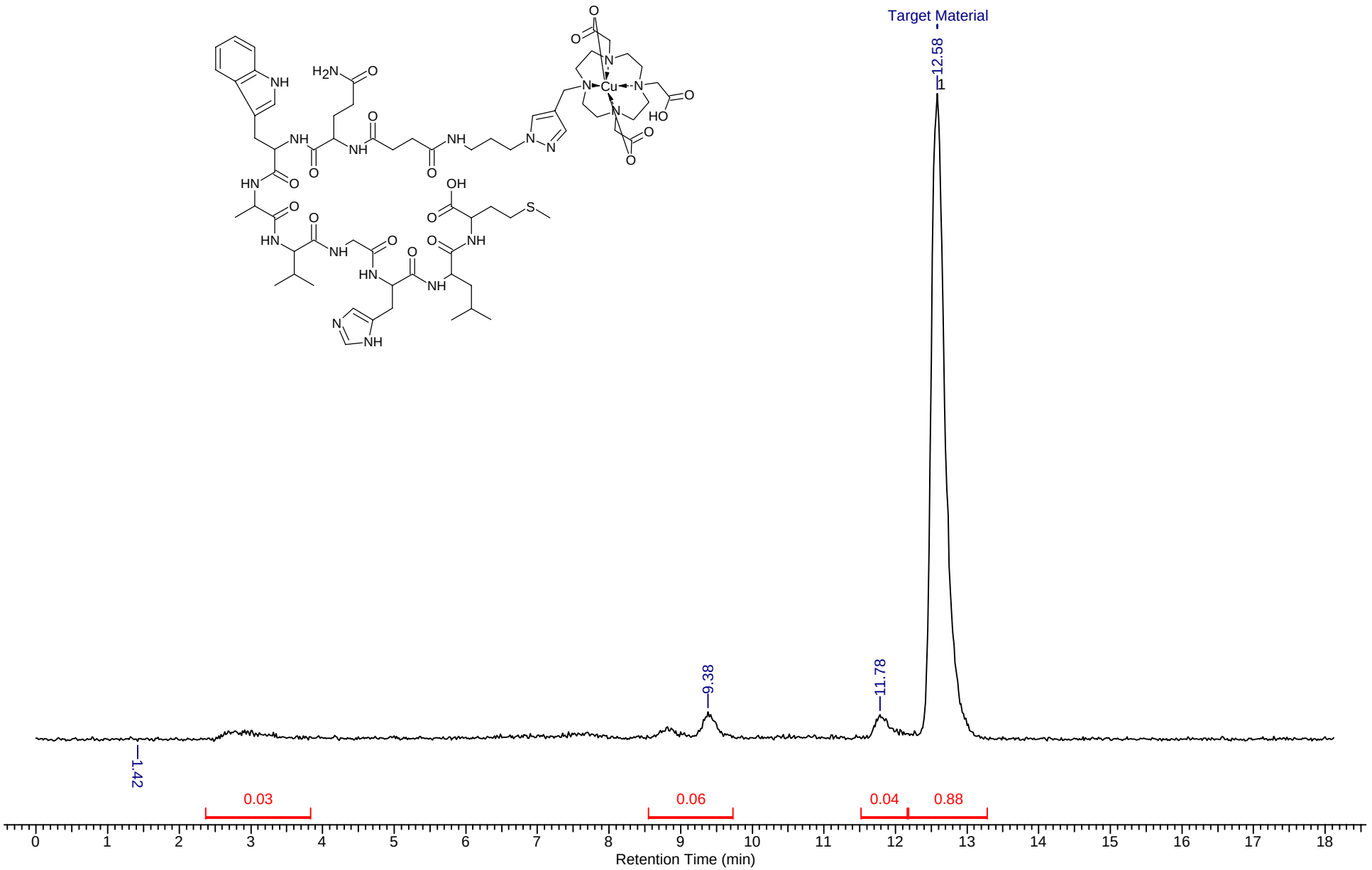
DOTA-GRP (7): ESI+



Protected DOTA-GRP (tBu-7): UPLC-MS (TIC)



^{64}Cu-DOTA-GRP (^{64}Cu-7): RadioHPLC; Reaction mixture



^{64}Cu-DOTA-GRP (^{64}Cu-7): RadioHPLC; Pure product