Supporting information for

Trifunctional ^{99m}Tc Based Radiopharmaceuticals: Metal-mediated Conjugation of a Peptide with a Nucleus Targeting Intercalator

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General information: The HPLC-MS data acquisition and processing were performed as described in experimental section. The spectral accuracy was calculated by MassWorks software (version 2.0, Cerno Bioscience, Danbury, CT, USA) using sCLIPS, which is a formula determination tool that performs peak shape calibration and matches calibrated experimental isotope pattern against possible theoretical ones using the spectral accuracy metric. The peak shape calibration is created using the monoisotope peak of the ion itself as the peak shape standard. As a standards the similar compounds with known elemental analysis were used.[14] The analysis were repeated under identical conditions to assure reproducibility. The red line represents the measured spectrum and the green line the fitted and calculated theoretical one. Mass errors and spectral accuracy are given in the following table.

* Corresponding author, E-mail: ariel@aci.uzh.ch.

Methyl 1-(4-bromobutyl)-1H-imidazole-4-carboxylate (2)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.4, 138.0, 134.3, 125.0, 51.9, 46.9, 32.4, 29.6, 29.5.

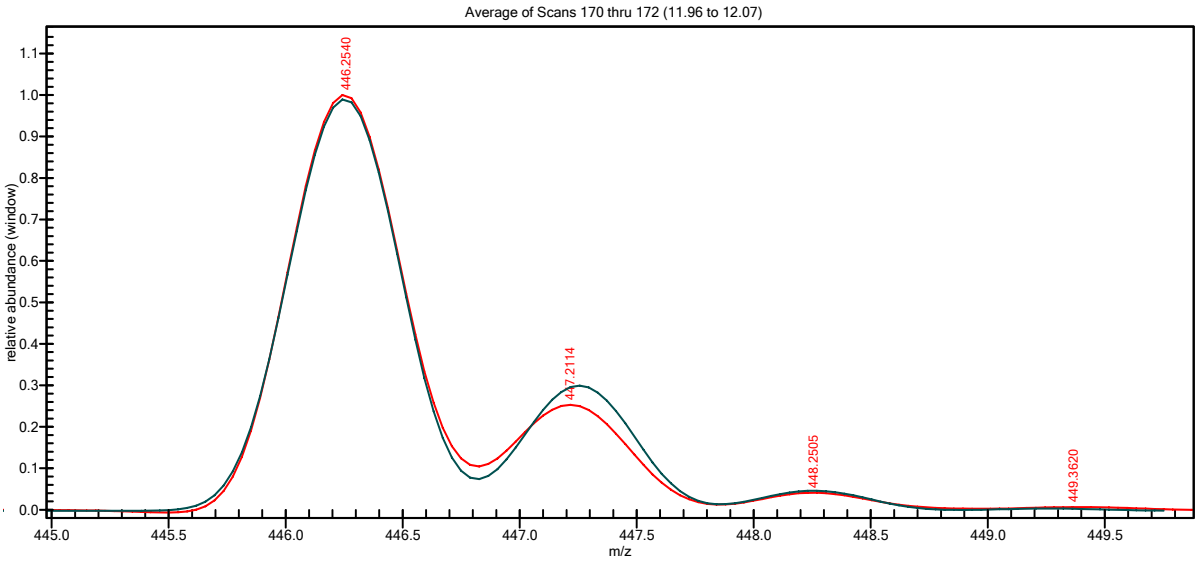
Methyl 1-(4-bromobutyl)-1H-imidazole-5-carboxylate (3)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.68 (s, 1H), 7.55 (s, 1H), 4.29 (t, ^{3}J = 7 Hz, 2H), 3.80 (s, 3H), 3.35 (t, ^{3}J = 6.4 Hz, 2H), 1.95-1.84 (m, 2H), 1.87-1.77 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.8, 142.1, 138.3, 122.3, 51.7, 46.2, 32.7, 29.9, 29.7. MS (ESI) m/z (%) 261.4 (100), 263.4(98) $[M + H]^+$. Calcd $C_9H_{14}BrN_2O_2^+$: 261.0 (100.0%), 263.0 (98.2%). TLC R_f 0.30 (EtOAc).^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.8, 142.1, 138.3, 122.3, 51.7, 46.2, 32.7, 29.9, 29.7.

3,6-bis(dimethylamino)-10-(4-(4-(methoxycarbonyl)-1H-imidazol-1-yl)butyl)acridinium (5)

^{13}C NMR (CD_3OD, 100 MHz): δ 156.5, 143.5, 143.2, 133.5, 117.6, 114.6, 92.6, 61.2,51.1, 46.9, 40.0, 28.2, 22.9.

Calculated HR-MS and spectral accuracy of compound **5**.



CLIPS Report - D:\Data\Karel\Cerno files\3.d\msd1. **09.07.2010 14:30:0**

Calibration File D:\Data\Karel\Cerno files\Pure AO Nikos calib.m
Calibration Time 09.07.2010 11:07:5

RT Windows
Average of Scans 170 thru 172 (11.96 to 12.(

CLIPS Parameters

Accurate Mass	446.254(
Charge	1
Mass Tolerance (mDa)	20.00
Electron State	Both
Double Bond Equivalent Rang	
Minimum:	-30.0(
Maximum:	50.00
Profile Mass Range (Da	
Start:	-5.00
End:	3.50

Element	Minimum	Maximum
C	26	26
N	5	5
O	2	2
H	32	32

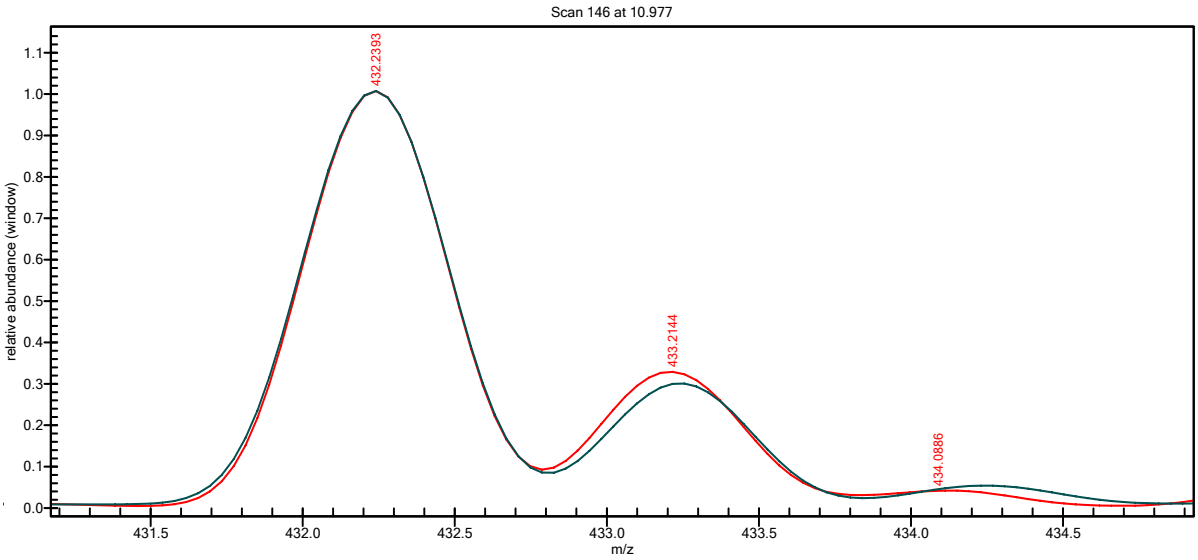
CLIPS Search Result

	Formula	Mono Isotope	Mass Error (mDa)	Mass Erro (PPM)	Spectral Accuracy	RMSE	DBE
1	C26N5O2H32	446.2556	1.6003	3.5861	94.2258	28'734	13.5

10-(4-(4-carboxy-1H-imidazol-1-yl)butyl)-3,6-bis(dimethylamino)acridinium (6)

^{13}C NMR ($CD_3CN + D_2O$, 100 MHz): δ 156.4, 143.7, 143.3, 133.65, 117.5, 114.9, 92.8, 47.0, 40.6, 27.5, 23.0.

Calculated HR-MS and spectral accuracy of compound **6**.



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Calibration Time 09.07.2010 11:07:5

RT Windows
Scan 146 at 10.97

CLIPS Parameters
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Mass Tolerance (mDa) 10.00
Electron State Both

Double Bond Equivalent Rang
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Maximum: 50.00

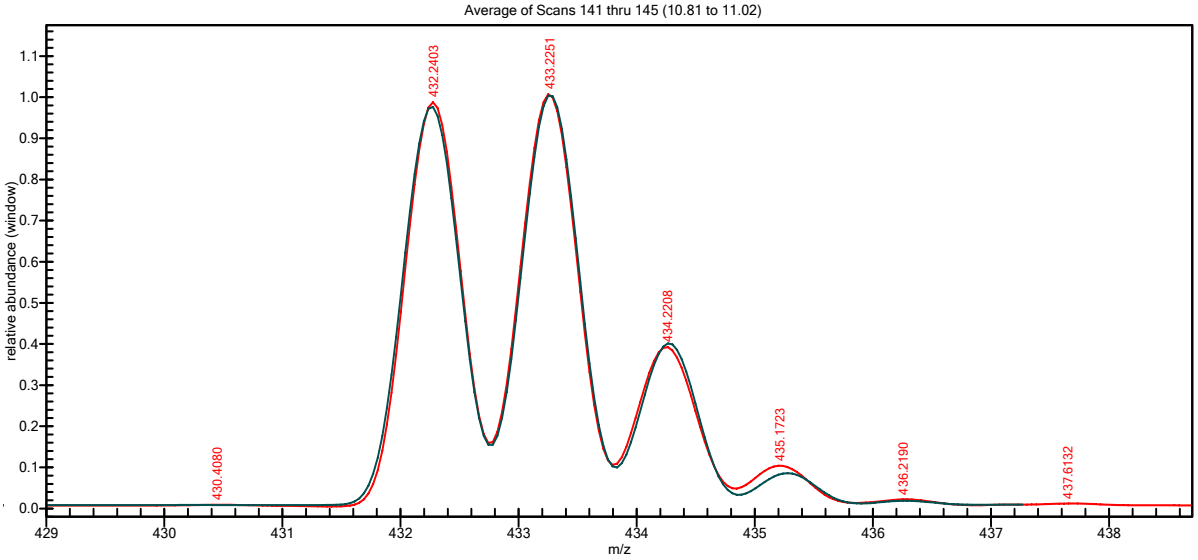
Profile Mass Range (Da
Start: -2.00
End: 3.00

Element	Minimum	Maximum
C	25	36
N	5	30
O	2	27
H	0	428

CLIPS Search Result

	Formula	Mono Isotope	Mass Error (mDa)	Mass Error (PPM)	Spectral Accuracy	RMSE	DBE
1	C25N5O2H30	432.2400	0.6502	1.5043	95.1814	41'862	13.5

Calculated HR-MS analysis measured from the NMR sample of **6** giving the isotopic masses.



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Calibration File D:\Data\Karel\Cerno files\Pure AO Nikos calib.m
Calibration Time 09.07.2010 11:07:5

RT Windows
Average of Scans 141 thru 145 (10.81 to 11.(

CLIPS Parameter:
Accurate Mass 432.240
Charge 1
Mass Tolerance (mDa) 100.0(
Electron State Both

Double Bond Equivalent Rang
Minimum: -30.0(
Maximum: 50.00

Profile Mass Range (Da
Start: -5.00
End: 5.00

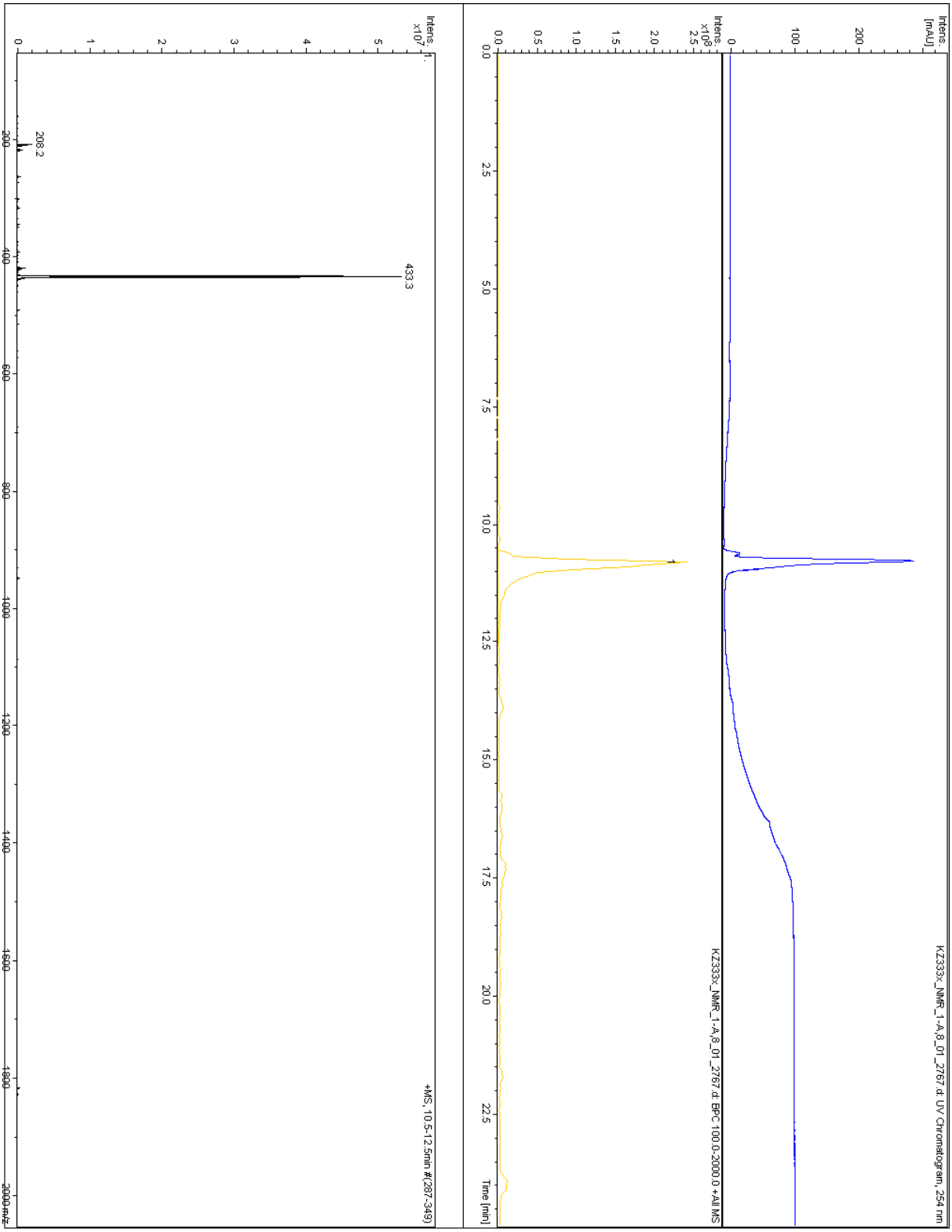
Element	Minimum	Maximum
C	25	25
H	30	30
O	2	2
N	5	5

Mixture Search
Formula 2 C25N5O2H29[2H
Formula 3 C25N5O2H28[2H]

CLIPS Search Result

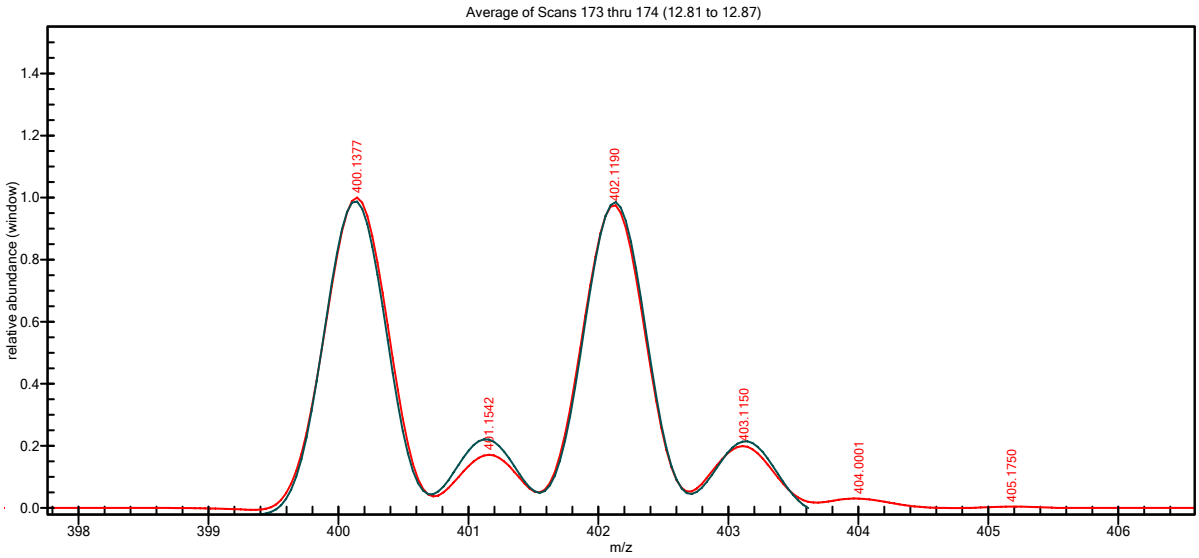
	Formula	Mono Isotope	Mass Error (mDa)	Mass Erro (PPM)	Spectral Accuracy	RMSE	DBE
1	C25H30O2N5	432.2400	-0.3498	-0.8092	95.6823	21'676	13.5

SI 1.The HPLC-MS trace of compound **6** (NMR sample, Gradient B).



10-(4-bromobutyl)-3,6-bis(dimethylamino)acridinium bromide (7)

Calculated HR-MS and spectral accuracy of compound **7**.



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Calibration Time 09.07.2010 11:07:5

RT Windows
Average of Scans 173 thru 174 (12.81 to 12.8

CLIPS Parameters
Accurate Mass 400.1377
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Mass Tolerance (mDa) 100.00
Electron State Both

Double Bond Equivalent Rang
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Maximum: 50.00

Profile Mass Range (Da
Start: -1.00
End: 3.50

Element	Minimum	Maximum
C	21	21
H	0	397
Br	1	4
N	3	3

CLIPS Search Result

	Formula	Mono Isotope	Mass Error (mDa)	Mass Error (PPM)	Spectral Accuracy	RMSE	DBE
1	C21H27BrN3	400.1388	1.1355	2.8377	94.6332	43'151	9.5

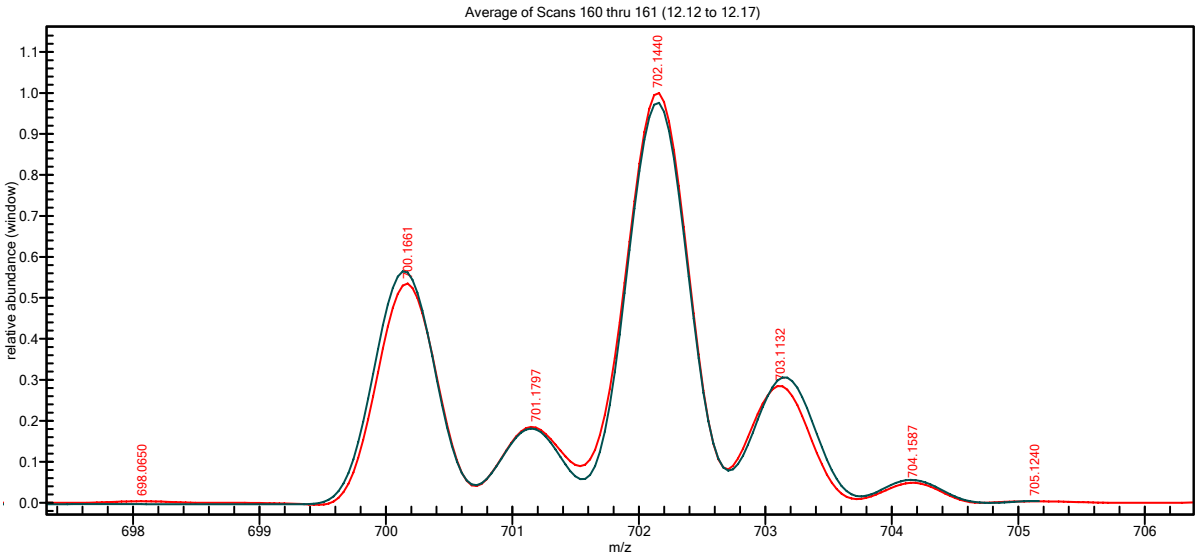
10-(4-(5-carboxy-1H-imidazol-1-yl)butyl)-3,6-bis(dimethylamino)acridinium (9)

^{1}H NMR (CD_3OD, 400 MHz): δ 8.92 (s, 1H), 8.57 (s, 1H), 8.06 (s, 1H), 7.82 (d, ^{3}J = 9.3 Hz, 2H), 7.19 (dd, ^{3}J = 9.2 Hz, ^{4}J = 2 Hz, 2H), 6.57 (s, 2H), 4.67 (bt, ^{3}J = 8 Hz, 2H), 4.58 (t, ^{3}J = 7.3 Hz, 2H), 3.24 (s, 12H), 2.14 (m, 2H), 1.99 (m, 2H). ^{13}C NMR (CD_3OD, 100 MHz): δ 157.6, 144.6, 144.2, 141.0, 134.6, 130.3, 118.7, 115.7, 93.7, 49.3, 47.9, 41.0, 29.16, 24.0. HPLC-MS (ESI, Gradient A) t_R: 13.8 min; m/z: 432.2 (100) $[M]^+$. Calcd for $C_{25}H_{30}N_5O_2^+$: 432.2 (100%).^{13}C NMR (CD_3OD, 100 MHz): δ 157.6, 144.6, 144.2, 141.0, 134.6, 130.3, 118.7, 115.7, 93.7, 49.3, 47.9, 41.0, 29.16, 24.0.

$[Re(H_2O)(6)(CO)_3]^+$ (10) and $[^{99m}Tc(H_2O)(6)(CO)_3]^+$ (11)

^{13}C NMR (CD_3CN, 100 MHz): δ 171.3, 156.5, 143.7, 143.5, 139.8, 122.5, 115.0, 92.8, 48.5, 47.2, 40.6, 27.8, 23.1. After the purification, the same product could be obtained from the reaction with the mixture of regioisomers **6**/**9**. The analyses were similar with compound **10**. Minor side product with additional **6** (or **9**) coordinated unit was also isolated (less then 1% see SI 5. and 6).

Calculated HR-MS and spectral accuracy of complex **10**.



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Calibration Time 09.07.2010 11:07:5

RT Windows
Average of Scans 160 thru 161 (12.12 to 12.

CLIPS Parameters

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Electron State	Both
Double Bond Equivalent Rang	
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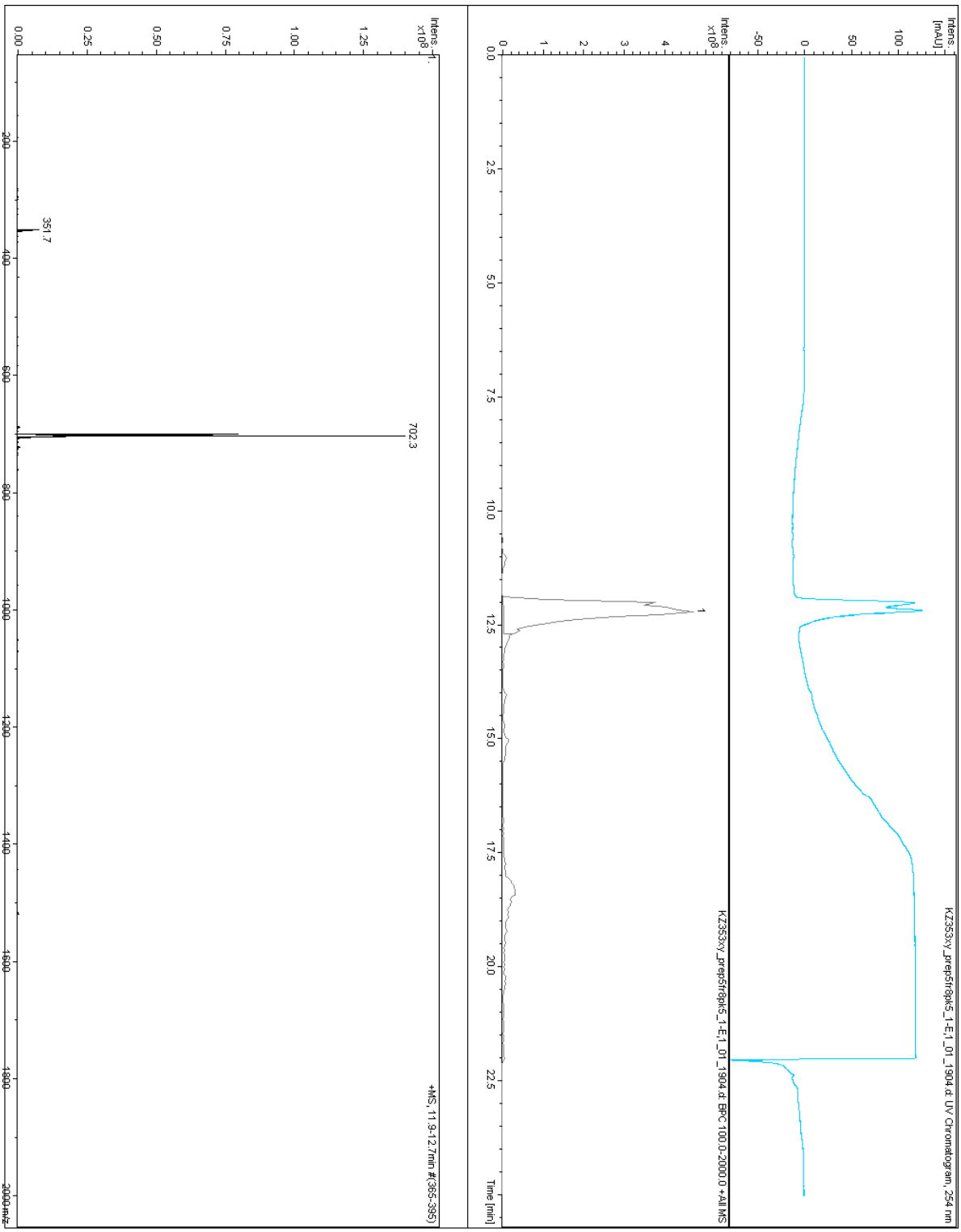
Profile Mass Range (Da	
Start:	-5.00
End:	5.00

Element	Minimum	Maximum
Re	1	1
C	28	28
N	5	5
O	5	5
H	0	694

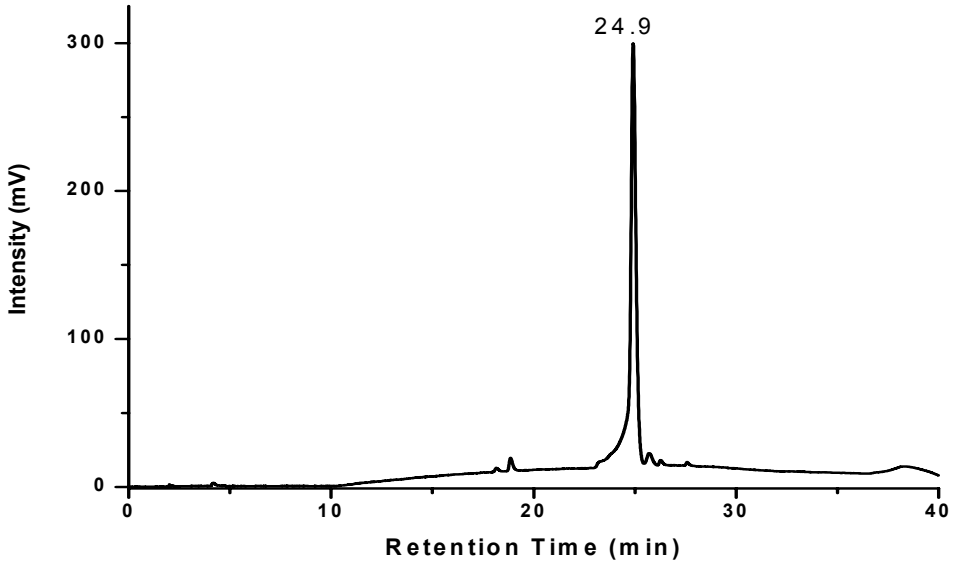
CLIPS Search Result

	Formula	Mono Isotope	Mass Error (mDa)	Mass Error (PPM)	Spectral Accuracy	RMSE	DBE
1	ReC28N5O5H29	700.1698	3.7248	5.3198	92.9869	37'087	16.5

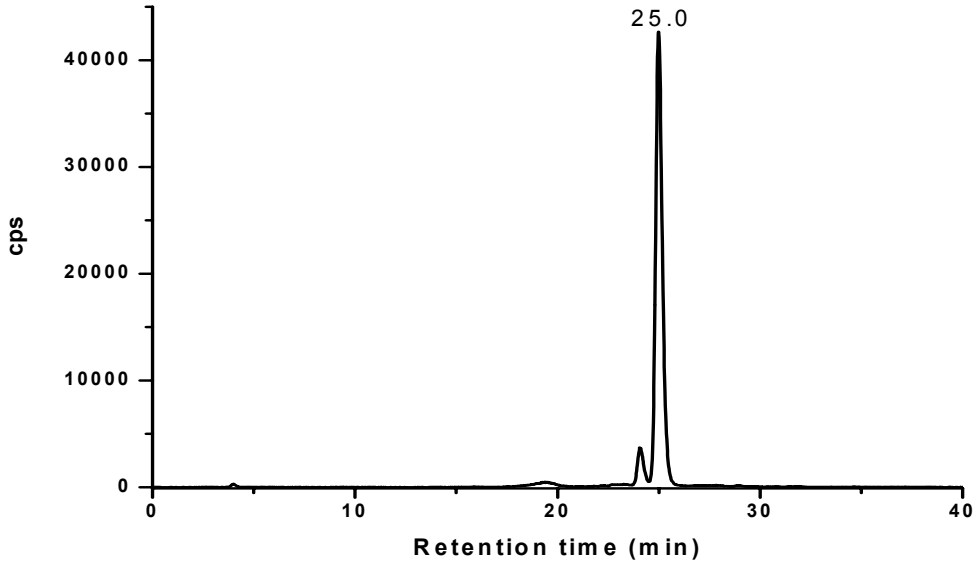
SI 2. The HPLC-MS trace of complex **10** (Gradient B).



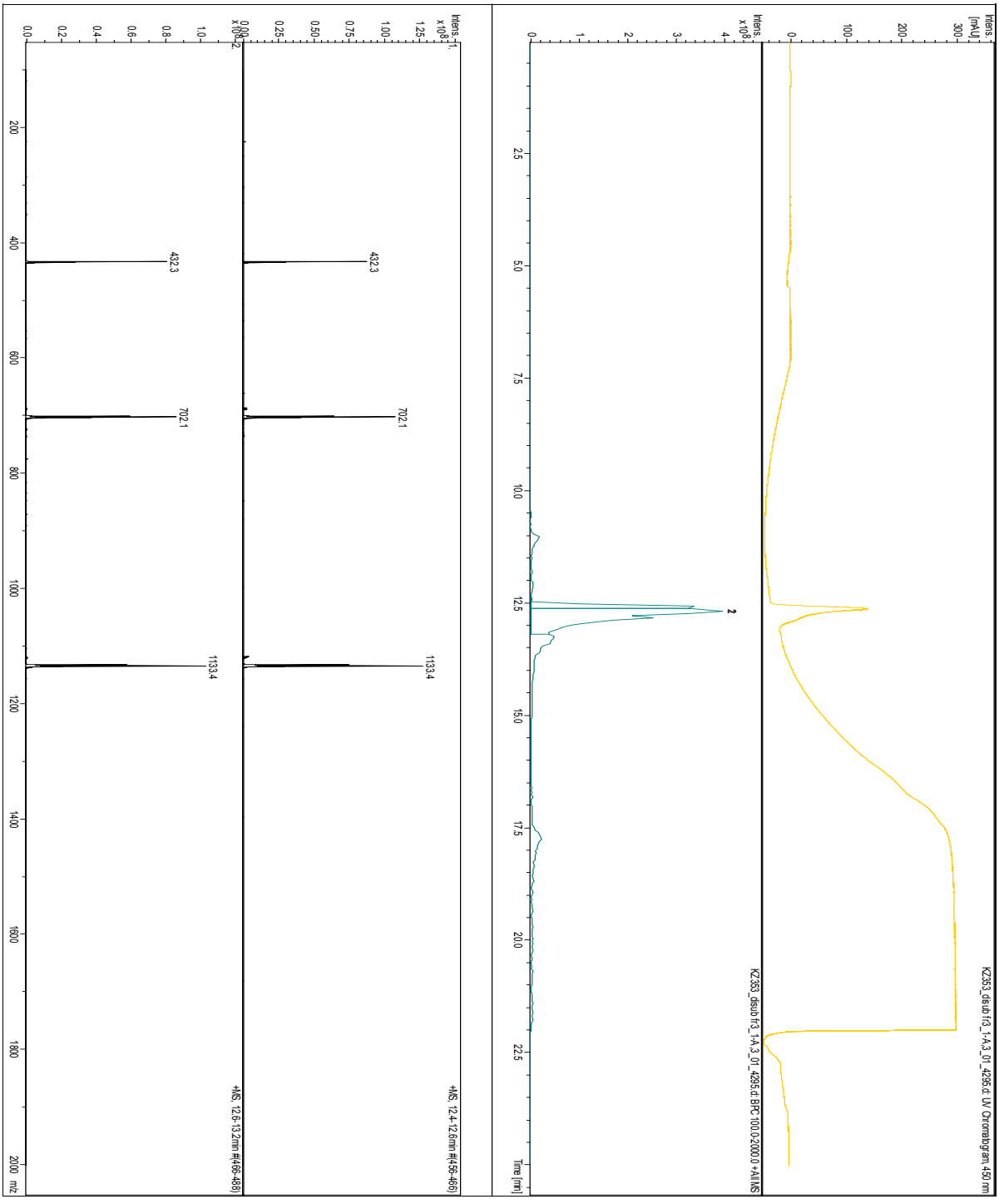
SI 3. The HPLC trace (UV) of complex **10** (Gradient D).



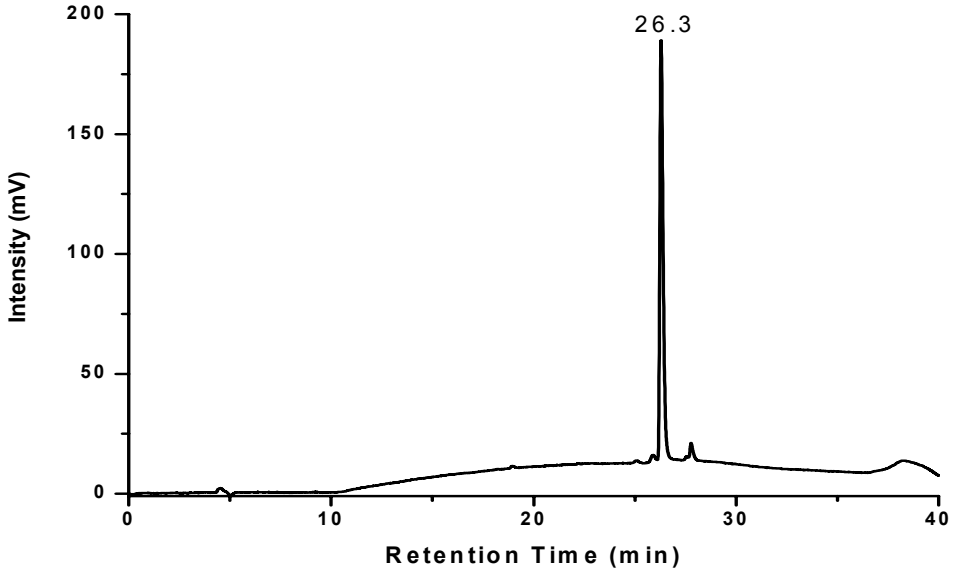
SI 4. The γ-trace HPLC of complex **11** (Gradient D).



SI 5. The HPLC-MS trace of complex **10** with an additional ligand **6**(or **9**) (Gradient B).

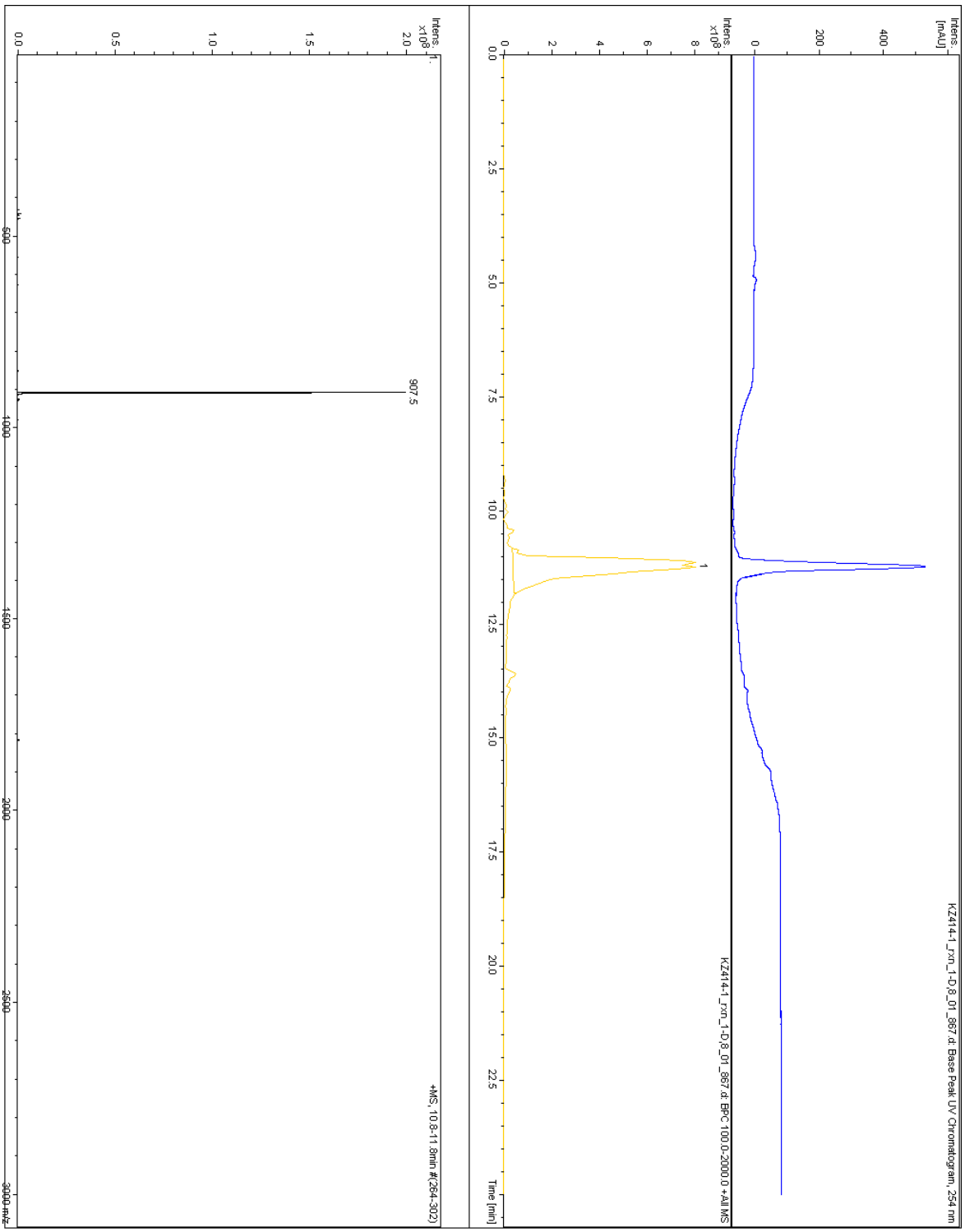


SI 6. The HPLC trace (UV) of complex **10** with an additional ligand **6**(or **9**) (Gradient D).



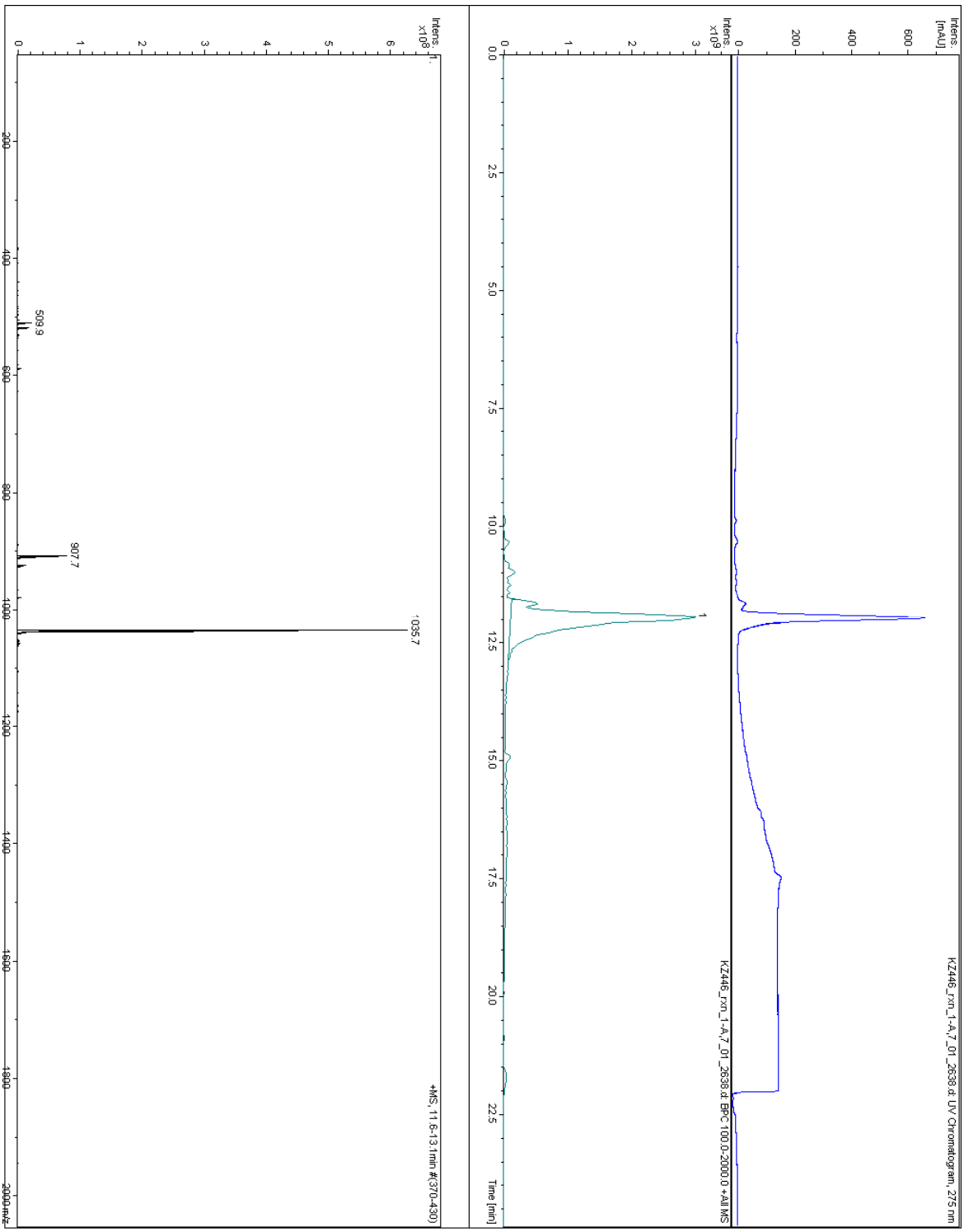
4-Isocyanobutyryl-bombesin(14)

SI 7. The HPLC-MS trace of complex **14** (Gradient B).



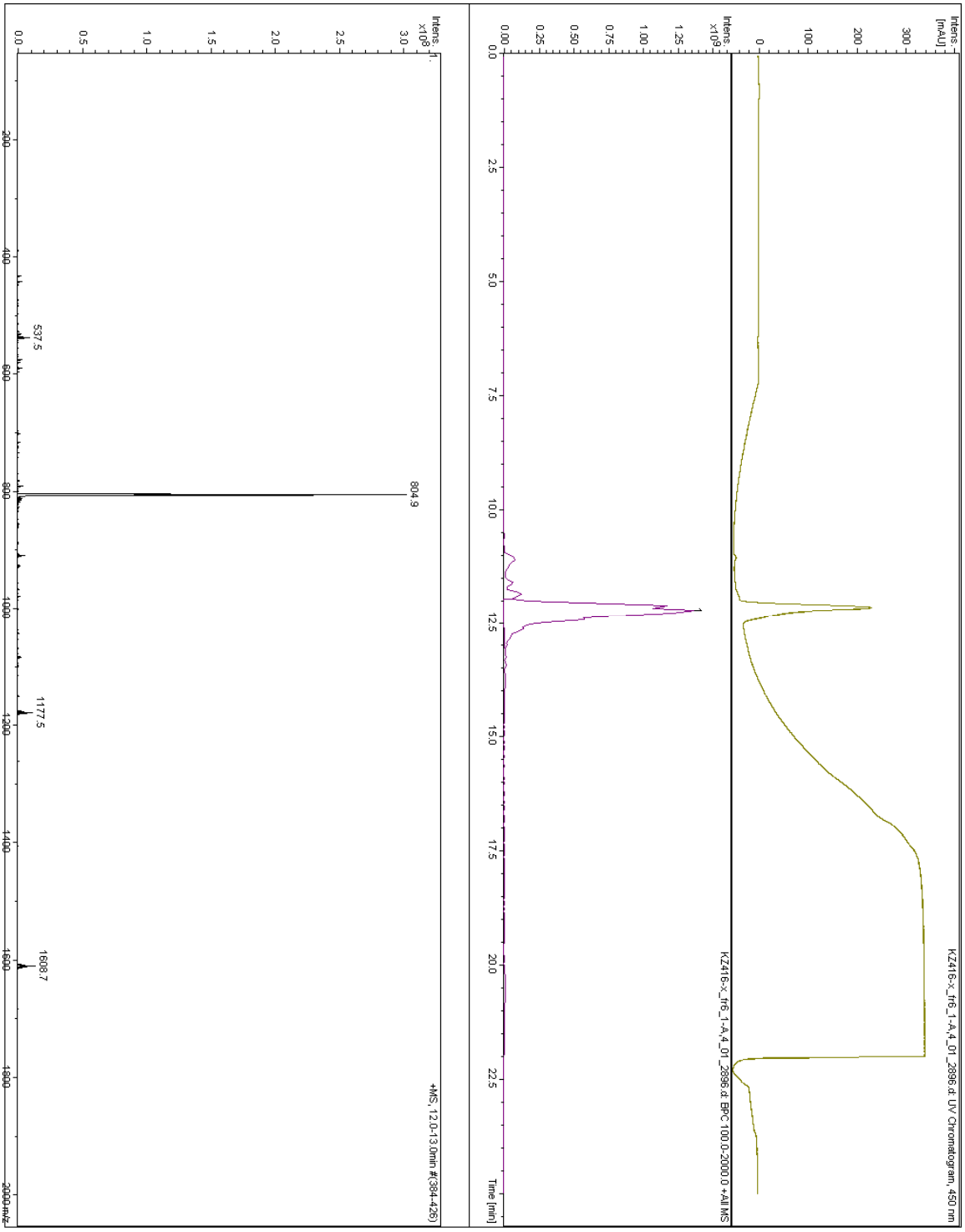
4-Isocyanobutyryl-bombesin(16)

SI 8. The HPLC-MS trace of complex **16** (Gradient B).

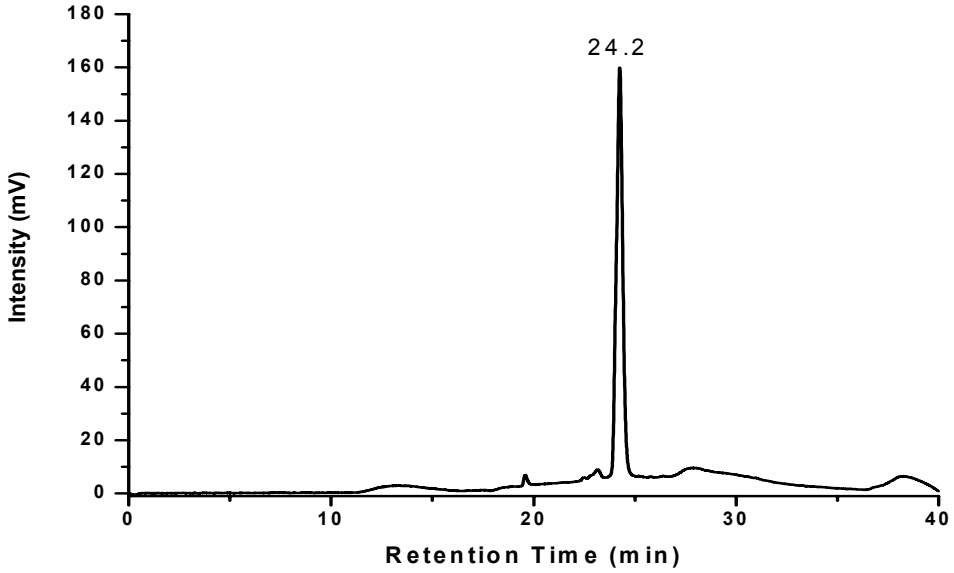


$[Re(14)(6)(CO)_3]^+$ (17) and $[^{99m}Tc(14)(6)(CO)_3]^+$ (18)

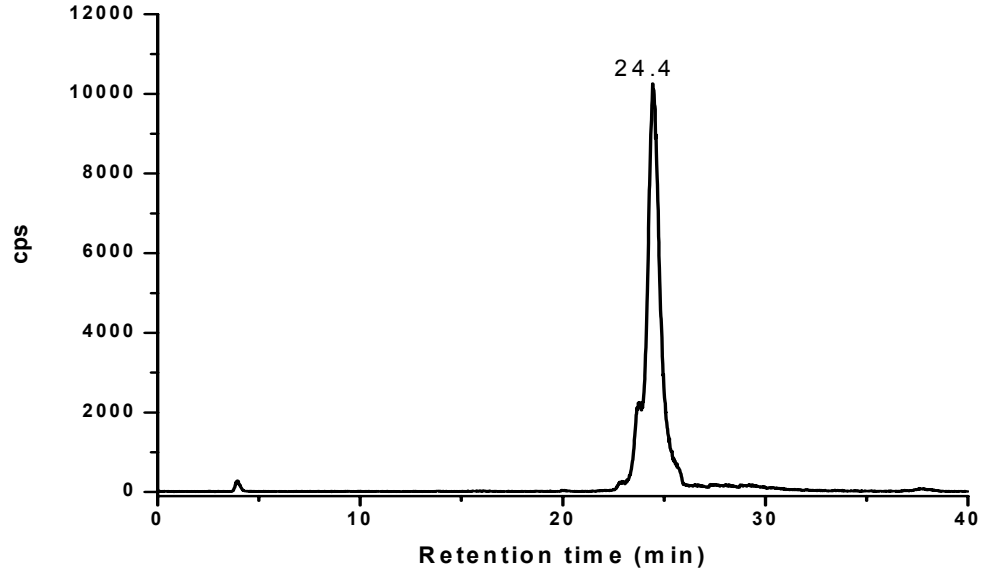
SI 9. The HPLC-MS trace of complex **17** (Gradient B).



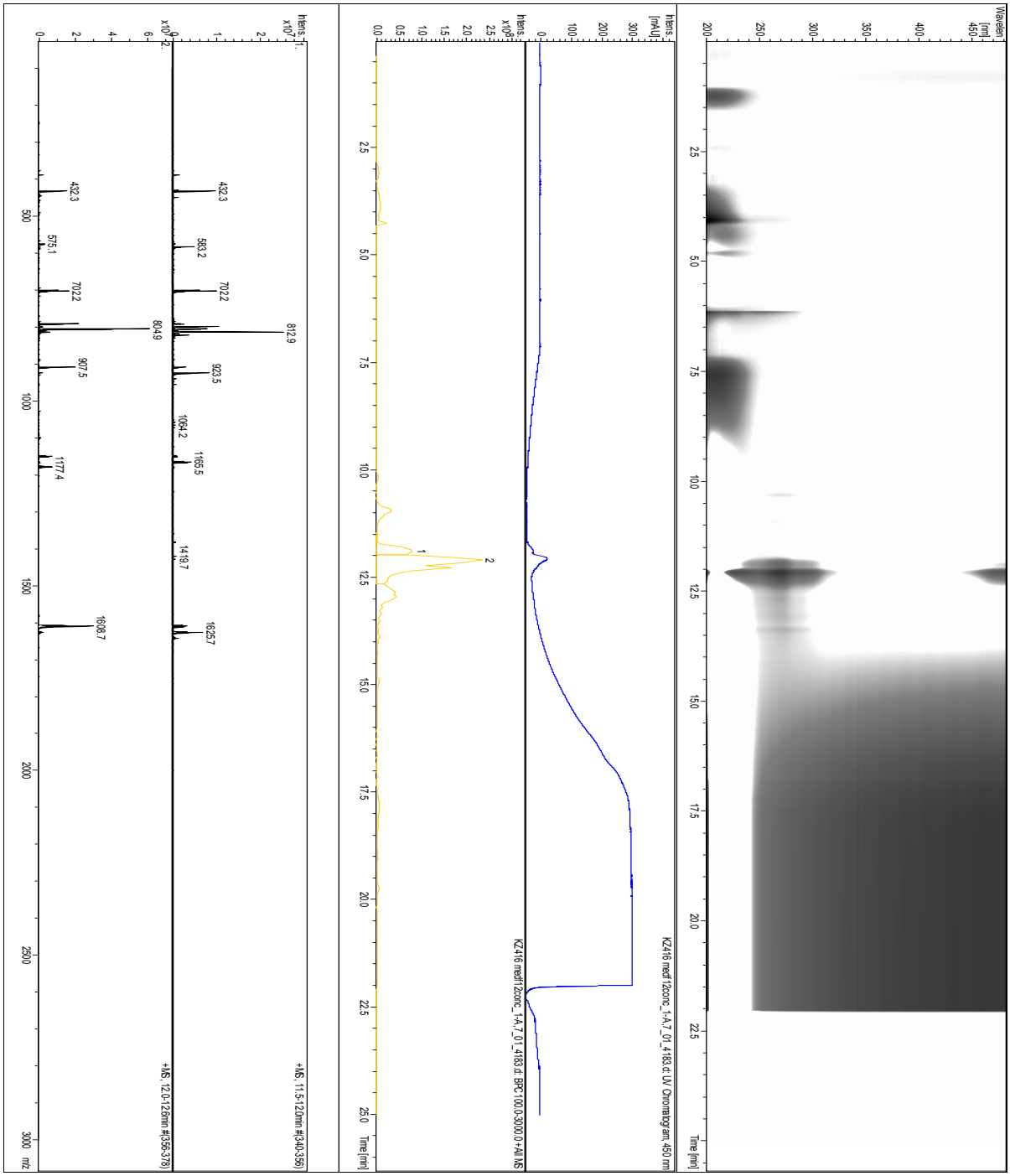
SI 10. The HPLC trace (UV) of complex **17** (Gradient D).



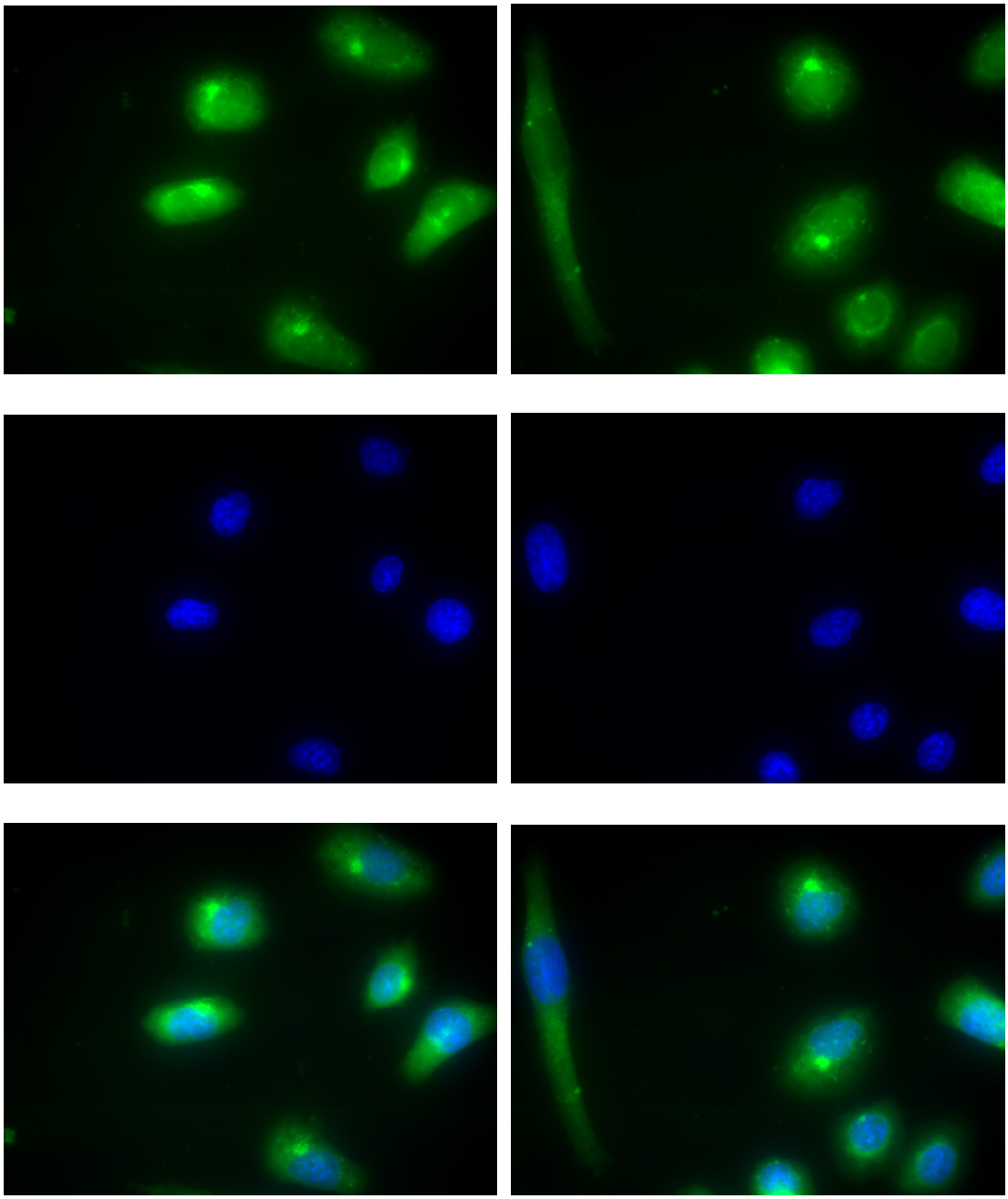
SI 11. The γ-trace HPLC of complex **18** (Gradient D).



SI 12. The HPLC-MS trace (and DAD) of complex **17** (Gradient B) after incubation over PC3 cells in F12 medium for 24 H (*c* = 1 mmol).

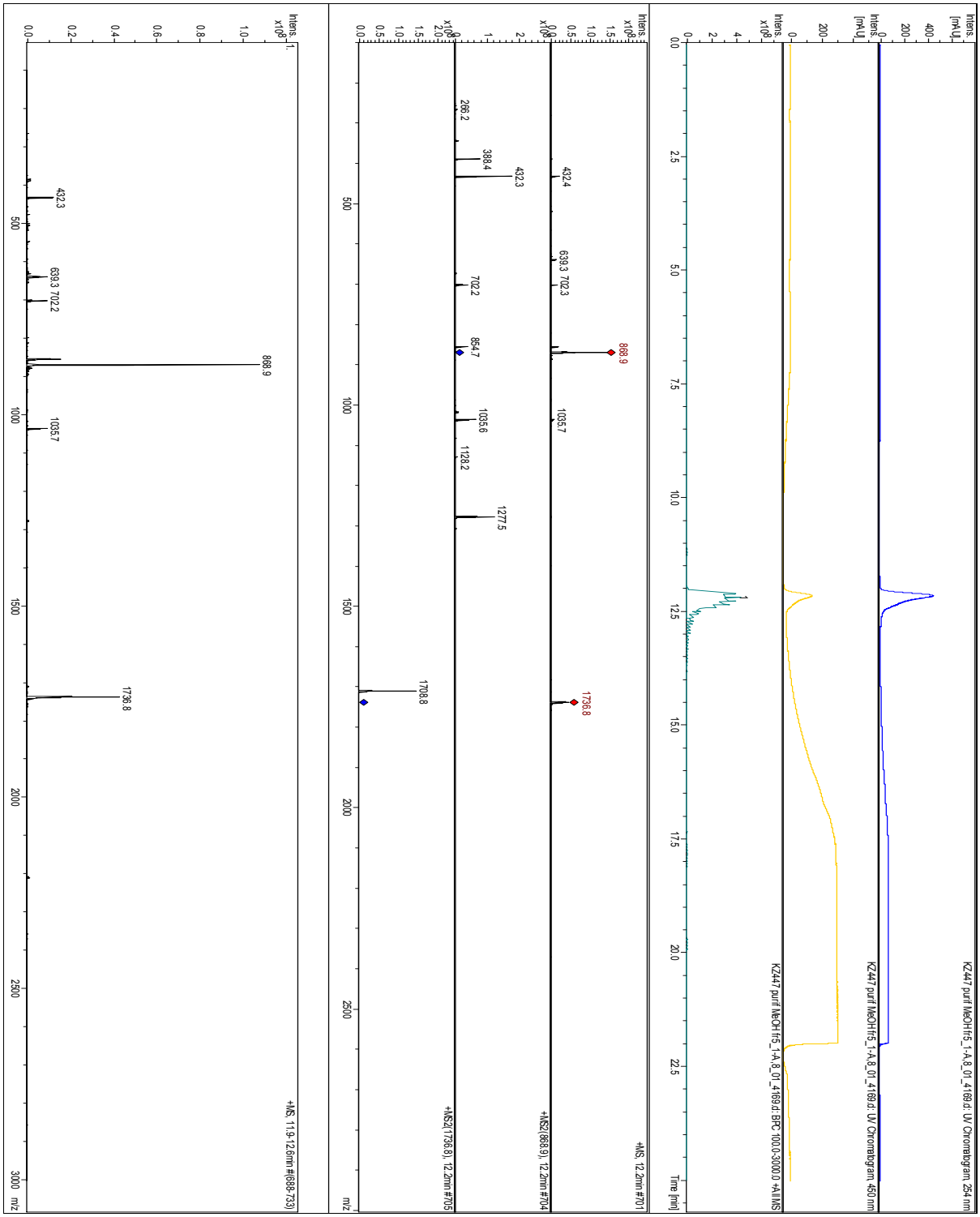


SI 13. Uptake of **17** by PC-3 cells after incubation for 12 H (*c* = 20 µmol), evaluated by fluorescence microscopy (green channel, blue channel, merged).

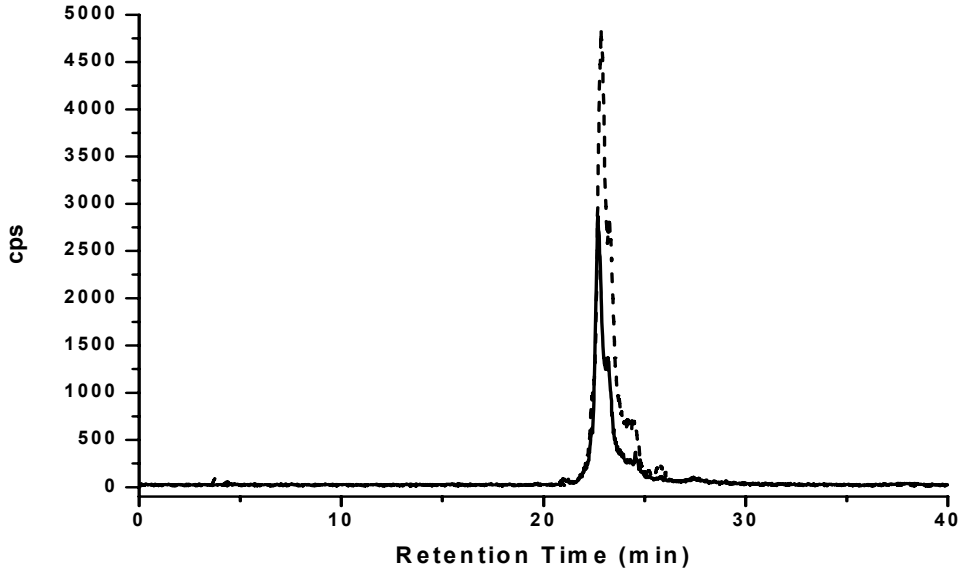


$[Re(16)(6)(CO)_3]^+$ (19) and $[^{99m}Tc(16)(6)(CO)_3]^+$ (20)

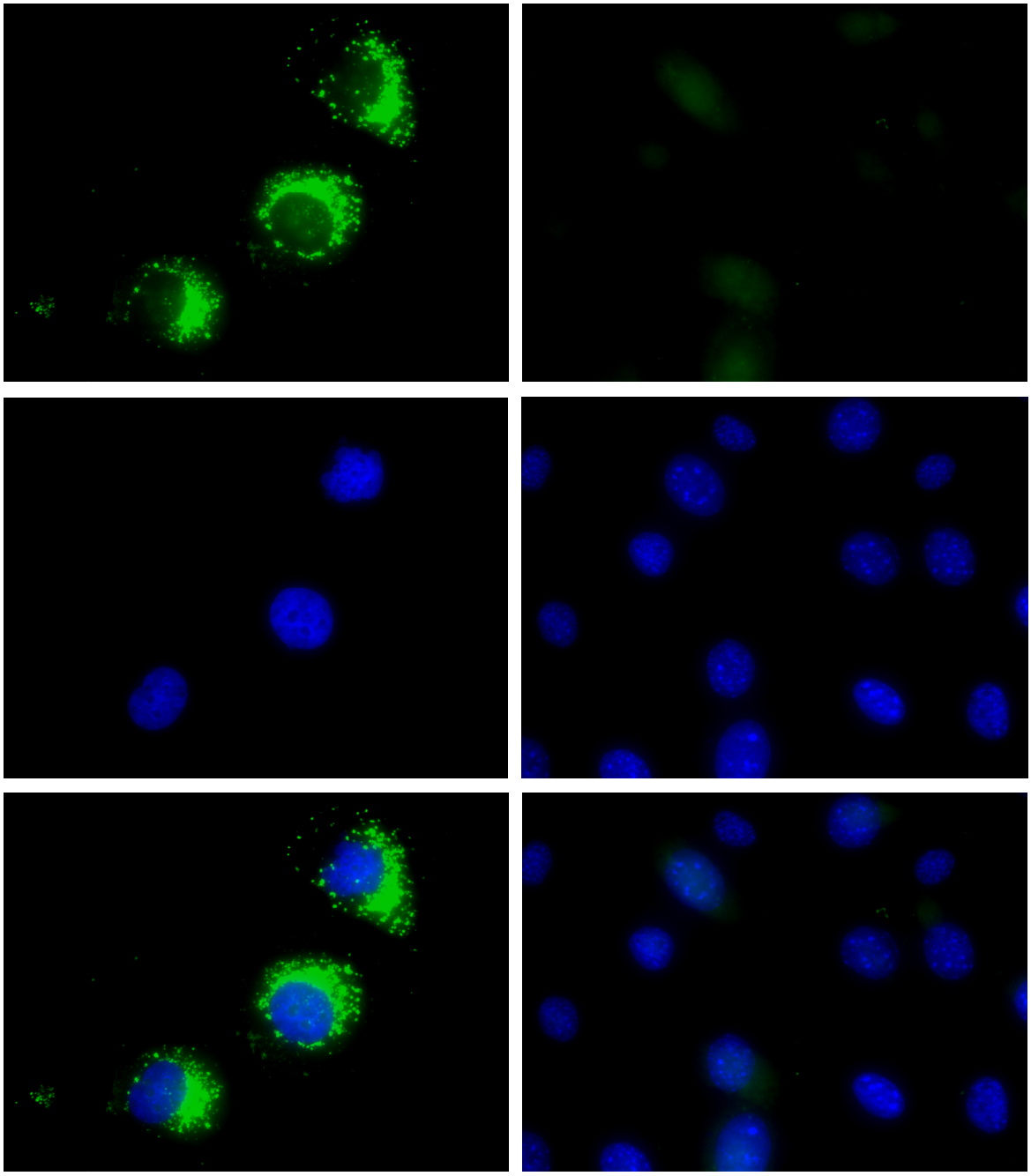
SI 14. The HPLC-MS (MS^2) trace of complex **19** (Gradient B).



SI 15. The radioHPLC trace of complex **20** (dashed line) and trace of complex **20** after incubation over PC3 cells in F12 medium for 6 H (solid line, Gradient D).



SI 16. Uptake of **19** by PC-3 (left) and B16 BL6 (right) cells after incubation for 6 H (c = 20 µmol), then 24 H fresh medium, evaluated by fluorescence microscopy (green channel, blue channel, merged). The pictures were taken with similar exposure time for the green channel.



Uptake of **19** by PC-3 (left) and B16 BL6 (right) cells. The pictures were taken without similar exposure time for the green channel (the exposure time for B16BL6 was considerably longer then for PC-3).