SUPPORTING INFORMATION

Centrally Active Multitarget Anti-Alzheimer Agents Derived from the Antioxidant Lead CR-6

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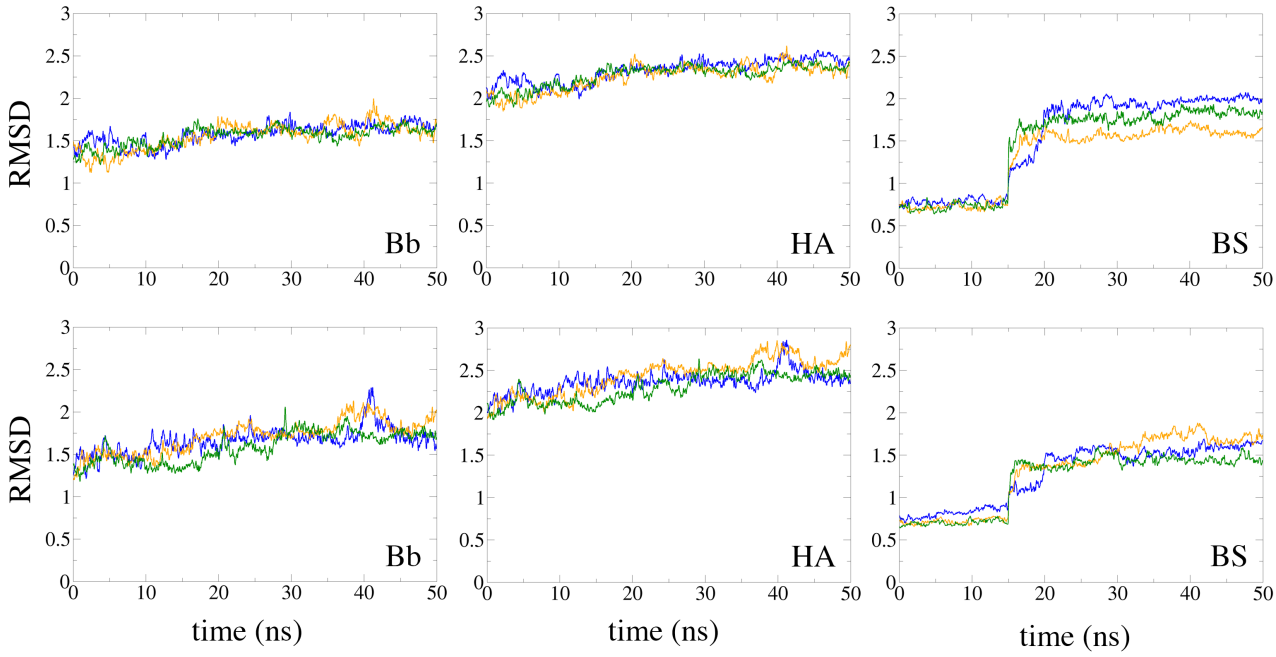


Figure S1. RMSD analysis of protein backbone (Bb), heavy atoms (HA) and residues in the binding site (BS) for the three independent MD simulations run for (*top*) (*R*)-**6b** and (*bottom*) (*S*)-**6b** bound to hAChE.

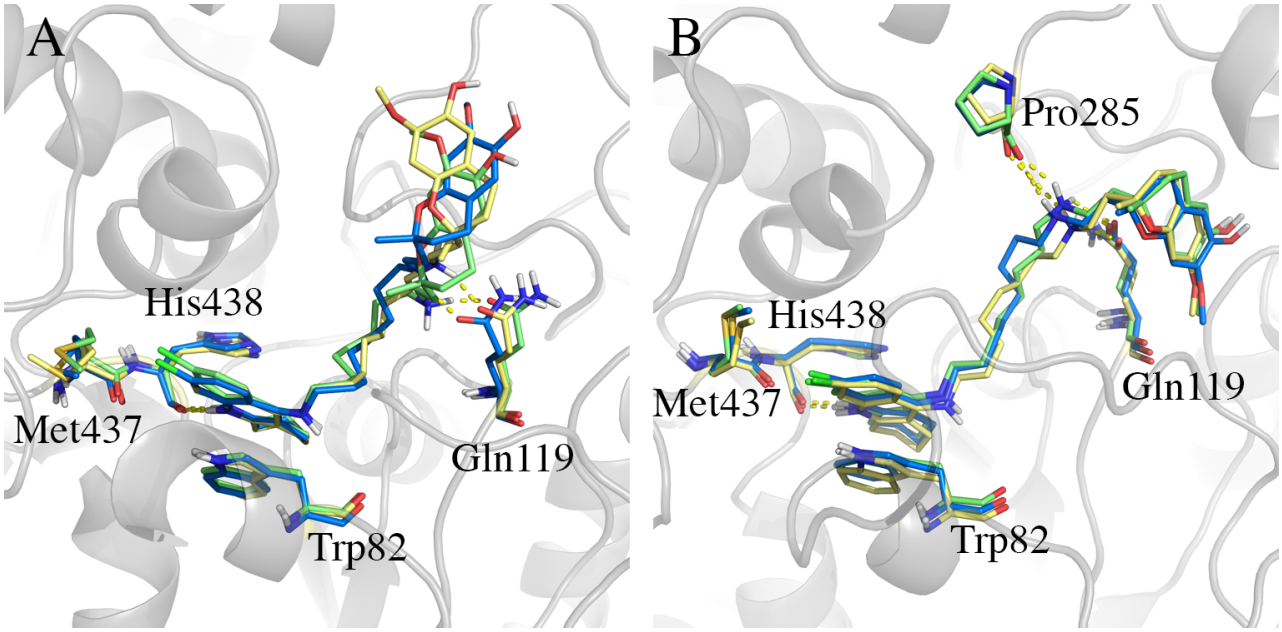


Figure S2. Protein–ligand complexes for (A) (*R*)-**6b** (A) and (B) (*S*)-**6b** bound to hBChE. MD-averaged complexes derived from three independent replicas run for each enantiomer are shown. Selected interactions are shown as yellow dashed lines.

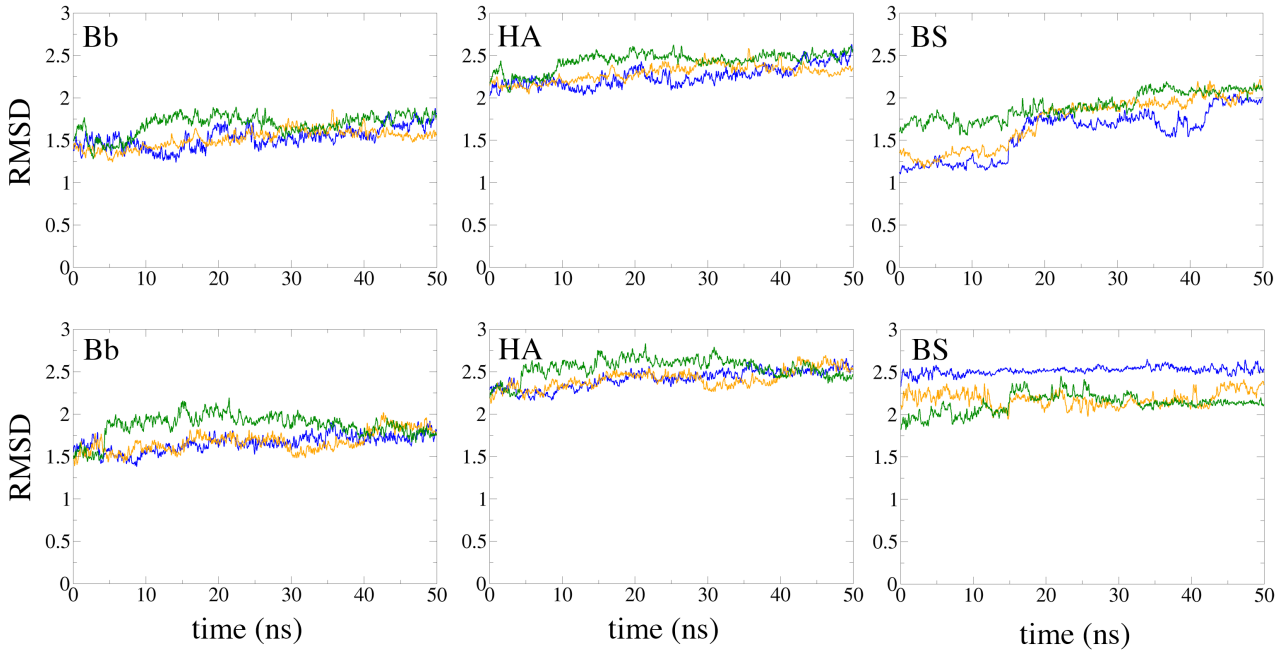


Figure S3. RMSD analysis of protein backbone (Bb), heavy atoms (HA) and residues in the binding site (BS) for the three independent MD simulations run for (*top*) (*R*)-**6b** and (*bottom*) (*S*)-**6b** bound to hBChE.

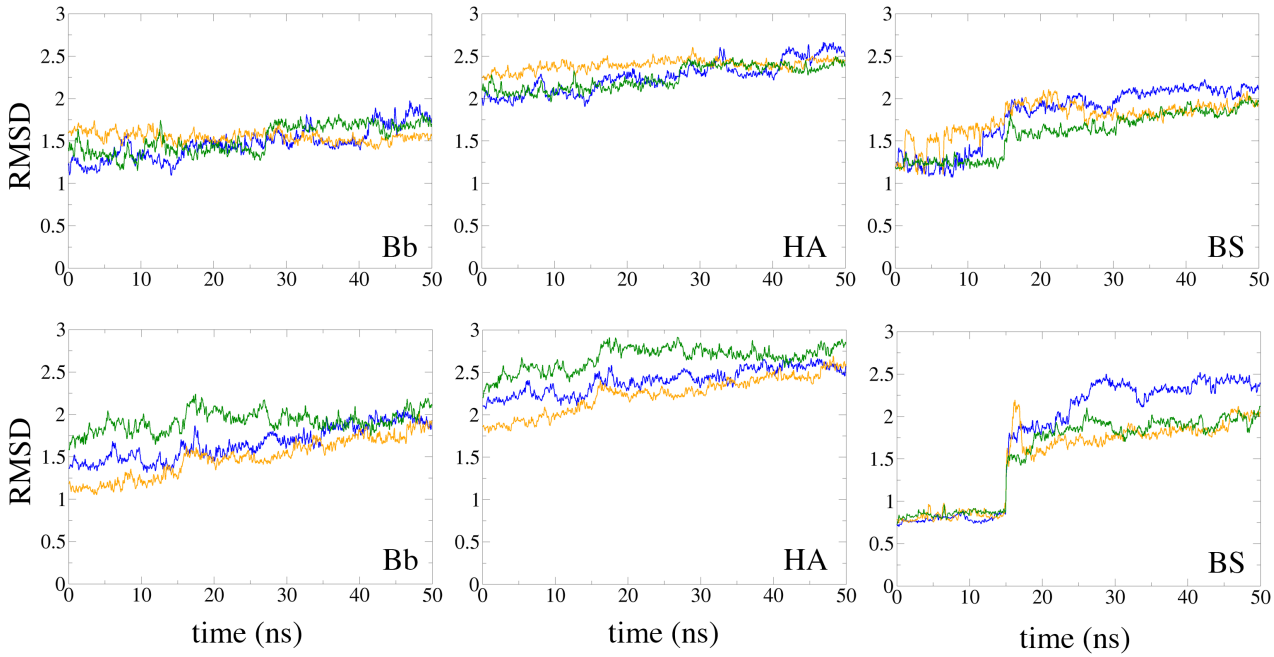


Figure S4. RMSD analysis of protein backbone (Bb), heavy atoms (HA) and residues in the binding site (BS) for the three independent MD simulations run for (*top*) (*R*)-**12a** and (bottom) (*S*)-**12a** bound to hBChE.

Table S1. Reported and Experimental Permeability (*Pe* 10^{-6} cm s^{-1}) Values in the PAMPA-BBB Assay of 14 Commercial Drugs Used for Assay Validation.

Compound	Bibliography value[a]	Experimental value (n=3) ± S.D.
Cimetidine	0.0	0.7 ± 0.1
Lomefloxacin	1.1	0.7 ± 0.1
Norfloxacin	0.1	0.9 ± 0.1
Ofloxacin	0.8	0.9 ± 0.1
Hydrocortisone	1.9	1.4 ± 0.1
Piroxicam	2.5	2.1 ± 0.1
Clonidine	5.3	6.5 ± 0.1
Corticosterone	5.1	6.7 ± 0.1
Imipramine	13.0	12.3 ± 0.1
Promazine	8.8	13.8 ± 0.3
Progesterone	9.3	16.8 ± 0.3
Desipramine	12.0	17.8 ± 0.1
Testosterone	17.0	25.6 ± 0.4
Verapamil	16.0	27.6 ± 0.5

[a] Taken from Di, L.; Kerns, E. H.; Fan, K.; McConnell, O. J.; Carter, G. T. High throughput artificial membrane permeability assay for blood-brain barrier. *Eur. J. Med. Chem.* **2003**, *38*, 223–232.

Table S2. Antibodies Used in Western Blot Studies.

Antibody	Host	Source/Catalog	WB dilution
ADAM10	Rabbit	Abcam	1:1000
BACE1	Rabbit	Abcam	1:1000
sAPPα	Rabbit	Covance	1:2000
sAPPβ	Rabbit	Covance	1:2000
NRF2	Rabbit	Cell Signaling	1:1000
Catalase	Rabbit	Abcam	1:1000
GPX1	Rabbit	Novus Biological	1:2000
GAPDH	Mouse	Millipore	1:5000

Table S3. Primers Used in qPCR Studies.

Target	Product size (bp)	Forward primer (5'-3')	Reverse primer (5'-3')
Adam10	54	GGGAAGAAATGCAAGCTGAA	CTGTACAGCAGGGTCCTTGAC
Bace1	128	AAGCTGCCGTCAAGTCCATC	GCGGAAGGACTGATTGGTGA
Hmox1	137	TGACACCTGAGGTCAAGCAC	GTCTCTGCAGGGGCAGTATC
iNOS	149	GAAGCGTTTCGGGATCTGAA	GAAGCGTTTCGGGATCTGAA
Actin	190	CAACGAGCGGTTCCGAT	GCCACAGGTTCCATACCCA

Table S4. Relative MS Peak Areas in the Identification of Metabolites in Human, Mouse, and Rat Liver Microsomes.[a]

	4b	**M1-M2**	**M3**	**M4**
HLM 60 min	857.611	2,642.590	2,220.529	1,103.357
HLM 40 min	5,207.464	6,172.992	7,965.484	732.660
HLM 20 min	35,515.574	4,020.527	4,858.155	291.824
HLM 10 min	45,318.645	2,091.129	2,030.786	248.183
HLM 0 min	49,641.340	1,155.693	452.668	183.363
MLM 60 min	30,259.844	2,589.753	3,069.414	255.269
MLM 40 min	42,688.520	2,559.730	1,906.893	261.439
MLM 20 min	49,374.652	1,572.661	834.982	185.967
MLM 10 min	50,930.750	1,269.113	596.771	184.181
MLM 0 min	48,678.672	1,115.285	386.902	159.195
RLM 60 min	16,605.932	5,805.929	6,085.236	375.713
RLM 40 min	30,782.176	5,457.648	5,528.240	317.334
RLM 20 min	45,931.359	2,319.008	1,858.295	205.586
RLM 10 min	48,575.387	1,495.265	798.099	211.890
RLM 0 min	46,383.352	[b]	449.619	140.174

[a] HLM, human liver microsomes; MLM, mouse liver microsomes; RLM, rat liver microsomes. Values are expressed as the mean of two independent experiments, each performed in triplicate. [b] Area under the limit of detection.

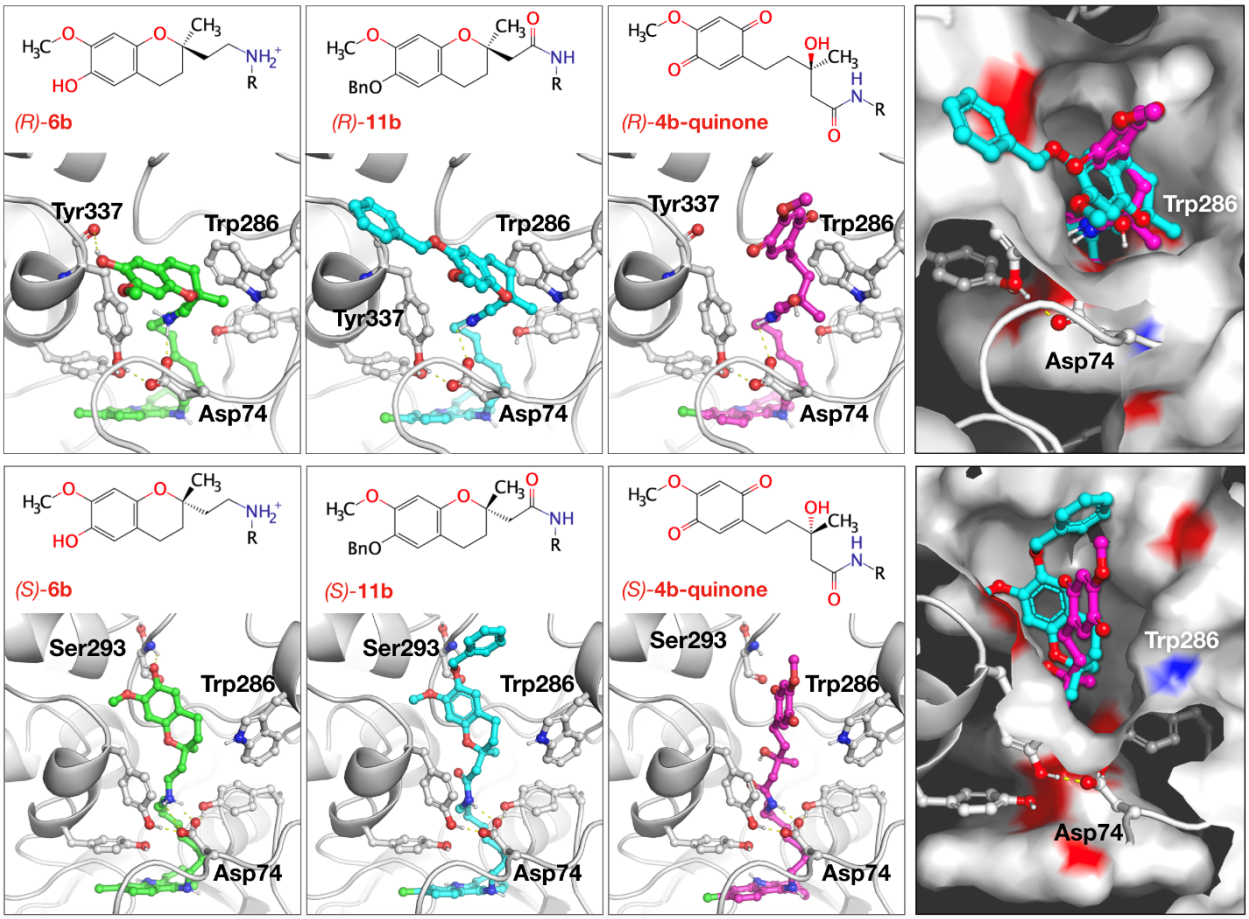
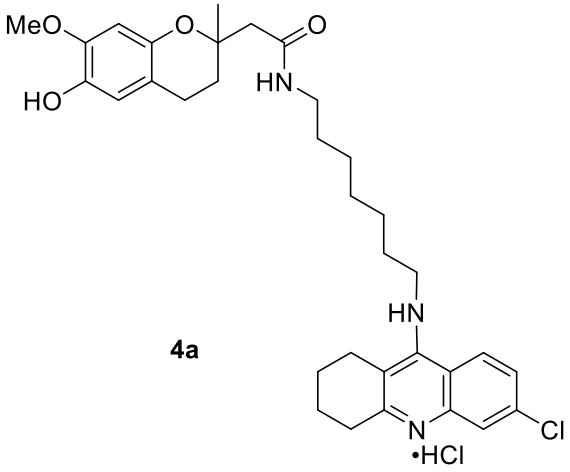


Figure S5. Representative snapshots of the complexes of (top) (*R*)-**6b** and (bottom) (*S*)-**6b** bound to hAChE, and the structurally related compounds **11b** and the *p*-benzoquinone metabolite of **4b**. For each complex selected residues relevant for interactions of the chlorotacrine unit at the catalytic site, and of the chroman scaffold (**6b**), the *O*-benzylated chroman scaffold (**11b**), and the ring-open *p*-benzoquinone (**4b** benzoquinone) at the peripheral site (characterized by Trp286) are highlighted. The panels on the right provide a representation of compounds **11b** and **4b** benzoquinone protruding from the gorge to the solvent, with the protein enclosed by the solvent-exposed surface.

Copies of ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100.6 MHz, CD_3OD) spectra and HPLC chromatograms of target compounds

N-{7-[(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)amino]heptyl}-2-(6-hydroxy-7-methoxy-2-methylchroman-2-yl)acetamide (**4a**)



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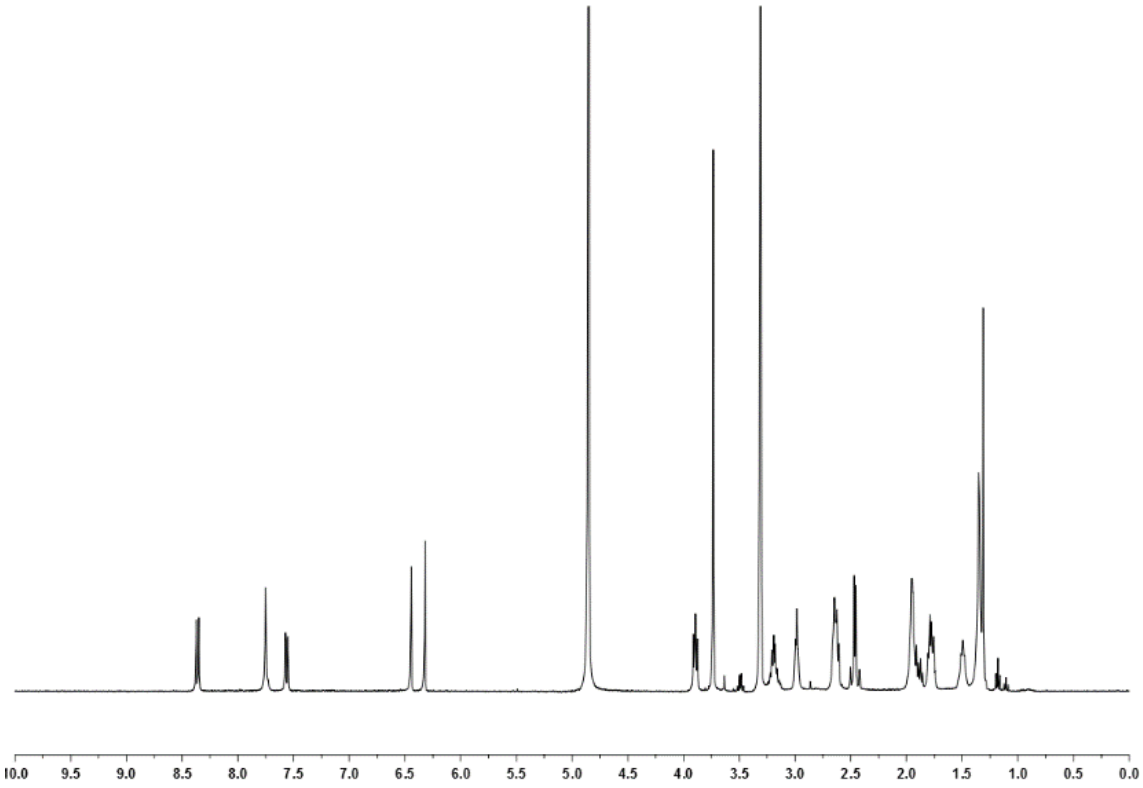

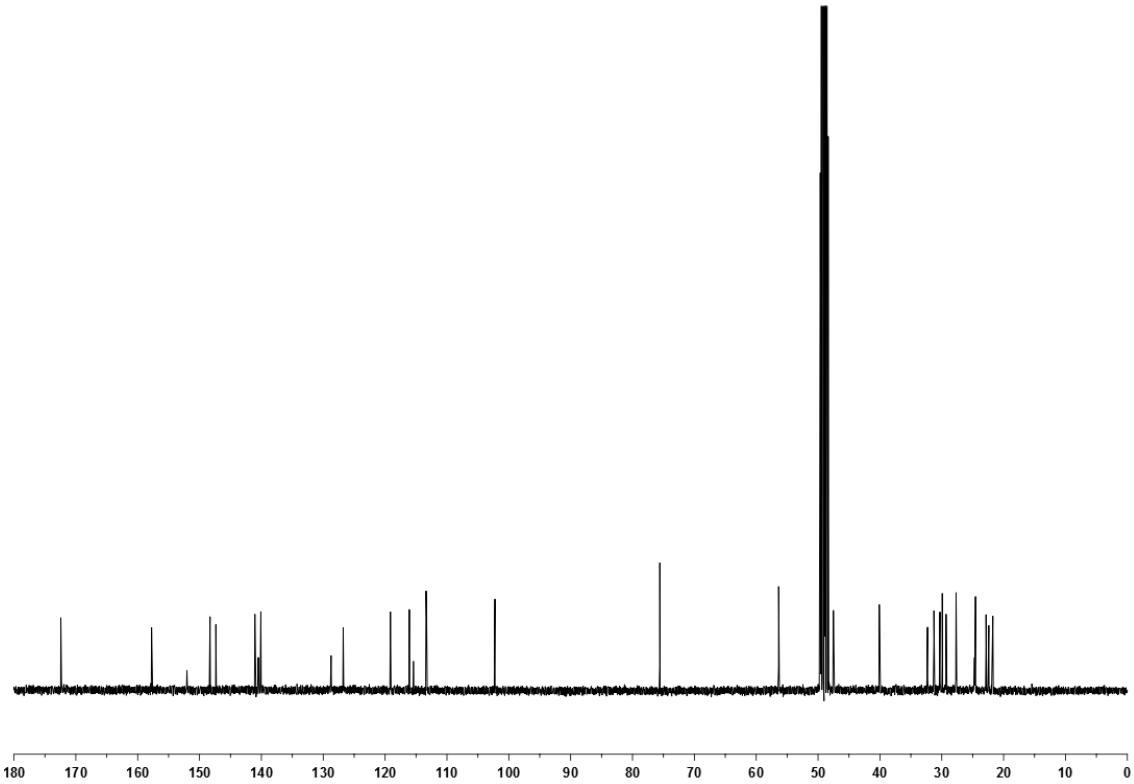
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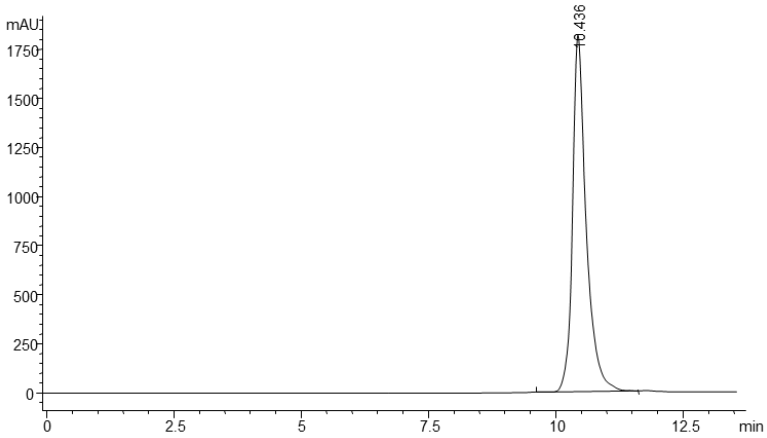
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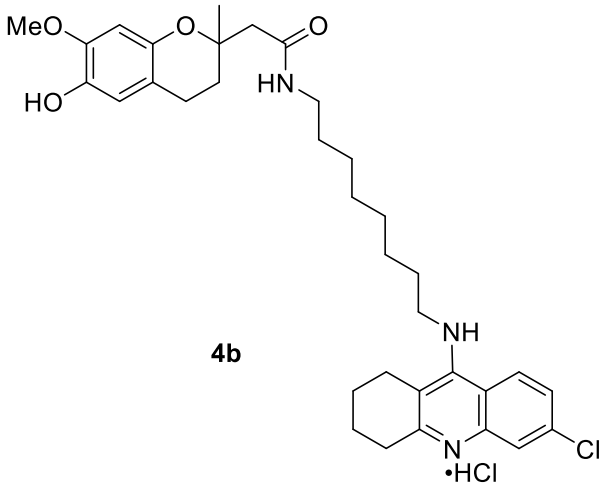
N-{8-[(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)amino]octyl}-2-(6-hydroxy-7-methoxy-2-methylchroman-2-yl)acetamide (**4b**)



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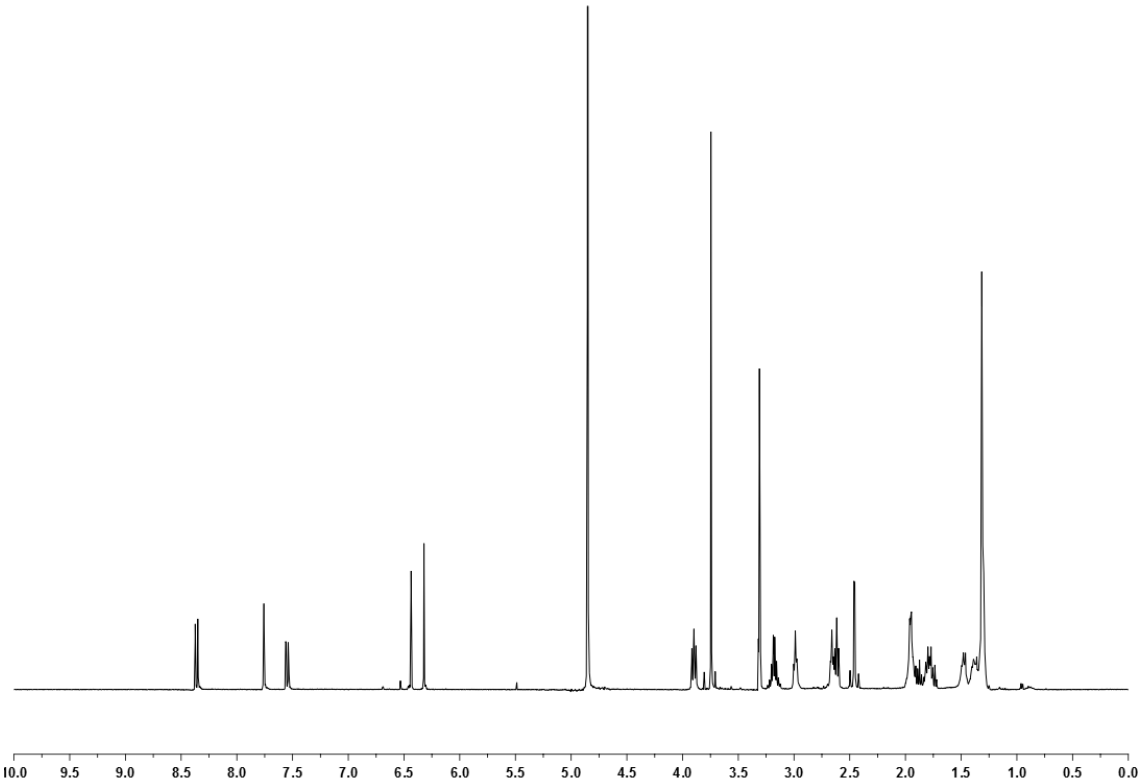

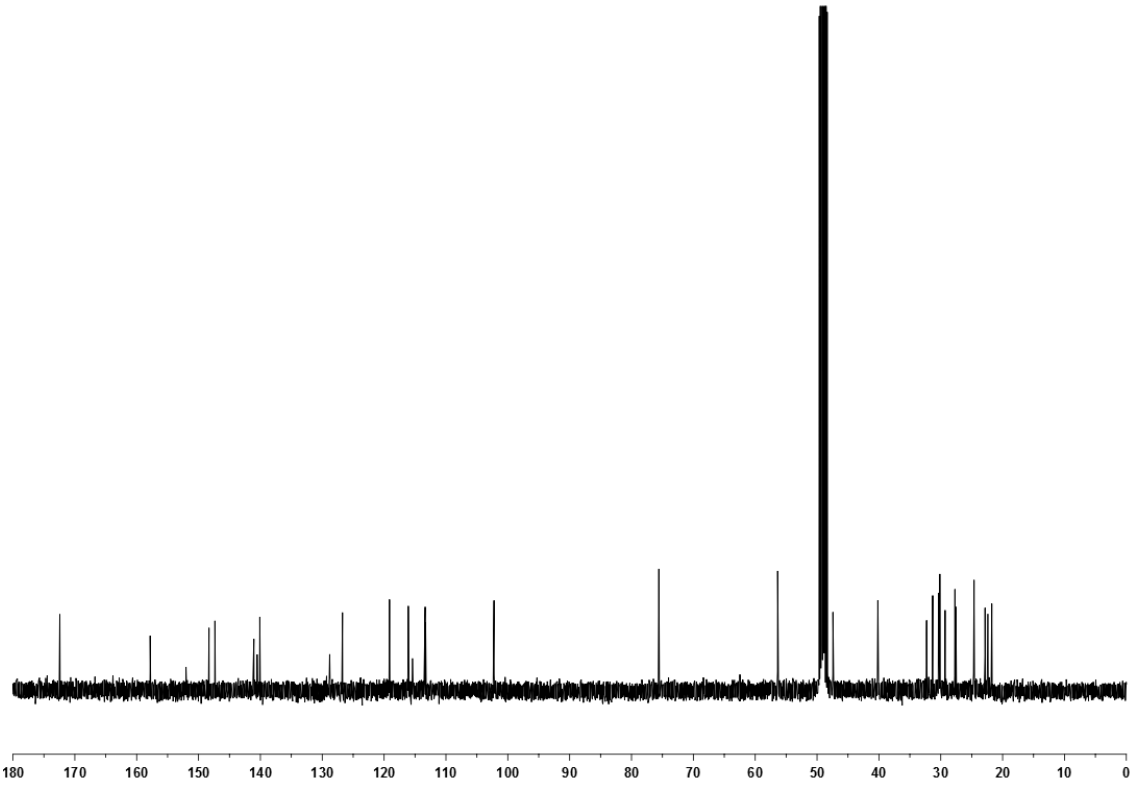
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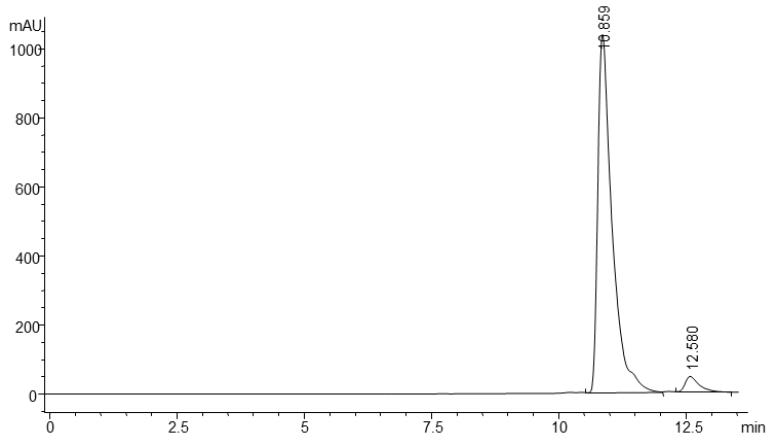
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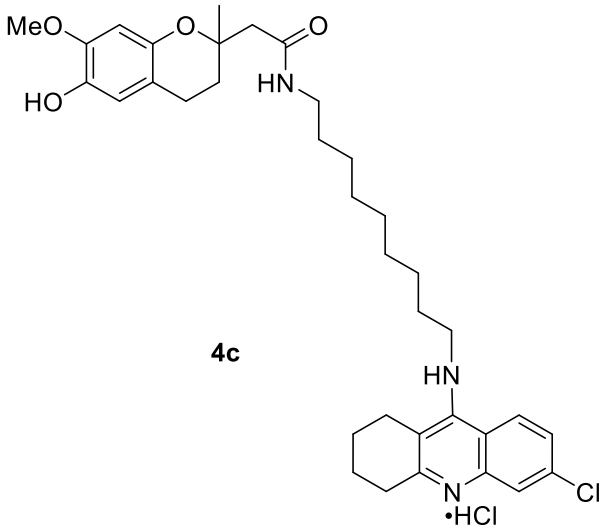
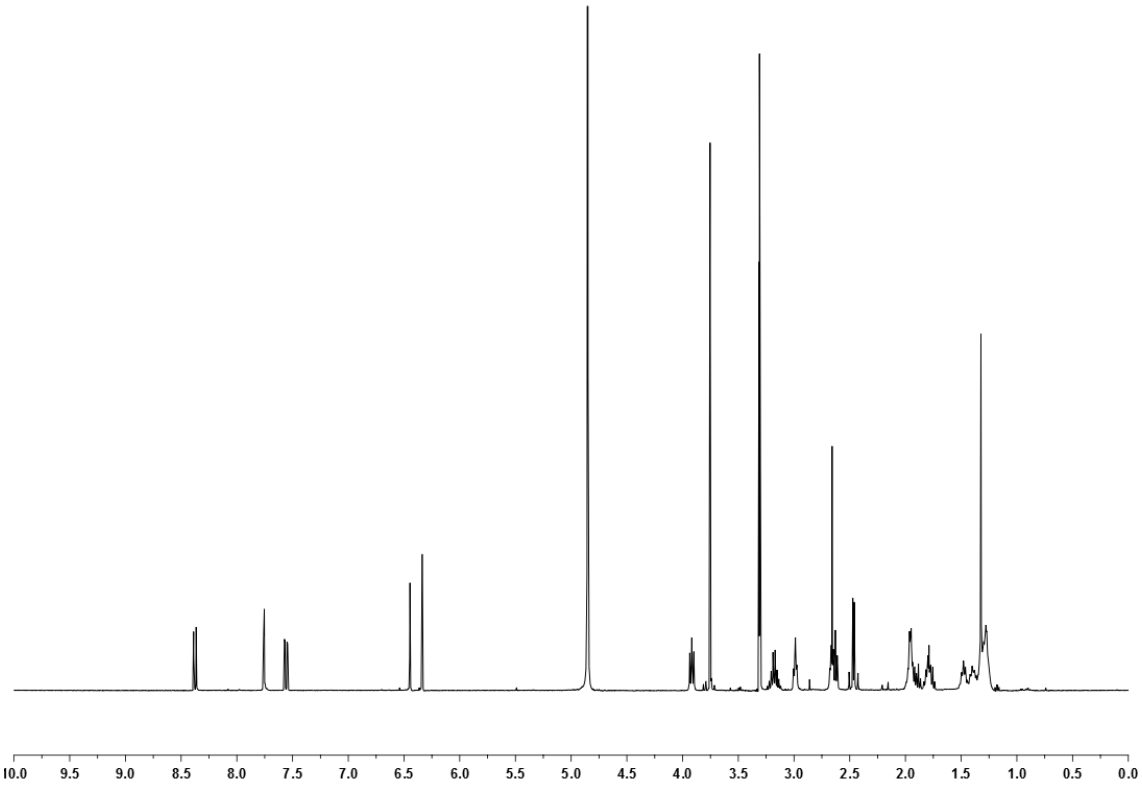
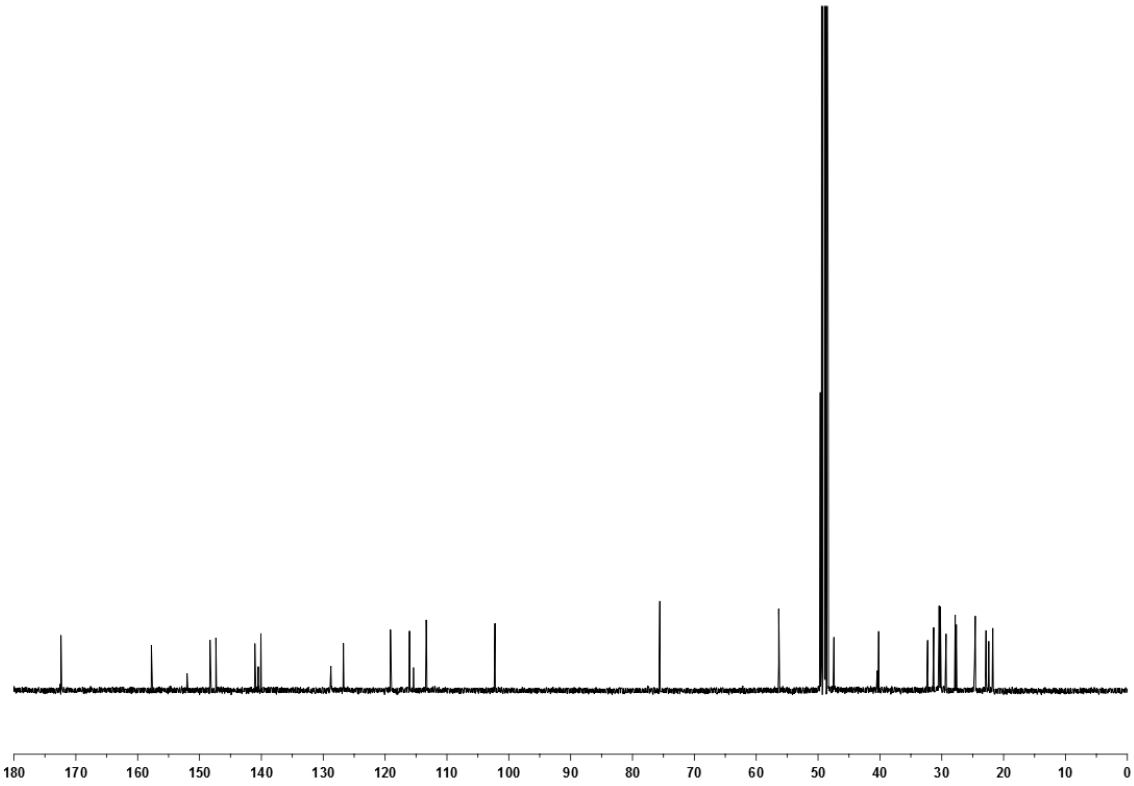
N-{9-[(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)amino]nonyl}-2-(6-hydroxy-7-methoxy-2-methylchroman-2-yl)acetamide (**4c)**



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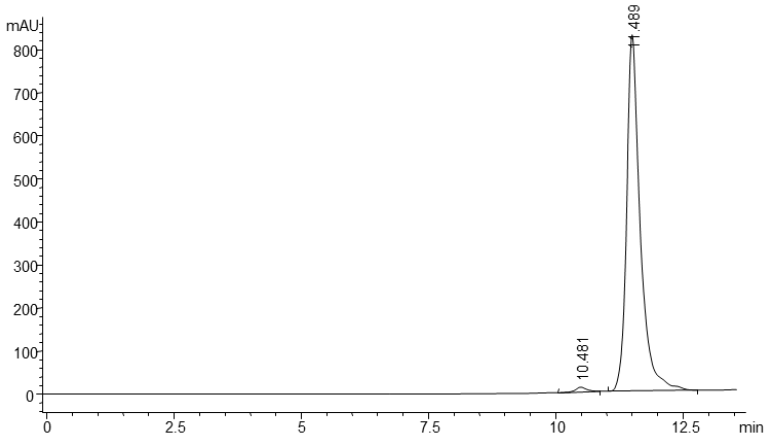
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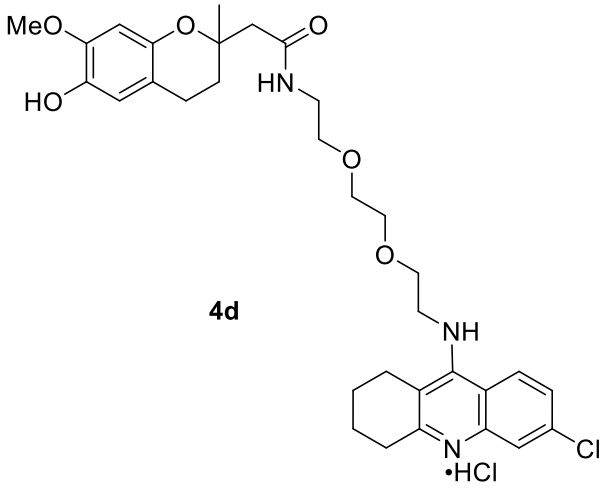
N-{8-[(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)amino]-3,6-dioxaoctyl}-2-(6-hydroxy-7-methoxy-2-methylchroman-2-yl)acetamide (**4d**)



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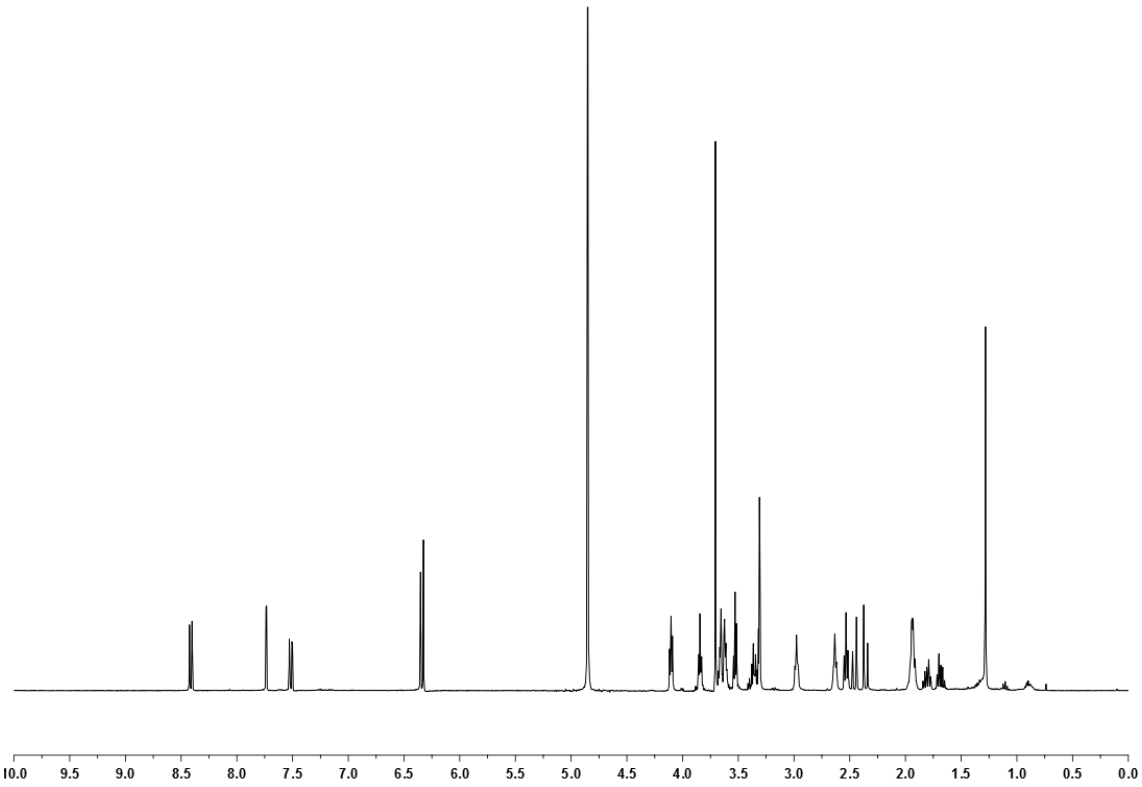

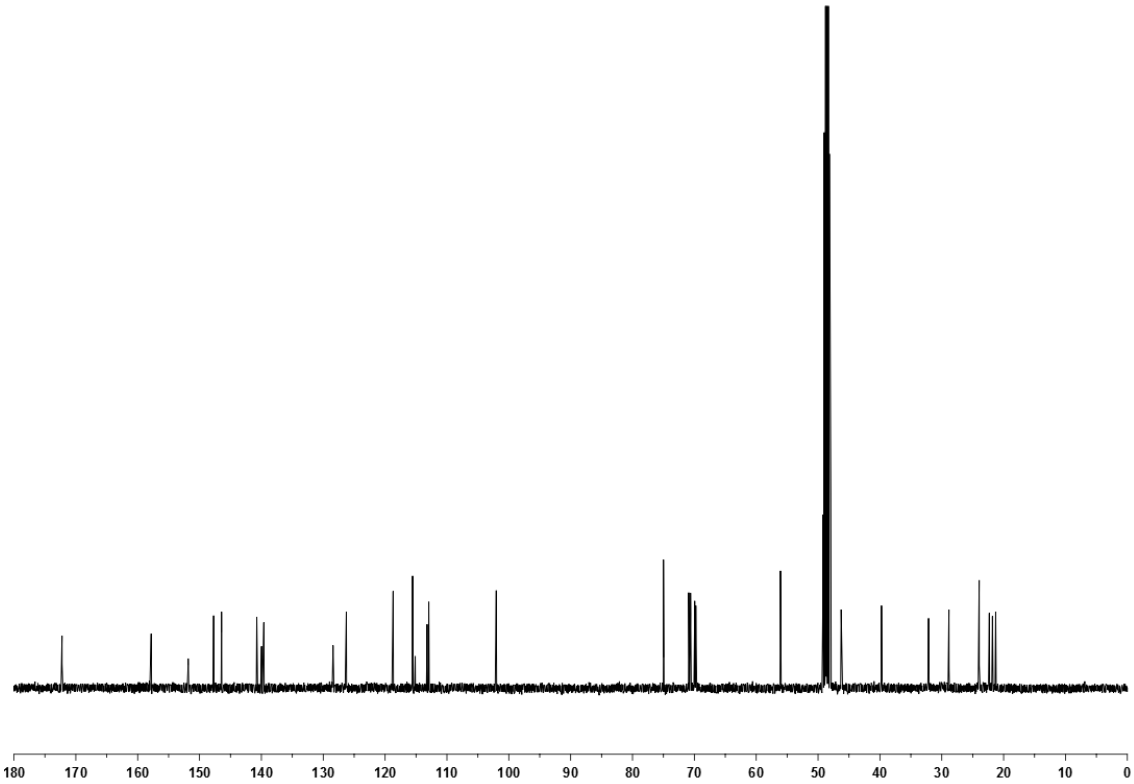
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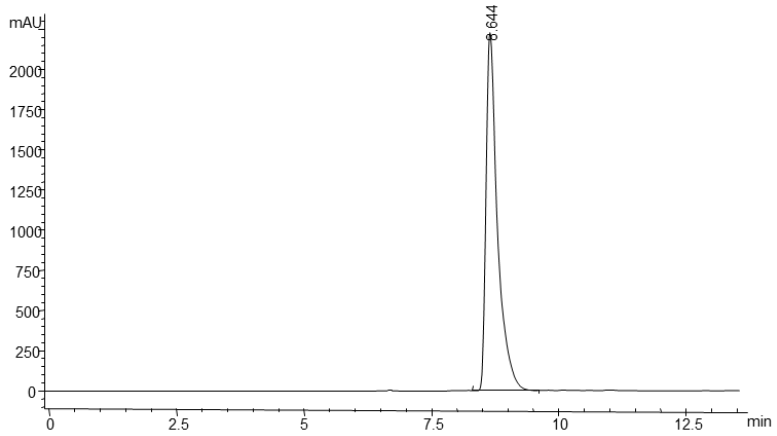
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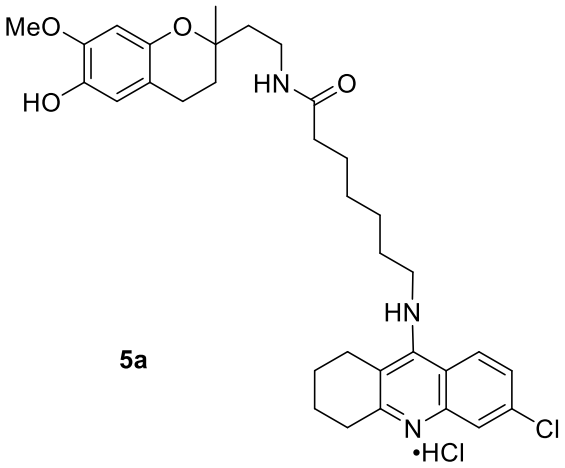
7-[(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)amino]-*N*-[2-(6-hydroxy-7-methoxy-2-methylchroman-2-yl)ethyl]heptanamide (**5a**)



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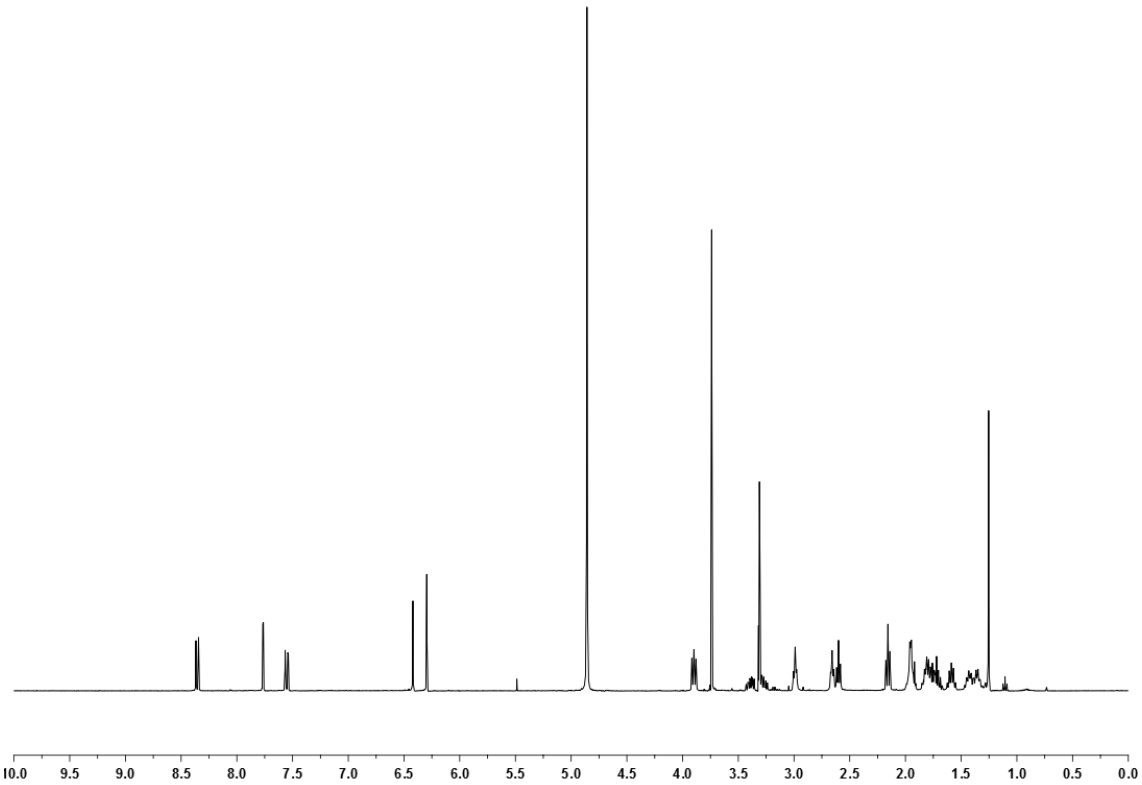

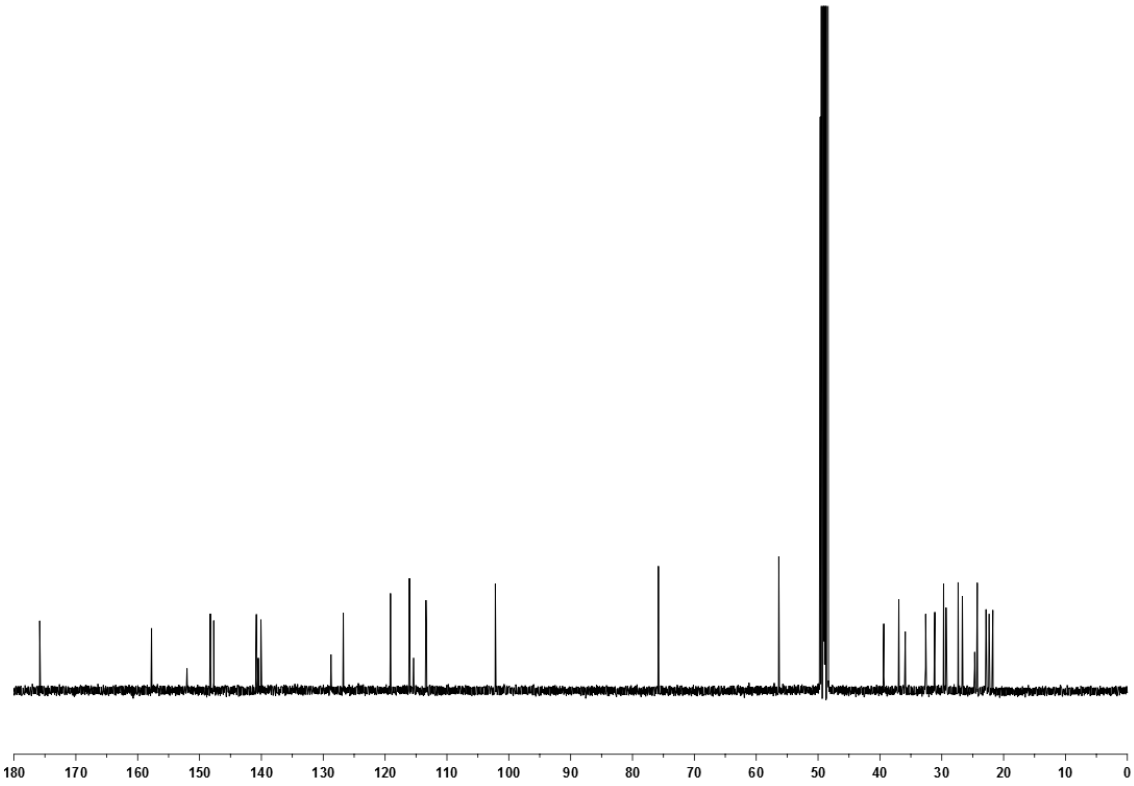
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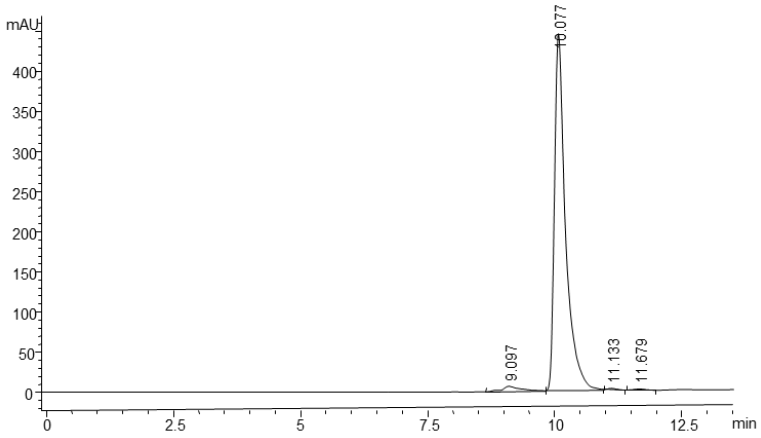
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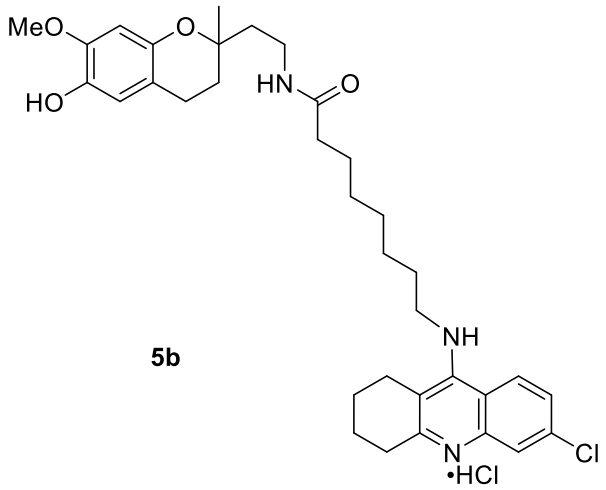
8-[(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)amino]-*N*-[2-(6-hydroxy-7-methoxy-2-methylchroman-2-yl)ethyl]octanamide (**5b**)



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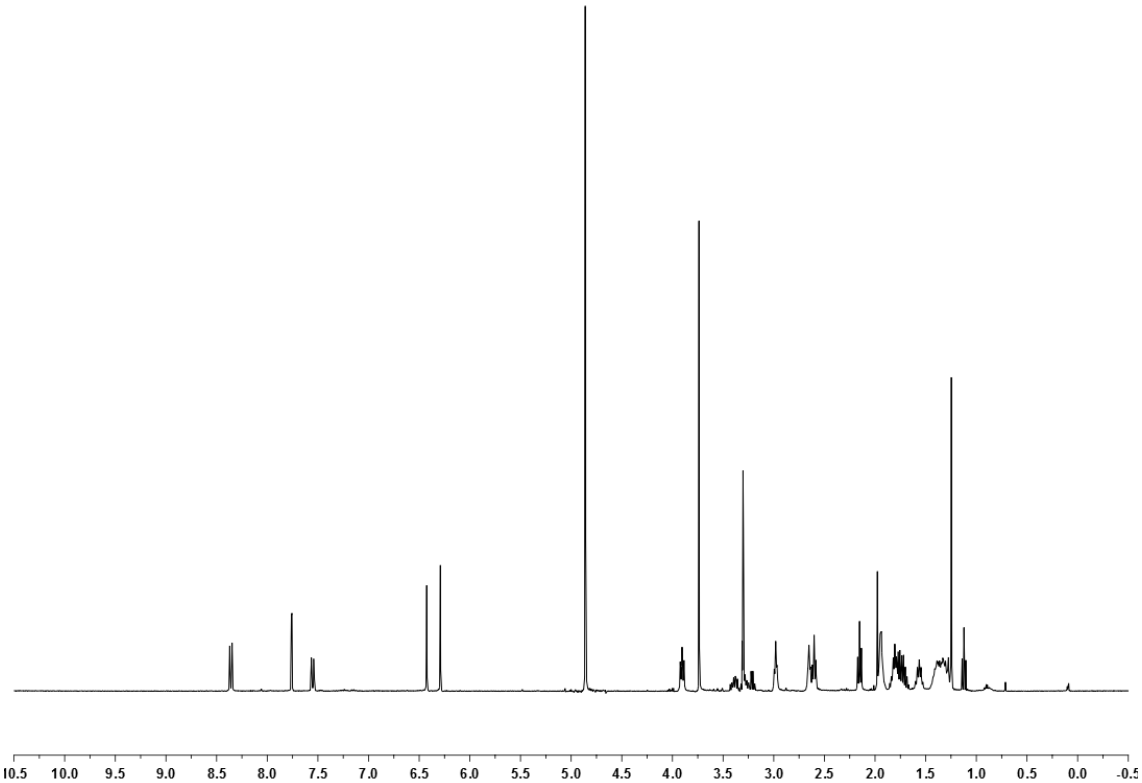

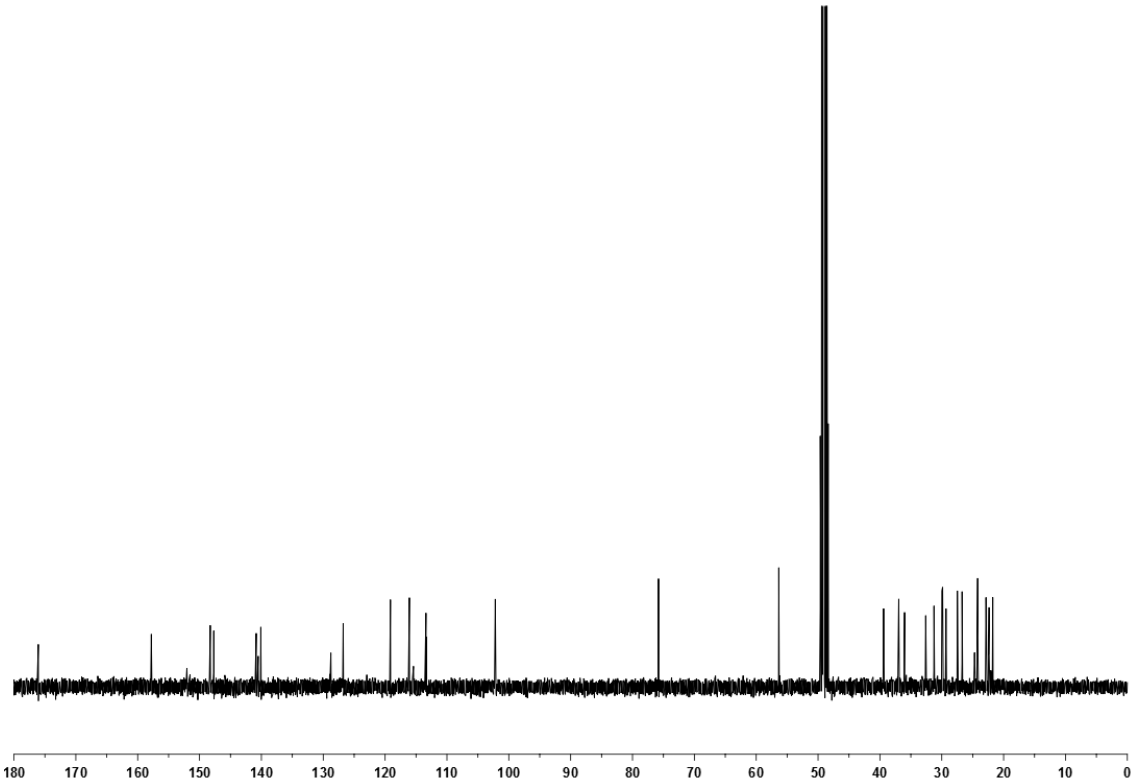
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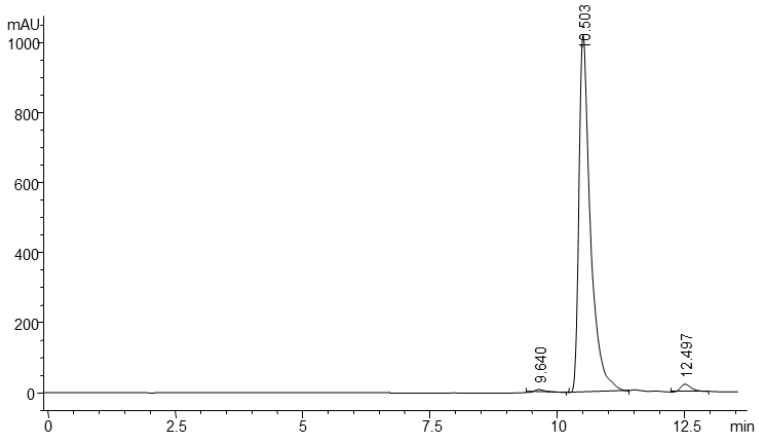
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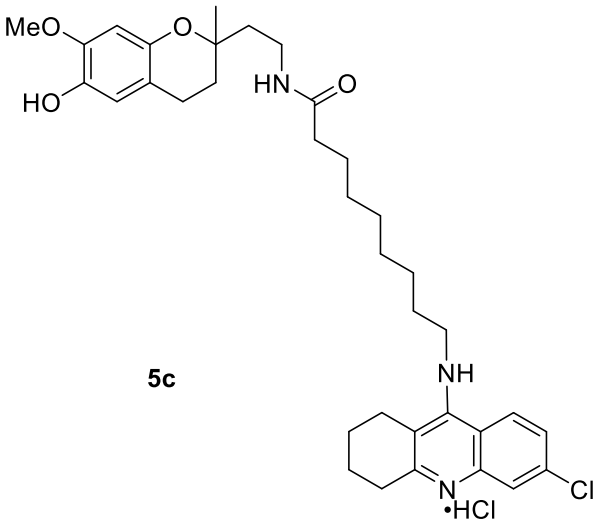
9-[(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)amino]-*N*-[2-(6-hydroxy-7-methoxy-2-methylchroman-2-yl)ethyl]nonanamide (**5c**)



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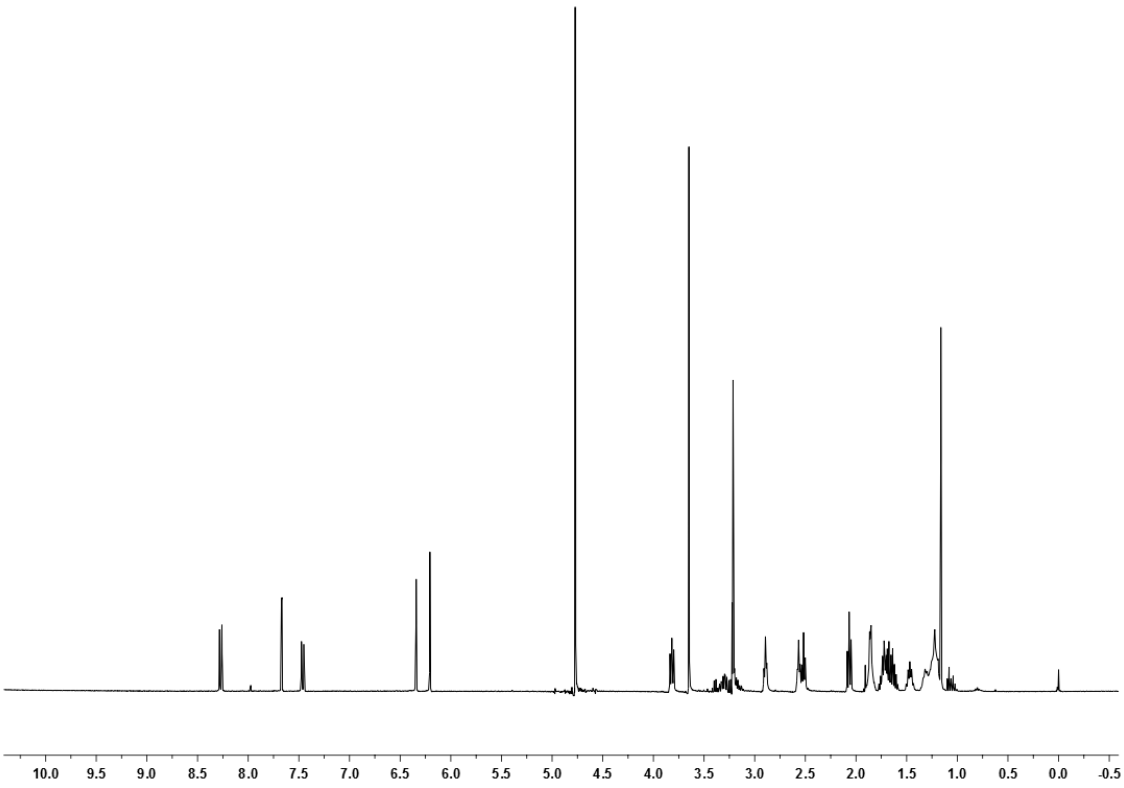

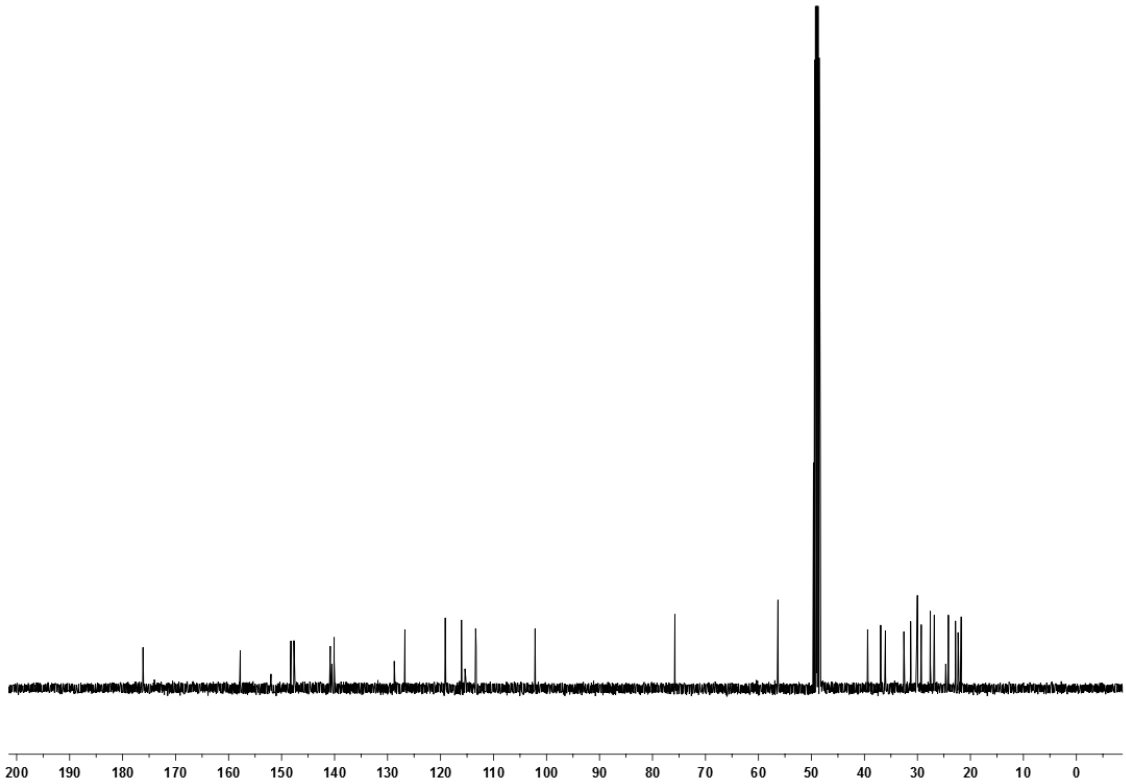
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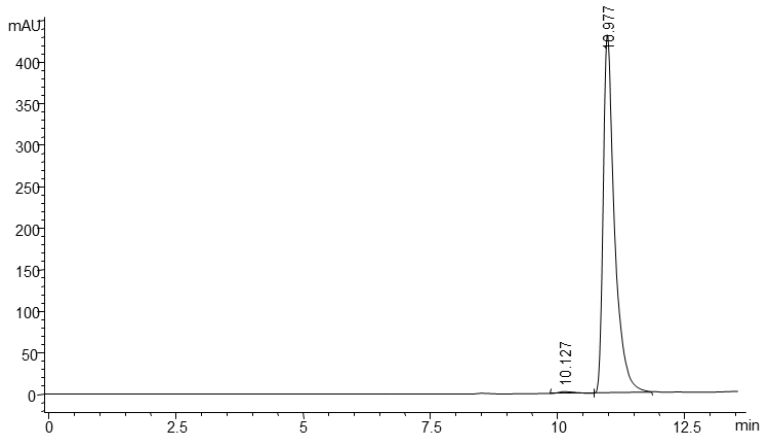
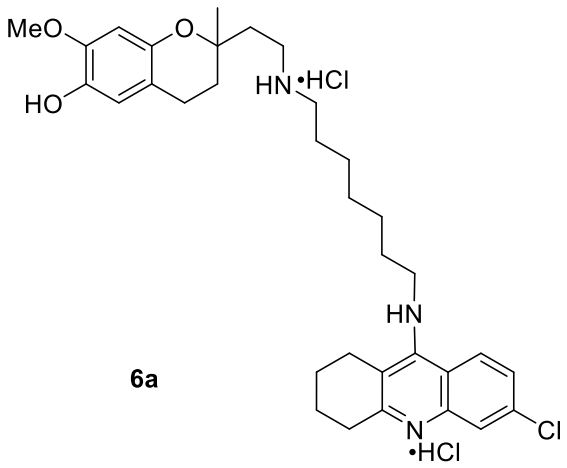
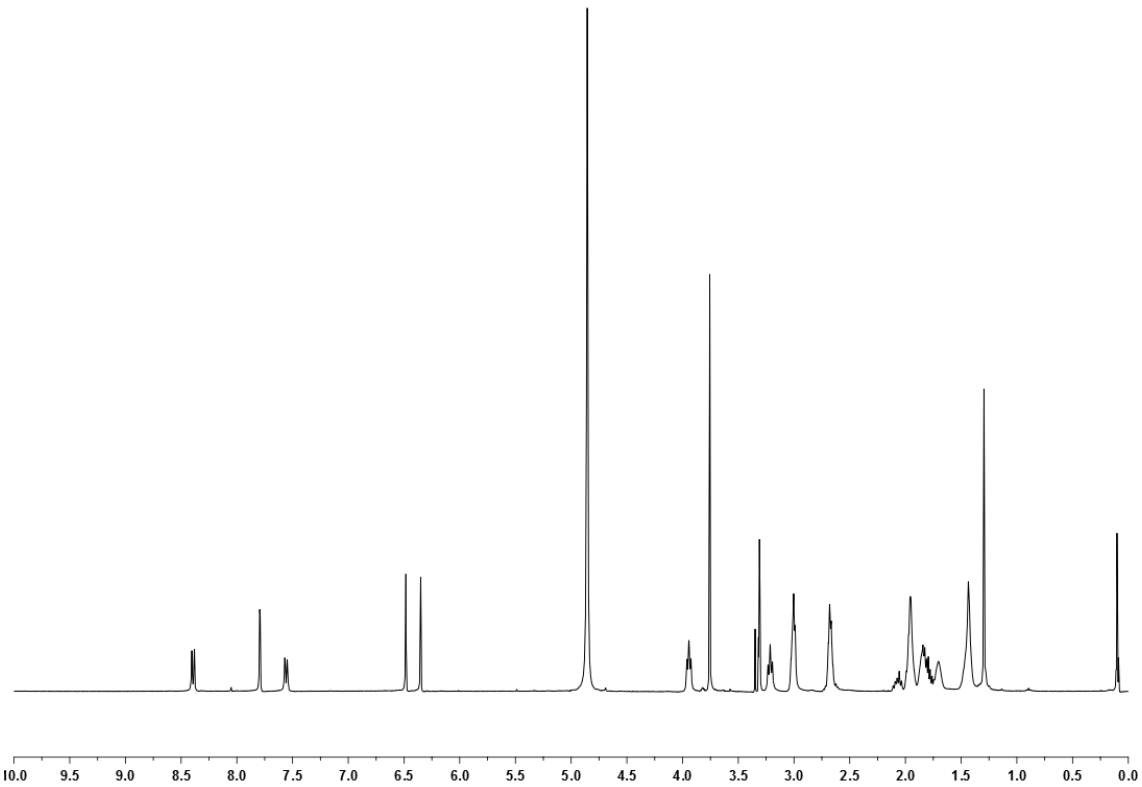
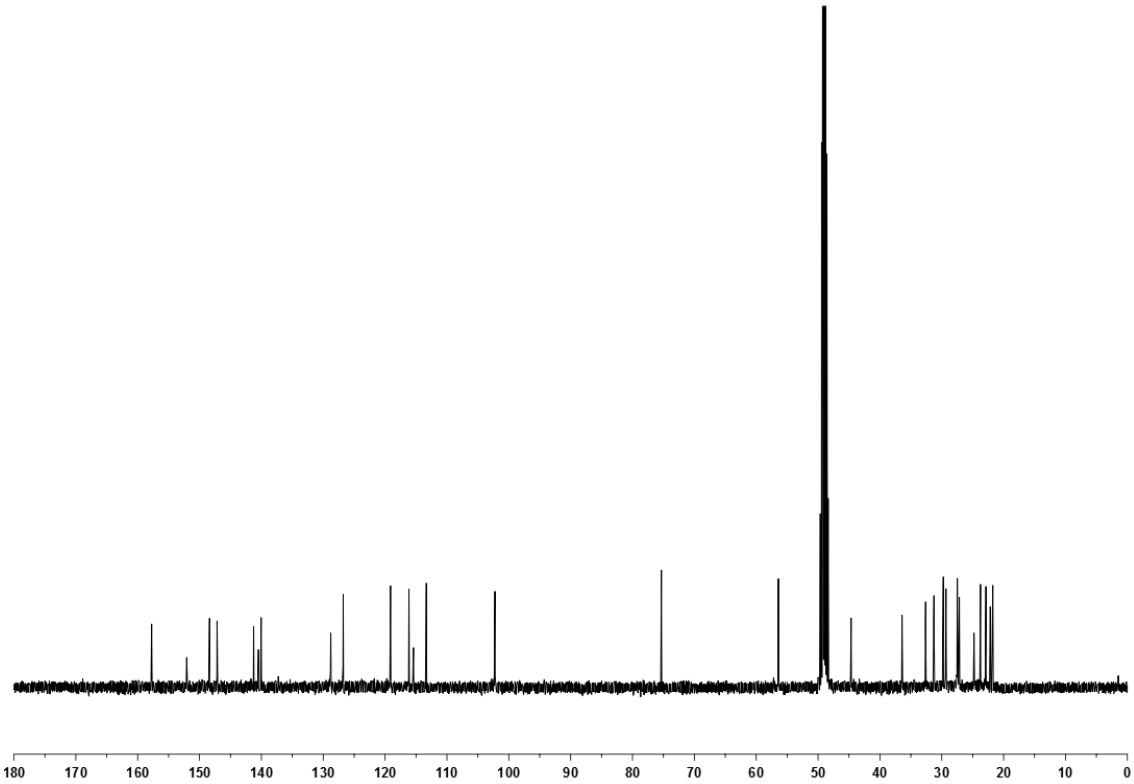

mAU
400
350
300
250
200
150
100
50
0
10.977
10.127
0
2.5
5
7.5
10
12.5
min

N-(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)-*N'*-[2-(6-hydroxy-7-methoxy-2-methylchroman-2-yl)ethyl]heptane-1,7-diamine (**6a**)



6a

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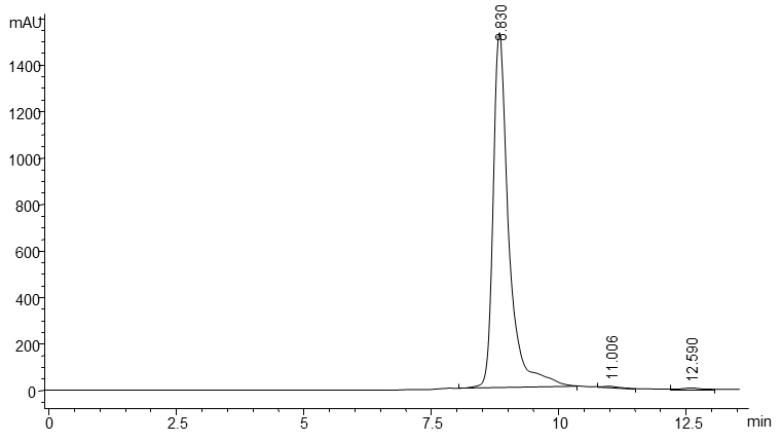
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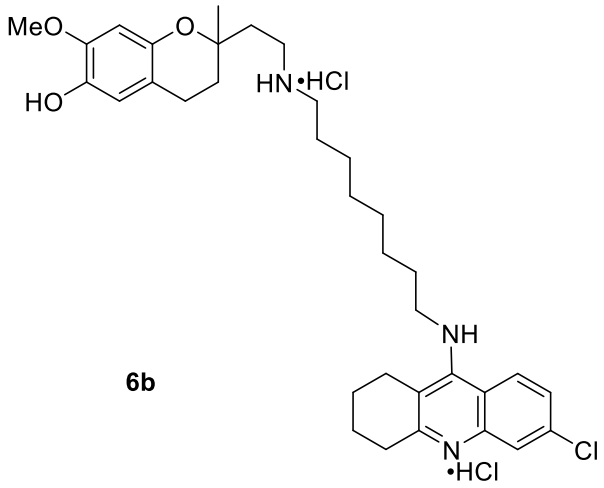
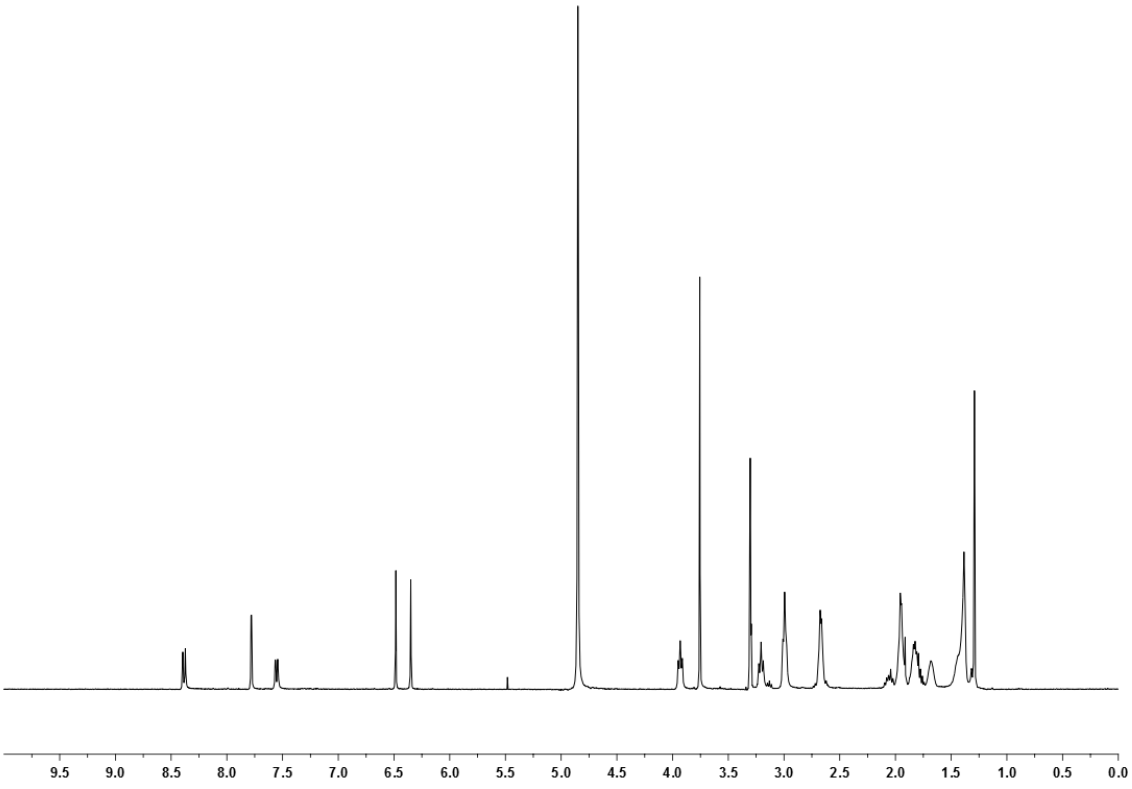
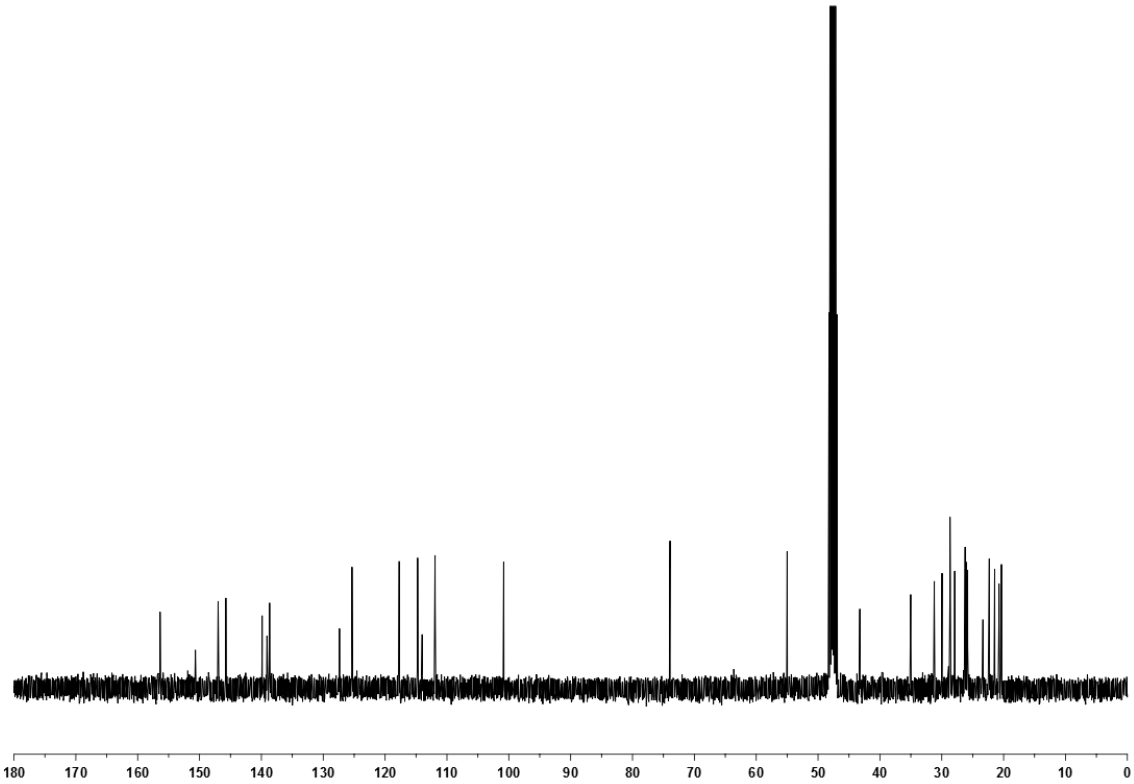
1400
1200
1000
800
600
400
200
0
8.830
11.006
12.590
0
2.5
5
7.5
10
12.5
min

N-(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)-*N'*-[2-(6-hydroxy-7-methoxy-2-methylchroman-2-yl)ethyl]octane-1,8-diamine (**6b**)



6b

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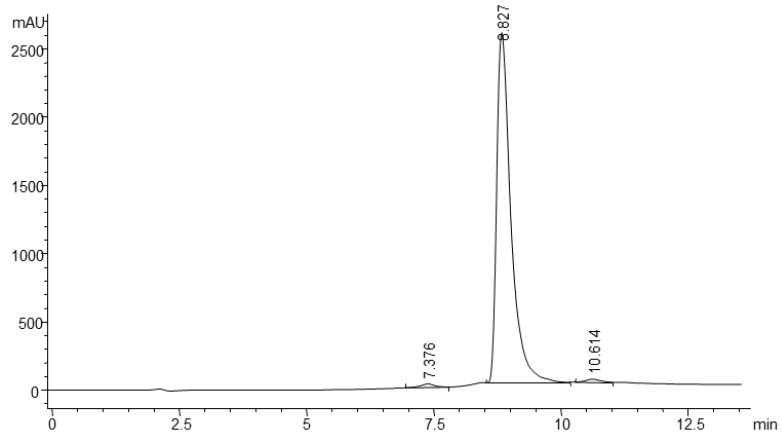
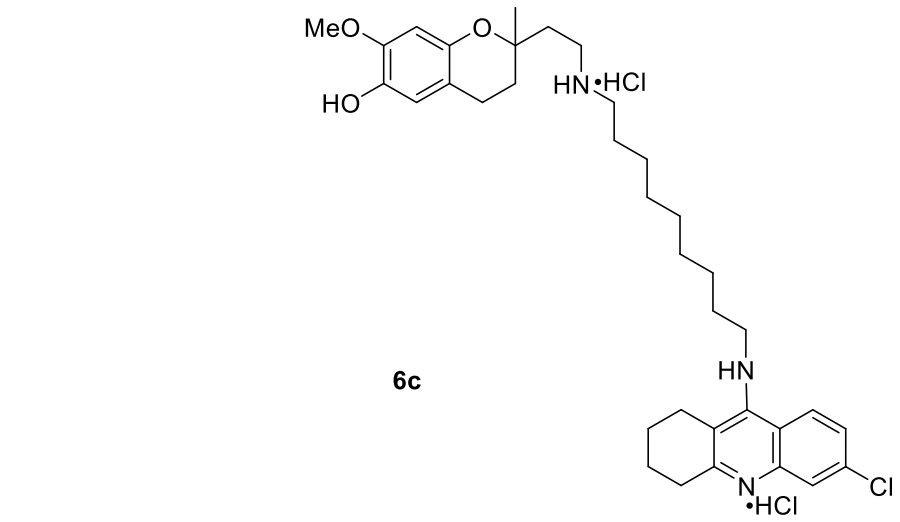
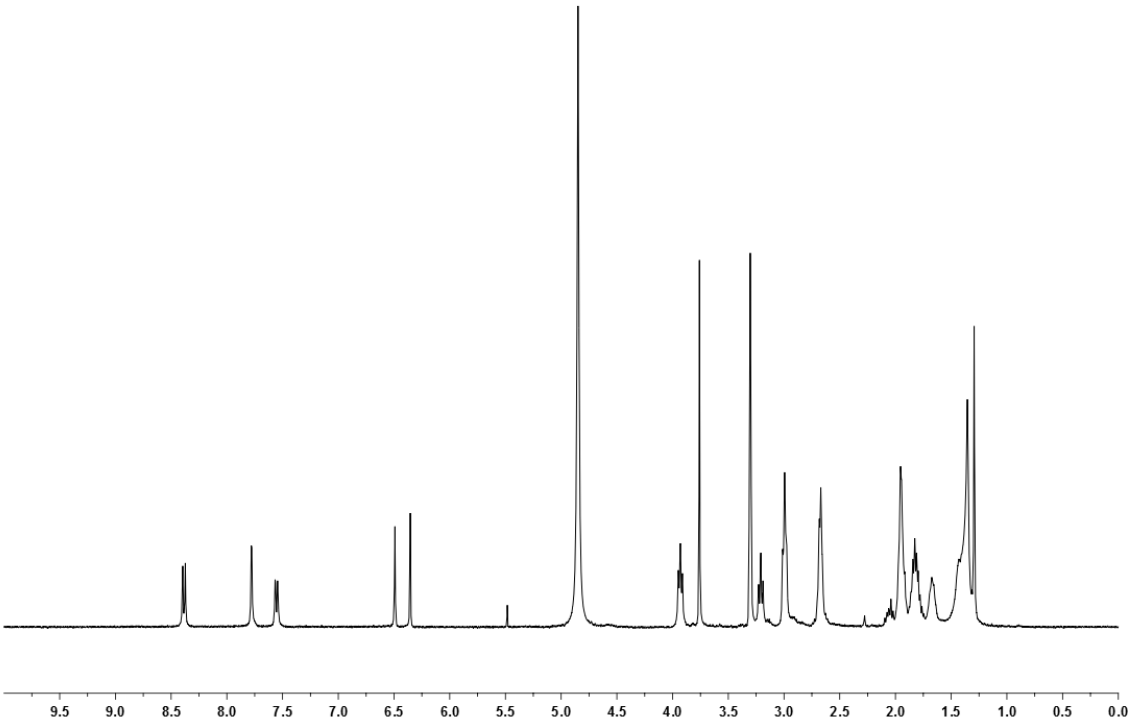
30
20
10
0

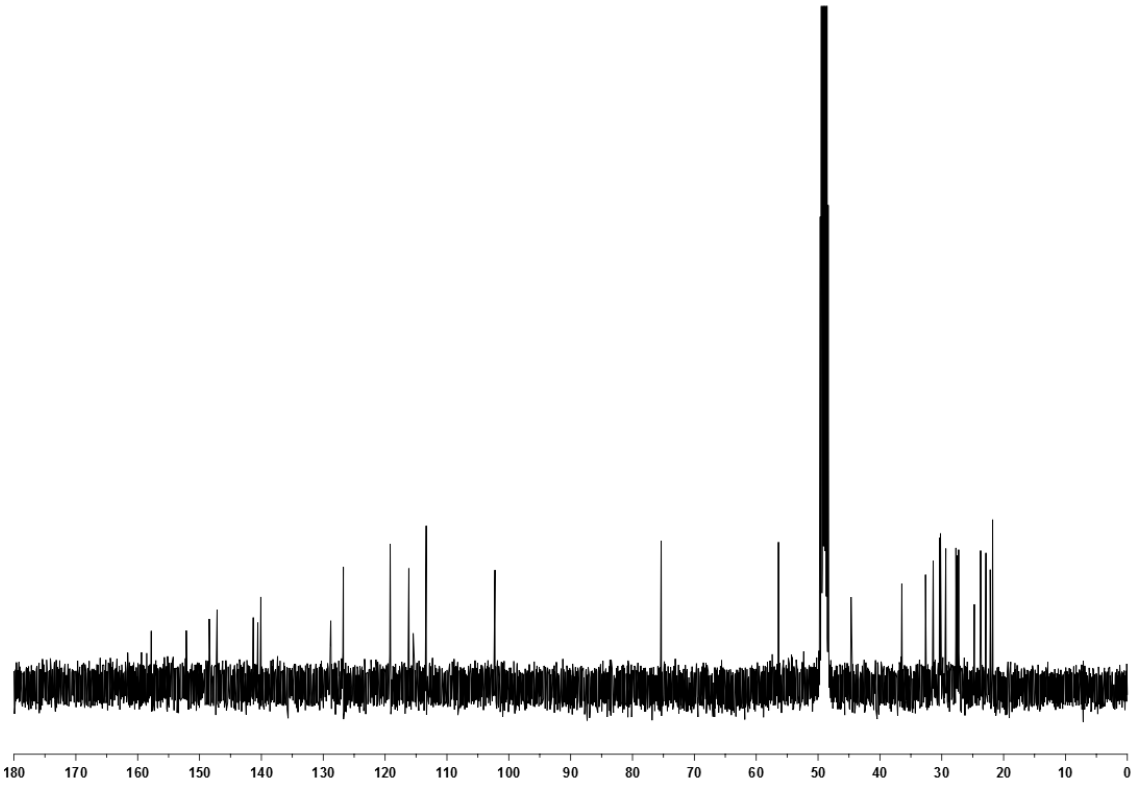

mAU
2500
2000
1500
1000
500
0
7.376
8.827
10.614
0
2.5
5
7.5
10
12.5
min

N-(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)-*N'*-[2-(6-hydroxy-7-methoxy-2-methylchroman-2-yl)ethyl]nonane-1,9-diamine (**6c**)



6c

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10
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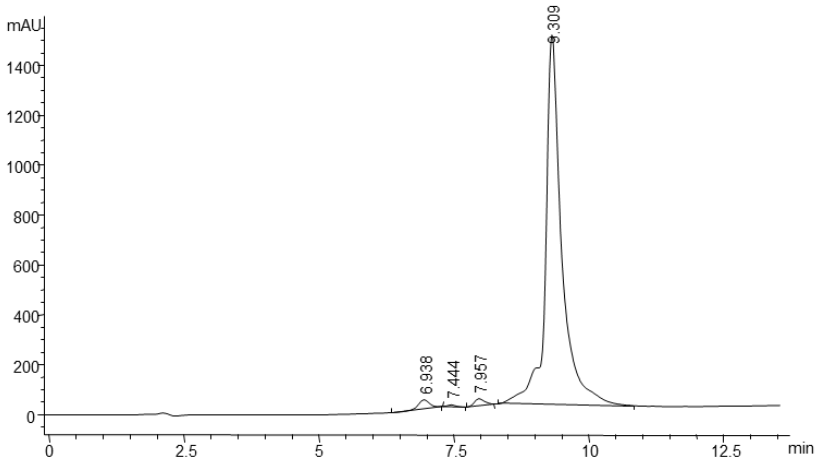
mAU
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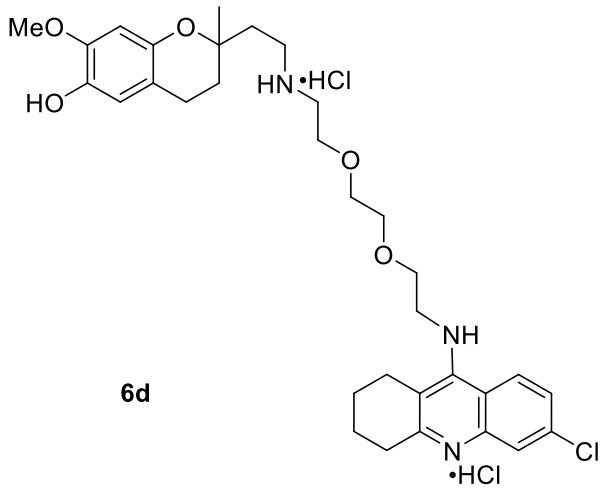
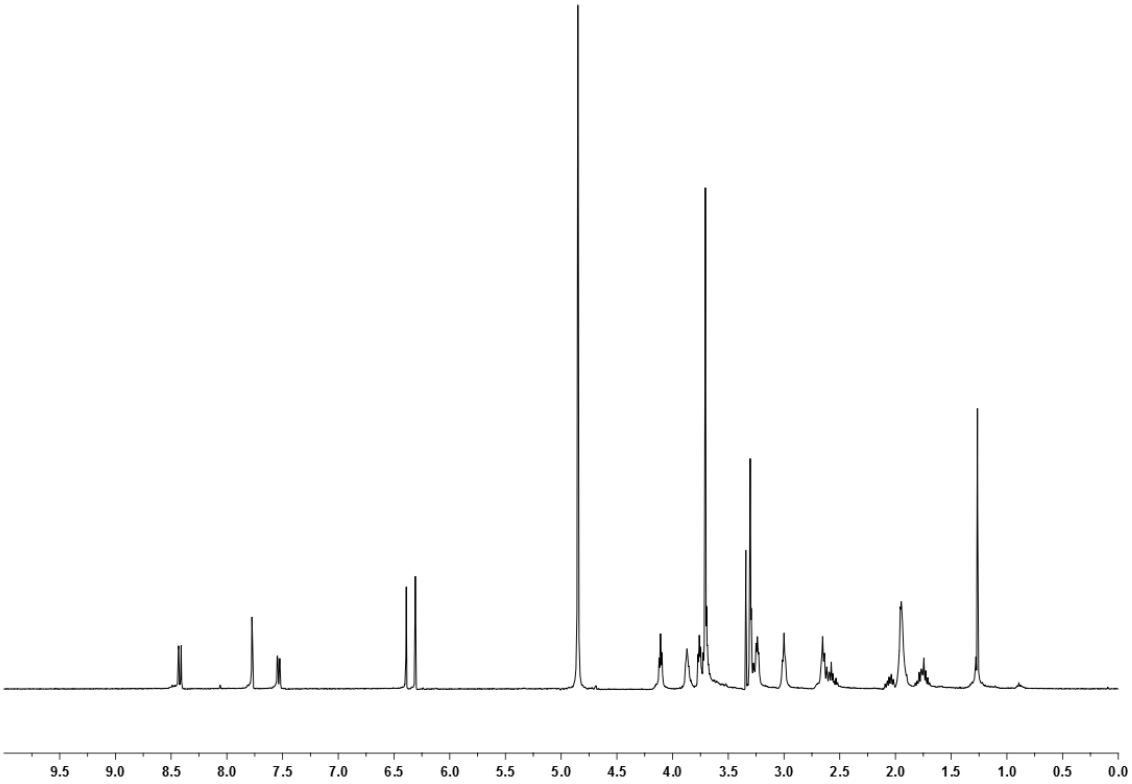
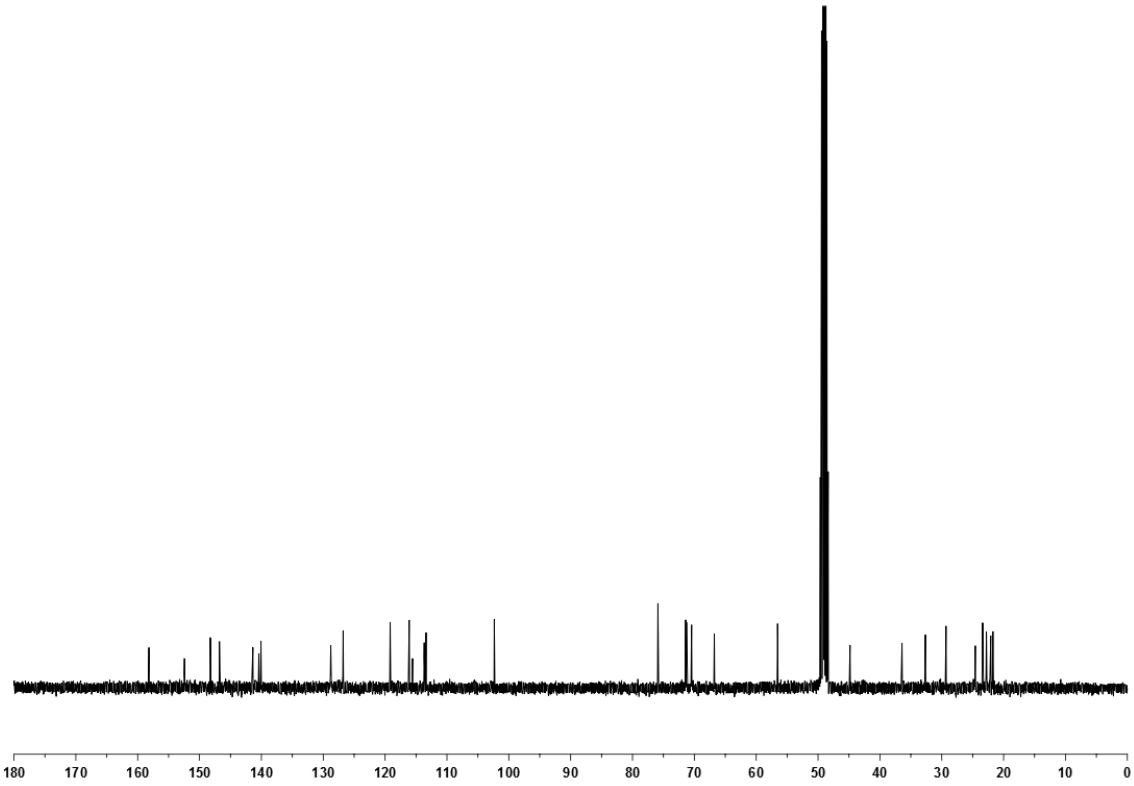
1200
1000
800
600
400
200
0
9.309
6.938
7.444
7.957
0
2.5
5
7.5
10
12.5
min

N-(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)-*N'*-[2-(6-hydroxy-7-methoxy-2-methylchroman-2-yl)ethyl]-3,6-dioxaoctane-1,8-diamine (**6d**)



6d

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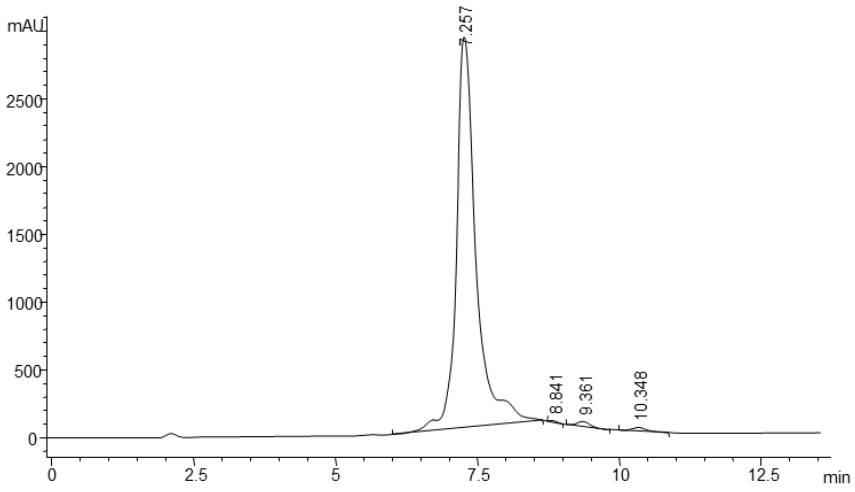
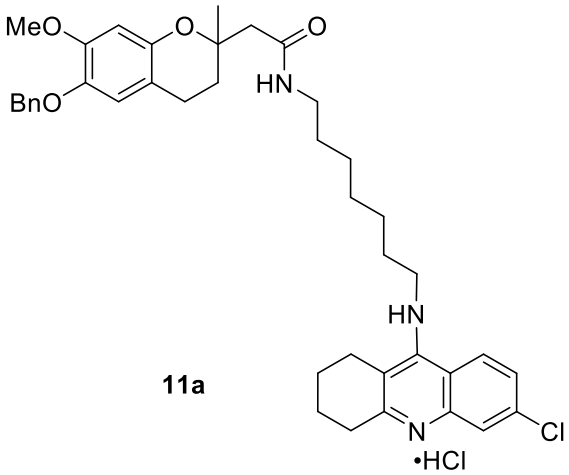
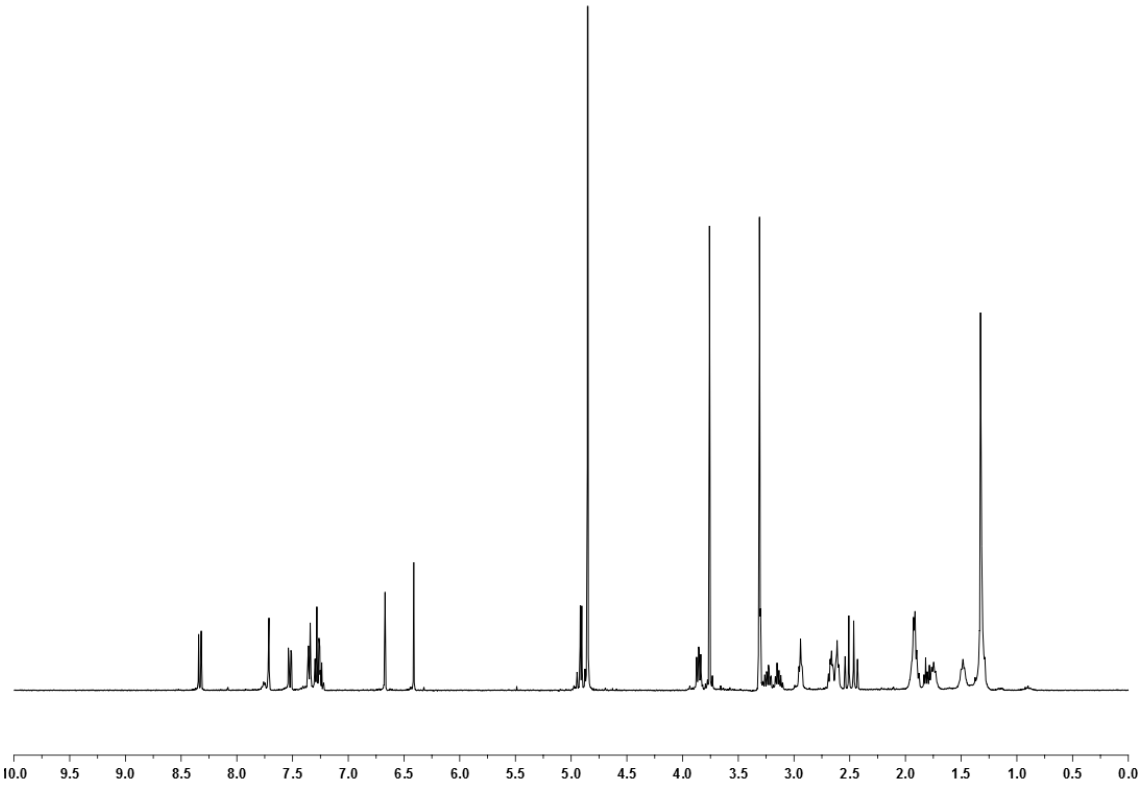
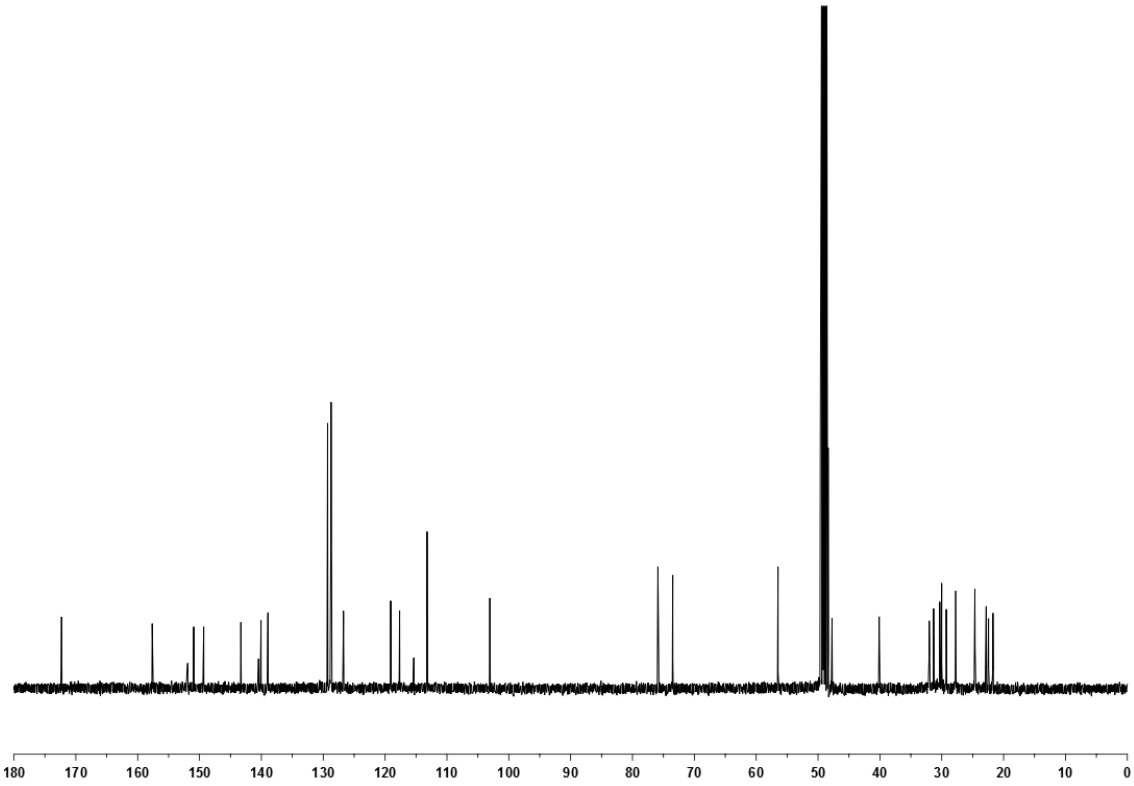

mAU
2500
2000
1500
1000
500
0
7.257
8.841
9.361
10.348
0
2.5
5
7.5
10
12.5
min

N-{7-[(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)amino]heptyl}-2-(6-benzyloxy-7-methoxy-2-methylchroman-2-yl)acetamide (**11a**)



11a

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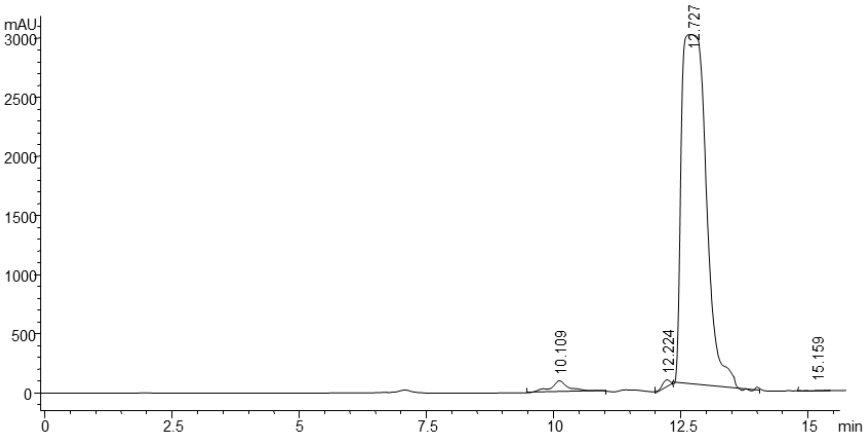
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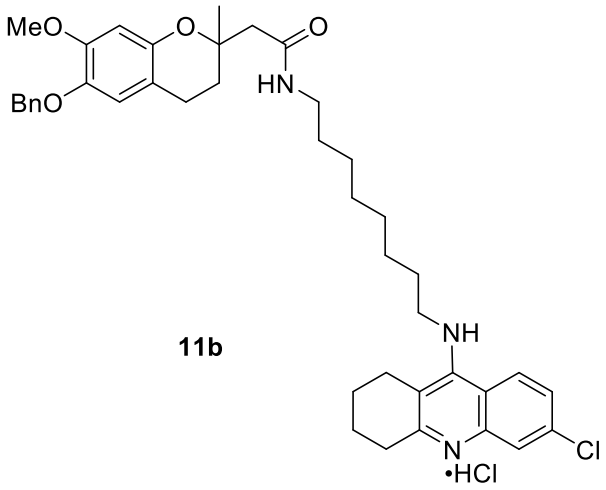
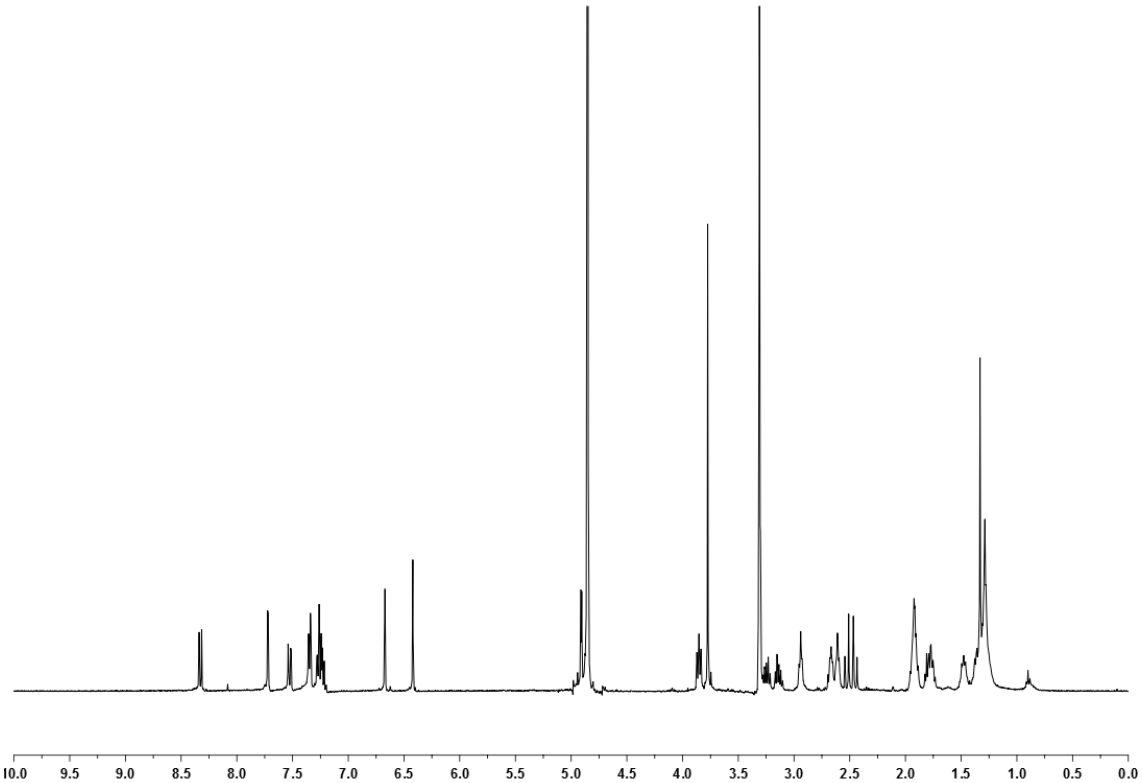
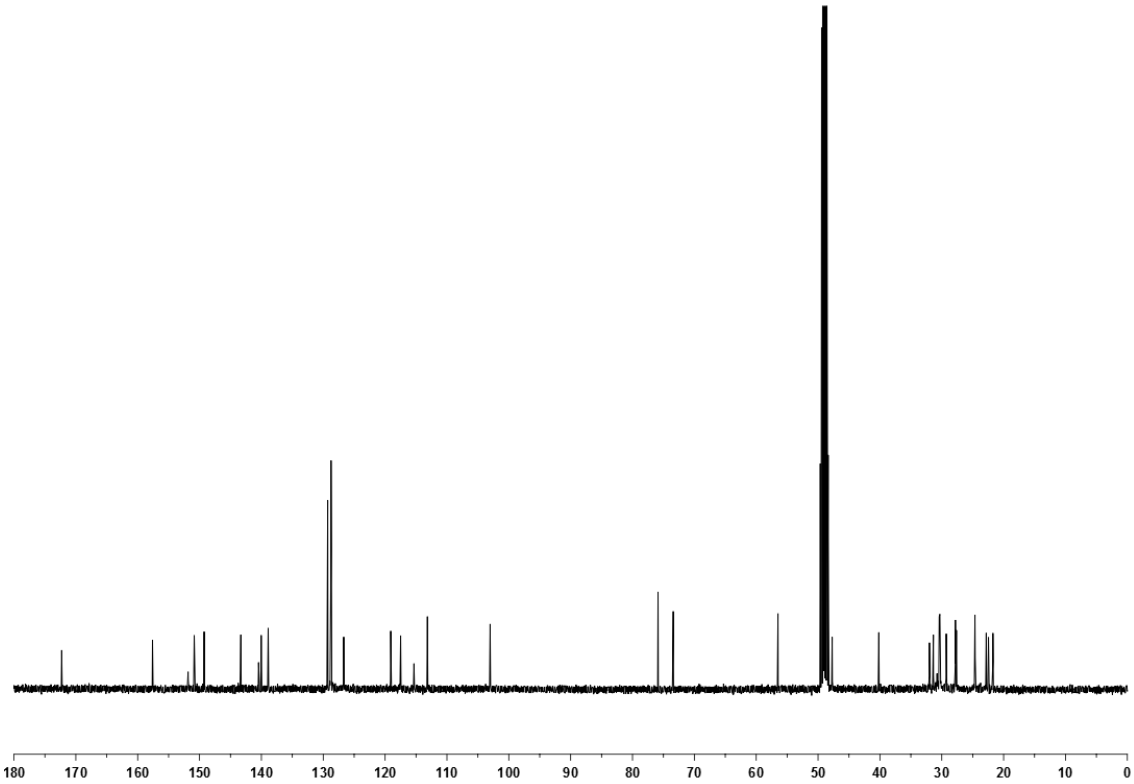
2500
2000
1500
1000
500
0
10.109
12.224
12.727
15.159
0
2.5
5
7.5
10
12.5
15
min

N-{8-[(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)amino]octyl}-2-(6-benzyloxy-7-methoxy-2-methylchroman-2-yl)acetamide (**11b**)



11b

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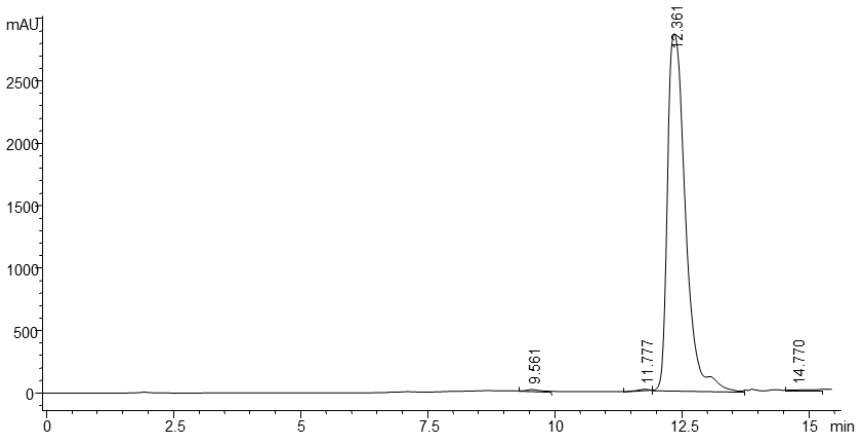
40
30
20
10
0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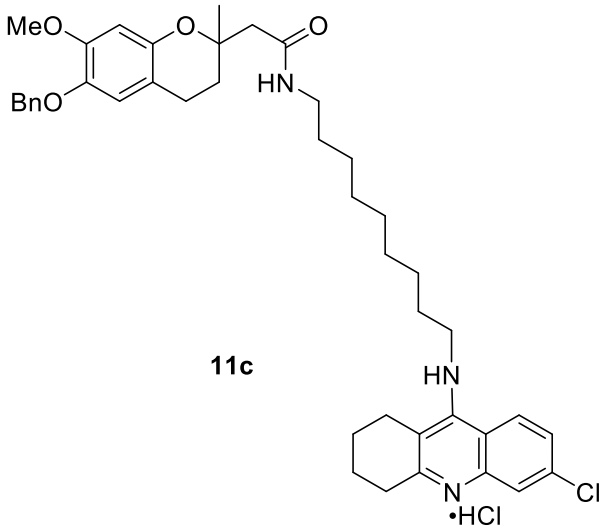
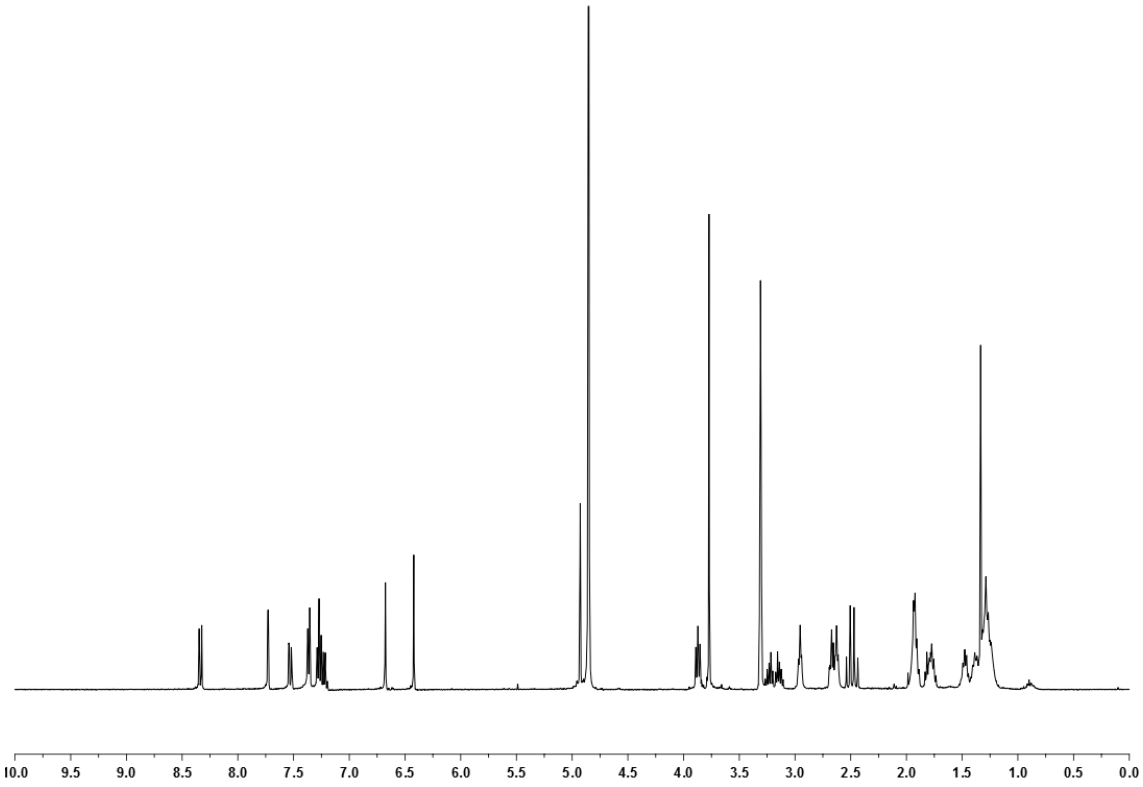
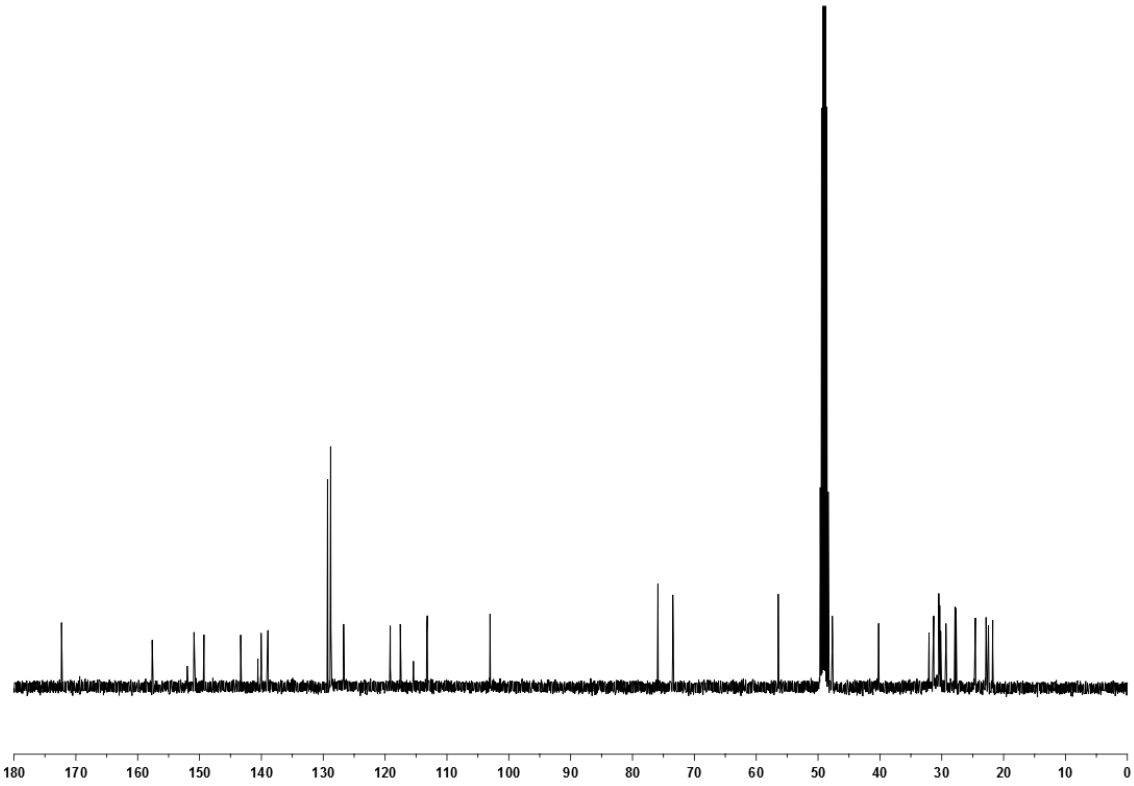
1500
1000
500
0
9.561
11.777
12.361
14.770
0
2.5
5
7.5
10
12.5
15
min

N-{9-[(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)amino]nonyl}-2-(6-benzyloxy-7-methoxy-2-methylchroman-2-yl)acetamide (**11c**)



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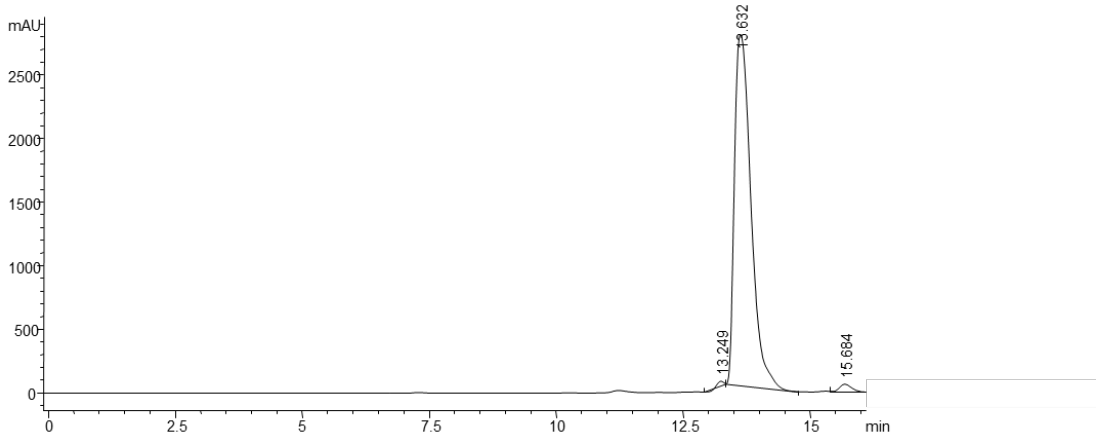
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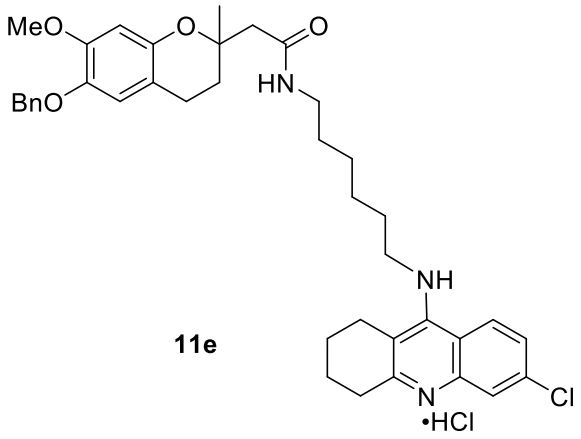
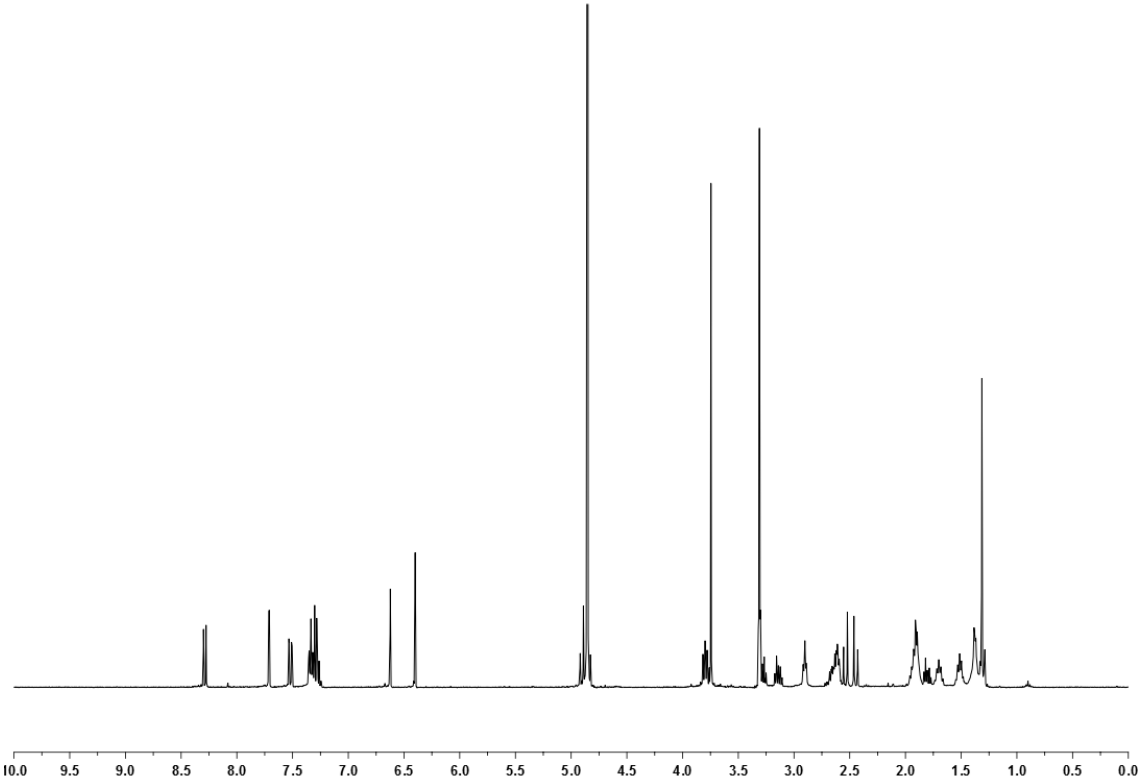
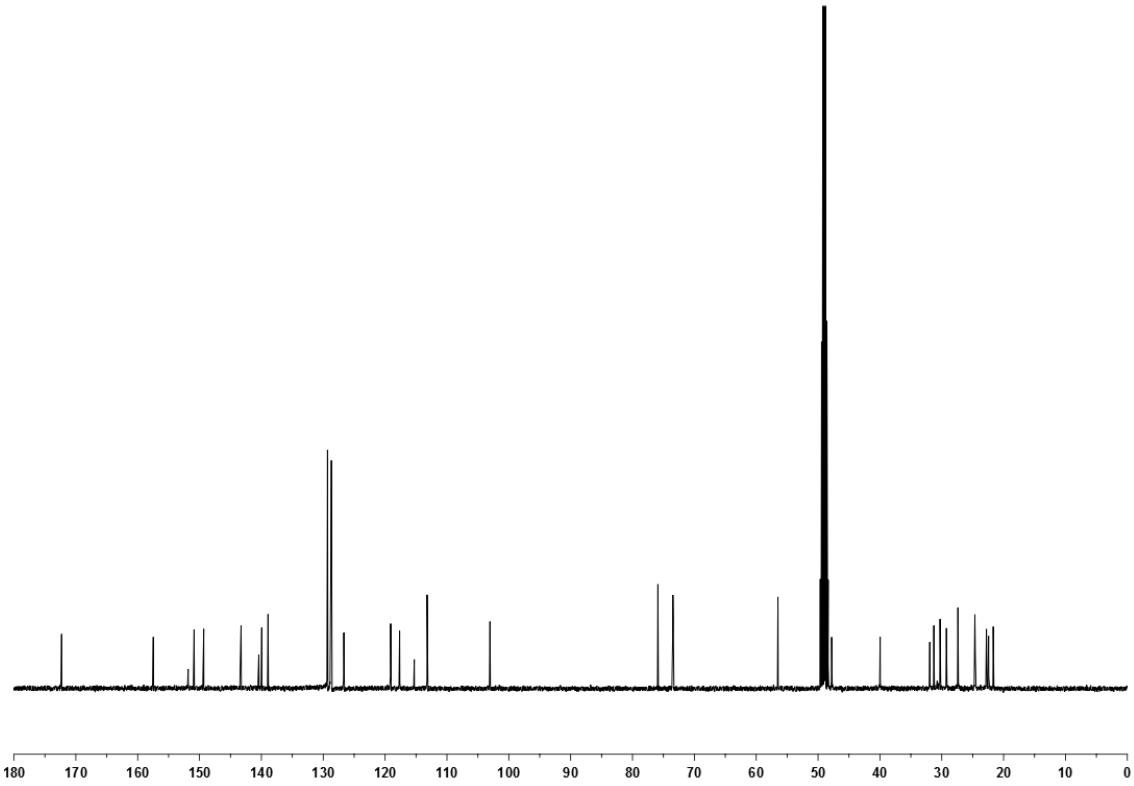
13.632
13.249
15.684
0
2.5
5
7.5
10
12.5
15
min

N-{6-[(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)amino]hexyl}-2-(6-benzyloxy-7-methoxy-2-methylchroman-2-yl)acetamide (**11e**)



11e

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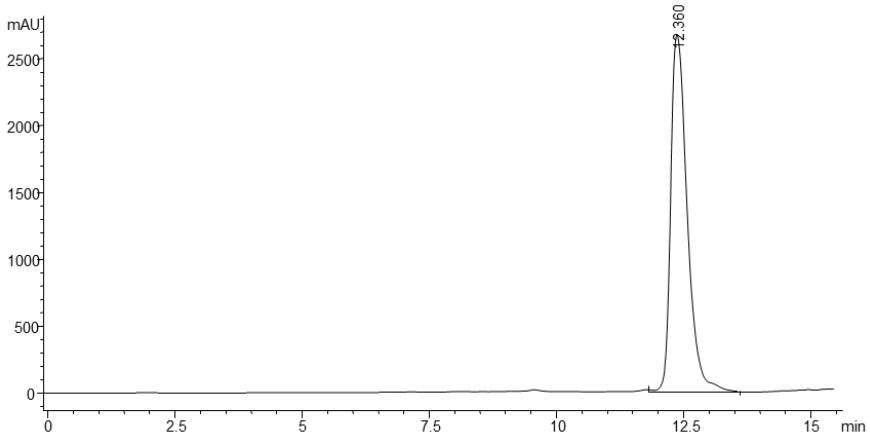
mAU
2500
2000
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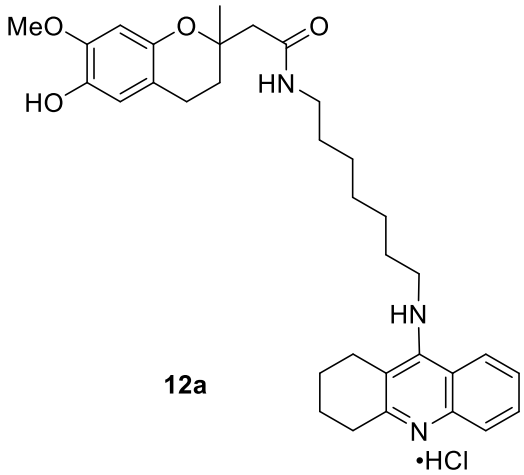
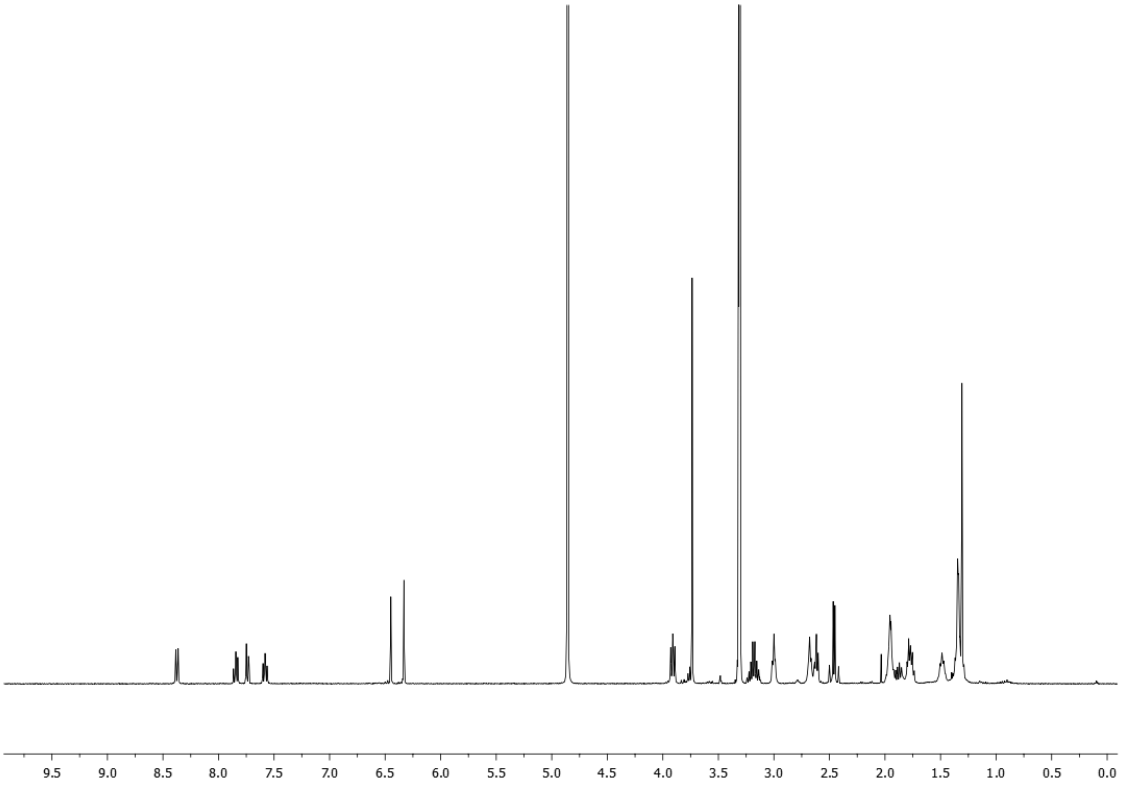
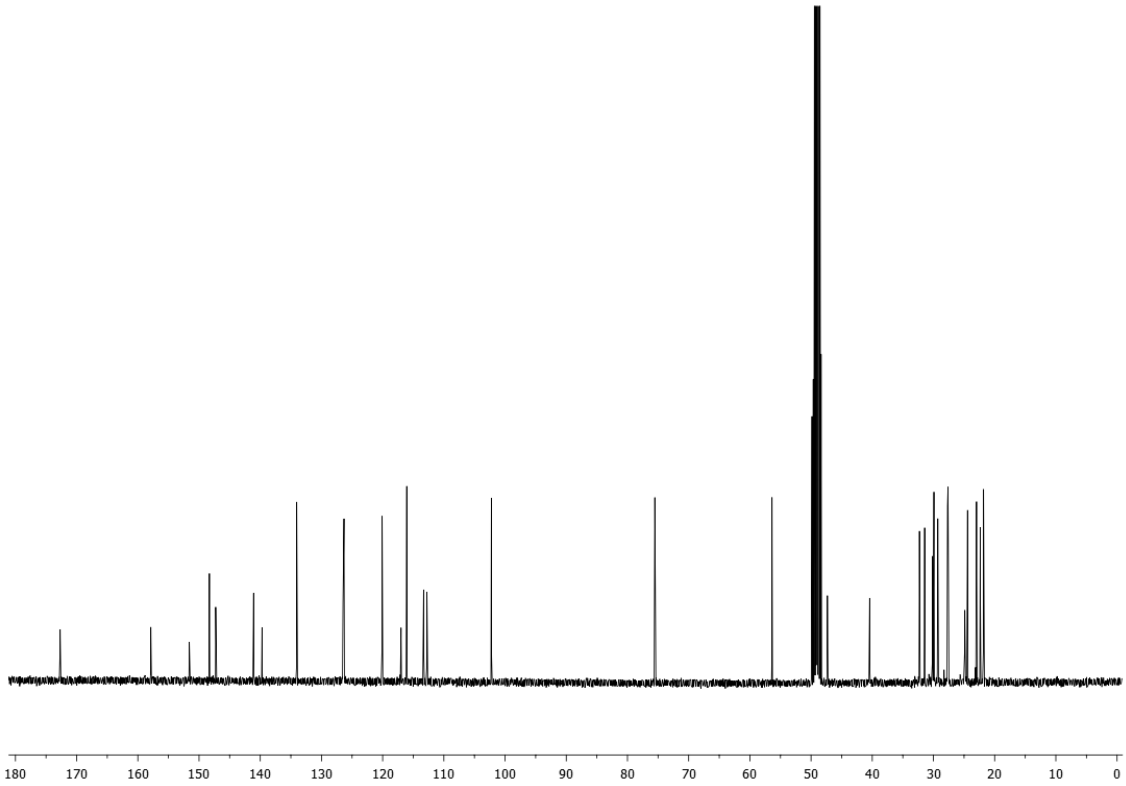
1000
500
0
12.360
0
2.5
5
7.5
10
12.5
15
min

N-{7-[(1,2,3,4-Tetrahydroacridin-9-yl)amino]heptyl}-2-(6-hydroxy-7-methoxy-2-methylchroman-2-yl)acetamide (**12a**)



12a

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30
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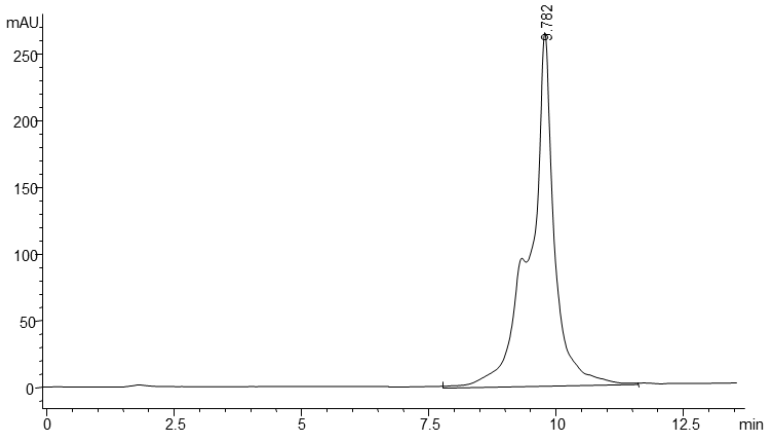
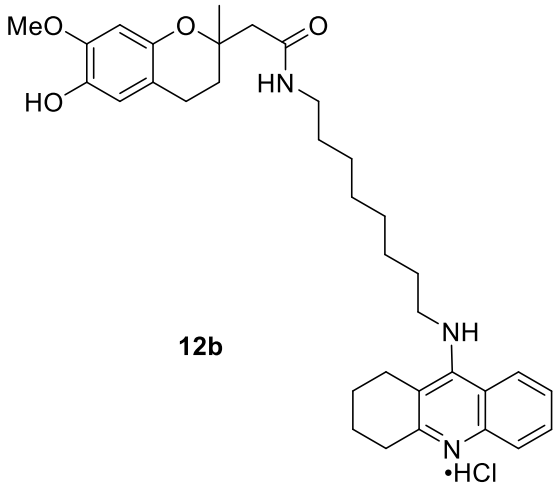
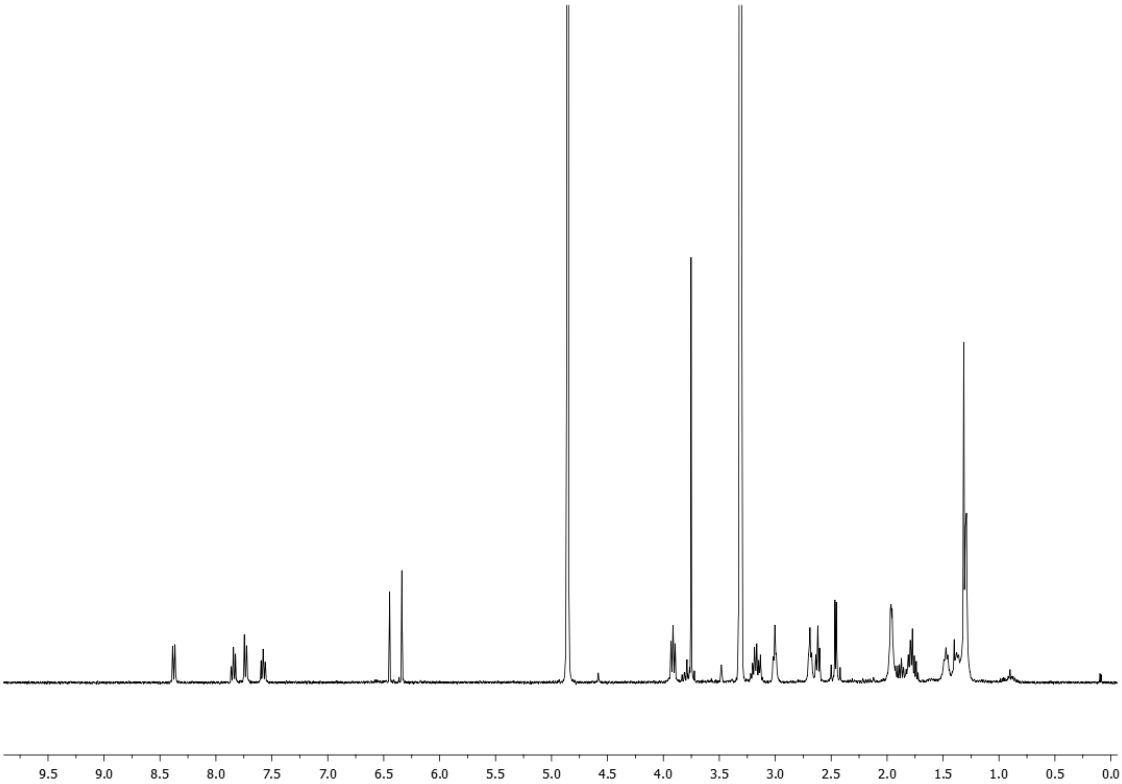
10
0

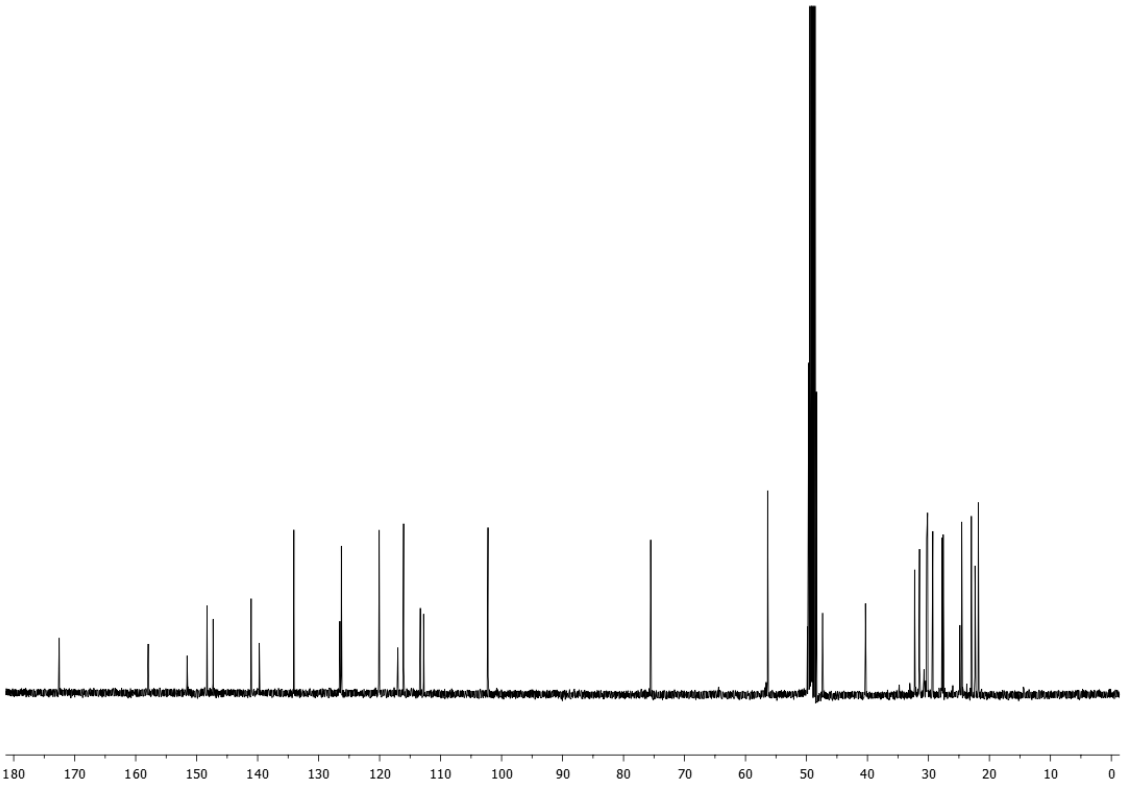

mAU
250
200
150
100
50
0
9.782
0
2.5
5
7.5
10
12.5
min

N-{8-[(1,2,3,4-Tetrahydroacridin-9-yl)amino]octyl}-2-(6-hydroxy-7-methoxy-2-methylchroman-2-yl)acetamide (**12b**)



12b

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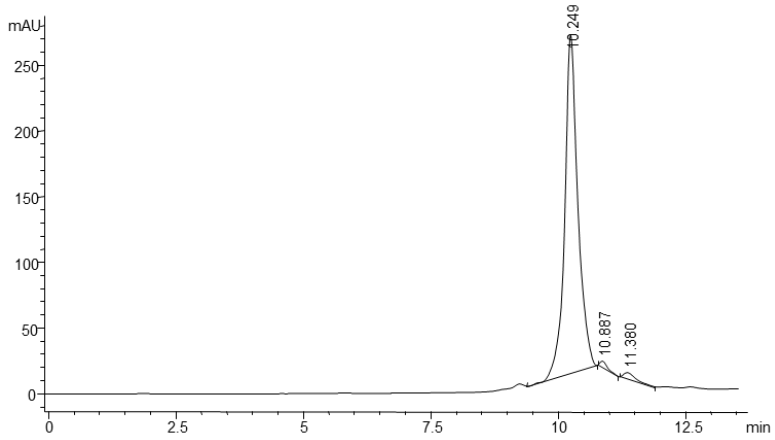
mAU
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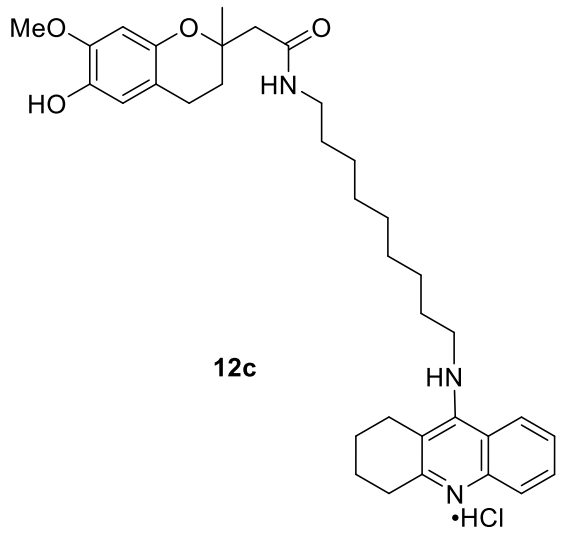
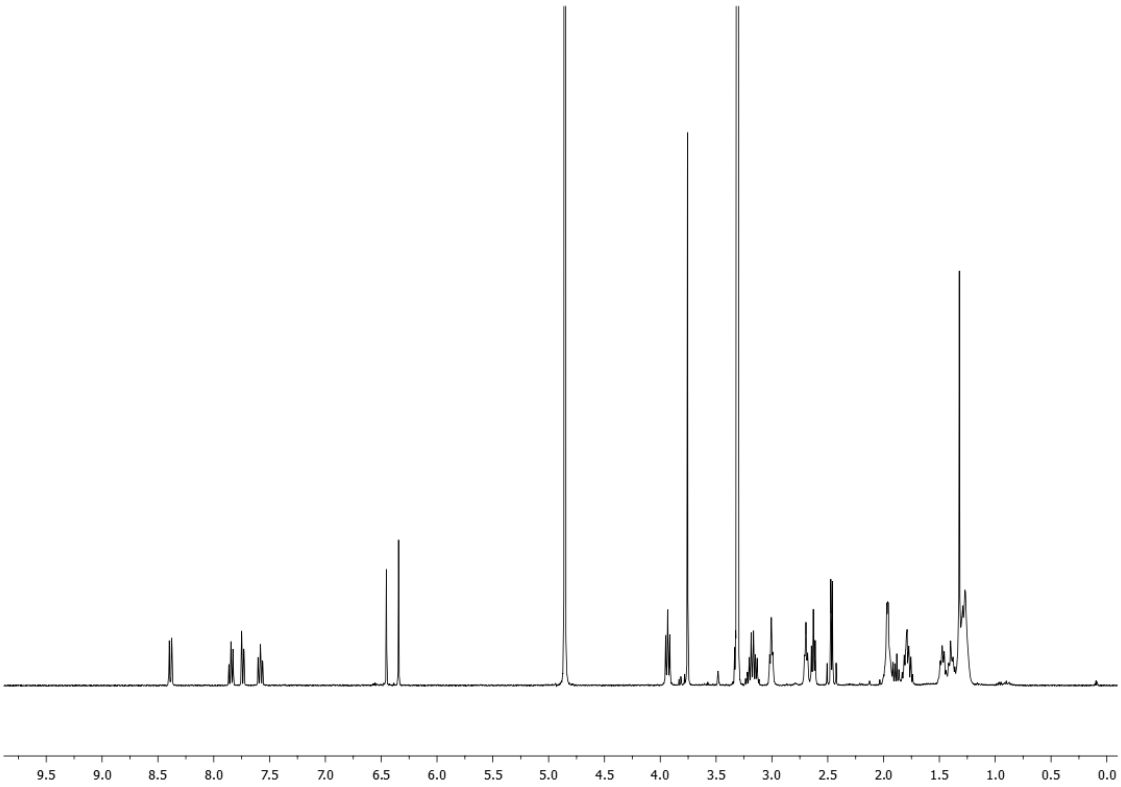
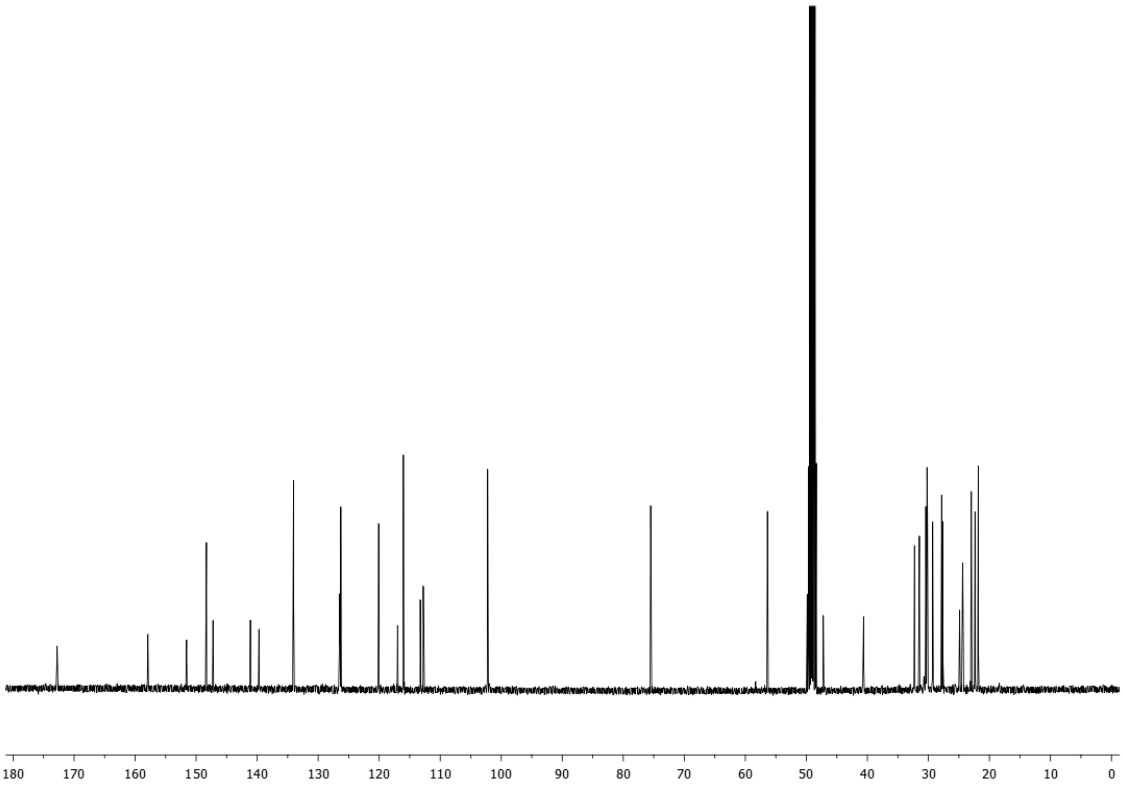
200
150
100
50
0
10.249
10.887
11.380
0
2.5
5
7.5
10
12.5
min

N-(9-((1,2,3,4-Tetrahydroacridin-9-yl)amino)nonyl)-2-(6-hydroxy-7-methoxy-2-methylchroman-2-yl)acetamide (**12c**)



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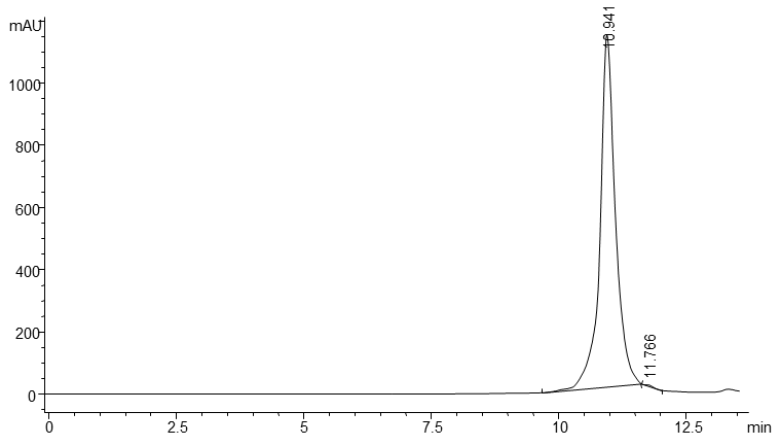
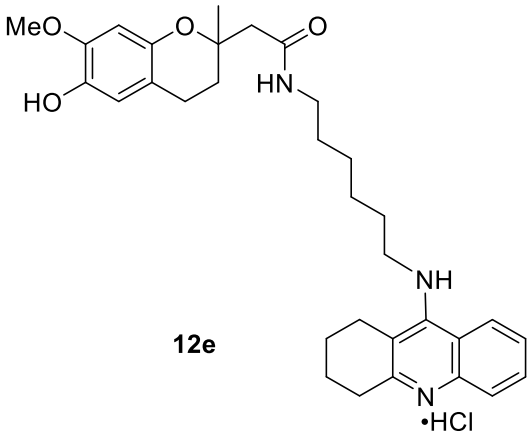
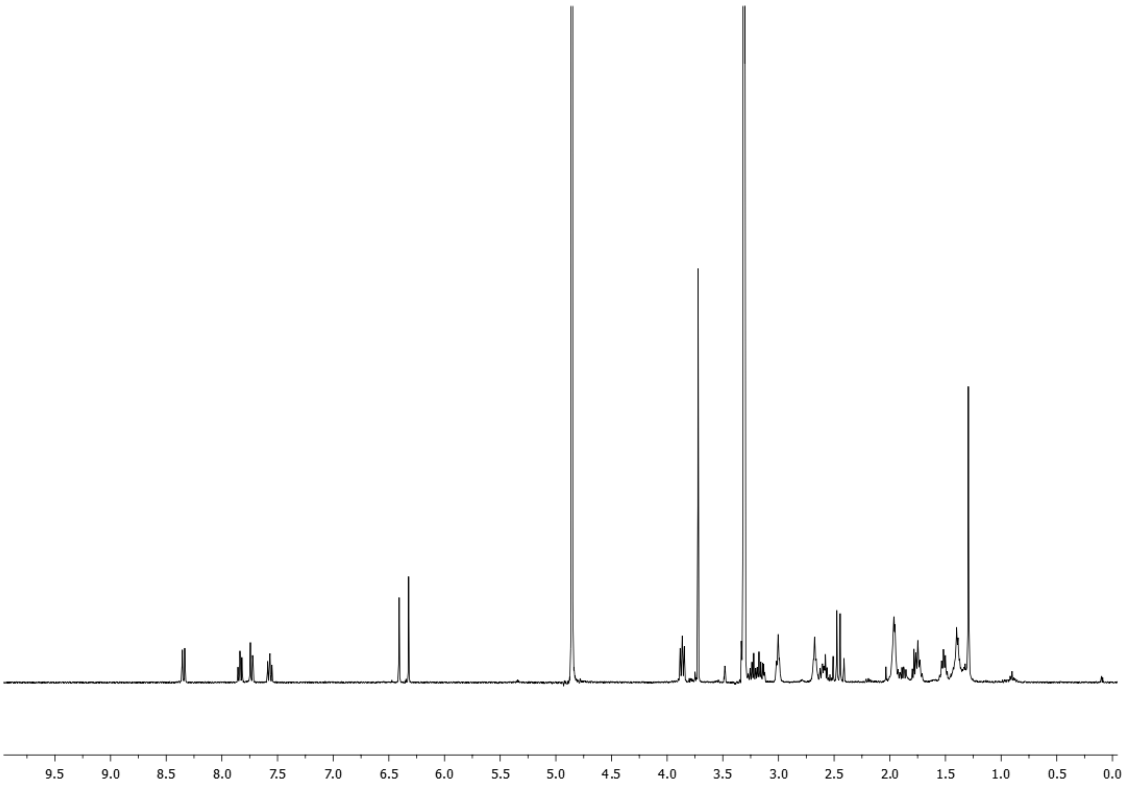
0

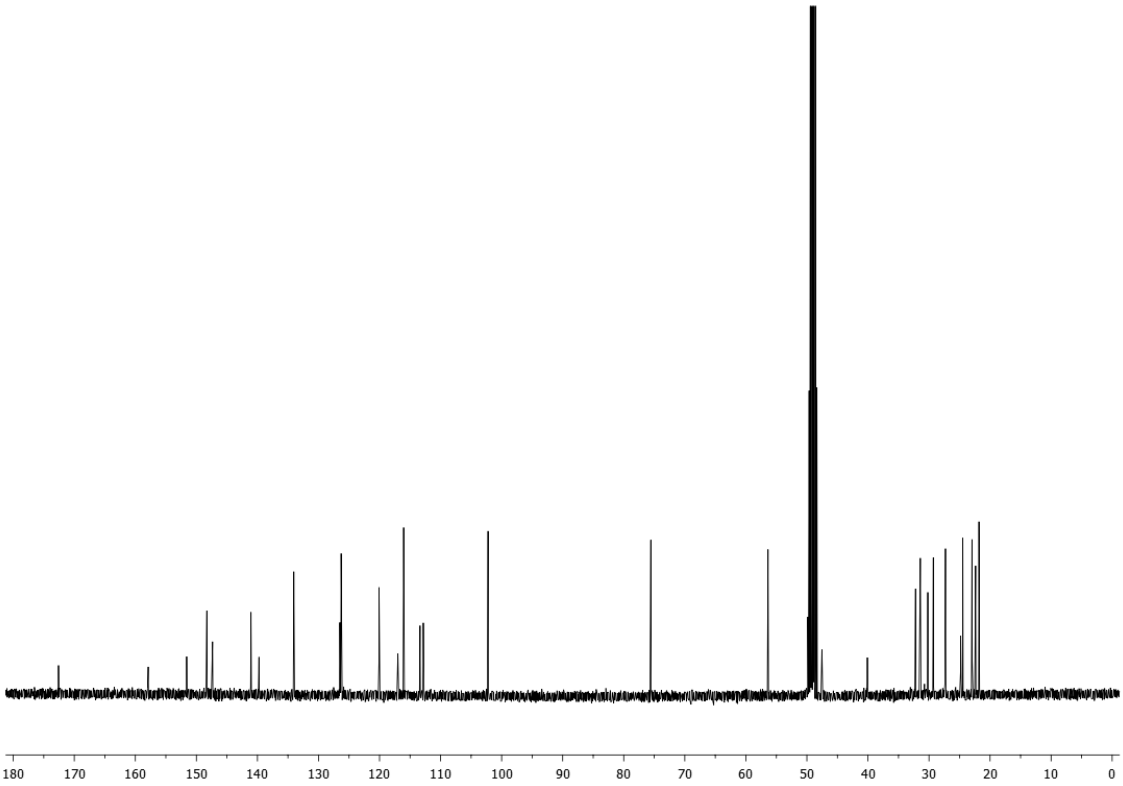

mAU
1000
800
600
400
200
0
10.941
11.766
0
2.5
5
7.5
10
12.5
min

N-{6-[(1,2,3,4-Tetrahydroacridin-9-yl)amino]hexyl]-2-(6-hydroxy-7-methoxy-2-methylchroman-2-yl)acetamide (**12e**)



12e

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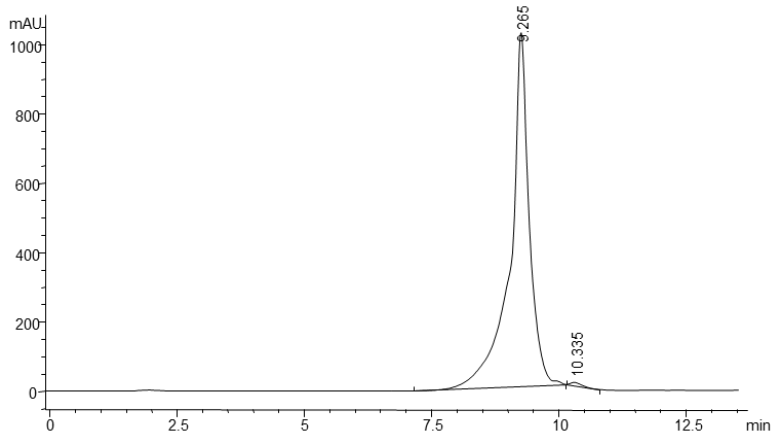
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0
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5
7.5
10
12.5
min