The Effect of gem-Difluorination on the Conformation and Properties of a Model Macrocyclic System

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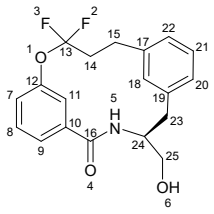
NMR data for macrocycle 4a



^{1}H / ^{19}F NMR data - d-DMSO 500 MHz

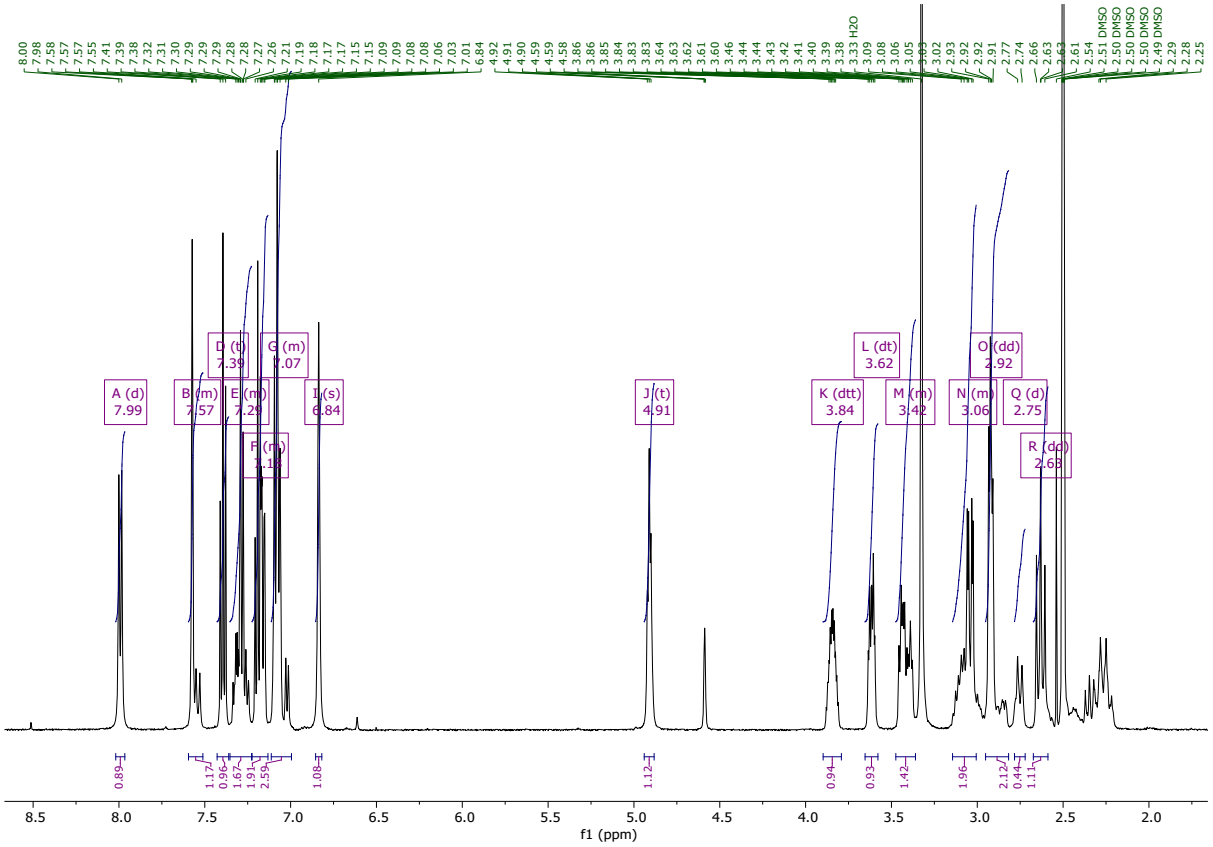
Assignment	Major conformer	Minor conformer
F	-66.5 (1F, d, J_F = 150.6 Hz)	-61.6 (1F, d, J_F = 150.4 Hz)
F	-65.0 (1F, d, J_F = 150.6 Hz)	-59.6 (1F, d, J_F = 150.4 Hz).
5	7.99 (d, *J* = 8.4 Hz, 1H)	7.54 (d, *J* = 11.0 Hz, 1H)
6 OH	4.91 (t, *J* = 5.1 Hz, 1H)	4.91 (t, *J* = *ca.* 5 Hz, 1H)
7	7.16 (dd, *J* = 2.0, 8.4 Hz, 1H)	7.08 – 7.10 (1H, m)
8	7.39 (1H, t, J_H = 7.9 Hz)	7.32 (t, J_H = 7.8 Hz)
9	7.28 (d, *J* = 7.6 Hz, 1H)	7.02 (d, *J* = 7.5 Hz, 1H)
11	6.84 (s, 1H)	4.59 (s, 1H)
14	2.19 – 2.32 (m, 1H)' 3.02 – 3.16 (m, 1H)''	2.38 - 2-48 (1H, m)' 2.56 – 2.63 (1H, m)''
15	2.89 – 2.97 (m, 2H)	2.74 – 2.80 (m, 1H)' 2.87 – 2.92 (m, 1H)''
18	7.57 (s, 1H)	6.83 (s, 1H)
20	7.09 (d, *J* = 8.2 Hz, 1H)	7.05 – 7.08 (m, 1H)
21	7.19 (t, *J* = 7.5 Hz, 1H),	7.31 (t, J_H = 7.4 Hz)
22	7.07 (d, *J* = 8.1 Hz, 1H)	7.25 (d, *J* = 7.6 Hz, 1H),
23	3.04 (dd, *J* = 13.1, 3.8 Hz, 1H)' 2.63 (t, *J* = 12.2 Hz, 1H)''	2.75 (d, *J* = 12.8 Hz, 1H)' 2.36 (t, *J* = 12.0 Hz, 1H)''
24	3.80 – 3.89 (1H, m)	2.96 – 3.02 (1H, m)
25	3.62 (dt, *J* = 10.4, 4.3 Hz, 1H)' 3.43 (dt, *J* = 10.7, 6.6 Hz, 1H)''	3.39 (t, *J* = 5.7 Hz, 2H)

^{13}C NMR data d-DMSO 125 MHz

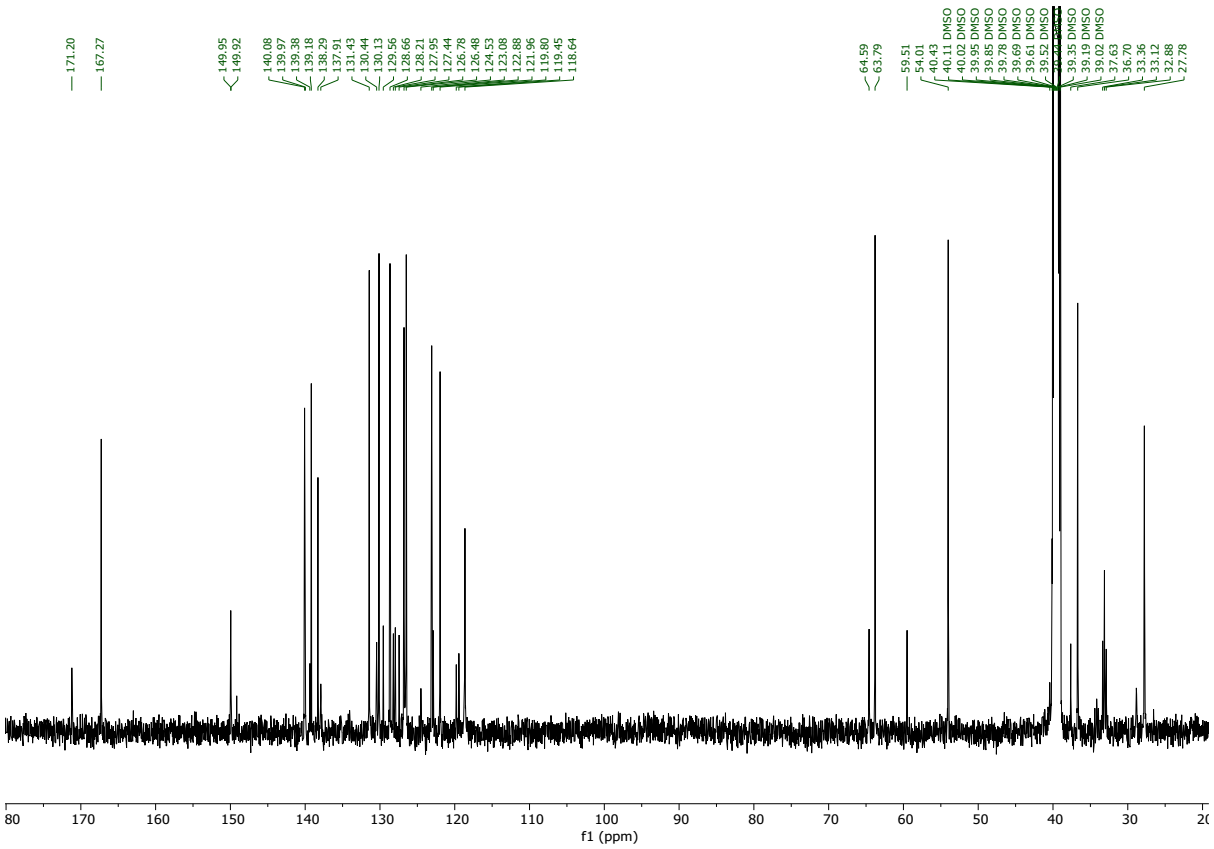
Assignment	Major conformer	Minor conformer
7	121.9	119.8
8	130.7	129.6

9	123.1	122.9
10	138.3	137.9
11	118.6	119.4
12	149.9 (t, *J* = 3.5 Hz)	149.1 (t, *J* = 3.1 Hz)
13	127.1 (t, *J* = 266 Hz)	126.2
14	33.1 (t, *J* = 30.3Hz)	34.2 (t, *J* = 28.7 Hz)
15	27.8	28.8
16	167.3	171.2
17	140.1	140
18	131.4	130.4
19	139.2	139.4
20	126.5	128.2
21	128.7	127.9
22	126.8	127.4
23	36.7	37.6
24	54	59.5
25	63.8	64.6

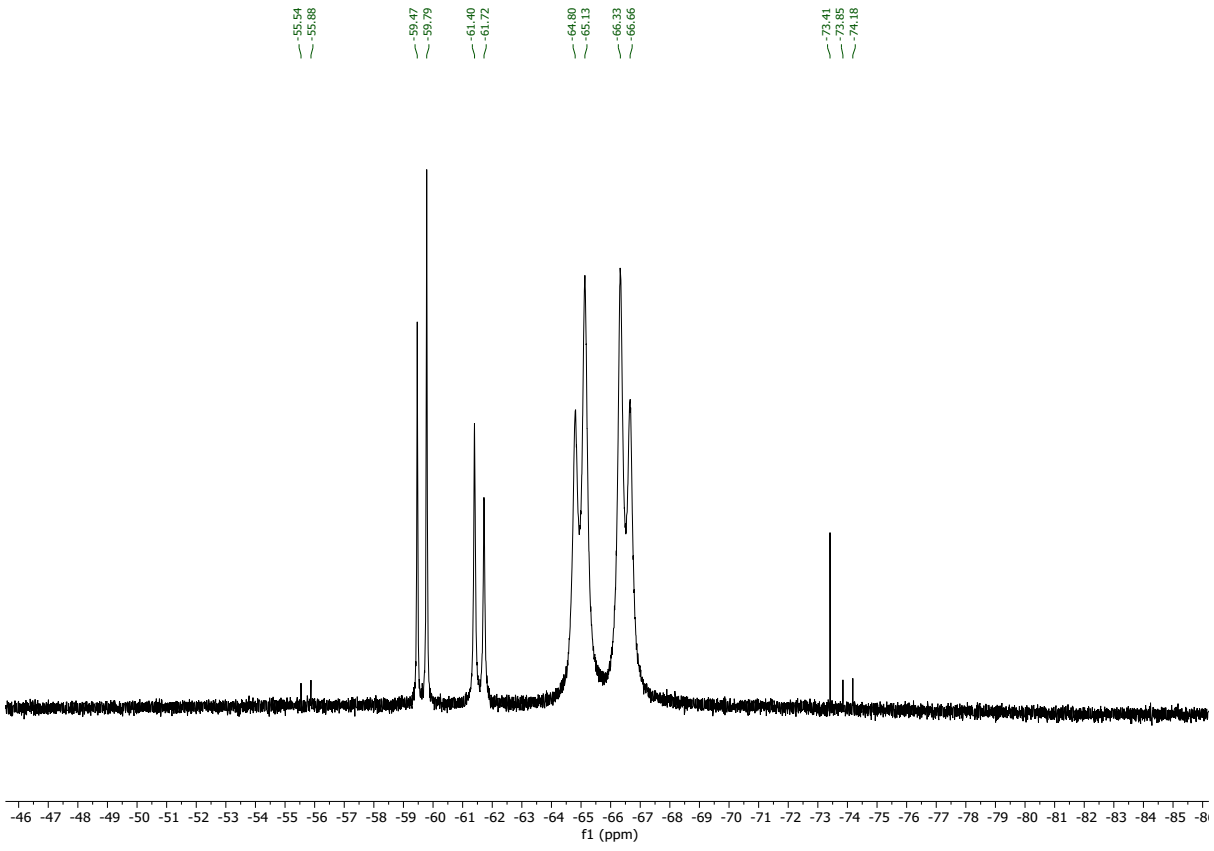
^{1}H NMR spectrum – d-DMSO, 500 MHz



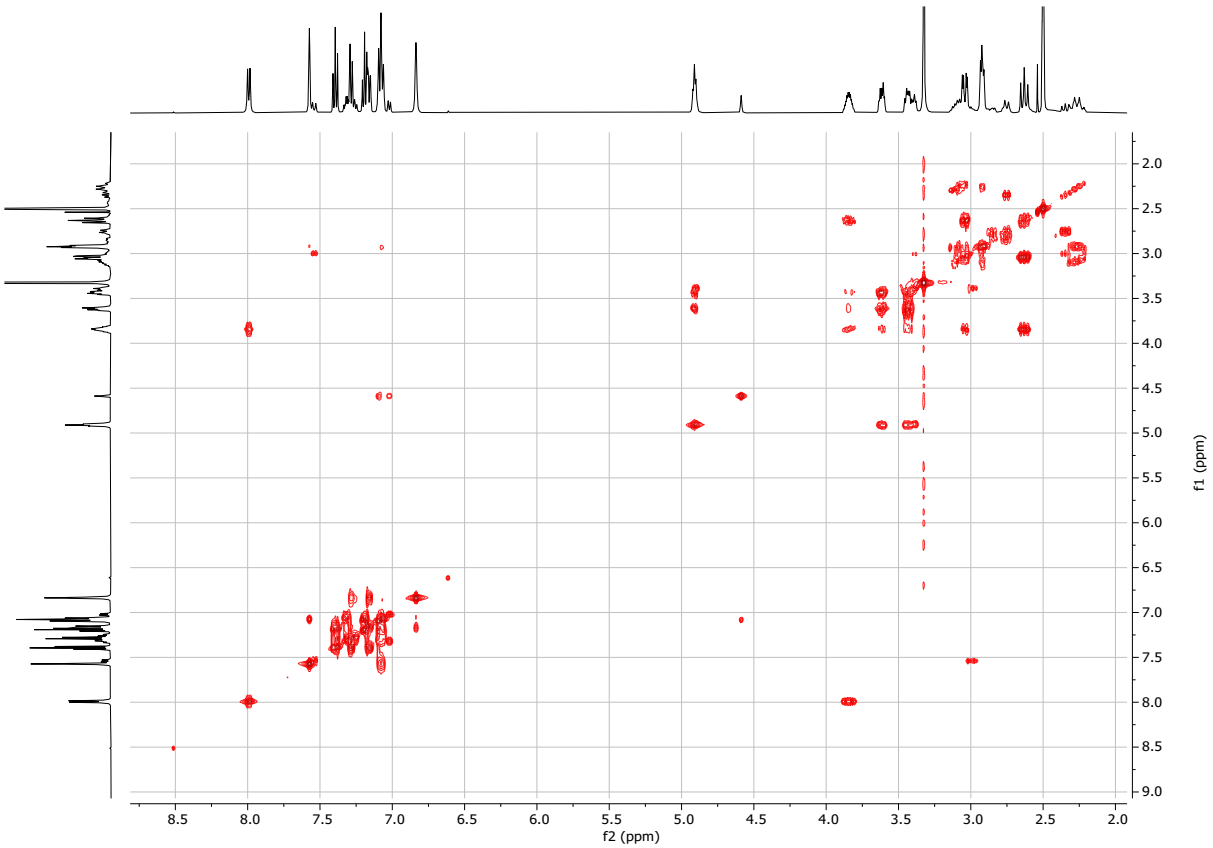
^{13}C NMR spectrum – d-DMSO, 125 MHz



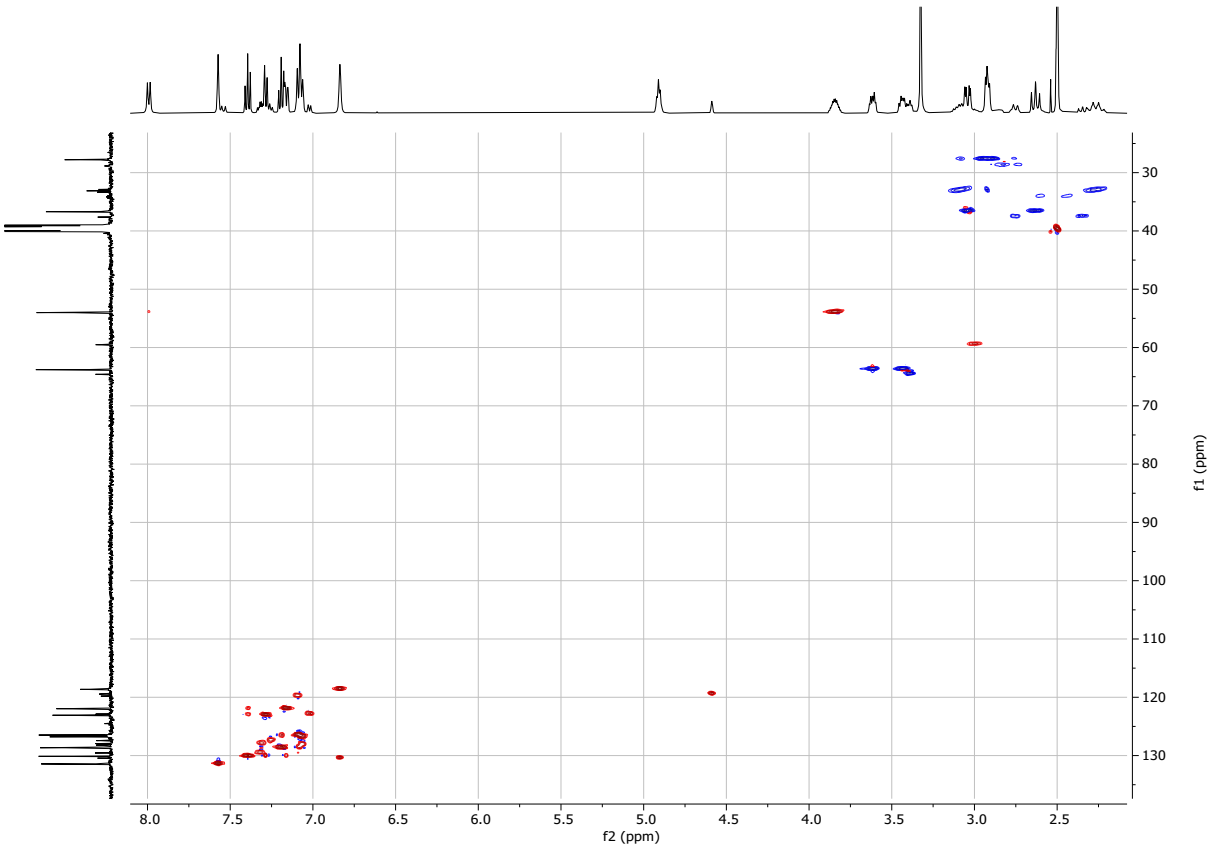
^{19}F NMR spectrum – d-DMSO, 470 MHz



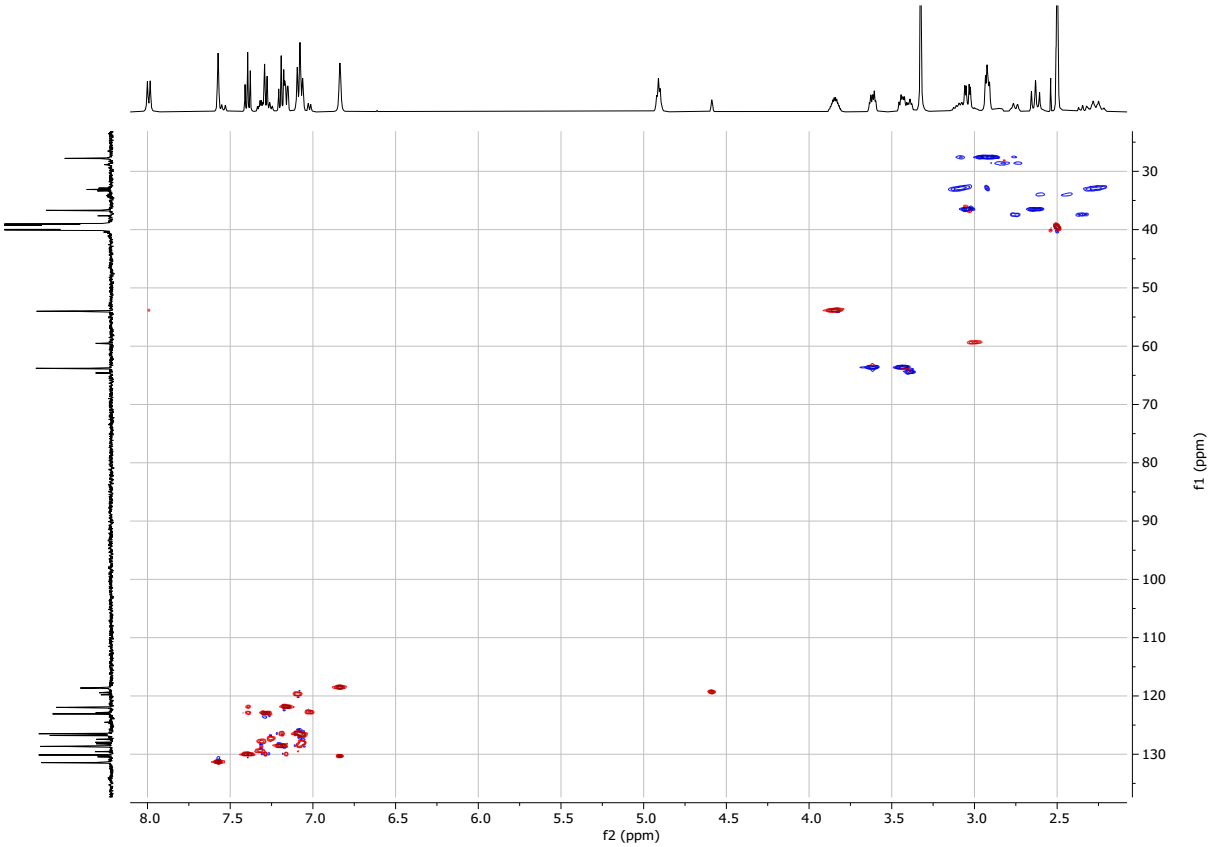
COSY spectrum – d-DMSO, 500 MHz



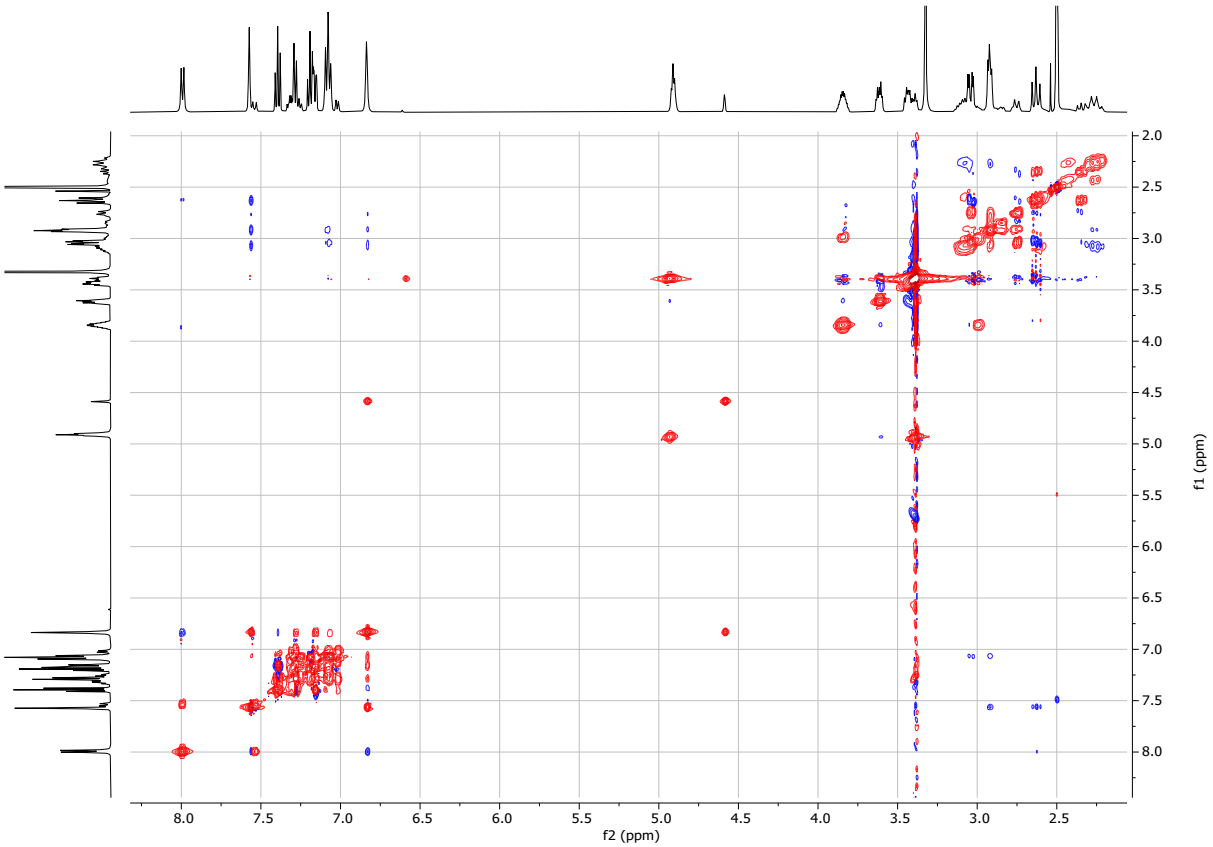
HSQC spectrum – d-DMSO, 500 MHz



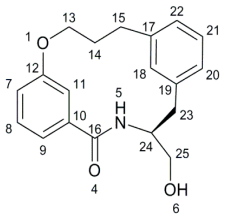
HMBC spectrum – d-DMSO, 500 MHz



ROESY spectra – d-DMSO, 500 MHz



NMR data for macrocycle 4b



^{1}H NMR/^{13}C NMR assignments Major conformer – d-DMSO, 500 MHz/125 MHz

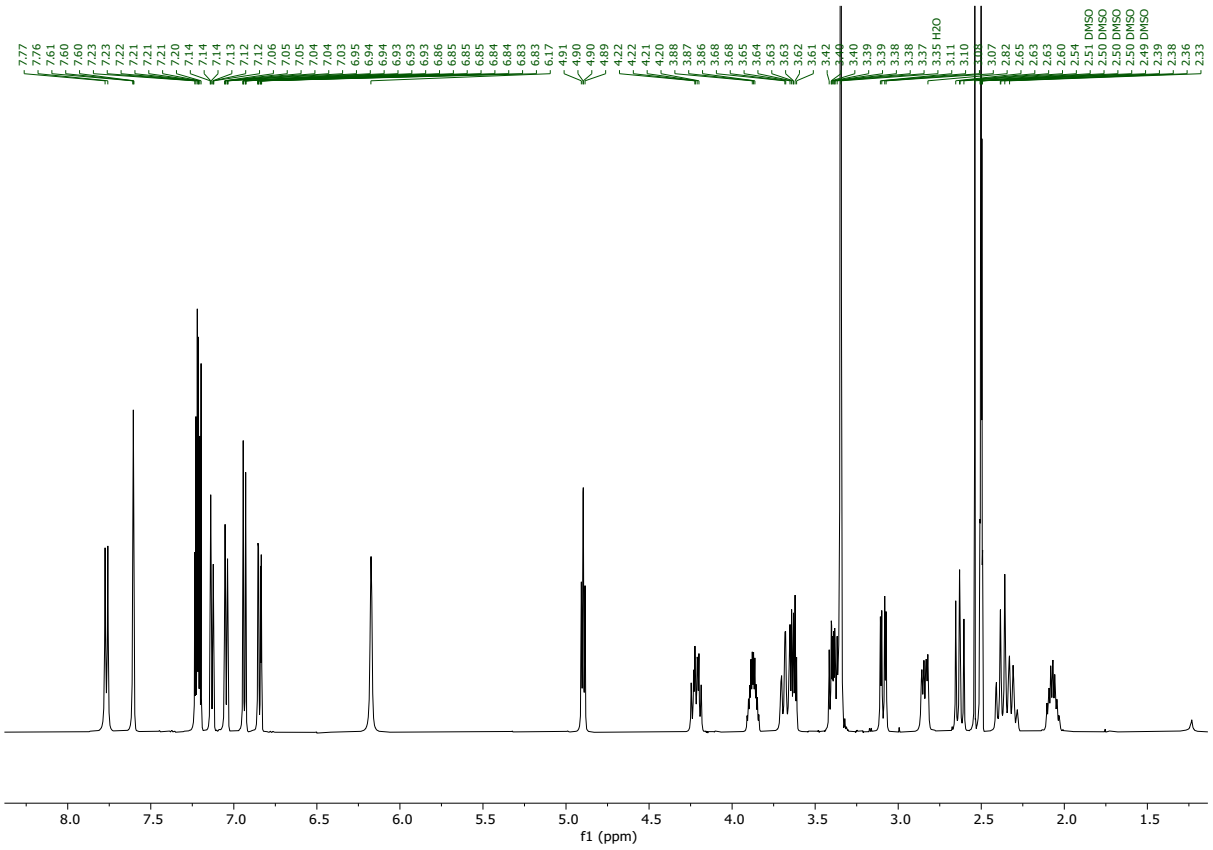
Assignment	Proton	Carbon
5 NH	7.77 (d, *J* = 8.1 Hz, 1H)	-
6 OH	4.90 (t, *J* = 5.6, 5.6 Hz, 1H)	-
7	6.84 (dd, *J* = 8.2, 1.9 Hz, 1H)	118.6
8	7.21 or (t, *J* = 7.8 Hz, 1H)	129.5
9	6.94 (d, *J* = 7.4 Hz, 1H)	118.3
10	-	138.9
11	6.17 (s, 1H)	111.3
12	-	157.5
13’ 13’’	4.22 (ddd, *J* = 12.3, 11.0, 7.1 Hz, 1H) 3.63 (dd, *J* = 12.2, 1.2 Hz, 1H)	65.4
14’ 14’’	2.32 – 2.27 (m, 1H) 2.07 (tt, *J* = 12.9, 12.9, 6.4, 6.4 Hz, 1H)	27.1
15’ 15’’	2.84 (dd, *J* = 12.4, 5.6 Hz, 1H) 2.38 (dt, *J* = 13.02, 1.5 Hz, 1H)	30.1
16	-	168.7
17	-	139.7
18	7.61 (s, 1H)	131.2
19	-	140.4
20	7.13 (d, *J* = 7.6 Hz, 1H)	126.4
21	7.22 (t, *J* = 7.5 Hz, 1H)	128.3
22	7.04 (d, *J* = 7.4 Hz, 1H)	127.6
23’ 23’’	3.09 (dd, *J* = 13.0, 4.0 Hz, 1H) 2.63 (dd, *J* = 13.1, 11.5 Hz, 1H)	36.7
24	3.92 – 3.84 (m, 1H)	53.7
25’ 25’’	3.68 – 3.59 (m, 1H) 3.44 – 3.35 (m, 1H)	63.9

^{1}H NMR/^{13}C NMR assignments Minor conformer – d-DMSO, 500 MHz/125 MHz

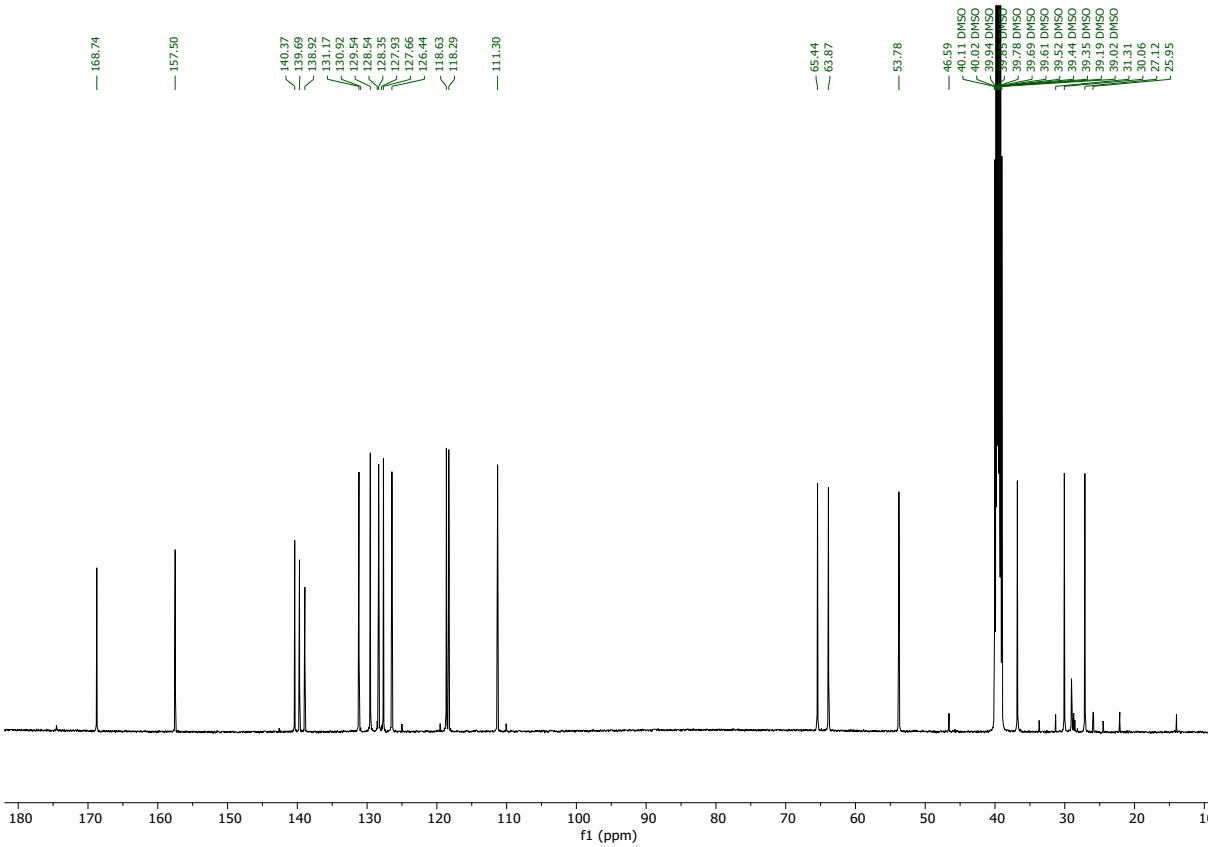
Assignment	Proton	Carbon
5 NH	7.48 (d, *J* = 10.7 Hz, 1H)	
6 OH		
7	6.85 – 6.83 (m, 1H)	
8		
9	6.73 (d, *J* = 7.1 Hz, 1H)	
10		
11	4.99 (s, 1H)	
12		
13’ 13’’	3.83 – 3.76 (m, 1H)	
14’ 14’’	1.77 – 1.67 (m, 1H)	
15’ 15’’	2.46 (t, *J* = 12.3 Hz, 1H) 2.63 (t, *J* = 12.1 Hz, 1H)	
16		
17		
18	6.94 (s, 1H)	
19		

20		
21		
22		
23’ 23’’	2.79 (d, *J* = 13.0 Hz, 1H) 2.37 (t, *J* = 11.9 Hz, 1H)	
24	3.42 – 3.38 (m, 1H)	
25’ 25’’		

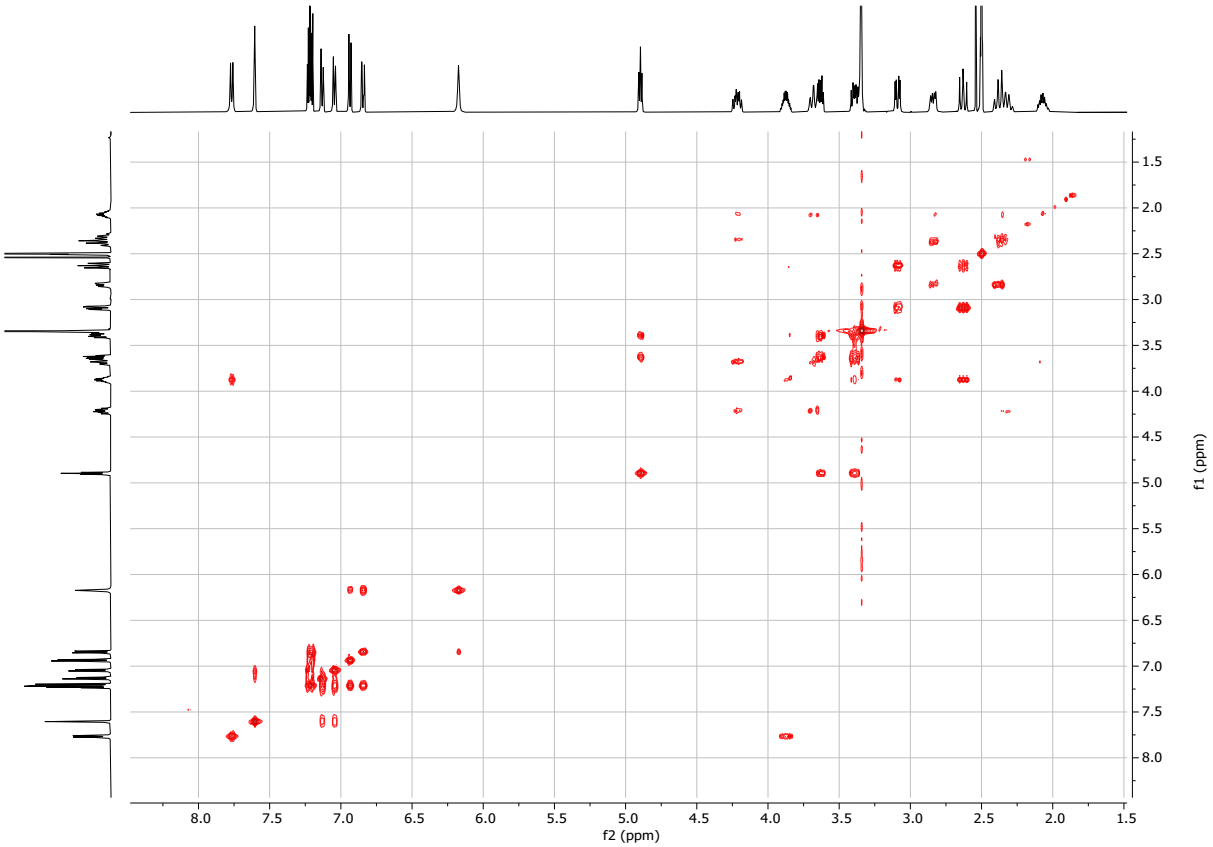
^{1}H NMR spectrum – d-DMSO, 500 MHz



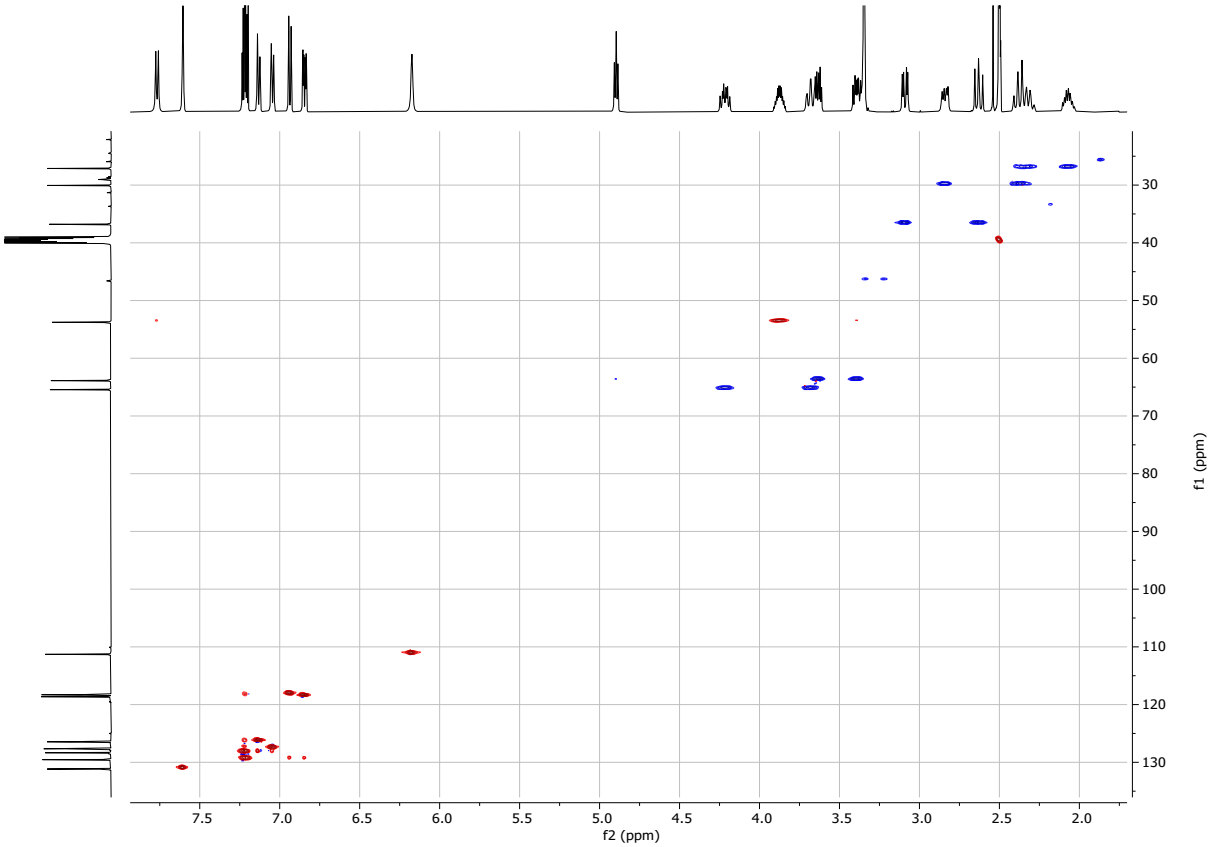
^{13}C NMR spectrum – d-DMSO, 125 MHz



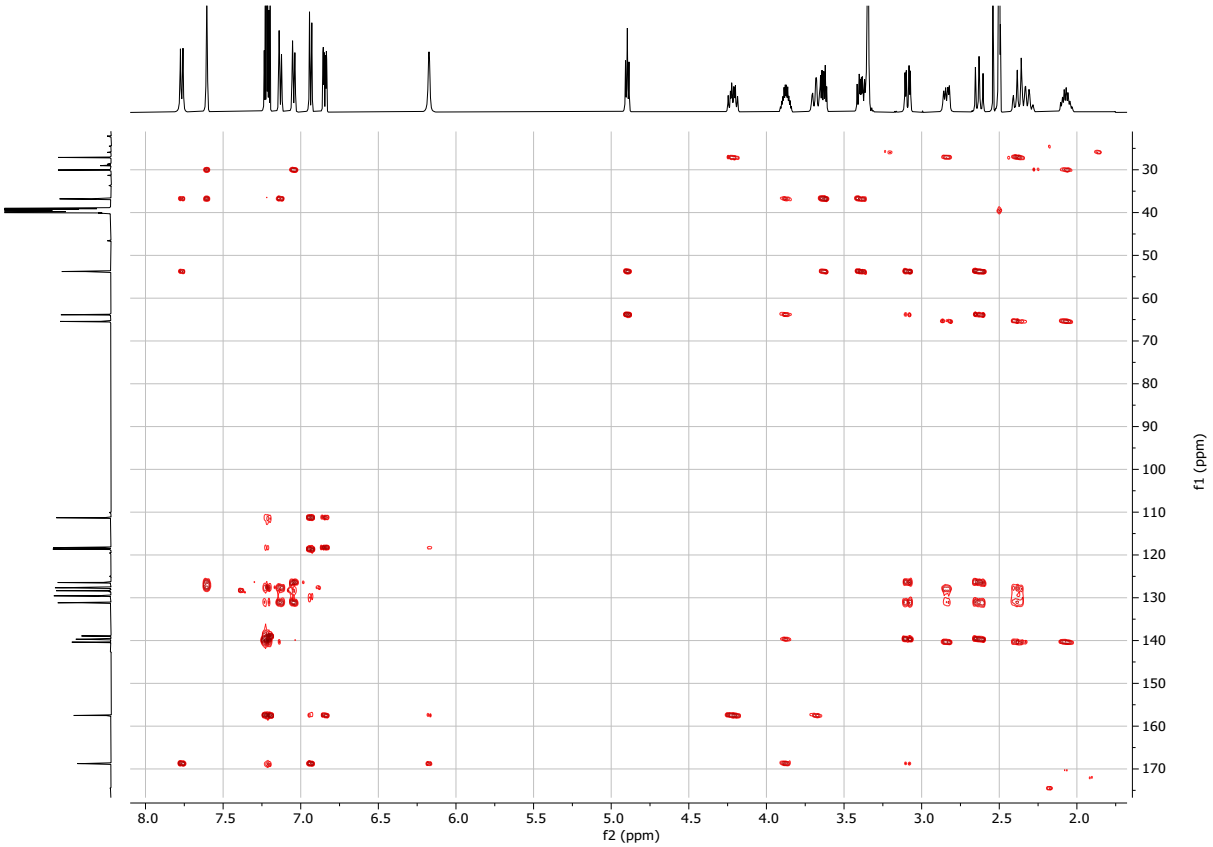
COSY spectrum – d-DMSO, 500 MHz



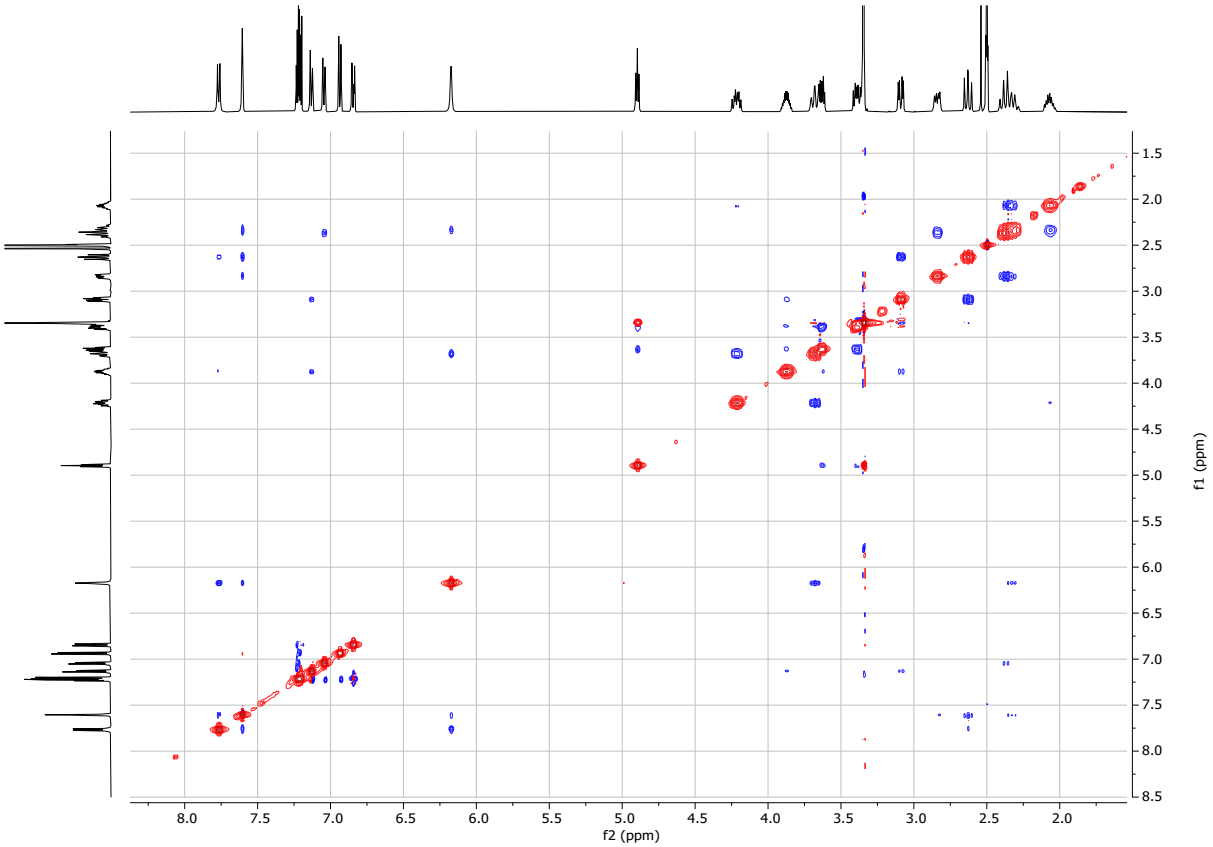
HSQC spectrum – d-DMSO, 500 MHz



HMBC spectrum – d-DMSO, 500 MHz



NOESY spectrum – d-DMSO, 500 MHz



Measurement of quantitative ROESY spectra and extraction of distance information.

Distances extracted from 1D selective NOEs, using geminal protons 23 as reference. A mixing time of 100 ms was used and the PANIC correction applied.[1] 10% benzene-d_6 was added, which moved signals such that there was better resolution in the spectrum. Only the NOEs were used that could be confidently assigned to either the major or minor conformation. Double exchange (ie from one conformation, to the other then back again) during the time course of the experiment was negligable and ignored.

Information from a proton – fluorine NOE experiment was used qualitatively. A strong NOE from fluorine to H11 in the major conformation was observed, but to H7 in the minor conformation.

Table S1. Experimentally derived ^{1}H-^{1}H NOE distances for the fluorinated macrocycle **4a**

Hydrogens	Distance (Å)	Hydrogens	Distance (Å)
H11-H14'	3.0	H23''-H25'	3.2
H11-H14''	2.5	H24-H25'	2.6
H11-H18	2.5	H7-H14'	4.4
H11-H23''	4.4	H8-H14'	4.4
H11-H24	4.4	NH5-H11	2.3
H14'-H18	3.3	NH5-H23'	3.7
H14'-H22	3.3	NH5-H23''	2.6
H18-H23''	2.4	NH5-H24	2.9
H18-H24	4.4	NH5-H25'	3.1
H20-H23''	3.5	NH5-OH6	3.9
H20-H24	2.9	OH6-H23''	3.5
H21-H24	4.4	OH6-H24	3.2
H23'-H25'	3.0	OH6-H25'	2.5
H23''-H24	2.6		

Table S2. Experimentally derived ^{1}H-^{1}H NOE distances for the non-fluorinated macrocycle **4b**

Hydrogens	Distance (Å)	Hydrogens	Distance (Å)
NH5-H11	2.3	H13'-H15''	2.6
NH5-H18	2.6	H13'-H14'	2.6
NH5-OH6	3.7	H13'-H14''	2.4
NH5-H24	2.8	H24-H20	2.7
NH5-H25'	3.1	H24-OH6	2.9
NH5-H25''	2.9	H24-H25'	3.1
NH5-H23'	3.9	H24-H23'	2.4
NH5-H23''	2.5	H24-H23''	3.1
NH5-H14'	3.5	H23'-H20	2.5
H18-H11	2.8	H23'-OH6	2.9
H18-H13'	3.7	H23'-H25'	2.9
H18-H13''	4.1	H23'-H25''	3.1
H18-H23'	3.4	H23''-H20	3.4
H18-H23''	2.4	H23''-OH6	3.1
H18-H24	4.2	H23''-H25'	3.1
H18-H14'	3.4	H23''-H25''	2.6
H22-H11	4.3	H23''-H14''	4.0
H22-H13'	3.8	H15'-H18	2.6
H22-H13''	3.1	H15'-H22	3.1
H22-H15'	3.2	H15'-H11	4.5
H22-H15''	2.5	H15'-H13''	3.4
H22-H14'	3.1	H15'-H13'	3.4
H22-H14''	4.3	H15'-H14''	2.5
H11-H24	4.1	H14''-NH5	4.8
H11-H13'	2.7	H14''-H8	4.8
H11-H13''	2.2	H14''-H7	4.5
H11-H23''	4.4	H14''-H13''	3.5
H11-H14''	3.2	H14''-H15''	2.4

Methods – conformational search and MSpin fitting

Calculations were performed within the Schrödinger Small Molecule Drug Discovery Suite.[2] Starting conformations of the two macrocycles were generated from smiles using LigPrep.[3] A macrocycle conformational search was performed using Prime-MCS with default settings.[4] The 200 conformations with lowest prime energy were submitted for further QM optimization using Gaussian 09 in gas phase at the B3LYP/6-311+G(2d,p) level of theory.[5] 57 unique conformations within 5 kcal of the minimum energy conformation were identified for **4a** and 52 conformations within 6 kcal of the minimum were identified for **4b**.

These conformations were provided to the MSpin program toegther with NOE distances, coupling constants and experimental and computed proton chemical shifts. The diastereotopic methylenes were assigned by comparing the quality of the data fits from all possible assignments. This procedure yielded clear assignments for all mathylene pairs with the exception of the hydroxyl methyl chain, the conformation of which should therefore be consided less well defined.

It is possible to *estimate* the energy penalty of having F in plane compared to protons in plane using the ratio of *trans* to *cis* conformer. We assume only that the exchange of F/H has no direct effect on the energetics of the remote amide bond. Using change in ratio from 200:1 (13.1 $kJmol^{-1}$ energy difference between *cis* and *trans* at room temperature) to 4:1 (3.4 $kJmol^{-1}$ difference) give an estimate of the minimum effect of ca. 10 $kJmol^{-1}$.

Coordinates of conformations:

4a$_{major}$_#1

0.3006	2.9476	-1.6304	C
-0.5809	3.1308	-0.5684	C
-0.3226	2.5574	0.6802	C
0.8435	1.8032	0.8398	C
1.7318	1.5967	-0.2215	C
1.4491	2.1803	-1.4589	C
2.9261	0.6849	-0.0661	C
2.7721	-0.6089	-0.8980	C
1.4689	-1.2055	-0.6715	N
0.4681	-1.1477	-1.5952	C
0.6585	-0.8942	-2.7764	O
-0.9152	-1.3990	-1.0601	C
-1.8700	-2.0099	-1.8705	C
-3.1621	-2.1938	-1.3820	C
-3.5152	-1.7382	-0.1130	C
-2.5575	-1.1065	0.6759	C
-1.2527	-0.9510	0.2180	C
-2.9744	-0.6736	1.9222	O
-2.4014	0.4499	2.4757	C
-2.5184	1.7194	1.6359	C
-1.3444	2.7122	1.7874	C
-3.0372	0.6156	3.6573	F
-1.1000	0.1812	2.8148	F
3.8634	-1.6157	-0.5660	C
3.6928	-1.9783	0.7972	O
2.8172	-0.3780	-1.9666	H
0.0868	3.3941	-2.5963	H
-1.4824	3.7232	-0.7097	H
1.0531	1.3471	1.8065	H
2.1193	2.0165	-2.2991	H
3.8447	1.1918	-0.3864	H
3.0554	0.4109	0.9864	H
1.2605	-1.5026	0.2722	H
-1.5904	-2.3252	-2.8701	H
-3.9101	-2.6819	-1.9983	H
-4.5221	-1.8529	0.2738	H
-0.5152	-0.4465	0.8266	H
-2.6262	1.4386	0.5848	H
-3.4615	2.1751	1.9483	H
-0.8757	2.5959	2.7686	H
-1.7511	3.7269	1.7481	H
3.7560	-2.4888	-1.2208	H
4.8501	-1.1609	-0.7298	H
4.2670	-2.7189	1.0144	H

4a$_{major}$_#2

-0.2900	6.8758	-0.7095	C
-1.4675	6.4940	-0.0682	C
-1.4225	5.7075	1.0829	C
-0.1712	5.3279	1.5814	C
1.0191	5.6752	0.9354	C
0.9446	6.4606	-0.2191	C
2.3444	5.1319	1.4121	C
2.7851	3.8971	0.6091	C
1.7314	2.8963	0.6593	N
1.6176	1.8976	-0.2598	C
2.5212	1.5890	-1.0229	O
0.2858	1.1976	-0.2465	C
0.2389	-0.1826	-0.4142	C
-0.9947	-0.8310	-0.3666	C
-2.1665	-0.1098	-0.1667	C
-2.1106	1.2776	-0.0322	C
-0.8875	1.9432	-0.0791	C
-3.3358	1.8914	0.1290	O
-3.4305	3.2467	0.3548	C
-2.9054	3.7216	1.7018	C
-2.6880	5.2425	1.7738	C
-2.8550	3.9198	-0.6806	F
-4.7535	3.5179	0.2732	F
4.1007	3.3252	1.1446	C
5.1596	4.2527	0.9895	O
2.9347	4.1764	-0.4443	H
-0.3383	7.4882	-1.6044	H
-2.4296	6.7929	-0.4749	H
-0.1224	4.7430	2.5023	H
1.8595	6.7437	-0.7338	H
3.1366	5.8809	1.3301	H
2.2749	4.8492	2.4710	H
0.9221	3.0952	1.2292	H
1.1622	-0.7284	-0.5760	H
-1.0470	-1.9085	-0.4836	H
-3.1348	-0.5968	-0.1268	H
-0.8350	3.0267	-0.0405	H
-3.6554	3.4032	2.4311	H
-1.9802	3.1896	1.9382	H
-2.6428	5.5203	2.8323	H
-3.5568	5.7563	1.3497	H
4.0011	3.1329	2.2184	H
4.3109	2.3774	0.6383	H
5.5091	4.1817	0.0947	H

$4a_{minor}$_#1

-0.9301	-2.7620	-1.8988	C
-2.0830	-2.3069	-1.2672	C
-2.0513	-1.9242	0.0787	C
-0.8394	-2.0052	0.7646	C
0.3325	-2.4529	0.1418	C
0.2752	-2.8370	-1.1974	C
1.6418	-2.4074	0.8904	C
2.2694	-1.0031	0.8448	C
2.7861	-0.7012	-0.4859	N
2.7754	0.5440	-1.0779	C
3.6520	0.9013	-1.8447	O
1.5779	1.4053	-0.7953	C
1.7468	2.7602	-0.5083	C
0.6297	3.5523	-0.2451	C
-0.6562	3.0110	-0.2808	C
-0.7991	1.6610	-0.5853	C
0.2978	0.8565	-0.8510	C
-2.0381	1.0366	-0.6832	O
-2.8630	1.0341	0.4054	C
-3.8633	-0.0977	0.3038	C
-3.3051	-1.4484	0.7814	C
-2.1380	0.9424	1.5579	F
-3.5104	2.2382	0.4968	F
3.3676	-0.8359	1.8993	C
3.9415	0.4517	1.8679	O
1.4827	-0.2734	1.0669	H
-0.9658	-3.0571	-2.9428	H
-3.0166	-2.2503	-1.8219	H
-0.8017	-1.6947	1.8082	H
1.1771	-3.1774	-1.6985	H
1.4781	-2.6827	1.9389	H
2.3516	-3.1314	0.4722	H
3.6378	-1.1990	-0.7313	H
2.7486	3.1782	-0.4982	H
0.7564	4.6052	-0.0154	H
-1.5304	3.6197	-0.0794	H
0.1385	-0.1880	-1.0990	H
-4.7281	0.1739	0.9149	H
-4.1869	-0.1422	-0.7399	H
-4.0981	-2.1920	0.6458	H
-3.1116	-1.3866	1.8561	H
2.9556	-1.0699	2.8915	H
4.1869	-1.5374	1.7041	H
3.2731	1.1079	2.0990	H

$4a_{minor}$_#2

-0.0884	2.6684	1.5896	C
-1.2273	2.4775	0.8096	C
-1.1207	1.9523	-0.4809	C
0.1487	1.6325	-0.9717	C
1.3023	1.8156	-0.2029	C
1.1686	2.3349	1.0883	C
2.6547	1.4266	-0.7508	C
2.9648	-0.0682	-0.5533	C
3.0872	-0.4288	0.8549	N
2.0632	-0.8082	1.6906	C
2.1319	-0.6528	2.8977	O
0.8684	-1.4406	1.0353	C
0.9782	-2.5708	0.2219	C
-0.1619	-3.0876	-0.3871	C
-1.4003	-2.4632	-0.2308	C
-1.4797	-1.3191	0.5614	C
-0.3654	-0.8333	1.2337	C
-2.6537	-0.6124	0.7570	O
-3.4554	-0.3219	-0.3192	C
-2.8022	0.3057	-1.5398	C
-2.3481	1.7603	-1.3444	C
-4.1144	-1.4594	-0.7131	F
-4.4103	0.4985	0.1745	F
4.2535	-0.4768	-1.2593	C
4.1070	-0.1732	-2.6331	O
2.1476	-0.6526	-0.9851	H
-0.1799	3.0715	2.5933	H
-2.2079	2.7290	1.2052	H
0.2458	1.2348	-1.9826	H
2.0525	2.4778	1.7051	H
2.7077	1.6378	-1.8232	H
3.4433	2.0146	-0.2624	H
3.8252	0.0423	1.3704	H
1.9464	-3.0404	0.0724	H
-0.0921	-3.9812	-0.9988	H
-2.2855	-2.8563	-0.7164	H
-0.4572	0.0472	1.8600	H
-1.9687	-0.3294	-1.8548	H
-3.5611	0.2660	-2.3265	H
-3.1810	2.3403	-0.9364	H
-2.1420	2.1646	-2.3414	H
5.1023	0.0730	-0.8216	H
4.4169	-1.5501	-1.0968	H
4.8999	-0.4377	-3.1101	H

4b_#1

0.5113	3.2205	-1.5947	C
-0.6468	3.3272	-0.8228	C
-0.7069	2.7529	0.4479	C
0.4243	2.0802	0.9269	C
1.5847	1.9444	0.1608	C
1.6162	2.5278	-1.1109	C
2.7388	1.1022	0.6491	C
2.7539	-0.2861	-0.0100	C
1.4629	-0.9249	0.1843	N
0.9909	-1.8779	-0.6694	C
1.7153	-2.4728	-1.4549	O
-0.4868	-2.1317	-0.5702	C
-0.9675	-3.4283	-0.7147	C
-2.3404	-3.6561	-0.6039	C
-3.2195	-2.6053	-0.3753	C
-2.7348	-1.2963	-0.2649	C
-1.3625	-1.0589	-0.3570	C
-3.6716	-0.3252	-0.0829	O
-3.2436	1.0298	-0.0436	C
-2.6827	1.4264	1.3204	C
-1.9725	2.7908	1.2764	C
3.8802	-1.1607	0.5455	C
5.1510	-0.6097	0.2496	O
2.9089	-0.1774	-1.0935	H
0.5462	3.6741	-2.5804	H
-1.5110	3.8595	-1.2125	H
0.3953	1.6476	1.9283	H
2.5112	2.4346	-1.7211	H
3.7003	1.5803	0.4420	H
2.6712	0.9716	1.7373	H
0.7958	-0.4715	0.7916	H
-0.2695	-4.2360	-0.9063	H
-2.7310	-4.6643	-0.7001	H
-4.2892	-2.7670	-0.2954	H
-0.9659	-0.0499	-0.3036	H
-2.5183	1.2155	-0.8458	H
-4.1396	1.6161	-0.2653	H
-3.5022	1.4519	2.0458	H
-1.9799	0.6594	1.6667	H
-1.7330	3.0979	2.3001	H
-2.6540	3.5483	0.8708	H
3.8006	-1.2061	1.6372	H
3.7717	-2.1746	0.1459	H
5.3964	-0.8574	-0.6484	H

4b_#2

-0.6439	6.2659	-1.4564	C
-1.8198	5.9021	-0.7984	C
-1.7874	5.4830	0.5326	C
-0.5505	5.4510	1.1888	C
0.6386	5.7917	0.5402	C
0.5784	6.2035	-0.7956	C
1.9720	5.6245	1.2293	C
2.7025	4.3480	0.7779	C
1.8034	3.2142	0.9166	N
1.9302	2.0782	0.1716	C
2.9463	1.8076	-0.4506	O
0.7185	1.1880	0.1804	C
0.8914	-0.1915	0.1716	C
-0.2359	-1.0145	0.2010	C
-1.5135	-0.4698	0.2182	C
-1.6840	0.9196	0.1929	C
-0.5639	1.7524	0.1785	C
-2.9717	1.3627	0.1716	O
-3.2047	2.7608	0.0677	C
-3.0634	3.4777	1.4090	C
-3.0348	5.0076	1.2447	C
3.9857	4.1070	1.5791	C
4.9132	5.1657	1.4291	O
2.9779	4.4361	-0.2812	H
-0.6830	6.5907	-2.4914	H
-2.7701	5.9409	-1.3252	H
-0.5173	5.1451	2.2357	H
1.4945	6.4708	-1.3164	H
2.6191	6.4807	1.0142	H
1.8244	5.5838	2.3179	H
0.9297	3.3565	1.4020	H
1.8956	-0.6004	0.1452	H
-0.1181	-2.0934	0.2077	H
-2.3984	-1.0973	0.2335	H
-0.6715	2.8314	0.1279	H
-2.5417	3.1940	-0.6916	H
-4.2310	2.8506	-0.2989	H
-3.8959	3.1842	2.0563	H
-2.1450	3.1464	1.9078	H
-3.0928	5.4712	2.2353	H
-3.9220	5.3357	0.6898	H
3.7252	3.9428	2.6354	H
4.4651	3.2032	1.1976	H
4.6869	5.8814	2.0314	H

4b_#3

0.6454	3.6618	-1.2584	C
-0.5625	3.6210	-0.5607	C
-0.7341	2.7454	0.5127	C
0.3318	1.9046	0.8645	C
1.5446	1.9222	0.1715	C
1.6901	2.8185	-0.8956	C
2.6581	0.9600	0.5128	C
2.8234	-0.1270	-0.5661	C
1.5584	-0.8392	-0.7733	N
1.0842	-1.8929	-0.0626	C
1.7785	-2.6050	0.6614	O
-0.3873	-2.1578	-0.2115	C
-0.8506	-3.4683	-0.1517	C
-2.2241	-3.7031	-0.2284	C
-3.1238	-2.6477	-0.3192	C
-2.6549	-1.3296	-0.3458	C
-1.2811	-1.0865	-0.3163	C
-3.5992	-0.3508	-0.3892	O
-3.1719	1.0002	-0.2553	C
-2.7548	1.3356	1.1754	C
-2.0439	2.6937	1.2727	C
4.0337	-1.0320	-0.2849	C
4.1836	-1.4123	1.0558	O
3.0255	0.3622	-1.5272	H
0.7694	4.3552	-2.0846	H
-1.3768	4.2809	-0.8490	H
0.2168	1.2159	1.7015	H
2.6319	2.8579	-1.4387	H
3.6121	1.4923	0.6087	H
2.4699	0.4697	1.4725	H
0.8833	-0.3540	-1.3479	H
-0.1396	-4.2790	-0.0367	H
-2.6004	-4.7207	-0.2003	H
-4.1950	-2.8160	-0.3531	H
-0.9015	-0.0713	-0.3036	H
-2.3661	1.2145	-0.9698	H
-4.0383	1.5977	-0.5498	H
-3.6456	1.3353	1.8117	H
-2.0966	0.5494	1.5606	H
-1.8591	2.9154	2.3298	H
-2.7053	3.4847	0.8992	H
3.9891	-1.9009	-0.9560	H
4.9274	-0.4529	-0.5401	H
3.4534	-2.0259	1.2439	H

4b_#4

0.4287	3.2390	-1.6401	C
-0.6869	3.3249	-0.8062	C
-0.6746	2.7331	0.4582	C
0.4857	2.0647	0.8665	C
1.6041	1.9515	0.0381	C
1.5647	2.5512	-1.2248	C
2.7909	1.1194	0.4623	C
2.8025	-0.2664	-0.2074	C
1.5027	-0.8850	-0.0378	N
0.9968	-1.7816	-0.9274	C
1.6685	-2.2744	-1.8238	O
-0.4625	-2.0838	-0.7384	C
-0.9304	-3.3798	-0.9236	C
-2.2890	-3.6396	-0.7335	C
-3.1675	-2.6191	-0.3914	C
-2.6972	-1.3091	-0.2420	C
-1.3373	-1.0416	-0.4067	C
-3.6353	-0.3648	0.0487	O
-3.2305	0.9968	0.0909	C
-2.5866	1.3760	1.4229	C
-1.8933	2.7482	1.3554	C
3.9241	-1.1606	0.3280	C
3.8235	-1.3694	1.7269	O
2.9577	-0.1565	-1.2885	H
0.4063	3.7041	-2.6207	H
-1.5754	3.8541	-1.1422	H
0.5116	1.6116	1.8584	H
2.4251	2.4727	-1.8848	H
3.7299	1.6293	0.2167	H
2.7647	0.9836	1.5506	H
0.9441	-0.6365	0.7654	H
-0.2348	-4.1622	-1.2071	H
-2.6695	-4.6483	-0.8589	H
-4.2273	-2.8055	-0.2526	H
-0.9450	-0.0335	-0.3185	H
-2.5630	1.2131	-0.7528	H
-4.1493	1.5712	-0.0573	H
-3.3574	1.3818	2.2001	H
-1.8562	0.6105	1.7099	H
-1.5998	3.0469	2.3675	H
-2.6036	3.5033	0.9971	H
3.8363	-2.1391	-0.1499	H
4.8984	-0.7300	0.0578	H
4.3043	-0.6792	2.1937	H

4b_#5

0.7693	3.6883	-1.0420	C
-0.4977	3.6262	-0.4614	C
-0.7768	2.6981	0.5432	C
0.2434	1.8218	0.9451	C
1.5144	1.8609	0.3664	C
1.7672	2.8125	-0.6300	C
2.5912	0.8690	0.7437	C
2.8168	-0.1631	-0.3809	C
1.5952	-0.9114	-0.6461	N
1.0140	-1.7521	0.2534	C
1.5764	-2.1516	1.2646	O
-0.4162	-2.1062	-0.0433	C
-0.8811	-3.4059	0.1316	C
-2.2408	-3.6628	-0.0498	C
-3.1323	-2.6330	-0.3354	C
-2.6633	-1.3227	-0.4664	C
-1.2953	-1.0690	-0.3648	C
-3.5943	-0.3481	-0.6677	O
-3.1853	1.0030	-0.4732	C
-2.8781	1.3070	0.9931	C
-2.1509	2.6463	1.1818	C
4.0190	-1.0700	-0.0986	C
4.2685	-1.9799	-1.1494	O
3.0281	0.3864	-1.3086	H
0.9758	4.4236	-1.8137	H
-1.2755	4.3127	-0.7861	H
0.0455	1.0881	1.7267	H
2.7562	2.8682	-1.0801	H
3.5349	1.3955	0.9304	H
2.3228	0.3316	1.6591	H
1.0546	-0.6611	-1.4599	H
-0.1881	-4.1906	0.4147	H
-2.6180	-4.6746	0.0604	H
-4.1972	-2.8160	-0.4335	H
-0.9079	-0.0602	-0.4490	H
-2.3297	1.2391	-1.1195	H
-4.0311	1.6016	-0.8202	H
-3.8171	1.3137	1.5559	H
-2.2685	0.5013	1.4148	H
-2.0562	2.8394	2.2564	H
-2.7620	3.4600	0.7733	H
3.8285	-1.6710	0.7904	H
4.8922	-0.4275	0.0879	H
4.5474	-1.4964	-1.9346	H

The conformation with the overall best fit to the ^{1}H-^{1}H NOE distances of **4a** (#208=209, Figure S2) also had a 2.25 Å distance between H11 and one of the fluorine substituents on C13 corresponding to a strong NOE as found experimentally. This conformation is proposed to be a preferred conformation of **4a** in solution. However, the second H11 to F distance was much larger corresponding to 3.95 Å for this particular conformation. Since both fluorine substituents displayed a strong ^{1}H-^{19}F NOE to H11 as observed experimentally it was suggested that this would come from a combination of more than one preferred conformation. A second solution is proposed from the conformations where the distance between H11 to the second fluorine is below 2.5 Å and the sum of the absolute ^{1}H-^{1}H difference between experiment and measured distance in the conformational ensemble is the lowest (#203, #204, Figure S3). The two conformations both have a trans amide bond and a HNCH torsional angle of -119.4 and -170.8°.

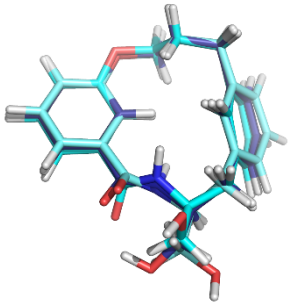


Figure S1. "Traditional" NOE fit compared to MSpin for the non-fluorinated macrocycle **4b**.

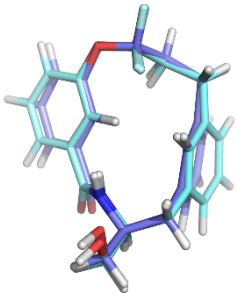


Figure S2. (#401-208) Conformation 2 compared to MSpin of **4a**.

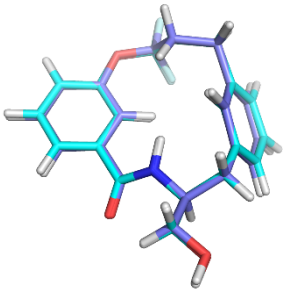
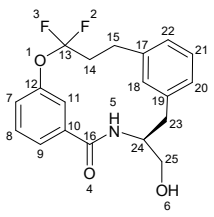
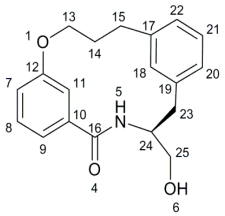


Figure S3. (#402-204) Conformation 1 compared to MSpin of **4a**.

Table S3. Torsion angles for the conformations identified as contributing to the solution conformations for Macrocycles **4a** and **4b**



4a



4b

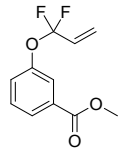
Conformation	10-16-N-24	N-24-23-19	17-15-14-13	15-14-13-O	13-O-12-7
4a$_{major}$_#1	-160.9	-48.6	97.3	-148.6	-148.5
4a$_{major}$_#2	-164.8	-55.1	-77.7	165.0	176.0
4a$_{minor}$_#1	-36.3	-71.8	-56.9	82.8	-59.6
4a$_{minor}$_#2	+26.5	-65.2	+73.2	-70.7	-45.7
4b_#1	-162.6	-54.1	-67.4	+168.7	-176.3
4b_#2	+164.2	-52.0	-67.3	+168.5	-176.4
4b_#3	+162.3	-55.9	-63.6	+169.0	+172.6
4b_#4	-167.6	-50.1	-68.0	+168.0	-174.4
4b_#5	+162.9	-60.2	-63.8	+167.5	+164.8

Synthetic experimental data

General Methods

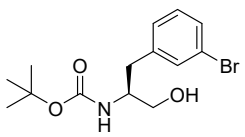
All reactions were performed in oven-dried glassware under an inert nitrogen atmosphere unless otherwise stated. The dried solvents including tetrahydrofuran (THF), dichloromethane (DCM), dimethylformamide (DMF) and methanol were purchased from Sigma Aldrich in 99.8% purity. All reagents were used as received, unless otherwise stated. Reactions were magnetically stirred and monitored by 19F NMR, LCMS or thin layer chromatography (TLC) using Merck Silica Gel 60 F254 plates and were visualized by fluorescence quenching at 254 nm. Solvents were evaporated under reduced pressure at 40 °C using a Buchi Rotavapor unless otherwise stated. Flash chromatography was performed using silica gel (Biotage® SNAP Cartridge KP-Sil) as the stationary phase. Optical rotation was measured with a polarimeter at 20 °C and 589 nm. Proton magnetic resonance spectra (1H NMR), fluorine magnetic spectra (19F NMR) and carbon magnetic resonance spectra (13C NMR) were respectively recorded at 500 MHz, 470 MHz and 125 MHz. Chemical shifts (δ) are reported in parts per million (ppm) and are referenced to the residual solvent peak. The order of citation in parentheses is (1) number of equivalent nuclei (by integration), (2) multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, sext = sextet, sept = septet, m = multiplet, br = broad, appt = apparent), (3) and coupling constant (J) quoted in Hertz to the nearest 0.1 Hz.

Methyl-3-((1',1'-difluoroallyl)oxy)benzoate, 5a



To a solution of methyl-3-hydroxybenzoate (1.0 g, 6.6 mmol) in THF (10 mL), was added sodium hydride (0.29 g, 7.2 mmol). After 5 minutes, $Pd(OAc)_2$ (0.02 g, 0.07 mmol), triphenylphosphine (0.07 g, 0.3 mmol) and THF (30 mL) were added sequentially. The reaction mixture was cooled to 0 °C, before a solution of 3-bromo-3,3-difluoroprop-1-ene (0.74 mL, 7.2 mmol) in THF (5 mL) was added and the reaction was heated to 40 °C until completion (20 h). The reaction was quenched with $NaHCO_3$ (30 mL), extracted with dichloromethane (3 × 20 mL) and the combined organics were dried over Na_2SO_4. After removal of the solvent under reduced pressure, the residue was purified by flash column chromatography (silica gel, 0 – 5% EtOAc in heptane) to give the desired product **5a** (1.4 g, 6.2 mmol, 95%) as a colourless oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 3.93 (3H, s), 5.62 (1H, d, J_H = 10.7 Hz), 5.94 (1H, dt, J_H = 1.9, 17.3 Hz), 6.07 (1H, ddt, J_{H+F} = 6.6, 10.6, 17.2 Hz), 7.37 – 7.47 (2H, m), 7.87 (1H, br s), 7.91 (1H, dd, J_H = 1.6, 7.2 Hz). ^{19}F NMR ($CDCl_3$, 470 MHz) δ: -68.9. ^{13}C NMR ($CDCl_3$, 126 MHz) δ: 52.5, 120.8 (t, J_F = 260.3 Hz), 122.2 (t, J_F = 6.5 Hz), 123.2 (t, J_F = 1.0 Hz), 126.5 (t, J_F = 1.26 Hz), 126.9, 129.3 (t, J_F = 33.5 Hz), 129.5, 131.8, 150.4 (t, J_F = 2.0 Hz), 166.3. *m/z* [EI (+ve)] 228.1[M+], HRMS found *m/z* $[M]^+$ 228.0588, $C_{11}H_{10}F_2O_3$ requires 228.0593. The spectral data is in agreement with the literature values.[6]

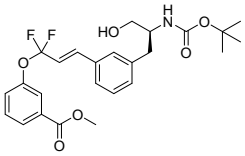
tert-Butyl (S)-(1-(3-bromophenyl)-3-hydroxypropan-2-yl)carbamate, 6



To a -10 °C solution of (*S*)-3-(3-bromophenyl)-2-((*tert*-butoxycarbonyl)amino)propanoic acid (2.5 g, 7.3 mmol) in THF (20 mL) were added *N*-methylmorpholine (0.80 mL, 7.3 mmol) and isobutyl chloroformate (0.95 mL, 7.3 mmol) and the reaction

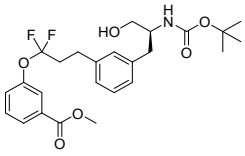
was stirred for 1 h. The precipitate was removed by filtration and the residue was washed with THF (3 × 10 mL). The filtrate was cooled to 0 °C, and $NaBH_4$ (0.55 g, 15 mmol) was added sequentially. The reaction mixture was stirred for 3 h before being quenched with 40 mL of water at 0 °C. The product precipitated on addition of H_2O and was isolated by filtration. The crude residue was washed with H_2O (2 × 15 mL) and heptane (2 × 15 mL), before drying *in vacuo* to yield alcohol **6** (2.1 g, 6.2 mmol, 85%) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.42 (9H, s), 2.13 – 2.20 (1H, m), 2.82 (2H, d, J_H = 7.1 Hz), 3.56 (1H, dt, J_H = 4.8, 10.5 Hz), 3.67 (1H, dt, J_H = 4.5, 9.2 Hz), 3.83 (1H, br s), 4.69 – 4.76 (1H, m), 7.13 – 7.19 (2H, m), 7.34 – 7.39 (2H, m). ^{13}C NMR (DMSO, 126 MHz) δ: 28.2 (3C), 36.3, 53.7, 63.1, 74.4, 121.3, 128.2, 128.6, 130.1, 131.8, 142.3, 156.1. HRMS found *m/z* $[M]^+$ 330.0705, $C_{14}H_{20}BrNO_3$ requires 330.0710. $[\alpha]_D$ = -14.0°.

Methyl-(*S*,*E*)-3-((3-(3-(2-((*tert*-butoxycarbonyl)amino)-3-hydroxypropyl)phenyl)-1,1-difluoroallyl)oxy)benzoate, 7a



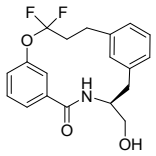
Alkene **5a** (0.89 g, 3.9 mmol) was dissolved in DMF (80 mL). Carbamate **6** (1.4 g, 4.1 mmol), $Pd(OAc)_2$ (0.09 g, 0.4 mmol), tri-*o*-tolylphosphane (0.24 g, 0.78 mmol) and Et_3N (1.63 mL, 11.7 mmol) where added sequentially and the reaction was heated to 110 °C in a sealed tube for 72 h. After this time, the reaction was quenched with $NaHCO_3$ (80 mL) and extracted with EtOAc (3 × 40 mL). The organics were washed with brine (4 × 30 mL), dried (Na_2SO_4) and the solvent was removed *in vacuo*. The crude material was purified by flash column chromatography (0 – 40% EtOAc in heptane) then repeated (0 – 30% EtOAc in heptane) to yield the *trans* alkene **7a** (1.57 g, 3.3 mmol, 84%) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.42 (9H, s), 2.09 – 2.17 (1H, m), 2.88 (2H, d, J_H = 7.5 Hz), 3.54 – 3.61 (1H, m), 3.65 – 3.72 (1H, m), 3.88 (1H, br s), 3.93 (3H, s), 4.62 – 4.77 (1H, m), 6.34 (1H, dt, J_{H+F} = 6.7, 15.9 Hz), 7.17 – 7.24 (2H, m), 7.31 – 7.38 (3H, m), 7.41 – 7.50 (2H, m), 7.88 – 7.94 (2H, m). ^{19}F NMR ($CDCl_3$, 470 MHz) δ: -65.8. ^{13}C NMR ($CDCl_3$, 126 MHz) δ: 28.5 (3C), 37.4, 52.5, 53.7, 64.3, 79.7, 119.6 (J_F = 33.5 Hz), 121.7 (t, J_F = 259.6 Hz), 123.1, 125.8, 126.5, 126.9, 128.4, 129.2, 129.6, 130.7, 131.8, 134.5, 136.4 (t, J_F = 6.5 Hz), 138.8, 150.5, 156.2, 166.4. HRMS found *m/z* $[M]^+$ 478.2119, $C_{25}H_{29}F_2NO_6$ requires 478.1996. $[\alpha]_D$ = -6.0°.

Methyl-(*S*)-3-(3-(3-(2-((*tert*-butoxycarbonyl)amino)-3-hydroxypropyl)phenyl)-1,1-difluoropropoxy)benzoate, 8a



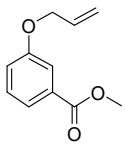
Alkene **7a** (1.0 g, 2.1 mmol) was dissolved in methanol (40 mL) and palladium on activated carbon (0.1 g, 10% bw) was added. The reaction was placed under 1 atmosphere of hydrogen and was stirred for 17 h. The resulting mixture was filtered through celite and the filtrate was evaporated *in vacuo*. The crude material was purified by flash column chromatography (0 – 30% EtOAc in heptane) to yield the saturated product **8a** (0.97 g, 2.0 mmol, 96%) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.42 (9H, s), 2.35 (1H, br s), 2.40 - 2.53 (2H, m), 2.75 – 2.89 (2H, m), 2.91 – 3.01 (2H, m), 3.51 – 3.61 (1H, m), 3.62 – 3.72 (1H, m), 3.86 (1H, br s), 3.93 (3H, s), 4.77 (1H, br s), 7.07 – 7.13 (2H, m), 7.21 – 7.31 (2H, m), 7.37 – 7.47 (2H, m), 7.83 – 7.95 (1H, m), 7.90 (1H, dt, J_H = 7.3 Hz). ^{19}F NMR ($CDCl_3$, 470 MHz) δ: -70.9. ^{13}C NMR ($CDCl_3$, 126 MHz) δ: 28.5 (3C), 28.9, 37.8 (t, J_F = 28.4 Hz), 52.5, 53.9, 64.5, 67.2, 79.9, 120.2, 123.0, 124.7 (t, J_F = 267.9 Hz), 126.4, 126.6, 126.7, 127.5, 129.0, 129.5, 129.6, 131.7, 138.4, 140.3, 150.5 (t, J_F = 1.7 Hz), 166.4. HRMS found *m/z* $[M]^+$ 480.2298, $C_{25}H_{31}F_2NO_6$ requires 480.2153. $[\alpha]_D$ = -13.2°.

(*S*)-3,3-Difluoro-8-(hydroxymethyl)-2-oxa-9-aza-1,6(1,3)-dibenzenacyclodecaphan-10-one, 4a



Sodium hydroxide (1M) was added to a solution of **8a** (0.10 g, 0.21 mmol) in MeOH (10 mL) until the pH reached 14 and the resulting mixture was stirred for 30 mins. Following this time, reaction was acidified with $KHSO_3$ until a pH of 2 was reached. The aqueous mixture was extracted with EtOAc (3 × 15 mL), the organics were dried (Na_2SO_4) and the solvent was removed *in vacuo*. The crude residue was redissolved in HCl (5 mL, 4M in dioxane) and the resulting mixture was stirred for 2 h. The solvent was removed *in vacuo* to give the crude amine. The crude amine (0.09 g, 0.2 mmol) was dissolved in CH_2Cl_2 (43 mL) along with pyBOP (0.34 g, 0.66 mmol). DIPEA (0.23 mL, 1.3 mmol) was added slowly, and the mixture was stirred for 17 h. The reaction mixture was washed with H_2O (2 × 20 mL) and brine (20 mL), before being dried (Na_2SO_4) and the solvent was removed *in vacuo*. The crude residue was purified by mass triggered HPLC (5 – 90% MeCN in H_2O, pH 10) to yield macrocycle **4a** (0.01 g, 0.04 mmol, 19%) as a white solid. Major conformation: ^{1}H NMR (DMSO, 500 MHz) δ: 2.19 – 2.32 (1H, m), 2.63 (1H, t, J_H = 12.2 Hz), 2.89 – 2.97 (2H, m), 3.02 – 3.16 (2H, m), 3.43 (1H, dt, J_H = 6.6, 10.7 Hz), 3.62 (1H, dt, J_H = 4.3, 9.0 Hz), 3.80 – 3.89 (1H, m), 4.91 (1H, t, J_H = 5.1 Hz), 6.84 (1H, s), 7.07 (1H, t, J_H = 8.1 Hz), 7.09 (1H, t, J_H = 8.2 Hz), 7.16 (1H, dd, J_H = 2.0, 8.4 Hz), 7.19 (1H, t, J_H = 7.5 Hz), 7.28 (1H, d, J_H = 7.6 Hz), 7.39 (1H, t, J_H = 7.9 Hz), 7.57 (1H, s), 7.99 (1H, dt, J_H = 8.4 Hz). ^{19}F NMR (DMSO, 470 MHz) δ: -66.5 (1F, d, J_F = 150.6 Hz), -65.0 (1F, d, J_F = 150.6 Hz). ^{13}C NMR (DMSO, 126 MHz) δ: 27.8, 33.1 (t, J_F = 30.1 Hz), 36.7, 54.0, 63.8, 118.6, 121.9, 123.1, 126.5, 126.8, 127.1 (t, J_F = 266 Hz), 128.7, 130.7, 131.4, 138.3, 139.2, 140.1, 149.9 (t, J_F = 3.5 Hz), 167.3. Minor conformation: ^{1}H NMR (DMSO, 500 MHz) δ: 2.36 (1H, t, J_H = 12.0 Hz), 2.38 – 2.48 (1H, m), 2.56 – 2.63 (1H, m), 2.75 (1H, d, J_H = 12.8 Hz), 2.74 – 2.80 (1H, m), 2.87 – 2.92 (1H, m), 2.96 – 3.02 (1H, t, J_H = 12.8 Hz), 3.39 (2H, t, J_H = 5.7 Hz), 4.59 (s, 1H), 4.91 (1H, t, J_H = 5.1 Hz), 6.83 (1H, s), 7.02 (1H, d, J_H = 7.5 Hz), 7.05 – 7.08 (1H, m), 7.08 – 7.10 (1H, m), 7.25 (1H, d, J_H =7.6 Hz), 7.31 (1H, t, J_H = 7.6 Hz), 7.32 (1H, t, J_H = 7.8 Hz), 7.54 (1H, d, J_H = 11.0 Hz). ^{19}F NMR (DMSO, 470 MHz) δ: -61.6 (1F, d, J_F = 150.4 Hz), -59.6 (1F, d, J_F = 150.4 Hz). ^{13}C NMR (DMSO, 126 MHz) δ: 28.8, 34.2 (t, J_F = 28.7 Hz), 37.6, 59.5, 64.6, 119.4, 119.8, 122.9, 126.2, 127.4, 127.9, 128.2, 129.6, 130.4, 137.9, 139.4, 140.0, 149.1 (t, J_F = 3.1 Hz), 171.2. General data: HRMS found *m/z* $[M]^+$ 348.1429, $C_{19}H_{19}F_2NO_3$ requires 348.1411. $[\alpha]_D$ = -7.2°.

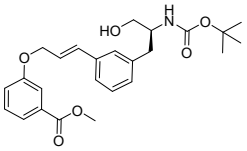
Methyl 3-(allyloxy)benzoate, 5b



Methyl 3-hydroxybenzoate (1.0 g, 6.6 mmol) and potassium carbonate (1.8 g, 13 mmol) were dissolved in acetone (50 mL). 3-bromoprop-1-ene (1.1 mL, 13 mmol) was added and the mixture was heated to 50 °C for 17 h. The reaction was quenched with $NaHCO_3$ (30 mL), extracted with EtOAc (3 × 20 mL) and washed with brine (5 × 20 mL). The organics were dried (Na_2SO_4), filtered and evaporated to dryness. The crude residue was purified by flash column chromatography (5% EtOAc in heptane) to yield alkene **5b** (1.1 g, 5.5 mmol, 84%) as a colourless oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 3.91 (3H, s), 4.58 (2H, dt, J_H = 1.5, 5.3 Hz), 5.30 (1H, dd, J_H = 1.4, 10.5 Hz), 5.43 (1H, dd, J_H = 1.6, 17.3 Hz), 6.06 (1H, ddt, J_H = 5.3, 10.5, 17.2 Hz), 7.12 (1H, ddd, J_H = 1.0, 2.7, 8.2 Hz), 7.34 (1H, t, J_H = 8.0 Hz), 7.57 (1H, dd, J_H = 1.5, 2.6 Hz), 7.63 (1H, dt, J_H = 1.2, 7.7 Hz). ^{13}C NMR ($CDCl_3$, 126

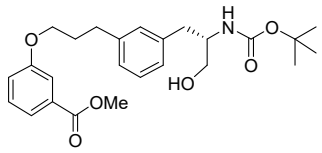
MHz) δ: 52.3, 69.0, 115.0, 118.1, 120.3, 122.3, 129.5, 131.5, 133.0, 158.6, 167.1. The spectral data is in agreement with the literature values.[7]

Methyl (*S*,*E*)-3-((3-(3-(2-((*tert*-butoxycarbonyl)amino)-3-hydroxypropyl)phenyl)allyl)oxy) benzoate, 7b



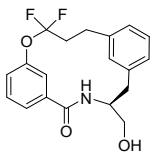
Alkene **5b** (1.1 g, 5.7 mmol) was dissolved in MeCN (20 mL). Carbamate **6** (0.75 g, 2.3 mmol), $Pd(OAc)_2$ (0.05 g, 0.2 mmol), tri-*o*-tolylphosphane (0.14 g, 0.45 mmol) and DIPEA (0.79 mL, 4.5 mmol) where added sequentially and the reaction was heated to 90 °C in a sealed tube for 2 d. After this time, the reaction was quenched with $NaHCO_3$ (40 mL) and extracted with EtOAc (3 × 20 mL). The organics were washed with brine (4 × 20 mL), dried and the solvent was removed *in vacuo*. The crude material was purified by flash column chromatography (0 – 40% EtOAc in heptane) to yield disubstituted alkene **7b** (0.28 g, 0.31 mmol, 28%) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.41 (9H, s), 2.84 (2H, d, J_H = 7.1 Hz), 3.56 (1H, dd, J_H = 5.0, 10.8 Hz), 3.67 (1H, dd, J_H = 3.1, 10.8 Hz), 3.87 (1H, br s), 3.92 (3H, s), 4.74 (2H, dd, J_H = 1.2, 5.8 Hz), 4.74 – 4.79 (1H, m), 6.41 (1H, dt, J_H = 5.7, 16.0 Hz), 6.72 (1H, d, J_H = 16.0), 7.10 – 7.14 (1H, m), 7.16 (1H, ddd, J_H = 0.79, 2.6, 8.2 Hz), 7.25 – 7.30 (3H, m), 7.35 (1H, t, J_H = 8.0), 7.62 (1H, dd, J_H = 1.5, 2.4), 7.65 (1H, d, J_H = 1.1, 8.2). ^{13}C NMR ($CDCl_3$, 126 MHz) δ: 28.5 (3C), 37.4, 52.5, 53.8, 64.4, 68.8, 79.9, 115.1, 120.3, 122.3, 124.3, 125.0, 127.7, 129.0, 129.1, 129.6, 131.6, 133.2, 136.8, 138.4, 156.3, 158.7, 167.1. HRMS found *m/z* $[M]^+$ 442.2230, $C_{25}H_{31}NO_6$ requires 442.2206. $[\alpha]_D$ = -10.4°.

Methyl (*S*)-3-(3-(3-(2-((*tert*-butoxycarbonyl)amino)-3-hydroxypropyl)phenyl)propoxy) benzoate, 8b



Alkene **7b** (0.23 g, 0.52 mmol) was dissolved in methanol (10 mL) and palladium on activated carbon (0.06 g, 0.5 mmol) was added. The reaction was placed under 1 atmosphere of hydrogen and was stirred for 17 h. The resulting mixture was filtered through celite and evaporated *in vacuo*. The crude material was purified by flash column chromatography (0 – 30% EtOAc in heptane) to yield saturated product **8b** (0.18 g, 0.41 mmol, 78%) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.42 (9H, s), 2.07 – 2.14 (2H, m), 2.39 (1H, br s), 2.76 – 2.85 (4H, m), 3.49 – 3.53 (1H, m), 3.61 – 3.65 (1H, m), 3.80 (1H, br s), 3.91 (3H, s), 3.99 (1H, t, J_H = 6.3 Hz), 4.78 (1H, br s), 7.04 – 7.09 (3H, m), 7.10 (1H, ddd, J_H = 0.89, 2.7, 8.2 Hz), 7.22 (1H, t, J_H = 8.0 Hz), 7.33 (1H, t, J_H = 7.9 Hz), 7.53 (1H, dd, J_H = 1.5, 2.6), 7.62 (1H, dt, J_H = 1.2, 7.7 Hz). ^{13}C NMR ($CDCl_3$, 126 MHz) δ: 28.5 (3C), 30.9, 32.2, 37.5, 52.4, 53.8, 64.3, 67.2, 79.8, 114.7, 120.2, 122.0, 126.8, 127.1, 128.8, 129.5, 129.8, 131.5, 138.1, 141.8, 156.3, 159.1, 167.2. HRMS found *m/z* $[M]^+$ 444.2386, $C_{25}H_{33}NO_6$ requires 444.2404. $[\alpha]_D$ = -13.2°.

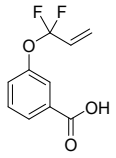
(*S*)-8-(Hydroxymethyl)-2-oxa-9-aza-1,6(1,3)-dibenzenacyclodecaphan-10-one, 4b



Sodium hydroxide (1M) was added to a solution of **8b** (0.16 g, 0.36 mmol) in MeOH (10 mL) until the pH reached 14 and the resulting mixture was stirred for 30 mins. Following this time, the reaction was acidified with $KHSO_3$ (1M) until a pH of 2 was

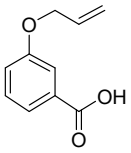
reached. The aqueous mixture was extracted with EtOAc (3 × 15 mL), the organics were dried (Na_2SO_4) and the solvent was removed *in vacuo*. The crude residue was redissolved in HCl (5 mL, 4M in dioxane) and the resulting mixture was stirred for 2 h. The solvent was removed *in vacuo* to give the crude amine. To a solution of the crude amine (0.13 g, 0.36 mmol) and PyBOP (0.57 g, 1.1 mmol) in DMF (60 mL), DIPEA (0.38 mL, 2.2 mmol) was added and the mixture was stirred for a further 17 hours. The solvent was removed *in vacuo* and redissolved in EtOAc (10 mL). The solution was washed with brine (4 × 20 mL), before being dried (Na_2SO_4), filtered and evaporated to dryness. The crude residue was purified flash column chromatography (0 – 100% EtOAc in heptane) to yield macrocycle **4b** (0.04 g, 0.1 mmol, 36%) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 2.07 (1H, tt, J_H = 6.4, 12.9 Hz), 2.27 – 2.32 (1H, m), 2.38 (1H, dt, J_H = 1.5, 13.0 Hz), 2.63 (1H, dd, J_H = 11.5, 13.1 Hz), 2.84 (1H, dd, J_H = 5.6, 12.4 Hz), 3.09 (1H, dd, J_H = 4.0, 13.0 Hz), 3.35 – 3.44 (1H, m), 3.59 – 3.68 (1H, m), 3.68 (1H, dd, J_H = 1.2, 12.2 Hz), 3.84 – 3.92 (1H, m), 4.22 (1H, ddd, J_H = 7.1, 11.0, 12.3 Hz), 4.90 (1H, t, J_H = 5.6 Hz), 6.17 (1H, s), 6.84 (1H, dd, J_H = 1.9, 8.2 Hz), 6.94 (1H, d, J_H = 7.4 Hz), 7.04 (1H, d, J_H = 7.4 Hz), 7.13 (1H, d, J_H = 7.6 Hz), 7.21 (1H, t, J_H = 7.8 Hz), 7.22 (1H, t, J_H = 7.5 Hz), 7.61 (1H, s), 7.77 (1H, d, J_H = 8.1 Hz). ^{13}C NMR ($CDCl_3$, 126 MHz) δ: 27.1, 30.1, 36.7, 53.7, 63.9, 65.4, 111.3, 118.3, 118.6, 126.4, 127.6, 128.3, 129.5, 131.2, 138.9, 139.7, 140.4, 157.5, 168.7. HRMS found *m/z* $[M]^+$ 312.1599, $C_{19}H_{21}NO_3$ requires 312.1614. $[\alpha]_D$ = -54.8°.

3-((1,1-Difluoroallyl)oxy)benzoic acid, 9a



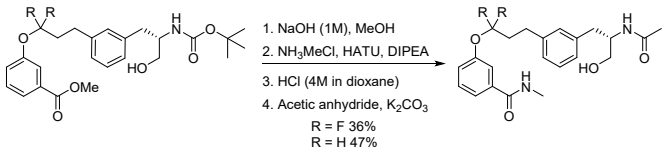
Methyl-3-((1,1-difluoroallyl)oxy)benzoate **5a** (0.03 g, 0.1 mmol) was dissolved in MeOH (2 mL). Sodium hydroxide (1M) was added until a pH of 14 was reached and the mixture was stirred at room temperature for 1 h. The solvent was removed and CH_2Cl_2 (20 mL) was added. HCl (3.8 M) was added slowly until all the solid was dissolved and the mixture was extracted with CH_2Cl_2 (2 × 10 mL), dried (Na_2SO_4), filtered and evaporated to give acid **9a** (0.03 g, 0.1 mmol, 99%) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 5.63 (1H, d, J_H = 10.7 Hz), 5.96 (1H, d, J_H = 17.3 Hz), 6.00 – 6.17 (1H, m), 7.45 – 7.45 (2H, m), 7.94 (1H, br s), 7.95 – 7.99 (1H, m). ^{19}F NMR ($CDCl_3$, 470 MHz) δ: -69.0. ^{13}C NMR ($CDCl_3$, 126 MHz) δ: 120.7 (t, J_F = 260.5 Hz), 122.2 (t, J_F = 6.6 Hz), 123.5 (t, J_F = 1.1 Hz), 127.2, 127.3, 129.0 (t, J_F = 33.3 Hz), 129.6, 130.7, 150.3 (t, J_F = 1.9 Hz), 171.0. HRMS found *m/z* $[M]^+$ 213.0367, $C_{10}H_8F_2O_3$ requires 213.0360.

3-(Allyloxy)benzoic acid, 9b

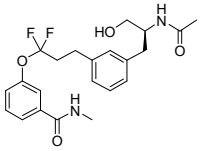


Methyl 3-(allyloxy)benzoate **5b** (0.52 g, 2.7 mmol) was dissolved in methanol (20 mL). sodium hydroxide (0.11 g, 2.7 mmol) was added and the mixture was stirred for 1 h. $KHSO_4$ (10 mL) was added and the mixture was extracted with EtOAc (3 × 10 mL). The organics were dried (Na_2SO_4), filtered and evaporated to give acid **9b** (0.48 g, 2.7 mmol, 100%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 4.61 (2H, dt, J_H = 1.5, 5.3 Hz), 5.32 (1H, dd, J_H = 1.3, 10.5 Hz), 5.44 (1H, dd, J_H = 1.5, 17.3 Hz), 6.07 (1H, ddt, J_H = 5.3, 10.5, 17.2 Hz), 7.18 (1H, ddd, J_H = 0.9, 2.7, 8.2 Hz), 7.38 (1H, t, J_H = 8.0 Hz), 7.63 (1H, dd, J_H = 1.5, 2.5 Hz), 7.72 (1H, dt, J_H = 1.2, 7.6 Hz). ^{13}C NMR ($CDCl_3$, 126 MHz) δ: 69.1, 115.5, 118.2, 121.3, 123.0, 129.7, 130.6, 132.9, 158.7, 172.1. HRMS found *m/z* $[M]^+$ 177.0557, $C_{10}H_{10}O_3$ requires 177.0552. The spectral data is in agreement with the literature values.[8]

Synthesis of linear match-pair analogues

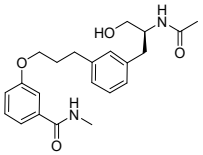


(*S*)-3-(3-(3-(2-Acetamido-3-hydroxypropyl)phenyl)-1,1-difluoropropoxy)-*N*-methylbenzamide, 10a



Ester **8a** (0.19 g, 0.39 mmol) was dissolved in methanol (10 mL) and NaOH (1M) was added until a pH of 13 was reached. The mixture was stirred for 30 mins before being acidified with $KHSO_3$ (1M) to pH 2. The aqueous mixture was extracted with EtOAc (3 × 10 mL) before being dried (Na_2SO_4), filtered and evaporated to dryness. The crude acid was redissolved in CH_2Cl_2 (5 mL) before HATU (0.22 g, 0.58 mmol), DIPEA (0.27 mL, 1.6 mmol) and methylamine hydrochloride (0.05 g, 0.8 mmol) were added sequentially. After stirring at room temperature for 2 h, the mixture was concentrated and purified by flash column chromatography (0 – 100% EtOAc in heptane) to yield the amide (0.10 g, 0.20 mmol, 52%) as a colourless oil. The amide (0.03 g, 0.05 mmol) was dissolved in HCl (1 mL, 4M in doxane) and stirred for 2 h. Following this time, the reaction mixture was concentrated *in vacuo* to yield the crude amine. To a solution of the crude amine in MeOH (1.5 mL) was added potassium carbonate (0.02 g, 0.2 mmol) and acetic anhydride (0.05 mL, 0.05 mmol) and the reaction was stirred at room temperature for 1 h. The reaction was quenched with $NaHCO_3$ (5 mL), extracted with EtOAc (3 × 5 mL) and washed with brine (5 mL). The organics were dried (Na_2SO_4), filtered and the solvent was removed *in vacuo*. The crude residue was purified by flash column chromatography (5% MeOH in CH_2Cl_2) to yield desired product **10a** (0.02 g, 0.03 mmol, 69%) as a colourless oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.92 (3H, s), 2.41 – 2.52 (2H, m), 2.87 (2H, dd, J_H = 2.9, 7.3 Hz), 2.94 (2H, dd, J_H = 7.0, 9.0 Hz), 2.97 (1H, t, J_H = 5.1 Hz), 3.01 (3H, d, J_H = 4.8 Hz), 3.58 (1H, dt, J_H = 4.8, 10.7 Hz), 3.67 (1H, dt, J_H = 4.0, 10.8 Hz), 4.16 (1H, dddd, J_H = 2.4, 4.4, 7.5, 11.8 Hz), 5.96 (1H, d, J_H = 7.6 Hz), 6.35 – 6.52 (1H, m), 7.06 – 7.13 (3H, m), 7.23 – 7.27 (1H, m), 7.31 (1H, ddt, J_H = 0.9, 1.2, 8.2 Hz), 7.40 (1H, t, J_H = 7.9 Hz), 7.53 (1H, t, J_H = 1.8 Hz), 7.58 (1H, ddd, J_H = 1.0, 1.6, 7.6 Hz). ^{19}F NMR ($CDCl_3$, 470 MHz) δ: -69.9, -69.9. ^{13}C NMR ($CDCl_3$, 126 MHz) δ: 23.5, 27.1, 29.0 (t, J_F = 3.3 Hz), 37.0, 37.4 (t, J_F = 28.3 Hz), 53.0, 64.1, 120.6, 123.8, 124.7, 124.8 (t, J_F = 267.4 Hz), 126.8, 127.4, 129.0, 129.6, 129.7, 136.2, 138.2, 140.4, 150.6 (t, J_F = 1.8 Hz), 167.6, 170.9. HRMS found *m/z* $[M]^+$ 421.1939, $C_{22}H_{26}F_2N_2O_4$ requires 421.1949. $[\alpha]_D$ = -6.8°.

(*S*)-3-(3-(3-(2-Acetamido-3-hydroxypropyl)phenyl)propoxy)-*N*-methylbenzamide, 10b

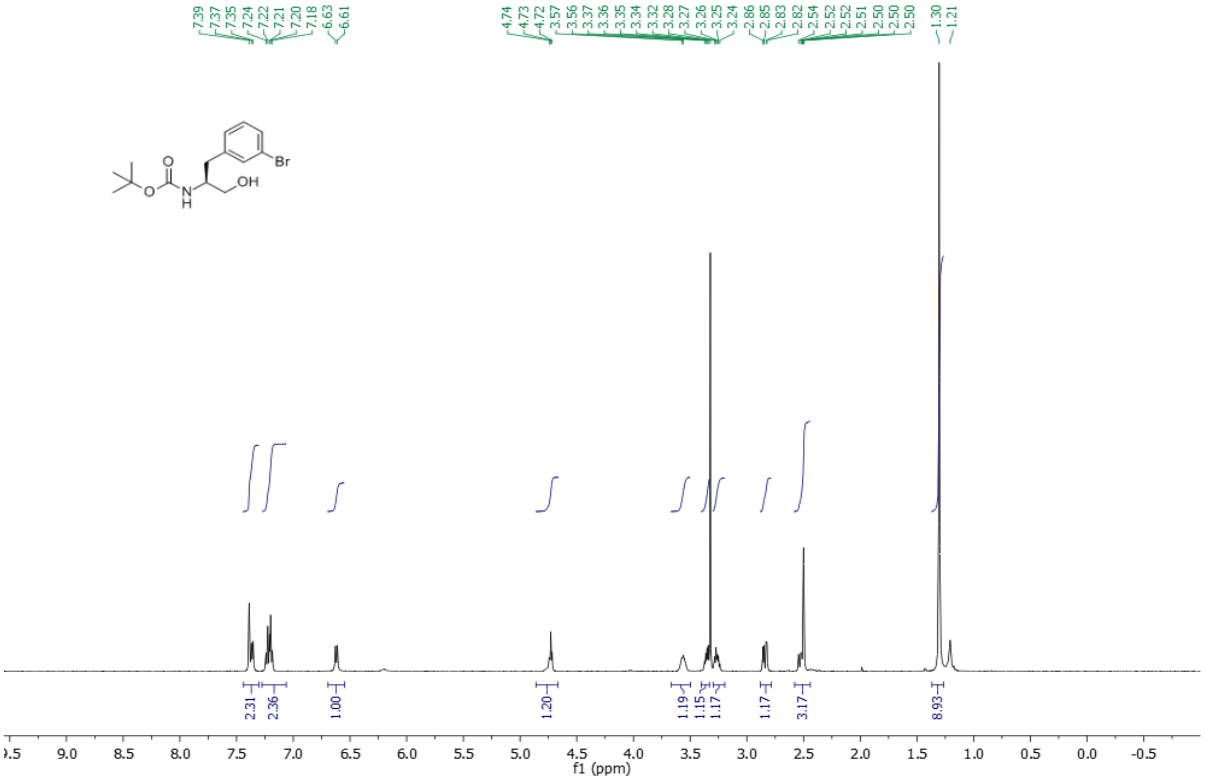


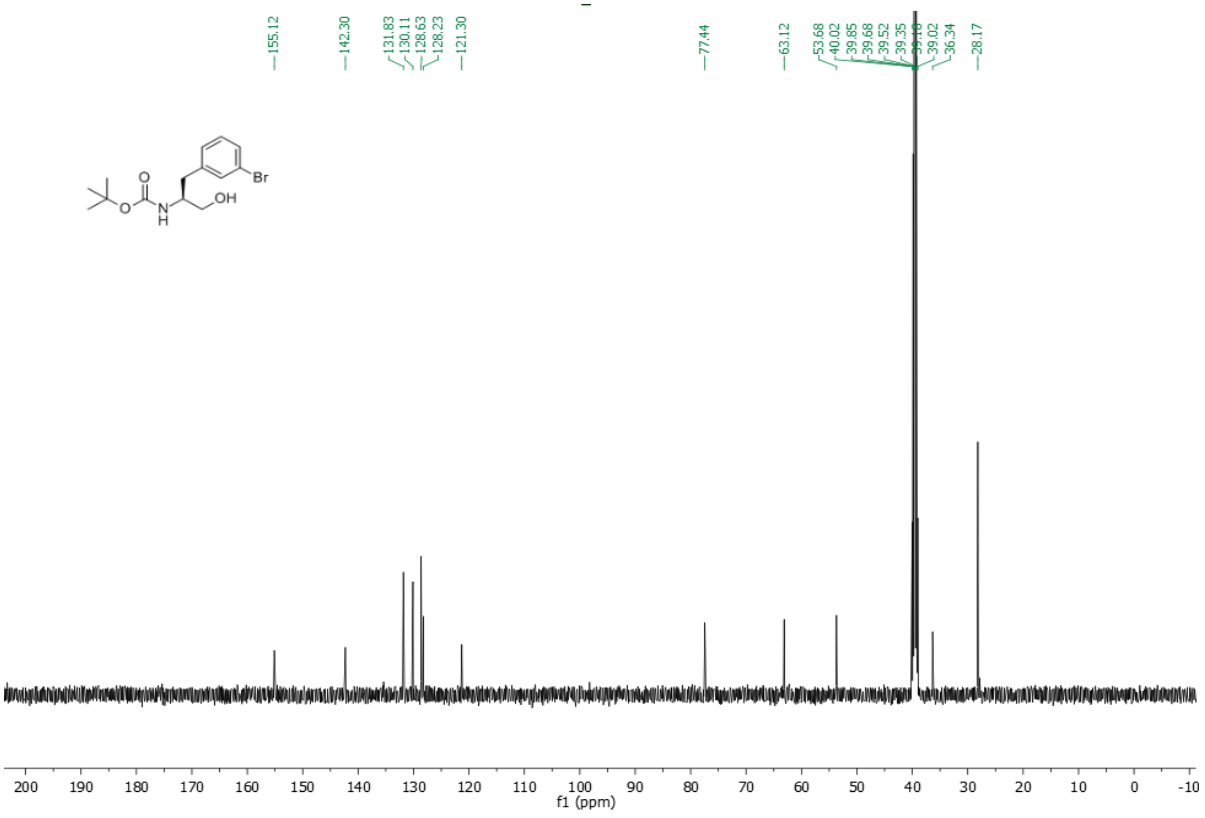
Ester **8b** (0.17 g, 0.38 mmol) was dissolved in methanol (10 mL) and NaOH (1M) was added until a pH of 13 was reached. The mixture was stirred for 30 mins before being acidified with $KHSO_3$ (1M) to pH 2. The aqueous mixture was extracted with EtOAc before being dried (Na_2SO_4), filtered and evaporated to dryness. The crude acid was redissolved in CH_2Cl_2 (5 mL) before HATU (0.22 g, 0.57 mmol), DIPEA (0.34 mL, 1.9 mmol) and methylamine hydrochloride (0.05 g, 0.8 mmol) were added

sequentially. After stirring at room temperature for 2 h, the mixture was concentrated and purified by flash column chromatography (0 – 100% EtOAc in heptane) to yield the amide intermediate (0.10 g, 0.22 mmol, 58%) as a colourless oil. The amide intermediate (0.09 g, 0.2 mmol) was dissolved in HCl (1 mL, 4M in dioxane) and stirred for 2 h. Following this time, the reaction mixture was concentrated *in vacuo* to yield the crude amine. To a solution of the crude amine in MeOH (4 mL) was added potassium carbonate (0.09 g, 0.7 mmol) and acetic anhydride (0.02 mL, 0.2 mmol) and the reaction was stirred at room temperature for 1 h. The reaction was quenched with $NaHCO_3$ (5 mL), extracted with EtOAc (3 × 5 mL) and washed with brine (5 mL). The organics were dried (Na_2SO_4) and the solvent was removed *in vacuo*. The crude residue was purified by flash column chromatography (5% MeOH in CH_2Cl_2) to yield the desired product **10b** (0.07 g, 0.2 mmol, 80%) as a colourless oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.92 (3H, s), 2.02 – 2.16 (2H, m), 2.71 – 2.82 (2H, m), 2.81 (2H, d, J_H = 7.3 Hz), 3.97 (3H, d, J_H = 4.8 Hz), 3.32 (1H, br s), 3.50 (1H, dd, J_H = 4.7, 10.7 Hz), 3.59 (1H, dd, J_H = 2.6, 10.7 Hz), 3.86 – 3.99 (2H, m), 4.10 – 4.14 (1H, m), 6.05 (1H, d, J_H = 7.7 Hz), 6.56 (1H, d, J_H = 3.8 Hz), 6.98 – 7.03 (2H, m), 7.03 – 7.08 (2H, m), 7.21 (1H, t, J_H = 7.5 Hz), 7.24 (1H, dt, J_H = 1.2, 7.6 Hz), 7.27 – 7.33 (2H, m). ^{13}C NMR ($CDCl_3$, 126 MHz) δ: 23.5, 27.0, 30.6, 32.0, 37.1, 52.9, 63.8, 66.8, 112.6, 118.7, 118.8, 126.9, 127.0, 128.8, 129.7, 130.0, 136.1, 138.1, 159.4, 167.6, 168.6, 170.7. HRMS found *m/z* $[M]^+$ 385.2127, $C_{22}H_{28}N_2O_4$ requires 385.2130. $[\alpha]_D$ = -8.4°.

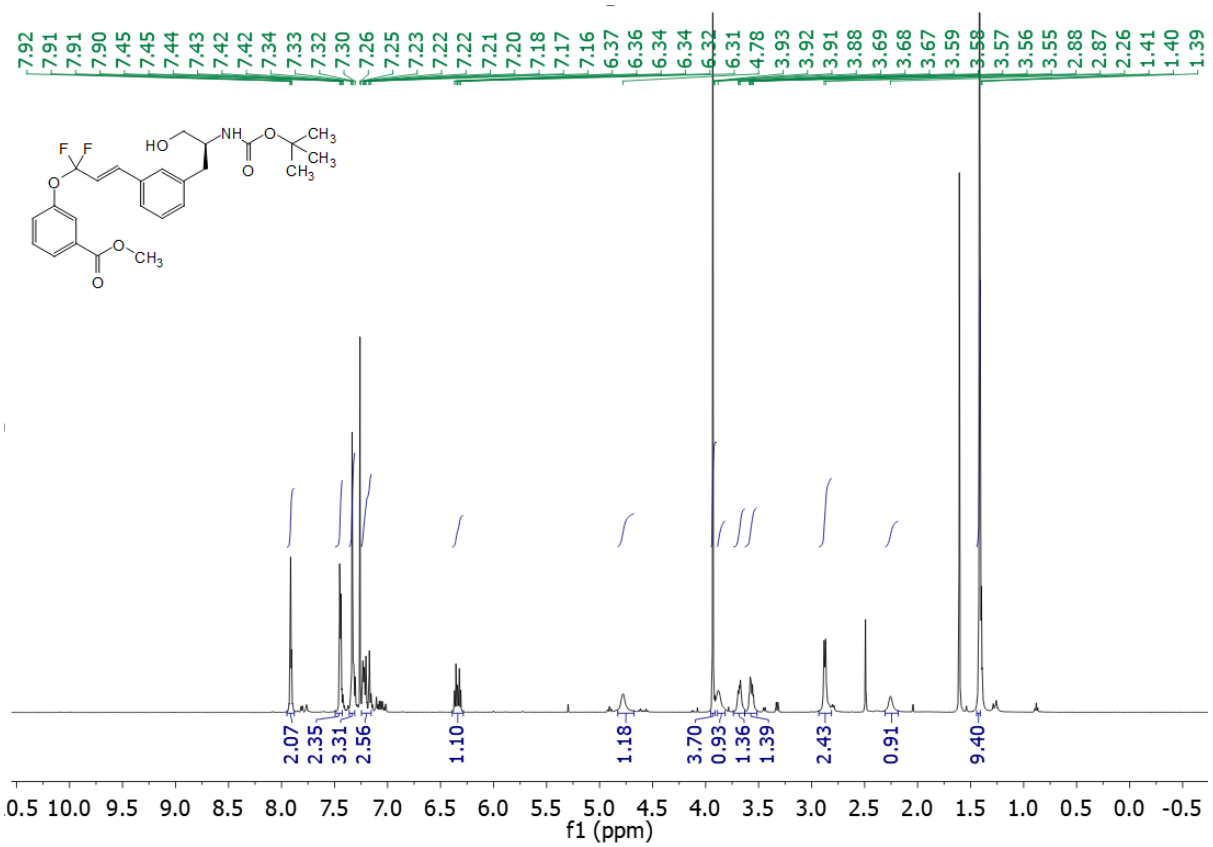
Synthetic spectral data

tert-Butyl (S)-(1-(3-bromophenyl)-3-hydroxypropan-2-yl)carbamate, 6

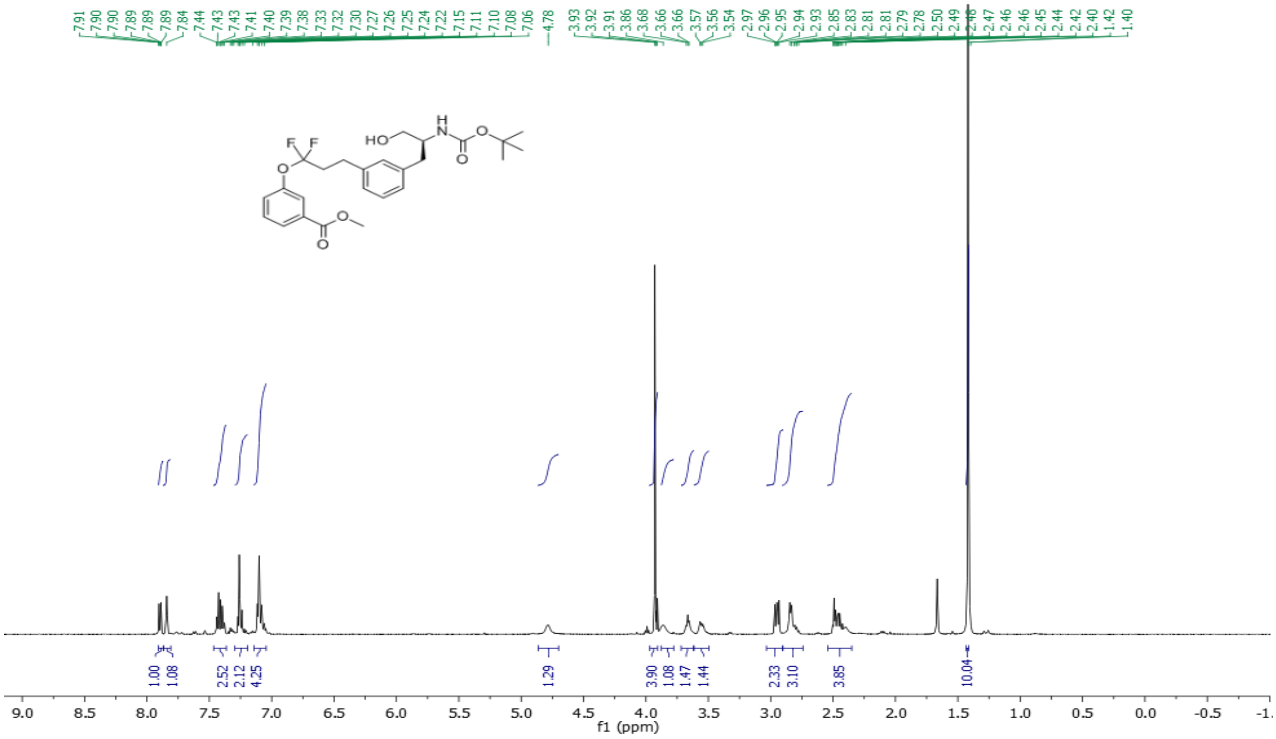


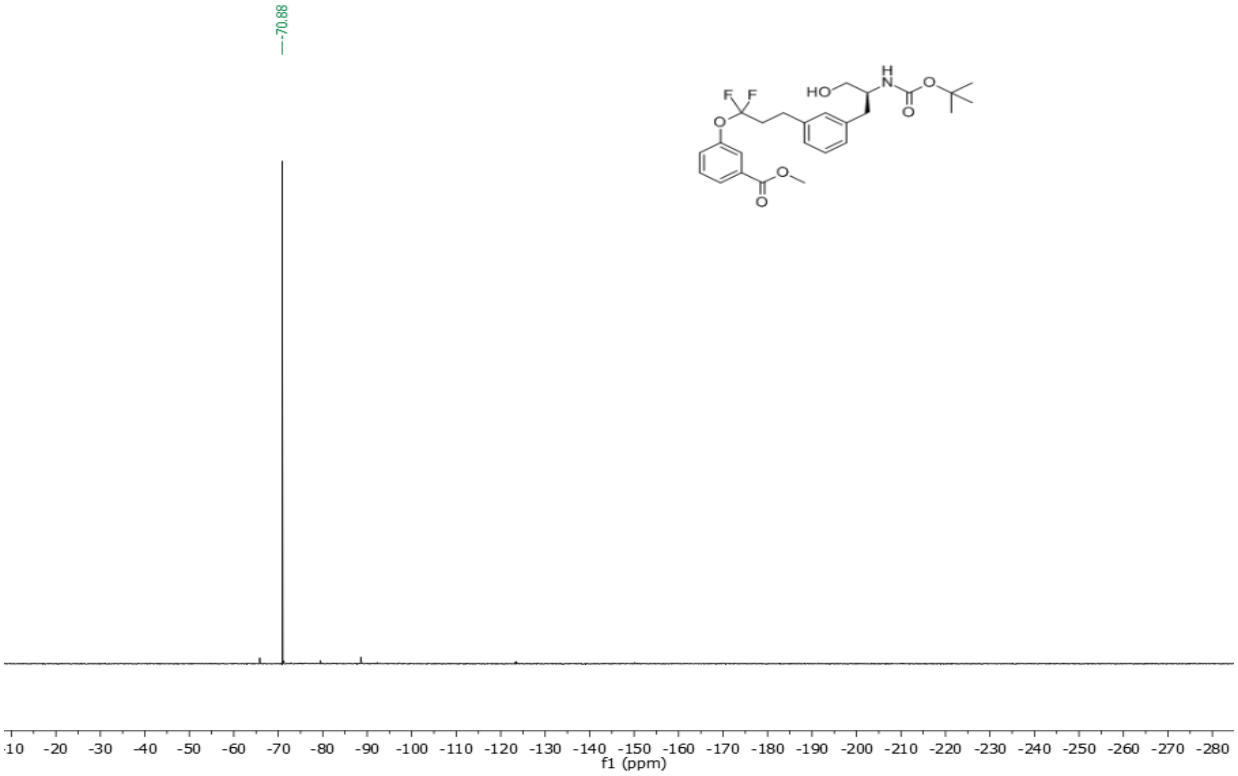


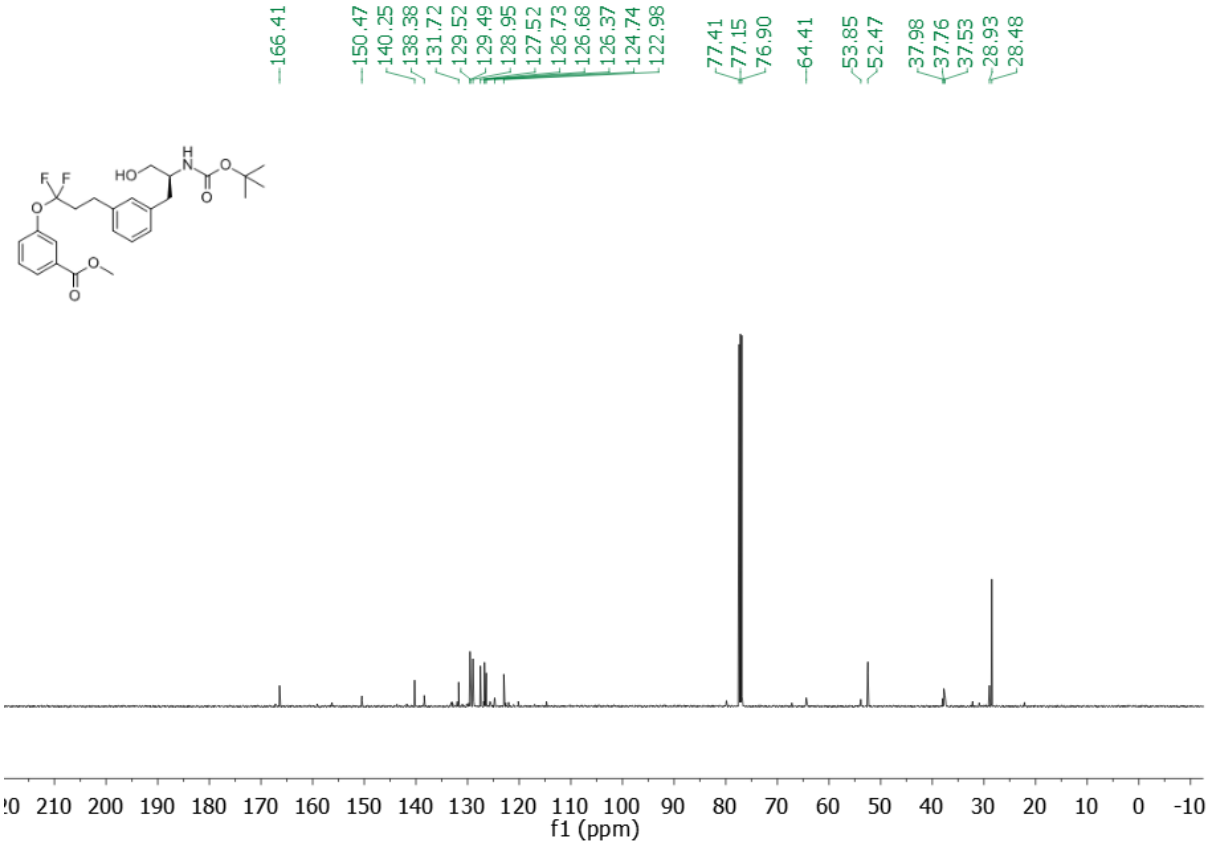
Methyl-(*S*,*E*)-3-((3-(3-(2-((*tert*-butoxycarbonyl)amino)-3-hydroxypropyl)phenyl)-1,1-difluoroallyl)oxy)benzoate, 7a



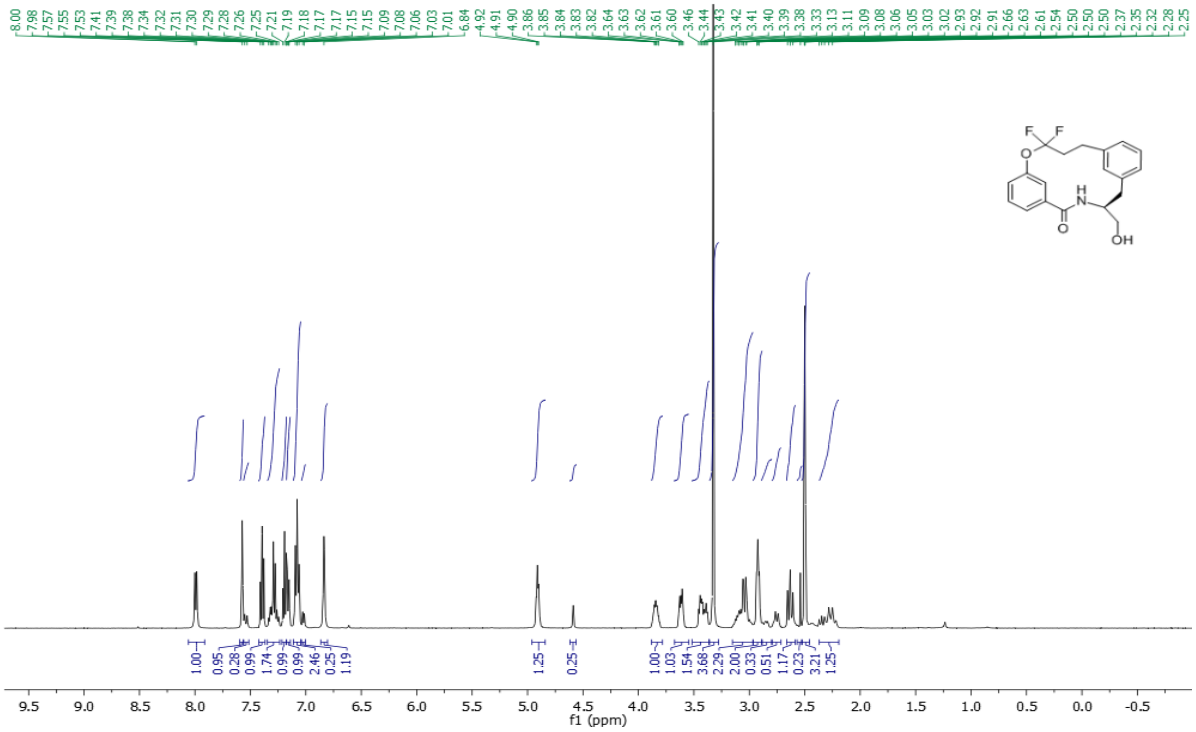
Methyl-(S)-3-(3-(3-(2-((tert-butoxycarbonyl)amino)-3-hydroxypropyl)phenyl)-1,1-difluoropropoxy)benzoate, 8a

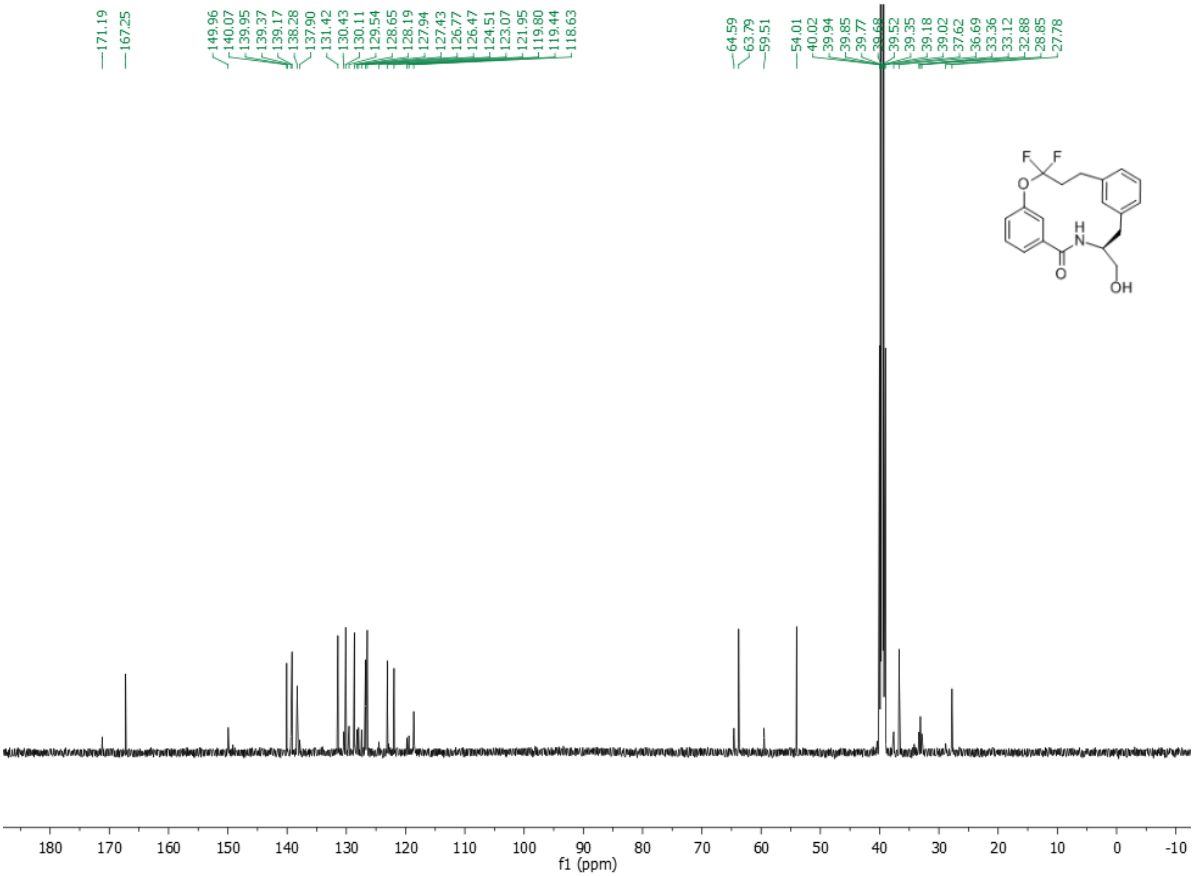


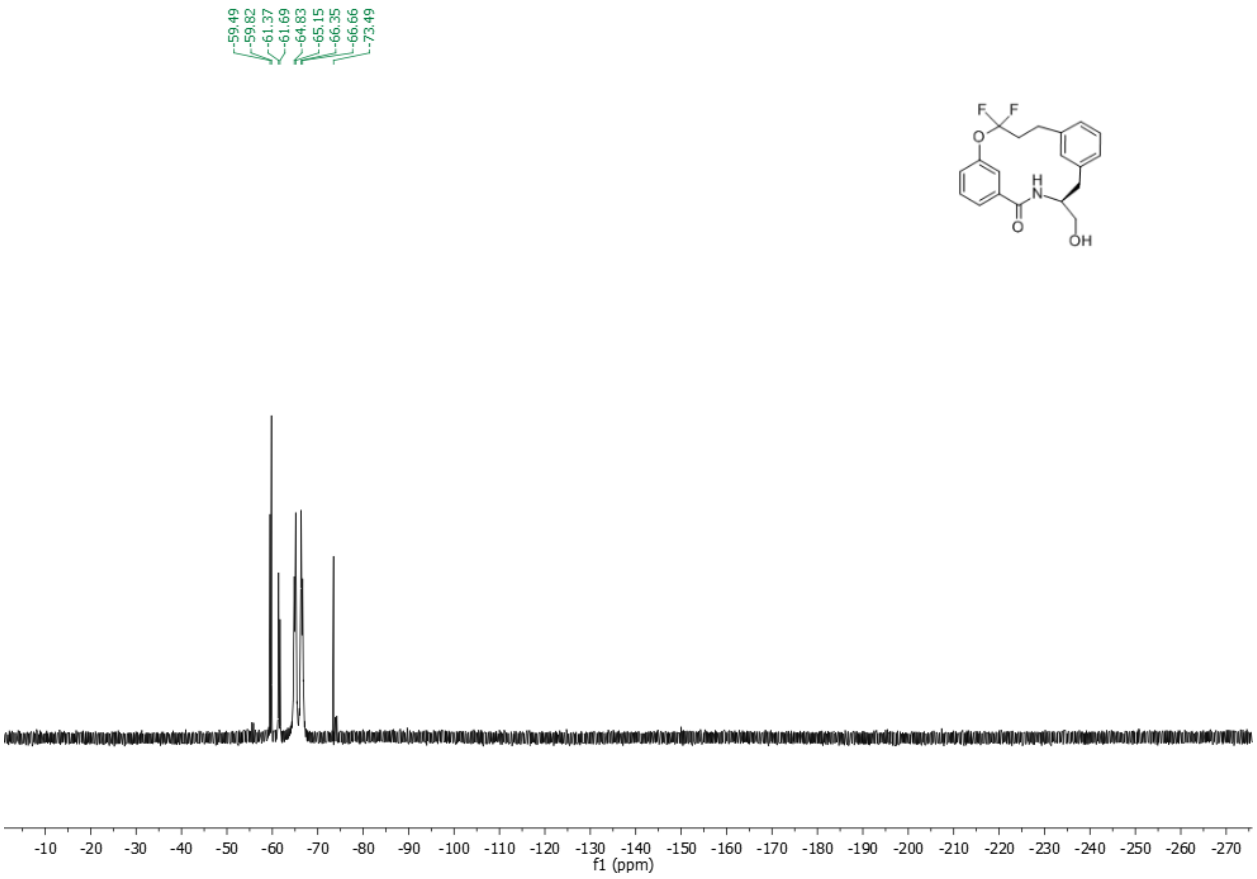

-70.88
F F
HO
H
N
O
O
O
O
O
O
-10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210 -220 -230 -240 -250 -260 -270 -280
f1 (ppm)


166.41
150.47
140.25
138.38
131.72
129.52
129.49
128.95
127.52
126.73
126.68
126.37
124.74
122.98
77.41
77.15
76.90
64.41
53.85
52.47
37.98
37.76
37.53
28.93
28.48
F F
HO
H
N
O
O
O
O
O
O
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

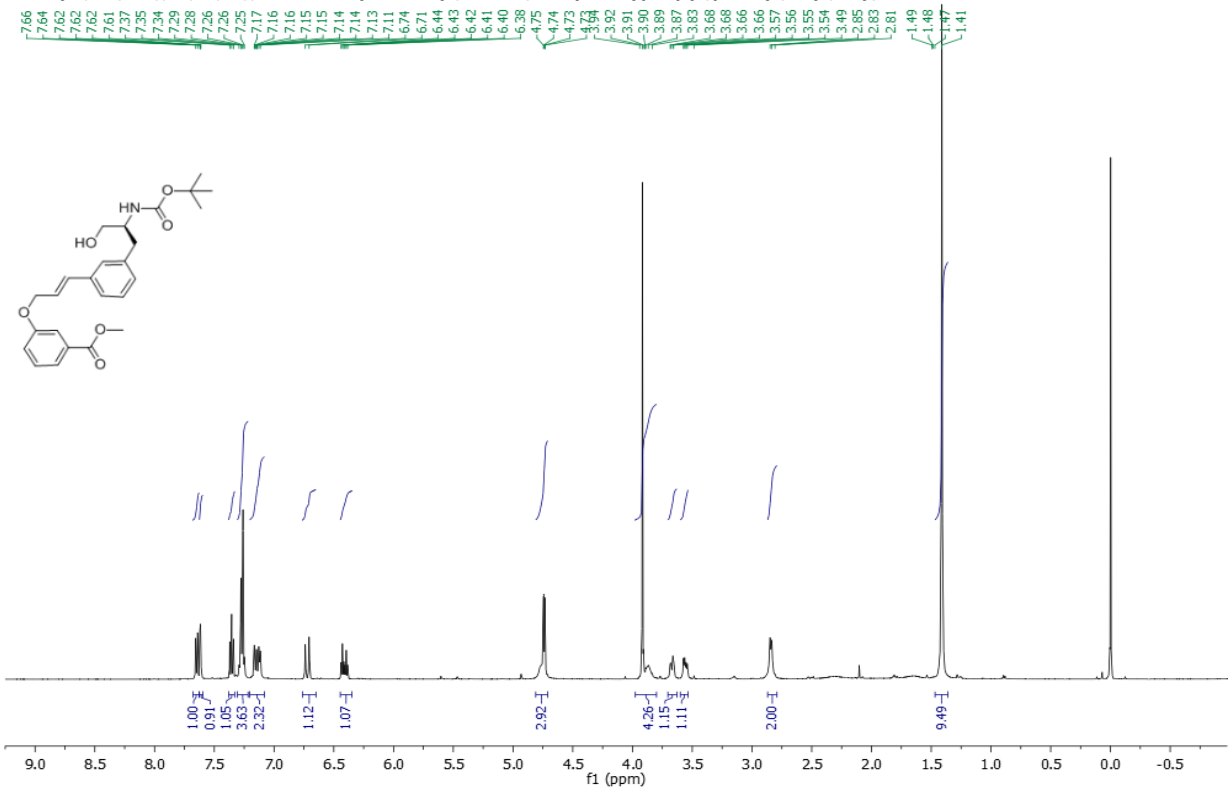
(S)-3,3-Difluoro-8-(hydroxymethyl)-2-oxa-9-aza-1,6(1,3)-dibenzenacyclodecaphan-10-one, 4a

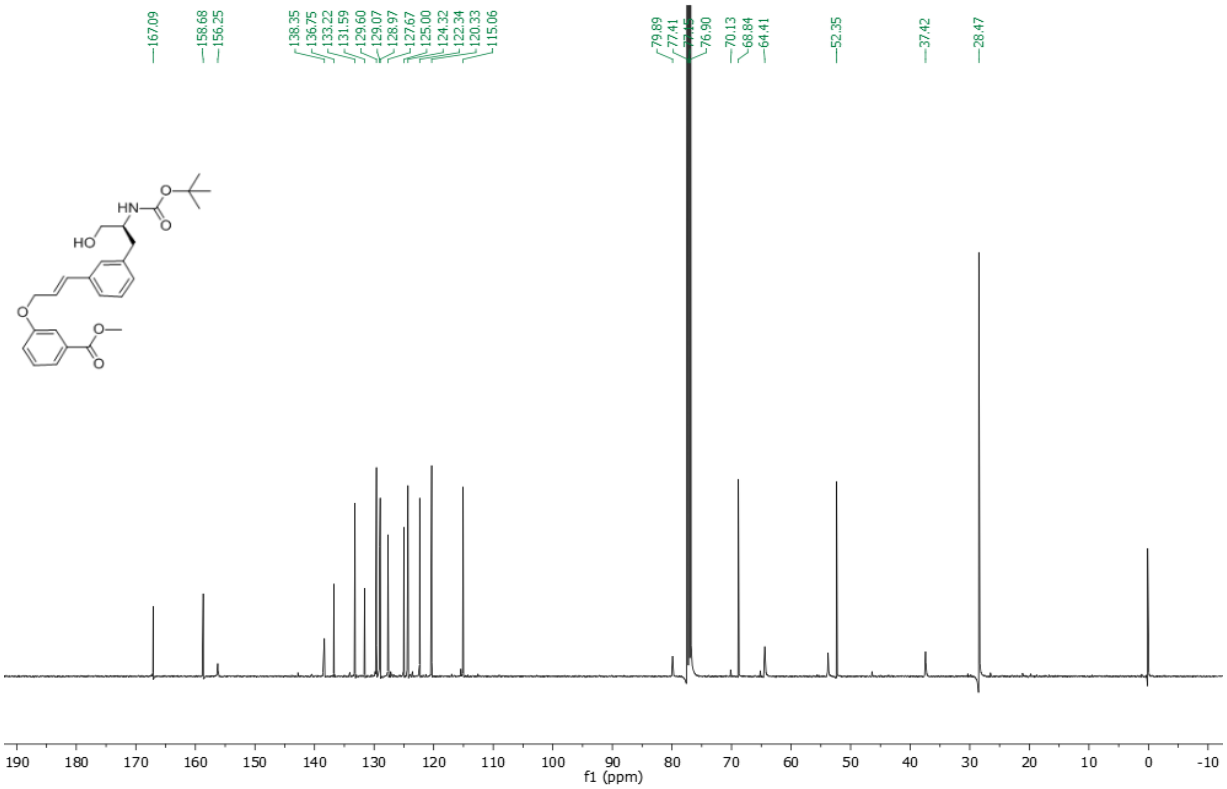




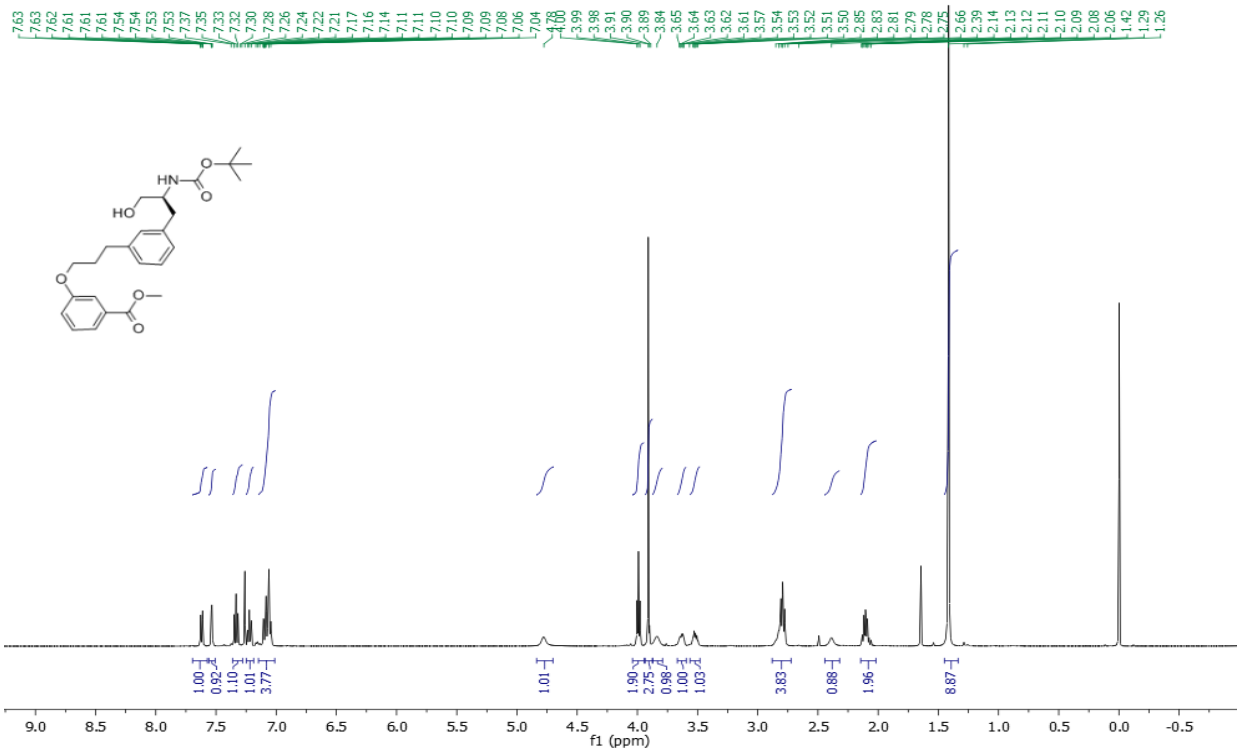


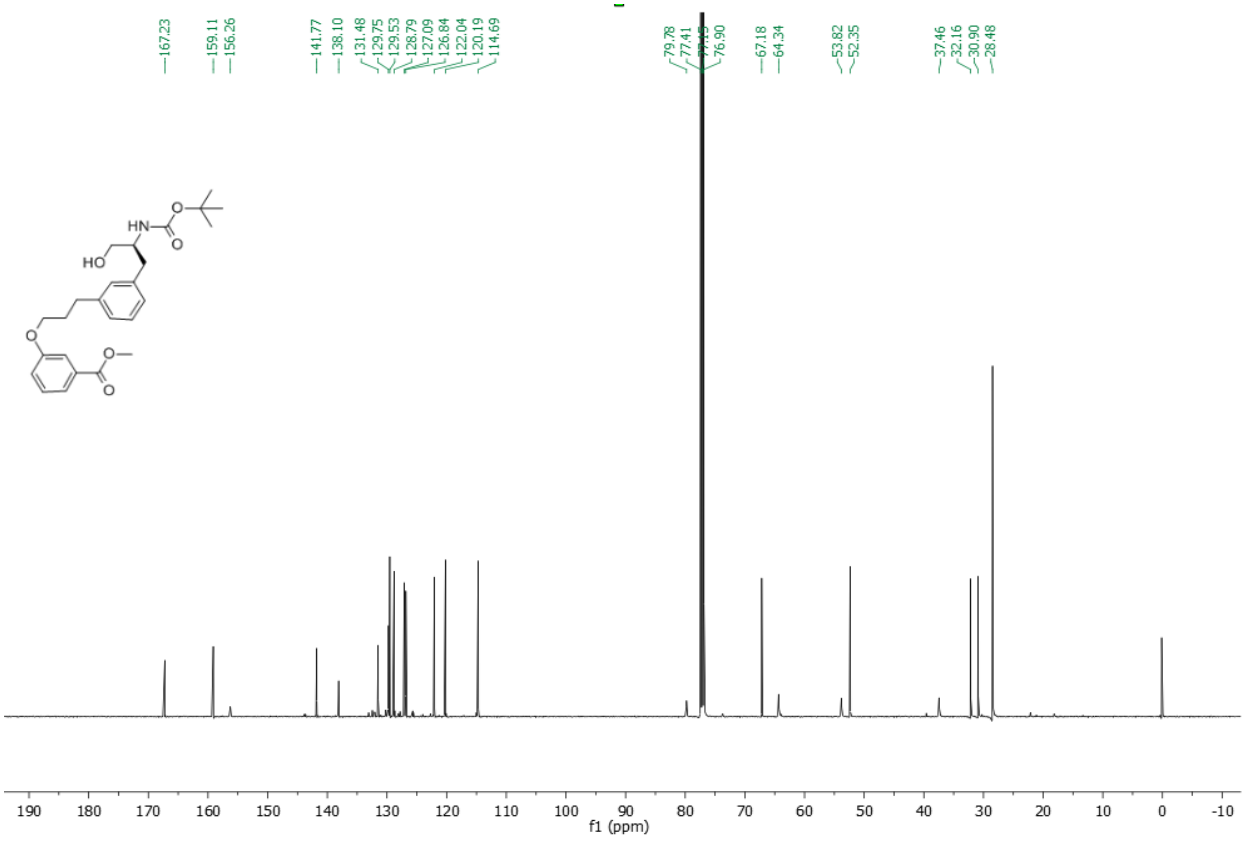
Methyl (*S*,*E*)-3-((3-(3-(2-((*tert*-butoxycarbonyl)amino)-3-hydroxypropyl)phenyl)allyl)oxy) benzoate, 7b



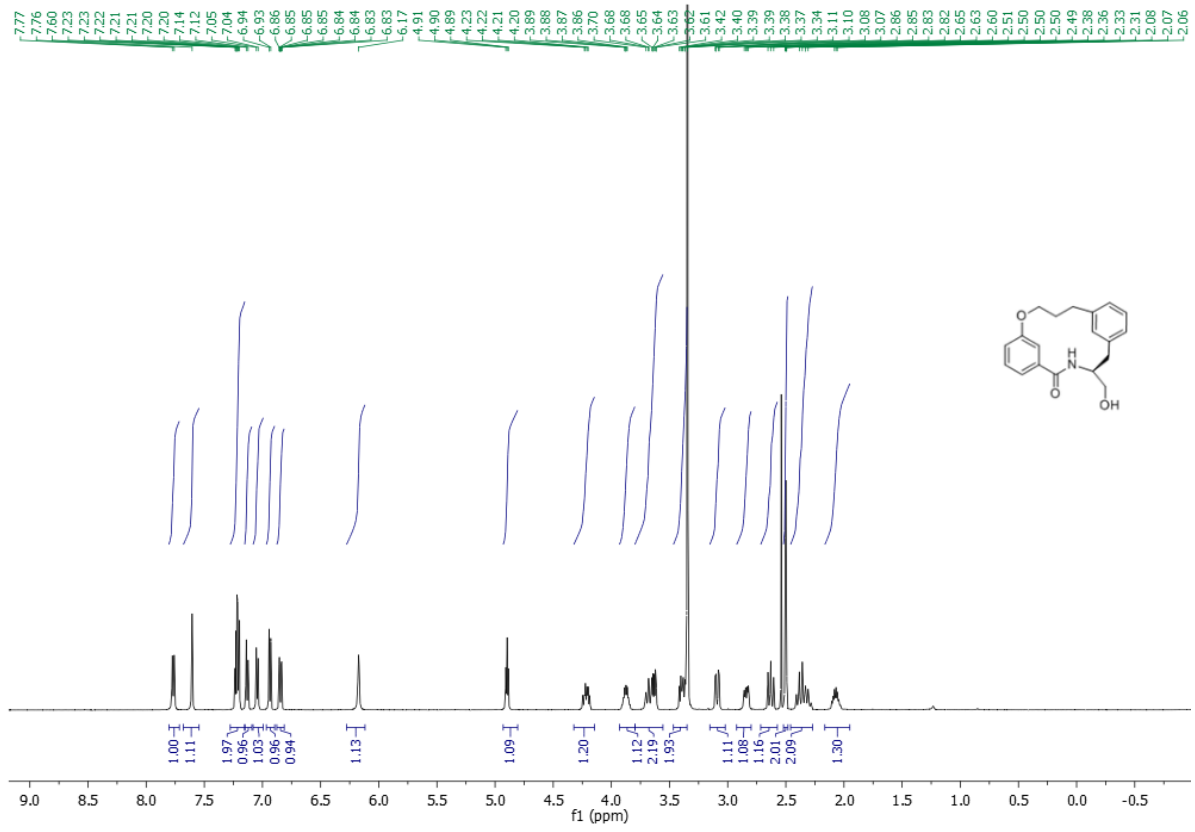


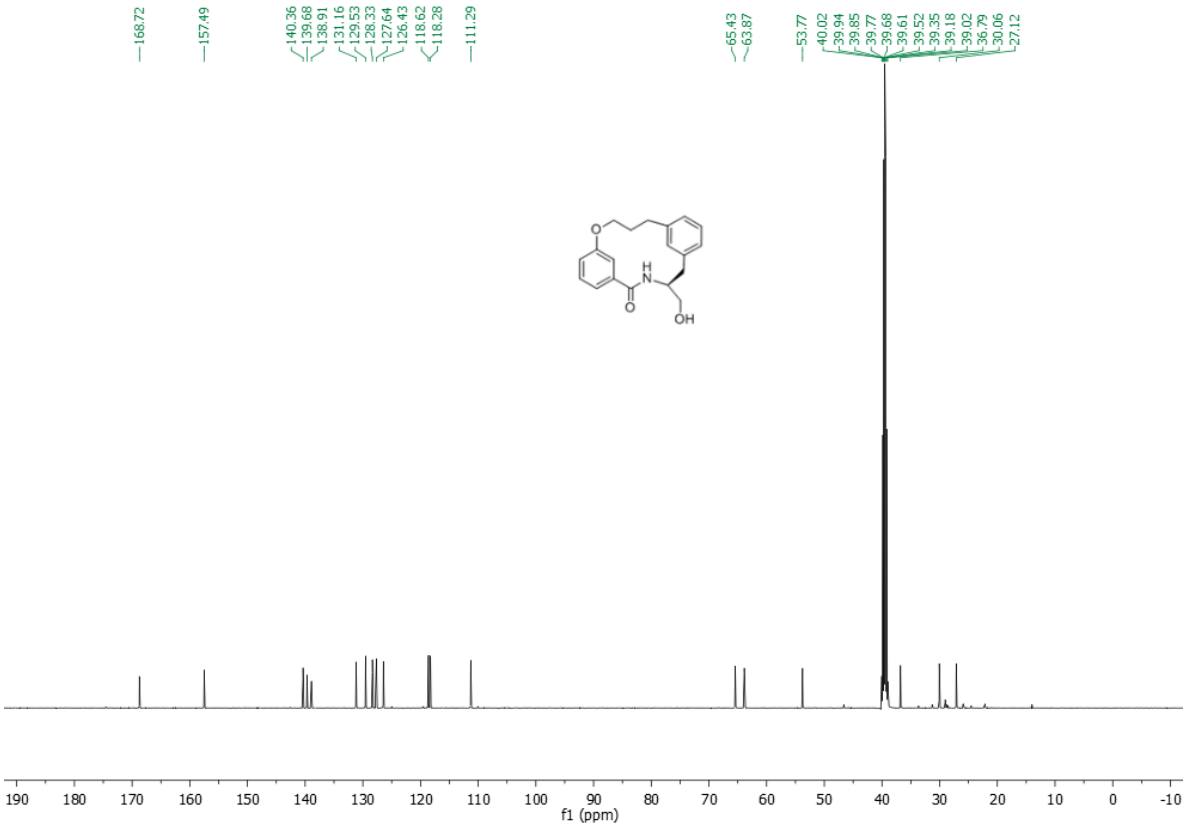
Methyl (*S*)-3-(3-(3-(2-((*tert*-butoxycarbonyl)amino)-3-hydroxypropyl)phenyl)propoxy) benzoate, 8b



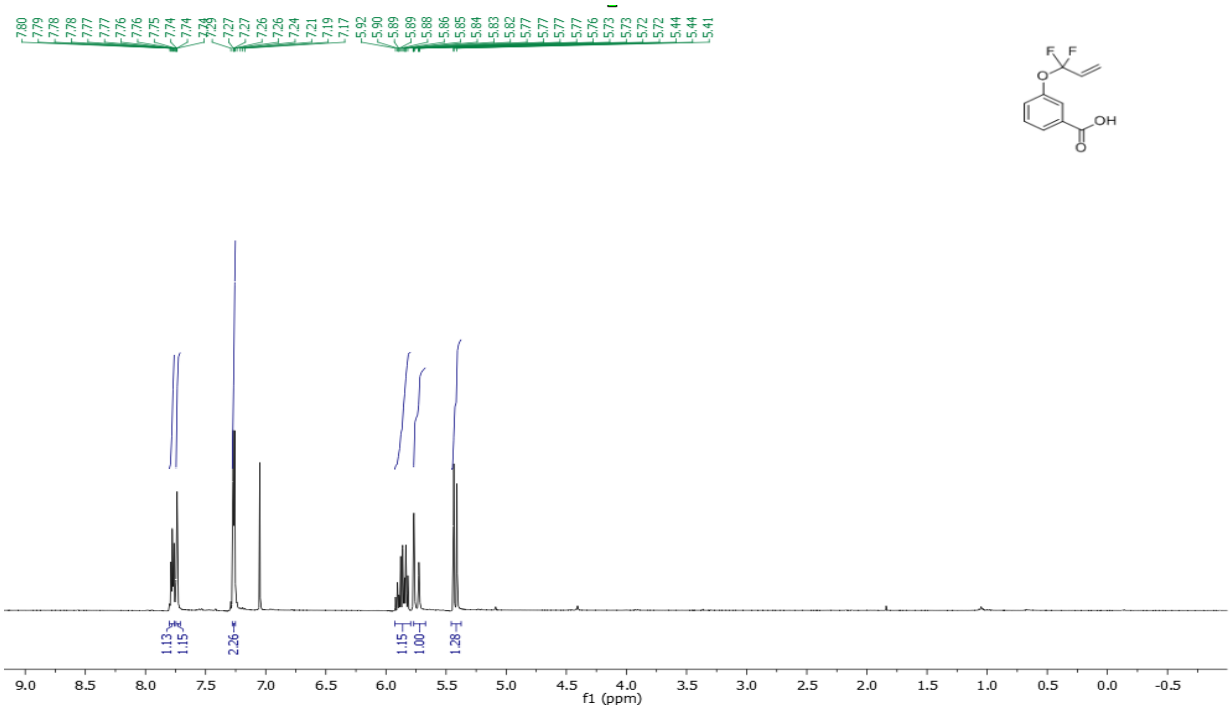


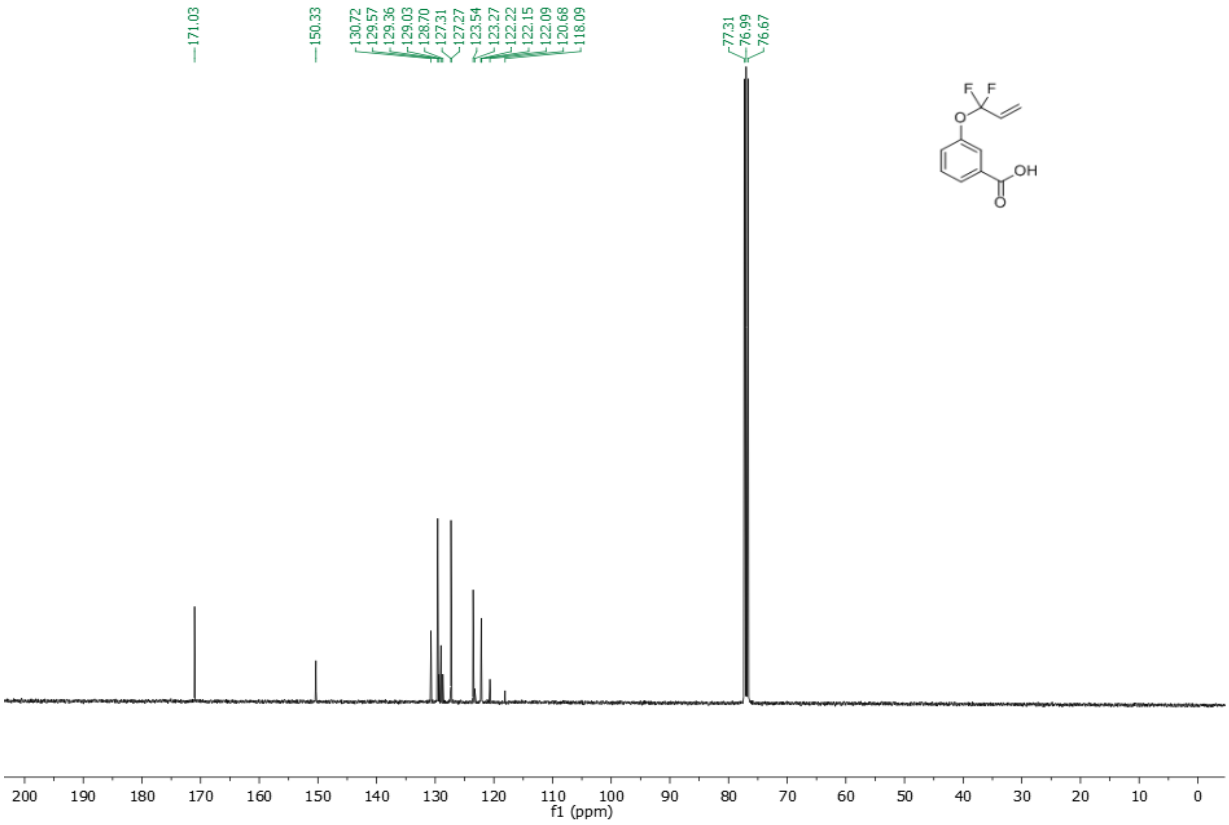
(*S*)-8-(Hydroxymethyl)-2-oxa-9-aza-1,6(1,3)-dibenzenacyclodecaphan-10-one, 4b



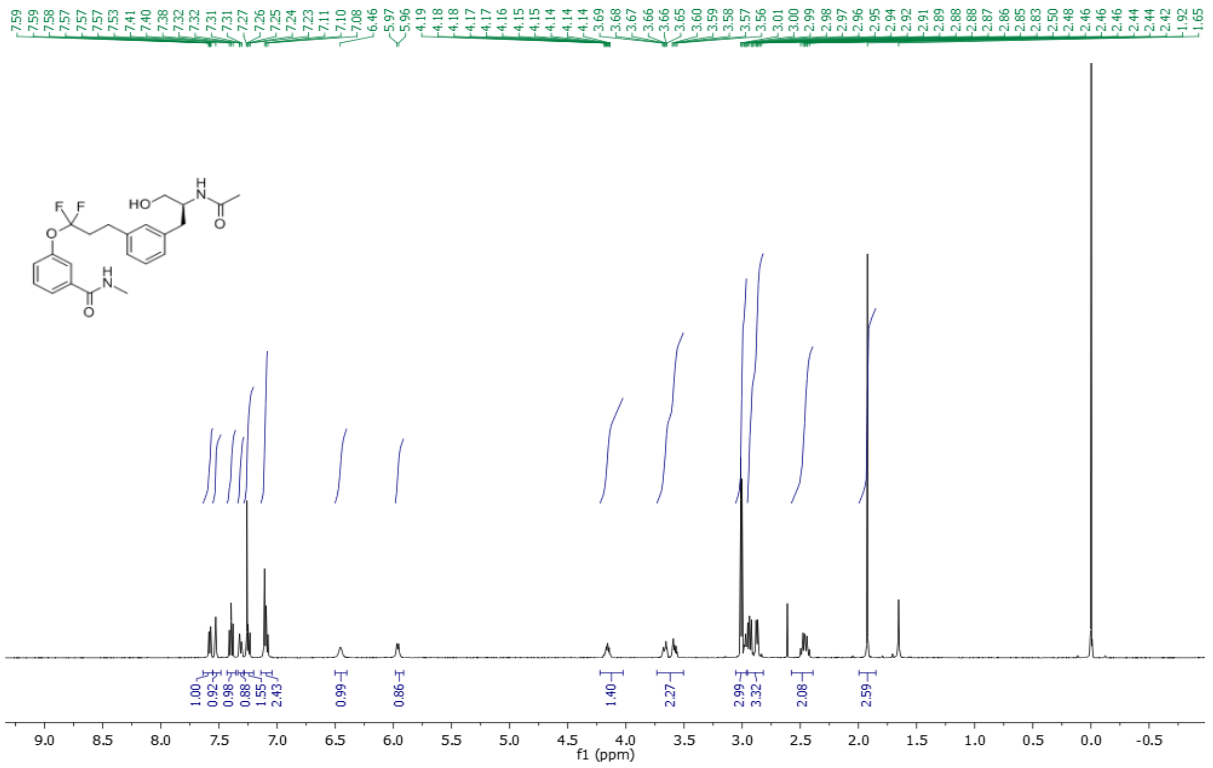


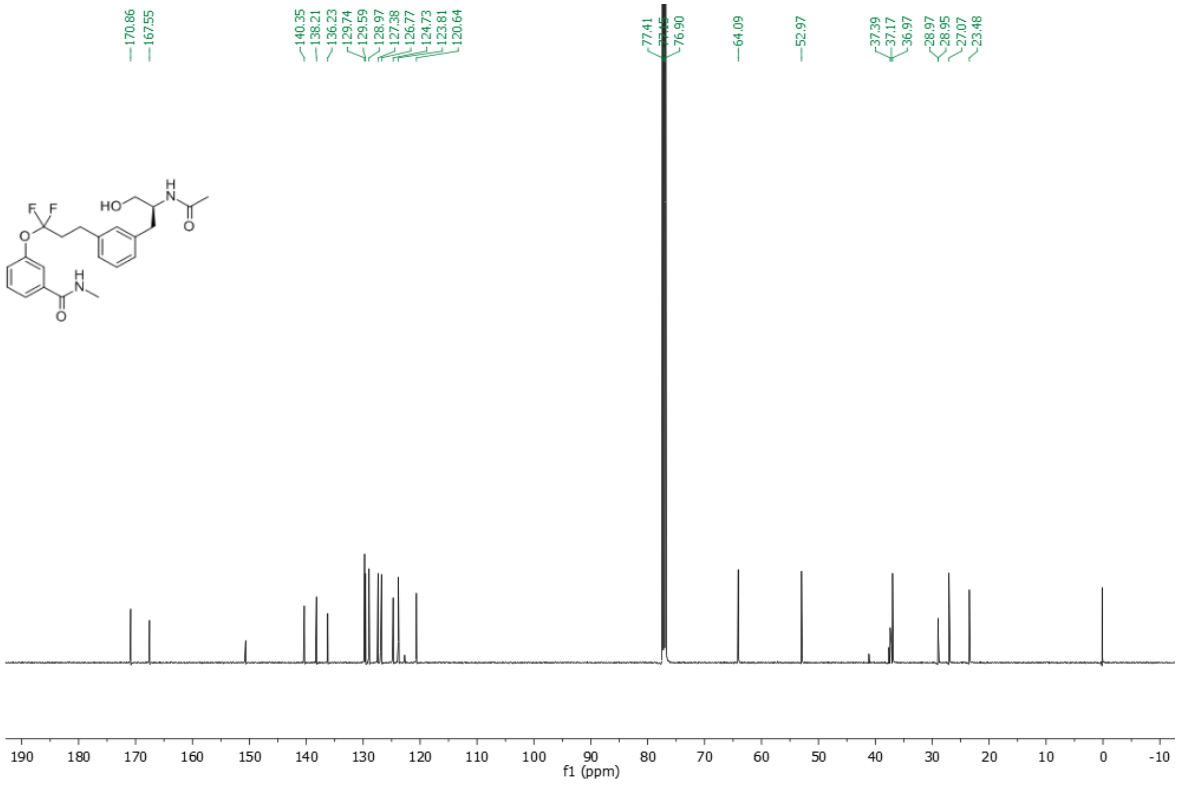
3-((1,1-Difluoroallyl)oxy)benzoic acid, 9a



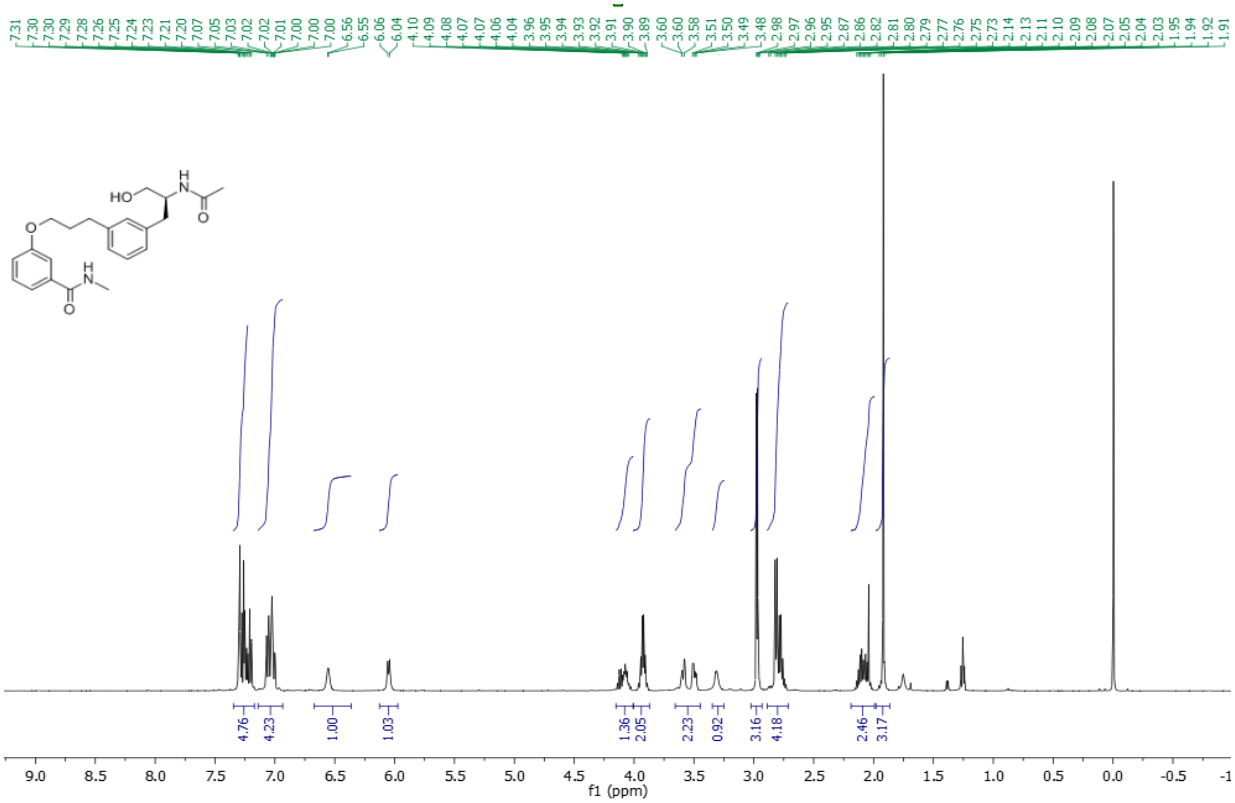


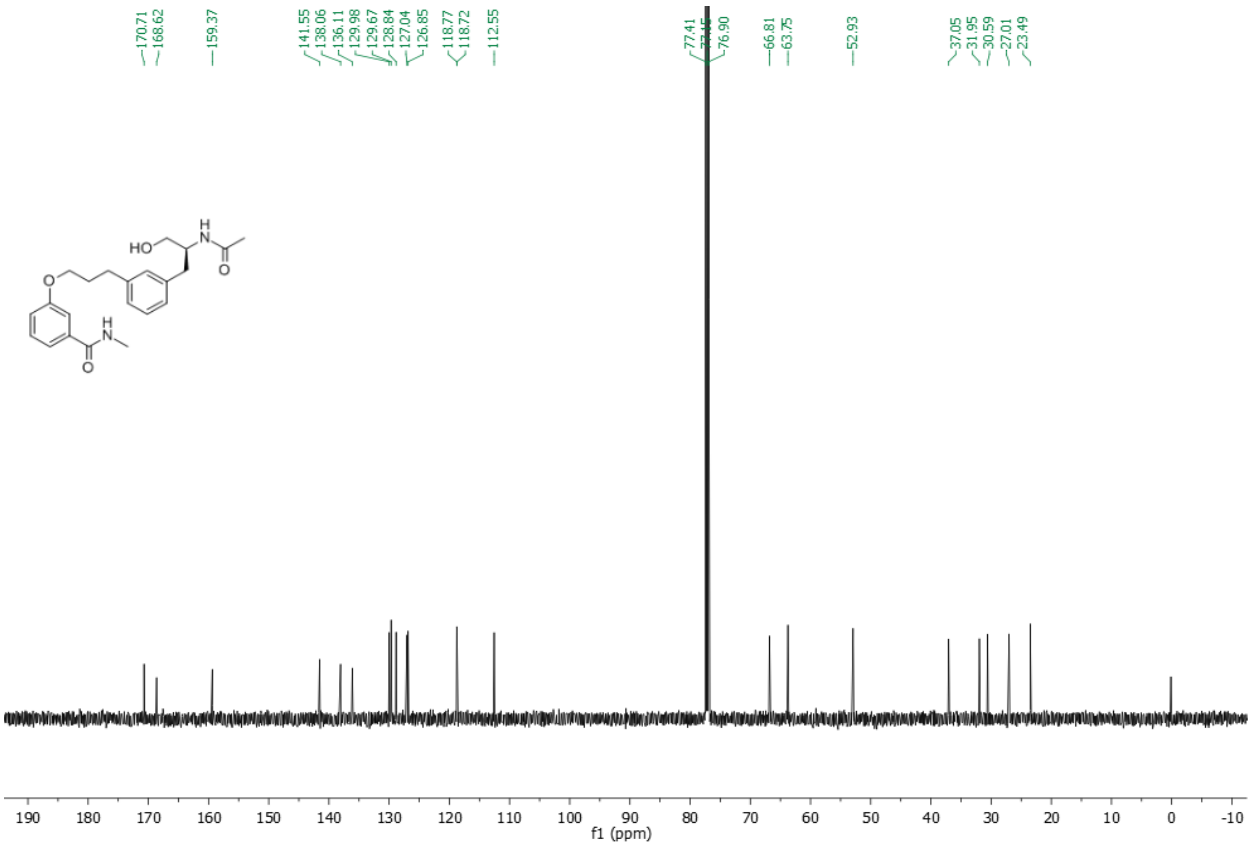
(*S*)-3-(3-(3-(2-Acetamido-3-hydroxypropyl)phenyl)-1,1-difluoropropoxy)-*N*-methylbenzamide, 10a



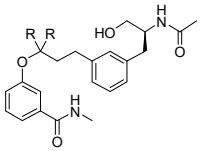


(*S*)-3-(3-(3-(2-Acetamido-3-hydroxypropyl)phenyl)propoxy)-*N*-methylbenzamide, 10b



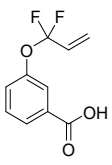


Matched pair analysis of linear analogs, 10a and 10b

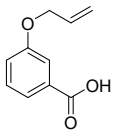


	Fluorinated linear analog 10a (R = F)	Non-fluorinated linear analog 10b (R = H)
log$D_{7.4}$	2.4	1.9
Rat hepatocytes metabolic stability $t_{½}$ (min)	13	33
Human liver microsomes metabolic stability $t_{½}$ (min)	22	65

Inductive effect difference estimation measuring pKas of *meta*-(allyloxy) and meta-(1',1'-difluoroallyloxy) benzoic acids, 9a and 9b



9a Measured pK_a : 3.7



9b Measured pK_a : 3.9

In vitro profiling protocols

$logD_{7.4}$

Lipophilicity, measured as the distribution coefficient (logD) between octanol and 10mM sodium phosphate buffer with pH adjusted to 7.4, is determined in a high-throughput assay based on the traditional shake flask method, using 96 well plates.[9] Up to ten compounds are pooled together, dissolved in the buffer and octanol phase and the two phases are mixed for two hours at room temperature. The concentrations in the octanol and buffer phase are analysed using liquid chromatography and quantitative tandem mass spectrometry (LC-MS/MS). One quality control compound with medium lipophilicity, cyclobenzaprine, is placed in each pool, whereas two other compounds, nicardipine (high logD) and caffeine (low logD), are placed randomly on the plate at each run. The range of logD values that can be determined by this method depends on a compound's solubility in octanol and buffer as well as its MS response, which is typically between 0 and 4.

DMSO/HBSS solubility measured at pH=7.4

Compounds, dissolved in DMSO and stored in 96-well plates, are transferred and diluted with buffer into new plates. The plates are shaken for 24 hours, then filtered, and the filtered solutions are analysed to give an estimate of the solubility. As standards for the concentration estimations, samples with the same degree of dilution are prepared, but using organic solvent (ethanol, acetonitrile, etc). The diluted samples, and the standards are analysed with LC-UV/MS.

Cl_{int} in Human Liver Microsomes

Incubations in human liver microsomes (BioreclamationIVT InVitroCYPTM, Brussels, Belgium) were performed on a Hamilton Microlab STAR Workstation (Hamilton Robotics AB, Kista, Sweden). Test compounds were incubated at 37°C in 96-well microtiter plates at 1 µM with human liver microsomes (1 mg/mL) and potassium phosphate buffer containing NADPH at a concentration of 1 mM. Aliquots of 40 µL were taken at 0.7, 6.0, 12, 17, 22 and 30 min and quenched 1:5 with ice-cold acetonitrile containing 0.8% (v/v) formic acid and 1 µM of internal standard/volume marker. The samples were then centrifuged at 3100 g for 20 min at 4 °C. Thereafter, 35 µL of the supernatant was diluted with 35 µL of water prior to analysis. The analyses were performed on an Acquity ultra-performance liquid chromatography (UPLC) system interfaced with an ACQUITY® Xevo TQS (Waters, Milford, MA, USA). The analytical column used for chromatographic separation was an Acquity UPLC HSS T3 column (1.8 µm, 2.1 × 30 mm). The mobile phases consisted of 0.1% (v/v) formic acid in water and acetonitrile, respectively. The LC gradient was as follow: 0 – 0.1 min 0.2% B, 0.1 – 0.7 95% B, 0.7 – 1.0 95%B, 1.0 – 1.01 0.2% B at a flow rate of 1.0 mL/min and the temperature set to 40°. Generic tune files were used. MassLynx 4.1 (Waters) was used for the data acquisition and the chromatographic peaks are integrated by Target Lynx software (Waters).

Clint in Rat Hepatocytes

The hepatocyte incubations were performed on a Hamilton Microlab STAR Workstation (Hamilton Robotics AB, Kista, Sweden). The incubation was made on a CAT orbital shaking plate heater (Hamilton Robotics AB, Kista, Sweden). Shaking speed of 900 rpm and a temperature of 37°C in the wells. The assay was run in a 96-deepwell plate with an incubation volume of 250 µl with 1 µM substrate concentration and rat hepatocytes (1 × 106 cells/mL, BioreclamationIVT, Brussels, Belgium). The incubation media used was L-15 Leibovitz. From each incubation 12.5 µL was taken out and quenched in 75 µL stop solution (acetonitrile including an internal standard). The time points used were 0.5, 5, 15, 30, 45, 60, 80, 100 and 120 min. The quenched samples were centrifuged at 3000 g for 15 min at 4 °C and the supernatant was diluted 1:1 with water prior to analysis.

The analyses were performed on an Acquity ultra-performance liquid chromatography (UPLC) system interfaced with an Ultima Platinum, Premier, Xevo TQS (Waters, Milford, MA, USA). The analytical column used for chromatographic separation was a Kinetex C18 column (2.6 µm, 2.1 × 50 mm). The mobile phases consisted of 0.1% (v/v) formic acid in water and methanol, respectively. The LC gradient was as follow: 0 – 0.1 min 5% B, 0.1 – 0.5 95% B, 0.5 – 0.95 95% B, 0.95 – 0.96 5% B at a flow rate of 800 µL/min and the temperature set to 60°C. Generic tune files were used. MassLynx 4.1 (Waters) was used for the data acquisition and the chromatographic peaks are integrated by Target Lynx software (Waters).

Metabolite identification in human hepatocytes

Samples from human hepatocyte incubations (120 mins, 4 µM substrate) were quenched with ice cold ACN (1 vol) and centrifuged at 4000 g for 15 mins at 4ºC. Aliquots (100 µL) were removed and further diluted with 0.1% formic acid in water (100µl) and metabolite identification analyses was performed on an Acquity UPLC system interfaced with a Synapt G2 QTOF mass spectrometer (Waters, Milford, MA, USA). The analytical column used for chromatographic separations was an Acquity UPLC BEH C18 column (1.7 µm, 2.1 × 100 mm). The mobile phases consisted of 0.1% formic acid in water (A) and acetonitrile (B), respectively. The LC gradient was as follows: 0 – 6 min 10 – 70% B, 6 – 6.7 min 90% B at a flow rate of 500 µL/min and the column oven temperature set to 45°C. The Synapt was operated in positive electrospray ionization (ESI) mode with a capillary voltage of 0.5 kV and cone voltage of 20 V. Two types of acquisitions, MSE and MSMS, were performed with a mass range of 80 – 1000 Da. For the low energy MSE acquisition, the trap and transfer energy were set to 4 V and 3 V, respectively. For the high energy MSE acquisition, the trap was ramped from 15 to 45 V and the transfer was held at a fixed energy of 12 V. Data was collected in centroid mode. Leucineenkephaline was used as a lock mass (*m/z* 556.2771) for internal calibration at a concentration of 250 pmol/µL and a flow rate of 40 µL/min. MassLynx 4.1 was used for the data acquisition. MS data was processed in Metabolynx V4.1(SCN871, 2012) to generate separate MSMS experiments. MSMS and MSE spectra were elucidated manually and using the MassFragment function. Biotansformation schemes were drawn in ACD/Labs Chemsketch and captured in the AZ Global Metabolite database using ACD Spectrus DB Enterprise (2017.1.3).

Caco2 P_{app}

An automated assay to study Caco-2 monolayer permeability in the apical to basolateral direction including a pH gradient (pH 6.5 in apical donor compartment and pH 7.4 in basolateral receiver compartment) was used.

pK_a measurement

The sample pK_as are investigated using the fast UV-metric method. This involves measuring the UV absorbance profile at each pH point during an acid/base titration using an *in situ* UV probe in the titration cell of a SiriusT3 instrument. Each sample is titrated in a triple titration over a nominal pH range of 2 – 12 in approximately 50, 40, 30 % methanol. Sample concentrations are typically in the range 35 15 µM. All titrations are carried out at 25 °C unless otherwise requested.

The pK_a(s) are determined by monitoring the change in UV absorbance with pH as the compound undergoes ionisation. This information is used to produce a 3D matrix of pH vs. Wavelength vs. Absorbance data. A mathematical technique called Target Factor Analysis is applied to the matrix to produce molar absorbance profiles for the different light absorbing species present in solution and also a Distribution of Species plot showing how the proportion of each species varies with pH. Sample pK_as are extrapolated to aqueous conditions using the Yasuda-Shedlovsky method.

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