Electronic Supplementary Information

The Discovery of Novel 10,11-Dihydro-5H-dibenz[b,f]azepine SIRT2 Inhibitors

Paolo Di Fruscia,[a] Ka-Kei Ho,[b] Sasiwan Laohasinnarong,[b] Mattaka Khongkow,[b] Sebastian H.B. Kroll,[a] Suhail A. Islam,[c] Michael J. E. Sternberg,[c] Karin Schmidtkunz,[d] Manfred Jung,[d] Eric W.-F. Lam,[b] and Matthew J. Fuchter[a*]

[a]Department of Chemistry, Imperial College London, London, United Kingdom
[b]Department of Surgery and Cancer, Imperial College London, London, United Kingdom
[c]Department of Bioinformatics, Imperial College London, London, United Kingdom
[d]Institute of Pharmaceutical Sciences, Albert-Ludwigs-Universität, Freiburg, Germany

*E-mail: m.fuchter@imperial.ac.uk

Table of Contents

A. Homology Modeling and Docking Studies

The human SIRT1 amino acid sequence was retrieved from the UniProt database (UniProt ID: Q96EB6)[1] and a 3D comparative model of the human SIRT1 enzyme was predicted by the PHYRE server using a yeast Sir2 X-ray crystal structure (PDB entry code: 2HJH, chain A)[2] as template. 2HJH showed high sequence identity with the target protein (Fig. S1) and high Blast[3] and SAS[4] scores (Table S1).

```
SIRT1  234  RKKRKDINTIEDAVKLLQECKKIIVLTGAGVSVSCGIPDFRSRDGIYARLAVDFPDLPDP  293
            R +  +  TI+  ++ L   +KI+VLTGAGVS S GIPDFRS +G Y++  +    L DP
Sir2   235  RLRLSNFFTIDHFIQKLHTARKILVLTGAGVSTSLGIPDFRSSEGFYSK--IKHLGLDDP  292

SIRT1  294  QAMFDIEYFRKDPRPFFKFAKEIYPGQFQPSLCHKFIALSDKEGKLLRNYTQNIDTLEQV  353
            Q +F+   F  DP  F+  A  + P +   S  H FI +   +GKLLRNYTQNID LE
Sir2   293  QDVFNYNIFMHDPSVFYNIANMVLPPEKIYSPLHSFIKMLQMKGKLLRNYTQNIDNLESY  352

SIRT1  354  AGIQ--RIIQCHGSFATASCLICKYKVDCEAVRGDIFNQVVPRCPRCPAD----------  401
            AGI   +++QCHGSFATA+C+ C + +  E +   I N  +P CP C
Sir2   353  AGISTDKLVQCHGSFATATCVTCHWNLPGERIFNKIRNLELPLCPYCYKKRREYFPEGYN  412

SIRT1  402  --------------------EPLAIMKPEIVFFGENLPEQFHRAMKYDKDEVDLLIVIGS  441
                                  ++KP+I FFGE LP +FH++++ D  E DLLI IG+
Sir2   413  NKVGVAASQGSMSERPPYILNSYGVLKPDITFFGEALPNKFHKSIREDILECDLLICIGT  472

SIRT1  442  SLKVRPVALIPSSIPHEVPQILINREPLPHLHFDVELLGDCDVIINELCHRLG  494
            SLKV PV+ I + +P  VPQ+LINR+P+ H  FD+ LLG CD I   +  + G
Sir2   473  SLKVAPVSEIVNMVPSHVPQVLINRDPVKHAEFDLSLLGYCDDIAAMVAQKCG  525
```

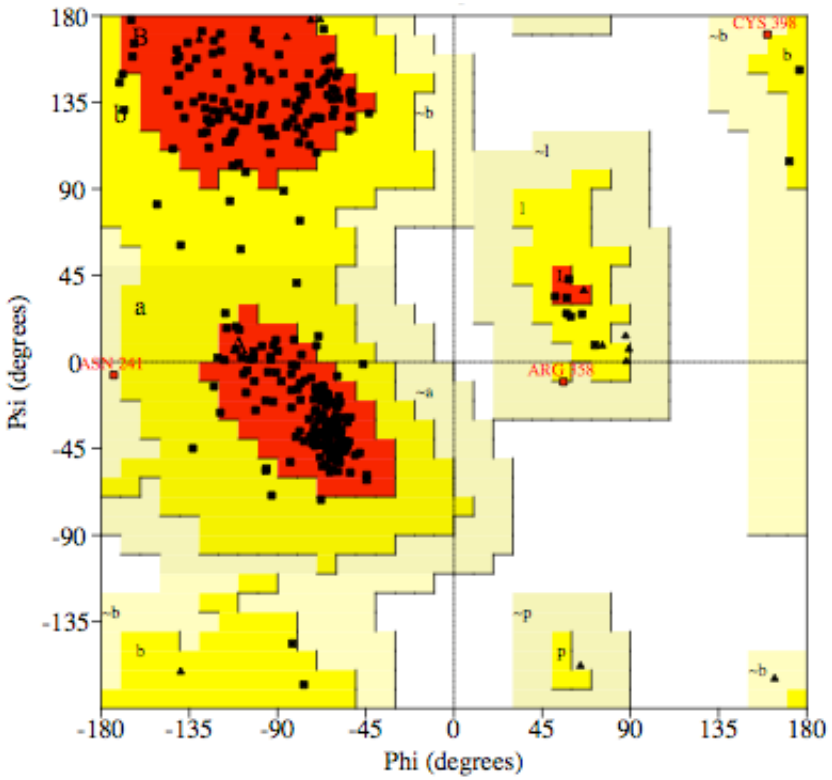
Fig. S1 Sequence alignment between target SIRT1 and template Sir2 catalytic domain.

Table S1 BLAST and SAS scoring of the selected template structure

PDB ID	BLAST		SAS					
	Score	E-Value	Smith-Waterman Score	%-Identity	aa-Overlap	Z-Score	Length	E-Value
2HJH_A	219	3e-57	764	43.8	276	405.4	325	8.4e-16

Dockings of inhibitor **8** were carried out using GOLD 4.0[5] The proteins used in the present study, SIRT2 X-ray crystal structure (PDB ID: 1J8F, chain B) and SIRT1 homology model, were initialised and optimised with GOLD. No degree of flexibility was adopted (fully rigid proteins). The molecular structure of **8** was generated with ChemBioDraw Ultra 12.0[6] and energy-minimized with ChemBio3D Ultra 12.0[6] using the MMFF94 force field. *GoldScore* was chosen as primary scoring function and *ChemScore* as rescoring function. The search efficiency of the genetic algorithm was set at 200%, which represents the highest efficiency the software can employ.

The binding site was defined on the C_α of His363 for SIRT1 and C_α of His187 for SIRT2, with a radius of 20 Å. Water molecules within the binding site definition of the SIRT2 protein were retained and considered in the docking studies, using the settings "toggle" and "spin". For the ligand, the maximum degree of flexibility (fully flexible ligand) was adopted, and 10 docking runs were performed. The top-ranked poses were visually analysed with PyMOL.[7] The stereochemical quality of the protein structures was evaluated with the PROCHECK[8] tool: the Ramachandran plot for the SIRT1 homology model yielded 88.6% of residues in the "most favoured regions", 10.3% in "additional allowed regions", 1.1% in "generously allowed regions" and 0.0% in "disallowed regions", indicating a general high quality of the model (Fig. S2).

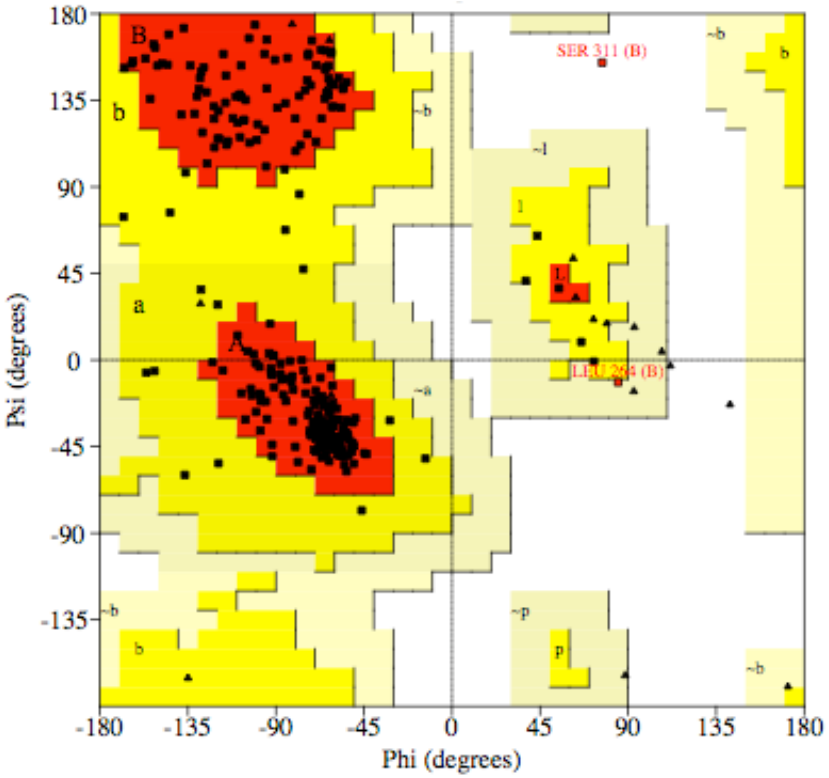


Plot statistics

Residues in most favoured regions [A,B,L]	241	88.6%
Residues in additional allowed regions [a,b,l,p]	28	10.3%
Residues in generously allowed regions [~a,~b,~l,~p]	3	1.1%
Residues in disallowed regions	0	0.0%
Number of non-glycine and non-proline residues	272	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	14	
Number of proline residues	28	
Total number of residues	316	

Fig. S2 Ramachandran plot of SIRT1 homology model.

The Ramachandran plot for the SIRT2 X-ray structure yielded 91.9% of residues in the "most favoured regions", 7.4% in "additional allowed regions", 0.4% in "generously allowed regions" and 0.4% in "disallowed regions". The 0.4% in "disallowed regions" represents Ser311, which is not located in the catalytic site (Fig. S3). Overall, it is expected that a homology model would have a lower quality Ramachandran plot than the template (X-ray crystal structure).



Plot statistics

Residues in most favoured regions [A,B,L]	250	91.9%
Residues in additional allowed regions [a,b,l,p]	20	7.4%
Residues in generously allowed regions [~a,~b,~l,~p]	1	0.4%
Residues in disallowed regions	1	0.4%
Number of non-glycine and non-proline residues	272	100.0%
Number of end-residues (excl. Gly and Pro)	3	
Number of glycine residues (shown as triangles)	21	
Number of proline residues	16	
Total number of residues	312	

Fig. S3 Ramachandran plot of SIRT2 crystal structure (PDB ID: 1J8F, chain B).

PDBsum server[9] was used to predict and analyse the secondary structure elements of SIRT1 (Fig. S4) and SIRT2 (Fig. S5) structures.

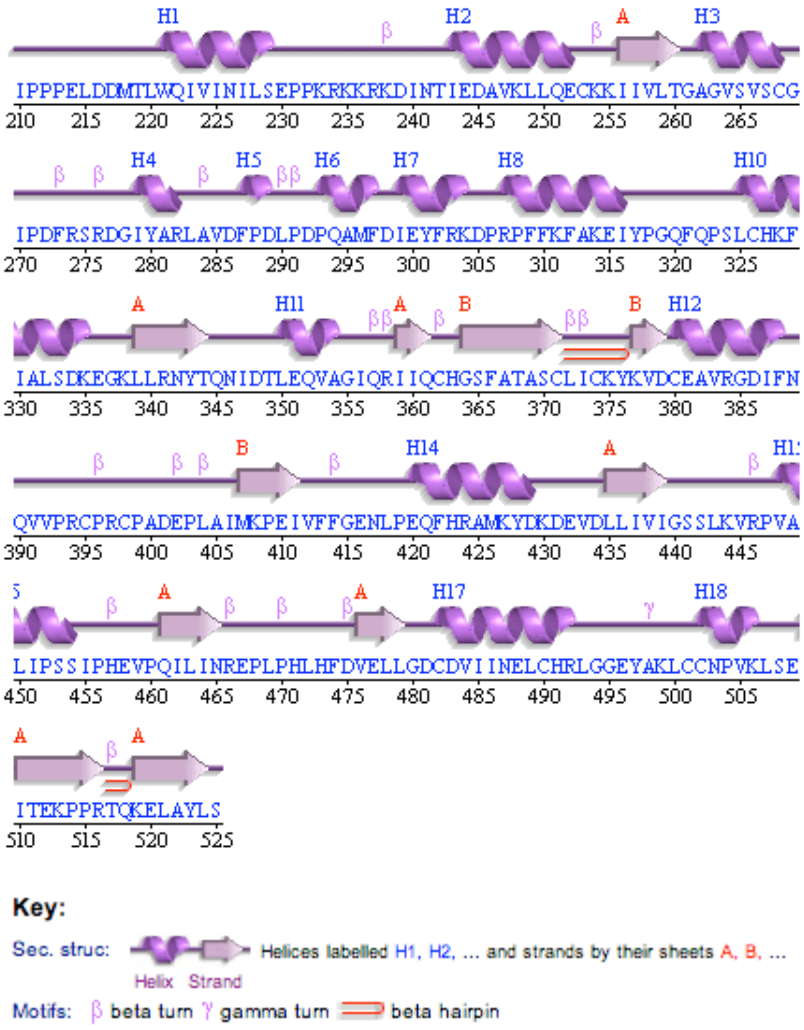


Fig. S4 Secondary structure elements of human SIRT1 homology model.

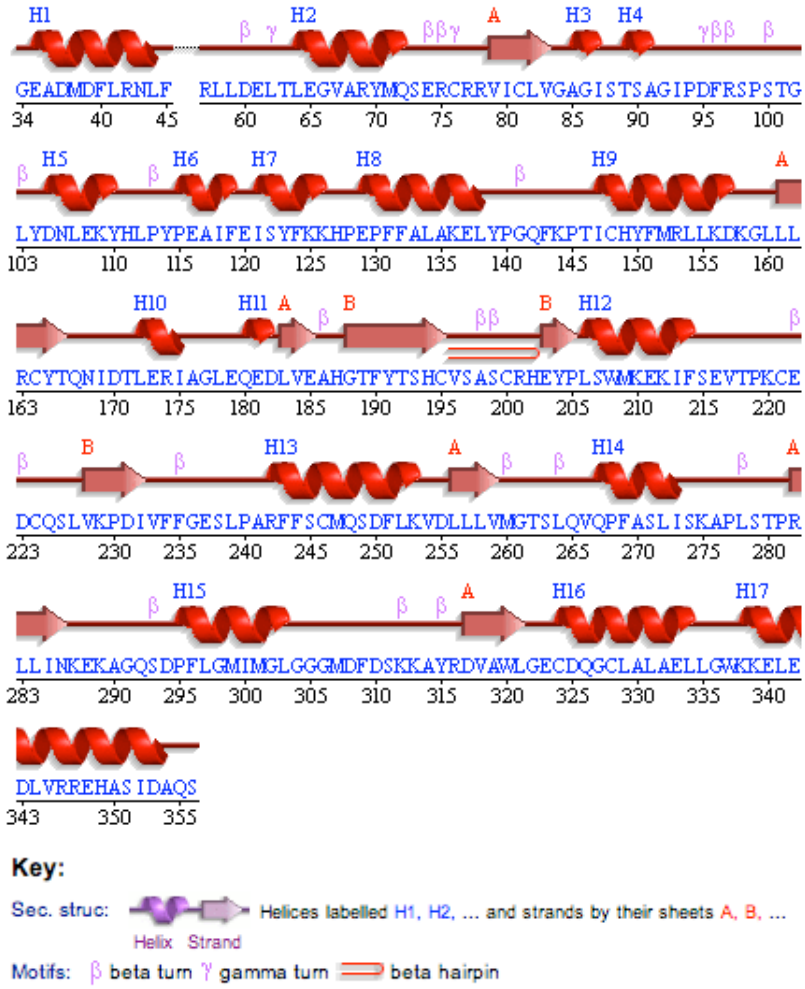


Fig. S5 Secondary structure elements of human SIRT2 crystal structure (PDB ID: 1J8F, chain B).

B. Enzymatic Screening Assays

All compounds were evaluated for their ability to inhibit recombinant sirtuins using a homogeneous fluorescent deacetylase assay. Stock solutions of inhibitors were prepared in DMSO. The assay was carried out in 96-well plates: 60 µL reaction volume contained the fluorescent histone deacetylase substrate ZMAL (10.5 µM), NAD^+ (500 µM), and SIRT2 or SIRT1. Total substrate conversion was driven to about 15% - 30% to assure initial state conditions. After 4 h incubation at 37 ˚C, the deacetylation reaction was stopped, and the metabolite formed (ZML, the deacetylated form of ZMAL) was developed using a tryptic digest for 20 min to form a different fluorophore. Finally, fluorescence was measured in a plate reader (BMG Polarstar) with excitation at λ=390 nm and emission at λ=460 nm. The amount of remaining substrate in the positive control with inhibitor versus negative control without inhibitor (only DMSO) was employed to calculate inhibition. All IC_{50} determinations were carried out in triplicate (pre-test determinations in duplicate). IC_{50} data were analyzed using GraphPad Prism software.

Materials, buffers and enzymes:

Fluorescent histone deacetylase substrate ZMAL, 12.6 mM in DMSO; stock solution of AMC (7-Amino-4-methylcoumarin; Fluka), 12.6 mM in DMSO; stock solution of nicotinamide, 120 mM in DMSO; all stored at -80 ˚C.

Sirtuin buffer pH 8.0: 25 mM Tris-HCl
137 mM NaCl
2.7 mM KCl
1 mM $MgCl_2$

Trypsin buffer pH 8.0: 50 mM Tris-HCl
100 mM NaCl

Recombinant hSIRT1 or recombinant hSIRT2 (in-house purification). Stock solution of trypsin from bovine pancreas (10000 BAEE units/mg) 6 mg/mL in trypsin buffer. Inhibitor solution in DMSO as 10 mM stock. Microplate reader Polarstar galaxy (BMG Labtechnologies, Germany) with an excitation filter of 390 nm an emission filter of 460 nm. Black 96-well micro plates (Greiner or PerkinElmer).

SIRT2

Plasmid pEV1440 (5.5 kb) was provided from the Lab of Prof. Dr. E. Verdin, Gladstone Institute, San Francisco, USA. Here, full length human SIRT2 cDNA was cloned into pHEX-2T with BamHI/EcoRI (original vector pGEX-2T from Phamacia in which the GST-encoding sequence was replaced by 6 x His). The resulting protein was an N-tagged 6 x His-Sirt2. We transformed pEV1440 into E. coli DH5α (Invitrogen) for plasmid purification and the purified plasmid was transformed into E. coli BL21 (DE3) for protein purification. First the protein was purified with affinity chromatography (Ni-NTA Superflow, Qiagen). In a second step the eluted protein was loaded on a PD10 desalting column (GE Healthcare) for buffer exchange. The activity was determined and the active fractions were pooled. The enzyme was analyzed with SDS page. The IC50 with NA was determined and the activity with or without NAD^+ was tested.

SIRT1

Plasmid pTe34 (6961 bp) was provided from Prof. Dr. A. Salminen (University Kuopio, Finland). Here, a human SIRT1 ORF fragment was inserted into pGEX2T (Amersham). The resulting protein was an N-tagged GST-SIRT1 fusion protein. Transformations for plasmid or protein purification were performed like described for SIRT2. The protein was purified by using Glutathione Sepharose 4B Beads (Amersham Biosciences) for affinity chromatography. Further purification and and analyses were performed like described before.

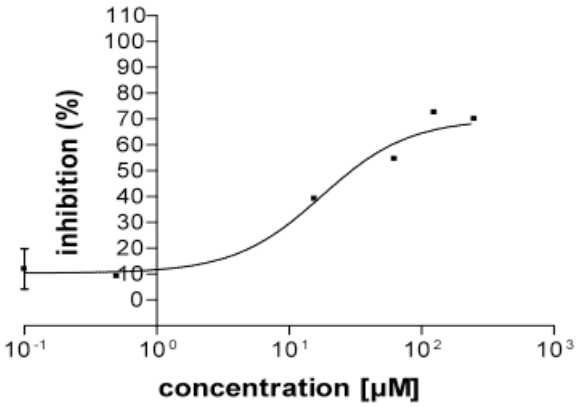


Fig. S6 Compound **8** concentration-SIRT2 inhibition curve.

Compound **8** (SIRT2 Fluorescence data)

		fluorescence data1	fluorescenze data	fluorescence data1-blank	fluorescence data2-blank	conversion [%] 1 related to 100% conversion	conversion [%] 1 related to 100% conversion	inhibition 1 related to conversion	inhibition 2 related to conversion	BMG Polarstar Ex: 390	Em: 460
blank		1253	1253	0	0					Tryp. Inkub [min]:	20
100% conversion		57982	57982	56728	56728					Inkub.time [min]:	240
conversion	DMSO	19114	19114	17861	17861	31.5	31.5			Inkub. Temp [°C]:	37
8	250 µM	6701	6478	5448	5225	9.6	9.2	69.5	70.7	Gain:	2151
8	125µM	6321	5976	5068	4723	8.9	8.3	71.6	73.6		
8	62,5 µM	9558	9199	8305	7946	14.6	14.0	53.5	55.5		
8	31,25 µM	14111	15632	12858	14379	22.7	25.3	28.0	19.5		
8	15,6 µM	12089	12143	10836	10890	19.1	19.2	39.3	39.0		
8	0,5 µM	17492	17492	16239	16239	28.6	28.6	9.1	9.1		
8	0,1µM	15582	18378	14329	17125	25.3	30.2	19.8	4.1		

Fig. S7 Fluorescence and percentage data, of SIRT2 inhibition, related to different concentrations of **8**.

C. Cellular Screens

SIRT inhibitors, EX-527, salermide and sirtinol were synthesized by us, while AK-7 was acquired from AKos GmbH. The inhibitors were dissolved in DMSO at a concentration of 20 mM for stock solutions and then appropriately diluted.

- *Cell viability analysis*

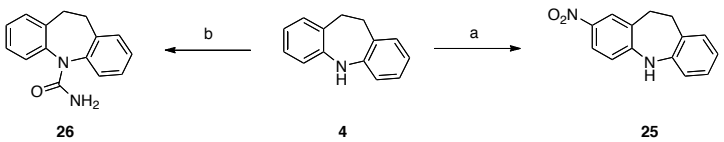
MCF-7 cells were incubated with growth media supplemented with DMSO or the indicated inhibitor at 30 µM for 0, 24, 48 and 72 hours. At each time point, cells were washed three times with PBS and once with EDTA, followed by trypsinisation. Cells were then suspended with PBS and cell numbers were determined using a haemocytometer. Representative data from three independent experiments are shown.

- *Cell cycle analysis*

Cell cycle analysis was performed by propidium iodide (PI) staining followed by FACS analysis. Both floating and adherent cells were harvested by trypsinisation, washed with PBS, fixed and permeablised with 90% cold ethanol. Cells were then stained with propidium iodide (0.2 mg/mL in PBS) containing 0.01 mg/mL RNase A. The stained cells were acquired using a Bectron Dickinson FACS flow cytometor and analysed for the cell cycle distribution by FacsDiva software (Becton Dickinson).

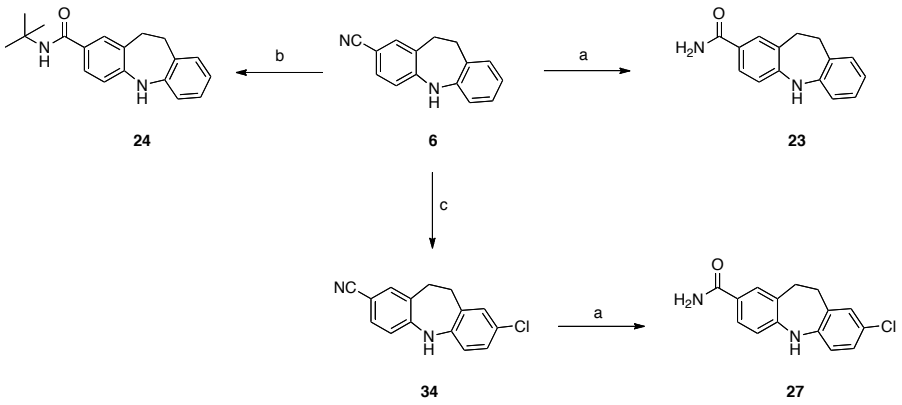
D. Synthetic Schemes

Scheme S1



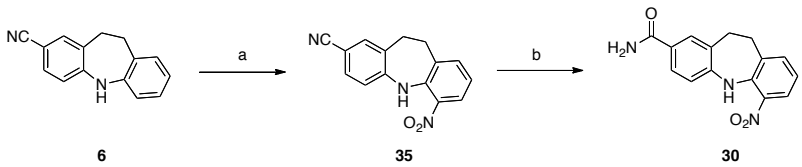
Reagents and conditions: (a) $ClSO_2NCO$, DCM, 0 ˚C, 1 h. (b) $AgNO_3$, BzCl, MeCN, rt, 16 h.

Scheme S2



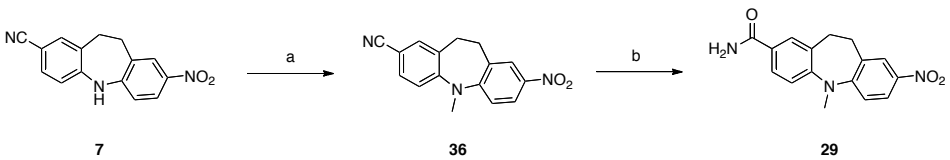
Reagents and conditions: (a) H_2O_2, KOH, EtOH, 60 ˚C, 2 h. (b) $EtCOO^tBu$, H_2SO_4, 42 ˚C, 1 h. (c) NCS, SiO_2, DCM, rt, 16 h.

Scheme S3



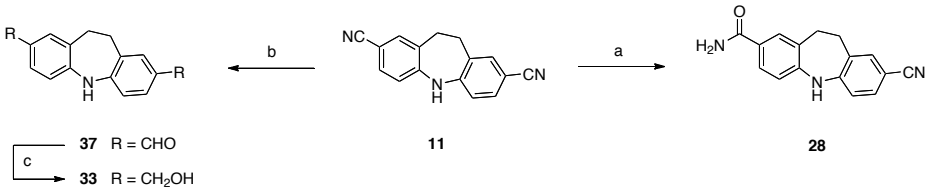
Reagents and conditions: (a) $AgNO_3$, BzCl, MeCN, rt, 16 h. (b) H_2O_2, KOH, EtOH, 60 ˚C, 2 h.

Scheme S4



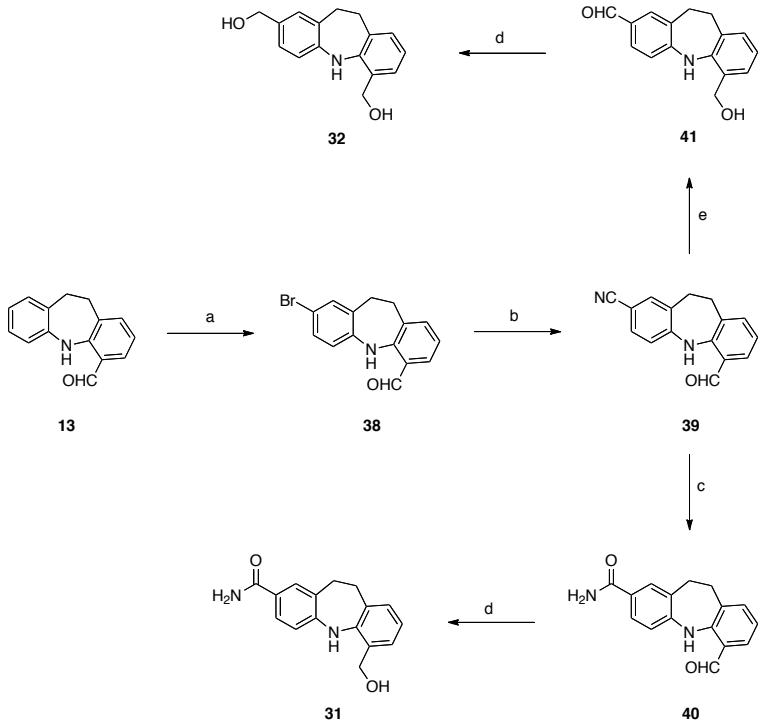
Reagents and conditions: (a) i) NaHMDS, THF, 0 ˚C 30 min; ii) MeI, rt, 16 h. (b) H_2O_2, KOH, EtOH, 60 ˚C, 2 h.

Scheme S5



Reagents and conditions: (a) H_2O_2, KOH, EtOH, 60 ˚C, 1 h. (b) DIBAL-H, DCM, 0 ˚C, 30 min. (c) $NaBH_4$, MeOH, rt, 1 h.

Scheme S6

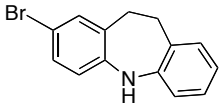


Reagents and conditions: (a) NBS, SiO_2, DCM, rt, 1 h. (b) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 90 ˚C, 2 h. (c) H_2O_2, KOH, EtOH, 60 ˚C, 2 h. (d) $NaBH_4$, MeOH, rt, 1 h. (e) DIBAL-H, DCM, 0 ˚C, 30 min.

E. Synthetic Procedures and Compound Characterization

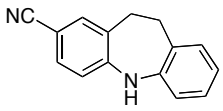
Melting points were obtained on a Reichert-Thermovar melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin Elmer (Spectrum Express Version 1.03.00) spectrometer with automated background substraction. Reported absorptions are strong or medium strength unless stated otherwise and given in wavenumbers (cm^{-1}). ^{1}H NMR spectra were recorded on a Bruker DRX-400 spectrometer operating at 400 MHz. ^{13}C NMR spectra were recorded on a Bruker DRX-400 spectrometer operating at 100 MHz. Chemical shifts (δ) are quoted in parts per million (ppm) and are referenced to a residual solvent peak. $CDCl_3$ (δ_H: 7.26, δ_C: 77.0), DMSO-d_6 (δ_H: 2.50, δ_C: 39.5). Low and high resolution mass spectrometry (EI, ESI) were recorded using a Micromass Platform II and Micromass AutoSpec-Q spectrometer. Elemental analyses were determined by the University of North London Analytical Service. All manipulations of air or moisture sensitive materials were carried out in oven or flame dried glassware under an inert atmosphere of nitrogen or argon. Syringes, which were used to transfer reagents and solvents, were purged with nitrogen prior to use. Reaction solvents were distilled from CaH_2 (dichloromethane, triethylamine), Na/Ph_2CO (tetrahydrofuran, diethyl ether) or obtained as dry or anhydrous from Sigma-Aldrich Chemical Company (*N*,*N*-dimethylformamide, acetonitrile) or BDH (ethanol). All reagents were obtained from commercial suppliers and used as obtained if purity was ≥98%. All flash-column chromatography was carried out on BDH silica gel 60, particle size 0.040 - 0.063 mm unless otherwise stated. Thin layer chromatography (TLC) was performed on pre-coated aluminium backed or glass backed plates (Merck Kieselgel 60 F_{254}), and visualised with ultraviolet light (254 nm) or potassium permanganate ($KMnO_4$), vanillin or phosphomolybdic acid (PMA) stains as deemed appropriate.

2-Bromo-10,11-dihydro-5H-dibenz[b,f]azepine (5)



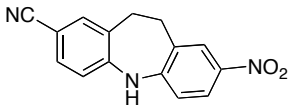
To a solution of **4** (5.00 g, 25.64 mmol) in DCM (2 L) were added NBS (4.60 g, 25.64 mmol) and SiO_2 (50.00 g). The resulting mixture was stirred at room temperature for 1 h. The suspension was filtered through a pad of celite and the filtrate washed with brine, dried over $MgSO_4$, filtered and evaporated under reduced pressure to afford the title compound (6.30 g) as a blue solid, which was used in the next step without purification: MS (ESI) m/z 274 $(M+H)^+$; HRMS (ESI) m/z calc for $C_{14}H_{13}BrN$ 274.0231, found: 274.0224.

10,11-Dihydro-5H-dibenz[b,f]azepine-2-carbonitrile (6)



Degassed DMF (30 mL) was added to a mixture of **5** (6.20 g, 22.71 mmol), $Zn(CN)_2$ (2.70 g, 22.71 mmol) and $Pd(PPh_3)_4$ (5.20 g, 4.54 mmol). The resulting suspension was stirred at 90 ˚C for 2 h. Sat. Na_2CO_3 (aq.) (200 mL) was added and the crude product extracted with Et_2O (x 2). The combined organics were washed with brine, dried over $MgSO_4$, filtered and the solvent evaporated under reduced pressure. The resultant residue was purified by silica gel flash-column chromatography with hexanes:DCM (4:6) as eluent to afford **6** (2.00 g, 36% over two steps) as a white solid: Elem. anal. calc for $C_{15}H_{12}N_2$: C, 81.79; H, 5.49; N, 12.72; found: C, 81.80; H, 5.41; N, 12.82; IR (neat) 3340, 2216 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 2.96 (*app*-s, 4H), 6.78 (*app*-t, *J* = 7.3 Hz, 1H), 7.01 - 7.13 (m, 4H), 7.41 - 7.46 (m, 2H), 8.99 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 34.8, 35.3, 99.0, 118.6, 119.3, 120.5, 120.6, 127.3, 127.8, 129.8, 130.7, 131.0, 135.1, 141.6, 147.3; MS (ESI) m/z 221 $(M+H)^+$; HRMS (ESI) m/z calc for $C_{15}H_{13}N_2$ 221.1079, found: 221.1067.

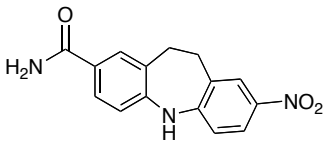
8-Nitro-10,11-dihydro-5H-dibenz[b,f]azepine-2-carbonitrile (7)



To a solution of **6** (480 mg, 2.18 mmol) in MeCN (10 mL) were added $AgNO_3$ (405 mg, 2.40 mmol) and BzCl (277 μL, 2.40 mmol) at 0 ˚C. The resulting mixture was stirred at room temperature for 16 h. MeCN was evaporated and the resultant residue dissolved in EtOAc and filtered under reduced pressure. The filtrate was washed with sat. Na_2CO_3 (aq.), brine, dried over $MgSO_4$, filtered and the solvent evaporated under reduced pressure. The crude material was purified by silica gel flash-column chromatography with hexanes:DCM (1:9) as eluent to afford **7** (200 mg, 35%) as an orange solid: Elem. anal. calc for $C_{15}H_{11}N_3O_2$: C, 67.92; H, 4.18; N, 15.84; found: C, 67.81; H, 4.09; N, 15.73; IR (neat) 3339, 2220, 1495, 1289 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 2.96 - 3.14 (m, 4H), 7.20 (*app*-t, *J* = 8.5 Hz, 2H), 7.52 - 7.62 (m, 2H), 7.97 - 8.06 (m, 2H), 9.80 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 34.4, 34.7,

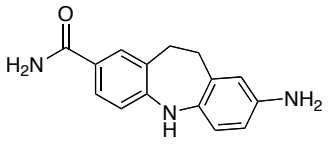
101.9, 119.2, 119.9, 120.1, 123.5, 126.9, 128.7, 130.3, 131.3, 134.9, 139.3, 145.5, 148.2; MS (EI) m/z 265 $M^{\bullet+}$; HRMS (EI) m/z calc for $C_{15}H_{11}N_3O_2$ 265.0851, found: 265.0848.

8-Nitro-10,11-dihydro-5H-dibenz[b,f]azepine-2-carboxamide (8)



To a solution of **7** (100 mg, 0.38 mmol) in EtOH (5 mL) were added KOH (192 mg, 3.42 mmol) and 35% wt. H_2O_2 (aq.) (0.8 mL, 9.50 mmol). The resultant mixture was stirred at 60 ˚C for 1 h. EtOH was evaporated and the residue partitioned between EtOAc and water. The organic layer was washed with brine, dried over $MgSO_4$, filtered and evaporated under reduced pressure to provide a residue which was purified by silica gel flash-column chromatography with DCM:EtOAc (3:7) as eluent to give the title compound (100 mg, 95%) as an orange crystalline solid: mp 248 ˚C (from EtOAc:MeOH 2:1); Elem. anal. calc for $C_{15}H_{13}N_3O_3$: C, 63.60; H, 4.63; N, 14.83; found: C, 63.66; H, 4.60; N, 14.83; IR (neat) 3447, 3339, 3296, 1643, 1496, 1310 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 3.00 - 3.14 (m, 4H), 7.09 - 7.19 (m, 3H), 7.64 - 7.69 (m, 2H), 7.71 - 7.80 (br s, 1H), 7.96 - 8.04 (m, 2H), 9.62 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 34.5, 34.7, 118.1, 118.7, 123.1, 126.0, 126.5, 126.6, 127.4, 128.7, 130.1, 138.0, 143.2, 148.5, 167.3; MS (ESI) m/z 284 $(M+H)^+$; HRMS (ESI) m/z calc for $C_{15}H_{14}N_3O_3$ 284.1035, found: 284.1023.

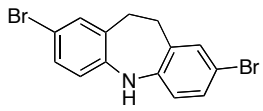
8-Amino-10,11-dihydro-5H-dibenz[b,f]azepine-2-carboxamide (9)



To a solution of **8** (60 mg, 0.21 mmol) in MeOH (5 mL) were added NH_4HCO_2 (134 mg, 2.10 mmol) and Pd/C (12 mg). The resulting mixture was stirred at 50 ˚C for 1 h. The mixture was filtered through a pad of celite and the filtrate evaporated. The residue was partitioned between EtOAc and water. The organic phase was washed with brine, dried over $MgSO_4$, filtered and the solvent evaporated under reduced pressure. The crude material was purified by silica gel flash-column chromatography with EtOAc as eluent to afford **9** (20 mg, 37%) as

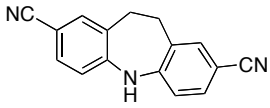
a brown solid: IR (neat) 3321, 3197, 1641 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 2.79 - 2.99 (m, 4H), 4.56 (s, 2H), 6.30 - 6.38 (m, 2H), 6.72 (d, *J* = 8.4 Hz, 1H), 6.82 (d, *J* = 8.4 Hz, 1H), 6.87 (s, 1H), 7.45 - 7.53 (m, 2H), 7.55 (s, 1H), 8.16 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 34.9, 35.9, 113.3, 115.8, 116.7, 120.0, 122.2, 124.6, 126.7, 131.2, 131.3, 132.6, 142.4, 146.8, 168.2; MS (ESI) m/z 254 $(M+H)^+$; HRMS (ESI) m/z calc for $C_{15}H_{16}N_3O$ 254.1293, found: 254.1299.

2,8-Dibromo-10,11-dihydro-5H-dibenz[b,f]azepine (10)



Following the procedure described for the preparation of 2-bromo-10,11-dihydro-5H-dibenz[b,f]azepine (**5**), compound **4** (1.00 g, 5.13 mmol) was treated with NBS (1.87 g, 10.52 mmol) and SiO_2 (20.00 g) in DCM (1 L) to give, after purification by silica gel flash-column chromatography with hexanes:DCM (7:3) as eluent and crystallisation from $CHCl_3$, the title compound (1.60 g, 88%) as white crystals: ^1H NMR (400 MHz, DMSO-d_6) δ 2.92 (s, 4H), 6.91 (d, *J* = 8.0 Hz, 2H), 7.17 - 7.21 (m, 4H), 8.58 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 34.6, 110.2, 120.5, 129.7, 130.7, 132.9, 142.3; MS (ESI) m/z 352 $(M+H)^+$; HRMS (ESI) m/z calc for $C_{14}H_{12}Br_2N$ 351.9336, found: 351.9330.

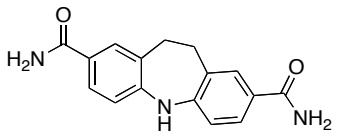
10,11-Dihydro-5H-dibenz[b,f]azepine-2,8-dicarbonitrile (11)



Following the procedure described for the preparation of 10,11-dihydro-5H-dibenz[b,f]azepine-2-carbonitrile (**6**), compound **10** (1.50 g, 4.25 mmol) was treated with $Zn(CN)_2$ (994 mg, 8.50 mmol) and $Pd(PPh_3)_4$ (982 mg, 0.85 mmol) in degassed DMF (5 mL) to give, after purification by silica gel flash-column chromatography with hexanes:DCM (1:9) as eluent, compound **11** (0.80 g, 77%) as a white solid: Elem. anal. calc for $C_{16}H_{11}N_3$: C, 78.35; H, 4.52; N, 17.13; found: C, 78.27; H, 4.47; N, 17.10; IR (neat) 3329, 2217 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 3.00 (s, 4H), 7.16 (d, *J* = 8.0 Hz, 2H), 7.51 - 7.57 (m, 4H), 9.53 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 34.5, 101.1, 119.7, 120.1, 129.6, 131.3,

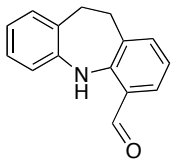
134.9, 146.0; MS (ESI) m/z 245 M^+; HRMS (ESI) m/z calc for $C_{16}H_{11}N_3$ 245.0953, found: 245.0945.

10,11-Dihydro-5H-dibenz[b,f]azepine-2,8-dicarboxamide (12)



Following the procedure described for the preparation of 8-nitro-10,11-dihydro-5H-dibenz[b,f]azepine-2-carboxamide (**8**), compound **11** (150 mg, 0.61 mmol) was treated with KOH (307 mg, 5.49 mmol) and 35% wt. H_2O_2 (aq.) (1.3 mL, 15.25 mol) to give, after purification by silica gel flash-column chromatography with EtOAc:MeOH (9:1) as eluent, the desired compound **12** (60 mg, 35%) as a white solid: IR (neat) 3439, 3458, 3316, 1643, 1598 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 2.98 - 3.02 (s, 4H), 6.96 - 7.10 (m, 4H), 7.56 - 7.63 (m, 4H), 7.69 (s, 2H), 8.99 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 35.5, 118.1, 124.7, 126.9, 127.6, 130.8, 145.1, 168.0; MS (ESI) m/z 282 $(M+H)^+$; HRMS (ESI) m/z calc for $C_{16}H_{16}N_3O_2$ 282.1243, found: 282.1237.

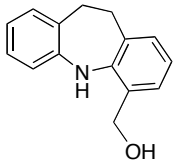
10,11-Dihydro-5H-dibenz[b,f]azepine-4-carbaldehyde (13)



To a solution of **4** (6.0 g, 30.77 mmol) in Et_2O (200 mL) was added *n*-BuLi (38 mL, 92.31 mmol) at -78 ˚C and the resulting mixture was stirred at room temperature for four days. DMF (3.6 mL, 46.16 mmol) was added at -78 ˚C and the reaction was allowed to stir at room temperature for additional 24 h. 0.5N HCl (aq.) (150 mL) was added. The organic phase was separated from the aqueous solution, washed with brine, dried over $MgSO_4$, filtered and the solvent evaporated under reduced pressure. The resultant residue was purified by silica gel flash-column chromatography with hexanes:DCM (7:3) as eluent to yield **13** (5.9 g, 85%) as an orange oil: Elem. anal. calc for $C_{15}H_{13}NO$: C, 80.69; H, 5.87; N, 6.27; found: C, 80.76; H, 5.84; N, 6.14; IR (neat) 1655 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.06 - 3.12 (m, 4H), 6.78 (*app*-t, *J* = 7.5 Hz, 1H), 6.87 (*app*-t, *J* = 7.2 Hz, 1H), 7.02 (d, *J* = 8.0 Hz, 1H), 7.07 (d, *J* = 7.6

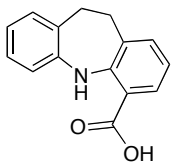
Hz, 1H), 7.16 (*app*-t, *J* = 8.2 Hz, 1H), 7.22 (d, *J* = 7.3 Hz, 1H), 7.42 (d, *J* = 7.8 Hz, 1H), 9.87 (s, 1H), 11.27 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.9, 36.0, 117.0, 120.0, 120.4, 121.0, 127.1, 129.2, 130.1, 130.8, 135.8, 136.9, 140.7, 145.8, 194.6; MS (ESI) m/z 224 $(M+H)^+$; HRMS (ESI) m/z calc for $C_{15}H_{14}NO$ 224.1075, found: 224.1073.

(10,11-Dihydro-5H-dibenz[b,f]azepin-4-yl)methanol (14)



To a solution of **13** (50 mg, 0.22 mmol) in MeOH (2 mL) was added $NaBH_4$ (8 mg, 0.22 mmol) and the resulting mixture was stirred at room temperature for 2 h. H_2O was added and the solution concentrated. The crude product was extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, filtered and the solvent evaporated under reduced pressure. The residue was purified by silica gel flash-column chromatography with hexanes:EtOAc (9:1) as eluent, to afford the title compound (45 mg, 90%) as a white solid: Elem. anal. calc for $C_{15}H_{15}NO$: C, 79.97; H, 6.71; N, 6.22; found: C, 80.07; H, 6.67; N, 6.15; IR (neat) 3381, 3216 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.76 (s, 1H), 3.00 - 3.15 (m, 4H), 4.78 (s, 2H), 6.74 (*app*-t, *J* = 7.1 Hz, 2H), 6.83 (d, *J* = 7.8 Hz 1H), 6.96 - 7.22 (m, 4H), 7.63 - 7.77 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 35.0, 35.4, 65.5, 118.7, 118.9, 119.1, 126.5, 126.8, 127.5, 127.6, 130.8, 130.9, 131.5, 142.4, 142.8; MS (ESI) m/z 226 $(M+H)^+$; HRMS (ESI) m/z calc for $C_{15}H_{16}NO$ 226.1232, found: 226.1230.

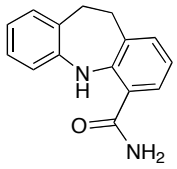
10,11-Dihydro-5H-dibenz[b,f]azepine-4-carboxylic acid (15)



To a solution of **13** (2.0 g, 8.97 mmol) in *t*-butanol (48 mL) and H_2O (12 mL) were added NaH_2PO_4 (9.6 g, 71.76 mmol), 2-methyl-2-butene (9.4 mL, 89.70 mmol) and $NaClO_2$ (3.2 g, 35.88 mmol). The mixture was stirred at room temperature for 16 h. *t*-Butanol was evaporated and the resulting residue partitioned between Et_2O and 2.0M NaOH (aq.). The aqueous phase

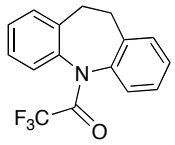
was washed with Et_2O (x 2), acidified with conc. HCl to acidic pH and filtered. The filter cake was washed with cold water and *n*-hexane, and purified by silica gel flash-column chromatography with DCM:MeOH (97:3) as eluent to give **15** (1.5 g, 71 %) as a yellow solid: Elem. anal. calc for $C_{15}H_{13}NO_2$: C, 75.30; H, 5.48; N, 5.85; found: C, 75.25; H, 5.40; N, 5.86; IR (neat) 2947, 1661 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 3.02 (*app*-s, 4H), 6.72 - 6.82 (m, 2H), 6.85 (d, J = 7.7 Hz, 1H), 7.05 - 7.15 (m, 2H), 7.29 (d, J = 7.4 Hz, 1H), 7.83 (d, J = 8.0 Hz, 1H), 10.98 (s, 1H), 13.15 - 13.32 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 34.9, 35.5, 113.5, 117.9, 119.7, 120.3, 127.4, 129.3, 130.5, 130.6, 130.9, 136.0, 141.5, 146.3, 171.3; MS (ESI) m/z 238 $(M-H)^-$; HRMS (ESI) m/z calc for $C_{15}H_{12}NO_2$ 238.0868, found: 238.0872.

10,11-Dihydro-5H-dibenz[b,f]azepine-4-carboxamide (16)



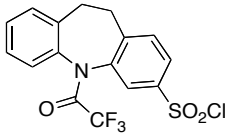
A solution of **15** (550 mg, 2.30 mmol), 0.5M NH_3 sol. in 1,4-dioxane (20 mL), HOBt (373 mg, 2.76 mmol) and EDC•HCl (527 mg, 2.76 mmol) was stirred at room temperature for 16 h. The solvent was evaporated and the residue partitioned between EtOAc and sat. Na_2CO_3 (aq.). The organic layer was washed with brine, dried over $MgSO_4$, filtered and the solvent evaporated under reduced pressure. The resultant residue was purified by silica gel flash-column chromatography with DCM:EtOAc (95:5) as eluent to yield **16** (400 mg, 82 %) as a yellow solid: Elem. anal. calc for $C_{15}H_{14}N_2O$: C, 75.61; H, 5.92; N, 11.76; found: C, 75.55; H, 5.86; N, 11.70; IR (neat) 3389, 3361, 3208, 1637 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 2.99 (*app*-s, 4H), 6.69 - 6.78 (m, 3H), 7.02 - 7.11 (m, 2H), 7.19 (d, J = 7.1 Hz, 1H), 7.50 - 7.62 (m, 2H), 8.06 - 8.16 (br s, 1H), 11.21 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 35.1, 35.2, 117.8, 118.0, 119.3, 119.4, 127.4, 127.8, 128.4, 131.0, 131.4, 133.8, 142.1, 144.7, 172.6; MS (ESI) m/z 239 $(M+H)^+$; HRMS (ESI) m/z calc for $C_{15}H_{15}N_2O$ 239.1184, found: 239.1183.

1-(10,11-Dihydro-5H-dibenz[b,f]azepin-5-yl)-2,2,2-trifluoroethanone (17)



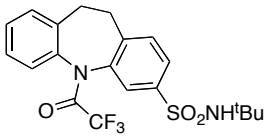
To a solution of **4** (3.00 g, 15.38 mmol) in DCM (30 mL) were added at 0 ˚C TFAA (4.3 mL, 30.77 mmol), DIPEA (6.7 mL, 38.46 mmol) and DMAP (cat.). The mixture was stirred at room temperature for 16 h. 1N HCl (aq.) (50 mL) was added. The organic phase was separated from the acidic aqueous solution, washed with brine, dried over $MgSO_4$, filtered and the solvent evaporated under reduced pressure. The resultant residue was purified by silica gel flash-column chromatography with hexanes:EtOAc (9:1) as eluent to yield **17** (3.28 g, 73%) as a yellow solid: Elem. anal. calc for $C_{16}H_{12}F_3NO$: C, 65.98; H, 4.15; N, 4.81; found: C, 65.94; H, 4.10; N, 4.76; IR (neat) 1695 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.82 - 2.99 (m, 2H), 3.33 - 3.58 (m, 2H), 7.13 - 7.46 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.7, 30.8, 116.5 (q), 127.0, 127.1, 127.3, 127.6, 128.5, 129.5, 129.7, 131.4, 134.2, 137.9, 138.8, 139.4, 156.7 (q); MS (ESI) m/z 292 $(M+H)^+$; HRMS (ESI) m/z calc for $C_{16}H_{13}F_3NO$ 292.0949, found: 292.0942.

1-(3-(Chlorosulphonyl)-10,11-dihydro-5H-dibenz[b,f]azepin-5-yl)-2,2,2-trifluoroethanone (18)



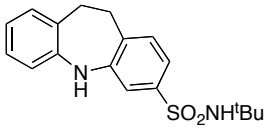
17 (150 mg, 0.52 mmol) was added to $ClSO_3H$ (2 mL) at 0 ˚C and the resultant mixture was allowed to stir at 0 ˚C for 3 h. The solution was poured dropwise onto crushed ice and the product extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, filtered and the solvent evaporated under reduced pressure. The resultant residue was purified by silica gel flash-column chromatography with hexanes:EtOAc (7:3) as eluent to yield the title compound (130 mg, 65%) as a white solid: ^1H NMR (400 MHz, $CDCl_3$) δ 2.85 - 3.09 (m, 2H), 3.38 - 3.62 (m, 2H), 7.17 - 7.61 (m, 5H), 7.82 - 8.12 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.7, 31.5, 116.5 (q), 127.0, 127.1, 127.3, 127.6, 128.5, 129.5, 129.7, 131.4, 134.2, 137.9, 139.2, 143.0, 156.7 (q).

***N-tert*-Butyl-5-trifluoroacetyl-10,11-dihydro-5H-dibenz[b,f]azepine-3-sulphonamide (19)**



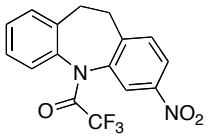
To a solution of **18** (80 mg, 0.21 mmol) in DCM (2 mL) was added t-BuNH$_2$ (88 μL, 0.84 mmol). The mixture was stirred at room temperature for 16 h. The solution was washed with brine, dried over $MgSO_4$, filtered and the solvent evaporated under reduced pressure to afford **19** (82 mg, 93%) as a white solid, which was used in the next step without purification.

***N-tert*-Butyl-10,11-dihydro-5H-dibenz[b,f]azepine-3-sulphonamide (20)**



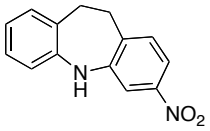
To a solution of **19** (60 mg, 0.14 mmol) in MeOH:THF:H_2O (2:1:1 mL) was added K_2CO_3 (580 mg, 4.20 mmol). The resultant mixture was stirred at room temperature for 16 h. The solvent was concentrated under reduced pressure and the resulting residue partitioned between EtOAc and H_2O. The organic phase was washed with brine, dried over $MgSO_4$, filtered and the solvent evaporated under reduced pressure. The crude material was purified by silica gel flash-column chromatography with DCM:EtOAc (97:3) as eluent to afford **20** (43 mg, 92%) as a white solid: Elem. anal. calc for $C_{18}H_{22}N_2O_2S$: C, 65.42; H, 6.71; N, 8.48; found: C, 65.58; H, 6.60; N, 8.44; IR (neat) 3366, 3261, 1299, 1151 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 1.13 (s, 9H), 2.91 - 3.04 (m, 4H), 6.70 (*app*-t, J = 7.3 Hz, 1H), 6.97 - 7.09 (m, 4H), 7.17 (d, J = 8.0 Hz, 1H), 7.36 (s, 1H), 7.49 (d, J = 1.8 Hz, 1H), 8.68 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 29.8, 34.4, 34.8, 53.1, 115.6, 115.7, 118.2, 119.0, 126.7, 128.1, 130.3, 130.8, 131.1, 142.2, 142.5, 142.9; MS (ESI) m/z 329 $(M+H)^-$; HRMS (ESI) m/z calc for $C_{18}H_{21}N_2O_2S$ 329.1329, found: 329.1333.

1-(3-Nitro-10,11-dihydro-5H-dibenz[b,f]azepin-5-yl)-2,2,2-trifluoroethanone (21)



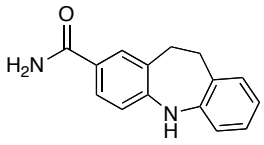
To a solution of **17** (200 mg, 0.69 mmol) in Ac_2O (3 mL) was added at 0 ˚C HNO_3 (1 mL). The resulting mixture was stirred at room temperature for 16 h, after which time the reaction was found to be complete (by TLC). The solution was poured dropwise onto crushed ice and the product extracted with EtOAc. The organic layer was washed with sat. $NaHCO_3$ (aq.), brine, dried over $MgSO_4$, filtered and the solvent evaporated under reduced pressure to give a crude product (250 mg) which was used in the next step without purification.

3-Nitro-10,11-dihydro-5H-dibenz[b,f]azepine (22)



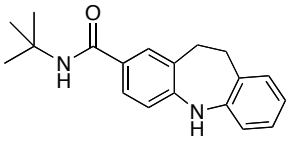
Following the procedure described for the preparation of *N-tert*-butyl-10,11-dihydro-5H-dibenz[b,f]azepine-3-sulphonamide (**20**), compound **21** (200 mg, crude material) was treated with K_2CO_3 (1.64 g) in MeOH:THF:H_2O (2:1:1 mL) to give, after purification by silica gel flash-column chromatography with hexanes:DCM (6:4) as eluent, the title compound (60 mg, 45% over two steps) as an orange solid: Elem. anal. calc for $C_{14}H_{12}N_2O_2$: C, 69.99; H, 5.03; N, 11.66; found: C, 69.89; H, 4.86; N, 11.54; IR (neat) 3366, 1489, 1318 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 2.93 - 3.11 (m, 4H), 6.75 (*app*-t, *J* = 7.2 Hz, 1H), 6.95 - 7.14 (m, 3H), 7.26 (d, *J* = 8.5 Hz, 1H), 7.46 (d, *J* = 7.2 Hz, 1H), 7.90 (s, 1H), 8.88 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 34.4, 35.5, 112.5, 112.5, 118.8, 120.1, 127.4, 129.0, 130.7, 132.2, 135.1, 142.2, 144.3, 147.0; MS (ESI) m/z 241 $(M+H)^+$; HRMS (ESI) m/z calc for $C_{14}H_{13}N_2O_2$ 241.0977, found: 241.0979.

10,11-Dihydro-5H-dibenz[b,f]azepine-2-carboxamide (23)



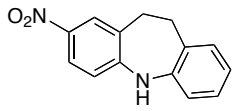
Following the procedure described for the preparation of 8-nitro-10,11-dihydro-5H-dibenz[b,f]azepine-2-carboxamide (**8**), compound **6** (100 mg, 0.45 mmol) was treated with KOH (0.22 g, 3.63 mmol) and 35% wt. H_2O_2 (aq.) (1.1 mL, 11.35 mol) in EtOH (2.5 mL) to give, after purification by silica gel flash-column chromatography with DCM:EtOAc (4:6) as eluent, the desired compound (104 mg, 96%) as a white solid: Elem. anal. calc for $C_{15}H_{14}N_2O$: C, 75.61; H, 5.92; N, 11.76; found: C, 75.51; H, 5.88; N, 11.64; IR (neat) 3359, 3153, 1638 cm^{-1}; ^1H NMR (400MHz, DMSO-d_6) δ 2.98 (*app*-s, 4H), 6.72 (*app*-t, J = 7.6 Hz, 1H), 6.93 - 7.13 (m, 5H), 7.54 - 7.74 (m, 3H), 8.65 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 35.2, 35.8, 117.6, 118.8, 119.7, 123.8, 126.6, 126.8, 127.2, 129.1, 130.7, 130.9, 142.5, 145.9, 168.1; MS (ESI) m/z 239 $(M+H)^+$; HRMS (ESI) m/z calc for $C_{15}H_{15}N_2O$ 239.1184, found: 239.1181.

***N-tert*-Butyl-10,11-dihydro-5H-dibenz[b,f]azepine-2-carboxamide (24)**



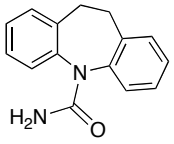
To a solution of **6** (40 mg, 0.14 mmol) in $EtCO_2t$-Bu (1 mL) was added conc. H_2SO_4 (cat.) and the resulting mixture was stirred at 42 ˚C for 1 h. Water (2 mL) was added and the crude product extracted with EtOAc (x2). The combined organics were washed with brine, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The resultant residue was purified by silica gel flash-column chromatography with hexanes:EtOAc (7:3) as eluent to afford **24** (10 mg, 19%) as a pale yellow solid: IR (neat) 3326, 1594 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, 9H), 2.88 - 3.28 (*app*-br s, 4H), 5.84 (s, 1H), 6.08 - 6.43 (br s, 1H), 6.52 - 6.95 (m, 3H), 6.99 - 7.16 (m, 2H), 7.35 - 7.53 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.0, 34.8, 35.3, 51.4, 117.5, 118.3, 120.3, 125.5, 126.0, 127.0, 127.4, 129.2, 130.0, 130.6, 141.5, 144.9, 166.5; MS (ESI) m/z 295 $(M+H)^+$; HRMS (ESI) m/z calc for $C_{19}H_{23}N_2O$ 295.1810, found: 295.1795.

2-Nitro-10,11-dihydro-5H-dibenz[b,f]azepine (25)



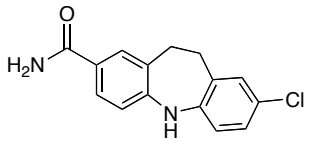
Following the procedure described for the preparation of 8-nitro-10,11-dihydro-5H-dibenz[b,f]azepine-2-carbonitrile (**7**), compound **4** (300 mg, 1.54 mmol) was treated with $AgNO_3$ (286 mg, 1.69 mmol) and BzCl (195 μL, 1.69 mmol) in anhydrous MeCN (10 mL) to give, after purification by silica gel flash-column chromatography with hexanes:DCM (6:4) as eluent, the title compound (100 mg, 27%) as an orange solid: Elem. anal. calc for $C_{14}H_{12}N_2O_2$: C, 69.99; H, 5.03; N, 11.66; found: C, 70.03; H, 4.96; N, 11.75; IR (neat) 3355, 1494, 1290 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 2.94 - 3.09 (m, 4H), 6.84 (*app*-t, *J* = 7.2 Hz, 1H), 7.05 - 7.18 (m, 4H), 7.91 - 7.98 (m, 2H), 9.39 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 34.7, 35.5, 118.0, 119.8, 121.4, 123.6, 126.9, 127.3, 127.4, 130.5, 130.6, 137.8, 141.0, 149.6; MS (ESI) m/z 241 $(M+H)^+$; HRMS (ESI) m/z calc for $C_{14}H_{13}N_2O_2$ 241.0977, found: 241.0968.

10,11-Dihydro-5H-dibenz[b,f]azepine-5-carboxamide (26)



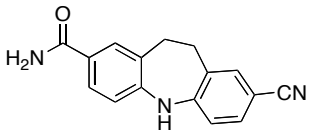
To a solution of dibenz[b,f]azepine **4** (100 mg, 0.50 mmol) in DCM (3.5 mL) was added $ClSO_2NCO$ (53 μL, 0.60 mmol) at 0 ˚C and the resulting mixture was stirred for 1 h. Water (3 mL) was added and the mixture allowed to stir for an additional 1 h. The organic phase was separated, washed with brine, dried over $MgSO_4$ and the solvent evaporated to give a residue which was purified by silica gel flash-column chromatography with DCM:MeOH (95:5) as eluent to afford **26** (95 mg, 80%) as a white solid: Elem. anal. calc for $C_{15}H_{14}N_2O$: C, 75.61; H, 5.92; N, 11.76; found: C, 75.56; H, 5.95; N, 11.84; IR (neat) 3471, 3352, 1648 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.84 (*app*-s, 2H), 3.42 (*app*-s, 2H), 4.82 (s, 2H), 7.18 - 7.28 (m, 6H), 7.36 - 7.44 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.7, 127.1, 128.1, 128.5, 130.4, 140.6, 157.6; MS (EI) m/z 238 M^+; HRMS (EI) m/z calc for $C_{15}H_{14}N_2O$ 238.1106, found: 238.1100.

8-Chloro-10,11-dihydro-5H-dibenz[b,f]azepine-2-carboxamide (27)



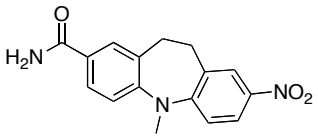
Following the procedure described for the preparation of 8-nitro-10,11-dihydro-5H-dibenz[b,f]azepine-2-carboxamide (**8**), compound **34** (20 mg, 0.08 mmol) was treated with KOH (36 mg, 0.64 mmol) and 35% wt. H_2O_2 (aq.) (0.2 mL, 2.00 mmol) in EtOH (1 mL) to give, after purification by silica gel flash-column chromatography with DCM:EtOAc (4:6) as eluent, the desired compound **27** (18 mg, 86%) as a white solid: IR (neat) 3469, 3324, 3182, 1635 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 2.97 (*app*-s, 4H), 6.88 - 7.18 (m, 5H), 7.49 - 7.74 (m, 3H), 8.78 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 34.8, 35.3, 117.7, 120.3, 122.9, 124.3, 126.7, 126.9, 127.0, 130.0, 130.9, 131.0, 141.6, 145.3, 168.1; MS (ESI) m/z 273 $(M+H)^+$; HRMS (ESI) m/z calc for $C_{15}H_{14}ClN_2O$ 273.0795, found: 273.0786.

8-Cyano-10,11-dihydro-5H-dibenz[b,f]azepine-2-carboxamide (28)



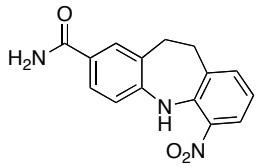
Following the procedure described for the preparation of 8-nitro-10,11-dihydro-5H-dibenz[b,f]azepine-2-carboxamide (**8**), compound **11** (150 mg, 0.61 mmol) was treated with KOH (307 mg, 5.49 mmol) and 35% wt. H_2O_2 (aq.) (1.3 mL, 15.25 mol) to give, after purification by silica gel flash-column chromatography with EtOAc:MeOH (9:1) as eluent, the desired compound **28** (40 mg, 25%) as a brown solid: IR (neat) 3421, 3345, 2216, 1649 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 2.99 (*app*-s, 4H), 6.99 - 7.16 (m, 3H), 7.43 - 7.53 (m, 2H), 7.59 - 7.67 (m, 2H), 7.73 (s, 1H), 9.28 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 35.0, 35.1, 100.0, 118.6, 119.1, 120.3, 125.7, 126.9, 128.4, 128.8, 130.7, 131.1, 135.0, 144.2, 146.7, 167.9; MS (ESI) m/z 264 $(M+H)^+$; HRMS (ESI) m/z calc for $C_{16}H_{14}N_3O$ 264.1137, found: 264.1128.

5-Methyl-8-nitro-10,11-dihydro-5H-dibenz[b,f]azepine-2-carboxamide (29)



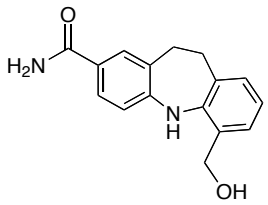
Following the procedure described for the preparation of 8-nitro-10,11-dihydro-5H-dibenz[b,f]azepine-2-carboxamide (**8**), compound **36** (25 mg, 0.09 mmol) was treated with KOH (45 mg, 0.81 mmol) and 35% wt. H_2O_2 (aq.) (0.2 mL, 2.25 mmol) in EtOH (1 mL) to give, after purification by silica gel flash-column chromatography with DCM:EtOAc (4:6) as eluent, the desired compound **29** (23 mg, 88%) as a yellow solid: Elem. anal. calc for $C_{16}H_{15}N_3O_3$: C, 64.64; H, 5.09; N, 14.13; found: C, 64.74; H, 5.00; N, 14.07; IR (neat) 3423, 3191, 1648, 1498, 1315 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 3.09 - 3.21 (m, 4H), 3.45 (s, 3H), 7.23 - 7.31 (m, 3H), 7.70 - 7.77 (m, 2H), 7.85 - 7.91 (br s, 1H), 7.98 - 8.06 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 31.6, 34.6, 42.6, 118.9, 121.3, 123.0, 126.4, 126.7, 128.7, 129.6, 130.8, 134.8, 140.2, 150.2, 152.9, 167.8; MS (ESI) m/z 298 $(M+H)^+$; HRMS (ESI) m/z calc for $C_{16}H_{16}N_3O_3$ 298.1192, found: 298.1186.

6-Nitro-10,11-dihydro-5H-dibenz[b,f]azepine-2-carboxamide (30)



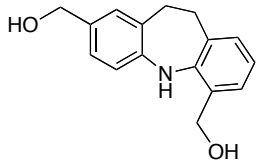
Following the procedure described for the preparation of 8-nitro-10,11-dihydro-5H-dibenz[b,f]azepine-2-carboxamide (**8**), compound **35** (30 mg, 0.11 mmol) was treated with KOH (55 mg, 0.99 mmol) and 35% wt. H_2O_2 (aq.) (0.25 mL, 2.75 mmol) in EtOH (1 mL) to give, after purification by silica gel flash-column chromatography with EtOAc as eluent, the title compound (23 mg, 73%) as a red solid: ^1H NMR (400 MHz, DMSO-d_6) δ 3.03 - 3.17 (m, 4H), 6.94 (*app*-t, J = 7.9 Hz, 1H), 7.09 (d, J = 8.2 Hz, 1H), 7.13 - 7.21 (br s, 1H), 7.50 (d, J = 7.0 Hz, 1H), 7.64 - 7.72 (m, 2H), 7.76 - 7.84 (br s, 1H), 8.01 (d, J = 8.0 Hz, 1H), 10.14 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 34.2, 34.6, 119.2, 119.8, 124.7, 126.3, 126.5, 128.2, 130.4, 133.8, 136.1, 136.5, 138.7, 142.3, 167.2; MS (ESI) m/z 284 $(M+H)^+$; HRMS (ESI) m/z calc for $C_{15}H_{14}N_3O_3$ 284.1035, found: 284.1037.

6-(Hydroxymethyl)-10,11-dihydro-5H-dibenz[b,f]azepine-2-carboxamide (31)



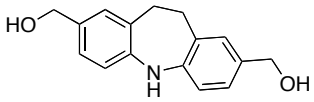
Following the procedure described for the preparation of (10,11-Dihydro-5H-dibenz[b,f]azepin-4-yl)methanol (**14**), compound **40** (40 mg, 0.15 mmol) was treated with $NaBH_4$ (6 mg, 0.15 mmol) in MeOH (1 mL) to give, after purification by silica gel flash-column chromatography with DCM:MeOH (9:1) as eluent, the desired compound **31** (35 mg, 87%) as colourless crystals: m.p. 201-203 ˚C (from EtOH); Elem. anal. calc for $C_{16}H_{16}N_2O_2$: C, 71.62; H, 6.01; N, 10.44; found: C, 71.68; H, 6.04; N, 10.40; IR (neat) 3360, 3193, 1665 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 3.01 (s, 4H), 4.64 (d, *J* = 5.1 Hz, 2H), 5.74 (t, *J* = 5.0 Hz, 1H), 6.76 (t, *J* = 7.4 Hz, 1H), 6.84 (d, *J* = 8.7 Hz, 1H), 6.96 - 7.11 (m, 3H), 7.55 - 7.63 (m, 2H), 7.67 (br s, 1H), 8.32 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 34.2, 35.3, 62.6, 117.6, 119.7, 123.5, 125.4, 126.5, 126.8, 128.9, 129.3, 130.7, 131.2, 140.8, 144.8, 167.5; MS (ESI) m/z 269 $(M+H)^+$; HRMS (ESI) m/z calc for $C_{16}H_{17}N_2O_2$ 269.1290, found: 269.1292.

(10,11-Dihydro-5H-dibenz[b,f]azepine-2,6-diyl)dimethanol (32)



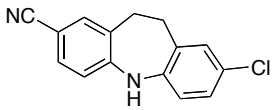
Following the procedure described for the preparation of (10,11-Dihydro-5H-dibenz[b,f]azepin-4-yl)methanol (**14**), compound **41** (90 mg, 0.36 mmol) was reduced with $NaBH_4$ (27 mg, 0.72 mmol) in MeOH (5 mL) to give, after purification by silica gel flash-column chromatography with DCM:MeOH (95:5) as eluent, compound **32** (80 mg, 88%) as a white solid: IR (neat) 3192 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 2.93 - 3.02 (m, 4H), 4.35 (d, *J* = 6.3 Hz, 2H), 4.61 (d, *J* = 5.2 Hz, 2H), 4.93 (t, *J* = 6.3 Hz, 1H), 5.66 (t, *J* = 5.2 Hz, 1H), 6.69 (*app*-t, *J* = 8.3 Hz, 1H), 6.78 (d, *J* = 8.2 Hz, 1H), 6.94 - 7.04 (m, 4H), 7.93 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 34.8, 35.4, 63.1, 63.3, 118.6, 119.2, 126.0, 126.9, 127.2, 128.6, 129.8, 129.9, 130.8, 132.8, 141.6, 142.4; MS (ESI) m/z 256 $(M+H)^+$; HRMS (ESI) m/z calc for $C_{16}H_{18}NO_2$ 256.1338, found: 256.1327.

(10,11-Dihydro-5H-dibenz[b,f]azepine-2,8-diyl)dimethanol (33)



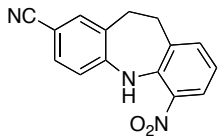
Following the procedure described for the preparation of (10,11-Dihydro-5H-dibenz[b,f]azepin-4-yl)methanol (**14**), compound **37** (50 mg, 0.20 mmol) was reduced with $NaBH_4$ (15 mg, 0.40 mmol) in MeOH (3 mL) to give, after purification by silica gel flash-column chromatography with DCM:Et_2O (1:1) as eluent, compound **33** (47 mg, 93%) as a yellow solid: IR (neat) 3367 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 2.94 (s, 4H), 4.34 (d, J = 5.8 Hz, 4H), 4.90 (t, J = 5.8 Hz, 2H), 6.86 - 6.99 (m, 6H), 8.18 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 35.4, 63.2, 118.0, 125.9, 127.6, 129.6, 132.6, 142.2; MS (ESI) m/z 256 $(M+H)^+$; HRMS (ESI) m/z calc for $C_{16}H_{18}NO_2$ 256.1338, found: 256.1330.

8-Chloro-10,11-dihydro-5H-dibenz[b,f]azepine-2-carbonitrile (34)



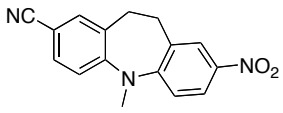
Following the procedure described for the preparation of 2-Bromo-10,11-dihydro-5H-dibenz[b,f]azepine (**5**), compound **6** (80 mg, 0.36 mmol) was treated with NCS (105 mg, 0.80 mmol) and SiO_2 (1.60 g) in DCM (20 mL) to give, after purification by silica gel flash-column chromatography with hexanes:Et_2O (6:4) as eluent, the desired compound **34** (30 mg, 33%) as a brown solid: IR (neat) 3347, 2212 cm^{-1}; ^1H NMR (400 MHz, MeOH-d_4) δ 3.00 (*app*-s, 4H), 6.88 (*app*-t, J = 8.0 Hz, 2H), 6.99 - 7.05 (m, 2H), 6.24 - 7.32 (m, 2H); ^{13}C NMR (100 MHz, MeOH-d_4) δ 34.5, 35.1, 99.5, 118.1, 120.0, 120.1, 125.0, 126.7, 127.4, 129.6, 130.7, 131.0, 134.8, 140.0, 146.7; MS (ESI) m/z 254 M^+; HRMS (ESI) m/z calc for $C_{15}H_{11}ClN_2$ 254.0611, found: 254.0597.

6-Nitro-10,11-dihydro-5H-dibenz[b,f]azepine-2-carbonitrile (35)



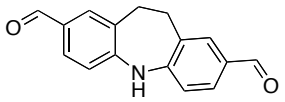
Following the procedure described for the preparation of 8-Nitro-10,11-dihydro-5H-dibenz[b,f]azepine-2-carbonitrile (**7**), compound **6** (480 mg, 2.18 mmol) was treated with $AgNO_3$ (405 mg, 2.40 mmol) and BzCl (277 μL, 2.40 mmol) in anhydrous MeCN (10 mL) to give, after purification by silica gel flash-column chromatography with hexanes:DCM (1:9) as eluent, the desired compound **35** (51 mg, 10%) as an orange solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.05 - 3.20 (m, 4H), 6.89 (*app*-t, *J* = 8.6 Hz, 1H), 7.02 (d, *J* = 8.4 Hz, 1H), 7.34 (d, *J* = 7.3 Hz, 1H), 7.38 (s, 1H), 7.43 (*app*-t, *J* = 8.3 Hz, 1H), 8.13 (d, *J* = 8.5 Hz, 1H), 10.79 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.6, 35.1, 103.8, 119.1, 119.7, 121.0, 125.4, 129.6, 131.1, 133.2, 134.7, 136.2, 136.8, 139.0, 143.9; MS (EI) m/z 265 $M^{\bullet+}$; HRMS (EI) m/z calc for $C_{15}H_{12}N_3O_2$ 265.0851, found: 265.0851.

5-Methyl-8-nitro-10,11-dihydro-5H-dibenz[b,f]azepine-2-carbonitrile (36)



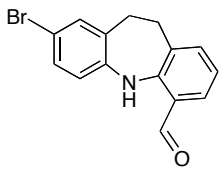
To a solution of **7** (100 mg, 0.38 mmol) in THF (3 mL) was added 1.0M (sol. in THF) NaHMDS (0.49 mL, 0.49 mmol) at 0 ˚C and the resulting mixture was stirred at 0 ˚C for 10 min. MeI (36 μL, 0.57 mmol) was added and the reaction mixture allowed to stir at room temperature for 2 h. Water (5 mL) was added and the crude product extracted with Et_2O. The organic phase was washed with brine, dried over $MgSO_4$, filtered and the solvent evaporated under reduced pressure. The resultant residue was purified by silica gel flash-column chromatography with hexanes:DCM (3:7) as eluent to yield the title compound (100 mg, 95%) as a yellow solid: IR (neat) 2225, 1480, 1316 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.16 - 3.25 (m, 4H), 3.49 (s, 3H), 7.13 (d, *J* = 9.0 Hz, 1H), 7.16 (d, *J* = 8.5 Hz, 1H), 7.42 (d, *J* = 1.9 Hz, 1H), 7.49 (dd, *J* = 8.7, 2.2 Hz, 1H), 8.00 (d, *J* = 2.7 Hz, 1H), 8.06 (dd, *J* = 9.0, 2.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 32.2, 33.5, 42.1, 106.1, 118.9, 119.1, 120.7, 122.7, 125.7, 131.0, 132.1, 133.3, 134.5, 141.7, 150.9, 152.3; MS (EI) m/z 279 $M^{\bullet+}$; HRMS (EI) m/z calc for $C_{16}H_{14}N_3O_2$ 279.1008, found: 279.1003.

10,11-Dihydro-5H-dibenz[b,f]azepine-2,8-dicarbaldehyde (37)



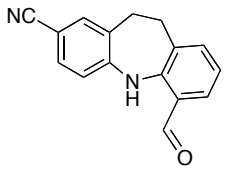
To a solution of **11** (200 mg, 0.82 mmol) in DCM (2 mL) was added 1.0M (sol. in DCM) DIBAL-H (4.1 mL, 4.10 mmol) at 0 ˚C. 2.0M NaOH (aq.) (10 mL) was added after 30 min and the mixture allowed to stir at room temperature for 1 h. The organic layer was washed with brine, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The resultant residue was purified by silica gel flash-column chromatography with DCM:Et_2O (9:1) as eluent to afford **37** (70 mg, 34%) as a yellow solid: IR (neat) 3313, 1663 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 3.08 (*app*-s, 4H), 7.22 (d, *J* = 8.5 Hz, 2H), 7.61 - 7.68 (m, 4H), 9.64 (s, 1H), 9.78 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 35.1, 119.6, 128.7, 128.9, 129.0, 133.5, 147.4, 191.2; MS (ESI) m/z 252 $(M+H)^+$; HRMS (ESI) m/z calc for $C_{16}H_{14}NO_2$ 252.1025, found: 252.1016.

8-Bromo-10,11-dihydro-5H-dibenz[b,f]azepine-4-carbaldehyde (38)



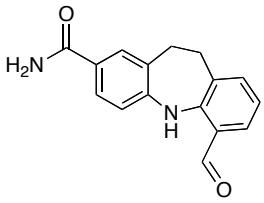
Following the procedure described for the preparation of 2-Bromo-10,11-dihydro-5H-dibenz[b,f]azepine (**5**), compound **13** (2.50 g, 11.21 mmol) was treated with NBS (2.10 g, 11.77 mmol) and SiO_2 (25.00 g) in DCM (300 mL) to give, after purification by silica gel flash-column chromatography with hexanes:DCM (7:3) as eluent, the desired compound **38** (2.10 g, 60%) as a yellow solid: Elem. anal. calc for $C_{15}H_{12}BrNO$: C, 59.62; H, 4.00; N, 4.64; found: C, 59.71; H, 4.05; N, 4.70; IR (neat) 1624 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.99 - 3.10 (m, 4H), 6.80 (*app*-t, *J* = 8.0 Hz, 1H), 6.87 (d, *J* = 8.1 Hz, 1H), 7.17 - 7.25 (m, 3H), 7.42 (d, *J* = 7.9 Hz, 1H), 9.86 (s, 1H), 11.24 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.6, 35.7, 113.2, 117.5, 120.2, 121.5, 129.0, 129.8, 132.5, 132.6, 135.9, 137.0, 140.0, 145.2, 194.7; MS (ESI) m/z 302 $(M+H)^+$; HRMS (ESI) m/z calc for $C_{15}H_{13}BrNO$ 302.0181, found: 302.0175.

6-Formyl-10,11-dihydro-5H-dibenz[b,f]azepine-2-carbonitrile (39)



Following the procedure described for the preparation of 10,11-dihydro-5H-dibenz[b,f]azepine-2-carbonitrile (**6**), compound **38** (1.70 g, 5.63 mmol) was treated with $Zn(CN)_2$ (660 mg, 5.63 mmol) and $Pd(PPh_3)_4$ (1.30 g, 1.13 mmol) in degassed DMF (15 mL) to give, after purification by silica gel flash-column chromatography with hexanes:DCM (3:7) as eluent, compound **39** (1.10 g, 75%) as a yellow solid: Elem. anal. calc for $C_{16}H_{12}N_2O$: C, 77.40; H, 4.87; N, 11.28; found: C, 77.53; H, 4.76; N, 11.20; IR (neat) 2213, 1656 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.89-3.23 (m, 4H), 6.93 (*app*-t, *J* = 8.3 Hz, 1H), 7.02 (d, *J* = 8.1 Hz, 1H), 7.29 (d, *J* = 8.0 Hz, 1H), 7.35 (s, 1H), 7.41 (d, *J* = 7.8 Hz, 1H), 7.49 (d, *J* = 8.2 Hz, 1H), 9.90 (s, 1H), 11.44 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.8, 35.2, 103.0, 119.0, 119.5, 120.5, 121.0, 129.8, 130.3, 131.0, 134.3, 135.9, 137.0, 144.1, 144.8, 195.0; MS (EI) m/z 248 $M^{\bullet+}$; HRMS (EI) m/z calc for $C_{16}H_{12}N_2O$ 248.0950, found: 248.0958.

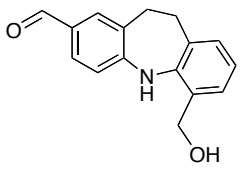
6-Formyl-10,11-dihydro-5H-dibenz[b,f]azepine-2-carboxamide (40)



Following the procedure described for the preparation of 8-nitro-10,11-dihydro-5H-dibenz[b,f]azepine-2-carboxamide (**8**), compound **39** (150 mg, 0.60 mmol) was treated with KOH (302 mg, 5.40 mmol) and 35% wt. H_2O_2 (aq.) (1.3 mL, 15.00 mmol) in EtOH (5 mL) to give, after purification by silica gel flash-column chromatography with DCM:MeOH (95:5) as eluent, the title compound (118 mg, 73%) as a yellow solid: Elem. anal. calc for $C_{16}H_{14}N_2O_2$: C, 72.16; H, 5.30; N, 10.52; found: C, 72.18; H, 5.30; N, 10.43; IR (neat) 3346, 3169, 1645, 1607 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.14 (*app*-s, 4H), 5.67 (br s, 1H), 5.85 (br s, 1H), 6.90 (*app*-t, *J* = 8.1 Hz, 1H), 7.05 (d, *J* = 8.0 Hz, 1H), 7.28 (s, 1H), 7.49 (d, *J* = 8.2 Hz, 1H), 7.57 (d, *J* = 8.3 Hz, 1H), 7.64 (s, 1H), 9.91 (s, 1H), 11.44 (br s, 1H); ^{13}C NMR (100 MHz, $CD_3OD/CDCl_3$) δ 35.2, 35.7, 118.7, 120.0, 120.9, 125.0, 126.7, 126.8, 130.0, 130.6,

136.2, 137.4, 144.4, 144.8, 170.6, 195.5; MS (ESI) m/z 267 $(M+H)^+$; HRMS (ESI) m/z calc for $C_{16}H_{15}N_2O_2$ 267.1134, found: 267.1125.

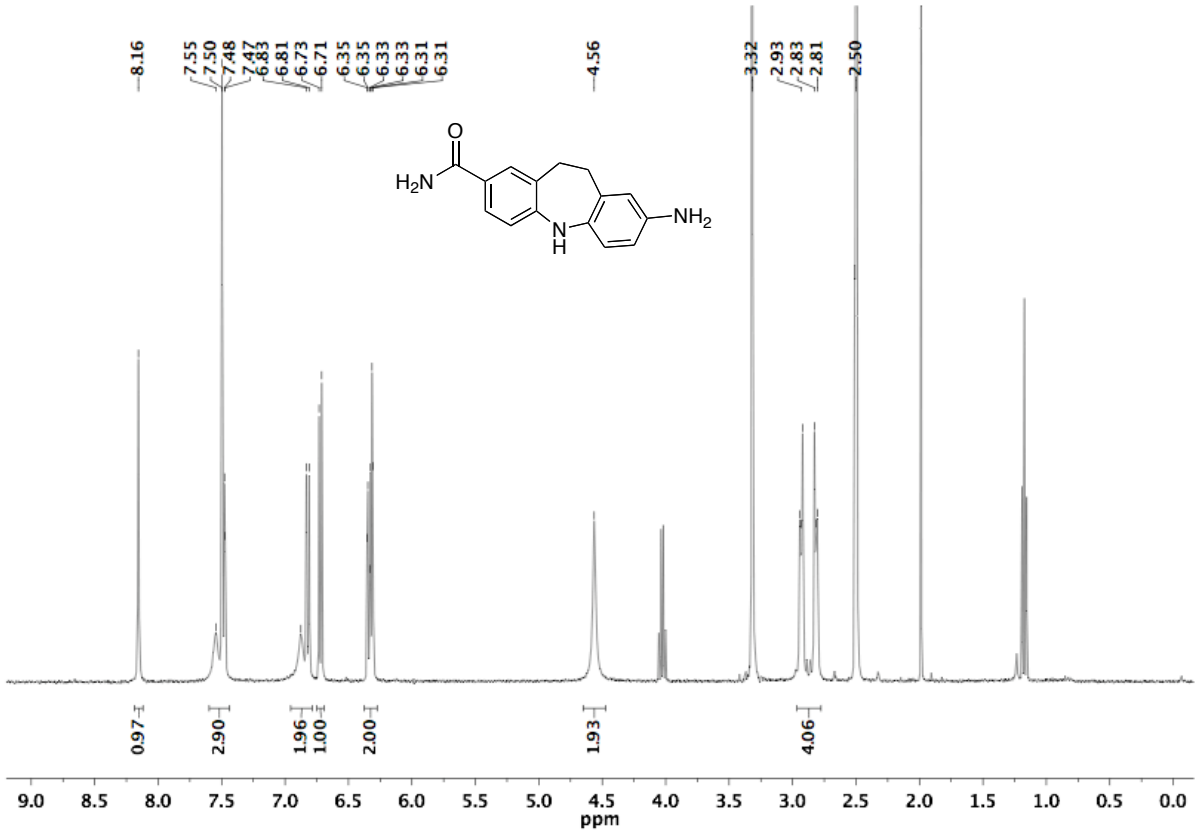
6-(Hydroxymethyl)-10,11-dihydro-5H-dibenz[b,f]azepine-2-carbaldehyde (41)



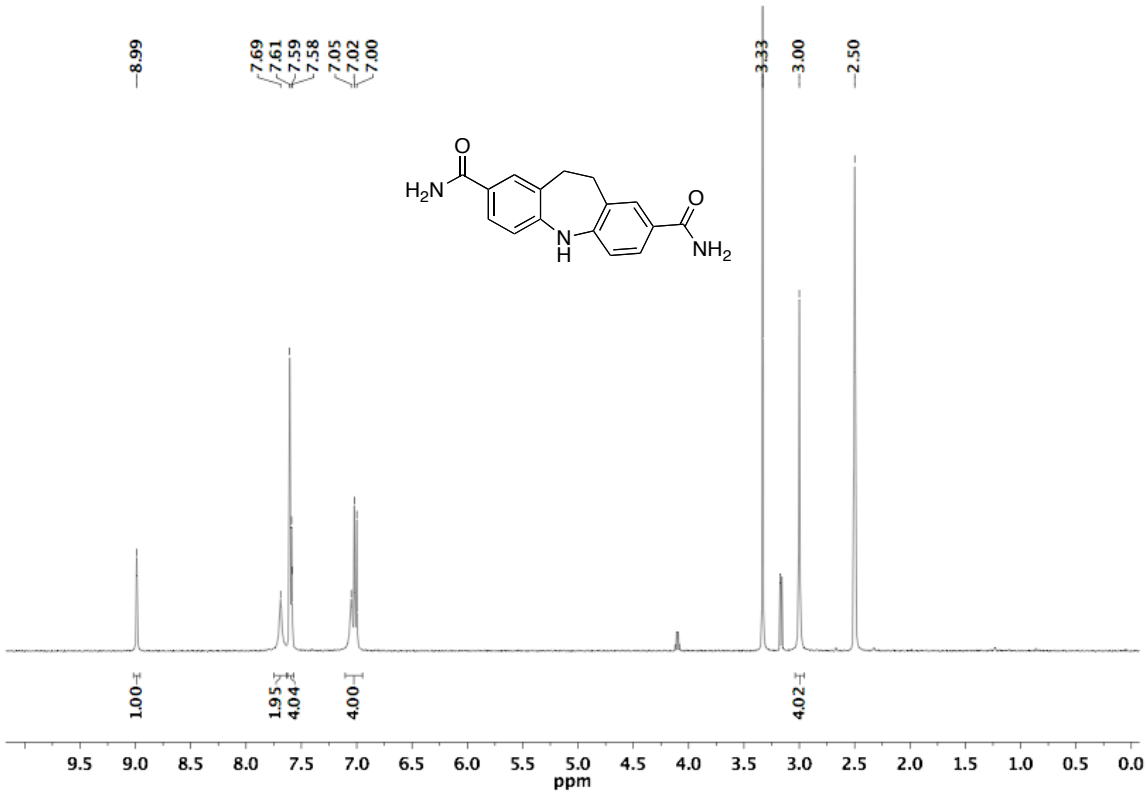
Following the procedure described for the preparation of 10,11-Dihydro-5H-dibenz[b,f]azepine-2,8-dicarbaldehyde (**37**), compound **39** (100 mg, 0.40 mmol) was treated with DIBAL-H (2.0 mL, 2.00 mmol) in DCM (2 mL) to give, after purification by silica gel flash-column chromatography with DCM:MeOH (95:5) as eluent, the title compound (90 mg, 88%) as a yellow oil: IR (neat) 3296, 1657 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.14 (*app*-s, 4H), 4.85 (s, 2H), 6.83 - 6.93 (m, 2H), 7.05 (d, J = 7.2 Hz, 1H), 7.11 (d, J = 7.1 Hz, 1H), 7.58 (s, 1H), 7.62 (d, J = 9.3 Hz, 1H), 8.48 (br s, 1H), 9.78 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.8, 35.8, 65.4, 118.8, 120.8, 126.6, 127.4, 127.5, 127.6, 129.0, 130.6, 132.5, 133.8, 141.1, 148.0, 190.6; MS (ESI) m/z 254 $(M+H)^+$; HRMS (ESI) m/z calc for $C_{16}H_{16}NO_2$ 254.1181, found: 254.1179.

F. ^{1}H NMR Spectra of Tested Compounds Without Elemental Analysis

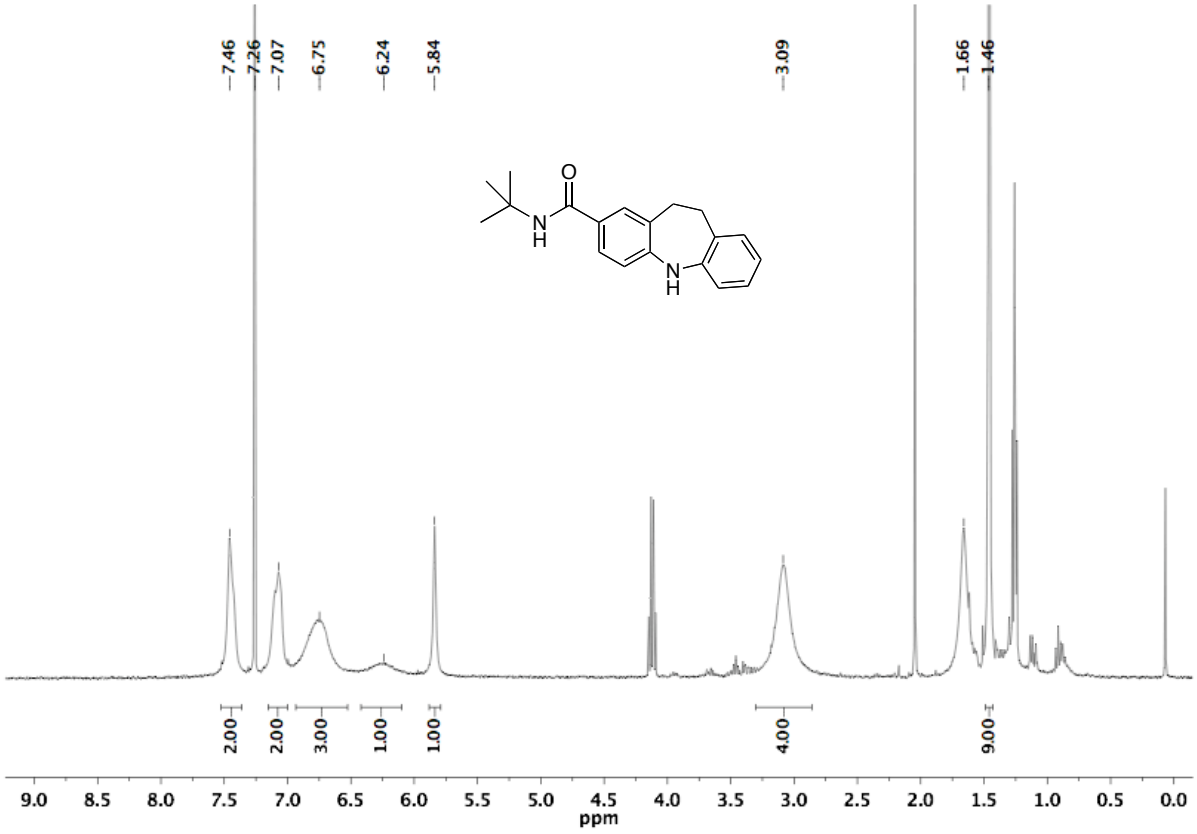
8-Amino-10,11-dihydro-5H-dibenz[b,f]azepine-2-carboxamide (9)



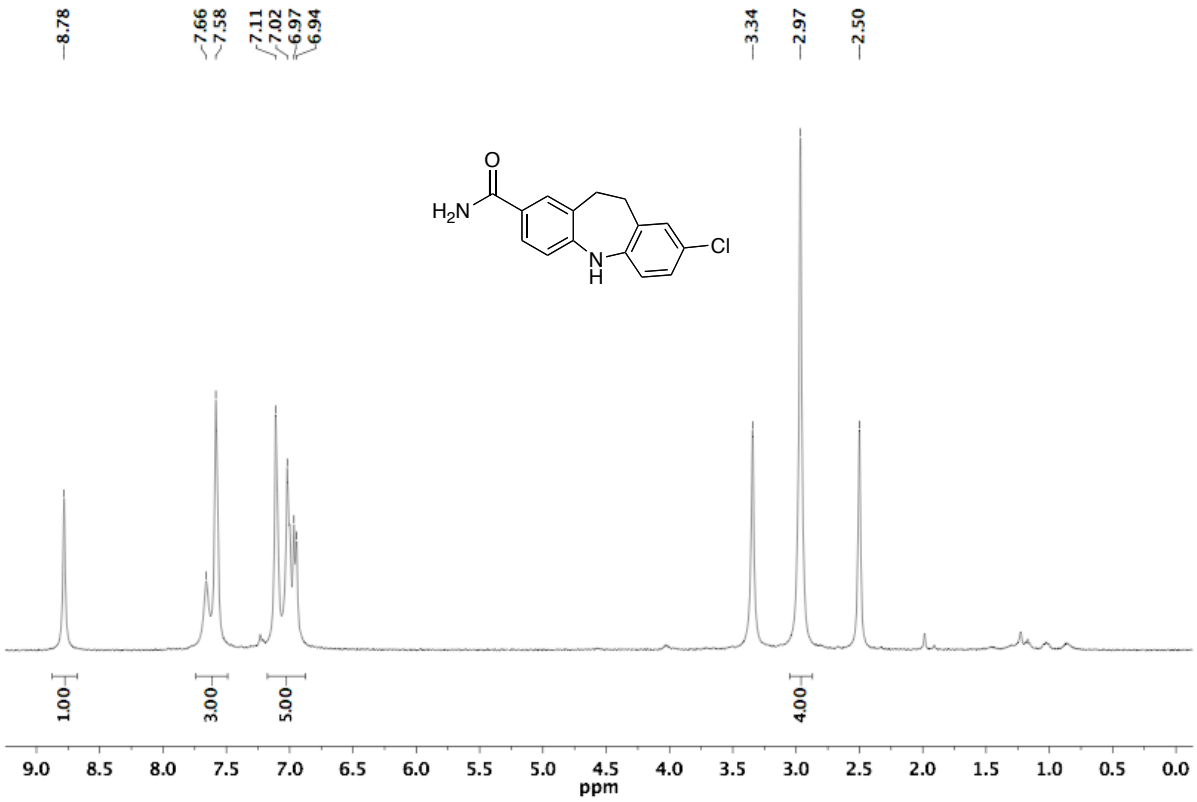
10,11-Dihydro-5H-dibenz[b,f]azepine-2,8-dicarboxamide (12)



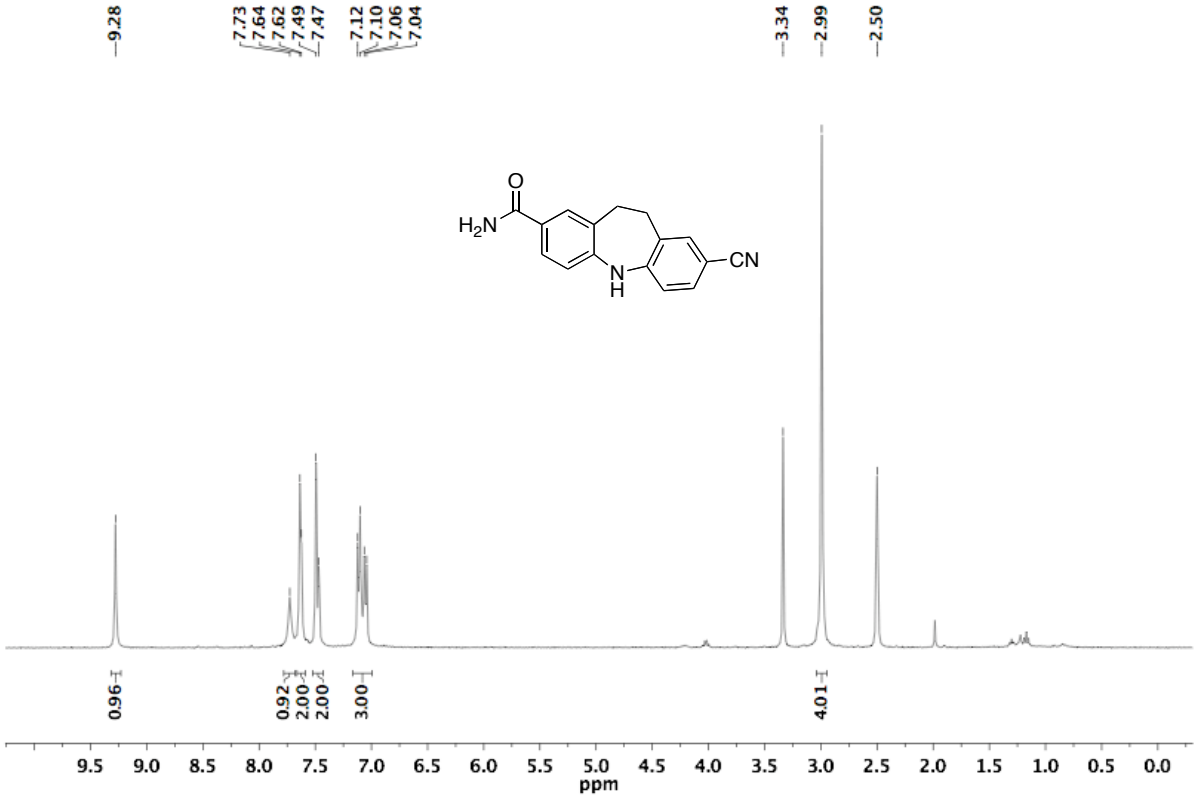
N-tert-Butyl-10,11-dihydro-5H-dibenz[b,f]azepine-2-carboxamide (24)



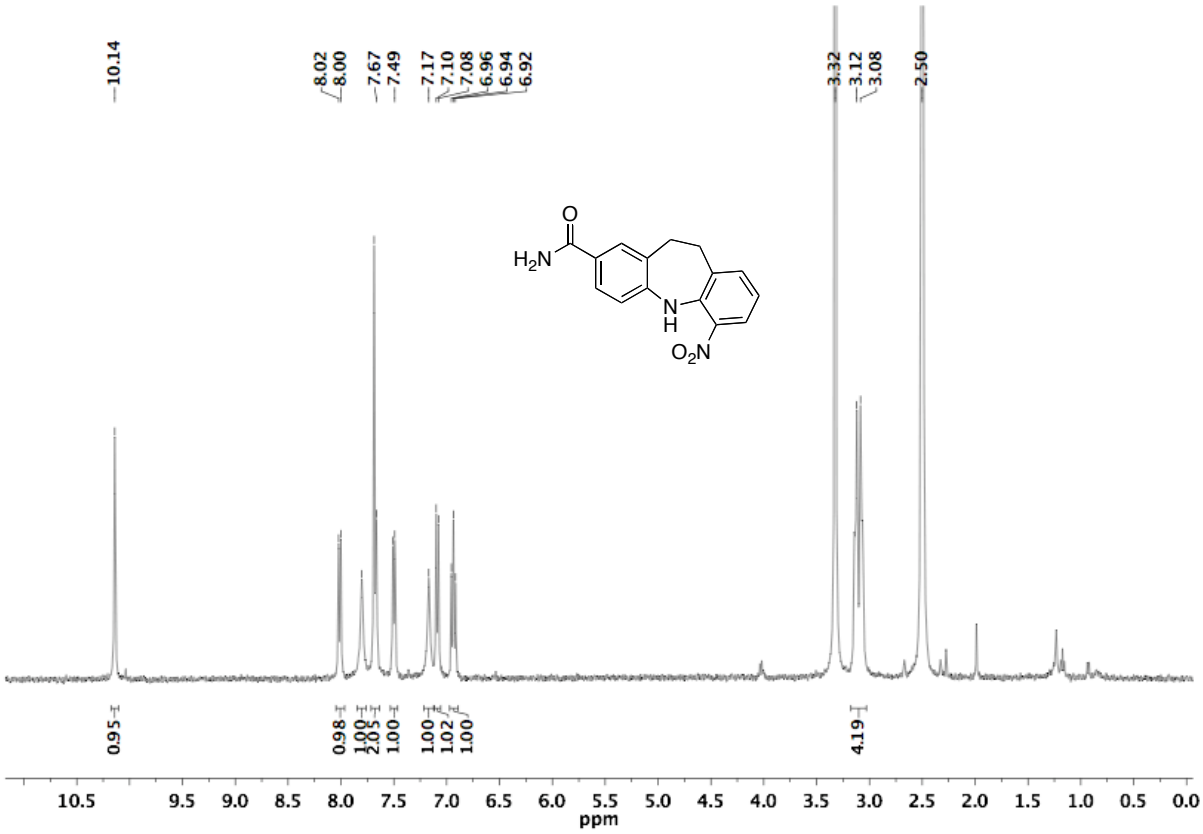
8-Chloro-10,11-dihydro-5H-dibenz[b,f]azepine-2-carboxamide (27)



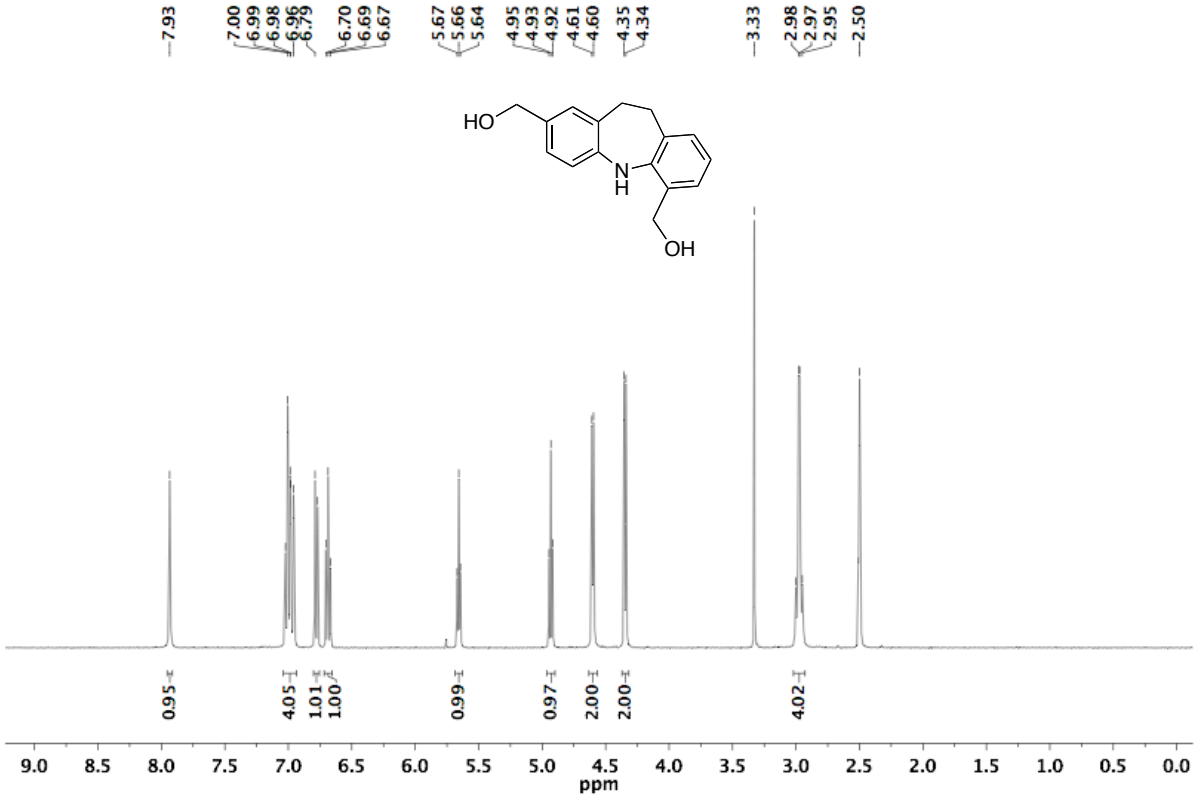
8-Cyano-10,11-dihydro-5H-dibenz[b,f]azepine-2-carboxamide (28)



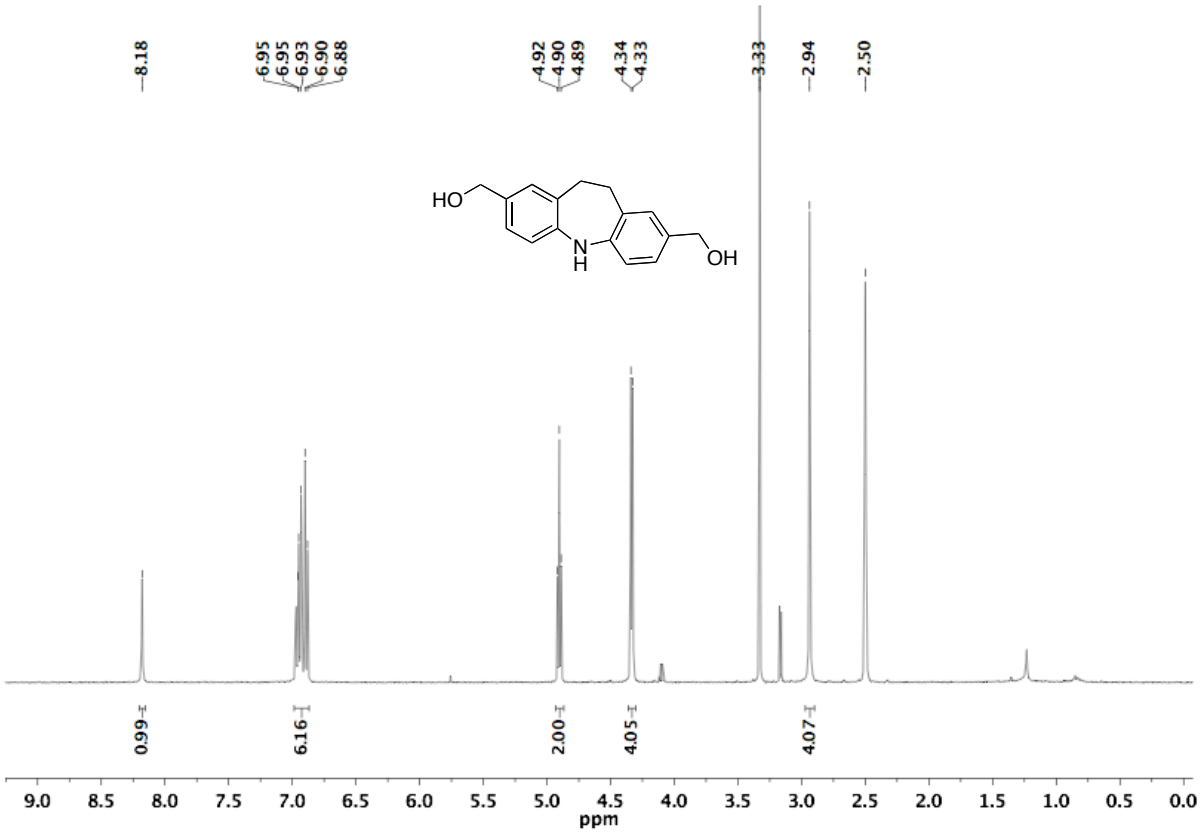
6-Nitro-10,11-dihydro-5H-dibenz[b,f]azepine-2-carboxamide (30)



(10,11-Dihydro-5H-dibenz[b,f]azepine-2,6-diyl)dimethanol (32)



(10,11-Dihydro-5H-dibenz[b,f]azepine-2,8-diyl)dimethanol (33)



G. References

1 Universal Protein Resource (http://www.uniprot.org/).

2 B. E. Hall, J. R. Buchberger, S. A. Gerber, A. L. B. Ambrosio, S. P. Gygi, D. Filman, D. Moazed, and T. Ellenberger, unpublished work.

3 Protein-protein BLAST (blastp), National Center for Biotechnology information, Bethesda, US (http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi/).

4 SAS, European Bioinformatics Institute, Cambridge, UK (http://www.ebi.ac.uk/thornton-srv/databases/sas/).

5 G. Jones, P. Willett, R. C. Glen, and R. Taylor, *J. Mol. Biol.,* 1997, **267**, 727-748.

6 CambridgeSoft, ChemBioOffice Ultra 2010 Suite (http://www.cambridgesoft.com/).

7 DeLano WL (2002) The PyMOL Molecular Graphics System. DeLano Scientific, Palo Alto, CA (http://www.pymol.org/).

8 R. A. Laskowski, M. W. MacArthur, D. S. Moss, and J. M. Thornton, *J. App. Cryst.,* 1993, **26**, 283-291.

9 PDBsum, European Bioinformatics Institute, Cambridge, UK (http://www.ebi.ac.uk/pdbsum/).