Supplementary Information for

Binding and orientation of tricyclic antidepressants within the central substrate site of the human serotonin transporter

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Supplementary Table 1. Eight setups were included in the initial induced fit docking study of imipramine binding in the hSERT, yielding 122 poses. The setups are described in Supplementary Methods.

Setup	Model	Scoring Function	Poses	Cluster1	Cluster2	Outlier	Vestibule
SI-I	**A**	SP/SP	10	4	1	1	4
SI-II	**A**	SP/SP	17				17
SI-III	**A**	SP/SP	19			4	15
SI-V	**A**	SP/XP	21	6	3	6	6
SI-VI	**A**	SP/XP	6	1			5
SI-IV	**A**	SP/XP	25	6	7	6	6
SI-VII	**A**	XP/XP	5				5
SI-VIII	**B**	SP/XP	19	2		10	7
Total			**122**	**19**	**11**	**27**	**69**

Supplementary Table 2. Induced fit docking of TCAs in the two homology models of the hSERT. The poses are sorted with respect to the binding mode, IFDScore, and GlideScore. Clusters 1 and 2 in the binding site are represented, as are clusters V1 and V2 in the extracellular vestibule. V1 corresponds to the binding mode observed in the LeuT structures[1,2], while V2 corresponds to the binding mode with the alkyl amine pointing towards the binding site.

Ligand	Model	IFDScore	GScore	EModel	Cluster	Dist TCA(N) ···Asp98(Oä2)
Desipramine	B	-808.1	-10.64	-50.09	1	2.80
Desipramine	B	-807.7	-10.44	-84.78	1	3.77
Desipramine	B	-806.4	-9.85	-62.22	1[a]	2.87
Desipramine	B	-806.2	-9.58	-56.94	1	4.42
Desipramine	B	-806.1	-9.92	-25.75	1	2.59
3-cyanoimipramine	B	-806.0	-10.46	-74.18	1[b]	4.22
Desipramine	B	-805.8	-9.29	-15.65	1	3.69
3-cyanoimipramine	B	-805.0	-11.55	-72.03	1[b]	4.52
3-cyanoimipramine	B	-804.9	-11.40	-75.00	1[b]	4.13
3-cyanoimipramine	B	-804.5	-9.07	-86.50	1[c]	3.18
3-cyanoimipramine	B	-804.0	-10.85	-10.03	1[a]	4.12
3-cyanoimipramine	B	-803.7	-10.29	-70.26	1[c]	3.79
Desipramine	B	-803.3	-6.56	-53.54	1	2.84
3-cyanoimipramine	B	-803.2	-8.11	-63.58	1[b]	4.87
3-cyanoimipramine	B	-802.0	-10.40	-74.60	1[b]	3.77
3-cyanoimipramine	B	-801.9	-10.44	-63.05	1[c]	4.58
3-cyanoimipramine	B	-801.6	-10.42	-60.38	1[b]	3.89
3-cyanoimipramine	B	-801.1	-10.29	-75.63	1[c]	2.93
Desipramine	A	-799.9	-9.95	-70.38	1	2.97
Clomipramine	B	-799.4	-9.70	-72.11	1[c]	3.71
Desipramine	A	-796.6	-8.32	-76.04	1	2.86
Imipramine	A	-795.9	-10.22	-69.31	1	4.33
Short imipramine	A	-793.7	-9.93	-78.18	1	3.82
Imipramine	A	-793.6	-8.05	-55.56	1[a]	3.97
Short imipramine	A	-793.4	-9.48	-44.25	1	4.17
Short imipramine	A	-793.3	-9.97	-64.42	1	2.98
Short imipramine	A	-793.3	-9.38	-62.66	1	3.24
Imipramine	A	-792.5	-9.24	-59.17	1	3.51
3-cyanoimipramine	A	-792.4	-9.75	-64.94	1[b]	3.01
Short imipramine	A	-792.3	-9.33	-35.36	1	2.72
Imipramine	A	-792.0	-9.00	-66.11	1	3.47
Short imipramine	A	-791.7	-8.21	4.86	1	2.86

Short imipramine	A	-790.9	-8.32	-36.65	1	3.09
Clomipramine	A	-790.6	-11.60	-16.87	1 [b]	3.98
Clomipramine	A	-789.9	-9.37	-75.45	1[c]	2.96
Clomipramine	A	-786.2	-8.82	-11.43	1 [b]	3.53
Desipramine	B	-807.1	-10.06	-42.23	2	3.30
Desipramine	B	-806.2	-8.99	-62.77	2	3.40
Desipramine	B	-804.5	-7.92	-24.55	2	4.04
3-cyanoimipramine	B	-801.4	-6.58	-7.73	2 [a]	4.25
Desipramine	A	-798.9	-9.66	-38.35	2	3.89
Desipramine	A	-797.8	-9.20	-65.83	2	2.98
Short imipramine	A	-791.8	-8.81	-53.32	2	4.38
Short imipramine	A	-791.4	-7.32	-39.96	2	4.64
Short imipramine	A	-790.5	-8.49	-34.97	2	3.92
Desipramine	B	-807.3	-9.27	-66.39		2.73
Desipramine	B	-805.8	-8.64	-47.70		2.75
Desipramine	B	-804.6	-7.78	-30.37		3.02
Desipramine	B	-803.3	-6.72	-15.16	[d]	7.36
Desipramine	B	-802.2	-5.44	-22.12	[d]	10.39
Desipramine	B	-802.0	-5.49	-28.78	[d]	9.83
Short imipramine	B	-801.1	-7.84	-47.96		3.02
Desipramine	A	-800.0	-10.38	-32.32		2.74
Short imipramine	B	-799.5	-8.03	-58.89		3.31
Clomipramine	B	-799.3	-10.36	-12.25	[d]	4.54
Imipramine	B	-794.9	-6.90	-23.80		3.46
Clomipramine	B	-794.2	-4.65	-7.85	[d]	9.33
Imipramine	B	-793.0	-6.46	0.56	[d]	7.21
Short imipramine	A	-792.3	-9.98	-27.45		3.47
Imipramine	A	-791.4	-9.35	-41.21		3.50
Short imipramine	A	-791.3	-9.05	-15.80		2.80
Imipramine	A	-790.7	-9.83	-39.33		2.94
Clomipramine	A	-789.7	-9.40	-0.95		4.48
Desipramine	A	-798.4	-9.45	-35.73	V1	
Desipramine	A	-797.4	-8.24	-47.07	V1	
Desipramine	A	-797.4	-7.87	-55.67	V1	
3-cyanoimipramine	A	-794.6	-7.17	-46.22	V1	
3-cyanoimipramine	A	-794.2	-9.92	-42.64	V1	
3-cyanoimipramine	A	-794.2	-7.69	-51.01	V1	
3-cyanoimipramine	A	-793.6	-8.81	-43.59	V1	
3-cyanoimipramine	A	-793.3	-7.21	-49.87	V1	
3-cyanoimipramine	A	-793.1	-8.78	-29.69	V1	
3-cyanoimipramine	A	-793.0	-8.51	-51.70	V1	
3-cyanoimipramine	A	-792.6	-7.16	-44.19	V1	
3-cyanoimipramine	A	-792.2	-7.16	-41.38	V1	
3-cyanoimipramine	A	-790.4	-7.02	-35.45	V1	

3-cyanoimipramine	A	-790.3	-7.62	-57.48	V1
3-cyanoimipramine	A	-789.4	-6.83	-56.02	V1
Imipramine	B	-788.3	-7.72	-42.25	V1
3-cyanoimipramine	A	-787.5	-4.90	-38.90	V1
3-cyanoimipramine	A	-785.5	-0.96	-40.15	V1
Desipramine	B	-808.5	-10.51	-62.15	V2
Desipramine	B	-808.3	-10.79	-62.48	V2
Desipramine	B	-807.6	-10.86	-69.87	V2
Desipramine	B	-807.1	-10.00	-75.46	V2
Desipramine	B	-806.9	-10.17	-69.39	V2
Desipramine	B	-806.7	-9.74	-60.64	V2
Desipramine	B	-806.5	-9.43	-50.73	V2
Desipramine	B	-806.4	-9.87	-53.01	V2
Desipramine	B	-806.2	-10.04	-35.42	V2
Desipramine	B	-806.0	-9.81	-66.19	V2
Desipramine	B	-805.6	-9.07	-61.12	V2
Desipramine	B	-805.5	-9.07	-58.55	V2
Desipramine	B	-805.1	-8.88	-66.79	V2
Desipramine	B	-804.6	-8.97	-52.81	V2
3-cyanoimipramine	B	-804.3	-10.06	-68.77	V2
Short imipramine	B	-801.4	-10.56	-45.27	V2
Clomipramine	B	-800.7	-8.84	-55.04	V2
Clomipramine	B	-800.6	-10.36	-58.68	V2
Desipramine	A	-799.7	-8.80	-50.86	V2
Short imipramine	B	-799.4	-9.96	-44.10	V2
Desipramine	A	-798.6	-9.19	-61.17	V2
Desipramine	A	-798.3	-8.51	-51.09	V2
Imipramine	B	-798.2	-9.39	17.35	V2
3-cyanoimipramine	B	-797.9	-8.52	-9.92	V2
Desipramine	A	-797.9	-8.48	-53.55	V2
Desipramine	A	-797.3	-7.91	-45.09	V2
Desipramine	A	-797.1	-6.82	-50.94	V2
Desipramine	A	-797.0	-7.86	-49.07	V2
Clomipramine	B	-796.8	-8.62	-45.01	V2
Clomipramine	B	-796.7	-5.84	-39.40	V2
Desipramine	A	-795.8	-6.92	-55.44	V2
3-cyanoimipramine	A	-792.9	-7.79	-51.70	V2
3-cyanoimipramine	A	-792.7	-7.07	-45.03	V2
3-cyanoimipramine	A	-791.0	-8.14	-58.06	V2
3-cyanoimipramine	A	-790.0	-7.79	-49.64	V2
Desipramine	A	-799.3	-8.52	-43.01	
Desipramine	A	-799.1	-8.42	-53.29	
Desipramine	A	-797.9	-8.30	-49.18	
Desipramine	A	-797.1	-7.99	-46.73	

Desipramine	A	-796.9	-7.48	-51.90
3-cyanoimipramine	A	-796.2	-10.95	-61.87
3-cyanoimipramine	A	-795.8	-9.29	-58.53
3-cyanoimipramine	A	-795.6	-10.03	-46.75
3-cyanoimipramine	A	-795.1	-9.75	-62.61
3-cyanoimipramine	A	-794.8	-9.61	-56.27
Imipramine	A	-794.4	-7.42	-47.01
3-cyanoimipramine	A	-794.2	-9.27	-40.53
Clomipramine	B	-794.2	-5.44	-63.03
3-cyanoimipramine	A	-794.1	-9.04	-54.92
3-cyanoimipramine	A	-793.9	-9.57	-53.48
3-cyanoimipramine	A	-793.6	-9.97	-46.26
3-cyanoimipramine	A	-793.4	-9.20	-61.64
3-cyanoimipramine	A	-793.0	-7.47	-49.94
3-cyanoimipramine	A	-792.6	-9.21	-31.49
3-cyanoimipramine	A	-792.0	-9.21	-41.95
3-cyanoimipramine	A	-791.3	-8.83	-59.29
3-cyanoimipramine	A	-791.1	-6.65	-44.15
3-cyanoimipramine	A	-790.7	-8.63	-43.67
3-cyanoimipramine	A	-790.5	-6.86	-52.55
3-cyanoimipramine	A	-789.6	-7.20	-50.12

[a] Some poses show a 180° rotation of the tricyclic moiety. [b] In these poses the substituent on the 3-position is pointing towards Ala173. [c] In these poses the substituent on the 3-position is pointing towards Phe335. [d] These poses have the TCA located inside the binding site, but without interactions to Asp98.

Supplementary Table 3. Mean K_i (nM) and 95% Confidence Intervals (in brackets) for inhibition of [^{3}H]-5-HT uptake in HEK293-MSR cells transiently transfected with hSERT wt and mutants. Values marked with * is the mean K_i from less than three experiments.

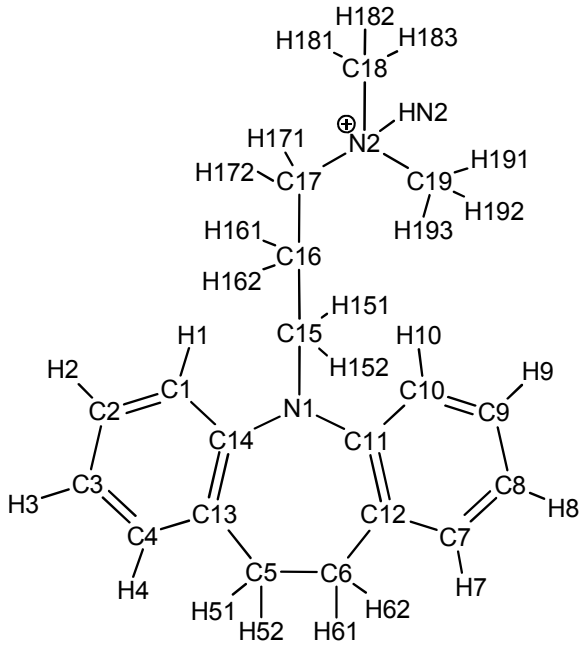
	Imipramine			Desipramine			Didesmethylimipramine			Short imipramine			Trimipramine		
	nM		n	nM		n	nM		n	nM		n	nM		n
hSERT	31.8	[21.6-46.8]	*34*	1870	[482-7290]	*30*	2223	[398-12420]	*25*	2020	[1472-2760]	*18*	3530	[2540-4920]	*18*
Asp98Glu	225	[45.7-1116]	*7*	2820	[905-8830]	*7*	200	[16.18-2500]	*5*	1581	[1016-2450]	*7*	8750	[5970-12850]	*4*
Ala169Ile	14.03	[1.77-111.4]	*3*	3310	[1416-7740]	*4*	21.04	[11.43-38.8]	*3*	50.9	[31.9-81.1]	*3*	186.2	[0.03-1208000]	*2*
Ala173Leu	4.07	[1.92-8.63]	*7*	174.2	[7.13-4260]	*8*	18.32	[4.72-71.1]	*3*	57.5	[42.1-78.7]	*6*	151.7	[84.3-274]	*7*
Ala173Met	7.31	[5.71-9.38]	*10*	48.0	[8.41-273]	*7*	71.9	[3.98-1297]	*3*	25.0	[9.75-63.7]	*6*	156.4	[91.1-268]	*3*
Ala173Cys	12.65	[6.35-25.2]	*5*	504	[111.7-2280]	*7*	382	[137.4-1062]	*5*	455	[206-1005]	*5*	1567	[16.83-145900]	*2*
Ala173Ser	11.40	[5.3-24.5]	*6*	766	[159.6-3670]	*5*	1374	[431-4390]	*5*	1076	[452-2560]	*5*	2260	[252-20300]	*2*
Ala173Thr	15.63	[7.14-34.2]	*5*	2270	[238-21700]	*5*	488	[154.5-1538]	*5*	488	[114.3-2080]	*5*	2820	[1570-5060]	*2*
Ala173Asp	11.83	[3.52-39.8]	*3*	807	[6.42-101900]	*3*	509	[154.9-1675]	*3*	242	[122.2-478]	*3*			
Tyr175Phe	40.6	[27.5-59.8]	*6*	277	[121.3-633]	*7*	993	[84.7-11610]	*3*	279	[136.1-570]	*6*	4930	[2030-12000]	*3*
Tyr176Phe	156.7	[98.4-249.5]	*6*	929	[689-1253]	*6*	607	[48.9-7550]	*3*	1091	[516-2310]	*6*	9480	[5040-17820]	*3*
Phe335Asn	61.0	[33.9-109.7]	*6*	614	[255-1479]	*6*	813	[24.3-27200]	*2*	4320	[2540-7350]	*6*	6680	[2750-16300]	*4*
Phe335His	57.02	[35.97-90.36]	*5*												
Phe335Ser	82.8	[18.6-370]	*6*	1089	[528-2240]	*6*	2590	[726-9250]	*3*	7000	[1991-24600]	*6*	9930	[2790-35500]	*4*
Phe335Ala	31.3	[11.09-88.5]	*8*	1191	[225-6310]	*5*	596	[37.0-9590]	*4*	1496	[361-6210]	*4*	7000	[1151-42600]	*4*
Phe335Gly	71.8	[42.9-120.2]	*5*	1259	[399-3970]	*5*	2240	*	*1*	3140	[1489-6640]	*4*	5200	[1791-15100]	*5*
Phe335Leu	17.10	[10.64-27.6]	*8*	492	[217-1114]	*6*				2450	[1675-3600]	*4*	3800	[2180-6620]	*5*
Val343Ala	75.5	[26.0-219]	*5*	2720	[94.2-78700]	*2*				4620	[2220-9620]	*2*	8610	[2310-32100]	*2*
Val343Leu	1538	[1191-1990]	*4*	2630	[335-20700]	*3*	3310	*	*1*	6120	[1746-21500]	*3*	4540	[2280-9020]	*3*
Val343Ile	152.4	[1.156-20200]	*3*	320	[26.6-3850]	*2*				700	[251-1950]	*2*	537	[69.3-4160]	*2*
Thr439Ala	14.72	[8.47-25.6]	*6*	1611	[369-7010]	*6*	697	[391-1245]	*3*	1611	[647-4010]	*3*	1114	[349-3560]	*4*
Thr439Ser	15.03	[8.09-27.9]	*7*	582	[401-843]	*6*	457	[26.4-7910]	*4*	1524	[1067-2180]	*6*	1556	[335-7230]	*5*
Thr439Val	14.96	[4.28-52.4]	*4*	832	[212-3250]	*5*	525	[165.2-1667]	*4*	1349	[511-3560]	*4*	2400	[53.8-106900]	*3*
Leu443Ser	24.3	[5.98-98.4]	*6*	1047	[740-1483]	*3*				2310	[2020-2640]	*3*	5090	[4280-6070]	*3*
Leu443Thr	16.03	[2.46-128.8]	*6*	1726	[1239-2400]	*3*				5710	[4500-7240]	*3*	8070	[5510-11800]	*3*
Cys473Glu	14.35	[0.241-1026]	*3*												
Cys473Met	34.7	[8.04-149.6]	*2*												
Cys473Leu	10.23	[1.290-80.9]	*4*	128.8	*	*1*									
Ala173Met-Phe335Leu	49.20	[29.2-82.8]	9	271.6	[220-335]	5	309	*	*1*	380	[204-706]	6	951	[577-1567]	5
Ala173Met-Phe335Ala	9.62	[3.93-23.77]	6	80.35	[8.38-769]	3				318	[16.29-6180]	3	530	[85.9-3270]	4

	2-hydroxymipramine			10-hydroxyimipramine			Clomipramine			3-cyanoimipramine			3,7-dicyanoimipramine		
	nM		n	nM		n	nM		n	nM		n	nM		n
hSERT	83.56	[58.08-120.0]	*26*	304	[167.9-552]	*3*	16.41	[10.45-25.7]	*24*	5.43	[1.54-19.14]	*33*	4.57	[2.37-8.83]	*3*
Asp98Glu	410	[299-565]	*6*				255	[148.3-441]	*4*	19.36	[11.07-34]	*4*			
Ala169Ile	18.20	[0.411-817]	*2*							1.079	[0.0232-65.2]	*3*			
Ala173Leu	2.45	[0.937-6.71]	*6*				28.2	[17.14-46.3]	*5*	7.87	[3.71-16.71]	*5*			
Ala173Met	7.69	[3.83-15.45]	*8*	37.4	[11.25-124.5]	*3*	34.91	[21.4-56.9]	*7*	18.79	[11.30-31.33]	*7*	140.3	[88.5-222]	*3*
Ala173Cys	9.48	[0.270-337]	*3*				15.14	[2.69-85.1]	*3*	3.57	[0.912-14.09]	*6*			
Ala173Ser	16.37	[5.69-47.1]	*5*				13.87	[2.88-66.7]	*4*	2.19	[0.570-8.49]	*7*			
Ala173Thr	8.41	[0.152-479]	*4*				41.9	[17.14-102.6]	*4*	2.24	[0.322-15.49]	*6*			
Ala173Asp	13.80	[0.412-462]	*2*				17.38	*	*1*	3.48	[0.851-14.35]	*4*			
Tyr175Phe	48.98	[5.21-460]	*3*				22.9	[3.63-144.5]	*3*	1.87	[0.398-8.89]	*6*			
Tyr176Phe	339	[86.7-1324]	*3*				37.8	[14.59-97.7]	*3*	1.14	[0.331-3.88]	*6*			
Phe335Asn	58.2	[20.9-161.8]	*4*				36.7	[9.64-140]	*4*	7.91	[1.290-48.5]	*5*			
Phe335His	59.29	[32.73-107.7]	*3*	885	[555-1409]	*3*	38.28	[22.8-64.4]	*3*	11.14	[9.10-13.61]	*3*	36.1	[14.03-92.7]	*3*
Phe335Ser	97.7	[8.32-1148]	*4*				27.2	[10.86-68.2]	*4*	6.79	[0.767-59.6]	*6*			
Phe335Ala	124.2	[32.0-483]	*7*	460	[154.2-1377]	*3*	14.39	[8.18-25.4]	*6*	3.71	[1.170-11.75]	*8*	18.49	[13.49-25.3]	*3*
Phe335Gly	126.5	[26.7-600]	*4*				61.2	[5.56-676]	*4*	3.24	[0.532-19.82]	*4*			
Phe335Leu	74.13	[60.7-90.6]	*7*	450	[152.8-1324]	*3*	35.32	[22.6-55.4]	*6*	12.19	[7.85-18.92]	*7*	30.4	[17.18-54.0]	*3*
Val343Ala	209	[58.8-743]	*5*				25.8	[4.38-152]	*5*	12.42	[4.83-31.9]	*5*			
Val343Leu	2690	[1091-6640]	*7*				447	[171.8-1161]	*5*	31.9	[13.46-75.7]	*5*			
Val343Ile	35.3	[29.1-42.8]	*5*				22.5	[0.928-568]	*3*	11.83	[7.93-17.7]	*3*			
Thr439Ala	23.4	[14.83-37.1]	*3*				7.82	[1.930-31.8]	*3*	1.11	[0.260-4.79]	*6*			
Thr439Ser	22.9	[20.8-25.3]	*3*				15.38	[1.934-122.2]	*3*	0.81	[0.162-4.21]	*7*			
Thr439Val	27.2	[23.5-31.6]	*2*				14.13	*	*1*	1.71	[0.991-2.94]	*5*			
Leu443Ser	57.0	[28.9-112.5]	*5*				36.1	[9.33-139.3]	*6*	4.12	[3.34-5.08]	*6*			
Leu443Thr	50.4	[15.28-166]	*5*				28.8	[6.47-128.5]	*6*	5.35	[2.43-11.91]	*6*			
Cys473Glu	83.2	[46.3-149.3]	*2*				49.3	[0.827-2920]	*3*	8.26	[5.52-12.33]	*3*			
Cys473Met	112.8	[14.83-889]	*2*				62.7	[1.210-3250]	*3*	13.58	[11.12-16.63]	*3*			
Cys473Leu	31.6	[1.542-650]	*3*				33.3	[2.31-481]	*4*	6.41	[0.672-61.4]	*4*			
Ala173Met-Phe335Leu	65.9	[42.46-102.3]	*9*	575	[117.0-2830]	*3*	362	[187.9-698]	*6*	187.1	[66.37-527]	*9*	1122	[136.1-9250]	*3*
Ala173Met-Phe335Ala	54.70	[2.48-1208]	*3*				121.3	[4.04-3640]	*2*	50.70	[0.311-8380]	*3*			

Supplementary Table 4. Mean K_M and V_{max} ±standard error mean (in brackets) for inhibition of [^{3}H]-5-HT uptake in HEK293-MSR cells transiently transfected with hSERT wt and mutants. The number of independent experiments (n) is also shown.

	Km	Vmax	n
	µM	pmol/well/min	
hSERT	1.34 ±0.20	1.92 ±0.47	15
Asp98Glu	0.87 ±0.03	0.72 ±0.30	3
Ala169Ile	5.4 ±1.40	1.65 ±0.68	4
Ala173Leu	8.4 ±1.27	0.25 ±0.053	4
Ala173Met	6.5 ±1.07	2.5 ±1.13	4
Ala173Cys	0.74 ±0.16	1.46 ±0.71	5
Ala173Ser	1.66 ±0.48	1.75 ±0.67	5
Ala173Thr	7.1 ±2.3	5.7 ±1.40	4
Ala173Asp	5.6 ±1.67	3.2 ±0.86	5
Tyr175Phe	1.46 ±0.35	2.2 ±1.41	3
Tyr176Phe	4.1 ±2.5	1.97 ±1.59	3
Phe335Asn	1.23 ±0.34	0.31 ±0.040	3
Phe335His	1.69 ±0.31	1.28 ±0.44	3
Phe335Ser	2.0 ±0.67	0.12 ±0.033	3
Phe335Ala	2.3 ±0.80	0.24 ±0.050	6
Phe335Gly	5.1 ±1.58	0.22 ±0.057	5
Phe335Leu	0.85 ±0.24	0.62 ±0.12	3
Val343Ala	2.8 ±0.54	4.0 ±1.13	3
Val343Leu	2.5 ±0.25	0.18 ±0.046	3
Val343Ile	1.65 ±0.16	0.32 ±0.139	3
Thr439Ala	11.8 ±1.59	3.9 ±1.57	5
Thr439Ser	3.3 ±0.73	0.28 ±0.147	5
Thr439Val	10.7 ±2.5	1.49 ±1.22	3
Leu443Ser	3.2 ±0.67	1.46 ±0.76	4
Leu443Thr	2.2 ±0.22	1.42 ±0.39	4
Cys473Glu	22 ±0.64	6.8 ±0.63	3
Cys473Met	8.5 ±1.65	4.0 ±0.52	3
Cys473Leu	4.1 ±1.18	3.0 ±1.11	5
Ala173Met/Phe335Leu	22 ±5.4	1.29 ±0.29	6
Ala173Met/Phe335Ala	10.1 ±0.61	0.26 ±0.037	6

Supplementary Table 5. Residue topology for imipramine.



RESI	IMI	1.0	
GROUP			
ATOM	N1	N	-0.0729
ATOM	C1	CA	-0.0995
ATOM	C2	CA	-0.0962
ATOM	C3	CA	-0.0962
ATOM	C4	CA	-0.0987
ATOM	C5	CT2	-0.1253
ATOM	C6	CT2	-0.1253
ATOM	C7	CA	-0.0987
ATOM	C8	CA	-0.0962
ATOM	C9	CA	-0.0962
ATOM	C10	CA	-0.0995
ATOM	C11	CA	0.0286
ATOM	C12	CA	0.0025
ATOM	C13	CA	0.0025
ATOM	C14	CA	0.0286
ATOM	C15	CT2	-0.0981
ATOM	C16	CT2	-0.1446
ATOM	C17	CT2	0.0154
ATOM	N2	NH3	-0.3805
ATOM	C18	CT3	-0.0846
ATOM	C19	CT3	-0.0846
ATOM	H1	HP	0.1151
ATOM	H2	HP	0.1151
ATOM	H3	HP	0.1151

```
ATOM    H4      HP      0.1151
ATOM    H51     HA      0.0962
ATOM    H52     HA      0.0962
ATOM    H61     HA      0.0962
ATOM    H62     HA      0.0962
ATOM    H7      HP      0.1151
ATOM    H8      HP      0.1151
ATOM    H9      HP      0.1151
ATOM    H10     HP      0.1151
ATOM    H151    HA      0.0957
ATOM    H152    HA      0.0957
ATOM    H161    HA      0.0967
ATOM    H162    HA      0.0967
ATOM    H171    HA      0.0944
ATOM    H172    HA      0.0944
ATOM    H181    HA      0.0965
ATOM    H182    HA      0.0965
ATOM    H183    HA      0.0965
ATOM    H191    HA      0.0965
ATOM    H192    HA      0.0965
ATOM    H193    HA      0.0965
ATOM    HN2     HC      0.3613
BOND    C14     C1
BOND    C14     C13
BOND    C14     N1
BOND    C1      C2
BOND    C1      H1
BOND    C2      C3
BOND    C2      H2
BOND    C3      C4
BOND    C3      H3
BOND    C4      C13
BOND    C4      H4
BOND    C13     C5
BOND    N1      C15
BOND    N1      C11
BOND    C5      C6
BOND    C5      H51
BOND    C5      H52
BOND    C15     C16
BOND    C15     H151
BOND    C15     H152
BOND    C16     C17
BOND    C16     H161
BOND    C16     H162
BOND    C17     N2
BOND    C17     H171
BOND    C17     H172
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BOND    N2      C18
BOND    N2      C19
BOND    N2      HN2
BOND    C18     H181
BOND    C18     H182
BOND    C18     H183
BOND    C19     H191
BOND    C19     H192
BOND    C19     H193
BOND    C7      C8
BOND    C7      C12
BOND    C7      H7
BOND    C8      C9
BOND    C8      H8
BOND    C9      C10
BOND    C9      H9
BOND    C10     C11
BOND    C10     H10
BOND    C11     C12
BOND    C12     C6
BOND    C6      H61
BOND    C6      H62
IMPH    C14     C1      C13     N1
IMPH    C1      C14     C2      H2
IMPH    C2      C1      C3      H2
IMPH    C3      C2      C4      H3
IMPH    C4      C3      C13     H4
IMPH    C13     C14     C4      C5
IMPH    C7      C8      C12     H7
IMPH    C8      C7      C9      H8
IMPH    C9      C8      C10     H9
IMPH    C10     C9      C11     H10
IMPH    C11     C10     C12     N1
IMPH    C12     C7      C11     C6
IC      C13     C14     C1      C2      1.41    119.59      0.02    120.29  1.41
IC      N1      C14     C1      C2      1.35    121.12   -179.83    120.29  1.41
IC      C13     C14     C1      H1      1.41    119.59    178.98    120.56  1.08
IC      C1      C13     *C14    N1      1.41    119.59    179.85    119.30  1.35
IC      C14     C2      *C1     H1      1.41    120.29   -178.98    119.14  1.08
IC      C14     C1      C2      C3      1.41    120.29      0.03    119.99  1.41
IC      H1      C1      C2      C3      1.08    119.14   -178.94    119.99  1.41
IC      C14     C1      C2      H2      1.41    120.29    179.51    120.06  1.08
IC      C1      C3      *C2     H2      1.41    119.99   -179.48    119.94  1.08
IC      C1      C2      C3      C4      1.41    119.99     -0.04    119.92  1.41
IC      H2      C2      C3      C4      1.08    119.94   -179.52    119.92  1.41
IC      C1      C2      C3      H3      1.41    119.99    179.71    120.02  1.08
IC      C2      C4      *C3     H3      1.41    119.92   -179.75    120.05  1.08
IC      C2      C3      C4      C13     1.41    119.92     -0.00    120.15  1.41
```

IC	H3	C3	C4	C13	1.08	120.05	-179.75	120.15	1.41
IC	C2	C3	C4	H4	1.41	119.92	179.79	119.25	1.08
IC	C3	C13	*C4	H4	1.41	120.15	-179.79	120.60	1.08
IC	C3	C4	C13	C14	1.41	120.15	0.05	120.06	1.41
IC	H4	C4	C13	C14	1.08	120.60	-179.74	120.06	1.41
IC	C3	C4	C13	C5	1.41	120.15	-179.49	120.22	1.52
IC	C14	C4	*C13	C5	1.41	120.06	-179.55	120.22	1.52
IC	C4	C13	C14	C1	1.41	120.06	-0.06	119.59	1.41
IC	C5	C13	C14	C1	1.52	119.73	179.49	119.59	1.41
IC	C4	C13	C14	N1	1.41	120.06	179.79	119.30	1.35
IC	C14	C13	C5	C6	1.41	119.73	63.82	113.73	1.54
IC	C4	C13	C5	C6	1.41	120.22	-116.63	113.73	1.54
IC	C14	C13	C5	H51	1.41	119.73	-58.96	107.92	1.09
IC	C14	C13	C5	H52	1.41	119.73	-173.54	109.65	1.09
IC	C13	C5	C6	C12	1.52	113.73	-51.97	119.70	1.54
IC	H51	C5	C6	C12	1.09	110.35	69.47	119.70	1.54
IC	H52	C5	C6	C12	1.09	109.25	-174.82	119.70	1.54
IC	C13	C5	C6	H61	1.52	113.73	68.88	107.48	1.09
IC	C13	C5	C6	H62	1.52	113.73	-177.06	108.72	1.09
IC	C5	C6	C12	C7	1.54	119.70	-177.16	115.40	1.41
IC	H61	C6	C12	C7	1.09	106.02	61.27	115.40	1.41
IC	H62	C6	C12	C7	1.09	108.30	-51.86	115.40	1.41
IC	C5	C6	C12	C11	1.54	119.70	4.57	125.56	1.43
IC	C7	C11	*C12	C6	1.41	119.02	178.22	125.56	1.54
IC	C11	C12	C7	C8	1.43	119.02	0.78	121.36	1.40
IC	C6	C12	C7	C8	1.54	115.40	-177.62	121.36	1.40
IC	C11	C12	C7	H7	1.43	119.02	179.31	119.88	1.08
IC	C8	C12	*C7	H7	1.40	121.36	178.53	119.88	1.08
IC	C12	C7	C8	C9	1.41	121.36	0.70	119.69	1.40
IC	H7	C7	C8	C9	1.08	118.75	-177.84	119.69	1.40
IC	C12	C7	C8	H8	1.41	121.36	178.85	120.17	1.08
IC	C7	C9	*C8	H8	1.40	119.69	-178.16	120.12	1.08
IC	C7	C8	C9	C10	1.40	119.69	-0.83	119.72	1.41
IC	H8	C8	C9	C10	1.08	120.12	-178.98	119.72	1.41
IC	C7	C8	C9	H9	1.40	119.69	178.05	120.08	1.08
IC	C8	C10	*C9	H9	1.40	119.72	-178.88	120.19	1.08
IC	C8	C9	C10	C11	1.40	119.72	-0.53	121.31	1.42
IC	H9	C9	C10	C11	1.08	120.19	-179.41	121.31	1.42
IC	C8	C9	C10	H10	1.40	119.72	178.58	118.13	1.08
IC	C9	C11	*C10	H10	1.41	121.31	-179.09	120.56	1.08
IC	C9	C10	C11	N1	1.41	121.31	-178.21	119.21	1.36
IC	H10	C10	C11	N1	1.08	120.56	2.70	119.21	1.36
IC	C9	C10	C11	C12	1.41	121.31	2.00	118.87	1.43
IC	N1	C10	*C11	C12	1.36	119.21	-179.79	118.87	1.43
IC	C10	C11	N1	C14	1.42	119.21	-119.95	121.30	1.35
IC	C12	C11	N1	C14	1.43	121.92	59.84	121.30	1.35
IC	C10	C11	N1	C15	1.42	119.21	40.67	120.88	1.47
IC	C14	C15	*N1	C11	1.35	115.16	-161.75	120.88	1.36

IC	C15	N1	C14	C1	1.47	115.16	-53.91	121.12	1.41
IC	C11	N1	C14	C1	1.36	121.30	107.75	121.12	1.41
IC	C15	N1	C14	C13	1.47	115.16	126.24	119.30	1.41
IC	C14	N1	C15	C16	1.35	115.16	-149.65	114.09	1.54
IC	C11	N1	C15	C16	1.36	120.88	48.60	114.09	1.54
IC	C14	N1	C15	H11	1.35	115.16	-28.10	109.06	1.09
IC	C14	N1	C15	H12	1.35	115.16	86.85	109.36	1.09
IC	N1	C15	C16	C17	1.47	114.09	56.98	113.12	1.54
IC	H11	C15	C16	C17	1.09	108.56	-64.84	113.12	1.54
IC	H12	C15	C16	C17	1.09	109.88	-179.79	113.12	1.54
IC	N1	C15	C16	H21	1.47	114.09	-67.23	109.10	1.09
IC	N1	C15	C16	H22	1.47	114.09	178.57	108.13	1.09
IC	C15	C16	C17	N2	1.54	113.12	-177.02	112.77	1.49
IC	H21	C16	C17	N2	1.09	111.11	-53.91	112.77	1.49
IC	H22	C16	C17	N2	1.09	109.61	62.24	112.77	1.49
IC	C15	C16	C17	H31	1.54	113.12	-54.97	110.75	1.09
IC	C15	C16	C17	H32	1.54	113.12	60.86	108.00	1.09
IC	C16	C17	N2	C18	1.54	112.77	75.67	112.83	1.49
IC	H31	C17	N2	C18	1.09	108.69	-47.53	112.83	1.49
IC	H32	C17	N2	C18	1.09	110.28	-163.50	112.83	1.49
IC	C16	C17	N2	C19	1.54	112.77	-157.40	112.05	1.48
IC	C16	C17	N2	HN2	1.54	112.77	-41.65	106.63	1.01
IC	C17	N2	C18	C181	1.49	112.83	-178.47	110.99	1.09
IC	C19	N2	C18	C181	1.48	111.57	54.35	110.99	1.09
IC	HN2	N2	C18	C181	1.01	107.11	-61.44	110.99	1.09
IC	C17	N2	C18	C182	1.49	112.83	-59.06	111.98	1.09
IC	C17	N2	C18	C183	1.49	112.83	61.42	111.31	1.09
IC	C17	N2	C19	H511	1.49	112.05	176.92	111.04	1.09
IC	C18	N2	C19	H511	1.49	111.57	-55.47	111.04	1.09
IC	HN2	N2	C19	H511	1.01	106.17	60.89	111.04	1.09
IC	C17	N2	C19	H512	1.49	112.05	-62.75	111.23	1.09
IC	C17	N2	C19	H513	1.49	112.05	57.66	111.13	1.09
IC	N1	C11	C12	C7	1.36	121.92	178.12	119.02	1.41
IC	C10	C11	C12	C7	1.42	118.87	-2.09	119.02	1.41
IC	N1	C11	C12	C6	1.36	121.92	-3.66	125.56	1.54

Supplementary Table 6. Added force field parameters for imipramine in CHARMM32.

Bond parameters:

CT2	N	200.00	1.4800	Same as CT2 NH3
CA	N	270.000	1.3700	Same as CA NY

Angle parameters:

HA	CT2	N	45.000	107.50	35.00	2.101	Same as HA CT2 NH3
CT2	CT2	N	67.700	110.00			Same as CT2 CT2 NH3
CA	CA	N	120.000	110.00	25.00	2.240	Same as NY CA CY
CA	N	CA	110.000	108.00			Same as CPT NY CA
CA	N	CT2	45.800	122.30			Same as CT2 CA CA
CT2	NH3	CT3	30.000	109.50	20.00	2.074	Same as HC NH3 C*
CT3	NH3	CT3	30.000	109.50	20.00	2.074	Same as HC NH3 C*

Torsion parameters:

N	CA	CA	CA	2.8000	2	180.00	Same as NY CPT CA CA
N	CA	CA	HP	3.5000	2	180.00	Same as NY CA CY HP
N	CA	CA	CT2	3.5000	2	180.00	Same as NY CA CY CT2
CA	CA	N	CT2	3.1000	2	180.00	Same as CA CA CA CT2
CA	CA	N	CA	3.1000	2	180.00	Same as CA CA CA CA
CA	N	CT2	CT2	0.2300	2	180.00	Same as CT3 CT2 CA CA
CA	N	CT2	HA	0.0000	6	0.00	Same as X CT2 CA X

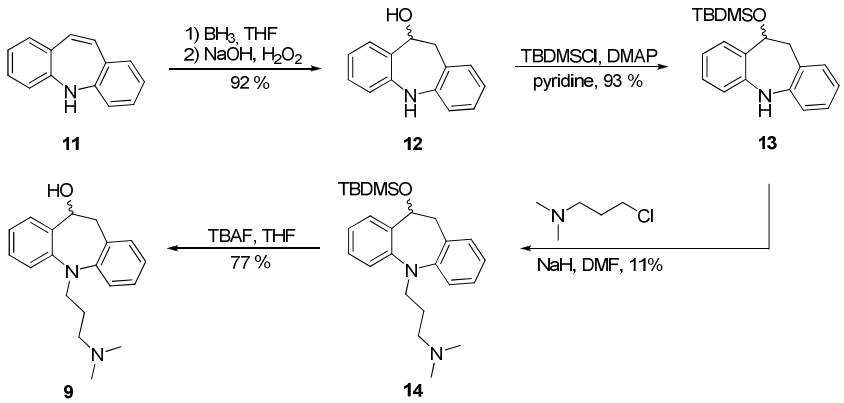
Improper parameters:

CA	CA	CA	N	100.0000	0	0.0000	Same as NY CA CY CPT

Supplementary Data

Modeling Studies. The initial induced fit docking studies of imipramine in the hSERT yielded a total of 122 poses. In 30 of these imipramine is bound in two different binding modes in the occluded central binding site, see Supplementary Table 1. Imipramine is also placed in the binding site in another 27 poses; here, however, it is randomly oriented and has no tendency towards a common binding pattern. Binding of imipramine in the extracellular vestibule is observed in 69 poses, only one of these show the same binding mode as the one observed in the LeuT[1,2]. The two identified binding modes of imipramine in the occluded binding site correspond to clusters 1 and 2 as described in the manuscript. They differ by a rotation of the hydrophobic ring-system inside the binding site. Both clusters include an ionic interaction to Asp98.

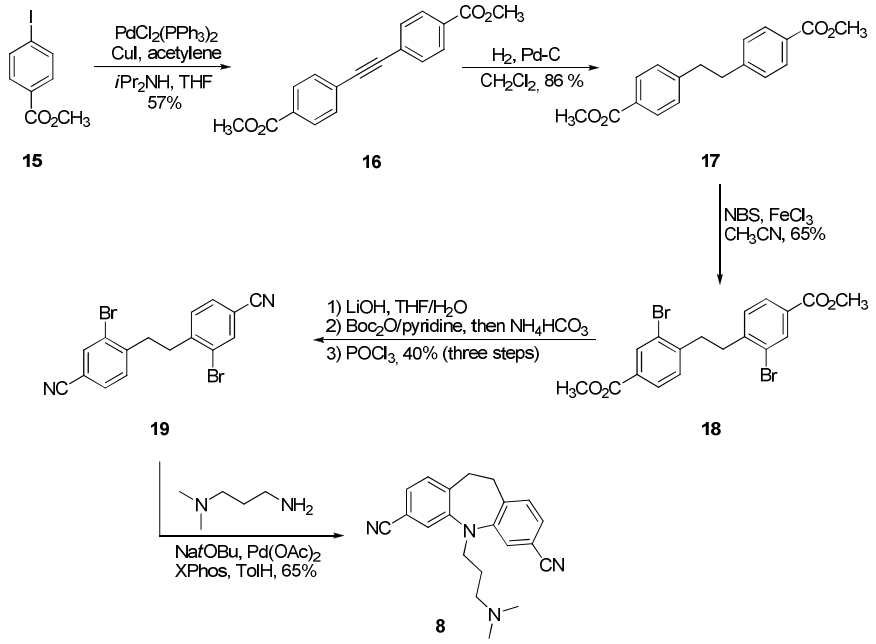
Organic Synthesis. Racemic 10-hydroxyimipramine (**9**, Supplementary Scheme 1) was prepared from iminostilbene (**11**), which underwent hydroboration with a BH_3:THF complex followed by oxidative work-up to give benzylic alcohol **12** in 92% yield. This was protected as its tert-butyldimethylsilyl (TBDMS)-ether using TBDMS-Cl in pyridine in 93% yield. The aniline function was then alkylated with 3-chloro-1-dimethylaminopropane and NaH in dimethylformamide (DMF) to give imipramine analogue **14** in 11% yield and then desilylated with tetrabutylammonium fluoride (TBAF) to give racemic 10-hydroxyimipramine



Supplementary Scheme 1. Synthesis of racemic 10-hydroxyimipramine from iminostilbene.

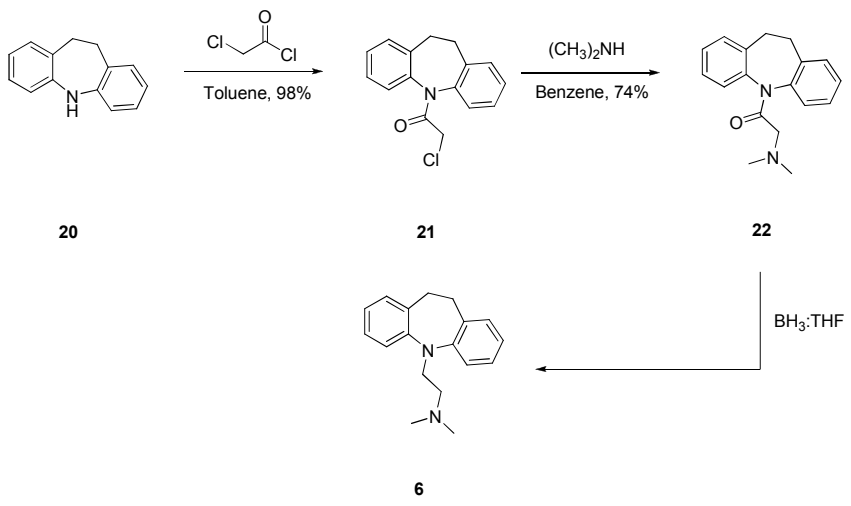
The synthesis of 3,7-dicyanoimipramine (**8**) (Supplementary Scheme 2) was accomplished from methyl p-iodobenzoate (**15**). This was dimerized with acetylene gas in a Pd/Cu-catalyzed Sonogashira coupling[3] to give a symmetrical alkyne (**16**) in a moderate yield (57%). The triple bond was reduced by H_2 over a Pd-C catalyst and regioselectively di-brominated with *N*-

bromosuccinimid (NBS)/$FeCl_3$ in acetonitrile to give compound **18**. The methyl ester functional groups were converted into cyano groups in a three-step procedure by saponification, primary amide formation, and dehydration with phosphorous oxychloride with an overall yield of 40%. The key Hartwig-Buchwald cyclization[4] smoothly produced 3,7-dicyanoimipramine in a yield of 65% in the presence of 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (XPhos)/$Pd(OAc)_2$ in toluene.



Supplementary Scheme 2. Synthesis of 3,7-dicyanoimipramine from methyl iodobenzoate.

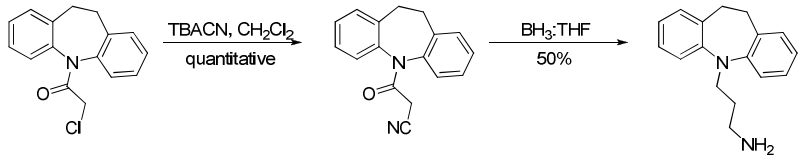
The synthesis of short imipramine (**6**) was undertaken as described in Supplementary Scheme 3. Iminodibenzyl (**20**) was reacted with chloroacetyl chloride to give chloroacetyl amide **21** in near quantitative yield. This was reacted with dimethylamine to give amino amide **22**, which was next reduced with borane to give the desired short imipramine (**6**).



20 21 22 6

Supplementary Scheme 3. Synthesis of short imipramine.

Didesmethylimipramine (**3**) was prepared as described in Supplementary Scheme 4 from chloroacetyl amide **21** (Supplementary Scheme 3). This was treated with tetrabutylammonium cyanide (TBACN) to give nitrile **23** in quantitative yield. One-pot reduction of both amide- and nitrile functions was carried out with borane to give didesmethylimipramine (**3**) in 50% yield.



21 23 3

Supplementary Scheme 4. Synthesis of didesmethylimipramine.

Supplementary Methods

Initial Induced Fit Docking of Imipramine in hSERT. Imipramine was docked into the hSERT binding site in models **A** and **B** employing the induced fit docking methodology from Schrödinger Inc.[5,6]. In both models the two sodium ions found in the crystal structure of the LeuT[7] are included. The central binding site in the two models is small relative to the bulky TCA, four protocols were therefore tested in an attempt to increase the binding site (see supplementary Table 1). First an induced fit docking protocol with default settings was tested (setup SI-I), then residues Tyr176 and Phe335 were mutated to alanine during the initial docking step (setup SI-II) to open the aromatic lid of the binding site. Including such mutations automatically changes the initial soft-docking protocol to a standard docking with no scaling of van der Waals radii, another setup was thus tested, enforcing the soft-docking approach (setup SI-III). Another possibility for increasing the size of a binding site is to allow for several rounds of side chain optimization after the soft-dockings step; this was tested in setup SI-IV. In the initial four setups the standard precision (SP) GlideScore[8] was applied in both the initial soft-docking and final re-docking steps; another possibility is to apply the extra precision (XP) GlideScore[9]. The XP GlideScore was therefore tested in the re-docking step (setup SI-V) where all residues were refined, as well as in a setup where the essential Asp98 was excluded from the side chain refinement step (setup SI-VI). Finally, the XP GlideScore was tested on both the initial soft-docking and the final re-docking steps (setup SI-VII). Setups SI-I to SI-VII all include model **A**, a single protocol was tested on model **B** (setup SI-VIII), namely one similar to the one in setup SI-V. In all setups the binding site was defined from residues Asp98 and Ile172; these two residues have been shown to interact with both 5-HT[10,11] and imipramine [10,12,13].

Molecular Dynamics Simulations. MD simulations were performed for imipramine bound as in cluster 1 of the hSERT as well as in the extracellular vestibule in a position similar to the one observed in the LeuT structures[1,2]. The hSERT was modeled as a dimer based on the crystal structure of LeuT and according to our previous simulations on the LeuT[14]. The hSERT dimer was embedded in a POPE membrane bilayer with 30 Å water slabs on each side of the membrane. The system was neutralized with a 0.2 M ion concentration (Na^+ and Cl^-), entailing approximately 195,000 atoms in the simulated systems. The complexes were minimized for 10,000 steps with a Conjugate Gradient algorithm, after which lipid tails were melted for 0.5 ns in a NVT simulation at 310 K while all other atoms were held fixed. Equilibration of the full system was then performed for 2 ns in an NPT ensemble and production dynamics performed for 3 ns in the NPT ensemble

imposing a constant area of the lipid-patch. A particle-mesh Ewald algorithm[15] was applied for long-range electrostatic interactions; constant temperature was achieved by employing Langevin dynamics with a damping constant of 0.1 ps^{-1} for melting the lipid tails and 0.5 ps^{-1} for the simulations. The Langevin Piston method[16] was employed to maintain a constant pressure of 1 atm with a piston period of 100 fs and a piston decay of 50 fs. Van der Waals interactions were accounted for to a cut-off distance of 12 Å and gradually dampened by use of a switching function from 10 Å. Simulations were performed in NAMD 2.6[17,18] with the CHARMM32 force field[19-21] including the CMAP corrections[22]. Force field parameters for imipramine were extracted from CHARMM32[19-21] and supplemented by Accelrys-CHARMm parameters as included in Quanta 2000[23]. Partial charges for imipramine were calculated with VCharge[24] by equalization of electronegativity. The topology file for imipramine is included as Supplementary Table 4, added parameters are included as Supplementary Table 5. Analysis of the computed trajectories was performed in VMD 1.8.6[25]; non-bonded energies for the full trajectories were calculated with the NAMDEnergy plugin to VMD 1.8.6 and NAMD 2.6.

Synthesis. All reagents, unless otherwise stated, were used as purchased without further purification. Solvents were dried according to standard procedures. Columns for flash chromatography were packed with silica gel (60 Å). TLC plates (Kieselgel 60 F_{254}) were visualized by use of aqueous $KMnO_4$ and heated until spots appeared or by UV-irradiation. ^1H and ^{13}C NMR experiments were recorded on a Varian Mercury 400 NMR instrument. Mass spectral data were carried out as electrospray experiments on a Micromass LC-TOF instrument. GC-MS analyses were carried out on a Hewlett-Packard 5890A gas chromatograph equipped with a 5971A MSD mass-selective detector. The GC column was an HP5 25m with an internal diameter of 0.25 mm, the injection temperature was 250 °C, the He-flow set to 1.0 mL/min, temperature program 40 °C for 5 min to 230 °C, rate 15 °C/min. Melting points were measured on a Büchi B-540.

(±) 10,11-dihydro-5*H*-dibenzo[*b,f*]azepin-10-ol (12). BH_3 in THF (1 M, 26.8 mL, 26.8 mmol) was slowly added to a solution of iminostilbene **11** (647 mg, 3.35 mmol) in dry THF (2 mL) at room temperature and stirred for 3 hours. The reaction mixture was cooled to 0 ºC before 6 M NaOH (1.12 mL, 6.69 mmol) and H_2O_2 (aq. 35%, 1.18 mL, 13.4 mmol) were carefully added. The reaction mixture was then allowed to warm to room temperature and stirred for an additional 4 hours before diluted with H_2O (25 mL) and extracted with Et_2O (25 mL). The organic phase was washed with saturated NaCl (25 mL), dried over $MgSO_4$, filtered, and concentrated under reduced

pressure. The crude product was purified by column chromatography (AcOEt/pentane 1:9) to afford the desired benzylic alcohol **12** (646 mg, 92 %) as a yellow oil. R_f 0.35 (AcOEt/pentane 1:4). ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H 7.35 (dd, 1H, *J* 1.2 Hz, *J* 7.6 Hz), 7.16 (m, 3H), 6.93 (dt, 1H, *J* 0.8 Hz, *J* 7.2 Hz), 6.86 (dt, 1H, *J* 0.8 Hz, *J* 7.2 Hz), 6.80 (dt, 1H, *J* 0.8 Hz, *J* 8.0 Hz), 6.21 (s, 1H), 5.11 (b d, 1H, *J* 4.8 Hz), 3.24 (m, 2H), 2.08 (b s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ_C 142.9, 141.7, 132.4, 131.4, 128.6, 128.5, 127.5, 124.2, 121.1, 119.3, 118.6, 118.2, 71.6, 40.9. HRMS(ES): *m/z* calcd. for $C_{14}H_{13}NONa$ 234.0895, found 234.0896.

(±) 10-(*tert*-butyldimethylsilyloxy)-10,11-dihydro-5*H*-dibenzo[*b,f*]azepine (13). To a stirred solution of benzylic alcohol **12** (447 mg, 2.11 mmol) in dry pyridine (4 mL) was added 4-dimethylaminopyridine (4-DMAP) (26 mg, 0.21 mmol) and TBDMS-Cl (382 mg, 2.54 mmol). The reaction mixture was stirred at room temperature for 24 hours then diluted with H_2O (10 mL) and stirred for additional 15 min. The phases were separated and the aqueous layer was extracted with CH_2Cl_2 (2·10 mL). The combined organic phases were dried over $MgSO_4$, filtered and concentrated under reduced pressure with toluene to afford crude silylated **13** (640 mg, 93 %) as a yellow oil, pure enough for further reaction. R_f 0.59 (AcOEt/pantane 1:9). ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H 7.59 (d, 1H, *J* 7.6 Hz), 7.21 (m, 3H), 6.99 (m, 2H), 6.80 (m, 2H), 6.11 (s, 1H), 5.24 (b d, 1H, *J* 7.8 Hz), 3.34 (m, 2H), 1.04 (s, 9H), 0.29 (s, 3H), 0.16 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ_C 142.1, 140.5, 131.8, 130.9, 128.2, 127.8, 126.9, 124.3, 119.9, 119.3, 118.2, 117.6, 71.5, 44.3, 25.9, 18.2, -4.5, -4.6. HRMS(ES): *m/z* calcd. $C_{20}H_{27}NOSiNa$ 348.1760, found 348.1750.

(±) 3-(10-(*tert*-butyldimethylsilyloxy)-10,11-dihydro-5*H*-dibenzo[*b,f*]azepin-5-yl)-*N,N*-dimethyl-propan-1-amine (14). Sodium hydride (60 % in mineral oil, 456 mg, 11.4 mmol) was washed three times with pentane (5 mL) under an atmosphere of N_2 before a solution of **13** (619 mg, 1.90 mmol) in dry DMF (10 mL) was added. The reaction mixture was stirred for 30 min. at room temperature before 3-chloro-*N,N*-dimethylpropan-1-amine (965 mg, 11.4 mmol) was added and the temperature raised to 80 ºC for 3.5 hours. The mixture was cooled to ambient temperature and transferred to a separation funnel containing AcOEt and ice water. The organic phase was washed four times with $NaHCO_3$ (aq. saturated) and NaCl (aq. saturated). The organic phase was then dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (AcOEt/pentane 1:19 containing 2.5% Et_3N) to afford the desired imipramine analogue **14** (88 mg, 11%) as a yellow oil. R_f 0.29 (AcOEt/pentane 1:9 containing 2.5% Et_3N). ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H 7.51 (d, 1H**,** *J* 6.8 Hz), 7.19 (m, 1H), 7.09 (m, 5H), 6.88 (m, 1H), 5.65 (dd, 1H, *J* 5.2 Hz, *J* 11.2 Hz), 3.78 (t, 2H, *J* 7.2 Hz), 3.36 (dd, 1H, *J* 5.2 Hz, *J* 15.8 Hz),

3.09 (dd, 1H, *J* 11.2 Hz, *J* 15.8 Hz), 2.32 (m, 2H), 2.18 (s, 6H), 1.75 (m, 2H), 1.00 (s, 9H), 0.16 (s, 3H), 0.14 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ_C 147.4, 146.3, 139.7, 131.2, 129.9, 127.1, 126.6, 125.8, 123.7, 121.8, 120.3, 118.8, 68.8, 57.9, 48.5, 45.7, 42.9, 26.4, 26.1, 18.5, -4.4.

(±) 5-(3-(dimethylamino)propyl)-10,11-dihydro-5*H*-dibenzo[*b,f*]azepin-10-ol (10-hydroxyimipramine) (9). To a solution of silylated imipramine analogue **14** (63 mg, 0.15 mmol) in dry THF (3 mL) was added tetrabutylammonium fluoride (1 M in THF, 0.17 mL, 0.17 mmol) at room temperature. The reaction mixture was stirred for 1 hour before concentrated under reduced pressure. The residue was taken up in AcOEt (10 mL) and washed with $NaHCO_3$ (aq. saturated 3·10 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (AcOEt/Et_3N 19:1) to give racemic 10-hydroxyimipramine (35 mg , 77 %) as a yellow oil. R_f 0.22 (AcOEt/Et_3N 19:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H 7.40 (d, 1H, *J* 7.2 Hz), 7.17 (m, 5H), 6.98 (dd, 2H, *J* 6.0 Hz, *J* 12.8 Hz), 5.08 (dd, 1H, *J* 3.8 Hz, *J* 7.2 Hz), 3.80 (t, 2H, *J* 6.4 Hz), 3.45 (dd, 1H, *J* 3.8 Hz, *J* 14.0 Hz), 3.21 (b dd, 2H, *J* 7.2 Hz, *J* 14.0 Hz), 2.34 (t, 2H, *J* 6.4 Hz), 2.14 (s, 6H), 1.76 (qv, 2H, *J* 6.4 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$) δ_C 148.9, 146.8, 134.6, 132.1, 130.8, 130.7, 128.1, 127.0, 123.4, 122.4, 120.6, 119.0, 70.4, 57.7, 48.7, 45.5, 40.0, 26.1. HRMS(ES): *m/z* calcd. for $C_{19}H_{25}N_2O$ 297.1967, found 297.1969.

Dimethyl 4,4`-(ethyne-1,2-diyl)dibenzoate (16). To a stirred solution of methyl 4-iodobenzoate **15** (10.12 g, 38.6 mol) in freshly distilled diisopropyl amine (55 mL) and THF (33 mL) was added $PdCl_2(PPh_3)_2$ (542 mg, 0.77 mmol) and CuI (294 mg, 1.55 mmol) at ambient temperature. The reaction vessel was filled with acetylene gas and then vigorously stirred 2 hours. The crude product was purified by column chromatography (pentane/CH_2Cl_2 1:2) to afford the desired alkyne as a yellow solid (3.22 g, 56 %). R_f 0.28 (pentane/CH_2Cl_2 1:2). ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H 8.03 (m, 4H), 7.59 (m, 4H), 3.93 (s, 6H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ_C 166.6, 132.6, 131.8, 129.7, 127.5, 91.5, 52.4. GC-MS (70 eV, EI): *m/z* calcd. for $C_{18}H_{14}O_4$ 294, found 294.

Dimethyl 4,4`-(ethane-1,2-diyl)dibenzoate (17). To a stirred solution of alkyne **16** (3.21 g, 10.9 mmol) in CH_2Cl_2 (90 mL) was added Pd/C (10 %, 500 mg). The reaction mixture was stirred under an atmosphere of H_2 (balloon) for 20 hours at room temperature before an additional portion of Pd/C (10 %, 500 mg) was added. After further 24 hours, TLC analysis (CH_2Cl_2) indicated reaction completion. The reaction mixture was filtered through a bed of Celite before purified by column chromatography (CH_2Cl_2) to give the desired alkane **17** as a slightly yellow solid (2.81 g, 86 %). R_f 0.23 (CH_2Cl_2). ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H 7.93 (d, 4H, *J* 8.2 Hz), 7.19 (d, 4H, *J* 8.2 Hz), 3.90

(s, 6H), 2.99 (s, 4H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ_C 167.2, 146.6, 129.8, 128.6, 128.2, 52.1, 37.5. HRMS(ES): *m/z* calcd. for $C_{18}H_{18}O_4Na$ 321.1103, found 321.1100.

Dimethyl 4,4`-(ethane-1,2-diyl)-bis-(3-bromobenzoate) (18). To a stirred solution of **17** (2.79 g, 9.36 mmol) in dry acetonitrile (28 mL) was added NBS (4.99 g, 28.1 mmol) and $FeCl_3$ (6.07 g, 37.4 mmol). The reaction mixture was stirred for 17 hours at reflux temperature before cooled to room temperature and added to water (100 mL). The aqueous layer was extracted with CH_2Cl_2 (3·100 mL) and the combined organic phased dried over $MgSO_4$.

The crude product was purified by column chromatography (CH_2Cl_2/pentane, 1:1) to give the desired dibromide **18** as a colorless solid (2.78 g, 65 %). R_f 0.46 (CH_2Cl_2). ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H 8.22 (d, 2H, *J* 2.0 Hz), 7.85 (dd, 2H, *J* 2.0 Hz, *J* 7.8 Hz), 7.17 (d, 2H, *J* 7.8 Hz), 3.91 (s, 6H), 3.10 (s, 4H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ_C 165.8, 145.3, 134.1, 130.7, 130.2, 128.7, 124.5, 52.5, 36.2. HRMS(ES): *m/z* calcd. for $C_{18}H_{16}{}^{79}Br^{81}BrO_4Na$ 478.9293, found 478.9283. Mp: (uncorr.) 180-182 ºC (CH_2Cl_2).

4,4'-(ethane-1,2-diyl)bis(3-bromobenzonitrile) (19). To a solution of **18** (732 mg, 1.60 mmol) in THF/H_2O (1:1, 20 mL) was added LiOH (384 mg, 16 mmol). The mixture was stirred for 2 hours at room temperature before concentrated under reduced pressure. The residue was acidified with HCl (aq., 3 M) and the white precipitates filtered off and washed with water and Et_2O to afford give crude dicarboxylic acid. The crude product was pure enough for further reaction. ^{1}H-NMR (400 MHz, DMSO-*d6*) δ_H 8.06 (d, 2H, *J* 1.6 Hz), 7.85 (dd, 2H, *J* 1.6 Hz, *J* 8.0 Hz), 7.43 (d, 2H, *J* 8.0 Hz), 3.07 (s, 4H).

To a suspension of crude dicarboxylic acid (633 mg, 1.48 mmol) in dioxane (6 mL) was added pyridine (dry, 0.12 mL, 1.49 mmol) and Boc_2O (0.75 mL, 3.27 mmol). The reaction mixture was stirred for 30 min at room temperature, whereupon NH_4HCO_3 (351 mg, 4.82 mmol) was added. The reaction mixture was stirred for 16 hours at room temperature before concentrated under reduced pressure. Diethyl ether was added to the residue and the resulting suspension was filtered and washed with more diethyl ether to afford the desired diamide as a colorless solid. The crude product was pure enough for further reaction. R_f 0.15 (AcOEt). ^{1}H-NMR (400 MHz, DMSO-*d6*) δ_H 8.07 (d, 2H, *J* 1.6 Hz), 8.06 (b s, 2H), 7.81 (dd, 2H, *J* 1.6 Hz, *J* 8.0 Hz), 7.47 (b s, 2H), 7.37 (d, 2H, *J* 8.0 Hz), 3.04 (s, 4H). ^{13}C-NMR (100 MHz, DMSO-*d6*) δ_C 166.2, 142.9, 134.3, 131.5, 130.6, 126.9, 123.7, 35.3. HRMS(ES): *m/z* calcd. for $C_{16}H_{14}{}^{79}Br^{81}BrN_2O_2Na$ 448.9299, found 448.9305.

$POCl_3$ (3 mL) was added to the primary amide (257 mg, 0.602 mmol) and was stirred for 48 hours at 70 ºC. The reaction mixture was cooled to room temperature and ice water was carefully

added. The mixture was extracted with CH_2Cl_2 (3·20 mL) and the combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The product was purified by column chromatography (CH_2Cl_2/pentane, 1:1) to afford the desired bis-nitrile **19** as a colorless solid (94 mg, 40 % over 3 steps). R_f 0.48 (CH_2Cl_2). ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H 7.85 (d, 2H, *J* 1.6 Hz), 7.52 (dd, 2H, *J* 1.6 Hz, *J* 7.8 Hz), 7.23 (d, 2H, *J* 7.8 Hz), 3.11 (s, 4H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ_C 145.4, 136.3, 131.2, 131.2, 124.9, 117.3, 112.3, 36.2. GC-MS(70 eV, EI): *m/z* calcd. for $C_{16}H_{10}{}^{79}Br^{81}BrN_2$ 390, found 390. Mp: (uncorr.) 217-219 ºC (CH_2Cl_2).

5-(3-(dimethylamino)propyl)-10,11-dihydro-5*H*-dibenzo[*b,f*]azepine-3,7-dicarbonitrile (3,7-dicyanoimipramine) (8). To a solution of $Pd(OAc)_2$ (16 mg, 0.0334 mmol) and XPhos (4 mg, 0.017 mmol) in dry toluene (1.5 mL) in a sealed vial under Ar was added bis-nitrile **19** (65 mg, 0.166 mmol), NaO*t*Bu (64 mg, 0.665 mmol) and *N*,*N*-dimethylpropane-1,3-diamine (0.02 mL, 0.183 mmol). The reaction mixture was stirred for 16 hours at 105 ºC on a heating block before it was allowed to cool to room temperature and poured into a solution of $NaHCO_3$ (aq., saturated, 20 mL). The aqueous layer was extracted with CH_2Cl_2 (3·20 mL) and the combined organic phases dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (AcOEt/pentane 2:5 containing 5 % Et_3N) to afford 3,7-dicyanoimipramine as a yellow oil (36 mg, 65 %). R_f 0.30 (AcOEt/pentane 1:1 containing 5 % Et_3N). ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H 7.36 (d, 2H, *J* 1.2 Hz), 7.24 (dd, 2H, *J* 1.2 Hz, *J* 7.6 Hz), 7.19 (d, 2H, *J* 7.6 Hz), 3.77 (t, 2H, *J* 7.2 Hz), 3.20 (s, 4H), 2.32 (t, 2H, *J* 7.2 Hz), 2.19 (s, 6H), 1.71 (qv, 2H, *J* 7.2 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$) δ_C 148.0, 139.5, 130.9, 126.8, 124.2, 118.8, 110.8, 57.2, 49.3, 45.5, 32.0, 25.9. HRMS(ES): *m/z* calcd. for $C_{21}H_{23}N_4$ 331.1923, found 331.1909.

3-(10,11-dihydro-dibenz[*b,f*]azepin-5-yl)acetyl chloride (21). Iminodibenzyl (98 mg, 0.5 mmol) was dissolved in dry toluene (1 mL) and acetyl chloride (0.08 mL, 1.0 mmol) was added. The reaction mixture was heated to reflux for 90 min before cooled to room temperature and the product isolated by crystallization and recrystallization from Et_2O to give chloroacetyl amide **21** (133 mg, 98%) as a white solid. R_f 0.3 (AcOEt/pentane 1:10). ^{1}H-NMR (200 MHz, $CDCl_3$) δ_H 7.40-7.14 (m, 8H), 4.02 (m, 2H), 3.58-3.28 (m, 2H), 2.95-2.76 (m, 2H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ_C 166.2, 140.9, 139.5, 137.9, 134.5, 130.8, 129.2, 128.2, 127.9, 127.6, 127.1, 126.7, 41.8, 30.8, 30.4. HRMS(ES): *m/z* calcd. for $C_{16}H_{14}NO^{35}ClNa$ 294.0662, found 294.0664.

3-(10,11-dihydro-dibenz[*b,f*]azepin-5-yl)acetyl dimethylamine (22). Chloride **21** (280 mg, 1.2 mmol) was dissolved in toluene (2 mL) and dimethylamine (1 mL, 10% solution in benzene) was added. The reaction mixture was stirred at ambient temperature for 18 hours and then refluxed

for 2 hours before cooled to ambient temperature and diluted with toluene. The organic layer was then washed with aqueous Na_2CO_3 (10%) and dried over $MgSO_4$ to give the desired amine (214 mg, 74%). ^{1}H-NMR (200 MHz, $CDCl_3$) δ_H 7.32-6.84 (m, 8H), 3.37-2.83 (m, 4H), 2.81-2.43 (m, 2H), 2.06 (s, 6H).

3-(10,11-dihydro-dibenz[*b,f*]azepin-5-yl)ethyl dimethylamine (6). Amido amine **21** (214 mg, 0.86 mmol) was dissolved in THF (1.5 mL) and cooled to 0 °C. Borane-THF complex (1 M, 3.4 mL, 3.4 mL) was added and the reaction mixture refluxed for 5 hours. The mixture was cooled to ambient temperature before hydrochloric acid (1 M, 3.4 mL, 3.4 mmol) was added and the mixture heated to reflux temperature for another hour. The mixture was alkalized at ambient temperature by addition of NaOH and the product isolated by extraction with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$, filtered and concentrated to give the desired didesmethylimipramine **6**. ^{1}H-NMR (200 MHz, $CDCl_3$) δ_H 7.18-6.87 (m, 8H), 3.82 (t, 2H, *J* 6.0 Hz), 3.16 (s, 4H), 2.85 (t, 2H, *J* 6.0 Hz), 1.96 (s, 6H).

3-(10,11-dihydro-dibenz[*b,f*]azepin-5-yl)acetyl cyanide (23). Tetrabutylammonium cyanide (2.74 g, 10.2 mmol) was dissolved in dry CH_2Cl_2 (6 mL) and added to chloride **21** (1.38 g, 5.1 mmol) in dry CH_2Cl_2 (1 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 2 hours before diluted with CH_2Cl_2 and washed with water. The organic layer was dried over $MgSO_4$ and concentrated to yield a solid orange compound which was purified by filtration through silica (AcOEt/pentane 1:3) to give the desired nitrile **22** (1.33 g) as a white solid in quantitative yield. R_f 0.43 (AcOEt/pentane 1:3). ^{1}H-NMR (200 MHz, $CDCl_3$) δ_H 7.40-7.12 (m, 8H), 3.55-3.25 (m, 4H), 2.97-2.79 (m, 2H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ_C 161.9, 140.6, 138.9, 137.9, 134.3, 130.9, 130.5, 129.7, 128.1, 128.0, 128.0, 127.1, 126.8, 113.9, 30.8, 30.1, 25.6. HRMS(ES): *m/z* calcd. for $C_{17}H_{14}N_2ONa$ 285.1004, found 294.0664.

3-(10,11-dihydro-dibenz[*b,f*]azepin-5-yl)propyl amine (3). Borane-THF complex (1 M, 2 mL, 2 mmol) was added to nitrile **22** (130 mg, 0.5 mmol) in THF (1.5 mL) at 0 °C. The reaction mixture was refluxed for 2.5 hours before cooled to ambient temperature before hydrochloric acid (1 M, 2 mL, 2 mmol) was added and the mixture refluxed for another hour. The mixture was alkalized with NaOH at ambient temperature and the product isolated by extraction with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated to give the desired product (**3**) (63 mg, 50%) as an oil. ^{1}H-NMR (200 MHz, $CDCl_3$) δ_H 7.20-6.80 (m, 8H), 3.78 (t, 2H, *J* 6.6 Hz), 3.14 (s, 4H), 2.74 (t, 2H, *J* 6.6 Hz), 1.75 (quint, 2H, *J* 6.6 Hz). HRMS(ES): *m/z* calcd. for $C_{17}H_{21}N_2$ 253.1705, found 253.1676.

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