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Probing binding interactions of cytisine derivatives to the α4β2 nicotinic acetylcholine receptor

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1. Supporting Figures S1-S5 and Tables S1-S13

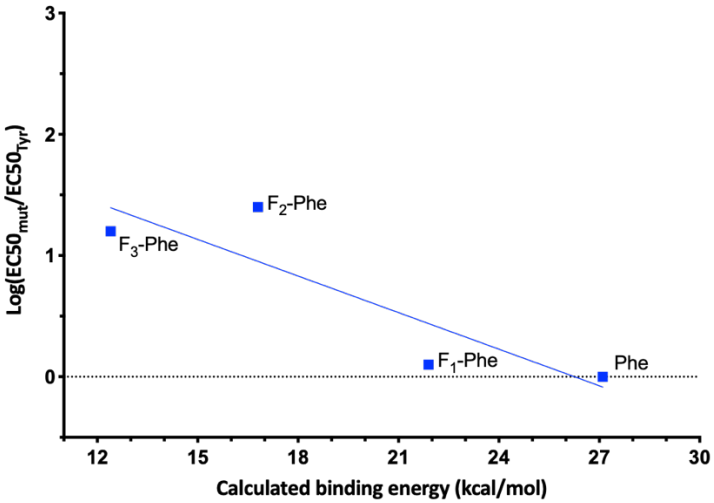


Figure S1. Fluorination plot for cytisine at TyrC2 in the A2B3 stoichiometry. (A) Fluorination plot showing F_n-Phe series in blue (R^2=0.77). The x-axis is the calculated binding energy between a Sodium ion and each side chain in the gas phase.[1] The y-axis is the log fold shift in EC_{50}.

Table S1. Cytisine EC_{50} values for non-canonical amino acids at TyrC2 in A2B3.

Residue	EC_{50} (μM)			n_H			Fold shift to WT	Fold shift to Phe	N	$\lvert I_{max} \rvert$
Tyr	0.0056	±	0.0001	1.4	±	0.04	1.0		13	0.11-1.9
Phe	0.012	±	0.0003	1.4	±	0.05	2.1	1.0	18	0.054-0.84
4-F_1-Phe	0.014	±	0.0005	1.4	±	0.07	2.5	1.2	16	0.022-0.54
3,5-F_2-Phe	0.34	±	0.02	1.3	±	0.1	60	28	8	0.009-0.10
3,4,5-F_3-Phe	0.20	±	0.009	1.3	±	0.07	36	17	13	0.034-0.30

Table S2. EC_{50} values for cytisine derivatives at WT A2B3.

Ligand		EC_{50} (nM)			n_H			Fold shift	N	Efficacy	$\lvert I_{max} \rvert$ (μA)
1	Cytisine	5.6	±	0.1	1.4	±	0.04	1.0	13	0.05	0.1 – 2.1
2	F	7.1	±	0.1	1.4	±	0.03	1.3	14	0.10	0.4 – 16
3	Me	13	±	0.2	1.4	±	0.03	2.3	12	0.16	0.5 – 8.7
4	OMe	76	±	2	1.4	±	0.04	14	13	0.08	0.3 – 1.0
5	CF_3	100	±	3	1.3	±	0.04	18	14	0.06	0.9 – 5.1
10	$NHCH_3$	260	±	4	1.1	±	0.02	44	12	0.19	1.1 – 4.5

[1] Duffy, N. H. Studies of the Serotonin Type 3A Receptor and the Chemical Preparation of TRNA. phd, California Institute of Technology, 2014. https://doi.org/Duffy, Noah Hanville (2014) Studies of the Serotonin Type 3A Receptor and the Chemical Preparation of tRNA. Dissertation (Ph.D.), California Institute of Technology. doi:10.7907/X1YA-DM13. http://resolver.caltech.edu/CaltechTHESIS:05062014-151417635

11	Br/ $NHCH_3$	230	±	6	1.2	±	0.03	37	13	0.29	0.35 - 2.2
6	Et	24	±	0.5	1.2	±	0.03	4.3	16	0.16	0.34 -3.9
7	Br/ Et	1.2	±	0.02	2.3	±	0.08	0.21	14	0.33	0.86 – 8.6
8	NH_2	30	±	0.8	1.2	±	0.03	5.4	16	0.18	0.40 - 3.7
9	Br/ NH_2	1.3	±	0.02	1.9	±	0.05	0.23	23	0.29	0.83 – 5.4

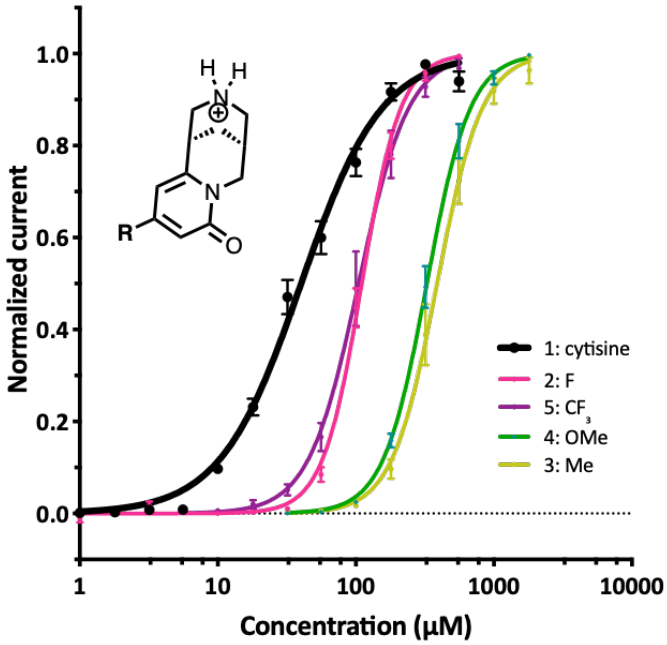


Figure S2. Dose-response curves of cytisine derivatives to the α7 nAChR. The cytisine structure indicated the position of the substituent at C(10). The EC_{50} of $NHCH_3$-cytisine was too far right-shifted to record a full dose-response relation.

Table S3. EC_{50} values for cytisine derivatives at WT α7. ND: not determined.

Ligand		**EC_{50} (µM)**			**n_H**			**Fold shift**	**N**	**Efficacy**	**$\|I_{max}\|$**
1	Cytisine	40	±	1	1.5	±	0.07	1.0	11	ND	0.86-14
2	F	110	±	2	3.1	±	0.2	2.8	6	ND	0.050-5.6
5	CF_3	110	±	4	2.4	±	0.2	2.6	7	0.16	0.22-4.1
4	OMe	330	±	8	2.8	±	0.2	8.1	8	0.37	0.37-18
3	Me	390	±	14	2.7	±	0.2	9.7	7	0.35	0.42-11

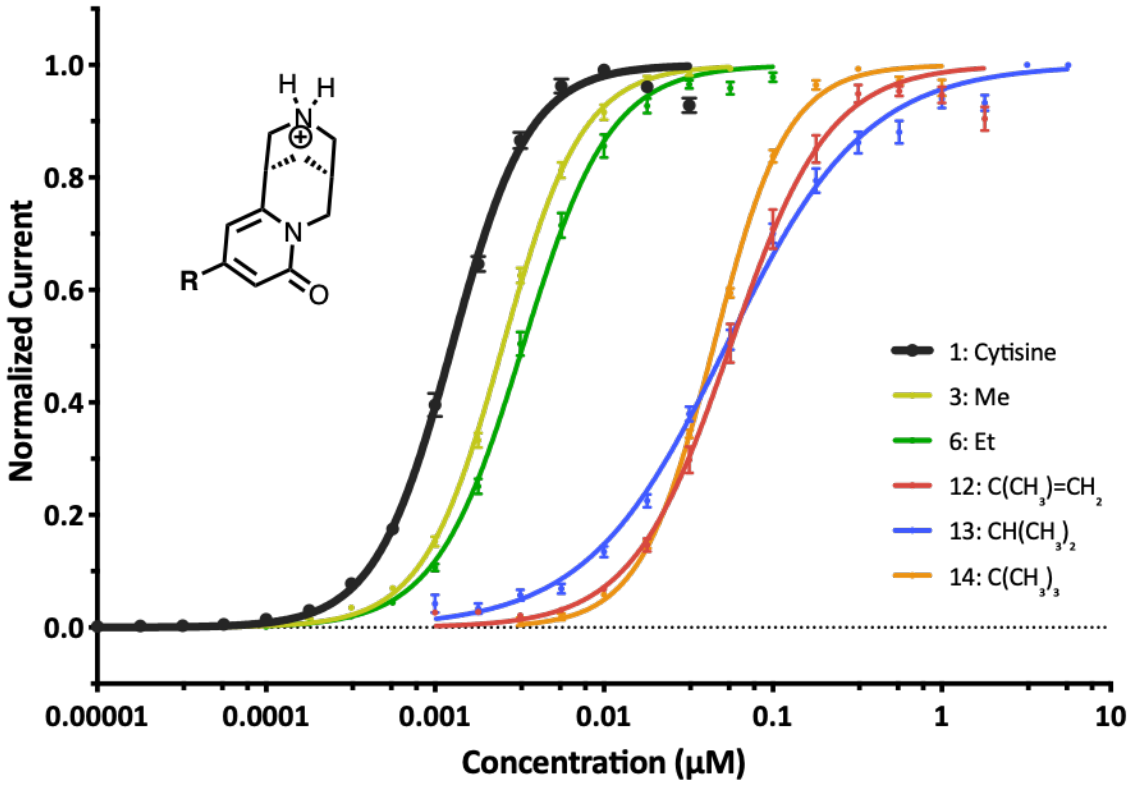


Figure S3. Investigation of steric effects on C(10)-substitution in A3B2. (A) Dose-response curves for C(10)-derivatives with increased steric bulk. Cytisine derivatives used in this series; 3 (Me), 6 (Et), 12 ($C(CH_3)CH_2$), 13 ($CH(CH_3)_2$), 14 ($C(CH_3)_3$).

Table S4. EC_{50} values for cytisine derivatives, sterics series, at WT A3B2. Volume of ligand was calculated after equilibrium geometry optimization using HF-6-31** in vacuum.

Ligand		EC_{50} (nM)	n_H	Fold shift	N	Efficacy	I_{max}	Volume (Å^3)
1	cytisine	1.3 ± 0.02	1.9 ± 0.05	1.0	12	0.73	0.22-27	198
3	Me	2.5 ± 0.2	1.8 ± 0.04	1.9	13	0.67	0.37-47	216
6	Et	3.3 ± 0.06	1.7 ± 0.04	2.5	16	0.51	1.1-36	234
12	$C(CH_3)CH_2$	56 ± 2	1.5 ± 0.06	44	12	0.14	0.28-5.8	249
13	$CH(CH_3)_2$	53 ± 2	1.4 ± 0.03	42	17	0.06	0.055-4.0	252
14	$C(CH_3)_3$	45 ± 0.5	2.0 ± 0.04	35	10	0.67	1.0-24	270

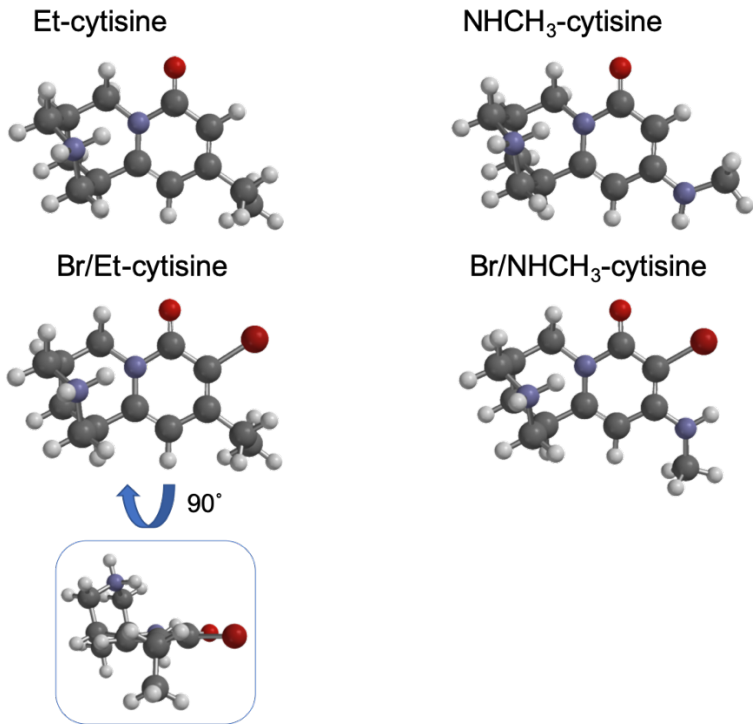


Figure S4. Conformational analysis of cytisine derivatives with $NHCH_3$ and Et-substituents at C(10). Lowest energy conformers of 6 (Et) and 7 (Br/Et) have the C(10)-substituent perpendicular to the pyridone ring, while 10 ($NHCH_3$) and 11 (Br/$NHCH_3$) have the substituent in the plane of the ring.

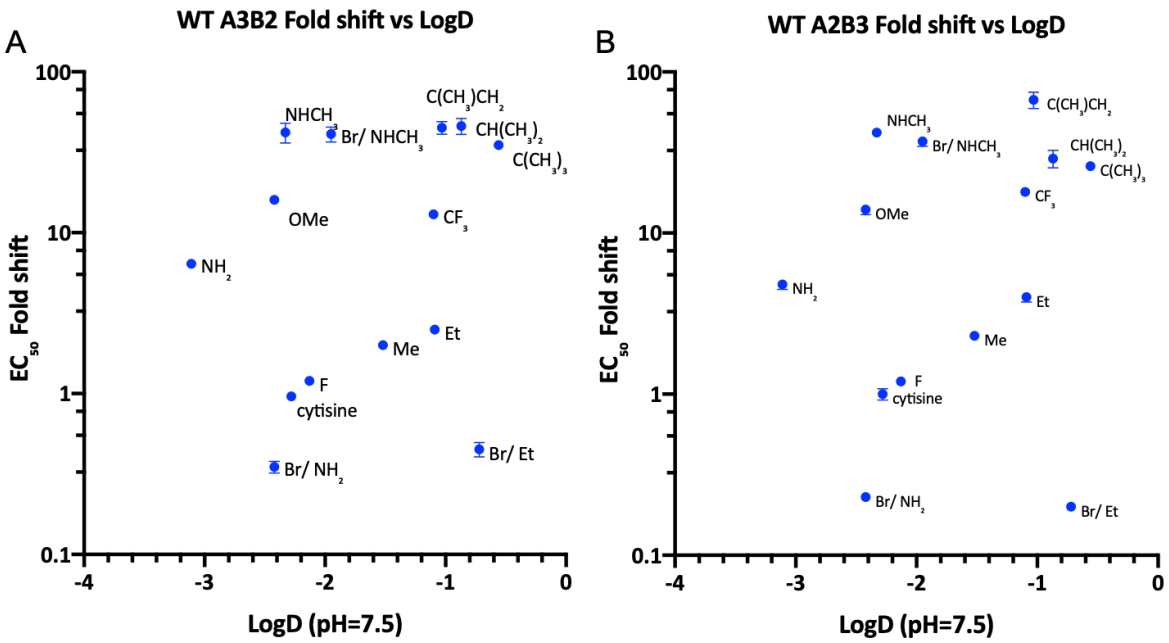


Figure S5. Scatterplot, EC_{50} fold shifts of C(10) derivatives vs $logD_{7.5}$. (A) Values for A3B2 stoichiometry and (B) values for A2B3 stoichiometry.

Table S5. Calculated electrostatic potentials of carbonyl and amine in cytisine derivatives, as well as the total volume of the ligand.

Ligand		Carbonyl (kcal/mol)	Amine (kcal/mol)	Volume (Å3)
5	CF_3	8.4	171	230
2	F	4.6	170	203
1	cytisine	2.9	168	198
3	Me	0.94	166	216
6	Et	0.44	166	234
12	$C(CH_3)CH_2$	0.35	166	249
13	$CH(CH_3)_2$	0.11	166	252
4	OMe	0.03	166	225
14	$C(CH_3)_3$	0.03	165	270
7	Br/ Et	-2.1	168	253
8	NH_2	-3.6	165	208
9	Br/ NH_2	-5.9	167	226
10	$NHCH_3$	-5.9	164	228
11	Br/$NHCH_3$	-8.1	166	246

Table S6. EC_{50} and n_H values for nonsense-suppression experiments at W154 in A3B2.

Ligand		Residue	EC_{50} (nM)			n_H			Fold shift	N	$\|I_{max}\|$
1	Cytisine	Trp	1.2	±	0.024	2.2	±	0.08		21	0.082 – 4.0
		F_4-Trp	14	±	0.81	1.1	±	0.07	12	10	0.075 – 0.15
10	$NHCH_3$	Trp	41	±	55	1.4	±	0.2		12	1.5 – 16
			1300	±	120	1.7	±	0.6			
		F_4-Trp	1100	±	51	1.1	±	0.06	27	14	0.080 – 0.70
5	CF_3	Trp	19	±	0.72	1.4	±	0.07		10	0.055 – 6.05
		F_4-Trp	780	±	82	0.91	±	0.08	41	10	0.087 – 0.54
4	OMe	Trp	15	±	0.39	1.9	±	0.08		17	0.18 – 8.8
		F_4-Trp	760	±	61	0.99	±	0.07	51	10	0.021 – 0.23
2	F	Trp	1.6	±	0.039	2.1	±	0.1		11	0.22 – 7.3
		F_4-Trp	150	±	7.9	1.1	±	0.05	96	11	0.069 – 0.64
3	Me	Trp	3.3	±	0.086	1.8	±	0.07		17	0.056 – 6.6
		F_4-Trp	140	±	8.1	1.1	±	0.07	46	10	0.087 – 0.56
6	Et	Trp	2.7	±	0.078	1.7	±	0.08		12	0.051 – 15
		F_4-Trp	460	±	12	1.0	±	0.03	170	11	0.063 – 0.58
8	NH_2	Trp	8.5	±	0.46	1.5	±	0.1		13	0.16 – 15
		F_4-Trp	550	±	72	1.0	±	0.1	65	9	0.033 – 0.78
14	$C(CH_3)_3$	Trp	44	±	1.1	1.7	±	0.06		15	1.9 – 29
		F_4-Trp	270	±	120	1.3	±	0.07	61	12	0.072 – 0.75
9	Br/NH_2	Trp	0.65	±	0.042	1.9	±	0.2		11	0.78 – 14
		F_4-Trp	21	±	0.98	1.0	±	0.05	33	11	0.063 – 0.97
7	Br/Et	Trp	0.41	±	0.023	2.4	±	0.3		9	1.3 – 17
		F_4-Trp	14	±	0.55	1.1	±	0.05	34	12	0.077 – 1.9
11	$Br/NHCH_3$	Trp	31	±	24	1.4	±	0.1		12	0.89 – 25
			2600	±	140	2.2	±	1			
		F_4-Trp	1900	±	94	1.2	±	0.07	61	13	0.074 – 0.22

Table S7. EC_{50} and n_H values for nonsense-suppression experiments at T155 in A3B2.

	Ligand	Residue	EC_{50} (nM)			n_H			Fold shift	N	$\|I_{max}\|$
1	Cytisine	Thr	1.7	±	0.030	1.7	±	0.05		12	0.26 - 12
		Tah	46	±	1.1	1.2	±	0.03	27	12	0.30 - 13
10	$NHCH_3$	Thr	40	±	78	1.7	±	0.44		14	0.26 – 17
			770	±	290	0.84	±	0.15			
		Tah	1000	±	33	1.4	±	0.06	25	11	0.28 - 14
5	CF_3	Thr	23	±	0.75	1.3	±	0.05		10	0.15 – 16
		Tah	1400	±	39	1.4	±	0.04	61	12	0.059 – 8.3
4	OMe	Thr	20	±	0.35	1.5	±	0.04		10	0.59 – 9.6
		Tah	430	±	15	1.3	±	0.05	22	10	0.015 – 9.3
2	F	Thr	1.8	±	0.029	1.5	±	0.03		12	0.72 – 7.0
		Tah	60	±	1.7	1.2	±	0.03	33	12	0.28 - 13
3	Me	Thr	3.4	±	0.088	1.5	±	0.06		13	0.098 – 8.2
		Tah	100	±	3.2	1.3	±	0.05	31	14	0.042 - 14
6	Et	Thr	3.1	±	0.10	1.4	±	0.06		12	0.086 – 7.3
		Tah	170	±	4.4	1.2	±	0.03	56	12	0.46 - 11
8	NH_2	Thr	13	±	0.37	1.3	±	0.04		13	0.15 – 7.6
		Tah	220	±	4.0	1.2	±	0.02	17	12	2.4 - 15
14	$C(CH_3)_3$	Thr	43	±	0.98	1.9	±	0.07		21	0.30 – 15
		Tah	1100	±	14	1.4	±	0.02	26	11	0.16 – 8.7
9	Br/NH_2	Thr	0.40	±	0.0089	2.2	±	0.10		13	0.031 – 6.0
		Tah	3.7	±	0.084	1.4	±	0.04	9.3	13	0.45 – 17
7	Br/Et	Thr	0.50	±	0.016	2.0	±	0.12		12	0.30 – 7.7
		Tah	5.6	±	0.13	1.3	±	0.03	11	11	1.6 – 20
11	$Br/NHCH_3$	Thr	38	±	47	1.3	±	0.13		20	0.13 – 34
			3400	±	150	1.4	±	0.43			
		Tah	97	±	31	1.3	±	0.05	25	10	0.97 - 12

Table S8. EC_{50} and n_H values for nonsense-suppression experiments at L119 in A3B2.

	Ligand	Residue	EC_{50} (nM)			n_H			Fold shift	N	$\|I_{max}\|$
1	Cytisine	Leu	1.2	±	0.023	1.9	±	0.06		13	0.069 – 2.8
		Lah	38	±	0.92	1.1	±	0.03	32	15	0.67 - 23
10	$NHCH_3$	Leu	41	±	91	1.5	±	0.3		11	0.064 – 1.1
			1300	±	150	1.1	±	0.4			
		Lah	1300	±	20	1.5	±	0.03	32	13	0.13 – 5.5
5	CF_3	Leu	17	±	0.66	1.2	±	0.06		10	0.012 – 1.3
		Lah	250	±	4.6	1.1	±	0.02	15	12	0.030 – 7.6
4	OMe	Leu	18	±	0.34	1.6	±	0.05		11	0.057 – 1.0
		Lah	530	±	13	1.2	±	0.03	30	12	0.10 – 7.4
2	F	Leu	2.0	±	0.029	2.0	±	0.05		14	0.057 – 0.90
		Lah	36	±	0.55	1.2	±	0.02	18	14	0.53 – 7.9
3	Me	Leu	2.8	±	0.074	1.8	±	0.07		11	0.11 – 1.0
		Lah	130	±	2.6	1.1	±	0.02	46	11	0.050 – 2.7
6	Et	Leu	4.5	±	0.24	1.2	±	0.07		11	0.075 – 1.4
		Lah	79	±	3.1	1.2	±	0.05	18	12	0.31 – 4.9
8	NH_2	Leu	9.5	±	0.19	1.4	±	0.03		15	0.48 – 16
		Lah	230	±	4.1	1.2	±	0.02	25	14	0.077 – 49
14	$C(CH_3)_3$	Leu	49	±	0.86	1.8	±	0.05		16	0.14 – 11
		Lah	950	±	17	1.4	±	0.03	19	16	0.79 - 33
9	Br/NH_2	Leu	0.41	±	0.0068	2.2	±	0.07		12	0.32 – 5.2
		Lah	2.4	±	0.043	1.6	±	0.04	5.9	11	1.1 – 28
7	Br/Et	Leu	0.40	±	0.020	1.4	±	0.1		13	0.055 – 0.47
		Lah	1.0	±	0.047	1.6	±	0.1	2.5	12	0.20 – 7.1
11	$Br/NHCH_3$	Leu	37	±	48	1.4	±	0.2		13	0.32 – 11
			2900	±	130	1.6	±	0.7			
		Lah	410	±	11	1.2	±	0.03	11	14	10 - 46

Table S9. EC_{50} and n_H values for nonsense-suppression experiments at Y202 in A3B2.

	Ligand	Residue	EC_{50} (nM)			n_H			Fold shift	N	$\|I_{max}\|$
1	Cytisine	Phe	6.6	±	0.14	1.4	±	0.04		13	0.064 – 22
		F_3-Phe	160	±	6.3	1.1	±	0.05	24	13	0.44 – 8.0
10	$NHCH_3$	Phe	53	±	71	1.3	±	0.3		18	0.10 – 12
			5700	±	69	1.4	±	0.3			
		F_3-Phe	5300	±	300	1.0	±	0.06	100	15	0.025 – 2.9
5	CF_3	Phe	6.9	±	0.78	0.78	±	0.06		12	0.64 – 8.6
		F_3-Phe	460	±	38	1.17	±	0.1	67	12	0.14 – 0.83
4	OMe	Phe	28	±	4.4	0.71	±	0.1		14	0.25 – 6.3
		F_3-Phe	1500	±	120	0.76	±	0.05	54	16	0.17 – 0.83
2	F	Phe	10	±	0.69	0.78	±	0.04		15	0.31 – 8.9
		F_3-Phe	190	±	10	0.88	±	0.04	19	16	0.21 – 1.1
3	Me	Phe	14	±	1.1	0.76	±	0.05		14	0.16 – 7.5
		F_3-Phe	140	±	8.4	1.0	±	0.06	10	13	0.059 – 0.50
6	Et	Phe	2.3	±	0.077	1.9	±	0.1		14	0.040 – 3.3
		F_3-Phe	160	±	6.9	1.3	±	0.07	70	10	0.15 – 1.3
8	NH_2	Phe	36	±	1.1	1.4	±	0.05		14	0.50 – 8.5
		F_3-Phe	2200	±	67	1.2	±	0.04	61	11	0.088 – 5.7
14	$C(CH_3)_3$	Phe	160	±	12	0.96	±	0.07		12	0.064 – 0.67
		F_3-Phe	6000	±	590	1.0	±	0.09	38	9	0.017 – 0.32
9	Br/NH_2	Phe	0.68	±	0.018	2.5	±	0.2		11	0.043 – 7.7
		F_3-Phe	30	±	0.84	1.1	±	0.03	44	6	0.30 – 2.4
7	Br/Et	Phe	0.41	±	0.020	2.6	±	0.3		15	0.053 – 7.6
		F_3-Phe	13	±	1.3	0.73	±	0.06	32	9	0.10 – 10
11	$Br/NHCH_3$	Phe	3.0	±	42	1.6	±	0.2		17	0.081 – 13
			260	±	110	1.2	±	0.4			
		F_3-Phe	280	±	52	1.0	±	0.1	93	16	0.49 – 5.4
			8900	±	100	2.7	±	2			

Table S10. EC_{50} and n_H values for nonsense-suppression experiments at W154 in A2B3.

	Ligand	Residue	EC_{50} (nM)			n_H			Fold shift	N	$\|I_{max}\|$
1	Cytisine	Trp	4.0	±	0.21	1.3	±	0.09		15	0.053 – 1.1
		F_4-Trp	97	±	3.5	0.97	±	0.03	23	9	0.057 – 0.17
10	$NHCH_3$	Trp	150	±	5.9	1.2	±	0.05		13	0.16 – 4.3
		F_4-Trp	1800	±	58	1.2	±	0.04	12	14	0.088 – 1.1
5	CF_3	Trp	70	±	2.2	1.2	±	0.05		14	0.18 – 4.5
		F_4-Trp	1100	±	38	1.3	±	0.05	16	10	0.14 – 0.75
4	OMe	Trp	43	±	2.2	1.5	±	0.1		14	0.10 – 2.6
		F_4-Trp	710	±	25	1.2	±	0.05	17	10	0.054 – 0.41
2	F	Trp	7.7	±	0.27	1.6	±	0.08		14	0.13 – 2.1
		F_4-Trp	200	±	7.9	1.1	±	0.05	26	13	0.051 – 0.22
3	Me	Trp	6.3	±	0.22	1.6	±	0.08		12	0.24 – 2.1
		F_4-Trp	150	±	6.8	1.2	±	0.06	24	11	0.023 – 0.26
6	Et	Trp	16	±	0.55	1.6	±	0.08		14	0.072 – 0.79
		F_4-Trp	380	±	32	1.1	±	0.1	24	11	0.011 – 0.84
8	NH_2	Trp	33	±	1.2	1.3	±	0.06		16	0.28 - 6.2
		F_4-Trp	590	±	20	1.0	±	0.03	18	9	0.023 – 0.85
14	$C(CH_3)_3$	Trp	140	±	5.9	1.4	±	0.07		11	0.088 – 2.3
		F_4-Trp	6200	±	550	1.2	±	0.1	44	17	0.015 – 1.2
9	Br/NH_2	Trp	1.9	±	0.11	1.8	±	0.2		12	0.15 - 11
		F_4-Trp	19	±	0.80	1.1	±	0.05	10	12	0.14 – 1.2
7	Br/Et	Trp	0.86	±	0.024	2.9	±	0.2		10	0.33 – 1.4
		F_4-Trp	16	±	0.74	1.1	±	0.05	19	6	0.060 – 0.25
11	Br/$NHCH_3$	Trp	140	±	3.9	1.2	±	0.04		11	0.21 – 3.2
		F_4-Trp	3000	±	190	1.2	±	0.08	21	18	0.017 – 0.19

Table S11. EC_{50} and n_H values for nonsense-suppression experiments at T155 in A2B3.

	Ligand	Residue	EC_{50} (nM)			n_H			Fold shift	N	$\|I_{max}\|$
1	Cytisine	Thr	7.2	±	0.18	1.4	±	0.04		12	0.19 – 0.56
		Tah	130	±	2.5	1.2	±	0.02	18	10	0.024 – 0.43
10	$NHCH_3$	Thr	180	±	3.3	1.33	±	0.03		13	0.048 – 0.49
		Tah	2800	±	52	1.4	±	0.03	16	12	0.23 – 2.3
5	CF_3	Thr	110	±	1.6	1.3	±	0.02		11	0.12 – 0.48
		Tah	3700	±	47	1.4	±	0.02	34	11	0.23 – 1.9
4	OMe	Thr	85	±	1.8	1.3	±	0.03		13	0.050 – 0.61
		Tah	1400	±	23	1.3	±	0.03	16	10	0.20 – 0.7.8
2	F	Thr	5.5	±	0.15	1.3	±	0.04		9	0.020 – 0.43
		Tah	170	±	2.5	1.2	±	0.02	31	11	0.023 – 1.8
3	Me	Thr	11	±	0.24	1.4	±	0.04		7	0.043 – 0.68
		Tah	330	±	4.1	1.3	±	0.02	30	19	0.38 – 2.5
6	Et	Thr	15	±	0.20	1.5	±	0.03		15	0.031 – 0.59
		Tah	680	±	12	1.3	±	0.03	45	14	0.11 – 0.94
8	NH_2	Thr	42	±	0.55	1.2	±	0.02		12	0.056 – 0.87
		Tah	850	±	11	1.2	±	0.02	20	13	0.053 – 0.47
14	$C(CH_3)_3$	Thr	230	±	5.4	1.3	±	0.03		14	0.093 – 0.42
		Tah	2000	±	55	1.2	±	0.03	8.7	15	0.052 – 1.6
9	Br/NH_2	Thr	1.4	±	0.032	1.8	±	0.07		14	0.099 – 1.8
		Tah	16	±	0.17	1.3	±	0.02	11	15	0.12 – 1.2
7	Br/Et	Thr	0.89	±	0.019	2.4	±	0.1		14	0.20 – 1.6
		Tah	18	±	0.37	1.3	±	0.03	20	15	0.23 – 3.3
11	$Br/NHCH_3$	Thr	190	±	3.0	1.2	±	0.02		13	0.22 – 0.94
		Tah	2300	±	37	1.3	±	0.02	12	15	0.20 – 2.7

Table S12. EC_{50} and n_H values for nonsense-suppression experiments at L119 in A2B3.

	Ligand	Residue	EC_{50} (nM)			n_H			Fold shift	N	$\|I_{max}\|$
1	Cytisine	Leu	4.9	±	0.096	1.3	±	0.03		11	0.078 – 1.3
		Lah	190	±	5.1	1.0	±	0.02	39	12	0.081 – 0.11
10	$NHCH_3$	Leu	160	±	6.7	1.0	±	0.04		7	0.21 – 1.9
		Lah	3800	±	76	1.4	±	0.04	24	11	0.079 – 1.0
5	CF_3	Leu	57	±	1.3	1.3	±	0.03		14	0.16 – 3.0
		Lah	990	±	20	1.4	±	0.03	17	12	0.59 – 3.7
4	OMe	Leu	65	±	1.9	1.2	±	0.04		10	0.043 – 0.31
		Lah	2100	±	30	1.2	±	0.02	32	13	0.036 – 3.1
2	F	Leu	5.2	±	0.13	1.5	±	0.05		11	0.031 – 0.47
		Lah	170	±	3.4	1.3	±	0.03	33	10	0.064 – 0.63
3	Me	Leu	8.7	±	0.29	1.3	±	0.05		11	0.11 – 2.0
		Lah	460	±	8.3	1.3	±	0.03	53	11	0.43 – 3.5
6	Et	Leu	18	±	0.40	1.4	±	0.04		11	0.097 – 1.1
		Lah	630	±	13	1.3	±	0.03	35	9	0.43 – 1.6
8	NH_2	Leu	43	±	0.85	1.1	±	0.02		15	0.3 – 2.4
		Lah	2200	±	39	1.2	±	0.02	51	14	0.044 – 8.2
14	$C(CH_3)_3$	Leu	160	±	3.9	1.3	±	0.04		12	0.030 – 0.29
		Lah	5300	±	91	1.3	±	0.03	33	15	0.11 – 0.95
9	Br/NH_2	Leu	1.5	±	0.024	1.9	±	0.05		14	0.59 – 3.1
		Lah	31	±	0.97	1.2	±	0.04	21	15	0.24 – 20
7	Br/Et	Leu	0.87	±	0.018	2.0	±	0.07		16	0.17 – 2.2
		Lah	8.1	±	0.22	1.4	±	0.05	9.3	11	0.96 – 4.6
11	$Br/NHCH_3$	Leu	160	±	4.6	1.3	±	0.04		12	0.080 – 0.81
		Lah	3000	±	41	1.2	±	0.02	19	15	0.12 – 2.7

Table S13. EC_{50} and n_H values for nonsense-suppression experiments at Y202 in A2B3.

	Ligand	**Residue**	**EC_{50} (nM)**			**n_H**			**Fold shift**	**N**	**$\|I_{max}\|$**
1	Cytisine	Phe	12	±	0.35	1.4	±	0.05		18	0.076 – 0.84
		F_3-Phe	200	±	9.2	1.3	±	0.07	17	13	0.034 – 0.30
10	$NHCH_3$	Phe	110	±	5.7	1.3	±	0.08		11	0.064 – 0.31
		F_3-Phe	8000	±	610	1.1	±	0.08	73	12	0.022 – 0193
5	CF_3	Phe	6.2	±	0.17	1.5	±	0.05		16	0.045 – 0.24
		F_3-Phe	720	±	53	1.2	±	0.1	116	14	0.008 – 0.040
4	OMe	Phe	42	±	3.2	1.4	±	0.1		10	0.017 – 0.086
		F_3-Phe	1400	±	100	1.4	±	0.1	33	11	0.010 – 0.19
2	F	Phe	7.9	±	0.53	1.2	±	0.09		12	0.036 – 0.16
		F_3-Phe	200	±	12	1.4	±	0.1	25	12	0.015 – 0.022
3	Me	Phe	8.6	±	0.30	1.2	±	0.05		12	0.036 – 0.30
		F_3-Phe	250	±	28	1.0	±	0.1	29	11	0.007 – 0.032
6	Et	Phe	7.9	±	1.4	1.0	±	0.1		9	0.017 – 0.072
		F_3-Phe	580	±	51	1.1	±	0.1	73	9	0.006 – 0.17
8	NH_2	Phe	85	±	3.4	1.4	±	0.07		5	0.054 – 0.136
		F_3-Phe	4000	±	230	1.3	±	0.1	47	10	0.010 – 0.096
14	$C(CH_3)_3$	Phe	300	±	19	1.5	±	0.1		11	0.014 – 0.13
		F_3-Phe	5000	±	880	1.4	±	0.3	17	6	0.005 – 0.017
9	Br/NH_2	Phe	2.1	±	0.10	1.6	±	0.1		10	0.064 – 1.6
		F_3-Phe	96	±	6.2	1.0	±	0.07	46	6	0.16 – 0.89
7	Br/Et	Phe	1.3	±	0.042	2.1	±	0.1		13	0.082 – 0.34
		F_3-Phe	26	±	1.5	1.1	±	0.07	20	13	0.025 – 0.27
11	$Br/NHCH_3$	Phe	10	±	0.46	1.4	±	0.08		10	0.21 -0.98
		F_3-Phe	560	±	27	1.3	±	0.07	56	11	0.023 – 0.16

2. Synthetic methods

2.1 General synthetic chemistry experimental protocols.

All reagents were purchased from commercial suppliers and used without further purification unless otherwise stated. Anhydrous solvents were obtained by distillation using standard procedures or by using the Anhydrous Engineering Ltd. double alumina and alumina-copper catalyzed drying columns. Reactions requiring anhydrous conditions were run under an atmosphere of dry nitrogen; glassware and needles were flamed-dried prior to use or placed in the oven (150 °C) for at least 2 h and allowed to cool in a desiccator. Thin layer chromatography was performed using aluminum-backed 60 F254 silica plates. Visualization was achieved by UV fluorescence or a basic $KMnO_4$ solution and heat. Flash column chromatography was performed on silica gel (Aldrich 40-63 μm, 230-400 mesh).

Infrared spectra were recorded using a Perkin Elmer Spectrum One FT-IR Spectrometer as solids or neat films in the range of 600-4000 cm^{-1}. NMR spectra were recorded using either a Varian 400 MHz or 500 MHz spectrometer. Chemical shifts are quoted in parts per million, coupling constants are given in Hz to the nearest 0.5 Hz. ^{1}H and ^{13}C NMR spectra are referenced to the appropriate residual peak. DEPT135, COSY, HSQC and HMBC were used in assigning NMR spectra. Melting points were determined using Reichert melting point apparatus. Mass spectra were determined by the University of Bristol mass spectroscopy service by electrospray ionization (ESI^{+}) using a Bruker Daltonics micrOTOF II spectrometer.

2.2 Structural assignment and numbering system.

Structural assignments follow from earlier work[2] to develop access to C(10) cytisine derivatives and only compounds not previously described are reported here.

The numbering system described below has been used in the Supplemental Information for NMR assignment and discussion of C(10)-monosubstituted and C(9),C(10)-disubstituted cytisine derivatives. For simplicity, the cytisine derivatives have been named using the scaffold as shown here.

[2]. Campello, H. R.; Del Villar, S. G.; Honraedt, A.; Viñas, T. M.; Oliveira, A. S. F.; Ranaghan, K. E.; Shoemark, D. K.; Bermudez, I.; Gotti, C.; Sessions, R. B.; Mulholland, A. J.; Wonnacott, S.; Gallagher, T. *Chem,* **2018**, *4*, 1710-1725.

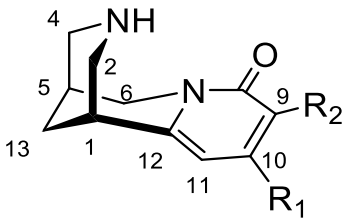
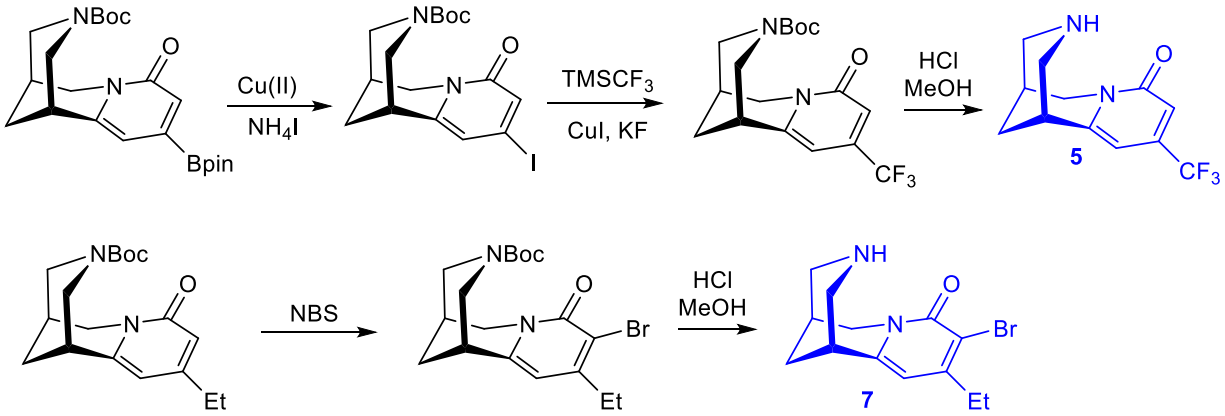


Figure S6. The numbering system for cytisine used here. [3]

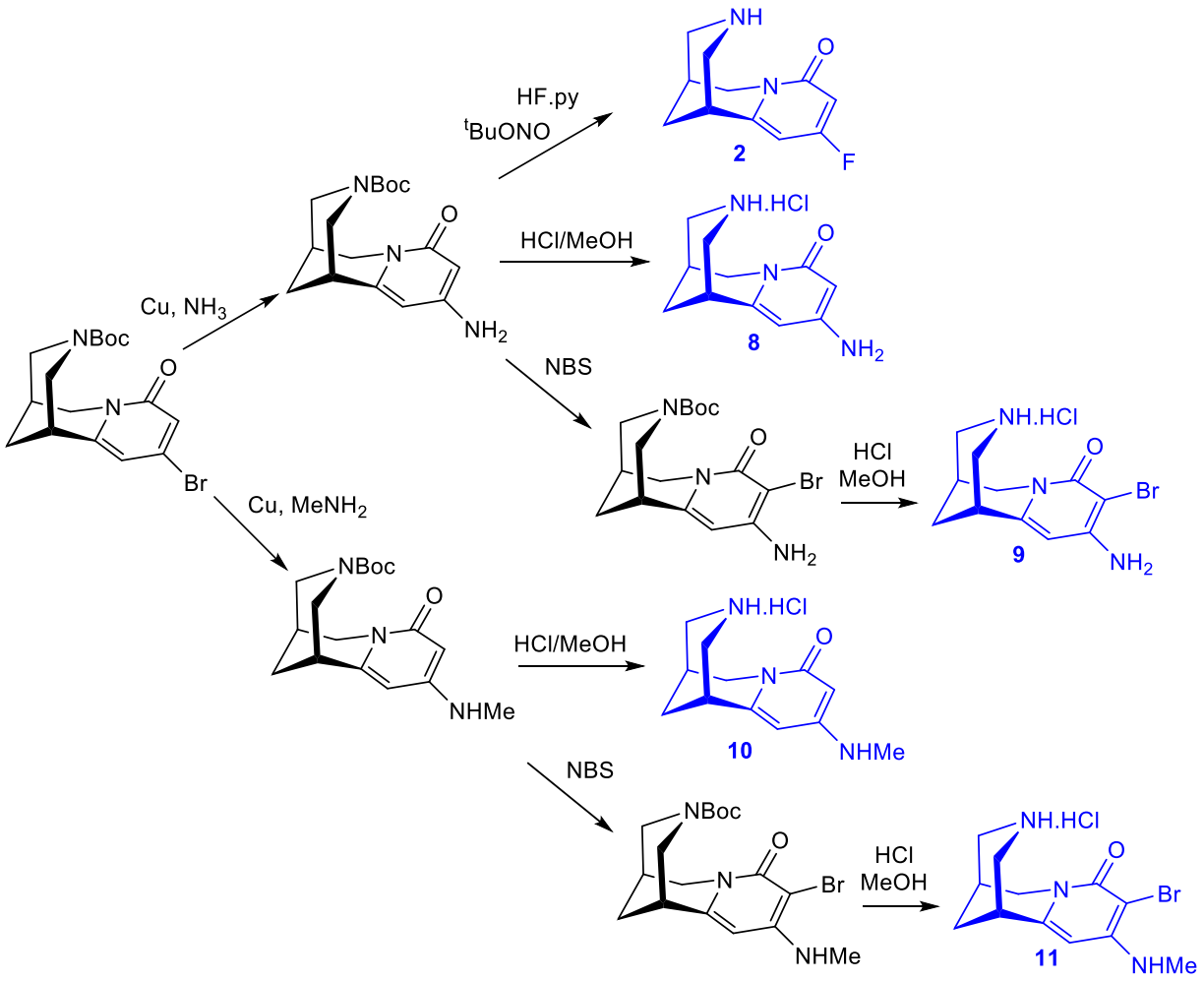
2.3 Summary and General procedures.

C(10)-functionalization of *N*-Boc cytisine (prepared from enantiomerically pure (-)-cytisine) via iridium-catalysed C–H activation/borylation has been described previously.[1] This chemistry was used previously to prepare 10-bromocytisine and C(10) ligands **3**, **4**, **6, 12**, **13** and **14**. In this paper, the C-H activation of N-Boc cytisine has been applied to generate new C(10) and C(9)/C(10) ligands using either N-Boc 10-bromocytisine[1] or N-Boc 10-iodocytisine (described here) as key intermediates. Synthetic details (summarized in schemes 1 and 2), including key intermediates and compound characterization data, for novel C(10) ligands **2**, **5**, **8** and **10** and the 9-bromo-10-substituted variants **7**, **9** and **11** are provided here.



Scheme S1. Transformations to provide ligands 5 and 7.

[3]. Rouden, J.; Lasne, M. C.; Blanchet, J.; Baudoux, J. *Chem. Rev.* **2014**, *114*, 712-778.



Scheme S2. Transformations from N-Boc 10-bromocytisine to provide derivatives 2, 8, 9, 10 and 11.

(i) General procedure A: C(9) Bromination of C(10)-substituted cytisine derivatives.

To a solution of the corresponding N-Boc C(10)-substituted cytisine derivative (specifically N-Boc precursors of **6**, **8** and **10**; 1 equiv.) in THF (0.05 M) was added N-bromosuccinimide (1 equiv.) and the reaction mixture was stirred at room temperature for 18 h. Water (10 volumes) was added, and the aqueous phase was extracted with EtOAc (3 x 10 volumes). The combined organic phases were dried over Na_2SO_4, filtered and concentrated. Purification of the crude reaction mixture by flash column chromatography afforded the corresponding N-Boc 9-bromo-10-substituted cytisine derivatives which were deprotected (see below) to provide **7**, **9** and **11**.

(ii) General procedure B: *N*-Boc deprotection and conversion to HCl salt.

The intermediate *N*-Boc C(10)-substituted (or C(9)-bromo-C(10)-disubstituted) cytisine derivative was dissolved in 0.5 M HCl in methanol (concentration of the substrate approx. 0.1

M) and the reaction mixture was stirred at room temperature for 72 h. The solvent was removed *in vacuo*, the residue was dissolved in the minimum amount of methanol, and acetone was added slowly to give approx. 1:10 v/v of methanol/acetone. After 2 h, the precipitated HCl salt was collected by filtration, washed with cold acetone and dried. This provided final products of good purity and the salts were not purified further.

3 Synthesis of C(10)-substituted cytisine derivatives.

3.1 (+)-10-Aminocytisine.HCl 8.

(i) N-Boc 10-aminocytisine.

A resealable tube was charged with *N*-Boc 10-bromocytisine[1] (1.85 g, 5 mmol), copper powder (32 mg, 10 mol%) and ammonium hydroxide (15 mL, conc. aq. sol.) was added. The tube was sealed the mixture was stirred at 100 °C (CAUTION) for 24 h, after which the solution was cooled to room temperature and extracted with DCM (5 x 50 mL). The combined extracts were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification of the crude reaction mixture by flash column chromatography [DCM/MeOH/NH_4OH: (89:10:1)] afforded the N-Boc 10-aminocytisine (1.30 g, 85%) as an off-white solid. During this procedure, some cleavage of the N-Boc residue (to give 10-aminocytisine **8** directly) was also observed by TLC.

R_f: 0.28 [DCM/MeOH (10% MeOH)]; **mp:** >200 °C (toluene); **FTIR (neat)** 3414, 3302, 3211, 2905, 1679, 1642, 1551 cm^{-1}; **^{1}H NMR (400 MHz, MeOD, δ)** 5.83 (s, 1H, C9-H), 5.51 (s, 1H, C11-H), 4.29-4.02 (m, 3H, C6-Ha, C2-H, C4-H), 3.68 (dd, 1H, *J* = 6.5, 14.5 Hz, C6-Hb), 3.34-2.93 (m, 3H, C1-H, C2-H, C4-H), 2.36 (s, 1H, C5-H), 1.98 (m, 2H, C13-H), 1.34-1.24 (m, 9H, Boc); **^{13}C NMR (100 MHz, MeOD, δ)** 165.2 (CO), 157.7 (CO Boc), 154.7 (C12), 149.0/148.8 (C10, rotamers), 99.6/99.3 (C9, rotamers), 91.9 (C11), 80.1/79.6 (Boc, rotamers), 51.4/50.2/50.1 (C2, C4, rotamers), 49.0 (C6), 34.9 (C1), 27.9/27.7 (C5, rotamers), 27.0 (Boc), 25.7 (C13); **HRMS (ESI$^+$) m/z** $[M+H]^+$ Calcd for $C_{16}H_{24}N_3O_3$ 306.1812; Found 306.1809.

(ii) 10-Aminocytisine.HCl **8**.

N-Boc 10-aminocytisine (1.0 mmol) was deprotected using the General Procedure B afforded (+)-*10-aminocytisine.HCl* **8** (273 mg, 98%) as an off-white solid.

mp: >200 °C; **$[\alpha]_D^{25}$** = +77 [c 1.0, MeOH]; **FTIR (neat)** 2929, 2790, 1649, 1533 cm^{-1}; **^{1}H NMR (400 MHz, D_2O, δ)** 6.26 (s, 1H, C11-H), 4.09 (d, 1H, *J* = 15.0 Hz, C6-Ha), 3.95 (dd, 1H, *J* = 6.5, 15.0 Hz, C6-Hb), 3.41-3.26 (m, 5H, C1-H, C2-H, C4-H), 2.68 (s, 1H, C5-H), 2.07-1.94 (m, 2H, C13-H); Note: C9-H was not observed in D_2O (see comment relating to ligand **10** below).

^{13}C NMR (100 MHz, D_2O, δ) 161.4 (CO), 159.1 (C10), 148.1 (C12),104.5 (C9), 91.5 (C11), 49.2 (C2 or C4), 48.8 (C6), 48.1 (C2 or C4), 31.2 (C1), 24.5 (C5), 22.5 (C13); **HRMS (ESI$^+$) m/z** $[M+H]^+$ Calcd for $C_{11}H_{16}N_3O$ 206.1288; Found 206.1292.

3.2 (–)-10-Fluorocytisine 2.

To a solution of *N*-Boc 10-aminocytisine (305 mg, 1.0 mmol) in HF-pyridine complex (70%, 2.0 mL) at -20 °C was slowly added ^{t}BuONO (0.18 mL, 1.5 mmol) over 1 min. The mixture was stirred at -20 °C for 30 min, warmed to room temperature and stirred for 2 h and finally heated at 60 °C overnight. The reaction mixture was cooled to 0 °C and taken to pH 10 with conc. aqueous NH_3. The mixture was diluted with EtOAC (10 mL), filtered to remove insoluble material, the aqueous phase was extracted with EtOAc (4 x 10 mL) and the combined organic layers were concentrated *in vacuo*. The crude reaction mixture was partitioned between 3M HCl (5 mL) and DCM (5 mL). The aqueous layer was washed with DCM (2 x 5 mL), basified with concentrated NH_4OH to pH 10 and extracted with DCM (5 x 5 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification of the crude by flash column chromatography [DCM/MeOH/NH_4OH (89:10:1)] gave (–)-10-fluorocytisine **2** (140 mg, 67%) as a pale-yellow solid. Note that N-Boc cleavage occurred under these reaction conditions and a pure sample of the free base was obtained by recrystallization in toluene.

R_f: 0.25 [DCM/MeOH (20% MeOH)]; **mp**: 143-145 °C (toluene); **$[\alpha]_D^{25}$** = -92 [c 1.0, EtOH]; **FTIR (neat)** 3391, 3289, 1644, 1552 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$, δ)** 6.06 (dd, 1H, *J* = 2.5, 11.0 Hz, C9-H), 5.87 (dd, 1H, *J* = 2.5, 7.0 Hz, C11-H), 4.04 (d, 1H, *J* = 15.5 Hz, C6-Ha), 3.83 (dd, 1H, *J* = 6.5, 15.5 Hz, C6-Hb), 3.08-2.95 (m, 4H, C2-H, C4-H), 2.89-2.86 (m, 1H, C1-H), 2.34-2.29 (m, 1H, C5-H), 1.93 (m, 2H, C13-H), 1.56 (br s, 1H, NH); **^{13}C NMR (100 MHz, $CDCl_3$, δ)** 169.9 (d, *J* = 264 Hz, C10), 164.8 (d, J = 19 Hz, CO), 153.5 (d, *J* = 13.5 Hz, C12), 99.6 (d, *J* = 16.5 Hz, C9), 96.5 (d, *J* = 26 Hz, C11), 53.7/52.9 (C2, C4), 49.8 (C6), 36.0 (d, *J* = 2 Hz, C1), 27.5 (C5), 26.2 (C13); **^{19}F NMR (376 MHz, $CDCl_3$, δ)** -99.6 (m); **HRMS (ESI$^+$) m/z** $[M+H]^+$ Calcd for $C_{11}H_{14}FN_2O$ 209.1090; Found 209.1095. **Anal. Calcd** for $C_{11}H_{13}FN_2O$: C, 63.45; H, 6.29; N, 13.45. Found: C, 63.06; H, 6.33; N, 13.20.

The corresponding HCl salt was prepared by dissolution of the free base in methanolic HCl and evaporation under vacuum for biological study.

3.3 (–)-10-Trifluoromethylcytisine.HCl 5.

(i) N-Boc 10-iodocytisine

N-Boc 10-(Bpin)cytisine was prepared on a 5 mmol scale as previously described.[1]

In a Schlenk flask, $Cu(NO_3)_2 \cdot 3H_2O$ (2.42 g, 10.0 mmol), NH_4I (1.45 g, 10.0 mmol) and 4 Å molecular sieves (500 mg) were added to the crude borylation mixture (see above). The Schlenk flask was placed under nitrogen and backfilled with oxygen for three times. The reaction mixture was dissolved in DMF (25 mL) and heated at 80 °C for 24 h under an oxygen atmosphere (balloon). The solvent was removed *in vacuo* and the residue was dissolved in DCM. The mixture was poured over ammonia (30 mL, 15% aq. sol.), the layers separated, and the aqueous phase was extracted with DCM (3 x 25 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. Purification of the crude reaction mixture by flash column chromatography [DCM/MeOH (2% MeOH)] gave N-Boc 10-iodocytsine (1.99 g, 95%) as a pale-yellow solid.

R_f: 0.21 [DCM:MeOH (3% MeOH)] ; **mp:** 139 - 140 °C, needles (toluene); **FTIR (neat)** 2920, 1679, 1632, 1523 cm^{-1}; **^{1}H NMR (500 MHz, $CDCl_3$, δ,** acquired at 52 °C to mitigate peak broadening) 6.90 (d, *J* = 2.0 Hz, 1H, C9-H), 6.37 (d, *J* = 2.0 Hz, 1H, C11-H), 4.39-4.04 (m, 3H, C2-H, C4-H, C6-H), 3.72 (dd, *J* = 16.0, 7.0 Hz, 1H, C6-H), 3.09-2.93 (m, 2H, C2-H, C4-H), 2.89 (s, 1H, C1-H), 2.39 (s, 1H, C5-H), 1.99-1.87 (m, 2H, C13-H), 1.26 (s, 9H, Boc); **^{13}C NMR (125 MHz, $CDCl_3$, δ, 52 °C)** 161.6 (CO), 154.3 (Boc), 148.8 (C12), 126.3 (C9), 114.3 (C11), 108.2 (C10), 80.1 (Boc), 50.4/49.3 (C2, C4), 48.8 (C6), 34.5 (C1), 28.1 (Boc), 27.5 (C5), 26.1 (C13) ; **HRMS (ESI^+) m/z** $[M+H]^+$ Calcd for $C_{16}H_{22}IN_2O_3$ 417.0670; Found 417.0672; **m/z** $[M+Na]^+$ Calcd for $C_{16}H_{21}IN_2NaO_3$ 439.0489; Found 439.0491.

(ii) N-Boc 10-trifluoromethylcytisine

A Schlenk flask was charged with *N*-Boc 10-iodocytisine (2.08 g, 5.0 mmol), copper iodide (4.52 g, 23.7 mmol), anhydrous potassium fluoride (1.38 g, 23.7 mmol) and trimethyl(trifluoromethyl)silane (3.5 mL, 23.7 mmol) in DMF (24 mL). The reaction mixture was placed under nitrogen and stirred at 50 °C for 16 h. After this time, the solvent was removed *in vacuo* and the residue distributed between DCM (20 mL) and ammonia (20 mL, 15% aq. sol.). The aqueous phase was extracted with DCM (3 x 20 mL) and the combined organic phases were dried over Na_2SO_4, filtered and the solvent was removed *in vacuo.* Purification of the crude reaction mixture by flash column chromatography [EtOAc/*n*-Hexane (4:1)] yielded N-Boc-10-trifluoromethylcytisine (1.52 g, 85%) as a colorless solid.

mp: 150-151 °C (toluene); **FTIR (neat)** 2981, 1680, 1664, 1547 cm^{-1}; **^{1}H NMR (500 MHz, $CDCl_3$, δ)** 6.73 (s, 1H, C9-H), 6.22 (s, 1H, C11-H), 4.48-4.15 (m, 3H, C2-H, C4-H, C6-H), 3.85 (dd, 1H, *J* = 16.0, 6.5 Hz, C6-H), 3.22-2.95 (m, 3H, C2-H, C4-H, C5-H), 2.49 (s, 1H, C1-H),

2.02 (m, 2H, C13-H), 1.43-1.15 (m, 9H, Boc); **^{13}C NMR (125 MHz, $CDCl_3$, δ)** 162.2 (CO), 154.5/154.0 (CO, rotamers), 151.4/151.0 (C12, rotamers), 141.0-139.5 (C10, poorly resolved due to amide resonance), 122.4 (q, *J* = 270 Hz, $\underline{C}F_3$), 114.2 (C9), 100.6/99.9 (C11, rotamers), 80.6/80.0 (Boc, rotamers), 51.5/50.5/50.1/49.2 (C2, C4, rotamers), 49.3 (C6), 35.3 (C1), 27.9 (Boc), 27.4 (C5), 25.9 (C13); **^{19}F NMR (470 MHz, $CDCl_3$, δ)** -66.5 (d, *J* = 102 Hz); **HRMS (ESI$^+$) m/z** $[M+H]^+$ Calcd for $C_{17}H_{22}F_3N_2O_3$ 359.1577; Found 359.1584, **m/z** $[M+Na]^+$ Calcd for $C_{17}H_{21}F_3N_2NaO_3$ 381.1396; Found: 381.1406; **Anal. Calcd** for C, 56.98; H, 5.91; N, 7.82. Found: C, 56.65; H, 5.55; N, 8.10.

(iii) (-)-10-Trifluoromethylcytisine.HCl **5**.

N-Boc 10-trifluoromethylcytisine (100 mg, 0.28 mmol) was deprotected using the General Procedure B afforded (-)-10-trifluoromethylcytisine.HCl **5** (55 mg, 77%) as a colorless solid.

mp: >200 °C; colorless powder; **$[\alpha]_D^{26}$** = -66 [c 0.5, MeOH]; **FTIR (neat):** 1658, 1551, 1278, 1166, 857 cm^{-1}; **^{1}H NMR (500 MHz, D_2O, δ)** 6.82 (s, 1H, C9-H), 6.70 (s, 1H, C11-H), 4.11 (d, 1H, *J* = 15.5 Hz, C6-H), 3.97 (d, 1H, *J* = 15.5, 6.5 Hz, C6-H), 3.53-3.30 (m, 5H, C2-H, C4-H, C1-H), 2.78 (s, 1H, C5-H), 2.05 (m, 2H, C13-H); **^{13}C NMR (125 MHz, D_2O, δ)** 164.3 (CO), 149.3 (C12), 141.5 (q, *J* = 34.0 Hz, C10), 122.2 (q, *J* = 273.0 Hz, $\underline{C}F_3$), 114.5 (C9), 104.4 (C11), 49.2/48.9 (C2, C4), 48.2 (C6), 31.8 (C1), 24.7 (C5), 22.3 (C13); **^{19}F NMR (376 MHz, D_2O, δ)** -66.2 (s); **HRMS (ESI$^+$) m/z** $[M+H]^+$ Calcd for $C_{12}H_{14}F_3N_2O$ 259.1053; Found: 259.1060.

3.4 (+)-10-(*N*-Methylamine)cytisine.HCl 10.

(i) N-Boc 10-(N-methylamino)cytisine

A resealable tube was charged with *N*-Boc 10-bromocytisine[1] (369 mg, 1.0 mmol) and copper (7 mg, 10 mol%), and $MeNH_2$ (2.0 mL, 40% aq. sol.) was added. The tube was sealed and mixture was stirred at 100 °C (CAUTION) for 24 h. The solution was cooled to room temperature and extracted with DCM (5 x 10 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification of the crude reaction mixture by flash column chromatography (DCM/MeOH (94:6)] gave N-Boc 10-(N-methylamino)cytisine (274 mg, 86%) as an off-white solid.

R_f: 0.58 [DCM/MeOH (10% MeOH)]; **mp:** 198-200 °C (toluene); **FTIR (neat)** 3266, 2928, 1684, 1641, 1571 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$, δ)** 5.49 (s, 1H, C9-H), 5.37 (s, 1H, C11-H), 4.53 (s, 1H, NH), 4.28-4.03 (m, 3H, C6-Ha, C2-H, C4-H), 3.72 (dd, 1H, *J* = 6.5, 15.0 Hz, C6-Hb), 3.02-2.70 (m, 6H, C1-H, C2-H, C4-H, NH$\underline{Me}$), 2.31 (s, 1H, C5-H), 1.93-1.81 (m, 2H, C13-H), 1.32-1.22 (br s, 9H, Boc); **^{13}C NMR (100 MHz, $CDCl_3$, δ)** 164.7 (CO), 156.0 (CO

Boc), 154.8/154.5 (C12, rotamers), 147.9/147.3 (C10, rotamers), 98.0/97.2 (C9, rotamers), 90.4/90.1 (C11, rotamers), 80.2/79.6 (Boc, rotamers), 51.7/50.7/50.4/49.4 (C2, C4, rotamers), 47.9 (C6), 34.8 (C1), 29.4 (NHMe), 28.1 (Boc), 27.6 (C5), 26.4 (C13); **HRMS (ESI$^+$) m/z** $[M+H]^+$ Calcd for $C_{17}H_{26}N_3O_3$ 320.1969; Found 320.1974.

(ii) (+)-10-(N-methylamino)cytisine.HCl **10**.

Following the general procedure B, *N*-Boc 10-(*N*-methylamino)cytisine (0.77 mmol) gave (+)-10-(*N*-methylamino)cytisine.HCl **10** (190 mg, 85%) as a colorless solid.

mp: >200 °C, colorless powder; **$[\alpha]_D^{25}$** = +66 [c 1.0, MeOH]; **FTIR (neat):** 3240, 2939, 2714, 2583, 1644, 1557 cm^{-1}; **^{1}H NMR (400 MHz, D_2O, δ)** 6.25 (s, 1H, C11-H), 4.09 (d, 1H, *J* = 15.0 Hz, C6-Ha,), 3.95 (dd, 1H, *J* = 6.5, 15.0 Hz, C6-Hb), 3.42-3.26 (m, 5H, C1-H, C2-H, C4-H), 2.71 (m, 4H, C5-H, NHMe), 2.05-1.94 (m, 2H, C13-H) Note: as with **8**, C9-H was not observed in D_2O. In D_6 DMSO we observed both C11-H and C9-H: 5.45 (d, 1H, *J* = 3.0 Hz) and 5.03 (d, 1H, *J* = 3.0 Hz) which suggests that C9-H undergoes H/D exchange; **^{13}C NMR (100 MHz, D_2O, δ)** 160.7 (CO), 158.7 (C10), 147.1 (C12),103.6 (C9), 88.4-87.9 (C11), 49.3 (C2 or C4), 48.7 (C6), 48.1 (C2 or C4), 31.3 (C1), 28.5 (NHMe), 24.5 (C5), 22.6 (C13); **HRMS (ESI$^+$) m/z** $[M+H]^+$ Calcd for $C_{12}H_{18}N_3O$ 220.1444; Found 220.1441.

4. Synthesis of 9-bromo-10-substituted cytisine derivatives.

4.1 9-Bromo-10-ethylcytisine.HCl 7.

(i) N-Boc 9-bromo-10-ethylcytisine .

N-Boc 9-bromo-10-ethylcytisine was synthesised from *N*-Boc 10-ethylcytisine[1] (341 mg, 1.07 mmol) according to the General Procedure A. Purification of the crude reaction mixture by flash column chromatography [DCM:MeOH (1% MeOH)] afforded *N-Boc 9-bromo-10-ethylcytisine* (296 mg, 70%) as a colorless solid.

FTIR (neat): 1676, 1638, 1590, 1425 cm^{-1}; **^{1}H NMR (400 MHz, $CDCl_3$, δ)** 6.00 (s, 1H, C11-H), 4.43-4.03 (m, 3H, C2-H, C4-H, C6-H), 3.85 (dd, *J* = 15.5, 6.5 Hz, 1H, C6-H), 3.18-2.87 (m, 3H, C1-H, C2-H, C4-H), 2.71-2.52 (m, 2H, CH_2), 2.41 (m, 1H, C5-H), 2.01-1.89 (m, 2H, C13-H), 1.44-1.04 (m, 12H, Boc, CH_3); **^{13}C NMR (100 MHz, $CDCl_3$, δ)** 159.3 (CO), 154.7/154.4 (Boc, rotamers), 147.0/146.6 (C12, rotamers), 112.9 (C9), 106.6/105.8 (C11, rotamers), 80.6/80.4 (Boc, rotamers), 51.6/ 50.6/50.4/49.3 (C2, C4, rotamers), 50.0 (C6), 34.6 (C1), 29.9

($\underline{C}H_2Me$), 28.6 (Boc), 27.4 (C5), 26.1 (C13), 12.7 ($CH_2\underline{C}H_3$), C10 was not observed; **HRMS (ESI$^+$) m/z** $[M+H]^+$ for $C_{18}H_{26}{}^{79}BrN_2O_3$ 397.1121; Found: 397.1121.

(ii) 9-Bromo-10-ethylcytisine.HCl **7**.

N-Boc 9-bromo-10-ethylcytisine (296 mg, 0.75 mmol) was deprotected (General Procedure B) affording 9-bromo-10-ethylcytsine.HCl **7** (221 mg, quant) as a colorless solid.
mp: > 200 °C; **FTIR (neat:** 2935, 1635, 1572, 1450 cm^{-1}; **^{1}H NMR (500 MHz, MeOD, δ)** 6.52 (s, 1H, C11-H), 4.44 (d, *J* = 15.5 Hz, 1H, C6-H), 4.01 (dd, *J* = 15.5, 6.5 Hz, 1H, C6-H), 3.62 (d, *J* = 13.5 Hz, 1H, C4-H), 3.52-3.44 (m, 4H, 2 x C2-H, C4-H, C1-H), 2.81 (s, 1H, C5-H), 2.71 (m, 2H, CH_2), 2.23 (d, *J* = 13.5 Hz, 1H, C13-H), 2.13 (d, *J* = 13.5 Hz, 1H, C13-H), 1.25 (t, *J* = 7.5 Hz, 3H, CH_3); **^{13}C NMR (125 MHz, MeOD, δ)** 159.9 (CO), 156.0 (C10), 144.9 (C12), 112.9 (C9), 108.8 (C11), 49.6 (C6), 49.3/48.4 (C2, C4), 31.6 (C1), 29.4 ($\underline{C}H_2Me$), 25.4 (C5), 22.9 (C13), 11.5 ($CH_2\underline{C}H_3$); **HRMS (ESI$^+$) m/z** $[M+H]^+$ Calcd for $C_{13}H_{18}{}^{79}BrN_2O$ 297.0597; Found: 297.0591.

4.2 9-Bromo-10-aminocytisine.HCl 9.

(i) *N*-Boc 9-bromo-10-aminocytisine.

N-Boc 9-bromo-10-aminocytisine was synthesized from *N*-Boc 10-aminocytisine (250 mg, 0.82 mmol) according to the General Procedure A. Purification of the crude product by flash column chromatography [DCM:MeOH, (6% MeOH)] afforded *N*-Boc 9-bromo-10-aminocytisine (216 mg, 68%) as a colorless solid.

mp: > 200 °C (*n*-Hex:DCM); **FTIR (neat):** 3441, 1682, 1633, 1591, 1533, 1442 cm^{-1}; **^{1}H NMR (500 MHz, $CDCl_3$, δ)** 5.70 (s, 1H, C11-H), 4.61 (s, 2H, NH_2), 4.43-4.05 (m, 3H, C2-H, C4-H, C6-H), 3.84 (dd, *J* = 13.5, 6.0 Hz, 1H, C6-H), 3.13-2.83 (m, 3H, C2-H, C4-H, C1-H), 2.38 (s, 1H, C5-H), 1.95 (d, *J* = 13.0 Hz, 1H, C13-H), 1.92 (d, *J* = 13.0 Hz, 1H, C13-H), 1.44-1.21 (m, 9H, Boc); **^{13}C NMR (125 MHz, $CDCl_3$, δ)** 159.8 (CO), 154.8 (Boc), 151.5 (C10), 147.0 (C12), 96.9 (C11), 91.4 (C9), 80.5 (Boc), 51.6/50.4 (C2, C4), 49.2 (C6), 34.7 (C1), 28.1 (Boc), 27.4 (C5), 26.4 (C13); **HRMS (ESI$^+$) m/z** $[M+Na]^+$ Calcd for $C_{16}H_{22}{}^{79}BrN_3NaO_3$ 406.0737; Found: 406.0741.

(ii) 9-Bromo-10-aminocytisine.HCl **9**.

N-Boc 9-bromo-10-aminocytisine (260 mg, 0.68 mmol) was deprotected using the General Procedure B affording bromide **9** (189 mg, 98%) as a colorless solid.

mp: > 200 °C; **FTIR (neat):** 3319, 2934, 2756, 1641, 1454 cm^{-1}; **^{1}H NMR (500 MHz, MeOD, δ)** 6.20 (s, 1H, C11-H), 4.41 (d, *J* = 15.5 Hz, 1H, C6-H), 3.95 (dd, *J* = 15.5, 6.5 Hz, 1H, C6-H), 3.59 (d, *J* = 13.0 Hz, 1H, C2-H), 3.52-3.41 (m, 3H, C2-H, C4-H), 3.35 (s, 1H, C1), 2.75 (s, 1H, C5), 2.19 (d, *J* = 13.0 Hz, 1H, C13), 2.10 (d, *J* = 13.0 Hz, 1H, C13); **^{13}C NMR (125 MHz, MeOD, δ)** 159.9 (CO), 154.7 (C10), 144.9 (C12), 100.2 (C11), 89.3 (C9), 49.2/48.3 (C2, C4), 48.9 (C6), 31.7 (C1), 25.4 (C5), 23.2 (C13); **HRMS (ESI$^+$) m/z** $[M+H]^+$ Calcd for $C_{11}H_{15}{}^{79}BrN_3O$ 284.0393; Found: 284.0390.

4.3 (+)-9-Bromo-10-(methylamino)cytisine.HCl 11.

(i) N-Boc 9-bromo-10-(methylamino)cytisine

N-Boc 9-bromo-10-(methylamino)cytisine was synthesised from *N*-Boc 10-(methylamine)cytisine (96 mg, 0.30 mmol) according to the General Procedure A. Purification of the crude reaction mixture by flash column chromatography on silica gel [DCM/MeOH (2% MeOH)] gave *N*-Boc 9-bromo-10-(methylamino)cytisine (84 mg, 70%), which was used in the next step without further purification.

FTIR (neat): 3290, 1633, 1493, 1206, 1100 cm^{-1}; **^{1}H NMR (500 MHz, $CDCl_3$, δ)** 5.68 (s, 1H, C11-H), 4.84 (s, 1H, NH), 4.36-4.05 (m, 3H, C2-H, C4-H, C6-H), 3.79 (dd, *J* = 6.5, 15.5 Hz, 1H, C6-H), 3.13-2.85 (m, 6H, C2-H, C4-H, C1-H, NHMe), 2.33 (s, 1H, C5-H), 1.91 (m, 2H, C13-H), 1.36-1.13 (m, 9H, Boc); **^{13}C NMR (125 MHz, $CDCl_3$, δ)** 159.2 (CO), 154.7/154.2 (C12, rotamers), 152.4 (CO Boc), 148.0/147.7 (C10, rotamers), 93.0/92.5 (C11), 90.4 (C9), 80.3/79.7 (Boc, rotamers), 51.6/50.6/50.2/49.3 (C2, C4 rotamers), 49.1 (C6), 35.0 (Me), 29.8 (C1), 28.6 (Boc), 27.4 (C5), 26.3 (C13).

(ii) *9-Bromo-10-(methylamino)cytisine.HCl* **11.**

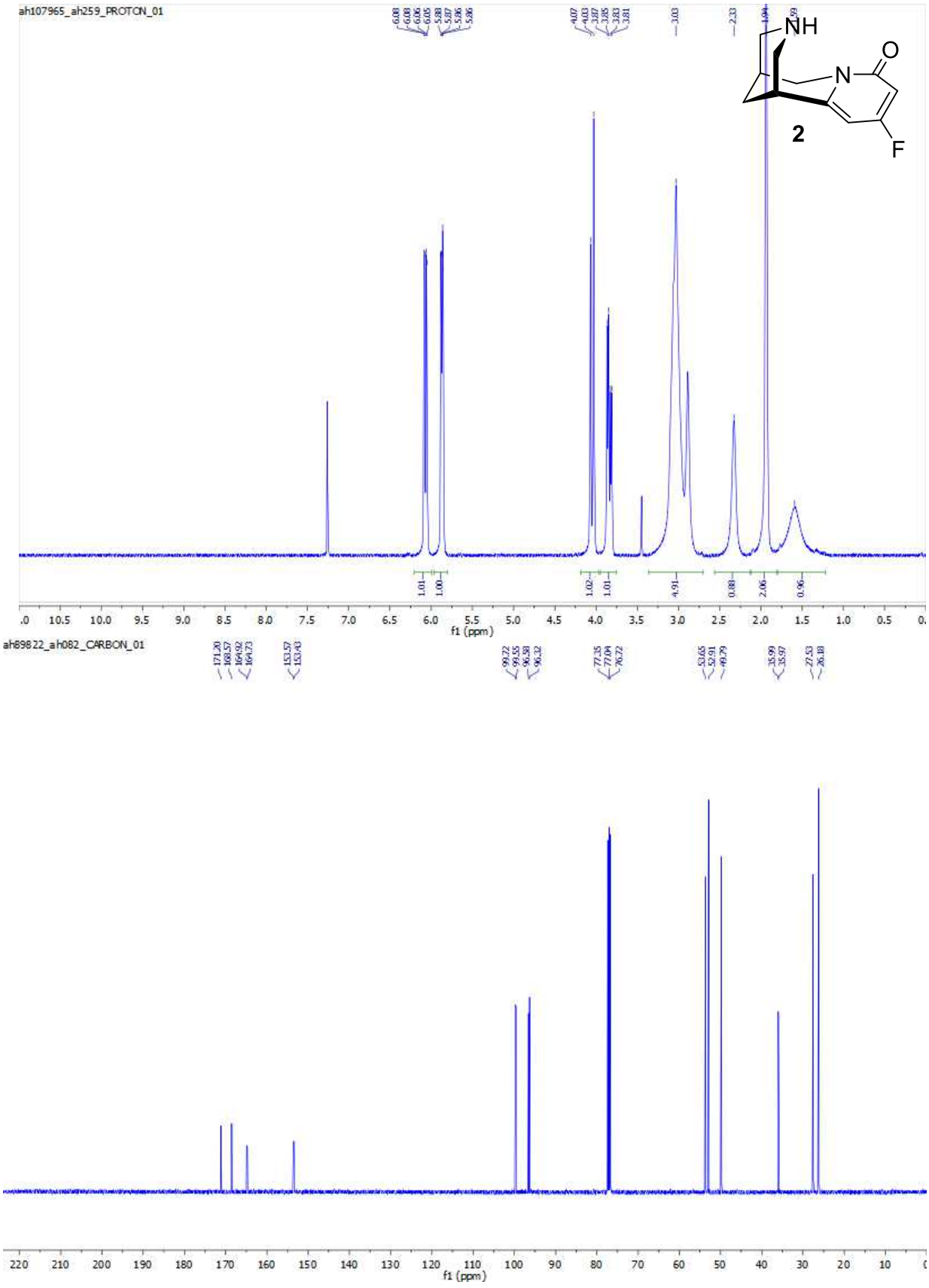
N-Boc 9-bromo-10-(methylamino)cytisine (67 mg, 0.21 mmol) was deprotected according to the General Procedure B affording 9-bromo-10-(methylamine)cytisine.HCl **11** (45 mg, 73%) as a colorless solid. Small amounts (<10%) of 10-(methylamino)-11-bromo-cytisine were observable but this component could not be separated cleanly or fully characterized.

$[\alpha]^D_{23}$ = +38 [c 0.5, MeOH]; **FTIR (neat):** 3291, 2949, 2761, 2624, 1634, 1583 cm^{-1}; **^{1}H NMR (500 MHz, D_2O, δ)** 6.14 (s, 1H, C11-H), 4.13 (d, *J* = 15.5 Hz, 1H, C6-H), 3.88 (dd, *J* = 6.5, 15.5 Hz, 1H, C6-H), 3.52-3.32 (m, 5H, C1-H, C2-H, C4-H), 2.84 (s, 3H, NHMe), 2.70 (s, 1H, C5-H), 2.04 (m, 2H, C13-H); **^{13}C NMR (125 MHz, D_2O, δ)** 160.5 (CO), 154.3 (C12), 145.6

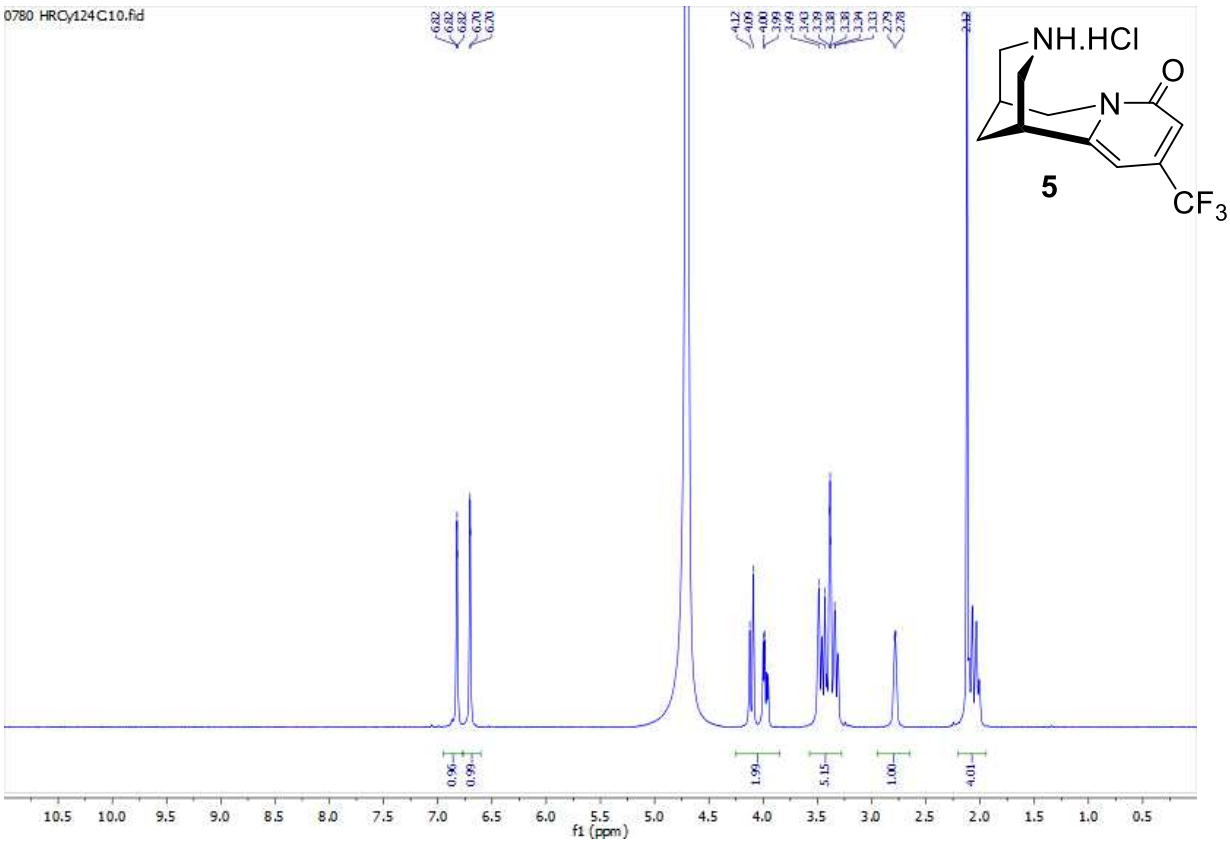
(C10), 97.0 (C9), 89.6 (C11), 49.3/48.5 (C2, C4), 48.9 (C6), 31.6 (C1), 28.9 (NHMe), 24.9 (C5), 22.9 (C13); **HRMS (ESI$^+$) m/z** $[M+H]^+$ Calcd for $C_{12}H_{17}{}^{79}BrN_3O$ 298.0549; Found: 298.0548.

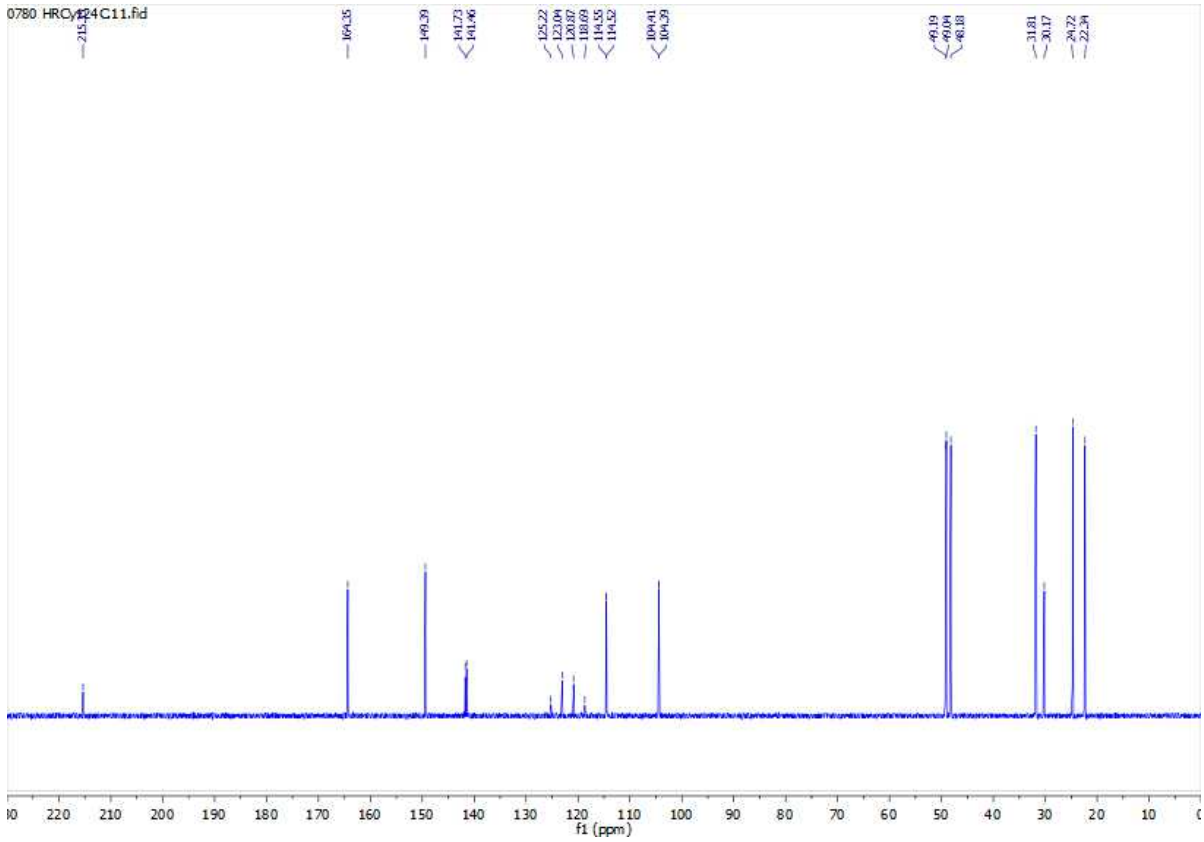
5. ^{1}H and ^{13}C NMR spectra.

(–)-10-Fluorocytisine **2:**

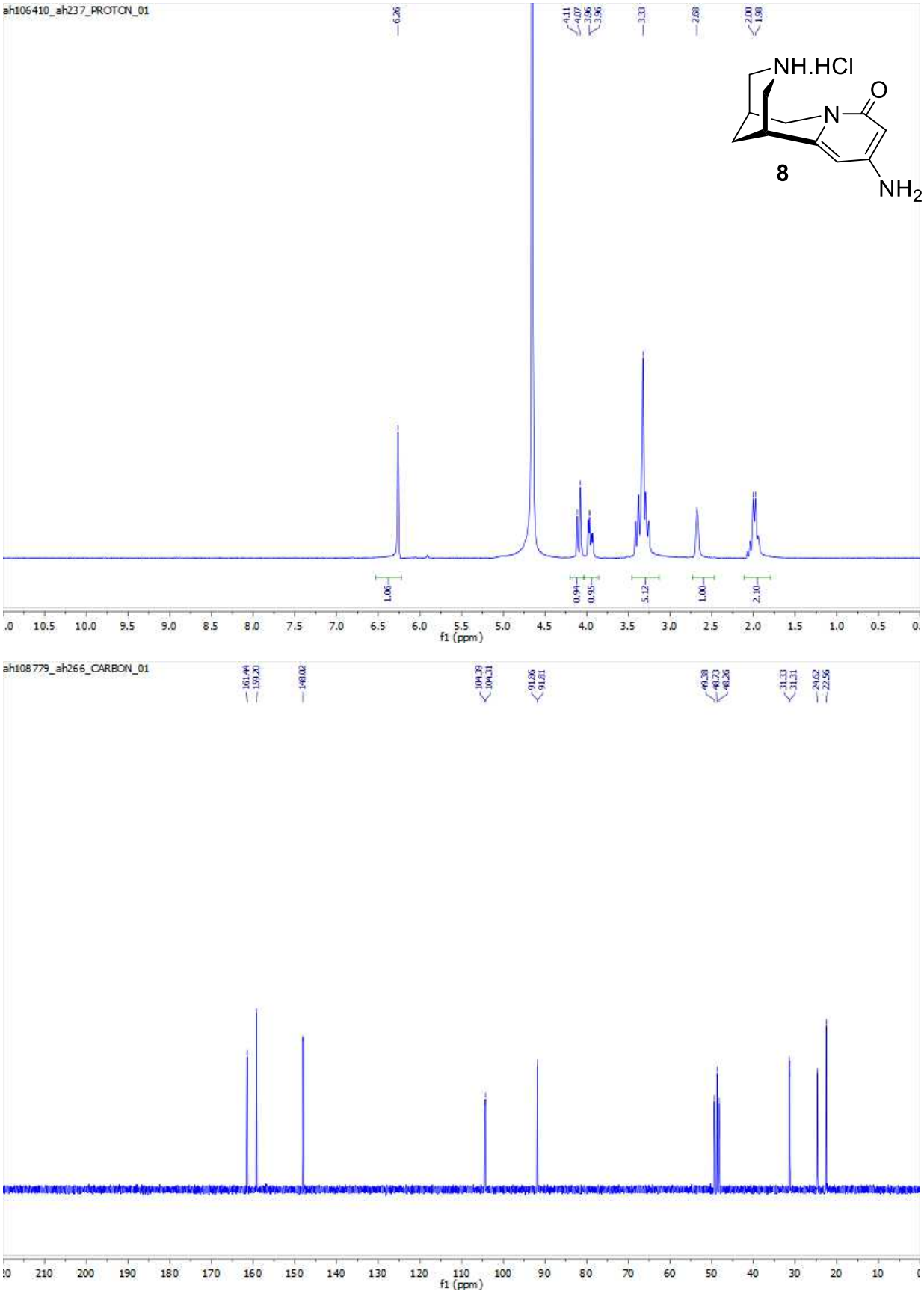


(–)-10-Trifluoromethylcytisine.HCl **5:**

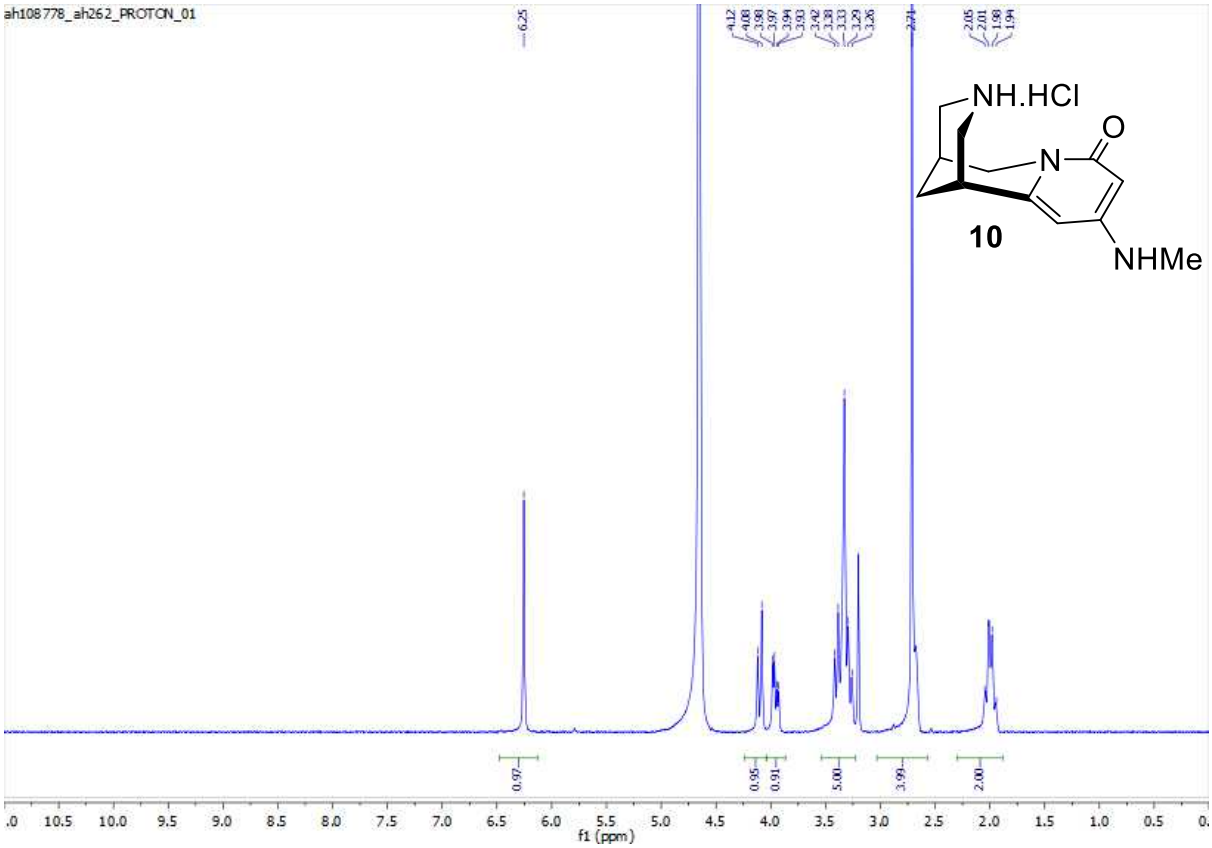


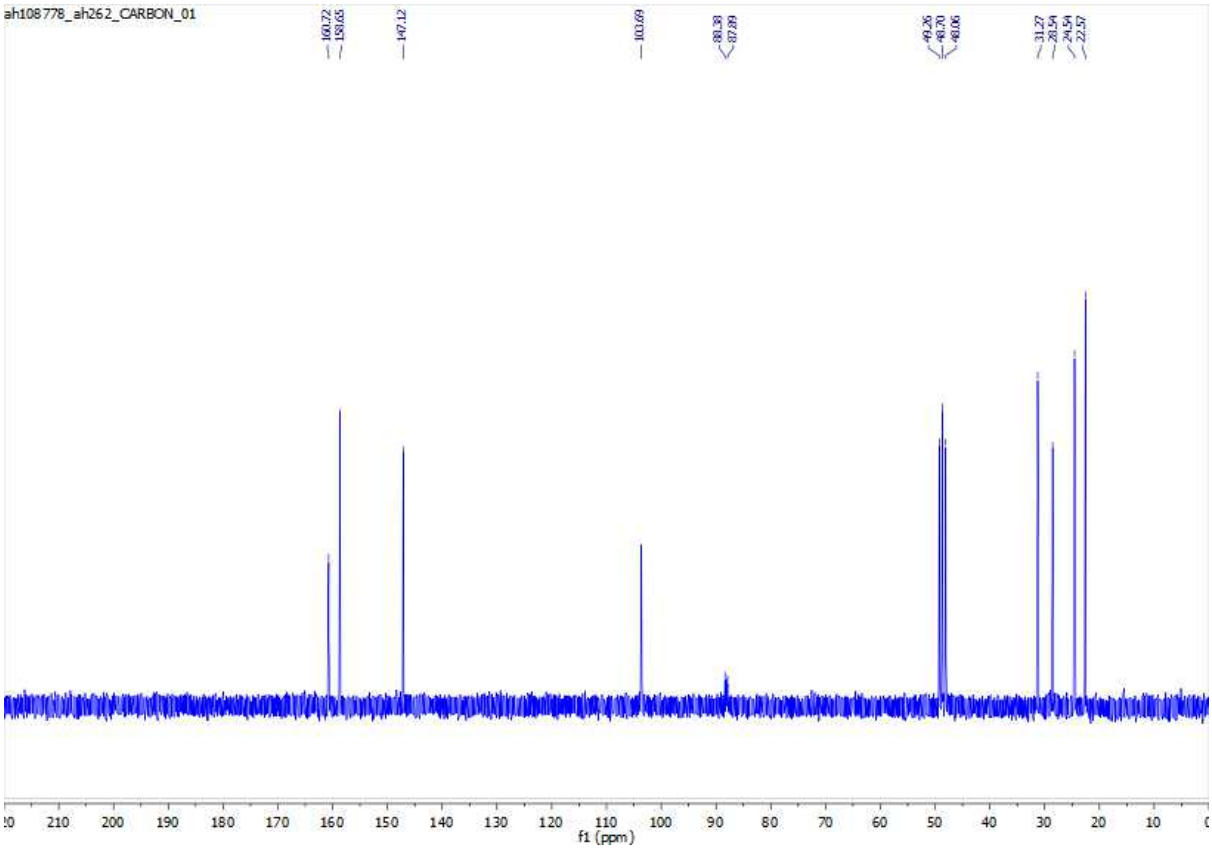


(+)-10-Aminocytisine.HCl **8:**

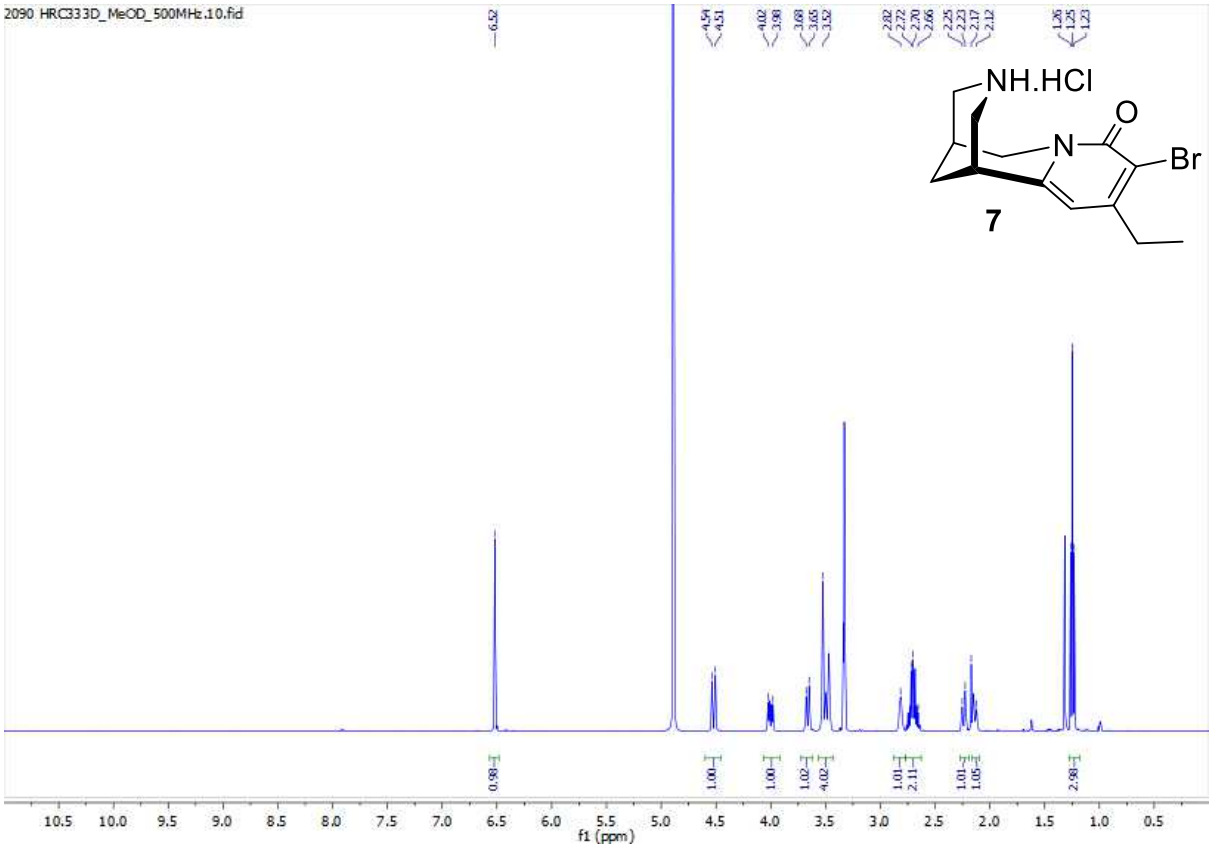


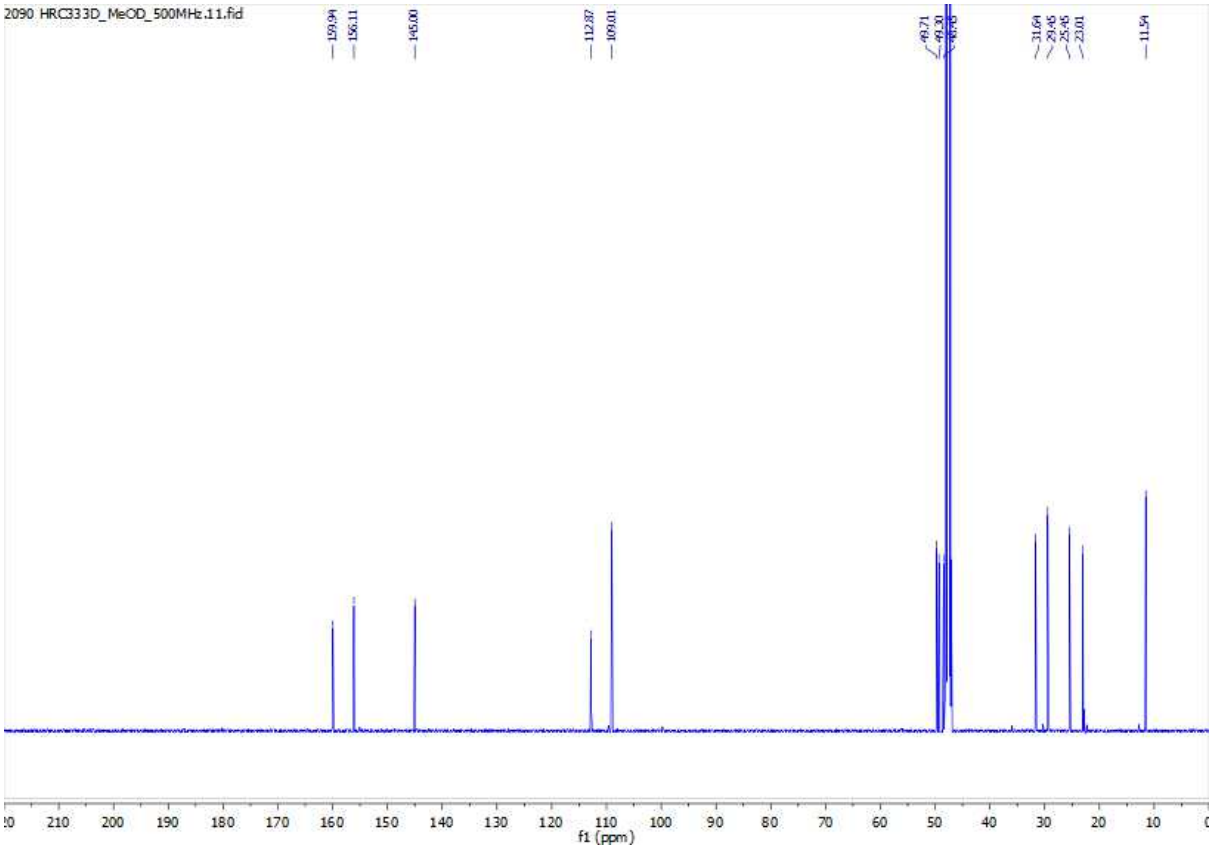
(+)-10-(*N*-Methylamine)cytisine.HCl **10:**



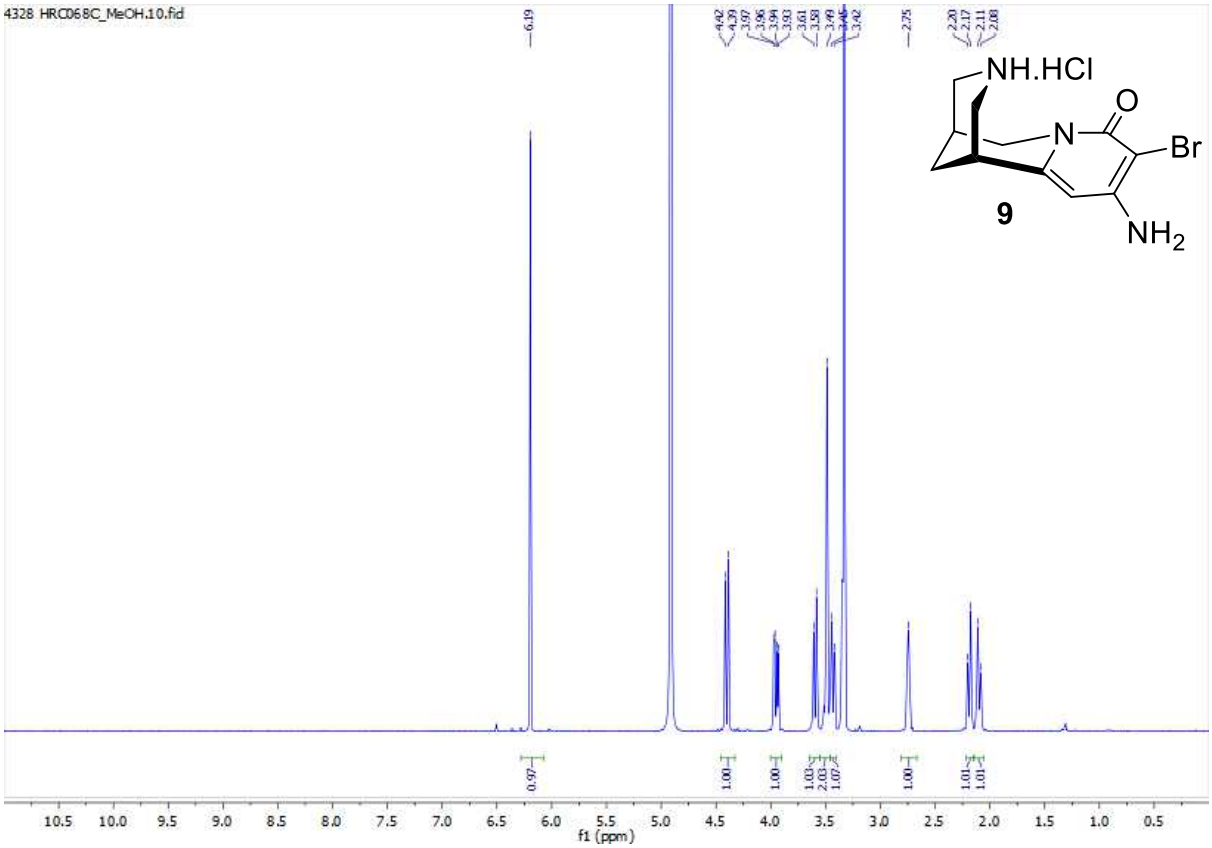


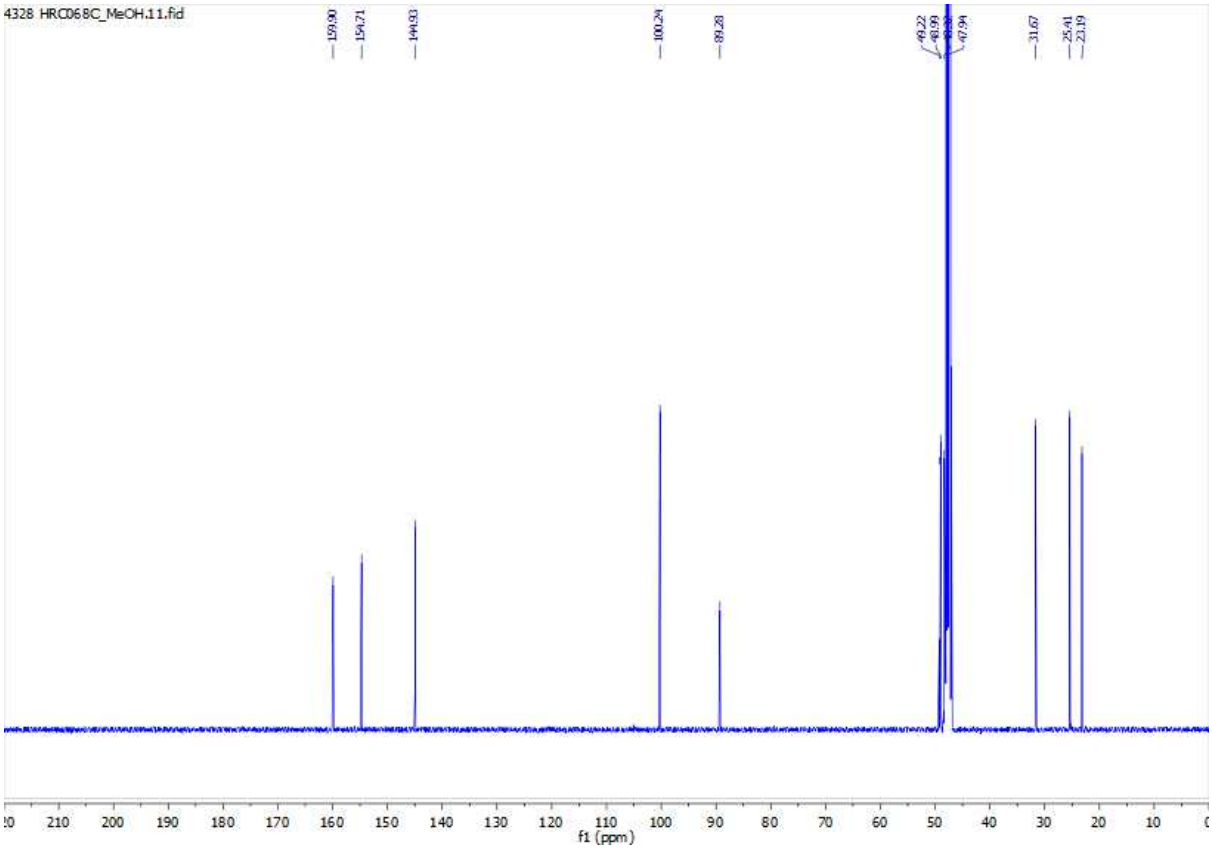
9-Bromo-10-ethylcytisine.HCl **7:**





9-Bromo-10-aminocytisine.HCl **9:**





9-Bromo-10-(methylamino)cytisine.HCl **11:**