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Identification of 9-fluoro substituted (−)-cytisine derivatives as ligands with high affinity for nicotinic receptors

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ABSTRACT

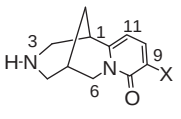
(−)-9-Fluorocytisine, (−)-9-methylcytisine and (−)-9-trifluoromethylcytisine were synthesized from the natural product (−)-cytisine. 9-Methyl and 9-trifluoromethyl cytisines display a remarkable affinity at the $\alpha_4\beta_2$ nicotinic receptor subtype (0.2 nM) with a high selectivity versus the α_7 nAChR subtype. Comparison of the affinity values suggests that the size of the substituent at the 9 position of (−)-cytisine seems more important than electronic factors for efficient binding and selectivity at $\alpha_4\beta_2$ nAChRs.

(1*R*,5*S*)-(−)-Cytisine **1** (Fig. 1) is a chiral natural product belonging to the lupin alkaloid family and large amounts are easily extracted from the seeds of *Laburnum anagyroides*. This compound, known for decades,[1] has recently been the lead in intensive studies[2–4] since it was recognized as a partial agonist of nicotinic cholinergic receptors (nAChRs) with a nanomolar affinity[5] and a high selectivity[6] for the $\alpha_4\beta_2$ subtype. This receptor subtype, present in high concentration in the central nervous system (CNS), may be involved in several neurodegenerative disorders including Alzheimer's disease and Parkinson's disease.[7] Therefore considerable work has focused on gaining insights into the exact role of these receptors in these disorders and more generally on their operating mode in the CNS using various molecular probes.[8] (−)-Cytisine, which is used as one of the main reference ligand in the process of identifying new structures of high affinity and selectivity for $\alpha_4\beta_2$ nAChR subtype, represents a valuable molecular platform from which to develop new derivatives with improved biological properties.

Numerous derivatives of (−)-cytisine have been prepared by substituting the 3,[9] 6,[10] 10,[11] 9 and 11[12] positions in order to evaluate their binding properties to nAChRs. These studies highlighted that 9-bromo-,[13] 9-iodo-,[12,14] 9-vinyl[12,11] 10-methyl[11]-cytisines display the highest affinities and selectivities, often higher than cytisine itself, required for the study of the neurotransmission process.

During continuing interest in the search of new nAChRs (radio)-ligands,[4,12b,d,15] we have focused on the 9-position. Since 9-bromocytisine **2** has a 10-fold higher affinity for $\alpha_4\beta_2$ nAChRs that (−)-cytisine itself, the aim of the present work was to compare the influence of the introduction of a fluorine atom or a trifluoromethyl group on the 9-position of cytisine, allowing the direct comparison of the binding affinities at nAChRs with that of their non-fluorinated counterparts, (−)-cytisine **1** and (−)-9-methylcytisine **5**. Indeed, fluorine is a halogen which often confers improved biological profiles compared to the hydrogenated analogue.[16] (−)-9-Fluorocytisine **3** and (−)-9-methylcytisine **5** have been briefly mentioned in a patent[2] but their chemical preparation and biological data have not been described. We herein report the synthesis and a preliminary pharmacological characterization of 9-fluoro-, 9-trifluoromethyl- and 9-methylcytisines for comparison of their affinities at $\alpha_4\beta_2$ and α_7 nAChRs.

One of most common methods to introduce a fluorine atom onto an aromatic ring is the Balz-Schieman reaction, substituting a diazonium group by fluoride (fluorodediazoniation).[17] Despite several drawbacks, this reaction is a regioselective method of fluorine introduction into aromatic and heterocycle systems. Starting



	X
1	H
2	Br
3	F
4	CF_3
5	CH_3

Figure 1. (−)-Cytisine derivatives.

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Table 1
Optimization of the reaction of selectfluor® with *N*-Boc-(−)-cytisine **6**

Entries	Solvent[a]	Temperature (°C)	Equiv	Conv[b] (%)	**7**[c] (%)	**8**[c] (%)
1	Dioxane	20	1	43	6	30
2	Dioxane/EtOH (1:1)	20	1	31	6	25
3	DMF	20	0.8	52	4	32
4	EtOH, 2M	20	1	52	9	29
5	H_2O	20	1	49	9	25
6	MeCN	20	1	41	1	25
7	**MeCN**	**20**	**0.5**	**49**	**9**	**25**
8	**DMF**	**20**	**0.8**	**54**	**9**	**31**
9	Dioxane	80	1	44	6	25
10	DMF	80	1	54	9	31
11	MeCN	80	1	43	6	30

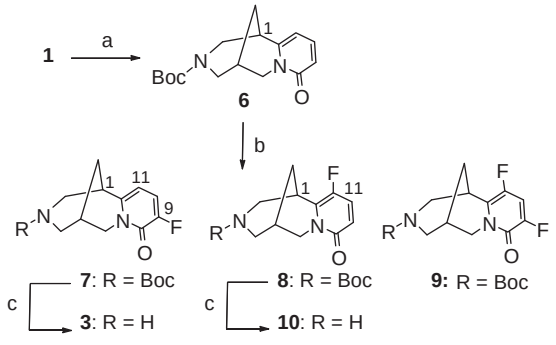
[a] 0.34–0.68 mmol of **6** in 3–5 mL of solvent.
[b] Determined by ^{1}H NMR spectroscopy.
[c] Isolated yields.

from (−)-9-aminocytisine,[4] easily obtained from the direct nitration of (−)-cytisine, all attempts to make the fluorinated derivative via the diazonium salt failed. We then attempted the direct electrophilic fluorination of (−)-cytisine.

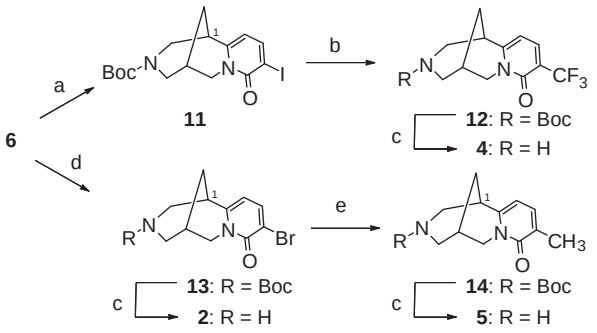
Due to the electron-rich character of the pyridone moiety of **1**, we first chose *N*-fluorobenzenesulfonamide as an electrophilic fluorinating reagent. However, its reaction with *N*-Boc protected cytisine **6**[12b] (THF, 48 h, room temperature) led to a mixture of compounds including 11-fluorocytisine **8**, in addition to a large amount of starting material (>50%) but not to 9-fluorocytisine **7**. *N*-Boc cytisine **6**[12] was then treated with selectfluor® [1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate) (F-TEDA-BF_4)], a safe and soluble salt,[18] under various solvent, temperature, and reagent stoichiometry conditions. The results presented in Table 1 show that in every case the reaction was sluggish. A mixture of fluorinated side products was formed with *N*-Boc protected 9- and 11-fluoro-substituted cytisines, **7** and **8**, respectively, and the difluorocompound **9**[19] identified (Scheme 1).

In order to avoid overfluorination of (−)-cytisine and to limit the presence of side compounds, we performed the reaction with a limited amount of selectfluor®, even though the reaction did not go to completion and large amounts of starting material were recovered. Under these conditions, it was possible to isolate the expected product **7**[20] with a 7–9% yield and its 11-regioisomer **8**[21] (25% yield). Deprotection of the secondary amine, carried out in TFA at room temperature, afforded pure (−)-9-fluorocytisine **3**[22] and (−)-11-fluorocytisine **10**[23] after chromatography.

To synthesize 9-trifluoromethylcytisine **4**, we first prepared the *N*-Boc-9-iodocytisine **11**[2,12b] (Scheme 2). Substitution of the iodide



Scheme 1. Reagents and conditions: (a) Boc_2O (1.2 equiv), Na_2CO_3 (1.2 equiv), THF, rt, 24 h, 96%; (b) selectfluor® (0.5 equiv), MeCN, **7**: 7–9%; **8**: 25%; (c) CF_3CO_2H, CH_2Cl_2, 16 h, rt, **3**: 55%, **10**: 50%.



Scheme 2. Reagents and conditions: (a) I_2, Ag_2SO_4, CH_2Cl_2, rt, 20 h, 51%; (b) CF_3CO_2Na (10 equiv), CuI (10 equiv), DMF, 150 °C, 16 h, 42%; (c) CF_3CO_2H, CH_2Cl_2, 16 h, rt, **2**: 79%, **4**: 60%, **5**: 56%; (d) NBS, CH_2Cl_2, 45 °C, 1.5 h, 57%; (e) Me_4Sn, HMPA, $PdCl_2(PPh_3)_2$, 120 °C, 30 min, 79%.

by a trifluoromethyl group was carried out under conditions described for iodoarenes.[24] Heating *N*-Boc-9-iodocytisine **11** in the presence of sodium trifluoroacetate and copper iodide in a polar solvent (DMF) triggered the substitution via the in situ formation of the reactive trifluoromethylcopper. The expected compound **12**[25] was isolated after 16 h at 150 °C in a 42% yield. Subsequent deprotection to 9-trifluorocytisine **4**[26] was carried out as previously in 60% yield.

The preparation of 9-methylcytisine **5**, incompletely characterized,[2] started by the synthesis of the known *N*-Boc-9-bromocytisine[2] **13** (Scheme 2). A Stille coupling with tetramethyltin in the presence of a palladium catalyst in HMPA afforded *N*-Boc-9-methylcytisine **14**[27] in 79% isolated yield. Deprotection of **14** led to the targeted compound **5**[28] in 56% yield. The known (−)-9-bromocytisine **2** was isolated similarly from **13**.

(−)-Cytisine **1**, (−)-9-methylcytisine **5** and their fluoro analogues **3** and **4** were evaluated for their binding affinities at $\alpha_4\beta_2$ and α_7 nAChRs by measuring the displacement of [^{3}H]cytisine and [^{125}I]-α -bungarotoxin in adult Wistar rat brain membranes, respectively.[29] Affinity of (−)-9-bromocytisine **2** was also determined for comparison of binding measurements. The K_i values for these compounds are summarized in Table 2 and compared to literature values for 9-bromo and 9-iodo cytisines.[12c,13,30]

(−)-9-Trifluoromethyl cytisine **4** exhibits a subnanomolar affinity at $\alpha_4\beta_2$ nAChRs, similar to that of (−)-9-methylcytisine **5** and four times higher than that of (−)-cytisine **1** itself measured under the same conditions. It is worth noting that these compounds have an affinity close to that of (−)-9-bromocytisine **2**, with a better specificity compared to α_7 subtypes (affinity ratio $\alpha_7/\alpha_4\beta_2$; 943, 538, 4545, 8333, respectively for compounds **1**, **2**, **4**, **5**). A drop of affinity (K_i 6.53 nM) for the $\alpha_4\beta_2$ subtype and selectivity $\alpha_4\beta_2/\alpha_7$ was observed for (−)-9-fluorocytisine **3**. These results are in good agreement with the previously observed higher affinity for the less electronegative and bulkier iodide[13,31] in the 9-halogenated

Table 2
Binding affinities of the synthesized compounds

Compound	$\alpha_4\beta_2$ K_i[a,b,30] (nM)		α_7 K_i[a](nM)
(−)-Cytisine **1**	1.06	(0.6)	1000
(−)-9-Bromocytisine **2**	0.116	(0.208)	40.1 (112)
(−)-9-Fluorocytisine **3**	**6.53**		
(−)-9-Iodocytisine		(0.165)	(115)
(−)-9-Trifluoromethylcytisine **4**	**0.22**		**1000**
(−)-9-Methylcytisine **5**	**0.24**		**2000**

[a] Binding affinities (K_i) are expressed as geometric means from 3 to 4 separate experiments.
[b] For comparison, known binding affinities[30] of (−)-cytisine, 3-bromo- and 3-iodocytisines are listed in parentheses.

cytisines. The selectivity operates in the following order: I-Cy ≈ Br-Cy < Cy < CF_3-Cy < CH_3-Cy), the highest value being observed for 9-methylcytisine. Comparison of the 9-halogenated cytisines with 9-methyl and 9-trifluoromethylcytisines suggests that the size of the halogen or of the substituent in the 9-position is more important than the electronic factors for increased binding and selectivity at $\alpha_4\beta_2$ nAChRs.

Finally, the affinities of 9-methylcytisine **5** and 9-trifluoromethyl cytisine **4** at $\alpha_4\beta_2$ nAChR subtype are similar to that of 10-methylcytisine and 10-vinylcytisine.[11a] By analogy with these 10-substituted derivatives, the introduction of a trifluoromethyl[32] or of a methyl group[11a] should also increase the lipophilicity of ligands **4** and **5** compared to cytisine itself. Thus, the absorption and blood–brain barrier penetration, crucial factors for in vivo experiments, should be improved as well. Compounds **4** and **5** could thus be good probes for biological studies of CNS nicotinic receptors. Furthermore, they could be easily radiolabeled with fluorine-18[33] or carbon-11[34] respectively for in vivo experiments using positron emission tomography.

Acknowledgments

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19. Due to the formation of a complex mixture, compound **9** was imperfectly purified. However spectral data of an enriched fraction suggest that **9** was formed. ^{19}F NMR ($CDCl_3$, 235 MHz) mixture of two conformers: δ −131.3 and −131.7; −149.8 and −151.7 ppm; HRMS (EI^+) calcd for $C_{16}H_{20}F_2N_2O_3$· 326.1441, found 326.1451.
20. *(−)-(1R,5S)-N-tert-Butoxycarbonyl-9-fluoro-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1,2-a][1,5]diazocin-8-one* (**7**). *N*-Boc-cytisine[12b] **6** (100 mg, 0.34 mmol), selectfluor® (55–60 mg, 0.17 mmol) were added to degassed and dried solvent (3 mL) under nitrogen. The mixture was stirred at room temperature for 24 h. NH_4OH was added and, after extraction with CH_2Cl_2, the combined organic layers were washed with water then brine, dried (Na_2SO_4), filtered and concentrated. The residue was purified by column chromatography on silica gel (eluents: acetone/heptanes, v/v from 60:40 to 90:10) to afford **7** (and **8**) as a white solid. R_f (acetone/heptanes: 1:1) 0.37; mp 126–127 °C; $[\alpha]_D^{22} = -187.6$ (*c* 0.5, $CHCl_3$); IR (KBr) 2974, 1686, 1572, 1240, 826 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 1.33 (br s, 9H), 1.97 (s, 2H), 2.43 (s, 1H), 3.0 (br s, 3H), 3.87 (dd, *J* = 15.6, 5.9 Hz, 1H), 4.19–4.30 (m, 3H), 5.98 (dd, *J* = 7.0, 4.5 Hz, 1H), 7.08 (dd, *J* = 9.2, 7.7 Hz, 1H) ppm; ^{13}C NMR ($CDCl_3$, 62.9 MHz), mixture of conformers, δ 26.3, 27.2, 28.1, 34.6, 49.5, 50.6 and 50.9, 51.9, 80.6, 102.3, 103.1, 119.9 (br), 149.1, 150.3 ($^1J_{CF}$ = 246 Hz), 154.6, 156.8 ($^2J_{CF}$ = 25.5 Hz) ppm; ^{19}F NMR ($CDCl_3$, 235 MHz) δ −135.6 and −135.8 (conformers) ppm; MS (EI^+) *m*/*z* (relative intensity%) 308 (46), 252 (26), 235 (26), 208 (57), 178 (13), 166 (21), 165 (100), 164 (49), 152 (15), 86 (30), 84 (30), 82 (36); HRMS (EI^+) calcd for $C_{16}H_{21}FN_2O_3$ 308,1535, found 308.1525.
21. *(−)-(1R,5S)-N-tert-Butoxycarbonyl-11-fluoro-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1,2-a][1,5]diazocin-8-one* (**8**). White solid; R_f (acetone/heptanes: 1:1) 0.30; mp 120–121 °C; $[\alpha]_D^{22} = -215$ (*c* 0.5, $CHCl_3$); IR (KBr) 826, 1240, 1572, 1686, 2974 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 1.32–1.38 (m, 9H), 1.97 (br s, 2H), 2.42 (br s, 1H), 3.0 (br s, 2H), 3.4 (br s,1H), 3.81 (dd, *J* = 15.5, 6.3 Hz, 1H), 4.15–4.25 (m, 3H), 6.41 (dd, *J* = 9.7, 5.2 Hz, 1H), 7.27 (dd, *J* = 9.7, 7.5 Hz, 1H) ppm; ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 25.7, 27.2, 27.9, 49.3, 80.4, 116.9, 117.0, 129.7 (br), 134.8 (br), 143.7 ($^1J_{CF}$ = 226 Hz), 154.3 (br), 161.2 ppm; ^{19}F NMR ($CDCl_3$, 235 MHz) δ −152.4 and −152.6 (conformers) ppm; MS (EI^+) *m*/*z* (relative intensity%) 308 (75), 252 (34), 235 (25), 208 (46), 165 (100), 127 (19), 82 (35); HRMS (EI^+) calcd for $C_{16}H_{21}FN_2O_3$ 308,1535, found 308.1525.
22. *(−)-(1R,5S)-9-Fluoro-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1,2-a][1,5] diazocin-8-one* (**3**). White solid; R_f (CH_2Cl_2/MeOH/NH_4OH: 8.7:1.2:0.1) 0.38; mp 156–158 °C; $[\alpha]_D^{22} = -62.2$ (*c* 0.5, $CHCl_3$); IR (KBr) 792, 1240, 1656, 2920 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 1.66 (s, NH), 2.0 (s, 2H), 2.3 (s, 1H), 2.9–3.1(m, 5H), 3.95(dd, *J* = 15.5, 6.5 Hz, 1H), 4.19 (d, *J* = 15.5 Hz, 1H), 7.16 (m, 1H) ppm; ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 26.8, 27.9, 35.6, 50.6, 53.3, 54.4, 102.4, 120.2 ($^2J_{CF}$ = 16.3 Hz), 146.5 ($^3J_{CF}$ = 5.0 Hz), 150.4 ($^1J_{CF}$ = 244 Hz), 157.3 ($^2J_{CF}$ = 24.5 Hz) ppm; ^{19}F NMR ($CDCl_3$, 235 MHz): δ −136.5 ppm; MS (EI^+) *m*/*z* (relative intensity%) 208 (78), 178 (19), 166 (32), 165 (95), 164 (100), 152 (25), 127 (15), 86 (20), 84 (31), 82 (28); HRMS (EI^+) calcd for $C_{11}H_{13}FN_2O$ 208.1011, found 208.1032.
23. *(−)-(1R,5S)-11-Fluoro-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1,2-a][1,5] diazocin-8-one* (**10**). White solid; R_f (CH_2Cl_2/MeOH/NH_4OH: 8.7:1.2:0.1) 0.34; mp 68–70 °C; $[\alpha]_D^{22} = -87.6$ (*c* 0.5, $CHCl_3$); IR (KBr) 816, 1250, 1660, 2946 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 1.66 (s, NH), 1.92–1.99 (m, 2H), 2.34 (s, 1H), 2.97–3.08 (m, 4H), 3.32 (s, 1H), 3.94 (dd, *J* = 6.5, 0.5 Hz, 1H), 4.11 (d, *J* = 15.6 Hz, 1H), 6.43 (dd, *J* = 9.8, 5.2 Hz, 1H), 7.30 (dd, *J* = 9.8, 8.3 Hz, 1H) ppm; ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 25.7, 27.3, 28.4, 29.6, 50.1, 51.5, 52.7, 116.4 (J_{CF} = 6.9 Hz), 129.6 ($^2J_{CF}$ = 23.9 Hz), 136.8 ($^2J_{CF}$ = 29.5 Hz), 143.8 ($^1J_{CF}$ = 224.5 Hz), 161.5 ppm; ^{19}F NMR ($CDCl_3$, 235 MHz) δ −152.4 ppm; MS (EI^+) *m*/*z* (relative intensity%) 208 (79), 178 (19), 166 (32), 165 (94), 164 (100), 152 (19), 127 (16), 86 (20), 84 (32), 82 (33); HRMS (EI^+) calcd for $C_{11}H_{13}FN_2O$ 208,10117, found 208,10166.
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25. *(−)-(1R,5S)-N-tert-Butoxycarbonyl-9-trifluoromethyl-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1,2-a][1,5]diazocin-8-one* (**12**). In a sealed tube, to a degassed solution of *N*-Boc-9-iodo-cytisine **11** (100 mg, 0.24 mmol, 1 equiv) in distilled DMF (5 mL) was added sodium trifluoromethylacetate (328 mg, 0.2.41 mmol, 10 equiv) and CuI (459 mg, 2.41 mmol, 10 equiv). The reaction mixture was heated at 150 °C for 16 h, then concentrated. The residue was dissolved in CH_2Cl_2 and washed with a saturated aqueous $NaHCO_3$ solution. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$), filtered and concentrated under vacuum. The residue was purified by flash chromatography (CH_2Cl_2/MeOH, 98:2) to give the pure product **12** (36 mg, 42%). $[\alpha]_D^{25} = -45$ (*c* 1.0, $CHCl_3$); IR (NaCl) 2932, 1667, 1564, 1425, 1320, 1127, 1129, 750 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 1.25–1.41 (s, 9H), 1.95–2.0 (m, 1H), 2.00–2.05 (m, 2H), 2.44–2.48 (m, 1H), 2.95–3.10 (m, 2H), 3.82 (dd, *J* = 15.7, 6.2 Hz, 1H), 4.2–4.3 (m, 3H), 6.12 (d, *J* = 7.3 Hz, 1H), 7.66 (d, *J* = 7.3 Hz, 1H) ppm; ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 25.9, 27.5, 28.1, 35.3, 49.3, 50.5, 51.2, 80.9, 104.1, 116.8 (q, $^2J_{CF}$ = 28.6 Hz), 123.2 (q, $^1J_{CF}$ = 257.3 Hz), 137.9 (br s), 153.9, 154.5, 159.3 ppm; ^{19}F NMR ($CDCl_3$, 235 MHz) δ −65.77 ppm; HRMS (ESI^+): calcd for $C_{17}H_{22}F_3N_2O_3$ 359.1583, found 359.1573.

26. *(−)-(1R,5S)-9-Trifluoromethyl-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1,2-a][1,5]diazocin-8-one* (**4**). A solution of compound **12** (24 mg, 0.07 mmol, 1 equiv) in CH_2Cl_2 (2 mL) was treated with trifluoroacetic acid (0.05 mL). The reaction mixture was stirred for 16 h. Na_2CO_3 (saturated aqueous solution) was added and the aqueous phase was extracted with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), filtered and concentrated under vacuum. The residue was purified by flash chromatography (CH_2Cl_2/MeOH, 98:2 and 1% NH_4OH) to afford the pure product **4** (10 mg, 56%). $[\alpha]_D^{25} = -34$ (*c* 0.5, $CHCl_3$); IR (KBr) 3331, 2934, 1653, 1562, 1319, 1123, 1091 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.25 (s, 1H), 1.90–1.95 (m, 2H), 2.35–2.40 (m, 1H), 2.90–3.10 (m, 5H), 3.92 (dd, *J* = 15.7, 6.7 Hz, 1H), 4.14 (d, *J* = 15.7 Hz, 1H), 6.05 (d, *J* = 7.3 Hz, 1H), 7.67 (d, *J* = 7.3 Hz, 1H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 26.1, 27.7, 36.1, 50.2, 53.0, 53.8, 103.2, 116.2 (q, $^2J_{CF}$ = 28.6 Hz), 123.4 (q, $^1J_{CF}$ = 285.6 Hz), 137.7, 156.4, 159.5 ppm; ^{19}F NMR ($CDCl_3$, 376 MHz) δ −65.95 ppm; HRMS (ESI^+) calcd for $C_{12}H_{14}F_3N_2O$ 259.1058, found 259.1070.
27. *(−)-(1R,5S)-N-tert-Butoxycarbonyl-9-methyl-cytisine* (**14**).[2] In a sealed tube, to a degassed solution of *N*-Boc-9-bromo-cytisine **13** (100 mg, 0.27 mmol, 1 equiv) in HMPA (2 mL) were added tetramethyltin (0.075 mL, 0.54 mmol, 2 equiv) and $PdCl_2(PPh_3)_2$ (10 mg, 0.01 mmol, 5 mol %). The reaction mixture was heated at 120 °C for 30 min. The reaction was filtered and the residue was washed with EtOAc. The filtrate was washed with a saturated aqueous $NaHCO_3$ solution. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$), filtered and concentrated under vacuum. The residue was purified by flash chromatography (CH_2Cl_2/MeOH, 98:2) to give the pure product **14** as a colorless oil (65 mg, 79%). $[\alpha]_D^{25} = -189$ (*c* 1.0, $CHCl_3$); IR (NaCl) 3463, 2927, 1686, 1643, 1560, 1423, 1238, 1163, 1150, 1129, 916, 802, 766 cm^{-1}; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 17.1, 26.4, 27.7, 28.0, 34.8, 49.1, 50.9, 51.9, 80.3, 104.6, 105.3, 126.0, 135.9, 136.4, 154.6, 163.6 ppm; HRMS (ESI^+) calcd for $C_{17}H_{25}N_2O_3$ 305.1865, found 305.1875.
28. *(−)-(1R,5S)-9-Methyl-cytisine* (**5**). A solution of compound **14** (40 mg, 0.13 mmol, 1 equiv) in CH_2Cl_2 (2 mL) was stirred with trifluoroacetic acid (0.10 mL) for 16 h. Na_2CO_3 (saturated aqueous solution) was added and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), filtered and concentrated under vacuum. The residue was purified by flash chromatography (CH_2Cl_2/MeOH, 98:2 and 1% NH_4OH) to afford the pure compound **5** (15 mg, 56%). $[\alpha]_D^{25} = -64$ (*c* 0.5, $CHCl_3$); IR (KBr) 3392, 2938, 1638, 1555 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.51 (br s, 1H), 1.9–2.0 (m, 2H), 2.13 (s, 3H), 2.25–2.30 (m, 1H), 2.80–2.85 (m, 1H), 2.95–3.05 (m, 4H), 3.89 (dd, *J* = 15.6, 6.6 Hz, 1H), 4.15 (d, *J* = 15.6 Hz, 1H), 5.91 (d, *J* = 6.9 Hz, 1H), 7.16 (d, *J* = 6.9 Hz, 1H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 17.2, 26.6, 27.9, 35.5, 50.0, 53.1, 54.3, 104.5, 125.5, 136.4, 148.0, 163.8 ppm; HRMS (ESI^+) calcd for $C_{12}H_{17}N_2O$ 205.1341, found 205.1305.
29. For labeling of $\alpha_4\beta_2$ receptors, purified rat brain cell membranes (250 μg/ml) were incubated with [^{3}H]-cystine (2 nM) for 2 h at room temperature. Non-specific binding was assessed by the incubation of membrane preparations with 10 μM (−)-(*S*)-nicotine. Marks, M. J.; Stitzel, J. A.; Collins, A. C. *Pharmacol. Exp. Ther.* **1985**, *235*, 619, and Ref. 15. For labeling of α7 receptors, purified rat brain cell membranes (500 μg/ml) were incubated with [^{125}I]alpha-bungarotoxin (2 nM) for 5 h at 37 °C. Non-specific binding was assessed by the incubation of membrane preparations with 1 μM alpha-bungarotoxin. Pabreza, L. A.; Dhawan, S.; Kellar, K. *Mol. Pharmacol.* **1990**, *39*, 9, and Ref. 15.
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31. See Ref. 12c for the affinities of 9-chloro, 9-bromo and 9-iodocytisines at $\alpha_4\beta_2$ nAChRs.
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