Alkaloid Synthesis

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Core Modification of Cytisine: A Modular Synthesis**

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(−)-Cytisine (**1**) has occupied a pivotal role in nicotinic pharmacology as a partial agonist at the α4β2 subtype of neuronal nicotinic acetylcholine receptor (nAChR).[1] More recently because of this partial agonist profile, cytisine provided the discovery lead for varenicline (**2**), Pfizer's smoking cessation agent which was launched in 2006.[2,3]

Recent interest in the chemistry of cytisine (**1**) has been high because of a lack of understanding as to the molecular basis of this underpinning partial agonist profile. In terms of analogues, manipulation of cytisine itself is readily achievable at C3 and C5,[4] C10,[5] and N12[6] but interest in cytisine as a target for total synthesis has also been significant.[7] Synthesis offers opportunities for variation within other regions of the heterocyclic scaffold,[8] and critically, access to more fundamentally, core-modified cytisine congeners.

To date, these have encompassed, for example, norcytisines (involving deletion of C8[9] or C13),[10] however, these specific modifications lead to substantial loss of nicotinic binding affinity. New insights linked to the detail of the ligand binding site can now offer a rationalization of these and related results. Crystallographic data for a number of ligands bound to acetylcholine binding protein (AChBP) have been recently published.[11] These protein complexes closely resemble the extracellular binding domain of the α4β2 nicotinic receptor[12] where part of the ligand binding site is characterized by a highly conserved "aromatic box" comprised of tyrosine and tryptophan residues. Taking this further, Abin-Carriquiry et al.[13] have very recently derived a model for cytisine (and 3- and 5-halocytisines) complexed to the AChBP binding site (Figure 1). This binding model was validated by correlation to experimental binding affinities and shows the bispidine moiety (and associated ammonium) of cytisine to be locked within the conserved "aromatic box"; this allows the lack of activity of norcytisines, where the bispidine scaffold has been modified, to be explained. In contrast, the pyridone component of **1** (and by analogy the quinoxaline ring of varenicline, **2**) is directed into a variable region of the binding site suggesting this as an area of molecular space that provides an opportunity for achieving (and rationalizing) nicotinic subtype selectivity.

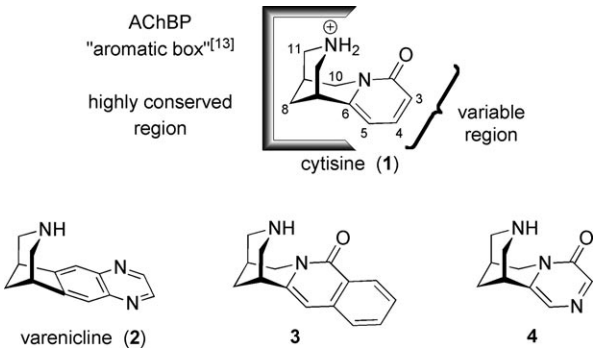

Figure 1. Cytisine (**1**), varenicline (**2**), and new core-modified derivatives **3** and **4**.

Our goal was to develop a modular synthetic approach towards cytisine to address, within the context of this variable region, the "*need to develop an approach to cytisine that delivers a late-stage intermediate that is equipped … for analogue preparation.*"[7] To challenge the strategy, a ring-extended derivative and a representative azacytisine, exemplified by ligands **3** and **4**, respectively, were targeted. In addition, these variants provide potential for new receptor binding interactions, unavailable by established and more conventional derivatizations of **1**, and an opportunity to elucidate those structural features of cytisine that contribute to its selective partial agonist profile.

We have recently reported an efficient and convergent entry to cytisine (as well as unnatural (+)-cytisine and other lupin alkaloids) based on the intramolecular addition of a lactam enolate to a pyridone in order to establish the tricyclic framework by construction of the C6–C7 bond.[14] This early approach, however, failed to secure core variants such as **3** and **4**.[15] As a result, an alternative and ultimately more robust and flexible strategy to cytisine (**1**) has been defined, the advantages of which are exemplified here by the successful synthesis of prototype analogues **3** and **4**.

The synthesis of cytisine (**1**) is shown in Scheme 1 where formation of the C6–C7 bond, based on bromoalkene **6b** as a pivotal intermediate, plays a central role. Bromide **6b** (readily available from lactam **5a**)[16] underwent smooth Stille coupling to the 6-stannylpyridine **7** to provide adduct **8** in 99% yield. Hydrogenation followed by desilylation generated lactams *cis*-**9** and *trans*-**9**. The diastereoselectivity of this transformation was not a concern. Alcohol activation (through mesylation) and thermal cyclization (through N alkylation and O demethylation),[17] with concomitant in situ epimerization, provided tricyclic lactam **10** in 91% overall yield. The latter was readily converted to **1** in two steps: selective lactam reduction and N debenzylation.[14] The key

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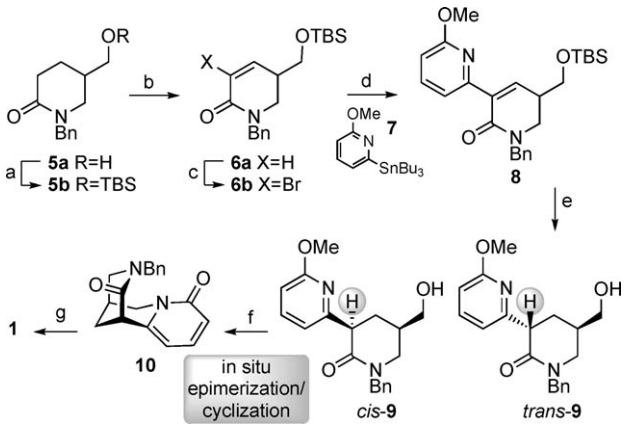
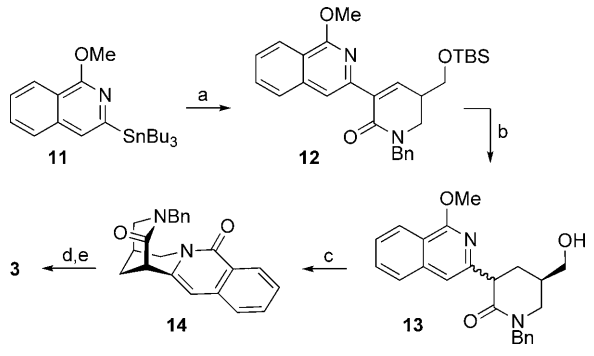


Scheme 1. Synthesis of cytisine (**1**): a) TBSCl, Et_3N, CH_2Cl_2, 37 h, 88%; b) 1. BuLi, PhSeCl, THF, −78°C to 0°C, 2. THF/MeOH/H_2O, $NaIO_4$, 14 h, 50%; c) 1. Br_2, CH_2Cl_2, 45°C, 3 h, 2. Et_3N, CH_2Cl_2, 45°C, 17 h, 63%; d) **7**, $[Pd(PPh_3)_4]$, CuCl, LiCl, THF, 70°C, 15 h, 99%; e) 1. H_2, Pd/C, MeOH, 6 h, 2. TBAF, THF/H_2O, 50°C, 36 h, 85% (*cis*/*trans* 1.5:1.0); f) 1. $MeSO_2Cl$, Et_3N, CH_2Cl_2, 0°C, 20 min, 2. PhMe/DMF (9:1), 110°C, 21 h, then LiHMDS (in THF) was added at RT, 110°C, 18 h, 91%; g) see Ref. [14]. Bn = benzyl, DMF = *N*,*N*′-dimethylformamide, HMDS = hexamethyldisilazide, TBAF = tetra-*n*-butylammonium fluoride, TBS = *tert*-butyldimethylsilyl, THF = tetrahydrofuran.

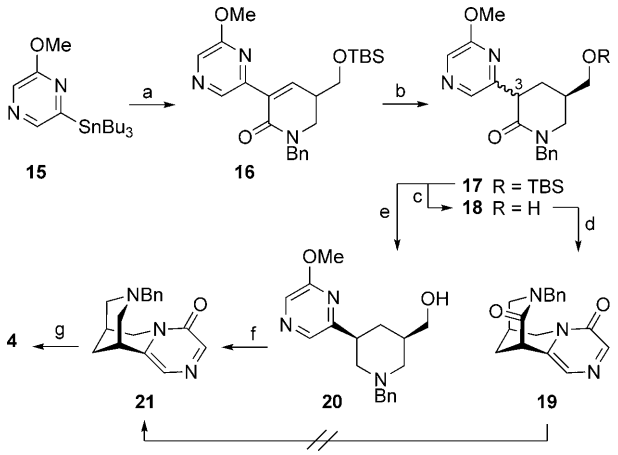
feature exploited here was facile interconversion of the mesylates derived from *cis*- and *trans*-**9** under basic conditions, thereby channelling both isomers through the cyclization step to provide tricycle **10** in high yield.

The ring-extended derivative **3** represents a bridge between cytisine (**1**) and varenicline (**2**), and such a hybrid offers a new vehicle for exploration of the partial agonist behavior associated with cytisine. The synthesis of the ring-extended isoquinolone **3** based on extrapolation of the approach devised for cytisine is shown in Scheme 2. Stille coupling of bromide **6b** with the requisite isoquinoline-based stannane **11** (see the Supporting Information) smoothly provided adduct **12**, which was reduced and simultaneously deprotected to afford alcohols **13** as an inseparable 1.7:1 mixture of *cis* and *trans* diastereomers. This mixture was subjected to mesylation followed immediately by in situ epimerization/cyclization to provide lactam **14** in 59% overall yield. Selective lactam reduction and N debenzylation yielded the target ring-extended isoquinolone **3**, the first example of a cytisine–varenicline hybrid, in 58% yield over two steps.



Scheme 2. Synthesis of isoquinolone **3**: a) **6b**, $[Pd(PPh_3)_4]$, CuCl, LiCl, THF, 70°C, 24 h, 92%; b) H_2, Pd/C, MeOH, 22 h, 100% (*cis*/*trans* 1.7:1.0); c) 1. $MeSO_2Cl$, Et_3N, CH_2Cl_2, 0°C, 20 min, 2. PhMe, 110°C, 15 h, then LiHMDS (in THF) was added at RT, 110°C, 24 h, 59%; d) BH_3, THF, 3 h, 85%; e) $Pd(OH)_2$, MeOH, HCl, H_2, 5 h, 68%.

The third case study reported here describes the first example of an aza-variant of cytisine, which represents a fundamental modification within the pyridone moiety that is only accessible by synthesis. The 4-aza isomer **4** was chosen as the exemplar and the synthesis of this target is shown in Scheme 3. Again, access to azacytisine **4** was also not achievable using our earlier[14] chemistry as the requisite pyrazine precursor (and also the corresponding pyrimidine-based substrate) was not tolerant to (and decomposed under) the conditions needed for lactam enolate formation.[15]



Scheme 3. Synthesis of 4-azacytisine (**4**): a) **6b**, $[Pd(PPh_3)_4]$, CuCl, LiCl, THF, 70°C, 15 h, 86%; b) $LiAlH_4$, THF, 0°C, 15 min, 89% (*cis*/*trans* 2.7:1.0); c) TBAF, THF/H_2O (9:1), 50°C, 36 h, 89% (*cis*/*trans* 1.5:1.0); d) 1. $MeSO_2Cl$, Et_3N, CH_2Cl_2, 0°C to RT, 2. Et_3N, PhMe/DMF (2:1), 110°C, 15 h, 79%; e) $LiAlH_4$, THF, 15 h, then TBAF, THF/H_2O (9:1), 50°C, 16 h, 49%; f) 1. $MeSO_2Cl$, Et_3N, CH_2Cl_2, 0°C to RT, 2. Et_3N, PhMe/DMF (4:1), 110°C, 15 h, 73%; g) 1. 1-chloroethyl chloroformate, $ClCH_2CH_2Cl$, 80°C, 17 h, 2. MeOH, 65°C, 3 h, 61%.

6-Methoxypyrazine tributylstannane **15** was conveniently prepared in two steps from 2,6-dichloropyrazine by consecutive nucleophilic substitution with NaOMe and Bu_3SnLi. Stille coupling of **15** with building block **6b** gave adduct **16** (86%), reduction of which was best achieved using $LiAlH_4$ to give lactam **17** as a 2.7:1 mixture of *cis*/*trans* diastereomers. Desilylation of **17** with commercially available tetrabutylammonium fluoride (TBAF; containing 5% H_2O) in THF led to epimerization at C3 and a 1:1 mixture of diastereomeric alcohols **18**. This was suppressed (but not totally) by addition of water and conducting the reaction in THF/H_2O (9:1); this produced **18** as a 1.5:1 *cis*/*trans* mixture. With the increased acidity of the proton at C3, epimerization/cyclization of the mesylates derived from alcohols **18** was possible under exceptionally mild conditions (with Et_3N) providing tricycle

19 in 79% overall yield. However, selective reduction of the lactam carbonyl in the presence of the pyrazinone moiety (i.e. **19**→**21**) was not successful, despite screening a range of reagents and conditions.[18] This issue was solved by $LiAlH_4$ reduction of lactam **17** (as a 2.7:1 mixture of *cis* and *trans* isomers), that is, prior to unmasking of the pyrazinone. This was, however, relatively inefficient, which we attributed to formation of aluminium complexes on basic workup. Optimal conditions involved $LiAlH_4$ reduction of **17** and direct treatment (with minimal workup) of the crude product with TBAF yielding the diastereomerically pure alcohol **20** in 49% yield (based on *cis*-**17**). Cyclization of **20** provided the *N*-benzyl 4-azacytisine **21** which was best deprotected under dealkylative conditions using 1-chloroethyl chloroformate followed by methanolysis to give the target 4-azacytisine **4** in 61% yield.[19]

In conclusion, we have defined a new, highly modular approach to the synthesis of cytisine, that is significantly more robust and adaptable, and allows access to previously inaccessible core-modified derivatives, exemplified by the cytisine–varencline hybrid **3** and the first example of an azacytisine, that is, pyrazinone **4**. This chemistry features the use of heteroarylstannanes, which are coupled with bromide **6b** (as the critical, common and late-stage intermediate), and unmasked (to release the pyridone moiety). Key to the efficient construction of the tricyclic core scaffold was use of a novel in situ epimerization/cyclization sequence that largely negated a requirement for *cis*/*trans* stereocontrol. Application of this methodology to other cytisine variants together with ligand-based studies exploiting the acetylcholine binding protein (AChBP) are underway.[20]

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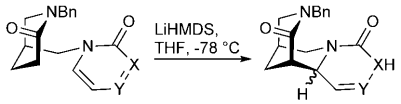

22a	X = CH, Y=CH	**23a**	88 %
22b	X = CH, Y=N	**23b**	0 %
22c	X = N, Y=CH	**23c**	0 %

[16] All compounds described here are racemic. The synthesis of (*R*)-**5a** and nonnatural (+)-cytisine has been reported; see Ref. [14] and also: M. H. Kim, Y. Park, B. S. Jeong, H. G. Park, S. S. Jew, *Org. Lett.* **2010**, *12*, 2826–2829.

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[19] Debenzylation of **21** under H_2 in the presence of Pd/C and HCl/MeOH led to pyrazinone reduction and attempts at oxidative debenzylation with ceric ammonium nitrate (CAN) in MeCN/H_2O caused degradation.
[20] Initial pharmacological evaluation of the ligands reported here has focused on the ring-extended isoquinolone **3**, as the first annulated cytisine and because of its structural relationship to varenicline. Using a whole-brain (heteromeric) assay with 200 pM [^{3}H]epibatidine, racemic isoquinolone **3** was a potent ligand with an IC_{50} of 85 nM (cf. (−)-cytisine 7.7 nM; A. Kennett, S. Wonnacott, C. Hirschhäuser, C. A. Haseler, T. Gallagher, unpublished results). Coe et al. have reported that varenicline is three-fold more potent than cytisine at α4β2 in rat cortex; see Ref. [2a].

