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Deconstructing cytisine: The syntheses of (±)-cyfusine and (±)-cyclopropylcyfusine, fused ring analogs of cytisine

Daniel Yohannes,*,† Kristen Procko,‡ Lorraine A. Lebel, Carol B. Fox and Brian T. O'Neill

Pfizer Global Research and Development, Groton Laboratories, Eastern Point Road, Groton, CT 06340, USA

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Abstract—A novel fused tricyclic analog (**11**) of cytisine has been prepared (coined 'cyfusine') and determined to have high affinity at neuronal nicotinic acetylcholine receptors. A [3 + 2] cycloaddition protocol permitted entry into a 3,4-differentially difunctionalized dihydropyrrole (**7**). The penultimate cyclization was accomplished using the modified Van Tamelen conditions developed in our earlier synthesis of (±)-cytisine. Sequential ring-forming reactions ([3 + 2] cycloaddition/cyclopropanation/pyridone cyclization) gives a unique cyclopropyl analog (**16**) possessing a skeleton isoatomic with that of cytisine.

Cytisine (**1**, Fig. 1), a natural product isolated over a century ago from legumes,[1] binds with high affinity to the $(\alpha4)_2(\beta2)_3{}^*$ neuronal nicotinic acetylcholine receptor (nAChR), the predominant heteromeric nAChR subtype in brain.[2] Cytisine has been shown to be a partial agonist at these receptors,[3] and the natural product had been evaluated in an early study for nicotine dependence without conclusive evidence of effect.[4] Three total syntheses of cytisine were published in the 1950s by Van Tamelen, Bohlmann, and Govindachari,[5] and 50 years passed before our work in this area resulted in two total syntheses.[6] The expanding interest in cytisine has led to additional racemic syntheses of the natural product[7] and the first asymmetric total syntheses of natural (−)-cytisine[8] as well as non-natural (+)-cytisine.[9] To date, some 10 total syntheses and one formal synthesis of cytisine have been reported.[10] Subsequent to our earlier efforts on cytisine came the identification of varenicline (**4**), a structurally related $(\alpha4)_2(\beta2)_3{}^*$ nAChR partial agonist now marketed for smoking cessation.[11]

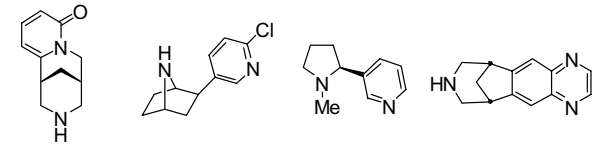

Figure 1. Natural products (**1**–**3**) and varenicline (**4**), ligands that act at neuronal nicotinic acetylcholine receptors.

Cytisine analogs have been prepared as a part of numerous synthetic[12] and drug discovery[13] efforts. However, the dearth of cytisine analogues disclosed that include carbocyclic skeletal modifications[14] (compared to skeletal analogs of epibatidine (**2**) or nicotine (**3**), for instance) has driven our interest in the development of a flexible synthesis of cytisine-type scaffolds.

A key element in any total synthesis of cytisine is the construction of the bridged bicyclic framework, a significant synthetic challenge, and a contributor to the length of some of the syntheses of the natural product. We therefore postulated that if the bridgehead methylene was removed and replaced by a bond between the carbons previously bridged, the resulting fused tricyclic **11** might retain the structural rigidity found in cytisine. In addition, the fused pyrrolidine tricyclic framework of **11** (which we have coined 'cyfusine') does appear to position the key pharmacophoric elements of cyfusine in

Keywords: Cytisine; Cyfusine; Nicotinic acetylcholine receptor; [3 + 2] cycloaddition; Pyridone; Cyclopropanation; (±)-Cytisine; (±)-Cyfusine; nAChR; $(\alpha3)_2(\beta4)_3$; $(\alpha4)_2(\beta2)_3{}^*$.
* Corresponding author. Tel.: +1 336 480 2190; fax: +1 336 480 2113; e-mail: daniel.yohannes@targacept.com
† Present address: Targacept, Inc., 200 East 1st Street, Suite 300, Winston-Salem, NC 27101, USA.
‡ Present address: Department of Chemistry and Biochemistry, The University of Texas, Austin, TX 78712, USA.

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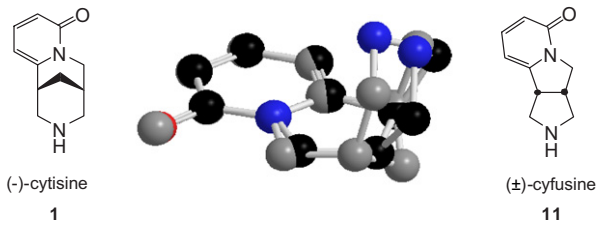


Figure 2. Spatial relationship of cytisine (**1**, gray) and cyfusine (**11**, black) with the pyridone rings of **1** and **11** superimposed.[15]

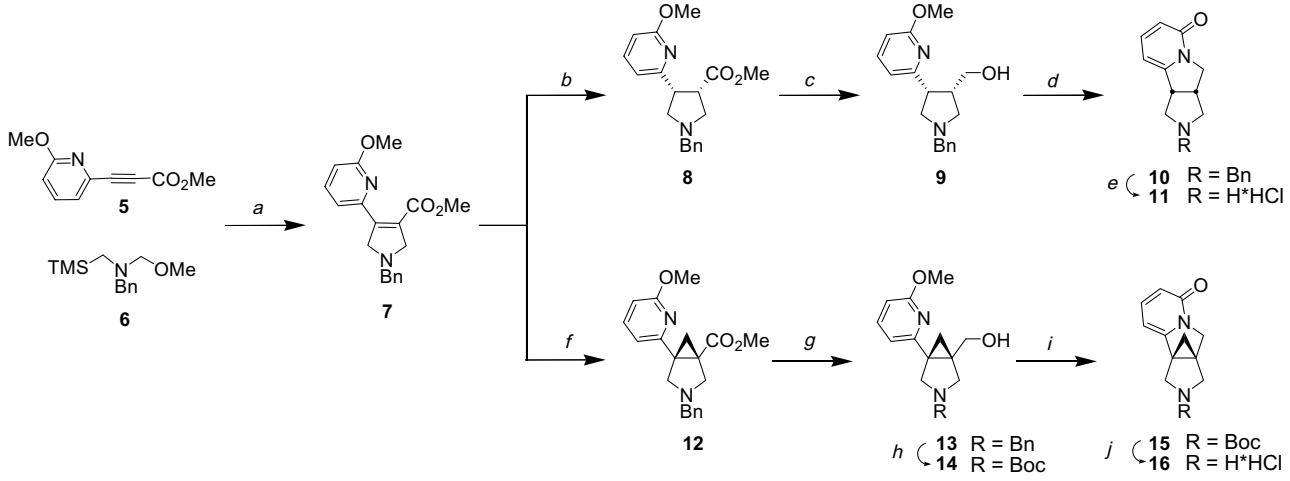
approximately the same spatial arrangement as in cytisine (Fig. 2). Furthermore, approaches to its synthesis hold the potential to be more flexible than current available methods to generate analogs of cytisine. We describe herein a novel synthesis of cyfusine (**11**), which, in principle, permits derivatization of each carbon of its molecular scaffold.

The synthetic approach relies on the potential of alkyne **5**[16] to generate the [3 + 2] adduct **7** when treated with commercially available dipole precursor **6**[17] under ambient conditions (Scheme 1). This adduct (**7**) presents a reactive double bond,[18] which holds promise for the introduction of additional functionality spanning the eventual pyridone and the basic amine moieties. Our exploitation of the ability of the olefin to participate in concerted reaction processes (i.e., hydrogenation, cyclopropanation, etc.) is described below.

Accordingly, the double bond of **7** underwent catalytic hydrogenation ($Pd(OH)_2$, 40 psi H_2) to produce saturated ester **8**. The syn-display of the methoxypyridyl and ester substituents of this fused analog makes it an ideal candidate for elaboration to a tricyclic pyridone utilizing the modified Van Tamelen approach described in our earlier synthesis of (±)-cytisine.[6a] Indeed, reduction of **8** with lithium aluminum hydride (LAH) provided alcohol **9**, which was treated with methanesulfonylchloride and diisopropylethylamine at 0 °C, followed by warming to room temperature to generate **10** in an overall yield of 67%. Debenzylation under conditions which permitted initial characterization of the free amine as its Boc carbamate (H_2, $Pd(OH)_2$, $(Boc)_2O$; 1 N HCl/MeOH) afforded (±)-cyfusine (**11**) as its hydrochloride salt.

Further reactivity of the α,β-unsaturated double bond of **7** was evidenced by its cyclopropanation to afford **12** in 61% yield. After reduction of the ester of **12** (95%), the benzyl group was exchanged for a Boc-group in very high yield (94%), with the anticipation that ultimate deprotection of the *N*-benzyl group while preserving an intact final cyclopropane would prove difficult. Repeating the mesylation–pyridone cyclization sequence on **14** as for **9** generated **15**. Boc-deprotection quantitatively liberated the secondary amine to produce cyclopropylcyfusine (**16**–HCl salt) in 82% yield from alcohol **14**.

To confirm the value of these analogs as ligands at the nicotinic acetylcholine receptor, we evaluated (±)-cyfusine (**11**) and (±)-cyclopropylcyfusine (**16**) in nAChR binding assays along with the natural product for comparison (Table 1). The nAChR subtypes chosen were the $(\alpha4)_2(\beta2)_3{}^*$, $(\alpha3)_2(\beta4)_3$, and α7 receptor subtypes. The α4β2 subtype is thought to be involved in important clinical indications such as addiction, while the α7 subtype is believed to be implicated in the neuroprotective effects of nicotine. The $(\alpha3)_2(\beta4)_3$ subtype represents one of the major ganglionic receptors and may mediate cardiovascular effects of nicotinic agents. Cyfusine (**11**) binds to the $(\alpha4)_2(\beta2)_3{}^*$ nAChR subtype with a K_i of 16 nM and exhibited good selectivity (at least 30-fold) for that subtype over the $(\alpha3)_2(\beta4)_3$ subtype, similar (in direction if not in magnitude) to that of natural (−)-cytisine. Cyclopropylcyfusine (**16**) was found to possess less affinity, with a K_i of 144 nM at $(\alpha4)_2(\beta2)_3{}^*$, and with a separation of at least 3- to 4-fold over the



Scheme 1. Reagents and conditions: TFA (0.1 equiv), CH_2Cl_2, 0 °C to rt, 2 h, 85%; (b) 40 psi $H_2/Pd(OH)_2$, MeOH, 2 h, 41%; (c) $LiAlH_4/Et_2O$ (1.6 equiv), Et_2O, 0 °C to rt, 1.5 h, 67%; (d) MsCl (1.5 equiv), DIPEA (4 equiv), CH_2Cl_2, 0 °C, warmed to rt, 67%; (e) (i) 45 psi $H_2/Pd(OH)_2$, $(Boc)_2O$, MeOH, quantitative; (ii) 1 N HCl, MeOH, rt, 16 h, quantitative; (f) 1 M dimethylsulfoxonium ylide in THF, THF, rt, 1.5 h, 61%; (g) $LiAlH_4/Et_2O$ (1.6 equiv), Et_2O, 0 °C to rt, 1.5 h, 95%; (h) 45 psi $H_2/Pd(OH)_2$, MeOH, $(Boc)_2O$, 94%; (i) MsCl (1.5 equiv), DIPEA (4 equiv), CH_2Cl_2, 0 °C, warmed to rt, 16 h, 82%; (j) 1 N HCl, MeOH/EtOAc/hexanes, rt, 16 h, quantitative.

Table 1. In vitro affinity of **11** and **16** at nAChR subtypes[19]

Compound	K_i (nM)		
	α4β2	α3β4	α7
(−)-Cytisine **1**	0.43	1560	5820
(±)-Cyfusine **11**	16	>500	>500
(±)-**16**	144	>500	>500

$(\alpha3)_2(\beta4)_3{}^*$ subtype. Additionally, both ligands demonstrate clear selectivity for the $(\alpha4)_2(\beta2)_3{}^*$ subtype over the α7 subtype. Thus, cyfusine (**11**) represents a novel scaffold that mimics the affinity and selectivity of cytisine for neuronal nicotinic acetylcholine receptors and as such, may serve as a novel starting point for drug discovery efforts directed at the nicotinic acetylcholine receptor.

In summary, we have completed a short (5-step) synthesis of (±)-cyfusine (**11**), which proceeds through two key steps: a [3 + 2] cycloaddition incorporating a pyridylpropiolate ester, followed by a pyridine → pyridone cyclization.[20] We have also generated an isoatomic analog of cytisine, cyclopropylcyfusine (**16**), by inserting a cyclopropanation step into the previous sequence of ring-forming reactions (cycloaddition → cyclopropanation → pyridine cyclization). The [3 + 2] adduct **7** represents a pluripotent synthon and a key intermediate for the possible preparation of other analogs of cytisine. The hypothesis that cyfusine could serve as a more synthetically tractable cytisine-like nicotinic ligand was supported by the affinity and selectivity of this compound.

In the recent exhaustive review of the synthetic approaches to cytisine, it was noted that 'there is still a need to develop an approach to cytisine that delivers a late-stage intermediate that is equipped with the appropriate functionality for analogue preparation.'[10] The chemistry outlined here potentially addresses this need in that it permits the functionalization of each aliphatic carbon in the skeletal framework of **11**, thereby enabling cyfusine to serve as a structurally equivalent surrogate scaffold for further skeletal interrogation of the SAR of cytisine (Fig. 3).

Our current efforts are directed toward a total synthesis of cytisine utilizing the cyclopropyl intermediate **12** in a ring-expansion operation to generate a piperidine, which later intersects with a known intermediate (**17**) in our previous synthesis of the natural product (Scheme 2). This approach offers promise for an enantiospecific synthesis of cytisine via stereospecific cyclopropyl ring formation. These efforts and our successes at the utilization

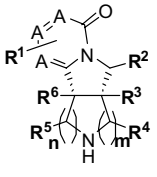
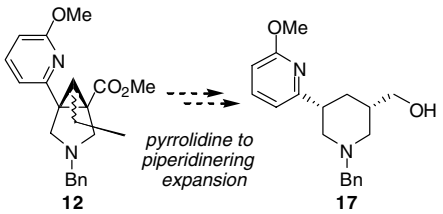


Figure 3. Systematic derivatization of (±)-cyfusine (**11**) enabled with the synthetic method described herein.



Scheme 2. Ongoing ring-expansion efforts.

of cycloadduct **7** to access substitution at all of the aliphatic carbons of (±)-cyfusine (**11**) and of (±)-cytisine (non-bridgehead atoms) will be detailed in subsequent publications.

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18. Compound **7** is oxidatively unstable, converting completely to the pyrrole over several days when exposed to air.
19. The assays were conducted according to the protocols in the supplementary information in Ref. 11.
20. *General procedure for the pyridone cyclizations:* To a 35-mL round-bottom flask was added 6-methoxypyridine substrate (either **9** or **14**). The mixture was cooled to 0 °C and treated with *N*,*N*-diisopropylethylamine (4 equiv), followed by the addition of methansulfonyl chloride (1.5 equiv). The reaction mixture was stirred at 0 °C for 1 h, then allowed to warm to room temperature. The reaction mixture was stirred at room temperature for 16 h then was partitioned between sodium bicarbonate and dichloromethane. The crude reaction mixture was dried with magnesium sulfate, and the solvent was evaporated. Flash chromatography was performed on silica gel using with 2.5–10% methanol/ethyl acetate to yield the pyridone (**10**: 64% yield; 1H NMR ($CDCl_3$, 400 MHz) 7.3 (dd, 1H), 7.25 to 7.17 (m, 5H), 6.35 (d, J = 9.1 Hz, 1H), 6.00 (d, J = 7.1 Hz, 1H), 4.29 (dd, J = 9.1 Hz, J = 13.3 Hz, 1 H), 3.95 (dd, 1H), 3.78 (t, J = 7.9 Hz, 1H), 3.60 (d, J = 12.9 Hz, 1H), 3.48 (d, J = 12.9 Hz, 1H), 3.02 (m, 1H), 3.54 (d, J = 9.1 Hz, 1H), 2.66 (t, 2H), 2.50 (t, 1H) ppm; mass spectrum (APCI) *m/e* 267 p + 1) (**15**: 82% yield; 1H NMR ($CDCl_3$, 400 MHz) 7.51 (t, 1H), 6.35 (t, 1H), 6.39 (d, J = 8.7 Hz, 1H), 4.23(d, J = 6.6 Hz, 2H), 3.90 to 3.82 (m, 2H), 3.65 to 3.53 (m, 2H), 1.49 (d, J = 5.8 Hz, 1H), 1.45 (s, 9H), 1.32 (d, J = 5.8 Hz, 1H) ppm. Mass spectrum (APCI) *m/e* 289 p + 1).