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First Total Synthesis of (±)-3-Hydroxy-11-norcytisine: Structure Confirmation and Biological Characterization

Daniel Yohannes,*,† Camilla P. Hansen,‡ Srinivasa Rao Akireddy,† Terry A. Hauser,† Melanie N. Kiser,† Nicholas J. Gurnon,† Cynthia S. Day,§ Balwinder Bhatti,*,† and William S. Caldwell†

Targacept, Inc., 200 East First Street, Suite 300, Winston-Salem, North Carolina 27101, Faculty of Pharmaceutical Sciences, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen, Denmark, and Chemistry Department, Wake Forest University, Salem Hall, Box 7486, Winston-Salem, North Carolina 27109

daniel.yohannes@targacept.com

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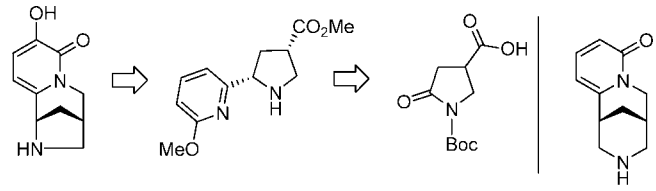
ABSTRACT



1 **2**

The first total synthesis of the natural product 3-hydroxy-11-norcytisine (1), structurally related to cytisine (2), a benchmark ligand at neuronal nicotinic acetylcholine receptors (NNRs), has been achieved. The synthesis permits the unambiguous confirmation of the structure originally proposed for 1 and has enabled initial biological characterization of 1 and its related compounds against NNRs.

Hydroxynorcytisine (**1**), an alkaloid from the legume family (*Laburnum anagyroides*), was isolated in 1989 by Hayman and Gray.[1] It is a naturally occurring skeletal congener of (−)-cytisine (**2**), which is prodigiously found in the Leguminosae family.[2] Despite its obvious structural and potential biosynthetic relationship to (−)-cytisine, hydroxynorcytisine has received little, if any, attention in the literature since the original isolation disclosure.

(−)-Cytisine (**2**) binds with high affinity to the α4β2 neuronal nicotinic acetylcholine receptor, the predominant heteromeric nicotinic acetylcholine receptor subtype in the brain.[3] Given our focus on the discovery of therapeutic agents acting at neuronal nicotinic receptors (NNRs), we were curious as to whether **1** would also display affinity at NNRs and, if so, what its selectivity profile would be across central and peripheral nicotinic acetylcholine receptor subtypes. Herein, we disclose the first total synthesis of hydroxynorcytisine (**1**), its unambiguous structure confirmation, and biological data at NNR subtypes for both **1** and its parent scaffold **3** (Figure 1).

† Targacept, Inc.
‡ University of Copenhagen.
§ Wake Forest University.

(1) Hayman, A. R.; Gray, D. O. *Phytochemistry* **1989**, *28*, 673–5.
(2) (a) Partheil, A. *Arch. Pharm. (Weinheim, Ger.)* **1894**, *232*, 161. (b) Ing, H. R. *J. Chem. Soc.* **1932**, 2778. (c) Leonard, N. J. *The Alkaloids*; Academic: New York, NY, 1953; Vol. III, p 120.
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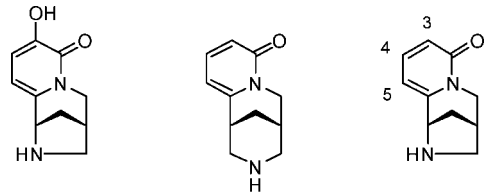
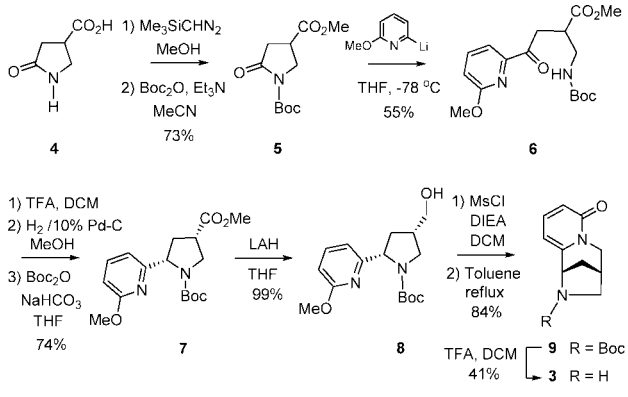


Figure 1. Structures of (±)-3-hydroxy-11-norcytisine (**1**), (−)-cytisine (**2**), and (±)-11-norcytisine (**3**).

Our preferred synthetic approach was to first access intermediate **9** for multiple reasons. Structure−function precedents on the pyridone of cytisine suggested to us that such an intermediate might be one on which we could rapidly elaborate to the hydroxypyridone, as well as other analogues substituted on the pyridone moiety. Additionally, we were interested in the nicotinic potential of **3** (derived from **9**, Scheme 1) as a direct comparator to natural cytisine, whose pyridone ring is similarly placed and unsubstituted. Finally, for the construction of the pyridone ring, we were relying on the precedent of our earlier total synthesis of cytisine which utilized a methoxypyridine → pyridone cyclization, a dependable method for construction of fused and bridged bicyclic pyridones.[4] As such, we were disinclined to complicate that cyclization with substituents on the pyridine ring (such as hydroxyl or an equivalent synthon) which may alter its reactivity toward cyclization.

In the event, we embarked on the synthesis utilizing the commercially available racemic **4**, hoping to position ourselves for a future asymmetric synthesis by using a starting material which possessed a stereogenic center. Diazomethane esterification of the carboxylic acid was followed by activation of the nitrogen with Boc anhydride (73% for the two steps). Addition of the 6-lithio-2-methoxypyridine to the lactam carbonyl was compromised by concomitant addition to the methyl ester, but a 55% yield of **6** was nevertheless obtained. Liberation of the free amine of **6** (excess TFA, then $NaHCO_3$) spontaneously closed the amino-ketone intermediate to the cyclic imine, the rapid hydrogenation (H_2/Pd−C) of which cleanly afforded the pyrrolidine. The free nitrogen of the crude pyrrolidine was immediately protected (Boc_2O, Na_2CO_3) to afford **7** in high overall yield (74% over 3 steps). Reduction of the ester to the primary alcohol (LAH) gave **8** in nearly quantitative yield, providing a compound well suited to participate in the modified Van Tamelen cyclization utilized in our earlier synthesis of cytisine.[4] Indeed, mesylation of the primary alcohol and subsequent heating (toluene reflux) afforded **9** in 84% yield. The intact scaffold of the title compound (sans hydroxyl) was revealed when **9** was deprotected (TFA) to give **3**.

Scheme 1



Cytisine has been extensively derivatized in efforts to delineate its structure−activity relationship.[5] Of relevance to our synthesis was the knowledge that cytisine could be readily nitrated or brominated.[6] Our expectation was that either of these approaches, applied to **9**, could provide the penultimate transformation that would give us access to the natural scaffold of **1**. The introduction of the hydroxyl substituent on intermediate **9**, containing the preformed pyridone ring, is described below (Scheme 2).

Our initial attempts at functionalization next to the pyridone carbonyl involved nitration of **9** (HNO_3, H_2SO_4; $(Boc)_2O$, 22%) followed by reduction to the amine **11** (H_2/Pd−C; 40%). However, preliminary attempts to convert the amine into the hydroxyl were unsuccessful.[7] Ultimately, we were most successful using the bromination of **9** (NBS, CCl_4), which provided the desired 3-substituted isomer (**12**) in 54% yield, along with a substantial amount of the

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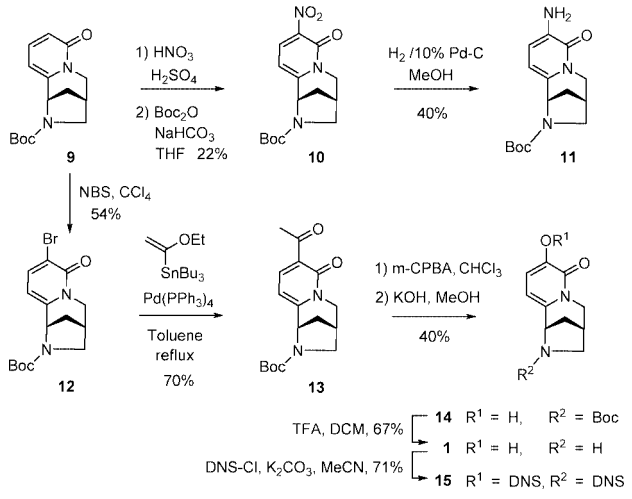
(7) The yields were very low for conversion of **11** to **14** through diazotization followed by copper-mediated displacement of diazonium by water.

undesired 5-substituted bromide (29% yield) and trace amounts of the 3,5-dibromopyridone (2−3% yield). Conversion of bromide **12** to the 3-acyl compound under Stille conditions (tributyl(1-ethoxyvinyl)stannane, cat. Pd(0), toluene, reflux) provided **13** in good isolated yield (70%), containing functionality that could be converted into the hydroxyl. The Bayer−Villager oxidation of ketone **13** (*m*-CPBA, $CHCl_3$) and subsequent saponification (KOH−MeOH) thus afforded **14** containing the hydroxyl functionality in the desired position (40%). Deprotection of the Boc group (TFA, CH_2Cl_2) cleanly gave what we believed to be the natural product (**1**).

Scheme 2



Gray et al. had characterized the natural product as its bisdansyl (DNS) adduct **15**,[1] by NMR. We therefore prepared a sample of **15** from **1** for direct spectral comparison and were pleased to find that our ^{1}H NMR of **15** was identical to that which was reported. As we did not have a sample of the isolated natural product compound with which to draw a direct comparison, we endeavored to generate high-quality crystals for X-ray determination of the structure. Our efforts were rewarded with an X-ray structure confirmation of the natural product characterized as its bisdansylated derivative **15** (Figure 2).[8]

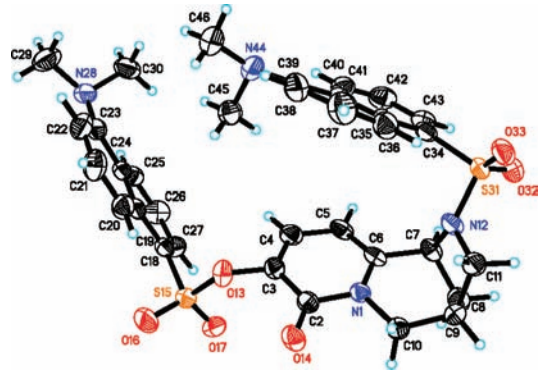


Figure 2. Displacement ellipsoids (50% probability) for the X-ray crystal structure of **15**, the bisdansyl derivative of 3-hydroxy-11-norcytisine (**1**).[9]

To assess the value of hydroxynorcytisine (**1**) as a ligand at the nicotinic acetylcholine receptor, we evaluated the natural product **1** and scaffold **3** in neuronal nicotinic acetylcholine receptor binding assays, along with cytisine (**2**) for comparison (Table 1). The NNR subtypes chosen were the rat α4*β*2 and α7 receptor subtypes. The α4*β*2 subtype is thought to be involved in important clinical indications such as addiction and dementia, while the α7 subtype is believed to be implicated in the neuroprotective effects of nicotine.[10] To our great surprise, the natural product **1** and its parent scaffold **3** displayed more than 4 orders of magnitude *less* affinity than cytisine (**2**) at α4*β*2 NNRs and at least an order of magnitude less affinity at α7 nicotinic receptors.[11] The disparate affinities at α4*β*2 nicotinic receptors of hydroxynorcytisine (**1**) and cytisine (**2**) illustrate the profound effect that minor differences in structure can exert at this receptor. Our current efforts are directed toward the molecular and structure−activity understanding of why such ostensibly minor structural variability between natural products **1** and **2** translates to vastly divergent affinities at the α4*β*2 nicotinic acetylcholine receptor.

To our knowledge, this disclosure represents the first total synthesis of 3-hydroxy-11-norcytisine (**1**). We have additionally provided unambiguous confirmation of the structure originally proposed for the natural product. Finally, we present herein the first characterization of **1** in biological screens, in particular the α4*β*2 nicotinic acetylcholine receptor, at which its well-known congener cytisine (**2**) is considered a benchmark ligand. Additional studies, including

Table 1. Affinities of Hydroxynorcytisine (**1**), Cytisine (**2**), and Analogue (**3**) at Neuronal Nicotinic Receptors (NNRs)[12]

	K_i (nM)	
compound	α4*β*2	α7
1	14000 ± 4000	260000 ± 31000
2	0.4 ± 0.1	1700 ± 300
3	41000 ± 7000	>100000

(8) The CIF for **15** has been deposited with the Cambridge Crystallographic Data Centre database, deposition code: 701795.

(9) Pale yellow single crystals of $C_{34}H_{34}N_4O_6S_2$ (**15**) are, at 193(2) K, monoclinic, space group $P2_1/n$ (an alternate setting of $P2_1/c - C_{2h}^5$ (No. 14)) with a = 6.933(2) Å, b = 22.473(5) Å, c = 20.118(4) Å, β = 96.409(4)°, V = 3115.1(12) Å^3, and Z = 4 {d_{calcd} = 1.405 g·cm^{-3}; μ_a(MoKα) = 0.225 mm^{-1}}. A full hemisphere of diffracted intensities (1868 30-second frames with a ω scan width of 0.30°) was measured for a single-domain specimen using graphite-monochromated MoKα radiation (λ = 0.71073 Å) on a Bruker SMART APEX CCD Single Crystal Diffraction System. X-rays were provided by a fine-focus sealed X-ray tube operated at 50 kV and 30 mA. For more information, see Supporting Information.

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(11) The estimate of >100000 is based on the failure of the compound to pass single concentration determination at 100000 nM.

molecular modeling and the full structure−activity work which addresses the disparate NNR profiles of **1** and **2**, will be delineated in a subsequent disclosure.

(12) Experimental details for the determination of affinities at $\alpha 4\beta 2$ and $\alpha 7$ NNRs can be found in the following reference: Marrero, M. B.; Papke, R. L.; Bhatti, B. S.; Shaw, S; Bencherif, M. *J. Pharmacol. Exp. Ther.* **2004**, *309*, 16–27.

Supporting Information Available: Experimental procedures and spectroscopic data for compounds **1**, **3**, **5**, **7**−**9**, and **12**−**15** and X-ray crystallographic information file (CIF) and detailed report for **15**. This material is available free of charge via the Internet at http://pubs.acs.org.

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