Expedited Articles

(−)-Spiro[1-azabicyclo[2.2.2]octane-3,5′-oxazolidin-2′-one], a Conformationally Restricted Analogue of Acetylcholine, Is a Highly Selective Full Agonist at the α7 Nicotinic Acetylcholine Receptor

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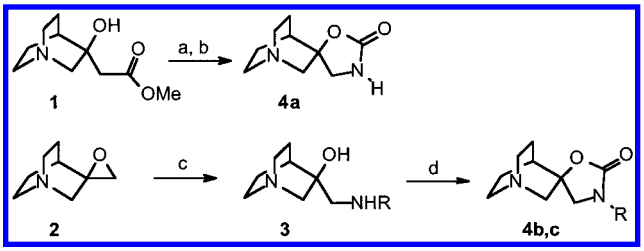
Neuronal nicotinic acetylcholine receptors are members of the ligand-gated ion channel receptor superfamily and may play important roles in modulating neurotransmission, cognition, sensory gating, and anxiety. Because of its distribution and abundance in the CNS, the α7 nicotinic receptor is a strong candidate to be involved in some of these functions. In this paper we describe the synthesis and in vitro profile of AR-R17779, (−)-spiro[1-azabicyclo[2.2.2]octane-3,5′-oxazolidin-2′-one] (**4a**), a potent full agonist at the rat α7 nicotinic receptor, which is highly selective for the rat α7 nicotinic receptor over the α4β2 subtype. Preliminary SAR of AR-R17779 presented here indicate that there is little scope for modification of this rigid molecule as even minor changes result in significant loss of the α7 nicotinic receptor affinity.

Introduction

Neuronal nicotinic acetylcholine receptors (nAChRs) are cation channels composed of five subunits and may be either homo-oligomeric (α subunits) or hetero-oligomeric (α and β subunits) in composition. Recent studies[1−8] have indicated that neuronal nicotinic receptors play important roles in modulating neurotransmission, cognition, sensory gating, and anxiety; thus, there has been renewed interest in the use of nicotinic agonists for treating neurodegenerative diseases,[9−12] such as Alzheimer's disease, in which there is cholinergic dysfunction. The subtypes which mediate nicotine's CNS actions are largely unknown; because of their distribution and abundance in the CNS,[10] the α7 and α4β2 subtypes are strong candidates to be involved in at least some of these functions.

While there are a number of nicotinic agonists known to be selective for the α4β2 subtype,[10,12] there are no reported agonists which bind the α7 nicotinic receptor selectively over other subtypes. Analogues of the marine worm toxin anabaseine[13,14] have been reported to be "functionally" selective for the α7 subtype because these compounds are partial agonists at the rodent α7 nicotinic receptor and antagonists at all other nicotinic receptor subtypes. However the therapeutic potential of these compounds as α7 nicotinic receptor agonist probes is compromised by the lack of significant binding selectivity for α7 over α4β2 (selectivity ratios of 10 or less); they also noncompetitively antagonize the α7 nicotinic receptor in a slowly reversible manner. In the current paper, we describe AR-R17779 [spirooxazolidinone **(−)-4a**[22]], a conformationally restricted analogue of acetylcholine. The compound is the first reported full agonist at the rat α7 subtype, with selective affinity for the α7 vs the α4β2 nAChR subtype and which also lacks noncompetitive antagonist activity.[15]

Scheme 1[a]



[a] Reagents: (a) NH_2NH_2/toluene; (b) $NaNO_2$, HCl/H_2O; (c) RNH_2/MeOH; (d) CDI/THF.

Chemistry

The spirooxazolidinones **4** were prepared as shown in Scheme 1. Reaction of hydroxy ester **1**[16] with hydrazine followed by Curtius rearrangement led to the desired carbamate **4a**. Resolution of **4a** was accom-

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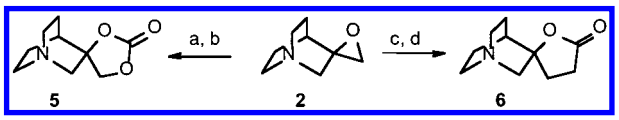
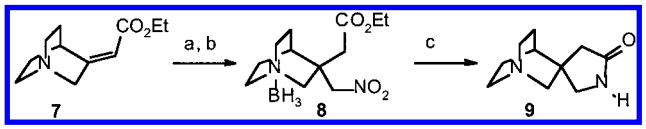
Scheme 2[a]



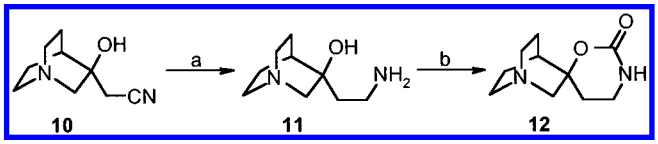
[a] Reagents: (a) 3 N $HClO_4$; (b) triphosgene; (c) NaH/diethyl malonate/toluene; (d) aq HCl/DMF.

Scheme 3[a]



[a] Reagents: (a) BH_3−THF; (b) $MeNO_2$/DBU; (c) Zn/HOAc.

Scheme 4[a]



[a] Reagents: (a) $LiAlH_4$/THF; (b) CDI/THF.

plished via the dibenzoyl-D(or L)-tartrate salt. Compounds **4b**,**c** were prepared from amino alcohols **3** by cyclization using carbonyldiimidazole (CDI). The amino alcohols **3** were prepared by reaction of epoxide **2**[17] with either methylamine or ethylamine in methanol.

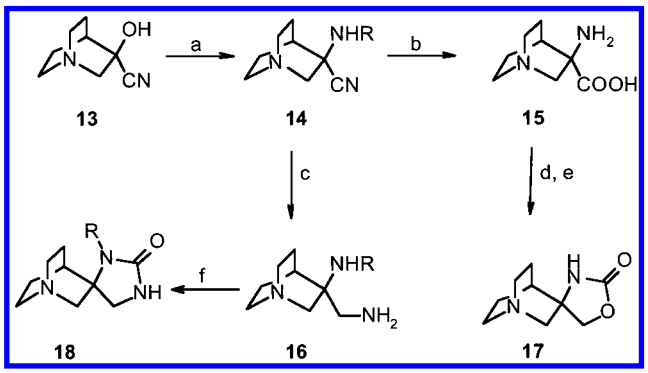
Compounds **5** and **6** were prepared from epoxide **2**[17] as shown in Scheme 2. The synthesis of the cyclic carbonate **5** was achieved by ring opening of the epoxide **2** using 3 N perchloric acid followed by ring closure with triphosgene. The lactone **6** was prepared via addition of the sodium anion of diethyl malonate to epoxide **2** followed by acid-catalyzed cyclization−decarboxylation.

Protection of the quinuclidine nitrogen as its borane adduct was necessary in order to prepare the spirolactam **9** (Scheme 3). Thus, reaction of the unsaturated ester **7**[18] with borane−THF, followed by Michael condensation with nitromethane, gave the intermediate nitro ester **8**; reduction of **8** using zinc in acetic acid and concomitant cyclization of the resulting amino ester/deprotection of the quinuclidine nitrogen provided the desired lactam **9**.

Reduction of the nitrile alcohol **10** (see Scheme 4), prepared by reaction of the lithium salt of acetonitrile with 3-quinuclidinone in THF, provided the amino alcohol **11**, which cyclized upon treatment with CDI to give homologue **12**.

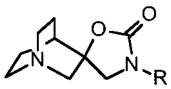
Synthesis of the 'reverse' spirooxazolidinone **17** and the spiroimidazolidinones **18** are presented in Scheme 5. Reaction of cyanohydrin **13**[19−21] with ammonia or methylamine provided the corresponding cyano amines **14** (R = H, Me). Hydrolysis of nitrile **14** (R = H) using aqueous HCl gave the amino acid **15**, which, upon borane reduction and cyclization with CDI, yielded the desired spirooxazolidinone **17**. Alternatively, Raney nickel hydrogenation of cyano amines **14** gave the triamines **16** (R = H, Me), which led to the spiroimidazolidinones **18** upon cyclization with CDI.

Scheme 5[a]



[a] Reagents: (a) RNH_2/MeOH; (b) aq HCl; (c) H_2/Ra Ni/NH_3/MeOH; (d) BH_3−THF; (e) CDI/$^i Pr_2NEt$/$CHCl_3$; (f) CDI/THF.

Table 1. Rat Affinity and Efficacy Data on Spirooxazolidinones



compd	R	K_i (nM) ± SEM[a] α4β2 affinity	K_i (nM) ± SEM[a] α7 affinity	α7 IA (%)	α7 potency EC_{50} (mM)
(−)-Nic		2.3 ± 0.6 (6)	480 ± 34 (6)	59	43
4a	H	16000 ± 4000 (3)	190 ± 10 (10)		
(+)-**4a**	H	30000 ± 10000 (4)	9400 ± 300 (3)	<10[c]	
(−)-**4a**	H	16000 ± 4000 (3)	92 ± 10 (4)	96	21
4b	Me	450 ± 80 (3)	250 ± 10 (3)		
(+)-**4b**	Me	24000[b] (2)	>32000 (3)	1	6
(−)-**4b**	Me	200 ± 80 (3)	220 ± 60 (4)	82	36
4c	Et	170 ± 10 (3)	690 ± 20 (3)		

[a] Number of determinations in parentheses. [b] Range = 1000. [c] @ 100 μM.

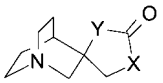
Results and Discussion

As presented in Table 1, the spirooxazolidinone **(−)-4a** (AR-R17779) is a potent *full* agonist (efficacy = 96%) for the α7 nicotinic receptor: it is twice as potent as (−)-nicotine, although (−)-nicotine is a partial α7 nicotinic receptor agonist (efficacy = 59%). Furthermore, AR-R17779 is the most selective α7 receptor agonist known thus far. This is especially apparent when one compares the selectivities of AR-R17779 and (−)-nicotine for the α7 receptor versus the α4β2 nicotinic receptor. Whereas nicotine is selective for the α4β2 nicotinic receptor (i.e., 0.005× "selective" for the α7 receptor), AR-R17779 is selective for the α7 receptor (175× selective for the α7 receptor − approximately 35000-fold more selective than (−)-nicotine). The unique potency and selectivity of AR-R17779 make this compound an important tool for the understanding of the function of the α7 nicotinic receptor.

When one examines the SAR of **4a** presented in Tables 1 and 2, it is readily apparent that there is little room for change in this rigid molecule. A clear stereogenic preference can be seen for the (−) versus the (+) enantiomer of **4**. In addition to the fact that **(+)-4a** has poor affinity for the α7 receptor, it also possesses poor intrinsic activity for the receptor. These results suggest that the location of the carbonyl functionality in AR-R17779 is crucial to mimicking acetylcholine in the α7 receptor. Addition of a simple methyl group to the amide nitrogen in **4** as found in **(−)-4b** reduces α7 receptor affinity and dramatically increases α4β2 receptor affinity. This trend is continued with the *N*-ethyloxazolidinone (**4c**), suggesting that there may be a lipophilic pocket in the α4β2 receptor that is lacking in the α7 receptor.

Several changes to the nature of the carbonyl functionality in **4a** cause an immediate loss in α7 receptor affinity. As seen in Table 2, changing the carbamate in

Table 2. Rat Affinity Data on Oxazolidinone Ring-Modified Derivatives



			K_i (nM) ± SEM[a]	
compd	X	Y	α4β2 affinity	α7 affinity
(−)-4a	NH	O	16000 ± 4000 (3)	92 ± 10 (4)
5	O	O	2200 (1)	560 ± 20 (3)
6	CH_2	O		3700 (1)
9	NH	CH_2		4000 (1)
12	CH_2NH	O		23000[b] (2)
17	O	NH		>4300 (1)
18a	NH	NH	11000 (1)	>13000 (1)
18b	NH	NCH_3	35000 (1)	>13000 (1)

[a] Number of determinations in parentheses. [b] Range = 1000.

4a to a carbonate as in **5**, to an ester as in **6**, or to an amide as in **9** all lead to a dramatic loss in α7 receptor affinity. These changes to the carbonyl made seemingly very minor changes to the electronic nature of the carbonyl but caused dramatic effects on binding to the α7 receptor. Insertion of a methylene into the oxazolidinone ring as seen in **12** or reversing the carbamate as seen in **17** also severely diminished α7 receptor affinity. All of these results further strengthen the hypothesis that the electronic nature and the location of the carbonyl group in AR-R17779 are linchpins for affinity and potency at the α7 nicotinic receptor. Although acetylcholine is a small, achiral receptor agonist, the molecule recognition features employed by the α7 nicotinic receptor likely are very well-defined and specific. This would correspondingly lead to a very narrow SAR with unnatural mimics. We are presently examining additional modification to, and analogues of, AR-R17779, and these results will be presented in due course.

Summary

The spirooxazolidinone **(−)-4a** is a unique and novel tool that will be useful for elucidating the use and function of the α7 nicotinic receptor. Its combination of receptor potency and nicotinic receptor selectivity makes **(−)-4a** the only known selective α7 nicotinic receptor agonist. **(−)-4a** is 5-fold more potent and 35000-fold more selective than (−)-nicotine for the α7 nicotinic receptor. Preliminary SAR of this compound has shown little opportunity for improvement as relatively minor changes result in significant loss of α7 nicotinic receptor affinity. We continue to examine the SAR around this unique discovery and are using the compound to probe the physiological function of the α7 nicotinic receptor.

Experimental Section

Melting points were determined in capillary tubes on a Thomas-Hoover apparatus and are uncorrected. Optical rotations were measured using a Jasco DIP-370 digital polarimeter. NMR spectra were determined in the indicated solvent on a Brucker AC 200 or Brucker AMX 500 NMR spectrometer at ambient temperature with tetramethylsilane (TMS) as the internal standard. Chemical shifts are given in ppm and coupling constants are in hertz. Splitting patterns are designated as follows: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Infrared spectra were recorded on a Nicolet 510P FTIR or Bruker Vector 22 FTIR spectrophotometer. Mass spectra were recorded by a Hewlett-Packard HP 5988A quadrupole mass spectrometer for desorption chemical ionization (CI) or by a Micromass Quattro 1 mass spectrometer for electrospray (ES). High-resolution mass spectra were obtained using a Micromass QTOF mass spectrometer. Elemental analyses were performed internally and were within 0.4% of the theoretical value when indicated by symbols of the elements, unless otherwise noted. Karl Fischer analyses were performed using a Brinkmann 684KF coulometric titrator.

Spiro[1-azabicyclo[2.2.2]octane-3,5′-oxazolidin-2′-one] (4a). Hydrazine (2.0 mL, 64 mmol) was added to a solution of 2.90 g (14.5 mmol) of methyl 3-hydroxy-1-azabicyclo-[2.2.2]octane-3-acetate[16] (**1**) in 10 mL of methanol. The resulting solution was refluxed under nitrogen for 1 h, allowed to cool, then concentrated in vacuo. The resulting solid was suspended in 50 mL of toluene and heated to reflux for 2 h in an apparatus equipped with a Dean−Stark trap. The reaction mixture was allowed to cool, and the precipitate was collected to give 1.82 g (63%) of the hydrazide as a tan solid. To a suspension of 910 mg (4.57 mmol) of the hydrazide in 7 mL of water was added 12 M HCl to give a solution with a pH = 1. The mixture was cooled to 0 °C and a 0 °C solution of 0.33 g of sodium nitrite in 5 mL of water was added. The solution was stirred at 0 °C for 20 min and then heated to 70 °C for an additional 20 min. The reaction mixture was cooled to ambient temperature, basified with 50% aqueous NaOH, saturated with NaCl and extracted with chloroform (4 × 20 mL). The combined organic extract was dried over $MgSO_4$ and concentrated in vacuo to give 730 mg (87%) of **4a** as an off-white solid: ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.36 (m, 1H), 1.48 (m, 2H), 1.77 (m, 1H), 1.91 (m, 1H), 2.64 (m, 4H), 3.33 (s, 2H), 3.28 & 3.53 (AB, 2H, J = 8.55), 7.42 (br s, 1H); IR (KBr, cm^{-1}) 1743; CIMS m/z 183.

A solution of 7.5 g (21 mmol) of dibenzoyl-L-tartaric acid in absolute ethanol was added to 3.8 g (21 mmol) of **4a** in absolute ethanol, followed by a small portion of ethyl acetate. The solid which formed upon standing was filtered (filtrate A). After recrystallization from ethanol, this solid was dissolved in aqueous NaOH, extracted with chloroform (3 × 50 mL), dried over $MgSO_4$ and concentrated in vacuo. The residue was dissolved in methanol and HCl gas was bubbled through the mixture until the pH < 2. Et_2O was added to the solution and the resulting white solid was filtered to give 630 mg of **(+)-4a**: mp >250 °C; chiral HPLC (Diacel OD, 4.6 × 250 mm, 4:1 hexane/ethanol, 1.00 mL/min) 8.69 min; $[\alpha]_D$ = +57.078 (c = 0.6570, MeOH); ^{13}C NMR (200 MHz, DMSO-d_6) δ 17.77, 18.10, 29.01, 44.39, 45.01, 48.74, 57.19, 78.20, 157.10; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.92 (m, 3H), 2.03 (m, 1H), 2.23 (m, 1H), 3.17 (m, 3H), 3.20 (m, 1H), 3.55 (m, 4H), 7.75 (s, 1H), 11.09 (br s, 1H). Anal. ($C_9H_{15}ClN_2O_2$) C, H, N.

Filtrate A was converted to the free base by concentrating the solution and then dissolving the residue in 20% aqueous NaOH; extraction with chloroform, followed by drying over $MgSO_4$ and concentration in vacuo, gave 1.21 g of residue, which was dissolved in absolute ethanol and combined with a solution of 2.38 g of dibenzoyl-D-tartaric acid in absolute ethanol. This solution was treated as above to give 210 mg of **(−)-4a**: mp >250 °C; chiral HPLC (Diacel OD, 4.6 × 250 mm, 4:1 hexane/ethanol, 1.00 mL/min) 9.78 min; $[\alpha]_D$ = −61.473 (c = 0.7727, MeOH); ^{13}C NMR (200 MHz, DMSO-d_6) δ 17.76, 18.10, 28.95, 44.47, 45.07, 48.73, 57.24, 78.18, 157.10; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.92 (m, 3H), 2.03 (m, 1H), 2.23 (m, 1H), 3.17 (m, 3H), 3.51 (s, 2H), 3.45 & 3.60 (AB, 2H, J = 9.60), 7.74 (s, 1H), 10.77 (br s, 1H). Anal. ($C_9H_{15}ClN_2O_2$) C, H, N.

3′-Methylspiro[1-azabicyclo[2.2.2]octane-3,5′-oxazolidin-2′-one] (4b). Methylamine (25 mL, 0.726 mol) was added to a solution of 10.2 g (0.0733 mol) of epoxide **2**[17] in 75 mL of methanol. The resulting solution was stirred at ambient temperature overnight, then concentrated in vacuo to give 12.42 g of amino alcohol **3** (R = Me). A mixture of 8.0 g of amino alcohol **3** (R = Me) and 9.15 g (0.056 mole, 1.2 equiv) of carbonyldiimidazole in 100 mL of tetrahydrofuran was refluxed for 3 h. Upon cooling to ambient temperature, the reaction was concentrated in vacuo and the residue was

dissolved in 200 mL of methylene chloride, washed successively with 100 mL of water, 50 mL of brine and dried over $MgSO_4$. Crystallization of the hydrochloride salt from 1:1 methanol/2-propanol gave 5.55 g (51%) of **4b** as its hydrochloride salt: mp 305−307 °C dec; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.82 (m, 3H), 1.98 (m, 1H), 2.22 (m, 1H), 2.75 (s, 3H), 3.15 (m, 4H), 3.48 (m, 2H), 3.53 & 3.66 (AB, 2H, J = 8.77), 10.64 (br s, 1H); IR (KBr, cm^{-1}) 1751; CIMS m/z 197.

Oxazolidinone **4b** as its HCl salt (500 mg) was converted to its free base via neutralization with Na_2CO_3, then dissolved in 5 mL of ethanol and treated with a solution of 900 mg of dibenzoyl-D-tartaric acid in 10 mL of ethanol to give, following recrystallization from ethanol, 177 mg of the dibenzoyl-D-tartrate salt of **4b**. Conversion to the free base with K_2CO_3 followed by salt formation using HCl in ether gave 93 mg of **(−)-4b** as its hydrochloride salt: $[\alpha]_D$ = −56.14 (c = 0.3438, MeOH); chiral HPLC (OD-H #139, 2.1 × 150 mm, 14.0% EtOH in hexane at 0.25 mL/min) 98.4% peak 1 at 5.68 min; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.79 (m, 2H), 1.88 (m, 1H), 2.03 (m, 1H), 2.25 (m, 1H), 2.77 (s, 3H), 3.14 (m, 2H), 3.31 (m, 2H), 3.48 & 3.70 (AB, 2H, J = 12.0), 3.53 (m, 2H), 10.76 (br s, 1H); IR (KBr, cm^{-1}) 1759; ESMS m/z 197. Anal. ($C_{10}H_{17}ClN_2O_2$) C, H, N, Cl.

Resolution of 5.60 g of **4b** using 10.20 g (1 equiv) of dibenzoyl-L-tartaric acid in ethanol gave, following 3 recrystallizations from ethanol, 529 mg of **(+)-4b**: chiral HPLC (OD-H #139, 2.1 × 150 mm, 14.0% EtOH in hexane at 0.25 mL/min) 98.7% peak 2 at 7.35 min.

3′-Ethylspiro[1-azabicyclo[2.2.2]octane-3,5′-oxazolidin-2′-one] (4c). Oxazolidinone **4c** was prepared from epoxide **2**[17] as above, using ethylamine, and isolated as its hydrochloride salt: mp 283−286 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.07 (t, 3H, J = 7.15), 1.82 (m, 3H), 2.02 (m, 1H), 3.15 (m, 4H), 3.29 (m, 2H), 3.42 & 3.55 (AB, 2H, J = 14.2), 3.58 & 3.68 (AB, 2H, J = 9.46), 11.18 (br s, 1H); IR (KBr, cm^{-1}) 1760; ESMS m/z 211. Anal. ($C_{11}H_{19}ClN_2O_2$) C, H, N.

Spiro[1-azabicyclo[2.2.2]octane-3,5′-dioxolan-2′-one] (5). A solution of 0.50 g (3.6 mmol) of epoxide **2**[17] and 4.8 mL (14.4 mmol) of 3 N perchloric acid in 10 mL of water was stirred at ambient temperature for 18 h, then made basic with sodium carbonate. The resulting solution was concentrated in vacuo and the solid residue was extracted with tetrahydrofuran; flash chromatography through neutral silica gel using a 95:5:0.1 mixture of methanol:ethyl acetate:concentrated ammonium hydroxide gave 0.27 g (48%) of the diol as a syrup which solidified on standing: ESMS m/z 158 (MH^+). Triphosgene (94 mg, 0.32 mmol) in 1.3 mL of methylene chloride was added to a suspension of 100 mg (0.64 mmol) of the above diol and 300 mg (3.8 mmol) of pyridine in 2 mL of methylene chloride, while stirring under nitrogen at −70 °C. The solution was allowed to warm to ambient temperature and made basic using sodium carbonate. Extraction with chloroform followed by removal of the solvent gave 48 mg of dioxolane **5** as a solid: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.53 (m, 4H), 1.72 (m, 1H), 2.77 (m, 2H), 2.91 (m, 3H), 3.28 (d, 1H, J = 16.6), 4.15 & 4.51 (AB, 2H, J = 8.8); IR (KBr, cm^{-1}) 1771 (s); ESMS m/z 184 (Karl Fischer: found, 1.00% H_2O). Anal. ($C_9H_{13}NO_3$) C, H, N.

Spiro[1-azabicyclo[2.2.2]octane-3,5′-2*H*-tetrahydrofuran-2-one] (6). Ethyl *tert*-butyl malonate (4.26 mL, 22.5 mmol) was added slowly to a suspension of 909 mg (22.5 mmol) of 60% sodium hydride in 50 mL of toluene, under nitrogen. The resulting suspension was heated to 90 °C and a solution of 1.04 g (7.5 mmol) of epoxide **2**[17] in 50 mL of toluene was added dropwise over 30 min. The reaction was stirred at 90 °C for 24 h, allowed to cool to ambient temperature, then poured into a mixture of 60 mL of 2.5 N hydrochoric acid and 100 g of ice. The layers were separated and the aqueous layer was basicified with $NaHCO_3$; extraction with chloroform gave 1.42 g (67%) of the α-*tert*-butoxycarbonyl lactone as a semisolid. A solution of 256 mg (0.91 mmol) of the α-*tert*-butoxycarbonyl lactone in 2 mL of concentrated HCl and 5 mL of DMF was heated to 80 °C and stirred for 17 h. Upon cooling to room temperature, the reaction was made basic with saturated $NaHCO_3$, then extracted with chloroform (4 × 20 mL). The combined organic extract was dried over $MgSO_4$ and concentrated in vacuo. The residue was flash chromatographed through neutral silica gel using a 97:3 mixture of chloroform: ammoniated methanol as the eluant to provide 34 mg (20%) of lactone **6**: ^{13}C NMR (200 MHz, $CDCl_3$) δ 21.269, 22.741, 28.741, 30.925, 32.087, 46.221, 46.560, 61.455; IR (KBr, cm^{-1}) 2361, 1772; HR-ESMS m/z calcd for $C_{10}H_{16}NO_2$, 182.1181; found, 182.1168.

Spiro[1-azabicyclo[2.2.2]octane-3,4′-pyrrolidin-2-one] (9). Under nitrogen, 4.8 mL of 1.0 M borane−THF was added to an ice-cold solution of 935 mg of ethyl 1-azabicyclo-[2.2.2]octane-3-acrylate[18] in 50 mL of THF. After 30 min, the solvent was removed in vacuo and the residue was dissolved in 1.3 mL of nitromethane, to which was added 0.74 mL of DBU. The reaction was stirred at ambient temperature for 4 days, then partitioned between 50 mL of ether and 50 mL of 0.1 N HCl. The acid layer was extracted twice with 25 mL of ether and the combined organic layer was dried over $MgSO_4$ and concentrated in vacuo. Flash chromatography, eluting with 3:1 hexane/ethyl acetate, followed by 2:1 hexane/ethyl acetate, gave 681 mg (52%) of ethyl 3-nitromethyl-1-azabicyclo-[2.2.2]octane-3-acetate (**8**) as a light yellow syrup: IR (KBr) 2365 (m), 2318 (m), 2270 (m), 1723 (s), 1547 (s); CIMS m/z 269 (M − H, 100%).

Zinc dust (1.3 g) was added to a solution of 666 mg (2.45 mmol) of ester **8** in 80 mL of acetic acid; the resulting suspension was heated to reflux and stirred for 20 h. An additional 1.2 g of zinc dust was then added and reflux was continued for an additional 48 h. Upon cooling to ambient temperature, the suspension was filtered through Celite. The filtrate was concentrated in vacuo and the residue dissolved in 50 mL of saturated aqueous Na_2CO_3. Extraction with a 3:1 mixture of chloroform/methanol gave, following flash chromatography using a 20:5:0.5 mixture of chloroform/methanol/concentrated ammonium hydroxide, 149 mg (34%) of lactam **9**: mp 162−165 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.58 (m, 4H), 1.73 (m, 1H), 2.22 & 2.43 (AB, 2H, J = 16.4), 2.85 (m, 6H), 3.26 & 3.42 (AB, 2H, J = 9.4), 6.09 (br s, 1H); IR (KBr, cm^{-1}) 1697; ESMS m/z 181 (Karl Fischer, found 1.0% H_2O). Anal. Calcd for $C_{10}H_{16}N_2O$ (adjusted for 1.0% H_2O): C, 65.97; H, 8.97; N, 15.39. Found: C, 65.02; H, 8.71; N, 14.95.

3-Hydroxy-1-azabicyclo[2.2.2]octane-3-acetonitrile (10). A solution of acetonitrile (4.26 mL, 81.8 mmol) in 80 mL of THF was added dropwise over 15 min to a solution of 35.5 mL (81.8 mmol) of 2.3 M ^{n}BuLi in hexane and 80 mL of THF, while stirring at −75 °C under nitrogen. After 1 h, a solution of 10.22 g (81.8 mmol) of 3-quinuclidinone in 80 mL of THF was added slowly. After an additional 2 h, the reaction was quenched with 50 mL of water, allowed to warm to 10 °C, and concentrated in vacuo. The residue was taken up in water, made basic with Na_2CO_3 and saturated with NaCl. Extraction with chloroform gave 12.21 g of a solid which crystallized from ethyl acetate/ethanol, providing 7.33 g (54%) of hydroxy nitrile **10**, mp 179−180 °C, which was used as such without further purification.

Spiro[1-azabicyclo[2.2.2]octane-2,6′-(3′,4′,5′,6′-tetrahydro-1′,3′-oxazin-2′-one)] (12). Hydroxy nitrile **10** (3.32 g, 20.0 mmol) was added to a suspension of 1.00 g (26.4 mmol) of lithium aluminum hydride in 50 mL of THF, while stirring under nitrogen in an ice bath. The reaction was then heated to reflux for 16 h, allowed to cool to ambient temperature, then quenched by cautious addition of sodium sulfate decahydrate, followed by 50 mL of THF and 1 mL of water. The mixture was stirred for 1 h, filtered and concentrated in vacuo to afford 3.49 g (100%) of 3-hydroxy-1-azabicyclo[2.2.2]octane-3-ethylamine (**11**) as a yellow oil, which was used as such without further purification.

A catalytic amount of (dimethylamino)pyridine was added to a solution of 1.58 g (9.28 mmol) of **11** and 3.00 g (18.5 mmol) of carbonyldiimidazole in 25 mL of THF, which was refluxed under nitrogen for 24 h. Upon cooling to ambient temperature, the reaction was flash chromatographed through 200 g of neutral silica gel using a 9:1:0.1 mixture of methylene chloride: methanol:ammonium hydroxide as the eluant, providing 344

mg of **12** as a yellow oil. Crystallization from ethyl acetate gave compound **12** as a white solid: 145 mg (8%); mp 171−175 °C; ^{13}C NMR (200 MHz, $CDCl_3$) δ 21.087, 23.859, 28.620, 29.914, 36.676, 46.740, 47.227, 61.831; IR (KBr, cm^{-1}) 3239, 1701; CIMS *m*/*z* 197 (M + H). Anal. ($C_{10}H_{16}N_2O_2$) C, H, N.

3-Amino-1-azabicyclo[2.2.2]octane-3-carbonitrile (14, R = H). 3-Hydroxy-1-azabicyclo[2.2.2]octane-3-carbonitrile[19−21] (**13**) (10.21 g, 67.1 mmol) was dissolved in methanolic ammonia (7 M, 100 mL) and the solution was stirred at 50 °C for 48 h, then allowed to cool and evaporated in vacuo. Crystallization from ethyl acetate/hexane gave amino nitrile **14** (R = H) as a colorless solid (7.67 g, 76%): ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.25 (1H, m), 1.67 (2H, m), 1.91 (2H, m), 2.66 (7H, m), 3.05 (1H, d); ESMS *m*/*z* 152 (100%, MH^+). Anal. ($C_8H_{13}N_3$) C, H, N.

3-*N*-Methylamino-1-azabicyclo[2.2.2]octane-3-carbonitrile (14, R = Me) was prepared from cyanohydrin **13**[19−21] as above using 2 M methylamine in methanol in place of ammonia. The residue was recrystallized from ethyl acetate/hexane to give **14** (R = Me) as a colorless solid (4.32 g, 74%): ^{1}H NMR (500 MHz, DMSO) δ 1.28 (1H, m), 1.63 (2H, m), 1.75 (1H, m), 2.00 (1H, m), 2.28 (3H, d, J = 4 Hz), 2.66 (6H, m), 3.02 (1H, d, J = 14 Hz); ESMS *m*/*z* 166. Anal. ($C_9H_{15}N_3$) C, H, N.

3-Amino-1-azabicyclo[2.2.2]octane-3-carboxylic Acid (15). Amino nitrile **14** (R = H) (20.05 g, 133 mmol) was dissolved in a 1:1 mixture of concentrated hydrochloric acid and water (200 mL). The solution was stirred at room temperature for 24 h, heated to reflux for 24 h, then allowed to cool and evaporated in vacuo. The residue was then dissolved in water and passed through a column of hydroxide form A26 ion-exchange resin (prepared by suspension of the resin in water, washing with aqueous potassium hydroxide solution and then with deionized water) using dilute aqueous acetic acid. Evaporation gave 9.04 g of amino acid **15**, acetate salt. A sample (403 mg) of the product was suspended in 4 M HCl in dioxane, and the solution was stirred overnight. Following evaporation, the residue was dissolved in the minimum volume of dilute aqueous hydrochloric acid and crystallized by the addition of THF to give, after drying, amino acid **15**, dihydrochloride hydrate as a colorless solid (178 mg): ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.73 (m, 1H), 1.92 (m, 2H), 2.22 (m, 1H), 2.44 (s, 1H), 3.06 (m, 1H), 3.25 (m, 2H), 3.39 (d, 1H), 3.50 (m, 1H), 3.83 (m, 1H), 9.29 (br m, 3H), 11.20 (br s, 1H); ESMS *m*/*z* 171 (100%, MH^+) (Karl Fisher analysis: found, 9.499% H_2O). Anal. ($C_8H_{16}Cl_2N_2O_2$) C, H, N.

Spiro[1-azabicyclo[2.2.2]octane-3,4′-oxazolidin-2′-one] (17). Borane (1 M in THF, 30 mL, 30 mmol) was added to a suspension of amino acid **15,** acetate salt (0.481 g, 2.82 mmol) in THF (20 mL), while stirring under nitrogen in an ice bath. The solution was heated briefly to reflux to dissolve the solid, and then stirred overnight at ambient temperature. Following workup with methanol, dilute hydrochloric acid was added to the residue; the resulting solution was stirred at room-temperature overnight and concentrated in vacuo. To 520 mg of the resulting residue, chloroform (10 mL) and diisopropylethylamine (2 mL, 1.48 g, 11.5 mmol) were added, followed by 1,1′-carbonyldiimidazole (1.84 g, 11.3 mmol). The solution was stirred overnight at room temperature, brine was added, and the solution was then extracted with chloroform. The combined organic extract was dried with magnesium sulfate and evaporated. The residue was purified by HPLC on a Waters Porasil column using a gradient of 0−50% 1:1 v/v 3.5 M NH_3 in MeOH/$CHCl_3$ in $CHCl_3$ to give compound **17** as a colorless solid: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.62 (m, 3H), 1.82 (m, 1H), 1.95 (m, 1H), 2.79 (m, 4H), 3.02 (m, 2H), 4.12 (d, 1H, J = 9), 4.48 (d, 1H, J = 9), 6.84 (br s, 1H); HR-ESMS calcd for $C_9H_{14}N_2O_2$, 183.1134; found, 183.1126.

Spiro[1-azabicyclo[2.2.2]octane-3,4′-2*H*-tetrahydroimidazolidin-2-one] (18a). 3-Amino-1-azabicyclo[2.2.2]octyl-3-carbonitrile (**14**, R = H) (160 mg, 1.06 mmol) was dissolved in 20 mL of 7 N methanolic ammonia, and Raney nickel (approximately 100 mg wet) was added. The resulting mixture was stirred under an atmosphere of hydrogen at 50 psi for 15 h. The solution was then filtered and evaporated under reduced pressure to afford triamine **16** (R = H) (132 mg, 80%) as an oil, which was used without further purification.

1,1′-Carbonyldiimidazole (446 mg, 2.74 mmol) was added to a solution of triamine **16** (R = H) (356 mg, 2.29 mmol) in dry THF (10 mL). The resulting solution was heated under reflux for 2 h. Following evaporation in vacuo, the residue was subjected to reverse-phase HPLC on a Waters Bondapak C18 column using a gradient of 0−10% acetonitrile and 0.1% aqueous trifluoroacetic acid. The product was dissolved in methanol and excess 1 M HCl in ether was added. The resulting salt was recrystallized from MeOH by vapor diffusion with ether to afford 29 mg (6%) of **18** (R = H) as a colorless solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.76−1.83 (3H, m), 1.96 (1H, s), 2.06 (1H,m), 3.11−3.33 (6H,m) 3.40 (1H, d, J = 9), 3.47 (1H, d, J = 13.5), 6.38 (1H, s), 7.11 (1H, s), 9.90 (1H, s); HR-ESMS calcd for $C_9H_{15}N_3O$, 182.1293; found, 182.1316.

3′-Methylspiro[1-azabicyclo[2.2.2]octane-3,4′-2*H*-tetrahydroimidazolidin-2-one] (18b). Amino nitrile **14** (R = Me) (422 mg, 2.55 mmol) was dissolved in methanolic ammonia (7 M, 20 mL), and Raney nickel (approximately 100 mg wet) was added. The resulting mixture was stirred under an atmosphere of hydrogen at 50 psi for 15 h. The solution was then filtered and concentrated in vacuo to afford **16** (R = Me) (392 mg, 91%) as an oil which was used without purification.

1,1′-Carbonyldiimidazole (501 mg, 3.09 mmol) was added to a solution of triamine **16** (R = Me) (392 mg, 2.32 mmol) in dry THF (10 mL). The resulting solution was heated under reflux for 1.5 h. The solvent was then evaporated in vacuo and the residue was subjected to reverse-phase HPLC on a Waters Bondapak C18 column using a gradient of 0−10% acetonitrile and 0.1% aqueous trifluoroacetic acid. The product was dissolved in methanol and excess 1 M HCl in ether was added. The resulting salt was recrystallized from MeOH by vapor diffusion with ether to afford 125 mg (23.2%) of **18b** as a colorless solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.74 (m, 1H), 1.84 (m, 1H), 1.94 (m, 1H), 2.12 (s, 1H), 2.21 (m, 1H), 2.83 (s, 3H), 3.17−3.41 (m, 8H), 6.54 (s, 1H), 9.91 (s, 1H); HR-ESMS calcd for $C_{10}H_{18}N_3O$, 196.1450; found, 196.1441. Anal. ($C_{10}H_{18}N_3ClO$) C, H, N.

α4β2 Nicotinic Receptor Binding Assay. Rat forebrains were homogenized in cold buffer (as above) and centrifuged at 12000*g* for 20 min. The washed membranes were incubated in buffer with 3 nM (−)-[^{3}H]nicotine for 1 h at 4 °C. Membranes were collected on GF/B filters treated with 0.01% PEI. Bound radioactivity was determined using a Beckman scintillation counter. Nonspecific binding was determined by 100 μM carbachol. IC_{50} values were determined from five concentrations of test compound (triplicate) using a nonlinear curve fitting program. K_i values were calculated using the Cheng−Prusoff equation: $K_i = IC_{50}/(((2 + [lig]/K_d)2)1/n) - 1$, where n = 1 and K_d = 1.7 nM.

α7 Nicotinic Receptor Binding Assay. Rat hippocampii were homogenized with a Polytron in 20 vol of cold buffer (mM: Tris 50; $MgCl_2$ 1; NaCl 120; KCl 5; $CaCl_2$ 2; pH 7.4). The homogenate was centrifuged (1000*g*, 5 min), reextracted and then the pooled supernatants were centrifuged at 12000*g* for 20 min. The washed membranes were incubated in buffer with 5 nM [^{125}I]α-bungarotoxin, 1 mg/mL BSA for 2 h at 21 °C. Membranes were collected on GF/C filters pretreated with 1% BSA/0.01% PEI. Bound radioactivity was determined using a Packard TopCount. Nonspecific binding was defined by 100 μM nicotine. IC_{50} values were determined from six concentrations (triplicate) of test compound using a nonlinear curve fitting program. K_i values were calculated using the Cheng−Prusoff equation: $K_i = IC_{50}/(((2 + [lig]/K_d)2)1/n) - 1$, where n = 2 and K_d = 1.67 nM.

α7 Nicotinic Receptor Functional Assay. Potency and intrinisic activity values were determined by measuring current activation in *Xenopus* oocytes expressing rat α7 nAChRs. Oocytes were injected with cRNA coding for nAChR α7. Oocytes were used 3−10 days postinjection. 100% intrinsic activity was defined for each egg by current elicited by 3 mM acetylcholine. Currents were measured from baseline to peak.

Oocytes were washed for 5 min after each application of agonist to allow receptors to fully recover from desensitization.

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