Journal of Medicinal Chemistry

ARTICLE

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Deconstruction of the α4β2 Nicotinic Acetylcholine Receptor Positive Allosteric Modulator Desformylflustrabromine

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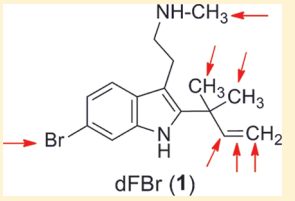
ABSTRACT: Desformylflustrabromine (dFBr; **1**), perhaps the first selective positive allosteric modulator of α4β2 neuronal nicotinic acetylcholine (nACh) receptors, was deconstructed to determine which structural features contribute to its actions on receptors expressed in *Xenopus* ooycytes using two-electrode voltage clamp techniques. Although the intact structure of **1** was found to be optimal, several deconstructed analogs retained activity. Neither the 6-bromo substituent nor the entire 2-position chain is required for activity. In particular, reduction of the olefinic side chain of **1**, as seen with **6**, not only resulted in retention of activity/potency but in enhanced selectivity for α4β2 versus α7 nACh receptors. Pharmacophoric features for the allosteric modulation of α4β2 nACh receptors by **1** were identified.

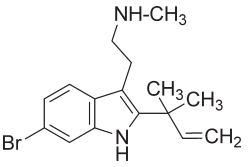


INTRODUCTION

The neurotransmitter acetylcholine (ACh) has been implicated in several neurological and neuropsychiatric disorders, such as Alzheimer's disease, autism, and schizophrenia, and might play a role in cognition, memory, and pain.[1−3] Receptors for ACh are of two broad types: muscarinic receptors and nicotinic receptors.[1,4] Muscarinic receptors (m1−m5) are G-protein coupled, whereas nicotinic acetylcholine (nACh) receptors are associated with ion channels and are composed of five membrane-spanning subunits.[1,4] Neuronal nACh receptors, an object of the present study, consist of either α or α and β subunits; multiple neuronal nACh receptor types are possible because several isoforms of α and β subunits exist (α2-α10 and β2-β4).[4] The two most prevalent neuronal nACh receptor subtypes in human brain are the heteromeric α4β2 receptors and the homomeric α7 receptors. A large number of nACh receptor agonists and antagonists have been identified (reviewed in ref 4), but a persistent problem is their low (or lack of) selectivity among the various nACh receptor subtypes.

Apart from direct-acting agonists and competitive antagonists that bind at the same (i.e., orthosteric) site as ACh, an alternate approach to influence or modulate the actions of ACh is via allosteric modulation of its receptors.[2,3,5] Positive allosteric modulators, for example, can enhance the actions of an endogenous ligand (e.g., a neurotransmitter such as ACh) by interaction at a binding site distinct from the orthosteric site. By definition, endogenous neurotransmitters and other orthosteric ligands normally do not interact with high affinity at an allosteric binding site(s), and conversely, allosteric ligands do not bind with high affinity at the orthosteric site. Consequently, due to lack of suitable design templates, it is virtually impossible, in the absence of any other available information (e.g., on some other allosteric agent that might bind in a similar fashion), to design allosteric agents de novo. Yet, the availability of allosteric agents can be advantageous, because they typically fail to desensitize receptors, are usually active only in the presence of the orthosteric agonist, and might achieve selectivity of effect if different subtypes of receptors possess different allosteric binding sites.[3]

Several years ago, a positive allosteric modulator of α4β2 nACh receptors was serendipitously discovered, desformylflustrabromine (dFBr, **1**).[6,7] Initially isolated from a North Sea marine organism,[6] **1** was later synthesized as a water-soluble salt.[8] In addition to increasing whole cell current when coapplied with effective concentrations of ACh to α4β2-containing preparations, **1** failed to potentiate the actions of ACh at α3β2, α3β4, α4β4, and α7 nACh receptors expressed in *Xenopus* oocytes.[7−9] As such, **1** can now serve as a template for the development of newer, more selective agents.

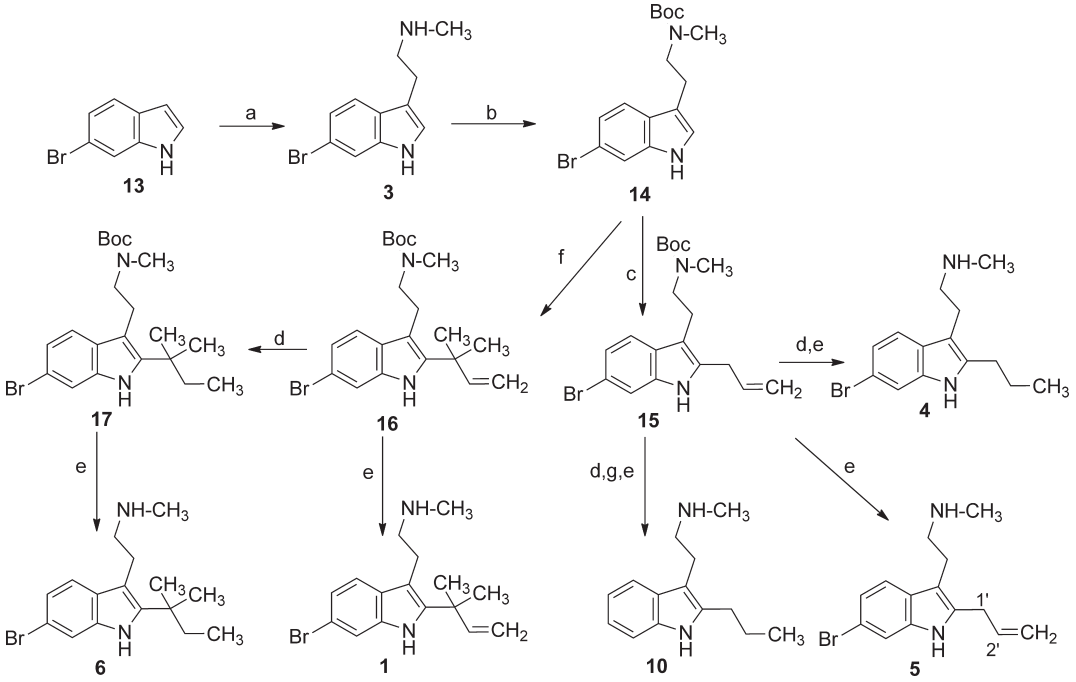


1 (dFBr)

Because of its actions as a positive allosteric modulator, desformylflustrabromine (**1**) represents a novel lead structure; however, nothing is known about its structure−activity relationships for producing this effect. In order to understand which structural features of desformylflustrabromine (**1**) are required for, or contribute to, this action, the structure of **1** was "deconstructed". Other goals are to identify a pharmacophore for the action of **1** as a positive allosteric modulator at α4β2 nACh receptors and, more long-term, to identify structures that retain this action but more readily lend themselves to convenient scale-up synthesis in order to have available sufficient

Received: June 27, 2011
Published: September 09, 2011

Scheme 1. Synthesis of Target Compounds 4−6 and 10[a]



[a] The synthesis of **1** has been previously reported and is shown here only for comparison of methods. Reagents: (a) (1) CO_2Cl_2, Et_2O; (2) $MeNH_2$; (3) dimethylethylamine alane, THF. (b) $(Boc)_2O$, Et_3N, DMF. (c) (1) t-BuOCl, THF; (2) allyltributyltin, $BF_3 \cdot Et_2O$, THF. (d) $NaBH_4$, $CoCl_2$, EtOH. (e) TFA, CH_2Cl_2. (f) (1) tBuOCl, THF; (2) prenyl 9-BBN, THF. (g) H_2 10% Pd/C.

quantities of compound for in vivo behavioral (and other) studies. We began our investigation by attempting to determine exactly what it is, structurally, that makes desformylflustrabromine (**1**) a positive allosteric modulator at $\alpha4\beta2$ nACh receptors.

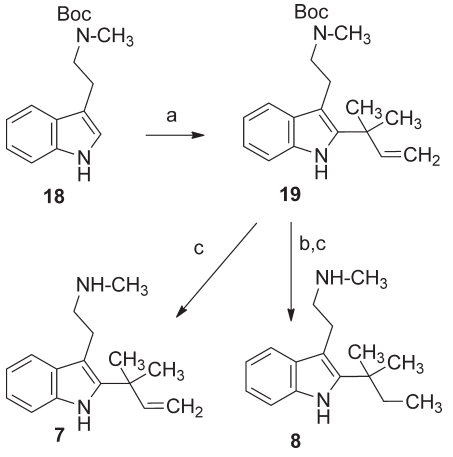
CHEMISTRY

Compound **1** (as its HCl salt) was prepared exactly as previously reported.[8] That is, 6-bromoindole (**13**; Scheme 1) was subjected to a Speeter/Anthony synthesis (i.e., reaction of an indole with oxalyl chloride and treatment of the glyoxylyl chloride product with methylamine, followed by reduction of the resulting glyoxylamide with a hydride reducing agent, dimethylethylamine alane in the case of **1**) to afford tryptamine **3**; protection of the basic amine of **3** with a Boc group (i.e., **14**) followed by prenylation (to **16**) and acidic deprotection provided **1**. The primary amine counterpart of **1** (i.e., **2**) was prepared in the same manner by prenylation of *N*-Boc-protected 6-bromotryptamine, rather than 6-bromo-*N*-methyltryptamine (**14**) used in the synthesis of **1**, followed by deprotection.

Compound **14**,[8] however, was a useful intermediate for the synthesis of several other desired targets (Scheme 1). Treatment of **14** with *tert*-butyl hypochlorite followed by allyl tributyltin and boron trifluoride afforded intermediate **15**. Reduction of the double bond of **15** using sodium borohydride and cobalt chloride,[10] followed by deprotection, provided **4**.

Deprotection of **15** without the intervening reduction step should have afforded **5**. It appears, however, that some double-bond migration occurred from the 2′-position to the 1′-position. That is, although elemental analysis for **5** was correct, the ^{1}H NMR spectrum of the product showed several minor spurious peaks, such as, for example, a small doublet of doublets at δ 1.85 (presumably a C−CH_3 signal). Elemental analysis and integration of the

Scheme 2. Synthesis of Target Compounds 7 and 8 [a]



[a] Reagents: (a) (1) t-BuOCl, THF; (2) prenyl 9-BBN, THF. (b) 10% Pd/C, H_2, 50 psi. (c) TFA, CH_2Cl_2.

^{1}H NMR signals indicated that two different positional isomers with the same elemental composition were obtained, with 86% of the product being the desired target **5** and 14% being the more conjugated 1′-alkenyl derivative. Neither isomer could be obtained in pure form. Compound **15** was also employed in the synthesis of **10** (Scheme 1); olefinic reduction of **15** and hydrogenolysis of the halogen afforded **10** following deprotection.

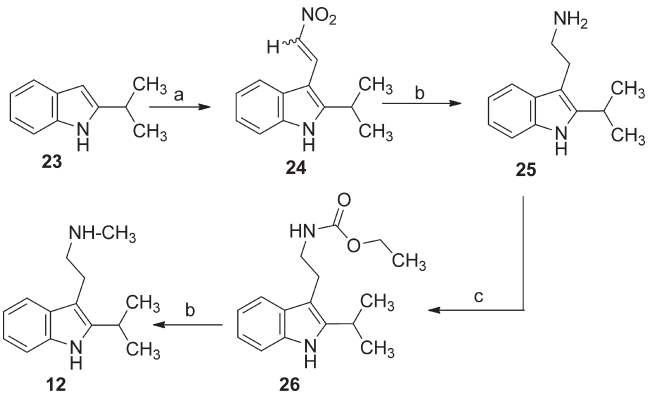
The double bond of **16**[8] was reduced using the same (i.e., sodium borohydride and cobalt chloride) reduction procedure described above (to yield **17**), and deprotection of **17** afforded the desired **6** (Scheme 1).

dx.doi.org/10.1021/jm200834x |*J. Med. Chem.* 2011, 54, 7259–7267

The desbromo analog of **1** (i.e., **7**) was obtained from **18** (Scheme 2). Compound **18** was prepared in modest yield according to a reported procedure.[11] However, upon changing the reaction solvent from dioxane to methylene chloride, the product could be obtained in 80% yield as a white solid (mp 86−89 °C) with a 3-h reaction time relative to that reported with dioxane (15 h). Compound **8** was prepared from the same intermediate (i.e., **19**) except that the double bond was catalytically reduced prior to deprotection.

Compounds **9** and **12** were prepared in a similar fashion (shown for **12** in Scheme 3). That is, the requisite indole (e.g., **23**) was treated with 1-dimethylamino-2-nitroethylene to afford nitroalkene **24**, which was readily reduced to primary amine **25**; acylation of **25** using ethyl chloroformate followed by reduction of the resulting carbamate **26** using lithium aluminum hydride provided the desired target **12**. Compound **9** was prepared from 2-*sec*-butylindole (**21**) in like manner. The *tert*-butyl derivative **11** was prepared via a classical Speeter/Anthony synthesis from 2-*tert*-butylindole.

Scheme 3. Synthesis of Compound 12 [a]



[a] Reagents: (a) 1-dimethylamino-2-nitroethylene, TFA. (b) $LiAlH_4$, THF. (c) Cl-$COOC_2H_5$, $CHCl_3$. The exact same method was employed to synthesize **9** from 2-*sec*-butylindole (**21**).

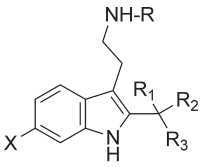
RESULTS AND DISCUSSION

A near-maximal effect in the present functional assays was consistently observed at an ACh concentration of 10 μM. The deconstructed analogs of **1** were examined using two-electrode voltage clamp recordings with $\alpha4\beta2$ nAChR receptors expressed in *Xenopus* oocytes in the presence and absence of this concentration of ACh. None of the analogs produced an effect in the absence of ACh. Several of the deconstructed analogs of **1** potentiated the effect achieved by ACh (Table 1). As with **1** itself, all deconstructed analogs that potentiated the action of ACh produced an inhibitory effect at high concentrations (representative tracings are shown in Figures 1 and 2). The mechanism underlying this action requires further investigation; however, data obtained with **1** suggest that the mechanism of the inhibitory component might be due to open channel blockade.[9]

At concentrations slightly higher than those required to potentiate its ACh-enhancing action in the $\alpha4\beta2$ receptor preparation, **1** inhibited (but failed to potentiate) the action of ACh in an $\alpha7$ nACh receptor preparation.[8] As an initial measure of selectivity, the compounds prepared in this investigation were also examined at $\alpha7$ nACh receptors in functional assays (Table 2).

Effect of Deconstructed Analogs of 1 on $\alpha4\beta2$ nACh Receptor Function. The pEC_{50} for **1** (6.48; Table 1; EC_{50} ca. 0.32 μM) for the potentiation of 100 μM ACh was quite comparable to what we originally reported (pEC_{50} ca. 6.8; EC_{50} = 0.12 μM);[8] the small difference likely reflects experimental variation and the much

Table 1. Effect of Deconstructed Analogs of Desformylflustrabromine (1) on the Action (potentiation or antagonism) of the Functional Activity of ACh at $\alpha4\beta2$ nACh Receptors Expressed in *Xenopus* Oocytes As Measured Using Two-Electrode Voltage Clamp Methods



1,**3**-**12** (R = -CH_3)
2 (R = -H)

	X	R_1	R_2	R_3	agonist pEC_{50} (±SEM)	antagonist pIC_{50} (±SEM)
1	−Br	$-CH_3$	$-CH_3$	$-CH{=}CH_2$	6.48 (0.28)	–[c]
2	−Br	$-CH_3$	$-CH_3$	$-CH{=}CH_2$	6.29 (0.24)	–[c]
3[a]	−Br	–	–	–	NP[b]	4.40 (0.06)
4	−Br	−H	−H	$-CH_2CH_3$	NP[b]	4.14 (0.10)
5	−Br	−H	−H	$-CH{=}CH_2$	NP[b]	5.45 (0.08)
6	−Br	$-CH_3$	$-CH_3$	$-CH_2CH_3$	6.86 (0.16)	–[c]
7	−H	$-CH_3$	$-CH_3$	$-CH{=}CH_2$	5.14 (3.45)	–[c]
8	−H	$-CH_3$	$-CH_3$	$-CH_2CH_3$	5.52 (1.03)	–[c]
9	−H	$-CH_3$	−H	$-CH_2CH_3$	NP[b]	4.78 (0.55)
10	−H	−H	−H	$-CH_2CH_3$	NP[b]	4.73 (0.06)
11	−H	$-CH_3$	$-CH_3$	$-CH_3$	5.40 (1.50)	–[c]
12	−H	$-CH_3$	−H	$-CH_3$	NP[b]	–[d]

[a] *N*-Methyl-6-bromotryptamine. [b] NP = No potentiation of the action of ACh. [c] pIC_{50} could not be reliably determined. [d] Not determined.

dx.doi.org/10.1021/jm200834x |*J. Med. Chem.* 2011, 54, 7259–7267

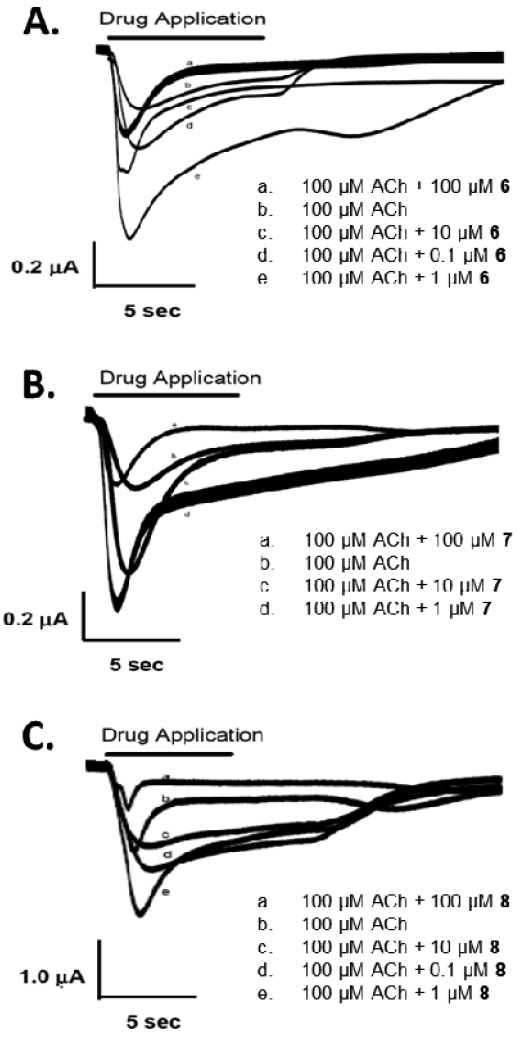


Figure 1. Potentiation of ACh-evoked responses by representative deconstructed analogs of desformylflustrabromine (**1**). Responses were elicited by application of 100 μM ACh to *Xenopus* oocytes expressing $\alpha4\beta2$ nicotinic receptors. Test compounds **6**–**8** were coapplied with ACh at the concentrations indicated. Each series of traces (A, B, and C for compounds **6**, **7**, and **8**, respectively) as shown here were elicited from a single oocyte for each compound. Concentration/response curves for these compounds are shown in Figure 2.

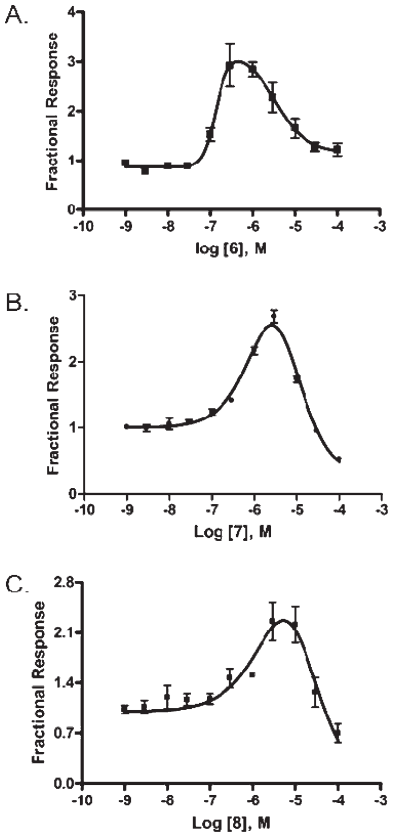
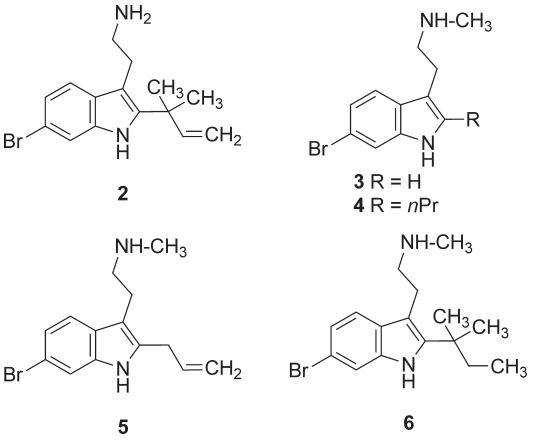


Figure 2. Concentration/response curves for representative deconstructed analogs of desformylflustrabromine (**1**). Deconstructed compounds **6** (A), **7** (B), and **8** (C) were coapplied with 100 μM ACh (i.e., a concentration of ACh that produced the maximal effect or functional response = 1) on human $\alpha4\beta2$ receptors. Peak amplitudes were normalized to peak currents obtained from 100 μM ACh in the absence of test compound. Curves were fit to a hormetic model. (A) For compound **6**, the half-maximal potentiation (EC_{50}) is 0.10 ± 0.04 μM; maximum potentiation was 290% of the controlled trace (100 μM ACh). (B) For compound **7**, EC_{50} = 6.77 ± 0.35 μM (250% of the control trace). (C) For compound **8**, EC_{50} = 1.22 ± 0.66 μM (260% of the control trace).

greater number of determinations that now have been performed with **1**. The *N*-desmethyl primary amine counterpart of **1** (i.e., **2**; pEC_{50} = 6.29; Table 1) was found to be about half as potent as **1** as a positive allosteric modulator at $\alpha4\beta2$ receptors. Hence, all subsequent compounds retained the *N*-methyl group associated with **1**.

The next several compounds addressed the role of the indole 2-position substituent. Complete elimination of the 2-position substituent (i.e., *N*-methyl-6-bromotryptamine, **3**) resulted in a compound that failed to enhance the action of ACh at the highest concentrations at which it was evaluated (Table 1). Similar results were obtained with the simple *n*-propyl analog **4** (Table 1). Either unsaturation and/or at least one of the *gem*-dimethyl groups is important for the ability of **1** to behave as a positive allosteric modulator at $\alpha4\beta2$ nACh receptors. The propenyl compound **5**, lacking the *gem*-dimethyl groups of **1**, also lacked potentiating activity, whereas the saturated *gem*-dimethyl analog of **1**, **6** (pEC_{50} = 6.86), was at least as potent, if not twice as potent, as **1**. Evidently, the *gem*-dimethyl groups, but not the unsaturation associated with the chain, is required for allosteric action.



dx.doi.org/10.1021/jm200834x |*J. Med. Chem.* 2011, 54, 7259–7267

Table 2. Potency of Examined Analogs of Desformylflustrabromine (1), Relative to 1, To Inhibit the Effect of ACh on Human α7 nACh Receptors Expressed in *Xenopus* Oocytes As Measured Using Two-Electrode Voltage Clamp Methods

	pIC_{50}	±SEM
1	5.73	0.05
2	5.24	0.14
3	4.96	0.32
4	4.68	0.10
5	4.53	0.14
6	4.59	0.24
7	5.11	0.14
8	4.69	0.20
9	5.01	0.10
10	4.35	0.19
11	5.32	0.11
12	ND[a]	

[a] ND = Not determined.

Before continuing to investigate 2-position substitution, the necessity of the 6-bromo group was examined. Desbromo **1** and **6** (i.e., **7** and **8**; pEC_{50} = 5.14 and 5.52, respectively) (Table 1) retained the allosteric action of their parents but were substantially less potent. Apparently, the bromo group contributes to potency, but its presence is not required for the compounds to behave as positive allosteric modulators at $\alpha4\beta2$ nACh receptors.

Further deconstruction of **7** and **8** provided analogs **9**−**12**. Again suggesting that the *gem*-dimethyl groups are important, the racemic monomethyl counterpart of **8** (i.e., **9**) (Table 1) was inactive (as a positive allosteric modulator). This is further supported by the inactivity of **10**. Interestingly, although the inactive compound **12** possesses a *gem*-dimethyl group, compound **11** (pEC_{50} = 5.40) is active (Table 1). It would seem that it is not simply the presence of the *gem*-dimethyl group but, rather, the quaternary nature of this carbon atom that contributes to activity.

Effect of Deconstructed Analogs of 1 on Blocking the Functional Effects of ACh at $\alpha4\beta2$ nACh Receptors. As previously shown for **1**,[8] high concentrations of all the deconstructed compounds blocked the action of 10 μM ACh at $\alpha4\beta2$ nACh receptors. For those compounds acting as positive allosteric modulators, pIC_{50} values could not be determined with accuracy. pIC_{50} values for compounds not acting as positive allosteric modulators ranged from 5.45 for **5** to 4.14 for **4**; additional results are shown in Table 1. The mechanism(s) whereby these analogs are able to functionally antagonize the actions of ACh at $\alpha4\beta2$ receptors remains to be elucidated.

Effect of deconstructed analogs of 1 on α7 nACh receptor function. As an initial measure of selectivity, compounds were examined for their ability to potentiate the electrophysiological effects of ACh at α7 receptors. None proved to be active at the highest concentrations examined. However, all of the examined compounds inhibited the action of 10 μM ACh at α7 receptors (Table 2). Interestingly, whereas **1** attenuated the action of ACh at α7 receptors at a concentration of only 6 times greater than that required for potentiation of ACh at $\alpha4\beta2$ receptors, its reduced counterpart **6** was 190-fold selective for the latter.

CONCLUSION

Deconstruction of the structure of the positive allosteric modulator **1** has shown, although several "deconstructed" analogs retained activity, that the intact structure of **1** seems optimal as a positive allosteric modulator at $\alpha4\beta2$ nACh receptors. The reduced counterpart of **1** (i.e., **6**), retains the action and potency of **1**; however, **6** offers no synthetic advantage in that it was synthesized by reduction of **1**. Also learned was that (a) *N*-demethylation of **1** to its primary amine reduces potency by 50%, (b) the 6-bromo group of **1** (and **6**) contributes to potency, not action, (c) the presence of the *gem*-dimethyl groups plays an important role in conferring activity, and (d) it is likely that the quaternary nature of the branched carbon atom, not simply the presence of *gem*-dimethyl groups, defines this action. Finally, as with **1**,[8] analogs **2**−**11** were capable of attenuating the action of ACh at α7 receptors; however, compound **6** was considerably more selective than **1** as a positive allosteric modulator at $\alpha4\beta2$ receptors. This might be an advantage for functional studies where both receptor types are present. In summary, structural features of **1** important for its action and potency as a positive allosteric modulator at $\alpha4\beta2$ nACh receptors were determined, pharmacophoric features were identified, and **6** was found to be at least as potent as, and more selective than, **1** in this regard.

EXPERIMENTAL SECTION

Chemistry. Melting points were taken in glass capillary tubes on a Thomas-Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded either with a Varian EM-390 or Bruker 400 MHz spectrometer, and peak position are given in parts per million (δ) downfield from tetramethylsilane as an internal standard. Purity of compounds (>95%) was established by elemental analysis; microanalyses were performed by Atlantic Microlab (GA) for the indicated elements, and the results are within 0.4% of theoretical values. Reactions and product mixtures were routinely monitored by thin-layer chromatography (TLC) on silica gel precoated F_{254} Merck plates, and chromatographic separations were performed using Aldrich Silica gel 60 columns unless otherwise stated.

6-Bromo-2-(1,1-dimethylallyl)tryptamine Hydrochloride (2). Following the addition of *tert*-BuOCl (0.10 g, 0.9 mmol) to a stirred solution of *tert*-butyl 2-(6-bromo-1*H*-indol-3-yl)ethylcarbamate[12] (0.25 g, 0.7 mmol) and Et_3N (0.90 g, 0.9 mmol) in THF (10 mL) at −78 °C, stirring was allowed to continue for 45 min. Freshly prepared prenyl 9-BBN (1.5 mmol)[13] was added in a dropwise manner over a 20-min period while the temperature was maintained at −78 °C. The reaction mixture was allowed to warm to room temperature and stirring was continued for an additional 2 h. Aqueous NaOH (3 M, 3 mL) and H_2O_2 (30%, 3 mL) were added in a dropwise fashion and stirring continued for another 1 h. The reaction mixture was diluted with Et_2O (100 mL), and the organic portion was washed with H_2O (3 × 30 mL) and brine (40 mL) and dried (Na_2SO_4). The solution was evaporated to dryness and the residue was purified by chromatography using hexanes/EtOAc (10:1) as eluent to afford 0.10 g (33%) of a white foam.

Gaseous HCl was bubbled into a stirred solution of the above compound (0.09 g, 0.22 mmol) in dry EtOAc (10 mL) at 0 °C; stirring was allowed to continue for 24 h, and the solvent was evaporated to yield a white solid. Recrystallization from EtOAc/MeOH provided 0.03 g (33%) of **2** as white crystals: mp 256−258 °C; ^{1}H NMR (DMSO-d_6) δ 1.49 (s, 6H, $2CH_3$), 2.85 (t, 2H, CH_2), 3.05 (t, 2H, CH_2), 5.05−5.10 (m, 2H, CH), 6.10−6.17 (m, 1H, CH), 7.12 (dd, 1H, ArH), 7.48−7.51 (m, 2H, ArH), 7.98 (br s, 3H, NH_3^+), 10.76 (s, 1H, NH). Anal. Calcd for ($C_{15}H_{19}BrN_2 \cdot HCl \cdot 0.25H_2O$) C, H, N.

***N*-Methyl-6-bromo-2-*n*-propyltryptamine Oxalate (4).** According to the procedure described for preparation of **17**, compound **15** (0.50 g, 1.27 mmol) was treated with $NaBH_4$ (0.10 g, 2.54 mmol) and $CoCl_2 \cdot 6H_2O$ (0.3 g, 1.27 mmol) to give 0.40 g (78%) of the reduced

compound as a white solid: mp 157−159 °C; ^{1}H NMR ($CDCl_3$) δ 1.01 (t, J = 7.2 Hz, 3H, CH_3), 1.42 (s, 9H, *t*-Bu), 1.62−1.77 (m, 2H, CH_2), 2.63−2.78 (m, 2H, CH_2), 2.80−2.96 (m, 2H, CH_2), 2.88 (s, 3H, N−CH_3), 3.33−3.46 (m, 2H, CH_2), 2.20 (dd, J = 1.5, 8.4 Hz, 1H, ArH), 7.35−7.47 (m, 2H, ArH), 7.86 (br s, 1H, NH).

The reduced product was deprotected as follows: TFA (0.8 mL) was added to a solution of the above compound (0.13 g, 0.33 mmol) in CH_2Cl_2 (5 mL) at room temperature, and the reaction mixture was allowed to stir for 0.5 h. An additional 10 mL of CH_2Cl_2 was added; the solution was cooled to 0 °C, treated with a saturated solution of $NaHCO_3$, and extracted with EtOAc (3 × 10 mL). The combined organic portion was dried (Na_2SO_4) and the solvent was removed. The crude product was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH 9:1:0.1) to give 0.10 g (98%) of the free base of **4** as a brown oil: ^{1}H NMR ($CDCl_3$) δ 0.96 (t, J = 7.2 Hz, 3H, CH_3), 1.59−1.74 (m, 2H, CH_2), 2.52 (s, 3H, N−CH_3), 2.63−2.73 (m, 2H, CH_2), 2.86−3.03 (m, 4H, 2CH_2), 4.65 (br s, 1H, amine NH), 7.16 (dd, J = 1.8, 8.4 Hz, 1H, ArH), 7.35−7.43 (m, 2H, ArH), 8.25 (br s, 1H, NH). The free base in Et_2O (5 mL) was treated with ethereal oxalic acid. The precipitated oxalate salt was collected by filtration, washed with anhydrous Et_2O (3 × 5 mL), and recrystallized from absolute EtOH/anhydrous Et_2O to afford 0.05 g (37%) of the salt as a off-white solid: mp 211−213 °C; ^{1}H NMR (DMSO-d_6) δ 0.91 (t, J = 7.2 Hz, 3H, CH_3), 1.58−1.72 (m, 2H, CH_2), 2.60 (s, 3H, N−CH_3), 2.63−2.69 (m, 2H, CH_2), 2.91−3.04 (m, 4H, 2CH_2), 7.09 (dd, J = 1.5, 8.4 Hz, 1H, ArH), 7.41−7.48 (m, 2H, ArH), 11.09 (br s, 1H, NH). Anal. Calcd for ($C_{14}H_{19}BrN_2 \cdot C_2H_2O_4$) C, H, N.

***N*-Methyl-2-allyl-6-bromotryptamine Oxalate (5).** According to the procedure described for the preparation of **4**, compound **15** (0.07 g, 0.17 mmol) was treated with TFA (0.5 mL) to give 0.04 g (74%) of the free base **5** as a pale-yellow oil which was treated with ethereal oxalic acid to afford the oxalate salt. Recrystallization from absolute EtOH/anhydrous Et_2O afforded 0.02 g (24%) of product as a pale-yellow solid: mp 198−203 °C (dec); ^{1}H NMR (DMSO-d_6) δ 2.62 (s, 3H, N−CH_3), 2.91−3.08 (m, 4H, 2CH_2), 3.50 (d, J = 6.0 Hz, 2H, allylic CH_2), 5.08−5.20 (m, 2H, vinylic H), 5.99 (m, 1H, vinylic H), 7.13 (br d, J = 8.7 Hz, 1H, ArH), 7.43−7.53 (m, 2H, ArH), 11.09 (br s, 1H, NH). Anal. Calcd for ($C_{14}H_{17}BrN_2 \cdot C_2H_2O_4$) C, H, N. Note: subsequent studies showed this product to be an isomeric mixture of positional isomers (see the text).

***N*-Methyl-6-bromo-2-(1,1-dimethylpropyl)tryptamine Oxalate (6).** Sodium borohydride (0.02 g, 0.37 mmol) was added in portions to a solution of **16**[8] (0.08 g, 0.18 mmol) and $CoCl_2 \cdot 6H_2O$ (0.04 g, 0.18 mmol) in EtOH (2 mL) at 0 °C. The reaction mixture was allowed to stir under a N_2 atmosphere at room temperature for 1 h and quenched by addition of H_2O (5 mL). The aqueous solution was extracted with Et_2O (3 × 10 mL). The combined organic portion was dried (Na_2SO_4) and solvent was removed under reduced pressure. The crude product was purified by column chromatography (hexane/EtOAc 5:1) to give 0.06 g (76%) of **17** as a white solid: mp 154−157 °C; ^{1}H NMR ($CDCl_3$) δ 0.79 (t, J = 7.2 Hz, 3H, CH_3), 1.47 (s, 6H, 2CH_3), 1.52 (s, 9H, *t*-Bu), 1.78 (q, J = 7.2 Hz, 2H, CH_2), 2.93 (s, 3H, N−CH_3), 3.02−3.09 (m, 2H, CH_2), 3.36−3.46 (m, 2H, CH_2), 7.19 (dd, J = 1.5, 8.4 Hz, 1H, ArH), 7.41−7.48 (m, 2H, ArH), 7.92 (br s, 1H, NH).

The reduced product (0.08 g, 0.18 mmol) was treated with TFA (0.45 mL) to give the free base of **6** (0.06 g, 96%) as a brown oil which was converted to the oxalate salt: mp 192−194 °C following recrystallization from absolute EtOH/anhydrous Et_2O; ^{1}H NMR (DMSO-d_6) δ 0.68 (t, J = 7.2 Hz, 3H, CH_3), 1.39 (s, 6H, 2CH_3), 1.70 (q, J = 7.2 Hz, 2H, CH_2), 2.63 (s, 3H, N−CH_3), 2.90−3.01 (m, 2H, CH_2), 3.06−3.16 (m, 2H, CH_2), 7.10 (dd, J = 1.5, 8.7 Hz, 1H, ArH), 7.44 (d, J = 1.5 Hz, 1H, ArH), 7.48 (d, J = 8.7 Hz, 1H, ArH), 10.70 (br s, 1H, NH). Anal. Calcd for ($C_{16}H_{23}BrN_2 \cdot C_2H_2O_4$) C, H, N.

***N*-Methyl-2-(1,1-dimethylallyl)tryptamine Hydrochloride (7).** *tert*-Butyl hypochlorite (0.19 mL, 2.15 mmol) was added in a dropwise manner to a stirred solution of **18**[11] (0.49 g, 1.8 mmol) and Et_3N (0.30 mL, 2.2 mmol) in anhydrous THF (7.0 mL) at −78 °C. The clear solution was allowed to stir for an additional 0.5 h before freshly prepared prenyl 9-BBN[13] (0.68 g, 3.59 mmol) solution in THF was added in a dropwise manner. After 30 min the reaction mixture was allowed to warm to room temperature, and stirring was continued for 1 h. The addition of 3 M NaOH (1.8 mL) and 30% H_2O_2 (1.8 mL) was followed by stirring for 1 h. The reaction mixture was diluted with Et_2O (30 mL), the organic layer was washed with 3 M NaCl solution (3 × 50 mL) and dried (Na_2SO_4), and solvent was removed under reduced pressure. The resultant residue was subjected to flash chromatography (hexanes/EtOAc 10:1) to give 0.30 g (40%) of **19** as an oil which crystallized upon standing: mp 141−143 °C; ^{1}H NMR ($CDCl_3$): δ 1.51 (s, 9H, Boc), 1.58 (s, 6H, 2CH_3), 2.94 (s, 3H, N−CH_3), 2.93−3.10 (m, 2H, CH_2), 3.38−3.52 (m, 2H, CH_2), 5.18−5.22 (m, 2H, vinylic H), 6.16 (dd, J = 10.5, 17.1 Hz, 1H, vinylic H), 7.16 (m, 2H, ArH), 7.30−7.35 (m, 1 H, ArH), 7.61 (m, 1H, ArH), 7.91 (br s, 1H, NH).

Gaseous HCl was bubbled through a solution of **19** (0.10 g, 0.29 mmol) in anhydrous EtOAc (10 mL). The salt was recrystallized from MeOH/anhydrous Et_2O to afford 0.04 g (50%) of **7** as an off-white solid: mp 223−225 °C; ^{1}H NMR (DMSO-d_6) δ 1.56 (s, 6H, 2CH_3), 2.61 (s, 3H, N−CH_3), 2.90−3.00 (m, 2H, CH_2), 3.02−3.12 (m, 2H, CH_2), 5.12 (dd, J = 1.2, 17.0 Hz, 2H, vinylic H), 6.16 (dd, J = 10.5, 17.0 Hz, 1H, vinylic H), 6.88−7.08 (m, 2H, ArH), 7.30 (d, J = 8.0 Hz, 1H, ArH), 7.59 (d, J = 8.0 Hz, 1H, ArH), 10.61 (br s, 1H, NH). Anal. Calcd for ($C_{16}H_{22}N_2 \cdot HCl$) C, H, N.

***N*-Methyl-2-(1,1-dimethylpropyl)tryptamine Hydrochloride (8).** A catalytic amount of 10% Pd/C (0.02 g) was added to **19** (0.09 g, 0.26 mmol) in MeOH (15 mL) and hydrogenated at ca. 50 psi for 3 h. The catalyst was removed by filtration, and the solvent was removed under reduced pressure. Compound **20** (0.09 g, 92%; mp 172−174 °C) was obtained as an off-white solid and used without further purification.

Gaseous HCl was bubbled through a solution of **20** (0.09 g, 0.25 mmol) in dry EtOAc (10 mL). The salt was recrystallized from MeOH/anhydrous Et_2O to afford 0.04 g (50%) of **8** as an off-white solid: mp 234−235 °C; ^{1}H NMR (DMSO-d_6) δ 0.69 (t, J = 7.5 Hz, 3H, CH_3), 1.41 (t, J = 12 Hz, 6H, 2CH_3), 1.73 (q, J = 7.5 Hz, 2H, CH_2), 2.58 (s, 3H, N−CH_3), 2.87−3.00 (m, 2H, CH_2), 3.05−3.23 (m, 2H, CH_2), 6.88−7.08 (m, 2H, ArH), 7.30 (d, J = 7.5 Hz, 1H, ArH), 7.59 (d, J = 7.5 Hz, 1H, ArH), 10.51 (br s, 1H, NH). Anal. Calcd for ($C_{16}H_{24}N_2 \cdot HCl \cdot 0.25H_2O$) C, H, N.

***N*-Methyl-2-*sec*-butyltryptamine Oxalate (9).** Ethyl chloroformate (0.06 mL, 0.65 mmol) was added in a dropwise manner to a solution of **22** (0.14 g, 0.65 mmol) in $CHCl_3$ (5 mL) at 0 °C, followed by addition of aqueous 4 M NaOH (0.17 mL, 0.65 mmol). The reaction mixture was allowed to stir at room temperature for 2 h and diluted with $CHCl_3$ (20 mL). The organic portion was separated, washed with H_2O (20 mL), and dried (Na_2SO_4), and solvent was removed under reduced pressure to afford 0.14 g (75%) of *N*-Boc-**22** as a brown oil: ^{1}H NMR ($CDCl_3$) δ 0.95 (t, J = 6 Hz, 3H, CH_3), 1.21−1.61 (m, 6H, 2CH_3), 1.62−2.12 (m, 2H, CH_2), 3.03 (m, 3H, CH_2, CH), 3.32−3.70 (m, 2H, CH_2), 3.95−4.30 (m, 2H, CH_2), 7.13−7.21 (m, 2H, ArH), 7.38 (d, J = 9 Hz, 1H, ArH), 7.62 (d, J = 9 Hz, 1H, ArH), 8.33 (br s,1H, NH). The crude reaction product was directly used in the next step.

A solution of the above product (0.14 g, 0.48 mmol) in anhydrous THF (5 mL) was added in a dropwise manner to a stirred suspension of $LiAlH_4$ (0.11 g, 3 mmol) in dry THF (10 mL) at 0 °C. The stirred mixture was heated at reflux under a N_2 atmosphere for 2 h; cooled to 0 °C; successively quenched with MeOH (1 mL), H_2O (1.5 mL), and NaOH (3 M, 1 mL); and diluted with CH_2Cl_2 (10 mL). The organic portion was dried (Na_2SO_4) and concentrated under reduced pressure. The crude residue was purified on a silica gel column using CH_2Cl_2/MeOH (9:1) → CH_2Cl_2/MeOH/Et_3N (9:1:0.1) as eluent to afford the amine as a brown oil. The oxalate salt was prepared and recrystallized from MeOH/anhydrous Et_2O to give 0.08 g (48%) of **9** as a beige solid: mp 180−181 °C; ^{1}H NMR (DMSO-d_6) δ 0.79 (t, J = 6.9 Hz, 3H, CH_3),

1.29 (d, J = 6.9 Hz, 3H, CH_3), 1.57−1.72 (m, 2H, CH_2), 2.57−2.67 (m, 4H, $2CH_2$), 2.62 (s, 3H, CH_3), 2.93−3.00 (m, 1H, CH), 6.95−7.08 (m, 2H, ArH), 7.29 (d, J = 7.5 Hz, ArH), 7.50 (d, J = 7.5 Hz, 1H, ArH), 10.79 (s, 1H, NH_3^+). Anal. Calcd for ($C_{15}H_{22}N_2 \cdot C_2H_2O_4 \cdot 0.25H_2O$) C, H, N.

***N*-Methyl-2-(*n*-propyl)tryptamine Oxalate (10).** A suspension of **4** (0.13 g, 0.33 mmol) in absolute EtOH (10 mL) was hydrogenated in the presence of 10% Pd/C (0.03 g) under a H_2 atmosphere (45 psi) at room temperature for 1 h. The catalyst was removed by filtration, and the filtrate was evaporated to give 0.10 g (100%) of a cream-colored solid: mp 142−144 °C; ^{1}H NMR ($CDCl_3$) δ 1.01 (t, J = 7.5 Hz, 3H, CH_3), 1.44 (s, 9H, $3CH_3$), 1.64−1.80 (m, 2H, CH_2), 2.74 (t, J = 7.8 Hz, 2H, CH_2), 2.82−3.00 (m, 2H, CH_2), 2.90 (s, 3H, N−CH_3), 3.43 (t, J = 7.8 Hz, 2H, CH_2), 7.10 (dt, J = 1.5, 6.9 Hz, 1H, ArH), 7.14 (dt, J = 1.5, 6.9 Hz, 1H, ArH), 7.30 (dd, J = 1.5, 6.9 Hz, 1H, ArH), 7.56 (m, 1H, ArH), 7.83 (br s, 1H, NH).

Using the procedure described for **4**, the amine (0.1 g, 0.31 mmol) was treated with TFA (0.8 mL) to give 0.06 g (93%) of a pale-yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.01 (t, J = 7.5 Hz, 3H, CH_3), 1.64−1.78 (m, 2H, CH_2), 2.47 (s, 3H, N−CH_3), 2.75 (t, J = 7.5 Hz, 2H, CH_2), 2.83−3.00 (m, 4H, $2CH_2$), 7.10 (dt, J = 1.5, 6.9 Hz, 1H, ArH), 7.15 (dt, J = 1.5, 6.9 Hz, 1H, ArH), 7.30 (m, 1H, ArH), 7.58 (dd, J = 1.5, 6.9 Hz, 1H, ArH), 8.00 (br s, 1H, NH). The free base in Et_2O (5 mL) was treated with an ethereal solution of oxalic acid to afford 0.07 g (69%) of **10**: mp 177−179 °C after recrystallization from absolute EtOH/anhydrous Et_2O; ^{1}H NMR (DMSO-d_6) δ 0.92 (t, J = 7.2 Hz, 3H, CH_3), 1.56−1.74 (m, 2H, CH_2), 2.60 (s, 3H, N−CH_3), 2.67 (t, J = 7.5 Hz, 2H, CH_2), 2.88−3.06 (m, 4H, $2CH_2$), 6.91−7.04 (m, 2H, ArH), 7.25 (br d, J = 7.8 Hz, 1H, ArH), 7.48 (br d, J = 7.8 Hz, 1H, ArH), 10.85 (br s, 1H, NH). Anal. Calcd for ($C_{14}H_{20}N_2 \cdot C_2H_2O_4$) C, H, N.

***N*-Methyl-2-(*tert*-butyl)tryptamine Hydrogen Oxalate (11).** Oxalyl chloride (0.2 mL, 2.308 mmol) was added in a dropwise manner at −5 °C to a stirred solution of 2-*tert*-butyl-1*H*-indole[14] (0.02 g, 1.154 mmol) in anhydrous Et_2O (10 mL). The reaction mixture was allowed to stir for 6 h at 0 °C; after evaporation of solvent under reduced pressure, methylamine (40% aqueous solution, 5 mL) was added at room temperature, and the reaction mixture was allowed to stir overnight. The precipitate was collected by filtration and recrystallized from MeOH to afford 0.17 g (56%) of the corresponding glyoxylamide as brown solid: mp 190−191 °C; ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 9H, $3CH_3$), 3.1 (s, 3H, N−CH_3), 6.77 (s, 1H, NH), 7.07−7.45 (m, 4H, ArH), 7.76 (br s, 1H, NH).

A solution of the glyoxylamide (0.17 g, 0.65 mmol) in dioxane (5 mL) was added to a stirred suspension of $LiAlH_4$ (0.25 g, 6.5 mmol) in dioxane (10 mL) at 60 °C and then heated at reflux overnight. The reaction mixture was cooled to room temperature and diluted with THF (10 mL), MeOH (3 mL), and NaOH (3N, 3 mL). The mixture was filtered and the filtrate was heated at reflux with THF (15 mL). The combined organic portion was evaporated to dryness under reduced pressure, and the residue was purified by column chromatography using CH_2Cl_2/MeOH (10:1) as eluent to afford the desired amine. The oxalate salt was prepared to give 0.03 g (56%) of **11** as white flakes after recrystallization from 2-PrOH: mp 194−195 °C; ^{1}H NMR (DMSO-d_6) δ 1.43 (s, 9H, $3CH_3$), 2.64 (s, 3H, N−CH_3), 2.94 (d, 2H, CH_2), 3.15 (d, 2H, CH_2), 6.96−7.51 (m, 4H, ArH), 10.55 (s, 1H, $R_2NH_2^+COO^-$). Anal. Calcd for ($C_{15}H_{22}N_2 \cdot (C_2H_2O_4) \cdot$2-PrOH) C, H, N.

***N*-Methyl-2-isopropyltryptamine Hydrochloride (12).** A solution of **26** (0.17 g, 0.61 mmol) in dry THF (5 mL) was added to a stirred suspension of $LiAlH_4$ (0.14 g, 3.7 mmol) in anhydrous THF (10 mL) at 0 °C, and the reaction mixture was heated at reflux for 2 h, cooled in an ice bath, and quenched with MeOH (1 mL), NaOH (15%, 1.5 mL) and H_2O (1 mL). The organic portion was separated and dried (Na_2SO_4), and solvent was evaporated under reduced pressure to yield an oily residue. Purification by column chromatography using CH_2Cl_2/MeOH (9:1) → CH_2Cl_2/MeOH/Et_3N (9:1:0.1) afforded 0.07 g of the amine as a light-yellow oil: ^{1}H NMR (DMSO-d_6) δ 1.28 (d, J = 9 Hz, 6H, $2CH_3$), 2.51 (s, 3H, CH_3), 2.60−2.70 (m, 2H, CH_2), 2.74−2.81 (m, 2H, CH_2), 3.22 (m, 1H, CH), 6.90−7.00 (m, 2H, ArH), 7.25 (d, J = 9 Hz, 1H, ArH), 7.48 (d, J = 9 Hz, 1H, ArH).

Gaseous HCl was bubbled through a solution of the amine in dry EtOAc (10 mL) to obtain 0.04 g (50%) of **12** as white crystals after recrystallization from MeOH/anhydrous Et_2O: mp 212−213 °C; ^{1}H NMR (DMSO-d_6) δ 1.30 (d, J = 6.9 Hz, 6H, 2 × CH_3), 2.59 (s, 3H, CH_3), 2.92−3.05 (m, 4H, $2CH_2$), 3.24 (m, 1H, CH), 6.95−7.08 (m, 2H, ArH), 7.29 (d, J = 7.5 Hz, 1H, ArH), 7.53 (d, J = 7.5 Hz, 1H, ArH), 9.00 (s, 1H, NH), 10.87 (br br s, 1H, NH_3^+). Anal. Calcd for ($C_{14}H_{20}N_2 \cdot$HCl) C, H, N.

***N*-[2-(2-Allyl-6-bromo-1*H*-indol-3-yl)ethyl]-*N*-methylcarbamic Acid *tert*-Butyl Ester (15).** *tert*-Butyl hypochlorite (0.58 mL, 5.10 mmol) was added in a dropwise manner to a stirred solution of **14**[8] (1.50 g, 4.25 mmol) and Et_3N (0.71 mL, 5.10 mmol) in THF (25 mL) at −78 °C under a N_2 atmosphere. After 1.5 h at −78 °C, allyltributyltin (2.61 mL, 8.49 mmol) was added followed by addition of $BF_3 \cdot Et_2O$ (1.1 mL, 8.49 mmol). The reaction mixture was allowed to stir for 0.5 h at the same temperature, and then quenched by addition of a saturated aqueous solution of $NaHCO_3$ (10 mL). Ethyl acetate (100 mL) and H_2O (100 mL) were added, and the aqueous portion was extracted with EtOAc (3 × 50 mL). The combined organic portion was dried (Na_2SO_4) and solvent was removed under reduced pressure. The residue was purified by column chromatography (hexane/EtOAc 7:1) to give 0.74 g (44%) of a white solid: mp 138−140 °C. The product was immediately used in the synthesis of **4**.

2-*sec*-Butyltryptamine (22). A solution of 2-*sec*-butylindole (**21**)[14] (0.35 g, 2 mmol) in CH_2Cl_2 (2 mL) was added to a stirred solution of 1-dimethylamino-2-nitroethylene (0.23 g, 2 mmol) in TFA (1.2 mL) at 0 °C. The reaction mixture was allowed to stir for 2 h, poured into ice-cold H_2O (50 mL), and extracted with EtOAc (2 × 20 mL). The combined organic portion was washed with brine (20 mL) and dried (Na_2SO_4), and solvent was removed under reduced pressure. The residue was recrystallized from EtOAc to yield the corresponding nitroalkene (0.27 g, 75% based on recovered starting material) as orange crystals: mp 141 °C; ^{1}H NMR ($CDCl_3$) δ 0.94 (t, J = 7.5 Hz, 3H, CH_3), 1.43 (d, J = 7.0 Hz, 3H, CH_3), 1.72−1.84 (m, 2H, CH_2), 3.28 (m, 1H, CH), 7.30−7.34 (m, 2H, ArH), 7.42−7.48 (m, 1H, CH), 7.72−7.78 (m, 1H, CH), 7.87 (d, J = 13 Hz, 1H, ArH), 8.40 (d, J = 13 Hz, 1H, ArH), 8.74 (br s, 1H, NH).

A solution of the alkene (0.27 g, 1.1 mmol) in dry THF (5 mL) was added in a dropwise manner at 0 °C to a stirred suspension of $LiAlH_4$ (0.26 g, 6.9 mmol) in anhydrous THF (10 mL). The reaction mixture was heated overnight at reflux under a N_2 atmosphere; cooled to 0 °C; quenched with MeOH (1 mL), H_2O (1.5 mL), NaOH (1 mL); and diluted with CH_2Cl_2 (30 mL). After acid (2 N HCl, 30 mL)/base (3 N NaOH, 30 mL) extraction, the organic portion was separated and dried (Na_2SO_4), and solvent was removed under reduced pressure to give 0.14 g (62%) of **22** as a light-brown oil: ^{1}H NMR ($CDCl_3$) δ 0.91 (t, J = 7.5 Hz, 3H, CH_3), 1.33 (d, J = 6.0 Hz, 3H, CH_3), 1.62−1.79 (m, 2H, CH_2), 2.84−2.97 (m, 2H, CH_2), 2.98−3.12 (m, 3H, CH, CH_2), 7.03−7.25 (m, 2H, ArH), 7.33 (d, J = 8.0 Hz, 1H, ArH), 7.82 (br s, 1H, NH).

2-Isopropyl-1*H*-indole (23). A solution of *n*BuLi (1.6 M in hexane, 14 mL, 22.4 mmol) was added to a stirred solution of *N*-(*o*-tolyl)isobutyramide[15] (2.0 g, 11 mmol) in anhydrous THF (20 mL) at 0 °C. After stirring for 1 h, the reaction mixture was allowed to warm gradually to room temperature. After an additional 8 h, EtOAc (15 mL) and then saturated aqueous NH_4Cl (5 mL) were added. The organic portion was separated and dried (Na_2SO_4) and solvent was removed under reduced pressure. The crude oily residue was purified by column chromatography using hexanes/EtOAc (20:1) as eluent to afford 0.70 g (66% based on recovered material) of **23** as an off-white solid: mp 75−76 °C (lit.[16] mp 73−74 °C); ^{1}H NMR ($CDCl_3$) δ 1.41 (d, J = 6 Hz, 6H, 2 × CH_3), 3.09−3.16 (m, 1H, CH), 6.31 (s, 1H, CH), 7.10−7.16

(m, 2H, ArH), 7.35 (d, J = 9 Hz, 1H, ArH), 7.60 (d, J = 9 Hz, 1H, ArH), 7.85 (br s, 1H, NH).

2-Isopropyltryptamine (25). A solution of **23** (0.30 g, 1.9 mmol) in CH_2Cl_2 (2 mL) was added to a stirred solution of 1-dimethylamino-2-nitroethylene (0.22 g, 1.9 mmol) in TFA (1.2 mL) at 0 °C. The reaction mixture was allowed to stir at room temperature for 2 h, poured into ice-cold water (20 mL), and extracted with EtOAc (20 mL). The organic portion was washed with brine (15 mL) and dried (Na_2SO_4), and the solvent was removed under reduced pressure. The crude product was purified recrystallized from EtOAc to afford 0.18 g (60%) of **24** as orange crystals: mp 159 °C; ^{1}H NMR ($CDCl_3$) δ 1.44 (d, J = 6.0 Hz, 6H, 2 × CH_3), 3.51−3.62 (m, 1H, CH), 7.28−7.35 (m, 2H, ArH), 7.42−7.46 (m, 1H, CH), 7.72−7.75 (m, 1H, CH), 7.84 (d, J = 13 Hz, 1H, ArH), 8.42 (d, J = 13 Hz, 1H, ArH), 8.74 (br s, 1H, NH).

A solution of **24** (0.26 g, 1.1 mmol) in anhydrous THF (5 mL) was added in a dropwise manner to a stirred suspension of $LiAlH_4$ (0.26 g, 6.9 mmol) in THF (10 mL) at 0 °C. The reaction mixture was heated at reflux overnight, cooled in an ice bath, and quenched with MeOH (1 mL), NaOH (15%, 1.5 mL), and H_2O (1 mL). After acid (3 N HCl, 10 mL)/base (3 N NaOH, 15 mL) extraction, the organic portion was dried (Na_2SO_4) and solvent was removed under reduced pressure to afford 0.14 g (61%) of **25** as a light-yellow oil: ^{1}H NMR (DMSO-d_6) δ 1.28 (d, J = 7.5 Hz, 6H, $2CH_3$), 1.73 (br s, 2H, NH_2), 2.73−2.84 (m, 4H, 2 × CH_2), 3.21 (m, 1H, CH), 6.89−7.01 (m, 2H, ArH), 7.27 (d, J = 7.5 Hz, 1H, ArH), 7.44 (d, J = 7.5 Hz, 1H, ArH), 8.78 (br s, 1H, NH). Compound **25** was used immediately in the synthesis of **26**.

Ethyl [2-(2-Isopropyl-1*H*-indol-3-yl)ethyl]carbamate (26). Ethyl chloroformate (0.07 mL, 0.70 mmol) was added in a dropwise manner at 0 °C to a stirred solution of **25** (0.14 g, 0.7 mmol) in $CHCl_3$ (5 mL), followed by addition of a 4 N aqueous solution of NaOH (0.20 mL, 0.70 mmol). The reaction mixture was allowed to stir for 2 h at room temperature and diluted with $CHCl_3$ (15 mL). The organic portion was dried (Na_2SO_4), and solvent was evaporated under reduced pressure to afford 0.17 g (88%) of **26** as an oily residue which was used in the preparation of **12** without further purification.

Electrophysiology. The cDNA sequences for human α4 (NCBI Reference Sequence: NM_000744.5), β2 (NCBI Reference Sequence: NM_000748.2), and α7 (NCBI Reference Sequence: NM_000746.3) nACh receptor subunits were used to synthesize a full-length cDNA for each subunit. cDNA synthesis was conducted by GeneArt Inc. (Burlingame, CA). The synthetic β2 cDNA was inserted into the pcDNA3.1/Zeo(+) expression vector and the α4 cDNA was inserted into the pcDNA3.1/hygromyocin expression (vectors procured from Invitrogen, Carlsbad, CA).

Ovarian lobes were surgically removed from *Xenopus laevis* frogs and washed twice in Ca^{2+}-free Barth's buffer (82.5 mM NaCl/2.5 mM KCl/1 mM $MgCl_2$/5 mM HEPES, pH 7.4) and then gently shaken with 1.5 mg/mL collagenase (Sigma type II, Sigma-Aldrich) for 1 h at 20−25 °C. Stage IV oocytes were selected for microinjection. Synthetic cRNA transcripts for human α7 and $\alpha4\beta2$ were prepared using the mMESSAGE mMACHINE High Yield Capped RNA Transcription Kit (Ambion, TX). Oocytes were injected with a total of 50 nL cRNA at a concentration of 0.2 ng/nL in appropriate subunit ratios and then incubated at 19 °C for 24−72 h prior to their use in voltage clamp experiments. Recordings were made using an automated two-electrode voltage-clamp system incorporating an OC-725C oocyte clamp amplifier (Warner Instruments, CT) coupled to a computerized data acquisition (Datapac 2000, RUN technologies) and autoinjection system (Gilson). Recording and current electrodes with resistance 1−4 MΩ were filled with 3 M KCl. Details of the chambers and methodology employed for electrophysiological recordings have been described earlier.[17] Oocytes were held in a vertical flow chamber of 200 μL volume and perfused with ND-96 recording buffer (96 mM NaCl/2 mM KCl/1.8 mM $CaCl_2$/1 mM $MgCl_2$/5 mM HEPES; pH 7.4) at a rate of 20 mL/min. Test compounds were dissolved in ND-96 buffer and injected into the chamber at a rate of 20 mL/min using the Gilson autosampler injection system. Compounds were coapplied with the EC_{75} concentration (100 μM) of ACh.

Data Analysis. Concentration/response curves were fit by nonlinear curve fitting and GraphPad Prism Software (San Diego, CA) using standard built-in algorithms. For IC_{50} determinations, data were fit to a single site competition model. For the potentiation/inhibition curves obtained for $\alpha4\beta2$ modulation, the data were fit to a bell-shaped dose−response equation as previously described.[9]

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ACKNOWLEDGMENT

This study was funded, in part, by the National Institutes of Health, National Institute of Neurological Disorders and Stroke [Grant 1R01NS066059] and by a Virginia Center on Aging. N.G. was partially supported by TA grant DA 007027.

ABBREVIATIONS USED

ACh, acetylcholine; dFBr, desformylflustrabromine; nACh, nicotinic acetylcholine; TFA, trifluoroacetic acid

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