# Synthesis and Nicotinic Binding Studies on Enantiopure Diazine Analogues of the Novel (2-Chloro-5-pyridyl)-9-azabicyclo[4.2.1]non-2-ene UB-165

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*Received May 23, 2001*

As part of our program aimed at optimizing therapeutic effects over toxic effects (as observed in the naturally occurring nicotinic acetylcholine receptor modulators (−)-nicotine, (−)-epibatidine, (−)-ferruginine, and (+)-anatoxin-a), we investigated the bioisosteric potential of diazines in the field of (+)-anatoxin-a-type structures. In the series of diazine analogues of deschloro-UB-165 (DUB-165, **6**), bioisosteric replacement of the 3-pyridyl pharmacophoric element by a 4-pyridazinyl, 5-pyrimidinyl, or 2-pyrazinyl moiety resulted in novel nAChR ligands **7**, **8**, and **9**. A palladium-catalyzed Suzuki cross-coupling of the 3-diethylboranylpyridine (**14**) and a Stille cross-coupling of the corresponding tributylstannyl diazines **15**−**17** with the vinyl triflate **13** of the N-protected 9-azabicyclo[4.2.1]nonan-2-one **12** constitute the key steps in the syntheses of these enantiopure anatoxinoids **6**−**9**. Studies of the in vitro affinity for $(\alpha4)_2(\beta2)_3$, $\alpha3\beta4$*, and α7* nAChR subtypes by radioligand binding assays demonstrated that the diazine analogues **7**−**9** can be considered as pharmacologically attractive bioisosteres of DUB-165 (**6**) but with different effects on the binding affinity with regard to the diazine moiety. The pyrimidine-containing bioisostere **8** turned out to be the most active diazine analogue, which interacts potently ($K_i$ = 0.14 nM) with the $(\alpha4)_2(\beta2)_3$ subtype and differentiates significantly among the nAChR subtypes investigated. The nitrogens in this anatoxinoid **8** show by far the most negative atomic charges (calculated using the AM1 Hamiltonian). This qualitatively correlates with the highest binding affinity observed for **8** for all subtypes under consideration.

## Introduction

The alkaloids (−)-nicotine (**1**), (−)-epibatidine (**2**), (−)-ferruginine (**3**), and (+)-anatoxin-a (**4**) are known as more or less potent but not selective modulators of the nicotinic acetylcholine receptors (nAChRs) (Chart 1).[1−4] Although these prototypical ligands differ in a number of respects concerning the ligand/nAChR interaction, all of them proved to be too toxic to be useful therapeutic agents. It was anticipated, for example, that novel bioisosteric analogues might possess improved pharmacodynamic profiles and safety over the natural alkaloids, possibly resulting from higher discrimination between the multifarious receptor subtypes.

On the basis of the finding that bioisosteric modification of the alkaloidal toxins (−)-nicotine (**1**) and (−)-epibatidine (**2**) provided nicotinic ligands with the most promising therapeutic utility in the treatment of a variety of central nervous system disorders[1,2] or with antinociceptive qualities,[1,5−8] a series of novel neuronal nAChR ligands has been developed in the past few years.[1−4] Among others, the highly potent nicotinic agonist with subtype selectivity UB-165 (**5**)[9,10] [in Schmitt's (based on the structure) classification scheme[3] a nAChR ligand with both the cationic center and the hydrogen bond acceptor π-moiety (HBA/π) within separate, nonfused rings] proved to be one of the most prominent ligands that has undergone advanced characterization as potential therapeutic agent and seemed to be suitable to serve as a lead compound. It represents a novel anatoxin-a/epibatidine hybrid in which the acetyl group of anatoxin-a (**4**) is replaced by a 5-(2-chloropyridyl) ring, thus combining the bulky azabicyclo-[4.2.1]nonene moiety of anatoxin-a (**4**) with the chloropyridyl pharmacophoric element of epibatidine (**2**).[9]

Interestingly UB-165 (**5**, Chart 1), having an absolute configuration corresponding to that of natural anatoxin-a, exhibits a significant degree of enantiospecificity and interacts in a stereoselective manner with the $(\alpha4)_2(\beta2)_3$ nAChR subtype exhibiting a binding affinity ($K_i$ = 0.04 nM) that is between the binding affinities of anatoxin-a (**4**) and epibatidine (**2**).[9,11]

Bioisosterism is an important concept in medicinal chemistry.[12] It serves as a valuable aid in structure−activity relationship (SAR) studies and the design of new drugs, successfully applied only recently for the development of several potent nAChR ligands.[13−17] Thus, it was anticipated that combining the steric volume close to the $sp^3$-hybridized nitrogen, as in the azabicycle of deschloro-UB-165 (DUB-165, **6**), with diazines such as pyridazine, pyrimidine, and pyrazine, could lead to bioisosteric structures possessing significant affinity for the nAChRs. Aimed at exploiting the scope and potential offered by the resulting ligands **6**−**9**

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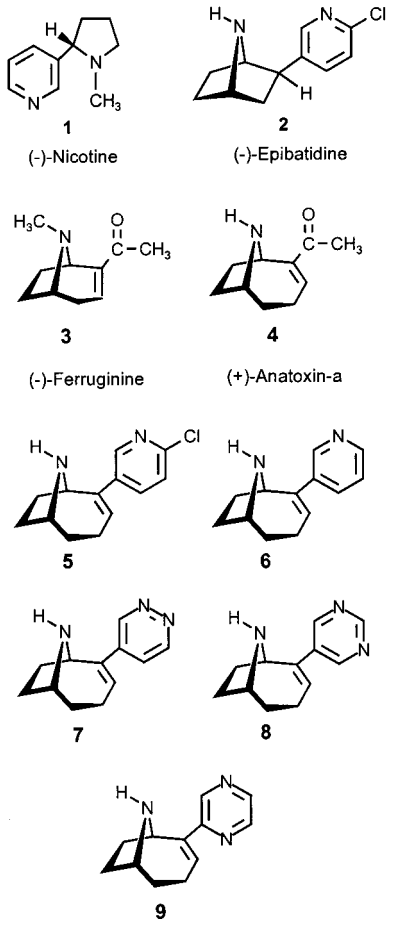
10.1021/jm010936y CCC: $22.00 
Published on Web 02/05/2002

**Chart 1.** Alkaloid nAChR Ligands Such as (−)-Nicotine (**1**), (−)-Epibatidine (**2**), (−)-Ferruginine (**3**), and (+)-Anatoxin-a (**4**) and Novel Analogues **6**−**9** of UB-165 (**5**)




(Chart 1), we report herein a new strategy for the syntheses of DUB-165 (**6**) and its bioisosteres **7**−**9** and describe a preliminary in vitro evaluation commonly used for the initial characterization of novel receptor ligands. The target ligands **6**−**9** are readily prepared utilizing enantiomerically pure N-protected (+)-2-tropanone **11** from the "chiral pool" as a versatile starting material and palladium-catalyzed cross-coupling reactions of the 3-diethylboranylpyridine (**14**) and the tributylstannyl diazines **15**−**17** with the vinyl triflate **13** of the azabicyclic ketone **12**.

## Results and Discussion

**Chemistry.** The synthetic route to DUB-165 (**6**), in which the 5-(2-chloropyridyl) pharmacophoric element of **5** is replaced by a 3-pyridyl moiety, started with the enantiomerically pure ketone **12** as chiral building block. This is easily accessible from (−)-cocaine hydrochloride (**10**) in a five-step route with the 2-tropanone **11** as intermediate (Scheme 1).[18,19] Most promising for the introduction of the 3-pyridyl unit into the bulky homotropane moiety seemed to be an approach utilizing a Suzuki-type cross-coupling,[20,21] the well-known palladium-catalyzed reaction of organoboron compounds with an organic electrophile as the pivotal step. Thus, the vinyl triflate **13** and 3-diethylboranylpyridine (**14**)[21] were examined as appropriate starting materials for the synthesis of the target ligand **6**. The vinyl triflate **13** was readily available from the ketone **12**.[19] When KHMDS in toluene was used at −78 °C, the corresponding potassium enolate of **12**[9,19] was generated and advantageously trapped with Comins' *N*-(5-chloro-2-pyridyl)triflimide to provide the requisite vinyl triflate **13** with 74% yield.[19] Then the 3-pyridyl group was introduced into the 2-position of the azabicycle by reacting triflate **13** with the borane **14** in THF using bis(triphenylphosphane)palladium(II) chloride as catalyst (0.01 equiv) and aqueous sodium carbonate as a nucleophilic activator. The reaction proceeded with satisfying success to give the target compound **18** in 38% isolated yield. Removal of the protecting group from carbamate **18** by treatment with aqueous hydrochloric acid (37%) afforded the desired DUB-165 (**6**), which could be isolated in 39% yield as an air-stable, pale-yellow oil. Because the Suzuki-type cross-coupling gave only fair yields of the target ligand **6**, another strategy for the syntheses of the diazine analogues of DUB-165 (**6**) was employed using the Stille cross-coupling[22−24] of the triflate **13** with the organostannanes **15**−**17**.

The optimal overall yield of the desired coupling product **19** was reproducibly achieved from triflate **13** and the organostannane **15**[25] in the presence of 10 mol % of $Pd(PhCN)_2Cl_2$ as the catalyst, in dry DMF as the solvent, and with the addition of 10 mol % CuI as a cocatalyst, essential for the success of the coupling.[22] The reaction was completed by heating the complex mixture for 3.5 h at 80 °C in the presence of 3 equiv of LiCl and of $Ph_3As$ as the ligand of choice, thereby increasing the yield of the N-protected DUB-165 analogue **19** to 89% after careful chromatographic purification. Removal of the carbamate group from compound **19** in the last step utilizing the synthetic protocol with $(CH_3)_3SiI$ in boiling $CHCl_3$[26] afforded the novel nAChR ligand **7** in 71% yield.

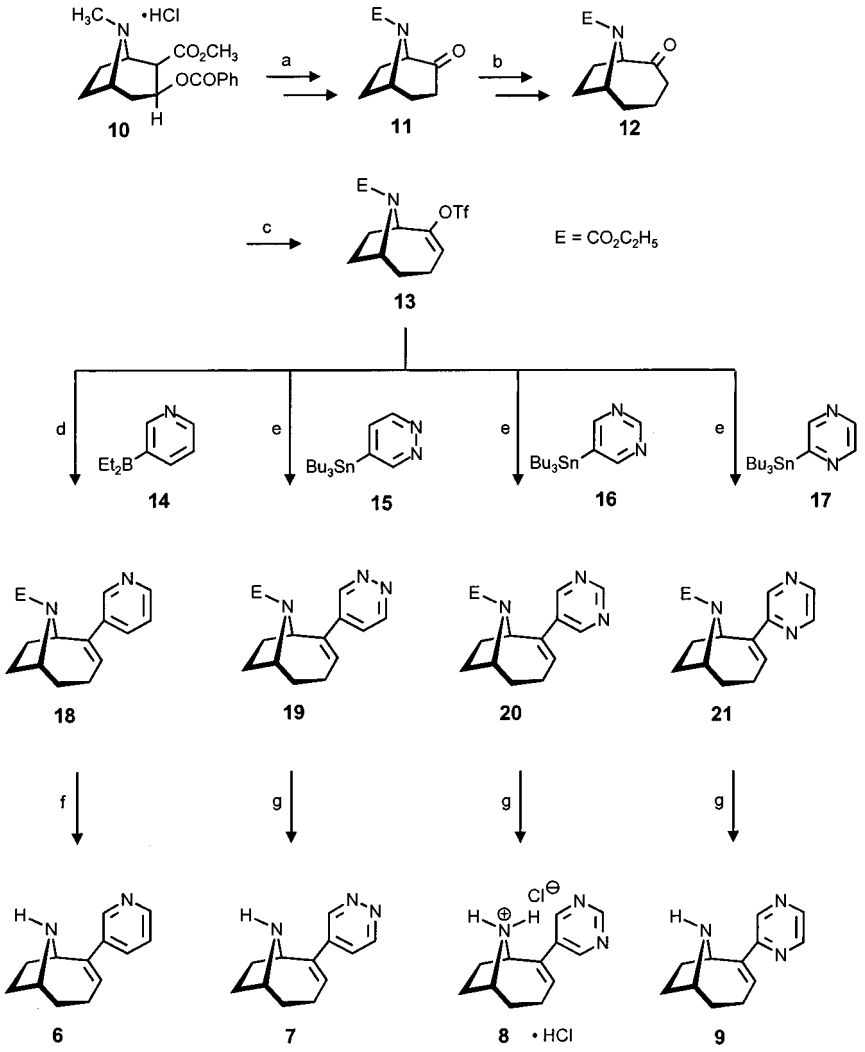
A similar approach for introduction of the pyrimidine nucleus into the bulky azabicyclo[4.2.1]nonane moiety utilized the stable organostannane **16** (see Supporting Information).[27−29] When $Pd(PhCN)_2Cl_2$ is employed as the catalyst, cross-coupling of the vinyl triflate **13** proceeded in 72% yield, again with $Ph_3As$ as the ligand to give the N-protected desired target compound **20**. After treatment with $CH_3OH$/HCl in $Et_2O$, deprotection of the carbamate **20** by $(CH_3)_3SiI$ in boiling $CHCl_3$ led to an intermediate methanol addition product[30] that is easily transformed to the hydrochloride of **8** by removal of the volatile components in vacuo.

The requisite organostannane **17**[29] (see Supporting Information) offered an elegant access to the pyrazine-substituted target ligand **9**. Cross-coupling with the vinyl triflate **13** could be achieved under similar conditions as described for the preparation of **7** and **8**. In this case the reaction mixture in DMF as the solvent was heated for 12 h at 85 °C, affording the coupling product **21** with 51% yield. Removal of the protecting group by the conventional protocol with $(CH_3)_3SiI$ in boiling $CHCl_3$ gave the bioisoster **9** with 50% yield.

The new nAChR ligands **6**−**9** exhibited the expected $^1H$ and $^{13}C$ NMR, IR, and mass spectral characteristics and gave satisfactory high-resolution mass spectral data.

**In Vitro Receptor Binding.** To address the issue of binding selectivity among nAChR subtypes, affinities

**Scheme 1**[a]

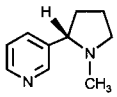
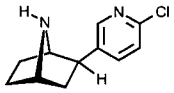
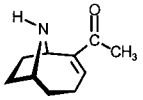
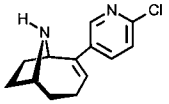
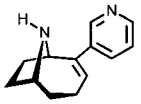
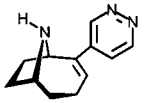
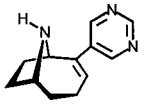
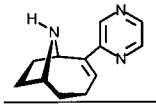



[a] Reagents and conditions: (a) lit. ref 26; (b) lit. ref 27; (c) KHMDS, DME, then 2-N($Tf_2$)-5-chloropyridine; (d) $Pd(PPh_3)_2Cl_2$, THF, 2 M aqueous $Na_2CO_3$, 80 °C; (e) $Pd(PhCN)_2Cl_2$, $Ph_3As$, CuI, LiCl, DMF, 80 °C; (f) 37% HCl, reflux; (g) $(CH_3)_3SiI$, $CHCl_3$, 80 °C; see Experimental Section.

of the UB-165 variants **6**−**9**, listed in Table 1, were measured in three different competition assays and compared with those of (−)-nicotine (**1**), (±)-epibatidine (**2**), (+)-anatoxin-a (**4**), and UB-165 (**5**). To determine the affinities for the $(\alpha4)_2(\beta2)_3$ nAChR subtype, a previously described competition assay[31] was used with (±)-[$^3$H]epibatidine and the P2 membrane fraction of the Sprague−Dawley rat forebrain. These studies demonstrated that the specific binding of (±)-[$^3$H]epibatidine to crude synaptic membranes of rat forebrain, at concentrations up to 800 pM, is characterized by a single population of binding sites with $K_d = 8 \pm 2$ pM.[31] To characterize binding of UB-165 (**5**) and each of the UB-165 variants **6**−**9** to the α7* nAChR subtype, [$^3$H]MLA and membrane fractions isolated from the rat brain were used, and [$^3$H]MLA bound to a single population of binding sites exhibited a $K_d$ value of 1.2 ± 0.2 nM ($n$ = 3). The affinity determined was in good agreement with previously published values[32] ($K_d$ = 1.86 nM). [$^3$H]-MLA bound to rat brain membranes with a regional distribution characteristic of α-BTX-sensitive, putative α7*-subunit-containing nAChRs.[32,33] To estimate the affinity for nAChRs containing α3 and β4 subunits, an assay using (±)-[$^3$H]epibatidine and a membrane fraction from pig adrenal glands was developed. This assay was based on a previous study that showed that (±)-[$^3$H]epibatidine, in addition to its high affinity for $(\alpha4)_2(\beta2)_3$ nAChRs in rat brain, bound to cells stably expressing receptors of the α3β4* subtype.[34,35] Further studies[36,37] suggested that the adrenal glands were rich in nAChR subtypes. Binding assays with (±)-[$^3$H]-epibatidine using pig adrenal gland membranes demonstrated a single population of binding sites (data not shown) with a $K_d$ value of 50 ± 7 pM ($n$ = 5), comparable to that from a previous study using rat adrenal glands.[38]

As shown in Table 1 the above characterized competition assays yielded $K_i$ values of 0.84 nM for (−)-nicotine (**1**) and 0.008 nM for (±)-epibatidine (**2**) for the $(\alpha4)_2(\beta2)_3$ subtype. These results are consistent with recently reported in vitro measurements of the alkaloids.[31,38] Compared to (±)-epibatidine (**2**), (+)-anatoxin-a (**4**) exhibited ca. 140-fold lower affinity ($K_i$ = 1.1 nM) for the $(\alpha4)_2(\beta2)_3$ subtype, approximately 20-fold lower affinity for the α7* subtype ($K_i$ = 90 nM), and a 45-fold lower affinity for the α3β4* subtype ($K_i$ = 19 nM). Remarkably, UB-165 (**5**) and DUB-165 (**6**) potently interact with all three nAChR subtypes under consideration with comparable high affinities, demonstrating

**Table 1.** Radioligand Binding Affinities of Several UB-165 Variants **6**−**9** to $(\alpha4)_2(\beta2)_3$, α7*, and α3β4* nAChRs in Comparison with (−)-Nicotine, (±)-Epibatidine, and (+)-Anatoxin-a[a]

| Structure | Compound | $(\alpha4)_2(\beta2)_3$[b] (±)-[$^3$H]-epibatidine rat brain $K_i$(nM) | α7*[b] [$^3$H]MLA rat brain $K_i$(nM) | α3β4*[b] (±)-[$^3$H]-epibatidine pig adr. gland $K_i$(nM) |
|---|---|---|---|---|
|  | (-)-Nicotine (**1**) | 0.84[38] ± 0.132 | 130 ± 10 [$^{125}$I]α-BTX[38] | 73 ± 2 |
|  | (±)-Epibatidine (**2**) | 0.008[38] ± 0.001 | 4.0 ± 0.5 [$^{125}$I] α-BTX[38] | 0.022 ± 0.0015 |
|  | (+)-Anatoxin-a (**4**) | 1.1 ± 0.2 | 90 ± 4[c] | 19 ± 1 |
|  | UB-165 (**5**) | 0.04 ± 0.004 | 12 ± 2.5 | 1.3 ± 0.1 |
|  | DUB-165 (**6**) | 0.051 ± 0.006 | 0.95 ± 0.05 | 6.2 ± 0.5 |
|  | **7** | 19.0 ± 2.5 | > 10.000 | 2.500 ± 150 |
|  | **8** | 0.14 ± 0.03 | 10.7 ± 1.3 | 20 ± 1 |
|  | **9** | 12.0 ± 1.8 | 250 ± 7.6 | 259 ± 21 |

[a] Values represent mean ± SEM obtained from *n* independent experiments where $n = 3-5$. [b] Naturally expressed nAChRs.[33] [c] (±)-Anatoxin-a exhibited $K_i = 707 \pm 144$ nM ($n = 4$) at [$^3$H]MLA binding sites.[32]

that the electron-withdrawing chloro atom of ligand **5** has only little effect on binding affinity. However, it is interesting to note that DUB-165 (**5**), compared to all ligands of Table 1, exhibits the highest affinity to the α7* subtype. The bioisosteric replacement of the 3-pyridyl moiety as the structural part of DUB-165 (**6**) by a 4-pyridazinyl-, 5-pyrimidinyl-, or 2-pyrazinyl pharmacophoric element led to anatoxinoids [structurally close to anatoxin-a (**4**)] that obviously interact with all three nAChR subtypes under consideration, although a more or less significant portion of affinity is lost. This demonstrates that the three isomeric diazine heterocycles are appropriate bioisosteres to the 3-pyridyl moiety of DUB-165 (**6**) but with varying results. Adding a second nitrogen in position 2 to the aromatic pyridine ring as in the 4-pyridazinyl analogue **7** proved to be deleterious, resulting in a significant drop in affinity for the $(\alpha4)_2(\beta2)_3$ nAChR subtype by a factor of ca. 370 (Table 1). The same order of decrease in affinity was observed for bioisoster **9** with formal incorporation of the additional nitrogen atom in position 4, thus characterized by a 2-pyrazinyl moiety. Similar results are obtained for both ligands **7** and **9** concerning the affinities for the α3β4* and the α7* subtypes. Surprisingly, introduction of a 5-pyrimidinyl substituent instead of the 3-pyridyl moiety resulted in a ligand that compares well with the lead compound **5** as well as with DUB-165 (**6**). Thus, the pyrimidine-containing bioisoster **8** turned out to be the most active ligand in the diazine series under consideration. It possesses high affinity and significant selectivity toward the $(\alpha4)_2(\beta2)_3$ nAChR subtype, being approximately 8-fold more potent than natural (+)-anatoxin-a (**4**) and only 3.5- and 2.7-fold less potent compared to UB-165 (**5**) or to DUB-165 (**6**). The relative affinities of ligand **8** for the three subtypes are 1:76:142 for $(\alpha4)_2(\beta2)_3$/α7*/α3β4*. That means that ligand **8** binds with ca. 140-fold lower affinity to the ganglionic α3β4* subtype, which mainly accounts for toxicity (e.g., of (−)-epibatidine[7]), than to the $(\alpha4)_2(\beta2)_3$ subtype.

A plausible interpretation for these findings seems possible in light of recently obtained results published by K. Brejc et al.[39] This research group, for the first time, succeeded in determining the three-dimensional structure (at the atomic level) of a molluscan acetylcholine-binding protein (AChBP) most closely related to the α-subunits of the nAChRs. The soluble, homopentameric AChBP can be considered as a structural and functional homologue of the N-terminal extracellular ligand-binding domain of a nAChR α-subunit, revealing the essential features and the organization of the key region of the agonist binding site. Thus, important insight of nAChR/ligand interactions was provided: on one hand, the key region of the nAChR binding domain does not appear to contain a negatively charged amino acid to bind the cationic pharmacophoric element of a ligand. Instead, it is shaped by five key aromatic residues of tryptophan and tyrosine,[39] and the cationic

moieties of nAChR ligands (in this case the N-protonated azabicyclo[4.2.1]nonene fragment) interact with these conserved clusters of the electron-rich aromatic amino acid side chains. Thus, Dougherty's earlier hypothesis was corroborated, that $\pi$-cation interactions[40−42] are a primary determinant in high-affinity binding. On the other hand, it is widely accepted[1,3,4] (although not proven) that the binding mode encompasses a hydrogen bond between a hydrogen bond donor in the receptor and a hydrogen bond acceptor (HBA) in the ligand, here one of the diazine nitrogens. Because all of the novel ligands **6**−**9**, including the lead compound **5**, belong to the 9-azabicyclo[4.2.1]nonene-based series, it is anticipated that the extent of $\pi$-cation interactions of the positively charged nitrogen atom at the bicyclic pharmacophoric element with the conserved clusters of the aromatic amino acid side chains within the receptor is at least similar if not equal. In view of the only moderate affinities of ligands **7** and **9** (compared to **8)** for all nAChR subtypes under consideration, the diazines **7** and **9** probably possess less favorable HBA capabilities at the heteroaromatic moieties. This can be attributed among other things to two reasons. The first is that presumably the conformational profiles of the ligands **5**−**9** are similar. The second is that the conformational flexibility of all these species is limited because they contain only one rotatable bond connecting the two pharmacophoric elements: the cationic and the aromatic HBA $\pi$-moiety. A conformational search of UB-165 (**5**) revealed that the low-energy conformers occur in pairs differing by approximately 180° in rotation of the 5-(2-chloropyridyl) ring. Thus, in contrast to the HBA pharmacophoric elements in **7** and **9**, the pyrimidine ring of **8** offers both its nitrogens (N-1 and N-3) for the putative interaction with the hydrogen bond donor of the receptor with equal probability; a 180° rotation around the central bond leads to a second equivalent low-energy conformation capable of hydrogen bond formation. In other words, it is twice as probable to find the pyrimidine-containing bioisoster **8** in an appropriate conformation for hydrogen bond formation with the receptor in comparison with both its diazine analogues **7** and **9**.

In addition to this entropical reason, it can be anticipated that bioisosteric substitution of the 3-pyridyl pharmacophoric element by the three diazines will influence the ligands' HBA capability and hence the binding affinity. The expected effect will crucially depend on the acceptor strength of the relevant diazine nitrogen atoms. According to recent studies of Nobeli et al.,[43] the energy of interaction of a hydrogen bond donor with a hetarene nitrogen is less favorable when a second nitrogen is introduced into the six-membered ring, as in the case of diazines. As anticipated, the mutual reduction of electron density at the ortho or para nitrogens in pyridazine or pyrazine leads to less favorable interactions with the H-bond donor of the receptor counterpart in comparison with the pyrimidine ring as HBA pharmacophoric element.[44] In the case of pyrimidine with the two nitrogens in the meta position, this effect is much less distinct. This is supported by the atomic ESP (electrostatical potential) charges of the two pyrimidine nitrogen atoms, calculated for fully optimized molecular geometries using the AM1 (Austin model 1) Hamiltonian[45] with default parameter settings as implemented in MOPAC and applying the method described by Besler et al.[46] As shown in Figure 1, the affinity of the anatoxinoids **5** and **6** of the pyridine series on one hand and of the diazine series (ligands **7**−**9**) on the other hand is obviously influenced by the negative atomic charge of the relevant hetarene nitrogens in that increased negative charge results in increased affinity. Thus, the slightly smaller negative atomic charge of the pyridine nitrogen in ligand **6** (compared to that in ligand **5**) elicits an only moderate reduction in binding affinity for anatoxinoid **6**. In the diazine series the nitrogen atoms of the pyrimidine moiety **8** show by far the most negative atomic charges, followed by the nitrogens in the hetarenes of **9** and **7**. Thus, also in the case of diazine-containing anatoxinoids **7**−**9**, this order *qualitatively* correlates well with the observed decrease in binding affinity.

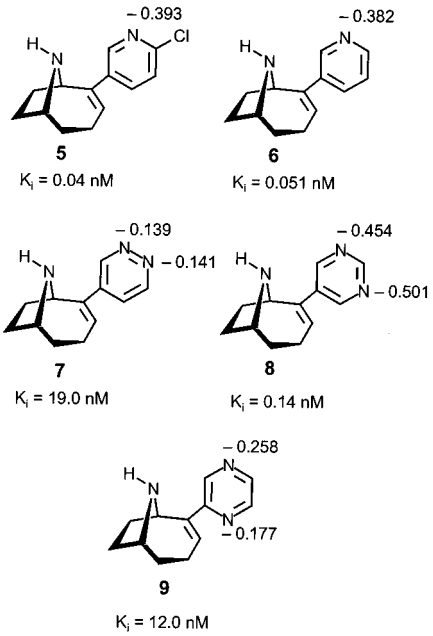



**Figure 1.** Atomic ESP charges of the pyridine and diazine nitrogen atoms of the nAChR ligands **5**−**9** and the in vitro affinities ($K_i$ values) for the $(\alpha4)_2(\beta2)_3$ nAChR subtype.

## Conclusion

Bioisosterism represents one approach used in medicinal chemistry for the rational modification of lead compounds into ligands with better ratios of pharmacological to toxicological activity. In our program aimed at the development of novel advantageous nAChR ligands, we investigated the bioisosteric potential of diazines and replaced the 3-pyridyl group of DUB-165 (**6**) by a 4-pyridazinyl, 5-pyrimidinyl, or 2-pyrazinyl moiety. When the homotropane-based vinyl triflate **13** is utilized as a versatile chiral building block, the enantiopure bioisosteres **7**−**9** of DUB-165 (**6**) are easily accessible without the need to effect resolutions. The Pd-catalyzed Suzuki or Stille cross-couplings constitute the key reactions. On the basis of the SARs observed in this study, it turned out that all the new ligands **6**−**9** retained affinity to nAChRs, $(\alpha4)_2(\beta2)_3$, $\alpha3\beta4$*, and $\alpha7$* subtypes, demonstrating that the three isomeric diazines are appropriate bioisosteres to the 3-pyridyl moiety of the lead compound but with different results. A

change from a 1,2- to a 1,3- or 1,4-diazine is connected with significantly different affinities for all nAChR subtypes investigated. The most active diazine analogue described, the pyrimidine-containing bioisoster **8** with $K_i$ values of 0.14 nM for the $(\alpha4)_2(\beta2)_3$, 20 nM for the ganglionic $\alpha3\beta4$*, and 10.7 nM for the $\alpha7$* subtype proves to be more potent and similarly selective compared to natural (+)-anatoxin-a (**4**). Thus, it might be of interest to utilize the bioisosteric potential of the pyrimidine moiety for further developments of nAChR ligands aimed at exploiting the scope and potential offered by these compounds.

## Experimental Section

**General Procedures.** Standard vacuum techniques were used in the handling of air-sensitive materials. Melting points are uncorrected ("Leitz-Heiztischmikroskop" HM-Lux). Solvents were dried and freshly distilled before use according to literature procedures. IR spectra were collected on Perkin-Elmer 257 and 398 spectrometers and on a Nicolet 510-P FT-IR spectrometer. Liquids were run as films and solids as KBr pellets. $^1$H and $^{13}$C NMR spectra were collected on JEOL JNM-GX 400 and LA 500 spectrometers; $\delta$/ppm = 0 for tetramethylsilane, and $\delta$/ppm = 7.24 for chloroform. MS spectra were collected on a Vacuum Generators 7070 (70 eV; $^{11}$B) spectrometer. For column chromatography, purifications were carried out on Merck silica gel 60 [70−260 (flash chromatography) or (200−400 mesh)]. Reactions were monitored by thin-layer chromatography (TLC) using plates of silica gel (0.063−0.200 mm, Merck) or silicagel-60-$F_{254}$ microcards (Riedel de Haen). UB-165 (**5**) was prepared according to literature procedures.[9]

**(1*R*)-2-Pyridin-3-yl-9-azabicyclo[4.2.1]non-2-ene-9-carboxylic Acid Ethyl Ester (18).** To a solution of triflate **13** (343 mg, 1 mmol) in dry THF (5 mL) were successively added bis(triphenylphosphane)palladium(II) chloride (8 mg, 0.01 mmol), 3-diethylboranylpyridine (**14**) (200 mg, 1.5 mmol), and 2 M aqueous $Na_2CO_3$ solution (2 mL). The mixture was heated at 85 °C for 3 h. Diethyl ether and water were added and the phases separated. The organic layer was dried with $Na_2SO_4$ and filtered, and the solvent was evaporated. The crude product was purified by column chromatography on silica gel (column, 3 cm × 15 cm; eluent, ethyl acetate) to yield a colorless oil (106 mg, 0.38 mmol, 38%). $R_f$ = 0.52 (eluent, ethyl acetate); $[\alpha]^{20}_D$ −49.6 (*c* 0.14, $CH_2Cl_2$). IR (film): $\nu$ (cm$^{-1}$) = 2975, 2871, 1695, 1566. $^1$H NMR (500 MHz, $CDCl_3$, 2 rotamers, ratio 1:1): $\delta$ 1.14 and 1.24 (2t, *J* = 7.1 Hz, 3H), 1.63−2.44 (m, 8H), 4.05−4.17 (m, 2H), 4.45−4.46 and 4.52−4.53 (2m, 1H), 4.80 and 4.84 (2d, *J* = 9.0 Hz, 1H), 5.82−5.87 (m, 1H), 7.20−7.22 (m, 1H), 7.67 (d, *J* = 7.9 Hz, 0,5H), 8.09 (d, *J* = 7.9 Hz, 0,5H), 8.43−8.46 (m, 1H), 8.56−8.59 (m, 1H). $^{13}$C NMR (500 MHz, $CDCl_3$, 2 rotamers, ratio 1:1): (a) $\delta$ 14.6, 24.1, 28.4, 30.9, 31.7, 56.2, 58.5, 61.0, 123.0, 129.0, 133.5, 138.1, 146.0, 147.7, 148.0, 153.6; (b) $\delta$ 14.8, 24.2, 29.2, 31.2, 32.1, 56.4, 59.0, 61.0, 123.1, 129.6, 134.5, 138.1, 146.7, 147.8, 148.1, 153.8. MS (70 eV), *m*/*z* (%): 272 (100, M$^+$). HRMS calcd for $C_{16}H_{20}N_2O_2$: 272.1525. Found: 272.1525.

**(1*R*)-2-Pyridin-3-yl-9-aza-bicyclo[4.2.1]non-2-ene (6).** In a sealed vessel, carbamate **18** (140 mg, 0.51 mmol) and degassed concentrated hydrochloric acid (37%, 5 mL) were heated under reflux for 1 h. The volatile components were removed in vacuo. Water was added, and the aqueous solution was washed with $CH_2Cl_2$ (5 mL). Then concentrated $NH_3$ was added until a pH of 9 was reached. The aqueous layer was extracted with $CH_2Cl_2$ (3 × 5 mL), the organic phase was dried with $Na_2SO_4$ and filtered, and the solvent was evaporated to yield the free base as a slightly yellow oil (40 mg, 39%). $R_f$ = 0.48 (eluent/$CH_2Cl_2$/MeOH/concentrated aqueous $NH_3$ = 90:10:1); $[\alpha]^{20}_D$ + 36.8 (*c* 0.22, $CH_3OH$). UV ($CH_3OH$): $\lambda_{max}$ (log $\epsilon$) = 241 nm (3.64). IR (film): $\nu$ (cm$^{-1}$) = 2920, 1653, 1565. $^1$H NMR (500 MHz, $D_3COD$): $\delta$ 1.57−1.68 (m, 2H), 1.70−1.77 (m, 1H), 1.82−1.94 (m, 2H), 2.12−2−20 (m, 1H), 2.26−2.39 (m, 2H), 3.64 (d, *J* = 3.7 Hz, 1H), 4.00 (d, *J* = 9.0 Hz, 1H), 5.81−5.85 (m, 1H), 7.23 (dd, *J* = 5.2 Hz, *J* = 7.6 Hz, 1H), 7.62−7.65 (m, 1H), 8.24 (d, *J* = 3.7 Hz, 1H), 8.34 (s, 1H); NH signal not visible. $^{13}$C NMR (125 MHz, $D_3COD$): $\delta$ 25.9, 30.8, 34.3, 34.4, 59.8, 62.0, 119.4, 125.2, 132.8, 135.8, 141.6, 147.8, 148.4. MS (70 eV), *m*/*z* (%): 200 (100, M$^+$). HRMS calcd for $C_{13}H_{16}N_2$: 200.1313. Found: 200.1325.

**(1*R*)-2-(4-Pyridazinyl)-9-azabicyclo[4.2.1]non-2-ene-9-carboxylic Acid Ethyl Ester (19).** A solution of bis(benzonitrile)palladium(II) chloride (38.3 mg, 100 $\mu$mol), CuI (38.0 mg, 200 $\mu$mol), $Ph_3As$ (63.3 mg, 200 $\mu$mol), LiCl (127 mg, 3.00 mmol), and triflate **13** (343 mg, 1.00 mmol) in anhydrous degassed DMF (1.0 mL) under Ar was immersed in an oil bath and maintained at a temperature of 75 °C, and a solution of the organostannane **15**[31] (400 mg, 1.02 mmol) in dry DMF (0.4 mL) was added. After the mixture was stirred for 3 h at 75 °C, the black slurry was allowed to cool to room temperature. A solution of KF (300 mg) in $CH_3OH$ (10 mL) was added, and the mixture was stirred for 12 h. After evaporation of the solvents in vacuo, the residue was purified by flash chromatography on silica gel (column, 15 cm × 2 cm; eluent, ethyl acetate) to provide **19** as a colorless oil (243 mg, 89%). $R_f$ = 0.13 (eluent, ethyl acetate); $[\alpha]^{20}_D$ −53 (*c* 0.2, $CH_2Cl_2$). $^1$H NMR (500 MHz, $CDCl_3$, 2 rotamers, ratio 4:3): $\delta$ 1.07 (t, *J* = 6.9 Hz, 1.4H), 1.19 (t, *J* = 6.9 Hz, 1.6H), 1.60−2.46 (m, 8H), 4.04 (q, *J* = 6.9 Hz, 0.92H), 4.11 (q, *J* = 6.9 Hz, 1.08H), 4.43−4.47 (m, 0.56H), 4.50 (t, *J* = 3.9 Hz, 0.44H), 4.79 (t, *J* = 2.1 Hz, 1H), 6.08 (t, *J* = 5.5 Hz, 0.44 H), 6.13 (t, *J* = 6.0 Hz, 0.56H), 7.37−7.38 (m, 0.44H), 7.83−7.86 (m, 0.56H), 9.02−9.04 (m, 1H), 9.12−9.16 (m, 1H). $^{13}$C NMR (125 MHz, $CDCl_3$, 2 rotamers): $\delta$ (major rotamer) 14.8, 24.4, 29.1, 31.1, 32.2, 56.4, 57.7, 61.2, 123.4, 133.3, 139.7, 145.3, 149.5, 151.2, 153.8; (minor rotamer) 14.6, 24.5, 29.3, 31.2, 32.0, 56.4, 57.7, 61.2, 122.1, 134.1, 139.8, 144.7, 149.2, 151.0, 153.8. MS (70 eV), *m*/*z* (%): 273 (100, M$^+$). HRMS calcd for $C_{15}H_{19}N_3O_2$: 273.1477. Found: 273.1474.

**(1*R*)-2-(4-Pyridazinyl)-9-azabicyclo[4.2.1]non-2-ene (7).** To a solution of carbamate **19** (20 mg, 73 $\mu$mol) in degassed $CHCl_3$ (1 mL) was added TMSI (14 $\mu$L, 19 mg, 95 $\mu$mol), and the resulting light-brown solution was heated under Ar in a sealed vessel at 80 °C for 3.5 h. After the mixture was cooled to ambient temperature and after evaporation of the volatile components in vacuo, a solution of sodium methoxide (4 mg, 73 $\mu$mol) in methanol (1 mL) was slowly added and the volatile components were evaporated in vacuo again. The residue was purified by column chromatography over (1) reversed-phase silica gel (ICN RP C-18, column 8 cm × 1 cm, eluting with *n*-hexane/methanol = 100:0 → 1:1) and (2) neutral alumina (ICN Alumina N, column 15 cm × 1 cm, eluting with (a) $CH_2Cl_2$/*n*-hexane = 1:1 and (b) $CH_2Cl_2$/2 N $NH_3$ in ethanol = 2:1). The product **6** (11 mg, 75%) is a light-yellow oil. $R_f$ = 0.17 (eluent, $CH_2Cl_2$/$CH_3OH$/concentrated aqueous $NH_3$ = 95:15:0.1; $[\alpha]^{20}_D$ +20.8° (*c* 0.1, $CH_3OH$). IR (film): $\nu$ (cm$^{-1}$) = 3218, 2964, 1589. $^1$H NMR (500 MHz, $CD_3OD$): $\delta$ 1.97−2.01 (m, 2H), 2.15−2.40 (m, 3H), 2.60−2.80 (m, 3H), 4.34 (bs, 1H), 4.78 (d, *J* = 8.6 Hz, 1H), 6.70−6.72 (m, 1H), 7.73−7.74 (dd, *J* = 5.5 Hz, *J* = 2.5 Hz, 1H), 9.14−9.15 (dd, *J* = 5.5 Hz, *J* = 1.1 Hz, 1H), 9.26 (dd, *J* = 2.5 Hz, *J* = 1.1 Hz, 1H); NH signal not visible. $^{13}$C NMR ($CD_3OD$, 125 MHz): $\delta$ 24.0, 27.1, 27.6, 30.6, 57.4, 59.8, 122.5, 137.8, 139.1, 139.8, 148.9, 149.6. MS (70 eV), *m*/*z* (%): 201 (70, M$^+$), 172 (100). HRMS calcd for $C_{12}H_{15}N_3$: 201.1266. Found: 201.1253.

**(1*R*)-2-(Pyrimidin-5-yl)-9-azabicyclo[4.2.1]non-2-ene-9-carboxylic Acid Ethyl Ester (20).** Bis(benzonitrile)palladium(II) chloride (25 mg, 0.07 mmol), CuI (14 mg, 0.12 mmol), $Ph_3As$ (42 mg, 0.13 mmol), LiCl (120 mg, 0.66 mmol), and triflate **13** (300 mg, 0.87 mmol) were placed in an argon-flushed flask and dissolved in dry DMF (0.5 mL). A solution of tributyltin pyrimidine **16**[34] (380 mg, 1 mmol) in dry DMF (0.5 mL) was added, and the mixture was heated for 16 h at 80 °C in a sealed flask under argon. The remaining mixture was worked up in the way described for **21**. The yield was 170 mg (72%) of a pale-yellow oil. $R_f$ = 0.52 (eluent, ethyl acetate); $[\alpha]^{20}_D$ −49.4° (*c* 0.16, $CH_3OH$). IR (film): $\nu$ (cm$^{-1}$) = 2936, 1670, 1560, 1438. $^1$H NMR (400 MHz, $CDCl_3$, 2 rotamers, ratio 1:1):

$\delta$ 1.11 and 1.22 (2t, $J$ = 7.1 Hz, 3H), 1.78−2.43 (m, 8H), 4.07−4.14 (m, 2H), 4.41 and 4.51 (2m, 1H), 4.73 and 4.78 (2d, $J$ = 9.0 Hz, 1H), 5.85−5.88 (m, 1H), 8.70 (s, 1H), 8.85 (s, 1H), 9.02 (s, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$, 2 rotamers, ratio 1:1): (a) $\delta$ 14.7, 24.3, 28.3, 30.6, 31.6, 56.2, 57.9, 61.1, 131.1, 135.6, 143.4, 153.4, 154.2 (2 C), 157.1; (b) $\delta$ 14.8, 24.4, 29.2, 31.1, 32.2, 56.5, 58.5, 61.1, 131.6, 135.6, 144.0, 153.9, 154,7 (2 C), 157.1. MS (70 eV), $m/z$ (%): 273 (85, $M^+$), 59 (100). HRMS calcd for $C_{15}H_{19}N_3O_2$: 273.1477. Found: 273.1471.

**(1*R*)-2-(Pyrimidin-5-yl)-9-azabicyclo[4.2.1]non-2-ene Hydrochloride (8·HCl).** To a solution of the carbamate **20** (150 mg, 0.55 mmol) in dry $CHCl_3$ (5 mL) was added TMSI (156 $\mu$L, 1.1 mmol), and the mixture was heated for 3 h under reflux and an atmosphere of argon. After the mixture was cooled to room temperature, the volatile components were evaporated in vacuo. A mixture of dry $CH_3OH$ (3 mL) and HCl in diethyl ether (0.3 mL, 2 M, 0.60 mmol) was added to the residue, and the resulting solution was stirred for 10 min at ambient temperature. After the solvent was removed in vacuo, the hydrochloride of **8** was obtained (80 mg, 61%) as very hygroscopic crystals. $R_f$ = 0.52 (eluent, $CH_2Cl_2$/MeOH/concentrated aqueous $NH_3$ = 95:5:1); $[\alpha]^{20}_D$ +22.5 ($c$ 0.16, $H_2O$). UV ($H_2O$): $\lambda_{max}$ (log $\epsilon$) = 228 nm (4.17). IR (KBr): $\nu$ ($cm^{-1}$) = 2938, 1619, 1418. $^1$H NMR (500 MHz, $D_2O$): $\delta$ 2.20−2.30 (m, 2H), 2.40−2.70 (m, 3H), 2.80−3.00 (m, 3H), 4.65−4.66 (d, $J$ = 6.0 Hz, 1H), 4.97−4.98 (d, $J$ = 9 Hz, 1H), 6.74−6.76 (m, 1H), 9.09 (s, 2H), 9.34 (s, 1H); NH signals not visible. $^{13}$C NMR (125 MHz, $D_2O$): $\delta$ 23.7, 27.4, 28.3, 31.0, 59.3, 60.3, 135.9, 136.2, 139.2, 154.8 (2 C), 156.2. MS (70 eV), $m/z$ (%): 201 (17, $M^+$), 59 (100). HRMS calcd for $C_{12}H_{15}N_3$ (free base): 201.1266. Found: 201.1273.

**(1*R*)-2-(Pyrazinyl-5-yl)-9-azabicyclo[4.2.1]non-2-ene-9-carboxylic Acid Ethyl Ester (21).** Bis(benzonitrile)palladium(II) chloride (30 mg, 0.08 mmol), CuI (17 mg, 0.15 mmol), $Ph_3As$ (50 mg, 0.15 mmol), LiCl (130 mg, 0.72 mmol), and triflate **13** (355 mg, 1.03 mmol) were placed in an argon-flushed flask dissolved in dry DMF (0.5 mL), and the mixture was heated under argon to 80 °C. A solution of tributyltin pyrazine[35] (450 mg, 1.2 mmol) in dry DMF (0.5 mL) was added dropwise. The flask was sealed, and the suspension was heated at 85 °C for 16 h. After the mixture was cooled to room temperature, a solution of KF (200 mg, excess) in $CH_3OH$ was added to the brown mixture. Stirring was continued for 40 min, the volatile components were removed in vacuo, and the residue was purified by column chromatography on silica gel (column, 2 cm × 18 cm; eluent, $n$-hexane/ethyl acetate = 2:1) to yield carbamate **21** as a colorless oil (145 mg, 51%). $R_f$ = 0.29 (eluent, $n$-hexane/ethyl acetate = 2:1); $[\alpha]^{20}_D$ −65.1 ($c$ 0.2, $CH_2Cl_2$). IR (film): $\nu$ ($cm^{-1}$) = 2983, 2954, 2930, 1685, 1559, 1483. UV ($CH_2Cl_2$): $\lambda_{max}$ (log $\epsilon$) = 224 nm (3.74), 291 nm (3.71). $^1$H NMR (400 MHz, $CDCl_3$, 2 rotamers, ratio = 2:3): $\delta$ 0.86−0.93, 1.20−1.28 (2m, 3H), 1.64−1.69, 1.79−1.82, 1.92−2.20, 2.41−2.50 (4m, 8H), 3.92−3.98, 4.07−4.12 (2m, 2H), 4.43−4.45, 4.50−4.52 (2m, 1H), 5.25 (d, $J$ = 8.9 Hz, 0.4 H), 5.34 (d, $J$ = 8.6 Hz, 0.6 H), 6.44−6.47 (m, 1H), 8.34−8.37 (m, 1H), 8.44 (d, $J$ = 1.8 Hz, 1H), 8.70 (s, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$, 2 rotamers): (a) $\delta$ 14.3, 24.2, 28.8, 31.0, 32.0, 56.0, 56.3, 60.7, 132.6, 133.0, 141.5, 142.0, 143.0, 145.8, 153.8; (b) $\delta$ 14.8, 24.2, 29.7, 31.3, 32.2, 55.8, 56.7, 60.9, 132.6, 133.0, 141.8, 142.0, 143.0, 153.7, 153.4. MS (70 eV), $m/z$ (%): 273 (100, $M^+$). HRMS calcd for $C_{15}H_{19}N_3O_3$: 273.1477. Found: 273.1507.

**(1*R*)-2-(Pyrazinyl-5-yl)-9-azabicyclo[4.2.1]non-2-ene (9).** To a solution of the carbamate **21** (140 mg, 0.51 mmol) in dry $CHCl_3$ (5 mL) saturated with argon was added TMSI (146 $\mu$L, 1.03 mmol), and the mixture was heated at 80 °C for 3 h. After the mixture was cooled to room temperature the volatile components were removed in vacuo and the resulting residue treated with a mixture of 2 M HCl in ethyl ether (0.3 mL, 0.6 mmol) and $CH_3OH$ (3 mL). After the solvent had been removed in vacuo, a solution of $NaOCH_3$ (36 mg, 0.66 mmol) in $CH_3OH$ (3 mL) was added, the solvent removed in vacuo, and the residue purified by column chromatography on silica gel (column, 2 cm × 15 cm; eluent, $CH_2Cl_2$/$CH_3OH$/concentrated aqueous $NH_3$ = 95:5:1) to yield the free base **8** (50 mg, 50%) as a colorless oil. $R_f$ = 0.2 (eluent, $CH_2Cl_2$/$CH_3OH$/concentrated aqueous $NH_3$ = 95:5:1); $[\alpha]^{20}_D$ +37.9 ($c$ 0.5, $CH_2Cl_2$). UV ($CH_2Cl_2$): $\lambda_{max}$ (log $\epsilon$) = 234 nm (4.02), 286 nm (3.81). IR (film): $\nu$ ($cm^{-1}$) = 2932, 1639, 1516, 1467. $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 1.65−1.80 (m, 3H), 1.80−1.90 (m, 1H), 2.00−2.10 (m, 1H), 2.20−2.35 (m, 1H), 2.40−2.50 (m, 2H), 3.15−3.35 (bs, NH, 1H), 3.85−3.86 (m, 1H), 4.65−4.67 (d, $J$ = 7.2 Hz, 1H), 6.33−6.36 (m, 1H), 8.30 (d, $J$ = 2.5 Hz, 1H), 8.38 (s, 1H), 8.60 (d, $J$ = 1.4 Hz, 1H). $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 24.9, 29.7, 32.7, 33.5, 57.6, 57.9, 134.0, 141.7, 142.0, 143.0, 147.8, 155.0. MS (70 eV), $m/z$ (%): 201 (48, $M^+$), 203 (100). HRMS calcd for $C_{12}H_{15}N_3$: 201.1266. Found: 201.1272.

**In Vitro Binding Studies. Materials.** (±)-[$^3$H]Epibatidine (33 Ci/mmol) was obtained from NEN Life Science Products (Cologne, Germany). [$^3$H]MLA (20 Ci/mmol) was purchased from Tocris/Biotrend (Cologne, Germany). All other chemicals used were obtained from Sigma-Aldrich (Deisenhofen, Germany). Frozen Sprague−Dawley rat brains and pig adrenals were purchased from Pel-Freez Biologicals (Rogers, AR).

**Membrane Preparation.** Frozen rat brains were thawed at 22 °C for 30−60 min before membrane preparation. Brain tissue used for binding studies was obtained by a single cut just behind the inferior colliculi to exclude the cerebellum and medulla. A crude membrane fraction (P2) was isolated as previously described.[31] On the day of assay, pellets were thawed, homogenized in 30 volumes of a HEPES-salt solution (HSS) containing HEPES (15 mM), NaCl (120 mM), KCl (5.4 mM), $MgCl_2$ (0.8 mM), and $CaCl_2$ (1.8 mM), and centrifuged at 35 000$g$ for 10 min. The resultant pellets were resuspended in a fresh HSS and used for binding assay. Total membrane fractions from frozen pig adrenal glands were isolated by homogenization with a Polytron homogenizer in 10−20 volumes of a HEPES−salt solution described above, followed by centrifugation at 40 000$g$ for 10 min. The pellets were washed five times with HSS through rehomogenization and centrifugation at the same settings.

**Binding Assays.** Assays for the $(\alpha4)_2(\beta2)_3$ and $\alpha7$* subtypes were carried out in HSS at 22 °C and were performed following published procedures[31,32] with minor modifications.[47] Nonspecific binding was determined in the presence of 300 $\mu$M (−)-nicotine except for the assays with [$^3$H]MLA, for which 1 $\mu$M MLA (methyllycaconitine) was used instead. For $\alpha3\beta4$* binding assay, each assay sample of a total volume of 0.5 mL contained 60 $\mu$g of membrane protein, 0.5 nM (±)-[$^3$H]epibatidine, and 0.2 mL of a test compound. The samples were incubated for 90 min. The incubation was terminated by vacuum filtration through Whatman GF/B glass fiber filters presoaked in 1% poly(ethylenimine) using a Brandel 48-channel cell harvester. The radioactivity was measured using a liquid scintillation counter (Tri-Carb 2100 TR, Packard, Dreieich, Germany).

**Data Analysis.** Competition binding data were analyzed using nonlinear regression methods. $K_i$ values were calculated by the Cheng−Prusoff[48] equation based on the measured $IC_{50}$ values and $K_d$ = 10 pM and $K_d$ = 50 pM for binding of (±)-[$^3$H]epibatidine to $(\alpha4)_2(\beta2)_3$ and $\alpha3\beta4$* subtypes, respectively, and $K_d$ = 1 nM for [$^3$H]MLA. The $K_d$ values were obtained from five independent experiments performed on the same membrane preparations that were used for the competition assays.

**Acknowledgment.** This work was generously supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. We also wish to thank the Merck AG, the Bayer AG, Hoechst AG, Degussa AG for gifts of various chemicals, the Boehringer Ingelheim Pharma KG for generous gifts of tropinone. S. Schwarz is grateful to the DPhG-Stiftung im Stifterverband für die Deutsche Wissenschaft for fellowship support.

**Supporting Information Available:** $^1$H NMR spectrum of UB-165 (**5**) and $^1$H and $^{13}$C NMR spectra of compounds **6**−**9**, **18**−**20**, and **21**, and the experimental protocols for the preparation of 5-tributylstannylpyrimidine (**16**) and 2-tribu-

tylstannylpyrazine (**17**). This material is available free of charge via the Internet at http://pubs.acs.org.

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JM010936Y