New Ligands with Affinity for the $\alpha_4\beta_2$ Subtype of Nicotinic Acetylcholine Receptors. Synthesis, Receptor Binding, and 3D-QSAR Modeling

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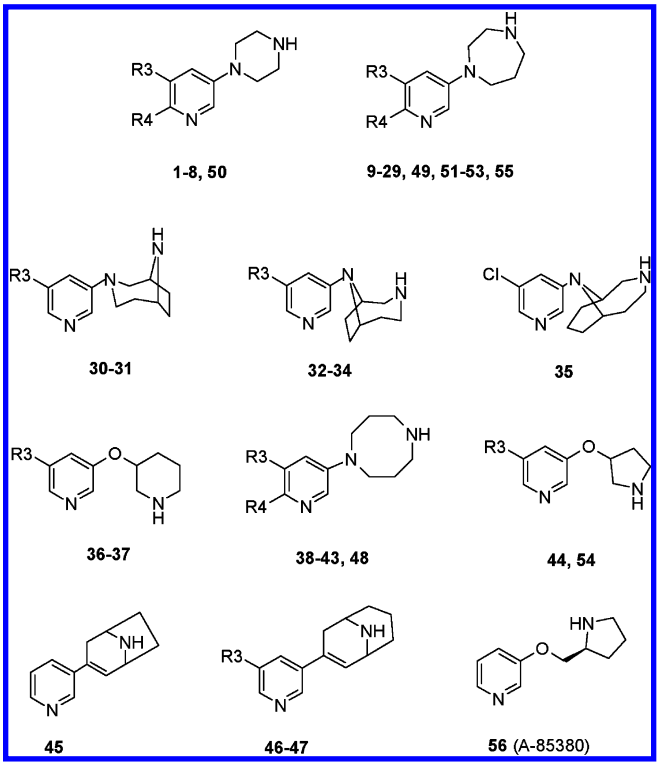
A new series of piperazines, diazepanes, diazocanes, diazabicyclononanes, and diazabicyclodecanes with affinity for the $\alpha_4\beta_2$ subtype of nicotinic acetylcholine receptors were synthesized on the basis of results from a previous computational study. A predictive 3D-QSAR model was developed using the GRID/GOLPE approach ($R^2 = 0.94$, $Q^2 = 0.83$, SDEP = 0.34). The SAR was interpreted in terms of contour maps of the PLS coefficients and in terms of a homology model of the $\alpha_4\beta_2$ subtype of the nicotinic acetylcholine receptors. The results reveal that hydrogen bonding from both hydrogens on the protonated amine and from the pyridine nitrogen to a water molecule as well as van der Waals interactions between the substituent bearing the protonated amine and the receptor is of importance for ligand affinity. The combination of 3D-QSAR and homology modeling proved successful for the interpretation of structure-affinity relationships as well as the validation of the individual modeling approaches.

Introduction

Neuronal nicotinic acetylcholine receptors (nAChRs) are members of a gene superfamily of ligand-gated ion channels that also comprises 5-HT_3, glycine, and $GABA_A$ receptors.[1] The nAChRs are composed of five subunits, each spanning the membrane four times and aligning around the central ion channel.[2] The ion channel of the nAChRs is cation-selective, and the nicotinic receptors participate in the mediation of fast excitatory transmission between neurons.[3] Furthermore, presynaptic nAChRs modulate the release of other neurotransmitters,[4−5] and novel ligands for neuronal nAChRs may have great potential as pharmaceuticals aiming at several diseases in the CNS.[6] Presently, six neuronal α ($\alpha_2-\alpha_7$) and three neuronal β ($\beta_2-\beta_4$) subunits have been cloned from humans.[7−9] Neuronal nAChRs containing α_7 subunits bind α-bungarotoxin with high affinity.[10,11] The current status of the nAChR nomenclatureture has recently been published.[12] Most nAChRs that bind nicotine with high affinity in the brain are composed of α_4 and β_2 subunits,[13] and the stoichiometry of the receptors has been shown to be $(\alpha_4)_2(\beta_2)_3$.[14]

In a previous article by Nielsen et al., a 3D-QSAR model based on 25 ligands, with affinity for the $\alpha_4\beta_2$ subtype of nAChRs, was described.[15] The analysis of the coefficient plots revealed that steric interactions between the target and the compounds are of major importance for describing the differences in affinity. All of the compounds studied include a pyridine ring and substituents in the pyridine 3-position, in particular, with bulky ring systems including an sp^3 nitrogen atom, were predicted to increase the affinity of the ligands. In the present article, we describe the synthesis of a series of 3-substituted pyridines designed on the basis of the 3D-QSAR model developed by Nielsen et al.[15] The novel compounds comprise piperazines, diazepanes, diazocanes, diazabicyclononanes, and diazabicyclodecanes (Chart 1 and Table 1)

Chart 1



because, according to the previous 3D-QSAR model, large substituents in the pyridine 3-position were predicted to lead to increased affinity. With the purpose of increasing the robustness of the previously developed 3D-QSAR model with regard to the area around the pyridine 3-position and thereby obtaining a more general model, we have improved the model by including the new compounds in the training set. The structure-affinity relationships for the compounds are interpreted using contour maps of the PLS coefficients obtained from the new 3D-QSAR analysis. In addition, the results are interpreted in light of a homology model of the $\alpha_4\beta_2$ receptor reported by Le Novere et al.[16]

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Table 1. Affinities of the Compounds[a]

compd	R3	R4	IC 50 (nM) obsd.	pred.
		training set		
1	OCH_3	H	500	340
2	$OCH{=}CH_2$	H	190	305
3	OCH_3	Br	180	209
4	Br	Cl	230	269
5	Cl	CH_3	520	313
6	C≡CH	H	110	269
7	Cl	H	310[b]	425
8	OCH_2CH_3	H	500[b]	247
9	*cis*$OCH{=}CHCH_3$	H	1.3	2.3
10	Cl	Br	1.4	2.0
11	Br	Cl	1.4	1.9
12	OH	I	22	5.7
13	cyclopropyl	H	5.8	6.4
14	3-anilinyl	H	4.0[b]	3.7
15	$OCH{=}CH_2$	H	5.0[b]	3.0
16	3-pyridyl	H	2.8[b]	3.5
17	SC_6H_5	H	4.0[b]	3.3
18	$CONH_2$	H	20[b]	16
19	OH	H	2.4[b]	11
20	OCH_2CH_2OH	H	1.8[b]	1.9
21	C_6H_5	H	3.0[b]	3.4
22	OCH_3	H	1.9[b]	3.2
23	H	Br	1.0[b]	2.5
24	H	SCH_2CH_3	170[b]	NI[b]
25	H	OCH_3	170[b]	61
26	OCH_2CH_3	Br	0.87[b]	0.59
27	OCH_3	OCH3	170[b]	135
28	OCH_3	Cl	0.68[b]	1.1
29	quinoline		6.6[b]	3.6
30	*cis*$OCH{=}CHCH_3$	H	1.9	1.8
31	OCH_2CH_3	H	6.5	2.8
32	*cis*$OCH{=}CHCH_3$	H	3.1	4.7
33	OCH_3	H	10	6.6
34	OCH_2CH_3	H	3.0	4.2
35	Cl	H	8.4	13
36	Cl	H	240	272
37	H	H	190[b]	265
38	OCH_3	H	1.0	0.89
39	$O(CH_2)_3CH_3$	H	1.8	1.0
40	H	H	0.9	1.0
41	H	Br	0.45	1.3
42	Cl	H	2.0	0.8
43	$OCH{=}CH_2$	Br	0.38	0.43
44	H	H	100[b]	92
45	H	H	6.7[b]	5.7
46	H	H	4.5[b]	3.3
47	OCH_3	H	3.0[b]	1.5
54	Cl	H	220[b]	140
		test set		
48	Br	H	2.8	1.3
49	$OCH{=}CH_2$	Br	0.64	0.94
50	H	Br	180	335
51	$OCH_2CH_2OCH_3$	H	2.4[b]	3.9
52	$OCH_2CH(CH_3)_2$	H	2.2[b]	4.1
53	$SCH_2C_5H_6$	H	8.0[b]	2.6
55	H	H	1.9	8.0
56	A-85380		1.5[b]	3.3

[a] Structures are shown in Chart. [b] Data from ref 15. NI = not included in training set.

Results and Discussion

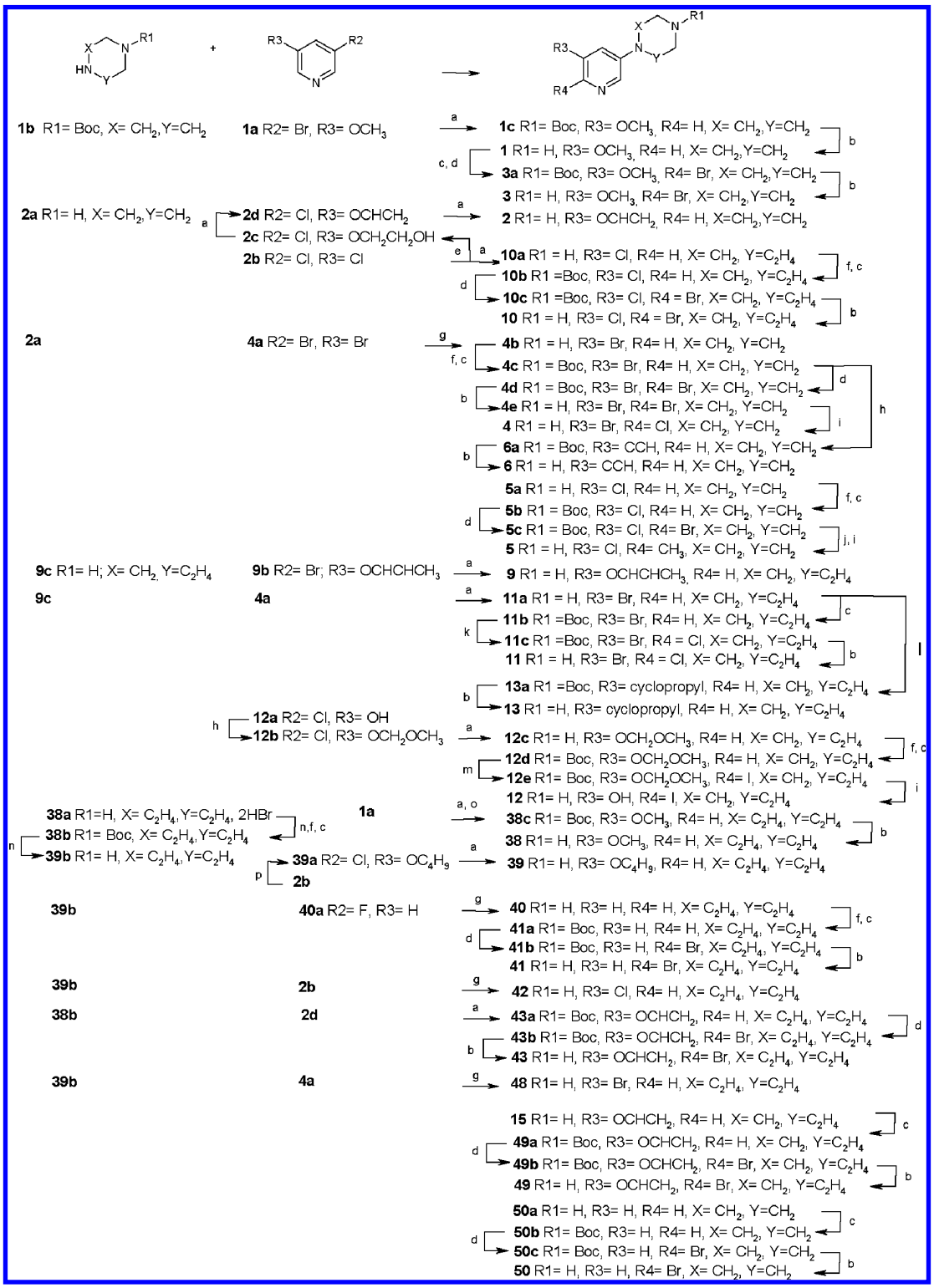
Chemistry. Compounds **7**−**8**, **14**−**29**, **37**, **44**−**47**, and **51**−**56** (Chart 1) have been previously reported,[15] and the preparation of compounds **1**−**6**, **9**−**13**, **30**−**36**, **38**−**43**, and **48**−**50** (Chart 1) is described in the Experimental Section. The key step in the preparation of all 3-pyridyl substituted diazacycles and diazabicycles (Chart 1) was the reaction of various diazacycloalkanes (piperazine, 1,4-diazepane and 1,5-diazocane) and diazabicycloalkanes (3,9-diazabicyclo[4.2.1]nonane and 3,10-diazabicyclo[4.3.1]-decane) with adequately substituted 3-halopyridines. The syntheses of seven different 3-pyridylpiperazines are described in Scheme 1. Compound **1** was prepared by a palladium catalyzed reaction of 1-*tert*-butoxycarbonylpiperazine (**1b**), 3-bromo-5-methoxypyridine (**1a**), and potassium-*tert*-butoxide, followed by deprotection with TFA (Scheme 1). 5-Vinyloxypyridyl analogue **2** was prepared in the absence of the palladium catalyst from 3-chloro-5-vinyloxypyridine (**2d**), potassium *tert*-butoxide, and unprotected piperazine (**2a**). Compounds **3** and **50** (Scheme 1) were prepared by NBS bromination of the *tert*-butoxycarbonyl protected piperazines **1c** and **50b**, respectively, followed by treatment with TFA. The 5-bromo-6-chloropyridine derivative, **4** (Scheme 1), was prepared from the corresponding 5-bromopyridine derivative **4b** by a sequence consisting of *tert*-butoxycarbonyl protection, NBS bromination, TFA deprotection, and finally heating in concentrated hydrochloric acid to replace the bromine in the 6-position with a chlorine. The 6-methylpyridyl substituted derivative, **5** (Scheme 1), was obtained from **5a** by *tert*-butoxycarbonyl protection followed by bromination to give **5c**. The methyl group in the pyridine 6-position was introduced via a palladium catalyzed methylation reaction, using tetramethyltin as the methyl source. *N*-formylation from solvent DMF required heating in concentrated hydrochloric acid to obtain the final product. The acetylenic derivative **6** (Scheme 1) was prepared from **4c** by a palladium catalyzed reaction with 2-methyl-3-butyn-2-ol followed by heating with sodium hydride, which resulted in a cleavage of the 3-carbon alcoholic fragment connected to the triple bond.

The 1,4-diazepanes **9**−**13** and **49** were prepared by similar procedures. Compound **9** (Scheme 1) was obtained from homopiperazine, (*Z*)-3-bromo-5-(prop-1-enyloxy)pyridine (**9b**) (Scheme 2), and potassium-*tert*-butoxide. The position of the double bond and the cis configuration of the final product **9** was confirmed by NMR. This pattern was seen for all of the *cis*-propene derivatives (**9**, **30**, and **32**). Bromo analogues **10** and **49** (Scheme 1) were both prepared by NBS bromination of precursors **10b** and **49a**, respectively, followed by reaction with TFA to remove the protection group. Chloro and iodo analogues **11** and **12** (Scheme 1) were prepared by halogenation of **11b** and **12d** using 1,3-dichlorodimethylhydantoine and *N*-iodo succinimide (NIS), respectively. Compound **13** (Scheme 1) was prepared from its corresponding *tert*-butoxycarbonyl protected bromo precursor **11a** by a palladium catalyzed reaction with tributylcyclopropylstannane followed by deprotection with TFA.

1,5-Diazocanes **38**−**43** and **48** (Scheme 1) were prepared in a manner analogous to that of the reactions above from two different precursors, either from *tert*-butoxycarbonyl derivative **38b**, obtained from [1,5]diazocane dihydrobromide[17] or from [1,5]diazocane **39b**, also obtained from [1,5]diazocane dihydrobromide by treatment with sodium methoxide.

Two different sets of isomers of the 3,9-diazabicyclo[4.2.1]-nonanes, **30** and **31**, were prepared (Scheme 3), which are connected in their 3-positions with the corresponding propenyloxy and ethoxypyridines and **32**−**34** (Scheme 4), connected in their 9-positions with the corresponding propenyloxy and alkyloxypyridines. The precursor of **30** and **31** was (±)-methyl-3,9-diazabicyclo[4.2.1] nonane (**30b**), prepared from commercial tropinone[18] (**30a**). The final step to give **30** and **31** was a demethylation reaction using diethyl azodicarboxylate. Compounds **32**−**34** were prepared from benzyltropinone (**32a**) as outlined in Scheme 4. 3,10-Diazabicyclo[4.3.1]decane (**35**) (Scheme 5), a homoanalogue of the 3,9-diazabicyclo[4.2.1]-nonanes with the pyridyl connected to its 3-position, was prepared as outlined in Scheme 5.

Scheme 1[a]



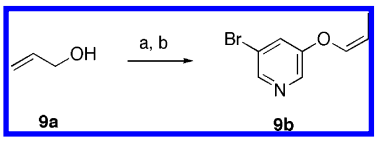
[a] Reagents: (a) *t*-BuOK; (b) TFA; (c) Boc_2O; (d) NBS; (e) Na, 1,2-ethanediol; (f) Et_3N; (g) 130°; (h) K_2CO_3, Cu(I)I, Pd/C, PPh_3; (i) HCl; (j) $PdCl_2(PPh_3)_2$, tetramethyltin; (k) 1,3-dichloromethylhydantoin; (l) $PdCl_2(PPh_3)_2$, tributylcyclopropylstannane; (m) NIS; (n) Na; (o) tetrakis(triphenylphosphine)palladium(0); (p) *n*-BuONa.

Pyridyl ether **36** (Scheme 6) was prepared by a Mitsunobu reaction with a Boc-protected alcohol followed by deprotection using TFA, analogous to previously described procedures.[15]

Receptor Binding. The predominant nAChR subtype found in brain tissue is composed of $\alpha_4\beta_2$ subunits, which can be selectively labeled by the nicotine agonist ^{3}H-cytisine.[19,20] The in vitro affinities of the compounds for the $\alpha_4\beta_2$ subtype of nAChRs were determined in a ^{3}H-cytisine binding assay using rat cerebral cortical membranes as described in the Experimental Section. The IC_{50} values are given in Table 1.

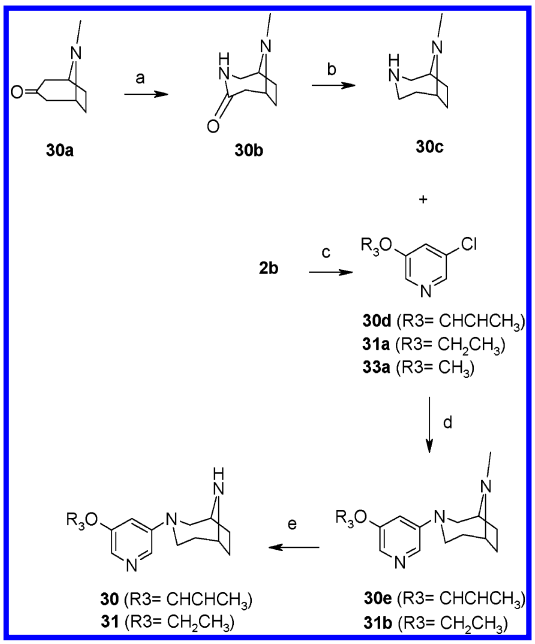
Comparing the affinities of compounds with a monocyclic ring system containing the sp^3 nitrogen atom and the same substitution in the pyridyl ring, the data in Table 1 shows that compound **38** containing an eight-membered ring displays a somewhat higher affinity (IC_{50} = 1.0 nM) than that of the previously studied seven-membered compound **22** (IC_{50} = 1.9

Scheme 2[a]



[a] Reagents: (a) Na; (b) **4a**.

Scheme 3[a]



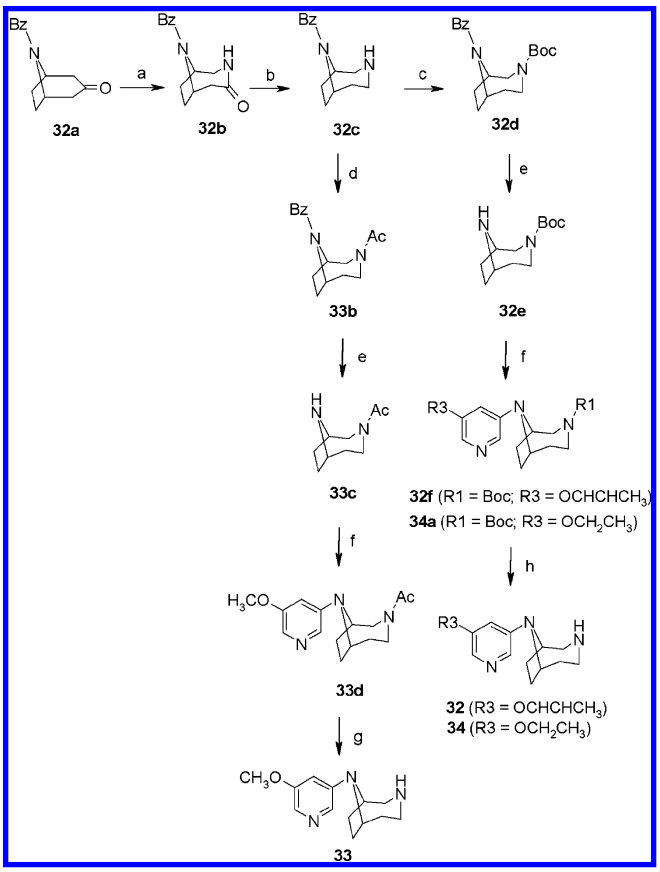
[a] Reagents: (a) H_2SO_4, NaN_3; (b) $LiAlH_4$; (c) R3ONa; (d) *t*-BuOK; (e) diethyl azodicarboxylate.

nM) and significantly higher affinity than that of the corresponding compound with a six-membered ring system (**1**, IC_{50} = 500 nM). Similarly, compound **41** (eight-membered ring, IC_{50} = 0.45 nM) displays a slightly higher affinity than **23** (seven-membered ring, IC_{50} = 1.0 nM) and an affinity that is 2 orders of magnitude higher than that of **50** (six-membered ring, IC_{50} = 180 nM). The eight-membered ring compound **43** displays the highest affinity (IC_{50} = 0.38 nM) in the present series of compounds. This is in agreement with the prediction from our previous 3D-QSAR study that a bulky ring system that includes the ammonium nitrogen atom is favorable for affinity.[15] Thus, larger monocyclic ring systems up to at least eight-membered rings display increased affinity, although the affinity differences between eight- and seven-membered rings are significantly smaller than that between seven- and six-membered rings.

Bicyclononanes **30**−**34** and bicyclodecane **35** have affinities (IC_{50}) in the range of 1.9−10 nM, which is similar to those observed for the previously studied bicyclooctene **45** and bicyclononenes **46** and **47** (Table 1). The large bicyclic nine- and ten-membered ring systems are well accommodated by the receptor but do not increase the affinity compared to that of the less bulky bicyclooctene and bicyclononenes and display lower affinities than that of the eight-membered monocyclic ring compounds.

Figure 1 shows a least-squares superimposition of compounds containing the previously studied[15] and the new rings systems. The superimposition is obtained according to the procedure described by Nielsen et al.[15] by superimposing the centroid of the pyridine ring, the pyridine nitrogen, the protonated nitrogen, and the two protons attached to this nitrogen using homopiperazine **55** as a template. The procedure is described below and in the Experimental Section in more detail. Figure 1 clearly

Scheme 4[a]



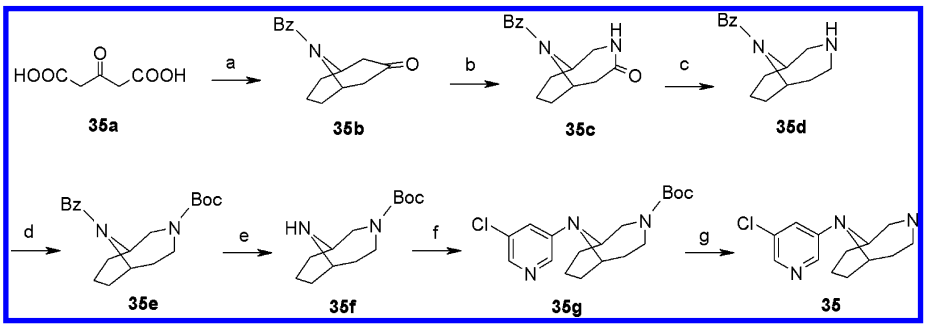
[a] Reagents: (a) NaN_3, H_2SO_4; (b) $LiAlH_4$; (c) Et_3N, Boc_2O; (d) Ac_2O; (e) Pd/C, H_2; (f) *t*-BuOK, **30d** (for compound **32f**), **33a** (for compound **33d**), **31a** (for compound **34a**); (g) HCl; (h) TFA.

shows that the new ringsystems are significantly more bulky than the previously studied ring systems and extend outside the volume spanning the these ringsystems.

3D-QSAR Analysis. With the purpose of increasing the robustness of the previous 3D-QSAR model and obtaining a more general model, we have improved the previous model by including the new compounds in the training set. The 3D-QSAR analysis is based on a training set of 47 compounds (**1**−**23**, **25**−**47**, **54**, and **56**) and tested with a validation set of 7 compounds (**48**−**53**, **55**). In contrast to the study by Nielsen et al.,[15] the thioethyl-substituted homopiperazine **24** was excluded from the training set. The substituent in this compound is one of a kind, and the bioactive conformation is ambiguous. The compounds were aligned to homopiperazine **55** in the proposed bioactive conformation calculated by Nielsen et al.[15] using the centroid of the pyridine ring, the pyridine nitrogen, the protonated nitrogen, and the two protons attached to this nitrogen as fitting points. For each compound, a conformational analysis was performed by using the Macromodel program.[21] The calculations were performed for the N-protonated compound in aqueous solution. The low energy conformer giving the best fit to template compound **55** was used for the 3D-QSAR analysis (for details see the Experimental Section). A superimposition of compounds **5**, **25**, **30**, **32**, **35**, **36**, **40**, **44**−**46**, and **56**, representing the 11 different structural classes included in the present study, is shown in Figure 1.

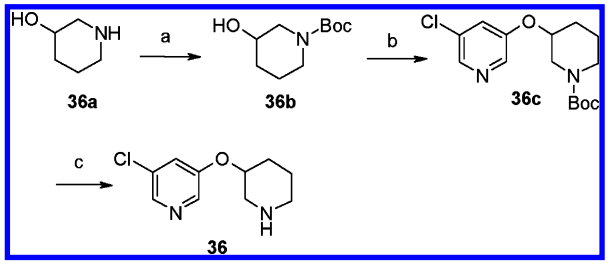
The molecular descriptors were generated with the GRID program[22] by calculating the interaction energies between two probes, water (OH2) and methyl (C3), and the target molecule as described by Nielsen et al.[15] Charged probes O^- and $N1^+$ do not improve the quality of the model.[15]

Scheme 5[a]



[a] Reagents: (a) benzylamine, glutaric dialdehyde, H_2O; (b) NaN_3, H_2SO_4; (c) $LiAlH_4$; (d) Et_3N, Boc_2O; (e) Pd/C, H_2; (f) 3,5-dicholoropyridine, *t*-BuOK; (g) TFA.

Scheme 6[a]



[a] Reagents: (a) Boc_2O, Et_3N, (b) diethyl azodicarboxylate, PPh_3, (c) TFA.

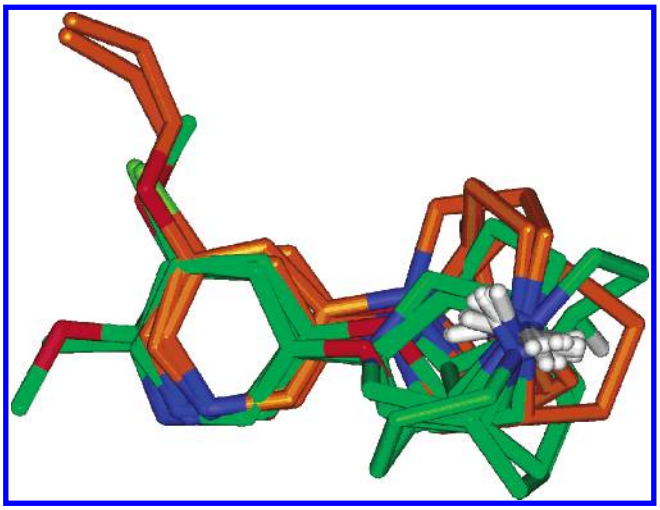


Figure 1. Alignment of compounds **5**, **25**, **30**, **32**, **35**, **36**, **40**, **44**−**46**, and **56**. Compounds **30**, **32**, **35**, and **40**, containing the new ring systems, are displayed with orange carbon atoms. Nonpolar hydrogens are removed for clarity.

Table 2. Properties of the 3D-QSAR Models

	no. of variables	LVS	R^2	q^2	SDEP[a]
after pretreatment	4314	3	0.92	0.68	0.39
after FFD selection	1413	3	0.94	0.83	0.34

[a] Standard deviation on error of prediction (SDEP) for external validation set (log(10) units), SDEP = $(\Sigma(Y_{Exp} - Y_{Calc})^2/N)$.

The 3D-QSAR model was calculated using the program GOLPE.[23,24] The properties of the calculated model are summarized in Table 2. A large number of the descriptors (variables) calculated by GRID do not contribute to the explanation of the observed differences in affinity and can, therefore, be characterized as noise. On the basis of the variable pretreatment protocol described in the Experimental Section, the initial 11 638 variables were reduced to 4314 without affecting the predictivity of the 3D-QSAR model (as judged by Q^2). Further variable

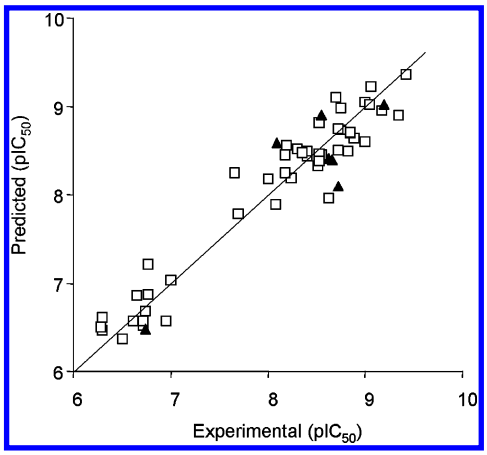


Figure 2. Experimental and predicted affinities (pIC_{50}) for the $\alpha_4\beta_2$ nAChR. Training set (□); validation set (▲).

selection was performed using the smart region definition[25] (SRD) in combination with fractional factoral design (FFD) procedures implemented in GOLPE. The SRD procedure groups the variables close in space and collapses the groups of variables containing virtually the same information to smart regions. These regions are then evaluated using the FFD procedure. In the FFD procedure, a large number of models are calculated with some regions left out to evaluate the importance of the individual regions. Only regions contributing in a positive manner to the predictivity of the model are included in the final model. The FFD procedure reduced the number of variables to 1413 with a significant improvement of the predictivity of the model as determined by Q^2 (from $Q^2 = 0.68$ to $Q^2 = 0.83$, Table 2). The model was further tested using an external validation set (i.e., a set of compounds not included in the training set). The affinities of these compounds were predicted with a standard deviation on error of prediction (SDEP) value of 0.34. The experimental and calculated affinities for the compounds in the training set and in the external validation set are listed in Table 1 and graphically shown in Figure 2.

Interpretation of Contour Plots. With the aim of interpreting the 3D-QSAR model, the weighed PLS coefficients from the most informative first component were plotted for both probes together with diazabicyclononane **30** to illustrate the size of the regions. The negative and positive fields for the water (OH2) probe are shown in Figure 3a, and the corresponding plot of the fields for the C3 probe are shown in Figure 3b. The two sets of plots appear similar to each other with only minor differences, suggesting that steric factors are of major importance in explaining the differences in the observed affinities of the molecules. The contribution of the OH2 probe to the PLS model is ambiguous because this probe, apart from the hydrogen

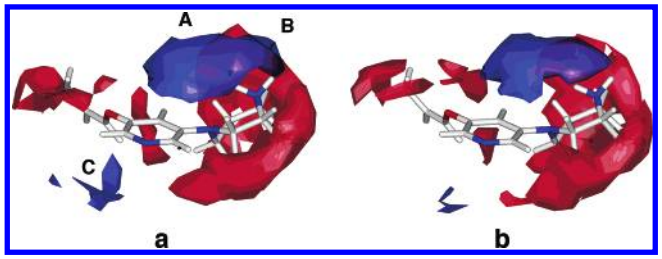


Figure 3. (a) Contour maps of the PLS coefficients from the first component for the OH2 probe. Negative coefficients are shown in blue (−0.001 level), and positive coefficients are shown in red (+0.0006 level). Diazabicyclononane **30** is shown to illustrate the size of the region. (b) Contour maps of the PLS coefficients from the first component for the C3 probe. Negative coefficients are shown in blue (−0.001 level), and positive coefficients are shown in red (+0.0006 level). Diazabicyclononane **30** is shown to illustrate the size of the region.

bonding component, also contains a steric component. Consequently, the regions of interest for the OH2 probe are the regions in space surrounding the molecules where the plot deviates from the corresponding plot of the C3 probe. The largest deviations, marked A, B, and C in Figure 3a, are seen in the regions with negative coefficients (blue regions). A favorable interaction (e.g., a hydrogen bonding interaction) between the molecule and a water probe in these regions is predicted to lead to increased affinity. The most important of these regions (most negative coefficients) is region A, which suggests that a hydrogen bond from the protonated amine to the receptor is of major importance in explaining the observed differences in affinity. Another major difference is region C, suggesting that the pyridine nitrogen has an important electrostatic interaction with the receptor and finally region B, suggesting that the second hydrogen on the protonated amine has a hydrogen bond to the receptor, although of lower importance compared to that of region A.

The most dominant regions in the plot for the C3 probe in Figure 3b are the positive regions (shown in red). An unfavorable interaction (steric clash) betwen the molecule and the probe in these regions is predicted to lead to increased affinity. The most important positive region surrounds the substituents holding the protonated amine and suggests that a large substituent in this position leads to increased affinity. This is evident when comparing piperazine **1** (six-membered ring) with corresponding diazocane **38** (eight-membered ring), the latter being more potent by a factor of 500. Another important positive region is in the area of the R3-substituents, suggesting that large substituents in this position are tolerated and could lead to a slightly increased affinity. This is evident when comparing homopiperazines **15** (R3: $OCH{=}CH_2$) and **9 (**R3: $OCH{=}CH_2$-CH_3**)**, the latter having a 4-fold higher affinity.

The present model complements and extends the previous model by Nielsen et al. by including more compounds with larger substituents containing the protonated nitrogen, thus improving the predictivity of the model with regard to such compounds. One major difference is the size of the region of negative coefficients for the C3 probe (shown in blue) around the R4-substituent, which is large in the model by Nielsen et al. and small in the present model. An unfavorable interaction between a molecule and the methyl probe in areas with negative coefficients is predicted to lead to decreased affinity. The difference between the two models is due to homopiperazine **24**, which was removed from the present study because of the ambiguous orientation of the thioethyl side chain.

Receptor Interactions. If the alignment of the compounds studied is a good representation of the binding modes and

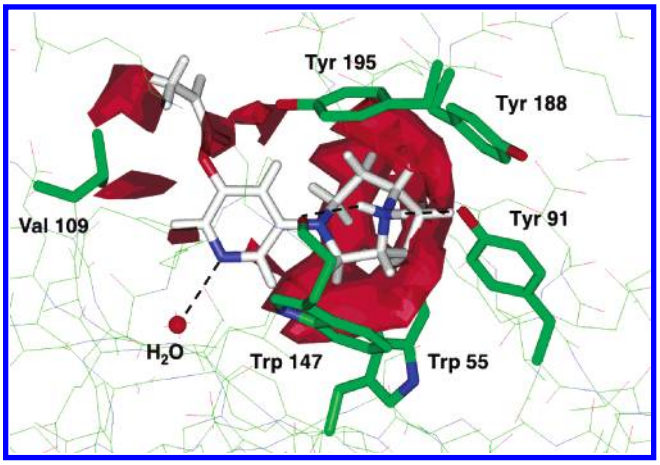


Figure 4. Contour map for the positive PLS coefficients (+0.0006 level) for the C3 probe and diazabicyclononane **30** manually docked into the binding pocket of the homology model of the $\alpha_4\beta_2$ nicotinic receptor. Amino acids Val 109, Tyr 195, Tyr 188, Tyr 91, Trp 55, and Trp 147 and a water molecule are highlighted.

bioactive conformations of the compounds, then the positive and negative regions calculated by GOLPE should represent interactions in the binding pocket of the $\alpha_4\beta_2$ receptor. To illustrate this, diazabicyclononane **30** was manually docked into the homology model of the $\alpha_4\beta_2$ receptor reported by Le Novere.[16] The docking was guided by the binding mode and interactions displayed by nicotine in the binding pocket of the acetylcholine binding protein (AChBP, pdb code: 1UW6).[26] The nicotine−AChBP complex displays a water molecule that is hydrogen bonding to the pyridyl nitrogen atom of nicotine. Thus, we introduced a water molecule at the corresponding position in the homology model of $\alpha_4\beta_2$. Compound **30** was docked into the binding pocket in the conformation employed in the 3D-QSAR analysis with the constraints of forming a hydrogen bond between its pyridyl nitrogen and the introduced water molecule and between the backbone carbonyl of Trp147 and one of the ammonium group hydrogen atoms of **30**. It is then observed that in this binding mode the ammonium group of **30** could also form a hydrogen bond to Tyr91. In Figure 4, compound **30** together with a plot of the positive PLS coefficients for the C3 probe is shown as docked into the binding cavity of $\alpha_4\beta_2$. The highlighted amino acids, Val109, Tyr195, Tyr 188, Tyr91, Trp55, Trp 147, and the water molecule form a cavity surrounding the plot of the positive PLS-coefficients displayed at the +0.0006 contour level. The Figure nicely illustrates that there are important lipophilic interactions between the five aromatic residues and the ringsystems containing the protonated nitrogen atom. The regions with positive coefficients around the R3 substituent extend into a small channel flanked by Val109. The size and shape of this channel extends beyond the borders of the present 3D-QSAR model, suggesting that the affinity could be increased further by extending the chain length of the R3 substituent.

The proposed binding mode and interactions of **30** are strongly supported by a recent crystal structure of a AChBP−epibatidine complex.[27] In particular, the ammonium group of epibatidine forms the two hydrogen bonds shown in Figure 4, and the pyridyl group is located in a position very close to that displayed by **30**. Because water molecules are not resolved in the crystal structure (3.4 Å resolution), the presence of a water molecule interacting with the pyridyl group cannot be confirmed by this structure. However, when comparing the position of the water molecule in Figure 4 with the negative region C in Figure 3a, it becomes obvious that region C represents a water molecule

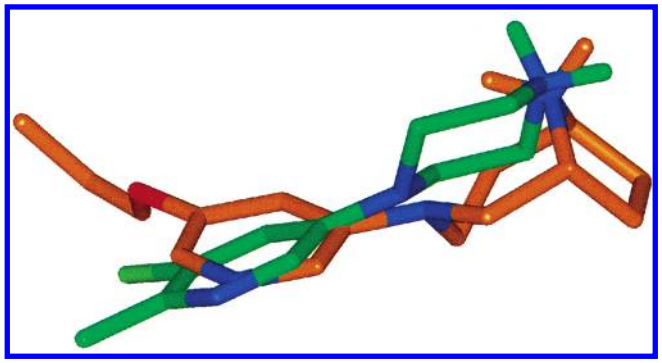


Figure 5. Molecular superimposition of low affinity piperazine compound **5** and high affinity diazabicyclononane **30**.

in the receptor. Furthermore, the hydrogen bonds from the protonated amine to the backbone carbonyl of Trp A147 and to the oxygen of Tyr A91 are represented in the QSAR model by regions A and B, respectively. The relative importance of region A compared to that of B may be explained by the backbone carbonyl being a much better hydrogen bond acceptor compared to the phenol of Tyr A91.

When interpreted in light of the 3D-QSAR model discussed above and in light of the homology model of the $\alpha_4\beta_2$ receptor, it is easy to understand why piperazines **1**−**8** and **50** all have low affinities. The hydrogens of the protonated amine has a nonoptimal direction for interaction with the two hydrogen bond acceptors in the receptor (corresponding to field A and B in Figure 3a) as displayed in Figure 5. Also, as shown in Figure 5, the piperazine ring lies closer to the areas of negative coefficients for the C3 probe (blue areas, Figure 3b) compared to the diazabicyclononane ring in **30**. The interaction between a molecule and the C3 probe in these areas is predicted to lead to decreased affinity. Similarly, the bulk of the piperidine ring in piperidin-3-yl-oxy derivatives **36** and **37** and the pyrrolidine ring of pyrrolidin-3-yl-oxy derivatives **44** and **54** are situated close to the negative areas, which may explain the low affinities (>100 nM) of these compounds.

The compounds substituted with the most bulky ring systems, that is, bicyclic derivatives **30**−**35** and **45**−**47**, all bind with high affinity to the $\alpha_4\beta_2$ receptor. These compounds have an almost perfect hydrogen-bonding geometry as indicated for diazabicyclo-nonane **30**, depicted in Figure 4. Furthermore, the compounds at least partly occupy the favorable area of positive coefficients for the C3 probe shown in Figure 3b. The compounds with the highest affinity are homopiperazine **28** and diazocanes **40**, **41**, and **43**, all binding to the $\alpha_4\beta_2$ receptor with subnanomolar affinity. Despite being slightly smaller than the bicyclic derivatives discussed above, these compounds also form almost perfect hydrogen bonds with the receptor and occupy space in the predicted favorable region shown in red in Figures 3b and 4.

Conclusions

In the present study, the preparation of a series of new ligands with affinity for the $\alpha_4\beta_2$ subtype of the nicotinic receptor is reported. The compounds were designed on basis of a previously reported 3D-QSAR model and revealed, in particular, a series of diazocanes, **40**, **41**, and **43**, with subnanomolar affinity for the $\alpha_4\beta_2$ subtype of the nicotinic receptor. A combination of 3D-QSAR and homology modeling was used to interpret the structure-affinity relationships of the ligands. These methods revealed that hydrogen bonding from both protons of the protonated amine to the receptor as well as from the pyridine nitrogen to a water molecule is important for ligand binding. Furthermore, lipopilic interactions between the ringsystems containing the protonated nitrogen seem to be of major importance with regard to the difference in the affinities of the described compounds. A previous 3D-QSAR study of $\alpha_4\beta_2$ ligands related to epibatidine, cytosine, anatoxin-a, and ferruginin using the CoMFA and CoMSIA methods resulted in high quality models.[30] However, the small variations in the data set with respect to the size of the ringsystems containing the sp^3 nitrogen atom could not properly map the importance of steric properties on the affinities of various ring systems.

The strength of the present study is the interpretability of the 3D-QSAR results. The combination of different methods has proven very successful for the validation of the individual modeling approaches. The previously reported homology model of the $\alpha_4\beta_2$ receptor and the coefficient plots obtained from the present 3D-QSAR study complement each other. When manually docked into the receptor model, the important fields of the coefficient plot overlay the areas of the homology model known from crystallographic ligand−receptor complexes to be important for ligand binding.

Experimental Section

Chemistry. 3-Bromo-5-methoxypyridine (1a). Compound **1a** was prepared from a mixture of **4a** (25.0 g, 105.0 mmol), sodium methoxide (2.91 g, 126.0 mmol), and DMSO (100 mL) stirred at 90 °C for 3 h. Aqueous NaOH (400 mL, 1 M) was added, and the mixture was extracted with diethyl ether (2 × 200 mL). Yield 12.1 g (61%); mp 35.2−36.8 °C.

4-(5-Methoxypyridin-3-yl)-piperazine-1-carboxylic Acid *tert*-Butyl Ester (1c). Procedure A. Compound **1c** was obtained from a mixture of tetrakis(triphenylphosphine)palladium(0) (0.11 g, 0.10 mmol), 1-*tert*-butoxycarbonylpiperazine (**1b**) (5.20 g, 28.0 mmol), and 3-bromo-5-methoxypyridine (**1a**) (5.30 g, 28.0 mmol), prepared according to Nielsen et al.,[15] and potassium *tert*-butoxide (*t*-BuOK) (6.20 g, 56.0 mmol) stirred in anhydrous toluene (40 mL) at 80 °C for 3 h. The reaction mixture was cooled to room temperature. Aqueous sodium hydroxide (NaOH) (80 mL, 1 M) was added, and the mixture was extracted with ethyl acetate (EtOAc) (2 × 80 mL). CC with dichloromethane (CH_2Cl_2), methanol (MeOH), and concentrated aqueous ammonia (89:10:1) gave **1c** as an oil. Yield 2.59 g (31%).

1-(5-Methoxypyridin-3-yl)-piperazine Fumaric Acid Salt (1). Procedure B. Compound **1** was prepared from a solution of **1c** (2.50 g, 8.50 mmol), TFA (19.4 g, 170.0 mmol), and CH_2Cl_2 (30 mL) stirred at 20 °C for 4 h. The mixture was evaporated to dryness, and aqueous NaOH (20 mL, 1 M) was added. The mixture was extracted with CH_2Cl_2 (4 × 20 mL). CC with CH_2Cl_2, MeOH, and concentrated aqueous ammonia (89:10:1) gave **1**. Yield 0.43 g (16%); mp 168.5−170.5 °C. The corresponding salt was obtained by the addition of a diethyl ether and MeOH mixture (9:1) saturated with fumaric acid. Anal. ($C_{10}H_{15}N_3O{\cdot}0.5C_4H_4O_4{\cdot}H_2O$) C, H, N.

2-(5-Chloropyridin-3-yloxy)-ethanol (2c). Compound **2c** was prepared using 1,2-ethanediol (135 mL) and sodium (6.20 g, 268.0 mmol) stirred at 80 °C for 3 h. 3,5-Dichloropyridine (**2b**) (33.0 g, 223.0 mmol) and DMSO (100 mL) were added, and the mixture was stirred at 90 °C overnight. Aqueous NaOH (200 mL, 1 M) was added and the compound was extracted with EtOAc (3 × 150 mL). Yield 14.5 g (36%); mp 41.6−43.6 °C.

3-Chloro-5-vinyloxypyridine (2d). Compound **2d** was prepared from a mixture of **2c** (14.5 g, 82.1 mmol) and thionyl chloride ($SOCl_2$) (58.6 g, 0.49 mol) stirred in THF (100 mL) at 50 °C for 1 h. The mixture was evaporated to dryness. Potassium hydroxide (KOH) (100 mL, 4 M) and *tert*-butyl alcohol (*t*-BuOH) (100 mL) were added, and the mixture was stirred at 100 °C overnight. The mixture was evaporated. Water (100 mL) was added and the mixture extracted with diethyl ether (2 × 100 mL). CC with CH_2Cl_2. The product was obtained as an oil. Yield 9.44 g (74%).

1-(5-Vinyloxypyridin-3-yl)-piperazine Fumaric Acid Salt (2). Compound **2** was prepared according to procedure A from a mixture

of **2d** (2.00 g, 12.9 mmol), piperazine (**2a**) (2.20 g, 25.7 mmol), *t*-BuOK (3.63 g, 25.7 mmol), and DME (50 mL). Yield 1.26 g (45%). The corresponding salt was obtained by the addition of a diethyl ether and MeOH mixture (9:1) saturated with fumaric acid; mp 136.4−138.2 °C. Anal. ($C_{11}H_{15}N_3O\cdot 1.2C_4H_4O_4$) C, H, N.

4-*tert*-Butoxycarbonyl-1-(6-bromo-5-methoxypyridin-3-yl)-piperazine (3a). Compound **3a** was prepared from compound **1** (23.1 g, 74.7 mmol), aqueous sodium hydrogen carbonate (224 mL, 1 M), and Boc_2O (16.3 g, 74.7 mmol) stirred in CH_2Cl_2 (200 mL) stirred at room temperature for 1 h. The phases were separated. NBS (1.80 g, 10.2 mmol) and acetonitrile (50 mL) were added to the organic phase at 0 °C. The reaction was allowed to warm to room temperature and stirred for 2 h. Aqueous NaOH (100 mL, 4 M) was added, and the mixture was extracted with EtOAc (2 × 100 mL). CC (petroleum ether/ EtOAc (3:1)) gave **3a.** Yield 2.59 g (59%); mp 169.9−171.3 °C.

1-(6-Bromo-5-methoxypyridin-3-yl)-piperazine Fumaric Acid Salt (3). Compound **3a** (2.30 g, 6.23 mmol) was deprotected according to procedure B with TFA (7.30 g, 64.0 mmol) and CH_2Cl_2 (30 mL). Yield 2.28 g (100%). The corresponding salt was obtained by the addition of a diethyl ether and MeOH mixture (9:1) saturated with fumaric acid; mp 202−218 °C. Anal. ($C_{10}H_{14}N_3OBr\cdot 1.2C_4H_4O_4\cdot 0.5H_2O$) C, H, N.

1-(5-Bromopyridin-3-yl)-piperazine (4b). Compound **4b** was prepared from a mixture of 3,5-dibromopyridine (**4a**) (100 g, 422.0 mmol) and **2a** (72.7 g, 844.0 mmol) stirred at 130 °C for 100 h. H_2O (200 mL) was added at 100 °C followed by HCl (4 M) to obtain pH 7. Aqueous NaOH (200 mL, 1 M) was added followed by extraction with EtOAc (3 × 600 mL). The product was isolated as an oil. Yield 44.0 g (43%).

1-(5-Bromopyridin-3-yl)-piperazine-1-carboxylic Acid *tert*-Butyl Ester (4c). Compound **4c** was prepared according to procedure D from a mixture of **4b** (3.00 g, 12.4 mmol), Et_3N (2.51 g, 24.8 mmol), Boc_2O (2.70 g, 12.4 mmol), and CH_2Cl_2 (30 mL). Yield 3.8 g (89%); mp 112.9−114.1 °C.

1-(5,6-Dibromopyridin-3-yl)-piperazine-1-carboxylic Acid *tert*-Butyl Ester (4d). Compound **4d** was prepared according to procedure F from a mixture of **4c** (3.75 g, 11.0 mmol), NBS (1.94 g, 11.0 mmol), and acetonitrile (60 mL) stirred at 0 °C for 3 h. Aqueous NaOH (100 mL, 1 M) was added, and the mixture was extracted with EtOAc (100 mL). Yield 4.25 g (91%); mp 140.9−142.7 °C.

1-(5,6-Dibromopyridin-3-yl)-piperazine (4e). Compound **4e** was prepared according to procedure B from a mixture of **4d** (4.20 g, 10.0 mmol), TFA (11.3 g, 99.7 mmol), and CH_2Cl_2 (40 mL). Yield 1.9 g (43%); mp 199.5 °C.

1-(5-Bromo-6-chloropyridin-3-yl)-piperazine Fumaric Acid Salt (4). Compound **4** was prepared from a mixture of **4e** (1.50 g, 4.70 mmol) and concentrated hydrochloric acid (30 mL) stirred for 52 h at reflux. The reaction mixture was evaporated, aqueous NaOH (100 mL, 1 M) was added, and the mixture was extracted with EtOAc (2 × 50 mL). Yield 1.00 g (54%). The corresponding salt was obtained by the addition of a diethyl ether and MeOH mixture (9:1) saturated with fumaric acid; mp 181.2−185.9 °C. Anal. ($C_9H_{11}N_3BrCl\cdot 1.2C_4H_4O_4$) C, H, N.

1-(5-Chloropyridin-3-yl)-4-*tert*-butoxycarbonylpiperazine (5b). Compound **5b** was prepared according to procedure D from a mixture of 1-(5-chloropyridin-3-yl)piperazine (**5a**) (8.10 g, 41.0 mmol), prepared according to Nielsen et al.,[15] Boc_2O (8.90 g, 41.0 mmol), Et_3N (8.30 g, 82.0 mmol), and CH_2Cl_2 (80 mL). The product was obtained as an oil. Yield 11.9 g (98%).

1-(6-Bromo-5-chloropyridin-3-yl)-4-*tert*-butoxycarbonylpiperazine (5c). Compound **5c** was prepared according to procedure F from a mixture of **5b** (11.9 g, 40.0 mmol), NBS (7.10 g 40.0 mmol), and acetonitrile (150 mL) stirred at 20 °C for 100 h. Aqueous NaOH (200 mL, 1 M) was added, and the product was extracted with diethyl ether (200 mL). Yield 10.7 g (71%); mp 107.4−109.2 °C.

1-(5-Chloro-6-methylpyridin-3-yl)-piperazine Fumaric Acid Salt (5). Compound **5** was prepared using **5c** (2.00 g, 5.30 mmol), tetramethyltin (1.90 g, 10.6 mmol), $PdCl_2(PPh_3)_2$ (0.18 g, 0.26 mmol), and DMF (2.0 mL) stirred at 160 °C in a sealed stainless steel vessel overnight. The reaction mixture was stirred in concentrated hydrochloric acid (30 mL) at reflux for 15 min. The mixture was evaporated. Aqueous NaOH (50 mL, 1 M) was added, and the mixture was extracted with CH_2Cl_2 (3 × 50 mL). CC (CH_2Cl_2, MeOH and concentrated ammonia (89:10:1)) gave compound **5.** Yield 0.70 g (43%). The corresponding salt was obtained by the addition of a diethyl ether and MeOH mixture (9:1) saturated with fumaric acid; mp 178.1−180.5 °C. Anal. ($C_{10}H_{14}N_3Cl\cdot 1.4C_4H_4O_4\cdot 0.5H_2O$) C, H, N.

1-(5-Ethynylpyridin-3-yl)-4-*tert*-butoxycarbonyl-piperazine (6a). Compound **6a** was prepared from a mixture of **4c** (53.5 g, 156.0 mmol), K_2CO_3 (54.0 g, 391.0 mmol), copper(I) iodide (5.90 g, 31.3 mmol), Pd/C 5% (20.0 g, H_2O, 50%), triphenylphosphine (PPh_3) (4.10 g, 15.6 mmol), 2-methyl-3-butyn-2-ol (131 g, 1.60 mol), NaH (100 mg), and dioxane (300 mL) stirred at reflux for 10 days. The crude mixture was filtered through Celite and isolated by CC (petroleum ether/EtOAc (1:3) to give the corresponding alcohol. The alcohol was stirred with sodium hydride (0.50 g, 23.8 mmol) in toluene (200 mL) at reflux 2 days. Compound **6a** was isolated by CC (petroleum ether/EtOAc (1:3). Yield 16.6 g (35%); mp 129−130.7 °C.

1-(5-Ethynyl-pyridin-3-yl)-piperazine Fumaric Acid Salt (6). Compound **6** was prepared according to procedure B from a mixture of **6a** (1.02 g, 3.50 mmol), TFA (4.00 g, 35.3 mmol), and CH_2Cl_2 (10 mL). Yield 0.60 g (91%). The corresponding salt was obtained by the addition of a diethyl ether and MeOH mixture (9:1) saturated with fumaric acid; mp 175.1 °C. Anal. ($C_{11}H_{13}N_3\cdot 1.2C_4H_4O_4$) C, H, N.

(*Z*)-3-bromo-5-(prop-1-enyloxy)pyridine (9b). Compound **9b** was prepared from allyl alcohol (**9a**) (12.2 g, 210.0 mmol) and sodium (Na) (1.20 g, 50.2 mmol) stirred at 80 °C for 3 h. Compound **4a** (10.0 g, 42.0 mmol) and DMSO (50 mL) were added to the mixture and stirred at 80 °C for 30 min. Aqueous NaOH (100 mL, 1M) was added, and the product was extracted with diethyl ether (2 × 100 mL). The product was obtained as an oil. Yield 8.0 g (65%).

***cis*-1-(5-Propen-1-yloxypyridin-3-yl)-[1,4]diazepane Fumaric Acid Salt (9).** Compound **9** was prepared according to procedure A from a mixture of **9b** (10.0 g, 46.7 mmol), homopiperazine (**9c**) (23.4 g, 233.0 mmol), *t*-BuOK (11.4 g, 93.4 mmol), and tetrakis-(triphenylphosphine)palladium(0) (20.0 mg, 18.0 mol) stirred in DME (100 mL) at reflux for 1.5 h. Yield 3.30 g (30%); mp 124−126 °C. The corresponding salt was obtained by the addition of a diethyl ether and MeOH mixture (9:1) saturated with fumaric acid. Anal. ($C_{13}H_{19}N_3O\cdot C_4H_4O_4$) C, H, N.

1-(5-Chloropyridin-3-yl)-[1,4]diazepane (10a). Procedure E. Compound **10a** was prepared from 3,5-dichloropyridine (**2b**) (60.0 g, 405.0 mmol), homopiperazine (40.0 g, 405.0 mmol), *t*-BuOK (68.0 g, 608.0 mmol), and DME (500 mL) stirred at room temperature for 1.5 h. Aqueous NaOH (100 mL, 1 M) was added, and the mixture was extracted with EtOAc (2 × 150 mL). The product was obtained as an oil. Yield 16.4 g (19%).

4-(5-Chloropyridin-3-yl)-[1,4]diazepane-1-carboxylic Acid *tert*-Butyl Ester (10b). Compound **10b** was prepared according to procedure D from a mixture of **10a** (16.4 g, 77.5 mmol), Boc_2O (16.9 g, 77.6 mmol) and Et_3N (7.90 g, 77.6 mmol). Yield 10.2 g (42%); mp 63.8−65.6 °C.

4-(6-Bromo-5-chloropyridin-3-yl)-[1,4]diazepane-1-carboxylic Acid *tert*-Butyl Ester (10c). Procedure F. Compound **10c** was prepared from a mixture of **10b** (2.30 g, 7.50 mmol) and NBS (1.34 g, 7.50 mmol) stirred in acetonitrile (75 mL) for 1 h at room temperature. Compound **10c** was obtained by CC (petroleum ether/ EtOAc; 2:1). Yield 2.50 g (85%); mp 113−114 °C.

1-(6-Bromo-5-chloropyridin-3-yl)-[1,4]diazepane Fumaric Acid Salt (10). Compound **10** was prepared according to procedure B from a mixture of **10c** (2.50 g, 6.40 mmol), TFA (7.30 g, 64.0 mmol), and CH_2Cl_2 (65 mL) for 6 h. Yield 1.75 g (94%). The corresponding salt was obtained by addition of a diethyl ether and MeOH mixture (9:1) saturated with fumaric acid; mp 213-216 °C. Anal. ($C_{10}H_{13}N_3BrCl\cdot C_4H_4O_4$) C, H, N.

4-(5-Bromopyridin-3-yl)-[1,4]diazepane (11a). Compound **11a** was prepared according to procedure E from a mixture of **4a** (60.0 g, 253.0 mmol), **9c** (25.0 g, 253.0 mmol), *t*-BuOK (42.0 g, 380.0 mmol), and DME (600 mL). The product was obtained as an oil. Yield 19.8 g (30%).

4-(5-Bromopyridin-3-yl)-[1,4]diazepane-1-carboxylic Acid *tert*-Butyl Ester (11b). Compound **11b** was prepared according to procedure E from a mixture of **11a** (19.0 g, 74.2 mmol), Boc_2O (16.2 g, 74.2 mmol), Et_3N (7.50 g, 74.2 mmol), and CH_2Cl_2 (200 mL). Yield 14.6 g (55%); mp 58.1−60.7 °C.

4-(5-Bromo-6-chloropyridin-3-yl)-[1,4]diazepane-1-carboxylic Acid *tert*-Butyl Ester (11c). Compound **11c** was prepared according to procedure A from a mixture of **11b** (2.68 g, 7.50 mmol), 1,3-dichlorodimethylhydantoin (0.88 g, 4.50 mmol), and acetonitrile (75 mL) for 45 min. Compound **11c** was obtained by CC (petroleum ether/EtOAc; 3:1). The product was isolated as an oil. Yield 0.80 g (51%).

4-(5-Bromo-6-chloropyridin-3-yl)-[1,4]diazepane Fumaric Acid Salt (11). Compound **11** was prepared according to procedure B from a mixture of **11c** (0.80 g, 2.00 mmol), TFA (2.10 g, 19.0 mmol), and CH_2Cl_2 (20 mL) stirred for 2 h. Compound **11** was isolated by CC. Yield 0.23 g (40%). The corresponding salt was obtained by the addition of a diethyl ether and MeOH mixture (9:1) saturated with fumaric acid; mp 188.2−189.2 °C. Anal. ($C_{10}H_{13}N_3ClBr\cdot1.2C_4H_4O_4\cdot0.1H_2O$) C, H, N.

3-Chloro-5-methoxymethoxypyridine (12b). Compound **12b** was prepared from a mixture of 5-chloro-3-hydroxypyridine (**12a**) (10.0 g, 77.2 mmol), bromomethoxymethane (10.6 g, 84.9 mmol), K_2CO_3 (10.6 g, 77.2 mmol), and DMF (100 mL) stirred 45 min at 70 °C. Aqueous NaOH (100 mL, 1 M) was added. The product was extracted with diethyl ether (3 × 100 mL) and isolated as an oil. Yield 10.0 g (75%). The product was extracted as an oil.

4-(5-Methoxymethoxypyridin-3-yl)-[1,4]diazepane (12c). Compound **12c** was prepared from a mixture of **12b** (10.0 g, 57.6 mmol), homopiperazine (28.8 g, 288.0 mmol), *t*-BuOK (12.9 g, 115.0 mmol), palladium-dichloro-bistriphenylphosphine ($PdCl_2(PPh_3)_2$) (0.18 g, 0.26 mmol), and DME (100 mL) stirred at reflux for 3 h. Aqueous NaOH (100 mL, 1 M) was added, and the product was extracted with EtOAc (2 × 150 mL). Yield 9.7 g (71%); mp 129−131 °C.

4-(5-Methoxymethoxypyridin-3-yl)-[1,4]diazepane-1-carboxylic Acid *tert*-Butyl Ester (12d). Compound **12d** was prepared from a mixture of **12c** (1.00 g, 2.80 mmol), Boc_2O (0.60 g, 2.80 mmol), sodium hydrogen carbonate (10 mL, 1 M), and CH_2Cl_2 (10 mL). Compound **12d** was obtained by CC (MeOH /EtOAc (1:9)). The product was obtained as an oil. Yield 0.79 g (83%).

4-(6-Iodo-5-methoxymethoxypyridin-3-yl)-[1,4]diazepane-1-carboxylic Acid *tert*-Butyl Ester (12e). Compound **12e** was prepared according to procedure F from a mixture of **12d** (0.80 g, 2.30 mmol), *N*-iodosuccinimide (0.50 g, 2.30 mmol), and acetonitrile (10 mL). The product was isolated as an oil. Yield 0.87 g (82%).

5-[1,4]Diazepan-1-yl-2-iodo-pyridin-3-ol Hydrochloric Acid Salt (12). Compound **12** was prepared from a mixture of **12e** (0.87 g, 1.80 mmol) and hydrochloric acid (10 mL, 4 M) stirred in MeOH (6 mL) for 30 min at 20 °C. The reaction mixture was evaporated. The crystalline product was triturated with diethyl ether and filtered. Yield 0.65 g (40%); mp 200 °C. Anal. ($C_{10}H_{14}N_3OI\cdot2HCl\cdot0.3H_2O$) C, H, N.

4-(5-Cyclopropylpyridin-3-yl)-[1,4]diazepane-1-carboxylic Acid *tert*-Butyl Ester (13a). Compound **13a** was prepared from a mixture of **11b** (20.0 g, 56.0 mmol), tributylcyclopropylstannane (46.4 g, 140.0 mmol), $PdCl_2(PPh_3)_2$ (1.90 g, 2.80 mmol), and DMF (20 mL) stirred at 120 °C for 24 h. Aqueous NaOH (20 mL, 1 M) was added followed by extraction with diethyl ether (3 × 100 mL). CC (petroleum ether/ EtOAc(1:4)) gave compound **13a**. Yield 4.21 g (9%); mp 91.3−93.8 °C.

1-(5-Cyclopropylpyridin-3-yl)-[1,4]diazepane Fumaric Acid Salt (13). Compound **13** was prepared according procedure B from a mixture of **13a** (2.80 g, 8.80 mmol), TFA (10.0 g, 88.2 mmol), and CH_2Cl_2 (30 mL). Yield 0.44 g (23%). The corresponding salt was obtained by the addition of a diethyl ether and MeOH mixture (9:1) saturated with fumaric acid; mp 155.7 °C. Anal. ($C_{13}H_{19}N_3\cdot1.5C_4H_4O_4$) C, H, N.

(±)-9-Methyl-3,9-diazabicyclo[4.2.1]nonan-4-one (30b). Compound **30b** was prepared according to Michaels et al.,[18] using tropinone (**30a**) (40.0 g, 287.0 mmol), sodium azide (NaN_3) (37.3 g, 574.0 mmol), and concentrated H_2SO_4 (100 mL). The product was obtained as an oil. Yield 18.5 g (42%).

(±)-9-Methyl-3,9-diazabicyclo[4.2.1]nonane (30c). Compound **30c** was prepared according to Michaels et al.[18] using **30b** (255 g, 1.66 mol), diethyl ether (7.0 L), and lithium aluminum hydride ($LiAlH_4$) (158 g, 4.16 mol). Yield 174 g (75%); bp 90 °C (18 mmHg).

3-Chloro-5-propen-*cis*-yloxypyridine (30d). Compound **30d** was prepared from allyl alcohol (12.2 g, 210.0 mmol) and Na (1.20 g, 50.2 mmol) stirred at 80 °C for 3 h. 3,5-Dichloropyridin (25.0 g, 168.0 mmol) and DMSO (75 mL) were added, and the mixture was stirred at 60 °C for 1 h. H_2O (150 mL) was added, and the product was extracted with diethyl ether (50 mL) and EtOAc (2 × 100 mL). The product was obtained as an oil. Yield 29 g (100%).

(±)-*cis*-9-Methyl-3-(5-propen-1-yl-oxypyridin-3-yl)-3,9-diazabicyclo [4.2.1]nonane (30e). Compound **30e** was prepared according to procedure A from a mixture of **30d** (3.63 g, 21.4 mmol), sodium *tert*-butoxide (2.70 g, 28.6 mmol), and **30c** (2.00 g, 14.3 mmol) stirred in DME (30 mL) at 20 °C for 1 h. *t*-BuOK (3.20 g, 28.6 mmol) was added, and the mixture was stirred at 20 °C for 72 h. Yield 0.92 g (24%); mp 132−134 °C.

(±)-*cis*-3-(5-Propen-1-yloxypyridin-3-yl)-3,9-diazabicyclo-[4.2.1]nonane Fumaric Acid Salt (30). Procedure C. Compound **30** was prepared from a mixture of **30e** (0.60 g, 2.20 mmol), diethyl azodicarboxylate (1.96 g, 11.2 mmol) and toluene (10 mL). The mixture was stirred at reflux for 30 min and cooled to room temperature. Aqueous hydrochloric acid (20 mL, 4 M) was added, followed by stirring for 5 min. Aqueous NaOH (25 mL, 4 M) was added, and the mixture was extracted with EtOAc (2 × 20 mL). Compound **30** was isolated by CC. Yield 0.15 g (27%). The corresponding salt was obtained by the addition of a diethyl ether and MeOH mixture (9:1) saturated with fumaric acid; mp 144−146. Anal. ($C_{15}H_{21}N_3O\cdot1.3C_4H_4O_4\cdot2H_2O$) C, H, N.

3-Chloro-5-ethoxypyridine (31a). Compound **31a** was prepared according to Nielsen et al.[15] using **2b** (295 g, 1.99 mol). Yield 278 g (89%); bp 95−105 °C (16 mmHg).

(±)-3-(5-Ethoxypyridin-3-yl)-9-methyl-3,9-diazabicyclo[4.2.1]-nonane (31b). Compound **31b** was prepared according to procedure A from a mixture of **30c** (20.0 g, 142.0 mmol), *t*-BuOK (32.0 g, 285.0 mmol), **31a** (45.0 g, 285.0 mmol), and DME (200 mL). The product was obtained as an oil. Yield 10.4 g (28%).

(±)-3-(5-Ethoxypyridin-3-yl)-3,9-diazabicyclo[4.2.1]nonane Fumaric Acid Salt (31). Compound **31** was prepared according to procedure C from a mixture of **31b** (1.00 g, 3.80 mmol) and diethyl azodicarboxylate (2.00 g, 11.5 mmol). Compound **31** was isolated by CC. Yield 0.19 g (20%). The corresponding salt was obtained by the addition of a diethyl ether and MeOH mixture (9:1) saturated with fumaric acid; mp 143−146 °C. Anal. ($C_{14}H_{21}N_3O\cdot1.5C_4H_4O_4\cdot0.5H_2O$) C, H, N.

(±)-9-Benzyl-3,9-diazabicyclo[4.2.1]nonane-4-one (32b). Compound **32b** was prepared from benzyltropinone (**32a**) (ABCD GmbH) (15.0 g, 69.7 mmol) added in chloroform ($CHCl_3$) (150 mL) at −5 °C. Concentrated H_2SO_4 (35 mL) was added dropwise to keep the temperature below 5 °C. NaN_3 (9.06 g, 139.0 mmol) was added, and the mixture was stirred at 20 °C overnight, followed by stirring for 2 h at 50 °C. The mixture was cooled to room temperature, and a mixture of H_2O/ice (120 mL) was slowly added. The mixture was neutralized with solid NaOH and stirred overnight at 25 °C. Aqueous NaOH (40 mL, 4 M) was added, and the product was extracted with CH_2Cl_2 (3 × 80 mL). Yield 12.9 g (81%); mp 110−111.5 °C.

(±)-9-Benzyl-3,9-diazabicyclo[4.2.1]nonane (32c). Compound **32c** was prepared by using $LiAlH_4$ (4.08 g, 107.0 mmol) in diethyl ether (250 mL) and **32b** (11.8 g, 51.2 mmol) stirred overnight at 20 °C. H_2O (15 mL) was slowly added under H_2, and the mixture

was extracted with diethyl ether (2 × 50 mL). The product was obtained as an oil. Yield 10.1 g (91%).

(±)-9-Benzyl-3,9-diazabicyclo[4.2.1]nonane 3-carboxylic Acid *tert*-Butyl Ester (32d) Procedure D: Compound **32d** was prepared from a mixture of **32c** (5.00 g, 23.1 mmol), triethylamine (Et_3N) (4.68 g, 46.2 mmol), and di-*tert*-butyl-dicarbonate (Boc_2O) (5.04 g, 23.1 mmol) stirred overnight in CH_2Cl_2 at 20 °C. The organic phase was washed with aqueous NaOH (50 mL, 1 M), and CC (CH_2Cl_2, MeOH, and concentrated ammonia (89:10:1)) gave the protected compound as an oil. Yield 7.79 g (100%).

(±)-3,9-Diaza-bicyclo[4.2.1]nonane-3-carboxylic Acid *tert*-Butyl Ester (32e). Compound **32e** was prepared using **32d** (7.60 g, 24.0 mmol) in ethanol (EtOH) (100 mL) stirred with palladium on carbon 5% (Pd/C) (0.80 g) for 28 h under a hydrogen atmosphere. CC (CH_2Cl_2, MeOH, and concentrated ammonia (89:10:1)) gave the title compound. Yield 4.51 g (83%); mp 66.7−69.7 °C.

(±)-*cis*-9-(5-Propen-1-yloxy-pyridin-3-yl)-3,9-diaza-bicyclo-[4.2.1]nonane-3-carboxylic Acid *tert*-Butyl Ester (32f). Compound **32f** was prepared according to procedure A from a mixture of **32e** (1.00 g, 4.40 mmol), **30d** (0.90 g, 5.30 mmol), *t*-BuOK (0.90 g, 8.80 mmol), and DME (20 mL) stirred at 20 °C for 1h. The product was obtained as an oil. Yield 0.86 g (55%).

(±)-*cis*-9-(5-Propen-1-yloxypyridin-3-yl)-3,9-diazabicyclo-[4.2.1]nonane Fumaric Acid Salt (32). Compound **32** was prepared according to procedure B from **32f** (0.18 g, 0.50 mmol), TFA (6.46 g, 56.6 mmol), and CH_2Cl_2 (10 mL). Yield 0.13 g (69%). The corresponding salt was obtained by the addition of a diethyl ether and MeOH mixture (9:1) saturated with fumaric acid; mp 161−163 °C. Anal. ($C_{15}H_{21}N_3O \cdot 1.1C_4H_4O_4$) C, H, N.

3-Chloro-5-methoxypyridine (33a). Compound **33a** was prepared from a mixture of sodium (18.6 g, 810.0 mmol) and MeOH (500 mL) followed by evaporation. Compound **2b** (100 g, 675.0 mmol) and DMSO (500 mL) were added, and the mixture was stirred 2 h at 70 °C. Aqueous NaOH (2.0 L, 1 M) was added, and the mixture was extrated with diethyl ether (3 × 500 mL). The product was isolated as an oil. Yield 90.1 g (77%).

(±)-1-(9-Benzyl-3,9-diazabicyclo-[4.2.1]non-3-yl)-ethanone (33b). Compound **33b** was prepared from **32c** (5.00 g, 23.1 mmol), acetic anhydride (3.54 g, 34.7 mmol), CH_2Cl_2 (50 mL) stirred for 3 h at 20 °C. Aqueous NaOH (20 mL, 1 M) was added, and the product was extracted with EtOAc (4 × 30 mL). The product was obtained as an oil. Yield 5.90 g (100%).

(±)-1-(3,9-Diazabicyclo-[4.2.1]non-3-yl)-ethanone (33c). Compound **33c** was prepared from a mixture of **33b** (5.90 g, 22.8 mmol), Pd/C 5% (600 mg), and EtOH (100 mL) stirred under an atmosphere of hydrogen for 24 h. The mixture was filtered and evaporated. The product was isolated as an oil.Yield 2.73 g (71%).

(±)-9-(5-Methoxypyridin-3-yl)-3,9-diazabicyclo[4.2.1]non-3-yl-ethanone (33d). Compound **33d** was prepared according to procedure A from a mixture of **33a** (0.80 g, 5.90 mmol), **33c** (1.00 g, 5.90 mmol), *t*-BuOK (1.30 g, 11.9 mmol), and DME (20 mL). The product was obtained as an oil. Yield 0.45 g (27%).

(±)-9-(5-Methoxypyridin-3-yl)-3,9-diazabicyclo[4.2.1]nonane Fumaric Acid Salt (33). Compound **33** was prepared from a mixture of **33a** (0.40 g, 1.50 mmol) and concentrated hydrochloric acid (25 mL, 25%) stirred at reflux for 72 h. The mixture was evaporated. Aqueous NaOH (30 mL, 1 M) was added followed by extraction with EtOAc (3 × 20 mL). CC (CH_2Cl_2, MeOH, and concentrated ammonia (89:10:1)) gave the title compound **33**. Yield 60 mg (17%). The corresponding salt was obtained by the addition of a diethyl ether and MeOH mixture (9:1) saturated with fumaric acid; mp 183−185 °C. Anal. ($C_{13}H_{19}N_3O \cdot 1.2C_4H_4O_4$) C, H, N.

(±)-9-(5-Ethoxypyridin-3-yl)-3,9-diazabicyclo[4.2.1]nonane-3-carboxylic Acid *tert*-Butyl Ester (34a). Compound **34a** was prepared according to procedure E from a mixture of **32e** (1.10 g, 4.90 mmol), *t*-BuOK (1.10 g, 8.70 mmol), **31a** (0.70 g, 4.90 mmol), and DME (20 mL). The product was obtained as an oil. Yield 0.24 g (14%).

(±)-9-(5-Ethoxypyridin-3-yl)-3,9-diazabicyclo[4.2.1]nonane Fumaric Acid Salt (34). Compound **34** was prepared according to procedure B from a mixture of **34a** (0.23 g, 0.70 mmol), TFA (0.75 g, 6.60 mmol), and CH_2Cl_2 (10 mL). Yield 0.60 g (21%). The corresponding salt was obtained by the addition of a diethyl ether and MeOH mixture (9:1) saturated with fumaric acid; mp 191−192 °C. Anal. ($C_{14}H_{21}N_3O \cdot 2C_4H_4O_4$) C, H, N.

9-Benzyl-9-azabicyclo[3.3.1]nonan-3-one (35b). Compound **35b** was prepared according to Mach[29] from a mixture of acetone-1,3-dicarboxylic acid (**35a**) (40.5 g, 249.0 mmol), aqueous glutaric dialdehyde (25%, 140 mL), benzylamine (24 mL), and H_2O (400 mL) stirred for 1 h at 60 °C. The product was obtained as an oil. Yield 34.3 g (60%).

(+)-10-Benzyl-3,10-diazabicyclo[4.3.1]decan-4-one (35c). Compound **35c** was prepared from a mixture of **35b** (15.0 g, 65.4 mmol) and $CHCl_3$ (150 mL) at −5 °C. Concentrated H_2SO_4 (33 mL) was added dropwise to keep the temperature below 5 °C. NaN_3 (8.50 g, 130.0 mmol) was added, and the mixture was stirred overnight at 20 °C, followed by stirring for 2 h at 50 °C. The mixture was cooled to room temperature, and a mixture of H_2O/ice (100 mL) was slowly added. The mixture was neutralized with solid NaOH and stirred overnight at 25 °C. Aqueous NaOH (40 mL, 10 M) was added, and the product was extracted with CH_2Cl_2 (2 × 100 mL). CC (CH_2Cl_2, MeOH and concentrated ammonia (89:10:1)) gave compound **35c**. Yield 9.6 g (60%); mp 149−153 °C.

(+)-10-Benzyl-3,10-diazabicyclo[4.3.1]decane (35d). Compound **35d** was prepared from a mixture of **35c** (9.50 g, 38.9 mmol), $LiAlH_4$ (3.09 g, 81.6 mmol), and diethyl ether (200 mL) stirred at 20 °C overnight. H_2O (15 mL) was added under N_2, and the solid mixture was extracted with diethyl ether (2 × 50 mL). Aqueous NaOH (100 mL, 4 M) was added, and the mixture was extracted with diethyl ether (100 mL). The product was obtained as an oil. Yield 6.47 g (72%).

(+)-10-Benzyl-3,10-diazabicyclo[4.3.1]decane-3-carboxylic Acid *tert*-Butyl Ester (35e). Compound **35e** was prepared according to procedure D from a mixture of **35d** (5.45 g, 23.7 mmol), Et_3N (2.39 g, 23.7 mmol), Boc_2O (5.17 g, 23.7 mmol), and CH_2Cl_2 (80 mL). The product was isolated as an oil. Yield 8.06 g (100%).

(±)-3,10-Diazabicyclo[4.3.1]decane-3-carboxylic Acid *tert*-Butyl Ester (35f). Compound **35f** was prepared from a mixture of **35e** (8.00 g, 24.2 mmol) and palladium on carbon 10% (1.00 g) stirred in ethanol (100 mL) under a hydrogen atmosphere. Yield 5.20 g (90%); mp 97.8−101.9 °C.

(±)-10-(5-Chloropyridin-3-yl)-3,10-diazabicyclo[4.3.1]decane-3-carboxylic Acid *tert*-Butyl Ester (35g). Compound **35g** was prepared according to procedure E from a mixture of **35f** (1.50 g, 6.20 mmol), *t*-BuOK (1.40 g, 12.5 mmol), 3,5-dichloropyridine (1.85 g, 12.5 mmol), and DME (30 mL). The product was obtained as an oil. Yield 0.21 g (10%).

(±)-10-(5-Chloro-pyridin-3-yl)-3,10-diaza-bicyclo[4.3.1]decane Fumaric Acid Salt (35). Compound **35** was prepared according to procedure B from a mixture of **35f** (0.20 g, 0.60 mmol), TFA (0.70 g, 6.00 mmol) and CH_2Cl_2 (10 mL). Yield 60 mg (29%). The corresponding salt was obtained by the addition of a diethyl ether and MeOH mixture (9:1) saturated with fumaric acid; mp 181.4 °C. Anal. ($C_{13}H_{18}ClN_3 \cdot 1.2C_4H_4O_4$) C, H, N.

(±)-3-Hydroxypiperidine-1-carboxylic Acid *tert*-Butyl Ester (36b). Compound **36b** was prepared from a mixture of (±)-3-hydroxypiperidine (**36a**) (10.0 g, 98.9 mmol), aqueous sodium hydrogen carbonate (15 mL, 2 M), Et_3N (9.90 g, 98.5 mmol), and Boc_2O (21.6 g, 98.9 mmol) stirred overnight in CH_2Cl_2 (150 mL) at 20 °C. Compound **36b** was isolated by CC (CH_2Cl_2, MeOH, and ammonia (89:10:1)). The product was obtained as an oil. Yield 21.5 g (100%).

(±)-3-Chloro-5-(piperidin-3-yloxy)-pyridine-1-carboxylic Acid *tert*-Butyl Ester (36c). Compound **36c** was prepared from a mixture of PPh_3 (19.6 g, 74.8 mmol) and diethyl azodicarboxylate acid (13.0 g, 74.8 mmol) stirred in THF (250 mL) at 20 °C for 30 min. 5-Chloropyridin-3-ol (9.70 g, 74.8 mmol) and **36b** (10.0 g 49.8 mmol) were added, and the mixture was stirred overnight at 60 °C. Aqueous NaOH (100 mL, 1 M) was added, and the mixture extracted with diethyl ether (2 × 100 mL). Compound **36c** was

isolated by CC (CH_2Cl_2/ ethanol (9:1)). The product was isolated as an oil. Yield 4.61 g (20%).

(±)-3-Chloro-5-(piperidin-3-yloxy)-pyridine Fumaric Acid Salt (36). Compound **36** was prepared according to procedure B from a mixture of **36c** (4.50 g, 14.4 mmol), TFA (16.2 g, 143.0 mmol), and CH_2Cl_2 (100 mL). Yield 2.70 g (50%). The corresponding salt was obtained by the addition of a diethyl ether and MeOH mixture (9:1) saturated with fumaric acid; mp 170.5−172.7 °C. Anal. ($C_{10}H_{13}ClN_2O \cdot C_4H_4O_4$) C, H, N.

[1,5]Diazocane-1-carboxylic Acid *tert*-Butyl Ester (38b). Compound **38b** was prepared from a mixture of NaOMe, prepared from Na (833 mg, 36.2 mmol), MeOH (30 mL), and [1,5]diazocane dihydrobromide (**38a**) prepared according to Hernandez-Mora[17] (5.00 g, 18.1 mmol), and stirred for 30 min at room temperature. Et_3N (3.66 g, 36.2 mmol), Boc_2O (3.16 g, 14.5 mmol), and CH_2Cl_2 (150 mL) were added. The mixture was stirred overnight at 20 °C, and aqueous NaOH (150 mL, 1 M) was added. Yield 2.02 g (10%); mp 92−96 °C.

5-(5-Methoxypyridin-3-yl)-[1,5]diazocane-1-carboxylic Acid *tert*-Butyl Ester (38c). Compound **38c** was prepared according to procedure A from a mixture of **1a** (1.70 g, 9.30 mmol), **38b** (2.00 g, 9.30 mmol), *t*-BuOK (2.10 g, 18.6 mmol), and tetrakis-(triphenylphosphine)palladium(0) (0.50 g) stirred in DME (50 mL) at reflux for 3 h. The product was isolated as an oil. Yield 0.3 g (10%).

1-(5-Methoxypyridin-3-yl)-[1,5]diazocane Fumaric Acid Salt (38). Compound **38** was prepared according to procedure B from a mixture of **38c** (0.30 g, 0.90 mmol), TFA (0.50 g, 4.70 mmol), and CH_2Cl_2 (10 mL). Yield 0.11 g (55%). The corresponding salt was obtained by the addition of a diethyl ether and MeOH mixture (9:1) saturated with fumaric acid; mp 158−160 °C. Anal. ($C_{12}H_{19}N_3O \times 1.5\ C_4H_4O_4$) C, H, N.

3-*n*-Butoxy-5-chloropyridine (39a). Compound **39a** was prepared from a mixture of sodium (3.89 g, 168.0 mmol) and *n*-butanol (200 mL) stirred at 80 °C for 3 h. DMSO (100 mL) and **2b** (20.0 g, 135.0 mmol) were added, and the mixture was stirred 10 h at 70 °C. Aqueous NaOH (400 mL, 1 M) was added, and the mixture was extracted with diethyl ether (200 mL). The product was obtained as an oil. Yield 28 g (100%).

[1,5]Diazocane (39b). Compound **39b** was prepared from sodium (3.67 g, 159 mmol) dissolved in MeOH (80 mL). Compound **38a** (20.0 g, 72.5 mmol) was added, and the mixture was stirred for 10 min at reflux and evaporated. The product was obtained as an oil. Yield 9.8 g (42%).

5-[(5-(1-Butoxy)-pyridin-3-yl]-[1,5]diazocane Fumaric Acid Salt (39). Compound **39** was prepared according to procedure E from a mixture of **39a** (1.50 g, 5.40 mmol), **39b** (1.50 g, 5.40 mmol), *t*-BuOK (1.20 g, 10.9 mmol), and DME (30 mL). Yield 90 mg (63%). The corresponding salt was obtained by addition of a diethyl ether and MeOH mixture (9:1) saturated with fumaric acid; mp 146−148 °C. Anal. ($C_{15}H_{25}N_3O \times 1.5C_4H_4O_4$) C, H, N.

1-Pyridin-3-yl-[1,5]diazocane Fumaric Acid Salt (40). Compound **40** was prepared from a mixture of 3-fluoropyridine (**40a**) (3.00 g, 30.9 mmol), **39b** (9.80 g, 30.9 mmol), and 1,4 dioxane (3.0 mL) heated to 160 °C in a sealed stainless steel vessel for 18 h. Aqueous NaOH (50 mL, 1 M) was added, and the mixture extracted with EtOAc (3 × 40 mL). Compound **40** was isolated by CC (CH_2Cl_2, MeOH, and concentrated ammonia (89:10:1)). Yield 70 mg (1.5%). The corresponding salt was obtained by the addition of a diethyl ether and MeOH mixture (9:1) saturated with fumaric acid; mp 137.7−141.1 °C. Anal. ($C_{11}H_{17}N_3 \cdot 2C_4H_4O_4 \cdot 0.3H_2O$) C, H, N.

4-(Pyridin-3-yl)-[1,5]diazocane Acid *tert*-Butyl Ester (41a). Compound **41a** was prepared according to procedure D from a mixture of **40** (0.25 g, 1.30 mmol), Et_3N (0.26 g, 2.60 mmol), Boc_2O (0.34 g, 1.60 mmol), and CH_2Cl_2 (10 mL). The product was obtained as an oil. Yield 0.60 g (11%).

5-(6-Bromopyridin-3-yl)-[1,5]diazocane-1-carboxylic Acid *tert*-Butyl Ester (41b). Compound **41b** was prepared according to procedure F from a mixture of **41a** (2.04 g, 0.70 mmol), NBS (1.37 g, 0.70 mmol), and acetonitrile (7.0 mL). The product was isolated as an oil. Yield 0.11 g (5%).

1-(6-Bromopyridin-3-yl)-[1,5]diazocane Fumaric Acid Salt (41). Compound **41** was prepared according to procedure B from a mixture of **41b** (0.10 g, 0.30 mmol), TFA (0.68 g, 6.00 mmol), and CH_2Cl_2 (5.0 mL). Yield 0.66 mg (82%). The corresponding salt was obtained by the addition of a diethyl ether and MeOH mixture (9:1) saturated with fumaric acid; mp 176−177 °C. Anal. ($C_{11}H_{16}N_3Br \cdot 1.5C_4H_4O_4$) C, H, N.

1-(5-Chloropyridin-3-yl)-[1,5]diazocane Fumaric Acid Salt (42). Compound **42** was prepared from a mixture of **2b** (2.70 g, 18.1 mmol) and **39b** (2.00 g, 18.1 mmol) stirred overnight at 150 °C. Aqueous NaOH (50 mL, 1 M) was added, and the mixture was extracted with EtOAc (2 × 30 mL). Yield 0.10 g (2.5%). The corresponding salt was obtained by the addition of a diethyl ether and MeOH mixture (9:1) saturated with fumaric acid; mp 174−176 °C. Anal. ($C_{11}H_{16}ClN_3 \cdot 1.6C_4H_4O_4$) C, H, N.

5-(5-Vinyloxypyridin-3-yl)-[1,5]diazocane-1-carboxylic Acid *tert*-Butyl Ester (43a). Compound **43a** was prepared according to procedure E from a mixture of **2d** (1.42 g, 9.10 mmol), **38b** (1.94 g, 9.10 mmol), *t*-BuOK (2.00 g, 18.2 mmol), and DME (40 mL). The product was obtained as an oil. Yield 0.14 g (4.6%).

5-(6-Bromo-5-vinyloxypyridin-3-yl)-[1,5]diazocane-1-carboxylic Acid *tert*-Butyl Ester (43b). Compound **43b** was prepared according to procedure F from a mixture of **43a** (0.14 g, 0.40 mmol), NBS (75.0 mg, 0.40 mmol), and acetonitrile (10 mL). Yield 86.0 mg (52%); mp 135.4−138 °C.

1-(6-Bromo-5-vinyloxypyridin-3-yl)-[1,5]diazocane Fumaric Acid Salt (43). Compound **43** was prepared according to procedure B from a mixture of **43b** (0.83 g, 0.20 mmol), TFA (0.23 g, 2.00 mmol), and CH_2Cl_2 (10 mL). Yield 30 mg (56%). The corresponding salt was obtained by the addition of a diethyl ether and MeOH mixture (9:1) saturated with fumaric acid; mp 155.7−158.9 °C. Anal. ($C_{13}H_{18}N_3OBr \cdot C_4H_4O_4 \cdot H_2O$) C, H, N.

1-(5-Bromopyridin-3-yl)-[1,5]diazocane Fumaric Acid Salt (48). Compound **48** was prepared from a mixture of **39b** (2.07 g, 18.1 mmol) and **4a** (4.28 g, 18.1 mmol) stirred for 48 h at 150 °C in the absence of solvent. The mixture was allowed to reach room temperature. Aqueous NaOH (100 mL, 1 M) was added, followed by extraction with EtOAc (2 × 30 mL). Compound **48** was isolated by CC (CH_2Cl_2, MeOH, and concentrated ammonia (89:10:1)). Yield 0.31 g (63%). The corresponding salt was obtained by the addition of diethyl ether and MeOH mixture (9:1) saturated with fumaric acid; mp 151−153 °C. Anal. ($C_{11}H_{16}N_3Br \cdot 0.7C_4H_4O_4 \cdot 0.8H_2O$) C, H, N.

1-(5-Vinylpyridin-3-yl)-[1,4]diazepane-1-carboxylic Acid *tert*-Butyl Ester (49a). Compound **49a** was prepared from a mixture of 1-(5-vinyloxy-pyridin-3-yl)-[1,4]diazepane fumarate (**15**) (1.00 g, 3.00 mmol), prepared according to Nielsen et al.,[15] aqueous sodium hydrogen carbonate (10 mL, 1 M), and Boc_2O (0.72 g, 3.30 mmol) stirred in CH_2Cl_2 (6.0 mL) at room temperature for 1 h. Compound **49a** was isolated by CC (CH_2Cl_2, MeOH, and concentrated ammonia (89:10:1)). The product was isolated as an oil.Yield 1.0 g (100%).

1-(6-Bromo-5-vinylpyridin-3-yl)-[1,4]diazepane-1-carboxylic Acid *tert*-Butyl Ester (49b). Compound **49b** was prepared according to procedure F from a mixture of **49a** (0.63 g, 2.00 mmol), NBS (0.42 g, 2.40 mmol), and acetonitrile (20 mL). Yield 0.64 g (80%); mp 115.2−116.8 °C.

1-(6-Bromo-5-vinylpyridin-3-yl)-[1,4]diazepane Fumaric Acid Salt (49). Compound **49** was prepared according to procedure B from a mixture of **49b** (0.64 g, 1.60 mmol), TFA (1.80 g, 16.0 mmol), and CH_2Cl_2 (25 mL). Yield 0.24 g (50%). The corresponding salt was obtained by the addition of a diethyl ether and MeOH mixture (9:1) saturated with fumaric acid; mp 128.6 °C. Anal. ($C_{12}H_{16}N_3BrO \cdot 1.2C_4H_4O_4$) C, H, N.

4-(Pyridin-3-yl)-piperazine-1-carboxylic Acid *tert*-Butyl Ester (50b). Compound **50b** was prepared from a mixture of 1-(pyridin-3-yl)-piperazine (**50a**) (2.50 g, 8.90 mmol), aqueous sodium hydrogen carbonate (25 mL, 1 M), Boc_2O (1.90 g, 8.90 mmol), and CH_2Cl_2 (25 mL) stirred overnight at room-temperature. The

organic phase was washed with NaOH (2 × 50 mL, 1 M). The product was isolated as an oil.Yield 1.97 g (84%).

4-(6-Bromopyridin-3-yl)-piperazine-1-carboxylic Acid *tert*-Butyl Ester (50c). Compound **50c** was prepared according to procedure F from a mixture of **50b** (1.90 g, 7.20 mmol), NBS (1.30 g, 7.20 mmol), and acetonitrile (20 mL). Yield 2.0 g (81%); mp 122.6−123.5 °C.

1-(6-Bromopyridin-3-yl)-piperazine Fumaric Acid Salt (50). Compound **50** was prepared according to procedure B from a mixture of **50c** (1.90 g, 5.60 mmol), TFA (0.63 g, 5.60 mmol), and CH_2Cl_2 (20 mL). Yield 0.20 g (15%). The corresponding salt was obtained by the addition of a diethyl ether and MeOH mixture (9:1) saturated with fumaric acid; mp 170.8−171.6 °C. Anal. ($C_9H_{12}N_3Br \cdot 0.8C_4H_4O_4 \cdot 0.5H_2O$) C, H, N.

In Vitro Inhibition of [^{3}H]-Cytisine Binding. Tissue Preparation. Preparations were performed at 0−4 °C unless otherwise indicated. Cerebral corticies from male Wistar rats[20] (150−250 g) were homogenized for 20 s in 15 mL of Tris HCl (50 mM, pH 7.4) containing 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 2.5 mM $CaCl_2$ using an Ultra-Turrax homogenizer. The homogenate was centrifuged at 27 000*g* for 10 min. The supernatant was discarded, and the pellet was resuspended in fresh buffer and centrifuged a second time. The final pellet was resuspended in fresh buffer (35 mL/g of original tissue) and used for binding assays. **Assay.** Aliquots of 500 μL of homogenate were added to 25 μL of drug solution and 25 μL of ^{3}H-cytisine (1 nM, final concentration), mixed, and incubated for 90 min at 2 °C. Nonspecific binding was determined using (−)-nicotine (100 μM, final concentration). After incubation, the samples were added to 5 mL of ice-cold buffer and poured directly onto Whatman GF/C glass fiber filters under suction and immediately washed with 2 × 5 mL of ice-cold buffer. The amount of radioactivity on the filters was determined by conventional liquid scintillation counting using a Trib-carb liquid scintillation analyzer with a counting efficiency of 58%. Specific binding was equal to total binding minus nonspecific binding.

Conformational Analysis. Monte Carlo sampling and the MMFF force field as implemented in Macromodel v. 9.0[21] were used for conformational analysis. In all cases, the calculations were performed on the N-protonated species in aqueous solution. The low energy conformer (<1 kcal/mol) that gave the best fit to the template molecule (**55**) was used to calculate the 3D-QSAR model.

Molecular Alignment. The fitting points used were the pyridine nitrogen atom, the centroid of the pyridine ring, the protonated nitrogen atom, and the two hydrogens attached to this atom.

GRID Calculations. The interaction energies were calculated using GRID version 22A.[22] The grid size ($X = 23$ Å, $Y = 23$ Å, $Z = 22$ Å) was automatically determined to exceed the inclusive grid box by 5 Å. Energies were calculated with a grid spacing of 1 Å for water (OH_2) and methyl (C3) probes.

GOLPE Analysis. The partial least-squares (PLS) models were calculated using GOLPE v. 4.5.[23,24] The dependent variables were imported as pIC_{50} values.

Variable Pretreatments. GOLPE automatically rejects *X*-variables having a total sum of square less than 10^{-7}. Further pretreatment was performed as follows. Absolute critical *X*-values below 0.05 were deleted. Minimum sd cutoff was set to 0.10 for all probes. Second, third, and fourth level *X*-variables were deleted. The initial PLS-model was build using these data.

Smart Region Definition (SRD).[25] The groups of variables were generated using the default number of seeds. The variables within a distance of 1 Å to the seeds were grouped. Neighboring groups within a distance of 2 Å containing the same information were collapsed.

Variable Selection FFD. The effect of the individual groups of variables were evaluated using the FFD procedure including 20% dummy variables. Only groups having a positive influence on the model larger than the average dummy variable were included in the final model.

Cross-Validation. Twenty reduced models were built and evaluated. Each time, 20% of the molecules were randomly chosen and left out of the model (leave five random groups out). The reduced models were then used to predict the affinity of the molecules that were left out. The final model was used to predict the affinities of an external validation set of seven compounds.

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Supporting Information Available: Experimental details, spectroscopic data, and elemental analysis results. This material is available free of charge via the Internet at http://pubs.acs.org.

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