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Article

Modifications at C(5) of 2-(2-Pyrrolidinyl)-Substituted 1,4-Benzodioxane Elicit Potent $\alpha4\beta2$ Nicotinic Acetylcholine Receptor Partial Agonism with High Selectivity over the $\alpha3\beta4$ Subtype

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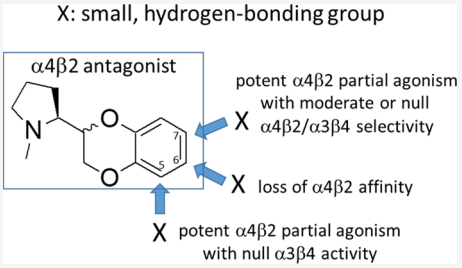
ABSTRACT: A series of diastereomeric 2-(2-pyrrolidinyl)-1,4-benzodioxanes bearing a small, hydrogen-bonding substituent at the 7-, 6-, or 5-position of benzodioxane have been studied for $\alpha4\beta2$ and $\alpha3\beta4$ nicotinic acetylcholine receptor affinity and activity. Analogous to C(5)H replacement with N and to a much greater extent than decoration at C(7), substitution at benzodioxane C(5) confers very high $\alpha4\beta2/\alpha3\beta4$ selectivity to the $\alpha4\beta2$ partial agonism. Docking into the two receptor structures recently determined by cryo-electron microscopy and site-directed mutagenesis at the minus $\beta2$ side converge in indicating that the limited accommodation capacity of the $\beta2$ pocket, compared to that of the $\beta4$ pocket, makes substitution at C(5) rather than at more projecting C(7) position determinant for this pursued subtype selectivity.



INTRODUCTION

For decades, much research has been aimed at developing ligands able to enhance the function of brain nicotinic acetylcholine receptors (nAChRs) as a treatment approach of cognitive deficits associated with neurological and psychiatric disorders and of some drug dependences.[1] Most efforts have been devoted to subtype selective agonists, in particular of the $\alpha4\beta2$ subtype, which constitutes the majority of brain nAChRs and is primarily involved in nicotine dependence and in antidepressant effects of nicotinic ligands.[2−4] However, their use has been limited by the narrow therapeutic range. It is not by chance that the only $\alpha4\beta2$ ligands approved for medical uses are not full but partial $\alpha4\beta2$ nAChR agonists, such as varenicline and cytisine, used as smoking cessation aids.[5,6] Nevertheless, $\alpha4\beta2$ ligands continue to be sought for their potential clinical applications.

New frontiers in this field are represented by the pharmacological characterization of the two $\alpha4\beta2$ isoforms with different α/β stoichiometries and the development of unorthodox $\alpha4$:$\alpha4$ agonists and $\alpha4\beta2$ allosteric modulators.[7−12] However, there are still matters deserving to be further deepened such as the structural features relevant for full or partial $\alpha4\beta2$ agonism and for $\alpha4\beta2$ versus $\alpha3\beta4$ selectivity,[13−15] two issues that remain critical in designing new $\alpha4\beta2$ ligands. Very recently, the structures of both human $\alpha4\beta2$ and $\alpha3\beta4$ receptors, the former in both the isoforms and the latter in the 2α:3β stoichiometry, have been determined by cryo-electron microscopy (cryo-EM).[16,17] Indeed, these are further potent investigation tools in comparison with previous X-ray 3D models[18] and homology models based on the crystal structure of AChBP,[19−23] suggesting new principles of agonist efficacy and subtype selectivity. Relying on these receptor architecture findings and on mutagenesis experiments aimed at defining residues governing receptor activation, we discuss here the (*S*,*S*)/(*S*,*R*) diastereomeric pairs **1**−**6** of pyrrolidinyl benzodioxanes, substituted at the 7-, 6-, and 5-benzodioxane positions (Chart 1). We have designed them in the past few years starting from the unsubstituted lead *N*-methyl-2-(2-pyrrolidinyl)-1,4-benzodioxane (*S*,*R*)-**7**,[24−26] a relatively rigid template endowed with moderate $\alpha4\beta2$ affinity, some $\alpha4\beta2$ versus $\alpha3\beta4$ selectivity, and as recently reported, potent $\alpha4\beta2$ antagonism,[13,27] in an effort to elicit $\alpha4\beta2$ partial agonism character and to optimize $\alpha4\beta2/\alpha3\beta4$ selectivity (Chart 1).

Such a ligand-based design started from aryl and heteroaryl ethers of prolinol, which are well-known, high-affinity $\alpha4\beta2$ ligands.[28] Initially, as shown in Chart 2, the (hetero)-aryloxymethyl portion was conformationally blocked into the benzodioxane substructure of **7**,[25] then it was made semirigid by α-methyl or *ortho*-methoxy substitution,[29] and finally, it

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Chart 1. *N*-Methyl Pyrrolidinyl Benzodioxanes Substituted at the 7-, 6-, and 5-Positions

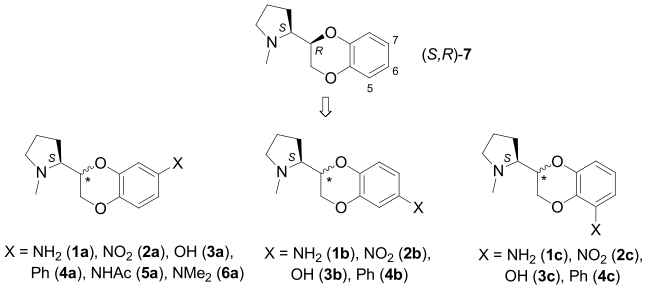
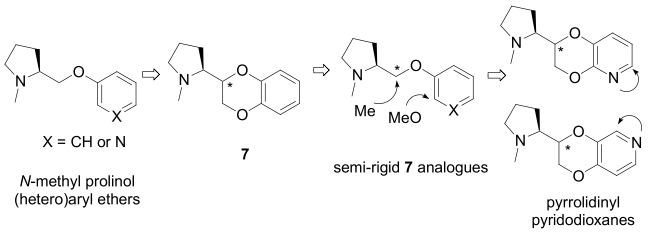


Chart 2. Conformational Restrictions of *N*-Methylprolinol (Hetero)aryl Ethers



was again completely rigidified, through challenging syntheses, into the four regioisomeric pyrrolidinyl pyridodioxanes.[13]

Among the (hetero)aryl ethers of prolinol, the *S* isomer of the 3-pyridyl ether of *N*-methylprolinol (**A**) is a potent $\alpha4\beta2$ full agonist with good $\alpha4\beta2/\alpha3\beta4$ selectivity[28] and its *meta*-hydroxy derivative (**B**), recently reported by us, is an $\alpha4\beta2$ superagonist with impressive efficacy and $\alpha4\beta2/\alpha3\beta4$ selectivity (Chart 3).[29] Under some conditions, partial (**C** and **D**) or complete [(*S*,*R*)-**3a** and (*S*,*R*)-**8**] rigidification of the aryloxymethyl substructure resulted in partial agonism and good $\alpha4\beta2/\alpha3\beta4$ selectivity (Chart 3).[13,29,30] In fact, both partial agonism and subtype selectivity are conditioned by the stereochemistry of the additional stereocenter, if present, and by the *meta*- or 7-OH substitution in semirigid prolinol phenyl ethers (**C**) and 2-pyrrolidinyl-1,4-benzodioxanes [(*S*,*R*)-**3a**], respectively, or by the 3- and 5-positions of pyridyl nitrogen in semirigid prolinol pyridyl ethers (**D**) and pyrrolidinyl pyridodioxanes [(*S*,*R*)-**8**], respectively. On the other hand, partial agonism and selectivity at the $\alpha4\beta2$ subtype can be seen also as a resultant from proper decoration of the aromatic ring of the rigid $\alpha4\beta2$ antagonist (*S*,*R*)-**7** (Chart 3).

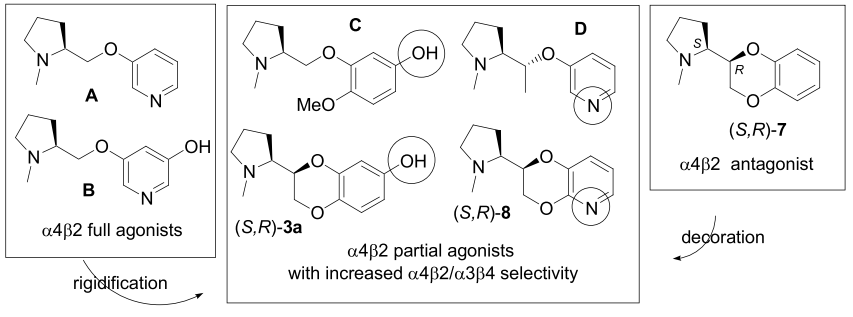
Indeed, 7-hydroxylation or C(5)H benzodioxane replacement with N converts the $\alpha4\beta2$ antagonist (*S*,*R*)-**7** into the $\alpha4\beta2$ partial agonists (*S*,*R*)-**3a** and (*S*,*R*)-**8** with higher $\alpha4\beta2/\alpha3\beta4$ selectivity than flexible prolinol (hetero)aryl ethers.[13,30] These latter findings were consistent with the *in vivo* effects described by our recent behavioral studies in zebrafish,[27] where we have determined that the antagonist (*S*,*R*)-**7** is able to block the nicotine-induced increase in spatial learning, memory, and attention and the partial agonist (*S*,*R*)-**3a** is able to reduce the nicotine-induced conditioned place preference, a behavioral model used to study the rewarding properties of nicotine. The results of such *in vivo* experiments, which are good evidences supporting the druggability of the biological target and the drug-likeness of pyrrolidinyl benzodioxanes, prompted us to systematically extend investigation over the 5-, 6-, and 7-substitutions at benzodioxane with groups, such as OH, NH_2, NO_2, NHAc, NMe_2, and by contrast, phenyl, adding new 12 pyrrolidinyl benzodioxane derivatives to the previously reported 7-phenyl and 7-hydroxy derivatives **3a** and **6a**, respectively.[29] Here, we present the nicotinic profile and discuss the SAR of the 14 diastereomeric pairs **1a**−**6a**, **1b**−**4b**, and **1c**−**4c** with the support of mutational and docking studies.

RESULTS

Chemistry. Scheme 1 reports the synthesis of the diastereomeric pairs of the three amino derivatives **1a**, **1b**, and **1c** with *S* configuration at the pyrrolidine stereocenter and epimers at the dioxane stereocenter. *N*-Boc-protected (*S*)-2-bromoacetyl-pyrrolidine was reacted with the potassium salt of *o*-benzyloxy phenol, bearing the nitro group at the 4-, 5-, or 6-position,[31] obtaining, without racemization, **9a**, **9b**, and **9c**, respectively. The carbonyl reduction with sodium borohydride in THF provided the diastereomeric mixtures (*S*,*S*)-**10a**/(*S*,*R*)-**10a**, (*S*,*S*)-**10b**/(*S*,*R*)-**10b**, and (*S*,*S*)-**10c**/(*S*,*R*)-**10c**, which were resolved by chromatography. Hence, parallel routes were followed. The hydroxyl function was mesylated; the benzyl protecting group was removed and the nitro function was reduced to amine by hydrogenation to give (*S*,*S*)-**12a**, (*S*,*S*)-**12b**, and (*S*,*S*)-**12c** and their epimers (*S*,*R*)-**12a**, (*S*,*R*)-**12b**, and (*S*,*R*)-**12c**. The subsequent cyclization of the three *S*,*S* stereoisomers by internal SN2 reaction afforded the pyrrolidinyl benzodioxanes (*S*,*R*)-**13a**, (*S*,*R*)-**13b**, and (*S*,*R*)-**13c**, which were converted into the final *N*-methylated compounds (*S*,*R*)-**1a**, (*S*,*R*)-**1b**, and (*S*,*R*)-**1c** by reduction of *N*-carbamate with $LiAlH_4$. Identically, (*S*,*R*)-**12a**, (*S*,*R*)-**12b**, and (*S*,*R*)-**12c** were cyclized to (*S*,*S*)-**13a**, (*S*,*S*)-**13b**, and (*S*,*S*)-**13c** and then reduced to *N*-methylated (*S*,*S*)-**1a**, (*S*,*S*)-**1b**, and (*S*,*S*)-**1c**.

Scheme 2 reports the synthesis of the diastereomeric pairs of the three nitro derivatives **2a**, **2b**, and **2c**, with *S* configuration at the pyrrolidine stereocenter and epimers at

Chart 3. $\alpha4\beta2$ Partial Agonists by Rigidification of Flexible $\alpha4\beta2$ Full Agonists or Decoration of Rigid $\alpha4\beta2$ Antagonists



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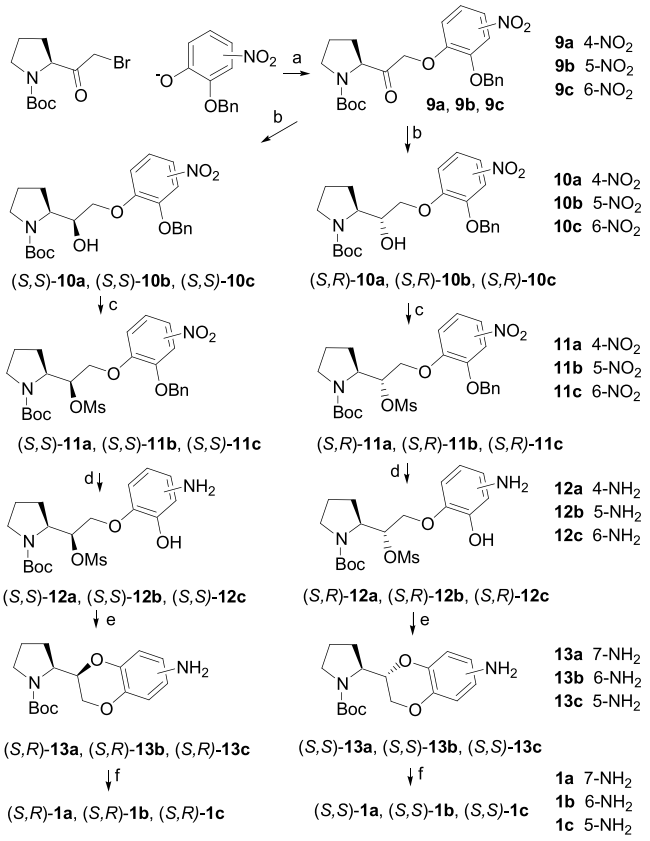
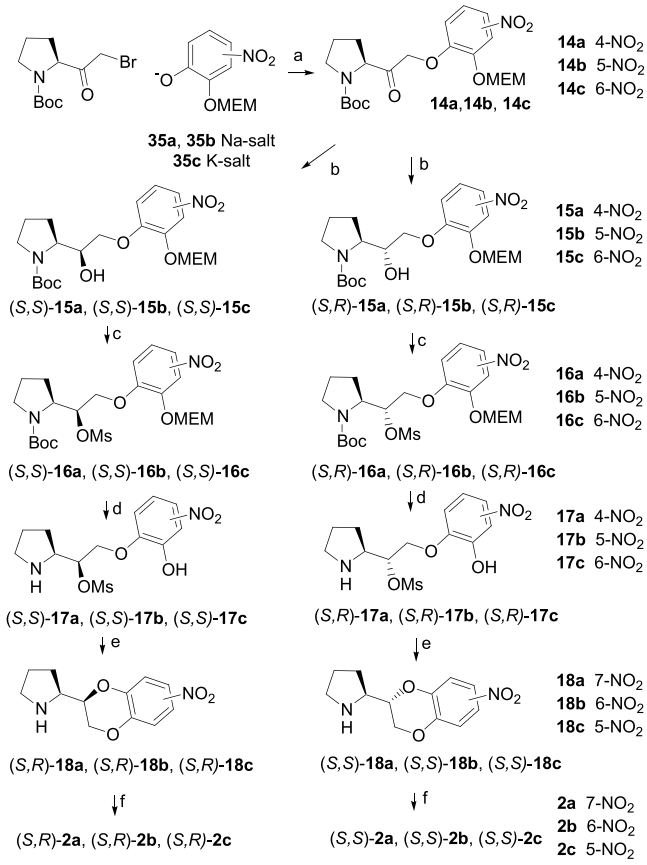
Scheme 1. Synthesis of Compounds 1a, 1b, and 1c[a]



[a]Reagents and conditions: (a) acetone, 25 °C, overnight; (b) $NaBH_4$, THF, 25 °C, 3 h, and then chromatographic separation of the diastereoisomers; (c) MsCl, TEA, DCM, 25 °C, 5 h; (d) H_2, 5% Pd/C, MeOH, 25 °C, 6 h; (e) K_2CO_3, DME, reflux, 6 h; (f) $LiAlH_4$, THF, reflux, 4 h.

Scheme 2. Synthesis of Compounds 2a, 2b, and 2c[a]



[a]Reagents and conditions: (a) acetone, 25 °C, overnight; (b) $NaBH_4$, THF, 25 °C, 3 h, and then chromatographic separation of the diastereoisomers; (c) MsCl, TEA, DCM, 25 °C, 5 h; (d) HCl, MeOH, 25 °C, 2 h; (e) K_2CO_3, DME and DMF, 85 °C, 6 h; (f) 37% CH_2O/H_2O, Aquivion-Fe, CPME, 40 °C, 4 h, then $NaBH_4$, MeOH, 0 °C, 30 min.

the dioxane stereocenter. *N*-Boc-protected (*S*)-2-bromoacetyl-pyrrolidine was reacted with MEM-monoprotected catechate bearing the nitro group at the 4-, 5-, and 6-positions (**35a**, **35b**, and **35c**, respectively), obtaining, without racemization, **14a**, **14b**, and **14c**, respectively. The carbonyl reduction with sodium borohydride in THF provided the diastereomeric mixtures (*S*,*S*)-**15a**/(*S*,*R*)-**15a**, (*S*,*S*)-**15b**/(*S*,*R*)-**15b**, and (*S*,*S*)-**15c**/(*S*,*R*)-**15c**, which were resolved by chromatography. Hence, parallel routes were followed. The hydroxyl function was mesylated and the amine function was deprotected to give (*S*,*S*)-**17a**, (*S*,*S*)-**17b**, and (*S*,*S*)-**17c** and their epimers (*S*,*R*)-**17a**, (*S*,*R*)-**17b**, and (*S*,*R*)-**17c**. The subsequent cyclization of the three *S*,*S* stereoisomers by internal SN2 reaction afforded the pyrrolidinyl benzodioxanes (*S*,*R*)-**18a**, (*S*,*R*)-**18b**, and (*S*,*R*)-**18c**, which were *N*-methylated to the final products (*S*,*R*)-**2a**, (*S*,*R*)-**2b**, and (*S*,*R*)-**2c** with formaldehyde and $NaBH_4$ using Aquivion-Fe as a catalyst in the green ether CPME.[32,33] Identically, (*S*,*R*)-**17a**, (*S*,*R*)-**17b**, and (*S*,*R*)-**17c** were cyclized to (*S*,*S*)-**18a**, (*S*,*S*)-**18b**, and (*S*,*S*)-**18c** and then *N*-methylated to give (*S*,*S*)-**2a**, (*S*,*S*)-**2b**, and (*S*,*S*)-**2c**.

N-Acetylation of (*S*,*R*)-**1a** and (*S*,*S*)-**1a** with acetyl chloride at room temperature in DCM afforded (*S*,*R*)-**5a** and (*S*,*S*)-**5a**, respectively. Dimethylation of the aniline function of the diastereomeric pairs of the intermediate **13a** by reductive amination with formaldehyde and subsequent reduction of carbamate to methyl with $LiAlH_4$ provided the final compounds (*S*,*R*)-**6a** and (*S*,*S*)-**6a**.

The synthesis of the 7-hydroxy derivatives **3a** was previously reported; those of the 6-hydroxy and 5-hydroxy derivatives, **3b** and **3c**, respectively, are shown in Schemes 3 and 4.

The synthesis of the two diastereoisomers of **3b** started from *N*-Boc-protected (*S*)-2-bromoacetyl-pyrrolidine and the sodium salt of 2,5-dibenzyloxy phenol **39**, which gave the intermediate **19** without racemization (Scheme 3). The carbonyl reduction with sodium borohydride in THF provided the diastereomeric mixture (*S*,*S*)-**20b**/(*S*,*R*)-**20b**, which was resolved by chromatography. Hence, parallel routes were followed. The alcoholic function was mesylated and the benzyl protections were removed by hydrogenolysis to give (*S*,*S*)-**22** and (*S*,*R*)-**22**. The subsequent cyclization to benzodioxane by intramolecular nucleophilic substitution afforded the intermediates (*S*,*R*)-**23b** and (*S*,*S*)-**23b**, which were converted into final (*S*,*R*)-**3** and (*S*,*S*)-**3b** by reduction of carbamate to methyl.

Analogously, the syntheses of the two diastereomers of **3c** started from *N*-Boc-protected (*S*)-2-bromoacetyl-pyrrolidine and the potassium salt of 1,3-diMEM-protected pyrogallol **41**, which gave the intermediate **24** without racemization (Scheme 4). The carbonyl reduction with sodium borohydride in THF provided the diastereomeric mixture (*S*,*S*)-**25**/(*S*,*R*)-**25**, which was mesylated, liberated from the MEM and Boc

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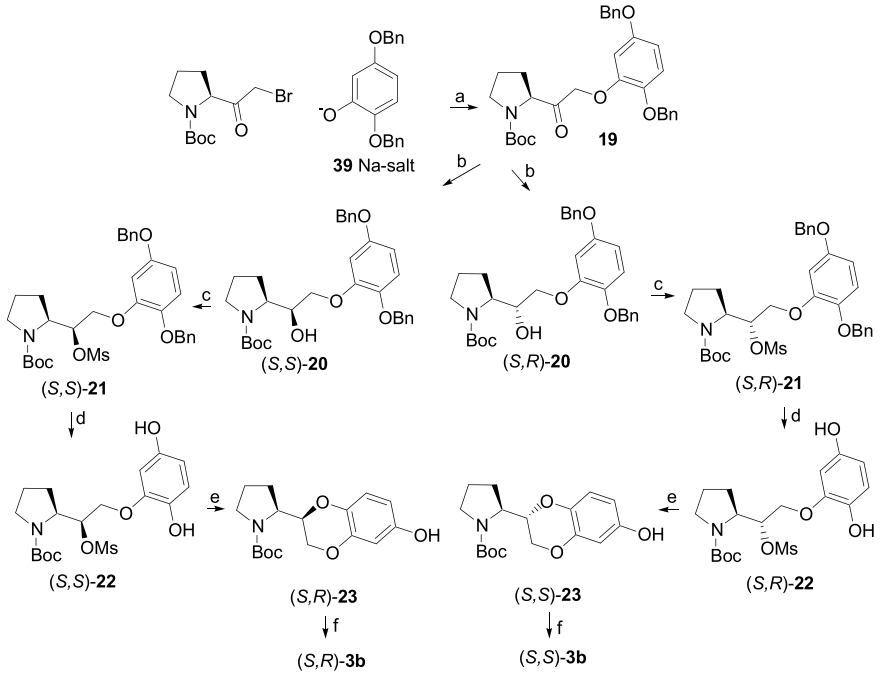
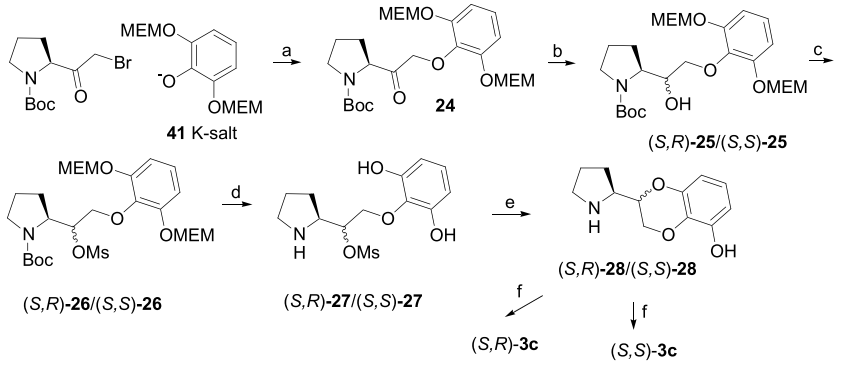
Scheme 3. Synthesis of Compounds 3b[a]



[a]Reagents and conditions: (a) acetone, 25 °C, overnight; (b) $NaBH_4$, THF, 25 °C, 3 h, and then chromatographic separation of the diastereoisomers; (c) MsCl, TEA, DCM, 25 °C, 5 h; (d) H_2, 5% Pd/C, 25 °C, 6 h; (e) K_2CO_3, DME and DMF, 85 °C, 6 h; (f) $LiAlH_4$, THF, reflux, 4 h.

Scheme 4. Synthesis of Compounds 3c[a]



[a]Reagents and conditions: (a) acetone, 25 °C, overnight; (b) $NaBH_4$, THF, 25 °C, 3 h; (c) MsCl, TEA, DCM, 25 °C, 5 h; (d) HCl, MeOH, 25 °C, 2 h; (e) K_2CO_3, DME and DMF, 85 °C, 6 h; (f) 37% CH_2O/H_2O, Aquivion-Fe, CPME, 40 °C, 4 h, then $NaBH_4$, MeOH, 0 °C, 30 min, and chromatographic separation of the diastereoisomers.

protections, and cyclized to the diastereomeric benzodioxanes (*S*,*R*)-**28** and (*S*,*S*)-**28**. After methylating pyrrolidine nitrogen, the two final diastereomers (*S*,*R*)-**3c** and (*S*,*S*)-**3c** were separated by chromatography.

The synthesis of the 7-phenyl derivatives **4a** was previously reported.[30] Scheme 5 shows the synthesis of the diastereomeric pairs of the two phenyl derivatives **4b**, with *S* configuration at the pyrrolidine stereocenter and epimers at the dioxane stereocenter. *N*-Boc-protected (*S*)-2-bromoacetylpyrrolidine was reacted with the sodium salt of 2-benzyloxy-5-phenylphenol,[34] obtaining **29b** without racemization. The carbonyl reduction with sodium borohydride in THF provided the diastereomeric mixture (*S*,*S*)-**30b**/(*S*,*R*)-**30b**, which was resolved by chromatography. Hence, parallel routes were followed. Benzyl protection was removed by hydrogenolysis and cyclization to a benzodioxane system was accomplished by the Mitsunobu reaction. The resultant intermediates (*S*,*S*)-**32b** and (*S*,*R*)-**32b** were converted into final (*S*,*R*)-**4b** and (*S*,*S*)-**4b** by reduction of carbamate to methyl.

The synthetic route to the phenyl derivatives **4c** was the same as to **1c**, but using the potassium salt of 2-benzyloxy-6-phenylphenol **46** instead of 2-benzyloxy-6-nitrophenol (Scheme 6). In sequence, the intermediates were the product of the bromine substitution product **29c**, the two alcohols (*S*,*S*)-**30c** and (*S*,*R*)-**30c** resulting from the reduction of **29c** and resolved by chromatography, the corresponding methanesulfonates (*S*,*S*)-**33** and (*S*,*R*)-**33**, the debenzylated intermediates (*S*,*S*)-**34** and (*S*,*R*)-**34**, and the cyclization products (*S*,*R*)-**32c** and (*S*,*S*)-**32c**. The final reduction of carbamate to methyl afforded (*S*,*R*)-**4c** and (*S*,*S*)-**4c**.

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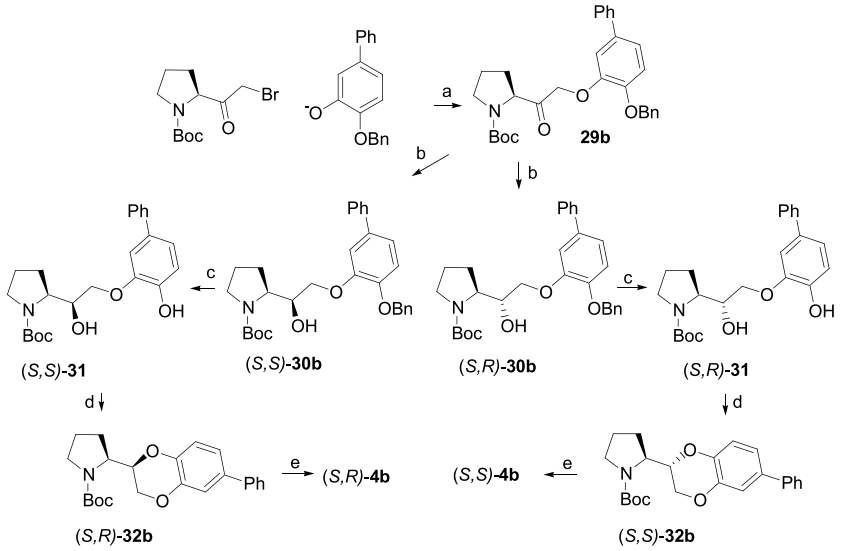
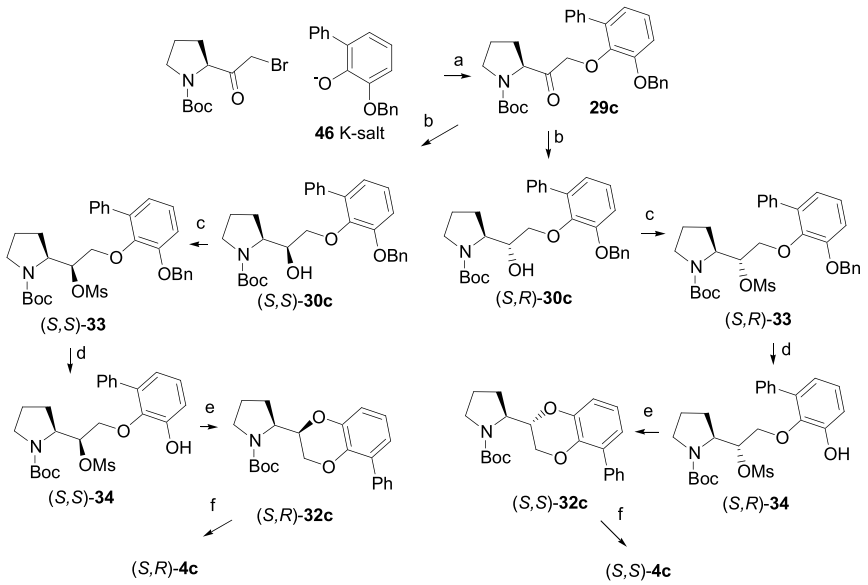
Scheme 5. Synthesis of Compounds 4b[a]



[a]Reagents and conditions: (a) acetone, 25 °C, overnight; (b) $NaBH_4$, THF, 25 °C, 3 h, and then chromatographic separation of the diastereoisomers; (c) H_2, 5% Pd/C, MeOH, 25 °C, 6 h; (d) Ph_3P, DIAD, THF, MW 130 °C (150 W), 10 min; (e) $LiAlH_4$, THF, reflux, 4 h.

Scheme 6. Synthesis of Compounds 4c[a]



[a]Reagents and conditions: (a) acetone, 25 °C, overnight; (b) $NaBH_4$, THF, 25 °C, 3 h, and then chromatographic separation of the diastereoisomers; (c) MsCl, TEA, DCM, 25 °C, 5 h; (d) H_2, 5% Pd/C, MeOH, 25 °C, 6 h; (e) K_2CO_3, DME, reflux, 6 h; (f) $LiAlH_4$, THF, reflux, 4 h.

The syntheses of the phenols **35a**–**35c**, **39**, **41**, and **46** to be reacted with *N*-Boc-protected (*S*)-2-bromoacetyl-pyrrolidine are shown in Scheme 7. The two regioisomeric MEM-monoprotected 4-nitrocathecols **35a** and **35b** were prepared both from 4-nitrocathecol and MEM chloride under different reaction conditions, exploiting the different acidity of the two hydroxyls. In fact, **35b** could be obtained by selective deprotonation of the only *p*-OH, while **35a** could be obtained by deprotonation of both hydroxyls with preferential MEM protection of the more nucleophilic phenolate function meta-positioned to the nitro group. The MEM-monoprotected 3-nitrocathecol **35c** was synthesized from 2-hydroxybenzaldehyde by MEM protection of the phenolic function, Baeyer–Villiger oxidation of the formyl group to phenol, and nitration. 2,5-Dibenzyloxyphenol (**39**) was prepared by dibenzylation of 2,5-dihydroxybenzaldehyde and subsequent Baeyer–Villiger oxidation. 1,3-MEM-diprotected pyrogallol (**41**) was obtained from MEM-diprotected resorcinol by treatment with *n*-butyl lithium, trimethyl borate, and Oxone. 2-Benzyloxy-6-phenylphenol (**46**) was synthesized from 2-phenylphenol by formylation to 2-hydroxy-3-phenylbenzaldehyde, MEM protection of phenol, Baeyer–Villiger oxidation of the formyl group, benzylation of the resultant phenol, and removal of the MEM protection.

Biology and Computational Docking. *Binding Affinities.* The synthesized compounds were assayed for binding

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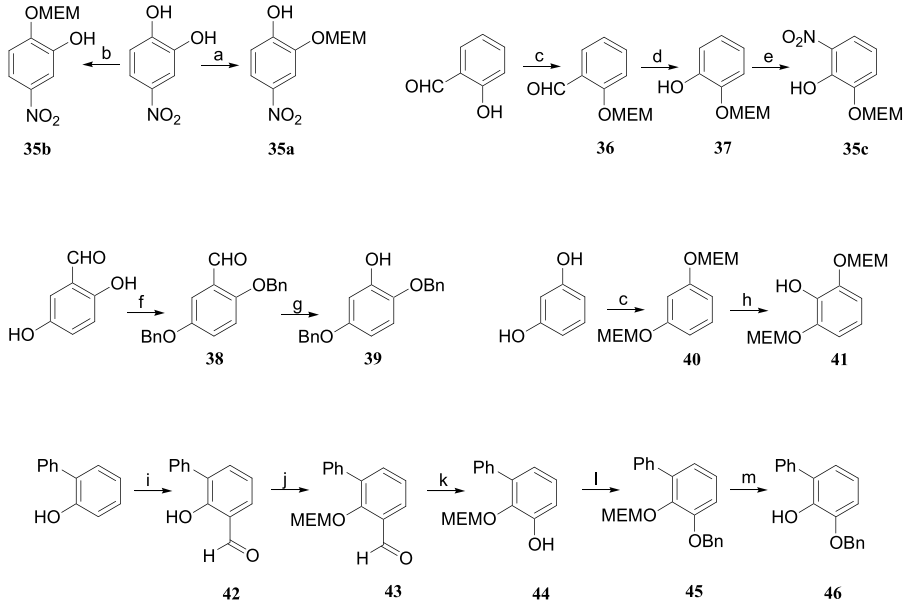
Scheme 7. Synthesis of Compounds 35a−35c, 39, 41, and 46[a]



[a]Reagents and conditions: (a) NaH, MEMCl, DMSO, 25 °C, 16 h; (b) K_2CO_3, MEMCl, DMF, 40 °C, 1 h; (c) DIPEA, MEMCl, DCM, 25 °C, 16 h; (d) *m*-CPBA, DCM, 25 °C, 48 h, then 2 M KOH, MeOH, 25 °C, 5 h; (e) CAN, CH_3CN, 10 °C, 3 h; (f) K_2CO_3, DMF, BnBr, 25 °C, 16 h; (g) *m*-CPBA, DCM, 24 h, then MeOH, 2.5 M NaOH, 25 °C, 2 h; (h) *n*-BuLi, THF, 0 °C, 1 h, then $B(OMe)_3$, 30 min, then 20% $NaHCO_3$ in aqueous acetone, Oxone, 25 °C, 5 min; (i) $MgCl_2$, TEA, THF, paraformaldehyde, reflux, 3 h; (j) NaH, THF, MEMCl, 25 °C, 16 h; (k) *m*-CPBA, EtOAc, 72 h, then MeOH, 2.5 M NaOH, 25 °C, 2 h; (l) K_2CO_3, DMF, BnBr, 50 °C, 1.5 h; (m) 37% HCl, MeOH, 60 °C, 1 h.

Table 1. Nicotine, Pyrrolidinyl Benzodioxane (*S,R*)-7, Pyrrolidinyl Pyridodioxane (*S,R*)-8, and Compounds 1a−6a, 1b−4b, and 1c−4c: Affinity for Native $\alpha4\beta2$ and $\alpha7$ nAChR Subtypes in Rat Membranes, Respectively Labeled by [^{3}H]Epibatidine and [^{125}I]α-Bungarotoxin, and for Heterologously Expressed Human $\alpha3\beta4$ nAChRs, Labeled by [^{3}H]Epibatidine, and $\alpha4\beta2$ vs $\alpha3\beta4$ Selectivity Ratio[a]

compound	$\alpha4\beta2$ [^{3}H]Epi K_i (μM)	$\alpha3\beta4$ [^{3}H]Epi K_i (μM)	$\alpha7$ [^{125}I]αBgtx K_i (μM)	$\alpha4\beta2/\alpha3\beta4$ selectivity	compound	$\alpha4\beta2$ [^{3}H]Epi K_i (μM)	$\alpha3\beta4$ [^{3}H]Epi K_i (μM)	$\alpha7$ [^{125}I]αBgtx K_i (μM)	$\alpha4\beta2/\alpha3\beta4$ selectivity
nicotine	0.004 (0.001)	0.261 (0.08)	0.234 (0.07)	65	(*S,R*)-**1b**	86 (31)	3.5 (1.3)	nd	0.04
(*S,R*)-**7**	0.26 (0.08)	1.2 (0.34)	21 (9.2)	4.6	(*S,S*)-**1b**	74 (25)	8.6 (3.8)	>10	0.1
(*S,R*)-**8**	0.41 (0.08)	16.2 (5.7)	89 (26.7)	40	(*S,R*)-**2b**	46 (6.9)	15 (4.05)	31.5 (9.5)	0.3
(*S,R*)-**1a**	0.022 (0.008)	0.019 (0.006)	2.6 (0.8)	0.9	(*S,S*)-**2b**	>100	4.8 (2.2)	13.5 (3.4)	<0.05
(*S,S*)-**1a**	0.14 (0.03)	0.18 (0.04)	19.1 (13.7)	1.3	(*S,R*)-**3b**	50 (31)	1.9 (0.8)	26.8 (9.4)	0.04
(*S,R*)-**2a**	14.0 (2.2)	4.5 (1.3)	10.4 (3.1)	0.3	(*S,S*)-**3b**	71 (42)	6.0 (2.3)	10.5 (3.7)	0.08
(*S,S*)-**2a**	33.0 (5.6)	2.1 (0.6)	42.2 (15)	0.06	(*S,R*)-**4b**	59 (35)	5.4 (2.2)	8.1 (2.6)	0.09
(*S,R*)-**3a**	0.012 (0.002)	0.31 (0.1)	0.43 (0.1)	26	(*S,S*)-**4b**	92 (64)	5.8 (2.2)	2.1 (1.0)	0.06
(*S,S*)-**3a**	0.42 (0.1)	0.70 (0.2)	20.0 (7.2)	1.7	(*S,R*)-**1c**	7.1 (5.9)	3.9 (1.3)	109 (53)	0.5
(*S,R*)-**4a**	35.0 (7.0)	nd	36.0 (16.0)		(*S,S*)-**1c**	0.131 (0.04)	13 (6.5)	44.5 (20)	100
(*S,S*)-**4a**	3.1 (0.7)	nd	51 (23.0)		(*S,R*)-**2c**	12.2 (4.9)	1.1 (0.3)	nd	0.09
(*S,R*)-**5a**	6.5 (2.7)	1.9 (0.8)	>10	0.3	(*S,S*)-**2c**	0.335 (0.14)	6.7 (2.7)	nd	20
(*S,S*)-**5a**	46.0 (14.3)	8.7 (3.3)	nd	0.2	(*S,R*)-**3c**	0.55 (0.05)	3.9 (2.1)	nd	7
(*S,R*)-**6a**	147 (85)	2.2 (0.6)	nd	0.015	(*S,S*)-**3c**	1.7 (0.9)	>100	nd	>59
(*S,S*)-**6a**	49.0 (29)	25 (6.5)	>10	0.5	(*S,R*)-**4c**	0.64 (0.07)	2.7 (1.4)	45.5 (19)	4.2
					(*S,S*)-**4c**	7.3 (2.7)	5.3 (2.1)	50.0 (20)	0.7

[a]The K_d and K_i values were derived from three [^{3}H]epibatidine and [^{125}I]α-bungarotoxin saturation and three competition binding experiments using rat cortex ($\alpha4\beta2$) and hippocampus ($\alpha7$) membranes and the membrane of human $\alpha3\beta4$-transfected cells, as described in refs 29 and 35. Data from saturation and competition binding curves were evaluated by one-site competitive binding curve-fitting procedures using GraphPad Prism version 5 (GraphPad Software, Inc., CA, USA). Each compound was tested in three separate competition binding curves for each subtype and the inhibition constant (K_i) was estimated in reference to the K_d of the radioligand according to the Cheng−Prusoff equation. The numbers in brackets represent the standard error. The affinities of compounds (*S,R*)-**7**, (*S,R*)-**8**, (*S,R*)-**3a**, (*S,S*)-**3a**, (*S,R*)-**4a**, and (*S,S*)-**4a** are those previously reported in refs 13, 25, and 30.

affinity on native rat $\alpha4\beta2$* nAChR present on rat cerebral cortex membranes and rat $\alpha7$* nAChR present on hippocampus membranes labeled by [^{3}H]epibatidine ([^{3}H]Epi) and [^{125}I]α-bungarotoxin ([^{125}I]αBgtx), respectively, and on heterologously expressed human $\alpha3\beta4$ receptors labeled with [^{3}H]Epi, as previously reported.[29] Nicotine was included in

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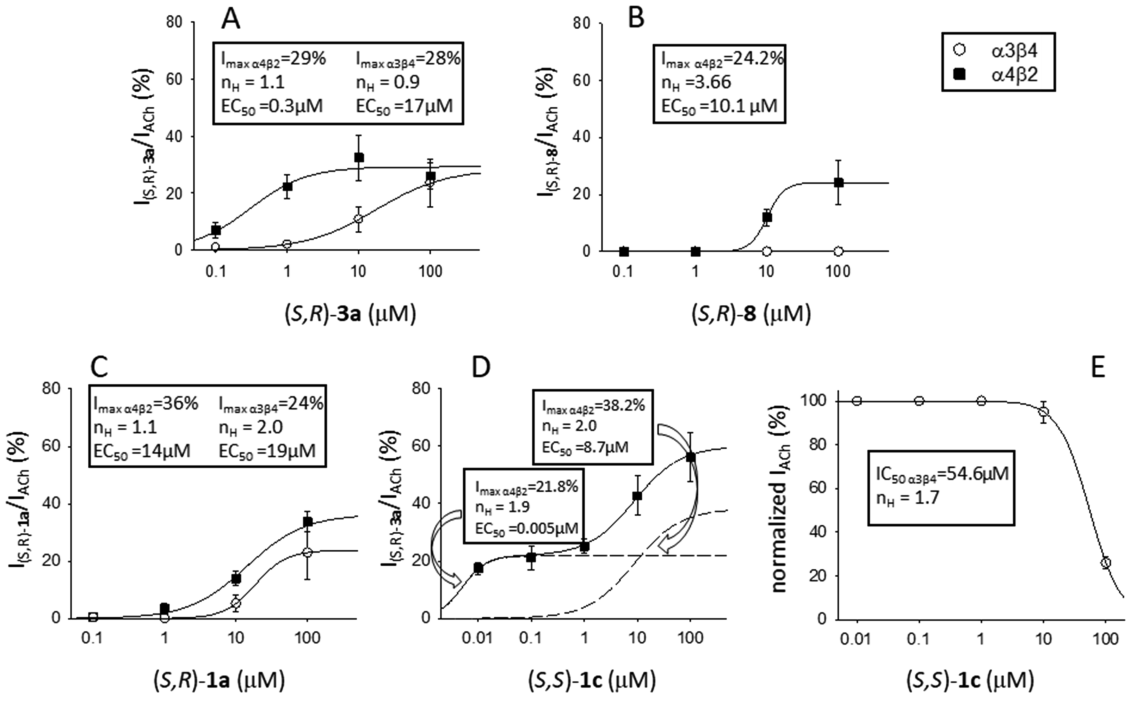

Figure 1. Agonist effects of (*S*,*R*)-**3a** (A), (*S*,*R*)-**8** (B), (*S*,*R*)-**1a** (C), and (*S*,*S*)-**1c** (D) and antagonist effect of (*S*,*S*)-**1c** (E) on transfected human $\alpha4\beta2$ and $\alpha3\beta4$ nAChRs. Human $\alpha4\beta2$ (solid square; $n = 8$, $n = 6$, $n = 9$, and $n = 6$ for (*S*,*R*)-**3a**, (*S*,*R*)-**8**, (*S*,*R*)-**1a**, and (*S*,*S*)-**1c**, respectively) and $\alpha3\beta4$ (open circle; $n = 4$, $n = 2$, $n = 7$, and $n = 2$ for (*S*,*R*)-**3a**, (*S*,*R*)-**8**, (*S*,*R*)-**1a**, and (*S*,*S*)-**1c**, respectively) nAChRs were transiently transfected into the GH4C1 rat anterior pituitary cell line and the activation responses are normalized to the maximal response to 1 mM ACh. The $\alpha3\beta4$ antagonist effect was normalized to that of 145 μM ACh. The $\alpha4\beta2$ and $\alpha3\beta4$ agonist effects of compounds (*S*,*R*)-**3a** and (*S*,*R*)-**8** are those previously reported in ref 29.

the series for comparison. The results are listed in Table 1, together with those previously reported for (*S*,*R*)-**7**, (*S*,*R*)-**8**, (*S*,*R*)-**3a**, (*S*,*S*)-**3a**, (*S*,*R*)-**4a**, and (*S*,*S*)-**4a**.

On the basis of their $\alpha4\beta2$ affinity, three groups of compounds can be distinguished.

The first includes four compounds with $\alpha4\beta2$ affinity of the 10−200 nM order: the 7-amino-substituted derivatives (*S*,*R*)-**1a** and (*S*,*S*)-**1a**, the 7-hydroxy derivative (*S*,*R*)-**3a**, and the 5-amino derivative (*S*,*S*)-**1c**. Of these compounds, the first two have no $\alpha4\beta2/\alpha3\beta4$ selectivity, while the third and fourth have moderate and high $\alpha4\beta2/\alpha3\beta4$ selectivities, respectively. Their $\alpha4\beta2/\alpha7$ selectivities are from good to very high, reaching the maximum for (*S*,*S*)-**1c**.

The second group includes six compounds with moderate submicromolar $\alpha4\beta2$ affinity. They are the previously reported (*S*,*R*)-**7**, (*S*,*R*)-**8**, and (*S*,*S*)-**3a** and three 5-substituted derivatives, the 5-hydroxy (*S*,*R*)-**3c**, the 5-nitro (*S*,*S*)-**2c**, and the 5-phenyl (*S*,*R*)-**4c**. The compound (*S*,*R*)-**8** shows good $\alpha4\beta2/\alpha3\beta4$ selectivity, while the other five benzodioxane derivatives show very modest to moderate $\alpha4\beta2/\alpha3\beta4$ selectivities. Within this group, the $\alpha7$ affinity was determined for (*S*,*R*)-**7**, (*S*,*R*)-**8**, (*S*,*S*)-**3a**, and (*S*,*R*)-**4c** and found negligible.

The other 20 compounds, including all the 6-substituted benzodioxanes (**1b**−**4b**), the 7-nitro, 7-phenyl, 7-dimethylamino, and 7-acetamido derivatives **2a**, **4a**, **5a**, and **6a**, respectively, and the distomers of the diastereomeric pairs of the four 5-substituted derivatives, exhibit low or very low $\alpha4\beta2$ affinities, almost always significantly lower than the corresponding $\alpha3\beta4$ and $\alpha7$ affinities.

Functional Assays. Binding studies indicate the 7-amino and 5-amino compounds (*S*,*R*)-**1a** and (*S*,*S*)-**1c**, respectively, as the most interesting derivatives: the former has high affinity for both $\alpha4\beta2$ and $\alpha3\beta4$ nAChRs, and the latter has high affinity for $\alpha4\beta2$ nAChRs and 100-fold lower affinity for $\alpha3\beta4$ nAChRs. We have previously reported that the 7-hydroxy compound (*S*,*R*)-**3a**, which shows 26-fold lower $\alpha3\beta4$ affinity than $\alpha4\beta2$ affinity, is a 50−200 times less potent partial agonist on the $\alpha3\beta4$ subtype than on the $\alpha4\beta2$ subtype. We have also reported that the pyrrolidinyl pyridodioxane (*S*,*R*)-**8**, which shows 40-fold lower $\alpha3\beta4$ affinity than $\alpha4\beta2$ affinity, is a partial agonist on the $\alpha4\beta2$ subtype with no activity at the $\alpha3\beta4$ subtype. Functional tests evidence greater $\alpha4\beta2/\alpha3\beta4$ selectivity than binding assays. To determine potency, efficacy, and selectivity toward the $\alpha4\beta2$ and $\alpha3\beta4$ subtypes, we tested functionally (*S*,*R*)-**1a** and (*S*,*S*)-**1c** and compared the results with those of (*S*,*R*)-**3a** and (*S*,*R*)-**8**. The effects of (*S*,*R*)-**1a** and (*S*,*S*)-**1c** on the two subtypes were determined via whole-cell patch clamp electrophysiology with GH4C1 cell line cells transiently expressing human $\alpha4\beta2$ and $\alpha3\beta4$ subtypes, as previously made for (*S*,*R*)-**3a** and (*S*,*R*)-**8**. Figure 1 shows the electrophysiological effects of (*S*,*R*)-**3a**, (*S*,*R*)-**8**, (*S*,*R*)-**1a**, and (*S*,*S*)-**1c** on heterologously expressed human $\alpha4\beta2$ and $\alpha3\beta4$ nAChRs. The first two graphs depict the above-mentioned $\alpha4\beta2$ partial agonism of (*S*,*R*)-**3a** (Figure 1A) and (*S*,*R*)-**8** (Figure 1B) with I_{max} values of 29 and 24% of that elicited by 1 mM Ach and with 0.3 and 10.1 μM EC_{50}, respectively, and the weaker $\alpha3\beta4$ activity of (*S*,*R*)-**3a** (28% I_{max}, 17 μM EC_{50}) (Figure 1A). Compound (*S*,*R*)-**8** does not elicit any $\alpha3\beta4$ response (Figure 1B). Quite different behaviors are shown by the two amino derivatives. In line with binding data, (*S*,*R*)-**1a** exhibits partial agonism toward both $\alpha4\beta2$ and $\alpha3\beta4$ subtypes with similar potency and efficacy (36 and 24% I_{max}, 14 and 19 μM EC_{50}) (Figure 1C). (*S*,*S*)-**1c** behaves as an $\alpha4\beta2$ partial

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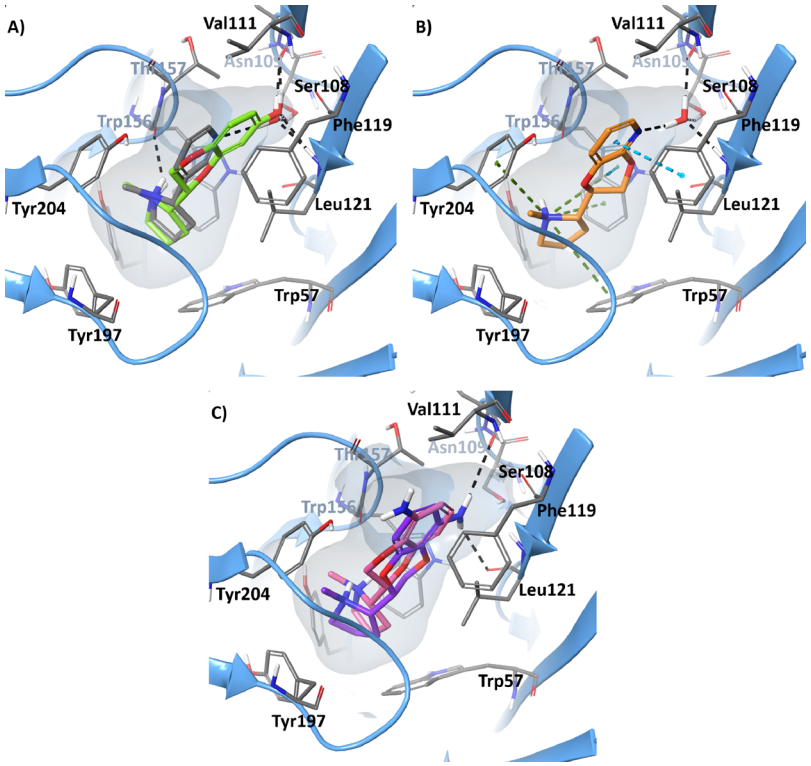

Figure 2. Proposed binding modes of the analogues of (*S*,*R*)-7 at the $\alpha4\beta2$ binding site (PDB ID: 5KXI). Black dashed lines represent H-bonds, while green and light blue dashed lines respectively represent π−cation and $\pi-\pi$ interactions. The inner receptor surface is depicted in faded azure. (A) Superimposition of (*S*)-nicotine (gray) with the docking pose of (*S*,*R*)-**3a** (faded green) displaying identical positioning of the pyrrolidine ring and same H-bond network between the nicotine-bound structural water and the 7-OH functional group with the residues of the β subunit. Only H-bonds are shown for clarity. (B) Binding pose of (*S*,*R*)-**8** (faded orange), showing that the pyridinic nitrogen establishes a H-bond to the structural water, similar to nicotine. (C) Superimposition of the docked poses of (*S*,*S*)-**1c** (violet) and (*S*,*R*)-**1c** (pink), showing a divergent positioning of the amino group. Only H-bonds are shown for clarity.

agonist with a bimodal dose−response curve, which can only be interpreted as the combination of different activities at the two stoichiometries of the $\alpha4\beta2$ subtype (Figure 1D). Particularly, the lower EC_{50} was calculated to be 5 nM with a maximal response of 20%, while the higher EC_{50} was 8.7 μM with a cumulative maximal response of 60%, which is almost twice the efficacy of (*S*,*R*)-**3a** and (*S*,*R*)-**8**. At sub-10 nM concentrations, (*S*,*S*)-**1c** elicits approximately 22% of the total current, while (*S*,*R*)-**3a** and (*S*,*R*)-**8** are nearly and completely inactive, respectively. Furthermore, (*S*,*S*)-**1c** is a very weak antagonist of the $\alpha3\beta4$ subtype with an IC_{50} of 54.6 μM (Figure 1E).

Computational Docking Studies. Binding and functional assays indicate that modifications at benzodioxane C(7) and C(5) are critical for $\alpha4\beta2$ interaction and activation. Particularly, functional groups providing a hydrogen-bonding network with no or minimal steric encumbrance increase are highly productive: higher $\alpha4\beta2$ affinity and partial agonist activity are attained when the hydroxyl or amino group is introduced into the 7-position of benzodioxane or when the 5-position of benzodioxane is modified by replacement of CH with N or by introduction again of an amino group.

Computational modeling was applied to shed light on the binding mode of the pyrrolidinyl benzodioxane analogues, presenting such advantageous modification, namely, (*S*,*R*)-**3a**, (*S*,*R*)-**8**, and (*S*,*S*)-**1c**. In detail, we performed docking in the orthosteric binding pocket of the $\alpha4\beta2$ receptor refined starting from the X-ray structure of the full-length human receptor (5KXI).[18] The $\alpha4\beta2$ dimer, containing nicotine in the binding site at the interface between the two subunits, was extracted from 5KXI and refined. Although a structural water molecule is notoriously known to be critical for nicotine−$\alpha4\beta2$ interaction,[36] the resolution of 5KXI is not high enough to detect it. Therefore, the X-ray structure of the homologous AChBP from *Lymnaea stagnalis* (1UW6), which contains the structural water, was superimposed to the $\alpha4\beta2$ structure and its water molecule was extracted from the binding site and merged with one duplicate of the above-prepared $\alpha4\beta2$ dimer.[37] Overall, two models of binding pocket were thus obtained: a water-free model and a water-containing model. Figure 2A shows the superimposition between the binding mode of nicotine in the water-containing $\alpha4\beta2$ binding site and (*S*,*R*)-**3a** in the water-free $\alpha4\beta2$ binding site. (*S*,*R*)-**3a** maintains all the principal interactions of nicotine and, in particular, it places its hydroxyl at C(7) in the small hydrophilic pocket of the minus $\beta2$ side, lined by a Leu121 −NH backbone, Asn109 −CO backbone, and Ser108 hydroxylated side chain, where nicotine orients its pyridine nitrogen. The position of the (*S*,*R*)-**3a** hydroxyl group perfectly overlaps that of the water molecule in the nicotine−receptor complex. The hydroxyl group of (*S*,*R*)-**3a** directly interacts with the backbone carbonyl of Asn109 and NH of Leu121, while the nicotine pyridine nitrogen does it through the water molecule. Furthermore, an additional interaction is established between (*S*,*R*)-**3a** and the hydroxyl group of Ser108 side chains. Similar to nicotine, the pyridine nitrogen of (*S*,*R*)-**8** indirectly interacts through a water molecule with the above-mentioned minus $\beta2$ side amino acid

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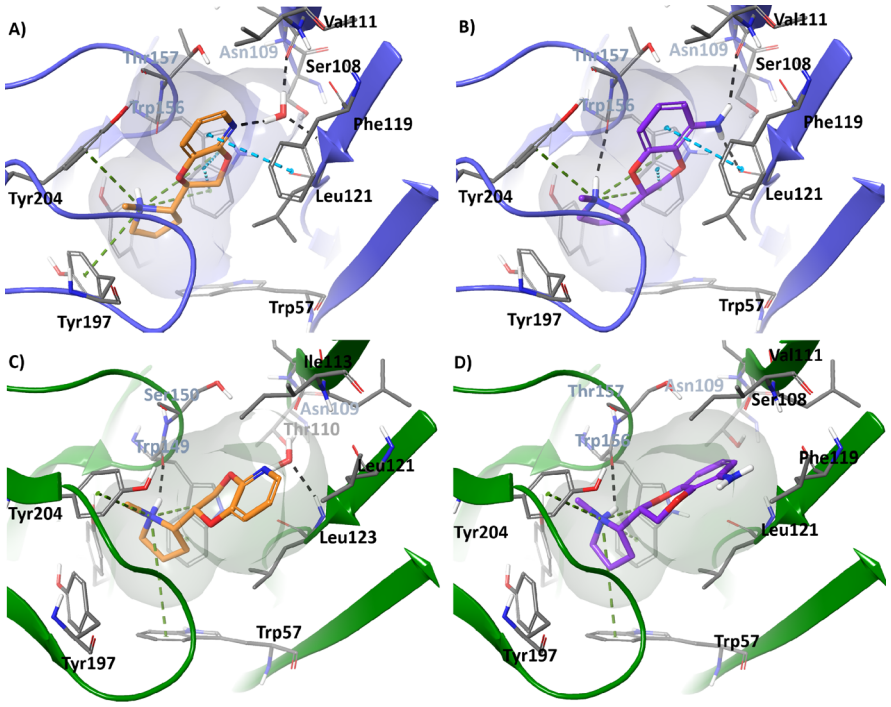

Figure 3. Rationalization of the $\alpha4\beta2/\alpha3\beta4$ selectivity of (*S*,*R*)-**8** and (*S*,*S*)-**1c** by docking them at the $\alpha4\beta2$ (PDB ID: 6CNJ) and $\alpha3\beta4$ (PDB ID: 6PV7) interfaces. Receptor backbone is represented by blue ($\alpha4\beta2$) or green ($\alpha3\beta4$) cartoons. Black dashed lines represent H-bonds, while green and cyan dashed lines respectively represent π−cation and $\pi-\pi$ interactions. The inner receptor surface is depicted in faded blue ($\alpha4\beta2$) or green ($\alpha3\beta4$). Binding modes of (*S*,*R*)-**8** (A) and (*S*,*S*)-**1c** (B) in the cryo-EM-derived $\alpha4\beta2$ binding site, confirming the same interactions previously identified with the X-ray-derived model. Docking poses of (*S*,*R*)-**8** (C) and (*S*,*S*)-**1c** (D) in the $\alpha3\beta4$ binding site, suggesting that the missing $\pi-\pi$ interactions (Phe119 to Leu121 replacement) and the disruption of the H-bond network with the β subunit could be responsible for low affinity at the $\alpha3\beta4$.

residues (Figure 2B). Instead, the 5-amino-substituted compound (*S*,*S*)-**1c** shows analogous interactions in the water-free binding pocket to those of (*S*,*R*)-**3a** despite having opposite configuration at the dioxane stereocenter (Figure 2C). To reach Asn109 and Leu121 residues with its 5-NH_2 substituent, (*S*,*S*)-**1c** twists the benzodioxane core of approximately 180° compared to (*S*,*R*)-**3a**. Oppositely, the other diastereomer (*S*,*R*)-**1c**, which has very modest $\alpha4\beta2$ affinity, is predicted to place the amino group at C(5) far from the hydrophilic $\beta2$ side binding pocket as a consequence of a pose of the benzodioxane core almost superimposable with that of (*S*,*R*)-**3a** (Figure 2C).

The second indication provided by binding and activity data was that the beneficial modifications we have considered above produce also high $\alpha4\beta2$ versus $\alpha3\beta4$ selectivity when accomplished at C(5) of the benzodioxane scaffold. The very recent cryo-EM structures of both human $\alpha4\beta2$ and $\alpha3\beta4$ nicotinic receptors are useful tools to investigate whether different binding modes at the two subtypes of the two most selective derivatives modified at the benzodioxane 5-position, namely, (*S*,*S*)-**1c** and (*S*,*R*)-**8**, can explain such a difference in affinity and even more in activity.[16,17] Comparison between the two orthosteric binding sites reveals a slightly outward displacement of loop C in the $\alpha3\beta4$ subtype, resulting in a more spacious binding pocket, in which replacement of Phe119 by a leucine residue (Leu121) is the most interesting difference from the $\alpha4\beta2$ subtype. $\beta2$-Asn109 and $\beta2$-Leu121, previously identified as key residues for the interaction with the 5- and 7-substituents, are conserved in the minus $\beta4$ side and are numbered Asn111 and Leu123. On the basis of these observations, the ability to accommodate larger ligands in the $\alpha3\beta4$ binding pocket and the absence of the $\beta4$-Phe119 engaging $\pi-\pi$ stacking interactions with the pyridine residue of nicotine may contribute to the explanation for the low $\alpha3\beta4$ affinity of nicotine compared to its $\alpha4\beta2$ affinity. To address the reasons behind the high $\alpha4\beta2/\alpha3\beta4$ selectivities of (*S*,*S*)-**1c** and (*S*,*R*)-**8**, the two compounds were docked in the $\alpha4\beta2$ and $\alpha3\beta4$ orthosteric binding sites (Figure 3). These were respectively obtained from the cryo-EM structures 6CNJ and 6PV7, refined as described for 5KXI. The binding modes of (*S*,*R*)-**8** and (*S*,*S*)-**1c** to the $\alpha4\beta2$ receptor site do not differ from those previously resulting from the docking into the X-ray structure (compare Figure 3A,B with Figure 2B,C). The positively charged nitrogen is accommodated in the "aromatic box", while the backbones of Leu121 and Asn109 create a H-bond network with pyridine nitrogen of (*S*,*R*)-**8**, mediated by a water molecule, and directly with 5-NH_2 of (*S*,*S*)-**1c**. Furthermore, the Phe119 lining the $\alpha4\beta2$ binding pocket establishes $\pi-\pi$ stacking interactions with the benzodioxane or pyridodioxane aromatic ring. When the same two compounds are docked within the $\alpha3\beta4$ binding pocket, at which they have very modest affinity and no agonist activity, they similarly accommodate the charged nitrogen in the aromatic box. However, the pyridine nitrogen of (*S*,*R*)-**8** and the 5-NH_2 of (*S*,*S*)-**1c** do not settle interactions with the Leu123 and Asn111 backbones. Furthermore, replacement of Phe119 with smaller and more flexible Leu121 abolishes the productive $\pi-\pi$ stacking interactions observed in the minus $\beta2$ side with the aromatic ring of the ligand and generates an extra pocket space toward which the pyridodioxane or benzodioxane core is diverted.

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Analysis by Site-Directed Mutagenesis. Docking of (*S*,*R*)-**3a**, (*S*,*S*)-**1c**, and (*S*,*R*)-**8** into the orthosteric $\alpha4\beta2$ binding site agreed in indicating that they are involved, through 7-OH, 5-NH_2, and pyridodioxane nitrogen, respectively, in hydrogen bond interactions with the backbone carbonyl of Asn109 and NH of Leu121. These residues line a small hydrophilic pocket of the minus $\beta2$ side together with the hydroxylated side chain of Ser108. Such interactions would be established also by the pyridine nitrogen of nicotine through a water molecule and their absence, in the case of the naked benzodioxane derivative (*S*,*R*)-**7**, would justify lower affinity and loss of agonist activity. Nicotine binding mode at the $\alpha4\beta2$ orthosteric site and, in particular, the interactions of its pyridinic nitrogen with the minus $\beta2$ side rest on a large body of experimental evidence: the structure of AChBP in complex with nicotine, mutations at the minus $\beta2$ side, and more recently, the nicotine-bound structures of the $\alpha4\beta2$ receptor obtained by X-ray crystallography and cryo-electron microscopy. For our compounds showing $\alpha4\beta2$ affinity and agonism and, in some cases, high $\alpha4\beta2/\alpha3\beta4$ selectivity, we needed some experimental evidence supporting the critical role ascribed to the elements decorating the aromatic ring in the interaction with the $\beta2$ small hydrophilic pocket lined by Asn109, Leu121, and Ser108. To assess the contribution of these residues to the binding and to provide further validation of the predicted binding mode, we opted for single mutations rather than backbone mutations, which can be generically disruptive to receptor function. In particular, we reasoned that, considering the small capacity of the pocket, the increase and decrease in the volume of Ser108 side chain should respectively disfavor and favor the accessibility to backbone CO of Asn109 and NH of Leu121. Therefore, we replaced the serine residue, on one side, with leucine and phenylalanine and, on the other, with alanine to augment and to reduce the steric encumbrance of the side chain, respectively. With regard to the suppression of the Ser108 hydroxyl resultant from such mutations, it is to be noted that, according to docking analysis, only (*S*,*R*)-**3a** establishes hydrogen bonds with the Ser108 hydroxyl as well as with the backbone carbonyl of Asn109 and NH of Leu121. Binding experiments were performed on the heterologously expressed human $\alpha4\beta2$ receptor and its three Ser108 → Leu, Ser108 → Phe, and Ser108 → Ala mutants. In addition to (*S*,*R*)-**3a**, (*S*,*S*)-**1c**, and (*S*,*R*)-**8**, the 7-amino derivative (*S*,*R*)-**1a** and the unsubstituted pyrrolidinyl benzodioxane (*S*,*R*)-**7** were included into the investigation. Although western blot analysis of the mutated receptors (data not shown) showed that none of the three mutations affected the expression of the receptors containing the mutated subunits, the replacement of Ser108 with leucine and phenylalanine in the $\beta2$ subunit completely abolished the binding of the ligand [^{3}H]epibatidine. This did not allow the determination of the K_i of our five compounds to the Ser108 → Leu and Ser108 → Phe mutated receptors. In contrast, as shown in Table 2, the mutation to alanine had a slightly ameliorative effect on the binding of the two 7-substituted derivatives (*S*,*R*)-**1a** and (*S*,*R*)-**3a** and it was irrelevant to the binding of the naked benzodioxane derivative (*S*,*R*)-**7** and of the two derivatives modified at the benzodioxane 5-position, (*S*,*S*)-**1c** and (*S*,*R*)-**8**. To assess whether conserved or slightly increased binding to the Ser108 → Ala mutated receptor corresponds to unaltered agonist activity, we tested functionally (*S*,*R*)-**3a**, (*S*,*S*)-**1c**, and (*S*,*R*)-**8** via whole-cell patch clamp electrophysiology with GH4C1 cell line cells transiently expressing the human $\alpha4\beta2$ subtype and its Ser108 → Ala mutant. As shown in Figure 4A–C, the graphs depict partial agonism responses of the three compounds with almost identical efficacies at the wild-type and mutated receptors, confirming the binding results.

Table 2. Pyrrolidinyl Benzodioxane (*S*,*R*)-7, Pyrrolidinyl Pyridodioxane (*S*,*R*)-8, and Compounds (*S*,*R*)-1a, (*S*,*R*)-3a, and (*S*,*S*)-1c: Affinity for Human $\alpha4\beta2$ nAChR Subtype and for Its Ser108 → Ala Mutant ($\alpha4\beta2$S108A) Labeled by [^{3}H]Epibatidine[a]

compound	wild-type $\alpha4\beta2$ nAChR [^{3}H]Epi; K_i (μM)	mutated $\alpha4\beta2$S108A nAChR [^{3}H]Epi; K_i (μM)	ratio (wild type/ mutant)
(*S*,*R*)-**7**	1.175 (0.16)	0.912 (0.29)	1.29
(*S*,*R*)-**8**	0.114 (0.026)	0.265 (0.091)	0.43
(*S*,*R*)-**1a**	0.046 (0.008)	0.006 (0.001)	7.7
(*S*,*R*)-**3a**	0.0067 (0.001)	0.0012 (0.0004)	5.6
(*S*,*S*)-**1c**	0.373 (0.049)	0.156 (0.017)	2.4

[a]The K_d and K_i values were derived from three [^{3}H]epibatidine saturation and three competition binding experiments using human WT $\alpha4\beta2$ and mutated $\alpha4\beta2$S108A subtypes. Data from saturation and competition binding curves were evaluated by one-site competitive binding curve-fitting procedures using GraphPad Prism version 5 (GraphPad Software, Inc., CA, USA). Each compound was tested in three separate competition binding curves for both wild-type and mutated receptors and the inhibition constant (K_i) was estimated in reference to the K_d of the radioligand according to the Cheng–Prusoff equation. The numbers in brackets represent the standard error.

DISCUSSION

The present results show that substitution at the 5-, 6-, and 7-positions of the aromatic ring of the *S*,*R* and *S*,*S* pyrrolidinyl-benzodioxane diastereomers greatly affects affinity and activity at $\alpha4\beta2$ and $\alpha3\beta4$ nAChR subtypes. We previously reported the *S*,*R* isomer of unsubstituted pyrrolidinyl benzodioxane, namely, (*S*,*R*)-**7**, as a potent and unselective $\alpha4\beta2$ and $\alpha3\beta4$ antagonist. Successively, we found that its 7-OH-substituted analogue (*S*,*R*)-**3a** is instead a potent $\alpha4\beta2$ partial agonist endowed with high $\alpha4\beta2$ affinity and good $\alpha4\beta2/\alpha3\beta4$ selectivity and that also its 5-aza-isostere, the pyridodioxane (*S*,*R*)-**8**, is an $\alpha4\beta2$ partial agonist but with much higher $\alpha4\beta2/\alpha3\beta4$ selectivity than (*S*,*R*)-**3a**. The effectiveness of both the modifications, 7-substitution and isosteric aza-replacement, was strictly conditioned by the dioxane stereochemistry and by electronic and steric factors, such as hydrogen-bonding capability and size of the substituent and position of the isosterically nitrogen-replaced CH. These observations indicated that subtle modifications at the benzene ring of (*S*,*R*)-**7** could generate potent and sometimes selective $\alpha4\beta2$ partial agonism and that an appropriately planned SAR study of these modifications, supported by mutagenesis and docking analysis, could shed light on the molecular bases of the $\alpha4\beta2$ vs $\alpha3\beta4$ selectivity. That is a topical issue, more challenging than $\alpha4\beta2/\alpha7$ selectivity, which is generally more inherent in both highly affinitive $\alpha4\beta2$ and $\alpha7$ ligands, as demonstrated, for instance, by very recent investigations on the $\alpha4\beta2$ partial agonist cytisine[14] and on the $\alpha7$ antagonist MG624.[38] In particular, in the case of cytisine, the $\alpha4\beta2$ affinity is 80-fold and 500-fold higher than the $\alpha3\beta4$ and $\alpha7$ selectivities, respectively, and some C(10)

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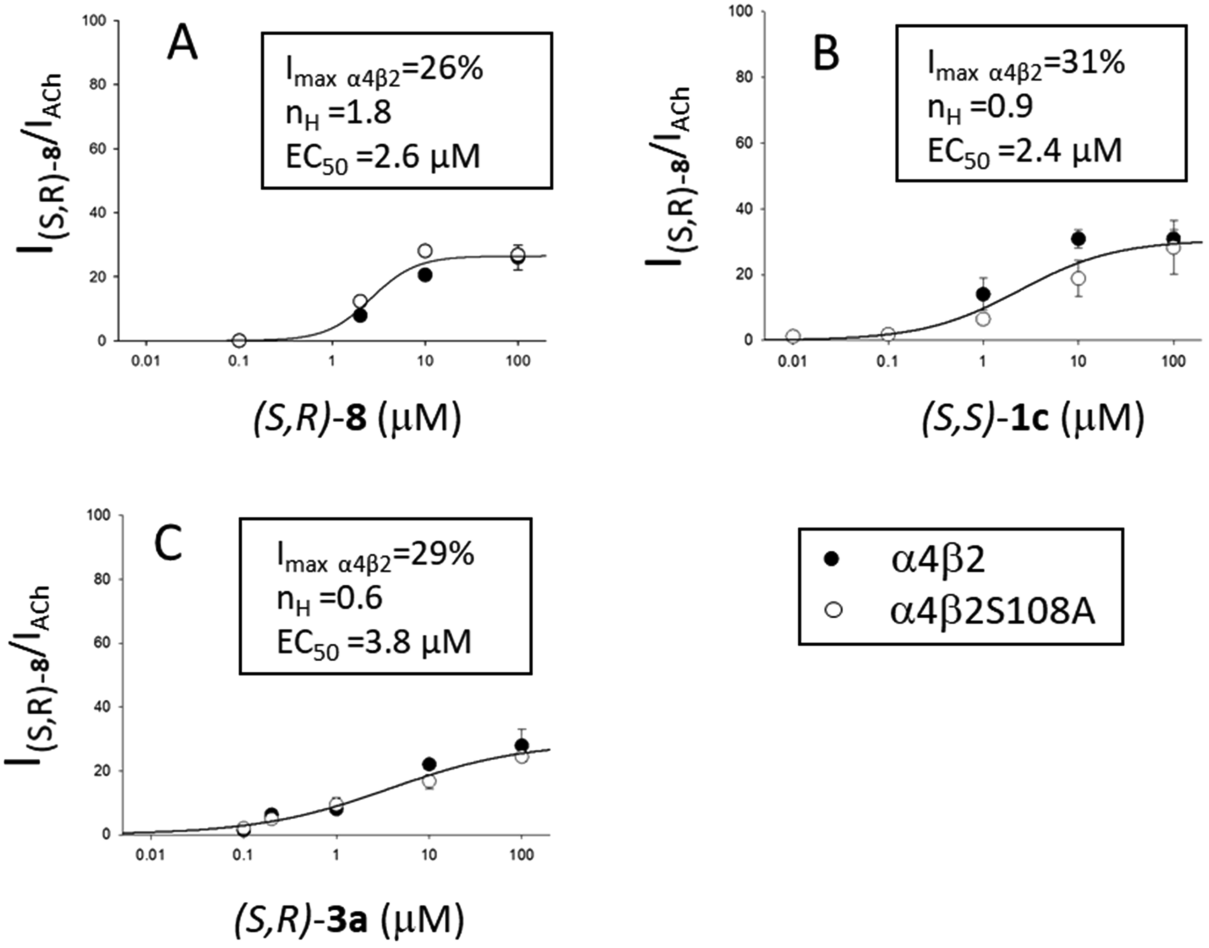

Figure 4. Agonist properties of (*S*,*R*)-**8** (A), (*S*,*S*)-**1c** (B), and (*S*,*R*)-**3a** (C) do not differ on human $\alpha4\beta2$ and $\alpha4\beta2$S108A nAChRs. (A) (*S*,*R*)-**8** dose−response relationship (normalized with respect to the response elicited by 100 μM ACh) for human $\alpha4\beta2$ nAChR (solid circle; n = 3) and for human $\alpha4\beta2$S108A nAChR (open circle; n = 2). A single Hill equation was best fitted to all data, yielding I_{max} = 26 ± 2%, EC_{50} = 2.6 ± 0.6 μM, and n_H = 1.8 ± 0.8. (B) (*S*,*S*)-**1c** dose−response relationship (normalized as in A) for human $\alpha4\beta2$ nAChR (solid circle; n = 3) and for human $\alpha4\beta2$S108A nAChR (open circle; n = 4). A single Hill equation was best fitted to all data, yielding I_{max} = 31 ± 5%, EC_{50} = 2.4 ± 1.7 μM, and n_H = 0.9 ± 0.4. (C) (*S*,*R*)-**3a** dose−response relationship (normalized as in A) for human $\alpha4\beta2$ nAChR (solid circle; n = 4) and for human $\alpha4\beta2$S108A nAChR (open circle; n = 8). A single Hill equation was best fitted to all data, yielding I_{max} = 29 ± 3%, EC_{50} = 3.8 ± 2.1 μM, and n_H = 0.6 ± 0.1.

substitutions elicit increased selectivity for the $\alpha4\beta2$ versus $\alpha3\beta4$ and $\alpha7$ nAChR subtypes.

Here, apart from phenyl, we considered small and both hydrogen bond-donating and hydrogen bond-accepting substituents (OH, NH_2, NO_2, NMe_2, and NHAc). We observed that all the 6-substitutions, which have the most elongating effect on the shape of the molecules, with the benzodioxane C(6) being the farthest carbon from pyrrolidine nitrogen, are deleterious, remarkably more for $\alpha4\beta2$ interaction than $\alpha3\beta4$ interaction. Otherwise, for the 7-substitution, the hydroxy and amino groups are productive for $\alpha4\beta2$ interaction and activation, but not other substituents and with an important difference: replacement of OH with NH_2 results in complete loss of $\alpha4\beta2/\alpha3\beta4$ selectivity. This was an intriguing result. The 5-position behaves oppositely to the 6-position but also to the 7-position. It is more tolerant: all the four substituents, OH, NH_2, NO_2, and even bulky Ph, maintain the submicromolar $\alpha4\beta2$ affinity of unsubstituted (*S*,*R*)-**7** with moderate to high $\alpha4\beta2/\alpha3\beta4$ selectivity, coincident, in the case of OH, NH_2, and NO_2, with *S* configuration of the dioxane stereocenter and not with *R* configuration as for the 7-hydroxy substitution. Furthermore, among the 5-substituted analogues, the 5-amino analogue (*S*,*S*)-**1c** behaves similarly to the pyridodioxane (*S*,*R*)-**8** but shows higher $\alpha4\beta2$ affinity and selectivity. The functional tests confirm such a trend: (*S*,*S*)-**1c** is an $\alpha4\beta2$ agonist more potent than (*S*,*R*)-**8** and both have no effect on the $\alpha3\beta4$ subtype. (*S*,*S*)-**1c** is even a weak $\alpha3\beta4$ antagonist.

This SAR analysis addressed our attention to the differences resulting from the introduction of the substituent at the 5-position relative to that at the 7-position: maintenance of $\alpha4\beta2$ affinity also with relatively bulky substituent and remarkably increased $\alpha4\beta2/\alpha3\beta4$ selectivity associated with opposite stereo-preference concerning the dioxane stereo-center. Visual inspection of the structure of pyrrolidinyl benzodioxane indicates that the same substituent has a more elongating effect on the shape of the molecule if positioned in the 7-position rather than in the 5-position. On the other hand, the cryo-EM structures of the $\alpha4\beta2$ and $\alpha3\beta4$ receptors show that the $\beta4$ binding pocket, although lined by nearly the same amino acid residues, is more spacious than the $\beta2$ binding pocket. Consequently, these observations suggest that small substituents, such as OH and NH_2, when positioned in the 5-position, do not efficiently interact with the amino acid

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residues of the wider minus β4 side. On the contrary, when positioned in the 7-position and thus more protrudent, the same substituents can establish productive interactions in the β4 binding pocket. On the other hand, the amino acid residues of the narrower β2 binding pocket would tightly interact with the same substituents when positioned in the 5-position but also in the 7-position. In the latter case, however, even minimal size increases of the substituent are not tolerated, the interaction already being "tailor-made", as indicated by the NO_2 substitution, which is highly pejorative in the 7-position and not in the 5-position. Such SAR considerations are supported by our site-directed mutagenesis experiments and docking analysis. Replacement of the hydroxymethyl side chain of Ser108, one of the amino acid residues lining the small hydrophilic β2 pocket, with the little less bulky methyl of Ala increases the affinity of the 7-OH and 7-NH_2 derivatives without significantly modifying that of the benzodioxane derivatives unsubstituted or 5-modified and, in any case, it does not affect α4β2 partial agonism. On the contrary, replacement of the hydroxymethyl side chain of Ser108 even just with the little bulkier isopropyl of Leu abolishes the labeled epibatidine binding so as to hinder α4β2 affinity determination. These results indicate that the small β2 pocket is well receptive to OH and NH_2 substituents and to pyridodioxane nitrogen, which are indispensable to elicit the agonist activity, but with very strict occupancy limits. If the mutational studies show what is detrimental for the α4β2 affinity and activity, which is reduction of the capacity of the β2 pocket, the docking into the binding sites of the cryo-EM α4β2 and α3β4 structures gives account of what is beneficial for both α4β2 affinity/activity and α4β2 vs α3β4 selectivity of our pyrrolidinyl benzodioxanes. This is the placement of a small hydrophilic substituent in the 5-position, in particular of the amino group, or the replacement of CH with nitrogen at the same position. Such modifications maintain the productive β2 interactions of the 7-hydroxy pyrrolidinyl benzodioxane analogue but are uninfluential to the β4 interactions and result into α3β4 null activity and antagonism, analogous to the unsubstituted pyrrolidinyl benzodioxane.

CONCLUSIONS

The present study confirms that pyrrolidinyl benzodioxane is a viable template for α4β2 ligands, whose activity and selectivity profile can be fruitfully managed through 7- and 5-substitutions at the benzodioxane nucleus. Compared to 7-OH and 7-NH_2 substitutions, replacement of C(5)H with nitrogen or attachment of NH_2 at this position elicits increased selectivity for α4β2 versus α3β4 while retaining or potentiating α4β2 partial agonism. Docking analysis, using the cryo-EM structures of the two nicotinic receptor subtypes, shows how pyrrolidinyl benzodioxanes functionalized at the benzodioxane 5-position can tightly interact with the small binding pocket of the minus β2 side through such a function, but not with the more spacious one of the minus β4 side. The same extent of discrimination is not achieved by analogous substitutions at the 7-position reasonably due to the capacity of the substituents at this position to reach also the farther amino acid residues lining the β4 binding pocket. Our mutational experiments prove the limited accommodation capacity of the β2 pocket, once again indicating that this is the distinctive feature of the orthosteric α4β2 binding site, which can be exploited to achieve α4β2 vs α3β4 selectivity.

EXPERIMENTAL SECTION

Chemistry. All chemicals and solvents were used as received from commercial sources or prepared as described in the literature. Flash chromatography purifications were performed using KP-Sil 32−63 μm 60 Å cartridges. TLC analyses were carried out on alumina sheets precoated with silica gel 60 F254 and visualized with UV light. Content of saturated aqueous solution of ammonia in eluent mixtures is given as v/v percentage. R_f values are given for guidance. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz using an FT-NMR spectrometer. Chemical shifts are reported in ppm relative to the residual solvent ($CHCl_3$, MeOH, or DMSO) as an internal standard. Melting points were determined by a Buchi Melting Point B-540 apparatus. Optical rotations were determined using a Jasco P-1010 polarimeter. Chiral HPLC analyses were performed using Hewlett Packard 1050 instrument. The purity of all tested compounds was determined by HPLC analysis and was higher than 95%. In each described preparation, the quantities of reagents are given for 1 mol of substrate. All reactions were conducted under a nitrogen atmosphere. The sodium or potassium salts of phenols were prepared by treatment of their methanol solution with an equimolar amount of 1 M aqueous NaOH or with 1 M aqueous KOH and successive removal of the solvents. 2-Benzyloxy-4-nitrophenol, 2-benzyloxy-5-nitrophenol, 2-benzyloxy-6-nitrophenol, and 2-benzyloxy-5-phenylphenol were prepared as previously described.[31,34]

General Procedure for Synthesis of Compounds (*S*)-9a−(*S*)-9c, (*S*)-14a−(*S*)-14c, (*S*)-19, (*S*)-24, (*S*)-29b, and (*S*)-29c. The *S* and *R* isomers of target compounds were synthesized, combining *N*-Boc-protected (*S*)- and (*R*)-2-bromoacetylpyrrolidine with the potassium salts of 2-benzyloxy-4-nitrophenol (**9a**), 2-benzyloxy-5-nitrophenol (**9b**), 2-benzyloxy-6-nitrophenol (**9c**), 2- methoxyethoxymethyloxy-6-nitrophenol **35c** (**14c**), 2,6-dimethoxyethoxymethyloxyphenol **41** (**24**), and 2-benzyloxy-6-phenylphenol **46** (**29c**) or with the sodium salts of 2-methoxyethoxymethyloxy-4-nitrophenol **35a** (**14a**), 2-methoxyethoxymethyloxy-5-nitrophenol **35b** (**14b**), 2,5-dibenzyloxyphenol **39** (**19**), and 2-benzyloxy-5-phenylphenol (**29b**). All the reactions were carried out in acetone at room temperature for 16 h using an excess of bromoketone (1.1 mol). Afterward, the solvent was evaporated under vacuum and the resulting residue was diluted with ethyl acetate and washed with water. The organic phase was dried over sodium sulfate and filtered and the solvent was evaporated under vacuum, affording a crude that was purified by flash chromatography.

*(S)-N-Boc-2-[2′-(2-benzyloxy-4-nitrophenoxy)acetyl]pyrrolidine [(S)-**9a**].* Obtained as a white amorphous solid in 86% yield after chromatography on silica gel (petroleum ether/acetone, 7:3): R_f = 0.33; $[\alpha]_D^{25}$ = −22.6 (*c* 1, $CHCl_3$); 99.60% e.e. (Chiralcel OJ; *n*-hexane/ethanol, 7:3; *F* = 1.5 mL/min; λ = 276 nm; Rt_S = 8.10 min; Rt_R = 5.74 min); ^{1}H NMR ($CDCl_3$) δ 7.89−7.82 (m, 2H), 7.48−7.34 (m, 5H), 6.96 (d, 0.75H, *J* = 8.8 Hz, rotamer), 6.83 (d, 0.25H, *J* = 9.1 Hz, rotamer), 5.20 (s, 2H), 5.05 (m, 1.5H, rotamer), 4.84 (s, 0.5H, rotamer), 4.52 (m, 1H), 3.60−3.40 (m, 2H), 2.30−2.05 (m, 1H), 2.00−1.75 (m, 3H), 1.47 (s, 6.75H, rotamer), 1.42 (s, 2.25H, rotamer).

*(S)-N-Boc-2-[2′-(2-benzyloxy-5-nitrophenoxy)acetyl]pyrrolidine [(S)-**9b**].* Obtained as a white amorphous solid in 85% yield after chromatography on silica gel (cyclohexane/EtOAc, 8:2): R_f = 0.18; $[\alpha]_D^{25}$ = −41.8 (*c* 1, $CHCl_3$); 99.50% e.e. (Kromasil 5-amycoat; *n*-hexane/ethanol, 8:2; *F* = 1 mL/min; λ = 276 nm; Rt_S = 9.21 min; Rt_R = 6.46 min); ^{1}H NMR ($CDCl_3$) δ 7.88 (m, 1H), 7.70 (m, 1H), 7.48−7.32 (m, 5H), 6.98 (m, 1H), 5.24 (s, 2H), 4.99 (m, 1.2H, rotamer), 4.83 (s, 0.8H, rotamer), 4.51 (m, 1H), 3.61−3.40 (m, 2H), 2.22−2.15 (m, 1H), 2.00−1.78 (m, 3H), 1.47 (s, 5.4H, rotamer), 1.41 (s, 3.6H, rotamer).

*(S)-N-Boc-2-[2′-(2-benzyloxy-6-nitrophenoxy)acetyl]pyrrolidine [(S)-**9c**].* Obtained as a yellow oil in 81% yield after chromatography on silica gel (cyclohexane/EtOAc, 7:3): R_f = 0.43; $[\alpha]_D^{25}$ = −15.4 (*c* 1, MeOH); 100% e.e. (Lux 3 μ; cellulose-2; *n*-hexane/ethanol, 8:2; *F* = 1 mL/min; λ = 276 nm; Rt_S = 8.20 min; Rt_R = 9.05 min); ^{1}H NMR ($CDCl_3$) δ: 7.38−7.31 (m, 6H), 7.20−7.09 (m, 2H), 5.17 (s,

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0.66H, rotamer), 5.12 (s, 1.34H, rotamer), 4.95 (s, 0.66H, rotamer), 4.86 (s, 1.34H, rotamer), 4.61 (m, 0.33H, rotamer), 4.52 (m, 0.67H, rotamer), 3.47−3.36 (m, 2H), 2.13−2.04 (m, 1H), 1.82−1.69 (m, 3H), 1.44 (s, 3H), 1.37 (s, 6H).

*(S)-N-Boc-2-[2′-(2-(2-methoxyethoxymethoxy)-4-nitrophenoxy)-acetyl]pyrrolidine [(S)-**14a**].* Obtained as a yellow light oil in 78% yield after chromatography on silica gel (dichloromethane/EtOAc, 8:2): R_f = 0.61; $[\alpha]_D^{25}$ = −16.0 (*c* 1, $CHCl_3$); 100% e.e. (Kromasil 5-amycoat; *n*-hexane/ethanol, 8:2; *F* = 1 mL/min; λ = 276 nm; Rt_S = 16.68 min; Rt_R = 13.13 min); ^{1}H NMR ($CDCl_3$) δ 8.08 (m, 1H), 7.88 (d, 1H, *J* = 8.8 Hz), 6.96 (d, 1H, *J* = 8.8 Hz), 5.38 (s, 2H), 5.16−4.89 (m, 2H), 4.51−4.47 (m, 1H), 3.90 (m, 2H), 3.59−3.42 (m, 4H), 3.38 (s, 3H), 2.25−1.94 (m, 4H), 1.47 (s, 9H).

*(S)-N-Boc-2-[2′-(2-(2-methoxyethoxymethoxy)-5-nitrophenoxy)-acetyl]pyrrolidine [(S)-**14b**].* Obtained as a yellow light oil in 55% yield after chromatography on silica gel (dichloromethane/acetonitrile, 95:5): R_f = 0.29; $[\alpha]_D^{25}$ = −36.8 (*c* 1, $CHCl_3$); 99.54% e.e. (Lux 3 μ; amylose-2; *n*-hexane/ethanol, 8:2; *F* = 0.8 mL/min; λ = 276 nm; Rt_S = 19.53 min; Rt_R = 18.45 min); ^{1}H NMR ($CDCl_3$) δ 7.89 (m, 1H), 7.7 (m, 1H), 7.29 (m, 1H), 5.42 (s, 2H), 5.03−4.88 (m, 2H), 4.54 (m, 1H), 3.89 (m,2H), 3.57−3.40 (m, 4H), 3.38−3.35 (s, 3H), 2.28−2.15 (m, 1H), 2.00−1.91 (m, 3H), 1.46 (s, 9H).

*(S)-N-Boc-2-[2′-(2-(2-methoxyethoxymethoxy)-6-nitrophenoxy)-acetyl]pyrrolidine [(S)-**14c**].* Obtained as a pale yellow oil in 42% yield after chromatography on silica gel (cyclohexane/EtOAc, 7:3): R_f = 0.28; $[\alpha]_D^{25}$ = −12.3 (*c* 1, MeOH); 100% e.e. (Lux 3 μ; cellulose-2; *n*-hexane/ethanol, 8:2; *F* = 1 mL/min; λ = 276 nm; Rt_S = 7.75 min; Rt_R = 8.45 min); ^{1}H NMR ($CDCl_3$) δ 7.46−7.39 (m, 2H), 7.20−7.13 (m, 1H), 5.35 (s, 0.9H, rotamers), 5.32 (s, 1.1H, rotamer), 4.91 (s, 1.1H, rotamer), 4.86 (s, 0.9H, rotamer), 4.71 (m, 0.45H, rotamer), 4.61 (m, 0.55H, rotamer), 3.84 (m, 2H), 3.51 (m, 4H), 3.36 (s, 3H), 2.26−2.14 (m, 1H), 2.08−2.02 (m, 1H), 1.94−1.85 (m, 2H), 1.45 (s, 4.95H, rotamer), 1.42 (s, 4.05H, rotamer).

*(S)-N-Boc-2-[2′-(2,5-dibenzyloxyphenoxy)acetyl]pyrrolidine [(S)-**19**].* Obtained as a white solid in 72% yield after chromatography on silica gel (petroleum ether/acetone, 8:2): mp = 91.14 °C; R_f = 0.24; $[\alpha]_D^{25}$ = −26.5 (*c* 1, $CHCl_3$); 98.51% e.e. (Chiralcel OJ; *n*-hexane/ethanol, 7:3; *F* = 1.5 mL/min; λ = 276 nm; Rt_S = 8.54 min; Rt_R = 5.88 min); ^{1}H NMR ($CDCl_3$) δ 7.46−7.27 (m, 10H), 6.90−6.81 (m, 1H), 6.64−6.46 (m, 2H), 5.06 (s, 2H), 4.99 (s, 2H), 4.82 (s, 1H), 4.70 (s, 1H), 4.67−4.55 (m, 1H), 3.55−3.35 (m, 2H), 2.25−2.03 (m, 1H), 1.97−1.70 (m, 4H), 1.44 (s, 4.5H), 1.38 (s, 4.5H).

*(S)-N-Boc-2-[2′-(2,6-bis(2-methoxyethoxymethoxy)phenoxy)-acetyl]pyrrolidine [(S)-**24**].* Obtained as a pale yellow oil in 77% yield after chromatography on silica gel (cyclohexane/EtOAc, 7:3): R_f = 0.3; 98.57% e.e. (Kromasil 5-amycoat; *n*-hexane/ethanol, 8:2; *F* = 1 mL/min; λ = 220 nm; Rt_S = 14.62 min; Rt_R = 7.51 min); ^{1}H NMR ($CDCl_3$) δ 6.99−6.90 (m, 1H), 6.89−6.83 (m, 2H), 5.27 (s, 4H), 4.71−4.66 (m, 2H), 3.84−3.81 (m, 5H), 3.56−3.53 (m, 6H), 3.36 (s, 6H), 2.38−1.98 (m, 4H), 1.42 (s, 9H).

*(S)-N-Boc-2-[2′-(2-benzyloxy-5-phenylphenoxy)acetyl]-pyrrolidine [(S)-**29b**].* Obtained as a white solid in 60% yield after chromatography on silica gel (petroleum ether/acetone, 8:2): mp = 81.44 °C; R_f = 0.32; $[\alpha]_D^{25}$ = −25.3 (*c* 0.5, $CHCl_3$); 99.79% e.e. (Chiralcel OJ; *n*-hexane/ethanol, 7:3; *F* = 1.5 mL/min; λ = 254 nm; Rt_S = 5.88 min; Rt_R = 4.11 min); ^{1}H NMR ($CDCl_3$) δ 7.57−7.29 (m, 10H), 7.26−7.14 (m, 2H), 7.05−6.97 (m, 1H), 5.18 (s, 1H), 5.16 (s, 1H), 4.93 (s, 1H), 4.78 (s, 1H), 4.68 (m, 1H), 3.52−3.39 (m, 2H), 2.22−2.10 (m, 1H), 1.96−1.75 (m, 3H), 1.43 (s, 4.5H, rotamer), 1.35 (s, 4.5H, rotamer).

*(S)-N-Boc-2-[2′-(2-benzyloxy-6-phenylphenoxy)acetyl]-pyrrolidine [(S)-**29c**].* Obtained as a pale yellow oil in 30% yield after chromatography on silica gel (cyclohexane/EtOAc, 9:1): R_f = 0.15; $[\alpha]_D^{25}$ = −5.88 (*c* 1, MeOH); 100% e.e. (Lux 3 m; cellulose-1; *n*-hexane/ethanol, 8:2; *F* = 0.5 mL/min; λ = 276 nm; Rt_S = 7.59 min; Rt_R = 8.45 min); ^{1}H NMR ($CDCl_3$) δ 7.54 (d, *J* = 7.1 Hz, 2H), 7.48−7.28 (m, 8H), 7.23−7.04 (m, 1H), 6.99 (m, 2H), 5.12 (s, 2H), 4.50 (m, 1H), 4.39 (m, 2H), 3.35 (m, 2H), 1.99−1.67 (m, 4H), 1.23 (s, 9H).

General Procedure for Synthesis of Compounds 10a−10c, 15a−15c, 20, 25, 30b, and 30c. The target compounds were obtained by treatment of the *S* isomers of **9a−9c**, **14a−14c**, **19**, **24**, **29b**, and **29c** with 1 mol of $NaBH_4$ in anhydrous THF. The reactions were carried out at room temperatures for 3 h. Afterward, the mixture was diluted with ethyl acetate and washed with a 1 M solution of HCl and then with brine. The organic phase was dried over sodium sulfate and filtered and the solvent was evaporated under vacuum. The resulting crudes were a diastereomeric mixture of (*S*,*S*) and (*S*,*R*) isomers and were separated by flash chromatography. The diastereomeric mixture of **25** was not resolved.

*(S,S)-N-Boc-2-[1′-hydroxy-2′-(2-benzyloxy-4-nitrophenoxy)-ethyl]pyrrolidine [(S,S)-**10a**].* Obtained as a pale yellow oil in 21% yield after chromatography on silica gel (dichloromethane/acetone, 95:5): R_f = 0.41; $[\alpha]_D^{25}$ = −53.8 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.91 (dd, 1H, *J* = 8.8 and 2.5 Hz), 7.82 (d, 1H, *J* = 2.5 Hz), 7.45−7.33 (m, 5H), 6.95 (d, 1H, *J* = 8.8 Hz), 5.15 (s, 2H), 4.65 (m, 1H), 4.18−3.95 (m, 4H), 3.55−3.40 (m, 1H), 3.30−3.10 (m, 1H), 2.17−2.05 (m, 1H), 2.05−1.85 (m, 1H), 1.80−1.65 (m, 2H), 1.46 (s, 9H).

*(S,R)-N-Boc-2-[1′-hydroxy-2′-(2-benzyloxy-4-nitrophenoxy)-ethyl]pyrrolidine [(S,R)-**10a**].* Obtained as a white solid in 48% yield after chromatography on silica gel (dichloromethane/acetone, 95:5): mp = 91.27 °C; R_f = 0.57; $[\alpha]_D^{25}$ = −28.3 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.90 (dd, 1H, *J* = 9.1 and 2.5 Hz), 7.81 (d, 1H, *J* = 2.5 Hz), 7.48−7.27 (m, 5H), 6.97 (d, 1H, *J* = 9.1 Hz), 5.17 (s, 2H), 4.20−4.07 (m, 3H), 4.00−3.85 (m, 1H), 3.48 (m, 1H), 3.32 (m, 1H), 2.00−1.74 (m, 4H), 1.48 (s, 9H).

*(S,S)-N-Boc-2-[1′-hydroxy-2′-(2-benzyloxy-5-nitrophenoxy)-ethyl]pyrrolidine [(S,S)-**10b**].* Obtained as a pale yellow oil in 28% yield after chromatography on silica gel (dichloromethane/acetone, 95:5): R_f = 0.27; $[\alpha]_D^{25}$ = +26.2 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.88 (dd, 1H, *J* = 8.9 and 2.8 Hz). 7.78 (d, 1H, *J* = 2.8 Hz), 7.36 (m, 5H), 6.95 (d, 1H, *J* = 8.9 Hz), 5.19 (s, 2H), 4.60−4.65 (bs, 1H, exchange with D_2O), 4.17−4.02 (m, 4H), 3.55−3.40 (m, 1H), 3.30−3.10 (m, 1H), 2.17−1.92 (m, 2H), 1.80−1.57 (m, 2H), 1.46 (s, 9H).

*(S,R)-N-Boc-2-[1′-hydroxy-2′-(2-benzyloxy-5-nitrophenoxy)-ethyl]pyrrolidine [(S,R)-**10b**].* Obtained as a pale yellow oil in 52% yield after chromatography on silica gel (dichloromethane/acetone, 95:5): R_f = 0.40; $[\alpha]_D^{25}$ = −22.5 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.87 (dd, 1H, *J* = 9.1 and 2.5 Hz), 7.81 (d, 1H, *J* = 2.5 Hz), 7.48−7.26 (m, 5H), 6.95 (d, 1H, *J* = 9.1 Hz), 5.21 (s, 2H), 4.16 (m, 3H), 3.95 (m, 1H), 3.45 (m, 1H), 3.32 (m, 1H), 1.95−1.69 (m, 4H), 1.48 (s, 9H).

*(S,S)-N-Boc-2-[1′-hydroxy-2′-(2-benzyloxy-6-nitrophenoxy)-ethyl]pyrrolidine [(S,S)-**10c**].* Obtained as a pale yellow oil in 26% yield after chromatography on silica gel (cyclohexane/ethyl acetate, 9:1): R_f = 0.42; $[\alpha]_D^{25}$ = −42.6 (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.44−7.34 (m, 6H), 7.16−7.06 (m, 2H), 5.16 (s, 2H), 4.37−4.30 (m, 1H), 4.14−4.08 (m, 2H), 3.85 (m, 1H), 3.48−3.39 (m, 1H), 3.29−3.21 (m, 1H), 2.03−1.98 (m, 1H), 1.92−1.67 (m, 3H), 1.42 (s, 9H).

*(S,R)-N-Boc-2-[1′-hydroxy-2′-(2-benzyloxy-6-nitrophenoxy)-ethyl]pyrrolidine [(S,R)-**10c**].* Obtained as a pale yellow oil in 74% yield after chromatography on silica gel (cyclohexane/ethyl acetate, 9:1): R_f = 0.51; $[\alpha]_D^{25}$ = −52.0 (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.46−7.35 (m, 6H), 7.16−7.05 (m, 2H), 5.16 (s, 2H), 4.36 (m, 1H), 4.14 (m, 1H), 4.02 (m, 1H), 3.92 (m, 1H), 3.45 (m, 1H), 3.28 (m, 1H), 1.93−1.80 (m, 3H), 1.78−1.72 (m, 1H), 1.44 (s, 9H).

*(S,S)-N-Boc-2-[1′-hydroxy-2′-(2-(2-methoxyethoxymethoxy)-4-nitrophenoxy)ethyl]pyrrolidine [(S,S)-**15a**].* Obtained as a pale yellow oil in 44% yield after chromatography on silica gel (dichloromethane/ethyl acetate, 85:15): R_f = 0.42; $[\alpha]_D^{25}$ = −27.3 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.02 (d, 1H, *J* = 2.6 Hz), 7.93 (dd, 1H, *J* = 2.6 and 9.1 Hz), 6.97 (d, 1H, *J* = 9.1 Hz), 5.33 (s, 2H), 4.19 (dd, 1H, *J* = 3.5 and 10.1 Hz), 4.12 (m, 2H), 3.96 (m, 1H), 3.89 (m, 2H), 3.58 (m, 2H), 3.52 (m, 1H), 3.37 (s, 3H), 3.31 (m, 1H), 2.06−1.79 (m, 4H), 1.47 (s, 9H).

*(S,R)-N-Boc-2-[1′-hydroxy-2′-(2-(2-methoxyethoxymethoxy)-4-nitrophenoxy)ethyl]pyrrolidine [(S,R)-**15a**].* Obtained as a pale yellow oil in 45% yield after chromatography on silica gel (dichloromethane/ethyl acetate, 85:15): R_f = 0.29; $[\alpha]_D^{25}$ = −28.3 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.03 (d, 1H, *J* = 2.6 Hz), 7.94 (dd, *J* = 2.6 and 8.1 Hz, 1H), 6.94 (d, 1H, J = 8.1 Hz), 5.33 (s, 2H),

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4.17−3.96 (m, 4H), 3.85 (m, 2H), 3.58 (m, 2H), 3.48(m, 1H), 3.37 (s, 3H), 3.36 (m, 1H), 2.13−1.79 (m, 4H), 1.46 (s, 9H).

*(S,S)-N-Boc-2-[1′-hydroxy-2′-(2-(2-methoxyethoxymethoxy)-5-nitrophenoxy)ethyl]pyrrolidine [(S,S)-**15b**].* Obtained as a pale yellow oil in 46% yield after chromatography on silica gel (dichloromethane/ethyl acetate, 95:5): R_f = 0.32; $[\alpha]_D^{25}$ = −27.7 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.86 (dd, 1H, *J* = 2.6 and 9.0 Hz), 7.80 (d, 1H, *J* = 2.6 Hz), 7.22 (d, 1H, *J* = 9.0 Hz), 5.38 (2H, s), 4.15−4.03 (m, 3H), 3.96 (m, 1H), 3.87 (m, 2H), 3.48 (m, 3H), 3.40−3.30 (m, 1H), 3.38 (s, 3H), 2.07−1.81 (m, 4H), 1.48 (s, 9H).

*(S,R)-N-Boc-2-[1′-hydroxy-2′-(2-(2-methoxyethoxymethoxy)-5-nitrophenoxy)ethyl]pyrrolidine [(S,R)-**15b**].* Obtained as a pale yellow oil in 54% yield after chromatography on silica gel (dichloromethane/ethyl acetate, 95:5): R_f = 0.29; $[\alpha]_D^{25}$ = −21.6 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.84 (dd, 1H, *J* = 2.6 and 9.0 Hz), 7.78 (d, 1H, *J* = 2.6 Hz), 7.22 (d, 1H, *J* = 9.0 Hz), 5.37 (s, 2H), 4.19−4.00 (m, 4H), 3.86 (m, 2H), 3.49 (m, 3H), 3.35 (s, 3H), 3.28 (m, 1H), 2.11−1.79 (m, 4H), 1.48 (s, 9H).

*(S,S)-N-Boc-2-[1′-hydroxy-2′-(2-(2-methoxyethoxymethoxy)-6-nitrophenoxy)ethyl]pyrrolidine [(S,S)-**15c**].* Obtained as a pale yellow-ochre oil in 72% yield after chromatography on silica gel (cyclohexane/ethyl acetate, 1:1): R_f = 0.62; $[\alpha]_D^{25}$ = −63.9 (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.43−7.36 (m, 2H), 7.10 (m, 1H), 5.34 (s, 2H), 4.33 (m, 1H), 4.15−4.08 (m, 2H), 3.88−3.85 (m, 3H), 3.57−3.54 (m, 2H), 3.54−3.40 (m, 1H), 3.37 (s, 3H), 3.37−3.30 (m, 1H), 2.00−1.89 (m, 3H), 1.83−1.79 (m, 1H), 1.45 (s, 9H).

*(S,R)-N-Boc-2-[1′-hydroxy-2′-(2-(2-methoxyethoxymethoxy)-6-nitrophenoxy)ethyl]pyrrolidine [(S,R)-**15c**].* Obtained as a pale yellow oil in 28% yield after chromatography on silica gel (cyclohexane/ethyl acetate, 1:1): R_f = 0.57; $[\alpha]_D^{25}$ = −19.65 (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.45−7.36 (m, 2H), 7.11 (t, *J* = 8.0 Hz, 1H), 5.34 (s, 2H), 4.34 (m, 1H), 4.12−4.02 (m, 2H), 3.87−3.84 (m, 3H), 3.57−3.54 (m, 3H), 3.44 (m, 1H), 3.37 (s, 3H), 2.10 (m, 1H), 1.97−1.79 (m, 3H), 1.45 (s, 9H).

*(S,S)-N-Boc-2-[1′-hydroxy-2′-(2,5-dibenzyloxyphenoxy)ethyl]-pyrrolidine [(S,S)-**20**].* Obtained as a pale yellow oil in 25% yield after chromatography on silica gel (toluene/ethyl acetate, 8:2): R_f = 0.39; $[\alpha]_D^{25}$ = −12.5 (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.45−7.12 (m, 10H), 6.83 (d, 1H, *J* = 8.5 Hz), 6.64 (d, 1H, *J* = 2.8 Hz), 6.47 (dd, 1H, *J* = 2.8 and 8.5 Hz), 5.02 (s, 2H), 4.99 (s, 2H), 4.52 (bs, 1H, exchange with D_2O), 4.20−3.80 (m, 4H), 3.60−3.35 (m, 1H), 3.35−3.15 (m, 1H), 2.20−2.02 (m, 1H), 2.02−1.65 (m, 3H), 1.58 (s, 2.2H, rotamer), 1.45 (s, 6.8H, rotamer).

*(S,R)-N-Boc-2-[1′-hydroxy-2′-(2,5-dibenzyloxyphenoxy)ethyl]-pyrrolidine [(S,R)-**20**].* Obtained as a white solid in 63% yield after chromatography on silica gel (toluene/ethyl acetate, 8:2): mp = 78.33 °C; R_f = 0.50; $[\alpha]_D^{25}$ = −28.4 (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.50−7.27 (m, 10H), 6.84 (d, 1H, *J* = 8.8 Hz), 6.66 (d, 1H, *J* = 2.2 Hz), 6.47 (dd, 1H, *J* = 8.8 and 2.2 Hz), 5.03 (s, 2H), 4.99 (s, 2H), 4.20−3.80 (m, 4H), 3.55−3.35 (m, 1H), 3.35−3.25 (m, 1H), 2.05−1.65 (m, 4H), 1.47 (s, 9H).

*(S,S)/(S,R)-N-Boc-2-[1′-hydroxy-2′-(2,6-bis(2-methoxyethoxymethoxy)phenoxy)ethyl]pyrrolidine [(S,S)/(S,R)-**25**].* Obtained as a pale yellow oil in 97% yield after chromatography on silica gel (cyclohexane/ethyl acetate, 6:4): R_f = 0.16; ^{1}H NMR ($CDCl_3$) δ 6.95−6.85 (m, 3H), 5.29 (s, 4H), 4.27−4.23 (m, 1H), 4.12 (m, 1H), 3.92 (m, 2H), 3.86−3.82 (m, 4H), 3.78−3.72 (m, 2H), 3.57−3.54 (m, 4H), 3.36 (s, 6H), 2.00−1.9 (m, 2H), 1.87−1.79 (m, 2H), 1.42 (s, 9H).

*(S,S)-N-Boc-2-[1′-hydroxy-2′-(2-benzyloxy-5-phenylphenoxy)-ethyl]pyrrolidine [(S,S)-**30b**].* Obtained as a white solid in 33% yield after chromatography on silica gel (dichloromethane/ethyl acetate, 95:5): mp = 133.09 °C; R_f = 0.20; $[\alpha]_D^{25}$ = −16.8 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.55−7.29 (m, 10H), 7.19 (d, 1H, *J* = 1.9 Hz), 7.15 (dd, 1H, *J* = 1.9 and 8.3 Hz), 7.00 (d, 1H, *J* = 8.3 Hz), 5.14 (s, 2H), 4.20−4.05 (m, 3H), 3.95 (m, 1H), 3.50 (m, 1H), 3.26 (m, 1H), 2.25−2.10 (m, 1H), 2.00−1.65 (m, 3H), 1.46 (s, 7.6H, rotamer), 1.32 (s, 1.4H, rotamer).

*(S,R)-N-Boc-2-[1′-hydroxy-2′-(2-benzyloxy-5-phenylphenoxy)-ethyl]pyrrolidine [(S,R)-**30b**].* Obtained as a white solid in 41% yield after chromatography on silica gel (dichloromethane/ethyl acetate, 95:5): mp = 111.74 °C; R_f = 0.32; $[\alpha]_D^{25}$ = −24.8 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.56−7.27 (m, 10H), 7.22 (d, 1H, *J* = 2.2 Hz), 7.15 (dd, 1H, *J* = 2.2 and 8.3 Hz), 7.00 (d, 1H, *J* = 8.3 Hz), 5.15 (s, 2H), 4.20−4.05 (m, 3H), 3.95 (m, 1H), 3.55 (m, 1H), 3.36 (m, 1H), 2.10−1.70 (m, 4H), 1.48 (s, 7.6H, rotamer), 1.35 (s, 1.4H, rotamer).

*(S,S)-N-Boc-2-[1′-hydroxy-2′-(2-benzyloxy-6-phenylphenoxy)-ethyl]pyrrolidine [(S,S)-**30c**].* Obtained as a pale yellow oil in 24% yield after chromatography on silica gel (toluene/ethyl acetate, 9:1): R_f = 0.33; $[\alpha]_D^{25}$ = −22.0 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.54 (d, 2H, *J* = 7.9 Hz), 7.50−7.27 (m, 8H), 7.07 (m, 1H), 7.02−6.89 (m, 2H), 5.16 (s, 2H), 3.72 (m, 2H), 3.65−3.41 (m, 2H), 3.27 (m, 1H), 3.13 (m, 1H), 1.73−1.50 (m, 4H), 1.30 (s, 9H).

*(S,R)-N-Boc-2-[1′-hydroxy-2′-(2-benzyloxy-6-phenylphenoxy)-ethyl]pyrrolidine [(S,R)-**30c**].* Obtained as a colorless oil in 62% yield after chromatography on silica gel (toluene/ethyl acetate, 9:1): R_f = 0.45; $[\alpha]_D^{25}$ = −20.6 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.57−7.53 (m, 2H), 7.48 (m, 2H), 7.45−7.29 (m, 6H), 7.09 (dd, 1H, *J* = 7.3 and 8.5 Hz), 6.94 (m, 2H), 5.15 (s, 2H), 3.83 (m, 2H), 3.61 (m, 2H), 3.31 (m, 1H), 3.12 (m, 1H), 1.95−1.55 (m, 4H), 1.36 (s, 9H).

General Procedure for Synthesis of Compounds 11a−11c, 16a−16c, 21, 26, and 33. The target compounds were obtained by treating, at 0 °C, a solution of the compounds **10a−10c**, **15a−15c**, **20**, **25**, and **30c** with 1.1 mol of methanesulfonylchloride and 1.1 mol of triethylamine. The reactions were warmed at room temperature and vigorously stirred for 5 h. Afterward, the mixture was diluted with ethyl acetate and washed with a 1 M solution of HCl and then with brine. The organic phase was dried over sodium sulfate and filtered and the solvent was evaporated under vacuum. The resulting crudes were purified by flash chromatography.

*(S,S)-N-Boc-2-[1′-mesyloxy-2′-(2-benzyloxy-4-nitrophenoxy)-ethyl]pyrrolidine [(S,S)-**11a**].* Obtained as a pale yellow oil in 82% yield after chromatography on silica gel (toluene/ethyl acetate, 9:1): R_f = 0.37; $[\alpha]_D^{25}$ = +22.4 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.90 (dd, 1H, *J* = 8.8 and 1.7 Hz), 7.86 (d, 1H, *J* = 1.7 Hz), 7.50−7.35 (m, 5H), 6.90 (d, 1H, *J* = 8.8 Hz), 5.25 (m, 1H), 5.18−5.02 (m, 2H), 4.35−4.13 (m, 2H), 4.02 (m, 1H), 3.38 (m, 2H), 2.72 (s, 3H), 2.20−1.80 (m, 4H), 1.47 (s, 9H).

*(S,R)-N-Boc-2-[1′-mesyloxy-2′-(2-benzyloxy-4-nitrophenoxy)-ethyl]pyrrolidine [(S,R)-**11a**].* Obtained as a white solid in 88% yield after chromatography on silica gel (toluene/ethyl acetate, 9:1): mp = 134.38 °C; R_f = 0.32; $[\alpha]_D^{25}$ = −76.9 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.93 (dd, 1H, *J* = 8.52 and 2.5 Hz), 7.87 (d, 1H, *J* = 2.5 Hz), 7.46−7.36 (m, 5H), 6.91 (d, 1H, *J* = 8.5 Hz), 5.16−5.00 (m, 2H), 4.42 (m, 1H), 4.25−4.08 (m, 3H), 3.50−3.30 (m, 2H), 2.74 (s, 3H), 2.20−2.08 (m, 3H), 1.95−1.80 (m, 1H), (s, 9H).

*(S,S)-N-Boc-2-[1′-mesyloxy-2′-(2-benzyloxy-5-nitrophenoxy)-ethyl]pyrrolidine [(S,S)-**11b**].* Obtained as a pale yellow oil in 83% yield after chromatography on silica gel (toluene/ethyl acetate, 9:1): R_f = 0.32; $[\alpha]_D^{25}$ = +18.9 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.95 (d, 1H, *J* = 8.8 Hz), 7.73 (s, 1H), 7.41 (s, 5H), 7.02 (d, 1H, *J* = 8.8 Hz), 5.30−5.07 (m, 3H), 4.21−4.01 (m, 3H), 3.41 (m, 2H), 2.70 (s, 3H), 2.36−1.86 (m, 4H), 1.48 (s, 9H).

*(S,R)-N-Boc-2-[1′-hydroxy-2′-(2-benzyloxy-5-nitrophenoxy)-ethyl]pyrrolidine [(S,R)-**11b**].* Obtained as a pale yellow oil in 90% yield after chromatography on silica gel (toluene/ethyl acetate, 9:1): R_f = 0.28; $[\alpha]_D^{25}$ = −74.4 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.95 (m, 1H), 7.76 (m, 1H), 7.38 (m, 5H), 7.00 (m, 1H), 5.21−5.02 (m, 3H), 4.45−4.05 (m, 3H), 3.50−3.32 (m, 2H), 2.74 (s, 3H), 2.03−2.19 (m, 3H), 1.85 (m, 1H), 1.50 (s, 9H).

*(S,S)-N-Boc-2-[1′-mesyloxy-2′-(2-benzyloxy-6-nitrophenoxy)-ethyl]pyrrolidine [(S,S)-**11c**].* Obtained as a viscous pale yellow oil in 86% yield after chromatography on silica gel (toluene/ethyl acetate, 9:1): R_f = 0.31; $[\alpha]_D^{25}$ = −24.5 (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.44−7.34 (m, 6H), 7.22−7.13 (m, 2H), 5.33 (m, 1H), 5.16 (s, 2H), 4.41 (m, 1H), 4.01 (m, 1H), 3.47−3.30 (m, 3H), 2.94 (s, 3H), 2.02−1.84 (m, 3H), 1.78−1.74 (m, 1H), 1.45 (s, 9H).

*(S,R)-N-Boc-2-[1′-mesyloxy-2′-(2-benzyloxy-6-nitrophenoxy)-ethyl]pyrrolidine [(S,R)-**11c**].* Obtained as a viscous pale yellow oil in 59% yield after chromatography on silica gel (cyclohexane/ethyl acetate, 7:3): R_f = 0.38; $[\alpha]_D^{25}$ = −46.5 (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.43−7.33 (m, 6H), 7.16−7.10 (m, 2H), 5.26 (m, 1H),

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5.13 (s, 2H), 4.48−4.42 (m, 1H), 4.29 (m, 1H), 4.08 (m, 1H), 3.40 (m, 1H), 3.23 (m, 1H), 3.02 (s, 3H), 1.90 (m, 2H), 1.76 (m, 2H), 1.47 (s, 9H).

*(S,S)-N-Boc-2-[1′-mesyloxy-2′-(2-(2-methoxyetoxymethoxy)-4-nitrophenoxy)ethyl]pyrrolidine [(S,S)-**16a**].* Obtained as a viscous pale yellow oil in 91% yield after chromatography on silica gel (cyclohexane/ethyl acetate, 7:3): R_f = 0.26; $[\alpha]_D^{25}$ = −44.5 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.03 (d, 1H, *J* = 2.6 Hz), 7.94 (dd, 1H, *J* = 2.6 and 9.0 Hz), 6.91 (d, 1H, *J* = 9.0 Hz), 5.31 (s, 2H), 5.20 (m, 1H), 4.41 (m, 1H), 4.25 (m, 2H), 3.83 (m, 2H), 3.58 (m, 2H), 3.45 (m, 2H), 3.36 (s, 3H), 3.25 (s, 3H), 2.13−2.04 (m, 3H), 1.87 (m, 1H), 1.48 (s, 9H).

*(S,R)-N-Boc-2-[1′-mesyloxy-2′-(2-(2-methoxyetoxymethoxy)-4-nitrophenoxy)ethyl]pyrrolidine [(S,R)-**16a**].* Obtained as a pale yellow oil in 45% yield after chromatography on silica gel (cyclohexane/ethyl acetate, 7:3): R_f = 0.25; ^{1}H NMR ($CDCl_3$) δ 8.04 (d, 1H, *J* = 2.6 Hz), 7.94 (dd, 1H, *J* = 9.6 and 2.6 Hz), 6.92 (d, 1H, *J* = 9.6 Hz), 5.31 (s, 3H), 4.25 (m, 2H), 4.08 (m, 1H), 3.84 (m, 2H), 3.58 (m, 2H), 3.39 (m, 4H), 3.18 (s, 3H), 2.16−1.85 (m, 4H), 1.47 (s, 9H).

*(S,S)-N-Boc-2-[1′-mesyloxy-2′-(2-(2-methoxyetoxymethoxy)-5-nitrophenoxy)ethyl]pyrrolidine [(S,S)-**16b**].* Obtained as a white solid in 76% yield after chromatography on silica gel (dichloromethane/acetonitrile, 95:5): mp = 118.78 °C; R_f = 0.42; $[\alpha]_D^{25}$ = −68.9 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.90 (m, 1H), 7.75 (d, 1H, *J* = 2.6 Hz), 7.24 (m, 1H), 5.35 (s, 2H), 5.23 (m, 1H), 4.26−4.05 (m, 3H), 3.82 (m, 2H), 3.56 (m, 2H), 3.42 (m, 2H), 3.36 (s, 3H), 3.23 (s, 3H), 2.12−2.00 (m, 3H), 1.89 (m, 1H), 1.51 (s, 9H).

*(S,R)-N-Boc-2-[1′-mesyloxy-2′-(2-(2-methoxyetoxymethoxy)-5-nitrophenoxy)ethyl]pyrrolidine [(S,R)-**16b**].* Obtained as a viscous pale yellow oil in 64% yield after chromatography on silica gel (dichloromethane/acetonitrile, 95:5): R_f = 0.48; $[\alpha]_D^{25}$ = +9.2 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.84 (dd, 1H, *J* = 2.6 and 9.0 Hz), 7.78 (d, 1H, *J* = 2.6 Hz), 7.22 (d, 1H, *J* = 9.0 Hz), 5.42 (m, 1H), 5.37 (s, 2H), 4.26 (m, 2H), 4.08 (m, 1H), 3.83 (m, 2H), 3.56 (m, 2H), 3.42 (m, 2H), 3.36 (s, 3H), 3.19 (s, 3H), 2.11−1.86 (m, 4H), 1.48 (s, 9H).

*(S,S)-N-Boc-2-[1′-mesyloxy-2′-(2-(2-methoxyetoxymethoxy)-6-nitrophenoxy)ethyl]pyrrolidine [(S,S)-**16c**].* Obtained as a viscous pale yellow-ochre oil in 58% yield after chromatography on silica gel (toluene/ethyl acetate, 1:1): R_f = 0.62; $[\alpha]_D^{25}$ = −31.8 (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.41 (m, 2H), 7.15 (m, 1H), 5.35 (s, 2H), 5.20 (m, 1H), 4.39 (m, 1H), 4.26 (m, 2H), 3.87−3.84 (m, 2H), 3.57−3.54 (m, 4H), 3.36 (s, 3H), 3.10 (s, 3H), 2.06 (m, 3H), 1.84 (m, 1H), 1.45 (s, 9H).

*(S,R)-N-Boc-2-[1′-mesyloxy-2′-(2-(2-methoxyetoxymethoxy)-6-nitrophenoxy)ethyl]pyrrolidine [(S,R)-**16c**].* Obtained as a viscous pale yellow oil in 66% yield after chromatography on silica gel (toluene/ethyl acetate, 1:1): R_f = 0.45; $[\alpha]_D^{25}$ = −20.8 (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.47−7.38 (m, 2H), 7.19−7.12 (m, 1H), 5.55 (m, 1H), 5.39 (s, 2H), 4.40 (m, 1H), 4.27 (m, 1H), 4.08 (m, 1H), 3.90 (m, 2H), 3.56 (m, 4H), 3.37 (s, 3H), 3.04 (s, 3H), 2.17−2.00 (m, 3H), 1.84 (m, 1H), 1.58 (s, 2.43H, rotamer), 1.47 (6.57H, rotamer).

*(S,S)-N-Boc-2-[1′-mesyloxy-2′-(2,5-dibenzyloxyphenoxy)ethyl]-pyrrolidine [(S,S)-**21**].* Obtained as a viscous pale yellow oil in 86% yield after chromatography on silica gel (toluene/ethyl acetate, 9:1): R_f = 0.37; $[\alpha]_D^{25}$ = +5.9 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.47−7.28 (m, 10H), 6.85 (d, 1H, *J* = 8.8 Hz), 6.59 (d, 1H, *J* = 2.8 Hz), 6.50 (dd, 1H, *J* = 2.8 and 8.8 Hz), 5.40−5.10 (m, 1H), 5.05−4.87 (m, 4H), 4.20−3.93 (m, 3H), 3.60−3.25 (m, 2H), 2.86 (s, 3H), 2.20−1.75 (m, 4H), 1.52 (s, 2.2H, rotamer), 1.47 (s, 6.8H, rotamer).

*(S,R)-N-Boc-2-[1′-mesyloxy-2′-(2,5-dibenzyloxyphenoxy)ethyl]-pyrrolidine [(S,R)-**21**].* Obtained as a white solid in 84% yield after chromatography on silica gel (toluene/ethyl acetate, 9:1): mp = 129.45 °C; R_f = 0.44; $[\alpha]_D^{25}$ = −62.9 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.50−7.27 (m, 10H), 6.85 (d, 1H, *J* = 8.8 Hz), 6.65−6.45 (m, 2H), 5.30−5.07 (m, 1H), 5.03−4.87 (m, 4H), 4.35−4.00 (m, 3H), 3.65−3.27 (m, 2H), 2.87 (s, 3H), 2.20−1.95 (m, 2H), 1.95−1.75 (m, 2H), 1.52 (s, 3.3H, rotamer), 1.48 (s, 5.7, rotamer).

*(S,S)/(S,R)-N-Boc-2-[1′-hydroxy-2′-(2,6-bis(2-methoxyethoxymethoxy)phenoxy)ethyl]pyrrolidine [(S,S)/(S,R)-**26**].* Obtained as a pale yellow oil in 54% yield after chromatography on silica gel (cyclohexane/ethyl acetate, 6:4): R_f = 0.21; ^{1}H NMR ($CDCl_3$) δ 6.99−6.89 (m, 1H), 6.86−6.82 (m, 2H), 5.27 (s, 4H), 4.21−4.11 (m, 1H), 4.12−3.97 (m, 3H), 3.86−3.82 (m, 4H), 3.59−3.51 (m, 6H), 3.37 (s, 6H), 3.22 (s, 1.5H), 3.18 (s, 1.5H), 2.10−1.9 (m, 2H), 1.83−1.79 (m, 2H), 1.42 (s, 9H).

*(S,S)-N-Boc-2-[1′-mesyloxy-2′-(2-benzyloxy-6-phenylphenoxy)-ethyl]pyrrolidine [(S,S)-**33**].* Obtained as a viscous pale yellow oil in 46% yield after chromatography on silica gel (toluene/ethyl acetate, 9:1): R_f = 0.41; $[\alpha]_D^{25}$ = −44.8 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.41 (m, 10H), 7.10 (m, 1H), 6.97 (m, 2H), 5.12 (s, 2H), 5.08−4.95 (m, 1H), 4.14−4.02 (m, 1H), 3.78 (m, 2H), 3.27 (m, 1H), 3.14 (m, 1H), 2.74 (s, 1.5H, rotamer), 2.67 (s, 1.5H, rotamer), 1.94−1.73 (m, 2H), 1.73−1.55 (m, 2H), 1.44 (s, 9H).

*(S,R)-N-Boc-2-[1′-hydroxy-2′-(2-benzyloxy-6-phenylphenoxy)-ethyl]pyrrolidine [(S,R)-**33**].* Obtained as a viscous colorless oil in 42% yield after chromatography on silica gel (toluene/ethyl acetate, 9:1): R_f = 0.39; $[\alpha]_D^{25}$ = −15.1 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.58−7.29 (m, 10H), 7.14−7.05 (m, 1H), 6.97 (m, 2H), 5.12 (s, 2H), 4.86 (m, 1H), 4.14 (m, 1H), 3.92 (m, 2H), 3.50−3.01 (m, 2H), 2.77 (s, 2H, rotamer), 2.70 (s, 1H, rotamer), 2.01−1.68 (m, 4H), 1.38 (s, 9H).

General Procedure for Synthesis of Compounds 12a−12c, 22, 31, and 34. The target compounds were obtained by treating a solution of the compounds **11a−11c**, **21**, **25**, **30b**, and **33** in methanol with 5% Pd/C under a H_2 atmosphere, at room temperature, for 6 h. Afterward, the catalyst was removed by filtration on a Celite pad and methanol was evaporated under vacuum. The resulting crudes were purified by flash chromatography.

*(S,S)-N-Boc-2-[1′-mesyloxy-2′-(2-hydroxy-4-aminophenoxy)-ethyl]pyrrolidine [(S,S)-**12a**].* Obtained as an orange oil in 92% yield after chromatography on silica gel (cyclohexane/ethyl acetate, 3:7): R_f = 0.28; $[\alpha]_D^{25}$ = −21.1 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.66 (d, 1H, *J* = 8.3 Hz), 6.60−6.45 (m, 1H), 6.40−6.25 (m, 1H), 5.31 (m, 2H, exchange with D_2O), 4.20−4.05 (m, 2H), 4.05−3.90 (m, 2H), 3.65−3.25 (m, 3H, 1H, exchange with D_2O), 3.07 (s, 3H), 2.10−1.80 (m, 4H), 1.47 (s, 9H).

*(S,R)-N-Boc-2-[1′-mesyloxy-2′-(2-hydroxy-4-aminophenoxy)-ethyl]pyrrolidine [(S,R)-**12a**].* Obtained as an orange oil in 83% yield after chromatography on silica gel (cyclohexane/ethyl acetate, 3:7): R_f = 0.25; $[\alpha]_D^{25}$ = −39.3 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.65−6.50 (m, 1H), 6.26 (d, 1H, *J* = 2.5 Hz), 6.07 (dd, 1H, *J* = 8.3 and 2.5 Hz), 5.25 (bs, 1H, exchange with D_2O), 5.09 (bs, 1H, exchange with D_2O), 4.30−4.05 (m, 3H), 4.00−3.85 (m, 1H), 3.60−3.35 (m, 2H, 1H, exchange with D_2O), 3.35−3.20 (m, 1H), 3.03 (s, 3H), 2.11−1.77 (m, 4H), 1.41 (s, 9H).

*(S,S)-N-Boc-2-[1′-mesyloxy-2′-(2-hydroxy-5-aminophenoxy)-ethyl]pyrrolidine [(S,S)-**12b**].* Obtained as an orange oil in 88% yield after chromatography on silica gel (cyclohexane/ethyl acetate, 3:7): R_f = 0.31; $[\alpha]_D^{25}$ = +4.6 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.74 (d, 1H, *J* = 7.4 Hz), 6.45 (m, 2H), 5.39 (m, 1H), 4.25−3.96 (m, 3H), 3.38−3.45 (m, 2H), 3.10 (s, 3H), 1.91 (m, 4H), 1.49 (s, 9H).

*(S,R)-N-Boc-2-[1′-hydroxy-2′-(2-hydroxy-5-aminophenoxy)-ethyl]pyrrolidine [(S,R)-**12b**].* Obtained as an orange oil in 82% yield after chromatography on silica gel (cyclohexane/ethyl acetate, 3:7): R_f = 0.35; $[\alpha]_D^{25}$ = +10.0 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.74 (d, 1H, *J* = 7.4 Hz), 6.25 (m, 2H), 6.25 (bs, 1H, exchange with D_2O), 5.35 (bs, 2H, exchange with D_2O), 5.19 (m, 1H), 4.25 (m, 2H), 4.05 (m, 1H), 3.59−3.38 (m, 2H), 3.10 (s, 3H), 2.19−1.91 (m, 4H), 1.49 (s, 9H).

*(S,S)-N-Boc-2-[1′-mesyloxy-2′-(2-hydroxy-6-aminophenoxy)-ethyl]pyrrolidine [(S,S)-**12c**].* Obtained as a pale yellow oil in 73% yield after chromatography on silica gel (cyclohexane/ethyl acetate, 1:1); R_f = 0.52; $[\alpha]_D^{25}$ = −37.4 (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) δ 6.80 (t, 1H, *J* = 8.0 Hz), 6.38 (dd, 1H, *J* = 1.7 and 8.0 Hz), 6.28 (dd, 1H, *J* = 1.7 and 8.0 Hz), 4.95 (m, 1H), 4.45 (m, 1H), 4.07 (m, 2H), 3.43 (m, 1H), 3.28 (m, 1H), 3.15 (s, 3H), 2.10 (m, 1H), 1.99−1.85 (m, 3H), 1.45 (s, 9H).

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*(S,R)-N-Boc-2-[1′-mesyloxy-2′-(2-hydroxy-6-aminophenoxy)-ethyl]pyrrolidine [(S,R)-**12c**].* Obtained as a viscous pale yellow oil in 82% yield after chromatography on silica gel (cyclohexane/ethyl acetate, 1:1): R_f = 0.58; $[\alpha]_D^{25}$ = −34.4 (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) δ 6.80 (t, 1H, *J* = 8.0 Hz), 6.38 (dd, 1H, *J* = 8.0 and 1.7 Hz), 6.30 (dd, 1H, *J* = 8.0 and 1.7 Hz), 5.09 (m, 1H), 4.48 (m, 1H), 4.15−4.00 (m, 2H), 3.50 (m, 1H), 3.34 (m, 1H), 3.12 (s, 3H), 2.20−2.05 (m, 1H), 2.04−1.85 (m, 3H), 1.48 (s, 9H).

*(S,S)-N-Boc-2-[1′-mesyloxy-2′-(2,5-dihydroxyphenoxy)ethyl]-pyrrolidine [(S,S)-**22**].* Obtained as a viscous pale yellow oil in 90% yield after chromatography on silica gel (cyclohexane/ethyl acetate, 1:1): R_f = 0.29; $[\alpha]_D^{25}$ = −23.5 (*c* 0.5, MeOH); ^{1}H NMR (DMSO-d_6, 100 °C) δ 8.39 (bs, 1H, exchange with D_2O), 7.83 (bs, 1H, exchange with D_2O), 6.62 (dd, 1H, *J* = 3.0 and 8.5 Hz), 6.43 (m, 1H), 6.27 (m, 1H), 5.15 (m, 1H), 4.20−4.00 (m, 4H), 3.30 (m, 1H), 3.25−3.09 (m, 1H), 3.18 (s, 3H), 2.05−1.75 (m, 4H), 1.42 (s, 9H).

*(S,R)-N-Boc-2-[1′-mesyloxy-2′-(2,5-dihydroxyphenoxy)ethyl]-pyrrolidine [(S,R)-**22**].* Obtained as a light yellow solid in 62% yield after chromatography on silica gel (cyclohexane/ethyl acetate, 1:1): mp = 153.26 °C; R_f = 0.31; $[\alpha]_D^{25}$ = −59.5 (*c* 0.5, MeOH); ^{1}H NMR (DMSO-d_6, 100 °C) δ 8.42 (bs, 1H, exchange with D_2O), 7.84 (bs, 1H, exchange with D_2O), 6.61 (dd, 1H, *J* = 1.7 and 8.5 Hz), 6.42 (m, 1H), 6.27 (m, 1H), 5.12 (m, 1H), 4.23−4.10 (m, 2H), 4.00 (m, 1H), 3.50−3.37 (m, 2H), 3.30−3.17 (m, 3H), 2.10−1.70 (m, 4H), 1.41 (s, 9H).

*(S,S)-N-Boc-2-[1′-hydroxy-2′-(2-hydroxy-5-phenylphenoxy)-ethyl]pyrrolidine [(S,S)-**31**].* Obtained as a spongy solid in 98% yield after chromatography on silica gel (toluene/ethyl acetate, 8:2): R_f = 0.19; $[\alpha]_D^{25}$ = +4.6 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.54 (m, 2H), 7.40 (m, 2H), 7.29 (m, 1H), 7.16 (m, 2H), 7.00 (d, 1H, *J* = 7.9 Hz), 4.18−4.04 (m, 3H), 3.95 (m, 1H), 3.52 (m, 1H), 3.27 (m, 1H), 2.15−1.75 (m, 4H), 1.47 (s, 9H).

*(S,R)-N-Boc-2-[1′-hydroxy-2′-(2-hydroxy-5-phenylphenoxy)-ethyl]pyrrolidine [(S,R)-**31**].* Obtained as a white solid in quantitative yield after chromatography on silica gel (toluene/ethyl acetate, 8:2): mp = 137.79 °C; R_f = 0.25; $[\alpha]_D^{25}$ = −17.4 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.55 (m, 2H), 7.43(m, 2H), 7.28 (m, 1H), 7.20 (m, 2H), 7.01 (d, 1H, *J* = 8.5 Hz), 4.17−4.00 (m, 3H), 3.87 (m, 1H), 3.53 (m, 1H), 3.32 (m, 1H), 2.10 (m, 1H), 1.95−1.65 (m, 3H), 1.49 (s, 9H).

*(S,S)-N-Boc-2-[1′-mesyloxy-2′-(2-hydroxy-6-phenylphenoxy)-ethyl]pyrrolidine [(S,S)-**34**].* Obtained partially pure as a viscous oil after chromatography on silica gel (toluene/ethyl acetate, 9:1): R_f = 0.13.

*(S,R)-N-Boc-2-[1′-hydroxy-2′-(2-hydroxy-6-phenylphenoxy)-ethyl]pyrrolidine [(S,R)-**34**].* Obtained partially pure as a viscous oil after chromatography on silica gel (toluene/ethyl acetate, 9:1): R_f = 0.37.

General Procedure for Synthesis of Compounds 13a−13c, 18a−18c, 23, 28, and 32c. The target compounds were obtained by treating with K_2CO_3 (2 mol) a solution of compounds **12a−12c** and **34** in dimethoxyethane or a solution of compounds **17a−17c**, **22**, and **27** in dimethoxyethane/dimethylformamide (1:1). The resulting mixture was heated at 85 °C under vigorous stirring for 6 h. Afterward, the mixture was diluted with ethyl acetate and washed with a 1 M solution of HCl and then with brine. The organic phase was dried over sodium sulfate and filtered and the solvent was evaporated under vacuum. The resulting crudes were purified by flash chromatography.

*(S,S)-2-(N-tert-Butoxycarbonyl-2′-pyrrolidinyl)-7-amino-1,4-benzodioxane [(S,S)-**13a**].* Obtained as a light yellow oil in 75% yield after chromatography on silica gel (cyclohexane/ethyl acetate, 1:1): R_f = 0.43; $[\alpha]_D^{25}$ = −72.5 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.68 (d, 1H, *J* = 8.5 Hz), 6.25 (d, 1H, *J* = 2.8 Hz), 6.20 (dd, 1H, *J* = 8.5 and 2.8 Hz), 4.23 (dd, 1H, *J* = 11.3 and 1.9 Hz), 4.20−3.80 (m, 3H), 3.55−3.30 (m, 4H, 2H, exchange with D_2O), 2.22 (m, 1H), 2.05−1.80 (m, 3H), 1.47 (s, 9H).

*(S,R)-2-(N-tert-Butoxycarbonyl-2′-pyrrolidinyl)-7-amino-1,4-benzodioxane [(S,R)-**13a**].* Obtained as a light yellow oil in 89% yield after chromatography on silica gel (cyclohexane/ethyl acetate, 1:1): R_f = 0.38; $[\alpha]_D^{25}$ = −44.1 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.66 (d, 1H, *J* = 8.3 Hz), 6.24 (d, 1H, *J* = 2.5 Hz), 6.19 (dd, 1H, *J* = 8.3 and 2.5 Hz), 4.38−4.11 (m, 3H), 3.92−3.77 (m, 1H), 3.66−3.20 (m, 4H, 2H, exchange with D_2O), 2.20−1.95 (m, 3H), 1.95−1.80 (m, 1H), 1.45 (s, 9H).

*(S,S)-2-(N-tert-Butoxycarbonyl-2′-pyrrolidinyl)-6-amino-1,4-benzodioxane [(S,S)-**13b**].* Obtained as a light yellow oil in 63% yield after chromatography on silica gel (cyclohexane/ethyl acetate, 1:1): R_f = 0.40; $[\alpha]_D^{25}$ = −33.7 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.67 (d, 1H, *J* = 8.4 Hz), 6.22 (m, 2H), 4.27 (d, 1H, *J* = 9.6 Hz), 4.08−3.80 (m, 3H), 3.55−3.32 (m, 2H), 2.21 (m, 1H), 2.05−1.90 (m, 3H), 1.47 (s, 9H).

*(S,R)-2-(N-tert-Butoxycarbonyl-2′-pyrrolidinyl)-6-amino-1,4-benzodioxane [(S,R)-**13b**].* Obtained as a light yellow oil in 76% yield after chromatography on silica gel (cyclohexane/ethyl acetate, 1:1): R_f = 0.39; $[\alpha]_D^{25}$ = −19.2 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.66 (d, 1H, *J* = 8.5 Hz), 6.22 (m, 2H), 4.36−4.09 (m, 3H), 3.92 (m, 1H), 3.53−3.31 (m, 2H), 2.08 (m, 3H), 1.95−1.80 (m, 1H), 1.45 (s, 9H).

*(S,S)-2-(N-tert-Butoxycarbonyl-2′-pyrrolidinyl)-5-amino-1,4-benzodioxane [(S,S)-**13c**].* Obtained as a light yellow oil in 84% yield after chromatography on silica gel (cyclohexane/ethyl acetate, 1:1): R_f = 0.78; $[\alpha]_D^{25}$ = −146.9 (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) δ 6.65 (t, 1H, *J* = 8.0 Hz), 6.36 (dd, 1H, *J* = 1.4 and 8.0 Hz), 6.33 (dd, 1H, *J* = 1.4 and 8.0 Hz), 4.36 (m, 1H), 4.08 (m, 1H), 3.97 (m, 2H), 3.49 (m, 1H), 3.38 (m, 1H), 2.21 (m, 1H), 2.02−1.91 (m, 3H), 1.48 (s, 9H).

*(S,R)-2-(N-tert-Butoxycarbonyl-2′-pyrrolidinyl)-5-amino-1,4-benzodioxane [(S,R)-**13c**].* Obtained as a light yellow oil in 78% yield after chromatography on silica gel (cyclohexane/ethyl acetate, 1:1): R_f = 0.73; $[\alpha]_D^{25}$ = −7.8 (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) δ 6.69 (t, 1H, *J* = 8.0 Hz), 6.53 (dd, 1H, *J* = 1.7 and 8.0 Hz), 6.44 (dd, 1H, *J* = 1.7 and 8.0 Hz), 4.46−4.37 (m, 1H), 4.29−4.21 (m, 2H), 3.93 (m, 1H), 3.55−3.34 (m, 2H), 2.05 (m, 3H), 1.89 (m, 1H), 1.46 (s, 9H).

*(S,S)-2-(2′-Pyrrolidinyl)-7-nitro-1,4-benzodioxane [(S,S)-**18a**].* Obtained as a light yellow oil in 55% yield after chromatography on silica gel [dichloromethane/methanol (95:5) + 0.20% NH_3]: R_f = 0.17; $[\alpha]_D^{25}$ = −78.5 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.29 (d, 1H, *J* = 2.4 Hz), 7.75 (dd, 1H, *J* = 2.4 and 8.8 Hz), 6.92 (d, 1H, *J* = 8.8 Hz), 4.46 (dd, 1H, *J* = 2.2 and 11.4 Hz), 4.13 (dd, 1H, *J* = 7.4 and 11.4 Hz), 3.96 (td, 1H, *J* = 7.4 and 2.2 Hz), 3.35−3.28 (m, 1H), 3.00−2.87 (m, 2H), 2.04−1.75 (m, 4H).

*(S,R)-2-(2′-Pyrrolidinyl)-7-nitro-1,4-benzodioxane [(S,R)-**18a**].* Obtained as a light yellow oil in 47% yield after chromatography on silica gel [dichloromethane/methanol (95:5) + 0.20% NH_3]: R_f = 0.14; $[\alpha]_D^{25}$ = +120.7 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.83 (d, 1H, *J* = 2.6 Hz), 7.76 (dd, 1H, *J* = 2.6 and 7.9 Hz), 6.92 (d, 1H, *J* = 7.9 Hz), 4.39 (m, 1H), 4.15 (m, 2H), 3.33 (m, 1H), 3.05 (m, 1H), 3.00 (m, 1H), 1.99−1.62 (m, 4H).

*(S,S)-2-(2′-Pyrrolidinyl)-6-nitro-1,4-benzodioxane [(S,S)-**18b**].* Obtained as a light yellow oil in 67% yield after chromatography on silica gel [dichloromethane/methanol (95:5) + 0.20% NH_3]: R_f = 0.27; $[\alpha]_D^{25}$ = −117.3 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.79 (m, 2H), 6.95 (m, 1H), 4.41 (dd, 1H, *J* = 2.2 and 11.4 Hz), 4.10 (dd, 1H, *J* = 7.0 and 11.4 Hz), 4.01 (td, 1H, *J* = 2.2 and 7.0 Hz), 3.32 (m, 1H), 2.99 (m, 2H), 2.01−1.92 (m, 1H), 1.89−1.73 (m, 3H).

*(S,R)-2-(2′-Pyrrolidinyl)-6-nitro-1,4-benzodioxane [(S,R)-**18b**].* Obtained as a light yellow oil in 49% yield after chromatography on silica gel [dichloromethane/methanol (95:5) + 0.20% NH_3]: R_f = 0.29; $[\alpha]_D^{25}$ = +125.7 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.80 (m, 2H), 6.99 (m, 1H), 4.38 (dd, 1H, *J* = 2.2 and 11.2 Hz), 4.14 (td, 1H, *J* = 2.2 and 7.9 Hz), 4.06 (dd, 1H, *J* = 7.9 and 11.2 Hz), 3.32 (m, 1H), 3.11−2.94 (m, 2H), 2.02−1.77 (m, 3H), 1.70−1.61 (m, 1H).

*(S,S)-2-(2′-Pyrrolidinyl)-5-nitro-1,4-benzodioxane [(S,S)-**18c**].* Obtained as a light yellow oil in 80% yield after chromatography on silica gel (ethyl acetate + 5% triethylamine): R_f = 0.42; $[\alpha]_D^{25}$ = −14.7 (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.47 (dd, 1H, *J* = 1.8 and 8.2 Hz), 7.10 (dd, 1H, *J* = 1.8 and 8.2 Hz), 6.88 (t, 1H, *J* = 8.2 Hz), 4.52 (dd, 1H, *J* = 2.3 and 11.7 Hz), 4.16 (dd, 1H, *J* = 7.6 and 11.7 Hz), 4.02 (dt, 1H, *J* = 2.3 and 7.6 Hz), 3.37 (m, 1H), 2.98 (m, 2H), 2.01−1.72 (m, 4H).

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*(S,R)-2-(2′-Pyrrolidinyl)-5-nitro-1,4-benzodioxane [(S,R)-**18c**].* Obtained as a dark yellow oil in 87% yield after chromatography on silica gel (ethyl acetate + 5% triethylamine): R_f = 0.40; $[\alpha]_D^{25}$ = −100.5 (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.47 (dd, 1H, *J* = 1.6 and 8.2 Hz), 7.11 (dd, 1H, *J* = 1.6 and 8.2 Hz), 6.89 (t, 1H, *J* = 8.2 Hz), 4.54−4.50 (m, 1H), 4.20−4.04 (m, 2H), 3.35 (m, 1H), 2.53 (m, 2H), 2.04−1.78 (m, 4H).

*(S,S)-2-(N-tert-Butoxycarbonyl-2′-pyrrolidinyl)-6-hydroxy-1,4-benzodioxane [(S,S)-**23**].* Obtained as a white solid in 67% yield after chromatography on silica gel (cyclohexane/ethyl acetate, 1:1): mp = 167.82 °C; R_f = 0.62; $[\alpha]_D^{25}$ = −153.9 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.71 (d, 1H, *J* = 8.5 Hz), 6.40 (d, 1H, *J* = 2.8 Hz), 6.34 (dd, 1H, *J* = 2.8 and 8.5 Hz), 5.17 (s, 1H, exchange with D_2O), 4.41 (d, 1H, *J* = 9.4 Hz), 4.10−3.87 (m, 3H), 3.60−3.30 (m, 2H), 2.30−2.10 (m, 1H), 2.05−1.80 (m, 3H), 1.48 (s, 9H).

*(S,R)-2-(N-tert-Butoxycarbonyl-2′-pyrrolidinyl)-6-hydroxy-1,4-benzodioxane [(S,R)-**23**].* Obtained as a light yellow solid in 71% yield after chromatography on silica gel (cyclohexane/ethyl acetate, 1:1): mp = 203.12; R_f = 0.57; $[\alpha]_D^{25}$ = −26.8 (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$) δ 6.69 (d, 1H, *J* = 8.5 Hz), 6.38 (d, 1H, *J* = 2.5 Hz), 6.32 (dd, 1H, *J* = 2.5 and 8.5 Hz), 4.86 (s, 1H, exchange with D_2O), 4.40−4.10 (m, 3H), 3.98−3.82 (m, 1H), 3.64−3.30 (m, 2H), 2.20−1.95 (m, 3H), 1.95−1.80 (m, 1H), 1.46 (s, 9H).

*(S,S)/(S,R)-2-(2′-Pyrrolidinyl)-5-hydroxy-1,4-benzodioxane [(S,S)/(S,R)-**28**].* Obtained as a light yellow oil in 25% yield after chromatography on silica gel [dichloromethane/methanol (97:3) + 1.5% NH_3]: R_f = 0.20; ^{1}H NMR ($CDCl_3$) δ 6.57 (t, *J* = 8.2 Hz, 1H), 6.50−6.43 (m, 2H), 4.36−4.15 (m, 1H), 4.12−3.98 (m, 2H), 3.35−3.22 (m, 1H), 3.15−2.84 (m, 3H), 1.98−1.59 (m, 4H).

*(S,S)-2-(N-tert-Butoxycarbonyl-2′-pyrrolidinyl)-5-phenyl-1,4-benzodioxane [(S,S)-**32c**].* Obtained as an oil in 49% yield after chromatography on silica gel (toluene/ethyl acetate, 9:1): R_f = 0.44; $[\alpha]_D^{25}$ = −88.37 (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.53 (d, 2H, *J* = 7.2 Hz), 7.41 (t, 2H, *J* = 7.2 Hz), 7.30 (m, 1H), 6.90 (m, 3H), 4.35 (dd, 1H, *J* = 1.7 and 11.4 Hz), 4.12 (m, 1H), 4.03−3.86 (m, 2H), 3.43−3.31 (m, 2H), 2.31−2.21 (m, 1H), 2.02−1.85 (m, 3H), 1.45 (s, 9H).

*(S,R)-2-(N-tert-Butoxycarbonyl-2′-pyrrolidinyl)-5-phenyl-1,4-benzodioxane [(S,R)-**32c**].* Obtained as a light brown oil in 34% yield after chromatography on silica gel (toluene/ethyl acetate, 9:1): R_f = 0.50; $[\alpha]_D^{25}$ = +5.86 (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.56 (m, 2H), 7.41 (t, 2H, *J* = 7.3 Hz), 7.34 (m, 1H), 6.92−6.86 (m, 3H), 4.27 (m, 3H), 3.91 (m, 1H), 3.63−3.29 (m, 2H), 2.18−2.03 (m, 3H), 1.97−1.83 (m, 1H), 1.44 (s, 9H).

General Procedure for Synthesis of Compounds 17a−17c and 27. The target compounds were obtained by treating an ice-cooled methanol solution of the compounds **16a−16c** and **26** with a 1.25 M solution of HCl in methanol (10 mol). The resulting mixture was warmed to room temperature and vigorously stirred for 2 h. Afterward, the solvent was evaporated and the residue was diluted with a saturated aqueous solution of sodium bicarbonate and extracted with ethyl acetate. The organic phase was dried over sodium sulfate and filtered and the solvent was evaporated under vacuum. The resulting crude products were used directly for the following reaction.

Synthesis of Compounds 32b. The target compounds were obtained using a Mitsunobu protocol. A solution of **31**, PPh_3 (1.2 mol), and DIAD (1.2 mol) in anhydrous THF was heated under microwave irradiation at 130 °C (150 W) for 10 min. Afterward, the solvent was evaporated and the resulting crudes were purified by flash chromatography.

*(S,S)-2-(N-tert-Butoxycarbonyl-2′-pyrrolidinyl)-6-phenyl-1,4-benzodioxane [(S,S)-**32b**].* Obtained as a white solid in 53% yield after chromatography on silica gel (cyclohexane/ethyl acetate, 9:1): mp = 100.40 °C; R_f = 0.55; $[\alpha]_D^{25}$ = −160.4 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.54 (m, 2H), 7.40 (m, 2H), 7.31 (m, 1H), 7.11 (m, 2H), 6.93 (d, 1H, *J* = 8.3 Hz), 4.36 (dd, 1H, *J* = 1.7 and 11.0 Hz), 4.24−3.92 (m, 3H), 3.60−3.34 (m, 2H), 2.30 (m, 1H), 2.10−1.85 (m, 3H), 1.49 (s, 9H).

*(S,R)-2-(N-tert-Butoxycarbonyl-2′-pyrrolidinyl)-6-phenyl-1,4-benzodioxane [(S,R)-**32b**].* Obtained as a white solid in 80% yield after chromatography on silica gel (cyclohexane/ethyl acetate, 9:1): mp = 166.46 °C; R_f = 0.70; $[\alpha]_D^{25}$ = +2.1 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.52 (m, 2H), 7.40 (m, 2H), 7.28 (m, 1H), 7.11 (m,2H), 6.92 (d, 1H, *J* = 8.3 Hz), 4.49−4.18 (m, 3H), 4.03−3.90 (m, 1H), 3.66−3.33 (m, 2H), 2.25−2.00 (m, 3H), 1.97−1.83 (m, 1H), 1.46 (s, 9H).

General Procedure for Synthesis of Compounds 1a−1c, 3b, 4b, and 4c. The target compounds were obtained by adding dropwise a solution of the compounds **13a−13c**, **23**, **32b**, and **32c** in anhydrous tetrahydrofuran to an ice-cooled suspension of $LiAlH_4$ (3 mol) in anhydrous tetrahydrofuran. The resulting mixture was warmed to room temperature and refluxed, under vigorous stirring, for 4 h. Afterward, the mixture was diluted with dichloromethane and the excess of $LiAlH_4$ was quenched by slowly adding water dropwise at 0 °C. The resulting suspension was filtered through a Celite pad and the solvent was evaporated under vacuum. The resulting crudes were purified by flash chromatography.

*(S,S)-2-(N-Methyl-2′-pyrrolidinyl)-7-amino-1,4-benzodioxane [(S,S)-**1a**].* Obtained as a light yellow oil in 47% yield after chromatography on silica gel [dichloromethane/methanol (95:5) + 0.5% NH_3]: R_f = 0.5; $[\alpha]_D^{25}$ = −69.1 (*c* 0.2, MeOH); ^{1}H NMR ($CDCl_3$) δ 6.68 (d, 1H, *J* = 8.5 Hz), 6.33 (d, 1H, *J* = 2.5 Hz), 6.19 (dd, 1H, *J* = 8.5 and 2.5 Hz), 4.20 (dd, 1H, *J* = 11.0 and 1.9 Hz), 4.09 (m, 1H), 3.94 (dd, 1H, *J* = 11.0 and 7.9 Hz), 3.39 (bs, 2H, exchange with D_2O), 3.12 (m, 1H), 2.53−2.45 (m, 1H), 2.42 (s, 3H), 2.35−2.15 (m, 1H), 2.00−1.70 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 144.27, 141.05, 136.65, 117.48, 108.56, 104.63, 74.42, 66.10, 65.54, 57.89, 42.24, 26.56, 23.58.

*(S,R)-2-(N-Methyl-2′-pyrrolidinyl)-7-amino-1,4-benzodioxane [(S,R)-**1a**].* Obtained as a light yellow solid in 56% yield after chromatography on silica gel [dichloromethane/methanol (95:5) + 0.5% NH_3]: R_f = 0.39; $[\alpha]_D^{25}$ = +21.5 (*c* 0.2, MeOH); mp = 113.84 °C; ^{1}H NMR ($CDCl_3$) δ 6.67 (d, 1H, *J* = 8.5 Hz), 6.28 (d, 1H, *J* = 2.5 Hz), 6.20 (dd, 1H, *J* = 8.5 and 2.5 Hz), 4.22 (d, 1H, *J* = 11.3 Hz), 4.12 (t, 1H, *J* = 7.2 Hz), 3.93 (dd, 1H, *J* = 11.3 and 7.2 Hz), 3.39 (bs, 2H, exchange with D_2O), 3.15−3.05 (m, 1H), 2.68−2.56 (m, 1H), 2.49 (s, 3H), 2.35−2.22 (m, 1H), 2.02−1.84 (m, 1H), 1.84−1.56 (m, 3H). ^{13}C NMR ($CDCl_3$) δ 143.85, 141.09, 136.57, 117.45, 108.65, 104.48, 76.98, 65.74, 64.95, 58.21, 42.97, 27.56, 23.66.

*(S,S)-2-(N-Methyl-2′-pyrrolidinyl)-6-amino-1,4-benzodioxane [(S,S)-**1b**].* Obtained as a light yellow oil in 90% yield after chromatography on silica gel [dichloromethane/methanol (95:5) + 0.5% NH_3]: R_f = 0.46; $[\alpha]_D^{25}$ = −38.4 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.75 (d, 1H, *J* = 8.5 Hz), 6.25 (d, 1H, *J* = 2.8 Hz), 6.21 (dd, 1H, *J* = 2.8 and 8.5 Hz), 4.23 (dd, 1H, *J* = 1.7 and 10.7 Hz), 4.04 (ddd, 1H, *J* = 1.7, 5.2, and 8.3 Hz), 3.97 (dd, 1H, *J* = 8.3 and 10.7 Hz), 3.38 (bs, 2H, exchange with D_2O), 3.15 (m, 1H), 2.51 (m, 1H), 2.42 (s, 3H), 2.19 (m, 1H), 1.93−1.72 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 144.02, 140.72, 136.69, 118.07, 109.00, 104.16, 73.66, 66.55, 65.59, 57.90, 42.15, 26.52, 23.53.

*(S,R)-2-(N-Methyl-2′-pyrrolidinyl)-6-amino-1,4-benzodioxane [(S,R)-**1b**].* Obtained as a light yellow oil in 72% yield after chromatography on silica gel [dichloromethane/methanol (95:5) + 0.5% NH_3]: R_f = 0.61; $[\alpha]_D^{25}$ = +9.0 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.69 (d, 1H, *J* = 8.4 Hz), 6.24 (d, 1H, *J* = 2.7 Hz), 6.20 (dd, 1H, *J* = 2.7 and 8.4 Hz), 4.25 (dd, *J* = 1.9, 10.9 Hz, 1H), 4.07 (t, 1H, *J* = 7.3 Hz), 3.96 (dd, 1H, *J* = 7.3 and 10.9 Hz), 3.39 (bs, 2H, exchange with D_2O), 3.10 (m, 1H), 2.57 (m, 1H), 2.49 (s, 3H), 2.32−2.17 (m, 1H), 1.97−1.61 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 144.01, 140.74, 136.39, 117.94, 109.01, 104.13, 76.21, 66.19, 65.01, 58.19, 42.92, 27.48, 23.67.

*(S,S)-2-(N-Methyl-2′-pyrrolidinyl)-5-amino-1,4-benzodioxane [(S,S)-**1c**].* Obtained as a beige solid in 84% yield after chromatography on silica gel [dichloromethane/methanol (95:5) + 0.5% $NH_{3(30\%\ in\ water)}$]: mp = 104.45 °C; R_f = 0.25; $[\alpha]_D^{25}$ = −127.8 (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) δ 6.64 (t, 1H, *J* = 8.1 Hz), 6.39 (dd, 1H, *J* = 1.5 and 8.1 Hz), 6.30 (dd, 1H, *J* = 1.5 and 8.1 Hz), 4.32 (dd, 1H *J* = 1.8 and 10.8 Hz), 4.10 (ddd, 1H, *J* = 8.0, 4.2 and 1.8 Hz), 4.02 (dd, 1H, *J* = 8.0 and 10.8 Hz), 3.72 (bs, 2H, exchange with

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D_2O), 3.15 (m, 1H), 2.54−2.48 (m, 1H), 2.43 (s, 3H), 2.30 (m, 1H), 1.94−1.74 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 143.82, 135.94, 131.53, 120.93, 107.65, 107.50, 74.07, 66.00, 65.29, 57.70, 42.10, 26.46, 23.37.

*(S,R)-2-(N-Methyl-2′-pyrrolidinyl)-5-amino-1,4-benzodioxane [(S,R)-**1c**].* Obtained as a beige solid in quantitative yield after chromatography on silica gel [dichloromethane/methanol (95:5) + 0.5% NH_3]: mp = 59.1 °C; R_f = 0.23; $[\alpha]_D^{25}$ = +26.9 (*c* 1, MeOH); ^1H NMR ($CDCl_3$) δ 6.64 (t, 1H, *J* = 7.8 Hz), 6.34 (dd, 1H, *J* = 1.5 and 7.8 Hz), 6.30 (dd, 1H, *J* = 1.5 and 7.8 Hz), 4.34 (dd, 1H, *J* = 2.1 and 11.1 Hz), 4.13 (dt, 1H, *J* = 2.1 and 7.3 Hz), 4.00 (dd, 1 H, *J* = 11.1 and 7.3 Hz), 3.72 (bs, 2H, exchange with D_2O), 3.12 (m, 1H), 2.60 (m, 1H), 2.48 (s, 3H), 2.27 (q, *J* = 8.6 Hz, 1H), 2.00−1.89 (m, 1H), 1.81−1.61 (m, 3H). ^{13}C NMR ($CDCl_3$) δ 143.50, 135.88, 131.48, 120.95, 107.74, 107.35, 76.29, 65.62, 64.74, 57.93, 42.65, 27.20, 23.44.

*(S,S)-2-(N-Methyl-2′-pyrrolidinyl)-6-hydroxy-1,4-benzodioxane [(S,S)-**3b**].* Obtained as a white solid in 88% yield after chromatography on silica gel [dichloromethane/methanol (95:5) + 0.5% NH_3]: mp 126.85 °C; R_f = 0.31; $[\alpha]_D^{25}$ = −108.0 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 6.75 (d, 1H, *J* = 8.8 Hz), 6.39 (d, 1H, *J* = 2.8 Hz), 6.31 (dd, 1H, *J* = 2.8 and 8.8 Hz), 4.24 (dd, 1H, *J* = 1.9 and 11.0 Hz), 4.08 (ddd, 1H, *J* = 1.9, 4.1 and 8.3 Hz), 3.97 (dd, 1H, *J* = 11.0 and 8.3 Hz), 3.14 (m, 1H), 2.50 (m, 1H), 2.43 (s, 3H), 2.28 (m, 1H), 2.00−1.70 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 150.69, 143.80, 137.39, 117.92, 108.92, 104.39, 72.95, 66.53, 65.81, 57.72, 41.99, 26.08, 23.28.

*(S,R)-2-(N-Methyl-2′-pyrrolidinyl)-6-hydroxy-1,4-benzodioxane [(S,R)-**3b**].* Obtained as a white solid in 86% yield after chromatography on silica gel [dichloromethane/methanol (95:5) + 0.5% NH_3]: mp = 166.40 °C; R_f = 0.20; $[\alpha]_D^{25}$ = +12.6 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 6.71 (d, 1H, *J* = 8.5 Hz), 6.37 (d, 1H, *J* = 2.8 Hz), 6.32 (dd, 1H, *J* = 2.8 and 8.5 Hz), 4.25 (dd, 1H, *J* = 1.9 and 11.0 Hz), 4.06 (dt, 1H, *J* = 1.9 and 7.2 Hz), 3.96 (dd, 1H, *J* = 7.2 and 11.0 Hz), 3.15 (m, 1H), 2.62 (m, 1H), 2.52 (s, 3H), 2.35 (m, 1H), 2.07−1.85 (m, 1H), 1.85−1.60 (m, 3H). ^{13}C NMR ($CDCl_3$) δ 150.69, 143.81, 136.96, 117.74, 109.09, 104.61, 76.34, 66.28, 65.12, 58.18, 43.21, 27.64, 23.30.

*(S,S)-2-(N-Methyl-2′-pyrrolidinyl)-6-phenyl-1,4-benzodioxane [(S,S)-**4b**].* Obtained as a white solid in 93% yield after chromatography on silica gel [dichloromethane/methanol (95:5) + 0.5% NH_3]: mp = 87.49 °C; R_f = 0.65; $[\alpha]_D^{25}$ = −132.4 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.54 (m, 2H), 7.40 (m, 2H), 7.29 (m, 1H), 7.11 (m, 2H), 7.02 (d, 1H, *J* = 8.3 Hz), 4.32 (dd, 1H, *J* = 1.9 and 11.0 Hz), 4.17 (ddd, 1H, *J* = 1.9, 4.1 and 7.9 Hz), 4.06 (dd, 1H, *J* = 7.9 and 11.0 Hz), 3.15 (m, 1H), 2.56 (m, 1H), 2.46 (s, 3H), 2.29 (m, 1H), 2.05−1.75 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 143.88, 143.48, 140.88, 134.73, 128.92, 127.01, 126.98, 120.43, 117.99, 115.79, 74.36, 66.36, 65.52, 57.93, 42.30, 26.67, 23.64.

*(S,R)-2-(N-Methyl-2′-pyrrolidinyl)-6-phenyl-1,4-benzodioxane [(S,R)-**4b**].* Obtained as a white solid in 94% yield after chromatography on silica gel [dichloromethane/methanol (95:5) + 0.5% NH_3]: mp = 95.77 °C; R_f = 0.21; $[\alpha]_D^{25}$ = +29.6 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.53 (m, 2H), 7.40 (m, 2H), 7.29 (m, 1H), 7.11 (m, 2H), 6.95 (d, 1H, *J* = 8.3 Hz), 4.34 (dd, 1H, *J* = 2.2 and 11.0 Hz), 4.22 (dt, 1H, *J* = 2.2 and 7.2 Hz), 4.04 (dd, 1H, *J* = 7.2 and 11.0 Hz), 3.19 (m, 1H), 2.75 (m, 1H), 2.52 (s, 3H), 2.32 (m, 1H), 2.08−1.65 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 143.8, 143.07, 140.84, 134.79, 128.91, 127.02, 126.96, 120.47, 117.83, 115.74, 76.60, 66.00, 65.12, 58.16, 42.91, 27.52, 23.67.

*(S,S)-2-(N-Methyl-2′-pyrrolidinyl)-5-phenyl-1,4-benzodioxane [(S,S)-**4c**].* Obtained as a light yellow oil in 85% yield after chromatography on silica gel [toluene/ethyl acetate (9:1) + 0.5% TEA]: R_f = 0.30; $[\alpha]_D^{25}$ = −64.7 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.53 (m, 2H), 7.45 (m, 2H), 7.32 (t, 1H, *J* = 7.3 Hz), 6.97 (dd, 1H, *J* = 4.2 and 5.7 Hz), 6.90 (d, 1H, *J* = 5.7 Hz), 6.89 (d, 1H, *J* = 4.2 Hz), 4.30 (dd, 1H, *J* = 2.2 and 11.2 Hz), 4.18 (ddd, 1H, *J* = 2.2, 4.1 and 8.0 Hz), 3.99 (dd, 1H, *J* = 8.0 and 11.2 Hz), 3.13 (m, 1H), 2.49 (m, 1H), 2.45 (s, 3H), 2.26 (q, 1H, *J* = 8.4 Hz), 1.99−1.70 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 143.89, 140.58, 137.69, 130.67, 129.83, 129.40, 128.01, 127.11, 122.57, 121.09, 116.81, 73.45, 65.99, 65.28, 57.64, 41.86, 29.71, 26.26, 23.33.

*(S,R)-2-(N-Methyl-2′-pyrrolidinyl)-5-phenyl-1,4-benzodioxane [(S,R)-**4c**].* Obtained as a light yellow oil in 85% yield after chromatography on silica gel [toluene/ethyl acetate (9:1) + 0.5% TEA]: R_f = 0.26; $[\alpha]_D^{25}$ = +14.1 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.55 (d, *J* = 7.3 Hz, 2H), 7.42 (t, *J* = 7.3 Hz, 2H), 7.33 (t, *J* = 7.3 Hz, 1H), 6.96−6.87 (m, 3H), 4.32 (dd, *J* = 11.2, 1.8 Hz, 1H), 4.22 (td, *J* = 7.2, 1.8 Hz, 1H), 4.00 (dd, *J* = 11.2, 7.2 Hz, 1H), 3.21−3.07 (m, 1H), 2.68 (m, 1H), 2.54 (s, 3H), 2.32 (m, 1H), 2.05−1.88 (m, 1H), 1.88−1.59 (m, 3H). ^{13}C NMR ($CDCl_3$) δ 143.58, 140.56, 137.66, 130.57, 129.83, 129.41, 128.02, 127.11, 125.53, 122.58, 121.09, 116.67, 76.12, 65.70, 64.88, 57.94, 42.72, 30.31, 29.71, 27.30, 23.42.

General Procedure for Synthesis of Compounds 2a−2c and 3c. The target compounds were obtained by treatment of a solution of the compounds **18a−18c** and **28** in CPME with Aquivion-Fe (5 mol %) and 37 wt % aqueous formaldehyde (10 mol). The resulting mixture was vigorously stirred at 40 °C for 4 h. After cooling down to 0 °C, $NaBH_4$ (1 mol) and MeOH were added and the mixture was stirred for 30 min. Afterward, water was added and the suspension was filtered to remove the Aquivion-Fe catalyst. The organic phase was separated and the aqueous one was extracted twice with ethyl acetate. The combined organic phases were dried over Na_2SO_4 and concentrated under vacuum to obtain a crude that was purified by flash chromatography.

*(S,S)-2-(N-Methyl-2′-pyrrolidinyl)-7-nitro-1,4-benzodioxane [(S,S)-**2a**].* Obtained as a light yellow oil in 93% yield after chromatography on silica gel [dichloromethane/methanol (95:5) + 0.5% NH_3]: R_f = 0.40; $[\alpha]_D^{25}$ = −141.6 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.83 (d, 1H, *J* = 2.6 Hz), 7.74 (dd, 1H, *J* = 2.6 and 9.0 Hz), 6.92 (d, 1H, *J* = 9.0 Hz), 4.43 (m, 1H), 4.14−4.06 (m, 2H), 3.10 (m, 1H), 2.62 (m, 1H), 2.43 (s, 3H), 2.34−2.26 (m, 1H), 1.96−1.75 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 149.32, 143.27, 141.87, 117.34, 116.92, 113.59, 74.72, 66.35, 64.87, 57.65, 42.33, 26.83, 23.61.

*(S,R)-2-(N-Methyl-2′-pyrrolidinyl)-7-nitro-1,4-benzodioxane [(S,R)-**2a**].* Obtained as a light yellow oil in 81% yield after chromatography on silica gel [dichloromethane/methanol (95:5) + 0.5% NH_3]: R_f = 0.53; $[\alpha]_D^{25}$ = +36.2 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.80 (m, 2H), 6.92 (d, 1H, *J* = 8.8 Hz), 4.41 (dd, 1H, *J* = 2.2 and 11.4 Hz), 4.19 (td, 1H, *J* = 1.8 and 7.9 Hz), 4.06 (dd, 1H, *J* = 7.9 and 11.4 Hz), 3.12 (m, 1H), 2.71 (m, 1H), 2.49 (s, 3H), 2.28 (m, 1H), 2.02−1.91 (m, 1H), 1.82−1.66 (m, 3H). ^{13}C NMR (CDCl3) δ 149.25, 143.07, 141.83, 117.48, 116.90, 113.47, 76.23, 66.12, 64.73, 57.81, 42.67, 27.20, 23.60.

*(S,S)-2-(N-Methyl-2′-pyrrolidinyl)-6-nitro-1,4-benzodioxane [(S,S)-**2b**].* Obtained as a white solid in 85% yield after chromatography on silica gel [dichloromethane/methanol (95:5) + 0.2% NH_3]: mp = 93.09 °C; R_f = 0.42; $[\alpha]_D^{25}$ = −171.3 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.79 (m, 2H), 7.00 (d, 1H, *J* = 7.0 Hz), 4.37 (dd, 1H, *J* = 2.2 and 11.4 Hz), 4.19 (m, 1H), 4.07 (dd, 1H, *J* = 11.4 and 7.9 Hz), 3.17 (m, 1H), 2.63 (m, 1H), 2.45 (s, 3H), 2.37 (m, 1H), 1.98−1.76 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 149.48, 143.21, 141.65, 117.71, 117.34, 113.21, 75.25, 65.83, 64.93, 57.62, 42.26, 26.71, 23.58.

*(S,R)-2-(N-Methyl-2′-pyrrolidinyl)-6-nitro-1,4-benzodioxane [(S,R)-**2b**].* Obtained as a light yellow solid in 64% yield after chromatography on silica gel [dichloromethane/methanol (95:5) + 0.5% NH_3]: mp = 91.22 °C; R_f = 0.47; $[\alpha]_D^{25}$ = +72.3 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.81 (m, 2H), 6.97 (m,1H), 4.39 (dd, 1H, *J* = 2.4 and 11.6 Hz), 4.26 (td, 1H, *J* = 2.4 and 7.5 Hz), 4.03 (dd, 1H, *J* = 7.5 and 11.6 Hz), 3.17 (m, 1H), 2.75 (m, 1H), 2.50 (s, 3H), 2.44−2.30 (m, 1H), 2.03−1.93 (m, 1H), 1.85−1.66 (m, 3H). ^{13}C NMR ($CDCl_3$) δ 149.18, 143.14, 141.75, 117.71, 117.24, 113.20, 77.10, 65.59, 64.81, 57.84, 42.70, 27.31, 23.61.

*(S,S)-2-(N-Methyl-2′-pyrrolidinyl)-5-nitro-1,4-benzodioxane [(S,S)-**2c**].* Obtained as a light yellow oil in 81% yield after chromatography on silica gel (ethyl acetate + 5% triethylamine): R_f = 0.72; $[\alpha]_D^{25}$ = −90.1 (*c* 1, MeOH). ^1H NMR ($CDCl_3$) δ 7.46 (dd, 1H, *J* = 1.6 and 8.3 Hz), 7.17 (dd, 1H, *J* = 1.6 and 8.3 Hz), 6.88 (t,

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1H, J = 8.3 Hz), 4.47 (dd, 1H, J = 2.0 and 11.3 Hz), 4.20 (ddd, 1H, J = 2.0, 3.9 and 8.3 Hz), 4.07 (dd, 1H, J = 8.3 and 11.3 Hz), 3.15 (m, 1H), 2.60 (m, 1H), 2.43 (s, 3H), 2.32−2.24 (m, 1H), 1.94−1.24 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 145.19, 139.09, 138.80, 122.20, 120.05, 117.59, 73.97, 66.57, 65.00, 57.56, 42.02, 26.36, 23.46.

*(S,R)-2-(N-Methyl-2′-pyrrolidinyl)-5-nitro-1,4-benzodioxane [(S,R)-**2c**].* Obtained as a light orange solid in 25% yield after chromatography on silica gel (ethyl acetate + 5% triethylamine): mp = 77.05 °C; R_f = 0.64; $[\alpha]_D^{25}$ = +40.39 (c 1, MeOH). ^{1}H NMR ($CDCl_3$) δ 7.48 (dd, 1H, J = 1.5 and 8.3 Hz), 7.12 (dd, 1H, J = 1.5 and 8.3 Hz), 6.88 (t, 1H, J = 8.3 Hz), 4.50 (dd, 1H, J = 2.1 and 11.3 Hz), 4.21 (dt, 1H, J = 2.1 and 8.0 Hz), 4.06 (dd, 1H, J = 8.0 and 11.3 Hz), 3.13 (m, 1H), 2.71 (m, 1H), 2.48 (s, 3H), 2.31 (q, 1H, J = 9.0 Hz), 1.91 (m, 1H), 1.82−1.65 (m, 3H). ^{13}C NMR ($CDCl_3$) δ 144.93, 138.99, 138.80, 122.11, 120.03, 117.71, 76.19, 66.28, 64.58, 57.88, 42.77, 27.30, 23.67.

*(S,S)-2-(N-Methyl-2′-pyrrolidinyl)-5-hydroxy-1,4-benzodioxane [(S,S)-**3c**].* Obtained as a white solid in 47% yield after chromatography on silica gel [dichloromethane/methanol (97:3) + 1.5% NH_3]: mp = 130.32 °C; R_f = 0.37; $[\alpha]_D^{25}$ = −57.2 (c 1, MeOH). ^{1}H NMR ($CDCl_3$) δ 6.71 (t, J = 8.3 Hz, 1H), 6.51 (d, J = 8.3, 2H), 5.50 (bs, 1H, exchange with D_2O), 4.36 (dd, J = 1.4 and 10.5 Hz, 1H), 4.12 (m, 1H), 4.05 (dd, J = 8.0 and 10.5 Hz, 1H), 3.13 (m, 1H), 2.56 (m, 1H), 2.44 (s, 3H), 2.37−2.18 (m, 1H), 1.95−1.75 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 145.5, 143.9, 131.4, 121.3, 109.1, 107.8, 74.1, 66.2, 64.6, 57.9, 42.8, 27.3, 23.7.

*(S,R)-2-(N-Methyl-2′-pyrrolidinyl)-5-hydroxy-1,4-benzodioxane [(S,R)-**3c**].* Obtained as a white solid in 36% yield after chromatography on silica gel [dichloromethane/methanol (97:3) + 1.5% NH_3]: mp = 105.22 °C; R_f = 0.30; $[\alpha]_D^{25}$ = +5.9 (c 1, MeOH). ^{1}H NMR ($CDCl_3$) δ 6.71 (t, J = 8.2 Hz, 1H), 6.50 (dd, J = 1.4 and 8.2 Hz, 1H), 6.46 (dd, J = 1.4 and 8.2 Hz, 1H), 5.56 (bs, 1H, exchange with D_2O), 4.36 (dd, J = 2.1 and 11.1 Hz, 1H), 4.20 (td, J = 2.1 and 7.2 Hz, 1H), 4.02 (dd, J = 7.2 and 11.1 Hz, 1H), 3.12 (m, 1H), 2.67 (m, 1H), 2.50 (s, 3H), 2.30 (q, J = 8.7 Hz, 1H), 2.04−1.90 (m, 1H), 1.83−1.68 (m, 3H). ^{13}C NMR ($CDCl_3$) δ 145.8, 143.9, 131.8, 121.3, 109.5, 107.9, 76.2, 66.1, 65.1, 57.9, 42.9, 27.1, 23.8.

Synthesis of Compounds 5a. The target compounds were obtained by treating a cooled solution of **1a** in dichloromethane with acetyl chloride (1 mol). The resulting suspension was warmed to room temperature and stirred for 30 min. Afterward, the desired compounds were obtained as hydrochlorides by simple filtration of the reaction mixture.

*(S,S)-2-(N-Methyl-2′-pyrrolidinyl)-7-acetamido-1,4-benzodioxane hydrochloride [(S,S)-**5a**·HCl].* Obtained as a white solid in 84% yield: mp = 125.50 °C; $[\alpha]_D^{25}$ = −109.3 (c 1, MeOH); ^{1}H NMR ($CDCl_3$) (free base) δ 7.16 (d, 1H, J = 2.5 Hz), 6.97 (bs, 1H, exchange with D_2O), 6.87 (dd, 1H, J = 2.5 and 8.8 Hz), 6.79 (d, 1H, J = 8.8 Hz), 4.25 (dd, 1H, J = 2.2 and 11.0 Hz), 4.08 (m, 1H), 3.98 (dd, 1H, J = 8.0 and 11.0 Hz), 3.11 (m, 1H), 2.51 (m, 1H), 2.42 (s, 3H), 2.27 (m, 1H), 2.14 (s, 3H), 1.95−1.70 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 168.27, 143.64, 140.62, 131.87, 117.12, 113.67, 110.29, 74.40, 66.03, 65.42, 57.88, 42.30, 26.82, 24.63, 23.63.

*(S,R)-2-(N-Methyl-2′-pyrrolidinyl)-7-acetamido-1,4-benzodioxane hydrochloride [(S,R)-**5a**·HCl].* Obtained as a white solid in 86% yield: mp = 130.84 °C; $[\alpha]_D^{25}$ = +10.8 (c 1, MeOH); mp = 113.84 °C; ^{1}H NMR ($CDCl_3$) (free base) δ 7.14 (d, 1H, J = 2.2 Hz), 7.10 (bs, 1H, exchange with D_2O), 6.84 (dd, 1H, J = 2.2 and 8.5 Hz), 6.77 (d, 1H, J = 8.5 Hz), 4.25 (dd, 1H, J = 2.2 and 11.0 Hz), 4.11 (dt, 1H, J = 2.2 and 7.1 Hz), 3.95 (dd, 1H, J = 7.1 and 11.0 Hz), 3.10 (m, 1H), 2.65 (m, 1H), 2.49 (s, 3H), 2.25 (m, 1H), 2.13 (s, 3H), 1.95−1.70 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 168.28, 143.48, 140.59, 131.79, 117.04, 113.58, 110.23, 76.77, 65.86, 64.98, 58.19, 42.97, 27.52, 24.68, 23.71.

Synthesis of Compounds 6a. The target compounds were obtained by treating a solution of **13a** in methanol with 37 wt % aqueous formaldehyde (4 mol) and 10% Pd/C under a hydrogen atmosphere. The mixture was stirred for 3 h and then filtered on a Celite pad to remove the Pd/C catalyst, and the solvent was evaporated under vacuum. The resulting crudes were purified by flash chromatography (cyclohexane/ethyl acetate, 8:2).

The obtained compounds were solubilized in anhydrous tetrahydrofuran and added dropwise to an ice-cooled suspension of $LiAlH_4$ (3 mol) in anhydrous tetrahydrofuran. The resulting mixture was warmed to room temperature and refluxed, under vigorous stirring, for 4 h. Afterward, the mixture was diluted with dichloromethane and the excess of $LiAlH_4$ was quenched by slowly adding water dropwise at 0 °C. The suspension was filtered through a Celite pad and the solvent was evaporated under vacuum. The resulting crudes were purified by flash chromatography.

*(S,S)-2-(N-Methyl-2′-pyrrolidinyl)-7-dimethylamino-1,4-benzodioxane [(S,S)-**6a**].* Obtained as a light yellow oil in 82% yield: R_f = 0.40; $[\alpha]_D^{25}$ = −161.4 (c 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.76 (d, 1H, J = 8.8 Hz), 6.42 (d, 1H, J = 2.8 Hz), 6.29 (dd, 1H, J = 2.8 and 8.8 Hz), 4.20 (d, 1H, J = 10.7 Hz), 4.17 (m, 1H), 3.92 (dd, 1H, J = 7.7 and 10.7 Hz), 3.13 (m, 1H), 2.83 (s, 6H), 2.42(s, 3H), 2.52−2.39 (m, 1H), 2.23 (m, 1H), 1.93−1.72 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 146.72, 144.10, 135.62, 117.22, 107.03, 103.02, 73.96, 66.32, 65.70, 57.85, 41.99, 41.79, 41.75, 26.24, 23.43.

*(S,R)-2-(N-Methyl-2′-pyrrolidinyl)-7-dimethylamino-1,4-benzodioxane [(S,R)-**6a**].* Obtained as a light yellow oil in 78% yield: R_f = 0.63; $[\alpha]_D^{25}$ = −3.8 (c 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.74 (d, 1H, J = 8.8 Hz), 6.32 (d, 1H, J = 2.7 Hz), 6.29 (dd, 1H, J = 2.7 and 8.8 Hz), 4.23 (dd, 1H, J = 2.2 and 11.0 Hz), 4.13 (dt, 1H, J = 2.2 and 7.2 Hz), 3.93 (dd, 1H, J = 7.2 and 11.0 Hz), 3.09 (m, 1H), 2.83 (s, 6H), 2.63 (m, 1H), 2.49 (s, 3H), 2.28 (m, 1H), 1.94 (m, 1H), 1.78−1.61 (m, 3H). ^{13}C NMR ($CDCl_3$) δ 146.73, 143.75, 135.68, 117.20, 107.10, 102.81, 76.84, 65.78, 65.15, 58.16, 42.93, 41.78, 41.73, 27.48, 23.70.

*2-Methoxyethoxymethyloxy-4-nitrophenol (**35a**).* Under a nitrogen atmosphere, at 0 °C, 4-nitrocathecol (2 g, 12.9 mmol) was added to a stirred suspension of NaH (0.68 mg, 28.4 mmol) in DMSO (8 mL). Upon stirring at 0 °C for 10 min, the mixture was warmed to room temperature and stirred for 1 h. Afterward, the mixture was cooled down to 15 °C and a solution of MEM chloride (1.61 g, 12.9 mmol) in DMSO (10 mL) was added dropwise over a 30 min period. Then, the mixture was warmed to room temperature and stirred overnight. Afterward, the reaction mixture was cooled down to 0 °C and diluted with diethyl ether and water. Upon phase separation, the aqueous phase was acidified with formic acid to pH 5 and extracted with diethyl ether (2 × 25 mL). The organic phases were combined, dried over anhydrous sodium sulfate, and filtered and the solvent was evaporated in vacuo, affording a crude that was purified through silica gel flash chromatography (cyclohexane/ethyl acetate, 7:3). The pure product **35a** was isolated as a pale yellow oil (1.30 g, 41%). TLC (cyclohexane/ethyl acetate, 7:3): R_f = 0.32. ^{1}H NMR ($CDCl_3$) δ 7.96−7.88 (m, 2H), 6.99 (d, 1H, J = 8.4 Hz), 5.32 (s, 2H), 3.92 (m, 2H), 3.64 (m, 2H), 3.41 (s, 3H).

*2-Methoxyethoxymethyloxy-5-nitrophenol (**35b**).* Under a nitrogen atmosphere, at 0 °C, 4-nitrocathecol (2.5 g, 16.10 mmol) was added to a stirred suspension of K_2CO_3 (2.67 mg, 19.3 mmol) in DMF (10 mL). Upon warming to 40 °C for 1 h, a solution of MEM chloride (2.40 g, 19.3 mmol) in DMF (5 mL) was added dropwise. The mixture was stirred at 40 °C for 1 h, then cooled down to room temperature, and diluted with water and ethyl acetate. Upon phase separation, the aqueous phase was extracted with ethyl acetate (2 × 25 mL); the organic phases were combined, dried over anhydrous sodium sulfate, and filtered and the solvent was evaporated in vacuo, affording a crude that was purified through silica gel flash chromatography (dichloromethane/ethyl acetate, 9:1). The pure product **35b** was isolated as a pale yellow oil (1.85 g, 47%). TLC (cyclohexane/ethyl acetate, 7:3): R_f = 0.6; ^{1}H NMR ($CDCl_3$) δ 7.81−7.75 (m, 2H), 7.14 (d, 1H, J = 8.8 Hz), 6.6 (bs, 1H), 5.38 (s, 2H), 3.88 (m, 2H), 3.58 (m, 2H), 3.42 (s, 3H).

*2-Methoxyethoxymethyloxy Benzaldehyde (**36**).* Under a nitrogen atmosphere, at 0 °C, *N*,*N*-diisopropylamine (9.97 mL, 57.26 mmol) was added to a stirred solution of 2-hydroxybenzaldehyde (5 g, 40.9 mmol) in dichloromethane (100 mL). Upon stirring at 0 °C for 10 min, MEM chloride (5.60 mL, 6.11 g, 49.08 mmol) was added

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dropwise and the reaction mixture was warmed to room temperature and stirred overnight. Afterward, the reaction mixture was quenched with an aqueous saturated solution of NH_4Cl. Upon phase separation, the aqueous phase was extracted with dichloromethane (2 × 25 mL) and the organic phases were combined and subsequently washed with a saturated solution of $NaHCO_3$ (3 × 10 mL), 1 M aqueous solution of NaOH (2 × 10 mL), and brine (2 × 10 mL). The organic phase was dried over anhydrous sodium sulfate and filtered and the solvent was evaporated in vacuo, providing the pure product **36** as a light yellow oil (7.43 g, 87%). TLC (cyclohexane/ethyl acetate, 7:3): R_f = 0.42. ^{1}H NMR ($CDCl_3$) δ 10.49 (s, 1H), 7.83 (dd, 1H, J = 1.8 and 7.8 Hz), 7.53 (dt, 1H, J = 1.8 and 8.0 Hz), 7.26 (m, 1H), 7.08 (dt, 1H, J = 0.8 and 7.8 Hz), 5.40 (s, 2H), 3.87 (m, 2H), 3.56 (m, 2H), 3.37 (s, 3H).

*2-Methoxyethoxymethyloxyphenol (**37**).* Under a nitrogen atmosphere, *m*-chloroperbenzoic acid (8.49 g, 49.18 mmol) was added to a solution of **36** (7.43 g, 35.38 mmol) in dichloromethane (30 mL). The resulting reaction mixture was stirred at room temperature for 48 h. Afterward, the mixture was cooled down to 0 °C and filtered through a Celite pad to remove the white solid. The filtrate was diluted with dichloromethane (20 mL) and washed with a saturated aqueous solution of $Na_2S_2O_3$ and then with brine. The organic phase was dried over anhydrous sodium sulfate and filtered and the solvent was evaporated in vacuo. The resulting residue was suspended in a methanolic (31 mL) solution of KOH (3.10 g, 55.20 mmol) and the resulting reaction mixture was stirred for 5 h at room temperature. Afterward, the mixture was concentrated in vacuo and the resulting residue was diluted with water (40 mL) and washed with diethyl ether (3 × 15 mL). Upon phase separation, the aqueous phase was acidified with formic acid until pH 5 and extracted with ethyl acetate (3 × 15 mL), which was in turn washed with a saturated solution of $NaHCO_3$. The organic phase was dried over anhydrous sodium sulfate and filtered and the solvent was evaporated in vacuo, providing the pure product **37** as a light yellow oil (3.93 g, 56%). TLC (cyclohexane/ethyl acetate, 7:3): R_f = 0.35. ^{1}H NMR ($CDCl_3$) δ 7.05 (m, 1H), 6.94 (m, 2H), 6.81 (m, 1H), 6.35 (bs, 1H), 5.26 (s, 2H), 3.88 (m, 2H), 3.60 (m, 2H), 3.41 (s, 3H).

*2-Methoxyethoxymethyloxy-6-nitrophenol (**35c**).* A solution of **37** (3.93 g, 19.86 mmol) in acetonitrile (30 mL) was cooled down to 10 °C and a solution of $(NH_4)_2Ce(NO_3)_6$ in acetonitrile (40 mL) was added dropwise. The reaction mixture was stirred at the same temperature for 3 h. Afterward, the mixture was diluted with water and extracted with ethyl acetate. The organic phase was dried over anhydrous sodium sulfate and filtered and the solvent was evaporated in vacuo, affording a crude that was purified through silica gel flash chromatography (cyclohexane/ethyl acetate, 8:2). The pure compound **35c** was obtained as a bright yellow oil (1.92 g, 40%). TLC (cyclohexane/ethyl acetate, 7:3): R_f = 0.33. ^{1}H NMR ($CDCl_3$) δ 10.73 (bs, 1H), 7.78 (dd, 1H, J = 1.5 and 8.5 Hz), 7.48 (dd, 1H, J = 1.5 and 8.5 Hz), 6.90 (t, 1H, J = 8.5 Hz), 5.36 (s, 2H), 3.90 (m, 2H), 3.57 (m, 2H), 3.36 (s, 3H).

*2,5-Dibenzyloxybenzaldehyde (**38**).* A solution of 2,5-dihydroxybenzaldehyde (2.5 g, 18.10 mmol) in DMF (10 mL) was added to a suspension of K_2CO_3 (5.508 mg, 39.82 mmol) in DMF (20 mL) and the resulting mixture was vigorously stirred for 20 min. Upon dropwise addition of benzyl bromide (4.73 mL, 39.82 mmol), the reaction mixture was stirred overnight. Afterward, the mixture was cooled down to room temperature and concentrated in vacuo. The resulting residue was diluted with diethyl ether and sequentially washed with brine, with a 2.5 M aqueous solution of NaOH (2 × 20 mL), with a 10% aqueous solution of HCl, and then with water. The organic phase was dried over anhydrous sodium sulfate and filtered and the solvent was removed in vacuo, providing a solid that was crystallized from aqueous ethanol. The pure compound **38** was obtained as a white solid (5.54 g, 96%). mp: 97.38 °C; TLC (cyclohexane/ethyl acetate, 7:3): R_f = 0.55. ^{1}H NMR ($CDCl_3$) δ 10.51 (s, 1H), 7.46−7.29 (m, 11H), 7.19 (dd, 1H, J = 3.3 and 8.8 Hz), 7.01 (d, 1H, J = 8.8 Hz), 5.16 (s, 2H), 5.06 (s 2H).

*2,5-Dibenzyloxyphenol (**39**).* At 0 °C, *m*-chloroperbenzoic acid (8.58 g, 34.80 mmol) was added to a solution of **38** (5.54 g, 17.40 mmol) in dichloromethane (50 mL). The resulting mixture was stirred at room temperature overnight. Afterward, the mixture was washed with a saturated solution of $NaHCO_3$ (3 × 20 mL) and water (10 mL). The organic phase was dried over anhydrous sodium sulfate and filtered and the solvent was evaporated in vacuo. The resulting crude was dissolved in MeOH and a 2.5 M aqueous solution of NaOH was added. Upon stirring for 2 h, methanol was removed in vacuo and the resulting aqueous residue was washed with diethyl ether. The aqueous phase was acidified to pH 5 with formic acid and the precipitated product was collected by filtration. Pure **39** was obtained as a white solid (4.05 g, 76%). mp = 94.37 °C; TLC (toluene/ethyl acetate, 95:5): R_f = 0.54. ^{1}H NMR (DMSO-d_6) δ 9.12 (bs, 1H, exchange with D_2O), 7.50−7.14 (m, 10H), 6.85 (d, 1H, J = 8.8 Hz), 6.49 (d, 1H, J = 3.3 Hz), 6.34 (dd, 1H, J = 3.3 and 8.8 Hz), 4.99 (s, 2H), 4.81 (s, 2H).

*1,3-Dimethoxyethoxymethyloxy Benzene (**40**).* Under a nitrogen atmosphere, at 0 °C, *N*,*N*-diisopropylamine (10.0 g, 80 mmol) was added to a solution of resorcinol (4.0 g, 36 mmol) in dichloromethane (20 mL). Upon stirring at 0 °C for 10 min, MEM chloride (10.0 g, 80 mmol) was added dropwise. The reaction mixture was warmed to room temperature and stirred overnight. Afterward, the mixture was diluted with dichloromethane and then sequentially washed with a 10% aqueous solution of HCl and a 1 M aqueous solution of NaOH. The organic phase was dried over anhydrous sodium sulfate and filtered and the solvent was evaporated in vacuo, affording a crude that was purified through silica gel flash chromatography (cyclohexane/ethyl acetate, 8:2). The pure product **40** was isolated as a pale yellow oil (4.7 g, 46%). TLC (cyclohexane/ethyl acetate, 8:2): R_f = 0.26. ^{1}H NMR ($CDCl_3$) δ 7.17 (t, 1H, J = 7.9 Hz), 6.75−6.70 (m, 3H), 5.24 (s, 4H), 3.83−3.80 (m, 4H), 3.57−3.54 (m, 4H), 3.39−3.36 (m, 6H).

*2,6-Dimethoxyethoxymethyloxy Phenol (**41**).* Under a nitrogen atmosphere, at 0 °C, 1.6 M *n*-butyllithium in hexane (1.9 mL, 3.0 mmol) was carefully added dropwise to a solution of **40** (1.0 g, 3.6 mmol) in anhydrous THF (12 mL). Upon stirring for 1 h at room temperature, the mixture was cooled down to 0 °C and trimethyl borate (620 mg, 6 mmol) was added; the resulting solution was stirred for further 30 min. Then, the mixture was concentrated in vacuo and the residue was dissolved in a solution of $NaHCO_3$ (3.5 g) in aqueous acetone (water/acetone, 8:2; 13 mL). Then, Oxone (0.9 g, 6 mmol) was added and, upon stirring for 5 min, $NaHSO_3$ (0.5 g) was added. Afterward, the reaction mixture was extracted with ethyl acetate (2 × 20 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, and evaporated in vacuo, affording a crude that was purified through silica gel flash chromatography (cyclohexane/ethyl acetate, 6:4). Pure **41** was obtained as a pale yellow oil (0.7 g, 65%). TLC (cyclohexane/ethyl acetate, 6:4): R_f = 0.28. ^{1}H NMR ($CDCl_3$) δ 6.87−6.84 (m, 2H), 6.76−6.70 (m, 1H), 5.29 (s, 4H), 3.90−3.87 (m, 4H), 3.60−3.55 (m, 4H), 3.39−3.36 (m, 6H).

*2-Hydroxy-3-phenylbenzaldehyde (**42**).* Procedure adapted from the literature.[39] Under an inert atmosphere, a solution of 2-phenylphenol (3.4 g, 20.0 mmol), anhydrous triethylamine (10 mL), and anhydrous $MgCl_2$ (2.9 g, 30.0 mmol) in anhydrous THF (60 mL) was stirred for 20 min. Paraformaldehyde (4.0 g) was added in eight portions (500 mg each) every 6 min and the reaction mixture was refluxed for 3 h. Afterward, upon cooling down to room temperature, a 2 M aqueous solution of HCl was added to reach pH 5 and the mixture was stirred for further 5 min. The product was extracted with ethyl acetate (3 × 40 mL) and the organic phase was washed with brine, dried over anhydrous sodium sulfate, and filtered and the solvent was evaporated in vacuo, affording a crude that was purified through silica gel flash chromatography (cyclohexane/ethyl acetate, 9:1). Pure **42** was isolated as a yellow solid (3.49 g, 88%). TLC (cyclohexane/ethyl acetate, 9:1): R_f = 0.41. mp: 45 °C. ^{1}H NMR ($CDCl_3$) δ 11.54 (s, 1H), 9.97 (s, 1H, exchange with D_2O), 7.70−7.53 (m, 4H), 7.46 (m, 2H), 7.38 (m, 1H), 7.12 (t, 1H, J = 7.6 Hz).

*2-Methoxyethoxymethyloxy-3-phenylbenzaldehyde (**43**).* Procedure adapted from the literature.[39] Under an inert atmosphere at 0 °C, a solution of **42** (3.0 g, 15.1 mmol) in THF (10 mL) was added

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dropwise to a stirred suspension of NaH (399 mg, 16.6 mmol) in THF (40 mL) and the resulting mixture was stirred for 30 min. Upon dropwise addition of MEM chloride (5.79 g, 5.31 mL, 46.5 mmol), the mixture was stirred overnight. Afterward, the solvent was evaporated in vacuo, the residue was diluted with a 2 M aqueous solution of KOH (50 mL), and the product was extracted with diethyl ether. The organic phase was dried over anhydrous sodium sulfate and filtered and the solvent was removed in vacuo, affording a crude that was purified through silica gel flash chromatography (gradient cyclohexane/ethyl acetate from 9:1 to 7:3). Pure **43** was obtained as a yellow oil (3.07 g, 71%). TLC (cyclohexane/ethyl acetate, 9:1): R_f = 0.18. ^{1}H NMR ($CDCl_3$) δ 10.49 (s, 1H), 7.87 (dd, 1H, J = 1.8 and 7.7 Hz), 7.61 (dd, 1H, J = 1.8 and 7.5), 7.58−7.50 (m, 2H), 7.50−7.28 (m, 4H), 4.81 (s, 2H), 3.56 (m, 2H), 3.35 (m, 2H), 3.29 (s, 3H).

*2-Methoxyethoxymethyloxy-3-phenylphenol (**44**).* At 0 °C, *m*-chloroperbenzoic acid (964 mg, 5.59 mmol) was added to a solution of **43** (640 mg, 2.24 mmol) in ethyl acetate (15 mL). The resulting mixture was stirred at room temperature for 72 h. Afterward, the solvent was evaporated in vacuo and the resulting crude was diluted in dichloromethane (15 mL) and washed with a saturated solution of $NaHCO_3$ (3 × 10 mL) and water (10 mL). The organic phase was dried over anhydrous sodium sulfate and filtered and the solvent was evaporated in vacuo. The resulting crude was dissolved in MeOH and a 2.5 M aqueous solution of NaOH was added. Upon stirring for 2 h, methanol was removed in vacuo and the resulting aqueous residue was washed with diethyl ether. The aqueous phase was acidified to pH 5 with aqueous formic acid and the product was extracted with ethyl acetate. The organic phase was dried over anhydrous sodium sulfate and filtered and the solvent was removed in vacuo, affording pure **44** as a yellow oil (450 mg, 73%). TLC (cyclohexane/ethyl acetate, 9:1): R_f = 0.19. ^{1}H NMR ($CDCl_3$) δ 7.56 (bs, 1H, exchange with D_2O), 7.54−7.47 (m, 2H), 7.40 (t, 2H, J = 7.6 Hz), 7.33 (m, 1H), 7.08 (dd, 1H, J = 7.6 and 8.1 Hz), 6.97 (dd, 1H, J = 1.7 and 8.1 Hz), 6.84 (dd, 1H, J = 1.7 and 7.6 Hz), 4.71 (s, 2H), 3.81 (m, 2H), 3.58 (m, 2H), 3.44 (s, 3H).

*2-Methoxyethoxymethyloxy-3-benzyloxy-biphenyl (**45**).* A solution of **44** (570 mg, 2.08 mmol) in DMF (10 ml) was added to a suspension of K_2CO_3 (574 mg, 4.16 mmol) in DMF (10 mL) and the resulting mixture was vigorously stirred for 20 min. Upon dropwise addition of benzyl bromide (462 mg, 0.32 mL, 2.7 mmol), the reaction mixture was warmed to 50 °C and stirred for 1.5 h. Afterward, the mixture was cooled down to room temperature and concentrated in vacuo. The resulting residue was diluted with diethyl ether and sequentially washed with brine, with a 2.5 M aqueous solution of NaOH (2 × 20 mL), with a 10% aqueous solution of HCl, and then with water. The organic phase was dried over anhydrous sodium sulfate and filtered and the solvent was removed in vacuo, providing pure **45** as an orange oil (590 mg, 78%). TLC (cyclohexane/ethyl acetate, 9:1): R_f = 0.29. ^{1}H NMR ($CDCl_3$) δ 7.58−7.50 (m, 2H), 7.49−7.27 (m, 8H), 7.13−7.05 (m, 1H), 7.00−6.93 (m, 2H), 5.13 (s, 2H), 4.99 (s, 2H), 3.23−3.16 (m, 5H), 3.14−3.05 (m, 2H).

*2-Benzyloxy-6-phenylphenol (**46**).* A concentrated solution of HCl (1 mL) was added dropwise to a solution of **45** (255 mg, 0.70 mmol) in methanol (50 mL). The resulting solution was vigorously stirred at 60 °C for 1 h. Afterward, the reaction mixture was cooled down to room temperature and quenched by carefully adding dropwise a saturated aqueous solution of $NaHCO_3$. Methanol was removed in vacuo and the resulting aqueous phase was extracted with ethyl acetate. The organic phase was dried over anhydrous sodium sulfate and filtered and the solvent was evaporated in vacuo, providing pure **45** as a white solid (186 mg, 96%). TLC (cyclohexane/ethyl acetate, 9:1): R_f = 0.5. mp: 86 °C. ^{1}H NMR ($CDCl_3$) δ 7.62 (m, 2H), 7.48−7.29 (m, 8H), 7.00 (dd, 1H, J = 3.2 and 5.2 Hz), 6.95−6.84 (m, 2H), 5.93 (s, 1H, exchange with D_2O), 5.17 (s, 2H).

Biological Assays. *Binding Studies.* The affinities (K_i) of the synthetized compounds for the $\alpha4\beta2$, $\alpha3\beta4$, and $\alpha7$ receptors were determined using [^{3}H]epibatidine (specific activity of 56−60 Ci/mmol; PerkinElmer, Boston, MA)-labeled rat cerebral cortex membranes ($\alpha4\beta2$) or membranes of HEK 243 cells stably transfected with human $\alpha3\beta4$ nAChR and [^{125}I]α-bungarotoxin (specific activity of 200−213 Ci/mmol, PerkinElmer, Boston, MA)-labeled rat hippocampus membranes ($\alpha7$), as previously described.[29,32] The K_d and K_i values were derived from three [^{3}H]epibatidine and [^{125}I]α-bungarotoxin saturation and three competition binding experiments using rat cortex ($\alpha4\beta2$) and hippocampus ($\alpha7$) membranes and the membrane of human $\alpha3\beta4$-transfected cells.[29,35]

Briefly, for saturation experiments, the membrane homogenate aliquots were incubated overnight at 4 °C with 0.01−2.5 nM concentrations of [^{3}H]epibatidine. Nonspecific binding was determined in parallel by adding to the incubation solutions 100 nM unlabeled epibatidine (Sigma-Aldrich). At the end of the incubation, the samples were filtered on a GFC filter soaked in 0.5% polyethylenimine and washed with 15 mL of ice-cold PBS and the filters were counted in a β counter. For [^{125}I]α-bungarotoxin saturation binding experiments, aliquots of the hippocampus membrane homogenates were incubated overnight with 0.1−10.0 nM concentrations of [^{125}I]α-bungarotoxin at r.t. Nonspecific binding was determined in parallel by including in the assay mixture 1 μM unlabeled α-bungarotoxin (Sigma-Aldrich). After incubation, the samples were filtered as described for [^{3}H]epibatidine binding.

[^{3}H]Epibatidine binding was determined by liquid scintillation counting in a β counter.

For competition studies, the inhibition of [^{3}H]epibatidine and [^{125}I]bungarotoxin binding was measured by incubating the membranes of the appropriate subtype with increasing concentrations of the compounds to be tested for 5 min followed by overnight incubation at 4 °C, with 0.1 nM [^{3}H]epibatidine for the rat cerebral cortex membrane ($\alpha4\beta2$ subtype), 0.25 nM [^{3}H]epibatidine for the $\alpha3\beta4$ subtype, or 2−3 nM [^{125}I]Bgtx at r.t. in the case of the $\alpha7$ subtype expressed on hippocampal membranes.

[^{125}I]Bungarotoxin binding was directly counted in a γ counter.

The K_d of the ligands for each subtype was determined by performing at least three to four separate saturation binding experiments for each subtype. K_i values were obtained by fitting three to four independent competition binding experiments, each performed in duplicate for each compound on each subtype. Data analysis, curve fitting, and K_d and K_i determination were calculated using GraphPad Prism software version 6 (GraphPad Software, Inc., San Diego, CA, USA)

Inhibition constants (K_i) were estimated in reference to the K_d of the radioligand according to the Cheng−Prusoff equation and are expressed as nM values ± SE.

Constructs of Mutated $\beta2$ Subunits. The amino acid S108 of the human $\beta2$ subunit, identified by modeling, corresponds to S133 (due to the presence of signal peptide of 25 amino acids) in the UniProt sequence. The mutants S133A, S133L, and S133F have been generated by site-directed mutagenesis using the QuikChange Lightning Site-directed mutagenesis kit (#210519, Agilent). The template used was pCDNA3-h$\beta2$ (a kind gift of Sergio Fucile, Università la Sapienza, Roma), which codes for the entire CDS of the human $\beta2$ subunits (protein sequence, ACHB2_HUMAN, UniProt database).

In pCDNA3-h$\beta2$-S133A, a TCC trinucleotide sequence (position 397−399) that codes for a serine is changed to GCC (one nucleotide mutated) that codes for an alanine. To obtain pCDNA3-h$\beta2$-S133L, the TCC is converted to CTC (two nucleotides mutated) that codes for a leucine, while it is converted to TTC (one nucleotide mutated) that codes for a phenylalanine in pCDNA3-h$\beta2$-S133F. To obtain these substitutions, we designed the following primers (the mutated oligonucleotides in bold):

S133A upper: 5′-CGAGGTGTCCTTCTAT**G**C-CAATGCCGTGGTCTCC-3′.

S133A lower: 5′-GGAGACCACGGCATTGG**C**ATAGAAGGA-CACCTCG-3′.

S133L upper: 5′-CGAGGTGTCCTTCTAT**CT**-CAATGCCGTGGTCTCC-3′.

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S133L lower: 5′-GGAGACCACGGC ATTG**AG**ATAGAAGGA-CACCTCG-3′.

S133F upper: 5′-CGAGGTGTCCTTCTATT**T**-CAATGCCGTGGTCTCC-3′.

S133F lower: 5′-GGAGACCACGGCATTG**A**AATAGAAGGA-CACCTCG-3′.

According to the protocol of the kit, 25 ng of the template was amplified using 125 ng of primers with the following PCR protocol: 1 cycle of 95 °C for 2 min (step 1), 18 cycles of 95 °C for 20 s, 60 °C for 10 min and 68 °C for 3.5 min (step 2), and 1 cycle of 68 °C for 5 min.

After the digestion of the parental methylated DNA with the DpnI enzyme, we transformed the mutated plasmid into XL10-GOLD Ultracompetent cells. The mutations were checked by sequencing the plasmid obtained.

Transfection and Binding Studies of WT and Mutated α4β2 Subtypes. HeLa cells were purchased from ATCC and grown in a high-glucose DMEM medium (Gibco, Thermo Fisher Scientific) supplemented with 10% heat-inactivated FBS (CARLO ERBA Reagents) of South American origin, 2 mM L-glutamine (Euro-Clone), 100 U/mL penicillin G, and 100 μg/mL streptomycin (EuroClone). The cells were maintained in a 5% CO_2 environment at 37 °C.

On the day before transfection, 2.2×10^6 to 2.5×10^6 cells for each condition were plated in a 100 mm Petri dish. Each dish was transfected with 15 μg of pcDNA (7.5 μg of pcDNA for each subunit: α4 and β2 for control α4β2; α4 and β2S133A for α4β2S133A subtype; α4 and β2S133L for α4β2S133L subtype; α4 and β2S133F for α4β2S133F subtype) by employing JetPEI DNA transfection reagent (Polyplus-transfection) diluted with a 150 mM NaCl solution by following the manufacturer's instructions. The culture medium was replaced with fresh medium just before transfection. After 24 h, the transfected cells were washed once with PBS, harvested, and immediately frozen.

For [^{3}H]epibatidine saturation binding experiments, aliquots of the membrane homogenate obtained from transfected HeLa cells were used and binding was performed as described above.

For competition studies, the inhibition of [^{3}H]epibatidine binding was measured by incubating the membranes of HeLa cells transfected with the appropriate subtype with increasing concentrations of compounds for 5 min followed by overnight incubation at 4 °C, with 0.1 nM [^{3}H]epibatidine. At the end of the incubation time, the samples were processed as described above.

[^{3}H]Epibatidine binding was determined by liquid scintillation counting in a β counter.

K_i values were obtained by fitting three independent competition binding experiments, each performed in duplicate for each compound on each subtype.

Electrophysiological Experiments. The human α4β2 and α4β2S108A nAChRs were expressed by transient transfection in the rat anterior pituitary GH4C1 cell line.[40] Transient transfection was achieved by adding to each well 0.5 μg of each subunit cDNA, along with 2 μL of Magnetofection reagent NeuroMag (OZ Biosciences, France). All culture media were purchased from Invitrogen (Italy). Whole-cell current recordings were performed 2−3 days after transfection. Recordings and data analysis were performed by using a borosilicate glass patch pipette (3 to 6 MΩ tip resistance) connected to an Axopatch 200A amplifier (Axon Instruments, Foster City, CA). Data were stored on a PC computer by using PCLAMP10 software (Molecular Devices). During the recording period, the cells were bathed in the following solution: 140 mM NaCl, 2 mM $CaCl_2$, 2.8 mM KCl, 2 mM $MgCl_2$, 10 mM Hepes/NaOH, and 10 mM glucose (pH 7.3). The patch pipettes were filled with a solution containing 140 mM CsCl, 2 mM MgATP, 10 mM Hepes/CsOH, and 5 mM BAPTA (pH 7.3). Whole-cell capacitance and patch series resistance (5−15 MΩ) were estimated from slow transient compensations. A series resistance compensation of 85−90% was obtained in all cases. The cells were voltage-clamped at a holding potential of −70 mV and continuously perfused with a gravity-driven system using independent external tubes for the control and agonist-containing solutions. These tubes were positioned 50−100 μm from the patched cell and connected to a fast exchanger system (RSC-160, BioLogic, France). Dose−response relationships were constructed by sequentially applying different concentrations of agonists and normalizing the obtained current amplitudes to the value obtained by applying 100 μM ACh on the same cell. For quantitative estimations of agonist actions, dose−response relationships were fitted by the equation $I = I_{max}([C]^{n_H}/(EC_{50}{}^{n_H} + [C]^{n_H}))$, where I is the peak current amplitude induced by the agonist at concentration [C], I_{max} is the maximum response of the cell, n_H is the Hill coefficient, and EC_{50} is the concentration for which a half-maximum response is induced. Dose−response curves shown in Figure 4 were fitted with separate or shared parameters for α4β2 and α4β2S108A nAChRs (I_{max}, EC_{50}, and n_H) and then compared with F statistical analysis. No significant difference was found; thus, a single dose−response curve was shown for each compound.

Computational Modeling. Ligand Preparation. Compounds (*S*,*S*)-**1c**, (*S*,*R*)-**1c**, (*S*,*R*)-**3a**, and (*S*,*R*)-**8** were constructed with the 2D sketch editor in Maestro[41] and the ionization states were assigned using LigPrep[41] with default settings. Two different absolute configurations of nitrogen are possible upon its protonation to an asymmetric ammonium. For each of the ligand, the (*R*) configuration at the nitrogen was chosen because it is the same absolute configuration of the nitrogen of nicotine in the X-ray crystal structure of 5KXI[18] and in the cryo-EM structures of 6CNJ[16] and 6PV7.[17]

Protein Preparation. The dimeric nicotine-containing α4β2 binding interface was obtained first from the X-ray crystal structure of the full-length human α4β2 receptor (5KXI). The X-ray crystal structure of the AChBP of *L. stagnalis* cocrystallized with nicotine (1UW6) was superimposed with the α4β2 dimer.[36] The structural water molecule interacting with the pyridine nitrogen of nicotine was extracted from 1UW6 and merged with the α4β2 dimer. The resulting water-containing dimer was preprocessed, the H-bond network was optimized, and the complex was subjected to constrained minimization with the Protein Preparation Wizard with default settings.[41]

The compound with the highest α4β2 binding affinity in the series, (*S*,*R*)-**3a**, was docked using the Induced Fit Protocol[42] to adapt the geometry of the binding site to the shape and size of our ligands. Water was removed and the docking center was defined by the current ligand, nicotine. Default settings were used with the following exceptions: both the receptor and ligand scaling factors were set to 1.0 to avoid excessive deformation of the binding site; redocking was performed with XP settings.[42] The pose with the best Induced Fit Docking score was selected, providing the so-called water-free binding site. The ligand was removed, and the structural water molecule was extracted from the nicotine-containing minimized complex and merged with the water-free binding site, providing the water-containing binding site.

When the newer cryo-EM structures of the full-length human α4β2 receptor (6CNJ) and of the full-length human α3β4 receptor (6PV7) were reported, the same procedure was applied to 6CNJ but imported the structural water molecule from 6PV7. Accordingly, the water-free and water-containing binding sites of the α3β4 receptor were obtained, starting from the full-length human α3β4 receptor (6PV7), using the compound with the highest α3β4 affinity in the series, (*S*,*R*)-**1a**, during the Induced Fit Protocol step.

Docking. Compounds (*S*,*S*)-**1c**, (*S*,*R*)-**1c**, and (*S*,*R*)-**3a** were docked in the water-free binding site and (*S*,*R*)-**8** in the water-containing binding site of both the α4β2 receptor (originated from 5KXI and 6CNJ) and α3β4 receptor (from 6PV7) using the Glide XP Ligand Docking Protocol in Schrodinger Maestro with default settings (van der Waals radius scaling factor of 0.8, partial charge cutoff of 0.15, XP precision, and flexible ligand sampling), with a grid centered on the ligand [(*S*,*R*)-**3a** for the water-free binding site and (*S*)-nicotine for the water-containing binding site], requiring to include maximum 10 poses per ligand.[42] Since all compounds are analogues of nicotine, they are expected to place the positively

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charged nitrogen within the aromatic box. Therefore, the best scoring output pose according to the docking score that placed the positively charged nitrogen at a distance not higher than 1.5 A from the corresponding nitrogen of nicotine was selected for each ligand. For all the compounds, this was the best scoring output pose, with the exception of the complex (*S*,*R*)-**8**/$\alpha4\beta2$, for which it was the second-best scoring.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.0c01150.

Fig. 2A - Supplementary docking information: water containing $\alpha4\beta2$ interface with nicotine and (*S*,*R*)-**3a** (PDB)

Fig. 2B - Supplementary docking information: water containing $\alpha4\beta2$ interface with (*S*,*R*)-**8** (PDB)

Fig. 2C - Supplementary docking information: water free $\alpha4\beta2$ interface with (*S*,*S*)-**1c** and (*S*,*R*)-**1c** (PDB)

Fig. 3A - Supplementary docking information: water containing $\alpha4\beta2$ interface from the 2α:3β stoichiometry with (*S*,*R*)-**8** (PDB)

Fig. 3B - Supplementary docking information: water free $\alpha4\beta2$ interface from the 2α:3β stoichiometry with (*S*,*S*)-**1c** (PDB)

Fig. 3c - Supplementary docking information: water containing $\alpha3\beta4$ interface with (*S*,*R*)-**8** (PDB)

Fig. 3D - Supplementary docking information: water free $\alpha3\beta4$ interface with (*S*,*S*)-**1c** (PDB)

^{1}H NMR and ^{13}C NMR spectra; HRMS and HPLC purity analyses of the final compounds; chiral HPLC analysis of compounds **9a**−**9c**, **14a**−**14c**, **19**, **24**, **29b**, and **29c** (PDF)

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Notes

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■ ABBREVIATIONS

αBgtx, α-bungarotoxin; AChBP, acetylcholine binding protein; CPME, cyclopentyl methyl ether; cryo-EM, cryo-electron microscopy; DIAD, diisopropyl azodicarboxylate; DIPEA, *N*,*N*-diisopropylethylamine; DMEM, Dulbecco's modified Eagle's medium; Epi, epibatidine; FBS, fetal bovine serum; F, flow; FT, Fourier transform; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; I_{max}, maximum inhibition; K_d, dissociation constant; MW, microwave; n_H, Hill coefficient; pcDNA, plasmid cloning DNA; TEA, triethylamine; XP, Extra Precision

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