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Research paper

From pyrrolidinyl-benzodioxane to pyrrolidinyl-pyridodioxanes, or from unselective antagonism to selective partial agonism at α4β2 nicotinic acetylcholine receptor

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ABSTRACT

Each of the four aromatic −CH= of (*S,R*)-2-pyrrolidinyl-1,4-benzodioxane [(*S,R*)-**6**] and of its epimer at the dioxane stereocenter (*S,S*)-**6**, previously reported as α4β2 nAChR ligands, was replaced with nitrogen. The resulting four diastereoisomeric pairs of pyrrolidinyl-pyridodioxanes were studied for the nicotinic affinity and activity at α4β2, α3β4 and α7 nAChR subtypes and compared to their common carbaisostere. It turned out that such isosteric substitutions are highly detrimental, but with the important exception of the *S,R* stereoisomer of the pyrrolidinyl-pyridodioxane with the pyridine nitrogen adjacent to the dioxane and seven atoms distant from the pyrrolidine nitrogen. Indeed, this stereo/regioisomer not only maintained the α4β2 affinity of [(*S,R*)-**6**], but also greatly improved in selectivity over the α3β4 and α7 subtypes and, most importantly, exhibited a highly selective α4β2 partial agonism. The finding that [(*S,R*)-**6**] is, instead, an unselective α4β2 antagonist indicates that the benzodioxane substructure confers affinity for the α4β2 nAChR binding site, but activation of this receptor subtype needs benzodioxane functionalization under strict steric requirements, such as the previously reported 7-OH substitution or the present isosteric modification.

1. Introduction

The α4β2 nicotinic acetylcholine receptors (nAChRs), the predominant nAChRs in the CNS, continue to raise interest as a target for selective ligands with therapeutic potential. Recent studies have intensively focused on selective α4β2 partial agonists, for some of which it has been evidenced also the ability of mitigating or inhibiting the α4β2 receptor activation by nicotine and acetylcholine [1–4]. Nicotine addiction and alcohol dependence as well as depression, anxiety and cognitive disorders are the main indications proposed for such partial agonists [1,5,6], whose prototypes are varenicline, approved by U.S. FDA as a smoking cessation aid [7–10], and sazetidine-A (Chart 1) [11,12]. In particular, the latter, structurally related to the potent α4β2 full agonist A-84543, [13] is the lead of a series of 3-pyridyl ethers of prolinol or azetidinylmethanol, differentially substituted at the 5-position of pyridine [1], that has recently included VMY-2-95, an α4β2 partial agonist/desensitizer evaluated in preclinical models for nicotine addiction and depression (Chart 1) [4,5].

The nature of the pyridine 5-substituent of these A-84543 derivatives is a determining factor in both increasing α4β2 selectivity and turning the profile from full agonist to partial agonist or inhibitor [1,14–17]. In previous papers [18,19], we too have investigated this issue. In particular, we have considered the OH substituent; as shown in Chart 2, we have evaluated the consequences of its introduction to the 5-position of A-84543 [(*S*)-**1**] and to the sumperimposable position of some analogues, that is the meta position of the A-8453 phenyl bioisostere [(*S*)-**3**] and of semi-

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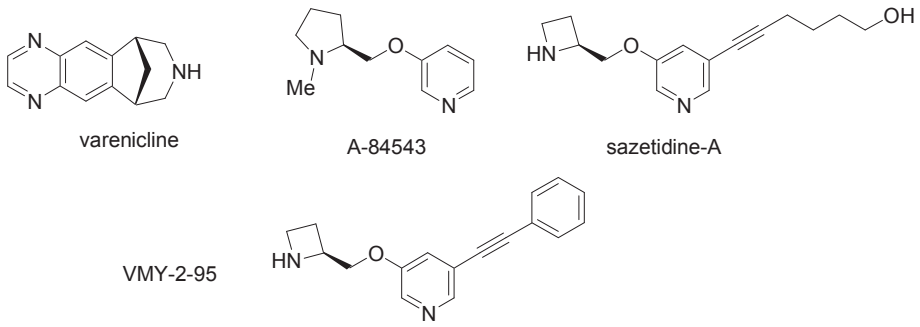


Chart 1. α4β2-nAChR agonists: Varenicline, 3-pyridyl ethers.

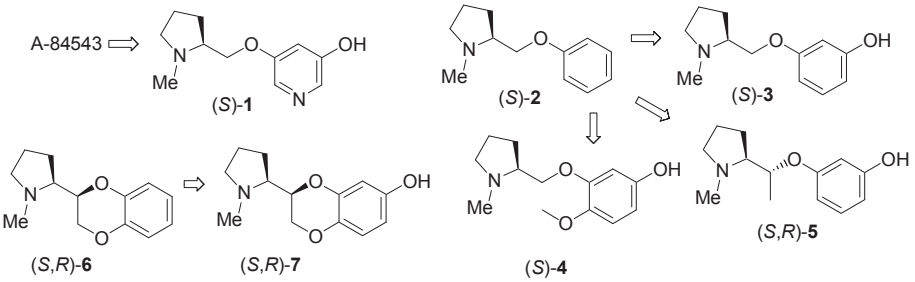


Chart 2. Hydroxylated derivatives of A-84543, (*S*)-**2** and (*S,R*)-**6**.

rigid analogues of this latter [(*S*)-**4** and (*S,R*)-**5**] and the 7-position of 2-pyrrolidinyl-1,4-benzodioxane [20], a rigidified analogue of the A-8453 phenyl bioisostere [(*S,R*)-**7**].

Biological tests have proven that such hydroxyl introductions are generally beneficial for α4β2 receptor affinity and activation, resulting, on the one hand, in full α4β2 agonism of non-constrained prolinol pyridyl and phenyl ethers (*S*)-**1** and (*S*)-**3** and, on the other hand, in partial and selective α4β2 agonism of rigid pyrrolidinyl-benzodioxane (*S,R*)-**7** and of semi-rigid opened analogues (*S*)-**4** and (*S,R*)-**5**. Such a different behavior is consistent with docking analysis, which shows that, apart from the pyrrolidine N^+, prolinol aryl ethers and pyrrolidinyl-benzodioxanes interact with the α4β2 receptor binding site through their pharmacophoric elements in a substantially different way despite the strict structural similarity [19,21,22]. Our more pronounced interest in constrained templates, such as pyrrolidinyl-benzodioxanes, and in their semi-rigid analogues than in flexible prolinol aryl ethers originates from these observations and it has been paralleled, over the same years, by the interest in pyrrolidinyl-pyridodioxanes, whose four positional isomers **8**, **9**, **10** and **11** present a much more severe challenge for synthesis than pyrrolidinyl-benzodioxanes (Chart 3) [19–21].

Pyridine is universally regarded and used as a benzene isostere and, in this case, phenyl and 3-pyridyl ethers of prolinol exhibit an analogous nicotinic profile, the pyridyl derivative however being a more potent α4β2 agonist [13,23]. Would we find the same analogy and the same trend between pyrrolidinyl-benzodioxane and pyrrolidinyl-pyridodioxanes regardless of the pyridine nitrogen position or depending on it? The question was intriguing. We had already experienced this bioisosteric replacement in compounds having other biological targets without dramatic changes in activity [24–26]. Here, however, many elements suggested that replacement, one by one, of each of the four benzodioxane CH with nitrogen would have some impact: (a) the known involvement of the aromatic system of nicotinoids in π-π and in HBA interactions, which are both strongly conditioned by the positioning of a heteroatom in that system [27,28], (b) the importance of the distance of the aromatic nitrogen from the charged aliphatic or alicyclic nitrogen, (c) our previous observation [19] of the significant affinity improvement resulting from OH substitution at the aromatic ring of (*S,R*)-**6** and, lastly, (d) the recently reported change in affinity resulting from repositioning pyridine nitrogen in a much more flexible molecule such as A-84543 [29,30] (Chart 4: compounds **12** and **13**) and the remarkable difference in α4β2 affinity observed between pyrrolidinyl-furopyridines **15** and **16**, on the one side, and pyrrolidinyl-benzofuran **14** and pyrrolidinyl-furopyridines **17** and **18** on the other side (Chart 4) [31,32].

Here, we report the synthesis of the diastereomeric pairs with *S* configuration at the pyrrolidine stereocenter of the pyrrolidinyl-pyridodioxanes **8** and **9**, regardable as constrained A-84543 analogues, and of the positional isomers **10** and **11**, their biological characterization and the SAR discussion comparing their nicotinoid profiles with those of pyrrolidinyl-benzodioxanes (*S,R*)-**6** and (*S,R*)-**7**.

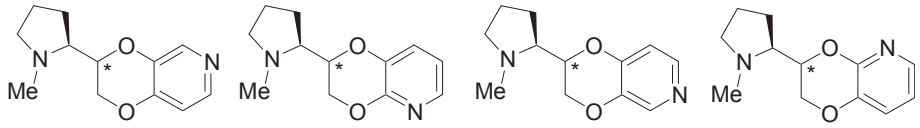


Chart 3. Target pyrrolidinyl-dihydrodioxino-pyridines.

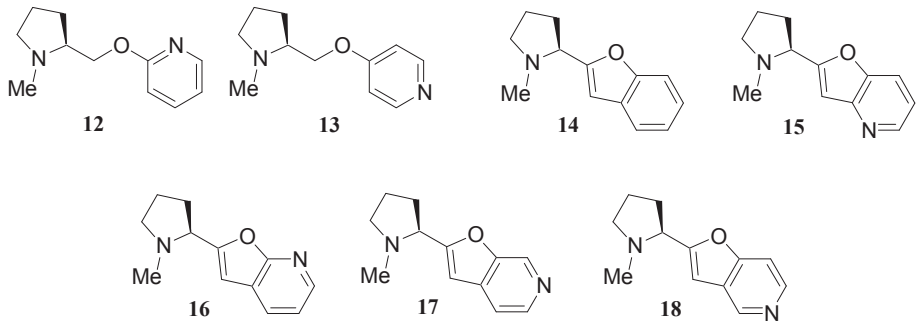


Chart 4. N-Positional isomers and constrained analogues of A-84543.

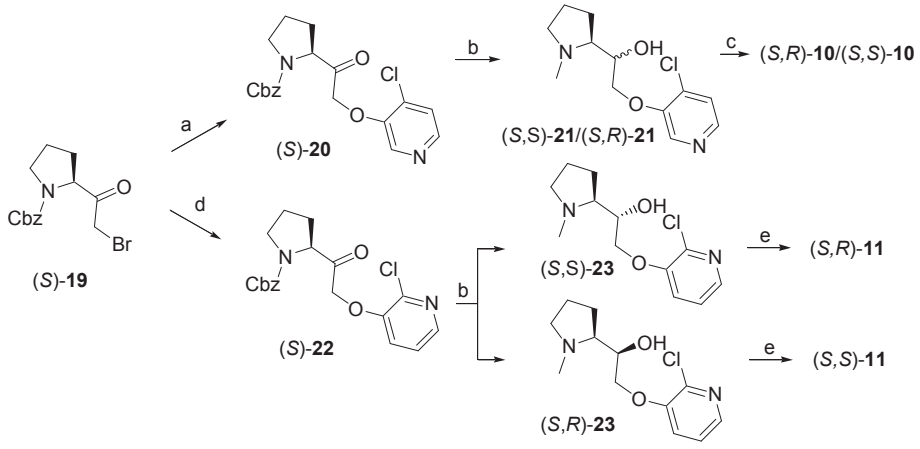
2. Chemistry

For each of the regioisomeric pyrrolidinyl-dihydrodioxino-pyridines **8**–**11**, the two diastereomers having *S* configuration at the pyrrolidine stereocentre were singly prepared, excepting **10** which was obtained as a near 60/40 mixture of the two diastereomers.

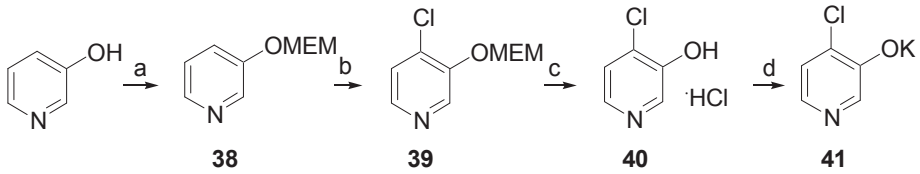
Scheme 1 reports the synthesis of the (*S,R*)-**10**/(*S,S*)-**10** mixture and that of (*S,R*)-**11** and (*S,S*)-**11**. N-Protected (*S*)-2-bromoacetylpyrrolidine (*S*)-**19** [19] was reacted, in acetone at room temperature, with the potassium salt of 4-chloro-3-hydroxypyridine, previously synthesized from *O*-MEM protected 3-hydroxypyridine by *para*-lithiation, lithium-chlorine exchange and removal of the *O*-protecting group (Scheme 2). The carbonyl and the Cbz group of the resultant enantiomerically pure 4-chloro-3-pyridyl ether (*S*)-**20** were reduced with $LiAlH_4$ to give a diastereomeric mixture of the secondary alcohols (*S,R*)-**21** and (*S,S*)-**21**, which were converted into the diastereomers (*S,R*)-**10** and (*S,S*)-**10** via intramolecular nucleophilic chlorine displacement by treatment with NaH in DMF. By the same route, but using the sodium salt of commercially available 2-chloro-3-hydroxypyridine, (*S*)-**19** was converted into the enantiopure 2-chloro-3-pyridyl ether (*S*)-**22**, which was then reduced to the secondary alcohols (*S,R*)-**23** and (*S,S*)-**23**. These were separated by chromatography and cyclized to (*S,S*)-**11** and (*S,R*)-**11**.

The same synthetic strategy was initially conceived also for **8** and **9**: etherifying the secondary OH, resultant from the reduction of carbonyl bound to pyrrolidine C(2), instead of the terminal primary OH, with 3-pyridyl residue, 4- and 2-Cl substituted respectively, and then cyclizing by intramolecular nucleophilic chlorine displacement. In fact, as retrosynthetically shown in Scheme 3, both **9** and **11** can be, in theory, obtained from 2-chloro-3-hydroxypyridine and 2-bromoacetilpyrrolidine, while both **8** and **10**, in theory, from 4-chloro-3-hydroxypyridine and 2-bromoacetylpyrrolidine.

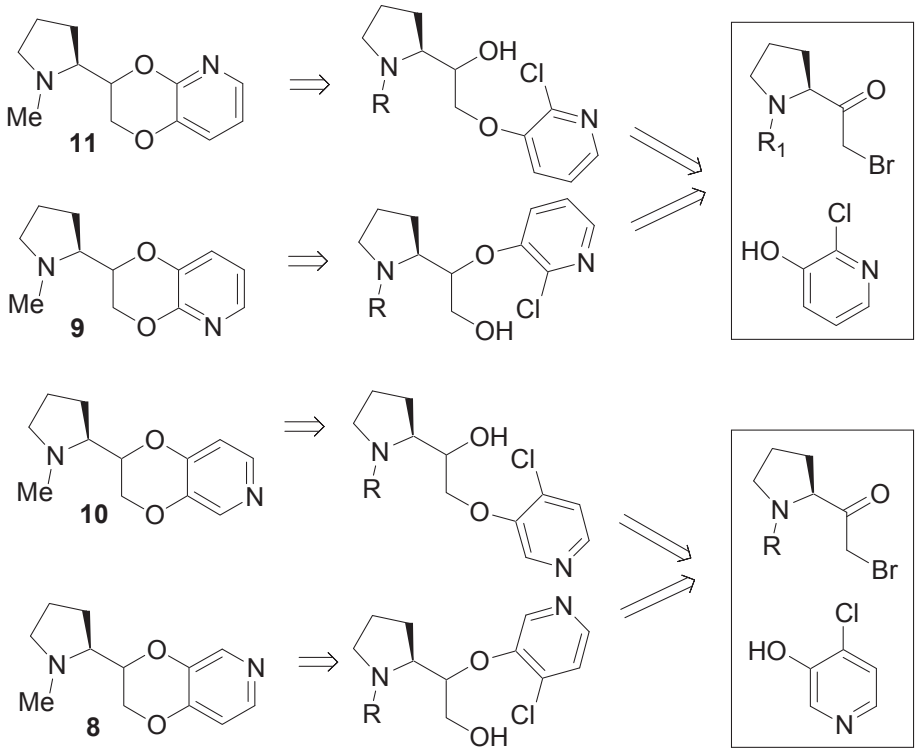
However, in practice, such a strategy, first applied to the synthesis of **9**, encountered many problems: attempts at etherifying the secondary OH in the presence of the *N*- and primary *O*-protecting groups Cbz and trityl were unsuccessful. On the other hand, after reduction of Cbz to methyl, the etherification took place but



Scheme 1. Synthesis of the *S,R* and *S,S* stereoisomers of compounds **10** and **11**. Reagents and conditions: (a) 4-Cloro-3-hydroxypyridine potassium salt, acetone, rt, 5 h; (b) $LiAlH_4$, THF, rt, 3 h; (c) NaH, DMF, reflux, 16 h; (d) 2-chloro-3-hydroxypyridine sodium salt, acetone, rt, 16 h; (e) NaH, DME, 120 °C, 1 h, MW.



Scheme 2. Synthesis of 4-chloro-3-hydroxypyridine. Reagents and conditions: (a) NaH, MEMCl, DMF, 0 °C, 3.5 h; (b) *t*-BuLi, C_2Cl_6, diethyl ether, −78 °C and then rt, 1 h; (c) HCl, MeOH, rt, 16 h; (d) KOH, MeOH, rt, 10 min.



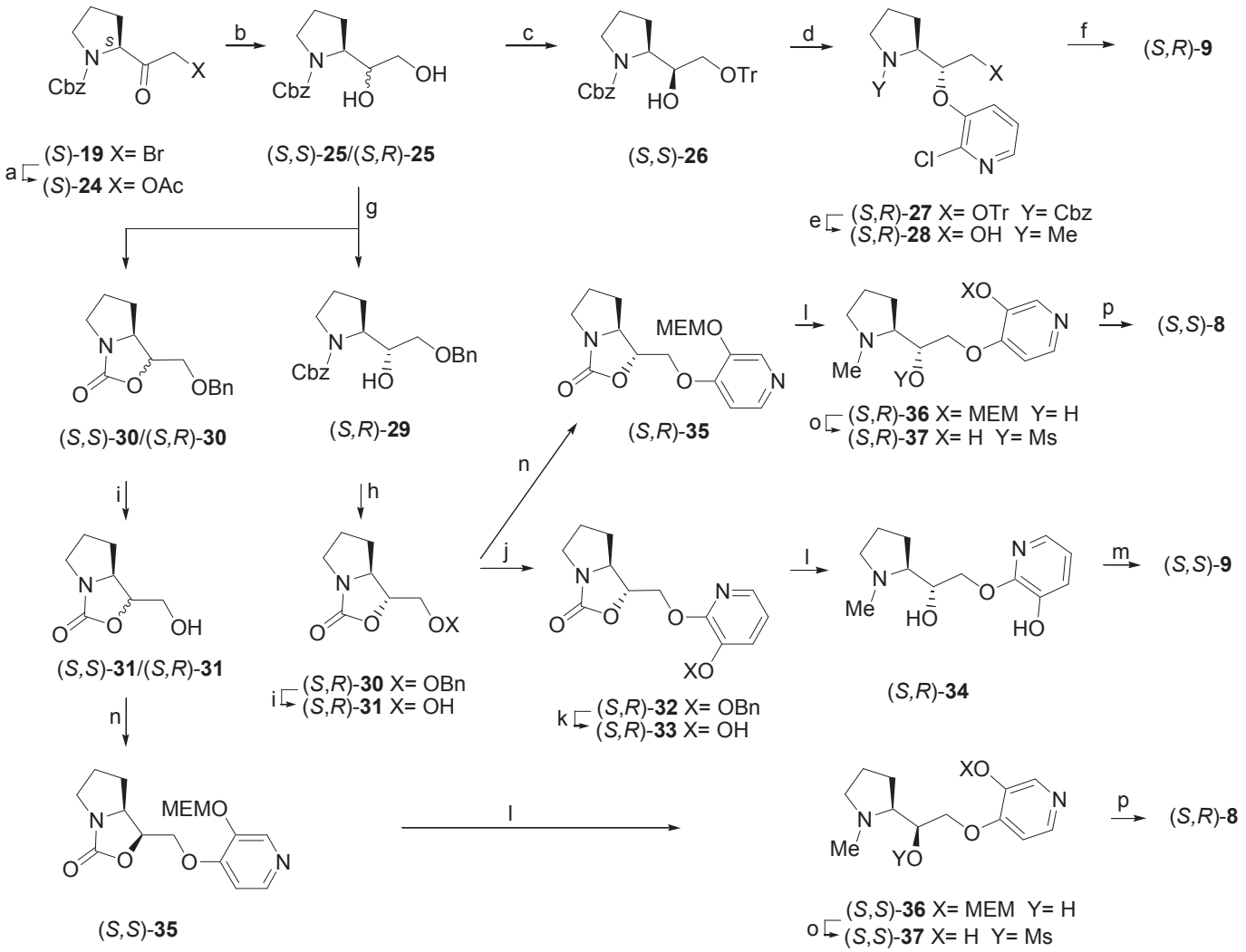
Scheme 3. Retrosynthetic analysis of the pyrrolidinyl-dihydrodioxino[2,3-*b*]pyridines **11** and **9** and of the pyrrolidinyl-dihydrodioxino[2,3-*c*]pyridines **10** and **8**.

the product was the 3-pyridyl ether of 3-hydroxypiperidine and not the ether at the secondary exocyclic hydroxyl. The pyridyloxypiperidine was produced by the pyrrolidine ring expansion through a bicyclic aziridinium ion intermediate, as we had previously experienced also in the chloride displacement from *N*-methyl-2-chloromethylpyrrolidine [33]. Indeed, etherification of the secondary exocyclic hydroxyl could be accomplished by the Mitsunobu reaction of the *N*-Cbz and *O*-trityl intermediate with 2-chloro-3-hydroxypyridine, but it was necessary to use trimethylphosphine and a higher temperature and, under such conditions, only one diastereoisomer was obtained. In detail, the route is shown in the upper part of Scheme 4. Without any racemization, (*S*)-**19** could be converted, by nucleophilic substitution of bromine with acetate in DMSO at room temperature, into enantiopure (*S*)-**24**, which was then reduced and desacetylated to (*S,S*)-**25**/(*S,R*)-**25** mixture. The primary OH of the two diastereoisomers was tritylated and the resultant trityl ethers (*S,S*)-**26** and (*S,R*)-**26** were separated by chromatography. The subsequent Mitsunobu reaction of (*S,S*)-**26** with 2-chloro-3-hydroxypyridine, trimethylphosphine and DEAD gave the pyridyl ether (*S,R*)-**27**, which was converted into (*S,R*)-**28** by reduction of Cbz to methyl and trityl removal a then cyclized to (*S,R*)-**9** by treatment with NaH in refluxing dimethoxyethane.

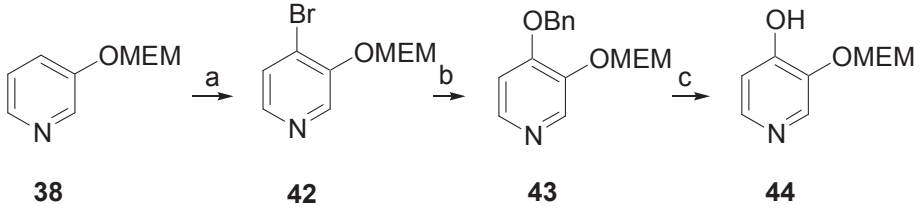
The same way could not be practiced to obtain (*S,S*)-**9**, because (*S,R*)-**26** did not react to give (*S,S*)-**27**. Therefore, to synthesize (*S,S*)-**9** and the two diastereomers of **8**, we abandoned the approach based on the 3-pyridylation of the secondary OH and focused on the 2-pyridylation and 4-pyridylation of the primary OH, the secondary hydroxyl being temporarily engaged in a cyclic carbamate with pyrrolidine nitrogen (Scheme 4). According to such a strategy, the new key intermediate was the bicyclic oxazolidinone **31**. However, this could not be obtained directly from the diol **25** through internal transcarbamoylation. The obstacle was overcome through the benzylation of the "tin acetal" of the (*S,S*)-**25**/(*S,R*)-**25** mixture, which afforded, after chromatographic purification, a 60/40 diastereomeric mixture of the two *O*-benzylated cyclic carbamates (*S,S*)-**30** and (*S,R*)-**30** and the benzyl ether at the primary position of (*S,R*)-**25**, that is (*S,R*)-**29**. This was transformed into (*S,R*)-**30** and, after debenzylation to (*S,R*)-**31**, 2-pyridylated with 3-benzyloxy-2-bromopyridine. The benzyl protection was removed from the pyridine 3-hydroxyl and the carbamate was reduced with $LiAlH_4$. The intramolecular Mitsunobu reaction between the two OH groups of the resultant (*S,R*)-**34** afforded (*S,S*)-**9**.

To synthesize the two diastereoisomers of **8**, it was necessary to prepare the suitable 4-pyridylating agent. This was found to be 3,4-dihydroxypyridine MEM protected at the 3-hydroxyl, which was obtained from 3-(2-methoxyethoxy) methoxypyridine by *para*-lithiation, lithium-bromine exchange, nucleophilic displacement of bromine with benzylate and debenzylation (Scheme 5). The Mitsunobu condensation of the resultant 4-hydroxy-3-(2-methoxyethoxy) methoxypyridine with (*S,R*)-**31** gave the 4-pyridyl ether (*S,R*)-**35**, whose carbamic function was reduced with $LiAlH_4$. Mesylation of the secondary OH and deprotection of the pyridine 2-hydroxyl provided the last intermediate (*S,R*)-**37**, which was cyclized to (*S,S*)-**8** by treatment with potassium carbonate (Scheme 4). The diastereomer (*S,R*)-**8** was synthesized from the (*S,S*)-**30**/(*S,R*)-**30** mixture through the same reactions sequence as (*S,S*)-**8** from (*S,R*)-**30** by accomplishing diastereomers chromatographic separation at the second step, namely after the Mitsunobu condensation with 4-hydroxy-3-(2-methoxyethoxy) methoxypyridine (Scheme 4).

The absolute configuration of the pyrrolidine stereocenter is *S* in all the final compounds and the intermediates and it is indicated first in their chemical names when there are two stereocenters, while that of the second stereocenter, namely that formed in the course of the synthesis and corresponding to dioxane C(2) in the final compounds, is either *R* or *S* and was assigned to the separated diastereoisomers of the final compounds and, retrospectively, to the same carbon of the respective precursors with the aid of ^1H NMR and conformational analysis as previously reported for the



Scheme 4. Synthesis of the *S,R* and *S,S* stereoisomers of compounds **8** and **9**. Reagents and conditions: (a) CH_3COOK, DMSO, rt, 2 h; (b) $NaBH_4$, THF, rt, 2 h and then CH_3ONa, MeOH rt, 1 h; (c) TrCl, TEA, DME, reflux, 4 h and then chromatographic separation from (*S,R*)-**26**; (d) 2-chloro-3-hydroxypyridine, $(Me)_3P$, DEAD, THF, 140 °C, 15 min, MW; (e) $LiAlH_4$, THF, reflux, 3 h and then HCl 1 M, rt, 1 h; (f) NaH, DME, reflux, 16 h; (g) Bu_2SnO, toluene, reflux, 5 h and then BnBr, TBAI, reflux, 16 h; (h) NaH, DMF, rt, 3 h; (i) H_2, Pd/C, MeOH, rt, 16 h; (j) 3-benzyloxy-2-bromopyridine, NaH, DMF, 80 °C, 4 h; (k) H_2, Pd/C, EtOH, rt, 2 h; (l) $LiAlH_4$, THF, rt, 3 h; (m) $(Ph)_3P$, DIAD, THF, 120 °C, 15 min, MW; (n) 4-hydroxy-3-(2-methoxyethoxy) methoxypyridine, $(Ph)_3P$, DIAD, THF, reflux, 12 h; (o) MsCl, TEA, DCM, rt, 30 min and then TFA, DCM, rt, 3 h; (p) K_2CO_3, DME, reflux, 16 h.



Scheme 5. Synthesis of 4-hydroxy-3-(2-methoxyethoxy) methoxypyridine. Reagents and conditions: (a) *n*-BuLi, THF, −78 °C, 1 h and then CBr_4, −90 °C, 1 h; (b) BnONa, DMSO, rt, 1 h; (c) H_2, Pd/C 10%, MeOH, 1 h.

benzodioxane analogues and the prolinol phenyl ethers [18–21]. Briefly, we assigned *R* configuration to the pyridodioxane methine, when H NMR spectrum showed that its proton is anti-disposed to the adjacent methyne proton, because such a disposition is conformationally disfavoured when the dioxane methine has *S* configuration. The anti disposition was revealed by the td (triplet of doublets) pattern of the dioxane methine signal resulting from the coupling with pyrrolidine methine proton and one of the two dioxane methylene protons with a similar large *J*, as shown in the H NMR spectra of (*S,R*)-**8** and (*S,R*)-**11** reported in the Experimental Section. However, such an approach could not be extended to the diastereomers of **9** because of signals overlapping. Therefore, we relied on the H NMR analysis of an (*S,S*)-**9** precursor, namely (*S,R*)-**30** (Scheme 4). In this precursor, where the two adjacent methine stereocenters are enclosed into a rigid bicyclic structure, the O-adjacent methine has its proton anti-disposed to the proton of the N-adjacent *S* stereocenter and therefore its configuration has to be *R*. The anti-disposition was revealed by the dt (doublet of triplets)

pattern of its signal resulting from the coupling to the N-adjacent methine proton with a large *J* and to the exocyclic methylene protons with little lower, nearly equal coupling costants (see the H NMR spectrum of (*S,R*)-**30** in the Experimental Section). As shown in Scheme 4, the absolute configuration of (*S,R*)-**30** is correlated not only to that of (*S,S*)-**9**, but also to that of (*S,S*)-**8** and this is congruent to the configuration assignment previously made to the other distereomer of **8**.

3. Biology

3.1. Binding studies

The synthetized compounds were assayed for binding affinity on native rat α4β2* nAChR present on rat cerebral cortex membranes, rat α7* nAChR present on hippocampus membranes and on human α3β4 nAChR transiently transfected into HEK 243 cells according to a previously described experimental protocol [18,19,34]. The α4β2 and α3β4 nAChRs were labeled by [^{3}H]-epibatidine ([^{3}H]-Epi) whereas the α7 nAChR by [^{125}I]-αBungarotoxin ([^{125}I]-αBgtx). The binding affinities (K_i) of the compounds were determined with competition binding experiments and the results are listed in Table 1. As controls in each experiment, we determined the affinity of (*S*)-(−)-nicotine for the different subtypes.

(*S,R*)-**6**, its diastereoisomer (*S,S*)-**6** and (*S,R*)-**9** exhibited the highest affinities for the α4β2 subtype with similar submicromolar K_i values (0.26, 0.47 and 0.41 μM respectively). For (*S,R*)-**9**, such a moderate α4β2 affinity was associated to very low α3β4 and α7 affinities (16.2 and 89 μM K_i respectively), the lowest ones among all the tested compounds. Therefore, (*S,R*)-**9** shows the highest selectivity towards the α4β2 subtype.

3.2. In vitro functional activity at nAChR

The binding studies indicate that (*S,R*)-**6** is the compound with the highest α4β2 affinity and with a much lower affinity towards the α3β4 subtype. In order to determine its effects on α4β2, and α3β4 nAChRs, in vitro, data were collected via whole-cell patch clamp electrophysiology with GH4CL cell line cells transiently expressing human α4β2 and α3β4 subtypes. As shown in Fig. 1D, (*S,R*)-**6** did not activate the α4β2, and α3β4 subtypes at any of the concentrations. We then measured its antagonistic activity; as shown in Fig. 1, (*S,R*)-**6** exhibited a significant antagonist effect towards both the α3β4 (Fig. 1F) (inhibitory concentration, IC_{50} 2.78 μM) and α4β2 (Fig. 1E) (IC_{50} 5.36 μM) subtypes, being able, at low micromolar concentration, to inhibit completely in both subtypes the inward currents elicited by ACh. Compound (*S,R*)-**9** was also tested and it showed no activity towards the α3β4 subtype, but a partial agonist activity with an Imax of 24.2% of that elicited by 1000 μM ACh with an effective concentration (EC_{50}) of 10.1 μM (Fig. 1C).

4. Discussion

The replacement of benzene with pyridine in the pyrrolidinyl-benzodioxane (*S,R*)-**6** and in its epimer at the dioxane stereocenter, (*S,S*)-**6** greatly affects nicotinic affinity depending on the position of nitrogen in the aromatic ring. As shown in Table 1, both the pyridodioxanes with nitrogen non adjacent to dioxane, namely **8** and **10**, exhibit, regardless of dioxane stereocenter configuration, modest supramicromolar α4β2 affinities, substantially lower than those of (*S,R*)-**6** and of (*S,S*)-**6**, with negligible α4β2 vs α3β4 selectivity. On the other hand, positioning of nitrogen adjacent to dioxane has dramatically divergent effects: (*S,R*)-**11** and (*S,S*)-**11** are almost devoid of nicotinic affinity, whereas **9**, but only the *S,R* stereoisomer, has a moderate submicromolar α4β2 affinity, similar to that of (*S,R*)-**6**, and high α4β2 vs α7 and α3β4 selectivities, significantly better than those of (*S,R*)-**6**. Therefore, these results indicate that the introduction of nitrogen into the aromatic ring of benzodioxane is invariably deleterious with the only exception of (*S,R*)-**9**. That may seem rather surprising considering that an opposite trend is generally observed for nicotinoids: the replacement of pyridine nitrogen with an sp^2 carbon drastically reduces the affinity, for instance, of nicotine, nornicotine, A-84543 and pyrrolidinyl-furopyridines **15** and **16** [31,32,35,36]. Such a decrease is usually imputed to the loss of the HBA properties of the sp^2 nitrogen and/or to weakening of π-stacking interactions with an aromatic amino acid residue of the binding site. It is known, in fact, that the presence of pyridine nitrogen can strengthen these interactions if a parallel-displaced orientation locating the nitrogen atom away from the benzene π-cloud is permitted [27,28]. Anyhow, as the pyridine orientation is determinant of both HBA and π-π interactions, SAR analysis of the aromatic nitrogen presence and of its position has to distinctly consider flexible prolinol aryl ethers from their constrained analogues and to compare the respective affinity and activity profiles. We had previously suggested the necessity of such a distinction when examining the effects of hydroxylation, namely the nicotinic profiles of meta-hydroxylated prolinol aryl ethers (*S*)-**1** and (*S*)-**3** compared with the 2-pyrrolidinyl-1,4-benzodioxane 7-hydroxylated derivative, (*S,R*)-**7**, and its semi-rigid analogues (*S*)-**4** and (*S,R*)-**5** [18]. In particular, we had emphasized the forcedly different pose of the aromatic ring of benzodioxane, which is distanced from those amino acid residues interacting with A-84543 pyridine through HBA and π-π interactions.

Now, if we consider nicotine and A-84543 and their respective phenyl bioisosteres, we see that replacement of pyridine nitrogen with −CH= results in an approximately 300-fold decrease in α4β2

Table 1
Nicotine and compounds **8**–**11**: affinity for native α4β2 and α7 nAChR subtypes, in rat brain membranes, respectively labeled by [^{3}H]-epibatidine and [^{125}I]-αBungarotoxin, and heterologously expressed human α3β4 nAChRs, labeled by [^{3}H]-epibatidine.

Compd	α4β2 nAChR [^{3}H]-Epi	K_i (μM) α7 nAChR [^{125}I]-αBgtx	α3β4 nAChR [^{3}H]-Epi	Compd	α4β2 nAChR [^{3}H]-Epi	K_i (μM) α7 nAChR [^{125}I]-αBgtx	α3β4 nAChR [^{3}H]-Epi
Nicotine	0.004 (18)	0.234 (29)	0.261 (30)	(*S,R*)-**9**	0.41 (20)	89	16.2 (35)
(*S,R*)-**6**	0.26 (32)	21 (44)	1.2 (20)	(*S,S*)-**9**	30.4 (34)	>10	22 (22)
(*S,S*)-**6**	0.47 (30)	14.6 (54)	8.2 (25)	(*S,R*)-**10**+(*S,S*)-**10**	2.5 (30)	–	12.3 (37)
(*S,R*)-**8**	1.64 (19)	–	5.8 (27)	(*S,R*)-**11**	43 (29)	24 (28)	–
(*S,S*)-**8**	3.6 (16)	–	8.9 (31)	(*S,S*)-**11**	30 (23)	12 (30)	–

The K_d and K_i values were derived from three [^{3}H]-epibatidine and [^{125}I]-αBungarotoxin saturation and three competition binding experiments using rat cortex (α4β2) and hippocampus (α7) membranes and the membrane of human α3β4 transfected cells as described in refs. [18,19] and [34]. The curves were fitted using a nonlinear least squares analysis program and the F test. The numbers in brackets represent the %CV. The α4β2 and α7 affinities of compounds (*S,R*)-**6** and (*S,S*)-**6** are those previously reported in ref. [21].

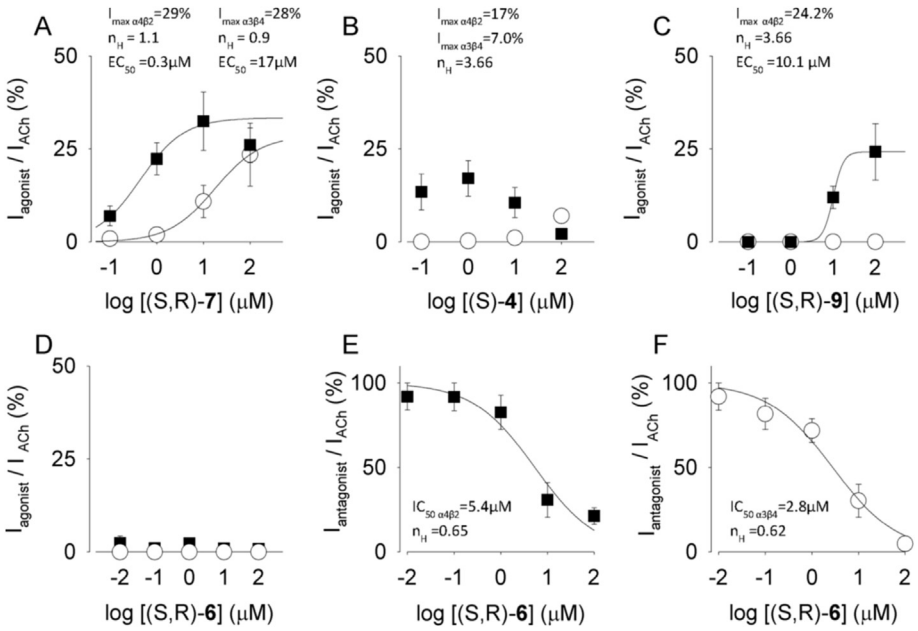


Fig. 1. Agonist effects of (*S,R*)-**7** (A), (*S*)-**4** (B), (*S,R*)-**9** (C), (*S,R*)-**6** (D) and antagonist effects of (*S,R*)-**6** (E and F) on transfected human α4β2 and α3β4 nAChRs. Human α4β2 ( ) and α3β4 ( ) nAChRs were transiently transfected into the GH4C1 rat anterior pituitary cell line and the activation responses are normalized to the maximal response to 1 mM ACh. The antagonist effects were normalized to that of 3 μM Ach for the α4β2 nAChR and to that of 145 μM ACh for the α3β4 nAChR. The α4β2 and α3β4 agonist effects of compounds (*S,R*)-**7** and (*S*)-**4** are those previously reported in ref. [18].

affinity in both cases and that the magnitude of such an affinity decrease is consistent with the transformation of the hydrogen-bonding complexation with pyridine (nitrogen base) into that with benzene (weak π base) [35,36]. On the other hand, if the nitrogen is repositioned in nicotine and in A-84543 instead of being replaced with −CH=, a further loss of affinity is observed: the 2-pyridil and 4-pyridyl analogues of A-84543 and nicotine show a 1000- or 10,000 fold lower α4β2 affinity [29]. That means that reintroducing nitrogen in other positions, besides not restoring HBA interactions with nitrogen, also weakens π-π interactions.

A different behavior is shown by the pyrrolidinyl-pyridodioxane (*S,R*)-**9** and its isostere pyrrolidinyl-benzodioxane (*S,R*)-**6**: replacement of pyridine nitrogen with −CH= does not decrease α4β2 affinity, but slightly increases it (cf. respective 0.41 and 0.26 μM K_i). This would indicate that nitrogen, in that position, is not involved in specific interactions and its presence is uninfluential on the interactions between the aromatic ring and the binding site. However, its repositioning in the cycle is detrimental or deleterious for α4β2 affinity and even the mere rearrangement of its position resulting from the configuration inversion of pyridodioxane stereocenter is prejudicial. In fact, (*S,S*)-**9** has a 70-fold lower α4β2 affinity than (*S,R*)-**9**, whereas the α4β2 affinities of the corresponding benzodioxane isosteres (*S,R*)-**6** and (*S,S*)-**6** are quite similar (Table 1). Significantly, the α4β2 affinity of pyrrolidinyl-furopyridine **15**, which is similar to our pyrrolidinyl-pyridodioxane **9** for pyridine nitrogen position and pyridyl moiety constraint, is also weakened by pyridine nitrogen repositioning [32]. Furthermore, it is noteworthy that replacement of pyridine nitrogen with −CH= in **15** causes a less drastic decrease of α4β2 affinity than in A-84543 (60-fold vs 280-fold) so that its benzofuran analogue **14** has an α4β2 affinity similar to that of our benzodioxane (*S,R*)-**6** (0.16 and 0.26 μM K_i respectively) [36].

After establishing that positioning nitrogen in the aromatic ring of (*S,R*)-**6** and of non-constrained nicotinoids has dissimilar consequences and that only (*S,R*)-**9** maintains the moderate α4β2 affinity of (*S,R*)-**6**, key issues are the selectivity of (*S,R*)-**9** over the α3β4 and α7 nicotinic subtypes and its activity profile. Is there a gain in replacing the carbon at position 5 of (*S,R*)-**6** with a nitrogen atom in comparison to (*S,R*)-**6**, to its 7-hydroxylated derivative (*S,R*)-**7** and to the recently reported opened analogues of this latter, (*S*)-**4** and (*S,R*)-**5**? The affinity ratios reported in Table 2 say yes showing that (*S,R*)-**9** has the best α4β2 vs α7 and vs α3β4 selectivities. Answering the same question about the activity profile required that the lead compound (*S,R*)-**6**, whose only nicotinic affinity had previously determined, was characterized also for functional activity and selectivity. Rather surprisingly, (*S,R*)-**6** was found devoid of both α4β2 and α3β4 agonistic activity and proved to be a relatively potent antagonist of both these receptors ($IC_{50\alpha4\beta2}$ = 5.4 μM, $IC_{50\alpha3\beta4}$ = 2.8 μM). In contrast, (*S,R*)-**9** behaves as a highly selective α4β2 partial agonist ($EC_{50\alpha4\beta2}$ = 10.1 μM, I_{max} = 24.2) having no effect on the α3β4 subtype. Furthermore, as shown in Fig. 1, the pyridodioxane (*S,R*)-**9**, compared to the 7-hydroxybenzodioxane derivative (*S,R*)-**7** and its opened analogue (*S*)-**4**, is less potent as an α4β2 partial agonist, but much more selective over the α3β4 subtype.

Above, we suggested that some parallelism can be drawn between the pair (*S,R*)-**9** – (*S,R*)-**6** and the pair furopyridine **15** – benzofuran isostere **14** for the α4β2 affinity. The functional tests confirm such an analogy: (*S,R*)-**9** and **15** are near equipotent α4β2 partial agonists without α3β4 functional activity and therefore, as underlined for **15** also by the same authors [31], both substantially differ from their common opened analogue A-84543, which is α4β2 full agonist and α3β4 partial agonist, and from the three other A-84543 stereoisomers, full α4β2 and α3β4 agonists with no selectivity over the α3β4 subtype.

5. Conclusions

Radical changes in α4β2 nicotinic affinity and activity result from replacing each of the four −CH= with nitrogen in the aromatic ring of *N*-methyl-2-pyrrolidinyl-1,4-benzodioxane, in particular of its epimers with *S* configuration at the pyrrolidine stereocenter (*S,R*)-**6** and (*S,S*)-**6**. The moderate α4β2 affinity of these two is maintained, among the resultant eight pyridodioxanes, only by

Table 2
Affinity ratios of pyridodioxane (*S,R*)-**9** compared with those of benzodioxanes (*S,R*)-**6**, of (*S,R*)-**7** and of the opened (*S,R*)-**7** analogues, (*S*)-**4** and (*S,R*)-**5**.

Compound	α7 K_i (μM)/α4β2 K_i (μM)	ratio	α3β4 K_i (μM)/α4β2 K_i (μM)	ratio
(*S,R*)-**9**	89/0.41	215	16.2/0.41	39
(*S,R*)-**6**	21/0.26	81	1.2/0.26	5
(*S,R*)-**7**	0.43/0.012	36	0.310/0.012	26
(*S*)-**4**	1.15/0.019	60	0.271/0.019	14
(*S,R*)-**5**	0.057/0.011	5	0.257/0.011	23

(*S,R*)-**9**, that is homochiral with respect to the most potent stereoisomer of **6** and has the pyridine N adjacent to dioxane and seven atoms distant from the pyrrolidine N like the prolinol 3-pyridyl ether A-84543, known α4β2 ligand. Accepted by the α4β2 nicotinic subtype but very regio- and stereoselectively, the presence of pyridine nitrogen is invariably detrimental for α3β4 affinity in comparison with (*S,R*)-**6** thus rendering (*S,R*)-**9** sensibly more α4β2 vs α3β4 selective than (*S,R*)-**6**. These affinity profiles are consistent with the finding that the isosteric modification of (*S,R*)-**6** into (*S,R*)-**9** coincides with the transformation of a relatively potent and α4β2 vs α3β4 unselective antagonist, (*S,R*)-**6**, into a highly selective α4β2 partial agonist, (*S,R*)-**9**. The naked structure of 2-pyrrolidinyl-linked benzodioxane binds to the receptor without activating it; only if suitably dressed up by benzene hydroxylation, as previously reported, or by −CH= replacement with nitrogen, it acquires α4β2 agonistic activity and high selectivity over the α3β4 subtype. As is to be expected, both the transformations into α4β2 selective partial agonists are strictly conditioned by the correct positioning of the pyridinic nitrogen or of the hydroxyl and can be advantaged, in the case of hydroxylation, by some flexibility increase of the system, as demonstrated by (*S*)-**4**, a semirigid analogue of 7-hydroxylated pyrrolidinyl-benzodioxane.

Overall, these results confirm that the benzodioxane and the pyridodioxane nucleus of these compounds interact with the α4β2 and α3β4 nAChRs in a different way from the pyridyloxymethyl residue of A-84543, which is in fact agonist at both the subtype receptors. On the other hand, analogies can be found between our pyrrolidinyl-dioxinopyridine (*S,R*)-**9** and the pyrrolidinyl-furopyridine **15**, which shares with (*S,R*)-**9** a profile of α4β2 partial agonist with no α3β4 effect.

6. Experimental section

6.1. 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 aluminum sheets precoated with silica gel 60 F254 and visualized with UV light; Rf values are given for guidance. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz using an FT-NMR spectrometer. Thermal analyses were performed on 2–5 mg samples in closed pans at 5 °C/min using DSC 2010 TA INSTRUMENTS. Melting points correspond to the peak maximum. Optical rotations were determined in a 1 dm cell of 1 mL capacity. Chiral HPLC analyses were performed on a Kromasyl Amycoat column or Daicel OJ columns using Hewlett Packard 1050 instrument. Electrospray mass spectra of all the final compounds [(*S,R*)-**8**, (*S,S*)-**8**, (*S,R*)-**9**, (*S,S*)-**9**, (*S,R*)-**10**/(*S,S*)-**10**, (*S,R*)-**11**, (*S,S*)-**11**] were superimposable and showed a singly charged base peak $[M+H]^+$ with an average *m/z* value of 221.12825 ± 0.00003. The accurate mass measurements match the theoretical value for the predicted molecules with an average error of −0.9 ppm. Elemental analyses (CHN) are within ±0.40% of theoretical values. The results of elemental analyses indicated that the purity of all tested compounds was higher than 95%. In the general procedures, the moles of reagents are given for one mole of substrate.

6.1.1. General procedure for the synthesis of compounds (S)-20, (S)-22 and (S)-24

The target compounds were synthesized combining *N*-Cbz protected (*S*)-2-bromoacetylpyrrolidine, (*S*)-**19**, with potassium salt of 4-chloro-3-hydroxypyridine (**41**), sodium salt of 2-chloro-3-hydroxypyrine or potassium acetate respectively. The reactions were carried out at room temperature in acetone for 5 h (**20),** for 16 h, (**22),** or in DMSO for 2 h (**24**) using an excess of bromoketone (1.1 mol).

6.1.2. (S)-N-Cbz-2-[(4-chloro-3-pyridyloxy)acetyl]pyrrolidine [(S)-20]

Obtained as a yellow oil in 48% yield after chromatography on silica gel (cyclohexane/EtOAc 8:2): R_f = 0.26, $[\alpha]_D^{25}$ = −30.9 (*c* 1, $CHCl_3$); e.e. = 99.50% (Chiracel OJ, hexane/EtOH 7:3, 1.5 ml/min, λ = 280 nm), ^{1}H NMR (DMSO-d_6, T = 100 °C) δ 1.85–2.04 (m, 3H), 2.10–2.31 (m, 1H), 3.40–3.49 (m, 2H), 4.56–4.64 (m, 1H), 5.07 (s, 2H), 5.13 (s, 2H), 7.26–7.35 (m, 5H), 7.47 (d, *J* = 5.0 Hz, 1H), 8.16 (d, *J* = 5.0 Hz, 1H),. 8.27 (s, 1H).

6.1.3. (S)-N-Cbz-2-[(2-chloro-3-pyridyloxy)acetyl]pyrrolidine [(S)-22]

Obtained as a white solid in 81% yield after chromatography on silica gel (cyclohexane/EtOAc 6:4): m.p. = 83.13 °C, R_f = 0.34, $[\alpha]_D^{25}$ = −31.7 (*c* 1, $CHCl_3$); e.e. = 100% (Chiracel OJ, hexane/EtOH 7:3, F = 1.5 ml/min, λ = 280 nm); ^{1}H NMR (DMSO-d_6) δ 1.77–1.91 (m, 3H), 2.00–2.08 (m, 1H), 3.38–3.49 (m, 2H), 4.53–4.68 (m, 1H), 4.97–5.30 (m, 4H), 7.10–7.15 (m, 1H), 7.23–7.38 (m, 5H), 7.90–7.98 (m, 1H), 7.92–8.02 (m, 1H).

6.1.4. (S)-N-Cbz-2-(acetoxyacetyl)pyrrolidine [(S)-24]

Obtained as a light oil in 98% yield: $[\alpha]_D^{25}$ = −79.4 (*c* 1, $CHCl_3$); e.e. = 99.28 (Kromasil Amycoat, hexane/EtOH 8:2, F = 1.5 ml/min, λ = 220 nm); ^{1}H NMR ($CDCl_3$) δ 1.86–2.23 (m, 4H), 2.15 (s, 3H), 3.46–3.62 (m, 2H), 4.37–4.41 (m, 0.4H rotamrs), 4.42–4.47 (m, 0.6H rotamers), 4.62 (s, 0.8H rotamers), 4.83 (s, 1.2H rotamers), 5.09–5.18 (m, 2H), 7.36–7.25 (m, 5H).

6.1.5. General procedure for the synthesis of compounds (S,S)/(S,R)-21, (S,R)-23, (S,S)-23, (S,R)-34, (S,R)-36, (S,S)-36

The target compounds were obtained by treatment of (*S*)-20, (*S*)-22, (*S,R*)-33, (*S,R*) and (*S,S*)-35 with LiAlH4 (3 mol) in THF at room temperature for 3 h.

6.1.6. (S,R)/(S,S)-N-Methyl-2-[1-hydroxy-2-(4-chloro-3-pyridyloxy) ethyl]pyrrolidine [(S,R)/(S,S)-21]

Obtained as a light yellow oil amorphous solid in 70% yield after chromatography on silica gel (DCM/MeOH/30%NH_3 90:10:0.5): ^{1}H NMR ($CDCl_3$) δ 1.74–1.85 (m, 3H), 1.86–2.06 (m, 1H), 2.35–2.46 (m, 1H), 2.43 (s, 1.8H), 2.47 (s, 1,2H), 2.52–2.83 (m, 1H), 3.10–3.21 (m, 1H), 3.73–3.83 (m, 0.6H), 4.00–4.08 (m, 0.4H), 3.99–4.14 (m, 1.2H),

4.20–4.28 (m, 0.8H), 7.22–7.31 (m, 1H), 8.12–8.18 (m, 1H), 8.32 (s, 1H).

6.1.7. (S,R)-N-Methyl-2-[1-hydroxy-2-(2-chloro-3-pyridyloxy) ethyl]pyrrolidine [(S,R)-23]

Obtained as a white solid in 43% yield after chromatography on silica gel (toluene/MeOH/TEA 90:10:3): m.p. = 66.69 °C, $R_f = 0.24$, $[\alpha]_D^{25} = -29.2$ (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$): 1.75–1.90 (m, 3H), 2.04–2.10 (m, 1H), 2.41–2.51 (m, 1H), 2.55 (s, 3H), 2.92–3.05 (m 1H), 3.20–3.31 (m, 1H), 3.40 (bs, 1H, exchange with D_2O), 3.83–3.97 (m, 1H), 4.11–4.02 (m, 2H), 7.15–7.19 (m, 1H), 7.22–7.31 (m, 1H), 7.93–8.06 (m, 1H).

6.1.8. (S,S)-N-Methyl-2-[1-hydroxy-2-(2-chloro-3-pyridyloxy) ethyl]pyrrolidine [(S,S)-23]

Obtained as a yellow oil in 26% yield after chromatography on silica gel (toluene/MeOH/TEA 90:10:3): $R_f = 0.42$, $[\alpha]_D^{25} = -25.5$ (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$): 1.67–1.80 (m, 3H), 1.84–2.00 (m, 1H), 2.28–2.34 (m, 1H), 2.42 (s, 3H), 2.52–2.63 (m, 1H), 3.08–3.13 (m, 1H), 3.15 (bs, 1H, exchange with D_2O), 3.98 (dd, $J = 6.0$, 9.4 Hz, 1H), 4.15 (dd, $J = 2.8$, 6.0 Hz 1H), 4.22–4.20 (m, 1H), 7.14 (dd, $J = 4.7$, 8.3 Hz, 1H), 7.22 (dd, $J = 1.7$, 8.3 Hz, 1H), 7.93 (dd, $J = 1.7$, 4.7 Hz, 1H).

6.1.9. General procedure for the synthesis of compounds (S,R)-9, (S,R)/(S,S)-10, (S,R)-11, (S,S)-11

The target compounds were obtained by treatment of (*S*,*R*)-28, (*S*,*S*)/(*S*,*R*)-21, (*S*,*S*)-23 and (*S*,*R*)-23 with NaH (1.05 mol). The reactions were carried out in DME at reflux for 16 h (9) or in DMF at reflux for 16 h (10) or in DME by microwave irradiation (120 °C, 300 W, 1 h) (11).

6.1.10. (S,R)/(S,S)-2-(N-Methyl-2-pyrrolidinyl)-2,3-dihydro-1,4-dioxino[2,3-c]pyridine [(S,S)/(S,R)-10]

Obtained as a light oil in 12% yield after chromatography on silica gel (DCM/MeOH/30%NH_3 90:10:2): $R_f = 0.46$; ^{1}H NMR ($CDCl_3$): 1.78–1.98 (m, 4H), 2.28–2.38 (m 1H), 2.42 (s, 3H), 2.45–2.60 (m 1H), 3.08–3.18 (m, 1H), 3.98–4.12 (m, 1H), 4.16–4.22 (m, 0.4H), 4.43–4.41 (m, 1.6H), 6.78 (d, $J = 5.3$ Hz, 0.4H), 6.83 (d, $J = 5.3$ Hz, 0.6H), 7.99–8.08 (m, 1H), 8.18 (s, 0.6H), 8.22 (s, 0.4H). ^{13}C NMR ($CDCl_3$) δ 23.49, 26.48, 26.68, 42.08, 42.21, 57.55, 57.64, 64.97, 65.03, 65.76, 66.53, 74.27, 75.07, 111.94, 112.40, 139.3, 139.80, 141.25, 143.06, 143.38, 146.75, 149.82, 149.99. Calcd for $C_{12}H_{16}N_2O_2$.

6.1.11. (S,R)-3-(N-Methyl-2-pyrrolidinyl)-2,3-dihydro-1,4-dioxino [2,3-b]pyridine [(S,R)-11]

Obtained as a light oil in 28% yield after chromatography on silica gel (EtOAc/TEA 98:2): $R_f = 0.35$; $[\alpha]_D^{25} = +8.3$ (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$): 1.78–1.93 (m, 3H), 1.95–2.05 (m, 1H), 2.28–2.36 (m, 1H), 2.52 (s, 3H), 2.72–2.83 (m 1H), 3.10–3.19 (m, 1H), 3.98 (dd, $J = 8.3$, 11.6 Hz, 1H), 4.35 (dd, $J = 2.2$, 11.6 Hz, 1H), 4.43–4.41 (td, $J = 2.2$, 8.3 Hz, 1H), 6.83 (dd, $J = 4.7$, 7.9 1H), 7.16 (dd, $J = 1.7$, 7.9 Hz, 1H), 7.80 (dd, $J = 1.7$, 4.7 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 23.49, 26.82, 42.36, 57.73, 64.97, 65.08, 76.48, 118.18, 124.37, 139.19, 139.85, 151.16. Calcd for $C_{12}H_{16}N_2O_2$.

6.1.12. (S,S)-3-(N-Methyl-2-pyrrolidinyl)-2,3-dihydro-1,4-dioxino [2,3-b]pyridine [(S,S)-11]

Obtained as a light yellow oil in 30% yield after chromatography on silica gel (EtOAc/TEA 98:2): $R_f = 0.36$; $[\alpha]_D^{25} = -95.9$ (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$): 1.78–1.93 (m, 3H), 1.75–2.04 (m, 1H), 2.31–2.40 (m 1H), 2.46 (s, 3H), 2.63–2.81 (m 1H), 3.02–3.13 (m, 1H), 4.15–4.05 (m, 2H), 4.35 (dd, $J = 2.5$, 9.4 Hz, 1H), 6.83 (dd, $J = 4.7$, 7.9 Hz, 1H), 7.16 (dd, $J = 1.6$, 7.9 Hz, 1H), 7.80 (dd, $J = 1.6$, 4.7 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 23.79, 27.72, 42.92, 57.81, 64.73, 65.25, 76.64, 118.01, 124.26, 139.25, 139.72, 151.12. Calcd for $C_{12}H_{16}N_2O_2$.

6.1.13. (S,R)/(S,S)-N-Cbz-2-(1,2-dihydroxyethyl)pyrrolidine [(S,R)/(S,S)-25]

A solution of (*S*)-**24** (505 mg, 1.65 mmol) in anhydrous THF (4 mL) was added dropwise to a suspension of $NaBH_4$ (66 mg, 1.74 mmol) in ahydrous THF (4 mL) at −10 °C. The reaction mixture was allowed to reach the room temperature and stirred for 3 h at this temperature. Then, dichloromethane was added and the organic phase was washed with water. The separated organic layer was dried over Na_2SO_4, filtered and concentrated under vacuum to give a residue that was dissolved in methanol (20 mL). Sodium methoxide (88 mg, 1.63 mmol) was added to the resulting solution at −10 °C and the reaction mixture was stirred for 1 h. Dichloromethane was added and the organic phase was washed with water, separated and filtered through a celite plug. The solvent was removed under vaccum to obtain (*S*,*S*)/(*S*,*R*)-**25** as a light yellow oil in 97% yield: ^{1}H NMR ($CDCl_3$) δ 1.71–2.11 (m, 4H), 3.05 (bs, 2H), 3.30–3.45 (m, 2H), 3.50–3.66 (m, 3H), 3.89–4.08 (m, 1H), 5.15 (s, 2H), 7.25–7.38 (m, 5H).

6.1.14. (S,S)-N-Cbz-2-(1-hydroxy-2-trityloxy)ethylpyrrolidine [(S,S)-26]

Triethylamine (5 mL) was added to a suspension of triphenylmethylchloride (5.8 g, 20.8 mmol) and (*S*,*S*)/(*S*,*R*)-**25** (4.61 g, 17.4 mmol) in DME (15 mL), under inert atmosphere. The resulting mixture was refluxed for 4 h. Dichloromethane was added and the mixture was washed with 10% HCl aqueous solution. The organic layer was dried over Na_2SO_4, filtered and concentrated under vacuum to give a residue that was purified by chromatography on silica gel (cyclohexane/EtOAc 8:2). (*S*,*S*)-**26** was obtained as a light yellow oil in 57% yield: $R_f = 0.20$, $[\alpha]_D^{25} = -31.5$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.60–1.85 (m, 4H), 2.99–3.04 (m, 1H), 3.25–3.40 (m, 2H), 3.47–3.56 (m, 1H), 3.60–3.72 (m, 1H), 4.12–4.25 (m, 1H), 4.66 (bs, 1H), 5.16 (s, 2H), 7.20–7.51 (m, 20H).

6.1.15. (S,R)-N-Cbz-2-[1-(2-chloro-3-pyridyloxy)-2-trytiloxyethyl] pyrrolidine [(S,R)-27]

(*S*,*S*)-**26** (400 mg, 0.79 mmol) was dissolved in anhydrous THF (3 mL). After adding a 1 M solution of trimethylphosphine in THF (1.58 mL, 1.58 mmol) and 2-chloro-3-hydroxypyridine (112 mg, 0.87 mmol), the reaction mixture was cooled at −10 °C and diethyl azodicarboxylate (151 mg, 0.87 mmol) was added. The reaction vessel was placed into a microwave reactor and irradiated at 140 °C for 15 min at 300 W. Dichloromethane was added to the reaction mixture, which was then washed with 1 M NaOH. The organic layer was separated, dried over Na_2SO_4, filtered and concentrated under vacuum to give a residue that was purified by chromatography on silica gel (cyclohexane/EtOAc 9:1) to give (*S*,*R*)-**27** as a light oil in 43% yield: $R_f = 0.28$, $[\alpha]_D^{25} = -46.6$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.70–1.87 (m, 2H), 2.08–2.21 (m, 2H), 3.17–3.62 (m, 4H), 4.00–4.11 (m, 0.6H rotamer), 4.19–4.30 (m, 0.4H rotamer), 4.91–5.19 (m, 2H), 5.25–5.42 (m, 1H), 6.68–6.81 (m, 0.5H rotamer), 6.90–7.01 (m, 1.5H rotamer), 7.11–7.47 (m, 20H), 7.86–7.94 (m, 1H).

6.1.16. (S,R)-N-Methyl-2-[1-(2-chloro-3-pyridyloxy)-2-hydroxyethyl]pyrrolidine [(S,R)-28]

A solution of (*S*,*R*)-**27** (495 mg, 0.8 mmol) in THF (5 mL) was added dropwise to a suspension of $LiAlH_4$ (91 mg, 2.40 mmol) in THF (10 mL) under inert atmosphere. The reaction mixture was refluxed for 3 h and cooled at room temperature, then dichloromethane was added. The suspension was filtered through a celite plug and the filtrate was concentrated under vacuum to give an oily residue that was dissolved in diethyl ether and stirred for 1 h after adding 10% aqueous HCl (5 mL). The acidic aqueous layer was

separated, basified with 3 M NaOH and extracted twice with ethyl acetate. The organic layer was dried over Na_2SO_4, filtered and concentrated under vacuum to give (*S,R*)-**28** as a light oil in 68% yield: $[\alpha]_D^{25} = -49.9$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.71–1.89 (m, 3H), 2.01–2.17 (m, 1H), 2.28–2.35 (m, 1H), 2.59 (s, 3H), 2.97–3.11 (m, 1H), 3.11–3.24 (m, 1H), 3.89 (dd, *J* = 12.4, 4.1 Hz, 1H), 4.01 (dd, *J* = 12.4, 3.0 Hz, 1H), 4.19–4.16 (m, *J* = 4.1, 3.0 Hz, 1H) 7.17 (dd, *J* = 8.0, 4.7 Hz, 1H), 7.31 (dd, *J* = 8.0, 1.6 Hz, 1H), 8.00 (dd, *J* = 4.7, 1.6 Hz, 1H).

6.1.17. (S,R)-2-(N-Methyl-2-pyrrolidinyl)-2,3-dihydro-1,4-dioxino [2,3-b]pyridine [(S,R)-9]

Obtained as described in the general procedure as a light oil in 75% yield after chromatography on silica gel (EtOAc/TEA 97:3): $R_f = 0.42$,: $[\alpha]_D^{25} = -70.8$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.74–1.93 (m, 4H), 2.18–2.29 (m, 1H), 2.42 (s, 3H), 2.42–2.49 (m, 1H), 3.07–3.16 (m, 1H), 4.05–4.18 (m, 2H), 4.42 (d, *J* = 10.5 Hz, 1H), 6.82 (dd, *J* = 8.0, 4.7 Hz, 1H), 7.21 (dd, *J* = 8.0, 1.7 Hz, 1H), 765. (dd, *J* = 4.7, 1.7 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 23.65, 26.58, 42.25, 57.81, 65.13, 66.77, 74.05, 118.62, 125.16, 139.47, 139.77, 151.36. Calcd for $C_{12}H_{16}N_2O_2$.

6.1.18. (S,R)-N-Cbz-2-(1-hydroxy-2-benzyloxy)ethylpyrrolidine [(S,R)-29] and (S,S)/(S,R)-1-(benzyloxymethyl)tetrahydro-1H,3H-pyrrolo[1,2-c]ossazol-3-one [(S,S)/(S,R)-30]

(*S,S*)/(*S,R*)-**25** (550 mg, 1.88 mmol) was dissolved in anhydrous toluene (10 mL) and dibutyltin oxide (609 mg, 2.45 mmol) was added. The reaction mixture was refluxed for 5 h and water was azeotropically removed by dean-stark apparatus. Then benzyl bromide (0.25 mL, 2.07 mmol) and tetrabutylammonium iodide (35 mg) were added and the mixture was refluxed for additional 16 h. The solvent was removed under vacuum and the resultant residue was purified by chromatography on silica gel (cyclohexane/EtOAc 1:1). *(S,R)*-**29** was obtained as a yellow oil in 43% yield: $R_f = 0.25$, $[\alpha]_D^{25} = -24.0$ (*c* 1, $CHCl_3$), ^{1}H NMR ($CDCl_3$) δ 1.82–2.15 (m, 4H), 3.32–3.60 (m, 4H), 3.85–4.10 (m, 2H), 4.38–4.60 (m, 2H), 5.09–5.16 (m, 2H), 7.26–7.46 (m, 10H). *(S,S)/(S,R)*-**30** was obtained as a yellow oil in 46%: $R_f = 0.30$, ^{1}H NMR ($CDCl_3$) δ 1.43–1.58 (m, 1.6H), 1.74–2.17 (m, 2.4H), 3.13–3.21 (m, 1H), 3.55–3.77 (m, 3.6H), 3.86–3.94 (m, 0.4H), 4.41–4.45 (q, *J* = 5.2 Hz, 0.6H), 4.52–4.65 (m, 2H), 4.82 (dt, *J* = 7.7, 6.1 Hz, 0.4H), 7.30–7.41 (m, 5H).

6.1.19. (S,R)-1-(Benzyloxymethyl)ltetrahydro-1H,3H-pyrrolo[1,2-c] ossazol-3-one [(S,R)-30]

A solution of (*S,R*)-**29** (1.5 g, 4.79 mmol) in anhydrous DMF (10 mL) was added dropwise to a suspension of NaH (115 mg, 4.79 mmol) in anhydrous DMF (5 mL) at −10 °C under inert atmosphere. The reaction was warmed to room temperature and stirred at the same temperature for 3 h. After adding dichloromethane, the reaction mixture was washed with water. The organic layer was separated, dried over Na_2SO_4, filtered and concentrated under vacuum to give a residue that was purified by chromatography on silica gel (cyclohexane/EtOAc 4:6) to give (*S,R*)-**30** as a yellow oil in 59% yield: $R_f = 0.45$, $[\alpha]_D^{25} = -23.9$ (*c* 2, $CHCl_3$), ^{1}H NMR ($CDCl_3$) δ 1.43–1.60 (m, 1H), 1.75–2.18 (m, 3H), 3.12–3.20 (m, 1H), 3.54–3.76 (m, 3H), 3.90 (ddd, *J* = 10.5, 7.7, 5.5 Hz, 1H), 4.57 (ABq, *J* = 11.83 Hz, 2H), 4.82 (dt, *J* = 7.7, 6.1 Hz, 1H), 7.30–7.41 (m, 5H). ^{13}C NMR ($CDCl_3$) δ 25.26, 25.80, 45.72, 62.10, 68.51, 73.97, 74.68, 128.06, 128.21, 128.75, 137.60, 161.21.

6.1.20. General procedure for the synthesis of compounds (S,R)-31, (S,S)/(S,R)-31 and (S,R)-33

Debenzylation of the target compounds was accomplished by stirring a methanol solution of (*S,R*)-**30** or of (*S,S*)/(*S,R*)-**30** for 16 h and an ethanol solution of (*S,R*)-**32** for 2 h in the presence of 10% Pd/C under H_2 atmosphere.

6.1.21. (S,R)-1-(Hydroxymethyl)tetrahydro-1H,3H-pyrrolo[1,2-c] ossazol-3-one [(S,R)-31]

Obtained as a white solid in 97% yield: m.p. = 60.04 °C, $[\alpha]_D^{25} = -8.6$ (c 0.4, $CHCl_3$); 1H NMR ($CDCl_3$) δ 1.60–2.20 (m and bs, 5H), 3.14–3.22 (m, 1H), 3.55–3.64 (m, 1H), 3.79–3.99 (m, 3H), 4.70–4.78 (m, 1H).

6.1.22. (S,S)/(S,R)-1-(Hydroxymethyl)tetrahydro-1H,3H-pyrrolo [1,2-c]ossazol-3-one [(S,S)/(S,R)-31]

Obtained as a light yellow oil in 80% yield: 1H NMR ($CDCl_3$) δ 1.37–1.59 (m, 1H), 1.66–2.13 (m, 3H), 3.08–3.40 (m and bs, 3H), 3.47–3.57 (m, 1H), 3.67–3.78 (m, 1.4H), 3.81–3.95 (m, 0.6H), 4.33–4.38 (m, 0.6H), 4.67–4.73 (m, 0.4H).

6.1.23. (S,R)-1-(3-Benzyloxy-2-pyridyloxymethyl)tetrahydro-1H,3H-pyirrolo[1,2-c]ossazol-3-one [(S,R)-32]

A solution of (*S,R*)-**31** (950 mg, 6.04 mmol) in anhydrous DMF (4 mL) was added dropwise to a suspension of NaH (148 mg, 6.04 mmol) in anhydrous DMF (1 mL) at −10 °C. After adding 2-bromo-3-benzyloxypyridine (1.6 g, 6.04 mmol) at the same temperature, the reaction mixture was stirred at 80 °C for 4 h. Afterwards the solvent was removed under vacuum to give a residue that was dissolved in diethyl ether and washed with water. The separated organic layer was dried over Na_2SO_4, filtered and concentrated under vacuum to give a residue that was purified by chromatography on silica gel (cyclohexane/EtOAc 1:1). (*S,R*)-**32** was obtained as a light oil in 34% yield: $R_f = 0.40$, $[\alpha]_D^{25} = -30.1$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.66–1.89 (m, 2H), 1.97–2.06 (m, 1H), 2.23–2.31 (m, 1H), 3.49–3.65 (m, 2H), 3.70–3.78 (dt, *J* = 9.9, 5.0 Hz, 1H), 4.10 (pt, 1H), 4.61 (dd, *J* = 10.5, 5.0 Hz, 1H), 5.12 (s, 2H), 5.26 (dt, *J* = 9.4, 5.0 Hz, 1H), 6.84 (dd, *J* = 7.7, 5.0 Hz, 1H), 7.12 (dd, *J* = 7.7, 1.4 Hz, 1H), 7.26–7.39 (m, 5H), 7.70 (dd, *J* = 5.0, 1.4 Hz, 1H).

6.1.24. (S,R)-1-(3-Hydroxy-2-pyridyloxymethyl)tetrahydro-1H,3H-pyrrolo[1,2-c]ossazol-3-one [(S,R)-33]

Obtained as a white solid in 99% yield: m.p. = 154.21 °C, $[\alpha]_D^{25} = -49.3$ (*c* 0.5, EtOH), ^{1}H NMR ($CDCl_3$) δ 1.66–1.86 (m, 2H), 1.98–2.06 (m, 1H), 2.23–2.30 (m, 1H), 3.48–3.73 (m, 3H), 4.10 (dd, *J* = 10.5, 9.1 Hz,1H), 4.56 (dd, *J* = 10.5, 4.7 Hz, 1H), 5.30 (dt, *J* = 9.1, 4.7 Hz, 1H), 6.12 (bs, 1H, exchange with D_2O), 6.86 (dd, *J* = 7.7, 5.0 Hz, 1H), 7.17 (dd, *J* = 7.7, 1.4 Hz, 1H), 7.64 (dd, *J* = 5.0, 1.4 Hz, 1H).

6.1.25. (S,R)-N-Methyl-2-[2-(3-hydroxy-2-pyridyloxy)-1-hydroxyethyl]pyrrolidine. [(S,R)-34]

A solution of (*S,R*)-**33** (200 mg, 0.8 mmol) in THF (4 mL) was added dropwise to a suspension of $LiAlH_4$ (92 mg, 2.40 mmol) in THF (4 mL) under inert atmosphere, at −10 °C. The reaction mixture was allowed to reach the room temperature and stirred for 2 h. After cooling, dichloromethane and water were added. The suspension was filtered through a celite plug and the filtrate was concentrated under vacuum to give *(S,R)*-**34** as a light oil in 77% yield: $[\alpha]_D^{25} = -17.0$ (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.85–2.02 (m, 3H), 2.12–2.19 (m, 1H), 2.41 (s, 4H), 2.42–2.57 (m, 1H), 3.24–3.32 (m, 1H), 3.66 (dd, *J* = 11.8, 2.2 Hz,1H), 3.93 (dd, *J* = 11.8, 8.8 Hz, 1H), 4.10 (dt, *J* = 8.8, 2.2 Hz, 1H), 5.60 (bs, 2H, exchange with D_2O), 6.99 (dd, *J* = 7.7, 4.7 Hz, 1H), 7.21 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.67 (dd, *J* = 4.7 1.6 Hz, 1H).

6.1.26. (S,S)-2-(N-Methyl-2-pyrrolidinyl)-2,3-dihydro-1,4-dioxino [2,3-b]pyridine [(S,S)-9]

(*S,R*)-**34** (150 mg, 0.63 mmol) was dissolved in anhydrous THF (2 mL) and triphenylphosphine (0.94 mmol) was added. The solution was cooled at −10 °C and diisopropyl azodicarboxylate (DIAD)

(0.19 mL) was added. The reaction vessel was placed into a microwave reactor and irradiated at 120 °C for 15 min at 300 W. The solvent was removed under vacuum to give a residue that was purified by chromatography on silica gel (DCM/MeOH/30% NH_3 95:5:3). (*S,S*)-**9** was obtained as a light yellow oil in 54% yield after chromatography on silica gel: $R_f = 0.15$, $[\alpha]_D^{25} = -51.5$ (*c* 0.5, $CHCl_3$), ^{1}H NMR (DMSO-d_6) δ 1.65–1.91 (m, 4H), 2.20–2.30 (m, 1H), 2.35 (s, 3H), 2.56–2.66 (m, 1H), 2.88–2.95 (m, 1H), 3.96 (dd, *J* = 11.3, 8.0 Hz, 1H), 4.23 (ddd, *J* = 8.0, 4.7, 2.2 Hz, 1H), 4.34 (dd, *J* = 11.3, 2.2 Hz, 1H), 6.90 (dd, *J* = 8.0, 4.7 Hz, 1H), 7.27 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.71 (dd, *J* = 4.7, 1.6 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 24.10, 28.09, 43.18, 58.16, 65.04, 65.53, 76.82, 118.40, 124.66, 139.56, 140.15, 151.38. Calcd for $C_{12}H_{16}N_2O_2$.

6.1.27. (S,R)-1-[3-(2-methoxyethoxy)methoxy-4-pyridyloxymethyl] tetrahydro-1H,3H-pyrrolo[1,2-c]oxazol-3-one [(S,R)-35]

4-hydroxy-3-(2-methoxyethoxy) methoxypyridine (633 mg, 3.18 mmol) was dissolved in anhydrous THF (5 mL). After adding triphenylphosphine (1.25 g, 4.77 mmol), the reaction mixture was cooled at −10 °C and a solution of (*S,R*)-**31** (500 mg, 3.18 mmol) in THF (5 mL) was added dropwise. Afterwards, diisopropyl azodicarboxylate (DIAD) (151 mg, 4.77 mmol) was added and the reaction mixture was refluxed for 12 h. The solvent was removed under vacuum to give a residue that was purified by chromatography on silica gel (DCM/MeOH 95:5). *(S,R)*-**35** was obtained as a yellow oil in 80% yield: $R_f = 0.31$, $[\alpha]_D^{25} = -7.6$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.54–1.61 (m, 1H), 1.82–1.98 (m, 2H), 2.09–2.17 (m, 1H), 3.16–3.24 (m, 1H), 3.35 (s, 3H), 3.53–3.57 (m, 2H), 3.58–3.67 (m, 1H), 3.84–3.88 (m, 2H), 4.00–4.15 (m, 1H), 4.11–4.25 (m, 1H), 4.22–4.35 (m, 1H), 5.00–5.12 (m, 1H), 5.25 (s, 2H), 6.81 (d, *J* = 5.5 Hz, 1H), 8.21 (d, *J* = 5.5 Hz, 1 H), 8.37 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 25.44, 25.94, 46.67, 59.31, 61.77, 66.85, 68.44, 71.71, 73.46, 95.47, 108.20, 140.21, 143.61, 145.61, 155.02, 160.47.

6.1.28. (S,S)-1-[3-(2-methoxyethoxy)methoxy-4-pyridyloxymethyl] tetrahydro-1H,3H-pyrrolo[1,2-c]oxazol-3-one [(S,S)-35]

Obtained as described for *(S,R)*-**35** from (*S,S*)-**31**/(*S,R*)-**31** as a yellow oil in 54% yield after chromatography on silica gel (DCM/MeOH 95:5): $R_f = 0.36$, $[\alpha]_D^{25} = -31.5$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.50–1.63 (m, 1H), 1.87–2.18 (m, 3H), 3.16–3.23 (m, 1H), 3.36 (s, 3H), 3.54–3.59 (m, 2H), 3.59–3.67 (m, 1H), 3.81–3.90 (m, 3H), 4.19–4.30 (m, 2H), 4.65–4.69 (m, 1H), 5.23 (s, 2H), 6.83 (d, *J* = 5.5 Hz, 1H), 8.22 (d, *J* = 5.5 Hz, 1H), 8.37 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 25.89, 30.90, 45.96, 53,32, 61.99, 68,44, 69.14, 71.72, 76.84, 95.67, 108.46, 140.84, 143.55, 145.76, 155.48, 160.64.

6.1.29. (S,R)-N-Methyl-2-[2-(3-(2-methoxyethoxy)methoxy-4-pyridyloxy)-1-hydroxyethyl]pyrrolidine [(S,R)-36]

Obtained as described for (*S,R*)-**34** as a light yellow oil in 86%: $[\alpha]_D^{25} = -13.2$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.68–1.85 (m, 4H), 2.25–2.44 (m, 1H), 2.37 (s, 3H), 2.33–2.46 (m, 1H), 3.08–3.19 (m, 1H), 3.38 (s, 3H), 3.52–3.57 (m, 2H), 3.82–3.87 (m, 2H), 3.88–3.97 (m, 1H), 4.06–4.18 (m, 2H), 5.27 (s, 2H), 6.84 (d, *J* = 5.5 Hz, 1H), 8.19 (d, *J* = 5.5 Hz, 1H), 8.35 (s, 1H).

6.1.30. (S,S)-N-Methyl-2-[2-(3-(2-methoxyethoxy)methoxy-4-pyridyloxy)-1-hydroxyethyl]pyrrolidine [(S,S)-36]

Obtained as described for (*S,R*)-**34** as a yellow oil in 95% yield: $[\alpha]_D^{25} = -18.0$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.37–1.83 (m, 3H), 1.95–2.26 (m, 1H), 2.38–2.46 (m, 1H), 2.50 (s, 3H), 2.69–2.75 (m, 1H), 3.08–3.14 (m, 1H), 3.36 (s, 3H), 3.54–3.60 (m, 2H), 3.78–3.84 (m, 1H), 3.87–3.90 (m, 2H), 3.99–4.06 (m, 2H), 5.27 (s, 2H), 6.84 (d, *J* = 5.5 Hz, 1H), 8.19 (d, *J* = 5.5 Hz, 1H), 8.34 (s, 1H).

6.1.31. (S,R)-N-Methyl-2-[2-(3-hydroxy-4-pyridyloxy)-1-mesyloxyethyl]pyrrolidine [(S,R)-37]

Mesyl chloride (0.1 mL, 0.846 mmol) was added to a solution of (*S,R*)-**36** (230 mg, 0.705 mmol) and triethylamine (0.09 mL, 0.846 mmol) in dichloromethane (5 mL) at −10 °C. After stirring for 30 min at room temperature, dichloromethane and 10% aqueous solution of $NaHCO_3$ were added. The organic phase was separated, dried, and concentrated to give a residue that was dissolved in dichloromethane (3 mL) and stirred with TFA at room temperature for 3 h. The solvent was removed under vacuum and the residue was purified by chromatography on silica gel (DCM/MeOH/30%NH_3 90:10:3) to give (*S,R*)-**37** as a yellow oil in 59%: $R_f = 0.47$, $[\alpha]_D^{25} = +9.2$ (*c* 1, MeOH); ^{1}H NMR (CD_3OD) δ 1.75–1.84 (m, 4H), 2.28–2.39 (m, 1H), 2.41 (s, 3H), 2.40–2.51 (m, 1H), 3.10–3.18 (m, 1H), 3.32 (s, 3H), 4.14–4.22 (m, 1H), 4.39 (dd, *J* = 2.4, 11.0 Hz, 1H), 4.71 (dd, *J* = 1.4, 11.0 Hz, 1H), 7.08 (d, *J* = 5.7 Hz, 1H), 7.93 (d, *J* = 5.7 Hz, 1H), 7.97 (s, 1H).

6.1.32. (S,S)-N-Methyl-2-[2-(3-hydroxy-4-pyridyloxy)-1-mesyloxyethyl]pyrrolidine [(S,S)-37]

Obtained as described for (*S,R*)-**37** as an oil in 40% yield after chromatography on silica gel (DCM/MeOH/30%NH_3 90:10:3): $R_f = 0.47$, $[\alpha]_D^{25} = -33.2$ (*c* 1, MeOH); ^{1}H NMR (CD_3OD) δ 1.90–2.12 (m, 4H), 2.31–2.39 (m, 2H), 2.39 (s, 3H), 2.88–2.93 (m, 1H), 3.29 (s, 3H), 4.16–4.37 (m, 2H), 4.59–4.68 (m, 1H), 7.04 (d, *J* = 3.0 Hz, 1H), 7.90–7.92 (m, 2H).

6.1.33. (S,R)-3-(N-Methyl-2-pyrrolidinyl)-2,3-dihydro-1,4-dioxino [2,3-c]pyridine [(S,R)-8]

A solution of (*S,R*)-**37** (50 mg, 0.16 mmol) in dimethoxyethane (4 mL) was added to a suspension of potassium carbonate (65 mg, 0.47 mmol) in dimethoxyethane (1 mL) under inert atmosphere and the resulting mixture was refluxed for 16 h. Then, dichloromethane was added and the resulting mixture was washed with a 10% $NaHCO_3$ aqueous solution. The separated organic layer was dried over Na_2SO_4, filtered and concentrated under vacuum to give a residue that was purified by chromatography on silica gel (DCM/MeOH/30%NH_3 90:10:3) to give (*S,R*)-**8** as a yellow oil in 69% yield: $R_f = 0.39$, $[\alpha]_D^{25} = +5.2$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.67–1.84 (m, 3H), 1.91–2.03 (m, 1H), 2.29–2.42 (m, 1H), 2.52 (s, 3H), 2.66–2.73 (m, 1H), 3.11–3.16 (m, 1H), 4.04 (dd, *J* = 7.7, 11.6 Hz, 1H), 4.21 (td, *J* = 2.2, 7.7 Hz, 1H), 4.38 (dd, *J* = 2.2, 11.6 Hz, 1H), 6.78 (d, *J* = 5.5 Hz, 1H), 8.01 (d, *J* = 5.5 Hz, 1H), 8.18 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 23.73, 27.39, 42.85, 58.01, 65.10, 66.47, 76.03, 112.20, 139.92, 141.18, 143.48, 149.96. Calcd for $C_{12}H_{16}N_2O_2$.

6.1.34. (S,S)-3-(N-Methyl-2-pyrrolidinyl)-2,3-dihydro-1,4-dioxino [2,3-c]pyridine [(S,S)-8]

Obtained as described for (*S,R*)-**8** as a white solid in 46% yield after chromatography on silica gel (DCM/MeOH/30%NH_3 90:10:3): m.p. = 57.88 °C, $R_f = 0.47$, $[\alpha]_D^{25} = -93.4$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.74–1.94 (m, 4H), 2.24–2.32 (m, 1H), 2.42 (s, 3H), 2.51–2.61 (m, 1H), 3.08–3.14 (m, 1H), 4.01–4.15 (m, 2H), 4.28–4.41 (m, 1H), 6.78 (d, *J* = 5.5 Hz, 1H), 8.00 (d, *J* = 5.5 Hz, 1H), 8.21 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 23.75, 26.94, 42.49, 57.91, 65.28, 66.78, 74.58, 112.17, 140.06, 141.52, 143.29, 150.05. Calcd for $C_{12}H_{16}N_2O_2$.

6.1.35. 3-[(2-Methoxyethoxy)methoxy]pyridine (38)

A solution of 3-hydroxypyridine (2.5 g, 26.3 mmol) in DMF (20 mL) was added dropwise to a stirred suspension of NaH (730 mg, 28.9 mmol) in DMF (10 mL) at −10 °C under nitrogen. After 10 min, (2-methoxyethoxy) methyl chloride (3.34 mL, 29.4 mmol) was added dropwise and temperature was increased to 0 °C. After 3.5 h, DMF was evaporated and the residue was treated with DCM and 10% aqueous NaOH. The organic phase was

separated, dried over anhydrous sodium sulphate, filtered and concentrated. The residue was purified by chromatography on silica gel (ethyl acetate/cyclohexane 7:3) to afford 3.99 g (83%) of **38** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 3.33 (s, 3H), 3.47–3.56 (m, 2H), 3.75–3.82 (m, 2H), 5.26 (s, 2H), 7.19 (ddd, J = 8.5, 5.0, 0.5 Hz, 1H), 7.37 (ddd, J = 8.5, 2.7, 1.4 Hz, 1H), 8.23 (dd, J = 5.0, 1.4 Hz, 1H), 8.38 (d, J = 2.7 Hz, 1H).

6.1.36. 4-Chloro-3-[(2-methoxyethoxy)methoxy]pyridine (39)

A 1.7 M solution of *tert*-butyllithium in *n*-pentane (3.4 mL, 5.78 mmol) was added dropwise to a stirred solution of **38** (1 g, 5.46 mmol) in diethyl ether (40 mL) at −78 °C. After 30 min, a solution of hexachloroethane (1.75 g, 6.55 mmol) in diethyl ether (5 mL) was added. After 30 min, temperature was increased to 20 °C. The reaction mixture was stirred for 1 h and then 10% aqueous $NaHCO_3$ was added. The organic phase was separated, washed with brine, dried over anhydrous sodium sulphate, filtered and concentrated. The residue was purified by chromatography on silica gel (40–60 petroleum ether/acetone 8:2) to afford 1.0 g (90%) of **39** as a dark yellow oil, which is stable if stored as a 50% v/v solution in MTBE at −28 °C: ^{1}H NMR ($CDCl_3$) δ 3.36 (s, 3H), 3.50–3.59 (m, 2H), 3.85–3.93 (m, 2H), 5.40 (s, 2H), 7.34 (d, J = 5.1 Hz, 1H), 8.20 (d, J = 5.1 Hz, 1H), 8.55 (s, 1H).

6.1.37. 4-Chloro-3-hydroxypyridine hydrochloride (40)

A 1.25 M hydrogen chloride solution in methanol (9.2 mL, 11.5 mmol) was added dropwise to a stirred solution of **39** (500 mg, 2.3 mmol) in methanol (5 mL) at −10 °C. After 1 h, the temperature was increased to 20 °C. The reaction mixture was stirred for 16 h and then concentrated. The crude product was crystallized from toluene to give 342 mg of **40** (90%) as a white crystalline solid: mp 205.9 °C; ^{1}H NMR (DMSO-d_6) δ 7.99 (d, J = 5.9 Hz, 1H), 8.30 (d, J = 5.9 Hz, 1H), 8.52 (s, 1H), 12.67 (br s, 2H).

6.1.38. 4-Chloro-3-hydroxypyridine potassium salt (41)

A 1 N KOH solution in methanol (5.3 mL, 5.3 mmol) was added to **40** (460 mg, 2.8 mmol) dissolved in a minimum volume of methanol. After 10 min, the temperature was lowered to 0 °C and the precipitate was collected by filtration and let dry in the air at room temperature to give 440 mg (95%) of **41** as a beige powder: ^{1}H NMR (DMSO-d_6) δ 6.81 (d, J = 4.8 Hz, 1H), 6.92 (d, J = 4.8 Hz, 1H), 7.44 (s, 1H).

6.1.39. 4-Bromo-3-[(2-methoxyethoxy)methoxy]pyridine (42)

A 2.7 M solution of *n*-BuLi in heptane (12.13 mL, 32.8 mmol) was added dropwise to a stirred solution of **38** (3.0 g, 16.4 mmol) in THF (60 mL) at − 78 °C. After 1 h, the reaction mixture was cooled to −90 °C and a solution of tetrabromoethane (10.86 g, 32.8 mmol) in THF (15 mL) was added dropwise. After 1 h at this temperature, water (20 mL) was added dropwise. The aqueous phase was extracted with ethyl acetate many times. The organic phases were combined, dried over anhydrous sodium sulphate, filtered and concentrated. The residue was purified by chromatography on silica gel (ethyl acetate/cyclohexane 7:3) to afford 3.1 g (72%) of **42** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 3.36 (s, 3H), 3.55–3.58 (m, 2H), 3.87–3.90 (m, 2H), 5.37 (s, 2H), 7.48 (d, J = 5.2 Hz, 1H), 8.08 (d, J = 5.2 Hz, 1H), 8.48 (s, 1H).

6.1.40. 4-Benzyloxy-3-[(2-methoxyethoxy)methoxy]pyridine (43)

A solution of benzyl alcohol (3.55 mL, 34.4 mmol) in DMSO (5 mL) was added dropwise to a stirred suspension of NaH (1.37 g, 57.2 mmol) in DMSO (15 mL) under nitrogen. The mixture was heated at 50 °C for 30 min and then cooled to 10 °C. After adding dropwise a solution of **42** (6.0 g, 22.9 mmol) in DMSO (40 mL), the temperature was increased to 20 °C and the whole reaction mixture was stirred at this temperature for 1 h. Diethyl ether and water were added. The organic phase was separated, washed with water, dried over anhydrous sodium sulphate, filtered and concentrated. The residue was purified by chromatography on silica gel (ethyl acetate/cyclohexane 7:3) to afford 5.4 g (82%) of **43** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 3.39 (s, 3H), 3.52–3.55 (m, 2H), 3.86–3.88 (m, 2H), 5.16 (s, 2H), 5.29 (s, 2H), 6.84 (d, J = 5.5 Hz, 1H), 7.31–7.41 (m, 5H), 8.17 (d, J = 5.5 Hz, 1H), 8.38 (s, 1H).

6.1.41. 4-Hydroxy-3-[(2-methoxyethoxy)methoxy]pyridine (44)

10% Pd/C (520 mg) was added to a solution of **43** (5.2 g, 18.4 mmol) in methanol (60 mL). The mixture was stirred under H_2 atmosphere for 1 h. The catalyst was filtered off and the filtrate was concentrated to give 3.57 g (98%) of **44** as a white solid: mp 89.0 °C; ^{1}H NMR ($CDCl_3$) δ 3.35 (s, 3H), 3.48–3.58 (m, 2H), 3.81–3.86 (m, 2H), 5.24 (s, 2H), 6.67 (d, J = 6.9 Hz, 1H), 7.69 (d, J = 6.9 Hz, 1H), 7.89 (s, 1H).

6.2. Binding studies. Biological assays

Details of the binding experiments to the nicotinic receptor subtypes have been previously reported [18]. The K_i values were obtained by simultaneously fitting three independent saturation and competition binding experiments for each compound on each subtype. The experimental data were analyzed by means of a non-linear least square procedure using the LIGAND program.

6.3. Electrophysiological experiments

The human α3β4 and α4β2 nAChRs were expressed by transient transfection in the rat anterior pituitary GH4C1 cell line [37]. Transient transfection was achieved as previously described [18].

Dose-response relationships were constructed by sequentially applying different concentrations of agonists, and normalizing the obtained current amplitudes to the value obtained by applying 1 mM ACh on the same cell. For quantitative estimations of agonist actions, dose-response relationships were fitted, when possible, by the equation

$$I = Imax\ ([C]^{nH}/(EC_{50}^{nH} + [C]^{nH})),$$

where I is the peak current amplitude induced by the agonist at concentration [C], Imax is the maximum response of the cell, nH is the Hill coefficient and EC_{50} is the concentration for which a half maximum response is induced.

For antagonist actions, dose-response relationships were constructed by sequentially applying ACh at the EC_{50} value (145 μM for α3β4, 3 μM for α4β2) or ACh plus different concentrations of the compounds (as indicated), and normalizing the obtained current amplitudes to the value obtained by ACh on the same cell. Antagonist dose-response relationship were fitted by the equation

$$I = Imax\ (IC_{50}^{nH}/(IC_{50}^{nH} + [C]^{nH})),$$

where IC_{50} is the concentration for which a half maximum response is observed.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2016.10.048.

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