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5-(2-Pyrrolidinyl)oxazolidinones and 2-(2-pyrrolidinyl)benzodioxanes: Synthesis of all the stereoisomers and α4β2 nicotinic affinity

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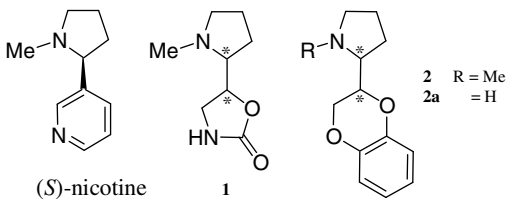
ABSTRACT

The four stereoisomers of 2-oxazolidinone 5-substituted with 1-methyl-2-pyrrolidinyl (**1**), of 1,4-benzodioxane 2-substituted with the same residue (**2**) and of the nor-methyl analogue of this latter (**2a**) were synthesized as candidate nicotinoids. Of the 12 compounds, two N-methylated pyrrolidinyl-benzodioxane stereoisomers, namely those with the same relative configuration at the pyrrolidine stereocentre as (*S*)-nicotine, bind at α4β2 nicotinic acetylcholine receptor with submicromolar affinity. Consistently with the biological data, docking analysis enlightens significant differences in binding site interactions not only between **1** and **2**, but also between **2** and **2a** and between the stereoisomers of **2** accounting for the critical role played, in the case of the pyrrolidinyl-benzodioxanes, by the chirality of both the stereolabile and stereostable stereogenic atoms, namely the protonated tertiary nitrogen and the two asymmetric carbons.

Neuronal nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels playing an important role in the regulation of neurotransmission in the CNS. Their dysfunction has been related to a number of severe brain pathologies, including Parkinson's and Alzheimer's diseases, schizophrenia, anxiety and some forms of epilepsy. Novel ligands for neuronal nAChRs, in particular for the two major α4β2 and α7 brain subtypes, may have a great therapeutic potential.[1,2] Subtype selective nAChR ligands have been developed over the last decade leading to the formulation of reliable α4β2 and α7 pharmacophores.[3–6] Furthermore, models of the ligand binding domain or full-length models for these nicotinic receptor subtypes have been published.[7–10] Recently, we have reported the *RS* and *SR* enantiomers of 5-(1-methyl-2-pyrrolidinyl)oxazolidinone (**1**) and 2-(1-methyl-2-pyrrolidinyl)-1,4-benzodioxane (**2**) as potential nicotinoids, where the typical nicotinoid 2-pyrrolidinyl residue is linked to a cyclic hydrogen bond acceptor and π-electron rich group (HBA/π), demonstrating that one of the two synthesized enantiomers of **2**, namely that with the same relative configuration at the pyrrolidine stereocentre as (*S*)-nicotine, displays a submicromolar α4β2 affinity.[11] This result and the intriguing feature of two vicinal stereocentres, positioned on the cationic head and on the HBA/π group, respectively, and connected by the only rotatable bond, prompted us to investigate also the *SS* and *RR* enantiomers of **1** and **2**. Therefore, a new synthetic strategy was designed to obtain all the four stereoisomers of pyrrolidinyl-oxazolidinone and of pyrrolidinyl-benzodioxane. Considering the promising results previously obtained for one enantiomer of **2**, we prepared also the four stereoisomers of **2a**, the nor-methyl derivative of **2**. In fact, it is known that the presence or the absence of the *N*-methyl group at the cationic head represents a critical feature for several nicotinoids resulting in unexpectedly drastic affinity changes, as well demonstrated by the opposite cases of nicotine, whose nor-methyl analogue displays an almost 100-fold lower affinity for α4β2 nAChR, and of epibatidine, which exhibits reduced α4β2 binding when N-methylated.[12]



The key step of the previous syntheses of **1** and **2** was the reaction of the diastereomeric mixture of *RS* and *SS* (1-carbobenzyloxy-2-pyr-

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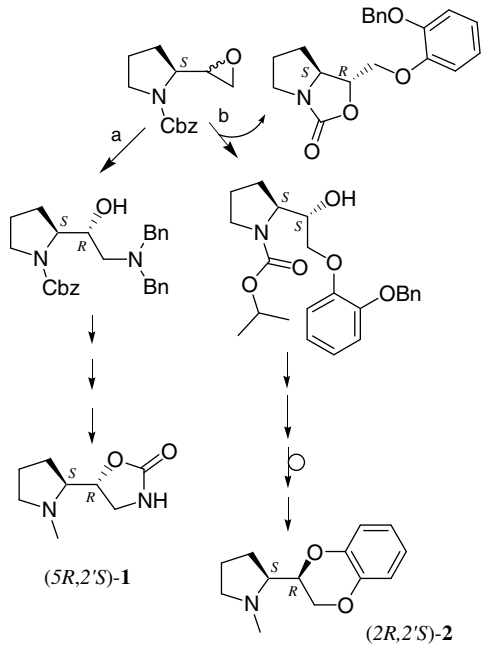
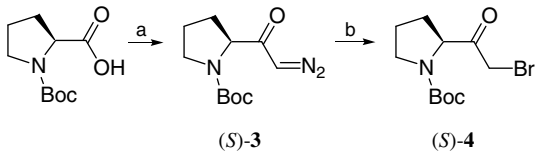
rolidinyl)oxiranes, epimers at the epoxide chiral centre prepared from (*S*)-(1-carbobenzyloxy-2-pyrrolidinyl)methanol, with dibenzylamine and 2-benzyloxyphenate, respectively.[11] However, such a reaction allows only one diastereomer of the resultant secondary alcohol to be isolated, namely that deriving from the *SS* pyrrolidinyloxirane and having *R* and *S* configuration at the exocyclic stereogenic carbon of the aminoalcohol and of the phenoxyalcohol, respectively (Scheme 1). Upon this reaction, the other oxirane, the *RS* diastereomer with *R* configuration at epoxide chiral centre, has a different fate, which is intramolecular cyclisation to tetrahydropyrroloxazolone, as demonstrated in the case of the synthesis of **2** by the isolation of the *o*-benzyloxyphenoxymethyl substituted tetrahydro-pyrroloxazolone shown in Scheme 1, and presumable, but not demonstrated, in the case of the synthesis of **1**.

Symmetrically, the specular diastereomeric mixture of *SR* and *RR* (1-carbobenzyloxy-2-pyrrolidinyl)oxiranes, epimers at the epoxide chiral centre prepared from (*R*)-(1-carbobenzyloxy-2-pyrrolidinyl)methanol, provides only the *SR* aminoalcohol and the *RR* phenoxyalcohol. As a consequence, only one pair of enantiomers of **1** and **2** could be obtained by these synthetic pathways, namely the *RS* and *SR* enantiomers.[11] The present synthesis, which yields all the stereoisomers of **1** and **2**, has 2-bromoacetylpyrrolidine in place of 2-pyrrolidinyloxirane as a key intermediate. (*S*)-*N*-*t*-Butoxycarbonyl-2-bromoacetylpyrrolidine [(*S*)-**4**] was prepared from (*S*)-*N*-*t*-butoxycarbonylproline. Treatment of the N-protected amino acid with triethylamine and isobutyl chloroformate and then with diazomethane yielded (*S*)-*N*-*t*-butoxycarbonyl-2-diazoacetylpyrrolidine [(*S*)-**3**],[13] which was converted into (*S*)-**4**[14] by reaction with aqueous HBr (Scheme 2).

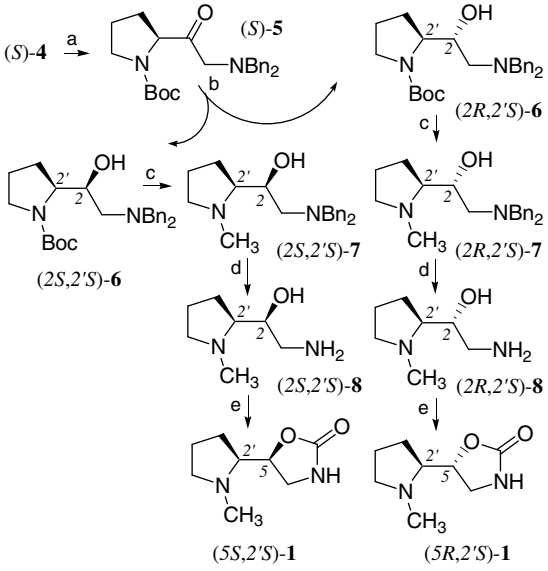
The nucleophilic substitution of bromide with dibenzylamine afforded the dibenzylaminomethylketone [(*S*)-**5**][15] (Scheme 3). Racemisation of bromoketone during the reaction was excluded on the basis of the chiral HPLC analysis of (*S*)-**5** and (*R*)-**5**, obtained from (*S*)-**4** and (*R*)-**4**, respectively. Consistently with this finding, no significant variation of optical rotation was observed after recrystallization of the two substitution products. The successive carbonyl reduction with lithium aluminium hydride yielded a 60:40 mixture of secondary aminoalcohols (2*S*,2′*S*)-**6**[16] and



Scheme 1. Reagents and conditions: (a) dibenzylamine, 2-propanol, MW irradiation (150 °C, 100 W), 15 min, 48%; (b) 2-benzyloxyphenol, potassium carbonate, 2-propanol, reflux, 24 h, 42%.



Scheme 2. Reagents and conditions: (a) *i*BuOCOCl, triethylamine, Et_2O, −10 °C, 20 min; CH_2N_2, 0 °C, 45 min and room temperature over-night, 78%; (b) aqueous HBr, Et_2O, 0 °C, 30 min and room temperature, 1 h, 71%.



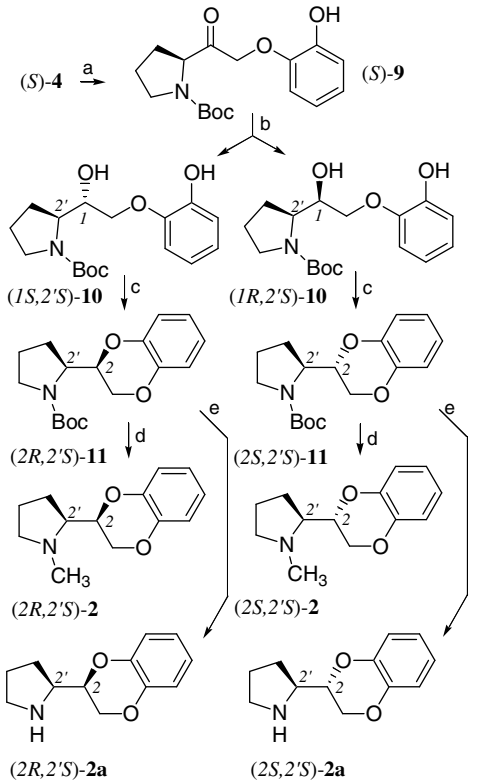
Scheme 3. Reagents and conditions: (a) dibenzylamine Et_2O, 0 °C, 2 h, 80%; (b) $LiAlH_4$, THF, 0 °C, 1 h, 51% [(2*S*,2′*S*)-**6**] and 35% [(2*R*,2′*S*)-**6**]; (c) $LiAlH_4$, THF, reflux, 4 h, 86% [(2*S*,2′*S*)-**7**] and 85% [(2*R*,2′*S*)-**7**]; (d) H_2–Pd/C, MeOH, 5 atm, over-night, 97% [(2*S*,2′*S*)-**8**] and 84% [(2*R*,2′*S*)-**8**]; (e) 1,1′-carbonyldiimidazole, THF, 3 h, 59% [(2*S*,2′*S*)-**1**] and 51% [(2*R*,2′*S*)-**1**].

(2*R*,2′*S*)-**6**,[17] which were separated by crystallization of the major *SS* diastereomer and chromatographic isolation of the minor *RS* diastereomer from the crystallization mother liquors. Absolute *R* configuration was assigned to the oxygen-bound asymmetric carbon of this latter diastereomer, second eluted in the chromatographic purification, and, consequently *S* configuration to the same carbon of the other diastereomer on the basis of the successive reduction of N-protecting group to methyl, which produced, when applied to the chromatographically purified diastereomer, diamine (2*R*,2′*S*)-**7**[18] with the same ^{1}H NMR as the *RS* diamine previously synthesized via pyrrolidinyloxirane and diamine (2*S*,2′*S*)-**7**[19] with a quite different ^{1}H NMR, when applied to the crystallized diastereomer. Hydrogenolitic debenzylation afforded the primary amines (2*S*,2′*S*)-**8**[20] and (2*R*,2′*S*)-**8**,[21] which were finally cyclised to (5*S*,2′*S*)-**1**[22] and (5*R*,2′*S*)-**1**[23] by reaction with 1,1′-carbonyldiimidazole.

Starting from (*R*)-*N*-*t*-butoxycarbonylproline, the same synthetic route[24–32] illustrated in Schemes 2 and 3 led to (5*R*,2′*R*)-**1**[33] and (5*S*,2′*R*)-**1**.[34]

The absolute configuration of the oxazolidinone stereocenter of the four stereoisomers of **1** was established by comparison with the *RS* and *SR* enantiomers previously synthesized via prolinal and 2-pyrrolidinyloxirane.[11]

The same synthetic strategy was followed to prepare the four stereoisomers of **2** reacting (*S*)-**4** with pyrocatechol in the presence of a base (Scheme 4). In this case, however, suitable reaction conditions had to be found to avoid the bromomethylketone racemisation, which was, for instance, complete using potassium carbonate



Scheme 4. Reagents and conditions: (a) pyrocatechol monosodium salt, acetone, 22 °C, over-night, 66%; (b) $NaBH_4$, THF, −10 °C, 2 h, 45% [(1*R*,2′*S*)-**10**] and 30% [(1*S*,2′*S*)-**10**]; (c) triphenylphosphine, DEAD, THF, reflux, over-night, 93% [(2*S*,2′*S*)-**11**] and 88% [(2*R*,2′*S*)-**11**]; (d) $LiAlH_4$, THF, reflux, 2 h, 89% [(2*S*,2′*S*)-**2**] and 94% [(2*R*,2′*S*)-**2**]; (e) TFA, DCM, 22 °C, 2 h, 83% [(2*S*,2′*S*)-**2a**] and 93% [(2*R*,2′*S*)-**2a**].

in acetone, as suggested by the almost null optical activity of isolated (*S*)-**9** and then proved by its chiral HPLC analysis. The use of preformed pyrocatechol monosodium salt in methanol, DMF, or acetone resulted in high enantiomeric excesses of (*S*)-**9**, generally not lower than 85%. In particular, the reaction in acetone at room temperature allowed (*S*)-**9** to be obtained in 66% yield and with >98% ee after 24 h. At 30 °C, the same reaction gave (*S*)-**9**[35] in 90% yield after 1.5 h, but with lower ee (85%). The successive carbonyl reduction with sodium borohydride in THF afforded a 60:40 mixture of secondary alcohols (1*R*,2′*S*)-**10**[36] and (1*S*,2′*S*)-**10**,[37] thus showing the same diastereopreference observed for the analogous reduction of (*S*)-**5**, which had produced a (2*S*,2′*S*)-**6**/(2*R*,2′*S*)-**6** mixture in 60/40 ratio. In fact, it is to be noticed that (1*R*,2′*S*)-**10** and (1*S*,2′*S*)-**10** have the same relative configuration of (2*S*,2′*S*)-**6** and (2*R*,2′*S*)-**6**, respectively. The two diastereomer were separated by chromatography on silica gel and the first eluted, solid *RS* diastereomer further purified by crystallization from diethyl ether. HPLC analysis demonstrated a >99.5% diastereomeric purity of the two separated secondary alcohols, while chiral HPLC analysis allowed to ascertain their unvaried enantiomeric excess with respect to (*S*)-**9**. The successive cyclisation by intramolecular Mitsunobu reaction yielded the pyrrolidinyl-benzodioxanes (2*R*,2′*S*)-**11**[38] and (2*S*,2′*S*)-**11**,[39] whose N-protecting group was reduced to methyl to give (2*R*,2′*S*)-**2**[40] and (2*S*,2′*S*)-**2**[41] or removed to give (2*R*,2′*S*)-**2a**[42] and (2*S*,2′*S*)-**2a**,[43] respectively.

Starting from (*R*)-*N*-*t*-butoxycarbonylproline, the same synthetic route[44–48] illustrated in Schemes 2 and 4 led to (2*S*,2′*R*)-**2**[49] and (2*R*,2′*R*)-**2**[50] and to the respective nor-methyl derivatives (2*S*,2′*R*)-**2a**[51] and (2*R*,2′*R*)-**2a**.[52]

As previously reported,[11] the absolute configuration of the benzodioxane C_2 was established on the basis of compared conformational and ^1H NMR analysis, recognizing the relative anti disposition of benzodioxane methine axial proton not only to one of the two benzodioxane methylene protons but also to pyrrolidine methine proton as indicative of an opposite configuration of benzodioxane C_2 to that of pyrrolidine stereocenter, since the anti disposition of the two methine protons is sterically forbidden when the two stereocenters have the same configuration. Consistently with this statement, which had allowed to establish the *RS* and *SR* configurations of the two enantiomers of **2** prepared via oxirane, the ^1H NMR spectra of the two new enantiomers of **2** obtained via bromoketone showed the anti disposition of benzodioxane methine proton only to one of the two benzodioxane methylene protons, but not to the pyrrolidine methine proton, thus proving their *SS* and *RR* configurations.

The optical powers and the melting points of the *RS* and *SR* forms of **1** and the optical powers of the *RS* and *SR* forms of **2** prepared via bromoketone, as described here, were significantly higher than those of the same stereoisomers synthesized from prolinal, as previously reported. This indicates that the synthesis via aldehyde implies partial racemisation.

We evaluated the affinity towards the α4β2 and the α7 subtypes present in rat cortex membranes by binding studies using, as ligands, [^{3}H]-epibatidine and [^{125}I]-αBungarotoxin, respectively.[53] Nicotine was included in the series for comparison as well as the *RS* and *SR* forms of **1** and **2**, considering their higher enantiomeric purity resulting from the new preparation method. As shown in Table 1 and as previously reported for (5*R*,2′*S*)-**1** and (5*S*,2′*R*)-**1**,[11] we found that the four pyrrolidinyl-oxazolidinones are virtually devoid of nicotinic affinity, with the exception of (5*S*,2′*S*)-**1**, which shows a modest micromolar α4β2 affinity. The two *RS* and *SR* stereoisomers, prepared by the new method, exhibit slightly higher, but always very low affinities compared with those previously found[11] for the same forms differently synthesized. On the contrary, two out of the four *N*-methyl pyrrolidinyl-benzodioxanes **2**, those with the same *S* configuration at the pyrrolidine stereocenter as (−)-nicotine, show a moderate submicromolar affinity for α4β2 nAChR and are 50- to 100-fold more potent than their enantiomers. In particular, a significant increase of enatioselectivity was observed for the newly synthesized *RS*–*SR* enantiomeric pair in comparison with the previously reported data[11] and this increment is consistent with the higher enantiomeric purity ensured by the new preparation method reported here. No enantiopreference

Table 1
Nicotine and compounds **1**, **2** and **2a**: affinity for native receptor subtypes, present in rat brain membranes, labelled by [^{3}H]-epibatidine, and [^{125}I]-αBungarotoxin and for α_2-ARs, present in rat cortex membranes, labelled by [^{3}H]RS 79948-197

	α4β2 nAChR [^{3}H]-Epi K_i (μM)	α7 nAChR [^{125}I]-αBgtx K_i (μM)	α_2-AR [^{3}H]RS 79948-197 K_i (μM)
(−)-Nicotine	0.004 (18)	0.234 (29)	
(5*R*,2′*S*)-**1**	35 (34)	100 (78)	
(5*S*,2′*R*)-**1**	37 (34)	43 (69)	
(5*S*,2′*S*)-**1**	2.7 (33)	21 (62)	
(5*R*,2′*R*)-**1**	40 (31)	>100	
(2*R*,2′*S*)-**2**·HCl	0.26 (32)	21 (44)	0.77 (6)
(2*S*,2′*R*)-**2**·HCl	12.5 (17)	35 (45)	0.47 (11)
(2*R*,2′*R*)-**2**·HCl	43.8 (16)	14.8 (35)	6.67 (20)
(2*S*,2′*S*)-**2**·HCl	0.47 (30)	14.6 (54)	1.67 (7)
(2*R*,2′*S*)-**2a**·HCl	2.1 (30)	36 (41)	1.35(3)
(2*S*,2′*R*)-**2a**·HCl	88 (18)	14.7 (42)	14 (27)
(2*R*,2′*R*)-**2a**·HCl	11.6 (17)	27 (38)	6.33 (6)
(2*S*,2′*S*)-**2a**·HCl	14.4 (32)	32.2 (45)	0.64 (5)
K_d (nM)	0.036 (22)	0.3 (35)	1.01 (0.5)

The K_d and K_i values were derived from [^{3}H]-epibatidine, [^{125}I]-αBungarotoxin and [^{3}H]RS 79948-197 saturation and two competition binding experiments on rat brain membranes as described in Refs. 53–55. The curves were fitted using a nonlinear least squares analysis program and the *F* test. The numbers in brackets represent the %CV.

was found in the case of the lower affinities shown by **2** stereoisomers for α7 nAChR.

The two stereoisomers of **2** with *S* configuration at pyrrolidine C_2 display a remarkable similar α4β2 versus α7 selectivity. The N-demethylation of **2** results in both modest α4β2 and α7 affinities: the K_i of all the nor-methyl derivatives are lower than 10 μM, excepting (2*R*,2′*S*)-**2a**, which shows, however, only a modest micromolar α4β2 affinity.

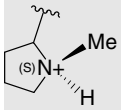
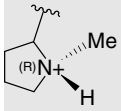
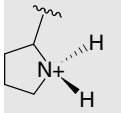
Furthermore, the structural analogy of benzodioxane derivatives to α_2-adrenergic receptor (α_2-AR) ligands such as efaroxan, imiloxan and, especially, idazoxan and the reported affinity of α_2-AR ligands for receptors labelled by [^{3}H]-epibatidine[54] induced to evaluate the binding of **2** and **2a** also to α_2-ARs. As previously reported for (2*R*,2′*S*)-**2** and (2*S*,2′*R*)-**2**,[11] also (2*S*,2′*S*)-**2a** proved to compete with [^{3}H]RS 79948-197 for rat cortex α_2-ARs with submicromolar affinity thus confirming such a coexistence of affinities for the two different receptor systems.[55,56]

Figure 1 depicts the best docked complexes of α4β2 nAChR binding site with the two most affinitive isomers of **2**, namely (2*R*,2′*S*)-**2** and (2*S*,2′*S*)-**2**, the chiral nitrogen of the ligands being in *S* configuration.[57] The protonated nitrogen of both isomers stabilizes a reinforced H-bond with the carbonyl group of Trp147(α4), while the carbon skeleton of their pyrrolidine ring contacts a set of apolar residues not displayed for clarity [e.g. Val109(β2), Phe117(β2), Leu119(β2), and Tyr195(α4)]. The two stereoisomers mainly differ in the benzodioxane interactions: H-bonds are formed by O(1) of (2*S*,2′*S*)-**2** with Lys77(β2) and Tyr195(α4), whereas the same amino acids form H-bonds with O(4) of (2*R*,2′*S*)-**2**. The greater distance between mutually repulsive Lys77 and pyrrolidine nitrogen resulting from the interaction with O(4) instead of O(1) may explain the higher affinity of (2*R*,2′*S*)-**2** and the same repulsive interaction, exalted by the demethylation of pyrrolidine nitrogen, could be responsible for the scarce affinity of the four stereoisomers of the nor-methyl derivative **2a**, even if these conserve the H-bond with Trp147. The two less affinitive isomers of **2**, namely those having the pyrrolidine stereocenter in *R* configuration, are unable to correctly arrange the pyrrolidine ring. For the pyrrolidinyl-oxazolidinones **1**, the docking analysis shows that the interaction of the protonated nitrogen with Trp147, also observed for the pyrrolidinyl-benzodioxanes, is not associated, differently from these latter, with significant contacts of the extra-pyrrolidine portion with Tyr195 and Lys77. Among the four oxazolidinones, the best docking score was obtained for the *SS* form, which is 13- to 15-fold more affinitive than the other stereoisomer.

Our previous investigations, based on the superposition of the lowest energy conformers of the four stereoisomers of **2** onto (*S*)-nicotine, had indicated the two *SR* and *RS* stereoisomers as the best candidates to display nicotinic affinity, with a preference for (2*R*,2′*S*)-**2**, thanks to the same chirality of (*S*)-nicotine and the maximum closeness of the pyridine nitrogen of nicotine to the benzodioxane O(4). The submicromolar α4β2 binding affinity of (2*R*,2′*S*)-**2**, significantly higher than that of (2*S*,2′*R*)-**2**, was consistent with those indications. Now, the additional α4β2 affinity data, submicromolar also for (2*S*,2′*S*)-**2**, complete the picture, while docking results better support SAR analysis enlightening (a) similar patterns of beneficial interactions of the charged *N*-methyl pyrrolidine residue for the two most affinitive *RS* and *SS* isomers of **2** and for (*S*)-nicotine, contrarily to the two modestly affinitive isomers with *R* configuration at the pyrrolidine stereocenter, and (b) different, but ever productive accommodations of the HBA moiety, namely the dioxane ring, for the two most affinitive *RS* and *SS* isomers of **2**. Such a different accommodation of the dioxane ring, which seems to interact with the same amino acids of the binding site through its O(4) in (2*R*,2′*S*)-**2** and through its O(1) in (2*S*,2′*S*)-**2**, would justify the failed prediction of the affinity of the latter by the previous conformational analysis, which had emphasized the maximum closeness of O(4) to pyridine nitrogen in the superposition onto nicotine undervaluing the interaction potentiality of O(1).[11] In the case of **1**, the same analysis had let us hope that (5*R*,2′*R*)-**1** could show a good or moderate nicotinic affinity. The present data deny such a prediction indicating that identical relative configuration to (*S*)-nicotine at the pyrrolidine stereocentre is more important than the other conformational properties we had previously considered for **1** stereoisomers.[11]

Finally, the poor affinities of the four nor-methyl derivatives **2a** cause to reflect over the role of the *N*-methyl group. It is known

Table 2
Docking scores (kcal/mol) for nicotine, nornicotine, **2** and **2a** in a α4β2 nicotinic receptor model

	(*S*)-Nicotine	(2*R*,2′*S*)-**2**	(2*S*,2′*S*)-**2**	(2*S*,2′*R*)-**2**	(2*R*,2′*R*)-**2**
	−76.03	−65.25	−63.25	−63.08	−59.66
	−47.57	−39.81	−39.22	−39.49	−34.76
	(*S*)-Nornicotine	(2*R*,2′*S*)-**2a**	(2*S*,2′*S*)-**2a**	(2*S*,2′*R*)-**2a**	(2*R*,2′*R*)-**2a**
	−55.67	−38.90	−44.22	−41.36	−39.58

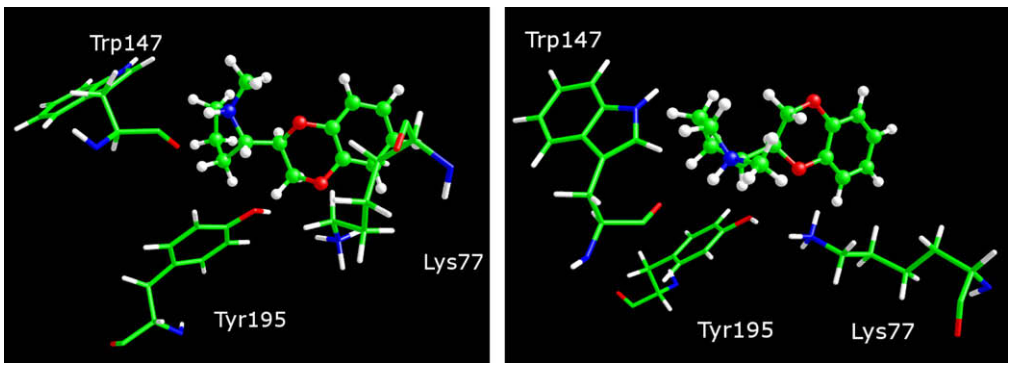


Figure 1. Main polar contacts stabilizing the putative complexes between α4β2 nAChR binding site and (2*R*,2′*S*)-**2** (left) and (2*S*,2′*S*)-**2** (right).

that N-demethylation of nicotinoids can enhance binding affinity, especially when the ligand molecule is relatively rigid and the configuration preferentially assumed by the chiral tertiary *N*-methyl ammonium leads to an unfavourable disposition of the nitrogen-bound proton and methyl.[58] Here, we observe the opposite trend: the N-demethylation of **2** reduces the binding affinity, as in the case of nicotine. We impute the reduced affinity of **2a** to a repulsive interaction of one of its two nitrogen protons with a binding site amino acidic residue. Interestingly, the same unfavourable interaction is shown by the unique nitrogen proton of **2** stereoisomers, when their chiral nitrogen assumes *R* configuration, and it is significant that **2a** and **2** with N^+ in *R* configuration have similar docking scores, worse than **2** with N^+ in *S* configuration (see Table 2). This would further address the key role, often neglected, of the protonation-induced chirality, whose implication in the interaction with the binding site transcends the mere stereoelectronic effects of *N*-methyl group.

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13. (*S*)-**3**: Isolated as a yellow solid after chromatographic separation on silica gel (eluent 7:3 cyclohexane/ethyl acetate); mp 45–46 °C; IR 2104 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.42, 1.43 and 1.46 (3s, 9H), 1.85–2.19 (m, 4 H), 3.42–3.50 (m, 2H), 4.19 and 4.28 (2br s, 1H), 5.38 and 5.47 (2br s, 1H).
14. (*S*)-**4**: Isolated as a pale yellow oil by chromatography on silica gel (eluent 7:3 cyclohexane/ethyl acetate); $[\alpha]_D^{25}$ −67.0 (*c*1, acetone); ^1H NMR ($CDCl_3$) δ 1.41 and 1.44 (2s, 9H), 1.87–2.03 (m, 3H), 2.12–2.29 (m, 1H), 3.41–3.56 (m, 2H), 4.02–4.17 (m, 2H), 4.45–4.55 (m, 1H).
15. (*S*)-**5**: Isolated as a white solid after chromatographic separation on silica gel (eluent 7:3 cyclohexane/ethyl acetate) and crystallization from hexane; mp 89–90 °C; $[\alpha]_D^{25}$ −44.6 (*c*1, methanol); ee 99.8% (by HPLC on a Kromasil AmyCoat column; 9:1 hexane/isopropanol, 1.5 ml/min at 254 nm; t_R 6.35 min); ^1H NMR (DMSO-d_6, 100 °C) δ 1.24 (s, 9H), 1.51–1.77 (m, 3H), 1.98–2.07 (m, 1H), 3.23–3.33 (m, 2H), 3.37 (s, 2H), 3.61–3.74 (2d, 4H), 4.27–4.32 (dd, 1H), 7.20–7.36 (m, 10H).
16. (2*S*,2′*S*)-**6**: Isolated as a white solid by crystallization from diisopropyl ether and furthermore from the crystallization mother liquors by chromatography on silica gel (eluent 7:3 cyclohexane/ethyl acetate); mp 101–102 °C; $[\alpha]_D^{25}$ −78.5 (*c*1, methanol); ^1H NMR (DMSO-d_6, 100 °C) δ 1.37 (s, 9H), 1.57–1.77 (m, 4H), 2.39–2.53 (m, 2H), 3.00–3.05 (m, 1H), 3.29–3.36 (m, 1H), 3.57–3.70 (m, 4H), 3.76–3.79 (m, 1H), 3.95–3.98 (m, 1H), 4.17 (br s, 1H), 7.17–7.34 (m, 10H).
17. (2*R*,2′*S*)-**6**: Isolated as a white solid by chromatography on silica gel (eluent 7:3 cyclohexane/ethyl acetate); mp 70–71 °C; $[\alpha]_D^{25}$ −34.5 (*c*1, methanol); ^1H NMR (DMSO-d_6, 100 °C) δ 1.35 (s, 6H), 1.41 (s, 3H), 1.42–1.96 (m, 4H), 2.38–2.47 (m, 2H), 3.07–3.15 (m, 1H), 3.28–3.36 (m, 1H), 3.43–3.48 (m, 2H), 3.69–3.74 (m, 2H), 3.77–3.79 (m, 1H), 4.12–4.15 (m, 1H), 4.16 (br s, 1H), 7.18–7.35 (m, 10H).
18. (2*R*,2′*S*)-**7**: isolated as a colourless oil by chromatography on silica gel (eluent 97:3 ethyl acetate/triethylamine); $[\alpha]_D^{25}$ −32.8 (*c*1, methanol); ^1H NMR spectrum as described in Ref. 11.
19. (2*S*,2′*S*)-**7**: Isolated as a colourless oil by chromatography on silica gel (eluent 97:3 ethyl acetate/triethylamine); $[\alpha]_D^{25}$ −71.4 (*c*1, methanol); ^1H NMR ($CDCl_3$) δ 1.34–1.39 (m, 1H), 1.62–1.77 (m, 3H), 2.17–2.33 (m, 2H), 2.35 (s, 3H), 2.48 (d, *J* = 6 Hz, 2H), 2.97–3.03 (m, 1H), 3.56–3.63 (m, 1H), 3.50 and 3.76 (2 d, *J* = 13.5 Hz, 4H), 7.21–7.32 (m, 10H).
20. (2*S*,2′*S*)-**8**: Isolated as an oil by concentration of the reaction solution in methanol after removal of the catalyst; ^1H NMR ($CDCl_3$) δ 1.53–1.56 (m, 1H), 1.70–1.77 (m, 2H), 1.85–1.94 (m, 1H), 2.31–2.42 (m, 2H), 2.43 (s, 3H), 2.58 (dd, *J* = 12.7, 8.3 Hz, 1H), 2.77 (dd, *J* = 12.7, 3.3 Hz, 1H), 3.01–3.07 (m, 1H), 3.20–3.26 (m, 1H).
21. (2*R*,2′*S*)-**8**: Isolated as an oil by concentration of the reaction solution in methanol after removal of the catalyst; ^1H NMR spectrum as described in Ref. 11.
22. (5*S*,2′*S*)-**1**: Isolated as a solid by chromatography on silica gel (eluent 8:2 toluene/methanol); mp 128–129 °C (dec); $[\alpha]_D^{25}$ −7.0 (*c*1, methanol); ^1H NMR ($CDCl_3$) δ 1.49–1.60 (m, 1H), 1.72–1.98 (m, 3H), 2.30 (pseudo q, *J* = 8.7 Hz, 1H), 2.45 (s, 3H), 2.63 (pseudo q, *J* = 8.7 Hz, 1H), 3.05–3.11 (m, 1H), 3.33 (pseudo t, *J* = 8.5 Hz, 1H), 3.57 (pseudo t, *J* = 8.5 Hz, 1H), 4.65 (pseudo q, *J* = 7.7 Hz, 1H), 5.40 (br s, 1H).
23. (5*R*,2′*S*)-**1**: Isolated as a solid by chromatography on silica gel (eluent 8:2 toluene/methanol); mp 173–174 °C; $[\alpha]_D^{25}$ −96.0 (*c*1, methanol); ^1H NMR spectrum as described in Ref. 11.
24. (*R*)-**3**: Mp and ^1H NMR identical to the enantiomer.
25. (*R*)-**4**: $[\alpha]_D^{25}$ +66.4 (*c*1, acetone); ^1H NMR identical to the enantiomer.
26. (*R*)-**5**: $[\alpha]_D^{25}$ +46.1 (*c*1, MeOH); ee 98.8% (by HPLC on a Kromasil Amycoat column; 9:1 hexane/isopropanol, 1.5 ml/min at 254 nm; t_R 3.90 min); mp and ^1H NMR identical to the enantiomer.
27. (2*R*,2′*R*)-**6**: $[\alpha]_D^{25}$ +77.7 (*c*1, MeOH); mp and ^1H NMR identical to the enantiomer.
28. (2*S*,2′*R*)-**6**: $[\alpha]_D^{25}$ +32.8 (*c*1, MeOH); mp and ^1H NMR identical to the enantiomer.
29. (2*R*,2′*R*)-**7**: $[\alpha]_D^{25}$ +74.4 (*c*1, MeOH); ^1H NMR identical to the enantiomer.
30. (2*S*,2′*R*)-**7**: $[\alpha]_D^{25}$ +35.4 (*c*1, MeOH); ^1H NMR identical to the enantiomer.
31. (2*R*,2′*R*)-**8**: ^1H NMR identical to the enantiomer.
32. (2*S*,2′*R*)-**8**: ^1H NMR identical to the enantiomer.
33. (5*R*,2′*R*)-**1**: $[\alpha]_D^{25}$ +6.9 (*c*1, MeOH); mp and ^1H NMR identical to the enantiomer.
34. (5*S*,2′*R*)-**1**: $[\alpha]_D^{25}$ +94.9 (*c*1, MeOH); mp and ^1H NMR identical to the enantiomer.
35. (*S*)-**9**: Isolated as an oil by chromatography on silica gel (eluent 7:3 cyclohexane/ethyl acetate); $[\alpha]_D^{25}$ −91.8 (*c*1, MeOH); ee 98.2% (by HPLC on a Chiralcel OJ column; 9:1 hexane/isopropanol, 0.6 ml/min at 276 nm; t_R 13.90 min); ^1H NMR ($CDCl_3$) δ 1.48 and 1.51 (2s, 9H), 1.61–2.21 (m, 4H), 3.34–3.59 (m, 2H), 3.94 (d, *J* = 11.0 Hz, 1H), 4.09–4.14 (m, 1.5H), 4.27–4.30 (m, 0.5H), 6.83–6.94 (m, 4H).
36. (1*R*,2′*S*)-**10**: Isolated as a white solid by chromatography on silica gel (eluent 7:3 toluene/ethyl acetate) and crystallization from diethyl ether; mp 105–106 °C; $[\alpha]_D^{25}$ −47.8 (*c*1, chloroform); de 99.0% (by HPLC on a LiChroCART LiChrospher Si 60 column, 5 μm; 65:35 hexane/ethyl acetate, 2 ml/min at 276 nm; t_R 4.74 min); ee 98.8% (by HPLC on a Chiralcel OD-H column; 9:1 hexane/isopropanol, 0.5 ml/min at 276 nm; t_R 12.23 min); ^1H NMR ($CDCl_3$) δ 1.48 (s, 9H), 1.50–2.10 (m, 4H), 3.26–3.34 (m, 1H), 3.48–3.58 (m, 1H), 3.81 (br s, 1H), 3.98 (dd, *J* = 10.5, 5.2 Hz, 1H), 4.05–4.12 (m, 2H), 6.74–6.80 (m, 1H), 6.90–6.96 (m, 3H).
37. (1*S*,2′*S*)-**10**: Isolated as an oil by chromatography on silica gel (eluent 7:3 toluene/ethyl acetate); $[\alpha]_D^{25}$ −14.2 (*c*1, chloroform); de 99.4% (by HPLC on a LiChroCART LiChrospher Si 60 column, 5 μm; 65:35 hexane/ethyl acetate, 2 ml/min at 276 nm; t_R 5.95 min); ee 97.9% (by HPLC on a Chiralcel OD-H column; 9:1 hexane/isopropanol, 0.5 ml/min at 276 nm; t_R 12.42 min); ^1H NMR ($CDCl_3$) δ 1.46 (s, 9H), 1.79–2.05 (m, 4H), 3.23–3.31 (m, 1H), 3.46–3.54 (m, 1H), 3.86–3.92 (m, 1H), 4.04–4.10 (m, 3H), 6.77–6.82 (m, 1H), 6.89–6.96 (m, 3H).
38. (2*R*,2′*S*)-**11**: Isolated as a viscous oil by chromatography on silica gel (eluent 95:5 toluene/ethyl acetate); $[\alpha]_D^{25}$ −45.1 (*c*1, chloroform); ^1H NMR ($CDCl_3$) δ 1.45 (s, 9H), 1.89–2.06 (m, 4H), 3.32–3.55 (m, 2H), 3.92–3.95 (m, 1H), 4.22–4.37 (m, 3H), 6.80–6.88 (m, 4H).
39. (2*S*,2′*S*)-**11**: Isolated as a viscous oil by chromatography on silica gel (eluent 95:5 toluene/ethyl acetate); $[\alpha]_D^{25}$ −152.0 (*c*1, chloroform); ^1H NMR ($CDCl_3$) δ 1.47 (s, 9H), 1.91–2.06 (m, 3H), 2.16–2.22 (br s, 1H), 3.39–3.49 (m, 2H), 3.93–4.10 (m, 3H), 4.29–4.33 (dd, *J* = 11.3, 1.9 Hz, 1H), 6.81–6.89 (m, 4H).
40. (2*R*,2′*S*)-**2**: Isolated as an oil by concentration of the reaction mixture after adding dichloromethane, washing with water and filtering through celite; ^1H NMR spectrum as described in Ref. 11. (2*R*,2′*S*)-**2** Hydrochloride: Mp and ^1H NMR spectrum as described in Ref. 11; $[\alpha]_D^{25}$ +33.3 (*c*2, EtOH).
41. (2*S*,2′*S*)-**2**: Isolated as an oil by concentration of the reaction mixture after adding dichloromethane, washing with water and filtering through celite; ^1H NMR ($CDCl_3$) δ 1.43–1.94 (m, 4H), 2.22–2.30 (m, 1H), 2.43 (s, 3H), 2.47–2.53 (m, 1H), 3.10–3.15 (m, 1H), 4.00 (dd, *J* = 11.0, 8.3 Hz, 1H), 4.10 (ddd, *J* = 8.3, 4.4, 1.9 Hz, 1H), 4.28 (dd, *J* = 11.0, 1.9 Hz, 1H), 6.79–6.96 (m, 4H). (2*S*,2′*S*)-**2** Hydrochloride: Mp 144–145 °C; $[\alpha]_D^{25}$ −107.5 (*c*2, EtOH); ^1H NMR (DMSO-d_6) δ 1.87–2.10 (m, 4H), 3.11–3.14 (m, 1H), 2.89 and 2.90 (2s, 3H), 3.50–3.60 (m, 1H), 3.70–3.80 (m, 1H), 3.94 (dd *J* = 11.6, 8.5 Hz, 1H), 4.42 (dd, *J* = 11.6, 2.2 Hz, 1H), 4.70–4.73 (m, 1H), 6.85–6.95 (m, 4H), 9.95 (br s, 1H).
42. (2*R*,2′*S*)-**2a**: Isolated as a colourless oil by diethyl ether/aq KOH extraction from the reaction mixture and concentration of the organic phase; ^1H NMR ($CDCl_3$) δ 1.53–1.65 (m, 1H), 1.73–1.97 (m, 3H), 2.28 (br s, 1H), 2.88–2.96 (m, 1H), 3.03–3.10 (m, 1H), 3.21–3.28 (m, 1H), 3.97–4.05 (m, 2H), 4.22–4.30 (m, 1H), 6.79–6.91 (m, 4H). (2*R*,2′*S*)-**2a** Hydrochloride: Mp 154–155 °C; $[\alpha]_D^{25}$ +68.3 (*c*2, EtOH); ^1H NMR (DMSO-d_6) δ 1.75–2.12 (m, 4H), 3.11–3.23 (m, 2H), 3.59–3.68 (m, 1H), 4.07 (dd, *J* = 11.8, 6.6 Hz, 1H), 4.32 (dd, *J* = 11.8, 2.5 Hz, 1H), 4.45–4.50 (m, 1H), 6.83–6.93 (m, 4H), 9.46 (br s, 2H).
43. (2*S*,2′*S*)-**2a**: Isolated as a colourless oil by diethyl ether/aq KOH extraction from the reaction mixture and concentration of the organic phase; ^1H NMR ($CDCl_3$) δ

1.72–1.99 (m, 4H), 2.28 (s, 1H), 2.93–3.02 (m, 2H), 3.26–3.33 (m, 1H), 3.99 (dt, *J* = 7.2, 2.2 Hz, 1H), 4.05 (dd, *J* = 11.0, 7.2 Hz, 1H), 4.34 (dd, *J* = 11.0, 2.2 Hz, 1H), 6.79–6.89 (m, 4H). (2*S*,2′*S*)-**2a** Hydrochloride: Mp 149–150 °C; $[\alpha]_D^{25}$ −83.3 (*c*2, EtOH); ^{1}H NMR (DMSO-d_6) δ 1.80–2.07 (m, 4H), 3.18 (br s, 2H), 3.70–3.82 (m, 1H), 4.02 (dd, *J* = 11.6, 7.4 Hz, 1H), 4.46 (dd, *J* = 11.6, 2.2 Hz, 1H), 4.52 (dt, *J* = 7.4, 2.2 Hz, 1H), 6.82–6.91 (m, 4H), 9.46 (br s, 1H), 10.10 (br s, 1H).

44. (*R*)-**9**: $[\alpha]_D^{25}$ +95.8 (*c*1, MeOH); ee 100.0% (by HPLC on a Chiralcel OJ column; 9:1 hexane/isopropanol, 0.6 ml/min at 276 nm; t_R 12.11 min); ^{1}H NMR identical to the enantiomer.
45. (1*R*,2′*R*)-**10**: $[\alpha]_D^{25}$ +13.4 (*c*1, chloroform); de 99.0% (by HPLC on a LiChroCART LiChrospher Si 60 column, 5 μm; 65:35 hexane/ethyl acetate, 2 ml/min at 276 nm; t_R 5.95 min); ee 98.7% (by HPLC on a Chiralcel OD-H column; 9:1 hexane/isopropanol, 0.5 ml/min at 276 nm; t_R 11.20 min); ^{1}H NMR identical to the enantiomer.
46. (1*S*,2′*R*)-**10**: $[\alpha]_D^{25}$ +49.0 (*c*1, chloroform); de 100.0% (by HPLC on a LiChroCART LiChrospher Si 60 column, 5 μm; 65:35 hexane/ethyl acetate, 2 ml/min at 276 nm; t_R 4.74 min); ee 100.0% (by HPLC on a Chiralcel OD-H column; 9:1 hexane/isopropanol, 0.5 ml/min at 276 nm; t_R 13.89 min); ^{1}H NMR identical to the enantiomer.
47. (2*S*,2′*R*)-**11**: $[\alpha]_D^{25}$ +43.3 (*c*1, chloroform); ^{1}H NMR identical to the enantiomer.
48. (2*R*,2′*R*)-**11**: $[\alpha]_D^{25}$ +157.0 (*c*1, chloroform); ^{1}H NMR identical to the enantiomer.
49. (2*S*,2′*R*)-**2**: ^{1}H NMR spectrum as described in Ref. 11. (2*S*,2′*R*)-**2** Hydrochloride: Mp and ^{1}H NMR spectrum as described in Ref. 11; $[\alpha]_D^{25}$ −35.5 (*c*2, EtOH).
50. (2*R*,2′*R*)-**2**: ^{1}H NMR identical to the enantiomer. (2*R*,2′*R*)-**2** Hydrochloride: Mp 145–146 °C; $[\alpha]_D^{25}$ +114.8 (*c*2, EtOH); ^{1}H NMR identical to the enantiomer.
51. (2*S*,2′*R*)-**2a**: ^{1}H NMR identical to the enantiomer. (2*S*,2′*R*)-**2a** Hydrochloride: Mp 154–155 °C; $[\alpha]_D^{25}$ −67.6 (*c*2, EtOH); ^{1}H NMR identical to the enantiomer.
52. (2*R*,2′*R*)-**2a**: ^{1}H NMR identical to the enantiomer. (2*R*,2′*R*)-**2a** Hydrochloride: Mp 150–151 °C; $[\alpha]_D^{25}$ +86.5 (*c*2, EtOH); ^{1}H NMR identical to the enantiomer.
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57. All the ligands were built by VEGA in their protonated form and the conformational space was explored by systematically rotating the unique rotatable bond connecting the two rings. The docking and scoring procedures involved extensive rigid-body sampling with the OpenEye Scientific Software package FRED, using the rat α4β2 nicotinic model (PDB Id 1OLE). The FRED-based sampling was performed in box defined by known residues implicated in ligand recognition (namely, Trp55, Lys77, Trp147, and Tyr188). The docking results were scored using the ChemGauss3 function (as compiled in Table 2) which accounts for interactions, steric fitting and desolvation.
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