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Synthesis and Cholinergic Affinity of Diastereomeric and Enantiomeric Isomers of 1-Methyl-2-(2-methyl-1,3-dioxolan-4-yl)-pyrrolidine, 1-Methyl-2-(2-methyl-1,3-oxathiolan-5-yl)pyrrolidine and of Their Iodomethylates†

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Abstract—Four out of the eight possible stereoisomers of 1-methyl-2-(2-methyl-1,3-dioxolan-4-yl)pyrrolidine, 1-methyl-2-(2-methyl-1,3-oxathiolan-5-yl)pyrrolidine and the corresponding iodomethylates have been synthesised. They were formally derived from hybridisation of potent though unselective agonists studied before, such as 1,3-dioxolane **1** and 1,3-oxathiolane **2**, with the structure of nicotine. It was expected that, by exalting the molecular complexity of the parent compounds, in particular through stereochemical complication in the proximity of the critical cationic head of the molecule, the chance to find agonists able to discriminate among cholinergic receptors subtypes would increase. The relative and absolute configuration of the compounds obtained has been established by means of NMR spectroscopy and X-ray crystallography. In preliminary studies, their binding affinity has been evaluated on rat brain nicotinic and muscarinic receptors. While none of the compounds showed any nicotinic affinity up to the dose of 10 μM, most of the iodomethylates were endowed with promising affinity for the muscarinic receptors.

Introduction

Cholinergic compounds have been studied widely in the past decades, to identify molecules useful for further characterisation of muscarinic[1] and nicotinic[2] receptor subtypes. However, while characterisation through antagonists has progressed satisfactorily, there are at present no agonists that are able to discriminate muscarinic or nicotinic subtypes. Thus, new agonists selective for one of the several muscarinic and nicotinic receptor subtypes would be extremely useful not only to further characterise the receptors but also for their therapeutic potential in neurodegenerative diseases like Alzheimer's disease,[3] and generally those of the central nervous system. The cholinergic hypothesis of Alzheimer's disease[4] has stimulated the synthesis and the pharmacological evaluation of hundreds of new compounds, most of them agonists or able to restore the central cholinergic tone. Initially, the research almost exclusively regarded muscarinic agonists[5] and several compounds were proposed for pre-clinical and clinical studies. However, clinical results were rather disappointing, mainly because of problems with toxicity attributed to poor selectivity against the M_1 muscarinic receptor subtype. Later on, the research seemed to have shifted toward the synthesis and the study of agonists of the nicotinic receptors[6] that appear to play a major role in the CNS, mainly by regulating the release of several important neurotransmitters. In both cases, the poor selectivity of the molecules studied has always represented the major problem. The possibility of using

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agonists with a dual action (nicotinic and muscarinic), as indeed happens for the natural mediator acetylcholine, seems to have been overlooked so far. Perhaps the reason lies in the difficulty of identifying compounds that are, at the same time, selective for muscarinic M_1 and nicotinic α2β4 or α7 receptors, which are the subtypes mainly involved in neurodegenerative diseases.[7]

For several years, we have been working with cholinergic agonists, characterised by a pentatomic cycle, among them 1,3-dioxolanes and 1,3-oxathiolanes.[8] Iodomethylates of both series such as **1** and **2** (Chart 1), show high agonistic activity on muscarinic as well as on nicotinic receptors.[9–12] However, both series of compounds show poor selectivity between receptor subtypes. We reasoned that by exalting the molecular complexity of the parent compounds, in particular through stereochemical complication in the proximity of the critical cationic head of the molecule, the chance of finding agonists able to discriminate among cholinergic receptors subtypes would increase. Therefore, we planned the synthesis of the series of compounds with three stereogenic centres shown in Chart 1, where the structure of nicotine is hybridised with that of 1,3-dioxolane **1** and 1,3-oxathiolane **2**. The choice of the pyrrolidine ring for molecular complication was due to the intent of increasing the nicotinic activity of the 1,3-dioxolanes and 1,3-oxathiolanes, while maintaining their high muscarinic activity. Moreover, we expected that the introduction of the pyrrolidine ring would induce activity also in the tertiary bases, as it happens for nicotine. As a matter of fact, the tertiary bases corresponding to 1,3-dioxolane **1** and 1,3-oxathiolane **2**, are much less potent than the iodomethylates which, in turn, are unsuitable as centrally acting drugs. In the present communication, we report the synthesis and the preliminary cholinergic binding profile of the four series of compounds shown in Chart 1.

Chemistry

Compounds **8** and **14**, and their corresponding methiodide derivatives **9** and **15** (Chart 1), possess three stereogenic centres and can exist as eight different isomers. For their synthesis we started from two chiral precursors, *S*- and *R*-prolinol, respectively. In both cases, only two

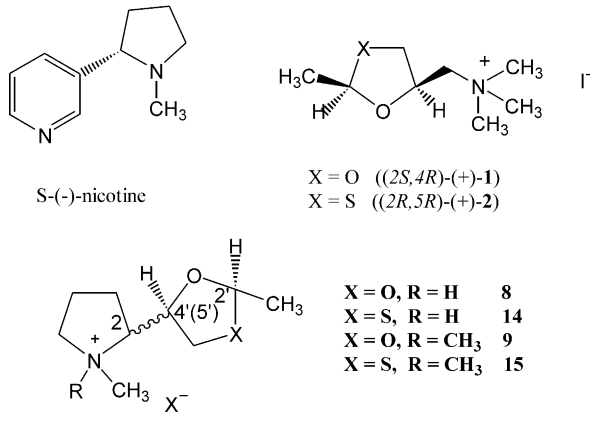


Chart 1.

out of the four possible diastereoisomers were obtained in reasonable yields, the other two being present only in traces which, so far, prevented their sound characterisation. Very luckily, the two isolated stereoisomers, both in the 1,3-dioxolane and 1,3-oxathiolane series, present a *cis* geometry of the side chains, that is identical to that of the most potent isomers of the parent compounds **1** and **2**. The situation parallels that of the parent compounds **1** and **2** for which the *cis* isomers are prevalent with respect to the *trans* ones (3:2[13] and 4:1,[12] respectively).

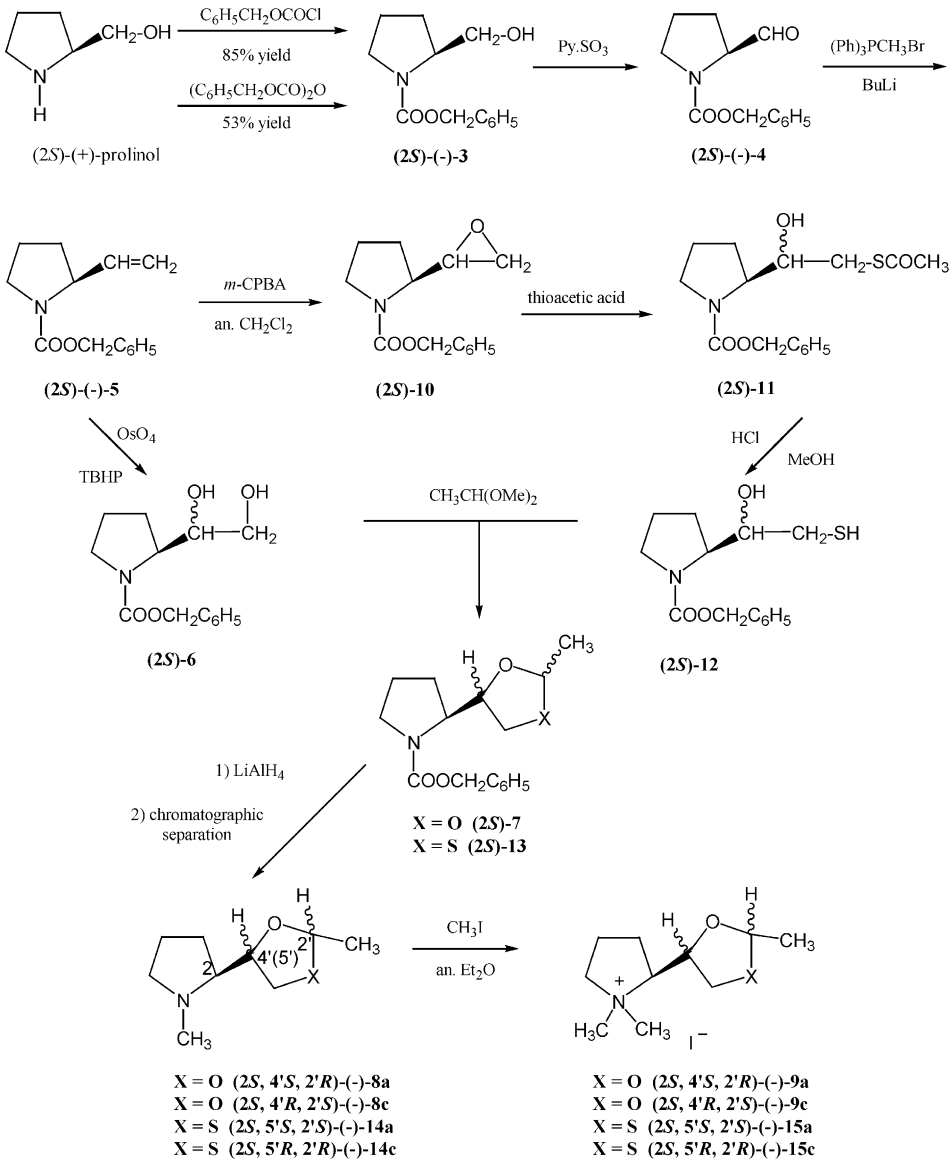
1,3-Dioxolanes

Starting from the (*2S*)-(+)-prolinol enantiomer [(*2S*)-(+)-pyrrolidinemethanol, Scheme 1], the pyrrolidine nitrogen was first protected as *N*-benzyloxycarbonyl (CBZ) derivative, using dibenzyldicarbonate[14] or (with better yields) benzylchloroformate.[15] On the protected derivative **(*2S*)-(−)-3**, reactions were then performed that allowed the building of the 1,3-dioxolane and the 1,3-oxathiolane rings, with retention of the starting prolinol configuration, as was verified by comparing the rotatory optical activity of our intermediates with the values already reported. In fact, compounds **(*2S*)-(−)-3**,[16] **(*2R*)-(+)-3**,[17] **(*2S*)-(−)-4**,[18,19] **(*2R*)-(+)-4**[17] and **(*2S*)-(−)-5**,[20,21] were known (see also the Experimental), even if not fully characterised or synthesised in a different way; in any case comparison with the reported rotatory optical activity confirmed the retention of configuration on the stereocentre. **(*2S*)-(−)-3** was therefore oxidized to aldehyde **(*2S*)-(−)-4** according to Parikh and Doering,[22] with low but acceptable yields, and without racemisation. Wittig reaction on this intermediate gave the corresponding alkene **(*2S*)-(−)-5**, that was oxidized, in the presence of TBHP (*t*-butyl hydroperoxide) and of a catalytic amount of OsO_4,[23] to diol **(*2S*)-6**, as mixture of two diastereoisomers. It is to note that one of the isomers present in our mixture was already described.[17] Subsequent cyclisation with acetaldehyde dimethylacetal[24] afforded the protected 1,3-dioxolane derivative **(*2S*)-7** as a mixture of diastereoisomers. The *N*-methyl derivatives **(*2S*)-8** were directly obtained from the protected compounds through reduction of the CBZ group with $LiAlH_4$.[25] The diastereomeric mixture **(*2S*)-8** was separated on an Al_2O_3 chromatographic column, in order to avoid the opening of the acid-sensitive 1,3-dioxolane ring, yielding two diastereomeric tertiary amines [**(*2S, 4′S, 2′R*)-(−)-8a** and **(*2S, 4′R, 2′S*)-(−)-8c**] plus traces of the other diastereoisomers (fraction b, see the Experimental). The isomers were also transformed into the corresponding methiodides **9**.

The same reaction sequence, performed on the (*2R*)-(−)-prolinol enantiomer [(*2R*)-(−)-pyrrolidinemethanol, not shown], afforded the enantiomers **(*2R, 4′R, 2′S*)-(+)-8a** and **(*2R, 4′S, 2′R*)-(+)-8c** (Chart 2) and the corresponding methiodides **9**.

1,3-Oxathiolanes

In order to synthesize the 1,3-oxathiolane derivatives (Scheme 1), starting again from the alkene derivative



Scheme 1. (a) The same reactions were performed also on the (2*R*)-(−)-prolinol (not shown).

(2*S*)-(−)-5, the key intermediate **(2*S*)-12** was obtained by a three-step procedure. By epoxidation with *m*-CPBA (*m*-chloroperbenzoic acid)[26] derivative **(2*S*)-10** was synthesised, which was then treated with thioacetic acid yielding intermediate **(2*S*)-11**, and hydrolysed in acidic conditions to the desired thiol-alcohol derivative **(2*S*)-12**. Cyclization afforded the diastereomeric mixture **(2*S*)-13**, which was treated with $LiAlH_4$, as described before for the 1,3-dioxolane compounds, yielding also in this case a mixture of the final compounds [**(2*S*)-14**] which were separated on Al_2O_3 chromatographic column. Two diastereomeric tertiary amines **(2*S*, 5′*S*, 2′*S*)-(−)-14a and (2*S*, 5′*R*, 2′*R*)-(−)-14c** were obtained, which were then transformed into the corresponding methiodides **15**.

Also, in this case, the same reactions were then performed also on the (2*R*)-(−)-prolinol enantiomer, yielding the two enantiomeric (2*R*)-1,3-oxathiolane amines **(2*R*, 5′*R*, 2′*R*)-(+)-14a** and **(2*R*, 5′*S*, 2′*S*)-(+)-14c** (Chart 2) and the corresponding methiodides **15**.

Stereochemistry and Configurational Analysis

The structure and the absolute configuration of the obtained compounds was assessed by 1D and 2D ^1H NMR (see Table 1 and the experimental part) and by X-ray crystallography (Fig. 1). Keeping in mind that, on the basis of Cahn, Ingold and Prelog rules, the priority on 1,3-oxathiolane and 1,3-dioxolane rings are different, the absolute stereochemistry of our derivatives is that reported in Chart 2. NMR experiments were performed on the tertiary amines, but their results can obviously be extended to the corresponding methiodides. The corresponding members of both series show fairly similar NMR spectra.

First of all, we identified and characterised the ^1H NMR signals of each proton. Toward this end, in addition to 200 MHz ^1H NMR spectra (see Experimental), 1D and COSY experiments were performed with a 600 MHz instrument on the two isomers of 1,3-dioxolane **(+)-8a**, **c** and on the corresponding 1,3-oxathiolanes **(+)-14a**, **c**,

which were obtained starting from *R*-prolinol and possess stereochemistry R on the C-2 of the pyrrolidine ring. Of course, conclusions reached can be extended to products **(−)-8** and **(−)-14**, derived from S-prolinol, since all the data indicate that they are their enantio-

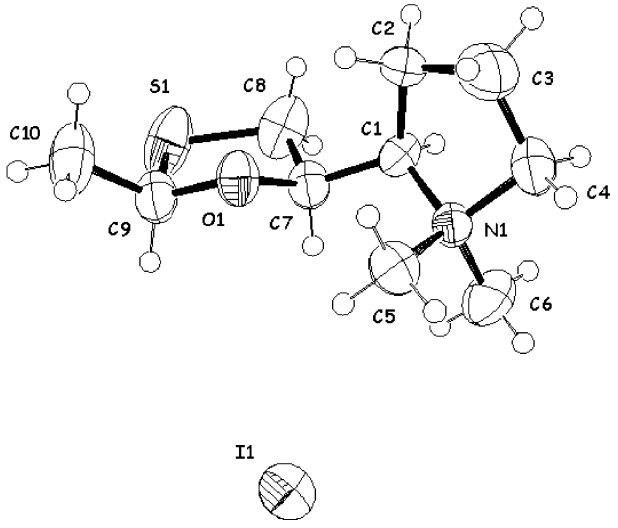


Figure 1. ORTEP drawing of compound (+)-**15a** showing one of the four independent molecules in the asymmetric unit.

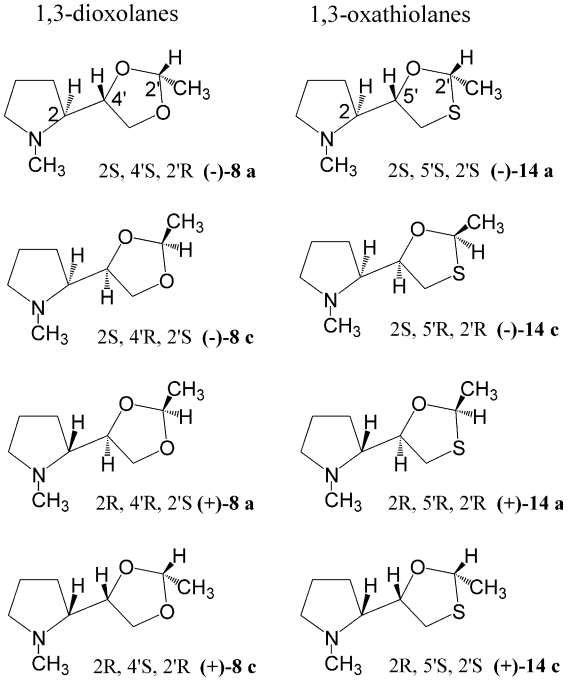
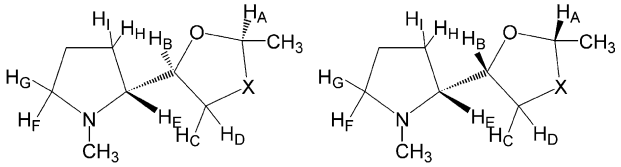


Chart 2. Absolute configuration of the obtained stereoisomers of **8** and **14**.

Table 1. ^{1}H NMR ($CDCl_3$) signals of the **(+)-8** and **(+)-14** isomers at 600 MHz: chemical shift (δ) and coupling constants (*J*)



	(+)-8 a	(+)-14 a	(+)-8 c	(+)-14 c
H_A	δ = 5.04 ppm $J_{A\text{-}Me}$ = 5.0 Hz	δ = 5.22 ppm $J_{A\text{-}Me}$ = 5.5 Hz	δ = 5.03 ppm $J_{A\text{-}Me}$ = 4.8 Hz	δ = 5.24 ppm $J_{A\text{-}Me}$ = 5.7 Hz
H_B	δ = 4.16 ppm J_{B-D} = 7.4 Hz J_{B-C} = 6.5 Hz J_{B-E} = 3.9 Hz	δ = 3.99 ppm J_{B-D} = 9.4 Hz J_{B-C} = 5.5 Hz J_{B-E} = 3.2 Hz	δ = 4.06 ppm J_{B-D} = 7.1 Hz J_{B-C} = 6.7 Hz J_{B-E} = 6.8 Hz	δ = 3.85 ppm J_{B-D} = 9.4 Hz J_{B-C} = 5.4 Hz J_{B-E} = 7.4 Hz
H_C	δ = 3.94 ppm $J_{ge\ C-D}$ = 7.5 Hz J_{B-C} = 6.5 Hz	δ = 3.03 ppm $J_{ge\ C-D}$ = 10.0 Hz J_{B-C} = 5.5 Hz	δ = 3.87 ppm $J_{ge\ C-D}$ = 7.8 Hz J_{B-C} = 6.7 Hz	δ = 3.05 ppm $J_{ge\ C-D}$ = 10.1 Hz J_{B-C} = 5.4 Hz
H_D	δ = 3.71 ppm $J_{ge\ C-D}$ = 7.5 Hz J_{B-D} = 7.4 Hz	δ = 2.87 ppm $J_{ge\ C-D}$ = 10.0 Hz J_{B-D} = 9.4 Hz	δ = 3.67 ppm $J_{ge\ C-D}$ = 7.8 Hz J_{B-D} = 7.1 Hz	δ = 2.80 ppm $J_{ge\ C-D}$ = 10.1 Hz J_{B-D} = 9.4 Hz
H_E	δ = 2.37 ppm J_{B-E} = 3.9 Hz J_{H-E} = 8.4 Hz J_{I-E} = 6.6 Hz	δ = 2.47 ppm J_{B-E} = 3.2 Hz J_{H-E} = 9.3 Hz J_{I-E} = 6.6 Hz	δ = 2.45 ppm J_{B-E} = 6.8 Hz	δ = 2.53 ppm J_{B-E} = 7.4 Hz
H_F	δ = 3.12 ppm $J_{ge\ F-G}$ = 9.3 Hz	δ = 3.10 ppm $J_{ge\ F-G}$ = 9.6 Hz	δ = 3.07 ppm $J_{ge\ F-G}$ = 9.0 Hz	δ = 3.09 ppm $J_{ge\ F-G}$ = 9.3 Hz
H_G	δ = 2.25 ppm $J_{ge\ F-G}$ = 9.3 Hz	δ = 2.24 ppm $J_{ge\ F-G}$ = 9.6 Hz	δ = 2.25 ppm $J_{ge\ F-G}$ = 9.0 Hz	δ = 2.28 ppm $J_{ge\ F-G}$ = 9.3 Hz
$\underline{CH_2}CH_ICH_H$			δ = 1.71–1.76 ppm	δ = 1.65–2.00 ppm
H_I	δ = 1.69–1.89 ppm	δ = 1.68–1.92 ppm	δ = 1.76–1.83 ppm	δ = 1.85–1.95 ppm
H_H			δ = 1.49–1.55 ppm	δ = 1.55–1.65 ppm
CH_3	δ = 1.41 ppm $J_{A\text{-}Me}$ = 5.0 Hz	δ = 1.57 ppm $J_{A\text{-}Me}$ = 5.5 Hz	δ = 1.39 ppm $J_{A\text{-}Me}$ = 4.8 Hz	δ = 1.59 ppm $J_{A\text{-}Me}$ = 5.7 Hz
N-CH_3	δ = 2.41 ppm	δ = 2.42 ppm	δ = 2.48 ppm	δ = 2.52 ppm

mers. The chemical shifts and the coupling constants of the protons of isomers **(+)-8a**, **c** and **(+)-14a, c** are reported in Table 1.

1,3-Dioxolanes

With regard to the 1,3-dioxolane derivatives **(+)-8a**, **c**, the ^{1}H NMR spectra of the two isomers show clear-cut differences in the chemical shift of some protons (Table 1). Among these, the most evident regards the H_H proton (*trans* with respect to H_E), that is remarkably shielded in **(+)-8c** with respect to the corresponding protons of isomer **(+)-8a**. 2D 600 MHz NOESY experiments were performed on the same substances, in order to obtain information on the spatial arrangement of the groups. The NOESY spectra indicate a clear interaction between H_A and H_B for the compounds **(+)-8a** and **(+)-8c**, whereas there is no interaction between H_B and the CH_3 group. These findings clearly indicate that, with respect to the 1,3-dioxolane ring, the substituents are in a *cis* geometry in compounds **(+)-8a** and **(+)-8c**. We also identified all other protons (Table 1).

1,3-Oxathiolanes

The 1D and 2D spectra of the 1,3-oxathiolane derivatives **(+)-14** give information that strictly parallels that of the corresponding 1,3-dioxolanes (Chart 2, Table 1). Thus, the evaluation of the COSY spectrum shows a shielding effect of about 0.3 ppm, with respect to the other isomer, on the H_H signal of the compound **(+)-14c**, as already shown by the corresponding 1,3-dioxolane.

The NOESY spectra also show in this case that substituents of compounds **(+)-14a** and **(+)-14c** present a *cis* geometry in the 1,3-oxathiolane ring; even if in the 1,3-oxathiolane isomers, unlike in the corresponding *cis* 1,3-dioxolane derivatives **(+)-8a** and **(+)-8c**, it was not possible to directly evaluate the interaction between H_A and H_C. This fact could be related to the greater dimensions of the sulphur with respect to the oxygen, that modify the structure of the pentatomic ring and send away H_A from H_C. The data of all other protons are reported in Table 1.

An attempt was made to establish the absolute stereochemistry of both 1,3-dioxolanes and 1,3-oxathiolanes on the basis of NOESY spectra, but the data did not allow any sound attribution. The absolute configuration of 1,3-oxathiolane compounds could be established by X-ray crystallography of methiodide **(+)-15a**, whose crystallographic structure is shown in Figure 1.

The crystal structure confirmed that the 2′ and 5′ side chains are in a *cis* geometry and shows that its absolute configuration is 2*R*, 5′*R*, 2′*R*. The structure of the other 1,3-oxathiolane methiodides and of the corresponding tertiary bases is straightforward. The absolute configuration of the 1,3-dioxolane compounds was attributed on the basis of the strict similarity of their ^{1}H NMR and ^{13}C NMR spectra with those of the corresponding 1,3-oxathiolanes.

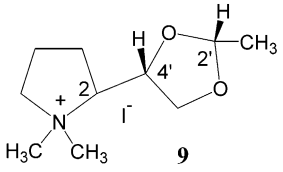
Results and Discussion

The binding affinity of the compounds synthesised on rat brain nicotinic and muscarinic receptors has been evaluated. The compounds did not show any appreciable affinity for nicotinic receptors labelled by [^{3}H] cytisine up to the dose of 10 μM. On the contrary, most of them showed affinity in the same range of carbachol for the muscarinic receptors labeled by [^{3}H] *N*-methyl scopolamine ([^{3}H] NMS). Among tertiary bases only **(+)-14c**, belonging to the 1,3-oxathiolane series, shows affinity for muscarinic receptors ($K_i = 21.7 \pm 2.6$ μM, $pK_i = 4.66$) comparable to that of carbachol. All other tertiary bases, did not show affinity up to the dose of 100 μM. As expected, methiodides were much more affinitive for muscarinic receptors, and their data are reported in Table 2.

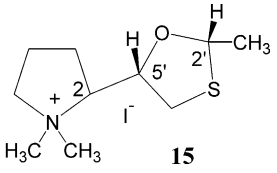
The first result to mention is the complete lack of affinity for the brain nicotinic receptors. From this point of view, our effort to design compounds endowed, at the same time, with nicotinic and muscarinic affinity has unexpectedly failed. Even if the comparison of the results is difficult, since the nicotinic activity of **1**[9] and **2**[12] was determined by functional assays on a peripheral nicotinic receptors model such as the frog rectus abdominis, stereochemical complication seems to have destroyed the nicotinic activity present in the parent compounds.

However, as far as muscarinic affinity is concerned the results were much more interesting. One tertiary base [**(+)-14c**] belonging to the 1,3-oxathiolane series showed a good affinity for the central muscarinic receptors which makes it promising as potential central acting drug. As

Table 2. Binding affinity of methyl iodide derivatives **9** and **15** on the rat brain muscarinic receptors



9



15

Drug	Stereochemistry binding[a]	K_i (μM) (±SEM)	ER[b]	pK_i
(−)-9a	2*S*, 4′*S*, 2′*R*	>100		—
			>7.94	
(+)-9a	2*R*, 4′*R*, 2′*S*	12.6±3.7		4.90
(−)-9c	2*S*, 4′*R*, 2′*S*	19.2±2.8		4.72
			>5.21	
(+)-9c	2*R*, 4′*S*, 2′*R*	>100		—
(−)-15a	2*S*, 5′*S*, 2′*S*	27.0±3.8		4.56
			4.58	
(+)-15a	2*R*, 5′*R*, 2′*R*	5.9±0.6		5.23
(−)-15c	2*S*, 5′*R*, 2′*R*	7.3±0.8		5.14
			1.25	
(+)-15c	2*R*, 5′*S*, 2′*S*	9.1±1.1		5.04
Carbachol		41.3±7.0		4.38

[a]On rat brain homogenates. The muscarinic receptors were labelled by [^{3}H] *N*-methyl scopolamine ([^{3}H] NMS). See the pharmacological experimental part for details ($n = 3$ experiments).
[b]Eudismic ratio. The eudismic ratio is the ratio: (affinity of the distomer)/(affinity of the eutomer).

expected, iodomethylates were much more affinitive for brain muscarinic receptors (Table 2). All 1,3-oxathiolane derivatives **15** showed affinity larger than that of carbachol, some of them being almost an order of magnitude more affinitive. At the contrary, only **(+)-9a** and **(−)-9c** among the 1,3-dioxolane derivatives showed affinity of the same order of magnitude of carbachol.

In principle, the molecular complication introduced into the structure of parent compounds could alter their pharmacological properties changing agonism into antagonism. This does not seem to be the case since, as shown by the dose–response curve reported in Figure 2, compound (+)-**9a** behaves as a full agonist on guinea pig ileum (M_3), showing an intrinsic activity of $\alpha = 0.95$ and a $-\log ED_{50} = 6.65$. Therefore, the changes introduced into the molecule seem to leave the intrinsic activity on muscarinic receptor unchanged. Of course, the functional pharmacological profile of the other members of the series need to be individually measured and this study will be reported in due time.

It is interesting to compare the absolute configuration of the most affinitive compounds with that of the parent compounds. The compounds of the 1,3-dioxolane series that show muscarinic affinity comparable to that of carbachol [**(+)-9a** and **(−)-9c**] have the 2′*S*, 4′*R* configuration, which is precisely that of the most potent enantiomer of **1** (2*S*,4*R*).[11,13] In the 1,3-oxathiolane series, the most affinitive compound **(+)-15a** has the 2′*R*,5′*R* absolute configuration, which is identical to that of the most active enantiomer of **2** (*2R,5R*), and the same happens for **(−)-15c** (2′*R*,5′*R*), which is only slightly less affinitive. However, its enantiomer **(+)-15c** (2′*S*, 5′*S*) shows almost the same affinity. In general, there is a fairly good correspondence between the absolute configuration of the most active enantiomers of the parent compounds and the affinity of the most affinitive representative of the new series. Minor discrepancies are probably due to the molecular complication introduced which might induce slight changes in the mode of binding of the ligands to the receptor recognition site.

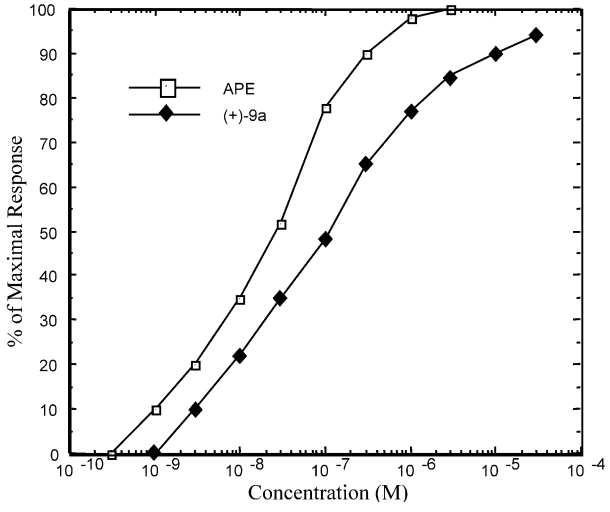


Figure 2. □ Dose–response curve of reference agonist arecaidine propargyl ester (APE); ◆ dose–response curve of agonist (+)-**9a** on guinea pig ileum (M_3). Each point is the mean of at least six independent observations. The SEMs are less than 10% and are not shown.

Of course, the results presented have to be considered preliminary. A screening on the five cloned human muscarinic subtypes (m_{1-5}) and the evaluation of the agonistic activity on available functional tests for muscarinic receptors (M_{1-4}), which are already under way, will be necessary to fully evaluate the pharmacological profile of this new set of compounds and to appreciate their usefulness for receptor characterisation and for drug development. Considering the large conservation in the structure of the recognising sites of the five known muscarinic receptors,[27,28] it is hoped that these stereochemically complex agonists, which are in principle able to detect subtle structural differences, will help to overcome the difficulties in designing subtype selective muscarinic agonists.

Experimental

Chemistry

All melting points were taken on a Büchi apparatus and are uncorrected. Infrared spectra were recorded with a Perkin-Elmer 681 spectrophotometer in Nujol mull for solids and neat for liquids. Unless otherwise stated, NMR spectra were recorded on a Gemini 200 spectrometer (200 MHz for ^{1}H NMR, 50.3 MHz for ^{13}C), and chromatographic separations were performed on a silica gel column by gravity chromatography (Kieselgel 40, 0.063–0.200 mm; Merck) or flash chromatography (Kieselgel 40, 0.040–0.063 mm; Merck). When necessary, chromatographic separation were performed on an Al_2O_3 column by gravity chromatography (Aluminium oxide 90 standardized, Merck). Yields are given after purification, unless otherwise stated. Where analyses are indicated by symbols, the analytical results are within ±0.4% of the theoretical values. Optical rotation was measured at a concentration of 1 g/100 mL ($c = 1$), unless otherwise stated, with a Perkin-Elmer polarimeter (accuracy ±0.002°).

(2*S*)-(−)-Phenylmethyl 2-(hydroxymethyl)pyrrolidine-1-carboxylate [*S*-(−)-3].[16] **Method A.** To a solution of 2.5 g (24.7 mmol) of L-prolinol [(2*S*)-(+)-pyrrolidinemethanol] in NaOH 1 N (25 mL) and dioxane (25 mL), dibenzyl dicarbonate (7.1 g, 24.8 mmol) in dioxane (25 mL) was added. After 5 h at room temperature, the bulk of dioxane was evaporated and the resulting aqueous solution acidified with H_2SO_4 1 N to pH 2 and extracted three times with ethyl acetate. The organic layer were dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography (eluent $CHCl_3$ 100). 3.1 g (13.2 mmol, 53% yield) of ***S*-(−)-3** were obtained.

Method B. An ice-cold solution of L-prolinol (2.5 g, 24.7 mmol) in a mixture of 2 M Na_2CO_3 aqueous solution/dioxane (4:1, 125 mL) was treated dropwise with vigorous stirring with benzyl chloroformate (8.6 mL, 60.24 mmol) dissolved in 50 mL dioxane. Simultaneously,

aqueous 2 M NaOH (31 mL) was added. After 3 h, most of the dioxane was evaporated, and the remaining suspension was extracted three times with diethyl ether. The combined extracts were dried over Na_2SO_4 and evaporated to afford a residue that was purified by flash chromatography (eluent $CHCl_3$ 100). 4.8 g (20.4 mmol, 85% yield) of title compound were obtained.

$[\alpha]_{20}^{D} = -40.2°$ ($CHCl_3$) ($[\alpha]_{20}^{D} = -42.4°$ ($CHCl_3$)[16]), IR (neat) ν (cm^{-1}): 3700–3200 (OH); 1690 (C=O), ^1H NMR ($CDCl_3$) δ (ppm): 1.30–2.10 (m, 4H, CH_2CH_2); 3.30–3.70 (m, 5H, $CH_2N + CH_2O + OH$); 3.90–4.06 (m, 1H, CHN); 5.18 (s, 2H, CH_2Ph); 7.39 (s, 5H, aromatics). Anal. ($C_{13}H_{17}NO_3$): C, H, N.

(2*R*)-(+)-Phenylmethyl 2-(hydroxymethyl)pyrrolidine-1-carboxylate [*R*-(+)-3].[17] Using procedure B described for *S*-(−)-3, and starting from 5 g (49.4 mmol) of D-prolinol [(2*R*)-(−)-pyrrolidinemethanol] and 17.2 mL (120.5 mmol) of benzyl chloroformate, crude *R*-(+)-3 was obtained. The residue was purified by flash chromatography (eluent $CHCl_3$) affording 10.2 g (43.4 mmol, 88% yield) of title compound.

$[\alpha]_{20}^{D} = +41.2°$ ($CHCl_3$) ($[\alpha]_{20}^{D} = +40°$ ($CHCl_3$)[17]), IR (neat) ν (cm^{-1}): 3650–3200 (OH); 1685 (C=O), ^1H NMR ($CDCl_3$) δ (ppm): 1.55–2.05 (m, 4H, CH_2CH_2); 3.30–3.70 (m, 5H, $CH_2N + CH_2O + OH$); 3.90–4.10 (m, 1H, CHN); 5.08 (s, 2H, CH_2Ph); 7.40 (s, 5H, aromatics). Anal. ($C_{13}H_{17}NO_3$): C, H, N.

(2*S*)-(−)-Phenylmethyl 2-formylpyrrolidine-1-carboxylate [*S*-(−)-4].[18,19] To 7.9 g (33.6 mmol) of ***S*-(−)-3**, a solution of 14.1 mL of an. triethylamine in 100 mL of anhyd dimethyl sulfoxide and a solution of 16.0 g (100.8 mmol) of sulfur trioxide–pyridine complex dissolved in 100 mL of anhyd dimethyl sulfoxide were added at rt. The reaction mixture was stirred for 2 h and poured into ice-water. The mixture was extracted twice with diethyl ether, and the combined organic layers were successively washed twice with 10% aqueous citric acid, water and saturated aqueous $NaHCO_3$, and dried over Na_2SO_4. The solvent was concentrated in vacuo to give 5.3 g (22.7 mmol) of ***S*-(−)-4**. Yield 67%.

$[\alpha]_{20}^{D} = -83.1°$ ($CHCl_3$) ($[\alpha]_{20}^{D} = -78.1°$ (CH_2Cl_2),[19] $[\alpha]_{20}^{D} = -40.8°$ (MeOH)[18]), IR (neat) ν (cm^{-1}): 1730 (C=O aldehyde); 1700 (C=O carbamate), ^1H NMR ($CDCl_3$) δ (ppm): 1.70–2.30 (m, 4H, CH_2CH_2); 3.40–3.65 (m, 2H, CH_2N); 4.20–4.40 (m, 1H, CHN); 5.14 (s, 0.5 + 0.5H) and 5.18 (s, 0.5 + 0.5H) (CH_2Ph); 7.20–7.40 (m, 5H, aromatics); 9.55 (d, $J = 21.0$ Hz, 0.5H) and 9.56 (d, $J = 21.0$ Hz, 0.5H) (CHO). Anal. ($C_{13}H_{15}NO_3$): C, H, N.

(2*R*)-(+)-Phenylmethyl 2-formylpyrrolidine-1-carboxylate [*R*-(+)-4].[17] Using the same procedure described for ***S*-(−)-4** and starting from 10.2 g (43.4 mmol) of ***R*-(+)-3,** 18 mL of anhyd triethylamine and 20.7 g (130.2 mmol) of sulfur trioxide–pyridine complex, 6.5 g (27.9 mmol) of ***R*-(+)-4** were obtained (yield 64%).

$[\alpha]_{20}^{D} = +82.1°$ ($CHCl_3$) ($[\alpha]_{20}^{D} = +83°$ ($CHCl_3$)[17]) IR (neat) ν (cm^{-1}): 1730 (C=O aldehyde); 1700 (C=O carbamate). ^1H NMR ($CDCl_3$) δ (ppm): 1.70–2.30 (m, 4H, CH_2CH_2); 3.40–3.60 (m, 2H, CH_2N); 4.07–4.18 (m, 1H, CHN); 5.14 (s, 0.5 + 0.5H) and 5.17 (s, 0.5 + 0.5H) (CH_2Ph); 7.20–7.40 (m, 5H, aromatics); 9.54 (d, $J = 20.6$ Hz, 0.5H) and 9.55 (d, $J = 20.6$ Hz, 0.5H) (CHO). Anal. ($C_{13}H_{15}NO_3$): C, H, N.

(2*S*)-(−)-Phenylmethyl 2-vinylpyrrolidine-1-carboxylate [*S*-(−)-5].[20,21] To a suspension of 8.1 g (22.7 mmol) of methyl triphenylphosphonium bromide in anhyd THF in ice-bath under nitrogen, a solution of 17.5 mL of *n*-BuLi 1.6 M in hexane was added. The resulting solution was maintained 20 min at 0 °C and 15 min at rt, and treated with 5.3 g (22.7 mmol) of ***S*-(−)-4** dissolved in anhyd THF. The mixture was stirred 2 h at rt, refluxed for 2 h, poured on ice and extracted with diethyl ether. The organic layer was washed twice with a saturated solution of NH_4Cl, dried over Na_2SO_4 and concentrated in vacuo to give a residue that was purified by flash chromatography on silica gel (eluent CH_2Cl_2/MeOH 98:2) yielding 2.5 g (10.8 mmol, 47% yield) of ***S*-(−)-5**.

$[\alpha]_{20}^{D} = -16.1°$ ($CHCl_3$) ($[\alpha]_{20}^{D} = -9°$ (CH_2Cl_2)[21]) IR (neat) ν (cm^{-1}): 1705 (C=O), ^1H NMR ($CDCl_3$) δ (ppm): 1.70–2.10 (m, 4H, CH_2CH_2); 3.40–3.57 (m, 2H, CH_2N); 4.31–4.50 (m, 1H, CHN); 4.95–5.15 (m, 4H, $CH_2Ph + CH_2=$); 5.65–5.90 (m, 1H, CH=); 7.25–7.40 (m, 5H, aromatics). Anal. ($C_{14}H_{17}NO_2$): C, H, N.

(2*R*)-(+)-Phenylmethyl 2-vinylpyrrolidine-1-carboxylate [*R*-(+)-5]. Using the same procedure described for ***S*-(−)-5**, and starting from 6.5 g (27.9 mmol) of ***R*-(+)-4**, 10.0 g (27.9 mmol) of methyl triphenylphosphonium bromide and 21.66 mL of *n*-BuLi 1.6 M in hexane, ***R*-(+)-5** crude was obtained, that was purified by flash chromatography (eluent CH_2Cl_2/MeOH 98:2). Yield 2.9 g (12.5 mmol, 45%). $[\alpha]_{20}^{D} = +17.3°$ ($CHCl_3$), IR (neat) ν (cm^{-1}): 1700 (C=O), ^1H NMR ($CDCl_3$) δ (ppm): 1.63–2.15 (m, 4H, CH_2CH_2); 3.40–3.59 (m, 2H, CH_2N); 4.35–4.45 (m, 1H, CHN); 4.97–5.21 (m, 4H, $CH_2Ph + CH_2=$); 5.68–5.90 (m, 1H, CH=); 7.30–7.45 (m, 5H, aromatics). Anal. ($C_{14}H_{17}NO_2$): C, H, N.

(2*S*)-(−)-Phenylmethyl 2-(1,2-dihydroxyethyl)pyrrolidine-1-carboxylate [(2*S*)-6].[17] 1.5 g (6.5 mmol) of ***S*-(−)-5**, 1.59 mL (16.5 mmol) of *t*-butyl hydroperoxide (TBHP) 70% and 480 mg (1.8 mmol) of tetraethylammonium acetate tetrahydrate ($Et_4NOAc \cdot 4H_2O$) were combined in 19 mL of acetone. The solution was cooled at 0 °C and 6.4 mL of the OsO_4 solution (250 mg of OsO_4 in 50 mL of *t*-butyl alcohol) were added in one portion. After 1 h, the ice bath was removed and the mixture was left for 10 h at rt. Then, 38.1 mL of diethyl ether were added to the solution, and the mixture was cooled by stirring in an ice-bath; 12.7 mL of 10% $NaHSO_3$ were added. The ice bath was then removed and stirring continued for 1 h. NaCl was added to saturate the aqueous layer and stirring was continued for 15 min. The phases were partitioned in a separatory funnel, the organic phase was washed with brine, and the aqueous layer re-extracted with ether. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo to afford a residue which, after flash chroma-

tography (eluent CH_2Cl_2/MeOH 98:2) gave 1.24 g (4.7 mmol, 72% yield) of **(2*S*)-6** as a mixture of two diastereoisomers.

$[\alpha]_{20}^{D} = -42.8°$ ($CHCl_3$) ($[\alpha]_{20}^{D} = -20°$ ($CHCl_3$) reported by Mazzini[17] for one of the two diastereoisomers), IR (neat) ν (cm^{-1}): 3700–3100 (OH); 1680 (C=O), ^{1}H NMR ($CDCl_3$) δ (ppm): 1.68–2.17 (m, 4H, CH_2CH_2); 2.60–2.98 (bs, 2H, OH + OH); 3.30–3.46 (m, 2H, CH_2N); 3.50–3.70 (m, 3H, CH_2O + CHN); 3.88–4.00 (m, 0.5H) and 4.00–4.15 (m, 0.5H) (CHO); 5.12 (s, 2H, CH_2Ph); 7.30–7.45 (m, 5H, aromatics). Anal. ($C_{14}H_{19}NO_4$): C, H, N.

(2*R*)-Phenylmethyl 2-(1,2-dihydroxyethyl)pyrrolidine-1-carboxylate [(2*R*)-6].[17] Using the same procedure described for **(2*S*)-6**, and using 2.0 g (8.6 mmol) of ***R*-(+)-5**, 2.12 mL (22.0 mmol) of TBHP 70%, 640 mg (2.4 mmol) of $Et_4NOAc{\cdot}4H_2O$ and 8.5 mL of the OsO_4 solution in *t*-butyl alcohol, **(2*R*)-6** was obtained and purified by flash chromatography (eluent CH_2Cl_2), affording 1.66 g (6.3 mmol, 72% yield) of product as a mixture of two diastereoisomers.

$[\alpha]_{20}^{D} = +41.8°$ ($CHCl_3$) ($[\alpha]_{20}^{D} = +66°$ ($CHCl_3$) reported by Mazzini[17] for one of the two diastereoisomers), IR (neat) ν (cm^{-1}): 3700–3100 (OH); 1685 (C=O), ^{1}H NMR ($CDCl_3$) δ (ppm): 1.70–2.20 (m, 4H, CH_2CH_2); 2.95–3.20 (bs, 2H, OH + OH), 3.25–3.74 (m, 5H, CH_2N + CH_2O + CHN); 3.85–4.00 (m, 0.5H) and 4.00–4.07 (m, 0.5H) (CHO); 5.08 (s, 2H, CH_2Ph); 7.30–7.45 (m, 5H, aromatics). Anal. ($C_{14}H_{19}NO_4$): C, H, N.

(2*S*)-Phenylmethyl 2-(2-methyl-1,3-dioxolan-4-yl)pyrrolidine-1-carboxylate [(2*S*)-7]. To a solution of 1.24 g (4.7 mmol) of **(2*S*)-6** in 26 mL of 2-propanol, 120 mg (0.6 mmol) of *p*-toluensulfonic acid monohydrate were added. To this solution, 3.6 mL (33.9 mmol) of acetaldehyde dimethyl acetal were added, and the mixture was kept to reflux for 2 h and concentrated in vacuo. The residue was dissolved in 60 mL of diethyl ether, and the organic layer was washed with 40 mL of water and dried over Na_2SO_4. Evaporation of the solvent gave an oily residue that was purified by column chromatography on Al_2O_3 as a mixture of isomers (eluent: $CHCl_3$/MeOH 99:1). Yield 1.13 g (3.9 mmol, 83%) of **(2*S*)-7**.

$[\alpha]_{20}^{D} = -60.5°$ ($CHCl_3$), IR (neat) ν (cm^{-1}): 1710 (C=O), ^{1}H NMR ($CDCl_3$) δ (ppm): 1.18–1.42 (m, 3H, CH_3CH); 1.70–2.08 (m, 4H, CH_2CH_2); 3.27–4.50 (m, 6H, CH_2O + CH_2N + CHO + CHN); 4.92–5.23 (m, 3H, CH_2Ph + CHCH$_3$); 7.30–7.45 (m, 5H, aromatics). Anal. ($C_{16}H_{21}NO_4$): C, H, N.

(2*R*)-Phenylmethyl 2-(2-methyl-1,3-dioxolan-4-yl)pyrrolidine-1-carboxylate [(2*R*)-7]. With the same procedure described for **(2*S*)-7**, using 1.66 g (6.3 mmol) of **(2*R*)-6**, 4.65 mL (44.0 mmol) of acetaldehyde dimethyl acetal, 1.56 g (5.4 mmol, 85% yield) of a mixture of isomers of **(2*R*)-7** were obtained.

$[\alpha]_{20}^{D} = +62.7°$ ($CHCl_3$), IR (neat) ν (cm^{-1}): 1705 (C=O), ^{1}H NMR ($CDCl_3$) δ (ppm): 1.22–1.40 (m, 3H, CH_3CH); 1.70–2.06 (m, 4H, CH_2CH_2); 3.15–4.52 (m, 6H, CH_2O + CH_2N + CHO + CHN); 4.92–5.20 (m, 3H, CH_2Ph + CHCH$_3$); 7.30–7.45 (m, 5H, aromatics). Anal. ($C_{16}H_{21}NO_4$): C, H, N.

(2*S*, 4′*S*, 2′*R*)-1-Methyl-2-(2-methyl-1,3-dioxolan-4-yl) pyrrolidine [(−)-8a] and (2*S*, 4′*R*, 2′*S*)-1-methyl-2-(2-methyl-1,3-dioxolan-4-yl)pyrrolidine [(−)-8c]. A solution of 1.13 g (3.9 mmol) of **(2*S*)-7** dissolved in the minimum amount of anhyd THF was added dropwise to a suspension of 0.97 g (25.5 mmol) of $LiAlH_4$ in anhyd THF at −18 °C under nitrogen. The mixture was allowed to reach room temperature, and after 4 h was treated with brine and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and concentrated in vacuo to afford an oil, **(2*S*)-8** as a mixture of three isomers, that was purified by column chromatography on Al_2O_3 (eluent CH_2Cl_2/MeOH 99:1). The chromatographic separation yielded 160 mg of isomer **a**, 120 mg of the isomer **c** and another fraction of unidentified isomers (fraction **b**), with a total yield of 320 mg (1.9 mmol, 48%). Elemental analysis and $[\alpha]_{20}^{D}$ of each isomer are reported in Table 3. Hydrogens are defined in Table 1.

(−)-8a. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.41 (d, J = 5.0 Hz, 3H, CH_3CH); 1.65–1.90 (m, 4H, CH_2CH_2); 2.15–2.40 (m, 2H, $CH H_G N$ and $CH_E N$), 2.41 (s, 3H, CH_3N), 3.08–3.16 (m, 1H, $CH_F HN$), 3.67–3.75 (m, 1H, $CH_D HO$), 3.90–3.97 (m, 1H, $CH H_C O$), 4.12–4.20 (m, OCH_B), 5.04 (q, J = 5.0 Hz, CHCH$_3$).

(−)-8c. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.39 (d, J = 5.0 Hz, 3H, CH_3CH); 1.45–1.85 (m, 4H, CH_2CH_2); 2.16–2.48 (m, 2H, $CH H_G N$ and $CH_E N$), 2.48 (s, 3H, CH_3N), 3.00–3.14 (m, 1H, $CH_E HN$), 3.67–3.75 (m, 1H, $CH_D HO$), 3.89–3.99 (m, 1H, $CH H_C O$), 3.98–4.08 (m, OCH_B), 5.00 (q, J = 5.0 Hz, CHCH$_3$).

Isomeric amines were transformed in the corresponding oxalate by treatment with one equivalent of oxalic acid in ethyl acetate (Table 3).

(2*R*, 4′*R*, 2′*S*)-1-Methyl-2-(2-methyl-1,3-dioxolan-4-yl) pyrrolidine [(+)-8a] and (2*R*, 4′*S*, 2′*R*)-1-methyl-2-(2-methyl-1,3-dioxolan-4-yl)pyrrolidine [(+)-8c]. With the same procedure described for **(2*S*)-8** and using 1.5 g (5.1 mmol) of **(2*R*)-7** and 1.3 g (34.2 mmol) of $LiAlH_4$, **(2*R*)-8** was obtained as a mixture of isomers, that were separated by column chromatography on Al_2O_3 (eluent CH_2Cl_2 100). Separation afforded 240 mg of isomer **a**, 110 mg of isomer **c** and another fraction of unidentified isomers (fraction **b**). Total yield 390 mg (2.3 mmol, 45%). Elemental analysis and $[\alpha]_{20}^{D}$ of each isomer are reported in Table 3. Hydrogens are defined in Table 1.

(+)-8a. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.41 (d, J = 5.0 Hz, 3H, CH_3CH); 1.64–1.89 (m, 4H, CH_2CH_2); 2.16–2.40 (m, 2H, $CH H_G N$ and $CH_E N$), 2.41 (s, 3H, CH_3N), 3.08–3.15 (m, 1H, $CH_F HN$), 3.66–3.75 (m, 1H, $CH_D HO$), 3.89–3.96 (m, 1H, $CH H_C O$), 4.12–4.20 (m,

Table 3. Chemical and physical characteristics of derivatives **8** and **14**

Compd	Stereochemistry	Mp (oxalate)[a] (°C)	$[\alpha]_{20}^{D}$ (CH_2Cl_2)	Analysis
(−)-8a	2*S*,4′*S*, 2′*R*	108–110	−16.7	$C_9H_{17}NO_2$
(−)-8c	2*S*,4′*R*, 2′*S*	96–98	−44.9	$C_9H_{17}NO_2$
(+)-8a	2*R*,4′*R*, 2′*S*	107–109	+17.2	$C_9H_{17}NO_2$
(+)-8c	2*R*,4′*S*, 2′*R*	94–96	+46.7	$C_9H_{17}NO_2$
(−)-14a	2*S*,5′*S*, 2′*S*	116–118	−24.5	$C_9H_{17}NOS$
(−)-14c	2*S*,5′*R*, 2′*R*	92–95	−62.1	$C_9H_{17}NOS$
(+)-14a	2*R*,5′*R*, 2′*R*	115–118	+25.4	$C_9H_{17}NOS$
(+)-14c	2*R*,5′*S*, 2′*S*	90–93	+60.3	$C_9H_{17}NOS$

[a]From abs ethanol/diethyl ether.

OCH_B), 5.04 (q, $J = 5.0$ Hz, $CHCH_3$); ^{13}C NMR ($CDCl_3$) δ (ppm): 19.90 (q), 23.52 (t), 26.27 (t), 41.89 (q), 58.23 (t), 67.24 (d), 67.82 (t), 77.17 (d), 102.03 (d).

(+)-8c. ^1H NMR ($CDCl_3$) δ (ppm): 1.39 (d, $J = 4.8$ Hz, 3H, CH_3CH); 1.45–1.85 (m, 4H, CH_2CH_2); 2.20–2.30 (m, 1H, $CH H_GN$), 2.31–2.45 (m, 1H, CH_EN), 2.48 (s, 3H, CH_3N), 3.00–3.13 (m, 1H, CH_FHN), 3.67–3.75 (m, 1H, CH_DHO), 3.88–3.99 (m, 1H, $CH H_CO$), 4.08–4.20 (m, OCH_B), 5.02 (q, $J = 4.8$ Hz, $CHCH_3$). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.21 (q), 23.90 (t), 27.27 (t), 42.55 (q), 58.37 (t), 67.48 (d), 67.64 (t), 80.22 (d), 102.24 (d).

Each isomeric amine was transformed in the corresponding oxalate by treatment with one equivalent of oxalic acid in ethyl acetate (Table 3).

(2*S*)-Phenylmethyl 2-oxiran-2-ylpyrrolidine-1-carboxylate [(2*S*)-10]. To a solution of 920 mg (4.0 mmol) of ***S*-(−)-5** in dry CH_2Cl_2 at 0 °C a solution of 1.38 g (8.0 mmol) of *m*-chloroperbenzoic acid (*m*CPBA) in dry CH_2Cl_2 was added. After being stirred at rt for 4 h, the mixture was kept at 50 °C for 1 h. The mixture was filtered, and the filtrate was washed successively with saturated Na_2CO_3, saturated $NaHCO_3$ and water, and dried over Na_2SO_4. The organic layer was concentrated in vacuo yielding 900 mg (3.61 mmol, 90% yield) of the title product **(2*S*)-10** as a mixture of two diastereoisomers.

$[\alpha]_{20}^{D} = -15.6°$ (benzene), ^1H NMR ($CDCl_3$) δ (ppm): 1.80–2.10 (m, 4H, CH_2CH_2); 2.45–3.10 (m, 3H, $CHCH_2$ epoxide); 3.30–3.55 (m, 2H, CH_2N); 3.55–3.65 (m, 0.5H, CHN); 3.65–3.80 (m, 0.5H, CHN); 5.13 (s, 0.5 + 0.5H) and 5.15 (s, 0.5 + 0.5H) (CH_2Ph); 7.30–7.45 (m, 5H, aromatics). Anal. ($C_{14}H_{17}NO_3$): C, H, N.

(2*R*)-Phenylmethyl 2-oxiran-2-ylpyrrolidine-1-carboxylate [(2*R*)-10]. Using the same procedure described for **(2*S*)-10**, and starting from 1.5 g (6.5 mmol) of ***R*-(+)-5** and 2.25 g (13.0 mmol) of *m*-chloroperbenzoic acid (*m*CPBA), 1.41 g (5.66 mmol, 86.5% yield) of **(2*R*)-10** were obtained as a mixture of two diastereoisomers.

$[\alpha]_{20}^{D} = +15.8°$ (benzene), ^1H NMR ($CDCl_3$) δ (ppm): 1.80–2.10 (m, 4H, CH_2CH_2); 2.45–3.10 (m, 3H, $CHCH_2$ epoxide); 3.36–3.55 (m, 2H, CH_2N); 3.55–3.66 (m, 0.5H, CHN); 3.66–3.80 (m, 0.5H, CHN); 5.12 (s, 0.5 + 0.5H) and 5.15 (s, 0.5 + 0.5H) (CH_2Ph); 7.30–7.45 (m, 5H, aromatics). Anal. ($C_{14}H_{17}NO_3$): C, H, N.

(2*S*)-Phenylmethyl 2-(1-hydroxy-2-mercaptoethyl)pyrrolidine-1-carboxylate [(2*S*)-12]. 900 mg (3.6 mmol) of **(2*S*)-10** were reacted with 270 mg (3.6 mmol) of thioacetic *S*-acid for 12 h at 60 °C in anhyd conditions. The excess thioacetic acid was removed under reduced pressure, the residue dissolved in $CHCl_3$ and washed twice with a saturated solution of Na_2CO_3 and twice with water, and dried over Na_2SO_4. Evaporation of the solvent gave 990 mg of **(2*S*)-phenylmethyl 2-(2-acetylsulfanyl-1-hydroxyethyl)pyrrolidine-1-carboxylate [(2*S*)-11]**. IR (neat) ν (cm^{-1}): 3600–3250 (OH); 1750 (C=O thioacetyl); 1700 (C=O carbamate). ^1H NMR ($CDCl_3$) δ (ppm): 1.80–2.05 (m, 4H, CH_2CH_2); 2.35 (s, 3H, $CH_3C=O$); 2.75–4.40 (m, 6H, $CH_2N + CH_2S + CHN + CHO$); 5.15 (s, 2H, CH_2Ph); 7.30–7.45 (m, 5H, aromatics). The TLC indicated the presence of two isomers. The compound was used as such without further characterization. **(2*S*)-11** was dissolved in 5.5 mL of MeOH and 0.22 mL of concd HCl were added. The solution was kept at 60 °C for 6 h. Evaporation of the solvent yielded 910 mg of **(2S)-12** as a mixture of two diastereoisomers. Yield 90%.

IR (neat) ν (cm^{-1}): 3600–3200 (OH); 1690 (C=O), ^1H NMR ($CDCl_3$) δ (ppm): 1.65–2.05 (m, 4H, CH_2CH_2); 2.45–2.80 (m, 2H, CH_2S); 3.30–3.45 (m, 2H, CH_2N); 3.50–4.17 (m, 2H, $CHO + CHN$); 4.22–4.50 (bs, 2H, $OH + SH$); 5.15 (s, 2H, CH_2Ph); 7.30–7.45 (m, 5H, aromatics). Anal. ($C_{14}H_{19}NO_3S$): C, H, N.

(2*R*)-Phenylmethyl 2-(1-hydroxy-2-mercaptoethyl)pyrrolidine-1-carboxylate [(2*R*)-12]. Using the same procedure described for **(2*S*)-11** and starting from 1.41 g (5.7 mmol) of **(2*R*)-10** and 430 mg (5.7 mmol) of thioacetic *S*-acid, 1.53 g of **(2*R*)-phenylmethyl 2-(2-acetylsulfanyl-1-hydroxyethyl)pyrrolidine-1-carboxylate [(2*R*)-11]** were obtained. The TLC showed the presence of two diastereoisomers. IR (neat) ν (cm^{-1}): 3600–3250 (OH); 1750 (C=O thioacetyl); 1695 (C=O carbamate). ^1H NMR ($CDCl_3$) δ (ppm): 1.75–2.15 (m, 4H, CH_2CH_2); 2.35 (s, 3H, $CH_3C=O$); 2.67–4.20 (m, 6H, $CH_2N + CH_2S + CHN + CHO$); 5.12 (s, 2H, CH_2Ph); 7.30–7.45 (m, 5H, aromatics). The compound was used as such without further characterization. **(2*R*)-11** was dissolved in 8.64 mL of MeOH and 0.33 mL of concd HCl were added. The solution was kept at 60 °C for 6 h. Evaporation of the solvent yielded 1.38 g (4.9 mmol) of **(2*R*)-12** as a mixture of two diastereoisomers in 86% yield.

IR (neat) ν (cm^{-1}): 3600–3200 (OH); 1690 (C=O), ^1H NMR ($CDCl_3$) δ (ppm): 1.60–2.05 (m, 4H, CH_2CH_2); 2.40–2.80 (m, 2H, CH_2S); 3.25–4.15 (m, 4H, $CH_2N + CHO + CHN$); 5.12 (s, 2H, CH_2Ph); 6.40–6.70 (bs, 2H, $OH + SH$); 7.30–7.45 (m, 5H, aromatics). Anal. ($C_{14}H_{19}NO_3S$): C, H, N.

(2*S*)-Phenylmethyl 2-(2-methyl-1,3-oxathiolan-5-yl)pyrrolidine-1-carboxylate [(2*S*)-13]. To a solution of 910 mg (3.3 mmol) of **(2*S*)-12** in 18.3 mL of 2-propanol, 90 mg (0.5 mmol) of *p*-toluensulfonic acid monohydrate were

added. To this solution, 2.7 mL (25.5 mmol) of acetaldehyde dimethyl acetal were added, and the mixture was kept to reflux for 2 h and concentrated in vacuo. The residue was dissolved in 45 mL of diethyl ether, and the organic layer was washed with 30 mL of water and dried over Na_2SO_4. Evaporation of the solvent gave an oily residue that was purified by column chromatography on Al_2O_3 (eluent: CH_2Cl_2/petroleum ether, 6:4). Yield 520 mg (1.7 mmol, 52%) of **(2*S*)-13**.

$[\alpha]_{20}^{D} = -67.0°$ (CH_2Cl_2), ^{1}H NMR ($CDCl_3$) δ (ppm): 1.56 (d, $J = 5.5$ Hz, 3H, CH_3CH); 1.75–2.18 (m, 4H, CH_2CH_2); 2.80–3.20 (m, 2H, CH_2S); 3.40–3.60 (m, 2H, CH_2N); 4.02–4.47 (m, 2H, CHO + CHN); 5.00–5.25 (m, 3H, $CH_2Ph + CHCH_3$); 7.30–7.45 (m, 5H, aromatics). Anal. ($C_{16}H_{21}NO_3S$): C, H, N.

(2*R*)-Phenylmethyl 2-(2-methyl-1,3-oxathiolan-5-yl)pyrrolidine-1-carboxylate [(2*R*)-13]. Using the same procedure described for **(2*S*)-13**, and starting from 1.27 g (4.5 mmol) of **(2*R*)-12** and 3.81 mL (36.0 mmol) of acetaldehyde dimethyl acetal, crude **(2*R*)-13** was obtained, that was purified by column chromatography on Al_2O_3 (eluent: CH_2Cl_2/petroleum ether 6:4), affording 650 mg (2.1 mmol, 47% yield) of title compound as a mixture of diastereoisomers.

$[\alpha]_{20}^{D} = +68.2°$ (CH_2Cl_2), ^{1}H NMR ($CDCl_3$) δ (ppm): 1.55 (d, $J = 5.5$ Hz, 3H, CH_3CH); 1.74–2.16 (m, 4H, CH_2CH_2); 2.80–3.20 (m, 2H, CH_2S); 3.35–3.70 (m, 2H, CH_2N); 4.02–4.47 (m, 2H, CHO + CHN); 5.08–5.20 (m, 3H, $CH_2Ph + CHCH_3$); 7.30–7.45 (m, 5H, aromatics). Anal. ($C_{16}H_{21}NO_3S$): C, H, N.

(2*S*, 5′*S*, 2′*S*)-1-Methyl-2-(2-methyl-1,3-oxathiolan-5-yl)-pyrrolidine [(−)-14a] and (2*S*, 5′*R*, 2′*R*)-1-methyl-2-(2-methyl-1,3-oxathiolan-5-yl)pyrrolidine [(−)-14c]. A solution of 520 mg (1.7 mmol) of **(2*S*)-13** dissolved in the minimum amount of anhyd THF was added dropwise to a suspension of 410 mg (10.8 mmol) of $LiAlH_4$ in an. THF at −18 °C under nitrogen. The mixture was allowed to reach room temperature, and after 4 h was treated with brine and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and concentrated in vacuo to afford an oily mixture of diastereoisomers **[(2*S*)-14]**. The chromatographic separation on Al_2O_3 (eluent $CHCl_3$/petroleum ether 3:7) yielded 70 mg of isomer **a**, 40 mg of the isomer **c** and another fraction of unidentified isomers (fraction **b**). Total yield 41%. Elemental analysis and $[\alpha]_{20}^{D}$ of each isomer are reported in Table 3. Hydrogens are defined in Table 1.

(−)-14a. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.58 (d, $J = 5.5$ Hz, 3H, CH_3CH); 1.66–1.96 (m, 4H, CH_2CH_2); 2.19–2.30 (m, 1H, $CH H_GN$), 2.41 (s, 3H, CH_3N), 2.43–2.51 (m, 1H, CH_EN), 2.84–2.92 (m, 1H, $CH H_DS$), 2.97–3.15 (m, 2H, CH_CHS and CH_FHN), 3.95–4.03 (m, 1H, OCH_B), 5.22 (q, $J = 5.5$ Hz, 1H, $CHCH_3$).

(−)-14c. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.58 (d, $J = 5.5$ Hz, 3H, CH_3CH); 1.56–2.00 (m, 4H, CH_2CH_2); 2.23–2.34 (m, 1H, $CH H_GN$), 2.51 (s, 3H, CH_3N), 2.45–2.58 (m, 1H, CH_EN), 2.75–2.83 (m, 1H, $CH H_DS$), 2.96–3.20 (m, 2H, CH_CHS and CH_FHN), 3.80–3.92 (m, 1H, OCH_B), 5.23 (q, $J = 5.5$ Hz, 1H, $CHCH_3$).

Each isomeric amine was transformed in the corresponding oxalate by treatment with one equivalent of oxalic acid in ethyl acetate (Table 3).

(2*R*, 5′*R*, 2′*R*)-1-Methyl-2-(2-methyl-1,3-oxathiolan-5-yl)pyrrolidine [(+)-14a] and (2*R*, 5′*S*, 2′*S*)-1-methyl-2-(2-methyl - 1,3 - oxathiolan - 5 - yl)pyrrolidine [(+)-14c]. Using the same procedure described for **(2*S*)-14**, and starting from 650 mg (2.1 mmol) of **(2*R*)-13** and 500 mg (13.2 mmol) of $LiAlH_4$, crude **(2*R*)-14** was obtained. The chromatographic separation on Al_2O_3 (eluent $CHCl_3$/petroleum ether 3:7) afforded 100 mg of the first isomer **a**, 70 mg of the isomer **c** and another fraction of unidentified isomers (fraction **b**). Total yield 56%. Anal. ($C_9H_{17}NOS$): C, H, N. Elemental analysis and $[\alpha]_{20}^{D}$ of each isomer are reported in Table 3. Hydrogens are defined in Table 1.

(+)-14a. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.57 (d, $J = 5.5$ Hz, 3H, CH_3CH); 1.66–1.94 (m, 4H, CH_2CH_2); 2.20–2.29 (m, 1H, $CH H_GN$), 2.42 (s, 3H, CH_3N), 2.43–2.51 (m, 1H, CH_EN), 2.83–2.92 (m, 1H, $CH H_DS$), 2.96–3.15 (m, 2H, CH_CHS and CH_FHN), 3.94–4.03 (m, 1H, OCH_B), 5.22 (q, $J = 5.5$ Hz, 1H, $CHCH_3$); ^{13}C NMR ($CDCl_3$) δ (ppm): 22.19 (q), 23.76 (t), 26.34 (t), 35.72 (t), 42.08 (q), 58.30 (t), 67.19 (d), 82.25 (d), 84.32 (d).

(+)-14c. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.59 (d, $J = 5.7$ Hz, 3H, CH_3CH); 1.55–2.00 (m, 4H, CH_2CH_2); 2.25–2.34 (m, 1H, $CH H_GN$), 2.52 (s, 3H, CH_3N), 2.45–2.58 (m, 1H, CH_EN), 2.75–2.84 (m, 1H, $CH H_DS$), 2.95–3.20 (m, 2H, CH_CHS and CH_FHN), 3.89–4.07 (m, 1H, OCH_B), 5.24 (q, $J = 5.7$ Hz, 1H, $CHCH_3$); ^{13}C NMR ($CDCl_3$) δ (ppm): 22.06 (q), 23.90 (t), 28.22 (t), 36.19 (t), 42.86 (q), 58.45 (t), 67.91 (d), 82.76 (d), 87.76 (d).

Each isomer was transformed in the corresponding oxalate by treatment with one equivalent of oxalic acid in ethyl acetate (Table 3).

General procedure for the synthesis of the methiodides

An anhyd ether solution of the suitable tertiary amine was treated with an excess of methyl iodide and kept for 1 night at rt in the dark. The obtained solid was filtered, dried under vacuum, and recrystallized (when necessary) from absolute ethanol. Compounds **(−)-9 a**, **c**, **(+)-9 a**, **c**, **(−)-15 a**, **c**, **(+)-15 a**, **c** have been prepared by this procedure. The chemical and physical characteristics of the compounds are reported in Table 4. Their ^{1}H NMR spectra are consistent with the proposed structures. The spectrum of **(+)-9a** is reported as an example: ^{1}H NMR ($CDCl_3$) δ (ppm): 1.42 (d, 3H, CH_3CH, $J = 4.8$ Hz); 2.05–2.45 (m, 4H, CH_2CH_2); 3.32 (s, 3H, CH_3N); 3.55 (s, 3H, CH_3N); 3.63–4.00 (m, 3H, $CHHO + CHHN +$ CHN); 4.10–4.22 (m, 1H, $CHHO$); 4.59–4.74 (m, 1H, $CHHN$); 4.70–4.74 (m, 1H, $OCHCH_2$); 5.05 (q, 1H, $OCHCH_3$, $J = 4.8$ Hz).

Crystal structure determination and refinement of (+)-15a. Diffraction data were collected at 293 K on a Siemens P4 Four circle diffractometer equipped with a graphite-monochromated CuK_α radiation, using a $\theta/2\theta$ scan technique.

The crystal data and details of the data collection and structure refinement are summarised in Table 5. During the data collection three standard reflections were measured every four hundred reflections collected as orientation and intensity control; significant intensity decay was not observed. Corrections for Lorentz and polarisation effects and for absorption by Ψ-scans were applied. The crystal structure was solved by direct methods using the SIR97 program[29] which gave the position of most of the non-hydrogen atoms. The remaining atoms were identified by successive Fourier difference syntheses. Four identical independent compound molecules and two water molecules were found in the asymmetric unit. Refinement was carried out on F^2 by full-matrix least square techniques, using the SHELX97 program package.[30] Hydrogen atoms were added in the model constrained to idealised positions and refined using a riding model with riding isotropic displacement parameters. Hydrogen atoms of the solvent water molecules were not added. All the non-hydrogen atoms were refined anisotropically. Atoms C5, C6, C7 in one of the four independent molecules in the asymmetric unit were disordered and were modelled as two different positions. Refinement of the Flack parameter [−0.0154 (0.0100)] confirmed that the right-handed diastereoisomer had been obtained in our initial structure determination. Least square superposition of the four independent molecules led to an average rms of 0.24 Å.

In Figure 1 (see above) an ORTEP representation of **(+)-15a** showing one of the four independent molecules is shown. A complete set of data has been deposited at the Cambridge Crystallographic Data Center with number CCDC 185530.

Table 4. Chemical and physical characteristics of derivatives **9** and **15**

Compd	Stereochemistry	Mp (°C)	$[\alpha]_{20}^{D}$	Analysis
(−)-9a	2*S*,4′*S*, 2′*R*	177–180	−32.5[a]	C_{10} H_{20} NJO_2
(−)-9c	2*S*,4′*R*, 2′*S*	107–108	−5.6[a]	C_{10} H_{20} NJO_2
(+)-9a	2*R*,4′*R*, 2′*S*	180–182	+26.5[b]	C_{10} H_{20} NJO_2
(+)-9c	2*R*,4′*S*, 2′*R*	105–106	+6.0[a]	C_{10} H_{20} NJO_2
(−)-15a	2*S*,5′*S*, 2′*S*	145–147	−41.5[a]	C_{10} H_{20} NJOS
(−)-15c	2*S*,5′*R*, 2′*R*	148–150	−12.2[b]	C_{10} H_{20} NJOS
(+)-15a	2*R*,5′*R*, 2′*R*	148–151	+42.2[a]	C_{10} H_{20} NJOS
(+)-15c	2*R*,5′*S*, 2′*S*	151–152	+13.8[a]	C_{10} H_{20} NJOS

[a]In $CHCl_3$.
[b]In MeOH.

Table 5. Crystal data and structure refinement for **(+)-15a**

Formula	C_{40} H_{80} I_4 N_4 O_6 S_4
Formula weight	1348.92
Space group	P 2_1
Unit cell dimensions	
a (Å)	14.4350(19)
b (Å)	7.8400(11)
c (Å)	25.503(3)
β (°)	99.637(9)
Volume (Å^3)	2845.5(7)
Z	8
Density (calcd) (g/cm^3)	1.574
Abs coeff (mm^{-1})	18.90
Radiation	CuK_α
Theta range for data collection (°)	1.76–64.52
Reflns collected	6456
No. of unique reflns	5702
Completeness to theta = 64.52° (%)	95.4
Value of R_{int}	0.043
Final *R* indices [$I > 2\sigma(I)$]	R1 = 0.0589, wR2 = 0.1630
R indices (all data)	R1 = 0.0659, wR2 = 0.1736
Absolute struct param	−0.014(10)
Extinction coeff	0.00202(16)
Largest diff. peak and hole (e Å^{-3})	1.848 and −1.400

Pharmacology

Binding studies. Membrane preparation. Male rats (Wistar, 200 g) were killed by decapitation and the brains were removed and immediately placed on ice. The brain was dissected, homogenised in 10 vol 0.32 M sucrose with a glass–teflon homogeniser. The homogenate was then centrifuged at 1000*g* for 15 min; the resulting supernatant was further centrifuged at 17,000*g* for 20 min and the pellet was stored at −80 °C until assayed.[31]

Binding experiments. The muscarinic receptors were labelled with 0.1 nM [^{3}H]*N*-methyl scopolamine ([^{3}H]NMS, 79.5 Ci/mmol from NEN-Dupont). The frozen pellet was suspended in Krebs–Hepes buffer pH 7.4 (composition, mM: NaCl 118, KCl 4.7, $NaHCO_3$ 5, KH_2PO_4 1.2, glucose 11, $MgSO_4$ 1.2, $CaCl_2$ 2.5 and Hepes 20), at 4 °C as previously described in Freedman et al.[31] Aliquots of cerebral membranes at a protein concentration of 100–150 μg/mL were incubated at 30 °C for 60 min in a final volume of 1 mL with the marker ligand and with different concentrations of unlabeled ligands (0.1 nM–0.1 mM); the incubation was terminated by filtration through Whatman GF/B glass fiber filters presoaked in 0.1% polyethyleneimine (PEI), using a Brandel M-48R 48-well cell harvester. Filters were washed twice with 10 mL of ice-cold 0.9% saline solution. Protein concentration was estimated using the Pierce protein assay reagent (Pierce ChemicalCo., Rockford, IL, USA) based on the method of Bradford[32] with bovine serum albumin as standard. In all experiments, the radioactivity retained by filters was measured in a liquid scintillation counter (TRI-CARB 1900TR, Packard) after the addition of 4 mL of scintillation fluid (Filter Count, Packard) and all measurements were obtained in duplicate. The binding data were evaluated quantitatively with non-linear least-square curve fittings using the computer programs ALLFIT[33] and LIGAND.[34]

Functional in vitro tests. Guinea-pig ileum. Male guinea pigs (200–300 g) were killed by cervical dislocation. Two-centimeter-long portions of terminal ileum were taken at about 5 cm from the ileum–cecum junction and mounted under 1 g of tension in 20-mL organ baths containing physiological salt solution [PSS; composition (mM): NaCl 118, $NaHCO_3$ 23.8, KCl 4.7, $MgSO_4.7H_2O$ 1.18, KH_2PO_4 1.18, $CaCl_2$ 2.52, and glucose 11.7] at 37 °C and aerated with 5% CO_2–95% O_2. Tension changes were recorded isotonically by means of a force–

displacement transducer connected to the MacLab system PowerLab/800 and to a two-channel Gemini polygraph (U. Basile). Tissues were equilibrated for 30 min and dose–response curves for arecaidine propargyl ester (APE) were obtained at 30-min intervals, the first one being discarded and the second one being taken as the control. The concentration of agonist in the organ bath was increased approximately 3-fold at each step, with each addition being made only after the response to the previous addition had attained a maximal level and remained steady. Following 30 min of washing, a new dose–response curve for the agonist under study was obtained. Responses were expressed as a percentage of the maximal response obtained in the control curve. The results are expressed in terms of $-\log ED_{50}$, the concentration of agonist required to produce 50% of the maximum contraction. Parallel experiments in which tissues received only the reference agonist were run in order to check any variation in sensitivity.

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