Stereoselective Synthesis and Biodistribution of Potent [^{11}C]-Labeled Antagonists for Positron Emission Tomography Imaging of Muscarinic Receptors in the Airways

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Quantitation of muscarinic receptors in the lungs in vivo with positron emission tomography (PET) is of clinical interest. For that purpose we decided to develop [^{11}C]-labeled ligands with a high affinity (K_D < 0.1 nM). Three quaternary muscarinic antagonists, racemic *N*-methylpiperidin-4-yl 2-cyclohexyl-2-hydroxy-2-phenylacetate methiodide **1a** (pK_B = 10.39), its (*R*)-isomer **1b** (pK_B = 11.08), and (*R*,*R*)-quinuclidin-3-yl 2-cyclohexyl-2-hydroxy-2-phenylacetate methiodide **2** (pK_B = 11.28), were labeled by reacting [^{11}C]CH_3I with their tertiary amine precursors. The enantiomerically pure tertiary amine precursors were prepared by stereoselective synthesis starting from (*R*)-(−)-mandelic acid. In vitro binding assay of **1b** and **2** demonstrated that both ligands bind with very high affinity to the muscarinic receptor subtypes M_1, M_2, and M_3. They are more potent than the muscarinic antagonist (*R*)-*N*-methylquinuclidinyl benzilate ((*R*)-MQNB). Distribution studies with **1a**, **1b**, and **2** in control and atropine-treated male Wistar rats demonstrated significant specific binding (90−99% of total issue uptake) in tissues containing cholinoceptors (heart, intestine, lung, pancreas, spleen, stomach, submandibular gland). Because the tissue/plasma concentration ratios of **1b** are most favorable, this ligand was used for further evaluation. Analysis of plasma samples showed a very rapid clearance ($t_{1/2}$ = 0.3 min) of the radioligand **1b** and a relatively slow appearance of a hydrophilic metabolite. At 15 min postinjection of **1b**, analysis of heart, lungs, and liver showed that respectively 99%, 88%, and 8% of the tissue radioactivity corresponded with the parent compound. Ligand **1b** appears to be an excellent candidate for PET studies of mAChR receptors in heart and lungs.

Introduction

Muscarinic cholinergic receptors (mAChR) are important in many physiological processes, such as smooth muscle contraction and exocrine and endocrine secretion.[1] Cloning studies have confirmed the heterogeneity of muscarinic receptors, and to date five different subtype genes (m_1, m_2, m_3, m_4, and m_5) have been identified.[2] Subtypes m_1, m_2, m_3, and m_4 correspond to the pharmacologically defined M_1, M_2, M_3, and M_4 receptors, respectively, and the m_5 subtype awaits pharmacological characterization.[3,4] Human lungs contain M_1, M_2, and M_3 receptors.[5] M_1 receptors facilitate neurotransmission through parasympathetic ganglia and enhance cholinergic reflexes. M_2 receptors act as autoreceptors on postganglionic cholinergic nerves and inhibit acetylcholine release. The M_3 subtype mediates smooth muscle contraction via phosphoinositide hydrolysis. Pulmonary mucus secretion is stimulated via the M_1 and M_3 subtypes. M_4 and M_5 receptors have not been identified in human airways.[5] Quantification of mAChRs in the lungs in vivo with positron emission tomography (PET) is of clinical interest, especially in patients suffering from asthma and chronic obstructive pulmonary diseases. The efficacy of β-agonists (sympathicomimetics) and muscarinic antagonists (parasympathicolytics) in such patients changes with the duration of treatment and with age. The suppression of bronchial hyperreactivity by sympathicomimetics is much better after a single administration than when the drugs are administered chronically. The protection and airway relaxation of juvenile asthma patients is much better after treatment with sympathicomimetics than after treatment with parasympathicolytics. However patients over 50 years of age respond better to parasympathicolytic drugs. These observations indicate an altered drug/receptor interaction after prolonged treatment and with increasing age. This could be due to a change of pulmonary receptor density[6,7] or to an altered coupling of the receptors to the contraction mechanism of the smooth muscle cells.

Although a few reports on labeled muscarinic ligands for quantification of the mAChRs in heart[8,9] and brain[10,11] with PET have appeared, little attention has been paid to the airways. A major obstacle in the quantitation of muscarinic receptors in the airways is the relatively low receptor density in this tissue. The concentrations in human peripheral lung range from 25 to 100 fmol/mg protein.[12−14] Because of this low density, high-affinity ligands (K_D < 10^{-10} M) must be employed to obtain reasonable (>10) signal to noise ratios in PET.[15] Muscarinic receptor subtype selective ligands like pirenzepine (M_1),[16] methoctramine (M_2),[16] AFDX 116 (M_2),[17] hexahydrosiladifenidol (M_3),[16,18] and 4-DAMP (M_3)[16,19,21]

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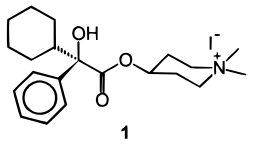
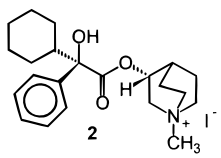
Scheme 1



1

N-Methyl-piperidin-4-yl-2-cyclohexyl-2-hydroxy-2-phenylacetate methiodide
a) racemate
b) (R)-isomer



2

(R,R)-Quinuclidin-3-yl-2-cyclohexyl-2-hydroxy-2-phenylacetate methiodide

are not potent enough to be used for PET imaging of cholinoceptors in the airways. Only a limited number of drugs have sufficiently high receptor affinity. (*R*)-*N*-Methylquinuclidinyl benzilate ((*R*)-MQNB) is a potent muscarinic antagonist,[21] but according to Delforge it is not extracted by the lungs.[22] Some esters of glycolic acid are among the most potent anti-acetylcholine drugs known. In 1973 Inch[23] reported the synthesis of various glycolic esters which were very potent antagonists in functional experiments. For instance compounds **1** and **2** (Scheme 1) antagonized the carbachol-induced contractions of guinea pig ileum in picomolar concentrations.[24] The (*R*)-isomer of **1** ($pK_B = 11.08$) and the (*R,R*)-isomer of **2** ($pK_B = 11.28$) were most potent in functional experiments.[24] Inch synthesized these potent antagonists by the degradation of carbohydrate derivatives. We explored a shorter route for the stereoselective synthesis of these potent cholinoceptor ligands.

To assess whether these antagonists are useful candidates for the quantitation of pulmonary muscarinic receptors, we reacted the tertiary amine precursors with CH_3I. The present paper describes the stereoselective synthesis and the binding of the ligands to the M_1, M_2, and M_3 muscarinic receptor subtypes in vitro as well as the tissue distribution, metabolism, and clearance from plasma of the [^{11}C]-labeled quaternized products **1a**, **1b**, and **2** in male Wistar rats.

Results and Discussion

Chemistry. Some methods have appeared in literature for the preparation of enantiomerically pure esters of α-hydroxy acids. These methods include the resolution of 2-cyclohexyl-2-hydroxy-2-phenylethanoic acid with quinine[25] or ephedrine[26] as the resolving agent, diastereoselective synthesis with 8-phenylmenthol as the chiral auxiliary,[27] and an asymmetric synthesis with (*S*)-(anilinomethyl)-2-pyrrolidine.[28] In 1984 the group of Seebach showed that protected α-hydroxy acids could be α-alkylated with various electrophiles (primary alkyl halides, allyl halides, and ketones) to produce 5-disubstituted dioxolanones in high yield and high diastereoselectivities. This process was called self-reproduction of chirality.[29−31] Although Inch synthesized the antagonists **1** and **2** by the degradation of carbohydrate derivatives, the route which we selected for the stereoselective synthesis of these potent cholinoceptor ligands is based on the method used by Seebach. This methodology is very attractive because all the isomers of compounds **1** and **2** are easily prepared in enantiomerically pure form, in a few, good- to high-yield steps, from readily available starting materials. Furthermore the substituents on the α-position of the α-hydroxy esters can be varied very easily, making this route particularly attractive for the synthesis of a variety of structurally related compounds. Therefore this method is highly interesting for pharmacological studies, e.g. structure−activity studies. The route is depicted in Scheme 2. (*R*)-(−)-Mandelic acid **3b** was condensed with pivalaldehyde **4** under azeotropic removal of the water formed to give a mixture of the *trans*- and *cis*-dioxolanones. ^{1}H NMR (200 MHz) showed a cis:trans ratio of 7.5:1. The *cis*-dioxolanone **5** was obtained in diastereomerically pure form after crystallization from pentane/ether in 71% yield. Deprotonation of the *cis*-dioxolanone at −80 °C with LDA did not result in formation of the lithium enolate, but by carrying out the reaction at higher temperatures (−40 to −10 °C), deprotonation went very smoothly.

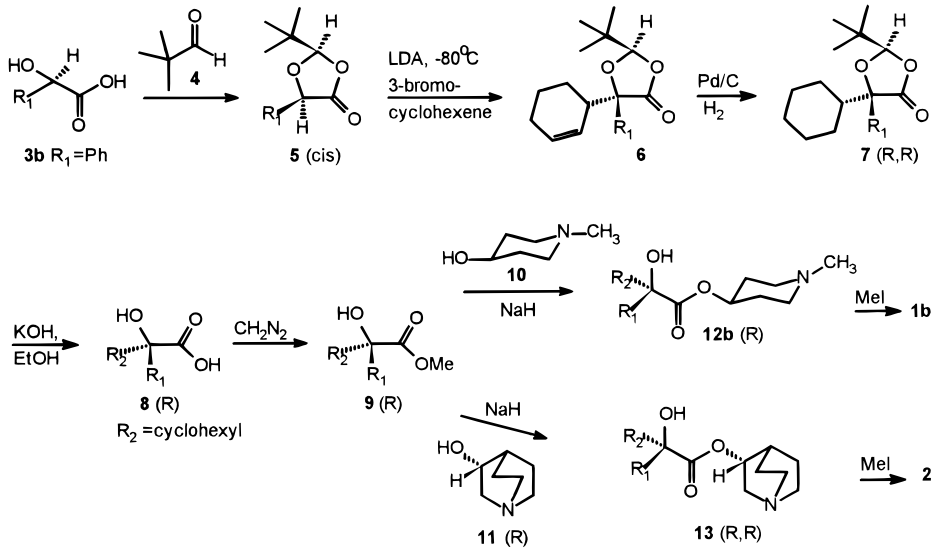
Reaction of the lithium enolate with bromocyclohexane did not afford any 5,5-disubstituted dioxolanone **7**. In this reaction step only starting material was isolated in quantitative yield even after performing the reaction at room temperature overnight. The lack of reactivity of the secondary alkyl halides is probably due to steric repulsion.[32] By using 3-bromocyclohexene which is an allyl halide and probably reacting via a S_N2' type of mechanism, the 5,5-disubstituted dioxolanone **6** could be isolated in good yields with a cis:trans ratio of 11:1. In this reaction step an additional stereogenic center is introduced (allylic carbon) with a selectivity ratio of 1:2. After crystallization from hexane at −18 °C, ^{1}H NMR showed that the trans isomers were no longer present. Subsequently the additional stereogenic center was destroyed again by reduction of the double bond with a catalytic amount of Pd/C and H_2, and compound **7** was obtained in 98% yield with a diastereomerical excess of >99%. It is also possible to reduce the double bond with Pd/C and H_2 first and to separate the cis and trans isomers afterward by crystallization from hexane at −18 °C.

Esterification of the 5,5-disubstituted dioxolanone with *N*-methyl-4-hydroxypiperidine under the influence of base (Na or NaH) as well as acid (*p*-TsOH) under various reaction conditions gave yields below 10%. However, deprotection with KOH at room temperature afforded α-cyclohexyl-α-hydroxy-α-phenylacetic acid **8** in 85% yield. The enantiomeric excess of **8** was determined by means of a chiral β-cyclodextrin column and appeared to exceed 99%.[33] Reaction of **8** with diazomethane afforded the methyl ester **9** in 95% yield. Transesterification with *N*-methyl-4-hydroxypiperidine **10** or (*R*)-3-quinuclidinol[34] **11** under the influence of NaH yielded the esters **12b** (*R*) and **13** (*R,R*) in 40% and 18% yield, respectively.

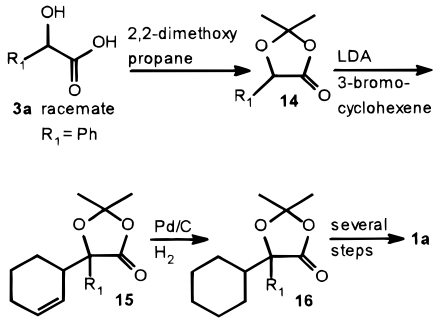
The racemic piperidyl ester **12a** was prepared, starting from racemic mandelic acid **3a**. Reaction of **3a** with 2,2-dimethoxypropane (Scheme 3) afforded the acetonide **14**, which after deprotonation with LDA was reacted with 3-bromocyclohexene to give the 5,5-disubstituted acetonide **15**. Again, reaction of the lithium enolate with bromocyclohexane did not give any result. Reduction of the double bond with Pd/C and H_2 yielded compound **16**, which was subsequently transformed in a few steps to the racemic piperidyl ester **12a**, analogous to the route as depicted in Scheme 2. Quaternization of the tertiary amines **12a**, **12b**, and **13** with [^{11}C]CH_3I in a closed vessel at 110 °C afforded the labeled muscarinic antagonists **1a**, **1b**, and **2** in 40−60% radiochemical yield and with specific activities of 11.1−44.4 TBq/mmol (300−1200 Ci/mmol), 40 min EOB (Scheme 4).

Dynamic PET Studies in Male Wistar Rats. To

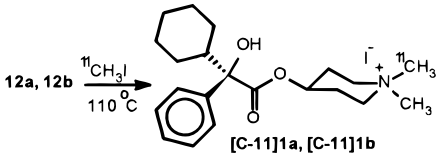
Scheme 2. Stereoselective Synthesis of Muscarinic Antagonists **1b** and **2**



Scheme 3. Synthesis of Racemic **1a**



Scheme 4. Quaternization with [^{11}C]Methyl Iodide[a]



[a] The same conditions were used for the labeling of compound **13**.

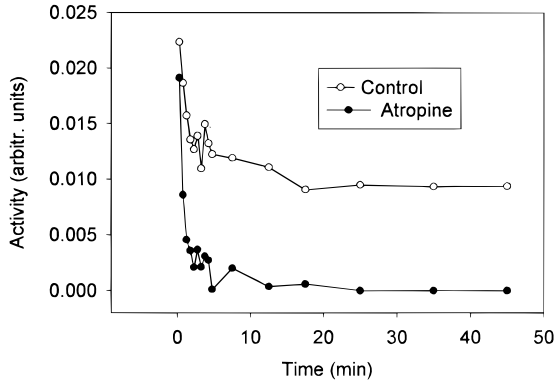


Figure 1. Dynamic PET study in male Wistar rats after injection of racemic **1a**, with and without pretreatment of atropine. The myocardial time activity curves are shown.

Table 1. Biodistribution of Muscarinic Antagonists **1a**, **1b**, and **2** in Male Wistar Rats, 15 min Postinjection[a]

controls	racemate **1a** ($N = 6$)	(*R*)-isomer **1b** ($N = 6$)	(*R*,*R*)-isomer **2** ($N = 6$)
bladder	4.71 ± 5.10	4.68 ± 1.53	5.11 ± 1.61
cerebellum	BLD	BLD	BLD
cortex	BLD	BLD	BLD
fat	0.50 ± 0.37	0.10 ± 0.08	0.44 ± 0.16
heart	8.45 ± 1.47	12.78 ± 1.17[b]	12.18 ± 1.31
intestine	3.39 ± 1.02	6.08 ± 2.23[b]	5.40 ± 2.40
kidney	2.48 ± 0.42	2.75 ± 0.57	3.10 ± 0.47
liver	6.27 ± 1.44	5.42 ± 1.00	5.73 ± 1.48
lung	0.67 ± 0.12	1.03 ± 0.22[b]	1.02 ± 0.10
muscle	0.08 ± 0.04	0.07 ± 0.04	0.31 ± 0.14
pancreas	1.97 ± 0.39	2.85 ± 0.50[b]	2.56 ± 0.51
plasma	0.14 ± 0.02	0.10 ± 0.01[b]	0.08 ± 0.01
red blood cells	BLD	BLD	BLD
spleen	0.97 ± 0.27	1.23 ± 0.15	1.41 ± 0.30
stomach	1.62 ± 0.40	2.76 ± 0.25[b]	1.90 ± 0.35
submandibular gland	1.68 ± 0.28	2.64 ± 0.39[b]	2.27 ± 0.34
testes	0.07 ± 0.02	0.12 ± 0.05	0.11 ± 0.04
trachea	0.54 ± 0.04	0.89 ± 0.15[b]	0.90 ± 0.22

[a] Data are listed as mean ± SD and expressed as standardized (body weight-corrected) uptake values. BLD = below limit of detection. [b] Significant difference (one-way ANOVA $p < 0.05$) between racemate **1a** and (*R*)-isomer **1b**. No significant difference between (*R*)-isomer **1a** and (*R*,*R*)-isomer **2** was observed.

obtain insight in the pharmacokinetics of the tracers, the time course of radioactivity in an organ with very high receptor density, i.e. the heart of male Wistar rats, was monitored over a 50 min period, using a Siemens ECAT 951/31 PET camera. In this preliminary study, male Wistar rats (240 g) were injected intravenously in the tail vein with the racemic piperidyl ester **1a**. Heart was visible in two planes, and the time−activity curve (Figure 1) showed an initial rapid, partial washout, followed by a slow decay. In the same figure, the myocardial time−activity curve of rats pretreated with atropine is shown. This curve demonstrates that the uptake of **1a** is nearly completely blocked by atropine. These results are a very good indication that the uptake of **1a** is receptor mediated and that there is low nonspecific binding. The curves also show that a plateau value is already reached within 10 min. Therefore, a distribution period of 15 min was used in the tissue distribution studies.

Tissue Distribution Studies in Male Wistar Rats. The distribution of radioligands **1a**, **1b**, and **2** in various tissues of male Wistar rats is shown in Table 1. The tissue/plasma ratios of some organs containing cholinoceptors are presented in Table 3. These ratios are important because they give a good indication of the signal to noise ratios which will be obtained in the PET images. Tissue uptake was determined by ex vivo counting, 15 min postinjection, and expressed as a

Table 2. Biodistribution of Muscarinic Antagonists **1a**, **1b**, and **2** in Male Wistar Rats, 15 min Postinjection with Pretreatment of the Muscarinic Antagonist Atropine 2.5 mg/kg[a]

blocked	racemate **1a** ($N = 5$)	(*R*)-isomer **1b** ($N = 5$)	(*R,R*)-isomer **2** ($N = 6$)
bladder	9.72 ± 9.77	5.92 ± 4.27	4.50 ± 2.82
cerebellum	BLD	BLD	BLD
cortex	BLD	BLD	BLD
fat	0.23 ± 0.20	0.37 ± 0.24	0.22 ± 0.15
heart	0.16 ± 0.02[b]	0.12 ± 0.01[b,c]	0.10 ± 0.02[b]
intestine	1.10 ± 0.52[b]	0.97 ± 0.75[b]	4.68 ± 2.32[d]
kidney	1.49 ± 0.06[b]	4.32 ± 0.67[b,c]	2.86 ± 0.72
liver	8.10 ± 0.78[b]	9.41 ± 1.18[b]	7.41 ± 1.83
lung	0.14 ± 0.02[b]	0.11 ± 0.02[b]	0.12 ± 0.04[b,d]
muscle	0.07 ± 0.05	0.07 ± 0.05	0.14 ± 0.13
pancreas	0.34 ± 0.12[b]	0.35 ± 0.11[b]	0.38 ± 0.16[b]
plasma	0.13 ± 0.02	0.19 ± 0.03[b]	0.12 ± 0.02[b]
red blood cells	B.L.D.	B.L.D.	B.L.D.
spleen	0.18 ± 0.03[b]	0.12 ± 0.02[b,c]	0.20 ± 0.08[b,d]
stomach	0.45 ± 0.28[b]	0.22 ± 0.07[b]	0.23 ± 0.07[b]
submandibular gland	0.20 ± 0.06[b]	0.23 ± 0.12[b]	0.25 ± 0.06[b,d]
testes	0.11 ± 0.06	0.16 ± 0.09	0.14 ± 0.05
trachea	0.24 ± 0.04[b]	0.26 ± 0.02[b]	0.31 ± 0.05[b,d]

[a] Data are listed as mean ± SD and expressed as standardized (body weight-corrected) uptake values. BLD = below limit of detection. [b] Significant difference (Student's *t*-test, $p < 0.05$) between controls and atropine treated animals. [c] Significant difference (one-way ANOVA, $p < 0.05$) between racemate 1a and (*R*)-isomer **1b**. [d] Significant difference (one-way ANOVA, $p < 0.05$) between (*R*)-isomer **1b** and (*R,R*)-isomer **2**.

differential absorption ratio (DAR) which is defined as (cpm recovered/g of tissue)/(cpm injected/g of body weight). These data demonstrate that the tissues rich in mAChR (heart, intestine, pancreas, submandibular gland) show the highest accumulation of radioactivity. Although there are many muscarinic receptors located in the brain, none of the ligands accumulated in this tissue. Probably because of the low lipophilicity, the quaternary antagonists cannot cross the blood/brain barrier. Uptake in erythrocytes was below the limit of detection. Injection of the more potent (*R*)-isomer (**1b**) instead of its racemate (**1a**) caused a significant increase in uptake in nearly all the organs containing cholinoceptors, and the plasma radioactivity was significantly lowered. This resulted in a doubling of the tissue to plasma ratios in these organs. Ligand **1b** demonstrated moderate lung/plasma ratios (10 ± 3) and lung/blood ratios (20 ± 3) at 15 min postinjection. Injection of (*R,R*)-quinuclidinyl ester (**2**) instead of the (*R*)-piperidyl ester (**1b**) did not improve uptake values and tissue/plasma ratios.

To prove that the uptake of the radioligands represents binding to cholinoceptors, blocking studies were performed in anaesthesized male Wistar rats. In these studies a solution of atropine (2.5 mg/kg) in saline was injected intravenously 1 min before administration of the radioligand. In Table 2 the results of the blocking experiments are shown. Radioactivity in the target organs (heart, intestine, kidney, liver, lung, pancreas, spleen, stomach, and trachea) was significantly reduced when the rats were pretreated with atropine. In heart and lungs, respectively 99% and 84% of the uptake was blocked by atropine, suggesting that the uptake in these organs is indeed receptor-mediated. Injection of the (*R,R*)-ligand **2** instead of the (*R*)-compound **1b** slightly increased nonspecific binding in intestine, lung, spleen, submandibular gland, and trachea.

Clearance from Plasma and Metabolism. Because radioligand **1b** was most promising in the biodistribution studies, this ligand was further evaluated in male Wistar rats. Its clearance from plasma was determined and shown to be very rapid (Figure 2). The clearance demonstrates a biphasic pattern with a half-life of 0.3 min for the initial rapid distribution phase and a half-life of 6.2 min for the slow phase. We have also studied the metabolism of radioligand **1b** in rat tissues and plasma in vivo. For quantitation of the receptor density it is important to know whether there are labeled metabolites in the target tissues and in blood. Therefore we analyzed extracts of heart, lung, and liver 15 min after intravenous administration of the antagonist **1b**. HPLC analyses of the tissue extracts indicated that respectively 99%, 88%, and 8% of radioactivity in heart, lungs, and liver was the parent compound. The parent compound eluted with a retention time of 7 min; one hydrophilic metabolite eluted with a retention time of 4.1 min. We also analyzed the time course of metabolism of ligand **1b** in plasma over a period of 40 min. The percentage of total plasma radioactivity which coeluted with the parent compound decreased from 99.5% at 0 min to 46% at 40 min postinjection.

Radioligand Binding Assays. Radioligand binding affinities and reference muscarinic agents are documented in Table 4. The binding studies were conducted in rat tissue and calf brain homogenates. The nonselective radioligand [^{3}H]-*N*-methylscopolamine was used to label muscarinic receptors in preparations of rat cortex (M_1), calf brain (M_1), and rat heart (M_2). Binding to the M_3 muscarinic subtype in guinea pig ileum was determined by Nova Screen (Hanover, MD) with [^{3}H]-*N*-methylscopolamine as the radioligand. In their assay, 4-DAMP was used as a reference compound. The results show that ligands **1b** and **2** have a very high affinity for all the muscarinic receptor subtypes. The antagonists are even more potent than (*R*)-MQNB. Compound **1b** shows the highest affinity for the M_1 and M_3 receptors. In rats 91% of the pulmonary cholinoceptors is of the M_2 subtype.[35] Cholinergic receptor densities are in the order of 16−35 fmol/mg of protein,[36,37] which is less than in human lungs. The receptor density in human lungs varies between 25 and 100 fmol/mg of protein.[12−14] In human lungs the dominant muscarinic receptors are of the M_1 (60%) and the remaining receptors are of the M_3 subtype. There is only a very small population of the M_2 subtype present.[13] So it is clear that the rat studies in comparison to human studies can be seen as a worst case scenario. Therefore lung/blood ratios in humans will probably be higher than those observed in rats, and we intend to evaluate compound **1b** in humans.

Conclusion

We have synthesized racemic *N*-methylpiperidin-4-yl 2-cyclohexyl-2-hydroxy-2-phenylacetate methiodide (**1a**), its more potent (*R*)-isomer **1b**, and (*R,R*)-quinuclidin-3-yl 2-cyclohexyl-2-hydroxy-2-phenylacetate methiodide (**2**) (most potent of the four possible isomers) by means of a novel stereoselective synthesis starting from (*R*)-(−)-mandelic acid. The binding affinity of the ligands **1b** and **2** to the M_1, M_2, and M_3 muscarinic receptor subtypes was determined in vitro. The compounds appeared to be more potent than (*R*)-MQNB. The tertiary amine precursors were labeled with [^{11}C]-CH_3I and tested in rats. The time course of myocardial radioactivity in male Wistar rats was monitored with a PET camera, and the time−activity curve showed an

Table 3. Tissue/Plasma Ratios of the Muscarinic Antagonists **1a**, **1b**, and **2** in Male Wistar Rats

	ligands					
organ	**1a** control	atropine	**1b** control	atropine	**2** control	atropine
heart	61.1 ± 8.5	1.3 ± 0.3	118.2 ± 24.4*	0.6 ± 0.1*	140.3 ± 9.9	1.0 ± 0.4
intestine	33.0 ± 20.2	8.7 ± 4.5	47.2 ± 24.4	5.3 ± 4.3	54.7 ± 19.0	33.5 ± 22.5#
lung	4.8 ± 0.7	1.1 ± 0.1	10.1 ± 2.9*	0.6 ± 0.1*	11.8 ± 1.1	1.0 ± 0.3#
pancreas	14.2 ± 1.5	2.3 ± 0.7	28.8 ± 5.6*	1.9 ± 0.8	30.3 ± 5.3	3.4 ± 1.7
spleen	6.3 ± 1.7	1.8 ± 1.1	11.0 ± 2.0*	0.6 ± 0.2*	15.6 ± 4.3	1.8 ± 0.8#
stomach	11.6 ± 1.8	3.0 ± 2.2	24.7 ± 3.6*	1.2 ± 0.4	22.6 ± 3.4	4.1 ± 3.2
sub. gland	12.1 ± 1.0	1.6 ± 0.5	26.3 ± 7.5*	1.3 ± 0.8	27.2 ± 4.9	2.7 ± 1.1#
trachea	4.0 ± 0.7	2.1 ± 0.6	8.0 ± 2.8*	1.6 ± 0.3	10.8 ± 3.1	3.0 ± 0.8#

* Significant difference ($p < 0.05$) between **1b** and **1a**. # Significant difference ($p < 0.05$) between **1b** and **2**.

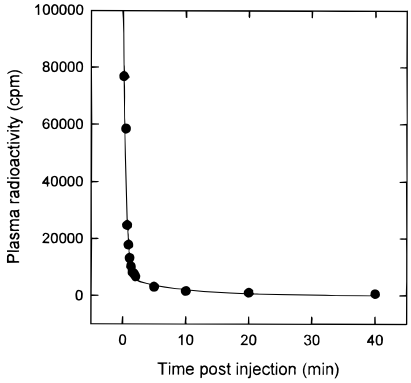


Figure 2. Clearance of radioactivity from plasma after administration of (*R*)-isomer **1b**. The solid line is a biexponential curve fit.

Table 4. Binding Affinities of **1b**, **2**, and Other Muscarinic Antagonists

	K_i (nM)			
compound	M_1 rat cortex	M_2 rat heart	M_3 guinea pig ileum	M_1 calf brain
1b	0.01	0.21	0.09[b]	0.02
2	0.02	0.10	0.12[b]	0.01
scopolamine	0.53	4.57		0.41
4-DAMP (MeI)			66.9[b]	
(*R*)-MQNB[a]	0.13[c]	0.43	0.65[d]	

[a] Reference 21. [b] Determined by NovaScreen, mean of two determinations. [c] In NB-OK-1-cells. [d] In rat pancreas.

initial rapid, partial washout followed by a slow decay. Biodistribution studies in untreated and atropine-treated rats demonstrated significant specific binding in tissues containing muscarinic receptors. Use of the active (*R*)-isomer (**1b**) instead of its racemate (**1a**) resulted in a doubling of the tissue to plasma concentration ratios in most organs. However use of the slightly more potent (*R,R*)-compound **2** instead of the (*R*)-compound (**1b**) did not improve tissue/plasma ratios, and it slightly increased nonspecific binding. Ligand **1b** demonstrated low but useful lung/plasma ratios (10 ± 3) and lung/blood ratios (20 ± 3) at 15 min postinjection. Compound **1b** was slowly metabolized in male Wistar rats. Tissue uptake of labeled metabolites was negligible in heart and 12% in lungs, 15 min postinjection. Ligand **1b** appeared to be an excellent candidate for PET studies of mACh receptors in heart and lungs.

Experimental Section

General Remarks. IR spectra were recorded with a Perkin-Elmer 841 infrared spectrometer. Melting points were determined with a Mettler FP-2 melting point apparatus, equipped with a Mettler FP-2 microscope (uncorrected). ^{1}H NMR spectra were recorded on a Varian Gemini 200 (200 MHz) spectrometer. Chemical shifts are denoted in δ units (ppm) and are determined relative to the solvent ($CDCl_3$) and converted to the TMS scale using δ ($CDCl_3$) = 7.26 ppm. ^{13}C NMR spectra (APT) were recorded on a Varian Gemini 200 (at 50.32 MHz). Chemical shifts are denoted in δ units (ppm) relative to the solvent and converted to the TMS scale using δ ($CDCl_3$) = 76.91 ppm. The splitting patterns are designated as follows: s (singlet), 2s (2× singlet), d (doublet), dd (doublet doublet), t (triplet), q (quartet), m (multiplet), and br (broad). Optical rotations were measured at the NaD-line on a Perkin-Elmer 241 MC polarimeter at room temperature. Elemental analyses were performed in the Microanalytical Department, University of Groningen. Mass spectra were recorded on an AEI-MS-902 mass spectrometer (EI). Chemicals were purchased from Merck, Janssen, or Aldrich. Ether, CH_2Cl_2, hexane, and pentane were distilled over P_2O_5 except when the solvent was used for extractions. Toluene and tetrahydrofuran (THF) were distilled over Na (benzophenone). Benzene was used without any further purification. Thin layer chromatography was performed using silica gel 60 F_{254} (Merck, art. no. 7734 or 9385). 3-Bromocyclohexene was synthesized according to a procedure described in literature.[38]

(2*R*,5*R*)-2-*tert*-Butyl-5-phenyl-1,3-dioxolan-4-one (5). A mixture of (*R*)-mandelic acid **3b** (13.24 g, 87 mmol), pivaldehyde **4** (15 g, 174 mmol), *p*-toluenesulfonic acid (0.25 g), and concentrated H_2SO_4 (two drops) in pentane (70 mL) was refluxed with azeotropic removal of the water formed. After the mixture was cooled, a white solid precipitated. The precipitate was dissolved in a large amount of ether (600 mL) and washed with water (2 × 100 mL). The organic layers were dried ($MgSO_4$) and evaporated. The crude material (cis:trans = 7.5:1) was recrystallized from hot ether/pentane, yielding **5** as colorless needles (13.59 g, 61.8 mmol, 71%, ds >99%): mp 142 °C (lit.[29] mp 140 °C); $[\alpha]_D = -88.7°$ ($c = 1.2$; $CHCl_3$) [lit.[29] $[\alpha]_D = +88.7°$ ($c = 1.2$; $CHCl_3$, (2*S*,5*S*)-isomer)]; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.51−7.39 (m, 5 H), 5.34 (d, 1 H, $J = 1.3$), 5.25 (d, 1 H, $J = 1.3$), 1.10 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 172.0 (s), 133.51 (s), 129.17 (d), 128.72 (d), 127.05 (d), 109.32 (d), 77.05 (d), 34.46 (s), 23.62 (q).

(2*R*,5*R*)-2-*tert*-Butyl-5-cyclohex-2-enyl-5-phenyl-1,3-dioxolan-4-one (6). A solution of diisopropylamine (4.48 mL, 32 mmol) in THF (200 mL) was cooled to −40 °C, and *n*-BuLi (20 mL, 32 mmol, 1.6 M solution in hexane) was added. The reaction mixture was warmed up to −10 °C during 15 min and then cooled to −80 °C. Subsequently a solution of **5** (7.0 g, 31.8 mmol) in THF (75 mL) was slowly added. The reaction mixture was allowed to warm up to −15 °C over a period of 1 h and then cooled to −80 °C again. Then 3-bromocyclohexene (3.7 mL, 31,9 mmol) was slowly added. The reaction mixture was allowed to warm up slowly to −20 °C during 3 h and subsequently poured into a half-saturated aqueous solution of ammonium chloride (100 mL) and extracted with ether (2 × 100 mL). The organic layers were dried ($MgSO_4$) and evaporated. The crude product (cis:trans = 12:1) was crystallized from hexane at −18 °C, yielding **6** (7.35 g, 24.50 mmol, 77%, de > 99%): mp 109.7−111.3 °C; $[\alpha]_D = -12.9°$ ($c = 1$, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.72−7.31 (m, 5 H), 5.94−5.37 (m, 2 H), 5.45 and 5.40 (2s, two diastereoisomers from additional stereogenic center, 1 H), 2.90−2.83 (m, 1 H), 1.99−1.41 (m, 6 H), 0.96 and 0.93 (2s, two diastereoisomers, 9 H); ^{13}C NMR ($CDCl_3$) δ 173.0 (s), 137.6 (2s), 132.30 (d), 130.96 (d), 128.71 (d), 127.93 (2xd), 125.57 (2xd), 110.53 (2xd), 84.25 (2s), 46.02 (2d), 35.06 (2s), 24.90 (t), 24.47 (t), 23.54 (q), 22.81 (t), 21.64 (t), 21.11 (t). (Most of the peaks are doubled due to the presence of an additional stereogenic center.)

(2*R*,5*R*)-2-*tert*-Butyl-5-cyclohexyl-5-phenyl-1,3-dioxolan-4-one (7). To a solution of **6** (15.8 g, 52.7 mmol) in THF (175 mL) was added Pd/C (10%, 10 mol %). The suspension was hydrogenated in a Parr apparatus at 5.0 bar of H_2 pressure until no hydrogen was adsorbed. After filtration, to remove the catalyst, the solvent was evaporated to afford **7** as a white crystalline compound (15.6 g, 51.7 mmol, 98%). Although the compound is pure enough for the next step, it can be recrystallized from hexane at −18 °C: mp 118.5−121 °C; $[\alpha]_D = -19.0°$ ($c = 1$, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.68−7.29 (m, 5 H), 5.41 (s, 1 H), 1.97−1.07 (m, 11 H), 0.94 (s, 9 H); ^{13}C NMR ($CDCl_3$) 173.90 (s), 137.82 (s), 127.83 (d), 127.51 (d), 125.35 (d), 110.63 (d), 85.14 (s), 48.40 (d), 35.54 (s), 28.02 (t), 26.09 (t), 25.93 (t), 23.52 (q); HRMS calcd 302.188, found 302.188. Anal. Calcd for $C_{19}H_{26}O_3$: C, 75.46; H, 8.67 Found: C, 75.57; H, 8.62.

2,2-Dimethyl-5-phenyl-1,3-dioxolan-4-one (14). A mixture of racemic mandelic acid **3a** (15.2 g, 0.10 mol), 2,2-dimethoxypropane (12.5 g, 0.12 mol), and benzene (100 mL) was refluxed for 2 h with azeotropic removal of methanol. After the mixture was concentrated at reduced pressure, **14** was obtained as a white solid (19.02 g, 99.1 mmol, 99%). The product was pure enough to be used in the next step: mp 40−42.5 °C (lit.[31] mp 42.5−43.5 °C); ^{1}H NMR ($CDCl_3$) δ 7.51−7.35 (m, 5 H), 5.41 (s, 1 H), 1.74 (s, 3 H), 1.68 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 171.48 (s), 134.48 (s), 128.94 (d), 128.71 (d), 126.43 (d), 110.94 (s), 75.90 (d), 27.20 (q), 26.13 (q).

2,2-Dimethyl-5-cyclohex-2-enyl-5-phenyl-1,3-dioxolan-4-one (15). The same synthetic procedure was used as described for **6** except that compound **14** was used instead of **5**. The crude product was obtained as an oil (1.35 g, 4.98 mmol, 99%) and was used in the next step without further purification: ^{1}H NMR ($CDCl_3$) δ 7.65−7.22 (m, 5 H), 5.92−5.23 (m, 2 H), 2.78 (m, 1 H), 2.20−1.20 (m, 6 H), 1.65 (2s, 3 H), 1.39 (2s, 3 H); ^{13}C NMR ($CDCl_3$) δ 171.00 (s), 138.36 (s), 130.90 (d), 128.17 (d), 128.10 (d), 125.60 (d), 125.35 (d), 110.26 (s), 85.30 (s). 45.44 (d), 27.94 (q), 27.23 (q), 24.90 (t), 24.46 (t), 21.45 (t). (Most of the peaks were doubled due to the presence of an additional stereogenic center.)

2,2-Dimethyl-5-cyclohexyl-5-phenyl-1,3-dioxolan-4-one (16). The same synthetic procedure was used as described for **7** except that compound **15** was used instead of **6**. Bulb to bulb distillation (0.02 mmHg, 125 °C) yielded the product as a clear viscous oil (7.86 g, 28.77 mmol, 77.5%): ^{1}H NMR ($CDCl_3$) δ 7.63−7.27 (m, 5 H), 1.89−0.89 (m, 11 H), 1.69 (s, 3 H), 1.41 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 173.20 (s), 138.84 (s), 128.02 (d), 127.68 (d), 125.45 (d), 110.08 (s), 86.13 (s), 47.29 (d), 28.02 (t), 27.95 (q), 27.29 (q), 26.27 (t), 26.01 (t); HRMS calcd 274.157, found 274.157. Anal. Calcd for $C_{17}H_{22}O_3$: C, 74.41; H, 8.09. Found: C, 74.30; H: 8.09.

(*R*)-2-Cyclohexyl-2-hydroxy-2-phenylacetic Acid (8). To a solution of **7** (8.0 g, 26.49 mmol) in a mixture of methanol (100 mL) and ether (100 mL) was added a solution of KOH (8.18 g, 145.8 mmol) in water (25 mL). The solution was stirred overnight, and the solvents were removed under vacuum. The residue was dissolved in water (20 mL) and acidified with HCl (6 M). The precipitate was taken up in ether (100 mL) and separated. The water layer was extracted with ether (2 × 30 mL). After the solvent was dried ($MgSO_4$) and evaporated, **8** was obtained as a white crystalline compound (5.28 g, 22.52 mmol, 85%): mp 139.5−141.0 °C; $[\alpha]_D = -26.5°$ ($c = 1$, EtOH) [lit.[33] mp 139.5−140.5 °C; $[\alpha]_D = -26.3°$ ($c = 1$, EtOH)]. The enantiomeric excess (>99%) was determined by means of a chiral β-cyclodextrin column (cyclobond I, 250 × 4.6, 5 μm, Astec) with the eluant $K_2HPO_4 \cdot 3H_2O$ (0.1 M, pH = 4); CH_3CN = 40:60, flow rate 1 mL/min; UV detection 205 nm, injected was 20 μL from a solution of 1 mg of **8** in 2 mL of eluant. The product was pure enough to be used in the next step: ^{1}H NMR ($CDCl_3$) δ 7.73−7.26 (m, 5H), 2.33−2.22 (m, 1 H), 2.02−1.00 (m, 10 H); ^{13}C NMR ($CDCl_3$) δ 180.68 (s), 139.86 (s), 128.18 (d), 127.70 (d), 125.95 (d), 80.99 (s), 45.67 (d), 27.33 (t), 26.23 (t), 26.09 (t), 25.39 (t).

(*R*)-Methyl 2-Cyclohexyl-2-hydroxy-2-phenylethanoate (9). To a solution of **8** (335 mg, 1.43 mmol) in ether (25 mL) was added diazomethane (3.3 mmol). The diazomethane was generated by adding a solution of NaOH (10%) to a yellow solution of diazald (1.0 g) in ethanol (30 mL). The NaOH solution was added until colorless. The generated diazomethane was led into the solution with acid by means of a stream of N_2. After the diazald solution became colorless, the reaction mixture was stirred for half an hour and then the ether was evaporated in vacuum. Bulb to bulb distillation (110 °C, 0.01 mmHg) gave the product **9** (350 mg, 1.41 mmol, 98%) as a clear oil which became solid on standing: mp 53.2−55.2 °C; $[\alpha]_D = -30°$ ($c = 2$, $CHCl_3$) [lit.[23] mp 53.5 °C; $[\alpha]_D = -30°$ ($c = 2$, $CHCl_3$)]; ^{1}H NMR ($CDCl_3$) δ 7.68−7.23 (m, 5 H), 3.79 (br, 3 H), 3.69 (s, 1 H), 2.24 (m, 1 H), 1.84−1.02 (m, 10 H); ^{13}C NMR ($CDCl_3$) δ 176.09 (s), 140.71 (s), 128.02 (d), 127.35 (d), 125.96 (d), 81.02 (s), 53.24 (q), 45.71 (d), 27.36 (t), 26.31 (t), 26.15 (t), 25.43 (t); HRMS calcd 248.141, found 248.141. Anal. Calcd for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12 Found: C, 72.17; H, 8.02.

(*R*)-*N*-Methylpiperidin-4-yl 2-Cyclohexyl-2-hydroxyphenylacetate (12b). *N*-Methyl-4-hydroxypiperidine **10** (165 mg, 1.43 mmol) was dissolved in heptane (20 mL) and refluxed for 30 min using a Dean−Stark reflux apparatus to remove any traces of moisture. After the mixture was cooled, NaH (10 mg, 60% dispersion in mineral oil) was added. After the mixture was stirred at room temperature for 45 min, compound **9** (350 mg, 1.43 mmol) was added. The reaction mixture was refluxed for 24 h, and subsequently the heptane was drained and discarded to a small volume (±10 mL). After the mixture was cooled in ice, ether (20 mL) was added. The organic layer was repeatedly washed with water until the washings were approximately pH 7. After the solvents were dried (Na_2SO_4) and evaporated, the crude product was obtained as an oil. Column chromatography (silica gel, $CHCl_3$:MeOH:NH_4OH (20%) = 90:9:1) afforded **12b** (187 mg, 0.56 mmol, 40%) as a clear oil: ^{1}H NMR ($CDCl_3$) δ 7.67−7.62 (m, 2H), 7.36−7.20 (m, 3H), 4.80 (m, 1H), 3.80 (br, 1H), 2.26 (s, 3H), 2.65−1.0 (m, 19H); ^{13}C NMR ($CDCl_3$) δ 175.01 (s), 140.87 (s), 127.92 (d), 127.26 (d), 126.03 (d), 80.70 (s), 71.66 (d), 52.30 (br, t), 46.18 (d), 45.69 (q), 30.56 (t), 30.21 (t), 27.27 (t), 26.33 (t), 26.16 (t), 25.48 (t).

(*R*)-*N*-Methylpiperidin-4-yl 2-Cyclohexyl-2-hydroxyphenylacetate Methiodide (1b). To a solution of **12b** (48 mg, 0.14 mmol) in ether (2 mL) was added MeI (0.2 mL, 3.17 mmol), and the mixture was stirred overnight. After evaporation of the excess methyl iodide and solvent, the product **1b** was crystallized from 2-butanone (2 mL) until one single peak was observed on HPLC (CN radialpak 10 μm, mobile phase: H_2O with 1% Et_3N (acidified to pH = 4 with HOAc):CH_3CN = 72:928, v/v) running at 4 mL/min. White crystals (50 mg, 0.11 mmol, 75%) were obtained: mp 169−172 °C (lit. racemate: mp 163−165 °C[39] and 165−170 °C[40]); ^{1}H NMR ($CDCl_3$) δ 7.63−7.24 (m, 5 H), 5.1 (m, 1 H), 3.80 (br, 1 H), 3.43 (s, 3 H), 3.51 (s, 3 H), 3.09−2.95 (dt, 1 H), 2.50−1.0 (m, 19 H); ^{13}C NMR ($CDCl_3$) δ 174.21 (s), 140.41 (s), 128.19 (d), 127.65 (d), 125.94 (d), 81.00 (s), 65.52 (d), 58.73 (t), 58.54 (t), 54.51 (q), 49.84 (q), 44.80 (d), 27.31 (t), 26.20 (t), 25.98 (t), 25.37 (t), 24.96 (t), 24.39 (t); HRMS calcd for $C_{21}H_{32}NO_3I$ 473.143, the parent peak was not observed. Immediate fragmentation of methyl iodide occurs. This results in the masses 141.96 and 331.11. MS (rel int) 331 (20), 189 (99), 142 (100), 127 (27), 98 (86), 96 (38), 55 (38), 28 (75).

(*R*,*R*)-Quinuclidin-3-yl 2-Cyclohexyl-2-hydroxyphenylacetate (13). The same synthetic procedure was used as described for **12b** except that (*R*)-quinuclidinol **11** was used instead of *N*-methyl-4-piperidinol **10**. Column chromatography (silica gel, $CHCl_3$:MeOH:NH_4OH (20%) = 90:9:1). The product was obtained as a clear, viscous oil: yield 18%; ^{1}H NMR ($CDCl_3$) δ 7.68−7.63 (m, 2 H), 7.40−7.26 (m, 3 H), 4.87 (m, 1 H), 3.6 (br, 1 H), 3.2 (m, 1 H), 2.90−1.10 (m, 21 H); ^{13}C NMR ($CDCl_3$) δ 175.5 (s), 140.68 (s), 128.02 (d), 127.38 (d), 125.97 (d), 80.80 (s), 73.48 (d), 54.89 (t), 47.25 (t), 46.28 (t), 45.51 (d), 27.39 (t), 26.33 (t), 26.15 (t), 25.52 (t), 25.30 (d), 24.37 (t), 19.67 (t).

(*R*,*R*)-Quinuclidin-3-yl 2-Cyclohexyl-2-hydroxyphenylacetate Methiodide (2). The same synthetic procedure was used as described for **1b** except that **13** was used instead of **12b**. The product **2** was crystallized from acetone until one peak was observed on HPLC (conditions as described for **1b**): yield 85%; mp 243.4−244.1 °C; ^{1}H NMR ($CDCl_3$) δ 7.62−7.59 (m, 2 H), 7.40−7.22 (m, 3 H), 5.16 (m, 1 H), 4.35 (m, 1 H), 3.95 (s, 1 H), 3.8 (m, 2 H), 3.45 (m, 2 H), 3.25 (s, 3 H), 1.10 (m, 17H); ^{13}C NMR ($CDCl_3$) δ 174.00 (s), 140.42 (s), 128.43 (d),

127.76 (d), 125.73 (d), 81.59 (s), 68.47 (d), 62.13 (t), 57.11 (t), 56.02 (t), 51.88 (q), 44.96 (t), 27.76 (t), 26.19 (t), 25.54 (t), 23.40 (d), 21.04 (t), 18.06 (t); HRMS calculated for $C_{22}H_{32}O_3IN$ 485.142, the parent peak was not observed. Immediate fragmentation of methyl iodide occurs. This results in the fragments 141.95 and 343.64. MS (rel intens) 343 (1), 189 (26), 142 (80), 126 (24), 105 (76), 77 (22), 28 (100); HRMS (CI, NH_3) m/e 486 (M + 1).

Radiosynthesis of [^{11}C]-Labeled Muscarinic Antagonists 1a, 1b, and 2. [^{11}C]CH_3I was prepared from [^{11}C]CO_2, which was generated by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction with 17 MeV protons. The [^{11}C]CO_2 was trapped in the reaction vessel which was previously filled with a solution of $LiAlH_4$ in THF (0.3 mL, a saturated solution of $LiAlH_4$ in THF (0.15 mL) was diluted with THF to a final volume of 1 mL) using an Anatech robotic system. After evaporation of the solvent at 130 °C, HI (57%, 0.8 mL) was added with the robot and the [^{11}C]CH_3I was formed. In this way 30 GBq [^{11}C]-labeled methyl iodide with a specific activity of 150 TBq/mmol was obtained 11 min EOB. The radiochemical yield was 70−90% (corrected for decay). The [^{11}C]CH_3I was trapped in a solution of the desmethyl precursor (1.4 mg) in CH_3CN (0.4 mL) in a stervial which was cooled to 0 °C with ice. After the [^{11}C]CH_3I was trapped, the stervial was capped and placed into an oil bath at 110 °C for 12 min. Then the stervial was cooled in an ice bath for 1 min and the solvents evaporated by rotary evaporation. Subsequently the radioligand was taken up in HPLC eluant (1 mL) and injected onto an HPLC column (CN-10 μm radialpak, mobile phase: H_2O with 1% Et_3N (acidified to pH = 4 with HOAc):CH_3CN = 72:928, v/v), running at 4 mL/min. The radiolabeled product was completely separated from the precursor: retention time precursor, 5 min; retention time product, 9 min. The radiochemical yield was 40−60% (corrected for decay), and the specific activity was 11.1−44.4 TBq/mmol, 40 min EOB. After evaporation of the solvent, the radioligand was dissolved in saline (0.9% NaCl) and injected in the tail vein of male Wistar rats.

Dynamic PET Studies in Male Wistar Rats. Animal handling was in accordance with the Law on Animal Experiments of The Netherlands. PET studies were carried out with a Siemens ECAT 951/31 positron camera. Data acquisition was performed using a dynamic protocol and in stationary mode. In this mode the in-plane spatial resolution amounts to 6 mm fwhm. During the reconstruction a zoom factor of 1.5 was applied and the matrix size was 128 × 128. The rats were anaesthetized with pentobarbital. The rats were positioned in the PET camera parallel to the transaxial plane of the tomograph, so sagittal sections were obtained. In the control rats, a saline solution of ligand **1b** (2.78 MBq, specific activity 25.9 TBq/mmol) was administered by injection in the tail vein. In the blocked group the rats were first pretreated with atropine followed by injection of a saline solution of the radioligand (2.78 MBq, specific activity 25.9 TBq/mmol). The following frames were defined: 10 × 30, 3 × 300, and 3 × 600 s. Data analysis was performed using the Siemens ECAT software (V6.5) on a Sun workstation.

Tissue Distribution Studies in Male Wistar Rats. The radioligands were intravenously injected in the tail of pentobarbital-anaesthetized (60 mg/kg body weight) male Wistar rats (240 g) as described previously by Van Waarde et al.[41] One minute before injection of the radioligand (1.85−2.78 MBq) which was dissolved in saline (0.4 mL), the rats were pretreated with saline (0,5 mL, controls) or saline containing atropine (0.5 mg/0.5 mL, blocking experiments). After 15 min the animals were killed by extirpation of the heart, and the following tissues were removed: bladder, cerebellum, cortex, fat, heart, intestine, kidney, liver, lung, muscle, pancreas, spleen, stomach, submandibular gland, testes, and trachea. Plasma was obtained from the blood by centrifugation (5 min, 2000g). Next the tissue samples were weighed, and the radioactivity was determined with a calibrated γ-counter (LKB-Wallac Compugamma 1282 CS, Turku, Finland). Differences between the various treatments were analyzed using Student's t-test or one-way analysis of variance (ANOVA); in both cases a probability of $p < 0.05$ was considered statistically significant.

Clearance from Plasma and Metabolism. The carotid artery of anaesthetized rats was cannulated, and arterial blood samples (0.2−0.3 mL) were drawn at 10, 30, 42, 55, 65, 80, 93, 108, 123, 300, 600, 1200, and 2400 s after injection of 5.55 MBq of labeled **1b**, with a specific activity of 20.35 TBq/mmol. Plasma and red blood cells were separated by centrifugation (2 min, 3000g). Plasma (100 μL) was then counted in a calibrated γ-counter. The clearance curve was analyzed by means of a commercial nonlinear regression program EnzFitter (Elsevier Biosoft, Cambridge, U.K.). For analysis of metabolites, pentobarbital-anaesthetized rats (280 g) were injected with radioligand **1b** (5.55 MBq in 0.4 mL of saline, specific activity 3.7 TBq/mmol) via a tail vein. After 15 min the rats were killed by extirpation of the heart. Lung, heart, and liver were quickly removed and homogenized in a mixture of saline (1 volume) and acetonitrile (2 volumes), using a Heidolph diax 600 apparatus (Salm & Kipp b.v., Breukelen, The Netherlands). Subsequently the extracts were centrifuged (5 min, 3000g), and the supernatant was injected onto an HPLC column (C-18 radialpak 5 μm, mobile phase NaH_2PO_4 (50 mM):CH_3CN:Et_3N = 50:50:2 (v/v); pH = 6.0; flow rate 1.5 mL/min). Fractions (0.75 mL) of the eluate were collected during 15 min and counted in a calibrated γ-counter. The recovery from the column exceeded 99%.

Radioligand Binding Assays. The binding of the radioligands **1b** and **2** to muscarinic M_1, M_2, and M_3 receptor subtypes was determined as follows: A male Wistar rat was anaesthetized with pentobarbital and sacrificed by extirpation of the heart. The heart and cortex were removed and frozen in liquid nitrogen and stored in the refrigerator at −20 °C. The following buffers were used: PB7, 50 mM phosphate buffer, pH 7.4, was prepared by dissolving 7.12 g of $Na_2HPO_4 \cdot 2H_2O$ and 1.3 g of $NaH_2PO_4 \cdot 2H_2O$ in 1 L of distilled water; PBS, the PB7 buffer was supplemented with sucrose to give a final concentration of the latter of 0.32 M. Subsequently the rat cortex was homogenized in 10 volumes (w/v) of ice-cold PBS using a glass−Teflon Potter-Elvejehem homogenizer (R. W. 18, Janke & Kunkel, Staufen i. Breisgau, FRG) at 1200 rpm, 10 up and down strokes. The homogenate was centrifuged for 10 min at 1000g, and the resulting supernatant was centrifuged for 60 min at 200000g. The pellet was washed with PB7 and centrifuged for 30 min at 100000g twice, and the final pellet was stored at −20 °C. For the determination of the affinity of the muscarinic antagonists **1b** and **2** to rat cortex, 2.72 mg of receptor material in 400 μL of buffer PB7 was used per sample. The preparation of the muscarinic receptor from calf brain was similar, though this preparation was lyophilized after reconstitution of the pellet in 5 volumes of PB7. In the receptor assay 0.5 mg of this preparation in 400 μL of PB7 was used per sample. For rat heart the procedure was as follows: the tissue was suspended in 25 mL of PB7 buffer and minced with an Ultraturrax apparatus. Subsequently the material was homogenized using a Potter-Elvejehem homogenizer at 1200 rpm, 10 up and down strokes. For the assay, 400 μL of receptor material was used per sample. The receptor suspensions were incubated with 50 μL (950 Bq) of labeled [^{3}H]NMS with a specific activity of 2960 Bq/pmol and 50 μL of the enantiomers **1b**, **2**, or scopolamine in concentrations ranging from 1×10^{-12} to 1×10^{-7} M. The mixtures were incubated at 37 °C for 45 min. All the incubations were terminated by the addition of 4 mL of ice-cold PB7 buffer and filtered through Whatman GF/B filters at a pressure of 500 mbar. The tubes were rinsed twice with 4 mL of ice-cold buffer, which was also filtered, and the filters were dispersed in 3.5 mL of Rialuma by shaking for 2 h. The vials were counted for 5 min in a liquid scintillation counter (Packard TriCarb 4000, Downers Grove, IL). The binding parameters were calculated using the RADLIG program (version 4.0), purchased from Elsevier/Biosoft. The dissociation constant of [^{3}H]NMS used was 0.1 nM for calf brain[42] and rat cortex[21,42] and 0.4 nM for rat heart.[21] Receptor concentration, N_1 (aspecific binding of labeled ligand), and dissociation constants were estimated by the computer program.

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References

(1) Goyal, R. Muscarinic receptor subtypes. Physiologic and clinical implications. *N. Engl. J. Med.* **1989**, *321*, 1022−1028.

(2) Buckley, N. J.; Bonner, T. I.; Buckley, C. M.; Brann, M. R. Antagonist binding properties of five cloned muscarinic receptors expressed in CHO-K1 cells. *Mol. Pharmacol.* **1989**, *35*, 469−476.

(3) Caulfield, M. P. Muscarinic receptors, characterization, coupling and function. *Pharmacol. Ther.* **1993**, *58*, 319−380.

(4) Lazareno, S.; Buckley, N. J.; Roberts, F. F. Characterization of muscarinic M4 binding sites in rabbit lung, chicken heart and NG108-15 cells. *Mol. Pharmacol.* **1990**, *38*, 805−815.

(5) Barnes, P. J. Muscarinic receptor subtypes in airways. *Life Sci.* **1993**, *52*, 521−527.

(6) Barnes, P. J.; Dollery, C. T.; MacDermot, J. Increased pulmonary α-adrenergic and reduced β-andrenergic receptors in experimental asthma. *Nature* **1980**, *285*, 569−571.

(7) Raaijmakers, J. A. M.; Terpstra, G. K.; Van Rozen, A. J.; Witter, A.; Kreukniet, J. Muscarinic cholinergic receptors in pheripheral lung tissue of normal subjects and of patients with chronic obstructive lung disease. *Clin. Sci.* **1983**, *66*, 585−590.

(8) Delforge, J.; Le Guludec, D.; Syrota, A.; Bendriem, B.; Crouzel, C.; Slama, M.; Merlet, P. Quantification of myocardial muscarinic receptors with PET in humans. *J. Nucl. Med.* **1993**, *34*, 981−991.

(9) Mulholland, G. K.; Chin, F. I. N.; Hutchins, G. D.; Fain, R.; Zipes, D.; New peripheral muscarinic receptor ligands N-[F-18] fluoroethyl-QNB and -TRB for parasympathetic imaging. *J. Nucl. Med.* **1995**, *36*, 137P−138P (abstract).

(10) Frey, K. A.; Koeppe, R. A.; Mulholland, G. K.; Jewett, D. M.; Hichwa, R. D.; Ehrenkaufer, R. L. E.; Carey, J. E.; Wieland, D. M.; Kuhl, D. E. In vivo muscarinic cholinergic receptor imaging in human brain with [C-11] scopolamine and positron emission tomography. *J. Cereb. Blood Flow Metab.* **1992**, *12*, 147−154.

(11) Frey, K. A.; Koeppe, R. A.; Mulholland, G. K.; Kuhl, D. E. Quantification of regional cerebral muscarinic receptors in brain with the use of C11 tropanyl benzilate and positron emission tomography. *J. Nucl. Med.* **1990**, *31*, 779.

(12) Joad, P. J.; Casale, T. B. [^{3}H]-Quinuclidinyl benzilate binding to the human lung muscarinic receptor. *Biochem. Pharmacol.* **1988**, *37*, 973−976.

(13) Mak, J. W. C.; Barnes, P. J. Muscarinic subtypes in human and guinea pig lung. *Eur. J. Pharmacol.* **1989**, *164*, 223−230.

(14) Casale, T. B.; Ecklund, P. Characterization of muscarinic receptor subtypes on human peripheral lung. *J. Appl. Physiol.* **1988**, *65* (2), 594−600.

(15) Eckelman, W. C.; Reba, R. C.; Gibson, R. E.; Rzeszotarski, W. J.; Vieras, F.; Mazaitis, J. K.; Francis, B. Receptor-binding radiotracers: A class of potential radiopharmaceuticals. *J. Nucl. Med.* **1979**, *20*, 350−357.

(16) Eglen, R. M.; Reddy, H.; Watson, N.; Challis, R. A. J. Muscarinic acetylcholine receptor subtypes in smooth muscle. *Trends Pharmacol. Sci.* **1994**, *15*, 114−119.

(17) Eberlein, W. G.; Engel, W.; Mihm, G.; Rudolf, K.; Wetzel, B.; Entzeroth, M.; Mayer, N.; Doods, H. N. Structure-activity relationships and pharmacological profile of selective tricyclic antimuscarinics. *Trends Pharmacol. Sci.* **1989**, Suppl. 50−54.

(18) Waelbroeck, M.; Camus, J.; Tastenoy, M.; Mutschler, E.; Strohmann, C.; Tacke, R.; Lambrecht, G.; Christophe, J. Binding affinities of hexahydrodifenidol and hexahydrosiladifenidol analogues at four muscarinic receptor subtypes: constitutional and stereochemical aspects. *Eur. J. Pharmacol.* **1991**, *206*, 95−103.

(19) Van Waarde, A.; Visser, G. M.; Visser, T. J.; Brouwer, J.; Paans, M. J.; Vaalburg, W. Rodent biodistribution and metabolism of tritiated 4-DAMP, a M3 subtype-selective cholinoceptor ligand. *Nucl. Med. Biol.* **1994**, *21*, 41−47.

(20) Waelbroeck, M.; Camus, J.; Tastenoy, M.; Mutschler, E.; Christophe, J. Binding properties of nine 4-diphenyl-acetoxy-N-methyl-piperidine (4-DAMP) analogues to M_1, M_2, M_3 and putative M_4 muscarinic receptor subtypes. *Br. J. Pharmacol.* **1992**, *105*, 97−102.

(21) Waelbroeck, M.; Tastenoy, M.; Camus, J.; Christophe, J. Binding kinetics of quinuclidinyl benzilate and methylquinuclidinyl benzilate enantiomers at neuronal (M_1), cardiac (M_2), and pancreatic (M_3) muscarinic receptors. *Mol. Pharmacol.* **1991**, *40*, 413−420.

(22) Delforge, J.; Janier, M.; Syrota, A.; Crouzel, C.; Vallois, J. M.; Cayla, J.; Lancon, J. P.; Mazoyer, B. M. Noninvasive quantification of muscarinic receptors in vivo with positron emission tomography in the dog heart. *Circulation* **1990**, *82*, 1494−1504.

(23) Inch, T.; Ley, R. V.; Rich, P. Asymmetric Synthesis. Part III. Stereospecific Synthesis of (R)-2-Hydroxy-2-phenylpropionic Acid, and (R)- and (S)-2-cyclohexyl-2-hydroxy-2-phenylacetic Acid. Configurational relationship between (R) (−)-2-Hydroxy-2-phenylpropionic Acid and (S) (+)-2-Phenylpropionic Acid. *J. Chem. Soc. C* **1968**, 1693−1699.

(24) Inch, T. D.; Green, D. M.; Thompson, P. B. J. The central and peripheral activities of anti-acetylcholine drugs. Some concepts of practical relevance. *J. Pharm. Pharmacol.* **1973**, *25*, 359−370.

(25) Schjelderup, L.; Harbitz, O.; Groth, P.; Aasen, A. J. The absolute configuration of oxyphencyclimine, a parasympatholytic drug. Synthesis of both enantiomers. *Acta Chem. Scand.* **1986**, *B40*, 601−603.

(26) Barlow, R. B.; Franks, F. M.; Pearson, J. D. M. Studies on the stereospecificity of closely related compounds which block postganglionic acetylcholine receptors in the guinea pig ileum. *J. Med. Chem.* **1973**, *16*, 439−446.

(27) Kiesewetter, D. O. Asymmetric synthesis of benzylic acid analogues using 8-phenylmenthol as a chiral auxiliary. *Tetrahedron: Asymmetry* **1993**, *4* (10), 2183−2198.

(28) Tambuté, A.; Collet, A. Dédoublement et détermination de la configuration absolute des énantiomères de l'acide (thiényl-3)-2-cyclohexyl-2-hydroxy-2-acétique. Application à la synthèse d'esters anticholinergiques du quinuclidinol-3. (Resolution and determination of the absolute configuration of the enantiomers of 2-(3-thienyl)-2-cyclohexyl-2-hydroxy acetic acid. Application to the synthesis of anticholinergic esters of 3-quinuclidinol.) *Bull. Soc. Chim. Fr.* **1984**, *11*, 77−82.

(29) Seebach, D.; Naef, R.; Calderari, G. α-Alkylation of α-heterosubstituted carboxylic acids without racemization; EPC-syntheses of tertiary alcohols and thiols. *Tetrahedron* **1984**, *40*, 1313−1324.

(30) Frater, G.; Müller, U.; Günther, W. Synthesis of enantiomerically enriched atrolactic acid and other α-hydroxy acids *Tetrahedron Lett.* **1981**, *22*, 4221−4224.

(31) Moorlag, H; Kellogg, R. M.; Kloosterman, M.; Kaptein, B.; Kamphuis, J.; Schoemaker, H. E. Pig liver esterase catalyzed hydrolysis of racemic α-substituted α-hydroxy esters. *J. Org. Chem.* **1990**, *55*, 5878.

(32) Hof, R. P. Enantioselective synthesis and (bio)catalysis. Routes to enantiomerically pure alcohols and thiols. Ph.D. Thesis, University of Groningen, The Netherlands, 1995.

(33) Feitsma, K. Enantiomers of oxyphenonium bromide, analytical and pharmacological aspects. Ph.D. Thesis, University of Groningen, The Netherlands, 1987.

(34) Ringdahl, B.; Resul, B.; Dahlbom, R. Facile preparation of the enantiomers of 3-acetoxyquinuclidine and 3-quinuclidinol. *Acta Pharm. Suec.* **1979**, *16*, 281−283.

(35) Wall, S. J.; Yasuda, R. P.; Li, M.; Wolfe, B. B. Development of an antiserum against m3 muscarinic receptors: distribution of m3 receptors in rat tissues and clonal cell lines. *Mol. Pharmacol.* **1991**, *40*, 783−789.

(36) Poaty, V.; Tavakoli, R.; Lockhart, A.; Frossard, N. Muscarinic receptors after syngeneic unilateral lung transplantation. *Life Sci.* **1993**, *52*, 613−620.

(37) Whitsett, J. A.; Hollinger, B. Muscarinic cholinergic receptors in developing rat lung. *Pediatr. Res.* **1984**, *18*, 1136−1140.

(38) Vogel, A. *Textbook of practical organic chemistry*; 4th ed.; Longman: London, p 401.

(39) Coan, S. B.; Jaffe, B.; Papa, D. Parasympathetic blocking agents. III. Phenylglycolic acid esters of N-alkyl-4-piperidinol. *J. Am. Chem. Soc.* **1956**, *78*, 3701−3703.

(40) Abramson, F. B.; Barlow, R. B.; Franks, F. M.; Pearson, J, D. M. Relationships between chemical structure and affinity for postganglionic acetylcholine receptors of the guinea pig ileum. *Br. J. Pharmacol.* **1974**, *51*, 81−93.

(41) Van Waarde, A.; Meeder, J. G.; Blanksma, P. K.; Bouwer, J.; Visser, G. M.; Elsinga, P. H.; Paans, A. M. J.; Vaalburg, W.; Lie, K. I. Suitability of CGP-12177 and CGP-26505 for quantitative imaging of β-adrenoceptors. *Nucl. Med. Biol.* **1992**, *19*, 711−718.

(42) Smisterova, J. Purification and solubilization of muscarinic receptors for the purpose of quantitative receptor assays of anticholinergics. Ph.D. Thesis, University of Groningen, The Netherlands, 1995.

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