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Discovery of Novel Quaternary Ammonium Derivatives of (3*R*)-Quinuclidinol Esters as Potent and Long-Acting Muscarinic Antagonists with Potential for Minimal Systemic Exposure after Inhaled Administration: Identification of (3*R*)-3-{[Hydroxy(di-2-thienyl)acetyl]oxy}-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane Bromide (Aclidinium Bromide)

María Prat,* Dolors Fernández,[†] M. Antonia Buil, María I. Crespo, Gaspar Casals, Manuel Ferrer, Laia Tort, Jordi Castro, Juan M. Monleón, Amadeu Gavaldà, Montserrat Miralpeix, Israel Ramos, Teresa Doménech, Dolors Vilella, Francisca Antón,[‡] Josep M. Huerta, Sonia Espinosa, Manuel López, Sonia Sentellas, Marisa González, Joan Albertí, Victor Segarra, Alvaro Cárdenas,[§] Jorge Beleta, and Hamish Ryder[∥]

Almirall, R&D Centre, Sant Feliu de Llobregat, Barcelona, Spain. [†]Present address: Thomsom Pharma, Barcelona, Spain. [‡]Present address: Laboratorios Lesvi, Sant Joan Despí, Barcelona, Spain. [§]Present address: UCB Pharma, Brussels, Belgium. [∥]Present address: Cancer Research Technology Ltd, London, U.K.

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The objective of this work was to discover a novel, long-acting muscarinic M_3 antagonist for the inhaled treatment of chronic obstructive pulmonary disease (COPD), with a potentially improved risk−benefit profile compared with current antimuscarinic agents. A series of novel quaternary ammonium derivatives of (3*R*)-quinuclidinol esters were synthesized and evaluated. On the basis of its overall profile, (3*R*)-3-{[hydroxy(di-2-thienyl)acetyl]oxy}-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane bromide (aclidinium bromide) emerged as a candidate for once-daily maintenance treatment of COPD. This compound is a potent muscarinic antagonist, with long duration of action in vivo, and was found to have a rapid hydrolysis in human plasma, minimizing the potential to induce class-related systemic side effects. Aclidinium bromide is currently in phase III development for maintenance treatment of patients with COPD.

Introduction

Chronic obstructive pulmonary disease (COPD[a]) is a major global health concern, which is characterized by airflow obstruction that deteriorates progressively and has a limited reversibility after bronchodilator therapy.[1] Projections from the World Health Organization predict that COPD will become the fourth most common cause of death by 2030 and the third most common cause of chronic disability by 2020.[2,3] Acetylcholine is involved in airway smooth muscle contraction and narrowing, and cholinergic tone appears to be the major reversible component of airway obstruction in COPD.[4−6]

Three subtypes of muscarinic receptor are expressed in the human airway: M_1, M_2, and M_3 receptors.[7] The M_3 receptor is the primary subtype responsible for bronchial and tracheal smooth muscle contraction.[8] M_2 receptors localized in cholinergic nerve endings act as negative feedback of acetylcholine release from the nerve.[9,10] Finally, M_1 receptors are localized to parasympathetic ganglia in the airways, facilitating ganglionic neurotransmission.[11]

Inhaled anticholinergics are an effective class of bronchodilators in the management of symptomatic patients with COPD: ipratropium is a short-acting agent requiring up to four doses per day, whereas tiotropium is a long-acting, once-daily treatment (Figure 1).[11−13] Certain systemic anticholinergic effects are associated with these compounds, including dry mouth (reported in 11.6% of patients treated with tiotropium bromide versus 3.5% of patients treated with placebo), glaucoma, constipation, increased heart rate, and urinary retention.[14,15] Therefore, a compound that has similar or improved bronchodilatory efficacy to these agents but causes fewer unwanted anticholinergic effects would be desirable as a monotherapy or as part of a combination therapy because of its lower potential risk of additive systemic pharmacology and drug−drug interactions.

The objective of our work was to discover a novel and potent inhaled muscarinic M_3 antagonist suitable for once-daily administration with an improved safety profile for the maintenance treatment of COPD.

Chemistry

3-Quinuclidinol esters are known to be potent muscarinic antagonists, and various examples of these compounds have been described in the literature.[16−22] Notably, the (3*R*)-quinuclidinol ester revatropate (Figure 1), an M_3, M_1 selective muscarinic antagonist, was developed as a potential treatment for COPD.[23−25] This compound entered phase II clinical trials, although the development of revatropate has since been discontinued, probably because of its short duration of action.[23,24]

*To whom correspondence should be addressed. Phone: +34 932 913816. Fax: +34 933 128635. E-mail: maria.prat@almirall.com.

[a] Abbreviations: CHO-K1, Chinese hamster ovary K1; COPD, chronic obstructive pulmonary disease; QNB, quinuclidinyl benzylate; MQNB, quinuclidinyl benzylate methiodide; PET, positron emission tomography.

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Quaternization of the tertiary amino function is a common chemical manipulation in this class of compounds, widely used to minimize absorption across membranes, resulting in low oral bioavailability and blood−brain barrier permeability.[26,27] Antimuscarinic activity has been reported for certain quaternary ammonium derivatives of quinuclidinyl benzylate (QNB), with aliphatic chains of different length on the N^+ of the azoniabicyclo ring,[28−30] although the potency and duration of action of these agents in the lung are unknown. Radiolabeled QNB and its methiodide salt MQNB are also used as muscarinic ligands in positron emission tomography (PET) to investigate receptor distribution and expression changes in several pathologies.[31,32]

Here we describe the synthesis of a variety of new (3*R*)-quinuclidinol esters and their corresponding quaternary ammonium derivatives, which were prepared with the objective of studying the potential of this scaffold to deliver the desired activity profile. These compounds were synthesized as outlined in Schemes 1−4.

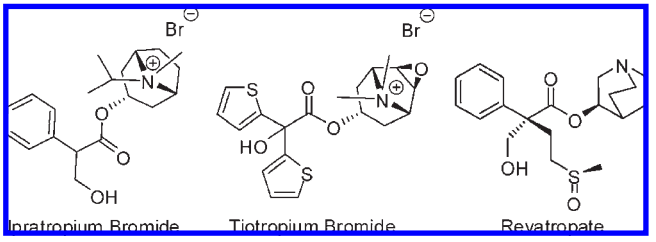


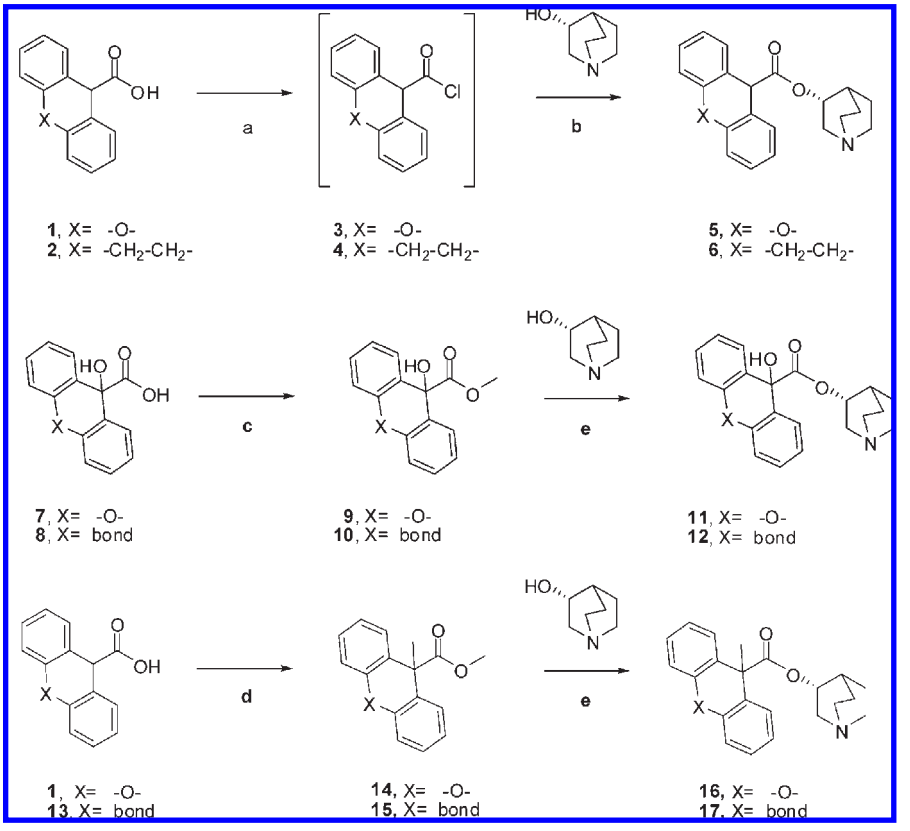
Figure 1

(3*R*)-Quinuclidinol esters **5**,[19,20] **6**, **11**,[33] **12**, **16**, and **17** were prepared from carboxylic acids **1**, **2**,[34] **7**,[35] **8**, and **13** (Scheme 1). Transformation of acids **1** and **2** into their acyl chloride derivatives (compounds **3** and **4**), followed by reaction with commercially available (3*R*)-quinuclidinol, led to the esters **5** and **6**. (3*R*)-Quinuclidinol esters **11** and **12** were obtained from their corresponding methyl ester derivatives, **9** and **10**, which were synthesized from α-hydroxy acids **7** and **8**, respectively. Acids **1** and **13** were methylated in the α position and esterified in a one-pot reaction to give methyl ester derivatives **14** and **15**. Transesterification of compounds **14** and **15** with (3*R*)-quinuclidinol yielded compounds **16** and **17**.

Scheme 2 shows the synthesis of (3*R*)-quinuclidinol esters with a dithienyl moiety (compounds **19**, **21**, **23**, and **26**). The reaction of dimethyl oxalate with 2-thienylmagnesium bromide and 3-thienylmagnesium bromide afforded methyl esters **18** and **22**, respectively.[36] Reduction of compound **18** by reaction with stannous chloride in acidic conditions[37] yielded the methyl ester **20**. The reaction of 2-oxopropanoic acid with thiophene in acidic media gave acid **24**,[38] which was transformed to its methyl ester **25** by standard methods. Reaction of synthesized methyl esters **18**, **20**, **22**, and **25** with (3*R*)-quinuclidinol by means of sodium hydride in refluxing toluene afforded the desired compounds **19**, **21**, **23**, and **26**, respectively.

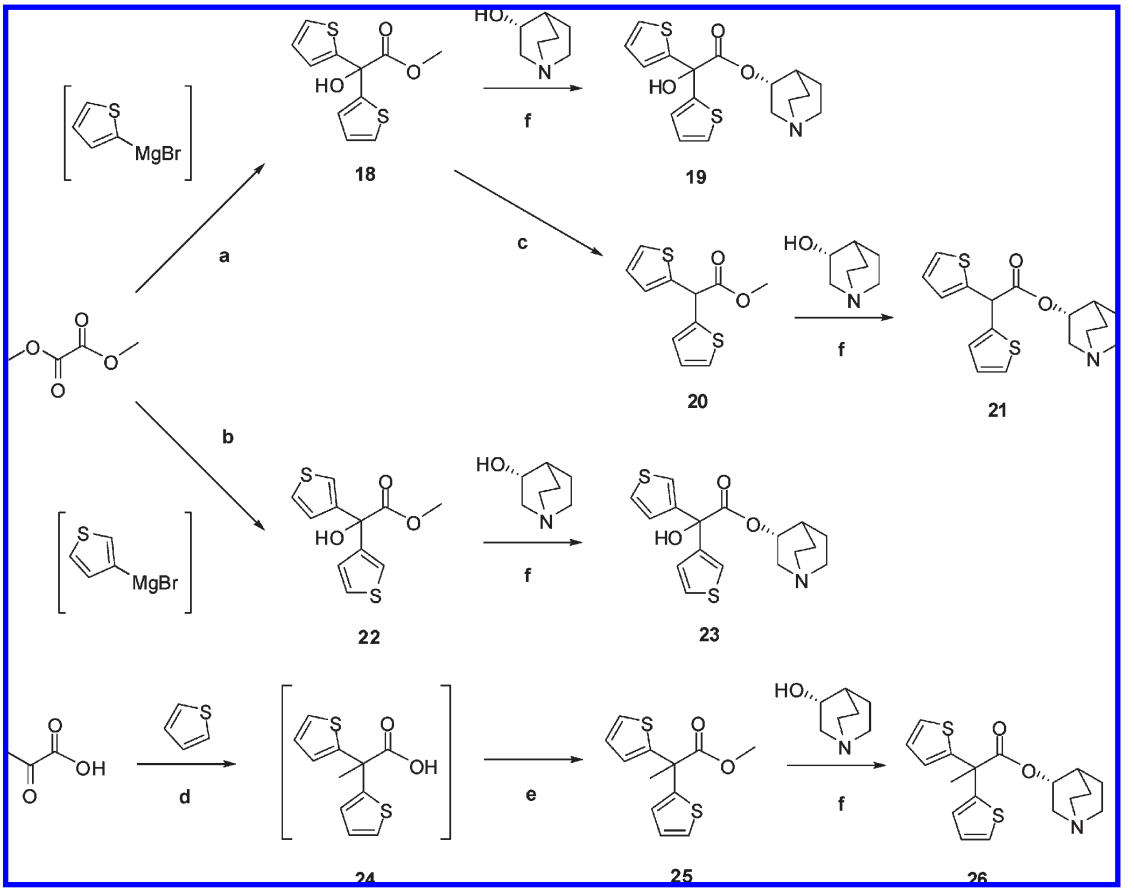
The synthesis of (3*R*)-quinuclidinol esters **33** ((*R*)-QNB[19]), **35**, **36**, **38**, and **39** are described in Scheme 3. Transformation of commercially available acids **27** and **28** to their corresponding acyl chloride derivatives **29** and **30**, followed by reaction with (3*R*)-quinuclidinol, afforded the glyoxalate derivatives

Scheme 1. Synthesis of (3*R*)-Quinuclidinol Esters **5**, **6**, **11**, **12**, **16**, and **17**[a]



[a] Reagents and conditions: (a) $CHCl_3$, oxalyl chloride, DMF (catalytic), room temp; (b) toluene, reflux, or $CHCl_3$, room temp; (c) MeOH, H_2SO_4 (catalytic), reflux, or acetone, K_2CO_3, $(CH_3O)_2SO_2$, reflux; (d) (i) THF, LDA (addition at 0 °C), reflux, (ii) CH_3I, room temp, (iii) MeOH, H_2SO_4, reflux; (e) toluene, HNa, reflux.

Scheme 2. Synthesis of (3*R*)-Quinuclidinol Esters **19**, **21**, **23**, and **26**[a]



[a] Reagents and conditions: (a) diethyl ether, reflux; (b) (i) diethyl ether, −30 °C, (ii) diethyl ether, 0−5 °C; (c) AcOH glacial, HCl gas, $Cl_2Sn \cdot 2H_2O$, 15 °C; (d) H_2O, H_2SO_4, 40−50 °C; (e) acetone, K_2CO_3, $(CH_3O)_2SO_2$, reflux; (f) toluene, HNa, reflux.

31[39] and **32**, respectively. Reaction of compounds **31** and **32** with commercially available solutions of different organometallic reagents yielded esters **33**, **34**, and **37**. Compounds **34** and **37** were both obtained as a mixture of diastereomers that were further separated by crystallization, yielding compounds **35**, **36**, **38**, and **39**, respectively.

The stereochemistry of the ester moiety in compounds **35**, **36**, **38**, and **39** was deduced from the configuration of acids **40**, **41**, and **42**, obtained by hydrolysis (Scheme 3). The configuration of these acids was elucidated by comparison of their optical properties (specific optical rotation [α], circular dichroism (CD) curve [Δε]) with published values of structurally related acids of known configuration.[40−42]

Quaternary ammonium derivatives **43**−**64** were obtained by reaction of selected (3*R*)-quinuclidinol esters with a variety of bromoalkyl or bromoalkylaryl derivatives (Scheme 4).

Results and Discussion

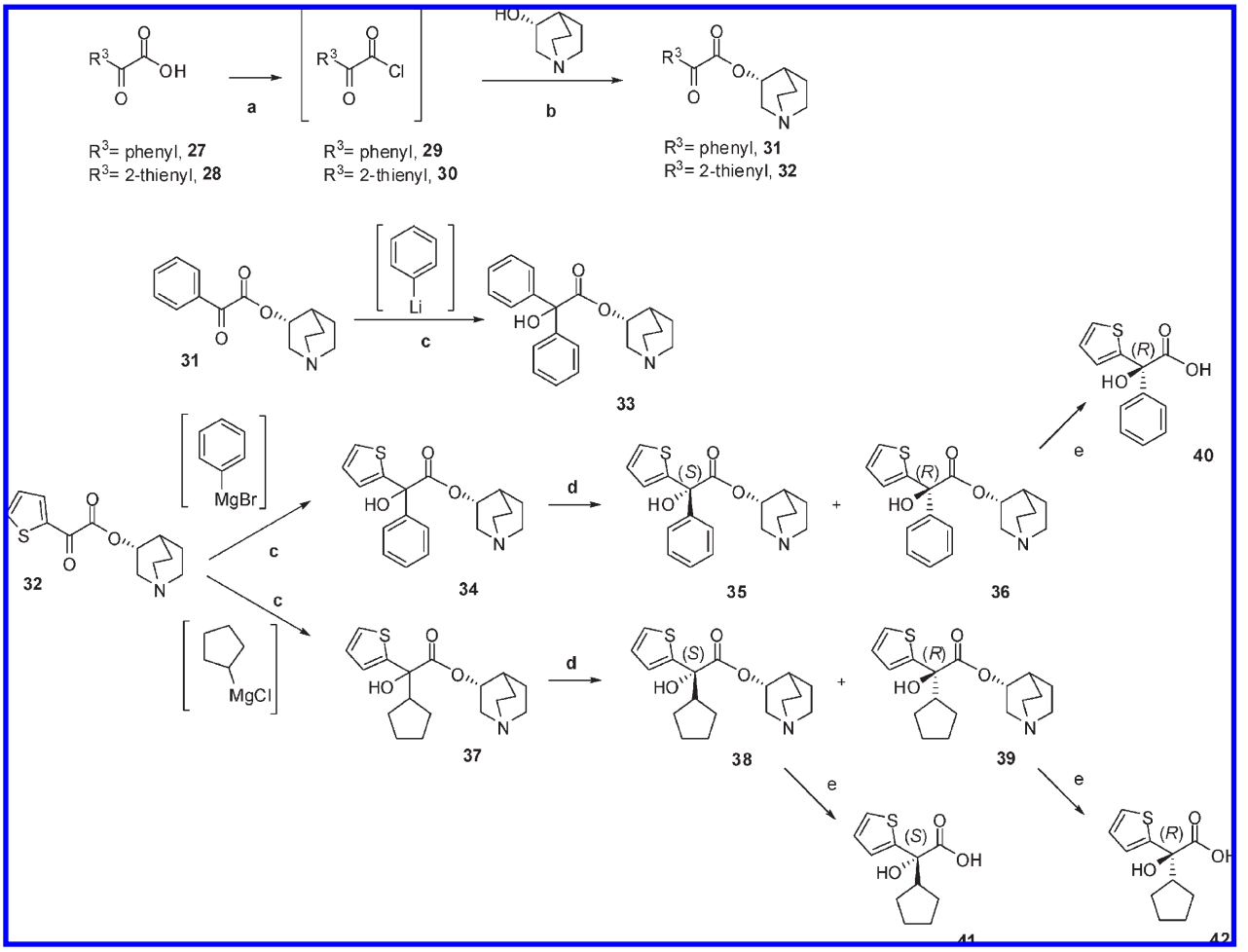
In Vitro Receptor-Binding Studies. We evaluated the muscarinic receptor-binding affinities of the synthesized compounds using membranes expressing the human M_1, M_2, and M_3 receptors transfected into Chinese hamster ovary-K1 (CHO-K1) cells. [^{3}H]-*N*-Methylscopolamine was used as a radioligand in displacement assays. Nonspecific binding was measured in the presence of atropine (1 μM).

The influence of R^1 in the binding affinity (IC_{50}, defined as the concentration required to induce 50% inhibition of muscarinic receptor activity) for some of the nonquaternized compounds previously described is shown in Table 1. The affinities of tiotropium bromide and ipratropium bromide were also determined for reference.[43] When R^1 was a noncyclized moiety, all tested derivatives were potent M_3 receptor ligands, with affinities at the low nanomolar range; however, like the reference compounds, they were not selective for M_3 receptors over M_1 and M_2 receptors. Among them, compound **19**, with two 2-thienyl substituents, had the highest affinity for the M_3 receptor, with an IC_{50} 1.5- and 9-fold lower than tiotropium bromide and ipratropium bromide, respectively. The tested R^1-cyclized subset of compounds maintained the low nanomolar muscarinic activity, with the exception of compound **6**.

To explore the SAR around R^2, we picked up two of the nonquaternized compounds previously described. We chose the most active derivative, compound **19**, and also compound **12** as a representative of the R^1-cyclized subset of compounds. Muscarinic receptor-binding affinities (IC_{50}) for their quaternized derivatives are shown in Table 2. Among the derivatives of compound **12** evaluated (compounds **46**−**48**), the highest binding affinity was obtained for compound **48**, with a phenoxypropyl chain. The binding affinity profile of compound **19** was retained for almost all of the quaternized derivatives tested (compounds **51**−**56**). The highest potencies were obtained for compound **54**, with a phenethyl chain, and compound **56**, with a phenoxypropyl chain also.

On the basis of the in vitro profile, compounds **12** and **19** and their active derivatives were selected for further evaluation.

Scheme 3. Synthesis of (3*R*)-Quinuclidinol Esters **33**, **35**, **36**, **38**, and **39**[a]



[a] Reagents and conditions: (a) $CHCl_3$, oxalyl chloride, DMF (catalytic), room temp; (b) $CHCl_3$, room temp; (c) THF, from $t \leq -40$ °C to room temp; (d) separation of diastereomers by crystallization; (e) MeOH, NaOH, room temp or EtOH, NaOH 2 N, room temp.

In Vivo Duration of Action Studies. The duration of action of selected compounds was evaluated by measuring the inhibition of bronchospasm induced by acetylcholine in anesthetized guinea pigs at different time points.[44] Duration of action results for compounds **12** and **19** and their corresponding quaternized derivatives are shown in Table 2, along with data for reference compounds, tiotropium bromide and ipratropium bromide.

Compound **12** had a short duration of action (3.9 h), comparable to that of ipratropium bromide (3.4 h). However, quaternization with a heptyl group (compound **46**), a phenethyl group (compound **47**), or a phenoxypropyl group (compound **48**) resulted in a longer duration of action (> 6 h), similar to that of tiotropium bromide. Most of the quaternized derivatives of compound **19** that were evaluated (compounds **51**, **52**, **54**, and **56**) retained the long duration of action observed for compound **19** (> 6 h). These results were comparable to those obtained for tiotropium bromide.

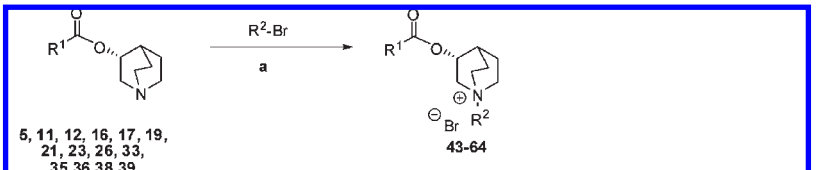
In conclusion, quaternization appeared to retain or improve the duration of action of these compounds relative to the corresponding unquaternized compounds.

On the basis of the results of M_3 receptor-binding affinity and duration of action studies, a series of compounds with phenethyl (compounds **43**, **44**, and **47**) or phenoxypropyl (compounds **45**, **48**, **60**, **63**, and **64**) as R^2 substituents and with diverse R^1 groups on the ester moiety were synthesized and evaluated. M_3 receptor-binding affinities and duration of action data for these compounds are presented in Table 3. All tested compounds showed high M_3 muscarinic affinity, ranging from 0.18 to 1.62 nM, and exhibited long durations of action (> 6 h). Compounds with the highest M_3 receptor-binding affinities (compounds **54**, **56**, and **63**) were selected for further evaluation.

In Vivo Characterization of Selected Compounds. Table 4 describes the duration of action and potency (IC_{50}) for compounds **54**, **56**, and **63**, reverting the bronchospasm induced by acetylcholine in guinea pigs. These compounds showed relevant potency in this in vivo model, comparable to tiotropium bromide and ipratropium bromide.

We further evaluated the preliminary in vivo safety profiles of compounds **54**, **56**, and **63** in mice using an observational test focusing on antimuscarinic side effects. Tiotropium bromide and ipratropium bromide were also evaluated for reference. Each compound was administered intraperitoneally at a dose of 30 mg/kg. Compound **56** did not produce mydriasis, a class-related adverse effect of muscarinic antagonists, and had the most favorable safety profile of the three compounds tested. For all of the other compounds tested in mice, including tiotropium bromide and ipratropium bromide, mydriasis was observed. At this dose, all the compounds tested showed no other adverse effects.

Scheme 4. Preparation of Quaternary Ammonium Derivatives **43** to **64**[a]



starting cmpd	quaternary ammonium derivative					
	R^2					
	CH_3-	$CH_3(CH_2)_6$-	$PhCH_2$-	$Ph(CH_2)_2$	$Ph(CH_2)_4$-	$PhO(CH_2)_3$-
5				**43**		
11				**44**		**45**
12		**46**		**47**		**48**
16						**49**
17						**50**
19	**51**	**52**	**53**	**54**	**55**	**56**
21						**57**
23						**58**
26						**59**
33						**60**
35 (3*R* , 2*S*)						**61**
36 (3*R* , 2*R*)						**62**
38 (3*R* , 2*S*)						**63**
39 (3*R* , 2*R*)						**64**

[a] Reagents and conditions: (a) THF reflux or $CHCl_3$ room temp or $CHCl_3$/acetonitrile room temp.

On the basis of its in vitro binding affinity, in vivo potency, duration of action, and preliminary safety profile, compound **56** was selected for further biological characterization.[45]

Human Plasma Stability Studies. To better understand the reasons for the favorable in vivo safety profile of compound **56** and to evaluate its potential for low systemic class-related side effects in humans, stability studies in human plasma for compounds **54**, **56**, and **63** were performed (Table 4). The plasma stability of tiotropium bromide and ipratropium bromide was also evaluated for reference. Each compound was incubated at 37 °C in human plasma, and the amount of remaining compound after 15 min were monitored. The degradation of this type of compound in human plasma would be expected to be mediated by ester cleavage. Surprisingly, compound **56** was almost fully degraded at the 15 min time point, providing a rationale for a wider safety margin of this compound. In order to elucidate the mechanism responsible of such a quick plasma degradation, we evaluated human plasma and chemical stability half-lives of these compounds at the same experimental conditions (37 °C and pH 7.4). In comparison with reference compounds, compounds **54** and **56** showed human plasma stability half-lives much shorter than the corresponding chemical stability half-lives, suggesting that plasma degradation of compounds **54** and **56** is mainly driven by enzymatic cleavage.

This interesting result led us to investigate the human plasma stability of several (3*R*)-quinuclidinol esters up to 60 min.

Table 5 shows the influence of R^2 substitution on plasma stability for analogues of compound **56**. All the quinuclidinol esters described in Table 5 were less stable in human plasma than tiotropium bromide and ipratropium bromide (scopine and tropine derivatives). Quaternized compounds **51**, **54**, and **56** were less stable than the unquaternized compound **19**. Thus, to achieve rapid hydrolysis in human plasma, quaternization of the (3*R*)-quinuclidinol ester **19** is required.

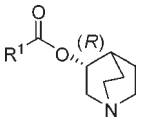
Tables 6 and 7 show the influence of the R^1 group on the rate of degradation in human plasma. The presence of two 2-thienyl substituents on the R^1 group (compound **56**) reduced stability in human plasma compared with the presence of two 3-thienyl substituents (compound **58**) or two phenyl substituents (compound **60**). Replacing one 2-thienyl group with a phenyl group (compounds **61** and **62**) or a cyclopentyl group (compounds **63** and **64**) increased plasma stability compared with compound **56**. For these compounds with a chiral α carbon, the 2*R*-enantiomers (compounds **62** and **64**) were less stable than the corresponding 2*S*-enantiomers (compounds **61** and **63**).

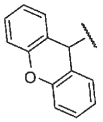
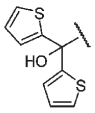
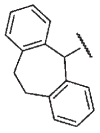
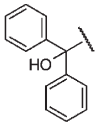
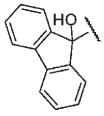
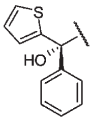
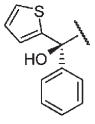
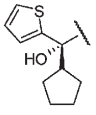
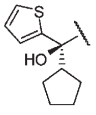
Bridging the two phenyl groups of compound **60** (compound **48**) resulted in lower stability. Substitution of the fluorenyl group (compounds **47** and **48**) by a xanthenyl group (compounds **44** and **45**) resulted in greater stability.

The human plasma stability was greater for compounds with a methyl substituent (compounds **49**, **50**, and **59**) than for compounds with a hydroxyl substituent (compounds **45**, **48**, and **56**), and compounds with a hydrogen substituent were the least stable (compound **43** vs compound **44**; and compound **57** vs compound **56**).

In summary, among the (3*R*)-quinuclidinol esters evaluated for human plasma stability, compounds **51**, **54**, **56**, and **57** were found to degrade most rapidly in human plasma.

Table 1. Effects of R^1 Substitution on M_1, M_2, and M_3 Muscarinic Receptor-Binding Affinities for (3*R*)-Quinuclidinol Ester Derivatives



R^1	cmpd	binding affinity[a] (IC_{50}, nM) M_3	M_2	M_1	R^1	cmpd	binding affinity[a] (IC_{50}, nM) M_3	M_2	M_1
	**5**	0.76 (0.01)	2.83 (0.37)	0.31 (0.04)		**19**	0.23 (0.01)	0.26 (0.001)	0.20 (0.02)
	**6**	94 (1)	79 (1)	15 (1)		**33**	0.90 (0.05)	0.40 (0.06)	0.36 (0.004)
	**12**	0.92 (0.13)	1.14 (0.18)	0.26 (0.02)		**35** (2*S*-isomer)	0.31 (0.03)	0.21 (0.02)	0.20 (0.04)
						**36** (2*R*-isomer)	0.42 (0.001)	0.26 (0.01)	0.23 (0.01)
						**38** (2*S*-isomer)	0.39 (0.05)	0.23 (0.04)	0.19 (0.02)
						**39** (2*R*-isomer)	0.70 (0.14)	0.54 (0.005)	0.24 (0.08)
	tiotropium bromide	0.35[b] (0.01)	0.21[b] (0.04)	0.22[b] (0.03)					
	ipratropium bromide	2.07[b] (0.03)	2.24[b] (0.16)	2.65[b] (0.13)					

[a] Values shown are the mean, $n = 2$ (SD). [b] Values shown are the mean, $n = 3$ (SD).

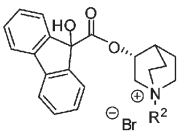
These results help us to understand the more favorable in vivo safety profile of compound **56** compared with the other compounds tested and reinforce the decision to take compound **56** forward as a candidate for further studies.

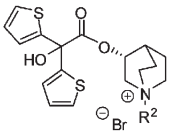
Conclusion

With the objective of identifying a novel, once-daily antimuscarinic agent for the maintenance treatment of COPD by inhalation, we synthesized a series of quaternized (3*R*)-quinuclidinol esters. In vitro and in vivo tests were then conducted to evaluate the potential beneficial characteristics of these compounds in terms of clinical efficacy (muscarinic receptor-binding affinity, duration of action) and low class-related side effects (preliminary in vivo safety, human plasma stability). On the basis of these preclinical results, compound **56** (aclidinium bromide) was selected as a candidate for a complete biological characterization. Aclidinium bromide showed high M_3 receptor-binding affinity and had duration of action longer than that of ipratropium bromide and comparable to that of tiotropium bromide. Aclidinium bromide also demonstrated a favorable in vivo safety profile likely to be due to its rapid human plasma hydrolysis, indicating low potential for systemic class-related side effects. The combination of high potency, long duration of action, low oral absorption, and rapid plasma degradation in the same molecule led us to a novel, effective, and long-acting anticholinergic compound with the potential for a wider safety margin than currently available inhaled anticholinergic therapies. Overall, this profile supports the potential for this compound as a safe and effective monotherapy or as part of a combination therapy for COPD.

Aclidinium bromide is currently in phase III development for maintenance treatment of patients with COPD and in phase II studies in combination with a long-acting β_2-agonist.

Table 2. Effects of R^2 Substitution on the M_1, M_2, and M_3 Muscarinic Receptor-Binding Affinities and in Vivo Duration of Action for Novel Quaternized (3*R*)-Quinuclidinol Ester Derivatives





		binding affinity[a] (IC_{50}, nM)			duration of action		binding affinity[a] (IC_{50}, nM)			duration of action
R^2	cmpd	M_3	M_2	M_1	*T* (h)	cmpd	M_3	M_2	M_1	*T* (h)
–	**12**	0.92 (0.13)	1.14 (0.18)	0.26 (0.02)	3.9[c]	**19**	0.23 (0.01)	0.26 (0.001)	0.20 (0.02)	> 6[c]
CH_3-						**51**	0.60 (0.01)	0.46 (0.003)	0.43 (0.12)	> 6[c]
$CH_3(CH_2)_6$-	**46**	1.68 (0.08)	4.50 (0.03)	3.10 (0.17)	> 6[d]	**52**	0.39 (0.003)	0.36 (0.09)	0.41 (0.04)	> 6[c]
$PhCH_2$-						**53**	200 (1)	12 (1)	100 (52)	NE[f]
$Ph(CH_2)_2$-	**47**	1.04 (0.12)	2.24 (0.28)	1.12 (0.02)	> 6[d]	**54**	0.20[b] (0.003)	0.18[b] (0.06)	0.14[b] (0.04)	> 6[c]
$Ph(CH_2)_4$-						**55**	0.45 (0.07)	0.38 (0.12)	0.29 (0.02)	6[d]
$PhO(CH_2)_3$-	**48**	0.71 (0.07)	1.08 (0.14)	0.63 (0.02)	> 6[c]	**56**	0.17[b] (0.05)	0.17[b] (0.05)	0.14[b] (0.03)	> 6[d]
						tiotropium bromide	0.35[b] (0.01)	0.21[b] (0.04)	0.22[b] (0.03)	> 6[d]
						ipratropium bromide	2.07[b] (0.03)	2.24[b] (0.16)	2.65[b] (0.13)	3.4[e]

[a] Values shown are the mean, $n = 2$ (SD). [b] Values shown are the mean, $n = 3$ (SD). [c] Administered concentration of 3 mg/mL, $n = 4-6$ animals. [d] Administered concentration of 1 mg/mL, $n = 4-6$ animals. [e] Administered concentration of 0.3 mg/mL, $n = 4-6$ animals. [f] NE = not evaluated. The duration of action for compound **53** was not evaluated because its muscarinic receptor binding affinity was too low.

Experimental Section

Biology: General. (3*R*)-Quinuclidinol ester compounds and tiotropium bromide were synthesized by the Department of Medicinal Chemistry (Laboratorios Almirall, Barcelona, Spain). Ipratropium bromide was purchased from Sigma Chemicals (Tres Cantos, Spain). All antagonists were dissolved in dimethyl sulfoxide.

In Vitro Receptor-Binding Studies. All binding assays were performed in 96-well plates (Nunc, Thermo Fischer Scientific, Roskilde, Denmark) with membrane preparations expressing human M_1, M_2, and M_3 receptors (transfected CHO-K1 cells; Membrane Target Systems, Perkin-Elmer Life and Analytical Sciences, Boston, MA). 1-[*N*-Methyl-^{3}H]scopolamine methyl chloride ([^{3}H]NMS specific activity of 82 Ci mmol) from Perkin-Elmer Life and Analytical Sciences (Boston, MA) was used in all binding affinity studies.

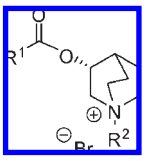
The membrane protein concentrations were 8.1, 5, and 4.9 μg/well for M_1, M_2, and M_3 receptors, respectively. The [^{3}H]NMS concentration was 0.3 nM for M_1 and 1 nM for both M_2 and M_3 assays. A range of antagonist concentrations ($10^{-14}-10^{-5}$ M) was tested to generate competition curves. Nonspecific binding was determined in the presence of 1 μM atropine. Reagents were dissolved in assay binding buffer (phosphate buffered saline with calcium and magnesium) to a total volume of 200 μL. After a 4 h incubation period at room temperature in order to reach equilibrium, 150 μL samples of the reaction mixtures were transferred to GF/C filter plates pretreated for 1 h with wash buffer containing 0.05% polyethylenimine. Bound and free [^{3}H]NMS samples were then separated by rapid vacuum filtration and four washes with ice-cold wash buffer. Samples were dried for 30 min before addition of 30 μL of OptiPhase Supermix (Perkin-Elmer Life and Analytical Sciences, Boston, MA), and radioactivity was quantified using a MicroBeta microplate scintillation counter (Trilux, Perkin-Elmer Boston, MA). The binding affinities (IC_{50} values) for each antagonist were determined for the three muscarinic receptors using GraphPad Prism software (San Diego, CA).

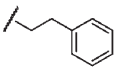
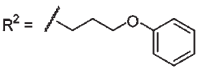
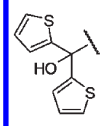
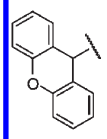
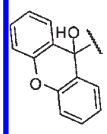
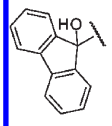
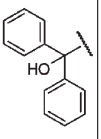
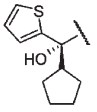
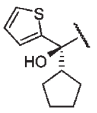
In Vitro Human Plasma Stability Studies. The stability of the test compounds was assessed using pooled human plasma samples from 10 healthy volunteers. Blood samples were collected in lithium heparin tubes, and the plasma was separated by centrifugation, pooled, and stored at −20 °C until use.

Stock solutions of test compounds (2 mM) were prepared by dissolving corresponding amounts of compounds in 0.4 mL of 0.1 N hydrochloric acid before adding acetonitrile to a final volume of 2 mL. Working solutions of test compounds were prepared by diluting from 2 mM stock solutions in water. All working solutions were prepared immediately before use. The final concentration of the test compounds was 1 μM.

Human plasma samples were incubated in triplicate for 5 min at 37 °C (Unitronic 320 OR, JPselecta, Spain) before the addition of the test compound to initiate the reaction (final concentration, 1 μM). At predetermined times during incubation (0, 15, and 60 min), aliquots of the incubation mixtures (100 μL) were transferred to another tube containing 300 μL of cold acetonitrile/1 N HCl (90/10, v/v) to stop the reaction. Samples were then centrifuged at 2800*g* for 10 min at 4 °C. Control incubations in the absence of the test compound were also performed. Additional incubations for the most unstable compounds (**51**, **56**, and **57**) were also performed at predetermined times (0, 5, 10, and 15 min) and assayed as indicated above.

Table 3. Effects of R^1 Substitution on the M_3 Muscarinic Receptor-Binding Affinities and the in Vivo Duration of Action for Novel Quaternized (3*R*)-Quinuclidinol Ester Derivatives (R^2 = Phenethyl; R^2 = Phenoxypropyl)



	R^2 = 					R^2 = 				
		binding affinity[a] (IC$_{50}$, nM)			duration of action		binding affinity[a] (IC$_{50}$, nM)			duration of action
R^1	cmpd	M_3	M_2	M_1	*T* (h)	cmpd	M_3	M_2	M_1	*T* (h)
	**54**	0.20[b] (0.003)	0.18[b] (0.06)	0.14[b] (0.04)	> 6[c]	**56**	0.17[b] (0.05)	0.17[b] (0.05)	0.14[b] (0.03)	> 6[d]
	**43**	0.31 (0.02)	1.82 (0.19)	0.44 (0.03)	> 6[d]					
	**44**	0.28 (0.12)	0.54 (0.15)	0.29 (0.10)	> 6[d]	**45**	0.42 (0.03)	0.36 (0.06)	0.23 (0.01)	> 6[d]
	**47**	1.04 (0.12)	2.24 (0.28)	1.12 (0.02)	> 6[d]	**48**	0.71 (0.07)	1.08 (0.14)	0.63 (0.02)	> 6[c]
						**60**	0.61 (0.03)	0.44 (0.01)	0.31 (0.05)	> 6[c]
						**63** (2*S*-isomer)	0.18[b] (0.02)	0.14[b] (0.04)	0.15[b] (0.01)	> 6[d]
						**64** (2*R*-isomer)	1.62 (0.21)	1.01 (0.29)	0.81 (0.17)	> 6[c]
	tiotropium bromide	0.35[b] (0.01)	0.21[b] (0.04)	0.22[b] (0.03)	> 6[d]					
	ipratropium bromide	2.07[b] (0.03)	2.24[b] (0.16)	2.65[b] (0.13)	3.4[e]					

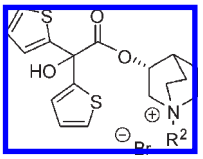
[a] Values shown are the mean, $n = 2$ (SD). [b] Values shown are the mean, $n = 3$ (SD). [c] Administered concentration of 3 mg/mL, $n = 4-6$ animals. [d] Administered concentration of 1 mg/mL, $n = 4-6$ animals. [e] Administered concentration of 0.3 mg/mL, $n = 4-6$ animals.

Table 4. Overall in Vitro and in Vivo Characterization of Selected Compounds

	binding affinity,[a] K_i (nM)			in vivo model		safety observational test	human plasma stability,[a,b]		chemical stability,[b,c]
compd	M_3	M_2	M_1	duration of action, T (h)	IC_{50} (μg/mL)	muscarinic-related side effects (30 mg/kg, ip)[d]	% disappearance (15 min)	$t_{1/2}$ (min)[e]	$t_{1/2}$ (min)[e]
54	0.08 (0.001)	0.08 (0.02)	0.08 (0.02)	>6[f]	54	mydriasis	68 (3)	7.9	56
56	0.07 (0.02)	0.08 (0.02)	0.08 (0.02)	>6[g]	140	none	99 (0)	2.2	60
63	0.07 (0.01)	0.06 (0.01)	0.09 (0.004)	>6[g]	120	mydriasis	<5	>60	>60
tiotropium bromide	0.14 (0.002)	0.10 (0.02)	0.12 (0.02)	>6[g]	45	mydriasis	5 (6)	>60	>60
ipratropium bromide	0.83 (0.01)	1.02 (0.07)	1.51 (0.08)	3.4[h]	68	mydriasis	<5	>60	>60

[a] Values shown are the mean, $n=3$ (SD). [b] Experimental conditions: pH 7.4, $T = 37$ °C. [c] Values shown are the mean, $n=2$ (SD). [d] ip = intraperitoneal. [e] Half-life calculated with at least three experimental time points. [f] Administered concentration of 3 mg/mL, $n = 4-6$ animals. [g] Administered concentration of 1 mg/mL, $n=4-6$ animals. [h] Administered concentration of 0.3 mg/mL, $n=4-6$ animals.

Table 5. Effect of R^2 Substitution on the in Vitro Human Plasma Stability of Compound **19** Derivatives



		binding affinity,[a] IC_{50} (nM)			human plasma stability % disappearance[b]			
R^2	compd	M_3	M_2	M_1	5 min	15 min	60 min	$t_{1/2}$ (min)[c]
	19	0.23 (0.01)	0.26 (0.001)	0.20 (0.02)	NE[d]	14 (5)	53 (6)	54.5
CH_3-	**51**	0.60 (0.01)	0.46 (0.003)	0.43 (0.12)	51 (1)	94 (0)	NE[d]	3.6
$Ph(CH_2)_2-$	**54**	0.20[b] (0.003)	0.18[b] (0.06)	0.14[b] (0.04)	NE[d]	68 (3)	99 (0)	7.9
$PhO(CH_2)_3-$	**56**	0.17[b] (0.05)	0.17[b] (0.05)	0.14[b] (0.03)	78 (2)	99 (0)	NE[d]	2.2
	tiotropium bromide	0.35[b] (0.01)	0.21[b] (0.04)	0.22[b] (0.03)	NE[d]	5 (6)	35 (3)	>60
	ipratropium bromide	2.07[b] (0.03)	2.24[b] (0.16)	2.65[b] (0.13)	NE[d]	<5	<5	>60

[a] Values shown are the mean, $n=2$ (SD). [b] Values shown are the mean, $n=3$ (SD). [c] Half-life calculated with at least three experimental time points. [d] NE = not evaluated.

The analyses of test compounds were performed by ultraperformance liquid chromatography (Acquity Ultra Performance LC, Waters, Milford, MA) with a Quattro Premier mass spectrometry detector operating in positive mode (Micromass Technologies, Waters, Milford, MA).

In all experiments, the stability of test compounds was determined as the percentage of remaining compound at the time point of interest (assuming 100% of the compound is present at time 0).

The test compound elimination half-life ($t_{1/2}$) in human plasma was estimated using the WinNonlin software, version 5.0.1. (Pharsight Corporation). The slope of the linear regression from log [S] versus time plot ($-k$) was determined, and the in vitro plasma half-life was determined as $t_{1/2} = -0.693/k$.

Chemical Stability Studies. The stability of the test compounds was assessed by monitoring for 24 h the 210 nm UV−UPLC chromatograms of 0.05 mg/mL compound solutions at 37 °C and pH 7.4.

An amount of 1 mg/mL test solution of each compound in DMSO (dimethyl sulfoxide) was prepared. At time zero, 50 μL of the test solution was added to 1 mL of pH 7.4 buffer solution (final concentration, 0.05 mg/mL). This solution was placed in the UPLC sample manager thermostabilized at 37 °C, and an amount of 5 μL was injected into the UPLC system (time zero). Final test solutions were prepared in duplicate. At predetermined times (0, 1, 4, 8, 12, 16, 20, and 24 h), an amount of 5 μL of each solution was injected in the UPLC system.

Control solutions for each compound were prepared using acetonitrile instead of pH 7.4 buffer solution. All compounds were stable in acetonitrile solutions.

The analyses of test compounds were performed by ultraperformance liquid chromatography (Acquity Ultra Performance LC, Waters, Milford, MA) with a PDA Acquity detector (Waters, Milford, MA).

In all experiments, the stability of test compounds was determined by integration of 210 nm extracted and blank subtracted chromatogram.

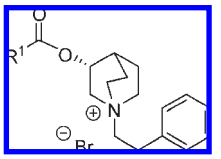
All obtained chromatograms were processed using Empower software (Waters, Milford, MA). The 210 nm chromatograms were extracted from 3D diode array signal, and a blank chromatogram was subtracted. Purity of compounds tested at each time point was determined by area normalization.

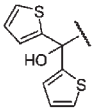
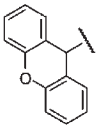
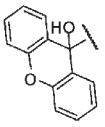
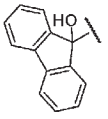
The test compound stability half-life ($t_{1/2}$) at 37 °C and pH 7.4 was estimated using the Kinetica software, version 4.2 (InnaPhase). The slope of the linear regression from log [S] versus time plot ($-k$) was determined, and the chemical stability half-life was determined as $t_{1/2} = -0.693/k$.

In Vivo Studies. Animals. Male Dunkin−Hartley guinea pigs (400−600 g) or male Swiss mice (20−25 g) were obtained from Harlan (Interfauna Ibérica, Spain). Animals were housed in groups of four or five at 20−24 °C under a 12 h light/dark cycle. Food and water were available ad libitum. All experiments were carried out with the approval of the Animal Ethical Committee of Almirall.

In Vivo Duration of Action Studies. Guinea pigs were anesthetized with an intraperitoneal injection of 1 g/kg urethane and 20 mg/kg sodium pentobarbital. Additional anesthetic was administered after 60 min, as required. The trachea was cannulated, and the lungs were artificially ventilated with a small rodent ventilator (Ugo Basile, Biological Research Apparatus, Comerio-Varese, Italy) at a rate of 60 strokes/min and a tidal volume of 10 mL/kg. Animals were maintained at 37 °C with a homeothermic blanket throughout the experiment. Blood pressure was measured from the carotid artery, and acetylcholine was administered via the jugular vein. Intrapulmonary pressure and blood pressure were measured by blood pressure transducers (MLT0699, ADInstruments-Panlab, Barcelona, Spain) connected to a bridge amplifier (PowerLab/8sp, ADInstruments-Panlab, Madrid, Spain).

Table 6. Effect of R^1 Substitution on the in Vitro Human Plasma Stability of Compound **54** Analogues



		binding affinity[a] (IC_{50}, nM)			human plasma stability (% disappearance)[b]			
R^1	cmpd	M_3	M_2	M_1	5 min	15 min	60 min	$t_{1/2}$ (min)[c]
	**54**	0.20[b] (0.003)	0.18[b] (0.06)	0.14[b] (0.04)	NE[d]	68 (3)	99 (0)	7.9
	**43**	0.31 (0.02)	1.82 (0.19)	0.44 (0.03)	NE[d]	22 (6)	53 (4)	56.1
	**44**	0.28 (0.12)	0.54 (0.15)	0.29 (0.10)	NE[d]	7 (11)	15 (3)	> 60
	**47**	1.04 (0.12)	2.24 (0.28)	1.12 (0.02)	NE[d]	35 (4)	90 (1)	17.6
	tiotropium bromide	0.35[b] (0.01)	0.21[b] (0.04)	0.22[b] (0.03)	NE[d]	5 (6)	35 (3)	> 60
	ipratropium bromide	2.07[b] (0.03)	2.24[b] (0.16)	2.65[b] (0.13)	NE[d]	< 5	< 5	> 60

[a] Values shown are the mean, $n = 2$ (SD). [b] Values shown are the mean, $n = 3$ (SD). [c] Half-life calculated with at least three experimental time points. [d] NE = not evaluated.

The data were recorded using Chart 5 software (ADInstruments-Panlab, Barcelona, Spain).

After induction of anesthesia and preparation, animals were allowed to stabilize for 10 min before bronchoconstriction was induced by an intravenous administration of acetylcholine. Acetylcholine (Sigma, Tres Cantos, Spain) was administered at a dose (10−60 μg/kg) that approximately doubled the basal intrapulmonary pressure. Repeated bolus injections of acetylcholine at the selected dose were administered until two reproducible responses were obtained; the mean of the two final responses before the addition of the test compound corresponded to the maximal response to acetylcholine and was used to evaluate the antibronchoconstrictor effect of the test compounds.

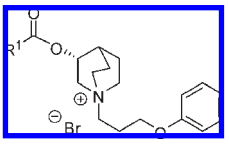
Five minutes after the last administration of acetylcholine that was used to calculate maximal bronchoconstriction, the test compound was administered via a nebulizer (5 s duration; Mumed ultrasonic nebulizer, Mumed Systems Ltd., London, U.K.) to investigate the reversal of the bronchoconstriction. Test compounds were delivered in the concentration range 0.3−3 mg/mL. Acetylcholine doses were then administered 5, 10, 20, 30, 60, 120, 180, 240, 300, and 360 min after the administration of the test compound to evaluate the antibronchoconstrictor effects of each compound. This was expressed as a percentage of the maximal response to acetylcholine. Duration of action (T, h) was defined as the time taken to recover 50% of the maximum inhibitory effect achieved by the test compound and was derived from a time-course bronchoconstriction curve and calculated with a one-phase exponential decay formula using GraphPad Prism software (San Diego, CA).

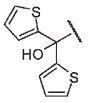
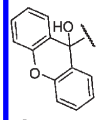
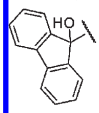
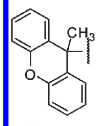
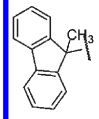
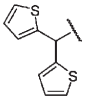
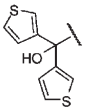
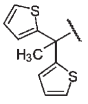
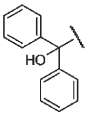
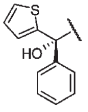
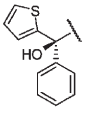
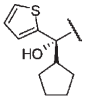
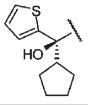
In Vivo Bronchospasm Inhibitory Activity. The in vivo potency (IC_{50}) of selected compounds was determined using the guinea pig model described above, at the t_{max} (data not shown), from a sigmoidal dose−response curve. T_{max} was defined as the time taken from the administration of the test compound to the time at which maximum inhibition of the acetylcholine-induced bronchoconstriction was achieved. The IC_{50} was calculated using GraphPad Prism software (San Diego CA).

In Vivo Safety Observational Test. Assessment of safety was performed for selected compounds administered intraperitoneally at a dose of 30 mg/kg, using an in vivo safety observational test focusing on antimuscarinic side effects (e.g., mydriasis, xerostomia) in Swiss mice. This assay comprises standardized observational tests that assess the neurobiological state of rodents and include measures of autonomic and sensorimotor functions, convulsive behavior, and excitability up to 24 h after drug administration.

Chemistry: General. Reagents, starting materials, and solvents were purchased from commercial suppliers and used as

Table 7. Effect of R^1 Substitution on the in Vitro Human Plasma Stability of Compound **56** Analogues



		binding affinity[a] (IC_{50}, nM)			human plasma stability (% disappearance)[b]			
R^1	cmpd	M_3	M_2	M_1	5 min	15 min	60 min	$t_{1/2}$ (min)[c]
	**56**	0.17[b] (0.05)	0.17[b] (0.05)	0.14[b] (0.03)	78 (2)	99 (0)	NE[d]	2.2
	**45**	0.42 (0.03)	0.36 (0.06)	0.23 (0.01)	NE[d]	6 (1)	30 (4)	> 60
	**48**	0.71 (0.07)	1.08 (0.14)	0.63 (0.02)	NE[d]	68 (5)	98 (0)	10.9
	**49**	0.24 (0.01)	0.27 (0.05)	0.28 (0.01)	NE[d]	< 5	< 5	> 60
	**50**	0.96 (0.11)	2.10 (0.10)	1.26 (0.05)	NE[d]	15 (12)	29 (3)	> 60
	**57**	0.37 (0.01)	0.35 (0.001)	0.34 (0.02)	100 (0)	100 (0)	NE[d]	0.6
	**58**	0.24 (0.12)	0.18 (0.03)	0.23 (0.01)	NE[d]	50 (13)	71 (1)	15.2
	**59**	0.59 (0.05)	0.73 (0.03)	0.60 (0.05)	NE[d]	32 (2)	82 (2)	23.9
	**60**	0.61 (0.03)	0.44 (0.01)	0.31 (0.05)	NE[d]	25 (7)	39 (3)	> 60
	**61** (2*S*-isomer)	0.25 (0.02)	0.17 (0.01)	0.16 (0.02)	NE[d]	26 (3)	79 (0)	26.5
	**62** (2*R*-isomer)	0.48 (0.004)	0.31 (0.01)	0.38 (0.01)	NE[d]	78 (1)	99 (0)	8.9
	**63** (2*S*-isomer)	0.18[b] (0.02)	0.14[b] (0.04)	0.15[b] (0.01)	NE[d]	< 5	< 5	> 60
	**64** (2*R*-isomer)	1.62 (0.21)	1.01 (0.29)	0.81 (0.17)	NE[d]	11 (5)	10 (3)	> 60
	tiotropium bromide	0.35[b] (0.01)	0.21[b] (0.04)	0.22[b] (0.03)	NE[d]	5 (6)	35 (3)	> 60
	ipratropium bromide	2.07[b] (0.03)	2.24[b] (0.16)	2.65[b] (0.13)	NE[d]	< 5	< 5	> 60

[a] Values shown are the mean, $n = 2$ (SD). [b] Values shown are the mean, $n = 3$ (SD). [c] Half-life calculated with at least three experimental time points. [d] NE = not evaluated.

received. Concentration refers to evaporation under vacuum using a Büchi rotatory evaporator. Reaction products were purified, when necessary, by flash chromatography on silica gel (40−63 μm) with the solvent system indicated. Melting points were recorded on a Büchi B-540 instrument. ^{1}H NMR spectra were performed using a Varian Gemini 2000 spectrometer operating at a frequency of 200 or 300 MHz. Samples were dissolved in deuterated chloroform ($CDCl_3$) or deuterated dimethyl sulfoxide (DMSO-d_6). Tetramethylsilane (TMS) was used as reference. The following abbreviations were used to assign spectra: (s) singlet, (d) doublet, (dd) double doublet, (ddd) double double doublet, (t) triplet, (dt) double triplet, (td) triple doublet, (m) multiplet, (br s) broad signal. HPLC−UV−MS chromatograms were acquired in a Waters Alliance 2695 chromatographer equipped with a Waters 2996 diode-array detector and a Waters ZQ mass spectrometer detector. HPLC analysis was conducted according to method A, B, or C, with the retention time (t_R) expressed in min. UV chromatograms were processed at 210 nm with blank subtraction. For HPLC method A, chromatography was performed on a Kromasil C18 column (100 mm × 4.6 mm, 5 μm). The mobile phase, at a flow of 1 mL/min, was a 30 min binary gradient (5−85%) of water (containing 0.01 M phosphoric acid at pH 3.0 with sodium hydroxide, 0.1 N) and acetonitrile. The total run time was 35 min. For HPLC method B, chromatography was performed on a Symmetry C18 column (100 mm × 2.16 mm, 3.5 μm). The mobile phase, at a flow of 0.4 mL/min, was a 20 min binary gradient of water (containing 0.01 M ammonic formiate at pH 3.0) and a mixture of acetonitrile−methanol 50:50 (containing 0.01 M ammonic formiate) (0−95%). The total run time was 26 min. For HPLC method C, chromatography was performed on a Symmetry C18 column (100 mm × 2.16 mm, 3.5 μm). The mobile phase, at a flow of 0.4 mL/min, was a 20 min binary gradient of water (containing 0.01 M ammonic formiate at pH 7.4) and a mixture of acetonitrile/methanol, 50:50 (containing 0.01 M ammonic formiate) (0−95%). The total run time was 26 min. Specific optical rotations were measured in a Perkin-Elmer 241 MC polarimeter at the wavelength of the D-line of sodium (λ = 589.3 nm) at 25 or 22 °C using a layer of 1 dm. Circular dichroism spectra were recorded on a Jasco J-720 spectropolarimeter controlled by J-700 software using the following acquisition conditions: optical path 0.1 cm, temperature 25 °C, purge gas nitrogen at 5 L/min, spectral width 1.0 nm, response time 4 s, wavelength 200−300 nm, scan speed 10 nm/min, number of scans 2. Samples were dissolved in methanol (concentration, 0.43 mM).

Tiotropium bromide was synthesized by the Department of Medicinal Chemistry (Laboratorios Almirall, Barcelona, Spain). Ipratropium bromide was purchased from Sigma Chemicals (Tres Cantos, Spain). The purity of these compounds was determined by HPLC−MS.

The methods used for the preparation of compounds **2**,[34] **7**,[35] **9**, **10**, **14**, and **15** (Scheme 1); **18**,[36] **20**, **22**,[36] and **25** (Scheme 2); and **31**[39] and **32** (Scheme 3) are available as Supporting Information.

Preparation of (3*R*)-Quinuclidinol Esters 5[19,20] **and 6 (Scheme 1). (3*R*)-1-Azabicyclo[2.2.2]oct-3-yl 9*H*-xanthene-9-carboxylate (5)**[19,20]**.** To a solution of the acid **1** (5 g, 22.1 mmol) in 65 mL of $CHCl_3$ (amylene stabilized), cooled to 0 °C, was added DMF (2 drops) followed by oxalyl chloride (3.1 mL, 36.6 mmol) dissolved in 5 mL of $CHCl_3$. After 1.5 h of stirring at room temperature, the reaction mixture was concentrated and the residue was coevaporated with $CHCl_3$ three times to give the acyl chloride **3** (100%). Compound **3**, dissolved in 30 mL of $CHCl_3$, was slowly added to a solution of (3*R*)-quinuclidinol (3.05 g, 23.9 mmol) in 50 mL of $CHCl_3$. The mixture was stirred at room temperature overnight. After this reaction time, solvent was evaporated and the residue was dissolved in toluene and extracted with 2 N HCl. The acid layers were combined and basified with solid K_2CO_3. The basic solution was extracted with $CHCl_3$. The organic layers were combined, washed with water, dried over $MgSO_4$, and concentrated. The obtained residue was purified by formation of the hydrochloride salt. The residue was dissolved in diethyl ether (150 mL), and a solution of EtOH/HCl (g) was slowly added. The solid obtained was filtered and washed with diethyl ether to give 5.6 g (68%) of the hydrochloride salt of compound **5**, mp 227 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.55−1.93 (m, 4 H), 2.06−2.17 (m, 1 H), 2.82−2.99 (m, 2 H), 2.98−3.15 (m, 2 H), 3.15−3.29 (m, 1 H), 3.48−3.63 (dd, J = 12.8, 9.2 Hz 1 H), 4.88−4.99 (m, 1 H), 5.27 (s,1 H), 7.11−7.28 (m, 4 H), 7.38 (dd, J = 8.2, 7.0 Hz, 2 H), 7.49 (dd, J = 7.0, 5.5 Hz, 2 H). HPLC (method B): 96.6%, t_R = 11.0 min. MS (ESI) 336 m/z $(M + H)^+$.

(3*R*)-1-Azabicyclo[2.2.2]oct-3-yl 10,11-dihydro-5*H*-dibenzo-[*a*,*d*][7]annulene-5-carboxylate (6) was prepared as described for compound **5** starting from the acid **2**[34] (2.15 g, 9.0 mmol) and (3*R*)-quinuclidinol (1.26 g, 9.9 mmol). The reaction conditions used in the second step were as follows: toluene, reflux, 2 h. Purification by column chromatography (silica gel, chloroform/methanol/NH_4OH, 95:5:0.5, as eluent) afforded 1.5 g (48%) of compound **6**, mp 112−113 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.07−1.36 (m, 2 H), 1.36−1.53 (m, 1 H), 1.54−1.69 (m, 1 H), 1.84−1.93 (m, 1 H), 2.47−2.58 (m, 2 H), 2.59−2.76 (m, 3 H), 2.80−3.00 (m, 2 H), 3.06−3.18 (ddd, J = 14.7, 7.9, 2.1 Hz, 1 H), 3.25−3.43 (m, 2 H), 4.75−4.83 (m, 1 H), 4.82 (s, 1 H), 7.09−7.33 (m, 8 H). HPLC (method B): 99.8%, t_R = 11.4 min. MS (ESI) 348 m/z $(M + H)^+$.

Preparation of (3*R*)-Quinuclidinol Esters 11,[33] **12, 16, and 17 (Scheme 1) and 19, 21, 23, and 26 (Scheme 2). (3*R*)-1-Azabicyclo[2.2.2]oct-3-yl 9-hydroxy-9*H*-xanthene-9-carboxylate (11)**[33]**.** To a stirred solution of the methyl ester **9** (2 g, 7.8 mmol) and (3*R*)-quinuclidinol (1.16 g, 9.1 mmol) in 50 mL of toluene was added NaH 60% in mineral oil (0.125 g, 3.12 mmol) in several portions. The mixture was refluxed with continuous removal of distillate and replacement with fresh toluene over 1.5 h. After this time, the reaction mixture was cooled to room temperature and extracted with 2 N HCl. The acid extracts were combined, washed with a small volume of AcOEt, basified by addition of solid K_2CO_3, and extracted with $CHCl_3$. The organic extracts were combined, washed with water, dried over Na_2SO_4, and concentrated. The obtained product was purified by dissolving in diethyl ether and repeating the acid/base extraction process to afford 1.86 g (68%) of compound **11**, mp 175 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.88−1.09 (m, 2 H), 1.22−1.49 (m, 2 H), 1.53−1.62 (m, 1 H), 1.88−1.98 (d, J = 14.3 Hz, 1 H), 1.99−2.14 (m, 1 H), 2.37−2.57 (m, 3 H), 2.81−2.93 (ddd, J = 14.6, 8.0, 2.4 Hz, 1 H), 4.49−4.58 (m, 1 H), 7.01−7.09 (br s, 1 H), 7.13−7.28 (m, 4 H), 7.33−7.45 (m, 2 H), 7.58−7.65 (dd, J = 7.6, 1.8 Hz, 2 H). HPLC (method A): 99.9%, t_R = 10.4 min. MS (ESI) 352 m/z $(M + H)^+$.

(3*R*)-1-Azabicyclo[2.2.2]oct-3-yl 9-hydroxy-9*H*-fluorene-9-carboxylate (12) was prepared as described for compound **11** starting from the methyl ester **10** (5 g, 20.8 mmol). The obtained product was purified by crystallization from $CHCl_3$/diisopropyl ether to afford 4.63 g (66%) of compound **12**, mp 217 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.90−1.08 (m, 2 H), 1.23−1.50 (m, 2 H), 1.54−1.67 (m, 1 H), 1.95−2.17 (m, 2 H), 2.35−2.58 (m, 3 H), 2.82−2.98 (dd, J = 14.3, 7.9 Hz, 1 H), 4.50−4.63 (m, 1 H), 6.74 (br s, 1 H), 7.27−7.38 (m, 2 H), 7.38−7.48 (m, 2 H), 7.51 (d, J = 6.41 Hz, 2 H), 7.81 (d, J = 7.32 Hz, 2 H). HPLC (method B): 99.7%, t_R = 8.0 min. MS (ESI) 336 m/z $(M + H)^+$.

(3*R*)-1-Azabicyclo[2.2.2]oct-3-yl 9-methyl-9*H*-xanthene-9-carboxylate (16) was prepared as described for compound **11** starting from the methyl ester **14** (2.65 g, 10.4 mmol). The yield was 1.91 g (53%) of **16** as an oil. Compound **16** (0.3 g, 0.86 mmol) was treated with oxalic acid (0.077 g, 0.86 mmol) in acetone/diethyl ether to give 0.25 g (66%) of the oxalate salt, mp 152 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.18−1.33 (m, 1 H), 1.39−1.56 (m, 1 H), 1.59−1.82 (m, 2 H), 1.90 (s, 3 H),

1.94−2.04 (m, 1 H), 2.53−2.67 (m, 1 H), 2.69−2.79 (d, J = 14.0 Hz, 1 H), 2.90−3.14 (m, 3 H), 3.42−3.55 (ddd, J = 14.2, 8.4, 2.7 Hz, 1 H), 4.84−4.96 (m, 1 H), 7.11−7.21 (m, 4 H), 7.30−7.40 (m, 2 H), 7.41−7.50 (m, 2 H), 7.80−10.80 (br s, 2 H). HPLC (method A): 100.0%, t_R = 15.1 min. MS (ESI) 350 m/z $(M + H)^+$.

(3*R*)-1-Azabicyclo[2.2.2]oct-3-yl 9-methyl-9*H*-fluorene-9-carboxylate (17) was prepared as described for compound **11** starting from the methyl ester **15** (3.15 g, 13.2 mmol). The obtained product was purified by column chromatography (silica gel, chloroform/isopropanol, 80:20, as eluent) to give 3.44 g (80%) of compound **17** as an oil. Compound **17** (0.3 g, 0.89 mmol) was treated with oxalic acid (0.080 g, 0.89 mmol) in acetone/diethyl ether to give 0.26 g (68%) of the oxalate salt, mp 186.3 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.34−1.61 (m, 2 H), 1.61−1.86 (m, 2 H), 1.75 (s, 3 H), 1.98−2.07 (m, 1 H), 2.72−2.87 (m, 1 H), 2.90−2.99 (d, J = 14.6 Hz, 1 H), 2.99−3.21 (m, 3 H), 3.47−3.59 (ddd, J = 14.0, 8.2, 2.4 Hz, 1 H), 4.82−4.95 (m, 1 H), 7.33−7.41 (m, 2 H), 7.42−7.50 (m, 2 H), 7.60−7.66 (d, J = 7.0 Hz, 1 H), 7.66−7.72 (d, J = 7.3 Hz, 1 H), 7.87−7.93 (d, J = 7.3 Hz, 2 H), 6.50−11.50 (br s, 2 H). HPLC (method A): 100.0%, t_R = 14.7 min. MS (ESI) 334 m/z $(M + H)^+$.

(3*R*)-1-Azabicyclo[2.2.2]oct-3-yl hydroxy(di-2-thienyl)acetate (19) was prepared as described for compound **11** starting from the methyl ester **18**[36] (6.0 g, 23.6 mmol). The obtained product was purified by treatment with diisopropyl ether to afford 4.11 g (50%) of compound **19**, mp 180 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.12−1.34 (m, 1 H), 1.37−1.68 (m, 3 H), 1.87−1.95 (m, 1 H), 2.34−2.75 (m, 5 H), 3.04−3.15 (ddd, J = 14.7, 8.1, 2.0 Hz 1 H), 4.76−4.87 (m, 1 H), 6.98−7.04 (m, 2 H), 7.09−7.12 (m, 2 H), 7.37 (br s, 1 H), 7.46 −7.54 (m, 2 H). HPLC (method B): 98.8%, t_R = 7.7 min. MS (ESI) 350 m/z $(M + H)^+$.

(3*R*)-1-Azabicyclo[2.2.2]oct-3-yl di-2-thienylacetate (21) was prepared as described for compound **11** starting from the methyl ester **20** (1.67 g, 7.0 mmol). The obtained product was purified by column chromatography (silica gel, chloroform/methanol/NH_4OH, 95:5:0.5, as eluent) to give 0.82 g (35%) of compound **21** as an oil. Compound **21** (0.82 g, 2.5 mmol) was treated with fumaric acid (0.144 g, 1.23 mmol) in acetone/diethyl ether to give 0.58 g (60%) of the fumarate salt, mp 122 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.34−1.52 (m, 1 H), 1.52−1.78 (m, 3 H), 1.98−2.07 (m, 1 H), 2.62−2.72 (d, J = 14.6 Hz, 1 H), 2.72−2.97 (m, 4 H), 3.27−3.37 (ddd, J = 14.5, 8.4, 2.1 Hz, 1 H), 4.84−4.96 (m, 1 H), 5.85 (s, 1 H), 6.55 (s, 2 H), 6.97−7.03 (dd, J = 5.2, 3.7 Hz, 2 H), 7.07−7.12 (m, 2 H), 7.47−7.52 (dd, J = 5.2, 1.2 Hz, 2 H). HPLC (method B): 98.7%, t_R = 10.7 min; MS(ESI) 334 m/z $(M + H)^+$.

(3*R*)-1-Azabicyclo[2.2.2]oct-3-yl hydroxy(di-3-thienyl)acetate (23) was prepared as described for compound **11** starting from the methyl ester **22**[36] (3.55 g, 14.0 mmol). The obtained product was purified by crystallization in acetonitrile. The resulting solid was filtered and washed with diethyl ether to afford 1.54 g (31.5%) of compound **23**, mp 151 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.10−1.31 (m, 1 H), 1.31−1.68 (m, 3 H), 1.82−1.94 (m, 1 H), 2.36−2.45 (d, J = 14.6 Hz, 1 H), 2.45−2.72 (m, 4 H), 3.02−3.15 (ddd, J = 14.6, 8.2, 2.3 Hz, 1 H), 4.73−4.83 (m, 1 H), 6.54−6.68 (br s, 1 H), 7.06−7.12 (dt, J = 5.2, 1.5 Hz, 2 H), 7.34−7.40 (td, J = 3.3, 1.4 Hz, 2 H), 7.46−7.54 (dt, J = 5.1, 3.4 Hz, 2 H). HPLC (method A): 99.0%, t_R = 10.0 min. MS (ESI) 350 m/z $(M + H)^+$.

(3*R*)-1-Azabicyclo[2.2.2]oct-3-yl 2,2-di-2-thienylpropanoate (26) was prepared as described for compound **11** starting from the methyl ester **25** (0.86 g, 3.4 mmol). The yield was 1.11 g (94.1%) of compound **26** as an oil. Compound **26** (0.25 g, 0.72 mmol) was treated with oxalic acid (65 mg, 0.72 mmol) in acetone/diethyl ether to give 0.25 g (79%) of the oxalate salt, mp 126.7−128.6 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.50−1.70 (m, 2 H), 1.69−1.93 (m, 2 H), 2.07 (s, 3 H), 2.14−2.24 (m, 1 H), 2.78−2.95 (m, 1 H), 2.95−3.24 (m, 4 H), 3.54−3.67 (ddd, J = 13.9, 8.4, 1.8 Hz, 1 H), 5.01−5.15 (m, 1 H), 6.97−7.04 (ddd, J = 5.2, 3.5, 1.7 Hz, 2 H), 7.04−7.10 (m, 2 H), 7.47−7.52 (m, 2 H), 7.75−10.75 (br s, 2 H). HPLC (method B): 99.3%, t_R = 11.1 min. MS (ESI) 348 m/z $(M + H)^+$.

Preparation of (3*R*)-Quinuclidinol Esters 33,[19] 34, 35, 36, 37, 38, and 39 (Scheme 3). (3*R*)-1-Azabicyclo[2.2.2]oct-3-yl hydroxy(diphenyl)acetate (33)[19]. To a solution of compound **31**[39] (1 g, 3.86 mmol) in 2 mL of THF, cooled to −40 °C, was slowly added phenyllithium (1.8 M solution in cyclohexane/diethyl ether, 2.4 mL, 4.32 mmol). The mixture was stirred for 10 min at −40 °C. Then it was warmed to room temperature and the stirring was continued for 1 h. The reaction mixture was poured over a saturated aqueous NH_4Cl solution and extracted with diethyl ether. The ether extracts were combined, washed with brine, dried over Na_2SO_4, and concentrated. The obtained residue was purified by treatment with diisopropyl ether to afford 0.46 g (35%) of compound **33**, mp 188 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.07−1.24 (m, 1 H), 1.24−1.40 (m, 1 H), 1.39−1.65 (m, 2 H), 1.82−1.90 (m, 1 H), 2.30−2.46 (m, 2 H), 2.52−2.67 (m, 3 H), 3.00−3.15 (ddd, J = 14.7, 8.1, 2.3 Hz, 1 H), 4.75−4.86 (m, 1 H), 6.59 (br s, 1 H), 7.16−7.47 (m, 10 H). HPLC (method B): 95.6%, t_R = 9.4 min. MS (ESI) 338 m/z $(M + H)^+$.

(3*R*)-1-Azabicyclo[2.2.2]oct-3-yl hydroxy(phenyl)2-thienylacetate (34). To a solution of compound **32** (16.6 g, 62.5 mmol) in 300 mL of THF, cooled to −70 °C, was added phenylmagnesium bromide (1.0 M solution in THF, 75 mL, 75 mmol). The mixture was stirred for 15 min at ≤−60 °C, and then it was warmed to room temperature and the stirring continued for 2 h. The reaction mixture was poured over a saturated aqueous NH_4Cl solution and extracted with ethyl acetate. The organic extracts were combined, washed with brine, dried over Na_2SO_4, and concentrated. The obtained solid was treated with isopropanol at room temperature, filtered, and washed with diethyl ether to yield 9.85 g (38%) of compound **34** as a mixture of diastereomers **35** and **36**, which were separated by crystallization. The diastereomeric resolution was monitored by ^{1}H NMR, and crystallizations were repeated until the signals of the minor diastereomer were negligible.

(3*R*)-1-Azabicyclo[2.2.2]oct-3-yl (2*R*)-hydroxy(phenyl)2-thienylacetate (36). After five successive crystallizations of compound **34** from boiling isopropanol, an amount of 2.08 g (16%) of diastereomer **36** was obtained. Diastereomeric purity by ^{1}H NMR was ≥95%, mp 174−175 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.07−1.27 (m, 1 H), 1.27−1.41 (m, 1 H), 1.41−1.64 (m, 2 H), 1.80−1.87 (m, 1H), 2.36−2.71 (m, 5 H), 3.03−3.15 (ddd, J = 14.6, 8.2, 1.9 Hz, 1 H), 4.74−4.86 (m, 1 H), 7.00 (s, 1 H), 7.01−7.10 (m, 2 H), 7.24−7.53 (m, 6 H). HPLC (method B): 99.9%, t_R = 8.6 min. MS (ESI) 344 m/z $(M + H)^+$.

The absolute configuration of the ester moiety of compound **36** was determined by correlation with the absolute configuration of (+)-hydroxy(phenyl)2-thienylacetic acid (compound **40**). Diastereomer **36** was hydrolyzed (MeOH, NaOH, 30 min, at room temperature) to give compound **40**, $[\alpha]^{25}_D$ = +25.4° (c = 2, EtOH). The *R* configuration for the acid **40** was assigned by comparison of its [α] value with the data described in the literature for (2*S*)-hydroxy(phenyl)2-thienylacetic acid: $[\alpha]^{25}_D$ = −20° (c = 2, EtOH).[40]

(3*R*)-1-Azabicyclo[2.2.2]oct-3-yl (2*S*)-hydroxy(phenyl)2-thienylacetate (35). The mother liquors from the first crystallization of compound **34** were enriched with the diastereomer **35**. The solution was concentrated and cooled. The precipitated solid was filtered and discarded. The filtrate was concentrated and treated with diethyl ether, and the resulting solid was filtered to give 0.89 g (7%) of compound **35**. Diastereomeric purity by ^{1}H NMR was ≥95%, mp 151.8−152.6 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.11−1.32 (m, 1 H), 1.35−1.64 (m, 3 H), 1.87−194 (m, 1 H), 2.28−2.46 (m, 2 H), 2.46−2.69 (m, 3 H), 2.97−3.15 (ddd, J = 14.7, 8.1, 2.0 Hz, 1 H), 4.71−4.86 (m, 1 H), 6.98 − 7.10 (m, 3 H), 7.26−7.52 (m, 6 H). HPLC (method B): 94.5%, t_R = 8.3 min. MS (ESI) 344 m/z $(M + H)^+$.

(3*R*)-1-Azabicyclo[2.2.2]oct-3-yl cyclopentyl(hydroxy)2-thienylacetate (37) was prepared as described for compound **34**

starting from a solution of compound **32** (39.0 g, 147 mmol) in THF (325 mL) and cyclopentyl magnesium chloride (2.0 M solution in diethyl ether, 86 mL, 172 mmol). Purification by column chromatography (silica gel, chloroform/methanol/NH_4OH, 97:3:0.5 to 97:4:0.5, as eluent) afforded 22.8 g (46%) of compound **37** as a mixture of diastereomers **38** and **39**, which were separated by crystallization. The diastereomeric resolution was monitored by ^{1}H NMR, and crystallizations were repeated until the signals of the minor diastereomer were negligible.

(3*R*)-1-Azabicyclo[2.2.2]oct-3-yl (2*S*)-cyclopentyl(hydroxy)2-thienylacetate (38). After successive macerations of the mixture **37** with diisopropyl ether (twice) and with diethyl ether (twice), an amount of 3.8 g (15%) of diastereomer **38** was obtained. Diastereomeric purity by ^{1}H NMR was ≥95%, mp 157.3−158.7 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.36−1.83 (m, 11 H), 1.83−1.97 (m, 1 H), 2.03−2.15 (m, 1 H), 2.50−2.64 (m, 1 H), 2.64−2.98 (m, 5 H), 3.09−3.23 (dd, J = 14.8, 8.1 Hz, 1 H), 4.08 (br s, 1 H), 4.87−4.98 (m, 1 H), 6.96−7.04 (m, 1 H), 7.12−7.19 (m, 1 H), 7.25−7.31 (m, 1 H). HPLC (method B): 99.2%, t_R = 9.8 min. MS (ESI) 336 m/z $(M + H)^+$.

The absolute configuration of the ester moiety of compound **38** was determined by correlation with the absolute configuration of (−)-cyclopentyl(hydroxy)2-thienylacetic acid[42] (compound **41**). Diastereomer **38** was hydrolyzed (EtOH/NaOH, 2 N, 2 h at room temperature and 2 h at 60 °C) to give compound **41**, $[\alpha]^{22}_D = -6.44°$ (c = 1, EtOH). CD curve: λ (nm) = 233, $\Delta\varepsilon$ (M^{-1} cm^{-1}) = −4.19 . Configuration *S* was assigned by comparison of the CD curve with that of the acid **68**[41] (see the Supporting Information for more details).

(3*R*)-1-Azabicyclo[2.2.2]oct-3-yl (2*R*)-cyclopentyl(hydroxy)2-thienylacetate (39). The mother liquors from the first treatment of compound **37** with diisopropyl ether were enriched with the diastereomer **39**. The solution was concentrated, and the residue was purified by column chromatography (silica gel, chloroform/methanol/NH_4OH, 97:3:0.5 to 97:4:0.5, as eluent). The obtained product was treated with diisopropyl ether (twice) and then with chloroform/diethyl ether to give 1.2 g (5%) of compound **39**. Diastereomeric purity by HPLC (method B) was 92.1%; mp was not determined. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.18−1.69 (m, 12 H), 1.80 −1.89 (m, 1 H), 2.40−2.89 (m, 6 H), 3.05−3.22 (dd, J = 14.5, 8.1 Hz, 1 H), 4.64−4.74 (m, 1 H), 5.99 (br s, 1 H), 6.95−7.00 (dd, J = 4.9, 3.7 Hz, 1 H), 7.10 (d, J = 3.7 Hz, 1 H), 7.40 (d, J = 4.9 Hz, 1 H). HPLC (method B): 95.7% (compound **39**, 88.1%; compound **38**, 7.5%), t_R = 9.5 min. MS (ESI) 336 m/z $(M + H)^+$.

The absolute configuration of the ester moiety of compound **39** was determined by correlation with the absolute configuration of (+)-cyclopentyl(hydroxy)2-thienylacetic acid[42] (compound **42**). Diastereomer **39** was hydrolyzed (EtOH/NaOH, 2 N, 2 h at room temperature and 2 h at 60 °C) to give compound **42**, $[\alpha]^{22}_D = +6.63°$ (c = 1, EtOH). CD curve: λ (nm) = 233, $\Delta\varepsilon$ (M^{-1} cm^{-1}) = +4.18. Configuration *R* was assigned by comparison of the CD curve with that of the acid **69**.[41] (see the Supporting Information section for more details).

Preparation of Quaternary Ammonium Derivatives 43−64 (Scheme 4). (3*R*)-1-(2-Phenylethyl)-3-[(9*H*-xanthen-9-ylcarbonyl)oxy]-1-azoniabicyclo[2.2.2]octane bromide (43). To a solution of compound **5** (5.05 g, 15.05 mmol) in 100 mL of THF was added (2-bromoethyl)benzene (6.88 g, 37.20 mmol). The mixture was refluxed for 9 h and then stirred at room temperature for 80 h. The precipitated solid was filtered and washed with diethyl ether to obtain 6.5 g (83%) of compound **43**, mp 258 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.65−2.03 (m, 4 H), 2.14−2.24 (m, 1 H), 2.89−3.10 (m, 2 H), 3.18−3.34 (m, 1 H), 3.35−3.55 (m, 5 H), 3.55−3.70 (m, 1 H), 3.85−3.98 (ddd, J = 13.7, 8.2, 2.1 Hz, 1 H), 5.01−5.12 (m, 1 H), 5.33 (s, 1 H), 7.14−7.22 (m, 3 H), 7.22 (s, 1 H), 7.24−7.42 (m, 7 H), 7.46−7.60 (ddd, J = 17.0, 7.6, 1.5 Hz, 2 H). HPLC (method B): 100.0%, t_R = 12.3 min. MS (ESI) 440 m/z $(M - Br)^+$.

(3*R*)-3-{[(9-Hydroxy-9*H*-xanthen-9-yl)carbonyl]oxy}-1-(2-phenylethyl)-1-azoniabicyclo[2.2.2]octane bromide (44). To a solution of compound **11** (0.3 g, 0.85 mmol) in 4 mL of acetonitrile and 6 mL of chloroform was added (2-bromoethyl)benzene (0.79 g, 4.25 mmol). The mixture was stirred at room temperature for 6 days. The solvents were evaporated, and the obtained residue was treated with diethyl ether. The resulting solid was filtered and washed with diethyl ether to obtain 0.29 g (64%) of compound **44**, mp 221 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.20−1.42 (m, 1 H), 1.51−1.73 (m, 1 H), 1.73−1.99 (m, 2 H), 2.01−2.14 (m, 1 H), 2.66−2.94 (m, 3 H), 2.98 (d, J = 13.7 Hz, 1 H), 3.23−3.54 (m, 5 H), 3.81 (dd, J = 12.1, 9.0 Hz, 1 H), 4.98−5.11 (m, 1 H), 7.13−7.39 (m, 10 H), 7.44 (t, J = 7.5 Hz, 2 H), 7.67 (t, J = 8.5 Hz, 2 H). HPLC (method B): 95.1%, t_R = 11.4 min. MS (ESI) 456 m/z $(M - Br)^+$.

(3*R*)-3-{[(9-Hydroxy-9*H*-xanthen-9-yl)carbonyl]oxy}-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane bromide (45) was prepared as described for compound **43** starting from compound **11** (0.95 g, 2.70 mmol) and (3-bromopropoxy)benzene (0.87 g, 4.05 mmol). The reaction time was 20 h at reflux temperature. Trituration with diethyl ether gave 1.33 g (87%) of compound **45**, mp 112.7−114.9 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.24−1.40 (m, 1 H), 1.56−1.71 (m, 1 H), 1.71−1.93 (m, 2 H), 1.91−2.13 (m, 3 H), 2.62−2.78 (m, 1 H), 2.86−2.98 (d, J = 13.7 Hz, 1 H), 3.19−3.48 (m, 5 H), 3.74−3.88 (ddd, J = 13.7, 7.9, 1.5 Hz, 1 H), 3.93−4.05 (t, J = 5.7 Hz, 2 H), 4.97−5.07 (m, 1 H), 6.91−7.02 (m, 3 H), 7.19 (s, 1 H), 7.19−7.37 (m, 6 H), 7.38−7.49 (m, 2 H), 7.64−7.71 (ddd, J = 6.4, 6.4, 1.2 Hz, 2 H). HPLC (method B): 98.7%, t_R = 12.1 min. MS (ESI) 486 m/z $(M - Br)^+$.

(3*R*)-1-Heptyl-3-{[(9-hydroxy-9*H*-fluoren-9-yl)carbonyl]oxy}-1-azoniabicyclo[2.2.2]octane bromide (46) was prepared as described for compound **44** starting from compound **12** (0.25 g, 0.75 mmol) and 1-bromoheptane (0.67 g, 3.75 mmol). The reaction time was 72 h. Trituration with diethyl ether gave 0.33 g (85.5%) of compound **46**, mp 217 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.89 (t, J = 7.0 Hz, 3 H), 1.13−1.70 (m, 12 H), 1.70−1.93 (m, 2 H), 2.03−2.11 (m, 1 H), 2.68−2.84 (m, 1 H), 2.98−3.19 (m, 3 H), 3.18−3.43 (m, 4 H), 3.78 (ddd, J = 13.6, 7.8, 2.4 Hz, 1 H), 4.97−5.05 (m, 1 H), 7.36 (dd, J = 7.5, 1.1 Hz, 2 H), 7.45 (dd, J = 7.3, 7.3 Hz, 2 H), 7.59 (d, J = 7.0 Hz, 1H), 7.64 (d, J = 7.6 Hz, 1 H), 7.84 (dd, J = 7.3, 1.1 Hz, 2 H). HPLC (method B): 99.6%, t_R = 12.6 min. MS (ESI) 434 m/z $(M - Br)^+$.

(3*R*)-3-{[(9-Hydroxy-9*H*-fluoren-9-yl)carbonyl]oxy}-1-(2-phenylethyl)-1-azoniabicyclo[2.2.2]octane bromide (47) was prepared as described for compound **43** starting from compound **12** (0.7 g, 2.1 mmol) and (2-bromoethyl)benzene (0.58 g, 3.15 mmol). The reaction time was 31 h at reflux and 6 days at room temperature. Trituration with diethyl ether/acetonitrile gave 0.89 g (81.5%) of compound **47**, mp 118 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.34−1.54 (m, 1 H), 1.60−1.76 (m, 1 H), 1.76−2.03 (m, 2 H), 2.07−2.15 (m, 1 H), 2.78−3.06 (m, 3 H), 3.21 (d, J = 13.4 Hz, 1 H), 3.28−3.58 (m, 5 H), 3.88 (dd, J = 5.3, 2.6 Hz, 1 H), 5.01−5.13 (m, 1 H), 6.87 (s, 1 H), 7.27−7.41 (m, 7 H), 7.47 (dd, J = 7.2 Hz, 2 H), 7.60 (d, J = 7.6 Hz, 1 H), 7.66 (d, J = 7.2 Hz, 1 H), 7.85 (d, J = 7.6 Hz, 2 H). HPLC (method B): 95.5%, t_R = 10.2 min. MS (ESI) 440 m/z $(M - Br)^+$.

(3*R*)-3-{[(9-Hydroxy-9*H*-fluoren-9-yl)carbonyl]oxy}-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane bromide (48) was prepared as described for compound **43** starting from compound **12** (2.2 g, 6.6 mmol) and (3-bromopropoxy)benzene (2.86 g, 13.3 mmol). The reaction time was 18 h at reflux temperature. Trituration with diethyl ether/acetonitrile gave 3.17 g (88%) of compound **48**, mp 108 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.35−1.54 (m, 1 H), 1.57−1.75 (m, 1 H), 1.75−1.96 (m, 2 H), 1.96−2.18 (m, 3 H), 2.77−2.96 (m, 1 H), 3.16 (d, J = 13.7 Hz, 1 H), 3.24−3.54 (m, 5 H), 3.86 (ddd, J = 8.1, 5.2, 2.0 Hz, 1 H), 4.01 (t, J = 5.8 Hz, 2 H), 4.96−5.08 (m, 1 H), 6.86 (s, 1 H), 6.90−7.03 (m, 3 H), 7.26−7.40 (m, 4 H), 7.45 (dd, J = 7.3 Hz, 2 H), 7.61 (d, J = 7.6 Hz, 1 H), 7.66 (d, J = 7.3 Hz, 1 H), 7.84 (d, J = 7.3 Hz, 2 H). HPLC (method A): 99.9%, t_R = 15.8 min. MS (ESI) 470 m/z $(M - Br)^+$.

(3*R*)-3-{[(9-Methyl-9*H*-xanthen-9-yl)carbonyl]oxy}-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane bromide (49) was prepared as described for compound **44** starting from compound **16** (0.10 g, 0.28 mmol) and (3-bromopropoxy)benzene (0.30 g, 1.40 mmol). The reaction time was 72 h. Trituration with diethyl ether gave 0.11 g (69%) of compound **49**, mp 195 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.33−1.48 (m, 1 H), 1.59−1.74 (m, 1 H), 1.74−1.96 (m, 2 H), 1.92 (s, 3 H), 1.97−2.18 (m, 3 H), 2.74−2.91 (m, 1 H), 3.05−3.18 (d, J = 13.7 Hz, 2 H), 3.23−3.50 (m, 5 H), 3.79−3.92 (m, 1 H), 3.97−4.07 (t, J = 5.8 Hz, 2 H), 5.00−5.10 (m, 1 H), 6.91−7.02 (m, 3 H), 7.12−7.23 (m, 4 H), 7.27−7.42 (m, 4 H), 7.44−7.50 (dd, J = 8.1, 1.7 Hz, 1 H), 7.50−7.55 (dd, J = 8.1, 1.4 Hz, 1 H). HPLC (method A): 98.4%, t_R = 21.3 min. MS (ESI) 484 m/z (M − Br)$^+$.

(3*R*)-3-{[(9-Methyl-9*H*-fluoren-9-yl)carbonyl]oxy}-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane bromide (50) was prepared as described for compound **44** starting from compound **17** (0.20 g, 0.6 mmol) and (3-bromopropoxy)benzene (0.64 g, 3.0 mmol). The reaction time was 72 h. Trituration with diethyl ether gave 0.20 g (61%) of compound **50**, mp 204 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.42−1.63 (m, 1 H), 1.63−1.97 (m, 3 H), 1.78 (s, 3 H), 1.97−2.25 (m, 3 H), 2.94−3.15 (m, 1 H), 3.20−3.60 (m, 6 H), 3.80−3.97 (m, 1 H), 3.96−4.18 (t, J = 5.5 Hz, 2 H), 4.91−5.05 (m, 1 H), 6.87−7.01 (m, 3 H), 7.24−7.50 (m, 6 H), 7.62−7.70 (d, J = 6.7 Hz, 1 H), 7.72−7.80 (d, J = 7.0 Hz, 1 H), 7.86−7.95 (d, J = 6.7 Hz, 2 H). HPLC (method A): 99.4%, t_R = 20.8 min. MS (ESI) 468 m/z (M − Br)$^+$.

(3*R*)-3-{[Hydroxy(di-2-thienyl)acetyl]oxy}-1-methyl-1-azoniabicyclo[2.2.2]octane bromide (51) was prepared as described for compound **44** starting from compound **19** (0.20 g, 0.57 mmol) and bromomethane (2.85 mL of a 1 M solution in acetonitile, 2.85 mmol). The reaction time was 18 h. Trituration with diethyl ether gave 0.20 g (79%) of compound **51**, mp 251 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.60−1.84 (m, 2 H), 1.82−2.07 (m, 2 H), 2.29 (m, 1 H), 3.02 (s, 3 H), 3.19−3.58 (m, 5 H), 3.90−4.03 (ddd, J = 13.3, 8.4, 2.1 Hz, 1 H), 5.15−5.27 (m, 1 H), 6.97−7.07 (dt, J = 5.0, 3.8 Hz, 2 H), 7.16−7.24 (ddd, J = 8.1, 3.5, 1.2 Hz, 2 H), 7.49 (s, 1 H) 7.50−7.55 (ddd, J = 5.1, 3.7, 1.2 Hz, 2 H). HPLC (method B): 99.7%, t_R = 7.7 min. MS (ESI) 364 m/z (M − Br)$^+$.

(3*R*)-1-Heptyl-3-{[hydroxy(di-2-thienyl)acetyl]oxy}-1-azoniabicyclo[2.2.2]octane bromide (52). To a solution of compound **19** (0.50 g, 1.43 mmol) in 10 mL of chloroform was added 1-bromoheptane (1.02 g, 5.52 mmol). The mixture was stirred at room temperature for 7 days. The solvent was evaporated, and the residue was treated with diethyl ether. The resulting solid was filtered and washed with diethyl ether to obtain 0.49 g (66%) of compound **52**, mp 134 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.88 (t, J = 6.7 Hz, 3 H), 1.16−1.39 (m, 8 H), 1.5−1.8 (m, 4 H), 1.83−2.06 (m, 2 H), 2.25−2.35 (m, 1 H), 3.03−3.54 (m, 7 H), 3.89 (ddd, J = 13.9, 8.2, 1.7 Hz, 1 H), 5.16−5.29 (m, 1 H), 7.02 (ddd, J = 5.1, 3.6, 2.3 Hz, 2 H), 7.17 (td, J = 3.6, 1.2 Hz, 2 H), 7.49 (s, 1 H), 7.52 (ddd, J = 5.1, 2.3, 1.2 Hz, 2 H). HPLC (method A): 99.5%, t_R = 17.9 min. MS (ESI) 448 m/z (M − Br)$^+$.

(3*R*)-1-Benzyl-3-{[hydroxy(di-2-thienyl)acetyl]oxy}-1-azoniabicyclo[2.2.2]octane bromide (53) was prepared as described for compound **44** starting from compound **19** (0.20 g, 0.57 mmol) and (bromomethyl)benzene (0.34 mL, 2.85 mmol). The reaction time was 18 h. Trituration with diethyl ether gave 0.23 g (77%) of compound **53**, mp 89.0−90.6 °C (dec). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.57−2.03 (m, 4 H), 2.26 (m, 1 H), 2.98−3.15 (m, 1 H), 3.22−3.54 (m, 4 H), 3.81−3.94 (m, 1 H), 4.47 (d, J = 12.8 Hz, 1 H), 4.54 (d, J = 12.8 Hz, 1 H), 5.21 (m, 1 H), 6.96 (dd, J = 8.6, 3.6 Hz, 2 H), 7.01−7.11 (d, J = 3.1 Hz, 2 H), 7.44 (s, 1H), 7.45−7.61 (m, 7 H). HPLC (method B): 95.6%, t_R = 10.3 min. MS (ESI) 440 m/z (M − Br)$^+$.

(3*R*)-3-{[Hydroxy(di-2-thienyl)acetyl]oxy}-1-(2-phenylethyl)-1-azoniabicyclo[2.2.2]octane bromide (54) was prepared as described for compound **44** starting from compound **19** (0.20 g, 0.57 mmol) and (2-bromoethyl)benzene (0.53 g, 2.86 mmol). The reaction time was 72 h. Crystallization from acetonitrile gave 0.18 g (59%) of compound **54**, mp 216 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.60−1.89 (m, 2 H), 1.89−2.13 (m, 2 H), 2.27−2.40 (m, 1 H), 2.89−3.12 (m, 2 H), 3.17−3.32 (m, 1 H), 3.37−3.67 (m, 6 H), 3.92−4.08 (m, 1 H), 5.22−5.34 (m, 1 H), 7.03 (dd, J = 3.4 Hz, 2 H), 7.19 (dd, J = 3.7 Hz, 2 H), 7.24−7.42 (m, 5 H), 7.49 (s, 1 H), 7.54 (dd, J = 3.4 Hz, 2 H). HPLC (method A): 99.6%, t_R = 15.5 min. MS (ESI) 454 m/z (M − Br)$^+$.

(3*R*)-3-{[Hydroxy(di-2-thienyl)acetyl]oxy}-1-(4-phenylbutyl)-1-azoniabicyclo[2.2.2]octane bromide (55) was prepared as described for compound **52** starting from compound **19** (0.50 g, 1.43 mmol) and (4-bromobutyl)benzene (1.22 g, 5.73 mmol). The reaction time was 7 days. Trituration with diethyl ether gave 0.32 g (41%) of compound **55**, mp 64 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.50−1.72 (m, 4 H), 1.72−2.12 (m, 5 H), 2.34−2.44 (m, 1 H), 2.61 (t, J = 6.6 Hz, 2 H), 3.34−3.59 (m, 5 H), 3.60−3.69 (m, 1 H), 3.74 (d, J = 13.7 Hz, 1 H), 4.20 (dd, J = 13.0, 8.4 Hz, 1 H), 5.18−5.32 (m, 1 H), 6.93 (dd, J = 5.0, 3.8 Hz, 2 H), 7.09−7.33 (m, 9 H). HPLC (method B): 98.6%, t_R = 12.5 min. MS (ESI) 482 m/z (M − Br)$^+$.

(3*R*)-3-{[Hydroxy(di-2-thienyl)acetyl]oxy}-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane bromide (56) was prepared as described for compound **44** starting from compound **19** (5.0 g, 14.33 mmol) and (3-bromopropoxy)benzene (15.97 g, 74.23 mmol). The reaction time was 18 h. Trituration with acetonitrile gave 7.20 g (89%) of compound **56**, mp 230 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.63−1.87 (m, 2 H), 1.85−2.06 (m, 2 H), 2.06−2.24 (m, 2 H), 2.26−2.38 (m, 1 H), 3.13−3.29 (m, 1 H), 3.32−3.64 (m, 6 H), 3.91−4.07 (m, 1 H), 4.03 (t, J = 5.8 Hz, 2 H), 5.19−5.31 (m, 1 H), 6.91−7.00 (m, 3 H), 7.00−7.06 (m, 2 H), 7.17−7.22 (m, 2 H), 7.28−7.36 (m, 2 H), 7.50 (s, 1H), 7.50−7.56 (m, 2 H). HPLC (method B): 99.8%, t_R = 11.9 min. MS (ESI) 484 m/z (M − Br)$^+$.

(3*R*)-3-[(Di-2-thienylacetyl)oxy]-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane bromide (57) was prepared as described for compound **44** starting from compound **21** (0.25 g, 0.75 mmol) and (3-bromopropoxy)benzene (0.81 g, 3.75 mmol). The reaction time was 24 h. Trituration with diethyl ether gave 0.34 g (83%) of compound **57**, mp 148 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.72−2.05 (m, 4 H), 2.05−2.23 (m, 2 H), 2.25−2.36 (m, 1 H), 3.20−3.34 (m, 1 H), 3.36−3.64 (m, 4 H), 3.40−3.48 (t, J = 7.9 Hz, 2 H), 3.90−4.03 (m, 1 H), 4.00−4.06 (t, J = 5.8 Hz, 2 H), 5.14−5.24 (m, 1 H), 5.91 (s, 1 H), 6.92−6.99 (m, 3 H), 6.99−7.04 (ddd, J = 5.0, 3.6, 1.2 Hz, 2 H), 7.12−7.18 (dd, J = 7.3, 3.4 Hz, 2 H), 7.26−7.36 (m, 2 H), 7.48−7.54 (d, J = 5.2 Hz, 2 H). HPLC (method B): 94.5%, t_R = 13.2 min. MS (ESI) 468 m/z (M − Br)$^+$.

(3*R*)-3-{[Hydroxy(di-3-thienyl)acetyl]oxy}-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane bromide (58) was prepared as described for compound **43** starting from compound **23** (0.5 g, 1.43 mmol) and (3-bromopropoxy)benzene (0.46 g, 2.15 mmol). The reaction time was 6 h at reflux and 72 h at room temperature. Trituration with diethyl ether gave 0.68 g (86%) of compound **58**, mp 219 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.50−1.66 (m, 1 H), 1.66−1.82 (m, 1 H), 1.82−2.03 (m, 2 H), 2.03−2.24 (m, 2 H), 2.25−2.37 (m, 1 H), 3.08−3.25 (m, 1 H), 3.28−3.60 (m, 6 H), 3.86−4.02 (m, 1 H), 4.01−4.08 (t, J = 5.7 Hz, 2 H), 5.15−5.26 (m, 1 H), 6.78 (s, 1 H), 6.91−7.01 (m, 3 H), 7.11−7.17 (d, J = 5.2 Hz, 2 H), 7.32 (m, 2 H), 7.42−7.48 (m, 2 H), 7.48−7.55 (m, 2 H). HPLC (method B): 99.7%, t_R = 11.8 min. MS (ESI) 484 m/z (M − Br)$^+$.

(3*R*)-3-[(2,2-Di-2-thienylpropanoyl)oxy]-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane bromide (59) was prepared as described for compound **44** starting from compound **26** (0.25 g, 0.72 mmol) and (3-bromopropoxy)benzene (0.77 g, 3.60 mmol). The reaction time was 72 h. Trituration with diethyl ether gave 0.35 g (92%) of compound **59**, mp 170 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.60−2.25 (m, 6 H), 2.10 (s, 3 H), 2.25−2.34 (m, 1 H), 3.02−3.21 (m, 1 H), 3.30−3.63 (m, 6 H), 3.90−4.08 (m, 1 H), 4.01−4.09 (t, J = 5.5 Hz,

2 H), 5.14−5.26 (m, 1 H), 6.92−7.00 (m, 3 H), 7.00−7.06 (dd, J = 5.0, 3.6 Hz, 2 H), 7.08−7.14 (m, 2 H), 7.26−7.36 (m, 2 H), 7.48−7.54 (d, J = 5.19 Hz, 2 H). HPLC (method B): 97.9%, t_R = 14.0 min. MS (ESI) 482 m/z $(M - Br)^+$.

(3*R*)-3-{[Hydroxy(diphenyl)acetyl]oxy}-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane bromide (60) was prepared as described for compound **44** starting from compound **33** (0.40 g, 1.18 mmol) and (3-bromopropoxy)benzene (1.23 g, 5.69 mmol). The reaction time was 18 h. Trituration with diethyl ether gave 0.28 g (42%) of compound **60**, mp 199 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.44−1.62 (m, 1 H), 1.61−1.79 (m, 1 H), 1.80−2.01 (m, 2 H), 2.01−2.22 (m, 2 H), 2.22−2.34 (m, 1 H), 2.99−3.18 (m, 1 H), 3.19−3.57 (m, 6 H), 3.85−3.99 (m, 1 H), 3.99−4.09 (t, J = 5.5 Hz, 2 H), 5.14−5.33 (m, 1 H), 6.89−7.02 (m, 3 H), 7.20−7.51 (m, 13 H). HPLC (method B): 94.9%, t_R = 12.6 min. MS (ESI) 472 m/z $(M - Br)^+$.

(3*R*)-3-{[(2*S*)-2-Hydroxy-2-phenyl-2-(2-thienyl)acetyl]oxy}-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane bromide (61) was prepared as described for compound **43** starting from compound **35** (0.5 g, 1.50 mmol) and (3-bromopropoxy)benzene (0.62 g, 2.92 mmol). The reaction time was 3 h at reflux and overnight at room temperature. The precipitated solid was filtered and washed with diethyl ether to obtain 0.66 g (79%) of compound **61**, mp 206.4−207.9 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.57−1.82 (m, 2 H), 1.82−2.03 (m, 2 H), 2.03−2.20 (m, 2 H), 2.25−2.35 (m, 1 H), 3.03−3.19 (m, 1 H), 3.24−3.55 (m, 6 H), 3.87−3.99 (ddd, J = 13.6, 8.2, 1.7 Hz, 1 H), 3.99−4.07 (t, J = 6.0 Hz, 2 H), 5.18−5.28 (m, 1 H), 6.91−7.00 (m, 3 H), 7.00−7.04 (dd, J = 5.0, 3.5 Hz, 1 H), 7.1−7.14 (dd, J = 3.7, 1.2 Hz, 1 H), 7.19 (s, 1 H), 7.27−7.43 (m, 5 H), 7.43−7.55 (m, 3 H). HPLC (method B): 99.7%, t_R = 12.3 min. MS (ESI) 478 m/z $(M - Br)^+$.

(3*R*)-3-{[(2*R*)-2-Hydroxy-2-phenyl-2-(2-thienyl)acetyl]oxy}-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane bromide (62) was prepared as described for compound **43** starting from compound **36** (0.5 g, 1.50 mmol) and (3-bromopropoxy)benzene (0.62 g, 2.92 mmol). The reaction time was 3 h at reflux and overnight at room temperature. The precipitated solid was filtered and washed with diethyl ether to obtain 0.69 g (82%) of compound **62**, mp 240.8−241.4 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.45−1.61 (m, 1 H), 1.63−1.79 (m, 1 H), 1.81−2.03 (m, 2 H), 2.03−2.23 (m, 2 H), 2.22−2.33 (m, 1 H), 3.05−3.21 (m, 1 H), 3.30−3.57 (m, 6 H), 3.88−4.01 (m, 1 H), 3.99−4.07 (t, J = 5.8 Hz, 2 H), 5.19−5.29 (m, 1 H), 6.92−7.00 (m, 3 H), 7.01−7.06 (dd, J = 4.9, 3.7 Hz, 1 H), 7.14−7.17 (dd, J = 3.5, 1.1 Hz, 1 H), 7.20 (s, 1 H), 7.27−7.41 (m, 5 H), 7.45−7.57 (m, 3 H). HPLC (method B): 99.9%, t_R = 12.2 min. MS (ESI) 478 m/z $(M - Br)^+$.

(3*R*)-3-{[(2*S*)-2-Cyclopentyl-2-hydroxy-2-(2-thienyl)acetyl]-oxy}-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane bromide (63) was prepared as described for compound **43** starting from compound **38** (1.0 g, 2.98 mmol) and (3-bromopropoxy)benzene (0.96 g, 4.44 mmol). The reaction time was 8.5 h at reflux and 16 h at room temperature. The precipitated solid was filtered and washed with diethyl ether to obtain 1.53 g (93%) of compound **63**, mp 168−170 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.27−1.67 (m, 8 H), 1.79−2.05 (m, 4 H), 2.05−2.19 (m, 2 H), 2.26−2.36 (m, 1 H), 2.75−2.91 (m, 1 H), 3.17−3.27 (d, J = 13.4 Hz, 1 H), 3.27−3.62 (m, 6 H), 3.85−3.97 (ddd, J = 13.4, 8.7, 2.3 Hz, 1 H), 4.04 (t, J = 5.8 Hz, 2 H), 5.11−5.19 (m, 1 H), 6.19 (s, 1 H), 6.91−6.98 (m, 3 H), 6.98−7.03 (dd, J = 4.9, 3.7, 1 H), 7.15−7.18 (dd, J = 3.7, 1.2 Hz, 1 H), 7.28−7.35 (dd, J = 7.8 Hz, 2 H), 7.42−7.45 (dd, J = 4.7, 1.1 Hz, 1 H). HPLC (method B): 99.7%, t_R = 13.1 min. MS (ESI) 470 m/z $(M - Br)^+$.

(3*R*)-3-{[(2*R*)-2-Cyclopentyl-2-hydroxy-2-(2-thienyl)acetyl]-oxy}-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane bromide (64) was prepared as described for compound **43** starting from compound **39** (1.0 g, 2.98 mmol) and (3-bromopropoxy)benzene (0.96 g, 4.44 mmol). The reaction time was 6 h at reflux and 12 h at room temperature. The precipitated solid was filtered and washed with diethyl ether to obtain 1.20 g (73%) of compound **64**, mp 181 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.27−1.67 (m, 8 H), 1.67−2.05 (m, 4 H), 2.05−2.20 (m, 2 H), 2.20−2.28 (m, 1 H), 2.76−2.95 (m, 1 H), 3.18−3.27 (d, J = 13.7 Hz, 1 H), 3.28−3.62 (m, 6 H), 3.95−3.97 (ddd, J = 13.7, 8.9, 2.1 Hz, 1 H), 4.05 (t, J = 5.5 Hz, 2 H), 5.10−5.19 (m, 1 H), 6.18 (s, 1 H), 6.93−6.99 (m, 3 H), 6.98−7.03 (dd, J = 4.9, 3.7, 1 H), 7.17−7.20 (dd, J = 3.7, 1.2 Hz, 1 H), 7.28−7.73 (dd, J = 7.8 Hz, 2 H), 7.42−7.46 (dd, J = 4.9, 1.2 Hz, 1 H). HPLC (method B): 99.8%, t_R = 13.1 min. MS (ESI) 470 m/z $(M - Br)^+$.

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Supporting Information Available: (i) Purity of compounds (HPLC); (ii) preparation of the acids **68** and **69**; (iii) assignment of the absolute configuration of acids **41** and **42** and circular dichroism (CD) curves; (iv) preparation of compounds **2**, **7**, **9**, **10**, **14**, and **15** (Scheme 1); (v) preparation of compounds **18**, **20**, **22**, and **25** (Scheme 2); (vi) preparation of compounds **31** and **32** (Scheme 3); (vii) references. This material is available free of charge via the Internet at http://pubs.acs.org.

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