Structure Elucidation Via Stereoselective Synthesis of the Acetate Center of 1-Azabicyclo[2.2.2]oct-3-yl α-Hydroxy-α-(1-iodo-1-propen-3-yl)-α-phenylacetate (IQNP). A High Affinity Muscarinic Imaging Agent for SPECT

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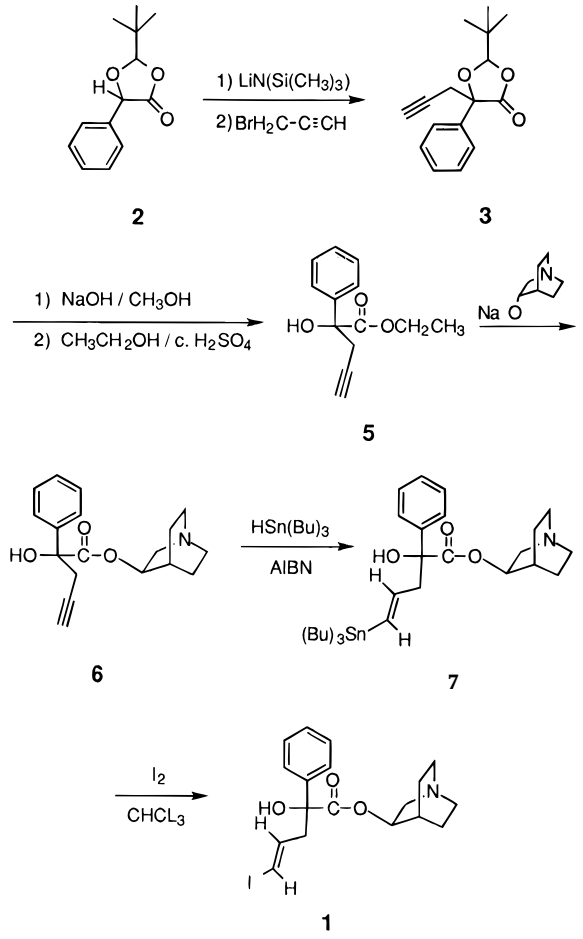
1-Azabicyclo[2.2.2]oct-3-yl α-hydroxy-α-(1-iodo-1-propen-3-yl)-α-phenylacetate (IQNP, **1**) has been developed as a new imaging agent for use in single photon emission computed tomography (SPECT). IQNP has been shown to be readily radioiodinated, to cross the blood−brain barrier and to localize in regions of the brain which contain varying concentrations of the muscarinic acetylcholine receptor (mAChR).[1] In addition, the various stereoisomers of IQNP demonstrated modest selectivity for the various subtypes of mAChR.[2−4]

The stereochemistry of 3-quinuclidinol is well established[5] and the (*R*)-(−) configuration of various 3-quinuclidinyl esters has been shown to impart mAChR receptor binding activity to the ligands.[6,7] The acetate moiety of IQNP (**1**) has been resolved as the (−)- and (+)-α-methylbenzylamine salts.[2] Biodistribution studies in rats have demonstrated that (*E*)- and (*Z*)-IQNP, which contain the (−) configuration of the acetate moiety, have significant uptake in areas of the brain rich in mAChR and the heart. However, IQNP is isolated as an oil and the *R*/*S* orientation of the acetate moiety has yet to identified due to the absence of a suitable crystal for analysis.

A stereoselective α-alkylation of α-heterosubstituted acids has been reported to afford diastereoselectivity of >95%.[8,9] The condensation of (*S*)-(+)-mandelic acid with pivalaldehyde affords *cis*-(2*S*,5*S*)-2-(*tert*-butyl)-5-phenyl-1,3-dioxolan-4-one (**2**). It has been demonstrated that after deprotonation with base, the electrophilic reaction with alkyl halides occurs on the less hindered face with retention of configuration.

Utilizing *cis*-(2*S*,5*S*)-**2** and *cis*-(2*R*,5*R*)-**2**, we have prepared the various stereoisomers of the acetate moiety and subsequently (*E*)-(*R*,*S*)- and (*E*)-(*R*,*R*)-IQNP[10] as shown in Scheme 1. A solution of *cis*-(2*S*,5*S*)-**2** in anhydrous tetrahydrofuran was treated with a solution of lithium 1,1,1,3,3,3-hexamethyldisilazane in anhydrous tetrahydrofuran at −78 °C. After addition was complete, the solution was stirred for 30 min at −78 °C and propargyl bromide was added. The solution was warmed to room temperature and stirred for 3 h to afford (2*S*,5*S*)-2-(*tert*-butyl)-5-phenyl-5-(1-propyn-3-yl)-1,3-dioxolan-4-one ((*S*,*S*)-**3**). (*S*,*S*)-**3** was then treated with a 2 M methanolic sodium hydroxide solution to afford (*S*)-(+)-α-hydroxy-α-phenyl-α-(1-propyn-3-yl)acetic acid ((*S*)-**4**). An analogous procedure utilizing (2*R*,5*R*)-**2** afforded (*R*,*R*)-**3** and (*R*)-**4**. (*R*)- and (*S*)-**4** were then esterified as described previously to afford (*R*)-(−)- and (*S*)-(+)-ethyl α-hydroxy-α-phenyl-α-(1-propyn-3-yl)acetate ((*R*)- and (*S*)-**5**), respectively.

Scheme 1. Stereoselective Synthesis of (*E*)-IQNP (1)



By comparison of the NMR analysis and measured specific rotation of (*S*)- and (*R*)-**4** and -**5** with those previously reported for **4** and **5** resolved by the use of the stereoisomers of α-methylbenzylamine (Table 1), the isomer with the measured (+)-specific rotation can be assigned the *S* configuration and the isomer with the measured (−)-specific rotation can be assigned the *R* configuration.

In addition, we have previously shown that the various *E*- and *Z*-stereoisomers of the acetate center of IQNP can be separated utilizing a normal phase column (mobile phase: methylene chloride:ethanol:triethylamine [97:3:0.03]). Also, the preparation of **1** from **5** as shown in Scheme 1 has been shown not to cause racemization of chiral centers.[2] Therefore, utilizing (*S*)- and (*R*)-**5**,

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(10) The assignment of stereochemistry refers to (*R*,*S*)-IQNP, for example, the first stereocenter designation as the quinuclidinyl moiety and the second as the acetate moiety.

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Table 1. Comparison of the Specific Rotation[a] of the E Isomers of IQNP (1) and Intermediates

compound	stereoselective synthesis	classical resolution[b]
(*R*,*R*)-**2**	−82.4°	
(*S*,*S*)-**2**	+87.3° [c]	
(*R*,*R*)-**3**	+27.2°	
(*S*,*S*)-**3**	−27.8°	
(*R*)-**4**	−20.6°	−10.7°
(*S*)-**4**	+20.8°	+12.7°
(*R*)-**5**	−24.9°	−13.6°
(*S*)-**5**	+25.2°	+18.4°
(*R*,*R*)-**6**	−13.4°	−4.5°
(*S*,*S*)-**6**	+40.6°	+41.8°
(*E*)-(*R*,*R*)-**7**	−17.5°	−12.5°
(*E*)-(R,S)-**7**	+30.7°	+29.0°
(*E*)-(R,R)-**1**	−17.2°	−20.2°
(*E*)-(R,S)-**1**	+42.4°	+39.5°

[a] Specific rotation measured in chloroform. [b] Reference 2. [c] Literature value +88.5°, reference 8.

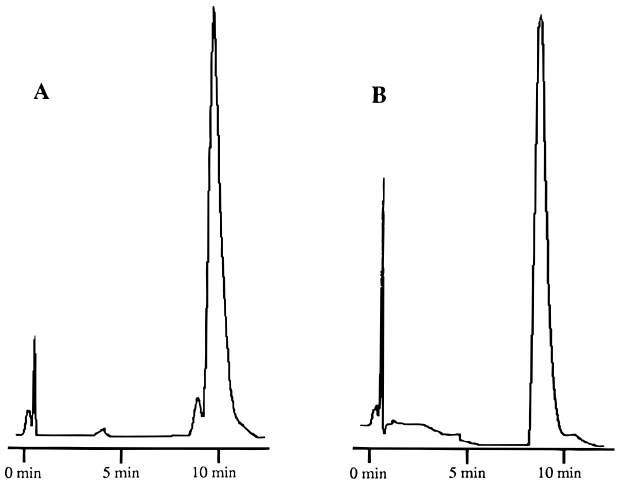

Figure 1. HPLC analysis of (*E*)-(*R*,*R*)-IQNP (A) and (*E*)-(*R*,*S*)-IQNP (B).

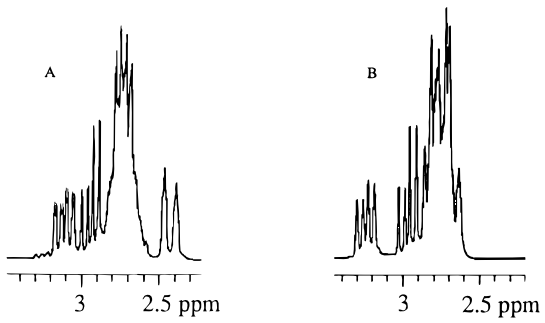

Figure 2. NMR spectra of (*E*)-(*R*,*R*)-IQNP (A) and (*E*)-(*R*,*S*)-IQNP (B).

respectively, (*E*)-(*R*,*S*)- and (*E*)-(*R*,*R*)-**1** were prepared to determine the diastereoselectivity of the electrophilic reaction of **2** with propargyl bromide. HPLC analysis of (*E*)-(*R*,*R*)- and (*E*)-(*R*,*S*)-**1** demonstrated the alkylation did indeed occur on the less hindered side of **2** with 94% and 98% enantiomeric excess, respectively (Figure 1).

A significant difference in the splitting pattern corresponding to hydrogen atoms α to the quinuclidinyl nitrogen was observed in the ^{1}H NMR analysis of (*E*)-(*R*,*R*)- and (*E*)-(*R*,*S*)-**1** synthesized from **5** by either the classical resolution of the acetate moiety or as described above (Figure 2). Therefore this apparent difference in the splitting pattern was also utilized to confirm the orientation of the acetate center as being either *R* or *S*.

In conclusion, a facile stereoselective synthesis of α-hydroxy-α-phenyl-α-(1-propyn-3-yl)acetic acid (**4**) in high enantiomeric excess has been developed and allows determination of the (*R*/*S*) conformation at this center. In addition, comparison of the specific rotation, HPLC, and NMR data of (*E*)-(*R*,*R*)- and (*E*)-(*R*,*S*)-IQNP to those prepared by a classical resolution of the acetate moiety allows the assignment of (*E*)-(*R*,*R*)-IQNP as the isomer demonstrating binding to the M_1 mAChR subtype and (*Z*)-(*R*,*R*)-IQNP as the isomer binding to both the M_1 and M_2 mAChR subtypes.

Experimental Section

General. Anhydrous THF, (*S*)-(+)-mandelic acid, (*R*)-(−)-mandelic acid, butyllithium (2.5 M in hexanes), 1,1,1,3,3,3-hexamethyldisilazane, propargyl bromide, and pivalaldehyde (trimethylacetaldehyde) were purchased from Aldrich Chemical Co. All other chemicals and solvents were analytical grade and were used without further purification. Thin layer chromatographic analyses (TLC) were performed using 250 μm layers of silica gel coated on glass (Alltech). High performance liquid chromatographic (HPLC) analysis was performed with a ISCO pump and ISCO Model A4 variable wavelength detector using a Waters Nova Pak (3.9 mm × 30 cm) column. Proton spectra are reported using tetramethylsilane as the internal standard, and carbon spectra are reported using $CHCl_3$ as the reference signal (77.0 ppm). Melting points are reported uncorrected.

***cis*-(2*S*,5*S*)-2-(*tert*-Butyl)-5-phenyl-1,3-dioxolan-4-one ((*S*,*S*)-2).** (*S*)-(+)-Mandelic acid (5.0 g, 33.2 mmol), pivalaldehyde (11.6 g, 134.7 mmol), and a catalytic amount of *p*-toluenesulfonic acid (73.6 mg) were added to anhydrous pentane (100 mL) under argon. A drop of sulfuric acid was added, and the solution was refluxed utilizing a Dean−Stark trap for 7 h. The solution was cooled, diluted with ether (200 mL), and washed twice with water (100 mL). The ether solution was dried over $MgSO_4$ and evaporated to dryness to afford a white solid. The solid was recrystallized from ether/hexane to afford *cis*-(*S*,*S*)-**2** (5.6 g, 76%). Mp 139.5−140 °C; ^{1}H NMR ($CDCl_3$) δ 7.44−7.38 (m, 5H), 5.32 (d, J = 1.4 Hz, 1H), 5.23 (d, J = 1.4 Hz, 1H), 1.08 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 171.7, 131.4, 129.1, 128.6, 127.0, 109.3, 77.0, 34.5, 23.7; $[\alpha]_D$ = +87.3° (c = 0.15 g/mL, $CHCl_3$).

***cis*-(2*R*,5*R*)-2-(*tert*-Butyl)-5-phenyl-1,3-dioxolan-4-one (*R*,*R*)-2.** (*R*,*R*)-**2** was prepared as above using (*R*)-mandelic acid (5.0 g, 32.9 mmol), pivalaldehyde (11.4 g, 134.9 mmol), and *p*-toluenesulfonic acid (79.0 mg) to afford *cis*-(*R*,*R*)-**2** as white needles (6.2 g, 85%). Mp 140 °C; ^{1}H NMR ($CDCl_3$) δ 7.43−7.38 (m, 5H), 5.31 (d, J = 1.3 Hz, 1H), 5.23 (d, J = 1.1 Hz, 1H), 1.07 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 172.0, 141.8, 129.1, 128.8, 127.0, 109.2, 77.0, 34.5, 23.7; $[\alpha]_D$ = −82.4° (c = 0.15 g/mL, $CHCl_3$).

(2*R*,5*R*)-2-(*tert*-Butyl)-5-phenyl-5-(1-propyn-3-yl)-1,3-dioxolan-4-one ((*R*,*R*)-3). A solution of 1,1,1,3,3,3-hexamethyldisilazane (0.8 g, 5.2 mmol) in anhydrous THF (25 mL) was cooled to −78 °C (dry ice/acetone) under argon. The solution was stirred and *n*-butyllithium (2.2 mL, 5.5 mmol) was slowly added. After addition was complete, the solution was stirred at −78 °C for 15 min followed by the dropwise addition of a solution of *cis*-(*R*,*R*)-**2** (1.1 g, 5.0 mmol) in anhydrous THF (40 mL). The solution was stirred at −78 °C for 30 min, and propargyl bromide (80% in toluene, 1.0 mL, 11.2 mmol) was added. The solution was then allowed to warm to rt over 3 h. The solution was poured into a cold 30% NH_4Cl solution (100 mL). The aqueous solution was washed with ether (200 mL), and the ether solution was washed with water (100 mL), dried over $MgSO_4$, and evaporated to dryness to afford an orange oil. The product was purified by Kugelrohr distillation under high vacuum (100−110 °C) to afford (*R*,*R*)-**3** as a pale yellow oil (0.9 g, 68%). ^{1}H NMR ($CDCl_3$) δ 7.71−7.66 (m, 2H), 7.41−7.26 (m, 3H), 5.68 (s, 1H), 3.03−2.75 (qd, J = 2.6 Hz, 2H), 2.10 (t, J = 2.6 Hz, 1H), 0.96 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 172.0, 137.4, 128.3, 124.6, 110.4, 81.6, 78.1, 71.7, 35.2, 31.1, 23.7; $[\alpha]_D$ = +27.2° (c = 0.088, $CHCl_3$); TLC (silica gel, hexane:ethyl acetate [8:2]) R_f = 0.72.

(2*S*,5*S*)-2-(*tert*-Butyl)-5-phenyl-5-(1-propyn-3-yl)-1,3-dioxolan-4-one ((*S*,*S*)-3). (*S*,*S*)-**3** was prepared as above using

1,1,1,3,3,3-hexamethyldisilazane (1.7 g, 10.4 mmol), anhydrous THF (50 mL), *n*-butyllithium (4.8 mL, 12.0 mmol), (*S*,*S*)-**2** (2.7 g, 22.6 mmol) in anhydrous THF (40 mL), and propargyl bromide (80% in toluene, 2.0 mL, 22.6 mmol) to afford (*S*,*S*)-**3** as a pale yellow oil (2.1 g, 82%). ^{1}H NMR ($CDCl_3$) δ 7.71−7.66 (m, 2H), 7.42−7.31 (m, 3H), 5.68 (s, 1H), 3.03−2.75 (qd, J = 2.6 Hz, 2H), 2.10 (t, J = 2.7 Hz, 1H), 0.96 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 172.0, 137.4, 128.2, 124.6, 110.4, 81.6, 78.0, 71.7, 35.1, 31.1, 23.5; $[\alpha]_D$ = −27.8° (c = 0.140 g/mL, $CHCl_3$); TLC (silica gel, hexane:ethyl acetate [8:2]) R_f = 0.77.

(*R*)-(−)-α-Hydroxy-α-phenyl-α-(1-propyn-3-yl)acetic acid ((*R*)-4). (*R*,*R*)-**3** (0.9 g, 3.4 mmol) was dissolved in methanol (25 mL), and a 1.8 M KOH solution (25 mL) was added. The solution was stirred at 60 °C for 20 min, cooled to rt, and diluted with water (100 mL). The aqueous solution was washed with ether (100 mL) and then made acidic with the slow addition of 1 N HCl (100 mL) and washed with ether (100 mL). The ether solution washed with water (100 mL), dried over $MgSO_4$, and evaporated to dryness to afford (*R*)-**4** as a pale orange oil (0.6 g, 98%). ^{1}H NMR ($CDCl_3$) δ 7.63−7.58 (m, 2H), 7.41−7.32 (m, 3H), 3.27−3.17 (dd, J = 2.6 Hz, 1H), 2.93−2.83 (dd, J = 2.6 Hz, 1H), 2.06 (t, J = 2.7 Hz, 1H); $[\alpha]_D$ = −20.6° (c = 0.063 g/mL, $CHCl_3$).

(*S*)-(+)-α-Hydroxy-α-phenyl-α-(1-propyn-3-yl)acetic acid ((*S*)-4). (*S*)-**4** (1.1 g, 100%) was prepared in the same manner as above from (*S*,*S*)-**3** (1.5 g, 5.8 mmol). ^{1}H NMR ($CDCl_3$) δ 7.61−7.56 (m, 2H), 7.38−7.28 (m, 3H), 3.22−3.13 (dd, J = 2.6 Hz, 1H), 2.92−2.82 (dd, J = 2.6 Hz, 1H), 2.01 (t, J = 2.6 Hz, 1H); $[\alpha]_D$ = +20.8° (c = 0.156 g/mL, $CHCl_3$).

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of *cis*-(2*S*,5*S*)-**2**, (2*S*,5*S*)-**3**, (+)-**5**, *cis*-(2*R*,5*R*)-**2**, (2*R*,5*R*)-**3**, (−)-**5** and ^{1}H NMR spectra of (+)-**4** and (−)-**4** (14 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of this journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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