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Synthesis of the Four Stereoisomers of 1-Azabicyclo[2.2.2]oct-3-yl-α-hydroxy-α-(4-phenylboronic acid)-α-phenylacetate (QNB-Boronic acid), including a preparative HPLC method to separate diastereoisomeric mixtures with high optical purity

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Abstract: The four stereoisomers of 1-azabicyclo[2.2.2]oct-3-yl-α-hydroxy-α-(4-phenylboronic acid)-α-phenylacetate (QNB boronic acids, 1a-1d) were synthesized initially via 1-azabicyclo[2.2.2]oct-3-yl-α-hydroxy-α-(4-iodophenyl)-α-phenylacetate (iodo-QNBs, 6a-6d) using an adaptation of a published procedure. The number of steps involved were reduced substantially using a distinctly different approach. This involved the synthesis of diastereoisomers of QNB-boronic acid, followed by separation and isolation of enantiomers by preparative reversed-phase HPLC. An improved method of synthesis of α-hydroxy-α-(4-aminophenyl)-α-phenyl-acetic acid (3a and 3b) from α-hydroxy-α-(4-nitrophenyl)-α-phenylacetic acid (2a and 2b) is also described.

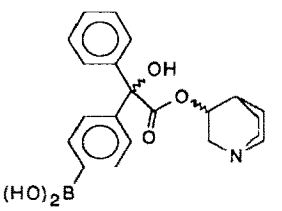
INTRODUCTION

Iodinated analogs of the muscarinic receptor (m-AChR) antagonist 3-quinuclidinyl benzilate (QNB)[1-3] have shown high affinity for muscarinic receptors *in vitro*[4,5], and radioiodinated QNB has proven to be a radiopharmaceutical candidate for the localization and quantitation of these receptors *in vivo*. For example, [I-123]Iodo-QNB has been examined for use in imaging m-AChR density in the myocardium.[4,6] The syntheses of a number of derivatives of QNB substituted with stable isotopes of the routinely used gamma-emitting radionuclides such as iodine-123 and iodine-125, bromine-75 and bromine-77 and fluorine-18 have been reported.[1-3] For reasons of cost, availability, and imaging characteristics, none of these isotopes are ideal for imaging purposes. The preferred radioisotope in diagnostic nuclear medicine is Tc-99m, and a m-AChR tracer labeled with this isotope would permit routine imaging of m-AChR density, occupancy, and kinetics.

The BATOs (Boronic acid Adducts of Technetium diQximes) are a series of technetium complexes which potentially could be adapted to be used as Tc-99m-labeled m-AChR tracers. The seven-coordinate technetium (III) BATO complexes contain one monodentate ligand and three vicinal dioximes, which are capped by one boronic acid derivative.[7-12] A variety of dioximes and boronic acids were used in the formation of BATO complexes, to evaluate their potential as heart and brain perfusion imaging agents,[7-9] and as protein labeling reagents.[11-12] Our approach to the development of a BATO for m-AChR imaging involved the attachment of QNB to the BATO 'cap' by way of a QNB derivative with a *p*-boronic acid substituent. This compound, 1-azabicyclo[2.2.2]oct-3-yl-α-hydroxy-α-(4-phenylboronic acid)-α-phenylacetate [QNB-boronic acid] (**1**) is shown below. QNB-boronic acid was prepared previously as a mixture of all four stereoisomers by Kabalka et al.[13] As it is known that the absolute configuration of QNB stereoisomers has an influence on m-AChR binding affinity,[14] we set out to synthesize the four stereoisomers of QNB-boronic acid with high stereochemical purity We now report the syntheses of the four QNB-boronic acid stereoisomers, including a route which involves the semi-preparative HPLC separation of diastereoisomeric mixtures.

RESULTS AND DISCUSSION

Partial details of the synthesis of QNB-boronic acid (**1**) have been published:[13] the precursor is (RS)-1-azabicyclo[2.2.2]oct-3-yl-(RS)-α-hydroxy-α-(4-iodophenyl)-α-phenylacetate [(RS)QN(RS)-iodo-benzilate]. As the synthesis of the resolved stereoisomers of quinuclidinyl-iodobenzilate have also been published,[13] our initial strategy was to use the published route to the individual iodo-QNB stereoisomers, then convert the quinuclidinyl-iodobenzilates to the desired QNB-boronic acid stereoisomers. The literature method[13] for the preparation of I-125 labelled QNB is shown in Scheme 1.



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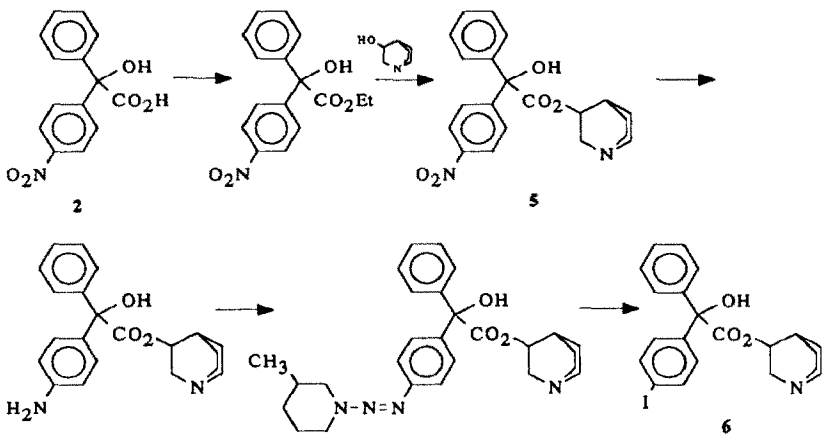
For the purpose of preparing non-radioactive iodo-QNB, a number of improvements to this method are possible. Conversion of the α-hydroxy-α-(4-nitrophenyl)-α-phenylacetic acid (**2**) to its quinuclidinyl ester (**5**) involved the preparation of the ethyl ester as an intermediate, followed by transesterification using a large excess of resolved 3-quinuclidinol. To eliminate both the intermediate step and the requirement of a large excess of the expensive resolved quinuclidinol, we opted to prepare **5** directly

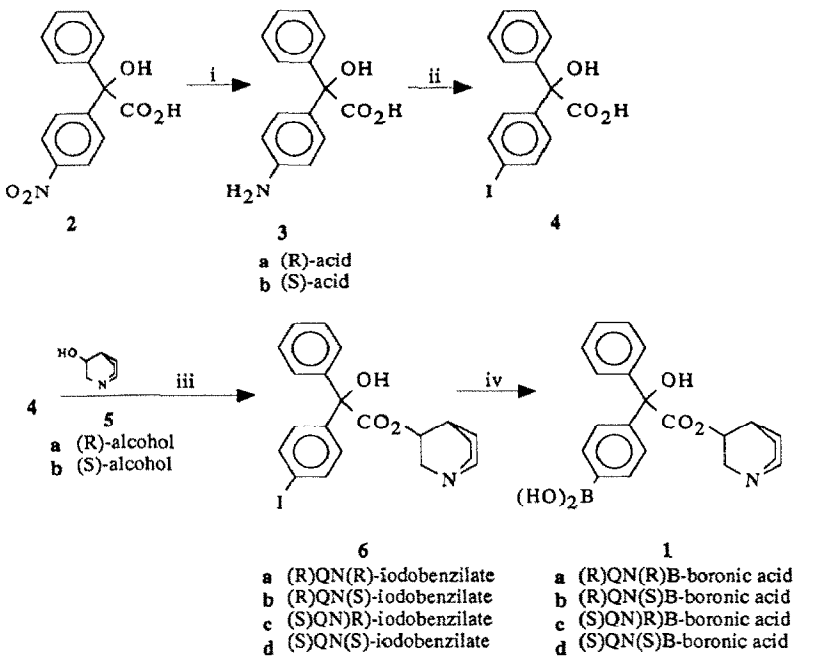
from 2 by a coupling reaction using 1,1'-carbonyldiimidazole. The subsequent reduction of the optically pure 3-quinuclidinyl-nitrobenzilate (**5**) with Pd-poly(ethylenimine) was slow (72 h) and, in our hands, did not yield a clean product. For this reason, and for economy in the use of resolved quinuclidinol, we modified the synthesis to carry out the nitro to iodo conversion early in the scheme, leaving the formation of the quinuclidinyl ester to the penultimate step. This modification, shown in scheme 2, also eliminated another step in the synthesis of iodo QNB (**6**); amine to iodo conversion was completed in one step, avoiding the preparation of a triazene intermediate.

Scheme 1



The resolved α-hydroxy-α-(4-nitrophenyl)-α-phenylacetic acids (**2a,b**) were reduced under milder conditions using Raney-Ni/hydrazine in ethanol. Reduction was found to give pure α-hydroxy-α-(4-aminophenyl)-α-phenylacetic acids (**3a** and **3b**) in very high yield (80-82%). The conventional method of synthesis of haloarenes through diazotization of α-hydroxy-α-(4-aminophenyl)-α-phenylacetic acid (**3a**) afforded (R)-α-hydroxy-α-(4-iodophenyl)-α-phenylacetic acid (**4a**). The acid **4a** was then esterified with 1.1 equivalent of the resolved (R)-quinuclidinol (**5a**) in the presence of 1,1'-carbonyldiimidazole in dry DMF to provide (R)-1-azabicyclo[2.2.2]oct-3-yl-(R)-α-hydroxy-α-(4-iodophenyl)-α-phenylacetate (**6a**) [(R)QN(S)-4-Iodobenzilate]. All the other 3-quinuclidinyl-iodobenzilate stereoisomers (**6b-6d**) were similarly obtained in good yields. (R)QN(R)-Iodobenzilate (**6a**) was then reacted with *n*-BuLi/triethyl borate to afford (R)-1-azabicyclo[2.2.2]oct-3-yl-(R)-α-hydroxy-α-(4-phenylboronic acid)-α-phenylacetate (**1a**) [(R)QN(R)B-boronic acid] in 4.4% yield.[15] Likewise, (R)-1-azabicyclo[2.2.2]oct-3-yl-(S)-α-hydroxy-α-(4-phenylboronic acid)-α-phenylacetate [(R)QN(S)B-boronic acid] (**1b**), (S)-1-

Scheme 2



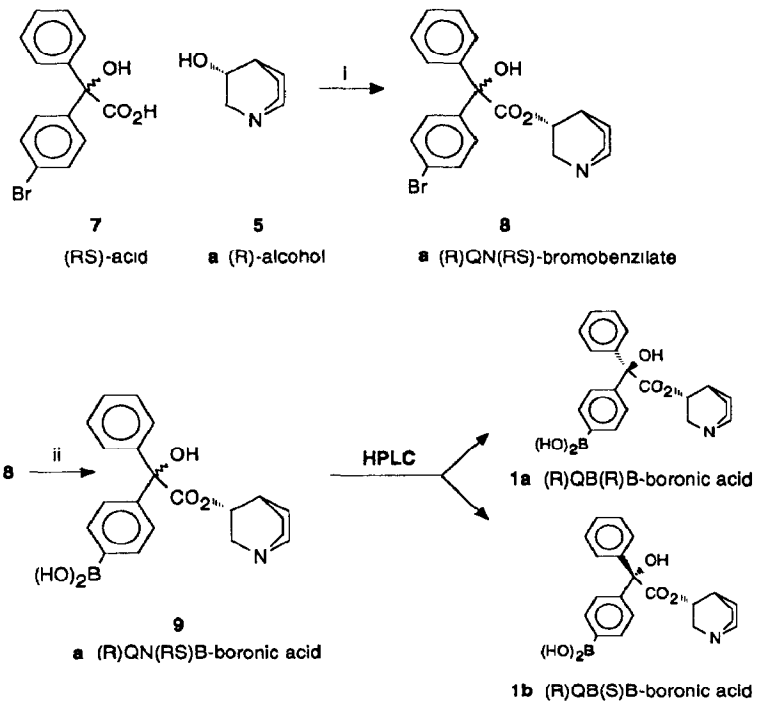
i) NH_2NH_2, Raney-Ni, ethanol; ii) $NaNO_2$, HCl; KI; iii) 1,1'-Carbonyldiimidazole, DMF, iv) *n*-BuLi, $B(OC_2H_5)_3$, THF.

azabicyclo[2.2.2]oct-3-yl-(R)-α-hydroxy-α-(4-phenylboronic acid)-α-phenylacetate [(S)QN(R)B-boronic acid] (**1c**) and (S)-1-azabicyclo[2.2.2]oct-3-yl-(S)-α-hydroxy-α-(4-phenylboronic acid)-α-phenylacetate [(S)QN(S)B-boronic acid] (**1d**) were prepared from their corresponding quinuclidinyl-iodobenzilate derivatives (**6b-6d**) in 11, 13 and 16% yields,[15] respectively. The retention times for the optically pure QNB-boronic acid on a reversed-phase HPLC system (Dynamax C_{18}, 0.46 x 25 cm, 8 micron; solvent: 82%H_2O (0.1%TFA):18%CH_3CN(0.1%TFA) are ~13.40 min for **1a** and **1d**, and ~15.70 min for **1b** and **1c**.

Although the synthetic scheme described for the preparation of all four stereoisomeric QNB-boronic acids appears to be efficient, it still required four separate four-step syntheses. We thought that this could be reduced to two separate syntheses yielding all four stereoisomers. The potential for such an approach was indicated by the analytical HPLC system described above. As the diastereoisomers of QNB-boronic acid displayed a difference in retention times of ~2.3 min, it may be possible to synthesize two pairs of

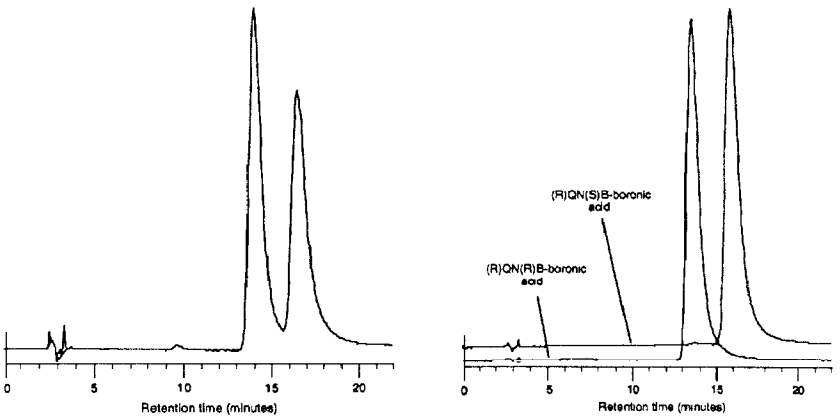
diastereoisomeric QNB boronic acids, and isolate all four enantiomers by preparative HPLC separation. This approach lead to the development of the route shown in Scheme 3. An additional improvement in the efficiency of the synthesis was achieved by the use of (RS)-α-hydroxy-α-(4-bromophenyl)-α-phenylacetic acid (7) as starting material, eliminating the initial nitro to amino to iodo conversion. Accordingly, (R)QN(RS)B- (**9a**) and (S)QN(RS)B-boronic acid (**9b**) were prepared from (RS)-α-hydroxy-α-(4-bromophenyl)-α-phenylacetic acid (**7**). Yields in the bromo to boronic acid conversions (~20%) were lower than corresponding iodo to boronic acid (~80%) conversions. The diastereoisomeric mixtures were separated by preparative reversed-phase HPLC. (R)QN(RS)B-boronic acid (**9a**) gave two peaks with a retention time difference similar to that found with the analytical system. From the retention times of authentic (R)QN(R)B- (**1a**) and (R)QN(S)B-boronic acid (**1b**), the peak with retention time ~13.40 min corresponds to the isomer **1a** and the peak with retention time ~15.70 min corresponds to the isomer **1b**.

Scheme 3



i) 1,1'-Carbonyldiimidazole, DMF; ii) *n*-BuLi, $B(OC_2H_5)_3$, THF.

Diastereoisomeric (S)QN(RS)B-boronic acid (**9b**) was similarly prepared from (RS)-α-hydroxy-α-(4-bromophenyl)-α-phenylacetic acid (**7**) and (S)-3-quinuclidinol (**5b**). HPLC analysis of the (S)QN(RS)B-boronic acid (**9b**) also gave two peaks at ~13.40 min and ~15.70 min. The analytical HPLC results suggested that it would be possible to separate diastereoisomeric QNB-boronic acids by preparative HPLC. In practice, it was found that on preparative HPLC there was considerable tailing of the (R)QN(R)B-boronic acid (**1a**) into the (R)QN(S)B-boronic acid (**1b**). So, while the first eluting isomer was obtained with high optical purity in a single HPLC run, multiple HPLC separations were required to obtain (R)QN(S)B-boronic acid (**1b**) which is free from (R)QN(R)B-boronic acid (**1a**). In fig. 1 are shown the analytical HPLC profiles of the diastereoisomeric mixture (R)QN(RS)B-boronic acid (**9a**) and the purified isomers (R)QN(R)B-boronic acid (**1a**) and (R)QN(S)B-boronic acid (**1b**).



i. (R)QN(RS)B-boronic acid (**9a**)

ii. Isolated (R)QN(R)B- and (R)QN(S)B-boronic acids (**1a** and **1b**)

Fig. 1: HPLC profiles of (i) the diastereoisomeric mixture (R)QN(RS)B-boronic acid (**9a**) and (ii) the pure isomers, (R)QN(R)B-boronic acid (**1a**) and (R)QN(S)B-boronic acid (**1b**).

CONCLUSIONS

All four stereoisomers of QNB-boronic acid (**1a-1d**) were prepared with high stereochemical purity. The initial route involved a modification of the literature[13] procedure for the preparation of iodo-QNB (**6**), a precusor to the desired boronic acid (**1**). However, this scheme required one separate four-step synthesis for each stereoisomer. A change in strategy greatly improved efficiency. Starting with 4-bromobenzophenone, and resolved 3-quinuclidinol, diastereoisomeric QNB-boronic acids were prepared

in three steps, and the individual stereoisomers resolved by semi-preparative HPLC. Thus, all four stereoisomers could be prepared using two three-step syntheses, with the first step shared by both syntheses.

EXPERIMENTAL

Melting points were taken on Thomas Hoover Capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a JEOL GSX/GX MULTIPLEXES (270 MHz) NMR Spectrometer and are reported in δ values relative to trimethylsilane. High resolution mass spectra were taken on a VG Analytical-ZAB-2F and/or a Finnigan TSQ Spectrometer. The optical rotations were measured on a Perkin Elmer 241 Polarimeter. Thin layer chromatography (TLC) was carried out on precoated Kieselgel 60 F_{254} on 0.25 mm glass plates and visualized by uv irradiation from a Mineralight shortwave UV lamp or in iodine chamber. All solvents were reagent grade and used directly as purchased except for THF which was distilled over sodium with benzophenone ketyl as indicator.

(RS)-α-Hydroxy-α-(4-nitrophenyl)-α-phenylacetic acid was prepared from 4-nitrobenzophenone and resolved into optically pure isomers, (R)-α-hydroxy-α-(4-nitrophenyl)-α-phenylacetic acid (**2a**) and (S)-α-hydroxy-α-(4-nitrophenyl)-α-phenylacetic acid (**2b**) by a reported method.[13] Similarly (RS)-α-hydroxy-α-(4-bromophenyl)-α-phenylacetic acid (**7**) was prepared[14b] from 4-bromobenzophenone in 19% yield. The (R)- (**5a**) and (S)- (**5b**) enantiomers of 3-quinuclidinol were isolated by methods reported by Grob et al[16] and Ringldhal et al.[17] Diastereoisomeric QNB-boronic acids were separated by semi-preparative HPLC on a reversed phase C_{18} column (4.14 x 25 cm, 8μ). Purified QNB-boronic acids were >98% pure as determined by HPLC monitored at two different wavelengths (230 and 254 nm) and all QNB boronic acids had appropriate molecular weights as assessed by high resolution FAB mass spectrometry. HPLC columns were obtained from Rainin Inc. HPLC grade acetonitrile and water were obtained from JT Baker Inc. and were filtered and degassed prior to use.

(R)-α-Hydroxy-α-(4-aminophenyl)-α-phenylacetic acid (3a). A solution of (R)-α-hydroxy-α-(4-nitrophenyl)-α-phenylacetic acid (**2a**) (2.0 g, 7.33 mmol) in ethanol (25 mL) was treated with hydrazine (1.0 mL) and Raney-Ni (0.8 g) and stirred at room temperature for 18 h under nitrogen atmosphere. Raney-Ni was filtered, washed with ethanol, the ethanol solution concentrated to a small volume and diluted with water (75 mL). The aqueous solution was extracted with ether (2 x 50 mL) and the ether layer was discarded. The aqueous solution was evaporated under vacuum to afford (R)-α-hydroxy-α-(4-aminophenyl)-α-phenylacetic acid (**3a**) as a light yellow solid. Yield: 1.46 g (82%); mp 139-140°C (dec); TLC[silica gel, acetone] R_f 0.20; ^{1}H NMR (D_2O) δ 6.75 and 7.18(2d, 4H, C_6H_4) and 7.31(s, 5H, C_6H_5).

(S)-α-Hydroxy-α-(4-aminophenyl)-α-phenylacetic acid (3b). Raney-Ni (0.8 g) was added to a solution of (S)-α-hydroxy-α-(4-nitrophenyl)-α-phenylacetic acid (**2b**) (2.0 g, 7.33 mmol) and hydrazine (1.0 mL) in ethanol (25 mL) and stirred at room temperature for 18 h under nitrogen atmosphere. Raney-Ni was removed by filtration, washed with ethanol, the ethanol layer concentrated to a small volume and diluted with water (75 mL). The aqueous solution was extracted with ether (2 x 50 mL) and the aqueous solution was evaporated under vacuum to provide a light yellow solid of (S)-α-hydroxy-α-(4-aminophenyl)-α-phenylacetic acid (**3b**) in 80% yield (1.40 g); mp 139-141°C (dec); TLC[silica gel, acetone] R_f 0.21; ^{1}H NMR (D_2O) δ 6.75 and 7.18(2d, 4H, C_6H_4) and 7.31(s, 5H, C_6H_5).

(R)-(+)-α-Hydroxy-α-(4-iodophenyl)-α-phenylacetic acid (4a). Sodium nitrite (1.7 g, 24.64 mmol) in water (5 mL) was slowly added to a stirred solution of (R)-α-hydroxy-α-(4-aminophenyl)-α-phenylacetic acid (**3a**) (3.0 g, 12.35 mmol) in 10%HCl (30 mL) at -5°C over a peroid of 30 min. Stirring was continued for an additional 30 min at 0°C and a solution of KI (4.09 g, 24.64 mmol) in water (5 mL) was added slowly. The reaction mixture was then stirred at 0°C for 1 h and at ambient temperature for 1 h.

The brown paste formed was extracted with ethyl acetate (3 x 75 mL). The ethyl acetate layer was then washed with sodium thiosulfate (10%, 2 x 50 mL), water (2 x 50 mL) and dried (Na_2SO_4). The organic layer was evaporated on a rotary evaporator to afford a brown paste which was adsorbed on silica gel (5 g) in 20 ml of ethyl acetate, and dried. The dry silica gel impregnated with the compound was loaded onto a silica gel column (40 g) and eluted with MeOH-dichloromethane (5:95). The fractions with the compound were pooled and evaporated under vacuum to afford (R)-(+)-α-hydroxy-α-(4-iodophenyl)-α-phenylacetic acid (**4a**) (2.05 g) in 46% yield as a light yellow solid; mp 80-82°C; TLC[silica gel, toluene-acetic acid 9:1] R_f 0.40; $[\alpha]^{25}_D$ +24.70(*c* 0.132, acetone). ^1H NMR ($CDCl_3$) δ 5.50(bs, 1H, O*H*), 7.21 and 7.69(2d, 4H, C_6H_4) and 7.41(s, 5H, C_6H_5).

(S)-(-)-α-Hydroxy-α-(4-iodophenyl)-α-phenylacetic acid (4b). The title compound **4b** was prepared from a solution of (S)-α-hydroxy-α-(4-aminophenyl)-α-phenylacetic acid (**3b**) (3.0 g, 12.35 mmol), sodiun nitrite (1.7 g, 24.64 mmol) in water (10 mL) and potassium iodide (4.09 g, 24.64 mmol) in water (10 mL) as outlined for the synthesis of (S)-(-)-α-hydroxy-α-(4-iodophenyl)-α-phenylacetic acid (**4a**) in 50% yield (2.18 g) as a light yellow solid; mp 80-81°C; TLC[silica gel, toluene-acetic acid 9:1] R_f 0.40; $[\alpha]^{25}_D$ -24.98 (*c* 0 134, acetone). ^1H NMR ($CDCl_3$) δ 5.50(bs, 1H, O*H*), 7.21 and 7.69(2d, 4H, C_6H_4) and 7.41(s, 5H, C_6H_5).

(R)-1-Azabicyclo[2.2.2]-oct-3-yl-(R)-α-hydroxy-α-(4-iodophenyl)-α-phenylacetate [(R)QN(R)-iodobenzilate] (6a). A solution of (R)-(+)-α-hydroxy-α-(4-iodophenyl)-α-phenylacetic acid (**4a**) (0.26 g, 0.74 mmol) in dry DMF (2 mL) was treated with 1,1'-carbonyldiimidazole (0.12 g, 0.74 mmol) in small portions. The reaction mixture was stirred at room temperature for 1 h under nitrogen atmosphere. To this light yellow colored solution, was added (R)-3-quinuclidinol (**5a**) (0.093 g, 0.73 mmol) and stirring was continued for an additional 15 h at room temperature. The reaction mixture was concentrated under vacuum and added to water (50 mL) and extracted with ether (3 x 30 mL). The combined ether extracts were washed with saturated sodium bicarbonate (2 x 20 mL), water (2 x 20 mL) and dried over anhydrous sodium sulfate. The residue obtained after the evaporation of the solvent was added to silica gel (0.5 g), ether (5 mL) and dried. The dry silica gel powder with compound was loaded onto a silica gel column (10 g) and eluted with 5% methanol in dichloromethane. The fractions with the compound were collected and evaporated on a rotary evaporator to afford **6a** as a cream colored solid in 73% yield (0.25 g); mp 120-122°C; TLC [silica gel, MeOH-NH_4OH 98:2] R_f 0.64; ^1H NMR ($CDCl_3$) δ 1.15-1.89(m, 4H), 2.01(s, 1H), 2.42-2.81(m, 5H), 3.21(m, 1H), 4.52(bs, 1H, O*H*), 5.01(m, 1H), and 7.15-7.82(m, 9H, Ar-*H*).

(R)-1-Azabicyclo[2.2.2]-oct-3-yl-(S)-α-hydroxy-α-(4-iodophenyl)-α-phenylacetate [(R)QN(S)-iodobenzilate] (6b). Compound **6b** was synthesized from (S)-(-)-α-hydroxy-α-(4-iodophenyl)-α-phenylacetic acid (**4b**) (0.26 g, 0.74 mmol), 1,1'-carbonyldiimidazole (0.12 g, 0.74 mmol and (R)-3-quinuclidinol (**5a**) (0.093 g, 0.73 mmol) as described for the synthesis of (R)-1-azabicyclo[2.2.2]-oct-3-yl-(R)-α-hydroxy-α-(4-iodophenyl)-α-phenylacetate (**6a**) as a light yellow solid in 75% yield (0.256 g); mp 123-125°C; TLC [silica gel, MeOH-NH_4OH 98:2] R_f 0.65; ^1H NMR ($CDCl_3$) δ 1.15-1.89(m, 4H), 2.01(s, 1H), 2.42-2.81(m, 5H), 3.21(m, 1H), 4.52(bs, 1H, O*H*), 5.01(m, 1H), 5.01(m, 1H) and 7.15-7.82(m, 9H, Ar-*H*).

(S)-1-Azabicyclo[2.2.2]-oct-3-yl-(R)-α-hydroxy-α-(4-iodophenyl)-α-phenylacetate [(S)QN(R)-iodobenzilate] (6c). The title compound **6c** was synthesized from a solution of (R)-(+)-α-hydroxy-α-(4-iodophenyl)-α-phenylacetic acid (**4a**) (0.26 g, 0.74 mmol), 1,1'-carbonyldiimidazole (0.12 g, 0.74 mmol), (S)-3-quinuclidinol (**5b**) (0.093 g, 0.73 mmol) in dry DMF as described for the synthesis of (R)-1-azabicyclo[2.2.2]-oct-3-yl-(R)-α-hydroxy-α-(4-iodophenyl)-α-phenylacetate (**6a**). The product was obtained as a light yellow solid in 80% yield (0.29 g); mp 124-127°C; TLC [silica gel, MeOH-NH_4OH 98:2] R_f 0.65; ^1H NMR ($CDCl_3$) δ 1.15-1.89(m, 4H), 2.01(s, 1H), 2.42-2.81(m, 5H), 3.21(m, 1H), 4.52(bs, 1H, O*H*), 5.01(m, 1H) and 7.15-7.82(m, 9H, Ar-*H*).

(S)-1-Azabicyclo[2.2.2]-oct-3-yl-(S)-α-hydroxy-α-(4-iodophenyl)-α-phenylacetate [(S)QN(S)-iodobenzilate] (6d). The title compound **6d** was prepared as a colorless paste in 70% yield (0.23 g) from (S)-(-)-α-hydroxy-α-(4-iodophenyl)-α-phenylacetic acid (**4b**) (0.26 g, 0.73 mmol), 1,1'-carbonyldiimidazole (0.12 g, 0.74 mmol), and (S)-3-quinuclidinol (**5b**) (0.093 g, 0.73 mmol) in dry DMF (2 mL) as described for the synthesis of (R)-1-azabicyclo[2.2.2]-oct-3-yl-(R)-α-hydroxy-α-(4-iodophenyl)-α-phenylacetate (**6a**); mp 124-126°C; TLC [silica gel, MeOH-NH_4OH 98:2]R_f 0.69; ^1H NMR ($CDCl_3$) δ 1.15-1.89(m, 4H), 2.01(s, 1H), 2.42- 2.81(m, 5H), 3.21(m, 1H), 4.52(bs, 1H, *OH*), 5.01(m, 1H) and 7.15-7.82(m, 9H, Ar-*H*).

(R)-1-Azabicyclo[2.2.2]-oct-3-yl-(R)-α-hydroxy-α-(4-phenylboronic acid)-α-phenylacetate (R)QN(R)B-boronic acid (1a). *n*-BuLi (0.2 g, 2.5M, 1.25 mL, 3.13 mmol) was added slowly *via* syringe to a solution of (R)-1-azabicyclo[2.2.2]-oct-3-yl-(R)-α-hydroxy-α-(4-iodophenyl)-α-phenylacetate (**6a**) (0.60 g, 1.3 mmol) in freshly distilled THF (2.0 mL) in dry 10 mL RB flask at -78°C. The mixture was stirred at the this temperature for 20 min, then freshly distilled triethyl borate (0.378 g, 0.441 mL, 2.59 mmol) was added, and the reaction mixture was stirred for an additional 1 h at -78°C. The reaction mixture was allowed to warm to room temperature and stirred for 18 h. The reaction mixture was then treated with few drops of water, decanted to remove the solution phase from the semi-solid which was then repeatedly washed with ether and finally triturated with ether to give a colorless solid. The solid was then dissolved in water containing <5% acetonitrile, filtered and loaded onto a reversed phase C_{18} HPLC column (Dynamax C_{18}, 4.14 x 25 cm, 8 micron) and eluted under isocratic condition with 12.5% acetonitrile containing 0.1%TFA in 0.1%TFA/H_2O. The fractions were analysed by an HPLC system (Dynamax C_{18}, 0.46 x 25 cm, 8 micron, 18% acetonitrile with 0.1%TFA in 0.1%TFA/water) and the fractions with the title compound **1a** in >99% purity were pooled, and freeze-dried to afford (R)QN(R)B-boronic acid (**1a**) as TFA salt; yield: 0.025 g (4.4%); ^1H NMR (CD_3OD) δ 1.68(m, 2H), 1.95(m, 1H), 2.35(s, 1H), 2.81-3.35(m, 5H), 3.80(m, 1H), 5.31(m, 1H) and 7.28-7.71(m, 9H, Ar-*H*). MS: Exact Mass (using gylcerol as the matrix) calcd. for $C_{24}H_{29}O_6NB$: 438.2088; found, 438.2092.

(R)-1-Azabicyclo[2.2.2]-oct-3-yl-(S)-α-hydroxy-α-(4-phenylboronic acid)-α-phenylacetate (R)QN(S)B-boronic acid (1b). (R)QN(S)B-boronic acid (**1b**) was obtained as a colorless solid in 11% yield (58 mg) from (R)-1-azabicyclo[2.2.2]-oct-3-yl-(S)-α-hydroxy-α-(4-iodophenyl)-α-phenylacetate (**6b**) (0.3 g, 0.65 mmol), *n*-BuLi (0.1 g, 2.5M, 0.63 mL, 1.56 mmol), and triethyl borate (0.19 g, 0.22 mL, 1.3 mmol) in dry THF at -78°C as described for the synthesis of (R)QN(R)B-boronic acid (**1a**); mp 208-211°C; ^1H NMR (CD_3OD) δ 1.68(m, 2H), 1.95(m, 1H), 2.35(s, 1H), 2.81- 3.35(m, 5H), 3.80(m, 1H), 5.31(m, 1H) and 7.28-7.71(m, 9H, Ar-*H*). MS: Exact Mass (using gylcerol as the matrix) calcd. for $C_{24}H_{29}O_6NB$: 438.2088; found, 438.2098.

(S)-1-Azabicyclo[2.2.2]-oct-3-yl-(R)-α-hydroxy-α-(4-phenylboronic acid)-α-phenylacetate (S)QN(R)B-boronic acid (1c). The QNB-boronic acid **1c** was obtained as a colorless solid (TFA salt form) in 13% yield (64 mg) from (S)-1-azabicyclo[2.2.2]-oct-3-yl-(R)-α-hydroxy-α-(4-iodophenyl)-α-phenylacetate (**6c**) (0.6 g, 1.3 mmol), *n*-BuLi (0.2 g, 2.5M, 1.25 mL, 3.13 mmol), and triethyl borate (0.378 g, 0.441 mL, 2.59 mmol) in dry THF at -78°C as described for the synthesis of (R)QN(R)B-boronic acid (**1a**); mp 208-211°C; ^1H NMR (CD_3OD) δ 1.68(m, 2H), 1.95(m, 1H), 2.35(s, 1H), 2.81- 3.35(m, 5H), 3.80(m, 1H), 5.31(m, 1H) and 7.28-7.71(m, 9H, Ar-*H*). MS: Exact Mass (using glycerol as the matrix) cald. for $C_{24}H_{29}O_6NB$: 438.2088; found, 438.1190.

(S)-1-Azabicyclo[2.2.2]-oct-3-yl-(S)-α-hydroxy-α-(4-phenylboronic acid)-α-phenylacetate (S)QN(S)B-boronic acid (1d). *n*-BuLi (0.1 g, 2.5M, 0.63 mL, 1.56 mmol) was added slowly *via* syringe to a solution of (S)-1-azabicyclo[2.2.2]-oct-3-yl-(S)-α-hydroxy-α-(4-iodophenyl)-α-phenylacetate (**6d**) (0.30 g, 0.65 mmol) in freshly distilled THF (2.0 mL) at -78°C. The reaction mixture was stirred at -78°C for 20 min, then freshly distilled triethyl borate (0.19 g, 0.22 mL, 1.3 mmol) was added, and the reaction mixture was stirred for an additional 1 h at -78°C. The reaction mixture was allowed to warm to room temperature and stirred for 18 h. Few drops of water was added to the reaction mixture and the solution was decanted. The

semi-solid formed was then repeatedly triturated with ether to afford a colorless solid of **1d** which was purified by the method described for **1a**; 0.068 g (16%); mp 208-211°C; ^{1}H NMR (CD_3OD) δ 1.68(m, 2H), 1.95(m, 1H), 2.35(s, 1H), 2.81- 3.35(m, 5H), 3.80(m, 1H), 5.31(m, 1H) and 7.28-7.71(m, 9H, Ar-*H*).

(R)-1-Azabicyclo[2.2.2]-oct-3-yl-(RS)-α-hydroxy-α-(4-bromophenyl)-α-phenylacetate (R)QN(RS)-bromobenzi-late (8a). 1,1'-Carbonyldiimidazole (0.810 g, 5.00 mmol) was added to a solution of (RS)-α-hydroxy-α-(4-bromophenyl)-α-phenylacetic acid (**7**) (1.53 g, 4.98 mmol) in dry DMF (5 mL) under nitrogen and the mixture was stirred for 1 h at 40°C. (R)-3-Quinuclidinol (**5a**) (0.8 g, 6.30 nmol) was added and the mixture was stirred at 40°C for 24 h. DMF was removed under vacuum and the residue was poured into water. The precipitated solid of **8a** was filtered and air dried. Yield: 1.20 g (58%). ^{1}H NMR (DMSO-d_6) δ 1.3-3.3(m, quinuclidinyl protons), 4.9(m, 1H, C*H*O), 7.3 7.7(m, H, Ar*H*). MS m/e 416 $(M+H)^+$.

(S)-1-Azabicyclo[2.2.2]-oct-3-yl-(RS)-α-hydroxy-α-(4-bromophenyl)-α-phenylacetate (S)QN(RS)-bromobenzilate (8b). 1,1'-Carbonyldiimidazole (0.810 g, 5.00 mmol) was added to solution of (RS)-α-hydroxy-α-(4-bromo-phenyl)-α-phenylacetic acid (**7**) (1.53 g, 4.98 mmol) in dry DMF (5 mL) under nitrogen atmosphere and the mixture was stirred for 1 h at 40°C. (S)-3-Quinuclidinol (**5b**) (0.8 g, .0063 mol) was added and the mixture was stirred at 40°C for 24 h. Isolation and purification as described for (R)-1-azabicyclo[2.2.2]-oct-3-yl-(RS)-α-hydroxy-α-(4-bromophenyl)-α-phenyl-acetate (**8a**) afforded 1.12 g (55%) of (S)-1-azabicyclo[2.2.2]-oct-3-yl-(RS)-α-hydroxy-α-(4-bromophenyl)-α-phenyl acetate (**8b**); mp 151-153°C; ^{1}H NMR (DMSO-d_6) δ 1.3-3.3(m, quinuclidinyl protons), 4.9(m, 1H, C*H*O), 7.3-7.7(m, 9H, Ar*H*). MS m/e 416 $(M+H)^+$.

(R)-1-Azabicyclo[2.2.2]-oct-3-yl-(RS)-α-hydroxy-α-(4-phenylboronic acid)-α-phenylacetate (R)QN(RS)B-boronic acid (9a). To a cooled (-78°C) solution of (R)-1-azabicyclo[2.2.2]-oct-3-yl-(RS)-α-hydroxy-α-(4-bromo-pheny)-α-phenylacetate (**8a**) (316 mg, 0.76 mmol) in dry THF (30mL) was added *n*-BuLi (2.5M solution in hexane, 0.72 mL, 0.12 g, 1.80 mmol) *via* a syringe. The mixture was stirred for 1 h at -78°C and triethylborate (**1** mL) was added at -78°C. The mixture was stirred for an additional 1 h at -78°C and 4 h at room temperature. THF was removed and the residue was treated with water and extracted with ethyl acetate. The EtOAc solution was dried (Na_2SO_4), and the solvent was evaporated to yield a semi-solid which was triturated with ether to give (R)QN(RS)B-boronic acid (**9a**) as a cream colored soild. Yield: 50 mg (17%); mp 242-244°C; ^{1}H NMR (DMSO-d_6) δ 1.30-3.3(m, quinuclidinyl protons), 4.9(m, 1H, C*H*O), 7.3-7.7(m, 9H, Ar*H*). MS: $(M+GLY-2H_2O+H)^+$ = 438.

(S)-1-Azabicyclo[2.2.2]-oct-3-yl-(RS)-α-hydroxy-α-(4-phenylboronic acid)-α-phenylacetate (S)QN(RS)B-boronic acid (9b). To a cooled (-78°C) solution of (S)-1-azabicyclo[2.2.2]-oct-3-yl-(RS)-α-hydroxy-α-(4-bromopheny)-α-phenylacetate (**8b**) (417 mg, 1.00 mmol) in dry THF (30 mL) was added *n*-BuLi (2.5M solution in hexane, 1.0 mL, 0.165 g , 2.50 mmol) *via* a syringe. The mixture was stirred for 1 h at -78°C and triethylborate (2 mL) was added at -78°C. The mixture was stirred for an additional 1 h at -78°C and 4 h at room temperature. Isolation and purification as described above for the synthesis of (R)QN(RS)B-boronic acid (**9a**) afforded 100 (26%) mg of (S)QN(RS)B-boronic acid (**9b**); mp. 241-243°C. ^{1}H NMR (DMSO-d_6) δ 1.30-3.30(m, quinuclidinyl protons), 4.9(m, 1H, C*H*O), 7.3-7.7(m, 9H, Ar*H*); MS: $(M+GLY-2H_2O+H)^+$ = 438.

Separation of (R)-1-azabicyclo[2.2.2]-oct-3-yl-(R)-α-hydroxy-α-(4-phenylboronic acid)-α-phenylacetate (1a) and (R)-1-azabicyclo[2.2.2]-oct-3-yl-(S)-α-hydroxy-α-(4-phenylboronic acid)-α-phenylacetate (1b) from (R)-1-azabicyclo[2.2.2]-oct-3-yl-(RS)-α-hydroxy-α-(4-phenylboronic acid)-α-phenylacetate (9a) (diastereoisomeric mixture) by semi-preparative HPLC. The solid (R)-1-azabicyclo[2.2.2]-oct-3-yl-(RS)-α-hydroxy-α-(4-phenylboronic acid)-α-pheny-lacetate (**9a**) (0.25 g, mmol) [prepared from (RS)-α-hydroxy-α-(4-bromophenyl)-α-phenylacetic acid (**7**)] was dissolved in water containing <5% acetonitrile, filtered and loaded onto a reversed phase C_{18} HPLC column (Dynamax C_{18}, 4.14 x 25 cm, 8 micron).

Fractions were collected during isocratic elution of the column (flow rate 10 mL/min) with 12.5% acetonitrile (containing 0.1% TFA) in 0.1% TFA/H_2O. The fractions were analysed by HPLC (Dynamax C_{18}, 0.46 x 25 cm, 8 micron, 18% CH_3CN with 0.1% TFA in 0.1% TFA/H_2O, 1.0 mL/min, 230 nm) and the fractions eluting at ~ 12.50 min in >99% purity were pooled, and freeze-dried. (R)QN(R)B-boronic acid (**1a**) was obtained as TFA salt; yield: 0.030 g (12%); mp 210-214°C (dec); $[\alpha]^{25}_D$ -17.54 (*c* 0.104, H_2O); ^1H NMR (D_2O) δ 1.33(m, 1H), 1.56(m 1H), 2.33(m, 1H), 2.75(m, 1H), 3.12(m, 4H), 3.63(m, 1H), 5.25(m, 1H), 7.35(m, 7H, Ar-*H*) and 7.70(d, 2H, Ar-*H*); FAB-MS (using glycerol as the matrix) produced the boronic ester: calcd. for $C_{24}H_{29}BNO_6$ 438.2088 $(M+H)^+$; found 438.2092.

The fractions with >80% of the (R)-1-azabicyclo[2.2.2]-oct-3-yl-(S)-α-hydroxy-α-(4-phenylboronic acid)-α-phenylacetate (**1b**) isomer were combined, concentrated under vacuum, and loaded onto the reversed phase C_{18} column. The column was eluted with a gradient solvent profile (100% water containing 0.1% TFA at t=0, with an increase of 0.2%/min of acetonitrile containing 0.1% TFA; flow rate: 10 mL/min; UV detection at 230 nm). The fractions were analysed by HPLC and the fractions with retention time at ~ 14.85 min in >99% purity were pooled and freeze-dried to give (R)QN(S)B-boronic acid (**1b**). TFA salt was obtained in 8% yield (0.020 g); mp 213-215°C (dec); $[\alpha]^{25}_D$ -31.76 (*c* 0.085, H_2O); ^1H NMR (D_2O) δ 1.39(m, 1H), 1.55(m 1H), 2.30(m, 1H), 2.77(m, 1H), 3.15(m, 4H), 3.62(m, 1H), 5.26(m, 1H), 7.34(m, 7H, Ar-*H*) and 7.72(d, 2H, Ar-*H*); FAB-MS (using glycerol as the matrix) produced the boronic ester: calcd. for $C_{24}H_{29}BNO_6$ 438.2088 $(M+H)^+$; found 438.2091.

Separation of (S)-1-azabicyclo[2.2.2]-oct-3-yl-(S)-α-hydroxy-α-(4-phenylboronic acid)-α-phenylacetate(1d) and (S)-1-azabicyclo[2.2.2]-oct-3-yl-(R)-α-hydroxy-α-(4-phenylboronic acid)-α-phenylacetate (1c) from (S)-1-azabicyclo[2.2.2]-oct-3-yl-(RS)-α-hydroxy-α-(4-phenylboronic acid)-α-phenylacetate (9b) (diastereoisomeric mixture by semi-preparative HPLC. The (S)-1-azabicyclo[2.2.2]-oct-3-yl-(S)-α-hydroxy-α-(4-phenylboronic acid)-α-phenylacetate(**1d**) and (S)-1-azabicyclo[2.2.2]-oct-3-yl-(R)-α-hydroxy-α-(4-phenylboronic acid)-α-phenylacetate (**1c**) were separated and isolated as a colorless solid in TFA salt form in 14% and 10%, respectively, from (S)-1-azabicyclo[2.2.2]-oct-3-yl-(RS)-α-hydroxy-α-(4-phenylboronic acid)-α-phenylacetate (**9b**) mixture [prepared from (RS)-4-bromobenzilic acid] using semi-preparative C_{18} HPLC method as described for the separation and isolation of (R)QN(R)B- (**1a**) and (R)QN(S)B-boronic acid (**1b**) from the (R)QN(RS)B-boronic acid (**9a**) mixture. Initial elution under isocratic condition as indicated above gave the (S)QN(S)B-boronic acid (**1d**) and the fractions with >99% of (S)QN(S)B-boronic acid (**1d**) isomer were pooled and lyophilized to provide a white solid; mp 212-215°C (dec); $[\alpha]^{25}_D$ +17.64 (*c* 0.102, H_2O); ^1H NMR (D_2O) δ 1.38(m, 1H), 1.55(m 1H), 2.33(m, 1H), 2.74(m, 1H), 3.15(m, 4H), 3.60(m, 1H), 5.25(m, 1H), 7.32(m, 7H, Ar-*H*) and 7.71(d, 2H, Ar-*H*); FAB-MS (using glycerol as the matrix) produced the boronic ester: calcd. for $C_{24}H_{29}BNO_6$ 438.2088 $(M+H)^+$; found 438.2073.

Repeat chromatography of the fractions rich in (S)-1-azabicyclo[2.2.2]-oct-3-yl-(R)-α-hydroxy-α-(4-phenylboronic acid)-α-phenylacetate (**1c**, >80%) on the HPLC column under linear gradient condition as described above and lyophilization of the fractions with >99% of (S)QN(R)B-boronic acid (**1c**) isomer afforded a white solid; mp 214-216°C (dec); $[\alpha]^{25}_D$ +32.00 (*c* 0.082, H_2O); ^1H NMR (D_2O) δ 1.39(m, 1H), 1.56(m 1H), 2.32(m, 1H), 2.77(m, 1H), 3.15(m, 4H), 3.61(m, 1H), 5.25(m, 1H), 7.34(m, 7H, Ar-*H*) and 7.72(d, 2H, Ar-*H*); FAB-MS (using glycerol as the matrix) produced the boronic ester: calcd. for $C_{24}H_{29}BNO_6$ 438.2088 $(M+H)^+$; found 438.2094.

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