An Improved Synthesis of a Protected (2*S*,3*R*)-3-Hydroxyaspartic Acid Suitable for Solid-Phase Peptide Synthesis

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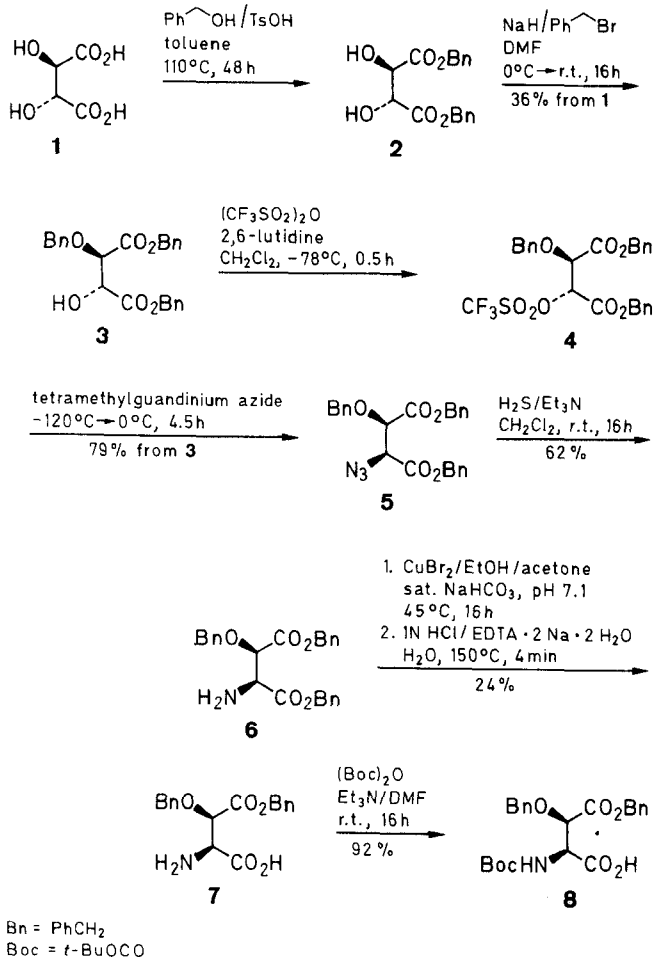
A tartrate based strategy was utilized for the synthesis of O^4-benzyl hydrogen (2*S*,3*R*)-*N*-(*tert*-butoxycarbonyl)-3-benzyloxyaspartate (**8**). The original synthetic strategy of Hansson and Kihlberg was adopted, however, several major alterations were found to be necessary in order to facilitate practical access to multigram quantities of **8**.

It is well known that β-hydroxy-α-amino acids play important roles in biological systems.[2] As part of an ongoing program on the development of biologically active peptides, we required multigram amounts of a suitably protected (2*S*,3*R*)-3-hydroxyaspartic acid for solid-phase peptide synthesis. Although all four optical isomers of 3-hydroxyaspartic acid were synthesized by Kaneko and Katsura in 1963,[3] it was not until recently that Hansson and Kihlberg described a preparation of one of these isomers, (2*S*,3*R*)-3-hydroxyaspartic acid, bearing protecting groups which were compatible with the Merrifield solid-phase methodology.[4]

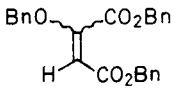
Though the sound synthetic approach developed by Hansson and Kihlberg was retained, we discovered that several of the steps needed major modification in order to provide multigram quantities of the final product **8**.[5]



Bn = $PhCH_2$
Boc = *t*-BuOCO

Thus, acid-catalyzed esterification of (*R*,*R*)-(+)-tartaric acid (**1**) with benzyl alcohol gave the benzyl ester **2**. While purification of **2** was found to be complicated by the formation of a gelatinous mass upon attempted recrystallization, the crude ester **2** was converted to the benzyl ether **3** by treating with sodium hydride in dimethylformamide, followed by the addition of benzyl bromide.

Introduction of the 2-(*S*)-amino group required S_N2 inversion at the carbon bearing the free hydroxyl substituent. Consistent with the chemistry of related systems, use of less active leaving groups than trifluoromethanesulfonate (triflate) led to low yields of product upon attempted azide displacement.[6] Thus the tosylate, *p*-bromophenylsulfonate or *p*-chlorophenylsulfonate, produced a small amount of azide **5** as well as the elimination product **9** as a major side product in the subsequent reaction. No attempt was made to assign the stereochemistry of the olefin.



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As the published procedure for the synthesis of triflate **4** provided a complex mixture of products,[4] it was necessary to develop an alternative method. Exceptionally clean conversion of alcohol **3** to triflate **4** was realized upon reaction with trifluoromethanesulfonic anhydride in the presence of 2,6-lutidine.[7] Moreover, nucleophilic substitution of the triflate with azide sources other than tetramethylguanidinium azide (TMGA),[8] including tetrabutylammonium azide,[4] provided significant amounts of **9**, and at best small quantities of **5**. From a practical standpoint, it was most convenient to generate the triflate **4** *in situ* and treat it immediately with tetramethylguanidinium azide to give the pure azide **5** in 79% yield. Reduction to the amine **6** was effected using hydrogen sulfide/triethylamine, although in lower yield (62%) than previously reported.[4] Regioselective saponification was readily achieved at pH 7.2 via copper-mediated hydrolysis of **6** to provide amino acid **7**. The amino group was then protected as the *tert*-butyl carbamate producing the final product **8** in high yield.

In summary, we have revealed a practical synthesis of O^4-benzyl hydrogen (2*S*,3*R*)-*N*-(*tert*-butoxycarbonyl)-3-benzyloxyaspartate (**8**) and have easily achieved the production of multigram quantities of the final product.

Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. ^{13}C-NMR spectra were recorded on a Varian XL-400 spectrometer. In addition to the data reported, each isolated compound was characterized by IR,

^{1}H-NMR and mass spectral analyses, which were identical to those reported.[4] Preparative HPLC was performed on silica gel Prep-Pak 500 cartridges using a Waters Associates Prep LC 500A. Flash chromatography was performed on silica gel (Kiesel gel 60, 70–230 mesh) supplied by E. Merck, Darmstadt under a N_2 pressure of 8–12 psi. DMF was dried over Linde 4A sieves; toluene, CH_2Cl_2, Et_3N, acetone and EtOH were supplied by Fisher and used without further purification. Concentration refers to removal of solvent under reduced pressure (< 1 mmbar) using a Büchi rotary evaporator.

Dibenzyl (2*R*,3*R*)-3-Benzyloxy-2-hydroxybutanedioate (Dibenzyl (2*R*,3*R*)-2-*O*-Benzyltartrate, 3):
(*R*,*R*)-(+)-Tartaric acid (34.5 g, 0.23 mol), benzyl alcohol (49.7 g, 0.46 mol) and TsOH (1.0 g) are stirred in refluxing toluene (500 mL) for 48 h with the azeotropic removal of water (8.3 mL). The mixture is concentrated under reduced pressure and the residual oil containing **2** is dissolved in DMF (230 mL). In a separate flask, a 60% dispersion of NaH in mineral oil (9.2 g, 0.23 mol) in DMF (230 mL) is cooled under Ar to 0 °C, and the solution containing **2** is added dropwise (20 min). After complete addition, the mixture is stirred an additional 90 min, whereupon benzyl bromide (27.6 mL, 0.23 mol) is added all at once. The ice is allowed to melt and the reaction is stirred at r.t. (16 h). After carefully pouring the mixture into 0.6 M aq NH_4Cl (2 L), it is extracted with Et_2O (3 × 1 L). The organic layers are washed with H_2O (1 L), brine (1 L), combined, dried ($MgSO_4$) and concentrated. The crude product is purified by flash chromatography on silica gel (hexanes/EtOAc, 4:1) to give **3** as a white solid; yield: 33.4 g (35%); mp 54.5–55.5 °C; $[\alpha]_D^{20}$ + 59.9° (*c* = 1.1, EtOH) (Lit.[4] mp 50–52 °C; $[\alpha]_D^{20}$ + 64.0° (*c* = 1.0., EtOH)).

^{13}C-NMR ($CDCl_3$/TMS): δ = 67.3, 67.7, 72.6, 73.1, 78.6, 128.2, 128.4, 128.5, 128.8, 135.1, 135.5, 136.9, 169.3, 171.1.

Dibenzyl (2*S*,3*R*)-2-Azido-3-benzyloxybutanedioate (Dibenzyl (2*S*,3*R*)-2-Azido-3-benzyloxysuccinate, 5):
To a mechanically stirred solution of **3** (33.4 g, 80 mmol) in CH_2Cl_2 (100 mL) under Ar trifluoromethanesulfonic anhydride (15.6 mL, 95 mmol) is added dropwise at − 78 °C. After 5 min, 2,6-lutidine (12.1 mL, 103 mmol) is added dropwise. The mixture is then stirred for 30 min, whereupon additional trifluoromethanesulfonic anhydride (1.5 mL, 9.4 mmol) and 2,6-lutidine (1.3 mL, 10.3 mmol) are added, and again stirred for 30 min. The mixture is chilled to − 120 °C, and solid TMGA (37 g, 238 mmol) is added over 15 min using a wormgear-driven solid addition funnel.[8] After complete addition, the mixture is warmed to − 78 °C, stirred for 15 min, and allowed to slowly warm to 0 °C (90 min), and stirred at 0 °C for 2.5 h. The mixture is then immediately filtered through a column of silica gel (hexane/EtOAc, 5.1) to separate the product from polar materials. All fractions containing compounds with R_f > 0.4 (hexanes/EtOAc, 3:1) are combined, concentrated and purified by HPLC on silica gel (hexanes/EtOAc, 9:1) to afford **5** as an oil; yield: 28.2 g (79%); $[\alpha]_D^{20}$ + 30.0° (*c* = 1.1, EtOAc) (Lit.[4] $[\alpha]_D^{20}$ + 50.3° (c = 1.0, EtOAc)).

^{13}C-NMR ($CDCl_3$/TMS): δ = 62.9, 67.3, 67.9, 73.4, 78.3, 128.1, 128.2, 128.4, 128.6, 134.6, 134.8, 136.2, 166.8, 168.2.

Dibenzyl (2*S*,3*R*)-3-Benzyloxyaspartate (6):
H_2S is bubbled through a solution of azide **5** (28.2 g, 63 mmol) and triethylamine (14.5 mL) in CH_2Cl_2 (925 mL) for 15 min. After 45 min, additional H_2S is passed through the mixture (15 min). The mixture is stirred at r.t. for 16 h and then washed with H_2O (700 mL), brine (700 mL) and sat. aq $NaHCO_3$ (700 mL). The aqueous layers are extracted with CH_2Cl_2 (700 mL) and the organic layers combined, dried (Na_2SO_4), and concentrated. The resulting residue (38 g) is purified by flash chromatography on silica gel using gradient elution (hexanes/EtOAc, 3:1, 2:1, 1:1, 1:2). Desired product **6** is eluted in the most polar fractions, which are combined and concentrated to dryness to provide pure **6** as an oil; yield: 16.4 g (62%); $[\alpha]_D^{20}$ + 38.3° (*c* = 1.0, EtOAC) (Lit.[4] $[\alpha]_D^{20}$ + 35.6° (*c* = 1.0, EtOAc)).

^{13}C-NMR ($CDCl_3$/TMS): δ = 56.9, 66.9, 67.2, 73.1, 80.0, 126.8, 127.3, 127.93, 127.98, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 128.8, 135.0, 137.0, 169.7, 171.5.

O^4-Benzyl Hydrogen (2*S*,3*R*)-3-Benzyloxyaspartate (7):
A solution of $CuBr_2$ (8.7 g, 39 mmol) in H_2O (100 mL) is added to a solution of diester **6** (16.4 g, 39 mmol) in EtOH (480 mL) and acetone (150 mL). The green mixture is warmed to 45 °C and carefully basified to pH 7.1 with aq sat. $NaHCO_3$. After 16 h the turquoise mixture is concentrated to a thick slurry, diluted with H_2O (225 mL), and acidified to pH 2.8 using 1 N HCl. Ethylenediaminetetraacetic acid disodium salt dihydrate (15.2 g, 41 mmol) is added and the flask containing this mixture is placed in a preheated oil bath (150 °C). The mixture is boiled (4 min) and then allowed to cool to r.t. The resulting slurry is chilled (5 °C) and the crude solid product collected by suction filtration. This is recrystallized from boiling H_2O (900 mL) to provide **7** as a white solid; yield: 3.1 g (24%); mp 188–189 °C; $[\alpha]_D^{20}$ + 48.6° (*c* = 0.8, DMSO) (Lit.[4] mp 182–184 °C; $[\alpha]_D^{20}$ + 49.1° (*c* = 0.8, DMSO)).

^{13}C-NMR ($CDCl_3$/TMS): δ = 58.0 (br), 68.2, 74.0, 79 (br), 129.1, 129.3, 129.4, 129.6, 137.2, 138.5, 171 (br).

O^4-Benzyl Hydrogen (2*S*,3*R*)-*N*-(*tert*-Butoxycarbonyl)-3-benzyloxyaspartate (8):
Amino acid **7** (3.1 g, 9.5 mmol) in DMF (23 mL) is treated at ambient temperature with Et_3N (1.5 mL, 10.3 mmol) and di-*tert*-butyl dicarbonate (2.3 g, 10.3 mmol) for 16 h at r.t. Removal of DMF *in vacuo* provides the crude product as an oil (ca. 5 g), which is dissolved in CH_3CN (5 mL) and purified in 2 separate injections on a Waters *Δ*-Prep HPLC using a C_{18} packed column (30 mm × 30 cm). The mixture is eluted (20 mL/min, det. 260 nm) first with water containing 0.2% AcOH up to 5 min, and for the next 30 min with CH_3CN and H_2O each containing 0.2% AcOH in the ratio of 60:40. The fractions containing product are pooled and concentrated to dryness. The residue is lyophilized from benzene to give the product **8** as a solid foam; yield: 3.7 g (92%); mp 39–41 °C; $[\alpha]_D^{20}$ + 74.6° (*c* = 0.8, EtOAc) (Lit.[4] oil; $[\alpha]_D^{20}$ + 74.7° (*c* = 1.0, EtOAc)).

$C_{23}H_{27}NO_7 \cdot 0.25H_2O$[9]	calc.	C 63.66	H 6.39	N 3.22	H_2O 1.03
(429.5)	found	63.71	6.49	3.30	1.09

^{13}C-NMR (acetone-d_6/TMS): δ = 28.5, 56.3, 67.3, 73.6, 79.3, 79.8, 128.5, 128.8, 129.1, 129.3, 136.9, 138.6, 169.9, 170.2.

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(9) Sample contains traces of water that are exceptionally difficult to remove.