Preparation of α-Hydroxy-β-Fmoc Amino Acids from *N*-Boc Amino Acids

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Abstract: A general method for the conversion of *N*-Boc amino acids into their homologated α-hydroxy-β-Fmoc amino acids is described. The protocol involved preparation of the amino aldehyde by reduction of the corresponding Weinreb amides, hydrocyanation, and hydrolysis of the nitrile group, followed by reprotection of the amino acid as the Fmoc derivative.

Key words: *N*-Boc amino acid, reduction, hydrocyanation, *N*-Fmoc amino acid

The development of combinatorial and parallel synthesis techniques in drug discovery has greatly increased the demand for reagents and scaffolds for the rapid assembly of new molecules for biological screening. Although these assays require small quantities of final compound, any solid-phase endeavor requires the proper choice of resins, loading requirements, and a ready supply of reagents.

In support of the preparation of libraries directed towards the discovery of novel statin-based **2** and α-keto amide inhibitors **1** of aspartyl and serines proteases,[3] respectively, we required the facile and scaleable synthesis of a series of Fmoc-protected β-amino acids **3** as intermediates in library formation (Figure 1). We developed a general method that maintained good control of the *R*-configuration of the β-amino acid, was robust enough to handle a range of R groups, and was scaleable to multi-gram quantities needed for combinatorial library development. There was no desire to control the hydroxy group stereochemistry in **3** since both *R*- and *S*-enantiomers of the hydroxy group of the statin can bind aspartyl proteases and would be oxidized to a carbonyl group after incorporation into the protease library. At this early juncture, there was no prerequisite on route selection to account for orthogonal protecting groups on R if other amino acid groups were to be considered. A method to prepare **3a**,**e**,**f** with a stereodefined hydroxy group using enantioselective addition of HCN catalyzed by an enzyme has been reported.[4] Considering our need to support library development with larger quantities, we chose to investigate an independent route with special attention to methods that were scaleable.

The general method used for the preparation of **3** is shown in Scheme 1. The amino acid pool was seen as an excellent source of chirality for our work. Thus, we focused on developing methodology that preserved the integrity of the chiral center along the route. Commercially available *N*-Boc-D-amino acids **4** were converted by the action of CDI/MeNH(OMe)·HCl into their corresponding Weinreb amides **5**[5–8] in 85–92% yield. The amides were smoothly reduced to the aldehydes **6**[5,6,9,10] with $LiAlH_4$ in 92–99% yield. Compound **6c** was prepared by a slightly different route: commercially available *N*-Boc-phenylglycinol was oxidized using Dess–Martin periodinane in quantitative yield.[11,12]

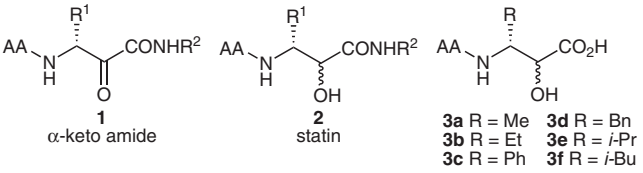

Figure 1 Retrosynthesis of aspartyl (statin) and serine (α-keto amide) protease libraries

With these aldehydes **6** in hand, we decided to convert them into their cyanohydrins **7**,[5,13–15] and Myers' method[16] (KCN, AcOH, EtOAc, MeOH) worked extremely well for the examples under investigation. As mentioned earlier, the formation of diastereomeric mixtures at the hydroxy center was not a concern because this group would be later transformed to the α-carbonyl group. It is worthwhile noting that for the easily epimerizable **6c**, a mixture of TFA and AcOH was used to effect conversion to **7c** (Scheme 2).

The hydrolysis of the cyanide **7** to the carboxylic acid **8**[4,17] was accomplished with concentrated HCl in 1,4-dioxane. It is important to note that anisole was beneficial to the reaction, presumably by acting as a cation scavenger, and improved the quality of the isolated product.[18] Running the reaction in aqueous 6 M HCl alone provided the product, but isolation in pure form or further reaction was problematic. However, **8** could be passed through an ion-exchange column for the purpose of purifying the material to analytical quality.

Finally, conversion of **8** into their Fmoc derivatives **3** was smoothly accomplished using Fmoc-OSuc under aqueous acetone conditions. The products **3** were isolated in sufficient purity by simple partitioning between water and ethyl acetate.

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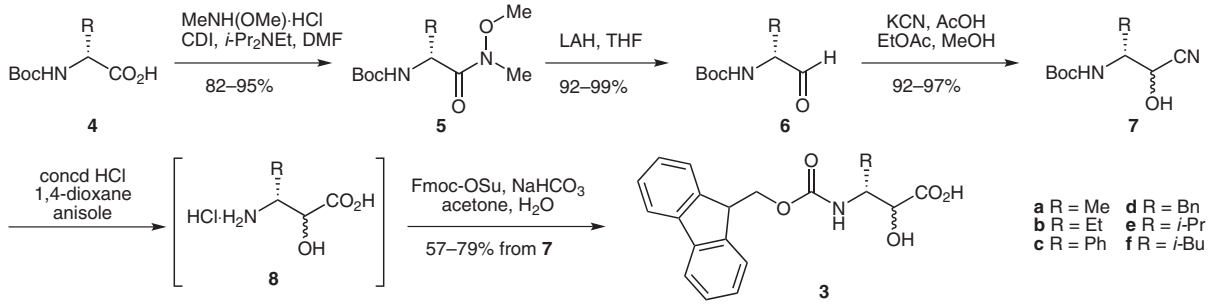
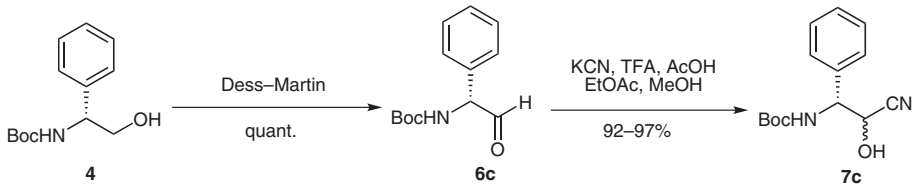


Scheme 1 General synthesis route



Scheme 2 Specific preparation of **6c** by Dess–Martin oxidation, and formation of **7c**

In summary, we have described a general method for the multi-gram preparation of epimeric Fmoc-protected α-amino-β-hydroxy acids **3** from commercially available Boc-amino acids **4**. Our method relies on high yielding formation of Weinreb amides **5**, chiral aldehydes **6**, cyanohydrins **7** and the key hydrolysis to acid **8**, and reprotection of the amino group. Although our method provides a mixture of hydroxy diastereomers, a potential benefit of this feature in library design would allow for more extensive coverage of the diversity space when screening for inhibitors in other proteases.[19] Use of reagents **3** in conjunction with combinatorial library development will be reported elsewhere.

Starting materials were used as supplied without further treatment unless otherwise noted. Work-ups requiring concentrations were done under reduced pressure. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Nuclear magnetic resonance spectra (^{1}H, ^{13}C) were obtained on a Varian VXR300S multinuclear spectrometer at 300 MHz and 75 MHz, respectively, and are referenced to Me_4Si (δ = 0.0 ppm). Infrared (IR) spectra were recorded as Nujol mulls (unless otherwise noted) on a Perkin-Elmer 783 infrared spectrometer and are reported in reciprocal wave numbers (cm^{-1}). Elemental analyses were performed by Robertson Microlit Labs, Inc., Madison, NJ. Melting points are uncorrected.

Experimental procedures are given for the preparation of **5a**, **6a**, **6c**, **7a**, **7c**, **8a**, and **3a**.

N-Boc-(*R*)-2-amino Weinreb Amides 5; General Procedure

The appropriate *N*-Boc-(*R*)-amino acid **4** (300 mmol, 1.00 equiv) was dissolved in anhyd THF (400 mL), and *N*,*N*′-carbonyldiimidazole (360 mmol, 1.20 equiv) was added portionwise over 1 h so as to control foaming. The mixture was stirred at r.t. for 1 h. A solution of *N*,*O*-dimethylhydroxylamine hydrochloride (330 mmol, 1.10 equiv) and *i*-Pr_2NEt (315 mmol, 1.05 equiv) in anhyd DMF (185 mL) was introduced over 0.5 h (with cooling as necessary to maintain the reaction temperature <30 °C). This mixture was stirred at r.t. for 18 h. Most of the solvent was removed, and the residue was partitioned between EtOAc (500 mL) and 1.67 M aq $NaHSO_4$ (500 mL, 833 mmol, 2.78 equiv). The separated aqueous layer (pH 1–2) was extracted with EtOAc (200 mL) and the combined organic phases were washed successively with H_2O (100 mL), sat. aq $NaHCO_3$ (200 mL), and H_2O (100 mL). Concentration and azeotropic drying with fresh EtOAc under reduced pressure afforded a syrupy product (255–276 mmol, 85–92%), suitable for use in the next step. These materials solidified after all traces of solvent had been removed.

(*R*)-*tert*-Butyl 1-[Methoxy(methyl)amino]-1-oxopropan-2-ylcarbamate (5a)

Mp 148.5–149.5 °C; $[\alpha]_D^{20}$ +28.5 (*c* = 1.0, MeOH).

IR (Nujol): 3290, 2900, 1710, 1650, 1300, 1250, 1065, 980 cm^{-1}.

^{1}H NMR ($CDCl_3$): δ = 5.33 (d, *J* = 8.0 Hz, 1 H), 4.68 (m, 1 H), 3.78 (s, 3 H), 3.21 (s, 3 H), 1.44 (s, 9 H), 1.31 (d, *J* = 6.9 Hz, 3 H).

^{13}C NMR ($CDCl_3$): δ = 173.5, 155.0, 79.2, 61.4, 46.4, 32.0, 28.1, 18.4.

HRMS: *m*/*z* calcd for $C_{10}H_{20}N_2O_4$: 233.1502; found: 233.1508 [M + H].

Anal. Calcd for $C_{10}H_{20}N_2O_4$: C, 51.71; H, 8.68; N, 12.06. Found: C, 51.75; H, 8.57; N, 11.94.

N-Boc-(*R*)-2-amino Aldehydes 6; General Procedure

A slurry of $LiAlH_4$ (250 mmol, 1.10 equiv) in anhyd THF (250 mL) agitated with an overhead stirrer was chilled to –15 °C. A solution of *N*-Boc-(*R*)-Weinreb amide (227 mmol, 1.00 equiv) in anhyd THF (250 mL) was added with cooling so as to keep the reaction temperature <5 °C. The reaction mixture was stirred for 45 min, quenched by slow addition of EtOAc (500 mL), keeping the internal temperature <5 °C, followed by treatment with aq 2 M $NaHSO_4$ (600 mL), keeping the internal temperature <5 °C. During the quench, the reaction mass gelled but later thinned to a slurry. The layers were separated and the gray aqueous phase was extracted with EtOAc (500 mL). The combined organic layers were washed successively with aq 1 M $NaHSO_4$ (100 mL), sat. aq $NaHCO_3$ (100 mL) and brine (100 mL). After drying ($MgSO_4$), filtration, and concentration, the product aldehyde (209–225 mmol, 92–99%) was obtained as an oil, which was used directly in the next step. Analytically pure material was not obtained unless the aldehyde crystallized or solvent was rigorously removed.

(*R*)-*tert*-Butyl 1-Oxopropan-2-ylcarbamate (6a)
Mp 89–89.5 °C; $[\alpha]_D^{20}$ +35.0 (*c* = 1.1, MeOH).

IR (Nujol): 3320, 2900, 1700, 1680, 1560, 1370, 1250, 1160 cm^{-1}.

^1H NMR ($CDCl_3$): δ = 9.56 (s, 1 H), 5.21 (m, 1 H), 4.21 (m, 1 H), 1.46 (s, 9 H), 1.33 (d, *J* = 7.3 Hz, 3 H).

^{13}C NMR ($CDCl_3$): δ = 199.7, 155.3, 79.9, 55.4, 28.2, 14.7.

HRMS: *m*/*z* calcd for $C_8H_{15}NO_3$: 174.1131; found: 174.1132 [M + H].

Anal. Calcd for $C_8H_{15}NO_3$: C, 55.47; H, 8.73; N, 8.09. Found: C, 55.57; H, 8.85; N, 7.96.

(*R*)-*N*-(*tert*-Butoxycarbonyl)-2-phenylglycinal (6c)
To a chilled slurry of *N*-Boc-(*R*)-phenylglycinol (23.7 g, 100 mmol) in H_2O-saturated CH_2Cl_2 (300 mL) was added portionwise, Dess–Martin periodinane (89.1 g, 210 mmol) over 0.5 h. The stirred mixture was allowed to warm to r.t. over 2 h. More H_2O-saturated CH_2Cl_2 (50 mL) was added and stirring was continued for 0.5 h. The reaction was quenched by the addition of a solution of $Na_2S_2O_3{\cdot}5H_2O$ (573 g, 2.31 mol) in sat. aq $NaHCO_3$ (1.5 L). When the mixture tested negative to starch-iodide paper, it was diluted with MTBE (300 mL), and the phases separated. The aqueous layer was extracted with MTBE (50 mL), and the combined organic phases were washed with H_2O (1 L). Concentration under reduced pressure and chasing the residual H_2O with EtOAc (100 mL) afforded a semi-solid yellow product (24.0 g, 102%) suitable for use directly in the next step; $[\alpha]_D^{20}$ –24.0 (*c* = 5.16, MeOH).

IR (neat): 3360, 3045, 3020, 2980, 2940, 2720, 1700, 1500, 1450, 1390, 1365, 1240, 1170, 1040, 860, 750, 700 cm^{-1}.

^1H NMR ($CDCl_3$): δ = 9.54 (s, 1 H), 7.34 (m, 5 H), 5.78 (br s, 1 H), 5.33 (d, *J* = 6.3 Hz, 1 H), 1.45 (s, 9 H).

^{13}C NMR ($CDCl_3$): δ = 191.8, 155.1, 133.0, 129.2, 128.7, 127.7, 81.1, 64.8, 28.3.

HRMS: *m*/*z* calcd for $C_{13}H_{17}NO_3$: 236.1287; found: 236.1275 [M + H].

Cyanohydrins 7; General Procedure
Crude *N*-Boc-(*R*)-amino aldehyde (250 mmol, 1.00 equiv) was dissolved in EtOAc (300 mL) and treated with a solution of KCN (275 mmol, 1.10 equiv) and AcOH (300 mmol, 1.2 equiv) in MeOH (425 mL) for 2 h. Concentration afforded a semi-solid, from which traces of AcOH and MeOH were removed by chasing with 1,4-dioxane (100 mL) under reduced pressure. The residue obtained was slurried in 1,4-dioxane (200 mL) and the solids were removed via filtration prior to use directly in the next step. Concentration gave the oily cyanohydrin (230–243 mmol, 92–97%) that typically crystallized after rigorous removal of solvent.

(*R*)-*tert*-Butyl 1-Cyano-1-hydroxypropan-2-ylcarbamate (7a)
Mp 74–82 °C.

IR (Nujol): 3460, 3330, 2900, 1680, 1250, 1170, 960 cm^{-1}.

^1H NMR ($CDCl_3$): δ = 5.53 (d, *J* = 7.7 Hz, 0.3 H), 4.87 (m, 1 H), 4.6 (s, 0.7 H), 4.58 (s, 0.6 H), 4.48 (dd, *J* = 2.5, 5.0 Hz, 0.4 H), 4.05 (m, 0.3 H), 3.88 (m, 0.7 H), 1.46 (s, 3.15 H), 1.45 (s, 5.85 H), 1.35 (d, *J* = 6.9 Hz, 2 H), 1.30 (d, *J* = 6.9 Hz, 1 H).

^{13}C NMR ($CDCl_3$): δ = 157.3, 156.0, 118.4, 117.9, 81.4, 80.9, 67.4, 65.0, 50.4, 49.5, 28.2, 28.1, 16.1, 14.9.

HRMS: *m*/*z* calcd for $C_9H_{16}N_2O_3$: 201.1240; found: 201.1233 [M + H].

Anal. Calcd for $C_9H_{16}N_2O_3$: C, 53.98; H, 8.05; N, 13.99. Found: C, 54.26; H, 7.79; N, 13.88

(*R*)-*tert*-Butyl 2-Cyano-2-hydroxy-1-phenylethylcarbamate (7c)
Crude *N*-Boc-(*R*)-phenylglycinal (22.10 g, 94.0 mmol) dissolved in EtOAc (150 mL), was treated with a solution of KCN (6.43 g, 98.7 mmol), TFA (9.64 g, 84.6 mmol), and AcOH (1.13 g, 18.8 mmol, 1.2 equiv) in MeOH (150 mL) for 2 h. Toluene (200 mL) was added, and the mixture was extracted with sat. aq $NaHCO_3$ (150 mL). The separated aqueous layer was extracted with toluene (100 mL) and the organics were washed with H_2O (500 mL), and concentrated to afford the product as a pale yellow oil (23.7 g, 96%) that eventually crystallized after refrigeration. This material was used directly in the next step.

IR (CCl_4): 3440, 3360, 3060, 3040, 2980, 2940, 1700, 1500, 1460, 1400, I370, 1250, 1170, 1090, 1050, 1030 cm^{-1}.

^1H NMR ($CDCl_3$): δ = 7.38 (s, 5 H), 5.63 (m, 0.6 H), 5.58 (m, 0.4 H), 5.32 (m, l H), 4.95 (m, 1 H), 4.70 (m, 0.6 H), 4.63 (m, 0.4 H), 1.45 (s, 2.7 H), 1.43 (s, 6.3 H).

^{13}C NMR ($CDCl_3$): δ = 156.0, 136.2, 135.8, 129.1, 128.9, 128.8, 127.9, 127.3, 127.0, 118.4, 117.9, 81.5, 81.0, 67.1, 65.1, 59.1, 57.9, 28.2.

HRMS: *m*/*z* calcd for $C_{14}H_{18}N_2O_3$: 263.1396; found: 263.1392 [M + H].

Anal. Calcd for $C_{14}H_{18}N_2O_3$: C, 64.10; H, 6.92; N, 10.68. Found: C, 64.39; H, 7.11; N, 10.57

***N*-Fmoc-3-(*R*)-amino-2-(*R*,*S*)-hydroxy Acids 3; General Procedure**
Crude **7** (243 mmol, 1.00 equiv) and anisole (34 mL) were dissolved in 1,4-dioxane (350 mL). Concd aq HCl (12.1 N, 350 mL) was added slowly and the mixture was heated at a gentle reflux for 4.5 h. Volatiles were removed under reduced pressure and the resulting residue was dissolved in H_2O (250 mL). After extraction with 50% toluene–EtOAc (100 mL), the pH was adjusted to 10.5–11 with solid NaOH. Water and NH_3 were removed under reduced pressure and the residual (3*R*,2*RS*)-3-amino-2-hydroxy acid sodium salt **8** was dissolved in 50% aq acetone (700 mL). This mixture was buffered with $NaHCO_3$ (243 mmol, 1.00 equiv) and Fmoc-*O*-succinimidyl ester (243 mmol, 1.00 equiv) and stirred at r.t. for 18 h. The reaction mixture was carefully poured into a mixture of aq $NaHSO_4$ (0.5 M, 1 L) and EtOAc (1 L), the layers were separated, and the aqueous phase was extracted with EtOAc (500 mL). The combined organic layers were washed with H_2O (500 mL) and concentrated to a residue, which was dissolved in 15% H_2O in acetone and applied to a column of Amberlyst A-21 ion exchange resin (210 g). Elution of impurities with the same mixture, followed by passage of a 3:4:16 H_2O–AcOH–acetone mixture through the column afforded essentially pure product (139–192 mmol, 57–79% yield, 54–74% from aldehyde) after concentration and drying under reduced pressure at 55 °C.

(*R*)-3-{[(9*H*-Fluoren-9-yl)methoxy]carbonylamino}-2-hydroxybutanoic Acid (3a)
Mp 120–132 °C.

IR (Nujol): 3500, 3310, 2900, 1680, 1530, 1250 cm^{-1}.

^1H NMR (acetone-d_6): δ = 7.85–7.29 (m, 8 H), 6.51 (d, *J* = 8.4 Hz, 0.4 H), 6.30 (d, *J* = 8.4 Hz, 0.6 H), 4.44–4.05 (m, 5 H), 1.28 (d, *J* = 6.8 Hz, 1.8 H), 1.16 (d, *J* = 6.8 Hz, 1.2 H).

^{13}C NMR (acetone-d_6): δ = 174.3, 174.0, 156.6, 145.3, 145.0, 144.9, 142.0, 128.4, 127.9, 127.8, 126.1, 126.0, 73.7, 73.4, 67.1, 50.3, 47.9, 17.8, 14.7.

HRMS: *m*/*z* calcd for $C_{19}H_{19}NO_5$: 342.1342; found: 342.1352 [M + H].

Anal. Calcd for $C_{19}H_{19}NO_5$: C, 66.85; H, 5.61; N, 4.10. Found: C, 66.70; H, 5.52; N, 3.85

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synthesis.

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