Synthesis of C-Protected 2,2-Dideutero β^3-Amino Acids

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Abstract: A simple three-step procedure for the preparation of C-protected 2,2-^{2}H-β^3-amino acids has been developed starting from the natural α-amino acids. Our synthetic path is based on the homologation reaction of α-amino acids through the formation of dideuterated alcohol intermediates obtained by heavy isotope reduction ($NaBD_4$) of the carboxylic function.

Key words: β^3-amino acids, deuterated amino acids, isotopically labeled compounds

The discovery of nonproteinogenic amino acids among natural products[1] has increased the level of interest in this family of molecules. β-Amino acids show interesting pharmacological properties either in free form, for instance emeriamine[2] (**1**), or as key components of a variety of bioactive molecules such as taxol[3] (**2**), one of the most active antitumor agents which contains phenylisoserine as its side chain (Figure 1). Furthermore, β-amino acids, although not as abundant as their α-analogues, are also segments in peptidic natural products with various biological activities, such as (*R*)-β-dopa (3,4-dihydroxy-β-phenylalanine, **3**) contained in mushroom *Cortinarius violaceus*.[4]

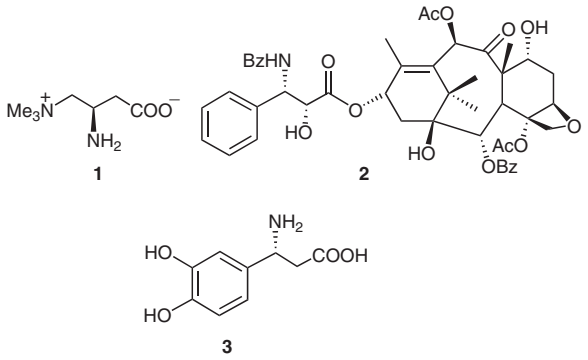
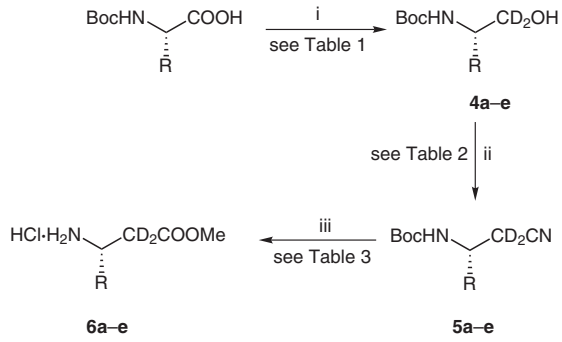


Figure 1 Naturally occurring compounds containing β-amino acid moiety

The incorporation of β-amino acids[5] has been successful in creating peptidomimetics[6] that not only have potent biological activity, but are also resistant to proteolysis. Nevertheless, metabolism and pharmacokinetics are other important aspects in addition to biological activity for the potential use of these compounds as drugs.

So far, only little information is available about the pharmacokinetic properties of bioactive β-peptides. Recently Seebach[7,8] and co-workers have performed an interesting study using ^{14}C-labelled peptides to follow their absorption, distribution, metabolism, and excretion (ADME). Pharmacokinetic studies traditionally used radiolabelled target compounds as a means for ADME studies; new analysis technologies now make it possible to use targets enriched with stable isotopes, such as carbon-13 and deuterium, as alternatives to radioisotopes.[9] The use of stable isotopes allows, ADME studies to be directly replicated in humans, providing unequivocal validation of animal models. Recently, deuterium labeling of proteins and peptides have also been used in quantitative proteomics analysis.[10]

As a part of our ongoing project on the preparation of a new ICAT (Isotope-Coded Affinity Tag) reagent[11] as a powerful tool for quantitative proteome analysis, containing a deuterated β-amino acidic linker moiety, we have applied a simple methodology to obtain C-protected 2,2-^{2}H-β^3-amino acids[12] starting from the natural α-amino acids. The current path consists of a homologation reaction by reduction of carboxylic function[13] and then substitution of the hydroxyl group of the corresponding primary hydroxyl function with a cyano group (Scheme 1). We have tested our methodology using various α-amino acids and the obtained results are reported in Tables 1– 3.



i) NMM, MeOCOCl in THF, then $NaBD_4$ in D_2O; ii) –PPh_2, I_2, HIm in CH_2Cl_2, then $Et_4N^+CN^-$ in CH_2Cl_2; iii) HCl in MeOH

Scheme 1

We began the synthesis of our deuterium labelled compounds by treating N-protected α-amino acids with methyl chloroformate. The corresponding carbomethoxy anhydrides formed in situ were next reduced and labelled by means of $NaBD_4$ (98% atom D) in D_2O (99.9%), affording the related 1,1-^{2}H-β-amino alcohols in high yield

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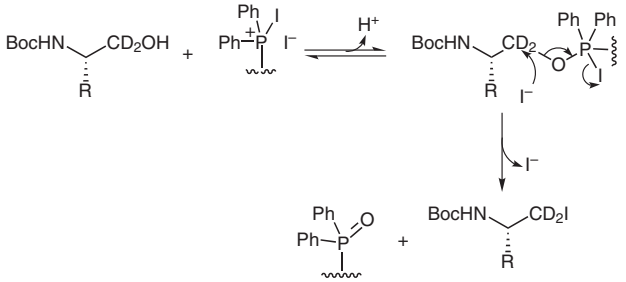
Table 1 1,1-Dideutero β-Amino Alcohols **4a–e** Prepared

Compound	Yield (%)	Mp (°C)	$[\alpha]_D^{25}$	^{1}H NMR (300 MHz, $CDCl_3$/TMS) δ, *J* (Hz)	^{13}C NMR (75 MHz, $CDCl_3$/TMS) δ
4a R = H	85	oil	–	1.43 (s, 9 H), 3.22 (d, *J* = 5.5, 2 H), 3.86 (br s, 1 H, exchangeable with D_2O), 5.10 (br t, *J* = 5.5, 1 H)	28.2, 42.8, 61.8 (CD_2), 79.6, 156.8
4b R = Me	94	58.2–59.1 (hexane)	–9.8 (*c* = 1.3, $CHCl_3$)	1.15 (d, *J* = 6.8, 3 H), 1.43 (s, 9 H), 2.95 (br s, 1 H, exchangeable with D_2O), 3.71 (br q, *J* = 6.8, 1 H), 4.75 (br d, *J* = 6.6, 1 H)	17.1, 28.2, 48.3, 66.5 (CD_2), 79.5, 156.2
4c R = CH_2OBn	89	64.9–65.7 (hexane)	+14.8 (*c* = 1.7, $CHCl_3$)	1.45 (s, 9 H), 2.60 (br s, 1 H, exchangeable with D_2O), 3.67 (dd, *J* = 4.3, 9.3, 1 H), 3.71 (dd, *J* = 3.8, 9.3, 1 H), 3.80–3.90 (m, 1 H), 4.38 (d, *J* = 12.0, 1 H), 4.62 (d, *J* = 12.0, 1 H), 5.22 (br s, 1 H), 7.27–7.42 (m, 5 H)	28.8, 51.7, 63.1 (CD_2), 70.6, 73.4, 79.5, 127.0, 127.2, 127.8, 136.9, 155.1
4d R = CH_2Ph	90	97.3–98.9 (hexane)	–23.1 (*c* = 1.2, $CHCl_3$)	1.43 (s, 9 H), 1.92 (br s, 1 H, exchangeable with D_2O), 2.82 (d, *J* = 7.1, 2 H), 3.78–3.92 (m, 1 H), 4.72 (br s, 1 H), 7.18–7.28 (m, 5 H)	28.2, 37.3, 49.1, 53.4 (CD_2), 79.6, 126.4, 128.4, 129.1, 137.6, 157.7
4e R = $(CH_2)_4NHBoc$	85	oil	–10.1 (*c* = 1.9, MeOH)	0.93–1.32 (m, 6 H), 1.43 (s, 18 H), 2.14 (br s, 1 H, exchangeable with D_2O), 2.78–2.98 (m, 2 H), 3.43–3.58 (m, 1 H), 4.13 (br s, 1 H), 4.51 (br s, 1 H)[a]	23.0, 28.7, 30.2, 30.9, 40.0, 52.6, 65.8 (CD_2), 79.4, 79.7, 156.6, 156.7

[a] Recorded in C_6D_6 at 75 °C.

(see Table 1) with a 98% deuterium incorporation (determined by ^{1}H NMR spectroscopy and MS analyses). On the basis of previously acquired knowledge,[14] the dideuterated amino alcohols **4a–e** were then converted into their corresponding amino iodides using a suspension of triphenylphosphine polymer-bound/iodine complex (polystyryl diphenyliodophosphonium iodide) in anhydrous dichloromethane.

The triphenylphosphine polymer-bound/halogen complex is a Lewis acid and a dehydrating agent widely employed in miscellaneous reactions[15] with low environmental impact. In fact it avoids contamination from by-products and use of non-environmentally friendly solvents in the purification processes. The phosphine oxide, which under our conditions is the only byproduct of the reaction, is linked to the polymeric matrix and can thus be easily separated by filtration (Scheme 2). Amino iodides so obtained[16] were directly engaged, after a simple filtration, in the next step in which the iodine atom was easily replaced with a cyano group (Table 2) by means of a suitable cyanide ion source ($Et_4N^+CN^-$). Finally, hydrolysis of nitriles **5a–e** using acid-catalyzed alcoholysis led to the corresponding 2,2-dideuterated amino esters **6a–e** (Table 3). The deuterium content percentage was maintained unchanged during the whole synthetic route, at a rate shown by MS spectrometry and ^{1}H NMR spectroscopy to be not less than 97%.



Scheme 2

^{1}H and ^{13}C NMR spectra: Varian Gemini 300 MHz and Varian Inova 500 MHz spectrometers. HRMS-EI: Micromass Q-TOF micro. Optical rotations: Jasco P-1010 (1.0 dm cell). Melting points are uncorrected and were determined with a capillary apparatus. Reactions were monitored by TLC (precoated silica gel plate F254, Merck). Column chromatography: Merck Kieselgel 60 (70–230 mesh). All moisture-sensitive reactions were conducted under dry N_2 using oven-dried glassware. THF was distilled from sodium/benzophenone immediately prior to use. Ph_3P polymer-bound was purchased from Fluka Chemical Co. D_2O minimum isotopic purity 99.9 atom% D and $NaBD_4$ 98 atom% D were used.

2,2-^{2}H-β^3-Amino Esters; Typical Procedures

1,1-^{2}H-*N*-Boc-β-amino Alcohol 4b

To a magnetically stirred solution of *N*-Boc-Ala (0.50 g, 2.6 mmol) in anhyd THF (20 mL) at 0 °C, was added *N*-methylmorpholine (0.35 mL, 3.2 mmol), followed by ethyl chloroformate (0.24 mL, 3.2 mmol). After 40 min, the mixture was filtered through a glass sinter funnel on a Celite pad and washed with THF. To the filtrate so obtained, at 0 °C and under magnetic stirring, was added a suspension of $NaBD_4$ (0.11 g, 2.6 mmol) in D_2O (2 mL) in one portion. The mixture was kept at r.t. for 10 min, then the solvent was removed under reduced pressure and the obtained residue dissolved with Et_2O and washed with H_2O until neutral. The organic layer was dried (Na_2SO_4) and evaporated under reduced pressure. Chromatography of the crude product on silica gel ($CHCl_3$–MeOH, 8:2)

Table 2 2,2-Dideutero β-Amino Nitriles **5a–e** Prepared

Compound	Yield (%)	Mp (°C)	$[\alpha]_D^{25}$	^{1}H NMR (300 MHz, $CDCl_3$/TMS) δ, *J* (Hz)	^{13}C NMR (75 MHz, $CDCl_3$/TMS) δ
5a R = H	88	42.4–45.0 (hexane)	–	1.45 (s, 9 H), 3.38 (d, *J* = 6.3, 2H), 4.90 (br s, 1 H)	28.1, 29.5 (CD_2), 36.5, 80.1, 118.0, 155.0
5b R = CH_3	90	68.4–70.0 (hexane)	–120 (*c* = 1.4, $CHCl_3$)	1.33 (d, *J* = 6.8, 3H), 1.43 (s, 9 H), 3.95–4.15 (m, 1 H), 4.69 (br d, *J* = 7.05, 1 H)[a]	19.4, 25.0 (CD_2), 28.3, 43.0, 80.1, 117.4, 154.8[b]
5c R = CH_2OBn	70	oil	–9.7 (*c* = 1.6, $CHCl_3$)	1.50 (s, 9 H), 3.60 (dd, *J* = 4.9, 9.6, 1 H), 3.70 (dd, *J* = 3.8, 9.6, 1 H), 4.05–4.18 (m, 1 H), 4.58 (s, 2 H), 5.10 (br d, *J* = 6.4, 1 H), 7.31–7.40 (m, 5 H)	28.1, 29.6 (CD_2), 46.8, 69.5, 73.4, 80.1, 117.1, 127.6, 127.9, 128.4, 137.1, 154.8
5d R = CH_2Ph	80	123.8–125.0 (hexane)	–18.2 (*c* = 1.2, $CHCl_3$)	1.43 (s, 9 H), 2.86 (dd, *J* = 7.8, 13.6, 1 H), 2.99 (dd, *J* = 5.8, 13.6, 1 H), 4.00–4.17 (m, 1 H), 4.72 (br s, 1 H), 7.18–7.28 (m, 5 H)	28.2, 29.9 (CD_2), 39.3, 48.3, 79.6, 117.3, 126.4, 128.4, 129.1, 137.6, 154.8
5e R = $(CH_2)_4NHBoc$	75	62.7–64.0 (hexane)	–45.2 (*c* = 0.6, $CHCl_3$)	0.72–1.18 (m, 6 H), 1.38 (s, 9 H), 1.44 (s, 9 H), 2.64–2.81 (m, 2 H), 3.29–3.42 (m, 1 H), 3.92 (br s, 1 H), 4.21 (br s, 1 H)[c]	23.0, 28.5, 28.6, 29.9 (CD_2), 30.0, 33.1, 40.0, 47.3, 79.5, 80.3, 117.5, 155.4, 156.4

[a] Recorded at 500 MHz.
[b] Recorded at 125 MHz.
[c] Recorded in C_6D_6 at 75 °C.

Table 3 2,2-Dideutero β[3]-Amino Ester Hydrochlorides **6a–e** Prepared

Compound	Yield (%)	Mp (°C)	$[\alpha]_D^{25}$	^{1}H NMR (300 MHz, CD_3OD/TMS) δ, *J* (Hz)	^{13}C NMR (75 MHz, CD_3OD/TMS) δ
6a R = H	93	104.4–106.0 ($CHCl_3$)	–	3.18 (br s, 2 H), 3.73 (s, 3 H)	32.1, 36.4 (CD_2), 52.7, 172.6
6b R = Me	90	250.0 (dec.) ($CHCl_3$)	+0.19 (*c* = 2.5, MeOH)	1.47 (d, *J* = 6.8, 3 H), 3.48 (q, *J* = 6.8, 1 H), 3.72 (s, 3 H)	18.3, 38.2 (CD_2), 44.7, 52.2, 170.8
6c R = CH_2OBn	85	oil	+0.68 (*c* = 0.7, MeOH)	3.63 – 3.78 (m, 4 H), 4.48 (d, *J* = 12.0, 1 H), 4.54 (d, *J* = 12.0, 1 H), 4.78 (m, 1 H), 7.31–7.40 (m, 5 H)	37.0 (CD_2), 48.0, 51.8, 72.4, 73.1, 127.2, 127.5, 127.8, 138.2, 171.1
6d R = CH_2Ph	87	255.0 (dec.) ($CHCl_3$)	+4.28 (*c* = 1.4, MeOH)	2.94 (dd, *J* =8 .3, 14.2, 1 H), 3.79 (dd, *J* = 6.3, 14.2, 1 H), 3.70 (s, 3 H), 3.82 (t, *J* = 7.3, 1 H), 7.05–7.38 (m, 5 H)[a]	37.0, 39.5 (CD_2), 48.3, 52.8, 128.7, 129.4, 130.1, 136.7, 171.2[b]
6e R = $(CH_2)_4NH_2$·HCl	83	oil	+8.49 (*c* = 0.6, MeOH)	1.48–1.58 (m, 2 H), 1.68–1.72 (m, 4 H), 2.93 (t, *J* = 7.8, 2 H), 3.57 (t, *J* = 6.8, 1 H), 3.75 (s, 3 H)[a]	23.0, 28.5, 30.0, 33.1 (CD_2), 40.0, 50.3, 52.8, 170.2[b]

[a] Recorded at 500 MHz.
[b] Recorded at 125 MHz.

gave the pure dideuterated *N*-Boc-amino alcohol **4b**; yield: 0.43 g (94%).

HRMS-EI: *m/z* calcd for $C_8H_{15}D_2NO_3$: 177.1332; found: 177.1342.

2,2-^{2}H-*N*-Boc-β-amino Nitrile 5b

To a magnetically stirred suspension of anhyd polystyryl diphenylphosphine (0.56 g, ~1.67 phosphine units) in anhyd CH_2Cl_2 (10 mL) at r.t., was added dropwise a solution of I_2 (0.42 g, 1.67 mmol) in the same solvent (10 mL) in the dark and under dry N_2. After 15 min, solid dideuterated *N*-Boc-amino alcohol **4b** (0.27 g, 1.52 mmol) was added in one portion to the suspension. The reaction was kept at 40 °C for 2 h (TLC monitoring: $CHCl_3$–MeOH, 8:2) until all the starting amino alcohol was completely consumed. The mixture was then filtered through a glass sinter funnel and washed with CH_2Cl_2. To the filtrate, under magnetical stirring, $Et_4N^+CN^-$ was added in one portion and the reaction kept at reflux for 3 h until complete consumption of the starting *N*-Boc-β-amino iodide. The cooled mixture was poured on a silica gel column (1:5) and eluted with CH_2Cl_2. The organic solvent evaporated under reduced pressure afforded the pure dideuterated *N*-Boc-β-amino nitrile **5b**; yield: 0.25 g (90%).

HRMS-EI: *m/z* calcd for $C_9H_{14}D_2N_2O_2$: 186.1335; found: 186.1346.

2,2-^{2}H-β^3-Amino Ester Hydrochloride 6b

To a magnetically stirred solution of **5b** (0.24 g, 1.3 mmol) in anhyd Et_2O (8 mL) at 0 °C, was added dropwise cold 12 M HCl in MeOH (3 mL). The reaction was kept at r.t. overnight. To the solution were added few drops of H_2O (28 μL, 1.56 mmol) and the solvent was co-evaporated with Et_2O (3 × 10 mL) affording the desired compound **6b** as a crystalline white solid; yield: 0.18 g (90%).

HREIMS: *m/z* calcd for $C_5H_{10}D_2ClNO_2$: 155.0680; found: 155.0691.

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