Synthesis of C-Protected 2,2-Dideutero β3-Amino Acids

Annalisa Guaragna,* Silvana Pedatella, Vittoria Pinto, Giovanni Palumbo
Università di Napoli Federico II, Dipartimento di Chimica Organica e Biochimica, Via Cynthia 4, 80126 Napoli, Italy
Fax +39(081)674119; E-mail: guaragna@unina.it
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Abstract: A simple three-step procedure for the preparation of C-protected 2,2-2Hβ3-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 di-deuterated alcohol intermediates obtained by heavy isotope reduction (NaBD4) of the carboxylic function.

Key words: β3-amino acids, deuterated amino acids, isotopically labeled compounds

The discovery of nonproteinogenic amino acids among natural products 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 (1), or as key components of a variety of bioactive molecules such as taxol (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

The incorporation of β-amino acids5 has been successful in creating peptidomimetics6 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 Seebach7,8 and co-workers have performed an interesting study using 14C-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) reagent11 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 2,2-2Hβ3-amino acids starting from the natural α-amino acids. The current path consists of a homologation reaction by reduction of carboxylic function 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.

We began the synthesis of our deuterium labelled compounds by treating N-protected α-amino acids with methylochloroformate. The corresponding carboxymethoxy anhydrides formed in situ were next reduced and labelled by means of NaBD4 (98% atom D) in D2O (99.9%), affording the related 1,1-2Hβ3-amino alcohols in high yield.
(see Table 1) with a 98% deuterium incorporation (determined by \(^1\)H NMR spectroscopy and MS analyses). On the basis of previously acquired knowledge, the dideuterated amino alcohols 4a–e were then converted into their corresponding amino iodides using a suspension of triphenylphosphine polymer-bound/halogen 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 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 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\(_4\)N\(^+\)CN\(^–\)). Finally, hydrolysis of nitriles was 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, P polymer-bound was purchased from Fluka Chemical Co. D\(_2\)O minimum isotopic purity 99.9 atom% D and NaBD\(_4\) 98 atom% D were used.

### Scheme 2

2,2-\(^2\)H-\(^3\)Boc-\(^4\)amino Alcohol 4b

To a magnetically stirred solution of 2,2-\(^2\)H-\(^3\)Boc-\(^4\)amino Alcohol 4b 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 \(\text{D}_2\text{O}\) (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\(_2\)O and washed with \(\text{H}_2\text{O}\) until neutral. The organic layer was dried (Na\(_2\)SO\(_4\)) and evaporated under reduced pressure. Chromatography of the crude product on silica gel (CHCl\(_3\)–MeOH, 8:2)

### Table 1 1,1-Dideutero \(\beta\)-Amino Alcohols 4a–e Prepared

<table>
<thead>
<tr>
<th>Compound</th>
<th>Yield (%)</th>
<th>Mp (°C)</th>
<th>([\alpha]_D^{25})</th>
<th>(^1)H NMR (300 MHz, CDCl(_3)/TMS) (\delta), J (Hz)</th>
<th>(^1)C NMR (75 MHz, CDCl(_3)/TMS) (\delta)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a R = H</td>
<td>85 oil</td>
<td>–</td>
<td>1.43 (s, 9 H), 3.22 (d, (J = 5.5), 2 H), 3.86 (br s, 1 H, exchangeable with D(_2)O), 5.10 (br t, (J = 5.5), 1 H)</td>
<td>28.2, 42.8, 61.8 (CD(_3)J), 79.6, 156.8</td>
<td></td>
</tr>
<tr>
<td>4b R = Me</td>
<td>94 58.2–59.1 (hexane)</td>
<td>–9.8 (c = 1.3, CHCl(_3))</td>
<td>1.15 (d, (J = 6.8), 3 H), 1.43 (s, 9 H), 2.95 (br s, 1 H, exchangeable with D(_2)O), 3.71 (br q, (J = 6.8), 1 H), 4.75 (br d, (J = 6.6), 1 H)</td>
<td>17.1, 28.2, 48.3, 66.5 (CD(_3)J), 79.5, 156.2</td>
<td></td>
</tr>
<tr>
<td>4c R = CH(_2)OBn</td>
<td>89 64.9–65.7 (hexane)</td>
<td>+14.8 (c = 1.7, CHCl(_3))</td>
<td>1.45 (s, 9 H), 2.60 (br s, 1 H, exchangeable with D(_2)O), 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)</td>
<td>28.8, 51.7, 63.1 (CD(_3)J), 70.6, 73.4, 79.5, 127.0, 127.2, 127.8, 136.9, 155.1</td>
<td></td>
</tr>
<tr>
<td>4d R = CH(_2)Ph</td>
<td>90 97.3–98.9 (hexane)</td>
<td>–23.1 (c = 1.2, CHCl(_3))</td>
<td>1.43 (s, 9 H), 1.92 (br s, 1 H, exchangeable with D(_2)O), 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)</td>
<td>28.2, 37.3, 49.1, 53.4 (CD(_3)J), 79.6, 126.4, 128.4, 129.1, 137.6, 157.7</td>
<td></td>
</tr>
<tr>
<td>4e R = (CH(_2))(_3)NHBoc</td>
<td>85 oil</td>
<td>–10.1 (c = 1.9, MeOH)</td>
<td>0.93–1.32 (m, 6 H), 1.43 (s, 18 H), 2.14 (br s, 1 H, exchangeable with D(_2)O), 3.43–3.58 (m, 1 H), 4.13 (br s, 1 H), 4.51 (br s, 1 H)</td>
<td>23.0, 28.7, 30.2, 30.9, 40.0, 52.6, 65.8 (CD(_3)J), 79.4, 79.7, 156.6, 156.7</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Recorded in C\(_6\)D\(_6\) at 75 °C.

\(^{1}\)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.
### Table 2  2,2-Dideutero β-Amino Nitriles 5a–e Prepared

<table>
<thead>
<tr>
<th>Compound</th>
<th>Yield (%)</th>
<th>Mp (°C)</th>
<th>$[^a]D_{25}$</th>
<th>$[^1]H$ NMR (300 MHz, CDCl$_3$/TMS) $\delta$, J (Hz)</th>
<th>$[^13]C$ NMR (75 MHz, CDCl$_3$/TMS) $\delta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a $R = H$</td>
<td>88</td>
<td>42.4–45.0 (hexane)</td>
<td>–</td>
<td>1.45 (s, 9 H), 3.38 (d, $J = 6.3$, 2H), 4.90 (br s, 1 H)</td>
<td>28.1, 29.5 (CD$_2$), 36.5, 80.1, 118.0, 155.0</td>
</tr>
<tr>
<td>5b $R = \text{CH}_3$</td>
<td>90</td>
<td>68.4–70.0 (hexane)</td>
<td>–120 ($c = 1.4$, CHCl$_3$)</td>
<td>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)$^a$</td>
<td>19.4, 25.0 (CD$_2$), 28.3, 43.0, 80.1, 117.4, 154.8$^b$</td>
</tr>
<tr>
<td>5c $R = \text{CH}_2\text{OBn}$</td>
<td>70</td>
<td>oil</td>
<td>–9.7 ($c = 1.6$, CHCl$_3$)</td>
<td>1.50 (s, 9 H), 3.60 (dd, $J = 9.6$, 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)</td>
<td>28.1, 29.6 (CD$_2$), 46.8, 69.5, 73.4, 80.1, 117.4, 127.6, 127.9, 128.4, 137.1, 154.8</td>
</tr>
<tr>
<td>5d $R = \text{CH}_2\text{Ph}$</td>
<td>80</td>
<td>123.8–125.0 (hexane)</td>
<td>–18.2 ($c = 1.2$, CHCl$_3$)</td>
<td>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)</td>
<td>28.2, 29.9 (CD$_2$), 39.3, 48.3, 79.6, 117.3, 126.4, 128.4, 129.1, 137.6, 154.8</td>
</tr>
<tr>
<td>5e $R = (\text{CH}_2)_4\text{NHBoc}$</td>
<td>75</td>
<td>62.7–64.0 (hexane)</td>
<td>–45.2 ($c = 0.6$, CHCl$_3$)</td>
<td>0.72–1.18 (m, 6 H), 1.38 (s, 9 H), 4.05–4.17 (m, 1 H), 4.72 (br s, 1 H), 4.21 (br s, 1 H)$^c$</td>
<td>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</td>
</tr>
</tbody>
</table>

$^a$ Recorded at 500 MHz.
$^b$ Recorded at 125 MHz.
$^c$ Recorded in C$_6$D$_6$ at 75 °C.

### Table 3  2,2-Dideutero β$^1$-Amino Ester Hydrochlorides 6a–e Prepared

<table>
<thead>
<tr>
<th>Compound</th>
<th>Yield (%)</th>
<th>Mp (°C)</th>
<th>$[^a]D_{25}$</th>
<th>$[^1]H$ NMR (300 MHz, CD$_3$OD/TMS) $\delta$, J (Hz)</th>
<th>$[^13]C$ NMR (75 MHz, CD$_3$OD/TMS) $\delta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>6a $R = H$</td>
<td>93</td>
<td>104.4–106.0 (CHCl$_3$)</td>
<td>–</td>
<td>3.18 (br s, 2 H), 3.73 (s, 3 H)</td>
<td>32.1, 36.4 (CD$_2$), 52.7, 172.6</td>
</tr>
<tr>
<td>6b $R = \text{Me}$</td>
<td>90</td>
<td>250.0 (dec.) (CHCl$_3$)</td>
<td>+0.19 ($c = 2.5$, MeOH)</td>
<td>1.47 (d, $J = 6.8$, 3, 3 H), 3.48 (q, $J = 6.8$, 1 H), 3.72 (s, 3 H)</td>
<td>18.3, 38.2 (CD$_2$), 44.7, 52.2, 170.8</td>
</tr>
<tr>
<td>6c $R = \text{CH}_2\text{OBn}$</td>
<td>85</td>
<td>oil</td>
<td>+0.68 ($c = 0.7$, MeOH)</td>
<td>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)</td>
<td>37.0 (CD$_2$), 48.0, 51.8, 72.4, 73.1, 127.2, 127.5, 127.8, 138.2, 171.1</td>
</tr>
<tr>
<td>6d $R = \text{CH}_2\text{Ph}$</td>
<td>87</td>
<td>255.0 (dec.) (CHCl$_3$)</td>
<td>+4.28 ($c = 1.4$, MeOH)</td>
<td>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)$^a$</td>
<td>37.0, 39.5 (CD$_2$), 48.3, 52.8, 128.7, 129.4, 130.1, 136.7, 171.2$^b$</td>
</tr>
<tr>
<td>6e $R = (\text{CH}_3)_2\text{NH}_2\text{HCl}$</td>
<td>83</td>
<td>oil</td>
<td>+8.49 ($c = 0.6$, MeOH)</td>
<td>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)$^a$</td>
<td>23.0, 28.5, 30.0, 33.1 (CD$_2$), 40.0, 50.3, 52.8, 170.2$^b$</td>
</tr>
</tbody>
</table>

$^a$ Recorded at 500 MHz.
$^b$ Recorded at 125 MHz.
gave the pure deuterated N-Boc-amino alcohol 4b; yield: 0.43 g (94%).

HRMS-EI: m/z calcld for C8H14D2NO2: 177.1332; found: 177.1342.

2.2-2H-N-Boc-β-amin Nitrile 5b
To a magnetically stirred suspension of anhyd polystyryl diphenylphosphine (0.56 g, 1.67 phosphate units) in anhyd CH2Cl2 (10 mL) at r.t., was added dropwise a solution of 1 (0.42 g, 1.67 mmol) in the same solvent (10 mL) in the dark and under dry N2. After 15 min, solid deuterated N-Boc-amino alcohol 4b (0.27 g, 1.52 mmol) was added in one portion to the suspension. The reaction was kept at 0 °C for 2 h (TLC monitoring: CHCl3–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 CH2Cl2. To the filtrate, under magnetical stirring, Et4N+CN– was added in one portion and the reaction kept at reflux for 3 h until complete consumption of the starting N-Boc-β-amin iodide. The cooled mixture was poured on a silica gel column (1:5) and eluted with CH3Cl. To the filtrate, under magnetical stirring, Et4N+CN– was added in one portion and the reaction kept at reflux for 3 h until complete consumption of the starting N-Boc-β-amin iodide. The cooled mixture was poured on a silica gel column (1:5) and eluted with CH3Cl. The organic solvent evaporated under reduced pressure afforded the pure deuterated N-Boc-β-amin nitrile 5b; yield: 0.25 g (90%).

HRMS-EI: m/z calcld for C8H14D2N2O2: 186.1335; found: 186.1346.

2.2-2H-β3-Amino Ester Hydrochloride 6b
To a magnetically stirred solution of 5b (0.24 g, 1.3 mmol) in anhyd Et2O (8 mL) at 0 °C, was added dropwise cold 12 M HCl in MeOH (8 mL) at r.t., was added dropwise a solution of 1 (0.42 g, 1.67 mmol) in the same solvent (10 mL) in the dark and under dry N2. After 15 min, solid deuterated N-Boc-amino alcohol 4b (0.27 g, 1.52 mmol) was added in one portion to the suspension. The reaction was kept at 0 °C for 2 h (TLC monitoring: CHCl3–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 CH2Cl2. To the filtrate, under magnetical stirring, Et4N+CN– was added in one portion and the reaction kept at reflux for 3 h until complete consumption of the starting N-Boc-β-amin iodide. The cooled mixture was poured on a silica gel column (1:5) and eluted with CH3Cl. The organic solvent evaporated under reduced pressure afforded the pure deuterated N-Boc-β-amin nitrile 5b; yield: 0.25 g (90%).

HRMS-EI: m/z calcld for C8H14D2N2O2: 186.1335; found: 186.1346.

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We thank Guido Smiraglia for collecting valuable results while performing his master thesis work. The authors would also like to thank Dr. Emiliano Manzo from Instituto di Chimica Biomolecolare, CNR Napoli for HRMS analysis. 1H and 13C NMR spectra were recorded at Centro Interdepartimentale di Metodologie Chimico-Fisiche, Università di Napoli Federico II. Varian Inova 500 MHz instrument is a property of Consortio Interuniversitario Nazionale La Chimica per l’Ambiente (INCA) and was used in the frame of a project by INCA and M.I.U.R. (L. 488/92, Cluster 11-A).

References
(1) Lelais, G.; Seebach, D. Biopolymers (Peptide Science) 2004, 76, 206.
(11) Unpublished results.
(12) β-Amino acids can be subdivided into β1, β2, and β3 amino acids, depending upon the position of the side chain(s) on the aminoalkanoic acid skeleton.
(16) Ph3P–iodine complex showed the same reactivity of the polymer bound/iodine complex towards all the tested amino acids.