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Chemoenzymatic synthesis of Fmoc-protected (2*S*,3*S*)-2-hydroxy-3-amino acids and their application in the synthesis of an α-hydroxylated β-hexapeptide

Reynier A. Tromp, Michael van der Hoeven, Alessia Amore, Johannes Brussee,* Mark Overhand, Gijs A. van der Marel and Arne van der Gen

Leiden Institute of Chemistry, Gorlaeus Laboratories, Leiden University, PO Box 9502, 2300 RA Leiden, The Netherlands

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Abstract—A chemoenzymatic and stereoselective synthesis of Fmoc-protected (2*S*,3*S*)-2-hydroxy-3-amino acids from 2-furaldehyde is described as well as their application, without prior hydroxyl protection, in the solid-phase synthesis of a novel completely α-hydroxylated β-hexapeptide.

1. Introduction

Over the last decade considerable attention has been directed toward the enantioselective synthesis of β-amino acids.[1–3] These compounds not only occur in various natural products, which exhibit interesting pharmaceutical properties, including paclitaxel[4,5] and bestatin,[6] they also function as precursors for the synthesis of β-lactams.[7,8] Apart from this, it has been demonstrated that oligomers composed of β-amino acids can fold in stable secondary structures similar to those of α-peptides, i.e. helices, sheets and turns.[9–11] Most intriguing was the discovery that only six β-amino acid residues are necessary for the formation of a stable helix,[12,13] whereas an α-amino acid oligomer normally requires 15–20 residues. Formation of the helical structure of β-peptides was strongly influenced by the nature and stereochemistry of the amino acid side chains at both the α- and the β-position.[14] It is important that the substituents of the β-amino acid residues are placed equatorial with respect to the helical axis to minimise steric hindrance with the adjacent turn.

So far, the effect of polar α-side chains on the stability of the secondary structures of β-peptides has scarcely been investigated. Seebach et al. synthesised helix forming oligopeptides containing two (2*R*,3*R*)-$\beta^{2,3}$-amino acids[15] with two serine or cysteine side chains.[16] Recently, Motorina and co-workers showed that a hexapeptide composed of (2*R*,3*S*)-2-silyloxy-3-amino acids formed stable β-strands.[17] Finally, Wen et al. have reported the synthesis of β-peptides containing guanidino side groups.[18] Our interest is focused on the influence of an unprotected α-hydroxyl function on the formation and stability of the secondary structures of β-peptides. In this paper we present a chemoenzymatic route towards a novel completely α-hydroxylated β-hexapeptide. For structural comparison, the α-hydroxylated analogue of the 3_{14} helix[19] forming β-hexapeptide H-($\beta^{2,3}$-Val(α-Me)-$\beta^{2,3}$-Ala(α-Me)-$\beta^{2,3}$-Leu(α-Me))$_2$-OH,[20] was synthesised.

2. Results and discussion

Several routes towards (2*S*,3*S*)-2-hydroxy-3-amino acids have been described in the literature.[2] An earlier route developed in our laboratories[21] proved to give low diastereoselectivity for hindered side groups (d.e. $\leq$20%).[22] Herein, we present a short chemoenzymatic route towards Fmoc-protected α-hydroxy-β-amino acids, starting from 2-furaldehyde, thereby increasing the d.e. and simultaneously decreasing the number of steps (Scheme 1).[23]

Enantioselective addition of HCN to 2-furaldehyde catalysed by R-oxynitrilase (E.C. 4.1.2.10) (as present in defatted almond meal)[24] afforded the cyanohydrin with high enantiomeric excess (e.e. = 98%). Subsequent silyl protection using TBS-Cl and imidazole in DMF

* Corresponding author. E-mail: brussee@chem.leidenuniv.nl

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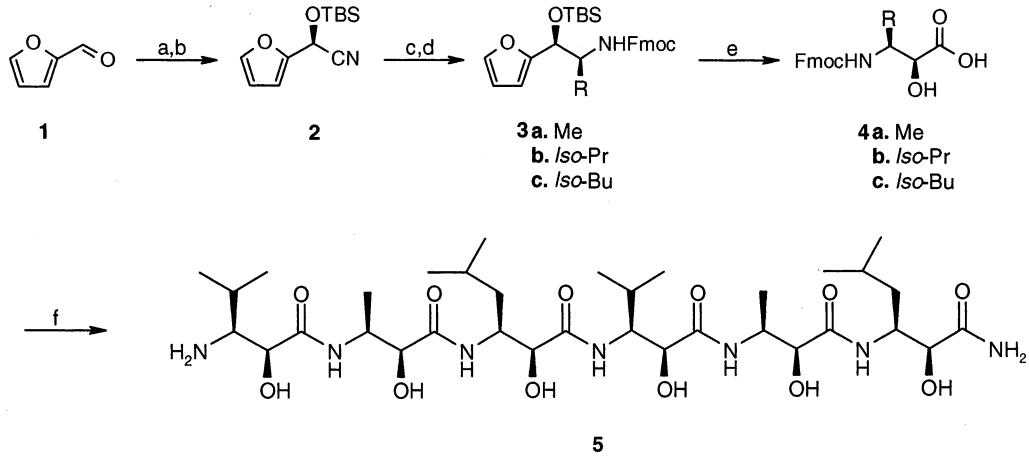

Scheme 1. (a) R-Oxynitrilase, HCN, MTBE, pH 5.5, 4°C; (b) TBS-Cl (1.2 equiv.), imidazole (2.4 equiv.), DMF, 0°C to rt; (c) 1. RMgX (1.2 equiv.), Et_2O, reflux; 2. MeOH, −20°C; 3. $NaBH_4$ (2 equiv.), −70°C to rt; (d) Fmoc-Cl (1.4 equiv.), CH_2Cl_2, $NaHCO_3$ (aq.), 0°C to rt; (e) 1. O_3, MeOH, −70°C; 2. THF/MeCN/H_2O, 40°C, 3 h; (f) Rink amide MBHA, HOBt, BOP, DIPEA and NMP.

gave **2** in high yield (88% from **1**).[25] Next, the amino acid side chains for the required α-hydroxylated β-alanine, β-valine and β-leucine were introduced by Grignard reaction. The silyl protected cyanohydrin **2** was dissolved in diethyl ether and a solution of the Grignard reagent RMgX (R = Me, *iso*-Pr, *iso*-Bu; X = I, Cl) was added dropwise. After 1 h of reflux, the mixture was cooled to −20°C. Dry methanol was added to destroy excess Grignard reagent and liberate the free imine. The solution was further cooled to −70°C, after which the amino function was obtained by reduction with sodium borohydride, which occurred with good selectivity (Table 1).[26,27] The crude amino alcohols were then protected with 9-fluorenylmethyloxocarbonyl (Fmoc) to give the fully protected amino alcohols **3a**–**3c**. Valine analogue **3b** was initially obtained in only 20–25% (from **2**). The yield was greatly improved (48%) by addition of a catalytic amount of Cu(I)Br, using THF as solvent,[28] and by using 2 equiv. of Fmoc-Cl and 1 equiv. of DIPEA in dry dichloromethane for protection of the amino group.[29]

Finally, the Fmoc-protected α-hydroxy-β-amino acids were obtained by a one-pot oxidation–deprotection procedure. Upon ozonolysis of the furan ring of compounds **3a**–**3c** at low temperature,[30] the TBS group partly migrated to the newly formed carboxylic acid, due to the presence of the amino group at the β-position.[31] To force the deprotection to completion, the solvent was evaporated and the crude product was left to stir for 3 h in THF/MeCN/H_2O (3/1/1) at 40°C. The required amino acids **4a**–**4c** could be obtained in pure form and with high d.e. by crystallisation from ethyl acetate and pentane in 61, 52 and 61% yield, respectively. After one recrystallisation, no diastereomeric impurities were visible in the ^1H NMR spectrum.

The *N*-protected α-hydroxy-β-amino acids **4a**–**4c** were used in the construction of a novel α-hydroxylated β-hexapeptide by solid-phase synthesis using Rink amide MBHA as resin, without prior protection of the hydroxyl function. Conventional procedures for solid-phase peptide synthesis were applied (Scheme 1).[32] Remarkably, after lyophilisation, the newly formed β-hexapeptide proved to be slightly soluble in most solvents, including water, methanol, acetonitrile, chloroform, ethyl acetate, 2,2,2-trifluoroethanol and hexafluoro-acetone. It could be dissolved in pyridine and TFA. So, impurities could be easily removed by washing the peptide with water/acetonitrile (80/20) yielding the pure β-hexapeptide (36%), as confirmed by LCMS and NMR spectroscopy.

^1H NMR spectra (in pyridine-d_5) showed all methyl groups resolved. Also, the protons of the methylene group of each leucine residue were found to have different chemical shifts, indicating restricted mobility.

Table 1. Diastereoselectivity of the Grignard addition–reduction sequence

	R	(2*S*,3*S*)/(2*S*,3*R*)-**3** (%)[a]
3a	Me	19/1 (80)
3b	*iso*-Pr	9/1 (48)
3c	*iso*-Bu	5/1 (61)

[a] (2*S*,3*S*)/(2*S*,3*R*)-Ratio determined by NMR.
Yields (in parenthesis) after purification from **2**.

Table 2. Chemical shifts and coupling constants of the amide and H_α protons of hexapeptide **5**

	δ (NH)[a]	J(NH,H_β)[b]	δ (H_α)[a]	J(H_α,H_β)[b]
Val[1]			5.10	4.4
Ala[2]	8.85	8.6	4.76	3.7
Leu[3]	8.42	9.4	4.81	3.0
Val[4]	8.32	9.7	4.70	4.4
Ala[5]	8.45	8.7	4.82	3.0
Leu[6]	8.39	9.3	4.81	3.0

[a] Chemical shift (δ) in ppm.
[b] Coupling constant J in Hz.

As has been found by other groups,[16,17,33,34] NH peaks all contained coupling constants ranging between 8.6 and 9.7 Hz (Table 2). The coupling constant between $H_{\alpha,i}$ and $H_{\beta,i}$ proved to be in the range of 3–4.4 Hz, unlike those reported for the helix forming β-peptides, including the α-methylated $\beta^{2,3}$-hexapeptide by Seebach et al. ($J = 9.3$–11.3 Hz).[20] Except for the NH_2 protons at the C-terminus, no interactions between amide protons were observed by NOESY experiments (Fig. 1). Furthermore, NOESY experiments indicated that no long-range interactions between residues *i* and *i*+2 or *i*+3 were present, as has been reported for folded β-peptides, including helices.[16,20,33–35]

Thus, although the stereochemistry of the residues was chosen to enable helix formation, first findings indicate that in pyridine a stable secondary structure is formed, which is probably not helical in character. Currently we are investigating the nature of the secondary structure using extensive NMR techniques (e.g. NOESY and ROESY) supported by calculations.

3. Conclusions

In summary, a new straightforward and stereoselective route towards Fmoc-protected (2*S*,3*S*)-2-hydroxy-3-amino acids has been developed, starting from 2-furaldehyde. It has been demonstrated that these α-hydroxy-β-amino acids can be used for solid-phase synthesis of α-hydroxylated β-oligopeptides without protection at the hydroxyl function. NMR studies on the resulting α-hydroxylated β-hexapeptide gave strong indications that in pyridine no helical structure was formed. Thus, the presence of unprotected α-hydroxyl groups have a great influence on the formation of the secondary structure.

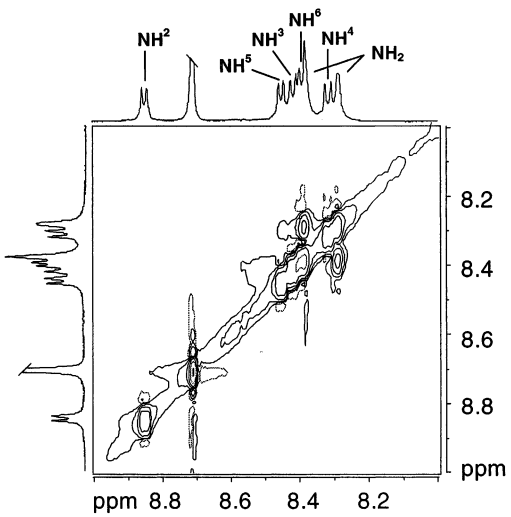

Figure 1. NH region of the NOESY spectrum (600 MHz) of hexapeptide **5** in pyridine-d_5.

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