Tetrahedron: *Asymmetry* 20 (2009) 1103–1105

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

journal homepage: www.elsevier.com/locate/tetasy

Asymmetric synthesis of suitably protected γ-hydroxy-aza-β^3-homothreonine building blocks

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ARTICLE INFO

Article history:
Received 26 February 2009
Accepted 18 March 2009
Available online 22 April 2009

ABSTRACT

An efficient and easily applicable method for the enantioselective synthesis of γ-Hydroxy aza-β^3-homothreonine (aza-β^3-Hyht) has been established. The method involves stereoselective reductive amination of glyoxylic acid and the corresponding Fmoc protected chiral hydrazine. A stereoselectivity of 99% was achieved for each step using (*R*)-2,3-*O*-isopropylideneglyceraldehyde as the chiral auxiliary. This new synthetic monomer is a useful building block for the solid-phase synthesis of new peptidomimetics.

1. Introduction

Hydroxylated aminoacids are biologically of major importance as natural products. They are components of various cyclopeptides such as vancomycin and cyclosporine.[1] The best characterized are γ-hydroxy-Pro (Hyp) and δ-hydroxy-Lys (Hyl), which are commonly found in collagen.[2] γ-Hydroxy-Arg (Hya) has been found as part of the sequence of polyphenolic proteins that form the adhesive plaques of marine mussel species.[3] The hydroxylation of Arg provides trypsin resistance to these mussel glue proteins. Recently, an unexpected modified residue, γ-hydroxy-D-valine (D-Hyv), was identified within ribosomally expressed polypeptide chains of four conopeptides from the venom of Conus gladiator and Conus mus.[4] These conopeptides were the first known examples of a naturally occurring polypeptide chain containing Hyv. In general, γ-hydroxy-amino acids are not that common in Nature (except γ-hydroxy-Pro) since a hydroxyl group in the γ-position can undergo intramolecular cyclization to form a lactone, cleaving the peptide bond.[5] The stability of Hyv within conopeptides has been explained by the configuration at the R-carbon in conjunction with specific interactions with the surrounding aminoacids.[4]

Although hydroxy threonine (Hyt) has been reported as an intermediate in the biosynthesis of vitamin B6[6] to the best of our knowledge lobocyclamide B, a cyclic natural peptide, was the first peptide exhibiting antifungal activity against fluconazole-resistant *Candida albicans*.[7] No analogues of such an amino acid have yet been described.

As part of our research program aimed at developing new peptide analogues with potentially useful biological properties, we have developed a synthesis strategy for aza-β^3-amino acids.[8–10] For our ongoing projects on the synthesis of aza-β^3-peptides[11–13] we need to have ample access to the aza-β^3-amino acid building blocks.

Aza-β^3-Hyht may be a useful building block for studying interactions in biological systems and would be the first monomer of this series with a stereogenic centeras well as peptoids containing chiral centers in their side chains, the existence of stable chiral conformations in solution could be demonstrated.[14–16] So, we have presently considered the synthesis of an aza-β^3-Hyht derivative suitably protected for Fmoc solid-phase chemistry and its subsequent incorporation into a peptide sequence.

2. Results and discussion

The synthesis of aza-β^3-Hyht derivative is not as complicated as Hyt by the fact that aza β^3-Hyht analogue contains only one asymmetric center.

(*R*)-2,3-*O*-Isopropylidene-D-glyceraldehyde **2** (Scheme 2) prepared[17] from di-*O*-isopropylidene-D-mannitol, was treated with Fmoc-carbazate **1** in diethyl ether.[18] The condensation leads to a crude product which was purified by chromatography on silica gel to give (*S*)-Fmoc-hydrazone **3** as one isomer in 98% yield from di-*O*-isopropylidene-D-mannitol. The ^{1}H NMR and ^{13}C NMR spectra were recorded and the structure of **3** was confirmed.[19] HPLC analysis (Chiralcel OD-H column) indicated that the (*S*)-Fmoc-hydrazone obtained was virtually enantiomerically pure (99.9%). The Fmoc-hydrazone **3** was then reduced in hydrazine **4**[20] with 1.5-fold excess of cyanoborohydride, according to a literature procedure.[12,13]

The corresponding protected aza-β^3-amino acid was then obtained by reductive amination of glyoxylic acid and N$^{\beta}$-Fmoc protected-N$^{\alpha}$-substituted hydrazine **4** (Scheme 1). Enantiomerically pure enantiomer Fmoc-aza-β^3-Hyht **5** was obtained in 90% yield.[21] Chiral GC analysis of purified compounds showed enantioselectivities of 99% ee.[22]

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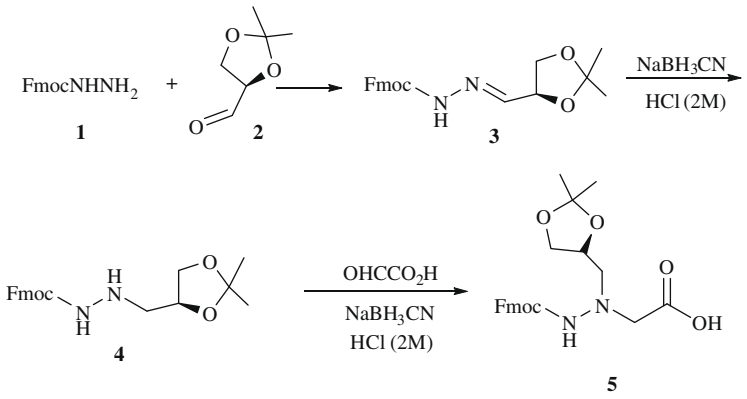
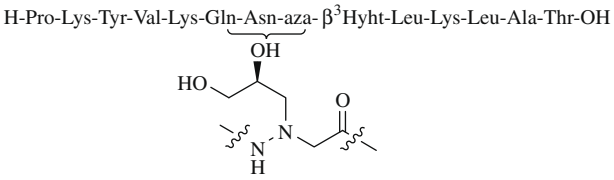
doi:10.1016/j.tetasy.2009.03.019



Scheme 1. Synthesis of Fmoc-γ-hydroxy aza-β^3-homoThr.

Fmoc-aza-β^3-Hyht-OH could be subsequently utilized for the solid-phase synthesis of a hybrid peptide using a Fmoc/*t*-Bu strategy. For this, coupling of such a monomer on the solid phase during the peptide construction must be efficient. The coupling of this new monomer was exemplified by the solid-phase synthesis of aza-β^3-peptide mimetics of a biologically active sequence corresponding to the antigenic peptide T307-319 derived from the influenza virus protein. Coupling aza-β^3-Hyht-OH or α-amino acids on a preloaded resin led to the expected peptide analogues with 52% yield and 99% purity (Scheme 2). Coupling (TBTU, DIEA), deprotection cycles (piperidine 20%), cleavage from the resin and removal of the protecting groups (TFA 95%/TIS 2.5%/H_2O 2.5%), and purification are identical to those for peptides.



Scheme 2. Synthesis of a peptide including γ-hydroxy aza-β^3-homoThr (aza-β^3-Hyht).

3. Conclusion

An efficient three-step synthesis of a chiral aza-β^3-Hyht building block, has been developed (Scheme 1). All of the steps lead to enantiomerically pure compounds. Moreover, these monomers could be coupled allowing the preparation of oligomers or mixed peptides on solid-phase support. The solid-phase synthesis of aza-β^3-Hyht-containing sequences was described and biological assays on hybrid peptides will be published later.

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19. *(4R)-2,2-Dimethyl-1,3-dioxolane, 7-fluorenyl methyl oxycarbonyl 4-hydrazone* **3**: To Fmoc-NH_2-NH_2 (Fmoc carbazate) (4.3 mmol; 5.62 g) dissolved in DCM, aldehyde **2** was added (4.3 mmol; 10.98 g). The mixture was stirred for 12 h under room temperature. After removal of DCM, ether was added to precipitate hydrazone **3**. The white powder was collected by filtration (15.37 g, 97%). Mp 174–175 °C; $[\alpha]_D^{20}$ +12.4 (*c* 0.005, EtOH); GC analysis: 22.86 min; ^{1}H NMR ($CDCl_3$, 200 MHz) δ ppm: 8.2 (s, 1H, CHβ), 7.28–7.80 (m, 8H, CH_{ar}), 4.67–4.55 (m, H, CHγ), 4.55 (d, *J* = 6.5 Hz, 2H, CH_{2Fmoc}), 4.29 (t, *J* = 7.2 Hz, H, CH_{Fmoc}), 4.19–4.29 (m, 2H, $CH_2\delta$), 1.49 (s, 3H, $CH_3\varepsilon$), 1.43 (s, 3H, $CH_3\varepsilon$); ^{13}C NMR (DMSO, 300 MHz) δ ppm: 153.7 (CO_{Fmoc}), 145.6 (CH_β) 144.1 141.3 ($2\times 2C_{ar(Fmoc)}$), 128.1 127.6 125.6 120.6 (CH_{ar}), 109.6 (C_ε), 75.5 (CH_γ), 67.1 ($CH_{2\delta}$), 66.2 (CH_{2Fmoc}), 47.0 (CH_{Fmoc}), 26.9 ($CH_{3\varphi}$), 25.8 ($CH_{3\varphi}$); HRMS (ESI) *m*/*z* calcd for $C_{21}H_{22}N_2O_4Na$ $[M+Na]^+$: 389.14773, found : 389.1469 ; for $C_{21}H_{22}N_2O_4$ $[M+K]^+$: 405.12167, found : 405.1222.
20. *(4R)-2,2-Dimethyl-1,3-dioxolane, 7-fluorenyl methyl oxycarbonyl 4-hydrazine* **4**: Hydrazone **3** (10.9 mmol, 4 g) was dissolved in a mixture of DCM/MeOH (1/2). Sodium cyanoborohydride (1.2 equiv, 13.1 mmol, 4 g) was added and pH was adjusted to 3 by slowly adding a solution of 2 M HCl. The mixture was stirred over a period of 2 h, then the pH was adjusted to 1. After 10 min of stirring, the solution was neutralized with solid $NaHCO_3$, the mixture was filtrated, concentrated under vacuum, and the residue was taken up with EtOAc and washed with water and brine. The organic layer was dried over Na_2SO_4 and concentrated and was removed to obtain a colorless oil: the corresponding hydrazine **4** (4 g, 99%). Mp 82–84 °C, $[\alpha]_D^{20}$ +4.6 (*c* 0.005, EtOH); GC analysis: 22.91 min; ^{1}H NMR ($CDCl_3$, 200 MHz) δ ppm : 7.29–7.82 (m, 8H, CH_{ar}), 6.61 (s, 1H, NH), 4.48 (d, 2H, *J* = 5.93 Hz, CH_{2Fmoc}) 4.25 (t, 1H, *J* = 6.7 Hz, CH_{Fmoc}), 3.91–4.09 (m, 2H, $CH_{2\beta}$), 3.66 (t, 1H, CHγ), 2.79–3.06 (m, 2H, $CH_{2\delta}$), 1.45 (s, 3H, $CH_{3\varepsilon}$) 1.38 (s, 3H, $CH_{3\varepsilon}$); ^{13}C NMR ($CDCl_3$, 300 MHz) δ ppm : 157.5 (CO_{Fmoc}), 143.8 141.3 ($2\times 2C_{ar(Fmoc)}$), 127.8, 127.1, 125.0, 120.0 (CH_{ar}), 109.3 (C_ε), 74.1 (CH_γ), 67.4 ($CH_{2\delta}$), 66.7 (CH_{2Fmoc}), 54.3 ($CH_{2\beta}$), 47.2 (CH_{Fmoc}), 26.9 ($CH_{3\varphi}$), 25.5 ($CH_{3\varphi}$); HRMS (ESI) *m*/*z* calcd for $C_{21}H_{25}N_2O_4$ $[M+H]^+$: 369.18143, found: 369.1836; $C_{21}H_{24}N_2O_4Na$ $[M+Na]^+$: 369.16338, found: 391.1637; for $C_{21}H_{24}N_2O_4K$ $[M+K]^+$: 407.13732, found: 407.1388.
21. *Fmoc-aza-β^3-Gly ((4R)-2,2-dimethyl-1,3-dioxolane)-OH or Fmoc-aza-β^3-Hyht* **5**: To hydrazine **4** (10.85 mmol, 4 g) dissolved in a mixture of DCM/MeOH (8/15), glyoxilic acid (1.2 equiv, 13.03 mmol, 1.2 g) was added and both were stirred. Sodium cyanoborohydride (1.5 equiv, 16.28 mmol, 1.2 g) was poured into the mixture under stirring for 2 h. The reaction was stopped by adjusting pH to 3 with the addition of some TFA drops. After 10 min, the pH was adjusted to 5 with a saturated solution of $NaHCO_3$; then, DCM and MeOH were removed by evaporation under vacuum. The residue was taken up with EtOAc and washed

with water and brine. The organic layer was dried over Na_2SO_4, concentrated under vacuum to give a yellow oil and purified by chromatography on silica gel (DCM/EtOAc 9/1 as eluent) to afford a crystalline solid **5** by concentration under reduced pressure (3.82 g, 82.6%). Mp 80–83 °C; $[\alpha]_D^{20}$ +5.8 (*c* 0.005, EtOH); ^{1}H NMR ($CDCl_3$, 200 MHz) δ ppm: 7.19–7.80 (m, 8H, CH_{ar}), 7.1 (s, 1H, NH), 4.35 (d, 2H, CH_{2Fmoc}), 4.06–4.27 (m, 1H, CH_{Fmoc}), 3.86–4.08 (m, 2H, $CH_{2\alpha}$), 3.56 - 3.73 (m, 1H, $CH\gamma$), 3.38–3.52 (m, 2H, $CH_{2\beta}$), 2.67–3.00 (m, 2H, $CH_{2\delta}$) 1.45 (s, 3H, $CH_{3\varepsilon}$) 1.32 (s, 3H, $CH_{3\varepsilon}$); ^{13}C NMR ($CDCl_3$, 300 MHz) δ ppm: 173.4 (*C*OOH), 156.9 (*C*O$_{Fmoc}$), 143.5, 141.3 (2 × 2C$_{ar(Fmoc)}$), 127.8, 127.1, 125.0, 120.0 (CH_{ar}), 109.6 (C_ε), 73.6 (CH_γ), 67.6 ($CH_{2\delta}$), 67.2 (CH_{2Fmoc}), 60.0 ($CH_{2\alpha}$), 59.1 ($CH_{2\beta}$), 47.1 (CH_{Fmoc}), 26.8 ($CH_{3\varphi}$), 25.4 ($CH_{3\varphi}$); HRMS (ESI) *m*/*z* calcd for $C_{23}H_{25}N_2O_6Na_2$ $[M-H+2Na]^+$: 471.1508, found 471.1482, for $C_{23}H_{26}N_2O_6Na$ $[M+Na]^+$: 449.1689, found: 449.1649.

22. Enantiomeric purities of all purified enantiomers were determined by chiral GC (CP-chirasil-dex Column operated at 120 °C and programmed to 200 °C at 1°/min. FID detector).