SUPPORTING INFORMATION

Synthesis and solid-phase application of suitably protected γ -hydroxyvaline building blocks

Mare Cudic, Frank Marí, and Gregg B. Fields*

Department of Chemistry & Biochemistry, Florida Atlantic University,
777 Glades Road, Boca Raton, FL 33431 U.S.A.
Phone 561-297-2093, Fax 561-297-2759, e-mail fieldsg@fau.edu

Table of Contents

General Experimental Methods	S3
Figure 1: One-dimensional ^1H -NMR spectrum of D-Hyv 1	S4
Figure 2: One-dimensional ^1H -NMR spectrum of Boc-D-Hyv lactone 2c	S5
Figure 3: One-dimensional ^1H -NMR spectrum of Cbz-D-Hyv lactone 3c	S6
Figure 4: One-dimensional ^1H -NMR spectrum of Cbz-D-Hyv lactone 3d	S7
Figure 5: One-dimensional ^1H -NMR spectrum of purified Fmoc-D-Hyv(<i>O</i> -TBDMS) 9a obtained using the 9-BBN complexation method	S8
Figure 6: RP-HPLC elution profile of purified Fmoc-D-Hyv(<i>O</i> -TBDMS) 9a obtained using the BBN complexation method	S9
Figure 7: One-dimensional ^1H -NMR spectrum of purified Fmoc-D-Hyv(<i>O</i> -TBDMS) 9a and 9b obtained using the copper complexation method	S10
Figure 8: MALDI-TOF mass spectrum of Fmoc-D-Hyv(<i>O</i> -TBDMS) 9a and 9b obtained using the copper complexation method	S11

Figure 9: RP-HPLC elution profile of purified Fmoc-D-Hyv(<i>O</i> -TBDMS) 9a and 9b obtained using the copper complexation method	S12
Figure 10: RP-HPLC elution profile of purified Fmoc-D-Hyv(<i>O</i> -TBDMS) 9a obtained following the copper complexation method	S13
Figure 11: RP-HPLC elution profile of purified Fmoc-D-Hyv(<i>O</i> -TBDMS) 9b obtained following the copper complexation method	S14
Figure 12: MALDI-TOF mass spectrum of purified Ala-Hyp-Ala-Asn-Ser-D-Hyv- Trp-Ser-NH ₂ ,	S15
Figure 13: RP-HPLC elution profile of purified Ala-Hyp-Ala-Asn-Ser-D-Hyv- Trp-Ser-NH ₂ , synthesized using Fmoc-D-Hyv(<i>O</i> -TBDMS) 9a and 9b	S16
Figure 14: One-dimensional ¹ H NMR spectra of gld- <u>Y</u> * from <i>Conus gladiator</i> and synthetic gld- <u>Y</u> *	S17

General Experimental Methods

All air- and moisture-sensitive reactions were performed under argon atmosphere and in the presence of molecular sieves (4 Å). The progress of reactions was monitored by TLC on silica gel 60 F₂₅₄ plates with detection by UV light and/or charring with ninhydrin. Flash column chromatography was performed on silica gel columns (60 Å, 230-400 mesh). ¹H NMR spectra were recorded with either a 400 MHz or 500 MHz NMR spectrometer at 298 K. ¹H chemical shifts were expressed relative to tetramethylsilane as an internal standard.

Analytical RP-HPLC was performed on a C₁₈ protein and peptide column (5 µm particle size, 120 Å pore size, 100 x 2.1 mm) at a flow rate of 1 mL/min. The elution gradient was 0-100% B over 20 min where A was 0.1% TFA in H₂O and B was 0.1% TFA in CH₃CN. Diode array detection was at λ = 220, 254, and 280 nm. Preparative RP-HPLC was performed with a C₁₈ column (15-20 µm particle size, 300 Å pore size, 250 x 22 mm) at a flow rate of 10.0 mL/min. The elution gradient was 0-60% B over 60 min where A was 0.1% TFA in H₂O and B was 0.1% TFA in CH₃CN. Detection was at λ = 220 nm.

Figure 1. One-dimensional ^1H -NMR spectrum of D-Hyv **1**. ^1H NMR (D_2O): δ 1.21 (d, 3H, **1a**-Hyv- CH_3), δ 1.12 (d, 3H, **1b**-Hyv- CH_3), 2.8 (m, 1H, **1a**-Hyv- CH_β), 3.0 (m, 1H, **1b**-Hyv- CH_β), 3.98-4.10 (m, 3H, **1a**-Hyv- CH_2 , **1b**-Hyv- CH_2 , **1a**-Hyv- CH_α), 4.51-4.62 (m, 3H, **1a**-Hyv- CH_2 , **1b**-Hyv- CH_2 , **1b**-Hyv- CH_α).

Figure 2. One-dimensional ^1H -NMR spectrum of Boc-D-Hyv lactone **2c**. ^1H NMR (CDCl_3): δ 1.21 (d, 3H, Hyv- CH_3), 1.43 (s, 9H, Boc- CH_3), 2.49 (m, 1H, Hyv- CH_β), 3.81 (t, 1H, Hyv- CH_2), 4.08 (t, 1H, Hyv- CH_α), 4.40 (t, 1H, Hyv- CH_2), 4.90 (br s, 1H, Hyv-NH).

Figure 3. One-dimensional ^1H -NMR spectrum of Cbz-D-Hyv lactone **3c**. ^1H NMR (CDCl_3): δ 1.18 (d, 3H, Hyv- CH_3), 2.45 (m, 1H, Hyv- CH_β), 3.80 (t, 1H, Hyv- CH_2), 4.18 (t, 1H, Hyv- CH_α), 4.38 (t, 1H, Hyv- CH_2), 5.12 (s, 2H, Cbz- CH_2), 5.21 (br s, 1H, Hyv-NH), 7.36 (s, Cbz-ArH).

Figure 4. One-dimensional ^1H -NMR spectrum of Cbz-D-Hyv lactone **3d**. ^1H NMR (CDCl_3): δ 0.98 (d, 3H, Hyv- CH_3), 2.90 (m, 1H, Hyv- CH_β), 4.38 (t, 1H, Hyv- CH_2), 4.54 (t, 1H, Hyv- CH_α), 4.85 (t, 1H, Hyv- CH_2), 5.18 (s, 2H, Cbz- CH_2), 5.42 (br s, 1H, Hyv-NH), 7.32 (s, Cbz-ArH).

Figure 5. One-dimensional ^1H -NMR spectrum of purified Fmoc-D-Hyv(*O*-TBDMS) **9a** obtained using the 9-BBN complexation method (Scheme 2). ^1H NMR (CDCl_3): δ 0.11 (s, 6H, TBDMS), 0.95 (s, 12H, TBDMS), 1.02 (d, 3H, Hyv- CH_3), 2.38 (m, 1H, Hyv- CH_β), 3.65 (t, 2H, Hyv- CH_2), 4.21-4.58 (m, 3H, Fmoc- CH_2 , Hyv- CH_α), 5.98 (br s, 1H, Hyv-NH), 7.25-7.81 (m, Fmoc-ArH).

Figure 6. RP-HPLC elution profile of purified Fmoc-D-Hyv(*O*-TBDMS) **9a** obtained using the BBN complexation method (Scheme 2). Fmoc-D-Hyv(*O*-TBDMS) **9a** elutes at 17.652 min. Conditions are given in the General Experimental Methods.

Figure 7. One-dimensional ^1H -NMR spectrum of purified Fmoc-D-Hyv(*O*-TBDMS) **9a** and **9b** obtained using the copper complexation method (Scheme 3). ^1H NMR (CDCl_3): **9a** (major) and **9b** (minor) isomers δ 0.10 (s, 6H, TBDMS), 0.93 (s, 12H, TBDMS), 1.02 (d, 3H, Hyv- CH_3), 1.12 (d, 3H, Hyv- CH_3), 2.38 (m, 1H, Hyv- CH_β), 2.40 (m, 1H, Hyv- CH_β), 3.60 (t, 2H, Hyv- CH_2), 3.85 (t, 2H, Hyv- CH_2), 4.22-4.59 (m, 3H, Fmoc- CH_2 , Hyv- CH_α), 5.95 (d 1H, Hyv-NH), 6.24 (d, 1H, Hyv-NH), 7.31-7.80 (m, Fmoc-ArH).

Figure 8. MALDI-TOF mass spectrum of Fmoc-D-Hyv(*O*-TBDMS) **9a** and **9b** obtained using the copper complexation method (Scheme 3). The mass observed at 492.52 corresponds to $[M+Na]^+$ for Fmoc-D-Hyv(*O*-TBDMS) (calculated $[M+Na]^+$ 492.62).

Figure 9. RP-HPLC elution profile of purified Fmoc-D-Hyv(*O*-TBDMS) **9a** and **9b** obtained using the copper complexation method (Scheme 3). Fmoc-D-Hyv(*O*-TBDMS) **9a** elutes at 17.673 min (see Figure 6), while **9b** elutes at 17.926 min. Conditions are given in the General Experimental Methods.

Figure 10. RP-HPLC elution profile of purified Fmoc-D-Hyv(*O*-TBDMS) **9a** obtained following the copper complexation method (Scheme 3). Conditions are given in the General Experimental Methods.

Figure 11. RP-HPLC elution profile of purified Fmoc-D-Hyv(*O*-TBDMS) **9b** obtained following the copper complexation method (Scheme 3). Conditions are given in the General Experimental Methods.

Figure 12. MALDI-TOF mass spectrum of purified Ala-Hyp-Ala-Asn-Ser-D-Hyv-Trp-Ser-NH₂, synthesized using Fmoc-D-Hyv(*O*-TBDMS) **9a** and **9b**. The masses observed at 863.49 and 885.48 correspond to [M+H]⁺ and [M+Na]⁺, respectively, for Ala-Hyp-Ala-Asn-Ser-D-Hyv-Trp-Ser-NH₂ (calculated [M+H]⁺ 862.89, [M+Na]⁺ 884.87).

Figure 13. RP-HPLC elution profile of purified Ala-Hyp-Ala-Asn-Ser-D-Hyv-Trp-Ser-NH₂, synthesized using Fmoc-D-Hyv(*O*-TBDMS) **9a** and **9b**. Conditions are given in the General Experimental Methods.

Figure 14. One-dimensional ^1H NMR spectra of (top) gld-V* from *Conus gladiator* and (bottom) synthetic gld-V*. Assignments for gld-V* were given previously in reference 3.