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A New Amino Acid Derivative with a Masked Side-chain Aldehyde and its Use in Peptide Synthesis and Chemoselective Ligation

JANE C. SPETZLER and THOMAS HOEG-JENSEN*

Novo Nordisk A/S, Bagsvaerd, Denmark

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Abstract: A new amino acid derivative with a diol side-chain, L-2-amino-4,5-dihydroxy-pentanoic acid (Adi), has been prepared from L-allylglycine by suitable protection, for use in peptide synthesis, as Fmoc-L-Adi(Trt)$_2$. This building block enables the introduction of a side-chain aldehyde at any position in a given peptide sequence without use of specialized side-chain protection schemes. The aldehyde is revealed by mild oxidation with sodium periodate, circumventing the problematic release of reactive peptidic aldehydes in TFA solution. Peptides with aldehyde side-chains are useful for chemo-selective ligation, reacting selectively with oxyamines to yield oxime links, while all other peptide functions can be left unprotected. The utility of the new building block has been demonstrated by the synthesis of peptide dimers and a cyclo-peptide. Copyright © 2001 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: Adi; ligation; oxime; oxyamine; peptide aldehyde

INTRODUCTION

Chemoselective ligation has been established as a powerful tool for the chemical synthesis of peptide constructs such as peptide dimers, templated peptides, cyclo-peptides, glyco-peptides and proteins [1,2]. Ligation techniques call for the synthesis of peptides bearing a functional group which under given conditions reacts selectively with a partner function, allowing all other functions to be left unprotected. Examples of useful partner functions for chemoselective ligation are thiols with alkyl bromides [3], aldehydes with oxyamines [4,5], and thioesters with 2-amino thiols [6,7].

The aldehyde-oxyamine combination yields an oxime bond, and this chemistry is well suited for use in the side-chain modification of peptides. Obviously, the required functional groups are not found in the commonly available amino acids. The oxyamine functionality has most often been introduced by acylation of peptide *N*-termini or lysine side-chains using a small building block such as protected aminoxy acetic acid [8]. Apart from the extra synthetic steps required in such procedures, the resulting side-chain constructs are relatively long and flexible, and this contrasts with the common desire to keep high rigidity in ligands for biological receptors. We have recently reported the synthesis of suitably protected *O*-aminoserine (Ams) [9], thus allowing the synthesis of peptides with side-chain oxyamines in any given position by straightforward Fmoc-based solid-phase peptide synthesis, without the need for specialized protecting group strategies. Furthermore, the Ams side-chain is very short and in compliance with the general desire for high rigidity. Homocanaline is an alternative, although the side-chain is longer and the amino acid synthesis more complex [10].

Peptide aldehydes are, apart from their use in ligations and other reactions, also known from work in fields such as protease inhibition [11]. For ligation purposes, they have usually been generated like oxyamino peptides, by coupling suitable building blocks to amino groups at *N*-terminal or side-chain positions [5,12–15].

* Correspondence to: Novo Nordisk A/S, Novo Alle 6BS.58, DK-2880 Bagsvaerd, Denmark; e-mail: tshj@novonordisk.com

We report here the design and synthesis of an amino acid building block with a built-in diol in a suitable position. By protecting the amino acid, 2-amino-4,5-dihydroxy-pentanoic acid (Adi), as Fmoc-Adi(Trt)$_2$, we enable the straightforward solid-phase synthesis of peptides with a short diol side-chain in any desired position; mild oxidation gives the corresponding peptide aldehyde. The peptide aldehydes so produced have been used in chemoselective ligation to form various model constructs.

MATERIALS AND METHODS

Analytical TLC was performed on Merck silica gel 60 F_{254} alumina sheets with detection by UV light or spray reagents, as indicated below. ^{1}H- and ^{13}C-NMR spectra were recorded on a 400 MHz Varian instrument. Chemical shifts are reported relative to TMS at 0 ppm. *J*-values are given in Hz. Mass spectra of amino acid derivatives were measured by ESMS. The molecular weights of the peptides were determined using MALDI-MS, recorded on a Voyager-DE (Perseptive Biosystems, Farmingham, MA, USA), using a matrix of cinnapinic acid. Analytical and semi-preparative HPLC was performed using a Waters RCM 8×10 module with C-18 columns, 19×300 mm and 25×300 mm, respectively. The solvent system for both analytical and semi-preparative HPLC was buffer A (0.1% TFA in water) and buffer B (0.07% TFA in acetonitrile) with UV detection at 215 nm. The gradient for analytical HPLC was 5–60% buffer B at 1.0 mL/min over 30 min and for semi-preparative HPLC 5–60% buffer B at 2.0 mL/min over 40 min, unless otherwise indicated.

Fmoc-L-allylglycine 9

L-Allylglycine (2.2 g, 19.1 mmol) and $NaHCO_3$ (4.8 g, 3 equivalents) in water (60 mL) was cooled with an ice-bath. Fmoc-OSu (7.09 g, 1.1 equivalents) in dioxan (75 mL) was added with stirring. The pH-value was adjusted to 8–9 by addition of Na_2CO_3. The mixture was left with stirring at room temperature overnight. TLC (AcOEt-hexane-AcOH, 25:25:1) showed that the reaction was complete. The reaction mixture was washed with ether and acidified with 4 M HCl to pH 2. The precipitated gum was extracted with AcOEt ($2 \times$), which was washed with water, dried over $MgSO_4$ and evaporated. The crude material was recrystallized from AcOEt-hexane, to give the Fmoc-amino acid **9** (5.80 g, 90%), m.p. 134–36°C (lit. [16], 126–30°C, lit. [17], 134–35°C).

δ_H ($CDCl_3$) 2.62 (2H, m, β-CH_2), 4.23 (1H, t, *J* 6.7, Fmoc-CH), 4.42 (2H, d, *J* 6.7, Fmoc-CH_2), 4.50 (1H, m, α-H), 5.19 (2H, dd, *J* 1.4 and 12.3, C=CH_2), 5.26 (1H, br d, NH), 5.73 (1H, m, CH=Me), 7.31 (2H, t, *J* 7.4, ArH), 7.40 (2H, t, *J* 7.4, ArH), 7.58 (2H, d, *J* 7.1, ArH), 7.76 (2H, d, *J* 7.1, ArH).

δ_C ($CDCl_3$) 36.7, 47.5, 53.4, 67.5, 119.9, 120.2, 125.2, 127.3, 127.9, 131.9, 141.5, 143.8, 144.0, 156.1, 176.5.

m/*z* (ESMS) 359.8 ($M + H^+$, 70%, $C_{20}H_{19}NO_4Na^+$ requires 360.1), 179.2 (100, dibenzofulvene).

p-Methylbenzyl Fmoc-L-allylglycinate 10

Fmoc-L-allylglycine **9** (1.86 g, 5.5 mmol) was dissolved in EtOH (50 mL) and cooled with an ice-bath. Cesium carbonate (0.896 g, 0.5 equivalent) in water (10 mL) was added dropwise and the mixture was stirred for 1 h. The pH-value was now 6–7. The solvent was removed in vacuum and the crude product was re-evaporated from toluene ($3 \times$) and dried in vacuum to give the cesium salt, 2.6 g (quantitative).

The cesium salt (2.6 g, 5.5 mmol) was dissolved in DMF (25 mL) and treated with *p*-methylbenzyl bromide (0.925 g, 5.00 mmol). The mixture was heated shortly to 40°C in order to help solubility. Precipitation of cesium bromide started after 5 min. The mixture was stirred at room temperature overnight. TLC (AcOEt-hexane, 1:3) was used to determine the reaction end-point. Excess ether was added and the organic solution was washed with 5% $NaHCO_3$ ($2 \times$), 0.5 M HCl ($2 \times$) and water. After drying over $MgSO_4$, the solvent was removed in vacuum to give the crude ester **10** (1.93 g, 80%). The compound was recrystallized from hexane-ether to give pure ester (1.46 g, 60%), m.p. 101–103°C.

δ_H ($CDCl_3$) 2.35 (3H, s, $ArCH_3$), 2.49–2.65 (2H, m, β-CH_2), 4.22 (1H, t, *J* 6.8, Fmoc-CH), 4.39 (2H, d, *J* 6.8, Fmoc-CH_2), 4.49–4.54 (1H, m, α-H), 5.08–5.19 (4H, m, Bzl-CH_2 and C=CH_2), 5.34 (1H, br d, NH), 5.61–5.72 (1H, m, CH=Me), 7.16 (2H, d, *J* 7.9, Bzl-H), 7.25 (2H, d, *J* 7.9, Bzl-H), 7.31 (2H, t, *J* 7.2, ArH), 7.40 (2H, t, *J* 7.2, ArH), 7.59 (2H, d, *J* 7.8, ArH), 7.77 (2H, d, *J* 7.8, ArH).

δ_C ($CDCl_3$) 21.6, 37.1, 47.5, 53.7, 67.4, 67.6, 119.6, 120.2, 125.3, 127.3, 127.9, 128.7, 129.5, 132.1, 132.4, 138.6, 141.5, 143.9, 155.8, 167.5.

m/*z* (ESMS) 442.2 ($M + H^+$, 60%, $C_{28}H_{28}NO_4^+$ requires 442.2), 283.0 (100).

Fmoc-L-allylglycinol 13

Fmoc-L-allylglycine **9** (3.37g, 10.0 mmol) was dissolved in DCM (30 mL) under argon and the solution was cooled with an ice-salt mixture (−10°C), which resulted in some precipitation. Pyridine was added (0.81 ml, 1.0 equivalent), resulting in the dissolution of all material. Cyanuric fluoride (1.80 mL, 20.0 mmol) was added and a slowly progressing precipitation was observed (cyanuric acid). After 1 h, more DCM was added (150 mL). The solution was washed with ice-water, dried (Na_2SO_4) and evaporated (to approximately 25 mL).

Sodium borohydride (760 mg, 20.0 mmol) was added in one portion, followed by dropwise addition of methanol (20 mL) over 15 min with stirring. A slight warming was observed. The mixture was neutralized with 1 M H_2SO_4 and evaporated. AcOEt and water was added, and the organic phase was washed with 1 M H_2SO_4, 5% $NaCO_3$ and water. The solution was dried and evaporated. TLC (AcOEt-hexane-AcOH, 25:25:1) showed two main products. Recrystallization from toluene-hexane isolated the lower eluting compound, which was identified by NMR as the desired alcohol **13** (2.16 g, 67%). The high eluting compound was identified as the methyl ester (NMR data not shown).

δ_H ($CDCl_3$) 2.20–2.36 (2H, m, β-CH_2), 3.63–3.76 (2H, m, CH_2O), 4.22 (1H, t, *J* 6.7, Fmoc-CH), 4.43 (2H, d, *J* 6.7, Fmoc-CH_2), 4.84–4.92 (1H, m, α-H), 5.11–5.15 (2H, m, C=CH_2), 5.34 (1H, br d, NH), 5.71–5.83 (1H, m, CH=Me), 7.32 (2H, t, *J* 7.8, ArH), 7.40 (2H, t, *J* 7.8, ArH), 7.59 (2H, d, *J* 7.9, ArH), 7.77 (2H, d, *J* 7.9, ArH).

δ_C ($CDCl_3$) 36.0, 47.4, 52.6, 64.9, 66.7, 118.1, 119.9, 124.9, 126.9, 127.6, 133.9, 141.2, 143.7, 156.9.

m/*z* (ESMS) 346.0 ($M + Na^+$, 30%, $C_{20}H_{21}NO_4Na^+$ requires 346.1), 178.2 (100, dibenzofulvene).

Fmoc-2,2-dimethyl-4-allyl-L-oxazolidine 14

Alcohol **13** (1.62 g) in acetone (20 mL) with 2,2-dimethoxypropane (6 mL) was treated with $BF_3.OEt_2$ (0.1 mL) with ice-cooling under an argon atmosphere. After 1 h, the solution was allowed to warm to room temperature. The solvent was partly evaporated, diluted with plenty of AcOEt and washed with 5% $NaHCO_3$ and dried (Na_2SO_4). Evaporation of the solvent and drying in vacuum gave **14** as an oily material, which solidified slowly upon storing at 5°C (1.81 g, 99%). NMR indicated the presence of two rotamers (3:1).

δ_H ($CDCl_3$) 0.84 (3H, s, CH_3, minor rotamer), 0.94 (3H, s, CH_3, minor rotamer), 1.42 (3H, s, CH_3, major rotamer), 1.55 (3H, s, CH_3, major rotamer), 1.98–2.08 (2H, m, β-CH_2, major rotamer), 2.10–2.20 (2H, m, β-CH_2, minor rotamer), 3.51–3.57 (2H, m, CH_2O, minor rotamer), 3.67–3.79 (2H, m, CH_2O, major rotamer), 4.22 (1H, t, *J* 6.7, Fmoc-CH), 4.58 (2H, d, *J* 6.7, Fmoc-CH_2), 4.67–4.78 (1H, m, α-H), 4.91–5.08 (2H, m, C=CH_2), 5.40–5.51 (1H, m, CH=Me), 5.63–4.72 (1H, br d, NH), 7.32 (2H, t, *J* 7.3, ArH), 7.38 (2H, t, *J* 7.3, ArH), 7.58 (2H, d, *J* 7.2, ArH), 7.76 (2H, d, *J* 7.2, ArH).

δ_C ($CDCl_3$) 23.5, 25.3, 26.8, 37.7, 47.8, 49.6, 53.9, 56.7, 66.2, 66.6, 117.9, 120.1, 124.8, 127.2, 127.8, 134.4, 141.7, 144.2, 158.8.

m/*z* (ESMS) 363.8 ($M + H^+$, 30%, $C_{23}H_{26}NO_3^+$ requires 364.2), 178.2 (100, dibenzofulvene).

Fmoc-L-allylglycyl *O*-acetyl (1S,2S)-(+)-ephedride 15

Fmoc-L-allylglycine (510 mg, 1.51 mmol) was dissolved in DCM (20 mL) and treated with HATU (632 mg, 1.1 equivalents), HOAt (226 mg, 1.1 equivalent) and (1S,2S)-(+)-ephedrine (500 mg, 2 equivalents). All the material dissolved within 5 min. The mixture was stirred overnight, diluted with AcOEt and washed with 5% $NaHCO_3$ (2 ×), 0.5 M HCl (2 ×) and water. After drying over $MgSO_4$, the solvent was removed in vacuum to give an oil. Recrystallization of the material was attempted from toluene-hexane, but the product always separated as an oil. It was isolated by decanting and drying in vacuum to give the *N*-acyl ephedride (295 mg, 40%). This amide (95 mg, 0.2 mmol) was dissolved in pyridine-acetic anhydride (1:1, 2 mL). TLC (AcOEt-hexane-AcOH, 25:25:1) after 3 h showed no starting material. The reaction mixture was diluted with AcOEt and washed with 5% $NaHCO_3$ (2 ×), 0.5 M HCl (2 ×) and water. Upon drying over $MgSO_4$, the solvent was removed in vacuum to give the acetate **15** as an oil (81 mg, 78%).

δ_H ($CDCl_3$) 1.06 (3H, d, *J* 6.8, CH_3), 2.22 (3H, s, CH_3N), 2.35–2.43 (2H, m, β-CH_2), 3.0 (3H, s, Ac), 4.20–4.50 (4H, m, Fmoc-CH, Fmoc-CH_2 and CH–O), 4.67–4.72 (1H, m, α-H), 5.07–5.15 (2H, m, C=CH_2), 5.70–5.80 (1H, m, CH=Me), 7.28–7.41 (9H, m, ArH), 7.59 (2H, d, *J* 7.9, ArH), 7.76 (2H, d, *J* 7.9, ArH).

m/*z* (ESMS) 527.0 ($M + H^+$, 90%, $C_{32}H_{35}N_2O_5^+$ requires 527.2), 289.0 (30), 178.2 (100, dibenzofulvene).

Fmoc-L-allylglycine indolinide 16

Fmoc-L-allylglycine (12.9 g, 38.2 mmol) in DCM (300 mL) was treated with TBTU (13.5 g, 1.1 equivalent), HOBt.H_2O (5.7 g, 1.1 equivalent), indoline (8.6 mL, 2 equivalents) and DIEA (6.54 mL, 1 equivalent). The mixture was stirred overnight, at which point TLC (AcOEt-hexane-AcOH, 25:25:1) indicated the reaction to be complete. The solvent was evaporated in vacuum and the material was re-dissolved in AcOEt, washed with 5% $NaHCO_3$ (2 ×), 0.5 M HCl (2 ×), water (2 ×) and brine. Drying over $MgSO_4$ and evaporation in vacuum yielded 16.5 g (97%). This material could be recrystallized from AcOEt to give the indolinide **16** (12.0 g, 72%), m.p. 149–50°C.

δ_H ($CDCl_3$) 2.50 (1H, p, *J* 7.1, β-CH), 2.50 (1H, p, *J* 7.1, β-CH′), 3.20–3.25 (2H, m, $PhCH_2$), 4.13 (1H, q, *J* 9.3, indoline-N-CH), 4.22 (2H, t, *J* 6.9, Fmoc-CH), 4.31 (1H, q, *J* 9.3, indoline-N-CH′), 4.35–4.41, (2H, d, *J* 6.9, Fmoc-CH_2), 4.68–4.73 (1H, m, α-H), 5.10–5.21 (2H, m, C=CH_2), 5.67 (1H, br d, NH), 5.77–5.87 (1H, m, CH=Me), 7.06 (1H, t, *J* 7.4, indoline-H), 7.20–7.33 (4H, m, indoline-H and Fmoc-H), 7.40 (2H, t, *J* 7.4, Fmoc-H), 7.60 (2H, d, *J* 7.4, ArH), 7.76 (2H, d, *J* 7.4, ArH), 8.23 (1H, d, *J* 7.2, indoline-H).

δ_C ($CDCl_3$) 28.4, 37.6, 47.5, 48.3, 53.1, 67.4, 117.6, 119.4, 120.1, 124.6, 125.3, 127.3, 127.7, 131.7, 132.5, 141.4, 142.6, 144.0, 156.1, 169.7.

m/*z* (ESMS) 439.4 ($M+H^+$, 100%, $C_{28}H_{27}N_2O_3^+$ requires 439.2), 178.6 (100, dibenzofulvene).

Fmoc-L-Adi indolinide 17

Indolinide **16** (12.0 g, 27.3 mmol) was dissolved in tetrahydrofuran (120 ml). NMO (50% in water, 22.6 mL, 2.0 equivalents) was added, followed by OsO_4 (280 mg, 0.04 equivalents) and the mixture was stirred at room temperature overnight. TLC (AcOEt-hexane-AcOH, 25:25:1) indicated that the reaction was complete and showed two overlapping product spots (diastereomers **17a**/**17b**). Crushed ice and saturated aqueous sodium bisulfite (60 mL) was added and the mixture was stirred for 1 h. Plenty of AcOEt was added and the mixture was washed with 5% $NaHCO_3$ (2 ×), 0.5 M HCl (2 ×), water (2 ×) and brine. Drying over $MgSO_4$ and evaporation in vacuum gave diol **17** (12.8 g, 99%).

HPLC analysis showed the diastereomeric ratio to be 60:40. Substitution of OsO_4-NMO with AD-mix α or β [18,19] gave ratios of 65:25 and 80:20, respectively.

δ_H ($CDCl_3$) 1.63 (1H, t, *J* 12.5, β-CH), 1.83 (1H, t, *J* 12.5, β-CH′), 1.87–1.93 (1H, m, β-CH, minor isomer), 1.98–2.06 (1H, m, β-CH′, minor isomer), 3.20 (2H, t, *J* 8.8, CH_2OH), 3.47–3.53 (1H, m, Ph-CH), 3.60–3.66 (1H, q, *J* 9.3, Ph-CH′), 3.77–3.85 (1H, m, CH-O), 4.20 (2H, t, *J* 6.8, Fmoc-CH), 4.25–4.31 (1H, m, indoline-N-CH), 4.37–4.48, (3H, m, Fmoc-CH_2 and indoline-N-CH′), 4.77–4.84 (1H, m, α-H), 6.11 (1H, br d, NH), 7.05 (1H, dd, *J* 6.8, indoline-H), 7.19 (2H, dd, *J* 8.2, indoline-H), 7.30 (1H, q, *J* 8.2, Fmoc-H), 7.38 (2H, q, *J* 7.5, Fmoc-H), 7.58 (2H, t, *J* 7.5, ArH), 7.74 (2H, d, *J* 7.5, ArH), 8.19 (1H, d, *J* 7.5, indoline-H).

δ_C ($CDCl_3$) 28.4, 39.5, 47.5, 48.2, 50.8, 58.2, 66.6, 67.7, 69.5, 117.5, 120.2, 124.8, 125.2, 127.3, 127.9, 130.8, 141.5, 142.4, 143.8, 148.1, 149.7, 151.1, 156.3, 162.2.

m/*z* (ESMS) 473.6 ($M+H^+$, 100%, $C_{28}H_{29}N_2O_5^+$ requires 473.2), 179.0 (100, dibenzofulvene).

Fmoc-L-Adi(5-Bu^t) lactone 18

Diol **17** (106 mg, 0.22 mmol) in diglyme (6 mL) was ice-cooled and treated with H_2SO_4 (0.6 mL), followed by liquid isobutene (3 mL). The flask was sealed and kept for 4 days at room temperature. The flask was cooled with ice and the content was poured into saturated $NaHCO_3$ and AcOEt. The organic solution was washed with 5% $NaHCO_3$ and water. Drying (Na_2SO_4) and evaporation in vacuum gave a colourless film (70 mg, 65%). Chromatography on silica isolated the lactone **18**.

δ_H ($CDCl_3$) 1.22 (9H, s, Bu^t), 1.95–2.03 (1H, m, β-CH), 2.72–2.81 (1H, m, β-CH′), 3.47 (1H, dd, *J* 10.2, δ-CH), 3.68 (1H, dd, *J* 10.2, δ-CH′), 4.23 (1H, t, *J* 7.3, Fmoc-CH), 4.46 (2H, d, J 7.3, Fmoc-CH_2), 4.54–4.66 (2H, m, CHO and α-H), 5.70 (1H, bd, NH), 7.32 (2H, t, *J* 8.1, Fmoc-H), 7.41 (2H, t, *J* 7.4, Fmoc-H), 7.59 (2H, d, *J* 7.6, Fmoc-H), 7.77 (2H, d, *J* 7.6, Fmoc-H).

δ_C ($CDCl_3$) 27.6, 32.4, 47.5, 50.3, 63.2, 63.7, 67.4, 74.4, 120.1, 124.3, 125.2, 127.2, 141.3, 144.0, 156.1, 175.6.

m/*z* (ESMS) 410.4 ($M+H^+$, 20%, $C_{24}H_{28}NO_5^+$ requires 410.1), 354.2 (80, M-Bu^{t+}), 179.0 (100, dibenzofulvene).

Fmoc-L-Adi(Trt)$_2$ indolinide 19

Indolinide **17** (11.7 g, 24.7 mmol) in dried acetonitrile (250 mL) was treated with lutidine (21.6 ml, 7.5 equivalents) and trityl bromide (23.9 g, 3 equivalents). The flask was fitted with a drying tube ($CaCl_2$) and the mixture refluxed for 2 h. TLC

(THF-hexane 1:3) was used to determine the reaction end-point. The mixture was cooled to room temperature, diluted with AcOEt and washed with 5% $NaHCO_3$ (2 ×), cold 5% citric acid (2 ×), water (2 ×) and brine. The crude product was isolated after drying (Na_2SO_4) and evaporation in vacuum, 30.0 g (in theory 30.1 g, from 23.6 g **19** and 6.43 g trityl alcohol). This material was either used directly in the following step, or purified by silica chromatography, using THF-hexane 1:6 to elute the trityl alcohol, and 1:2 to elute the tritylated diol **19** (21.2 g, 90%).

δ_H ($CDCl_3$) 2.01–2.09 (1H, m, β-CH), 2.17–2.24 (1H, m, β-CH′), 2.69–2.77 (1H, m, Ph-CH), 2.81–2.92 (1H, m, Ph-CH′), 2.97–3.05 (2H, m, CH_2-O), 3.27–3.32 (1H, m), 3.56–3.65 (2H, m), 3.79–3.86 (1H, m, CH-O), 4.03–4.35 (5H, m), 4.56–4.62 (1H, m), 4.77–4.81 (1H, m), 5.42–5.46 (1H, bd, NH), 6.97–7.05 (2H, m), 7.08–7.50 (38H, m), 7.74–7.77 (2H, m), 8.09 (1H, d, *J* 7.8), 8.20 (1H, d, *J* 7.6).

δ_C ($CDCl_3$) 26.0, 30.7, 34.6, 47.6, 67.4, 68.3, 120.0, 124.2, 124.6, 124.7, 125.7, 127.2, 127.3, 127.4, 127.8, 127.9, 128.0, 128.7, 128.8, 128.9, 129.3, 141.4, 143.7, 143.9, 144.6, 156.1, 186.3.

m/*z* (ESMS) 697.6 (M-Trt-OH^+, 15%, $C_{47}H_{41}N_2O_4^+$ requires 697.3), 243.4 (100), 179.0 (80, dibenzofulvene).

Fmoc-L-Adi(Trt)$_2$ indolide 20

Tritylated Adi **19** (23.6 g, 24.7 mmol) in toluene (60 mL) was treated with DDQ (16.8 g, 3 equivalents) and heated to 80°C. TLC (AcOEt-hexane, 1:3) was used to monitor the reaction closely. The separation of starting material and product was small, but adequate (ΔR_f appr. 0.1). Spraying of the TLC-plate with xanthydrol-HCl [20] and heating of the plate to 110°C coloured the indolide via yellow to purple, whereas the indolinide stayed yellow. The reaction was allowed to run until all starting material had disappeared. Some detritylated by-products were generated, but it was easier to separate this material from the desired product, then it was to separate residual starting material. The reaction was complete after 2–3 h. The product was worked up by addition of chloroform and filtering through a silica plug, which was washed with further chloroform. The resulting solution was washed with 5% $NaHCO_3$ until the dark colour was removed (usually 2 ×), and with water and brine. Drying (Na_2SO_4) and evaporation gave 19.8 g (84%). This material was either used directly in the next step or purified by silica chromatography, eluting with AcOEt-hexane, 1:3, to give the indolide **20** (14.2 g, 60%).

δ_H ($CDCl_3$) 2.06–2.16 (2H, m, β-CH), 3.06 (1H, dd, J_1 5.5, J_2 9.1, CH_2-O), 3.18 (1H, dd, J_1 5.7, J_2 8.8, CH'_2-O), 4.09–4.16 (1H, m, CH-O), 4.26 (1H, t, *J* 6.9, Fmoc-CH), 4.32–4.42 (2H, m, Fmoc-CH_2), 4.74 (1H, m, α-H), 5.43–5.48 (1H, NH), 6.79 (1H, d, *J* 4.0, indole-CH), 7.21–7.50 (32H, m), 7.63 (1H, d, *J* 7.5), 7.74 (2H, t, *J* 7.2), 7.86 (2H, d, *J* 7.2), 7.97 (1H, d, *J* 3.8), 8.47 (1H, d, *J* 7.8).

δ_C ($CDCl_3$) 26.0, 30.0, 30.8, 52.9, 59.7, 68.2, 125.6, 126.9, 127.1, 127.3, 127.4, 127.5, 127.9, 128.0, 128.1, 128.2, 128.3, 128.7, 128.8, 128.9, 129.0, 129.2, 129.5, 141.3, 144.7, 147.0, 149.8, 174.8.

m/*z* (ESMS) 737.2 (M-Trt + Na^+, 5%, $C_{47}H_{40}N_2O_5Na^+$ requires 735.3), 243.4 (100), 179.0 (30, dibenzofulvene).

Fmoc-L-Adi(Trt)$_2$ 22

Indolide **20** (8.3 g, 8.7 mmol) in THF (120 mL) and water (100 mL) was treated with LiOH-hydrate (3.7g, 10 equivalents) and the mixture was stirred for 3 h, monitoring by TLC (THF-hexane 1:3). The crude lithium salt **21** was worked up by addition of AcOEt and water, and the organic solution was washed with water and brine, dried over Na_2SO_4 and evaporated. The crude material was dissolved in THF (300 mL) and treated with Fmoc-OSu (3.22 g, 1.1 equivalents). DIEA was added until pH 8–9 (approx. 1.5 mL, 0.9 equivalents), with further DIEA-adjustment after 1 h to reach pH 8–9. The mixture was stirred overnight and worked up from AcOEt by washing with cold 5% citric acid (2 ×), water (2 ×) and brine. Upon drying (Na_2SO_4) and evaporation, the product was isolated by silica chromatography, eluting initially with AcOEt-hexane 1:1, followed by AcOEt-hexane-AcOH 25:25:1. The fractions containing the desired product were washed with water to remove AcOH and evaporated to give Fmoc-Adi(Trt)$_2$ **22** (3.0 g, 40%).

δ_H (acetone-d_6) 2.28–2.38 (2H, m, β-CH_2), 2.85–2.91 (2H, m, CH_2-O), 3.48–3.53 (1H, m, CH-O), 4.19–4.24 (1H, t, *J* 7.0, Fmoc-CH), 4.32–4.40 (2H, m, Fmoc-CH_2), 4.48–4.54 (1H, m, α-H), 6.53 (1H, bd, NH), 7.14–7.46 (34H, m), 7.64–7.69 (2H, m), 7.84–7.88 (2H, m).

δ_C (acetone-d_6) 35.6, 47.3, 51.3, 54.8, 66.7, 70.9, 120.1, 125.5, 127.1, 127.2, 127.8, 128.5, 127.7,128.8, 128.9, 129.1, 141.3, 144.1, 144.4, 145.0, 145.2, 156.1, 173.7.

m/*z* (ESMS) 878.6 (M + Na^+, 10%, $C_{58}H_{49}NO_6Na^+$ requires 878.4), 242.8 (100), 179.0 (50, dibenzofulvene).

SOLID-PHASE PEPTIDE SYNTHESIS GENERAL PROTOCOL

All peptides were synthesized manually in plastic syringes using preloaded Rink amide linker (RAM) [21] on TentaGel (0.21 mmol g^{-1}). Three equivalents of N^{α}-Fmoc amino acids were used and the Fmoc-protecting group was removed after each cycle by 30% piperidine in *N*-methylpyrrolidone (NMP). The amino acids were activated in NMP using DIC (3 equivalents) and HOAt (3 equivalents). The completion of the acylation reaction was monitored visual by the use of bromophenol blue [22]. The peptides were deprotected and cleaved from resin by treatment with 95% aqueous TFA containing 4 equivalents of TIS for 1.5 h. The resins were rinsed with 95% aqueous acetic acid (4 ×). TFA and acetic acid were evaporated and the peptides were precipitated in diethyl ether and lyophilized overnight.

Synthesis of Peptide 23

The precursor H-Asp(OBu^t)-Leu-Trp(Boc)-Gln(Trt)-Lys(Boc)-RAM-TentaGel was assembled as described above. Fmoc-Adi(Trt)$_2$ **22** (20 mg, 23 μmol) was coupled to the protected peptide resin (10.5 μmol) using DIC (3.5 μL, 23 μmol) and HOAt (3 mg, 23 μmol), overnight at room temperature. Deprotection and cleavage as described above gave the crude peptide **23** (Table 1), t_R 11.4 min.

Synthesis of Peptide 24

Fmoc-Adi(Trt)$_2$ 22 (100 mg, 115 μmol) was coupled to the RAM TentaGel (200 mg, 0.23 mmol g^{-1}) using DIC (17.8 μL, 116 μmol) and HOAt (15.7 mg, 116 μmol). Peptide build-up and deprotection as described above gave **24** (Table 1), t_R 11.5 min.

Synthesis of Peptide 25

The resin-bound, protected precursor H-Asp(OBu^t)-Leu - Trp(Boc) - Gln(Trt) - Lys(Boc) - Ams(Boc) - RAM-Tentagel was coupled with Fmoc-Adi(Trt)$_2$ to give **25**, t_R 11.8 min, purified by semi-preparative HPLC (Table 1).

Synthesis of Peptide 26

H-Asp(OBu^t)-Leu-Trp(Boc)-Gln(Trt)-Lys(Boc)-RAM-TentaGel was coupled with Fmoc-Ams(Boc) (91,2 mg, 208 μmol) to give **26**, t_R 12.3 min, purified by semi-preparative HPLC (Table 1).

Preparation of Peptide 27 and Dimer 28

Peptide **23** (2 mg, 1.8 μmol) was dissolved in 50 mM phosphate buffer, pH 7.4/DMSO (9:1, v/v) (6 μL) and $NaIO_4$ (1.4 mg, 6.6 μmol) was added. The reaction was left for 5 min at room temperature. The aldehyde peptide **27** was analysed (t_R 11.8 min) (Figure 3(a)) and purified by HPLC and isolated by lyophilization. Peptide **27** (0.5 mg, 0.62 μmol) was reacted with 1,10-diamino-tris(ethyleneglycol) (18.5 μg, 0.1 μmol) [23] in 20 mM NaOAc buffer, pH 5.1/DMSO (9:1, v/v) (1.75 μL). The solution was left for 1 h at 38°C and progress of the reaction was monitored by MALDI-MS. Dimer **28** showed up as a single product in analytical HPLC (t_R 17.8 min) along with excess starting material (Figure 3(b)).

Table 1 Expected and Observed Molecular Masses and Yields for Peptide Amides **23**–**27**, Peptide Dimers **28**–**29** and Cyclic Peptide **30**

Compound	Structure	Molecular formular	m/z ($M+H^+$), found (required)	Yield (%)[a]
23	Adi-DLWQK-NH_2	$C_{37}H_{58}N_{10}O_{11}$	817.4 (818.9)	65[b]
24	DLWQK-Adi-NH_2	$C_{37}H_{58}N_{10}O_{11}$	817.3 (818.9)	60[b]
25	Adi-DLWQK-Ams-NH_2	$C_{40}H_{64}N_{12}O_{13}$	918.3 (921.0)	55[b]
26	Ams-DLWQK-NH_2	$C_{35}H_{55}N_{11}O_{10}$	789.1 (789.5)	63[b]
27	Aob-DLWQK-NH_2	$C_{36}H_{54}N_{10}O_{10}$	786.6 (786.9)	46
28	(CH_2O-Et-ON = Aob-DLWQK-NH_2)$_2$	$C_{78}H_{120}N_{22}O_{22}$	1716.9 (1716.9)	80[c]
29	H_2N-KQWLD-Ams = Aob-DLWQK-NH_2	$C_{71}H_{107}N_{21}O_{19}$	1561.2 (1558.8)	70
30	Ams=Aob-NH_2 | | DLWQK	$C_{39}H_{58}N_{12}O_{11}$	869.7 (870.0)	37

[a] Obtained after purification by HPLC.
[b] Based on the loading of the resin.
[c] Based on the amount of limiting component (PEG-linker).

Yield and MALDI-MS data for peptide **27** and peptide dimer **28** are presented in Table 1.

Preparation of Peptide Dimer 29

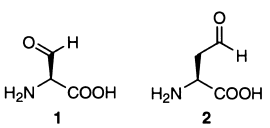
Peptide **27** (0.4 mg, 0.51 μmol) and peptide **26** (0.56 mg, 0.71 μmol) were dissolved in 20 mM NaOAc buffer, pH 5.1/DMSO (9:1, v/v) (1.5 μL). The solution was left for 1 h at 38°C and the reaction was monitored by MALDI-MS. Crude peptide dimer **29** was purified by HPLC and characterized by MALDI-MS (Table 1).

Preparation of Cyclic Peptide 30

Peptide **25** (2 mg, 2.2 μmol) was dissolved in 50 mM phosphate buffer, pH 7.4/DMSO (9:1, v/v) (10 mL) and $NaIO_4$ (1.9 mg, 8.8 μmol) was added. The solution was left 5 min at room temperature and the product was purified by semi-preparative HPLC. Analytical HPLC showed a single main peak (t_R 11.8 min) and MALDI-MS confirmed the desired cyclic peptide **30** (Table 1).

RESULTS AND DISCUSSION

The shortest possible side-chain in an amino acid aldehyde is found in α-formyl glycine **1** (2-amino-3-oxo-propionic acid) [24].



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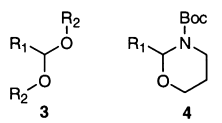

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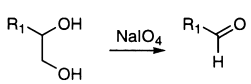


5 **6**

However, the α-proton in this molecule is quite acidic and the stereochemical integrity of the amino acid would surely be problematic during peptide synthesis. We therefore targeted an amino acid with a side-chain of one more carbon, namely 2-amino-4-oxo-butyric acid **2**, in short Aob. Derivatives of Aob have previously been reported (under various names including aspartate semi-aldehyde) [25–28], but none of these previous seemed suitable for peptide synthesis. For protection or masking of the Aob aldehyde function, we considered a number of different strategies. In classical organic synthesis, aldehydes are often protected as *O*,*O*-acetals **3**, but this function has found little use in peptide synthesis. Depending on their exact structure, *O*,*O*-acetals may not deprotect under standard peptide deprotection conditions, e.g. concentrated HCl has been used for peptide acetal deprotection [12]. *N*,*O*-acetals are more labile, and often too labile in themselves. However, *N*-Boc-protected *N*,*O*-acetals (**4**) are attractive [29], since the TFA used for final deprotection in standard Fmoc-based peptide synthesis will cleave the Boc-group, thus exposing the *N*,*O*-acetal and allowing it to hydrolyse easily.

Discouragingly, the inherent reactivity of aldehydes is pronounced in acidic TFA, and TFA-promoted reactions of aldehydes with peptide functions are known, e.g. the Pictet–Spengler reaction [30–32]. Also, cycloadditions in TFA solution of neighbouring amide groups towards *N*-terminal peptide aldehydes have been observed, and this side-reaction could only be eliminated by protecting the relevant amide groups as e.g. *N*-methyl amides [33]. Accordingly, generation of peptide aldehyde functions in a step separate from TFA cleavages appear attractive.

By using a Weinreb-type linker, *C*-terminal peptide aldehydes can be generated via reduction of the Weinreb amide with various hydride reagents [34,35]. This chemistry has recently been extended to side-chain Weinreb-derivatives of aspartic or glutamic acid, thus allowing aldehydes in other positions than the *C*-terminal [36]. This chemistry appears promising, but it has been suggested that this only works with short peptides [35]. Additionally, avoidance of side products appears difficult [37].

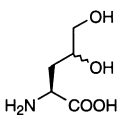
C-terminal peptide aldehydes may alternatively be prepared by preparation of an *N*-protected α-amino aldehyde and linking this compound as e.g. an *O*,*N*-acetal (oxazolidine) [38]. However, α-amino aldehydes and *C*-terminal peptide aldehydes are often described as being rather sensitive towards racemization [39].

Numerous oxidative routes to aldehydes are available and several have been successfully used with peptides. Ozonolysis of double bonds is a possibility [40–42], but ozone is reported to destroy tryptophan, cysteine and methionine, even under the mildest conditions [35].

Primary alcohols may be oxidized to aldehydes using a range of reagents [43], but since larger peptides often have several alcohols (serines and threonines), general use of this method would require specialized protection group strategies.

1,2-Diols **5** and 1,2-amino alcohols are not common in peptides, and these groups may be selectively oxidized to aldehydes **6** under mild conditions using sodium periodate. This has been utilized for peptides with *N*-terminal serine to give the *N*-terminal aldehyde [14,44], and similarly for serines mounted on e.g. a lysine side-chain [15]. The periodate oxidation is quick and mild, but it is problematic with peptides containing cysteine and methionine residues. Nevertheless, $NaIO_4$ oxidations on proteins without destruction of cystines (S-S bridges) [15] and methionines have been reported, e.g. by using auxiliary methionine as a scavenger [45]. Furthermore, $NaIO_4$ is known to leave tryptophan unaffected.

The above considerations taken together, we found 2-amino-4,5-dihydroxy-pentanoic acid **7** (Adi) to be the most attractive equivalent of an α-amino acid aldehyde.

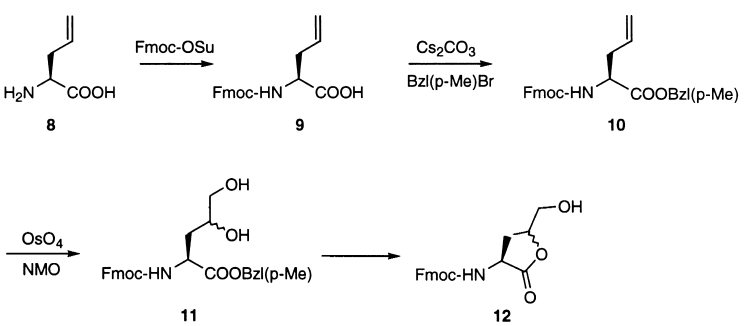


7 (L-Adi)

Various derivatives of this amino acid have appeared in the literature, but none of these appear suitable for solid-phase peptide synthesis [25,46–55]. We here combine N^{α}-Fmoc-protection of Adi with TFA-labile protection of the diol, thus allowing standard Fmoc-based solid-phase peptide assembly and standard protecting group cleavage, followed by a mild and fast oxidation step to the peptide aldehyde.

We considered preparation of Adi by Schöllkopf chemistry and other known methods of asymmetric synthesis [56], but found commercially available L-allylglycine to be the most convenient starting point. Noteworthy, the enzymatic separation of L-allylglycine from its racemate is very well described [57]. The 4,5-diol functionality of Adi should expectedly be introducible by OsO_4-promoted oxidation of the double bond [25,58,59]. However, two problems in the planned synthesis were expected. Firstly, the establishment of the 4-hydroxy group would introduce a new chiral carbon in Adi **7**. This is only a problem in passing, because the chirality of C-4 will be eliminated on oxidation of the diol to the aldehyde. Nevertheless, this point is problematic, because the peptide diols will be diastereomers if the chirality cannot be controlled. For analysis and purification at the peptide diol step, the presence of closely related diastereomers could be annoying. On the other hand, larger peptides with a single hydroxy group in either of two orientations are very closely related, and we suspected that standard HPLC analysis would often not separate such diastereomers.

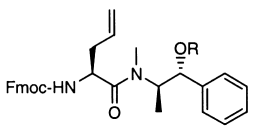
The second problem arises from the tendency of 4-hydroxy carboxylates and their esters to form lactones. It could thus be expected that simple ester protection of the carboxyl during amino acid build-up would be problematic. Nevertheless, since the route via an ester would be the simplest, this possibility was pursued first (Scheme 1). L-allylglycine **8** was *N*-protected by use of Fmoc-OSu to give **9** [16,17]. The carboxy function was subsequently protected as the *p*-methylbenzyl ester (**10**), which was prepared via the caesium carboxylate and *p*-methylbenzyl bromide [60]. Attempts to dihydroxylate the double bond using a catalytic amount of OsO_4 with *N*-methyl-morpholine-*N*-oxide (NMO) in acetone-water or dioxane-water looked promising initially (TLC analysis), but at the reaction end-point the result was always a mixture of several Fmoc-derivatives, presumably the diastereomeric diols and their lactones. Furthermore, all attempts to protect the crude diol with *tert*-butyl or trityl groups resulted in further formation of lactone **12**, for which reason the route via the ester was abandoned.



Scheme 1 Undesired lactonification of Adi ester.

Subsequently, an attempt was made to circumvent the lactone problem by reducing the carboxyl group to the alcohol [61] and protecting the resulting Fmoc-allylglycinol **13** as the 2,2-dimethyloxazolidine **14** (Scheme 2) [62]. The allyl oxazolidine dihydroxylated neatly, but on subsequent attempts to protect the alcohol groups this material was unstable.

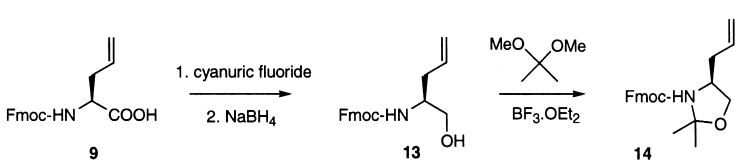
Attention was then turned to amides as protection for the carboxy group. Discouraging, deprotection/cleavage of simple amides require heating in strong base for prolonged periods. Acyl ephedrides cleave more easily, due to participation of the ephedrine hydroxy group [17]. Besides, ephedrides might be advantageous for the subsequent dihydroxylation step, because their inherent stereochemistry could help the dihydroxylation step to favour a particular stereoisomer. Unfortunately, Fmoc-L-allylglycyl ephedrides and their acetylated derivatives (**15**) were oils, which were difficult to purify.



15 R = H, Ac

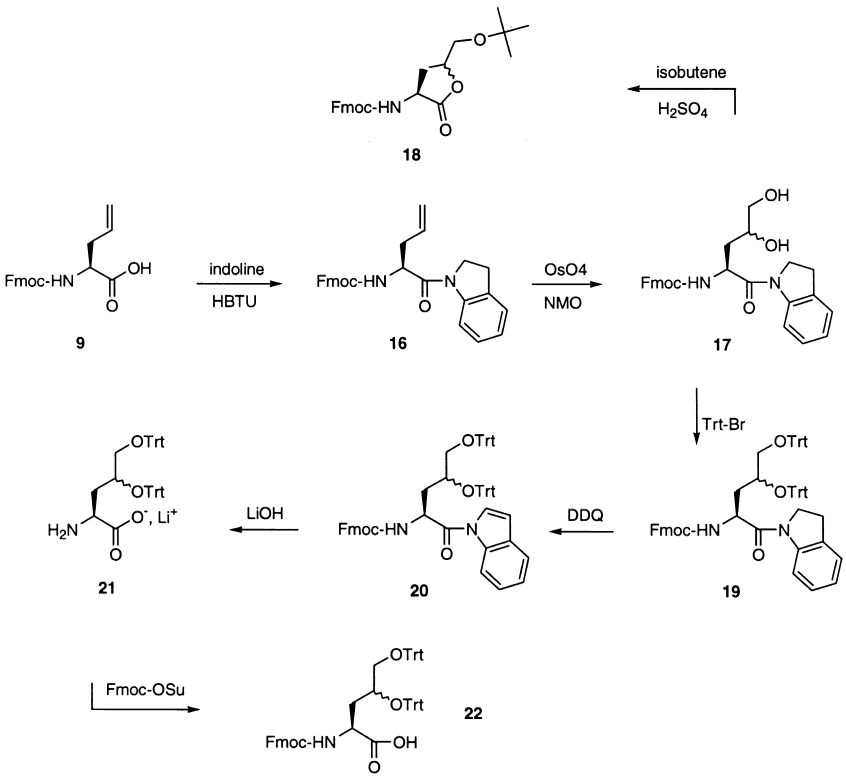
Furthermore, the dihydroxylation step gave heterogeneous products, possibly due to migration of the acetyl group from ephedrine to the Adi 4- and 5-hydroxy groups.

Acyl indolines ('indolinides') are robust towards nucleophilic attack, and thus expectedly robust towards lactone formation, but they are relatively easily aromatized by mild oxidants to acyl indoles ('indolides'), which cleave under mild conditions [63,64]. Fmoc-L-allylglycine indolinide **16** was prepared by use of TBTU, HATU [65] or DCC (Scheme 3). This compound was crystalline and easy to handle. A drawback of the indolinide class of compounds is the splittings of NMR signals, due to the presence of cis/trans rotamers about the CO-N bond, as is well known from other cyclic secondary amides e.g. acyl prolines. Encouragingly, **16** underwent dihydroxylation smoothly, and TLC suggested two overlapping spots (the expected diastereomers **17a** and **17b**). From HPLC analysis, the diastereoisomeric ratio was 60:40 (see below). Attempts to protect the diol with *tert*-butyl groups by reaction with H_2SO_4 and isobutene resulted in formation of lactone **18**. This was not surprising considering the strongly acidic conditions. However, tritylation of the diol proceeded smoothly to give **19** using trityl bromide and lutidine in acetonitrile at reflux. The high temperature was necessary in order to drive the introduction of two neighbouring trityl groups to completion. The Fmoc-group was not affected under these weakly basic conditions, even during heating. Fmoc-Adi(Trt)$_2$-indolinide **19** was either purified by column chromatography, or the crude material was used directly in the next step. Oxidation of the indolinide **19** to indolide **20** was performed with DDQ in toluene at 80°C [63,66]. This step was somewhat tricky, since longer reaction times resulted in significant cleavage of the trityl groups. During the redox process, DDQ is reduced to the dihydroquinone (DDQH), and this phenolic compound is apparently acidic enough to initiate trityl-cleavage under the prevailing conditions. Experimentation with addition of base (pyridine or sodium carbonate) resulted in inhibition of the aromatization reaction. Use of more polar solvents than toluene led to even more trityl cleavage. Attempts to perform the oxidation using either manganese-(III)-acetate[67,68] or the milder quinone chloranil [64] were unsuccessful. Manganese-(III)-acetate oxidations are usually performed in hot acetic acid, but such conditions are incompatible with the trityl groups, and attempts with this chemistry in pyridine or dioxane-sodium acetate gave no reaction. Luckily, satisfactory results could be achieved using careful monitoring of the DDQ-oxidation, although yields were moderate. Curiously, the DDQ-promoted aromatization reaction worked better on crude **19** than on purified **19**. Crude **19** is contaminated with trityl alcohol and since trityl radicals and cations are known to promote aromatizations [69], this finding is not really surprising. Cleavage of the indolide could be achieved with LiOH in THF-water, simultaneously cleaving the Fmoc-group. Attempts to preserve the



9 **13** **14**

Scheme 2 Synthesis of oxazolidine **14**.



Scheme 3 Synthetic route to Fmoc-Adi(Trt)$_2$.

Fmoc-group by using $CaCl_2$-NaOH in isopropanol [70] during the amide hydrolytic step were not successful. The crude Adi(Trt)$_2$ **21** was therefore re-protected using Fmoc-OSu to give **22**, which was purified using column chromatography. The overall yield of **22** from **9** was 15–20%.

Peptide synthesis with the new building block suggested that the mentioned problem of Adi diastereomers could be ignored. All prepared peptides showed up as relatively homogeneous; the analytical method (HPLC) did not separate the closely related diastereomers. By HPLC analysis of Fmoc-Adi-indolinide **17a**/**17b**, with careful optimizing of the chromatographic gradient, the amino acid isomers were separated and it was found that dihydroxylation with OsO_4 gave a product ratio of 60:40 (Figure 1). The absolute configuration of the diastereomers has not been assigned, but analogies with other dihydroxylations are available [25,58,59]. Attempts to improve the ratio towards a single stereoisomer (**17a** or **17b**) by substituting OsO_4 with the known asymmetric dihydroxylation reagents AD-mix-α and AD-mix-β [19,71] met with limited success. Product ratios of 65:35 and 80:20 were obtained, respectively. We were not able to separate **17a** and **17b** or their later derivatives, on a preparative scale. Accordingly, we currently use **22** as the diastereoisomeric mixture. Since the resulting peptides are more stable at the diol stage than the aldehyde stage, we usually purify the peptides at the diol stage, and we have yet to see an Adi

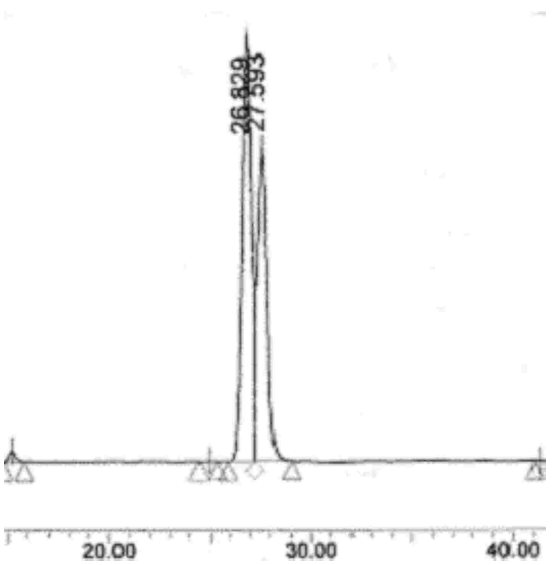


Figure 1 HPLC separation of **17a**/**17b**.

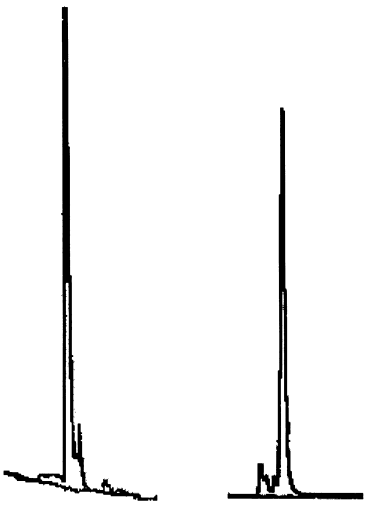


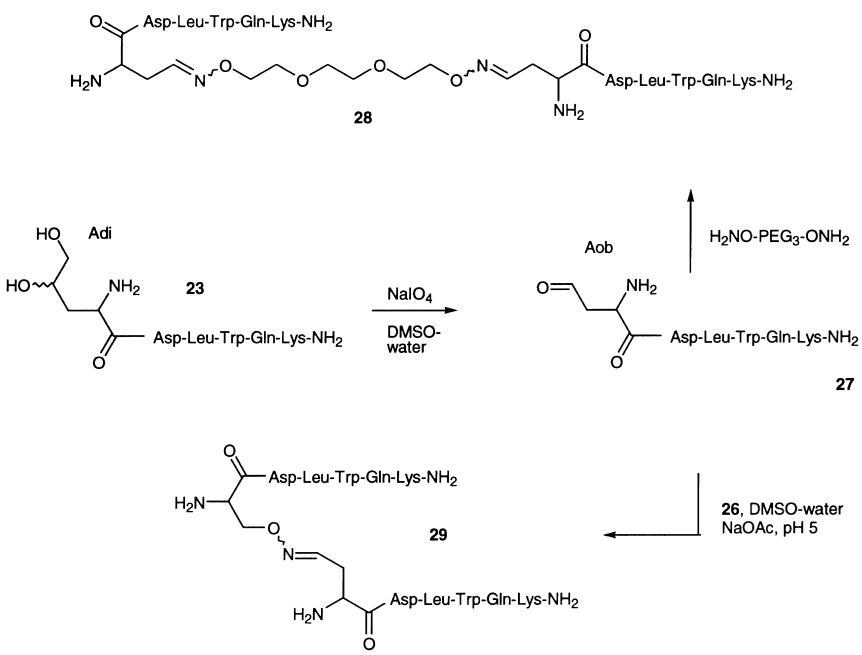
Figure 2 HPLC profiles of crude peptides **23**–**24**

peptide that separates into the diastereomers in the HPLC analysis. As mentioned above, on going to the aldehyde stage the problem disappears (the 4-carbon becomes achiral), but since peptide aldehydes can be expected to be more unstable than peptide diols, we prefer purifying and storing peptides at the diol stage and using the aldehydes immediately after formation.

Three Adi-containing peptide amides were synthesized, Table 1, **23**–**25**. The Adi residue was placed either at the *N*- or *C*-terminus of the peptides **23** and **24**. The peptides were deliberately made as amides, in order to hinder lactone formation, which could otherwise result from peptides with *C*-terminal Adi residues. For preparation of an end-to-end cyclic peptide [72,73], Adi was complemented by *O*-amino serine (Ams) [9] in peptide **25**. As a building block for peptide hetero dimers, the Ams-containing **26** was also synthesized. The applied model peptide sequence Asp-Leu-Trp-Gln-Lys has been isolated from the ultra-filtrate of a uremic patient [74]. The peptides were prepared by the stepwise solid-phase synthesis [75] using Fmoc-chemistry with DIC-HOAt activation on a Rink resin [21]. The peptides were deprotected and cleaved from the resin using 95% TFA containing TIS. Analytical HPLC gave in each case a single major peak (Figure 2) and MALDI-MS showed the expected masses (Table 1). From HPLC purifications, the linear peptides were obtained in yields of 55–65%.

Mild periodate oxidation [8], using 3.6 equivalents of $NaIO_4$ in 10% DMSO pH 7.4 phosphate buffer for 5 min at room temperature, converted the Adi residue into the 2-amino-4-oxo-butyrate residue (Aob) (Scheme 4). Analytical HPLC showed the oxidations to proceed cleanly giving **27**.

The peptide dimers **28** and **29** were prepared by ligation chemistry (Scheme 4). The unprotected and oxidized peptide **27** was dimerized on 1,10-diamino-tris(ethyleneglycol) [23,76] at pH 5 for 1 h at 38°C, to form **28**. In order to drive this type of dimer



Scheme 4 Oxime ligation with formation of peptide dimers.

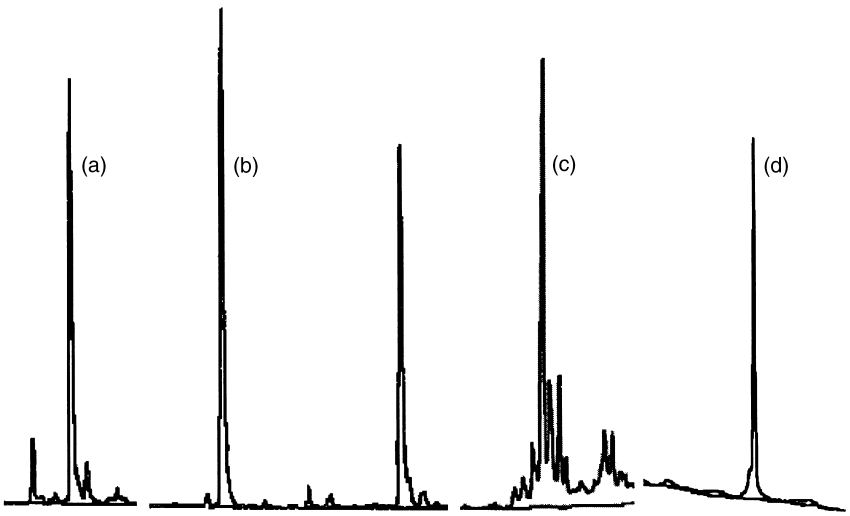
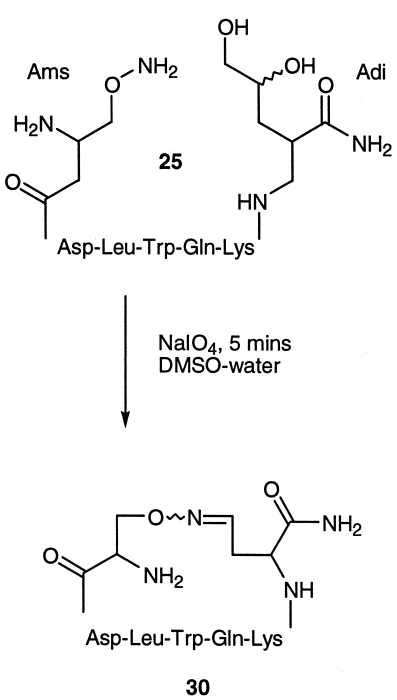


Figure 3 HPLC profiles of crude peptide **27** (a), crude peptide dimer **28** (b, with excess starting material on the left), crude cyclic peptide **30** (c) and purified cyclic peptide **30** (d).

formation to completion, it was necessary to use an excess of peptide **27**. Analytical HPLC (Figure 3(b)) thus shows excess starting material **27** along with the product **28** (Table 1). The requirement for excess peptide is a drawback for this type of dimer, even though the excess starting material could be recycled. A different type of dimer (**29**, Scheme 4) was obtained from the reaction of the peptide building blocks **26** and **27** in stochiometric amounts (Table 1). Finally, the end-to-end cyclic peptide **30** was formed from the unprotected linear peptide precursor **25** by intramolecular ligation (Scheme 5). Upon oxidation of **25**, the intra-molecular reaction happened rapidly to give **30** in 37% isolated yield (Figure 3(d) and Table 1).



Scheme 5 Peptide cyclization by oxime ligation.

CONCLUSION

A new amino acid derivative with a short side-chain masked aldehyde has been prepared and protected in a manner suitable for standard peptide synthesis. Fmoc-Adi(Trt)$_2$ thus enables the insertion of a masked aldehyde at any position in a given peptide, without use of multi-dimensional protection schemes. The peptide diol function can be converted to the aldehyde by $NaIO_4$ oxidation, circumventing release of reactive aldehydes in TFA. Two peptide dimers and a cyclic peptide have been prepared by oxime chemoselective ligation. Since aldehyde electrophiles can be used in a number of other reactions, Fmoc-Adi(Trt)$_2$ should be applicable in the construction of other peptide isosteres and mimetics.

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