Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 14 (2006) 6197–6201
Bioorganic & Medicinal Chemistry

Peptide–polyethylene glycol conjugates: Synthesis and properties of peptides bearing a C-terminal polyethylene glycol chain

Jessica Grun, Jefferson D. Revell, Matteo Conza and Helma Wennemers*

Department of Chemistry, University of Basel, St. Johanns Ring 19, CH-4056 Basel, Switzerland

Received 28 February 2006; accepted 30 May 2006
Available online 19 June 2006

Dedicated to Professor Bernd Giese

Abstract—The introduction of a polyethylene glycol chain has become a popular tool for increasing water solubility and bioavailability. Our interest in the development of catalytically active peptides and the selective recognition of peptides has led us to investigate strategies to increase the solubility of peptides in organic solvents. Specifically, we became interested in the introduction of solubilizing moieties at the C-terminus of two peptides. Here we present different synthetic strategies for the preparation of peptide–polyethylene glycol conjugates and discuss the effect of the polyethylene glycol chain on the solubility and other properties, such as the catalytic activity of these peptides.

1. Introduction

Low solubility often hampers the conventional study and analysis of molecular properties in solution phase. Limited solubility in aqueous solution is commonly overcome by the introduction of a polyethylene glycol (PEG) chain.[1] Far less explored are strategies to increase solubility in organic solvents. In recent years, we have been interested in the development of catalytically active peptides and the study of selective receptor–peptide interactions.[2–5] With regard to the development of catalytically active peptides, we have identified the peptide H-Pro-Pro-Asp-NH_2 **1** as a highly active and selective catalyst for asymmetric aldol reactions.[2,3] Only 1 mol% of the peptide is sufficient to catalyze aldol reactions with enantioselectivities of up to 91% and good to excellent yields. The applicability of the peptidic catalyst is however limited by its rather low solubility in many organic solvents such as CH_2Cl_2, dioxane, toluene, acetone or $CHCl_3$, commonly used for aldol and related reactions. In the area of selective receptor–peptide interactions, we have identified a highly selective non-covalent interaction between the peptide Ac-D-Val-D-Val-D-His-NH-resin **2a** (resin = polystyrene) and the diketopiperazine receptor **3** in chloroform.[4,5] Combinatorial binding studies demonstrated that peptide **2a** is selected among ≈2000 other peptides by the receptor with a binding affinity of $\Delta G = -5.8$ kcal mol^{-1}. Further studies of the binding mode of this receptor–peptide complex, for example, by NMR spectroscopy, were thwarted by the poor solubility of the non-solid-supported peptide Ac-D-Val-D-Val-D-His-NH_2 **2b** in $CHCl_3$. Thus, to allow for studying the interaction between receptor **3** and peptide **2** as well as to increase the versatility of the catalytically active peptide **1**, we sought to improve the solubility of both peptides in organic solvents. Here we present synthetic routes for the functionalization of **1** and **2** by a PEG chain at their respective C-termini (see Fig. 1).

2. Results and discussion

Since the catalytic activity of H-Pro-Pro-Asp-NH_2 **1** is based upon initial formation of an enamine by the secondary amine of the N-terminal proline, structural modifications at this position lead to loss of catalytic activity. Likewise, modifications at the N-terminus of **2** lead to reduced binding affinities toward receptor **3**. Thus, the solubilizing group had to be attached at the C-termini of both peptides.

Initially we attempted to increase the solubility of peptide **2** by conjugation with hydrophobic and/or

Keywords: Peptides; Polyethylene glycol; Asymmetric catalysis; Epimerization; Molecular recognition.
* Corresponding author. Tel.: +41 61 267 1146; fax: +41 61 267 0976; e-mail: Helma.Wennemers@unibas.ch

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.05.079

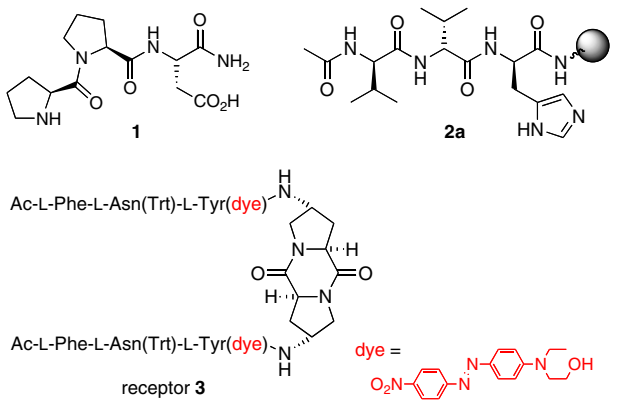


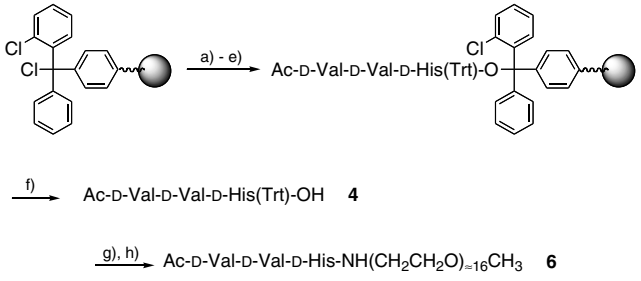
Figure 1. H-Pro-Pro-Asp-NH_2 **1** an efficient asymmetric catalyst for aldol reactions and Ac-D-Val-D-Val-D-His-NH-resin **2a** selectively bound by diketopiperazine receptor **3**.

sterically demanding residues like octylamine, 3,3-diphenylpropylamine or 3,4-dibenzyloxyphenethylamine, designed to hinder intermolecular aggregation of the peptides. However, none of these residues rendered the modified peptides significantly more soluble in chloroform. Hence, we started to explore the possibility of conjugation with PEG chains of different lengths as solubilizing moieties.

Two strategies were pursued to obtain the pegylated peptides Ac-D-Val-D-Val-D-His-NH-PEG_n and H-Pro-Pro-Asp-NH-PEG_n: (a) conjugation in solution phase by coupling of peptides with a carboxylic acid at their C-termini with a PEG bearing a terminal amino group and (b) synthesis of the peptide–PEG conjugates on solid phase.

2.1. Conjugation in solution phase

This strategy encompassed coupling of the peptides Ac-D-Val-D-Val-D-His(Trt)-OH **4** and Boc-Pro-Pro-Asp(O*t*Bu)-OH **5** with a polyethylene glycol bearing a terminal amino group. Peptides **4** and **5** with a carboxylic acid at their C termini and acid labile protecting groups on the side-chain functional groups were prepared on 2-chlorotrityl chloride resin (Scheme 1).



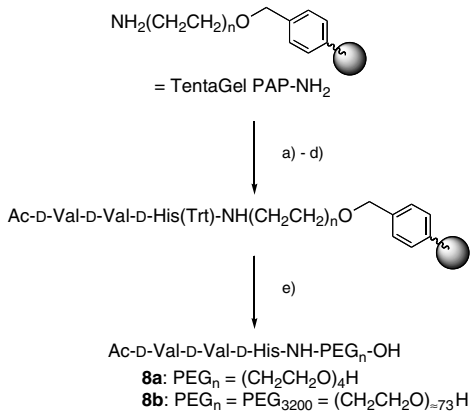
Scheme 1. Synthesis of the peptide–PEG conjugate **6** in solution phase (H-Pro-Pro-Asp-Ahx-NH$(CH_2CH_2O)_3CH_3$ **7** was prepared analogously)[3]. Reagents: (a) 2 equiv Fmoc-D-His(Trt)-OH, 4 equiv $^iPr_2NEt$, CH_2Cl_2; (b) piperidine:DMF 1:4; (c) 3 equiv Fmoc-D-Val-OH, 3 equiv DIC, 3 equiv HOBt, CH_2Cl_2; (d) repeat steps (b), (c) and (b); (e) 5 equiv Ac_2O, 5 equiv NEt_3, CH_2Cl_2; (f) CF_3CH_2OH/AcOH/CH_2Cl_2 1:1:8; (g) 0.8 equiv $H_2N(CH_2CH_2O)_{\approx 16}CH_3$, 1.5 equiv DEPBT, 2 equiv $^iPr_2NEt$, THF/CH_2Cl_2; (h) TFA/CH_2Cl_2 1:5, 45% yield overall.

The first amino acids (Fmoc-D-His(Trt)-OH and Fmoc-Asp(O*t*Bu)-OH) were attached to the solid support using Hünig's base, the following amino acids were coupled following the general Fmoc/*t*Bu protocol using piperidine for Fmoc-deprotections and DIC/HOBt as coupling reagent with *N*-α-Fmoc-protected amino acids. The peptides were removed from the 2-chlorotrityl resin under mildly acid conditions (CF_3CH_2OH/AcOH/CH_2Cl_2 1:1:8)[6] affording the side-chain-protected peptides **4** and **5**. Conjugation of peptides **4** and **5** with PEG was accomplished by coupling either with amino-functionalized triethylene glycol H_2N-$(CH_2CH_2O)_3CH_3$ (peptide **5**) or H_2N-PEG_{750} (peptide **4**).[7,8] However, use of the coupling reagents EDC/HOBt,[9] HATU/$^iPr_2NEt$,[9] TBTU/$^iPr_2NEt$[9] or an activated pentafluorophenyl ester led to considerable epimerization at the chiral centers of the C-terminal amino acids histidine and aspartic acid, respectively. Only the use of DEPBT/$^iPr_2NEt$[9,10] proceeded without detectable epimerization for the coupling of peptide **4**, based on analysis by ^1H NMR spectroscopy. Under the same conditions, a ratio of diastereomers of 12:1 was still observed for the coupling with peptide **5**. To avoid epimerization at the C-terminal Asp of peptide **5** we introduced aminocaproic acid (Ahx) as an achiral linker between the peptide and the PEG chain to afford H-Pro-Pro-Asp-Ahx-NH $(CH_2CH_2O)_3CH_3$ **7**.

2.2. Conjugation on a solid support functionalized with PEG

This strategy involved the synthesis of the peptides on a polystyrene (PS) based solid support where an amino-functionalized PEG chain is attached via an acid labile benzylether linker (TentaGel® PAP-NH_2).[11] Peptide **2** was prepared on solid supports bearing either tetraethylene glycol or PEG_{3200} (Scheme 2).[8] The syntheses were performed as described above by following the standard Fmoc/*t*-Bu-protocol.

The peptide–PEG conjugates were removed from the solid support using a mixture of 99% CF_3CO_2H



Scheme 2. Synthesis of the peptide–PEG conjugates **8a** and **8b** on TentaGel® PAP-NH_2. Reagents: (a) 3 equiv Fmoc-D-His(Trt)-OH, 3 equiv DIC, 3 equiv HOBt, CH_2Cl_2; (b) piperidine:DMF 1:4; (c) repeat steps (a) and (b) twice with 3 equiv Fmoc-D-Val-OH; (d) 5 equiv Ac_2O, 5 equiv NEt_3, CH_2Cl_2; (e) TFA/TMSBr 99:1.

(TFA) and 1% trimethylsilylbromide (TMSBr) and isolated by precipitation with diethyl ether. As judged by NMR-spectroscopic, HPLC, and MS analysis the purity of the peptide–PEG conjugates **8a** and **8b** was $\geqslant$90%. This second approach yields peptide–PEG conjugates where the PEG chain terminates with a hydroxy group in comparison to the methylether obtained by the conjugation of the peptides with PEG in solution phase. The risk of epimerization is significantly reduced since only single amino acids are coupled, hence avoiding the risk of oxazolidinone formation.

2.3. Properties of the peptide–PEG conjugates

Peptide H-Pro-Pro-Asp-Ahx-NH$(CH_2CH_2O)_3CH_3$ **7** bearing only a triethylene glycol chain proved soluble in for example, THF, $CHCl_3$, CH_2Cl_2, and dioxane up to concentrations of at least 100 mM. Compound **7** catalyzes aldol reactions with the same high enantioselectivities as the un-modified peptide **1**, demonstrating that the C-terminal PEG modification did not effect the conformation of the peptide necessary for high selectivities.[3] At the same time, the higher solubility of the pegylated peptide **7** allows for the use of a smaller amount of the catalyst (0.5 mol%) while still maintaining efficient substrate to product conversion and enantioselectivities.

In contrast, the tetraethylene glycol chain of peptide **8a** is not sufficient to render it fully soluble in $CHCl_3$ (maximal solubility $\approx$9 mM). Only the peptide–PEG conjugates **6** and **8b** with PEG_{750} and PEG_{3200},[8] respectively, showed significantly improved solubility compared to the non-pegylated peptide **2b**. These peptides are soluble in $CHCl_3$ in concentrations of at least 100 mM (**6**) and 150 mM (**8b**). Upon mixing each of these peptides with receptor **3** in chloroform solution, the formation of gels was observed, indicating the assembly of supramolecular aggregates.

The side chain protected peptide–PEG conjugates with tri- and tetraethylene glycol chains as well as with PEG_{750} still allow for purification by normal phase column flash chromatography. Peptide–PEG conjugates with PEG_{3200} could only be purified by precipitation from, for example, Et_2O or cold CH_3OH. For these peptide–PEG conjugates, the synthesis on solid phase is significantly superior to the solution phase approach since simultaneous precipitation of the coupling reagent, for example DEPBT, renders the purification difficult.

3. Conclusions

We have presented two strategies for the C-terminal conjugation of two example peptides with PEG chains in solution and on solid phase. Pegylation with PEG chains consisting of $\geqslant$16 units of ethylene glycol rendered the examined peptides soluble to at least a concentration of 100 mM in organic solvents like chloroform or THF. The example of peptide–PEG conjugate **7** demonstrated that even a short triethylene glycol chain can be sufficient to significantly increase the solubility in organic solvents. Currently, we are investigating the catalytic properties of the pegylated peptide **7** in a range of different solvents and the supramolecular assembly formed between the pegylated peptides **6** and **8b** and receptor **3**.

4. Experimental

4.1. General

Materials and reagents were of the highest commercially available grade and used without further purification. Reactions were monitored by thin-layer chromatography using Merck silica gel 60 F_{254} plates. Compounds were visualized by UV, ceric ammonium molybdate (CAM), and ninhydrin. Flash chromatography was performed using Merck silica gel 60. Ion exchange chromatography was performed using Dowex 1×2-400 from Sigma–Aldrich. ^1H and ^{13}C NMR spectra were recorded on Bruker DPX 400 and DPX 500 spectrometers. Chemical shifts are reported in ppm using the solvent residue signals as reference. Bruker esquire 3000plus and Finnigan MAT LCQ instruments were used for electrospray ionization (ESI) and a Voyager-DE PRO BioSpectrometry Workstation from Applied Biosystems for MALDI-ToF mass spectrometry. HPLC analyses were carried out on LiChrosphere 100 RP-18e 5 μm (250 mm × 4.6 mm) from Merck. TentaGel-PAP-NH_2[11] was purchased from Rapp Polymere GmbH, Tübingen Germany.

4.2. General procedure for the functionalization of 2-chlorotrityl chloride resin with carboxylic acids

The *N*-α-Fmoc-protected amino acid Fmoc-Xxx-OH (3 equiv) and $^iPr_2NEt$ (3 equiv) were added to the suspension of 2-chlorotrityl chloride resin (11.2 g, 1.80 mmol g^{-1}) in dry CH_2Cl_2 (110 mL). The reaction mixture was agitated for 2 h, drained, and washed with DMF (3×) and CH_2Cl_2 (5×). A quantitative Fmoc-test indicated a functionalization of the resin of 96% using Fmoc-Ahx-OH and 58% with Fmoc-D-His-OH.

4.3. General procedure for peptide couplings

Fmoc-Xxx-OH (3 equiv) and 1-hydroxybenzotriazole (3 equiv) dissolved in the minimum amount of DMF necessary were added to the suspension of amino-functionalized resin in CH_2Cl_2. The mixture was agitated for 2 min before adding DIC (3 equiv) and agitated for a further 2 h at room temperature. The suspension was washed with DMF (3×) and CH_2Cl_2 (5×).

4.4. General procedure for Fmoc-deprotections

To the resin (pre-swollen in DMF) was added piperidine in DMF (1:4) and the reaction mixture was agitated for 3 min, drained and the piperidine treatment repeated for 10 min. Finally, the resin was washed with DMF (7×) and CH_2Cl_2 (5×). Couplings and deprotections were both monitored by the qualitative Kaiser test.[12] Quantitative Fmoc-monitoring was carried out as spot checks.

4.5. General procedure for acetylation

NEt_3 (5 equiv) followed by Ac_2O (5 equiv) were added to the resin suspended in CH_2Cl_2 and the reaction mixture was agitated for 1 h. The resin was then washed with DMF (3×), and CH_2Cl_2 (5×) and coupling efficiencies were checked qualitatively by the Kaiser Test.

4.6. General procedure for the removal of side-protected peptides from 2-chlorotrityl resin

The solid-supported peptides on 2-chlorotrityl resin were treated with $AcOH/CF_3CH_2OH/CH_2Cl_2$ (1:1:8) and agitated for 45 min at room temperature. Following filtration of the supernatant solution, the resin was washed (3×) with the cleavage solution. Filtrates were combined and AcOH and TFE were removed by repeated azeotropic distillation with hexanes, yielding the side-chain protected peptides which were triturated with Et_2O and coupled with amino-functionalized PEG without further purification.

4.7. General procedure for the removal of peptides from TentaGel-PAP resin

The solid-supported peptides on TentaGel-PAP resin were treated with TFA/TMSBr (99:1) and agitated for 6 h at room temperature. Following filtration, the resin was washed with the cleavage solution (2×) and CH_2Cl_2 (3×). Filtrates were combined and after removal of all volatiles at reduced pressure, the residues were precipitated with Et_2O to yield the peptide–PEG conjugates.

4.8. Ac-D-Val-D-Val-D-His(Trt)-OH, (4)

The peptide was synthesized on 2-chlorotrityl chloride resin following the general procedures for peptide couplings, Fmoc-deprotection, and removal of peptides from 2-chlorotrityl resin. 625 mg (60% based on the initial coupling of Fmoc-D-His(Trt)-OH onto 2-chlorotrityl chloride resin) of **4** was isolated as a white solid.

^{1}H NMR (500 MHz, d_6-DMSO, 25 °C): δ = 8.07 (d, J = 7.2 Hz, 1H), 7.90 (d, J = 8.8 Hz, 1H), 7.65 (d, J = 8.9 Hz, 1H), 7.38 (m, 10H; Trt), 7.24 (s, 1H; His), 7.05 (m, 5H; Trt), 6.71 (s, 1H; His), 4.36 (ψq, J = 7.0 Hz, 1H; His-Hα), 4.18 (m, 2H; Val-Hα), 2.86 (dd, J = 15.2, 6.0 Hz, 1H; His-Hβ), 2.77 (dd, J = 15.2, 8.2 Hz, 1H; His-Hβ), 1.97 (m, 2H; Val-Hβ), 1.91 (*s*, 3H; Ac), 0.79 (m, 12H; Val-Hγ); ^{13}C NMR (125.6 MHz, DMSO, 25 °C): δ = 172.8, 171.0, 170.6, 169.3, 147.3, 137.7, 136.5, 129.3, 128.2, 119.1, 74.5, 57.9, 57.1, 52.5, 30.9, 30.0, 29.8, 29.8, 19.3, 19.2, 18.2, 17.7; MS (ESI): *m*/*z* (%): 638 (100) $[M+H]^+$.

4.9. Ac-D-Val-D-Val-D-His(Trt)-NH-$(CH_2CH_2O)_{16}CH_3$, (4a)

A solution of DEPBT (130 mg, 0.42 mmol) and ^{i}Pr$_2$NEt (100 μl, 0.56 mmol) in a 1:1 mixture of CH_2Cl_2/THF (2 mL) was added to the side-chain-protected peptide **4** (217 mg, 0.34 mmol). After 1 min the solution was added to a solution of anhydrous $H_2N(CH_2CH_2O)_{\approx 16}OCH_3$ (H_2N-PEG_{750}) (210 mg, 0.28 mmol) in anhydrous CH_2Cl_2 (2 mL) and the reaction mixture was allowed to stir at RT for 1 h. After removal of all volatiles at reduced pressure, the residue was purified by flash chromatography on silica gel (gradient of CH_2Cl_2/CH_3OH from 99.5:0.5 to 98:2) to afford 230 mg (0.17 mol, 60%) of **4a** as a colorless oil.

^{1}H NMR (500 MHz, CD_3OD, 25 °C): δ = 7.39 (s, 1H; His), 7.38 (m, 10H; Trt), 7.12 (m, 5H; Trt), 6.76 (s, 1H; His), 4.59 (ψt, J = 7.2 Hz, 1H; His-Hα), 4.22 (d, J = 6.6 Hz, 1H; Val-Hα), 4.17 (d, J = 7.7 Hz, 1H; Val-Hα), 3.76–3.28 (m, 64H; PEG), 2.97 (s, 3H; OCH_3), 2.97 (dd, J = 14.7, 6.4 Hz, 1H; His-Hβ), 2.88 (dd, J = 14.7, 7.3 Hz, 1H; His-Hβ), 2.06 (m, 2H; Val-Hβ), 1.94 (s, 3H; Ac), 0.89 (m, 12H; Val-Hγ); ^{13}C NMR (125.6 MHz, CD_3OD, 25 °C): δ = 174.1, 173.6, 173.2, 173.1, 143.8, 139.7, 137.6, 131.2, 129.7, 121.6, 73.7, 72.9, 71.4, 71.3, 71.3, 71.5, 70.4, 70.2, 60.5, 59.8, 59.1, 54.8, 40.5, 40.4, 32.1, 31.6, 31.5, 22.4, 20.0, 19.8, 18.9, 18.5; MS (ESI): *m*/*z* (%): 1377 (100) $[M+Na]^+$ (distribution of signals due to the polydispersity of PEG).

4.10. Ac-D-Val-D-Val-D-His-NH-$(CH_2CH_2O)_{16}CH_3$, (6)

Compound **4a** (780 mg, 0.57 mmol) was dissolved in a mixture of CH_2Cl_2/TFA (5:1, 30 mL) and allowed to stir at rt for 1 h. After removal of all volatiles at reduced pressure, the oily residue was dissolved in water and eluted over a bed of ion exchange resin (Dowex 1 × 2-400-acetate form) to afford after removal of all volatiles **6** (460 mg, 71%) as a colorless sticky oil.

^{1}H NMR (500 MHz, CD_3OD, 25 °C): δ = 7.29 (s, 1H; His), 6.56 (s, 1H; His), 4.26 (dd, J = 7.8, 1.5 Hz, 1H; His-Hα), 3.85 (d, J = 3.8 Hz, 1H; Val-Hα), 3.84 (d, J = 3.7 Hz, 1H; Val-Hα), 3.31–2.94 (m, 64H; PEG), 3.04 (s, 3H; OCH_3), 2.73 (dd, J = 14.9, 6.2 Hz, 1H; His-Hβ), 2.63 (dd, J = 14.8, 7.9 Hz, 1H; His-Hβ), 1.72 (m, 2H; Val-Hβ), 1.68 (s, 3H; Ac), 0.60 (m, 12H; Val-Hγ); ^{13}C NMR (125.6 MHz, CD_3OD, 25 °C): δ = 174.0 173.4, 173.1, 173.0, 136.3, 73.0, 71.5, 71.4, 71.3, 70.4, 60.5, 60.2, 59.1, 54.7, 40.4, 31.9, 31.6, 30.4, 22.4, 19.8, 19.7, 18.9, 18.9; MS (ESI): *m*/*z* (%): 1113 (100) $[M+H]^+$ (distribution of peaks due to the polydispersity of the PEG).

4.11. H-Pro-Pro-Asp-Ahx-NH$(CH_2CH_2O)_3CH_3$ (7)[3]

Peptide–PEG conjugate **7** was prepared via an analogous route as described for peptide **6**.[3] The ^{1}H and ^{13}C NMR spectra show a double set of peaks in a ratio of 5:1 due to s-*trans* and s-*cis* conformers around the tertiary amide. Major conformer: ^{1}H NMR (500 MHz, D_2O, 23 °C): δ = 4.49 (dd, J = 8.8 Hz, 6.6 Hz, 1 H; Pro^1-Hα), 4.33 (dd, J = 9.0 Hz, 5.2 Hz, 1H; Asp-Hα), 4.25 (dd, J = 8.3 Hz, 5.0 Hz, 1H; Pro^2-Hα), 3.57–3.50 (m, 2H; Pro^2-Hδ, 6H; CH_2O), 3.49–3.40 (m, 1H; Pro^1-Hδ, 4H; CH_2O), 3.39–3.20 (m, 1H; Pro^1-Hδ, 3H; CH_3, 2H; CH_2O), 3.00 (m, 2H, Ahx), 2.58 (dd, J = 14.8 Hz, 5.0 Hz, 1H; Pro^1-Hβ), 2.44 (dd, J = 14.8 Hz, 8.3 Hz, 2H; Asp-Hβ), 2.20–2.15 (m, 1H; Pro^2-Hβ), 2.10 (t, J = 7.5 Hz, 2H; Ahx), 2.04–1.79 (m, 6H; Pro^1-Hβ,

Pro^2-Hβ, Pro^1-2Hγ, Pro^2-2Hγ), 1.44 (m, 2H; Ahx), 1.35 (m, 2H; Ahx), 1.16 (m, 2H; Ahx); ^{13}C NMR (125.6 MHz, D_2O, 23 °C): δ = 177.0, 176.6, 172.6, 172.3, 168.3, 70.9, 69.5, 69.4, 69.3, 68.8, 60.7, 59.1, 58.0, 52.2, 47.6, 46.5, 39.2, 38.8, 38.2, 35.5, 29.2, 28.4, 27.9, 25.5, 24.9, 24.4, 23.8. Minor conformer: ^{1}H NMR (500 MHz, D_2O, 23 °C): δ = 4.38 (dd, J = 8.8 Hz, 3.3 Hz, 1H; Asp-Hα), 4.33 (obscured, 1H; Pro^2-Hα), 4.12 (t, J = 8.0 Hz, 1H; Pro^1-Hα), 3.57–3.50 (m, 2H; Pro^2-Hδ, 6H; CH_2O), 3.49–3.40 (m, 1H; Pro^1-Hδ, 4H; CH_2O), 3.39–3.20 (m, 1H; Pro^1-Hδ, 3H; CH_3, 2H; CH_2O), 3.00 (m, 2H, Ahx), 2.67 (dd, J = 14.8 Hz, 4.3 Hz, 1H; Pro^1-Hβ), 2.48 (dd, J = 14.8 Hz, 10.3 Hz, 2H; Asp-Hβ), 2.20–2.15 (m, 1H; Pro^2-Hβ), 2.10 (t, J = 7.5 Hz, 2H; Ahx), 2.04–1.79 (m, 6H; Pro^1-Hβ, Pro^2-Hβ, Pro^1-2Hγ, Pro^2-2Hγ), 1.44 (m, 2H; Ahx), 1.35 (m, 2H; Ahx), 1.16 (m, 2H; Ahx); ^{13}C NMR (125.6 MHz, D_2O, 23 °C): δ = 177.0, 176.6, 172.6, 172.4, 168.5, 70.9, 69.5, 69.4, 69.3, 68.7, 60.4, 59.0, 58.0, 52.5, 47.8, 46.6, 38.9, 38.8, 37.6, 35.5, 29.2, 28.4, 27.9, 25.5, 24.9, 24.4, 24.0. MS (ESI): m/z (%): 586.4 (100) $[M+H]^+$, 587.4 (29) $[M+2H]^+$.

The peptide–PEG conjugates **8a** and **8b** were prepared following the general procedures for peptide couplings, deprotections, and removal from TentaGel-PAP resin:

4.12. Ac-D-Val-D-Val-D-His-NH-$(CH_2CH_2O)_4H$, (8a)

^{1}H NMR (500 MHz, CD_3OD, 25 °C): δ = 8.83 (s, 1H; His), 7.40 (s, 1H; His), 4.68 (ψt, J = 6.3 Hz, 1H; His-Hα), 4.16 (d, J = 7.3 Hz, 1H; Val-Hα), 4.08 (d, J = 7.8 Hz, 1H; Val-Hα), 3.72–3.40 (m, 16H; PEG), 3.22 (dd, J = 14.5, 8.7 Hz, 1H; His-Hβ), 3.12 (dd, J = 6.7, 15.1 Hz, 1H; His-Hβ), 2.05 (m, 2H; Val-Hβ), 2.01 (s, 3H; Ac), 0.95 (m, 12H; Val-Hγ). ^{13}C NMR (125.6 MHz, CD_3OD, 25 °C): δ = 174.2, 173.5, 173.4, 171.5, 135.1, 130.5, 118.8, 73.6, 71.6, 71.5, 71.3, 71.1, 70.3, 62.1, 60.6, 60.5, 53.4, 40.4, 31.6, 31.5, 28.3, 22.4, 19.7, 19.6, 19.1, 18.9; MS (ESI): m/z (%): 571 (100) $[M+H]^+$.

4.13. Ac-D-Val-D-Val-D-His-NH-$(CH_2CH_2O)_{73}H$, (8b)

^{1}H NMR (500 MHz, CD_3OD, 25 °C): δ = 8.90 (s, 1H; His), 7.43 (s, 1H; His), 4.66 (ψt, J = 6.6 Hz, 1H; His-Hα), 4.17 (d, J = 7.6 Hz, 1H; Val-Hα), 4.10 (d, J = 7.5 Hz, 1H; Val-Hα), 3.82–3.25 (m, 360H; PEG), 3.23 (dd, J = 14.9, 6.5 Hz, 1H; His-Hβ), 3.12 (dd, J = 15.1, 6.3, Hz, 1H; His-Hβ), 2.06 (m, 2H; Val-Hβ), 2.01 (s, 3H; Ac), 0.96 (m, 12H; Val-Hγ); ^{13}C NMR (125.6 MHz, CD_3OD, 25 °C): δ = 174.4, 173.7, 173.6, 171.7, 135.9, 130.5, 119.1, 74.0, 71.7, 71.5, 70.6, 62.5, 60.5, 60.8, 53.8, 40.7, 32.0, 31.9, 28.6, 22.7, 20.1, 20.0, 19.3, 19.2; MS (ESI): m/z (%): 3609 (100) $[M+H]^+$ (distribution of peaks due to the polydispersity of the PEG).

Acknowledgments

This work was supported by BACHEM and the Swiss National Science Foundation. H.W. is grateful to the BACHEM company for an endowed professorship. We thank Dr. Wolfgang Rapp (Rapp Polymere, Tübingen) for samples of the TentaGel PAP-NH_2 resins.

References and notes

1. for example, see: (a) Roberts, M. J.; Bentley, M. D.; Harris, J. M. *Adv. Drug Deliv. Rev.* **2002**, *54*, 459–476; (b) Veronese, F. M. *Biomaterials* **2001**, *22*, 405–417.
2. (a) Krattiger, P.; Kovasy, R.; Revell, J. D.; Ivan, S.; Wennemers, H. *Org. Lett.* **2005**, *7*, 1101–1103; (b) Krattiger, P.; Kovàsy, R.; Revell, J. D.; Wennemers, H. *QSAR Comb. Sci.* **2005**, *24*, 1158–1163.
3. Revell, J. D.; Gantenbein, D.; Krattiger, P.; Wennemers, H. *Biopolymers Pept. Sci.* **2006**, *84*, 105–113.
4. (a) Wennemers, H.; Conza, M.; Nold, M.; Krattiger, P. *Chem. Eur. J.* **2001**, *7*, 3342–3347; (b) Wennemers, H.; Nold, M.; Conza, M.; Kulicke, K. J.; Neuburger, M. *Chem. Eur. J.* **2003**, *9*, 442–448.
5. Conza, M.; Wennemers, H. *Chem. Commun.* **2003**, 866–867.
6. Barlos, K.; Gatos, D.; Kallitsis, J.; Papaphotiu, G.; Sotiriu, P.; Wenqing, Y.; Schäfer, W. *Tetrahedron Lett.* **1989**, *30*, 3943–3946.
7. Dombi, K.; Griesang, N.; Richert, C. *Synthesis* **2002**, 816–824.
8. PEG_{750} and PEG_{3200} refer to PEG chains with average molecular weights of M = 750 and M = 3200, respectively. Their average chain lengths comprise 16 and 73 units ethyleneglycol ($DP_{n,PEO} \approx 16$ and $DP_{n,PEO} \approx 73$, respectively).
9. EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, HOBt = 1-hydroxybenzotriazole, HATU = *O*-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium-hexafluorophosphate, TBTU = *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium-tetrafluoroborate, DEPBT = 3-(Diethoxy-phosphoryloxy)-3*H*-benzo[*d*][1,2,3] triazin-4-one.
10. Li, H.; Jiang, X.; Ye, Y.; Fan, C.; Romoff, T.; Goodman, M. *Org. Lett.* **1999**, *1*, 91–93.
11. (a) Rapp Polymere, Tübingen (Germany); (b) Hudson, D. *J. Comb. Chem.* **1999**, *1*, 403–457.
12. Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595–598.