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Stereoselective Synthesis of Ψ[CH_2O] Pseudodipeptides and Conformational Analysis of a PheΨ[CH_2O]Ala Containing Analogue of the Drug Desmopressin

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Abstract—A method for synthesis of XaaΨ[CH_2O]Ala/Gly pseudodipeptides in good yields and excellent diastereoselectivity from azido alcohols and (*R*)-2-chloropropionic acid or *tert*-butyl bromoacetate has been developed. Insertion of one of the pseudodipeptide building blocks in the peptide drug desmopressin revealed that methylene ether isosteres may have only a minor influence on the secondary structure of peptides.

Replacement of backbone amide bonds by isosteric units stabilizes biologically active peptides towards enzymatic degradation and may also alter other pharmacokinetic properties in a favourable manner.[1] In addition, incorporation of amide bond isosteres is a convenient way to elucidate the role of selected amide bonds in receptor binding, as well as their influence on the secondary structure of peptides. The methylene ether isostere constitutes an interesting amide bond surrogate since the calculated C_α^i–C_α^{i+1} distance of Ψ[CH_2O] pseudodipeptides (3.7 Å) is almost identical to that in a dipeptide (3.8 Å).[2] Ψ[CH_2O] pseudodipeptides are, however, substantially more flexible than normal dipeptides. Several synthetic routes towards Ψ[CH_2O] pseudodipeptides suitable for incorporation into peptides have been reported.[2–6] The most straightforward of these methods is the Williamson's ether synthesis that involves intermolecular[2] or intramolecular[3] substitution of an α-halo acid with an alkoxide obtained from an amino-alcohol. The intermolecular route has predominantly been employed for synthesis of XaaΨ[CH_2O]Gly pseudodipeptides,[2] but was found to give poor diastereoselectivity when applied to a LeuΨ[CH_2O]Ala pseudodipeptide.[3] Unfortunately, in our hands both the intermolecular and the intramolecular route led to formation of diastereomeric mixtures as well as to difficulties in purification of the target pseudodipeptides (cf., below). We have therefore developed an improved procedure for synthesis of XaaΨ[CH_2O]Ala/Gly pseudodipeptide building blocks and incorporated two of these in biologically active peptides. Conformational studies of one of the resulting modified peptides are also reported.

Studies based on NMR spectroscopy and/or energy calculations have revealed that peptide hormones, or their analogues, such as the drug desmopressin[7] (Mpa-Tyr-Phe-Gln-Asn-Cys-Pro-D-Arg-Gly-NH_2, Mpa and Cys form a disulphide bridge), Leu-enkephalin[8,9] (Tyr-Gly-Gly-Phe-Leu) and LHRH[10] (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) may adopt γ- or β-turn structures in aqueous solution (residues involved in turns have been underlined). We have therefore become interested in introduction of turn mimetics in the above peptides in attempts to establish if turns are also involved in their interactions with G-protein coupled receptors.[11,12] Our group has also investigated the role of a glycopeptide from type II collagen (Ac-Ile-Ala-Gly-Phe-Hyl(β-D-Gal)-Gly-Glu-Gln-NH_2: CII260–267) in induction of rheumatoid arthritis in a mouse model.[13] In both of these projects it was essential to determine the role of certain amide bonds in receptor binding and initially we focused our attention on preparation of PheΨ[CH_2O]Ala, TyrΨ[CH_2O]Gly, and IleΨ[CH_2O]Ala pseudodipeptides. Since it is known that Gln4 in desmopressin can be replaced by Ala without

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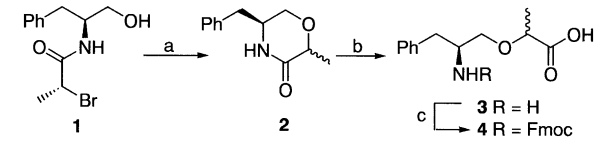
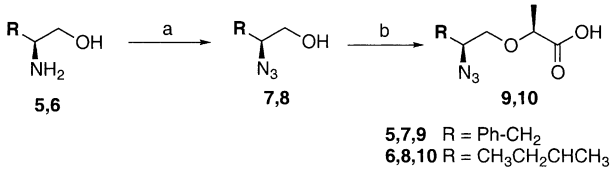
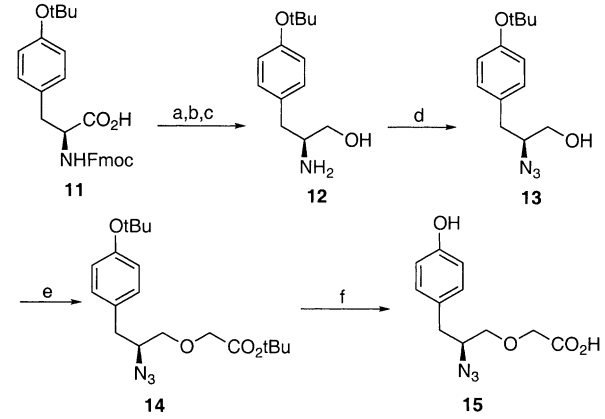
having a major effect on the biological activity,[14] the PheΨ[CH_2O]Ala pseudodipeptide was to be incorporated instead of residues Phe3-Gln4 of desmopressin. The TyrΨ[CH_2O]Gly pseudodipeptide can be incorporated both in Leu-enkephalin and LHRH, whereas IleΨ[CH_2O]Ala matches the first two residues of the CII260–267 glycopeptide. Synthesis of a PheΨ[CH_2O]Ala pseudodipeptide was first attempted by substitution of (*R*)-2-bromopropionic acid with L-phenylalaninol under basic conditions, but the product was obtained in poor yield and as a mixture of diastereomeres that could not be separated.[15] Intramolecular formation of methylene ether isosteres has been reported to proceed without formation of diastereomers[3] and the next attempt therefore involved an intramolecular route (Scheme 1). Compound **1** was prepared and then treated with potassium hydride in THF to give morpholinone **2** in good yield (74%).[15] Unfortunately, ^{1}H NMR spectroscopy revealed that **2** was obtained as a distereomeric mixture which was difficult to purify. Ring-opening of **2** in refluxing 6 M HCl gave **3** which was then protected with an Fmoc group to give **4**. Compounds **3** and **4** were both very difficult to handle during workup and purification by flash column chromatography, and neither of them could be obtained in diastereomerically pure form.



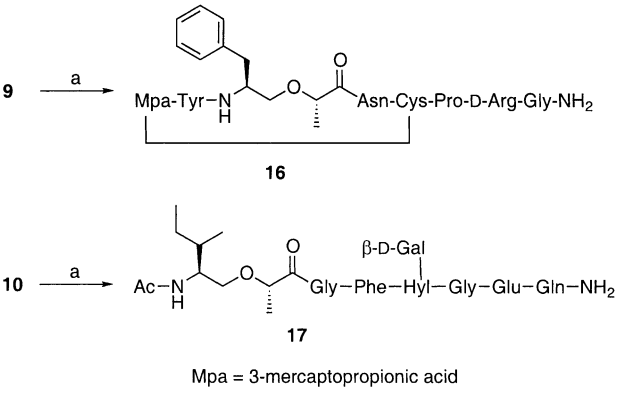
Scheme 1. (a) KH, THF/DMF, 0 °C; 74%; (b) 6 M aq HCl, 100 °C; (c) Fmoc-Cl, 10% aq Na_2CO_3, dioxane, 0 °C.



Scheme 2. (a) TfN_3, DMAP, $CuSO_4$, CH_2Cl_2, rt; 68% for **7** and 90% for **8;** (b) (*R*)-2-chloropropionic acid, NaH, dioxane, rt; 48% for **9** and 63% for **10**.



Scheme 3. (a) *i*BuOCOCl, NMM, THF, −10 °C; (b) $NaBH_4$, MeOH, 0 °C; 90% over two steps; (c) morpholine, rt; 81%; (d) TfN_3, DMAP, $CuSO_4$, CH_2Cl_2, rt; 64%; (e) $BrCH_2CO_2tBu$, NaOH, $QHSO_4$, benzene, rt, 98%; (f) HCOOH, rt, 68%.



Scheme 4. (a) Solid-phase peptide synthesis.

The difficulties encountered in preparation of **2**–**4** prompted us to find an improved synthetic approach to XaaΨ[CH_2O]Ala/Gly pseudodipeptides (Schemes 2 and 3). To circumvent the workup problems encountered with **3** and **4**, an azido group was chosen as protective group for the α-amino group of the pseudodipeptides. The limited size of the azido group also serves to reduce steric hindrance in subsequent reactions. In addition, the azido group can easily be reduced to an amine after the pseudodipeptide has been coupled to a peptide.[16] Aminoalcohols **5** and **6** were converted to azides **7** and **8** in 68 and 90% yields, respectively, via a copper-catalyzed diazo transfer reaction.[17] In a first attempt, alkylation of azidoalcohol **7** with (*R*)-2-bromopropionic acid was carried out in dioxane with sodium hydride as base. This gave pseudodipeptide **9** in 68% yield, but the product was found to consist of a mixture of diastereomers (~4:1) according to ^{1}H and ^{13}C NMR spectroscopy. In an attempt to improve the diastereoselectivity in this reaction, the bromopropionic acid was exchanged for (*R*)-2-chloropropionic acid.[18] Under conditions that were otherwise identical to when 2-bromopropionic acid was used as alkylating agent pseudodipeptides **9**[19,20] and **10**[21] could be obtained in 48 and 63% yields, respectively, with only 1–3% of the diastereomeric PheΨ[CH_2O]-D-Ala and IleΨ[CH_2O]-D-Ala being formed as side-products according to ^{1}H NMR spectroscopy. TyrΨ[CH_2O]Gly (**15**) was synthesized in a similar manner as **9** and **10** (Scheme 3). Thus, conversion of Fmoc-Tyr(*t*Bu)-OH (**11**) into a mixed anhydride by treatment with isobutylchloroformate in the presence of *N*-methyl morpholine, followed by reduction using sodium borohydride,[22] and Fmoc-deprotection with morpholine gave L-tyrosinol (**12**, 72%). Compound **12** was then converted into azide **13** by diazo transfer[17] (64%) after which *O*-alkylation of **13** with *tert*-butyl bromoacetate under phase-transfer catalysis gave protected methylene ether isostere **14** (98%). Deprotection of the *tert*-butyl ester, as well as the *tert*-butyl ether, in formic acid gave TyrΨ[CH_2O]Gly pseudodipeptide **15** (68%).[23] Finally, pseudodipeptides **9** and **10** were incorporated in desmopressin analogue **16**[24] and the type II collagen derived glycopeptide **17**[25] on solid phase using conditions described previously (Scheme 4; 23 and 28% yields, respectively).[11,13] After attachment of pseudodipeptides **9** and **10** to the solid phase, the azido

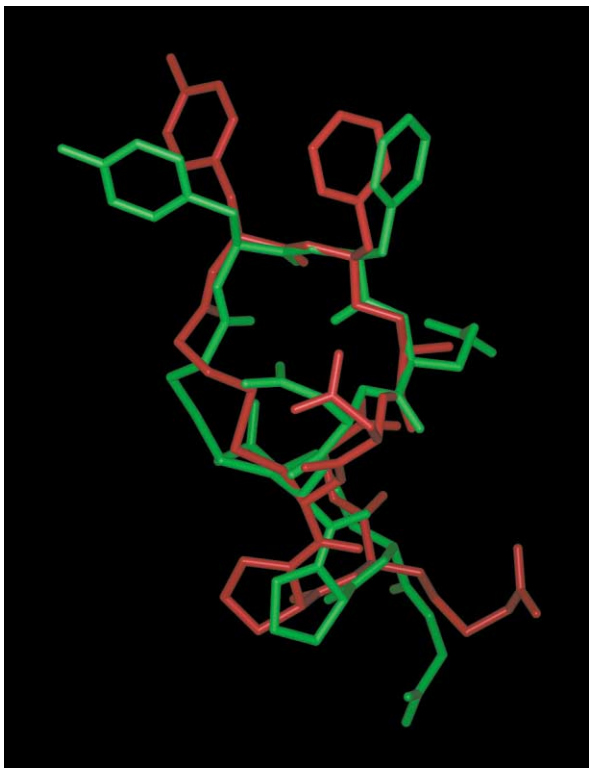


Figure 1. Comparison of the lowest energy structures found in aqueous solution for desmopressin and peptide **16**. Desmopressin is represented in green and **16** in red. The backbone atoms (N, Cα, CO) of residues 1–6 were used for the superimposition and the rmsd calculations.

groups were conveniently reduced[16] by treatment with tin(II) chloride in the presence of thiophenol and triethylamine. However, it was found to be essential to wash the resin with 20% piperidine in DMF before proceeding with the synthesis. If washing with piperidine was omitted, incorporation of the following amino acid did not reach completion. This was assumed to be due to complexation of tin-salts to the liberated amino group in the attached pseudodipeptide.

Little is known on how replacement of amide bonds by methylene ether isosteres affects the secondary structure of peptides. The structure of desmopressin (Mpa-Tyr-Phe-Gln-Asn-Cys-Pro-D-Arg-Gly-NH_2, Mpa and Cys form a disulphide bridge) in aqueous solution has been determined by NMR spectroscopy.[7] Investigation of the structure of analogue **16** would therefore allow an assessment of the structural influence of insertion of a methylene ether isostere into a peptide. In desmopressin the backbone of residues Phe3-Cys6 was found to be rigid and contained an inverse γ-turn centered at Gln4. Residues Mpa1 and Tyr2 were somewhat more mobile, whereas the disulphide bridge and Pro7-Gly9 in the acyclic tail were even more flexible. The structure of peptide **16** was determined in aqueous solution under the same conditions as for desmopressin using two-dimensional NMR techniques. The ^{1}H NMR resonances of **16** were assigned according to standard techniques using COSY, TOCSY and ROESY spectra.[26,27] A total of 55 distance restraints were then derived from the ROESY spectrum and used in simulated annealing calculations using the program X-PLOR.[28] As revealed by comparison of the lowest energy structures of **16** and desmopressin the two peptides have a high degree of similarity (Fig. 1). The rmsd when superimposing all backbone atoms in the macrocyclic ring of the two lowest energy conformations is only 0.66 Å. The methylene ether isostere in **16** does not allow formation of the hydrogen bond between Phe3 CO and Asn5 NH, which is found in the inverse γ-turn of desmopressin. Despite this the backbone conformation is almost identical for residues Tyr2-Cys6 in the two peptides. In addition, the positions of the side chains of residues Tyr2-Cys6 deviate only slightly between **16** and desmopressin. Altogether this suggests that insertion of methylene ether isosteres into peptides may not have a major influence on their conformations. Methylene ether isosteres, as well as, for example, aminomethylene, ketomethylene, and vinylic amide bond replacements, should therefore be useful tools in attempts to establish the role of amide bonds in interactions between peptides and receptors.[29–31] Future studies in our laboratory will focus on incorporation of methylene ether isosters in biologically active peptides such as hormones, and the influence this has on structural features and receptor binding.

In conclusion, an improved method for synthesis of XaaΨ[CH_2O]Ala/Gly pseudodipeptides in good yields and excellent diastereoselectivity has been developed. This method is based on reaction of azido alcohols, which are readily available from amino acids, with (*R*)-2-chloropropionic acid or *tert*-butyl bromoacetate. The versatility of the pseudodipeptide building blocks prepared in this manner was demonstrated by incorporation in the drug desmopressin and a T cell stimulatory glycopeptide from type II collagen. Conformational studies of the resulting desmopressin analogue revealed a close similarity to desmopressin, suggesting that replacement of amide bonds in peptides by methylene ether isosteres can be done without having a major effect on the structure of the substituted peptide.

Acknowledgements

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19. Conditions used for synthesis of pseudodipeptide **9**: A mixture of **7** (89 mg, 0.50 mmol), (*R*)-2-chloropropionic acid (125 mg, 1.15 mmol) and NaH (50% in mineral oil, 610 mg, 12.7 mmol) in 1,4-dioxane (25 mL) was stirred for 16 h at rt. Then H_2O (9 mL) was added to destroy the excess of NaH. The upper phase was was washed with heptane, acidified with 2 M HCl, and extracted with CH_2Cl_2. The resulting organic phase was then dried with $MgSO_4$. After filtration and concentration in vacuo, the crude product was purified by flash chromatography (heptane/ethyl acetate 6:1 + 2% HOAc) on silica gel to give **9** as a colorless oil (60 mg, 48%).
20. Compound **9** had: ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.11 (br s, 1H, COO*H*), 4.05 (q, J = 6.9 Hz, 1H, C*H*CH_3), 3.83 (m, 2H, CH_2O and CHN_3), 3.41 (dd, J = 10.3 and 8.0 Hz, 1H, CH_2O), 2.77 (dd, J = 13.5 and 8.0 Hz, 1H, PhCH_2), 2.68 (dd, J = 13.5 and 5.8 Hz, 1H, PhCH_2), 1.52 (d, J = 7.0 Hz, 3H, CH_3). FAB MS m/z calcd for $C_{12}H_{15}N_3O_3$ ($M + H^+$) 250.119, found 250.119.
21. Compound **10** had: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97 (br s, 1H, COO*H*), 4.07 (q, J = 6.9 Hz, 1H, C*H*OCH_3), 3.88 (dd, J = 9.0 and 2.2 Hz, 1H, CH_2O), 3.47 (dd, J = 9.0 and 8.4 Hz, 1H, CH_2O), 3.50 (m, 1H, CHN_3), 1.58 (m, 1H, C*H*CH_2CH_3), 1.51 (d, J = 6.9 Hz, 3H, CHOCH_3), 1.48 (m, 1H, CHC$H_2$$CH_3$), 1.22 (m, 1H, CHC$H_2$$CH_3$), 0.92 (d, J = 6.8 Hz, 3H, CHCH_3), 0.90 (t, J = 7.6 Hz, CH_2CH_3). FAB MS m/z calcd for $C_9H_{17}N_3O_3$ ($M + H^+$) 216.135, found 216.136.
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23. Compound **15** had: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.09 (d, J = 8.5 Hz, 2H, Ph), 6.80 (d, J = 8.5 Hz, 2H, Ph), 4.21 (s, 2H, OCH_2CO), 3.75 (m, 1H, C*H*N_3), 3.67 (dd, J = 3.7 and 9.8 Hz, 1H, CHN_3CH_2O), 3.55 (dd, J = 6.7 and 9.8 Hz, 1H, CHN_3CH_2O), 2.83 (dd, J = 6.5 and 14.0 Hz, CH_2PhOH), 2.77 (dd, J = 7.5 and 14.0 Hz, CH_2PhOH). FAB MS m/z calcd for $C_{11}H_{13}N_3O_3$ ($M + Na^+$) 274.079, found 274.083.
24. Peptide **16** had: FAB MS calcd for $C_{44}H_{63}N_{12}O_{11}S_2$ 999 (M + H), found 999.
25. Glycopeptide **17** had: ES MS calcd for $C_{46}H_{74}N_{10}O_{18}$ 1055 (M), found 1055.
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