Dihydropyran-2-carboxylic acid, a novel bifunctional linker for the solid-phase synthesis of peptides containing a C-terminal alcohol

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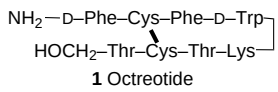
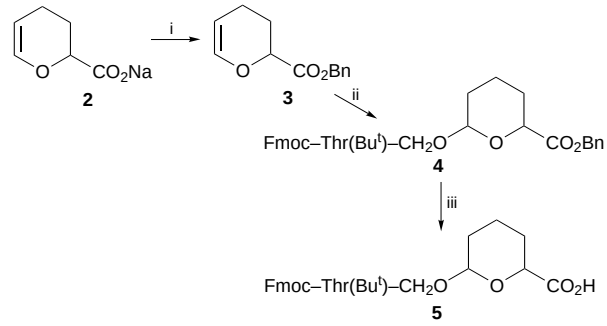
Dihydropyran-2-carboxylic acid, a novel compound used to link an amino alcohol and an amine resin, is utilised for the solid-phase synthesis of peptide alcohols *via* the Fmoc strategy; this bifunctional linker was effectively applied to the synthesis of Octreotide.

Peptides that contain a C-terminal alcohol, such as Octreotide,[1] Enkephalins,[2] Gramicidin[3] and Trichoderma species,[4] have been the focus of recent research, because of these peptides bind with high affinity to their receptors and have a long half-life *in vivo*. Peptides containing a C-terminal alcohol cannot be synthesized by conventional solid-phase peptide synthesis (SPPS), because present protocols require a free carboxy group to attach onto the resin. Octreotide **1**, a metabolically stable somatostatin analog, inhibits the growth of tumor cells by binding to surface somatostatin receptors.[5] Many methods have been reported for its synthesis,[1a,6a,b,c] however[5,6] these methods have drawbacks including low yields and complicated procedures. We describe here a new procedure using dihydropyran-2-carboxylic acid as a bifunctional linker for the synthesis of peptide alcohols by SPPS.



1 Octreotide

Sodium 3,4-dihydro-2*H*-pyran-2-carboxylate **2** was prepared according to established procedures.[7,8] Compound **2** is a key component of the bifunctional linker which easily undergoes self-lactonization under acidic conditions,[9,10] and the carboxy group of **2** must be protected before the dihydropyran portion couples with the amino alcohol. Compound **2** was reacted with BnBr in DMF at 25 °C in the presence of Cs_2CO_3 to form benzyl 3,4-dihydro-2*H*-pyran-2-carboxylate **3**, which was further reacted with Fmoc-Thr(Bu^t)OH[11] in the presence of TsOH in CH_2Cl_2 to yield Fmoc-Thr(Bu^t)-THP-2-CO_2Bn **4**. Selective catalytic transfer hydrogenation to remove the benzyl group of **4** using Pd/C–cyclohexa-1,4-diene yielded Fmoc-Thr(Bu^t)-THP-2-CO_2H **5** (90% yield) without interfering with the other three protection groups. Enzymes such as alcalases and lipases or chemical methods such as K_2CO_3 in THF–H_2O under mild hydrolytic conditions were ineffective for removal of the benzyl ester, due to low reactivity and low conversion. Catalytic hydrogen transfer, however, effectively cleaved the benzyl ester without deprotecting the Fmoc group, while the Fmoc group could be cleaved by hydrogenation over Pd/C or $Pd(OAc)_2$.[12] In a similar manner, Fmoc-Gly-THP-2-CO_2H **6** and Fmoc-Phe-THP-2-CO_2H **7**, the C-terminal amino alcohols of the Grami-



Scheme 1 *Reagents and conditions*: i, Cs_2CO_3, BnBr, room temp., 24 h (80%); ii, TsOH, CH_2Cl_2, Fmoc-Thr(Bu^t)-ol, 12 h (85%); iii, Pd/C, MeOH, cyclohexa-1,4-diene, 2 h (90%)

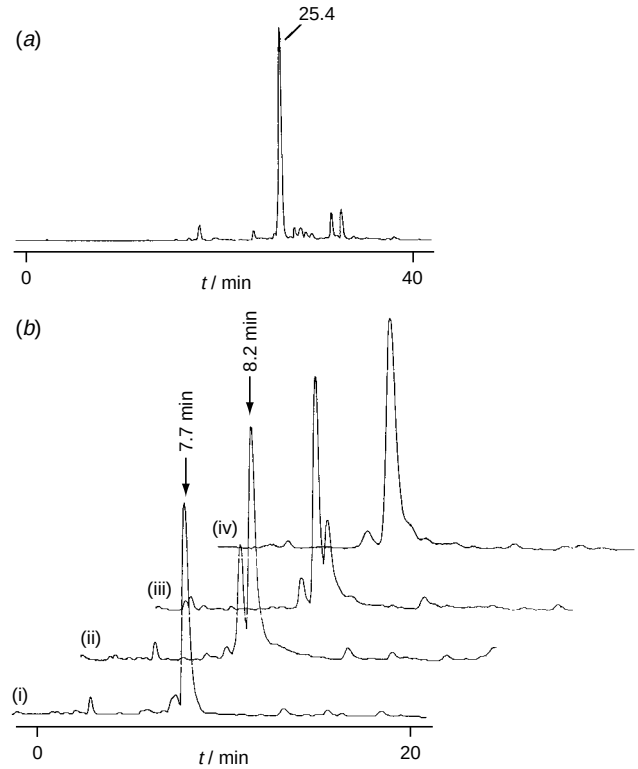
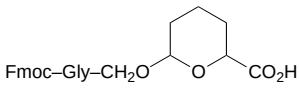


Fig. 1 (*a*) Analysis of reduced Octreotide (retention time 25.4 min) by HPLC. Sample was taken from the aq. AcOH (5%) used to extract the peptide immediately after the elongated peptide was cleaved from the resin. Conditions for HPLC: RP-18 column, 4.6 × 250 mm; gradient 0 → 30 min, 0 → 100% B; 30 → 35 min, 100% B; 35 → 40 min, 100 → 0% B; flow rate 1.0 ml min^{-1}; A = 5% MeCN in water containing 0.1% TFA; B = 90% MeCN in water containing 0.1% TFA; UV detection at 214 nm. (*b*) The time course for the folding of reduced Octreotide: (i) 0, (ii) 8, (iii) 32 and (iv) 46 h. The progress of the peptide folding was followed by analytic reverse-phase HPLC. The retention time for the reduced Octreotide was 8.2 min and for the folded Octreotide was 7.7 min. Conditions for HPLC: RP-18 column, 4.6 × 250 mm; gradient 0 → 20 min, 50 → 60% B; 20 → 25 min, 60% B; 25 → 30 min, 60 → 50% B; flow rate 1.0 ml min^{-1}; A = 50% MeCN in water containing 0.1% TFA; B = 90% MeCN in water containing 0.1% TFA; UV detection at 214 nm.



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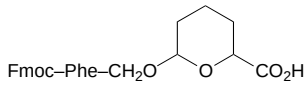

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cidin[3] and Trichoderma species,[4] were also obtained from Fmoc-Gly-ol[13] and Fmoc-Phe-ol[13] in yields of 72 and 78%, respectively (see Scheme 1).

The novel linker was used successfully to synthesize Octreotide. Fmoc-Thr(Bu^t)-THP-2-CO_2H was attached to an amine resin and the peptide Fmoc-D-Phe-Cys(Trt)-Phe-D-Trp(Boc)-Lys(Boc)-Thr(Bu^t)-Cys(Trt)-Thr(Bu^t)-CH_2O-THP-CO-amide resin was synthesized stepwise using an Fmoc protocol. After completion of the peptide synthesis, simultaneous cleavage of Octreotide from the resin and deprotection of the side-chains was performed using the TFA method (TFA–thioanisole–ethanedithiol–H_2O = 0.9 : 0.5 : 0.25 : 0.25). Dilsulfide bond formation was achieved by air oxidation in aqueous phosphate buffer at 25 °C pH 7 for 48 h monitored by HPLC. Pure Octreotide was isolated *via* preparative reverse-phase HPLC (70% total yield). Fig. 1 illustrates the time course of the disulfide bond formation. The reduced form of Octreotide and the final product appeared as major peaks (Fig. 1). The presence of Octreotide was confirmed by amino acid analysis and FAB mass spectrometry (*m*/*z* 1019.5 [M^+ + 1]).

The high yields and purity (Fig. 1) of Octreotide produced using the novel bifunctional linker demonstrate that the linker is stable under basic Fmoc/Bu^t synthetic conditions as well as acid-labile and easily removed from the resin to regenerate the desired C-terminal alcohol. Furthermore, because the DHP-2-CO_2H linker first couples with the C-terminal amino alcohol to form the first residue for SPPS, the linker can more easily couple to any primary, secondary or even tertiary alcohols compared to Ellman's DHP-resin bound system.[6] In conclusion, the novel bifunctional linker can be used successfully in the synthesis of peptide C-terminal alcohols in addition to the preparation and screening of combinatorial peptide and non-peptide libraries *via* solid-phase synthesis techniques.[14]

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Notes and References

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