Solid-Phase Peptide Synthesis

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Synthesis of Peptide Alcohols on the Basis of an O–N Acyl-Transfer Reaction**

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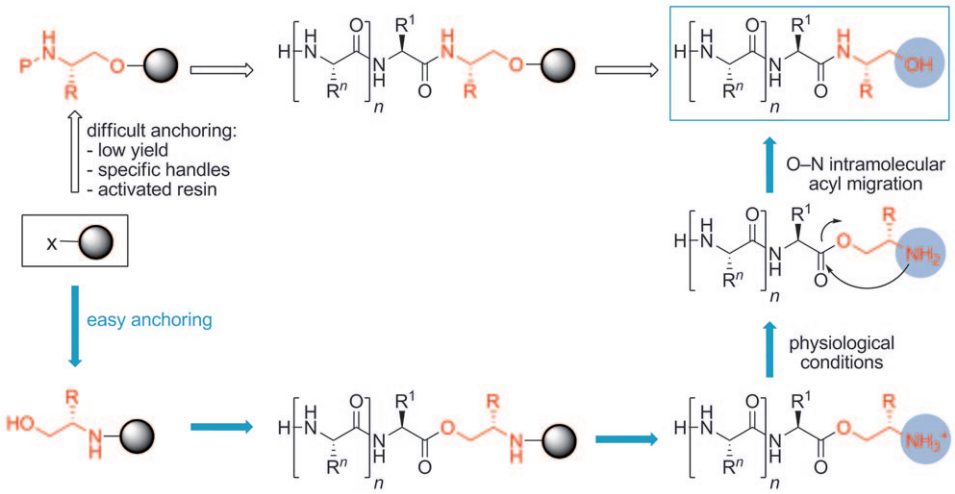
Peptides containing a C-terminal alcohol function (C-terminal peptide alcohols) constitute an important class of compounds. They exhibit a range of biological activities, such as the antibiotic properties of the peptaibols[1,2] and the potent and unique biological activity of the metabolically stable somatostatin analogue octreotide (Sandostatin), which is used clinically for the diagnosis and treatment of a variety of neuroendocrine tumors and gastrointestinal disorders.[2] Other examples of biologically active C-terminal peptide alcohols are the potent enkephalin analogue Tyr-D-Ala-Gly-MePhe-Met(O)-ol,[3] the gramicidins,[4] and compounds isolated from *Trichoderma* species.[5,6] Furthermore, peptide alcohols serve as precursors for the synthesis of peptide aldehydes, an important class of protease inhibitors and versatile synthetic intermediates.[7] C-terminal peptide alcohols cannot be synthesized by conventional solid-phase peptide synthesis (SPPS) because of the absence of a free carboxylic group to attach to the resin. However, other SPPS methods have been developed that involve reductive cleavage of a peptide ester linkage,[8,9] the use of redox-sensitive resins,[10] aminolysis of a peptide ester linkage with a β-amino alcohol,[11] or standard cleavage of a peptide alcohol ester linkage.[12] Except when the chlorotrityl resin is used,[13] anchoring of the hydroxy function requires derivatization of the linker or C-terminal β-amino alcohol with a specific handle, such as that found in tetrahydropyranyl-based linkers,[14,15] hemisuccinate linkers,[16,17] or polymeric diphenyldiazomethane;[18] alternatively, activated resins can be used.[19,20] There is still a strong need for new methodology for the preparation of peptide alcohols by the conventional 9-fluorenylmethoxycarbonyl (Fmoc)/*t*Bu SPPS strategy with commercially available resins that would enable the recovery of a nonprotected or totally protected peptide alcohol. According to these requirements, the trityl-type resin is the most promising. Unfortunately, the attachment of the alcohol group to this support remains difficult and proceeds in low yield.

To overcome the drawbacks of these methods, we developed a new strategy based on an O–N acyl-transfer reaction (Scheme 1). A C-terminal β-amino alcohol residue was anchored to a trityl chloride resin through its β-amino function. The free hydroxy function afforded the starting point for Fmoc/*t*Bu SPPS after an esterification reaction with a Fmoc-protected amino acid. At the end of the synthesis, cleavage with trifluoroacetic acid (TFA) released the unprotected isopeptide as the TFA salt. The peptide alcohol was obtained through an intramolecular O–N acyl transfer of the isopeptide. This reaction occurred in both aqueous and organic media.[21–29] This methodology could be used as a prodrug strategy for the generation of peptide alcohols from the corresponding isopeptides as described for taxoids.[30]



Scheme 1. General strategy for the synthesis of peptide alcohols on the basis of an O–N acyl migration.

To validate the strategy, three peptide alcohols were synthesized: a fragment of gramicidin A (**1a**), a fragment of

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the antimicrobial peptide trichogin GA IV (**1b**), and octreotide (**1c**). The approach combined the synthesis of isopeptides on a solid support and the O–N acyl-transfer reaction in solution (Scheme 2). *N*-Fmoc β-amino alcohols **2a–c** were obtained by the $NaBH_4$ reduction of mixed anhydrides of *N*-Fmoc amino acids in an organic/aqueous medium.[31]

The *N*-Fmoc β-amino alcohols **2a–c** were subjected to a one-pot deprotection (removal of Fmoc) and resin-loading reaction with a 2% solution of DBU[32] in anhydrous DMF to afford the β-amino alcohol functionalized resins **3a–c**. The free hydroxy function was then acylated with a suitable N-protected amino acid in the presence of DIC/DMAP to afford the supported compounds **4a**,**b** and **4c′**. No epimerization was detected in the DIC/DMAP acylation with Fmoc-Trp(Boc)-OH and Fmoc-Ile-OH to produce **1a** and **1b**. However, in the synthesis of octreotide, a substantial degree of epimerization (about 30%) of the especially sensitive cysteine residue occurred. The extent of epimerization was analyzed by reversed-phase (RP) HPLC on a chiral phase after the treatment of **4c′** with TFA/TIS/H_2O/EDT (Figure 1).

In the search for a general epimerization-free anchoring system, treatment of the polymer-supported β-amino alcohol **3c** with Fmoc-(L or D)-Cys(Trt)-OH in a Mitsunobu reaction[33] afforded compounds **4c** and **4d**, respectively, in enantiomerically pure form (Figure 1). This esterification system is reported to lead to a low level of substitution[34] but to preserve chiral integrity.[35] As expected, epimerization was not detected (by HPLC of compounds **7c** and **7d**, formed

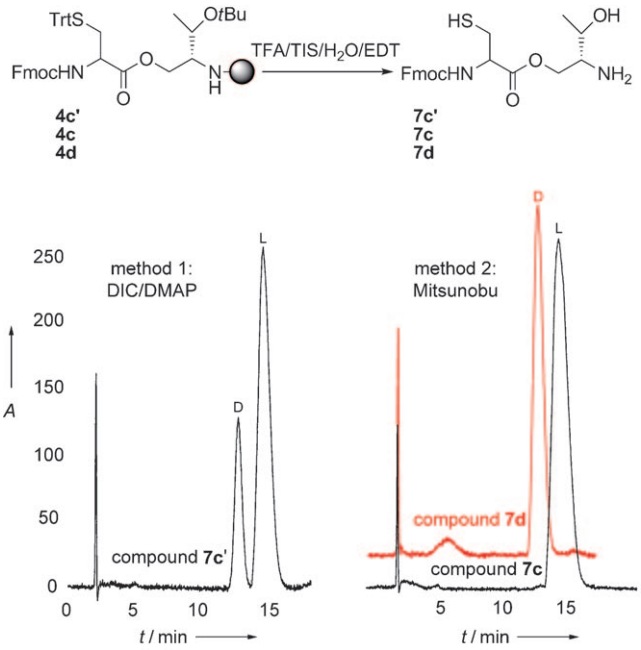
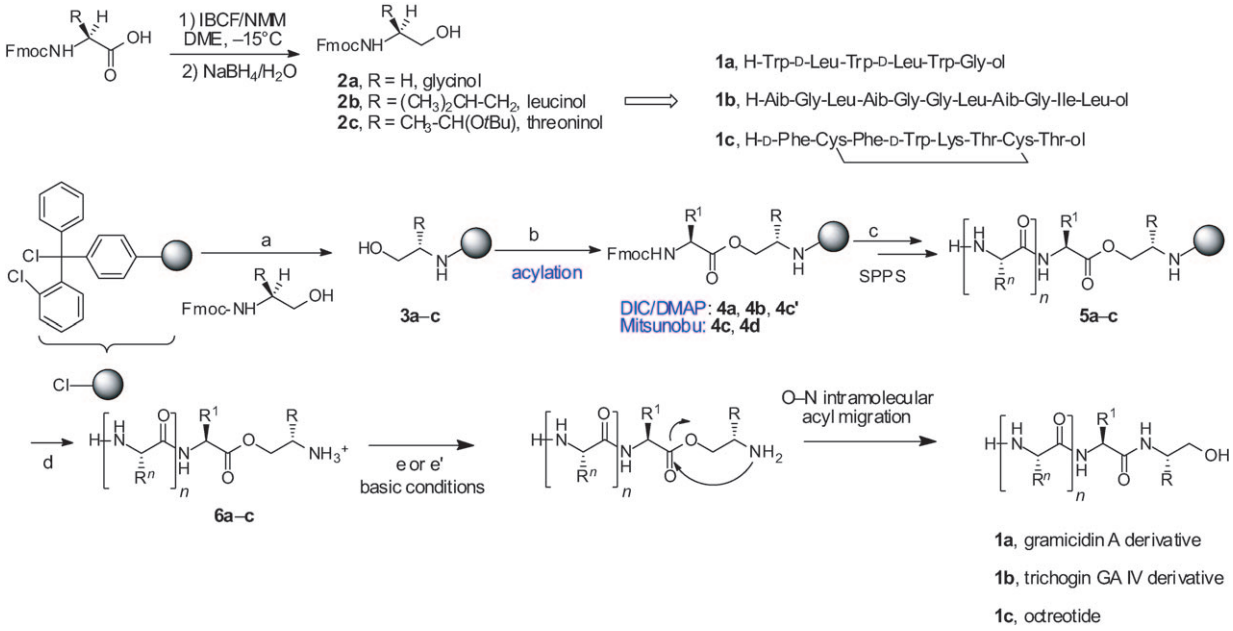


Figure 1. Investigation of the epimerization of cysteine during esterification by method 1 or 2 by HPLC (chiralcel OD-RH column; detection at 214 nm; elution of crude Fmoc-(L/D)-Cys-Thr-ol (**7c′**), Fmoc-Cys-Thr-ol (**7c**), and Fmoc-D-Cys-Thr-ol (**7d**) under isocratic conditions with a mixture of H_2O/0.1% TFA and CH_3CN/0.1% TFA (70:30) for 30 min at a flow rate of 0.8 mL min^{-1}). Absorbance is given in mAU. AU = absorbance units, Trt = trityl (triphenylmethyl).



Scheme 2. General conditions for the synthesis of peptide alcohols: a) Fmoc-β-amino alcohol (3 equiv), 2% DBU in anhydrous DMF, room temperature, 12 h; b) method 1 (DIC/DMAP acylation) for **4a**,**b** and **4c′**: Fmoc-AA1-OH (6 equiv), DIC (3 equiv), DMAP (0.3 equiv), anhydrous CH_2Cl_2/DMF (50:50), room temperature, 12 h; method 2 (Mitsunobu conditions) for **4c**,**d**: Fmoc-(L or D)-Cys(Trt)-OH (3 equiv), PPh_3 (3 equiv), DEAD (3 equiv), anhydrous THF, room temperature, 2×6 h; c) SPPS: deprotection: DMF/piperidine (80:20); coupling: Fmoc-AA-OH (3 equiv), HBTU (3 equiv), DIEA (3 equiv), DMF; d) for **5a**: TFA/TIS/H_2O (95:2.5:2.5 v/v/v), 1 h; for **5b**: TFA, 1 h; for **5c**: TFA/EDT/anisole/thioanisole (90:5:2.5:2.5 v/v/v/v), 2 h; e) for **6a**,**b** (O–N acyl shift): DMF/piperidine (80:20); e′) for **6c** (O–N acyl shift and oxidation): aqueous phosphate buffer (pH 7.4), oxyfold resin (5 equiv). DEAD = diethyl azodicarboxylate, DIC = diisopropylcarbodiimide, DIEA = *N*,*N*-diisopropylethylamine, DMAP = 4-dimethylaminopyridine, DME = dimethoxyethane, DMF = *N*,*N*-dimethylformamide, EDT = 1,2-ethanedithiol, HBTU = *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, IBCF = isobutylchloroformate, NMM = *N*-methylmorpholine, TIS = triisopropylsilane.

from **4c** and **4d** by removal from the resin and cleavage of the *tert*-butyl ether and trityl thioether; Figure 1). After treatment at room temperature for two periods of 6 hours, the estimated peptidyl resin loading was the same as that observed with DIC/DMAP (0.47 mmol g^{-1}, 64% yield; see calculation in the Supporting Information). Moreover, epimerization of the cysteine residue linked to a resin through an ester bond during peptide elongation was investigated by the synthesis of D-Cys[7]-containing octreotide: no epimerization was observed (see the Supporting Information).

During the synthesis of **1a**, which contains an unfavorable L/D combination, the risk of diketopiperazine formation[36] was analyzed (see the Supporting Information). Upon treatment with DMF/piperidine for 1 hour, no release of the diketopiperazine [D-Leu-L-Trp] was observed.

Further elongation by the HBTU/DIEA coupling strategy yielded the resin-anchored isopeptides **5a–c**. Cleavage from the resin by treatment with TFA afforded the free isopeptides **6a–c** with high purity (80–95%). Compounds **6a–c** were then subjected to the O–N acyl-migration reaction. They were dissolved and stirred in aqueous phosphate buffer at pH 7.4 or in the organic solvent mixture DMF/piperidine (80:20),[22] and the intramolecular O–N acyl migration was monitored for each compound by RP HPLC and LC/MS analysis.

The intramolecular transposition reaction was quantitative in organic solvents at room temperature; it reached completion within 1 hour to afford the crude compounds **1a,b** with good purity (average, 90%; see the Supporting Information). The yields of the recovered peptides were between 80 and 90% on the basis of the calculated resin loading of **4a,b**. For octreotide (**1c**), the O–N acyl shift and oxidation of **6c** were performed under slightly alkaline conditions at room temperature in a phosphate buffer, either in the presence of oxyfold resin (5 equiv)[37] for 6 hour or without the oxidizing reagent for 2 days. Octreotide (**1c**) was recovered in high yield with high purity (Figure 2). Notably, complete conversion of the isopeptide **6c** into the corresponding peptide alcohol occurred prior to the formation of the disulfide bond.

We have reported a simple method for the synthesis of challenging peptide-alcohol targets from β-amino alcohol starting materials through the use of an O–N acyl-transfer reaction. This methodology proved to be efficient for the loading of the β-amino alcohols onto the resin through their amino function, and no epimerization was observed when the Mitsunobu coupling protocol was used for acylation with a cysteine derivative.

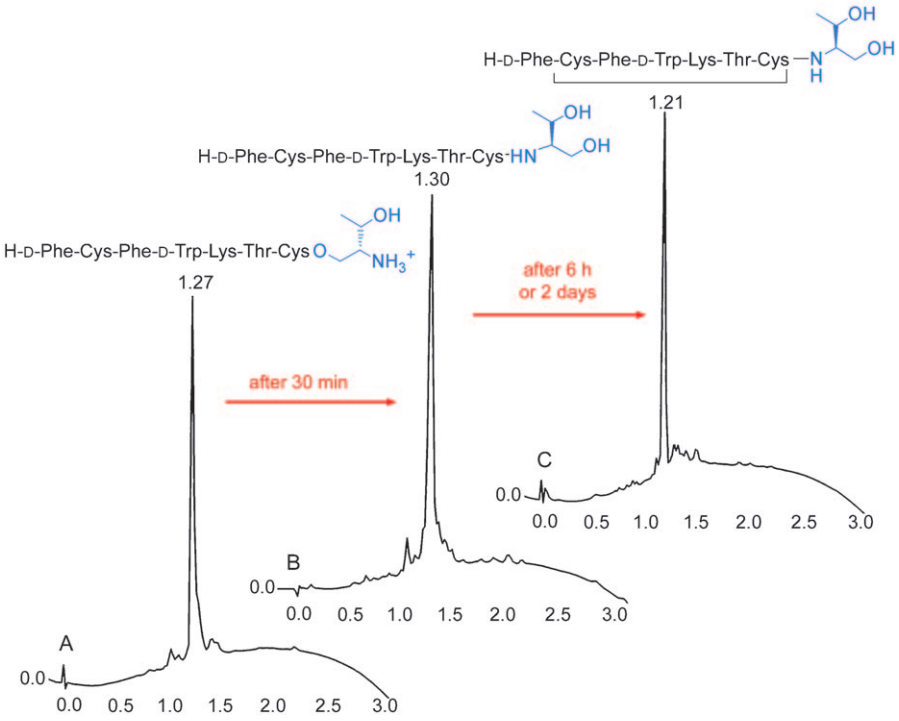


Figure 2. Monitoring of the formation of octreotide (**1c**). HPLC profiles A) of crude isooctreotide **6c**, B) for the conversion of **6c** into the corresponding peptide alcohol, and C) of octreotide (**1c**). (HPLC conditions: VWR chromolith column; detection at 214 nm; elution: 0–100% B linear gradient over 3 min at a flow rate of 5 mL min^{-1}; solvent A: H_2O/0.1% TFA; solvent B: CH_3CN/0.1% TFA).

Experimental Section

3a–c: The Fmoc-β-amino alcohol **2** (3 equiv) was dissolved in a solution of DBU (2%) in anhydrous DMF, and the resulting solution was added to the 2-chlorotrityl chloride resin (1.55 mmol g^{-1}). The reaction mixture was stirred at room temperature for 12 h, and the resin was then removed by filtration and washed with DMF (×3), 10% DIEA in MeOH (×3), DMF (×3), and CH_2Cl_2 (×3).

4a,b and 4c′: DIC (3 equiv) and DMAP (0.3 equiv) were added to a solution of Fmoc-AA1-OH (6 equiv relative to resin substitution) in a mixture of anhydrous CH_2Cl_2 and DMF (50:50) at 0°C (3 mL mmol^{-1}). The resulting mixture was stirred with the β-amino alcohol functionalized resin for 6 h at room temperature. This procedure was repeated twice, and then the loading was determined by Fmoc titration: **4a**: 0.46 mmol g^{-1}; **4b**: 0.42 mmol g^{-1}; **4c′**: 0.47 mmol g^{-1}

4c: A solution of PPh_3 (3 equiv) and Fmoc-Cys(Trt)-OH (3 equiv) in anhydrous THF (20 mL per gram of resin) was added to the resin. A 40% solution of DEAD in toluene (3 equiv) was then added, and the mixture was stirred for 6 h at room temperature. This procedure was repeated twice. The loading was determined by Fmoc titration: 0.47 mmol g^{-1}.

Elongation and cleavage of the isopeptides were performed by conventional SPPS (see the Supporting Information).

1c: Isopeptide **6c** was dissolved in phosphate buffer (pH ≈ 7.4), and the mixture was stirred at room temperature. The reaction was monitored by HPLC and LC/MS. After 30 min, conversion of the isopeptide **6c** into the corresponding peptide alcohol was observed: *m/z* 1021.8 $[M+H]^+$. After 48 h, the filtrate was concentrated under reduced pressure and lyophilized for analysis by HPLC and LC/MS. HPLC: $t_R = 1.21$ min (89% purity for the crude compound); LC/MS: monoisotopic mass calculated for $C_{49}H_{66}N_{10}O_{10}S_2$: 1018.4 Da; found:

m/*z* 1019.6 $[M+H]^+$; yield of **1c** after HPLC purification: 80% (purity > 99%).

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