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Use of Trichloroacetimidate Linker in Solid-Phase Peptide Synthesis

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Abstract: A solid-phase method for the preparation of C-terminal amino-alcohol-containing peptides using activated Wang resin is presented. A diverse set of (fluorenylmethoxy)carbonyl (Fmoc) protected amino alcohols was found to load rapidly and efficiently. The synthetic utility of this approach was demonstrated through the direct synthesis of the peptide drug octreotide with excellent yield and purity. These results suggest that the use of trichloroacetimidate activated resins offers an attractive alternative in the preparation of this class of peptides.

The successful execution of a solid-phase procedure requires the covalent attachment of the starting material to a solid support, usually polystyrene or poly(ethylene glycol), through an appropriate linker or "handle". The classic C-terminal anchoring strategy for peptide synthesis pioneered by Merrifield based on benzyl and benzhydrylamine linkers for Boc/Bzl chemistry[1−3] as well as the more labile alkoxybenzyl[4] or 2,4-dimethoxybenzhydrylamine[5] versions used in Fmoc/tBu protocols is generally restricted in its application to the synthesis of peptides with either free carboxyl or carboxyl amide termini. While C-terminal anchoring permits modifications to the N-terminus of a peptide, modifications to the C-terminus itself tend to be more challenging and may require use of specialized linkers, prior cleavage from support, or other indirect methods. An example of such a modification is a C-terminal alcohol shared by the antibacterial peptide alamethicin,[6] a number of cholecystokinin antagonists, growth hormone secretagogues,[7,8] and, most prominently, the growth hormone inhibitor octreotide.[9] Several specialized anchoring strategies have been devised in order to synthesize octreotide and related analogues by the solid-phase method. Albericio and coworkers[10] reported the use of an active carbonate resin to anchor Boc-threoninol (OBzl) as part of a Boc/Bzl synthetic strategy, while Arano et al.[11] chose 2-chlorotrityl linker[12] for attachment of the C-terminal threoninol in their preparation of a diethylene-triaminepentacetic acid (DPTA) conjugated octreotide by (fluorenylmethoxy)-carbonyl (Fmoc)/tBu strategy. Additionally, a creative solution was provided by the Novartis group, which involved the use of a cyclic acetal linkage between Fmoc-threoninol and a 4-formylphenoxy resin in a solid-phase synthesis of a radiotherapeutic analogue of octreotide.[13] Rivier and co-workers recently reported a series of C-terminal peptide alcohols as gonadotropin-releasing hormone antagonists, prepared by using a solution-coupling method to attach the amino acid alcohol to the preassembled peptide fragment.[14]

TABLE 1. Loading of Amino Alcohol

Fmoc AA	substitution[a]	yield (%)[b]
glycinol	0.45	64.3
alaninol	0.42	60.2
D-alaninol	0.52	74.6
argininol (Pbf)	0.42	74.5
leucinol	0.39	57.6
phenylalaninol	0.40	60.4
D-phenylalaninol	0.49	74.0
prolinol	0.42	61.3
tryptophanol	0.36	55.8
lysinol (Boc)	0.35	55.7
threoninol (OtBu)	0.48	73.0

[a] Substitution levels were determined as described in the text and are expressed in millimoles substrate per gram polystyrene. [b] Yields are based on the percent of maximal theoretical substitution.

The recent reports by Hanessian[15] describing the use of trichloroacetimidate modified resins in organic synthesis demonstrated the straightforward preparation, substrate loading, and facile cleavage as well as stability of this linker strategy. These findings encouraged us to investigate the application of trichloroacetimidate resins in the solid-phase synthesis of peptides containing an amino alcohol at the C-terminus.

Initial feasibility studies with a number of diverse Fmoc protected amino alcohols (obtained either commercially or prepared according to the procedure of Rodriquez et al.[16] from the corresponding carboxylic acids) confirmed the validity of this approach. Investigation of loading conditions including time, equivalents of Lewis acid, and substrate indicated an optimized protocol involving exposure of the resin to a 3-fold excess of the Fmoc amino alcohol in dry THF in the presence of 0.2 equiv of $BF_3 \cdot Et_2O$ for 1 h. The loading performance for a representative set of amino alcohols using the above set

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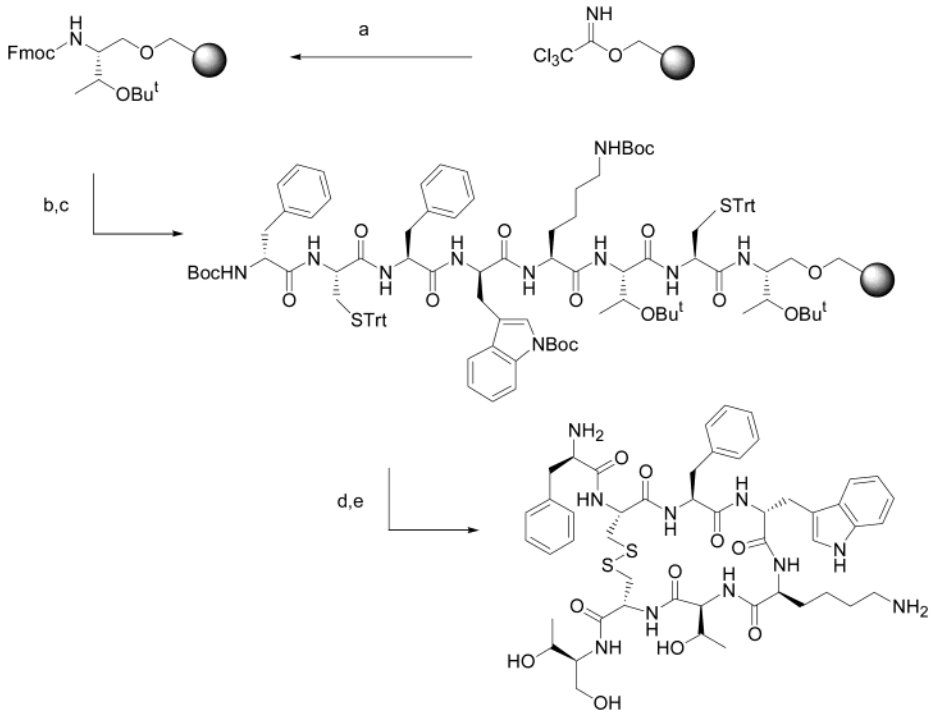
SCHEME 1. Solid-Phase Synthesis of Octreotide[a]



[a] Reagents and conditions: (a) Fmoc−threoninol(OtBu), $BF_3{\cdot}Et_2O$ in dry THF; (b) 20% piperidine, DMF; (c) 3 equiv FmocAA−OH, DCC, HOBt; (d) TFA/DCM cleavage; (e) air oxidation.

of conditions is summarized in Table 1. Substitution levels, determined by the method of Meienhofer[17] through spectrophotometric detection of dibenzofulvene from resin aliquots, revealed a consistent range of loading from 0.3 to 0.50 mmol/g and were largely independent of the amino acid side chain. Subsequent studies showed that the conditions could be further modified to reduce the amount of $BF_3{\cdot}Et_2O$ to 0.05 equiv for a 1 h reaction time. Similarly, the reaction time could be shortened to as little as 1.0 min, using 0.5 equiv of catalyst. Neither the use of additional $BF_3{\cdot}Et_2O$ nor extension of the reaction time beyond 1 h resulted in a significant increase in the substitution levels. The use of an alternative Lewis acid catalyst was investigated using $AlCl_3$. The performance of this reagent was found to be inferior to that of $BF_3{\cdot}Et_2O$ in terms of maximal substitution (approximately 50% of $BF_3{\cdot}Et_2O$).

A direct, solid-phase synthesis of octreotide, shown in Scheme 1, was carried out in order to fully demonstrate the utility of this linker strategy. By use of the above conditions, a manual loading of commercially available trichloroacetimidate resin (commercially available, initial substitution: 0.77 mmol/g) with Fmoc-threoninol(OtBu) produced a substitution level of 0.48 mmol/g within 1 h, a yield of 73%. In comparison, loading of 2-chlorotrityl linker with an initial loading of 1.39 mmol/g with Fmoc-threoninol(OtBu), according to published procedures,[11] produced a substitution level of 0.15 mmol/g after 22 h, a yield of only 14.6%. The octreotide primary sequence was assembled using a synthesizer utilizing a single coupled dicyclohexylcarbodiimide/hydroxybenztriazole activation protocol, a 4-fold excess of each amino acid residue, and a conventional protecting group scheme: Fmoc−Thr(OtBu), Fmoc−Cys(Trt), Fmoc−Lys(Boc), Fmoc−Trp(Boc). The peptide was simultaneously deprotected and cleaved from the resin using a mixture of 45% TFA, 45% methylene chloride, 5% water, and 5% thioanisole as scavenger for 1 h, by following the assembly of the primary sequence. The yield of recovered peptide after purification was 37.8%, based on the initial resin loading. Air oxidation of the free cysteines resulted in nearly quantitative disulfide bond formation and an overall 36.3% yield of octreotide after HPLC purification.

These results suggest that the use of trichloroacetimidate activated Wang resin offers a convenient alternative to existing linker strategies and is compatible with all aspects of Fmoc/tBu based solid-phase chemistry. The major advantages are the relatively rapid and efficient loading, mild cleavage conditions, and low cost associated with the use of this resin. This strategy has additionally been found to be applicable to the side-chain-supported synthesis of serine and threonine containing cyclic peptides, and the results of this work will be reported independently.

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Supporting Information Available: A detailed resin loading procedure as well as HPLC trace of octreotide is available free of charge via the World Wide Web at http://pubs.acs.org.

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