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Solid-Phase Peptide Synthesis in the Reverse (*N* → *C*) Direction

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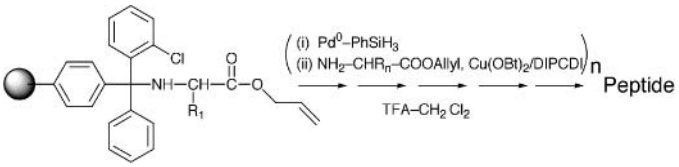
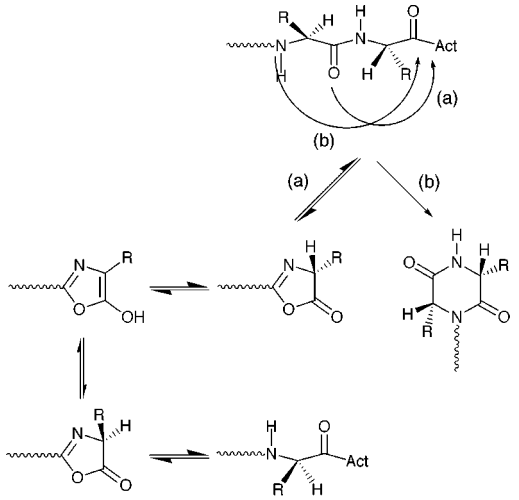
ABSTRACT



A new strategy for SPPS in the reverse direction based on the use of 2-Cl-trityl resin, an allyl ester as the temporary protecting group, and Cu(OBt)$_2$/DIPCDI or HATU/DIEA as the coupling method is described. These conditions ensure good yields with minimal racemization of the *C*-terminal residue.

SPPS is typically carried out in the *C* → *N* direction by sequential incorporation of N^{α}-urethane-protected amino acids followed by removal of the protecting group.[1] Conversely, the assembly in the reverse *N* → *C* direction has not been widely studied.[2] The main advantage of the reverse strategy consists of the possibility of directly generating *C*-terminal modified peptides, which are abundant in nature and have potential application in a therapeutic context.[3] Furthermore, such peptides could be readily employed in fragment condensations for the assembly of large peptides or proteins.[1] Finally, in the emerging field of combinatorial chemistry, it is important to be able to elongate a peptide or peptidomimetic chain from both ends (*N* and *C*). The main drawback associated with the *N* → *C* (reverse) strategy is the fact that the carboxyl-activated species is anchored to the resin, the result of which is side reactions that can occur via the two cyclization pathways described by Henkel et al. (Scheme 1).[2d] The activated carboxylic acid could suffer attack by either the oxygen from the carbonyl group (a) or

Scheme 1



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the nitrogen of the amine (b) from the β residue to form either the 5(4*H*)-oxazolone[1] or the diketopiperazine (DKP), respectively, leading to epimerization of the α residue or to capping of the growing chain.

In this Letter, we describe a strategy for SPPS in the $N \rightarrow C$ direction involving the following new orthogonal protection scheme: the use of allyl esters as a temporary protecting group for the carboxyl group, acid-labile resin (2-Cl-Trt resin)[4] for the anchoring of the first residue through its α-amino function, and side-chain protecting groups (*t*-Bu and related groups).[5] Regarding the activation of the carboxyl group, reagents comprising aminium and phosphonium salts[6] as well as diisopropylcarbodiimide (DIPCDI) in conjunction with a bis(1-hydroxybenzotriazole) copper complex [Cu(OBt)$_2$] have been investigated.[7] Our study has been carried out on a solid support in the context of peptide synthesis, but this new approach to peptide elongation could also be used for other types of chemistry conducted on solid supports, combinatorial chemistry in particular.

Allylic protecting groups [allyl (All) for carboxylic acids, Alloc for amines] may be removed under specific conditions through palladium-catalyzed transfer of the allyl entity to various nucleophilic species (oxygen, nitrogen, or sulfur nucleophiles, hydride donors).[8] We have recently shown that phenylsilane ($PhSiH_3$), used as an allyl group scavenger, offers a wide scope for utilization,[9] similar to that for tributyltin hydride but without the disadvantage often associated with the use of tin compounds (toxicity, difficulty in elimination of byproducts). Furthermore, the neutral conditions required for allyl group removal provide orthogonality with the TFA-labile groups.

The use of the hindered 2-Cl-Trt resin has several advantages. In contrast to other carbamate-based resin, the bond (secondary amine) between the resin and the first residue is lacking a carbonyl group, preventing the formation of a 5(4*H*)-oxazolone. Furthermore, the lability of this resin under weakly acid conditions would allow the cleavage of the protected peptide from the resin, which can be further used in a convergent strategy.[4]

As activation reagents (Figure 1), we utilized the aminium HATU and the phosphonium PyAOP salts derived from 7-aza-1-hydroxybenzotriazole (HOAt),[6] as well as the phosphonium salts PyDOP[10] derived from 1-oxo-2-hydroxydihydrobenzotriazine (HODhbt or HOOBt)[6] and PyPyOOP[11]

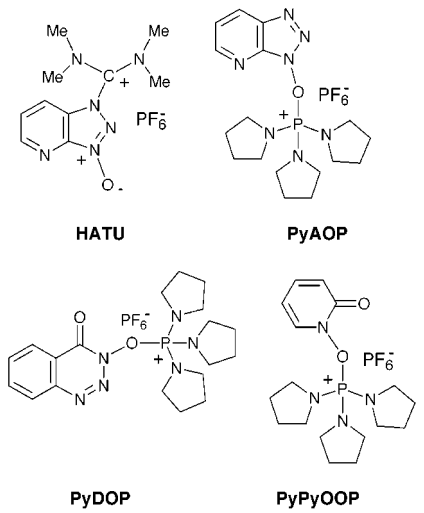

Figure 1.

from *N*-hydroxy-1,2-dihydro-2-oxopyridine (HOPyr).[6] HATU and PyAOP have been shown to be effective for solid-phase activation of a pendant carboxyl group and coupling in the preparation of *C*-terminal-modified peptides using a backbone amide linker (BAL) strategy.[12] PyDOP has been shown to be efficient for activation of monothio acid groups as a means of obtaining thioamide derivatives. The use of PyPyOOP appeared to be attractive due to the small size of the leaving group and its solubility properties.[13] Among the copper complexes available for peptide synthesis in solution, Cu(OBt)$_2$ has recently been developed by Blodgett et al. and shown to reduce racemization in solution peptide segment coupling involving DIPCDI.[7] Accordingly, it also appeared to be attractive and innovative to attempt $N \rightarrow C$ synthesis on a solid support using DIPCDI activation and this type of copper complex.

The synthesis of the demanding peptide H-Phe-Leu-Val-Ile-OH was chosen as a model, and several experimental conditions were investigated.[14]

Results in Table 1 show that, in terms of HPLC purity as proxy for yield of the desired peptide product, activation was more effective with uronium than with phosphonium salts

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(14) All syntheses in the reverse direction were performed at 25 °C according to the following procedure: (i) Incorporation of the first allyl ester amino acid (0.5 equiv) was performed in CH_2Cl_2 in the presence of *N*,*N*-diisopropyletylamine (DIEA, 10 equiv) for 1 h. (ii) Removal of the allyl group was accomplished under an Ar atmosphere with Pd(PPh$_3$)$_4$ (20 mol %, based on the substitution of the resin) and $PhSiH_3$ (24 equiv) in CH_2Cl_2 (2 × 15 min).[9c] After filtering the Pd solution, the resin was washed with CH_2Cl_2 (6 × 30 s), H_2O−dioxane (1: 9) (2 × 1 min) and DMF (6 × 30 s). (iii) The coupling reaction was conducted by adding first the allyl ester amino acid (5 equiv) dissolved in DCM−DMF (9:1) and then the activating reagent (5 equiv) as a solid. (iv) The cleavage of the unprotected peptides was carried out with TFA−CH_2Cl_2 (9:1) for 30 min.

Table 1. Synthesis of H-Phe-Leu-Val-Ile-OH

entry	coupling reagent	DIEA equiv	coupling time	purity[a]	extent of racemization[d]
1	HATU	20	1 × 2 h	48%[b]	4%
2	HATU	20	2 × 1.5 h	64%[b]	8%
3	HATU	11	1 × 2 h	73%[b]	2.5%
4	PyOAP	20	1 × 2 h	20%[c]	*e*
5	PyDOP	20	1 × 2 h	21%[c]	*e*
6	PyPyOOP	20	2 × 1.5 h	20%[c]	*e*
7	$Cu(OBt)_2$/DIPCDI		1 × 2 h	56%[b]	0.6%

[a] Determined by HPLC of the crude H-Phe-Leu-Val-Ile-OH. HPLC conditions: Nucleosil C_{18}-silica column (4 × 250 mm), flow rate 1 mL/min, absorbance at 220 nm; eluents 0.1% of TFA in water (A) and 0.09% of TFA in CH_3CN (B). [b] Linear gradient from 20% B to 50% in 30 min and then 100% B during 5 min. [c] Linear gradient from 20% B to 65% in 30 min and then 100% B during 5 min. [d] To estimate the racemization, all diastereoisomers were synthesized on a 2-Cl-trityl resin using Fmoc chemistry. Only H-Phe-DLeu-Val-Ile-OH was detected. [e] Not determined.

(entry 1 vs 4, 5, and 6). Impurities, in all cases, have been identified by mass spectroscopy as peptides of deletion (lack of Leu, Val, Ile or a combination thereof) and cyclopeptides (probably occurring at the cleavage from the resin step). Some improvement was observed by repeating the coupling step with HATU (entry 2 vs 1), but more significantly, HPLC purity was found to be quite dependent on the quantity of the tertiary amine used (entry 3). This could be explained by several factors. A large excess of base should favor the formation of the corresponding guanidine, which would be expected to prevent the amino acid from reacting.[12,15] Less base should reduce the tendency for intramolecular cyclization and subsequent DKP formation, which is responsible for terminating peptidic chain elongation. Consistent with the argument that less base leads to less intramolecular cyclization in general is the lower extent of racemization that is observed when less base is used [thereby indicating less formation of the 5(4*H*)-oxazolone]. With the $Cu(OBt)_2$/DIPCDI method (entry 7), an HPLC purity comparable to that associated with HATU activation is obtained, but more importantly, the reaction proceeds with minimal racemization.

For allyl group removal, a longer exposure of the substrate to $Pd(PPh_3)_4$ and $PhSiH_3$ (1 h instead of 2 × 15 min) did not alter the HPLC purity of the final product nor the extent of racemization (purity 46%, racemization 4%, vs 48% and 4%, respectively, in entry 1). Similarly, no improvement was observed as a result of replacing $PhSiH_3$ with the new allyl group scavenger $NHMe_2BH_3$[5] (purity 48%, racemization 4%). Therefore, the purity of the final product was essentially unaffected by the deprotection step.

Finally, Leu-enkephalin (H-Tyr-Gly-Gly-Phe-Leu-NH_2) was synthesized according to this new strategy using both HATU/DIEA and $Cu(OBt)_2$/DIPCDI as activating reagents. For this sequence, synthesis with $Cu(OBt)_2$/DIPCDI afforded a higher purity product than that obtained using HATU/DIEA, i.e., 57% vs 21% as determined by HPLC (data not shown). Most important of all, however, was the total suppression of racemization during peptide elongation involving $Cu(OBt)_2$/DIPCDI (a significant 5% of the only diastereoisomer[16] possible H-Tyr-Gly-Gly-(D)Phe-Leu-OH was detected by HPLC when HATU was used). For comparison, Bayer and collaborators[2d] synthesized this peptide using amino acid fluorenylmethyl esters with HOBt/DIPCDI or TBTU/NMM as coupling reagents. In their case, the coupling yield was improved by using TBTU/NMM but the epimerization of the Phe residue increased dramatically (40.5% with TBTU vs 8.2% with HOBt/DIPCDI).

In conclusion, solid-phase peptide assembly in the reverse $N \rightarrow C$ direction can be conducted efficiently using a synthesis scheme based on the use of 2-Cl-trityl resin, an allyl ester as a temporary protecting group, and either $Cu(OBt)_2$/DIPCDI or HATU/DIEA as a coupling method. 2-Cl-trityl resin allows the flexibility of obtaining both protected and unprotected peptides. The convenience of the allyl group as a temporary protecting group is similar to that displayed by Boc, Fmoc, or Alloc in the traditional $N \rightarrow C$ strategy. Furthermore, the neutral conditions associated with the allyl deprotection step [$Pd(PPh_3)_4$ and $PhSiH_3$] do not require an additional neutralization step, which must be performed with other base labile esters.[2d] Finally, similar to the results obtained in solution, the use of $Cu(OBt)_2$/DIPCDI permits a racemization level from reasonable to nil according to the difficulty of the desired peptide.

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(16) Racemization of the Phe residue is expected to be very low due to the structure of 2-Cl-trityl moiety (there is no possibility of oxazolone formation and the steric hindrance retards the abstraction of the α-proton).