New and Efficient Coupling Method for the Synthesis of Peptides Bearing the Norstatine Residue and Their Analogs

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Summary: Novel ring-opening coupling reactions of enantiomerically pure (3*R*,4*S*)-1-(*tert*-butoxycarbonyl)-3-hydroxy β-lactams **1** with various (*S*)-amino acid esters **2** including proline methyl ester proceed smoothly at ambient temperature to give the corresponding dipeptides **3** bearing the norstatine residue and its analogs in excellent yields. This new coupling method is applicable to solid-phase peptide syntheses.

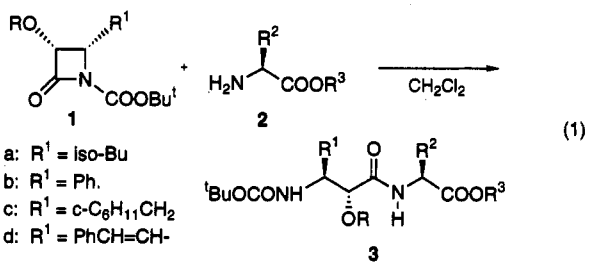
We have been applying our β-lactam synthon method to the synthesis of various nonprotein amino acids and dipeptides containing unusual amino acid residues, which are potential enzyme inhibitors, fragments of peptide hormone analogs, and the C-13 side chain of Taxol and Taxotère.[1,2] It has been shown that 1-benzoyl- or 1-(*tert*-butoxycarbonyl)-(3*R*,4*S*)-3-(1-ethoxyethoxy)-4-phenylazetidin-2-one reacts with the C-13 hydroxy group of a protected baccatin III in the presence of an appropriate base to form Taxol or Taxotère in excellent yield after deprotection.[2-5] This strongly suggests that 1-acyl-3-hydroxy-4-substituted β-lactams can serve as acylating agents for alcohols and amines. If carbamates such as *tert*-butoxycarbonyl (BOC) groups can be used for coupling with amino esters, a new method for peptide coupling based on the ring-opening of β-lactams would be developed. Since norstatine, i.e., (2*R*,3*S*)-3-amino-2-hydroxy-5-methylhexanoic acid, and its analogs are important amino acid residues for inhibitors of enzymes such as renin and HIV protease,[6,7] facile incorporation of various α-hydroxy-β-amino acid residues into peptides is particularly significant. We describe here our preliminary results on the ring-opening coupling method for the synthesis of dipeptides bearing α-hydroxy-β-amino acid residues (eq 1).

We carried out the reactions of (3*R*,4*S*)-1-BOC β-lactams **1**[4,8] with (*S*)-α-amino esters **2** under neutral conditions in CH_2Cl_2. Results are summarized in Table 1. As Table 1 shows, the ring-opening coupling of 3-hydroxy β-lactams **1a–1d** proceeds smoothly at 25 °C to give the corresponding *N*-BOC-dipeptides **3** in excellent yields (entries 3–5, 8, and 10–12). It is apparent from the results shown in Table 1 that the steric hindrance at the 3-position of the 1-BOC β-lactam **1** exerts a marked influence on the rate of the coupling. For example, the reaction of 1-(*tert*-butoxycarbonyl)-3-[(triisopropylsilyl)oxy]-4-isobutyl β-lactam **1a**–TIPS (TIPS = triisopropylsilyl) with (*S*)-Phe-OMe·HCl in the presence of *N*-methylmorpholine (NMM) gives the coupling product, 3-(*tert*-butoxycarbonyl)-amino-2-[(triisopropylsilyl)oxy]-5-methylhexanoyl-Phe-OMe (*O*-TIPS-**3aa**-OMe), in 92% yield after refluxing the mixture in CH_2Cl_2 for 30 h (entry 1). 1-BOC-3-(EE-O) β-lactam **1a**-EE (EE = ethoxyethyl) reacts with Phe-OMe·HCl in the presence of NMM sluggishly in refluxing CH_2Cl_2, but without decomposition, to give **3aa**-OMe in 90% yield after citric acid workup (entry 2). The reactions of **1a**-TIPS and **1a**-EE virtually did not proceed at 25 °C for 18 h. It should be noted that 1-(*tert*-butoxycarbonyl)-3-[(*tert*-butoxycarbonyl)oxy] β-lactam **1a**-BOC reacts with (*S*)-PheOMe smoothly at 25 °C to give the coupling product *O*-BOC-**3aa**-OMe in excellent yield (entries 6 and 7). The bulky C-4 substituent of **1** as well as R^2 of **2** also affects the coupling rate to some extent. For instance, the coupling of **1b** (R^1 = Ph) with (*S*)-Val-OMe appears to be

Table 1. Ring-Opening Coupling of 1-BOC β-Lactams 1 with (*S*)-α-Amino Acid Esters[a]

entry	β-lactam 1	R	2	reaction time (h)	isolated yield (%)
1	1a	TIPS	Phe-OMe	30	92[b]
2	1a	EE	Phe-OMe	30	90[b]
3	1a	H	Phe-OMe	5	94
4	1a	H	Leu-OBu^t	5	94
5	1a	H	Trp-OMe	24	91
6	1a	BOC	Phe-OMe	8	93
7	1a	BOC	Trp-OMe	44	91
8	1b	H	Leu-OMe	5	85
9	1b	H	Val-OMe	18	63
10	1c	H	Phe-OMe	5	94
11	1c	H	Trp-OMe	16	92
12	1d	H	Phe-OMe	5	92

[a] All reactions were run with **1** (0.047 mmol) and amino acid ester **2** (0.094 mmol) in CH_2Cl_2 (2.0 mL) at room temperature unless otherwise noted. The reaction mixture was washed with 10% citric acid, dried over anhydrous $MgSO_4$, concentrated *in vacuo*, and purified on a short silica gel column using hexane/EtOAc (4/1) as the eluant. [b] Refluxed in CH_2Cl_2. (*S*)-Phenylalanine methyl ester (Phe-OMe) was generated *in situ* by treating Phe-OMe·HCl with *N*-methylmorpholine.



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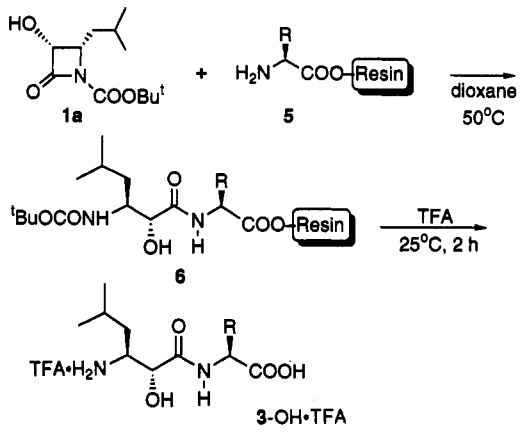
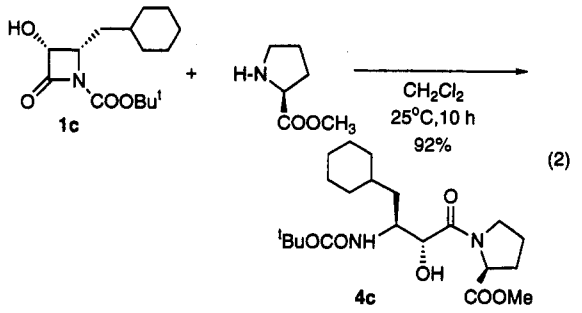
0022-3263/94/1959-1249$04.50/0

Scheme 1



slower than others, due to the bulky phenyl and isopropyl groups (entry 9). (*S*)-Tryptophan methyl ester also reacts somewhat slower than less bulky amino esters (entries 5, 7, and 11).

It is worth mentioning that (*S*)-Pro-OMe (a secondary amine) reacted with **1c** smoothly at 25 °C to give the corresponding dipeptide **4c** in 92% yield (eq 2). In the same manner, the reaction of (*S*)-Pro-OMe with **1a** (R^1 = isobutyl) also gave **4a** in 91% yield.



(2)

Next, we applied this novel peptide coupling to a solid-phase peptide synthesis system. A preliminary study using the "Wang resin"[9] was very encouraging (Scheme 1). For example, the coupling of **1a** with the resin-bound glycine (**5a**) and phenylalanine (**5b**) at 50 °C gave the corresponding resin-bound dipeptides **6a** (2 h) and **6b** (30 h) in high yields, which were treated with trifluoroacetic acid (TFA) at 25 °C for 2 h to give the TFA salt of dipeptides **3ae**-OH and **3aa**-OH in 72% and 78% isolated yields, respectively.[10] Although reactions are sluggish at 25 °C and thus some activation protocol should be developed, the coupling process is very clean and warrants further investigation.

Salient features of this new coupling method include the reaction conditions being mild and neutral, no racemization detected, and no coupling agent required. Further studies on the applications of this methodology for incorporating a wide variety of norstatine analog fragments into peptides by means of solution as well as solid-phase peptide synthesis are actively underway.

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Supplementary Material Available: General experimental procedures for the ring-opening peptide coupling reactions and the characterization data for **3** and **4** (4 pages). This material is contained in libraries on microfiche, immediately follow this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(9) The "Wang resin" is a commercially available polystyrene-based polymer resin bearing a (hydroxymethyl)phenoxy]methyl tether so that peptides synthesized can be cleaved readily by treatment with trifluoroacetic acid.

(10) Reactions were run with **1a**[4,8] (0.082 mmol) and α-amino acid bonded to the Wang resin (0.042 mmol) in dioxane (2.0 mL) in a peptide-coupling cartridge equipped with a filter. After the reaction, the coupling product bonded to the Wang resin was washed with DMF (5 mL) and methanol (5 mL) and treated with trifluoroacetic acid (5.0 mL) at 25 °C for 2 h. The resulting TFA salt of dipeptide **3**-OH was isolated from the filtrate as a white solid. Identification of dipeptides **3**-OH was performed based on HPLC analysis using authentically prepared samples as well as ^1H and ^{13}C NMR spectroscopies.