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Improved synthesis of D-allothreonine derivatives from L-threonine

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ABSTRACT

The improved synthesis of protected D-allothreonine derivatives [Fmoc-D-alloThr(^{t}Bu)-OH (**1**) and Fmoc-D-alloThr-O^{t}Bu (**2**)] is described. The epimerization of cheap L-threonine (L-Thr) (**3**) with catalytic salicylaldehyde afforded a mixture of L-Thr (**3**) and D-alloThr (**4**) and separation of ammonium salt gave D-alloThr (**4**) in 96% de. The chemoselective deprotection of *tert*-butyl ether or *tert*-butyl ester of Fmoc-D-alloThr(^{t}Bu)-O^{t}Bu (**5**) easily succeeded in converting Fmoc-D-alloThr(^{t}Bu)-OH (**1**) or Fmoc-D-alloThr-O^{t}Bu (**2**), respectively.

1. Introduction

Recently, peptidyl natural products containing non-proteinogenic amino acids were isolated from marine sponges and showed a broad spectrum of biological activities, including cytotoxic, antitumor, antifungal, and anti-HIV. Non-proteinogenic amino acids, which are important building blocks composed of bioactive compounds, are expected to be resistant to proteases. D-alloThr (**4**), one of the non-proteinogenic amino acids, contains callipeltins.[1] PCM1206, and plipastatin, which showed potent anti-HIV activity and/or cytotoxicity. In the course of a synthetic and structure–activity relationship study of callipeltins.[2] D-alloThr (**4**) was found to be an important constituent because it is highly expensive and difficult to obtain in large quantities.[3–5] The strategy used by the Genet group[3] was based on the electrophilic amination of di-*tert*-butyl azodicarboxylate to (*R*)-ethyl 3-hydroxy butanoate followed by separation of L-Thr. In contrast, Kobayashi et al.[5] employed stereo-inversion of hydroxyl group of D-Thr via oxazolidinone intermediate. However, these methods were against the large-scale synthesis for our group because the starting materials were high expensive in both cases.

For the reasons, we planned the synthesis of D-allothreonine using cheap L-amino acids. Although we have previously achieved the synthesis of D-alloThr (**4**) using Garner's aldehyde prepared from L-Ser, there were complicated problems with gram-scale synthesis.[6] Therefore, Fmoc-D-alloThr-OH (**6**) was prepared to use the method using L-Thr in previous literature,[7,8] for the solid phase total synthesis of callipeltin E.[2] Though the Yajima's procedure afforded D- and L-alloThr from diastereoisomeric mixtures, which were obtained by simply epimerizing L- or D-Thr,[7,8] it was consequently difficult for us in hand to purify them as efficiently as the preparation of D-alloThr (**4**) to give Fmoc-D-alloThr-OH (**6**) in 9% overall yield.

On the other hand, Fmoc-D-alloThr(^{t}Bu)-OH (**1**) and Fmoc-D-alloThr-O^{t}Bu (**2**) were required to construct cyclic depsipeptides callipeltins A and B. Protection of hydroxy groups with the ^{t}Bu group generally used H_2SO_4/2-methylpropene attached to a special apparatus; therefore, ^{t}Bu protection has been avoided in synthetic schemes. Fischer and Sandosham synthesized Fmoc-D-alloThr(^{t}Bu)-OH (**1**) through the protection of hydroxy groups with the ^{t}Bu using H_2SO_4/2-methylpropene and deprotection of ^{t}Bu ester by 25% $Cl_2CHCOOH$ in 8% yield (three steps).[9] In the present study, we attempted to reconsider the synthesis of D-alloThr (**4**) and simple protection of the side chain or C-terminus of Fmoc-D-alloThr-OH (**6**) with the ^{t}Bu group, respectively (Fig. 1).

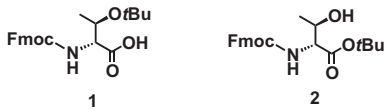

Fig. 1. Fmoc-D-alloThr(^{t}Bu)-OH (**1**) and Fmoc-D-alloThr-O^{t}Bu (**2**).

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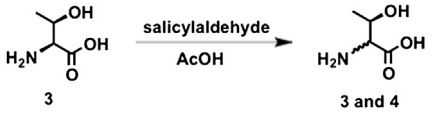
2. Results and discussion

First, epimerization of the C-2 position of L-Thr (**3**) based on reported protocol was attempted.[7] Treatment of L-Thr (**3**) with catalytic salicylaldehyde (10 mol %) in AcOH (0.5 M) at 90 °C for 2 h obtained a mixture of L-Thr (**3**) and D-alloThr (**4**) (1:0.30) in poor yield (20%). We supposed that the compounds were decomposed by rapidly heating at 90 °C. Consequently, the reaction mixture became brown and the chemical yield and ratio of L-Thr (**3**) and D-alloThr (**4**) were disappointing (entry 1). After attempting several conditions, we optimized the method using the 0.2 M solution in the presence of 20% catalytic salicylaldehyde heating at 70 °C for 7 h to give L-Thr (**3**) and D-alloThr (**4**) mixtures in a yield of 61% with the epimerized ratio of 1:0.74 (entry 6). These results suggested that heating at over 70 °C caused a decrease in chemical yield. In contrast, the reaction mixture at 60 °C was suspension to insolubility of L-Thr (**3**) to give L-Thr without epimerization practically (entry 7). These results showed controlling of reaction temperature at ~70 °C progressed the epimerized ratio (Table 1).

Table 1
Epimerization condition of L-Thr (**3**)



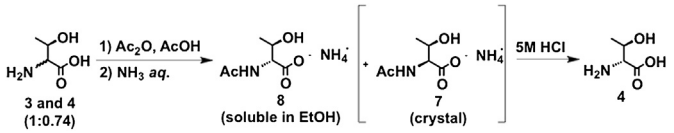
Entry	Concn (mol/l)	Salicylaldehyde (mol %)	Temp (°C)	Time (h)	Yield[c] (%)	Ratio[d] 2(L-/D-allo)
1	0.5	10	90	2	20	1:0.30
2	0.5	10	90	42[a]	52	1:0.60
3	0.5	12.5	90	41[a]	45	1:0.73
4	0.4	10	90	46[a]	53	1:0.67
5	0.3	10	80	25[a]	47	1:0.73
6	0.2	20	70	7[b]	61	1:0.74
7	0.4	10	60	20[b]	71	1:0.10

[a] Reaction mixtures were gradually warmed from room temperature to preset temperatures over 22 h.
[b] Reaction mixtures were gradually warmed from room temperature to preset temperatures over 3 h.
[c] Isolation yields.
[d] The ratio of L-form and D-all-form was determined by the signal of methyl groups on ^{1}H NMR spectra.

For the purpose of separating the L-Thr (**3**) and D-alloThr (**4**) mixtures (1:0.74), N-acetylation of the mixtures with Ac_2O/AcOH at 90 °C followed by the filtration of adjusting pH 7 solution and deacetylation afforded the D-alloThr (**4**) in 74% yield. The diastereomeric excess of D-alloThr (**4**) checked by the ^{1}H NMR spectra was determined to be 50% (entry 1).[7] Consequently, the prudent investigation of the reaction sequence conducted the efficient conditions. After the N-acetylation of these mixtures with Ac_2O/AcOH at 50 °C for 2.5 h, treatment with concd NH_3 aq (pH 10) afforded the ammonium salts of Ac-L-Thr NH_3 (**7**) and Ac-D-alloThr NH_3 (**8**), which showed a clearly different nature in EtOH solution. In brief, Ac-L-Thr NH_3 (**7**) as a solid was given by filtration and the filtrate contained Ac-D-alloThr NH_3 (**8**). After the deprotection of acetyl group of Ac-D-alloThr NH_3 (**8**) using 5 M HCl, the residue in cold EtOH was treated with Et_3N (pH 6) followed by filtration, washing with cold EtOH to give D-alloThr (**4**) in 56% yield and 96% de (entry 5). The obtained D-alloThr (**4**) was easily purified by recrystallization with 80% EtOH to afford optically pure D-alloThr (**4**) in 43% overall yield. As a result, we accomplished the improvement of chemical yields by comparison of our previous report[2,12] (Table 2).

After the quantitative Fmoc protection of D-alloThr (**4**), the hydroxy group of the side chain or C-terminus carboxylic acid would be protected with the ^{t}Bu group to employ Fmoc-SPPS, especially

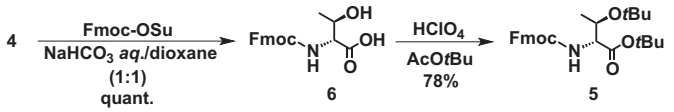
Table 2
Preparation of D-alloThr (**4**)



Entry	Acetylation		Precipitation of ammonium salt[a] (pH)	Yield (%)	de[b] (%)
	Temp (°C)	Time (h)			
1	90	0.5	7	74	50
2	90	2	8	37	69
3	90	2	9	55	74
4	50	2.5	8	64	64
5	50	2.5	10	56	96

[a] The pH of the solution of ammonium salts **7** and **8** was justified with 25% ammonium solution.
[b] Diastereomeric excess (de) of **4** was determined by the signal of methyl groups on ^{1}H NMR spectra.

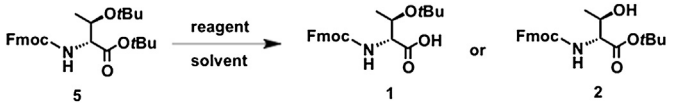
the synthesis of cyclic depsipeptides. Barge et al. reported that protection of the hydroxy group as ^{t}Bu ether could be easily performed using $HClO_4$ in AcO^tBu at room temperature/ambient pressure.[10] These reagents were easily obtained by comparison of another *tert*-butylating reagents. For example, *tert*-butyl 2,2,2-trichloroacetimidate, a high expensive reagent, is available with protection of ^{t}Bu. Fmoc-D-alloThr-OH (**6**) was found to protect with the ^{t}Bu group with 60% $HClO_4$ aq (0.2 equiv)/AcO^tBu (0.04 M solution) at room temperature for 13.5 h to give Fmoc-D-alloThr(^{t}Bu)-O^{t}Bu (**5**) in 78% yield (Scheme 1).



Scheme 1. Synthesis of Fmoc-D-alloThr(^{t}Bu)-O^{t}Bu (**5**).

To reach both Fmoc-D-alloThr(^{t}Bu)-OH (**1**) and Fmoc-D-alloThr-O^{t}Bu (**2**), chemoselective deprotection of *tert*-butyl group was attempted as depicted in Table 3. First, the treatment of **5** by the

Table 3
Synthesis of Fmoc-D-alloThr(^{t}Bu)-OH (**1**) or Fmoc-D-alloThr-O^{t}Bu (**2**)



Entry	Reagent (equiv)	Solvent[b]	Temp	Time (h)	Products (%)			
					1	**2**	**5**	**6**
1	SiO_2[a]	Toluene	Reflux	0.5	39	0	4	15
2	SiO_2[a]	MeCN	Reflux	51	0	0	50	0
3	$HClO_4$ (0.2)	MeCN	rt	1.7	7	43	10	39
4	$HClO_4$ (0.14)	MeCN	0 °C	1.5	3	39	49	2
5	$HClO_4$ (0.2)	CH_2Cl_2	rt	4	8	49	30	12
6	$HClO_4$ (0.2), SiO_2 (1)	MeCN	rt	0.5	2	5	0	16
7	$HClO_4$ (0.2), TIPS (1)	CH_2Cl_2	rt	8	2	49	5	29
8	$HClO_4$ (0.2), Et_3SiH (2)	CH_2Cl_2	rt	24	3	35	1	20

[a] SiO_2 was used by the ratio of weight/substrate weight: SiO_2/**5**=10 (entry 1), (entry 2).
[b] Entry 4 was performed the concentration of 0.014 mol/l, and entries 3, 5–8 were performed the concentration of 0.04 mol/l.

SiO_2 in toluene at reflux based on Jackson's procedure[11] afforded Fmoc-D-alloThr(^{t}Bu)-OH (**1**) in 39% yield. In this condition chemoselective deprotection of ^{t}Bu ether was achieved and consequently Fmoc-D-alloThr-O^{t}Bu (**2**) was not detected by HPLC analysis (entry 1). Deprotection of ^{t}Bu ether with SiO_2 using another solvents was