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A CONVENIENT AND HIGHLY EFFICIENT SYNTHESIS OF ONE KIND OF PEPTIDE NUCLEIC ACID MONOMER

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ABSTRACT. *S*-Thyminyl-*L*-cysteine methyl ester hydrochloride (compound **1**), a non-classical peptide nucleic acid monomer, was synthesized through the key intermediate, *N*-*tert*- butoxycarbonyl-*S*-thyminyl-*L*-cysteine (compound **3**), which afforded from the reaction of *S*-thyminyl-*L*-cysteine hydrochloride (compound **2**) with di-*tert*-butyl dicarbonate (Boc_2O). This was followed by the esterification and deprotection of compound **3** at an overall yield of 82%. The mixture of thionyl chloride and methanol was found as an efficient reagent for simultaneous deprotection of *tert*-butoxycarbonyl (Boc) group and esterification of carboxy group of compound **3**. This high-yield two-step method was also applied to other analogues of compound **1** successfully. The chemical structures of four new compounds (**5a**-**5d**) were confirmed by ^{1}H NMR and ^{13}C NMR.

KEY WORDS: Thionyl chloride, Esterification, Deprotection, Peptide nucleic acid

INTRODUCTION

Peptide nucleic acid (PNA), a DNA mimic with pseudo peptide backbone, was introduced by Nielsen in 1991 [1], and is still attracting broad attention from both chemists and biologists due to its superior characters, such as the stronger specificity and selectivity of binding towards complementary DNA and RNA [2, 3]. Over the past two decades, a growing number of PNA derivatives have been synthesized and successfully applied in molecular biology, diagnostics and therapeutics [4-7]. Recently, a novel non-classical PNA monomer, *S*-thyminyl-*L*-cysteine hydrochloride (compound **2**), which consists of natural chiral cysteine and 5-hydroxymethyl-uracil [8], was reported by Yu and his co-workers [9, 10]. Compound **2** can be conveniently conjugated with certain cyclens, and the resulting PNA monomer-cyclen conjugates can bind metal ions and show moderate catalytic activity on DNA cleavage [10]. For further study the activity of other peptides containing this PNA monomer, *S*-thyminyl-*L*-cysteine methyl ester hydrochloride (compound **1**) is an important intermediate. Up to now, however, only one method was developed to synthesize the intermediate with low yield of 40% (Route A**,** Scheme 1) [11]. Therefore, it is highly desirable to develop a new effective method for preparation of compound **1**. Herein, we wish to report a new synthetic route which gave the compound **1** with the overall yield of 82% (Route C**,** Scheme 1).

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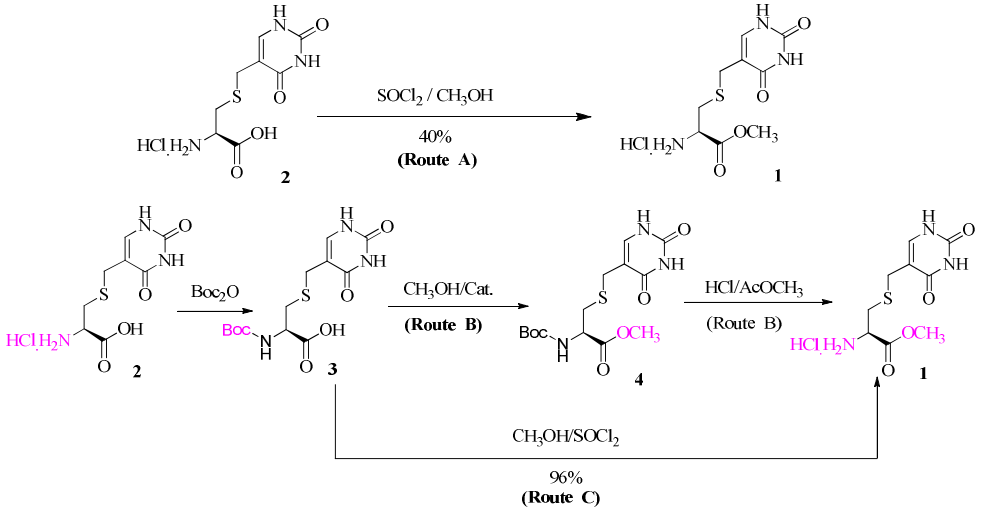


Scheme 1. Reported and designed preparative routes of compound **1**.

EXPERIMENTAL

Measurements

Melting points were detected by X-6 sophisticated micro-melting point apparatus. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV300 spectrometer in D_2O-d_2 with tetramethylsilane (TMS) as an internal standard. The data of electro-spray ionization mass spectrum (ESI MS) was obtained on an Agilent 1946B instrument. IR spectra were carried out on Perkin-Elmer, Spectrum GX, USA, using KBr pellets. The progress of the reactions was monitored by thin-layer chromatography (TLC).

General procedure for synthesis

The compound **2** was prepared according to the previously reported method [9] in the range of 83-91% yield. Its chemical structure was identified by IR and ESI MS.

*Synthesis of N-tert-butoxycarbonyl-S-thyminyl-L-cysteine (compound **3**)*. Compound **2** (8.445 g, 30 mmol) was added to an aqueous solution of potassium hydroxide (5.601 g, 100.0 mmol). The solution was cooled to 0 °C in ice bath with vigorous stirring. Then the acetone solution (20 mL) of di-*tert*-butyl dicarbonate (Boc_2O) (9.845 g, 45 mmol) was added dropwise. After being kept in ice bath for 2 h, the solution was allowed to warm up to ambient temperature. The reaction progress was monitored by TLC ($CHCl_3$:CH_3OH = 4:1, v/v). After the reaction completed, the mixture was concentrated under reduced pressure to remove the acetone. Then excess Boc_2O was removed by extracting with ether (3×30 mL), and the pH was adjusted to 3-4 with 2 N HCl in ice bath. Compound **3** (8.789 g) was obtained as a white powder through filtering, washing with water (2×20 mL) and drying over P_2O_5 in vacuum. m.p. 120.3-121.2 °C (lit. 118-119 °C [9]).

*Synthesis of S-thyminyl-L-cysteine methyl ester hydrochloride (compound **1**)*. Thionyl chloride ($SOCl_2$, 5 mL) was added dropwise to methanol (50 mL, 0 °C) in a round-bottom flask. The solution was stirred for 0.5 h in ice bath, prior to the addition of compound **3** (8.625 g, 25 mmol). The temperature of this reaction mixture was allowed to rise to ambient temperature. The progress was monitored by TLC ($CHCl_3$:CH_3OH = 4:1, v/v). Upon completion, the resulting mixture was concentrated under reduced pressure to remove methanol and the obtained crude product was scattered in ether for approximately 2 h at ambient temperature. The analytically pure compound **1** then was collected by filtration as a white powder.

*Synthesis of compounds **5a-5d***. Thionyl chloride (6 mmol) was added dropwise to the selected alcohol (ethanol, 1-propanol, 1-butanol or 1-pentanol) (5 mL) in a round-bottom flask at 0 °C. After keep stirring for 0.5 h, compound **3** (2 mmol) was added. The progress was monitored by TLC ($CHCl_3$:CH_3OH = 4:1, v/v), the temperature of this solution was allowed to rise to 45-50 °C. After completion of the reaction, appropriate amount of petroleum ether was added to precipitate the target compounds, and finally the white solid was collected by filtration and dried in vacuo to give the analytically pure compounds **5a**-**5d**, respectively.

RESULTS AND DISCUSSION

Initial study was undertaken using the reported one-step method (Route A, Scheme 1). Comparable to the literature [11], the same result was obtained and the target compound was gained only in 40% yield with tedious workup. On the assumption that the low yield was probably attributed to the poor solubility of compound **2** in methanol, we designed a new synthetic method (Route B, Scheme 1). The synthesis of compound **1** in route B was accomplished through three-step sequence. Protection of **2** with Boc_2O produced **3** [12] which was converted into **4** through esterification. Finally, removal of the Boc group produced **1** [13]. Indeed, the solubility of compound **3** in methanol was improved substantially. The esterification of carboxy group of compound **3** and the deprotection of Boc group of compound **4**, unexpectedly, took place simultaneously in the mixture of thionyl chloride and methanol, as such the compound **1** was gained from compound **3** in one-step rather than two-step reaction. Thus, we found a high-yield two-step synthetic method of compound **1** (Route C, Scheme 1).

To obtain compound **3** in high yield, we selected Boc_2O as the protecting reagent. As shown in Table 1, the reaction of compound **2** (30 mmol), Boc_2O (45 mmol) and KOH (100 mmol) gave the product with the yield of 85% (entry 3).

Table 1. The synthetic results of compound **3**.

Entry	Reactants (g/mmol)			Time (h)	Weight (g)	Yield (%)
	2	Boc_2O	KOH			
1	0.562/2	0.542 /2.4	0.337/6.0	24	0.484	70
2	5.610 /20	5.410 /24	3.370/60.0	20	5.729	83
3	8.445/30	9.845 /45	5.601/100.0	23	8.798	85

Considering the importance of the step in esterification of carboxy group and removal of Boc group in compound **3**, we mainly investigated the effect of molar ratio of compound **3** to $SOCl_2$ on the reaction. As shown in Table 2, the molar ratio of **3**/$SOCl_2$ had a remarkable influence on the yield. When the ratio of **3**/$SOCl_2$ was changed from 1/0.4 to 1/0.6 (entry 1 and 2), the compound **3** was not converted into the compound **1** completely. When the ratio surpassed 1/0.8 (entry 3-9), the conversion of compound **3** performed completely and the yield was up to 80-91%. Along with larger ratio of **3**/$SOCl_2$, the reaction afforded compound **1** in

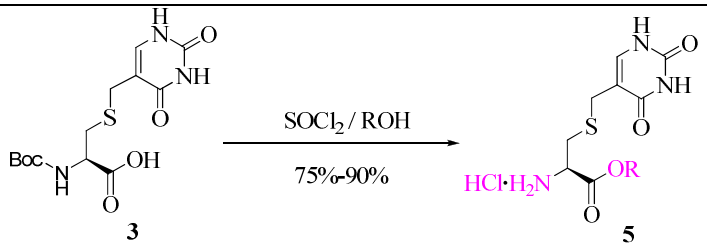
higher yield in shorter reaction time. And then the large scale preparation of compound **1** was carried out in the best yield of 96% within 6 hours (entry 11).

Table 2. The synthetic results of compound **1**.

Entry	Scale (mmol)	**3** (g/mmol)	$SOCl_2$ (mL/mmol)	3/$SOCl_2$ molar ratio	Time (h)	Weight (g)	Yield (%)
1	4	1.380/4.0	0.114/1.6	1.0:0.4	43.0	-	-
2	5	1.725/5.0	0.213/3.0	1.0:0.6	41.0	-	-
3	5	1.728/5.0	0.284/4.0	1.0:0.8	41.0	1.181	80
4	5	1.725/5.0	0.391/6.0	1.0:1.2	24.0	1.286	87
5	5	1.727/5.0	0.530/7.5	1.0:1.5	21.7	1.326	90
6	5	1.723/5.0	0.710/10.0	1.0:2.0	18.0	1.297	88
7	5	1.735/5.0	0.850/12.0	1.0:2.4	19.0	1.340	91
8	5	1.727/5.0	0.920/13.0	1.0:2.6	19.3	1.320	89
9	5	1.725/5.0	1.00/15.0	1.0:3.0	15.0	1.344	91
10	25	8.650/25.0	5.00/75.0	1.0:3.0	10.0	6.746	91
11	25	8.630/25.0	5.00/75.0	1.0:3.0	6.0	7.130	96

To expand upon the scope of the reaction, several other alcohols were also used. Generally, these alcohols afforded the corresponding products (**5a-5d**) in the yield of 75-90% (Table 3) and the chemical structures of these new compounds have been confirmed by NMR. The result proved that the $SOCl_2$-alcohol system is a general reagent for simultaneous deprotection of the Boc group and esterification of the carboxylic acid.

Table 3. The synthetic results of compound **5a-5d**.



3 **5**

Compd.	R	3 (g/mmol)	$SOCl_2$ (mL/mmol)	Temp. (°C)	Time (h)	Weight (g)	Yield (%)
5a	CH_3CH_2	0.698/2.0	0.43/6.0	45	25	0.549	89
5b	$CH_3(CH_2)_2$	0.695/2.0	0.43/6.0	50	51	0.558	86
5c	$CH_3(CH_2)_3$	0.694/2.0	0.43/6.0	50	51	0.608	90
5d	$CH_3(CH_2)_4$	0.693/2.0	0.43/6.0	50	34	0.533	75

Characterization of compounds

*Compound **1***. m.p. 193.7-195.0 °C; ESI MS: 260.1 ($[M-Cl]^+$, 100).

*Compound **2***. m.p. 239.3-241.3 °C (lit. 238-240 °C [9]). IR (cm^{-1}, KBr): 3432, 3268, 3229, 3073, 2966, 2816, 1752, 1722, 1701, 1638, 1175; ESI MS: m/z = 268.0 $[M+Na-HCl]^+$; m/z = 246.0 $[M+H-HCl]^+$.

The compounds **5a-5d** were characterized by ^{1}H NMR, ^{13}C NMR. Spectral and analytical data are as follows.

5a. White powder; m.p. 217.3-219.7 °C; ^{1}H NMR (D_2O, 300 MHz) δ: 1.17 (t, 3H, *J* = 7.2 Hz, CH_2CH_3), 2.93 (dd, 1H, *J* = 7.8, 15 Hz, CH_2CH), 3.10 (dd, 1H, *J* = 4.8, 15 Hz, CH_2CH), 3.40 (s, 2H, SCH_2), 4.15-4.22 (m, 2H, OCH_2), 4.26-4.29 (m, 1H, CH), 7.46 (s, 1H, thymine-6-CH); ^{13}C NMR (D_2O, 75MHz) δ: 168.6, 165.8, 152.8, 141.0, 109.5, 63.9, 52.4, 31.0, 27.5, 13.2.

5b. White powder; m.p. 205.6-208.5 °C; ^{1}H NMR (D_2O, 300MHz) δ: 0.80 (t, 3H, *J* = 7.2 Hz, CH_2CH_3), 1.51-1.63 (m, 2H, CH_2CH_3), 2.95 (dd, 1H, *J* = 7.2, 14.7 Hz, CH_2CH), 3.10 (dd, 1H, *J* = 4.5, 15 Hz, CH_2CH), 3.41 (s, 2H, SCH_2), 4.10 (t, 2H, *J* = 6.6 Hz, OCH_2), 4.29-4.32 (m, 1H, CH), 7.47 (s, 1H, thymine-6-CH); ^{13}C NMR (D_2O, 75MHz) δ: 168.7, 165.7, 152.7, 141.0, 109.5, 69.2, 52.4, 31.0, 27.5, 21.2, 9.6.

5c. White powder; m.p. 163.1-166.7 °C; ^{1}H NMR (D_2O, 300 MHz) δ: 0.76 (t, 3H, *J* = 7.5 Hz, CH_3), 1.17-1.29 (m, 2H, CH_2CH_3), 1.48-1.57 (m, 2H, $CH_2CH_2CH_3$), 2.94 (dd, 1H, *J* = 7.5, 15.3 Hz, CH_2CH), 3.08 (dd, 1H, *J* = 4.2, 14.7 Hz, CH_2 CH), 3.40 (s, 2H, SCH_2), 4.14 (t, 2H, *J* = 6.0 Hz, OCH_2), 4.27-4.31 (m, 1H, CH), 7.46 (s, 1H, thymine-6-CH); ^{13}C NMR (D_2O, 75MHz) *δ*: 166.5, 163.5, 150.5, 138.8, 107.3, 65.2, 50.1, 28.8, 27.5, 25.2, 16.2, 10.6.

5d. White powder; m.p. 189.0-191.8 °C; ^{1}H NMR (D_2O, 300 MHz) δ: 0.73 (t, 3H, *J* = 6.6 Hz, CH_2CH_3), 1.17-1.19 (m, 4H, CH_2 CH_2CH_3), 1.50-1.57 (m, 2H, CH_2CH_2 CH_2CH_3), 2.95 (dd, 1H, *J* = 7.2, 14.7 Hz, CH_2CH), 3.07 (dd, 1H, *J* = 4.5, 15 Hz, CH_2CH), 3.34 (s, 2H, SCH_2), 4.10-4.18 (m, 2H, OCH_2), 4.28-4.32 (m, 1H, CH), 7.47 (s, 1H, thymine-6-CH). ^{13}C NMR (D_2O, 75MHz) δ: 168.7, 165.8, 152.8, 141.0, 109.5, 67.7, 61.8, 52.3, 31.0, 27.4, 27.2, 21.5, 13.2.

CONCLUSIONS

In summary, the mixture of thionyl chloride and methanol was found to be an efficient reagent for simultaneous deprotection of Boc group and esterification of carboxy group of compound **3**. Adopting this kind reagent system, the target compound **1** and its analogues could be synthesized by two-step method in high yield. It might be a practical and potential approach for the synthesis of other related molecules.

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