Journal of Zhejiang University-SCIENCE A (Applied Physics & Engineering)
ISSN 1673-565X (Print); ISSN 1862-1775 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Accelerated effect on Mitsunobu reaction via bis-N-tert-butoxycarbonylation protection of 2-amino-6-chloropurine and its application in a novel synthesis of penciclovir

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Received July 10, 2013; Revision accepted Sept. 3, 2013; Crosschecked Sept. 12, 2013

Abstract: Solubility of 2-amino-6-chloropurine in Mitsunobu solvents could be significantly improved after its exocyclic amino group is protected via N-tert-butoxycarbonylation. The bis-Boc protected 2-amino-6-chloropurine also shows excellent activity and N9 selectivity in the coupling with various alcohols by a Mitsunobu reaction. Then, a new practical and efficient method is established for the synthesis of penciclovir (PCV) from bis-Boc-2-amino-6-chloropurine **9** and the side chain of 5-(2-hydroxyethyl)-2,2-dimethyl-1,3-dioxane **5**—the latter being a more easily prepared cyclic precursor of the diacetate side chain used in the conventional process. The coupling of **9** with **5** proceeded regioselectively at a N9 position of purine derivative for a good yield under Mitsunobu conditions.

Key words: 2-amino-6-chloropurine, Mitsunobu reaction, bis-Boc protection, Penciclovir (PCV)
doi:10.1631/jzus.A1300238 **Document code:** A **CLC number:** X5

1 Introduction

Numerous nucleoside analogues in which the sugar residues have been replaced by acylic side-chains have been found to exhibit high antiviral activity (De Clercq, 1991). Purine derivatives (Fig. 1), in the majority N9 position, represent a plurality of important active substances endowed with antiviral activity. This group of compounds includes acyclovir (ACV) **1** (Schaeffer *et al.*, 1978), ganciclovir (GCV) **2** (Ogilvie *et al.*, 1982; Martin *et al.*, 1983), penciclovir (PCV) **3** (Harnden *et al.*, 1985; 1987; Harnden and Jarvest, 1987), and famciclovir (FCV) **4** (Green *et al.*, 1992), and so on. Since Schaeffer *et al.* (1978) discovered that acyclovir is a promising anti-herpes virus agent, several groups have undertaken intensive studies to develop still more potent and effective acylic nucleoside analogues (Ashton *et al.*, 1982; Smith *et al.*, 1982; Martin *et al.*, 1983). As a result, penciclovir (PCV) **3** and its pro-drug famciclovir (FCV) **4** were found to be potent and highly selective antiviral agents against both the herpes simplex virus (HSV) and the vari-cella-zoster virus (VZV) (Tippie *et al.*, 1984). It was also reported that **3** exhibits anti hepatitis B virus (HBV) and duck hepatitis B virus (DHBV) activity (Korba and Boyd, 1996; Shaw *et al.*, 1994).

To synthesize **3** and **4**, 2-amino-6-chloropurine (ACP) is commonly used as a starting material, coupling with alkyl halide side chains (Geen *et al.*, 1990; Geen *et al.*, 1992; Kim *et al.*, 1998; Brand *et al.*, 1999; Toyokuni *et al.*, 2003). However, considering its isomerization at N7 and N9 positions under acidic or alkaline conditions, the most challengeable issue is the selectivity of a N-alkylation at the N7 or N9

position of ACP. Normally, alkylation takes place at the N9 position as well as at the N7 position of the purine moiety, and the N9/N7 ratio is usually less than 6:1 (Kim *et al.*, 1998). Accordingly, to improve this ratio, several approaches have been reported, mainly involving changing the structure of the side chains (Geen *et al.*, 1992) and modification of the ACP (Brand *et al.*, 1999). For example, as reported by Zheng *et al.* (2004) (Fig. 2), a side chain **6** was synthesized and separated readily at 0 °C. After coupling **6** with 2-amino-6-chloropurine **7**, the ratio of the product 9-isomer purine (**8a**) and the 7-isomer purine (**8b**) could reach about 10:1. However, the reaction temperature must be strictly controlled as **6** decomposes easily even at room temperature and then an extra careful column chromatography separation procedure would be required to obtain pure **8a**. Thus, finding a more practical and efficient method, which could avoid the formation of N7-alkylated compound and shorten the synthetic steps to obtain ACP, becomes attractive.

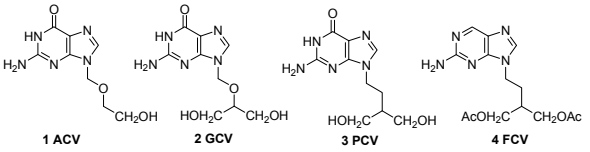


Fig. 1 Purine derivatives

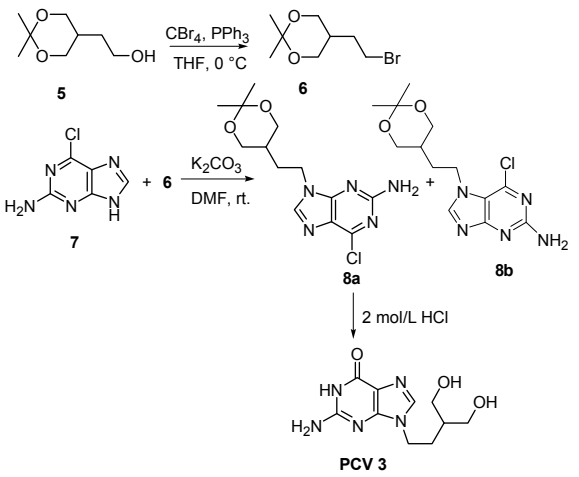


Fig. 2 Synthesis of penciclovir (PCV) with conventional method

The Mitsunobu reaction might be an alternative (potential) approach (Mitsunobu, 1981; Swamy *et al.*, 2009). This reaction has become a very popular chemical transformation due to its mildness, occurring under essentially neutral conditions, and its stereospecificity, proceeding with complete Walden inversion of stereochemistry (Mitsunobu, 1981). Moreover, it permits C-O, C-S, C-N, or C-C bonds formed by the condensation of an acidic component with a primary or a secondary alcohol. Actually, some literature has already reported successful Mitsunobu coupling of ACP and adenine with allylic and benzylic alcohol, showing a good N9 selectivity (Yang *et al.*, 2005; Kitade *et al.*, 2006; Yin *et al.*, 2006). However, a poor to modest yield (20%–50%) and a limited substrate scope were observed. In order to improve these yields, Lu *et al.* (2007) developed a modified Mitsunobu method to couple purine with alcohols in a higher temperature (70 °C), along with two rounds of the Mitsunobu reaction; yet its long reaction procedure and poor atom economy weaken its potential. The poor solubility of ACP or its derivatives in THF, the preferred solvent for Mitsunobu reactions, is likely the primary reason for these defects being observed.

A possible process to improve the solubility of ACP is to make use of the tert-butoxycarbonyl group (Boc), which can serve as the protection of the exocylic amino groups functionality and increase the lipophilicity of the base portion of the purine. Another advantage of the Boc protection group is that its acidolytic removal is less sensitive to steric factors and can also be removed under neutral conditions (Hwu *et al.*, 1996; Siro *et al.*, 1998). In contrast, a few studies have recently been reported that apply the Boc group in the protection of nucleobase (Sikchi and Hultin, 2006; Porcheddu *et al.*, 2008). As described by Porcheddu *et al.* (2008), solubility of nucleobases, including guanine, was increased in some organic solvents after protected by Boc groups. In addition, some results in our previous study (Yang *et al.*, 2011) demonstrated a very good improvement in coupling purine derivatives under Mitsunobu conditions. Thus, it could be safer to presume that protecting amino groups of ACP with Boc would be an ideal way for its application in the synthesis of PCV **3** and offer similar results as shown under Mitsunobu conditions.

In this study, we firstly synthesized a bis-Boc protected ACP, namely, bis-Boc-2-amino-6-chloropurine **9** (Fig. 3) and investigated its solubility in several different Mitsunobu solvents, then coupling

bis-Boc-2-amino-6-chloropurine **9** with a large scope of alcohols confirmed its good reactivity for a Mitsunobu reaction and successfully developed a new and efficient method for the preparation of PCV using Mitsunobu coupling reaction as the key step.

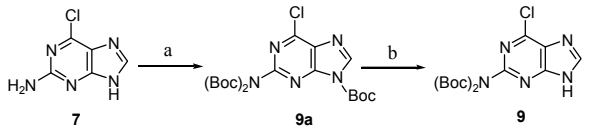


Fig. 3 Synthesis of bis-Boc-6-chloropurine 9

a: 2-amino-6-chloropurine, 4,4-dimethylaminopyridine (DMAP), THF and Boc_2O, 25 °C, N_2; b: MeOH, $NaHCO_3$, 55 °C

2 Experimental

2.1 General

Acetic ether and hexane, used for extraction and chromatography, were distilled. Absolute anhydrous THF used in the Mitsunobu reactions were prepared by distillation over a drying agent (Na/benzophenone). All other reagents were purchased and used without further purification. Thin-layer chromatography (TLC) analyses were conducted on the Merck Kieselgel 60 F254 plates. Flash chromatography was performed using a silica gel Merck 60 (particle size 0.040–0.063 mm). All ^{1}H NMR and ^{13}C NMR spectra were recorded on the BRUKER AVANCE DX500 (BRUKER AVANCE, Germany), using $CDCl_3$ or d6-DMSO as solvent at room temperature. Chemical shifts are given in 10^{-6} relative to tetramethylsilane (TMS) and the coupling constants J are given in Hz. TMS served as an internal standard (δ=0) for ^{1}H NMR, and $CDCl_3$ was used as an internal standard (δ=77.0×10^{-6}) for ^{13}C NMR. Melting points (mp) were obtained on a Melting Point WRR (Shanghai Precision & Scientific Instrument Co., Ltd., China).

2.2 Bis-Boc-2-amino-6-chloropurine (9)

1. *t*-Butyl-2-[bis(t-butoxycarbonyl)amino]-6-chloro-9H-purine-9-carboxylate (**9a**)

To a 250 ml N_2-flushed flask with dry THF (100 ml), equipped with a magnetic stir bar, 2-amino-6-chloropurine (2.0 g, 11.8 mmol) and DMAP (0.14 g, 1.18 mmol) were added. Boc_2O (10.3 g, 47.2 mmol) was added to the stirred suspension under an N_2 atmosphere, then the reaction mixture was stirred for 6 h at room temperature (TLC analysis indicated the disappearance of 2-mino-6-chloropurine). The excess amount of THF was removed, and the crude product was dissolved in AcOEt (400 ml), washed with HCl aqueous (2 mol/L, 1×30 ml) and brine (2×50 ml), dried with Na_2SO_4 and concentrated in vacuo to give a white solid (5.2 g, 94.5%). mp 51–52 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ=1.47 (s, 18H, $C(CH_3)_3$), 1.69 (s, 9H, $C(CH_3)_3$), 8.58 (s, 1H, CH); ^{13}C NMR (125 MHz, $CDCl_3$) δ=153.8, 152.0, 151.8, 150.6, 145.5, 144.7, 130.8, 88.0, 83.9, 28.0.

2. Bis-Boc-2-amino-6-chloropurine (**9**)

A solution of the white solid obtained above (14 g, 30 mmol) in MeOH (400 ml) was added to saturated $NaHCO_3$ aqueous (200 ml), then the turbid solution was stirred at 55 °C for 2 h, at which point clean conversion to bis-Boc protected adenine was observed by TLC. After evaporation of MeOH, the residue mixture was cooled, added 5 mol/L hydrochloric acid to get pH=7 (approximate). A large amount of white solid formed, the reaction mixture was filtrated and then dried under a vacuum to give a white solid **9** (10.5 g, 95.5%). mp 101.3–103.3 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ=1.50 (s, 18H, $C(CH_3)_3$), 8.41 (s, 1H, CH); ^{13}C NMR (125 MHz, $CDCl_3$) δ=153.5, 151.9, 151.6, 151.3, 145.6, 128.5, 82.7, 28.5.

2.3 5-(2-hydroxyethyl)-2,2-dimethyl-1,3-dioxane (5)

2-hydroxymethyl-1,4-butanediol **11** (8.10 g, 67.4 mmol) and 2,2-dimethoxypropane (13 ml, 105.7 mmol) were dissolved in dry THF (20 ml). The mixture was stirred and *p*-toluenesulfonic acid monohydrate (0.64 g, 3.4 mmol) was added, the clear solution was stirred at room temperature for 12 h, triethylamine (10 ml) was added to quench the reaction, and the solution was stirred for 30 min. Then solvents were removed to leave a colorless liquid, the residue was subject to column chromatography on silica gel eluted with 2:1 EtOAc/hexane to give a colorless liquid **5** (6.2 g, 61.5%), R_f=0.46 (2:1 EtOAc/hexane). ^{1}H NMR (500 MHz, $CDCl_3$): δ=3.99 (dd, 2H, Heq. J_1=11.80 Hz, J_2=4.45 Hz, CH_2); 3.80 (t, 2H, J=6.71 Hz, CH_2), 3.34 (dd, 2H, Hax. J_1=11.80 Hz, J_2=8.11 Hz, CH_2), 1.90–1.98 (m, 2H, CH and OH), 1.62 (q, 2H, J=6.85 Hz, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$): δ=100.5, 69.8, 60.4, 31.9, 30.3, 21.2.

2.4 Bis-Boc-2-amino-6-chloro-9-[2-(2,2-dimethyl-1,3-dioxan-5-yl)ethyl] purine (12)

Bis-Boc-2-amino-6-chloropurine **9** (1.0 equivalent) was added to a solution of the side chain **5** (1.1 equivalent) and phosphine reagent (1.1 equivalent) in anhydrous THF under N_2 atmosphere at 0 °C, the resulting solution was treated with di-*p*-nitrobenzyl azocarboxylate (DNAD) (1.1 equivalent) dropwise and the reaction mixture was continued at room temperature for 8 h, then the solvent was evaporated and the residue dissolved in cyclohexane. The triphenylphosphane oxide precipitated and was filtered off and then the filtrate evaporated under reduced pressure. The product was purified by a column chromatography on silica gel to obtain the pure products as a white solid. mp>280 °C (dec); ^{1}H NMR (500 MHz, $CDCl_3$): δ=8.36 (s, 1H, CH), 4.02 (t, 2H, J=7.23 Hz, CH_2), 3.79 (dd, 2H, Heq. J_1=11.57 Hz, J_2=4.46 Hz, CH_2), 3.56 (dd, 2H, Hax. J_1=11.57 Hz, J_2=8.77 Hz, CH_2), 1.67 (q, 2H, J=7.22 Hz, CH_2), 1.53–1.61 (m, 1H, CH), 1.47 (s, 18H, $C(CH_3)_3$), 1.39 (s, 3H, CH_3), 1.36 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ=154.3, 151.7, 151.5, 151.1, 128.0, 104.8, 81.7, 71.5, 50.8, 33.7, 28.6, 26.2, 25.7.

2.5 2-amino-6-chloro-9-[2-(2,2-dimethyl-1,3-dioxan-5-yl) ethyl]purine (8a)

A mixture of compound **12** (2.56 g, 5.0 mmol), 2,6-dimethyl pyridine (1.18 ml, 10 mmol) and dry DCM (20 ml) was stirred at 0 °C, then TBTMS-OTf was added dropwise; after the addition, the reaction mixture was stirred at room temperature until TLC showed that compound **12** had completely disappeared. Then 30 ml saturated ammonium chloride solution was added, separated the organic layer, extracted with DCM (2×20 ml), combined and washed by saturated NaCl (2×40 ml), dried with anhydrous sodium sulfate and evaporated to give a white solid (1.21 g, 78%). mp 125–126 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ=8.07 (s, 1H, CH) , 6.99 (s, 2H, NH_2), 4.12 (t, 2H, J=7.31 Hz, CH_2), 3.82 (dd, 2H, 4′-Heq, J_1= 11.79 Hz, J_2=4.50 Hz, CH_2), 3.53 (dd, 2H, 4′-Hax, J_1 =11.79 Hz, J_2=8.80 Hz, CH_2), 1.74 (q, 2H, J=7.30 Hz, CH_2), 1.53–1.65 (m, 1H, CH), 1.36 (s, 3H, CH_3), 1.31 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ=159.94, 150.31, 150.26, 141.84, 132.11, 100.52, 68.14, 52.90, 31.32, 26.84, 26.05.

2.6 9-[4-hydroxy-3-(hydroxymethyl)butyl] guanine (PCV 3)

Compound **12** (5.12 g, 10 mmol) was dissolved in THF (20 ml) hydrochloric acid (2 mol/L, 20 ml). The mixture was stirred for 2 h at 70 °C, and then slowly warmed to reflux for 2 h. After evaporation of the THF under reduced vacuum, 10% aqueous NaOH solution was added to neutralize the residual liquid, and a large amount of off-white solid formed, filtered, washed with acetone and then water, and dried under vacuum to give an off-white solid **3** (2.07 g, 82%). mp 274.6–276.9 °C.

3 Results and discussion

To begin with, bis-Boc-2-amino-6-chloropurine **9** was synthesized from 2-amino-6-chloropurine **7** in high yield followed by Subhakar's procedure (Dey and Garner, 2000). The solubility of bis-Boc-2-amino-6-chloropurine **9** was investigated and the results are shown in Table 1. Unlike 2-amino-6-chloropurine **7**, known for its notorious insolubility in most common solvents, the solubility of **9** in DCM, methylbenzene, acetonitrile, and especially in THF was increased dramatically.

Table 1 Mole fraction solubility x of bis-Boc-2-amino-6-chloropurine 9 in different Mitsunobu solvents

T (K)	Solubility x^a (%)			
(±0.05 K)	THF[b]	DCM[b]	Methylbenzene[b]	Acetonitrile[b]
273.15	0.1141	0.0493	0.0213	0.0150
278.15	0.1191	0.0552	0.0253	0.0178
283.15	0.1251	0.0613	0.0303	0.0210
288.15	0.1299	0.0664	0.0349	0.0244
293.15	0.1352	0.0734	0.0405	0.0288
298.15	0.1399	0.0809	0.0470	0.0347
303.15	0.1463	0.0894	0.0544	0.0417
308.15	0.1523	0.0983	0.0634	0.0501
313.15	0.1581	0.1081	0.0734	0.0617

a: the solubility of bis-Boc-2-amino-6-chloropurine **9** was measured by our previous method with temperature ranging from 273.15 K to 313.15 K (Wang *et al.*, 2008) at atmospheric pressure. The laser monitoring observation technique was used to determine the disappearance of the solid phase in a solid and liquid mixture. b: all the solvents were further purified by distillation in dry agent (Na/benzophenone) and the sample bis-boc-2-amino-6-chloropurine **9** was dried in vacuum for over 2 d

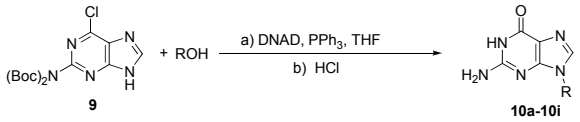
As shown in Table 1, THF, which is the most common solvent in Mitsunobu reaction, has great

solubility for bis-Boc-2-amino-6-chloropurine **9**. Afterwards, the best solvent THF was taken for coupling **9** with a number of alcohols under normal Mitsunobu conditions to investigate its reactivity. The results were illustrated in Table 2. We clearly learned that bis-Boc-2-amino-6-chloropurine **9**, as an excellent nucleophilic precursor, was able to react with a large number of alcohols, including primary alcohol, secondary alcohol, allyl alcohol, benzyl alcohol, etc., with high N9 selectivity and yields. Moreover, tert-Butyl alcohol still could not react with a protected purine as in the previous study (Yang *et al.*, 2011), owing to its steric hindrance in tertiary carbon.

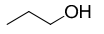
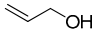
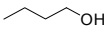
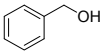
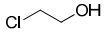
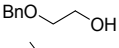
According to the research results above, it is more reasonable and assuring to prepare PCV via a Mitsunobu reaction. This novel method for the preparation of PCV is indicated in Fig. 4. First, the side chain of 5-(2-hydroxyethyl)-2,2-dimethyl-1,3-dioxane **5** was achieved through the commercially available starting material 2-hydroxymethyl-1,4-butanediol **11** reacting with 2,2-dimethoxypropane catalyzed by *p*-toluenesulfonic acid. The free –OH group of compound **5** is not necessary to be converted to the other leaving group such as chlorine, tosylate or methanesulphonate, which is always taken as a necessary step in the previous method or many other previous studies for the preparation of PVC till now (Harnden and Jarvest, 1985; Harnden *et al.*, 1987; Zheng *et al.*, 2004), making the synthesis of the side chain part of our method much more convenient and practical.

Our next objective was the synthesis of PCV. As was expected, bis-Boc-2-amino-6-chloropurine **9** combined with the side chain **5** (1.1 equivalent) under normal Mitsunobu conditions successfully obtained the desired N9-alkylated compound **12** in 92% yield without the undesired N7 alkylation by-product being formed. Importantly, the reaction conditions were significantly milder than those reported in recent studies (Geen *et al.*, 1990; 1992; Kim *et al.*, 1998; Brand *et al.*, 1999; Toyokuni *et al.*, 2003), requiring only 1.1 equivalent of each of the alcohol, PPh_3 and DNAD, and proceeding to completion within 60 min at room temperature. This is mainly due to the enhanced solubility of the compound **9** as mentioned above. By process c in Fig. 4, compound **8a** was obtained under neutral conditions. It is ^{1}H and ^{13}C NMR spectra further indicated that no 7-isomer purine (**8b**) was formed. Subsequently, we could obtain PCV **3** in an acid condition as procedure e; or directly starting from **12**, where hydrolytic dechlorination and deprotection step(s) were accomplished in one pot under mild acid conditions (2mol/L, hydrochloric acid in THF at room temperature) to afford the target PCV **3** in 80%–85% yield (process d). The overall yield of PCV from **11** was 44.5% higher than that in previous study (16%) (Zheng *et al.*, 2004).

Table 2 Investigation of the reactivity of bis-Boc-2-amino-6-chloropurine 9 with different alcohols



9 **10a-10i**

Entry	Alcohol	Product	Isolated yield (%)
1		**10a**	90.2
2		**10b**	86.6
3		**10c**	83.3
4		**10d**	84.8
5		**10e**	86.4
6		**10f**	81.2
7		**10g**	81.5
8		**10h**	80.7
9		**10i**	0

a): a mixture of **9** (1.0 equivalent), alcohol (1.1 equivalent) and phosphine reagent (1.1 equivalent) in anhydrous THF stirring under N_2 atmosphere at 0 °C, then treated with azo-reagent DNAD (1.1 equivalent) warmed to room temperature; b): the mixture of the products from procedure a, THF (20 ml) and aqueous hydrochloric acid (2 mol/L, 20 ml) was refluxed for 2 h at 70 °C

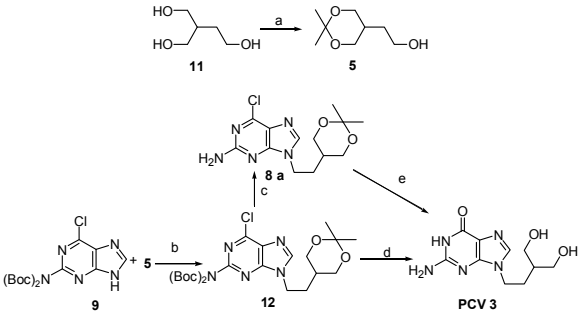


Fig. 4 Synthesis of penciclovir (PCV) with new method
a: 2,2-dimethoxypropane, *p*-toluenesulfonic acid, THF; b: 1.1 equivalent of the side chain **5**, 1.1 equivalent of PPh_3, and 1.1 equivalent of azodicarboxylate reagent at rt. in THF; c: TBDMS-OTf, DCM; d: aqueous hydrochloric acid (2 mol/L), THF; e: aqueous hydrochloric acid (2 mol/L)

4 Conclusions

In this study, ACP was protected with a bis-Boc carbamate group and showed a significant increase of solubility in the favorite Mitsunobu solvents. Coupling bis-Boc-2-amino-6-chloropurine **9** with different alcohols indicated a higher N9 selectivity and good reactivity in a Mitsunobu reaction. The results provided a convenient and practical protocol to prepare PCV from ACP, avoiding the presence of undesired N7 by-product and requiring only a few synthetic steps with higher yields.

Acknowledgements

We are grateful to Prof. Jun-quan SUN of Department of the Chemical and Biological Engineering of Zhejiang University, China for his insightful advice into our research work. We also would like to express our appreciation to Dr. Shi-chao YU and others for language polishing.

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