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Protection Process of the *tert*-Butyl Group as a Non-Polar Moiety of D-Serine: Unexpected Rearrangement

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The use of amino acid derivatives as building blocks in peptide synthesis is increasingly being recognized as a potential route for the development of pharmaceutical agents. Side chain protection of polyfunctional amino acids such as Ser, Thr, Tyr is viewed as being particularly important. Although these derivatives are commercially listed, they are expensive and not widely available. We describe here a practical large-scale synthesis of *t*-butyl introduced D-serine, one of the building blocks of zoladex, a peptide drug.

Key words: D-Serine, Peptide synthesis, Protecting group, *t*-Butyl ether, *t*-Butyl ester

INTRODUCTION

Synthetic peptides are increasingly been recognized as potential pharmaceutical agents and are of particular interest in the design, synthesis and evaluation of peptide libraries (Carbrele *et al.*, 1999). To meet the growing demand for amino acid derivatives, for use as building blocks in peptide synthesis, synthetic peptides should not only be available in chemically and stereochemically pure forms, but should also be available at reasonable prices. Therefore, their large-scale preparation must be both practical and convenient.

The side chain protection of polyfunctional amino acids such as Ser, Thr, Tyr is difficult to achieve, which is reflected by their prices and availabilities (Xi and Macro, 1995; Nefkens and Zwaneburg, 1983).

Traditionally, in peptide synthesis, the side chain hydroxyl functions in Ser and Thr have been protected as Bzl ethers, using N-Boc protection, and as *t*-butyl ethers in combination with an Fmoc group as the N-protecting group (Wang *et al.*, 1996). In published procedures, the amino group has been blocked by a benzyloxycarbonyl group and the carboxyl group by a *p*-nitrobenzyl ester, which is removed by hydrogenation after *t*-butylation of the hydroxyl groups (Wang *et al.*, 1997).

Our synthetic approach is based on a procedure using *t*-butyl trichloroacetimidate, made *in situ* in the *t*-butylation step. The side chain hydroxy function in Ser was pro-

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tected as the *t*-butyl ether in combination with a benzyloxycarbonyl group as the N-protecting group. *t*-Butyl ether is stable under the basic conditions used in the Cbz and Fmoc methodologies and easily removed under acidic conditions (Roby and Voyer, 1997). Traditional methods for the preparation of *t*-butyl ethers and esters rely upon the use of isobutylene in the presence of strong acid to generate the *t*-butyl cation. Recently a milder effective method for the conversion of the hydroxyl group directly to the corresponding *t*-butyl derivatives was reported, which uses *t*-butyl trichloroacetimidate in the presence of a catalytic amount of boron trifluoride etherate. This reagent is more convenient to handle than gaseous isobutylene and easily prepared *in situ* (Alan *et al.*, 1988).

MATERIALS AND METHODS

Materials

Reactions were generally carried out under a positive pressure of dried nitrogen gas; for moisture sensitive reactions the glassware was flame-dried under a stream of dried nitrogen gas. Dried nitrogen gas was supplied by Shin Yang Co., dried through Silica gel, blue(Shinyo Pure Chemicals Co. LTD., practical grade)and inlet to the reactions vessel by the silicon tubing connected a stainless steel needle. Air and moisture sensitive liquid reagents were transferred by dispossable sylinge and were introduced into reaction vessels through Suba Seal white-rubber septa(William Freeman LTD.) Solid reagents were added either in a nitrogen-filled glove bags or under a stream of dried nitrogen gas. All reactions were stirred with a Teflon covered magnetic stirring bar. Anhydrous solvents were

distilled before use. They were typically purchased from Oriental Chemical Ind. (extra pure grade). Dichloromethane (Yakuri chemical) was distilled from calcium hydride (Sigma) powder under a stream of dried argon gas(Shin Yang Co.). Flash chromatography following the method of Still (Still *et al.*, 1978) employed Merck silica gel (Kieselgel 60, 200-400 mesh). Analytical thin-layer chromatography (TLC) was performed with commercial silica gel glass plates (Sigma-Aldrich, 250 m layer tickness, 5-17 m particle size, 60 pore size, fluorescent indicator). Proton nuclear magnetic resonance (1H NMR) spectra were recorded on a Varian Gemini 2000 instrument (200 MHz). Chemical shifts are reported in parts per million(ppm) downfield from the internal standard, tetramethysilane. All drawing chemical structure were generated by CS ChemDraw ProTM Version 4.0.1.

Synthetic procedures

Benzyloxycarbonyl-D-serine(2)

A solution of D-serine (10.0 g, 0.095 mol) in 2N NaOH (50 ml) was stirred and cooled in an ice-water bath. Benzyl chlorocarbonate (17 ml, 0.124 mol) and 2N K_2CO_3 (70 ml) were added alternately, each in about ten approximately equal portions. Stirring was continued for ca. 30min, and the alkaline solution was extracted with ethyl acetate. Citric acid was then added to the cooled aqueous layer until its pH fell to 3. The aqueous solution was then extracted with ethyl acetate and the extracted organic layer was washed with water, brine and dried over anhydrous magnesium sulfate. The solvent was removed by vacuum and the white solid so obtained collected (10g, 44%). R_f=0.5 ($CHCl_3$:MeOH:AcOH = 85:10:5, v/v); ^{1}H NMR (200MHz, DMSO-d_6):7.36(s, 5H), 5.04(s, 2H), 4.04 (m, 1H), 3.66-3.63(d, J=4.8 Hz, 1H), 3.18-3.15 (d, J = 5.2 Hz, 1H); 13C-NMR(200MHz, DMSO-d_6) δ: 56.6, 61.3, 65.4, 127.6 (2 × C), 127.7, 128.2 (2 × C), 136.9, 155.9, 172.0; HRMS (EI) calc. for $C_{11}H_{13}NO_5$ (M^+) 239.0794 found 239.0794; $[\alpha]_D^{23}$ =-5.19 (c=0.05 in AcOH).

Benzyloxycarbonyl-*p*-nitrobenzyl-D-serine(3)

Benzyloxycarbonyl-D-serine (10.0 g, 41.8 mmol) dissolved in ethyl acetate (165 ml) was treated with triethylamine (5.8 ml) and with *p*-nitrobenzyl bromide (13.5 g, 62.7 mmol). The mixture was kept in a oil bath at 80°C for ca. 8 h and then cooled to room temperature. The solution was filtrated through cellite and then washed with 2N HCl, water, 10%NaHCO₃, and finally again with water. It was the dried over anhydrous MgSO₄ and evaporated *in vacuo*. The residue was dissolved in a small volume of ethyl acetate and diluted with hexane until turbid. When crystallization was complete, the product was collected, washed with a mixture of ethyl acetate and hexane and dried (15g, 82%). Rf = 0.5(Hexanes : Ethyl acetate = 1 : 1); ^{1}H NMR(200MHz, CDCl₃) δ: 8.24-8.20(d, J = 8.8 Hz,

2H), 7.53-7.49(d, J = 8.8 Hz, 2H), 7.36(s, 5H), 5.69(m, 1H), 5.14(s, 2H), 4.52(m, 1H), 4.07-4.01(d, J = 12.6 Hz, 2H); ^{13}C NMR(200MHz, CDCl₃) : 56.1, 63.2, 65.8, 67.3, 123.9(2 × C), 128.2(2 × C), 128.3, 128.4(2 × C), 128.6 (2 × C), 135.9, 142.3,147.9,160.1,170.2; HRMS (EI) calc. for $C_{18}H_{18}N_2O_7$ (M^+) 374.1114 found 374.1102 ; $[\alpha]_D^{24}$ = 10.22 (c= 0.009 in MeOH).

O-*t*-Butylbenzyloxycarbonyl-*p*-nitrobenzyl-D-serine(4)

Benzyloxycarbonyl-*p*-nitrobenzyl-D-serine (15 g, 34 mmol) was dissolved in a mixture of dichloromethane (50 ml) and hexane (50ml). The mixture was the cooled in an iced water bath. *t*-butyl trichloroacetimidate (7 ml, 39 mmol) and boron trifluoride etherate (0.6 ml) were added and stirred for ca. 24 h at room temperature. The solution was neutralized with solid NaHCO₃ and filtrated through a mixture of cellite and silica. The solvent was removed under vacuum and the residue dissolved in a small volume of ethyl acetate, diluted with hexane until some yellow oilseparation was evident. The clear solution was decanted off and the solvent was removed under vacuum. The product was a white solid (15 g, 86%). R_f=0.4(Hexanes :Ethyl acetate = 1:1); ^{1}H NMR(200MHz, CDCl₃) δ: 8.23-8.19(d, J = 9.0 Hz, 2H), 7.53-7.48(d, J = 8.8 Hz, 2H), 7.37(s, 5H), 5.67-5.63(d, J=8.6 Hz, 1H), 5.39-5.21(q, J=6.6 Hz, 2H), 5.14(s, 2H), 4.58-4.54(d, J=8.8 Hz, 1H), 3.91-3.85(dd, J=11.6, 2.8 Hz, 1H), 3.64-3.58(dd, J=8.9, 3.1 Hz, 1H); ^{13}C NMR(200MHz, CDCl₃): 27.3 (3C), 54.7, 65.4,65.8,67.1,73.6,123.7(2C),128.2(3C),128.3(2C),128 .6(2C),136.2,142.9,144.5,156.1,170.4239.0794;$[\alpha]_D^{26}$ = 11.78 (c=0.0325 in MeOH).

O-*t*-Butyl-benzyloxycarbonyl-D-serine(5)

O-*t*-Butyl-benzyloxycarbonyl-*p*-nitrobenzyl-D-serine (5 g, 10 mmol) was dissolved in Ethanol (60 ml) and 1N NaOH was added, and the mixture stirred for about 5 h at room temperature. The solution was evaporated under vacuum, and diluted with water before the alkaline solution was extracted with ethyl acetate. Citric acid was added to the cooled aqueous layer until the pH reached 3. The aqueous solution was then extracted with ethyl acetate and extracted organic layer washed with water, brine and dried over anhydrous magnesium sulfate. The solvent was removed under vacuum and a pale yellow oil collected (2.4 g, 78%). R_f=0.37(Hexanes:Ethyl acetate = 1:1); ^{1}H NMR(200MHz, CD₃OD) δ: 7.28-7.24(m, 5H), 5.04(s, 2H), 4.24(m, 1H), 3.75-3.54(m, 2H), 1.09(s, 9H); ^{13}C NMR (200MHz, CD₃OD) δ :25.6 (3 × C), 59.5, 65.7, 68.8, 72.5, 126.7, 127.0 (2 × C), 127.4 (2 × C), 136.2, 160.7, 171.8; HRMS (EI) calc. for $C_{15}H_{21}NO_5$ (M^+) 295.1420 found 295.1423; $[\alpha]_D^{23.6}$ = -16.37 (c=0.024 in MeOH).

RESULTS AND DISCUSSION

In this method, the protected intermediates were

prepared from the free-form D-serine. The side chain protected D-serine product was realized, after full protection of the side chain and selective de-protection of the intermediate. This side chain-protected D-serine is one of building blocks of zoladex, a peptide drug. An initial synthetic attempt involved the direct introduction of *t*-butyl trichloroaceti-midate into Cbz-D-serine. *t*-Butyl trichloroacetimidate was prepared using a previously published procedure (Alan et al., 1988). D-serine was converted to the correspond-ing *t*-butyl ether by treatment with *t*-butyl trichloroacetimi-date (1.1 equiv.) in a mixed solvent (cyclohexane: 2 μl/mmol of D-serine, dichloromethane:1 ml/mmol of D-serine) at room temperature, and with a catalytic amount of boron trifluoride etherate (20 ml/mmol) added.

This reaction did not produce the desired product as expected, because the reaction between the t-butyl cation and the carboxyl moiety dominated, which was surprising, because the hydroxyl group is known to be more neucleophilic than the carboxyl group. The reaction was *t*-butyl trichloroacetimidate concentration dependent (Fig. 1).
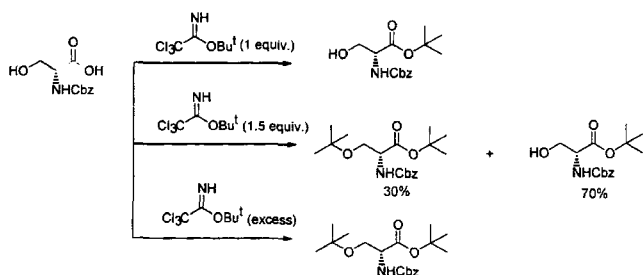

Fig. 1. The direct introduction of the *t*-butyl to the Cbz-D-serine

We then examined the possibility of performing selective *t*-butyl ester cleavage after fully protecting both hydroxyl and carboxyl functions by using an excess of *t*-butyl trichloro-acetimidate (2.5 equiv.) in the mixed solvent at room temperature. D-Ser was fully protected and a selective *t*-butyl ester cleavage was carried out(Fig. 2).

Using trifluoroacetic acid, the *t*-butyl ester was selectively cleaved in a short time and a crude compound was obtained. However, the expected product was not obtained
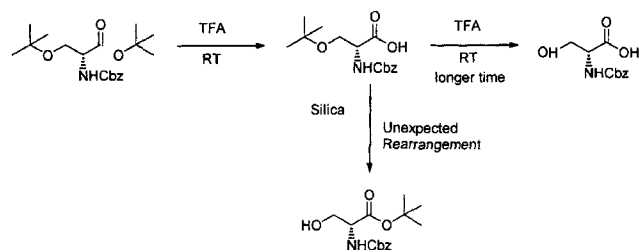

Fig. 2. Selective cleavage of *t*-butyl ester

after chromatography separation. Instead an unusual pro-duct, which was identified as a *t*-butyl ester was obtained. This unexpected result could have been caused by the rearrangement shown in Fig. 3.
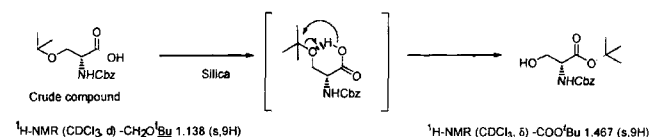

Fig. 3. Unexpected rearrangement

The initial trial is believed to have failed because of this rearrangement. Finally, the desired product was success-fully obtained by blocking the carboxyl moiety with *p*-nitro-benzyl bromide, which is easily removed by saponification after *t*-butylation (Fig. 4). Product separation and purifica-tion were performed by crystallization and work-up.
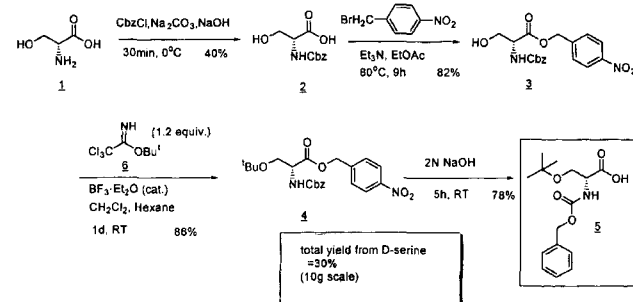

Fig. 4. Conclusive synthetic scheme

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