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Synthesis of N^{α}-(9-Fluorenylmethoxycarbonyl)-Allothreonine *t*-Butyl Ether *via* Threonine Oxazolines

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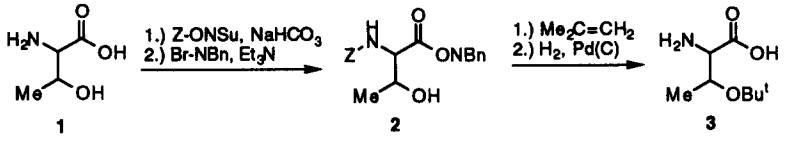
Abstract: New procedures for the preparation of allothreonine, suitably protected for solid phase peptide synthesis, were developed. Stereochemical inversion in the side chain of threonine *via* thionyl chloride-induced oxazoline cyclisation and subsequent hydrolysis, followed by protection of the allothreonine amino and hydroxyl functions provided Fmoc-D-alloThr(Bu^t)-OH from benzoyl-D-threonine phenacyl ester in only five steps. Alternatively, it was shown that temporary carboxyl protection for introduction of the *t*-butyl ether group can be circumvented by direct selective ester acidolysis from Fmoc-Thr(Bu^t)-OBu^t.

We were interested in the synthesis of alloThr derivatives suitable for solid-phase peptide synthesis by the Fmoc/*t*-butyl protection strategy.[1] In the standard method,[2] Thr **1** is protected with comparatively acid-stable temporary amino- and carboxyl-blocking groups, which are required for the acid-catalysed *t*-butyl etherification of **2**.[3] Finally the benzyl protecting groups (typically benzyloxycarbonyl (Z) and *p*-nitrobenzyl (NBn) as shown in *Scheme 1*) are removed simultaneously to yield the *t*-butyl ether **3**, which can then be further transormed to the Fmoc-derivative.

Scheme 1



1 **2** **3**

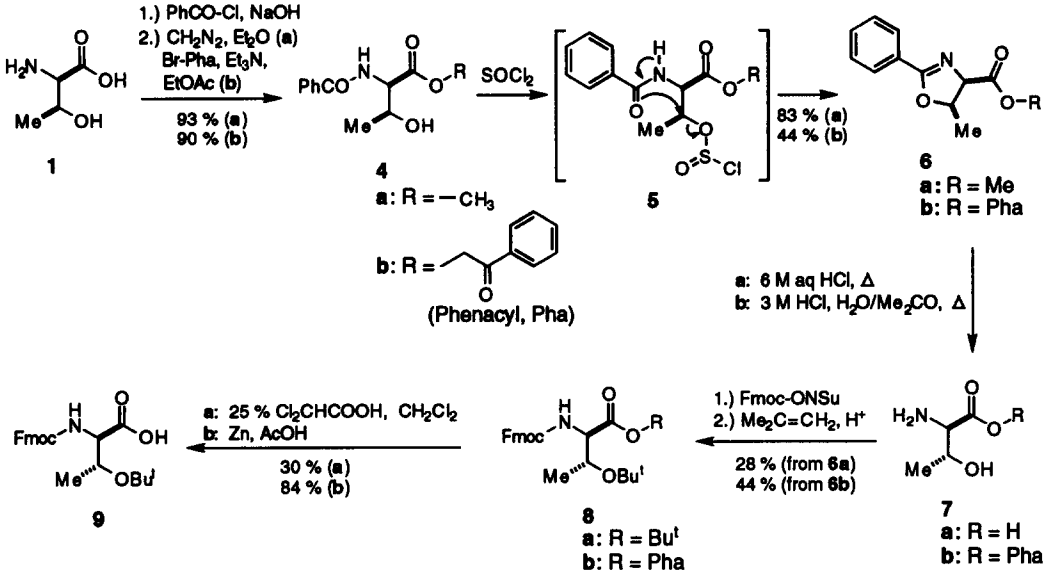
While direct etherification of Fmoc-Thr-OBn is of course possible,[4] hydrogenolytic debenzylation in the presence of the Fmoc group is equivocal.[5] Due to the very high cost of the starting material alloThr, it was thus attractive to develop more direct methods for the synthesis of our target Fmoc-D-alloThr(Bu^t)-OH **9** (*Scheme 2*). In some solid-phase syntheses the problem of Thr side-chain protection can be circumvented by employing derivatives lacking an hydroxyl protecting group altogether, as we have shown.[6] Furthermore, we have demonstrated recently that *t*-butyldimethylsilyl (Tbdms) ethers of hydroxyamino acids, including Thr, are suitable for Fmoc-based solid-phase peptide synthesis and that they offer abbreviated protection protocols since selective ester desilylation of the intermediate Fmoc-Thr(Tbdms)-OTbdms is possible.[7] In the present study we show that an analogous approach with Fmoc-Thr(Bu^t)-OBu^t **8a**, although not as efficient, is nevertheless possible.

A stereoselective method for converting benzoyl-Thr methyl esters **4a** to alloThr **7a** *via* 2-phenyl-5-methyl-Δ^2-oxazoline-4-carboxylic esters **6a** was described some time ago.[8] (*Scheme 2*, D-Thr to D-alloThr is

shown). Our ultimate aim was to combine this stereoconversion principle with the introduction of the amino- and hydroxyl-protecting groups we needed for peptide synthesis purposes.

In our hands the 4-step reaction sequence from Thr **1** to the ether **3** is possible in overall yields of *ca.* 65 %. In the alloThr series, on the other hand, both the acid-catalysed addition of **2** to isobutene, and particularly the hydrogenolysis reaction to **3**, proceed less satisfactorily and we have not been able to achieve acceptable yields. For this reason we investigated alternative methods of preparing protected alloThr derivatives:

Scheme 2



Oxazoline esters. The hypothetical intermediate **5** is obtained by treatment of esters **4** with thionyl chloride.[9] In the oxazoline reaction the attack of the carbonyl oxygen on the threonine-C^{β} leading to cyclisation appears to proceed by a strict S_N2 mechanism and therefore with complete optical inversion at that chiral center in **6**. Thus reaction of either L- or D-threonine derivatives gives rise exclusively to oxazolines with the stereochemistry of L- or D-allothreonine, respectively. The limiting factor in generating optical purity is the isolation procedures of the oxazolines **6** from the reaction mixtures. The cyclisation reactions cannot be driven to completion because side reactions which are attendant become dominant under forcing conditions. It is therefore inevitable that the oxazoline reaction mixtures always contain some quantities of unreacted starting materials with the undesired C^{β}-stereochemistry. In our experience traditional Kugelrohr distillation of the reaction mixtures[8] does not offer sufficient discrimination in this respect and we have therefore developed chromatographic methods. Short column silica gel chromatography in the standard or flash modes is suitable.[10] In the methyl ester series **6a** we use an EtOAc - hexane (1:1) solvent system, while in the phenacyl ester series **6b** we prefer Et_2O - CH_2Cl_2 (1:12) due to different solubility properties. Using these solvent systems on TLC we oberserve ΔR_f values of 0.22 (methyl esters) and 0.19 (phenacyl esters) for the starting materials and oxazoline products. Using these methods for the methyl oxazoline esters we have been able to obtain materials with significantly higher optical rotatory power than previously described.[11] In both

the methyl and phenacyl ester[12] series we observe no traces of diastereomeric threonines after oxazoline hydrolysis, complete deprotection and chromatographic analysis of a suitable derivative (refer *Fig. 1*).

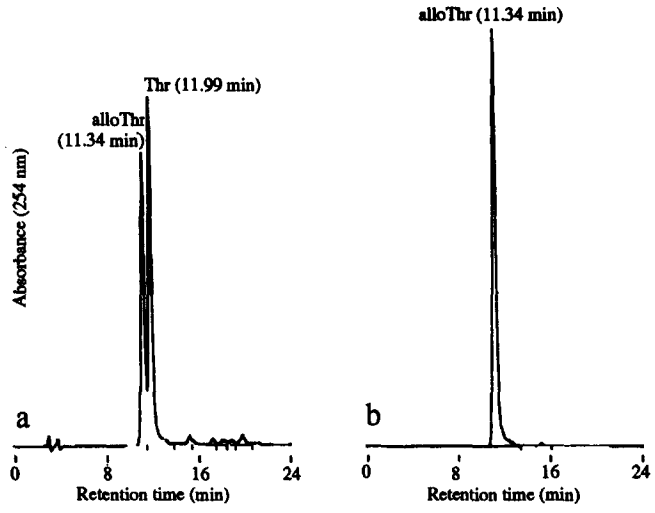


Figure 1. ***Reversed-Phase HPLC Analysis of Oxazoline Ester Hydrolysis Product.*** An aliquot of compound **6b** was gas-phase hydrolysed (6 M HCl, 1 % PhOH; 156°, 1 h), evaporated, neutralised and derivatised with phenylisothiocyanate (using standard procedures applied for amino acid analysis according to the Pico.Tag method[13]) and chromatography of the resulting phenylthiocarbamoyl derivatives (Phenomenex PTC column, 2 x 250 mm; 0.2 mL/min, 2 to 16 % MeCN over 16 min in 0.1 M Et_3N/NaOAc buffer, pH 4). **a**; A standard mixture of authentic alloThr and Thr was used, **b**; the derivative from **6b** hydrolysate.

Selective t-*butyl ether deprotection.* Since preparation of Fmoc-Thr(Bu^t)-OH's **9** *via* the *t*-butyl ether/ester **8a** would shorten significantly the synthetic route, we investigated if it was possible to remove the *t*-butyl ester in the presence of the *t*-butyl ether function. It was found that both the *t*-butyl ether and ester functions were particularly stable towards HCl in dioxane. It was hoped that addition of water, *i.e.* shifting from an acidolytic to an hydrolytic mechanism, might improve selectivity. In fact this was not the case and the reaction was observed to become even more sluggish. CF_3COOH in CH_2Cl_2, *i.e.* the standard acidolysis reagent for *t*-butyl groups, was investigated. The lowest concentration of CF_3COOH at which some deprotection was observed after 15 min reaction time was around 2 %. The main product was the desired ether/acid derivative but the product from deprotection of both *t*-butyl groups was already visible. If substantial conversion of the starting material was permitted, either by increasing reaction time or acid strength, the main product was always the fully de-*t*-butylated derivative. Modulation of the acid strength improved the situation somewhat. While CCl_3COOH was not found to be suitable for our purposes, $CHCl_2COOH$ looked more promising. Some selectivity was clearly observed here and it was possible to choose reaction conditions (25 % $CHCl_2COOH$ in CH_2Cl_2, 16 h) which gave a reasonable yield of the desired mono-*t*-butyl derivative. It is interesting to note that at that point almost all starting material had been reacted and only a trace of the fully de-*t*-butylated product was present. Separation of the ester and ether derivatives using conventional silica gel column chromatography methods (CH_2Cl_2/MeOH/AcOH solvent systems) presented no problem and the desired Fmoc-Thr(Bu^t)-OH derivative was obtained in isolated yields of up to 30 % and the by-products could be recycled into the *t*-butylation reaction. Unlike the halogenated acetic acids,

both HCl in trifluoroethanol and boron trifluoride etherate complex in CH_2Cl_2 appeared to exhibit some selectivity for *t*-butyl ether cleavage.

Optimised synthesis of Fmoc-alloThr(Bu^t)-OH. Apart from the possibility of selective *t*-butyl ester/ether cleavage, we find the route *via* phenacyl esters **6b** to the protected alloThr **9** particularly convenient. The phenacyl ester function in oxazoline **6b** is sufficiently acid stable to permit selective hydrolysis of the oxazoline ring. This ester group can thus subsequently serve as carboxyl protection during introduction of the amino- and hydroxyl protecting groups. The phenacyl ester is readily and selectively removed from **8b** with zinc in acetic acid. It is thus possible to obtain *e.g.* pure Fmoc-D-alloThr(Bu^t)-OH **9** in 15 % overall yield[14] from readily available and inexpensive H-D-Thr-OH.

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9. With excess freshly distilled pre-cooled (-20°C) neat $SOCl_2$. Reaction with $CaCl_2$ guard tube during 2 h at room temperature and overnight at 4°C for **4a**, during 3 h at room temperature for **4b**. Work-up by evaporation and partioning between aq Na_2CO_3 soln. and $CHCl_3$. Analytical data for **4b**: M.p. 104 -106°, $[\alpha]_D^{20}$ = +25.2 (*c* = 1, CH_2Cl_2).
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11. Compound **6a**: M.p. 74°C (Lit.[8] 75-76°C), $[\alpha]_D^{20}$ = -76.5 (*c* = 1, EtOH) [Lit.[8] $[\alpha]_D^{25}$ = -68.9 (*c* = 8.4, EtOH); Greenstein, J. P.; Winitz, M. *Chemistry of the Amino Acids*; Robert E. Krieger Publishing Company, Inc.: Malabar, FL, 1984, p. 2255: $[\alpha]_D$ = -69], ^{1}H-NMR (300 MHz; $CDCl_3$): 1.36 (*d*, $J_{C\beta H,Me}$ = 6.3 Hz, C*H*$_3$, 3 H); 3.75 (*s*, COOC*H*$_3$, 3 H); 4.95-5.10 (*m*, Cα*H* & Cβ*H*, 2 H); 7.36-7.57 (*m*, Ar-*H*, 3 H); 7.95-7.98 (*m*, Ar-*H*, 2 H). ^{13}C-NMR (50 MHz; $CDCl_3$): 16.17 (*C*H$_3$); 51.99 (OC*H*$_3$); 71.71 (*C*β); 77.58 (*C*α); 127.19 (aromatic *C*); 128.22, 128.45, 131.70 (aromatic *C*H); 166.05 (*C*=N); 170.31 (*C*=O).
12. Compound **6b**: M.p. 139°C, $[\alpha]_D^{20}$ = +34.6 (*c* = 1, CH_2Cl_2). ^{1}H-NMR (300 MHz; $CDCl_3$): 1.59 (*d*, $J_{C\beta H,Me}$ = 4.5 Hz, C*H*$_3$, 3 H); 5.13-5.21 (*m*, Cα*H* & Cβ*H*, 2 H); 5.37-5.52 (*q*, OC*H*$_2$CO, 2 H); 7.38-7.52 (*m*, Ar-*H*, 5 H); 7.56-7.62 (*tm*, Ar-*H*, 1 H); 7.88-7.92 (*dm*, Ar-*H*, 2 H); 7.98-8.02 (*dm*, Ar-*H*, 2 H). ^{13}C-NMR (50 MHz; $CDCl_3$): 16.36 (*C*H$_3$); 66.28 (OC*H*$_2$CO); 71.52 (*C*β); 77.99 (*C*α); 127.24, 134.13 (aromatic *C*); 127.70, 128.22, 128.30, 128.81, 131.72, 133.84 (aromatic *C*H); 166.32 (*C*=O ester); 169.38 (*C*=N); 191.44 (*C*=O ketone). $C_{19}H_{17}NO_4$ = 323.35, FAB-MS $[M + H]^+$ = 324.2. Enantiomer of **6b**: M.p. 139°C, $[\alpha]_D^{20}$ = -34.2 (*c* = 1, CH_2Cl_2).
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14. Compound **9**: M.p. 98°C (from EtOAc/hexane), $[\alpha]_D^{20}$ = -4.5 (*c* = 0.5, CH_2Cl_2). ^{1}H-NMR (300 MHz; d_6-DMSO): 1.02 (*m*, Cγ*H*$_3$, 3 H); 1.10 (*s*, Bu^t ether, 9 H); 4.03 (*m*, Cα*H* & Cβ*H*, 2 H), 4.22 (*m*, 9-fluorenyl*H* & Ar-C*H*$_2$, 3 H), 7.27 - 7.88 (*m*, Ar-*H*, 8 H). ^{13}C-NMR (50 MHz; d_6-DMSO): 19.00 (*C*H$_3$); 28.43 (C(*C*H$_3$)$_3$); 47.14 (*C*β); 60.84 (*C*α); 66.06 (Ar-*C*H$_2$); 67.70 (9-fluorenyl*C*); 73.59 (*C*(CH$_3$)$_3$); 120.45, 125.70, 127.45, 127.99 (aromatic *C*H); 141.09. 144.29 (aromatic *C*); 156.26 (*C*=O, urethane). Treatment of an aliquot of this material with CF_3COOH for 1 h, followed by evaporation of solvent and crystallisation from CH_2Cl_2/hexane, afforded Fmoc-D-alloThr-OH, m.p. 176-177°C, $[\alpha]_D^{20}$ = -0.05 (*c* = 1, CH_2Cl_2/DMF). Further treatment for several hours with Et_2NH in CH_2Cl_2, followed by evaporation of solvent and crystallisation from EtOH/H_2O, afforded material indistinguishable from authentic H-D-alloThr-OH.

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