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Simple Methods for the Preparation of Protected Derivatives of D-*allo*- and L-*allo*-Threonine

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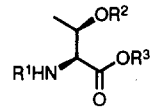
Abstract: *Practical syntheses of protected derivatives of the non-proteinogenic allo-threonines are decribed. The key step is enzymatic resolution of threonine diastereomers, produced on controlled epimerization of the α-carbon atom. The protected allo-threonines are obtained in good overall yield and can be used in standard peptide synthesis protocols.*

Of the four diastereoisomers of 2-amino-3-hydroxybutanoic acid, threonine (Thr) **1** [(2S, 3R)] is one of the 20 DNA-encoded amino acids found in proteins. The other three, D-Thr **2** [(2R, 3S)], D-*allo*-Thr **3** [(2R, 3R)], and L-*allo*-Thr **4** [(2S, 3S)], occur much less extensively in nature, but are components of several fungal and marine peptides, some of which show interesting biological activity.[1, 2] The D-*allo*-isomer **3** is also required for the application of the retro-enantio concept[3, 4] to the design of new peptides and protein domains, since it has the opposite configuration to L-Thr at the α-stereogenic carbon atom, while maintaining the native configuration at the β-position. Synthetic routes to suitably protected derivatives of the non-proteinogenic *allo* threonines are desirable, therefore, and a number have been reported.[5-9] Generally speaking, however, they require a considerable number of chemical steps and, in some cases, the protecting groups used are not compatible with standard solid-phase peptide synthesis protocols.

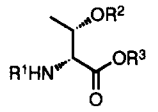
Our interest in the generality of the retro-inverso concept, especially when applied to helical peptides,[10, 11] led us to investigate simpler methods for the preparation of protected derivatives of both D-*allo*- and L-*allo*-Thr, especially those required for use in either or both of the main protection schemes employed in contemporary solid-phase peptide synthesis,[12] in addition to in peptide synthesis in solution. We have previously described[13] short and practical syntheses of N^{α}-protected-D-*allo*-isoleucine derivative, using L-isoleucine as starting material, and now report on the extension of the method to the synthesis of protected derivatives of both D-*allo*- and L-*allo*-Thr. These derivatives can be used directly in standard Boc/Bzl-SPPS.[14]

Commercially available H-L-Thr(Bzl)-OH **5** can be converted into Ac-L-Thr(Bzl)-OH **7**, m.p.180-182 °C, $[\alpha]_D$ +33.0 (*c* 1, MeOH)[15] on treatment with acetic anhydride.[16] Similarly, commercially available H-D-Thr(Bzl)-OBzl **6** can be transformed into Ac-D-Thr(Bzl)-OH **8**, m.p.184-186 °C, $[\alpha]_D$ -32.5 (*c* 1, MeOH), by acetylation and saponification.[16] (Yields >90% in each case) Epimerization of the C_{α} stereogenic atom of both **7** and **8** can be brought about by treatment with *N*-ethyl-*N'*-3-dimethylaminopropyl carbodiimide [water-soluble carbodiimide (EDC)], following the method described by Benoiton.[17] Such epimerization proceeds *via* formation of the corresponding 5(4H)-oxazolones **9** (in the case of Thr derivative **7**) and **10** (in the case of **8**),

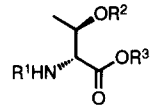
both of which were sufficiently stable to permit their characterization by ^{1}H- and ^{13}C-NMR spectroscopy. Each of the oxazolones **9** and **10** can be produced, in an optically pure form (as judged by high-field ^{1}H-NMR spectrocopy) on short (2-3 min) exposure of derivatives **7** or **8** to EDC followed by work-up. Longer reaction times (15-20 min) with the carbodiimide gave oxazolones that were epimeric mixtures at C_{α}. Hydrolysis of epimerized **9** using 10% aqueous hydrochloric acid then provided a 57:43 mixture of Ac-L-Thr(Bzl)-OH **7** and Ac-D-*allo*-Thr(Bzl)-OH **11**. Hydrolysis of epimeric **10** gave a similar mixture of Ac-D-Thr(Bzl)-OH **8** and Ac-L-*allo*-Thr(Bzl)-OH **12**.



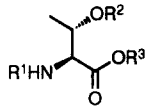
1, $R^1=R^2=R^3=H$
5, $R^1=R^3=H$, $R^2=Bzl$
7, $R^1=Ac$, $R^2=Bzl$, $R^3=H$



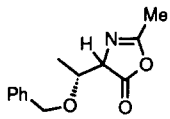
2, $R^1=R^2=R^3=H$
6, $R^1=H$, $R^2=R^3=Bzl$
8, $R^1=Ac$, $R^2=Bzl$, $R^3=H$



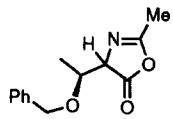
3, $R^1=R^2=R^3=H$
11, $R^1=Ac$, $R^2=Bzl$, $R^3=H$
13, $R^1=H$, $R^2=Bzl$, $R^3=H$
15, $R^1=Boc$, $R^2=Bzl$, $R^3=H$



4, $R^1=R^2=R^3=H$
12, $R^1=Ac$, $R^2=Bzl$, $R^3=H$
14, $R^1=H$, $R^2=Bzl$, $R^3=H$
16, $R^1=Boc$, $R^2=Bzl$, $R^3=H$



9



10

These mixtures can be resolved enzymatically[13, 18] on treatment with hog kidney acylase I, at pH 8 and 38 °C. (See Experimental, below) For the epimers **7** and **11**, enzyme catalyzed hydrolysis of Ac-L-Thr(Bzl)-OH **7** is much more rapid than of Ac-D-*allo*-Thr(Bzl)-OH **11**, which remains essentially unchanged throughout. The resulting mixture of H-L-Thr(Bzl)-OH **5** and Ac-D-*allo*-Thr(Bzl)-OH **11** can be separated from the enzyme by centrifuging, followed by gel-filtration (Sephadex G-10, 0.1 M AcOH) of the supernatant. The components of the mixture of **5** and **11** are then easily separated from one another by simple extraction of the resulting aqueous solution with ethyl acetate, since **11** is soluble in the organic solvent. An advantage of the method is that the H-L-Thr(Bzl)-OH **5** recovered from the aqueous phases can be recycled, allowing all of the initial L-form starting material to be converted into the desired D-*allo*-derivative by repeated cycles of acetylation, epimerization and resolution. The pure Ac-D-*allo*-Thr(Bzl)-OH **11**, m.p.172-174 °C, $[\alpha]_D$ -12.4 (*c* 1.5, MeOH), obtained in this way can then be subjected to acetyl group removal, which occurs smoothly, without detectable epimerization,[19] on refluxing in 2M hydrochloric acid for 120 min, giving H-D-*allo*-Thr(Bzl)-OH **13** (85% yield). Conversion of this into the desired Boc-D-*allo*-Thr(Bzl)-OH **15**, m.p.113-117 °C, $[\alpha]_D$ -17.9 (*c* 1, CH_2Cl_2), was accomplished by treatment with *tert*-butyl pyrocarbonate, (90% yield) following a standard protocol.[16]

A similar procedure can be applied to the resolution of the mixture of **8** and **12**. In this case enzymatic hydrolysis of **12** occurs much more rapidly than of **8**. The rate of hydrolysis of **12**, in fact, appreciably more rapid[20] than of **7**, indicating sensitivity[21] to the configuration at C_β. The resulting mixture of **8** and **14** was separated from the enzyme by centrifuging followed by gel filtration, as described above, and the components of the mixture were then separated by extraction of the aqueous solution with ethyl acetate. In this case, the desired H-L-*allo*-Thr(Bzl)-OH **14** remains in the aqueous phase, while Ac-D-*allo*-Thr-OH **8** is soluble in the organic solvent. Recycling of **8** allows the progressive conversion of all the D-starting material into the L-*allo*-derivative, by successive epimerization and resolution steps. The H-L-*allo*-Thr(Bzl)-OH **14** isolated was then converted (90% yield) into Boc-L-*allo*-Thr(Bzl)-OH **15**, m.p.110-117 °C, $[\alpha]_D$ +15.7 (*c* 1, CH_2Cl_2), as described above.

These syntheses are quite efficient and operationally simple. In each case, conversion of the commercially available starting material into the desired *allo* product can be achieved in high overall yield by recycling. As reported here, they have been carried out with hundreds of milligrams of material but further scale up should be possible, in principle.

Typical Experimental Procedure for the Enzymatic Resolution of Threonine Diastereoisomers— A 57:43 mixture (540 mg) of Ac-L-Thr(Bzl)-OH **7** and Ac-D-*allo*-Thr(Bzl)-OH **11** was suspended in H_2O (55 mL) and the pH taken to 8 (indicator paper) by dropwise addition of 1M LiOH. Hog kidney acylase I [Fluka, Activity 2000-3000 Umg^{-1}, (273 mg)] was added, and the mixture placed under argon and stirred vigorously for five days at 38 °C. The pH of the medium was checked daily and, if necessary, adjusted to 8 (indicator paper) by adding 1M LiOH. In order to monitor the progress of the enzymatic hydrolysis, an aliquot of the mixture was removed, centrifuged to precipitate the enzyme and analyzed by HPLC (Vydac C18 column with 10 μm particle size; gradient elution starting from 25% B in A, taken over 20 min to 35% B in A, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA, at a flow rate of 1 $mLmin^{-1}$; detection by ultraviolet absorption at 220 nm). After 5 days the reaction was 80% complete. Hog kidney acylase (137 mg) was added and the mixture again stirred under argon at 38 °C and pH 8.0 for a further 3 days. HPLC analysis indicated that hydrolysis was complete after this time. The mixture was centrifuged and the supernatant subjected to gel filtration on Sephadex G-10, eluting with 0.1 M AcOH at a flow rate of 23 mLh^{-1}. The fractions containing Ac-D-*allo*-Thr(Bzl)-OH were combined (total volume 140 mL), taken to pH 2-3 (indicator paper) by the addition of 5% aqueous HCl and extracted with ethyl acetate (5 x 20 mL). The combined extracts were washed with H_2O (5 x 20 mL) and dried. Filtration, followed by solvent removal gave Ac-D-*allo*-Thr(Bzl)-OH **11** as a white solid (170 mg, 73 % [of the D-*allo*-threonine derivative present in the original mixture]). Column fractions containing H-L-Thr(Bzl)-OH were combined and the solvent removed. The white solid obtained was treated with acetic anhydride[16] and Ac-L-Thr(Bzl)-OH was recovered by standard work-up, for recycling.

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14. Abbreviations for amino acids used in this paper are those recommended by the IUPAC-IUPAB Commission of Biochemical Nomenclature, and published in *J. Biol. Chem.*, 1972, **247**, 977-983. Additional abbreviations used are: Ac, acetyl; Boc, *tert*-butoxycarbonyl; Bzl, benzyl; HPLC, high performance liquid chromatography; SPPS, solid-phase peptide synthesis; TFA, trifluoroacetic acid.
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19. Product purity was checked by analytical HPLC, either using conditions similar to those described in the experimental section or the method reported by Buck and Krummen.[22] This latter allowed all four diastereomers of Thr to be cleanly separated. Synthetic *allo*-threonines were judged to contain less than 0.5 % of their corresponding enantiomers.
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