Pergamon

Tetrahedron, Vol. 53, No. 9, pp. 3369-3382, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(97)00059-8

Synthetic Studies on Threonines. The Preparation of Protected Derivatives of D-*allo*- and L-*allo*-Threonine for Peptide Synthesis

Paul Lloyd-Williams,* Agustí Sánchez, Natàlia Carulla, Teresa Ochoa and Ernest Giralt*

Department of Organic Chemistry, University of Barcelona, E-08028 Barcelona, Spain

Abstract: *N*-Acetylated threonine derivatives, having *tert*-butyl or benzyl-based side-chain protection, form isolable 5(4H)-oxazolones on treatment with *N*-ethyl-*N'*-3-dimethylaminopropyl carbodiimide. *N*-chloroacetylated threonine derivatives, on the other hand, do not form oxazolones so readily. The *N*-acetylated oxazolones are easily epimerized and lead to diastereoisomeric mixtures of threonine derivatives on hydrolysis with dilute aqueous acid. The components of these mixtures can be separated chromatographically, but a useful alternative for the *O*-benzylated mixture is selective enzymatic hydrolysis using hog kidney acylase. These chemical transformations provide the basis for practical syntheses of protected derivatives of the non-proteinogenic *allo*-threonines, suitable for use in peptide synthesis.

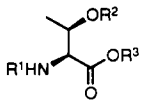
INTRODUCTION

Of the four diastereoisomers of 2-amino-3-hydroxybutanoic acid, L-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,[1] some of which show interesting biological activity.[2, 3] The D-*allo*-isomer **3** is also required for the application of the retro-enantio concept[4, 5] 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.[6-10] Generally speaking, however, they are rather involved and in some cases the products cannot be used in standard SPPS protocols, owing to protecting group incompatibility.

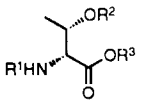
Our interest in the generality of the retro-inverso concept, especially when applied to helical peptides,[11, 12] led us to investigate simpler methods for the preparation of the protected derivatives of both D-*allo*- and L-*allo*-Thr that are required for use in either or both of the major protection schemes used in

† 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; ClAc, chloroacetyl; EDC, *N*-ethyl-*N'*-3-dimethylaminopropyl carbodiimide; HPLC, high performance liquid chromatography; SPPS, solid-phase peptide synthesis; TFA, trifluoroacetic acid; UV, ultra violet

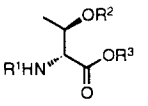
contemporary solid-phase methods.[13] In Boc/Bzl-synthesis, N^{α}-amino protection is provided by the *tert*-butoxycarbonyl group and side-chain protection is based on the benzyl group, whereas in Fmoc/*t*Bu-synthesis, the 9-fluorenyloxycarbonyl group and *tert*-butyl based protection, respectively, are used. Both of these protecting group combinations can also be used in peptide synthesis in solution. Threonine is seldom incorporated without side-chain hydroxyl protection, since this can be the cause of several unwanted side reactions.[14]



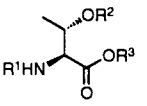
1, R^1=R^2=R^3=H
5, R^1=R^3=H, R^2=Bzl
6, R^1=R^3=H, R^2=*t*Bu
7, R^1=Ac, R^2=H, R^3=H
8, R^1=Ac, R^2=Bzl, R^3=H
9, R^1=Ac, R^2=*t*Bu, R^3=H
10, R^1=ClAc, R^2=Bzl, R^3=H
11, R^1=ClAc, R^2=*t*Bu, R^3=H
12, R^1=ClAc, R^2=H, R^3=H



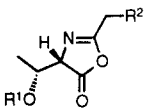
2, R^1=R^2=R^3=H
23, R^1=H, R^2=R^3=Bzl
24, R^1=Ac, R^2=Bzl, R^3=Bzl
25, R^1=Ac, R^2=Bzl, R^3=H



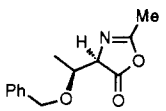
3, R^1=R^2=R^3=H
18, R^1=Ac, R^2=Bzl, R^3=H
19, R^1=Ac, R^2=*t*Bu, R^3=H
20, R^1=Ac, R^2=R^3=H
21, R^1=R^3=H, R^2=Bzl
22, R^1=Boc, R^2=Bzl, R^3=H



4, R^1=R^2=R^3=H
27, R^1=Ac, R^2=Bzl, R^3=H
28, R^1=R^3=H, R^2=Bzl
29, R^1=Boc, R^2=Bzl, R^3=H



13, R^1=R^2=H
14, R^1=Bzl, R^2=H
15, R^1=*t*Bu, R^2=H
16, R^1=Bzl, R^2=Cl
17, R^1=*t*Bu, R^2=Cl



26

The approach adopted[15] was based on our previously described[16] synthesis of Boc-D-*allo*-Ile-OH, which was required both as a precursor to isostatine for our total synthesis of the didemnins[17] and for application in retro-enantio studies. Epimerization of suitable *N*-acylated L- or D-threonine derivatives gives diasteromeric products that can either be separated chromatographically or resolved by hog kidney acylase-mediated enzymatic hydrolysis.[18] In the latter case, the component having L-stereochemistry at the α-stereogenic carbon atom is hydrolyzed much more rapidly than that having D-stereochemistry. The resulting mixture of free- and *N*-acylated- amino acids can then be separated by simple extraction with a suitable organic solvent such as ethyl acetate.

RESULTS AND DISCUSSION

1. Preparation and Enzymatic Hydrolysis of N-Acylthreonine Derivatives

In order to optimize the conditions for the selective enzymatic hydrolysis of pairs of threonine epimers at the α-carbon atom, the effect of both the *N*-acyl group and the *O*-protecting group on the rate of hydrolysis was investigated. *N*-Acetyl-L-threonine **7**, *N*-acetyl-*O*-benzyl-L-threonine **8**, *N*-acetyl-*O*-*tert*-butyl-L-threonine **9**,

N-chloroacetyl-*O*-benzyl-L-threonine **10**, *N*-chloroacetyl-*O*-*tert*-butyl-L-threonine **11**, were prepared by acylation of L-Thr **1** or its *O*-Bzl or *O*-*t*Bu-protected derivatives (**5** and **6**, respectively), as appropriate. *N*-Chloroacetyl-L-threonine **12**, could not be prepared by direct chloroacetylation of **1**, but was obtained by trifluoroacetic acid treatment of **11**. The crude compound was, however, difficult to purify and apart from its partial characterization as described in the Experimental section, its properties were not investigated further.

Aqueous solutions of the acetylated L-Thr derivatives **7**—**11**, adjusted to pH 8 by the addition of 1M lithium hydroxide, were submitted to hog kidney acylase 1 mediated hydrolysis, at 38 °C. Much larger quantities of enzyme, compared to those required[16] to hydrolyze Ac-L-Ile-OH, were necessary for hydrolysis of *O*-protected threonines. In all these preliminary experiments 22000 U of enzyme per mmol of amino acid substrate were employed, where 1U is defined by the suppliers§ as being the amount of enzyme required to hydrolyze 1μM of Ac-L-Met-OH per minute at pH 7 and 25 °C. The rate of reaction was followed by reversed-phase HPLC (for typical conditions, see the Experimental section), since the *N*-acetylated compounds had quite different retention times to those having a free amino group. In cases where the free amino acid derivative did not have a suitable chromophore, a known quantity of H-Phe-OH was added to the hydrolysis mixture and acted as an internal standard.

Large differences in the rate of hydrolysis were observed between the various derivatives, under a given set of conditions. Hydrolysis was most rapid in the case of Ac-L-Thr-OH **7**, being complete within 40 min. The rate of hydrolysis of this compound was similar to that of Ac-L-Ile-OH, which underwent 79% hydrolysis after 10 min and 100% after 4 hours, under similar conditions. Hydrolysis of the *tert*-butyl protected derivatives **9** and **11**, on the other hand, was not appreciable even after 72 hours. The benzyl protected derivatives **8** and **10** underwent 52% and 99% hydrolysis, respectively after this time.

Overall, the results indicate that *O*-protected acetyl-L-threonines are not especially good substrates in acylase-catalyzed hydrolysis, with *tert*-butyl protected derivatives being the poorest. Although hydrolysis was fairly slow in the case of the *O*-benzylated derivatives **8** and **10**, the rate was still judged to be sufficiently rapid, particularly in the case of **10**, to allow the procedure to be synthetically useful.

2. Oxazolone Formation and Epimerization of N-Acylthreonine Derivatives

The major epimerization pathway in peptide synthesis proceeds *via* the formation of 5(4H)-oxazolones, which are formed easily when *N*-acylated amino acid derivatives are activated for coupling. Abstraction of a proton from the oxazolone then leads to epimerization at the stereogenic centre concerned. Normally, of course, these processes are highly undesirable but in the present work the controlled epimerization of threonine derivatives is a key step in the production of the *allo*-threonines. Oxazolones **14** and **15** could be formed from compounds **8** and **9**, respectively, on treatment with one equivalent of *N*-ethyl-*N'*-3-dimethylaminopropyl carbodiimide [water-soluble carbodiimide (EDC)], following the method described by Benoiton.[19] Both of these oxazolones were sufficiently stable to permit their isolation and characterization by ^{1}H-NMR, ^{13}C-NMR and IR spectroscopy and, in the case of **15**, by mass spectrometry in the CI mode. The compounds were not, however, sufficiently stable to allow them to be isolated in a pure state and fully characterized. The physical data that were obtained are given in the Experimental section.

§ See the entry for Acylase I in Fluka, Sigma or Aldrich catalogues for the current year

Oxazolones **14** and **15** could be produced, either in their optically pure forms, as judged from their ^1H NMR spectra, or as mixtures of epimers at C_α, depending upon the reaction time of L-Thr derivatives **8** or **9** with the carbodiimide. Short (1-2 min) exposure of **8** or **9** to EDC, followed by work-up gave an optically pure oxazolone, whereas longer reaction times (15-20 min) gave the epimerized oxazolone. Hydrolysis of epimerized **14** using 10% aqueous hydrochloric acid then provided a 56:44 mixture of Ac-L-Thr(Bzl)-OH **8** and Ac-D-*allo*-Thr(Bzl)-OH **18**, as judged from the respective peak areas on HPLC analysis (conditions are given in the Experimental section). Similar hydrolysis of oxazolone **15** gave a 38:62 mixture of Ac-L-Thr(*t*Bu)-OH **9** and Ac-D-*allo*-Thr(*t*Bu)-OH **19**. Oxazolone **13**, derived from Ac-L-Thr-OH **7**, could not be produced in an optically pure form under the conditions used. The formation of the epimerized oxazolone was verified by ^1H NMR and IR spectroscopy. Hydolysis of this oxazolone presumably led to a mixture of Ac-L-Thr-OH **7** and Ac-D-*allo*-Thr-OH **20**. These compounds, however, are soluble in water and their isolation by repeated extraction with organic solvents was not possible. No other attempts were made to recover **7** and **20** from the aqueous phase, where likely contaminants would include unreacted carbodiimide and the carbodiimide-derived urea.

Treatment of *N*-chloroacetyl derivatives **10** and **11** with EDC, under similar conditions to those applied in the cases of *N*-acetylated derivatives **7**, **8** and **9**, did not lead to clean oxazolone formation. ^1H NMR and IR spectroscopy indicated that, in both cases, mixtures containing oxazolones **16** and **17** and starting materials **10** and **11**, in addition to other unidentified components, were produced. Use of longer reaction times or larger excesses of carbodiimide led to extensive decomposition of the starting materials **10** or **11**. These results ruled out the option of taking advantage of the more rapid enzymatic hydrolysis of chloroacetyl derivative **10**, and dictated instead the use of acetyl derivative **8**, whose hydrolysis is slower.

3. Separation of Epimeric Protected Threonine Mixtures. Preparation of Boc-D-allo-Thr(Bzl)-OH and Boc-L-allo-Thr(Bzl)-OH

The pairs of *N*-acylated-*O*-protected threonine epimers, **8** and **18** and **9** and **19** could be separated chromatographically, the most efficient method being reversed-phase medium pressure liquid chromatography. However, an alternative method for the *O*-benzyl protected derivatives **8** and **18** is enzymatic resolution. Selective enzymatic hydrolysis of only the Ac-L-Thr(Bzl)-OH **8** in a 56:44 mixture of Ac-L-Thr(Bzl)-OH **8** and Ac-D-*allo*-Thr(Bzl)-OH **18**, produced on opening epimerized oxazolone **14**, provided a simple way of isolating **18**. The progress of the reaction could be followed by HPLC, since the epimers could easily be separated using standard operating conditions (see the Experimental section for typical elution conditions). When hydrolysis of **8** was complete, centrifugation of the mixture precipitated the major part of the enzyme and the rest was removed by gel-filtration through Sephadex G-10 using 0.1 M AcOH as eluent. After chromatography, separation of the two threonine derivatives **5** and **18** could be achieved simply by extraction with ethyl acetate. The free amino component **5** remained in aqueous solution whereas **18** was soluble in the organic phase. 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. Removal of the acetyl group from **18** was achieved in moderate yield, without detectable epimerization, on refluxing with 4M hydrochloric acid for 2h, affording **21** as its hydrochloride. Conversion of **21** into the desired Boc derivative **22** was then carried out using a standard protocol.[20]

A similar procedure, but starting from D-Thr, was applied to the formation of the corresponding L-*allo*-Thr derivative. Conversion of the commercially-available **23** into **24** and then **25** was achieved by acetylation with acetic anhydride followed by saponification.[20] Epimerization of **25** proceeded *via* the oxazolone **26**, whose hydrolysis led to a 57:43 mixture of Ac-D-Thr(Bzl)-OH **25** and Ac-L-*allo*-Thr(Bzl)-OH **27**. In the enzymatic resolution of this mixture, the L-*allo*-product **27** was selectively hydrolyzed and Ac-D-Thr(Bzl)-OH **25** remained unchanged. In fact, the hydrolysis of Ac-L-*allo*-Thr(Bzl)-OH **27** was significantly faster than that of Ac-L-Thr(Bzl)-OH **8**, indicating that the enzyme is sensitive to the stereochemistry of the β-carbon atom.[21] As in the case of the D-*allo*-derivative, after removal of the enzyme, separation of the components of the mixture of Ac-D-*allo*-Thr-OH **25** and H-L-*allo*-Thr(Bzl)-OH **28** was accomplished by extraction with ethyl acetate, **25** being soluble in the organic solvent. Recycling of **25** allows the progressive conversion of all the D-starting material into the L-*allo*-derivative, by successive epimerization and resolution steps. Conversion of **28** into the desired Boc derivative **29** was again done using a standard protocol.[20]

Enzymatic hydrolysis of *N*-acetylated-*O*-*tert*-butyl protected Thr derivatives was too slow to be useful for the separation of the 38:62 mixture of Ac-L-Thr(*t*Bu)-OH **9** and Ac-D-*allo*-Thr(*t*Bu)-OH **19**, produced on acidolytic opening of oxazolone **15**. These products were separated by reversed-phase medium pressure chromatography, as outlined in the Experimental section. Conversion of Ac-D-*allo*-Thr(*t*Bu)-OH **19** into the Fmoc N^{α}-protected derivative, however, is not possible owing to the lability of the *tert*-butyl ether. Removal of the chloroacetyl group from ClAc-L-Thr(*t*Bu)-OH **11** could, on the other hand, be done in almost quantitative yield, following the method described by Masaki[22] and provides the basis for a synthesis of Fmoc-protected *allo*-threonines. The poor susceptibility of the chloroacetylated L-Thr derivative **11** to undergo oxazolone formation and epimerization, under the conditions used, precluded the realization of such a route in the present work.

The purity of the *allo*-threonine derivatives was checked by analytical HPLC, either using standard operating conditions or the method reported by Buck and Krummen.[23] This latter procedure 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.

CONCLUSIONS

The procedures described here provide simple access to the protected derivatives of the non-proteinogenic *allo* threonines required for Boc/Bzl-SPPS. Such protected derivatives can also be used in peptide synthesis in solution. The methods are operationally simple and in each case, conversion of the commercially available starting material, **5** or **23**, into the desired *allo* product **22** or **29** can be achieved in high overall yield by recycling. As reported here, synthesis has been carried out with hundreds of milligrams of material but further scale up should be possible, in principle.

EXPERIMENTAL

All organic solutions were dried over sodium sulfate. Chemical shifts are quoted in δ values downfield from tetramethylsilane and *J* values are given in Hz. All melting points are uncorrected. HPLC was performed using a Shimadzu apparatus and a Vydac C18 column (25 x 0.4 cm, 10 μm, 120 Å).

N-Acetyl-L-threonine **7** L-Threonine (2.01 g, 16.8 mmol) was suspended in glacial acetic acid (25 cm^3) and acetic anhydride (2 cm^3, 20.16 mmol). The suspension was heated to 40 °C and stirred for 15 hours, after which time a clear solution had formed. The solvent was removed by high vacuum rotatory evaporation and the residue was dissolved in acetone (5 cm^3) and filtered. Rotatory evaporation of the filtrate, chasing with toluene, gave a greenish oil that was crystallized by dissolving it in acetone, adding hexane until turbidity and cooling to 4 °C. The product was formed as a white solid (1.79 g, 66%), m.p.118-119 °C, which had: $[\alpha]_D$ +1.5 (*c* 1, H_2O); δ_H (200 MHz, CD_3OD) 1.27 (3H, d, *J* 6.6), 2.14 (3H, s), 4.39 (1H, qd, J_1 6.4, J_2 3.2), 4.51 (1H, d, *J* 2.8); δ_C (50 MHz, CD_3OD) 20.41, 22. 43, 59.21, 68.34, 173.71, 173.83; ν_{max} (KBr disk)/cm^{-1} 3402, 3330, 3050, 2979, 1742, 1652, 1530, 1312, 1202, 1153, 1090, 1153, 1202, 1012; *m/z* (CI) 179, [(*M*+NH_4)$^+$, 100], 162 [(*M*+H)$^+$, 24]. (Found: C, 44.51; H, 7.41; N, 8.37. Calc. for $C_6H_{11}NO_4$: C, 44.72; H, 6.83; N, 8.70).

N-Acetyl-O-benzyl-L-threonine **8** *O*-Benzyl-L-threonine (6.02 g, 28.7 mmol) was dissolved in 2M sodium hydroxide solution (14.35 cm^3, 28.7 mmol) at 0 °C and acetic anhydride (4.07 cm^3, 43.05 mmol) and 2M sodium hydroxide solution (21.25 cm^3, 43.05 mmol) were added alternately, each in six separate portions at 10 min intervals. After addition was complete, the reaction was stirred at 0 °C for 90 min and a pH of 8-9 (indicator paper) was maintained by addition of 2M sodium hydroxide solution, as necessary. The solution was adjusted to pH 2 (indicator paper) by addition of concentrated hydrochloric acid and the white precipitate formed was recovered by filtration and washed with 10% hydrochloric acid. The filtrate was extracted with ethyl acetate (3 x 10 cm^3) and the combined extracts dried, filtered and subjected to solvent removal, affording a white solid. These two solids were combined and dried in an oven at 50 °C. The product (6.57 g, 91%), m.p.180-182 °C had: $[\alpha]_D$ +33.0 (*c* 1, MeOH); δ_H (200 MHz, $CDCl_3$) 1.22 (3H, d, *J* 6.2), 2.07 (3H, s), 4.21 (1H, qd, J_1 6.2, J_2 2.2), 4.51 (2H, ABq, *J* 11.8), 4.69 (1H dd, J_1 8.9, J_2 2.1), 6.47 (1H, d, *J* 8.8), 7.28-7.31 5H, m), 8.68 (1H, bs); δ_C (50 MHz, $CDCl_3$) 16.35, 22.92, 56.55, 71.30, 74.33, 77.00, 127.79, 127.90, 128.39, 137.45, 171, 57, 173.59; ν_{max} (KBr disk)/cm^{-1} 3350, 2977, 2879, 1706, 1629, 1538, 1235, 1132, 1088, 1050; *m/z* (CI, CH_4) 252[(*M*+H)$^+$, 100]. (Found: C, 61.75; H, 7.26; N, 5.56. Calc. for $C_{13}H_{17}NO_4$: C, 62.15; H, 6.77; N, 5.58). HPLC analysis (gradient elution starting from 12.5% B in A, taken to 70% B in A over 20 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA; flow rate of 1 cm^3min^{-1}; detection by U/V absorbance at 220 nm) showed a single peak with a retention time of 12.4 min.

N-Acetyl-O-tert-butyl-L-threonine **9** *O-tert*-Butyl-L-threonine (2 g, 11.4 mmol) was dissolved in 2M sodium hydroxide solution (5.71 cm^3, 11.4 mmol) at 0 °C, and acetic anhydride (1.62 cm^3, 17.1 mmol) and 2M sodium hydroxide solution (8.35 cm^3, 17.1 mmol) were added alternately, each in six separate portions at 10 min intervals. After addition was complete, the reaction was stirred at 0 °C for 90 min and a pH of 8-9 (indicator paper) was maintained by addition of 2M sodium hydroxide solution, as necessary. The solution was adjusted

to pH 5-6 (indicator paper) by addition of 10% hydrochloric acid and the solution was extracted with ethyl acetate (4 x 5 cm^3) and the combined extracts dried, filtered and subjected to solvent removal. The product was obtained as a white solid (2.23 g, 90%), m.p.132-133 °C. It had: $[\alpha]_D$ +84.8 (*c* 1, $CHCl_3$); δ_H (200 MHz, $CDCl_3$) 1.15 (3H, d, *J* 6.2), 1.25 (9H, s), 2.09 (3H, s), 4.33 (1H, qd, J_1 6.6, J_2 3.4), 4.52 (1H, dd, J_1 7.4, J_2 3.2), 6.52 (1H, d, *J* 7.2), 8.88 (1H, bs); δ_C (50 MHz, $CDCl_3$) 19.30, 22.86, 28.09, 57.40, 66.70, 75.51, 171.32, 172.92; ν_{max} (KBr disk)/cm^{-1} 3346, 2976, 1727, 1615, 1534, 1380, 1283, 1212, 1086; *m/z* (CI) 218 $[M+H)^+$, 32], 162 (100). (Found: C, 54.90; H, 8.61; N, 6.42. Calc. for $C_{10}H_{19}NO_4$: C, 55.30; H, 8.75; N, 6.45). HPLC analysis (gradient elution starting from 12.5% B in A, taken to 70% B in A over 20 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA; flow rate of 1 cm^3min^{-1}; detection by U/V absorbance at 220 nm) showed a single peak with a retention time of 9.6 min.

N-Chloroacetyl-O-benzyl-L-threonine **10** *O*-Benzyl-L-threonine (1.60 g, 7.66 mmol) was suspended in dry acetonitrile (30 cm^3) and chloroacetyl chloride (0.55 cm^3, 6.89 mmol) was added. The suspension was placed under nitrogen atmosphere, heated to 40 °C and stirred for 15 hours, after which time a clear solution resulted. The solvent was removed by high vacuum rotatory evaporation and the residue was dissolved in chloroform (5 cm^3) and washed with 1M sodium hydrogensulfate solution (2 x 15 cm^3). Drying, filtration and solvent removal gave a white solid (1.77 g, 93%), m.p.72-73 °C. It had: $[\alpha]_D$ +29.2 (*c* 1, $CHCl_3$); δ_H (200 MHz, $CDCl_3$) 1.25 (3H, d, *J* 6.4), 4.14 (2H, s), 4.24 (1H, qd, J_1 6.4, J_2 2.2), 4.55 (2H, ABq, *J* 11.4), 4.66 (1H, dd, J_1 8.8, J_2 2.6), 7.29-7.35 (5H, m); δ_C (50 MHz, $CDCl_3$) 16.28, 42.26, 56.95, 70.94, 73.74, 127.60, 127.81, 128.30, 137.31, 167.62, 174.01; ν_{max} (KBr disk)/cm^{-1} 3400, 2939, 2620, 1629, 1546, 1729, 1414, 1240, 1088; *m/z* (CI) 288 $[(M+H)^+$, 21], 286 $[(M+H)^+$, 100]. (Found: C, 54.16; H, 6.00; Cl, 12.90; N, 4.85. Calc. for $C_{13}H_{16}ClNO_4$: C, 54.64; H, 5.60; Cl, 12.43; N, 4.90). HPLC analysis (gradient elution starting from 12.5% B in A, taken to 70% B in A over 20 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA; flow rate of 1 cm^3min^{-1}; detection by U/V absorbance at 220 nm) showed a single peak with a retention time of 15.1 min.

N-Chloroacetyl-O-tert-butyl-L-threonine **11** *O-tert*-Butyl-L-threonine (1.26 g, 7.21 mmol) was suspended in a 1:1 mixture of acetone-acetonitrile (40 cm^3) at -78 °C and chloroacetyl chloride (0.55 cm^3, 6.89 mmol) was added. The suspension was placed under nitrogen atmosphere, and the mixture allowed to come to room temperature over 120 min. The solvent was removed by high vacuum rotatory evaporation and the solid residue was dissolved in chloroform (5 cm^3) and washed with 1M sodium hydrogensulfate solution (2 x 15 cm^3). Drying, filtration and solvent removal gave a white solid (1.01 g, 59%), m.p.102-103 °C. It had: $[\alpha]_D$ +66.3 (*c* 1, $CHCl_3$); δ_H (200 MHz, $CDCl_3$) 1.18 (3H, d, *J* 6.3), 1.25 (9H, s), 4.12 (2H, s), 4.35 (1H, qd, J_1 6.4, J_2 3.2), 4.48 (1H, dd, J_1 7.3, J_2 3.1), 7.39 (1H, d, *J* 7.2), 8.02 (1H, bs); δ_C (50 MHz, $CDCl_3$) 19.21, 28.09, 42.32, 57.60, 66.36, 75.93, 166.93, 172.59; ν_{max} (KBr disk)/cm^{-1} 3398, 2981, 1750, 1632, 1546, 1405, 1200 1076; *m/z* (CI) 254 $[(M+H)^+$, 16], 252 $[(M+H)^+$, 43], 196 (100). (Found: C, 47.40; H, 7.16; N, 5.62. Calc. for $C_{10}H_{18}ClNO_4$: C, 47.71; H, 7.16; N, 5.57). HPLC analysis (gradient elution starting from 12.5% B in A, taken to 70% B in A over 20 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA; flow rate of 1 cm^3min^{-1}; detection by U/V absorbance at 220 nm) showed a single peak with a retention time of 12.9 min.

N-Chloroacetyl-L-threonine **12** *N*-Chloroacetyl-*O-tert*-butyl-L-threonine **11** (0.10 g, 0.39 mmol) was dissolved in trifluoroacetic acid (1 cm^3) at 0 °C. After stirring for 90 min at 0 °C, the solvent was removed by high vacuum rotatory evaporation, leaving a reddish oil (0.08 g). The crude product had: δ_H [200 MHz, $(CD_3)_2CO$] 1.23 (3H, d, *J* 6.6), 4.29 (2H, s), 4.44 (1H, qd, J_1 6.2, J_2 3.1), 4.52 (1H, d, *J* 7.6); δ_C [50 MHz, $(CD_3)_2CO$] 20.52, 29.80, 58.44, 67.74, 167.21, 172.03; *m/z* (CI) 215 [$(M+NH_4)^+$, 30], 213 [$(M+H)^+$, 100]. Attempts to purify this product by crystallization or chromatography led to its decomposition.

4-[1-(R)-Hydroxyethyl]-2-methyl-4(H)-(RS)-oxazol-5-one **13** A suspension of *N*-acetyl-L-threonine **7** (0.021 g, 0.13 mmol) and *N*-ethyl-*N'*-3-dimethylaminopropyl carbodiimide (0.025 g, 0.13 mmol) was suspended in deuterated chloroform (0.5 cm^3) and stirred for 30 min. The resulting solution was washed successively with water (1 cm^3), 5% sodium hydrogen carbonate solution (1 cm^3) and water (1 cm^3) at 0 °C. The organic phase was dried and the solvent removed giving a clear oil (0.015 g, 90%) whose spectroscopic data were consistent with those expected for an approximately 50(S):50(R) mixture of epimers at C-4 of the oxazolone **13**. It had: δ_H (200 MHz, $CDCl_3$) 1.32 (3H, d, *J* 7.0), 1.35 (3H, d *J* 6.6), 2.18 (3H, d, *J* 1.8), 2.19 (3H, d, *J* 2.0), 4.05-4.11 (2H, m), 4.15-4.41 (2H, m); ν_{max} (MeCN)/cm^{-1} 3350, 2988, 1827, 1720, 1690, 1500, 1240, 1100, 1045. Attempts to purify the products, by crystallization or chromatography, led to their decomposition.

4-[1-(R)-tert-Butoxyethyl]-2-methyl-4(H)-(S/R)-oxazol-5-one **15** A solution of *N*-acetyl-*O-tert*-butyl-L-threonine **9** (1.16 g, 5.33 mmol) and *N*-ethyl-*N'*-3-dimethylaminopropyl carbodiimide (1.35 g, 7.04 mmol) in dichloromethane (40 cm^3) was stirred at 0 °C for 120 min. The resulting solution was washed successively with water (15 cm^3), 5% sodium hydrogen carbonate solution (15 cm^3) and water (15 cm^3) at 0 °C. The organic phase was dried and the solvent removed giving a clear oil (0.79 g, 75%) whose spectroscopic data were consistent with those expected for a 41(S):59(R) mixture of epimers at C-4 of the oxazolone **14**. It had: δ_H (300 MHz, $CDCl_3$) 1.15 (9H, s), 1.16 (9H, s), 1.29 (3H, d, *J* 6.3), 1.37 (3H, d, *J* 6.3), 2.22 (3H, d, *J* 1.8), 2.24 (3H, d, *J* 2.1), 4.05-4.09 (2H, m), 4.13 (1H, m), 4.22 (1H, qd, J_1 6.3, J_2 2.8); δ_C (75 MHz, $CDCl_3$) 14.96, 15.15, 18.81, 20.59, 28.31, 28.33, 66.55, 67.26, 70.37, 71.47, 74.10, 74.33, 163.26, 163.38, 176.23, 177.33; ν_{max} (CH_2Cl_2)/cm^{-1} 2979, 2937, 1831, 1686, 1438, 1370, 1119, 1070; *m/z* (CI) 217 [$(M+NH_4)^+$, 86], 200[$(M+H)^+$, 100].

A similar procedure, but using only 1 equivalent of carbodiimide and a reaction time of 2-3 min, gave a product whose spectroscopic data were consistent with the presence of only one diastereoisomer, *4-[1-(R)-tert-butoxyethyl]-2-methyl-4(H)-(R)-oxazol-5-one* **15**. Attempts to purify the products, by crystallization or chromatography, led to their decomposition.

Mixture of N-acetyl-O-tert-butyl-L-threonine **9** *and N-acetyl-O-tert-butyl-D-allo-threonine* **19** 0.01 M Hydrochloric acid (10 cm^3) was added to the epimeric oxazolones **15** (0.70 g, 3.52 mmol) and the mixture stirred for 60 min at 0 °C. After extraction with ethyl acetate (4 x 5 cm^3) the combined extracts were dried. Filtration and solvent removal gave a white solid (0.59 g, 77%) whose physical data were consistent with it being a 38:62 mixture of **8** and **18**. It had: δ_H (200 MHz, $CDCl_3$) 1.14 (3H, d, *J* 6.6), 1.20 (9H, s), 1.26 (3H, d, *J* 6.6), 1.27 (9H, s), 2.07 (3H, s), 2.09 (3H, s), 4.07 (1H, qd, J_1 6.5, J_2 3.9), 4.35 (1H, qd, J_1 6.5, J_2 3.5), 4.51 (1H, dd, J_1 7.0, J_2 3.6), 4.61 (1H, dd, J_1 7.7, J_2 3.7), 6.42 (1H, d,*J* 8.0), 6.46 (1H, d, *J* 7.2), 9.17 (2H, bs); δ_C (50 MHz, $CDCl_3$) 19.21, 22.88, 28.09, 57.40, 57.62, 66.65, 67.81, 74.64, 75.62, 170.95,

171.35, 172.65, 172.79. HPLC analysis (gradient elution starting from 12.5% B in A, taken to 70% B in A over 20 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA; flow rate of 1 cm^3min^{-1}; detection by U/V absorbance at 220 nm) showed two peaks with retention times of 8.9 min and 10.4 min.

4-[1-(R)-Benzoxyethyl]-2-methyl-4(H)-(SR)-oxazol-5-one **14** A solution of *N*-acetyl-*O*-benzyl-L-threonine **8** (1.01 g, 3.98 mmol) and *N*-ethyl-*N'*-3-dimethylaminopropyl carbodiimide (0.99 g, 5.18 mmol) in dichloromethane (30 cm^3) was stirred at 0 °C for 120 min. The resulting solution was washed successively with water (15 cm^3), 5% sodium hydrogen carbonate solution (15 cm^3) and water (15 cm^3) at 0 °C. The organic phase was dried and the solvent removed giving a clear oil (0.83 g, 90%) whose spectroscopic data were consistent with those expected for a 54(S):46(R) mixture of epimers at C-4 of the oxazolone **14**. It had: δ_H (200 MHz, $CDCl_3$) 1.36 (3H, *J* 6.5), 1.40 (3H, *J* 6.3), 2.19 (3H, d, *J* 2.0), 2.22 (3H, d *J* 1.9), 3.97-4.11 (2H, m), 4.14 (1H, m), 4.18 (1H, m), 4.43-4.68 (4H, m), 7.30 (10, m); δ_C (50 MHz, $CDCl_3$) 15.08, 15.29, 15.68, 16.54, 69.23, 70.19, 71.30, 71.58, 73.44, 74.21, 127.33, 127.66, 128.27, 137.58, 137.95; ν_{max} (CH_2Cl_2)/cm^{-1} 3348, 3000, 1829, 1685, 1500, 1240, 1100, 1045.

A similar procedure, but using only 1 equivalent of carbodiimide and a reaction time of 2-3 min, gave a product whose spectroscopic data were consistent with the presence of only one diastereoisomer, *4-[1-(R)-benzoxyethyl]-2-methyl-4(H)-(S)-oxazol-5-one* **14**. Attempts to purify the products, by crystallization or chromatography, led to their decomposition.

Mixture of N-acetyl-O-benzyl-L-threonine **8** *and N-acetyl-O-benzyl-D-allo-threonine* **18** 0.01 M Hydrochloric acid (10 cm^3) was added to the epimeric oxazolones **14** (0.75 g, 3.22 mmol) and the mixture stirred for 120 min at 0 °C. After extraction with ethyl acetate (4 x 5 cm^3) the combined extracts were dried. Filtration and solvent removal gave a white solid (0.48 g, 60%) whose physical data were consistent with it being a 56:44 mixture of **8** and **18**. It had: δ_H (200 MHz, $CDCl_3$) 1.21 (3H, d, *J* 6.6), 1.28 (3H, d, *J* 6.6), 1.97 (3H, s), 2.06 (3H, s), 3.90 (1H, qd, J_1 6.4, J_2 3.4), 4.21 (1H, qd, J_1 6.2, J_2 2.2), 4.40-4.61 (4H, m), 4.69 (1H, dd, J_1 9.2, J_2 2.2), 4.82 (1H, dd, J_1 8.3, J_2 3.5), 6.50-6.58 (2H, m), 7.23-7.33 (10H, m), 10.04 (bs, 2H); δ_C (50 MHz, $CDCl_3$) 16.13, 16.37, 22.77, 55.36, 56.58, 70.94, 71.25, 74.40, 74.67, 127.75, 127.95, 120.02, 128.39, 137.45, 137.71, 171.24, 171.84, 172.65, 173.45; *m/z* (CI) 252 [$(M+H)^+$, 100]. HPLC analysis (isocratic elution with 20% B in A, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA; flow rate of 1 cm^3min^{-1}; detection by U/V absorbance at 220 nm) showed two peaks having retention times of 12.4 min and 15.5 min.

N-Acetyl-O-benzyl-D-allo-threonine **18** A 57:43 mixture of Ac-L-Thr(Bzl)-OH **7** and Ac-D-*allo*-Thr(Bzl)-OH **11** (540 mg) was suspended in water (55 cm^3) and the pH taken to 8 (indicator paper) by dropwise addition of 1M lithium hydroxide solution. 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 lithium hydroxide solution. 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 (gradient elution starting from 25% B in A, taken over 20 min to 35% B in A, where A is water/0.045% TFA and B is acetonitrile/0.036% TFA, at a flow rate of 1 cm^3min^{-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 acetic acid solution at a flow rate of 23 cm^3h^{-1}. The relevant fractions were combined (total volume 140 cm^3), taken to pH 2-3 (indicator paper) by the addition of 5% hydrochloric acid and extracted with ethyl acetate (5 x 20 cm^3). The combined extracts were washed with water (5 x 20 cm^3) and dried. Filtration, followed by solvent removal gave Ac-D-*allo*-Thr(Bzl)-OH **11** as a white solid (170 mg, 73 %), m.p.185-187 °C. It had: $[\alpha]_D$ -12.4 (*c* 1.5, MeOH); δ_H (200 MHz, CD_3OD) 1.23 (3H, d, *J* 6.2), 2.01 (3H, s), 3.95 (1H, m), 4.56 (2H, ABq, *J* 11.5), 4.82, (1H, d, *J* 4.6), 7.29-7.35 (5H, m); δ_C (50 MHz, CD_3OD) 56.81, 72.24, 76.09, 128.99, 129.30, 129.63, 139.04, 173.65, 173.70; ν_{max} (KBr disk)/cm^{-1} 3434, 2909, 2369, 1713, 1620, 1551, 1265, 1111, 1026; *m/z* (CI) 269 [(*M*+NH_4)$^+$, 100], [(*M*+H)$^+$, 52]. (Found: C, 61.73; H, 6.87; N, 5.71. Calc. for $C_{13}H_{17}NO_4$: C, 62.15; H, 6.77; N, 5.58). HPLC analysis (isocratic elution with 20% B in A, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA; flow rate of 1 cm^3min^{-1}; detection by U/V absorbance at 220 nm) showed a single peak with a retention time of 12.4 min.

O-Benzyl-D-allo-threonine **21** A suspension of *N*-acetyl-*O*-benzyl-D-*allo*-threonine **18** (230 mg, 0.92 mmol) in 1M hydrochloric acid (5.5 cm^3, 5.52 mmol) was refluxed for 150 min. Best yields were obtained on pre-heating the oil bath to approx. 150 °C, prior to commencing reflux. After cooling the resulting solution was washed with chloroform (2 x 5 cm^3) and the aqueous phase was submitted to high vacuum rotatory evaporation, giving the hydrochloride of the product as a white solid (219 mg, 97%), m.p.250-255 °C. It had: $[\alpha]_D$ -4.0 (*c* 0.7, H_2O); δ_H (200 MHz, D_2O) 1.12 (3H, d, *J* 6.4), 4.25 (1H, m), 4.15 (1H, d, *J* 3.4), 4.48 (2H, s), 7.26 (5H, s); δ_C (50 MHz, D_2O) 17.29, 58.90, 73.81, 75.19, 131.31, 131.35, 131.66, 139.74, 172.49; ν_{max} (KBr disk)/cm^{-1} 3429, 2981, 1735, 1225, 1086, 1067; *m/z* (CI) 227 [(*M*+NH_4)$^+$, 100], 210 [(*M*+H)$^+$, 44]. HPLC analysis (isocratic elution with 20% B in A, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA; flow rate of 1 cm^3min^{-1}; detection by U/V absorbance at 220 nm) showed a single peak with a retention time of 5.0 min.

N-tert-Butoxycarbonyl-O-benzyl-D-allo-threonine **22** *tert*-Butylpyrocarbonate (78 mg, 0.36 mmol) was added to solution of *O*-benzyl-D-*allo*-threonine **21** (68 mg, 0.32 mmol), in *tert*-butanol (6.5 cm^3) and 1M sodium hydroxide (3.2 cm^3, 0.32 mmol), at 0 °C. The mixture was stirred for 16 h, during which time it was allowed to reach room temperature. The mixture was extracted with hexanes (2 x 5 cm^3), acidified to pH 3 (indicator paper) by addition of 5% hydrochloric acid and extracted with ethyl acetate (4 x 5 cm^3). The ethyl acetate fractions were combined and washed with brine (2 x 5 cm^3). Drying, followed by filtration and solvent removal gave the product as a white solid, (74 mg, 74%), m.p.113-117 °C. It had: $[\alpha]_D$ -17.9 (*c* 1, CH_2Cl_2); δ_H (300 MHz, $CDCl_3$) 1.21 (3H, d, *J* 6.6), 1.38 (9H, s), 3.85 (1H, m), 4.27 (1H, m), 4.52 (2H, s), 5.27 (1H, d, *J* 8.4), 7.22-7.27 (5H, m); δ_C (75 MHz, $CDCl_3$) 16.06, 28.23, 56.65, 70.94, 74.88, 80.19, 127.66, 127.72, 128.34, 137.76, 155.59, 174.65; ν_{max} (KBr disk)/cm^{-1} 3448, 3934, 1734, 1653, 1512, 1420, 1375, 1204, 1165, 1061; *m/z* (CI) 327 [(*M*+NH_4)$^+$, 40], 310 [(*M*+H)$^+$, 54]. (Found: C, 62.05; H, 7.63; N, 4.73. Calc. for $C_{16}H_{23}NO_5$: C, 62.14; H, 7.44; N, 4.53). HPLC analysis (gradient elution starting from 12.5% B in A, taken to 70% B in A over 20 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA; flow rate of 1 cm^3min^{-1}; detection by U/V absorbance at 220 nm) showed a single peak with a retention time of 19.3 min.

N-Acetyl-O-benzyl-D-threonine benzyl ester **24** *O*-Benzyl-D-threonine benzyl ester hemioxalate (1.5 g, 3.85 mmol) was dissolved in 1M sodium hydroxide solution (11.56 cm^3, 11.56 mmol) at 0 °C and acetic anhydride (0.17 cm^3, 1.79 mmol) and 1M sodium hydroxide solution (0.76 cm^3, 0.76 mmol) were added alternately, each in six separate portions at 10 min intervals. After addition was complete, the reaction was stirred at 0 °C for 90 min and a pH of 8-9 (indicator paper) was maintained by addition of 1M sodium hydroxide solution, as necessary. The solution was adjusted to pH 2 (indicator paper) by addition of 5% hydrochloric acid and was extracted with ethyl acetate (3 x 10 cm^3). The combined extracts were dried, filtered and subjected to solvent removal, affording the product as a pale yellow oil (1.24 g, 94%), which was used without further purification. It had: $[\alpha]_D$ +16.0 (*c* 1, CH_2Cl_2); δ_H (200 MHz, $CDCl_3$) 1.21 (3H, d, J 6.2), 2.07 (3H, s), 4.16 (1H, qd, J_1 6.2, J_2 2.2), 4.34 (2H, ABq, *J* 11.6), 4.75 (1H, dd, J_1 9.2, J_2 2.2), 5.11 (2H ABq, *J* 11.8), 6.4 (1H, d, *J* 9.2), 7.15-7.38 (10H, m); δ_C (50 MHz, $CDCl_3$) 16.82, 23.58, 57.24, 67.76, 71.42, 74.96, 128.25, 128.36, 128.88, 128.94, 128.97, 129.08, 135.69, 138.01, 171.56, 171.93; ν_{max} (film)/cm^{-1} 3303, 3033, 2981, 1748, 1663, 1499, 1454, 1379, 1314, 1271, 1209, 1157, 1092, 1028; *m/z* (CI) 359 [$(M+NH_4)^+$, 100], 342 [$(M+H)^+$, 31]; (HRFABMS) Found: 342.1691. $C_{20}H_{24}NO_4$ requires: 342.1705. HPLC analysis (isocratic elution with 50% B in A, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA; flow rate of 1 cm^3min^{-1}; detection by U/V absorbance at 220 nm) showed a single peak with a retention time of 9.6 min.

N-Acetyl-O-benzyl-D-threonine **25** *N*-Acetyl-*O*-benzyl-D-threonine benzyl ester **24** (1.24 g, 3.62 mmol) was stirred in 4M sodium hydroxide (18 cm^3, 72.4 mmol) at 0 °C. The suspension was allowed to come to room temperature, as the ice-water bath melted, and stirring was continued until no solid remained (4 h). The resulting solution was taken to pH 2 (indicator paper) by addition of 5% hydrochloric acid and was extracted with ethyl acetate (4 x 5 cm^3). The combined extracts were dried, filtered and subjected to solvent removal, gaving a yellow oil. This was taken up in ethyl acetate (10 cm^3) and the solution washed with 5% aqueous sodium bicarbonate (3 x 5 cm^3). The combined aqueous phases were acidified to pH 2 (indicator paper) and extracted with ethyl acetate (4 x 5 cm^3). The combined organic extracts were dried and filtered. Solvent removal gave the product as a white solid (0.84 g, 92%), m.p.184-186 °C. It had: $[\alpha]_D$ -32.5 (*c* 1, MeOH); δ_H (200 MHz, $CDCl_3$) 1.19 (3H, d, *J* 6.6), 2.03 (3H, s), 4.19 (1H, qd, J_1 6.6, J_2 2.5), 4.48 (2H, ABq, *J* 11.4), 4.63 (1H, dd, J_1 9.2, J_2 2.5), 6.11 (1H, bs), 6.48 (1H, d, J 9.2), 7.27-7.34 (5H, m); δ_C (50 MHz, $CDCl_3$) 16.92, 23.38, 57.11, 71.83, 75.04, 128.43, 128.94, 128.01, 138.04, 172.28, 174.03; ν_{max} (KBr disk)/cm^{-1} 3349, 2878, 1705, 1624, 1539, 1381, 1317, 1232, 1132, 1088, 1051; *m/z* (CI) 269 [$(M+NH_4)^+$, 100], 252 [$(M+H)^+$, 40]. (Found: C, 61.84; H, 6.96; N, 5.72. Calc. for $C_{13}H_{17}NO_4$: C, 62.15; H, 6.77; N, 5.58). HPLC analysis (isocratic elution with 20% B in A, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA; flow rate of 1 cm^3min^{-1}; detection by U/V absorbance at 220 nm) showed a single peak with a retention time of 15.8 min.

4-[1-(S)-Benzoxyethyl]-2-methyl-4(H)-(SR)-oxazol-5-one **26** A solution of *N*-acetyl-*O*-benzyl-D-threonine **25** (200 mg, 0.79 mmol) and *N*-ethyl-*N'*-3-dimethylaminopropyl carbodiimide (153 mg, 0.80 mmol) in dichloromethane (10 cm^3) was stirred at 0 °C for 30 min. The resulting solution was washed successively with water (10 cm^3), 5% sodium hydrogen carbonate solution (10 cm^3) and water (10 cm^3) at 0 °C. The organic phase was dried and the solvent removed giving a clear oil (180 mg, 97%) whose spectroscopic data were

consistent with those expected for a 43(S):57(R) mixture of epimers at C-4 of the oxazolone **26**. It had: δ_H (200 MHz, $CDCl_3$) 1.34 (3H, d, *J* 6.2), 1.38 (3H, d, *J* 6.2), 2.19 (3H, d, *J* 1.8), 2.22 (3H, d, *J* 1.8), 3.99 (1H, m), 4.05 (1H, m), 4.12 (1H, m), 4.16 (1H, m), 4.21-4.61 (4H, m),, 7.29-7.37 (10H, m); δ_C (50 MHz, $CDCl_3$) 13.35, 14.10, 14.70, 15.56, 64.28, 68.28, 68.20, 69.17, 70.31, 70.58, 72.42, 73.19, 125.94, 126.36, 126.56, 126.68, 127.30, 127.49, 137.44, 139.91, 162.24, 164.35, 175.29, 176.11.

Mixture of N-acetyl-O-benzyl-D-threonine **25** *and N-acetyl-O-benzyl-L-allo-threonine* **27** 10% Citric acid (20 cm^3) was added to the epimeric oxazolones **26** (180 mg, 7.73 mmol) and the mixture stirred for 30 min at 0 °C. After extraction with ethyl acetate (4 x 5 cm^3) the combined extracts were dried. Filtration and solvent removal gave a white solid (160 mg, 82%) whose physical data were consistent with it being a 57:43 mixture of **25** and **27**. It had: δ_H (200 MHz, CD_3OD) 1.22 (3H, d, *J* 6.2), 1.25 (3H, d, *J* 6.4), 1.99 (3H, s), 2.03 (3H, s), 3.97 (1H, m), 4.2 (1H, qd, J_1 6.2, J_2 2.6), 4.42-4.65 (4H, m), 4.62 (1H, d, *J* 2.6), 4. 83 (1H, d, *J* 4.6), 7.31-7.33 (10H, m); δ_C (50 MHz, CD_3OD) 16.81, 17.03, 22.83, 22.91, 56.86, 58.50, 72.24, 72.58, 76.15, 76.29, 129.05, 129.12, 129.28, 129.34, 129.67, 129.71, 139.85, 139.88, 173.63, 173.74, 174.02, 174.20. HPLC analysis (isocratic elution with 20% B in A, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA; flow rate of 1 cm^3min^{-1}; detection by U/V absorbance at 220 nm) showed two peaks having retention times of 12.3 min and 15.3 min.

O-Benzyl-L-allo-threonine **28** A 57:43 mixture of Ac-D-Thr(Bzl)-OH **25** and Ac-L-*allo*-Thr(Bzl)-OH **27** (277 mg) was suspended in water (27 cm^3) and the pH taken to 8 (indicator paper) by dropwise addition of 1M lithium hydroxide solution. Hog kidney acylase I [Fluka, Activity 2000-3000 Umg^{-1}, (103 mg)] was added, and the mixture placed under argon and stirred vigorously at 38 °C. The pH of the medium was checked daily and, if necessary, adjusted to 8 (indicator paper) by adding 1M lithium hydroxide solution. HPLC analysis (gradient elution starting from 25% B in A, taken over 20 min to 35% B in A, where A is water/0.045% TFA and B is acetonitrile/0.036% TFA, at a flow rate of 1 cm^3min^{-1}; detection by ultraviolet absorption at 220 nm) indicated that hydrolysis was complete after three days. The mixture was centrifuged and the supernatant subjected to gel filtration on Sephadex G-10, eluting with 0.1 M acetic acid solution at a flow rate of 23 cm^3h^{-1}. The relevant fractions were combined (total volume 100 cm^3), taken to pH 2-3 (indicator paper) by the addition of 5% hydochloric acid and extracted with ethyl acetate (5 x 20 cm^3). The organic combined extracts were washed with water (5 x 20 cm^3) and dried. The combined aqueous phases were subjected to high vacuum rotatory evaporation affording the hydrochloride of the product as a white solid (68 mg, 83%), m.p. 225-230 °C, that was used without further purification. It had: $[\alpha]_D$ +0.7 (*c* 0.7, H_2O); δ_H (200 MHz, D_2O) 1.12 (3H, d, *J* 6.6), 3.49 (1H, d, *J* 3.9), 4.02 (1H, qd J_1 6.6, J_2 4.0), 4.41 (2H, ABq, *J* 11.6), 7.23 (5H, m); δ_C (50 MHz, D_2O) 16.79, 59.78, 73.63, 75.68, 131.24, 131.26, 131.64, 139.97, 174.51; ν_{max} (KBr disk)/cm^{-1} 3432, 2902, 1636, 1522, 1404, 1383, 1360, 1329, 1119, 1030; *m/z* (CI) 227 [$(M+H)^+$, 100]. HPLC analysis (isocratic elution with 20% B in A, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA; flow rate of 1 cm^3min^{-1}; detection by U/V absorbance at 220 nm) showed a single peak having a retention time of 5.3 min.

N-tert-Butoxycarbonyl-O-benzyl-L-allo-threonine **29** *tert*-Butylpyrocarbonate (82 mg, 0.37 mmol) was added to solution of *O*-benzyl-L-*allo*-threonine **28** (72 mg, 0.34 mmol), in *tert*-butanol (6.5 cm^3) and 1M sodium hydroxide (3.4 cm^3, 0.34 mmol), at 0 °C. The mixture was stirred for 16 h, during which time it was allowed to reach room temperature. The mixture was extracted with hexanes (2 x 5 cm^3), acidified to pH 3 (indicator paper) by addition of 5% hydrochloric acid and extracted with ethyl acetate (4 x 5 cm^3). The ethyl acetate fractions were combined and washed with brine (2 x 5 cm^3). Drying, followed by filtration and solvent removal gave the product as a white solid, (94 mg, 88%), m.p.110-117 °C. It had: $[\alpha]_D$ +15.7 (*c* 1, CH_2Cl_2); δ_H (300 MHz, $CDCl_3$) 1.24 (3H, d, *J* 6.6), 1.41 (9H, s), 3.90 (1H, m), 4.36 (1H, m), 4.54 (2H, s), 5.27 (1H, d, *J* 8.4), 7.22-7.31 (5H, m), 8.79 (bs); δ_C (75 MHz, $CDCl_3$) 16.05, 28.24, 56.65, 70.93, 74.84, 80.18, 127.68, 127.75, 128.35, 137.75, 155.61, 174.76; ν_{max} (KBr disk)/cm^{-1} 2981, 2934, 2363, 1717, 1506, 1456, 1395, 1369, 1254, 1163, 1107, 1028; *m/z* (FAB) 209 [(*M*-Boc+H)$^+$, 100]. (Found: C, 61.31; H, 8.08; N, 4.93. Calc. for $C_{16}H_{23}NO_4$: C, 62.14; H, 7.44; N, 4.53). HPLC analysis (gradient elution starting from 12.5% B in A, taken to 70% B in A over 20 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA; flow rate of 1 cm^3min^{-1}; detection by U/V absorbance at 220 nm) showed a single peak with a retention time of 19.4 min.

ACKNOWLEDGEMENTS

We thank Josep Maria Caba for technical assistance. Financial support was provided by Ministerio de Educación y Ciencia, Madrid (Grant no PB95-1131) and Generalitat de Catalunya, Barcelona [Grup Consolidat (1995SGR 494) i Centre de Referència en Biotecnologia].

REFERENCES

1. Hamann, M. T., Scheuer P. J., *J. Am. Chem. Soc.*, **1993**, 115, 5825.
2. Rinehart, K. L., Holt, T. G., Fregeau, N. L., Keifer, P. A., Wilson, G. R., Perun, T. J., Sakai, R., Thompson, A. G., Stroh, J. G., Shield, L. S., Seigler, D. S., Li, L. H., Martin, D. G., Grimmelikhuijzen, C. J. P., Gäde, G., *J. Nat. Prod.*, **1990**, 53, 771.
3. Wipf, P., *Chem. Rev.*, **1996**, 95, 2115.
4. Goodman, M., Chorev, M., *Acc. Chem. Res.*, **1979**, 12, 1.
5. Chorev, M., Goodman, M., *Acc. Chem. Res.*, **1993**, 26, 266.
6. Pons, D., Savignac, M., Genet, J.-P., *Tetrahedron Lett.*, **1990**, 31, 5023.
7. Beaulieu, P. L., *Tetrahedron Lett.*, **1991**, 32, 1031.
8. Blaskovich, M. A., Lajoie, G. A., *Tetrahedron Lett.*, **1993**, 34, 3837.
9. Jackson, R. F. W., Palmer, N. J., Wythes, M. J., *Tetrahedron Lett.*, **1994**, 35, 7433.
10. Fischer, P. M., Sandosham, J., *Tetrahedron Lett.*, **1995**, 36, 5409.
11. Sánchez, Y. M., Haack, T., González, M. J., Ludevid, M. D., Giralt, E., In *Peptides. Chemistry, Structure and Biology. Proceedings of the 14th American Peptides Symposium*, Kaumaya, P.T.P., Hodges, R.S., Eds., Mayflower Scientific Ltd, Kingswinford, UK, 1996, p. 558.

12. Guichard, G., Muller, S., van Regenmortel, M., Briand, J. P., Mascagni, P., Giralt, E., *Trends in Biotechnology*, **1996**, 14, 44.
13. Lloyd-Williams, P., Albericio, F., Giralt, E., *Chemical Approaches to the Synthesis of Peptides and Proteins*, CRC Press, Boca Raton, FL, in press.
14. Bodanszky, M., Martinez, J., *Synthesis*, **1981**, 333.
15. Lloyd-Williams, P., Carulla, N., Giralt, E., *Tetrahedron Lett.*, in press.
16. Lloyd-Williams P., Monerris, P., Gonzalez, I., Jou, G., Giralt, E., *J. Chem. Soc., Perkin Trans. 1*, **1994**, 1969.
17. Jou, G., González, I., Albericio, F., Lloyd-Williams, P., Giralt, E., *J. Org. Chem.*, in press.
18. Greenstein, J. P., *Methods Enzymol.* **1957**, 3, 554.
19. Chen, F. M. F., Kuroda, K., Benoiton, N. L., *Synthesis*, **1979**, 230.
20. Bodanszky, M., Bodanszky, A., *The Practice of Peptide Synthesis*, Springer Verlag, Berlin, 1984.
21. Winitz, M., Bloch-Frankenthal, L., Izumiya, N., Birnbaum, S. M., Baker, C. G., Greenstein, J. P., *J. Am. Chem. Soc.*, **1956**, 78, 2423.
22. Masaki, M., Kitahara, T., Kurita, H., Ohta, M., *J. Am. Chem. Soc.*, **1968**, 90, 4508.
23. Buck, R. H., Krummen, K., *J. Chromatogr.*, **1987**, 387, 255.

(*Received in UK* 5 *December* 1996; *revised* 14 *January* 1997; *accepted* 16 *January* 1997)