Simple Synthesis of *cis*-4-Hydroxy-L-Proline and Derivatives Suitable for Use as Intermediates in Peptide Synthesis†

Dionissios Papaioannou,*[a] George Stavropoulos,[a] Kostas Karagiannis,[a] George W. Francis,[b] Trond Brekke[b] and Dagfinn W. Aksnes[b]

[a]Department of Chemistry, University of Patras, Patras, Greece and [b]Department of Chemistry, University of Bergen, N-5007 Bergen, Norway

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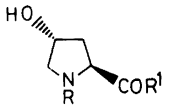
An intramolecular Mitsunobu reaction in the presence of triphenylphosphine – diethyl azodicarboxylate (TPP–DEAD) resulted in the conversion of *trans*-4-hydroxy-*N*-trityl-L-proline to 5-triphenylmethyl-2-oxa-5-aza-bicyclo[2.2.1]heptan-3-one. This bicyclic lactone proved to be a key intermediate in the synthesis of *cis*-4-hydroxy-L-proline and derivatives thereof. TPP–DEAD-catalysed methanolysis of the key lactone gave the methyl ester of *cis*-4-hydroxy-*N*-trityl-L-proline, while ammonolysis in isopropyl alcohol provided the corresponding amide. *p*-Toluenesulfonic acid treatment of the lactone, ester and amide led to detritylation and formation of the corresponding *p*-toluenesulfonates. Saponification of the key intermediate provided *cis*-4-hydroxy-*N*-trityl-L-proline which was first benzylated and then elaborated to the 1-hydroxybenzotriazolyl ester. This last ester and the three *p*-toluenesulfonates preparared above were utilised for the incorporation of *cis*-4-hydroxy-L-proline in the synthesis of model peptides.

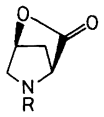
cis-4-Hydroxy-L-proline (cHyp, **1**) occurs in plants either in the free state or, as originally isolated,[2] bound within toxic cyclopeptides. The incorporation of **1** inhibits the formation of the natural triple-helix structure in collagen.[3,4] On the other hand replacement of L-proline (Pro) in the 7-position of arginine-vasopressin (AVP) by cHyp gave the analogue d[cHyp[7]]AVP which showed increased antidiuretic and uterine activity and a considerable decrease in pressor activity compared with AVP.[5]

These results prompted us to investigate the effect of replacing Pro by cHyp in a number of other biologically important peptides and this required the preparation of derivatives of **1** suitable for use in peptide synthesis. The structures of all of the compounds may be found in Scheme 1. To the best of our knowledge the only derivatives so far reported which allow the incorporation of cHyp into peptide chains are *cis*-*N*-tert-butyloxycarbonyl-4-hydroxy-L-proline (Boc-cHyp)[5] (**2**) and the *O*-benzylated derivative (**3**) thereof.[6] The derivatives **2** and **3** have been synthesised from cHyp which, although commercially available, was considered prohibitively expensive for use on a preparative scale.

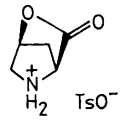
The present paper reports on short and simple syntheses



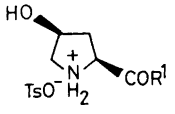
4 R = H; R¹ = OH
5 R = Trt; R¹ = OH
8 R = Trt; R¹ = OMe



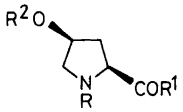
6 R = Trt
11 R = Trt–Leu
18 R = Boc



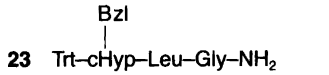
14



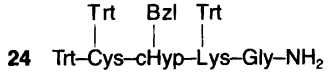
15 R¹ = OMe
16 R¹ = NH₂



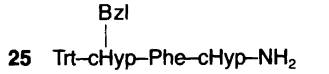
1 R = R² = H; R¹ = OH
2 R = Boc; R¹ = OH; R² = H
3 R = Boc; R¹ = OH; R² = Bzl
7 R = Trt; R¹ = OMe; R² = H
9 R = Trt; R¹ = OMe; R² = Ts
10 R = Trt; R¹ = NH₂; R² = H
12 R = Trt–Leu; R¹ = OMe; R² = H
13 R = Trt–Leu; R¹ = NH₂; R² = H
17 R = Trt; R¹ = OH; R² = H
19 R = Boc; R¹ = Gly–OBzl; R² = H
20 R = Trt; R¹ = OH; R² = Bzl
21 R = Trt; R¹ = OBt; R² = Bzl
22 R = H; R¹ = OH; R² = Bzl



23 Trt–cHyp–Leu–Gly–NH₂



24 Trt–Cys–cHyp–Lys–Gly–NH₂

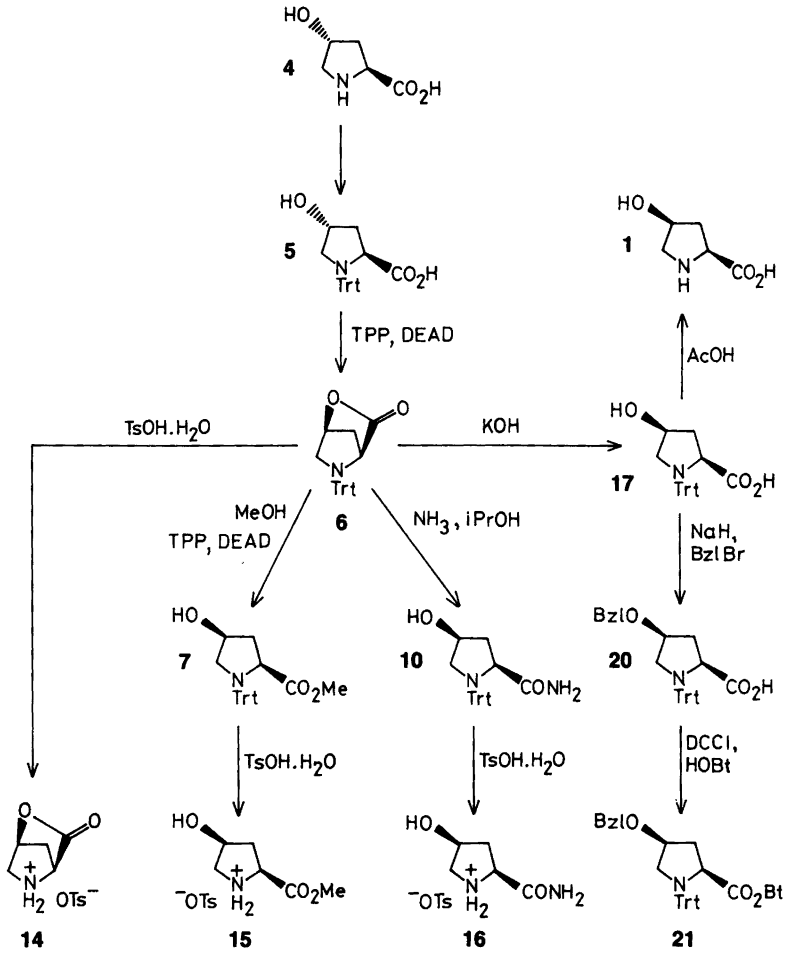


25 Trt–cHyp–Phe–cHyp–NH₂

Scheme 1. Structures of compounds discussed in this report.

† Presented in part at the 20th European Peptide Symposium, Tübingen, West Germany, September 4–9, 1988 (see Ref. 1).

* Post-doctoral fellow on leave from Department of Chemistry, University of Patras, Greece.



Scheme 2. The synthesis of *cis*-4-hydroxy-L-proline derivatives from *trans*-4-hydroxy-L-proline.

of cHyp and derivatives starting from the inexpensive, commercially available, *trans*-4-hydroxy-L-proline (**4**). The key step in the sequence of reactions employed was the inversion of configuration at C-4 of the *N*-tritylated derivative **5** by means of an intramolecular Mitsunobu reaction.[7] This reaction provided the common precursor, the bicyclic lactone **6**, which was easily converted into the final products (Schemes 1 and 2). The triphenylmethyl (trityl, Trt) group was chosen for protection of the amino group since it confers lability to mild acids[8] and shows excellent resistance to racemisation.[9,10] It is thus a highly attractive protecting group in peptide synthesis and chiral α-amino acid transformations.

N-Tritylation of **4** utilising the previously reported one-pot sequence of pertrimethylsilylation, selective aminodesilylation with isopropyl alcohol, and finally reaction with trityl chloride, afforded *trans*-4-hydroxy-*N*-triphenylmethyl-L-proline (**5**).[11] Treatment of **5** with triphenylphosphine (TPP) and diethyl azodicarboxylate (DEAD)[12] provided the key intermediate, namely the lactone **6**, in 60% yield. The structure of **6**, and hence the desired inversion of configuration at C-4, was confirmed spectroscopically (IR, ^{1}H NMR, MS) and proved[13] unambiguously by X-ray crystallography (Fig. 1). The crystal structure also explained the observation that one of the methylene protons of the bridge resonates almost as far up-field (0.070 ppm) as $SiMe_4$, whereas the other resonates at 1.453 ppm. The same protons in otherwise *N*-substituted lactones resonate[12] in the range 1.95–2.33 ppm. The bulky trityl group must be orientated in **6**, even in solution, towards the

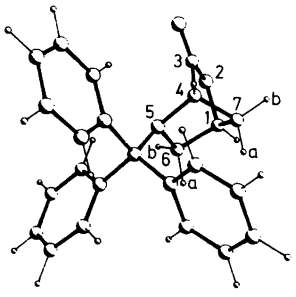


Fig. 1. ORTEP-drawing of the lactone **6**.

methylene bridge to avoid steric congestion. As a result, one of the methylene bridge protons, i.e. H-7a (Fig. 1), is situated inside the shielding cone of one of the aromatic rings of the trityl moiety. The dihedral angles (X-ray) involving the bridgehead protons and the vicinal methylene protons are in the range 52–70°. The observed zero coupling between these protons in the NMR spectrum should thus be due to either a deformation of the bicyclic structure in solution and/or the presence of the electronegative substituents. The bridgehead protons appear as singlets and the methylene protons as doublets in the ^{1}H NMR spectrum of **6**. Additional long-range coupling of the order of 0.9 Hz is observed for the methylene bridge protons.

Yields of the lactone **6** varied according to the rate of crystallisation from the crude reaction during initial non-aqueous work-up of the Mitsunobu reaction. The mother liquors contained a new product, more polar than **6**, not present in the reaction mixture before work-up. This product was isolated by flash chromatography (FC) and spectroscopically identified as the ester **7**. The epimeric ester **8** (*R*-configuration at C-4) was prepared for comparison by esterification of **4** by means of sequential treatment with 2,2-dimethoxypropane/HCl[14] and tritylation with TrtCl/Et_3N. The esters **7** and **8** had different physical properties and, most importantly, completely different ^{1}H NMR spectra (Table 1) in the non-aromatic region. While more examples from the *trans* series are required, special diagnostic value appears to attach to the chemical shift of H-4 which resonates at higher field (ca. 0.5 ppm) for compounds of the *cis* series: this is probably due to the shielding effect of the trityl groups (see e.g. Fig. 2). The configuration at C-4 for various 4-substituted *N*-trityl-L-proline methyl esters can readily be deduced, as for similar but otherwise *N*-protected derivatives,[15] from their ^{1}H NMR spectra.

The ^{1}H NMR spectrum of the ester **7** shows that all vicinal couplings for the protons of the heterocyclic ring have values either of zero or of the order of 1 Hz. Since the corresponding dihedral angles as seen in the crystal structure[16] of **7** (Fig. 2) are well away from 90°, the ring must be deformed away from an envelope conformation in solution, i.e. it acquires a twist conformation due to hydrogen bond-

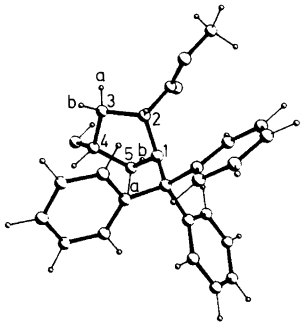


Fig. 2. ORTEP-drawing of the ester **7**.

Table 1. Chemical shifts, splitting patterns and coupling constants for the lactone **6**, esters **7**, **8** and **9**, and amide **10**. Characteristic fragments in the mass spectra are also given, together with tentative identifications: values in parentheses are percentage relative intensities.

^{1}H NMR	Mass spectrum
Compound **6**[a]	
7.558–7.537 (6 H, M, ArH)	355, *M* (only at 9 eV)
7.277–7.216 (6 H, m, ArH)	277, *M*–PhH (6.4)
7.217–7.179 (3 H, m, ArH)	243, Ph_3C (100)
4.736 (1 H, s, H-1)	241, 243–H_2 (13)
3.865 (1 H, s, H-4)	239, 241–H_2 (12)
3.368 (1 H, d, *J* 10.7 Hz, H-6b)	228, (14)
2.857 (1 H, d, *J* 10.7 Hz, H-6a)	215, 243–C_2H_4 (9)
1.453 (1 H, dd, *J* 11.0 and 0.9 Hz, H-7b)	202, 228–C_2H_2 (6)
0.070 (1 H, dd, *J* 11.0 and 1.0 Hz, H-7a)	165, 243–PhH (39)
Compound **7**[b]	
7.554–7.530 (6 H, m, ArH)	387, *M* (2)
7.273–7.235 (6 H, m, ArH)	369, *M*–H_2O (1)
7.181–7.142 (3 H, m, ArH)	355, *M*–MeOH (1)
3.871 (1 H, dd, *J* 10.0 and 1.0 Hz, H-4)	328, *M*–CO_2CH_3 (3)
ca. 3.9 (2 H, unresolved m, H and OH)	310, *M*–Ph (10)
3.641 (3 H, s, OCH_3)	277, 355–PhH (3)
3.453 (1 H, dd, *J* 11.5 and 1.0 Hz, H-5a)	243, Ph_3C (100)
2.810 (1 H, dd, *J* 11.5 and 1.9 Hz, H-5b)	228, (16)
1.616 (1 H, d, *J* 13.8 Hz, H-3a)	215, 243–C_2H_4 (13)
1.200 (1 H, m, unresolved m, H-3b)	202, 228–C_2H_2 (5)
	165, 243–PhH (55)
Compound **8**[c]	
7.574–7.552 (6 H, m, ArH)	387, *M* (2)
7.290–7.244 (6 H, m, ArH)	355, *M*–MeOH (1)
7.190–7.154 (3 H, m, ArH)	328, *M*–CO_2CH_3 (2)
4.374 (1 H, deceptive quintet, *J* 7.2, 6.4 and 5.0 Hz, H-4)	310, *M*–Ph (12)
	243, Ph_3C (100)
3.930 (1 H, dd, *J* 8.9 and 3.2 Hz, H-2)	228, (13)
3.729 (1 H, dd, *J* 11.4 and 6.4 Hz, H-5b)	215, 243–C_2H_4 (8)
3.851 (3 H, s, OCH_3)	202, 228–C_2H_2 (5)
2.667 (1 H, dd, *J* 11.4 and 5.0 Hz, H-5a)	165, 243–PhH (36)
1.902 (1 H, ddd, *J* 12.8, 6.4 and 3.2 Hz, H-3b)	
1.126 (1 H, ddd, *J* 12.8, 8.9 and 7.2 Hz, H-3a)	
Compound **9**[c]	
7.628–7.507 (8 H, m, ArH)	541, *M* (absent)
7.306–7.160 (11 H, m, ArH)	464, *M*–Ph (3)
3.925 (1 H, dd, *J* 9.6 and 3.0 Hz, H-2)	385, *M*–Tol·SO_2H (1)
3.740 (4 H, OCH_3 and H-4)	369, *M*–Tol·SO_3H (3)
3.482 (1 H, dd, *J* 13.9 and 5.4 Hz, H-5a)	310, 369–CO_2Me (3)
3.320 (1 H, dd, *J* 13.9 and 6.9 Hz, H-5b)	291, 369–PhH (3)
2.443 (3 H, s, $ArCH_3$)	243, Ph_3C (100)
1.758 (1 H, dt, *J* 14.6 and 3.0 Hz, H-3b)	228, (8)
1.299 (1 H, ddd, *J* 14.6, 19.6 and 7.9 Hz, H-3a)	215, 243–C_2H_4 (8)
	202, 228–C_2H_2 (4)
	165, 243–PhH (16)
Compound **10**[c,d]	
7.626–7.574 (6 H, m, ArH)	372, *M* (absent)
7.360–7.321 (6 H, m, ArH)	354, *M*–H_2O (1)
7.266–7.230 (3 H, m, ArH)	328, *M*–$CONH_2$ (10)
3.634 (1 H, dd, *J* 9.9 and 3.0 Hz, H-2)	295, *M*–Ph (4)
3.209 (1 H, t, *J* 7.4 Hz, H-4)	243, Ph_3C (100)
3.113–3.064 (2 H, m, H-5 and OH)	228, (19)
1.378 (1 H, dt, *J* 13.3 and 3.0 Hz, H-3a)	215, 243–C_2H_4 (15)
1.179–1.093 (1 H, m, H-3b)	202, 228–C_2H_2 (9)
	165, 243–PhH (65)

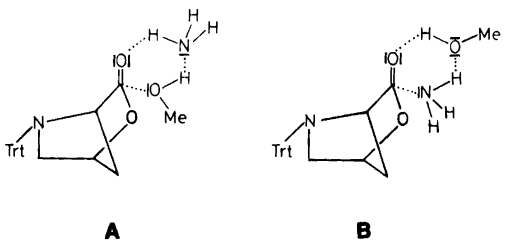
[a]Numbering of protons is given in Fig. 1. [b]Numbering of protons is given in Fig. 2. [c]Numbering of protons as for **7**: spectral parameters for this compound were obtained by iterative computer simulation. [d]NMR spectrum recorded in DMSO.

ing. In accordance with this hypothesis values in excess of 3 Hz are found for the corresponding vicinal coupling constants in the ^{1}H NMR spectrum of the ester **9**.

The formation of the ester **7** was attributed to methanol transesterification of the strained lactone **6**, a reaction catalysed by excess TPP–DEAD used in the preparation of **6**. Methanol transesterification of a variety of esters has in fact been shown to proceed by TPP–DEAD catalysis.[17] This observation prompted us to develop a mild and neutral method for the production of **7**. A solution of **6** in THF–MeOH was left for 2 days at room temperature in the presence of TPP–DEAD and this led to the clean formation of **7** which was ultimately isolated by FC in 87 % yield. As would be expected from the known stereochemistry of TPP–DEAD-catalysed transesterification,[17] no trace of the diastereoisomer **8** could be detected in the product (TLC with genuine **8** as the standard). Treatment of **7** with *p*-toluenesulfonyl chloride (TsCl) and pyridine in $CHCl_3$[18] for 4 days at 0 °C provided, with no sign of lactonisation (TLC), a 90 % yield of the corresponding *p*-toluenesulfonate **9**. Since forcing conditions are normally used[19] for the displacement of the TsO group from similar substrates bearing strongly electron-withdrawing protecting groups, by various nucleophiles/bases, the *N*-trityl protected derivative **7** should be considered highly advantageous in the preparation of a variety of *trans*-4-substitued-L-proline derivatives.

Ammonolysis of lactone **6** in THF–MeOH produced a mixture of two products in the ratio 1:3. Separation by FC and spectroscopic investigation of the products revealed that the minor, more polar (TLC), product was the expected amide **10**, while the major product was the ester **7**. The relative amounts of ester **7** and amide **10** formed are presumably the result of the different steric requirements for the two possible orientations of the reactants during the nucleophilic attack at the carbonyl function (Fig. 3).

In a separate experiment, the ester **7** failed to produce any detectable (TLC) amount of amide **10** under reaction conditions identical with those used in the ammonolysis of **6**. Although the presence of the bulky trityl group alone might be thought to explain the reluctance of **7** to undergo ammonolysis, the X-ray structure reveals that this group can at best screen, by orientating itself *trans* to the hydroxy and ester functions, only one face of the carbonyl group at a time from nucleophilic attack. The lack of reactivity of



A **B**

Fig. 3. Relative orientation of reactants during the formation of the ester **7** (A) and amide **10** (B).

the carbonyl function must thus presumably be due, at least in part, to screening of the other face by the heterocyclic part of the molecule and/or to reduced electrophilicity caused by the presence of the trityl amino group in the α-position. In accordance with this hypothesis, ammonolysis of the related dipeptide lactone **11**, under identical reaction conditions, produced initially a mixture of two products, the corresponding ester **12** and amide **13**. However, prolonging the reaction time led to conversion of **12** into **13** as judged by TLC. Thus, removal of the bulky trityl group from the vicinity of the carbonyl function and replacement with the electron-withdrawing acyl group rendered the carbonyl function of the intermediate ester **12** susceptible to nucleophilic attack by ammonia. It should be pointed out that no reaction was observed on attempted ammonolysis of **6** in neat THF. Ammonolysis of **6** in liquid ammonia failed due to lack of solubility. However, clean formation of the amide **10** resulted when the more sterically demanding isopropyl alcohol replaced methanol and **10** was actually isolated in 95 % yield.

Treatment of the *N*-tritylated compounds **6**, **7** and **10** with *p*-toluenesulfonic acid monohydrate ($TsOH \cdot H_2O$) produced the corresponding crystalline salts **14**, **15** and **16** in yields of 88, 85 and 80 %, respectively. Formation of the salts **15** and **16** was clean and no lactone formation was evident by TLC examination of the reaction mixtures.

Saponification of the lactone **6** with 2 M KOH in DMSO–MeOH at room temperature gave the expected *cis*-4-hydroxy-*N*-trityl-L-proline (**17**) which was isolated in crystalline form as the corresponding diethylammonium salt in 68 % yield. Treatment of **17** with glacial acetic acid for a few minutes at room temperature afforded a 90 % yield of stereochemically pure *cis*-4-hydroxy-L-proline (**1**) as demonstrated by the recording of physical constants identical with those previously reported.[20] The present four-step transformation of *trans*- into *cis*-4-hydroxy-L-proline provides simple access to the latter in about 30 % overall yield.

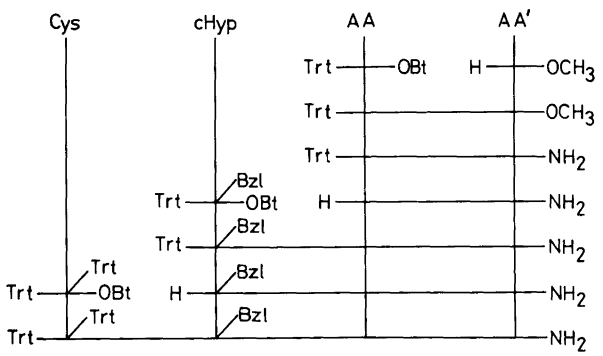
Attempts to use the strained lactone **6** for the incorporation of cHyp as the *N*-terminal amino acid in peptide chains were unsuccessful. Even simple esters of glycine required forcing conditions to effect sluggish coupling and this to such a small degree that dioxopiperazine formation was in effective competition. Since Boc-cHyp (**2**), activated by dicyclohexylcarbodiimide (DCCI) and 1-hydroxybenzotriazole (HOBt), has been successfully applied in solid-phase peptide synthesis,[5] we turned our attention to the corresponding *N*-trityl derivative **17**. However, activation of **17** with DCCI and HOBt and subsequent coupling with an amino acid ester failed due to the very fast, energetically favourable, conversion of **17** into the lactone **6**, as shown by TLC monitoring. As has already been shown **6** does not couple with carboxy components.

Prompted by these results we prepared Boc-cHyp (**2**) and the corresponding lactone **18** according to reported procedures.[5,12] Treatment of either **18** or **2**, activated *in situ* with DCCI/HOBt, with glycine benzyl ester afforded the

expected dipeptide **19** in comparable yields. Since lactone formation was evident (TLC) in the latter reaction, it is plausible to suggest that coupling of **2** with carboxy components takes place via the lactone **4** which is activated by the electron-withdrawing Boc group.

It was thus apparent that successful application of **17** in peptide synthesis required protection of the hydroxy group. In practice, treatment of **17** with excess NaH and benzyl bromide at −5 °C gave the *O*-benzyl derivative **20**, isolated in 60 % yield as the corresponding DEA salt. Finally, treatment of **20** with DCCI and HOBt[21] afforded the activated derivative **21** in 90 % yield. As revealed by the IR spectrum, **21** was of the pure ester type[21] and thus exhibited excellent acylating properties. It is worth noting that **20** may be readily transformed by a simple two-step sequence into the corresponding Boc protected derivative **3** if required. Thus treatment of **20** with glacial acetic acid at room temperature provided *cis*-*O*-benzyl-4-hydroxy-L-proline (**22**) in 92 % yield. t-Butoxycarbonylation[22] of the latter gave **3** in 85 % yield. For comparison **3** has been prepared from cHyp by *N*-t-butoxycarbonylation followed by *O*-benzylation with sodium in liquid ammonia.[6]

The applicability of the derivatives **14**, **15**, **16** and **21** in liquid-phase peptide synthesis was tested by the preparation of the model dipeptides **11**, **12** and **13**, and oligopeptides **23**, **24** and **25**. The latter represent analogues, or *C*-terminal fragments, of peptides of considerable biological interest in which L-Pro has been replaced by cHyp. Thus, the tripeptide Pro-Leu-Gly-NH_2 exhibits a number of effects on the central nervous system,[23] and the peptides Cys-Pro-Lys-Gly-NH_2 and Pro-Phe-Pro-NH_2 are related to lysine-vasopressin[24] and morphiceptin[25] respectively. The latter exhibited potent and specific opioid activity. Generally, in the syntheses of Scheme 3, the trityl group was employed for the protection of the amino and sulfhydryl functions and couplings were effected by using isolated 1-hydroxybenzotriazolyl esters[21] of the appropriate amino acids (Trt-AA-OBt). Deprotection of the intermediate fragments was carried out using either $TsOH \cdot H_2O$ or 1 % trifluoroacetic acid in CH_2Cl_2 for selective N^{α}-trityl deprotection[26] in the case of the tetrapeptide **24**. In relation to the use of *p*-toluenesulphonates **14**, **15** and **16** as synthons for the incorporation of cHyp at the *C*-terminal of a peptide chain, it was of interest to examine their reactivity towards Trt-AA-OBt, taking as a model, Trt-Leu-OBt, and to discover whether protection of the hydroxy function was needed during coupling. Furthermore, possible lactonisation at the *C*-terminus during either coupling or deprotection was considered. Coupling of Trt-Leu-OBt with either **15** or **16** proceeded rapidly with a 10 % molar excess of the carboxy component, giving the corresponding dipeptides **12** and **13** in good yields and showing no evidence of either *O*-acylation or lactonisation. The coupling of lactone **14**, while giving the corresponding dipeptide in good yield, was slow even with a 30 % molar excess of **14**, presumably the result of steric hindrance. Similar results were obtained when the dipeptide Trt-Phe-cHyp-NH_2 was prepared either directly from Trt-Phe-OBt and **16**, or as indicated in Scheme 3. Deprotection of this dipeptide with $TsOH \cdot H_2O$ proceeded without lactonisation (IR).



Scheme 3. Peptide synthesis (schematic representation).

As has already been indicated, the active ester **21** showed excellent acylating behaviour and coupled in a fast and clean manner to the appropriate dipeptides in all three syntheses depicted in Scheme 3, to give the corresponding peptides in high yields. Further applications of the presently reported derivatives **15**, **16** and **21** of cHyp in the synthesis of biologically important peptides incorporating cHyp in place of Pro and evaluation of their biological activity is now in progress, while the coupling of **15**, **16** and **21** with esters of the sterically demanding amino acids Val and Ile is the subject of a separate study.

Experimental

General. Capillary melting points were taken on a Buchi SMP-20 apparatus and are uncorrected. Optical rotations were determined with a Carl–Zeiss precision polarimeter. IR spectra were recorded as Nujol mulls on a Perkin–Elmer 457 grating spectrophotometer. ^1H NMR spectra were obtained at 400.13 MHz on a Bruker AM400 spectrophotometer, using $CDCl_3$ as the solvent unless noted otherwise and tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in δ units, parts per million (ppm) downfield from TMS. Mass spectra were recorded at 70 eV a JEOL JMS D-100 instrument, using a source temperature of 180 °C and the minimum probe temperature required for volatilisation (150–200 °C). Flash chromatography (FS) was performed on Merck silica gel 60 (230–400 mesh) and TLC on Merck silica gel 60 F_{254} films (0.2 mm) precoated on aluminium foil. The solvent systems used were: (A) $CHCl_3$/MeOH (9:1), (B) toluene/ethyl acetate (8:2), (C) toluene/hexane/ethyl acetate (7:3:2), (D) $CHCl_3$/MeOH (7:3), (E) BuOH/AcOH/H_2O (4:1:1), (F) BuOH/AcOH/pyridine/H_2O (30:6:20:24), (G) BuOH/AcOH/H_2O (4:1:5, upper phase) and (H) $CHCl_3$/MeOH/AcOH

(85:10:5). Spots were visualised by UV light at 254 nm, with ninhydrin, and chlorine/KI/starch reagent. All solvents were dried and/or purified according to standard procedures[27] prior to use. DEAD was used as purchased (Merck).

In general, 1-hydroxybenzotriazolyl esters of *N*-tritylamino acids such as Trt-Leu-OBt, Trt-(Trt)Lys-OBt, Trt-Phe-OBt, Trt(Trt)Cys-OBt and **21** were prepared and coupled with carboxy components according to reported[11,26] general procedures. The peptides **23**, **24** and **25** were prepared according to the methodology described in Refs. 26 and 28; see Scheme 3.

Amino acid analyses were performed on a Beckmann Model 120C amino acid analyser using a three-buffer column system. It should be noted that cHyp is detected solely as the corresponding lactone as was confirmed by comparison with an authentic sample (i.e. **14**). Yields, physical constants and spectral data for the compounds described below and of the peptides **23**, **24** and **25** are given in Table 2.

5-Triphenylmethyl-2-oxa-5-aza-bicyclo[2.2.1]heptan-3-one (**6**). *trans*-4-Hydroxy-*N*-trityl-L-proline DEA salt[11] (11.2 g, 25 mmol) was partitioned between a precooled (0 °C) 5 % aqueous solution of citric acid (200 ml) and ethyl acetate (100 ml), and the aqueous layer was re-extracted with a

Table 2. Yields, physical constants and spectral data for derivatives of *cis*-4-hydroxy-L-proline.[a]

Cpd.	Yield (%)	M.p./°C	$[\alpha]_D^{30}$	TLC/R_f[b]		Analysis[c]	IR/cm^{-1}[d]
3	85	109–110	−33.4 (*c* 1, MeOH)	0.25 (A)	0.77 (G)	$C_{17}H_{23}NO_5$	3200–2400, 1720, 1630
6	60	230–235	+104.6 (*c* 1, $CHCl_3$)	0.77 (A)	0.55 (C)	$C_{24}H_{21}NO_2$	1785
7	85	129–130	−35.3 (*c* 1, $CHCl_3$)	0.68 (A)	0.34 (B)	$C_{25}H_{25}NO_3$	3550–3100, 1750, 1715
8	70	144–145	−24.2 (*c* 1, MeOH)	0.62 (A)	0.29 (B)	$C_{25}H_{25}NO_3$	3455, 1710
9	85	168–170	+13.7 (*c* 1, $CHCl_3$)	0.70 (A)	0.61 (C)	$C_{32}H_{31}NO_5S$	1750, 1360 and 1180 (S=O)
10	95	202–203	+11.8 (*c* 2, $CHCl_3$)	0.29 (A)	0.73 (E)	$C_{24}H_{24}N_2O_2$	3500–3100, 1670
11	83	138–140 (soft. 95)	+58.8 (*c* 1, MeOH)	0.69 (H)	0,70 (A)	$C_{30}H_{32}N_2O_3$	1800, 1650
12	92	76–78	+38.4 (*c* 1, MeOH)	0.51 (A)	0.78 (H)	$C_{31}H_{36}N_2O_4$	3600–3150, 1740, 1640
13	89	125–126 (soft. 80)	+23.0 (*c* 1, MeOH)	0.30 (A)	0.59 (H)	$C_{30}H_{35}N_3O_3$	3500–3100, 1665, 1620
14	86	154–156 (soft. 152)	+18.6 (*c* 1, MeOH)	0.46 (E)	0.10 (D)	$C_{12}H_{15}NO_5S$	3400–2200, 1790
15	85	89–91	−9.3 (*c* 1, MeOH)	0.24 (E)	0.42 (F)	$C_{13}H_{19}N$	3350, 3200–2200, 1745
16	80	117–119	−31.0 (*c* 1, MeOH)	0.14 (E)	0.26 (F)	$C_{12}H_{18}N_2O_5S$	3500–2200, 1690
17[e]	86	226 (decomp.)	−23.1 (*c* 3, MeOH)	0.12 (A)		$C_{28}H_{34}N_2O_3$	3260–3150, 2800–2200, 1630–1550
19	84	Oil	−21.7 (*c* 1, MeOH)	0.72 (E)	0.75 (H)	$C_{19}H_{25}N_2O_6$	3600–3150, 1750, 1720–1650, 1540
20[e]	60	142–145	−2.2 (*c* 2, MeOH)	0.39 (A)	0.18 (B)	$C_{35}H_{40}N_2O_3$	2800–2200, 1630–1520
21	90	79–80	−50.0 (*c* 1, $CHCl_3$)	0.76 (A)	0.65 (B)	$C_{37}H_{32}N_4O_3$	1820
22	92	220–221 (decomp.)	+6.9 (*c* 1, AcOH)	0.28 (E)	0.45 (F)	$C_{12}H_{15}NO_3$	3200–2000, 1630–1520
23	78[f]	96–98	−40.9 (*c* 1, MeOH)	0.36 (A)	0.76 (E)	cHyp: 0.95 Leu: 1.09 Gly: 1.00	3500–3100, 1690–1610
24	67[f]	168–169 (soft. 119)	+57.0 (*c* 1, DMF)	0.82 (E)	0.84 (F)	Cys: 1.12 cHyp: 1.02 Lys: 0.92 Gly: 1.05	3400–3200, 1690–1620
25	81[f]	102–104 (soft. 98)	−20.6 (*c* 1, MeOH)	0.36 (A)	0.74 (E)	cHyp: 1.93 Phe: 1.08	3500–3150, 1670, 1640

[a]Data for *trans*-4-hydroxy-*N*-trityl-L-proline methyl ester included. [b]R_f-values (solvent system). [c]Combustion analysis (C, H, N) except for peptides **23**, **24** and **25** which were analysed by amino acid analysis (cHyp was detected exclusively as the lactone). [d]Only amino, hydroxy and carbonyl group frequencies are given except where otherwise noted. [e]DEA salt. [f]Total yields.

further portion of ethyl acetate (100 ml). The combined organic layers were washed with brine (3×100 ml), dried ($MgSO_4$) and evaporated under reduced pressure at a bath temperature not exceeding 40 °C to yield **5** as a white solid. The solid was dissolved in dry THF (70 ml), TPP (7.9 g, 30 mmol) added and the resulting solution was treated dropwise with DEAD (4.7 ml, 30 mmol) at 0 °C. After 5 min at this temperature, the solvent was removed by evaporation under reduced pressure and the residual oil subjected directly to FC (10 g silica per 1 g mixture) using benzene as the eluant. The fractions containing **6** were pooled and evaporated to dryness to leave a pure crystalline product (5.3 g) which was recrystallised from acetone. An alternative work-up procedure takes advantage of the solubility of **6** in MeOH. Thus, the residual oil obtained on evaporation after completion of the reaction is dissolved in ethyl acetate and subjected to the following washing procedure: 5 % aqueous citric acid (50 ml), H_2O (2×50 ml), 5 % aqueous $NaHCO_3$ (50 ml) and H_2O (2×50 ml). Drying the organic phase and evaporation left an oil which on trituration with MeOH afforded the crystalline product on refrigeration (4.9 g, 55 % yield). This latter work-up procedure, while very simple and economic, cannot be applied to otherwise *N*-protected lactones, e.g. **18**, where careful FC is required for separation from other polar by-products of the reaction, including triphenylphosphine oxide.

cis-*4-Hydroxy*-N-*triphenylmethyl*-L-*proline methyl ester* (**7**). Lactone **6** (5 g, 14 mmol) was dissolved in a mixture of dry THF (80 ml) and anhydrous MeOH (20 ml) and treated sequentially at 0 °C with TPP (1.84 g, 7 mmol) and DEAD (1 ml, 7 mmol). The resulting solution was kept at room temperature for 2 days, the solvents removed and the residual oil subjected to FC with toluene/ethyl acetate (9:1) to give pure **7** (4.7 g). An analytical sample was prepared by recrystallisation from diisopropyl ether/petroleum ether b.p. 60–80 °C.

trans-*4-Hydroxy*-N-*triphenylmethyl*-L-*proline methyl ester* (**8**). Conc. HCl (3 ml) was added to a suspension of **4** (0.4 g, 3 mmol) in 2,2-dimethoxypropane (40 ml) and the mixture was stirred overnight at room temperature. Evaporation of the solvent from the resultant brown solution and crystallisation of the residue from MeOH/Et_2O gave the crystalline hydrochloride of *trans*-4-hydroxy-L-proline methyl ester (0.45 g, 83 %). This was dissolved in dry CH_2Cl_2 (10 ml) and treated sequentially with triethylamine (0.75 ml, 6 mmol) and trityl chloride (0.7 g, 2.5 mmol). The reaction mixture was stirred at room temperature for a further 5 min, diluted with CH_2Cl_2 (25 ml) and washed with water. Drying and evaporation of the solvent left a residue which crystallised from diisopropyl ether/hexane after refrigeration (4 days) to give **8** (0.75 g).

cis-*4-Hydroxy*-N-*triphenylmethyl*-L-*proline amide* (**10**). Isopropyl alcohol (150 ml) was added to a solution of the lactone **6** (5 g, 14 mmol) in THF (250 ml) which was then saturated with ammonia at 0 °C. After 3 days at room temperature, the solvent evaporated and the residue was triturated with diethyl ether to give **10** (4.9 g) as a white precipitate which could be recrystallized from ethyl acetate/petroleum ether b.p. 60–80 °C. Amide **10** was also obtained after FC from the admixture with ester **7** obtained on ammonolysis of the lactone **6** in the presence of MeOH as hydroxylic counter-solvent. The compounds **10** and **7** were recovered in the ratio 1:3 after FC.

cis-*4-Hydroxy*-O-*(4-tolylsulfonyl)*-N-*triphenylmethyl*-L-*proline methyl ester* (**9**). Ester **7** (1.29 g, 3.3 mmol) was treated with 4-toluenesulfonyl chloride and pyridine in $CHCl_3$ for 4 days at 0 °C, according to the literature procedure.[18] Dilution with diethyl ether gave a white precipitate which was filtered and washed with water, methanol and diethyl ether in that order. Crystallization from CH_2Cl_2/hexane gave the product **9** (1.53 g).

3-Oxo-2-oxa-5-azoniabicyclo[2.2.1]heptane 4-toluenesulfonate (**14**). Lactone **6** (3.6 g, 10 mmol) was dissolved in THF (40 ml) and isopropyl alcohol (50 ml) under reflux. To the resulting hot solution was added TsOH·H_2O (2.9 g, 15 mmol) with stirring. This solution was allowed to attain room temperature, whereupon the product crystallised. Filtration of the cooled (0 °C) mixture and washing with diethyl ether gave pure **14** (2.5 g).

cis-*4-Hydroxy*-L-*proline methyl ester 4-toluenesulfonate* (**15**). The mixture resulting from the addition of TsOH·H_2O (2.9 g, 15 mmol) to a solution of ester **7** (3.9 g, 10 mmol) in THF (6 ml) and isopropyl alcohol (9 ml) was stirred at room temperature for 15 min. Trituration with diethyl ether (20 ml) and refrigeration overnight gave, on filtration, pure crystalline **15** (3.1 g).

cis-*4-Hydroxy*-L-*prolinamide 4-toluenesulfonate* (**16**). Amide **10** (3.72 g, 10 mmol) was dissolved under reflux in a mixture of THF (45 ml) and isopropyl alcohol (15 ml), treated with TsOH·H_2O (2.9 g, 15 mmol) for 2 min at room temperature and a further portion of isopropyl alcohol (8 ml) was added. The resulting solution was kept at room temperature for 5 min and the solvents removed under reduced pressure. The residue was treated with diethyl ether and refrigerated overnight. The solvent was then decanted and the oily residue shaken well with a fresh portion of diethyl ether. The solvent was decanted again and the residue was crystallized from isopropyl alcohol to give the pure amide **16** (2.4 g).

Preparation of dipeptides **11**, **12** and **13**. These dipeptides were prepared by coupling Trt-Leu-OBt[11] with the corresponding *p*-toluenesulphonates **14**, **15** and **16**, respectively, as in the following paragraphs. Yields and physical data are given in Table 2.

5-(N-Triphenylmethyl-L-leucyl)-2-oxa-5-azabicyclo[2.2.1]-heptan-3-one (**11**). TsOH·cHyp-lactone (**14**, 297 mg, 1.04 mmol) was dissolved in DMF and neutralised with *N*-methylmorpholine at 0 °C, and Trt-Leu-OBt (390 mg, 0.8 mmol) added. The reaction mixture was stirred for 24 h at room temperature and diluted with brine. The resulting precipitate was extracted into ethyl acetate and the organic phase then washed with 5 % aqueous citric acid (2×50 ml), H_2O (2×50 ml), 5 % aqueous $NaHCO_3$ (50 ml) and finally H_2O (2×50 ml). Drying with Na_2SO_4 and evaporation of the solvent gave a solid residue **11** which, after crystallisation from hexane, amounted to 310 mg.

N-*Triphenylmethyl-L-leucyl*-cis-*4-hydroxy-L-proline methyl ester* (**12**). This dipeptide was prepared in a similar way to that above except that only a 10 % molar excess of reagent was required and reaction time was reduced to 4 h.

N-*Triphenylmethyl-L-leucyl-4-hydroxy-L-prolinamide* (**13**). This dipeptide was prepared identically with the dipeptide **12**.

Ammonolysis of dipeptide **11**: *Preparation of dipeptide* **13**. Ammonolysis of **11** was carried out under the same reaction conditions as used for ammonolysis of lactone **6** in the presence of MeOH. Reaction for 3 days at room temperature gave the dipeptide **13** (90 %).

Diethylammonium salt of cis-N-*triphenylmethyl-4-hydroxy-L-proline* (**17**). Lactone **6** (4.3 g, 12 mmol) was suspended in a mixture of DMSO (20 ml) and MeOH (12 ml) [water bath] and a solution of KOH (3.2 g, 57 mmol) in H_2O (8 ml) was added. An additional portion of DMSO (16 ml) was then added. Stirring of the resulting mixture at room temperature for 10 min resulted in dissolution and saponification was complete after a further 50 min. The solvents were removed under reduced pressure and the residue dissolved in H_2O. The solution was adjusted to pH 6 at 0 °C by the dropwise addition of glacial AcOH and extracted with ethyl acetate (2×100 ml). The combined organic layers were washed with ice-cold water (50 ml) and brine (2×50 ml), and dried. After filtration, diethylamine (1.25 ml, 12 mmol) was added and the product (4.6 g) collected after overnight refrigeration.

cis-*4-Hydroxy-L-proline* (**1**). *cis*-4-Hydroxy-*N*-trityl-L-proline (**17**) obtained from the corresponding DEA salt (4.5 g, 10 mmol) as described for the *trans*-isomer (see the preparation of **6**) was dissolved in glacial AcOH (20 ml) and left to stand at room temperature for 15 min. The resulting solution was diluted with H_2O (80 ml) and extracted with diethyl ether (2×30 ml). The aqueous layer was evaporated to dryness under reduced pressure and the residue was crystallized from H_2O/MeOH/acetone (1:1:3) to give **1** (1.2 g).

Diethylammonium salt of cis-O-*benzyl-4-hydroxy*-N-*triphenylmethyl-L-proline* (**20**). The DEA salt of **17** (5 g, 11.2 mmol) was added in portions to a suspension of NaH (3.3 g, 140 mmol) and imidazole (0.08 g, 1.1 mmol) at −15 °C with vigourous stirring and under nitrogen. The resulting mixture was stirred at −5 °C for a further 1 h and then treated with benzyl bromide (7.2 ml, 60 mmol) for 1 h. A further portion of NaH (2 g, 85 mmol) was introduced at −15 °C and the mixture was stirred for a further hour at −5 °C. Finally, more benzyl bromide (3.6 ml, 30 mmol) was added. The reaction was then complete within 1 h (TLC). Excess NaH was destroyed with glacial AcOH and the product transferred into diethyl ether (2×50 ml). The ethereal extracts were combined, washed with H_2O and dried ($MgSO_4$). Trituration of the filtered solution with diethylamine (1.2 ml, 11.2 mmol) and evaporation of the solvent gave a residue which was crystallised from acetone/hexane to give the product (3.9 g).

cis-O-*Benzyl-4-hydroxy*-N-*triphenylmethyl-L-proline 1-hydroxybenzotriazolyl ester* (**21**). Trityl amino acid **20**, obtained from the corresponding DEA salt (3.2 g, 6 mmol), was allowed to react with DCCI and 1-HOBt according to the literature procedure,[11] to give the active ester (3 g) as a foam.

cis-N-*tert-Butoxycarbonyl-4-hydroxy-L-prolylglycine benzyl ester* (**19**). TsOH·Gly-OBzl (0.74 g, 2.2 mmol) was dissolved in DMF and neutralised at 0 °C with *N*-methylmorpholine. Boc-lactone **18** (0.46 g, 2 mmol) was then added and the resulting mixture was stirred overnight at room temperature. When the reaction was complete (TLC), the solvent was evaporated and the residual oil was dissolved in ethyl acetate and washed with 5 % aqueous citric acid (2×50 ml), H_2O (2×50 ml), 5 % aqueous $NaHCO_3$ (50 ml) and finally H_2O (2×50 ml). Drying and evaporation of the organic solvent afforded an oil (635 mg, pure by TLC). The same compound was collected in comparable yield when TsOH·Gly-OBzl was coupled with Boc-cHyp (**2**) using DCCI/HOBt.

cis-O-*Benzyl-4-hydroxy-L-proline* (**22**). Trityl amino acid **20**, obtained from the corresponding DEA salt (5.4 g, 10 mmol), was dissolved in glacial AcOH (25 ml). The resulting solution was kept at room temperature for 1 h and then diluted with diethyl ether. The precipitated crystalline product (**22**) was washed with acetone and then weighed (2 g).

cis-O-*Benzyl*-N-*tert-butoxycarbonyl-4-hydroxy-L-proline* (**3**). The amino acid (**22**) (1.8 g, 8 mmol) was converted into the product **3** according to the procedure of Nagasawa *et al.*[22]

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References

1. Papaioannou, D., Stavropoulos, G. and Karagiannis, K. In: Jung, G. and Bayer, E., Eds., *Peptides 1988; Proceedings of the 20th European Peptide Symposium,* Walter de Gruyter, Berlin 1989, pp. 91–93.
2. Mauger, A. B. and Witkop, B. *Chem. Rev. 66* (1966) 47.
3. Inoye, K., Sakakibara, S. and Prockop, D. *Biochem. Biophys. Acta 420* (1976) 133.
4. Uitto, J. and Prockop, D. *Arch. Biochem. Biophys. 181* (1977) 293.
5. Buku, A., Schwartz, I., Yamin, N., Wyssbrod, H. and Gazis, D. *J. Med. Chem. 30* (1987) 1509.
6. Sakakibara, S. and Shudo, K. *Japan Kokai 7451* (1974) 266; *Chem. Abstr. 82* (1975) 17123h.
7. Mitsunobu, O. *Synthesis* (1983) 1.
8. Zervas, L. and Theodoropoulos, D. *J. Am. Chem. Soc. 78* (1956) 1359.
9. Barlos, K., Papaioannou, D., Patrianakou, S. and Tsegenidis, T. *Liebigs Ann. Chem.* (1986) 1950.
10. Baldwin, J. E., North, M., Flinn, A. and Moloney, M. G. *Tetrahedron 45* (1989) 1453 and references therein.
11. Barlos, K., Papaioannou, D. and Theodoropoulos, D. *J. Org. Chem. 47* (1982) 1324.
12. Bowers-Niemia, M. M. and Joullié, M. M. *Heterocycles 20* (1983) 817.
13. Papaioannou, D., Stavropoulos, G., Nastopoulos, V., Voliotis, S. and Leban, I. *Acta Crystallogr., Sect. C* (1989). *In press.*
14. Rachele, J. R. *J. Org. Chem. 28* (1963) 3898.
15. Andreatta, R. H., Nair, V. and Robertson, A. V. *Aust. J. Chem. 20* (1967) 2701.
16. Stavropoulos, G., Papaioannou, D., Karagiannis, K., Vlassi, M. and Nastopoulos, V. *Acta Crystallogr., Sect. C* (1989). *In press.*
17. Bittner, S., Berneis, Z. and Felix, S. *Tetrahedron Lett.* (1975) 3871.
18. Kabalka, G. W., Varma, M. and Varma, R. S. *J. Org. Chem. 51* (1986) 2386.
19. Andreatta, R. H., Nair, V., Robertson, A. V. and Simpson, W. R. J. *Aust. J. Chem. 20* (1967) 1493.
20. Leuchs, H. and Bernmann, K. *Ber. Dtsch. Chem. Ges. 52* (1919) 2086.
21. Barlos, K., Papaioannous, D. and Theodoropoulos, D. *Int. J. Pept. Protein Res. 23* (1984) 300.
22. Nagasawa, T., Kuroiwa, K., Narita, K. and Isowa, Y. *Bull. Chem. Soc. Jpn. 46* (1973) 1269.
23. Vander Elst, P., Elseviers, M., De Cock, E., Van Marsenille, M., Tourwé, D. and Van Binst, G. *Int. J. Pept. Protein Res. 27* (1986) 633.
24. Meienhofer, J. and Du Vigneaud, V. *J. Am. Chem. Soc. 82* (1960) 2279.
25. Chang, K.-J., Killian, A., Hazum, E. and Cuatrecasas, P. *Science 213* (1981) 75.
26. Barlos, K., Mamos, P., Papaioannou, D., Patrianakou, S., Sanida, C. and Schäfer, W. *Liebigs Ann. Chem.* (1987) 1025.
27. Perrin, D. D., Armarego, W. L. F. and Perrin, D. R. *Purification of Laboratory Chemicals,* 2nd ed., Pergamon Press, Oxford 1980.
28. Barlos, K., Papaioannou, D. and Sanida, C.*Liebigs Ann. Chem.* (1984) 1308.

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