# SYNTHESIS OF *CIS* AND *TRANS* ISOMERS OF 4-CHLORO-L-PROLINE, 4-BROMO-L-PROLINE, AND 4-AMINO-L-PROLINE

By R. H. ANDREATTA,*† V. NAIR,*‡ A. V. ROBERTSON,* and W. R. J. SIMPSON*

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*Summary*

*cis*- and *trans*-4-Chloro- and 4-bromo-L-prolines have been synthesized stereospecifically, the key step being $S_N2$ displacement of a free or substituted 4-hydroxyl group in suitably protected 4-hydroxy-L-prolines. Similar displacements with azide ion followed by reduction provide convenient routes to *cis*- and *trans*-4-amino-L-proline. A less satisfactory pathway to *cis*-4-aminoproline is reduction of a 4-oximinoproline derivative. In the course of the syntheses, which involve a variety of protecting groups, 45 new L-proline derivatives have been prepared. Unexpected side reactions were the formation of *cis*-4-hydroxyprolinamide by the action of ammonia on *trans*-4-bromoproline, and the reduction by sodium borohydride of *N*-benzyloxycarbonyl-4-oximinoproline methyl ester to *N*-benzyloxycarbonyl-4-oximinoprolinol.

## INTRODUCTION

Mauger and Witkop have recently reviewed proline and hydroxyproline chemistry with particular emphasis on analogues and homologues and their various biological ramifications.[1] We present here syntheses of the *cis* and *trans* isomers of 4-chloro-L-proline, 4-bromo-L-proline, and 4-amino-L-proline. A main aim has been to devise routes of preparative significance and this has required exploration of a variety of pathways. Starting material for all syntheses was natural *trans*-4-hydroxy-L-proline (I). Careful selection of the most appropriate protecting group for the secondary amino function was vital because reactions which proceed satisfactorily on monofunctional compounds often failed or gave unstable products in this series. Frequently, it was also necessary to mask the carboxyl group to avoid unwanted side reactions as well as to permit chromatographic purification of these very polar compounds. Preliminary announcement of some of our results was included in Mauger and Witkop's review.[1]

## DISCUSSION

$S_N2$ displacement of the secondary hydroxyl group in a suitably protected 4-hydroxy-L-proline would provide direct access to stereochemically defined 4-haloprolines. In general, for alcohols in which the transition states are unhindered, phosphorus halides produce inversion by an $S_N2$ mechanism, and thionyl halides substitute with retention by an $S_Ni$ mechanism.[2] The presence of base in reactions

* Department of Organic Chemistry, University of Sydney.

† U.S.P.H.S. Postdoctoral Fellow, 1964–65.

‡ U.S.P.H.S. Postdoctoral Fellow, 1966.

[1] Mauger, A. B., and Witkop, B., *Chem. Rev.*, 1966, **66**, 47.

[2] Shoppee, C. W., Bellas, T. E., and Lack, R. E., *J. chem. Soc.*, 1965, 6450, and references therein.

with thionyl chloride promotes the $S_N2$ mechanism at the expense of $S_Ni$, although elimination may be a major competing reaction.

*N*-Tosyl*-*trans*-4-hydroxy-L-proline methyl ester (III) was readily prepared from the known[3] *N*-tosyl acid (II). The action of phosphorus pentachloride in chloroform on (III) gave an 83% yield of a chloro product, formulated as (XVII) in the *cis* (= allo) series on the above mechanistic grounds. Thionyl chloride in pyridine generated the same compound in 54% yield. Both to confirm the stereochemical assignment and to determine the yield with another reagent, the two-step replacement of OH by Cl using the method of Cramer and co-workers[4] was examined. This involves conversion of the alcohol group into its trichloroacetimidate by interaction of the alkoxide anion with $CCl_3$–CN. Treatment of the acetimidate with hydrogen chloride in ether forms the chloro compound with elimination of trichloroacetamide. In all known cases under these reaction conditions, this displacement proceeds with virtually quantitative inversion.[4]

Preparation of the trichloroacetimidate (XXIV) of the alcohol (III) proved difficult, and of many bases tried only sodium hydride was satisfactory. The action of hydrogen chloride in ether on (XXIV) gave a 54% yield of the chloro compound (XVII), and a further 30% of starting material was accounted for as the trichloroacetate (XXV) which must have been formed by reaction with water in the work-up. Hydrolysis of (XXIV) with aqueous acid provided an authentic specimen of (XXV).

The unprotected amino acid *cis*-4-chloro-L-proline (XV) was obtained almost quantitatively from its *N*-tosyl methyl ester (XVII) by alkaline hydrolysis to the acid (XVI) followed by detosylation with HBr/HOAc.

The key intermediate in the synthesis leading to the *trans*-4-chloro series was the known compound *N*-tosyl-*cis*-4-hydroxy-L-proline methyl ester (VIII).[5] This compound was obtained here by a more direct route through chromic acid oxidation of *N*-tosyl-*trans*-4-hydroxyproline (II) to the keto acid (XXVIII), reduction of which with sodium borohydride gave *N*-tosyl-*cis*-4-hydroxyproline (VII) as the single product in 90% yield. Such stereospecificity in hydride reductions of *N*-protected 4-oxoprolines is well known.[6] The *cis*-hydroxy acid (VII) was further characterized by cyclization to its lactone (XXVII) with dicyclohexylcarbodiimide. Esterification with diazomethane gave the ester (VIII). Thionyl chloride in pyridine or phosphorus pentachloride in chloroform converted this *cis*-hydroxy ester (VIII) into the corresponding *trans*-chloro derivative (XIV) in 92% and 88% yields respectively. Noticeably higher yields in these $S_N2$ reactions compared with those leading to the *cis*-series reflect differences in steric hindrance to chloride attack. Generation

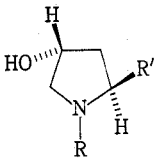
* Tosyl = *p*-toluenesulphonyl.

[3] McChesney, E. W., and Swan, W. K., *J. Am. chem. Soc.*, 1937, **59**, 1116; Portoghese, P. S., and Mikhail, A. A., *J. org. Chem.*, 1966, **31**, 1059.

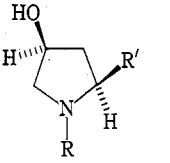
[4] Cramer, F., Pawelzik, K., and Baldauf, H. J., *Chem. Ber.*, 1958, **91**, 1049; Cramer, F., Pawelzik, K., and Lichtenthaler, F. W., *Chem. Ber.*, 1958, **91**, 1555; Cramer, F., and Baldauf, H. J., *Chem. Ber.*, 1959, **92**, 370.

[5] Fujita, Y., Gottlieb, A., Peterkovsky, B., Udenfriend, S., and Witkop, B., *J. Am. chem. Soc.*, 1964, **86**, 4709.

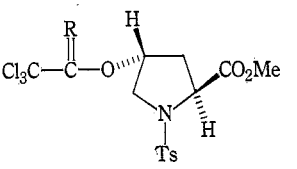
[6] Robertson, A. V., Katz, E., and Witkop, B., *J. org. Chem.*, 1962, **27**, 2676, and references therein.



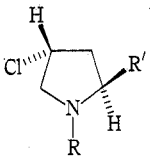
| | R | R′ |
|---|---|---|
| (I) | H | $CO_2H$ |
| (II) | Ts | $CO_2H$ |
| (III) | Ts | $CO_2Me$ |
| (IV) | Ts | $CONH_2$ |
| (V) | $PhSO_2$ | $CO_2H$ |



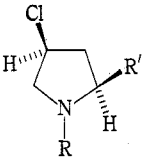
| | R | R′ |
|---|---|---|
| (VI) | H | $CONH_2$ |
| (VII) | Ts | $CO_2H$ |
| (VIII) | Ts | $CO_2Me$ |
| (IX) | $PhSO_2$ | $CO_2H$ |
| (X) | $PhSO_2$ | $CO_2Me$ |
| (XI) | $PhSO_2$ | $CONH_2$ |



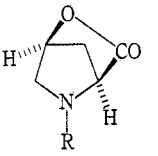
(XXIV) R = NH
(XXV) R = O



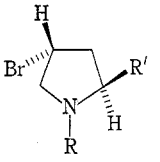
| | R | R′ |
|---|---|---|
| (XII) | H | $CO_2H$ |
| (XIII) | Ts | $CO_2H$ |
| (XIV) | Ts | $CO_2Me$ |



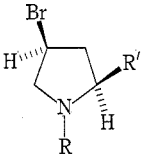
| | R | R′ |
|---|---|---|
| (XV) | H | $CO_2H$ |
| (XVI) | Ts | $CO_2H$ |
| (XVII) | Ts | $CO_2Me$ |



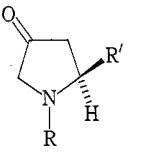
(XXVI) R = H
(XXVII) R = Ts



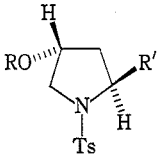
| | R | R′ |
|---|---|---|
| (XVIII) | H | $CO_2H$ |
| (XIX) | Ts | $CO_2H$ |
| (XX) | Ts | $CO_2Me$ |



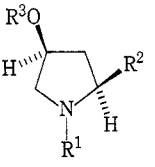
| | R | R′ |
|---|---|---|
| (XXI) | H | $CO_2H$ |
| (XXII) | Ts | $CO_2H$ |
| (XXIII) | Ts | $CO_2Me$ |



| | R | R′ |
|---|---|---|
| (XXVIII) | Ts | $CO_2H$ |
| (XXIX) | Ts | $CO_2Me$ |
| (XXX) | Ts | $CONH_2$ |
| (XXXI) | $PhSO_2$ | $CO_2H$ |
| (XXXII) | Cbz | $CO_2H$ |
| (XXXIII) | Cbz | $CO_2Me$ |
| (XXXIV) | Cbz | $CH_2OH$ |



| | R | R′ |
|---|---|---|
| (XXXV) | Ts | $CO_2H$ |
| (XXXVI) | Ts | $CO_2Me$ |
| (XXXVII) | Ts | $CONH_2$ |
| (XXXVIII) | $MeSO_2$ | $CO_2Me$ |



| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| (XXXIX) | Ts | $CO_2Me$ | Ts |
| (XL) | $PhSO_2$ | $CONH_2$ | $PhSO_2$ |

of the *trans*-series requires attack from the unhindered face of the heterocyclic ring opposite to the $CO_2Me$ group. Hydrolysis of (XIV) and detosylation gave *trans*-4-chloro-L-proline (XII), but in this case the intermediate *N*-tosyl-4-chloro acid (XIII) was very hygroscopic and difficult to manipulate.

Similar reactions led to both isomers of 4-bromo-L-proline. The *trans*-4-hydroxy derivative (III) was converted into the *N*-tosyl-*cis*-4-bromoproline methyl ester (XXIII) in 60% yield using phosphorus pentabromide in chloroform, and 14% yield with phosphorus tribromide in benzene. Removal of the protecting groups gave *cis*-4-bromo-L-proline (XXI) via the *N*-tosyl bromo acid (XXII).

Phosphorus pentabromide and the *cis*-4-hydroxy derivative (VIII) gave a 64% yield of the *trans*-4-bromo ester (XX), from which *trans*-4-bromo-L-proline (XVIII) was prepared in excellent yield via the *N*-tosyl bromo acid (XIX).

Other studies leading to the 4-bromo series involved the *trans*- and *cis*-*N*,*O*-ditosyl-4-hydroxy-L-proline methyl esters (XXXVI) and (XXXIX). These known compounds[5] were prepared here by treatment of the *N*-tosyl-4-hydroxy esters (III) and (VIII) with tosyl chloride in pyridine. The action of lithium bromide in acetone for 3 days on both (XXXVI) and (XXXIX) gave a 70% conversion into the 4-bromo series, but in each case the product was a mixture of the *cis* epimer (XXIII) and *trans* epimer (XX) in the ratio *c.* 2 : 5 respectively. Although the substitution of *O*-tosyl by bromide can be expected to proceed with inversion, further displacement of the 4-bromo atom by the excess of bromide leads to an equilibrium mixture of epimers. This is analogous to the displacement of *O*-tosyl groups by iodide ion in the *N*-Cbz*-4-hydroxyproline series.[5] Monitoring of the lithium bromide reactions by thin-layer chromatography revealed that the equilibration reaction proceeds at a similar rate to the *O*-tosyl displacement reaction so that this route cannot yield a single 4-bromo epimer. On the other hand the composition of the product mixture must be near the true thermodynamic equilibrium ratio.

So far the stereochemical assignment of these 4-haloprolines rests not on direct proof but on the expectation that the mechanism of displacement of secondary hydroxyl under the conditions used will follow its normal $S_N2$ course. Other observations support the assignments. Firstly, the higher relative yields in the displacement steps leading to *trans*-isomer (less hindered attack) rather than *cis*-isomer, and secondly, the greater relative proportion of the less hindered *trans*-4-bromo epimer in the thermodynamically equilibrated mixture of (XX) and (XXIII). Nuclear magnetic resonance spectra have been recorded for all the compounds in this paper in the expectation that correlations defining the *cis* and *trans* stereochemical families would emerge. This hope has been justified and the results fully confirm the stereochemistry already allotted. The detailed spectroscopic analyses and the ramifications concerning coupling constants and the Karplus equation in this series will be published separately.

The four new halogenated free amino acids (XII), (XV), (XVIII), and (XXI) are all well-crystalline, stable compounds, and are not hygroscopic.

Two obvious routes for the synthesis of 4-aminoprolines are $S_N2$ displacement of a suitable leaving group by a nitrogenous nucleophile, and reduction of the oxime

* Cbz = benzyloxycarbonyl.

of a protected 4-oxoproline. Success has been achieved by both routes but only after extensive variation of reaction conditions and protecting groups.

The use of ammonia as a nucleophile was unsatisfactory. When *trans*-4-bromoproline (XVIII) was treated with concentrated ammonium hydroxide, bromide ion was released quantitatively, but the product obtained was shown to be the hydrobromide of the hitherto unknown *cis*-4-hydroxyprolinamide (VI). This crystalline salt was too hygroscopic to be recrystallized, and the free base (VI) liberated by ion-exchange techniques was crystalline but unstable. It was characterized as its *N*,*O*-dibenzenesulphonyl derivative (XL). Proof of this unforeseen conversion of (XVIII) into (VI) was provided by an unequivocal synthesis of (XL). The known *N*-benzenesulphonyl-*trans*-4-hydroxy-L-proline (V)[7] was converted into its *cis* epimer (IX) by chromic acid oxidation to the keto acid (XXXI) followed by stereospecific sodium borohydride reduction. Diazomethane and methanol/ammonia gave in turn the *N*-benzenesulphonyl-*cis*-4-hydroxy ester (X) and amide (XI). The latter compound was not isolated but was treated directly with benzenesulphonyl chloride in aqueous base to yield authentic (XL).

Formation of *cis*-4-hydroxyprolinamide from *trans*-4-bromoproline and ammonium hydroxide is considered to take place by the following mechanism. Rather than $S_N2$ replacement of Br by $NH_2$, neighbouring group displacement by the *trans*-carboxylate anion occurs to yield the lactone (XXVI) which then undergoes ammonolysis to the hydroxy amide (VI). Provided the bromide displacement by carboxylate is a synchronous $S_N2$ process, this reaction supplies a chemical proof of stereochemistry for the 4-bromoprolines. Analogies exist for $\gamma$-lactone formation by intramolecular displacement of a *trans*-4-*O*-tosyl group by carboxylate anion.[8] There is negative evidence in our case to support the $S_N2$ mechanism over the alternative of $S_N1$ release of bromide ion followed by carboxylate attack on the C4 carbonium ion. When *cis*-4-bromoproline (XXI) was treated with ammonium hydroxide as for (XVIII), bromide ion was again released quantitatively, but the reaction product was a dark oil yielding no crystalline free base or solid derivative after benzenesulphonylation. Had an $S_N1$ mechanism operated with this epimer, some (VI) should have been formed and been isolable as (XL). There are always several competing reactions in the ammonolysis of halo acids to amino acids. Such side reactions do not prevent the process being a general method for the preparation of $\alpha$-amino acids from $\alpha$-halo acids, but they dominate our experiments.

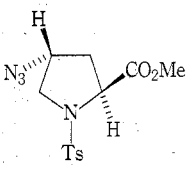
Displacement reactions with ammonia on the *N*,*O*-ditosyl methyl ester (XXXVI) were also unsuccessful, despite the fact that *O*-tosyl is typically a better leaving group than bromide[9] and that no complications due to carboxylate anion are possible. When (XXXVI) was treated with a solution of ammonia in methanol under mild pressure, only *N*,*O*-ditosyl-4-hydroxyprolinamide (XXXVII) was isolated. Repetitions of this reaction at pressures up to 100 atm and temperatures up to 100° failed to cause any displacement at C4. Neither did the reaction between (XXXVI) and sodium amide in liquid ammonia.

[7] Milne, H. B., and Peng, C.-H., *J. Am. chem. Soc.*, 1957, **79**, 639.

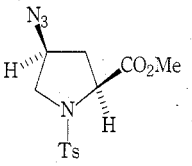
[8] Patchett, A. A., and Witkop, B., *J. Am. chem. Soc.*, 1957, **79**, 185.

[9] Gould, E. S., "Mechanism and Structure in Organic Chemistry." p. 261. (Holt, Rinehart & Winston: New York 1959.)

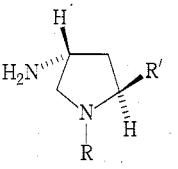
Azide ion, a more potent nucleophile, was satisfactory. Displacement with azide ion proceeds with inversion although the detailed mechanism probably involves an ion-pair intermediate rather than the classical $S_N2$ transition state.[10] The *trans*-*N*,*O*-ditosyl methyl ester (XXXVI) reacted with sodium azide in moist dimethylformamide to give the stable, crystalline, *N*-tosyl-*cis*-4-azido methyl ester (XLII) in 88% yield. The analogous reaction using exactly the same conditions on the corresponding 4-*O*-methanesulphonyl compound (XXXVIII), an oil prepared from (III) in the usual way, gave only a 54% yield of (XLII). Catalytic hydrogenation of the azide



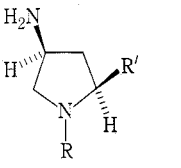
(XLI)



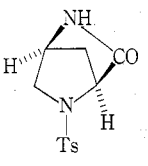
(XLII)



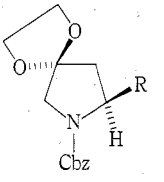
| | R | R′ |
|---|---|---|
| (XLIII) | H | $CO_2H$ |
| (XLIV) | Ts | $CO_2H$ |
| (XLV) | Ts | $CO_2Me$ |



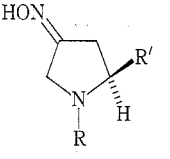
| | R | R′ |
|---|---|---|
| (XLVI) | H | $CO_2H$ |
| (XLVII) | Ts | $CO_2H$ |
| (XLVIII) | Ts | $CO_2Me$ |



(XLIX)



(L) R = $CO_2Me$
(LI) R = $CH_2OH$



| | R | R′ |
|---|---|---|
| (LII) | Ts | $CO_2H$ |
| (LIII) | Ts | $CO_2Me$ |
| (LIV) | Cbz | $CO_2Me$ |
| (LV) | Cbz | $CH_2OH$ |

group in (XLII) yielded the expected *N*-tosyl-*cis*-4-amino ester (XLVIII) as an unstable viscous oil which was hydrolysed by alkali at room temperature to *N*-tosyl-*cis*-4-amino-L-proline (XLVII), a solid of high melting point. Reductive cleavage of the *N*-tosyl residue with hydrobromic acid/acetic acid gave *cis*-4-amino-L-proline (XLVI). This new dimino acid is crystalline, but is hygroscopic and discolours in air in a few days. Its spectroscopic properties are in full accord with the assigned structure. The hydrobromide salt is hygroscopic, and although the hydrochloride can be manipulated more readily it is not stable. Satisfactory analyses for the expected dihydrochloride have not been obtained. Repeated recrystallization causes it to decompose with formation of ammonium chloride. The free diamino acid is best preserved under nitrogen in sealed vessels after purification by ion-exchange methods.

[10] Weiner, H., and Sneen, R. A., *J. Am. chem. Soc.*, 1962, **84**, 3599; 1965, **87**, 292; Weiner, H., and Sneen, R. A., *Tetrahedron Lett.*, 1963, 1309; Larsen, J. W., and Sneen, R. A., *J. Am. chem. Soc.*, 1966, **88**, 2593.

Direct confirmation of the *cis* stereochemistry was sought by cyclization reactions which would yield the *N*-tosyl lactam (XLIX). A variety of intramolecular cyclization attempts on the 4-amino ester (XLVIII) failed; the natural instability of this oil is apparently due to intermolecular polymerization. Attempts to induce cyclization of *N*-tosyl-*cis*-4-aminoproline (XLVII) with dicyclohexylcarbodiimide in organic solvents were also unsuccessful and the lactam (XLIX) was finally obtained by dehydration of (XLVII) in an aqueous medium using the water-soluble *N*-cyclohexyl-*N'*-(β-morpholinyl-4-ethyl)carbodiimide methyl *p*-toluenesulphonate. The product crystallized directly out of the aqueous reaction mixture.

The synthesis of *trans*-4-amino-L-proline paralleled that of the *cis* isomer. Displacement of the 4-*O*-tosyl group in the *cis*-*N*,*O*-ditosyl ester (XXXIX) by azide ion also proceeded in 88% yield to the crystalline *trans*-azido ester (XLI). Hydrogenation of (XLI) generated the oily, unstable *trans*-4-amino ester (XLV). Removal of protecting groups as before gave *N*-tosyl-*trans*-4-amino-L-proline (XLIV) and finally *trans*-4-amino-L-proline (XLIII). The stabilities of this compound and its hydrochloride and hydrobromide salts are similar to those of the *cis* compounds.

In other experiments it was found that the halogen atom in both *cis*- and *trans*-*N*-tosyl-4-chloroproline methyl esters (XVII) and (XIV) was not displaceable by azide ion, and that, although a reaction occurred when the *N*,*O*-ditosyl acid (XXXV) was treated with sodium azide in wet dimethylformamide, no azide absorption was present in the infrared spectrum of the reaction product.

*cis*-4-Aminoproline is also accessible, but in lower yield, by a route involving reduction of a 4-oximino group. *N*-Tosyl-4-oxoproline (XXVIII) readily formed the crystalline oxime (LII). Catalytic hydrogenation with platinum in ethanol/hydrochloric acid proceeded stereospecifically from the face of the proline ring opposite to the hindering carboxyl group and yielded (55%) the hygroscopic solid hydrochloride of *N*-tosyl-*cis*-4-aminoproline. Removal of HCl on an ion-exchange column gave the free base (XLVII), identical with that obtained above from the azide approach. The compound was further characterized as the hydrochloride of its methyl ester (XLVIII).

Problems associated with the choice of protecting groups are illustrated by the following results. The solid oxime (LIII) of *N*-tosyl-4-oxoproline methyl ester (XXIX) did not afford any satisfactory product despite an extensive series of reductive reactions including catalytic hydrogenation. This 4-keto ester (XXIX) was prepared from *N*-tosyl-*trans*-4-hydroxy-L-proline (II) both by oxidation to the keto acid (XXVIII) followed by esterification, and by the reverse order of reactions via the hydroxy ester (III); the former sequence gave a much higher overall yield. *N*-Tosyl-4-oxoprolinamide (XXX), prepared from the 4-hydroxy ester (III) by ammonolysis to the hydroxy amide (IV) followed by oxidation with chromic acid, not only failed to form an oxime, but decomposed under the reaction conditions.

*N*-Benzyloxycarbonyl was not useful as a protecting group for the oxime route. *N*-Benzyloxycarbonyl-4-oxo-L-proline (XXXII)[8] failed to yield an oxime, in contrast to the easy formation of the *N*-tosyl analogue (LII). The methyl ester (XXXIII) of the acid (XXXII) was prepared but it was an unstable oil. However,

it did yield the *N*-Cbz oxime (LIV), also as a relatively unstable oil. Like its crystalline *N*-tosyl relative (LIII) it failed to give any satisfactory product upon catalytic hydrogenation under a variety of conditions. On the other hand, sodium borohydride in buffered propan-2-ol reduced (LIV) to a white crystalline product having the correct molecular formula for *N*-benzyloxycarbonyl-4-amino-proline. Its spectroscopic properties did not, however, correspond to those expected for this amino compound, nor was it soluble in aqueous acid. The i.r. spectrum showed bands more in keeping with O–H rather than N–H stretching frequencies and no carbonyl absorption for an ester or a carboxyl group. Only two exchangeable protons were observable in the n.m.r. spectrum, and the other bands indicated the compound to be *N*-benzyloxycarbonyl-4-oxoprolinol oxime (LV), an isomer of *N*-benzyloxycarbonyl-4-aminoproline. An independent synthesis confirmed this structure. *N*-Benzyloxycarbonyl-4-oxoproline methyl ester (XXXIII) was converted into its ketal (L). Lithium borohydride reduction of the ester group in (L) yielded the ketalized prolinol (LI). Acid hydrolysis gave the free ketone (XXXIV) which on treatment with hydroxylamine hydrochloride in pyridine produced authentic (LV) identical to the sample above. Reduction of esters to alcohols by sodium borohydride is uncommon, but has been observed for molecules having electron-withdrawing α-substituents.[11]

## Experimental

Melting points were determined on a Büchi melting point apparatus, and are uncorrected. Infrared spectra were recorded on a Perkin–Elmer 221 instrument as Nujol mulls unless otherwise indicated. Rotations were determined on a Hilger Mark III polarimeter with a 0·5-dm cell. Spence type H and neutral Woelm aluminas were used for chromatography. Microanalyses were carried out by Miss B. J. Stevenson of this Department and by the Australian Microanalytical Service, Melbourne. Nuclear magnetic resonance spectra have been recorded on a Varian Associates A60 instrument for all compounds described. In every case only absorption of expected chemical shift and area was observed. As implied above, the analysis of fine structure is complicated and the n.m.r. results form the subject matter of another paper. Microanalyses were not done on compounds (XI), (XIII), (XXXVIII), (XLIII), (XLV), (XLVI), and (XLVIII) because of difficulties in manipulation, and their n.m.r. spectra provided both the main criterion of purity, and the most direct structure confirmation. Identity of samples prepared by alternative routes was established by m.p., mixed m.p., and superposability of their i.r. and n.m.r. spectra.

### (*a*) N-*Tosyl*-trans-*4-hydroxy*-L-*proline Methyl Ester* (*III*)

A solution of *N*-tosyl-*trans*-4-hydroxyl-L-proline[3] (II) (11·4 g) was treated with an excess of an ethereal solution of diazomethane. The reaction mixture was worked up in the usual way. N-*Tosyl*-trans-*4-hydroxy*-L-*proline methyl ester* crystallized from benzene as white needles (12·0 g, 90%), m.p. 103–104°, $[\alpha]_D^{20}$ −98·5° (1% in $CHCl_3$) (Found: C, 52·2; H, 5·8. $C_{13}H_{17}NO_5S$ requires C, 52·2; H, 5·7%). $\nu_{max}$ 3500, 1750 $cm^{-1}$.

### (*b*) N-*Tosyl*-cis-*4-chloro*-L-*proline Methyl Ester* (*XVII*)

(i) Phosphorus pentachloride (4·5 g, 0·025 mole) was stirred into a solution of the ester (III) (3 g, 0·01 mole) in A.R. chloroform (30 ml). The reaction mixture was warmed gently, and, when all the phosphorus pentachloride had dissolved, it was heated under reflux for 0·5 hr. It was then cooled, diluted with chloroform (150 ml), washed with 5% sodium carbonate solution,

[11] House, H. O., "Modern Synthetic Reactions." p. 32. (Benjamin: New York 1965.); Gaylord, N. G., "Reduction with Complex Metal Hydrides." p. 500. (Interscience: New York 1956.)

brine, and dried ($MgSO_4$). The solvent was removed and the residue was purified by chromatography over deactivated alumina. N-*Tosyl*-cis-*4-chloro*-L-*proline methyl ester* crystallized from benzene/cyclohexane as white needles (2·65 g, 83%), m.p. 96·5–97·5°, $[\alpha]_D^{20}$ −46·6° (1·5% in $CHCl_3$) (Found: C, 49·2; H, 5·2; Cl, 10·9. $C_{13}H_{16}ClNO_4S$ requires C, 49·1; H, 5·1; Cl, 11·2%).

(ii) The ester (III) (3·0 g, 0·01 mole) was treated with dry pyridine (2 ml) followed by thionyl chloride (30 ml). The solution was heated under reflux for 2 hr and the excess of thionyl chloride was removed under reduced pressure. The residue was extracted with ether and the combined ethereal extracts were washed with brine and dried ($MgSO_4$). Removal of the solvent and crystallization of the yellow residue from benzene/cyclohexane gave the *cis*-chloro ester (XVII) as white needles (1·7 g, 54%), m..p 98–99°.

(iii) The finely powdered ester (III) (6·0 g) was suspended in dry ether (400 ml). The reaction mixture was refluxed under dry nitrogen until the supernatant was saturated. A suspension of 50% sodium hydride in paraffin oil (1·0 g) was added and the reaction mixture was heated under reflux for 1 hr. About half the volume of ether was removed by distillation. The white slurry produced was cooled to room temperature and trichloroacetonitrile (6 ml) was added to it with vigorous stirring. Chloroform (10 ml) was then introduced and the reaction mixture was stirred at room temperature for 3 hr before being filtered. Removal of the solvents from the filtrate gave a yellow oil which was dissolved in benzene/cyclohexane (1 : 1) and chromatographed over deactivated alumina. Elution with benzene/cyclohexane (5 : 1) gave N-*tosyl*-trans-*4-trichloroacetimidoxy*-L-*proline methyl ester* (XXIV) which crystallized from benzene/cyclohexane as colourless needles (6·95 g, 78·5%), m.p. 104–105°, $[\alpha]_D^{20}$ −78·5° (1·7% in $CHCl_3$) (Found: C, 40·8; H, 3·9; Cl, 23·9; N, 6·4. $C_{15}H_{17}Cl_3N_2O_5$ requires C, 40·6; H, 3·9; Cl, 24·0; N, 6·3%). $\nu_{max}$ ($CHCl_3$) 3340, 1745, 1665 $cm^{-1}$.

The foregoing compound (XXIV) (2·2 g) was dissolved in dry ether (100 ml) and a stream of pure dry nitrogen was bubbled through the solution. After 15 min the nitrogen stream was replaced by a slow stream of dry HCl gas. A white material which separated out during the first few minutes almost completely disappeared after 5 hr. The reaction mixture was heated under reflux for 3 hr and a stream of nitrogen was then passed through the turbid solution to remove excess of HCl. The ethereal solution was washed repeatedly with brine until neutral, and then dried ($MgSO_4$). The solvent was removed and the crystalline residue obtained was treated with cold ether (15 ml) and filtered from undissolved trichloroacetamide (0·44 g, 61%). Concentration of the filtrate and chromatography of the residue over deactivated alumina gave two fractions. Fraction 1 (eluted with benzene/cyclohexane, 4 : 1) gave *N*-tosyl-*cis*-4-chloro-L-proline methyl ester which crystallized from benzene/cyclohexane as colourless needles (0·86 g, 54%), m.p. 98–99°. Fraction 2 (eluted with benzene/ether, 1 : 1) gave *N-tosyl*-trans-*4-trichloroacetoxy*-L-*proline methyl ester* (XXV) which crystallized from benzene as colourless needles (0·67 g, 30%), m.p. 127–128°, $[\alpha]_D^{20}$ −82·8° (1·7% in $CHCl_3$) (Found: C, 40·8; H, 3·7; Cl, 23·9. $C_{15}H_{16}Cl_3NO_6S$ requires C, 40·5; H, 3·6; Cl, 23·5%). $\nu_{max}$ ($CHCl_3$) 1760, 1750 $cm^{-1}$.

(*c*) N-*Tosyl*-trans-*4-trichloroacetoxy*-L-*proline Methyl Ester* (*XXV*)

*N*-Tosyl-*trans*-4-trichloroacetimidoxy-L-proline methyl ester (XXIV) (0·44 g) was dissolved in ether (50 ml) and to this solution was added with stirring over 4 hr a 10% solution of acetic acid (50 ml). The ethereal phase was separated, washed with brine, and dried ($MgSO_4$). Removal of solvent and crystallization of the residue from benzene gave the trichloroacetate (XXV) as colourless needles (0·32 g, 72%), m.p. 127–128°.

(*d*) N-*Tosyl*-cis-*4-chloro*-L-*proline* (*XVI*)

*N*-Tosyl-*cis*-4-chloro-L-proline methyl ester (XVII) (3·2 g) in methanol (25 ml) was stirred at 0° while 1M NaOH (11 ml) was slowly added (10 min). After 1 hr the ice-bath was removed, and the reaction mixture was stirred at room temperature until all the solid had dissolved (3 hr). Methanol was removed under vacuum and the solution was cooled to 0° and acidified with 1M HCl. The resulting crystalline precipitate was collected and washed with a little ice-water. Crystallization from ethanol/water gave N-*tosyl*-cis-*4-chloro*-L-*proline* as colourless needles (2·9 g,

95%), m.p. 174–175°, $[\alpha]_D^{20}$ −49·6 (2% in EtOH) (Found: C, 47·4; H, 4·7; Cl, 11·5; N, 4·3. $C_{12}H_{14}ClNO_4S$ requires C, 47·4; H, 4·7; Cl, 11·5; N, 4·3%).

(*e*) cis-*4-Chloro*-L-*proline* (*XV*)

*N*-Tosyl-*cis*-4-chloro-L-proline (XVI) (2·8 g, 0·01 mole) and phenol (2·0 g, 0·02 mole) were dissolved in acetic acid containing hydrogen bromide (45%, 20 ml). The reaction flask was sealed and allowed to stand at room temperature for 26 hr.[12] The reaction mixture was poured into a stirred flask of dry ether (200 ml). The white, crystalline precipitate formed was filtered off, dissolved in water (50 ml), and adsorbed on a Dowex 50 cation-exchange column in the $H^+$ form. The column was washed thoroughly with distilled water and the amino acid was eluted with 0·4M $NH_4OH$. Removal of water under vacuum and crystallization of the residue from water/acetone gave cis-*4-chloro*-L-*proline* as white prisms (1·35 g, 98%), m.p. 224–225° (dec.), $[\alpha]_D^{20}$ −29·5° (1·5% in $H_2O$) (Found: C, 40·4; H, 5·4; Cl, 23·1; N, 9·5. $C_5H_8ClNO_2$ requires C, 40·2; H, 5·4; Cl, 23·7; N, 9·5%).

(*f*) N-*Tosyl-4-oxo*-L-*proline* (*XXVIII*)

Chromium trioxide in sulphuric acid solution[13] (32 ml) was added dropwise to a stirred solution of *N*-tosyl-*trans*-4-hydroxy-L-proline (II) (8·64 g) in A.R. acetone (150 ml), care being taken to ensure that the reaction temperature did not rise above 30°. Stirring was continued for a further 1 hr and excess of oxidant was destroyed by careful addition of methanol. Precipitated chromium salts were removed by filtration. The filtrate was concentrated (50 ml), diluted with chloroform (150 ml), washed with water (2 × 50 ml), and dried ($NaSO_4$). Removal of solvent gave N-*tosyl-4-oxo*-L-*proline* as a white solid which crystallized from ethyl acetate as white needles (7·1 g, 84%), m.p. 176–178°, $[\alpha]_D^{20}$ −1·2° (1% in EtOH) (Found: C, 50·7; H, 4·5; N, 4·8. $C_{12}H_{13}NO_5S$ requires C, 50·8; H, 4·6; N, 5·0%). $\nu_{max}$ 1750, 1710 $cm^{-1}$.

(*g*) N-*Tosyl*-cis-*4-hydroxy*-L-*proline* (*VII*)

The keto acid (XXVIII) (9·5 g, 0·034 mole) was dissolved in methanol (200 ml) and the solution was cooled to 0°. A cold solution of sodium borohydride (4 g) in water (15 ml) was added dropwise with stirring. The reaction mixture was left at 0° for 1 hr and the methanol was then removed. The residue was treated with 1M NaOH (70 ml) at room temperature for 0·5 hr and a clear solution resulted. This was cooled to 0°, acidified with 10M HCl, and extracted with ethyl acetate (3 × 100 ml). The combined ethyl acetate extracts were washed with brine and dried ($MgSO_4$). On reduction of volume, *N*-tosyl-*cis*-4-hydroxy-L-proline crystallized as shiny white prisms (8·6 g, 90%), m.p. 145–146°, $[\alpha]_D^{20}$ −74·8° (2·5% in EtOH) (lit.[5] m.p. 146–147°, $[\alpha]_D^{20}$ −43·9±1·0° (1% in EtOH)).

(*h*) N-*Tosyl*-cis-*4-hydroxy*-L-*proline Lactone* (*XXVII*)

The foregoing *cis*-hydroxy acid (VII) (0·86 g, 0·003 mole) was dissolved in methylene chloride (100 ml) and stirred with anhydrous magnesium sulphate (0·5 g) for 30 min. *N,N'*-Dicyclohexylcarbodiimide (0·62 g, 0·003 mole) was added and after 24 hr the reaction mixture was filtered. The residue obtained by evaporation of the filtrate was dissolved in benzene (15 ml) and chromatographed on deactivated alumina. The *lactone* (XXVII) crystallized from benzene/cyclohexane as colourless prisms (0·46 g, 57%), m.p. 106–107°, $[\alpha]_D^{20}$ +27·7° (3% in $CHCl_3$) (Found: C, 54·0; H, 5·0; N, 5·1. $C_{12}H_{13}NO_4S$ requires C, 54·0; H, 4·7; N, 5·2%). $\nu_{max}$ ($CHCl_3$) 1800 $cm^{-1}$.

(*i*) N-*Tosyl*-cis-*4-hydroxy*-L-*proline Methyl Ester* (*VIII*)

The *cis*-hydroxy acid (VII) (5·8 g) in methanol (50 ml) was esterified at 0° with excess of ethereal diazomethane. The reaction mixture was worked up in the usual way, and the *cis*-hydroxy

[12] Weisblat, D. I., Magerlein, B. J., and Myers, D. R., *J. Am. chem. Soc.*, 1953, **75**, 3630.
[13] Bladon, P., Fabian, J. M., Henbest, H. B., Koch, H. P., and Wood, G. W., *J. chem. Soc.*, 1951, 2407.

ester (VIII) crystallized from benzene as white prisms (5·5 g, 90%), m.p. 100–101° raised to m.p. 104° by recrystallization, $[\alpha]_D^{20}$ −69·0° (3% in $CHCl_3$) (lit.[5] m.p. 103–104°, $[\alpha]_D^{20}$ −66·5±1·0° (1% in $CHCl_3$)).

(*j*) N-*Tosyl*-trans-*4-chloro*-L-*proline Methyl Ester* (*XIV*)

(i) Treatment of the *cis*-hydroxy ester (VIII) (3 g) with thionyl chloride and pyridine as in (*b*)(ii) gave N-*tosyl*-trans-*4-chloro*-L-*proline methyl ester* which crystallized from benzene/cyclohexane as white needles (2·9 g, 92%), m.p. 113–114°, $[\alpha]_D^{20}$ −110·3° (1·8% in $CHCl_3$) (Found: C, 49·0; H, 5·0; N, 4·5. $C_{13}H_{16}ClNO_4S$ requires C, 49·1; H, 5·1; N, 4·4%).

(ii) Treatment of the *cis*-hydroxy ester (VIII) (3 g) with phosphorus pentachloride in chloroform as in (*b*)(i) gave the *trans*-chloro compound which crystallized from benzene/cyclohexane as white needles (2·8 g, 88%), m.p. 113–114°.

(*k*) trans-*4-Chloro*-L-*proline* (*XII*)

The *trans*-chloro ester (XIV) (4·8 g) was hydrolysed as described above in (*d*). N-*Tosyl*-trans-*4-chloro*-L-*proline* (XIII) was obtained as a hygroscopic solid (4·5 g, 98%) which was very difficult to manipulate.

Reductive detosylation of this compound (XIII) was carried out as described above in (*e*). trans-*4-Chloro*-L-*proline* crystallized from water/acetone as white prisms [1·65 g, 73% from (XII)], m.p. 194–195° (dec.), $[\alpha]_D^{20}$ −55·6° (2% in $H_2O$) (Found: C, 40·4; H, 5·5; Cl, 22·9; N, 9·5. $C_5H_8ClNO_2$ requires C, 40·2; H, 5·4; Cl, 22·9; N, 9·5%).

(*l*) N-*Tosyl*-cis-*4-bromo*-L-*proline Methyl Ester* (*XXIII*)

(i) *N*-Tosyl-*trans*-4-hydroxy-L-proline methyl ester (III) (3·0 g, 0·01 mole) was dissolved in dry benzene (30 ml) and phosphorus tribromide (2·0 g, 0·007 mole) was added to this solution dropwise over a period of 10 min. The reaction mixture was allowed to stand at room temperature for 2 hr and then heated under reflux for 1 hr. It was cooled, washed with cold water, 5% $Na_2CO_3$, brine, and dried ($MgSO_4$). Solvent was removed and the residue was chromatographed over deactivated alumina. Two fractions were obtained. Fraction 1 (eluted with benzene/chloroform, 10 : 1) gave N-*tosyl*-cis-*4-bromo*-L-*proline methyl ester* which crystallized from benzene/cyclohexane as white needles (0·5 g, 14%), m.p. 78–79°, $[\alpha]_D^{20}$ −36·9° (2% in $CHCl_3$) (Found: C, 43·3; H, 4·6; Br, 22·3; N, 3·9. $C_{13}H_{16}BrNO_4S$ requires C, 43·1; H, 4·5; Br, 22·1; N, 3·9%). Fraction 2 (eluted with chloroform) gave the starting material (III) (2·1 g), m.p. 103–104°.

(*ii*) Phosphorus pentabromide (5·0 g, 0·01 mole) was slowly added with stirring and warming to a solution of the *trans*-hydroxy compound (III) (3·0 g, 0·01 mole) in A.R. chloroform (30 ml). When all the reagent had dissolved the flask was sealed, left at room temperature for 3 hr, and then heated to 60° for 0·5 hr. The reaction mixture was cooled, washed with 5% $Na_2CO_3$, brine, and then dried ($MgSO_4$). The solvent was removed and the residue was purified by chromatography over deactivated alumina. The *cis*-bromo compound (XXIII) crystallized from benzene/cyclohexane as white needles (2·2 g, 60%), m.p. 78–79°.

(*m*) cis-*4-Bromo*-L-*proline* (*XXI*)

The hydrolysis of the *cis*-bromo compound (XXIII) (4·35 g) was performed as described in (*d*). N-*Tosyl*-cis-*4-bromo*-L-*proline* (XXII) crystallized from acetone/ethanol/water as white prisms (3·85 g, 92%), m.p. 172–173°, $[\alpha]_D^{20}$ −34·3° (2% in EtOH) (Found: C, 41·7; H, 4·3; Br, 23·1. $C_{12}H_{14}BrNO_4S$ requires C, 41·4; H, 4·1; Br, 23·0%).

Reductive detosylation of compound (XXII) was carried out as described in (*e*). cis-*4-Bromo*-L-*proline* crystallized from water/ethanol/acetone as white needles (1·9 g, 96%), m.p. 167–168° (dec.), $[\alpha]_D^{20}$ −17·5° (2·5% in $H_2O$) (Found: C, 30·9; H, 4·2; Br, 41·4; N, 7·0. $C_5H_8BrNO_2$ requires C, 31·0; H, 4·2; Br, 41·2; N, 7·2%).

(*n*) N-*Tosyl*-trans-*4-bromo*-L-*proline Methyl Ester* (*XX*)

Synthesis of this compound from *N*-tosyl-*cis*-4-hydroxy-L-proline methyl ester (VIII) (6 g) and phosphorus pentabromide was carried out as described in (*l*)(ii) to give the trans-

*bromo ester* (XX) as white needles (4·65 g, 64%), m.p. 93–94°, $[\alpha]_D^{20}$ −90·3% (1·5% in $CHCl_3$) (Found: C, 43·5; H, 4·7; Br, 22·2; N, 3·8. $C_{13}H_{16}BrNO_4S$ requires C, 43·1; H, 4·5; Br, 22·1; N, 3·9%).

(*o*) trans-*4-Bromo*-L-*proline* (*XVIII*)

Hydrolysis of the *trans*-bromo ester (XX) (4·35 g) was accomplished as described in (*d*). N-*Tosyl*-trans-*4-bromo*-L-*proline* (XIX) crystallized from chloroform/light petroleum as colourless needles (4·0 g, 96%), m.p. 119–120°, $[\alpha]_D^{20}$ −92·5 (2% in EtOH) (Found: C, 41·2; H, 4·2; Br, 23·1; N, 4·1. $C_{12}H_{14}BrNO_4S$ requires C, 41·3; H, 4·1; Br, 23·0; N, 4·0%).

The reductive detosylation of compound (XIX) was performed as described in (*e*). trans-*4-Bromo*-L-*proline* crystallized from water/ethanol/acetone as white needles (1·9 g, 96%), m.p. 166–167° (dec.), $[\alpha]_D^{20}$ −38·2 (2% in $H_2O$) (Found: C, 31·0; H, 4·3; Br, 41·1; N, 7·0. $C_5H_8BrNO_2$ requires C, 31·0; H, 4·2; Br, 41·1; N, 7·2%).

(*p*) N,O-*Ditosyl*-trans-*4-hydroxy*-L-*proline Methyl Ester* (*XXXVI*)

*N*-Tosyl-*trans*-4-hydroxy-L-proline methyl ester (III) (8·5 g, 0·028 mole) was dissolved in dry A.R. pyridine (25 ml) and the solution was chilled in an ice-bath. A cold solution of *p*-toluenesulphonyl chloride (5·7 g, 0·03 mole) in dry A.R. pyridine (20 ml) was added and the reaction mixture was left at 0° for 3 days. Ice-cold 2M HCl (170 ml) was added and the resulting crystalline precipitate was collected and washed with 2M HCl (50 ml). Crystallization from ethyl acetate/ether (charcoal) gave *N,O*-ditosyl-*trans*-4-hydroxy-L-proline methyl ester as white needles (8·5 g, 66%), m.p. 95°, $[\alpha]_D^{20}$ −54·8° (2% in $CHCl_3$) (lit.[5] m.p. 94–95·5°, $[\alpha]_D^{20}$ −54·1±1·0° (1% in $CHCl_3$)).

(*q*) N,O-*Ditosyl*-cis-*4-hydroxy*-L-*proline Methyl Ester* (*XXXIX*)

Tosylation of the *cis*-4-hydroxy derivative (VIII) (4·5 g) was carried out as described in (*p*) to give the *cis*-ditosyl compound (XXXIX) as white needles (5·1 g, 75%), m.p. 94–95° raised to m.p. 98–99° by recrystallization, $[\alpha]_D^{20}$ −24·8° (2% in $CHCl_3$) (lit.[5] m.p. 123–124°, $[\alpha]_D^{20}$ −25·0±1·0° (1% in $CHCl_3$)) (Found: C, 53·0; H, 5·3. Calc. for $C_{20}H_{23}NO_7S_2$: C, 53·0; H, 5·1%).

(*r*) *Reaction of* trans- *and* cis-N,O-*Ditosyl-4-hydroxy*-L-*proline Methyl Esters with Lithium Bromide*

(i) The *trans*-ditosyl compound (XXXVI) (4·5 g, 0·01 mole) in acetone (30 ml) was treated with lithium bromide (2·5 g, 0·03 mole). The reaction mixture was refluxed for 3 days and the precipitated lithium *p*-toluenesulphonate (1·4 g) was filtered off. The filtrate was evaporated to dryness and the residue was dissolved in chloroform and chromatographed on alumina. Crystallization of the chromatographed material from benzene/cyclohexane gave as the first crop almost pure *N*-tosyl-*trans*-4-bromo-L-proline methyl ester (XX) (2·1 g) which was obtained pure after one further crystallization (1·9 g, 53%), m.p. 92–93°. The combined mother liquors gave, after addition of a little cyclohexane, *N*-tosyl-*cis*-4-bromo-L-proline methyl ester (XXIII) (0·9 g) which was obtained pure after one further crystallization (0·8 g, 21%), m.p. 77–78°.

(ii) The reaction of the *cis*-ditosyl compound (XXXIX) (4·5 g) with lithium bromide was carried out as described in (i) to give the *cis*-bromo ester (XXIII) (0·9 g, 25%), m.p. 77–78°, and the *trans*-bromo ester (XX) (1·8 g, 50%), m.p. 92°.

(*s*) *Reaction of* trans-*4-Bromo*-L-*proline with Aqueous Ammonia: Formation and Characterization of* (*VI*)

*trans*-4-Bromo-L-proline (XVIII) (0·5 g) was dissolved in conc. ammonium hydroxide (50 ml) and the reaction flask was sealed and allowed to stand at room temperature for 1 week. The solvent was removed under vacuum below 40° and the residual oil was dried over $P_2O_5$ under vacuum. After a few days the oil (0·56 g) began to crystallize in long needles. Attempts to recrystallize the extremely hygroscopic material failed. It was dissolved in water (10 ml) and adsorbed on a Dowex 50 cation-exchange column in the $H^+$ form. The column was washed with water until the eluate was neutral and then with 0·5M $NH_4OH$. The acidic water washings containing HBr were titrated with 0·1M NaOH. Consumption was 98% of the theoretically calculated

amount. The ammonium hydroxide eluate was concentrated under vacuum and the oily residue was dried under vacuum over $P_2O_5$. After 3 days the material started to crystallize in long plates (0·33 g). The compound was found to be unstable and therefore difficult to manipulate.

The foregoing compound (VI) (0·33 g) was dissolved in 1M NaOH (10 ml) and a solution of benzenesulphonyl chloride (1·0 g) in ether (15 ml) was added. The reaction mixture was shaken mechanically for 4 hr and the precipitated material was filtered, washed with ether, water, and then dried under vacuum. Crystallization from chloroform/light petroleum gave N,O-*dibenzenesulphonyl*-cis-*4-hydroxy*-L-*prolinamide* (XL) as white needles (0·84 g, 79% from (XVIII)), m.p. 184–185·5°, $[\alpha]_D^{25}$ −70·2° (1·8% in $CHCl_3$) (Found: C, 49·6; H, 4·6; N, 6·8. $C_{17}H_{18}N_2O_6S_2$ requires C, 49·7; H, 4·4; N, 6·8%). $\nu_{max}$ ($CHCl_3$) 3510, 3400, 1690 $cm^{-1}$.

(*t*) N-*Benzenesulphonyl*-cis-*4-hydroxy*-L-*proline Methyl Ester* (*X*)

*N*-Benzenesulphonyl-*trans*-4-hydroxy-L-proline (V) was prepared by a known method[7] in 85% yield, m.p. 150–151°, $[\alpha]_D^{20}$ −96·2° (2·5% in EtOH) (lit.[7] m.p. 143–144°) (Found: C, 48·9; H, 5·0; N, 5·1. Calc. for $C_{11}H_{13}NO_5S$: C, 48·7; H, 4·8; N, 5·2%).

Chromic acid oxidation of (V) (5·45 g) was carried out as described in (*f*). The *4-keto acid* (XXXI) crystallized from ethyl acetate/light petroleum as white needles (3·9 g, 73%), m.p. 175–176°, $[\alpha]_D^{20}$ −4·8° (2·5% in EtOH) (Found: C, 49·0; H, 4·3; N, 5·2. $C_{11}H_{11}NO_5S$ requires C, 49·0; H, 4·1; N, 5·2%).

Reduction of (XXXI) (4·05 g) with sodium borohydride was carried out as described in (*g*). The cis-*4-hydroxy derivative* (IX) crystallized from ethyl acetate/light petroleum as white needles (3·0 g, 74%), m.p. 113·5–114·5°, $[\alpha]_D^{20}$ −72·4 (2·5% in EtOH) (Found: C, 48·9; H, 5·1; N, 4·9. $C_{11}H_{13}NO_5S$ requires C, 48·7; H, 4·8; N, 5·2%).

Esterification of (IX) (2·7 g) was accomplished with diazomethane. N-*Benzenesulphonyl*-cis-*4-hydroxy*-L-*proline methyl ester* (X) crystallized from benzene/cyclohexane as colourless prisms (2·4 g, 85%), m.p. 104–105°, $[\alpha]_D^{25}$ −70·3° (2·6% in $CHCl_3$) (Found: C, 50·6; H, 5·5; N, 4·4. $C_{12}H_{15}NO_5S$ requires C, 50·5; H, 5·3; N, 4·9%).

(*u*) N,O-*Dibenzenesulphonyl*-cis-*4-hydroxy*-L-*prolinamide* (*XL*)

The ester (X) (1·43 g) was dissolved in methanol (20 ml) and the solution was added dropwise to liquid ammonia (40 ml) over a period of 20 min. The reaction mixture was stoppered and allowed to stand at room temperature for 2 days and then evaporated to dryness under vacuum to give a colourless material (1·3 g). Attempts to crystallize this *4-hydroxy amide* (XI) failed.

The compound (XI) (1·3 g) was dissolved in 1M NaOH (15 ml) and water (10 ml). To this solution benzenesulphonyl chloride (1·76 g) in ether (30 ml) was added, and the reaction mixture was shaken mechanically for 4 hr. The precipitated material was collected, dissolved in chloroform (100 ml), and the solution was dried ($MgSO_4$). Removal of solvent and crystallization of the residue from chloroform/light petroleum gave the prolinamide (XL) as white needles (1·72 g, 84% overall yield), m.p. 184·5–185·5°.

(*v*) *Reaction of* N,O-*Ditosyl*-trans-*4-hydroxy*-L-*proline Methyl Ester with Ammonia: Formation of* N,O-*Ditosyl*-trans-*4-hydroxy*-L-*prolinamide* (*XXXVII*)

Interaction of the ditosyl methyl ester (XXXVI) (1·2 g) with liquid ammonia was carried out as described in (*u*). Crystallization of the reaction product from methylene chloride gave the *prolinamide* (XXXVII) as white needles (0·94 g, 85%), m.p. 217·5° (Found: C, 52·2; H, 5·3; N, 6·2. $C_{19}H_{22}N_2O_5S_2$ requires C, 52·1; H, 5·1; N, 6·4%). $\nu_{max}$ 3445, 3320, 3270, 1670 $cm^{-1}$.

(*w*) N-*Tosyl*-cis-*4-azido*-L-*proline Methyl Ester* (*XLII*)

(i) The *trans*-N,O-ditosyl methyl ester (XXXVI) (3·0 g, 0·007 mole) was dissolved in dimethylformamide (30 ml), and sodium azide (0·6 g, 0·009 mole) in water (3 ml) was added. The reaction mixture was heated at 70° for 5 hr. It was cooled, and poured into a mixture of

saturated brine (500 ml) and water (100 ml), and extracted with ether (3 × 150 ml). The combined ether extracts were washed with saturated brine and dried ($MgSO_4$). Removal of the ether and crystallization of the residue from ether/light petroleum gave the cis-*azido ester* (XLII) as colourless needles (1·89 g, 88%), m.p. 69–70°, $[\alpha]_D^{21}$ −47·8° (2% in $CHCl_3$) (Found: C, 47·8; H, 5·1; N, 17·2. $C_{13}H_{16}N_4O_4S$ requires C, 48·1; H, 4·9; N, 17·3%). $\nu_{max}$ ($CHCl_3$) 2108, 1750 $cm^{-1}$.

(ii) The preparation of N-*tosyl*-O-*methanesulphonyl*-trans-*4-hydroxy*-L-*proline methyl ester* (XXXVIII) (5·1 g, 81%) from the *N*-tosyl methyl ester (III) (5·0 g) and methanesulphonyl chloride (2·0 g) was carried out as described in (*p*) for the *N,O*-ditosyl compound (XXXVI). The azide displacement reaction of this oily ester (XXXVIII) (2·0 g) was performed as in (i) above. The *cis*-azido ester (XLII) crystallized from ether/light petroleum as needles (0·93 g, 54%), m.p. 67–68°.

(*x*) N-*Tosyl*-cis-*4-amino*-L-*proline* (*XLVII*) *via the Azide Route*

The *cis*-azido ester (XLII) (1·0 g) in methanol (50 ml) was hydrogenated in the presence of 20% palladium on charcoal (0·12 g) at room temperature and 1 atm pressure. After 2 hr the catalyst was filtered off and the solvent was removed under vacuum to give N-*tosyl*-cis-*4-amino*-L-*proline methyl ester* (XLVIII) as a viscous oil (0·75 g, 82%), $n_D^{18}$ 1·6450. The compound was found to be very unstable and was hydrolysed immediately after preparation.

The 4-amino ester (XLVIII) (3·0 g) in methanol (100 ml) was hydrolysed with 2·5M NaOH (15 ml) at room temperature for 6 hr. The reaction mixture was then chilled and acidified with 2M HCl to pH 6. It was concentrated and partitioned between water (pH 2) and chloroform. The aqueous phase was percolated through a Dowex 50 cation-exchange column in the $H^+$ form. The column was washed with distilled water (250 ml) and eluted with 2M $NH_4OH$ containing 20% ethanol. Removal of solvent under vacuum from the ammoniacal eluate gave N-*tosyl*-cis-*4-amino*-L-*proline* as prisms (2·7 g, 94%), m.p. 278–280° (dec.). Crystallization from aqueous ethanol gave white needles, m.p. 284–285° (dec.), $[\alpha]_D^{20}$ −72° (0·4% in EtOH/$H_2O$) (Found: C, 50·2; H, 5·7; N, 9·9. $C_{12}H_{16}N_2O_4S$ requires C, 50·7; H, 5·6; N, 9·9%). $\nu_{max}$ 3580, 3360, 2750, 1610, 1580 $cm^{-1}$.

(*y*) cis-*4-Amino*-L-*proline* (*XLVI*)

The reductive detosylation of the amino compound (XLVII) (9·0 g) was carried out as described in (*e*) to give cis-*4-amino*-L-*proline* as a yellow solid (3·5 g, 85%). The diamino acid crystallized with difficulty from aqueous ethanol as white prisms, m.p. 191–193° (dec.), $[\alpha]_D^{20}$ −57·4° (3% in $H_2O$), $\nu_{max}$ 3400, 3310, 3220, 1600 $cm^{-1}$. It was found to be hygroscopic and unstable and could not be analysed satisfactorily. A mass spectrum confirmed the molecular weight of 130 (MS9 instrument). The *dihydrochloride* of (XLVI), m.p. 247°, prepared by evaporation of a solution in aqueous HCl, was also found to be unstable.

(*z*) N-*Tosyl*-cis-*4-amino*-L-*proline Lactam* (*XLIX*)

*N*-Cyclohexyl-*N'*-(β-morpholinyl-4-ethyl)carbodiimide methyl *p*-toluenesulphonate (0·465 g) was added to a stirred solution of N-tosyl-*cis*-4-amino-L-proline (XLVII) (0·285 g) in 30% aqueous ethanol (10 ml) at 70°, and the reaction mixture was maintained at this temperature for 7·5 hr. The crystalline product was filtered off, and the filtrate was extracted with chloroform (2 × 5 ml). Removal of solvent from the dried ($MgSO_4$) extract gave a white solid which was combined with the filtered crystals. Crystallization from ethanol/benzene gave the *lactam* (XLIX) as white prisms (0·15 g, 56%), m.p. 250–251°, $[\alpha]_D^{25}$ +26·7° (0·3% in EtOH) (Found: C, 53·9; H, 5·3; N, 10·2. $C_{12}H_{14}N_2O_3S$ requires C, 54·1; H, 5·3; N, 10·5%). $\nu_{max}$ 3220, 1710 $cm^{-1}$.

(*aa*) N-*Tosyl*-trans-*4-azido*-L-*proline Methyl Ester* (*XLI*)

Reaction of the *cis-N,O*-ditosyl ester (XXXIX) (3·0 g) with sodium azide in moist dimethylformamide was accomplished as described in (*w*)(i) to give the trans-*azido ester* (XLI) as white slender needles (1·9 g, 88·4%), m.p. 123°, $[\alpha]_D^{20}$ −45·5° (2% in $CHCl_3$) (Found: C, 48·2; H, 5·1; N, 17·4. $C_{13}H_{16}N_4O_4S$ requires C, 48·1; H, 4·9; N, 17·3%). $\nu_{max}$ ($CHCl_3$) 2119, 1750 $cm^{-1}$.

(*bb*) N-*Tosyl*-trans-*4-amino*-L-*proline* (*XLIV*)

The *trans*-azido ester (XLI) (4·0 g) in methanol (100 ml) was hydrogenated for 1 hr with 20% palladium on charcoal catalyst at room temperature and 5 atm hydrogen pressure to give N-*tosyl*-trans-*4-amino*-L-*proline methyl ester* (XLV) as a viscous oil (3·04 g, 82·5%), $n_D^{22}$ 1·6490. This unstable amino ester (3·0 g) was hydrolysed as described in (*x*) to give N-*tosyl*-trans-*4-amino*-L-*proline* as a white solid (2·8 g, 96·5%). Crystallization from aqueous ethanol gave white prisms, m.p. 275–276° (dec.), $[\alpha]_D^{20}$ −62·7° (0·3% in EtOH/$H_2O$) (Found: C, 50·4; H, 5·6; N, 9·5. $C_{12}H_{16}O_4N_2S$ requires C, 50·7; H, 5·6; N, 9·9%). $\nu_{max}$ 3580, 3370, 2800, 1610, 1580 $cm^{-1}$.

(*cc*) trans-*4-Amino*-L-*proline* (*XLIII*)

Reductive detosylation of the amino compound (XLIV) (2·2 g) was performed as described in (*e*). trans-*4-Amino*-L-*proline* was obtained as an oil which crystallized slowly to white prisms (0·75 g, 80%), m.p. 229–230° (dec.), $[\alpha]_D^{21}$ −57·8° (1·2% in $H_2O$), $\nu_{max}$ 3400, 3305, 3220, 1610 $cm^{-1}$. It was found to be hygroscopic and unstable. A mass spectrum confirmed the molecular weight of 130. The *dihydrochloride* of (XLIII), m.p. 257–258°, was found to be unstable.

(*dd*) N,O-*Ditosyl*-trans-*4-hydroxy*-L-*proline* (*XXXV*)

The *trans-N,O*-ditosyl ester (XXXVI) (5·0 g) in methanol (100 ml) was hydrolysed in 1·2M NaOH (15 ml) at 0° for 48 hr. The reaction mixture was acidified to pH 4 at 0°. The crude product which precipitated was collected, dissolved in ethyl acetate, and the solution was washed with brine. Removal of solvent from the dried ($MgSO_4$) solution and crystallization of the residue from ethyl acetate/light petroleum gave N,O-*ditosyl*-trans-*4-hydroxy*-L-*proline* as white needles (3·1 g, 64%), m.p. 147–148°, $[\alpha]_D^{20}$ −60·2° (1% in EtOH) (Found: C, 51·9; H, 5·0. $C_{19}H_{21}NO_7S_2$ requires C, 51·9; H, 4·8%). $\nu_{max}$ ($CHCl_3$) 1725 $cm^{-1}$.

(*ee*) N-*Tosyl-4-oximino*-L-*proline* (*LII*)

(i) *N*-Tosyl-4-oxo-L-proline (XXVIII) (2 g, 0·007 mole) and hydroxylamine hydrochloride (2·5 g, 0·03 mole) were dissolved in a mixture of pyridine (10 ml) and absolute ethanol (10 ml) and the reaction mixture was heated under reflux for 2·5 hr. After removal of solvents the residue was partitioned between ethyl acetate (3×100 ml) and 1·3M HCl (120 ml) at 0°. The combined organic layers were washed with brine (2×50 ml) and dried ($MgSO_4$). Removal of solvent and crystallization of the residual oil from ethyl acetate/cyclohexane gave the oxime (LII) as white needles (1·7 g, 79%), m.p. 152–153°, $[\alpha]_D^{25}$ +4° (1% in EtOH) (Found: C, 48·4; H, 5·0. $C_{12}H_{14}N_2O_5S$ requires C, 48·3; H, 4·7%). $\nu_{max}$ ($CHCl_3$) 3685, 3600, 3050, 1760 $cm^{-1}$.

(ii) The keto acid (XXVIII) (1·5 g, 0·005 mole), methanol (120 ml), and water (4 ml) were placed in a flask which was flushed continuously with nitrogen. To the stirred solution was added hydroxylamine hydrochloride (1 g, 0·012 mole) and sodium bicarbonate (1·3 g). The reaction mixture was heated under reflux for 3 hr, and then stirred at room temperature for 24 hr. The methanol was removed and the residue was diluted with water and extracted with ethyl acetate (3×100 ml). The combined ethyl acetate extracts were washed with brine and dried ($MgSO_4$). Removal of the solvent and crystallization of the colourless residue from ethyl acetate/cyclohexane gave the oxime (LII) as white needles (1·2 g, 75%), m.p. 152–153°.

(*ff*) N-*Tosyl*-cis-*4-amino*-L-*proline* (*XLVII*) *and* cis-*4-Amino*-L-*proline* (*XLVI*) *via the Oxime Route*

The oxime (LII) (0·6 g) was dissolved in ethanol (20 ml) and 1M HCl (2 ml) and shaken with platinum oxide (0·2 g) under 5 atm hydrogen pressure for 5·5 hr. The reaction mixture was filtered and the solvent was removed. Crystallization of the residue from ethanol/ether gave N-*tosyl*-cis-*4-amino*-L-*proline hydrochloride* as white hygroscopic needles (0·35 g, 55%), m.p. 184–185° (Found: C, 44·7; H, 5·8; ionic Cl, 11·2; N, 8·4. $C_{12}H_{17}ClN_2O_4S$ requires C, 44·9; H, 5·3; ionic Cl, 11·1; N, 8·7%).

The above reaction was repeated on a 3·24-g scale and the crude hydrochloride was passed through an ion-exchange column as described in (*x*) to give the monosubstituted diamino acid (XLVII) (1·65 g, 54%), m.p. 281° (dec.). Electrophoresis at pH 1·9, −5°, and 1900 V in a buffer of acetic acid/formic acid/water (15 : 5 : 80) showed only a single spot 23 cm from the starting line after 2 hr. Colour was developed by the usual ninhydrin method.

Compound (XLVII) (0·86 g) was dissolved in 0·8M HCl in methanol (50 ml) and the solution was heated under reflux for 2 hr. Solvent was removed and crystallization of the residue from methanol/ether gave N-*tosyl*-cis-*4-amino*-L-*proline methyl ester hydrochloride* as needles 0·76 g, 75%), m.p. 221–222° (Found: C, 46·7; H, 5·8; N, 8·2. $C_{13}H_{19}ClN_2O_4S$ requires C, 46·6; H, 5·7; N, 8·4%).

Reductive detosylation of compound (XLVII) was then carried out as described in (*e*) and (*y*) to give the free diamino acid (XLVI).

(*gg*) N-*Tosyl-4-oxo*-L-*proline Methyl Ester* (*XXIX*)

(i) The 4-hydroxy ester (III) (3·5 g) was oxidized as described in (*f*) to give the *keto ester* (XXIX) which crystallized from benzene/cyclohexane as white needles (1·6 g, 48%), m.p. 103–104°, $[\alpha]_D^{20}$ +7·7° (2% in $CHCl_3$) (Found: C, 52·5; H, 5·1. $C_{13}H_{15}NO_5S$ requires C, 52·5; H, 5·1%). $\nu_{max}$ 1750, 1710 cm$^{-1}$.

(ii) The keto acid (XXVIII) (3·6 g) was esterified with ethereal diazomethane to give the keto ester (XXIX) (3·6 g, 96%), m.p. 103–104°.

(*hh*) N-*Tosyl-4-oximino*-L-*proline Methyl Ester* (*LIII*)

This preparation, from the keto ester (XXIX) (1 g) and hydroxylamine hydrochloride, was carried out as described in (*ee*)(i) to give the *oxime* (LIII) which crystallized from benzene/cyclohexane as white needles (0·81 g, 78%), m.p. 110–111°, $[\alpha]_D^{25}$ +9° (0·4% in EtOH) (Found: C, 50·2; H, 5·4; N, 8·5. $C_{13}H_{16}N_2O_5S$ requires C, 50·0; H, 5·2; N, 9·0%).

(*ii*) N-*Tosyl-4-oxo*-L-*prolinamide* (*XXX*)

Ammonolysis of the 4-hydroxy ester (III) (2·64 g) was performed as described in (*u*). The *hydroxy prolinamide* (IV) crystallized from methanol as white prisms (2·2 g, 88%), m.p. 204°, $[\alpha]_D^{25}$ −80° (0·3% in EtOH) (Found: C, 50·3; H, 5·5; N, 9·6. $C_{12}H_{16}N_2O_4S$ requires C, 50·7; H, 5·7; N, 9·9%). $\nu_{max}$ 3480, 3290, 3230, 1680 cm$^{-1}$.

Chromic acid oxidation of (IV) (1 g) in acetone/acetic acid was done as in (*f*) to give N-*tosyl-4-oxo*-L-*prolinamide* which crystallized from methanol/water as prisms (0·44 g, 44%), m.p. 154·5–155·5° (Found: C, 51·1; H, 5·3. $C_{12}H_{14}N_2O_4S$ requires C, 51·2; H, 5·0%). $\nu_{max}$ 3395, 3295, 3240, 1760, 1700 cm$^{-1}$.

(*jj*) N-*Benzyloxycarbonyl-4-oximino*-L-*proline Methyl Ester* (*LIV*)

Esterification of the keto acid[8] (XXXII) (10 g) with ethereal diazomethane gave the *keto ester* (XXXIII) as a colourless oil (10·2 g, 97%) (Found: C, 60·7; H, 5·5; N, 5·3. $C_{14}H_{15}NO_5$ requires C, 60·6; H, 5·5; N, 5·1%).

Interaction of the keto ester (XXXIII) (11·5 g) with hydroxylamine hydrochloride was carried out as described in (*ee*)(i) to give the *oxime* (LIV) as a colourless oil (9·2 g, 75%) (Found: C, 57·5; H, 5·7; N, 9·4. $C_{14}H_{16}N_2O_5$ requires C, 57·5; H, 5·5; N, 9·6%).

(*kk*) N-*Benzyloxycarbonyl-4-oximino*-L-*prolinol* (*LV*)

(i) A solution of sodium borohydride (0·5 g) in propan-2-ol (30 ml) was added to a solution of the oxime (LIV) (1·5 g) in propan-2-ol (20 ml). Disodium hydrogen phosphate (1·3 g) was added, and the mixture was stirred at room temperature for 16 hr. The reaction mixture was concentrated and partitioned between chloroform (3 × 50 ml) and water (50 ml). Removal of the solvent from the dried organic phase gave the *oximino prolinol* (LV) which crystallized from ethyl acetate/cyclohexane as white plates (0·83 g, 60%), m.p. 138–140°, $[\alpha]_D^{25}$ +11·3° (1·6% in

EtOH) (Found: C, 59·3; H, 6·1; N, 10·2; O, 24·5. $C_{13}H_{16}N_2O_4$ requires C, 59·1; H, 6·1; N, 10·6; O, 24·2%). $\nu_{max}$ 3470, 3300 $cm^{-1}$.

(ii) The keto ester (XXXIII) (0·72 g) and *p*-toluenesulphonic acid (0·07 g) were dissolved in ethylene glycol (50 ml) and the solution was heated at 100° for 1·5 hr. After partial removal of solvent the concentrate (10 ml) was poured into 20% aqueous $Na_2CO_3$ (30 ml). The oil which formed was extracted into ether (2 × 50 ml), and the combined extracts were dried ($MgSO_4$). Removal of ether and chromatography of the residue on deactivated alumina gave *N-benzyloxycarbonyl-4,4-ethylenedioxy-*L*-proline methyl ester* (L) as a colourless oil (0·54 g, 64%) (Found: C, 59·8; H, 6·1; N, 4·6. $C_{16}H_{19}NO_6$ requires C, 59·8; H, 6·0; N, 4·4%). $\nu_{max}$ ($CHCl_3$) 1745 $cm^{-1}$.

A solution of lithium borohydride (0·14 g) in 1,2-dimethoxyethane (10 ml) was slowly added to a chilled solution of (L) (0·7 g) in 1,2-dimethoxyethane (10 ml). The reaction mixture was left at 0° for 2 hr before addition of 7% aqueous $NaHCO_3$ (25 ml). It was extracted with ether (4 × 50 ml) and the combined extracts were dried ($MgSO_4$). Removal of solvent and chromatography of the residue on deactivated alumina gave *N-benzyloxycarbonyl-4,4-ethylenedioxy-*L*-prolinol* (LI) as a colourless oil (0·54 g, 84%) (Found: C, 60·9; H, 6·8. $C_{15}H_{19}NO_5$ requires C, 61·4; H, 6·5%). $\nu_{max}$ ($CHCl_3$) 3400 $cm^{-1}$.

The foregoing compound (LI) (1·3 g) in methanol (10 ml) and 1M HCl (8 ml) was heated under reflux for 1 hr. After removal of methanol the aqueous phase was diluted with water (20 ml) and extracted with chloroform (2 × 50 ml). Removal of solvent from the dried extracts and chromatography of the residue over deactivated alumina gave *N-benzyloxycarbonyl-4-oxo-*L*-prolinol* (XXXIV) as an oil (1·1 g, 100%) (Found: C, 62·2; H, 6·3; N, 5·8. $C_{13}H_{15}NO_4$ requires C, 62·6; H, 6·1; N, 5·6%). $\nu_{max}$ ($CHCl_3$) 3400, 1760 $cm^{-1}$.

The oxime (LV) was prepared from (XXXIV) (0·47 g) as described in (*ee*)(i). It crystallized from ethyl acetate/cyclohexane as white plates (0·15 g, 31%), m.p. 138–140°.

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