Tetrahedron Vol. 40, No 19, pp 3695 to 3708, 1984
Printed in the U.S.A

0040-4020/84 $3.00+.00
Pergamon Press Ltd.

STEREOSPECIFIC SYNTHESIS OF TABTOXIN

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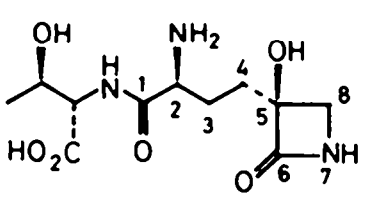
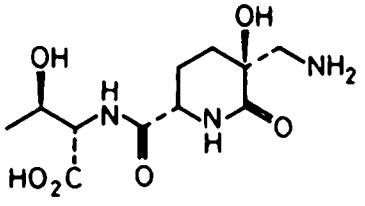
(*Received in UK 12 June 1984*)

Abstract - Tabtoxin (1), the exotoxin from Pseudomonas tabaci (the organism responsible for Wildfire disease of tobacco plants), has been synthesised by a stereospecific route. The cycloaddition of an acylnitroso compound to a cyclohexadiene proceeded regioselectively to give the bicyclic ester (6), which was transformed to the amine (24) through reductive amination of the aldehyde (10). Oxidative cleavage of the olefin (25) gave the corresponding diacid (26), whose functional groups were appropriately differentiated by formation of the peptide (29). Cyclisation of the β-amino acid (30) and subsequent hydrogenolysis of the resulting spiro β-lactam (31) gave tabtoxin.

Wildfire is an infectious disease of tobacco plants causing circular yellow leafspots, first recognised in 1917[1]. An infecting agent, Pseudomonas tabaci, was isolated from the centre of the chlorotic lesions and the production of an exotoxin, namely tabtoxin (1), by the bacterium was found to be responsible for the disease. To exert its effect on plants the toxin appears to inhibit glutamine synthetase in the photorespiratory nitrogen cycle; symptoms being considered to be due to the subsequent accumulation of NH_3, the substrate of the inhibited enzyme.[2] Relatively recently the structure[3] of (1), including stereochemistry[4], was revealed to be a dipeptide consisting of L-threonine or L-serine and an unusual amino acid containing a monocyclic β-lactam with two asymmetric centres. In addition to its stereochemical complexity the molecule possesses a marked instability ($t_{\frac{1}{2}}$ 24 hr at pH 7, 15 min at pH 8.2); the β-lactam moiety of the toxin undergoing a facile transacylation to the stable, inactive isotabtoxin (2) under mild basic conditions. We detail herein the first total synthesis of tabtoxin which has been reported in a preliminary form.[5]

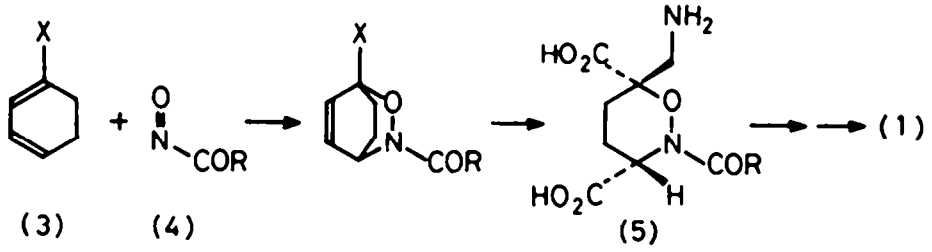


(1)



(2)

Recognising the chiral 1,4-aminoalcohol part as the key structural feature of the molecule, we settled for a Diels-Alder strategy, starting with an appropriate cyclohexadiene (3) and an acylnitroso compound (4)[6], leading to the amino diacid (5), a logical precursor of the toxin (Scheme 1). Owing to a preferential interaction between the LUMO of the nitroso dienophile and the HOMO of the diene the desired regiochemistry of the cycloaddition was anticipated. Particularly noteworthy is that the crucial relative stereochemical relationship between C(2) and C(5) could be achieved during the simultaneous formation of C(2)-N and C(5)-O bonds.

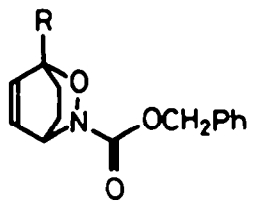


Scheme 1. E.g. R = OCH_2Ph ; X = CO_2Et

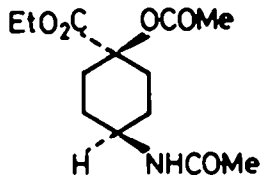
The cycloaddition was shown to be a quite efficient process. The starting diene, ethyl cyclohexa-1,3-dienecarboxylate (3, X = CO_2Et), was prepared according to a conventional and high yield procedure.[7] It was reacted with benzyl nitrosoformate (4, R = OCH_2Ph), generated *in situ* from benzyl *N*-hydroxycarbamate[8] and tetraethylammonium periodate[9], resulting in exclusive formation of a single regioisomer (6) in 93% yield. In order to confirm the regiochemistry the adduct (6) was subjected to hydrogenation (H_2,Pd-C, EtOH) and acetylation (Ac_2O, pyridine), successively. The resulting compound (7) exhibited an amide hydrogen splitting [($CDCl_3$) 5.36 (J 5 Hz)] from a single methine hydrogen on ^1H n.m.r. Furthermore, the correct regiochemistry of the adduct was unambiguously established by X-ray crystallography of the *N*-chloroacetyl derivative (14), as will be described below.

Transformation of the ester group of (6) to an aminomethyl group was next investigated. Sodium borohydride reduction of (6) gave the corresponding alcohol (8) quantitatively. For the subsequent displacement reaction, the alcohol (8) was then converted into the mesylate (9) in 96% yield. However, it was found that reaction of various nitrogen nucleophile with (9) is not possible presumably because of the neopentylic nature of the reaction site. Thus, the alcohol (8) was further transformed to the aldehyde (10) by the Moffat oxidation in 68% yield. Although direct amination of (10) gave rise to the formation of the undesired diastereomeric secondary amines (11), on the other hand, the crystalline *O*-methyl oxime (12), obtained in 87% yield from (10), was readily reduced with sodium cyanoborohydride[10] to give the corresponding hydroxylamine derivative (13) in 97% yield. The methoxyamino group in (13) was protected as the *N*-chloroacetyl derivative (14) (87%).

X-ray diffraction analysis of (14) was carried out, verifying the regiochemistry of the Diels-Alder reaction, as shown by the general view of the molecule (Figure 1). The distance for all atoms generally agree with the accepted value. It is noted that the bicyclic skeleton has slightly staggered D_3 pseudo symmetry, when viewed down the C(5)-C(8) axis and that the configuration of both N(1) and N(2) atoms are pyramidal.[11] The displacement of the nitrogen atoms from the plane were: N(1) 0.260 A; N(2) 0.356 A.



(6) R = CO_2Et
(8) R = CH_2OH
(9) R = CH_2OSO_2Me
(10) R = CHO
(12) R = CH=NOMe
(13) R = CH_2NHOMe
(14) R = $CH_2N(OMe)(COCH_2Cl)$



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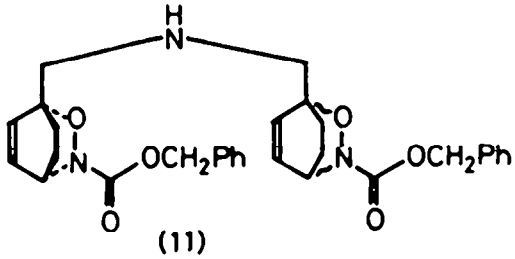

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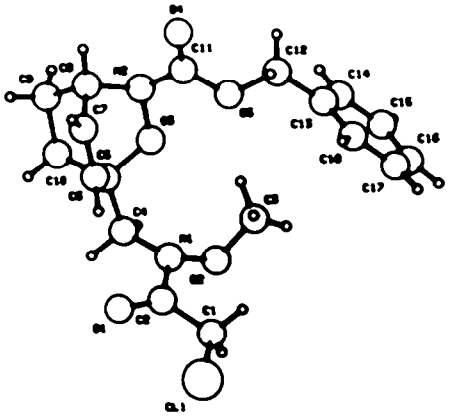
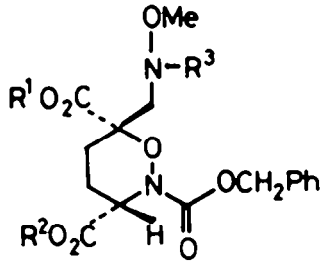


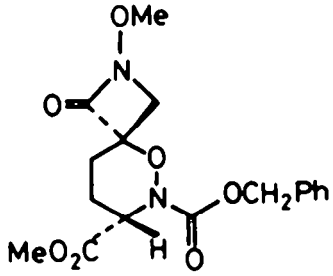
Figure 1. Perspective view of the molecule (14) with atomic numbering.

Oxidation of the double bond in (14) to the corresponding diacid was straightforward. The use of potassium permanganate under phase-transfer condition[12] effected a facile olefin cleavage, affording the diacid (15) in 58% yield. For the cyclisation to ß-lactam the N-chloroacetyl group was then removed with thiourea in ethanol, followed by sodium bicarbonate[13] to the methoxyamino diacid (16) (78%). However, all of the attempts to obtain ß-lactam from (16) were unsuccessful. Accordingly, in order to try cyclisation from ß-amino ester (17) diacid (15) was transformed by esterification with diazomethane in 80% yield and the successive treatment with thiourea and sodium bicarbonate gave diester (18) in 57% yield. The compound (18) also failed to cyclise to the ß-lactam.

Differentiation of the two carboxyl functions seemed essential for efficient cyclisation. Thus, the diacid (15) was reacted with one equivalent of diphenyldiazomethane, followed by diazomethane. All four possible products (19a), (19b), (19c), and (17) were obtained, including the desired (19b) in 20% yield. The two benzhydryl esters, (19b) and (19c), were distinguished by their ^{1}H n.m.r. spectra, in particular, by the chemical shifts and coupling constants for the C-6 *N*-chloroacetyl-*N*-methoxyamino substituent i.e., one benzhydryl methyl ester strongly resembled the dimethyl ester (17) and the other one closely resembled di(benzhydryl) ester (19a). This assignment was confirmed when the compound assigned (19b) was elaborated to a β-lactam as follows. Removal of the *N*-chloroacetyl group of (19b) with thiourea and sodium bicarbonate gave (20) (71%), which was further treated with trifluoroacetic acid for the selective cleavage of the benzhydryl ester, affording the monoester (21) in 94% yield. All of the functional groups in (21) are now suitably differentiated and protected and compound (21) was successfully cyclised to the spiro β-lactam (22) in 53% yield by treatment with potassium bicarbonate, methanesulphonyl chloride and tetra-*n*-butylammonium bisulphate catalyst[14]. This reasonably satisfactory entry into the β-lactam seemed to allow us to reach a major subgoal to tabtoxin. However, a serious problem was encountered in the removal of the *N*-methoxy substituent. Hydrogenolysis using Raney Nickel W-2 gave no desired products presumably due to overreduction. The choice of methoxy group seemed therefore not ideal.

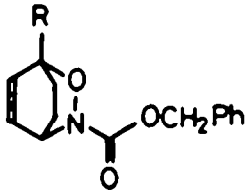


(15) $R^1 = R^2 = H$, $R^3 = COCH_2Cl$
(16) $R^1 = R^2 = R^3 = H$
(17) $R^1 = R^2 = Me$, $R^3 = COCH_2Cl$
(18) $R^1 = R^2 = Me$, $R^3 = H$
(19a) $R^1 = R^2 = CHPh_2$, $R^3 = COCH_2Cl$
(19b) $R^1 = CHPh_2$, $R^2 = Me$, $R^3 = COCH_2Cl$
(19c) $R^1 = Me$, $R^2 = CHPh_2$, $R^3 = COCH_2Cl$
(20) $R^1 = CHPh_2$, $R^2 = Me$, $R^3 = H$
(21) $R^1 = H$, $R^2 = Me$, $R^3 = H$

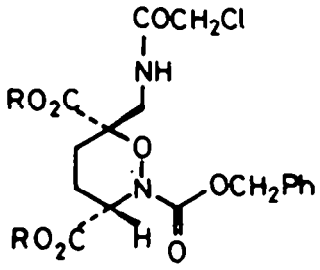


(22)

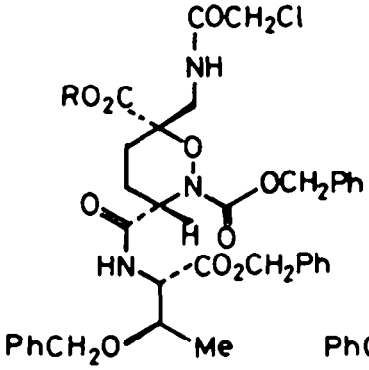
Accordingly, attention was centred on the exploration of other appropriate N-substituent and the problem was solved by a re-examination of the reductive amination of the aldehyde (10). Use of a primary amine whose alkyl group could be easily removed under mild acid conditions seemed preferable; 4,4'-dimethoxybenzhydryl group[15] satisfies this constraint. Thus, the aldehyde (10) was condensed with 4,4'-dimethoxydiphenylmethylamine and reduced using sodium cyanoborohydride. The product (23), obtained in 59% yield, was readily deprotected with trifluoroacetic acid to the free amine (24) (89%), which was then converted into the

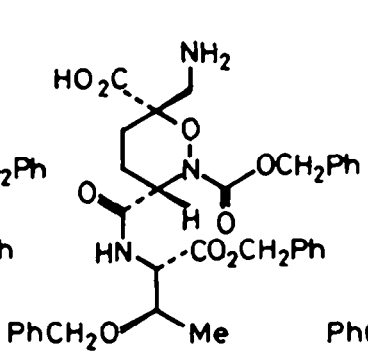


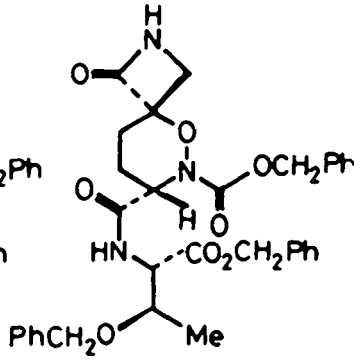
(23) R = $CH_2NHCH(4\text{-}MeO\text{-}C_6H_4)_2$
(24) R = CH_2NH_2
(25) R = $CH_2NHCOCH_2Cl$



(26) R = H
(27) R = $COBu^t$



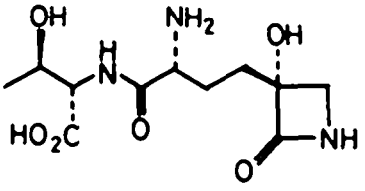




(28) R = H
(29) R = $CHPh_2$

(30)

(31)



(32)

N-chloroacetamide (25) in 86% yield. The potassium permanganate oxidation in a two-phase system also effected the cleavage of the olefin (25) to the diacid (26) (58%). The use of *N*-methoxy substituent was thus avoided.

It was gratifying that selective differentiation of the two carboxyl groups was successfully achieved *via* pivaloyl mixed anhydride methodology. The diacid (26) was reacted with pivaloyl chloride (2 equiv) in the presence of triethylamine (2 equiv). The mixed anhydride (27) obtained was reacted *in situ* with *O*-benzyl-L-threonine benzyl ester[16] to give the product (28), resulting from selective attack at the less hindered of the two carbonyl groups, as a mixture of diastereoisomers. The absolute stereochemistry of only one of the two diastereoisomers is depicted here.

Separation of the diastereoisomers (28) was carried out by conversion to the crystalline benzhydryl esters by treatment with diphenyldiazomethane. One diastereoisomer was crystallised from ethyl acetate (now known to be (29), m.p. 180-182°C, 25% from (26)), and the other from ether (m.p. 98-100°C, 28% from (26)). Both isomers were carried through the rest of the synthesis. The higher m.p. isomer (29) was deprotected with trifluoroacetic acid to the monoacid (28) in 90% yield. The *N*-chloroacetyl group was removed by careful treatment with thiourea in acetonitrile-ethanol at exactly 40°C, affording the β-amino acid (30) in 41% yield. Although DCC or the two-phase reaction[14] did not effect the cyclisation of (30), the spiro β-lactam (31) was obtained in acceptable yield (14%) by employing the 2,2'-dithiodipyridine-triphenylphosphine-acetonitrile system.[17] In contrast to the case of *N*-methoxy β-lactam (23) deprotection of the benzyloxycarbonyl group and reductive cleavage of the perhydro-1,2-oxazine ring of (31) was smoothly carried out simultaneously *via* catalytic hydrogenolysis, affording tabtoxin (1) in 100% yield. Stereochemical integrity of the compounds were confirmed *via* biological tests. The synthetic tabtoxin (1) showed the same biological activity on the tobacco plant, the same glutamine synthetase and *E. coli* assay and had an identical 300 MHz ^{1}H n.m.r. spectrum (Figure 2) to the natural tabtoxin obtainerd from *P. tabaci*. The stereoisomer (32), obtained from the lower m.p. isomer of (29) by the same procedure, showed virtually no significant activity in the biological tests.

The total synthesis of tabtoxin was thus achieved for the first time.

EXPERIMENTAL

All reagents and chemicals were purified and dried by a standard procedure. Melting points were determined on a Kofler block and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 257 spectrometer. ^{1}H n.m.r. spectras were recorded on a Bruker WH300 spectrometer at 300 MHz. Mass spectra were recorded on Vacuum Generators Micromass ZAB 1F and 16F instruments.

Ethyl 3-benzyloxycarbonyl-2-oxa-3-azabicyclo[2.2.2]oct-5-ene-1-carboxylate (6). - To a stirred solution of ethyl cyclohexa-1,3-dienecarboxylate[7] (31.63g, 0.21mol) and benzyl *N*-hydroxycarbamate[8] (38.2g, 0.23mol) in CH_2Cl_2 (350ml), a solution of Et_4NIO_4[9] (73.5g, 0.23mol) in CH_2Cl_2, was added over 1 hr whilst maintaining the temperature at -5±2°C. After stirring for a further 20 min the solution was washed with aq. sodium bisulphite (15%, 3 x 200ml), saturated aq. $NaHCO_3$ (2 x 150ml) and brine (150ml), (dried $MgSO_4$) and concentrated *in vacuo*. The resulting brown oil was purified by flash chromatography (stepwise elution with CH_2Cl_2, 5% EtOAc-CH_2Cl_2) to give (6) as a viscous yellow oil (61.29g, 93%). ν_{max}($CHCl_3$) 3000, 1745, 1715, 1455 and 1390 cm^{-1}; δ_H($CDCl_3$) 1.3(3H, t, J 7Hz, CH_2CH_3), 1.3-2.5(4H, m, ring CH_2), 4.25(2H, q, J 7Hz, CH_2CH_3), 4.85(1H, m, ring CH), 5.1(2H, s, benzylic), 6.6(2H, m, vinylic) and 7.25(5H, m, phenyl); m/e 317(M^+).

Ethyl t-4-acetamido-t-1-acetoxycyclohexane-r-1-carboxylate (7). - The ester (6) (317mg, 1mmol) was dissolved in EtOH (10ml) and 10% Pd-C (95mg) was added. The mixture was stirred for 18 hr under H_2. The catalyst was filtered off and the filtrate was concentrated *in vacuo*. The resulting crude aminoalcohol was dissolved in pyridine (2ml) and Ac_2O (2ml) was added. The solution was stirred for 18 hr at room temperature, then water (10ml) was added. After stirring for 1 hr at room temperature EtOAc (50ml) was added. The EtOAc solution was washed with HCl (M, 10 x 5ml), saturated aq. $NaHCO_3$ (20ml) and brine (20ml), dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by preparative t.l.c. (Et_2O) to give (7) as white crystals (43.2mg,

16%). m.p. 128-133°C(EtOAc); ν_{max}(KBr) 3290, 2960, 2930, 1735, 1635 and 1545 cm^{-1}; $\delta_H(CDCl_3)$ 1.25(3H, t, J 7.5Hz, CH_2CH_3), 1.3-1.5(2H, m, ring CH_2), 1.78-2.40(6H, m, ring CH_2), 1.97(3H, s, CH_3), 2.11(3H, s,CH_3), 3.82(1H, m, ring CH), 4.18(2H, q, J 7.5Hz, CH_2CH_3) and 5.36(1H, br d, J 5Hz,NH); m/e 272(MH^+).

3-Benzyloxycarbonyl-2-oxa-3-azabicyclo[2.2.2]oct-5-ene-1-methanol (8). - A solution of the ester (6) (31.03g, 98mmol) in MeOH (100ml) was added to solid $NaBH_4$ (11.2g, 294mmol) over 5 min with stirring. The mixture frothed and boiled. The solution was allowed to cool to room temperature and was partitioned between water (300ml) and CH_2Cl_2 (300ml). The aq. layer was extracted with CH_2Cl_2 (100ml). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo to give (8) as a viscous yellow oil (26.92g, 100%), $\nu_{max}(CHCl_3)$ 3400, 2940, 1750, 1715, 1455 and 1400 cm^{-1}; $\delta_H(CDCl_3)$ 1.2-1.4(4H, m, ring CH_2), 3.75(2H, s, CH_2OH), 3.75(1H, br, OH and 2H, s, CH_2OH), 4.75(1H, m, ring CH), 5.10(2H, s, benzylic), 6.4-6.7(2H, m, vinylic) and 7.25(5H, m, phenyl); m/e 275(M^+) and 276(MH^+).

(3-Benzyloxycarbonyl-2-oxa-3-azabicyclo[2.2.2]oct-5-en-1-yl)methyl methanesulphonate (9). - A solution of alcohol (8) (181mg, 0.6mmol) in dry pyridine (1ml) was added to a solution of methanesulphonyl chloride (0.049ml, 0.63mmol) in dry pyridine (1ml) over 15 min at 0°C. The solution was stirred for 16 hr at room temperature and then diluted with cold Et_2O (10ml). The solution was washed with cold HCl (2M, 2 x 10ml), saturated aq. $NaHCO_3$ (2 x 10ml) and brine (10ml), dried (Na_2SO_4) and concentrated in vacuo to give (9) as a white solid (202mg, 96%). m.p. 86-88°C (CH_2Cl_2 - light petroleum); ν_{max}(KBr) 3040, 2970, 1710, 1350 and 1178 cm^{-1}; $\delta_H(CDCl_3)$ 1.2-2.4(4H, m, ring CH_2), 2.95(3H, s, CH_3), 4.4(2H, s, CH_2OSO_2), 4.85(1H, m, ring CH), 5.1(2H, s, benzylic), 6.6(2H, m, vinylic) and 7.25(5H, m, phenyl); m/e 354(MH^+).

3-Benzyloxycarbonyl-2-oxa-3-azabicyclo[2.2.2]oct-5-ene-1-carbaldehyde (10).- Alcohol (8) (26.92g, 98mmol) was dissolved in dry dimethylsulphoxide(150ml) and dry PhH (150ml) containing dry pyridine (7.74g, 98mmol) and dry trifluoroacetic acid (5.59g, 49mmol). N,N'-dicyclohexylcarbodiimide (60.56g, 294mmol) was added. After stirring for 18 hr at room temperature a solution of oxalic acid (26.46g, 294mmol) in MeOH (250ml) was added cautiously over 20 min. After further 40 min Et_2O (1l) and water (1l) were added. The mixture was filtered to remove N,N'-dicyclohexylurea and the filter cake was washed with Et_2O (2 x 200ml). The combined organic solutions were concentrated to 500 ml in vacuo, washed with saturated aq. $NaHCO_3$ (3 x 150ml), water (3 x 150ml) and brine (150ml), dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash chromatography (stepwise elution with 5% and 33% EtOAc-CH_2Cl_2) to give a mxiture of aldehyde (10) and its hydrate (ca. 80% hydrate) as a viscous pale yellow oil (19.46g, 68%). $\nu_{max}(CHCl_3)$ 3400, 2940, 1740, 1720, 1455 and 1390 cm^{-1}; $\delta_H(CDCl_3)$ 1.1-2.4 (5.6H, m, ring CH_2 and 80% $CH(OH)_2$),4.8(1H, m, ring CH), 5.0(0.8H, br s, 80% $CH(OH)_2$), 5.1(2H, s, benzylic), 6.5(2H, m, vinylic), 7.25(5H, m, phenyl) and 9.7(0.2H, s, 20% CHO).

bis[[(1RS, 4SR)-3-Benzyloxycarbonyl-2-oxa-3-azabicyclo[2.2.2]-oct-5-en-1-yl]methyl]amine and [[(1RS, 4SR)-3-benzyloxycarbonyl-2-oxa-3-azabicyclo[2.2.2]oct-5-en-1-yl]methyl][[(1SR, 4RS)-3-benzyloxycarbonyl-2-oxa-3-azabicyclo[2.2.2]oct-5-en-1-yl]methyl]amine (11). - Aldehyde (10) (8.48g, 2.9mmol) was dissolved in dry MeOH (100ml) containing dry ammonium acetate (22.3g, 0.29mol). Molecular sieves 3A (20g) and $NaBH_3CN$ (1.83g, 2.9mmol) were added successively. The mixture was stirred for 7 days at room temperature and filtered. The pH was adjusted to 2 with methanolic HCl (2M) and the solution was concentrated in vacuo. The residue was triturated with Et_2O to remove nonbasic organics and then partitioned between brine (200ml) and Et_2O (200ml). The aq. layer was adjusted to pH 10 with solid KOH and extracted with Et_2O (3 x 200ml). The combined organic solution were dried (K_2CO_3) and concentrated in vacuo. The residue was separated by flash chromatography (column packed with 200:15 dry CH_2Cl_2-EtOAc and eluted with 200:15:1 dry CH_2Cl_2-EtOAc-.880 ammonia) to give two pairs of diastereomeric secondary amines (A) (1.17g, 15%) and (B) (1.00g, 13%). (A), $\nu_{max}(CHCl_3)$ 3400, 3000, 1705, 1455 and 1390 cm^{-1}; $\delta_H(CDCl_3)$ 1.4-2.3(8H, m, ring CH_2), 2.91(2H, AB quartet, J 13.5Hz, CH_2N), 3.11(2H, AB quartet, J 13.5Hz, CH_2N), 4.76(2H, m, ring CH), 5.12(4H, s, benzylic), 6.6(4H, m, vinylic) and 7.3(10H, m, phenyl); $\delta_C(CDCl_3)$ 21.06(t, $C_{7,7'}$ or $C_{8,8'}$), 26.94(t, $C_{8,8'}$ or $C_{7,7'}$), 50.51(d, $C_{4,4'}$), 53.43(t, CH_2N), 67.43(t, benzylic), 79.64(s, C_1), 127.65, 127.87, and 128.24 (all m, phenyl), 131.27(d, $C_{5,5'}$ or $C_{6,6'}$), 133.58(d, $C_{6,6'}$ or $C_{5,5'}$), 135.90(s, phenyl) and 158.51 (s, carbonyl). (B), $\nu_{max}(CHCl_3)$ 3400, 3000, 1705, 1455 and 1390 cm^{-1}; $\delta_H(CDCl_3)$ 1.4-2.3(8H, m, ring, CH_2), 3.00(2H, AB quartet, J 13.0Hz, CH_2N), 3.07(2H, AB quartet, J 13.0Hz, CH_2N), 4.76(2H, m, ring CH), 5.12(4H, s, benzylic) 6.6(4H, m, vinylic) and 7.3(10H, m, phenyl); $\delta_C(CDCl_3)$ 21.12(t, $C_{7,7'}$ or $C_{8,8'}$), 26.78(t,$C_{8,8'}$ or $C_{7,7'}$), 50.46(d, $C_{4,4'}$), 53.54(t, CH_2N), 67.34(t, benzylic) 79.32(s, $C_{1,1'}$), 127.71, 127.87 and 128.24 (all m, phenyl), 131.53(d, $C_{5,5'}$ or $C_{6,6'}$), 133.32(d, $C_{6,6'}$ or $C_{5,5'}$), 135.90(s, phenyl) and 158.29(s, carbonyl).

3-Benzyloxycarbonyl-1-[(methoxyimino)methyl]-2-oxa-3-azabicyclo[2.2.2]oct-5-ene (12). - Aldehyde (10) (19.46g, 67.1mmol) and methoxyammonium chloride (6.72g, 80.5mmol) were dissolved in dry pyridine (120ml). After stirring for 18 hr at room temperature the reaction mixture was poured into water (400ml) and extracted with CH_2Cl_2 (3 x 100ml). The combined extracts were washed with HCl (2M, 6 x 50ml), saturated aq. $NaHCO_3$ (50ml) and brine (50ml),dried ($MgSO_4$) and concentrated in vacuo. The residue was taken up in Et_2O (500ml), filtered and concentrated

in vacuo to give (12) as crystals (17.7g, 87%). m.p.58.5-59.5°C (Et_2O-light petroleum); $\nu_{max}(CHCl_3)$ 3000, 1705, 1455 and 1390 cm^{-1}; $\delta_H(CDCl_3)$ 1.3-2.5(4H, m, ring CH_2), 3.85(3H, s, OCH_3), 4.8(1H, m, ring CH), 5.1(2H, s, benzylic), 6.6(2H, m, vinylic), 7.25(5H, m, phenyl) and 7.4(1H, s, CH=N); (Found: C, 63.55; H, 5.9; N, 9.15%. $C_{16}H_{18}N_2O_4$ requires C, 63.55; H, 6.0; N, 9.25%).

3-Benzyloxycarbonyl-1-[(methoxyamino)methyl]-2-oxa-3-azabicyclo[2.2.2]oct-5-ene (13). - Methoxime (12) (16.4g, 54.3mmol) and $NaCNBH_3$ (10.2g, 163mmol) were dissolved in MeOH (160ml) containing a trace of methyl orange. Methanolic HCl (2M) was added to maintain the pH at 3 (indicator just pink). The reaction was complete (no more HCl required) after 30 min. The solution was partitioned between EtOAc (400ml) and saturated aq. $NaHCO_3$ (400ml). The aq. layer was extracted with EtOAc (2 x 100ml). The combined organic solutions were dried ($MgSO_4$) and concentrated *in vacuo* to give (13) as a pale yellow oil (16.0g, 97%). $\delta_H(CDCl_3)$ 1.2-2.4(4H, m, ring CH_2), 3.25(2H, s, CH_2N), 3.5(3H, s, OCH_3), 4.75(1H, m, ring CH), 5.1(2H, s, benzylic), 6.55(2H, m, vinylic) and 7.25(5H, m, phenyl).

3-Benzyloxycarbonyl-1-[[N-(chloroacetyl)-N-methoxyamino]methyl]-2-oxa-3-azabicyclo[2.2.2]-oct-5-ene (14). - To a solution of the hydroxylamine derivative (13) (1.52g, 5.0mmol) in CH_2Cl_2 (10ml), Et_3N (0.835ml, 6.0mmol) and chloroacetyl chloride (0.478ml, 6.0mmol) were successively added at 0°C. After stirring for further 1 hr at the same temperature the mixture was partitioned between EtOAc (40ml) and HCl (2M, 20ml). The organic layer was washed with saturated aq. $NaHCO_3$ (20ml) and brine (20ml) then dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by flash chromatography (7% EtOAc-CH_2Cl_2) to give an off white solid. Recrystallisation from Et_2O gave (14) as fine white crystals (1.657g, 87%). m.p. 78.0-79.0°C; $\nu_{max}(CHCl_3)$ 3000, 1695, 1455 and 1395 cm^{-1}; $\delta_H(CDCl_3)$ 1.2-2.4(4H, m, ring CH_2), 3.7(3H, s, OCH_3), 3.7(1H, AB quartet, J 16Hz, CH_2Cl), 4.2(1H, AB quartet, J 16Hz, CH_2Cl), 4.25(2H, s, CH_2N), 4.8(1H, m, ring CH), 5.1(2H, s, benzylic), 6.5(2H, m, vinylic) and 7.3(5H, m, phenyl); (Found: C, 56.45; H, 5.55; N, 7.5% $C_{18}H_{21}N_2O_5Cl$ requires C, 56.75; H, 5.55; N, 7.35%).

(3S*,6S*)-Benzyloxycarbonyl-6-[[N-(chloroacetyl)-N-methoxyamino]methyl]perhydro-1,2-oxazine-3,6-dicarboxylic acid (15). - A solution of the amide (14) (12.94g, 34.0mmol) in PhH (130ml) was stirred with aq. $KMnO_4$ (0.2M, 453.3ml, 90.67mmol) and aq. n-Bu_4NHSO_4 (0.1M, 34ml, 3.4mmol)for 16 hr at room temperature. The mixture was filtered to remove MnO_2 and the filter cake was washed four times with alternate portions of water (50ml) and EtOAc (50ml). The combined aq. solution was adjusted to pH 2 with HCl (2M) and extracted with EtOAc (2 x 100ml). All the organic solutions were combined, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was crystallised from toluene-Et_2O to give (15) as a white powder (8.84g, 58%). m.p. 108-111°C(dec); ν_{max}(nujol) ca. 3000 (partly obscured), 2600, 1755, 1700 and 1460 cm^{-1}; δ_H(acetone-d_6) 1.7-2.6(4H, m,ring CH_2), 3.7(3H, s, OCH_3), 3.7-4.5(4H, m, CH_2N and CH_2Cl), 4.8(1H, m, ring CH), 5.2(2H, s, benzylic), 7.3(5H, m, phenyl) and 8.5(2H, br s, CO_2H); m/e 446(M^+).

(3S*,6S*)-2-Benzyloxycarbonyl-6-[(methoxyamino)methyl]perhydro-1,2-oxazine-3,6-dicarboxylic acid (16). - A solution of the diacid (15) (795.6mg, 1.79mmol) and thiourea (272mg, 3.58mmol) in EtOH (30ml) was stirred for 16 hr at 40°C. The solution was allowed to cool to room temperature and saturated aq. $NaHCO_3$ (20ml) was added. The resulting suspension was stirred for 15 min at room temperature, then partitioned between EtOAc (50ml) and water (50ml). The aq. layer was adjusted to pH 4 with HCl (2M) and extracted with EtOAc (5 x 50ml). The combined extracts were dried ($MgSO_4$) and concentrated *in vacuo*. The resulting orange semisolid was taken up in acetone (6ml) and PhH (18ml) was added. The solution was filtered and concentrated *in vacuo* to give (16) (514.9mg, 78%). m.p. 110-116°C(dec); δ_H(DMSO-d_6) 1.5-2.4(4H, m, ring CH_2), 3.3(5H, s, CH_2N and OCH_3), 4.7(1H, m, ring CH), 5.2(2H, s, benzylic), 5.8(2H, br s, NH_2^+), 7.1(1H, br s, CO_2H) and 7.35(5H, m, phenyl).

Dimethyl (3S*,6S*)-2-benzyloxycarbonyl-6-[[N-(chloroacetyl)-N-methoxyamino]methyl]-perhydro-1,2-oxazine-3,6-dicarboxylate (17). - A distilled solution of diazomethane in Et_2O was added to a solution of the diacid (15) (691.3mg, 1.56mmol) in EtOAc (10ml) until a yellow colour just persisted. The excess diazomethane was destroyed with two drops of methanolic HCl (M) and the solution was concentrated *in vacuo*. The residue was purified by flash chromatography (13% EtOAc-CH_2Cl_2) to give (17) as a colourless oil (587.6mg, 80%). $\nu_{max}(CHCl_3)$ 3000, 1740, 1690(sh), 1450 and 1405 cm^{-1}; $\delta_H(CDCl_3)$ 1.82(H, m, ring CH_2), 1.97(1H, m, ring CH_2),2.26(2H, m, ring CH_2), 3.62(3H, s, OCH_3), 4.10(1H, AB quartet, J 15.0Hz, CH_2Cl), 4.22(1H, AB quartet, J 13.8Hz, CH_2N), 4.28(1H, AB quartet, J 13.8Hz, CH_2N), 4.58(1H, AB quartet, J 15.0Hz, CH_2Cl), 4.76(1H, m, ring CH), 5.16(1H, d, J 12.7Hz, benzylic), 5.28(1H, d, J 12.7Hz, benzylic) and 7.3(5H, m, phenyl).

Dimethyl (3S*,6S*)-2-benzyloxycarbonyl-6-[(methoxyamino)methyl]perhydro-1,2-oxazine-3,6-dicarboxylate (18). - A solution of the diester (17) (587.6mg, 1.24mmol)and thiourea (189.0mg, 2.48mmol) in EtOH (40ml) was stirred for 16 hr at 40°C. The solution was allowed to cool to room temperature and saturated aq. $NaHCO_3$ (40ml) was added. The resulting suspension was stirred for 20 min at room temperature, then partitioned between Et_2O (100ml) and HCl (2M, 40ml). The organic layer was extracted with HCl (2M, 3 x 25ml). The combined aq. layers were adjusted to pH 8 with saturated aq. $NaHCO_3$ and extracted with CH_2Cl_2 (4 x 80ml). The combined organic

extracts were dried (K_2CO_3) and concentrated in vacuo. The residue was purified by flash chromatography (20% EtOAc-CH_2Cl_2) to give (18) as a colourless oil (282.5mg, 57%). ν_{max}($CHCl_3$) 3000, 1740, 1440 and 1410 cm^{-1}; δ_H(acetone-d_6) 1.25(1H, m, ring CH_2), 1.76(1H, m, ring CH_2), 2.07(2H,m, ring CH_2), 3.33(3H, s, OCH_3), 3.33(1H, AB quartet, J 14.3Hz, CH_2N), 3.41(1H, AB quartet, J 14.3Hz, CH_2N), 4.72(1H, t, J 6.0Hz, ring CH), 5.15(1H, AB quartet, J 12.2Hz, benzylic), 5.33(1H, AB quartet, J 12.2Hz, benzylic) and 7.4(5H, m, phenyl); m/e 396(M^+).

Di(Benzhydryl) (3S*, 6S*)-2-benzyloxycarbonyl-6-[[N-(chloroacetyl)-N-methoxyamino]-methyl]perhydro-1,2-oxazine-3,6-dicarboxylate (19a), methyl (3S*,6S*)-6-benzhydryl-oxycarbonyl-2-benzyloxycarbonyl-6-[N-(chloroacetyl)-N-methoxyamino]methyl]perhydro-1,2-oxazine-3-carboxylate (19b), benzhydryl (3S*, 6S*)-2-benzyloxycarbonyl-6-[[N-(chloroacetyl)-N-methoxyamino]methyl]-6-methoxycarbonylperhydro-1,2-oxazine-3-carboxylate (19c), and (17). - A solution of diphenyldiazomethane (60% pure; 5.34g, 16.5mmol) in toluene (20 ml) was added to a solution of diacid (15) (6.67g, 15.0mmol) in EtOH (100ml). When the purple colour had been discharged a distilled solution of diazomethane in Et_2O was added until a yellow colour just persisted. The solution was concentrated in vacuo. The residue was separated by flash chromatography (14% and 20% EtOAc-toluene) to give (19a) (3.66g, 31%), (19b) (1.87g, 20%), (19c) (2.34g, 25%) and (17) (1.10g, 16%). (19a), m.p. 159.0-160.5°C (toluene-Et_2O); ν_{max}($CHCl_3$) 3000, 1735, 1680(sh), 1495, 1455 and 1410 cm^{-1}; δ_H($CDCl_3$) 1.88(2H, m, ring CH_2), 2.34(2H, m, ring CH_2), 3.09(3H, s, OCH_3), 4.08(2H, s, CH_2N), 4.11(1H, AB quartet, J 14.6Hz, CH_2Cl),4.70(1H, AB quartet, J 14.6Hz, CH_2Cl), 4.92(1H, m, ring CH), 5.12(1H, AB quartet, J 12.38Hz, benzylic), 5.18(1H, AB quartet, J 12.38Hz, benzylic), 6.85(1H, s, $CHPh_2$), 6.92(1H, s, $CHPh_2$) and 7.3(25H, m, phenyl); (Found: C, 67.8; H, 5.3; N, 3.7%. $C_{44}H_{41}N_2O_9Cl$ requires C, 68.0; H, 5.3; N, 3.6%). (19b), m.p. 133.0-133.5°C (toluene-Et_2O); ν_{max}($CHCl_3$) 3000, 1740, 1680(sh), 1450 and 1405 cm^{-1}; δ_H($CDCl_3$) 1.81(1H, m, ring CH_2), 2.02(1H, m, ring CH_2), 2.25(2H, m, ring CH_2), 2.99(3H, s, $NOCH_3$), 3.68(3H, s, CO_2CH_3), 4.03(2H, s, CH_2N), 4.04(1H, AB quartet, J 15.04Hz. CH_2Cl), 4.56(1H, AB quartet, J 15.04Hz, CH_2Cl), 4.78(1H, m, ring CH), 5.22(1H, AB quartet, J 12.38Hz, benzylic), 5.24(1H, AB quartet, J 12.38Hz, benzylic), 6.84(1H, s, $CHPh_2$) and 7.3(15H, m, phenyl); (Found: C, 61.7; H, 5.2; N, 4.6%. $C_{32}H_{33}N_2O_9Cl$ requires C, 61.5; H, 5.3; N, 4.5%). (19c), m.p. 143.0-144.0°C (toluene-Et_2O); ν_{max}($CHCl_3$) 3000, 1735, 1680, 1450 and 1405 cm^{-1}; δ_H($CDCl_3$) 1.83(2H, m, ring CH_2), 2.30(2H, m, ring CH_2), 3.65(3H, s, OCH_3), 3.78(3H, s, OCH_3), 4.04(1H, AB quartet, J 14.59Hz, CH_2Cl), 4.20(1H, AB quartet, J 13.71Hz, CH_2N),4.30(1H, AB quartet, J 13.71Hz, CH_2N), 4.74(1H, AB quartet, J 14.59Hz, CH_2Cl), 4.84(1H, m, ring CH), 4.96(1H, AB quartet, J 12.38Hz, benzylic), 5.08(1H, AB quartet, J 12.38Hz, benzylic), 6.85(1H, s, $CHPh_2$) and 7.3(15H, m, phenyl); (Found: C, 61.4; H, 5.3; N, 4.7%. $C_{32}H_{33}N_2O_9Cl$ requires C, 61.5; H, 5.3; N, 4.5%). (17), identical to the material derived from (15).

Methyl (3S*, 6S*)-6-benzhydryloxycarbonyl-2-benzyloxycarbonyl-6-[(methoxyamino)methyl]-perhydro-1,2-oxazine-3-carboxylate (20). - A solution of diester (19b) (313mg, 0.5mmol) and thiourea (76mg, 1mmol) in EtOH (50ml) and EtOAc (1ml) was stirred for 18 hr at 60°C then allowed to cool to room temperature and saturated aq. $NaHCO_3$ (25ml) was added. The resulting suspension was stirred for 20 min then partitioned between water (25ml)-brine (25ml) and $CHCl_3$ (50ml). The aq. layer was extracted with $CHCl_3$ (3 x 30ml). The combined organic extracts were dried (K_2CO_3) and concentrated in vacuo. The residue was purified by flash chromatography (15% EtOAc-CH_2Cl_2) to give (20) as a colourless oil (195.4mg, 71%). δ_H($CDCl_3$) 1.80(1H, m, ring CH_2), 2.07(3H, m, ring CH_2), 3.04(3H, s, $NOCH_3$), 3.39(1H, AB quartet, J 13.71Hz, CH_2N), 3.44(1H, AB quartet, J 13.71Hz, CH_2N), 3.72(3H, s, CO_2CH_3), 4.73(1H, m, ring CH), 5.21(1H, AB quartet, J 11.96Hz, benzylic), 5.30(1H, AB quartet, J 11.96Hz, benzylic), 6.93(1H, s, $CHPh_2$) and 7.3(15H, m, phenyl).

(3S*, 6S*)-2-Benzyloxycarbonyl-6-[(methoxyamino)methyl]-3-methoxycarbonylperhydro-1,2-oxazine-6-carboxylic acid (21). - A solution of the benzhydryl ester (20) (138.7mg, 0.253mmol) in trifluoroacetic acid (5ml) was stirred for 45 min at room temperature then concentrated in vacuo. The residue was taken up in EtOAc (15ml). The solution was extracted with 1:5 saturated aq. $NaHCO_3$-water (3 x 6ml). The combined extracts were adjusted to pH 6 with HCl (2M) and then to pH 4.5 with saturated aq. NaH_2PO_4. The aq. solution was extracted with EtOAc (4 x 25ml). The combined extracts were dried ($MgSO_4$) and concentrated in vacuo to give (21) as a colourless oil (90.6mg, 94%) which was used without further purification.

Methyl (4S*, 7S*)-6-benzyloxycarbonyl-2-methoxy-1-oxo-5-oxa-2,6-diazaspiro[3.5]nonane-7-carboxylate (22). - A mixture of the methoxyamino acid (21) (273mg, 0.71mmol), $KHCO_3$ (355mg, 3.55mmol), aq. tetra-n-butylammonium bisulphate (0.1M, 1.07ml, 0.107mmol) in water (20ml) and $CHCl_3$ (20ml) was stirred at room temperature. After all material had dissolved a solution of methanesulphonyl chloride (0.11ml, 1.42mmol) in $CHCl_3$ (2ml) was added. The mixture was stirred for 20 hr at room temperature and saturated aq. $NaHCO_3$ (10ml) was added. The organic layer was separated, dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash chromatography (12% EtOAc-CH_2Cl_2) to give (22) as a colourless oil (138.7mg, 53%). ν_{max}($CHCl_3$) 3000,1790, 1740, 1715 and 1480 cm^{-1}; δ_H($CDCl_3$) 1.68[1H, m, H(9)], 1.90[1H, m, H(8)], 2.14[1H, m, H(9)], 2.43[1H, m, H(8)], 3.43[1H, AB quartet, J 4.78, H(3)], 3.63(3H, s, $NOCH_3$), 3.69[1H, AB quartet, J 4.78, H(3)], 3.71(3H, s, CO_2CH_3), 4.78(1H, dd, J 2.0, 5.7Hz, ring CH), 5.13(1H, AB quartet, J 12.13Hz, benzylic),5.23(1H, AB quartet, J 12.13Hz, benzylic), and 7.3(5H,m, phenyl);

δ_C($CDCl_3$) 21.80[t, C(9)],23.65[t,C(8)],52.88(q, $NOCH_3$), 56.03[t, C(3)], 56.27[d, C(7)], 63.04 (q, ester CH_3), 68.16(t, benzylic) 87.18[s, C(4)], 128.16, 128.43 and 128.55 (all d, phenyl), 135.62(s, phenyl), 155.33(s, carbonyl),159.70(s, carbonyl) and 168.99(s, ester carbonyl); m/e 364(M^+); (Found: C, 56.30; H, 5.45; N, 7.65%; MH^+, 365.1356. $C_{17}H_{20}N_2O_7$ requires C, 56.05; H, 5.55; N, 7.70%; MH^+,365.1349).

N-[(3-Benzyloxycarbonyl-2-oxa-3-azabicyclo[2.2.2]oct-5-en-1-yl)methyl]-N-(4,4'-dimethoxydiphenylmethyl)amine (23). - A solution of 4,4'-dimethoxydiphenylmethylamine (8.74g, 35.8mmol) in dry MeOH (30ml) was added to a solution of the aldehyde (10) (4.90g, 17.9mmol) in dry MeOH (30ml). The mixture was adjusted to pH 6 with methanolic HCl (M). Ammonium acetate (400mg, 5.2mmol) and activated molecular sieves 3A (5g) were added and after stirring for 2 hours at room temperature under Ar $NaCNBH_3$ (1.22g, 19.3mmol) was added. The mixture was stirred for 24 hours then filtered. The filtrate was adjusted to pH 3 with methanolic HCl (M) and concentrated *in vacuo*. The residue was triturated with Et_2O then partitioned between Et_2O and aq. K_2CO_3. The aq. layer was extracted with Et_2O (3 x 150ml). The combined Et_2O solutions were dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by flash chromatography (Et_2O:light petroleum 3:1) to give (23) as a colourless oil (5.28g, 59%). ν_{max} ($CHCl_3$) 1710, 1610, 1590 and 1510cm^{-1}; δ_H($CDCl_3$) 1.28-2.15(5H,m,ring CH_2 and NH), 2.87(2H,s,CH_2N), 3.77(3H,s,OCH_3), 3.79(3H,s, OCH_3), 4.78(1H,s,$CHAr_2$), 4.80-4.82(1H,m, ring CH), 5.14(2H,s,benzylic), 6.56-6.66(2H,m, vinylic), 6.79-7.37(8H,m, aromatic) and 7.29(5H,s,phenyl); m/e 500(M^+).

[(3-Benzyloxycarbonyl-2-oxa-3-azabicyclo[2.2.2]oct-5-en-1-yl)methyl]amine (24). - A solution of protected amine (23) (1.31g, 2.62mmol) in anisole (2ml) and trifluoroacetic acid (15ml) was stirred for 1 hour at room temperature under Ar. The solution was concentrated *in vacuo* and the residue was partitioned between Et_2O (25ml) and aq. HCl (M, 25ml). The aq. layer was extracted with Et_2O basified with concentrated aq. K_2CO_2 and extracted with EtOAc (8 x 25ml). The combined EtOAc solutions were dried (Na_2SO_4) and concentrated *in vacuo* to give (24) as a colourless oil (640mg, 89%). ν_{max}($CHCl_3$) 3390, 1710, 1615 and 1590 cm^{-1}; δ_H($CDCl_3$) 1.24-2.21(6H,m,ring CH_2 and NH_2), 3.01(2H,s,CH_2N), 4.78-4.82(1H,m,ring CH), 5.15(2H,s,benzylic), 6.44-6.63(2H,m,vinylic) and 7.34(5H,s,phenyl); (Found: M^+, 274.1320; $C_{15}H_{18}N_2O_3$ requires M^+, 274.1317).

N-[(3-Benzyloxycarbonyl-2-oxa-3-azabicyclo[2.2.2]oct-5-en-1-yl)methyl]chloroacetamide (25). - Et_3N (0.4ml, 2.5mmol) and chloracetyl chloride (0.23ml, 2.4mmol) were added to a solution of the amine (24) (640mg, 2.3mmol) in CH_2Cl_2 (10ml) at 0°C. The solution was stireed for 90 minutes at the same temperature. Water (10ml) was added and the organic layer was separated. The aq. layer was extracted with CH_2Cl_2 (3 x 10 ml). The combined organic solutions were dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by preparative t.l.c. (Et_2O) to give (25) as a colourless oil (700mg, 86%). m.p. 65-66°C(Et_2O-light petroleum); ν_{max}($CHCl_3$) 3430, 1710, 1680, 1535 and 1500 cm^{-1}; δ_H($CDCl_3$) 1.32-2.21(4H,m,ring CH_2), 3.33(1H,dd,J14,3.5Hz,CH_2N), 4.02 (2H,s,CH_2Cl), 4.06(1H,dd,J14,8Hz,CH_2N), 4.79-4.83(1H,m,ring CH), 5.13(1H,AB quartet, J12Hz,benzylic), 5.18(1H,AB quartet,J12Hz,benzylic), 6.30-6.62(2H,m, vinylic), 7.13(1H,br s,NH) and 7.34(5H,s,phenyl); (Found: C,58.57;H,5.41;N,7.83%. $C_{17}H_{19}N_2O_4Cl$ requires C,58.21; H,5.46;N,7.99%).

(3S*, 6S*)-2-Benzyloxycarbonyl-6-[(chloroacetamido)methyl]perhydro-1-2-oxazine-3,6-dicarboxylic acid (26). - To an ice-cooled mixture of chloroacetamide (25) (1,186g, 3.39mmol) in PhH (60ml), aq. n-Bu_4NHSO_4 (0.1M, 3.74ml, 0.374mmol) and aq. $KMnO_4$ (0.2m, 64.4ml, 12.88mmol) was added over 5 minutes with stirring. After stirring for 30 minutes at room temperature concentrated aq. sodium sulphite (10ml) was added with external cooling. The mixture was filtered and the filter cake washed with water and EtOAc. The combined filtrate was basified with concentrated aq. $NaHCO_3$. The aq. layer was separated, acidified with HCl (M) and extracted with EtOAc (5 x 200ml). The combined extracts were dried (Na_2SO_4) and concentrated *in vacuo*. The resulting colourless oil was crystallised from EtOAc to give (26) as white crystals (810mg, 58%). m.p. 178-179°C(dec); ν_{max}(CH_3CN) 3200, 1755, 1720, 1685 and 1535cm^{-1}; δ_H(CD_3CN) 1.79-2.23 (4H,m, ring CH_2), 3.48(1H,dd,J14,3Hz,CH_2N), 3.92(2H,s,CH_2Cl), 4.05(1H,dd,J14,8Hz,CH_2N), 4.74(1H,dd,J4.6,ring CH), 5.23(2H,s,benzylic), 7.16(1H,br s,NH) and 7.39(5H,s,phenyl); (Found: C,49.01;H,4.66;N,6.69%. $C_{17}H_{19}N_2O_8Cl$ requires C,49.22;H,4.62;N,6.76 %).

N-[(3S,6S)-6-Benzhydryloxycarbonyl-2-benzyloxycarbonyl-6-[(chloroacetamido)methyl]-perhydro-1,2-oxazine-3-carbonyl]-O-benzyl-L-threonine benzyl ester (29) and its diastereoisomer. - Et_3N (418µl, 3.0mmol) was added to a suspension of the diacid (26) (624mg, 1.5mmol) in dry CH_3CN (6ml) at 0°C under Ar and the mixture was stirred until it became homogeneous. Pivaloyl chloride (366µl, 3.0mmol) was added and the mixture was stirred for 10 minutes at 0°C then 30 minutes at room temperature. The mixture was again cooled to 0°C and O-benzyl-L-threonine benzyl ester [16] (448mg, 1.5mmol) in CH_3CN (4ml) was added. After stirring for 30 minutes at 0°C Et_3N (210µl, 1.5mmol) was added. The solution was stirred for 30 minutes at 0°C then 2 hours at room temperature. Saturated aq. $NaHCO_3$ (20ml) and water (20ml) were added and the resulting mixture was stirred vigorously for 30 minutes. The mixture was extracted with Et_2O (2 x 10ml). The aq. layer was acidified with HCl (M) and extracted with EtOAc (5 x 25ml). The combined EtOAc solutions were dried (Na_2SO_4) and concentrated *in vacuo*. The resulting crude acid (28) was dissolved in $CHCl_3$ (25ml) and diphenyldiazomethane in CH_2Cl_2 was added dropwise with stirring

until the purple colour just persisted. After stirring for 30 minutes at room temperature the excess diphenyldiazomethane was destroyed with a few drops of AcOH. The solution was concentrated in vacuo. The residue was crystallised from EtOAc to give (29) as white crystals (320mg, 25%). m.p. 180-182°C; ν_{max}($CHCl_3$) 1745, 1680 and 1525 cm^{-1}; δ_H($CDCl_3$) 1.06(3H,d,J6.2Hz, CH_3), 1.9-2.2(4H,m,ring CH_2), 3.39(2H,coincident d,J15Hz,CH_2N and CH_2Cl), 3.6(1H,AB quartet,J15Hz,CH_2Cl), 4.1(1H,dq,J6.3,2.5Hz,$CHCH_3$), 4.2(1H,d,J11.9Hz,$CHOCH_2Ph$), 4.25(1H,dd,J15,9.5Hz,CH_2N), 4.4(1H,d,J11,9Hz,$CHOCH_2Ph$), 4.61(1H,dd,J2.5,9Hz,$NHCHCO_2$), 4.7(1H,t,J6.5Hz,ring CH), 5.05(1H,AB quartet,J12Hz,benzylic), 5.10(1H,AB quartet,J12Hz,benzylic), 5.15(1H,AB quartet,J12Hz,benzylic) 5.20(1H,AB quartet,J12Hz,benzylic), 6.87(1H,s,$CHPh_2$), 7.04(1H,d,J9Hz,NH), and 7.1-7.4(26H,m,phenyl and NH); (Found: C,66.65;H,5.57;N,4.87%. $C_{48}H_{48}N_3O_{10}Cl$ requires C,66.85;H,5.61;N,4.87%). The mother liquor was concentrated in vacuo. The residue was crystallised from Et_2O to give the diastereoisomer of (29) (365mg, 28%). m.p.98-100°C; δ_H($CDCl_3$) similar to the above, notable exceptions being, 1.09(3H,d,J6.2Hz, CH_3), 3.35(1H,d,J15Hz,CH_2Cl), 4.95(1H,AB quartet,J12Hz,benzylic) and 5.02(1H,AB quartet,J12Hz,benzylic).

O-Benzyl-N-[(3S,6S)-2-benzyloxycarbonyl-6-carboxy-6-[(chloroacetemido)methyl]perhydro-1,2-oxazine-3-carbonyl]-L-threonine benzyl ester (28). - A solution of the benzhydryl ester (29), m.p. 180-182° (115mg, 0.13mmol) in trifluoroacetic acid (5ml) was stirred for an hour at room temperature under Ar. The solution was concentrated in vacuo and the residue was partitioned between Et_2O and dilute aq. $NaHCO_3$. The aq. layer was extracted with Et_2O (2 x 15ml), acidified with HCl (M) and extracted with EtOAc (5 x 20 ml). The combined EtOAc solutions were dried (Na_2SO_4) and concentrated in vacuo to give (28) (984mg, 90%). m.p. 154-156°C; δ_H(CD_3CN) 1.12(3H,d,J6.2Hz,CH_3), 1.9-2.22(4H,m,ring CH_2), 3.39(1H,ABX,J14.5,2.8Hz,CH_2N), 3.92(2H,s,CH_2Cl), 4.07(1H,ABX,J14.5,9.5Hz,CH_2N), 4.16(1H,dq,J6.3,2.5Hz,CH_3CH), 4.29(1H,AB quartet,J,11.7Hz, $CHOCH_2Ph$), 4.52(1H,AB quartet,J11.7Hz, $CHOCH_2Ph$), 4.61(1H,dd,J2.5,9Hz,$NHCHCO_2$), 4.68(1H,t,6.5Hz, ring CH), 5.12(2H,s,benzylic), 5.17(2H,s,benzylic), 6.95(1H,d,J9.1Hz,NH) and 7.21-7.37(16H,m,phenyl and NH); (Found: C,60.50;H,5.57;N,5.77%. $C_{35}H_{38}N_3O_{10}Cl$ requires C,60.38;H,5.50;N,6.03%).

N-[(3S,6S)-6-Aminomethyl-2-benzyloxycarbonyl-6-carboxyperhydro-1,2-oxazine-3-carbonyl]-O-benzyl-L-threonine benzyl ester (30). - Thiourea (24mg, 0.32mmol) was added to a solution of the acid (28) (49mg, 0.07mmol) in dry CH_3CN (1ml) and dry EtOH (3.5ml) under Ar. The solution was stirred for 3 days at exactly 40°C. After cooling to room temperature water (6ml) and saturated aq. $NaHCO_3$ (6ml) were added and the resulting mixture was stirred for 30 minutes at room temperature. The solution was then acidified to pH 6 with HCl (M) and extracted with EtOAc (5 x 10ml). The combined EtOAc solutions were dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by preparative t.l.c. (15% MeOH-$CHCl_3$) to give (30) (18mg, 41%). ν_{max}(CH_3CN) 3640, 3540, 1735, 1700, 1670 and 1630 cm^{-1}.

O-Benzyl-N-[(4S,7S)-6-benzyloxycarbonyl-1-oxo-5-oxa-2,6-diazaspiro[3.5]nonane-7-carbonyl]-L-threonine benzyl ester (31). - A mixture of the β-amino acid (30) (15mg, 0.024mmol), PPh_3 (7.6mg, 0.029mmol), 2,2'-dithiodipyridine (6.5mg, 0.029mmol) and dry CH_3CN (3ml) was refluxed for 6 hours under Ar. The mixture was concentrated in vacuo and the residue was purified by preparative t.l.c. (EtOAc) to give (31) as a colourless oil (2mg, 14%). ν_{max}(neat) 1780, 1745 and 1680 cm^{-1}; δ_H($CDCl_3$) 1.27(3H,d,J6.3Hz,CH_3), 1.7-2.5(2H,m,ring CH_2), 3.5(1H,AB quartet,J5.7Hz,CH_2N), 3.68(1H,br AB quartet, J5.7Hz,CH_2N), 4.19(1H,dq,6.3,2.4Hz,CH_3CH), 4.30(1H,AB quartet,J12Hz,$CHOCH_2Ph$), 4.54 (1H,AB quartet, J12Hz,$CHOCH_2Ph$), 4.73(1H,dd,J9.1, 2.4Hz,$NHCHCO_2$), 4.92(1H,dd,J5.7,2.4Hz,ring CH), 5.07(1H,AB quartet, J12Hz,benzylic),5.12(1H,AB quartet,J12Hz,benzylic), 5.14(1H,AB quartet,J12Hz,benzylic), 5.2(1H,AB quartet,J12Hz, benzylic), 5.8(1H,br s,NH) and 7.0-7.4(16H,m,phenyl and NH); m/e 602(MH^+).

Tabtoxin (1) and its diastereoisomer (32). - The spiro β-lactam (31) was dissolved in dry EtOH (2ml) and 10% Pd-C (10mg) was added. The mixture was stirred for 16 hours under H_2. The mixture was filtered through celite and the celite was washed with EtOH (4ml). The filtrate was concentrated in vacuo to give tabtoxin (1) as a colourless oil (1mg, 100%). δ_H(D_2O) 1.02(3H,d,J7Hz,CH_3), 1.66-1.92(4H,m,ring CH_2), 3.16(1H,d,J6Hz,CH_2N), 3.30(1H,d,J6Hz,CH_2N) and 4.0-4.12(3H,m,CH_3CHO,CHOH and $CHNH_2$) (identical to natural tabtoxin, Figure 2 (A) (B) (C)). The diastereoisomer (32) was prepared in an identical manner starting with the lower m.p. isomer of the benzhydryl ester (29). Noteworthy is the fact that in this case the yield of the β-lactam formation was 30% and that the stereoisomer of the spiro β-lactam (31) exhibited a methyl doublet at δ 1.1 which is significantly different from that of (31) (Figure 2 (D)).

Crystal Data. $C_{18}H_{21}N_2O_5Cl$, M = 380.9, monoclinic, a = 5.977(1), b = 19.945(4), c = 16.059(2)Å, β = 98.80(2)°, U = 1892 $Å^3$, Z = 4, Dc = 1.34g cm^{-3}, μ (MoK_α) = 2.36 cm^{-1}, space group $P2_1/C$.

A colourless crystal of (14) was obtained by slow evaporation of CH_2Cl_2 - hexane. Data collection was performed with a CAD - 4F computer controlled diffractometer equipped with graphite crystal monochromator. The cell dimensions were derived by least-squares calculations from angular settings of 25 reflections measured at $8 \leq \Theta < 16°$. Integrated intensities of 2575 reflections with $\Theta < 25°$ were collected using $\theta/2\theta$ mode. 1755 reflections were assumed to be observed by the criterion $I > 3(I)$ and used for further calculations. Data were corrected for Lorentz and polarisation effects but not for absorption.

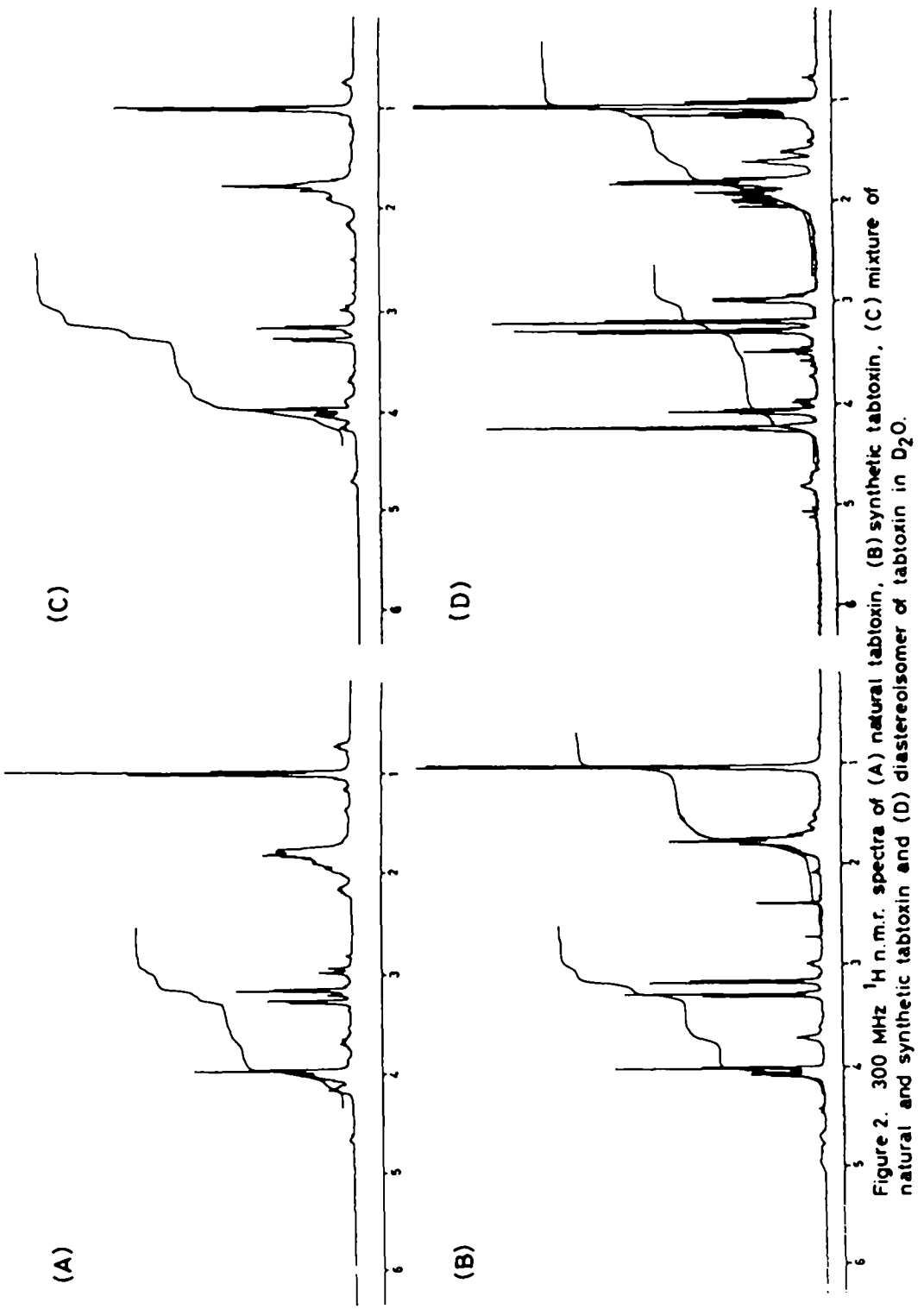


Figure 2. 300 MHz ^1H n.m.r. spectra of (A) natural tabtoxin, (B) synthetic tabtoxin, (C) mixture of natural and synthetic tabtoxin and (D) diastereoisomer of tabtoxin in D_2O.

The structure was solved using MULTAN 80[18] and refined with isotropic temperature factors. All hydrogens except those attached to the phenyl ring were located on a difference fourier map and included in the refinement (co-ordinates and isotropic temperature factors). Hydrogens attached to the phenyl ring were placed geometrically with a C-H distance 1.0Å. The weight for each reflection was calculated[19] from the Chebyshev series $W = [392.5 \text{ to } (X) + 586.0\ t_1(X) + 236.4\ t_2(X) + 41.0\ t_3(X)]^{-1}$ where $X = Fo/(Fo)_{max}$. Least squares refinement converged with conventional $R = 0.039$ and $R_w = 0.049$ for observed reflections. In the final cycle all shifts in parameters were less than 0.5 of their e.s.d's. The difference Fourier map calculated after the last cycle of refinement showed no peaks higher than $0.4eÅ^{-3}$.

All calculations were carried out on the Oxford University Chemical Crystallogrphy VAX 11/750 computer using MULTAN 80, CRYSTALS[20] and CHEMGRAF[21] packages of programs. Atomic scatterings were taken from reference 22.

Tables of bond distances and angles have been deposited at the Cambridge Crystallographic Data Centre, University Chemical Laboratories, Lensfield Road, Cambridge CB2 1EN.

ACKNOWLEDGEMENT. - We are indebted by Dr. J. G. Turner, School of Biological Sciences, University of East Anglia, for a sample of natural tabtoxin and also for the biological assays. We would also like to thank the S.E.R.C. for support (P.M.W.).

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