Tetrahedron Vol. 42, No. 12, pp. 3097 to 3110, 1986
Printed in Great Britain.

0040-4020/86 $3.00+.00
Pergamon Journals Ltd.

SYNTHETIC STUDIES ON TABTOXIN. SYNTHESIS OF A NATURALLY OCCURING INHIBITOR OF GLUTAMINE SYNTHETASE, TABTOXININE-β-LACTAM, AND ANALOGUES†

JACK E. BALDWIN*, MASAMI OTSUKA AND PHILIP M. WALLACE.

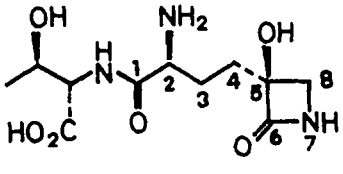
The Dyson Perrins Laboratory, University of Oxford, South Parks Road, Oxford, OX1 3QY, U.K.

(*Received in UK* 28 *November* 1985)

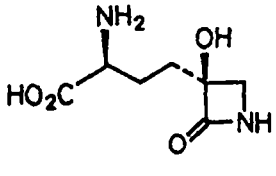
Abstract- (±)- Tabtoxinine-β-lactam 2, a potent inhibitor of glutamine synthetase, and related compounds, have been synthesised by a cycloaddition approach. The cycloaddition of an acylnitroso compound to cyclohexadienes proceeded regioselectively to give the bicyclic, ester 8 (X- CO_2Et) or nitrile 24. These were converted into the cyclic diacids 15, 17, 27 and 33 by permanganate oxidation. A benzyl chloroformate mediated esterification procedure was developed to enable differentiation of the two carboxyl groups to provide mono esters 16a, 19, 28a and 34. Treatment of hydroxy diacid 20 with benzyl chloroformate gave the spiro β-lactone 11. Cyclisation of β-amino acids 29 and 35 followed by hydrogenation gave (±)- tabtoxinine-β-lactam 2 and a methoxy derivative 5 respectively. Alternatively spiro β-lactams 45a and 45b were obtained in a ratio of 41 :59 by a regioselective 1,3-dipolar cycloaddition of nitrone 39 to the exomethylene β-lactam 44.

Introduction

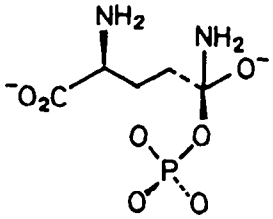
Tabtoxin 1 is a dipeptide exotoxin produced by Pseudomonas tabaci, the organism responsible for the Wildfire disease of tobacco plants.[1] When hydrolysed by peptidases in vivo, this exotoxin releases tabtoxinine-β-lactam 2, which inhibits glutamine synthetase of the photorespiratory nitrogen cycle, causing chlorosis and death of the tobacco plant.[2] As the dipeptide 1 itself does not inhibit purified glutamine synthetase in vitro,[2c] the amino acid 2 is considered to be the active form of 1 and hence the actual toxin of Wildfire disease. It seems likely that this inhibition is the result of tight binding of tabtoxinine-β-lactam 2 to the enzyme as an analogue of the postulated tetrahedral intermediate 3 involved in the enzyme reaction.[1a] The inhibition of



1



2

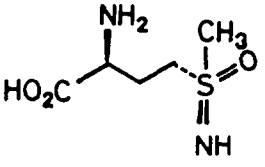


3

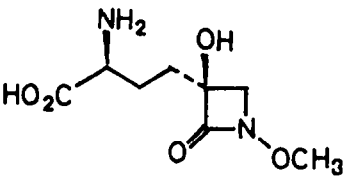
glutamine synthetase by L-methionine-S-sulphoximine 4 and related compounds has previously been documented and extensively studied.[3] Since the detailed mechanism of glutamine synthetase inhibition by tabtoxinine-β-lactam attracts current interest, a synthetic approach to the toxin 2 and its analogues is of increasing importance. In 1983 we achieved a stereospecific synthesis of the dipeptide toxin[4] but found that this approach was not amenable to the synthesis of the actual

† This paper is dedicated to Professor R. Raphael on the occasion of his 65th birthday.

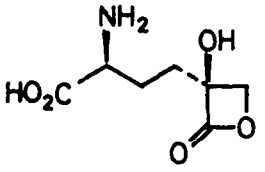
toxin, tabtoxinine-β-lactam 2, nor was the hydrolysis of 1, under acidic[1e] or enzymatic[2b] conditions, a satisfactory source of 2. Consequently we investigated a new and efficient route to the toxin 2. Herein we described in detail the synthesis of (±)- tabtoxinine-β-lactam 2 [3] and its N-methoxy analogue 5, as well as synthetic studies towards the β-lactam analogue 6 and the lower homologue 7.



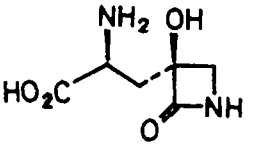
4



5



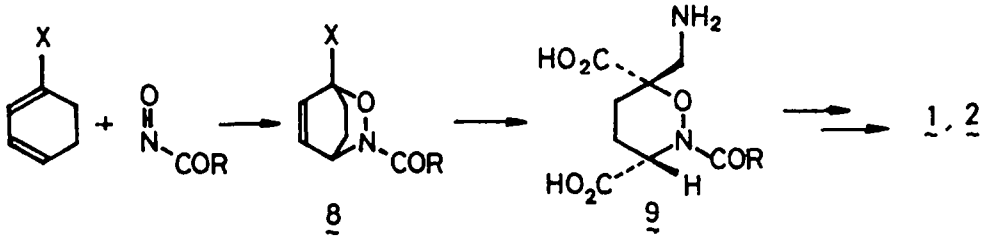
6



7

β-Lactone Approach

In our synthesis of tabtoxin[4] the crucial stereochemical relationship of the 1,4-amino alcohol moiety was achieved by the simultaneous formation of C(2)-N and C(5)-O bonds in a nitroso Diels-Alder reaction[5] (Scheme 1, X= CO_2Et). The formation of perhydro-1,2-oxazine ring in 9



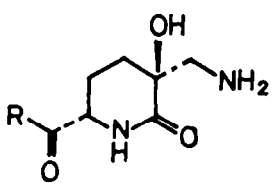
8

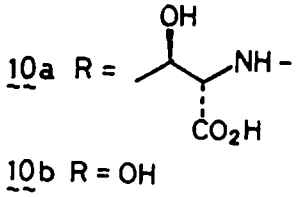
9

R = OCH_2Ph

X = CO_2Et, CN

Scheme 1

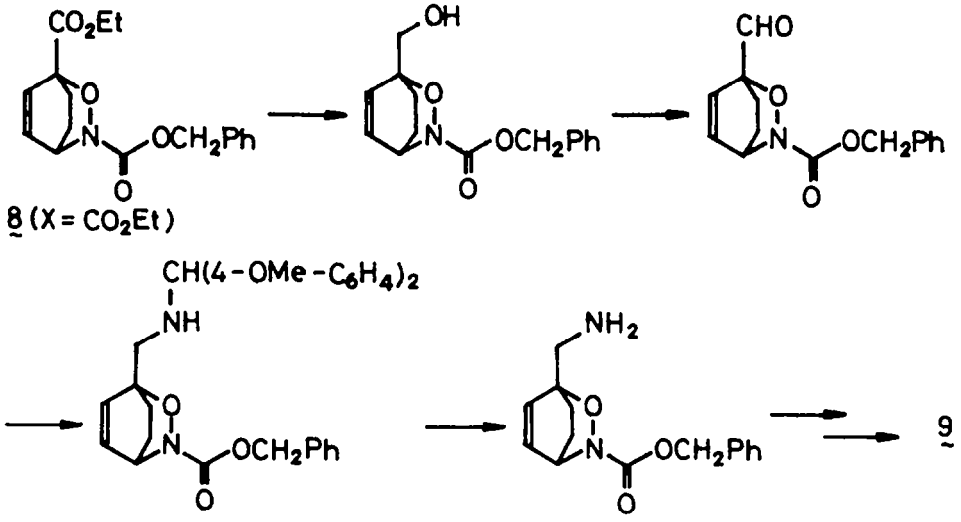




10a R =

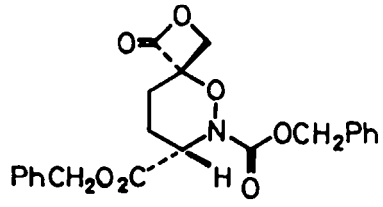
10b R = OH

avoided the intramolecular transacylation of the toxin to the stable, inactive, δ-lactam, such as isotabtoxin 10 or tabtoxinine-δ-lactam 10b.[1b] This Diels-Alder approach seemed also applicable to the synthesis of amino acid 2. However, the intermediate cyclic amino acid 9 was not easily accessible by the previous route[4] involving a complicated multistep procedure to introduce the amino group into the adduct 8 (Scheme 2). Direct amination of 8 was not possible

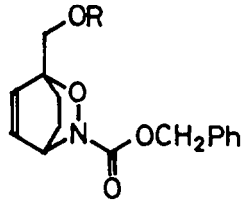


Scheme 2 [4]

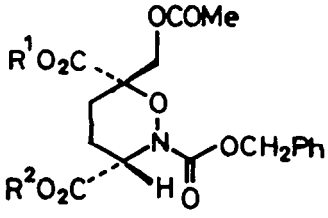
probably because of the neopentylic nature of the substituent X. Moreover, we confronted a serious difficulty in the differentiation of the functional groups of the amino diacid (9). Attempts to cyclise 9 to the corresponding β-lactam failed,[7] and so selective protection of one of the two carboxyl groups seemed essential for successful β-lactam closure. However, treatment of an amino-protected derivative of 9 with diphenyldiazomethane (1 eq.) followed by diazomethane yielded all of the four possible regioisomers of mixed benzhydryl-methyl diesters nonselectively.[8b] Therefore we investigated an alternate route involving spiro β-lactone 11 as a key intermediate. It was considered that the β-lactone 11 could be ring opened with ammonia to a β-amino acid or β-hydroxy amide and subsequently cyclised to a β-lactam. Also compound 11 could be regarded as a reasonable precursor for the β-lactone analogue 6 which might be more reactive than the naturally occuring toxin 2.



11

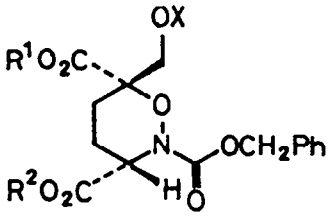


12 R = H
13 R = COMe
14 R = $SiMe_2Bu^t$

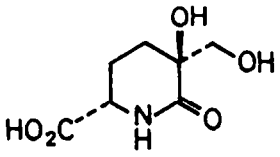


15 $R^1 = R^2 = H$
16a $R^1 = H$, $R^2 = CH_2Ph$
16b $R^1 = R^2 = CH_2Ph$

The starting bicyclic alcohol, 12, had already been prepared in a good yield[9] from 8 (X-CO_2Et),and was now transformed into the corresponding acetate 13 (90%). The double bond in the bicyclic system was smoothly cleaved according to the procedure of Starks[8], affording acetyl diacid 15 (80%). In preparation for subsequent β-lactone closure the selective esterification of the diacid to a monoester was attempted. When 15 was treated with benzyl bromide (2.4 eq.) in the presence of tetra-n-butylammonium bromide in a two-phase system[8] dibenzyl ester 16b was obtained (70%). This method afforded the diester exclusively and use of 1 equivalent of benzyl bromide resulted in the formation of diester (12%) no monoester being obtained. Monobenzyl ester 16a was first obtained nonselectively as follows. Treatment of the diacid 15 with toluene-p-sulphonyl chloride (2 eq.) in the presence of pyridine at 0°C followed by benzyl alcohol (1 eq.) afforded monoester 16a and diester 16b (23% and 21%, respectively). Use of 3 equivalents of toluene-p-sulphonyl chloride resulted in exclusive formation of the diester 16b. After extensive investigation a satisfactory new method for the differentiation was devised. Thus, on treatment of the diacid 15 with benzyl chloroformate (1.5 eq.) in the presence of pyridine in dichloromethane, monoester 16a was obtained as a major product (67%) along with a small amount of diester 16b (4.9%). To the best of our knowledge[10] this decarboxylative esterification is a new procedure for the preparation of benzyl esters. However, in spite of the successful differentiation of the diacid, attempts to cleave the acetyl group of 16a were entirely unsuccessful. It seemed likely that this ring system, containing a N-O single bond and sensitive functional groups in addition to acidic hydrogens, undergoes facile skeletal rearrangement in various ways under basic conditions.



17 $R^1=R^2=H$, $X=SiMe_2Bu^t$
18a $R^1=H$, $R^2=CH_2Ph$, $X=SiMe_2Bu^t$
18b $R^1=R^2=CH_2Ph$, $X=SiMe_2Bu^t$
19 $R^1=X=H$, $R^2=CH_2Ph$
20 $R^1=R^2=X=H$



21

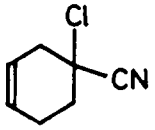
For this reason a silyl protecting group for this alcohol was tried. Thus, the alcohol (12) was treated with tert-butylchlorodimethylsilane in the usual manner[11] to give the silyl derivative 14 (91%). Permanganate oxidation[8] of 14 was straightforward and silyl diacid 17 was isolated as a stable colourless powder (94%). The differentiation of the two carboxyl groups of the silyl diacid (17) was achieved by the benzyl chloroformate procedure described above, monoester 18a and diester 18b being obtained in a ratio of 4 : 1. Whereas the silyl group of the diacid 17 was remarkably stable the monoacid 18a was quite labile. On the other hand, when the mixture of 18a and 18b obtained from 17 as above was directly treated with acetic acid, β-hydroxy acid 19 and silyl diester 18b were obtained (43% and 13%, respectively). Treatment of 19 with N,N'-dicyclohexyl-carbodiimide gave a crude mixture which exhibit a β-lactone absorption above 1800 cm^{-1} in its ir spectrum. This confirmed the desired β-hydroxy acid structure of 19 resulting from the preferential esterfication of the less hindered carboxyl group. However, the β-lactone now formed was found to be highly labile and was decomposed during silica gel chromatography. Consequently an alternate procedure for β-lactone formation was required and was developed as follows. The silyl protecting group of 17 was smoothly cleaved with tetra-n-butylammonium fluoride to yield hydroxy diacid 20 quantitatively. Simultaneous formation of β-lactone and benzyl ester was successfully

accomplished by treatment of 20 with benzyl chloroformate (2.5 eq.) and pyridine (3 eq.) in dichloromethane at room temperature, yielding spiro β-lactone 11 directly. Most of the side-products and unreacted reagents were removed by extractive work up and evaporation to yield pure β-lactone (11) (39%).

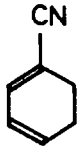
Attempts to transform 11 into a β-lactam were unsuccessful, presumably due to the instability and facile rearrangement of the molecule. Hydrogenation of 11 gave a product which was probably δ-lactam 21.

Synthesis of (±)- Tabtoxinine-β-lactam by an Improved Strategy

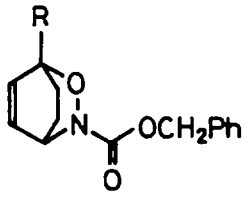
Although the β-lactone analogue was not obtained the usefulness of the new differentiation procedure for the tabtoxin skeleton was demonstrated. In order to avoid the previous difficult introduction of the β-amino group in the synthesis of β-amino acid 29 we, attempted to make use of a cyano-diene in the Diels-Alder strategy (Scheme 1, X= CN). The cyano group in 8 (X= CN) might be transformed into the corresponding amino group by a single reduction step.



22



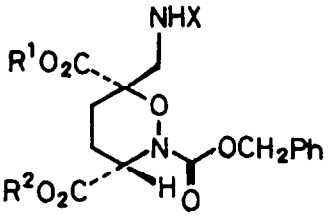
23



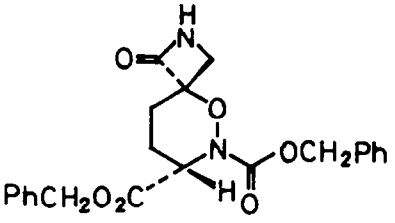
24 R = CN
25 R = CH_2NH_2
26 R = $CH_2NHCO_2Bu^t$

Thus, 2-chloroacrylonitrile was treated with butadiene in a sealed tube to give cyclohexene 22[12] (85%). Dehydrochlorination of 22 was effected by heating it in pyridine at reflux and cyano-diene 23 was obtained (72%). The cycloaddition of diene 23 to benzyl nitrosoformate[a] (generated in situ from benzyl N-hydroxycarbamate[13] and tetraethylammonium periodate[14] in dichloromethane) proceeded regioselectively to afford the adduct 24 (73%) as a single regioisomer. The nitrile function of 24 was smoothly reduced with $NaBH_2(OCOCF_3)$[15] to the primary amine 25 (48%), which was identical to that synthesised previously by a more complex procedure (Scheme 2)[a], confirming the desired regiochemistry of the Diels-Alder step. The high regioselectivity was substantially better than the usual moderate ratios reported.[16]

The amine 25 was then protected with a tert-butoxycarbonyl group [17] to 26 (83%) and oxidative cleavage of the double bond accomplished as before to afford diacid 27 (98%). The key differentiation of the two carboxyl groups of 27 was successfully achieved by decarboxylative esterification with benzyl chloroformate (1.5 eq.) and pyridine in dichloromethane to yield monoester 28a (57%) along with a small amount of diester (28b) (4.8%). Removal of the primary amino protection of 28a (98% formic acid [18]) gave the penultimate precursor, β-amino acid 29 (99%).



27 $R^1=R^2=H$, $X=CO_2Bu^t$
28a $R^1=H$, $R^2=CH_2Ph$, $X=CO_2Bu^t$
28b $R^1=R^2=CH_2Ph$, $X=CO_2Bu^t$
29 $R^1=X=H$, $R^2=CH_2Ph$

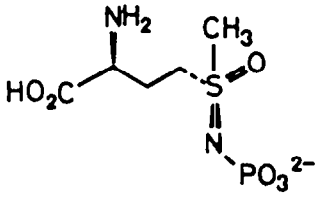


30

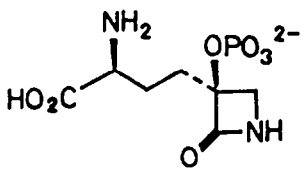
The β-lactam closure was achieved by use of a Ph_3P- (Py S)$_2$- CH_3CN system[19] to yield spiro β-lactam 30 (63%). Complete deprotection and reductive cleavage of the N-O bond of 30 was affected in one step by catalytic hydrogenation to produce (±)- tabtoxinine-β-lactam (2) (quantitative). The synthetic sample was identical (500MHz n.m.r.) to sample isolated from *P.tabaci* and was an active glutamine synthetase inhibitor, *in vitro* and *in vivo*.[2a]

Synthesis of Methoxy Derivative

It has been established that glutamine synthetase requires ATP, ammonia and 2 equivalent of divalent metal to form glutamine and ADP.[3] Initial formation of γ-glutamyl phosphate and subsequent ammonolysis to the phosphorylated tetrahedral intermediate 3 were postulated. Analogously, inhibition of glutamine synthetase by L-methionine-S-sulphoximine was attributed to the formation of N-phosphate 31. Tight binding of phosphorylated tabtoxinine-β-lactam has been



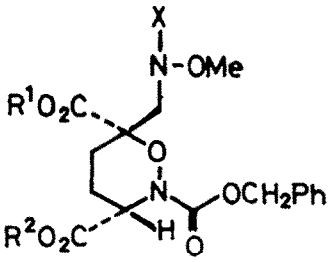
31



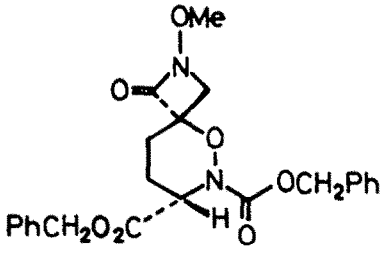
32

suggested. Although tabtoxinine-β-lactam possesses two possible sites for phosphorylation, i.e., the hydroxyl group and the β-lactam nitrogen, only O-phosphorylated species 32 has been proposed.[1] In order to find the position of phosphorylation we designed a methoxy derivative 5 whose β-lactam nitrogen is blocked, but still retains a reactive carbonyl group and the hydroxyl group.

The starting N-methoxy-N-chloroacetyl diacid (33) had been previously prepared in good yield.[4] The carboxyl groups in 33 were smoothly differentiated by our standard benzyl chloroformate procedure to give monoester 34 (50%). The chloroacetyl protection was cleaved with thiourea in ethanol, affording methoxyamine 35 (91%). β-Lactam closure was effected by Mukaiyama's two-phase procedure[20] and methoxy β-lactam 36 was obtained in satisfactory yield (39%). Hydrogenation of 36 afforded the N-methoxy derivative of (±)- tabtoxinine-β-lactam, 5, quantitatively. Glutamine synthetase inhibition by 5 is currently under investigation.



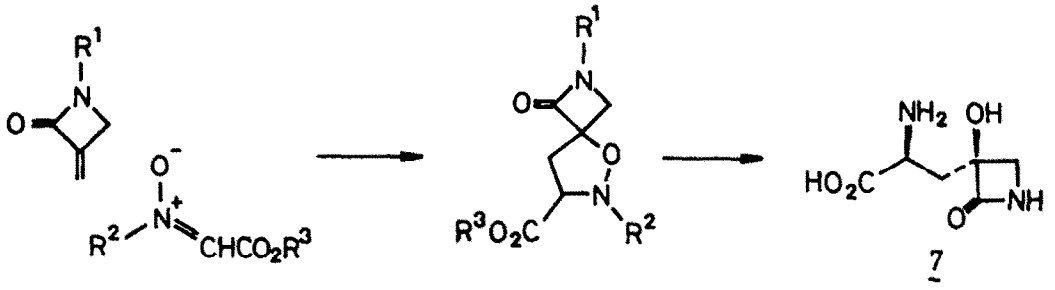
33 $R^1 = R^2 = H$, $X = COCH_2Cl$
34 $R^1 = H$, $R^2 = CH_2Ph$, $X = COCH_2Cl$
35 $R^1 = X = H$, $R^2 = CH_2Ph$



36

1,3-Dipolar Cycloaddition Approach to Lower Homologues

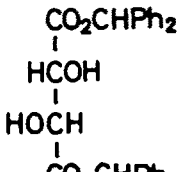
The present nitroso Diels-Alder strategy was shown to provide an efficient route for the tabtoxin skeleton with correct regio- and stereo- chemistry. However, it was considered that a 1,3-dipolar cycloaddition could rapidly furnish the lower homologue of tabtoxinine-β-lactam 7 (Scheme 3). The cycloaddition of nitrones to α, β-unsaturated esters has previously been studied in these laboratories and moderate regioselectivity (2-3 : 1 ratio) was observed.[21] In striking



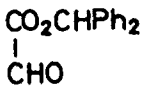
Scheme 3

contrast to these results we now achieved an efficient 1,3-dipolar addition with complete regioselectivity.

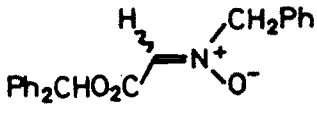
The nitrone was prepared as follows. L-Tartaric acid was esterified with diphenyldiazomethane to dibenzhydryl L-tartrate 37 (95%). The glycol 37 was cleaved with lead tetraacetate in benzene to give glyoxylate 38. Treatment of aldehyde 38 with N-benzylhydroxylamine afforded nitrone 39 as an equilibrium mixture of E and Z isomers (63 : 37). One of the isomers of the nitrone 39 was crystallised from benzene-hexane and the stereochemistry shown to be Z, based on an n.O.e. experiment.



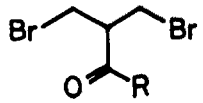
37



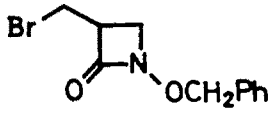
38



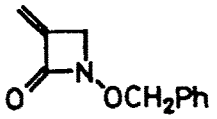
39



40 R = OH
41 R = Cl
42 R = NHOCH$_2$Ph



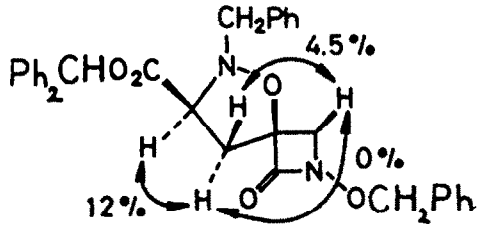
43



44

The dipolarophile, exomethylene β-lactam 44, was synthesised as follows. Dibromo acid 40 [22] was converted to the corresponding acid chloride 41 (thionyl chloride, 91%) followed by reaction with O-benzylhydroxylamine to give hydroxamate 42 (76%). Subsequent β-lactam closure was achieved with sodium hydride. Thus, treatment of β-bromo hydroxamate 42 with sodium hydride (1 eq.) in tetrahydrofuran gave bromomethylazetidinone 43, which was then converted into exomethylene β-lactam 44 by dehydrobromination with triethylamine (95%). β-Lactam 44 could also be obtained directly from 42 by treatment with 2 equivalents of sodium hydride in tetrahydrofuran (90%).

The E and Z mixture of 39 was reacted with exomethylene β-lactam 44 in chloroform at 50°C to yield a mixture of stereoisomers 45a and 45b (quantitative). These isomers were separated by chromatotron and were crystallised, to give a 41 : 59 ratio of 45a to 45b. The nitrone E and Z isomers were found to equilibrate faster than reaction with the alkene, hence, no conclusions could be made about the geometry of the addition, nor could the yield of the required isomer 45a be improved. The use of pure Z isomer of nitrone 39 gave the same 41 : 59 mixture of adducts 45a and 45b as using the mixture of nitrones. The desired regiochemistry of cycloaddition was confirmed by an ABX type coupling in the 300 MHz n.m.r. spectrum and the stereochemistry of each isomer, 45a and 45b, was rigorously established by n.O.e. experiments (Figure 1).



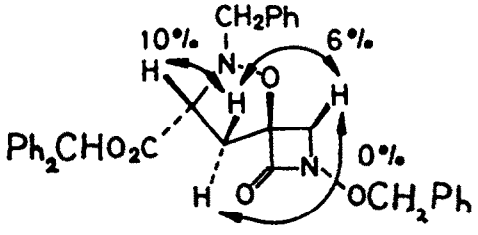
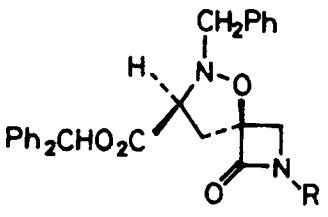
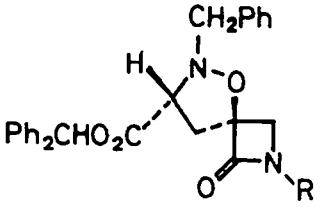


Figure 1

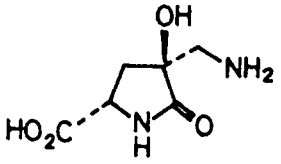
Conversion of the cycloadduct 45a into the lower homologue was performed by debenzylation of 45a by hydrogenation to N-hydroxy β-lactam 46a (75%). Removal of the hydroxyl group on the β-lactam nitrogen was achieved by the Miller's titanium trichloride procedure[23], affording 47a (80%). Further hydrogenation of 47a gave a product assigned the structure 7 on the basis of its



45a R = OCH_2Ph
46a R = OH
47a R = H



45b R = OCH_2Ph
46b R = OH
47b R = H



48

spectral characteristics (i.r. and n.m.r.) though its tendency to lactamise to 48 prevented characterisation.

EXPERIMENTAL

All reagents and chemicals were purified and dried by standard procedures. Melting points were determined on a Kofler block and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 257 spectrometer or a Perkin-Elmer 1750 FT spectrometer. ^1H n.m.r. spectra were recorded on a Bruker WH 300 spectrometer and Mass spectra were recorded on VG Micromass ZAB 1F and 16F instruments.

1-[(Acetoxy)methyl]-3-benzyloxycarbonyl-2-oxa-3-azabicyclo[2.2.2]oct-5-ene(13).

A solution of alcohol 12* (1.559g, 5.67 mmol) in pyridine (4.5 ml) was added to an ice-cooled mixture of acetic anhydride (4.5 ml) and pyridine (2 ml). The solution was stirred at room temperature for 2 days, then poured into iced water. The mixture was extracted with EtOAc. The EtOAc solution was washed three times with H_2O and the combined aqueous layer was extracted with EtOAc. The combined EtOAc solution was dried over sodium sulphate and concentrated in vacuo to give 13 as white crystals (1.61g, 90%), m.p. 91-92°C (CH_2Cl_2 - light petroleum); ν_{max} (KBr) 2955, 1750 and 1735 cm^{-1}; δ_H ($CDCl_3$) 1.23-2.22 (4H,m), 2.07 (3H,s), 4.34 (2H,s), 4.84 (1H,m), 5.10-5.29 (2H,m), 6.39 (1H,m), 6.61 (1H,m), 7.34 (5H,m); m/z (DCI,NH_3) 318 (MH^+).

3-Benzyloxycarbonyl-1-[[(tert-butyldimethylsilyl)oxy]methyl]-2-oxa-3-azabicyclo[2.2.2]oct-5-ene (14).

A solution of tert-butylchlorodimethylsilane (1.103g, 7.32mmol) in CH_2Cl_2 (2.5 ml), triethylamine (741mg, 7.32mmol) and a solution of 4-dimethylaminopyridine (447mg, 3.66mmol) in CH_2Cl_2 (2.5ml) were successively added to a solution of alcohol 12* (1.007g, 3.66mmol) in CH_2Cl_2 (10ml) under argon. The solution was stirred at room temperature for 1 day and then purified by flash chromatography (CH_2Cl_2) to give 14 as a colourless oil (1.098g, 91%); ν_{max} ($CHCl_3$) 2950, 2930, 2855 and 1690 cm^{-1}; δ_H ($CDCl_3$) 0.07 (3H,s), 0.08 (3H,s), 0.90 (9H,s), 1.51 (2H,m), 1.97 (1H,m), 2.15 (1H,m), 3.83 (2H, AB , J=10 and 25 Hz), 4.83 (1H,br,s), 5.14 (2H, AB, J=10 and 15 Hz), 6.53 (2H,m), 7.23-7.38 (5H,m); m/z (DCI,NH_3) 390 (MH^+), 407 (MNH_4^+).

(3S*, 6S*)-6-[(Acetoxy)methyl]-2-benzyloxycarbonylperhydro-1,2-oxazine-3,6-dicarboxylic acid (15).

A mixture of acetate 13 (317mg, 1mmol) in benzene (20ml), aq. $KMnO_4$ (0.2 M, 15ml) and aq. n-Bu_4NHSO_4 (0.1 M, 1ml) was vigorously stirred overnight at room temperature. The mixture was filtered and the filter cake was washed four times with alternate portions of H_2O and EtOAc. The combined organic solution was retained. The combined aqueous solution was adjusted to pH2 with aq. HCl (1 M) and extracted with EtOAc. All the organic layers were combined, dried over sodium sulphate and concentrated in vacuo to give 15 as crystals (304mg, 80%), m.p. 166-167°C ($CHCl_3$); ν_{max} (KBr) 3060, 2940, 1760, 1725 and 1695 cm^{-1}; δ_H (acetone - d_6) 1.82 (3H,s), 1.83-2.36 (4H,m), 4.24 (1H, AB, J=12 Hz), 4.71 (1H, AB , J=12 Hz), 4.80 (1H,m), 5.18 (2H,s), 7.31-7.51 (5H,m); m/z (DCI,NH_3) 382 (MH^+).

(3S*,6S*)-6-[(Acetoxy)methyl]-2,3-bis(benzyloxycarbonyl)perhydro-1,2-oxazine-6-carboxylic acid (16a) and dibenzyl (3S*,6S*)-6-[(Acetoxy)methyl]-2-benzyloxycarbonylperhydro-1,2-oxazine-3,6-dicarboxylate (16b).

[A] A mixture of diacid 15 (38mg, 0.1mmol), n-Bu_4NBr (64mg, 0.2mmol), benzyl bromide (41 mg, 0.24mmol), saturated aq. $NaHCO_3$ (0.2ml) and CH_2Cl_2 (0.2ml) was vigorously stirred at room temperature for 2 days. The mixture was partitioned between CH_2Cl_2 (2ml) and H_2O (2ml). The aqueous layer was acidified with aq. HCl and extracted with EtOAc. The EtOAc solution was dried over sodium sulphate and concentrated in vacuo to recover the unreacted 15 (10.7mg, 28%). The CH_2Cl_2 layer was dried over sodium sulphate and concentrated in vacuo. The residue was purified by preparative t.l.c. (CH_2Cl_2 : MeOH = 95 : 5) to give 16b as colourless crystals (27.9mg, 70%).

[B] A mixture of diacid 15 (38mg, 0.1mmol), n-Bu_4NBr (32mg, 0.1mmol), 1M benzyl bromide in CH_2Cl_2 (0.1ml, 0.1mmol) and saturated aq. $NaHCO_3$ (0.1ml) was vigorously stirred overnight at room temperature. The mixture was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 layer was dried over sodium sulphate and concentrated in vacuo. The residue was purified by preparative t.l.c. (CH_2Cl_2 : MeOH = 95 : 5) to give 16b (6.8 mg,12%).

[C] Toluene-p-sulphonyl chloride (38mg, 0.2mmol) was added to an ice-cooled solution of diacid 15 (38mg, 0.1mmol) and pyridine (0.1ml) in CH_3CN (5ml). The solution was stirred at 0°C for 15 min, then at room temperature for 30 min. The solution was again cooled to 0°C and 10% solution of benzyl alcohol in CH_3CN (0.1ml, 0.1mmol) was added. After being stirred at 0°C for 15 min and at room temperature for 3 hr the solution was concentrated in vacuo. The residue was dissolved in EtOAc (10ml), washed with aq. HCl (M, 3 x 2ml) and brine (2ml), dried over sodium sulphate and concentrated in vacuo. The residue was separated by preparative t.l.c. to give oily 16a (11.0mg, 23%) and crystalline 16b (11.7mg, 21%).

[D] A solution of toluene-p-sulphonyl chloride (57mg, 0.3mmol) in CH_3CN (1ml) was added to an ice-cooled solution of diacid 15 (38mg, 0.1mmol) and pyridine (0.1ml) in CH_3CN (5ml). The solution was stirred at 0°C for 15 min and then at room temperature for 30 min. The solution was again cooled to 0°C and a 10% solution of benzyl alcohol in CH_3CN (0.1ml, 0.1mmol) was added. The solution was stirred for 4 hr at room temperature, then concentrated in vacuo. The residue was purified by preparative t.l.c. (Et_2O : light petroleum = 2 : 1) to give 16b (21.1mg, 38%).

[E] To a solution of 15 (145mg, 0.3mmol) and pyridine (0.06ml) in CH_2Cl_2 (12ml) a solution of benzyl chloroformate (77mg, 0.45mmol) in CH_2Cl_2 (3ml) was added. The solution was stirred at room temperature for 2 days, then concentrated in vacuo. The residue was separated by preparative t.l.c. (Et_2O) to give 16a (94.7mg, 67%) and 16b (8.2mg, 4.9%).

16a, ν_{max} ($CHCl_3$) 3400, 3020, 2950 and 1740 cm^{-1}; δ_H ($CDCl_3$) 1.79-2.32 (4H,m) 1.95 (3H,s), 4.15(1H,AB , J=12Hz), 4.75 (1H,m), 4.78 (1H,AB, J=12Hz), 5.03 (~1H,br s), 5.15 (4H,m), 7.20-7.47 (10H,m); m/z (DCI, NH_3) 472 (MH^+), 489 (MNH_4^+).

16b, m.p. 84°C (aq.MeOH); ν_{max} ($CHCl_3$) 3035, 1750 and 1715 (sh) cm^{-1}; δ_H ($CDCl_3$) 1.70-2.29 (4H,m), 1.72 (3H,s), 4.26 (1H,AB, J=12.5Hz), 4.69 (1H,AB , J=12.5Hz), 4.79 (1H,m), 5.10 (1H, AB, J=12.5Hz), 5.19 (2H,s), 5.20 (1H, AB, J=12.5Hz), 5.24 (2H,s), 7.25-7.44 (15H,m); m/z 562 (MH^+).

(3S*,6S*)-2-Benzyloxycarbonyl-6-[[(tert-butyldimethylsilyl)oxy]methyl]perhydro-1,2-oxazine-3,6-dicarboxylic acid (17).

A mixture of a solution of olefin 14 (988mg, 2.53mmol) in benzene (50ml), of $KMnO_4$ (1.12g, 7.59mmol) in H_2O (38ml) and aq. n-Bu_4NHSO_4 (0.1 M, 2.5ml) was vigorously stirred overnight at room temperature. The mixture was filtered through celite. The aqueous layer was separated and acidified with aq. HCl (M) and extracted with EtOAc (3 x 50ml). The combined organic solution was dried over sodium sulphate and concentrated in vacuo to give 17 as crystals (1.075g, 94%), m.p. 136-139°C (dec.) (CH_2Cl_2 - light petroleum); ν_{max} (KBr) 3500, 2960, 2030 and 1725 cm^{-1}; δ_H (CD_3OD) 0.01 (3H,s), 0.02 (3H,s), 0.85 (9H,s), 1.75-2.27 (4H,m), 3.81 (1H, AB, J=10Hz), 4.09 (1H,AB , J=10Hz), 4.70 (1H,m), 5.21 (2H,s), 7.25-7.50 (5H,m).

(3S*,6S*)-2,3-Bis(benzyloxycarbonyl)-6-hydroxymethylperhydro-1,2-oxazine-6-carboxylic acid (19) and dibenzyl (3S*,6S*)-2-benzyloxycarbonyl-6-[[(tert-butyldimethylsilyl)oxy]methyl]perhydro-1,2-oxazine-3,6-dicarboxylate (18b).

A solution of pyridine (0.04ml) in CH_2Cl_2 (2ml) and a solution of benzyl chloroformate (52mg, 0.3mmol) in CH_2Cl_2 (2ml) were successively added to a solution of diacid 17 (91mg, 0.2mmol) in CH_2Cl_2 (8ml). The solution was stirred a room temperature for 1 day and then concentrated in vacuo. The residue was dissolved in acetic acid (2ml). The solution was allowed to stand for 1 hr at room temperature and then concentrated in vacuo. The residue was dissolved in EtOAc (30ml) and the solution was washed with brine (3 x 10ml), dried over sodium sulphate and concentrated in vacuo. The residue was separated by preparative t.l.c. (CH_2Cl_2 : MeOH = 9 : 1) to give 19 (37mg, 43%) and 18b (16.5mg, 13%). 19 δ_H ($CDCl_3$) 1.53-2.38 (4H,m), 3.88 (2H, br, AB), 4.65 (1H, br s), 4.75-5.75 (2H,br), 5.13 (4H,m), 7.15-7.33 (10H,m). 18b ν_{max} ($CHCl_3$) 3035, 2950, 2930, 2860, 1740 and 1700 cm^{-1}; δ_H ($CDCl_3$) -0.05 (3H,s), -0.02 (3H,s), 0.81 (9H,s), 1.83-2.29 (4H,m), 3.88 (1H, ABq, J=10Hz), 4.08 (1H, AB, J=10Hz), 4.75 (1H,m), 5.09-5.34 (6H,m), 7.22-7.37 (15H,m).

(3S*,6S*)-2-Benzyloxycarbonyl-6-hydroxymethylperhydro-1,2-oxazine-3,6-dicarboxylic acid (20).

A solution of n-Bu_4NF (1M in THF, 0.6ml) was added to a solution of silyl diacid 17 (181mg, 0.4mmol) in THF (8ml). The solution was stirred for 2 hr at room temperature. EtOAc (30ml) was added to the solution. The resulting solution was washed with 1N HCl (3 x 10ml) and brine (10ml), dried over sodium sulphate and concentrated in vacuo to give 20 as an oil (138mg, quantitative); ν_{max} (neat) 3440, 2960, 2880 and 1740 cm^{-1}; δ_H (CD_3OD) 1.67-2.25 (4H,m), 3.75 (1H, AB, J=7Hz), 3.93 (1H, AB, J=7Hz), 4.70 (1H,m), 5.20 (2H, ABq, J=10 and 15Hz), 7.21-7.53 (5H,m); m/z (DCI, NH_3) 340 (MH^+), 357 (MNH_4^+).

Benzyl (4S*,7S*)-6-benzyloxycarbonyl-1-oxo-2,5-dioxa-6-azaspiro[3.5]nonane-7-carboxylate (11).

A solution of pyridine (21mg, 0.264mmol) in CH_2Cl_2 (1ml) and a solution of benzyl chloroformate (38mg, 0.22mmol) in CH_2Cl_2 (1ml) were successively added to a solution of hydroxy diacid 20 (30mg, 0.088mmol) in CH_2Cl_2 (4ml). The solution was stirred overnight at room temperature and then partitioned between CH_2Cl_2 (20ml) and H_2O (10ml). The organic layer was washed with H_2O (2 x 10ml), dried over sodium sulphate and concentrated in vacuo. The residue was purified by Sephadex LH-20 (EtOAc) to give 11 (14.1mg, 39%); ν_{max} (neat) 3035, 2955, 2925, 2855, 1840, 1745 and 1715 cm^{-1}; δ_H ($CDCl_3$) 1.92-2.76 (4H,m), 4.31 (1H, AB, J=6Hz), 4.53 (1H, AB, J=6Hz) 4.90 (1H,m), 5.08-5.33 (4H,m), 7.27-7.37 (10H,m); m/z (DCI, NH_3) 412 (MH^+), 429 (MNH_4^+).

4-Chlorocyclohexene-4-carbonitrile (22).

α-Chloroacrylonitrile (7.16g, 82mmol) was added to a sealable bulb containing a stirrer and hydroquinone (560mg). This was then frozen in liquid nitrogen and 1,3-butadiene (5.32g, 98mmol) was distilled into the bulb which was then sealed under vacuum. The mixture was then stirred for 24 h at 90-100°C. The sealed tube was then frozen in liquid nitrogen before being opened. The

contents, a colourless liquid were then distilled under reduced pressure (80-85°C, 17mmHg) to give 22 as a colourless mobile liquid (8.2g 85%); ν_{max} (neat) 3020, 2970, 2850, 2250, 1705, 1435 and 735 cm^{-1}; δ_H ($CDCl_3$) 2.17-2.42 (4H,m), 2.66-2.75 and 2.87-2.95 (2H,m), 5.55-5.63 (1H,m), 5.80-5.84 (1H,m); m/z (EI) 141 (M^+), 143 ($M^{37}Cl^+$).

1,3-Cyclohexadiene-1-carbonitrile (23).

A solution of cyclohexene 22 (1.62g, 11.4mmol) in pyridine (25ml) was heated at reflux. The reaction was monitored by n.m.r. and when the starting material had disappeared the reaction mixture was cooled and added to ice cold dil. HCl. The pH was checked to ensure acidity and the acidic layer was extracted with Et_2O. The organic layer was dried over sodium sulphate and concentrated in vacuo. The residual, a colourless liquid was purified by Kugelröhr distillation (60-65°C, 15mmHg) to give 23 (864mg, 72%); ν_{max} (neat) 3050, 2950, 2895, 2840, 2205 and 1570 cm^{-1}; λ_{max} 282 nm (ε = 715 x 10^3); δ_H ($CDCl_3$) 2.1-2.4 (4H,m), 5.9-6.0 (1H,m), 6.1-6.2 (1H,m), 6.64 (1H,d, J=7Hz).

3-Benzyloxycarbonyl-2-oxa-3-azabicyclo[2.2.2]oct-5-ene-1-carbonitrile (24).

To a stirred solution of diene 23 (510mg, 4.8mmol) and benzyl N-hydroxycarbamate[18] (1.63g, 9.76mmol) in CH_2Cl_2 (10ml), a solution of Et_4NIO_4[19] (3.53g, 1.1mmol) in CH_2Cl_2(10ml) was added over 1 hr whilst maintaining the temperature at 0°C.[6] The solution was allowed to warm to room temperature and stirred for 3 hr. The solution was again cooled to 0°C and sodium bisulphite solution (3g in 25ml) was added. The resulting mixture was stirred for a further 15 min. The organic layer was separated, washed with aq. $NaHCO_3$ (2 x 25ml), H_2O (25ml) and brine (25ml), dried over sodium sulphate and concentrated in vacuo. The residue was purified by flash chromatography (3% EtOAc in CH_2Cl_2) to give 24 as a white solid (890mg, 73%); ν_{max} ($CHCl_3$) 3960, 3050, 2400, 1740 and 1715 cm^{-1}; λ_{max} 246 (ε = 5.4 x 10^3); δ_H ($CDCl_3$) 1.55-2.57 (4H,m), 4.89 (1H,m), 5.13 (1H, AB, J=12.3Hz), 5.22 (1H, AB, J=12.3Hz), 6.56 (1H, dd, J=2 and 8.5Hz), 6.70 (1H, dd, J=6.3 and 8.5Hz), 7.34-7.40 (5H,m).

[(3-Benzyloxycarbonyl-2-oxa-3-azabicyclo[2.2.2]oct-5-en-1-yl)methyl]amine (25).

Trifluoroacetic acid (20.8µl) was added to $NaBH_4$ (10.3mg) in THF (160µl) at 0°C. The solution was allowed to warm to room temperature and a solution of nitrile 24 (74.5mg, 0.272mmol) in THF (1ml) was added. This was stirred at room temperature for 14 hr. The reaction was quenched by the addition of aq. HCl (2M, 1ml) at 0°C and stirred for 30 min before addition of Et_2O and partition. The Et_2O layer was extracted with aq. HCl (2M) and the combined aqueous layer was made alkaline with K_2CO_3 and extracted with EtOAc (five times). The combined EtOAc extracts was dried over sodium sulphate and concentrated in vacuo to give 25 as a yellow oil (36mg, 48%), identical in all respects to that prepared previously.[6]

3-Benzyloxycarbonyl-1-[[(tert-butoxycarbonyl)amino]methyl]-2-oxa-3-azabicyclo[2.2.2]oct-5-ene (26).

To a solution of amine 25 (85mg, 0.25mmol) in CH_2Cl_2 (3ml) was added 2-[(tert-butoxycarbonyl)-imino]-2-phenylacrylonitrile[17] (123mg, 0.5mmol) under argon. The mixture was stirred at room temperature. At the completion of the reaction the solvent was removed in vacuo. The residue was purified by flash chromatography (CH_2Cl_2 : EtOAc = 9 : 1) to give 26 as a white solid (100mg, 83%), m.p. 115.5°C; ν_{max} 3330, 2920, 2880, 2820 and 1650 cm^{-1}; δ_H ($CDCl_3$) 1.24-2.19 (13H,m), 3.48 (1H,ABX, J=5 and 15Hz), 3.58 (1H,ABX, J=10 and 15Hz), 4.79 (1H,m), 5.06 (1H,br), 5.36 (1H,AB, J=12Hz), 5.42 (1H,AB, J=12Hz), 6.38 (1H,dd, J=8 and 15Hz), 6.58 (1H,m, J=8Hz), 7.35 (5H,m); m/z (NH_3 D.C.I.) 375 (NH^+), 392 (MNH_4^+); (Found : C, 64.4; H, 7.3; N, 7.3%. $C_{20}H_{26}O_5N_2$ requires C, 64.2; H, 7.0; N, 7.5%).

(3S*,6S*)-2-Benzyloxycarbonyl-6-[[(tert-butoxycarbonyl)amino]methyl]perhydro-1,2-oxazine-3,6-dicarboxylic acid (27).

Olefin 26 (374.4mg, 1mmol) was dissolved in benzene (20ml) and to this was added aq. $n\text{-}Bu_4NHSO_4$ (0.1M, 1ml). The mixture was cooled to 0°C before dropwise addition of aq. $KMnO_4$ (0.2M, 15ml). The mixture was allowed to warm to room temperature after the addition was complete and stirred overnight. The reaction mixture was filtered and the filter cake was washed with H_2O (10ml) and EtOAc (10ml). The filtrate emulsion was acidified with aq. HCl (2M) then the organic layer was collected. The aqueous layer was extracted with EtOAc (3 x 30ml). The combined organic solution was dried over sodium sulphate and concentrated in vacuo to give 27 as a colourless foam (430.1mg, 98%); ν_{max} 3450, 2500-3100 and 1730 cm^{-1}; δ_H (acetone d_6) 1.4-2.2 (13H,m), 3.4-3.8 (2H,ABX), 4.63 (1H,m), 5.1 (1H,AB, J=12.5Hz), 5.2 (1H,AB, J=12.5), 7.4 (5H,m).

(3S*,6S*)-2,3-Bis(benzyloxycarbonyl)-6-[[(tert-butoxycarbonyl)amino]methyl]perhydro-1,2-oxazine-6-carboxylic acid (28a) and dibenzyl.
(3S*,6S*)-2-benzyloxycarbonyl-6-[[(tert-butoxycarbonyl)amino]methyl]perhydro-1,2-oxazine-3,6-dicarboxylate (28b).

A solution of benzyl chloroformate (150mg, 0.88mmol) in CH_2Cl_2 (6ml) was added to a solution of diacid 27 (256.5mg, 0.585mmol) and pyridine (0.12mmol) in CH_2Cl_2 (24ml). The solution was stirred overnight at room temperature then concentrated in vacuo. The residue was dissolved in EtOAc (50ml), washed with aq. HCl (10%, 2 x 30ml) and brine (2 x 30ml), dried over sodium sulphate and concentrated in vacuo. The residue was separated by flash chromatography (CH_2Cl_2 : MeOH = 9 : 1) to give monoester 28a (177.4mg, 57%) and diester 28b (17.5mg, 4.8%). 28a, δ_H ($CDCl_3$) 1.37 (9H,s), 1.7-2.4 (4H,m), 3.45 (1H,br), 3.95 (1H,br), 4.67 (1H,br), 5.08 (2H,br s), 5.15 (2H, br s), 5.80 (1H,br), 7.0-7.5 (10H,m). 28b, ν_{max} ($CHCl_3$) 3440, 3030, 2980, 1745, 1710 cm^{-1}; δ_H ($CDCl_3$) 1.42 (9H,s), 1.65-2.22 (4H,m), 3.42 (1H,ABX, J=2.5 and 15Hz), 3.92 (1H,ABX, J=10 and 15Hz), 4.75 (1H,t, J=5.0Hz), 5.05-5.38 (7H,m), 7.25-7.45 (15H,m); m/z (DCI,NH_3) 619 (MH^+), 636 (MNH_4^+).

(3S*,6S*)-6-[(Amino)methyl]-2,3-bis(benzyloxycarbonyl)perhydro-1,2-oxazine-6-carboxylic acid (29).

A solution of monoester 28a (165.5mg, 0.313mmol) in 98% formic acid (6ml) was stirred at room temperature for 2 hr. The reagent was removed by evaporation. The residue was dissolved in EtOAc (50ml), washed with brine (30ml), dried over sodium sulphate and concentrated in vacuo to give 29 as a colourless oil (132.6mg, 99%); δ_H (CD_3OD). 1.58-1.77 (1H,m), 2.05-2.30 (3H,m), 3.30-3.47 (2H,br), 4.78 (1H,br m), 5.03-5.30 (4H,m), 7.20-7.38 (10H,m).

Benzyl (4S*,7S*)-6-benzyloxycarbonyl-1-oxo-5-oxa-2,6-diazaspiro[3.5]nonane-7-carboxylate (30).

β-Amino acid 29 (15.8mg, 0.0369mmol) and $(PyS)_2$ (8.1mg, 0.369mmol) were dissolved in CH_3CN (7.9ml) under argon. The solution was heated at 80°C. A solution of Ph_3P (9.7mg, 0.0369mmol) in CH_3CN (7.9ml) was added dropwise. The solution was stirred at 80°C for 2 hrs, then concentrated in vacuo. The residue was purified by Sephadex LH-20 (EtOAc) to give 30 as a colourless oil (9.5mg, 63%), ν_{max} ($CHCl_3$) 3420, 3030, 3010, 2960, 2930, 1780, 1740, 1710 cm^{-1}; δ_H ($CDCl_3$) 1.77-1.88 (1H,m), 1.93-2.08 (1H,m), 2.10-2.25 (1H,m), 2.43-2.55 (1H,m), 3.33 (1H,ABq, J=5Hz), 3.56 (1H,AB, J=5Hz), 4.88 (1H, br s), 5.08-5.32 (4H,m), 6.21 (1H, br s), 7.22-7.40 (10H,m); δC ($CDCl_3$) 21.55(t), 23.98(t), 49.01(t), 56.74(d), 67.54(t), 68.23(t), 92.38(s), 127.87(d), 128.10(d), 128.26(d), 128.31(d), 128.49(d), 128.55(d), 135.26(s), 135.50(s), 155.73(s), 165.07(s), 168.56(s); m/z (DCI,NH_3) 411 (MH^+), 428 (MNH_4^+).

(+)-Tabtoxinine-β-lactam (2).

Spiro β-lactam 30 (10mg) was dissolved in EtOH (5ml) and then degassed before addition of 10% Pd-C (2mg). The suspension was efficiently stirred at room temperature under an atmosphere of H_2 (1atm). After 24 hr the suspension was filtered through celite which was then thoroughly washed with EtOH. The solvent was removed in vacuo to yield (+)-tabtoxinine-β-lactam 2 as a white solid (quantitative); ν_{max} (D_2O) 1736 cm^{-1}; δ_H (D_2O) 1.62-2.00 (4H,m), 3.15 (1H,d, J=6Hz), 3.28 (1H,d, J=6Hz), 3.60 (1H,m).

(3S*,6S*)-2,3-Bis(benzyloxycarbonyl)-6-[[N-(chloroacetyl)-N-methoxyamino]methyl]perhydro-1,2-oxazine-6-carboxylic acid (34).

A solution of benzyl chloroformate (256mg, 1.5mmol) in CH_2Cl_2 (10ml) was added to a solution of diacid 33* (443.3mg, 0.997mmol) and pyridine (0.2ml) in CH_2Cl_2 (40ml). The solution was stirred overnight at room temperature then concentrated in vacuo. The residue was dissolved in EtOAc (50ml), washed with aq. HCl (10%, 3 x 20ml) and brine (20ml), dried over sodium sulphate and concentrated in vacuo. The residue was purified by dry column flash chromatography[24] (CH_2Cl_2 : MeOH = 9 : 1) to give 34 as an oil (264.2mg, 50%); ν_{max} ($CDCl_3$) 3450, 3040, 2940, 1740, 1675 cm^{-1}; δ_H ($CDCl_3$) 1.75-2.40 (4H,br), 3.66 (3H, br s), 4.33 (2H, br s), 4.62 (1H, br s), 4.72 (2H, br s), 5.13 (4H, br m), 7.27 (10H, br m).

(3S*,6S*)-2,3-Bis(benzyloxycarbonyl)-6-[(methoxyamino)methyl]perhydro-1,2-oxazine-6-carboxylic acid (35).

A solution of chloroacetamide 34 (264.2mg, 0.494mmol) and thiourea (74mg, 0.972mmol) in EtOH (16ml) was stirred overnight at 35-40°C. The solution was allowed to cool to room temperature then saturated aq. $NaHCO_3$ (5.5ml) was added. The resulting suspension was stirred for 20 min and partitioned between EtOAc (30ml) and H_2O (30ml). The aqueous layer was acidified with aq. HCl (2M) and extracted with EtOAc (3 x 30ml). The combined EtOAc solution was washed with brine (30ml), dried over sodium sulphate and concentrated in vacuo. The residue was partially purified by dry column flash chromatography[24] (CH_2Cl_2 : MeOH = 9 : 1) to give 35 as a foam (206.1 mg, 91%) which was used without further purification.

Benzyl (4S*,7S*)-6-benzyloxycarbonyl-2-methoxy-1-oxo-5-oxa-2,6-diazaspiro[3.5]nonane-7-carboxylate (36).

A mixture of methoxyamino acid 35 (99mg, 0.216mmol), $KHCO_3$ (108mg, 1.08mmol), aq. $n\text{-}Bu_4NHSO_4$ (0.1M, 0.32ml) in H_2O (6ml) and $CHCl_3$ (6ml) was stirred at room temperature. After all material had dissolved a solution of methanesulphonyl chloride (49mg, 0.432mmol) in $CHCl_3$ (1ml) was added. The mixture was stirred overnight at room temperature and saturated aq. $NaHCO_3$ (5 ml) was added. The organic layer was separated, dried over sodium sulphate and concentrated in vacuo. The residue was purified by preparative t.l.c. (EtOAc) to give 36 as a foam (36.8mg, 39%); ν_{max} ($CHCl_3$) 3020, 2940, 1785, 1740 and 1715 cm^{-1}; δ_H ($CDCl_3$) 1.70-1.82 (1H,m), 1.92-2.08 (1H,m), 2.15-2.30 (1H,m), 2.45-2.58 (1H,m), 3.46-3.70 (2H,m), 3.80 (3H,s), 4.88 (1H,br), 5.07-5.28 (4H,m), 7.20-7.43 (10H,m); m/z (DCI,NH_3) 441 (MH^+), 458 (MNH_4^+).

(S*)-2-Amino-4-[(S*)-3-hydroxy-1-methoxy-2-oxoazetidin-3-yl]butanoic acid (5).

Spiro methoxy β-lactam 36 (10mg) was dissolved in EtOH (5ml) and then degassed before addition of 10% Pd-C (2mg). The suspension was efficiently stirred at room temperature under an atmosphere of H_2 (1atm). After 24 hr the suspension was filtered through celite which was then thoroughly washed with EtOH. The solvent was removed in vacuo to yield (+) 5 as a white solid (quantitative); ν_{max} (D_2O) 1732 cm^{-1}; δ_H (D_2O) 1.65-2.25 (4H,m), 3.39 (3H,s), 3.47 (1H,m), 3.67 (1H,m), 4.03 (1H,m).

Dibenzhydryl-L-tartrate (37)

To a slurry of L-tartaric acid (10g, 66mmol) in ethyl acetate diphenyldiazomethane was added, until a purple colour persisted. The solvent was removed in vacuo and the resultant solid was recrystallised from dichloromethane to give 37 (30g, 95%), m.p. 110.5-111°C (CH_2Cl_2/Hexane) (Found: C, 74.4; H, 5.4 $C_{30}H_{26}O_6$ requires C, 74.7; H, 5.4%; ν_{max} ($CHCl_3$) 3700, 3070, 1745, 1245 and 700 cm^{-1}; δ ^1H(300MHz, $CDCl_3$) 7.4-7.3 (10H, m, phenyl), 7.02 (1H, s, $CHPh_2$), 4.78 (1H, d, J 7Hz, CHOH) and 3.2 (1H, d, J 7Hz, CHOH); m/z (NH_3 D.C.I.) 483 (MH^+).

Benzhydryl glyoxylate (38)

To an ice-cooled solution of tartrate 37 (1.5g, 3.1mmol) in dry benzene (25cm^3) under argon, lead tetraacetate (1.5g, 3.4mol) was added in portions at a rate such that the temperature did not rise above 20°C. After the addition was complete the temperature was allowed to rise to room temperature and the solution was stirred for a further 1h. The solution was quickly filtered through celite which was washed with benzene (25cm^3). The solvent was removed in vacuo and the product pumped under high vacuum for 4h to give as a colourless foam 38; ν_{max} $CHCl_3$ 1755 and 1715 cm^{-1}; δ ^1H(300MHz, $CDCl_3$) 8.5 (1H, s, CHO), 7.4-7.2 (10H, m, phenyl) and 7.0 (1H, s, $CHPh_2$); this material was reacted without purification.

N-[(Benzhydryloxycarbonyl)methylidene]benzylamine-N-oxide (39)

To a suspension of benzyhydryl glyoxylate (3gm, 12.5mmol) and calcium chloride (5gm) in chloroform (20cm³), N-benzylhydroxylamine (1.6mg, 12.8mmol) was added. The reaction mixture was then stirred at room temperature for 4 hours under argon. The solids were removed by filtration through anhydrous sodium sulphate and the filtrate evaporated to give white solid. Recrystallisation from benzene afforded, as a white solid, 39 (3.1gm, 71%) m.p. 145-146°C (benzene/hexane) (Found: C, 77.0; H, 5.7; N, 3.9 $C_{22}H_{19}O_3N$ requires C, 76.70; H, 5.55; N, 4.06%); ν_{max} (KBr) 1720, 1555, 1225, 1200 and 1180cm^{-1}; λ_{max} (EtOH) 272nm (ε 7146 dm³mol^{-1}cm^{-1}); δ ^1H(300MHz, $CDCl_3$) 7.50-7.28 (10H, m, phenyl), 7.27 (~.8H, s, vinyl E isomer), 7.01 (0.37H, s, $CHPh_2$ Z isomer), 6.98 (1H, s, $CHPh_2$, E isomer), 5.68 (1.26H, s, CH_2Ph) and 5.01 (0.74H, s, CH_2Ph); m/z (NH_3 D.C.I.) 346 (MH^+); fractional crystallisation from benzene gave 39; δ ^1H(300MHz $CDCl_3$) 7.5-7.3 (10H, m, phenyl), 7.18 (1H, s, vinyl), 7.01 (.6H, s, $CHPh_2$) and 5.01 (2H, s, CH_2Ph); δC (62.9MHz, $CDCl_3$) 73.4 (t, CH_2Ph), 77.9 (d, $CHPh_2$), 124.8 (d, vinyl), 126.4-129.5 (phenyl), 131.5, 139.5 (s, quarternary phenyl) and 150.0 (s, C=O); m/z 346 (MH^+).

3-Bromo-2-(bromomethyl)propionyl chloride (41)

A solution of acid 40 (22g, 0.089 mol) in thionyl chloride (50cm³) was refluxed overnight under argon. The excess thionyl chloride was removed by distillation at atmospheric pressure. The residue was then distilled under reduced pressure to give, as a colourless liquid, 41 (19.9g, 91%, b.p. 60-62°C, 0.8 mm Hg); ν_{max} 1780 (br), 1600, 1337 and 1290cm^{-1}; δ ^1H(300MHz, $CDCl_3$) 4.88-4.71 (4H, m, CH_2Br) and 4.61-4.56 (1H, m).

O-Benzyl-3-bromo-2-(bromomethyl)propionohydroxamic acid (42)

To a vigorously stirred solution of acid chloride 41 (9.6g, 38mmol) in dry ethyl acetate O-benzylhydroxylamine (9.6g, 76mmol) was added at 0°C over 30 minutes, under argon. After the addition was complete the solution was allowed to warm to room temperature and stirred for a further 18 hr. The solution was filtered and concentrated in vacuo to yield a white solid which was recrystallised from hot chloroform to give, as white flakes, 42 (10.3g, 76%) m.p. 129-129.5°C ($CHCl_3$) (Found C, 37.6; H, 3.7; N, 3.9; Br, 44.9. $C_{11}H_{13}NBr_2O_2$ requires C, 37.6; H, 3.7; N, 4.0; Br, 45.5%); ν_{max} (KBr) 3300-3000 and 1710 cm^{-1}; δ ^1H(300MHz, $CDCl_3$) 8.4 (1H, br, NH), 7.42-7.36 (5H, m, phenyl), 5.05 (2H, s, CH_2Ph), 3.54 (2H, 2 x ABX, J_{AB} 12Hz, CH_2Br), 3.42 (2H, 2 x ABX, J_{BA} 12Hz, CH_2Br) and 2.75 (1H, m, X of ABX, $CHCH_2$) m/z (NH_3 D.C.I.) 350/352/354 (1:2:1)[MH^+, $MH^{+81}Br^+$, $MH^{+81}Br_2^+$].

1-Benzyloxy-3-bromomethyl-2-azetidinone

A solution of hydroxamate 42 (1.7g, 4.8mmol) and sodium hydride (50% dispersion in oil, 150mg, 1 equiv) in THF (50cm³) was stirred at room temperature under argon for 1h. The solution was filtered through celite, which was washed with ethyl acetate (25cm³), before removal of the solvent in vacuo. The residue was purified by chromatotron (4 mm, 200cm³ 1:1 CH_2Cl_2: hexane then CH_2Cl_2) to yield a colourless oil 43 (1.23g, 95%); ν_{max} ($CHCl_3$) 3060 and 1770 cm^{-1}; δ ^1H(300MHz, $CDCl_3$) 7.45-7.35 (m, 5H, phenyl), 5.00 (2H, s, CH_2Ph), 3.63-3.58 (1H,A of ABX, J_{AB} 10.7Hz, J_{AX} 3.8Hz, CH_2Br), 3.47-3.46 (2H, B of ABX, J_{BX} 10.7Hz and C of CDX, J_{CD} 4.9Hz), 3.33-3.27 (1H, m, X, CH_2CH) and 3.19-3.17 (1H, D of CDX, J_{DC} 4.9Hz, J_{DX} 2.4Hz); δC (62.9MHz, $CDCl_3$) 28.5 (t, CH_2Br), 46.7 (d, CH), 50.6 (t, CH_2N), 77.9 (t, CH_2Ph), 128.2-129.4 (m, phenyl), 134.8 (s, quarternary phenyl) and 162.4 (s, C=O); m/z (NH_3 D.C.I.) 270 (MH^+), 272 ($MH^{+81}Br$), 287 (MNH_4^+) and 289 ($NH_4^{+81}Br$).

1-Benzyloxy-3-methylene-2-azetidinone (44)

Method [A]

A solution of dibromide 42 (1.72g, 6.6mmol) and sodium hydride (50% dispersion in oil, 320mg, 2.1 equiv) in dry THF (50cm³) was stirred at room temperature for 4 hours under argon. The solution was then filtered through celite which was washed with ethyl acetate (50cm³). The combined organic fractions were concentrated in vacuo. The residue was purified by chromatotron (4mm, 200cm³ 1:1 CH_2Cl_2/hexane then CH_2Cl_2) to give, as a colourless oil, 44 (840mg, 90%); ν_{max} 2910, 1770 and 1570 (br) cm^{-1}; λ_{max} (EtOH) 236nm (sh) (ε 2,263 dm^{-3} mol^{-1} cm^{-1}); δ ^1H(300MHz, $CDCl_3$) 7.45-7.35 (m, 5H, phenyl), 5.83 (1H, m, vinylic), 5.26 (1H, d, J 2Hz, vinylic), 5.03 (2H, s, CH_2Ph) and 3.72 (2H, m, allylic); δC (62.9 MHz, $CDCl_3$) 53.0 (t, allylic), 78.2 (t, CH_2Ph), 112.0 (t, α vinylic), 128.5-129.3 (m, phenyl) 134.1 (s, quarternary phenyl), 140.2 (s, β vinylic) and 161.7 (s, C-O); m/z (NH_3 D.C.I.) 190 (MH^+).

Method [B]

A solution of β-lactam bromide 43 (1.0g, 3.7mmol) and triethylamine (0.51cm³, 3.7mmol) in dichloromethane (25cm³) was refluxed overnight, under argon. The solution was then poured into ice-cooled HCl solution (20cm³, 1M) and the organic layer separated, and washed with a further solution of HCl (20cm³, 1M). Purification by chromatotron (4mm, 200cm³ 1:1 hexane/CH_2Cl_2 then CH_2Cl_2) to give, as a colourless oil 44 (0.62g, 95%), identical to that prepared by the above method.

Diphenylmethyl(4S*,7S*)-6-benzyl-2-benzyloxy-1-oxo-5-oxa-2,6-diazaspiro[3,4]octane-7-carboxylate (45a) and Diphenylmethyl(4S*,7R*)-6-benzyl-2-benzyloxy-1-oxo-5-oxa-2,6-diazaspiro [3,4]-octane-7-carboxylate (45b)

A solution of exomethylene-β-lactam 44 (177mg, 0.94mmol) and nitrone (39) (323mg, 1 equiv) in chloroform (4cm³) was heated at 50°C overnight. The solvent was removed in vacuo to give a quantatative yield of adducts 45a and 45b by proton n.m.r. (41:59). The isomers were separated using a chromatotron (4 mm, 1:1 CH_2Cl_2 : hexane) to yield, as white granules, 45a (187mg, 37%) m.p. 150-151.5°C (CH_2Cl_2/EtOAc) (Found: C, 74.1; H, 5.8; N, 4.9 $C_{33}H_{30}N_2O_5$ requires C, 74.1; H, 5.6; N, 5.2%); ν_{max} ($CHCl_3$) 1778 and 1737cm^{-1}; δ ^1H(300MHz, $CDCl_3$) 7.46-7.31 (15H, m, phenyl), 6.85 (H, s, $CHPh_2$), 5.05 (1H, ABq, J_{AB} 11.5 Hz, OCH_2Ph), 4.90 (1H, AB, J_{BA} 11.5Hz, OCH_2Ph), 4.3 (1H, AB, J_{AB} 13Hz, NCH_2Ph), 4.03-3.99 (2H, m, B of AB, and X of ABX), 3.38 (2H, m,$CONCH_2$), 2.87 (1H, ABX, J_{AB} 13.5Hz J_{AX} 7.5Hz, $CHCH_2OH$), 2.71 (1H, ABX, J_{BA} 13.5Hz, $CHCH_2COH$); δC(62.9 MHz, $CDCl_3$) 36.6 (t,

CH_2CH), 60.8, 62.6 (t, 2 x CH_2Ph), 66.0 (d, $CHCH_2$), 77.8 (t, $CONCH_2$), 78.1 (d, $CHPh_2$) 87.5 (s, spiro C), 126.6-129.2 (phenyl), 134.6, 135.7, 139.5 (br) (quarternary phenyl), 163.2 and 167.7 (s, 2 x C=O); m/z (NH_3 D.C.I.) 539 (MH^+) and, as white flakes, 45b (258mg, 51%) m.p. 129°C (Et_2O/-hexane) (Found C, 74.1; H, 5.4; N, 4.9 $C_{33}H_{30}N_2$), reauires C, 74.1; H, 5.6; N, 4.9%); ν_{max} ($CHCl_3$) 1781, 1741, 1497 and 1456 cm^{-1}; δ ^1H(500MHz, $CDCl_3$) 7.42-7.26 (15H, m, phenyl), 6.90 (1H, s Ph_2CH), 5.03 (1H, AB, J_{AB} 10.9Hz, OCH_2Ph), 4.90 (1H, AB, J_{BA} 10.9Hz, OCH_2Ph), 4.14 (1H, AB, J_{AB} 13.5Hz, NCH_2Ph), 3.84 (1H, ABq, J_{BA} 13.5Hz, NCH_2Ph) 3.82 (1H, br, X of ABX, O_2CCH), 3.37 (1H, AB, J_{AB} 4.7Hz, $CONCH_2$), 3.26 (1H, AB, J_{BA} 4.7 Hz, $CONCH_2$), 3.10 and 2.60 (2H, A and B of ABX, $CHCH_2COH$); δC (62.9 MHz, $CDCl_3$) 34.6 (t, $CHCH_2$), 60.3, 62.7 (t, 2 x CH_2Ph), 66.4 (d, $CHCH_2$), 77.9 (t, $CONCH_2$), 80.0 (d, $CHPh_2$), 87.5 (s, spiro C), 126.7-129.5 (phenyl), 134.6, 136.2, 139.4, 139.6 (quarternary phenyl), 164.3 and 169.0 (s, 2 x C=O); m/z (F.D.) 539 (MH^+).

Diphenylmethyl (4S*,7R*)-6-benzyl-2-hydroxy-1-oxo-5-oxa-2,6-diazaspiro [3.4] octane-7-carboxylate (46a)

A solution of cycloadduct 45a (60mg, 0.11mmol) in methanol ($1cm^3$) was degassed, then Pd-C (5mg) added. The reaction mixture was stirred under an atmosphere of hydrogen (1atm) for 30 mins, then filtered through celite before the solvent was removed in vacuo to give as a white solid, 46a (37mg, 75%); ν_{max} (CH_3CN) 1772 and 1732cm^{-1}; δ_H (300MHz), CD_3CN) 7.31 (15H, m, phenyl), 6.92 (1H, s, $CHPh_2$). 4.06 (1H, ABq, J_{AB} 12Hz, CH_2Ph, 4.00 (1H, AB, J_{AB} 12Hz, CH_2Ph), 3.85 (1H, br m, $CHCH_2$) 3.71 (1H, AB, J_{AB} 7Hz, $CONCH_2$), 3.65 (1H, AB, J_{BA} 7Hz, $CONCH_2$), 3.06 (1H, m, CH_2CH), 2.68 (1H, m, CH_2CH) and 1.67 (br, OH).

Diphenyl (4S*,7S*)-6-benzyl-1-oxo-5-oxa-2,6-diazaspriо[3.4]octane-7-carboxylate (47a)

N-Hydroxy lactam 46a (45mg, 0.1mmol) was dissolved in THF under argon. A solution of sodium acetate (90mg, 0.6mmol) in water (5cm^3) was added, followed by the dropwise addition of 15% $TiCl_3$ (0.2mmol) in water. After 2h at room temperature the resulting blue suspension was poured into ethyl acetate ($10cm^3$), extracted with 0.1M soldium hydroxide solution (2x5cm^3) and brine ($10cm^3$), dried over sodium sulphate, filtered and evaporated to give a white solid 47a (35mg, 80%); ν_{max} 3440, 1778 and 1740cm^{-1}; δ_H(300MHz, CD_3CN) 7.4-7.2 (15H, m, phenyl), 6.91 (1H, s, Ph_2CH), 5.8 (1H, br,NH), 4.3-3.7 (3H, m, CH_2Ph and $CHCH_2$), 3.56 (1H, AB, J_{AB} 6Hz, $CONCH_2$) and 2.68-2.51 (2H, AB of ABX, $CHCH_2$).

(S*)-2-amino-3-[(S*)-3-hydroxy-2-oxoazetidin-3-yl]propionic acid (7)

A solution of β-lactam 47a (15mg, 0.034mmol) in methanol ($2cm^3$) was degassed and Pd-C (2mg) added. The reaction mixture was stirred under hydrogen (1atm) for 18h. The reaction mixture was filtered through celite and the solvent removed. The residue was triturated with water and the aqueous extracts lyophilised to give, as a white powder, 7 (6mg, 65%); ν_{max} (D_2O) 1742cm^{-1}; δ 1H(300MHz, D_2O) 4.15 (distorted by HOD suppression), 3.60 (2H, m, $CONCH_2$), 3.1 (1H, A of AB, CH_2CH) and 2.68 (1H, B of ABX, CH_2CH).

Acknowledgement: We are grateful to Dr. J.G. Turner, University of East Anglia, for a sample of natural tabtoxinine-β-lactam and for biological tests, to Professor R.D. Durbin, University of Wisconsin, for ^1H n.m.r. spectrum of natural tabtoxinine-β-lactam and Professors M. Ohno and S. Kabayashi, University of Tokyo, for helpful advice on the β-lactam formation procedure.

References

1. (a) W.W. Stewart, Nature, 1971, 229, 174; (b) D.L. Lee and H. Rapoport, J.Org.Chem., 1975, 40, 3491; (c) P.A. Taylor, H.K. Schnoes and R.D. Durbin, Biochim. Biophys.Acta, 1972, 286, 107; (d) J.P. Scannell, D.L. Pruess, J.F. Blount, H.A. Ax, M. Kellett, F. Weiss, T.C. Demny, T.H. Williams and A. Stempel, J.Antibiot., 1975, 28, 1; (e) D.W. Woolley, G. Schaffner and A.C. Braun, J.Biol.Chem., 1952, 197, 807.
2. (a) J.G. Turner, personal communication; (b) T.F. Uchytil and R.D. Durbin, Experientia, 1980, 36, 301; (c) M.D. Thomas, P.J. Langston-Unkefer, T.F. Uchytil and R.D. Durbin, Plant Physiol., 1983, 71, 912.
3. (a) A. Meister, The Enzymes, 3rd Ed., 1974, Vol.X, 699; (b) T.D. Meek and J.J. Villafranca, Biochemistry, 1980, 19, 5513.
4. (a) J.E. Baldwin, P.D. Bailey, G. Gallacher, K.A. Singleton and P.M. Wallace, J.Chem.Soc.,-Chem.Commun., 1983, 1049; (b) J.E. Baldwin, P.D. Bailey, G. Gallacher, M. Otsuka, K.A. Singleton, P.M. Wallace, K. Prout and W.M. Wolf, Tetrahedron, 1984, 40, 3695.
5. The synthesis of (±)-2 has been reported in a preliminary form; J.E. Baldwin, M. Otsuka and P.M. Wallace, J.Chem.Soc.,Chem.Commun., submitted for publication.
6. (a) G.W. Kirby, Chem.Soc.Rev., 1977, 6, 1; (b) G.W. Kirby, J.W.M. MacInnon and R.P. Sharma, Tetrahedron Lett., 1977, 215; (c) G.W. Kirby, H. McGuigan, J.W.M. MacInnon, D. McLean and R.P. Sharma, J.Chem.Soc. Perkin Trans 1., 1985, 1437.
7. J.E. Baldwin, G. Gallacher, unpublished.
8. C.M. Starks, J.Am.Chem.Soc., 1971, 93, 195.
9. V. Bocchi, G. Casnati, A. Dossena and R. Marcelli, Synthesis, 1979, 957.
10. T.W. Greene, Protective Groups in Organic Synthesis, John Wiley & Sons, New York, 1979, 957.
11. S.K. Chaudhary and O. Hernandez, Tetrahedron Lett., 1979, 99.
12. D.P. Wyman, I.H. Song, J.Org.Chem., 1967, 32, 4139.
13. E. Boyland and R. Nery, J.Chem.Soc.(C), 1966, 354
14. A.K. Quersi and B. Sklarz, J.Chem.Soc.(C), 1966, 412.
15. N. Umino, T. Iwakuma and N. Itoh, Tetrahedron Lett., 1976, 2875.
16. (a) D.L. Boyer and M. Patel, J.Org.Chem., 1984, 49, 4098; (b) D.L. Boyer, M. Patel and F. Takusagawa, J.Org.Chem., 1985, 50, 1911.
17. M. Itoh, D. Hagiyama and T. Kamiya, Bull.Chem. Soc.Japan, 1977, 50, 718.
18. B. Halpern and D.E. Nitecki, Tetrahedron Lett., 1967, 3031.
19. S. Kobayashi, T. Iimori, T. Izawa and M. Ohno, J.Am.Chem.Soc., 1981, 103, 2406.
20. Y. Watanabe and T. Mukaiyama, Chem.Lett., 1981, 443.
21. (a) J.E. Baldwin, M.F. Chan, G. Gallacher, P. Monk and K. Prout, J.Chem.Soc.,Chem.Commun., 1983, 250; (b) J.E. Baldwin, M.F. Chan, G. Gallacher, M. Otsuka, P. Monk and K. Prout, Tetrahedron, 1984, 40, 4513.
22. W. Kloetzer, Monatsh.Chem., 1973, 104, 415.
23. P.G. Mattingley and M.J. Miller, J.Org.Chem., 1980, 45, 410.
24. L.M. Harwood, Aldrichimica Acta, 1985, 18, 25.