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Evidence for an Insertion-Homolysis Mechanism for Carbon-Sulphur Bond Formation in Penicillin Biosynthesis; 2. Incubation and Interpretation

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Abstract: Evidence to support an insertion-homolysis mechanism for the event leading to carbon-sulphur bond formation in penicillin biosynthesis has been obtained by incubation of a series of cyclopropane-containing stereochemical probes.

In the accompanying paper,† we described the synthesis of stereospecifically labelled tripeptides **1-4** (figure 1). In this paper we reveal the results of incubating these substrates with isopenicillin N synthase (IPNS) and show how these results are consistent with an insertion-homolysis mechanism for the thiazolidine ring closure in penicillin biosynthesis.

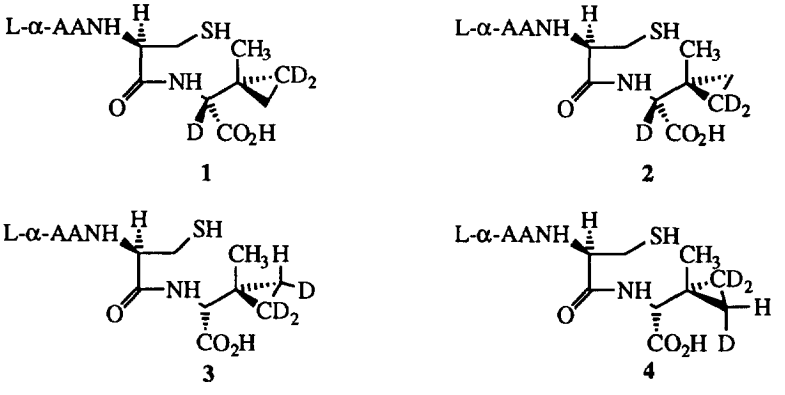


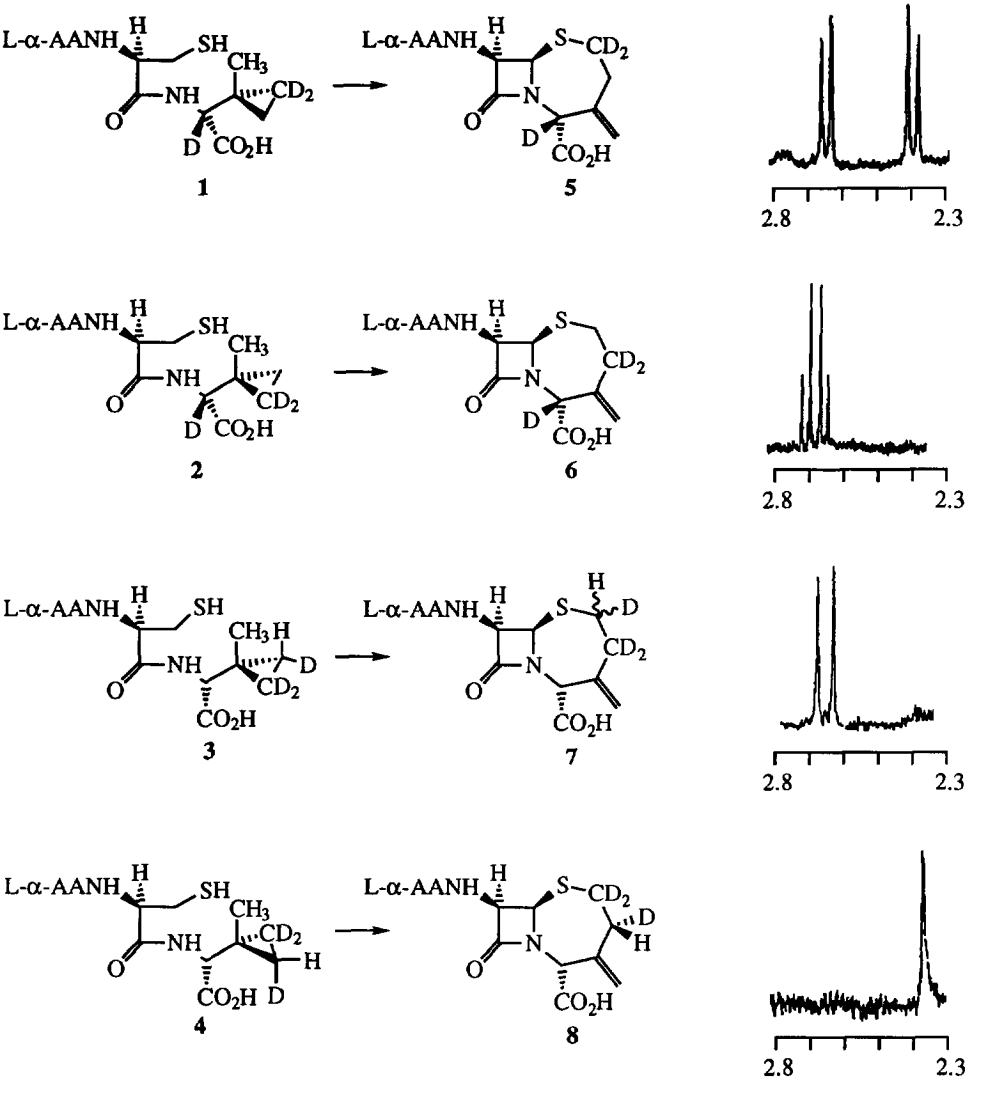
Figure 1

Incubation of **1** and **2** with IPNS gave respectively the labelled 3-exomethylene homocephams **5** and **6**. Examination of the region corresponding to -SCX_2CY_2- in the ^1H NMR (500MHz, D_2O) of the metabolites demonstrated that each isotopomer delivered a single regiospecifically labelled 3-exomethylene homocepham (scheme 1). Confirmation of the -SCX_2CY_2- connectivity in the 3-exomethylene homocepham

† Preceding paper in this issue

was gained from the observation in a 2D-COSY spectrum of an allylic coupling between the lowfield C4 proton in **5** and the lowfield olefinic proton. No equivalent coupling between the SCH_2CD_2 signal and the olefinic region was observed in **6**.

^{1}H NMR (500MHz, D_2O) spectra of -SCH_2CH_2- region



Scheme 1

Likewise analysis of nuclear Overhauser experiments provided results consistent with this assignment; thus irradiation of the -SCH_2- AB quartet in **6a** (see accompanying paper) gave n.O.e. to 7-H (12%) and 2-H (6%), whilst irradiation of either -SCD_2CH_2- hydrogen in **5** gave only a strong geminal n.O.e. without detectable n.O.e. to 7-H (figure 2).

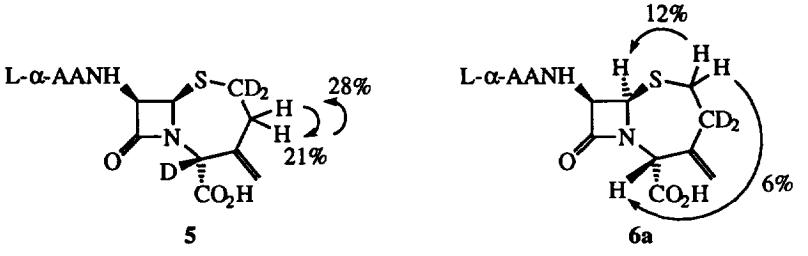


Figure 2. Nuclear Overhauser enhancements in **5** and **6a**

Incubation of **3** and **4** with IPNS gave the labelled 3-exomethylene homocephams **7** and **8** respectively. Examination of the region corresponding to -SCX2CY2- in the ^{1}H NMR (500MHz, D2O) of the metabolites demonstrated that the regiochemistry of the isotope labels was as anticipated. However, the single deuteron at C5 in **7** was present as a 1:1 mixture of epimers. In contrast the single deuteron at C4 in **8** had retained its stereochemical integrity (scheme 1).

If cyclopropylcarbinyl radicals were intermediates in the processing of substrates **1-4** by IPNS then some external element would have to control the stereochemistry of the cyclopropylcarbinyl-homoallyl radical rearrangement, in order to form the regiochemically pure 3-exomethylene homocephams **5-8**. To investigate whether the chiral centre adjacent to the cyclopropane could, due to its diastereotopic influence, dictate the course of ring-opening in such a manner, we decided to generate chemically the two diastereomers of a radical such as **9** (where R and R' are protecting groups) and to observe whether any inherent selectivity for cleavage of one carbon-carbon bond over the other existed.

We thus required a method that would allow the first-formed homoallylic radical from **9** to be trapped without the reverse reaction taking place. As the rearrangement of cyclopropylcarbinyl radicals is known to be reversible at room temperature, resulting in scrambling of a deuterium label,[1] a precursor that would allow us to generate **9** by photolysis at low temperature was required; for this reason selenide **10** was selected as a synthetic target (figure 3).

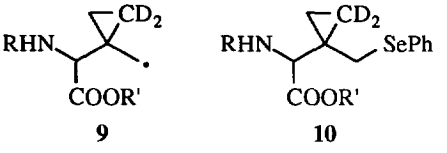
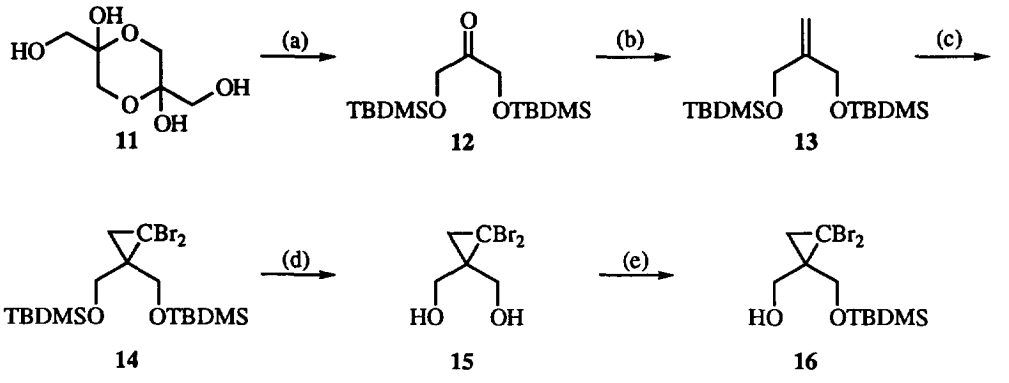


Figure 3

The route selected to prepare **10** was similar to that used for compounds **3** and **4**, with the deuterium atoms being introduced at a late stage *via* radical debromination, and the selenide added in the final step by a nucleophilic displacement. Thus dihydroxyacetone dimer **11** was treated with *tert*-butyldimethylsilyl chloride to afford the bis-silyl ether **12** (scheme 2). Wittig methylenation afforded olefin **13**, and cyclopropanation was effected using bromoform and potassium *t*-butoxide to give **14**.[2] It proved impossible to selectively remove one of the two silyl ethers, so both were removed using ammonium fluoride in methanol[3] to afford **15**, and monoprotection of this diol performed using the method of McDougal *et al.*,[4] to give monosilylated compound **16**.

Oxidation of the primary alcohol **16** using the TPAP/NMO system[5, 6] afforded aldehyde **17** in good yield (scheme 3). As with previous syntheses, the amino-acid functionality was introduced using the Greenlee modification of the Strecker reaction.[7] In this case, no diastereoselectivity was observed, and the

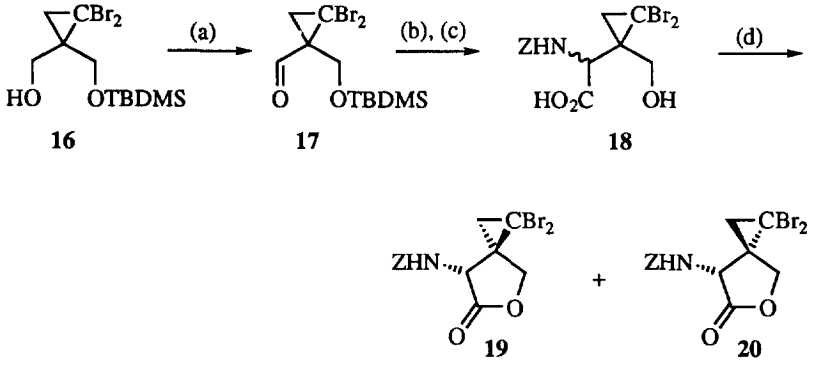
amino-acid was obtained as a 1:1 mixture of diastereomers at the α-centre (estimated by ^{1}H NMR). It proved essential to purify the amino acid by ion-exchange chromatography on Dowex®-1 (acetate form) for subsequent steps to proceed reproducibly.



(a) TBDMSCl, Et_3N, DMAP, CH_2Cl_2 (100%) (b) Ph_3PMeBr, BuLi, THF, 0°C (70%)
(c) $CHBr_3$, KO^{t}Bu, pentane, -20°C to r.t. (72%) (d) NH_4F, MeOH, 50°C (80%) (e) NaH, THF then TBDMSCl (84%).

Scheme 2

The resulting free amino acid was *N*-protected[8] to give compound **18**, which was then closed to the mixture of lactones **19** and **20** by heating in toluene under Dean-Stark conditions, using a sulphonic acid resin as catalyst. At this stage, the diastereomers could be separated by column chromatography. Recrystallisation of **19** from ethyl acetate / petroleum ether gave a crystal suitable for X-ray diffraction analysis, thus allowing assignment of the relative stereochemistry of **19** and **20** (figure 4).



(a) TPAP, NMO, CH_2Cl_2 (86%) (b) $(p\text{-}MeOC_6H_4)_2CHNH_2$; TMSCN; 6M HCl, reflux; Ion-exchange chromatography on Dowex®-1 (acetate form) (83%)
(c) *N*-(benzyloxycarbonyloxy)succinimide, Na_2CO_3, H_2O, acetone
(d) Dowex®-50 (H^+ form), toluene, Dean-Stark (20% of **19**, 17% of **20**)

Scheme 3

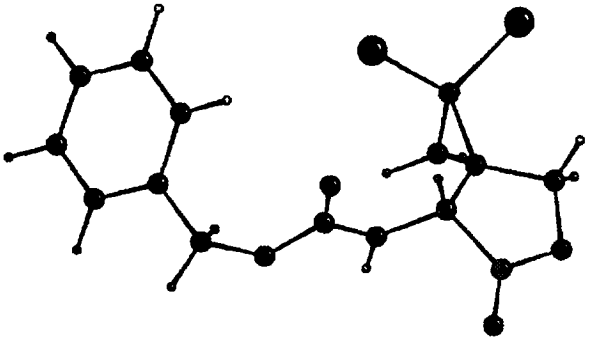
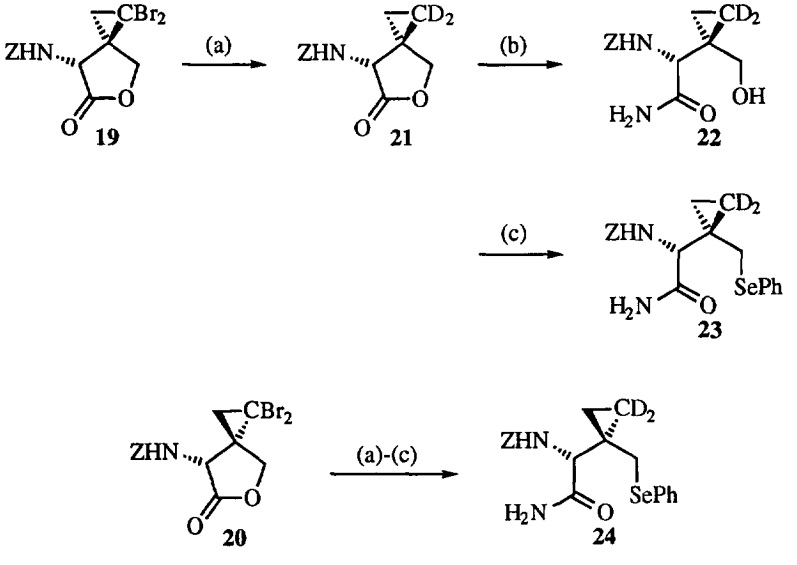


Figure 4. X-ray structure of **19**

Radical debromination of **19** was effected using triphenyltin deuteride, to give dideuterocyclopropane **21** (scheme 4).[9-11] At this stage attempts were made to introduce the selenide directly *via* opening of the lactone by alkyl-oxygen cleavage, using phenylselenide anion;[12, 13] this, however, led to none of the desired product. While lactone opening could be performed using lithium hydroxide, attempts to protect the so-formed hydroxy acid led to reclosure to the starting lactone. Irreversible ring opening could, however, be effected in high yield by reaction with liquid ammonia, to afford hydroxyamide **22**. Direct conversion of the alcohol to an aryl selenide was achieved using *N*-phenylselenophthalimide (NPSP) and tributylphosphine in THF,[14, 15] yielding selenide **23** which was used as the photolysis substrate. The diastereomeric selenide **24** was obtained in 39% overall yield from **20** by the same sequence. Each selenide was shown to be free from contamination by the other by ^{1}H NMR.

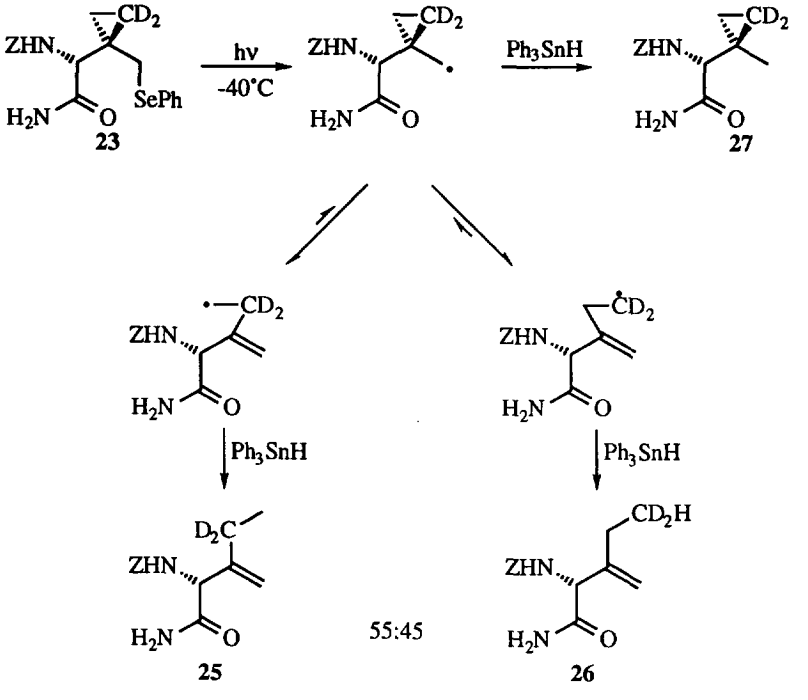


(a) Ph_3SnD, AIBN, benzene, reflux (81%); (b) NH_3(l) (77%);
(c) NPSP, Bu_3P, THF (35%)

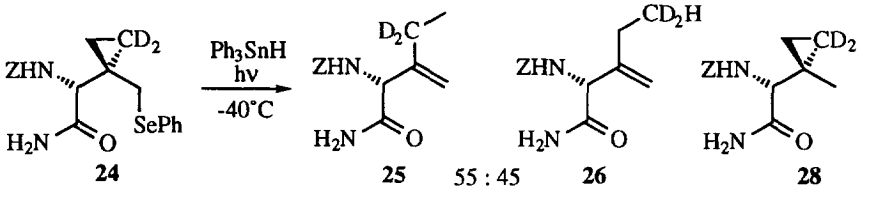
Scheme 4

Photolysis of **23** at -42°C using a medium-pressure mercury lamp, with a large excess of triphenyltin hydride as radical trapping agent afforded an inseparable mixture of the two olefinic products **25** and **26**, together with non-ring opened material **27** which had retained its stereochemical integrity in the cyclopropane (scheme 5). Observation of the latter product strongly suggests that the trapping reactions to form **25** and **26** were sufficiently fast to prevent reclosure of the homoallylic radicals and thus scrambling of the deuterium label. The observed ratio of ring-opened products was thus equal to the ratio of cleavage rates of the two diastereotopic bonds.

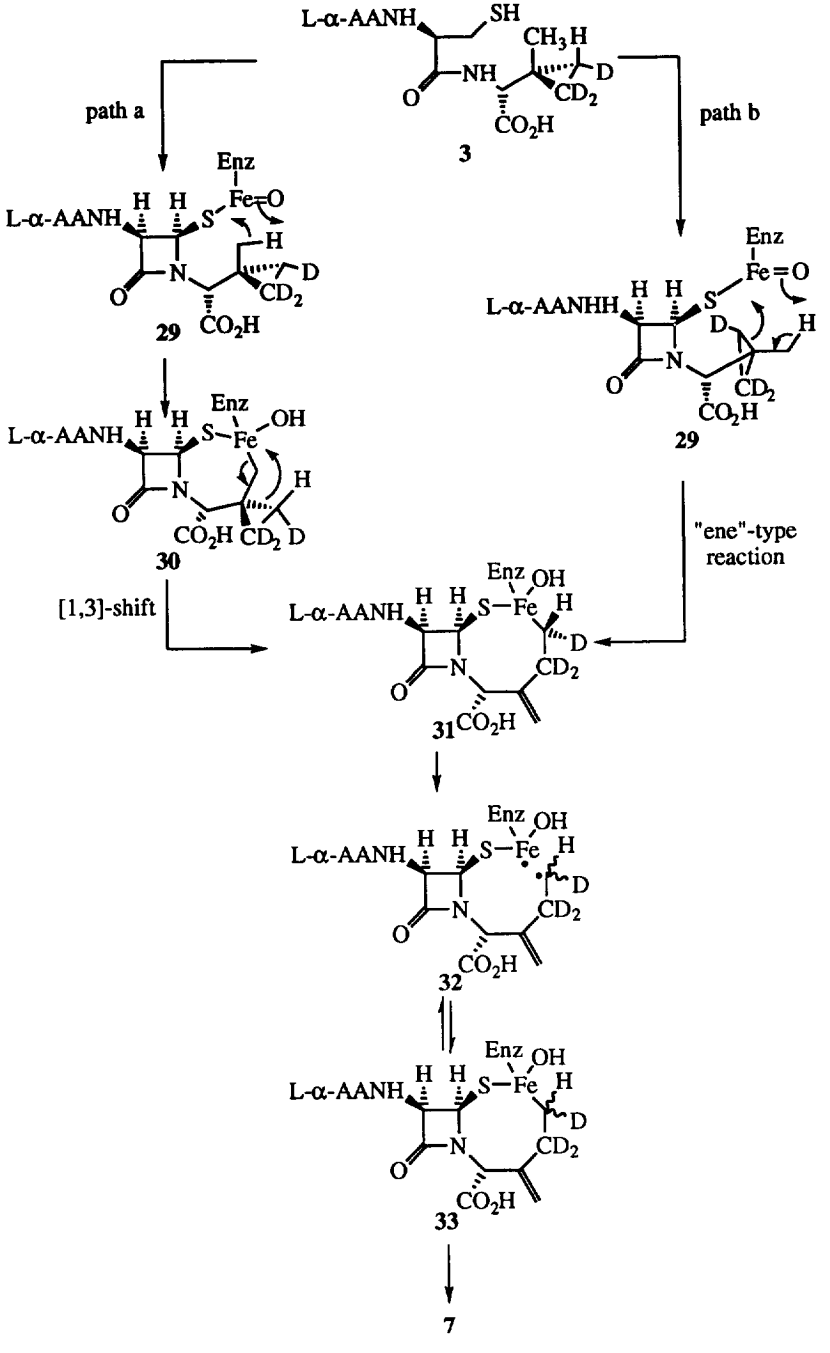
By integration of signals in the ^1H NMR spectrum of the product mixture, the ratio **25**:**26** was estimated to be 55:45. Similarly, photolysis of **24** afforded a 55:45 ratio of **25** and **26**, together with cyclopropane **28** (scheme 6).



Scheme 5



Scheme 6


L-α-AANH
SH
CH3 H
NH
D
CD2
CO2H
3
path a
path b
Enz
Fe=O
H H
S
H
D
CD2
CO2H
29
L-α-AANHH
OH
Fe
30
[1,3]-shift
"ene"-type
reaction
31
32
33
7

Scheme 7

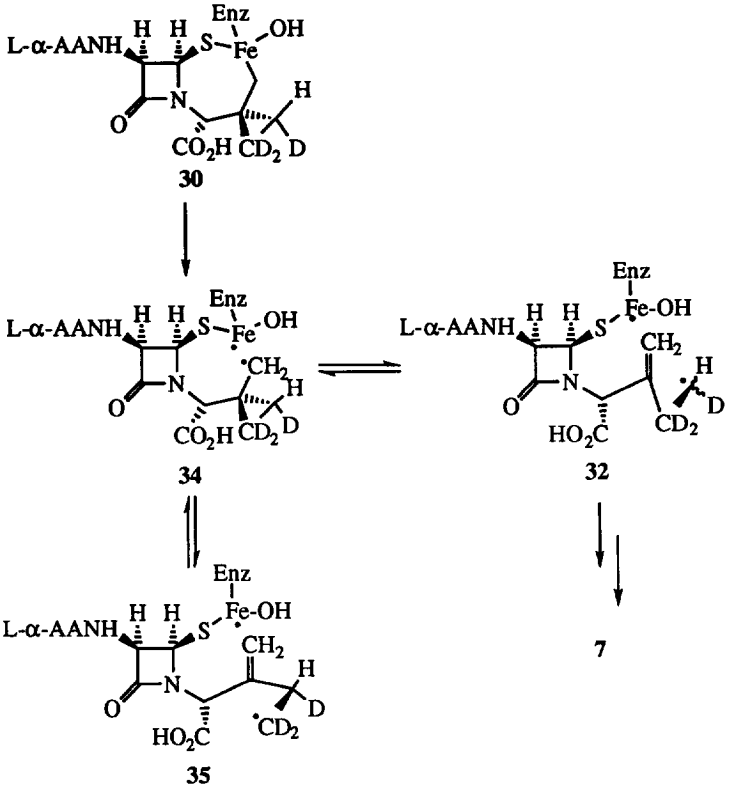
Positive identification of products **25-28** in the photolysis mixtures was made by comparison with authentic samples; thus thermally initiated radical rearrangement of **24** in the presence of triphenyltin hydride led to a 1:1 mixture of **25** and **26**. The unlabelled analogue of **27** and **28** was synthesised in 4 steps from 1-methylcyclopropanemethanol (see experimental section).

We can thus conclude that the chiral centre adjacent to the cyclopropane exerts little or no effect on the regioselectivity of radical ring-opening, and that the imbalance observed between products **25** and **26** may be due to a small secondary kinetic isotope effect. While the effect of more remote chiral centres in the substrate has not been addressed, we consider it extremely unlikely that they could influence the diastereoselectivity of ring-opening.

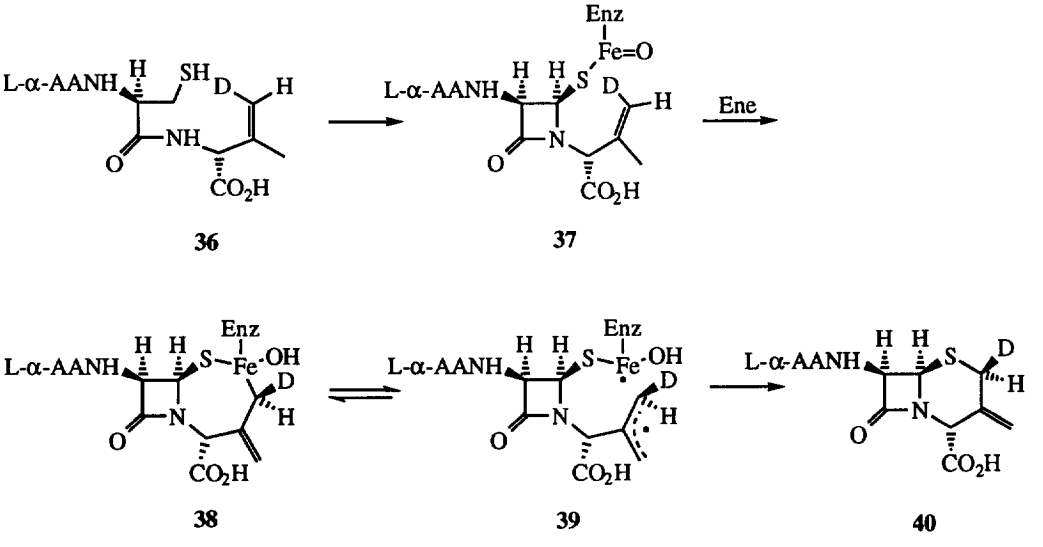
These results indicate that formation of a free cyclopropylcarbinyl radical in the processing of substrates **1-4** by IPNS is unlikely, as it is hard to envisage such a radical opening with complete regioselectivity at room temperature to provide products **5-8**. Instead, we propose that an ordered progression of a concerted followed by a free radical pathway operates during the conversion by IPNS of the deuterated cyclopropyl substrates to their corresponding 3-exomethylene homocepham products. Thus it is assumed that a β-lactam bound iron oxene, e.g. **29** from **3**, is first generated (scheme 7), followed by either a C-H insertion and a concerted [1, 3] shift (path a), or a metallo-cyclopropyl-ene reaction (path b). In either case, only the pro-*S* bond of the cyclopropane can be correctly oriented to undergo reaction, accounting for the complete regiochemical control observed in the enzymic conversion of substrates **1-4**. The so-formed homoallylic iron-carbon bonded intermediate **31** then equilibrates with a free radical form **32** which, after rotation of the labelled methylene group, closes to provide a stereorandomised C5 monodeuterated product **7** (scheme 7). As predicted by this mechanism the analogous iron-carbon bonded intermediate derived from tripeptide **4** cannot undergo scrambling of its stereochemical information and as a result the C4 deuterium label in the 3-exomethylene homocepham **8** was observed to remain intact.

Careful examination of these results deems unlikely the situation where an equilibrium is established between diradicals such as **34**, **35** and **32** (scheme 8), with only **32** being converted to the 3-exomethylene homocepham. If this were the case the diradical **35** resulting from the ring opening of the pro-*R* bond would need to be differentiated from **32** and unable to form an exomethylene homocepham product. The mechanistic probes **1**, **2** and **3** do not indicate whether this pathway operates since reversible homolytic cleavage of the pro-*R* bond would not be detected. However, for the corresponding diradical derived from tripeptide **4**, homolytic cleavage of the pro-*R* bond would necessitate scrambling of its stereochemical information *via* a single bond rotation in the diradical corresponding to **35**. Such a process is not consistent with the observation of retention of stereochemical integrity in **8**.

It is implicit in this analysis of these results that free radical character is only manifested after cyclopropane ring cleavage, when it is revealed by stereorandomisation of the methylene group in **7** and retention of stereochemistry in **8**. It is of interest to compare this situation with that previously reported[16] for substrate **36** in which the carbon-sulphur bond in the product **40** is formed without scrambling of the deuterium label. In this case, homolysis of the iron-carbon bond in metallocycle **38**, formed by a proposed stereospecific ene reaction of the iron-oxene intermediate, leads to an allylic radical **39**. As the barrier to rotation in such a radical is likely to be larger than that in a primary alkyl radical the high stereospecificity observed would become understandable (scheme 9).



Scheme 8



Scheme 9

In conclusion the synthesis of four LLD-ACV analogues containing a modified valine moiety has been described. The substitution of the valine residue by a series of α-amino acids containing a specifically deuterated cyclopropane ring has allowed us to probe the existence of radicals on the pathway of the second ring closure in penicillin biosynthesis. Incubation of these tripeptides with IPNS and ^{1}H NMR analysis of the resulting 3-exomethylene homocepham metabolites established the regiochemistry and stereochemical integrity of the labels following enzymatic reaction. Involvement of a cyclopropylcarbinyl radical has been ruled out by observing the fate of a related, chemically generated radical species. From consideration of these results, an ordered progression of a concerted followed by free radical pathway appears to operate during the conversion of the deuterated cyclopropyl substrates to their corresponding 3-exomethylene homocepham products. These experiments support the stepwise and ultimately radicaloid nature of the carbon-sulphur bond formation process in the second ring closure of penicillin biosynthesis.

EXPERIMENTAL

Melting points were obtained using a Leica Galen™ III hot stage apparatus and are uncorrected. All other general experimental procedures were as described in the accompanying paper.

4,4'-Dimethoxybenzhydrylamine,[7] triphenyltin hydride, triphenyltin (^{2}H)-hydride[9] and 1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1*H*)-one,[17] were prepared by literature methods. All other reagents were purified in accordance with the instructions in D. D. Perrin and W. L. F. Armarego, "Purification of Laboratory Chemicals", Pergamon Press, Third edition, 1988 or used as obtained from commercial sources.

Recombinant IPNS was obtained by the published procedure.[18]

High pressure liquid chromatography (HPLC) was performed on a twin Waters 512 pump, a Rheodyne 7125 injector, a Waters model 441 absorbance detector, a Waters Automated Gradient Controller and a 250mm x 4.2mm column packed with Zorbax Hypersil ODS.

Photolyses were performed using a Hanovia 125W medium-pressure mercury lamp.

Standard IPNS Incubation Procedure: Biosynthesis of (2R,5'S,7R,8R)-(2,5,5-$^2H_3$)-8-(5'-amino-5'-carboxypentanamido)-1-aza-3-methylidene-9-oxo-6-thiabicyclo[5.2.0]nonane 2-carboxylic acid (5) from (1).

A Pharmacia PD-10® gel exclusion column packed with Sephadex G-25M®, was equilibrated with 25mM NH_4HCO_3 and a solution of IPNS from *Cephalosporium Acremonium* CO278 (15.71Iu ml^{-1}, 0.87Iu mg^{-1}) in TRIS [$(HOCH_2)_3CNH_2$] buffer (2ml) was applied and eluted to exchange the TRIS buffer for 25 mM NH_4HCO_3 (3ml). A solution of δ-(L-α-aminoadipoyl)-L-cysteinyl-[(2*R*,1'*S*)-(2-^{2}H)-2-[(1'-methyl-(2',2'-$^2H_2$)-cyclopropyl)glycine] (**1**) (*c.* 3mg) in 25mM NH_4HCO_3 (1.76ml) was treated with aqueous solutions of dithiothreitol (100mM, 80 μl), ascorbate (50 mM, 80μl) and iron (II) sulphate (50mM, 80μl), and the solution of IPNS in 25mM NH_4HCO_3 (2ml) to give a total of 4ml. The solution was split into two portions and each was incubated at 27°C (250 rpm). After 40 minutes further dithiothreitol solution (100mM, 20μl) was added to each fraction and the reaction incubated for a further 20 minutes. The solutions were then combined, and the enzyme was precipitated by addition of acetone (10ml), and centrifuged (10 000 rpm, 10 minutes). The supernatant was then decanted off, concentrated *in vacuo*, filtered and lyophilised to afford a white solid. Observation of ^{1}H NMR resonances (500MHz, D_2O) in the β-lactam fingerprint region showed one AB quartet, confirming that the tripeptide had been converted to a single β-lactam product. Reverse phase HPLC (250mm x 4.2mm reverse phase ODS-hypersil C18 (5 microns) column, 25 mM NH_4HCO_3, 214nm detection, 2ml min^{-1} flow rate) afforded *(2R,5'S,7R,8R)-(2,5,5-$^2H_3$)-8-(5'-amino-5'-carboxypentanamido)-1-aza-3-methylidene-9-oxo-6-thiabicyclo[5.2.0] nonane 2-carboxylic acid (5)*, retention time 14 minutes; δ_H (500MHz; D_2O) 1.52 to 1.77 (4H, m, $(CH_2)_2CH_2CONH$), 2.23 (2H, br t, *J* 6Hz, CH_2CONH), 2.40 and 2.66

(2H, AB, J_{AB} 14Hz, $SCD_2C\underline{H}_2$), 3.44 (1H, t, J 7Hz, adipoyl αC$\underline{H}$), 5.02 (1H, s) and 5.09 (1H, s, C=C$\underline{H}_2$), 5.15 and 5.22 (2H, AB, J_{AB} 4Hz, β-lactam C$\underline{H}$). Irradiation of the doublet at 2.40 p.p.m. gave a 21% nuclear Overhauser enhancement to the geminal hydrogen at 2.66 p.p.m. and irradiation of the doublet at 2.66 p.p.m. generated a 28% enhancement to the geminal hydrogen at 2.40 p.p.m. No enhancement to the olefin or β-lactam regions could be detected in either Overhauser experiment. A 2D-COSY NMR indicated cross-peaks between the signals at 1.52 p.p.m-1.77 p.p.m. and 3.44 p.p.m. (vicinal coupling), at 2.40 p.p.m. and 2.66 p.p.m. ($SCD_2C\underline{H}_2$ geminal coupling), at 2.66 p.p.m. and 5.09 p.p.m.(allylic coupling), at 5.02 p.p.m. and 5.09 p.p.m. (olefinic geminal coupling) and at 5.15 p.p.m. and 5.22 p.p.m. (β-lactam vicinal coupling). ; *m/z* (fast atom bombardment, +ve Argon) 375 (MH^+, 100%).

(2R,5'S,7R,8R)-(2,4,4-$^2H_3$)-8-(5'-amino-5'-carboxypentanamido)-1-aza-3-methylidene-9-oxo-6-thiabicyclo [5.2.0]nonane 2-carboxylic acid (6) from (2).

δ_H (500MHz; D_2O) 1.53 to 1.69 (4H, m, $(C\underline{H}_2)_2CH_2CONH$), 2.23 (2H, br t, J 6Hz, $C\underline{H}_2CONH$), 2.65 and 2.71 (2H, AB, J_{AB} 14Hz, $SC\underline{H}_2CD_2$), 3.46 (1H, t, J 7Hz, adipoyl αC$\underline{H}$), 5.02 (1H, s) and 5.09 (1H, s, C=C$\underline{H}_2$), 5.14 and 5.22 (2H, AB, J_{AB} 4Hz, β-lactam C$\underline{H}$); A 2D-COSY NMR indicated cross-peaks between the signals at 1.53 p.p.m. to 1.69 p.p.m. and 3.46 p.p.m. (vicinal coupling), at 2.65 p.p.m. and 2.71 p.p.m. ($SC\underline{H}_2CD_2$ geminal coupling), at 5.02 p.p.m. and 5.09 p.p.m. (olefinic geminal coupling) and at 5.14 p.p.m. and 5.22 p.p.m. (β-lactam vicinal coupling); *m/z* (fast atom bombardment, +ve Argon) 375 (MH^+, 100%).

(2R,5'S,7R,8R)-(4,4-$^2H_2$)-8-(5'-amino-5'-carboxypentanamido)-1-aza-3-methylidene-9-oxo-6-thiabicyclo [5.2.0]nonane 2-carboxylic acid (6a)

δ_H (500MHz; D_2O) 1.54 to 1.72 (4H, m, $(C\underline{H}_2)_2CH_2CONH$), 2.23 (2H, br t, J 6Hz, $C\underline{H}_2CONH$), 2.66 and 2.71 (2H, AB, J_{AB} 14Hz, $SC\underline{H}_2CD_2$), 3.47 (1H, t, J 7Hz, adipoyl αC$\underline{H}$), 5.03 (1H, s) and 5.09 (1H, s, C=C$\underline{H}_2$), 5.16 and 5.21 (2H, AB, J_{AB} 4Hz, β-lactam C$\underline{H}$), C2-H signal obscured by HOD peak; δ_H (500MHz; D_2O: CD_3CN 1: 5) 2.33 (2H, br t, J 6Hz, $C\underline{H}_2CONH$), 2.80 and 2.86 (2H, AB, J_{AB} 14Hz, $SC\underline{H}_2CD_2$), 3.44 (1H, br s, adipoyl αC$\underline{H}$), 4.78 (1H, s, $C\underline{H}CO_2H$), 5.17 (2H, s, C=C$\underline{H}_2$), 5.33 and 5.46 (2H, AB, J_{AB} 3Hz, β-lactam $(C\underline{H}_2)_2CH_2CONH$ signals obscured by H_2O peak. Irradiation of the $SC\underline{H}_2CD_2$ signal at 2.80 p.p.m. and 2.86 p.p.m. in D_2O: CD_3CN 1: 5 generated a 6% enhancement to the $C\underline{H}CO_2H$ signal at 4.78 p.p.m. and a 12% enhancement to the β-lactam C$\underline{H}$S signal at 5.46 p.p.m. ; *m/z* (fast atom bombardment, +ve Argon) 374 (MH^+, 100%).

(2R,5RS,5'S,7R,8R)-(4,4,5-$^2H_3$)-8-(5'-amino-5'-carboxypentanamido)-1-aza-3-methylidene-9-oxo-6-thia bicyclo[5.2.0]nonane 2-carboxylic acid (7) from (3).

δ_H (500MHz; D_2O) 1.54 to 1.74 (4H, m, $(C\underline{H}_2)_2CH_2CONH$), 2.23 (2H, br t, J 6Hz, $C\underline{H}_2CONH$), 2.63 (0.5H, s) and 2.68 (0.5H, s, $SC\underline{H}DCD_2$), 3.54 (1H, t, J 7Hz, adipoyl αC$\underline{H}$), 5.02 (1H, s) and 5.08 (1H, s, C=C$\underline{H}_2$), 5.14 and 5.21 (2H, AB, J_{AB} 4Hz, β-lactam C$\underline{H}$), C2-H signal obscured by HOD peak; *m/z* (positive electrospray) 375 (MH^+, 100%).

(2R,4R,5'S,7R,8R)-(4,5,5-$^2H_3$)-8-(5'-amino-5'-carboxypentanamido)-1-aza-3-methylidene-9-oxo-6-thia bicyclo[5.2.0]nonane 2-carboxylic acid (8) from (4).

δ_H (500MHz; D_2O) 1.52 to 1.72 (4H, m, $(C\underline{H}_2)_2CH_2CONH$), 2.22 (2H, br t, J 6Hz, $C\underline{H}_2CONH$), 2.37 (1H, s, $SCD_2C\underline{H}D$), 3.50 (1H, t, J 7Hz, adipoyl αC$\underline{H}$), 5.00 (1H, s) and 5.06 (1H, s, C=C$\underline{H}_2$), 5.13 and 5.19 (2H, AB, J_{AB} 4Hz, β-lactam C$\underline{H}$), C2-H signal obscured by HOD peak; *m/z* (positive electrospray) 375 (MH^+, 100%).

*Synthesis of 1,3-di-(*tert*-butyldimethylsilyloxy)acetone (12).*[19]

To a stirred solution of *tert*-butyldimethylsilyl chloride (70.7g, 0.47mol) and 1,3-dihydroxyacetone dimer (**11**) (21.1g, 0.12mol) in anhydrous CH_2Cl_2 (350ml) under nitrogen were added triethylamine (100ml, 0.72mol) and 4-(*N,N*-dimethylamino)pyridine (2.2g, 18mmol). The mixture was stirred for 18 hours then washed with water (2x200ml) and 2M HCl (2x100ml), dried ($MgSO_4$), filtered and concentrated *in vacuo* to yield *1,3-di-(*tert*-butyldimethylsilyloxy)acetone (12)* (76.3g, quantitative) as a yellow oil. An analytical sample was obtained by flash chromatography (SiO_2, petroleum ether 30-40: ether 95:5); (Found: C, 56.6; H, 11.0. $C_{15}H_{34}O_3Si_2$ requires C, 56.55; H, 10.75%); (R_f 0.55, petroleum ether 30-40: ether; 90:10); ν_{max} (liquid film)/cm^{-1} 2955s, 2931s, 2887s, 2859s (CH), 1743s (C=O), 1473m, 1464m, 1256s, 1190m, 1139s, 1103s, 839s and 780s; δ_H (200MHz; $CDCl_3$) 0.09 (12H, s, $Si(CH_3)_2$), 0.92 (18H, s, $C(CH_3)_3$), 4.42 (4H, s, CH_2OSi); δ_C (50MHz; $CDCl_3$) -5.78 ($Si(CH_3)_2$), 18.17 ($C(CH_3))_3$, 25.63 ($C(CH_3)_3$), 67.90 (CH_2OSi), 209.14 (C=O);*m/z* (chemical ionisation, NH_3) 319 (MH^+, 100%), 303 (28), 261 (MH^+-^{t}Bu, 68), 132 (35), 129 (46), 106 (64), 90 (43), 73 (57).

Synthesis of O,O'-*di-(*tert*-butyldimethylsilyl)-2-methylidenepropan-1,3-diol (13).*[20]

To a suspension of methyltriphenylphosphonium bromide (26.5g, 74mmol) in anhydrous THF (150ml) at 0°C under nitrogen was added *n*-butyllithium (2.38M in hexane, 30ml, 71mmol). The mixture was stirred for 50 minutes before dropwise addition of 1,3-di-(*tert*-butyldimethylsilyloxy)acetone (**12**) (12.5g, 39mmol) as a solution in dry THF (20ml). The reaction was stirred for 3 hours and the solvent removed *in vacuo*. Petroleum ether 30-40 (150ml) was added and the mixture filtered through a pad of Celite®, washing through with further petroleum ether (3x100ml). The combined organic solutions were concentrated *in vacuo*. Flash chromatography (SiO_2, petroleum ether 30-40: ether 90: 10) afforded O,O'-*di-(*tert*-butyldimethylsilyl)-2-methylidenepropan-1,3-diol (13)* (8.7g, 70%) as a colourless oil; (Found: C, 60.55; H, 11.7. $C_{16}H_{36}O_2Si_2$ requires C, 60.7; H, 11.45%); (R_f 0.55, petroleum ether 30-40: ether 95:5); ν_{max} (liquid film)/cm^{-1} 2956s, 2930s, 2887m, 2858s (CH), 1473m, 1464m, 1256m, 1083s, 837s and 776m; δ_H (200MHz; $CDCl_3$) 0.07 (12H, s, $Si(CH_3)_2$), 0.92 (18H, s, $C(CH_3)_3$), 4.17 (4H, s, CH_2OSi), 5.09 (2H, s, $C=CH_2$); δ_C (50MHz; $CDCl_3$) -5.60 ($Si(CH_3)_2$), 18.21 ($SiC(CH_3)_3$), 25.77 ($SiC(CH_3)_3$), 63.88 (CH_2O), 109.16 ($C=CH_2$), 148.14 ($C=CH_2$); *m/z* (chemical ionisation, NH_3) 317 (MH^+, 100%), 149 (61), 147 (23), 73 (27).

*Synthesis of 2,2-dibromo-*O,O'-*di(*tert*-butyldimethylsilyl)cyclopropane-1,1-dimethanol (14).*

A stirred solution of *O,O'*-di-(*tert*-butyldimethylsilyl)-2-methylidenepropan-1,3-diol (**13**) (2.94g, 9.3mmol) in dry pentane (30ml) under argon was cooled to -20°C. Potassium *tert*-butoxide (1.35g, 12.0mmol) was added followed by bromoform (1.0ml, 11.5mmol). The mixture was stirred at -20°C for 30 minutes then allowed to warm to room temperature over 30 minutes. The process of addition and stirring were repeated a further 4 times. The reaction was then quenched with water (20ml) and the organic layer removed. The aqueous layer was extracted with Et_2O (2x20ml) and the combined organic extracts washed with water (2x20ml) then dried ($MgSO_4$), filtered and concentrated *in vacuo* to yield a brown oil. Flash chromatography (SiO_2, petroleum ether 30-40: ether 98:2) afforded *2,2-dibromo-*O,O'-*di(*tert*-butyldimethylsilyl)cyclopropane-1,1-dimethanol (14)* (3.27g, 72%) as a slightly yellow oil; (Found: C, 41.95; H, 7.55. $C_{17}H_{36}Br_2O_2Si_2$ requires C, 41.8; H, 7.45%); (R_f 0.55, petroleum ether 30-40: ether 95:5); ν_{max} (liquid film)/cm^{-1} 2955s, 2930s, 2884m, 2858s (CH), 1472m, 1255s, 1105s, 839s and 777s; δ_H (200MHz; $CDCl_3$) 0.07 (6H, s, $Si(CH_3)$) and 0.09 (6H, s, $Si(CH_3)$), 0.92 (18H, s, $C(CH_3)_3$), 1.54 (2H, s, CH_2CBr_2), 3.82 and 3.89 (4H, AB, J_{AB} 10.5Hz, CH_2OSi); δ_C (50MHz; $CDCl_3$) -5.54 ($Si(CH_3)_2$), 18.14 ($C(CH_3)_3$), 25.77 ($C(CH_3)_3$), 29.68 (CH_2CBr_2), 32.76 and 35.79 (CBr_2 and $CCBr_2$), 64.72 (CH_2OSi);*m/z* (chemical ionisation, NH_3) 487, 489, 491 (MH^+, 46, 100, 50%), 429, 431, 433 (M^+-^{t}Bu, 5, 10, 6), 245 (41), 132 (43), 106 (48), 90 (58), 73 (54).

Synthesis of 2,2-dibromocyclopropane-1,1-dimethanol (15).

A stirred solution of ammonium fluoride (5.95g, 161mmol) and 2,2-dibromo-*O,O'*-di-(*tert*-butyldimethylsilyl)cyclopropane-1,1-dimethanol (**14**) (22.26g, 46mmol) in methanol (500ml) was heated to 50°C for 80 hours, then concentrated *in vacuo*. Water (100ml) was added and the product extracted with ethyl acetate (3x250ml). The combined organic extracts were dried (Na_2SO_4) and concentrated *in vacuo* to afford *2,2-dibromocyclopropane-1,1-dimethanol (15)* (9.47g, 80%) as a pale brown solid, sufficiently pure for use directly in the next step. An analytical sample could be obtained by flash chromatography (SiO_2, ethyl acetate); m.p. 101-103°C (ethyl acetate); (Found: C, 23.25; H, 2.95. $C_5H_8Br_2O_2$ requires C, 23.1; H, 3.1%); (R_f 0.55, ethyl acetate); ν_{max} (KBr disc)/cm^{-1} 3246 br s (OH), 2037m (CH), 1064m, 1024s, 1012s and 679m; δ_H (200MHz; $CDCl_3$) 1.63 (2H, s, $C\underline{H}_2CBr_2$), 2.55 (2H, br s, O$\underline{H}$), 3.92 (2H, A of ABX, J_{AB} 12Hz, J_{AX} 3Hz, 1x$C\underline{H}_2OH$), 4.13 (2H, B of ABX, J_{AB} 12Hz, J_{BX} 4.5Hz, 1x$C\underline{H}_2OH$); δ_C (50MHz; $CDCl_3$) 29.10 ($\underline{C}H_2CBr_2$), 32.01 and 35.29 ($\underline{C}Br_2$ and $\underline{C}CBr_2$), 63.82 ($\underline{C}H_2OH$); *m/z* (chemical ionisation, NH_3) 276, 278, 280 (MNH_4^+, 50, 100, 50%), 118 (39), 70 (38), 56 (54), 55(93).

*Synthesis of 2,2-dibromo-*O*-(tert-butyldimethylsilyl)cyclopropane-1,1-dimethanol (16).*

Sodium hydride (60% dispersion in mineral oil, 0.33g, 8.3mmol) was washed with dry petroleum ether 30-40 then suspended in anhydrous THF (10ml) under nitrogen. 2,2-dibromocyclopropane-1,1-dimethanol (**15**) (2.18g, 8.4mmol) was added dropwise as a solution in THF (20ml) and the mixture stirred for 35 minutes, after which time *tert*-butyldimethylsilyl chloride (1.29g, 8.6mmol) was added dropwise as a solution in THF (10ml). The reaction was stirred for 18 hours, then concentrated *in vacuo* to afford a yellow oil, which was dissolved in ether (50ml) and washed with water (20ml). The organic solution was dried (Na_2SO_4), filtered and concentrated *in vacuo*. Flash chromatography (SiO_2, petroleum ether 30-40: ether 25:75) afforded *2,2-dibromo-*O*-(tert-butyldimethylsilyl)cyclopropane-1,1-dimethanol (16)* (2.62g, 84%) as a pale yellow oil; (Found: C, 35.6; H, 6.15. $C_{11}H_{22}Br_2O_2Si$ requires C, 35.3; H, 5.9%); (R_f 0.45, petroleum ether 30-40: ether 25:75); ν_{max} (liquid film)/cm^{-1} 3430 br m (OH), 2955s, 2930s, 2884m, 2858s (CH), 1471m, 1464m, 1256s, 1102s, 838s and 778s; δ_H (200MHz; $CDCl_3$) 0.12 (3H, s, Si($C\underline{H}_3$)), 0.14 (3H, s, Si ($C\underline{H}_3$)), 0.94 (9H, s, C($C\underline{H}_3$)$_3$), 1.55 and 1.63 (2H, AB, J_{AB} 7.5Hz, $C\underline{H}_2CBr_2$), 2.86 (1H, t, *J* 6Hz, O$\underline{H}$), 3.81 (1H, A of ABX, J_{AB} 12Hz, J_{AX} 6Hz, 1x$C\underline{H}_2OH$), 4.11 (1H, B of ABX, J_{AB} 12Hz, J_{BX} 6Hz, 1x$C\underline{H}_2OH$), 3.92 and 4.05 (2H, AB, J_{AB} 10.5Hz, $C\underline{H}_2OSi$); δ_C (50MHz; $CDCl_3$) -5.50 (Si($\underline{C}H_3$)$_2$), 18.03 ($\underline{C}$(CH_3)$_3$), 25.70 (C($\underline{C}H_3$)$_3$), 30.14 ($\underline{C}H_2CBr_2$), 32.11 and 34.84 ($\underline{C}Br_2$ and $\underline{C}CBr_2$), 67.42 and 68.03 ($\underline{C}H_2OSi$ and $\underline{C}H_2OH$); *m/z* (chemical ionisation, NH_3) 373, 375, 377 (MH^+, 49, 100, 50%), 131 (23), 92 (77), 74 (33).

Synthesis of 2,2-dibromo-1-(tert-butyldimethylsilyloxymethyl)cyclopropane-1-carbaldehyde (17).

To a stirred solution of 2,2-dibromo-*O*-(*tert*-butyldimethylsilyl)cyclopropane-1,1-dimethanol (**16**) (2.62g, 7.0mmol) in dry CH_2Cl_2 (30ml), under an inert atmosphere of nitrogen, were added *N*-methylmorpholine-*N*-oxide (1.52g, 13.0mmol) and powdered 4Å molecular sieves (~2g), and the mixture was stirred for 1 hour. Tetra-*n*-propylammonium perruthenate (0.12g, 0.3mmol) was added and the mixture stirred for 5 hours, then filtered through a short plug of Florisil® (CH_2Cl_2 eluent) and concentrated *in vacuo* to afford *2,2-dibromo-1-(tert-butyldimethylsilyloxymethyl)cyclopropane-1-carbaldehyde (17)* (2.24g, 86%) as a colourless oil; (R_f 0.4, petroleum ether 30-40: ether 95:5); ν_{max} (liquid film)/cm^{-1} 2954s, 2930s, 2857s (CH), 1719s (C=O), 1471m, 1255m, 1106s and 837s; δ_H (200MHz; $CDCl_3$) 0.10 (6H, s, Si($C\underline{H}_3$)$_2$), 0.90 (9H, s, C($C\underline{H}_3$)$_3$), 2.01 and 2.38 (2H, AB, J_{AB} 8Hz, $C\underline{H}_2CBr_2$), 3.95 and 4.41 (2H, AB, J_{AB} 11Hz, $C\underline{H}_2OSi$), 9.51 (1H, s, C$\underline{H}$O); δ_C (50MHz; $CDCl_3$) -5.63(Si($\underline{C}H_3$)$_2$), 18.08 (Si$\underline{C}$(CH_3)$_3$), 25.67 (SiC($\underline{C}H_3$)$_3$), 26.19 ($\underline{C}Br_2$), 29.89 ($\underline{C}H_2CBr_2$), 41.90 ($\underline{C}CBr_2$), 62.91 ($\underline{C}H_2OSi$), 197.04 ($\underline{C}$=O); *m/z* (chemical ionisation, NH_3) 371, 373,

375 (MH^+, 48, 100, 53%), 313, 315, 317 (M^+-^{t}Bu, 34, 66, 35), 236 (46), 213 (49), 155 (80), 132 (46), 97 (39), 74 (41).

Synthesis of 2-[2', 2'-dibromo-1'-(hydroxymethyl)cyclopropyl]glycine

2,2-Dibromo-1-(*tert*-butyldimethylsilyloxymethyl)cyclopropane-1-carbaldehyde (**17**) (6.87g, 18mmol) was dissolved in dry CH_2Cl_2 (125ml) under an inert atmosphere of nitrogen, and 4,4'-dimethoxybenzhydrylamine[7] (4.64g, 19mmol) and powdered 4Å molecular sieves (8g) were added. The mixture was stirred for 4 hours, after which an aliquot (~0.5ml) was removed and concentrated *in vacuo* to afford N-*[di-(4-methoxyphenyl)methyl]-[2',2'-dibromo-1'-(*tert*-butyldimethylsilyloxymethyl)cyclopropyl] methanimine*; ν_{max} (liquid film)/cm^{-1} 2954m, 2930m, 2856m (CH), 1656m, 1610m, 1510s, 1464m, 1303m, 1249s, 1174m, 1105s, 1037s, 910m, 837s, 779m and 734s; δ_H (200MHz; $CDCl_3$) 0.04 (3H, s, Si(CH_3)), 0.05 (3H, s, Si(CH_3)), 0.88 (9H, s, C(CH_3)$_3$), 1.82 and 2.35 (2H, AB, J_{AB} 7.5Hz, CH_2Br_2), 3.80 (6H, s, OCH_3), 3.98 and 4.23 (2H, AB, J_{AB} 10.5 Hz, CH_2OSi), 5.50 (1H, s, $CHAr_2$), 6.85 (4H, d, *J* 7.5Hz) and 7.17-7.24 (4H, m, aromatic CH), 7.85 (1H, s, CH=N); δ_C (50MHz; $CDCl_3$) -5.57 (Si(CH_3)$_2$), 18.10 (SiC(CH_3)$_3$), 25.71 (SiC(CH_3)$_3$), 29.78 (CH_2CBr_2), 31.07 (CBr_2), 38.04 ($CCBr_2$), 55.21 (OCH_3), 66.23 (CH_2OSi), 75.82 ($CHAr_2$), 113.78, 113.89, 128.05, 129.01, 135.55, 135.88 and 158.78 (aromatic C), 161.89 (CH=N); *m/z* (chemical ionisation, NH_3) 596, 598, 600 (MH^+, 1, 2, 1%), 227 (Ar_2CH^+, 100).

Trimethylsilyl cyanide (12.5ml, 94mmol) was then added dropwise, and the reaction stirred for 19 hours, after which a second aliquot (~0.5ml) was removed and concentrated *in vacuo* to afford *2-{[di-(4-methoxyphenyl)methyl](trimethylsilyl)amino}-2-[2',2'-dibromo-1'-(*tert*-butyldimethylsilyloxymethyl)cyclopropyl] ethanenitrile*; δ_H (200MHz; $CDCl_3$) 0.03 (3H, s) and 0.05 (3H, s, Si(CH_3)$_2$), 0.39 (9H, s, Si(CH_3)$_3$), 0.77 (9H, s, C(CH_3)$_3$), 1.59 (2H, s, CH_2CBr_2), 3.41 and 3.56 (2H, AB, J_{AB} 12.5Hz, CH_2OSi), 3.76 (3H, s, OCH_3), 3.78 (3H, s, OCH_3) 4.27 (0.5H, s, CHCN), 4.33 (0.5H, s, CHCN), 5.04 (1H, s, $CHAr_2$), 6.79-6.87 (4H, m) and 7.40-7.49 (4H, m, aromatic H).

The mixture was filtered through Celite®, the solvent removed *in vacuo*, and 6M HCl (500ml) added to the residue. The reaction was heated under reflux for 26 hours, then cooled, washed with ether (2x500ml), concentrated *in vacuo* and lyophilised. The crude material was purified by chromatography on Dowex®-1 (acetate form) and lyophilised to afford *2-[2',2'-dibromo-1'-(hydroxymethyl)cyclopropyl]glycine* (4.64g, 83% from **17**) as a mixture of diastereomers (*ca.* 1:1); ν_{max} (KBr disc)/cm^{-1} 3414br s (OH), 3155 br s (NH), 3051s, 1679s (C=O), 1572m, 1528m, 1398s, 1366s; δ_H (200MHz; D_2O) 1.49-1.53 (1H, m) and 1.66-1.70 (1H, m, CH_2CBr_2), 3.1-3.8 (3H, m, CH_2OH and $CHNH_3^+$); δ_C (50MHz; D_2O) 31.02, 32.11, 34.78, 35.07, 37.38, 37.89 (cyclopropyl C), 62.00, 63.17, 64.52, 65.82 ($CHNH_3^+$ and CH_2OH), 179.71 (C=O);*m/z* (desorption chemical ionisation, NH_3) 302, 304, 306 (MH^+, 9, 15, 7%), 284, 286, 288 (MH^+-H_2O, 64, 100, 52), 208 (30), 206 (38), 162 (41), 160 (42), 126 (56), 82 (52), 80 (39), 55 (79); HRMS found 285.8901, $C_6H_8{}^{79}Br^{81}BrNO_2$ (MH^+-H_2O) requires 285.8902.

Synthesis of (±)-(3R,4R*)-N-benzyloxycarbonyl-4-amino-1,1-dibromo-6-oxaspiro[4.2]heptan-5-one (19) and (±)-(3R*,4S*)-N-benzyloxycarbonyl-4-amino-1,1-dibromo-6-oxaspiro[4.2]heptan-5-one (20) via N-benzyloxycarbonyl-2-[2',2'-dibromo-1'-(hydroxymethyl)cyclopropyl]glycine (18).*

To a stirred solution of 2-[2',2'-dibromo-1'-(hydroxymethyl)cyclopropyl]glycine (420mg, 1.4mmol) in acetone: water 1:1 (8ml) were added *N*-benzyloxycarbonyloxysuccinimide (410mg, 1.7mmol) and sodium carbonate (240mg, 2.2mmol). The mixture was stirred at room temperature for 24 hours and the acetone removed *in vacuo*. The residue was diluted with water (15ml) and washed with CH_2Cl_2 (3 x 15ml). The aqueous layer was then acidified to pH 2 by dropwise addition of 1M $KHSO_4$ solution and extracted with ethyl acetate (4 x 20ml). The organic extracts were dried (Na_2SO_4) and concentrated *in vacuo* to afford N-*benzyloxycarbonyl-2-[2',2'-dibromo-1'-(hydroxymethyl)cyclopropyl]glycine (18).* as a yellow oil.

This oil was dissolved in toluene (15ml), and Dowex®-50 (H^+ form, ~80mg) added. The solution was heated to reflux under Dean and Stark conditions for 90 minutes. The solution was filtered then concentrated *in vacuo* to yield a brown oil. Flash chromatography (SiO_2, petroleum ether 30-40: ether 50:50→40:60, gradient elution) afforded *(±)-(3R*,4R*)-N-benzyloxycarbonyl-4-amino-1,1-dibromo-6-oxaspiro[4.2]heptan-5-one (19)* (120mg, 20% from free amino acid) as a white solid; m.p. 110-112°C (ethyl acetate, petroleum ether 30-40); (Found: C, 40.4; H, 3.05. $C_{14}H_{13}Br_2NO_4$ requires C, 40.15; H, 3.15%); (R_f 0.15, petroleum ether 30-40: ether 60:40); ν_{max} (KBr disc)/cm^{-1} 3326br m (NH), 3033w, 2961w (CH), 1795s (lactone C=O), 1708s (carbamate C=O), 1520s, 1266s, 1181s, 1042s and 1019s; δ_H (200MHz; $CDCl_3$) 1.77 and 1.82 (2H, AB, J_{AB} 9Hz, $C\underline{H}_2CBr_2$), 4.23 (1H, d, *J* 7.5Hz, C<u>H</u>NH), 4.33 and 4.62 (2H, AB, J_{AB} 10Hz, $C\underline{H}_2O$), 5.11 (2H, s, $C\underline{H}_2Ph$), 5.79 (1H, d, *J* 7.5Hz, N<u>H</u>), 7.35 (5H, br s, $C_6\underline{H}_5$); δ_C (50MHz; $CDCl_3$) 30.10 ($\underline{C}CBr_2$), 31.45 ($\underline{C}H_2CBr_2$), 33.64 ($\underline{C}Br_2$), 55.48 ($\underline{C}HNH$), 67.79 ($\underline{C}H_2O$), 72.11 ($\underline{C}H_2Ph$), 128.39, 128.74 and 128.88 (aromatic <u>C</u>H), 135.70 (*ipso*-<u>C</u>), 155.79 (carbamate <u>C</u>=O), 173.33 (lactone <u>C</u>=O); *m/z* (chemical ionisation, NH_3) 435, 437, 439 (MNH_4^+, 20, 39, 19%), 418, 420, 422 (MH^+, 6, 11, 6), 108 (58), 91 (100); and *(±)-(3R*,4S*)-N-benzyloxycarbonyl-4-amino-1,1-dibromo-6-oxaspiro[4.2]heptan-5-one (20)* (100mg, 17% from free amino acid) as a white solid; m.p. 150-152°C; (Found: C, 40.4; H, 2.9. $C_{14}H_{13}Br_2NO_4$ requires C, 40.15; H, 3.15%); (R_f 0.10, petroleum ether 30-40: ether 60:40); ν_{max} (KBr disc)/cm^{-1} 3293s (NH), 3068w, 3009w, 2796w (CH), 1781s (lactone C=O), 1689s (carbamate C=O), 1551s, 1303s, 1261s, 1173s, 1046s, 1005s, 971s and 757s; δ_H (200MHz; $CDCl_3$) 2.02 and 2.14 (2H, AB, J_{AB} 8Hz, $C\underline{H}_2CBr_2$), 4.11 (1H, d, *J* 7.5Hz, C<u>H</u>NH), 4.05 and 4.89 (2H, AB, J_{AB} 9Hz, $C\underline{H}_2O$), 5.14 and 5.22 (2H, AB, J_{AB} 12Hz, $C\underline{H}_2Ph$), 5.72 (1H, d, *J* 7.5Hz, N<u>H</u>), 7.37 (5H, s, $C_6\underline{H}_5$); δ_C (50MHz; $CDCl_3$) 24.29 and 32.87 ($\underline{C}CBr_2$ and $\underline{C}Br_2$), 37.09 ($\underline{C}H_2CBr_2$), 57.05 ($\underline{C}HNH$), 67.87 ($\underline{C}H_2O$), 75.12 ($\underline{C}H_2Ph$), 128.50, 128.65 and 128.83 (aromatic <u>C</u>H), 135.86 (*ipso*-<u>C</u>), 155.68 (carbamate <u>C</u>=O), 172.85 (lactone <u>C</u>=O); *m/z* (chemical ionisation, NH_3) 435, 437, 439 (MNH_4^+, 19, 39, 19%), 418, 420, 422 (MH^+, 6, 11, 6), 108 (59), 91 (100).

Slow diffusion of petroleum ether 30-40 into an ethyl acetate solution of *(±)-(3R*,4R*)-N-benzyloxycarbonyl-4-amino-1,1-dibromo-6-oxaspiro[4.2]heptan-5-one (19)* afforded a crystal suitable for X-ray diffraction analysis, allowing assignment of the relative stereochemistry.

Synthesis of (±)-(3R,4R*)-N-benzyloxycarbonyl-(1,1-$^2H_2$)-4-amino-6-oxaspiro[4.2]heptan-5-one (21).*

To a stirred solution of (±)-(3*R**,4*R**)-*N*-benzyloxycarbonyl-4-amino-1,1-dibromo-6-oxaspiro[4.2]heptan-5-one (**19**) (0.59g, 1.4mmol) and triphenyltin (^2H)-hydride (1.52g, 4.3mmol) in dry benzene (50ml) under nitrogen was added AIBN (8mg, 50μmol). The solution was heated under reflux for 1.75 hours then further AIBN (4mg, 25μmol) added and the mixture heated under reflux for a further hour. The reaction was then concentrated *in vacuo* to afford a white solid. Flash chromatography (SiO_2, petroleum ether 30-40: ether 60:40) afforded *(±)-(3R*,4R*)-N-benzyloxycarbonyl-(1,1-$^2H_2$)-4-amino-6-oxaspiro[4.2]heptan-5-one (21)* (0.30g, 81%); m.p. 128-129°C (ether, petroleum ether 30-40); (R_f 0.25, petroleum ether 30-40: ether 60:40); ν_{max} ($CHCl_3$) 1786s (lactone C=O) and 1726s (carbamate C=O); δ_H

(200MHz; $CDCl_3$) 0.52 and 0.86 (2H, AB, J_{AB} 5Hz, CH_2CD_2), 4.03 and 4.50 (2H, AB, J_{AB} 9Hz, CH_2O), 4.73 (1H, d, J 8Hz, CHNH), 5.11 (3H, br s, NH and $PhCH_2$), 7.37 (5H, s, C_6H_5); δ_C (125MHz, $CDCl_3$) 5.36 (quintet, J_{CD} 25Hz, CD_2), 8.42 (CH_2CD_2), 22.36 (CCD_2), 53.09 (CHNH), 67.46 (CH_2O), 73.05 (CH_2Ph), 128.11, 128.32 and 128.57 (aromatic CH), 135.81 (*ipso*-C), 156.40 (carbamate C=O), 174.53 (lactone C=O); *m/z* (chemical ionisation, NH_3) 281 (MNH_4^+, 51%), 264 (MH^+, 14), 173 (MH^+-$PhCH_2$, 100), 108 (23), 91 ($C_7H_7^+$, 15); HRMS found 264.1205; $C_{14}H_{14}D_2NO_4$ (MH^+) requires 264.1203.

Synthesis of (±)-(1'R,2R*)-2-(benzyloxycarbonylamino)-2-[(2',2'-$^2H_2$)-1'-(hydroxymethyl)cyclopropyl] ethanamide (22).*

(±)-(3*R**,4*R**)-*N*-benzyloxycarbonyl-(1,1-$^2H_2$)-4-amino-6-oxaspiro[4.2]heptan-5-one (**21**) (30mg, 0.11mmol) was dissolved in liquid ammonia (50ml) and stirred at -78°C for 30 minutes. The reaction vessel was then allowed to warm to room temperature and stirred until all the ammonia had evaporated to leave a white solid residue. Flash chromatography (SiO_2, ethyl acetate) afforded *(±)-(1'R*,2R*)-2-(benzyloxycarbonylamino)-2-[(2',2'-$^2H_2$)-1'-(hydroxymethyl)cyclopropyl]ethanamide (22)* (24mg, 77%); ν_{max} (KBr disc) 3362s, 3266m, 3186m, 1689s (carbamate C=O), 1660s (amide C=O), 1401m, 1354m, 1322m, 1090m and 1031m; δ_H (200MHz, $CDCl_3$) 0.58 and 0.68 (2H, AB, J_{AB} 5Hz, CH_2CD_2), 2.77 (1H, br s, OH), 3.18 (1H, A of AB, J_{AB} 11Hz, 1xCH_2OH), 3.95-4.05 (2H, m, 1xCH_2OH and CHNH), 5.12 (2H, s, $PhCH_2$), 5.66 (1H, br s) and 6.49 (1H, br s, $CONH_2$), 6.22 (1H, d, J 7.5Hz, NHZ), 7.36 (5H, s, aromatic CH); *m/z* (desorption chemical ionisation) 281 (MH^+, 100%), 237 (MH^+-$CONH_2$, 16), 173 (MH^+-C_7H_7OH, 17), 108 (15), 91 (19).

Synthesis of (±)-(1'R,2R*)-2-(benzyloxycarbonylamino)-2-[(2',2'-$^2H_2$)-1'-(phenylselanylmethyl)cyclopropyl] ethanamide (23).*

To a stirred solution/suspension of *N*-(phenylseleno)phthalimide (131mg, 0.43mmol) and (±)-(1'*R**,2*R**)-2-(benzyloxycarbonylamino)-2-[(2',2'-$^2H_2$)-1'-(hydroxymethyl)cyclopropyl]ethanamide (**22**) (46mg, 0.16mmol) in dry THF (2ml) was added tributylphosphine (105µl, 0.42mmol). The solution was stirred for 24 hours then concentrated *in vacuo*. Flash chromatography (SiO_2, petroleum ether 30-40: ethyl acetate 50:50) afforded *(±)-(1'R*,2R*)-2-(benzyloxycarbonylamino)-2-[(2',2'-$^2H_2$)-1'-(phenylselanylmethyl) cyclopropyl]ethanamide (23)* (24mg, 35%) as a white solid; m.p. 143-145°C (THF, petroleum ether 30-40); (R_f 0.3, petroleum ether 30-40: ethyl acetate 50:50); ν_{max} (KBr disc) 3402m, 3335m, 1697m (carbamate C=O), 1658s (amide C=O), 1550m, 1285m, 1257m, 1051m, 745m and 695m; δ_H (500MHz; THF-d_8) 0.62 and 0.96 (2H, AB, J_{AB} 5Hz, CH_2CD_2), 3.15 and 3.47 (2H, AB, J_{AB} 12.5Hz, CH_2SePh), 4.40 (1H, d, J 8.5Hz, CHNH), 5.20 and 5.24 (2H, AB, J_{AB} 12.5Hz, CH_2Ph), 6.79 (1H, br s) and 7.03 (1H, br s, $CONH_2$), 6.86 (1H, br d, J 8.5Hz, NHZ), 7.33-7.67 (10H, m, aromatic CH); δ_C (125MHz, THF-d_8) 11.41 (CH_2CD_2), 23.31 (CCD_2), 34.56 (CH_2SePh), 59.36 (CH_2Ph), 127.12, 128.31, 128.58, 128.90, 129.53, 132.47, 133.03 and 138.30 (aromatic C), 156.87 (carbamate C=O), 172.23 (amide C=O); CD_2 of too low intensity to be observed; CHNH obscured by THF resonance; *m/z* (desorption chemical ionisation, NH_3) 419, 421 (MH^+, 11, 21%), 311, 313 (MH^+-C_7H_7OH, 15, 30), 240, 242 (18, 39), 174 (39), 173 (55), 157 (54), 108 (100), 91 ($C_7H_7^+$, 94), 78 (62); HRMS found 421.0997; $C_{20}H_{21}D_2N_2O_3{}^{80}Se$ (MH^+) requires 421.0997.

Synthesis of (±)-(3R,4S*)-N-benzyloxycarbonyl-(1,1-$^2H_2$)-4-amino-6-oxaspiro[4.2]heptan-5-one.*

To a stirred solution of (±)-(3*R* *,4*S**)-*N*-benzyloxycarbonyl-4-amino-1,1-dibromo-6-oxaspiro[4.2]heptan-5-one (**20**) (0.23g, 0.55mmol) and triphenyltin (^2H)-hydride (0.85g, 2.4mmol) in dry benzene (20ml) under nitrogen was added AIBN (9mg, 55µmol). The solution was heated under reflux for 70 minutes then concentrated *in vacuo* to afford a white solid. Flash chromatography (SiO_2, petroleum ether 30-40: ether 60:40) afforded *(±)-(3R*,4S*)-N-benzyloxycarbonyl-(1,1-$^2H_2$)-4-amino-6-oxaspiro[4.2]heptan-5-*

one (0.11g, 74%); m.p. 127-128°C (ether, petroleum ether 30-40); (R_f 0.25, petroleum ether 30-40: ether 60:40); ν_{max} ($CHCl_3$) 1785s (lactone C=O) and 1726s (carbamate C=O); δ_H (500MHz; $CDCl_3$) 0.73 and 0.95 (2H, AB, J_{AB} 5Hz, CH_2CD_2), 4.02 and 4.48 (2H, AB, J_{AB} 9Hz, CH_2O), 4.71 (1H, d, J 7.5Hz, CHNH), 5.00 (1H, br s, NH), 5.12 (2H, s, $PhCH_2$), 7.32-7.39 (5H, m, C_6H_5); δ_C (125MHz, $CDCl_3$) 5.47 (quintet, J_{CD} 25Hz, CD_2), 8.41 (CH_2CD_2), 22.55 (CCD_2), 53.07 (CHNH), 67.57 (CH_2O), 72.96 (CH_2Ph), 128.17, 128.38 and 128.60 (aromatic CH), 135.71 (ipso C), 156.34 (carbamate C=O), 174.37 (lactone C=O); *m/z* (chemical ionisation, NH_3) 281 (MNH_4^+, 39%), 264 (MH^+, 18), 173 (MH^+-$PhCH_2$, 100), 130 (24), 108 (41), 91 ($C_7H_7^+$, 31), 83 (37); HRMS found 264.1205; $C_{14}H_{14}D_2NO_4$ (MH^+) requires 264.1203.

Synthesis of (±)-(1'R,2S*)-2-(benzyloxycarbonylamino)-2-[(2',2'-$^2H_2$)-1'-(hydroxymethyl)cyclopropyl] ethanamide.*

(±)-(3*R**,4*S**)-*N*-benzyloxycarbonyl-(1,1-$^2H_2$)-4-amino-6-oxaspiro[4.2]heptan-5-one (60mg, 0.23mmol) was dissolved in liquid ammonia (50ml) and stirred at -78°C for 6 hours. The reaction vessel was then allowed to warm to room temperature and stirred until the ammonia had evaporated to afford *(±)-(1'R*,2S*)-2-(benzyloxy carbonylamino)-2-[(2',2'-$^2H_2$)-1'-(hydroxymethyl)cyclopropyl]ethanamide* (63mg, 98%) as a white solid which was used without further purification; ν_{max} (KBr disc) 3363s, 3266m, 3186m, 1688s (carbamate C=O), 1661s (amide C=O), 1401m, 1354m, 1322m, 1090m and 1036m; δ_H (200MHz, $CDCl_3$) 0.55 and 0.77 (2H, AB, J_{AB} 5.5Hz, CH_2CD_2), 3.20 (1H, A of AB, J_{AB} 11Hz, 1xCH_2OH), 3.93-4.03 (2H, m, 1xCH_2OH and OH), 4.73 (1H, br s, CHNH), 5.12 (2H, s, $PhCH_2$), 5.55 (1H, br s) and 6.47 (1H, br s, $CONH_2$), 6.20 (1H, br d, J 7Hz, NHZ), 7.35 (5H, s, aromatic CH); *m/z* (desorption chemical ionisation) 281 (MH^+, 55%), 190 (MH^+-C_7H_7, 28), 173 (MH^+-C_7H_7OH, 100), 130 (32), 129 (40), 128 (31), 108 (44), 91 (61).

Synthesis of (±)-(1'R,2S*)-2-(benzyloxycarbonylamino)-2-[(2',2'-$^2H_2$)-1'-(phenylselanylmethyl)cyclopropyl] ethanamide (24).*

To a stirred solution/suspension of *N*-(phenylseleno)phthalimide (172mg, 0.57mmol) and (±)-(1'*R**,2*S**)-2-(benzyloxycarbonylamino)-2-[(2',2'-$^2H_2$)-1'-(hydroxymethyl)cyclopropyl]ethanamide (58mg, 0.21mmol) in dry THF (2ml) under an inert atmosphere of nitrogen, was added tributylphosphine (145µl, 0.58mmol). The solution was stirred for 24 hours then concentrated *in vacuo*. Flash chromatography (SiO_2, petroleum ether 30-40: ethyl acetate 50:50) afforded *(±)-(1'R*,2S*)-2-(benzyloxycarbonylamino)-2-[(2',2'-$^2H_2$)-1'-(phenylselanylmethyl) cyclopropyl]ethanamide (24)* (47mg, 54%) as a white solid; m.p. 145-147°C (THF, petroleum ether 30-40); (R_f 0.3, petroleum ether 30-40: ethyl acetate 50:50); ν_{max} (KBr disc) 3402m, 3335m, 1697m (carbamate C=O), 1658s (amide C=O), 1549m, 1285m, 1258m, 1051m, 745m, 695m; δ_H (500MHz; THF-d_8) 0.60 and 0.97 (2H, AB, J_{AB} 5Hz, CH_2CD_2), 3.11 and 3.43 (2H, AB, J_{AB} 12.5Hz, CH_2SePh), 4.35 (1H, d, J 8Hz, CHNH), 5.16 and 5.20 (2H, AB, J_{AB} 12.5Hz, CH_2Ph), 6.64 (1H, br s) and 6.88 (1H, br s, $CONH_2$), 6.76 (1H, br d, J 8Hz, NHZ), 7.26-7.62 (10H, m, aromatic CH); δ_C (125MHz, THF-d_8) 11.66 (CH_2CD_2), 23.45 (CCD_2), 34.74 (CH_2SePh), 59.47 (CH_2Ph), 127.25, 128.43, 128.70, 129.02, 129.65, 132.59, 133.18 and 138.44 (aromatic C), 156.97 (OCONH), 172.23 ($CONH_2$); CD_2 of too low intensity to be observed; CHNH obscured by THF resonance; *m/z* (desorption chemical ionisation, NH_3) 419, 421 (MH^+, 13, 25%), 311, 313 (MH^+-C_7H_7O, 14, 26), 173 (55), 155 (41), 108 (72), 91 ($C_7H_7^+$, 100), 78 (39); HRMS found 421.0999; $C_{20}H_{21}D_2N_2O_3{}^{80}Se$ (MH^+) requires 421.0997.

Photolysis of (±)-(1'R,2R*)-2-(benzyloxycarbonylamino)-2-[(2',2'-$^2H_2$)-1'-(phenylselanylmethyl) cyclopropyl] ethanamide (23).*

A solution of (±)-(1'*R**,2*R**)-2-(benzyloxycarbonylamino)-2-[(2',2'-$^2H_2$)-1'-(phenylselanylmethyl) cyclopropyl]ethanamide (**23**) (5.6mg, 13μmol) and triphenyltin hydride (51mg, 145μmol) in dry THF (1ml) under nitrogen was cooled to -42°C and irradiated with a medium-pressure mercury lamp for 8.5 hours, then concentrated *in vacuo* to yield a white solid. Flash chromatography (SiO_2, petroleum ether 30-40: ethyl acetate 80:20→ 0:100; gradient elution) afforded an inseparable mixture of *(±)-2-(benzyloxycarbonylamino)-3-methylidene-(4,4-$^2H_2$)-pentanamide (**25**), (±)-2-(benzyloxycarbonylamino)-3-methylidene-(5,5-$^2H_2$)-pentanamide (**26**),* and *(±)-(1'R*,2R*) -2-(benzyloxycarbonylamino)-2-[(2',2'-$^2H_2$)-1'-methylcyclopropyl]ethanamide (**27**)*; slightly contaminated with triphenyltin residues; the following diagnostic peaks were observed: δ_H (500MHz, $CDCl_3$) 0.42 and 0.90 (2H, AB, J_{AB} 5Hz, CH_2CD_2 of **27**), 1.05 (5H, br s, CH_3 of **25** and **27**, CD_2H of **26**), 1.98 (0.5H, A of ABX, J_{AB} 17Hz, J_{AX} 7.5Hz, 1xCH_2CD_2H of **26**), 2.10 (0.5H, B of ABX, J_{AB} 17Hz, J_{BX} 7.5Hz, 1xCH_2CD_2H of **26**), 3.68 (1H, d, *J* 6.5Hz, CHNH of **27**), 4.77 (1H, d, *J* 5Hz, CHNH of **25** and **26**), 5.08-5.15 (5H, m, $PhCH_2$ of **25**, **26** and **27**, 1xC=CH_2 of **25** and **26**), 5.27 (1H, s, 1xC=CH_2 of **25** and **26**), 5.51 (2H, br s), 5.70 (1H, br s), 5.81 (2H, br s) and 5.90 (1H, br s, 6xNH); aromatic protons obscured by triphenyltin peaks. Integration of these signals allowed the following ratios to be measured: (**25** + **26**) : **27** = 50:50 ; **25** : **26** = 55:45.

An clean sample of the olefinic products **25** and **26** was obtained by thermolysis; a clean sample of the unlabelled cyclopropane product was obtained by a 4-step sequence (*vide infra*).

Photolysis of (±)-(1'R,2S*)-2-(benzyloxycarbonylamino)-2-[(2',2'-$^2H_2$)-1'-(phenylselanylmethyl)cyclopropyl] ethanamide (24).*

A solution of (±)-(1'*R**,2*S**)-2-(benzyloxycarbonylamino)-2-[(2',2'-$^2H_2$)-1'-(phenylselanylmethyl) cyclopropyl]ethanamide (**24**) (5.3mg, 13μmol) and triphenyltin hydride (48.6mg, 138μmol) in dry THF (1ml) under nitrogen was cooled to -40°C and irradiated with a medium-pressure mercury lamp for 7 hours, then concentrated *in vacuo* to yield a white solid. Flash chromatography (SiO_2, petroleum ether 30-40: ethyl acetate 80:20→ 0:100; gradient elution) afforded an inseparable mixture of *(±)-2-(benzyloxycarbonylamino)-3-methylidene-(4,4-$^2H_2$)-pentanamide (**25**), (±)-2-(benzyloxycarbonylamino)-3-methylidene-(5,5-$^2H_2$)-pentanamide (**26**),* and *(±)-(1'R*,2S*)-2-(benzyloxycarbonylamino)-2-[(2',2'-$^2H_2$)-1'-methylcyclopropyl]ethanamide (**28**)*; slightly contaminated with triphenyltin residues; the following diagnostic peaks were observed: δ_H (500MHz, $CDCl_3$) 0.52 and 0.69 (2H, AB, J_{AB} 4.5Hz, CH_2CD_2 of **28**), 1.06 (5H, br s, CH_3 of **25** and **27**, CD_2H of **26**), 1.98 (0.5H, A of ABX, J_{AB} 17Hz, J_{AX} 7.5Hz, 1xCH_2CD_2H of **26**), 2.10 (0.5H, B of ABX, J_{AB} 17Hz, J_{BX} 7.5Hz, 1xCH_2CD_2H of **26**), 3.68 (1H, d, *J* 7Hz, CHNH of **28**), 4.77 (1H, d, *J* 5Hz, CHNH of **25** and **26**), 5.08-5.15 (5H, m, $PhCH_2$ of **25**, **26** and **28**, 1xC=CH_2 of **25** and **26**), 5.27 (1H, s, 1xC=CH_2 of **25** and **26**), 5.47 (2H, br s), 5.70 (1H, br s), 5.79 (2H, br s) and 5.88 (1H, br s, 6xNH); aromatic protons obscured by triphenyltin peaks. Integration of these signals allowed the following ratios to be measured: (**25** + **26**) : **28** = 55:45 ; **25** : **26** = 55:45.

*Preparation of an authentic 1:1 mixture of (±)-2-(benzyloxycarbonylamino)-3-methylidene-(4,4-$^2H_2$)-pentanamide (**25**), (±)-2-(benzyloxycarbonylamino)-3-methylidene-(5,5-$^2H_2$)-pentanamide (**26**) by thermolysis.*

To a stirred solution of (±)-(1'*R* *,2*S**)-2-(benzyloxycarbonylamino)-2-[(2',2'-$^2H_2$)-1'-(phenylselanylmethyl) cyclopropyl]ethanamide (**24**) (6.6mg, 16μmol) and triphenyltin hydride (17.8mg, 51μmol) in dry THF (1ml) under nitrogen was added AIBN (2-3 crystals) and the mixture stirred under reflux for 90 minutes. The mixture was then concentrated *in vacuo*. Repeated flash chromatography (SiO_2, petroleum ether 30-40: ethyl acetate 50:50) afforded a 1:1 mixture of *(±)-2-(benzyloxycarbonylamino)-3-*

methylidene-(4,4-$^2H_2$)-pentanamide (25), (±)-2-(benzyloxycarbonylamino)-3-methylidene-(5,5-$^2H_2$)-pentanamide (26) (4.6mg); (R_f 0.3, petroleum ether 30-40: ethyl acetate 40:60); ν_{max} ($CHCl_3$)/cm^{-1} 3520w, 3470m, 2973w, 1698s, 1647w, 1586m, 929m; δ_H (500MHz; $CDCl_3$) 1.06 (2H, br s, CH_3 of **25** and CD_2H of **26**), 1.98 (0.5H, A of ABX, J_{AB} 17Hz, J_{AX} 7.5Hz, 1xCH_2CD_2H of **26**), 2.10 (0.5H, B of ABX, J_{AB} 17Hz, J_{BX} 7.5Hz, 1xCH_2CD_2H of **26**), 4.77 (1H, d, *J* 5Hz, CHNH), 5.08-5.15 (3H, m, $PhCH_2$ and 1x$C=CH_2$), 5.27 (1H, s, 1x$C=CH_2$), 5.46 (1H, br s), 5.80 (1H, br s) and 5.89 (1H, br s, 3xNH), 7.30-7.52 (5H, m, aromatic CH); δ_C (125MHz; $CDCl_3$) 11.55 (CD_2CH_3 of **25**), 23.61 (CH_2CD_2H of **26**), 60.60 (CHNH), 66.97 ($PhCH_2$), 114.09 ($C=CH_2$), 128.06, 128.14 and 128.50 (aromatic CH), 136.25 (*ipso*-C), 147.65 ($C=CH_2$), 155.65 (carbamate C=O), 171.28 (amide C=O); *m/z* (chemical ionisation, NH_3) 265 (MH^+, 100%), 221 (MH^+-$CONH_2$, 35), 174 (MH^+-C_7H_7, 40), 157 (MH^+-$PhCH_2OH$, 80), 108 (50), 91 ($C_7H_7^+$, 87), 81 (53); HRMS found 265.1521, $C_{14}H_{17}D_2N_2O_3$ (MH^+) requires 265.1519.

Preparation of authentic (±)-2-(benzyloxycarbonylamino)-2-(1'-methylcyclopropyl)ethanamide.

To a stirred solution of 1-methylcyclopropanemethanol (0.131g, 1.52mmol) in dry CH_2Cl_2 (2ml) under nitrogen was added 1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1*H*)-one[17,21] (0.791g, 1.86mmol). Water (30µl, 1.67mmol), solvated in CH_2Cl_2 (5ml), was then added dropwise over 15 minutes[22] and stirring was continued for 2 hours. Further CH_2Cl_2 (10ml) was added, followed by saturated aqueous sodium bicarbonate (10ml) and sodium thiosulphate (2.5g in 10ml water). The organic layer was removed and dried (Na_2SO_4) to afford *1-methylcyclopropanemethanal* as a solution in CH_2Cl_2; δ_H (200MHz; $CDCl_3$) 0.92-0.95 (2H, m) and 1.14-1.20 (2H, m, $(CH_2)_2$), 1.24 (3H, s, CH_3), 8.63 (1H, s, CHO).

This solution was placed under nitrogen, and treated with 4,4'-dimethoxybenzhydrylamine (0.379g, 1.56mmol) and powdered 4Å molecular sieves (1g) and stirred for 24 hours. Trimethylsilyl cyanide (0.5ml, 3.7mmol) was then added and stirring continued for a further 24 hours. The mixture was then concentrated *in vacuo*, treated with 6*M* HCl (35ml) and stirred under reflux for 19 hours, then cooled, filtered through Celite®, washed with CH_2Cl_2 (3x30ml), concentrated *in vacuo* and lyophilised. The crude material was partially purified by chromatography on Dowex®-1 (acetate form) to afford *(±)-2-(1'-methylcyclopropyl)-glycine* as a pale brown solid; δ_H (200MHz; D_2O) 0.29-0.46 (3H, m) and 0.59-0.70 (1H, m, $(CH_2)_2$), 0.81 (3H, s, CH_3), 3.63 (1H, s, CHN). This material was not further purified but dissolved in water (15ml) and cooled to 0°C. 1*M* NaOH (4ml) was added, followed by benzyl chloroformate (0.3ml, 2.1mmol). The reaction was stirred at 0°C for 10 minutes then at room temperature for 3.5 hours, with occasional dropwise addition of 1*M* NaOH to keep the pH above 10. The reaction mixture was then acidified to pH 4 (1*M* $KHSO_4$) and extracted with ethyl acetate (3x40ml). The organic extracts were dried (Na_2SO_4), filtered and concentrated *in vacuo* to afford a brown oil. Flash chromatography (SiO_2, Et_2O) afforded *(±)-N-benzyloxycarbonyl-2-(1'-methylcyclopropyl)glycine* as a white solid (36mg, 9% from 1-methylcyclopropanemethanol); (R_f 0.15, Et_2O); δ_H (200MHz; $CDCl_3$) 0.36-0.55 (2H, m), 0.67-0.79 (1H, m) and 0.84-0.93 (1H, m, $(CH_2)_2$), 1.05 (3H, s, CH_3), 3.71 (1H, d, *J* 8Hz, CHNH), 5.11 (2H, s, $PhCH_2$), 5.80 (1H, d, *J* 8 Hz, NH), 7.37 (5H, br s, $PhCH_2$); δ_C (50MHz; $CDCl_3$) 12.04 and 12.35 (cyclopropyl CH_2), 18.72 (CH_3), 19.23 (CCH_3), 61.04 (CHNH), 67.05 ($PhCH_2$), 128.24, 128.38, 128.74 (aromatic CH), 136.42 (ipso C), 156.73 (NHCOO), 173.01 (COOH).

To a stirred solution of (±)-*N*-benzyloxycarbonyl-2-(1'-methylcyclopropyl)glycine (19mg, 72µmol) in dry CH_2Cl_2 (1ml) under nitrogen, cooled to 0°C, were added triethylamine (15µl, 108µmol) and isobutyl chloroformate (14µl, 108µmol). The mixture was stirred at 0°C for 30 minutes then ammonia bubbled through the cooled solution for 55 minutes. The reaction was then allowed to stir for 14 hours and concentrated *in vacuo*. Flash chromatography (SiO_2, petroleum ether 30-40:ethyl acetate 40:60) afforded *(±)-2-(benzyloxycarbonylamino)-2-(1'-methylcyclopropyl)ethanamide* (16mg, 83%) as a white solid; m.p. 132-134°C (THF, petroleum ether 30-40); (R_f 0.3, petroleum ether 30-40: ethyl acetate 40:60); ν_{max} (KBr disc)

3392s, 3328s, 2948w, 1660s, 1541s, 1250s; δ_H (500MHz; $CDCl_3$) 0.39-0.43 (1H, m), 0.48-0.52 (1H, m), 0.69-0.73 (1H, m) and 0.86-0.91 (1H, m, (CH_2)$_2$), 1.05 (3H, s, CH_3), 3.70 (1H, br s, CHNH), 5.11 (2H, s, $PhCH_2$), 5.78 (2H, br s) and 5.95 (1H, br s, 3xNH), 7.32-7.39 (5H, m, C_6H_5); δ_C (125MHz, $CDCl_3$) 12.18 and 12.52 ((CH_2)$_2$), 18.82 (CH_3), 19.35 (CCH_3), 61.09 (CHNH), 67.03 ($PhCH_2$), 128.02, 128.15 and 128.52 (aromatic CH), 136.24 (ipso C), 156.40 (NHCOO), 172.48 ($CONH_2$); *m/z* (chemical ionisation, NH_3) 263 (MH^+, 67%), 155 (39), 108 (32), 91 (100); HRMS found 263.1396; $C_{14}H_{19}N_2O_3$ (MH^+) requires 263.1396.

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