Tetrahedron Vol. 47, No. 29, pp. 5603-5614, 1991
Printed in Great Britain

0040-4020/91 $3.00+.00

The Synthesis Of Substituted Aryl Diazirines. A Bifunctional Reagent Suitable For Application To Photoaffinity Labelling Studies.

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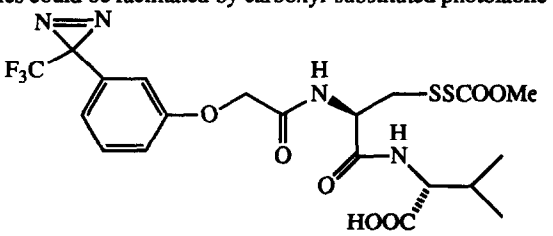
(*Received in UK* 10 *April* 1991)

Key Words: photoaffinity; diazirine; bifunctional; carbene; penicillin

Abstract: The 1,3,5-trisubstituted aryl diazirine(2) has been synthesised as a photoaffinity probe and has been elaborated to a reagent suitable for application to studies of penicillin and cephalosporin biosynthetic enzymes.

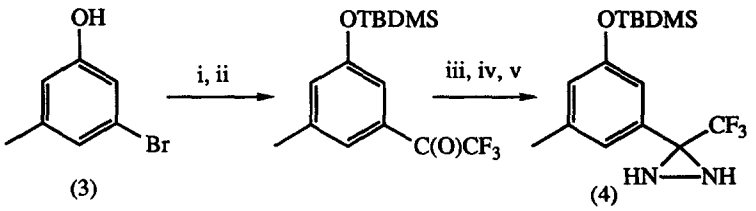
We recently reported the synthesis of diazirine(1) and described its incorporation into a dipeptide suitable for photoaffinity labelling studies[1] which was successfully applied to the study of the side chain binding site of Isopenicillin N Synthetase (IPNS)[2,3]. Thus, the diazirine function was converted readily to the corresponding carbene upon laser photolysis at 355nm, leading to inactivation of IPNS. Detailed analysis of this inactivated enzyme demonstrated that two regions of the peptide chain were apparently involved in the interaction between IPNS and substrate peptides. In order to provide supporting evidence of these results, and to extend this work to other β-lactam biosynthetic enzymes, more highly substituted side chains were required. Since IPNS and the ring expandase/hydroxylase enzyme (REXH), which is responsible for the biosynthesis of cephems in fungi, both show preferences for side chain substituents which contain carboxylic acid functions[4], photoaffinity labelling studies could be facilitated by carboxyl-substituted photolabile side chains.



(1)

Thus, the synthesis of diazirine(2) (Scheme 2) was undertaken. This required a convenient preparation of a 3,5-disubstituted phenol in which the carboxyl substituent could be easily deprotected, but which was also resistant to nucleophilic (ammonia or hydroxylamine) attack. Two routes, which used "latent" carboxyl groups, were investigated.

The first route involved elaborating 1-bromo-3-hydroxytoluene[5] using chemistry similar to that already described (Scheme 1). Phenol (3) was protected by silylation[6], and converted to the α,α,α-trifluoroacetophenone in a one-pot procedure upon treatment with *n*-butyllithium followed by methyl trifluoroacetate.[1] In this reaction, with careful temperature control, the initial adduct formed by attack of the aryllithium upon the ester does not decompose to a ketone, thereby preventing a second addition of the aryllithium species, and allowing isolation of the desired trifluoroacetophenone.[1] Elaboration of the ketone to generate diaziridine(4) *via* the oxime and tosyl oxime was effected by sequential treatment with hydroxylamine, tosyl chloride and ammonia. This intermediate possessed the requisite functional group precursors for the formation of the desired diazirine(2). Although the oxidation of benzylic methyl groups of similar compounds has been reported using potassium permanganate in pyridine[7], no useable benzoic acid products could be recovered after treatment of (4) with this reagent. In view of the difficulties in carrying out the oxidation of the benzylic methyl group, an alternative was sought which would allow oxidation to be carried out under significantly milder conditions.

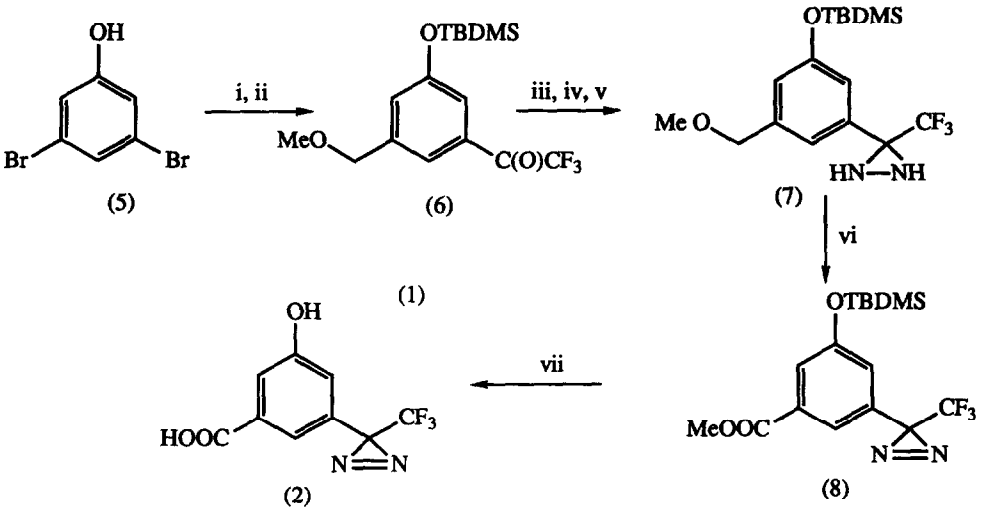


(i) TBDMSCl,DBU,CH_2Cl_2 ; (ii) (a) BuLi (b) CF_3COOMe ; (iii) NH_2OH, py; (iv) TsCl,py; (v) NH_3

Scheme 1

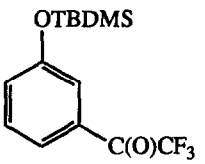
This second approach made use of the oxidation of benzylic ethers to the corresponding benzoate esters with ruthenium dioxide[8]. The required substrate, a 1,3,5-trisubstituted aromatic compound, was prepared from the silyl ether of 3,5-dibromophenol(5)[9]. Lithiation with subsequent acylation using methyl trifluoroacetate, as above, was followed, without work-up, by a second lithiation and finally trapping of the second lithiated species with chloromethyl methyl ether (Scheme 2). This one pot procedure generated an aromatic ketone of the desired substitution pattern in good yield (79%). One by-product from this reaction was trifluoroacetyl compound (9), which arose from quenching of the intermediate aryllithium by hydrochloric acid present in the chloromethyl methyl ether. The yield of (9) was minimised by standing a solution of the alkylating agent in tetrahydrofuran over sodium carbonate immediately before use. Elaboration of the ketone was performed by the same sequence of reactions as those described above (hydroxylamine, tosyl chloride, and ammonia) to generate diaziridine(7) in 42% overall yield. Treatment with ruthenium dioxide/sodium metaperiodate[8] oxidised both the methyl benzyl ether to the corresponding methyl benzoate, and the diaziridine to the diazirine, to give the product (8) in 90% yield. Hydrolysis of the ester with comcomitant cleavage of the silyl ether was

accomplished using lithium hydroxide in aqueous methanol[9] leading to the desired diazirine (2) in 77% yield. This photolabile precursor is capable of attachment to a suitable enzymic substrate or probe through either the phenolic or carboxylic functionality.



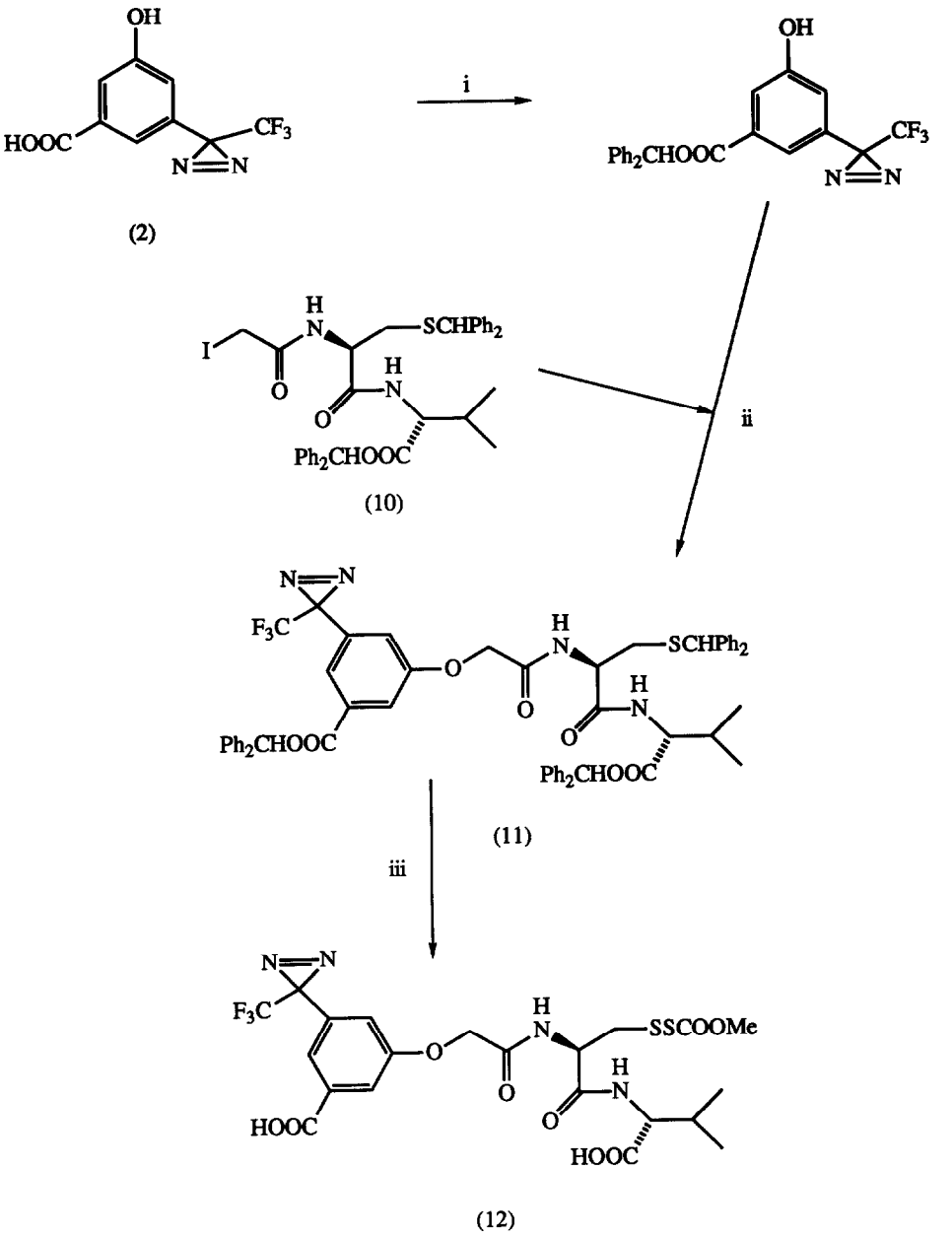
(i) TBDMSCl,DBU,CH_2Cl_2 ; (ii) (a) BuLi (b) CF_3COOMe ; (c) BuLi (d) $ClCH_2OCH_3$ (iii) NH_2OH,py; (iv) TsCl,py; (v) NH_3; (vi) RuO_2, $NaIO_4$; (vii) LiOH, MeOH, H_2O.

Scheme 2



(9)

The applicability of diazirine(2) to photoaffinity labelling studies was demonstrated by its incorporation into a dipeptide substrate suitable for inhibition studies of IPNS. Thus, esterification of diazirine(2) with diphenyldiazomethane[11] followed by reaction with iodoacetylpeptide(10) in the presence of potassium carbonate under previously reported conditions[1] gave intermediate(11) in 90% yield, which was deprotected in a two-step sequence to give the tripeptide(12) in low yield. This compound was found to be a substrate for IPNS, under the standard incubation conditions, and further evaluation of this compound as a photolabile probe for penicillin and cephalosporin biosynthetic enzymes is under examination.



(i) Ph_2CN_2; (ii) K_2CO_3/acetone; (iii) (a) ClSCOOMe (b) TFA

Scheme 3

EXPERIMENTAL

Standard experimental procedures were as described[1]. Melting points were determined with a Buchi 510 capillary apparatus and are uncorrected. IR spectra were obtained in chloroform or methanol solution, or as Nujol mulls, and were recorded on a Perkin Elmer 681 spectrophotometer; broad (b), weak (w), medium (m), and strong (s) bands are reported. UV spectra were recorded on a Perkin Elmer 555 UV-VIS spectrophotometer, and the solution solvents are stated in parenthesis. ^{1}H n.m.r. spectra were recorded on a Bruker WH 300 MHz, Bruker AM 500 MHz, or Varian Gemini 200 MHz spectrometers using the solvent ($CDCl_3$, CD_3OD, D_2O) as internal standard. ^{13}C n.m.r. were recorded at 50 MHz on a Varian Gemini 200 MHz or at 62.5 MHz on a Bruker AM 250 MHz spectrometer using $CDCl_3$ = 77.0 p.p.m. as internal reference. Only selected ^{13}C n.m.r. signals are assigned. Multiplicities are recorded as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Mass spectra were recorded on VG Analytical Ltd. ZAB1F or MM30F Mass spectrometers [for Ammonia Desorption Chemical Ionization (DCI NH_3), positive argon Fast Atom Bombardment (FAB), and Electron Impact (EI)]. Microanalyses were performed by Mrs. V. Lamburn, Dyson Perrins Laboratory, University of Oxford.

All starting materials, reagents and solvents were purified and dried unless otherwise stated. Tetrahydrofuran was distilled from sodium/benzophenone. Light petroleum refers to that in the boiling range 40-60°C and was redistilled before use. Dimethylformamide was dried and distilled from barium oxide. Dichloromethane was distilled from phosphorus pentoxide. Flash chromatography was performed using Merck silica gel 60H (40-63 mm, 230-400 mesh).

1-Bromo-3-(*t*-butyldimethylsilyloxy)-5-methylbenzene

A solution of 1-bromo-3-hydroxytoluene[5] (3) (0.5 g, 2.9 mmol) and *t*-butyldimethylsilyl chloride (0.5 g, 3.2 mmol) in dichloromethane was treated dropwise with a solution of 1,8-diazabicyclo[5.4.0]undec-7-ene (0.5 g, 3.4 mmol) in dichloromethane (5 ml), and the mixture stirred for 1h at room temperature[6]. The resulting solution was washed with water, 0.1M hydrochloric acid, saturated aqueous sodium hydrogen carbonate solution, water, and dried over sodium sulphate. The solvent was removed, and the crude product distilled (Kugelrohr) b.p. 100-110°C (1mmHg) to give the protected alcohol as a colourless liquid (0.77 g, 90%). (Found C, 51.5; H, 6.95; $C_{13}H_{21}BrOSi$ requires C, 51.8, H, 7.0%); $\upsilon_{max}(CHCl_3)$ 2960(m), 2860(m), 1600(s), 1570(s), 1460(m), 1440(m), 1290(s), 1250(m), 1160(s), 1020(m), 550(s) cm^{-1}; δ_H (200 MHz; $CDCl_3$) 0.21 (6H, s, $Si(CH_3)_2$), 1.00 (9H, s, $(CH_3)_3CSi$), 2.28 (3H, s, CH_3), 6.59 (1H, s, H4), 6.82 (1H, s, H2), 6.94 (1H, s, H6); δ_C (50MHz; $CDCl_3$) -4.47 (q, $Si(\underline{C}H_3)_2$), 18.2 (s, $\underline{C}(CH_3)_3$), 21.1 (q, CH_3), 25.6 (q, $(\underline{C}H_3)_3CSi$), 119.7 (d), 120.4 (d), 122.1 (s,C1), 125.2 (d), 140.8 (s), 156.3 (s); *m/z* (ACE, NH_3) 302 (15%, M^++2), 300 (16,M^+), 245 (98), 243 (100), 164 (34), 163 (30), 149 (30), 139 (24), 137 (23), 107 (18), 91 (60), 77 (24), 73 (25), 57 (28).

1-(3-*t*-Butyldimethylsilyloxy-5-methylphenyl)-2,2,2-trifluoroethanone

1-Bromo-3-(*t*-butyldimethylsilyloxy)-5-methylbenzene prepared above (0.5g, 1.7mmol) in tetrahydrofuran (20ml) was treated with *n*-butyllithium (1.2ml, 1.5M) at -78°C, and the solution was stirred

for 1h. A solution of methyl trifluoroacetate (0.23g, 1.8mmol) in tetrahydrofuran (20ml) was then added at -78°C, and the mixture was stirred for 2h[12]. The mixture was warmed to room temperature, the solvent removed, ether was added, and the organic phase was washed with saturated aqueous ammonium chloride, water, and dried over sodium sulphate. The solvent was removed, and the product obtained as a colourless liquid after distillation (Kugelrohr), (0.3g, 55%), b.p.85-95°C/0.5mmHg. (Found: C, 56.6; H, 6.65. $C_{15}H_{21}F_3SiO_2$ requires C, 56.6; H, 7.0%); υ_{max} ($CHCl_3$) 1710(m), 1590(m), 1350(m), 1280(s), 1260(s), 1150(s) cm^{-1}; δH(200MHz, $CDCl_3$) 0.23 (6H, s, $(CH_3)_2Si$), 1.01 (9H, s, $(CH_3)_3C$), 2.40 (3H, s, CH_3), 7.01 (1H, s, ArH), 7.32 (1H, s, ArH), 7.49 (1H, s, ArH); δ_C (50MHz; $CDCl_3$) -4.48 (q, $Si(CH_3)_3$), 18.2 (q, $C(\underline{C}H_3)_3$), 25.6 (q, $(CH_3)_3\underline{C}Si$), 114.4 (s), 118.3 (d), 119.0 (s), 123.8 (d), 128.3 (d), 131.0 (s), 140.6 (s), 156.1 (s), 180.2 (s); *m/z* (EI) 318 (M^+, 16%), 262 (26), 261 (95), 211 (100), 183 (41), 105 (25), 77 (60), 57 (26).

1-(3-*t*-Butyldimethylsilyloxy-5-methylphenyl)-2,2,2-trifluoroethanone oxime

1-(3-*t*-Butyldimethylsilyloxy-5-methylphenyl)-2,2,2-trifluoroethanone prepared above (0.50g, 1.57mmol) and hydroxylamine hydrochloride (0.13g, 1.89mmol) were refluxed in a mixture of pyridine (10ml) and ethanol (5ml) for 4h. The solvent was removed, the residue dissolved in ether, the solution washed with water, dried over sodium sulphate, and the solvent removed. The crude material was purified by flash chromatography (50% chloroform/light petroleum then dichloromethane) and the oxime was obtained as a colourless liquid (0.55g, 100%). υ_{max} ($CHCl_3$) 3560(m), 3280(m) 2960(s), 2930(s), 2860(s), 1590(s), 1470(s), 1465(s), 1440(m), 1360(m), 1350(s), 1290(s), 1260(s), 1190(s), 1160(s), 1065(m), 1020(s), 975(s), 840(s) cm^{-1}; δ_H(200MHz, $CDCl_3$) 0.22 (6H, s, $Si(CH_3)_2$), 1.00 (9H, s, $C(CH_3)_3$), 2.35 (3H, s, Me), 6.80 (2H, s, ArH), 6.92 (1H, s, ArH), 8.98 and 9.12 (1H, s, *syn-* and *anti-* OH); δ_C (50MHz; $CDCl_3$) -4.5 (q, $Si(\underline{C}H_3)_2$), 18.2 (s, $Si\underline{C}(CH_3)_3$), 21.3 (q, CH_3), 25.6 (q, CH_3), 117.4 (d, ArC), 122.0 (d, ArC), 123.0 (s), 123.2 (d, ArC), 126.7 (s), 130.9 (s), 139.9 (s), 155.6 (s); *m/z* (EI) 333 (M^+,21%), 276 (49), 210 (30), 190 (35), 77 (56), 75 (100).

O-(4-Toluenesulphonyl)-1-(3-*t*-butyldimethylsilyloxy-5-methylphenyl)-2,2,2-trifluoroethanone oxime

1-(3-*t*-Butyldimethylsilyloxy-5-methylphenyl)-2,2,2-trifluoroethanone oxime prepared above (0.15g, 0.90mmol) in pyridine (10ml) was treated with 4-toluenesulphonyl chloride (172mg, 0.90mmol) and the mixture refluxed for 2h. The solvent was removed, the residue dissolved in ether, and the organic phase washed with water and dried over sodium sulphate, and the solvent removed. The crude product was purified by flash chromatography (50% dichloromethane/light petroleum) to give the tosyl oxime as a colourless oil. (Found: C, 54.0; H, 5.89; N, 2.69. $C_{22}H_{28}F_3NO_4SSi$ requires C, 54.2; H, 5.79; N, 2.87%). υ_{max} ($CHCl_3$) 2960(w), 2930(w), 2840(w), 1600(m), 1590(m), 1390(s), 1350(s), 1300(s), 1260(m), 1195(s), 1180(s), 1165(m), 1155(m), 1095(w), 895(m), 840(s), 815(m) cm^{-1}; δ_H (200MHz; $CDCl_3$) 0.24 (6H, s, $Si(CH_3)_2$), 1.01 (9H, s, $SiC(CH_3)_3$), 2.34 (3H, s, (CH_3)), 2.48 (3H, s, (CH_3)), 6.67 (1H, s, ArH), 6.78 (1H, s, ArH),

7.39 (2H, d, ArH m- to OSO_2), 7.90 (2H, d, ArH o- to OSO_2); *m/z* (EI) 488 (M^+, 3%), 430 (75), 316 (40), 229 (32), 190 (68), 155 (60), 149 (26), 91 (100), 77 (27), 73 (58), 65 (45).

3-(3-*t*-Butyldimethylsilyloxy-5-methylphenyl)-3-trifluoromethyl-3H-diaziridine(4)

O-(4-Toluenesulphonyl)-1-(3-*t*-butyldimethylsilyloxy-5-methylphenyl)-2,2,2-trifluoroethanone oxime obtained above (1.0g, 2.1 mmol) in ether (10ml) at -78°C was added ammonia (20ml), the solution stirred for 6h, and then allowed to warm to room temperature overnight, when all of the ammonia had evaporated. The solution was diluted with ether, washed with water, dried over sodium sulphate, and the solvent removed. The crude product was purified by flash chromatography (50% dichloromethane/light petroleum) to give the diaziridine (4) as a yellow liquid (250mg, 38%). (Found: C, 54.4; H, 7.27; N, 8.28. $C_{15}H_{23}F_3N_2OSi$ requires C, 54.2; H, 6.97: N, 8.43%). υ_{max} ($CHCl_3$) 3280(m), 2960(s), 2930(s), 2860(s), 1600(s), 1470(s), 1460(s), 1450(s), 1400(s), 1320(s), 1280(s), 1250(s), 1175(s), 1145(s), 1100(s), 1035(s), 960(m), 840(s) cm^{-1}; δ_H (200MHz; $CDCl_3$) 0.21 (6H, s, $Si(CH_3)_2$), 0.99 (9H, s, $SiC(CH_3)_3$), 2.22 (1H, bs, NH), 2.33 (3H, s, CH_3), 2.75 (1H, bs, NH), 6.73 (1H, s, ArH), 6.89 (1H, s, ArH), 7.02 (1H, s, ArH); *m/z* (ACE, NH_3) 333 (M^++1, 20%), 332 (M^+, 50), 275 (45), 209 (30), 183 (100), 180 (40), 179 (38), 165 (55), 77 (90), 73 (60), 66 (41).

O-(*t*-Butyldimethylsilyl)-3,5-dibromophenol

A solution of 3,5-dibromophenol (5)[9](5.0 g, 20 mmol) and *t*-butyldimethylsilyl chloride (3.5 g, 23.2 mmol) in dichloromethane was treated dropwise with a solution of 1,8-diazabicyclo[5.4.0]undec-7-ene (3.5 g, 23.0 mmol) in dichloromethane (10 ml), and the mixture stirred for 1 h at room temperature[6]. The resulting solution was washed with 0.1M hydrochloric acid, saturated aqueous sodium hydrogen carbonate solution, water, and dried over sodium sulphate. The solvent was removed, and the crude product distilled (Kugelrohr) b.p. 160°C (1mmHg) to give silyl ether (5) as a colourless liquid (5.9 g, 81%). δ_H (200 MHz; $CDCl_3$) 0.23 (6H, s, $Si(CH_3)_2$), 0.99 (9H, s, $SiC(CH_3)_3$), 6.95 (2H, s, ArH), 7.28 (1H, s, ArH).

(*t*-Butyldimethylsilyloxy)-3-(trifluoroacetyl)-5-(methoxymethyl)benzene (6)

To a solution of silyl ether (5) (2.0 g, 5.5 mmol) in tetrahydrofuran (20 ml) at -78°C was added *n*-butyllithium (1.4M, 4.0 ml, 1 equiv.) and the mixture was stirred for 0.5h under nitrogen. A solution of methyl trifluoroacetate (0.7 g, 5.5 mmol) in tetrahydrofuran (10 ml) was added at -78°C, and the mixture stirred for 0.5h. Another equivalent of *n*-butyllithium was added and the mixture stirred for a further 0.5h. A solution of chloromethyl methyl ether (1.0 g, 12.4 mmol) in tetrahydrofuran (10 ml) (which had been treated with sodium carbonate to remove any hydrogen chloride present) was added to the reaction mixture which was then stirred for 1h. The reaction was quenched with saturated aqueous ammonium chloride/methanol (1:3 mixture, 8 ml) at -78°C, and the mixture warmed to room temperature. The solvent was removed, the residue dissolved in ether, washed with saturated aqueous ammonium chloride solution, water, and dried over sodium sulphate. The solvent was evaporated and the crude material was purified by flash chromatography (2-10% ethyl acetate/light petroleum). The liquid obtained was distilled (Kugelrohr) b.p. 135°C (0.6 mmHg) to give

ketone (6) (1.5g, 79%). (Found: C, 55.0; H, 6.8. $C_{16}H_{23}F_3O_3Si$ requires C, 55.2; H, 6.6%); υ_{max} ($CHCl_3$) 2960(m), 2940(s), 2860(m), 1720(s), 1595(s), 1470(m), 1460(m), 1450(m), 1385(m), 1365(m), 1350(m), 1285(s), 1260(s), 1200(s), 1150(bs), 1100(m), 1040(w), 1015(m), 1000(m), 840(bs), 825(m), 810(m), 715(w) cm^{-1}; δ_H (200 MHz; $CDCl_3$) 0.24 (6H, s, $Si(CH_3)_2$), 1.01 (9H, s, $SiC(CH_3)_3$), 3.42 (3H, s, OCH_3), 4.48 (2H, s, OCH_2Ar), 7.20 (1H, s, ArH), 7.44 (1H, s, ArH), 7.63 (1H, s, ArH); δ_C (50 MHz; $CDCl_3$) -5.02 (q, $(\underline{C}H_3)_2Si$), 17.85 (s, $(CH_3)_3\underline{C}Si$), 25.2 (q, $(\underline{C}H_3)_3CSi$), 57.9 (q, OCH_3), 73.3 (t, OCH_2Ar), 113.8 (s), 119.6 (s), 120.1 (d, ArH), 121.8 (d, ArH), 126.3 (d, ArH), 131.1 (s), 141.6 (s), 156.5 (s); *m/z* [CI(NH_3)] 366 ($M+NH_4^+$, 75%), 348 (22), 308 (69), 277 (22), 276 (100), 265 (17), 259 (20), 91 (30).

The major side product formed was found to be a monoacylated product, 1-(*t*-butyldimethylsilyloxy)-3-(trifluoroacetyl)benzene (9). υ_{max} ($CHCl_3$) 2980(s), 2950(s), 2860(m), 1720(s), 1600(m), 1580(m), 1490(m), 1475(m), 1465(m), 1440(m), 1395(w), 1365(w), 1345(m), 1320(w), 1265 (s), 1205(s), 1175(s), 1155(s), 1005(m), 990(m), 910(s), 845(s), 830(s), 810(m); δ_H (200 MHz; $CDCl_3$) 0.25 (6H, s, $Si(CH_3)_2$), 1.02 (9H,s, $SiC(CH_3)_3$), 7.20 (1H, dd, *J* 8.0 Hz, ArH), 7.42 (1H, m, ArH), 7.55 (1H, s, ArH), 7.69 (1H, d, *J* 7.7 Hz, ArH); δ_C (50 MHz; $CDCl_3$) -4.9 (q, $CH_3)_2Si$), 17.95 (s, $(CH_3)_3\underline{C}Si$), 25.3 (q, $(\underline{C}H_3)_3CSi$), 113.8 (s), 119.64 (s), 121.1 (d, ArH), 123.2 (d, ArH), 127.7 (d, ArH), 130.3 (d, ArH), 131.3 (s), 156.5 (s); *m/z* [CI(NH_3)] 322 ($M+NH_4^+$, 7%), 265 (32), 264 (100), 235 (12), 198 (12), 197 (46), 91 (68), 74 (39).

{[3-(*t*-Butyldimethylsilyloxy)-5-(methoxymethyl)]phenyl}-2,2,2-trifluoroethanone Oxime

Ketone (6) (453.4 mg, 1.3 mmol) and hydroxylamine hydrochloride (107.6 mg, 1.5 mmol) were refluxed in a solution of pyridine (20 ml) and absolute ethanol (10 ml) for 4 h. The solvent was removed *in vacuo*, the residue dissolved in ether, washed with water, and dried over sodium sulphate. The solvent was removed to give the crude product, which was purified by flash chromatography (5% ethyl acetate /dichloromethane). The oxime was obtained as a colourless oil (380 mg, 81%). (Found: C, 52.6; H, 6.7; N, 4.2. $C_{16}H_{24}F_3NO_3Si$ requires C, 52.9; H, 6.6; N, 3.9%); υ_{max} ($CHCl_3$) 3560(w), 3250(bm), 2930(bs), 1595(s), 1470(s), 1465(s), 1435(s), 1380(s), 1365(s), 1350(s), 1285(s), 1260(s), 1190(s), 1155(bs), 1100(s), 1060(m), 1005(bs), 975(bs), 840(bs), 815(m) cm^{-1}; δ_H (200 MHz; $CDCl_3$) 0.21 (6H, s, $Si(CH_3)_2$), 0.97 (9H, s, $SiC(CH_3)_3$), 3.42 (3H, s, OCH_3), 4.48 (2H, s, OCH_2Ar), 6.93 (2H, m, ArH), 6.94 (1H, s, ArH), 9.62 and 9.93 (1H, 2xs, *syn* and *anti* OH); δ_C (50 MHz; $CDCl_3$) -4.80 (q, $(CH_3)_2Si$), 18.0 (s, $(CH_3)_3\underline{C}Si$), 25.4 (q, $(\underline{C}H_3)_3CSi$), 58.0 (q, OCH_3), 74.0 (t, OCH_2Ar), 115.6 (s), 119.6 (d, ArH), 119.8 (d, ArH), 120.9 (d, ArH), 121.3 (d, ArH), 121.5 (d, ArH), 127.5 (s), 131.6 (s), 139.8 (s), 146.6 (s), 155.9 (s); *m/z* [CI(NH_3)] 381 ($M+NH_4^+$, 100%), 348 (69), 295 (35).

(4-Toluenesulphonyl)-1-{[3-(*t*-butyldimethylsilyloxy)-5-(methoxymethyl)]-phenyl}-2,2,2-trifluoroethanone Oxime

{[3-(*t*-Butyldimethylsilyloxy)-5-(methoxymethyl)]phenyl}-2,2,2-trifluoroethanone oxime prepared above (1.3 g, 3.6 mmol) in pyridine (20 ml) was treated with 4-toluenesulphonyl chloride (1.1 g, 5.7 mmol) and the mixture refluxed for 3.5 h. The solvent was removed *in vacuo*, the residue dissolved in ether,

washed with water, and dried over sodium sulphate. The solvent was removed and the crude material purified by flash chromatography (50% dichloromethane/light petroleum, then 75% dichloromethane/light petroleum) to give the tosyl oxime as a colourless oil (1.5 g, 81%). (Found: C, 53.1; H, 6.1; N, 2.6. $C_{23}H_{30}F_3NO_5SSi$ requires C, 53.4; H, 5.8; N, 2.7%); υ_{max} ($CHCl_3$) 2940(bm), 1600(s), 1465(bm), 1390(s), 1350(m), 1300(s), 1260(m), 1200(s), 1185(s), 1155(s), 1095(m), 1020 (bw), 895(s), 840(s), 820(s) cm^{-1}; δ_H(200Hz; $CDCl_3$) 0.22 (6H, s, $Si(CH_3)_2$), 0.99 (9H, s, $SiC(CH_3)_3$), 2.49 (3H, s, CH_3Ar), 3.40 (3H, s, OCH_3), 4.43 (2H, s, OCH_2Ar), 6.76 (1H, s, ArH), 6.92 (1H, s, ArH), 6.97 (1H, s, ArH), 7.40 (2H, AA'BB' system, J_{AB} + AB' 8.3 Hz, ArH *meta* to OSO_2), 7.89 (2H, AA'BB' system, J_{AB} + AB' 8.3 Hz, ArH *ortho* to OSO_2); δ_C (50 MHz; $CDCl_3$) -4.80 (q, $(CH_3)_2Si$), 18.0 (s, $(CH_3)_3\underline{C}Si$), 21.5 (q, CH_3Ar), 25.4 (q, $(\underline{C}H_3)_3CSi$), 58.2 (q, OCH_3), 73.5 (t, OCH_2Ar), 117.0 (s), 119.2 (d, ArH), 119.8 (d, ArH), 122.1 (d, ArH), 125.6 (s), 129.3 (d, ArH), 130.0 (d, ArH), 131.4 (s), 141.3 (s), 146.4 (s), 153.6 (s), 156.2 (s); *m/z* [DCI(NH_3)] 535 ($M+NH_4^+$, 9%), 460 (100), 346 (22), 333 (25), 188 (23), 91 (38).

{[3-(*t*-Butyldimethylsilyloxy)-5-(methoxymethyl)]phenyl}-3-trifluoromethyl-3H-diaziridine (7)

(4-Toluenesulphonyl)-1-{[3-(*t*-butyldimethylsilyloxy)-5-(methoxymethyl)]-phenyl}-2,2,2-trifluoro-ethanone oxime (1.2 g, 2.3 mmol) prepared above was dissolved in dry ether (10 ml), cooled to -78°C, and ammonia (15 ml) added. The mixture was stirred at -78°C for 8 h, and the ammonia allowed to evaporate overnight. The residue was diluted with ether, washed with water, dried over sodium sulphate, and the solvent removed. The crude product was purified by flash chromatography (20% ether/ dichloromethane) to give diaziridine (7) as a colourless liquid (0.85 g, 98%). (Found: C, 52.9; H, 7.2; N, 7.5. $C_{16}H_{25}F_3N_2O_2Si$ requires C, 53.0; H, 6.9; N, 7.7%); υ_{max} ($CHCl_3$) 3280(m), 2960(bs), 1605(s), 1475(m), 1465(m), 1450(m), 1405(s), 1385(m), 1365(m), 1320(s), 1285(s), 1260(s), 1180(bs), 1145(s), 1105(s), 1035 (m), 1005(m), 940(m), 865(m), 845(s), 810(m), 705(m) cm^{-1}; δ_H (200 MHz; $CDCl_3$) 0.21 (6H, s, $Si(CH_3)_2$), 0.99 (9H, s, $SiC(CH_3)_3$), 2.28 (1H, d, *J* 8.4 Hz, NH), 2.79 (1H, d, *J* 8.6 Hz, NH), 3.39 (3H, s, OCH_3), 4.42 (2H, s, OCH_2Ar), 6.91 (1H, s, ArH), 7.02 (1H, s, ArH), 7.18 (1H, s, ArH); δ_C (50 MHz; $CDCl_3$) -4.90 (q, $CH_3)_2Si$), 17.9 (s, $(CH_3)_3\underline{C}Si$), 25.4 (q, $(\underline{C}H_3)_3CSi$), 57.8 (q, OCH_3), 73.7 (t, OCH_2Ar), 119.1 (d, ArH), 120.0 (d, ArH), 120.7 (d, ArH), 126.4 (s), 133.2 (s), 140.6 (s), 156.0 (s), 157.0 (s); *m/z* [CI(NH_3)] 381 (25%), 380 ($M+NH_4^+$, 100), 363 (49), 348 (16), 270 (12).

{[3(*t*-Butyldimethylsilyloxy)-5-(methyloxycarbonyl)]phenyl}-3-trifluoromethyl-3H-diazirine (8)

The diaziridine (7) (0.31 g, 0.86 mmol) and sodium periodate (1.8 g, 8.4 mmol, 10 equiv.) in a mixture of tetrachloromethane (2 ml), acetonitrile (2 ml), and water (3 ml) was treated with ruthenium dioxide[8] (10 mg), and stirred overnight at room temperature. The mixture was filtered through celite, diluted with dichloromethane and water, and the layers separated. The aqueous phase was washed with dichloromethane, the combined organic extracts washed with water, dried over sodium sulphate, and the solvent removed to give diazirine (8) (0.29 g, 90%). υ_{max} ($CHCl_3$) 3020(w), 2960(m), 2935(m), 2860(m), 1725(s), 1595(s), 1460(m), 1435(m), 1365(s), 1300(s), 1255(s), 1240(s), 1200(s), 1180(s), 1160(s),

1110(w), 1015(m), 1005(m), 935(m), 840(s) cm^{-1}; λ_{max} 240nm (log ε 3.4), 300 (2.7), 353 (2.4); δ_H (200 MHz; $CDCl_3$) 0.23 (6H, s, $Si(CH_3)_2$), 0.99 (9H, s, $SiC(CH_3)_3$), 3.91(3H, s, OCH_3), 6.92 (1H, s, ArH), 7.38 (1H, s, ArH), 7.53 (1H, s, ArH); δ_C (50 MHz; $CDCl_3$) -4.78 (q, $CH_3)_2Si$), 18.0 (s, $(CH_3)_3\underline{C}Si$), 25.4 (q, $(\underline{C}H_3)_3CSi$), 52.4 (q, OCH_3), 120.3 (d, ArH), 122.4 (d, ArH), 122.7 (d, ArH), 130.9 (s), 132.5 (s), 156.4 (s), 166.0 (s); *m/z* [CI(NH_3)] 393 (20%), 392 ($M+NH_4^+$, 100), 363 (12), 346 (9).

[(3-Hydroxy-5-carboxy)phenyl]-3-trifluoromethyl-3H-diazirine (2)

Diazirine (8) (200 mg, 0.53 mmol) in methanol (25 ml) was treated with aqueous 1M lithium hydroxide solution[10] (10 ml) and the mixture stirred for 3.5 h. The solvent was removed *in vacuo*, and the residue dissolved in water. The aqueous phase was washed with ether, then acidified with 10% citric acid. The resulting precipitate was extracted with ethyl acetate, and the solvent evaporated to give crude acid (2) (100 mg, 77%). δ_H (200 MHz; $CDCl_3$) 6.85 (1H, s, ArH), 7.28 (1H, s, ArH), 7.50 (1H, s, ArH).

[(3-Hydroxy-5-diphenylmethyloxycarbonyl)phenyl]-3-trifluoromethyl-3H-diazirine

A solution of acid (2) (80 mg, 0.32 mmol) in acetone (10 ml) was treated with diphenyldiazomethane[11] (69 mg, 0.35 mmol) and the mixture stirred at room temperature for 2 h. The solvent was removed and the crude material purified by flash chromatography (50% light petroleum/dichloromethane, then dichloromethane) to give the ester (86 mg, 65%). υ_{max} ($CHCl_3$) 3590(w), 3260(bw), 3030(w), 1720 (s), 1600(m), 1495(m), 1450(m), 1370(m), 1290(s), 1215(bs), 1180(s), 1160(s), 1115(m), 1080(w), 1030 (w), 980(m), 910(m), 880(w), 810(w), 700(s) cm^{-1}; δ_H (200 MHz; $CDCl_3$) 6.62 (1H, s, OH), 6.80 (1H, s, $CHPh_2$), 6.90-7.90 (13H, m, 3ArH + 10ArH in $CHPh_2$); δ_C (50 MHz; $CDCl_3$) 78.4 (d, $CHPh_2$), 118.1 (d, ArH), 118.5 (d, ArH), 119.8 (d, ArH), 127.1 (s), 127.1 (d, ArH), 127.7 (s), 128.4 (d, ArH), 128.7 (d, ArH), 128.8 (d, ArH), 131.4 (s), 132.4 (s), 139.6 (s), 139.8 (s), 156.8 (s). *m/z*(CI/NH_3) 429 (M^++NH_3, 10%), 412 (M^+,17), 377 (27), 275 (73), 258 (43), 200 (27), 167 (100).

[3-(Diphenylmethyloxycarbonyl)-5-(3-trifluoromethyl-3H-diazirin-3-yl)phenoxy]acetyl-L-(S-diphenylmethyl)cysteinyl-D-valine Diphenylmethyl Ester (11)

A solution of [(3-hydroxy-5-diphenylmethyloxycarbonyl)phenyl]-3-trifluoromethyl-3H-diazirine prepared above (30.7 mg, 0.07 mmol) and dipeptide(10)[1] (53.7 mg, 0.07 mmol) and potassium carbonate (10.3 mg, 0.07 mmol) were stirred in acetone (10 ml) in the dark overnight. The solvent was removed and the crude material purified by flash chromatography (dichloromethane, then 10% ethyl acetate/dichloromethane) to give (11) as a colourless oil (75 mg, 98%). υ_{max} ($CHCl_3$) 3410(w), 3030(bw), 1730(s), 1680(s), 1605(m), 1520(m), 1500(m), 1455(m), 1440(w), 1375(bm), 1295(s), 1205(bs), 1180(bs), 960(bm) cm^{-1}; λ_{max}(MeOH) 206nm (log ε 4.1), 300(3.4), 350sh(2.35). δ_H (200 MHz; $CDCl_3$) 0.77 (3H, d, *J* 6.9 Hz, CH_3), 0.90 (3H, d, *J* 7.0 Hz, CH_3), 2.19-2.28 (1H, m, $C\underline{H}(CH_3)_2$), 2.80-2.84 (2H, m, AB of ABX), 4.46 (1H, d, *J* 14.4 Hz, OCHCO), 4.52 (1H, d, *J* 14.4 Hz, OCH'CO), 4.60-4.71 (2H, m, α–Cys and α–Val), 5.34 (1H, s, $SC\underline{H}Ph_2$), 6.50-7.80 (35H, m, 30 ArH in $CHPh_2$, 2x$C\underline{H}Ph_2$ and 3ArH); δ_C (62.5 MHz, $CDCl_3$) 17.3 (q, CH_3), 19.1 (q, CH_3), 31.3 (d, β–C Val), 34.5 (t, β–C Cys), 52.0 (d, α–C), 54.7 (d), 57.5 (d), 67.4 (t, OCH_2), 78.1 (d, $Ph_2\underline{C}H$), 78.4 (d, $Ph_2\underline{C}H$), 116.5 (d), 118.2 (d), 121.6 (d), 126.6

(d, ArH), 127.0 (d, ArH), 127.2 (d, ArH), 127.4 (d, ArH), 127.5 (d, ArH), 128.1 (d, ArH), 128.2 (d, ArH), 128.4 (d, ArH), 128.5 (d, ArH), 128.6 (d, ArH), 128.7 (d, ArH), 128.8 (d, ArH), 131.4 (s), 132.9 (s), 140.7 (s), 140.9 (s), 157.4 (s), 163.9 (s), 167.1 (s), 169.5 (s), 170.4 (s), 218.6 (s).*m/z*(FAB) 167(100%).

[3-(Oxycarbonyl)-5-(3-trifluoromethyl-3H-diazirin-3-yl)phenoxy]acetyl-L-(S-carbomethoxysulphenyl)cysteinyl-D-valine (12)

A solution of protected peptide (11) (17mg, 0.017mmol) in chloroform (0.5ml) and methanol (0.25ml) at 0°C was treated with redistilled methoxycarbonylsulphenyl chloride (10mg, 0.079mmol). The mixture was stirred for 1.5h until all the starting material had been consumed, as indicated by t.l.c. (5% ethyl acetate/dichloromethane). The solvent was removed *in vacuo* and dried under high vacuum, to give disulphide (12) which was used without further purification. δ_H (200 MHz; $CDCl_3$) 0.89(3H, d, *J* 6.8Hz, CH_3), 0.99 (3H, d, *J* 6.8Hz, CH_3), 2.29-2.39 (1H, m, Me_2CH), 2.76-2.87 (2H, m, AB of ABX), 3.95 (5H, s, OCH_3 and OCH_2CO), 4.60-4.79 (2H, m, α-Cys and α-Val), 6.90 (1H, s, $COOCHPh_2$), 7.00-7.40 (24H, m, ArH and $COOCHPh_2$).

To this crude disulphide was added anisole (1 drop) and redistilled trifluoracetic acid, and the mixture was stirred until all of the starting material had been consumed (0.25h) as indicated by t.l.c.(dichloromethane). The solvent was removed *in vacuo*, and the residue treated with toluene (3 times). The crude product was redissolved in dichloromethane, and washed twice with 0.19M sodium carbonate in D_2O. The aqueous extracts were then freeze-dried to give the disodium salt of the diacid (12). δ_H (200 MHz; $CDCl_3$) 0.76(3H, d, *J* 6.8Hz, CH_3), 0.89 (3H, d, *J* 6.8Hz, CH_3), 2.30-2.45 (1H, m, Me_2CH), 2.70-2.90 (2H, m, AB of ABX), 3.95 (5H, s, OCH_3 and OCH_2CO), 4.60-4.79 (2H, m, α-Cys and α-Val), 6.70-7.40 (3H, m, ArH); *m/z*(FAB) 594 (M^++2Na, 12%), 573 (80), 551 (100).

Bioassay

The bioassay was performed using the standard hole-plate assay[13], and gave a zone which was positive against *Staphylococcus aureus* N. C. T. C. 6571.

ACKNOWLEDGEMENTS

We gratefully acknowledge both the SERC and Glaxo Group Research for financial support.

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