Syntheses of Penicillin N, [6 α - 3 H]Penicillin N and [10- 14 C,6 α - 3 H]Penicillin N

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1. Penicillin N was synthesized by coupling α -amino- α -*p*-nitrobenzyl-*N*-*p*-nitrobenzyloxycarbonyl-D-adipate with 6-aminopenicillanic acid benzyl ester, followed by removal of the protecting groups through hydrogenolysis. 2. α -Amino- α -*p*-nitrobenzyl-*N*-*p*-nitrobenzyloxycarbonyl-D-[5- 14 C]adipate was prepared by treating α -*p*-nitrobenzyl-*N*-*p*-nitrobenzyloxycarbonyl-D-glutamic acid with [14 C]diazomethane followed by rearrangement with silver trifluoromethanesulphonate. 3. Coupling of α -amino- α -*p*-nitrobenzyl-*N*-*p*-nitrobenzyloxycarbonyl-D-[5- 14 C]adipate with 6-aminopenicillanic acid benzyl ester gave triprotected [10- 14 C]penicillin N. 4. 3 H was introduced at C-6 of the Schiff's base derivative (10) by oxidation followed by reduction with NaB 3 H $_4$. 5. The so-derived (6 α - 3 H)-labelled Schiff's base was hydrolysed to give 6-amino[6 α - 3 H]penicillanic acid benzyl ester *p*-toluenesulphonic acid salt, which after coupling as the free amine with α -amino- α -*p*-nitrobenzyl-*N*-*p*-nitrobenzyloxycarbonyl-D-adipate and then hydrogenolysis, yielded [6 α - 3 H]penicillin N. 6. Triprotected [10- 14 C]penicillin N and triprotected [6 α - 3 H]penicillin N in admixture were hydrogenolysed to give [10- 14 C,6 α - 3 H]penicillin N.

Penicillin N (1a; R = H) was shown to stimulate the production of cephalosporin(s) by Kohsaka & Demain (1976) in a cell-free system obtained from *Cephalosporium acremonium* CW-19. The identity of the enhanced product was shown to be deacetoxycephalosporin C (2; Yoshida *et al.*, 1978) by paper electrophoresis, paper chromatography and t.l.c. The present paper describes the synthesis of penicillin N (1a; 10-C; R = H)[6 α - 3 H]penicillin N (1c; 10-C; R = 3 H) and [10- 14 C,6 α - 3 H]penicillin N (1d; 10- 14 C; R = 3 H), which were used in the incorporation experiments described in the following paper (Baldwin *et al.*, 1980).

(1a) 10-C; R = 1 H

(1b) 10- 14 C; R = 1 H

(1c) 10-C; R = 3 H

(1d) 10- 14 C; R = 3 H

Penicillin N has been synthesized by two groups (Vanderhaeghe *et al.*, 1974; Usher *et al.*, 1975). The synthesis described in the present paper involves the coupling of two halves, α -amino- α -*p*-nitrobenzyl-*N*-*p*-nitrobenzyloxycarbonyl-D-adipate and 6-amino-penicillanic acid benzyl ester in the presence of 2-ethoxy-*N*-ethoxycarbonyl-1,2-dihydroquinoline to give the triprotected (i.e. the functional groups are blocked) penicillin N (9a, 10-C, R = H) as a stable compound that could be purified by preparative t.l.c. on silica. The advantage of this procedure is that 14 C can be introduced in the α -amino-D-adipic acid moiety and 3 H into the nucleus.

D-Glutamic acid (3) was converted into the *N*-*p*-nitrobenzyloxycarbonyl derivative (4; yield 64.4%) by treatment with *p*-nitrobenzyl chloroformate, which in turn was converted into a diprotected glutamic acid derivative (5) in 46.4% yield with *p*-nitrobenzylbromide (Scheme 1). The mixed anhydride obtained from the diprotected

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Scheme 1. Stages in the syntheses of penicillin N, [6α-³H]penicillin N and [10-¹⁴C, 6α-³H]penicillin N

glutamic acid (5) with triethylamine/ethyl chloroformate (Nefkins *et al.*, 1964) was treated with diazomethane to give the diazoketone (6a) in 44.4% yield after chromatography (Moore *et al.*, 1973). This substance, on treatment with silver trifluoromethanesulphonate (silver triflate), gave the rearranged diprotected α-amino-D-adipic acid (7a; 70% yield). The diprotected derivative (7a) was coupled with 6-aminopenicillanic acid benzyl ester (8a) in the presence of 2-ethoxy-N-ethoxycarbonyl-1,2-dihydroquinoline to yield a triprotected peni-

cillin N (9a; 55.7% yield) after chromatography. Hydrogenation of this derivative (9a) with 10% Pd-C in the presence of 1 equiv. of NaHCO₃ gave penicillin N monosodium salt (1a) in 78.4% yield. This material was judged 30–40% pure by bioassay on agar plates seeded with *Escherichia coli* Ess or *Pseudomonas aeruginosa* Pss. The crude material after purification on a Sephadex G-25 column (Smith *et al.*, 1967) gave an off-white solid of approx. 80% purity by bioassay.

The diprotected glutamic acid (5) was treated with

triethylamine/ethyl chloroformate followed by [^{14}C]-diazomethane to yield the (6- ^{14}C)-labelled diazomethane (6b; 28% yield) after chromatography. This substance, on rearrangement with silver triflate, gave a diprotected α -amino[5- ^{14}C]adipic acid (7b; 91% yield), which after coupling with 6-aminopenicillanic acid benzyl ester gave the triprotected [10- ^{14}C]penicillin N (9b) in 65.7% yield, after chromatography.

The Schiff's base (10) was obtained by refluxing 6-aminopenicillanic acid benzyl ester (8a) with 3,5-di-*t*-butyl-4-hydroxybenzaldehyde (99.6% yield). This compound, on oxidation with freshly prepared PbO_2 , afforded the quinonoidal compound (11) (Yanagisawa *et al.*, 1975) in quantitative yield as a red unstable oil (Scheme 2). The reduction of compound (11) with $\text{Na}(\text{CN})\text{BH}_3$ at pH 3 gave the over-reduced product (12) in 88.8% yield. However, when the reduction was carried out with 8 hydride

equivalents of NaBH_4 , a 1:1 mixture of epimers at C-6 of Schiff's base (10) was obtained. By reducing the amount of NaBH_4 to 1.3 equivalents it was possible to obtain Schiff's base (10) exclusively. When 1.5 equivalents of NaB^2H_4 was used, the (6 α - ^2H)-labelled Schiff's base (13a) was obtained cleanly. This substance, on hydrolysis in the presence of *p*-toluenesulphonic acid monohydrate, yielded 6-amino[6 α - ^2H]penicillanic acid benzyl ester tosylammonium salt (14a; 75% yield) (Bohme *et al.*, 1973).

Having established the stereospecificity of this deuteration, NaB^3H_4 was substituted in the reduction of quinonoidal compound (11) to give (6 α - ^3H)-labelled Schiff's base (13b), which after hydrolysis with *p*-toluenesulphonic acid monohydrate provided 6-amino[6 α - ^3H]penicillanic acid benzyl ester tosylammonium salt (14b). The free amine corresponding to compound (14b) was coupled with α -amino-

Scheme 2. Introduction of ^3H into the nucleus of penicillin N

α - *p* - nitrobenzyl - 4 - *p* - nitrobenzyloxycarbonyl-D-adipate (7a) in the presence of 2-ethoxy-*N*-ethoxycarbonyl-1,2-dihydroquinoline and gave the triprotected [6 α -³H]penicillin N (9c; 40% yield), which, on hydrogenation over 10% Pd-C with 1 equiv. of NaHCO₃, gave [6 α -³H]penicillin N monosodium salt (1c) in 78.4% yield (sp. activity 20.67 μ Ci/ μ mol). Thus the overall yield of [6 α -³H]penicillin N monosodium salt (1c) was 15.5% from the Schiff's base (10).

For a doubly labelled penicillin N, the triprotected [6 α -³H]penicillin N (9c, 5 mg, 6.55 μ mol, sp. activity 61.5 μ Ci/ μ mol) was mixed with triprotected [10-¹⁴C]penicillin N (9b, 9 mg, 11.79 μ mol, sp. activity 0.250 μ Ci/ μ mol) and the protecting groups were removed with 10% Pd-C in the presence of NaHCO₃ to provide doubly labelled penicillin N monosodium salt (1d) in 80% yield (³H sp. activity 22.06 μ Ci/ μ mol; ¹⁴C sp. activity 0.166 μ Ci/ μ mol).

Experimental

Materials

6-Aminopenicillanic acid was a gift from Eli Lilly and Co., Indianapolis, IN, U.S.A. *p*-Nitrobenzyl chloroformate was obtained from Eastman-Kodak, Rochester, New York, U.S.A. Sephadex G-25 and cation-exchange resin (Dowex 50W; X8) were received from Sigma Chemical Co., St. Louis, MO, U.S.A. Scintiverse liquid was obtained from Fisher Scientific Co., Pittsburgh, PA, U.S.A. *N*-[¹⁴C]-Methyl-*N*-nitroso-*p*-toluenesulphonamide and NaB³H₄ were purchased from New England Nuclear, Boston, MA, U.S.A. 2-Ethoxy-*N*-ethoxycarbonyl-1,2-dihydroquinoline and other chemicals were obtained from Aldrich Chemical Co., Milwaukee, WI, U.S.A.

Radioactive measurements

The radioactivity was measured with a Beckman LS-8100 liquid-scintillation counter. Weighed amounts of penicillin N sample were dissolved in 15 ml of Scintiverse liquid for counting. The counting efficiencies were 50–53% for ³H and 89% for ¹⁴C.

Antibacterial activities

These were determined by the hole-plate method (Smith *et al.*, 1967) or paper-disc method with *Escherichia coli* Ess or *Pseudomonas aeruginosa* Pss (with cephalosporin C as the standard). These β -lactam supersensitive-mutant strains are about equally sensitive to deacetoxycephalosporin C, deacetylcephalosporin C, cephalosporin C and penicillin N.

Methods

Melting points were taken on a Thomas-Hoover capillary melting-point apparatus or on a Kofler Micro Hot-Stage block and are uncorrected. Microanalyses were performed by Midwest Microlab Inc., Indianapolis, IN, U.S.A.

I.r. spectra were recorded on a Perkin-Elmer model 700 infrared spectrophotometer and were calibrated against polystyrene. N.m.r. spectra were obtained on a Varian Associates T-60 spectrometer, a Perkin-Elmer R-24B spectrometer or a Perkin-Elmer R-22 spectrometer. Chemical shifts are reported in parts per million relative to internal tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulphonate, with the notations giving the multiplicity of the signal. Mass spectra were determined on a Varian Associates MAT-44 spectrometer. High-resolution mass spectra were by courtesy of Dr. Catherine E. Castello and field-desorption spectra were by courtesy of Dr. R. T. Aplin. Optical rotations were measured with a Perkin-Elmer 141 polarimeter.

Silica gel for column chromatography was Merck silica gel 60 (no. 7734). Analytical t.l.c. was performed on Baker-flex silica gel IB-F or Merck pre-coated silica gel t.l.c. plates 60F-254. Preparative thin-layer separations were carried out on Merck silica gel GF 254 (type 60), no. 7730 on layers (200 mm $\times$ 200 mm $\times$ 1.25 mm). Solvents were purified and dried by using standard procedures.

N-p-Nitrobenzyloxycarbonyl-D-glutamic acid (4). D-Glutamic acid (10 g, 0.07 mol) was dissolved in 2 M-NaOH (75 ml, 0.14 mol) and dioxan (30 ml) was added. This was cooled to 0°C with vigorous stirring while a solution of *p*-nitrobenzyl chloroformate (22.5 g, 0.1 mol) in dioxan (115 ml) and a solution of 2 M-NaOH (75 ml) was added dropwise from two separate dropping funnels over 30 min. The resulting mixture was stirred at room temperature for 30 min. The precipitate was filtered and washed with water (225 ml). The filtrate and washing were combined and extracted with ethyl acetate (500 ml). The aqueous layer was acidified to pH 3 and extracted with ethyl acetate (300 ml). The combined ethyl acetate extract was washed with saturated NaCl, dried (MgSO₄) and concentrated *in vacuo* leaving a yellow oil, which was crystallized from ethyl acetate/light petroleum (b.p. 35–60°C) as yellow crystals (14.28 g, 64.4%); m.p. 154–155°C; δ (p.p.m.) ([²H]dimethyl sulphoxide) 1.65–2.4 (m, 4H); 4.10 (m, 1H), 5.25 (s, 2H), 6.3 (broad s, 2H), 7.63, 8.25 (AB_q, *J*_{AB} 10 Hz, 4H), 7.7 (s, 1H); mass spectrum (70 eV) *M*⁺ 326, [α]_D²⁰ +11.52 (in methanol).

α -*p*-Nitrobenzyl-*N-p*-nitrobenzyloxycarbonyl-D-glutamic acid (5). To a solution of *N-p*-nitrobenzyloxycarbonyl-D-glutamic acid (4; 3.26 g, 0.01 mol) in *NN*-dimethylformamide (25 ml) at 75–

85°C (inner temperature) was added dicyclohexylamine (2 ml, 0.01 mmol). This hot solution was stirred for 5 min and to it was added a solution of *p*-nitrobenzylbromide (2.16 g, 0.01 mmol) in *NN*-dimethylformamide (6 ml) in a single portion. Within seconds a precipitate separated, the mixture was cooled to room temperature and ethyl acetate was added. The precipitate was filtered and washed with ethyl acetate. The filtrate was washed with water, saturated NaCl, dried (MgSO₄) and concentrated *in vacuo* to leave a yellow solid, which was recrystallized from ethyl acetate/light petroleum (b.p. 35–60°C) as yellow clumps of needles (5; 2.14 g, 46.4%); m.p. 149–50°C; δ (p.p.m.) ([²H]dimethyl sulphoxide) 1.65–2.7 (m, 4H), 3.4 (broad s, 1H), 4.20 (m, 1H), 5.22 (s, 2H), 5.30 (s, 2H), 7.60, 8.20 (AB_q, *J*_{AB} 8 Hz, 8H), 7.93 (s, 1H); mass spectrum (70 eV) (*M*⁺ 461; [α]_D²⁰ –0.76 (in tetrahydrofuran).

p-Nitrobenzyl *S*-6-diazo-2-*p*-nitrobenzyloxycarbonylamino-5-oxohexanoate (6a). The acid (5, 5.43 g, 11.8 mmol) was dissolved in dry tetrahydrofuran (120 ml) and triethylamine (1.65 ml, 11.8 mmol) was added. The cloudy solution was cooled to –5°C and ethyl chloroformate (4.6 ml, 59 mmol, 5 equiv.) was added in one portion with vigorous stirring. After stirring for 30 min at room temperature, the mixture was filtered and washed with diethyl ether. The filtrate was evaporated to dryness to give an oil. This was dissolved in tetrahydrofuran (35 ml) and added to an ethereal solution of diazomethane (160 mmol, 13-fold excess). The resulting solution was kept at 0°C for 50 h and the excess diazomethane was distilled off, keeping the temperature of the distillation flask at 0°C. The residue was dissolved in diethyl ether, filtered, washed with diethyl ether and concentrated *in vacuo* to leave a yellow oil. The crude product was purified by column chromatography on silica with toluene/ethyl acetate (1:1) as eluent, when the diazoketone (6a) was obtained as a yellow oil (2.54 g, 44.4% yield); δ (p.p.m.) ([²H]chloroform) 2.00–2.75 (m, 4H), 4.4 (m, 1H), 5.20 (s, 2H), 5.30 (s, 2H), 5.33 (s, 1H), 6.03 (d, *J* 8 Hz, 1H), 7.55, 8.20 (AB_q, *J*_{AB} 8 Hz, 8H); ν (chloroform) 3400; 2150, 1720 cm^{–1}.

α -Amino- α -*p*-nitrobenzyl-*N*-*p*-nitrobenzyloxycarbonyl-D-adipate (7a). *S*-6-Diazo-*p*-nitrobenzyl-2-*p*-nitrobenzyloxycarbonylamino-5-oxohexanoate (6a; 2.6 g, 5.75 mmol) was dissolved in tetrahydrofuran (60 ml) and water (30 ml), triethylamine (0.8 ml) was added and the resulting solution was cooled to 0°C. To this cold stirred solution was added a solution of silver triflate (0.75 g, 2.9 mmol) in tetrahydrofuran (26 ml) dropwise over 15 min. Within 5 min, the reaction turned dark and a gas was evolved. The mixture was stirred at room temperature for 45 min. This was acidified with 10% HCl, filtered and ethyl acetate was added to the filtrate. The layers were

separated and the ethyl acetate extract was washed with water, saturated NaCl, dried (MgSO₄) and concentrated *in vacuo* to leave a colourless syrup (1.7 g, 70% yield); δ (p.p.m.) ([²H]chloroform) 1.65–2.00 (m, 4H), 2.36 (t, *J* 6 Hz, 2H), 4.43 (m, 1H), 5.20 (s, 2H), 5.26 (s, 2H), 5.63 (d, *J* 8 Hz, 1H), 7.47, 8.15 (AB_q, *J*_{AB} 8 Hz, 8H), 9.53 (broad s, 1H); ν (chloroform) 3800–3500, 1710, 1520 cm^{–1}; mass spectrum (70 eV) *M*⁺ 475.

In subsequent experiments, the crude diazoketone (6a; 5.2 g, 11.5 mmol) was rearranged with silver triflate as described above, the crude acid was flushed through a column (15 cm × 3 cm) of silica with ethyl acetate/dichloromethane (1:1, v/v) when a clean mixture of the acid (7a) and the side product (a lactam) was obtained (3.4 g). This was dissolved in ethyl acetate and purified as its dicyclohexylammonium salt (3.5 g) by stirring with a cation-exchange resin [Dowex 50W (X8); 7 ml dry volume] in acetone (27 ml) and water (24 ml) at room temperature for 1.5 h. The reaction mixture was filtered, washed with acetone, concentrated and extracted with ethyl acetate, dried (MgSO₄) and evaporated to dryness to give the acid [7a; 1.5 g, 26.8% from the diprotected glutamic acid (5)].

n-Butyl-D-4-*p*-nitrobenzyloxycarbonyl-4-*p*-nitrobenzyloxycarbonylamino penicillin benzyl ester (9a). A suspension of 6-aminopenicillanic acid benzyl ester tosylammonium salt in ethyl acetate was washed with aq. NaHCO₃, water, saturated NaCl, dried (MgSO₄) and concentrated *in vacuo* to give the corresponding free amine (8a; 220 mg, 0.72 mmol). This was dissolved in dichloromethane (6 ml) and α -amino- α -*p*-nitrobenzyl-*N*-*p*-nitrobenzyloxycarbonyl-D-adipate (7a; 331.9 mg, 0.72 mmol) and 2-ethoxy-*N*-ethoxycarbonyl-1,2-dihydroquinoline (178 mg, 0.72 mmol) were added. The resulting solution was stirred at room temperature for 15 h and concentrated *in vacuo*. The residue was dissolved in ethyl acetate, washed with 1 M-HCl, aq. NaHCO₃, water and saturated NaCl and then dried (MgSO₄) and concentrated *in vacuo*. The residue was purified by preparative t.l.c. on silica with ethyl acetate/dichloromethane (3:7) as eluent to give the triprotected penicillin N (9a, 300 mg, 55.7%) as a white foam; δ (p.p.m.) ([²H]chloroform) 1.40 (s, 3H), 1.60 (s, 3H), 1.60–2.00 (m, 4H), 2.2–2.4 (t, *J* 6 Hz, 2H), 4.45 (s, 1H), 4.25–4.60 (broad s, 1H), 5.20 (s, 4H), 5.3 (s, 2H), 5.50–5.80 (m, 2H), 6.35 (d, *J* 8 Hz, 1H), 7.35 (s, 5H), 7.50, 8.20 (AB_q, *J*_{AB} 8 Hz, 8H); ν (chloroform) 3400, 1780, 1730 (broad), 1680 cm^{–1}; field-desorption mass spectrum *M*⁺ 761.

Penicillin N monosodium salt (1a). The triprotected penicillin N (9a, 42 mg) was added to tetrahydrofuran (5 ml) and water (5 ml), and hydrogenated with 10% Pd–C (84 mg) in the presence of NaHCO₃ (6 mg, 1 equiv.) at room temperature for 2.5 h at 101 kPa (1 atm). The suspension was filtered

through filter-cell Celite while keeping the receiving flask cold and the residue was washed with ethanol (5 ml) and water (5 ml). The filtrate was freeze-dried to leave an off-white solid, which was washed with diethyl ether (5 ml $\times$ 5 ml) and dried to give penicillin N monosodium salt (1a, 16 mg, 78.4% yield); δ (p.p.m.) ($^2\text{H}_2\text{O}$; 2,2-dimethyl-2-silapentane-5-sulphonate as external standard) 1.50 (s, 3H), 1.60 (s, 3H), 1.70–2.00 (m, 4H), 2.33 (t, J 5 Hz, 2H), 3.36 (m, 1H), 4.20 (s, 1H), 5.4–5.56 (m, 2H). This was 30–40% pure by bioassay on agar plates seeded with *E. coli* Ess as the test organism (cephalosporin C as the reference standard).

In one experiment, crude penicillin N (105 mg) was purified on Sephadex G-25 (fine) at 4°C. Sephadex G-25 (10 g) was added slowly to water (200 ml) with constant stirring. The slurry was kept in a boiling-water bath for 2 h and cooled to room temperature over a 1 h period. The supernatant and the fines were decanted. The swollen Sephadex was mixed with 4–5 times its volume of 70% (v/v) propan-2-ol, stirred, allowed to stand and the supernatant and fines were decanted. This was repeated twice, the slurry was made up to a suitable consistency, the column (30 cm $\times$ 1.5 cm) was packed and allowed to equilibrate overnight. The compound was dissolved in 70% (v/v) propan-2-ol and applied to the column; 1 ml fractions/20 min were collected and bioassayed by using *E. coli* Ess. The fractions having antibiotic activity were pooled, extracted with diethyl ether and the aqueous layer was freeze-dried to give penicillin N as an off-white solid (20.6 mg, 80% pure by bioassay).

S-6-Diazo-p-nitrobenzyl-2-p-nitrobenzyloxycarbonylamino-5-oxo[6- ^{14}C]hexanoate (6b). The acid (5; 68 mg, 0.15 mmol) was dissolved in tetrahydrofuran (1.5 ml) and triethylamine (20 μl , 0.15 mmol). The cloudy solution was cooled to -5°C and ethyl chloroformate (60 μl , 0.75 mmol) was added. The resulting suspension was stirred for 30 min while warming up to room temperature. It was filtered, washed with diethyl ether and concentrated *in vacuo* to give a colourless syrup. This dissolved in tetrahydrofuran (3 ml), added to an ethereal solution of [^{14}C]diazomethane (prepared as described below) and kept at 0°C for 40 h. The reaction mixture was filtered through Celite, washed with diethyl ether and concentrated *in vacuo*. The residue was purified by preparative t.l.c. on silica with benzene/ethyl acetate (2:1, v/v) as eluent, when the diazoketone (6b; 20 mg, 28% yield) was obtained as a colourless oil (n.m.r. and i.r. spectra were identical with those for the cold diazoketone (6a)).

Preparation of [^{14}C]diazomethane. *N*-[^{14}C]-Methyl-*N*-nitroso-*p*-toluenesulphonamide (11.3 mg, 500 μCi) was dissolved in diethyl ether (1 ml) and mixed with a solution of *N*-methyl-*N*-nitroso-*p*-toluenesulphonamide (214 mg) in diethyl ether

(2 ml). The mixed solution was added slowly to 2-(2-ethoxyethoxy)ethanol (0.7 ml) and 10.7 M-KOH solution (0.2 ml), heated to 65°C and the diazomethane was distilled off. Diethyl ether (6 ml) was added and the remaining diazomethane was distilled off.

*6 β -(3,5-Di-*t*-butyl-4-hydroxy)benzylideneamino-penicillin 3 α -benzyl ester (10).* To a solution of 6-aminopenicillanic acid benzyl ester (8a; 1.19 g, 3.9 mmol) in dry dichloromethane (40 ml) were added, 3,5-di-*t*-butyl-4-hydroxybenzaldehyde (0.9 g, 3.9 mmol), absolute ethanol (40 ml) and anhydrous Na_2SO_4 (6 g). The reaction mixture was refluxed for 4 h, cooled to room temperature, filtered and concentrated *in vacuo* leaving a yellow foam (10; 2.02 g, 99.6%), δ (p.p.m.) ($[\text{H}]$ chloroform) 1.43 (s, 21H), 1.6 (s, 3H), 4.33 (s, 1H), 5.1 (s, 2H), 5.26 (d of d, J 1.5, 3 Hz, 1H), 5.46 (d, J 3 Hz, 1H), 5.56 (s, 1H), 7.23 (s, 5H), 7.46 (s, 2H), 8.36 (d, J 1.5 Hz, 1H); ν (chloroform) 3570, 1770, 1740, 1630 cm^{-1} .

Quinonoidal compound (11). The Schiff's base (10; 60 mg, 0.11 mmol) was stirred with freshly prepared PbO_2 (50 mg, 0.2 mmol) in dry benzene (3 ml) at room temperature for 1 h. The reaction mixture was filtered through Celite and the solvent was removed under reduced pressure leaving an orange-red foam (60 mg, quantitative yield), δ (p.p.m.) ($[\text{H}]$ chloroform) 1.3 (s, 18H), 1.46 (s, 3H), 1.53 (s, 3H), 4.63 (s, 1H), 5.13 (s, 2H), 5.8 (s, 1H), 6.86 (d, J 2 Hz, 1H), 7.23 (s), 7.26 (s, 7H), 7.76 (d, J 2 Hz, 1H); ν (chloroform) 1770, 1740, 1640, 1610 cm^{-1} .

*6 β -(3,5-Di-*t*-butyl-4-hydroxy)benzylaminopenicillin benzyl ester (12).* The quinonoidal compound (11; 120 mg, 0.23 mmol) was stirred with $\text{Na}(\text{CN})\text{BH}_3$ (19 mg, 0.3 mmol) in benzene (pH 3, acetic acid/water) at room temperature for 30 min. The reaction was transferred to a separating funnel and the layers were separated. The organic layer was washed with water, saturated NaCl, dried (MgSO_4) and concentrated *in vacuo* giving a yellow foam (12; 97 mg, 88.8% yield). δ (p.p.m.) ($[\text{H}]$ chloroform) 1.43 (s, 24H), 1.63 (s, 3H), 3.73 (d, J 4 Hz, 1H), 4.33 (s, 1H), 4.4 (d, J 4 Hz, 2H), 5.1 (broad s, 1H), 5.26 (d, J 4 Hz, 4H), 7.0 (s, 2H), 7.23 (s, 5H); ν (chloroform) 3580, 1770, 1740, 1670 cm^{-1} ; electron-impact mass spectrum M^+ 524.

*6 β -(3,5-Di-*t*-butyl-4-hydroxy)benzylideneamino-[6- ^2H]penicillin 3 α -benzyl ester (13a).* The quinonoidal compound (11; 110 mg, 0.21 mmol) was dissolved in dry tetrahydrofuran (8 ml) and stirred with NaB^2H_4 (3.4 mg, 0.08 mmol) at room temperature for 5 min. Benzene was added and the reaction mixture was washed with water (2 $\times$ 4 ml), saturated NaCl (1 $\times$ 4 ml), dried (MgSO_4) and concentrated *in vitro* giving a yellow foam (105 mg, quantitative yield). δ (p.p.m.) ($[\text{H}]$ chloroform) 1.46 (s, 3H), 1.5 (s, 21H), 4.4 (s, 1H), 5.2 (s, 2H), 5.53 (s,

1H), 5.6 (s, 1H), 7.36 (s, 5H), 7.6 (s, 2H), 8.5 (s, 1H); ν (chloroform) 3600, 1770, 1740, 1630 cm^{-1} ; mass spectrum (high resolution), calc. for $\text{C}_{30}\text{H}_{37}\text{N}_2\text{O}_4^2\text{HS}$, 523.26151; found, 523.26199.

6 β -Amino-[6 α - ^3H]penicillanic acid benzyl ester tosylammonium salt (14a). The Schiff's base (13a; 110 mg, 0.21 mmol) was stirred with *p*-toluenesulphonic acid monohydrate (55.1 mg, 0.29 mmol) in ethyl acetate (3 ml) and water (0.056 ml, 3.1 mmol) at room temperature, when a white precipitate separated. The reaction mixture was stirred for 3 h, the precipitate was filtered, washed with ice-cold ethyl acetate and dried (75 mg, 75%); m.p. 141–142°C; ν (Nujol) 1780, 1730 cm^{-1} .

[6 α - ^3H]Penicillin N monosodium salt (1c). The Schiff's base (10; 135 mg, 0.25 mmol) was oxidized with PbO_2 to the quinonoidal compound as described before and the solvent was removed under reduced pressure. The residue was dissolved in tetrahydrofuran (10 ml) and stirred with NaB^3H_4 (5 mg, 0.10 mmol, 25 mCi) for 10 min. The reaction mixture was transferred to a separating funnel with benzene and washed with water (1 $\times$ 5 ml), saturated NaCl (1 $\times$ 5 ml), dried (MgSO_4) and concentrated *in vacuo* to give a yellow foam (13b; 108 mg, quantitative yield). N.m.r. and i.r. spectra were identical with those for the 6-protio compound (10).

The (6 α - ^3H)-labelled Schiff's base (13b; 108 mg, 0.206 mmol) was stirred with *p*-toluenesulphonic acid monohydrate (55 mg, 0.29 mmol) in ethyl acetate (3 ml) in the presence of water (0.05 ml) at room temperature for 3 h when a white precipitate separated. The precipitate was filtered, washed with cold ethyl acetate and dried. This was suspended in ethyl acetate (5 ml), washed with aq. NaHCO_3 , water, saturated NaCl, dried (MgSO_4) and concentrated *in vacuo* to give a yellow oil (45 mg) of the free amine corresponding to compound (14b).

The 6-amino[6 α - ^3H]penicillanic acid benzyl ester (45 mg, 0.146 mmol) was dissolved in dichloromethane (3 ml) and a solution of α -amino- α -*p*-nitrobenzyl-*N*-*p*-nitrobenzyloxycarbonyl-D-adipate (7a; 70 mg, 0.146 mmol) in dichloromethane (4 ml)

was added, followed by 2-ethoxy-*N*-ethoxycarbonyl-1,2-dihydroquinoline (36 mg, 0.146 mmol). The reaction mixture was stirred at room temperature for 15 h. The solvent was removed under reduced pressure, the residue was dissolved in ethyl acetate (5 ml) and washed with aq. NaHCO_3 (1 $\times$ 2 ml), 10% HCl (1 $\times$ 2 ml), water (1 $\times$ 2 ml), saturated NaCl (1 $\times$ 2 ml), dried (MgSO_4) and concentrated *in vacuo*. The crude product was purified by preparative t.l.c. on silica with dichloromethane/ethyl acetate (7:3, v/v) as eluent, when the triprotected [6 α - ^3H]penicillin N was obtained as a white foam (45 mg, 40% yield).

Triprotected [6 α - ^3H]penicillin N (9c; 45 mg) was hydrogenated with 10% Pd-C (90 mg) in tetrahydrofuran (5 ml) and water (5 ml) in the presence of NaHCO_3 (1 equiv.) at room temperature for 2.5 h at 101 kPa (1 atm). The reaction mixture was filtered through Celite as described previously and the filtrate was freeze-dried to leave an off-white solid (1c; 16 mg, 75.5% yield, sp. activity 20.67 $\mu\text{Ci}/\mu\text{mol}$).

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