Pergamon

Tetrahedron, Vol. 52, No. 16, pp. 5983-5988, 1996

0040-4020/96 $15.00 + 0.00

S0040-4020(96)00228-1

A *N*-Nitrosochloroethyl-cephalosporin Carbamate Prodrug for Antibody-Directed Enzyme Prodrug Therapy (ADEPT)

Rikki P. Alexander[1], Robert W. Bates[2], Andrew J. Pratt[2a]* and (in part) James A.E. Kraunsoe[2]

[1]Celltech Research, 216 Bath Road, Slough, SL1 4EN, Berkshire, UK

[2]The Dyson Perrins Laboratory, South Parks Road, Oxford, OX1 3QY, UK

[a] Current address: Department of Chemistry, University of Canterbury, Christchurch, NZ.

Abstract: *N*-Nitrosochloroethyl-cephem **7** was prepared via acylation and selective nitrosation of a cephalothin derivative. This cephalosporin is an efficient substrate for *Enterobacter cloacae* P99 β-lactamase. Kinetic parameters were determined for enzyme-catalysed hydrolysis. This cephalosporin is a potential prodrug for the delivery of bis-alkylating chloroethyldiazo species to tumours by antibody-β-lactamase conjugates. Copyright © 1996 Elsevier Science Ltd

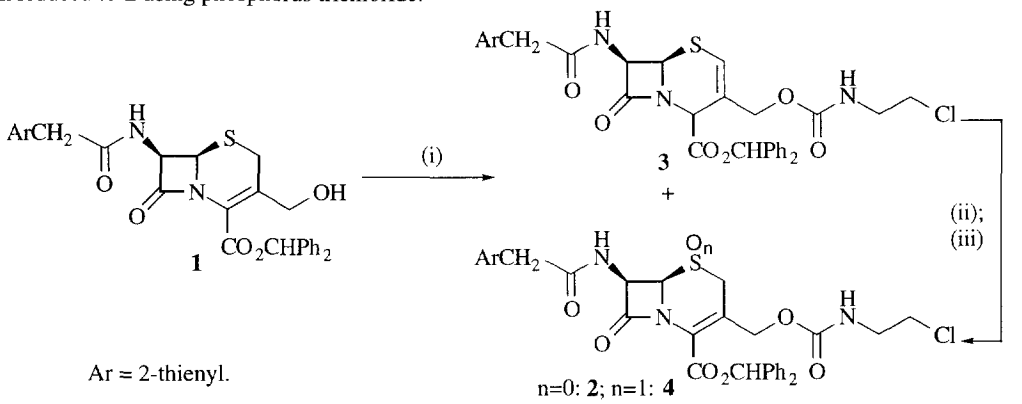
INTRODUCTION

Enzymes, covalently attached to tumour selective antibodies, can be used to catalyse the conversion of biologically inactive prodrugs to active antitumour agents selectively at tumour cells[1]. The release of toxic agents local to tumours by Antibody-Directed Enzyme Prodrug Therapy (ADEPT) may result in diminished toxic side effects relative to conventional chemotherapeutic approaches. Because of the therapeutic promise of this methodology,[2] new ADEPT strategies and prodrugs are being pursued vigorously.[3] β-Lactamase is an attractive enzyme for such a drug delivery system[4] since hydrolysis of appropriately substituted cephalosporins can result in expulsion of leaving groups from the 3-methyl position, remote from the immediate recognition site[5]. The low substrate specificity of β-lactamases with respect to the 3-methyl substituent[6] allows enzyme-catalysed release of a variety of toxic agents[7]. Cephalosporins appropriately functionalised at the the 3-methyl position have been synthesised and evaluated as prodrugs for the delivery of a range of toxic antitumour agents[8]. *N*-Nitrosochloroethyl ureas are known potent cytotoxic agents which are believed to act via conversion to chloroethyldiazotate and/or related bis-alkylating agents[9]. We set out to prepare cephalosporin *N*-nitrosochloroethylcarbamate derivative **7** which is capable of releasing this toxic agent on β-lactamase catalysed hydrolysis. Our synthetic strategy relied upon acylation of a suitably protected cephem alcohol **1** and selective nitrosation of the carbamate function thereby introduced.

RESULTS AND DISCUSSION

Diphenylmethyl cephem alcohol **1**[10] was acylated by 2-chloroethyl isocyanate in pyridine. Under these conditions a mixture of the Δ3 and Δ2 cephem isomers **2** and **3** was formed. The desired isomer **2** predominated and, whilst it proved impossible to separate it cleanly from the minor isomer **3** by chromatography, we succeeded in obtaining pure samples of **2** by fractional crystallisation. In order to increase the efficiency of this

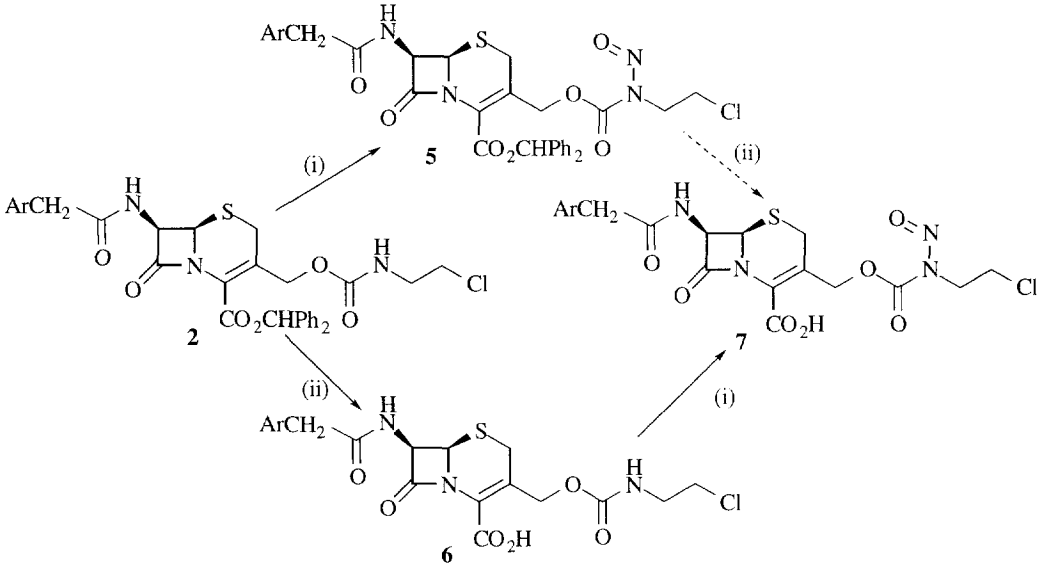
route to **2** we isomerised the side product **3,** to **2,** by the approach developed at Eli Lilly[11]. Impure samples of **3** were oxidised using *m*-CPBA. The resulting sulfoxide[12] isomerises *in situ* to the Δ3 isomer **4**. Sulfoxide **4** was then reduced to **2** using phosphorus trichloride.



Ar = 2-thienyl.

Reagents: (i) $ClCH_2CH_2NCO$, C_5H_5N; (ii) *m*-CPBA, CH_2Cl_2 (**3** ->**4**); (iii) PCl_3, CH_2Cl_2 (**4** ->**2**).

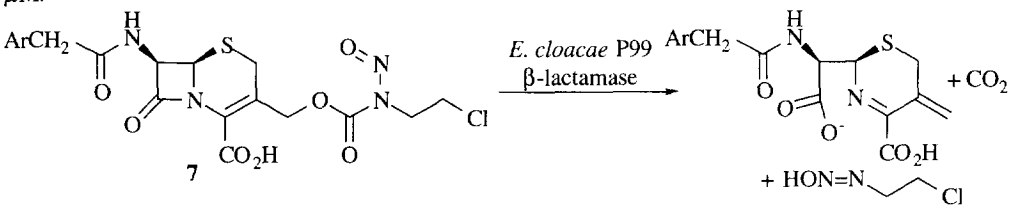
The selective nitrosation of the carbamate nitrogen of **2** was essential to our synthetic strategy. It is known that the side chain amide functionality of penicillins and cephalosporins can be nitrosated using dinitrogen tetroxide at between -5 and 0°C[13]. We elected to undertake nitrosations with the same reagent but at lower temperatures. By carrying out the reaction at -23°C we observed clean mononitrosation of carbamate **2** to give **5**. The site of nitrosation was identified primarily on the basis of NMR shift data. This assignment was supported by the fact that the diphenylmethyl ester of cephalothin, similar to **2**, but lacking the carbamate functionality, failed to undergo nitrosation under these conditions.



Reagents: (i) N_2O_4, NaOAc, CH_2Cl_2; (ii) CF_3CO_2H, CH_2Cl_2, PhOMe or Me_3SiI, CH_2Cl_2.

We attempted deprotection of **5** with trifluoroacetic acid and with trimethylsilyl iodide. In both cases decomposition preceded deprotection. In view of the difficulties in deprotection of nitrosocarbamate **5** and the ease of the nitrosation of its precursor **2**, we decided to change to order of steps in the synthesis. Carbamate **2**

was converted to free acid **6** on treatment with either trifluoroacetic acid or trimethylsilyl iodide. Low temperature nitrosation of this acid proceeded smoothly to generate the target free nitrosocarbamate **7**.
Nitrosocarbamate **7** was found to be an efficient substrate for the *Enterobacter cloacae* P99 β-lactamase and Michaelis-Menton analysis of the rate of enzyme-catalysed hydrolysis of **7** gave a Michaelis constant of 427μM.



Nitrosocarbamate **7** is therefore a candidate prodrug for the release of chloroethyldiazotate to tumour cells utilising β-lactamase dependant ADEPT.

ACKNOWLEDGEMENTS

We gratefully acknowledge financial support from Celltech and constructive discussions with several scientists there.

EXPERIMENTAL

Melting points were determined on a Gallenkamp hot stage apparatus, and are uncorrected. I.r. spectra were recorded on a Perkin-Elmer 1750 fourier transform instrument. Microanalysis was performed in the Dyson Perrins Laboratory by Mrs. V. Lamburn. ^{1}H n.m.r. (at 200MHz) and ^{13}C n.m.r. (at 50.3 MHz) spectra were recorded on a Gemini 200. Mass spectra were recorded on a V.G. Micromass ZAB IF (FAB$^+$, DCI$^+$), and V.G. Trio 1 (GCMS-CI$^+$, EI$^+$) by Dr R. Aplin and R. Proctor. Optical rotations are quoted in units of 10^{-1} deg cm^{-2} g^{-1}. Evaporation of organic solvents was carried out under reduced pressure. Organic solutions resulting from conventional aqueous work up procedures were dried with $MgSO_4$ and filtered prior to evaporation. Solutions of N_2O_4 were prepared by bubbling NO_2(g) through dry CH_2Cl_2.

Diphenylmethyl 3-(2-chloroethylcarbamoyl)methyl-7β-(2-thienylacetamido)-3-cephem-4-carboxylate **2** *and Diphenylmethyl 3-(2-chloroethylcarbamoyl)methyl-7β-(2-thienylacetamido)-2-cephem-4-carboxylate* **3**

2-Chloroethyl isocyanate (1.13 ml, 13.2 mmol) was added to a stirred solution of cephem **1** (2.25 g, 4.36 mmol) in dry pyridine (5 ml) at -23 °C. After 0.5 h the mixture was warmed to room temperature, stirred for a further 4 h and evaporated. The residue was taken up in $CHCl_3$ (100 ml), washed with 1 M HCl, saturated aq. $NaHCO_3$ and water, dried and evaporated. Flash silica chromatography (85 % CH_2Cl_2/15 % EtOAc), gave a 1:2 mixture of *carbamates* **2** and **3** (2.25 g, 3.60 mmol, 83 %, R_f 0.5). Selective crystallisation from EtOAc gave pure *carbamate* **2** (1.5 g, 2.40 mmol, 55 %) as a white solid. M.p. 194-195 °C; $[\alpha]^{20}_D$ +27.57° (c 1.03, DMF); ν_{max} ($CHCl_3$)/cm^{-1} 1791(s) (β-lactam), 1728(s) (ester), 1687(s) (amide), 1589(m) cm^{-1}; δ_H (d_6-DMSO) 3.28-3.56 (6H, m, C-2H_2 and CH_2CH_2Cl), 3.74 (2H, s, C-7CH_2), 4.66 and 4.85 (2H, ABq, J 13.4 Hz, C-10H_2), 5.08 (1H, d, J 4.8 Hz, C-6*H*), 5.73 (1H, dd, J 4.8 and 8.2 Hz, C-7*H*), 6.87-6.91 (3H, m, Ph$_2$C*H* and thiophene H_2), 7.22-7.47 (12H, m, thiophene *H*, (C_6H_5)$_2$ and C-10N*H*), 9.15 (1H, d, J 8.2 Hz, C-7N*H*); δ_C (d_6-DMSO) 26.14 (t, *C*-2), 36.23 (t, *C*-7), 42.80 (t, *C*H$_2$Cl), 43.42 (t, *C*H$_2$N), 58.07 (d, *C*-6H), 59.57 (d, *C*-7H), 62.96 (t, *C*-10H$_2$), 79.40 (d, *C*-9), 125.27 (s), 126.83 (s), 127.09 (d), 127.23 (d), 127.53 (d), 128.40 (d), 128.89 (d), 129.02 (d), 137.31 (s), 140.20 (s), 156.54 (s, *C*=O), 161.19 (s, *C*=O), 165.71 (s, *C*=O), 170.80 (s,

C=O); *m/z* (FAB$^+$) 648 (MNa$^+$, 10 %), 503 (M$^+$-$CO_2NHCH_2CH_2Cl$, 12 %), 458 (M$^+$-Ph_2CH, 5 %), 167 (Ph_2CH^+,100 %); (Found C 57.54 %, H 4.51 %, N 6.71 %; $C_{30}H_{28}ClN_3O_6S_2$ requires C 57.60 %, H 4.24 %, N 6.71 %). *Carbamate* **3**: δ_H ($CDCl_3$) 3.48-3.72 (4H, m, CH_2CH_2Cl), 3.86 (2H, s, C-7CH_2), 4.85-5.02 (2H, m, C-10H_2), 5.19 (1H, s, C-2*H*), 5.23 (1H, d, J 4.8 Hz, C-6*H*), 5.65 (1H, dd, J 4.8 and 9.6 Hz, C-7*H*), 6.28 (1H, s, C-4*H*), 6.55 (1H, d, J 9.6 Hz, C-7N*H*), 6.91 (1H, s, Ph_2CH), 6.92-7.08 (2H, m, thiophene *H*), 7.25-7.49 (12H, m, thiophene *H*, $(C_6H_5)_2$ and C-10N*H*).

Diphenylmethyl 3-(2-chloroethylcarbamoyl)methyl-7β-(2-thienylacetamido)-3-cephem-4-carboxylate 1-sulfoxide **4**

2-Propanol (4 ml) was added to a solution of carbamates **2** and **3** (0.75 g, 1.20 mmol) in $CHCl_3$ (20 ml). The mixture was cooled to 0 °C and *m*-CPBA (50-60 %; 0.37 g, 1.17 mmol) in $CHCl_3$ (25 ml) was added over a period of 2 h. The reaction mixture was washed with saturated aq. $NaHCO_3$ and saturated aq. NaCl, dried and evaporated. Flash silica chromatography (50 % EtOAc/50 % CH_2Cl_2), gave *sulfoxide* **4** (0.59 g, 0.92 mmol, 77 %, R_f 0.25) as a white solid. M.p. 180-182 °C (dec.); $[\alpha]^{20}_D$ +34.09 ° (c 0.18, DMF); ν_{max} ($CHCl_3$)/cm^{-1} 1806(s), 1731(s), 1688(s), 1589(w); δ_H (d_6-DMSO) 3.22-3.60 (6H, m, CH_2CH_2Cl and C-2H_2), 3.84 (2H, s, C-7H_2), 4.58 and 5.03 (2H, ABq, J 13.5 Hz, C-10H_2), 4.96 (1H, d, J 4.2 Hz, C-6*H*), 5.93 (1H, dd, 4.2 and 8.5 Hz, C-7*H*), 6.94-6.98 (3H, m, thiophene *H* and Ph_2CH), 7.23-7.61 [11H, m, $(C_6H_5)_2C$ and thiophene *H*], 8.49 (1H, d, J 8.5 Hz, C-7N*H*); δ_C (d_6-DMSO) 36.05 (t, *C*-7), 42.81 (t, *C*-Cl), 43.74 (t, CH_2NH), 45.70 (t, *C*-2), 58.40 (d), 63.34 (t), 66.93 (d), 79.60 (d, *C*-9), 123.30 (s), 124.66 (s), 125.78 (d), 127.23 (d), 127.46 (d), 128.54 (d), 129.05 (d), 129.20 (d), 137.52 (s), 140.48 (s), 156.50 (s, *C*=O), 160.57 (s, *C*=O), 165.31 (s, *C*=O), 170.94 (s, *C*=O); *m/z* (FAB$^+$) 664 (MNa$^+$, 16 %), 519 (M$^+$-$CO_2NHCH_2CH_2Cl$, 20 %), 167 (Ph_2CH^+, 100 %).

Diphenylmethyl 3-(2-chloroethylcarbamoyl)methyl-7β-(2-thienylacetamido)-3-cephem-4-carboxylate **2**

A solution of sulfoxide **4** (0.59 g, 0.92 mmol) in CH_2Cl_2 (25 ml) was added to a refluxing solution of PCl_3 (1.34 g, 9.90 mmol) in CH_2Cl_2 (10 ml). After 4 h at reflux the solution was allowed to cool then neutralised with aq. $NaHCO_3$, washed with water, dried and evaporated. Flash silica chromatography (20 % EtOAc/80 % CH_2Cl_2), gave *carbamate* **2** (0.28 g, 0.45 mmol, 49 %) identical to a previously prepared sample.

Diphenylmethyl 3-(2-chloroethyl-N-nitrosocarbamoyl)methyl-7β-(2-thienylacetamido)-3-cephem-4-carboxylate **5**

A solution of N_2O_4 in CH_2Cl_2 (1.8 ml, 12.6 mmol) was added to a slurry of sodium acetate (340 mg, 4.15 mmol) in CH_2Cl_2 (2 ml) at -78 °C under nitrogen. A solution of carbamate **2** (260 mg, 0.42 mmol) in CH_2Cl_2 (30 ml) was added dropwise over a few minutes. The mixture was stirred at -78 °C for 15 min, warmed to -23 °C and after a further 15 min the reaction was quenched by the addition of ice cold water (30 ml). The organic layer was separated, washed with aq. $NaHCO_3$ (30 ml) and water (30 ml), dried and evaporated. Flash silica chromatography (40 % EtOAc/60 % pet. ether), gave *nitrosocarbamate* **5** (239 mg, 0.37 mmol, 87 %, R_f 0.7) as a colourless oil. ν_{max} ($CHCl_3$)/cm^{-1} 1806(s), 1751(s), 1731(s), 1688(s), 1510(s) (N-NO); δ_H ($CDCl_3$) 3.44 and 3.63 (2H, ABq, J 18.6 Hz, C-2H_2), 3.45 (2H, t, J 6.5 Hz, CH_2Cl), 3.86 (2H,s, C-7CH_2), 4.05 (2H, t, J 6.5 Hz, CH_2NNO), 4.99 (1H, d, J 5 Hz, C-6*H*), 5.2 and 5.52 (2H, ABq, J 13.6 Hz, C-10H_2), 5.91 (1H, dd, J 5 and 9.1 Hz, C-7*H*), 6.56 (1H, d, J 9.1 Hz, C-7N*H*), 6.95- 7.03 (3H, m, thiophene H_2 and C-9C*H*), 7.26-7.48 (11H, m, thiophene *H* and $(C_6H_5)_2C$); δ_C ($CDCl_3$) 26.30 (t, *C*-2), 36.88 (t, *C*-7), 38.93 (t, *C*-Cl), 41.38 (t, CH_2N), 57.42 (d, *C*-6), 59.16 (d, *C*-7), 66.78 (t, *C*-10), 80.18 (d, *C*-9), 126.00 (d), 126.34 (s), 127.21 (d), 127.87 (d), 128.49 (d), 128.72 (d), 128.86 (d), 135.19 (s), 139.22 (s), 139.33 (s), 153.61 (s, *C*=O), 160.95 (s, *C*=O), 165.43

(s, *C*=O), 170.82 (s, *C*=O); *m/z* (FAB$^+$) 677 (MNa$^+$, 7 %), 503 (M$^+$-$CO_2N(NO)CH_2CH_2Cl$, 15 %), 488 (M$^+$-Ph_2CH, 7 %), 167 (Ph_2CH^+, 100 %).

Deprotection of Diphenylmethyl 3-(2-chloroethyl-carbamoyl)methyl-7β-(2-thienylacetamido)-3-cephem-4-carboxylate **2** *with trifluoroacetic acid*

Distilled anisole (2.5 ml) and freshly distilled CF_3CO_2H (2.5 ml) were added to a suspension of carbamate **2** (476 mg, 0.76 mmol) in dry CH_2Cl_2 (20 ml) at -23 °C. After 15 min the reaction was warmed to 0 °C and, after a further 15 min, the solvent was evaporated and traces of CF_3CO_2H and anisole were removed by trituration with isopropyl ether and benzene respectively. The residue was taken up in $CHCl_3$ (10 ml) and *3-(2-chloroethylcarbamoyl)methyl-7β-(2-thienylacetamido)-3-cephem-4-carboxylic acid* **6** (315 mg, 0.68 mmol, 90 %) was precipitated with pet. ether, as a white solid, filtered and dried *in vacuo*. M.p. 150-152 °C; $[\alpha]^{25}_D$ +81.73 ° (c 0.32, DMF); ν_{max} ($CHCl_3$) 2928(m), 1793(m), 1733(s), 1602(s) cm^{-1}; δ_H (d_6-DMSO) 3.17-3.66 (6H, m, CH_2CH_2Cl and C-2H_2), 3.74 (2H, s, C-7CH_2), 4.64 and 4.95 (2H, ABq, J 12.7 Hz, C-10H_2), 5.08 (1H, d, J 4.8 Hz, C-6*H*), 5.66 (1H, dd, J 4.8 and 8.2 Hz, C-7*H*), 6.91-6.96 (2H, m, thiophene *H*), 7.35 (1H, m, thiophene *H*), 7.54 (1H, t, J 5.3 Hz, C-10N*H*), 9.12 (1H, d, J 8.2 Hz, C-7N*H*); δ_C (d_6-DMSO) 25.83 (t, *C*-2), 36.14 (t, *C*-7), 42.77 (t, *C*-Cl), 43.83 (t, *C*-N), 57.80 (d, *C*-6), 59.51 (d, *C*-7), 63.25 (t, *C*-10), 124.97 (s), 125.66 (d, thiophene *C*-H), 126.75 (s), 126.97 (d, thiophene *C*-H), 127.27 (d, thiophene *C*-H), 137.53 (s, thiophene *C*-2), 156.71 (s, *C*=O), 163.63 (s, *C*=O), 165.42 (s, *C*=O), 170.79 (s, *C*=O); *m/z* (FAB$^+$) 482 (MNa$^+$, 38 %), 337 (M$^+$-$CO_2NHCH_2CH_2Cl$, 48 %), 97 (100 %); (Found C 44.53 %, H 3.97 %, N 9.06 %; $C_{17}H_{18}ClN_3O_6S_2$ requires C 44.39 %, H 3.94 %, N 9.14 %).

Deprotection of Diphenylmethyl 3-(2-chloroethyl-carbamoyl)methyl-7β-(2-thienylacetamido)-3-cephem-4-carboxylate **2** *with trimethylsilyl iodide*

Trimethylsilyl iodide (5.2 µl, 0.04 mmol) was added dropwise to a solution of carbamate **2** (22.7 mg, 0.04 mmol) in dry CH_2Cl_2 (5 ml) at -23 °C. After 30 min the reaction was warmed to 0 °C in an ice bath and a further portion of trimethylsilyl iodide (5.2 µl. 0.04 mmol) was added. After a further 15 min the solvent was evaporated, the residue was taken up in $CHCl_3$ (10 ml) and the *acid* **6** (15 mg, 0.03 mmol, 80 %), identical to a previously prepared sample, was precipitated with pet. ether, filtered and dried *in vacuo*.

3-(2-Chloroethyl-N-nitrosocarbamoyl)methyl-7β-(2-thienylacetamido)-3-cephem-4-carboxylic acid **7**

A solution of N_2O_4 (0.39 g, 4.2 mmol) in CH_2Cl_2 (5 ml) was added to a slurry of anhydrous sodium acetate (150 mg, 1.83 mmol) in dry CH_2Cl_2 (5 ml) at -78 °C to give a pale blue solution. Free acid carbamate **6** (82 mg, 0.18 mmol) was suspended in dry CH_2Cl_2 (5 ml) and taken into solution by the addition of CF_3CO_2H (0.1 ml). This solution was cooled to 0 °C and added to the N_2O_4 solution dropwise over a period of a few minutes. After 15 min the reaction was warmed to -23 °C and stirred for a further 20 min. The solvent was evaporated; the residue was triturated with isopropyl ether, taken up in $CHCl_3$ (30 ml), washed with brine, dried and evaporated. Purification by reverse phase HPLC (linear gradient of 80 % H_2O (0.1 % CF_3CO_2H)/20 % CH_3CN (0.1 % CF_3CO_2H) to 5 % H_2O (0.1 % CF_3CO_2H)/95 % CH_3CN (0.1 % CF_3CO_2H) over 30 min) gave *nitroso acid* **7** (60 mg, 0.13 mmol, 73 %, retention time of 21.8 min) as a colourless oil. ν_{max} ($CHCl_3$)/cm^{-1} 1793(s), 1734(s), 1688(s), 1621(m), 1508(s) (N-NO); δ_H ($CDCl_3$) 3.49-3.76 (4H, m, CH_2Cl and C-2H_2), 3.79 (2H, s, C-7CH_2), 4.07 (2H, t, J 6.4 Hz, CH_2NNO), 5.07 (1H, d, J 4.9 Hz, C-6*H*), 5.17 and 5.47 (2H, ABq, J 12.9 Hz, C-10H_2), 5.79 (1H, dd, J 4.9 and 8.6 Hz, C-7*H*), 6.96-7.00 (2H, m, thiophene *H*), 7.28-7.31 (1H, m, thiophene *H*), 7.38 (1H, d, J 8.6 Hz, C-7N*H*); δ_C ($CDCl_3$) 25.95 (t, *C*-2), 36.75 (t, *C*-7),

39.03 (t, CH_2Cl), 41.80 (t, *C*-N), 57.23 (d, *C*-6), 59.10 (d, *C*-7), 67.04 (t, *C*-10), 125.02 (s), 125.58 (d, thiophene *C*-5), 126.24 (d, thiophene *C*-3H), 126.56 (s), 127.15 (d, thiophene *C*-4H), 139.00 (s, thiophene *C*-2), 153.85 (s, *C*=O), 163.05 (s, *C*=O), 165.14 (s, *C*=O), 170.81 (s, *C*=O); *m/z* (FAB$^+$) 511 (MNa^+, 35 %), 337 [M^+-$CO_2N(NO)CH_2CH_2Cl$].

Evaluation of K_m for hydrolysis of nitrosocarbamate **7** *by* Enterobacter cloacae *P99 β-Lactamase*

The rate of hydrolysis of nitrosocarbamate **7** was measured by following the absorbance change at 265 nm in 0.1 M phosphate buffer, pH 7.0 at 37 °C. The change in extinction coefficient at 265nm was assumed to be equal to that of cephalothin i.e. 6.5 x 10^{-3} m M cm^{-1}. Lineweaver-Burk analysis gave a value for the Michaelis constant, K_m, of 427 μM.

REFERENCES AND NOTES

1 Bagshawe, K.D. *Br. J. Cancer*, **1987**, *56*, 531. Recent reviews include: Bagshawe, K.D.;. Sharma, S.K; Springer, C.J.; Rogers, G.T. *Annals of Oncology*, **1994**, *5*, 879; Jungheim, L.N.; Shepherd, T.A. *Chem. Rev.*, **1994**, *94*, 1553.

2 See *e.g.* Kerr, D.E.; Schreiber, G.J.; Vrudhula, V.M.; Svensson, H.P.; Hellstrom, I.; Hellstrom, K.E.; Senter, P.D. *Cancer Res.*, **1995**, *55*, 3558.

3 Recent studies include: Hay, M.P.; Wilson, W.R.; Denny, W.A. *Bioorg. Med. Chem. Lett.*, **1995**, *5*, 2829; Leenders, R.G.G.; Gerrits, K.A.A.; Ruijtenbeek, R.; Scheeren, H.W.; Haisma, H.J.; Boven, E. *Tetrahedron Lett.*, **1995**, *36*, 1701.

4 Eaton, M.A.W.; Pratt, A.J.; Alexander, R.P. Eur. Pat. 90303681.2. (1990); Shepherd, T.A.; Jungheim, L.N.; Meyer, D.L.; Starling, J.J. *Bioorg. Med. Chem. Lett.*, **1991**, *1*, 21; Meyer, D.L.; Jungheim, L.N.; Law, K.L.; Mikolajczyk, S.D.; Shepherd, T.A.; Mackenson, D.G.; Briggs, S.L.; Starling, J.J. *Cancer Res.*, **1993**, *53*, 3956; Goshorn, S.C.; Svensson, H.P.; Kerr, D.E.; Somerville, J.E.; Senter, P.D.; Fell, H.P. *Cancer Res.*, **1993**, *53*, 2123; Vrudhula, V.M.; Svensson, H.P.; Senter, P.D. *J. Med. Chem.*, **1995**, *38*, 1380.

5 Faraci, W.S.; Pratt, R.F. *J. Am. Chem. Soc.*, **1984**, *106*, 1489.

6 Hamilton-Millar, J.M.T.; Newton, G.G.F.; Abraham, E.P. *Biochem. J.*, **1979**, *116*, 371.

7 See *e.g.* Mobashery, S.; Lerner, S.A.; Johnston, M. *J. Am. Chem. Soc.*, **1986**, *108*, 1685; Brown, R.F.; Kinnick, M.D.; Morin, J.M.; Vasileff, R.T.; Counter, F.T.; Davidson, E.O.; Ensminger, P.W.;. Eudaly, J.A; Kasher, J.S.; Katner, A.S.; Koehler, R.E.; Kurtz, K.D.; Lindstrom, T.D.; Lunn, W.H.W.; Preston, D.A.; Turner, J.L.; Webber, J.A.; Wright, W.E.; Zimmerman, K.M. *J. Med. Chem.*, **1990**, *33*, 2114; Albrecht, H.A.; Beskid, G.; Chan, K.K.; Christenson, J.G.; Cleeland, R.; Deitcher, K.H.; Georgopapadakou, N.H.; Keith, D.D.; Pruess, D.L.; Sepinwell, J.; Specian, A.C.; Then, R.L.; Weigele, M.; West, F.; Yang, R. *J. Med. Chem.*, **1990**, *33*, 77.

8 Alexander, R.P.; Beeley, N.R.A.; O'Driscoll, M.; O'Neill, F.P.; Millican, T.A.; Pratt, A.J.; Willenbrock, F.M. *Tetrahedron Lett.*, **1991**, *32*, 3269; Jungheim, L.N.; Shepherd, T.A.; Meyer, D.L. *J. Org. Chem.*, **1992**, *57*, 2334; Jungheim, L.N.; Shepherd, T.A.; Kling, J.K. *Heterocycles*, 1993, **35**, 339; Meyer, D.L.; Law, K.L.; Payne, J.K.; Mikolajczyk, S.D.; Zarrinmayeh, H.; Jungheim, L.N.; King, J.K.; Shepherd, T.A.; Starling, J.J. *Bioconjugate Chem.*, **1995**, *6*, 440; Svensson, H.P.;. Vrudhula, V.M; Emswiler, J.E.; MacMaster, J.F.; Cosand, W.L.; Senter, P.D.; Wallace, P.M. *Cancer Res.*, **1995**, *55*, 2357; Rodrigues, M.L.; Carter, P.; Wirth, C.; Mullins, S.; Lee, A.; Blackburn, B.K. *Chemistry and Biology*, **1995**, *2*, 223; Hudyma, T.W.; Bush, K.; Colson, K.L.; Firestone, R. A.; King, H.D. *Bioorg. Med. Chem. Lett.*, **1993**, *3*, 323; Hanessian, S.; Wang, J.C. *Can. J. Chem.*, **1993**, *71*, 896.

9 Johnston, T.P.; McCaleb, G.S.; Montgomery, J.A. *J. Med. Chem.*, **1963**, *6*, 669; Johnston, T.P.; McCaleb, G.S.; Montgomery, J.A. *J. Med. Chem.*, **1966**, *9*, 892; Reed, D.J. *Cancer Res.*, **1975**, *35*, 568.

10 Mobashery, S.; Johnston, M. *J. Biol. Chem.*, **1986**, *261*, 7879.

11 Kaiser, G.V.; Cooper, R.D.G.; Koehler, R.E.; Murphy, C.F.; Webber, J.A.; Wright, I.G.; Van Heyningen, E.M. *J. Org. Chem.*, **1970**, *35*, 2430.

12 Cooper, R.D.G.; DeMarco, P.V.; Cheng, J.C.; Jones, N.D. *J. Am. Chem. Soc.*, **1969**, *91*, 1408.

13 Sheehan, J.C.; Lo, Y.S.; Loliger, J.; Podewell, C.C. *J. Org. Chem.*, **1974**, *39*, 1444.

(*Received in UK* 29 *January* 1996; *revised* 19 *February* 1996; *accepted* 22 *February* 1996)