Design and synthesis of a cephalosporin–carboplatinum prodrug activatable by a β-lactamase

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The design and syntheses of two cephalosporin–carboplatinum prodrugs that can be released by a β-lactamase are described. The hydrolysis of cephalosporins catalyzed by a β-lactamase with acetyl or DACCP as 3′-leaving groups is studied by ^{1}H nuclear magnetic resonance in deuterated buffer solutions. These notions provide a new approach to the use of platinum complexes for antitumor therapy.

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Deux agents antitumoraux du type «pro-drug» qui consistent en une unité cephalosporine et une unité de carboplatine ont été synthétisés. Les molécules ont le potentiel d'être hydrolyses par une β-lactamase qui libérerait l'agent antitumoral (DACCP). L'action enzymatique a été étudiée par la résonance magnétique nucléaire du proton.

Introduction

The development of chemotherapeutic agents for the treatment of neoplastic diseases has been an area of immense interest and importance during the last decades (1). The discovery of hybridoma technology (2) and the advent of monoclonal antibodies has also revolutionized many aspects of drug development (3). An obvious application has been in tumor immunotherapy (4), where monoclonal antibodies have been used as directed messengers through conjugation, delivery, and, ultimately, release of drugs at the tumor site.

There are, however, many problems remaining that have limited the more widespread use of this technique in clinical trials (5). Factors affecting the activity of immunotoxin conjugates include the number of target antigens on the cell surface (6), the route of entry into the cell by the conjugate (7), the limited numbers of drug molecules coupled to a single antibody (8), the heterogeneity of antigen expression (9), and maintaining the antigen recognition sites of the antibody after chemical conjugation to the drug. Most of the cytotoxic agents exert their activities once inside the cell, requiring that the monoclonal antibody carrier facilitate the delivery of the drug to its precise site of activity within the cell. Many monoclonal antibodies directed against tumor antigens are not effective in this regard (10).

An exciting development in this area of research has involved the conjugation of specific enzymes to monoclonal antibodies, and the enzymatic release of a drug at the tumor site. The advantage of this approach is that one can, in principle, deliver many drug molecules to the tumor site with each immunoconjugate molecule, and some of the limitations mentioned above could perhaps be overcome.

The mechanism by which most β-lactamases inactivate β-lactam antibiotics is through acylation of a serine residue at the active site of the enzyme (11). In the case of a cephalosporin, a potential leaving group (X) at the 3′-position is eliminated during the enzymatic reaction, which most probably proceeds in two steps (12).

In another context, the leaving group could be an antibacterial agent having a different mode of action than the cephalosporin. Thus, the enzymatic acylation of the cephalosporin moiety by a serine residue in endogenous proteins such as penicillin-binding proteins releases the antibacterial agent, hence the term dual action antibiotics (13).

Recently, the monoclonal antibody – enzyme conjugate technique has been applied to design the prodrugs related to vinca alkaloids (14), and to nitrogen mustards (15) using β-lactamases. The enzyme was obtained from *Enterobacter cloacae*, which offers a number of advantages (16), particularly in being tolerant of a wide variety of substituents at the 3′-position of cephalosporins.

As a general program aimed at the development of novel and effective *cis*-platinum analogs (17, 18), we have been investigating the prospects of applying the above-mentioned strategy to the design of a prodrug consisting of a cephalosporin–platinum complex. Figure 1 schematically illustrates the approach to cancer chemotherapy with a *cis*-platinum prodrug.

Results and discussion

We chose two platinum complexes, 4′-carboxyphthalato (1,2-cyclohexanediamine) platinum (DACCP), which is a potent antitumor agent (19), and a carboplatinum complex that contains a hydrophobic ester linking arm, as the releasable portion of the prodrug. The synthesis of the cephalosporin–DACCP prodrug **8** is illustrated in Scheme 1.

The synthesis started with the commercially available 7-aminocephalosporanic acid (7-ACA), which was converted to the known ester **2** (20). This was then coupled with trimellitic anhydride chloride to afford **3** as a mixture of Δ^2- and Δ^3-acyloxy methyl cephems (ratio 1.5:1). The Δ^2 isomer could be converted to the Δ^3 isomer via the sulfoxide **4**, a process that capitalizes on the fact that β,γ-unsaturated sulfoxides are thermodynamically more stable than the corresponding α,β-unsaturated sulfoxides (21). Reduction (22) of the Δ^3-cephem sulfoxide **4** by phosphorus trichloride gave Δ^3-cephem **5**. The diphenyl methyl ester was cleaved with trifluoroacetic acid (TFA) in the presence of anisole and the resulting carboxylic acid derivative **6** was subsequently converted into the monopotassium compound **7**. Complex formation with *trans*-1,2-diamino cyclohexane platinumII dinitrate (23) afforded the desired product **8** as a white amorphous powder, which was moderately soluble in DMSO and carbonate buffer (~2 mg/mL, pH 10), but very slightly soluble in water (<0.2 mg/mL).

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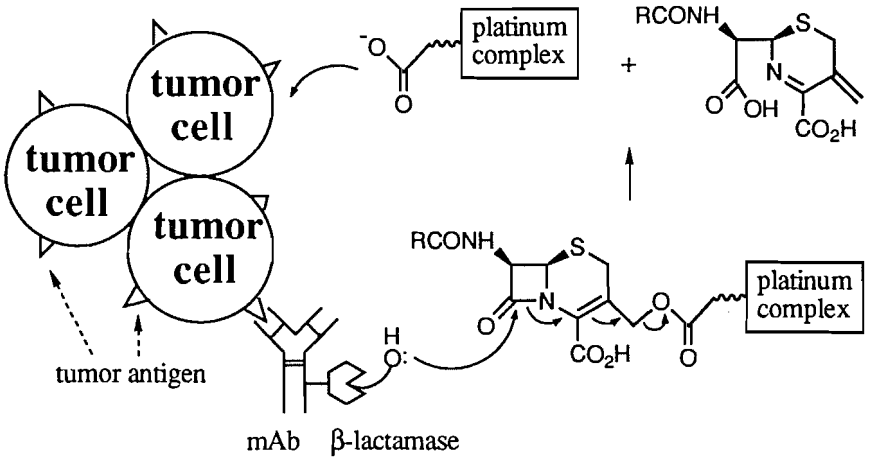
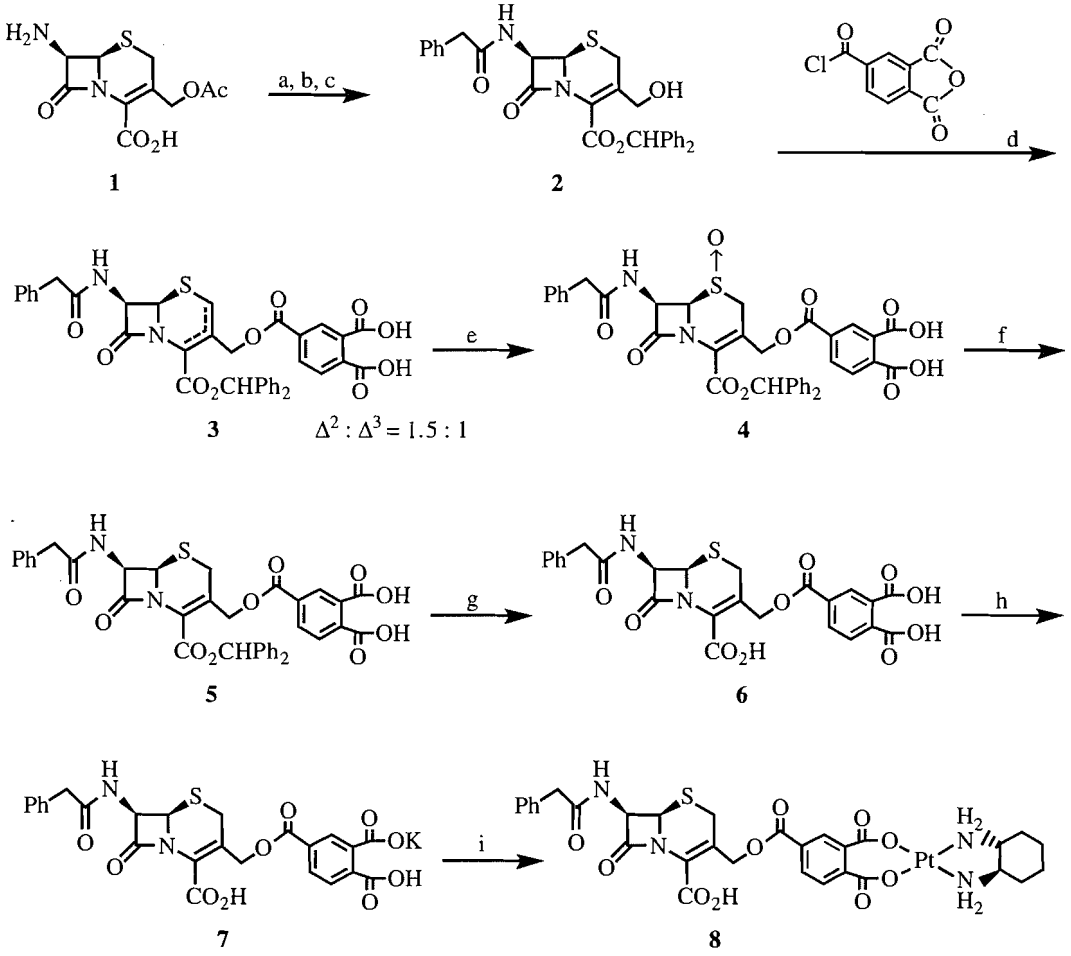


FIG. 1

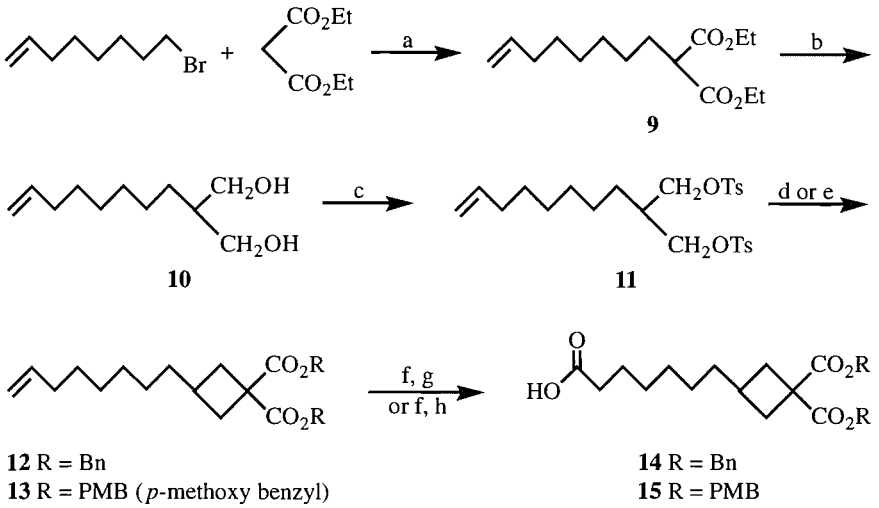


(a) aq NaOH, 0°C, 15 min; (b) $PhCH_2COCl$, acetone, 0°C, 1 h; (c) Ph_2CN_2, EtOAc, r.t., 2 h; (d) Et_3N, THF, 0°C, 1 h; (e) *m*-CPBA, EtOAc, 0°C, 3 h; (f) PCl_3, DMF, −25°C, 3 h; (g) TFA, anisole, CH_2Cl_2, 0°C, 2 h; (h) 0.1 N KOH; (i) dinitro(cyclohexane-1,2-diamine-*N*,*N*)platinum(II), H_2O, r.t., 3 h

SCHEME 1

We then turned our attention to the synthesis of a second type of cephalosporin – DACH platinum complex. Here, we chose to introduce a hydrophobic linker to provide some flexibility and "distance" between the *cis*-platinum component and the activatable units. The synthesis of the linker is illustrated in Scheme 2.

Alkylation of malonic acid diethyl ester with 8-bromo-1-octene gave compound **9**, which was then reduced to the diol **10** with lithium aluminum hydride. Tosylation of **10** in dichloromethane in the presence of triethylamine gave the ditosyl derivative **11**. The formation of the cyclobutane ring was carried out by treatment of malonic acid dibenzyl ester



(a) NaH, THF, 55°C, 2 days; (b) LAH, THF, r.t., 30 min; (c) TsCl, Et_3N, CH_2Cl_2, r.t., 2 days; (d) dibenzyl malonate, $NaN(SiMe_3)_2$, dioxane, reflux, 1 day; (e) di-*p*-methoxybenzyl malonate, $NaN(SiMe_3)_2$, dioxane, reflux, 1 day; (f) O_3, CH_2Cl_2, −78°C, 5 h; (g) R=Bn: PDC, DMF, r.t., 14 h; (h) R=PMB: 2-methyl-2-butene, *t*-BuOH, $NaH_2PO_4 \cdot H_2O$, $NaClO_2$, r.t., 30 min.

SCHEME 2

and **11** with sodium bis(trimethylsilyl) amide in dioxane to give **12**, which was subsequently oxidized to the acid **14** in a two-step process. It is noteworthy that the normal conditions for malonate condensation (NaH, dioxane) (21) were not effective in the case of **11**. Unfortunately, after esterification of **14** with the Δ^3-cephem **2**, the dibenzyl ester of the resulting compound could not be cleaved by hydrogenation, and only several unidentified products were obtained. Other attempts at debenzylation either gave no reaction or complete degradation. We then decided to repeat the previous synthesis using the *p*-methoxybenzyl ester derivative (24) with *p*-methoxybenzyl chloride to give dimethoxybenzyl malonate. Condensation with ditosyl derivative **11** led to the cyclobutane compound **13**. Ozonolysis of **13** followed by oxidation with sodium chlorite in the presence of 2-methyl-2-butene and sodium dihydrogen phosphate (25) afforded the carboxylic acid **15** in good yield.

Esterification of **15** with **2** by using DCC and DMAP gave **16** as a mixture of two isomers (Δ^2:Δ^3 1.5:1) (Scheme 3). The Δ^2 isomer was converted to the Δ^3 isomer **18** by oxidation with *m*-CPBA and reduction with phosphorus trichloride via sulfoxide **17**. The protecting groups were cleaved with trifluoroacetic acid in the presence of anisole in one step to give tricarboxylic acid **19**. Treatment of this compound with 1 equivalent of aqueous 0.1 N potassium hydroxide solution at 5°C followed by lyophilization led to the desired monopotassium compound **20** and the Δ^2 isomer as a by-product. Finally, aqueous solutions of **20** and dinitrocyclohexane-1,2-diamine platinum(II) were mixed in an equimolar ratio to form the complex **21**, which was obtained as a white amorphous powder.

Model studies of enzymatic hydrolysis by ^1H nuclear magnetic resonance

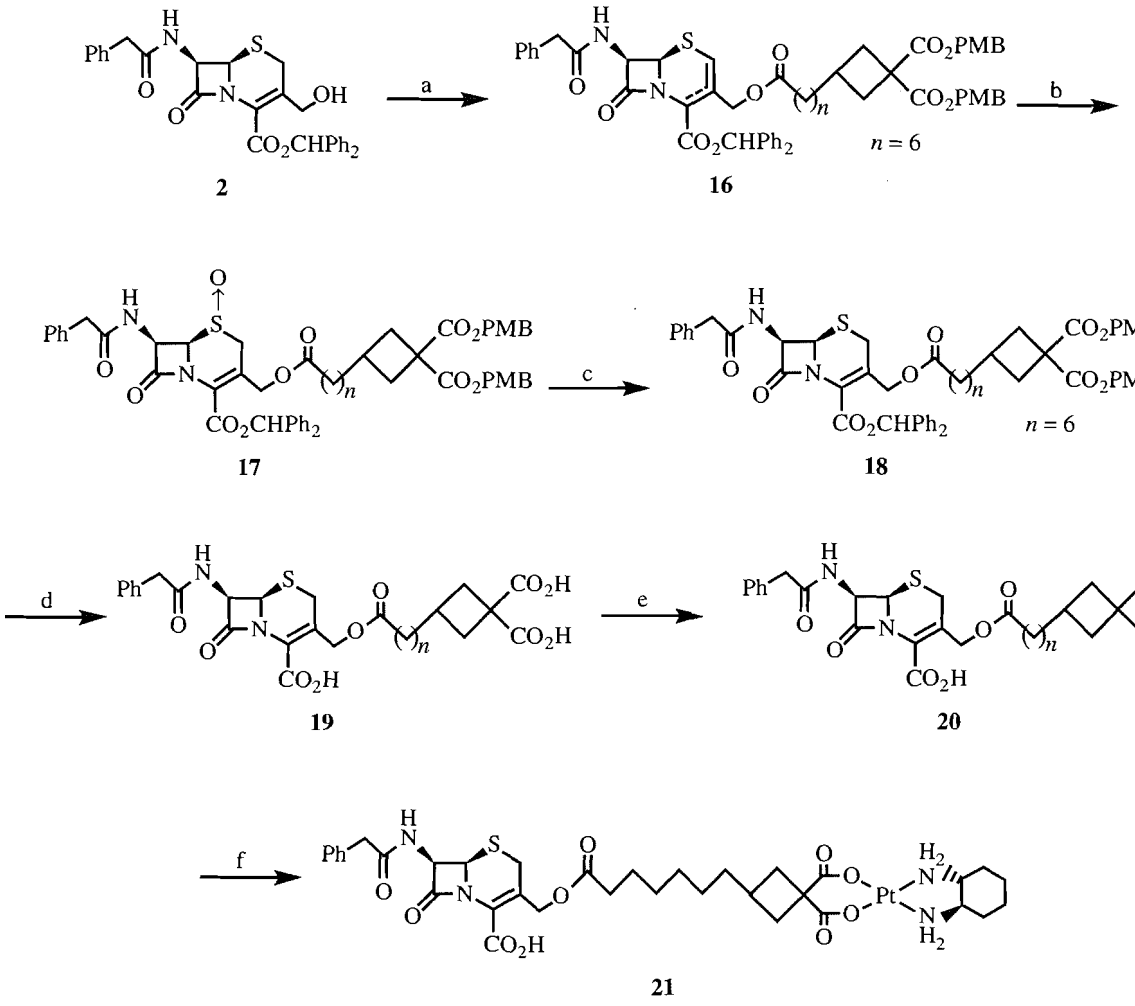
Previous studies by Faraci and Pratt (12) showed that the enzymatic hydrolysis of cephaloridine and other cephem derivatives at pD 6.4 and pD 10.2 can be followed by ^1H nmr spectroscopy. Accordingly, a model study of the enzymatic hydrolysis was carried out utilizing 3-acetoxymethyl-7-phenylacetylamino cephem potassium salt **22** in phosphate buffer at pD 7 and in carbonate buffer at pD 10.1 (Scheme 4). In the presence of sufficient amounts of β-lactamase (EC 3.5.2.6, type III, from *Enterobacter cloacae*), the initial spectrum changed rapidly (<2 min) to that of the eliminated products (12, 26). Encouraged by, this we proceeded to test the enzymatic hydrolysis with the *cis*-platinum conjugate **8**.

Because of the poor solubility of complex **8** in neutral phosphate buffer solution, a carbonate buffer solution (pD 10.1) was used for the ^1H nmr study of β-lactamase catalyzed hydrolysis. In the presence of sufficient β-lactamase (from *E. cloacae*), the ^1H nmr spectrum of complex **8** changed rapidly to that of the eliminated compounds **23** and **25** (Scheme 5).

Figure 2 shows the ^1H nmr spectra of the cephalosporin–cisplatinum ester **8**, before and after treatment with the enzyme (A and B respectively). The product resulting from compound **22** is shown in C. It can be seen that B contains peaks from the products of enzymatic cleavage as expected. In a control experiment under the same conditions, **8** was stable to hydrolysis for over 2 h.

Conclusion

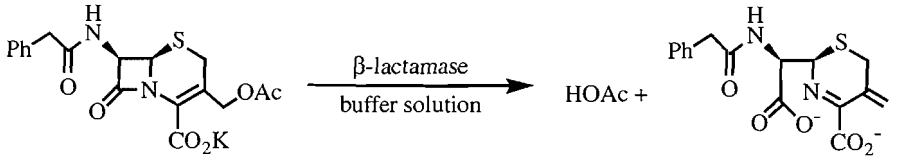
We have shown that carboplatinum prodrugs can be linked to a cephalosporin via an ester linkage as shown in structures **8** and **21**. The β-lactamase catalyzed hydrolysis of the cephalosporin derivative **8** with DACCP as the 3′-leaving group was studied by ^1H nmr in deuterated buffer solutions, showing that the carboplatinum subunit is indeed released as expected. These results could pave the way to further studies involving the effectiveness of monoclonal antibody – β-lactamase conjugates in releasing the platinum complexes in vivo and the obvious potential in tumor directed chemotherapy (27).



2 16 17 18 19 20 21

(*a*) **15**, DCC, DMAP, CH_2Cl_2, r.t., overnight; (*b*) *m*-CPBA, EtOAc, 0°C, 3 h; (*c*) PCl_3, DMF, −20°C, 1 h; (d) TFA, anisole, CH_2Cl_2, 0°C, 2 h; (e) 0.1 N KOH, H_2O, 0°C; (f) dinitro(cyclohexane-1,2-diamine-*N*,*N*′)platinum(II), H_2O, r.t., 3 h

SCHEME 3



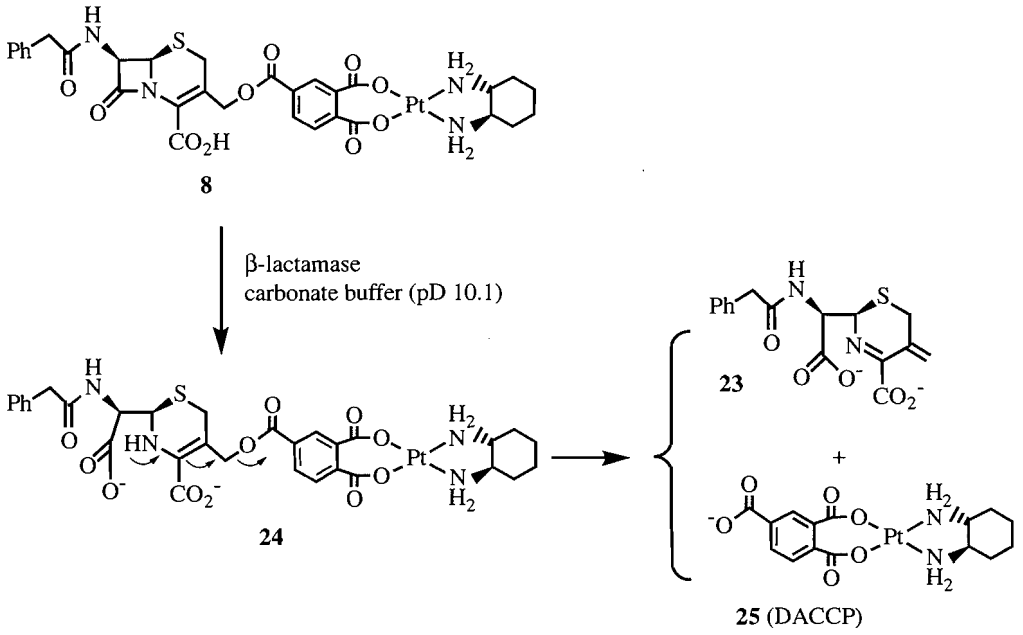
22 23

SCHEME 4

Experimental

Melting points (mp) were determined on a Buchi melting point apparatus and are uncorrected. Optical rotations were measured at 25°C using a Perkin–Elmer model 241 automatic polarimeter. Proton magnetic resonance (^{1}H nmr) spectra were determined on either a 300 MHz Varian or a 400 MHz Bruker spectrometer in deuterated chloroform, methanol-d_4, dimethyl sulfoxide-d_6, or deuterium oxide (D_2O) with $CHCl_3$ (δ_{ppm} = 7.265), Me_4Si (δ_{ppm} = 0.00), or dioxane (δ_{ppm} = 67.40 for ^{13}C) as references. Coupling constants are expressed in hertz (Hz). The abbreviations used for the description of the peaks are as follows: (s, singlet; d, doublet; dd, doublet of doublets; dt, doublet of triplets; ddd, doublet of doublet of doublets; t, triplet; q, quartet; m, multiplet; br, broad). The ^{13}C nmr spectra were recorded on a Varian VXR 300 at 75 MHz. Low-resolution mass spectra were determined on a VG Micro Mass 1212 mass spectrometer by using desorption chemical ionization (DCI), or by fast atom bombardment (FAB). High-resolution mass spectra were determined on a Kratos MS-50 TCTA mass spectrometer by using electron impact (EI) at 70 eV or by the fast atom bombardment (FAB) techniques. Infrared spectra (ir) were recorded on a Perkin–Elmer 781 infrared spectrophotometer. The spectra of solids were recorded as Nujol mulls. Liquid samples were determined as films between sodium chloride plates. Combustion analyses were performed by Guelph Laboratories Ltd., Guelph, Ontario.

Benzene-1,2,4-tricarboxylic acid 4-[[2R-(2α,6α,7β]-(2-benzhydryloxycarbonyl-8-oxo-7-phenylacetylamino-5-thia-1-azabicyclo[4.2.0]oct-3-en-3-yl)methyl] ester (Δ^2 isomer, 3a)



SCHEME 5

*and benzene-1,2,4-tricarboxylic acid 4-[(6*R-trans*)-(2-benzhydryloxycarbonyl-8-oxo-7-phenylacetylamino-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)methyl] ester (Δ³ isomer, 3b)*

To a cold (ice-water bath) solution of **2** (420 mg, 0.816 mmol) and DMAP (123 mg, 1.00 mmol) in 15 mL of dry THF was added a solution of trimellitic anhydride chloride (210 mg, 0.987 mmol) in 5 mL of dry THF. The mixture was stirred at that temperature for 30 min. After removing the solvent under vacuum, the residue was dissolved in 50 mL of ethyl acetate and acidified with 5% HCl. The ethyl acetate layer was washed with water and brine, dried ($MgSO_4$), and evaporated to give a slightly brown foam (560 mg, 99%). The crude product was purified by flash chromatography, eluting with ethyl acetate – hexanes (1:3) in the presence of 1% AcOH to give an inseparable mixture of the Δ^2 and Δ^3 isomers (ratio 1:1) of **3** as a foam; ir ($CHCl_3$): 3350, 1855, 1780 (C=O, β-lactam), 1730 (COOR), 1670 (CONH) cm^{-1}; ^1H nmr (300 MHz, $CDCl_3$) for Δ^3 isomer, δ: 3.42, 3.62 (ABq, 2H, J_{gem} = 18.7 Hz, SCH_2), 3.66 (m, 2H, $PhCH_2$), 5.00 (d, 1H, J = 4.9 Hz, H-6), 5.08, 5.34 (ABq, 2H, J_{gem} = 13.3 Hz, CH_2O), 5.91 (dd, 1H, J = 4.9 Hz, 9.1 Hz, H-7), 6.03 (d, 1H, J = 9.1 Hz, NH), 6.95 (s, 1H, $OCHPh_2$), 7.24–7.41 (m, 15H, PhH), 8.07 (d, 1H, J = 7.9 Hz, aromatic H-6), 8.45 (dd, 1H, J = 1.3 Hz, 7.9 Hz, aromatic H-5), 8.53 (s, 1H, aromatic H-3). For Δ^2 isomer, δ: 3.65 (m, 2H, $PhCH_2$), 4.84, 4.96 (ABq, 2H, J_{gem} = 12.6 Hz, OCH_2), 5.14 (d, 1H, J = 1.5 Hz, H-2), 5.20 (d, 1H, J = 3.9 Hz, H-6), 5.60 (dd, 1H, J = 3.9 Hz, 8.5 Hz, H-7), 6.18 (d, 1H, J = 8.5 Hz, NH), 6.58 (d, 1H, J = 1.5 Hz, H-4), 6.88 (s, 1H, $OCHPh_2$), 7.24–7.41 (m, 15H, PhH), 8.01 (d, 1H, J = 7.9 Hz, aromatic H-6), 8.37 (dd, 1H, J = 1.3 Hz, 7.9 Hz, aromatic H-5), 8.48 (s, 1H, aromatic H-3); ms (FAB), m/e: 729 $(M + Na)^+$, 707 $(M + 1)^+$.

*Benzene-1,2,4-tricarboxylic acid 4-[(6*R-trans*)-(2-benzhydryloxycarbonyl-5,8-dioxo-7-phenylacetylamino-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)methyl] ester (4)*

A solution of **3** (500 mg, 0.726 mmol) in 10 mL of ethyl acetate was cooled to 0°C and *m*-chloroperbenzoic acid (50–60%, 250 mg) in 5 mL of ethyl acetate was added dropwise. After stirring at this temperature for 3 h, the solution was diluted with 60 mL of ethyl acetate and washed with water and brine. The organic phase was dried ($MgSO_4$) and evaporated to give **4** (495 mg, 97%) as a pale yellow residue that was used for next reaction without further purification. However, a small sample was dissolved in a mixed solvent of dioxane (10 mL) and deionized water (1 mL) and stirred at room temperature for 15 min. The solvent was removed under vacuum and the residue was purified by flash chromatography, eluting with dichloromethane–methanol – acetic acid (90:10:1) to afford the pure product **4**; $[\alpha]_D$ +37 (*c* 0.57, methanol); ir ($CHCl_3$): 3300, 1850, 1800, 1780 (C=O, β-lactam), 1720 (COOR), 1710 (CONH) cm^{-1}; ^1H nmr (300 MHz, $CDCl_3$) δ: 3.29, 3.93 (ABq, 2H, J_{gem} = 18.8 Hz, SCH_2), 3.65 (s, 2H, $PhCH_2$), 4.53 (d, 1H, J = 4.1 Hz, H-6), 5.03, 5.53 (ABq, 2H, J_{gem} = 13.7 Hz, CH_2O), 6.11 (dd, 1H, J = 4.1 Hz, 9.6 Hz, H-7), 6.96 (s, 1H, $OCHPh_2$), 6.94 (d, 1H, J = 9.6 Hz, NH), 7.27 (m, 15H, PhH), 8.01 (d, 1H, J = 8.0 Hz, aromatic H-6), 8.40 (dd, 1H, J = 1.3 Hz, 7.9 Hz, aromatic H-5), 8.59 (s, 1H, aromatic H-3), 11.28 (br s, 2H, COOH); ^{13}C nmr (75 MHz, CD_3OD) δ: 43.22, 60.07, 65.48, 68.13, 81.40, 97.19, 122.15, 128.04, 128.11, 128.61, 128.98, 129.46, 129.52, 129.67, 130.30, 130.51, 131.07, 133.21, 133.82, 133.91, 135.42, 140.58, 161.35, 165.75, 168.20, 169.57, 170.60, 174.20; ms (FAB), m/e: 745 $(M + Na)^+$, 723 $(M + 1)^+$. Exact Mass calcd. for $C_{38}H_{30}N_2NaO_{11}S$: 745.1469; found: 745.1549.

*Benzene-1,2,4-tricarboxylic acid 4-[(6*R-trans*)-(2-benzhydryloxycarbonyl-8-oxo-7-phenylacetylamino-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)methyl] ester (5)*

Phosphorus trichloride (128 μL, 1.46 mmol) was added slowly to a solution of **4** (470 mg, 0.667 mmol) in 5 mL of dry DMF at −22°C. After stirring at a similar temperature for 3 h, the reaction mixture was poured onto 50 mL of ice-water. The precipitate was collected by filtration, washed with cold water, and dried over P_2O_5 under vacuum in a desiccator to afford **5** (430 mg, 93%) as a powder. The product could be purified by flash chromatography, eluting with ethyl acetate – hexanes (5:1, containing 1% acetic acid); $[\alpha]_D$ −9 (*c* 2.26, $CHCl_3$); ir ($CHCl_3$): 3290, 1850, 1780 (C=O, β-lactam), 1720 (COOR), 1670 (CONH) cm^{-1}; ^1H nmr (300 MHz, $CDCl_3$) δ: 3.41, 3.61 (ABq, 2H, J_{gem} = 18.5 Hz, SCH_2), 3.66 (d, 2H, J = 4.9 Hz, $PhCH_2$), 5.00 (d, 1H, J = 5.1 Hz, H-6), 5.08, 5.34 (ABq, 2H, J_{gem} = 13.2 Hz, CH_2O), 5.91 (dd, 1H, J = 5.1 Hz, 9.1 Hz, H-7), 6.28 (d, 1H, J = 9.1 Hz, NH), 6.95 (s, 1H, $OCHPh_2$), 7.22–7.45 (m, 15H, PhH), 8.06 (d, 1H, J = 7.7 Hz, aromatic H-6), 8.44 (dd, 1H, J = 1.3 Hz, 7.9 Hz, aromatic H-5), 8.53 (s, 1H, aromatic H-3); ^{13}C nmr (75 MHz, $CDCl_3$) δ: 26.42, 43.00, 57.22, 59.04, 64.08, 79.84, 126.07, 126.84, 126.91,

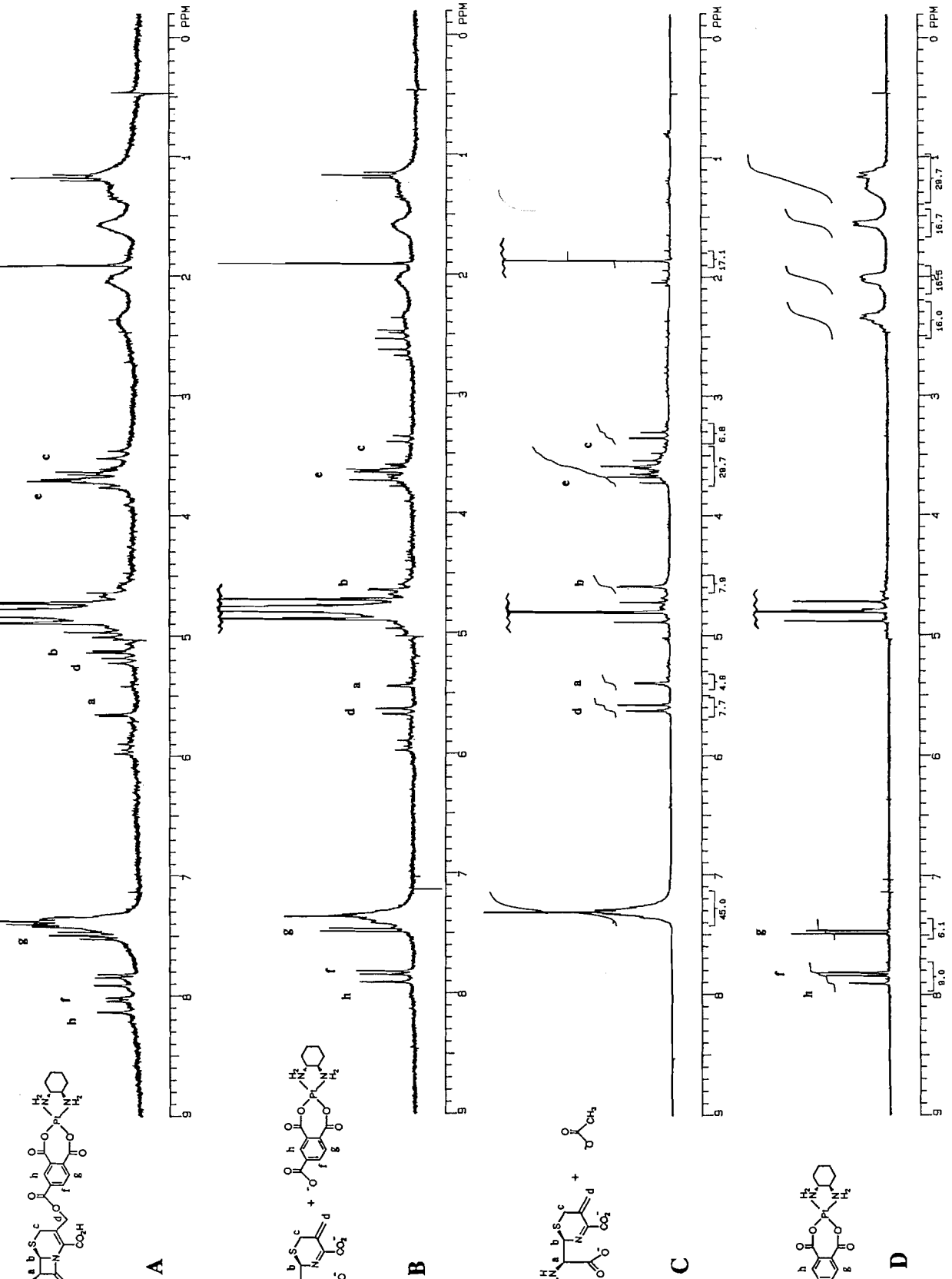


FIG. 2. The ^{1}H nmr spectra in deuterated buffer solution: (A) **8** in the absence of β-lactamase; (B) **8** in the presence of β-lactamase; (C) **22** in the presence of β-lactamase; (D) **25** (DACCP).

127.45, 127.59, 128.03, 128.11, 128.33, 128.44, 128.56, 128.94, 129.02, 129.33, 130.87, 131.40, 133.06, 133.45, 137.33, 138.43, 138.74, 138.86, 160.52, 164.18, 164.77, 167.68, 169.70, 171.66; ms (FAB), *m/e*: 729 $(M + Na)^+$, 707 $(M + 1)^+$. Exact Mass calcd. for $C_{38}H_{30}N_2NaO_{10}S$: 729.1520; found: 729.1599.

*Benzene-1,2,4-tricarboxylic acid 4-[(6*R-trans*)-(2-carboxy-8-oxo-7-phenylacetylamino-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)methyl] ester (**6**)*

Trifluoroacetic acid (1.5 mL, 19.5 mmol) was added dropwise to a solution of compound **5** (565 mg, 0.800 mmol) in anisole (0.52 mL, 4.80 mmol) and dichloromethane (12 mL) at 0°C (ice-water bath), and the mixture was stirred at a similar temperature for 2 h. After removal of the solvent under vacuum the residue was treated with 3 mL of ether and the resulting white precipitate was filtered and washed with ether (5 × 3 mL). This was dried under reduced pressure in a desiccator to give product **6** (410 mg, 95%) as white powder. The compound was used for the next reaction without further purification; $[\alpha]_D$ +25 (c 0.45, DMF); ir (Nujol): 3500–2500 (br, COOH, NH), 1780 (C=O, β-lactam), 1725 (COOR), 1660 (CONH) cm^{-1}; ^1H nmr (300 MHz, methanol-d_4–D_2O 1:1) δ: 3.49, 3.71 (ABq, 2H, J_{gem} = 18.0 Hz, SCH_2), 3.60 (m, 2H, $PhCH_2$), 5.07, 5.29 (ABq, 2H, J_{gem} = 12.5 Hz, $COOCH_2$), 5.08 (d, 1H, J = 4.6 Hz, H-6), 5.65 (d, 1H, J = 4.6 Hz, H-7), 7.22–7.35 (m, 5H, PhH), 7.75–8.42 (m, 3H, aromatic H); ms (FAB), *m/e*: 563 $(M + Na)^+$, 541 $(M + 1)^+$.

*Potassium benzene-1,2,4-tricarboxylic acid 4-[(6*R-trans*)-(2-carboxy-8-oxo-7-phenylacetylamino-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)methyl] ester (**7**)*

To a suspension of tricarboxylic acid **6** (100 mg, 0.185 mmol) in 10 mL of deionized water was slowly added 0.105 N aqueous potassium hydroxide (1.8 mL, 0.189 mmol) at 0°C, at which point the pH value was 6–7. The mixture was stirred at this temperature until most of the suspension had dissolved (pH ~4). The insoluble residue was filtered and the filtrate was lyophilized to give monopotassium salt **7** (102 mg, 95%) as a white powder; $[\alpha]_D$ +83 (*c* 0.9, H_2O); ^1H nmr (300 MHz, D_2O) δ: 3.50, 3.74 (ABq, 2H, J_{gem} = 17.9 Hz, SCH_2), 3.68, 3.74 (ABq, 2H, J_{gem} = 7 Hz, $PhCH_2$), 4.99, 5.20 (ABq, 2H, J_{gem} = 12.5 Hz, $COOCH_2$), 5.13 (d, 1H, J = 4.6 Hz, H-6), 5.67 (d, 1H, J = 4.6 Hz, H-7), 7.35–7.42 (m, 5H, PhH), 7.53 (d, 1H, J = 8.1 Hz, aromatic H-5), 8.05 (dd, 1H, J = 1.6 Hz, 8.1 Hz, aromatic H-6), 8.16 (d, 1H, J = 1.6 Hz, aromatic H-2).

*(SP-4-2)-Dichloro[(1*R,2R*)-cyclohexane-1,2-diamine*-N,N′*] platinum*

A quantity of (1*R*,2*R*)-(−)-1,2-cyclohexanediamine (28) (0.923 g, 8.08 mmol), was dissolved in 8 mL of deionized water at room temperature, and the solution was then added to a solution of K_2PtCl_4 in 40 mL of deionized water. The red colored solution was stirred manually, and was left standing in the dark until the color became yellow or orange yellow. The resulting yellow precipitate was filtered, washed with deionized water 5 times, and dried over P_2O_5 in the absence of light under vacuum to give the platinum complex (2.71 g, 88.2%) as bright yellow crystals: $[\alpha]_D$ +85.3 (*c* 0.66, DMF); ir (Nujol): 3270, 3180, 3100 (NH) cm^{-1}; ^1H nmr (300 MHz, DMSO-d_6) δ: 0.86–1.01 (m, 2H, CH_2), 1.14–1.21 (m, 2H, CH_2), 1.40–1.42 (m, 2H, CH_2), 1.80–1.84 (m, 2H, CH_2), 2.07–2.10 (m, 2H, H-1, -2), 5.00–5.06 (m, 2H, NH), 5.54–5.57 (m, 2H, NH); ^{13}C nmr (75 MHz, DMSO-d_6) δ: 24.19, 31.39, 62.77.

*(SP-4-2)-Dinitro [(1*R,2R*)-cyclohexane-1,2-diamine*-N,N′*] platinum*

To a suspension of the above complex (150 mg, 0.395 mmol) in 50 mL of deionized water was added a solution of silver nitrate (132 mg, 0.777 mmol) in 3 mL of deionized water. The yellow suspension was stirred vigorously in the dark at room temperature for 1 day. The resulting white suspension was filtered with the aid of Celite and the filtrate was concentrated to 12.5 mL. The colorless solution of the platinum complex (0.0315 mmol/mL) was used directly for the next reaction. A small sample was lyophilized to dryness to afford a white powder: $[\alpha]_D$ + 64 (*c* 1.00, H_2O); ir (Nujol): 3240, 3205, 3140 (NH_2) cm^{-1}; ^1H nmr (300 MHz, D_2O) δ: 1.14–1.36 (m, 4H, H-4, -5), 1.58–1.61 (m, 2H, H-3, -6), 2.02–2.08 (m, 2H, H-3, -6), 2.35–2.42 (m, 2H, H-1, -2).

*(SP-4-2)-[4-[(6*R-trans*)-(2-Carboxy-8-oxo-7-phenylacetylamino-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)methoxycarbonyl] benzene-1,2-dicarboxylato][(1*R,2R*)-cyclohexane-1,2-diamine*-N,N′*] platinum (**8**)*

(SP-4-2)-Dinitro (1,2-cyclohexanediamine *N,N′*) platinum (76 mg, 0.176 mmol) was dissolved in 7 mL of deionized water and traces of insoluble material were removed by centrifugation. The supernatant was transferred into a 20-mL vial, and a solution of potassium salt **7** (102 mg, 0.176 mmol) in 3 mL of deionized water was added. The mixture was stirred at room temperature in the dark for 3 h, and the resulting precipitate was filtered, washed with deionized water, and dried over P_2O_5 under vacuum to give the platinum complex **8** (115 mg, 76%) as white powder: $[\alpha]_D$ +60 (*c* 0.33, DMSO); ir (Nujol): 3500–2500 (br, COOH, NH), 2400, 2290, 1780 (C=O, β-lactam), 1720 (COOR), 1640 (CONH) cm^{-1}; ^1H nmr (300 MHz, deuterated carbonate buffer, pD 10.1) δ: 1.09–1.36 (br m, 4H, cyclohexane CH_2), 1.53–1.62 (br m, 2H, cyclohexane CH_2), 1.90–2.07 (br m, 2H, cyclohexane CH_2), 2.38 (br m, 2H, cyclohexane H-1, -2), 3.49, 3.74 (ABq, 2H, J_{gem} = 17.5 Hz, SCH_2), 3.61–3.76 (m, 2H, $PhCH_2$), 4.98, 5.20 (ABq, 2H, J_{gem} = 12.3 Hz, $COOCH_2$), 5.13 (d, 1H, J = 4.6 Hz, H-6), 5.66 (d, 1H, J = 4.6 Hz, H-7), 7.32–7.45 (m, 5H, PhH), 7.51 (d, 1H, J = 8 Hz, aromatic H-5), 8.03 (dd, 1H, J = 1.5 Hz, 7.9 Hz, aromatic H-6), 8.13 (d, 1H, J = 1.4 Hz, aromatic H-2); ms (FAB), *m/e*: 847 $(M)^+$. Anal. calcd. for $C_{31}H_{32}N_4O_{10}PtS \cdot 4H_2O$: C 40.48, H 4.38, N 6.09; found: C 40.62, H 4.21, N 6.24.

*Malonic acid bis(*p*-methoxybenzyl) ester*

A mixture of dipotassium malonate (8.00 g, 45.0 mmol), *p*-methoxybenzyl chloride (13.0 mL, 2.1 equiv.), and dry DMF (160 mL) was stirred at 60 °C for 2 days and then diluted with 700 mL of ether, washed with water, 10% HCl, water, saturated $NaHCO_3$ solution, water, and brine. The organic phase was dried (Na_2SO_4) and evaporated to give a syrup that was purified by flash column chromatography, eluting with ethyl acetate – hexanes (1:3, then 1:1) to give the ester (7.20 g, 47%) as a colorless syrup that was crystallized at 0°C in a refrigerator; mp 28–29°C; ir ($CHCl_3$): 1745, 1730 (C=O) cm^{-1}; ^1H nmr (300 MHz, $CDCl_3$) δ: 3.44 (s, 2H, H-2), 3.82 (s, 6H, OCH_3), 5.11 (s, 4H, $PhCH_2O$), 6.89 (m, 4H, aromatic H), 7.28 (m, 4H, aromatic H); ^{13}C nmr (75 MHz, $CDCl_3$) δ: 41.50, 55.12, 66.96, 113.79, 127.16, 130.06, 159.59, 166.24; ms (FAB), *m/e*: 345 $(M + 1)^+$, 344 $(M)^+$. Exact Mass calcd. for $C_{19}H_{20}O_6$: 344.1260; found: 344.1226.

*2-(Oct-7-enyl)malonic acid diethyl ester (**9**)*

Sodium hydride (1.07 g, 27.0 mmol) was washed with dry THF twice and then suspended in 60 mL of dry THF. Diethyl malonate (4.2 mL, 27.0 mmol) was added dropwise at −20°C. The resulting clear solution was stirred for 5 min before adding 8-bromo-1-octene (2.9 mL, 17 mmol). The reaction was stirred at 55–60°C for 2 days and quenched with ethanol. After addition of ether (350 mL), the resulting white precipitate was filtered, the filtrate was washed with cold water, brine, and dried (Na_2SO_4). Evaporation of the solvent gave a oil that was purified by a flash column (ethyl acetate – hexanes 1:6, then 1:4) to give product **9** (4.50 g, 99%) as a colorless oil; ^1H nmr (300 MHz, $CDCl_3$) δ: 1.27 (t, 6H, J = 7.1 Hz, CH_3), 1.26–1.40 (m, 8H, H-4, -5, -6, -7), 1.88 (m, 2H, H-3), 2.03 (m, 2H, H-8), 3.31 (t, 1H, J = 7.6 Hz, H-2), 4.19 (q, 4H, J = 7.1 Hz, $COOCH_2$), 4.91–5.02 (m, 2H, CH_2=), 5.80 (ddt, 1H, J = 6.8 Hz, 10.5 Hz, 17.3 Hz, C=CH).

*2-(Oct-7-enyl)propane-1,3-diol (**10**)*

To a cold (ice-water bath) solution of **9** (4.50 g, 16.6 mmol) in 100 mL of dry ether was added dropwise lithium aluminum hydride (1 M in THF solution, 34 mL, 34 mmol). After addition was

complete the mixture was stirred at room temperature for 30 min. The reaction was quenched by slow addition of water at 0°C until effervescence stopped. The mixture was neutralized with 6 N HCL (~25 mL), saturated with solid NaCl, and then extracted with ether (3 × 60 mL). The combined extract was washed with saturated $NaHCO_3$ solution and brine, dried (Na_2SO_4), and evaporated to afford **10** (3.01 g, 98%) as a syrup that was used directly for the next reaction; ir ($CHCl_3$): 3350 (br, OH) cm^{-1}; ^1H nmr (300 MHz, $CDCl_3$) δ: 1.19–1.41 (m, 10H, H-3, -4, -5, -6, -7), 1.71–1.82 (m, 1H, H-2), 2.00–2.07 (m, 2H H-8), 2.56 (s, 2H, OH), 3.65 (dd, 2H, J = 7.6 Hz, 10.7 Hz, H-1), 3.81 (dd, 2H, J = 3.8 Hz, 10.7 Hz, H-1), 4.95–5.03 (m, 2H, CH_2=), 5.81 (ddt, 1H, J = 6.7 Hz, 10.2 Hz, 17.1 Hz, C=CH-).

*2-(Oct-7-enyl)propane-1,3-diol di-*p*-toluenesulfonate (**11**)*

To a cold (ice-water bath) solution of **10** (162 mg, 0.870 mmol), triethylamine (0.44 mL, 3.1 mmol), and a catalytic amount of DMAP in 5 mL of dry dichloromethane was added slowly a solution of tosyl chloride (500 mg, 2.60 mmol) in 5 mL of dichloromethane. After stirring at room temperature for 2 days, the reaction was quenched by the addition of cold water (0.5 mL) at 0°C. The organic phase was dried over Na_2SO_4 and evaporated to give a syrup that was purified by flash chromatography on silica gel (ethyl acetate – hexanes 1:5, then 1:3, then 1:1) to give pure compound **11** (350 mg, 81%) as a syrup that crystallized on standing in a refrigerator; mp 49–50°C; ir ($CHCl_3$): 1640, 1600, 1465, 1360, 1190, 1175, 1095, 970, 815, 665 cm^{-1}; ^1H nmr (300 MHz, $CDCl_3$) δ: 1.11–1.34 (m, 10H, H-3, -4, -5, -6, -7), 1.92–2.04 (m, 3H, H-2, -8), 2.46 (s, 6H, $ArCH_3$), 3.94 (dq, 4H, J = 4.6 Hz, 6.1 Hz, 9.9 Hz, CH_2OSO_2), 4.91–5.02 (m, 2H, CH_2=), 5.79 (ddt, 1H, J = 6.7 Hz, 10.2 Hz, 17.1 Hz, C=CH-), 7.33–7.36 (m, 4H, ArH), 7.72–7.76 (m, 4H, ArH); ^{13}C nmr (75 MHz, $CDCl_3$) δ: 21.46, 26.01, 26.61, 28.50, 28.57, 29.05, 33.48, 37.71, 68.56, 114.12, 127.67, 129.75, 132.23, 138.72, 144.85.

*3-(Oct-7-enyl) cyclobutane-1,1-dicarboxylic acid bis(*p*-methoxybenzyl) ester (**13**)*

To a cold solution of dimethoxybenzyl malonate (5.10 g, 14.8 mmol) in 100 mL of dry dioxane was added dropwise 15 mL of sodium bis(trimethylsilyl)amide (1 M in THF) at 0°C. The solution was stirred at this temperature for 5 min, at which point a solution of the ditosyl derivative **11** (7.35 g, 14.9 mmol) in 100 mL of dry dioxane was slowly added, followed by 15 mL of sodium bis(trimethylsilyl)amide THF solution. The reaction mixture was stirred at 12°C for 15 min, then heated to reflux for 16 h, diluted with 700 mL of ether, washed with 1 N HCl, water, saturated $NaHCO_3$, water, and brine, dried with anhydrous sodium sulfate, and evaporated to give a syrup. Purification by flash chromatography (ethyl acetate – hexanes 1:5, then 1:2) afforded the cyclobutane derivative **13** (3.80 g, 52%) as a colorless syrup; ir ($CHCl_3$): 1725 (C=O) cm^{-1}; ^1H nmr (300 MHz, $CDCl_3$) δ: 1.15–1.40 (m, 10H, H-1′, -2′, -3′, -4′, -5′), 1.99–2.12 (m, 2H, H-6′), 2.15–2.19 (m, 2H, H-2, -4), 2.31 (m, 1H, H-3), 2.57–2.64 (m, 2H, H-2, -4), 3.80 (s, 6H, $PhOCH_3$), 4.91–5.03 (m, 2H, C=CH_2), 5.04 (s, 2H, $PhCH_2O$), 5.07 (s, 2H, $PhCH_2O$), 5.81 (ddt, 1H, J = 6.8 Hz, 10.3 Hz, 17.0 Hz, C=CH-), 6.81–6.87 (m, 4H, aromatic H), 7.16–7.27 (m, 4H, aromatic H); ^{13}C nmr (75 MHz, $CDCl_3$) δ: 26.53, 28.71, 28.92, 29.15, 29.52, 33.63, 34.70, 36.31, 49.55, 55.08, 66.62, 66.75, 113.67, 114.02, 127.59, 129.70, 139.01, 159.39, 171.45, 171.78; ms, m/e: 494 $(M)^+$. Exact Mass calcd. for $C_{30}H_{38}O_6$: 494.2669; found: 494.2676.

*3-(6-Carboxyhexyl) cyclobutane-1,1-dicarboxylic acid bis(*p*-methoxybenzyl) ester (**15**)*

A solution of compound **13** (0.500 g, 1.01 mmol) in 15 mL of dry dichloromethane was cooled to −78°C, and ozone was bubbled into it over 1 h at this temperature. After passing argon through the solution for 30 min, 0.4 mL of dimethyl sulfide was added, followed by 0.5 mL of triethylamine. The solution was stirred for 10 min, and warmed to 0°C for 1 h until almost all of the ozonide disappeared (monitored by tlc). The reaction mixture was diluted with 50 mL of dichloromethane and washed with saturated $NaHCO_3$, water, and brine. The organic phase was dried (Na_2SO_4) and evaporated to dryness to give the aldehyde. The crude aldehyde was dissolved in 20 mL of *tert*-butyl alcohol and 5 mL of 2-methyl-2-butene, then a solution of sodium chlorite (0.810 g, 8.96 mmol) and sodium dihydrogenphosphate hydrate (0.940 g, 6.81 mmol) in 8 mL of deionized water was added dropwise over a 15 min period. The yellow mixture was stirred at room temperature for 30 min. After removal of the volatile components under vacuum, the yellow liquid residue was extracted with 20 mL of ether. The aqueous phase was acidified to pH 3 with 6 N HCl and extracted with ether (2 × 10 mL). The combined ether extracts were washed with water and brine, dried ($MgSO_4$), and evaporated to give a syrup that was purified by flash chromatography on a silica gel column, eluting with ethyl acetate – hexanes (1:3, then 1:2) in the presence of 0.5% acetic acid to give carboxylic acid **15** (0.405 g, 79%, two steps from olefin **13**) as white crystals; mp 56–57°C; ir ($CHCl_3$): 3400–2500 (br, COOH), 1725, 1705 (C=O) cm^{-1}; ^1H nmr (300 MHz, $CDCl_3$) δ: 1.16–1.38 (m, 8H, H-1′, -2′, -3′, -4′), 1.58–1.63 (m, 2H, H-5′), 2.10–2.19 (m, 2H, H-2, -4), 2.33 (t, 2H, J = 7.4 Hz, H-6′), 2.20–2.35 (m, 1H, H-3), 2.57–2.64 (m, 2H, H-2, -4), 3.80 (s, 6H, $PhOCH_3$), 5.05 (d, 4H, J = 9.2 Hz, OCH_2Ph), 6.81–6.86 (m, 4H, aromatic H), 7.16–7.27 (m, 4H, aromatic H); ^{13}C nmr (75 MHz, $CDCl_3$) δ: 24.42, 26.35, 28.81, 28.89, 29.45, 33.84, 34.65, 36.20, 49.53, 55.05, 66.63, 66.74, 113.66, 127.56, 129.68, 159.37, 171.42, 171.74, 179.80; ms (FAB), m/e: 535 $(M + Na)^+$, 513 $(M + 1)^+$. Exact Mass calcd. for $C_{29}H_{36}NaO_8$: 535.2309; found: 535.2248.

*[2R-(2α,6α,7β)-3-[[6-(2-Benzhydryloxycarbonyl-8-oxo-7-phenylacetylamino-5-thia-1-azabicyclo[4.2.0]oct-3-en-3-yl)methoxycarbonyl]hexyl]cyclobutane-1,1-dicarboxylic acid bis(*p*-methoxybenzyl) ester (Δ^2 isomer, **16**a) and (6*R*-trans)-3-[[6-(2-Benzhydryloxycarbonyl-8-oxo-7-phenylacetylamino-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)methoxycarbonyl]hexyl]cyclobutane-1,1-dicarboxylic acid bis(*p*-methoxybenzyl) ester (Δ^3 isomer, **16**b)*

To a cold (ice-water bath) solution of **15** (350 mg, 0.683 mmol) in 50 mL of dry dichloromethane was added DCC (210 mg, 1.02 mmol), DMAP (20 mg), and then a solution of **2** (420 mg, 0.816 mmol) in 150 mL of dry dichloromethane. The mixture was stirred at room temperature overnight, and washed with water, 10% HCl, water, saturated $NaHCO_3$ solution, water, and brine. The organic layer was dried (Na_2SO_4) and evaporated to give a residue that was applied to a column of flash silica gel. Elution with ethyl acetate – hexanes (2:1) gave an inseparable mixture of the Δ^2 and the Δ^3 isomers (ratio 3:2) of **16** (532 mg, 64.6%); For Δ^2 isomer, ir ($CHCl_3$): 3320 (NH), 1780 (C=O, β-lactam), 1730 (COOR), 1680 (CONH) cm^{-1}; ^1H nmr (300 MHz, $CDCl_3$) δ: 1.15–1.58 (m, 10H, aliphatic CH_2), 2.13–2.34 (m, 5H, CH_2COOR, cyclobutane-CH_2, CH), 2.59–2.66 (m, 2H, cyclobutane-CH_2), 3.64 (ABq, 2H, J = 16.0 Hz, $PhCH_2CON$), 3.80 (s, 6H, $PhOCH_3$), 4.53, 4.59 (ABq, 2H, J_{gem} = 12.8 Hz, 3′-CH_2OCOR), 5.07 (d, 4H, J = 8.9 Hz, $COOCH_2Ph$), 5.09 (s, 1H, H-2), 5.19 (d, 1H, J = 4.0 Hz, H-6), 5.62 (dd, 1H, J = 4.0 Hz, 8.7 Hz, H-7), 6.35 (d, 1H, J = 8.7 Hz, CONH), 6.38 (d, 1H, J = 1.7 Hz, H-4), 6.82–6.87 (m, 4H, PMB-aromatic H), 6.89 (s, 1H, $COOCHPh_2$), 7.16–7.22 (m, 4H, PMB-aromatic H), 7.25–7.44 (m, 15H, aromatic H); ^{13}C nmr (75 MHz, $CDCl_3$) δ: 24.48, 26.35, 28.84, 29.43, 33.70, 34.64, 36.19, 43.08, 49.50, 50.13, 53.26, 55.04, 60.15, 65.14, 66.61, 66.73, 78.99, 113.66, 119.12, 121.66, 126.58–129.66 (multiple peaks), 133.49, 138.74, 159.37, 164.11, 165.81, 170.84, 171.37, 171.69, 172.92; ms (FAB), m/e: 1031 $(M + Na)^+$, 1009 $(M + 1)^+$. Exact Mass calcd. for $C_{58}H_{61}N_2O_{12}S$: 1009.3947; found: 1009.3824. For Δ^3 isomer, all the data, see **18**.

*(6R-trans-3-[[6-(2-Benzhydryloxycarbonyl-5,8-dioxo-7-phenylacetylamino-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)-methoxycarbonyl]hexyl]cyclobutane-1,1-dicarboxylic acid bis(*p*-methoxybenzyl) ester (**17**)*

A solution of **16** (230 mg, 0.228 mmol) in 25 mL of ethyl acetate was cooled to 0°C and a solution of *m*-chloroperbenzoic acid (50–60%, 88 mg) in 10 mL of ethyl acetate was added dropwise. After stirring at that temperature for 3 h, the solution was diluted with 50 mL of ethyl acetate and washed with saturated $NaHCO_3$ solution, brine, and dried ($MgSO_4$). The solvent was evaporated to give a syrup that was purified by flash chromatography. Elution with ethyl acetate and hexane (1:1, then 3:1) gave a colorless syrup **17** (180 mg, 77%) that crystallized on standing in a refrigerator; $[\alpha]_D$ + 18.9 (*c* 1.35, $CHCl_3$); mp 123–124 °C; ir ($CHCl_3$): 3280 (NH), 1795 (C=O, β-lactam), 1730 (COOR), 1690 (CONH) cm^{-1}; ^1H nmr (300 MHz, $CDCl_3$) δ: 1.14–1.58 (m, 10H, aliphatic CH_2), 2.15 (m, 2H, cyclobutane-CH_2), 2.25 (t, 2H, J = 7.6 Hz, CH_2COOR), 2.30 (m, 1H, cyclobutane-CH), 2.61 (m, 2H, cyclobutane-CH_2), 3.17, 3.78 (ABq, 2H, J_{gem} = 19.0 Hz, SCH_2), 3.61, 3.68 (ABq, 2H, J_{gem} = 5.6 Hz, $PhCH_2CON$), 3.81 (2 × s, 6H, $PhOCH_3$), 4.45 (dd, 1H, J = 1.5 Hz, 4.8 Hz, H-6), 4.37, 5.29 (ABq, 2H, J_{gem} = 14.3 Hz, 3′-CH_2OCOR), 5.06 (d, 4H, J = 9.5 Hz, $COOCH_2Ph$), 6.10 (dd, 1H, J = 4.8 Hz, 9.9 Hz, H-7), 6.76 (d, 1H, J = 9.9 Hz, CONH), 6.82–6.87 (m, 4H, PMB-aromatic H), 6.95 (s, 1H, $COOCHPh_2$), 7.17–7.20 (m, 4H, PMB-aromatic H), 7.27–7.48 (m, 15H, aromatic H); ^{13}C nmr (75 MHz, $CDCl_3$) δ: 24.55, 26.37, 28.86, 29.46, 33.73, 34.68, 36.18, 43.26, 45.64, 49.54, 55.11, 58.91, 62.99, 66.62, 66.68, 66.80, 80.21, 113.70, 121.34, 125.01, 126.94, 127.42, 127.48, 127.56, 128.05, 128.10, 128.37, 128.48, 128.92, 129.24, 129.71, 133.46, 138.75, 138.85, 159.43, 159.50, 163.97, 171.11, 171.43, 171.74, 172.98; ms (FAB), *m/e*: 1047 $(M + Na)^+$, 1025 $(M + 1)^+$.

*(6*R-trans*)-3-[[6-(2-Benzhydryloxycarbonyl-8-oxo-7-phenylacetylamino-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)methoxycarbonyl]hexyl]cyclobutane-1,1-dicarboxylic acid bis(*p*-methoxybenzyl) ester (**18**)*

Phosphorus trichloride (12 μL, 0.138 mmol) was added to a solution of **17** (70 mg, 0.068 mmol) in 1.5 mL of dry DMF at −22°C. After stirring at this temperature for 1 h, the slightly red solution was diluted with 20 mL of ether, and washed with cold water (2 × 5 mL) and brine. The organic phase was dried ($MgSO_4$) and evaporated to give a syrup that was purified by flash chromatography. Elution with ethyl acetate – hexanes (1:3, then 1:2) afforded the desired product **18** (66.5 mg, 96%) as a colorless syrup; $[\alpha]_D$ −1.2 (*c* 3.05, $CHCl_3$); ir ($CHCl_3$): 3320 (NH), 1790 (C=O, β-lactam), 1730 (COOR), 1680 (CONH) cm^{-1}; ^1H nmr (300 MHz, $CDCl_3$) δ: 1.16–1.59 (m, 10H, aliphatic CH_2), 2.17 (m, 2H, cyclobutane-CH_2), 2.25 (t, 2H, J = 7.6 Hz, CH_2COOR), 2.32 (m, 1H, cyclobutane-CH), 2.62 (m, 2H, cyclobutane-CH_2), 3.31, 3.50 (ABq, 2H, J_{gem} = 18.7 Hz, SCH_2), 3.61, 3.68 (ABq, 2H, J_{gem} = 16.0 Hz, $PhCH_2CON$), 3.81 (s, 6H, $PhOCH_3$), 4.79, 5.04 (ABq, 2H, J_{gem} = 13.7 Hz, 3′-CH_2OCOR), 4.95 (d, 1H, J = 4.8 Hz, H-6), 5.07 (d, 4H, J = 8.9 Hz, $COOCH_2Ph$), 5.88 (dd, 1H, J = 4.8 Hz, 9.1 Hz, H-7), 6.28 (d, 1H, J = 9.1 Hz, CONH), 6.84 (m, 4H, PMB-aromatic H), 6.94 (s, 1H, $COOCHPh_2$), 7.19 (m, 4H, PMB-aromatic H), 7.26–7.45 (m, 15H, aromatic H); ^{13}C nmr (75 MHz, $CDCl_3$) δ: 24.55, 26.22, 26.37, 28.87, 29.44, 33.73, 34.65, 39.19, 43.11, 49.50, 55.07, 57.15, 58.90, 62.63, 66.65, 66.77, 79.58, 113.66, 125.22, 126.86, 126.97, 127.48, 127.95, 128.04, 128.28, 128.40, 129.02, 129.31, 129.69, 133.47, 138.82, 138.99, 159.37, 160.42, 164.64, 171.01, 171.41, 171.72, 173.03; ms (FAB), *m/e*: 1031 $(M + Na)^+$, 1009 $(M + 1)^+$.

*(6*R-trans*)-3-[[6-(2-Carboxy-8-oxo-7-phenylacetylamino-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)methoxycarbonyl]hexyl]-cyclobutane-1,1-dicarboxylic acid (**19**)*

Trifluoroacetic acid (0.95 mL, 12.3 mmol) was added dropwise to a solution of compound **18** (250 mg, 0.248 mmol) in anisole (0.40 mL, 3.68 mmol) and dichloromethane (6 mL) with cooling (ice-water bath) and the mixture was stirred at that temperature for 2 h. After removal of the volatile components, the residue was washed with dichloromethane and ether to give white powder **19**, which was used immediately for the next reaction without purification.

*(6*R-trans*-3-[[6-(2-Carboxy-8-oxo-7-phenylacetylamino-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)methoxycarbonyl]hexyl]-cyclobutane-1,1-dicarboxylic acid potassium salt (**20**)*

To a mixture of compound **19** (0.25 mmol), ethyl acetate (3 mL), and deionized water (10 mL) was slowly added 0.105 N aqueous potassium hydroxide (2.4 mL, 0.25 mmol) at 0°C. The aqueous solution was then separated and washed with ether twice before lyophilizing to give a white powder **20** (80 mg). The organic phase was treated with 0.105 N KOH aqueous solution until pH 6.5. The aqueous layer was separated and washed with ether twice, then lyophilized to yield a further 58 mg of white powder **20**, bringing the total yield to 138 mg (87%, two steps from **18**); ^1H nmr showed that this powder was a mixture of the Δ^2 and Δ^3 (ratio 1:1.4) isomers; ^1H nmr (300 MHz, D_2O) δ: 1.24–1.68 (m, 20H, aliphatic CH_2), 2.08–2.17 (m, 4H, cyclobutane-CH_2), 2.24–2.38 (m, 2H, cyclobutane-CH), 2.46 (t, 4H, J = 7.4 Hz, CH_2COOR), 2.52–2.59 (m, 4H, cyclobutane-CH_2), 3.39, 3.66 (ABq, 2H, J_{gem} = 17.9 Hz, Δ^3-SCH_2), 3.76 (m, 4H, $PhCH_2$), 4.73, 4.89 (ABq, 2H, J_{gem} = 9.1 Hz, Δ^2-3′-CH_2O), 4.79, 4.98 (ABq, 2H, J_{gem} = 12.5 Hz, Δ^3-3′-CH_2O), 4.86 (d, 1H, J = 1.1 Hz, Δ^2-H-2), 5.15 (d, 1H, J = 4.7 Hz, Δ^2-H-6), 5.35 (d, 1H, J = 3.8 Hz, Δ^3-H-6), 5.47 (d, 1H, J = 3.8 Hz, Δ^3-H-7), 5.70 (d, 1H, J = 4.7 Hz, Δ^2-H-7), 6.52 (s, 1H, Δ^2-H-4), 7.41–7.51 (m, 10H, ArH).

*(SP-4-2)-[4-[(6*R-trans*)-(2-Carboxy-8-oxo-7-phenylacetylamino-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)methoxycarbonyl]hexyl]cyclobutane-1,1-dicarboxylato][(1*R,2R*)-cyclohexane-1,2-diamine-*N,N′*] platinum (**21**)*

(SP-4-2)-Dinitro (1,2-cyclohexanediamine-*N,N′*) platinum (16 mg, 0.036 mmol) was dissolved in 1 mL of deionized water and the trace of insoluble residue was removed by centrifugation. The supernatant was transferred into a 20-mL vial, then a solution of potassium salt **20** (23 mg, 0.036 mmol) in 1 mL of deionized water was added. The mixture was stirred at room temperature in darkness for 3 h and the resulting precipitate was filtered and washed with deionized water, then dried over P_2O_5 under vacuum to give the platinum complex **21** (21 mg, 64%) as a white powder; $[\alpha]_D$ +122 (*c* 0.50, DMSO); ^1H nmr (300 MHz, DMSO-d_6) δ: 1.00–1.50 (m, 16H, hexanyl H-1, -2, -3, -4, -5, cyclohexanyl H-4, -5, Hax-3, -6), 1.88–2.02 (m, 4H, cyclobutane H-2, -4, cyclohexanyl Heq-3, -6), 2.12–2.55 (m, 7H, cyclobutane H-2, -3, -4, hexanyl H-6, cyclohexanyl H-1, -2), 3.3–3.7 (m, 4H, $PhCH_2$, SCH_2), 4.55–6.45 (m, 8H, H-6, H-7, RCO_2CH_2, NH_2), 7.2–7.35 (m, 5H, ArH). Anal. calcd. for $C_{35}H_{46}N_4O_{10}PtS \cdot 3H_2O$: C 43.61, H 5.44, N 5.81; found: C 43.82, H 5.29, N 5.56.

*3-Acetoxymethyl-8-oxo-7-phenylacetylamino-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid potassium salt (**22**)*

To a suspension of 7-aminocephalosporanic acid (7-ACA) (1.20 g, 4.40 mmol) in 40 mL of mixed solvent (acetone–water 1:1) was carefully added $NaHCO_3$ (0.930 g, 11.0 mmol) with cooling (ice-water bath). After the addition was complete, the mixture was warmed to room temperature and stirred until the suspended solid had dissolved in the solvent. The reaction mixture was cooled (0°C) again and a solution of phenyl acetyl chloride (0.87 mL, 6.6 mmol) in 20 mL of acetone was slowly added. Stirring was continued at room temperature overnight. After removal of the solvent, the residue was overlaid with ethyl acetate and acidified with 1 N HCl. The separated aqueous phase was extracted with ethyl acetate three times and the combined extracts were washed with cold water and brine, dried with anhydrous sodium sulfate, and evaporated to give the crude phenylacetylaminocephalosporanic acid. To the residue, dissolved in 150 mL of ethyl acetate, was added, with manual stirring, potassium 2-ethyl hexanoate (0.5 M solution in ethyl acetate) (8.8 mL, 4.4 mmol). The resulting precipitate was filtered and washed with ethyl acetate, then dried under vacuum to afford the desired product **22** (1.56 g, 83%) as yellow crystals; ^1H nmr (300 MHz, deuterated carbonate buffer, pD 10.1) δ: 2.09 (s, 3H, CH_3), 3.34, 3.60 (ABq, 2H, J_{gem} =

17.9 Hz, SCH_2), 3.65, 3.72 (ABq, 2H, J_{gem} = 14.9 Hz, $PhCH_2$), 4.69, 4.86 (ABq, 2H, J_{gem} = 12.7 Hz, CO_2CH_2), 5.07 (d, 1H, J = 4.7 Hz, H-6), 5.62 (d, 1H, J = 4.7 Hz, H-7), 7.32–7.43 (m, 5H, ArH); ^{13}C nmr (75 MHz, D_2O) δ: 18.68, 23.80, 40.37, 55.70, 57.56, 62.53, 114.52, 125.80, 127.31, 127.60, 129.84, 133.01, 163.13, 166.84, 172.40, 173.45; ms (FAB), *m/e*: 428 $(M)^+$. Exact Mass calcd. for $C_{18}H_{17}KN_2O_6S$: 428.0445; found: 428.0468.

Preparation of 0.2 M phosphate buffer (pD 7.2)

A solution containing Na_2HPO_4 (204.5 mg, 1.44 mmol) and $NaH_2PO_4 \cdot 2H_2O$ (87.4 mg, 0.56 mmol) in 2 mL of deuterated water was lyophilized to dryness and the resulting white crystalline residue was dissolved in 10 mL of deuterated water.

Preparation of 0.25 M carbonate buffer (pD 10.1)

A solution containing Na_2CO_3 (159 mg, 1.50 mmol) and $NaHCO_3$ (84 mg, 1.00 mmol) in 2 mL of deuterated water was lyophilized to dryness and the resulting white residue was dissolved in 10 mL of deuterated water.

Preparation of β-lactamase buffer solution

The commercially available (Sigma Chemical Co.) penicillinase (EC 3.5.2.6) type III, from *Enterobacter cloacae* (67 units/mg protein for cepholoridine) was used as hydrolytic enzyme. To the original vial containing 0.15 mg (~10 units) protein was added 0.6 mL of deuterated phosphate buffer (pD 7.2, 0.2 M). The solution (10 units/0.6 mL) was kept in 0–5°C and was used for nmr studies. One unit will hydrolyze 1.0 μmol of cephaloridine per minute at pH 7.0 at 25°C.

Enzymatic hydrolysis of **22** *in 0.2 M phosphate buffer (pD 7.2)—(^1H nmr study)*

Potassium cephalosporinate **22** (5 mg, 11.6 μmol) was dissolved in 0.2 M deuterated sodium phosphate buffer (0.6 mL, pD 7.2) at 20°C. The ^1H nmr spectrum was taken at this temperature and then 0.2 mL (3.3 units) of β-lactamase buffer solution was added. The ^1H nmr spectrum at 20°C was taken immediately; ^1H nmr (300 MHz, deuterated phosphate buffer, pD 7.2) δ: 1.88 (s, 3H, CH_3), 3.35, 3.72 (ABq, 2H, J_{gem} = 14.7 Hz, SCH_2), 3.59, 3.65 (ABq, 2H, J_{gem} = 14.8 Hz, $PhCH_2$), 4.61 (d, 1H, J = 3.0 Hz, H-6), 5.41 (d, 1H, J = 3.0 Hz, H-7), 5.60 (s, 1H, C=CH_2), 5.65 (s, 1H, C=CH_2), 7.27–7.40 (m, 5H, PhH).

Enzymatic hydrolysis of **22** *in 0.25 M carbonate buffer (pD 10.1)—(^1H nmr study)*

Potassium cephalosporinate **22** (5 mg, 11.6 μmol) was dissolved in 0.25 M deuterated sodium carbonate buffer (0.6 mL, pD 10.1) at 20°C. The ^1H nmr spectrum was taken immediately and, subsequently, after 40 min. Then 0.2 mL (3.3 units) of β-lactamase buffer solution was added and the ^1H NMR spectrum was taken immediately (Fig. 2 **C**).

Enzymatic hydrolysis of **8** *in 0.25 M carbonate buffer (pD10.1)—(^1H nmr study)*

A suspension containing 10 mg of platinum complex **8** was stirred in 0.8 mL of deuterated carbonate buffer (pD 10.1) at 0–5°C for 30 min and the mixture was centrifuged. The supernatant was lyophilized and the resulting white powder was dissolved in 0.6 mL of D_2O. The ^1H nmr spectrum was taken immediately (Fig. 2 **A**). To this sample was added 0.1 ml (5 units) of β-lactamase buffer solution. The ^1H nmr was taken immediately at room temperature (Fig. 2 **B**): ^1H nmr (300 MHz, deuterated carbonate buffer, pD 10.1) δ: 1.1–1.4 (br m, 4H, cyclohexane-CH_2), 1.53–1.68 (br m, 2H, cyclohexane-CH_2), 1.95–2.14 (br m, 2H, cyclohexane-CH_2), 2.35 (br m, 2H, cyclohexane-H-1,2), 7.48 (d, 1H, J = 7.8 Hz, aromatic H-5), 7.83 (dd, 1H, J = 1.6 Hz, 7.8 Hz, aromatic H-6), 7.91 (d, 1H, J = 1.5 Hz, aromatic H-2); and 3.93, 3.75 (ABq, 2H, J_{gem} = 14.8 Hz, SCH_2), 3.60–3.71 (m, 2H, $PhCH_2$), 4.64 (d, 1H, J = 3.0 Hz, H-6), 5.44 (d, 1H, J = 3.0 Hz, H-7), 5.63 (s, 1H, C=CH_2), 5.68 (s, 1H, C=CH_2), 7.30–7.42 (m, 5H, ArH).

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