Brief Articles

A Light-Inactivated Antibiotic

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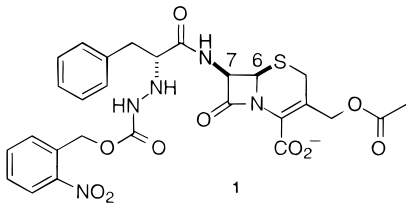
Sodium 7β-[(*R*)-2-(N^b-*o*-nitrobenzyloxycarbonyl)hydrazino-3-phenylpropanamido]cephalosporanate (**1**) is described as a new type of β-lactam antibiotic, which undergoes light-induced destruction of its β-lactam moiety and hence becomes biologically inactive. This type of antibiotic holds the promise of self-destruction over a number of hours of exposure to light, so that it would not allow selection of resistance in the environment.

Introduction

Antibiotics are among the most prescribed pharmaceuticals worldwide. Approximately 160 million prescriptions for antibiotics were written in 1996 in the United States alone, and in excess of 50 million pounds of these drugs were produced for animal feeds and human use.[1] Antibiotics, in general, are not metabolized in the body of animals or humans and are subsequently introduced to the environment. These antibiotics continue to kill the nonresistant bacteria in the environment (sewer, soil, etc.) and favor selection of resistant organisms. In the United States, as well as other parts of the globe, resistant fecal strains of bacteria are distributed to agricultural lands by the spread of manure from the antibiotic-fed farm animals as fertilizer. It is in this manner that the spread of resistant bacteria into the ecosystem is accelerated. Ultimately, the resistant organisms—now effectively selected—return to human populations as pathogens that are difficult to treat. Levy accounts a narrative that antibiotics from animal feed ultimately selected resistant bacteria, which were traced from farm animals to farm workers to agricultural products (i.e., fruits and vegetables) and even to a common house fly.[2] The fact that massive quantities of antibiotics are used annually and that these antibiotics survive in the environment for extended periods of time presents a problem.

We present here the concept for a new type of β-lactam antibiotic that has in its structure a functionality that unmasks *over several hours* of exposure to light (e.g., artificial light or sunshine). This light-induced unmasking generates a reactive functionality, which destroys the β-lactam moiety and hence abolishes its antibiotic property. In principle, this property of this type of antibiotic would limit its availability and exposure time to the environment and hence would make minimal the undue selection pressure for resistance. The concept is general, and one may envision other factors than light that could trigger such a self-destructive reaction (vide infra).



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Compound **1** is one such antibiotic of our design. This compound is an analogue of cephalosporanic acid, which has been modified at the $C_{7\beta}$ position with a moiety that contains a hydrazine function protected by the *o*-nitrobenzyloxycarbonyl (i.e., *o*-nitrobenzylcarbamate, *o*-NBC) group. The *o*-NBC group may be removed over several hours (typically 10−16 h) by irradiation at the UV−vis wavelength range;[3] the λ_{max} for the chromophore centers at 320 nm, but the chromophore trails into the visible range. As a result, *o*-NBC-protected compounds are slightly yellow. In the presence of light (e.g., sunshine), the *o*-NBC group is removed in several hours. The unprotected hydrazine is a supernucleophile,[4] which would readily react with the lactam carbonyl in an intramolecular fashion in dilute solutions. Such a reaction would result in the scission of the β-lactam moiety, which would abolish the antibacterial property (**1** → **2** → **3**). We decided in favor of the hydrazine moiety instead of an amine because of supernucleophilicity of the former. The same reaction with an amine would take place,[5] however, since the β-lactam moiety of the cephalosporin is relatively resistant to nucleophilic addition—because of the conjugation of the lactam nitrogen lone pair of electrons into the lactam carbonyl and the olefin π systems—the reaction with hydrazine would be considerably faster.[6,7]

Results and Discussion

Compound **1** was synthesized according to Scheme 1, which employs a stereoselective amination of an Evans-type chiral enolate as its central feature.[8] The lithium enolate of **4** underwent reaction virtually instantaneously with di-*tert*-butyl azodicarboxylate (DBAD) at −78 °C to give **5** in good yield. Compound **5** was then hydrolyzed to provide **6**. After deprotection of **6** by

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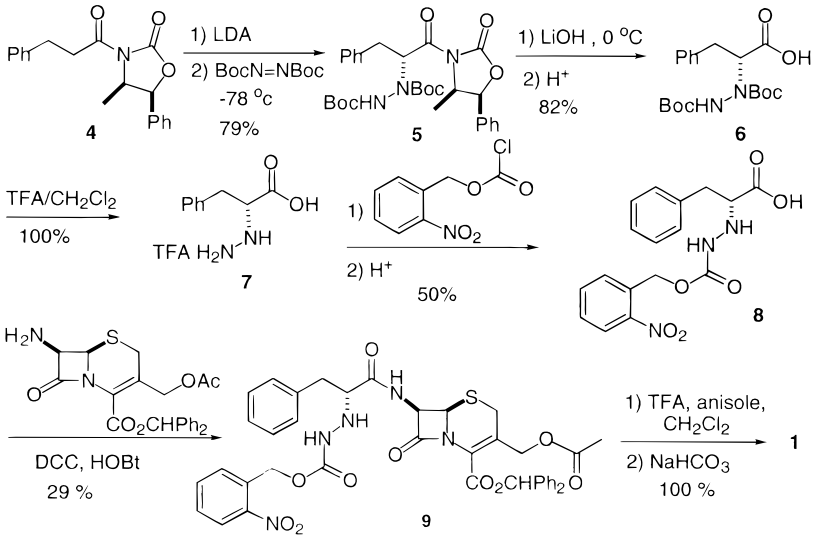
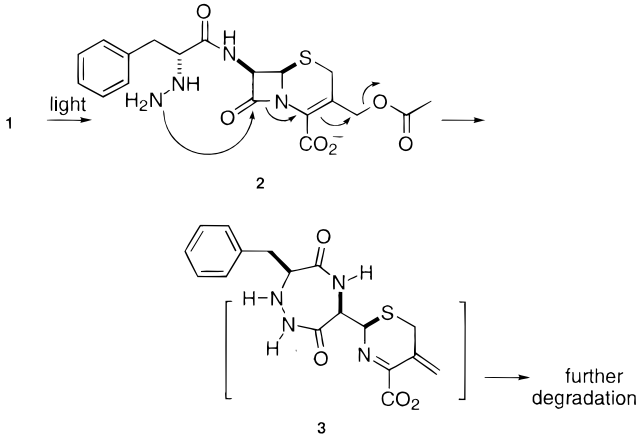
Scheme 1





trifluoroacetic acid (TFA), the resulting hydrazine **7** was protected with 2-nitrobenzyloxycarbonyl chloride, which was in turn prepared by reaction of 2-nitrobenzyl alcohol and phosgene.[9] A dicyclohexylcarbodiimide (DCC) coupling between the resulting acid **8** and diphenylmethyl ester of 7β-aminocephalosporanic acid, followed by removal of the diphenylmethyl group with TFA, produced the desired **1**.

As anticipated, compound **1** was photolabile. The compound was irradiated at 350 nm in a Pyrex vessel (RPR-100 photochemical reactor, Rayonet, Hg lamps; 1.0 mL of 10 μM **1** in 100 mM phosphate buffer, pH 7.0). Within 10 h of irradiation, the product mixture was analyzed by HPLC. The end result of irradiation showed no trace of the starting material but was constituted of a complex mixture of several compounds. NMR of the crude product was also complex, and one did not see any peaks corresponding to the cephalosporin nucleus. HPLC (Vydac C_4 column, acetonitrile/water as solvent system) was employed to separate the mixture into three major, but unidentifiable, components. These observations are consistent with a complicated sequence of events ensuing the opening of the β-lactam moiety of cephalosporins.[10]

We also monitored the time course of the irradiation experiment (Figure 1). During the photolysis experiment, the *o*-NBC protective group is removed by being converted to *o*-nitrosobenzaldehyde. One sees the formation of the aldehydic proton during the NMR experiment (δ 9.82). Another feature of the time-course experiment is the diminution and the ultimate disappearance of the two doublets at δ 5.61 and 4.98 for the C_7 and C_6 protons, respectively. This observation is indicative of the loss of the β-lactam moiety, which is consistent with earlier literature reports.[5,10] In general, the signals corresponding to compound **1** disappear as a function of time, corresponding to the chemical events that are set in motion by the scission of the β-lactam moiety. The signals disappeared as the resultant fragments from the cephalosporin nucleus are prone to polymerization.[5,10] An identical experiment was carried out with cephalothin (the structure is similar to **1**, except the $C_{7\beta}$ substituent is the thienylacetamido group). There was no change in the spectrum of cephalothin after the same duration of exposure to irradiation.

Compound **1** behaves in many ways as a typical cephalosporin. It served as a substrate for β-lactamases, bacterial resistance enzymes which degrade β-lactam drugs. The turnover kinetics for the class A TEM-1 β-lactamase and the class C Q908R β-lactamase, respectively, are as follows: $k_{cat} = 7.4 \pm 0.7\ s^{-1}$, $K_m = 80 \pm 10\ \mu M$, and $k_{cat}/K_m = (9.3 \pm 1.5) \times 10^4\ M^{-1}\ s^{-1}$; and $k_{cat} = 1769 \pm 304\ s^{-1}$, $K_m = 62 \pm 14\ \mu M$, and $k_{cat}/K_m = (2.8 \pm 0.8) \times 10^7\ M^{-1}\ s^{-1}$. The compound also exhibited antibacterial property against laboratory strains of several bacteria, as evaluated by the values for the minimal inhibitory concentrations (MIC). The organisms and the corresponding MIC (in μg/mL) were *Klebsiela pneumoniae* ATCC 10031, 32; *Enterococcus faecalis* ATCC 29212, >32; *Staphylococcus aureus* ATCC 25923, 1; and *Bacillus subtilis* ATCC 33608, 2. The compound shows more activity against the Gram-positive organisms (the latter two) than the Gram-negative ones.

Compound **1**, disclosed herein, represents the prototype molecule of its kind. The concept behind the design of compound **1** is general and can be exploited in many different ways, and the present report is a demonstration of the proof of concept. One can envision molecules that are sensitive to prolonged exposure to moisture or may be sensitive to the pH of the environment to which they are exposed, among other possibilities. Antibiotics

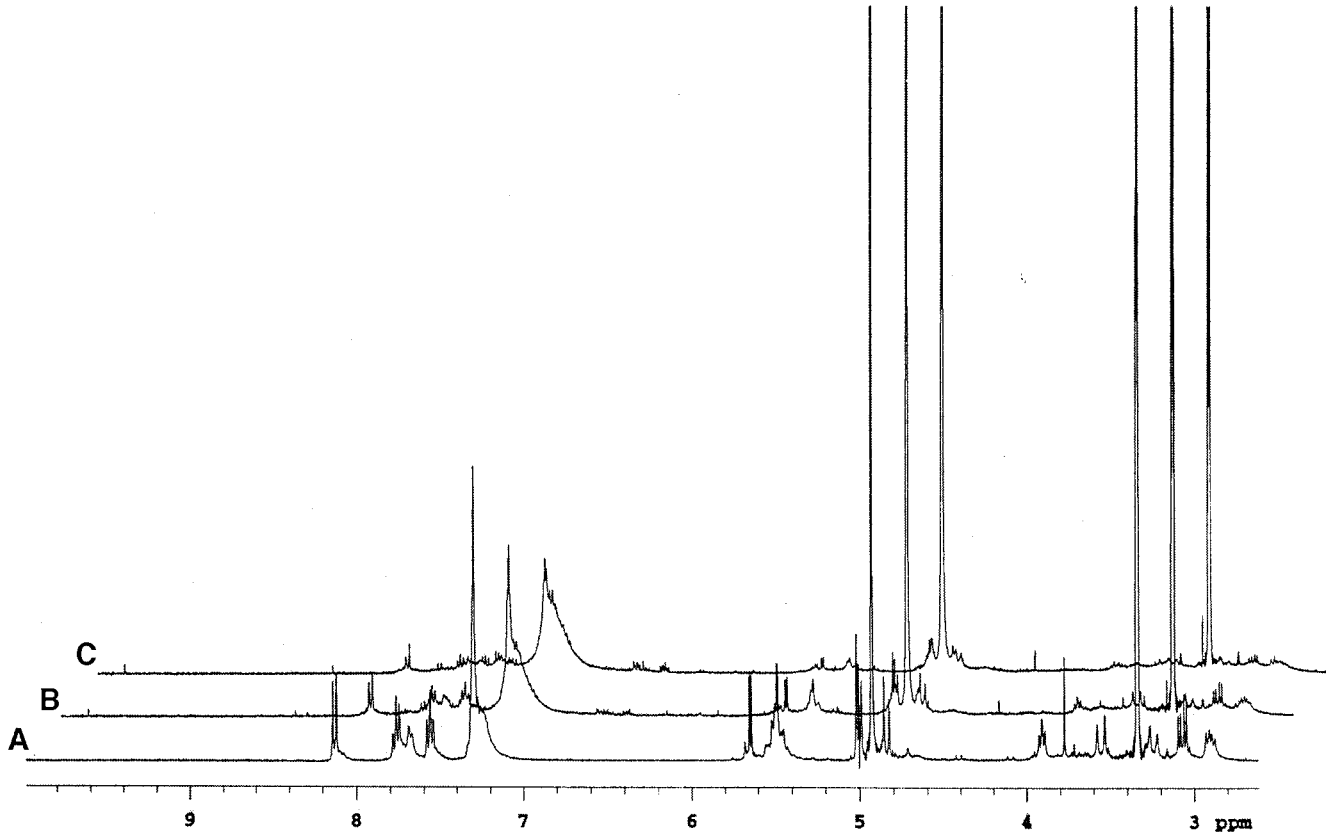


Figure 1. (A) Proton NMR of 18 mM compound **1** in methanol-d_4 (δ 3.33 and 4.93 signals correspond to those of methanol; for a complete assignment of the resonances consult the Experimental Section). Spectra for the solution of **1** after 165 min (B) and 420 min (C) of photolysis are also depicted. The resonances corresponding to **1** disappear as a function of time, and a few new signals appear as cephalosporin fragmentation results in a mixture of components, which ultimately polymerize.

of this type can be prepared where the functionality at the $C_{7\beta}$ position of cephalosporin may be incorporated with groups that impart resistance to the action of the resistance enzymes, such as seen in expanded-spectrum cephalosporins.[11] The concept is, of course, generalizable to other classes of antibiotics as long as suitable intramolecular reactions could be identified, which would destroy the antibiotic nucleus, rendering it inactive and incapable of selection of resistant organisms in nature.

Experimental Section

^{1}H and ^{13}C NMR spectra were recorded on either a Varian Gemini-300 or a Varian Unity-500 spectrometer. Chemical shifts are reported in ppm from tetramethylsilane on the δ scale. Infrared and mass spectra were recorded on Nicolet 680 DSP and Kratos MS 80RFT spectrometers, respectively. Melting points were taken on an Electrothermal melting point apparatus and are uncorrected. Thin-layer chromatography was performed with Whatman Reagents 0.25-mm silica gel 60-F plates. The wild-type TEM-1 β-lactamase was purified according to the method of Zafaralla et al.,[12] and the Q908R β-lactamase was purified by the procedure of Ross.[13] Kinetic measurements were carried out on a Hewlett-Packard Vectra XM instrument. All other reagents were purchased from the Aldrich Chemical Co. Enzyme kinetic parameters were determined according to procedures described elsewhere.[12]

(4*R*,5*S*)-3-((*R*)-2-*N*,*N*′-Bis-*tert*-butoxycarbonylhydrazino-3-phenyl-1-oxopropyl)-4-methyl-5-phenyl-2-oxazolidinone (5). *n*-Butyllithium (2.5 M in hexanes, 2.2 mL, 5.5 mmol) was added dropwise to a solution of diisopropylamine (0.77 mL, 5.5 mmol) in anhydrous THF (40 mL) at −20 °C, and the mixture was stirred at −20 °C for 30 min. The solution was then cooled to −78 °C and a solution of *N*-acyloxazolidinone **(4)**[8a] (1.545 g, 5 mmol) in dry THF (5 mL) was added via cannula. The mixture was stirred at −78 °C for 1 h, at which time a precooled solution of di-*tert*-butyl azodicarboxylate (1.38 g, 6 mmol) in dry CH_2Cl_2 (30 mL) was added to it via cannula over 10 min, followed by an additional 5 min of stirring. Glacial acetic acid (0.7 mL, 12.5 mmol) was added to quench the reaction. The solution was concentrated in vacuo. The residue was dissolved in EtOAc, washed with water, 5% $NaHCO_3$, and saturated NaCl, and then dried over $MgSO_4$. The solvent was removed in vacuo and the residue was purified by column chromatography (silica gel, hexane:EtOAc, 4:1) to give a pale yellow solid (2.14 g, 79%): mp 181−184 °C; IR (KBr) 3326, 2980, 2914, 1783, 1741 1724, 1698 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.84 (d, J = 6.3 Hz, 3H), 1.32−1.46 (m, 18H), 3.22 (m, 2H), 4.49 (m, 1H), 5.22 (br m, 1H), 6.33 (br m, 1H), 7.2−7.4 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 14.9, 28.8, 28.9, 36.3, 55.7, 61.1, 79.7, 79.9, 81.6, 81.8, 82.8, 126.3, 127.6, 129.0, 129.4, 129.5, 130.0, 130.4, 133.8, 137.0, 152.8, 155.6, 156.5; EI HRMS 539.2631 (M^+, calcd for $C_{29}H_{37}N_3O_7$ 539.2631).

(*R*)-2-*N*,*N*′-Bis-*tert*-butoxycarbonylhydrazino-3-phenylpropanoic Acid (6). A solution of LiOH (0.33 g, 7.766 mmol) in water (15 mL) was slowly added to a solution of the hydrazide **5** (1.82 g, 3.38 mmol) in THF (30 mL) at 0 °C, and the mixture was stirred at that temperature for 3 h. The organic solvent was removed in vacuo and the aqueous solution was washed with CH_2Cl_2, acidified with HCl to pH 2, and then extracted with EtOAc. The combined EtOAc portion was washed with water and saturated NaCl and dried over $MgSO_4$. Removing the solvent in vacuo gave a white solid (1.06 g, 82%): mp 93−95 °C; IR (KBr) 3281, 2971, 2929, 1717, 1338, 1146 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.40−1.49 (m, 18H), 3.14 (br t, J = 14 Hz, 1H), 3.40 (dd, J = 14, 4.5 Hz, 1H), 4.32 (br s, 1H), 6.24 (br s, 1H), 7.15−7.35 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 28.7, 36.1, 54.2, 85.1, 85.2, 127.6, 129.4, 129.5, 138.1, 154.2, 160.0, 171.9; EI HRMS 280.1420 ($M^+ - C_5H_8O_2$, calcd for $C_{14}H_{20}N_2O_4$ 280.1423).

(*R*)-2-Hydrazino-3-phenylpropanoic Acid TFA Salt (7).

Trifluoroacetic acid (10 mL) was added to a solution of **6** (0.98 g, 2.58 mmol) in CH_2Cl_2 (10 mL) at room temperature, followed by 50 min of stirring at that temperature. The solvent was removed in vacuo to give the product as a solid (0.54 g, 100%): mp 215−218 °C; IR (KBr) 3311, 3065−2513 (br), 1614, 1594, 1428, 1355 cm^{-1}; ^1H NMR (CD_3OD) δ 2.98 (dd, J = 14, 8 Hz, 1H), 3.17 (dd, J = 14, 5 Hz, 1H), 3.91 (dd, J = 8, 5 Hz, 1H), 7.22−7.29 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 37.5, 63.3, 128.1, 129.6, 130.5, 137.6, 174.4; EI HRMS 180.0895 (M^+, calcd for $C_9H_{12}N_2O_2$ 180.0899).

(*R*)-2-*N*^{b}-*o*-Nitrobenzyloxycarbonylhydrazino-3-phenylpropanoic Acid (8). A solution of **7** (90 mg, 0.5 mmol) in 1:1 dioxane/water (10 mL) and 1 N NaOH (0.5 mL, 0.5 mmol) was stirred at ice−water temperature. Additional 1 N NaOH (0.55 mL, 0.55 mmol) and a solution of 2-nitrobenzyl chloroformate[9] (0.12 g, 0.55 mmol in 7 mL of dioxane) were added to the mixture gradually. After the addition of the reagents, the solution was stirred at that temperature for an additional 30 min. Dioxane was removed in vacuo, the aqueous solution was acidified to pH 2 with 2 N HCl, and the product was extracted into CH_2Cl_2. The combined organic portion was washed with saturated NaCl and was dried over $MgSO_4$. The solvent was removed in vacuo to give the crude product (90 mg, 50%), which was used in the next step without further purification: ^1H NMR (CD_3OD) δ 3.03 (br m, 2H), 3.87 (br s, 1H), 5.49 (s, 2H), 7.22−7.29 (m, 5H), 7.55−8.12 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 37.12, 63.81, 64.59, 125.07, 125.16, 126.98, 128.71, 129.04, 129.15, 129.68, 134.29, 137.78, 148.02.

Benzhydryl 7β-((*R*)-2-*N*^{b}-*o*-Nitrobenzyloxycarbonylhydrazino-3-phenylpropanamido)cephalosporanate (9). A solution of **7** (0.15 g, 0.42 mmol) in THF (8 mL) was added to a solution of benzhydryl 7-aminocephalosporate[14] (0.20 g, 0.45 mmol), DCC (0.08 g, 0.41 mmol), and HOBt (0.056 g, 0.42 mmol) in CH_2Cl_2 (12 mL), and the mixture was stirred at room temperature for 24 h. The precipitate was filtered and the filtrate was concentrated under reduced pressure. A 50-mL portion of EtOAc was added to precipitate another crop of DCU, which was then filtered out. The product was passed through a short column of silica gel to give the crude product as a white solid (95 mg, 29%), which was used in the next step without further purification: ^1H NMR ($CDCl_3$) δ 2.00 (s, 3H), 2.75 (dd, J = 14, 10.5 Hz, 1H), 3.25 (dd, J = 14, 4 Hz, 1H), 3.37 (d, J = 18 Hz, 1H), 3.53 (d, J = 18 Hz, 1H), 3.91 (m, 1H), 4.79 (d, J = 13.5 Hz, 1H), 5.01 (m, 2H), 5.46 (s, 2H), 5.79 (dd, J = 9, 5 Hz, 1H), 6.53 (br s, 1H), 6.95 (s, 1H), 7.28−8.06 (m, 19H); ^{13}C NMR ($CDCl_3$) δ 20.6, 26.6, 37.3, 57.6, 59.2, 62.9, 64.0, 65.4, 79.8, 124.9, 126.8, 127.0, 127.4, 127.6, 128.1, 128.2, 128.4, 128.5, 128.8, 129.0, 133.8, 135.9, 139.0, 139.2, 156.3, 160.6, 164.2, 170.5, 172.6.

Sodium 7β-((*R*)-2-*N*^{b}-*o*-Nitrobenzyloxycarbonylhydrazino-3-phenylpropanamido)cephalosporanate (1). Anisole (1 mL) and TFA (2 mL) were added to a solution of **9** (40 mg) in CH_2Cl_2 (10 mL) at 0 °C, and the mixture was stirred for 3 h at that temperature. After removal of solvent in vacuo, $NaHCO_3$ (5 mg) in water (5 mL) was added to the residue and the mixture was stirred at room temperature for 10 min. Water was removed in vacuo to give the product as a white solid (40 mg, 99%): mp 140 °C dec; IR (KBr) 3158−3680 (br), 1760, 1724, 1701, 1604, 1520 cm^{-1}; ^1H NMR (CD_3OD) δ 2.03 (s, 3H, CH_3CO-), 2.87 (dd, J = 14, 7 Hz, 1H**,** benzyl CH*H*), 3.05 (dd, J = 14, 7 Hz, 1H**,** benzyl C*H*H), 3.22 (d, J = 17.5 Hz, 1H, C_2-H), 3.53 (d, J = 17.5 Hz, 1H, C_2-H′), 3.88 (br t, J = 7 Hz, 1H, CH on C_7-side chain), 4.82 (d, J = 12.5 Hz, 1H, C_{10}-H), 4.98 (m, 2H, C_{10}-H′ and C_6-H), 5.46 (br s, 2H, *o*-nitrobenzyl CH_2), 5.61 (d, J = 4.5 Hz, 1H, C_7-H), 7.21−8.11 (m, 9H, aromatic); ^{13}C NMR (CD_3OD) δ 19.99, 26.03, 37.75, 57.96, 59.70, 63.74, 64.73, 115.79, 125.10, 127.19, 128.96, 129.10, 129.59, 133.37, 134.46, 164.06, 172.03, 174.90; HRFAB 612.1374 (M^- + H, calcd for $C_{27}H_{27}N_5O_{10}S$ 612.1400). Anal. ($C_{27}H_{34}N_5NaO_{14}S$−compound plus 4 waters of hydration) C, N; H: calcd, 4.84; found, 4.09.

Determination of MICs. The MICs for **1** were determined by the broth microdilution method with inocula of 5×10^5 colony-forming units per milliliter in Mueller-Hinton broth.[15] The bacterial strains used were *K. pneumoniae* ATCC 10031, *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, and *B. subtilis* ATCC 33608. The MIC was defined as the lowest concentration of antibiotic that prevented growth, which was determined by the appearance of turbidity after 24 h of incubation at 37 °C.

Photodegradation of Compound 1. Compound **1** (10 mg) dissolved in 1 mL of phosphate buffer (100 mM, pH 7.0) in a Pyrex vessel was irradiated at 350 nm (64 W, Hg lamp) in a Rayonet RPR-100 photoreactor for 10 h at ∼35 °C (normal operating temperature in the reactor). The resultant yellow reaction mixture was concentrated to one-half volume in vacuo, treated with active charcoal, and filtered, and the filtrate was evaporated to dryness to obtain the crude product. The TLC and HPLC showed no trace of starting material, but it was constituted of several different products. The NMR gave a complex pattern for which no specific structure could be assigned.

NMR Experiment of the Photoreaction. A CD_3OD solution of compound **1** (10 mg in 0.8 mL) in an NMR tube (Pyrex) was irradiated at 350 nm (64 W) in a Rayonet RPR-100 photoreactor (Hg lamp) at ∼35 °C. The light-induced degradation of the compound was monitored by taking its NMR spectra every 30 min until the spectrum showed the absence of **1**. Whenever this compound was handled outside the photoreactor, it was protected from light.

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