(s, 3 H), 3.3–3.1 (m, 2 H), 2.0 (s, 3 H); ^{13}C NMR δ 169.6, 158.9, 140.8, 133.3, 132.8, 128.2, 127.2, 126.4, 126.0, 114.5, 54.9, 52.8, 41.6, 22.7; MS *m/z* (relative intensity) 301 (4), 244 (2), 243 (7), 242 (42), 197 (3), 162 (5), 148 (25), 106 (100), 43 (10). Anal. Calcd for $C_{17}H_{19}NO_2S$: C, 67.75; H, 6.35; N, 4.65. Found: C, 67.61; H, 6.41; N, 4.70.

2-Acetamido-1-[(4-methoxyphenyl)thio]octane (2): mp 83–85 °C; ^{1}H NMR δ 7.32 and 6.75 (AA′BB′, 4 H), 5.9 (d, 1 H, *J* = 9.0 Hz), 4.1–3.9 (m, 1 H), 3.7 (s, 3 H), 2.9 (d, 2 H, *J* = 5.5 Hz), 1.7 (s, 3 H), 1.6–1.0 (m, 10 H), 0.8 (t, 3 H, *J* = 7.0 Hz); ^{13}C NMR δ 169.4, 158.9, 132.9, 126.5, 114.6, 55.1, 49.0, 40.5, 33.4, 31.5, 28.9, 25.7, 23.0, 22.4, 13.8; MS *m/z* (relative intensity) 309 (9), 252 (4), 251 (12), 250 (69), 170 (28), 142 (3), 141 (6), 140 (46), 139 (30), 114 (100), 43 (39). Anal. Calcd for $C_{17}H_{27}NO_2S$: C, 65.98; H, 8.79; N, 4.53. Found: C, 66.06; H, 8.70; N, 4.49.

3-Acetamido-4-[(4-methoxyphenyl)thio]hexane (3): mp 56–58 °C; ^{1}H NMR δ 7.38 and 6.82 (AA′BB′, 4 H), 5.63 (d, 1 H, *J* = 9.1 Hz), 4.1 (ddt, 1 H, *J* = 3.5, 9.1, and 9.9 Hz), 3.8 (s, 3 H), 3.05 (ddd, 1 H, *J* = 3.5, 6.1, and 8.4 Hz), 1.71 (s, 3 H), 1.8–1.3 (m, 4 H), 1.07 (t, 3 H, *J* = 7.2 Hz), 0.9 (t, 3 H, *J* = 7.2 Hz); ^{13}C NMR δ 169.5, 159.2, 134.1, 126.8, 114.7, 58.9, 55.2, 53.8, 26.3, 23.1, 22.7, 12.3, 10.7; MS *m/z* (relative intensity) 281 (9), 224 (4), 223 (10), 222 (68), 182 (11), 181 (15), 142 (15), 141 (4), 140 (16), 139 (74), 100 (39), 58 (100), 43 (17). Anal. Calcd for $C_{15}H_{23}NO_2S$: C, 64.02; H, 8.24; N, 4.98. Found: C, 64.11; H, 8.31; N, 5.04.

1-Acetamido-2-[(4-methoxyphenyl)thio]cyclohexane (4): mp 131–134 °C; ^{1}H NMR δ 7.4 and 6.85 (AA′BB′, 4 H), 5.76 (d, 1 H, *J* = 7.6 Hz), 3.74 (s, 3 H), 3.61 (ddt, 1 H, *J* = 4.0, 7.6, and 10.4 Hz), 2.64 (dt, 1 H, *J* = 3.6 and 10.4 Hz), 2.25–1.95 (m, 2 H), 2.0 (s, 3 H), 1.8–1.6 (m, 2 H), 1.5–1.2 (m, 4 H); ^{13}C NMR δ 169.3, 159.8, 136.4, 123.1, 114.5, 55.2, 52.5, 52.2, 33.6, 33.0, 25.9, 24.5, 23.5; MS *m/z* (relative intensity) 280 (1), 279 (9), 225 (5), 221 (15), 220 (100), 140 (40), 139 (14), 98 (29), 81 (26), 43 (18). Anal. Calcd for $C_{15}H_{21}NO_2S$: C, 64.49; H, 7.58; N, 5.01. Found: C, 64.38; H, 7.52; N, 4.96.

2-[[(4-Methoxyphenyl)thio]methyl]tetrahydrofuran (5):[12] oil; ^{1}H NMR δ 7.38 and 6.8 (AA′BB′, 4 H), 3.95 (quintet, 1 H, *J* = 6.4 Hz), 3.92–3.65 (m, 2 H), 3.75 (s, 3 H), 3.04 (dd, 1 H, *J* = 5.8 and 13.0 Hz), 2.83 (dd, 1 H, *J* = 6.8 and 13.0 Hz), 2.1–1.8 (m, 3 H), 1.7–1.5 (m, 1 H); ^{13}C NMR δ 158.8, 133.0, 126.5, 114.5, 77.8, 68.0, 55.1, 40.9, 30.8, 25.7; MS *m/z* (relative intensity) 226 (2), 225 (5), 224 (39), 154 (17), 139 (12), 71 (100), 43 (22).

2-Methyl-5-[[(4-methoxyphenyl)thio]methyl]tetrahydrofuran (6): oil; ^{1}H NMR (from the spectrum of the mixture the absorptions due to the two isomers could be distinguished with the help of decoupling experiments) δ 7.38 and 6.72 (AA′BB′, 4 H), 4.2–4.05 (m, 2 H), 3.8 (s, 3 H), 3.05 (dd, 1 H, *J* = 5.5 and 13.0 Hz), 2.81 (dd, 1 H, *J* = 7.3 and 13.0 Hz), 2.2–1.8 (m, 2 H), 1.6–1.3 (m, 2 H), 1.19 (d, 3 H, *J* = 6.2 Hz); δ 7.38 and 6.72 (AA′BB′, 4 H), 4.05–3.9 (m, 2 H), 3.8 (s, 3 H), 3.07 (dd, 1 H, *J* = 5.4 and 13.1 Hz), 2.84 (dd, 1 H, *J* = 7.2 and 13.1 Hz), 2.2–1.8 (m, 2 H), 1.8–1.6 (m, 2 H), 1.23 (d, 3 H, *J* = 6.1 Hz); ^{13}C NMR δ 158.8, 132.9, 132.6, 126.6, 114.5, 78.0, 77.3, 75.8, 75.1, 55.2, 41.3, 33.7, 32.8, 31.6, 30.8, 21.3, 21.1; MS *m/z* (relative intensity) 238 (1), 173 (45), 172 (65), 157 (6), 156 (17), 155 (100), 123 (41), 108 (31), 83 (24), 82 (27), 55 (82); 238 (1), 173 (47), 172 (65), 157 (5), 156 (15), 155 (100), 123 (39), 108 (31), 83 (24), 82 (27), 55 (81). Anal. Calcd for $C_{13}H_{18}O_2S$: C, 65.51; H, 7.61. Found: C, 65.55; H, 7.57.

2-[[(4-Methoxyphenyl)thio]methyl]tetrahydro-2*H*-pyran (7):[12] oil; ^{1}H NMR δ 7.32 and 6.78 (AA′BB′, 4 H), 4.05–3.9 (m, 1 H), 3.75 (s, 3 H), 3.5–3.25 (m, 2 H), 2.95 (dd, 1 H, *J* = 6.5 and 13.1 Hz), 2.78 (dd, 1 H, *J* = 5.9 and 13.1 Hz), 1.9–1.1 (m, 6 H); ^{13}C NMR δ 153.7, 127.7, 122.0, 109.4, 71.4, 63.3, 50.0, 36.6, 26.0, 20.8, 18.2; MS *m/z* (relative intensity) 240 (2), 239 (6), 238 (37), 154 (22), 139 (12), 85 (100), 67 (14), 57 (12), 43 (15).

2-[[(4-Methoxyphenyl)thio]methyl]-2,3-dihydrobenzofuran (8) and 3-[(4-methoxyphenyl)thio]-2,3-dihydrobenzopyran (9): oil; ^{1}H NMR δ 7.7–7.55 (m, 4 H), 7.2–6.7 (m, 12 H), 5.4–5.2 (m, 1 H), 4.95–4.8 (m, 1 H), 3.9 (s, 6 H), 3.55–2.85 (m, 8 H); ^{13}C NMR δ 128.4, 128.3, 126.2, 125.8, 125.0, 121.0, 115.0, 109.9, 109.0, 96.2, 77.2, 76.6, 64.5, 62.4, 55.5, 35.6, 35.3; MS *m/z* (relative intensity) 272 (100), 154 (54), 140 (87), 139 (70), 133 (76), 131 (70), 119 (58), 105 (29), 91 (49), 77 (35), 44 (69); 272 (94), 156 (96), 155 (65), 154 (61), 153 (67), 139 (83), 133 (100), 131 (72), 119 (63), 105 (30), 91 (47), 77 (40), 44 (57). Anal. Calcd for $C_{16}H_{16}O_2S$: C, 70.56; H, 5.92. Found: C, 70.48; H, 5.89.

2-Phenyl-5-[[(4-methoxyphenyl)thio]methyl]-4,5-dihydrooxazole (10): oil; ^{1}H NMR δ 7.84 and 6.8 (AA′BB′, 4 H), 7.5–7.3 (m, 5 H), 4.77 (ddt, 1 H, *J* = 5.5, 7.0, and 9.4 Hz), 4.12 (dd, 1 H, *J* = 9.4 and 15.0 Hz), 3.83 (dd, 1 H, *J* = 6.9 and 15.0 Hz), 3.76 (s, 3 H), 3.18 (dd, 1 H, *J* = 5.5 and 13.7 Hz), 2.94 (dd, 1 H, *J* = 7.1 and 13.7 Hz); ^{13}C NMR δ 163.6, 159.4, 134.0, 131.1, 128.1, 127.7, 125.1, 114.7, 78.4, 59.5, 55.2, 40.3; MS *m/z* (relative intensity) 301 (6), 300 (19), 299 (94), 146 (100), 130 (68), 118 (46), 105 (79), 91 (67), 77 (63). Anal. Calcd for $C_{17}H_{17}NO_2S$: C, 68.20; H, 5.72; N, 4.68. Found: C, 68.25; H, 5.78; N, 4.62.

4-[(4-Methoxyphenyl)thio]tetrahydrofuran-2-one (11): oil; ^{1}H NMR δ 7.4 and 6.88 (AA′BB′, 4 H), 4.48 (dd, 1 H, *J* = 6.7 and 9.8 Hz), 4.18 (dd, 1 H, *J* = 5.5 and 9.8 Hz), 3.9–3.75 (m, 1 H), 3.82 (s, 3 H), 2.84 (dd, 1 H, *J* = 8.0 and 17.9 Hz), 2.48 (dd, 1 H, *J* = 6.4 and 17.9 Hz); ^{13}C NMR δ 174.7, 160.6, 136.3, 122.0, 115.1, 96.2, 72.4, 55.4, 42.6, 34.9; MS *m/z* (relative intensity) 226 (6), 225 (13), 224 (100), 166 (7), 142 (3), 141 (10), 140 (69), 139 (97), 125 (32), 96 (10), 85 (9), 45 (7). Anal. Calcd for $C_{11}H_{12}O_3S$: C, 58.91; H, 5.39. Found: C, 59.03; H, 5.34.

5-[[(4-Methoxyphenyl)thio]methyl]tetrahydrofuran-2-one (12):[12] oil; ^{1}H NMR δ 7.4 and 6.85 (AA′BB′, 4 H), 4.65–4.48 (m, 1 H), 3.8 (s, 3 H), 3.25 (dd, 1 H, *J* = 4.9 and 13.8 Hz), 2.95 (dd, 1 H, *J* = 7.7 and 13.8 Hz), 2.6–2.25 (m, 3 H), 2.1–1.9 (m, 1 H); ^{13}C NMR δ 176.2, 159.7, 134.0, 125.0, 114.9, 78.7, 55.3, 40.6, 28.4, 26.9; MS *m/z* (relative intensity) 240 (6), 239 (13), 238 (96), 155 (5), 154 (14), 153 (100), 139 (22), 138 (22), 109 (19), 85 (35), 45 (12).

8-[(4-Methoxyphenyl)thio]-2-oxabicyclo[3.3.0]octan-3-one (13): oil; ^{1}H NMR δ 7.4 and 6.83 (AA′BB′, 4 H), 4.76 (d, 1 H, *J* = 6.5 Hz), 3.8 (s, 3 H), 3.72–3.63 (m, 1 H), 3.2–3.0 (m, 1 H), 2.8 (dd, 1 H, *J* = 9.9 and 18.3 Hz), 2.3 (dd, 1 H, *J* = 2.5 and 18.3 Hz), 2.35–2.0 (m, 2 H), 1.85–1.65 (m, 1 H), 1.65–1.45 (m, 1 H); ^{13}C NMR δ 176.5, 159.6, 134.3, 124.2, 114.8, 89.5, 55.2, 52.9, 37.1, 35.6, 31.9, 29.8; MS *m/z* (relative intensity) 266 (5), 265 (15), 264 (100), 142 (4), 141 (9), 140 (90), 139 (36), 125 (28), 41 (12). Anal. Calcd for $C_{14}H_{16}O_3S$: C, 63.62; H, 6.10. Found: C, 63.57; H, 6.16.

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Registry No. 1, 141248-72-8; 2, 141248-73-9; 3, 141248-74-0; 4, 141248-75-1; 5, 111017-45-9; 6, 141248-76-2; 7, 111017-46-0; 8, 111017-50-6; 9, 141248-77-3; 10, 141248-78-4; 11, 141248-79-5; 12, 111017-49-3; 13, 141248-80-8; bis(4-methoxyphenyl) disulfide, 5335-87-5; ammonium peroxydisulfate, 7727-54-0; styrene, 100-42-5; 1-octene, 111-66-0; (*E*)-3-hexene, 13269-52-8; cyclohexene, 110-83-8; 4-penten-1-ol, 821-09-0; 5-hexen-2-ol, 626-94-8; 5-hexen-1-ol, 821-41-0; 2-allylphenol, 1745-81-9; allylbenzamide, 10283-95-1; 3-butenoic acid, 625-38-7; 4-pentenoic acid, 591-80-0; 2-cyclopentene-1-acetic acid, 13668-61-6.

A Facile and General Synthesis of 3-(Acyloxy)cephalosporins

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The ease of isomerization of the cephem double bond has been a significant hindrance to the chemical modification of cephalosporin antibiotics. A facile base-catalyzed double-bond isomerization occurs when the 4-carboxylic acid is esterified or otherwise blocked (e.g., as the mixed anhydride or chloride).[1-3] Since purification of these Δ^2/Δ^3

(1) Cocker, J. D.; Eardley, S.; Gregory, G. I.; Hall, M. E.; Long, A. G. *J. Chem. Soc.* **1966**, 1142.
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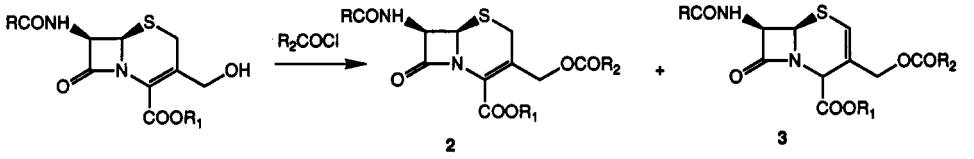
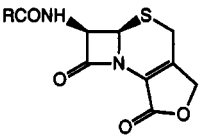
0022-3263/92/1957-4027$03.00/0

Scheme I

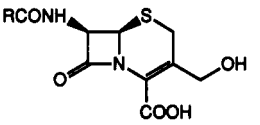


1, R_1 = Protecting Group

2 3

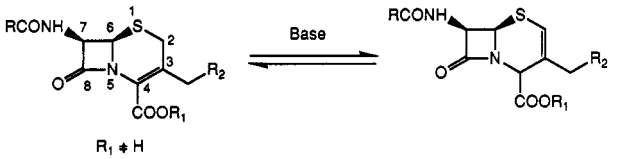


4



5

isomeric mixtures is rarely attainable by chromatographic methods or by crystallization, an oxidation–reduction sequence developed by Kaiser and co-workers[4] has been routinely used to restore the double bond to the desired Δ^3-position through the intermediacy of the Δ^3-sulfoxide. Recently, we have been involved with the preparation of a new class of cephalosporins which have a catechol-containing ester at the 3-methyl position (e.g., **9**). In conjunction with this work, we have developed a new synthetic strategy which provides a general route to the preparation of 3-[(acyloxy)methyl]-3-cephems and avoids the problem of double-bond isomerization.



$R_1 \neq H$

Δ^3-Isomer Δ^2-Isomer

Generally, 3-[(acyloxy)methyl]-3-cephems have been synthesized either by the acylation of a suitably protected 3-(hydroxymethyl)-3-cephem or by the nucleophilic displacement of a 3-(halomethyl)-3-cephem with carboxylate ion. The acylation of cephem alcohol **1** (Scheme I) leads to a mixture of Δ^2- and Δ^3-products **3** and **2**, along with varying amounts of the lactone **4**.[5] Although the formation of the undesired products, **3** and **4**, can be minimized through careful control of reaction conditions[6] (e.g., by using pyridine as the base and closely monitoring the reaction), the necessity for protecting-group manipulations as well as the oxidation–reduction scheme makes this approach cumbersome. Direct acylation of the deacetyl cephalosporanic acid **5** using a large excess of acid chloride at pH 8 in aqueous acetone has been reported,[7] but this procedure is limited to aroyl chlorides. The nucleophilic displacement reaction of 3-(halomethyl)cephems[8,9] also requires the appropriate use of protecting groups and can lead to double-bond migration depending on the basicity of the carboxylate and the nature of the halide. The use of 3-(diazomethyl)cephems[10] to form esters has been described, but is not practical as a general procedure due to the complexity of chemistry.

Our new approach is to acylate the cephem alcohol **5** without protecting the carboxyl group. Under basic conditions, deprotonation of the carboxylic acid moiety reduces the acidity of the protons at the C-2 position. As a result, the formation of a carbanion at C-2 is made more difficult and, even if it does occur, reprotonation α to the carboxylate to form Δ^2-isomer will not be favored. This presumption is in agreement with the observation that cephalosporanic acids undergo only a very slow double-bond isomerization in pyridine.[1]

For this approach to be successful, the acylating agent employed must meet several requirements with regard to reactivity. It must not react rapidly with solvent. To avoid solvolysis, an aprotic solvent and a tertiary amine base are utilized. In addition, it must react more rapidly with the 3-hydroxymethyl residue than with the carboxylate ion. The product from reaction with carboxylate might lead to unacceptible amounts of double-bond migration and/or the formation of lactone. Our efforts toward achieving the required selective reactivity using Mukaiyama's reagent[11,12] for activation to form the acylating agent are described below.

As shown in Scheme II, 3,4-bis(acetyloxy)benzoic acid (1.2 equiv) and triethylamine (3.6 equiv) in dry methylene chloride at 0 °C were treated with N-methyl-2-fluoropyridinium tosylate. Subsequent addition of cephem alcohol **6** (1.08 equiv) at 0 °C to the activated carboxylic acid gave the desired 3-[[3,4-bis(acetyloxy)benzoyl]oxy]-3-cephem **7** in 76% yield. A minor amount of the lactone was observed by thin-layer chromatography in the reaction mixture but was easily removed by extraction. Furthermore, there was no detectable Δ^2-isomeric ester in the products. Without further purification, **7** was converted to the catechol cephalosporin antibiotic **9** in an overall yield of 49.8%. These transformations involved the deprotection with trifluoroacetic acid, N-acylation, and removal of the acetyl protecting groups on the catechol. To emphasize the practical aspects of this sequence, we note that **9** was prepared in hundred-gram quantities by this route.

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(11) Mukaiyama, T.; Usui, M.; Shimada, E.; Saigo, K. *Chem. Lett.* **1975**, 1045.
(12) Saigo, K.; Usui, M.; Kikuchi, K.; Shimada, E.; Mukaiyama, T. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 1863.

Scheme II

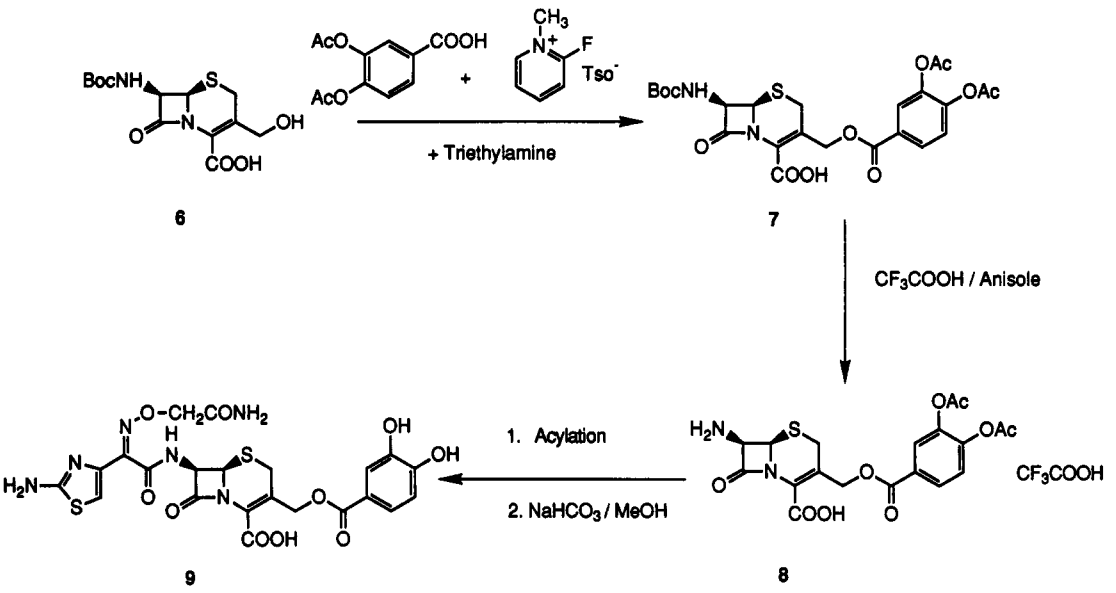
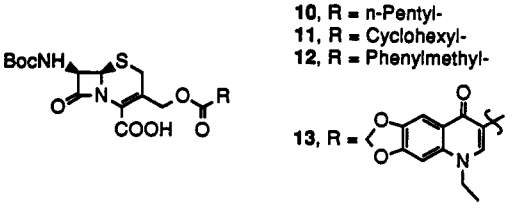


Table I. Reaction Conditions for the Preparation of 3-[(Acyloxy)methyl]-3-cephems

carboxylic acid	temp, (°C)	reaction time	amine	product[a] (%)
3,4-bis(acetyloxy)benzoic acid	0	30 min	triethylamine	7 (75)
hexanoic acid	25	16 h	triethylamine	10 (79)
cyclohexanecarboxylic acid	25	20 h	triethylamine	11 (68)
phenylacetic acid	25	3 h	triethylamine	12 (70)
oxolinic acid	25	3 h	4-(dimethylamino)pyridine	13 (32)

[a] The structures are provided below.



Encouraged by this finding, we then examined the application of this method to other carboxylic acids since the previously reported direct acylation of cephem alcohols **5** with acid chlorides was limited to aroyl examples only.[7] As listed in the Table I, aliphatic acids also gave good yields of the desired product with the new procedure. For aliphatic acids, the reaction temperature was somewhat higher, and a longer time was required for completion of the coupling. In the case of oxolinic acid a rather low yield was obtained and, instead of triethylamine, 4-(dimethylamino)pyridine was necessary for this reaction to proceed at all.

The present procedure provides an improved method for the preparation of 3-[(acyloxy)methyl]cephems without the usual problems of double-bond isomerization. In addition, the reaction is straightforward, does not require protecting-group manipulations of the 4-carboxylic acid, and appears to be general for acylation with both aromatic and aliphatic acids. Because of these attributes, we feel it represents a useful addition to cephalosporin chemistry.

Experimental Section

Physical Chemistry. Infrared spectra were recorded on either a Digilab FTS 15-E or a FTS-14 spectrometer. Mass spectra were obtained on a VG7070-HF mass spectrometer in the positive-ion fast atom bombardment mode using glycerol or thioglycerol as the solvent. Proton nuclear magnetic resonance spectra were obtained on a Varian XL-400 instrument, and chemical shifts (δ) are expressed in parts per million (ppm) downfield from tetramethylsilane, with coupling constants (J) in hertz (Hz).

(6*R*,7*R*)-3-[[[3,4-Bis(acetyloxy)benzoyl]oxy]methyl]-7-[[(1,1-dimethylethoxy)carbonyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (7).[13] Triethylamine (52.5 mL, 0.376 mol) was added to a solution of 3,4-bis(acetyloxy)benzoic acid (29.75 g, 0.125 mol) in dry CH_2Cl_2 (625 mL) under an argon atmosphere. The solution was cooled to 0 °C (ice bath), and *N*-methyl-2-fluoropyridinium tosylate (29.78 g, 0.105 mol) was added portionwise. After the addition, the reaction was stirred at 0 °C for 1 h and *N*-(*tert*-butyloxycarbonyl)-3-deacetylcephalosporanic acid[14] (**6**, 37.78 g, 0.114 mol) was added. The resulting solution was stirred for an additional 20 min at 0 °C and allowed to warm to room temperature. The mixture was then poured into a mixture of AcOEt (1.5 L) and 1.0 N aqueous HCl (1.5 L). The organic phase was separated and was washed successively with 2% aqueous $NaHCO_3$ (1.5 L) and brine (1.8 L). The unreacted benzoic acid was removed by such extraction, but the product remained in the organic phase. A slow separation of the phases occurred, and the organic layer, after separation, was washed with 0.1 N HCl (1 L) and then brine solution (1.5 L), dried over Na_2SO_4, and filtered. Removal of the solvent in vacuo gave **7** (47.5 g) in 75% yield with a purity of 88% based on ^1H NMR: ^1H NMR (DMSO-d_6) δ 1.41 (s, 9 H, *t*-Bu), 2.30 (s, 3 H, CH_3), 2.31 (s, 3 H, CH_3), 3.62, 3.73 (AB, 2 H, J_{gem} = 17.5 Hz, SCH_2), 4.96, 5.29 (AB, 2 H, J_{gem} = 13 Hz, OCH_2), 5.05 (d, 1 H, J = 4.3 Hz), 5.49 (br m, 1 H), 7.46 (d, 1 H, J = 8.4 Hz, Ar), 7.84 (d, 1 H, J = 1.9 Hz, Ar), 7.92 (dd, 1 H, J = 1.9, 8.4 Hz, Ar), 8.02 (d, 1 H, J = 8.9 Hz, NH), 13.74 (br, 1 H, COOH); IR (KBr) 3344, 1778, 1722 cm^{-1}; HR-MS m/z 551.1314 (M + H^+, $C_{24}H_{27}N_2O_{11}S$ requires 551.1335).

Compounds **10**, **11**, **12**, and **13** were prepared from their respective carboxylic acids and the cephem alcohol **6** in a similar manner to that described above for the preparation of **7**. The reaction time after the addition of **6** was varied to optimize the yield of each product.

Trifluoroacetic Acid Salt of (6*R*,7*R*)-3-[[[3,4-Bis(acetyloxy)benzoyl]oxy]methyl]-7-amino-8-oxo-5-thia-1-azabi-

(13) This nomenclature is indexed in *Chemical Abstracts*. Note that the numbering system is different from that used in the majority of the chemical literature.

(14) This compound was prepared by a procedure similar to that reported in ref 5.

cyclo[4.2.0]oct-2-ene-2-carboxylic Acid (8). To a stirred solution of 7 (25.7 g, 0.047 mol) and anisole (25 mL) in dry CH_2Cl_2 (250 mL) was added trifluoroacetic acid (250 mL) under an argon atmosphere. Stirring was continued at room temperature for 3 h, and the reaction was then evaporated in vacuo. The resulting oily residue was triturated with AcOEt (200 mL) for 20 min to induce precipitation. Anhydrous ether (650 mL) was added to this suspension, and the mixture were stirred for a further 20 min. Due to the hygroscopic nature of the product, the precipitate was filtered under a nitrogen atmosphere to afford 20.25 g of 8 (76%): ^{1}H NMR (DMSO-d_6) δ 2.30 (s, 3 H, CH_3), 2.31 (s, 3 H, CH_3), 3.65, 3.76 (AB, 2 H, J_{gem} = 18 Hz, SCH_2), 4.91 (d, 1 H, J = 5.0 Hz, CH), 5.07 (d, 1 H, J = 5.0 Hz, CH), 4.96, 5.29 (AB, 2 H, J_{gem} = 12.8 Hz, OCH_2), 7.46 (d, 1 H, J = 8.4 Hz, Ar), 7.85 (d, 1 H, J = 1.7 Hz, Ar), 7.93 (dd, 1 H, J = 1.7, 8.4 Hz, Ar); IR (KBr) 1800, 1775, 1720, 1680 cm^{-1}; HR-MS m/z 473.0675 (M + Na$^+$, $C_{19}H_{18}N_2O_9SNa$ requires 473.0631).

[6*R*,7*R*(*Z*)]-7-[[[(2-Amino-2-oxoethoxy)imino](2-amino-4-thiazolyl)acetyl]amino]-3-[[(3,4-dihydroxybenzoyl)oxy]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid Sodium Salt (9). To the suspension of 8 (25.2 g, 0.0446 mol) in THF (390 mL) was added a solution of $NaHCO_3$ (7.3 g, 0.087 mol) in H_2O (500 mL). The resulting mixture was stirred until a clear solution occurred. To this was added *S*-(2-benzothiazolyl) (2-amino-4-thiazolyl)-(*Z*)-[(2-amino-2-oxoethoxy)imino]acetate (23.3 g, 0.059 mol), and the reaction was stirred at room temperature for 5 h. The resulting solution was poured into a mixture of AcOEt (1.9 L) and H_2O (0.4 L) containing $NaHCO_3$ (3.7 g) and then extracted thoroughly. The aqueous layer, after separation and filtration through Celite, was further washed with AcOEt (1.9 L). The aqueous solution was then treated with MeOH (95.6 mL) and $NaHCO_3$ (19.7 g) at room temperature for 2 h. The organic solvents were removed under reduced pressure, and the reaction was acidified with 1.0 N HCl to pH 2.8 while cooling in an ice bath. The precipitate which formed was filtered and dried in vacuo to give 26.02 g of the free acid of 9.

To a 10% solution of H_2O in acetone (800 mL) was added 26.02 g (0.044 mol) of the free acid of 9, and the mixture was stirred for 30 min until most of the sample dissolved. The solution was diluted with acetone to a volume of 3 L and filtered. To the filtrate was slowly added a solution of sodium 2-ethylhexanoate (8.5 g, 0.051 mol) in acetone (1 L) with stirring over a period of 2 h. The precipitate was filtered and crystallized from H_2O (150 mL) to give 17.5 g of 9 (65%): ^{1}H NMR (DMSO-d_6) δ 3.30, 3.57 (AB, 2 H, J_{gem} = 17.1 Hz, SCH_2), 4.41 (s, 2 H, OCH_2), 4.89, 5.17 (AB, 2 H, J_{gem} = 12.0 Hz, OCH_2), 5.05 (d, 1 H, J = 4.7 Hz, CH), 5.64 (dd, 1 H, J = 4.7, 8.0 Hz, CH), 6.8 (d, 1 H, J = 8.2 Hz, Ar), 6.85 (s, 1 H, Ar), 7.12 (s, 1 H, NH_2), 7.30 (br s, 3 H, NH_2 and Ar), 7.36 (s, 1 H, Ar), 7.50 (s, 1 H, NH_2), 9.75 (d, 1 H, J = 8.0 Hz, NH), 9.46 (br, 1 H, OH), 9.91 (br, 1 H, OH); IR (KBr) 3300, 1755, 1708, 1680, 1655 cm^{-1}; HR-MS m/z 615.0599 (M + H$^+$, $C_{22}H_{20}N_6O_{10}S_2Na$ requires 615.0580).

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Registry No. 6, 37051-06-2; 7, 139290-97-4; 8, 141344-49-2; 9, 122005-95-2; 9 free acid, 122005-44-1; 10, 141344-50-5; 11, 141344-51-6; 12, 141344-52-7; 13, 141344-53-8; 3,4-bis(acetyloxy)benzoic acid, 58534-64-8; hexanoic acid, 142-62-1; cyclohexanecarboxylic acid, 98-89-5; phenylacetic acid, 103-82-2; oxolinic acid, 14698-29-4; *N*-methyl-2-fluoropyridinium tosylate, 58086-67-2; *S*-(2-benzothiazolyl) (2-amino-4-thiazolyl)-(*Z*)-[(2-amino-2-oxoethoxy)imino]thioacetate, 89876-15-3.

Supplementary Material Available: ^{1}H NMR spectra for compounds 7–13 (7 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

The Biogenetic Origin of the Carbon Skeleton and the Oxygen Atoms of Elaiophylin, a Symmetric Macrodiolide Antibiotic

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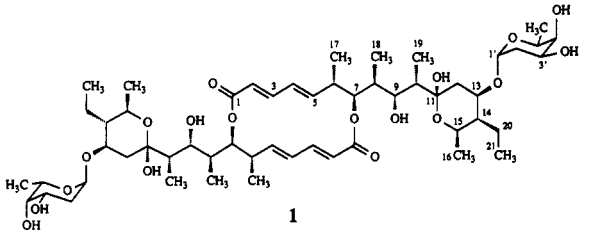
Elaiophylin (1),[1-4] a 16-membered macrodiolide antibiotic exhibiting C_2 symmetry was originally isolated from *Streptomyces melanosporus*, later as azalomycin B from *Streptomyces hygroscopicus* var. *azalomyceticus*, and also from *Streptomyces violaceoniger* (Tü 905).[5,6] A high-producing strain (*Streptomyces* sp., DSM 3816), which also biosynthesizes the antibiotics niphimycin and nigericin as well as two novel niphimycin analogues,[7] was detected in the course of our chemical screening program.[8] Elaiophylin exhibits antibacterial as well as in vivo anthelminthic activity. Because of its multifunctionalized polyketide aglycon moiety deriving from two identical halves, elaiophylin (1) attracted our interest in studying modern



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hypotheses for polyketide biosynthetic pathways (see also Discussion).[9-11] On the basis of detailed NMR spectroscopic analysis,[1] we present here the first results of our biosynthetic studies, namely the biogenetic assembly of elaiophylin (1) formed via a mixed biogenesis from polyketide and carbohydrate building blocks.

Experimental Section

Fermentation. The two-step fermentations of *Streptomyces* sp. (DSM 4137) were carried out using a loopful of agar slants (medium: 2% soybean meal; degreased, 2% mannitol, 1.5% agar; incubation time: 6 weeks at 30 °C to gain highest productivity) shaken in 5 × 100 mL of culture medium (2% soybean meal, degreased, 2% mannitol) in 250-mL Erlenmeyer flasks at 180 rpm at 30 °C for 3 days; i.e., the fermentation scale in each experiment was 0.5 L. An aliquot of this seed culture (10%) was used to inoculate the same medium for production (harvest after 5 days). The production time course was examined during a 50-L fermentation. Samples of 100 mL were taken every 6 h, extracted with 300 mL of ethyl acetate each, and analyzed by HPLC using a reversed-phase column (KONTRON Spherisorb 10μm, ODS RP-18, 25 cm × 4.6 mm, methanol, detection at 265 nm; retention time of 1: 3.1 min at 1 mL/min). In a typical fermentation, the production of elaiophylin (1) started 40 h after inoculation and increased by further cultivation; after ca. 100 h the concentration of 1 remained constant (maximum yield: ca. 300 mg/L).

Feeding Experiments. Feeding experiments were carried out by addition of labeled precursors in equal portions 45, 50, 55, and 60 h after inoculation (total amounts: see Table I). The feeding experiment with [1-^{13}C]-D-glucose was accomplished with replacement culture techniques because glucose is the major carbon source of the microorganism. Seventy-two hours after inoculation the culture was centrifuged (9000 rpm, 20 min). The cells were reincubated in a medium containing 10 g/L of soybean meal

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