Synthesis and Antimicrobial Activity of Novel 3-[(Aminopyrimidiniumyl)thio]methyl Cephalosporins

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A series of novel cephalosporin compounds which have 3-[(aminopyrimidiniumyl)thio]methyl substituents was synthesized. They show high antimicrobial activity against various bacterial species including *Pseudomonas aeruginosa*. Structure–activity relationships with various thiopyrimidines, thiopyrimidiniums, bicyclic thiotriazolopyrimidiniums, and bicyclic thioimidazolopyrimidiniums as 3′-substituents were also studied; cephalosporins with quarternized pyrimidinium moieties have better antimicrobial activities than neuteral pyrimidine cephalosporins, and stabilization of the positive charge on the pyrimidinium moieties is essential for better activity. According to semiempirical PM3 calculations, amino and alkylthio substituents on the pyrimidinium rings play a major role in charge stabilization and delocalization.

The cephalosporin antimicrobial agents continue to play a major role in the treatment of bacterial infection. Among significant advances, the recent introduction of positively-charged substituents at the 3′-position of the cephem nucleus has improved antibacterial activity against a wide variety of Gram-positive and Gram-negative pathogens, including *Pseudomonas aeruginosa*, and yielded third-generation antimicrobial agents.[1] The substituents are usually quaternary ammonium or pyridinium salts; examples include pyridinium in ceftazidime, cyclopentenopyridinium in cefpirome, and *N*-methylpyrrolidinium in cefepime.[2] To discover antimicrobial agents that show increased Gram-positive activity while maintaining the excellent Gram-negative spectrum of agents having positively-charged 3′-substituents, we have synthesized a series of novel 3-[(aminopyrimidiniumyl)thio]methyl cephalosporins, where the thiopyrimidinium moieties have both positive charges and charge-stabilizing substituents. Here we compare the antimicrobial activities of neutral thiopyrimidinyl cephalosporins and quarternized [(pyrimidiniumyl)thio]methyl cephalosporins and present a study on the stabilization of positive charge developed on the pyrimidinium rings.

Chemistry

3-(Pyrimidiniumylthio)methyl cephalosporins **1**–**41** were synthesized by introducing alkoxyimino thiazole substituents at the 7-position and various thiones ($\mathbf{S}_{10-41}$) and corresponding 2-mercaptopyrimidines ($\mathbf{S}_{1-9}$) at the 3′-position of cephems (Scheme 1). The triethyl ammonium salts of the (aminothioazolyl)iminoacetic acids were reacted with *p*-methoxybenzyl 7-amino-3-(chloromethyl)-Δ^3-cephem-4-carboxylate (7-ACLE) and phosphoryl chloride in the presence of pyridine to yield *p*-methoxybenzyl 3-(chloromethyl)-7-[(*Z*)-2-[2-[(triphenylmethyl)amino]thiazol-4-yl]-2-[(carboxyprop-2-oxy)imino]acetamido]-Δ^3-cephem-4-carboxylate.[4] Then the 3′-chloride was displaced with a thione in dimethylformamide, and deprotection was performed

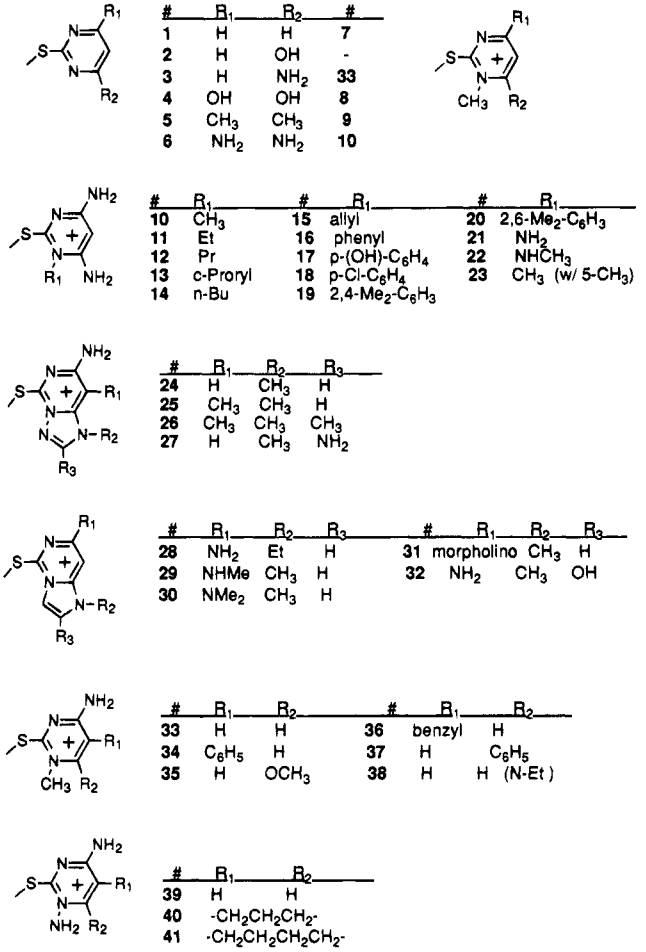

Figure 1. Novel cephalosporin compounds with various thiopyrimidiniums ($\mathbf{S}_{1-41}$).

to give a final 3′-substituted cephalosporin, **1**–**41**, as shown in Scheme 1.

The 3′-substituent moieties, various pyrimidinethiones ($\mathbf{S}_{7-23}$ and $\mathbf{S}_{33-41}$), were synthesized from malononitrile derivatives and substituted thioureas in the presence of a base such as sodium methoxide (Scheme

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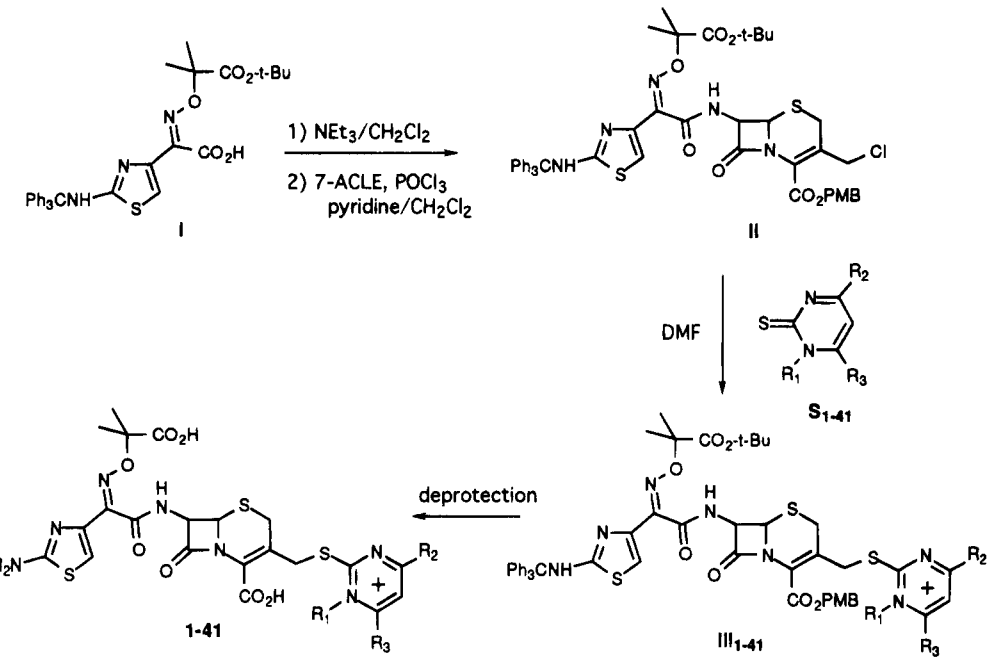
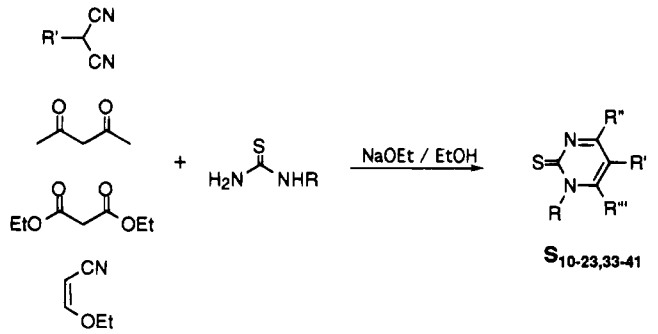
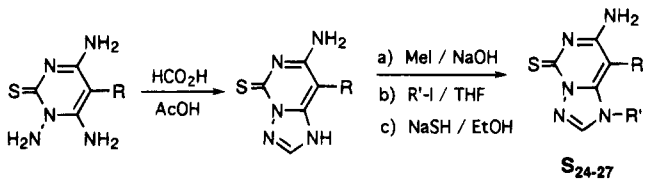
Scheme 1



Scheme 2



Scheme 3



2).[3] The corresponding thioureas could be obtained from ammonia and alkyl or aryl thiocyanates, and malononitrile derivatives were prepared from reaction of malononitrile with the corresponding alkyl halides. Bicyclic triazolopyrimidinethiones $\mathbf{S_{24-27}}$ were synthesized by condensation of *N*-aminopyrimidinethiones and formic acid (Scheme 3). Bicyclic imidazopyrimidinethiones $\mathbf{S_{28-32}}$ were prepared from amino(methylthio)-pyrimidine and chloroacetaldehyde as described in Scheme 4.

Bioactivity

In order to determine minimal inhibitory concentration (MIC) of compounds **1**–**41** for Gram-positive and Gram-negative bacterial species, we used an agar dilution method.[5] Ceftazidime was used as a reference, since it has the same (2-carboxyprop-2-oxy)imino moiety at the 7-position. In general, the antibacterial activity of substituted diaminopyrimidinium cephalosporins is similar to or better than that of the reference and is illustrated in Table 1.

Cephalosporins with neutral thiopyrimidinyl substituents, **1**–**6**, are less potent than most of those with positively-charged thiopyrimidiniumyl substituents. When the ring nitrogen of compound **1** was quarternized by introducing a methyl group to give compound **7**, the positively-charged **7** became more potent against *P. aeruginosa* than the corresponding neutral compound **1**. The same is true in **3** vs **33**, **5** vs **9**, and **6** vs **10**. However, both the neutral (**4**) and positively-charged (**8**) thiopyrimidine with hydroxy substituents are poorly active, suggesting that the pyrimidine of compound **8** exists in the form of a neutral pyrimidinone rather than a quarternized hydroxypyrimidine. When there are additional methyl substituents which inductively stabilize the charge, as in **9**, the MIC gets better throughout the antibacterial spectra. Introduction of amino substituents instead of methyl groups improves the bioactivity even more because these substituents are expected to delocalize more the pyrimidinium charge. The positive charge is also expected to be stabilized by the electron-donating 2-thio group. Both the positive charge and its delocalization within the pyrimidini-

Scheme 4

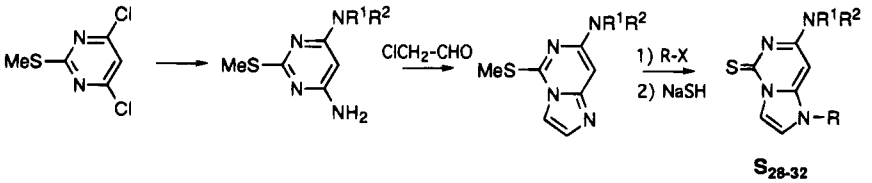


Table 1. In Vitro Antibacterial Activities of Various 3-[(Aminopyrimidiniumyl)thio]methyl Cephalosporins against Clinical Isolates (μg/mL)

no.	*S. aureus* ATCC-6538p	*S. epidermidis* ATCC-12228	*C. freundii* ATCC-8090	*E. aerogenes* ATCC-29751	*E. cloacae* ATCC-27508	*E. coli* ATCC-10536	*K. pneumoniae* ATCC-10031	*P. vulgaris* ATCC-6059	*S. typhimurium* ATCC-14028	*S. marcescens* ATCC-27117	*P. aeruginosa* ATCC-27853	*P. aeruginosa* ATCC-10145
1	16	8	1	16	0.5	1	0.5	0.063	2	2	16	32
2	>128	>128	8	32	4	8	8	2	64	16	>128	>128
3	32	16	1	8	0.5	1	0.5	0.063	1	1	8	16
4	>128	>128	8	32	1	4	2	0.25	8	4	32	32
5	32	8	0.5	8	0.5	0.5	0.5	0.25	4	2	32	64
6	32	8	1	2	0.25	0.25	0.25	0.031	0.5	0.5	2	4
7	128	64	8	16	0.25	1	0.5	0.13	1	2	8	8
8	>128	>128	4	16	1	4	2	0.5	16	16	16	32
9	8	8	0.25	4	0.06	0.13	0.13	0.06	0.5	0.25	4	4
10	2	1	0.063	1	0.016	0.063	0.063	0.031	0.13	0.063	1	2
11	2	1	0.13	2	0.03	0.06	0.06	0.03	0.25	0.13	1	2
12	2	2	0.25	2	0.03	0.13	0.13	0.06	0.25	0.25	2	4
13	4	2	0.13	2	0.03	0.13	0.06	0.13	0.5	0.25	2	4
14	2	1	0.13	1	0.03	0.13	0.06	0.25	0.5	0.5	4	4
15	4	2	0.13	2	0.03	0.13	0.06	0.06	0.25	0.13	2	2
16	4	2	0.25	0.5	0.06	0.25	0.13	0.13	0.5	0.5	2	4
17	4	2	0.25	2	0.06	0.25	0.13	0.13	0.5	0.5	1	2
18	4	2	0.25	1	0.13	0.25	0.13	0.25	0.5	1	2	4
19	4	2	2	2	0.25	0.5	0.13	0.5	2	2	2	8
20	4	2	1	2	0.25	0.5	0.25	0.5	2	2	4	8
21	4	2	0.06	1	0.02	0.06	0.06	0.02	0.13	0.06	1	1
22	32	16	0.5	16	0.13	0.5	0.25	0.03	1	1	8	8
23	4	2	0.13	2	0.06	0.06	0.06	0.06	0.25	0.13	2	2
24	8	1	0.13	1	0.02	0.06	0.06	0.06	0.25	0.25	2	4
25	8	2	0.06	1	0.02	0.13	0.06	0.06	0.25	0.25	2	4
26	8	2	0.13	1	0.03	0.25	0.13	0.13	0.25	0.5	4	8
27	4	1	0.06	1	0.02	0.06	0.13	0.06	0.25	0.25	2	2
28	4	2	0.06	2	0.02	0.06	0.06	0.06	0.13	0.13	2	4
29	8	4	0.13	4	0.03	0.13	0.25	0.13	0.5	0.5	4	8
30	2	1	0.25	4	0.06	0.13	0.13	0.25	0.5	0.5	4	8
31	8	2	0.5	8	0.06	0.25	0.25	0.25	0.5	0.5	4	8
32	16	4	0.25	4	0.03	0.13	0.13	0.06	0.5	0.5	4	4
33	4	2	0.03	1	≤0.008	0.03	0.03	0.06	0.13	0.06	1	2
34	8	4	0.13	2	0.03	0.25	0.13	0.5	0.5	1	8	16
35	4	1	0.13	4	0.02	0.06	0.06	0.13	0.25	0.25	2	4
36	8	2	0.06	1	0.03	0.25	0.06	0.5	0.5	0.5	8	16
37	4	1	0.5	4	0.13	0.25	0.13	0.5	1	2	4	16
38	4	2	0.06	1	0.02	0.06	0.06	0.13	0.25	0.13	2	2
39	8	2	0.03	0.5	0.02	0.06	0.03	0.03	0.13	0.13	1	2
40	4	2	0.03	0.25	0.02	0.06	0.06	0.06	0.13	0.13	2	2
41	4	2	0.06	0.5	0.02	0.06	0.06	0.06	0.13	0.25	2	4
ceftazidime	8	4	0.13	4	0.03	0.06	0.06	0.06	0.25	0.13	1	2

umylthio groups at the 3′-position of these cephalosporin compounds seem to be major factors that improve the antibacterial activity. The positively-charged pyrimidines show especially high efficacy against *Staphylococcus aureus* and *P. aeruginosa*.

When the size of the 1-substituents of 4,6-diamino-2-thiopyrimidinium become bulkier in the methyl, ethyl, propyl, cyclopropyl, and *n*-butyl analogs, **10**–**14**, their bioactivities decrease slightly. With an aryl substituent, it further decreased by 1/4 that of **10** (R = methyl). Compound **23**, with a 5-methyl group, has less potency than **10** (5-unsubstituted); alkyl groups at the 5-position induce steric congestion at the two neighboring amino groups, which reduces both the planarity and the resonance stabilization by the amino groups. When the 1-substituent is NH_2 as in compound **21**, its efficacy is high and broad.

Compounds with bicyclic triazolopyrimidiniums, **24**–**27**, also show excellent activities against Gram-negative organisms but are slightly less potent against Gram-positive organisms than the monocyclic diamino compound **21**. Cephalosporins with imidazolopyrimidiniums, **28**–**32**, show similar antibacterial activities to triazolopyrimidiniums. Their activities are equal or slightly less potent against Gram-positive and Gram-negative organisms than that of the monocyclic compound **10**.

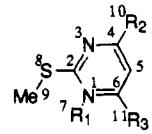
The compounds having 4-monoamino substituents, **33**–**41**, also show excellent bioactivities. When compared with **10** (4,6-diamino substituted), compound **33** is even more potent against *P. aeruginosa*. Bulky 5-substituents (phenyl and benzyl in **36** and **37**, respectively), again decrease activity, especially against Gram-positive organisms and *P. aeruginosa*. The 6-membered ring moiety of bicyclic compound **41** would generate slightly more steric hindrance to the 4-NH_2 group than the 5-membered ring **40**, making the 4-NH_2 group of compound **41** more out of plane and reducing the charge-stabilizing ability of the NH_2 and bioactivity.

In summary, the positive charge developed on pyrimidinium rings is an essential factor for better MIC values, and substituents which can stabilize the charge are also important. In this pyrimidinium series, the 4-NH_2 group is an important substituent for the latter purpose.

Charge Stabilization by Amino Substituents

To understand the charge stabilization of these pyrimidinium substituents, we performed semiempirical calculations on several model (methylthio)pyrimidiniums with the PM3 method (MOPAC).[6] Calculated

Table 2. Calculated Atomic Charges at Each Position of (Methylthio)pyrimidiniums **pyrim1–8**



	pyrim1	pyrim2	pyrim3	pyrim4	pyrim5	pyrim6	pyrim7	pyrim8
R_1	–	–	NH_2^+	NH_2^+	NH_2^+	CH_3^+	CH_3^+	CH_3^+
R_2	NH_2	NH_2	NH_2	NH_2	H	NH_2	NH_2	H
R_3	H	NH_2	NH_2	H	NH_2	NH_2	H	NH_2
1	−0.128	−0.179	0.130	0.195	0.170	0.233	0.291	0.304
2	−0.068	−0.033	−0.108	−0.128	−0.159	−0.104	−0.128	−0.168
3	−0.192	−0.229	−0.189	−0.196	−0.118	−0.200	−0.199	−0.114
4	−0.002	0.028	0.063	0.031	0.105	0.058	0.026	0.102
5	−0.262	−0.311	−0.365	−0.234	−0.258	−0.331	−0.213	−0.236
6	0.020	0.022	0.022	0.002	−0.017	−0.029	−0.060	−0.072
7	–	–	0.002	0.016	−0.007	−0.135	−0.136	−0.153
8	0.140	0.131	0.176	0.201	0.186	0.202	0.222	0.220
9	−0.190	−0.188	−0.159	−0.161	−0.155	−0.171	−0.172	−0.168
10	0.108	0.104	0.284	0.320	–	0.286	0.309	–
11	–	0.107	0.206	–	0.273	0.156	–	0.195

atomic charges of the energy-minimized structures are shown in Table 2. The positive charge developed by addition of NH_2 or CH_3 groups at N-1 is delocalized over several ring atoms. The atomic charges at N-1 were changed from −0.179 (**pyrim2**) to 0.130–0.304 (**pyrim3–8**). This charge introduction by the amino or methyl groups at the N-1 position results in improvement of bioactivity as in **10** and **21** vs **6**.

The developed charge is expected to be stabilized by the methylthio group at the C-2 position and the amino group at the 4- or 6-position. When the neutral **pyrim2** is compared with the positive **pyrim3–8**, the atomic charge of S-8 of the latter pyrimidiniums became more positive by 0.05–0.07 with introduction of a NH_2 group at the N-1 position and even more positive by 0.07–0.09 with a methyl group at the same position. To evaluate the charge-stabilizing effect of substituents in detail, we compared the atomic charge at each position of the neutral **pyrim2** and the cationic **pyrim3**. The major differences in atomic charge appear at nitrogen atoms such as the N-1 atom, 4-NH_2, and 6-NH_2 (0.21, 0.18, and 0.10, respectively). It is noteworthy that the positive charge developed at the N-1 atom is stabilized better by a 4-NH_2 than by a 6-NH_2 substituent. When there is only one amino substituent at either the 4- or 6-position (**pyrim4** or **pyrim5**), the 4-NH_2 has more positive charge and seems to stabilize the positive charge better than the 6-NH_2. The same happens with a CH_3 group at the N-1 position instead of a NH_2 group (**pyrim6–8**). The positive charge is localized even more by the 4-NH_2 group in this case. These calculations indicate that the amino group at the 4-position is essential in stabilizing the positve charge introduced by the NH_2 or CH_3 groups at N-1. This is well correlated with the biological activities of **34–44** which have only one amino group at the 4-position.

In the NMR spectra, the two amino protons of the 4-NH_2 group are equivalent but those of the 6-NH_2 groups are not; this indicates that the 6-NH_2 group is not completely free to rotate and is not coplanar with the pyrimidine ring because of steric hindrance by the 1-substituents. This nonplanarity of the 6-NH_2 group coincides with less charge stabilization as indicated by the charge calculation. Calculated structures of the 1-NH_2 groups of **pyrim3–5** are almost perpendicular to the pyrimidine rings, which could also explain that the charge stabilization by the 1-NH_2 group is weak.

Experimental Section

Various 2-mercaptopyrimidines and 7-amino-3-(chloromethyl)-Δ^3-cephem-4-carboxylates were used as received. ^{1}H-NMR spectra were obtained with either JEOL-270, Bruker ARX300, or JEOL-500 spectrometers. GC-MS spectra were taken from a JEOL JMS-DX300 mass spectrometer. All final compounds were purified on preparative HPLC (a Waters Associates system; UV detection at 245 nm), using Bondapak C_{18}, 15–20 μm particle size, 125 Å pore size columns, 19 × 300 mm. Elemental analyses were performed on a Perkin-Elmer 2400 CHN elemental analyzer. Where indicated by the element symbols, analyses were within +/-0.4% of theoretical values.

General procedure for the preparation of thiones (Schemes 1–3) are described in the following pages. Triamino-2(1*H*)-pyrimidinethiones from Scheme 1 were further cyclized with formic acid to give triazolopyrimidinethiones, and diaminopyrimidine derivatives were reacted with chloroacetaldehyde to yield imidazopyrimidinethiones.

Antimicrobial activity was determined by a two-fold dilution method, i.e., the two-fold serial dilutions of a compound were made and dispersed in Muller–Hinton agar medium, and then 2 μL of standard test strain which had 10^7 CFU/mL was inoculated on the medium, which was incubated at 37 °C for 20 h.

Structures and charges of the model **pyrim-8** were calculated as follows; initial structures were obtain from force field calculation (SYBYL 6.0 implemented on ESV/3; Evans & Sutherland). Then the semiempirical method (MOPAC 5.0) was applied to obtain molecular structures and atomic charges, where the keyword PRECISE was used in geometry optimization. All semiempirical calculations were performed on VAX-6610.

(1) Synthesis of 1-Substituted-4,6-diamino-2(1*H*)-pyrimidinethiones (Scheme 1). Various malononitriles and various *N*-substituted-thioureas were refluxed for 24 h in the presence of sodium ethoxide. After cooling and neutralization, the precipitate was filtered, washed, and dried to give a pale yellow solid as products (**S_{7-23}**).[3,7]

4,6-Diamino-1-methyl-2(1*H*)-pyrimidinethione.[3] Sodium metal (4.6 g) was added to anhydrous ethyl alcohol (100 mL) and refluxed for an hour. After *N*-methylthiourea (9 g) and malononitrile (6.6 g) were added, the reaction mixture was refluxed for 24 h. The reaction mixture was cooled to room temperature and neutralized with concentrated HCl solution. The precipitate was filtered, washed with water (20 mL) and

ethyl alcohol (50 mL), and dried in vacuo to give the above-indicated compound (8.1 g) as a pale yellow solid. ^{1}H-NMR (D_2O + acetone-d_6): δ 3.80 (s, 3H), 5.39 (s, 1H). MS (EI): 156 (M^+), 126. IR (KCl, cm^{-1}): 3441, 3335 (N—H), 1682 (C=N), 1095 (C=S).

1,4,6-Triamino-2(1*H*)-pyrimidinethione.[3] Sodium metal (4.6 g) was added to anhydrous ethyl alcohol (100 mL) and refluxed for an hour. After malononitrile (6.6 g) and thiosemicarbazide (9.1 g) were added, the reaction mixture was refluxed for 24 h and then cooled to room temperature. The precipitate was filtered, washed with ethyl alcohol (50 mL), and dried under reduced pressure to give the above-indicated compound (8.3 g) as a white solid. ^{1}H-NMR (D_2O + acetone-d_6): δ 5.42 (s, 1H). MS (EI): 157 (M^+), 126. IR (KCl, cm^{-1}): 3440, 3420 (N—NH_2), 3310, 3260 (N—H), 1645 (C=N), 1138 (C=S).

(2) Synthesis of 1,8-Disubstituted-7-amino-1*H*-[1,2,4]-triazolo[1,5-*c*]pyrimidine-5-thiones (Scheme 2). 5-Substituted-1,4,6-triamino-2(1*H*)-pyrimidinethiones were refluxed with formic acid in AcOH to give 8-substituted-7-aminotriazolopyrimidinethiones, and then triazolopyrimidinethiones were converted to the methyl thioethers with methyl iodide. These thiomethyl ethers were further quarternized with methyl iodide at the N-1 positions, and then finally the methylthio group was converted to the thiol group with sodium hydrosulfide to give the above-mentioned 1,8-disubstituted-7-amino-1*H*-[1,2,4]triazolo[1,5-*c*]pyrimidine-5-thiones **S**$_{24-27}$.

(3) Synthesis of 1-Substituted7-amino-1*H*-imidazo[1,2-*c*]−pyrimidine-5-thiones (Scheme 3). 2-(Methylthio)pyrimidine 4,6-dichloride was aminated at the 4- and 6-positons. The diaminated pyrimidines were reacted with chloroacetaldehyde to yield imidazopyrimidines, which were further quarternized with either methyl iodide or ethyl bromide. Finally the (methylthio)pyrimidiniums were converted to the thiones **S**$_{28-32}$ with sodium hydrosulfide.

(4) Synthesis of 1,4-Diamino-1,5,6,7-Tetrahydrocyclopentapyrimidine-2-thione (S$_{40}$) and 1,4-Diamino-1,5,6,7-tetrahydro-1*H*-quinazoline-2-thione (S$_{41}$). 1,4-Diamino-1,5,6,7-tetrahydrocyclopentapyrimidine-2-thione (**S**$_{40}$) was synthesized in the following way.

6,7-Dihydro-5*H*-cyclopentapyrimidine-2,4-diol. To a solution of 100 g of ethyl 2-oxocyclopentane carboxylate dissolved in 100 mL of ethyl alcohol was added 120 g of urea. The reaction solution was heated to reflux at 160° for 5 h and cooled to room temperature to yield precipitates, which were washed with 200 mL of distilled water, and the resulting suspension was slowly stirred at room temperature for 10 h and filtered to obtain a solid, which was washed with 50 mL of distilled water and 100 mL of acetone and dried to obtain 40 g of the title compound as a white powder. ^{1}H-NMR DMSO-d_6): δ 1.92 (m, 2H), 2.42 (t, 2H), 2.61 (t, 2H), 10.70 (s, 1H), 11.02 (s, 1H).

2,4-Dichloro-6,7-dihydro-5*H*-cyclopentapyrimidine. The above diol (20 g) was charged into 100 mL of phosphorus oxychloride. The reaction mixture was heated under reflux for 2 h and distilled under reduced pressure to remove the solvent. The residue was poured into 500 mL of ice−water and neutralized with 28% ammonium hydroxide. The resultant solution was extracted with 200 mL of ethyl acetate, and the organic layer was dried over anhydrous magnesium sulfate and distilled under reduced pressure to obtain 19 g of the title compound as a white solid. ^{1}H-NMR (DMSO-d_6): δ 2.06 (m, 2H), 2.89 (t, 2H), 3.01 (t, 2H).

(2-Chloro-6,7-dihydro-5*H*-cyclopentapyrimidin-4-yl)-amine. The above dichloropyrimidine (10 g) was dissolved in 300 mL of ethyl alcohol. To the resulting solution was added 150 mL of 28% ammonium hydroxide, and the solution was stirred at 40 °C for 14 h. The solution was then distilled under reduced pressure to remove the ethanol and filtered to collect solids, which were washed with 20 mL of distilled water and 20 mL of hexane to obtain 8 g of the title compound as a white powder. ^{1}H-NMR (DMSO-d_6): δ 1.93 (m, 2H), 2.57 (t, 2H), 2.68 (t, 2H), 7.01 (s, 2H).

1,4-Diamino-1,5,6,7-tetrahydrocyclopentapyrimidine-2-thione (S$_{40}$). The above compound (5 g) was dissolved in 100 mL of dichloromethane, and the resulting solution was cooled to a temperature ranging from −50 to −60 °C. To the cold solution was slowly added over 10 min a solution of (mesitylenesulfonyl)hydroxylamine (10 g) dissolved in 20 mL of dichloromethane. The resulting solution was warmed to room temperature, stirred for an additional 10 min, and filtered to collect a solid, which was washed with 20 mL of diethyl ether and dried to obtain a white solid. The solid was dissolved in 100 mL of methanol, and then 2.5 g of sodium hydrogen sulfide was added in the same solution. The resulting solution was stirred at room temperature for 30 min and filtered to provide a solid, which was washed with 10 mL of distilled water and 10 mL of acetone and dried to obtain 3 g of the title compound as a white powder. ^{1}H-NMR (DMSO-d_6): δ 2.01 (m, 2H), 2.69 (t, 2H), 2.93 (t, 2H), 6.53 (s, 2H), 7.91 (bs, 2H). MS (FAB, M + 1): 183. Anal. ($C_7H_{10}N_4S_1$) Calcd C, 46.13; H, 5.53; N, 30.74. Found C, 45.97; H, 5.72; N, 30.53.

In a similar way, 1,4-diamino-1,5,6,7-tetrahydro-1*H*-quinazoline-2-thione (**S**$_{41}$) was synthesized from ethyl 2-cyclohexanone carboxylate instead of ethyl 2-oxocyclopentane carboxylate. ^{1}H-NMR (DMSO-d_6): δ 1.63 (m, 4H), 2.19 (t, 2H), 2.38 (t, 2H), 6.45 (s, 2H), 6.9−7.2 (bs, 2H). MS (FAB, M + 1): 197. Anal. ($C_8H_{12}N_4S_1$) Calcd C, 48.71; H, 6.13; N, 28.40. Found C, 48.55; H, 6.38; N, 28.19.

(5) Synthesis of 7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-[(2-carboxyprop-2-oxy)imino]acetamido]-3-[[(4,6-diamino-1-methylpyrimidinium-2-yl)thio]methyl]-Δ^3-cephem-4-carboxylate (10). (*Z*)-2-[[2-(*tert*-Butoxycarbonyl)prop-2-oxy]imino]-2−[2-[(triphenylmethyl)amino]thizol-4-yl]acetic acid and *p*-methoxybenzyl 3-(chloromethyl)-7-[(*Z*)-2-[2-[(triphenylmethyl)-amino]thiazol-4-yl]-2-[[2-(*tert*-butoxycarbonyl)prop-2-oxy]imino]acetamido]-Δ^3-cephem-4-carboxylate were synthesized via a known method.[4]

***p*-Methoxybenzyl 7-[(*Z*)-2-[2-[(Triphenylmethyl)amino]thiazol-4-yl]-2-[[2-(*tert*-butoxycarbonyl)prop-2-oxy]-imino]acetamido]-3-[[(4,6-diamino-1-methylpyrimidinium-2-yl)thio]methyl]-Δ^3-cephem-4-carboxylate (III$_{10}$).** *p*-Methoxybenzyl 3-(chloromethyl)-7-[(*Z*)-2-[2-[(triphenylmethyl)amino]thiazol-4-yl]-2-[[2-(*tert*-butoxycarbonyl)prop-2-oxy]imino]-acetamido]-Δ^3-cephem-4-carboxylate (0.23 g, 10 mmol) and 4,6-diamino-1-methyl-2(1*H*)-pyrimidinethione (1.71 g, 10 mmol) were dissolved in dimethylformamide (40 mL) and stirred for an hour at room temperature. The reaction mixture was concentrated under reduced pressure, and to the residue was added ethyl acetate (300 mL). The solution was washed twice with saturated sodium chloride solution (300 mL). The separated organic layer was dried with anhydrous magnesium sulfate and concentrated under reduced pressure, and finally the residue was triturated with ethyl ether (100 mL). The solid was filtered, washed with diethyl ether (50 mL), and dried to give the title compound (9.85 g, 90%) as a yellow solid. ^{1}H-NMR ($CDCl_3$): δ 1.42 (s, 9H), 1.51 (d, 6H), 3.57 (ABq, 2H), 3.63 (s, 3H), 3.84 (s, 3H), 4.43 (ABq, 2H), 5.12 (d, 1H), 5.23 (ABq, 2H), 5.29 (dd, 1H), 5.62 (s, 1H), 6.85 (s, 1H), 6.92 (s, 1H), 7.02−7.28 (m, 23H).

7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-[(2-carboxyprop-2-oxy)imino]acetamido]-3-[[(4,6-diamino-1-methylpyrimidinium-2-yl)thio]methyl]-Δ^3-cephem-4-carboxylate. *p*-Methoxybenzyl 7-[(*Z*)-2-[2-[(triphenylmethyl)amino]thiazol-4-yl]-2-[[2-(*tert*-butoxycarbonyl)prop-2-oxy]imino]acetamido]-3-[[(4,5-diamino-1-methylpyrimidinium-2-yl)thio]methyl]-Δ^3-cephem-4-carboxylate (10.93 g, 10 mmol) was dissolved in trifluoroacetic acid (16 mL) and anisole (26 mL) at 0 °C. The solution temperature was raised to 30 °C, and the solution was stirred for 3 h. The solution was cooled to 0 °C, and the precipitate was filtered and washed with the mixture of trifluoroacetic acid and anisole (30 mL, 2:8, v/v) and with acetone (100 mL). The dried solid was dissolved in methanol (20 mL), and after stirring for a few minutes, the solution was filtered. To the filtrate was added water (20 mL), and the solution was adjusted to pH 4−5 by sodium bicarbonate solution. After removing methanol under reduced pressure, the formed solid was filtered, washed with water (10 mL), and dried to give the title compound, **10** (2.9 g, 51.3%), as a white solid. MP: 151 °C dec. ^{1}H-NMR (D_2O + $NaHCO_3$): δ 1.50 (s, 6H), 3.50 (s, 3H), 3.59 (ABq, 2H), 4.29 (ABq, 2H), 5.17 (d, 1H), 5.58 (s, 1H), 5.79 (d, 1H), 6.95 (s, 1H). MS (FAB, M + 1): 624. IR (KBr, cm^{-1}): 1761 (β-lactam), 1660, 1580, 1550.

Other final cephalosporins were synthesized via a similar method with various pyrimidinethiones and purified by preparative HPLC.

Supplementary Material Available: Spectroscopic data (^{1}H-NMR, MS, IR) of the final cephalosporin compounds **1-41** and calculated geometries of **pyrim1−8** (16 pages). Ordering information is given on any current masthead page.

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