# CEPHALOSPORINS. I
# CEPHALOGLYCIN ANALOGS WITH SIX-MEMBERED HETEROCYCLES IN THE C-3 SIDE CHAIN

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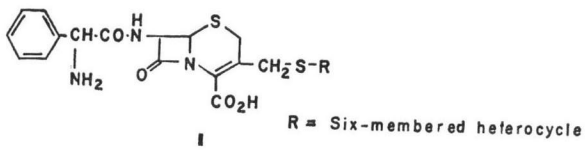
Cephaloglycin analogs with six-membered heterocycles in the C-3 side chain have been prepared by nucleophilic substitution of 7-aminocephalosporanic acid with appropriate azine thiols followed by 7-N-acylation with phenylglycine by the mixed anhydride method. Seventeen thiols of non-substituted or substituted pyridines, pyridazines, pyrimidines, pyrazines and triazines were used as the S-nucleophiles. In general, pyridazine thiols gave cephalosporins possessing good antimicrobial activity against both gram-positive and gram-negative bacteria. Among them 6-hydroxypyridazine-3-thiol gave the most active compound of this series, BB-S 118 (**1f**), which was significantly more active than cephalexin and cephaloglycin *in vitro* against gram-positive and gram-negative bacteria.

Among numerous structural manipulations at the 3-position of the cephem nucleus, modification of the C-3 acetoxy group by direct replacement with sulfur nucleophiles has been reported by many workers[1~16]. Five-membered heteroaromatic thiols have mostly been used as the S-nucleophile[5~14] to give excellent cephalosporin derivatives with enhanced activity superior to the parent acetoxy derivatives. There have been reported only a few examples of cephalosporin derivatives in which the acetoxy group has been replaced by 6-membered heteroaromatic thiols[1,2,3,15].

This paper describes analogs of 7-[D(−)-α-aminophenylacetamido]-3-cephem-4-carboxylic acid (cephaloglycin) possessing 6-membered heterocycles in the C-3 side chain shown by the following general formula (**1**).

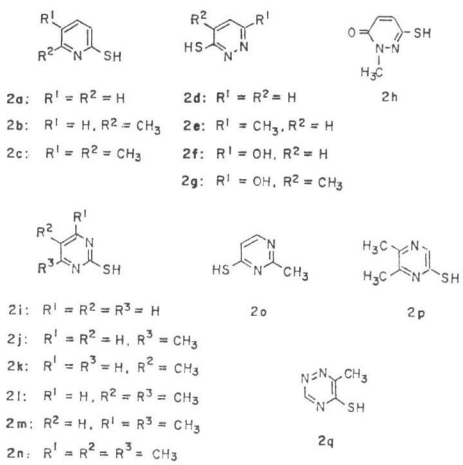



**1**

## Chemistry

Six-membered heterocyclic thiols used in this study were prepared by either one of three known procedures. Heating of the azine halides and alkali hydrogen sulfide in an appropriate alcohol gave pyridine thiols **2a**, **2b** and **2c**, pyridazine thiols **2f**, **2g** and **2h**, and pyrimidine thiols **2i** and **2o**. Reaction of the hydroxy azines and phosphorus pentasulfide in the presence of pyridine was used for the preparation of pyridazine thiols **2d** and **2e**, 2,3-dimethylpyrazine-6-thiol (**2p**) and 6-methyl-as-triazine-thiol (**2q**). Pyrimidine-2-thiols, **2j**, **2k**, **2l**, **2m** and **2n**, were prepared by heating the appropriate β-diketones and thiourea in a mixture of hydrochloric acid and alcohol.

Scheme 1 illustrates the general route to prepare the 3-substituted cephaloglycin analogs (**1**) from 7-aminocephalosporanic acid (7-ACA) (**3**). Nucleophilic displacement of the acetoxyl group in the C-3 side chain of 7-ACA (**3**) with appropriate azine thiols (**2**) was performed by heating for 2~5 hours in 0.1 M phosphate buffer (pH 6.4) to give the corresponding 3-substituted 7-ACA (**4**) in 50~79% yield (Table 1). The $\beta$-lactam carbonyl stretching absorption band of **4** appeared in a fairly high wave number region (1800~1805 $cm^{-1}$).




The 7-amino function of **4** was acylated with sodium D(−)-α-(N-1-ethoxycarbonyl-1-propen-2-yl)aminophenylacetate[17] by the mixed anhydride method using ethyl chloroformate. The N-blocking group was removed by treating with formic acid at 0°C to afford the desired products (**1**) listed in Table 2. The $\beta$-lactam carbonyl band of **1** shifted to lower wave-number by 30~40 $cm^{-1}$ compared with the corresponding 7-amino derivative **4**.

As shown in Tables 1 and 2, compounds **1** and **4** gave UV spectra which reflected the absorption due to the cephem nucleus around 265~270 nm[18] and that due to the heterocycles in the C-3 side chain. In the pyridazine series, the 7-amino derivatives, **4d** and **4e**, and the corresponding 7-N-acyl derivatives, **1d** and **1e**, exhibited maxima at 265~271 nm, while the hydroxypyridazines, **1f**, **1g**, **4f** and **4g**, gave maxima at 243~250 nm, similar to those of the N-methylpyridazinone derivatives, **1h**

Table 1. 3-Substituted 7-aminocephalosporanic acids (**4**)

| Compound | Yield, % | Mp, °C (dec.) | $\lambda_{max}$ in 1%$NaHCO_3$, nm (ε)[c] | Formula | Analyses[a] |
|---|---|---|---|---|---|
| **4a** | 75 | 225~235 | 246 (13200), 275 (12600), 291 (9900) | $C_{13}H_{13}N_3O_3S_2 \cdot \frac{1}{2}H_2O$ | C, H, N |
| **4b** | 77 | 228~230 | 250*(14000), 273 (15000), 292*(11500) | $C_{14}H_{15}N_3O_3S_2$ | S |
| **4c** | 69 | 221~223 | 248 (19000), 268 (18500), 300*(10000) | $C_{15}H_{17}N_3O_3S_2 \cdot \frac{1}{2}H_2O$ | C, H, N, |
| **4d** | 50 | >300 | 271 (13000) | $C_{12}H_{12}N_4O_3S_2 \cdot \frac{1}{2}H_2O$ | C, H, N, S |
| **4e** | 52 | >300 | 265 (11800) | $C_{13}H_{14}N_4O_3S_2 \cdot \frac{1}{2}H_2O$ | C, H, N, S |
| **4f** | 71 | 290~300 | 249 (19400) | $C_{12}H_{12}N_4O_4S_2 \cdot \frac{1}{2}H_2O$ | C, H, N, S |
| **4g** | 79 | >300 | 246 (20500) | $C_{13}H_{14}N_4O_4S_2 \cdot H_2O$ | C, H, N, S |
| **4h** | 58 | 205~215 | 250 (18800) | $C_{13}H_{14}N_4O_4S_2 \cdot \frac{1}{2}H_2O$ | C, H, N, S |
| **4i** | 62 | 214~216 | 271 (21000) | $C_{12}H_{12}N_4O_3S_2 \cdot \frac{1}{2}H_2O$ | C, H, N, S |
| **4j** | 58 | 200~210 | 270 (19600) | $C_{13}H_{14}N_4O_3S_2 \cdot \frac{1}{2}H_2O$ | C, H, N, S |
| **4k** | 61 | 239~246 | 268 (18000) | $C_{13}H_{14}N_4O_3S_2 \cdot \frac{1}{2}H_2O$ | C, H, N |
| **4l** | 50 | 200~210 | 269 (19200) | $C_{14}H_{16}N_4O_3S_2 \cdot \frac{3}{2}H_2O$ | C, H, N |
| **4m** | 58 | 210~215 | 270 (25300) | $C_{14}H_{16}N_4O_3S_2 \cdot \frac{1}{2}H_2O$ | C, H, N, S |
| **4n** | 52 | 200~210 | 269 (19200) | $C_{15}H_{18}N_4O_3S_2 \cdot \frac{1}{2}H_2O$ | C, H, N |
| **4o** | 68 | 207~210 | 291 (11600) | $C_{13}H_{14}N_4O_3S_2 \cdot \frac{1}{2}H_2O$ | C, H, N, S |
| **4p** | 66 | 235~245 | 270 (13600), 324 (6100) | $C_{14}H_{16}N_4O_3S_2 \cdot \frac{1}{2}H_2O$ | C, H, N, S |
| **4q** | 61 | >300 | 268 (12300), 304 (9000) | $C_{12}H_{13}N_5O_3S_2 \cdot \frac{1}{2}H_2O$ | C, H, N[b] |

a: Symbols of the elements indicate that analyses are coincident with the calculated value within ±1% deviation.

b: N, calcd, 18.66; Found, 17.51.

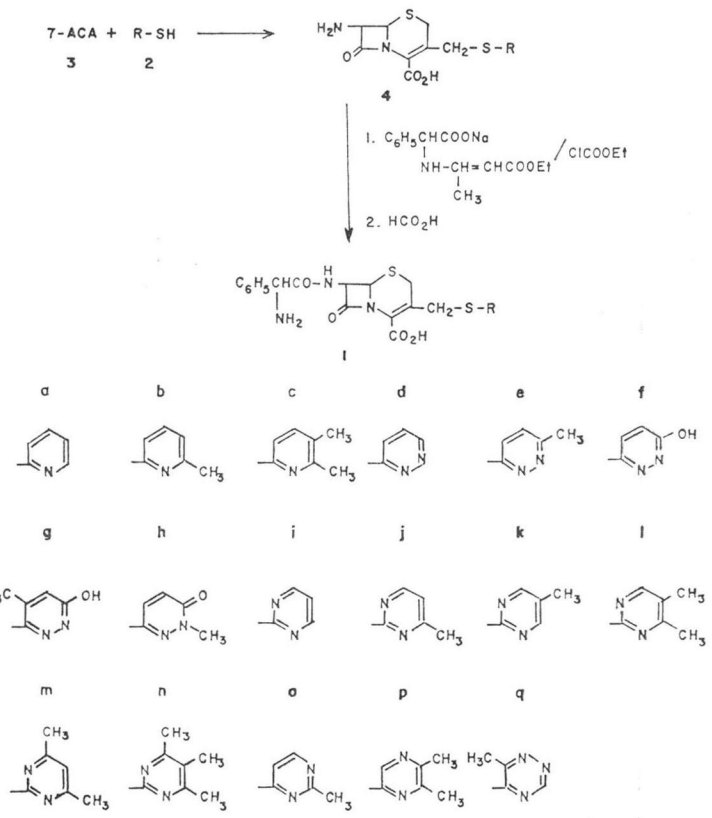
c: Asterisk (*) indicates a shoulder.

Table 2. 3-Substituted 7-[D(−)-α-aminophenylacetamido]cephalosporanic acids (**1**)

| Compound | Yield, % | Mp, °C (dec) | $\lambda_{max}$ in 1%$NaHCO_3$, nm (ε) | Formula | Analyses[a] |
|---|---|---|---|---|---|
| **1a** | 32 | 165~170 | 250 (11500), 265 (11000), 289 (8050) | $C_{21}H_{20}N_4O_4S_2 \cdot H_2O$ | C, H, N, |
| **1b** | 33 | 168~169 | 250 (11200), 272 (12000), 292 (9300) | $C_{22}H_{22}N_4O_4S_2 \cdot H_2O$ | C, H, N, S |
| **1c** | 54 | 167~170 | 245 (11900), 265 (11900), 295 (7700) | $C_{23}H_{24}N_4O_4S_2 \cdot 2H_2O$ | C, H, N, S |
| **1d** | 65 | 190~200 | 270 (12400) | $C_{20}H_{19}N_5O_4S_2 \cdot 3H_2O$ | C, H, N[b] |
| **1e** | 56 | 190~200 | 265 (13000) | $C_{21}H_{21}N_5O_4S_2 \cdot 3H_2O$ | C, H, N |
| **1f** | 58 | 245~250 | 248 (12400) | $C_{20}H_{19}N_5O_5S_2 \cdot 3H_2O$ | C, H, N |
| **1g** | 75 | 250~255 | 243 (17800) | $C_{21}H_{21}N_5O_5S_2 \cdot 2.5H_2O$ | C, H, N, S |
| **1h** | 76 | 230~240 | 249 (15700) | $C_{21}H_{21}N_5O_5S_2 \cdot 3H_2O$ | C, H, N, S |
| **1i** | 26 | 190~200 | 270 (18500) | $C_{20}H_{19}N_5O_4S_2 \cdot 2H_2O$ | C, H, N |
| **1j** | 21 | 180~190 | 270 (18600) | $C_{21}H_{21}N_5O_4S_2 \cdot 3H_2O$ | C, H, N |
| **1k** | 52 | 215~220 | 270 (21700) | $C_{21}H_{21}N_5O_4S_2 \cdot 1.5H_2O$ | C, H, N, S |
| **1l** | 51 | 180~190 | 268 (17800) | $C_{22}H_{23}N_5O_4S_2 \cdot 1.5H_2O$ | C, H, N, S |
| **1m** | 56 | 180~190 | 270 (19200) | $C_{22}H_{23}N_5O_4S_2 \cdot 2H_2O$ | C, H, N |
| **1n** | 68 | 190~195 | 268 (18800) | $C_{23}H_{25}N_5O_4S_2 \cdot 1.5H_2O$ | C, H, N, S |
| **1o** | 17 | 216~218 | 285 (17100) | $C_{21}H_{21}N_5O_4S_2 \cdot 1.5H_2O$ | C, H, N |
| **1p** | 50 | 210~220 | 270 (8700) | $C_{22}H_{23}N_5O_4S_2 \cdot 4.5H_2O$ | C, H[c], N |
| **1q** | 17 | >300 | 275 (8900) | | |

a: see, footnote a of Table 1. b: N, calcd, 13.64; found, 12.56. c: H, calcd, 5.70; found, 4.14

Scheme 1. Preparation of 3-substituted cephaloglycin analogs



and **4h**. This shows that **1f**, **1g**, **4f** and **4g** are present as the keto form rather than the enol form, at least in 1% sodium bicarbonate solution. The pyrimidin-2-ylthiomethyl derivatives, **1i**~**1n** and **4i**~**4n**, showed absorption maxima at 268~271 nm, whereas the pyrimidin-4-ylthiomethyl derivatives **1o** and **4o** had maxima at longer wave length by about 20 nm (285~291 nm).

## Antimicrobial Activity

The minimum inhibitory concentrations (MIC) of this series of cephalosporins (**1**) were determined by the two-fold serial tube dilution method using nutrient broth (Eiken) against 4 strains each of gram-positive and gram-negative bacteria, and the results are shown in Table 3 along with those of cephaloglycin (CEG) and cephalexin (CEX).

All of the compounds **1** showed strong inhibitory activity against gram-positive organisms generally superior to that of CEG and CEX. Activity of **1** against gram-negative organisms varied considerably depending upon the type of heterocycle in the 3-side chain with the following order in activity: pyridazine>pyridine>triazine≈pyrimidine>pyrazine. The hydroxy pyridazine derivatives **1f** and **1g** were the most active members of this series and showed good gram-negative activity equivalent to that of CEG and superior to that of CEX. The pyridazine derivatives **1d** and **1e** are the second most active group, but the pyridazinone derivative **1h** was considerably less active than **1f** and **1g**. This suggested that the latter compounds would interact with organisms in the enol form, but not with the keto form which was predominant in the equilibrium mixture. The pyrimidin-4-ylthiomethyl derivative **1o** was the most active one of the pyrimidine group (**1i**~**1o**) and approximately 4 times more active than the isomeric pyrimidin-2-ylthiomethyl derivative **1j** in gram-negative activity.

Table 3. *In vitro* activity of 3-substituted 7-[D(−)-α-aminophenylacetamido]cephalosporanic acid derivatives by tube dilution method in nutrient broth

| Compound | MIC (mcg/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Staphylococcus aureus* Smith | *Staphylococcus aureus* BX-1633-2 | *Streptococcus pyogenes* A 9604 | *Streptococcus pneumoniae* A-9585 | *Escherichia coli* NIHJ | *Escherichia coli* Juhl | *Klebsiella pneumoniae* A 9977 | *Salmonella enteritidis* A 9531 |
| **1a** | 0.4 | 1.6 | 0.16 | 0.31 | 3.1 | 12.5 | 12.5 | 3.1 |
| **1b** | 0.2 | 0.8 | 0.08 | 0.31 | 0.8 | 6.3 | 6.3 | 1.6 |
| **1c** | 0.2 | 1.6 | 0.16 | 0.31 | 1.6 | 12.5 | 6.3 | 3.1 |
| **1d** | 0.8 | 1.6 | 0.16 | 0.31 | 3.1 | 3.1 | 3.1 | 1.6 |
| **1e** | 0.8 | 1.6 | 0.16 | 0.31 | 3.1 | 3.1 | 6.3 | 1.6 |
| **1f** | 0.2 | 0.8 | 0.16 | 0.16 | 1.6 | 3.1 | 1.6 | 1.6 |
| **1g** | 0.2 | 1.6 | 0.63 | 1.25 | 0.8 | 12.5 | 3.1 | 1.6 |
| **1h** | 0.4 | 0.8 | 0.63 | 1.25 | 12.5 | 50 | 25 | 12.5 |
| **1i** | 0.8 | 1.6 | 0.63 | 1.25 | 6.3 | 25 | 12.5 | 3.1 |
| **1j** | 0.4 | 1.6 | 0.31 | 0.31 | 3.1 | 50 | 25 | 6.3 |
| **1k** | 0.8 | 1.6 | 0.63 | 2.5 | 6.3 | 25 | 25 | 12.5 |
| **1l** | 0.4 | 0.8 | 0.31 | 1.25 | 3.1 | 25 | 25 | 12.5 |
| **1m** | 0.8 | 1.6 | 0.63 | 0.63 | 6.3 | 100 | 25 | 12.5 |
| **1n** | 0.4 | 0.8 | 0.16 | 0.63 | 3.1 | 50 | 50 | 12.5 |
| **1o** | 0.2 | 1.6 | 0.31 | 1.25 | 0.8 | 12.5 | 3.1 | 1.6 |
| **1p** | 0.8 | 1.6 | 0.16 | 1.25 | 6.3 | 50 | 50 | 12.5 |
| **1q** | 1.6 | 3.1 | 0.63 | 1.25 | 6.3 | 25 | 25 | 6.3 |
| CEG | 0.8 | 1.6 | 0.63 | 1.25 | 3.1 | 3.1 | 1.6 | 1.6 |
| CEX | 0.8 | 3.1 | 0.31 | 5.0 | 12.5 | 6.3 | 6.3 | 6.3 |

Table 4. *In vivo* activity of BB-S 118, cephaloglycin and cephalexin

| Organism | Route | $PD_{50}$ (mg/kg, mice) | | |
|---|---|---|---|---|
| | | BB-S 118 | Cephaloglycin | Cephalexin |
| *S. aureus* Smith | sc | 1.4 | 1.2 | 0.8 |
| | po | 1.9 | 1.8 | 0.6 |
| *E. coli* Juhl | sc | 38 | 32 | 25 |
| | po | 50 | 32 | 18 |

Compound **1f** designated BB-S 118 was selected for the comparative *in vivo* evaluation with CEG and CEX. Table 4 compares $PD_{50}$ values of BB-S 118 with those of reference cephalosporins against experimental mice infections of *S. aureus* Smith and *E. coli* Juhl, indicating that BB-S 118 was as effective as CEG but less active than CEX when administered orally.

## Experimental

Azine thiols (**2**)

Three general methods were used for the synthesis of **2**. Method A was the reaction of an azine halide and an alkali hydrogen sulfide in an appropriate alcohol and was applied for preparation of **2a**[19], **2b**, **2c**[20], **2f**[21], **2g**[22], **2h**, **2i**[23] and **2o**[24]. Method B which required the reaction of a hydroxy azine and phosphorous pentasulfide in the presence of pyridine was used to obtain **2d**[25], **2e**[25], **2p** and **2q**[26]. Method C was the procedure for preparation of pyrimidine-2-thiols **2j**[27], **2k**[28], **2l**, **2m**[29] and **2n** and consisted of heating a $\beta$-diketone and thiourea in a mixture of hydrochloric acid and alcohol. Representative preparations are given below for each of the three methods.

6-Methylpyridine-2-thiol (**2b**)—Method A

To a hot solution of 11 g (0.152 mol) of KSH in 30 ml of propylene glycol was added dropwise 9.3 g (0.054 mol) of 6-methyl-2-bromopyridine[30]. The mixture was heated at 150~170°C for 24 hours with stirring and then kept to stand at room temperature to separate KBr, which was filtered off and washed with acetone and ethyl acetate. The filtrate was combined with the washings and evaporated *in vacuo*. The residue was dissolved in 25 ml of water, treated with active carbon and filtered. The filtrate was adjusted to pH 4 with glacial acetic acid to give **2b**, which was collected by filtration and washed three times with 20 ml of water. Recrystallization from ethanol gave 4.6 g (68%) of pure **2b** melting at 153~156°C.

Anal. Calcd. for $C_6H_7NS$: C, 57.56; H, 5.64; N, 11.19; S, 25.61.
Found: C, 57.69; H, 5.58; N, 10.97; S, 26.15.

1-Methyl-6(1H)-pyridazinone-3-thiol (**2h**)

According to Method A, **2h** was prepared from 3-chloro-1-methyl-6-(1H)-pyridazinone. Yield 53.5%. M.p. 102~106°C.

Anal. Calcd. for $C_5H_6N_2OS$: C, 42.24; H, 4.25; N, 19.70; S, 22.50.
Found: C, 42.66; H, 3.89; N, 20.08; S, 22.18.

5,6-Dimethylpyrazine-2-thiol (**2p**)—Method B

A mixture of 5.0 g (0.14 mol) of 5,6-dimethyl-3-hydroxypyrazine[31] and 7.0 g (0.03 mol) of $P_2S_5$ in 40 ml of pyridine was refluxed for 2 hours. After adding 100 ml of water, the reaction mixture was evaporated *in vacuo* to remove pyridine and extracted with chloroform. The chloroform extracts were treated with active carbon and evaporated under reduced pressure. The residue was crystallized from ethanol - *n*-hexane to give 0.85 g (15%) of **2p**. M.p. 168~169°C.

Anal. Calcd. for $C_6H_8N_2S$: C, 51.40; H, 5.75; N, 19.98; S, 22.87.
Found: C, 51.52; H, 5.89; N, 19.90; S, 22.77.

4,5-Dimethylpyrimidine-2-thiol (**2l**)—Method C

A mixture of 28.7 g (0.165 mol) of $\alpha$-methyl-$\beta$,$\beta$-diethoxyethyl methyl ketone[32] and 13.2 g

(0.173 mol) of thiourea in 55 ml of conc. HCl and 300 ml of ethanol was stirred at room temperature for 2 hours. The hydrochloride of **2l** which separated during the reaction was collected by filtration and dissolved in 30 ml of water. The aqueous solution was treated with active carbon and neutralized with dil. NaOH to give free **2l**, which was crystallized from ethanol - water to afford pale yellow needles. Mp. 235~240°C (in a sealed tube).

Anal. Calcd. for $C_6H_8N_2S$: C, 51.40; H, 5.75; N, 19.98; S, 22.87.
Found: C, 51.31; H, 5.54; N, 20.23; S, 23.03.

3-Substituted 7-aminocephalosporanic acids (**4**) (Table 1)

The syntheses of **4a**~**4q** listed in Table 1 have been achieved by the general procedure given below and the results are summarized in Table 1.

A mixture of 0.01 mole of 7-ACA, 0.02 mole of sodium bicarbonate and 0.012 mole of an azine thiol (**2**) in 40 ml of 0.1 M phosphate buffer (pH 6.4) was heated for 2~4 hours at about 60°C. The mixture was cooled to room temperature and adjusted to pH 4 with acetic acid to give the desired product **4**.

3-Substituted 7-[D(−)-α-aminophenylacetamido]cephalosporanic acids (**1**) (Table 2)

The syntheses of **1a**~**1q** listed in Table 2 have been achieved by the general procedure given below with the results summarized in Table 2.

To a stirred suspension of 0.01 mole of sodium D(−)-α-(N-1-ethoxycarbonyl-1-propen-2-yl)-aminophenylacetate[17] in 40 ml of dry acetonitrile containing one drop of N,N-dimethylbenzylamine was added 0.01 mole of ethyl chloroformate at −15°C and the mixture stirred for 0.5 hour at the same temperature. A mixture of 0.01 mole of **4** and 0.01 mole of triethylamine in 20 ml of 50% acetonitrile was added in one portion to the mixed anhydride solution at 0~5°C. The reaction mixture was evaporated under reduced pressure below 30°C; the resulting aqueous solution was covered with 40 ml of methyl isobutyl ketone and treated with 3 ml of formic acid at 0°C. The mixture was stirred for 0.5 hour at the same temperature to give the final product **1**, which was collected by filtration, washed successively with water and acetonitrile and dried *in vacuo*.

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