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NOTES

Synthesis of cefepime-d_3 and cefepime-d_8

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SUMMARY

Synthesis of cefepime-d_3 (**6a**) and cefepime-d_8 (**6b**) is described. Diphenylmethyl 7-benzylideneamino-3-chloromethyl-3-cephem-4-carboxylate (**2**) was treated with sodium iodide to afford the iodide **3**, which was, without isolation, allowed to react with N-methyl-d_3-pyrrolidine to give the quaternary salt **4a**. Deblocking of **4a** with HCO_2H and HCl gave 7-amino-3-(N-methyl-d_3-pyrrolidinio)methyl-3-cephem-4-carboxylate hydrochloride (**5a**). Acylation of **5a** with benzotriazol-1-yl (Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetate followed by treatment with dil. H_2SO_4 afforded **6a** sulfate. In the same way, **6b** was synthesized using N-methyl-pyrrolidine-d_8.

Key Words: cephalosporin, deuterium, cefepime-d_3, cefepime-d_8, N-methyl-pyrrolidine

INTRODUCTION

Cefepime (BMY-28142)(**1**) is a novel semi-synthetic cephalosporin which was synthesized in our Institute[1] and is now under development for clinical use. It shows a broad antibacterial spectrum against Gram-positive and Gram-negative bacteria[2,3] and slow hydrolysis by β-lactamases[4,5] which is important for resistance to enzymatic hydrolysis. This report describes the synthesis of cefepime derivatives with a deuterium labelled (N-methylpyrrolidinio)methyl group for metabolism studies.

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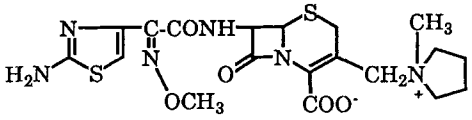
Fig. 1 Structure of cefepime



1, Cefepime (BMY-28142)

RESULTS AND DISCUSSION

Deuterium-labelled cefepimes were synthesized by an optimized method employed in the synthesis of non-deuterated cefepime[1] as shown in Scheme 1. In the course of the preparation, N-methyl-d_3-pyrrolidine (deuterium enrichment: 97.7 % d_3, 1.4 % d_2 and 0.9 % d_1) and N-methyl-d_8-pyrrolidine (deterium enrichment: 94.3 % d_8, 4.9 % d_7 and 0.8 % d_6) were used instead of N-methylpyrrolidine to give the quaternary salt **4a** and **4b**, respectively, both of which were converted to the 7-aminocephems (**5a** and **5b**) and subsequently N-acylated to afford deuterated cefepimes (**6a** and **6b**), respectively. Proton NMR spectra, elemental analyses and mass spectra supported the sturucture of **6a** and **6b**. Deuterium enrichment of **6a** was determined by accurate mass spectrometry (FAB) (Table 1). The apparent relative peak areas of $(M+H)^+$ of cefepime-d_3 and cefepime-d_2 were 100 and 10.1, respectively. Since the relative peak areas of $(M+H)^+$ and M^+ of cefepime were 100 and 8.4, respectively, the $(M+H)^+$ peak area of cefepime-d_2 was corrected to be 1.7 by subtraction of the peak area due to M^+ of cefepime-d_3 (8.4) from the apparent area of $(M+H)^+$ of cefepime-d_2 (10.1). Thus, deuterium enrichment of **6a** was determined to be 98 % d_3 and 2 % d_2. Deuterium enrichment of **6b** was determined to be 94% d_8 and 6% d_7 by a correction similar to that described above.

Scheme 1 Preparation of cefepime-d_3 (6a) and cefepime-d_8 (6b)

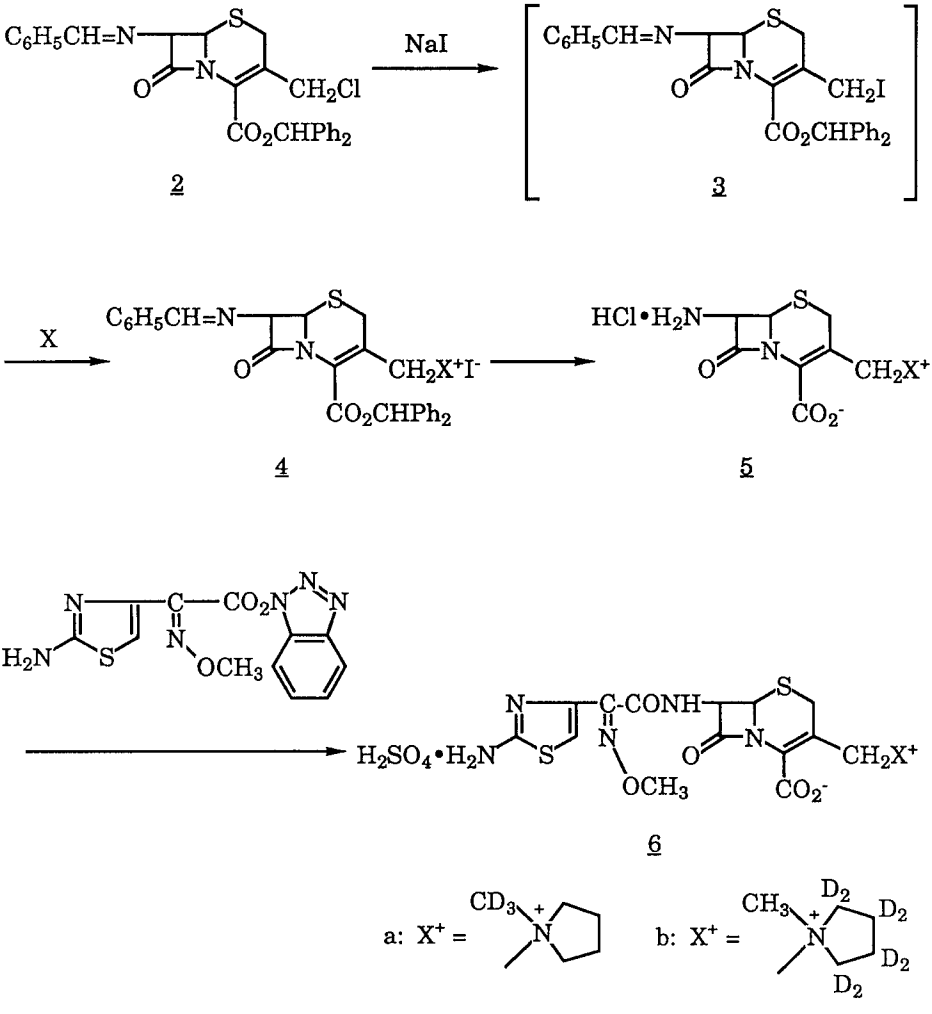


Table 1 Determination of deuterium enrichment of **6a** and **6b** by mass spectroscopy

a) Cefepime-d_3 (**6a**)

$(M+H)^+$	Formula	Mass Unit (m/z) Calc'd	Found	Relative Peak Area	Enrichment (%)
d_3	$C_{19}H_{22}N_6O_5S_2D_3$	484.1516	484.1480	100	98
d_2	$C_{19}H_{23}N_6O_5S_2D_2$	483.1454	483.1464	1.7*	2

b) Cefepime-d_8 (**6b**)

$(M+H)^+$	Formula	Mass Unit (m/z) Calc'd	Found	Relative Peak Area	Enrichment (%)
d_8	$C_{19}H_{17}N_6O_5S_2D_8$	489.1830	489.1853	100	94
d_7	$C_{19}H_{18}N_6O_5S_2D_7$	488.1768	488.1777	6.9*	6

* Corrected value by subtraction of M^+ relative peak area of cefepime

EXPERIMENTAL

Melting points were determined with a Yanagimoto micro-hot stage apparatus and are uncorrected. IR spectra were recorded on an Analect fx-6160 and UV spectra on a Shimadzu UV-260 spectrophotometer. NMR spectra were recorded on a Jeol GX-400 and mass spectra on a Jeol JMS-AX505H equipped with 5 KeV Xe^0 FAB ion gun. N-Methyl-d_3-pyrrolidine (deuterium enrichment 97.7 % d_3, 1.4 % d_2, 0.9 % d_1) and N-methyl-pyrrolidine-d_8 (deuterium enrichment 94.3 % d_8, 4.9 % d_7, 0.8 % d_6) were purchased from Daiichi Pure Chemicals Co. (Japan).

Diphenylmethyl 7-benzylideneamino-3-(N-methyl-d_3-pyrrolidinio)methyl-3-cephem-4-carboxylate iodide (**4a**)

To a stirred suspension of diphenylmethyl 7-benzylideneamino-3-chloromethyl-3-cephem-4-carboxylate (**2**) (8.66g, 17.2 mmol) in CCl_4 (174 ml) was added a solution of sodium iodide (3.4 g, 22.6 mmol) in acetone (44 ml); the mixture was stirred for 1 hr. at 20 °C. The reaction mixture was filtered and insolubles were washed with CCl_4 (11 ml). The filtrate and washings were combined, washed with aq. sat. $Na_2S_2O_3$ (100 ml) and water (100 ml x 2)

successively. The organic layer was dried over anhydrous sodium sulfate and filtered. To the filtrate was added dropwise under vigorous stirring a solution of N-methyl-d_3-pyrrolidine (1.67 g, 18.9 mmol) in CCl_4 (40 ml) at -3 °C to 0 °C over a period of 15 min. The mixture was stirred for 1 hr. at -2 °C to 0 °C to precipitate the quaternized product, which was collected by filtration, washed with CCl_4 (40 ml) and dried to give 11.80 g (quantitative) of **4a**. M.p. 120-122 °C (dec.). IR ν_{max} (KBr) cm^{-1} 1779, 1728. UV λ_{max} (EtOH) nm (ε) 258 (24300). ^1H NMR ($CDCl_3$, δ in ppm), the N-Me-pyrrolidine moiety 3.55 (4H, m, NCH_2), 2.15 (4H, m, CH_2CH_2), no N-CH_3 signal.

7-Amino-3-(N-methyl-d_3-pyrrolidinio)methyl-3-cephem-4-carboxylate (5a)

To a stirred mixture of **4a** (11.5 g, 17.3 mmol) in 98 % formic acid (11.5 ml) was added concentrated hydrochloric acid (7.67 ml). The mixture was stirred for 1 hr. at room temperature and poured into acetone (460 ml) with stirring to precipitate the crude product, which was collected by filtration. The precipitate was dissolved in water (60 ml) and the solution was diluted with acetone (300 ml) to crystallize the product as needles after seeding with a few crystals of the non-deuterated analog[1]. The crystals were collected by filtration, washed with acetone (80 ml) and dried to give 2.72 g (49 %) of **5a**. M.p. 170-190 °C (grad. dec.). IR ν_{max} (KBr) cm^{-1} 1793, 1618. UV λ_{max} (pH 7 buffer) nm (ε) 266 (8900). MS (FAB, glycerol) m/z 301 $(M+H)^+$. ^1H NMR (D_2O, δ in ppm), the N-Me-pyrrolidine moiety 3.50 (4H, m, N-CH_2), 2.20 (4H, m, CH_2CH_2), no N-CH_3 signal. *Anal.* calcd for $C_{13}H_{16}D_3N_3O_3S \cdot HCl \cdot 1/2H_2O$: C, 45.15; N, 12.15; S, 9.27; Cl, 10.25. Found: C, 45.08; N, 12.15; S, 9.39; Cl, 10.45.

Cefepime-d_3(6a)

To a cold suspension of **5a** (2.62 g, 7.0 mmol) in DMF (52 ml) and water (26 ml) was added sodium bicarbonate (1.18 g, 14.0 mmol). Benzotriazol-1-yl (Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetate6) (3.34 g, 10.6 mmol) was added and the mixture was stirred for 1 hr. at room temperature. A solution of 4*N* H_2SO_4 (1.80 ml) was added and the insoluble materials were removed by filtration. The filtrate was poured into acetone (720 ml) with stirring. The

precipitate was collected by filtration and dissolved in water (30 ml). Charcoal (200 mg) was added to the solution and filtered off. The filtrate was treated with 4*N* H_2SO_4 (14 ml) and seeded with a few crystals of non-deuterated cefepime to give 2.85 g (65 %) of **6a** as colorless needles. M.p. >210 °C (grad. dec.). IR ν_{max} (KBr) cm^{-1} 1792, 1679, 1649. UV λ_{max} (pH 7 buffer) nm (ε) 235 (17700) 258 (17500). ^1H NMR (D_2O, δ in ppm), the N-Me-pyrrolidine moiety 3.50 (4H, m, N-CH_2), 2.20 (4H, m, CH_2CH_2), no N-CH_3 signal. *Anal*. calcd for $C_{19}H_{21}D_3N_6O_5S_2 \cdot H_2SO_4$: C, 39.23; N, 14.45; S, 16.54. Found: C, 39.03; N, 14.30; S, 16.60.

Cefepime-d_8 (6b)

Cefepime-d_8 (**6b**) was synthesized by a procedure similar to that described above using N-methyl-pyrrolidine-d_8 *via* **4b** and **5b**.

Compound **4b**; Yield 11.83 g (quantitative yield). M.p. 122-125 °C (dec.). IR ν_{max} (KBr) cm^{-1} 1780, 1728. UV λ_{max} (EtOH) nm (ε) 258 (21600). ^1H NMR ($CDCl_3$, δ in ppm) the N-Me-pyrrolidine moiety 2.82 (3H, s, N-Me), no CH_2 signals of pyrrolidine.

Compound **5b**; Yield 2.99 g (47 %). M.p. 170-190 °C (grad. dec.). IR ν_{max} (KBr) cm^{-1} 1795, 1618. UV λ_{max} (pH 7 buffer) nm (ε) 266 (8300). MS (FAB, glycerol) m/z 306 $(M+H)^+$. ^1H NMR (D_2O, δ in ppm), the N-Me-pyrrolidine moiety 2.99 (3H, s, N-Me), no CH_2 signals of pyrrolidine. *Anal*. calcd for $C_{13}H_{11}D_8N_3O_3S \cdot HCl \cdot H_2O$: C, 43.39; H, 11.68; S, 8.91; Cl, 9.85. Found: C, 43.38; H, 11.63; S, 8.97; Cl, 9.96.

Compound **6b** (cefepime-d_8); Yield 3.00 g (64 %). M.p. > 200 °C (grad. dec.). IR λ_{max} (KBr) cm^{-1} 1792, 1679, 1650. UV_{max} (pH 7 buffer) nm (ε) 235 (18000), 258 (17800). ^1H NMR (D_2O, δ in ppm), the N-Me-pyrrolidine moiety 2.98 (3H, s, N-Me), no CH_2 signals of pyrrolidine. *Anal*. calcd for $C_{19}H_{16}D_8N_6O_5S_2 \cdot H_2SO_4$: C, 38.90; H, 14.32; S, 16.39. Found: C, 39.01; H, 14.13; S, 16.37.

Determination of deuterium enrichment of 6 by mass spectroscopy

Determination of deuterium enrichment of **6a** and **6b** was carried out by

using FAB accurate mass spectroscopy (sample, dissolved in 50 % aqueous DMSO; matrix, glycerol; accelerating voltage, 3 KV). The results are summarized in Table 1, in which deuterium enrichment of d_2 and d_7 were corrected by relative peak area of M^+ (8.4 %) to $(M+H)^+$ of cefepime.

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