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A rapid procedure to prepare cefotaxime

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Abstract

A rapid procedure is reported for the synthesis of cefotaxime, by acylation of the 7-amino cephalosporanic acid with the 2-mercaptobenzothiazolyl thioester of (*Z*)-2-[2-aminothiazol-4-yl]-2-methoxyimino acetic acid (MAEM) that is a commercial reagent. The reaction was carried out at room temperature for 1 h, obtaining 95% yield. 2-Mercaptobenzothiazole was recovered as a side-product with a high purity and yield. The proposed method differentiates from those reported previously for a shorter time and very mild reaction condition, as well as for a ready for use reagent.

Keywords: Cefotaxime; MAEM; Synthesis; 7-Aminocephalosporanic acid

1. Introduction

Cefotaxime (7β-(2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyimino acetamido)-3-acetoxymethyl-3-cephem-4-carboxylic acid or its sodium salt) was the first cephalosporin of the third generation developed. This antibiotic displays a high antimicrobial potency, a broad antibacterial spectrum, high resistance against the action of β-lactamases, as well as a low index of side effects [1,2]. For these reasons it is largely used in the treatment of several infections including, meningitis, septicemia, peritonitis, infections of the genito-urinary and breathing tracts, infections of the skin, bones, articulations, etc. [2]. Moreover, cefotaxime can be used as the main intermediary in the synthesis of cefpodoxime proxetil, a third generation cephalosporin for oral administration, introduced recently into the medical practice [3,4]. Cefotaxime contains a 2-aminothiazol-4-yl moiety linked to position 7 of 7-amino cephalosporanic acid (7-ACA) through a methoxyimino acetamido bridge (Fig. 1). This moiety can be introduced either by acylation of 7-ACA either with (*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid (ATMA) suitably activated [5] or by reaction in a modified form, such as acid chloride, anhydride, etc. [6–9]. The high reactivity of these derivatives makes it necessary to protect and de-protect the amino function of ATMA, to avoid the formation of side products [1]. The development of alternative forms for ATMA, such as esters and/or amides of the hydroxybenzotriazole (HOBT) [10–12] and different thioesters [13], allowed a selective and advantageous acylation of 7-ACA, without necessity of blocking the amino function of ATMA [1].

Cefotaxime is poorly soluble in water, therefore for its clinical use it is transformed into the soluble sodium salt, prepared by neutralization with sodium hydrogen carbonate [8,14] or by reaction with salts of carboxylic acids of higher pK_a, as sodium acetate or sodium 2-ethylhexanoate. In both cases, different water–alcoholic mixtures [8] are used currently as reaction media in order to isolate and purify the product.

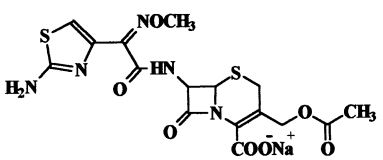

Fig. 1. Cefotaxime sodium salt.

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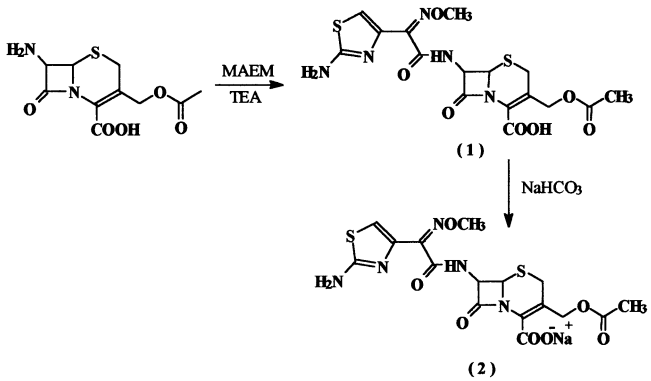
The purpose of this paper is to report a new procedure for the synthesis of cefotaxime, starting from the 7-ACA and a thioester of ATMA (MAEM), a commercially available reagent that does not need preliminary modifications to react.

2. Results and discussion

The new procedure developed for cefotaxime (Scheme 1) consists in the reaction between 7-ACA and MAEM in dichloromethane as a solvent and using TEA both to dissolve 7-ACA and catalyze the reaction (Scheme 1).



Scheme 1.

The process was carried out at room temperature, monitoring by TLC the advance of the reaction, which was found complete within 1 h.

Cefotaxime was obtained in the form of the corresponding triethylammonium salt with yield 95%; separated from the reaction mixture by means of a simple extractions with water, while 2-mercaptobenzothiazole, obtained as by-product, remained in the organic phase. The aqueous extracts were acidified to obtain the acid form and washed with water, ethanol and diethyl ether. Addition of sodium hydrogen carbonate in aqueous ethanol gives the sodium salt of cefotaxime [8,15,16].

A brief summary is shown in Table 1 of the different methods reported for the synthesis of cefotaxime, taking into account the reaction conditions, the reagents used and the yields obtained in each process, for comparison.

The methods A–E use acylation of 7-ACA with acid chloride or anhydride of ATMA (except E). Because of the high reactivity of these derivatives it is necessary, in all of the cases, to block the ATMA amino group to avoid the formation of side reactions. Although the acylation reaction is fast (1–2 h), the final deprotection requires much time, and decreases the yield below 65%.

In the last four procedures F–I, ATMA esters and/or active amides are used as reagents. The lower reactivity of these derivatives makes it possible to carry out the process without blocking the ATMA amino group and undesirable side reactions are less probable. As a consequence, the final yield is higher than 90%; the time required is shorter than that necessary for other methods, despite a slower reaction rate.

Two main advantages of the proposed method J are, that acylation takes place in 1 h and protection of the ATMA amino function is no more necessary. As a result a higher yield is obtained with a shorter time of reaction (1 h). Moreover, an additional advantage of J is the use of MAEM, a commercial reagent, as opposed to the hydroxybenzotriazole esters and/or amides of ATMA. In the procedure I, where MAEM is also used, a catalyst (BSA) was necessary and, despite this, the reaction time needs a longer time than that necessary

Table 1
Reported methods in synthesis of cefotaxime [a]

Method [references]	Reactants	*t* (h)	*T* (°C)	Yield (%)
A [6,10]	*t*-Butylester of 7-ACA, CATMA, dichloromethane, pyridine, thiourea, anisole, TFA.	16	25	31
B [8]	7-ACA, tritylated derivative of ATMA, DCC, TEA, dichlorometane, formic acid.	3	20	65
C [9]	7-ACA, acid chloride CATMA, dichloromethane, TEA, thiourea.	8	20	54
D [11]	7-ACA, acid chloride of CATMA, TEA, thiourea tetrahydrofuran.	16	25	24
E [16]	7-ACA, formyl derivative of ATMA, diphenyl phosphite, pyridine, dioxane.	2	25	72
F [12]	7-ACA, mixture of ester and active amide of ATMA, dichloromethane, tetrahydrofuran, TEA.	16	25	69
G [13]	7-ACA, active ester of ATMA, acetonitrile, sodium hydrogen carbonate.	6	25	93
H [14]	7-ACA, active amide of ATMA, acetonitrile sodium hydrogen carbonate.	4	25	95
I [15]	7-ACA, MAEM, BSA.	15	25	92
J [present work]	7-ACA, MAEM, dichloromethane, TEA.	1	25	95

[a] 7-ACA, 7-aminocephalosporanic acid; ATMA, (*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid; TFA, trifluoroacetic acid; DCC, dicyclohexylcarbodiimide; TEA, triethylamine; CATMA, 7β-[2-(2-chloroacetylaminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid; MAEM, 2-mercaptobenzothiazolyl thioester of (*Z*)-2-[2-aminothiazol-4-yl]-2-methoxyimino acetic acid; BSA, *N*,*O*-bis(trimethylsilyl)acetamide.

with the method J. Finally the proposed method allows the recovery of 2-mercaptobenzothiazole with a high degree of purity and high yield, constituting an additional advantage of this method.

3. Experimental

3.1. General methods

TLC analysis was performed on precoated silica gel Merck GF-254 plates and using mixtures of ethyl acetate–ethanol–water–formic acid (65:25:15:1, v/v) (a) and ethyl acetate–*n*-hexane (1:1, v/v) (b) as mobile phases. The spots were visualized in a UV CAMAG lamp at $\lambda = 254$ nm. ^{1}H NMR spectra were recorded at 250 MHz in a Bruker BHZ AC 250F using DMSO-d_6 as solvent and TMS as internal standard. Chemical shifts are expressed in ppm (δ) downfield from TMS. MS spectra were recorded in a quadrupolar mass spectrometer TRIO 1000 (Fisons Instrument) based on the electronic impact technique with EI = 70 eV and DMK 400 V. pH measurements were carried out in aqueous solution on 10% w/v at 25°C in a Crison micropH 2001. Melting points were determined in a Gallenkamp apparatus and are uncorrected.

The HPLC techniques were carried out in a Merck Hitachi, LaChrom model, performed by a pump L7100, a UV detector model L-7400 and an injector with a loop of 20 ml, using the Biochrom software (CIGB, Cuba). The stationary phase was a LiChrosorb RP-18 (5 mm; Merck) column of 250 × 4 mm coupled with a pre-column RP-18 (Merck). As mobile phase was used a mixture of methanol–water–acetic acid (30:70:0.1, v/v) adjusted to pH 3.40 with glacial acetic acid. The work-flow was 0.75 ml min^{-1} and detection was made at $\lambda = 254$ nm.

Prepared cefotaxime sodium salt and recovered 2-mercaptobenzothiazole were compared with authentic samples.

3.2. Synthesis of cefotaxime free acid form (*1*)

A suspension of 7-ACA 62.9 g (231 mmol) in 755 ml of dichloromethane, was chilled to 5–10°C and 71.0 ml (513 mmol) of TEA were added with stirring. The mixture was heated to room temperature, 89.6 g (256 mmol) of MAEM was added and the resulting mixture stirred for 1 h. After that the mixture was extracted twice with 320 and 160 ml of water. The combined aqueous extracts were adjusted to pH 2.9 by adding 47 ml of 6 M hydrochloric acid with continuous stirring. The suspension was chilled to 0–5°C, the precipitate was isolated by vacuum filtration and washed successively with water (60 ml), ethanol (60 ml) and ethyl ether (2 × 80 ml). The solid was dried for 4 h at 40°C, affording 100 g (95% yield) of **1**.

3.3. Synthesis of cefotaxime sodium salt (*2*) [8]

A suspension of 50.0 g (110 mmol) of **1** in a mixture of 110 ml of water and 90 ml of ethanol was added of 8.78 g (105 mmol) sodium hydrogen carbonate suspended in 25 ml of ethanol. The resulting solution was treated with 5 g of activated charcoal and stirred during 15 min. The mixture was vacuum filtered, the filter was washed successively with 250 ml of ethanol and 100 ml of water, the filtrates were combined and evaporated to dryness. The residue dissolved in 110 ml of methanol was poured into 2.2 l of diethyl ether under stirring and then filtered. The precipitate washed with diethyl ether (2 × 50 ml) was vacuum dried during 3–4 h at 35–40°C affording 50 g (95.4% yield). ^{1}H: 9.61 (1H, d, NH); 7.25 (2H, s, NH_2); 6.75 (1H, s, thiazole ring); 5.80 (1H, dd, H7); 5.16 (1H, d, H6); 5.0 (2H, d, CH_2O); 4.70 (2H, d, H_2); 3.86 (3H, s, OCH_3); 2.07 (3H, s, CH_3COO) [16], pH (solution 10% w/v): 5.0 [17], HPLC: purity: 98%; retention time: 6.42 min [17], TLC: R_f: 0.74 (a) as eluent mixture [18].

3.4. Procedure for the recovery of 2-mercaptobenzothiazole

The residual organic layer obtained, after extraction of cefotaxime with water, was washed with 140 ml of 2 M sodium hydroxide solution; the aqueous layer was then acidified with 43 ml of 6 M hydrochloric acid. The precipitate was filtered, washed with water (3 × 50 ml) and dried during 1 h at 100°C. Yield 34.6 g (89.6%). TLC: R_f: 0.78 (b) as eluent mixture, m.p.: 176–178°C (for 95% 2-mercaptobenzothiazole: 174–178°C) [19], MS m/z 167 [M^+], 140, 122, 108, 95, 82, 76, 69, 63, 59, 50, 45, 38 and 32 [17].

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