POSSIBILITIES TO ACYLATE 7-AMINOCEPHALOSPORANIC ACID TO OBTAIN SOME CEPHALOSPORINS

Cătălina Daniela Stan¹, Maria Drăgan¹, D.E. Diaconu³, Alina Ştefanache²
University of Medicine and Pharmacy “Grigore T. Popa” - Iaşi
Faculty of Pharmacy
1. Drug Industry and Pharmaceutical Biotechnology Department
2. General and Inorganic Chemistry Department
3. Antibiotice S. A. – Iaşi

POSSIBILITIES TO ACYLATE 7-AMINOCEPHALOSPORANIC ACID TO OBTAIN SOME CEPHALOSPORINS (Abstract): Aim: Acylation of 7-aminocephalosporanic acid with an adequate acyl chloride in order to obtain cefotaxime sodium salt. Material and methods: Cefotaxime sodium salt was synthesized by acylating 7-amino cephalosporanic acid with 2-[2′-chloracetamidothiazole-4-yl]-2-(syn)-methoxy-imino acetic chloride in four steps. The melting point was determined, and IR spectral analysis and elemental analysis were performed to confirm cefotaxime structure. The quantitative determination was performed. Results: The reaction conditions were established. The yield of the synthesis phases (73-80%) and actual yield (45-47%) were very good. The structure of the obtained cefotaxime sodium salt was confirmed by the IR spectral analysis and by elemental analysis (C, H, N). The melting point was 163°C. The purity of the synthesized cefotaxime sodium salt was 98.9%. Conclusions: Cefotaxime sodium salt was synthesized by acylation of 7-aminocephalosporanic acid with 2-[2′-chloracetamidothiazole-4-yl]-2-(syn)-methoxy-imino acetic chloride, in aqueous solution, then transformed into sodium salt with sodium 2-ethylhexanoate. The method proved to be very good, yields were good, it is reproducible and simple, and does not involve high risks, so it is also safe. Keywords: 7-AMINOCEPHALOSPORANIC ACID, CEFOTAXIME, CEPHALOSPORINS, ACYLATION.

Cephalosporins are often used to treat severe infections with Gram-negative and Gram-positive aerobic and anaerobic microorganisms (1). Cefotaxime is a third generation cephalosporin, being a broad spectrum beta-lactam antibiotic with high resistance to beta-lactamase inactivation. In therapeutic doses it has no toxicity, and it is used as antibacterial agent in severe infections caused by susceptible strains of Gram-positive and Gram-negative bacilli, Gram-positive and Gram-negative cocci. It is a parenteral cephalosporin used as “ex temporae” solution of cefotaxime sodium salt, 1-2 g/day every 8-12 hours. It can be used to treat severe infections in adults, children and infants (2).

Cephalosporins could be obtained by acylation of the 7-amino group of 7-amino cephalosporanic acid (7ACA) with acyl chlorides, mixed anhydrides, activated esters, activated acids, enzymatic acylation and transacylation (3, 4, 5, 6).

This paper presents the possibilities of acylating 7-aminocephalosporanic acid (7ACA) with an adequate acyl chloride in
order to obtain cefotaxime, a third generation cephalosporin.

**MATERIAL AND METHODS**

**Apparatus:**
- Infrared Spectrophotometer UNICAM SP200;
- Melting point apparatus Mel-Temp Barnstead/Thermolyne 1002 (USA);
- Exeter-Analytical, CE-440 elemental analyzer with thermal conductivity detection.

**Materials and reagents:**
- 7-aminocephalosporanic acid; 2-[2’-chloracetamidothiazole-4-yl]-2-(syn)-methoxy-imino acetic acid; thiourea; sodium 2-ethylhexanoate; phosphorus pentachloride; standard reagents and organic solvents (*p.a.* quality) purchased from Sigma Aldrich Company.

**Chemistry**

Cefotaxime sodium salt synthesis was performed in four steps:

1. 2-[2’-chloracetamidothiazol-4-yl]-2-(syn)-methoxy-imino acetic (CATMA) chloride obtained by treating the methylene chloride solution of the CATMA acid with phosphorus pentachloride at −5°C (fig. 1);
2. 7ACA sodium salt acylation with CATMA chloride, obtained in the first step, at 10–15°C, when a protected cefotaxime was formed (fig. 2);
3. Deprotection of the second step obtained compound, by treating it with thiourea and then with formic acid until pH = 1.9–2.2 at −5°C, when cefotaxime free acid was formed (fig. 3);
4. Cefotaxime sodium salt obtained by treating the cefotaxime free acid with an isopropanol solution of sodium 2-ethylhexanoate (fig. 4).

---

![Fig. 1. Synthesis of CATMA chloride](image1)

![Fig. 2. Synthesis of protected cefotaxime](image2)
In the first phase the CATMA chloride was obtained by treating the methylene chloride solution of the CATMA acid with phosphorus pentachloride at \(-5^\circ C...-8^\circ C\). We suspended 5 g of 2-[2\(^\prime\)-chloracetamidothiazol-4-yl]-2-(syn)-methoxy-imino acetic acid in 125 ml of methylene chloride and cooled the suspension to \(-5^\circ C...-8^\circ C\). Then, 5.8 g of phosphorus pentachloride were added, and after 30 minutes of stirring 63 ml of \(n\)-hexane were added. The suspension was filtered and the precipitate washed 2-3 times with \(n\)-hexane and then dried.

In the second phase, the 7ACA sodium salt was acylated with the previously obtained CATMA chloride. Four g of 7ACA acid were suspended in 72 ml of distilled water. The suspension was cooled to 10–15\(^\circ C\) and then 4.7 g of NaHCO\(_3\) were slowly added. In 15–20 minutes a solution of 7ACA sodium salt was obtained. The CATMA chloride was slowly added and at
Possibilities to acylate 7-aminocephalosporanic acid to obtain some cephalosporins

room temperature the acylation reaction was performed for 3 hours. The protected cefotaxime sodium salt was obtained.

In the third phase, the compound obtained in phase 2 was deprotected by treating it with thiourea and then with formic acid until pH=1.9–2.2 at −50°C; cefotaxime free acid was formed. Over the solution obtained in phase 2, protected cefotaxime sodium salt, 2.8 g thiourea were added and stirred for 6 hours at room temperature. The solution was discolored with activated carbon charcoal and the suspension was filtered. The obtained filtrate was diluted with distilled water, cooled to −50°C and acidulated with 80% formic acid solution until pH=1.9–2.2. The obtained suspension was filtered, and the precipitate was washed with cold water. The crystals were suspended in 95% ethanol, stirred for 2 hours, then filtered and dried for 3 hours at 25–40°C.

In the fourth phase the cefotaxime sodium salt was obtained. The methanolic solution of cefotaxime (5 g of cefotaxime dissolved in 17 ml of methanol) was treated with stoechiometric quantities of isopropanolic solution of sodium 2-ethylhexanoate. The mixture was stirred for 10 minutes and then discolored with activated carbon, for other 10 minutes. The activated carbon charcoal was filtered and washed with methanol. After that, over 200 ml of isopropanol the cefotaxime sodium salt methanolic solution was added and the crystallization process of the cefotaxime sodium salt started. The suspension was stirred for 60 minutes and then filtered. The crystals were washed with 10 ml of isopropanol and dried.

The product was purified by recrystallization from 95% ethanol. The pure cefotaxime sodium salt was dried for 5 hours at 40–45°C.

The IR spectra of the synthesized cefotaxime sodium salt and of the standard one were recorded with an Infrared Spectrophotometer UNICAM SP200. The spectra were acquired over the range of 650 and 5000 cm⁻¹. The elemental analysis was performed with an Exeter-Analytical CE-440. The quantitative determination of the synthesized cefotaxime sodium salt was carried out by titration in non-aqueous medium with perchloric acid in dioxane, according to pharmacopoeia (7, 8).

RESULTS AND DISCUSSION
Using the presented method we obtained cefotaxime sodium salt as a white, white-yellowish crystalline powder, with characteristic odor, bitter taste, and very slightly soluble in water.

The compound was characterized by melting point and elemental analysis (C, H, N) (tab. I).

<table>
<thead>
<tr>
<th>No</th>
<th>Substance</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Melting point (°C)</th>
<th>C, H, N calculated/ found (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Cefotaxime sodium salt</td>
<td>C₁₆H₁₆N₅NaO₇S₂</td>
<td>Mr=477.45</td>
<td>163</td>
<td>C:40.24/40.18</td>
</tr>
</tbody>
</table>
<pre><code>                    |                               |                   |                  |                                | H:3.37/3.34                 |
                    |                               |                   |                  |                                | N:14.66/14.61                |
</code></pre>
The yields of the process phases were very good, over 70% for each phase, and also the overall yield of the general process was very good, over 45% (tab. II).

**TABLE II**

The yields of the process

<table>
<thead>
<tr>
<th>Phase</th>
<th>η (%)</th>
<th>η (%) average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 1</td>
<td>77.96</td>
<td>77.78</td>
</tr>
<tr>
<td>Phase 3</td>
<td>79.61</td>
<td>80.89</td>
</tr>
<tr>
<td>Phase 4</td>
<td>73.07</td>
<td>73.46</td>
</tr>
<tr>
<td>General process</td>
<td>45.35</td>
<td>46.21</td>
</tr>
</tbody>
</table>

These suggest that the method used to obtain cefotaxime sodium salt is a good one, is reproducible, and the obtained amounts are large. The method is easy to perform, is simple and does not involve high risks, so it is also safe.

The structure of the cefotaxime sodium salt obtained by the above described method was confirmed by IR spectral analyses and compared with the IR spectrum of cefotaxime sodium salt standard. The characteristic absorption bands of all functional groups were recorded (tab. III).

In the IR spectrum of the obtained cefotaxime sodium salt the characteristic absorption bands of all functional groups were observed. The presence of absorption bands at 1760 cm\(^{-1}\), characteristic of CO lactonic group, proved the integrity of the β-lactam ring. The presence of absorption bands at 3320 cm\(^{-1}\) proved the presence of the free amine group in the 3-position of the thiazole ring.

The quantitative determination was done by titration in non-aqueous medium with perchloric acid in dioxane. In order to correspond to pharmacopoeia provisions, the obtained cefotaxime sodium salt must contain at least 98.0% and no more than 102.0% cefotaxime sodium salt related to the anhydrous substance (7, 8). The obtained cefotaxime sodium salt had a purity of 98.9%, so it meets the provisions of pharmacopoeia (tab. IV).

**TABLE III**

Characteristic spectral bands of cefotaxime sodium salt

<table>
<thead>
<tr>
<th>Substance</th>
<th>IR characteristic band (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthesized cefotaxime sodium salt</td>
<td>-NH-</td>
</tr>
<tr>
<td></td>
<td>3320</td>
</tr>
<tr>
<td>Cefotaxime sodium salt standard</td>
<td>3350</td>
</tr>
</tbody>
</table>
Possibilities to acylate 7-aminocephalosporanic acid to obtain some cephalosporins

TABLE IV
Cefotaxime sodium salt concentration

<table>
<thead>
<tr>
<th>No.</th>
<th>Substance</th>
<th>Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Cefotaxime sodium salt</td>
<td>98.9</td>
</tr>
</tbody>
</table>

CONCLUSIONS
In this study we synthesized cefotaxime sodium salt by acylation of 7ACA sodium salt with CATMA chloride, in aqueous solution, when cefotaxime free acid was formed, followed by the transformation in sodium salt with sodium 2-ethylhexanoate. The method proved to be a very good one, having good yields, is reproducible, and the obtained amounts are large. It is not difficult to perform, is simple, and does not involve high risks, so it is safe.

REFERENCES
3. Tippa DMR, Singh N. Synthesis of cefotaxime from DAMA (diethyl thiophosphoryl [(z)-(2-aminothiazol-4-yl)-2-(methoxyimino) and 7-ACA (7-amino cephalosporinic acid). IJPSR 2011; 2(8): 2178-2182.
8. *** European Pharmacopoeia. 7.0 edition, on line, 2011.