Research Article

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Efficient pilot-scale synthesis of the key cefonicid intermediate at room temperature

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Abstract: Cefonicid is a common second-generation cephalosporin, and the 7-amino-3-[sulphomethyl-1-*H*-tetrazol-5-yl-thiomethyl]-3-cephem-4-carboxylate monosodium salt is a key synthetic intermediate in its preparation. Despite the considerable international demand for this antibiotic, its preparation is hampered by low synthetic yield, long reaction time, and time-consuming industrial filtration over charcoal after the purification step. In the context of the industrial production of pharmaceutical intermediates, in which the balance between streamlining and enhancing productivity is necessary in order to compete in the global active pharmaceutical ingredients (API) market, we have investigated an efficient and practical procedure for the synthesis of a key cefonicid intermediate that features a telescopic route whose synthetic steps are all performed at room temperature; from the displacement of the acetoxy group with boron trifluoride to crystallization without treatment with charcoal. In other words, a simpler, scalable, cost-effective and energy-saving protocol is herein reported as a means of moving towards commercial manufacturing. The optimization of the process parameters and the industrial-scale impact assessment should pave the way for industrialization.

Keywords: API production, cephalosporin, mild reaction conditions, pilot-scale method, energy saving

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1 Introduction

β-Lactams (and β-lactamase inhibitors) are the most frequently employed class of bactericidal antibiotics, accounting for more than 60% of all antibiotics use with an annual expenditure of approx. 15 billion USD. These compounds share the 4-membered 2-azetidinone subunit as a common structural motif and can be classified into two subgroups according to their structural environment: (a) conventional β-lactams (penicillin (penams), cephalosporins, cephamycins, cephabacins) and (b) nonconventional β-lactams (clavams, carbapenems, nocardicins, monobactams) (see refs [1,2]; other relevant references are listed in the article of Southgate and Elson [3]). In the conventional β-lactams group, penams and cephalosporins deserve particular mention. Cephalosporins are reminiscent of penicillins in which the 5-membered thiazolidine ring of the penams is replaced by a 6-membered dihydrothiazine ring, and are categorized in five groups, or generations, depending on their activity spectrum and resistance to enzymatic hydrolysis.

7-Aminocephalosporanic acid (7-ACA) is the lead compound in the booming synthesis of cephalosporin, in which complex congeners (structures) are made in the hope of increasing their antibacterial activity (broad or selective) while minimizing inactivation by β-lactamase and cross-reactivity with cephalosporins and penicillins. Structure optimization is performed via the empirical (trial-and-error) systematic addition of a new chain at position 7 and/or the modification of the 3′-side chains embodied in the 7-ACA scaffold [4].

Cefonicid (CFND – [7-D-mandelamido-3-(1-sulfomethyl-tetrazol-5-yl-thiomethyl)-3-cephem-4-carboxylic acid, as disodium salt]) is a broad-spectrum second-generation cephalosporin that is resistant to β-lactamase, parenterally administered and used in the management of urinary tract infections, lower respiratory tract infections, and soft tissue and bone infections [5]. CFND (SK&F 75073) was patented by GlaxoSmithKline in 1978 [6], approved for medical use by the US Food and Drug Administration on July 26, 1993, and marketed under the brand name Monocid. CFND shares

the same 7-acylamino side chain as cefamandole but differs in the presence of an SO_3H group that is appended at the 1-Me of the tetrazole ring. Although CFND is no longer marketed (Feb 2002) in the U.S., because of a lack of commercial significance, it is still commonly used in medium and low-income countries. It has the longest half-life (4.6 h) of all the second-generation agents and, consequently, its once-daily dosage regimen makes CFND truly cost-effective [7,8].

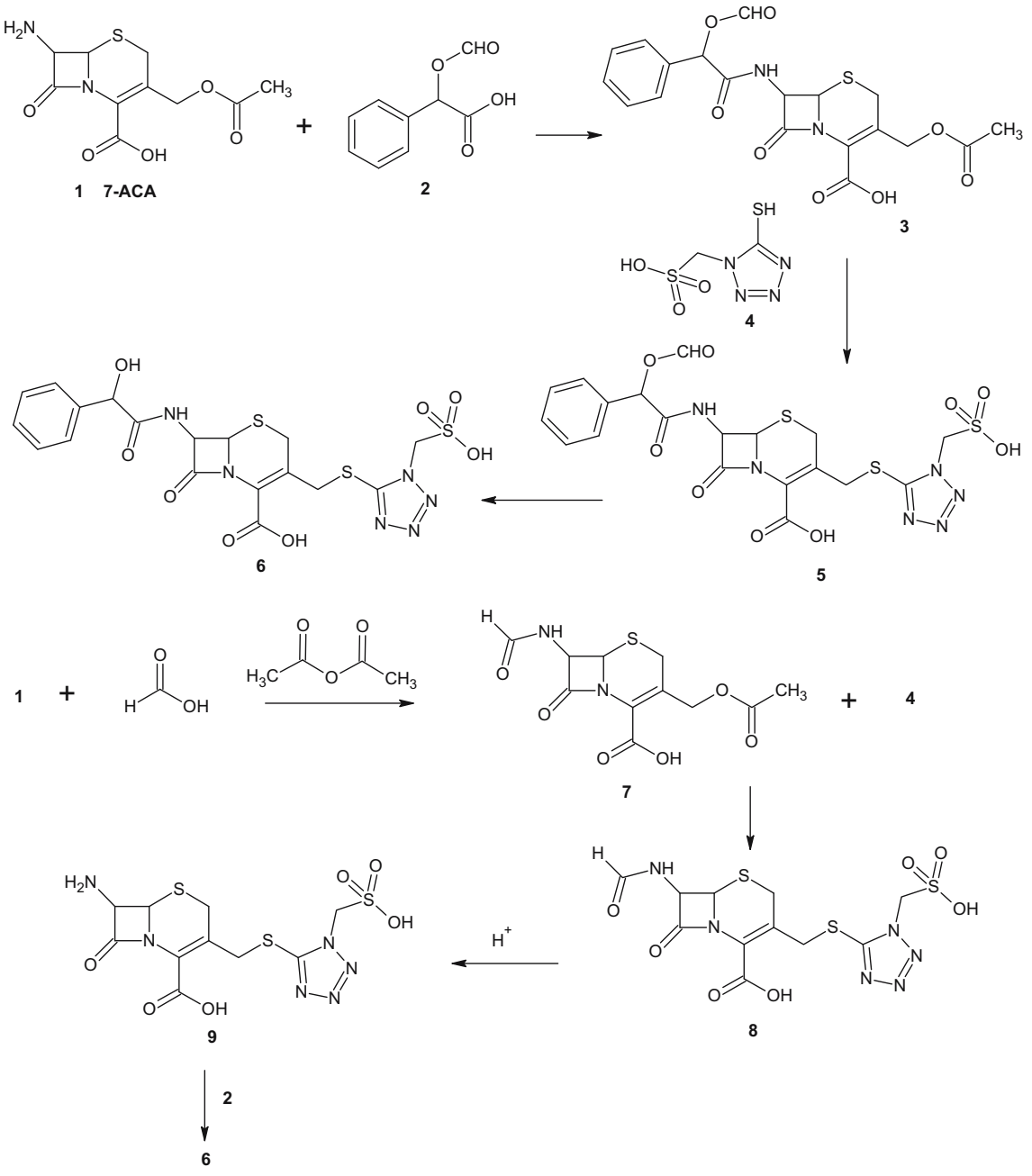
Recently, there has been a remarkable increase in demand for CFND in the pharmaceutical market. This fact has prompted us to investigate improved synthetic protocols and their scalability to production levels.

Synthetic approaches to the target CFND can be divided into two classes: those involving amidation at the 7-position, followed by the displacement of the 3′-acetoxy group of 7-ACA by the incoming S-nucleophile; and those where the sequence is reversed (first 3′-nucleophilic substitution and then 7-amidation). Accordingly, the first path entails acylation at the 7-amino (**1**) with a properly O-protected mandeloyl chloride (**2**), the subsequent displacement of the 3′-acetoxy group with sulfomethyl tetrazole thiol (**4**), and, lastly, the removal of the O-protective group to give the desired pharmaceutical molecule (**6**). In the second approach, the 7-formamidocephalosporanic acid (**7**) is prepared via the reaction of 7-aminocephalosporanic acid (7-ACA, **1**) with formic acid and acetic anhydride, followed by 3′-derivatization with a substituted tetrazole thiol (**4**) and then by deformylation of the formyl group with an acid to obtain the 3-substituted-thiomethyl-7-aminocephalosporin intermediate (**9**), which is the intermediate under investigation. The subsequent deprotection of the O-protected mandeloyl moiety in the 7-position gives the active pharmaceutical ingredient (**6**) [9–11] (Scheme 1). The second has been chosen as the most straightforward of the two strategies in this work. In this context, almost all of the synthetic efforts *en route* to CFNC have employed the pivotal intermediate 7-amino-3-[sulfomethyl-1-*H*-tetrazol-5-yl-thiomethyl]-3-cephem-4-carboxylate monosodium salt, subsequently called 7-SACA, which has led to the need for high-yielding as well as economically and environmentally sound processes. The earliest report of a 3′-OAc displacement from a cephalosporin by a S-nucleophile (i.e., thiosulfate) is probably the proposed formation of the Bunte salt in 1963 [12]. Since then, unquestionably due to the high polarizability of the sulfur atom, a plethora of S-nucleophiles (e.g., aliphatic and het (aromatic) thiols) have been observed to react smoothly, at least in an aqueous medium, to give the corresponding 3′-thio-substituted compounds in moderate and acceptable yields [13]. Hatfield et al. have studied the chemistry of the nucleophilic displacement of the acetoxy group as promoted by a variety of nitrogen and sulfur compounds in aqueous solution at near-neutral pH, and in organic solvents with acid as a catalyst. This demonstrated that conditions in water lead to a higher degradation of the cephalosporanic nucleus than nonaqueous solvents and directed academic and industrial research toward replacing the acetoxy group in the presence of strong Lewis acids [14].

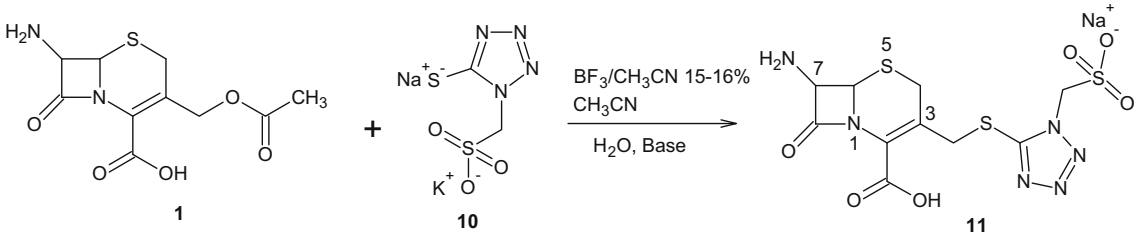
The procedures of that age therefore suffered from shortcomings that ranged from long reaction times, non-ambient temperatures, strictly controlled pH conditions, the formation of side-products arising from the hydrolysis of β-lactam, and double-bond isomerization to 2-cephems and unwanted lactone formation. The age of these procedures, the intricate and cumbersome synthetic chemical steps, and the purification procedures that require ionic exchange resins have been the limitations to the industrialization of these types of cephalosporins using this chemistry.

The seminal paper by Saikawa et al. on the successful application of protic and Lewis-acid catalysis in non-aqueous solvents [15–17] at 30°C, thereby avoiding undesirable by-products, was a significant breakthrough in the synthesis of 3′-modified cephems. In the first part, the authors studied a number of acids to investigate the substitution reaction between 1-methyl tetrazole thiol and 7-ACA, and it was shown that BF_3, with acetonitrile as a solvent, demonstrated the best performance in terms of yield and quality under mild conditions. In the second part, various thiols were utilized to study the previously developed reaction conditions. Moving this study to the present day, the industrial applicability of the other acids studied in the article ($BF_3{\cdot}(C_2H_5)_2O$, $BF_3{\cdot}(C_4H_9)_2O$, $SnCl_4$, $ZnCl_2$, F_3CSO_3H, etc.) would have a severe environmental impact, which API producers are trying to avoid, and for this reason, boron trifluoride provides the perfect blend of technical advantages and environmentally friendly features.

More recently, researchers at Farmabios [18] have shown that the BF_3-catalyzed displacement of 3′acetoxy in 7-ACA by TSA, as a disodium salt (**10**), worked well on a lab scale. However, it had not been optimized for the production of commercial quantities of 7-SACA. Compared to the old synthetic routes, the use of a boron trifluoride complex in acetonitrile allows the 7-aminocephalosporanic acid to be directly converted into intermediate **11** without protection on the 7-position on the amine, providing a more efficient synthesis and preserving the stability of the β-lactam ring, as indicated in Scheme 2.



Scheme 1: Two synthetic pathways for compound **6**.



Scheme 2: Boron trifluoride complex pathway to obtain **11**.

2 Materials and methods

7-ACA, the TSA (Na, K) salt (**10**) and solvents were kindly provided by ACS Dobfar. All other reagents were purchased from Merck KGaA (Darmstadt, Germany).

The analytical instrumentation used to identify and analyze the product was the Agilent 1200 Series HPLC system, NMR (Bruker 400) and Karl-Fischer titration. Boric acid and its salts were analyzed with an ion chromatography system.

2.1 Experimental procedures

1-Sulfomethyl-5-mercapto-1,2,3,4-tetrazole TSA (Na, K) salt (**10**). ^{1}H NMR (400 MHz, DMSO-d_6): 5.48 (s); ^{13}C NMR (100 MHz, DMSO-d_6): 60.3 (CH_2), 167.0(C) 7-amino-3-[sulfomethyl-1*H*-tetrazol-5-yl-thiomethyl]-3-cephem-4-carboxylate monosodium salt (**11**).

Method: About 122.4 g of a 16% boron trifluoride complex in acetonitrile (9.83 equiv.) was added to a mixture of 7.7 g of 1-sulfomethyl-5-mercapto-1,2,3,4-tetrazole monosodium and monopotassium salt (1.023 equiv.), 8.0 g of 7-ACA (1.0 equiv.) and 60 mL of acetonitrile (7.5 V) at 25–28°C. After 30–40 min under stirring, the solution was poured into 72 mL of water (9.0 V) at room temperature, yielding a precipitate that was filtered and washed with a mix of acetonitrile and water (the weight of the dried product was 3.3–3.4 g). The product was precipitated with 15% sodium hydroxide by adjusting the pH to 3.0 ± 0.1 at room temperature, and, after 2 h, the precipitate was filtered and washed with 32 mL of acetone to give 8.6 g (yield of 68%) of 7-amino-3-[sulfomethyl-1*H*-tetrazol-5-yl-thiomethyl]-3-cephem-4-carboxylate monosodium salt (**11**). HPLC assay 101.0% (as sodium on the anhydrous basis).

^{1}H NMR (400 MHz, DMSO-d_6): 3.50(1H, d, ^{2}J 17.6 Hz), 3.74(1H, d, ^{2}J 17.6 Hz), 4.08(1H, d, ^{2}J 13.6 Hz), 4.42(1H, d, ^{2}J 13.6 Hz), 5.20(1H, d, ^{3}J 5.2 Hz), 5.40(1H, d, ^{3}J 5.2 Hz), 5.87(2H, s). ^{13}C NMR (100 MHz, DMSO-d_6): 26.25(CH_2), 36.0(CH_2), 53.3(CH), 57.6(CH), 60.4(CH_2), 119.8 (C), 130.0(C), 154.9(C), 159.6(C), 161.8(C).

In the same way, operating with 72 mL of acetonitrile (9.0 V), the product is obtained with the same quality and 72% yield (9.1 g).

3 Results

First, we defined the optimal equivalent ratio to be 16% of the boron trifluoride complex in acetonitrile to act as a Lewis acid for the synthesis and the correct amount of acetonitrile as solvent. In the displacement reaction of the cephalosporin substrate (7-ACA), in the absence of water, BF_3 activates the OAc group at the 3′-position by producing a more electrophilic species. Accordingly, kinetic evidence has been provided suggesting that this process occurs via an allylic-stabilized betaine in a S_N1 reaction [14,19]. Moreover, it has been well established that the presence of even a small amount of water leads to the opening of the β-lactam, while the desacetyl and lactone compound drop at zero [13,19,20].

The inert environment and the Karl Fischer method to detect the amount of water in the solvent and avoid the destruction of the 4-membered ring will be fundamental to any proposed industrial application of these concepts.

4 Discussion

Given that our substrates, 7-ACA (**1**) and 1-sulfomethyl-5-mercapto-1,2,3,4-tetrazole (Na, K) salt (**10**), are poorly soluble in highly polar solvents, such as acetonitrile, the contribution of both factors – protonation by acid and solvolysis – is necessary to completely dissolve them and to promote the S_N1 pathway. As summarized in Table 1, in which we present different molar ratios of the Lewis acid and substrate (**1**), using the boron trifluoride complex in acetonitrile as is, we have shown that the data for the yield and assay increase dramatically as we gradually increase the ratio and, in parallel, the volume of acetonitrile contained in the complex. This is because the substrates dissolve, facilitating the attack of the S-nucleophile.

We always used a tiny excess of (Na, K) salt **10**, with respect to 7-ACA (1.02 vs 1.00 equiv.), in a 15% sodium hydroxide solution as an alkali base to crystallize product **11** as a monosodium salt, and all steps were performed between 23°C and 28°C. To decrease the raw-material costs of the total process, we used the (Na, K) salt of

Table 1: Effects of equivalents of the 16% BF_3/CH_3CN complex and volume of acetonitrile on the yield and assay of compound **11**

Entry	Mole BF_3/ mole of 7-ACA	Yield (%)	Assay (in % as sodium on the anhydrous basis)
1	4.9	23.2	5.1
2	9.8	23.2	2.7
3	14.7	72.6	65.2
4	19.6	76.3	69.2

Table 2: Influence of the amount of acetonitrile on the reaction time and equiv. of BF_3

Entry	Mole BF_3/mole of 7-ACA	Volume of solvent/ weight of 7-ACA ($mL \cdot g^{-1}$)	Time (min)	Yield (%)	Assay (in % as sodium on the anhydrous basis)
5	4.9	5.4	240	61.7*	69.6
6	9.8	5.4	120	71.9*	67.7
7	9.8	6.5	60	81.4*	69.9
8	9.8	7.5	60	79.9*	69.4
9	9.8	9.0	60	50.6	102.4

*Without the filtration of boric acid and its salt after reaction quenching.

sulfomethyl tetrazole thiol **10**, as confirmed by ion chromatography (IC) analysis (found: Na 8.4% and K 14.4% vs calcd.: Na 8.9% and K 15.2%), rather than the more common disodium salt, which allowed our supplier to reduce, by one step, the complicated synthesis of this side chain in the 3′-position.

Aiming to optimize the synthetic yield of **11**, the influence of BF_3·MeCN equivalents and the dilution in MeCN were investigated (Table 1). The experiments were carried out at room temperature and quenched after 120 min without filtration of boric acid (and/or borates).

In entry 1 and, to a lesser extent, in entry 2, the solution remained turbid during the reaction because of the small volume of solvent. However, working with a huge excess of complexed Lewis acid, with a greater volume of acetonitrile, as in entries 3 and 4, provided a clear solution, which indicated the activation of nucleophilic substitution. Despite the turbid appearance of the reaction mixture, owing to the residual starting materials, the boric acid salt precipitated from the clear solution after quenching with water (for all entries the amount of water was calculated by the equation 15.2 g of 7-ACA). This relevant point undoubtedly demonstrates that it is impossible to avoid the filtration of boric acid or its salts, as supported by the assay data in entry 9 (Table 2) in which we filtered the by-product after the quenching step, respect at all entries. An analysis of the filtered and dried solid using ion chromatography also allows us to confirm the precipitation of the potassium salt (30.8%), a tiny percentage of sodium borate (0.2%) and free boric acid (see Figure A1 in Appendix).

In order to cut costs and minimize the waste of boric acid salts after quenching, we investigated reducing the loading of the boron trifluoride complex. The acid-catalyzed displacement was completed with an equimolar ratio of BF_3 and 7-ACA (entries 3 and 4). We also focused our study on the use of acetonitrile as a polar solvent to reduce the Lewis acid amount. Bearing in mind the worst cases in Table 1 in terms of yield and assay – entries 1 and 2 – we could only improve both by adding a minimum amount of acetonitrile to dissolve the substrates in association with the acid, as demonstrated in entries 5 and 6 of Table 2.

The remarkable difference in reaction times between entries 5 and 7–9, and the obtained 10% increase in yield, further supports that Lewis acid plays a crucial role in cooperation with solvent.

Considering the difference to reach the peak of concentration of **11** in solution (Figure A2) in association with our goal to obtain a very fast process with a high level of productivity, we permanently chose and developed the procedure with 9.8 equiv. of boron trifluoride complex.

To explain why the dual contribution of the Lewis acid and polar solvent is so crucial in this type of reaction, we have studied the kinetics of all of the entries reported in Table 2. We noticed that the concentration of **11** remains constant over the reaction time when

Table 3: Effects of the water content on the quenching step and loss of product in mother liquor

Entry	10	11	12	13	14
Volume of water/weight of 7-ACA ($mL \cdot g^{-1}$)	15.2	12	19	9	6
Loss in mother liquor (g per activity)	3.4	2.7	5.0	1.8	Failure
Yield (%)	59.1	60.1	45.9	68.0	
Assay (in % as sodium on the anhydrous basis)	102.4	100.6	100.9	101.0	

Table 4: Crystallization temperature and product features

Entries	Temperature of crystallization (°C)	Yield (%)	Within production specifications
15	25–28	72.0	Yes
16	10–13	74.3	No
17	2–5	75.1	No

Table 5: Current protocol and US 5 625 058 patent comparison (features, *pros* and *cons*)

Parameters	US 5 625 058 features		Current protocol features	
Temperature	0°C	Low productivity, long reaction time for the nucleophilic substitution and high energy costs	Room temperature	High productivity, short reaction time, energy and cost savings
Reaction time	480 min		30–40 min	
Volume of acetonitrile as the reaction solvent	3.3 g of 7-ACA	The low acetonitrile volume makes the purification over charcoal before crystallization necessary	7.5 or 9.0 g of 7-ACA	The higher acetonitrile volume allows a reduction in the BF_3 complex equiv., making charcoal treatment unnecessary
BF_3 complex equiv.	Not reported	—	9.8	This amount gave 83% molar yield in solution in a very short time
BF_3 complex in acetonitrile Percentage (Lewis acid)	Not reported	—	9.1–11.6%	Lower percentage of BF_3 gas in solution leading to a safer process
1-Sulfomethyl-5-mercapto-1,2,3,4-tetrazole salt	Disodium salt	High raw material costs with low productivity	mono (K, Na) salts	The side chain as mono (K, Na) salts allows a reduction in the raw material costs of the entire process
Amounts of reagents used in trials lab	10.0 g of 7-ACA as a limiting reagent	Such small amounts of starting materials needs a second lab trial for scaling up	50.0 g of 7-ACA as a limiting reagent	Reliable procedure well suited for industrialization
Water volume for quenching	1 g of 7-ACA	The small volume of water makes the treatment with charcoal necessary to clarify the solution before crystallization	9 g of 7-ACA	Higher water volumes make the charcoal treatment and subsequent time-consuming filtration unnecessary
Charcoal treatment step	Yes		No	
Quenching and crystallization temperature	0°C	High energy costs	Room temperature	Energy saving
Alkali base	Ammonium hydroxide solution	Release of ammonia gas (toxic and irritating)	Sodium hydroxide solution	Safer work up
Molar yield	83% as monoammonium salt	Higher yield but much lower productivity	72% as monosodium salt	Lower yield but much higher productivity

working as in entry 5, while when we employed the double equivalents of the boron trifluoride complex in acetonitrile, but with the same volume of solvent as in entry 6, the molar yield in solution increased by 43% in the first 30 min and started to decrease slightly after 1 h (Figure A2). Another important aspect is that the peak of concentration of **11** in the first 30 min does not change when we changed the volume of the solvent with the same equivalents of Lewis acid (as in entries 6–9), allowing us to reduce the reaction time to 30–40 min.

By referring to earlier studies, we were able to set an optimal combination of factors and define the best amount of water vs 7-ACA to quench the reaction mixture and remove the need for a charcoal purification step. The pre-crystallization solution provided a high-quality 7-SACA sodium salt **11** and a little loss of product in the mother liquor. This is a relevant goal for our study in the perspective of industrializing the process of **11**, as it enables us to highlight the convenience and technical feasibility of our process compared to old procedures. This telescopic downstream simplifies industrial operations and cuts the waste of the process.

In detail, we have found that a large volume of water (entry 10) corresponds to a superior loss of product in the mother liquor, while, working with a lower water volume at a selected volume of acetonitrile (entry 14), we obtained a gummy formation with water quenching, as indicated in Table 3.

At this point, after having successfully defined the moles of Lewis acid and the volume of acetonitrile, we were able to efficiently optimize the amount of water (entry 13), leading to high yield and quality.

We also investigated the possibility of changing the alkali base to crystallize the product, and the influence of crystallization time to evaluate, once again, the loss in the mother liquor. Crystallizing the product with 14% ammonium hydroxide, we found that the product precipitates mainly as a monoammonium salt (found 3.4% vs calcd. 4.2% by IC analysis) (Figure A3), proving that the cation of the alkali base precipitates the corresponding salt. When using the 15% NaOH solution, we always obtained the monosodium salt as confirmed by IC analysis (found 4.9% vs calcd. 5.3%).

Performing the crystallization in 2 h or overnight does not change the amount of product loss in the mother liquor. The use of a sodium hydroxide solution, rather than an ammonia solution, makes the process safer, adding another advantage to our process. Moreover, at a lower concentration of sodium hydroxide (5%) the product yield decreases due to a higher amount of water that worsens the crystallization efficiency. Although our goal is to industrialize the room temperature method, we notice a slight yield increase at lower temperatures but the product was out of specifications (Table 4).

After having studied and enhanced all of the operational parameters in the development of this new cost-effective and environmentally friendly process, we wish to summarize the differences between our method and the well-known standard approach [15], highlighting *pros* and *cons* (Table 5).

5 Conclusion

In conclusion, we have successfully developed a novel synthetic protocol for the synthesis of 7-amino-3-[sulfomethyl-1*H*-tetrazol-5-yl-thiomethyl]-3-cephem-4-carboxylate monosodium salt (**11**), a key intermediate in the synthesis of cefonicid, in a telescopic route and in about 70% overall yield from readily accessible 7-ACA. This process appears to be more compatible with the industrial scale and has some evident advantages over the existing synthetic procedures. By virtue of fine balance between equiv. of 16% BF_3·MeCN and volume of MeCN (83% molar solution) the reaction occurred in only 30–40 min.

The work-up avoids charcoal purification and loss in the mother liquor. A well-defined amount of water was sufficient to quench the reaction mixture. These improvements dramatically reduced the reaction time and increased productivity, making it more attractive for industrial production. In the worldwide market for the production of intermediates for APIs, where companies try to improve their processes with the aims of reducing waste and making them more reliable in terms of strict environmental concerns, we are sure that this is a competitive process.

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Author contributions: Marziale Comito: investigation, writing – original draft, Riccardo Monguzzi: formal analysis, project administration; Silvia Tagliapietra: conceptualization, writing – original draft, writing – review and editing; Giovanni Palmisano: writing – original draft, supervision; Giancarlo Cravotto: supervision, writing – review and editing.

Conflict of interest: The corresponding author of this article (Giancarlo Cravotto) is a member of the Editorial Board of Green Processing and Synthesis.

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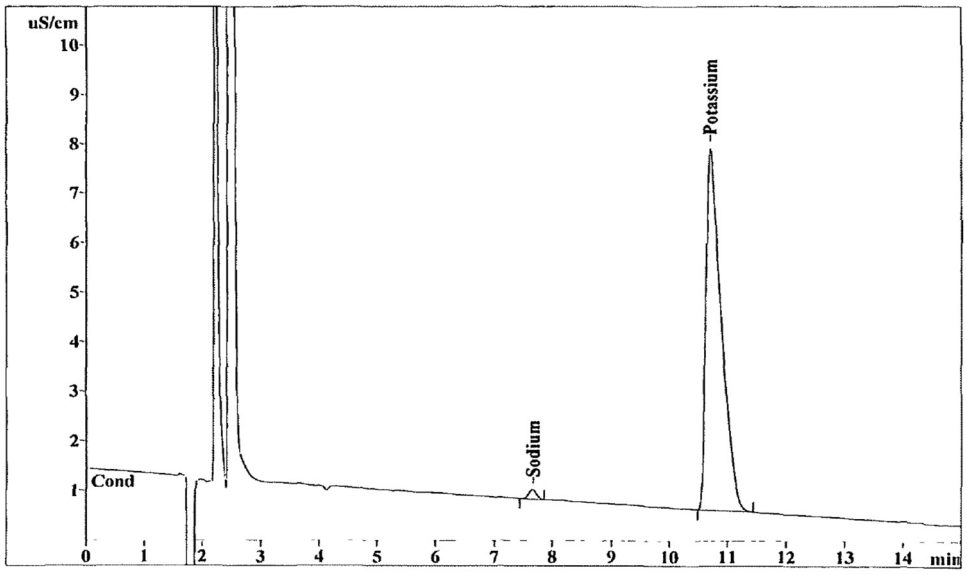
Appendix



Quantitation method: Custom

No	Retention min	Height uS/cm	Area uS/cm*sec	RF	Conc. mg/L	Name
1	7.66	0.19	1.808	3.966	1830.912	Sodium
2	10.71	7.32	132.903	9.071	307866.688	Potassium
2	15.00	7.51	134.711	450.139	309697.600	

Figure A1: IC analysis of the by-product boric acid.

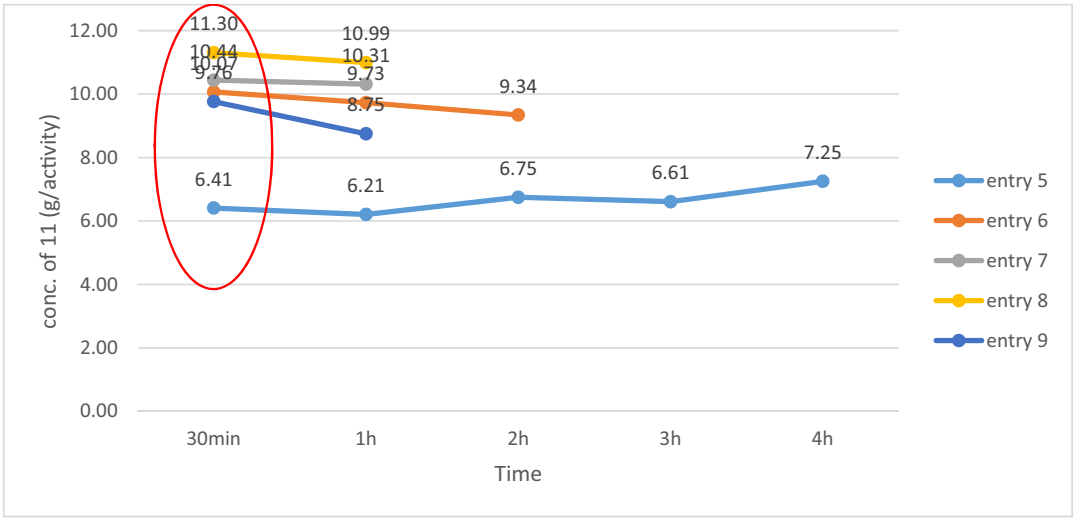
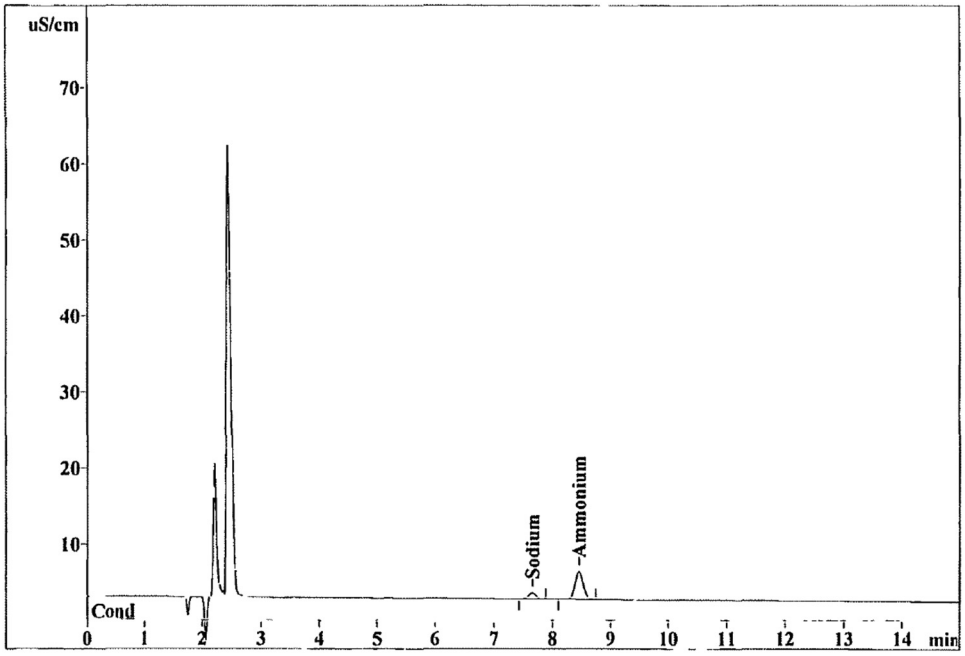


Figure A2: Kinetic assessment of **11** in solution.



Quantitation method: Custom

No	Retention min	Height uS/cm	Area uS/cm*sec	RF	Conc. mg/L	Name
1	7.65	0.78	6.974	4.159	7003.090	Sodium
2	8.45	3.64	36.127	3.870	33754.391	Ammonium
2	15.00	4.42	43.101	195.838	40757.480	

Figure A3: IC analysis of 7-amino-3-[sulfomethyl-1-*H*-tetrazol-5-yl-thiomethyl]-3-cephem-4-carboxylate ammonium salt.