Synthesis of Cephalosporin-Type Antibiotics by Coupling of Their β-Lactam Nucleus and Racemic Amino Acid Side Chains Using a Clathration-Induced Asymmetric Transformation

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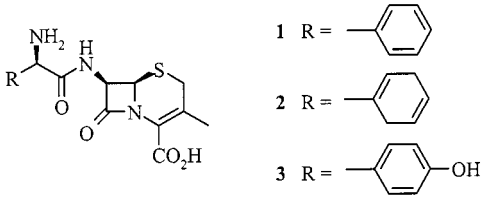
Keywords: Asymmetric synthesis / Clathrates / Antibiotics / Amino acids

The cephalosporin-type antibiotics Cephalexin, Cephradine and Cefadroxil have been prepared by coupling of their β-lactam nucleus and racemic amino acid side chain precursors. The initially obtained mixture of cephalosporin epimers is subjected to a clathration-induced asymmetric transformation which results in the epimerization of the *epi*-cephalosporin into the cephalosporin with the correct diastereomeric configuration.

Introduction

Cephalexin **1**, Cephradine **2** and Cefadroxil **3** are widely used antibiotics belonging to the class of the cephalosporins. They are semi-synthetic antibiotics consisting of a β-lactam nucleus and a D-amino acid side chain. The β-lactam nucleus of these cephalosporins can be obtained by chemical modification of the penicillin nucleus[1] and recently also by direct fermentation.[2] The amino acid side chains of cephalosporins **1**−**3** can be prepared from basic organic chemicals.[3−6] The final step in the preparation of cephalosporins **1**−**3** is the coupling of the β-lactam nucleus to the respective side chain. Although the coupling of nucleus and side chain can be accomplished enzymatically,[7] most manufacturers still use the conventional chemical process for this purpose. Since the yields of the chemical coupling reactions are fully optimized, the use of cheaper starting materials becomes an interesting option for further cost reduction. In this context, the use of racemic amino acids as starting materials for the side chains may offer an attractive cheaper alternative for the currently used enantiopure compounds. However, coupling of the β-lactam nucleus with an appropriate racemic amino acid derivative, leads to an epimeric mixture of cephalosporins of which only the derivative from the D-amino acid exhibits the desired antibiotic activity. Therefore, epimerization of the stereogenic center in the amino acid side chain of the *epi*-cephalosporins is a must to make the coupling starting from racemic amino acids an economically feasible one. In a preliminary report we showed that Cefadroxil can be epimerized under very mild conditions by a Schiff-base-mediated process.[8] It was also shown that by addition of 2,7-dihydroxynaphthalene to the mixture of equilibrating epimeric Cefadroxils, a selective clathration takes place with the antibiotic having the correct stereochemistry. In fact, an asymmetric transformation was achieved by means of this selective clathrate formation. The question now arises as to whether this clathration-induced asymmetric transformation can be incorporated in the synthetic sequence leading to cephalosporins, starting from racemic amino acids. The results for the three target cephalosporins, *viz.* Cephalexin **1**, Cephradine **2** and Cefadroxil **3**, will be reported in this paper.



Results and Discussion

The coupling of the β-lactam nucleus and the appropriate side chain is conducted by the so-called Dane salt route, as shown in Scheme 1.[9] For the preparation of the Dane salt, the racemic amino acid is heated with ethyl acetoacetate in the presence of potassium hydroxide. The thus-obtained Dane salt is then activated by conversion into the mixed anhydride of pivalic acid by reaction with pivaloyl chloride. This anhydride is subsequently used in the actual coupling reaction with the β-lactam nucleus, 7-ADCA. In order to make 7-ADCA more soluble in dichloromethane its tetramethylguanidine salt was prepared prior to its addition to the mixed anhydride. After hydrolytic removal of the Dane protecting group, an aqueous solution containing an epimeric mixture of the cephalosporin was obtained in yields ranging from 80 to 88%. The lowest conversion was found for Cefadroxil, while Cephradine gave the highest conversion. It should be noted that the Dane salt route is also used in the industrial production of cephalosporins.

The amino acid side chain of Cephradine, *viz.* cyclohexadienylglycine, is not commercially available in racemic

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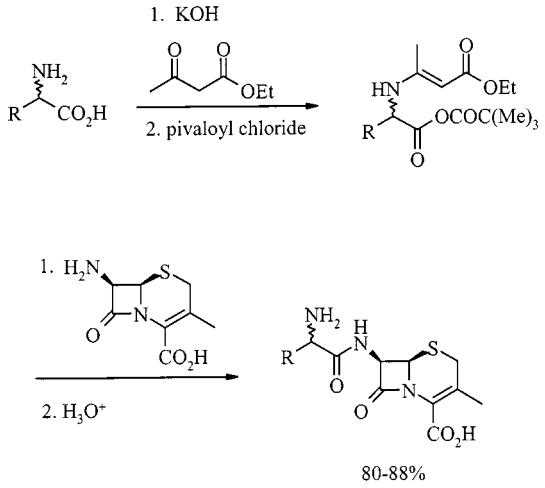
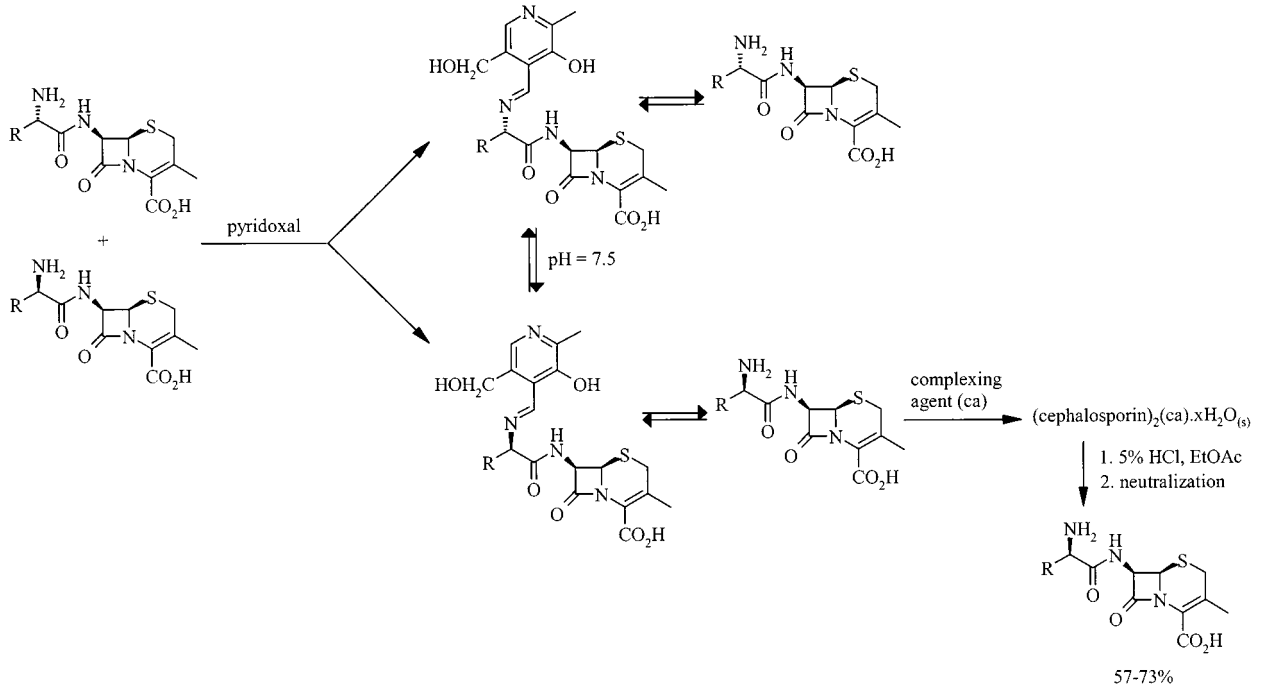


R = phenyl (Cephalexin **1**), cyclohexadienyl (Cephradine **2**), *p*-hydroxyphenyl (Cefadroxil **3**)

Scheme 1

form. The racemate needed for this study was prepared by racemization of the enantiopure material by warming an aqueous solution of this compound with 1.1 equivalents of sodium hydroxide in the presence of a catalytic amount of salicylic aldehyde. Clearly, this racemization proceeds via the intermediacy of a Schiff base, which readily racemizes under basic conditions. The thus-formed racemic cyclohexadienylglycine was converted into its Dane salt by treatment with sodium hydroxide instead of potassium hydroxide because the potassium salt refused to crystallize. The potassium Dane salts of racemic phenylglycine and *p*-hydroxyphenylglycine were prepared by reported procedures.[9]

The epimeric mixture of cephalosporins obtained above contained the *epi*-cephalosporin as the major component, implying that the L-amino acid derivative had reacted considerably faster in the coupling reaction than the corresponding D-form. This remarkable observation has the interesting consequence that, in principle, a higher conversion can be achieved in the coupling of the racemic side chains compared with that of the commonly used enantiopure side chains. The required epimerization of the *epi*-cephalosporin (Scheme 2) could be readily accomplished under the conditions reported earlier for Cefadroxil,[8] *viz.* by treatment of the aqueous solution of epimers with 10 mol % of pyridoxal at a pH of 7.5. In this epimerization, a Schiff base of cephalosporin and pyridoxal is formed, which undergoes isomerization by a sequence of deprotonation/reprotonation reactions. Schiff-base-mediated racemization of amino acids is a well documented process.[10] It was found that the epimerization of Cephalexin and Cephradine proceeds smoothly under these conditions similar to Cefadroxil. The next step is to drive the equilibrium of epimeric cephalosporins to the side of the desired epimer with the antibiotic activity. Selective clathration with a complexing agent could be accomplished for Cephalexin and Cephradine by adding α-naphthol to the mixture of equilibrating epimers. For Cefadroxil, 2,7-dihydroxynaphthalene gave the best performance in this selective clathration, which was still less effect-



R = phenyl (Cephalexin **1**), cyclohexadienyl (Cephradine **2**), *p*-hydroxyphenyl (Cefadroxil **3**)

Scheme 2

ive than with the other two cephalosporins. After one night, the complexed cephalosporin had crystallized and could simply be collected by filtration. Decomplexation was achieved by hydrolysis of the complex with aqueous dilute acid and subsequent removal of the complexing agent by extraction with ethyl acetate. Neutralization of the highly concentrated aqueous solution of cephalosporin resulted in precipitation of the ultimate product. The overall yields after decomplexation based on 7-ADCA amounted to 68%, 73% and 57%, for Cephalexin, Cephradine and Cefadroxil, respectively. The products were analyzed by HPLC to determine the purity and by X-ray powder diffraction to ascertain that the desired cephalosporin monohydrate had been obtained.

Conclusion

The objective of preparing the cephalosporin antibiotics Cephalexin, Cephradine and Cefadroxil from racemic side chain precursors has successfully been accomplished. This novel methodology makes use of a Schiff-base-mediated epimerization in combination with selective clathration of the cephalosporins. This new approach to the synthesis of cephalosporins employing racemic amino acid as the starting material for the side chains may have attractive prospects for cost reduction in the manufacture of these life saving antibiotics.[11]

Experimental Section

General: Racemic phenylglycine and *p*-hydroxyphenylglycine, D-cyclohexadienylglycine and 7-aminocephalosporanic acid (7-ADCA) were obtained from DSM Life Sciences Group (The Netherlands). Pyridoxal was obtained from Fluka. α-Naphthol and 2,7-dihydroxynaphthalene were purchased from Acros. For the HPLC analysis a Pharmacia LKB.LCC 2252 HPLC was used with a reversed phase column (Merck 50983 LiChrospher 100RP18, 5 μm, 250×4 mm). For detection a Farmacia LKB.UV-MII UV detector (λ = 254 nm) was used. An appropriate eluent for the analysis was a mixture of acetonitrile (HPLC grade) and a 50 mM phosphoric acid buffer with a pH of 2.7. The pH stat apparatus used was a Schot Geräte Titrator TR154.

D,L-Cyclohexadienylglycine: D-Cyclohexadienylglycine (20 g, 0.130 mol) and sodium hydroxide (5.7 g, 0.140 mol) were dissolved in water (80 mL). Salicylic aldehyde (1.6 g, 0.013 mol) was added to this stirred solution and the resulting mixture was heated to 80 °C for 2 h. The reaction mixture was then allowed to cool and diluted with water (300 mL). Next, the reaction mixture was acidified with 95% sulfuric acid to a pH of 2. After 10 min. the reaction mixture was neutralized with ammonia (5%) whereupon the product precipitated. The product was thoroughly washed with water and acetone. After drying under a flow of nitrogen, racemic cyclohexadienylglycine was obtained in nearly quantitative yield (≈20 g). Optical rotation: $[\alpha]_D = 0°$. The optically pure D-cyclohexadienylglycine has $[\alpha]_D^{25} = -89$.[4]

***N*-[2-ethoxycarbonyl-1-methylvinyl]-α-aminophenylacetic Acid Potassium Salt (Dane Salt of Racemic Phenylglycine):** Racemic phenylglycine (16.1 g, 0.107 mol) and potassium hydroxide (6.2 g, 0.110 mol) were suspended in methanol (120 mL). The reaction was heated under reflux and stirred until the solution was clear. While stirring under reflux, ethyl acetoacetate (15 g, 0.115 mol) in methanol (25 mL) was added. The reaction mixture was stirred and heated under reflux for 30 min. The reaction mixture was concentrated to approximately 60 mL and allowed to cool. The product crystallized as a white solid which was filtered and washed with methanol. After drying 27.3 g (85%) of racemic phenylglycine Dane salt was obtained.

***N*-[2-ethoxycarbonyl-1-methylvinyl]-α-aminocyclohexadienylacetic Acid Sodium Salt (Dane Salt of Racemic Cyclohexadienylglycine):** Racemic cyclohexadienylglycine (19.45 g, 0.127 mol) was suspended in isopropyl alcohol (120 mL). To this solution a suspension of sodium hydroxide (5.1 g, 0.128 mol) was gradually added. While stirring, the reaction mixture was heated under reflux. After both components had dissolved, ethyl acetoacetate (17.0 g, 0.131 mol) in isopropyl alcohol (25 mL) was added and the reaction mixture was heated under reflux for 3 h. After cooling the product appeared as a white crystalline solid. The crystals were collected, washed with isopropyl alcohol and subsequently dried under a flow of nitrogen. The desired product was obtained in a yield of 34.6 g (95%).

***N*-[2-ethoxycarbonyl-1-methylvinyl]-α-amino-(4-hydroxyphenyl)-acetic Acid Potassium Salt (Dane Salt of Racemic *p*-Hydroxyphenylglycine):** Racemic *p*-hydroxyphenylglycine (15 g, 0.0898 mol) and potassium hydroxide (5 g, 0.090 mol) were dissolved in methanol (120 mL). The solution was stirred and heated under reflux. A solution of ethyl acetoacetate (12.9 g, 0.099 mol) in methanol (25 mL) was gradually added. The reaction mixture was heated under reflux for 10 min. The product crystallized upon cooling. The crystals were collected and washed with methanol. After drying the product was obtained in a yield of 21.9 g (77%).

Synthesis of Cephalexin from 7-ADCA and Racemic Phenylglycine Dane Salt: The potassium Dane salt of racemic phenylglycine (3 g, 10.0 mmol) was suspended in a mixture of dichloromethane (15 mL) and dimethylformamide (2 mL). To this suspension γ-picoline (50 mg) was added. The suspension was cooled to −25 °C and pivaloyl chloride (1.27 g, 11.0 mmol) was added as quickly as possible. The reaction mixture was stirred for 30 min. at a temperature between −20 and −25 °C. After this time, the reaction mixture was diluted with dichloromethane (15 mL) and cooled to −50 °C. A solution of 7-ADCA (1.9 g, 9.0 mmol) and tetramethylguanidine (1.1 g, 9.4 mmol) in dichloromethane (10 mL) at −10 °C was added to the reaction mixture in approximately 30 min. The reaction mixture was stirred for 5 h while the temperature was allowed to rise slowly to −30 °C. When conversion of 7-ADCA to Cephalexin had been completed (according to HPLC), the reaction mixture was allowed to warm to 0 °C after which water was added (10 mL). While stirring, the reaction mixture was acidified to a pH between 0 and 1 with 37% HCl. The reaction mixture was stirred for 10 min. while the temperature was maintained at 0 °C. The dichloromethane layer was separated and washed with 5% HCl (5 mL). Pyridoxal hydrochloride (160 mg, 0.80 mmol) was added to the combined aqueous layers. The pH was adjusted to 7.5 with 5% ammonia and the aqueous reaction mixture was allowed to warm to ambient temperature. A solution of α-naphthol (1.3 g, 9.0 mmol) in ether (10 mL) was then added and the reaction mixture was stirred overnight. The precipitated Cephalexin/α-naphthol complex was filtered off and successively washed with ether (5 mL) and water (5 mL). After drying the Cephalexin/α-naphthol complex was obtained in a yield of 3.1 g (73%).

The complex (2 g) was suspended in ethyl acetate (10 mL). While stirring the suspension 5% HCl (≈2 mL) was added until both layers had turned completely clear. The aqueous layer was separated and washed with ethyl acetate (5 mL). The thus-obtained aqueous Cephalexin solution was cooled below 5 °C. The pH was raised to 3 with 25% ammonia and then to a pH of 4 with 5% ammonia, resulting in precipitation of the Cephalexin. The mixture was maintained at a temperature below 5 °C for another 30 min., after which the product was collected by filtration. After drying under a flow of nitrogen 1.45 g of Cephalexin monohydrate (94% for the decomplexation, 68% overall for 7-ADCA) was obtained as a white solid. The product had a purity of 97% according to HPLC. X-ray powder diffraction confirmed that the desired Cephalexin monohydrate had indeed been obtained.

Synthesis of Cephradine from 7-ADCA and Racemic Cyclohexadienylglycine Dane Salt: This procedure was essentially the same as that described for Cephalexin except that the sodium Dane salt of racemic cyclohexadienylglycine (2.87 g, 10.0 mmol) was used as a suspension in dichloromethane (15 mL). The amounts of the remaining ingredients were the same. α-Naphthol was again used as the clathrating agent (1.3 g, 9.0 mmol) and was added as a solution in diethyl ether (10 mL). The Cephradine/α-naphthol complex was obtained in a yield of 77% (3.3 g). After decomplexation, which was carried out in exactly the manner as described for Cephalexin, the desired Cephradine monohydrate was obtained as a white solid (1.5 g; 95% for the decomplexation, 73% overall for 7-ADCA).

Synthesis of Cefadroxil from 7-ADCA and Racemic *p*-Hydroxyphenylglycine Dane Salt: This procedure was essentially the same as that described for Cephalexin except that the potassium Dane salt of racemic *p*-hydroxyphenylglycine (3.17 g, 10.0 mmol) was used as a suspension in dichloromethane (15 mL) and dimethylformamide (2 mL). The amounts of the remaining ingredients were the same. 2,7-Dihydroxynaphthalene was used as the clathrating agent (1.4 g, 9.0 mmol) and was added as a solution in diethyl ether (10 mL). The Cefadroxil/2,7-dihydroxynaphthalene complex was obtained in a yield of 60% (2.70 g). After decomplexation, which was carried out in exactly the manner as described for Cephalexin, the desired Cefadroxil monohydrate was obtained as a white solid (1.45 g; 95% for the decomplexation, 57% overall for 7-ADCA).

Acknowledgments

This project was financially supported by DSM Life Sciences Group (Geleen, The Netherlands) and by the Dutch ministry of economical affairs (Senter). Dr. Ben de Lange (DSM Research, Geleen, The Netherlands) is kindly acknowledged for valuable discussions.

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Received November 27, 2000
[O00596]