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Novel Synthesis of C-3 Vinylic Cephem Systems

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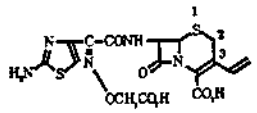
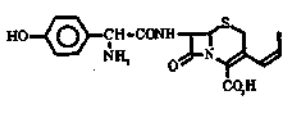
The 3-formyl-2-cephem **4**, available from 7-aminocephalosporanic acid has been converted to C-3 vinylic cephems. The reactions involved are the Grignard addition to **4**, the conversion of the resulting alcohols to mesylates, and the elimination of the mesyl group by LiCl. When ethylmagnesium iodide is used, only 3-[(E)-1-propenyl] cephem is obtained, which is not easily available by conventional Wittig reaction.

Introduction

Cefixime is the first cephalosporin antibiotic having a vinyl group at C-3 position, which was developed by Fujisawa company as an orally administrable drug in 1983.[2] Since then, the syntheses of cefixime analogs have been reported in the literature. For example, Bristol-Myers company reported the preparation of (Z)-1-propenyl derivative named BMY-28100.[3]

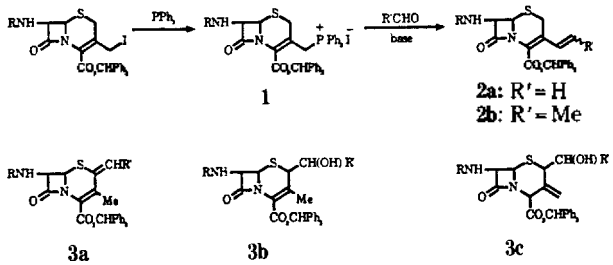


Cefixime



BMY-2810

Literature preparations of cefixime and BMY-28100 involve a Wittig-type reaction, namely a coupling of cephem-derived triphenylphosphonium salt **1** with formaldehyde and acetaldehyde in the presence of base, respectively, as shown in Scheme 1.[2,3]



Scheme 1

In the Wittig reaction with acetaldehyde, the major (Z)-product **2b** is contaminated with ca. 15-20% of the minor (E)-isomer. The separation of this two isomers requires the tedious preparative HPLC method.

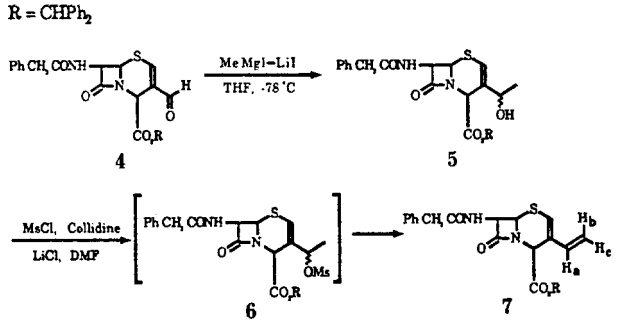
Also, it is generally known that the cephem ylide is less reactive to higher aliphatic aldehydes than to formaldehyde, giving various C-2 substituted compounds **3a** to **3c** as well as the expected Wittig product.[4]

We thought that 3-formyl-2-cephem **4** (Scheme 2), easily available starting from 7-aminocephalosporanic acid could serve as a useful starting material for the preparation of 3-vinylic cephem system.[5]

We wish to report a transformation of **4** to 3-vinylic cephem in 2-cephem system.

Result and Discussion

When 3-formyl-2-cephem **4** was treated with a large ex-

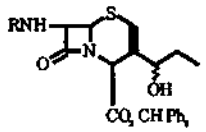


Scheme 2

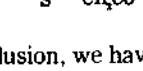
cess of methylmagnesium iodide in THF in the presence of lithium chloride at -78 °C, secondary methyl carbinol **5** was obtained in 65% purified yield as a nearly 1:1 mixture of two diastereoisomers (Scheme 2).[6]

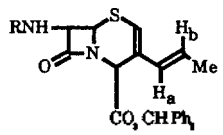
Dehydration[7] was performed by in-situ formation of the mesylate **6** (methanesulfonyl chloride, collidine in N,N-dimethylforamide), followed by elimination of the mesyl group using lithium chloride[8] to give the 3-vinylic cephem **7**, mp 201-3 °C in 38% purified yield. In the original paper[8], a combination of methanesulfonyl chloride, collidine and lithium chloride in DMF was used for the conversion of allylic alcohols to allylic chlorides without rearrangement. However, in our hand these reagents failed to convert the carbinol **5** to the corresponding chloride, instead giving an elimination product **7**, probably due to the secondary nature of our carbinol. In a proton NMR spectrum (acetone-d_6) signals for vinylic protons appear at: for H_a proton, δ 6.37 (dd, J = 18, 11 Hz); for H_b proton, δ 5.28 (d, J = 18 Hz); for H_c proton, δ 4.95 (d, J = 11 Hz), thus confirming the vinylic structure.

Next, we focused our attention to 1-propenyl system. As before, the aldehyde **4** was treated with ethylmagnesium iodide in THF at -78 °C to give the ethyl carbinol **8a** as an 1:1 mixture of two diastereoisomers. Dehydration[9] was carried out *via* mesylate as in the case of vinyl system, to give the 3-(1-propenyl)-2-cephem **9**, mp 137-8 °C in 40% purified yield. The proton NMR (acetone-d_6) showed that the coupling constant of two vinylic protons H_a and H_b is 18 Hz, thus suggesting the (E)-configuration of the double bond. NMR signals for vinylic H_a and H_b protons appear at δ 6.06 and 5.76, respectively. Thus, the present method permits an easy preparation of (E)-1-propenyl cephem, which is not easily available by Wittig reaction.



8a: R = $PhCH_2CO$
8b: R =





9: R = $PhCH_2CO$

In conclusion, we have found an alternative way to 3-vinyl cephem and especially, 3-(E)-1-propenyl cephem starting from the easily available 3-formyl-2-cephem **4**.

Experimental

Material and Analysis of Products. The 3-formyl-2-cephem (**4**) was prepared according to the literature procedure[5], starting from 7-aminocephalosporanic acid available from Loneza, Italy. All the reagents were purchased from Aldrich or Fluka and used without further purification. Tetrahydrofuran (THF) was dried over sodium. Melting points were measured on a Thomas Hoover Capillary Melting Point Apparatus and are uncorrected. Thin layer chromatography (TLC) was performed by using glass-backed silica gel plates (E. Merck 60 F-254, 0.25 mm). Developed plates were visualized by UV light or by staining with *p*-anisaldehyde. Flash chromatography was performed with Merck Silica gel 60 (230~400 mesh) as described.[11] Proton NMR spectra were recorded on a Bruker WM-300 (300 MHz) spectrometer using TMS as an internal standard.

Diphenylmethyl 7-phenylacetamido-3-(1-hydroxyethyl)-2-cephem-4-carboxylate (5). A solution of 1.00g (1.95 mmol) of 3-formyl-2-cephem **4** in 50 m*l* of dry THF was treated with 0.68g (16 mmol) of anhydrous lithium chloride. The mixture was cooled in dry ice-acetone bath and treated with 8 m*l* (16 mmol) of 2M methylmagnesium iodide solution in ether for 5 min. After half an hour's stirring, the reaction was quenched with 20 m*l* of 1M hydrochloric acid at -78 °C. The reaction mixture was allowed to warm to room temperature and diluted with 50 m*l* of ethyl acetate and 30 m*l* of water. The lower aqueous phase was discarded and the organic phase was washed with brine. Drying with sodium sulfate followed by concentration gave a red oil, which was purified by flash chromatography [toluene-ethyl acetate (1:1)] to give 670 mg (65%) of **5** as a solid. TLC[toluene-ethyl acetate (1:1)] of the crude product showed two spots of equal size (R_f = 0.33 and 0.27) for the alcohols **5** and a small spot for the lactone (R_f = 0.12). For the starting aldehyde, R_f = 0.52; ^{1}H-NMR (acetone-d_6): δ 8.08 (d, 1H, J = 8 Hz, NH), 6.89 (s, 1H, $CHPh_2$), 6.49, 6.42 (s, 1H, C-2), 5.58-5.51 (m, 1H, C-7), 5.30, 5.12 (s, 1H, C-4), 5.19, 5.18 (d, 1H, J = 4 Hz, C-6), 4.41-4.22 (m, 1H, CH(OH)), 3.69, 3.64 (ABq, 2H, J = 14 Hz, $PhCH_2$), 1.24 (d, 3H, J = 7 Hz, CH_3).

Diphenylmethyl 7-phenylacetamido-3-(1-hydroxypropyl)-2-cephem-4-carboxylate (8a). Diphenylmethyl 7-phenylacetamido-3-(1-hydroxypropyl)-2-cephem-4-carboxylate (**8a**) was similarly prepared by the reaction of **4** with ethylmagnesium iodide; ^{1}H-NMR (acetone-d_6): δ 8.10 (d, 1H, J = 8 Hz, NH), 6.88, 6.67 (s, 1H, $CHPh_2$), 6.47, 6.42 (s, 1H, C-2), 5.59-5.51 (m, 1H, C-7), 5.22, 5.18 (d, 1H, J = 4 Hz, C-6), 5.18, 5.11 (s, 1H, C-4), 4.19-4.17, 4.06-4.02 (m, 1H, CH(OH)), 3.67, 3.64 (ABq, 2H, J = 15 Hz, $PhCH_2$), 1.62-1.52 (m, 2H, CH_2CH_3), 0.81 (t, 3H, J = 7 Hz, CH_2CH_3).

Diphenylmethyl 7-phenylacetamido-3-vinyl-2-cephem-4-carboxylate (7). A solution of 0.30g (0.57 mmol) of the alcohol **5** in 5 m*l* of DMF was treated with 30 mg (0.71 mmol) of lithium chloride followed by 45 u*l* (67 mg, 0.58 mmol) of methanesulfonyl chloride and 85 u*l* (78 mg, 0.64 mmol) of collidine at 0 °C. The mixture was stirred at room temperature overnight. Then, the mixture was diluted with 30 m*l* of ethyl acetate and 30 m*l* of water. The organic phase was separated and washed with 1N hydrochloric acid followed by brine. Drying with magnesium sulfate and concentration gave a crude product which was purified by flash chromatography [toluene-ethyl acetate (7:1)] to give 110 mg (38%) of 3-vinyl-2-cephem **7** as a solid, mp 201-3 °C; ^{1}H-NMR (acetone-d_6); δ 8.15 (d, 1H, J = 8 Hz, NH), 6.88 (s, 1H, $CHPh_2$), 6.61 (s, 1H, C-2), 6.37 (dd, 1H, J = 18, 11 Hz, H_a), 5.54 (dd, 1H, J = 8, 4 Hz, C-7), 5.39 (s, 1H, C-4), 5.28 (d, 1H, J = 18 Hz, H_b), 5.28 (d, 1H, J = 4 Hz, C-6), 4.95 (d, 1H, J = 11 Hz, H_c), 3.67, 3.65 (ABq, 2H, J = 14 Hz, $PhCH_2$).

Diphenylmethyl 7-phenylacetamido-3-[(E)-1-propenyl]-2-cephem-4-carboxylate (9). Diphenylmethyl 7-phenylacetamido-3-[(E)-1-propenyl]-2-cephem-4-carboxylate (**9**) was similarly prepared in 40% purified yield, mp 137-8 °C; ^{1}H-NMR (acetone-d_6); δ 8.11 (d, 1H, J = 8 Hz, NH), 6.89 (s, 1H, $CHPh_2$), 6.06 (A part of ABX_3, 1H, J_{AB} = 16 Hz, H_a), 5.76 (B part of ABX_3, 1H, J_{AB} = 16 Hz, J_{BX} = 7 Hz, H_b), 5.54 (dd, 1H, J = 8, 4 Hz, C-7), 5.32 (s, 1H, C-4), 5.25 (d, 1H, J = 4 Hz, C-6), 3.66, 3.63 (ABq, 2H, J = 14

Hz, $PhCH_2$), 1.65 (X part of ABX_3, 3H, J_{BX} = 1 Hz, CH_3).

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Applications of the Fourier Deconvolution Procedure For Quantitative Analysis of Raman Spectra of Biomolecules

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The constrained, iterative Fourier deconvolution procedure was applied to quantitatively analyze the overlapped bands in the Raman spectra of biomolecules. When applied to Raman spectra of lysozyme and α-amylase, this procedure resolved the amide I band into five component peaks. The relative intensities of the resolved peaks can possibly provide the composition of secondary structure elements in proteins. The deconvolution procedure was also useful in monitoring the small changes in relative intensities of C-S stretching modes due to different conformers of L-methionine in aqueous solutions at different pH values. The implemented procedure is generally applicable to the problem of resolution enhancement of spectroscopic, chromatographic, and electrophoretic data.

Introduction

Raman spectra of aqueous solutions of biomolecules often show broad, overlapped bands. Interpretation of these low resolution Raman spectra are greatly facilitated by the ability to resolve the broad bands into their component peaks. Three approaches have been generally followed to achieve the necessary resolution enhancement of the spectral data. The curve-fitting method[1] is conceptually simple and straightforward to implement on a personal computer. However, it requires some a priori imformation about the number of component bands, their shape and width, and the forms of the baselines. The maximum entropy method[2] is capable of providing an exellent resolution enhancement but the computational requirement makes its implementation on a personal computer impractical at present.

Recently, the constrained, iterative Fourier deconvolution method for the analyses of overlapped bands in Raman spectra has been developed[3,4]. The Fourier deconvolution method is computationally efficient and neither a knowledge about the number of components in the complex band nor the starting guesses for peak ampitudes and locations are required.

In this paper, we demonstrate the potential application of the constrained, iterative Fourier deconvolution procedure implemented on a personal computer for quantitative analysis of the overlapped bands in the Raman spectra of some biomolecules.

Experimental

Sample Preparation and Raman Spectroscopy. Lysozyme from hen egg white, α-amylase from *Bacillus amyloliquefaciens*, and L-methionine were purchased from Sigma Chemical Co. An aqueous solution containing lysozyme at 100 mg/ml concentration was maintained at pH 7.0 with 50 mM potassium phosphate buffer. An aqueous solution of α-amylase at 0.6 mM concentration was maintained at pH 7.0 with 5 mM tris buffer. The pH values of aqueous solutions of L-methionine were adjusted with HCl and NaOH. The samples were inserted into glass capillary cells and the cell was sealed at both ends.

The Raman spectra were obtained with the Japan Spectroscopic Company model R-300 laser Raman spectrometer

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