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Syntheses of Highly Antibacterial 3-Vinylcephalosporin Derivatives

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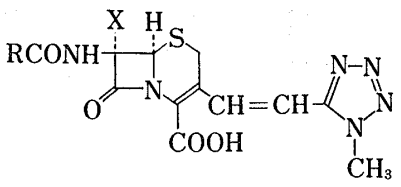
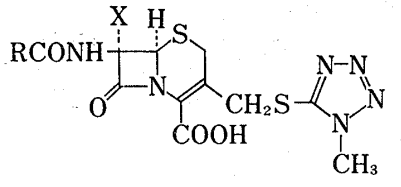
7-Acylamido-3-[2-(1-methyl-1H-tetrazol-5-yl)vinyl]-3-cephem-4-carboxylic acids including the 7-methoxy derivatives were prepared by two synthetic methods. The first method involves the reaction of the 3-bromomethylcephalosporin derivative (III) with 5-methylsulfinylmethyl-1-methyl-1H-tetrazole (IV) followed by thermal elimination leading to an olefinic compound (VI). An alternative olefin formation was carried out by utilizing the Wittig reaction of the 3-formylcephalosporin derivative (X). Among the 3-tetrazolylvinylcephalosporin derivatives, the mandelamido derivative (XVa) showed the best *in vitro* antibacterial activities against gram negative bacteria.

Keywords——3-vinylcephalosporin; antibacterial activity; thermal elimination; Wittig reaction; tetrazole

A variety of modifications of naturally occurring cephalosporin compounds have been made in search of better antibiotics. Our work on cephalosporin antibiotics has been concerned with the introduction of a new substituent at the 3-position, since a 3-substituent may play a crucial role in the antibacterial action of cephalosporin compounds. Recent works[2] indicate that the electronic factor of 3-substituents is related to the chemical reactivity of the β-lactam ring and an electron-withdrawing group at the 3-position enhances the antibacterial activity of the β-lactam antibiotics. In an earlier paper,[3] we dealt with the total synthesis of 3-trifluoromethylcephalosporin derivatives which was undertaken in view of the great electron-withdrawing power of the CF_3 group. This paper reports our success in obtaining highly antimicrobial 3-vinylcephalosporin derivatives (I).



I: X=H, OCH_3



IIa: R=PhCH(OH)–, X=H
IIb: R=$CNCH_2SCH_2$–, X=H
IIc: R=$CNCH_2SCH_2$–, X=OCH_3

Chart 1

From the viewpoint of electronic effects, a number of unsaturated groups have been introduced at the 3-position so as to be conjugated with Δ^3-double bond in the cephem ring. These groups participate in the delocalization of nitrogen's lone pair electrons thereby enhanc-

1) Location: *1-2-58 Hiromachi, Shinagawa-ku, Tokyo.*
2) J.M. Indelicato, T.T. Norvilas, R.R. Pfeiffer, W.J. Wheeler, and W.L. Wilham, *J. Med. Chem.*, **17**, 523 (1974); R.B. Hermann, *J. Antibiot.*, **26**, 223 (1973).
3) T. Watanabe, Y. Kawano, T. Tanaka, T. Hashimoto, M. Nagano, and T. Miyadera, *Tetrahedron Lett.*, **1977**, 3053.

ing the reactivity of the β-lactam amide bond. The linking of a cephem ring and either an aromatic ring or an unsaturated functional group through a double bond forms an extended conjugated system. Although this type of 3-vinylcephalosporin derivatives has been prepared by two research groups,[4] there is no literature concerning heterocycle-substituted vinyl derivatives. Based on structure-activity relationships, an electron-withdrawing heterocycle, 1-methyltetrazole was chosen as the substituent at the double bond. The heterocyclic moiety seemed to be suitable in lipophilicity and steric requirement for enzyme inhibition in light of potent antimicrobial activities of the cephalosporin derivatives such as cefamandole(IIa),[5] SQ-67590(IIb)[6] and CS-1170(IIc)[7] bearing (1-methyltetrazol-5-yl)thiomethyl group as a 3-substituent. However, it should be noted that there may be a great difference between chemical or biological behavior of the two substituents, irrespective of the structural similarity. The tetrazolylthio group can be released chemically as a mercaptan, while such a bond cleavage is impossible in the case of the 3-tetrazolylvinyl group.

The 3-tetrazolylvinylcephalosporin derivatives were prepared by the following two synthetic methods. The first method involves the reaction of a 3-bromomethyl-3-cephem derivative (III) with 1-methyl-5-methylsulfinylmethyl-1H-tetrazole (IV) followed by thermal elimination of the resulting product (V) to an olefinic compound (VI). The bromomethyl derivative (III) was prepared by bromination of *tert*-butyl 7-phenoxyacetamido-3-methyl-3-cephem-4-carboxylate-1-oxide with 1,3-dibromo-5,5-dimethylhydantoin.[8] The other starting material IV was

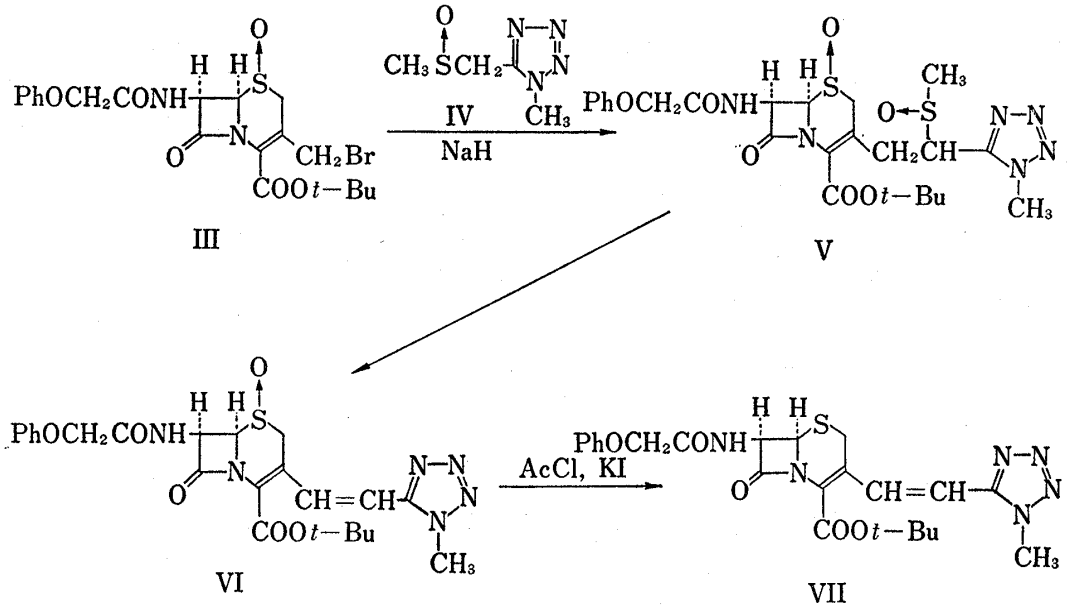


Chart 2

obtained from the displacement reaction of 5-chloromethyl-1-methyl-1H-tetrazole with methylmercaptan sodium salt followed by oxidation with *m*-chloroperbenzoic acid. The active methylene group of IV was initially activated with sodium hydride and reacted with

4) *a*) J.A. Webber, J.L. Ott, and R.T. Vasileff, *J. Med. Chem.*, **18**, 986 (1975); *b*) A.H. Shingler and N.G. Weir, "Recent Advances in the Chemistry of β-Lactam Antibiotics," ed. by J. Elks, The Chemistry Society, Burlington House, London, 1977, pp. 153—160.
5) W.E. Wick and D.A. Preston, *Antimicrobial Agents Chemother.*, **1**, 221 (1972).
6) H. Breuger, M.D. Treuner, D. Isaacson, and H.H. Gadebusch, Abstracts of Papers, The 9th International Congress of Chemotherapy, London, July 13–18, 1975, M-66, M-67; G. Molgora, G. Biasoli, P.N. Girald, G. Meinardi, and I. de Garneri, *Arzneim.-Forsch.*, **25**, 1665 (1975).
7) H. Nakao, H. Yanagisawa, B. Shimizu, M. Kaneko, M. Nagano, and S. Sugawara, *J. Antibiot.*, **29**, 554 (1976).
8) B. Laundon, B.R. Cowley, and D.C. Humber, Brit. Patent 1326531 (1973).

the bromide III to give the tetrazolylethyl derivative (V). The crude product was expected to contain at least four stereoisomers, since two chiral centers are introduced into the cephem derivative. In fact, the reaction mixture showed several spots on thin-layer chromatography (TLC), but separation and characterization of the products were not performed. The isomeric mixture was submitted to the following elimination reaction with the disappearance of the chiral centers. The elimination of CH_3SOH[9] from V was carried out by refluxing the benzene solution to form the *trans* olefinic compound VI. The presence of the double bond was confirmed by the nuclear magnetic resonance (NMR) spectrum exhibiting two doublets (J= 16 Hz) at 8.07 and 6.50 ppm. The reduction of the sulfoxide VI to the sulfide (VII) was performed with potassium iodide in the presence of acetyl chloride according to the usual procedure.[10] The reduction product VII can be utilized for the following two purposes: i) removal of the protecting group to give a carboxylic acid; ii) cleavage of the acyl group to the 7-amino derivative (XIII) for acylations with a variety of carboxylic acid derivatives.

The second method for preparing the above-mentioned olefinic cephalosporins started from 7-aminocephalosporanic acid (7-ACA), while the first synthetic method was possible starting from 7-aminodeacetoxycephalosporanic acid (7-ADCA). The 3-formyl derivative (X) which can be prepared *via* five steps from 7-ACA[4a] was reacted with a phosphorane (IX) in tetrahydrofurane (THF) below 0° to give an olefinic compound (XI) corresponding to VI. The Wittig reaction was unfavorable at higher temperatures because of poorer yields. The phosphorane IX was prepared by treating the phosphonium chloride (VIII) with aq. sodium hydroxide solution over a short period of time with ice-cooling. The phosphorane IX was hydrolyzed slowly in water to give triphenylphosphine oxide. For this reason the base treatment of VIII was carried out quickly in water and the resulting precipitate was filtered and immediately dried under reduced pressure.

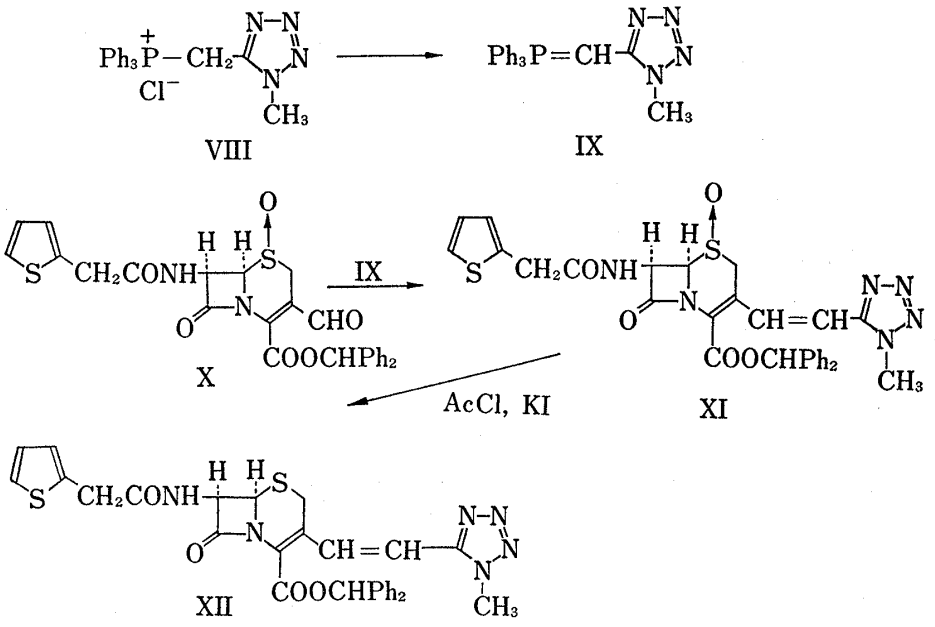
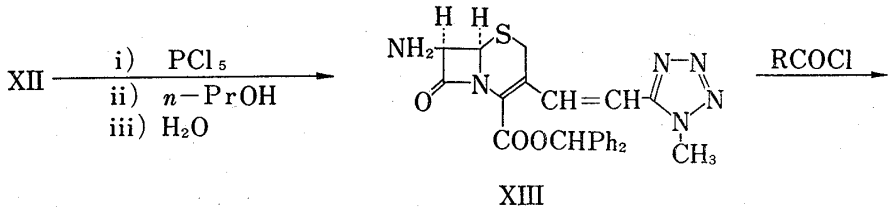


Chart 3

The key intermediate XIII for preparing 7-acyl derivatives was formed by way of an iminochloride and iminoether by treating XII with phosphorus pentachloride, *n*-propyl

9) B.M. Trost, W.P. Conway, P.E. Strenge, and T.J. Dietsche, *J. Am. Chem. Soc.*, **96**, 7165 (1974).
10) G.V. Kaiser, R.D.G. Copper, R.E. Koehler, C.F. Murphy, J.A. Webber, I.G. Wright, and E.M. Van Heyningen, *J. Org. Chem.*, **35**, 2430 (1970).

alcohol and water according to the usual method.[11] The acylation of XIII with acyl chlorides in the presence of a base afforded a number of acyl derivatives. The 7-methoxylated 3-vinylcephalosporin derivatives (XVII) were prepared by the method involving lead dioxide oxidation of a 3,5-di-*tert*-butyl-4-hydroxybenzylideneamino moiety and methanol addition to the resulting imino compound.[12] Chart 5 illustrates the methoxylation of XIII leading to



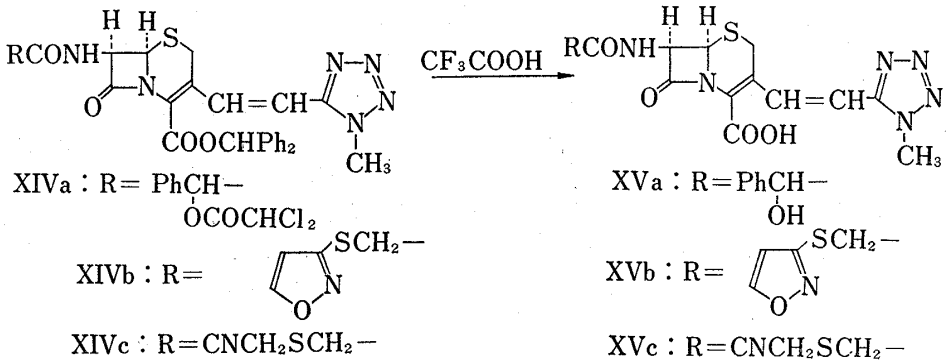
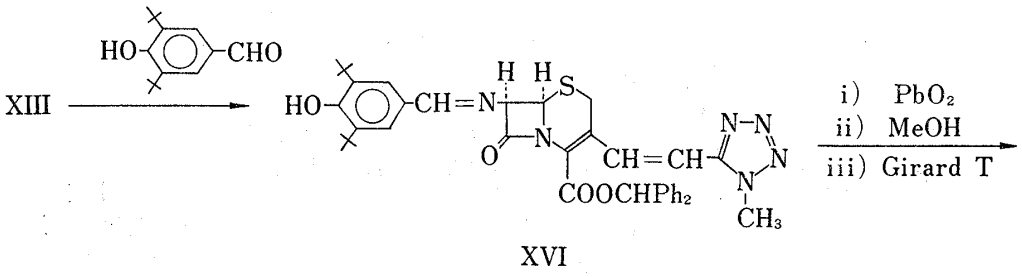


Chart 4



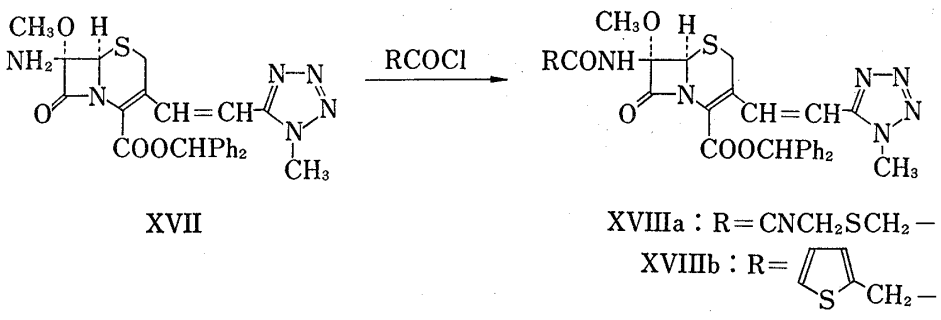


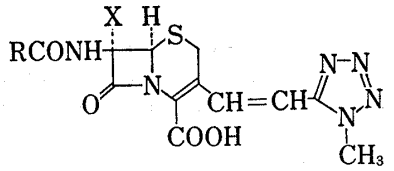
Chart 5

11) R.R. Chauvette, P.A. Pennington, C.W. Ryan, R.D.G. Copper, F.L. Jose, I.G. Wright, E.M. Van Heyningen, and G.W. Huffman, *J. Org. Chem.*, **36**, 1259 (1971).
12) H. Yanagisawa, M. Fukushima, A. Ando, and H. Nakao, *Tetrahedron Lett.*, **1975**, 2705.

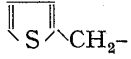
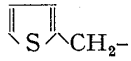
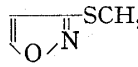
7-methoxycephalosporins. The reaction process from XIII to XVIII was carried out without isolation and purification of the intermediates.

The *tert*-butyl and diphenylmethyl groups were removed by treating in an organic solvent containing trifluoroacetic acid. The desired acids thus obtained were tested for antibacterial activities and a few compounds were found to exhibit strong inhibition against gram positive

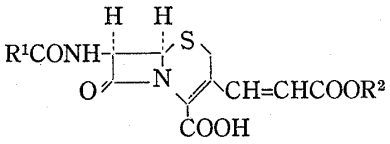
Table I. Antibacterial Activities of 3-Tetrazolylvinylcephalosporins



XIX

Compound	R	X	Minimal inhibitory concentrations (mcg/ml) Organism: *Staph. aureus* 209P	*Escherichia coli* NIHJ	*Shigella flexneri* 2a	*Klebsiella* 806	*Proteus vulgaris*
XIXa		H	0.025	0.4	0.4	0.4	0.4
XIXb		OCH_3	6.2	50	50	100	12.5
XVc	$CNCH_2SCH_2-$	H	0.05	0.8	0.8	1.5	6.2
XIXc	$CNCH_2SCH_2-$	OCH_3	12.5	>50	>50	>50	>50
XVa	PhCH(OH)– (D)	H	0.05	0.1	0.05	0.4	0.05
XVb		H	≦0.1	0.4	0.8	0.8	50
XIXd	$PhOCH_2-$	H	≦0.1	12.5	12.5	12.5	50
cf.							
	Cephalothin		0.05	6.2	12.5	6.2	6.2
	Cefamandole (IIa)		≦0.1	0.8	0.4	0.8	0.8
	XXc		≦0.1	3.1	3.1	3.1	6.2

and gram negative bacteria. The *in vitro* activities of the 3-tetrazolylvinylcephalosporin derivatives are summarized in Table I. The introduction of a methoxy group at the 7-position resulted in marked decrease of antibacterial activities as is seen in Table I. The 7-phenylglycylamido derivative could not be obtained in pure form probably because of its instability. Among these derivatives the mandelamido derivative (XVa) was most antibacterial and approximately eight times as active against *E. coli* and some other gram negative bacteria as cefamandole (IIa) and sixty-four times more active than cephalothin. To the best of our knowledge the mandelamido compound has the best MIC value among 3-vinylcephalosporin derivatives so far synthesized. According to the paper reported by Webber and coworkers,[4a] 3-(2-carboxyvinyl)- and 3-(2-ethoxycarbonylvinyl)-3-cephem-4-carboxylic acid derivatives, XXa and XXb, are 17 and 9 times, respectively, as active against *E. coli* as cephalothin. For comparison, 3-(2-methoxycarbonylvinyl)-7-(2-thienylacetamido)-3-cephem-4-carboxylic acid



XXa: R^1=PhCH(OH)–, R^2=H

XXb: R^1=PhCH(OH)–, R^2=C_2H_5

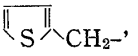
XXc: R^1=  , R^2=CH_3

Chart 6

(XXc) was similarly prepared and tested for antibacterial activity. The corresponding 3-tetrazolylvinyl derivative (XVa) was proved to be more active than the carbomethoxy derivative (XXc).

Experimental

Melting points were obtained on a Yanagimoto micro melting point apparatus and are uncorrected. The NMR spectra were taken on a Varian A-60D or HA-100 spectrometer using tetramethylsilane as an internal standard. The chemical shifts of various compounds are given in δ units.

1-Methyl-5-methylthiomethyl-1H-tetrazole——To an ice-cooled solution of 5-chloromethyl-1-methyl-1H-tetrazole[13] (2.65 g, 20.0 mmol) in N,N-dimethylformamide (DMF, 30 ml) was added dropwise aq. 20% CH_3SNa (7.01 g, 20.0 mmol). The mixture was stirred at 0° for 1 hr and stood overnight at room temperature. The reaction mixture was poured into ice-H_2O, and extracted with AcOEt. The extract was washed with saturated brine, dried over Na_2SO_4, and evaporated *in vacuo* to give 2.43 g of 1-methyl-5-methylthiomethyl-1H-tetrazole as crystals, which was distilled at 140—142°/1 mmHg to give pure crystals, mp 32—34°. NMR ($CDCl_3$) δ: 4.10 (3H, s, NCH_3), 3.93 (2H, s, SCH_2), 2.05 (3H, s, CH_3S). *Anal.* Calcd. for $C_4H_8N_4S$: C, 33.31; H, 5.59; N, 38.85; S, 22.23. Found: C, 33.34; H, 5.63; N, 39.04; S, 22.40.

1-Methyl-5-methylsulfinylmethyl-1H-tetrazole (IV)——To an ice-cooled solution of 1-methyl-5-methylthiomethyltetrazole (1.44 g, 10 mmol) in $CHCl_3$ (40 ml) was added dropwise a solution of *m*-chloroperbenzoic acid (1.97 g, 9.7 mmol) in $CHCl_3$ (30 ml). After stirring for 5 min, the reaction mixture was extracted with saturated aq. $NaHCO_3$. The extract was evaporated to dryness *in vacuo*, and to the residue was added AcOEt. After removal of an insoluble material, the solvent was distilled off to leave 1.04 g of an oil, which was chromatographed on a column of silica gel eluting with AcOEt–MeOH (10: 1) to afford 0.96 g of IV as colorless crystals, mp 61—62°. IR ν_{max}^{Nujol} cm^{-1}: 1035 (S→O); NMR ($CDCl_3$) δ: 4.48, 4.14 (2H, 2×d, J=14.0 Hz, SCH_2), 4.14 (3H, s, NCH_3), 2.64 (3H, s, SCH_3). *Anal.* Calcd. for $C_4H_8N_4OS$: C, 29.99; H, 5.03; N, 34.97; S, 20.01. Found: C, 30.12; H, 4.86; N, 34.75; S, 19.88.

***tert*-Butyl 3-[2-(1-Methyl-1H-tetrazol-5-yl)vinyl]-7β-(2-phenoxyacetamido)-3-cephem-4-carboxylate-1-oxide (VI)**——To a suspension of NaH (50% mineral oil dispersion, 216 mg, 4.5 mmol) in DMF (18 ml) was added IV (288 mg, 1.8 mmol) under N_2. The mixture was stirred at room temperature until the evolution of hydrogen gas ceased, cooled to −20° in a CCl_4-dry ice bath, and then III (900 mg, 1.8 mmol) was added in one portion. The mixture was stirred at that temperature for 1.5 hr and quenched with AcOH (1 ml). The mixture was poured into ice-H_2O and extracted with AcOEt. The extract was washed successively with aq. 5% $NaHCO_3$, H_2O and saturated brine, dried over Na_2SO_4 and freed of the solvent *in vacuo*. To the residue was added 200 ml of dry benzene, and the mixture was refluxed for 120 hr. After removal of the solvent, the residual crude product was chromatographed on a column of silica gel using AcOEt as an eluent to yield 225 mg of VI, mp 145—147° (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3370 (NH), 1795 (β-lactam CO), 1705 (ester CO), 1690 (amide CO); NMR (DMSO-d_6) δ: 8.07, 6.50 (2H, 2×d, J=16 Hz, olefinic protons), 7.73 (1H, d, J=9.5 Hz, CONH), 7.3—6.6 (5H, m, aromatic protons), 4.12, 3.28 (2H, 2×d, J=19 Hz, 2-H_2), 6.02 (1H, d–d, J=9.5, 5.0 Hz, 7-H), 4.60 (1H, d, J=5 Hz, 6-H), 3.98 (3H, s, NCH_3), 1.49 (9H, s, *tert*-Bu).

***tert*-Butyl 3-[2-(1-Methyl-1H-tetrazol-5-yl)vinyl]-7β-(2-phenoxyacetamido)-3-cephem-4-carboxylate (VII)**——To a cold (0°) solution of VI (258 mg, 0.5 mmol) in DMF were added KI (332 mg, 2.0 mmol) and AcCl (157 mg, 2.0 mmol). The mixture was stirred at 0° for 30 min, poured into ice-H_2O, and then extracted with AcOEt. The extract was washed successively with aq. $K_2S_2O_5$, H_2O, aq. 5% $NaHCO_3$, and saturated brine, and dried over Na_2SO_4. The solvent was removed *in vacuo* to leave a residue, which was chromatographed on a column of silica gel with benzene–AcOEt (1: 1) affording 203 mg of VII, mp 110—115° (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3330 (NH), 1785 (β-lactam CO), 1710 (ester CO); NMR ($CDCl_3$) δ: 8.15, 6.49 (2H, 2×d, J=16 Hz, olefinic protons), 7.78 (1H, d, J=9 Hz, CONH), 7.4—6.75 (5H, m, aromatic protons), 5.94 (1H, d–d, J=9, 5 Hz, 7-H), 5.04 (1H, d, J=5 Hz, 6-H), 4.56 (2H, s, $PhOC\underline{H}_2$), 3.99 (3H, s, NCH_3), 1.52 (9H, s, *tert*-Bu).

3-[2-(1-Methyl-1H-tetrazol-5-yl)vinyl]-7β-(2-phenoxyacetamido)-3-cephem-4-carboxylic Acid (XIXd)——To a solution of VII (75 mg) in CH_2Cl_2 (0.3 ml) was added CF_3COOH (1 ml) at room temperature. After stirring the mixture for 10 min, the solvent was distilled off *in vacuo* at room temperature. The residue was dissolved in AcOEt (10 ml) and extracted with two portions of 10 ml of aq. 3% $NaHCO_3$. The extract was layered with ethyl acetate, adjusted to pH 2.1 with 4 N HCl and extracted with AcOEt 3 times. The extracts were combined and dried over Na_2SO_4, and evaporated *in vacuo* to give 29 mg of XIXd as colorless crystals, mp 134—135°, IR ν_{max}^{Nujol} cm^{-1}: 1782 (β-lactam CO); NMR (CD_3OD) δ: 8.12, 6.82 (2H, 2×d, J=16 Hz, olefinic protons), 7.4—6.8 (5H, m, aromatic protons), 5.85 (1H, d, J=5 Hz, 7-H), 5.18 (1H, d, J=5 Hz, 6-H), 4.60 (2H, s, $PhOC\underline{H}_2$), 4.07 (3H, s, NCH_3). From the first AcOEt layer, 32 mg of the starting material (VII) was recovered.

(1-Methyl-1H-tetrazol-5-yl)methyltriphenylphosphonium Chloride (VIII)——Triphenylphosphine (7.98 g) and 1-methyl-5-chloromethyl-1H-tetrazole (3.98 g) were dissolved in benzene (45 ml), and the mixture was

13) E.K. Harvill, R.M. Herbst, and E.G. Schreiner, *J. Org. Chem.*, **17**, 1597 (1952).

refluxed for 17 hr. The precipitate was collected by filtration, washed with benzene and dried to yield 11.15 g of VIII, which was recrystallized from EtOH–acetone to give colorless crystals, mp 148—149°. *Anal.* Calcd. for $C_{21}H_{20}ClN_4P \cdot 1/2H_2O$: C, 62.45; H, 5.24; Cl, 8.77; N, 13.87; P, 7.66. Found: C, 62.41; H, 5.47; Cl, 8.77; N, 13.84; P, 7.47.

(1-Methyl-1H-tetrazol-5-yl)methylenetriphenylphosphorane (IX)——To a solution of VIII (8.1 g) in water (200 ml) was added over 1 min with stirring and ice-cooling an aqueous solution (20 ml) of NaOH (804 mg). After addition, the mixture was stirred for 1 min and filtered leaving the precipitate. The precipitate was washed with cold H_2O and immediately dried under reduced pressure to yield 5.7 g of IX, which was recrystallized from benzene to give colorless crystals, mp 241—243°. *Anal.* Calcd. for $C_{21}H_{19}N_4P$: C, 70.37; H, 5.34; N, 15.63; P, 8.64. Found: C, 70.66; H, 5.19; N, 15.66; P, 8.34.

Diphenylmethyl 3-[2-(1-Methyl-1H-tetrazol-5-yl)vinyl]-7β-[2-(2-thienyl)acetamido]-3-cephem-4-carboxylate-1-oxide (XI)——A mixture of diphenylmethyl 3-formyl-7β-[2-(2-thienyl)acetamido]-3-cephem-4-carboxylate-1-oxide (X, 5.35 g) and IX (4.3 g) in THF (130 ml) was stirred at −5——10° for 5 hr. The reaction mixture was poured into a mixture of AcOEt and ice-H_2O and made acidic with 5% HCl (10 ml). The organic layer was washed with 5 portions of aq. saturated NaCl and dried over Na_2SO_4. The solvent was distilled off *in vacuo* to leave a solid residue. The residue was, after addition of MeOH (*ca.* 30 ml), stirred at room temperature for 20 min and filtered leaving 3.62 g of XI as a powder. The filtrate was evaporated *in vacuo* to dryness, and the residue was chromatographed on silica gel using AcOEt as an eluent to give an additional amount (600 mg) of XI. mp 159—162°. IR ν_{max}^{Nujol} cm^{-1}: 3300 (NH), 1790 (β-lactam CO), 1720 (ester CO). NMR (DMSO-d_6) δ: 3.34, 3.67 (2H, 2×d, J=18.0 Hz, 2-H_2), 3.87 (2H, s, CH_2CO), 4.05 (3H, s, NCH_3), 5.08 (1H, d, J=5.0 Hz, 6-H), 6.00 (1H, d–d, J=5.0, 8.0 Hz, 7-H), 6.50, 8.05 (2H, 2×d, J=15.0 Hz, olefinic protons), 6.99 (1H, s, C<u>H</u>Ph_2), 6.9—7.8 (13H, m, C_6H_5×2, thienyl protons), 8.83 (1H, d, J=8.0 Hz, NH).

Diphenylmethyl 3-[2-(1-Methyl-1H-tetrazol-5-yl)vinyl]-7β-[2-(2-thienyl)acetamido]-3-cephem-4-carboxylate (XII)——To a solution of XI (1.3 g) in 25 ml of DMF were added with stirring and cooling with ice-H_2O KI (2.0 g) and AcCl (0.7 ml), and the mixture was stirred for 5 min. The mixture was stirred for another 90 min at room temperature and worked up as described for the preparation of VII. The crude product was chromatographed on a column of silica gel eluting with benzene–AcOEt (1: 1) to give 1.1 g of XII. IR ν_{max}^{KBr} cm^{-1}: 1780 (β-lactam CO), 1720 (ester CO); NMR ($CDCl_3$) δ: 3.55 (2H, broad s, 2-H_2), 3.77 (2H, s, CH_2CO), 3.80 (3H, s, NCH_3), 4.96 (1H, d, J=5.5 Hz, 6-H), 5.91 (1H, d–d, J=5.5, 9.0 Hz, 7-H), 6.40, 8.05 (2H, 2×d, J=16 Hz, olefinic protons), 6.95 (1H, s, C<u>H</u>Ph_2), 6.8—7.6 (14H, m, NH, phenyl, thienyl protons).

Diphenylmethyl 7β-Amino-3-[2-(1-methyl-1H-tetrazol-5-yl)vinyl]-3-cephem-4-carboxylate (XIII)——To a solution of PCl_5 (1.218 g) in $CHCl_3$ (15 ml) was added quinoline (0.929 g) at 10—25°. After stirring for 5 min, XII (2.7 g) was added with cooling at −10——20°. The mixture was stirred at that temperature for 10 min and at room temperature for additional 1 hr. The reaction mixture was cooled to 0°, to which was added *n*-propanol (5.4 ml). The mixture was stirred at room temperature for 1 hr and poured into $CHCl_3$–ice-H_2O. The precipitate was collected by filtration and dried to give 1.3 g of XIII–HCl. The hydrochloride was treated with a mixture of AcOEt and aq. $NaHCO_3$. From the dried (Na_2SO_4) AcOEt solution the free amino compound (XIII) was obtained. IR ν_{max}^{Nujol} cm^{-1}: 3340, 3400 (NH_2), 1780 (β-lactam CO), 1720 (ester CO); NMR ($CDCl_3$) δ: 2.03 (2H, broad s, NH_2), 3.61 (2H, broad s, 2-H_2), 3.82 (3H, s, NCH_3), 7.88, 6.50 (2H, 2×d, J=16.5 Hz, olefinic protons), 7.00 (1H, s, C<u>H</u>Ph_2), 7.20—7.65 (10H, m, 2×C_6H_5), 4.85, 5.07 (2H, 2×d, J=7 Hz, 6-H, 7-H).

Diphenylmethyl 7β-D-(−)-O-Dichloroacetylmandelamido-3-[2-(1-methyl-1H-tetrazol-5-yl)vinyl]-3-cephem-4-carboxylate (XIVa)——To a solution of XIII (204 mg) in THF (15 ml) were added at −10° a solution of N,N-diethylaniline (149 mg) in THF (2 ml) and a solution of D(−)-O-dichloroacetylmandelyl chloride (168.9 mg) in THF (2 ml). The mixture was stirred at −10° for 30 min. After completion of the reaction, an appropriate amount of AcOEt was added to the reaction mixture. The mixture was then washed successively with aq. $KHSO_4$, aq. $NaHCO_3$ and H_2O, and dried over Na_2SO_4. The solvent was distilled off *in vacuo* to give XIVa in a quantitative yield. IR ν_{max}^{KBr} cm^{-1}: 1780 (β-lactam CO), 1720 (ester CO), 1680 (amide CO); NMR ($CDCl_3$) δ: 3.46 (2H, broad s, 2-H_2), 3.78 (3H, s, NCH_3), 4.94 (1H, d, J=5.0 Hz, 6-H), 5.78 (1H, d–d, J=5.0, 9.0 Hz, 7-H), 6.11, 6.20 (2H, 2×s, C<u>H</u>OCOC<u>H</u>Cl_2), 6.93 (1H, s, C<u>H</u>Ph_2), 7.1—7.7 (16H, m, NH, 3×C_6H_5).

Diphenylmethyl 7β-[2-(Isoxazol-3-ylthio)acetamido]-3-[2-(1-methyl-1H-tetrazol-5-yl)vinyl]-3-cephem-4-carboxylate (XIVb)——3-Isoxazolylthioacetyl chloride (52.2 mg) and XIII (140 mg) were similarly reacted in the presence of N,N-diethylaniline (44 mg) in THF (10 ml) and the resulting mixture was worked up in the same manner as described above. The crude product was purified by preparative silica gel TLC (AcOEt) to give 100 mg of XIVb as colorless fine crystals, mp 105—108°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3140 (NH), 1790 (β-lactam CO), 1720 (ester CO), 1690 (amide CO). NMR ($CDCl_3$) δ: 3.42, 3.74 (2H, 2×d, J=18.0 Hz, 2-H_2), 3.84 (3H, s, NCH_3), 3.86 (2H, s, SCH_2), 4.97 (1H, d, J=5.0 Hz, 6-H), 5.86 (1H, d–d, J=5.0, 8.0 Hz, 7-H), 6.25, 8.28 (2H, 2×d, J=1.5 Hz, isoxazolyl protons), 6.50, 7.96 (2H, 2×d, J=15.0 Hz, olefinic protons), 6.95 (1H, s, C<u>H</u>Ph_2), 7.05—7.56 (10H, m, C_6H_5×2), 7.73 (1H, d, J=8.0 Hz, NH). *Anal.* Calcd. for $C_{29}H_{25}N_7O_5S_2$: C, 56.57; H, 4.09; N, 15.92; S, 10.42. Found: C, 56.43; H, 4.05; N, 15.96; S, 10.28.

Diphenylmethyl 7β-(2-Cyanomethylthioacetamido)-3-[2-(1-methyl-1H-tetrazol-5-yl)vinyl]-3-cephem-4-carboxylate (XIVc)——Cyanomethylthioacetyl chloride (115 mg) and XIII (244 mg) were similarly reacted in the presence of N,N-diethylaniline (115 mg) in THF (20 ml) and the reaction mixture was worked up in the same manner as described above. The crude product was purified by silica gel TLC (AcOEt) to give 50 mg of XIVc. IR ν_{max}^{KBr} cm^{-1}: 3300 (NH), 1800 (β-lactam CO), 1720 (ester CO). NMR (DMSO-d_6) δ: 3.35 (2H, s, $C\underline{H}_2CO$), 3.43 (2H, broad s, 2-H_2), 3.56 (2H, s, $CNCH_2$), 3.78 (3H, s, NCH_3), 4.95 (1H, d, J=5.0 Hz, 6-H), 5.84 (1H, d-d, J=5.0, 9.0 Hz, 7-H), 6.42, 8.02 (2H, 2×d, J=16 Hz, olefinic protons), 6.92 (1H, s, $C\underline{H}Ph_2$), 7.2—7.6 (10H, m, 2×C_6H_5), 7.70 (1H, d, J=9.0 Hz, NH).

3-[2-(1-Methyl-1H-tetrazol-5-yl)vinyl]-7β-[2-(2-thienyl)acetamido]-3-cephem-4-carboxylic Acid (XIXa)——CF_3COOH (0.5 ml) was added to a solution of XII (100 mg) in dichloroethane (3 ml) with stirring at −12° and the mixture was stirred for 60 min. After completion of the reaction, the solvent was distilled off *in vacuo* at room temperature. The residue was dissolved in AcOEt and extracted with 2 portions of 10 ml of aq. 10% K_2HPO_4. The extract was washed with AcOEt and adjusted to pH 2 with 10% HCl. The precipitated carboxylic acid was taken up in AcOEt and dried over anhydrous Na_2SO_4. Upon evaporation of the solvent, there was obtained 50 mg of XIXa as fine crystals. IR ν_{max}^{KBr} cm^{-1}: 3300 (NH), 1780 (β-lactam CO), 1720 (COOH); NMR (DMSO-d_6) δ: 3.78 (2H, s, 2-H_2), 4.08 (3H, s, NCH_3), 5.24 (1H, d, J=5.0 Hz, 6-H), 5.76 (1H, d-d, J=5.0, 9.0 Hz, 7-H), 7.00, 7.90 (2H, 2×d, J=16.0 Hz, olefinic protons), 6.8—7.4 (3H, m, thienyl protons), 9.19 (1H, d, J=9.0 Hz, NH).

7β-D(−)-Mandelamido-3-[2-(1-methyl-1H-tetrazol-5-yl)vinyl]-3-cephem-4-carboxylic Acid (XVa)——Diphenylmethyl ester XIVa (287 mg) and CF_3COOH (1.2 ml) were similarly reacted in dichloroethane (10 ml) and work-up as described above gave 155 mg of XVa, mp 150—152°. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 3350 (NH), 1715 (CO_2H), 1790 (β-lactam CO); NMR (CD_3OD) δ: 3.70, 4.02 (2H, 2×d, J=18 Hz, 2-H_2), 4.09 (3H, s, NCH_3), 5.14 (1H, s, $C\underline{H}OH$), 5.17 (1H, d, J=5.0 Hz, 6-H), 5.78 (1H, d, J=5.0 Hz, 7-H), 6.84, 8.11, (2H, 2×d, J=16 Hz, olefinic protons), 7.2—7.6 (5H, m, C_6H_5).

7β-[2-(Isoxazol-3-ylthio)acetamido]-3-[2-(1-methyl-1H-tetrazol-5-yl)vinyl]-3-cephem-4-carboxylic Acid (XVb)——According to the above procedure, XVb (22 mg) was obtained from XIVb (67 mg), mp 150—154° (dec.). IR ν_{max}^{KBr} cm^{-1}: 3400 (NH), 1780 (β-lactam), 1720 (CO_2H). NMR (DMSO-d_6) δ: 3.68, 4.10 (2H, 2×d, J=18.0 Hz, 2-H_2), 3.95 (3H, s, NCH_3), 4.08 (2H, s, SCH_2), 5.22 (1H, d, J=5.0 Hz, 6-H), 5.74 (1H, d-d, J=5.0 Hz, 8.0 Hz, 7-H), 6.67, 8.90 (2H, 2×d, J=1.5 Hz, isoxazolyl protons), 6.96, 7.91 (2H, 2×d, J=16.0 Hz, olefinic protons), 9.26 (1H, d, J=8.0 Hz, NH). UV $\lambda_{max}^{H_2O(pH=6.8)}$ nm (ε): 324 (23500).

7β-(2-Cyanomethylthioacetamido)-3-[2-(1-methyl-1H-tetrazol-5-yl)vinyl]-3-cephem-4-carboxylic Acid (XVc)——According to the above procedure, XVc (50 mg) was obtained from XIVc (244 mg). IR ν_{max}^{KBr} cm^{-1}: 3300 (NH), 1800 (β-lactam CO); NMR (DMSO-d_6) δ: 3.46 (2H, s, CH_2CO), 3.66 (2H, broad s, 2-H_2), 3.78 (2H, s, CH_2CN), 4.06 (3H, s, NCH_3), 5.20 (1H, d, J=5.0 Hz, 6-H), 5.66 (1H, d-d, J=5.0, 7.6 Hz, 7-H), 6.78, 7.98 (2H, 2×d, J=16.2 Hz, olefinic protons), 9.24 (1H, d, J=7.6 Hz, NH).

Diphenylmethyl 7β-Amino-7α-methoxy-3-[2-(1-methyl-1H-tetrazol-5-yl)vinyl]-3-cephem-4-carboxylate (XVII)——To a solution of the Schiff base (XVI, 248 mg) in benzene (10 ml) was added PbO_2 which was freshly prepared from $Pb(OAc)_4$ (500 mg), and the mixture was stirred at room temperature for 2 hr. After the insoluble material was filtered off, the filtrate was concentrated to *ca.* 2 ml, and MeOH (10 ml) was added. The mixture was stirred at room temperature for 1 hr, and then MeOH (4 ml) and Girard T (500 mg) were added. The mixture was stirred at room temperature until the Schiff base had disappeared. After the solvent was distilled off *in vacuo*, the residue was shaken with a mixture of AcOEt–H_2O. The organic layer was evaporated *in vacuo* to give 228 mg of the crude XVII. The product was used for acylation without further purification.

Diphenylmethyl 7β-(2-Cyanomethylthioacetamido)-7α-methoxy-3-[2-(1-methyl-1H-tetrazol-5-yl)vinyl]-3-cephem-4-carboxylate (XVIIIa)——To a solution of the crude XVII (228 mg) in 1,2-dichloroethane (8 ml) were added with cooling at −10° N,N-diethylaniline (101 mg) and cyanomethylthioacetyl chloride (101 mg). After stirring for 1 hr, AcOEt was added to the reaction mixture. The mixture was washed successively with aq. $KHSO_4$, aq. $NaHCO_3$, and H_2O, dried over Na_2SO_4, and then evaporated *in vacuo*. The residual oil was purified by silica gel TLC (AcOEt) to give 63.6 mg of XVIIIa. NMR ($CDCl_3$) δ: 8.05, 6.59 (2H, 2×d, J=16.5 Hz, olefinic protons), 7.98 (1H, s, NH), 7.1—7.7 (10H, m, 2×C_6H_5), 7.00 (1H, s, $C\underline{H}Ph_2$), 5.05 (1H, s, 7-H), 3.90 (3H, s, NCH_3), 3.58 (5H, broad s, OCH_3, $NCCH_2$), 3.47 (2H, s, CH_2CO), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1780 (β-lactam CO), 1730 (ester CO), 1690 (amide CO).

7β-(2-Cyanomethylthioacetamido)-7α-methoxy-3-[2-(1-methyl-1H-tetrazol-5-yl)vinyl]-3-cephem-4-carboxylic Acid (XIXc)——To a cooled (−7°) solution of XVIIIa (63.6 mg) in 1,2-dichloroethane (2.5 ml) was added CF_3COOH (1.2 ml), and the mixture was stirred for 1 hr. The reaction mixture was worked up as described for the preparation of XIXd. Recrystallization from MeOH afforded 5.7 mg of XIXc. The mother liquor was evaporated *in vacuo*, and the residue was purified by preparative TLC using $CHCl_3$–MeOH (2:1) as a developing solvent to give an additional amount (12 mg) of XIXc. IR ν_{max}^{KBr} cm^{-1}: 1780 (β-lactam), 3300 (NH).

Diphenylmethyl 7α-Methoxy-3-[2-(1-methyl-1H-tetrazol-5-yl)vinyl]-7β-[2-(2-thienyl)acetamido]-3-cephem-4-carboxylate (XVIIIb)——(2-Thienyl)acetyl chloride and XVII were reacted in the presence of N,N-diethylaniline as described for XVIIIa. The crude product was purified by silica gel TLC (benzene-

AcOEt (1: 1)) to give XVIIIb. IR ν_{max}^{Nujol} cm^{-1}: 1780 (β-lactam CO), 1725 (ester CO), 1695 (amide CO), NMR ($CDCl_3$) δ: 3.40 (2H, broad s, 2-H_2), 3.43 (3H, s, OCH_3), 3.76 (3H, s, NCH_3), 3.79 (2H, s, CH_2CO), 5.00 (1H, s, 6-H), 6.46, 7.82 (2H, 2×d, J=16 Hz, olefinic protons), 6.8—7.5 (14H, m, C_6H_5×2, thienyl protons, C<u>H</u>Ph_2).

7α-Methoxy-3-[2-(1-methyl-1H-tetrazol-5-yl)vinyl]-7β-[2-(2-thienyl)acetamido]-3-cephem-4-carboxylic Acid (XIXb)——According to the procedure described for XIXc, CF_3COOH and XVIIIb were reacted and the reaction mixture was worked up as described above to give XIXb. IR ν_{max}^{KBr} cm^{-1}: 3400 (broad, NH), 1770 (β-lactam CO), 1680 (amide CO).

3-(2-Methoxycarbonylvinyl)-7β-[2-(2-thienyl)acetamido]-3-cephem-4-carboxylic Acid (XXc)——To a solution of X (160 mg) in THF (10 ml) was added carbomethoxymethylenetriphenylphosphorane (208.8 mg). The mixture was stirred at room temperature for 3 days, poured into ice-H_2O and extracted with CH_2Cl_2. The extract was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was purified by preparative TLC (AcOEt) to give 116 mg of diphenylmethyl 3-(2-methoxycarbonylvinyl)-7β-[2-(2-thienyl)acetamido]-3-cephem-4-carboxylate-1-oxide. IR ν_{max}^{Nujol} cm^{-1}: 1780 (β-lactam CO), 1060 (S→O). To a cold (0°) solution of the sulphoxide prepared above (177 mg, 0.3 mmol) in DMF (3 ml) were added KI (0.33 mg, 2 mmol) and AcCl (1.0 ml) and the mixture was stirred for 1.5 hr. The reaction mixture was worked up as described for the preparation of XII. The crude product was purified by preparative TLC using benzene–AcOEt (1: 1) as a solvent to give 75.6 mg of the benzhydryl ester of XXc. NMR ($CDCl_3$) δ: 3.50 (2H, s, 2-H_2), 3.71 (3H, s, OCH_3), 3.83 (2H, s, CH_2CO), 4.99 (1H, d, J=6 Hz, 6-H), 5.86 (1H, d-d, J=6, 8 Hz, 7-H), 6.40 (1H, d, J=8 Hz, NH), 5.99, 7.86 (2H, 2×d, J=16 Hz, olefinic protons), 6.9—7.6 (14H, m, 2×C_6H_5, C<u>H</u>Ph_2, thienyl protons). IR ν_{max}^{Nujol} cm^{-1}: 1785 (β-lactam CO), 1725 (ester CO), 1700 (broad, ester and amide CO).

According to the procedure described for XIXa, 627 mg of the carboxylic acid (XXc) was obtained from 1.207 g of benzhydryl ester of XXc. IR ν_{max}^{KBr} cm^{-1}: 3290 (NH), 1780 (β-lactam), 1680 (broad, amide and ester CO), 980 (C=C).

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