PROTECTION OF HYDROXYL IN THE SYNTHESIS OF SEMISYNTHETIC β-LACTAM ANTIBIOTICS

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The 2-methoxypropan-2-yl group fulfills the need of a hydroxyl protecting group generally suitable for the synthesis of β-lactam antibiotics, satisfying the criteria of low-cost, convenience and selectivity in formation, and, above all, ease of deprotection under conditions compatible to the highly sensitive β-lactam function and without contamination of the final products. The use of this protecting group has enabled the successful attachment of 6-[4-(*N*-acetyl-4-hydroxyl-L-prolylamino)phenyl]-1,2-dihydro-2-oxo-3-pyridinecarboxyl group, through an amide linkage, to amoxicillin, cephaloglycin, and the 3-[[(1-carboxymethyl)-1-*H*-tetrazol-5-yl]thio]methyl analogue of the latter, yielding broad-spectrum antibiotics with notably good activities against strains of *Pseudomonas aeruginosa*.

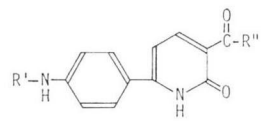
As part of our drug discovery program in semisynthetic β-lactam antibiotics we have prepared a series of penicillins (*e.g.*, **4a**, **4b**) and cephalosporins (*e.g.*, **4c**, **4d**) which incorporate, as part of the side chain, the 6-[4-(*N*-acylamino)phenyl]-1,2-dihydro-2-oxo-3-pyridinecarboxyl group (*cf.* **1**), which is attached through an amide linkage to amoxicillin (*cf.* **2**), cephaloglycin (*cf.* **3a**), or its various C-3 analogs (*cf. e.g.*, **3b**).[1,2,3] A number of these compounds have shown good activity against Gram-negative bacteria, especially against strains of *Pseudomonas aeruginosa*.

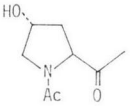
An essential sequence in the synthesis of various members of **4** involves the activation of the carboxyl group of **1** by converting it to the imidazolide (*e.g.*, **5c**), *N*-hydroxysuccinimide ester,[4] or mixed anhydride,[5] followed by coupling to amoxicillin, cephaloglycin, or various C-3 analogs of the latter. A hydroxyl group present in the R′ acyl group may require protection prior to the activation of the carboxyl. Thus, while the side chain having a *N*-acetylserinyl group could be coupled without hydroxyl protection to give reasonably pure product (*e.g.*, **4a***), the corresponding side chain with a *N*-acetyl-4-hydroxyl-L-prolyl group, without hydroxyl protection, invariably led to side-products through competitive acylation of the hydroxyl group.

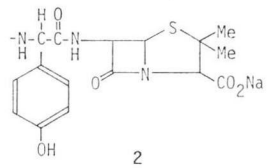
The major requirement of a suitable hydroxyl protecting group in the context described above, besides the ability to protect selectively the hydroxyl in the presence of the carboxyl group, is that the conditions for its removal must not lead to decomposition of the highly sensitive β-lactam ring system. Groups that are removed by catalytic hydrogenolysis may fulfil this requirement but are inconvenient to use on β-lactams containing divalent sulfur, since large quantities of the catalyst have to be used and low yields are often obtained. Protecting groups removed by mild alkaline (*e.g.*, formate ester[6]) or acid treatment (*e.g.*, tetrahydropyranyl ether,[7] substituted triarylmethyl ethers[8]), while applicable to cephalosporins, in which the β-lactam ring possesses a higher degree of stability, may not be tolerated by other more labile β-lactam compounds such as penicillins and thienamycin.[9] The trifluoroacetate ester,[10] while likely to be compatible with the more sensitive β-lactams, was found to be unsuitable

* Compound **4a** was prepared according to the methods in Reference 3 by Dr. E. D. NICOLAIDES.

Fig. 1. Structures of some β-lactam antibiotics (**4a**~**d**), showing the common side chain feature (*cf.* **1**) and the β-lactam moieties (**2**, **3a**, or **3b**).

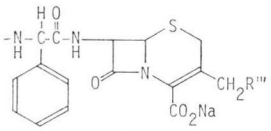


Compound	R'	R''
1	Acyl (various types)	-OH
4	*N*-Acetylaminoacyl	2,3a or 3b
4a	$HOCH_2$-C(AcNH)-C(=O)-	2
4b		2
4c		3a
4d		3b



2

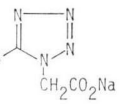
(H-2 = Amoxicillin sodium)



3

3a R''' = -OCMe

(H-3a = Cephaloglycin sodium)

3b R''' = 

because of excessive lability towards other nucleophiles present in the reactions. Thus attempts to obtain an imidazolide from the *O*-trifluoroacetate ester **5d** (**5a**, HO- = CF_3COO-), pure enough to be useful, invariably failed, undoubtedly because of side-reactions initiated by the deprotective attack of imidazole molecules generated in the reaction. Similarly, attempted coupling of **5d** with an amine *via* the mixed anhydride route also led to a mixture of products. Hence, in spite of the numerous hydroxyl protecting groups available to the organic chemists,[11] it is still necessary to find one which is generally suitable for β-lactam synthesis, especially one that is inexpensive and easy to introduce or remove with selectivity. Such a group could, undoubtedly, also be useful in peptide synthesis.

The 2-methoxypropan-2-yl (1-methoxy-1-methylethyl) protecting group, first devised by REESE *et al.*,[12] for use in nucleoside synthesis, where, however, it was found too labile to be useful,[11] appeared to fulfil the above-mentioned criteria for hydroxyl protection and was indeed found ideally suitable for our purpose. Thus, **5a** was converted to **5b** by treatment with 2-methoxypropene[13] (**6**) in *N*,*N*-dimethylacetamide (DMA), the carboxylic hydroxyl of **5a** serving as the acid catalyst. The protected compound **5b** could be isolated in crystalline form but was somewhat unstable when subjected to high vacuum, slowly losing the protecting group. Hence, **5b** was converted, without extensive prior drying, to the imidazolide **5c**, in a solvent mixture containing just enough DMA to allow all reactants to dissolve and the product to crystallize out subsequently. The imidazolide **5c** was coupled with amoxicillin in DMA to give the protected penicillin product, which was then deprotected by gradual acidification in cold aqueous solution. Partial deprotection was observed already at a pH of 6, and further deprotection occurred as the pH was lowered to 2 to give the insoluble free acid form of the desired product, which was isolated and converted to the sodium salt **4b**.

The product **4b** thus obtained generally contained only small amounts (*e.g.*, 1.4%) of a decomposition product, presumably the penicilloate (**7**) arising from β-lactam cleavage, as determined by HPLC using as reference a sample of **4b** previously treated with β-lactamase. The iodometric β-

Scheme 1.

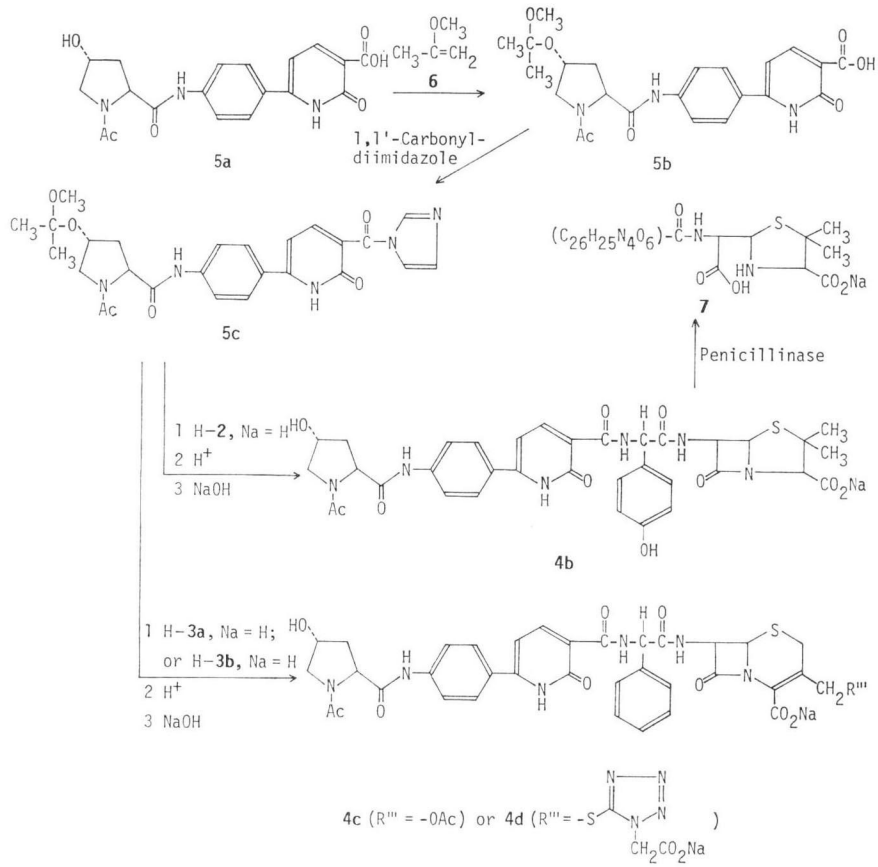


Table 1. Comparative *in vitro* activity.

Test organisms	MIC (μg/ml)*				
	4b	Piperacillin	**4c**	**4d**	Cefoperazone
Pseudomonas aeruginosa #28	1.6	1.6	12.5	6.3	3.1
Pseudomonas aeruginosa BRK 12-4-4	3.1	3.1	12.5	12.5	3.1
Pseudomonas aeruginosa UI-18	0.8	1.6	12.5	6.3	3.1
Escherichia coli Brig.	3.1	1.6	12.5	3.1	0.1
Escherichia coli Vogel	0.8	0.8	3.1	0.8	0.4
Proteus vulgaris	1.6	0.4	25.0	1.6	0.4
Enterobacter cloacae	3.1	0.8	12.5	3.1	0.2
Serratia marcescens	6.3	0.8	>50	12.5	0.4
Klebsiella pneumoniae	12.5	3.1	6.3	1.6	0.1
Streptococcus faecalis	1.6	1.6	25	>50	25
Staphylococcus aureus UC-76	0.8	0.8	0.8	6.3	1.6
Staphylococcus aureus S-18713**	>50	>50	3.1	25.0	3.1

* Microtitration broth dilution in TSB. Final inoculum approximately 10^4 CFU/ml for Gram-negative bacteria and 10^6 CFU for *S. faecalis* and *Staph. aureus*.

** β-Lactamase producing, multi-resistant isolate.

Table 2. Comparative *in vivo* data.

Compound	PD_{50} Values in mg/kg in mice*				LD_{50}** mg/kg
	Pseudomonas aeruginosa		*Entero. cloacae*	*Klebsiella pneumoniae*	
	(UI-18)	(BRK 12-4-4)	(IMM-11)	(MGH-2)	
4b	20	16	16	64	>2000
Piperacillin	150	100	20	38	>2000
4c	62	74	54	42	>2000
4d	50	50	10	7	>2000
Cefoperazone	94	54	0.3	3	

* Total of two doses administered s.c.; 0 and 2 hours post infection.
** Single dose given i.v.

lactam assay[14] generally gave a higher value (*e.g.*, 7.1%, as "blank") undoubtedly as a result of interfering reactions. The ring-opened product was also recognized qualitatively by the upfield proton NMR shift shown by one of the *C*-methyls in the penicillin nucleus.

In like manner, the protected imidazolide **5c** was successfully coupled with cephaloglycin (H-**3a**, Na=H) and its carboxymethylthiotetrazole analog (H-**3b**, Na=H) to give the corresponding cephalosporins **4c** and **4d**.

Biological Activities

The comparative biological activities of **4b** to **4d**, together with those of piperacillin* and cefoperazone,* are shown in Tables 1 and 2. Compound **4b** shows particularly good *in vivo* activities against strains of *P. aeruginosa*.

Experimental

Thin-layer chromatography (TLC) was performed with EM Silica Gel 60 F-254, 0.25 mm, on 10 cm-long glass plate (EM Laboratories), fluorescence-quenching at 253.7 nm, and solvent systems of chloroform - methanol - triethylamine (CMT) at vatious proportions as indicated.

Liquid chromatography (LC) was performed with commercial prepacked columns using the following systems: 1) C_{18} silylated, EM Hibar, LiChrosorb RP-18, 10 μm, C_{18} hydrocarbon phase chemically bonded to silica gel, 25 cm×4.6 mm ID, 0.005 M PIC-A (tetrabutylammonium phosphate)[15] in acetonitrile - water (3: 7), EM Laboratories; 2) μPorisil, deactivated, 10 μm, silica, 30 cm×3.9 mm ID, acetonitrile - tetrahydrofuran - water (50: 35: 15) buffered with sodium acetate (0.1%) and sodium bicarbonate (0.01%), Waters Associates; 3) SAX, Partisil-10 SAX, strong anion exchanger, quarternary nitrogen, 25 cm×4.6 mm ID, 0.05 M pH 7 phosphate buffer - methanol (90: 10). Assays were based on integrated UV absorption at 254 nm.

All filtration was done with suction through Whatman Glass Microfiber Filters GF/A unless otherwise indicated.

2-Methoxypropene (**6**)

2-Methoxypropene, now commercially available, was prepared according to NEWMAN and ZWAN,[13] stored at −30°C, and freshly distilled through a 8 cm Vigreux column before use. According to NMR, the reagent showed only a slight increase in impurities after 50 days at −30°C but was appreciably more unstable at 0°C.

6-[4-[*N*-Acetyl-4-(1-methoxy-1-methylethoxy)-L-prolylamino]phenyl]-1,2-dihydro-2-oxonicotinic acid (**5b**)

* *Cf.* abstracts of papers presented at the 19th Intersci. Congr. Antimicr. Agents & Chemoth., Oct. 1~5, 1979

A mixture of 17.5 g (45.4 mmole) of **5a**, derived originally from 4-hydroxy-L-proline which showed $[\alpha]_D^{23}$ −75.35° (*c* 20, water),[3] and 56 ml of DMA was stirred with 25 ml (266 mmole) of 2-methoxypropene (**6**) at 23~27°C for 26 hours. TLC (2 μl triethylamine, 0.5 μl reaction mixture, 2 μl triethylamine applied successively to plate, dried in high vacuum, then developed in CMT, 72: 18: 10) showed one spot, Rf *ca.* 0.5. Acetonitrile (110 ml) was then added rapidly with stirring so that a clear solution resulted momentarily before crystallization started. After 1.5 hours the crystalline product (**5b**) was filtered and washed on the filter with 45 ml of acetonitrile, followed by 35 ml of ether. The compound was generally used directly, without complete removal of ether, for subsequent reaction with 1,1′-carbonyldiimidazole to give **5c**.

Compound **5b** decomposed partially to the strating acid **5a** upon drying in high vacuum overnight; it also slowly decomposed upon standing as a solution in dimethyl sulfoxide-d_6 (extensive decomposition after 4 days according to NMR).

A 252-mg sample of **5b** which had partially lost the protecting group was reprotected by a new treatment with 0.575 ml of DMA and 0.22 ml of 2-methoxypropene (**6**) according to the procedure above. The filtered product (**5b**), after drying for 10 minutes at 16 mmHg, showed a high degree of purity by TLC; mp 280~286°C (dec.); ^{1}H NMR (DMSO-d_6) δ 1.30 (s, C-CH_3), 3.12 (s, O-CH_3).

Anal. Calcd. for $C_{23}H_{27}N_3O_7$ (mol. wt 457.47): C, 60.38; H, 5.95; N, 9.19.
Found: C, 59.80; H, 5.91; N, 9.09.

6-[4-[*N*-Acetyl-4-(1-methoxy-1-methylethoxy)-L-prolylamino]phenyl]-1,2-dihydro-2-oxonicotinic acid imidazolide (**5c**)

Compound **5b**, prepared from 8.783 g (22.79 mmole) of **5a**, was added as an ether-moist filter cake to a solution of 8.094 g (49.9 mmole) of 1,1′-carbonyldiimidazole in 125 ml of acetonitrile containing 1.0 ml of DMA. The mixture was stirred vigorously in an oil bath at 52°C; any lumps were broken up mechanically. All of **5b** dissolved after 30 minutes, and heating was continued for another 5 minutes. The warm solution was filtered and the residue was washed with 2×3 ml of acetonitrile. Crystallization occurred in the combined filtrates after about 40 minutes. After standing for about 2.5 days at 3°C, the crystals were removed by filtration, washed successively with 10 ml of cold acetonitrile, 25 ml of acetonitrile - ether (1: 4), 20 ml of ether, and dried at 16 mmHg for 15 minutes and finally at 1 atm to give 7.11 g of **5c** (61.5% overall yield from **5a**), which by TLC was of high purity (*cf.* below). ^{1}H NMR (DMSO-d_6) δ 1.28 (s, C-CH_3), δ 3.10 (s, O-CH_3).

A 97-mg sample of the imidazolide **5c** was warmed and stirred with 0.28 g of DMA at 50°C. The solid, recovered by centrifugation, was washed with 0.7 ml of acetonitrile, filtered, and washed with acetonitrile and then ether to give an analytical sample, mp 67°C.

Anal. Calcd. for $C_{26}H_{29}N_5O_6$ (507.53): C, 61.53; H, 5.76; N, 13.80.
Found: C, 60.97; H, 5.33; N, 13.98.

Compound **5c**, 4.8 mg, in 0.09 ml of DMA, was converted into the *n*-propylamide by treatment with 0.01 ml of *n*-propylamine for 15 minutes. TLC of the reaction mixture, in CMT (43: 2: 5) showed one strong spot, Rf *ca.* 0.5, with minute traces of impurities.

N-[6-[4-(*N*-Acetyl-4-hydroxy-L-prolylamino)phenyl-1,2-dihydro-2-oxonicotinyl]-6-[D-2-amino-2-(4-hydroxyphenyl)acetamido]penicillanic acid, monosodium salt (**4b**)

A mixture of 6.11 g (12.04 mmole) of **5c** and 38 ml of DMA was stirred for 8 minutes, when most of the solid dissolved, then cooled to 0°C. Amoxicillin-DMSO complex (free acid form),[16] 9.84 g (14.62 mmole) was added during about 8 minutes. The stirred reaction became clear after 15 minutes, and TLC indicated that almost all of the imidazolide had disappeared after 4 hours at 0°C (2 μl of *n*-propylamine, 1/3 μl of reaction mixture, and 2 μl of *n*-propylamine once applied successively to the same spot on the TLC plate, which was then developed in CMT (43: 2: 5). After a total of 5 hours at 0°C, the reaction mixture was added to 200 ml of ice-cold water, adjusted to pH 6.8 by dropwise addition of 0.5 ml of 1 N acetic acid, and washed at 3°C or below with 250, 125, and 110 ml of cold ethyl acetate. The aqueous phase was then acidified gradually with stirring, at 0°C, to pH 2.6 by dropwise addition of 95 ml of 0.2 N hydrochloric acid during 1.5 hours. The precipitated product (**4b**, free acid form) was removed by filtration at 2°C and washed with 30 ml of cold 0.001 N hydrochloric

acid, followed by 180 ml of cold water in portions.

The filter cake was stirred in 60 ml of water (0°C), and 69 ml of ice-cold 0.15 N sodium hydroxide was added dropwise with stirring, raising the pH from 3.8 to 6.0. After filtration, the clear solution was freeze-dried to give 8.22 g of **4b**. TLC, Rf 0.30 in CMT (60: 30: 10), with small amounts of high-Rf impurities and little, if any, penicilloate (**7**, Rf<0.1). Karl Fisher water analysis 5.4%; β-lactam assay (corrected for 5.4% water) 93.4%; blank, 7.1%. LC: μPorisil, 92.6% (10.38 minutes); C_{18}, PIC-A, 91.4% (5.03 minutes), 1.34% (7.74 minutes, the penicilloate **7**). In the ^{1}H NMR spectrum the *O*-methyl singlet belonging to the 2-methoxypropen-2-yl group was absent, showing that the latter had been removed. IR (KBr) 1770 cm^{-1}. UV (pH 7 phosphate buffer) λ_{max} 358 nm ($E^{1\%}_{1cm}$ 384), 270 (146). $[\alpha]_D^{23}$ −460° (*c* 0.95, pH 7 phosphate buffer).

Anal. Calcd. for $C_{31}H_{35}N_6O_{10}SNa \cdot 3H_2O$ (808.8): C, 51.97; H, 5.11; N, 10.39; S, 3.97.
Found: C, 51.70; H, 4.79; N, 10.32; S, 3.64.

A solution of 99 mg of **4b** (0.126 mmole) in 3.2 ml of deuterium oxide was treated with 40 μl of Bacto penase concentrate, penicillinase, 100,000 units/ml (Difco). After 2.5 hours at room temperature, TLC (CMT, 60: 30: 10) showed that very little β-lactam cleavage had occurred. More penicillinase was added (total of 1825 units/mmole of **4b**), and after a total reaction time of 7 hours at room temperature the reaction mixture was freeze-dried to give 101 mg of a mixture containing **4b** and the penicilloate **7**. LC: C_{18}, PIC-A, 19.5% of **4b** (4.8 minutes) and 73.4% of **7** (7.57 minutes). IR (KBr), small β-lactam peak at 1770 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 1.14 (s, one C-3-CH_3 of **7**), with greatly diminished intensities of peaks characteristic of **4b**, namely, C-3-CH_3 (δ 1.40, s), H-7 (δ 5.23, d), H-6 (δ 5.38, dd).

N-[6-[4-(*N*-Acetyl-4-hydroxy-L-prolylamino) phenyl]-1,2-dihydro-2-oxonicotinyl]-7-[D-2-amino-2-phenylacetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid, monosodium salt (**4c**)

To a stirred mixture of 2.49 g (4.90 mmole) of **5c**, 15 ml of DMA, and 0.65 ml of 2-methoxypropene (**6**) at 0°C was added 0.8 ml (5.74 mmole) of triethylamine, followed by 2.38 g (5.81 mmole) of cephaloglycin (**H-3a**, Na=H), and 0.75 ml of dimethyl sulfoxide. After stirring for 11 hours at 0°C and standing 9 hours at −30°C, the cold reaction mixture (absence of **5c** by TLC) was filtered during 40 minutes into 300 ml of ethyl acetate stirred at 0°C, followed by washing of the solid on the filter with 0.5 ml of DMA. Cold ether, 200 ml, was added with stirring to the ethyl acetate mixture, which was then filtered. The filter cake was washed with 50 ml of ethyl acetate - ether (1: 1), followed by 50 ml of ether, dried and dissolved in 75 ml of cold water. Gradual addition during 75 minutes of 0.3 N hydrochloric acid to pH 2.5 while stirring at 0°C gave a copious precipitate which was centrifuged off at 9000 rpm at 0°C for 15 minutes, washed with 50 ml of cold water and again centrifuged. The light-orange solid was triturated with 25 ml of cold water, filtered, suspended and stirred in 25 ml of cold water while the pH was adjusted from 3.65 to 6.7 by dropwise addition of 0.25 N sodium hydroxide. The solution, after clarification by filtration, was freeze-dried to give 3.42 g of **4c**. LC, μPorisil, 85.8% (9.33 minutes). ^{1}H NMR indicated the absence of O-CH_3 and hence the removal of the protecting group. UV (pH 7 phosphate buffer) λ_{max} 358 nm ($E^{1\%}_{1cm}$ 364), 262 (222). $[\alpha]_D^{23}$ −276° (*c* 0.53, pH 7 phosphate buffer). IR (KBr) 1770 cm^{-1}.

Anal. Calcd. for $C_{17}H_{35}N_6O_{11}SNa \cdot 5H_2O$ (888.84): C, 50.22; H, 5.12; N, 9.50; S, 3.62.
Found: C, 50.01; H, 5.06; N, 9.69; S, 4.12.

N-[6-[4-(*N*-Acetyl-4-hydroxy-L-prolylamino) phenyl]-1,2-dihydro-2-oxonicotinyl]-7-[D-2-amino-2-phenylacetamido]-3-[[[1-(carboxymethyl)-1-*H*-tetrazol-5-yl]thio]methyl]-3-cephem-4-carboxylic acid, disodium salt (**4d**)

The trifluoroacetate salt of H-**3b**, (Na=H 87.1% pure by LC, SAX),[17] 4.018 g (5.67 mmole), was stirred with 20 ml of cold DMA. Triethylamine (2.2 ml, 15.8 mmole) was added during 5 minutes, followed by 2.80 g (5.52 mmole) of **5c**. After 4 hours of stirring at 0°C, 0.4 ml (2.85 mmole) of triethylamine was added, and the reaction mixture was added to 320 ml of cold ethyl acetate with stirring. After stirring for 20 minutes the mixture was filtered and the solid washed with 80 ml of cold ethyl acetate in portions, then with 50 ml of cold ether. A solution of the solid in 80 ml of cold water, pH 7.6, was adjusted to pH 2.4 with 50 ml of 0.2 N hydrochloric acid by dropwise addition during 80

minutes, to give a copious precipitate which was centrifuged at 9000 rpm at 0°C, washed with 55 ml of 0.0032 N hydrochloric acid, and then two times with 80-ml portions of cold water. The solid suspended in 40 ml of cold water was stirred, while the pH was adjusted from 3.4 to 6.0 by drowise addition of 47.5 ml of 0.15 N sodium hydroxide. The solution obtained was freeze-dried to give 4.19 g of **4d**. LC: C_{18}, PIC-A, 85% (29.8 minutes); μPorisil, 78% (15 minutes). IR (KBr) 1767 cm^{-1}. ^1H NMR indicated the absence of *O*-methyl singlet and hence the removal of the protecting group.

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