A NEW AMINOGLYCOSIDE ANTIBIOTIC COMPLEX—THE SELDOMYCINS

IV. THE STRUCTURE OF SELDOMYCIN FACTOR 5++

JAMES B. MCALPINE, ARTHUR C. SINCLAIR, RICHARD S. EGAN, R. LARRY DE VAULT, RUTH S. STANASZEK, MOMIR CIROVIC, SANDRA L. MUELLER, PAUL C. GOODLEY, ROBERT J. MAURITZ and NORMAN E. WIDEBURG

Division of Antibiotics and Natural Products, Abbott Laboratories
North Chicago, Illinois 60064, U.S.A.

LESTER A. MITSCHER* and KUNIKATSU SHIRAHATA+

College of Pharmacy, The Ohio State University,
Columbus, Ohio 43210, U.S.A.

HIDEO MATSUSHIMA, SEIJI SATO and TAKAO IIDA

Tokyo Research Laboratory, Kyowa Hakko Kogyo Company, Ltd.
3-6-6 Asahimachi, Machidashi, Tokyo, Japan

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Seldomycin factor 5 is shown to be 4-O-[2,6-diamino-2,4,6-trideoxy-α-D-*xylo*-hexopyranosyl]-6-O-[2,3-diamino-2,3-dideoxy-4-O-methyl-α-D-*xylo*-pyranosyl]-2-deoxystreptamine.

Seldomycin factor 5 is the most active antibiotic isolated from the fermentation broth of *Streptomyces hofunensis*. The taxonomy and fermentation of this organism have been described.[1] The isolation procedure and the physical properties[2] of seldomycin factor 5 characterize this antibiotic as a water-soluble basic compound of the aminoglycoside class. The antibacterial spectrum of seldomycin factor 5 indicates that this compound is probably a close relative of the kanamycin-gentamicin group. Moreover, the activity or lack of activity of seldomycin factor 5 against a number of isolates having known mechanisms of resistance, involving in each case the modification of a functional group, was strongly suggestive of the absence or presence of these groups in the structure of seldomycin factor 5.[2] Spectrometric analysis of seldomycin factor 5 and a number of degradation products has allowed for the definition of the structure and stereochemistry of this antibiotic.

Results and Discussion

The mass spectrum of the free base of seldomycin factor 5 (Table 1) shows an M+1 peak at 451 amu in agreement with the molecular formula $C_{18}H_{38}N_6O_7$.[2] The degradation pattern is indicative of a diglycoside of 2-deoxystreptamine in which each of the three basic carbohydrate units has the same molecular formula, namely $C_6H_{14}N_2O_3$. The per-N-acetyl-per-O-trimethylsilyl derivative[3] of seldomycin factor 5 was prepared and the mass spectrum obtained from this derivative (Table 2, Fig. 1) indicates the presence of six primary or secondary amino groups in the parent molecule, two in each of the subunits. Also, each of two adjacent subunits of the parent bears an hydroxyl group.

++ Also known as XK-88-5

* Present address: Department of Medicinal Chemistry, University of Kansas, Lawrence, Kansas 66044, U.S.A.

+ On leave from the Kyowa Hakko Kogyo Company

The parameters from the CMR spectrum of seldomycin factor 5 as the free base are given in Table 3. It shows the expected 18 lines in agreement with the mass spectral and microanalytical conclusions. Of particular use in the assignment of lines and in structural interpretation of aminoglycosides has been the changes of line positions in the CMR spectra that occur on protonation of these compounds.[4,5,6,7]

Fig. 1. Mass spectral degradations of per-N-acetyl-per-O-trimethylsilylseldomycin factor 5

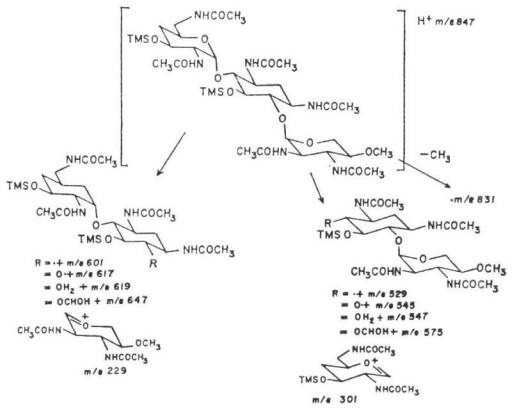


Fig. 2. Change in chemical shift (CMR spectra) with deuterium ion concentrations
Part A Seldomycin factor 5
Part B Seldomycin factor 2

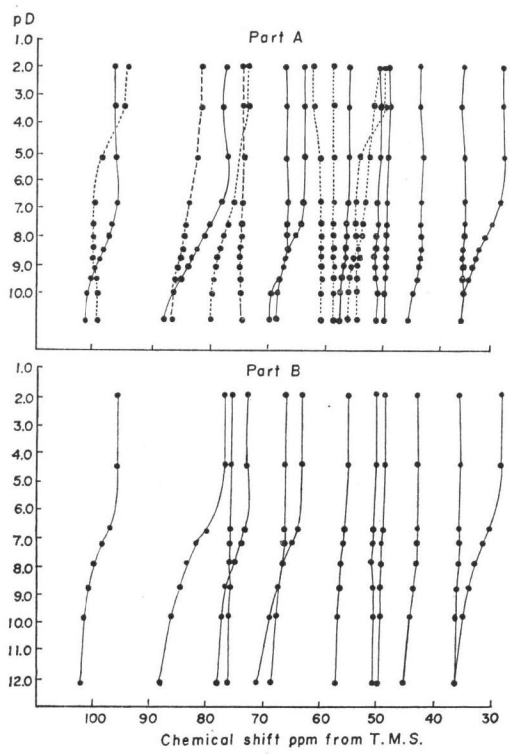


Table 1. Mass spectrum of seldomycin factor 5 (free base)

m/e	Intensity (%)	*m/e*	Intensity (%)
451	0.3	204	4
433	0.3	187	5
376	1	186	8
347	5	163	17
335	6	145	100
317	5	126	9
307	11	110	12
289	29	98	17
215	4	86	61

Table 2. Mass spectrum of per-N-acetyl-per-O-trimethyl-silylseldomycin factor 5 (*m/e* >200)

m/e	Intensity (%)	*m/e*	Intensity (%)
847	<1	512	8
831	3	455	7
647	17	410	5
619	8	347	11
617	<1	319	13
601	5	301	96
575	12	285	16
547	11	229	100
545	1	201	50
529	8		

Fig. 2 shows the change of the CMR spectral line positions with deuterium ion concentration for seldomycin factors 5 and 2.[8] The 10 solid lines in part (*A*) of Fig. 2 are virtually superimposable on lines occcurring in part (*B*). The two broken lines in part (*A*) can be assigned to carbon 5 and carbon 6 of 2-deoxystreptamine showing the expected small upfield and large downfield shifts respectively from their position in part (*B*), which results from attachment of another sugar to the C6 hydroxyl group of 2-deoxystreptamine. The dotted lines in Fig. 2, part (*A*), are then assigned to the signals of this third sugar. Four of these lines show β-shifts, one of which at 100.3 ppm* is the anomeric carbon of this sugar and another of which at 80.2 ppm must be assigned to a carbon bearing oxygen. The remaining two lines at 56.2 and 54.8 ppm showing apparent β-shifts must be assigned to carbons bearing amino groups. This unusual situation can only arise if the two amino groups are attached to the adjacent carbons C2 and C3 of the sugar.

Table 3. The ^{13}CMR spectrum of seldomycin factor 5

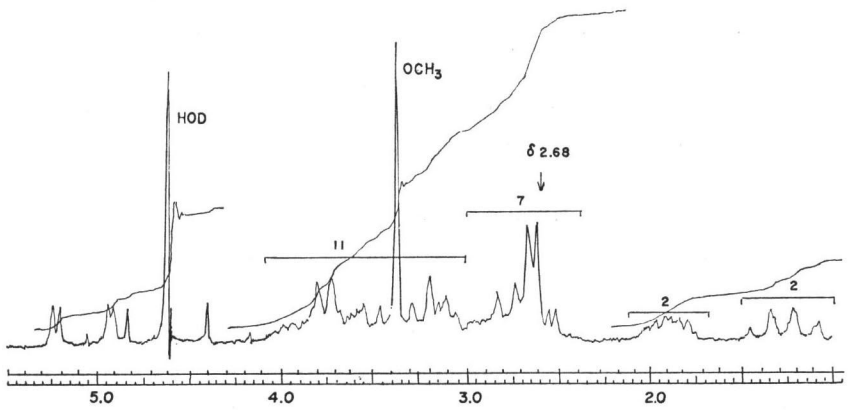
C_1	51.1	C_1'	102.3	C_1''	100.3
C_2	36.5	C_2'	57.7	C_2''	56.2
C_3	50.1	C_3'	69.1	C_3''	54.8
C_4	88.1	C_4'	36.9	C_4''	80.2
C_5	75.1	C_5'	71.3	C_5''	60.8
C_6	87.0	C_6'	45.7	OCH_3	58.7

The proton magnetic resonance spectrum of seldomycin factor 5 in D_2O is presented in Fig. 3. The sharp singlet at 3.55 ppm is assigned to the protons of a methoxyl group. The two anomeric proton doublets at 5.11 ppm and 5.45 ppm were collapsed by irradiation at 2.68 ppm, confirming that both of these sugars were 2-amino-2-deoxy sugars.

The spectrometric data presented suggest structural features of seldomycin factor 5 which are summarized as follows: 4′-Deoxyneamine (seldomycin factor 2) is in glycosidic linkage at the C6 hydroxyl group with a 2,3-diamino-2,3-dideoxy-O-methylpentose. The ring size of this sugar is undetermined and the only stereochemical inferences concerning it to be drawn from this data is that the small J_{H1H2} coupling requires that the substituents at C1 and C2 in this sugar cannot both be in equatorial orientation.

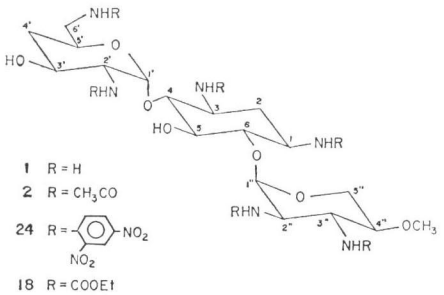
Further definition of this structure and information on the streochemistry was sought from the chemical degradations of seldomycin factor 5. Brief treatment of seldomycin factor 5 with sodium metaperiodate followed by quenching the reaction with ethylene glycol and subsequent treatment with

Fig 3. Proton magnetic resonance spectrum of seldomycin factor 5 (free base)



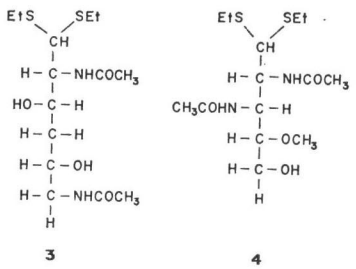
* δ values will be those of the free base.

Chart 1.



1 R = H
2 R = CH_3CO
24 R = $2,4-(NO_2)_2C_6H_3$
18 R = COOEt

Chart 2.



3 4

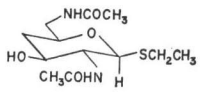


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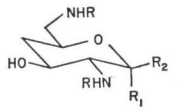
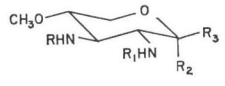
Chart 3.

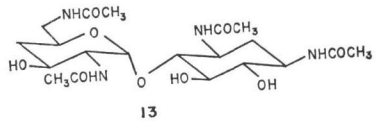




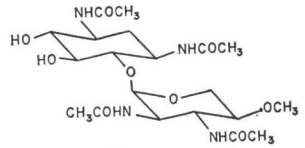
6 R = R_2 = H, R_1 = OCH_3
11 R = CH_3CO, R_1 = OCH_3, R_2 = H
12 R = CH_3CO, R_1 = H, R_2 = OCH_3
21 R = COOEt, R_1 = OCH_3, R_2 = H
22 R = COOEt, R_1 = H, R_2 = OCH_3

7 R = R_1 = R_3 = H, R_2 = OCH_3
8 R = R_1 = R_2 = H, R_3 = OCH_3
9 R = R_1 = CH_3CO, R_3 = H, R_2 = OCH_3
10 R = R_1 = CH_3CO, R_3 = OCH_3, R_2 = H
16 R = R_3 = H, R_1 = CH_3CO, R_2 = OCH_3
17 R_1 = R_3 = H, R = CH_3CO, R_2 = OCH_3
19 R = R_1 = COOEt, R_3 = H, R_2 = OCH_3
20 R = R_1 = COOEt, R_3 = OCH_3, R_2 = H

Chart 4.

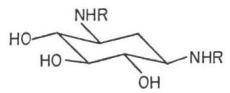


13



14

Chart 5.



15 R = CH_3CO
23 R = COOEt

mineral acid gave, as the only detected product, 2-deoxystreptamine. Quantitative determination of the periodate uptake indicated that seldomycin factor 5 slowly consumed three moles of periodate per mole of antibiotic. Compounds containing a -$CH(NH_2)$-$CH(OCH_3)$- grouping are known to be oxidized by periodate ion in a "non-Malapradian" manner,[9] and confident interpretation of this data is difficult but it may be considered as supportive of a pyranose formulation of the pentose.

The sequential fragmentation of dithiodiacetals of 2-acetamido sugars during mass spectral determination has been particularly useful in ascertaining the positions of substituents.[10,11] Mercaptolysis of seldomycin factor 5 per-N-acetate **2** with ethanethiol and concentrated hydrochloric acid gave, after chromatographic separation, the two dithiodiacetals **3** and **4** and the thiolglycoside **5**. The structures of these compounds were assigned on the basis of their proton magnetic resonance and mass spectra (see experimental section). The mass spectrum of **3** confirms the known structure of the hexose[12] and that of **4** establishes the pentose as a 2,3-diamino-2,3-dideoxy-4-O-methylpentose.

Prolonged methanolysis of seldomycin factor 5 per-N-acetate **2** yielded after chromatography methyl 2,6-diamino-2,4,6-trideoxy-α-*xylo*-hexapyranoside **6** and a mixture of the α and β-methyl pentosides **7** and **8** (named methyl α- and β-seldosides, respectively). Numerous attempts to separate **7** and **8** by chromatography were unsuccessful.

Methanolysis of seldomycin factor 5 per-N-acetate for a short time followed by N-acetylation of the reaction mixture gave after chromatographic separation each of the methylpentoside N,N'-diacetates

9 and **10**, each of the methylhexoside N,N′-diacetates **11** and **12**, each of the two possible pseudo-disaccharide-per-N-acetates **13** and **14**, a small quantity of recovered seldomycin factor 5-per-N-acetate **2** and 2-deoxystreptamine-N,N′-diacetate **15**.

Per-N-acetylation of seldomycin factor 2 gave a compound identical in all respects with **13** obtained from seldomycin factor 5, thus confirming the conclusions drawn above from the spectral data.

The near coincidence of the chemical shifts of all the ring protons of **9** and **10** precluded any analysis of the coupling constants in either of these compounds.

The N,N′-diacetate of the major anomer, **9**, proved to be remarkably stable to basic hydrolysis. Whereas **6** could be regenerated by treatment of **11** with barium hydroxide under reflux overnight, treatment of **9** under the same conditions for two weeks gave a 25% yield of recovered starting material, **9**, together with each of the possible mono-N-acetates **16** and **17**. The proton magnetic resonance parameters for **16** and **17** are given in Table 4. A computer simulated spectrum using the parameters for the spectrum of methyl-α-seldoside-2-N-acetate **16** together with the actual spectrum is shown in Fig. 4. The only noticeable difference between the two spectra is that the lines assigned to H_2 and H_3 are of lower peak height in the actual spectrum than in the simulated spectrum. This is attributed to nitrogen quadrupole broadening. The large $H_{2,3}$ and $H_{3,4}$ coupling constants in the spectra of **16** and **17** require that the pentose have *xylo*-configuration.

In order to obtain the unprotected methyl seldosides in reasonable yield, it has been necessary to use a more base-labile N-protecting group. The per-N-carboethoxy derivative of seldomycin factor 5 **18** was prepared and methanolysed. Silica gel chromatography of the reaction product gave the α and β-methyl N, N′-dicarboethoxy pentosides **19** and **20**, the α and β-methyl N,N′-dicarboethoxy hexosides **21** and **22** and N,N′-dicarboethoxy-2-deoxystreptamine **23**. Removal of the carboethoxy groups from **19** was effected with alkaline hydrolysis to afford the unprotected methyl α-seldoside **7**, the PMR spectral parameters of which

Table 4. Proton magnetic resonance parameters of methyl α-seldoside-2-N-acetate (**16**) and methyl α-seldoside-3-N-acetate **17**†

	16*	17**		16	17
H_1	4.60	4.91	$J_{1,2}$	3.5	3.2
H_2	3.98	3.08	$J_{2,3}$	10.8	10.9
H_3	2.82	4.57	$J_{3,4}$	9.8	9.7
H_4	3.09	3.70	$J_{4,5a}$	10.0	10.0
H_{5a}	3.41	3.57	$J_{4,5e}$	5.0	5.0
H_{5e}	3.81	3.90	$J_{5a,5e}$	−11.0	−11.0
CH_3CO	2.05	2.14			
CH_3O	3.38, 3.47	3.32, 3.39			

† The trivial name seldose has been chosen for the unusual diamino pentose of seldomycin factor 5.
* Solvent $CDCl_3+D_2O$
** Solvent $C_5D_5N+D_2O$

Fig. 4. ^1H NMR spectra of methyl α-D-seldoside-2-N-acetate
Part A Measured spectrum
Part B Synthesized spectrum

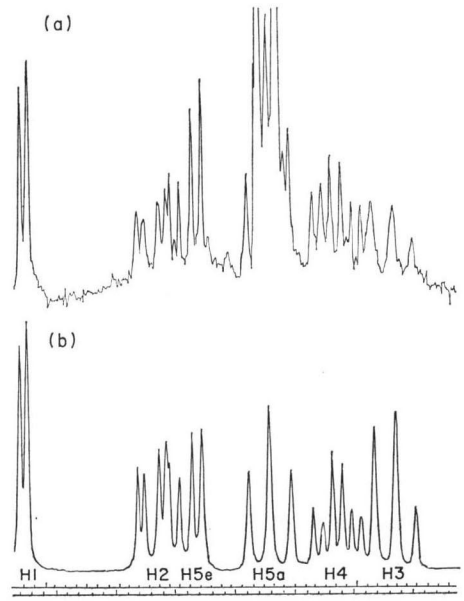


Fig. 5 Ultraviolet and circular dichroism spectra of seldomycin factor 5-per-N-2,4-dinitrophenyl derivative **24**

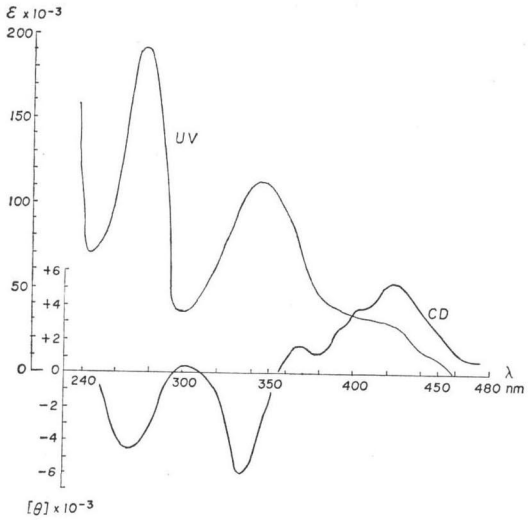
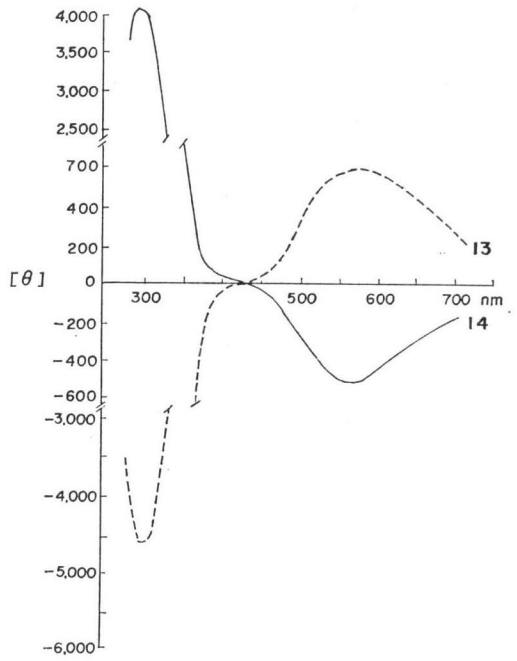


Fig. 6. CD spectra of seldomycin 5 derivatives in Cupra A solution containing 20% of EtOH



(see Experimental.) are further evidence for the *xylo*-configuration of the pentose.

A recent study[13] of the circular dichroism of the 2,4-dinitrophenyl derivatives of diamines establishes a rule for correlating the chirality of a diamino system with the COTTON effects in the CD spectrum of its 2,4-dinitrophenyl derivative. The per-N-2,4-dinitrophenyl derivative of seldomycin factor 5 **24** was prepared and the circular dichroism and ultraviolet spectra of this derivative is shown in Fig. 5. The +—pattern of the COTTON effect in the vicinity of 350 nm requires that the 1,2-diamino system have the *R*-chirality. Thus seldose is defined as having the D-*xylo*-configuration and seldomycin factor 5 determined to be 4-O-[2,6-diamino-2,4,6-trideoxy-α-D-*xylo*-hexopyranosyl]-6-O[2,3-diamino-2,3-dideoxy-4-O-methyl-α, D-*xylo*-pyranosyl]-2-deoxystreptamine, **1**.

Table 5. $\Delta\delta$ CMR from pH 9 to pH 2

	C-1′	C-4	C-1″	C-6
Seldomycin factor 1	3.2	6.4	4.7	4.3
Seldomycin factor 2	4.5	8.4	—	3.6
Seldomycin factor 5				
(to pH 6.4)	4.1	7.0	−0.2	2.9
(to pH 2)	4.1	7.9	5.2	4.8
Gentamicin B*	3.9	8.5	−0.9	4.2

* Reference 14

The circular dichroism curves of **13** and **14** in Cupra A solution containing 20% ethanol are shown in Fig. 6. The CD curve of **13** has a COTTON effect with a +—pattern in the vicinity of 450 nm requiring an *R*-chirality for the 5,6 diol system, whereas the curve of **14** requires an *S*-chirality for the 4,5 diol system. Thus the 4,6 attachment of the hexose and pentose, respectively, to 2-deoxystreptamine is confirmed.

An inspection of Fig. 2 reveals that the 3″ amino group (see Table 3) accepts a charge fairly early in the titration but in so doing repells a charge from the 2″ amine. The acceptance of a charge by this amine occurs very abruptly and in addition to the expected β shifts a small but distinct downfield shift

of the C5″ carbon signal and an upfield shift of the O-methyl carbon signal occur. This is interpreted as evidence that the pentose ring has inverted from the $^4C_1$ chair-form shown in structure **1** to the alternate $^1C_4$ chair-form in which the two charged ammonium groups are in the *trans*-diaxial conformation. In support of this interpretation it was found that proton magnetic resonance signal of H_1'' underwent significant changes in chemical shift only in the latter stages of the titration and at the same time underwent a change in coupling to H_2'' from 3.0 Hz to less than 1.5 Hz.

The optical rotation ($[\alpha]_D^{24}$ *c* 1.05 as free base, H_2O) of seldomycin factor 5 was found to be +133°, +142° and +113° at pH 10.0, 6.8 and 2.5 respectively.

NAGABHUSHAN and DANIELS have recently correlated $\Delta\delta$ CMR values for C_4, C_1' and C_6,C_1'' on acidification with the stereochemistry of the two sugar moieties.[14] Seldomycin factors 1, 2 and 5 have $\Delta\delta$ CMR values for C_4 and C_1' in good agreement with the NAGABHUSHAN and DANIELS rule (Table 5). It would appear that changes associated with acceptance of a charge by a 2″ amine may provide an exception to the rule. Seldomycin factor 1 does not fit the rule at C_1''. The $\Delta\delta$ CMR values for C_6 and C_1'' of seldomycin factor 5, calculated to pH 6.4, are in good agreement with the rule. As the rule only includes axially linked sugar, $\Delta\delta$ CMR values calculated at pD 1 for seldomycin factor 5 would be inapplicable.

Experimental

General Experimental

Mass spectra were obtained on an A.E.I. MS-902 spectrometer at 50 eV using the direct insertion probe. PMR spectra were measured on a Virian Associates HA-100 or a Varian Associates XL-100-15 spectrometer in the solvent described and are reported as downfield from internal TMS. Those measured in D_2O were determined with an external TMS reference and have been corrected (−0.42 ppm) to the commonly applied internal TSP scale. CMR spectra were measured on a Varian Associates XL-100-15 spectrometer in D_2O with dioxane as internal reference (67.4 ppm) or in DMSO with TMS as internal reference. Chemical shifts are reported in ppm downfield for TMS. The symbols s, d, t, q and m refer to singlet, doublet, triplet, quartet and multiplet respectively. Melting points were determined with a Thomas-Hoover Uni-Melt apparatus. Optical rotations were measured with a Hilger and Watts polarimeter. CD spectra were obtained at 29°C (cell compartment temperature) in a Durrum-Jasco ORD/4V/CD5 instrument operating under constant nitrogen flush. Tlc's were performed on Analtech plates precoated with silica gel GF at 250 micron thickness.

Treatment of Seldomycin Factor 5 with Sodium Metaperiodate

(1) Isolation of 2-deoxystreptamine: Seldomycin factor 5 sulfate (300 mg) in water (25 ml) was treated with sodium metaperiodate (500 mg) for 25 hours at room temperature. Ethylene glycol (0.2 ml) was added and after 50 minutes concentrated hydrochloric acid (3 ml) was added and the solution was allowed to stand at room temperature for 20 hours, then neutralized with sodium bicarbonate, concentrated, filtered and the filtrate was chromatographed on a column (1.5×48 cm) of Bio Rex® 70, 200~400 mesh, in the ammonium form eluted with a gradient of ammonium hydroxide. RYDON-SMITH positive fractions were combined and after concentration on a rotary evaporator yielded 2-deoxystrepramine 23.7 mg. Identical with an authentic sample by tlc, glc and pmr analyses.

(2) Quantitative uptake procedure: Seldomycin factor 5 (base) (20.0 mg) was treated with 0.015 N sodium metaperiodate (25 ml) in acetate buffer (pH 4.0) at 5°C in the dark. Aliquots (1 ml) were sampled at intervals and diluted 200-fold with water. The optical density was measured at 223 nm with a Hitachi-Perkin Elmer Model 139 spectrophotometer. Seldomycin factor 5 consumed 2 moles of periodate per mole during 6 hours. An additional 1 mole of periodate was consumed in the succeeding 37 hours, after which time, uptake ceased.

Seldomycin Factor 5-per-N-acetate

Seldomycin factor 5 free base (155 mg) in methanol (10 ml) was treated with acetic anhydride (1 ml), allowed to stand at room temperature for one hour and concentrated. In a number of experiments a heterogeneous crude product was obtained. A homogeneous product was obtained by passing this crude product in water through Dowex® AG 1×2 resin—concentrating and reacetylating as above. Seldomycin factor 5, per-N-acetate had $[\alpha]_D^{24}+100°$ (*c* 1.05, H_2O).

CMR Spectrum (D_2O)

C_1	50.1	C_1'	98.7	C_1''	96.8
C_2	34.0	C_2'	55.9	C_2''	52.6
C_3	48.9	C_3'	66.1	C_3''	51.8
C_4	79.7	C_4'	36.6	C_4''	77.4
C_5	76.1	C_5'	67.9	C_5''	60.9
C_6	79.4	C_6'	43.5	OCH_3	58.7
CH_3	22.8				
CO	175.3, 175.2, 174.6, 173.9, 173.8				

Anal. Calcd. for $C_{30}H_{50}N_6O_{13}\cdot3/2\ H_2O$: C, 49.37; H, 7.32, N, 11.51
Found: C, 49.16; H, 7.33; N, 11.21

Mercaptolysis of Seldomycin Factor 5 per-N-acetate

Seldomycin factor 5 per-N-acetate (157 mg) in ethanethiol (1 ml) was treated with concentrated hydrochloric acid (1 ml) and allowed to stand at room temperature for 22 hours. The mixture was concentrated on a rotary evaporator, and the residue was digested in water and passed through a short column of Dowex AG 1×2 (OH^- form). The eluate was concentrated and the residue was digested in methanol (5 ml) and treated with acetic anhydride (0.5 ml). The mixture was allowed to stand for 30 minutes at room temperature and concentrated. Ethanol and benzene were added to the residue and removed on a rotary evaporator several times. The oily residue was triturated with chloroform, the insoluble fraction (65 mg) co-chromatographed with an Rf identical with that of N,N'-diacetyl-2-deoxystreptamine (Rf 0.74 solvent system, H_2O). The chloroform-soluble fraction (116 mg) was chromatographed on a size A, E. M. Merck, prepacked silica gel 60 column eluted with a gradient from chloroform (300 ml, mixing chamber) and chloroform : methanol (4/1, v/v 300 ml, reservoir).

Fractions were collected, combined as follows, and concentrated to yield:

Fractions 22~27: 20.7 mg, 2,3-diacetamido-2,3-dideoxy-4-O-methyl-D-xylose-diethyldithioacetal 4 M^+ *m/e* 352.1489. $C_{16}H_{28}N_2O_4S_2$ requires *m/e* 352.1489.

PMR spectrum (D_2O): 1.29, t and 1.30 t, CH_3CH_2 (total 6H); 2.06, s, and 2.09 s CH_3CO (total 6H); 2.75 and 2.77 CH_3-CH_2 (total 4H); 3.51 s OCH_3 superimposed on 3.44~3.72 m C4H and $C5H_2$ (total 6H), 4.07 d C1H (1H); 4.46 t C2H (1H), 4.66; d of d's C3H (1H).

Mass spectrum: Significant peaks at *m/e*: 352, 323, 293, 232, 218, 217, 199, 170, 167, 158, 146, 138, 135, 128, 126, 101, 100, 86, 75.0450 ($C_3H_7O_2$) and 75.0270 (C_3H_7S).

Fractions 38~41: 37.5 mg, 2,6-diacetamido-2,4,6-trideoxy-D-*xylo*-hexopyranose-diethyldithioacetal 3 M^+ *m/e* 352.1500. $C_{14}H_{28}N_2O_4S_2$ requires *m/e* 352.1489.

PMR spectrum (D_2O): 1.29, t and 1.30 t, CH_3CH_2 (total 6H); 1.58 m $C4H_2$ (2H); 2.06 s, CH_3CO (3H); 2.12 s CH_3CO (3H); 2.76 overlapping CH_3CH_2 (4H); 3.29 m C3H and C5H (2H); 4.05 m, C1H and $C6H_2$ (3H); 4.55 m, C2H (1H).

Mass spectrum: Significant peaks at *m/e*: 352, 323, 293, 264, 246, 232, 217, 199, 158, 146, 140, 139, 135, 128, 122, 116, 104, 103, 102.

Fractions 52~57: 8.0 mg, ethylthio-2,6-diacetamido-2,4,6-trideoxy-β-D-*xylo*-hexopyranoside.

PMR spectrum (D_2O): 1.28 t, CH_3CH_2 (3H); 1.58 m, C4Ha (1H); 2.04 s, CH_3CO (3H); 2.06 s, CH_3CO (3H); 2.22 m, C4He (1H); 3.73 q and 3.75 q, CH_3CH_2 (total 4H); 3.36 m, $C6H_2$ (2H); 3.71 m, C2H, C3H and C5H (total 3H); 4.58 d C1H (1H) $J_{1,2}=10.4$ Hz.

Mass spectrum: Significant peaks at *m/e* 290, 261, 231, 229, 213, 170, 152, 128, 124, 110, 100, 99, 84.

Methanolysis of Seldomycin Factor 5-per-N-acetate

Seldomycin factor 5 per-N-acetate (4 g) in methanol (200 ml) saturated with hydrogen chloride was heated under reflux overnight. The mixture was concentrated and N-acetylated according to the procedure for the preparation of seldomycin factor 5-per-N-acetate. The product mixture was extracted

with chloroform to give extract A and residue B.

A in chloroform (plus a trace of methanol) was chromatographed on an E. M. Merck prepacked silica gel 60 column (size B) eluted with a gradient from chloroform (400 ml, mixing chamber) and chloroform: methanol (2/1, v/v, 400 ml reservoir). Fractions were collected, combined as follows and concentrated to yield:

Fractions 15~18: 48.8 mg methyl 2,3-diacetamido-2,3-dideoxy-4-O-methyl-β-D-*xylo*-pyranoside $[\alpha]_D^{18.5}-113°$ (*c* 0.27, CH_3OH), sublimes from 260°C to 305°C, unchanged by tlc, (M^+-MeOH) 228.083, Calcd. for $C_{10}H_{16}N_2O_4$ 228.111.

PMR spectrum ($CDCl_3$): 1.97 s, $2\times CH_3CO$ (6H); 3.43 s, OCH_3 (3H); 3.49 s, OCH_3 (3H); 4.56 m, C1H (1H); 1 proton multiplets occur at 3.23, 3.74, 3.82, 4.01 and 4.14. All discernible ring proton couplings are small. This is taken to indicate that this ring is in the conformation in which all four substituents are trans-diaxial. The presence of a large number of long range (presumable W type) couplings makes ring proton assignments tenuous.

Mass spectrum: Significant peaks at *m/e* 228, 201, 196, 169, 142, 141, 138, 115, 100.

Fractions 26~45: 125.8 mg methyl 2,3-diacetamido-2,3-dideoxy-4-O-methyl-α-D-*xylo*-pyranoside $[\alpha]_D^{18.5}+36°$ (*c* 0.86, CH_3OH), sublimes 301~302°C (unchanged by tlc).

Anal. Calcd. for $C_{11}H_{20}N_2O_5$: C, 50.76; H, 7.74; N, 10.76
Found C, 49.16; H, 7.33; N, 11.21

PMR spectrum ($CDCl_3$): 1.94 s and 1.95 s, $2\times CH_3CO$ (6H); 3.37 s and 3.39 s, $2\times OCH_3$ (6H), 3.1~4.2 ring protons other than C1H; 4.60 d, C1H (1H) $J_{1,2}=3.2$ Hz. Attempts to render the ring proton coupling pattern decipherable by use of other solvents (CD_3OD, D_2O, C_5D_5N) were unsuccessful.

Fractions 46~54: 288.7 mg methyl 2,6-diacetamido-2,4,6-trideoxy-β-D-*xylo*-hexopyranoside.

PMR spectrum (D_2O): 1.47 broad q, C4Ha (1H); 2.05 s and 2.07 s, $2\times CH_3CO$, overlapping 2.1 m, C4He (total 7H); 3.7~4.1 m, C2H, C3H, C5H (total 3H); 4.81 d, C1H (1H) $J_{1,2}=3.0$ Hz.

Fractions 55~64: 121 mg methyl 2,6-diacetamido-2,4,6-trideoxy-α-D-*xylo*-hexopyranoside.

PMR spectrum (D_2O): 1.47 broad q, C4Ha (1H); 2.06 s and 2.08 s, $2\times CH_3CO$ overlapping 2.1 m, C4He (total 7H); 3.41 m $C6H_2$ (2H); 3.53 s OCH_3 (3H); 3.61 broad d; C2H (1H); 3.7~4.0 m, C3H and C5H (2H); 4.38 d, C1H (1H) $J_{1,2}=8.0$ Hz.

Residue B above was extracted with the lower phase of a mixture of equal parts of chloroform, methanol and concentrated ammonium hydroxide and the extract was chromatographed over a column (56×2.5 cm) of "Woelm" silica gel (0.032~0.064 mm diameter) in the same solvent system. Fractions were collected, combined as follows and concentrated to yield:

Fractions 35~56: 391.6 mg seldomycin factor 5 per-N-acetate.

PMR identical with that of an authentic sample.

Fractions 62~89: 130 mg 6-O-(α-D-2,3-diacetamido-2,3-dideoxy-4-O-methyl-*xylo*-pyranosyl)-2-deoxystreptamine-N,N′-diacetate.

PMR spectrum (D_2O): 1.42 rough q, H_2 (1H); 1.93 s, 2.01 s, 2.03 s, CH_3CO, on 2.0 m, (H_2e) (total 13H), 3.46 s, OCH_3 on 3.4~4.2 m (total 13H); 5.12 d, H_1 (1H).

CMR spectrum (D_2O):

C_1	50.2		C_1'	96.8
C_2	34.0		C_2'	51.9
C_3	50.2		C_3'	51.9
C_4	63.4		C_4'	77.5
C_5	75.2		C_5'	60.8
C_6	79.1		OCH_3	58.9
	CH_3	22.8		
	CO	175.4, 174.7, 173.8		

Fractions 102~128: 43 mg seldomycin factor 2 per-N-acetate $[\alpha]_D^{24}+106$ (*c* 1.15, H_2O) (authentic seldomycin factor 2 per-N-acetate had $[\alpha]_D^{24}+107$ (*c* 1.16, H_2O)).

PMR spectrum, identical with that of an authentic sample.

Fractions 270~330: 145 mg 2-deoxystreptamine-N,N′-diacetate.

PMR identical with that of an authentic sample.

Barium Hydroxide Hydrolysis of Methyl 2,3-diacetamido-2,3-dideoxy-4-O-methyl-α-D-*xylo*-pyranoside

Methyl 2,3-diacetamido-2,3-dideoxy-4-O-methyl-α-D-*xylo*-pyranoside (125 mg) in carbon dioxide free water (40 ml) was treated with barium hydroxide octahydrate (1 g) and the mixture was protected from carbon dioxide and heated under reflux for fourteen days. The mixture was cooled, saturated with carbon dioxide, filtered through a pad of Celite and the filtrate was chromatographed on a column (57×1.5 cm) of E. M. Merck silica gel 60 in chloroform- methanol- concentrated ammonium hydroxide (8.5:1.5:0.1, v/v/v). Fractions were collected, combined as follows and concentrated to yield:

Fractions 8~14: 30.1 mg methyl 2,3-diacetamido-2,3-dideoxy-4-O-methyl-α-D-xylopyranoside. PMR spectrum identical with that of the starting material.

Fractions 19~26: 94 mg methyl 2-acetamido-3-amino-2,3-dideoxy-4-O-methyl-α-D-xylopyranoside. M^+ 218.1263, calcd. for $C_9H_{18}N_2O_4$ 218.1267.

PMR spectrum as described in Table 4.

Mass spectrum: Significant peaks at *m/e* 219, 218, 186, 159, 154, 128, 115, 100, 86, 73, 58.

Fractions 34~61: 24.1 mg methyl 3-acetamido-2-amino-2,3-dideoxy-4-O-methyl-α-D-*xylo*-pyranoside. M^+ 218.1263, calcd. for $C_9H_{18}N_2O_4$ 218.1267.

PMR spectrum as described in Table 4.

Mass spectrum: Significant peaks at *m/e* 218, 186, 159, 129, 115, 100, 86, 73, 58.

Methanolysis of per-N-carboethoxyseldomycin factor 5

Per-N-carboethoxyseldomycin factor 5 (400 mg) was treated with 7.85 N dry methanolic hydrogen chloride for 17 hours at 18°C. Solvent was removed and the residue was chromatographed on a column of silica gel (33 g) eluted with a gradient of chloroform-methanol from 30:1, v/v to 5:1 v/v. Usual work up of the appropriate fractions gave methyl 2,3-dideoxy-2,3-diethoxycarbamido-4-O-methyl-α-D-*xylo*-pyranoside **19** $[\alpha]_D^{22}+40°$ (*c* 0.133, CH_3OH), the corresponding β isomer **20**, methyl 2,6-diethoxycarbamido-2,4,6-trideoxy-α-D-*xylo*-hexopyranoside **21**, the corresponding β isomer **22** and N,N'-dicarboethoxy-2-deoxystreptamine **23***.

Sodium Hydroxide Hydrolysis of Methyl-2,3-dideoxy-2,3-diethoxycarbamido-4-O-methyl-α-D-*xylo*-pyranoside

Methyl 2,3-dideoxy-2,3-diethoxycarbamido-4-O-methyl-α-D-*xylo*-pyranoside (60 mg) in methanol (5 ml) and 2 N sodium hydroxide solution (5 ml) was heated in a sealed tube at 110°C for 2 hours. The mixture was neutralized with hydrochloric acid and solvent was removed. The residue was extracted with the lower phase of chloroform, methanol, 17% ammonium hydroxide (2/1/1, v/v/v) and chromatographed on a column of silica gel (10 g) using the same solvent system as eluent to yield methyl 2,3-diamino-2,3-dideoxy-4-O-methyl-α-D-*xylo*-pyranoside (40 mg), m.p. 86~88°C.

PMR spectrum: 2.48, C2H (1H); 2.68 C3H (1H); 3.00 C4H; 3.35 s, OCH_3 (3H); 3.40 s, OCH_3 (3H); 3.42 C5Ha (1H); 3.75 C5He (1H); 4.49 d C1H (1H). $J_{1,2}$ 3.2; $J_{2,3}$ 10.6; $J_{3,4}$ 8.4; $J_{4,5a}$ 8.4; $J_{4,5e}$ 4.9; $J_{5a,5e}$ 10.6 Hz.

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