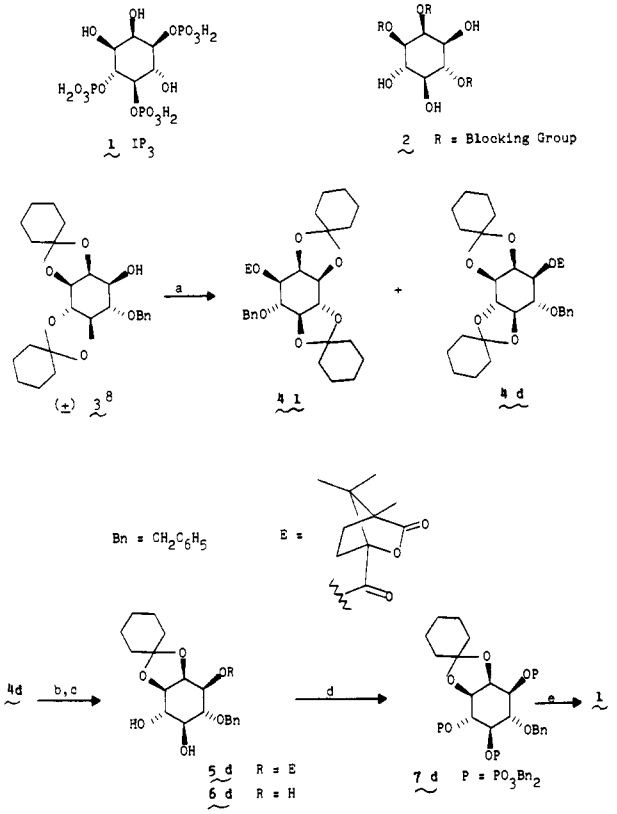
Scheme I[a]



[a] (a) (S)-(-)-camphanic acid chloride, NEt_3, DMAP, CH_2Cl_2, 25 °C, 1 h; (b) MeOH/EtOAc, (1:5) catalytic HCl (gas), room temperature, 3 h; (c) 10 equiv of LiOH, DME/H_2O (2:1), room temperature 1 h; (d) 4 equiv of KH, THF, 60 °C, 30 min then 4 equiv of tetrabenzyl pyrophosphate, room temperature 4 h; (e) H_2, 50 psig, 10% Pd/C, 95% EtOH, room temperature 3 h then AcOH/H_2O, room temperature 16 h.

hydroxy substituent with (*S*)-(-)-camphanic acid chloride[9] (DMAP, Et_3N, CH_2Cl_2, 1 h, 25 °C) yielded a mixture of two diastereomers (combined yield = 90%) that were chromatographically separated (CH_2Cl_2/Et_2O, 98:2; SiO_2) to give **4d** (mp 152-153 °C)[10] and **4l** (mp 121-123 °C).[10] The diastereomeric purity of each compound exceeded 98% as determined both by HPLC and ^1H NMR. Selective hydrolysis of the trans ketal of **4d** with a catalytic amount of gaseous HCl in a mixture of ethyl acetate-methanol (5:1, 3 h) gave diol **5d** (mp 176-178 °C, $[\alpha]^{20}_D$ -32°, $CHCl_3$, c = 1 mg/mL)[10] in 76% yield. Basic hydrolysis of the ester group of **5d** (10 equiv of LiOH, DME/H_2O 2:1, 25 °C, 1 h) afforded triol **6d** (88%, mp 137-139 °C, $[\alpha]^{20}_D$ +21°, $CHCl_3$, c = 1 mg/mL).[10]

The crucial step of the synthesis relied on successfully phosphorylating triol **6d**, which contains the troublesome 4,5-vicinal diol. It was also essential that the phosphate groups be introduced in a form easily deprotected prior to hydrolysis of the 1:2-cyclohexylidene ketal in order to minimize the facile migration of phosphate esters from the 1- to the 2-hydroxyl group.[11] We explored a variety of methods to trisphosphorylate **6d** under neutral conditions, including the use of phosphite triesters,[12] but none were satisfactory. Phosphorylation of the third hydroxyl oxygen is kinetically very slow, allowing side reactions to predominate. In order to enhance the reaction rate, we explored the possibility of phosphorylating alkoxide salts. The preformed tripotassium alkoxide of triol **6d** (4 equiv of KH, THF, 60 °C, 30 min) reacted cleanly with tetrabenzyl pyrophosphate[13,14] (4 equiv of tetrabenzyl pyrophosphate, THF, 4 h, 25 °C) to give compound **7d** (oil, $[\alpha]^{20}_D$ -4.2°, $CHCl_3$, c = 1 mg/mL)[10a] in 65% yield. No trace of a 4,5-cyclic phosphate, often the predominant product in other phosphorylation procedures,[4] could be found under these conditions (Scheme I).

Hydrogenolysis of the seven benzyl groups of trisphosphate **7d** (H_2, 50 psig, 10% Pd/C, 95% EtOH, 3 h) and subsequent hydrolysis of the cyclohexylidene ketal (AcOH/H_2O 1:1, 16 h, 25 °C) in a one-pot procedure gave D-*myo*-inositol 1,4,5-trisphosphate (**1**) in 95% isolated yield. The compound was characterized as its hexasodium salt: $[\alpha]^{20}_D$ -30°, H_2O (pH 9.5), c = 1.6 mg/mL (lit.[3] $[\alpha]^{20}_D$ -28°, H_2O (pH 9.5), c = 1.6 mg mL); FAB mass spectrum, m/e M + H = 421, M + 22 = 443; C, H anal. Both the ^1H (300 MHz) and ^{31}P NMR of **1** was identical with those recently reported by Lindon[15] and Williamson[16] for natural **1**. None of the 2,4,5-trisphosphate was detected. Ester **4l** was transformed in the same manner as described for ester **4d** to give L-*myo*-inositol 1,4,5-trisphosphate which was characterized as its free acid $[\alpha]^{20}_D$ + 35°, H_2O (pH 10.0), c = 1 mg/mL; FAB mass spectrum, m/e M + 1 = 421, M + 22 = 443; C, H anal.).

We have successfully used this phosphorylation procedure for the synthesis of (±)-*myo*-inositol 1,3,4-trisphosphate and (±)-*myo*-inositol 1,3,4,5-tetrakisphosphate. The details will be published elsewhere.

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^{57}Fe NMR of Heme Proteins. The Chemical Shift Anisotropy of Ferrocytochrome *c*

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Iron-57 has a spin $I = {}^1/_2$ and a large chemical shift range that makes it very useful for the study of heme-ligand interactions. We have recently demonstrated the correlation between the ^{57}Fe NMR chemical shift and the free energy of ligand binding in carbonyl iron porphyrin complexes.[1] Also, we and others, have shown by direct[2,3] and indirect[4] determination of the ^{57}Fe NMR chemical shift[2-4] and relaxation parameters[2,3] of myoglobin-CO (MbCO) that ^{57}Fe NMR is a powerful tool for the study of heme proteins. In this paper, we report the first ^{57}Fe NMR observation of a cytochrome, ferrocytochrome *c*, together with its chemical

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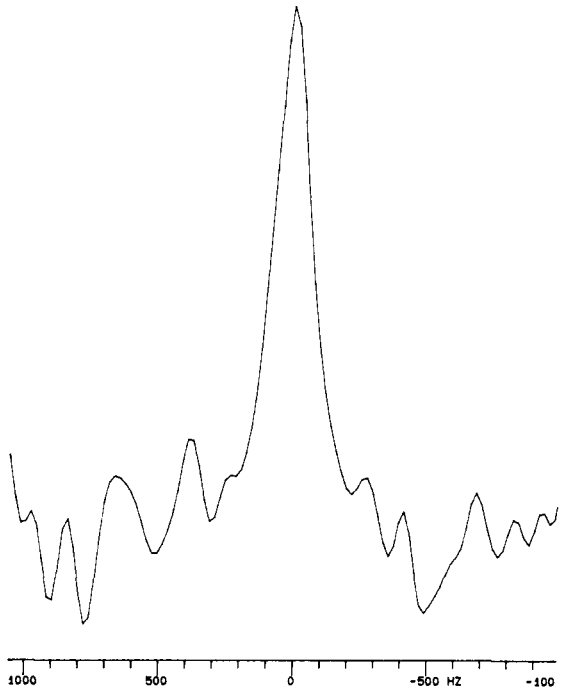

Figure 1. ^{57}Fe NMR spectrum of 3 mM cytochrome *c* in H_2O–D_2O (3:1), pH 7, 298 K; line broadening, 50 Hz.

shift and relaxation parameters. Also, since cytochrome *c* is a protein involved in the transfer of electrons, the effect on ^{57}Fe NMR spectra of diamagnetic molecules by the presence of paramagnetic iron is considered. For horse heart cytochrome *c* in the available concentration range the self-exchange rate is too slow to cause exchange broadening. For small molecules, ferric iron complexes may be used as convenient shiftless relaxation reagents for the observation of the corresponding ferrous complexes.

The ^{57}Fe NMR spectra were recorded at 9.4 T (400 MHz, ^{1}H frequency, 13.09-MHz ^{57}Fe frequency) on a Varian XL 400 spectrometer, equipped with a solenoid-coil probe (Cryomagnet Systems Inc.). Nonspinning sample tubes, 15 mm in diameter, contained 3.1 mL of a 3 mM solution of horse heart ferrocytochrome *c* (Sigma) in 0.075 M phosphate buffer at pH 7 and 298 K. The protein was enriched to 90% in ^{57}Fe by using the published procedure.[5] For efficient use of ^{57}Fe NMR, pulse repetition times of less than 5 ms may be used. For the determination of the natural line width, a pulse repetition time of 33 ms was used, together with a sweep width of 20 KHz. To avoid the effects of acoustic ringing ("rolling base line"), a preacquisition delay of 1 ms was used. In a total experimental time of 24 h, 2.6 $\times$ 10^6 transients were acquired.

The chemical shift of cytochrome *c*, Figure 1, at 298 K is 11197 ppm downfield from $Fe(CO)_5$, and the temperature dependence is +3.0 ppm/°C. The known chemical shift range of iron porphyrins is thereby extended to 4000 ppm and the resonance is 3000 ppm downfield from that of MbCO. The transverse relaxation time, T_2, of 3 ms was estimated from the observed natural line width, 105 Hz, under the usual assumption of negligible field inhomogeneity. The longitudinal relaxation time, T_1, is expected to be close to the "T_1-minimum" and only slightly longer than $^7/_6T_2$, which is the theoretical value under extreme narrowing conditions.[6] It is therefore not possible to calculate the chemical shift anisotropy (CSA) and the rotational correlation time, τ_c, from determinations of T_1 and T_2, as for MbCO.[2,3] Instead, the published value of τ_c of 2.7 mM cytochrome *c*, 6 ns,[7] and the experimental value of T_2 are used to obtain the chemical shift anisotropy. Insertion into the usual formula[6] gives a CSA of 7630 ppm, under the assumption of dominant CSA relaxation, a much larger CSA than any reported so far for an iron compound. This is in agreement with the large chemical shift and will allow an estimate to be made of the components of the chemical shift tensor. A calculation of T_1 from these numbers gives 4 ms.

The observed chemical shift is described by the trace of the chemical shift tensor.[6] The screening constant, σ, for an axially symmetric molecule, is given by $\sigma = {}^1/_3(2\sigma_\perp + \sigma_\parallel)$, where $\sigma_\parallel$ is the component along the major molecular axis and $\sigma_\perp$ is that perpendicular to it. The CSA is given by $\Delta\sigma = \sigma_\parallel - \sigma_\perp$. Although the porphyrin plane is not strictly symmetric, the approximation is probably good. From the chemical shift, the calculated CSA, and the expressions for σ and $\Delta\sigma$, two equations with two unknowns are obtained. Unfortunately, only the absolute value of the CSA can be calculated, and in solving these equations, we get two sets of solutions, $\sigma_\perp = -13\,739$, $\sigma_\parallel = -6112$ ppm and $\sigma_\perp = -8655$, $\sigma_\parallel = -16\,282$ ppm. The shielding constants and the chemical shifts have opposite signs in accordance with the δ convention.

Assignment or σ for an iron porphyrin is attractive since it further increases our arsenal for predicting ^{57}Fe NMR shifts and relaxation properties. Correlation with $1/\Delta E$ is, for example, not feasible due to the difficulty in identifying iron d–d transitions. Since the difference in $\sigma_\perp$ between the two sets of solutions is 5000 ppm, a comparison with other ^{57}Fe NMR porphyrin chemical shifts will allow assignment of the screening constants. It seems reasonable that $\sigma_\perp$ is similar for the porphyrins. In a porphyrin complex of high symmetry, such as $Fe(TPP)(py)_2$ or $Fe(TPP)(pyrr)_2$,[8] the observed chemical shift is therefore "close" to $\sigma_\perp$, certainly closer than 5000 ppm. The observed ^{57}Fe NMR chemical shifts for these two compounds are both in the vicinity of 7300 ppm and $\sigma_\perp$ for cytochrome *c* may rather safely be assigned as −8655 ppm. Similar arguments in the case of MbCO suggest that $\sigma_\perp$ is −9400 ppm. Between the several experimental errors involved, an estimate of $\sigma_\perp$ for the porphyrins is roughly −9000 ppm.

We now turn to the question of paramagnetism and ^{57}Fe NMR. Since much of the chemistry of iron is redox chemistry, the effect of paramagnetic iron on the ^{57}Fe NMR spectrum of diamagnetic iron is interesting. First we consider some model systems and then we discuss the spectrum of cytochrome *c*. Ferric relaxation reagents have been successfully used in organic chemistry and should provide an efficient relaxation mechanism also for ferrous complexes. Since complex pulse sequences have been adopted for the study of slowly relaxing ferrous compounds and long relaxation times have been quoted as obstructing the efficient use of ^{57}Fe NMR, it should be pointed out that the corresponding redox partner may serve as a convenient, clean, and shiftless relaxation reagent for the diamagnetic compound. We measured the chemical shift and T_1 of $Fe(bpy)_3Cl_2$, enriched to 60% in ^{57}Fe, which is known to have unfavorable relaxation properties.[9] At a 45 mM concentration in D_2O in the presence of 2.5 mM $Fe(bpy)_3Cl_3$, the observed T_1 is 0.43 s, considerably shorter than the reported value of 5 s at 11.74 T in the absence of paramagnetics. The chemical shift is unchanged within 2 ppm. In contrast, 1 M ferrocene in the presence of 6 mM ferricinium hexafluorophosphate is not observable by ^{57}Fe NMR. Here, the electron exchange rate is high enough to cause exchange broadening beyond recognition. Also, porphyrin complexes are known to have fast electron-transfer rates.

In horse heart cytochrome *c* the observed electron transfer rate constant for the self-exchange reaction is 10^3–10^4 M^{-1} s^{-1}. In the concentration range that is available, the exchange is slow on the NMR time scale, and the diamagnetic protein may be observed in the presence of its paramagnetic redox partner. On the other hand, with heme proteins with rate constants on the order of 10^6

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M^{-1} s^{-1}, of which there are several, ^{57}Fe NMR may provide a convenient method of studying electron transfer reactions.

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Biosynthetic Studies on Validamycins: A $C_2 + C_2 + C_3$ Pathway to an Aliphatic C_7N Unit[1]

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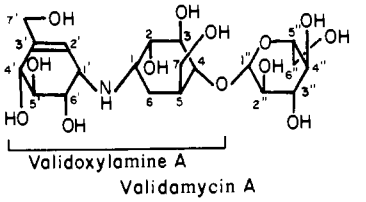
Validamycin A[3] is the major component of the validamycin complex, used in the Orient to treat sheath blight disease in rice. The discovery of validamycins, followed by the isolation of the antibacterial pseudo-α-galactopyranose,[4] introduced novel (hydroxymethyl)cyclitols including validamine, hydroxyvalidamine,[5] valienamine, valiolamine,[6] and carbocyclic analogues of hexopyranoses (pseudosugars).[7] The valienamine and hydroxyvalidamine units have also been identified as building blocks of recently discovered microbial α-glucosidase inhibitors[8] (Chart I).

Nothing has been reported previously about the biosynthesis of these (hydroxymethyl)cyclitols. In principle, validamine, hydroxyvalidamine, valienamine, and valiolamine units could be considered aliphatic analogues of the "*m*-C_7N" units,[9] widely found in quinonoid antibiotics and related compounds. However, we present evidence here derived from feeding ^{13}C-labeled precursors to *Streptomyces hygroscopicus* var. *limoneus*[10] indicating that both the validamine and valienamine units are, on the contrary, biosynthesized by a pathway involving a seven-carbon sugar which is formed by a C_2-group transfer ($C_2 + C_2 + C_3$) related to the pentose phosphate pathway.[11]

Chart I. Structures of Validamycin A and (Hydroxymethyl)cyclitols



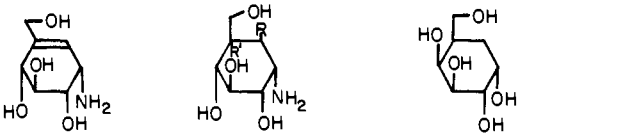
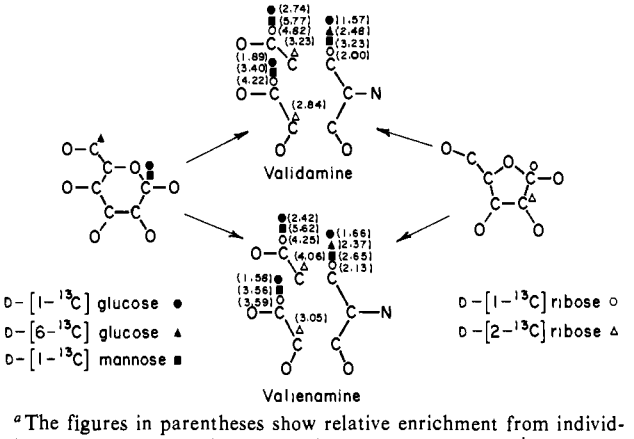


Table I. ^{13}C NMR Signals[a] for the Validoxylamine A Unit of Validamycin A Derived from *D*-[U-^{13}C]Glucose

C-*n*	δ, ppm[b]	pattern	J_{C-C}, Hz	changes in C-*n* on irradiating C-*m*	C-*m*
C-1	56.2	t + d + s	38.5, 35.5	→ d + s	C-6
C-2	~75.5	*c*			
C-3	75.2	d + s	37.6	→ s	C-4
C-4	86.8	d + s	37.6		
C-5	39.9	d + s	37.4		
C-6	29.4	d + s	35.5		
C-7	64.3	d + s	37.4	→ s	C-5
C-1′	54.9	t + d + s	42.3, 39.5	→ d + s	C-2′
C-2′	125.5	d + s	42.3		
C-3′	141.7	d + s	47.5		
C-4′	74.0	d + s	41.1		
C-5′	~76.0	*c*			
C-6′	72.0	d + s	39.5		
C-7′	64.1	d + s	47.5	→ s	C-3′

[a] Spectra were recorded in deuterium oxide on an NSF-250 spectrometer. [b] See ref 10. [c] Due to overlap of these signals, the splitting patterns could not be determined.

Scheme I. Labeling of C_7 Units by Carbohydrate Precursors[a,b]



[a] The figures in parentheses show relative enrichment from individual precursors (see Table III, supplementary material). [b] Precursors were added to the production media, which contain 1% D-glucose, after 24-h incubation (see ref 10).

The *m*-C_7N units of geldanamycin[9b,12a] and pactamycin[9b,12b] are constructed from C_4 and C_3 units and the cyclopentanoid unit of pactamycin[13] from C_6 and C_1 units, and analogous mechanisms

(1) Presented in part at Antibiotics 86, Granada, Spain, June 19-24, 1986; Abstr., pp 36-37.
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