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STUDIES OF THE SYNTHESIS OF SUGAR PHOSPHONATES RELATED TO 3-DEOXY-D-*manno*-2-OCTULOSONIC ACID (KDO)*

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ABSTRACT

In attempting to synthesize the analogue of β-Kdo (2*R*)-2-carboxy-6-(1′,2′-dihydroxyethyl)-4,5-dihydroxy-D-*manno*-1,2λ^5-oxaphosphorinan-2-one (**6**) as an inhibitor of the enzyme CMP–Kdo synthetase, which is involved in the biosynthesis of the lipopolysaccharide component of the outer membrane of Gram-negative bacteria, (2*R*)-6-(1′,2′-dihydroxyethyl)-2-ethoxy-3,4,5-trihydroxy-4,5:1′,2′-di-*O*-isopropylidene-D-*glycero*-D-*talo*-*1,2*λ^5-oxaphosphorinan-2-one (**8**) was converted into (2*S*)-6-(1′,2′-dihydroxyethyl)-4,5-dihydroxy-4,5:1′,2′-di-*O*-isopropylidene-2-vinyl-D-*manno*-1,2λ^5-oxaphosphorinan-2-one (**16**), but alkene cleavage to give the target carboxyphosphonate failed. Reduction–oxidation–Arbuzov reaction on the intermediate (2*R*)-6-(1′,2′-dihydroxyethyl)-2-ethoxy-4,5-dihydroxy-4,5:1′,2′-di-*O*-isopropylidene-D-*manno*-1,2λ^5-oxaphosphorinan-2-one (**11**) gave the 2*S* isomer of the protected target compound, but removal of the protecting groups gave the acyclic product dilithium (D-*manno*-2,3,4,5,6-pentahydroxyhexyl)phosphinatoformate (**24**). N.m.r. studies of the intermediates allowed assignment of stereochemistry at P for all compounds via $^2J_{P,H}$ coupling constants.

INTRODUCTION

Inhibition of the biosynthesis of the outer membrane in Gram-negative bacteria by blocking the incorporation of 3-deoxy-β-D-*manno*-octulopyranosonic acid (β-Kdo, **1**) provides a route to new types of antibacterial agents[1]. Such Kdo-analogues as **2** and **3** are good inhibitors of the enzyme CMP–Kdo synthetase (CKS)[2,3].

The search for other inhibitors has resulted in the synthesis of several Kdo-analogues, *e.g.*, **4**[4] and **5**[5], but most of these compounds have weak activities, and it seems that the ring oxygen of Kdo should be retained for activity. Modification at position 2 in Kdo was also considered desirable since it is the reacting centre

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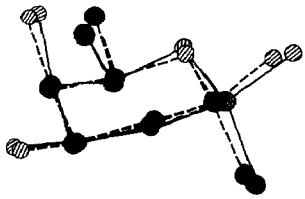
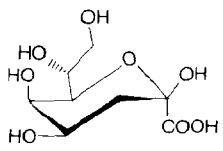
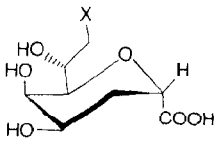


Fig. 1. AM1-calculated structures of **1** (---) and **6** (——). All hydrogens, the oxygens of the caboxylate group, and part of the side-chain have been omitted for clarity.

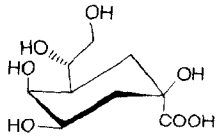
during the incorporation of Kdo into the developing lipopolysaccharide of the outer membrane[6]. The ring phosphorus analogue **6** was considered to be a possible Kdo mimic since the ring oxygen is retained and it also carries an oxygen in the 2-β position.



1



2, X = OH
3, X = NH_2



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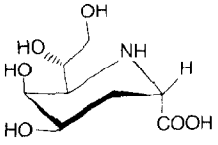

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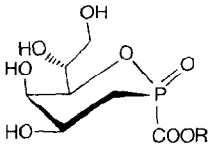


6, R = H
7, R = e^-

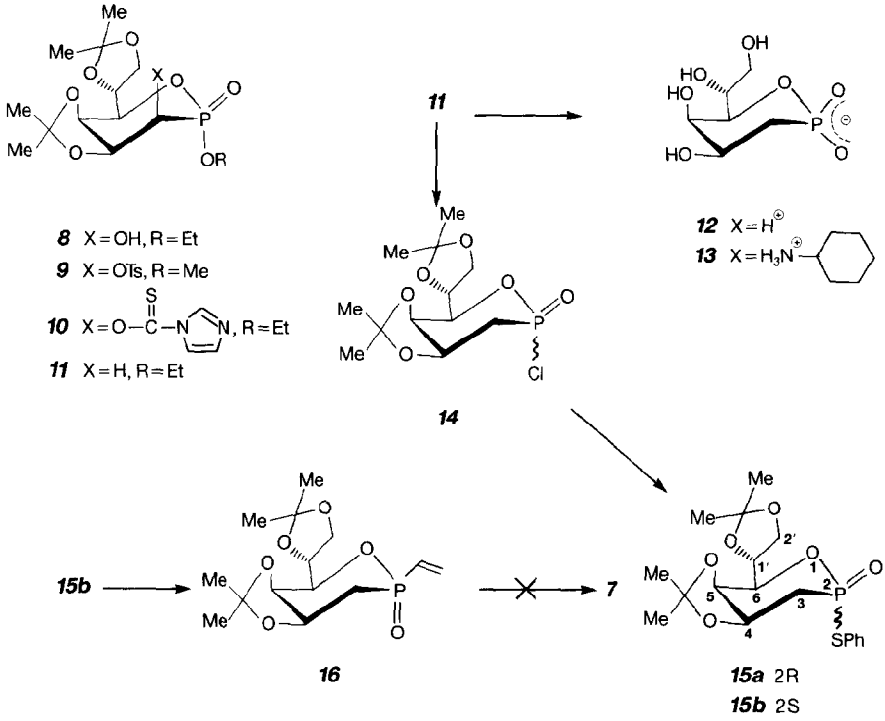
Computer modelling studies[7] (AM1) on **6** further indicated that its stereochemical features were similar to those of β-Kdo (**1**). On superposition of the rings of the AM1-calculated structures of **1** and **6**, the 2-β oxygens are seen to be 0.3 Å apart (Fig. 1). Thus, the structure **6** is a close mimic of β-Kdo and therefore its synthesis was undertaken.

RESULTS AND DISCUSSION

Synthesis. — The synthesis started from the oxaphosphorinane **8**[8], which was obtained in a yield of ~40%, either by the published procedure, or by treatment of 2,3:5,6-di-*O*-isopropylidene-D-mannose with the lithium salt of diethyl phosphite in tetrahydrofuran.

Conversion of **8** into the thiocarbonylimidazolyl derivative **10** with thiocarbonyldi-imidazole in tetrahydrofuran followed by reduction[9] with tributyltin hydride in toluene gave 80% of **11**. Removal of the protecting groups from **11** gave 81% of the cyclic phosphonate monoester salt **13** via conversion into the silyl ester, hydrolysis, and then titration with cyclohexylamine.

Two approaches for introducing the carboxylate function in **6** were considered, namely, nucleophilic substitution[10] at P and reduction at P followed by an Arbuzov reaction.



In order to obtain a compound with a leaving group at P that could easily be purified, **11** was converted into the thiophenyl ester **15** via the sequence: reaction with Me_3SiBr, treatment with oxalyl chloride in the presence of a catalytic amount of imidazole (to give **14**), and reaction with thiophenol in the presence of base to give **15a** (28%) and **15b** (23%) after chromatography. In seeking to introduce a carboxylate function at P, **15a** or **15b** was treated with ethoxyvinyl-lithium[11] under various conditions, but no useful products were obtained. Modification of the reagent with, for example, Cu[12], Zn, Mg, or Ce[13] did not result in any improvement. However, treatment of **15b** with vinylmagnesium bromide in tetrahydrofuran gave 81% of the vinyl derivative **16**. Under the same conditions, **15a** yielded no useful products. Attempts to cleave **16** oxidatively with permanganate/periodate[14] or

with ozone[15] were unsuccessful, and the nucleophilic substitution approach was abandoned.

In the second approach, initial attempts to convert **11** into a P(III) derivative included conversion into the thionophosphonate **17** by treatment with Lawesson's reagent followed by desulphuration with triphenylphosphine[16], hexachlorodisilane[17], or Raney Ni. However, these reagents were ineffective and attention was turned to hydride reduction at P.

Reduction of **11** by sodium bis(methoxyethoxy)aluminium hydride[18] in toluene gave 70% of the primary phosphine **18**. Oxidation of **18** with 2 equiv. of hydrogen peroxide in 2-propanol also caused cyclisation to give the phosphinate ester isomers **19a** (22%) and **19b** (28%) after chromatography. It was necessary to use freshly prepared **18** in order to obtain good yields of **19**. When a solution of **19b** in $CDCl_3$ was stored at room temperature, slow epimerisation into **19a** occurred.

Attempted *O*-deisopropylidenation of **19a**, using aqueous trifluoroacetic acid, resulted in ring opening and gave the phosphinic acid **20** in quantitative yield.

In order to introduce the carboxylate function at P via an Arbuzov reaction, **19a** was converted into the silyl phosphonite **21** by heating with hexamethyldisilazane in $CDCl_3$. Treatment of **21** with benzyl chloroformate at room temperature then gave 72% of the carboxylate ester **22** after chromatography. The progress of the silylation and Arbuzov reactions could be followed conveniently by ^{13}C-n.m.r. spectroscopy.

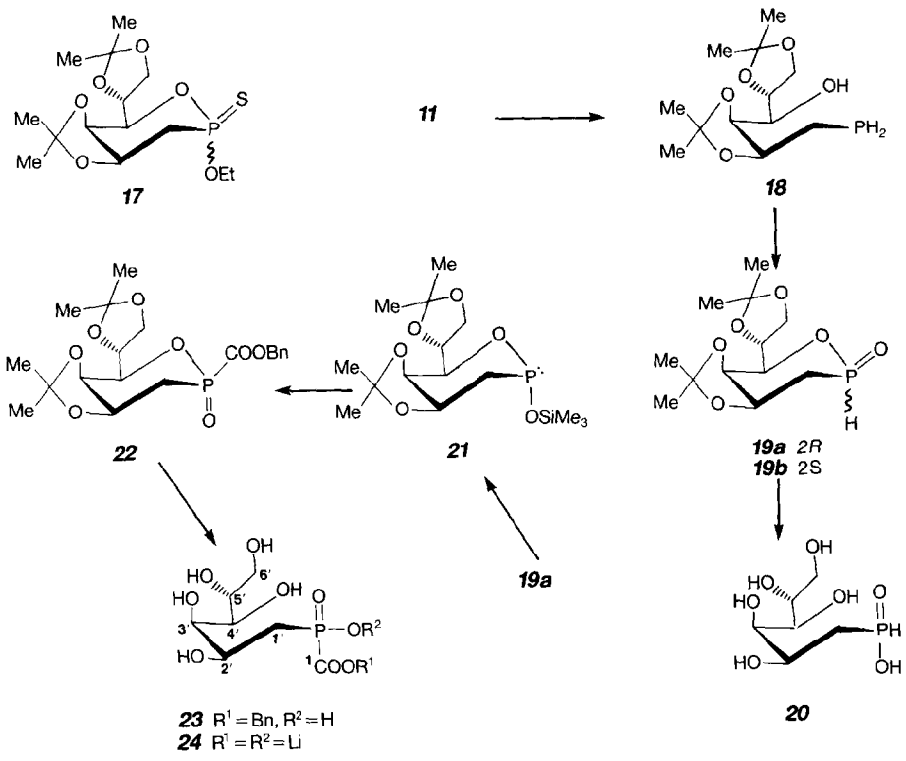
Attempts to convert the epimer **19b** into the corresponding silyl phosphonite and then to the carboxylate ester did not give the desired results. The silylation reaction was sluggish and gave, according to the ^{13}C-n.m.r. spectrum, a mixture of products which, on Arbuzov reaction with benzyl chloroformate, gave only 10% of **22**.

It proved to be impossible to remove the protecting groups from **22** to give the cyclic phosphinate ester/carboxylate **7**. Thus, removal of the benzyl group by catalytic hydrogenolysis over Pd/C in the presence of either pyridine or 1,8-diazabicyclo[5.4.0]undec-7-ene resulted in decarboxylation to give **19a**. Mild hydrolysis of **22** with trifluoroacetic acid resulted in ring opening to give 47% of the phosphinic acid **23**. The benzyl ester could then be removed by hydrolysis with LiOH to give the unstable ring-opened dilithium salt **24** in good yield, contaminated by ~25% of the Li salt of **20**.

The deprotected compounds **13, 20,** and **24** were not inhibitors of the CKS enzyme.

N.m.r. spectroscopy. — Differential isotope shift[19] (DIS) experiments were performed on **13** and **20**, and the data in Table IV indicate cyclic and acyclic structures, respectively.

The assignment of the ^{13}C and ^{1}H resonances of **11** was performed with the aid of H–H and C–H correlated 2D-spectra. Spectral assignment for all other cyclic compounds was straightforward by comparison with the data obtained for **11**. The ^{1}H- and ^{13}C-n.m.r. data for cyclic compounds are summarised in Tables I and II,



respectively. It can be seen from the data in Table I that the conformations of the cyclic compounds are similar. In particular, the value of $^3J_{H\text{-}4,P}$ is a sensitive indicator of conformational changes. Each compound has a $^3J_{H\text{-}4,P}$ value close to 30 Hz, indicating H-4 and P to be *trans*-diaxial and a boat-type conformation for each compound. There is also excellent agreement between the ^{1}H-n.m.r. data obtained for the compounds in Table I and those reported by Thiem *et al.*[8] for **9**. Thus, it can be assumed that ring conformations for the compounds in Table I are in accord with the X-ray structure reported[8] for **9**. With these data at hand, the stereochemistry at P could be assigned from the $^2J_{H\text{-}3,P}$ values. Such couplings show a Karplus-like relationship with respect to either the lone pair of electrons on P in P(III) compounds or the phosphoryl oxygen in phosphoryl compounds[20]. Thus, for compounds in which there is a *syn* relationship between the vicinal proton and the phosphoryl oxygen, the P–H coupling constant has a larger value than for compounds in which there is an *anti* relationship between the proton and the phosphoryl oxygen[20,21]. The data in Table I show that, for each compound where both P-isomers were obtained, the size of the $^2J_{H\text{-}3,P}$ values for H-3*a* and H-3*e* are characteristic. For example, for **19a**, $J_{H\text{-}3a,P}$ is 21.5 Hz, and $J_{H\text{-}3e,P}$ is 6.1 Hz, whereas the values for **19b** are 4.6 and 15.4 Hz, respectively. Thus, **19a** and **19b** have the *R* and *S* configurations, respectively, at P. It follows that **22, 15b,** and **16** each has the *S* configuration, whereas **15a** has the *R* configuration at P.

TABLE 1

^{1}H-N.M.R. DATA[a] FOR CYCLIC COMPOUNDS

Compound	*Chemical shifts (p.p.m.)*									*Coupling constants (Hz)*										
	H-3a	*H-3e*	*H-4*	*H-5*	*H-6*	*H-7*	*H-8*	*H-8'*	*Others*	$J_{3a,3e}$	J_{3a4}	$J_{3a,P}$	$J_{3e,4}$	$J_{3e,P}$	$J_{4,5}$	$J_{4,P}$	$J_{5,6}$	$J_{6,7}$	$J_{7,8-8}$	*Others*
8	n.r.	—	4.76	4.50	3.85	n.r.	3.97	n.r.		—	3.2	n.r.	—	—	7.3	31.2	1.5	7	4.4	$J_{6,P}$ 5
9[b]	5.01	—	4.85	4.53	3.84	4.33	3.91	4.09		—	2.8	10.1	—	—	7.3	28.8	1.7	7.5	4.0, 9.1	$J_{6,P}$ 5.3
11[c]	2.09	2.45	4.71	4.35	3.98	4.42	4.03	4.18		16.1	3.9	16.1	4.9	17.8	6.5	29	n.r.	6	6, 6	
15a	2.31	2.74	4.82	4.5	4.1	4.5	4.1	4.1		16.1	3.9	8.3	4.6	16.3	7	29	n.r.	n.r.	n.r.	
15b	2.20	2.72	4.76	4.4	4.1	4.4	4.1	4.1		16.6	3.4	16.8	3.9	11.0	7	33	n.r.	n.r.	n.r.	
16	2.12	2.53	4.76	4.43	4.05	4.36	4.1	4.1	vinyl H 6.0–6.7	16.6	2.7	19.5	3.7	6.4	7.8	30	1.5	8.5	4.9	$J_{6,P}$ 6.5
19a	2.00	2.43	4.79	4.46	4.09	4.38	4.14	4.14	2, 7.34	16.6	2.7	21.5	3.7	6.1	7.6	27.6	1.5	8.3	4.9	$J_{2,3a}$ 5.6 $J_{2,4}$ 1.0 $J_{5,P}$ 1.0 $J_{6,P}$ 6.5 $J_{2,P}$ 607
19b	1.96	2.56	4.79	4.44	3.84	4.41	4.06	4.15	2, 7.40	16.1	3.4	4.6	3.9	15.4	7	26	1.7	7.8	4, 6	$J_{2,4}$ 2.7 $J_{6,P}$ 6.3 $J_{2,P}$ 555
22[d]	2.13	3.06	4.74	4.4	4.1	4.4	4.1	4.1	PhCH_2 5.24	16.8	2.4	19.3	3.9	9.3	7	30.4	1.5	6.5[e]	4.9	$J_{6,P}$ 5.8[e]

[a]For solutions in $CDCl_3$ (internal Me_4Si) at 200 MHz except where noted. [b]Data from Thiem *et al.*[8]. [c]400 MHz, [d]500 MHz. [e]Assignments may be interchanged.

TABLE II

^{13}C-N.M.R. DATA[a] FOR CYCLIC COMPOUNDS

Compound	*C-3*	*C-4*	*C-5*	*C-6*	*C-7*	*C-8*	*-C-*	*$(CH_3)_2$*	*Others*
8	65.9 (145.2)	76.6 (6.1)	74.5	77.2	73.9 (9.8)	66.6	110.0, 111.2	25.3, 25.4, 26.2, 27.1	OEt 62.6 (7.3), 16.8 (4.9)
10	73.7 (157.5)	74.1 (3.7)	74.2	77.0 (7.3)	73.5 (9.8)	66.4	109.9, 111.8	25.0, 25.1, 26.0, 26.9	OEt 62.0 (7.3), 16.5 (6.1) Im 118.3, 131.3, 137.6 CO 183.3
11	24.8 (131.8)	71.8 (7.3)	72.6 (4.9)	76.6 (6.1)	74.0 (9.8)	66.4	109.6, 109.8	25.1, 25.2, 26.5, 26.9	OEt 61.3 (7.3), 16.5 (6.1)
12	27.3 (118)	69.3	68.0	76.6 (4.9)	70.1 (11.0)	63.2			
13	28.0 (116)	69.8	68.3	75.9 (2.4)	70.3 (13.4)	63.4			$C_6H_{13}N$ 51.3, 31.3, 25.2, 24.7
14	33.6 (107.4)	71.8 (8.5)	72.7 (3.7)	78.8 (7.3)	73.2 (11.0)	66.1	109.5, 109.9	24.7, 24.9, 26.2, 26.7	
15a	30.4 (89.1)	71.7 (7.3)	72.2 (6.1)	77.5 (8.6)	73.9 (9.8)	66.1	109.7, 109.9	24.8, 25.2, 26.3, 27.0	SPh 125.0 (6.1), 129.4, 129.6, 135.2 (3.7)
15b	29.8 (90.3)	71.3 (8.6)	72.3 (4.9)	76.6 (4.9)	73.6 (9.8)	66.7	109.6, 110.0	24.5, 25.1, 26.0, 27.0	SPh 125.7 (6.1), 129.3, 135.9 (4.9)
16	26.6 (87.9)	71.0 (8.6)	73.0 (3.7)	75.1 (6.1)	73.5 (9.8)	67.0	109.5, 110.1	24.7, 25.2, 26.0, 27.1	Vinyl 130.5 (133.1), 136.7
19a	26.0 (72.0)	71.2 (7.3)	72.8 (3.7)	75.8 (7.3)	73.4 (9.8)	66.6	109.4, 109.9	24.3, 25.1, 25.7, 27.0	
19b	27.4 (79.3)	72.1 (9.8)	72.1 (4.9)	75.5 (6.1)	73.8 (11.0)	66.5	109.8, 110.1	24.8, 25.1, 26.3, 27.0	
21	33.7 (30.5)	71.5	70.1 (8.6)	74.6	74.0	67.2	109.1, 109.4	25.3, 25.4, 26.4, 27.1	
22	23.8 (86.7)	70.8 (8.5)	72.6 (2.4)	76.8 (6.1)	73.2 (9.8)	66.5	109.5, 109.9	24.3, 24.8, 25.0, 26.9	$PhCH_2$ 67.4 (4.9), COO 166.8 (211), Ph 128.4, 128.5, 128.7, 134.3

[a]Coupling constants to phosphorus are noted in parentheses. All spectra were obtained for solutions in $CDCl_3$ at 50.3 MHz except for **12** and **13** which were run in D_2O. For $CDCl_3$ solutions, the centre peak of $CDCl_3$, taken as 77.16 p.p.m., was used as the reference. For D_2O solutions, the methyl resonance of (added) *tert*-butyl alcohol, taken as 30.6 p.p.m., was used as the reference.

TABLE III

^{13}C-N.M.R. DATA[a] FOR ACYCLIC COMPOUNDS

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*Others*
18	14.7 (11.0)	76.5 (2.4)	79.1 (3.7)	70.6	76.2	67.1	Me_2C 24.8, 25.3, 26.9, 27.0, Me_2C 108.0, 109.4
20	35.1 (94.0)	67.1 (3.7)	73.7 (11.0)	71.9	69.8	64.0	
	C-1'	*C-2'*	*C-3'*	*C-4'*	*C-5'*	*C-6'*	*Others*
23	33.7 (98.9)	67.7 (3.7)	73.5 (12.2)	72.0	70.0	64.0	$PhCH_2$ 68.0, Ph 129.2, 129.6, 129.7, 136.2
24	34.8 (84.2)	68.2 (2.4)	73.5 (12.2)	72.1	70.3	64.1	PCOO 181.8 (167)

[a]Coupling constants to phosphorus are noted in parentheses. All spectra were obtained for solutions in D_2O at 50.3 MHz except for **18** which was run in $CDCl_3$. For $CDCl_3$ solutions, the centre peak of $CDCl_3$, taken as 77.16 p.p.m., was used as the reference. For D_2O solutions, the methyl resonance of (added) *tert*-butyl alcohol, taken as 30.6 p.p.m., was used as the reference.

TABLE IV

DEUTERIUM-INDUCED SHIFTS (DIS) IN THE ^{13}C-N.M.R. SPECTRA OF **13** AND **20**

Compound	*C-3*	*C-4*	*C-5*	*C-6*	*C-7*	*C-8*	
13	<0.05	0.12	0.12	0.05	0.12	0.17	(found)
	0.03	0.17	0.17	0.06	0.17	0.18	(calc.[19])
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	
20	0.25	0.22	0.15	0.15	0.15	0.15	(found)
	0.20	0.20	0.20	0.20	0.20	0.20	(calc.)

EXPERIMENTAL

(2R)-6-(1',2'-Dihydroxyethyl)-2-ethoxy-3,4,5-trihydroxy-4,5:1',2'-di-O-isopropylidene-D-glycero-D-talo-*1,2λ⁵-oxaphosphorinan-2-one* (**8**). — This compound was prepared either by the procedure described by Thiem *et al.*[8] or by the following method. To a solution of diethyl phosphite (39.6 mL, 307.3 mmol) in toluene (200 mL) under N_2 over 30 min was added 1.59M BuLi in hexane (193.3 mL, 307.3 mmol) at −40°. The mixture was stirred for 1 h and then a solution of 2,3:5,6-di-*O*-isopropylidene-D-mannose (80 g, 307.3 mmol) in toluene (2.5 L) was added over 1 h at −40°. The mixture was stirred overnight and allowed to reach room temperature. Trimethylammonium chloride (44 g, 461 mmol) was added, and the mixture was stirred for 15 min, then extracted with M $KHCO_3$ (4 × 500 mL) and water (400 mL). The combined aqueous extracts were extracted with chloroform (5 × 400 mL), and the combined organic extracts were concentrated to give **8** as a white solid (45 g, 41%). T.l.c. (EtOAc) and ^{13}C-n.m.r. spectroscopy indicated contamination by ~15% of starting material. Column chromatography on silica gel (EtOAc) gave **8**, R_F 0.25 (EtOAc), m.p. 156–157°, $[\alpha]_D^{20}$ +43° (*c* 1, chloroform). See Tables I and II for the n.m.r. data. F.a.b.-mass spectrum: *m/z* 353 (M + H$^+$).

(2R)-6-(1',2'-Dihydroxyethyl)-2-ethoxy-4,5-dihydroxy-4,5:1',2'-di-O-isopropylidene-D-manno-*1,2λ⁵-oxaphosphorinan-2-one* (**11**). — To a solution of **8** (7.9 g, 22.4 mmol) in tetrahydrofuran (120 mL) under nitrogen was added thiocarbonyldi-imidazole (6.0 g, 33.7 mmol). The mixture was stirred at 80° for 4 h in a sealed vessel, stored at 5° overnight, then concentrated. Column chromatography (EtOAc) of the residue gave (2*R*)-6-(1',2'-dihydroxyethyl)-2-ethoxy-3,4,5-trihydroxy-3-*O*-(1-imidazolylthiocarbonyl)-4,5:1',2'-di-*O*-isopropylidene-D-*glycero*-D-*talo*-1,2λ⁵-oxaphosphorinan-2-one (**10**; 9.08 g, 87%), R_F 0.25 (EtOAc). See Table II for the ^{13}C-n.m.r. data.

To a boiling mixture of tributyltin hydride (8.0 mL, 29.7 mmol) and dry toluene (1 L) was added a solution of **10** (9.01 g, 19.5 mmol) in toluene (400 mL) during 45 min. The solution was boiled under reflux for 2 h and then concentrated. The residue was triturated with acetonitrile (3 × 200 mL), and the resulting solution was washed with *n*-hexane (4 × 200 mL), then concentrated to give **11** (5.9 g, 91%), R_F 0.25 (EtOAc). Recrystallisation from ether–hexane gave material with m.p. 106–106.5°, $[\alpha]_D^{20}$ +12° (*c* 1.1, chloroform); ν_{max}^{KBr} 1255 (P=O) 1032, 1042 cm^{-1} (P–O). See Tables I and II for the ^{1}H- and ^{13}C-n.m.r. data. ^{31}P-N.m.r. ($CDCl_3$): δ 24.2. F.a.b.-mass spectrum: *m/z* 337 (M + H$^+$).

Anal. Calc. for $C_{14}H_{25}O_7P$: C, 50.00; H, 7.49; P, 9.21. Found: C, 49.85; H, 7.58; P, 9.09.

Cyclohexylammonium 6-(1',2'-dihydroxyethyl)-4,5-dihydroxy-4,5:1',2'-di-O-isopropylidene-D-manno-*1,2λ⁵-oxaphosphorinan-2-one-2-oxide* (**13**). — To a solution of **11** (359 mg, 1.07 mmol) in $CDCl_3$ (2 mL) under nitrogen was added bromotrimethylsilane (0.148 mL, 1.12 mmol) at room temperature, the mixture was stirred, and the progress of the reaction was followed by ^{1}H-n.m.r. spectroscopy.

Two more portions of bromotrimethylsilane were added after 5 h (0.03 mL, 0.23 mmol) and 8 h (0.02 mL, 0.15 mmol), and the mixture was then stored at 5° overnight. Evaporation of the solvent gave the trimethylsilyl derivative (495 mg), which was hydrolyzed by dissolution in D_2O (1 mL) to give 6-(1',2'-dihydroxyethyl)-2,4,5-trihydroxy-D-*manno*-1,2-λ^5-oxaphosphorinan-2-one (**12**) (see Table II for the ^{13}C-n.m.r. data). This solution was then concentration, and a solution of the residue in H_2O was washed with several portions of ether, then concentrated to give **12** (273 mg). Dissolution in water followed by titration to pH 7.5 with cyclohexylamine (0.125 mL, 1.09 mmol), then concentrated, and recrystallisation of the residue from methanol–2-propanol gave **13** (286 mg, 81%), m.p. 203–207°, $[\alpha]_D^{19.5}$ +34.5° (*c* 1, chloroform). ^{31}P-N.m.r. (D_2O): δ 19.8.

Anal. Calc. for $C_{12}H_{26}NO_7P$: C, 44.03; H, 8.00; P, 9.46. Found: C, 43.93; H, 8.09; P, 9.34.

6-(1',2'-Dihydroxyethyl)-4,5-dihydroxy-4,5:1',2'-di-O-isopropylidene-2-phenylthio-D-manno-*1,2*λ^5*-oxaphosphorinan-2-one* (**15**). — To a solution of **11** (2.0 g, 5.95 mmol) in $CDCl_3$ (6 mL) under N_2 was added bromotrimethylsilane (0.817 mL, 6.19 mmol). The mixture was kept at 50° until ^{13}C-n.m.r. spectroscopy indicated complete reaction of **11** (2.5 h). Oxalyl chloride (0.621 mL, 7.14 mmol) was added followed by a solution of imidazole (40 mg, 0.59 mmol) in $CDCl_3$ (0.1 mL). The mixture was left overnight at room temperature, then heated for 2.5 h at 50°, filtered, and concentrated to give crude 2-chloro-6-(1',2'-dihydroxyethyl)-4,5-dihydroxy-**4**,5:1',2'-di-*O*-isopropylidene-D-*manno*-1,2λ^5-oxaphosphorinan-2-one (**14**; 1.75 g, 90%) as a white foam (^{13}C-n.m.r. data in Table II).

To a solution of crude **14** (798 mg, 2.44 mmol) in tetrahydrofuran (3.5 mL) under N_2 was added thiophenol (0.500 mL, 4.88 mmol) with stirring followed by a solution of triethylamine (0.340 mL, 2.44 mmol) in tetrahydrofuran (1.15 mL) at room temperature during ~10 min. The mixture was kept at 45–65° for 10 h, then stored overnight at room temperature and concentrated. Column chromatography (EtOAc) gave **15a** (270 mg, 28%), R_F 0.45 (EtOAc), m.p. 142–143°, $[\alpha]_D^{25}$ +37° (*c* 1, $CDCl_3$). See Tables I and II for the n.m.r. data. ^{31}P-N.m.r. ($CDCl_3$): δ 43.4.

Anal. Calc. for $C_{18}H_{25}O_6PS$: C, 53.99; H, 6.29; P, 7.74; S, 8.00. Found: C, 54.04; H, 5.89; P, 7.81; S, 7.96.

Eluted second was **15b** (229 mg, 23%), R_F 0.25 (EtOAc), m.p. 137–139°, $[\alpha]_D^{25}$ +93° (*c* 1, $CDCl_3$). See Tables I and II for the n.m.r. data. ^{31}P-N.m.r. data ($CDCl_3$): δ 46.1.

Anal. Calc. for $C_{18}H_{25}O_6PS$: C, 53.99; H, 6.29; P, 7.74; S, 8.00. Found: C, 54.18; H, 6.17; P, 7.84; S, 7.95.

6-(1',2'-Dihydroxyethyl)-4,5-dihydroxy-4,5:1',2'-di-O-isopropylidene-2-vinyl-D-manno-*1,2*λ^5*-oxaphosphorinan-2-one* (**16**). — To a solution of **15b** (302 mg, 0.76 mmol) in tetrahydrofuran (3 mL) under N_2 at −20° was added a M solution of vinylmagnesium bromide (0.83 mL, 0.83 mmol) in tetrahydrofuran during 5 min at −20°. The temperature was allowed to reach 0° during 30 min and the mixture was stored overnight at 0°. EtOAc (5 mL) was added followed by saturated aqueous

ammonium chloride (1 mL, pH 8) with vigorous stirring. The organic phase was separated and the aqueous phase extracted with EtOAc (5 mL). The combined organic extracts were dried and concentrated. Column chromatography (EtOAc–MeOH–toluene, 7:1:3) gave **16** (126 mg, 52%), R_F 0.25, 155–157°, $[\alpha]_D^{20}$ +4.9° (*c* 0.5, dichloromethane). See Tables I and II for the n.m.r. data. ^{31}P-N.m.r. ($CDCl_3$): δ 34.9. F.a.b.-mass spectrum: Calc. for $C_{14}H_{24}O_6P$ (M + H$^+$): *m/z* 319.1310. Found: *m/z* 319.1285.

Anal. Calc. for $C_{14}H_{23}O_6P$: C, 52.83; H, 7.28; P, 9.73. Found: C, 53.20; H, 7.09; P, 8.78.

Also eluted was **15b** (111 mg, 37%). The yield of **16**, based on recovered **15b**, was 81%.

2,3:5,6-Di-O-isopropylidene-D-manno-*2,3,4,5,6-pentahydroxyhexylphosphine* (**18**). — To a solution of **11** (3.36 g, 10.0 mmol) in dry oxygen-free toluene (40 mL) under N_2 at ~ −10° was added sodium bis(methoxyethoxy)aluminium hydride (7.05 mL of a 70% solution in toluene, 50.0 mmol) via a syringe over 30 min. The mixture was stirred for 1.5 h while the temperature was allowed to reach 0°. The mixture was then stored overnight at −25°, water (5 mL) was added slowly with vigorous stirring at −5° to +2°, followed by *n*-hexane (60 mL), and stirring was continued for 10 min. The organic phase was decanted and the aqueous phase was extracted with a mixture of toluene (20 mL) and hexane (30 mL). The organic extracts were combined (under N_2), dried, and concentrated to give **18** (1.78 g, 70%) as a viscous oil, R_F 0.70 (EtOAc–^{i}PrOH, 9:1), $[\alpha]_D^{20}$ −10° (*c* 0.8, toluene); $\nu_{max}^{CHCl_3}$ 2270 cm^{-1} (P–H). ^{31}P-N.m.r. ($CDCl_3$): δ −152.6 (t, *J* 200 Hz). See Table I for the ^{13}C-n.m.r. data. A f.a.b.-mass spectrum could not be obtained for this compound due to oxidation.

6-(1',2'-Dihydroxyethyl)-4,5-dihydroxy-4,5:1',2'-di-O-isopropylidene-D-manno-*1,2λ^5-oxaphosphorinan-2-one* (**19**). — To a stirred mixture of **18** (1.78 g, 6.39 mmol), sodium hydrogencarbonate (54 mg), and 2-propanol (40 mL) at 0° under N_2 was added hydrogen peroxide (1.30 mL, 2 equiv.) dropwise over 20 min. T.l.c. (EtOAc–^{i}PrOH, 9:1) after the addition of 1 equiv. of H_2O_2 indicated almost complete conversion into a new product, R_F 0.07. The stirred mixture was allowed to attain room temperature over 2 h. T.l.c. then indicated complete conversion into two products, R_F 0.15 and 0.25. Excess of sodium hydrogencarbonate (1.0 g, 11.9 mmol) was added together with toluene (100 mL), and the mixture was concentrated. Column chromatography (EtOAc–^{i}PrOH, 9:1) of the residue gave **19a** (417 mg, 22%), R_F 0.25, m.p. 120–122°, $[\alpha]_D^{20}$ −37° (*c* 1.2 chloroform); $\nu_{max}^{CHCl_3}$ 2400 (broad, P–H), 1380 cm^{-1} (geminal CH_3), F.a.b.-mass spectrum: Calc. for $C_{12}H_{22}O_6P$ (M + H$^+$): *m/z* 393.1154. Found: *m/z* 293.1175. See Tables I and II for the n.m.r. data. ^{31}P-N.m.r. ($CDCl_3$): δ 24.8 (d, *J* 610 Hz).

Anal. Calc. for $C_{12}H_{21}O_6P$: C, 49.31; H, 7.24. Found: C, 49.24; H, 7.11.

Eluted second was **19b** (531 mg, 28%), R_F 0.15, m.p. 134–137°, $[\alpha]_D^{20}$ −27° (*c* 1, chloroform); ν_{max}^{KBr} 2380 (broad, P–H), 1380 cm^{-1} (geminal CH_3). F.a.b.-mass spectrum: Calc. for $C_{12}H_{22}O_6P$ (M + H$^+$): *m/z* 393.1154. Found: *m/z* 293.1174.

See Tables I and II for the n.m.r. data. ^{31}P-N.m.r. ($CDCl_3$): δ 25.4 (d, J 556 Hz).

Anal. Calc. for $C_{12}H_{21}O_6P$: C, 49.31; H, 7.24. Found: C, 49.22; H, 7.09.

D-manno-*2,3,4,5,6-Pentahydroxyhexylphosphinic acid* (**20**). — To a solution of **19a** (103 mg, 0.35 mmol) in tetrahydrofuran (2 mL) and water (0.5 mL) was added trifluoroacetic acid (1 mL) and the mixture was stirred for 4 h at room temperature and then stored overnight at −20°. Repeated concentration of the mixture with toluene yielded **20** (74 mg, 100%) as a colourless glass. Trituration with tetrahydrofuran gave **20** as an amorphous powder, m.p. 89–91°, $[\alpha]_D^{20}$ +16° (*c* 0.5, water). See Table II for the ^{13}C-n.m.r. data. ^{31}P-N.m.r. (H_2O + 10% D_2O): δ 32.6. F.a.b.-mass spectrum: Calc. for $C_6H_{14}O_7P$ ($M - H^-$): *m/z* 229.0477. Found: *m/z* 229.0452.

Anal. Calc. for $C_6H_{15}O_7P$: C, 31.31; H, 6.56. Found: C, 31.30; H, 6.21.

2-Benzyloxycarbonyl-6(1',2'-dihydroxyethyl)-4,5-dihydroxy-4,5:1',2'-di-O-isopropylidene-D-manno-*1,2λ^5-oxaphosphorinan-2-one* (**22**). — To a solution of **19a** (176 mg, 0.60 mmol) in $CDCl_3$ (1 mL) was added hexamethyldisilazane (0.138 mL, 0.66 mmol), and the solution was kept at 55° overnight. The ^{1}H- and ^{13}C-n.m.r. spectra of the mixture then indicated that ~17% of **19a** remained and heating was continued for 7 h at 70°. The n.m.r. spectra then indicated >95% conversion into 6-(1',2'-dihydroxyethyl)-4,5-dihydroxy-4,5:1',2'-di-*O*-isopropylidene-2-trimethylsilyloxy-D-*manno*-1,2λ^3-oxaphosphorinane (**21**). See Table II for the ^{13}C-n.m.r. data. ^{31}P-n.m.r. data: δ 149.4.

The mixture was allowed to cool to room temperature, benzyl chloroformate (0.170 mL, 1.20 mmol) was added, and, after 30 min, the ^{13}C-n.m.r. spectrum indicated complete conversion into **22**. The mixture was stored overnight at room temperature, excess of sodium hydrogencarbonate (323 mg, 3.8 mmol) was added, and the mixture was concentrated. Column chromatography (EtOAc–hexane, 5:1) of the residue (708 mg) afforded **22** (184 mg, 72%), m.p. 187–188°, $[\alpha]_D^{20}$ +18° (*c* 0.5, chloroform); ν_{max}^{KBr} 1718 (carboxyl ester) 1373, 1383 cm^{-1} (geminal CH_3). F.a.b.-mass spectrum: Calc. for $C_{20}H_{27}O_8P$ ($M + H^+$): *m/z* 427.1522. Found: *m/z* 427.1546. See Tables I and II for the n.m.r. data. ^{31}P-N.m.r. ($CDCl_3$): δ 22.1.

Anal. Calc. for $C_{20}H_{26}O_8P$: C, 56.47; H, 6.16. Found: C, 56.28; H, 6.26.

Dilithium (D-manno-*2,3,4,5,6-pentahydroxyhexyl)phosphinatoformate* (**24**). — To a solution of **22** (105 mg, 0.25 mmol) in tetrahydrofuran (5 mL) and water (1 mL) was added trifluoroacetic acid (2.5 mL). The mixture was stirred at room temperature until t.l.c. (^{i}PrOH–EtOAc–water, 5:3:1) indicated complete disappearance of **22** (15 h). Co-concentration of the mixture several times with toluene and trituration of the residue with ethyl acetate gave benzyl (D-*manno*-2,3,4,5,6-pentahydroxyhexyl)phosphinicoformate (**23**; 43 mg, 47%) as an amorphous solid, R_F 0.20 (^{i}PrOH–EtOAc–water, 5:3:1), $[\alpha]_D^{20}$ +7.5° (*c* 0.5, water); ν_{max}^{KBr} 1712 cm^{-1} (carboxylic ester). See Table II for the ^{13}C-n.m.r. data. ^{31}P-N.m.r. (D_2O): δ 21.8. F.a.b.-mass spectrum: Calc. for $C_{14}H_{22}O_9P$ ($M + H^+$): *m/z* 363.0845. Found: *m/z* 363.0875.

To a solution of **23** (38 mg, 0.107 mmol) in water (7 mL) was added M LiOH

(0.225 mL, 2.1 equiv.) with stirring to give a pH of 11.6. The mixture was stirred for 1 h at room temperature, stored overnight at 0°, then concentrated. A solution of the residue in water (0.5 mL) was extracted with EtOAc (5 mL) and then repeatedly co-concentrated with tetrahydrofuran–toluene to give **24** (32 mg, 100%); ν_{max}^{KBr} 1578, 1640, 1420 (carboxylate), 1150 (P=O$^-$), 1040 cm^{-1} (P–O$^-$). The ^{13}C-n.m.r. spectrum indicated contamination, by ~25% of the decarboxylation product (the Li salt of **20**). See Table II for the ^{13}C-n.m.r. data. ^{31}P-N.m.r. (D_2O): δ 26.3. F.a.b.-mass spectrum: Calc. for $C_7H_{14}Li_2O_9P$ (M + H$^+$): *m/z* 287.0696. Found: *m/z* 287.0661.

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