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SYNTHESIS OF A NON-ISOSTERIC, ISOPOLAR MONOPHOSPHONATE ANALOGUE OF β-D-FRUCTOSE 2,6-BISPHOSPHATE*

AIMÉE DESSINGES AND ANDREA VASELLA†

Organisch-Chemisches Institut der Universität Zürich, Winterthurerstr. 190, CH-8057 Zürich (Switzerland)

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ABSTRACT

A synthesis of the non-isosteric, isopolar glyculosylmonophosphonate analogue **6** of β-D-fructose 2,6-bisphosphate (**1**) is described. Treatment of 2-*O*-acetyl-1,3,4,6-tetra-*O*-benzyl-α,β-D-fructofuranose (**8**) with trialkyl or triaryl phosphite in the presence of trimethylsilyl trifluoromethanesulfonate gave the glyculosylphosphonates **9**, **11**, and **13**, respectively, with the 2,3-*cis*-configuration, as the major anomers, and **10**, **12**, and **14**, respectively, as the minor anomers. The structures of **9**–**14** were deduced from the ^{1}H-, ^{13}C-, and ^{31}P-n.m.r. spectra, which indicated a $^4T_3$ conformation for each conformer. Selective acetolysis of **13** gave diphenyl (6-*O*-acetyl-1,3,4-tri-*O*-benzyl-β-D-fructofuranosyl)phosphonate (**15**). Base-catalysed transesterification of **15** gave diphenyl (1,3,4-tri-*O*-benzyl-β-D-fructofuranosyl)phosphonate (**17**), which cyclised easily to give the phostone **18**. Phosphorylation of **17** followed by hydrogenolysis gave the β-D-fructofuranosylphosphonate **6** characterised as the cyclohexylammonium salt **20**.

INTRODUCTION

The role of β-D-fructose 2,6-bisphosphate[1] (**1**, Fru-2,6-P_2) as a regulator of glycolysis and gluconeogenesis in animals[2] and in plants[3] has generated growing interest during the past six years. Fru-2,6-P_2 promotes glycolysis by stimulating 6-phosphofructo-1-kinase (EC 2.7.1.11), the enzyme that catalyses the conversion of D-fructose 6-phosphate into D-fructose 1,6-bisphosphate, and it attenuates gluconeogenesis by inhibiting fructose 1,6-bisphosphatase (EC 3.1.3.11), the enzyme that catalyses the reverse reaction[4].

The easy hydrolysis of Fru-2,6-P_2 to D-fructose 6-phosphate has led to syntheses of analogues which are stable towards hydrolysis and thus useful for biochemical studies. The synthesis of α- and β-D-arabinose 1,5-bisphosphate (**2**), analogues of Fru-2,6-P_2 lacking the anomeric substituent, has been described[5] and

*Dedicated to Professor Hans Paulsen.
†To whom correspondence should be addressed.

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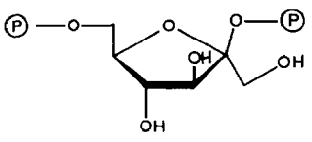

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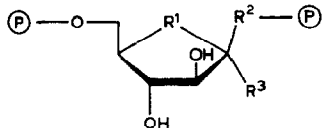
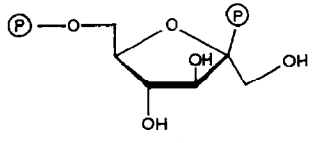


2 $R^1 = O, R^2 = O, R^3 = H$
3 $R^1 = CH_2, R^2 = O, R^3 = CH_2OH$
4 $R^1 = O, R^2 = CH_2, R^3 = H$
5 $R^1 = O, R^2 = CH_2, R^3 = CH_2OH$



6

their relative biological activity confirmed the β-D configuration of Fru-2,6-P_2. Preliminary results indicated that the carbocyclic analogue[6] **3** of Fru-2,6-P_2 was also a strong positive effector of phosphofructokinase. Two syntheses of the monophosphonate **4**, an isosteric analogue of β-D-arabinose 1,5-bisphosphate, have been reported, one of the stereochemically pure compound[7], and the other of a mixture of anomers[8]. The synthesis of the monophosphonate **5** (and of its α-D anomer), an isosteric analogue of **1**, has been described[9].

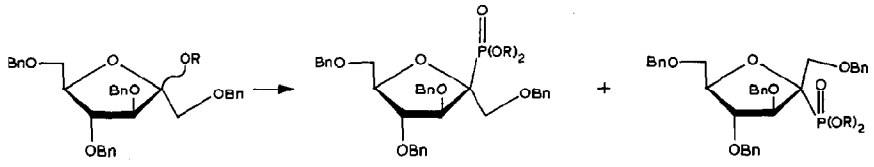
We now report the synthesis of the glyculosylphosphonate **6**, a non-isosteric but isopolar* analogue of Fru-2,6-P_2. Paulsen and Thiem[11] prepared the first compounds carrying a phosphono group at the anomeric center, and Vasella and Meuwly[12] have reported a general synthesis of non-isosteric phosphonate analogues of aldose 1-phosphates with the 1,2-*cis*-configuration by the reaction of benzylated 1-*O*-acetylaldoses with trialkyl phosphites in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf). The preponderant formation of 1,2-*cis*-phosphonates was rationalised[13,14] by postulating an equilibrium between the anomeric phosphonium salt intermediates and a stabilisation of the salts with the *cis*-configuration through the formation of a pentaco-ordinated species by participation of the neighbouring benzyloxy group. It was anticipated that the analogous reaction of a 2-*O*-acetylulose would lead preferentially to a 2,3-*cis*-glyculosylphosphonate. Although both AlkO-1 and AlkO-3 can interact with the phosphonium salt group, to a first approximation, only the AlkO-3 will determine the relative configuration of the product.

RESULTS AND DISCUSSION

Treatment of 2-*O*-acetyl-1,3,4,6-tetra-*O*-benzyl-α,β-D-fructofuranose[15] (**8**; αβ-ratio 5.5:1) with 1.5 equiv. of $P(OMe)_3$ and 1.2 equiv. of TMSOTf in CH_2Cl_2 at room temperature[12] gave dimethyl (1,3,4,6-tetra-*O*-benzyl-β-D-fructofuranosyl)-

*The isopolar character is anticipated, since α-D-glucose 1-phosphate and the corresponding glucosylphosphonate possess approximatively the same pK_a values[10].

phosphonate (**9**, 72%), with the 2,3-*cis*-configuration, and its α-anomer **10** (10%). Under similar conditions, **8** reacted with $P(OEt)_3$ to yield the diethyl phosphonate **11** (78%), with the 2,3-*cis*-configuration, and its anomer **12** (9%), and with $P(OPh)_3$ to give the diphenyl phosphonates **13** (67%) and **14** (6%) (Table I). The anomers were separated by flash chromatography and the configuration at C-2 was deduced from their n.m.r. spectra.



7 R = H
8 R = Ac

9 R = Me (72%)
11 R = Et (78%)
13 R = Ph (67%)

10 R = Me (10%)
12 R = Et (9%)
14 R = Ph (6%)

The ^{1}H-n.m.r. spectra (Tables II and III) showed similar and relatively large values for $J_{3,4}$ and $J_{4,5}$ (6.7–7.0 and 7.0–8.0 Hz, respectively) for the phosphonates **9–14**, irrespective of their anomeric configuration, indicating that each has the $^4T_3$ conformation. However, the similarity of the ^{1}H-n.m.r. spectra does not allow assignment of the anomeric configurations. A *T* (twist) conformation is not unexpected considering the similar A values of the dimethoxyphosphoryl[18] (2.0 kcal.mol^{-1}) and the hydroxymethyl group[19] (1.8 kcal.mol^{-1}), and the weak anomeric effect of a dimethoxyphosphoryl group[19] (0.56 kcal.mol^{-1}).

The configuration at C-2 of **11** and **12**, however, was deduced from n.O.e. measurements. After irradiation of the C-1 methylene groups, a n.O.e. of 8% was observed on the signal for H-3 of **11**, but there was no effect on the signal for H-3 of **12**, indicating the β-D configuration, as expected, for the major product.

The configuration at the anomeric center was also deduced from the $^3J_{P,H\text{-}3}$ (Table III) and $^3J_{P,C\text{-}4}$ values (Table V). A Karplus-type relation[16,17] between the vicinal ^{31}P–C–C–^{1}H coupling constants and the corresponding dihedral angle of phosphonates indicates maxima at 0° and 180° of ~18 and ~41 Hz, respectively, and a minimum at 90°. The values of $^3J_{P,H\text{-}3}$ of 16–22.2 Hz for **10**, **12**, and **14**, and 21.5–22.2 Hz, for **9**, **11**, and **13** correspond to vicinal angles ϕ(P,H) of ~0° or

TABLE I

TOTAL YIELDS AND RATIOS OF THE GLYCULOSYLPHOSPHONATES **9–14**[a]

Phosphite	*Products*	*αβ-Ratio*	*Total yield (%)*
$P(OMe)_3$	**9** and **10**	1:7	82
$P(OEt)_3$	**11** and **12**	1:8	87
$P(OPh)_3$	**13** and **14**	1:11	73

[a]Obtained by treatment of 2-*O*-acetyl-1,3,4,6-tetra-*O*-benzyl-α,β-D-fructofuranose (**8**) with trialkyl (or triaryl) phosphites (1.5 equiv.) and TMSOTf (1.2 equiv.).

TABLE II

^{1}H-N.M.R. CHEMICAL SHIFT DATA (P.P.M.)

Hydrogen	*α*-D *Series*			*β*-D *Series*						
	10[a]	**12**[a]	**14**[a]	**9**[a]	**11**[b]	**13**[a]	**15**[a,c]	**17**[b,d]	**18**[a]	**19**[a]
1a	3.92 dd	3.86 dd	4.04 t	3.84 dd	3.87 dd	4.09 dd	4.20–3.94 m	4.07 dd	4.26–4.21 m	4.05 dd
1b	3.84–3.72 m	3.76 dd	3.93 t	3.78 t	3.80 dd	3.99 dd		4.02 t		3.96 t
3	4.66 dd	4.65 dd	4.93 dd	4.69 dd	4.70 dd	4.80 dd	4.81 dd	4.84 dd	↑	4.81 dd
4	4.22 dd	4.20 m	4.28 dd	4.36 dd	4.39 dd	4.45 t	4.50 t	4.75–4.52 m	4.86–4.43 m	↑
5	4.10 ddd	4.08 ddd	4.20 ddd	4.20 m	4.19 m	4.30 m	4.22 m	4.16 m	↓	4.50–4.30 m
6a	3.67 dd	3.66 dd	3.68 dd	3.76–3.60 m	3.75 dd	3.65 AB	4.35 dd	3.75 ddd	4.03 dd	↓
6b	3.59 dd	3.58 dd	3.60 dd		3.69 dd	3.60 AB	4.16 t	3.64 ddd	3.95 dd	
Benzylic	4.90–4.46 m (8 H)	4.92–4.40 m (8 H)	4.90–4.44 m (8 H)	4.80–4.45 m (8 H)	4.75–4.50 m (8 H)	4.90–4.40 m (8 H)	4.80–4.50 m (6 H)	4.75–4.52 m (6 H)	4.86–4.42 m (6 H)	4.90–4.42 m (6 H)
Aromatic	7.40–7.15 m (20 H)	7.40–7.10 m (20 H)	7.40–7.03 m (30 H)	7.40–7.15 m (20 H)	7.20–7.08 m (20 H)	7.40–7.00 m (30 H)	7.40–7.20 m (25 H)	7.40–7.05 m (25 H)	7.30–7.10 m (20 H)	7.38–7.00 m (35 H)
OCH_3	3.81 d 3.80 d			3.70 d 3.65 d						
OCH_2CH_3		4.30–4.04 m (4 H) 1.30 t (6 H)			4.15–3.98 m (4 H) 1.19 t (6 H)					

[a]At 200 MHz. [b]At 400 MHz. [c]AcO-6, δ 1.90 s. [d]HO-6, δ 2.65 dd.

TABLE III

^{1}H-N.M.R. SPIN-COUPLING DATA (Hz)

J	α-D *Series*			β-D- *Series*						
	10	**12**	**14**	**9**	**11**	**13**	**15**[a]	**17**	**18**	**19**
$J_{1a,1b}$	10.7	10.7	10.5	10.7	10.8	10.8		10.7		10.8
$J_{3,4}$	7.0	7.0	6.5	6.7	7.0	7.0	7.0	7.0		5.9
$J_{4,5}$	8.0	8.0	8.0	7.8	7.7	7.0	7.0			
$J_{5,6a}$	3.2	3.0	3.5		6.2		3.0	1.8	8.0	
$J_{5,6b}$	4.2	4.8	5.0		4.0		10.0	4.5	6.5	
$J_{6a,6b}$	10.9	10.1	11.0		10.6	11.0	10.0	9.8	11.0	
$^3J_{P,1a}$	9.0	9.0	10.5	4.0	4.2	6.0		5.5		6.0
$^3J_{P,1b}$		9.0	10.5	10.7	7.6	10.8		10.7		10.8
$^3J_{P,3}$	22.2	22.2	16.0	21.5	22.0	22.5	30.4	24.0		21.5
$^3J_{P,CH_3}$	10.4			10.3						
$^3J_{P,CH_3}$	10.5			10.7						

[a] $J_{6a,OH}$ 3.6, $J_{6b,OH}$ 8.8 Hz.

~150°. The ^{13}C-n.m.r. spectra of **9–14** show a clear dependency of the $^3J_{P,C-4}$ values on the anomeric configuration (*cf.* Table V). A "Karplus-like" correlation between $^3J_{P,C}$ and θ(P,C) for phosphonate derivatives and the influence of the electronegativity of the substituents and their orientation, of the ring strain, and of bond-angle distortions has been discussed[16,20,21]. The large $^3J_{P,C-4}$ values for **10, 12,** and **14** correspond to dihedral angles of ~120 or ~40°, and the zero values of the vicinal coupling constants for **9, 11,** and **13** to a dihedral angle close to 90° (Fig. 1). The $^4T_3$ conformation of the phosphonates **9, 11,** and **13,** deduced from the ^{1}H-n.m.r. spectra, imply that the β-D anomers possess P,H-3 angles of ~150° and P,C-4 angles of ~90°, and the α-D anomers P,H-3 angles of ~0° and P,C-4 angles of ~120°. The $^3J_{P,H-3}$ and $^3J_{P,C-4}$ values found for the two series of anomers are compatible only with a β-D configuration for **9, 11,** and **13** and an α-D configuration for **10, 12,** and **14,** confirming the result of the n.O.e. experiments.

The chemical shifts of the signals for C-2 of the α-D anomers appeared at a higher field than those of the corresponding β-D anomers (*cf.* Table IV). A similar situation has been found for partially deoxygenated glycosylphosphonates[18].

The $^1J_{P,C}$ values depend on the axial or equatorial orientation of the dialkoxyphosphoryl group, with $^1J_{C,Pe} > {}^1J_{C,Pa}$[18,22]. A $^1J_{P,C}$ value of 166.7–167.7 Hz was found for the α-D-fructofuranosylphosphonates and a value of 163.4–165.9 Hz for the β-D anomers, indicating a pseudo-equatorial orientation of the P-substituent for the α-D-phosphonates and a pseudo-axial orientation for the β anomers.

Selective acetolysis[23] of the least sterically hindered benzyl ether group of **13,** catalysed by boron trifluoride etherate, yielded the monoacetate **15** (65%). The reaction was stopped when t.l.c. showed that the formation of the diacetate **16** was beginning. In the ^{13}C-n.m.r. spectra, the signal for C-6 of **15** occurred at 63.62

TABLE IV

^{13}C- AND ^{31}P-N.M.R. CHEMICAL SHIFT DATA (P.P.M.)

C or P	*α*-D *Series*			*β*-D *Series*								
	10	**12**	**14**	**9**	**11**	**13**	**15**[a]	**17**	**18**	**19**	**20**[b]	**22**[c]
C-1	69.63	69.83	69.61	70.82	70.84	70.26	70.56	72.73	74.50	70.44	65.16	63.27
C-2	82.86	82.60	82.94	83.60	85.21	85.69	85.57	85.74	87.68	86.13	85.71	84.02
C-3	85.65	85.78	85.53	84.38	84.24	84.69	84.49	84.75	87.30	84.53	80.32	77.94
C-4	82.91	83.12	83.04	83.51	83.58	83.60	82.49	81.72	85.67	83.04	78.05	75.90
C-5	79.07	79.05	79.37	80.80	80.60	81.63	79.68	83.34	79.93	80.77	82.97	81.24
C-6	69.52	69.77	69.57	70.63	70.90	70.73	69.62	62.38	67.45	68.41	66.04	61.79
Benzylic	73.85	73.82	73.84	73.74	73.71	73.84	73.88	73.85	73.96	73.81		
	73.47	73.49	73.65	73.46	73.32	73.48	73.73	73.34	72.92	73.59		
	73.19	73.19	73.17	73.26	73.28	73.22	72.84	73.25	71.52	72.60		
	72.70	72.65	72.67	72.76	72.67	72.64						
C_{ar} (s)	138.21	138.32	138.19	138.18	138.24	138.20	137.67	137.77	137.40	137.53		
of Bn	138.13	138.32	138.00	138.01	138.07	137.88	137.45	137.54	137.23	137.39		
	137.99	138.15	137.94	137.89	137.99	137.75	137.45	137.41	137.09	137.22		
	137.95	138.15	137.77	137.73	137.92	137.58						
C_{ar} (s)			150.46			150.91	150.9	150.9	150.08	150.64		
of OPh			150.42			150.36	150.0	150.1		150.41		
										150.34		
										150.27		
C_{ar} (d)	128.26–127.33	128.26–127.27	129.54–120.58	128.28–127.40	128.32–127.49	129.44–120.91	129.50–120.89	129.51–120.68	129.63–120.25	128.33–119.96		
OCH_3	53.66			54.22								
	53.37			54.92								
OCH_2CH_3		62.99			63.41							
		62.86			62.24							
		16.56			16.46							
		16.45			16.34							
P-2	25.72	23.21	16.29	22.07	19.56	12.59	11.99	13.21	7.70	12.44	16.30	15.30
										–11.35		
PO-6											4.07	

[a]AcO-6, 170.75 and 20.73. [b]In CD_3OD, signals of cyclohexylammonium: 51.27, 32.00 (2×), 26.02, and 25.51 (2×). [c]In D_2O, signals of cyclohexylammonium: 50.66, 30.74 (2×), 24.70, and 24.21 (2×).

TABLE V

^{13}C–^{31}P SPIN-COUPLING DATA (Hz)

J	α-D *Series*			β-D *Series*								
	10	**12**	**14**	**9**	**11**	**13**	**15**	**17**	**18**[a]	**19**	**20**	**22**
$^{2}J_{P,C-1}$	10.2	11.5	11.3	13.9	14.0	11.3	14.4	15.6	10.0	13.1	10.9	11.1
$^{1}J_{P,C-2}$	166.7	166.7	167.7	165.9	164.1	163.4	168.1	167.1	142.5	168.2	146.3	152.0
$^{2}J_{P,C-3}$	4.0	3.9	5.2	2.6	2.9	2.5	2.6	3.4	0	2.5	2.1	4.9
$^{3}J_{P,C-4}$	6.8	7.1	7.1	0	0	0	0	0	0	0	0	0
$^{3}J_{P,C-5}$	2.7	2.8	2.2	3.4	3.4	3.8	3.7	3.2	0	4.1	0	0
$^{3}J_{P',C-5}$										8.3	2.2	
$^{2}J_{P',C-6}$										6.4	3.5	
$^{2}J_{PO,C}$	7.0	6.8	10.3	6.5	6.8	9.7	~10	~10	10.4	10.6		
	7.3	6.8	10.4	7.3	7.4	9.7	~10	~10		10.6		
										7.0		
										7.0		
$^{3}J_{POC,C}$		2.4			3.9							
		2.7			3.7							

[a] $^{3}J_{P,C-6}$ 10.3 Hz.

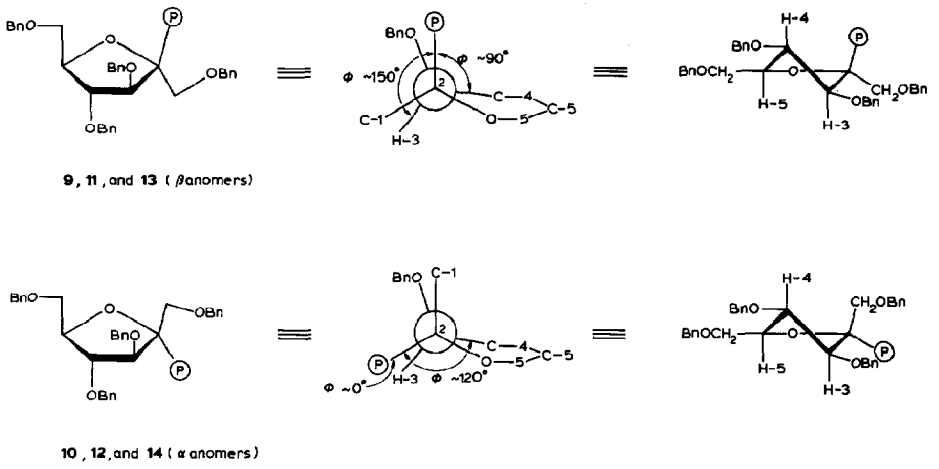
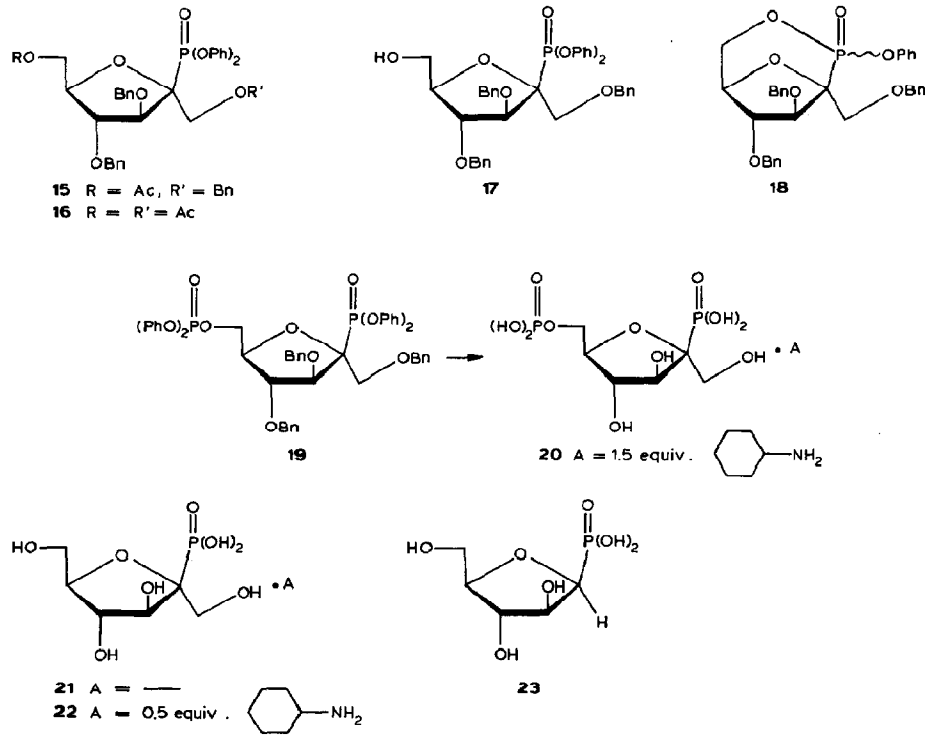


Fig. 1. Conformations of the glyculosylphosphonates **9**–**14**.



p.p.m. and that of **13** at 70.73 p.p.m. Deacetylation of **15** with methanolic sodium 3-nitrophenoxide[24] gave the alcohol **17** (major product) and a cyclic ester **18** with unknown configuration at phosphorus. These products were separated by flash chromatography, during which more **17** cyclised to **18**. The ^{31}P-n.m.r. spectra of

compounds **17** and **18** contained signals at +13.21 p.p.m. and +7.70 p.p.m., respectively. The i.r. spectrum of **18** showed the absence of hydroxyl and carbonyl groups. The ^{1}H- and ^{13}C-n.m.r. spectra confirmed the presence of one phenoxy group. Moreover, in the ^{13}C-n.m.r. spectrum, the signal of C-6 at 67.45 p.p.m. showed a significant $^2J_{P,C\text{-}6}$ coupling of 10.4 Hz. The structure of **18** was confirmed by the c.i.-mass spectrum, which contained a peak for ($M^+ + 1$) at *m/z* 573, and the elemental analysis. The formation of **18** is compatible only with a β-D configuration of **17**.

The crude mixture of **17** and **18** was treated with diphenyl phosphorochloridate in pyridine[25] to give the phosphonate **19** (40% from **15**), the ^{31}P-n.m.r. spectrum of which contained signals at +12.44 p.p.m. for CPO_3Ph_2 and at −11.35 p.p.m. for OPO_3Ph_2. Hydrogenolysis of **19** in the presence of 10% Pd/C removed the three benzyloxy groups, then in the presence of PtO_2 removed the four phenyl ester groups. The phosphonic acid **6** was isolated as the cyclohexylammonium salt **20** (75%). The structure of **20** was confirmed by its ^{1}H-, ^{13}C-, and ^{31}P-n.m.r. spectra. The ^{1}H-n.m.r. spectrum reflected the loss of all the groups and the presence of 1.5 mol of cyclohexylamine. Particularly significant signals in the ^{13}C-n.m.r. spectrum occurred at 66.04 (C-6, $^2J_{P,C\text{-}6}$ 3.5 Hz) and at 85.71 p.p.m. (C-2, $^1J_{P,C\text{-}2}$ 146.3 Hz). The ^{31}P-n.m.r. spectrum contained signals at +16.30 p.p.m. for CPO_3H_2 and at +4.07 p.p.m. for OPO_3H_2. The structure was confirmed by the f.a.b.-mass spectrum, which contained a peak for ($C_6H_{14}O_{11}P_2^+ + 1$) at *m/z* 325. The elemental analysis ($C_6H_{14}O_{11}P_2 \cdot 1.5C_6H_{13}N \cdot H_2O$) was consistent with the relative amount of cyclohexylamine determined by ^{1}H-n.m.r. spectroscopy.

Back-titration of the crystalline cyclohexylammonium salt of **6** showed only one inflection point at pH 6.67. Back-titration of the cyclohexylammonium salt of the phosphonate **21** indicated a pK'(2) value of 6.05, similar to the values for the *arabino*-phosphonic acid[10] **23** (6.10), α-D-glucose 1-phosphate (6.22), and β-D-glucose 1-phosphate (6.00) (ref. 26).

EXPERIMENTAL

General methods. — Melting points were determined on a Büchi 510 apparatus and are uncorrected. Qualitative t.l.c. was effected on Kieselgel 60 F_{254} (Merck) with detection with a 0.02M solution of I_2 in aqueous 10% H_2SO_4 or ethanolic 10% phosphomolybdic acid at ~200°. Flash chromatography was carried out on Kieselgel 60 (Merck, 230–400 mesh). I.r. spectra were recorded for 3% solutions in $CHCl_3$ with a Perkin–Elmer 298 spectrometer. N.m.r. spectra were recorded with a Varian-XL-200 [^{1}H (200 MHz), ^{13}C (50.3 MHz), ^{31}P (80 MHz)] or Bruker-AM-400 spectrometer [^{1}H (400 MHz), ^{13}C (100.6 MHz)] for solutions in $CDCl_3$ unless otherwise specified; internal Me_4Si for ^{1}H and ^{13}C, and external H_3PO_4 for ^{31}P. Mass spectra were recorded on Varian 112S (e.i. 70 eV; c.i. isobutane) and Varian 711 spectrometers (f.a.b., 8 keV Xe atoms, glycerol matrix). Determinations of pK_a were made by using a Mettler DL 40 GP Memo Titrator pH meter.

Dimethyl (1,3,4,6-tetra-O-benzyl-β- (**9**) *and -α-*D*-fructofuranosyl)phosphonate* (**10**). — Treatment of 2-*O*-acetyl-1,3,4,6-tetra-*O*-benzyl-α,β-D-fructofuranose (**8**; 485 mg, 0.87 mmol; αβ-ratio 5:1) with $P(OMe)_3$ (162 mg, 0.15 mL, 1.30 mmol) and trimethylsilyl trifluoromethanesulfonate (232 mg, 0.19 mL, 1.04 mmol) in CH_2Cl_2 (4.3 mL) gave, after chromatography (50 g of SiO_2; AcOEt–CH_2Cl_2–hexane, 3:3:4), **9** (405 mg, 72%) and **10** (56 mg, 10%).

Compound **9** had $[\alpha]_D^{25}$ +10.5° (*c* 1.3, chloroform), R_F 0.25; ν_{max} 2960, 2910, 2860, 1455, 1365, 1240 (P=O), 1095 (P–O–C), 1030, and 1000 cm^{-1}. Mass spectrum (c.i.): *m/z* 633 ($M^+ + 1$).

Anal. Calc. for $C_{36}H_{41}O_8P$ (632.64): C, 68.35; H, 6.53; P, 4.89. Found: C, 68.59; H, 6.71; P, 4.63.

Compound **10** had $[\alpha]_D^{25}$ −8° (*c* 0.8, chloroform), R_F 0.10; ν_{max} 2960, 2920, 2875, 1455, 1360, 1240 (P=O), 1105 (P–O–C), 1060, 1045, 1000, and 860 cm^{-1}. Mass spectrum (c.i.): *m/z* 633 ($M^+ + 1$).

Anal. Found: C, 68.25; H, 6.57; P, 4.65.

*Diethyl (1,3,4,6-tetra-O-*benzyl-*β-* (**11**) *and -α-*D*-fructofuranosyl)phosphonate* (**12**). — Treatment of **8** (1.47 g, 2.65 mmol, αβ-ratio 5:1) with $P(OEt)_3$ (660 mg, 0.68 mL, 3.97 mmol) and trimethylsilyl trifluoromethanesulfonate (707 mg, 0.58 mL, 3.18 mmol) in CH_2Cl_2 (5 mL) gave, after chromatography (150 g of SiO_2; AcOEt–CH_2Cl_2–hexane, 3:3:4), **11** (1.30 g, 78%) and **12** (155 mg, 9%).

Compound **11** had $[\alpha]_D^{25}$ −2.5° (*c* 0.8, chloroform), R_F 0.33; ν_{max} 2995, 2935, 2920, 2860, 1495, 1450, 1360, 1240 (P=O), 1095 (P–O–C), 1030, 995, 950, 690, and 660 cm^{-1}. Mass spectrum (c.i.): *m/z* 661 ($M^+ + 1$).

Anal. Calc. for $C_{38}H_{45}O_8P$ (660.74): C, 69.07; H, 6.86; P, 4.68. Found: C, 68.79; H, 6.85; P, 4.51.

Compound **12** had $[\alpha]_D^{25}$ −9° (*c* 1.1, chloroform), R_F 0.20; ν_{max} 3000, 2930, 2910, 2860, 1450, 1360, 1240 (P=O), 1095 (P–O–C), 1090, 1050, 995, 970, 690, and 660 cm^{-1}. Mass spectrum (c.i.): *m/z* 611 ($M^+ + 1$).

Anal. Found: C, 69.31; H, 6.86; P, 4.69.

Diphenyl (1,3,4,6-tetra-O-benzyl-β- (**13**) *and -α-*D*-fructofuranosyl)phosphonate* (**14**). — Treatment of **8** (24.50 g, 44.17 mmol, αβ-ratio 5:1) with $P(OPh)_3$ (20.60 g, 17.40 mL, 66.25 mmol) and trimethylsilyl trifluoromethanesulfonate (11.78 g, 9.60 mL, 53.0 mmol) in CH_2Cl_2 (260 mL) gave, after chromatography (500 g of SiO_2; AcOEt–hexane, 1.5:8.5), **13** (22.50 g, 67%) and **14** (2.00 g, 6%).

Compound **13** had $[\alpha]_D^{25}$ −12° (*c* 1.1, chloroform), R_F 0.35 (AcOEt–hexane, 3:7); ν_{max} 3005, 2920, 2870, 1595, 1490, 1455, 1365, 1240 (P=O), 1105 (P–O–C), 1030, 1010, 940 (aromatic C–H), and 690 cm^{-1}. Mass spectrum (c.i.): *m/z* 757 ($M^+ + 1$).

Anal. Calc. for $C_{46}H_{45}O_8P$ (756.88): C, 72.99; H, 6.00; P, 4.09. Found: C, 72.73; H, 6.21; P, 3.89.

Compound **14** had $[\alpha]_D^{25}$ +2° (*c* 0.8, chloroform), R_F 0.30; ν_{max} 3000, 2910, 2870, 1590, 1490, 1450, 1365, 1240 (P=O), 1105 (P–O–C), 1030, 940 (aromatic C–H), and 685 cm^{-1}. Mass spectrum (c.i.): *m/z* 757 ($M^+ + 1$).

Anal. Found: C, 72.71; H, 5.87; P, 3.94.

Diphenyl (6-O-acetyl-1,3,4-tri-O-benzyl-β-D-fructofuranosyl)phosphonate (**15**). — A catalytic amount of boron trifluoride etherate was added to a stirred solution of **13** (10.3 g, 13.61 mmol) in acetic anhydride (80 mL) under N_2 at −40°. The mixture was allowed to warm up to 0° during 3 h, and then kept for 3 h at 0° when t.l.c. indicated the presence of diphenyl (1,6-di-*O*-acetyl-3,4-di-*O*-benzyl-β-D-fructofuranosyl)phosphonate [**16**; R_F 0.18 (AcOEt–hexane, 3:1)]. Water was added to the mixture which was then stirred until the acetic anhydride had hydrolysed. After the usual work-up, the residual syrup was purified by chromatography (500 g of SiO_2; AcOEt–hexane, 1.5:8.5) to give **13** (1.5 g, 14.5%) and **15** (6.5 g, 67%) as an oil, $[\alpha]_D^{25}$ +4° (*c* 1.2, chloroform), R_F 0.24 (AcOEt–hexane, 3:7); ν_{max} 2910, 1740 (C=O), 1595, 1455, 1390, 1370, 1240 (P=O), 1185, 1160, 1105 (P–O–C), 1030, 940 (aromatic C–H), and 905 cm^{-1}. Mass spectrum (c.i.): *m/z* 709 ($M^+ + 1$).

Anal. Calc. for $C_{41}H_{41}O_9P$ (708.78): C, 69.47; H, 5.84; P, 4.37. Found: C, 69.28; H, 6.04; P, 4.24.

Diphenyl (1,3,4-tri-O-benzyl-β-D-fructofuranosyl)phosphonate (**17**) *and phenyl (1,3,4-tri-O-benzyl-6-deoxy-β-D-fructofuranosyl-6-yloxy)phosphonate* (**18**). — To a solution of **15** (3.6 g, 5.1 mmol) in MeOH (100 mL) was added sodium 3-nitrophenoxide (160 mg, 1.0 mmol). The solution was stirred under reflux for 3 h, MeOH was evaporated under vacuum, CH_2Cl_2 was added, the precipitate was removed, and the filtrate was concentrated under vacuum. The crude mixture (1.6 g) of **17** and **18** was phosphorylated without purification. For spectroscopy and analysis, pure samples of **17** and **18** were obtained by flash chromatography (AcOEt–hexane, 1:4).

Compound **17** had $[\alpha]_D^{25}$ −13° (*c* 1.1, chloroform), R_F 0.20 (AcOEt–hexane, 2:3); ν_{max} 3580 (OH), 3440 (OH), 3075, 3005, 2930, 2880, 1595, 1490, 1460, 1455, 1360, 1250 (P=O), 1185, 1160, 1105 (P–O–C), 1030, 950 (aromatic C–H), 905, and 690 cm^{-1}. Mass spectrum (c.i.): *m/z* 667 ($M^+ + 1$), 572 ($M^+ - C_6H_6O$).

Anal. Calc. for $C_{39}H_{39}O_8P$ (666.72): C, 70.25; H, 5.91; P, 4.65. Found: C, 70.28; H, 5.89; P, 4.49.

Compound **18** had $[\alpha]_D^{25}$ +23.5° (*c* 1.2, chloroform), R_F 0.29; ν_{max} 3075, 3010, 2960, 2890, 1595, 1490, 1455, 1395, 1360, 1285 (P=O), 1170, 1095 (P–O–C), 1060, 930 (aromatic C–H), and 690 cm^{-1}. Mass spectrum (c.i.): *m/z* 573 ($M^+ + 1$).

Anal. Calc. for $C_{33}H_{33}O_7P$ (573.28): C, 69.25; H, 5.81; P, 5.40. Found: C, 69.08; H, 5.77; P, 5.19.

Diphenyl (2,3,4-tri-O-benzyl-6-O-diphenoxyphosphoryl-β-D-fructofuranosyl)phosphonate (**19**). — To a solution of the mixture (3.6 g) of crude **17** and **18** in dry pyridine (30 mL) was added diphenyl phosphorochloridate (2.05 g, 1.58 mL, 7.62 mmol) at 0° under N_2. After stirring for 30 min at 0°, the mixture was worked-up as usual. The residue was purified by flash chromatography (500 g of SiO_2; AcOEt–hexane, 3:7) to give **19** (1.8 g, 40%) and **18** (960 mg, 32%). Compound **19** had $[\alpha]_D^{25}$ −9.5° (*c* 1.2, chloroform), R_F 0.35 (AcOEt–hexane, 2:3); ν_{max} 3075,

3010, 2940, 2880, 1600, 1490, 1460, 1360, 1280 (P=O), 1185, 1160, 1100 (P=O), 1030, 1015, 1000, 950 (P–O–C), and 690 cm^{-1}. Mass spectrum (c.i.): *m/z* 899 ($M^+ + 1$).

Anal. Calc. for $C_{51}H_{48}O_{11}P_2$ (898.93): C, 68.14; H, 5.39; P, 6.89. Found: C, 67.89; H, 5.16; P, 6.70.

Cyclohexylammonium (β-D-fructofuranosylphosphonate 6-phosphate) (**20**). — A solution of **19** (200 mg, 0.22 mmol) in MeOH (15 mL) was hydrogenolysed at room temperature and 9 atm. in the presence of 10% Pd/C (250 mg). Within 6 h, the benzyl groups had been removed, and hydrogenation was continued until the disappearance of the product with R_F 0.45 (AcOEt) was complete. After the addition of PtO_2 (150 mg), hydrogenolysis was continued at 9 atm. for 2 h. T.l.c. (1-propanol–NH_4OH–H_2O, 4:3:1) then revealed a single phosphorus-containing substance (R_F 0.18) that did not absorb u.v. light. The catalyst was collected and washed with methanol, and the combined filtrate and washings were concentrated under vacuum. A solution of the residual oil in water (1 mL) was treated with freshly distilled cyclohexylamine to pH 9 and then concentrated under vacuum to dryness. To a solution of the residue in MeOH (2 mL) was added acetone to give **20** (98 mg, 71%), m.p. 173°, $[\alpha]_D^{25}$ +21° (*c* 0.4, methanol), R_F 0.18; ν_{max}^{KBr} 3400 (OH), 2940 (NH_3^+ st.), 1630 (NH_3^+), 1530 (NH_3^+), 1450 (NH_3^+), 1390, 1150, 1070 (P=O), 1035, 980, 925, and 820 cm^{-1}. ^{1}H-N.m.r. data (200 MHz, CD_3OD): δ 4.34–3.78 (m, 7 H, H-1a,1b,3,4,5,6a,6b), 3.04 (s, 1.5 H, CH–N), and 2.12–1.08 (m, 18 H, cyclohexylamine CH_2). Mass spectrum (f.a.b.): *m/z* 325 ($C_6H_{14}O_{11}P_2^+ + 1$) and 347 ($C_6H_{14}O_{11}P_2^+ + Na$).

Anal. Calc. for $C_6H_{14}O_{11}P_2 \cdot 1.5\ C_6H_{13}N \cdot H_2O$ (487.0): C, 36.96; H, 7.28; N, 4.31; P, 12.73. Found: C, 36.78; H, 7.41; N, 4.61; P, 12.50.

Cyclohexylammonium β-D-fructofuranosylphosphonate (**22**). — A solution of **13** (1.1 g, 1.45 mmol) in MeOH (50 mL) was hydrogenolysed at room temperature and 9 atm. in the presence of 10% Pd/C (800 mg); within 6 h, the benzyl groups had been removed. Hydrogenation was continued until the disappearance of the product with R_F 0.5 (AcOEt) was complete. After the addition of PtO_2 (400 mg), hydrogenolysis was continued at 9 atm. for 2 h. T.l.c. (1-propanol–NH_4OH–H_2O, 4:3:1) then revealed a single phosphorus-containing substance (R_F 0.28) that did not absorb u.v. light. The mixture was worked-up as described for **20**, to give **22** (416 mg, 89%), m.p. 175°, $[\alpha]_D^{25}$ −3° (*c* 0.7, water), R_F 0.28; ν_{max}^{KBr} 3400 (OH), 2940 (NH_3^+ st.), 2860 (NH_3^+ st.), 1630 (NH_3^+), 1530 (NH_3^+), 1450 (NH_3^+), 1390, 1125, 1065 (P=O), 950, 925, 890, and 845 cm^{-1}. ^{1}H-N.m.r. data (200 MHz, D_2O): δ 4.46–4.28 (m, 2 H), 3.98–3.65 (m, 5 H), 3.14 (s, 0.5 H, CH–N), and 2.10–1.06 (m, 6 H, cyclohexylamine CH_2). Mass spectrum (f.a.b.): *m/z* 245 ($C_6H_{13}O_8P^+ + 1$) and 100 ($C_6H_{13}N^+ + 1$).

Anal. Calc. for $C_6H_{13}O_8P \cdot 0.5\ C_6H_{13}N$ (262.0): C, 41.22; H, 7.44; N, 2.67; P, 11.83. Found: C, 41.32; H, 7.59; N, 2.71; P, 11.62.

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