Enzymatic Resolution of Acetoxyalkenylphosphonates and Their Exploitation in the Chemoenzymatic Synthesis of Phosphonic Derivatives of Carbohydrates

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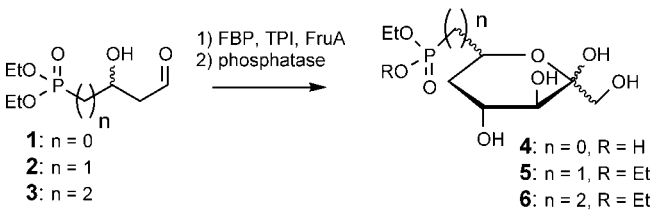
Abstract: The resolution of racemic α-, β-, and γ-hydroxy-ω-alkenylphosphonates was achieved by enzymatic hydrolysis of the corresponding acetates. The optically active alcohols were transformed into β-hydroxyaldehydes and allowed to react with dihydroxyacetone phosphate (DHAP) *via* enzymatic aldol addition catalyzed by rabbit muscle fructose 1,6-bisphosphate aldolase ($FruA_{rab}$). After enzymatic dephosphorylation, a set of novel sugar phosphonates was obtained.

Keywords: aldol reaction; carbohydrates, enzymatic resolution, hydrolases; phosphonates

Introduction

In a recent communication[1] we have reported on the enzyme-catalyzed aldol addition of dihydroxyacetone phosphate (DHAP) [*in situ* generated from fructose 1,6-bisphosphate (FBP)] to various racemic β-hydroxyaldehydes **1** – **3** bearing a phosphonate group at the ω-position (Scheme 1). This reaction, promoted by fructose 1,6-bisphosphate aldolase from rabbit muscle ($FruA_{rab}$), allowed the production, in moderate to good yields, of a set of unnatural ω-phosphonic deoxysugars **4** – **6** endowed with potential bioactivity as inhibitors or regulators of metabolic processes. Since $FruA_{rab}$ is notoriously completely stereoselective for the two newly generated stereocenters at C3 and C4, starting from racemic aldehydes two epimers at C6 were expected. However, using an excess of aldehydes **1** – **3**, one of the two possible epimers turned out to be favored in part (in the case of **1**) or completely (for **2** or **3**).



Scheme 1.

We thought that it was quite worthwhile to repeat these experiments starting from the same aldehydes, but in enantiomeric pure forms, for various reasons: *a*) it would have allowed the separate synthesis of the two epimers of **4**, which were formed in mixture starting from racemic **1**; *b*) the use of (*S*)-**2** and (*S*)-**3** would have given the epimers not observed in the previous work; *c*) starting from enantiomerically pure aldehydes, it would have no longer been necessary to employ an excess of substrate; *d*) we hoped that the results with enantiomerically pure aldehydes would have helped in rationalizing the behavior observed with the racemic ones.

Unfortunately, there are very few examples of phosphorylated chiral building blocks belonging to the so called "chiral pool". While in the literature we could find some examples of enantioselective synthesis of α-hydroxyphosphonates, either *via* chemical[2,3] or biological methods,[4,5] only few reports dealt with the isolation in enantiomerically pure form of β-[3,6] or γ-hydroxyphosphonates.[3] Thus, for the synthesis of both enantiomers of the optically pure aldehydes **1** – **3** we decided to set up a novel methodology, based on a lipase-catalyzed kinetic resolution of the acetates **10**, **13**, and **17**. The utility of the resulting optically active phosphonates is not limited to the synthetic application described in this paper. They can indeed be viewed as new, useful chiral building blocks for the enantioselective synthesis of various phosphorus-containing bioactive compounds.

We report here the successful enzymatic kinetic resolution of acetates **10**, **13**, **17**, the conversion of the

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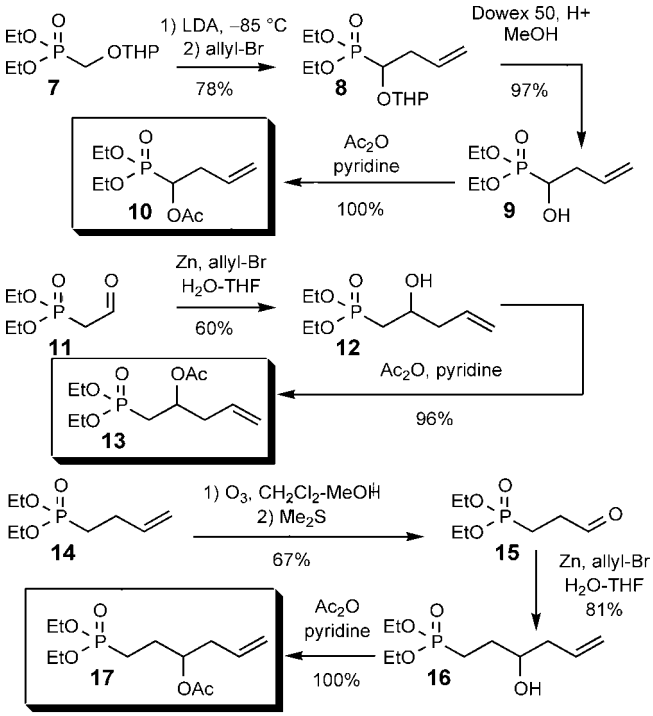
resulting alcohols **9**, **12**, and **16** into optically active β-hydroxyaldehydes **1** – **3**, and finally the exploitation of the latter in FruA-catalyzed aldol additions.

Results and Discussion

Synthesis of the Substrates for Enzymatic Kinetic Resolution

The syntheses of acetates **10**, **13**, and **17** is reported in Scheme 2. They are all new compounds. Acetate **10** was prepared by acetylation of known alcohol **9**.[7] For the synthesis of the latter we preferred a route different from the one described.[7] The key-step was the electrophilic allylation of the α-anion of the known tetrahydropyranyl ether **7**.[8] In the case of **13** the key step was the Barbier-Grignard allylation (in aqueous solvent) of the known aldehyde **11**.[9] A similar route was employed for converting aldehyde **15** into **17**. The former was obtained through ozonolysis of known alkene **14**.[10]



Scheme 2.

Lipase-Catalyzed Kinetic Resolution of Acetates 10, 13, and 17

The results of enzymatic resolutions of diacetates **10**, **13**, and **17** are reported in Table 1. In all three cases we could find conditions which allowed the preparation of the acetate or of the alcohol (depending on conversion) in high ee (> 92%) and in good isolated yield. In the case of **10** and **17** the best enzyme turned out to be Amano P lipase (from *Pseudomonas cepacia*) (entries 1 and 11). In the case of **17**, however, the reaction was considerably slower (compare entries 1 and 9). This problem was solved by employing a higher amount of enzyme and by addition of a cosolvent (*i*-Pr_2O) (entry 11). In both cases the more reactive enantiomer was the one expected by Kazlauskas' model[11] supposing the allyl group as medium and the phosphorus substituted alkyl as large groups [on passing from **10** to **17** the main alcohol becomes (*R*) instead of (*S*) only because of the different priority or-

Table 1. Enzymatic hydrolysis of racemic acetates **10**, **13**, and **17**.[a]

Entry	Subst.	Enzyme	Time	Conv.[b]	Alcohol			Acetate		
					Yield	ee[c]	Conf.	Yield	ee[c],[d]	Conf.
1	**10**	Amano PL (200 mg/mmol)	24 h	50%	44%	96.6%	(*S*)	46%	90.2%	(*R*)
2	**13**	Amano PL (200 mg/mmol)	36 h	10%	–	–	–	–	–	–
3	**13**	Amano PL[e] (400 mg/mmol)	26 h	15%	16%	97%[f]	(*R*)	74%	15%	(*S*)
4	**13**	PFL[e] (400 mg/mmol)	15 h	10%	–	–	–	–	–	–
5	**13**	CAL (400 mg/mmol)	48 h	15%	–	–	–	–	–	–
6	**13**	PLE (400 μl/mmol)	0.67 h	50%	37%	92%[f]	(*S*)	42%	88%	(*R*)
7	**13**	PLE (90 μl/mmol)	3 h	47%	36%	87%[f]	(*S*)	40%	91%	(*R*)
8	**13**	PLAP (150 mg/mmol)	1 h	50%	45%	89%[f]	(*S*)	40%	96%	(*R*)
9	**17**	Amano PL (200 mg/mmol)	40 h	10%	–	–	–	–	–	–
10	**17**	Amano PL (400 mg/mmol)	30 h	15%	–	–	–	–	–	–
11	**17**	Amano PL[e] (400 mg/mmol)	30 h	44%	42%	92%	(*R*)	54%	78%	(*S*)
12	**17**	CAL[e] (400 mg/mmol)	30 h	20%	20%	88%	(*R*)	73%	28%	(*S*)
13	**17**	PLE (70 μl/mmol)	24 h	50%	46%	72%	(*S*)	54%	28%	(*R*)

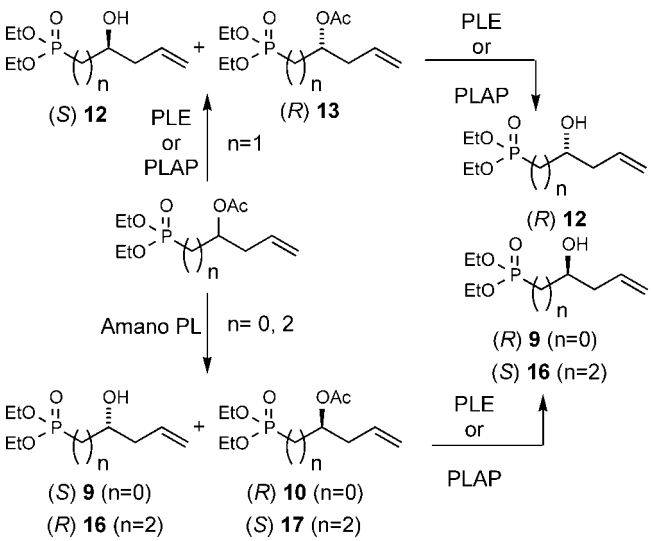
[a] Reactions were carried out at room temperature on 0.1 M solution of substrate in phosphate buffer (pH 7). The pH was maintained constant by an automatic burette. For the meaning of the enzyme abbreviations see the experimental part.
[b] Conversion, determined by the consumption of NaOH.
[c] Determined by chiral GC.
[d] Determined by ^{1}H NMR in the presence of Eu(hfc)$_3$.
[e] In the presence of *i*-Pr_2O (20%).
[f] Determined by ^{31}P NMR in the presence of Eu(hfc)$_3$.

der of substituents]. The decrease of enantioselectivity on passing from **10** to **17** can be attributed to the lower steric requirements of the phosphorus substituent when it is more distant.

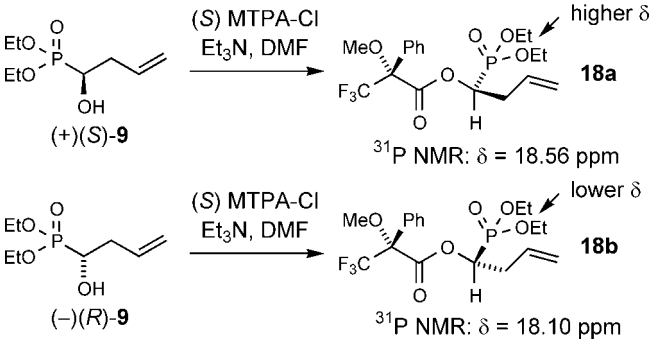
On the other hand in the case of **13** the reaction became too sluggish, even in the presence of *i*-Pr_2O. After a screening of various lipases and esterases, we found that best results were achieved with the latter, especially PLE (entries 6 and 7) and pig liver acetone powder (PLAP) (entry 8), which is a crude form of the same esterase. A high reactivity was accompanied by an excellent enantioselectivity. Interestingly, while **13** is less reactive than **17** towards Amano PL (entries 3 and 11), it seems to be more reactive with PLE (entries 7 and 13).

In all cases, by stopping the reaction at an appropriate conversion, we could obtain the alcohols (*S*)-**9**, (*S*)-**12**, (*R*)-**16**, and the acetates (*R*)-**10**, (*R*)-**13**, (*S*)-**17** with an ee higher than 92%.

In order to have access also to alcohols (*R*)-**9**, (*R*)-**12**, and (*S*)-**16**, the corresponding enantiomerically enriched acetates were hydrolyzed to the corresponding alcohols. By using ordinary chemical methods (e.g., Et_3N in MeOH or KOH in MeOH) the reactions either turned out to be too slow or furnished several by-products, deriving most likely by attack at the phosphonate moiety. We finally found that the best method was to carry out an enzymatic hydrolysis with PLE. In the case of acetate (*S*)-**17** this method was particularly advantageous since PLE has an opposite selectivity compared to Amano P lipase. Thus, the hydrolysis of the major enantiomer, (*S*), was faster, allowing an increase of the ee by stopping the reaction just before completion. The enantioselective preparation of all enantiomers of alcohols **9**, **12**, and **16** is summarized in Scheme 3.



Scheme 3.



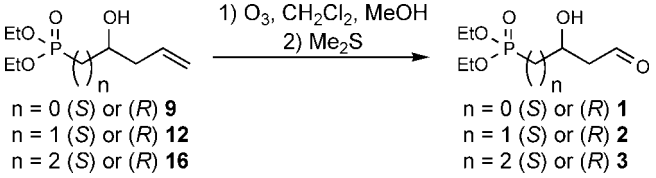
Scheme 4.

It is interesting to note that a possible alternative strategy for the kinetic resolution, involving acetylation of alcohols **9**, **12**, and **16**, was abandoned after preliminary tests which indicated a very low reactivity of our substrates even with notoriously efficient enzymes like *Candida antarctica* lipase. This fact is maybe due to the low lipophilicity of these substrates.

In the case of alcohols (+)- and (–)-**9** the absolute configuration was assigned on the basis of Mosher's method (Scheme 4).[12] This method had been previously employed for the absolute configuration of α-hydroxyphosphonates,[5,13] through observation of the ^{31}P NMR chemical shifts. In the hypothesis that the esters assume the conformation expected for the Mosher's esters of α-hydroxy esters, in compound **18a** the phosphorus nucleus should resonate at higher frequencies, as observed in our case, because of the effect of the phenyl group. This assignment was further corroborated by the results of FruA-promoted aldol additions (see below). Since this enzyme is well known to absolutely control the configuration of the newly created stereogenic centers,[14] the relative configuration of the adduct obtained by such an addition represents clear proof of the absolute configuration of the starting β-hydroxyaldehyde. In the case of alcohols **12** and **16** the absolute configuration was proved only by this latter method.

FruA-Catalyzed Addition of Dihydroxyacetone Phosphate to Optically Active Phosphonic β-Hydroxyaldehydes

Having in hand the homoallylic alcohols **9**, **12**, and **16** in both enantiomeric pure forms, we converted them straightforwardly into aldehydes **1** – **3** (Scheme 5) by ozonolysis followed by treatment with dimethyl sulfide. The aldehydes were not isolated, but directly used as crude products for the following enzymatic aldol additions (Scheme 6). As enzyme we employed fructose 1,6-bisphosphate aldolase from rabbit muscle ($FruA_{rab}$, EC 4.1.2.13). The actual reagent dihydroxyacetone phosphate (DHAP) was generated *in situ* from fructose 1,6-bisphosphate (FBP), which, in

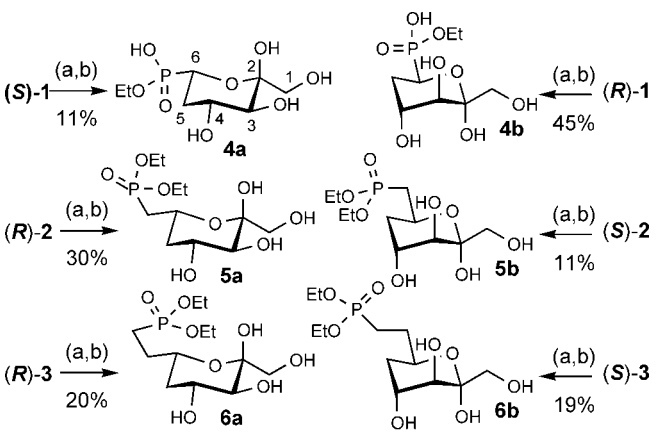


Scheme 5.

the presence of FruA, is in equilibrium with DHAP and glyceraldehyde 3-phosphate (G-3-P). Triose phosphate isomerase (TPI, EC 5.3.1.1.) was also included in the reaction mixture, in order to convert also G-3-P into DHAP. We preferred to use this combination of reagents, instead of directly employing DHAP, first for the lower cost of FBP, and secondly because of the long reaction times (2 – 7 days) that would have needed repeated additions of the unstable DHAP to the reaction mixture. At the end of reaction (when the starting aldehyde had disappeared by TLC), the solutions were acidified to pH 4.5 and treated with acid phosphatase to give the dephosphorylated ketopyranoses **4** – **6**, which were then isolated by silica gel chromatography. The isolated yields shown in Scheme 6 are based on starting alkenes **9**, **12**, and **16**.

All the reactions turned out to be completely stereoselective, as expected, giving only one stereoisomer. Although the stereochemistry at the anomeric center is not fixed, all these six adducts are present in aqueous solution as a single anomer, which are most likely the most stable α ones, as shown in Scheme 6.

When the substrates were the aldehydes (*S*)- or (*R*)-**1**, we isolated the monoethyl phosphonates **4a,b**. The unexpected phosphonate monohydrolysis takes place most likely during the treatment with acid phosphatase. Actually, in the case of (*S*)-**1**, upon a shorter treatment with phosphatase, we were able to isolate an about 1:1 mixture of **4a** and the corresponding



(a) FruA, FBP, TPI (pH = 7); (b) acid phosphatase
Yields are calculated from alkenes **9**, **12**, **16**.

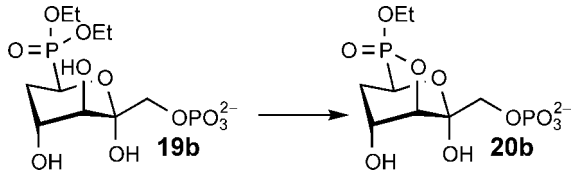
Scheme 6.

diethyl phosphonate. This mixture was quantitatively converted into **4a** by further treatment with the same enzyme (or also with alkaline phosphatase at pH 10). We could find some literature precedents of phosphonate monohydrolysis catalyzed by alkaline phosphatase.[15] The yields observed in the two reactions reflect in some way the kinetic preference of FruA towards the two enantiomeric aldehydes. In fact, we observed that aldehyde (*R*)-**1** reacted faster than its (*S*) stereoisomer. In the former case reaction was complete after 2 days, using 7 U of FruA per mmol aldehyde; in the latter case it took 7 days with 100 U/mmol. Accordingly, when we carried out the same reaction using an excess of racemic aldehyde, we obtained a **4b**:**4a** ratio of 2:1, with an overall yield of 50% based on FBP, which is the limiting agent.[1]

In the case of aldehydes **2** and **3** we did not observe any appreciable difference in reactivity between the two enantiomers. It is worth noting that in these two cases no hydrolysis of the phosphonate group took place and only the diethyl esters have been isolated. Thus, the exclusive formation of compounds **5a** and **6a** observed in the previously reported additions involving racemic aldehydes, may be due to a thermodynamic preference. Actually **5a** and **6a** having all the substituents (except from the anomeric OH) equatorial appear to be more stable.

The results obtained with racemic aldehydes **2** and **3** seem to be in agreement with previous studies, where a thermodynamic control was proposed in the enzyme-catalyzed aldol addition of DHAP to α- or β-hydroxyaldehydes.[16] On the other hand, the result obtained starting from racemic aldehyde **1** is more puzzling. The preferential formation of **4b** through a thermodynamic control seems in this case less likely, since compound **4b** is expected to be thermodynamically less stable. The higher reactivity of (*R*)-**1** suggests that the control may be kinetic. It has been previously shown that FruA displays efficient kinetic resolution of racemic α-hydroxyaldehydes with a negative charge displaced four or five atoms from the aldehydic center, showing a preference for the (D) compound.[17] More recently, a kinetic control was suggested for rationalizing the preferential reaction of the (D) enantiomer in the addition of a β-hydroxyaldehyde, namely 3-hydroxy-3-carboxypropanal, to DHAP, catalyzed by $FruA_{rab}$.[18] The presence of the phosphonyl group can be beneficial in improving the enantioselectivity, since it resembles the phosphate group at O3 of the natural substrate (D-glyceraldehyde 3-phosphate).

If in this case the thermodynamic control is not operating, this means that for some reasons the retro-aldol reaction is more difficult, making the attainment of equilibrium composition slower. A possible explanation for this fact was already proposed in our preliminary communication.[1] It is based on an irreversi-



Scheme 7.

ble intramolecular transphosphorylation carried out by the anomeric hydroxy, that is expected to be faster for the adduct **19b** (corresponding to the final compound **4b**) (Scheme 7). The resulting cyclic phosphonate **20b** is no longer capable of undergoing a retroaldol reaction, thus withdrawing the products from the enzymatic equilibrium.

The relative configurations of compounds **4 – 6** was established by ^{1}H NMR in analogy with previous work on the addition of DHAP to β-hydroxyaldehydes. The assignment is also based on the usual stereoselectivity of FruA from rabbit muscle, which always gives (D)-*threo* adducts. In the case of compounds **5** and **6** the relative configuration represents also a proof for the absolute configuration of the starting aldehydes **2** and **3** and thence of the alcohols and acetates **12**, **13**, **16**, **17**.

In the case of **4a,b** particularly diagnostic are the coupling constants between protons 3 and 4 (9.4 Hz for **4a** and 1.8 Hz for **4b**), 4 and 5 (**4a**: J_{trans} = 12.0 Hz, J_{cis}: not determined; **4b**: $J_{trans} \approx J_{cis}$ = 3.0 Hz), 5 and 6 (**4a**: J_{trans} = 10.0 Hz, J_{cis} = 2.2 Hz; **4b**: J_{trans} = 10.0 Hz, J_{cis} = 2.0 Hz). These data clearly indicate that in both compounds the phosphonyl group is equatorial, while the OH at C4 is equatorial (and thus *cis* to the phosphonyl) in **1a** and axial (*trans* to the phosphonyl) in **1b**. Therefore, in **1b** the bulky phosphonyl group forces the two OH groups at C3 and C4 into an axial position.

In the case of **5a,b** the assignment was based especially on the observation of the protons bonded at C5. Useful information is given not only by the coupling constants, but also by the chemical shifts. In a series of similar adducts, derived by aldol addition of DHAP to various β-hydroxyaldehydes, and differentiated only by the substituent at C6, it has indeed been observed that the difference of chemical shifts between axial and equatorial H5 protons is very small in *trans* isomers (like **5b**, **6b**), but it is around 0.5 ppm for *cis* isomers (like **5a**, **6a**).[19] We have observed $\Delta\delta \approx$ 0.6 ppm (**5a**), <0.15 ppm (**5b**), ≈ 0.75 ppm (**6a**), <0.4 ppm (**6b**). Moreover, in **5a** and **6a** the $J_{3,4}$ (equal respectively to 12.0 and 11.8 Hz.) and the $J_{4,5ax}$ (12.0 and 11.8 Hz.) indicate that the phosphonyl group and the OH at C3 are both equatorial (and therefore *cis*). In **5b** $J_{3,4}$ is much lower (3.9 Hz.) (for **6b** it was not possible to measure it).

We have no direct proof for the enantiomeric purity of compounds **4 – 6**. However the formation of the enantiomers of **4 – 6** would require two concurrent events: *a*) racemization of starting aldehydes **1 – 3** and *b*) an incomplete enantioselectivity of the enzyme. These two circumstances are quite unlikely since β-hydroxyaldehydes are usually configurationally stable and it is well known that $FruA_{rab}$ shows a complete preference for (D)-*threo* adducts. Moreover, if one of these two events or both would have taken place, mixture of diastereisomers would have been observed. On the contrary, within the detection limits, we observed only one product for all the reactions shown in Scheme 6.

Conclusions

Using enzyme-catalyzed kinetic resolution of the corresponding acetates, we have developed efficient methodologies for the preparation in high ee of both enantiomers of a series of 3-hydroxyaldehydes bearing a phosphonyl group bonded at C3, C4, or C5. These aldehydes are valuable chiral building blocks for the synthesis of various phosphorus-containing analogues of biologically active substances. In particular, in this work they have been employed in $FruA_{rab}$-catalyzed aldol additions with dihydroxyacetone phosphate to give carbohydrate analogues bearing a phosphonic substituent. The use of optically active aldehydes represents an advantage compared to the previously described addition with racemic aldehydes.[1] First of all, also isomers **5b** and **6b**, not previously obtained, can be synthesized. Secondly, **4a** and **4b** can be obtained independently, thus avoiding their troublesome separation. Finally, there is no more need to use an excess of the aldehydes. Although the obtained yields may seem in some cases only modest, it should be stressed that highly complex carbohydrate-like structures like **4 – 6**, possessing three stereogenic centers (not counting the anomeric one) may be assembled in just 3 – 4 steps in enantio- and diastereoisomerically pure form starting from easily available racemic acetates **9**, **13**, and **17**. This goal has been achieved through an atom-economical, environmental friendly process, which uses, with the exception of the ozonolysis step, a series of 2 or 3 enzymatic reactions in aqueous media, without any need of protecting groups.

Experimental Section

General Methods

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ (internal standard: tetramethylsilane) or D_2O (internal standard: CH_3CN) on a Varian Gemini 200 spectrometer at 200 MHz and 50 MHz, respectively. ^{31}P NMR spectra were recorded

on a Varian FT 80 spectrometer at 32 MHz, using H_3PO_4 as external standard. Chemical shifts are reported in ppm (δ scale), coupling constants are reported in Hertz. The presence of a * on the coupling constant means that the value was obtained through double resonance experiments. GC-MS were carried out on an HP-5971A instrument, using a HP-1 column (12 m long, 0.2 mm diameter), electron impact at 70 eV and a mass temperature of approx. 167 °C. Analyses were performed with a constant He flow (0.9 mL/min), starting at 70 °C for 2 min, and then raising the temperature by 20 °C/min. GC were performed with a Carlo Erba HGRC 5300 instrument equipped with a MEGA Dmet.Terbut.SBeta column (25 m long, 0.25 mm diameter) and an FID detector. Analyses were performed with a constant He flow (1.9 mL/min), and constant temperature (100 – 135 °C). Optical rotations were determined with a Jasco DIP-181 polarimeter in $CHCl_3$ at $c = 2$ unless otherwise stated. TLC was carried out on silica gel plates, which were developed by dipping into the solutions A, B, or C and warming. Solution A: $(NH_4)_4MoO_4{\cdot}4\,H_2O$ (21 g) and $Ce(SO_4)_2{\cdot}4\,H_2O$ (1 g) in H_2SO_4 (31 mL) and H_2O (469 mL). Solution B: 2% aqueous $KMnO_4$. Solution C: anisaldehyde (5.5 mL) in EtOH (200 mL), AcOH (2.2 mL) and H_2SO_4 (7.5 mL). Chromatographies were carried out on 220 – 400 mesh silica gel using the 'flash' methodology. Petroleum ether (40 – 60 °C) is abbreviated as PE. In extractive work-up, aqueous solutions were always re-extracted three times with the appropriate organic solvent. Organic extracts were dried over Na_2SO_4 and filtered, before evaporation of the solvent under reduced pressure. All reactions employing dry solvents were carried out under a nitrogen atmosphere. The following enzymes were employed in this work: FruA [EC 4.1.2.13], TPI [EC 5.3.1.1], acid phosphatase [EC 3.1.3.2], PPL [EC 3.1.1.3](52 U/mg with triacetine), and PLAP purchased from Sigma. PLE [EC 3.1.1.1] (1.22 U/μL) and PFL purchased from Fluka. CAL was a kind gift from Novo Nordisk (lot No. LCC0013–1, 7.4 U/mg). Amano P lipase (30 U/mg) was kindly donated by Amano.

(*rac*)-Diethyl 1-[(Tetrahydropyranyloxy)-methyl]but-3-enylphosphonate (8)

A solution of diisopropylamine (9.3 mL, 65.0 mmol) in THF (50 mL) was cooled at –15 °C and treated with a hexane solution of *n*-butyllithium (37 mL of 1.6 M solution). After 30 min the resulting solution was cooled to –85 °C and treated with a solution of diethyl tetrahydropyranyloxymethylphosphonate (**7**)[8] (7.5 g, 29.73 mmol) in THF (50 mL) which was added dropwise over 30 min through a dropping funnel. After 90 min a solution of allyl bromide (5.1 mL, 59.5 mmol) in THF (50 mL) was added dropwise over 30 min through a dropping funnel. The reaction mixture was stirred overnight at –85 °C. Quenching with saturated NH_4Cl solution, followed by extraction with Et_2O and chromatography (AcOEt) gave a diastereomeric mixture of (±)-**8** as a colorless oil; yield: 6.76 g (78%). R_f = 0.5 (AcOEt:PE, 8:2; detection: A); ^{1}H NMR ($CDCl_3$): δ = 1.35 and 1.36 [2 × 3H, 2 t, ($CH_3CH_2O)_2$P, J = 7.0], 1.4 – 1.9 [6H, m, $CH_2CH_2CH_2$], 2.4 – 2.7 [2H, m, $CH_2CH{=}CH_2$], 3.4 – 3.6 and 3.8 – 4.0 [2H, m, CH_2CH_2O J^*_{AB} = 11.2], 4.0 – 4.1 [1H, m, PC*H*], 4.10 – 4.25 [4H, m, ($CH_3CH_2O)_2$P], 4.65 [1H, s, OC*H*O minor diast.], 5.00 [1H, s, OC*H*O main diast.], 5.05 – 5.25 [2H, m, CH=CH_2], 5.90 [1H, ddt, C*H*=CH_2, J = 17.1 (d), 10.2 (d), 7.0 (t)].

(*rac*)-Diethyl 1-Hydroxybut-3-enylphosphonate (9)

A diastereomeric mixture of (±)-**8** (5.13 g, 17.49 mmol) was dissolved in MeOH (75 mL), and Dowex resin (H^+ form, 1.25 g) was added. The reaction mixture was stirred overnight at room temperature, then the resins were removed by filtration, the solvent was evaporated to dryness, and the crude product purified by chromatography (AcOEt:MeOH, 9:1) to give (±)-**9** as a colorless oil; yield: 3.50 g (97%). R_f = 0.30 (AcOEt:PE, 8:2; detection: A). ^{1}H NMR ($CDCl_3$): δ = 1.35 [6H, t, P($OCH_2CH_3)_2$, J = 7.0], 2.4 – 2.7 [2H, m, $CH_2CH{=}CH_2$], 3.35 [1H, t, O*H*, J = 7.0], 3.8 – 4.0 [1H, m, PC*H*], 4.18 [4 H, quint, P($OCH_2CH_3)_2$, J = 7.0], 5.15 – 5.26 [2H, m, CH=CH_2], 5.90 [1H, ddt, C*H*=CH_2 J = 17.1 (d), 10.1 (d), 7.0 (t)]. ^{13}C NMR ($CDCl_3$): δ = 16.25 [d, ($CH_3CH_2O)_2$P, J = 5.5], 35.77 [d, $CH_2CH{=}CH_2$, J = 1.9], 62.52 and 62.66 [2 d, ($CH_3CH_2O)_2$P, J = 5.3], 66.99 [d, *C*HOH, J = 161.5], 117.53 [s, CH=CH_2], 133.81 [d, *C*H=CH_2, J 14.3]. ^{31}P NMR ($CDCl_3$): δ = 24.6. GC-MS: R_t = 6.04; m/z = 208 (M^+, 2%), 179 (9), 167 (14) 138 (29) 111 (100), 93 (9), 82 (56), 65 (34), 41 (11).

(*rac*)-Diethyl 1-Acetoxybut-3-enylphosphonate (10)

A solution of (±)-**9** (1.30 g, 6.2 mmol) in pyridine (3 mL) was cooled at 0 °C and treated with Ac_2O (1.2 mL, 12.8 mmol). The reaction was stirred for 12 h at rt, then the solvent was evaporated to dryness. The crude product was chromatographed (AcOEt:PE, 55:45) to give pure (±)-**10** as a colorless oil; yield: 1.5 g (100%). R_f = 0.66 (AcOEt; detection: B). ^{1}H NMR ($CDCl_3$): δ = 1.33 and 1.35 [2 × 3H, 2 t, ($CH_3CH_2O)_2$P, J = 7.2], 2.10 [3H, s, CH_3CO], 2.4 – 2.7 [2H, m, $CH_2CH{=}CH_2$], 4.08 – 4.25 [4H, m, P($OCH_2CH_3)_2$], 5.15 – 5.20 [2H, m, CH=CH_2], 5.31 [1H, ddd, PC*H*, J = 12.9, 8.8, 4.1], 5.75 [1H, ddt, C*H*=CH_2 J = 17.2 (t), 10.0 (t), 7.0 (d)]. ^{13}C NMR ($CDCl_3$): δ = 16.25 [d, ($CH_3CH_2O)_2$P, J = 5.5], 20.55 [s, CH_3CO], 33.89 [s, $CH_2CH{=}CH_2$], 62.64 and 62.79 [2 d, ($CH_3CH_2O)_2$P, J = 4.9], 66.71 [d, PCH, J = 168.1], 118.30 [s, CH=CH_2], 132.51 [d, *C*H=CH_2, J = 13.7], 169.56 [s, CH_3CO]. ^{31}P NMR ($CDCl_3$): δ = 20.2. GC-MS: R_t = 6.52; m/z = 250 (M^+, 1%), 207 (M^+ – 43, 8), 191 (12), 167 (14), 138 (49), 111 (58), 81 (31), 65 (21), 43 (100).

(*rac*)-Diethyl 2-Hydroxypent-4-enylphosphonate (12)

Aldehyde **11** (3.96 g, 22.0 mmol) was suspended in a saturated solution of NH_4Cl (88 mL) and THF (88 ml) in a flask equipped with a cooling system. The suspension was treated with zinc powder (5.75 g, 88.0 mmol) and allyl bromide (7.6 mL, 88.0 mmol). The reaction started vigorously and the developed heat maintained the reflux. After consumption of the zinc, the reaction was stirred at rt for 2 h. The solid was filtered over a Büchner funnel, the solution was extracted with CH_2Cl_2, to give, after evaporation and chromatography (AcOEt) pure (±)-**12** as a colorless oil; yield: 2.9 g (60%) .R_f = 0.45 (AcOEt; detection: A). ^{1}H NMR ($CDCl_3$): δ = 1.37 [6H, t, ($CH_3CH_2O)_2$P, J = 7.0], 1.85 – 2.15 [2H, m, PCH_2], 2.30 – 2.40 [2H, m, $CH_2CH{=}CH_2$], 3.50 [1H, bs, O*H*], 4.00 – 4.20 [5H, m, ($CH_3CH_2O)_2$P and C*H*OH], 5.10 – 5.20 [2H, m, CH=CH_2], 5.83 [1H, ddt, C*H*=CH_2, J = 17.7 (d), 9.5

(d), 7.0 (t)]. ^{13}C NMR ($CDCl_3$): δ = 16.18 [d, ($CH_3CH_2O)_2P$, *J* = 6.4], 32.62 [d, PCH_2, *J* = 138.3], 42.33 [d, $CH_2CH{=}CH_2$, *J* = 16.2], 61.78 and 61.91 [2 d, ($CH_3CH_2O)_2P$, *J* = 4.5], 65.70 [d, CHOH, *J* = 5.0], 117.92 [s, $CH{=}CH_2$], 133.81 [s, $CH{=}CH_2$]. ^{31}P NMR ($CDCl_3$): δ = 30.2. GC-MS: R_t = 6.5; *m/z* = 181 (M^+ – 41, 37%), 153 (19), 125 (100), 123 (5), 107 (24), 97 (7), 81 (18), 67 (7), 65 (11) ,45 (7), 41 (10), 32 (25).

(*rac*)-Diethyl 2-Acetoxypent-4-enylphosphonate (13)

It was prepared in 96% yield from (±)-**12** following the same procedure already described for the synthesis of **10**. R_f = 0.45 (AcOEt:PE, 7:3; detection: A). ^{1}H NMR ($CDCl_3$): δ = 1.35 [6H, t, ($CH_3CH_2O)_2P$, *J* = 7.0], 2.05 [3H, s, CH_3CO], 2.10 [2H, dd, PCH_2, *J* = 18.6, 6.9], 2.38 – 2.51 [2H, m, $CH_2CH{=}CH_2$], 4.10 [2H, quint, ($CH_3CH_2O)_2P$, *J* = 7.2], 5.05 – 5.20 [2H, m, $CH{=}CH_2$], 5.21 [1H, quint, CHOAc, *J* = 5.8], 5.75 [1H, ddt, $CH{=}CH_2$, *J* = 17.5 (d), 9.6 (d), 7.1 (t)]. ^{13}C NMR ($CDCl_3$): δ = 16.31 [d, ($CH_3CH_2O)_2P$, *J* = 6.0], 21.00 [s, CH_3CO], 30.14 [d, PCH_2, *J* = 140.4], 39.16 [d, $CH_2CH{=}CH_2$, *J* = 9.8], 61.66 and 61.78 [2 d, ($CH_3CH_2O)_2P$, *J* = 3.6], 68.09 [s, CHOAc], 118.65 [s, $CH{=}CH_2$], 132.65 [s, $CH{=}CH_2$], 170.01 [s, CH_3CO]. ^{31}P NMR ($CDCl_3$): δ = 26.6. GC-MS: R_t = 7.1; *m/z* = 223 (M^+ – 41, 0.8%), 221 (M^+ – 43, 2.3), 204 (12), 181 (100), 177 (10), 153 (24), 149 (14), 125 (83), 123 (8), 109 (19), 107 (14), 97 (13), 94 (11), 81 (24), 68 (7), 67 (21), 66 (11), 65 (14), 43 (63), 41 (16), 39 (8).

General Procedure for Ozonolysis

A solution of alkene (1.00 mmol) in dry MeOH (10 mL) and dry CH_2Cl_2 (10 mL) was cooled to –78 °C. Ozone was bubbled into the solution until persistence of gray/blue color. After further bubbling of O_2 for 3 min, Me_2S (0.5 mL) was added. The reaction mixture was allowed to warm to rt, and stirred for 2 h. Evaporation of the solvent, followed by codistillation with MeOH (3 × 20 mL) for removing formaldehyde, afforded crude aldehydes which were usually employed for the following steps without further purification.

Diethyl 3-Oxopropylphosphonate (15)

It was prepared in 67% yield from **14** following the general procedure of ozonolysis, followed by chromatography. R_f = 0.20 (AcOEt; detection: B). ^{1}H NMR ($CDCl_3$): δ = 1.33 [6H, t, ($CH_3CH_2O)_2P$, *J* = 7.0], 1.90 – 2.15 [2H, m, PCH_2], 2.8 [2H, dt, CH_2CHO, *J* = 12.0 (t), 7.2 (d)], 4.11 [4 H, quint, ($CH_3CH_2O)_2P$, *J* = 7.0], 9.8 [1H, bs, CH_2CHO]. ^{13}C NMR ($CDCl_3$): δ = 16.7 [d, ($CH_3CH_2O)_2P$, *J* = 5.9], 18.5 [d, PCH_2, *J* = 145.0], 38 [d, CH_2CHO, *J* = 4.1], 62 [d, ($CH_3CH_2O)_2P$, *J* = 6.4], 200 [d, CH_2CHO, *J* = 15.7]. ^{31}P NMR ($CDCl_3$): δ = 31.0. ^{31}P NMR (D_2O): δ = 35.4. GC-MS: R_t = 5.7; *m/z* = 194 (M^+, 91%), 166 (75), 149 (4.6), 138 (100), 121 (93), 111 (98), 83 (27), 82 (67), 65 (25), 57 (14), 41 (11), 32 (7).

(*rac*)-Diethyl 3-Hydroxyhex-5-enylphosphonate (16)

It was prepared in 81% yield from (±)-**15** following the same procedure already described for the synthesis of **12**. R_f = 0.20 (AcOEt; detection: B). ^{1}H NMR ($CDCl_3$): δ = 1.33 [6H, t, ($CH_3CH_2O)_2P$, *J* = 7.0], 1.60 – 2.00 [4H, m, PCH_2CH_2], 2.20 – 2.40 [2H, m, $CH_2CH{=}CH_2$], 2.60 [1H, bs, OH], 3.60 – 3.80 [1H, m, CHOH], 4.00 – 4.20 [4H, m, ($CH_3CH_2O)_2P$], 5.10 – 5.20 [2H, m, $CH{=}CH_2$], 5.82 [1H, ddt, $CH{=}CH_2$, *J* = 17.0 (d), 9.5 (d), 7.0 (t)]. ^{13}C NMR ($CDCl_3$): δ = 16.28 [d, ($CH_3CH_2O)_2P$, *J* = 5.9], 21.77 [d, PCH_2CH_2, *J* = 140.7], 29.22 [d, PCH_2CH_2, *J* = 4.5], 41.50 [s, $CH_2CH{=}CH_2$], 61.49 [d, ($CH_3CH_2O)_2P$, *J* = 6.4], 70.20 [d, CHOH, *J* = 14.7], 117.53 [s, $CH{=}CH_2$], 134.52 [s, $CH{=}CH_2$]. ^{31}P NMR (D_2O): δ = 33.1. GC-MS: R_t = 7.2; *m/z* = 195 (M^+ – 41, 32%), 167 (5), 166 (6), 149 (59), 139 (11), 138 (5), 121 (100), 111 (7), 83 (7), 82 (9), 81 (15), 80 (5), 79 (7), 65 (14), 57 (27), 55 (10), 45 (9), 41 (26), 39 (8), 31 (12).

(±)-Diethyl 3-Acetoxyhex-5-enylphosphonate (17)

It was prepared in 100% yield from (±)-**16** following the same procedure already described for the synthesis of **10**. R_f = 0.50 (AcOEt:PE, 1:1, detection: B). ^{1}H NMR ($CDCl_3$): δ = 1.33 [6H, t, ($CH_3CH_2O)_2P$, *J* = 7.1], 1.6 – 2.0 [4H, m, PCH_2CH_2], 2.05 [3H, s, CH_3CO], 2.32 [2H, t, $CH_2CH{=}CH_2$, *J* = 7.0], 4.09 [4H, quint, ($CH_3CH_2O)_2P$, *J* = 7.2], 4.90 [1H, quint, CHOAc, *J* = 7.0], 5.72 [2H, m, $CH{=}CH_2$], 5.73 [1H, ddt, $CH{=}CH_2$, *J* = 17 (d), 9.5 (d), 7.0 (t)]. ^{13}C NMR ($CDCl_3$): δ = 16.19 [d, ($CH_3CH_2O)_2P$, *J* = 6.0], 20.89 [s, CH_3CO], 21.43 [d, PCH_2CH_2, *J* = 142.5], 26.19 [d, PCH_2CH_2, *J* = 4.5], 34.06 [s, $CH_2CH{=}CH_2$], 61.92 [d, ($CH_3CH_2O)_2P$, *J* = 6.6], 72.54 [d, CHOAc, *J* = 18.0], 117.99 [s, $CH{=}CH_2$], 132.78 [s, $CH{=}CH_2$], 170.39 [s, CH_3CO]. ^{31}P NMR ($CDCl_3$): δ = 31.7. GC-MS: R_t = 7.84; *m/z* = 278 (M^+, 0.09%), 277 (M^+ – 1, 0.2), 235 (M^+ – 43, 2), 218 (17), 195 (100), 191 (9), 167 (8), 163 (6), 149 (16), 139 (13), 138 (7), 121 (40), 111 (9), 109 (8), 82 (12), 81 (27), 80 (16), 79 (13), 65 (11), 57 (10), 43 (56), 41 (17).

General Procedure for Enzymatic Kinetic Resolution of Racemic Acetates 10, 13, 17

Racemic acetates **10**, **13** and **17** were suspended in a 0.1 M pH 7 buffer solution (KH_2PO_4-K_2HPO_4) and cosolvent, if required, was added. The suspension was treated with the enzyme (quantity indicated in Table 1). The pH was maintained constant at 7.00 by continuous addition of 0.1 N NaOH from an automatic burette. After consumption of the required amount of NaOH, the crude mixture was diluted with AcOEt, filtered over celite, extracted with AcOEt, and evaporated to dryness. The crude product was chromatographed (AcOEt) to separate the alcohol and acetate.

$[\alpha]_D$: (*S*)-**9**: +14.4 [obtained using Amano PL at 48% conversion; ee: 96.6% (GC)]. (*R*)-**10**: –3.4 [obtained using Amano PL at 54% conversion; ee: 99.5% (GC)]. (*R*)-**12**: +12.0 [obtained using PLE at 45% conversion; ee: 93% (^{31}P NMR)]. (*S*)-**13**: –6.4 [obtained using PLE at 53% conversion; ee = 96% (^{1}H NMR)]. (*S*)-**16**: –8.5 [obtained using Amano PL at 44% conversion; ee: 92% (GC)]. (*R*)-**17**: +12.3 [obtained using Amano PL at 56% conversion; ee: 92% (GC)].

General Procedure for Enzymatic Hydrolysis of Optically Active Acetates 10, 13, 17

(*R*)-(–)-**10**, (*S*)-(+)-**13**, and (*R*)-(–)-**17** were suspended in a 0.1 M pH 7 buffer solution (KH_2PO_4-K_2HPO_4) and PLE (50 μL/mmol) or PLAP (150 mg/mmol) was added. The pH

was maintained constant at 7.00 by continuous addition of 0.1 N NaOH from an automatic burette. After consumption of 1 equivalent of NaOH, the crude mixture was diluted with AcOEt, filtered over celite and evaporated to dryness. The crude product was chromatographed (AcOEt) to give respectively pure (*R*)-(–)-**9**, (*S*)-(+)-**12**, and (*R*)-(–)-**16** as colorless oils.

(±)-, (*R*)-, or (S)-Diethyl 1-Hydroxy-3-oxopropylphosphonates (1)

They were obtained starting from (±)-, (*R*)-, or (*S*)-**1** following the general procedure for ozonization. An analytical sample was obtained by chromatography (AcOEt). R_f = 0.18 (AcOEt; detection: B). ^{1}H NMR ($CDCl_3$): δ = 1.45 [6H, t, $P(OCH_2CH_3)_2$, *J* = 7.0], 2.85 [2H, m, CH_2CHO], 4.20 and 4.21 [2 × 2H, 2 quint, $P(OCH_2CH_3)_2$, *J* = 7.0], 4.45 – 4.55 [1H, m, PC*H*OH], 9.85 [1H, bs, CH_2CHO]. ^{13}C NMR ($CDCl_3$): δ = 18 [d, $P(OCH_2CH_3)_2$, *J* = 5.5], 46 [s, CH_2CHO], 63.5 [d, $P(OCH_2CH_3)_2$, *J* = 7.0], 63.2 [d, PCHOH, *J* = 167.0], 200 [d, CH_2CHO, *J* = 15.7]. ^{31}P NMR ($CDCl_3$): δ = 26.1.

(±)-, (*R*)-, or (*S*)-Diethyl 2-Hydroxy-4-oxobutylphosphonates (2)

These compounds were obtained starting from (±)-, (*R*)-, or (*S*)-**2** following the general procedure for ozonization. An analytical sample was obtained by chromatography (AcOEt). R_f = 0.16 (AcOEt; detection: B). ^{1}H NMR ($CDCl_3$): δ = 1.29 and 1.27 [2 × 3H, 2 t, $(CH_3CH_2O)_2P$, *J* = 7.0], 1.85 – 2.15 [4H, m, PCH_2 and CH_2CHO], 3.85 [1H, s, CHO*H*], 3.9 – 4.2 [6H, m, $(CH_3CH_2O)_2P$], 4.43 [1H, quint., C*H*OH, *J* = 6.2], 9.8 [1H, s, CH_2CHO]. ^{13}C NMR ($CDCl_3$): δ = 17.16 [d, $(CH_3CH_2O)_2P$, *J* = 5.6], 33.90 [d, PCH_2 *J* = 138.3], 51.75 [d, CH_2CHO, *J* = 13.6], 62.86 [d, $(CH_3CH_2O)_2P$, *J* = 6.4], 63.1 [d, CHOH *J* = 3.7], 201.94 [s, CH_2CHO]. ^{31}P NMR ($CDCl_3$): δ = 28.0.

(±)-, (*R*)-, or (*S*)-Diethyl 3-Hydroxy-5-oxopentylphosphonate (3)

These compounds were obtained starting from (±)-, (*R*)-, or (*S*)-**3** following the general procedure for ozonization. An analytical sample was obtained by chromatography (AcOEt). R_f = 0.50 (AcOEt:MeOH, 8:2; detection: A and B). ^{1}H NMR ($CDCl_3$): δ = 1.32 [6H, t, $(CH_3CH_2O)_2P$, *J* = 7.1], 1.6–2,0 [4H, m, PCH_2CH_2], 2.60 – 2.70 [2H, m, CH_2CHO], 4.00 – 4.25 [5H, m, $(CH_3CH_2O)_2P$ and C*H*OH], 9.8 [1H, s, -CH_2-*C*HO]. ^{31}P NMR ($CDCl_3$): δ = 32.0.

General Procedure for Enzymatic Aldol Addition and Dephosphorylation

Crude aldehydes (*S*)- or (*R*)-**1**, -**2**, or -**3** (1 mmol) were dissolved in distilled H_2O (10 mL) to give a concentration 0.1 M, fructose 1,6-bisphosphate trisodium salt (FBP) (1.5 – 4.0 mmol, see below) was added, and the pH was adjusted to 7.00 by addition of 1 M NaOH. After addition of the appropriate amount of D-fructose 1,6-bisphosphate aldolase from rabbit muscle (FruA) (see below) and triose phosphate isomerase (20 U), the mixture was incubated at rt and analyzed for conversion by TLC. After the time indicated below, the pH of the reaction was adjusted to 4.8 and acid phosphatase (the amount indicated below) was added for the time indicated below. After filtration over active charcoal, the solvent was evaporated and the residue was purified by flash chromatography ($CHCl_3$:MeOH:H_2O, 20:10:1), to give pure aldol adducts **4 – 6**.

6-(Ethylphosphono)-L-*xylo*-5-deoxyhex-2-ulopyranose (4a)

It was obtained as a slightly yellow foam in 11% yield from (*S*)-**1** using 100 U of FruA, 1.5 mmol of FBP, and 7 days of reaction time. Treatment with acid phosphatase (160 U) lasted 5 days. R_f = 0.05 ($CHCl_3$:MeOH:H_2O, 20:10:1; detection C). Elemental analysis: found: C 35.2, H 6.4%; $C_8H_{17}O_8P$ requires: C 35.30, H 6.30%. $[\alpha]_D$: –34.3 (*c* 2.35, H_2O). ^{1}H NMR (D_2O): δ = 1.20 [3H, t, $(CH_3CH_2O)_2P$, *J* = 7.1], 1.60 [1H, quart. d, H-5ax, *J* = 12.0 (q), 9.0 (d)], 2.10 [1H, m, H-5eq, J^*_{AB} = 12.8], 3.41 [1H, d, H-3ax, *J* = 9.4], 3.67 and 3.61 [2H, AB system, H-1, J_{AB} = 11.7], 3.80 – 3.90 [1H, m, H-4ax], 3.92 [2H, quint, $(CH_3CH_2O)_2P$, *J* = 7.1], 4.03 [1H, ddd, H-6, *J* = 12.8, 10.0, 2.2]. ^{13}C NMR (D_2O): δ = 16.51 [d, $(CH_3CH_2O)_2P$, *J* = 5.4], 34.07 [s, C-5], 62.27 [d, $(CH_3CH_2O)_2P$, *J* = 5.5], 64.29 [s, C-1], 65.11 [d, C-6, *J* = 165.60], 68.74 [d, C-4, *J* = 18.80], 72.34 [s, C-3], 99.07 [d, C-2, *J* = 13.2]. ^{31}P NMR (D_2O): δ = 18.7.

6-(Ethylphosphono)-D-*arabino*-5-deoxyhex-2-ulopyranose (4b)

It was obtained as a white foam in 45% yield from (*R*)-**1** using 67 U of FruA, 1.5 mmol of FBP, and 2 days of reaction time. Treatment with acid phosphatase (160 U) lasted 2.5 days. R_f = 0.12 ($CHCl_3$:MeOH:H_2O, 20:10:1; detection C). Elemental analysis: found: C 35.1, H 6.45%; $C_8H_{17}O_8P$ requires: C 35.30, H 6.30%. $[\alpha]_D$: +14.0 (*c* 2.2, H_2O). ^{1}H NMR (D_2O): δ = 1.19 [3H, t, $(CH_3CH_2O)_2P$, *J* = 7.1], 1.71 [1H, d quart, H-5eq, *J* = 14.6 (q), 3.0 (d)], 2.11 [1H, dddd, H-5ax, *J* = 14.3, 13.6, 10.3, 3.2], 3.41 and 3.59 [2H, AB system, H-1, J_{AB} = 12.1], 3.6 [1H, d, H-3eq, *J* = 1.8], 3.92 [2H, quint, $(CH_3CH_2O)_2P$, *J* = 7.1], 4.04 [1H, quint, H-4, *J* = 3.0], 4.26 [1H, ddd, H-6, *J* = 13.3, 11.4, 2.3]. ^{13}C NMR (D_2O): δ = 16.52 [d, $(CH_3CH_2O)_2P$, *J* = 5.3], 27.22 [s, C-5], 61.31 [d, C-6, *J* = 166.5], 62.10 [d, $(CH_3CH_2O)_2P$, *J* = 5.6], 65.14 [s, C-1], 66.40 [s, C-3], 67.90 [d, C-4, *J* = 15.3], 97.93 [d, C-2, *J* = 11.2]. ^{31}P NMR (D_2O): δ = 19.8.

7-(Diethylphosphono)-L-*xylo*-7,5-dideoxyhept-2-ulopyranose (5a)

It was obtained as a slightly yellow foam in 30% yield from (*R*)-**2** using 40 U of FruA, 3.0 mmol of FBP, and 4 days of reaction time. Treatment with acid phosphatase (100 U) lasted 2 days. R_f = 0.04 ($CHCl_3$:MeOH:H_2O, 20:10:1; detection: C). Elemental analysis: found: C 42.3, H 7.5%; $C_{11}H_{23}O_8P$ requires: C 42.04, H 7.38%. $[\alpha]_D$: –21.9 (*c* 1.5, H_2O). ^{1}H NMR (D_2O): δ = 1.20 [6H, t, $(CH_3CH_2O)_2P$, *J* = 7.0], 1.44 [1H, quart, H-5ax, *J* = 12.0], 2.0–2.1 [1H, m, H-5eq, $J^*_{5eq/4}$ = 4.8], 2.10 – 2.25 [2H, m, H-7, $J^*_{P/H\text{-}7}$ = 16.0], 3.43 [1H, d, H-3, *J* = 9.5], 3.6 – 4.0 [3H, m, H-4 and H-1], 4.08 [4H, quint, $(CH_3CH_2O)_2P$, *J* = 7.5], 4.26 [1H, m, H-6, $J^*_{6/5ax} = J^*_{P/H\text{-}7}$ = 11.0]. ^{13}C NMR (D_2O): δ = 16.08 [d, $(CH_3CH_2O)_2P$, *J* = 4.3], 30.74 [d, C-7, *J* = 138.8],

39.74 [d, C-5, J = 12.4], 63.82 and 64.13 [2d, $(CH_3CH_2O)_2P$, J = 5.9, 6.2], 64.47 [s, C-6], 67.02 [s, C-1], 68.15 [s, C-4], 72.22 [s, C-3], 98.10 [d, C-2, J = 9.3]. ^{31}P NMR (D_2O): δ = 31.3.

7-(Diethylphosphono)-D-*arabino*-7,5-dideoxy-hept-2-ulopyranose (5b)

It was obtained as a yellow foam in 11% yield from (*S*)-**2** using 50 U of FruA, 5.0 mmol of FBP, and 5 days of reaction time. Treatment with acid phosphatase (63 U) lasted 2 days. R_f = 0.04 ($CHCl_3$:MeOH:H_2O, 20:10:1; detection: C). Elemental analysis: found: C 42.35, H 7.3%; $C_{11}H_{23}O_8P$ requires: C 42.04, H 7.38%. $[\alpha]_D$: –1.6 (*c* 0.49, H_2O). ^1H NMR (D_2O): δ = 1.28 [3H, t, $(CH_3CH_2O)_2P$, J = 7.0], 1.75 – 1.95 [2H, m, H-5, $J^*_{4/5}$ = 2.5 and 2.1, J^*_{AB} = 14.4], 2.09 – 2.22 [2H, m, H-7, $J^*_{P/H}$ = 18.7], 3.63 [1H, d, H-3, J = 3.9], 3.36 and 3.65 [2H, AB system, H-1, J_{AB} = 11.6], 4.00 – 4.10 [1H, m, H-4], 4.11 [4H, quint, $(CH_3CH_2O)_2P$, J = 7.1], 4.45 – 4.51 [1H, m, H-6]. ^{13}C NMR (D_2O): δ = 16.08 [d, $(CH_3CH_2O)_2P$, J = 5.7], 31.27 [d, C-7, J = 138.9], 33.45 [d, C-5, J = 12.0], 60.89 and 65.25 [2s, C-6 and C-3], 63.77 and 64.00 [2d, $(CH_3CH_2O)_2P$, J = 6.4 and 6.0], 67.50 [s, C-1], 68.11 [s, C-4], 96.47 [d, C-2, J = 8.7 Hz]. ^{31}P NMR (D_2O): δ = 31.5.

8-(Diethylphosphono)-L-*xylo*-8,7,5-trideoxy-oct-2-ulopyranose (6a)

It was obtained as a white foam in 20% yield from (*R*)-**3** using 130 U of FruA, 5.0 mmol of FBP, and 3 days of reaction time. Treatment with acid phosphatase (83 U) lasted 2 days. R_f = 0.05 ($CHCl_3$:MeOH:H_2O, 20:10:1; detection: C). Elemental analysis: found: C 44.15, H, 7.75%; $C_{12}H_{25}O_8P$ requires: C 43.90, H 7.68%. $[\alpha]_D$: –42.0 (*c* 1.1, H_2O). ^1H NMR (D_2O): δ = 1.27 [6H, t, $(CH_3CH_2O)_2P$, J = 6.9], 1.36 [1H, td, H-5ax, J = 11.8 (t), 5.0 (d)], 1.6 – 2.1 [5H, m, H-5eq and CH_2CH_2P], 3.44 [1H, d, H-3, J = 11.7], 3.58 – 3.78 [1H, m, H-4], 3.39 and 3.64 [2H, AB system, H-1, J_{AB} = 11.6], 3.91 [1H, qd, H-6, J = 10.3 (q), 5.6 (d)], 4.09 [4H, quint, $(CH_3CH_2O)_2P$, J = 7.3]. ^{13}C NMR (D_2O): δ = 16.71 [d, $(CH_3CH_2O)_2P$, J = 5.8], 20.85 [d, C-8, J = 138.7], 27.52 [d, C-7, J = 4.4], 38.44 [s, C-5], 63.76 [d, $(CH_3CH_2O)_2P$, J = 6.3], 67.30 [s, C-1], 68.50 and 72.86 [s, C-3 and C-4], 68.89 [d, C-6, J = 18.0], 97.90 [d, C-2, J = 8.8]. ^{31}P NMR (D_2O): δ = 36.05.

8-(Diethylphosphono)-D-*arabino*-8,7,5-trideoxy-oct-2-ulopyranose (6b)

It was obtained as a slightly yellow foam in 19% yield from (*S*)-**3** using 31 U of FruA, 2.0 mmol of FBP, and 5 days of reaction time. Treatment with acid phosphatase (46 U) lasted 1 day. R_f = 0.10 ($CHCl_3$:MeOH:H_2O, 20:10:1; detection: C). Elemental analysis: found: C 44.3, H 7.8%; $C_{12}H_{25}O_8P$ requires: C 43.90, H 7.68%. $[\alpha]_D$: –9.5 (c 0.52, H_2O). ^1H NMR (D_2O): δ = 1.26 [6H, t, $(CH_3CH_2O)_2P$, J = 7.0], 1.50 – 2.20 [6H, m, *H*-5 and CH_2CH_2P], 3.44 [1H, d, H-3, J = 11.7], 3.58 – 3.78 [1H, m, H-4], 3.40 – 4.10 [5H, m, *H*-1, *H*-3, *H*-4, *H*-6], 4.07 [4H, quint. $(CH_3CH_2O)_2P$, J = 7.3]. ^{13}C NMR (D_2O): δ = 16.12 [d, $(CH_3CH_2O)_2P$, J = 5.6], 20.48 [d, C-8, J = 139.0], 27.79 [d, C-7, J = 4.1], 32.01 [s, C-5], 63.55 [d, $(CH_3CH_2O)_2P$, J = 6.3], 64.86 [d, C-6, J = 17.6], 65.64 and 68.27 [s, C-3 and C-4], 67.46 [s, C-1], 98.20 [d, C-2, J = 8.8]. ^{31}P NMR (D_2O): δ = 35.98.

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References

[1] G. Guanti, L. Banfi, M. T. Zannetti, *Tetrahedron Lett.* **2000**, *41*, 3181–3185.

[2] F. Hammerschmidt, H. Völlenkle, *J. Liebigs Ann. Chem.* **1989**, 577–583; T. Yokomatsu, S. Shibuya, *Tetrahedron: Asymmetry* **1992**, *3*, 377–378; T. Yokomatsu, T. Yamagishi, S. Shibuya, *Tetrahedron: Asymmetry* **1993**, *4*, 1779–1782; T. Yokomatsu, T. Yamagishi, S. Shibuya, *Tetrahedron: Asymmetry* **1993**, *4*, 1783–1784; H. Wynberg, A. A. Smaardijk, *Tetrahedron Lett.* **1983**, *24*, 5899–5900; N. P. Rath, C. D. Spilling, *Tetrahedron Lett.* **1994**, *35*, 227–230; T. Arai, M. Bougauchi, H. Sasai, M. Shibasaki, *J. Org. Chem.* **1996**, *61*, 2926–2927; V. Sum, T. P. Kee, *J. Chem. Soc., Perkin Trans. 1* **1993**, 2701–2711; V. Sum, T. P. Kee, M. Thornton-Pett, *J. Chem. Soc., Chem.Commun.* **1994**, 743–744; T. Gajda, *Tetrahedron: Asymmetry* **1994**, *5*, 1965–1972.

[3] C. Meier, W. H. G. Laux, *Tetrahedron: Asymmetry* **1995**, *6*, 1089–1092.

[4] M. Drescher, Y.-F. Li, F. Hammerschmidt, *Tetrahedron* **1995**, *51*, 4933–4946; M. Drescher, F. Hammerschmidt, H. Kählig, *Synthesis* **1995**, 1267–1272; M. Drescher, F. Hammerschmidt, *Tetrahedron* **1997**, *53*, 4627–4636; G. Eidenhammer, F. Hammerschmidt, *Synthesis* **1996**, 748–754; T. Khushi, K. J. O'Toole, J. T. Sime, *Tetrahedron Lett.* **1993**, *34*, 2375–2378.

[5] Y.-F. Li, F. Hammerschmidt, *Tetrahedron: Asymmetry* **1993**, *4*, 109–120.

[6] E. Zymanczyk-Duda, B. Lejczak, P. Kafarski, J. Grimaud, P. Fischer, *Tetrahedron* **1995**, *51*, 11809–11814.

[7] M. Gulea-Porcarescu, E. About-Jaudet, N. Collignon, *J. Organomet. Chem.* **1994**, *464*, C14–C16.

[8] A. F. Kluge, I. S. Cloudsdale, *J. Org. Chem.* **1979**, *44*, 4847–4852.

[9] W. Nagata, T. Wakabayashi, Y. Hayase, *Org. Synth., Coll. Vol. VI*, 448–451.

[10] J. Kabak, L. De Filippe, R. Engel, *J. Med. Chem.* **1972**, *15*, 1074–1075.

[11] R. J. Kazlauskas, A. N. E. Weissfloch, A. T. Rappaport, L. A. Cuccia, *J. Org. Chem.* **1991**, *56*, 2656–2665.

[12] I. Ohtani, T. Kusumi, Y. Kashman, H. Kakisawa, *J. Am. Chem. Soc* **1991**, *113*, 4092–4096; D. Parker, *Chem. Rev.* **1991**, *91*, 1441–1457; J. K. Kozlowski, N. P. Rath, C. D. Spilling, *Tetrahedron* **1995**, *51*, 6385–6396; G. Barth, W. Voelter, H. S. Mosher, E. Bunnenberg, C. Djerassi, *J. Am. Chem. Soc.* **1970**, *92*, 875–886.

[13] F. Hammerschmidt, Y.-F. Li, *Tetrahedron* **1994**, *50*, 10253–10264.

[14] W.-D. Fessner, C. Walter, *Top. Curr. Chem.* **1996**, *184*, 97–194.

[15] I. A. Natchev, *Bull. Chem. Soc. Jpn.* **1988**, *61*, 3711–3715; I. A. Natchev, *Bull. Chem. Soc. Jpn.* **1988**, *61*, 3699–3704; I. A. Natchev, *Tetrahedron* **1991**, *47*, 1239–1248.
[16] J. R. Durrwachter, C.-H. Wong, *J. Org. Chem.* **1988**, *53*, 4175–4181.
[17] W. J. Lees, G. M. Whitesides, *J. Org. Chem.* **1993**, *58*, 1887–1894.
[18] M. T. Zannetti, C. Walter, M. Knorst, W.-D. Fessner, *Chem. Eur. J.* **1999**, *5*, 1882–1890.
[19] C. Walter, *Ph. D. thesis*, Rheinisch-Westfälische Technische Hochschule (Aachen), **1996**.