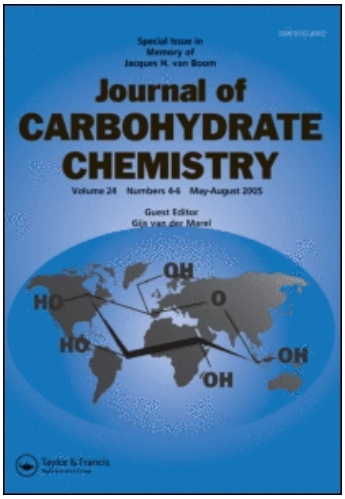

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SYNTHESIS OF PROTEIN CONJUGATES OF 2-CARBOXY-L-ARABINITOL 5-PHOSPHATE AND 2-CARBOXY-L-RIBITOL 5-PHOSPHATE

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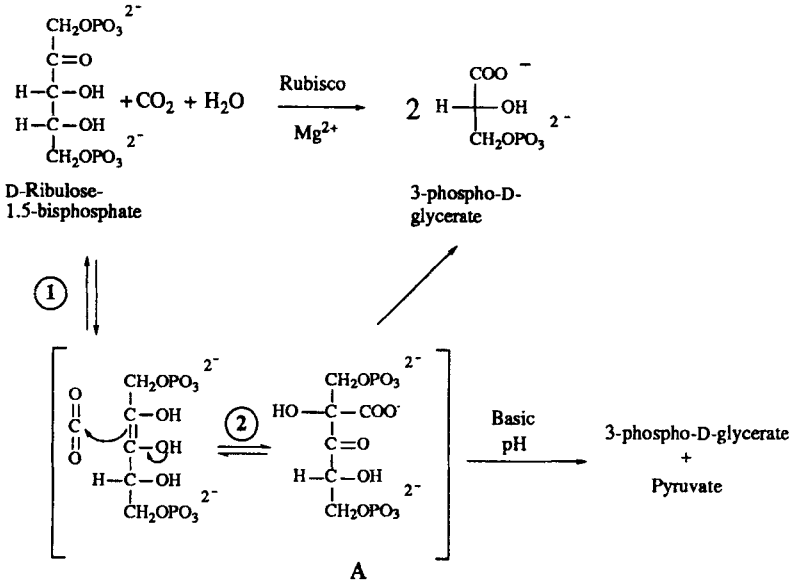
ABSTRACT

The synthesis of 5-*O*-[5-(pentanoic acid)]-phosphono-L-*erythro*-pent-2-ulose **2** was successfully achieved by coupling benzyl 5-hydroxypentanoate **9b** and 1-*O*-benzyl-L-*erythro*-pent-2-ulose ethane-1,2-diyl dithioacetal **13** with the enediol pyrophosphate **18**. Compound **2** was coupled to carrier proteins, porcine thyroglobuline and bovine serum albumin and the conjugates were treated with KCN to give conjugates of the hapten **1**, designed to raise catalytic antibodies.

INTRODUCTION

The protein conjugates which are described here were designed with the hope of eliciting catalytic antibodies. Many examples of this new class of biological catalysts, which accelerate a number of chemical transformations are now described: hydrolysis of esters, amides, enol ethers, lactonisation, transacylation, elimination and redox reactions etc.[1] However, only a few reactions involving C-C bond formation have been catalyzed by antibodies: this includes intramolecular reactions such as Claisen,[2] oxy-Cope[3] and dienone-phenol rearrangements,[4] Diels-Alder reactions,[5] cationic condensations[6] and aldol condensations.[7]

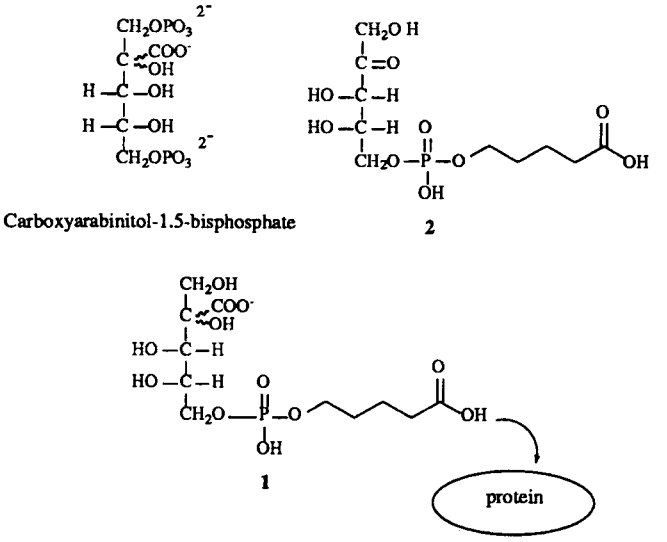


Scheme 1

Our goal is to obtain antibodies capable of catalyzing the formation of an intermolecular carbon-carbon bond, a process which has been rarely described except for Diels-Alder reactions. The process that we have chosen to mimic corresponds to the first two steps (enolisation and carboxylation) of the carboxylation of ribulose-1,5-bisphosphate, catalyzed in plants by ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco), the key enzyme of photosynthesis[8] (Scheme 1). We do not expect to achieve the complete reaction, but the antibody could yield the β-ketoacid intermediate **A** which is unstable and can be cleaved at basic pH into 3-phospho-D-glycerate and pyruvate[9] (Scheme 1).

Catalytic antibodies are classically generated by immunisation with stable analogues of the transition state of the reaction. A well-known excellent competitive inhibitor of Rubisco, which can be considered as a transition-state analogue of step ② is D-carboxyarabinitol 1,5-bisphosphate (CABP) ($K_i < 10^{-11}$ M)[10] (Scheme 2).

We have chosen to employ an analog of CABP, compound **1**, as hapten to elicit antibodies (Scheme 2). The strategy that we have adopted was to prepare first a derivative of L-ribulose 5-phosphate **2** (L-ribulose is cheaper than D-ribulose) with a carboxy terminal chain and to couple it with two proteins, porcine thyroglobuline (PTG) and bovine serum albumin (BSA), the first one for immunization, the second one for antibody screening. The



Scheme 2

hydroxy acid moiety was then installed on the conjugates by addition of KCN to the carbonyl group.

This leads to a mixture of arabinitol and ribitol derivatives. This is not important here since two easily separable families of antibodies recognizing each of the diastereoisomers are expected.

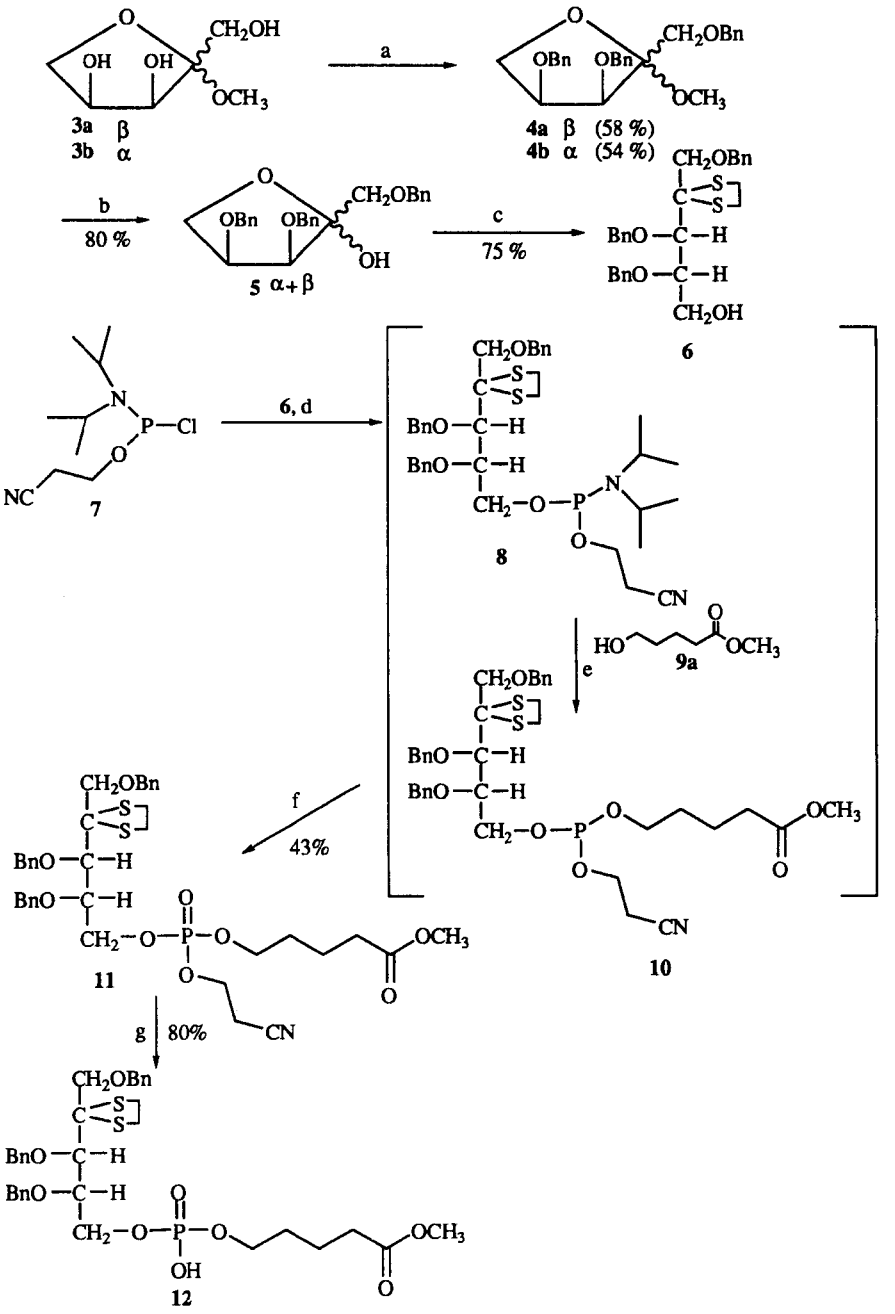
RESULTS AND DISCUSSION

Synthesis of compounds 2 and 1. Two routes were tested to synthesise **2**.

1) Phosphite triester procedure.

The first route (Scheme 3) uses the phosphite triester procedure introduced by Letsinger *et al.*[11] and requires the protection of all the functions except the alcohol at C-5. The carbonyl group has also to be protected to avoid the hemiaketal formation.

This was achieved starting from L-*erythro*-pentulofuranosides **3a** or **3b** as prepared by Stankovic *et al.*[12] **3a** or **3b**, treated with NaH and benzyl bromide in the presence of $Bu_4N^+ I^-$ [13] gave, respectively, the methyl 1,3,4-tri-*O*-benzyl-L-*erythro*-pentulofuranosides, **4a** and **4b**. After removal of the methyl group by 1N HCl in THF,[14] **5** was obtained as a mixture of anomers and was then treated with ethanedithiol in the presence of $BF_3 \cdot Et_2O$ in CH_2Cl_2,[5] giving the thioketal **6**.



a) NaH,$C_6H_5CH_2Br$, $Bu_4N^+I^-$; b) HCl 1N, THF; c) HS-$(CH_2)_2$SH, BF_3•Et_2O, CH_2Cl_2; d) iPr_2NH, CH_3CN; e) tetrazole; f) I_2, THF, H_2O, Pyridine; g) $NaOCH_3$, CH_3OH

Scheme 3

A one pot phosphite triester procedure was used to obtain **11**.[16] The chlorophosphoramidite **7** was obtained from PCl_3 by treatment with β-cyanoethanol and then with diisopropylamine.[17]

7 reacted with **6** in the presence of *N,N*-diisopropylethylamine to afford the novel phosphoramidite **8** which was subsequently treated with methyl 5-hydroxypentanoate **9a**[18] in the presence of tetrazole as catalyst to yield **10**. (When the order of the addition was reversed, the reaction of **7** with **9** gave a phosphoramidite which did not react with the protected pentulose **6**). **10** was oxidized *in situ* by a 0.1 M solution of iodine in a mixture THF, H_2O, pyridine, at -10 °C.[19] (The temperature and the pH of the solution were essential factors to avoid the formation of H-phosphonate which was found at higher temperature and/or without pyridine). The resulting phosphotriester **11** was treated with sodium methoxide at room temperature to release the cyanoethyl group, giving **12**.

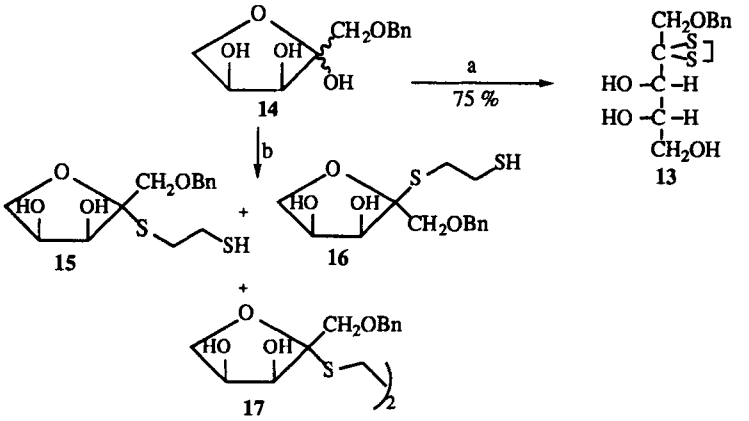
Unfortunately all attemps to remove the thioketal of **12** were unsuccessful. With heavy metals salts, such as $HgCl_2/HgO$,[20] $AgNO_3$,[21] $CuCl_2$ /CuO[22] no deprotection occurred (reaction was tested only on the pentulofuranoside **6**). With HgO/HBF_4[23] or ICH_3/Na_2CO_3[24] the phosphate group was cleaved as well as the dithioketal. Assuming that the very difficult deprotection of the thioketal could be due to the steric hindrance of the benzyl groups we tried another route leaving free the secondary hydroxyl groups. We had to change the phosphorylating agent since the phosphite triester procedure has no selectivity for primary alcohols.

2) Enediol pyrophosphate procedure.

We used the pyrophosphate **18**[25] to selectively phosphorylate primary alcohols in the presence of unprotected secondary alcohols. This second route, described in Scheme 5, uses compound **13** in which only the primary alcohol at C-1 is protected by a benzyl group. The ketone was also protected, as the thioketal, to avoid hemiacetal formation and keep free the alcohol at C-5.

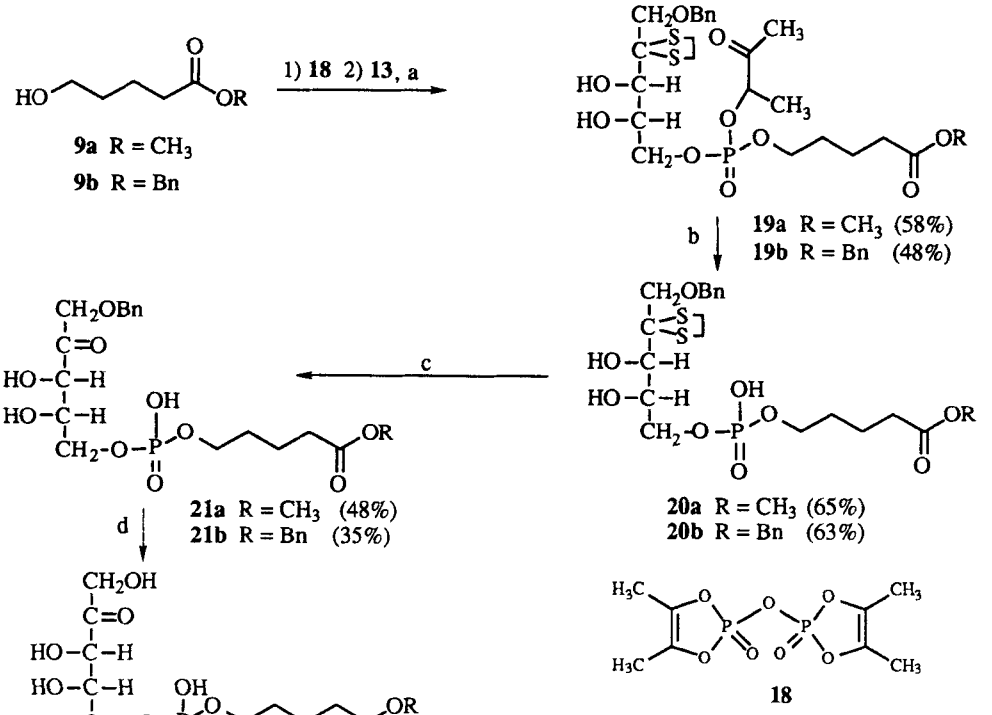
13 was obtained from **14** prepared from L-ribulose as reported by Vanhessche *et al.* [26] (Scheme 4). The thioketalisation occurred in a good yield using ethanedithiol in concentrated hydrochoric acid.[27] With ethanedithiol and BF_3•Et_2O in dichloromethane,[28] only compounds **15**, **16** and **17** were obtained.

The double phosphorylation was carried out according to the one pot procedure of Ramirez *et al.*[29] (Scheme 5). **9a** was first reacted with pyrophosphate **18**, then with **13** to give the phosphotriester **19a**. The 3-oxobutyl phosphate protective group was hydrolyzed under basic conditions with Na_2CO_3 into **20a**. The methods employed to deprotect **6** or **12** were tried to cleave the thioketal of **20a**. The only successful method was treatment with



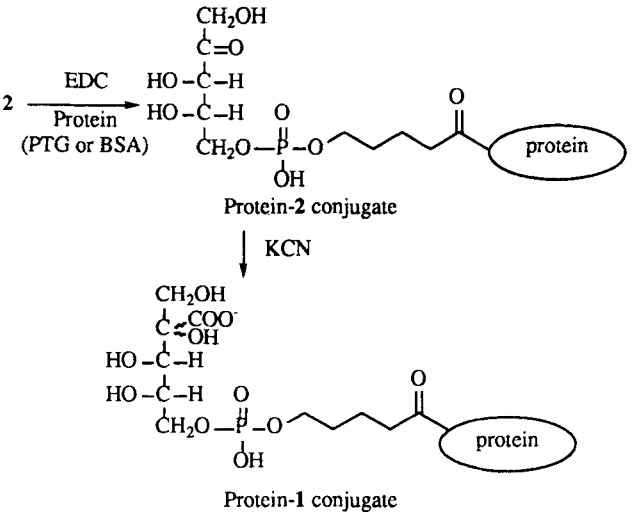
a) $HS(CH_2)_2SH$, HCl conc.; b) $HS(CH_2)_2SH$, $BF_3 \cdot Et_2O$

Scheme 4



a) Et_3N, CH_2Cl_2; b) Na_2CO_3, H_2O-CH_3CN 2:1;
c) CH_3I, CH_3CN - Phosphate buffer pH 7.6; d) H_2, Pd/C

Scheme 5



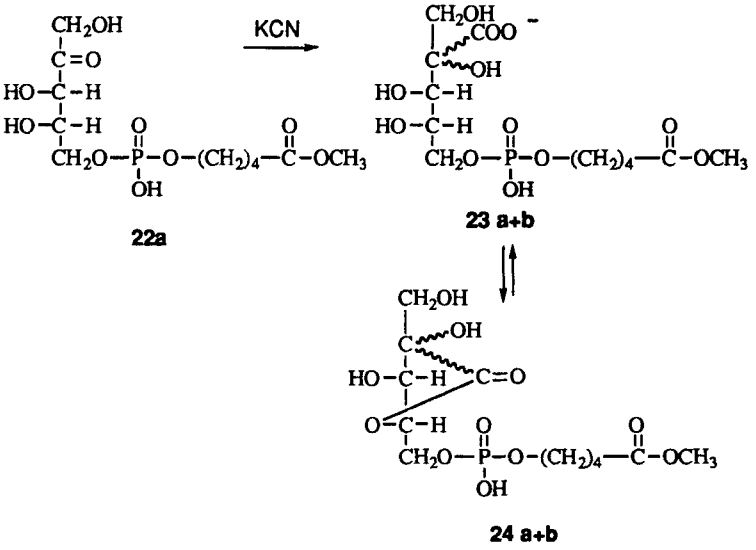
Scheme 6

CH_3I in a mixture CH_3CN-phosphate buffer pH 7.6.[24] The benzyl protecting group was removed from **21a** by hydrogenolysis[30] to give **22a**. However, saponification of the methyl ester of **22a** could not be carried out. Even with a mild base such as LiOH,[31] only the decomposition of **22a** was observed.

We had thus to change the protecting group of the carboxylic acid and the same sequence of reactions was carried out starting with benzyl 5-hydroxypentanoate **9b** leading to **21b**. The benzyl ether and ester of **21b** were easily deprotected in one step by hydrogenolysis to give **2**.

Formation and characterisation of the conjugates. The protein-hapten conjugates are formally derived from hapten **1**. They must be formed by covalent attachment between the terminal carboxylic acid and the free amino groups of the carrier protein. Although the carboxylic acid at C-2 is more sterically hindered than the one at the end of the arm, we wanted to make sure that the hapten was fixed by the latter position. Thus compound **2** was first conjugated, using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide) hydrochloride (EDC) as activating agent.[32] We obtained conjugates with the two common carrier proteins: porcine thyroglobuline (PTG) for immunisations and bovine serum albumin (BSA) for ELISA test.

Protein-**2** conjugates were freed from the excess of EDC and unbound **2** by extensive dialysis in water. Then the hapten was directly formed on the protein adducts by addition of KCN (Scheme 6).



Scheme 7

We determined with compound **22a** that using the procedure described by Pearce *et al.*[10] for the synthesis of carboxyarabinitol, the intermediate cyanhydrins were directly hydrolyzed into the acids **23a** and **23b**[33] (Scheme 7).

Indeed, according to these authors, the acids are in equilibrium with the corresponding γ lactones and treatment with a cation exchange resin (H^+ form) completes the lactonisation. After such a treatment, we isolated the mixture of lactones **24a + 24b.** The reaction was then repeated on protein-**2**.

To evaluate the number of haptens bound to the protein, we carried out the addition of KCN with radio labelled [^{14}C]KCN as tracer. After extensive dialysis to free the conjugate from the excess KCN, the remaining radioactivity on the protein was counted: on average, 15 haptens have been fixed on PTG and 9 on BSA.

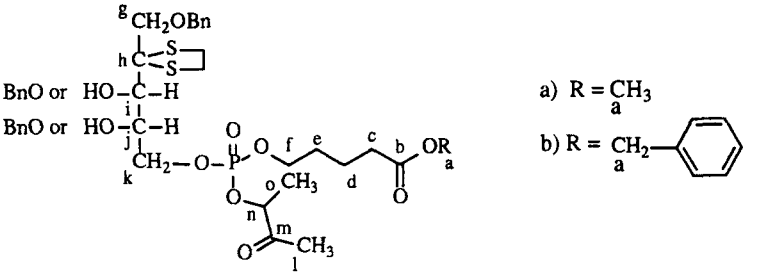
Another problem concerns the possible equilibrium between the hydroxyacids and the lactones (Scheme 7). Of course, antibodies against the hydroxyacids were searched for. To simplify the NMR study of this equilibrium, **22** was treated with 20 % [^{13}C] enriched KCN. The signals of the carbonyl group of the diastereoisomeric lactones **24a** and **24b** were well separated (177.2 and 178.2 ppm). After 2 h at pH 9 at 4 °C, these signals were replaced by two other ones, at 179.2 and 179.3 ppm corresponding to the open forms. When this mixture was neutralized at pH 7.4 the hydroxyacid forms remained largely predominant even after 3 days at 37 °C.

The PTG-haptene was thus treated in a pH 9 buffer for 2 hours and then neutralized to pH 7.4 before injection to mice. Immunisations are in progress.

This synthesis leads to a diastereoisomeric mixture at C-2 of protein-hapten conjugates. The mixture PTG-**1** has been used for immunizations. If antibodies which catalyze the carboxylation are produced, two populations could *a priori* be found, corresponding to both isomers at C-2. If the ELISA tests are also conducted with the diastereoisomeric mixture, BSA-**1**, the two populations will be detected.

EXPERIMENTAL SECTION

General. Reaction progress was monitored by analytical TLC on silica gel 60F-254 plates or reversed phase plates RP18 F254S from Merck and visualized first with UV light, and then with phosphomolybdic acid in ethanol. Column chromatography was performed using either Merck silica gel 230(0,04-0,063 mm, flash) or Merck silica gel 90(0,63-0,2 mm, flash). Chromatography on reversed phase was performed on silica gel Merck Lichroprep RP 18 (25-40 μm). Melting points were measured on a Kofler apparatus. FAB mass spectra were recorded on a NERMAG R10-10 mass spectrometer at "Laboratoire de chimie structurale organique et bioorganique" from University Paris 6, using triethanolamine (TEA), Glycerol (Gly), nitrobenzylalcohol (Noba) or thioglycerol (TG) as matrix. ^{1}H NMR spectra were recorded on a Jeol GSX400, a Bruker ARX400 or a Bruker AC200. ^{13}C NMR spectra were recorded on a Jeol GSX400 or on a Brucker ARX400 at 100 MHz and on a Bruker AC200 at 50 MHz. ^{31}P NMR spectra were recorded on a Brucker ARX 400 and Bruker AM 500. Chemical shifts are given in ppm downfield from internal tetramethylsilane for ^{1}H NMR and ^{13}C NMR (from phosphoric acid for ^{31}P NMR). Coupling constants were obtained by spin decoupling. Protons and carbons of compounds **12**, **19a,b**, **20a,b**, **21a,b**, **22a**, **2** are designated as shown in the scheme below :



Compounds **7**, **9a**, **14** and **18** were synthesized as previously described.[17,18,26,34] [^{14}C]KCN was purchased from NEN Research Product, specific activity 55 mCi /mmol.

Counting was performed with a 1214 Rackbeta scintillation counter, using Aquasol-2 from Packard as scintillation liquid (counting yield 94.4%).

Methyl 1,3,4-tri-*O*-benzyl-β-L-*erythro*-pent-2-ulofuranoside (4a). Sodium hydride (80 % in mineral oil) (673 mg, 22.4 mmol) was added to a solution of methyl β-L-*erythro*-pent-2-ulofuranoside (819 mg, 5 mmol) in anhydrous *N,N*-dimethylformamide (30 mL). After stirring 15 min at room temperature, tetrabutylammonium iodide (2.7 g, 7.48 mmol) and benzyl bromide (2.67 mL, 22.4 mmol) were added. The mixture was stirred at 80 °C for 4 h, then at room temperature, CH_2Cl_2 (100 mL) was added and the solution was washed with H_2O to pH = 7, and with 1N HCl (40 mL) and H_2O to pH = 7. The organic layer was dried over $MgSO_4$ and concentrated under vacuum. Purification by chromatography on silica-gel (cyclohexane : ethyl acetate 9:1) yielded 1.3 g (58 %) of **4a** as a white powder which crystallised from ether. mp 52 °C; $[\alpha]_D^{20}$ -220 (*c* 1.55, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 3.12 (s, 3H, $-OCH_3$), 3.53 (d, 1H, J_{gem} = 10.4 Hz, H_{1a}), 3.65 (d, 1H, J_{gem} = 10.4 Hz, H_{1b}), 3.80 (dd, 1H, J_{gem} = 8.3 Hz, J_{H5a-H4} = 7.1 Hz, H_{5a}), 3.88 (d, 1H, J_{H3-H4} = 4.4 Hz , H_3), 3.91 (dd, 1H, J_{gem} = 8.3 Hz, J_{H5b-H4} = 7.7 Hz, H_{5b}), 4.33 (m, 1H, H_4), 4.33 (d, 1H, J_{gem} = 12.1 Hz, $-CH_2\phi_a$), 4.42 (d, 1H, J_{gem} = 9.3 Hz, $-CH_2\phi_b$), 4.45 (d, 1H, J_{gem} = 9.3 Hz, $-CH_2\phi_b$), 4.54 (d, 1H, J_{gem} = 12.1 Hz, $-CH_2\phi_a$), 4.64 (s, 2H, $-CH_2\phi_c$), 7.19-7.25 (m, 15H, H aromatic); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 48.45 ($-OCH_3$), 65.00 (C_1), 69.28 (C_5), 72.37-73.45-73.72 ($-CH_2\phi$), 78.51 (C_3), 79.94 (C_4), 108.63 (C_2), 127.53-127.62-127.74-127.98-128.07-128.20-128.33 137.64-137.97-138.26 (C aromatic).

Anal. Calcd for $C_{27}H_{30}O_5$: C, 74.65; H, 6.91. Found: C, 74.94; H, 6.95.

Methyl 1,3,4-tri-*O*-benzyl-α-L-*erythro*-pent-2-ulofuranoside (4b). Methyl-α-L-*erythro*-pent-2-ulofuranoside (970 mg, 5.9 mmol) treated according to the above procedure gave 1.4 g (54 %) of **4b** as a white powder which crystallized from ether. mp 64 °C; $[\alpha]_D^{20}$ +60° (*c* 1.55, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz) : δ 3.35 (s, 3H, $-OCH_3$), 3.47 (d, 2H,H_1), 3.89 (d, 1H, J_{H3-H4} = 1.9 Hz, H_3), 3.89 (m, 2H, H_5), 3.97 (m, 1H, H_4), 4.44 (d, 1H, J_{gem} = 12 Hz, $-CH_2\phi_a$), 4.50 (d, 1H, J_{gem} = 12 Hz, $-CH_2\phi_a$), 4.53 (d, 1H, J_{gem} = 12 Hz, $-CH_2\phi$b), 4.60 (d, 1H, J_{gem} = 12 Hz, $-CH_2\phi_b$), 4.63 (s, 2H, $-CH_2\phi_c$), 7.17-7.3 (m, 15H, H aromatic);^{13}C NMR ($CDCl_3$, 100 MHz): δ 49.91 ($-OCH_3$), 70.10 (C_1), 70.72 (C_5), 72.13-72.61-73.47 ($-CH_2\phi$), 74.73 (C_3), 78.09 (C_4), 104.45 (C_2), 127.62-127.69-127.87-128.13-128.29 137.89-138.06-138.17(C aromatic).

Anal. Calcd for $C_{27}H_{30}O_5$: C, 74.65; H, 6.91. Found: C, 74.93; H, 6.94.

1,3,4-Tri-*O*-benzyl-α,β-L-*erythro*-pent-2-ulofuranose (5). 1N HCl (2 mL) was added to a solution of **4a** or **4b** (178.5 mg, 0.41 mmol) in THF (2 mL). After heating at 80 °C for 5 h, 5 % NH_4OH (6 mL) was added, then THF was evaporated under

vacuum and the product was extracted from H_2O with CH_2Cl_2 (40 mL). The organic phase was dried over $MgSO_4$ and concentrated under vacuum. Silica gel chromatography (cyclohexane : ethyl acetate, 8:2) yielded 141 mg (80 %) of **5** as a colorless oil, mixture of α and β anomers. ^1H NMR ($CDCl_3$, 400 MHz): δ 3.66 (d, 1H, J_{gem} = 10.4 Hz, H_{1a} maj. anom.), 3.71 (d, 1H, J_{gem} = 10.4 Hz, H_{1b} maj. anom.), 3.83 (d, 1H, J_{gem} = 10 Hz, H_{1a} min. anom.), 3.90 (d, 1H, J_{gem} = 10 Hz, H_{1b} min. anom.), 3.97 (dd, 1H, J_{gem} =9.9 Hz, J_{H5a-H4} = 4.4 Hz, H_{5a} maj. anom.), 4.07 (m, 1H, H_4 maj. anom.), 4.14-4.22 (m, 5H, H_{5b} maj. and min. anom. -H_{5a} min. anom-H_3 maj. anom-H_4 min. anom.), 4.33 (d, 1H, H_3 min. anom.), 4.54-4.85 (m, 6H, -$CH_2\phi$) ; 7.20-7.27 (m, 15H, H aromatic); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 65 (C_5 min. anom.), 69.08 (C_5 maj. anom.), 70.67 (C_1 maj. anom.), 71.69 (C_1 min. anom.), 72.24-72.70-73.49(-$CH_2\phi$ maj. anom.), 73.19-73.9 (-$CH_2\phi$ min. anom.), 76.45 (C_3 maj. anom.), 77.09 (C_4 maj. anom.), 77.84 (C_3 min. anom.), 82.06 (C_4 min. anom.), 102.40 (C_2 maj. anom.), 126.91 (C_2 min. anom.), 127.73-127.78-127.91-128.04-128.30-128.40-128.46-137.36-137.46-137.99 (C aromatic).

Anal. Calcd for $C_{26}H_{28}O_5$: C, 74.28; H, 6.66. Found: C, 74.16; H, 6.80.

1,3,4-Tri-*O*-benzyl-L-*erythro*-pent-2-ulose ethane-1,2-diyl dithioacetal (6). To a solution of **5** (1.05 g, 2.5 mmol) in anhydrous CH_2Cl_2 (10 mL) was added ethanedithiol and then BF_3•Et_2O (325 μL, 2.5 mmol). After stirring 3 h, 0.2 N NaOH (2 mL) was added to destroy BF_3•Et_2O. The organic phase was washed with H_2O to pH = 7, dried over $MgSO_4$ and concentrated under vacuum. Silica gel column chromatography (cyclohexane : ethyl acetate, 8:2) yielded 933 mg (75 %) of **6** as a colorless oil. ^1H NMR ($CDCl_3$, 400 MHz): δ 1.49 (s, 1H, -OH), 3.15 (m, 4H, -CH_2S-), 3.63 (d, 1H, J_{gem} = 9.3 Hz, H_{1a}), 3.76 (dd, 1H, J_{gem} = 12 Hz, J_{H5a-H4} = 4 Hz, H_{5a}), 3.80 (d, 1H, J_{gem} = 9.3 Hz, H_{1b}), 3.92 (m, 2H, H_{5b} and H_4), 4.24 (d, 1H, J_{H3-H4} = 2.7 Hz, H_3), 4.45 (d, 1H, J_{gem} = 12.1 Hz, -$CH_2\phi_a$), 4.48 (d, 1H, J_{gem} = 12.1 Hz, -$CH_2\phi_a$), 4.51 (d, 1H, J_{gem} = 11.5 Hz, -$CH_2\phi$b), 4.61 (d, 1H, J_{gem} = 11 Hz, -$CH_2\phi_c$), 4.66 (d, 1H, J_{gem} = 11.5 Hz, -$CH_2\phi_b$), 4.77 (d, 1H, J_{gem} = 11 Hz, -$CH_2\phi_c$), 7.18-7.30 (m, 15H, H aromatic); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 38.76 (-CH_2S-), 38.90 (-CH_2S-), 62.40 (C_5), 71.38 (-$CH_2\phi$), 72.53 (C_2), 73.25 (-$CH_2\phi$), 75.37 (-$CH_2\phi$), 75.97 (C_1), 81.51 (C_4), 83.30 (C_3), 127.54-127.62-127.74-127.82-128.20-128.26-128.33-137.78-137.99-138.24 (C aromatic).

Anal. Calcd for $C_{28}H_{32}O_4S_2$: C, 67.74; H, 6.45. Found: C, 67.87; H, 6.55.

1,3,4-Tri-*O*-benzyl-5-*O*[2-cyanoethyl-1-(4-methoxycarbonylbutyl)]-phosphono-L-*erythro*-pent-2-ulose ethane-1,2-diyl dithioacetal (11). *N,N*-diisopropylethylamine (95 μL, 0.546 mmol) and 2-cyanoethyl *N,N*-

diisopropylaminochlorophosphine[17] **7** (177 mL, 0.39 mmol) were added to a solution of **6** (194 mg, 0.39 mmol) in anhydrous acetonitrile (5 mL). After stirring 30 min at room temperature, tetrazole (120 mg, 1.7 mmol) was added and then methyl 5-hydroxypentanoate **9a**[18] (57 mg, 4.31 mmol). The mixture was stirred 2 h at room temperature and then a 0.1 M solution of iodine in a mixture of THF:pyridine:H_2O, 80:40:2 (10 mL, 1 mmol) was added. After stirring 1 h at room temperature CH_2Cl_2 (100 mL) was added and the organic phase was washed with a 20 % aqueous solution of sodium thiosulfate (20 mL), twice with H_2O, and then dried over $MgSO_4$. Silica gel column flash chromatography (cyclohexane : ethyl acetate, 6:4) yielded **11** (124 mg, 43 %) as a colorless oil. ^1H NMR ($CDCl_3$, 400 MHz): δ 1.62 (m, 4H, H_d and H_e), 2.25 (m, 2H, H_c), 2.49 (m, 2H, CH_2CN), 3.22 (m, 4H, CH_2S), 3.62 (s, 3H, H_a), 3.66 (d, 1H, J_{gem} = 10 Hz, H_g), 3.85 (d, 1H, J_{gem} = 10 Hz, $H_{g'}$), 3.98 (m, 4H, H_f and $C\underline{H}_2$-CH_2CN), 4.18 (m, 2H, H_j and H_k), 4.26 (d, 1H, J_{Hi-Hj} = 4.4 Hz, H_i), 4.49 (d, 1H, J_{gem} = 12.1 Hz, $CH_2\phi_a$), 4.55 (d, 1H, J_{gem} = 12.1 Hz, $CH_2\phi_a$), 4.62 (d, 1H, J_{gem} = 11 Hz $CH_2\phi_b$), 4.64-4.73 (m, 1H, $H_{k'}$), 4.7 (s, 2H, $CH_2\phi_c$), 4.80 (d, 1H, J_{gem} = 11 Hz, $CH_2\phi_b$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 19.19 (d, J_{C-P} = 7.3 Hz, CH_2CN), 20.67 (d), 29.30 (d, J_{C-P} = 7.4 Hz, e), 33.09 (c), 38.68 and 38.90 (-CH_2S-), 51.41 (a), 61.30 (J_{C-P} = 5.5 Hz, $\underline{C}H_2CH_2CN$), 61.32 (J_{C-P} = 5.5 Hz, $\underline{C}H_2CH_2CN$), 67.48 (J_{C-P} = 5,5 Hz, f isom. 1), 67.61(f isom. 2), 69.13 (J_{C-P} = 5.5 Hz, k isom. 1), 69.24 (J_{C-P} = 5.7 Hz, k isom. 2), 71.69 ($CH_2\phi$), 72.15 (h), 73.12 ($CH_2\phi$), 75.22 ($CH_2\phi$), 75.77 (g), 80.16 (d, J_{C-P} = 7.4 Hz, j isom 1), 80.29 (d, J_{C-P} = 7.3 Hz, j isom. 2), 83.20 (i), 116.3 and 116.42(CN), 127.45-127.60-127.89-128.15-137.71-137.82-138.02 and 138.15 (C aromatic), 173.39 (b).

1,3,4-Tri-*O*-benzyl-5-*O*[1-(4-methoxycarbonyl-butyl)]-phosphono-L-*erythro*-pent-2-ulose ethane-1,2-diyl dithioacetal (12). Sodium methoxide (30 % in methanol) (200 μL) was added to a solution of **11** (143 mg, 0.19 mmol) in anhydrous methanol (6 mL). After stirring 30 min at room temperature, the mixture was neutralized with DOWEX H $^{\oplus}$ resin. After filtration and washing of the resin with methanol, the solvent was evaporated under vacuum yielding **12** (105 mg, 80 %) as a colorless oil. ^1H NMR ($CDCl_3$, 400 MHz): δ 1.83 (m, 4H, H_d and H_e), 2.05 (m, 2H, H_c), 3.00-3.11 (m, 4H, -CH_2S-), 3.38 (s, 1H, -OH), 3.46 (d, 1H, J_{gem} = 10 Hz, H_g), 3.47 (s, 3H, H_a), 3.71 (m, 2H, H_f), 3.76 (d, 1H, J_{gem} = 10 Hz, Hg'), 4.03 (m, 2H, H_k and H_j), 4.17 (d, 1H, J_{Hi-Hj} = 2.2 Hz, H_i), 4.34 (d, 1H, J_{gem} = 12.1 Hz, $CH_2\phi_a$), 4.41 (d, 1H, J_{gem} = 12.1 Hz, -$CH_2\phi_a$), 4.50 (m, 2H, H_{5b}, $CH_2\phi_c$), 4.56 (d, 1H, J_{gem} = 11.5 Hz, -$CH_2\phi_b$), 4.66 (d, 1H, J_{gem} = 11.5 Hz, $CH_2\phi_b$), 4.70 (d, 1H, J_{gem} = 11 Hz, -$CH_2\phi_c$), 7.15-7.28 (m, 15H, H aromatic); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 21.07 (d), 29.70 (e), 33.44 (c), 38.85 and

39.05 ($-CH_2S-$), 51.52 (a), 65.18 (f), 67.17 (k), 71.97 ($-CH_2\phi$), 72.19 (h), 73.10 ($-CH_2\phi$), 74.93 ($-CH_2\phi$), 74.93 ($-CH_2\phi$), 76.30 (g), 80.96 (d, JC-P = 7.4 Hz, j), 82.70 (i), 127.25-127.42-127.53-127.93-127.96-128.15-128.22-128.79-138.15-138.26-138.64 (C aromatic), 174.36 (b).

1-*O*-Benzyl-L-*erythro*-pent-2-ulose ethane-1,2-diyl dithioacetal (13). A concentrated HCl solution (1.4 mL) was first added at 0 °C to **14**[26] (1 g, 19 mmol) followed by ethanedithiol (352 µL, 4.19 mmol). After stirring for 30 min at 0 °C, the solution was neutralized with a saturated $NaHCO_3$ solution, diluted with water and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, the solvent evaporated to give an oil which was purified by flash chromatography on silica gel (cyclohexane-AcOEt, 2:8) to yield **13** (1g, 75%). ^1H NMR ($CDCl_3$, 400 MHz): δ 3.09 to 3.25 (m, 4H, $-CH_2S-$), 3.37 (d, 1H, J = 5.5 Hz, -OH), 3.43 (d, 1H, J = 4,9 Hz, -OH), 3.76 to 3.81 (m, 5H, H_{5a}, H_{5b}, H_{1a}, H_{1b}, H_4), 3.91 (dd, 1H, J = 7.1, 5.5 Hz, H_3), 4.56 (m, 2H, $-CH_2\phi$), 7.26 to 7.29 (m, 5H, aromatic H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 37.90 and 39.05 ($-CH_2S-$), 64.20 (C_5), 72.76 (C_4), 73.21 (C_2), 73.67 ($-CH_2\phi$), 76.14 (C_1), 77.22 (C_3), 127.80-128.15-128.60-136.50 (aromatic C); mp 84 °C; $[\alpha]_D^{20}$-53° (*c* 1, $CHCl_3$).

Anal. Calcd for $C_{14}H_{20}O_4S_2$: C, 53.14; H, 6.7. Found: C, 53.23; H, 6.41.

2-(1-*O*-Benzyl-β-L-*erythro*-pent-2-ulofuranosyl thio)ethane thiol (15). 2-(1-*O*-Benzyl-α-L-*erythro*-pent-2-ulofuranosyl thio)ethane thiol (16). 1,2-Bis-(1-*O*-benzyl-L-*erythro*-pent-2-ulofuranosyl thiol)ethane (17). Ethanedithiol (1.2 mL, 14.25 mmol) and $BF_3 \cdot Et_2O$ (233 µL, 1.9 mmol) were added to a solution of **14** (2.28 g, 9.5 mmol) in CH_2Cl_2 (18 mL). The mixture was stirred for 1 h at room temperature. The solution was neutralized with a 10% NaOH solution (3 mL), extracted with CH_2Cl_2. The organic phase was washed with water, dried over $MgSO_4$ and the solvent was evaporated. Flash chromatography on silica gel (cyclohexane-AcOEt : 3-7), gave a mixture of **15** + **16** (1.5 g, 58%) as a colourless oil and compound **17** (123 mg, 2 %) as a white solid. The mixture **15** + **16** was purified by chromatography on silica gel (CH_2Cl_2-AcOEt, 9:1) to give **15** (or **16**) (827 mg, 28%) and **16** (or **15**) (255 mg, 8%) as colourless oils. Compound **15** or **16** $[\alpha]_D^{20}$ + 281° (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ 2.53-2.66 (m, 2H, $-C\underline{H}_2SH$), 2.67-2.81 (m, 2H, $-CH_2$ S-), 3.30 (d, 1H, J = 8.8 Hz, -OH), 3.44 (d 1H, J = 9.9 Hz, -OH), 3.65 (d, 1H, Jgem = 10.4 Hz, H_{1a}), 3.74 (d, 1H, J_{gem} = 10.4 Hz, H_{1b}), 3.81 (dd, 1H, J_{gem} = 10. 4 Hz, J = 1. 1 Hz, H_{5a}), 3.88 (dd, 1H, J_{gem} = 10.4 Hz, J = 3.3 Hz, H_{5b}), 4.02 to 4.12 (m, 2H, H_4, H_3), 4.50 (d, 1H, J_{gem} = 11.5 Hz, $-CH_2\phi$), 4.58 (d, 1H, J_{gem} = 11.5 Hz, $-CH_2\phi$), 7.25 à 7.29 (m, 5H, aromatic H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 25.46 Hz ($-CH_2SH$), 26.76 ($-CH_2S-$), 71.31 (C_1), 71.35 (C_5), 71.66 (C_4), 73.87 ($-CH_2\phi$), 80.40 (C_3), 92.58 (C_2), 127.91-128.22-128.59-136.43 (aromatic C).

Anal. Calcd for $C_{14}H_{20}O_4S_2$: C, 53.14; H, 6.37. Found: C, 53.15; H, 6.34.

Compound **16** or **15**: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.60 to 2.65 (m, 2H, -C$\underline{H}_2$SH), 2.66 to 2.79 (m, 2H, -CH_2S-), 3.02 (br, 2H, -OH), 3.61 (d, 1H, J_{gem} = 11 Hz, H_{1a}), 3.65 (d, 1H, J_{gem} = 11 Hz, H_{1b}), 3.86 (dd, 1H, J_{gem} = 10.2, 2.5 Hz; H_{5a}), 4.18 to 4.49 (m, 3H, $H_{5b\text{-}H4}$ and H_3), 4.51 (d , 1H, J_{gem} = 11.5 Hz, -CH_2ϕ), 4,58 (d, 1H, J_{gem} = 11.5 Hz, CH_2ϕ), 7.24 to 7.30 (m, 5H, aromatic H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 25.86 (-CH_2SH), 31.42 (CH_2S-), 70.93 (C_3), 73.11 (C_1), 73.72 (C_4), 74.01 (-CH_2ϕ), 74.45 (C_5), 95.28 (C_2), 128.01-128.16-128.76 -137.87 (aromatic C).

Anal. Calcd for $C_{14}H_{20}O_4S_2$: C, 53.14; H, 6.37. Found: C, 53.24; H, 6.40.

Compound **17**: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.59 to 2.67 (m, 1H, -CH_2S-), 2.74 to 2.80 (m, 2H, -CH_2S-), 3.18 (d, 1H, J = 9.9 Hz, -OH), 3.66 (d, 1H, J_{gem} = 10.4 Hz, H_{1a}), 3.76 (d, 1H, J_{gem} = 10.4 Hz, H_{1b}) ; 3.80 (dd, 1H, J_{gem} = 10.2 Hz, J = 1.1 Hz, H_{5a}), 3.81 (dd, 1H, J_{gem} = 10.2 Hz, J = 2.7 Hz, H_{5b}), 4.05 to 4,10 (m, 2H, H_4 and H_3), 4.50 (d, 1H, J_{gem} = 11.5 Hz, -CH_2ϕ), 4.60 (d, 1H, J_{gem} = 11.5 Hz, -CH_2ϕ), 7.25 to 7.31 (m, 5H, aromatic H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 30.02 (2C, -CH_2S-), 71.37 (C_5 or C_1), 71.42 (C_1 or C_5), 73.96 (-CH_2ϕ), 80.53 (C_3), 92.60 (C_2), 127.93-128.30-128.66-136.4 (aromatic C).

Anal. Calcd for $C_{26}H_{34}O_8S_2$: C, 57.97; H, 6.36. Found: C, 58.08; H: 6.41.

1-*O*-Benzyl-5-*O*-[1-(4-methoxycarbonylbutyl)-2-(3-oxobutyl)]-phosphono-L-*erythro*-pent-2-ulose ethane-1,2-diyl dithioacetal (19a).

A solution of **9a** (625 mg , 4.73 mmol) and triethylamine (470 µL, 4.73 mmol) in anhydrous CH_2Cl_2 was added to a stirred solution of **18**[34] (1,34 g, 4.73 mmol) in anhydrous CH_2Cl_2 (3.3 mL). The solution was stirred for 1 h at room temperature. A solution of **13** (1 g, 3.6 mmol) and triethylamine (470 µL, 4.73 mmol) in anhydrous CH_2Cl_2 (2.9 mL) was added. After 6 h at room temperature, the solution was diluted with CH_2Cl_2 (35 mL), washed with 5% Na_2CO_3 solution, 5% HCl solution and water. The organic phase was dried over $MgSO_4$ and the solvent evaporated. Chromatography on reversed phase silica gel (CH_3CN-H_2O, 1:1) gave **19a** (1.08 g, 58%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.43 (d, 3H, J = 7.1 Hz, H_o maj. isomer), 1.44 (d, 3H, J = 7.1 Hz, H_o min. isomer), 1.66 (m, 4H, H_d and H_e), 2.19 (s, 3H, H_l maj. isomer), 2.20 (s, 3H, H_l min. isomer), 2.29 (m, 2H, H_c), 3.10 to 3.25 (2m, 4H, -CH_2S-), 3.63 (s, 3H, H_a), 3.75 (m, 2H, -OH), 3.82 (d, 1H, J_{gem} = 10 Hz, H_g), 3.91 (d, 1H, J_{gem} = 10 Hz, $H_{g'}$), 3.96 (m, 2H, H_i and H_j), 4.09 (m, 2H, H_f), 4.31 (m, 2H, H_k), 4.58 (s, 2H, -CH_2ϕ), 4.69 (m, 1H, H_n), 7.25 to 7.33 (m, 5H, aromatic H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 18.08 (d, J = 5.5 Hz , o min. isomer), 18.13 (d, J = 4 Hz , o maj. isomer), 20.84 (d), 25.46 (l), 29.43 (J = 7.3 Hz, e), 33.22 (c), 37.83 and 39.22 (-CH2S-), 51.51 (a), 67.89 (d, J = 3.7 Hz, f), 70,09 (d, J = 3.7 Hz, k), 72,41 (d, J = 3.7 Hz, j), 73.08 (h), 73.72 (-CH2ϕ), 75.61 (i), 76.39 (g), 78.64 (n), 127.71-127.89-128.44-137.20 (aromatic C); 173.59 (b), 212.07 (m); ^{31}P NMR ($CDCl_3$, 202 MHz): 0.31 (3 %), 0.35 (9 %), 0.41 (21 %) ; 0.42 (67 %).

Anal Calcd for $C_{24}H_{37}O_{10}PS_2$: C : 49.64 ; H : 6.42. Found : C : 49.19 ; H : 6.47.

1-*O*-Benzyl-5-*O*-[1-(4-methoxycarbonylbutyl)]-phosphono-L-*erythro*-pent-2-ulose ethane-1,2-diyl dithioacetal (20a). Na_2CO_3 (256 mg, 2.41 mmol) was added to a solution of **19a** (701.8 mg, 1.21 mmol) in a mixture of H_2O-CH_3CN, 2:1. After 1 h of stirring at room temperature, the solution was diluted with water (3 mL) and washed with CH_2Cl_2. The aqueous phase was acidified 1N HCl solution to reach pH 1 and extracted with AcOEt. The organic phase was dried over $MgSO_4$, the solvent was evaporated. The resulting oil was purified by chromatography on reversed phase silica gel (CH_3CN-H_2O, 4:6) to give **20a** (400 mg, 65%). ^1H NMR ($CDCl_3$, 400 MHz): δ 1.61 (m, 4H, H_d and H_e), 2.24 (m, 2H, H_c), 2.98-3.18 (m, 4H, -CH_2S-), 3.56 (s, 3H, H_a), 3.75 (d, 1H, J_{gem} = 9.9 Hz, H_g), 3. 84 (d, 1H, J_{gem} = 9.9 Hz, $H_{g'}$), 3.94 (m, 4H, H_f, H_i, H_j), 4.22 (m, 2H, H_k), 4.53 (s, 2H, $CH_2\phi$), 6. 05 (br, 3H, OH), 7.18 to 7.26 (m, 5 aromatic H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 21.18 (d), 29.72 (d, J = 7.3 Hz, e), 33.60 (c), 38.06 and 39.58 (-CH_2S-), 51.78 (a), 67.18 (d, J = 5.5 Hz, f), 69.33 (d, J = 3.7 Hz, k), 72.67 (d, J = 5.5 Hz, j), 73.28 (h), 73.87 ($CH_2\phi$), 76.32 (i), 76.47 (g), 127.91-127.97-128.48-128.68-137.68 (aromatic C), 174.18 (b); ^{31}P NMR ($CDCl_3$, 202 MHz): 1.41. MS of the triethyl ammonium salt : (FAB^+, Noba) : MH^+ = 612.

1-*O*-Benzyl-5-*O*-[1-(4-methoxycarbonylbutyl)]-phosphono-L-*erythro*-pent-2-ulose (21a). Methyl iodide (6.6 mL, 106 mmol) was added to a solution of **20a** (465 mg ; 0.91 mmol) in CH_3CN (4.5 mL) and phosphate buffer pH =7.6 (14 mL). The mixture was stirred at 50 °C for 18 h and cooled to room temperature. The solution was diluted with 60 mL of water and washed with 15 mL of CH_2Cl_2. The aqueous phase was lyophilized. Chromatography of the resulting oil on reversed phase silica gel (CH_3CN-H_2O, 2:8) gave **21a** (189 mg, 48%). ^1H NMR (CD_3OD, 400 MHz): δ 1.58 (m, 4H, H_d and H_e), 2.25 (t, 2H, J = 7 Hz, H_c), 3.54 (s, 3H, OCH_3), 3.92 (m, 5H, H_f-H_j and H_k), 4.23 (d, 1H,J = 4.8 Hz, H_i), 4.37 (d, 1H, J_{gem} = 18.3 Hz, H_g), 4.43 (d, 1H, J_{gem} = 18.3 Hz, $H_{g'}$), 4.48 (s, 2H, $CH_2\phi$), 7.24-7.27 (aromatic H); ^{13}C NMR (CD_3OD, 100 MHz): δ 22.23 (d), 25.019 (d, J = 8.1 Hz, e), 34.19 (c), 52.04 (a), 67.41 (k), 67.77 (j), 73.09 (d, J = 7.6 Hz, f), 74.18 ($CH_2\phi$), 75.01 (g), 76.42 (i), 128.91-129.12-129-129.42-138.96 (aromatic C), 175.61 (b), 210.60 (h); ^{31}P NMR (CD_3OD, 162 MHz): δ 1.03. Mass (FAB^+;TEA):(M^+ Na^+) = 457 ; $(M-H+2Na)^+$ = 479.

5-*O*-[1-(4-methoxycarbonylbutyl)]-phosphono-L-*erythro*-pent-2-ulose (22a). A solution of **21a** (85 mg, 0.195 mmol) in methanol (0.5 mL) was added to a suspension of 10 % Pd/C (50 mg) and glacial acetic acid (475 µL) in methanol (2.5 mL). The mixture was stirred under an atmosphere of H2 at room temperature for 1 h. The solution was centrifuged in a conical tube, the Pd/C was washed twice with methanol and centrifuged again. The methanol layers were combined, filtered on small reversed phase silica gel column. The solvent was evaporated to yield **22a** (44 mg, 65%). ^1H NMR (CD_3OD, 400 MHz): δ1.63 (m, 4H, H_e and H_d), 2.40 (t, 2H, J = 6.1 Hz, H_c), 3.67 (s, 3H, H_a), 4.00 (d, m, 5H, H_f-H_k and H_j), 4.26 (d, 1H, J = 3.8 Hz, H_i), 4.37 (d, 1H, J_{gem}

= 20 Hz, H_g), 4.44 (d, 1H, J_{gem} = 20 Hz, $H_{g'}$); ^{13}C NMR (CD_3OD, 400 MHz): δ 22.3 (d), 30.81 (e), 34.20 (c), 52.01 (a), 67.34 (k), 67.64 (j), 68.02 (f), 73.21 (g), 76.53 (i), 175.69 (b), 212.72 (h); ^{31}P NMR (CD_3OD, 162 MHz): δ 0.68. Mass (FAB⁻;TEA): (M-H)- = 343.

Benzyl 5-hydroxypentanoate (9b). Benzyl chloride (2.08 mL, 17.2 mmol) was added to a stirred suspension of sodium 5-hydroxypentanoate (2.4 g, 14.5 mmol) in DMF (30 mL). The solvent was evaporated and the residue was dissolved in 60 mL of water and extracted with 200 mL of ether. The organic phase was dried over MgSO4 and the solvent evaporated. Flash chromatography on silica gel (cyclohexane-AcOEt, 6:4) gave **9b** (1.2 g, 40%). ^1H NMR ($CDCl_3$, 200 MHz): δ 1. 52 to 1.80 (m, 4H, H_e and H_d), 2.39 (t, 2H, J = 7.1 Hz, H_c), 3.61 (t, 2H, J = 6.1 Hz, H_f), 5.10 (s, 2H, H_a), 7.33 (m, 5H, aromatic H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 21.12 (d), 32.06 (e), 33.94 (c), 62.23 (f), 66.29 (a), 128-128.62-136.02 (aromatic C), 173.64 (b).

Anal Calcd for $C_{12}H_{16}O_3$: C, 69.23; H, 7.69. Found: C, 69.17; H, 7.72.

1-*O*-Benzyl-5-*O*-[1-(4-benzyloxycarbonylbutyl)-2-(3-oxobutyl)]-phosphono-L-*erythro*-pent-2-ulose ethane-1,2-diyl dithioacetal (19b). A solution of **9b** (197 mg, 0.94mmol) and triethylamine (94 μL, 0.94 mmol) in anhydrous CH2Cl2 (700 μL) was added to a solution of **18** (267 mg, 0.94 mmol) in anhydrous CH2Cl2 (700 μL). The mixture was stirred for 2 h at room temperature. A solution of **13** (200 mg, 0.63 mmol) and triethylamine (94 μL, 0.94 mmol) in anhydrous CH_2Cl_2 (500 μL) was added. After stirring for 6 h at room temperature, the solution was treated under the conditions used for **19a**. Chromatography on reversed phase silica gel (CH3CN-H_2O, 6:4) gave **19b** (200 mg, 48%). ^1H NMR ($CDCl_3$, 200 MHz): δ 1.44 (d, 3H, J = 7.0 Hz, H_o), 1.71 (m, 4H, H_d and H_e), 2.19 (s, 3H, H_l), 2.35 (m, 2H, H_c), 3.18 (m, 4H, -CH_2S-), 3.80 (d, 1H, J_{gem} = 9.8 Hz, H_g), 3.88 (d, 1H, J_{gem} = 9.8 Hz, $H_{g'}$), 3.91 (m, 2H, H_i and H_j), 4.08 (m, 2H, H_f), 4.31 (m, 2H, H_k), 4.59 (s, 2H, $CH_2\phi$), 4.75 (m, 1H, H_n), 5.09 (s, 2H, H_a), 7.32 (m, 10H, aromatic H); ^{13}C NMR ($CDCl_3$, 50 MHz): 18.20 (Jc-p = 4.5 Hz, o), 20.93 (d), 25.54 (e), 29.52 (Jc-p = 7.14 Hz, e), 33.54 (c), 37.93- 39.29 (CH_2-S), 66.26 (a), 67.93 (Jc-p = 6.24 Hz, g), 70.08 (Jc-p = 6.1 Hz, j), 73.19 (h), 73.81 ($CH_2\phi$), 75.66 (i), 76.43 (g), 78.78 (Jc-p = 5.34 Hz, n), 127.78, 127.97, 128.21, 128.24, 128.53, 128.57, 128.66, 135.95, 137.28 (aromatic), 173 (b), 206.16 (m); ^{31}P NMR ($CDCl_3$, 162 MHz): δ 0.54 (5%), 0.60 (16%), 0.66 (29%), 0.68 (50%).

Anal Calcd for $C_{30}H_{41}O_{10}PS_2$: C, 54.86; H, 6.29. Found: C, 54.83; H, 6.32.

1-*O*-Benzyl-5-*O*-[1-(4-benzyloxycarbonylbutyl)]-phosphono-L-*erythro*-pent-2-ulose ethane-1,2-diyl dithioacetal (20b). Solid Na_2CO_3 (147 mg, 1.38 mmol) was added to a solution of **19b** (456 mg, 0.69 mmol) in a mixture of H_2O-CH_3CN, 1:1. The experimental procedure was identical to that used for **20a**. A

chromatography on reversed phase silica gel (CH_3CN-H_2O, 1:1) gave **20b** (255.3 mg, 63%). ^1H NMR (CD_3OD, 400 MHz): δ 1.76 (m, 4H, H_e and H_d), 2.47 (t, 2H, J = 7 Hz, H_c), 3.25 (m, 4H, -CH_2S-), 3.92 (d, 1H, J_{gem} = 9.6 Hz, H_g), 3.95 (d, 1H, J_{gem} = 9.6 Hz, $H_{g'}$), 4.04 (m, 2H, H_f), 4.09 (m, 1H, H_j), 4.12 to 4.15 (m, 2H, H_i and H_k), 4.37 (dd, 1H, J_{gem} = 9.3 Hz, J = 5 Hz, $H_{k'}$), 4.65 (s, 2H, $CH_2\phi$), 5.17 (s, 2H, H_a), 7.32 to 7.44 (aromatic H); ^{13}C NMR (CD_3OD, 100 MHz): δ 22.29 (d), 30.79 (d, J = 6 Hz, e), 34.05 (c), 38.89 and 40.18 (-CH_2S-), 67.16 (a), 67.23 (d, J = 7.5 Hz, f), 69.46 (d, J = 6.3 Hz, k), 73.77 (d, J = 7.9 Hz, j), 74.52 ($CH_2\phi$), 74.81 (h), 77.25 (g), 77.37 (i), 128.65-128.79-129.17-129.52-137.65-139.43 (aromatic C), 174.88 (b); ^{31}P NMR (CD_3OD, 162 MHz): δ 1.45. Mass (FAB^+, TG) : $(M+Na)^+$ = 609 ; $(M-H+2Na)^+$ = 631.

1-*O*-Benzyl-5-*O*-[1-(4-benzyloxycarbonylbutyl)]-phosphono-L-*erythro*-pent-2-ulose (21b). Methyl iodide (2.7 mL, 43 mmol) was added to a solution of **20b** (255.3 mg, 0.43 mmol) in CH_3CN (5 mL) and phosphate buffer pH = 7.6 (7 mL). The mixture was stirred at 50 °C for 7 h and cooled to room temperature. The solution was then treated under the same conditions as **21a**. Chromatography on reversed phase silica gel (CH_3CN-H_2O, 4:6) gave **21b** (50 mg, 35%). ^1H NMR (CD_3OD, 400 MHz): δ 1.56 (m, 2H, H_e), 1.62 (m, 2H, H_d), 2.29 (t, 2H, J = 7.2 Hz, H_c), 3.74 (m, 2H, H_f), 3.85 (m, 3H, H_k and H_j), 4.15 (d, 1H, J = 5.4 Hz, H_i), 4.35 (d, 1H, J_{gem} = 18.3 Hz, H_g), 4.41 (d, 1H, J_{gem} = 18.3 Hz, $H_{g'}$), 4.47 (d, 1H, J_{gem} = 11.8 Hz, $CH_2\phi$), 4.49 (d, 1H, J_{gem} = 11.8 Hz, $CH_2\phi$), 4.99 (s, 2H, H_a), 7.22 (m, 10H, aromatic H); ^{13}C NMR (CD_3OD, 100 MHz): δ 22.51 (d), 31.06 (d, J = 7.5 Hz, e), 34.64 (c), 66.14 (d, J = 5.7 Hz, f), 66.79 (d, J = 4.9 Hz, k), 67.16 (a), 73.53 (d, J = 6.3 Hz , j), 74.21 ($CH_2\phi$), 75.04 (g), 76.4 (i), 128.38-128.57-128.93-129.29-129.46-137.69-138.94 (aromatic C); ^{31}P NMR (CD_3OD, 162 MHz): δ 2.35. Mass (FAB^-, TEA) : $(M-H)^-$ = 509.

5-*O*-[5-(pentanoic acid)]-phosphono-L-*erythro*-pent-2-ulose (2). 10% Pd/C (3 mg) was added to a solution of **21b** (60 mg, 0.117 mmol) and acetic acid (200 μL) in methanol (2 mL). The suspension was stirred under H_2 atmosphere at room temperature for 1 h. The reaction was then treated under the same conditions as **22a** to give **2** (36.3 mg, 94%). ^1H NMR (CD_3OD, 400 MHz): δ 1.59 (m, 4H, H_e and H_d), 2.25 (t, 2H, J = 6.4 Hz, H_c), 3.72 to 3.90 (m, 5H, H_f, H_k and H_j), 4.18 (m, 1H, H_i), 4.43 (s, 2H, H_g); ^{13}C NMR (CD_3OD, 100 MHz): δ 22. 49 (d), 31.03 (d, J = 3.9 Hz, e), 34.50 (c), 66.23 (d, J = 5.4 Hz, k), 66.39 (d, J = 3.8 Hz, g), 67.90 (g), 73.43 (d, J = 5.6 Hz, j), 76.76 (i), 175.28 (b), 213.19 (h); ^{31}P NMR (CD_3OD, 162 MHz): δ 1.92. Mass (FAB^-, TEA):$(M-H)^-$ = 329.

Lactones 24a + 24b. A 0.5 M aqueous solution of KCN (106.8 μL, 53.4 μmol) was added to a solution of **22a** (18.4 mg, 53.4 μmol) in 750 μL of water. The mixture was stirred 48 h at room temperature. Then an excess of Dowex 50 (H^+) WX2

was added. After further stirring for 15 min, filtration and lyophilization, 19 mg (95 %) of **24** were obtained. Mass (FAB, positive mode, matrix TEA): $(M+K^+)$ = 411 ; $(M+Na^+)$ = 395; ^{1}H NMR (CD_3OD, 400 MHz): δ 1.89 (m, 4H, H_d, H_e), 2.37 (m, 2H, H_c), 3.73 (s, 3H, H_a), 3.61-4.42 (m, 8H, H_f, H_g, H_i, H_j, H_k).

Formation of the BSA-2 conjugate. A solution of **2** (15 mg, 45 μmol) in 0.83 mL of water was added to a solution of BSA (50 mg, 0.75 μmol) in water (2.4 mL). A solution of EDC (43 mg, 220 μmol) in 1 mL of water was added. The mixture was stirred overnight at 4 °C. The solution was divided into three fractions of 1.26 mL each and one of 0.41 mL, which were dialysed 12 h in 2 L of water.

Formation of the BSA-1 conjugate. A 0.15 M aqueous solution of KCN (45 μL, 6.9 μmol) was added to a solution of BSA-**2** conjugate (15 mg, 0.23 μmol) in 1 mL of water. The mixture was stirred 48 h at 4 °C and dialysed in 1 L of water overnight.

Estimation of the number of haptens fixed per molecule of BSA by ^{14}C labelling. An aqueous solution of [^{14}C]KCN (10 μL, 0.87 μmol, 65 eq/BSA, S.A. : 0.465 mCi/mmol) was added to a solution of BSA-**2** (0.93 mg, 13.5 nmol) in 500 μL of water. The mixture was stirred 48 h at 4 °C and dialysed in 3 L of water overnight and then twice 12 h in 1 L of water. The radioactivity remaining on the protein was counted: 126 525 dpm in the total volume, 1 nmole of hapten corresponding to 1025 dpm, the average number of fixed hapten molecules is about 9 ± 1/mole of BSA.

Formation of the PTG-2 conjugate. A solution of **2** (0.75 mg, 2.27 μmol) in 150 μL of water was added to a solution of PTG (10 mg, 1.51 10^{-2} μmol) in water (1 mL). A solution of EDC (2.17 mg, 50 μmol) in 50 mL of water was added. The mixture was stirred overnight at 4 °C. The solution was divided into two fractions of 500 μL each (5 mg of PTG) which were dialysed 12 h in 1 L of water.

Formation of the PTG-1 conjugate. An aqueous solution of KCN (14 μL, 1.07 μmol) was added to a solution of PTG-**2** conjuguate (5 mg, 7.57x10^{-3} μmol) in 500 μL of water. The mixture was stirred 48 h at 4 °C and dialysed in 1 L of water overnight.

Estimation of the number of haptens fixed per molecule of PTG. An aqueous solution of KCN (14 μL, 1.07 μmol, 141 eq/PTG) was added to a solution of PTG-**2** conjugate (5 mg, 7.59x10^{-3} μmol) in 500 mL of water. 172 μL of a 7.75 μCi/mL [^{14}C]KCN solution were added (1.33 μCi, 2.96x10^6dpm).The mixture was stirred for 48 h at 4 °C and dialysed in 1 L of water overnight. The remaining radioactivity on the protein was counted: 302475 dpm in the total volume. Each equivalent of hapten represents 20992 dpm. Thus, each PTG molecule has fixed on average 14.4 haptens.

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