This article was downloaded by: [New York University] On: 02 May 2013, At: 06:50 Publisher: Taylor & Francis Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



# Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information: http://www.tandfonline.com/loi/lcar20

# Synthesis of Protein Conjugates of 2-Carboxy-L-Arabinitol 5-Phosphate and 2-Carboxy-L-Ribitol 5-Phosphate

Nelly Gourlaouën <sup>a</sup> , Dominique Florentin <sup>a</sup> & Andrée Marquet <sup>a</sup> <sup>a</sup> Laboratoire de Chimie Organique Biologique, URA CNRS 493 Université, P. et M. Curie - 4 place Jussieu, Tour 44-45, 75252, Paris Cedex 05, France Fax: E-mail: Published online: 16 Aug 2006.

To cite this article: Nelly Gourlaouën , Dominique Florentin & Andrée Marquet (1998): Synthesis of Protein Conjugates of 2-Carboxy-L-Arabinitol 5-Phosphate and 2-Carboxy-L-Ribitol 5-Phosphate, Journal of Carbohydrate Chemistry, 17:8, 1219-1238

To link to this article: <u>http://dx.doi.org/10.1080/07328309808001895</u>

## PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <u>http://www.tandfonline.com/page/terms-and-conditions</u>

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae, and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand, or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

#### J. CARBOHYDRATE CHEMISTRY, 17(8), 1219-1238 (1998)

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

Nelly Gourlaouën, Dominique Florentin and Andrée Marquet\*

Laboratoire de Chimie Organique Biologique, URA CNRS 493 Université P. et M. Curie - 4 place Jussieu, Tour 44-45, 75252 Paris Cedex 05, France e. mail : marquet@ccr.jussieu.fr - Fax : + 33 1 44 27 71 50

Received November 20, 1997 - Final Form May 19, 1998

#### 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-Lerythro-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.<sup>1</sup> However, only a few reactions involving C-C bond formation have been catalyzed by antibodies: this includes intramolecular reactions such as Claisen,<sup>2</sup> oxy-Cope<sup>3</sup> and dienone-phenol rearrangements,<sup>4</sup> Diels-Alder reactions,<sup>5</sup> cationic condensations<sup>6</sup> and aldol condensations.<sup>7</sup>



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<sup>8</sup> (Scheme 1). We do not expect to achieve the complete reaction, but the antibody could yield the  $\beta$ -ketoacid intermediate A which is unstable and can be cleaved at basic pH into 3-phospho-D-glycerate and pyruvate<sup>9</sup> (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) ( $K_i < 10^{-11} \text{ M}$ )<sup>10</sup> (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.*<sup>11</sup> 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.*<sup>12</sup> **3a** or **3b**, treated with NaH and benzyl bromide in the presence of Bu<sub>4</sub>N<sup>+</sup> I<sup>-13</sup> 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,<sup>14</sup> **5** was obtained as a mixture of anomers and was then treated with ethanedithiol in the presence of BF<sub>3</sub>·Et<sub>2</sub>O in CH<sub>2</sub>Cl<sub>2</sub>,<sup>5</sup> giving the thioketal **6**.



a) NaH,C<sub>6</sub>H<sub>5</sub>CH<sub>2</sub>Br, Bu<sub>4</sub>N<sup>+</sup>I<sup>-</sup>; b) HCl 1N, THF; c) HS-(CH<sub>2</sub>)<sub>2</sub>SH, BF<sub>3</sub>•Et<sub>2</sub>O, CH<sub>2</sub>Cl<sub>2</sub>; d)  $iPr_2NH$ , CH<sub>3</sub>CN; e) tetrazole; f) I<sub>2</sub>, THF, H<sub>2</sub>O, Pyridine; g) NaOCH<sub>3</sub>, CH<sub>3</sub>OH

Scheme 3

A one pot phosphite triester procedure was used to obtain  $11.^{16}$  The chlorophosphoramidite 7 was obtained from PCl<sub>3</sub> by treatment with  $\beta$ -cyanoethanol and then with diisopropylamine.<sup>17</sup>

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<sub>2</sub>O, pyridine, at -10 °C.<sup>19</sup> (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  $HgCl_2/HgO$ ,<sup>20</sup>  $AgNO_3$ ,<sup>21</sup>  $CuCl_2$  / $CuO^{22}$  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.*<sup>26</sup> (Scheme 4). The thioketalisation occurred in a good yield using ethanedithiol in concentrated hydrochoric acid.<sup>27</sup> With ethanedithiol and  $BF_3 \cdot Et_2O$  in dichloromethane,<sup>28</sup> only compounds 15, 16 and 17 were obtained.

The double phosphorylation was carried out according to the one pot procedure of Ramirez *et al.*<sup>29</sup> (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



Scheme 4



Scheme 5



Scheme 6

 $CH_3I$  in a mixture  $CH_3CN$ -phosphate buffer pH 7.6.<sup>24</sup> The benzyl protecting group was removed from 21a by hydrogenolysis<sup>30</sup> to give 22a. However, saponification of the methyl ester of 22a could not be carried out. Even with a mild base such as LiOH,<sup>31</sup> 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.<sup>32</sup> 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<sup>33</sup> (Scheme 7).

Indeed, according to these authors, the acids are in equilibrium with the corresponding  $\gamma$  lactones and treatment with a cation exchange resin (H<sup>+</sup> 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 % [<sup>13</sup>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. <sup>1</sup>H NMR spectra were recorded on a Jeol GSX400, a Bruker ARX400 or a Bruker AC200. <sup>13</sup>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. <sup>31</sup>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 <sup>1</sup>H NMR and <sup>13</sup>C NMR (from phosphoric acid for <sup>31</sup>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.<sup>17,18,26,34</sup>  $[^{14}C]$ 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-B-L-erythro-pent-2-ulofuranoside (4a). Sodium hydride (80 % in mineral oil) (673 mg, 22.4 mmol) was added to a solution of methyl  $\beta$ -L-erythro-pent-2-ulofuranoside (819 mg, 5 mmol) in anhydrous N,Ndimethylformamide (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<sub>2</sub>Cl<sub>2</sub> (100 mL) was added and the solution was washed with H<sub>2</sub>O to pH = 7, and with 1N HCl (40 mL) and H<sub>2</sub>O to pH = 7. The organic layer was dried over MgSO<sub>4</sub> 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;  $[\alpha]_{n}^{20}$  -220 (c 1.55, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  3.12 (s, 3H, -OCH<sub>3</sub>), 3.53 (d, 1H,  $J_{gem} = 10.4 \text{ Hz}$ ,  $H_{1a}$ ), 3.65 (d, 1H,  $J_{gem} = 10.4 \text{ Hz}$ ,  $H_{1b}$ ), 3.80 (dd, 1H,  $J_{gem} = 8.3$ Hz,  $J_{H_{5a}-H_4} = 7.1$  Hz,  $H_{5a}$ ), 3.88 (d, 1H,  $J_{H_3-H_4} = 4.4$  Hz ,  $H_3$ ), 3.91 (dd, 1H,  $J_{gen} = 8.3$ 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,  $J_{gem} = -2.3$  Hz,  $-CH_2\phi_b$ ), 4.54 (d, 2H, 2H) 12.1 Hz,  $-CH_2\phi_1$ , 4.64 (s, 2H,  $-CH_2\phi_2$ ), 7.19-7.25 (m, 15H, H aromatic); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz): δ 48.45 (-OCH<sub>3</sub>), 65.00 (C<sub>1</sub>), 69.28 (C<sub>5</sub>), 72.37-73.45-73.72 (-CH<sub>2</sub> $\phi$ ), 78.51 (C<sub>3</sub>), 79.94 (C<sub>4</sub>), 108.63 (C<sub>2</sub>), 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<sub>27</sub>H<sub>30</sub>O<sub>5</sub>: 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<sub>3</sub>). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) : δ 3.35 (s, 3H, -OCH<sub>3</sub>), 3.47 (d, 2H,H<sub>1</sub>), 3.89 (d, 1H, J<sub>H3-H4</sub> = 1.9 Hz, H<sub>3</sub>), 3.89 (m, 2H, H<sub>5</sub>), 3.97 (m, 1H, H<sub>4</sub>), 4.44 (d, 1H, J<sub>gen</sub> = 12 Hz, -CH<sub>2</sub>φ<sub>b</sub>), 4.50 (d, 1H, J<sub>gen</sub> = 12 Hz, -CH<sub>2</sub>φ<sub>b</sub>), 4.63 (s, 2H, -CH<sub>2</sub>φ<sub>c</sub>), 7.17-7.3 (m, 15H, H aromatic);<sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz): δ 49.91 (-OCH<sub>3</sub>), 70.10 (C<sub>1</sub>), 70.72 (C<sub>5</sub>), 72.13-72.61-73.47 (-CH<sub>2</sub>φ), 74.73 (C<sub>3</sub>), 78.09 (C<sub>4</sub>), 104.45 (C<sub>2</sub>), 127.62-127.69-127.87-128.13-128.29 137.89-138.06-138.17(C aromatic ).

Anal. Calcd for C<sub>27</sub>H<sub>30</sub>O<sub>5</sub>: C, 74.65; H, 6.91. Found: C, 74.93; H, 6.94.

1,3,4-Tri-O-benzyl- $\alpha$ , $\beta$ -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<sub>4</sub>OH (6 mL) was added, then THF was evaporated under

vacuum and the product was extracted from H<sub>2</sub>O with CH<sub>2</sub>Cl<sub>2</sub> (40 mL). The organic phase was dried over MgSO<sub>4</sub> and concentrated under vacuum. Silica gel chromatography (cyclohexane : ethyl acetate, 8:2) yielded 141 mg (80%) of 5 as a colorless oil, mixture of  $\alpha$  and  $\beta$  anomers. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  3.66 (d, 1H, J<sub>gen</sub> = 10.4 Hz, H<sub>1a</sub> 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<sub>gem</sub> = 10 Hz, H<sub>1b</sub> min. anom.), 3.97 (dd, 1H, J<sub>gem</sub> =9.9 Hz,  $J_{H5_{a}-H4} = 4.4 \text{ Hz}, H_{5_{a}} \text{ maj. anom.}, 4.07 \text{ (m, 1H, H}_{4} \text{ maj. anom.}, 4.14-4.22 \text{ (m, 5H, H}_{5_{b}} \text{ H}_{5_{b}})$ maj. and min. anom. -H<sub>5</sub>, min. anom-H<sub>3</sub> maj. anom-H<sub>4</sub> min. anom.), 4.33 (d, 1H, H<sub>3</sub> min. anom.), 4.54-4.85 (m, 6H, -CH<sub>2</sub> $\phi$ ); 7.20-7.27 (m, 15H, H aromatic); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  65 (C<sub>5</sub> min. anom.), 69.08 (C<sub>5</sub> maj. anom.), 70.67 (C<sub>1</sub> maj. anom.), 71.69 (C, min. anom.), 72.24-72.70-73.49(-CH, maj. anom.), 73.19-73.9 (-CH<sub>2</sub>\$\phi min. anom.), 76.45 (C<sub>3</sub> maj. anom.), 77.09 (C<sub>4</sub> maj. anom.), 77.84 (C<sub>3</sub> min. anom.), 82.06 (C<sub>4</sub> min. anom.), 102.40 (C<sub>2</sub> maj. anom.), 126.91 (C<sub>2</sub> 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<sub>26</sub>H<sub>28</sub>O<sub>5</sub>: 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<sub>2</sub>Cl<sub>2</sub> (10 mL) was added ethanedithiol and then BF3•Et,O (325 µL, 2.5 mmol). After stirring 3 h, 0.2 N NaOH (2 mL) was added to destroy BF<sub>3</sub>•Et<sub>2</sub>O. The organic phase was washed with H<sub>2</sub>O to pH = 7, dried over MgSO<sub>4</sub> and concentrated under vacuum. Silica gel column chromatography (cyclohexane : ethyl acetate, 8:2) yielded 933 mg (75 %) of 6 as a colorless oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  1.49 (s, 1H, -OH), 3.15 (m, 4H, -CH<sub>2</sub>S-), 3.63 (d, 1H,  $J_{gem} = 9.3$  Hz,  $H_{1s}$ ), 3.76 (dd, 1H,  $J_{gem} = 12$  Hz,  $J_{H5s-H4} = 4$  Hz,  $H_{5s}$ ), 3.80 (d, 1H,  $J_{gem} = 9.3 \text{ Hz}$ ,  $H_{1b}$ ), 3.92 (m, 2H,  $H_{5b}$  and  $H_4$ ), 4.24 (d, 1H,  $J_{H3-H4} = 2.7 \text{ Hz}$ ,  $H_3$ ), 4.45 (d, 1H,  $J_{gem} = 12.1$  Hz,  $-CH_2\phi_*$ ), 4.48 (d, 1H,  $J_{gem} = 12.1$  Hz,  $-CH_2\phi_*$ ), 4.51 (d, 1H,  $J_{gem} = 11.5$  Hz, -CH<sub>2</sub> $\phi$ b), 4.61 (d, 1H,  $J_{gem} = 11$  Hz, -CH<sub>2</sub> $\phi$ c), 4.66 (d, 1H,  $J_{gem} = 11.5 \text{ Hz}, -CH_2\phi_b$ , 4.77 (d, 1H,  $J_{gem} = 11 \text{ Hz}, -CH_2\phi_c$ ), 7.18-7.30 (m, 15H, H aromatic); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz): δ 38.76 (-CH<sub>2</sub>S-), 38.90 (-CH<sub>2</sub>S-), 62.40 (C<sub>5</sub>), 71.38 (-CH<sub>2</sub> $\phi$ ), 72.53 (C<sub>2</sub>), 73.25 (-CH<sub>2</sub> $\phi$ ), 75.37 (-CH<sub>2</sub> $\phi$ ), 75.97 (C<sub>1</sub>), 81.51 (C<sub>4</sub>), 83.30  $(C_{1}),$ 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<sub>28</sub>H<sub>32</sub>O<sub>4</sub>S<sub>2</sub>: 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,Ndiisopropylethylamine (95  $\mu$ L, 0.546 mmol) and 2-cyanoethyl N,N-

diisopropylaminochlorophosphine<sup>17</sup> 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 5hydroxypentanoate 9a<sup>18</sup> (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<sub>2</sub>O, 80:40:2 (10 mL, 1 mmol) was added. After stirring 1 h at room temperature CH<sub>2</sub>Cl<sub>2</sub> (100 mL) was added and the organic phase was washed with a 20 % aqueous solution of sodium thiosulfate (20 mL), twice with H<sub>2</sub>O, and then dried over MgSO<sub>4</sub>. Silica gel column flash chromatography (cyclohexane : ethyl acetate, 6:4) yielded 11 (124 mg, 43 %) as a colorless oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 1.62 (m, 4H, H<sub>d</sub> and H<sub>e</sub>), 2.25 (m, 2H, H<sub>e</sub>), 2.49 (m, 2H, CH<sub>2</sub>CN), 3.22 (m, 4H, CH<sub>2</sub>S), 3.62 (s, 3H, H<sub>2</sub>), 3.66 (d, 1H,  $J_{gem} = 10 \text{ Hz}, H_g$ ), 3.85 (d, 1H,  $J_{gem} = 10 \text{ Hz}, H_g$ ), 3.98 (m, 4H,  $H_f$  and  $C\underline{H}_2$ -CH<sub>2</sub>CN), 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<sub>k</sub>), 4.7 (s, 2H, CH<sub>2</sub> $\phi_c$ ), 4.80 (d, 1H, J<sub>gen</sub> = 11 Hz, CH<sub>2</sub> $\phi_h$ ); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  19.19 (d, J<sub>C-P</sub> = 7.3 Hz, CH<sub>2</sub>CN), 20.67 (d), 29.30 (d, J<sub>C-P</sub> = 7.4 Hz, e), 33.09 (c), 38.68 and 38.90 (-CH<sub>2</sub>S-), 51.41 (a), 61.30 ( $J_{C-P} = 5.5$  Hz, <u>C</u>H<sub>2</sub>CH<sub>2</sub>CN), 61.32 (J<sub>C-P</sub> = 5.5 Hz, <u>C</u>H<sub>2</sub>CH<sub>2</sub>CN), 67.48 (J<sub>C-P</sub> = 5,5 Hz, f isom. 1), 67.61(f isom. 2), 69.13 ( $J_{C-P} = 5.5 \text{ Hz}$ , k isom. 1), 69.24 ( $J_{C-P} = 5.7 \text{ Hz}$ , k isom. 2), 71.69 (CH<sub>2</sub> $\phi$ ), 72.15 (h), 73.12 (CH<sub>2</sub> $\phi$ ), 75.22 (CH<sub>2</sub> $\phi$ ), 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-Lerythro-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<sup> $\odot$ </sup> 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. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 1.83 (m, 4H, H<sub>d</sub> and H<sub>e</sub>), 2.05 (m, 2H, H<sub>c</sub>), 3.00-3.11 (m, 4H, -CH<sub>2</sub>S-), 3.38 (s, 1H, -OH), 3.46 (d, 1H, J<sub>gem</sub> = 10 Hz, H<sub>g</sub>), 3.47 (s, 3H, H<sub>a</sub>), 3.71 (m, 2H, H<sub>f</sub>), 3.76 (d, 1H, J<sub>gem</sub> = 10 Hz, Hg'), 4.03 (m, 2H, H<sub>k</sub> and H<sub>j</sub>), 4.17 (d, 1H, J<sub>Hi-Hj</sub> = 2.2 Hz, H<sub>i</sub>), 4.34 (d, 1H, J<sub>gem</sub> = 12.1 Hz, CH<sub>2</sub>φ<sub>a</sub>), 4.41 (d, 1H, J<sub>gem</sub> = 12.1 Hz, -CH<sub>2</sub>φ<sub>b</sub>), 4.50 (m, 2H, H<sub>5b</sub>, CH<sub>2</sub>φ<sub>c</sub>), 4.56 (d, 1H, J<sub>gem</sub> = 11.5 Hz, -CH<sub>2</sub>φ<sub>b</sub>), 4.66 (d, 1H, J<sub>gem</sub> = 11.5 Hz, CH<sub>2</sub>φ<sub>b</sub>), 4.70 (d, 1H, J<sub>gem</sub> = 11 Hz, -CH<sub>2</sub>φ<sub>c</sub>), 7.15-7.28 (m, 15H, H aromatic); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz): δ 21.07 (d), 29.70 (e), 33.44 (c), 38.85 and 39.05 (-CH<sub>2</sub>S-), 51.52 (a), 65.18 (f), 67.17 (k), 71.97 (-CH<sub>2</sub> $\phi$ ), 72.19 (h), 73.10 (-CH<sub>2</sub> $\phi$ ), 74.93 (-CH<sub>2</sub> $\phi$ ), 74.93 (-CH<sub>2</sub> $\phi$ ), 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<sub>3</sub> solution, diluted with water and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The organic layer was dried over MgSO<sub>4</sub>, 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%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  3.09 to 3.25 (m, 4H, -CH<sub>2</sub>S-), 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<sub>54</sub>, H<sub>55</sub>, H<sub>14</sub>, H<sub>15</sub>, H<sub>4</sub>), 3.91 (dd, 1H, J = 7.1, 5.5 Hz, H<sub>3</sub>), 4.56 (m, 2H, -CH<sub>2</sub> $\phi$ ), 7.26 to 7.29 (m, 5H, aromatic H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  37.90 and 39.05 (-CH<sub>2</sub>S-), 64.20 (C<sub>5</sub>), 72.76 (C<sub>4</sub>), 73.21 (C<sub>2</sub>), 73.67 (-CH<sub>2</sub> $\phi$ ), 76.14 (C<sub>1</sub>), 77.22 (C<sub>3</sub>), 127.80-128.15-128.60-136.50 (aromatic C); mp 84 °C; [ $\alpha$ ]<sup>20</sup><sub>2</sub>-53° (c 1, CHCl<sub>3</sub>).

Anal. Calcd for C<sub>14</sub>H<sub>20</sub>O<sub>4</sub>S<sub>2</sub>: 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).  $2 \cdot (1 - O - Benzy - \alpha - L - erythro-pent - 2 - ulofuranosyl thio) ethane thiol (16). 1, 2 -$ Bis-(1-O-benzyl-L-erythro-pent-2-ulofuranosyl thiol)ethane (17). Ethanedithiol (1.2 mL, 14.25 mmol) and BF<sub>3</sub>•Et<sub>2</sub>O (233 µL, 1.9 mmol) were added to a solution of 14 (2.28 g, 9.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (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<sub>2</sub>Cl<sub>2</sub>. The organic phase was washed with water, dried over MgSO<sub>4</sub> 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<sub>2</sub>Cl<sub>2</sub>-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<sub>3</sub>). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 2.53-2.66 (m, 2H, -CH<sub>2</sub>SH), 2.67-2.81 (m, 2H, -CH<sub>2</sub>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,  $J_{gem} = 10.4 \text{ Hz}$ ,  $H_{1b}$ ), 3.81 (dd, 1H,  $J_{gem} = 10.4 \text{ Hz}$ , J = 1.1 Hz,  $H_{5e}$ ), 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,  $J_{gem} = 11.5 \text{ Hz}$ , -CH<sub>2</sub> $\phi$ ), 4.58 (d, 1H,  $J_{gem} = 11.5 \text{ Hz}$ , -CH<sub>2</sub> $\phi$ ), 7.25 à 7.29 (m, 5H, aromatic H ); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz): δ 25.46 Hz (-CH<sub>2</sub>SH), 26.76 (-CH<sub>2</sub>S-), 71.31 (C<sub>1</sub>), 71.35 (C<sub>5</sub>), 71.66 (C<sub>4</sub>), 73.87 (-CH<sub>2</sub> $\phi$ ), 80.40 (C<sub>3</sub>), 92.58 (C<sub>2</sub>), 127.91-128.22-128.59-136.43 (aromatic C).

Anal. Calcd for C<sub>14</sub>H<sub>20</sub>O<sub>4</sub>S<sub>2</sub>: C, 53.14; H, 6.37. Found: C, 53.15; H, 6.34.

Compound 16 or 15: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  2.60 to 2.65 (m, 2H, -CH<sub>2</sub>SH), 2.66 to 2.79 (m, 2H, -CH<sub>2</sub>S-), 3.02 (br, 2H, -OH), 3.61 (d, 1H, J<sub>gem</sub> = 11 Hz, H<sub>1a</sub>), 3.65 (d, 1H, J<sub>gem</sub> = 11 Hz, H<sub>1b</sub>), 3.86 (dd, 1H, J<sub>gem</sub> = 10.2, 2.5 Hz; H<sub>5a</sub>), 4.18 to 4.49 (m, 3H, H<sub>5b-H4</sub> and H<sub>3</sub>), 4.51 (d, 1H, J<sub>gem</sub> = 11.5 Hz, -CH<sub>2</sub> $\phi$ ), 4,58 (d, 1H, J<sub>gem</sub> = 11.5 Hz, CH<sub>2</sub> $\phi$ ), 7.24 to 7.30 (m, 5H, aromatic H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  25.86 (-CH<sub>2</sub>SH), 31.42 (CH<sub>2</sub>S-), 70.93 (C<sub>3</sub>), 73.11 (C<sub>1</sub>), 73.72 (C<sub>4</sub>), 74.01 (-CH<sub>2</sub> $\phi$ ), 74.45 (C<sub>5</sub>), 95.28 (C<sub>2</sub>), 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: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  2.59 to 2.67 (m, 1H, -CH<sub>2</sub>S-), 2.74 to 2.80 (m, 2H, -CH<sub>2</sub>S-), 3.18 (d, 1H, J = 9.9 Hz, -OH), 3.66 (d, 1H, J<sub>gen</sub> = 10.4 Hz, H<sub>1a</sub>), 3.76 (d, 1H, J<sub>gen</sub> = 10.4 Hz, H<sub>1b</sub>); 3.80 (dd, 1H, J<sub>gen</sub> = 10.2 Hz, J = 1.1 Hz, H<sub>5a</sub>), 3.81 (dd, 1H, J<sub>gen</sub> = 10.2 Hz, J = 2.7 Hz, H<sub>5b</sub>), 4.05 to 4,10 (m, 2H, H<sub>4</sub> and H<sub>3</sub>), 4.50 (d, 1H, J<sub>gen</sub> = 11.5 Hz, -CH<sub>2</sub> $\phi$ ), 4.60 (d, 1H, J<sub>gen</sub> = 11.5 Hz, -CH<sub>2</sub> $\phi$ ), 7.25 to 7.31 (m, 5H, aromatic H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  30.02 (2C, -CH<sub>2</sub>S-), 71.37 (C<sub>5</sub> or C<sub>1</sub>), 71.42 (C<sub>1</sub> or C<sub>5</sub>), 73.96 (-CH<sub>2</sub> $\phi$ ), 80.53 (C<sub>3</sub>), 92.60 (C<sub>2</sub>), 127.93-128.30-128.66-136.4 (aromatic C).

Anal. Calcd for C<sub>26</sub>H<sub>34</sub>O<sub>8</sub>S<sub>2</sub>: 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<sub>2</sub>Cl<sub>2</sub> (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 CH2Cl2 (2.9 mL) was added. After 6 h at room temperature, the solution was diluted with CH2Cl2 (35 mL), washed with 5% Na2CO3 solution, 5% HCl solution and water. The organic phase was dried over MgSO4 and the solvent evaporated. Chromatography on reversed phase silica gel (CH<sub>3</sub>CN-H<sub>2</sub>O, 1:1) gave 19a (1.08 g, 58%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  1.43 (d, 3H, J = 7.1 Hz, H<sub>o</sub> maj. isomer), 1.44 (d, 3H, J = 7.1 Hz, H<sub>o</sub> min. isomer), 1.66 (m, 4H,  $H_d$  and  $H_e$ ), 2.19 (s, 3H,  $H_1$  maj. isomer), 2.20 (s, 3H,  $H_1$  min. isomer), 2.29 (m, 2H, H<sub>c</sub>), 3.10 to 3.25 (2m, 4H, -CH<sub>2</sub>S-), 3.63 (s, 3H, H<sub>1</sub>), 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<sub>i</sub> and H<sub>i</sub>), 4.09 (m, 2H, H<sub>f</sub>), 4.31 (m, 2H, H<sub>k</sub>), 4.58 (s, 2H, -CH<sub>2</sub>\$, 1H, H<sub>n</sub>), 7.25 to 7.33 (m, 5H, aromatic H);  $^{13}C$  NMR (CDCl\_3, 100 MHz):  $\delta$  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 (-CH2 $\phi$ ), 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); <sup>31</sup>P NMR (CDCl<sub>3</sub>, 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-erythropent-2-ulose ethane-1,2-diyl dithioacetal (20a). Na<sub>2</sub>CO<sub>3</sub> (256 mg, 2.41 mmol) was added to a solution of 19a (701.8 mg, 1.21 mmol) in a mixture of H<sub>2</sub>O-CH<sub>3</sub>CN, 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<sub>4</sub>, 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%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  1.61 (m, 4H,  $H_d$  and  $H_e$ ), 2.24 (m, 2H,  $H_c$ ), 2.98-3.18 (m, 4H, -CH<sub>2</sub>S-), 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,  $H_f$ ,  $H_i$ ,  $H_i$ ), 4.22 (m, 2H, H<sub>k</sub>), 4.53 (s, 2H, CH<sub>2</sub>¢), 6. 05 (br, 3H, OH ), 7.18 to 7.26 (m, 5 aromatic H ); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  21.18 (d), 29.72 (d, J = 7.3 Hz, e), 33.60 (c), 38.06 and 39.58 (-CH<sub>2</sub>S-), 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<sub>2</sub> $\phi$ ), 76.32 (i), 76.47 (g), 127.91-127.97-128.48-128.68-137.68 (aromatic C), 174.18 (b); <sup>31</sup>P NMR (CDCl<sub>3</sub>, 202 MHz): 1.41. MS of the triethyl ammonium salt :  $(FAB^{\dagger}, Noba)$  : MH<sup>+</sup> = 612.

1-*O*-Benzyl-5-*O*-[1-(4-methoxycarbonylbutyl)]-phosphono-L-erythropent-2-ulose (21a). Methyl iodide (6.6 mL, 106 mmol) was added to a solution of 20a (465 mg; 0.91 mmol) in CH<sub>3</sub>CN (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<sub>2</sub>Cl<sub>2</sub>. The aqueous phase was lyophilized. Chromatography of the resulting oil on reversed phase silica gel (CH<sub>3</sub>CN-H<sub>2</sub>O, 2:8) gave 21a (189 mg, 48%). <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz): δ 1.58 (m, 4H, H<sub>d</sub> and H<sub>e</sub>), 2.25 (t, 2H, J = 7 Hz, H<sub>e</sub>), 3.54 (s, 3H, OCH<sub>3</sub>), 3.92 (m, 5H, H<sub>f</sub>-H<sub>j</sub> and H<sub>k</sub>), 4.23 (d, 1H, J = 4.8 Hz, H<sub>i</sub>), 4.37 (d, 1H, J<sub>gen</sub> = 18.3 Hz, H<sub>g</sub>), 4.43 (d, 1H, J<sub>gen</sub> = 18.3 Hz, H<sub>g</sub>), 4.48 (s, 2H, CH<sub>2</sub>φ), 7.24-7.27 (aromatic H ); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 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<sub>2</sub>φ), 75.01 (g), 76.42 (i), 128.91-129.12-129-129.42-138.96 (aromatic C), 175.61 (b), 210.60 (h); <sup>31</sup>P NMR (CD<sub>3</sub>OD, 162 MHz): δ 1.03. Mass (FAB<sup>+</sup>;TEA):(M<sup>+</sup> Na<sup>+</sup>) = 457 ; (M-H+2Na)<sup>+</sup> = 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  $\mu$ L) 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%). <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz):  $\delta 1.63$  (m, 4H, H<sub>e</sub> and H<sub>d</sub>), 2.40 (t, 2H, J = 6.1 Hz, H<sub>c</sub>), 3.67 (s, 3H, H<sub>a</sub>), 4.00 (d, m, 5H, H<sub>f</sub>-H<sub>k</sub> and H<sub>j</sub>), 4.26 (d, 1H, J = 3.8 Hz, H<sub>j</sub>), 4.37 (d, 1H, J<sub>gen</sub>

= 20 Hz, H<sub>g</sub>), 4.44 (d, 1H, J<sub>gem</sub> = 20 Hz, H<sub>g</sub>); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 400 MHz):  $\delta$  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); <sup>31</sup>P NMR (CD<sub>3</sub>OD, 162 MHz):  $\delta$  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%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 200 MHz):  $\delta$  1. 52 to 1.80 (m, 4H, H<sub>e</sub> and H<sub>d</sub>), 2.39 (t, 2H, J = 7.1 Hz, H<sub>e</sub>), 3.61 (t, 2H, J = 6.1 Hz, H<sub>f</sub>), 5.10 (s, 2H, H<sub>a</sub>), 7.33 (m, 5H, aromatic H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 50 MHz):  $\delta$  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<sub>12</sub>H<sub>16</sub>O<sub>3</sub>: 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, 0.94 mmol) and triethylamine (94  $\mu$ L, 0.94 mmol) in anhydrous CH2Cl2 (700 µL) was added to a solution of 18 (267 mg, 0.94 mmol) in anhydrous CH2Cl2 (700  $\mu$ L). 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<sub>2</sub>Cl<sub>2</sub>  $(500 \,\mu\text{L})$  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%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 200 MHz):  $\delta$  1.44 (d, 3H, J = 7.0 Hz, H<sub>a</sub>), 1.71 (m, 4H, H<sub>d</sub> and H<sub>e</sub>), 2.19 (s, 3H, H<sub>b</sub>), 2.35 (m, 2H, H<sub>c</sub>), 3.18 (m, 4H, -CH<sub>2</sub>S-), 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  $H_{i}$ , 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<sub>2</sub>), 7.32 (m, 10H, aromatic H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 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); <sup>31</sup>P NMR (CDCl<sub>3</sub>, 162 MHz): δ 0.54 (5%), 0.60 (16%), 0.66 (29%), 0.68 (50%).

Anal Calcd for C<sub>30</sub>H<sub>41</sub>O<sub>10</sub>PS<sub>2</sub>: C, 54.86; H, 6.29. Found: C, 54.83; H, 6.32.

1-O-Benzyl-5-O-[1-(4-benzyloxycarbonylbutyl)]-phosphono-Lerythro-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<sub>2</sub>O-CH<sub>3</sub>CN, 1:1. The experimental procedure was identical to that used for 20a. A chromatography on reversed phase silica gel (CH<sub>3</sub>CN-H<sub>2</sub>O, 1:1) gave **20b** (255.3 mg, 63%). <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz):  $\delta$  1.76 (m, 4H, H<sub>e</sub> and H<sub>d</sub>), 2.47 (t, 2H, J = 7 Hz, H<sub>e</sub>), 3.25 (m, 4H, -CH<sub>2</sub>S-), 3.92 (d, 1H, J<sub>gem</sub> = 9.6 Hz, H<sub>g</sub>), 3.95 (d, 1H, J<sub>gem</sub> = 9.6 Hz, H<sub>g</sub>), 4.04 (m, 2H, H<sub>f</sub>), 4.09 (m, 1H, H<sub>j</sub>), 4.12 to 4.15 (m, 2H, H<sub>i</sub> and H<sub>k</sub>), 4.37 (dd, 1H, J<sub>gem</sub> = 9.3 Hz, J = 5 Hz, H<sub>k</sub>), 4.65 (s, 2H, CH<sub>2</sub> $\phi$ ), 5.17 (s, 2H, H<sub>s</sub>), 7.32 to 7.44 (aromatic H); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 100 MHz):  $\delta$  22.29 (d), 30.79 (d, J = 6 Hz, e), 34.05 (c), 38.89 and 40.18 (-CH<sub>2</sub>S-), 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<sub>2</sub> $\phi$ ), 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); <sup>31</sup>P NMR (CD<sub>3</sub>OD, 162 MHz):  $\delta$  1.45. Mass (FAB<sup>+</sup>, TG) : (M+Na)<sup>+</sup> = 609 ; (M-H+2Na)<sup>+</sup> = 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<sub>3</sub>CN (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<sub>3</sub>CN-H<sub>2</sub>O, 4:6) gave 21b (50 mg, 35%). <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz):  $\delta$  1.56 (m, 2H, H<sub>e</sub>), 1.62 (m, 2H, H<sub>d</sub>), 2.29 (t, 2H, J = 7.2 Hz, H<sub>c</sub>), 3.74 (m, 2H, H<sub>f</sub>), 3.85 (m, 3H, H<sub>k</sub> and H<sub>j</sub>), 4.15 (d, 1H, J = 5.4 Hz, H<sub>i</sub>), 4.35 (d, 1H, J<sub>gen</sub> = 18.3 Hz, H<sub>g</sub>), 4.41 (d, 1H, J<sub>gen</sub> = 18.3 Hz, H<sub>g</sub>), 4.47 (d, 1H, J<sub>gen</sub> = 11.8 Hz, CH<sub>2</sub> $\phi$ ), 4.49 (d, 1H, J<sub>gen</sub> = 11.8 Hz, CH<sub>2</sub> $\phi$ ), 4.99 (s, 2H, H<sub>s</sub>), 7.22 (m, 10H, aromatic H ); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 100 MHz):  $\delta$  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<sub>2</sub> $\phi$ ), 75.04 (g), 76.4 (i), 128.38-128.57-128.93-129.29-129.46-137.69-138.94 (aromatic C); <sup>31</sup>P NMR (CD<sub>3</sub>OD, 162 MHz):  $\delta$  2.35. Mass (FAB<sup>+</sup>, TEA) : (M-H)<sup>-</sup> = 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<sub>2</sub> 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%). <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz):  $\delta$  1.59 (m, 4H, H<sub>e</sub> and H<sub>d</sub>), 2.25 (t, 2H, J = 6.4 Hz, H<sub>e</sub>), 3.72 to 3.90 (m, 5H, H<sub>f</sub>, H<sub>k</sub> and H<sub>j</sub>), 4.18 (m, 1H, H<sub>i</sub>), 4.43 (s, 2H, H<sub>g</sub>); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 100 MHz):  $\delta$  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); <sup>31</sup>P NMR (CD<sub>3</sub>OD, 162 MHz):  $\delta$  1.92. Mass (FAB<sup>-</sup>, TEA):(M-H) = 329.

**Lactones 24a + 24b.** A 0.5 M aqueous solution of KCN (106.8  $\mu$ L, 53.4  $\mu$ mol) was added to a solution of 22a (18.4 mg, 53.4  $\mu$ mol) in 750  $\mu$ L of water. The mixture was stirred 48 h at room temperature. Then an excess of Dowex 50 (H<sup>+</sup>) 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):  $(M+K^*) = 411$ ;  $(M+Na^*) = 395$ ; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz):  $\delta$  1.89 (m, 4H, H<sub>d</sub>, H<sub>e</sub>), 2.37 (m, 2H, H<sub>c</sub>), 3.73 (s, 3H, H<sub>4</sub>), 3.61-4.42 (m, 8H, H<sub>e</sub>, H<sub>e</sub>, H<sub>i</sub>, H<sub>i</sub>, H<sub>k</sub>).

Formation of the BSA-2 conjugate. A solution of 2 (15 mg, 45  $\mu$ mol) in 0.83 mL of water was added to a solution of BSA (50 mg, 0.75  $\mu$ mol) in water (2.4 mL). A solution of EDC (43 mg, 220  $\mu$ mol) 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  $\mu$ L, 6.9  $\mu$ mol) was added to a solution of BSA-2 conjugate (15 mg, 0.23  $\mu$ mol) 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 <sup>14</sup>C labelling. An aqueous solution of [<sup>14</sup>C]KCN (10  $\mu$ L, 0.87  $\mu$ mol, 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  $\mu$ L 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  $\mu$ mol) in 150  $\mu$ L of water was added to a solution of PTG (10 mg, 1.51 10<sup>-2</sup>  $\mu$ mol) in water (1 mL). A solution of EDC (2.17 mg, 50  $\mu$ mol) in 50 mL of water was added. The mixture was stirred overnight at 4 °C. The solution was divided into two fractions of 500  $\mu$ L 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  $\mu$ L, 1.07  $\mu$ mol) was added to a solution of PTG-2 conjuguate (5 mg, 7.57x10<sup>-3</sup>  $\mu$ mol) in 500  $\mu$ L 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  $\mu$ L, 1.07  $\mu$ mol, 141 eq/PTG) was added to a solution of PTG-2 conjugate (5 mg, 7.59x10<sup>-3</sup>  $\mu$ mol) in 500 mL of water. 172  $\mu$ L of a 7.75  $\mu$ Ci/mL [<sup>14</sup>C]KCN solution were added (1.33  $\mu$ Ci, 2.96x10<sup>6</sup>dpm).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.

#### ACKNOWLEDGMENTS

We thank Dr. R. Burgada and Dr. J.M. Valéry for helpful discussions.

#### REFERENCES

- 1. P.G. Schultz, R.A. Lerner and S.J. Benkovic, C&EN, 28, 26 (1990). R. A. Lerner, S. J. Benkovic, P. G. Schultz, Science, 252, 659, (1991).
- D.Y. Jackson, J.W. Jacobs, R. Sugasawara, S.H. Reich, P.A. Bartlett and P.G. Schultz, J. Am. Chem. Soc., 110, 4841 (1988). D. Hilvert and K.D. Nared, J. Am. Chem. Soc., 110, 5593 (1988). D.Y. Jackson, M.N. Liang, P.A. Bartlett and P.G. Schultz, Angew. Chem. Int. Engl., Ed., 31, 182 (1992).
- 3. A.C. Braisted and P.G. Schultz, J. Am. Chem. Soc., 116, 2211 (1994). H.D. Ulrich, E.M.G. Driggers and P.G. Schultzt, Acta Chem. Scand., 50, 328 (1996).
- 4. Y. Chen, J.L. Reymond and R.A. Lerner, Angew. Chem. Int. Engl., Ed. 33, 1607 (1994).
- D. Hilvert, K.W. Hill, K.D. Nared and M.N. Auditor, J. Am. Chem. Soc., 111, 926 (1989).
  A.C. Braisted and P.G. Schultz, J. Am. Chem. Soc., 112, 7430 (1990).
  V.E. Gouverneur, K.N. Houk, B. de Pascual-Teresa, B. Beno, K.D. Janda and R.A. Lerner, Science, 262, 204 (1993).
  C.J. Sucking, M.C. Tedford, L.M. Bence, J.I. Irvine and W.H. Stimson, Biomed. Chem. Lett., 2, 49 (1992).
- T. Li, R.A. Lerner, K.D. Janda, Acc. Chem. Res., 30, 115 (1997). J. Hasserodt, K.D. Janda, R.A. Lerner, J. Am. Chem. Soc., 118, 11654 (1996).
- T. Koch, J.L. Reymond and R.A. Lerner, J. Am. Chem. Soc., 117, 9383 (1995).
  J. Wagner, R.A. Lerner and C.F. Barbas III, Science, 270, 1797 (1995).
  G. Zhong, T. Hoffmann, R.A. Lerner, S. Danishefsky and C.F. Barbas III, J. Am. Chem. Soc., 119, 8131 (1997).
- 8. M.H. Miziorko and G.H. Lorimer, Ann. Rev. Biochem., 52, 507 (1983). F.C. Hartman and M.R. Harpel, Ann. Rev. Biochem., 63, 197 (1994).
- G.H. Lorimer, T.J. Andrews, J. Pierce and J.V. Schloss, *Philos. Trans. R. Soc. Lond.*, B 313, 397 (1986). J. Pierce, T.J. Andrews and G.H. Lorimer, *J. Biol. Chem.*, 261, 10248 (1986). A. Jaworoski, F.C. Hartman and I.A. Rose, *J. Biol. Chem.*, 259, 6783 (1984).
- 10. J. Pierce, N.E. Tolbert and R. Barker, Biochemistry, 19, 934 (1980).
- 11. R. L. Letsinger and K. K. Ogilvie, J. Am. Chem. Soc., 91, 3350 (1969).
- 12. L. Stankowic, K. Linek and M. Ferodenko, Carbohydr. Res., 35, 242 (1976).
- S. Czernecki, C. Georgoulis and C. Provelenghiou, *Tetrahedron Lett.*, 39, 3535 (1976).
- J. N. BeMiller, Advances in Carbohydrate Chemistry; Academic Press: New York, 1967, Vol 22, p 25.
- 15. R. P. Hatch, J. Shringarpure and S. M. Weinreb, J. Org. Chem., 43, 4172 (1978).
- 16. S.L. Beaucage and R.P. Tyer, Tetrahedron, 48, 2223 (1992).
- 17. N. D. Sinha, J. Biernat, J. Mc Manus and H. Köler, Nucleic Acids Res., 12, 4539 (1984).
- 18. F. Frappier, M. Jouany, A. Marquet, A. Olesker and J.C. Tabet, J. Org. Chem., 77, 2257 (1982).
- 19. P. Mc Cullis, J. Chem. Soc. Chem. Commun., 1510 (1984).
- 20. E.J. Corey, D. Seebach and R. Freedman, J. Am. Chem. Soc., 89, 34 (1967).
- 21 C.A. Reece, J.O. Rodin, R.G. Brownlee, W.G. Duncan and R.M. Silverstein, Tetrahedron, 24, 4249 (1968).
- 22. P. Stütz and P.A. Stadler, Organic Syntheses, John Wiley and Sons, Eds.; New York, 1988, Vol 6, p 108.
- 23. B.C. Ranu, S. Bhar and R. Chakraborti, J. Org. Chem., 57, 7349 (1992).

- 24. H.L. Wang Chang, Tetrahedron Lett., 19, 1989 (1972).
- F. Ramirez and J.F. Marecek, Synthesis, 449 (1985). F. Ramirez, J.F. Marecek and H. Okasaki, J. Am. Chem. Soc., 98, 5310 (1976).
- 26. K. Vanhessche, E. Van der Eycken and M. Vandewalle, Tetrahedron Lett., 31, 2337 (1990).
- H.L. Wolfrom and A. Thompson, Methods in Carbohydrate Chemistry; Academic Press: New York, 1963, Vol. 2, p 427.
- 28. R.P. Hatch, J. Shringarpure and S.M. Weinreb, J. Org. Chem., 43, 4172 (1978).
- 29. F. Ramirez, J.F. Marecek and H. Okasaki, J. Am. Chem. Soc., 97, 7181 (1975).
- 30. S.F. Martin, J.A. Josey, Y.L. Wong and D.W. Dean, J. Org. Chem., 59, 4805 (1994).
- 31. E.J. Corey, I. Szekely and C.S. Shiner, Tetrahedron Lett., 40, 3529 (1977).
- B.F. Erlanger, Methods in Enzymology; Academic Press: New York, 1980, Vol 70, p 85. K.D. Janda, S.J. Benkovic and R.A. Lerner, Science, 244, 437 (1989).
- B.R. Rabin, D.F. Shaw, N.G. Pon, J.M. Anderson and M. Calvin, J. Am. Chem. Soc., 80, 2528 (1958).
- 34. F. Ramirez, J.F. Marecek and I.J. Ugi, J. Am. Chem. Soc., 97, 3809 (1975).