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SYNTHESIS OF POLYPRENYL PYROPHOSPHATE SUGARS FROM UNPROTECTED MONO- AND OLIGO-SACCHARIDE PHOSPHATES

LEONID L. DANILOV, SERGEI D. MALTSEV, VLADIMIR N. SHIBAEV, AND NIKOLAI K. KOCHETKOV
N. D. Zelinsky Institute of Organic Chemistry, Academy of Sciences of the U.S.S.R., Moscow (U.S.S.R.)
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ABSTRACT

New syntheses of polyprenyl pyrophosphate sugars are reported. Moraprenyl phosphate, prepared by chemical phosphorylation of a polyprenol from mulberry leaves, was treated with *N,N'*-sulfinyldi-imidazole, and the resulting imidazolidate **2** was treated, without purification, with α-D-galactopyranosyl or α-D-glucopyranosyl phosphate, to give the P^1-moraprenyl, P^2-glycosyl pyrophosphates **7** or **8**. The method was used to prepare the biologically active, oligosaccharide polyprenyl derivatives **9** and **10**, which are intermediates in the biosynthesis of O-specific polysaccharides of *Salmonella* serological group E. The polyprenyl pyrophosphate sugars were purified by ion-exchange chromatography, and their structures were confirmed by analytical data and specific degradations. Sensitive variants of the analytical procedures for characterising polyprenyl pyrophosphate sugars and a modified procedure for purification of moraprenyl phosphate are reported.

INTRODUCTION

The significance of polyprenyl pyrophosphate derivatives of mono- and oligosaccharides as intermediates in the biosynthesis of carbohydrate chains of bacterial cell-wall or extracellular polymers and viral or eucaryotic glycoproteins is now well-established. Chemical synthesis of these derivatives is an important step for a more complete understanding of these processes, since the availability of polyprenyl pyrophosphate sugars from natural sources or through enzymic reactions is very limited.

The only reported procedure[1] for the synthesis of polyprenyl pyrophosphate sugars is based on the interaction of P^1-polyprenyl, P^2-diphenyl pyrophosphates with fully acetylated glycosyl phosphates, followed by deprotection of the resulting pyrophosphate diester. In this way, polyprenyl pyrophosphate sugars derived from α-D-galactopyranose[1], α-D-mannopyranose[2], 2-acetamido-2-deoxy-α-D-glucopyranose[3,4], and some oligosaccharides having 2-acetamido-2-deoxy-α-D-glucopyranose at the reducing end of a chain were synthesised[5]. In most cases, this approach is very useful, but it is not satisfactory for the preparation of pyrophosphate derivatives in which HO-2 of the glycosyl residue is equatorial, *i.e.*, is sterically suited for the formation of 1,2-cyclic phosphate derivatives with splitting of the pyrophosphate linkage.

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Examples include α-D-galactopyranosyl or α-D-glucopyranosyl derivatives, and deacetylation of the protected pyrophosphate intermediate is then difficult, because of the extreme alkali-lability of the product[6].

An α-D-galactopyranosyl residue is present at the "reducing" end of oligosaccharide chains in many biosynthetic polyprenyl pyrophosphate intermediates, particularly those involved in the biosynthesis of *Salmonella*[6-8] and *Citrobacter* O-antigens[9], as well as *Klebsiella aerogenes* capsular polysaccharide[10]. The existence of α-D-glucopyranosyl pyrophosphate intermediates and oligosaccharides derived therefrom has been demonstrated in bacteria[11] and plants[12].

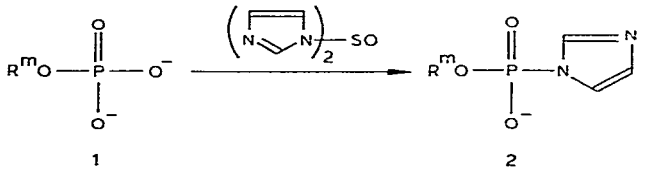
We now report on a method for the synthesis of polyprenyl pyrophosphate sugars which uses unprotected glycosyl phosphates, and the preparation of polyprenyl pyrophosphate derivatives of α-D-galactopyranose, α-D-glucopyranose, and di- and tri-saccharides corresponding to the structure of intermediates in the biosynthesis of O-specific polysaccharides of *Salmonella* group E by this procedure. We have used moraprenol, a readily available polyprenol from mulberry leaves, which is similar in chain length to bacterial undecaprenol but contains three instead of two *trans*-internal isoprene units[13]. Ficaprenol, a polyprenol from *Ficus* leaves, has been used in a similar synthesis[1,3]; a recent paper[14] showed that ficaprenol and moraprenol are identical. Moraprenyl derivatives are good substrate analogues[8,15] for the enzymes involved in O-antigen biosynthesis in *Salmonella*. Part of the material in this paper has been reported in preliminary form[16,17].

RESULTS AND DISCUSSION

The need for protection of hydroxyl groups in glycosyl phosphates during the synthesis of polyprenyl pyrophosphate sugars is not obvious, as nucleoside diphosphate sugars may be easily prepared from P^1-nucleoside-5′, P^2-diphenyl pyrophosphates and unprotected glycosyl phosphates[18]. Nevertheless, Warren and Jeanloz observed[1] the formation of a complex mixture of products when P^1-farnesyl, P^2-diphenyl pyrophosphate was treated with α-D-galactopyranosyl phosphate, probably because of degradation of the polyprenyl pyrophosphate esters through nucleophilic attack on the allylic carbon atom by the pyridine present as a catalyst for pyrophosphate-linkage formation. An efficient way to suppress this side reaction involves the use of activated phosphomonoester derivatives that can form pyrophosphates without pyridine catalysis. Polyprenyl phosphoimidazolidates seemed to be plausible candidates. Nucleoside 5′-phosphoimidazolidates, which have been used for the preparation of "nucleoside diphosphate sugars"[19], may be obtained conveniently by the reaction of nucleoside 5′-phosphates with *N,N′*-carbonyldi-imidazole[20]; uses of the more-active *N,N′*-sulfinyldi-imidazole[21] have been reported[22].

When ammonium moraprenyl phosphate[13] (**1**) was treated with ~3 equiv. of *N,N′*-sulfinyldi-imidazole (prepared *in situ* from thionyl chloride and imidazole[21]) in tetrahydrofuran, it was converted rapidly into a product of higher chromatographic mobility, presumably the moraprenyl phosphoimidazolidate **2**; the reaction was

inhibited by traces of moisture. The product **2** was used for pyrophosphate synthesis after decomposition of the excess of *N,N'*-sulfinyldi-imidazole with methanol, and without further purification because of its instability.



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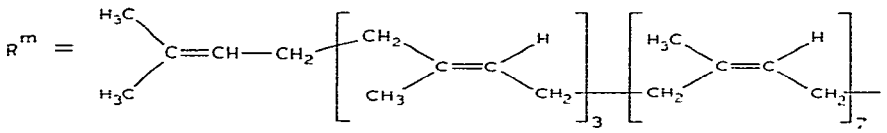

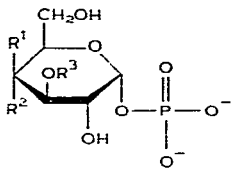

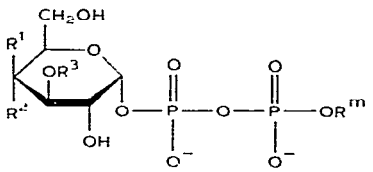

3 R^1 = OH, R^2 = R^3 = H
4 R^1 = R^3 = H, R^2 = OH
5 R^1 = OH, R^2 = H, R^3 = α-L-rhamnopyranosyl
6 R^1 = OH, R^2 = H, R^3 = 4-*O*-β-D-mannopyranosyl-α-L-rhamnopyranosyl

7 R^1 = OH, R^2 = R^3 = H
8 R^1 = R^3 = H, R^2 = OH
9 R^1 = OH, R^2 = H, R^3 = α-L-rhamnopyranosyl
10 R^1 = OH, R^2 = H, R^3 = 4-*O*-β-D-mannopyranosyl-α-L-rhamnopyranosyl

α-D-Galactopyranosyl phosphate (**3**) was used in the preliminary experiments on the synthesis of moraprenyl pyrophosphate sugars. Satisfactory solubilisation of the reaction components was achieved by using tetrahydrofuran–methyl sulfoxide (1 : 1) as the solvent and the bis(tri-octylammonium) salt of the glycosyl phosphate. When mixtures of **2** and **3** in the molar ratios 1 : 2, 1 : 1, and 1 : 0.5 were incubated for 16 h at room temperature, t.l.c. revealed, in each case, a product with R_F 0.20 (solvent *A*), a simultaneous decrease or disappearance of **2**, and formation of **1** and several by-products. The best results for formation of the slow-moving product were achieved in the experiment with an excess of the glycosyl phosphate.

Ion-exchange chromatography on DEAE-cellulose (AcO^-), with a linear gradient of ammonium acetate in methanol, was used for further analysis of the reaction mixtures and preparative isolation of the products. Five phosphorus-con-

taining products were generally present, and the following preliminary identification was possible on the basis of their position of elution, colorimetric determinations of phosphorus and unsaturated derivatives, and t.l.c. analysis.

(*a*) Two poorly resolved peaks appeared at the beginning of the salt gradient centered at 8–10 and 15–17mM salt, respectively. T.l.c. showed the presence of **2** and **1**, together with several minor by-products, in these peaks. When an excess of **2** was used, an additional product was present.

(*b*) The peak centered at 40–45mM salt contained the main reaction-product, R_F 0.15–0.20 (solvent *A*); it contained 2 moles of phosphorus per moraprenyl residue. The identification of this product as **7** is presented below.

(*c*) The peak eluted at ~60mM salt contained 1 mole of phosphorus per moraprenyl residue and was tentatively identified as dimoraprenyl pyrophosphate. It was present in significant amounts only in experiments with **2** and **3** in the molar ratio 1 : 1 or 1 : 0.5.

(*d*) The last peak, centered at 75–80mM salt, contained the glycosyl phosphate **3** and some glycosyl pyrophosphate, which was probably formed by splitting of the polyprenyl–phosphate linkage in **7**; the presence of **7** was demonstrated by paper electrophoresis of the reaction mixtures prior to separation.

With an excess of the phosphate **3**, the product present in peak (*b*) was readily isolated, in chromatographically pure form, in a yield of 47%. The resulting solution may be desalted after dilution with benzene and extraction with water or by using gel filtration on Sephadex LH-20 with chloroform–methanol mixtures. The product was stable in methanol containing ammonium acetate and may be stored for months at −20°, but decomposed quickly in desalted benzene or chloroform solutions, or after their evaporation. Thus, the preparation of an analytical sample was not feasible, and the following technique was used. The product was stored in solution after ion-exchange chromatography without desalting, concentration of the solutions was determined by using phosphate analysis, and the purity of the product was checked by t.l.c. To confirm the structure of the product, its solutions were desalted and subjected to specific degradations as outlined below.

Brief treatment with aqueous phenol is a known method for cleaving the allylic phosphate bond in polyprenyl pyrophosphate sugars (see, for example, ref. 11*b*). Galactosyl pyrophosphate was formed on treatment of the substance present in peak (*b*) with 40% aqueous phenol for 10 min at 70°. When the same product was treated with ammonia in methanol–benzene, rapid degradation occurred, yielding moraprenyl phosphate and galactose 1,2-cyclic phosphate. These results confirmed the presence of a pyrophosphate bridge between moraprenyl and galactopyranosyl residues in the reaction product, and consequently proved the structure **7**.

The conditions used for the synthesis of **7** were used for the preparation of other moraprenyl pyrophosphate sugars. Treatment of bis(tri-octylammonium) α-D-glucopyranosyl phosphate (**4**) with the imidazolidate **2** gave the pyrophosphate **8** (isolated in a yield of 65%). Its structure was confirmed by colorimetric analyses and by specific degradations with phenol and ammonia. An analogous experiment

performed with α-D-[^{14}C]glucopyranosyl phosphate gave moraprenyl pyrophosphate D-[^{14}C]glucose. In this case, additional evidence for the structure of the reaction product was the ratio of D-[^{14}C]glucose to phosphorus, which was close to 1:2 in accordance with the structure **8**.

The method was further applied to the synthesis of polyprenyl pyrophosphate oligosaccharides that contained a D-galactopyranosyl residue at the "reducing" end of the oligosaccharide chains. The disaccharide phosphate **5** and the trisaccharide phosphate **6** (ref. 23), which correspond to fragments of the O-antigens of *Salmonella* serogroup E, were used as starting materials. Due to limited availability of the oligosaccharide phosphates, the pyrophosphate synthesis was performed on a smaller scale. The pyrophosphate derivatives **9** and **10** were isolated in 25–30% yields, using ion-exchange chromatography and an appropriately higher concentration of ammonium acetate. Their structures were confirmed by the methods described above.

The synthetic polyprenyl pyrophosphate sugars **7**, **9**, and **10** were active substrate analogues for the enzymes participating in the biosynthesis of *Salmonella* O-antigens. These derivatives were very unstable after removal of ammonium acetate, but the presence of the salt at low concentration did not interfere with assay of the glycosylation reactions. In fact, addition of the polyprenyl derivatives to incubation mixtures from solution in methanol containing ammonium acetate gave better solubilisation of the substrates than use of desalted solutions in non-polar solvents. The ability of **7** to serve as an acceptor for rhamnosyl transferase in *S. anatum* has been reported[16,24]. The ability[17] of **7**, **9**, and **10** to serve as substrates for enzymes catalysing consecutive reactions of O-antigen biosynthesis in *S. senftenberg* will be described in detail elsewhere.

EXPERIMENTAL

Chromatographic methods. — T.l.c. was performed on precoated plates of Silica Gel G (Merck) with chloroform–methanol–water, 60:25:4 (*A*) or 10:10:1 (*B*). The spray reagent described by Vaskovsky *et al.*[25] was used to detect phosphate esters (blue spots after leaving at room temperature) or organic substances (dark spots after heating the plate). Unsaturated derivatives were detected with 1% potassium permanganate in 2% aqueous sodium carbonate.

Anion-exchange chromatography was performed with Whatman DE-52 DEAE-cellulose (AcO^- form) equilibrated with methanol[26], and a linear gradient of ammonium acetate in methanol (pH 7.0) was used for elution. Fractions were assayed for acid-labile phosphate and moraprenol (see below).

Filtrak FN-16 paper and 0.05M triethylammonium hydrogencarbonate (pH 8.5) were used for paper electrophoresis, and phosphate esters were detected with the Hanes–Isherwood spray[27].

Colorimetric methods. — Acid-labile phosphate was determined by a modification[23] of the Hess and Derr procedure[28]. A modified procedure[29] for olefins was used to determine moraprenol in moraprenyl pyrophosphate sugars: 2.0 mL of 1.7μM

HBr_3 in 90% acetic acid (prepared according to the described procedure[29], but diluted 600-fold) was placed in a 1-cm spectrophotometric cell, and an aliquot (15–40 nmol) of the moraprenyl derivative in methanol was added. After mixing and completion of bromide consumption (20 min at room temperature), the absorbance at 290 nm was measured against a suitable blank. Moraprenol was used for calibration; ammonium acetate and glycosyl phosphates did not interfere.

Moraprenyl phosphate (**1**). — The phosphate **1** was prepared by procedure B described by Vergunova *et al.*[13]. The fractions containing **1** after ion-exchange chromatography were concentrated, chloroform–methanol (2:1; 0.5 mL) was added, and the resulting solution was applied to a column (40 × 1 cm) of Sephadex LH-20 equilibrated with chloroform–methanol (2:1). The column was eluted with the same mixture, and the conductivity of each fraction was measured (Conductivity Meter CDM-3, Radiometer). Ammonium moraprenyl phosphate was eluted immediately after the void volume of the column (30 mL), well ahead of ammonium acetate, and the recovery was quantitative.

Ammonium P^1-moraprenyl, P^2-α-D-galactopyranosyl pyrophosphate (**7**). — A solution of freshly distilled thionyl chloride (10 μL) in dry tetrahydrofuran (0.4 mL) was added to a solution of imidazole (54 mg) in tetrahydrofuran (0.4 mL), the mixture was stored for 1 h at room temperature, and the supernatant solution was used as a solution of sulfinyldi-imidazole (170mM).

Ammonium moraprenyl phosphate (6.6 μmol) was dried by twice freeze-drying from dry benzene and dissolved in a freshly prepared solution (0.1 mL) of sulfinyldi-imidazole (17 μmol). After 2 h at room temperature, t.l.c. (solvent *A*) showed conversion of **1** (R_F 0.45) into **2** (R_F 0.70). Methanol (15 μL) was added and, after 15 min, evaporated *in vacuo*. The residual **2** was used for pyrophosphate synthesis without purification.

Bis(tri-octylammonium) α-D-galactopyranosyl phosphate was prepared by passing an aqueous solution of the dipotassium salt of **3** through a cooled column of Dowex 50W-X8 (H^+) resin into alcoholic tri-octylamine (2 equiv. or more), the mixture was shaken, and excess of amine was extracted with hexane. An aliquot of the residual solution (13 μmol of **3**, as determined by phosphate analysis) was evaporated, the residue was dissolved in benzene (2 mL), the solution was freeze-dried, and this operation was repeated. The residue was dissolved in 0.4 mL of tetrahydrofuran–methyl sulfoxide (1:1), and a solution of **2** (6.5 μmol) in the same solvent (0.4 mL) was added. The mixture was kept for 16 h at room temperature, and t.l.c. then revealed a product having R_F 0.20 (solvent *A*). The mixture was diluted with chloroform–methanol (2:1; 30 mL) and applied to a column (8 × 1 cm) of DE-52 equilibrated with the same solvent. The column was washed with chloroform–methanol (2:1; 30 mL) and methanol (30 mL), and eluted (36 mL/h) with a linear gradient (0→100mM, 75 mL in each vessel) of ammonium acetate in methanol (6-mL fractions). Fractions 8–14 contained **7** (3.1 μmol, 47%), R_F 0.20 (*A*), 0.80 (*B*). The ratio moraprenol–acid-labile phosphate was 1:1.8 (calc. 1:2).

Ammonium P^1-moraprenyl, P^2-α-D-glucopyranosyl pyrophosphate (**8**). — The

analogous reaction of 8.8 μmol of **2** and 20 μmol of bis(tri-octylammonium) α-D-glucopyranosyl phosphate (prepared from the potassium salt of **4**) was performed in 0.6 mL of tetrahydrofuran–methyl sulfoxide (1 : 1) for 18 h. The product was isolated by chromatography on a column (8 × 0.5 cm) of DE-52 with a gradient (0→100mM) of ammonium acetate (50 mL in each vessel). Fractions (3 mL) 8–13 yielded **8** (5.7 μmol, 65%), R_F 0.20 (*A*). The ratio moraprenol–acid-labile phosphate was 1 : 2.3 (calc. 1 : 2).

Ammonium P¹-moraprenyl, P²-α-D-[U-¹⁴C]glucopyranosyl pyrophosphate. — The reaction of 5 μmol of **2** and 6 μmol of bis(tri-octylammonium) α-D-[U-¹⁴C]glucopyranosyl phosphate (11 mCi/mole), as described for **8**, gave the title product (1.05 μmol, 21%), R_F 0.20 (*A*). The ratios moraprenol–acid-labile phosphate-[¹⁴C]glucose were 1 : 2.3 : 1.1 (calc. 1 : 2 : 1).

Ammonium P¹-moraprenyl, P²-3-O-α-L-rhamnopyranosyl-α-D-galactopyranosyl pyrophosphate (**9**). — The reaction of 2 μmol of **2** and 4 μmol of the bis(tri-octylammonium) salt of the phosphate **5** (prepared[23] from triethylammonium **5** with the use of pyridinium Dowex-50) in 0.8 mL of tetrahydrofuran–methyl sulfoxide (1 : 1), essentially as described for **7**, gave **9** (0.5 μmol, 25%), R_F 0.10 (*A*). The ratio moraprenol–acid-labile phosphate was 1 : 1.8 (calc. 1 : 2).

Ammonium P¹-moraprenyl, P²-3-O-(4-O-β-D-mannopyranosyl-α-L-rhamnopyranosyl)-α-D-galactopyranosyl pyrophosphate (**10**). — The reaction of 5 μmol of **2** and 10 μmol of the bis(tri-octylammonium) salt of **6** (prepared from the triethylammonium salt[23] of **6** as above) in 0.8 mL of tetrahydrofuran–methyl sulfoxide was performed as described for **7**. The product was isolated by ion-exchange chromatography on a column (15 × 1 cm) of DE-52 with a gradient (0→120mM) of ammonium acetate (100 mL in each vessel: 6-mL fractions). Fractions 23–29 yielded **10** (1.5 μmol, 30%), R_F 0.05 (*A*), 0.60 (*B*). The ratio moraprenol–acid-labile phosphate was 1 : 1.8 (calc. 1 : 2).

Desalting of solutions of polyprenyl pyrophosphate sugars. — (*a*) Portions (0.3–0.5 mL) of the fractions after ion-exchange chromatography, containing **7**–**10**, were each diluted with benzene (2 mL), and ammonium acetate was extracted with water (5 × 0.3 mL). The resulting benzene solution was used for phenol or ammonia degradations.

(*b*) Combined fractions, after ion-exchange chromatography, containing 4 μmol of **8** were concentrated, and a solution of the residue in chloroform (0.2 mL) was applied to a column (30 × 1 cm) of Sephadex LH-20 equilibrated with chloroform–methanol (15 : 1). The column was eluted (20 mL/h) with the same mixture: after the void volume of the column, fractions (1 mL) were collected and assayed for conductivity and acid-labile phosphate. The pyrophosphate **8** (3.5 μmol) was present in fractions 4–7, separated from ammonium acetate.

Degradation of polyprenyl pyrophosphate sugars with phenol. — A desalted solution of each of the pyrophosphates **7**–**10** (0.3 μmol) in benzene (0.3 mL) was treated with 80% aqueous phenol (50 μL) and concentrated. Water (50 μL) was added, the mixture was heated for 10 min at 70° and then cooled, and the aqueous

layer was separated, washed with carbon tetrachloride, and analysed by paper electrophoresis. $R_{Glc\text{-}1\text{-}P}$ values for glycosyl pyrophosphates: 0.78 (from **7** and **8**), 0.75 (from **9**), and 0.71 (from **10**).

Degradation of polyprenyl pyrophosphate sugars with ammonia.— A desalted solution of each of the pyrophosphates **7–10** (0.3 μmol) in benzene (0.3 mL) was treated with conc. aqueous ammonia (0.1 mL), and methanol was added until homogeneity. After 40 min at room temperature, the solution was concentrated to 0.1 mL and extracted with chloroform (3 × 50 μL). Each chloroform layer was analysed by t.l.c.; moraprenyl phosphate (R_F 0.45) was present as a main product. Each aqueous layer was analysed by paper electrophoresis. $R_{Glc\text{-}1\text{-}P}$ values for glycosyl cyclophosphates: 0.63 (from **7** and **8**), 0.60 (from **9**), and 0.55 (from **10**).

REFERENCES

1 C. D. Warren and R. W. Jeanloz, *Biochemistry*, 11 (1972) 2565–2572.
2 C. D. Warren and R. W. Jeanloz, *Biochemistry*, 14 (1975) 412–419.
3 C. D. Warren, Y. Konami, and R. W. Jeanloz, *Carbohydr. Res.*, 30 (1973) 257–279.
4 C. D. Warren and R. W. Jeanloz, *Carbohydr. Res.*, 37 (1974) 252–260.
5 (*a*) C. D. Warren, A. Herscovics, and R. W. Jeanloz, *Carbohydr. Res.*, 61 (1978) 181–196; (*b*) E. Walker-Nasir and R. W. Jeanloz, *ibid.*, 68 (1979) 343–363.
6 M. Dankert, A. Wright, W. S. Kelley, and P. W. Robbins, *Arch. Biochem. Biophys.*, 116 (1966) 425–435.
7 M. J. Osborn, M. A. Cynkin, J. M. Gilbert, L. Müller, and M. Singh, *Methods Enzymol.*, 28 (1972) 583–601.
8 (*a*) V. N. Shibaev, T. N. Druzhinina, A. N. Popova, N. K. Kochetkov, S. S. Rozhnova, and V. A. Kilesso, *Bioorg. Khim.*, 5 (1979) 1071–1082; (*b*) V. N. Shibaev, T. N. Druzhinina, A. N. Popova, S. S. Rozhnova, and V. A. Kilesso, *Eur. J. Biochem.*, 101 (1979) 309–316.
9 R. Y. Tse-Yuen and B. Horecker, *J. Bacteriol.*, 95 (1968) 2242–2248.
10 F. A. Troy, F. E. Frerman, and E. C. Heath, *J. Biol. Chem.*, 246 (1971) 118–133.
11 (*a*) I. W. Sutherland and M. Norwal, *Biochem. J.*, 120 (1970) 567–576; (*b*) R. C. Garcia, E. Recondo, and M. Dankert, *Eur. J. Biochem.*, 43 (1974) 93–105; (*c*) J. G. Johnson and D. B. Wilson, *J. Bacteriol.*, 129 (1977) 225–236.
12 H. E. Hopp, P. A. Romero, G. R. Daleo, and R. P. Lezica, *Eur. J. Biochem.*, 84 (1978) 561–571.
13 G. I. Vergunova, I. S. Glukhoded, L. L. Danilov, G. I. Eliseeva, N. K. Kochetkov, M. F. Troitsky, A. I. Usov, A. S. Shashkov, and V. N. Shibaev, *Bioorg. Khim.*, 3 (1977) 1484–1492.
14 J. Tanaka and M. Takagi, *Biochem. J.*, 183 (1979) 163–165.
15 V. N. Shibaev, Yu. Yu. Kusov, T. N. Druzhinina, N. A. Kalinchuk, N. K. Kochetkov, V. A. Kilesso, and S. S. Rozhnova, *Bioorg. Khim.*, 4 (1978) 47–56.
16 V. N. Shibaev, L. L. Danilov, V. N. Chekunchikov, Yu. Yu. Kusov, and N. K. Kochetkov, *Bioorg. Khim.*, 5 (1979) 308–309.
17 L. L. Danilov, T. N. Druzhinina, V. N. Shibaev, and N. K. Kochetkov, *Bioorg. Khim.*, 6 (1980) 468–470.
18 A. M. Michelson, *Biochim. Biophys. Acta*, 91 (1964) 1–13.
19 V. N. Shibaev, G. I. Eliseeva, and N. K. Kochetkov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1973) 2095–2099.
20 (*a*) F. Cramer, H. Schaller, and H. A. Staab, *Chem. Ber.*, 94 (1961) 1612–1621; (*b*) D. E. Hoard and D. G. Ott, *J. Am. Chem. Soc.*, 87 (1965) 1785–1788.
21 H. A. Staab and K. Wendel, *Justus Liebigs Ann. Chem.*, 694 (1966) 86–90.
22 (*a*) L. I. Kolobushkina, A. M. Krytzin, and V. L. Florent'ev, *Khim. Geterotsikl. Soedin.*, (1973) 996–1000; (*b*) A. M. Krytzin, S. M. Mihailov, L. I. Kolobushkina, N. S. Padjukova, and V. L. Florent'ev, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1975) 1846–1850.
23 L. L. Danilov, M. F. Troitzky, N. S. Utkina, V. N. Shibaev, and N. K. Kochetkov, *Carbohydr. Res.*, 87 (1980) 141–146.

24 YU. YU. KUSOV, E. V. KISELEVA, L. L. DANILOV, V. N. SHIBAEV, N. K. KOCHETKOV, S. S. ROZHNOVA, AND V. A. KILESSO, *Bioorg. Khim.*, 5 (1979) 1863–1872.
25 V. E. VASKOVSKY, E. Y. KOSTETSKY, AND I. M. VASENIN, *J. Chromatogr.*, 114 (1975) 129–141.
26 G. ROUSER, G. KRITCHEVSKY, AND A. YAMAMOTO, *Lipid Chromatographic Analysis*, Vol. 1, Dekker, New York, 1967, pp. 447–463.
27 C. S. HANES AND F. A. ISHERWOOD, *Nature (London)*, 164 (1949) 1107–1109.
28 H. H. HESS AND J. E. DERR, *Anal. Biochem.*, 63 (1975) 607–613.
29 J. S. FRITZ AND G. E. WOOD, *Anal. Chem.*, 40 (1968) 134–139.