Synthesis of a Phosphorylated Disaccharide Fragment of the O-Specific Polysaccharide of *Vibrio cholerae* O139, Functionalized for Conjugation

by **Bart Ruttens** and **Pavol Kováč***

National Institutes of Health, NIDDK, LMC, Carbohydrates, Bldg. 8, Rm. B1A25, 8 Center Drive, Bethesda, MD, 20892-0815, USA
(phone: +1301-496-3569; fax: +1301-402-0589; e-mail: kpn@helix.nih.gov)

The title disaccharide, 2-{2-{2-[(2-ethoxy-3,4-dioxocyclobut-1-en-1-yl)amino]ethoxy}ethoxy}ethyl 2-*O*-(3,6-dideoxy-α-L-*xylo*-hexopyranosyl)-β-D-galactopyranoside cyclic 4,6-(potassium phosphate) (**2**), was synthesized from the two isomeric linker-equipped galactose acceptors **9** and **10**, obtained by phosphorylation of 2-[2-(2-azidoethoxy)ethoxy]ethyl 3-*O*-benzyl-β-D-galactopyranoside (**8**), which were glycosylated with ethyl 2,4-di-*O*-benzyl-3,6-dideoxy-1-thio-β-L-*xylo*-hexopyranoside (**12**; *Scheme*). Mainly the fully protected α-(1 → 2)-linked products **13*α*** and **14*α*** were formed. Catalytic hydrogenolysis/hydrogenation effected global deprotection, thereby removing the chirality at the P-atom, and simultaneously converted the azido group in the linker to an amino group (→ **15**). Final treatment with diethyl squarate (=3,4-diethoxycyclobut-3-ene-1,2-dione) gave target compound **2**, amenable for conjugation to proteins.

Introduction. – Cholera [1] is an enteric disease caused by *Vibrio cholerae*. Ingestion of contaminated water or food can lead to severe diarrhea, dehydration, and even hypotensive shock and death. Current vaccines give only short-term protection and produce an inadequate serological response, particularly in young children. Thus, cholera is a persistent problem for the developing and, occasionally, the developed world. Before 1992, only the O1 serogroup was associated with epidemic and pandemic cholera, other strains merely caused sporadic illness. Since its appearance in India in 1992, *Vibrio cholerae* O139 has emerged as a new serious threat to public health [2]. The lack of protective immunity against this new strain of the adult population in *V. cholerae* O1 endemic regions comprises its epidemic and pandemic potential. Research indicates that serotype O139 has evolved from serotype O1 through acquisition of new genes encoding for enzymes involved in the biosynthesis of O-specific polysaccharides (O-PS) [3]. As a result, the main differences between both serotypes are found in the constitution of the cell surface [4]. As opposed to *V. cholerae* O1, serotype O139 expresses a capsular polysaccharide (CPS), whose repeating unit is identical to the monomeric O-chain of the lipooligosaccharide (LOS, *Fig. 1*) [4][5]. Both the CPS and the O-chain of the LOS were shown to be virulance factors [4], and a critical level of serum immunoglobulin G (IgG) antibodies against these surface saccharides is required for immunity to the parent *Vibrio* [6].

Carbohydrates, classified as T-cell-independent (TI) antigens, are poor immunogens [7]. They are capable of activating antibody-producing B cells but fail to generate memory B cells. In contrast, T-cell-dependant (TD) antigens, such as proteins, not only initiate immunologic memory but also educe a stronger and earlier antibody response.

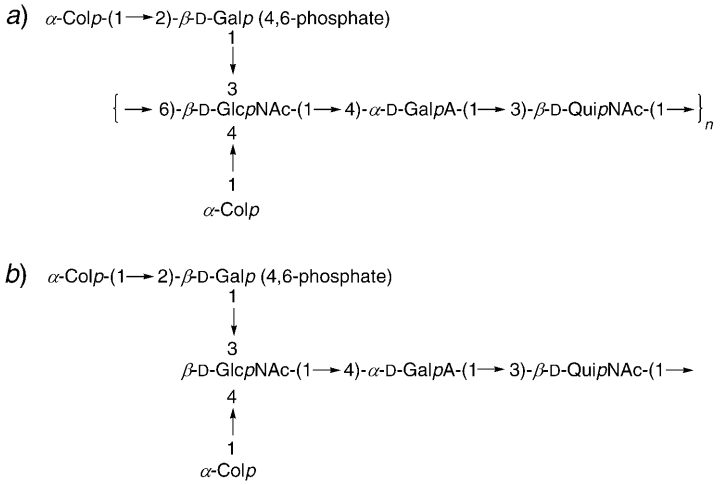


Fig. 1. *Schematic representation of* a) *the capsular polysaccharide* (CPS) *and* b) *the O-specific polysaccharide* (O-PS) *of* V. cholerae *O139*. (Colitose = Col*p*, galactose = Gal*p*, *N*-acetylglucosamine = Glc*p*NAc, galacturonic acid = Gal*p*A, and *N*-acetylquinovosamine = Qui*p*NAc).

Avery and *Goebel* [8] first demonstrated that chemical conjugation to proteins converted carbohydrates into TD antigens, or rather, elicited anti-carbohydrate antibodies in T-dependant fashion. Although the underlying cellular mechanisms are still not fully understood, synthetic glycoconjugates have found wide application as carbohydrate immunogens [9]. Initially, this concept was used mainly to produce vaccines where the hapten was harvested from pathogens. More recently, the progress in oligo- and polysaccharide synthesis sparked efforts to prepare conjugate vaccines from synthetic carbohydrate antigens [10]. Clinical trials with the first synthetic conjugate vaccine demonstrated the feasibility of a synthetic approach [11].

For *V. cholerae* O139, only cellular vaccines [12][13] and conjugate vaccines based on detoxified CPS [14] or LOS [15] have been reported. Compared to these pathogen-derived vaccines, conjugate vaccines from synthetic O-PS, although more difficult to obtain, would offer significant advantages when it comes to purity, reproducibility of preparation, homogeneity, shelf life, and potential side effects. In addition, access to fragments of the O-PS allows mapping of the epitopes that are crucial for protective capacity.

Recently, as an extension of our earlier and ongoing work on a conjugate vaccine for cholera caused by *V. cholerae* O1 [16], we started a project focused on the development of a conjugate vaccine **1** for cholera (*Fig. 2*) from the synthetic hexasaccharide that represents the O-antigenic region of the LOS of *V. cholerae* O139. *Oscarson* and co-workers [17] were the first to synthesize fragments of the *V. cholerae* O139 O-PS, but the reported tri- and tetrasaccharides lacked the phosphate group and needed further derivatization to allow conjugation with a carrier protein. Here, we describe the first synthesis of a phosphate-containing, *V. cholerae* O-PS related disaccharide fragment, equipped with a linker functionalized for conjugation.

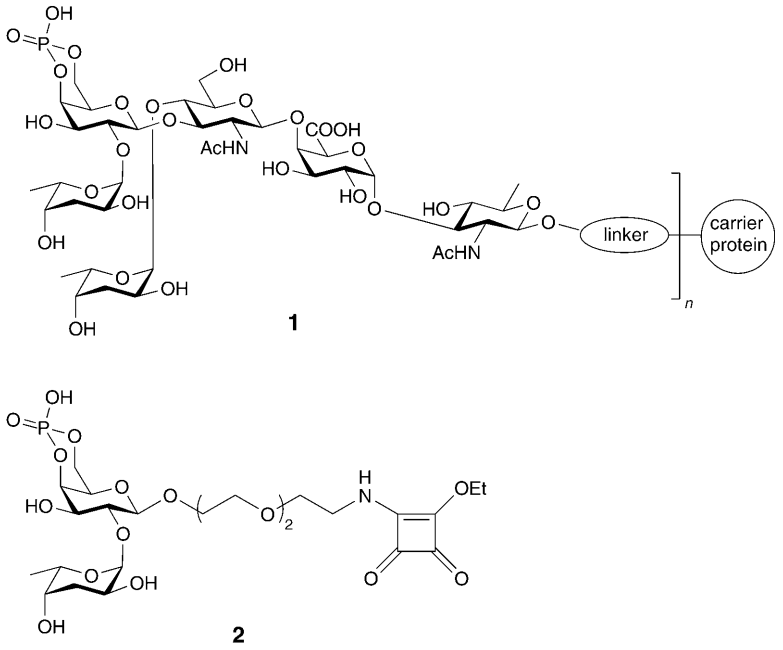


Fig. 2. *General structure of a conjugate vaccine* **1** *against* V. cholerae *O139 and the upstream terminal disaccharide fragment* **2** *for conjugation*

Because interaction of antigenic oligo- and polysaccharide with antibodies often occurs through the terminal saccharide units at the upstream [18] end, we concentrated our initial efforts on the synthesis of disaccharide fragment **2**, which is designed for conjugation by the squaric acid diester methodology originally developed by *Tietze et al.* [19]. It allowed us to research some of the key issues in the preparation of **1**, such as formation of the cyclic phosphate and the stability of the labile colitosidic bond. The 8-amino-3,6-dioxaoctyl (=2-[2-(2-aminoethoxy)ethoxy]ethyl) linker [20] provides good chemical inertness and, if required, allows length variation without fundamental changes to the chemistry.

Results and Discussion. – The synthesis of fragment **2** (see *Scheme*) was based on results of preliminary experiments aimed at probing cyclic 4,6-phosphate, *i.e.*, 1,3,2-dioxaphosphorinane oxide, formation, stability, and deprotection. The 1,3,2-dioxaphosphorinane 2-oxides with electronegative 2-substituents preferentially adopt chair conformations with axially oriented 2-aryloxy or 2-alkoxy groups [21]. However, for the formation of 1,3,2-dioxaphosphorinane 2-oxides by condensation of a phosphoric dihalide (=phosphorodihalogenidate) and a diol, a kinetic preference for the thermodynamically less stable isomer is generally observed [22], leading to a phosphate isomer mixture. The final isomer ratio depends on steric interactions in transition intermediates and the relative stabilities of the chair conformers. Indeed, reaction of methyl 2,3-di-*O*-benzyl-β-D-galactopyranoside with alkyl or aryl phosphorodichloridate reagents in the presence of pyridine as base and catalyst (not described in the *Exper.*

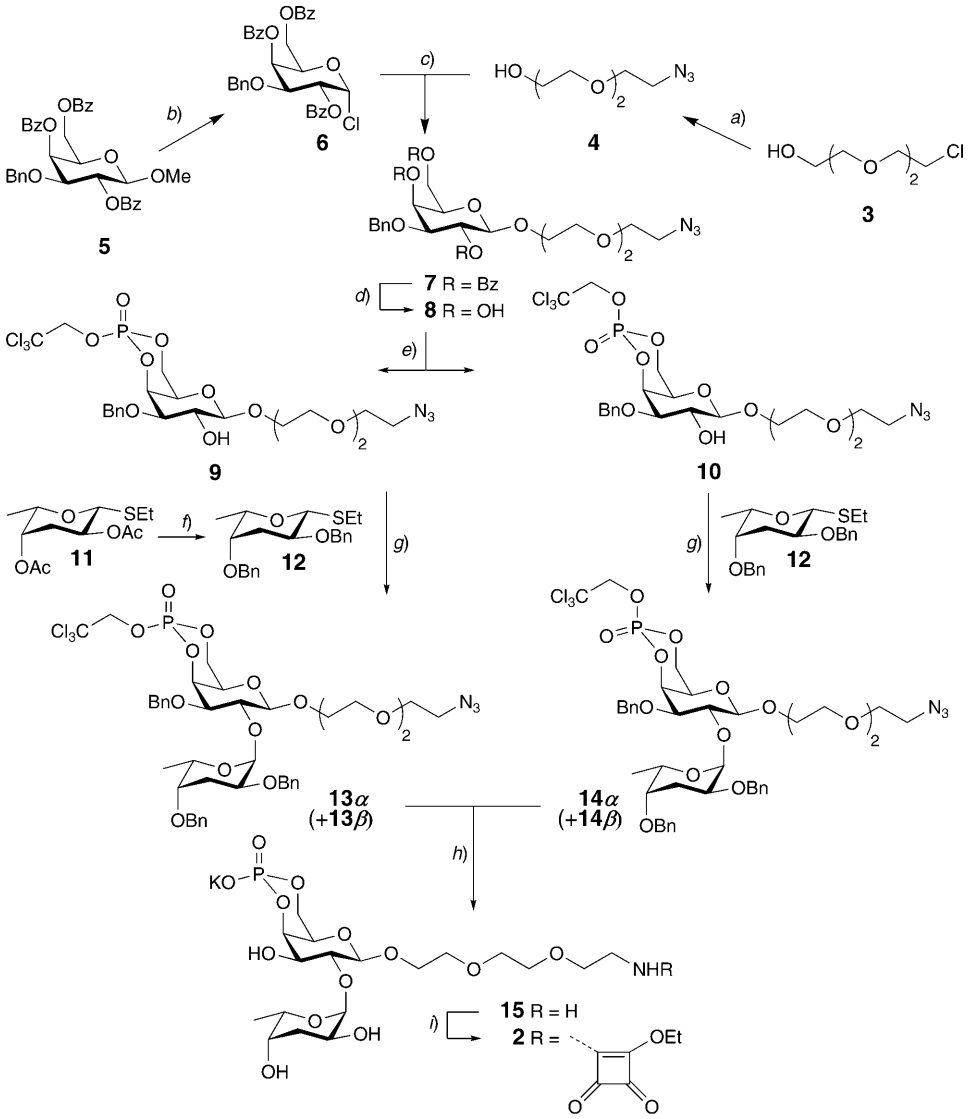
Part) gave virtually theoretical yields of a mixture of isomeric cyclic phosphates. They showed good acid stability but were unstable under basic conditions. Treatment with KOH in aqueous solution, *e.g.*, led quickly to deprotection of the cyclic phosphate or ring opening. The acyclic phosphate was subsequently converted slowly into the deprotected cyclic derivative by intramolecular substitution of the phosphate protecting group, rather than further hydrolyzed to the acyclic dibasic phosphate. These observations (not described in the *Exper. Part*) can be explained by the considerable difference in rates of alkaline hydrolysis of phosphate esters [23] and are consistent with earlier reports [24]. Due to their base lability, basic reaction conditions such as for deacetylations and debenzoylations are incompatible with protected phosphates, and isomer formation suggests the phosphate be introduced as late as possible in the synthesis, ideally just before deprotection. We do not follow the latter advice because it would lenghten our synthesis and could potentially lead to problems (see further).

The amine function, necessary for conjugation by dialkyl squarate chemistry, was generated at the end of the synthesis by catalytic reduction of an azide precursor (*Scheme*). A common approach to obtain the 8-azido-3,6-dioxaoctan-1-ol (=2-[2-(2-azidoethoxy)ethoxy]ethanol; **4**) linker relies on monosulfonylation of triethylene glycol (=2,2′-[ethane-1,2-diylbis(oxy)]bis[ethanol]), followed by nucleophilic displacement of the leaving group with azide ion [19][25]. The major drawback of this approach is the moderate selectivity for monoactivation of the OH groups in the starting glycol. Therefore, we opted for direct substitution of the Cl-atom in commercially available 2-[2-(2-chloroethoxy)ethoxy]ethanol (**3**), by a modified procedure of *Rensen et al.* [26]. Since monitoring of this conversion by TLC (normal or reversed phase) was difficult, it was done by GC. Purification by destillation led to significant losses due to thermal decomposition of the azide derivative **4**. Equally pure **4** was obtained more efficiently by chromatography on silica gel.

The lability of the 3-*O*-benzyl group under the conditions reported [27] for the preparation of galactosyl chloride **6** from **5** led to considerable fluctuations in yield (60–80%), especially when working on small scale. Three factors appeared to contribute to this problem, *i.e.*, the reaction temperature, the workup procedure, and, in particular, the amount of $ZnCl_2$ catalyst. To make addition of freshly fused $ZnCl_2$ to the reaction mixture more accurate, the catalyst was added from a stock solution in AcOH. This $ZnCl_2$ solution has a shelf life of at least 8 months, and we found its activity superior to the commercially available solutions in Et_2O or THF. When the reaction was carried out at room temperature instead of 60° [27] and the crude mixture was washed with an ice-cold saturated hydrogen carbonate solution before evaporation, **6** was obtained from **5** [28] in a consistent yield of 85%. Glycosidation of **6** with acceptor **4**, followed by debenzoylation of the formed 8-azido-3,6-dioxaoctyl β-D-galactoside **7**, gave triol **8**.

The most obvious sequence to convert triol **8** into disaccharide **2** starts with selective 4,6-protection, followed by O(2) glycosylation and release of the 4,6-diol to introduce the phosphate. In this way, formation of the phosphate is the last step before deprotection. However, reports that formation of six-membered cyclic phosphates is favored over polymerization [29] prompted us to explore a more straightforward approach, namely phosphorylation of **8**. From the few commercially available phosphoric dihalide reagents, 2,2,2-trichloroethyl phosphorodichloridate was selected, due to

Scheme. *Preparation of a Terminal Upstream Disaccharide Fragment* **2** *of the O-PS of* V. cholerae *O139 Designed for Conjugation by the Squaric Acid Diester Methodology*



a) NaN_3, NaI, aq. EtOH, 125°. *b*) $ZnCl_2$, Cl_2CH_2OMe, CH_2Cl_2, r.t. *c*) AgOTf, *sym*-collidine, CH_2Cl_2, r.t. *d*) K_2CO_3, MeOH, r.t. *e*) $Cl_3CCH_2OP(O)Cl_2$, pyridine, CH_2Cl_2, −15°. *f*) 1. K_2CO_3, MeOH, r.t.; 2. NaH, BnBr, DMF, 0° to r.t. *g*) Powdered 4 Å mol. sieves, AgOTf, NIS, *sym*-collidine, toluene/dioxane 3:1, 0°. *h*) Pd/C, H_2 (65 psi), ^{i}PrOH, potassium phosphate buffer (pH 7), r.t. *i*) $KHCO_3$, diethyl squarate, abs. EtOH, r.t.

the wide variety of methods for removal of the 2,2,2-trichloroethyl group [30]. From **8** and phosphorylating reagent, a 35:65 mixture (NMR) of the two phosphate isomers **9**

and **10** was obtained in virtually theoretical yield. Concomitant phosphorylation at HO–C(2) can be avoided by portionwise addition of the reagent based on monitoring by TLC. Phosphorylation this early in the sequence requires separate glycosylation of phosphates **9** and **10**, but it avoids possible problems with deprotection of a temporary 4,6-*O* protecting group in the presence of the labile colitosidic bond.

The α/β-L ratio of the glycosylation of acceptor **10** with β-L thioglycoside **12** [31] under various conditions was determined by isolation of both anomers after coupling (*Table*). Reasonable α-L-selectivity was observed in CH_2Cl_2/toluene at room temperature (*Entry 1*), yet an improved α-L-directional solvent was found in dioxane/toluene [32] (*Entry 2*). Performing the glycosylation at 0° (*Entry 3*) did not change the α/β-L ratio, but a cleaner reaction mixture was formed. Since acceptor **10** is insoluble in Et_2O, THF was tested as a substitute for dioxane for coupling at lower temperatures. However, the reaction in THF/toluene at 0° (*Entry 4*) was less selective. Iodonium dicollidine triflate (=bis(2,4,6-trimethylpyridine-κN)iodine(1+) trifluoromethanesulfonate; IDCT) [33] (*Entry 5*) gave the best anomeric selectivity, but the reaction was sluggish, and a fraction of the donor remained unchanged, even with a large excess of promoter. Although the use of the NIS/AgOTf (=*N*-iodosuccinimide/$AgOSO_2CF_3$) promoter system led to formation of *N*-succinimidyl colitoside (=2,5-dioxopyrrolidin-1-yl 3,6-dideoxy-L-*xylo*-hexoside) by-products [17], this side reaction was only minor and did not seem to affect the yield of the glycosylation. Thus, glycosylation of **10** with β-L-thioglycoside **12** in dioxane/toluene at 0° in the presence of *sym*-collidine (=2,4,6-trimethylpyridine), powdered molecular sieves, and NIS/AgOTf gave a mixture of **14*α*** and **14*β*** in 68% and 11% yield, respectively, in an overall yield of 95%, based on consumed acceptor **10**. Glycosylation of alcohol **9** with donor **12** under the same conditions furnished **13*α*** in 76% and **13*β*** in 19% yield. The lower α/β-L ratio and complete consumption of the acceptor suggest a higher reactivity of **9** compared to its phosphate isomer **10**. It is noteworthy that β-L-thioglycoside **12** slowly anomerized and decomposed, even when kept at −20°. Therefore, it is advisable to prepare it from its stable crystalline diacetate **11** [31] as required, following the procedure given in the *Exper. Part*, which is a slight modification of that reported [31].

Table. *Glycosylation of Acceptor* **10** *with* β-L-*Donor* **12** *in the Presence of Powdered 4 Å Molecular Sieves and* sym-*Collidine*

Entry	Activator[a])	Solvent (*v*/*v*)	Temperature	α/β-L
1	NIS/AgOTf	CH_2Cl_2/toluene 1:1	r.t.	4:1
2	NIS/AgOTf	dioxane/toluene 3:1	r.t.	6:1
3	NIS/AgOTf	dioxane/toluene 3:1	0°	6:1
4	NIS/AgOTf	THF/toluene 3:1	0°	3:1
5	IDCT	dioxane/toluene 3:1	0° to r.t.	8:1

[a]) NIS=*N*-Iodosuccinimide, AgOTf=$AgOSO_2CF_3$, IDCT=iodonium dicollidine triflate.

Hydrogenolysis/reduction of the benzyl ethers, the trichloroethyl group [34], and the azide group was accomplished in one step in the presence of Pd/C catalyst, but reaction conditions had to be carefully optimized. Partial decomposition of the disaccharide caused by HCl generated during cleavage of the trichloroethyl group was observed

when the reaction was carried out in EtOH/H_2O in the absence of a proton scavenger. In a mixture of EtOH and a potassium phosphate buffer (pH 7), cleavage of the glycosidic bonds was suppressed but the reaction was incomplete. At elevated temperature (50°), required to push the reaction to completion, *N*-ethylation of the formed amine was observed [35]. When ^{i}PrOH instead of EtOH was used, *N*-alkylation did not occur, but another side reaction of the amine took place, namely transamination. This led to the formation of the dimer of the desired deprotected disaccharide [36]. The mechanism of dimerization involves dehydrogenation and release of ammonia [37], suggesting this side reaction could be minimized under higher H_2 pressure. Indeed, at room temperature and 60–70 psi H_2 pressure, a reasonably clean reaction was observed (TLC), and amino derivative **15** was isolated in 65–70% yield. The two phosphate isomers **13*α*** and **14*α*** gave the same material **15** (^{1}H-NMR, ^{13}C-NMR, ^{31}P-NMR, TLC) after deprotection.

Amino derivative **15** displayed an atypical reactivity toward squaric acid diesters [19] in that nucleophilicity seemed subdued at slightly acidic and neutral pH, possibly by internal salt formation with the phosphate. This had important consequences for purification and derivatization. Purification could be accomplished on regular silica gel without addition of a base to the solvent because, apparently, little protonation of the amine by the acidic silanol groups occurred. Derivatization required slightly basic conditions to be efficient. Formation of squarate **2**, *e.g.*, was sluggish in a pH 7 potassium phosphate buffer or in anhydrous EtOH, the two classic methods [19]. In anhydrous EtOH in the presence of solid $KHCO_3$ [38], however, the reaction proceeded smoothly with smaller excess of reagent. The slightly basic conditions led to some hydrolysis of the formed monoethyl squarate, but **2** was obtained in excellent yield (90–95%).

No elemental analyses or optical rotations were collected for the disaccharide phosphate salts **15** and **2**. The structure of these compounds follows unequivocally from the mode of synthesis and the *m/z* values found in their low- and high-resolution mass spectra, and their purity was verified by TLC and NMR spectroscopy.

This research was supported by the Intramural Research Program of the *NIH*, NIDDK.

Experimental Part

General. HPLC-Grade solvents were used, and reactions requiring anh. conditions were carried out under N_2 or Ar. A *Parr* hydrogenator was used for reactions under H_2. Dioxane was distilled from Na/benzophenone, and toluene from Na. *N*-Iodosuccinimide was recrystallized from dioxane/CCl_4 and stored in a desiccator. Dichloromethyl methyl ether (Cl_2CH_2OMe), 2,2,2-trichloroethyl phosphorodichloridate ($d \approx 1.7$ g/ml at 20°, density roughly determined by differential weighing of 1 ml of reagent) and 2-[2-(2-chloroethoxy)ethoxy]ethanol (**3**) were purchased from *Aldrich* and used as provided. *Strata-SPE-C18* cartridges were purchased from *Phenomenex*. The 5% Pd/C catalyst (*Escat 103*) was a product of *Engelhard Industries*. *sym*-Collidine was stored over KOH pellets, pyridine over CaH_2. Conversion of **3** to **4** was monitored by GC, all other reactions by TLC. Unless stated otherwise, solns. in org. solvents were dried with anh. Na_2SO_4 and concentrated at *ca.* 40°/2 kPa. GC: *Finnigan GCQ* gas chromatograph/mass spectrometer equipped with a *ZB-5* column (5% phenyl, 95% dimethyl polysiloxane, 30 m; 100° to 280°, 10°/min). TLC: silica gel *60* glass slides; visualized by heating after treatment with 5% H_2SO_4 in EtOH (*v*/*v*) or 5% ninhydrine in EtOH (*w*/*v*) for primary amines. Column chromatography (CC): for purification of **6**, silica gel *60* was dried overnight at 160° and cooled under a stream of Ar.

M.p.: *Kofler* hot stage. $[\alpha]_D$: *Perkin-Elmer 341* automatic polarimeter. NMR Spectra: *Varian Mercury* spectrometer, at 300 MHz for ^{1}H, 75 MHz for ^{13}C, and 121 MHz for ^{31}P; *Bruker Avance* spectrometer, at 600 MHz for ^{1}H and 150 MHz for ^{13}C; assignments by first-order analysis and, when feasible, supported by homonuclear decoupling experiments or homonuclear and heteronuclear 2-dimensional correlation spectroscopy, run with the software supplied with the spectrometers; chemical shifts δ in ppm rel. to $SiMe_4$ as internal standard (^{1}H,^{13}C) or rel. to H_3PO_4 in D_2O as an internal standard (stem coaxial insert; ^{31}P), coupling constants J in Hz. Arbitrary primed atom numbering (C(1′), C(2′), C(4′), C(5′), C(6′)) for the 8-azido-3,6-dioxaoctyl spacer, and serial disaccharides numbering with superscripts I (unit bearing the aglycon) and II; J(P,C) was observed for all phosphates; some ^{13}C-NMR signals of the squaric acid derivative **2** were split due to the effect of the vinylogous amide group [19].

8-Azido-3,6-dioxaoctan-1-ol (=2-[2-(2-Azidoethoxy)ethoxy]ethanol; **4**). A mixture of 2-[2-(2-chloroethoxy)ethoxy]ethanol (**3**; (42.015 g, 250 mmol), NaN_3 (24.400 g, 375 mmol), NaI (3.750 g, 25 mmol), and 95% aq. EtOH (250 ml) was heated in a closed vessel at 125°. After 24 h (GC monitoring), the mixture was filtered and the precipitate washed with 95% aq. EtOH (3×100 ml). The combined filtrate was evaporated and the residue treated with Et_2O. The precipitate was filtered off, washed several times with Et_2O, and the combined filtrate evaporated. The residue was subjected to CC (petroleum ether/acetone 3:1): **4** (38.185 g, 87%). Sligthly yellow liquid.

2,4,6-Tri-O-*benzoyl-3*-O-*benzyl-α*-D-*galactopyranosyl Chloride* (**6**). A soln. of freshly fused $ZnCl_2$ in AcOH (0.62M; 40 µl, 3.4 mg, 24.8 µmol) was added to a stirred soln. of **5** [28] (2.0 g, 3.355 mmol) in a mixture of CH_2Cl_2 (6 ml) and Cl_2CH_2OMe (6 ml). The flask was equipped with a drying tube, and the reaction was monitored by TLC while stirring was continued at r.t. After 2–5 h, the mixture was diluted with CH_2Cl_2 (100 ml) and washed with an ice-cold, sat. $NaHCO_3$ soln. (100 ml) and H_2O (100 ml). The org. layer was dried and evaporated at r.t. and the residue subjected to CC ($CHCl_3$/AcOEt 99:1): **6** (1.713 g, 85%). Foam.

2-[2-(2-Azidoethoxy)ethoxy]ethyl 2,4,6-Tri-O-*benzoyl-3*-O-*benzyl-β*-D-*galactopyranoside* (**7**). A soln. of **6** (1.7 g, 2.833 mmol) and *sym*-collidine (375 µl, 2.833 mmol) in CH_2Cl_2 (22.5 ml) was added to a stirred mixture of AgOTf (800 mg, 3.116 mmol) and **4** (496 mg, 2.833 mmol) in CH_2Cl_2 (7.5 ml). Stirring was continued in the dark. After 2 h, the mixture was treated with Et_3N, filtered through a *Celite* pad, and the solids were washed with CH_2Cl_2. The combined filtrate (*ca.* 100 ml) was washed with 10% aq. $Na_2S_2O_3$ soln. (100 ml) and H_2O (100 ml), dried, and concentrated, and the residue was subjected to CC (hexane/AcOEt 3:1): **7** (1.771 g, 85%). Colorless oil. $[\alpha]_D^{25} = +251.3$ ($c = 0.515$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 300 MHz): 8.14–8.20 (*m*, 2 H); 7.97–8.08 (*m*, 4 H); 7.53–7.64 (*m*, 3 H); 7.40–7.52 (*m*, 6 H); 7.04–7.20 (*m*, 5 H, *Ph*CH_2O–C(3)); 5.93 (br. *d*, J=2.8, H–C(4)); 5.56 (*dd*, J=8.1, 10.1, H–C(2)); 4.72 (*d*, J=8.1, partial overlap, H–C(1)); 4.71 (*d*, J=12.6, partial overlap, 1 H, PhCH_2O); 4.62 (*dd*, J=6.9, 11.3, H_a–C(6)); 4.52 (*d*, J=12.6, 1 H, PhCH_2O); 4.44 (*dd*, J=6.0, 11.3, H_b–C(6)); 4.10 (br. *t*, J=6.5, H–C(5)); 4.00 (*dt*, J=4.1, 11.3, H_a–C′(1)); 3.83 (*dd*, J=3.4, 10.1, H–C(3)); 3.75 (*ddd*, J=3.9, 7.2, 11.3, H_b–C(1′)); 3.56–3.63 (*m*, 2 H); 3.42–3.52 (*m*, 4 H); 3.30–3.37 (*m*, 2 H); 3.23–3.30 (*m*, 2 H, CH_2N_3). ^{13}C-NMR ($CDCl_3$, 75 MHz): 166.06, 165.78, 165.06 (3 Ph*C*O); 137.13; 133.34; 133.19; 133.0; 130.02 (2 C); 129.86; 129.76 (2 C); 129.66 (2 C); 129.47; 129.26; 128.43 (2 C); 128.39 (2 C); 128.24 (2 C); 128.15 (2 C); 127.83 (2 C); 127.58; 101.53 (C(1)); 76.06 (C(3)); 71.26 (C(5)); 71.08 (C(2)); 70.86 (Ph*C*H_2); 70.53, 70.35, 70.22, 69.73, 69.21 (C(1′), C(2′), C(3′), C(4′), C(5′)); 66.57 (C(4)); 62.52 (C(6)); 50.45 (CH_2N_3). ES-TOF-MS (pos.): 757.3 ($[M+NH_4]^+$), 762.3 ($[M+Na]^+$). Anal. calc. for $C_{40}H_{41}N_3O_{11}$ (739.27): C 64.94, H 5.59, N 5.68; found: C 65.01, H 5.59, N 5.70.

2-[2-(2-Azidoethoxy)ethoxy]ethyl 3-O-*Benzyl-β*-D-*galactopyranoside* (**8**). Anh. K_2CO_3 (331 mg, 2.394 mmol) was added to a stirred soln. of **7** (1.770 g, 2.394 mmol) in MeOH (25 ml). When TLC indicated complete debenzoylation (*ca.* 8 h), the mixture was neutralized with *IR-120*(H^+) resin. The resin was filtered off and washed with MeOH, the combined filtrate concentrated, and the residue subjected to CC (CH_2Cl_2/MeOH 97:3): **8** (975 mg, 95%). Colorless oil. $[\alpha]_D^{25} = -31.5$ ($c = 0.54$, $CHCl_3$). ^{1}H-NMR (C_5D_5N, 300 MHz): 7.54–7.62 (*m*, 2 H); 7.26–7.37 (*m*, 3 H); 5.03, 4.98 (2*d*, J=12.1, PhCH_2); 4.80 (*d*, J=7.7, H–C(1)); 4.68 (br. *d*, J=3.3, H–C(4)); 4.57 (*dd*, J=7.7, 9.3, H–C(2)); 4.48 (*dd*, J=6.3, 11.0, partial overlap, H_a–C(6)); 4.43 (*dd*, J=6.1, 11.0, partial overlap, H_b–C(6)); 4.26 (*dt*, J=5.0, 10.4, H_a–C(1′)); 3.99 (br. *t*, J=6.2, partial overlap, H–C(5)); 3.93 (*dt*, J=5.1, 10.4, partial overlap, H_b–C(1′)); 3.87 (*dd*, J=3.3, 9.3, partial overlap, H–C(3)); 3.72 (br. *t*, J=5.1, 2 H); 3.56–3.67 (*m*, 6 H); 3.33 (br. *t*, J=5.0,

CH_2N_3). ^{13}C-NMR (C_5D_5N, 75 MHz): 140.38; 129.03 (2 C); 128.62 (2 C); 128.09; 105.78 (C(1)); 83.55 (C(3)); 77.21 (C(5)); 72.38 (Ph*C*H_2); 71.86 (C(2)); 71.27, 71.25, 71.14, 70.61, 69.39 (C(1′), C(2′), C(3′), C(4′), C(5′)); 67.36 (C(4)); 62.66 (C(6)); 51.38 (CH_2N_3). ES-TOF-MS (pos.): 450.1 ([M+Na]$^+$). Anal. calc. for $C_{19}H_{29}N_3O_8$ (427.20): C 53.39, H 6.84, N 9.83; found: C 53.68, H 6.95, N 9.73.

*(2-[2-(2-Azidoethoxy)ethoxy]ethyl 3-*O*-benzyl-β-*D*-galactopyranoside Cyclic [P(*R*)]-4,6-(2,2,2-Trichloroethyl Phosphate*) (**9**) *and 2-[2-(2-Azidoethoxy)ethoxy]ethyl 3-*O*-Benzyl-β-*D*-galactopyranoside Cyclic [P(*S*)]-4,6-(2,2,2-Trichloroethyl Phosphate*) (**10**). The 2,2,2-trichloroethyl phosphorodichloridate (425 µl, 2.711 mmol) was added dropwise to a cooled (−15°; ice/acetone bath) and stirred soln. of **8** (965 mg, 2.259 mmol) and pyridine (1.83 ml, 22.590 mmol) in CH_2Cl_2 (25 ml). After 5 min, TLC indicated incomplete reaction, and more 2,2,2-trichloroethyl phosphorodichloridate (212 µl, 1.356 mmol) was added while stirring was continued at −15°. One additional portion of reagent (106 µl, 0.678 mmol) was required to drive the reaction to completion. The reaction was quenched with MeOH (1 ml), the mixture concentrated, and the residue treated with AcOEt. The precipitate was filtered off and washed with AcOEt, the combined filtrate concentrated, and the residue chromatographed (hexane/acetone 65:35 → 1:1): **9** followed by **10**. Minor impurities in both fractions were removed by CC (*Strata C18 SPE*, MeOH/H_2O 1:1): pure **9** (460 mg, 33%) and **10** (854 mg, 61%).

Data for **9**: White solid. M.p. 67.8–68.5° (CH_2Cl_2/Et_2O). $[\alpha]_D^{25} = -3.6$ (c = 0.56, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 600 MHz): 7.36–7.39 (*m*, 2 H_o); 7.32–7.36 (*m*, 2 H_m); 7.27–7.31 (*m*, 1 H_p); 4.79 (br. *d*, J = 3.1, partial overlap, H−C(4)); 4.78, 4.75 (2*d*, J = 12.2, partial overlap, PhCH_2); 4.61 (*d*, J = 6.9, CCl_3CH_2); 4.53 (br. *d*, J = 1.4, partial overlap, H_a−C(6)); 4.51 (*ddd*, J = 1.4, 12.3, 31.4, partial overlap, H_b−C(6)); 4.40 (*d*, J = 7.9, H−C(1)); 4.05 (*dt*, J = 4.3, 11.3, H_a−C(1′)); 3.96 (*dd*, J = 7.9, 9.7, H−C(2)); 3.77 (*dt*, J = 4.9, 11.3, H_b−C(1′)); 3.64–3.75 (*m*, CH_2(2′), C(3′), C(4′), C(5′)); 3.55 (br. *s*, partial overlap, H−C(5)); 3.53 (*ddd*, J = 3.3, 4.3, 9.7, partial overlap, H−C(3)); 3.40 (*dt*, J = 5.2, 13.2, partial overlap, CH_aN_3); 3.37 (*dt*, J = 5.0, 13.2, partial overlap, CH_bN_3). ^{13}C-NMR ($CDCl_3$, 150 MHz): 137.61; 128.42 (2 C); 127.84; 127.66 (2 C); 103.21 (C(1)); 94.99 (*d*, J = 10.2, CCl_3); 77.53 (*d*, J = 7.2, C(3)); 76.73 (*d*, J = 4.2, CCl_3CH_2); 76.43 (*d*, J = 7.2, C(4)); 72.02 (Ph*C*H_2); 70.79 (*d*, J = 7.7, C(6)); 70.44, 70.40, 70.15, 69.86, 68.37 (C(1′), C/2′), C(3′), C(4′), C(5′)); 69.37 (C(2)); 66.13 (*d*, J = 6.6, C(5)); 50.52 (CH_2N_3). ^{31}P-NMR ($CDCl_3$): −11.18. ES-TOF-MS (pos.): 620, 622, 624 ([M+H]$^+$), 642, 644, 646 ([M+Na]$^+$). Anal. calc. for $C_{21}H_{29}Cl_3N_3O_{10}P$ (619.07): C 40.63, H 4.71, N 6.77; found: C 40.70, H 4.58, N 6.69.

Data for **10**: Slightly yellow oil. $[\alpha]_D^{25} = +9.2$ (c = 0.53, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 600 MHz): 7.38–7.41 (*m*, 2 H_o); 7.33–7.37 (*m*, 2 H_m); 7.27–7.31 (*m*, 1 H_p); 4.95 (br. *d*, J = 3.0, H−C(4)); 4.81, 4.71 (2*d*, J = 12.0, PhCH_2); 4.67 (br. *dt*, J = 2.7, 12.3, H_a−C(6)); 4.63 (*dd*, J = 1.1, 7.4, CCl_3CH_2); 4.47 (*ddd*, J = 1.6, 12.3, 20.8, H_b−C(6)); 4.41 (*d*, J = 7.9, H−C(1)); 4.04 (*dt*, J = 4.3, 11.3, H_a−C(1′)); 3.88 (*dd*, J = 7.9, 9.7, H−C(2)); 3.77 (*m*, H_b−C(1′)); 3.61–3.72 (*m*, CH_2(2′), CH_2(3′), CH_2(4′), CH_2(5′)); 3.57 (br. *d*, J = 1.1, H−C(5)); 3.53 (*ddd*, J = 3.2, 4.3, 9.7, H−C(3)); 3.39 (*dt*, J = 4.9, 13.3, partial overlap, CH_aN_3); 3.36 (*dt*, J = 5.1, 13.3, partial overlap, CH_bN_3); 3.15 (br. *s*, HO−C(2)). ^{13}C-NMR ($CDCl_3$, 150 MHz): 137.26; 128.48 (2 C); 127.94; 127.86 (2 C); 103.19 (C(1)); 94.46 (*d*, J = 10.9, CCl_3); 77.65 (*d*, J = 5.3, CCl_3CH_2); 77.42 (*d*, J = 7.0, C(3)); 74.96 (*d*, J = 5.3, C(4)); 71.62 (Ph*C*H_2); 70.49, 70.42, 70.17, 69.91, 68.59 (C(1′), C(2′), C(3′), C(4′), C(5′)); 70.16 (*d*, J = 5.8, partial overlap, C(6)); 69.53 (C(2)); 66.53 (*d*, J = 7.0, C(5)); 50.51 (CH_2N_3). ^{31}P-NMR ($CDCl_3$): −8.82. ES-TOF-MS (pos.): 620, 622, 624 ([M+H]$^+$), 642, 644, 646 ([M+Na]$^+$). Anal. calc. for $C_{21}H_{29}Cl_3N_3O_{10}P$ (619.07): C 40.63, H 4.71, N 6.77; found: C 40.79, H 4.80, N 6.72.

*Ethyl 2,4-Di-*O*-benzyl-3,6-dideoxy-1-thio-β-*L*-*xylo*-hexopyranoside* (**12**). Anh. K_2CO_3 (125 mg, 0.906 mmol) was added to a stirred soln. of **11** [31](1.0 g, 3.622 mmol) in MeOH (14.5 ml). After 16 h, the mixture was neutralized with *IR-120*(H$^+$) resin. The resin was filtered off and washed with MeOH, the combined filtrate concentrated, and the residue concentrated from MeCN (3×25 ml), and dried. NaH (60% dispersion in mineral oil; 435 mg, 10.866 mmol) was added with stirring at 0° to a soln. of the intermediate diol in DMF (14.5 ml), followed by BnBr (1.3 ml, 10.866 mmol). The cooling was removed and, after 2 h, MeOH (0.5 ml) was added to destroy excess of BnBr. The mixture was partitioned between Et_2O (100 ml) and H_2O (100 ml), the aq. phase extracted with Et_2O (2×100 ml), the combined org. phase washed with brine (100 ml), dried, and concentrated, and the residue chromatographed (hexane/AcOEt 95:5): **12** (1.296 g, 96%). Nearly colorless oil. The α-L-anomer, formed by slow anomerization, can be separated from **12** by chromatography (hexane/acetone 95:5).

*Data for Ethyl 2,4-Di-*O*-benzyl-3,6-dideoxy-1-thio-α-*L*-xylo-hexopyranoside*: ^{1}H-NMR ($CDCl_3$, 300 MHz): 7.26–7.40 (*m*, 10 H); 5.50 (br. *d*, *J* = 4.9, H–C(1)); 4.67, 4.47 (2*d*, *J* = 11.7, PhCH_2); 4.62, 4.42 (2*d*, *J* = 12.1, PhCH_2); 4.24 (*dq*, *J* = 1.6, 6.6, H–C(5)); 4.13 (*dt*, *J* = 4.7, 12.1, H–C(2)); 3.45 (br. *s*, H–C(4)); 2.62 (*dq*, *J* = 7.4, 12.9, partial overlap, 1 H, MeCH_2S); 2.54 (*dq*, *J* = 7.4, 12.9, partial overlap, 1 H, MeCH_2S); 2.16 (*dddd*, *J* = 1.4, 4.5, 4.5, 13.7, H_{eq}–C(3)); 1.76 (*ddd*, *J* = 2.7, 12.1, 13.7, H_{ax}–C(3)); 1.30 (*t*, *J* = 7.4, *Me*CH_2S); 1.19 (*d*, *J* = 6.6, Me(6)). ^{13}C-NMR ($CDCl_3$, 75 MHz): 138.22; 137.99; 128.31 (2 C); 128.23 (2 C); 127.87 (2 C); 127.75 (2 C); 127.65; 127.55; 83.91 (C(1)); 75.31 (C(4)); 71.12, 70.56 (2 PhCH_2); 70.43 (C(2)); 66.16 (C(5)); 29.63 (C(3)); 23.45 (MeCH_2S); 16.29 (C(6)); 15.00 (*Me*CH_2S).

*2-[2-(2-Azidoethoxy)ethoxy]ethyl 3-*O*-Benzyl-2-*O*-(2,4-di-*O*-benzyl-3,6-dideoxy-α-*L*-xylo-hexopyranosyl)-β-*D*-galactopyranoside Cyclic [P(*R*)]-4,6-(2,2,2-Trichloroethyl Phosphate)* (**13*α***). Powdered 4 Å molecular sieves (1.570 g) were added to a stirred soln. of **9** (485 mg, 0.783 mmol), **12** (438 mg, 1.175 mmol), and *sym*-collidine (260 µl, 1.959 mmol) in 31.5 ml of dioxane/toluene (*v*/*v*; 3 : 1). After 30 min, the flask was covered with aluminium foil, AgOTf (353 mg, 1.371 mmol) was added, and the mixture was cooled to 0°. NIS (309 mg, 1.371 mmol) was added (5 portions in 15-min intervals). Stirring was continued for 1 h at 0°, and the mixture was filtered through a *Celite* pad, directly into. 10% aq. $Na_2S_2O_3$ soln./sat. aq. $NaHCO_3$ soln. 2 : 1 (*v*/*v*; 150 ml). The solids were washed with CH_2Cl_2 (200 ml) and the layers were separated. The aq. phase was extracted with CH_2Cl_2 (2 × 100 ml) and the combined org. layer dried and evaporated. The residue was subjected to CC (petroleum ether/acetone 4 : 1 → 3 : 2): **13*β*** (138 mg, 19%) followed by **13*α*** (553 mg, 76%).

Data for **13*α***: Nearly colorless oil. $[\alpha]_D^{25} = -42.6$ (*c* = 0.51, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 600 MHz): 7.21–7.32 (*m*, 13 H); 7.14 (*m*, 2 H); 5.48 (*d*, *J* = 3.1, H–C(1^{II})); 4.84 (*d*, *J* = 3.1, H–C(4^{I})); 4.75 (*d*, *J* = 11.6, 1 H, PhCH_2); 4.56–4.61 (*m*, 4 H, H–C(1^{I}), PhCH_2, CCl_3CH_2); 4.47–4.55 (*ddd*, *J* = 1.6, 12.2, 26.9, partial overlap, H_a–C(6^{I})); 4.53 (*m*, H_b–C(6^{I})); 4.52, 4.33 (2*d*, *J* = 12.3, PhCH_2); 4.49, 4.29 (2*d*, *J* = 12.3, PhCH_2); 4.35 (*dq*, *J* = 1.7, 6.5, partial overlap, H–C(5^{II})); 4.21 (*dd*, *J* = 7.8, 9.4, H–C(2^{I})); 3.99 (*ddd*, *J* = 4.1, 5.4, 10.5, H_a–C(1′)); 3.81 (br. *dt*, *J* = 4.0, 12.2, H–C(2^{II})); 3.77 (*m*, H–C(3^{I})); 3.70 (*ddd*, *J* = 4.2, 6.5, 10.5, H_b–C(1′)); 3.55–3.67 (*m*, CH_2(2′), CH_2(3′), CH_2(4′), CH_2(5′)); 3.54 (br. *s*, H–C(5^{I})); 3.38 (br. *s*, H–C(4^{II})); 3.35 (br. *t*, *J* = 5.1, CH_2N_3); 2.07 (br. *dt*, *J* = 4.1, 13.0, H_{eq}–C(3^{II})); 1.84 (*dt*, *J* = 2.3, 13.0, H_{ax}–C(3^{II})); 1.15 (*d*, *J* = 6.5, Me(6^{II})). ^{13}C-NMR ($CDCl_3$, 150 MHz): 138.25; 138.01; 137.27; 128.31 (2 C); 128.09 (2 C); 128.08 (2 C); 127.69 (2 C); 127.60 (2 C); 127.58; 127.40; 127.38; 126.89 (2 C); 101.59 (C(1^{I})); 96.40 (C(1^{II})); 94.89 (*d*, *J* = 10.3, CCl_3); 80.00 (*d*, *J* = 7.2, C(3^{I})); 76.64 (*d*, *J* = 4.3, CCl_3CH_2); 75.53 (C(4^{II})); 75.37 (*d*, *J* = 7.0, C(4^{I})); 71.06 (PhCH_2); 71.00 (C(2^{I})); 70.77 (PhCH_2); 70.74 (*d*, *J* = 7.9, partial overlap, C(6^{I})); 70.57 (PhCH_2); 70.43, 70.30, 70.09, 69.80 (C(2′), C(3′), C(4′), C(5′)); 68.23 (C′(1)); 65.94 (C(5^{II})); 65.67 (*d*, *J* = 6.7, C(5^{I})); 50.46 (CH_2N_3); 27.23 (C(3^{II})); 16.28 (C(6^{II})). ^{31}P-NMR ($CDCl_3$): −11.25. ES-TOF-MS (pos.): 952.2, 954.2, 956.2 ([*M* + Na]$^+$). Anal. calc. for $C_{41}H_{51}Cl_3N_3O_{13}P$ (929.22): C 52.88, H 5.52, N 4.51; found: C 52.98, H 5.26, N 4.56.

*Data for 2-[2-(2-Azidoethoxy)ethoxy]ethyl 3-*O*-Benzyl-2-*O*-(2,4-di-*O*-benzyl-3,6-dideoxy-β-*L*-xylo-hexopyranosyl)-β-*D*-galactopyranoside Cyclic [P(*R*)]-4,6-(2,2,2-Trichloroethyl Phosphate)* (**13*β***): Colorless oil. ^{1}H-NMR ($CDCl_3$, 600 MHz): 7.42 (*m*, 2 H); 7.21–7.34 (*m*, 13 H); 4.88, 4.79 (2*d*, *J* = 12.0, PhCH_2); 4.84 (*d*, *J* = 7.6, H–C(1^{II})); 4.80 (br. *d*, *J* = 3.6, partial overlap, H–C(4^{I})); 4.78, 4.58 (2*d*, *J* = 11.9, PhCH_2); 4.62 (*dd*, *J* = 6.6, 11.4, partial overlap, 1 H, CCl_3CH_2); 4.60 (*dd*, *J* = 6.9, 11.4, partial overlap, 1 H, CCl_3CH_2); 4.59, 4.42 (2*d*, *J* = 12.0, PhCH_2); 4.46–4.55 (*ddd*, *J* = 1.7, 12.4, 31.6, partial overlap, H_a–C(6^{I})); 4.52 (*m*, partial overlap, H_b–C(6^{I})); 4.43 (*d*, *J* = 7.8, partial overlap, H–C(1^{I})); 4.23 (*dd*, *J* = 7.8, 9.5, H–C(2^{I})); 3.93 (*ddd*, *J* = 4.3, 6.3, 10.7, H_a–C(1′)); 3.53–3.69 (*m*, H–C(3^{I}), H–C(2^{II}), H–C(5^{II}), H_b–C(1′), CH_2(2′), CH_2(3′), CH_2(4′), CH_2(5′)); 3.51 (br. *d*, *J* = 1.4, H–C(5^{I})); 3.35 (*m*, H–C(4^{II}), CH_2N_3); 2.36 (*ddd*, *J* = 3.1, 4.8, 13.8, H_{eq}–C(3^{II})); 1.48 (*ddd*, *J* = 2.8, 11.7, 13.8, H_{ax}–C(3^{II})); 1.23 (*d*, *J* = 6.4, Me(6^{II})). ^{13}C-NMR ($CDCl_3$, 150 MHz): 139.18; 138.58; 138.15; 128.16 (6 C); 127.80 (2 C); 127.54 (2 C); 127.49 (2 C); 127.44; 127.36; 127.30; 104.10 (C(1^{II})); 103.46 (C(1^{I})); 95.01 (*d*, *J* = 10.8, CCl_3); 77.34 (*d*, *J* = 7.1, C(4^{I})); 76.84 (*d*, *J* = 7.2, partial overlap, C(3^{I})); 76.71 (*d*, *J* = 4.2, CCl_3CH_2); 75.44 (C(4^{II})); 74.43 (C(2^{I})); 73.83 (C(2^{II})); 73.34 (C(5^{II})); 72.71, 72.43, 71.08 (3 PhCH_2); 70.66 (*d*, *J* = 7.6, C(6^{I})); 70.52, 70.39, 70.16, 69.84 (C(2′), C(3′), C(4′), C(5′)); 69.04 (C(1′)); 65.88 (*d*, *J* = 6.7, C(5^{I})); 50.57 (CH_2N_3); 33.00 (C(3^{II})); 16.66 (C(6^{II})). ^{31}P-NMR ($CDCl_3$): −11.41. ES-TOF-MS (pos.): 902.1, 904.1, 906.1 ([*M* − N_2 + H]$^+$); 954.1, 956.1, 958.1 ([*M* + Na]$^+$).

*2-[2-(2-Azidoethoxy)ethoxy]ethyl 3-*O*-Benzyl-2-*O*-(2,4-di-*O*-benzyl-3,6-dideoxy-α-*L*-xylo-hexopyranosyl)-β-*D*-galactopyranoside Cyclic [P(S)]-4,6-(2,2,2-Trichloroethyl Phosphate)* (**14*α***). As described for **13*α***, with molecular sieves (4.120 g), **10** (1.275 g, 2.060 mmol), **12** (1.151 g, 3.090 mmol), *sym*-collidine (681 µl, 5.150 mmol), dioxane/toluene 3:1 (82.5 ml), AgOTf (926 mg, 3.605 mmol), and NIS (811 mg, 3.605 mmol). CC (CH_2Cl_2/acetone 10:1 → 2:1) gave **14*β*** (210 mg, 11%), then **14*α*** (1.306 g, 68%), followed by unreacted **10** (204 mg, 16%).

Data for **14*α***: Nearly colorless oil. $[\alpha]_D^{25} = -9.1$ ($c = 0.58$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 600 MHz): 7.22–7.32 (*m*, 13 H); 7.12–7.16 (*m*, 2 H); 5.49 (*d*, $J = 3.4$, H–C(1^{II})); 4.97 (*d*, $J = 3.1$, H–C(4^I)); 4.80, 4.53 (2*d*, $J = 11.5$, PhCH_2); 4.70 (*dd*, $J = 7.7$, 11.4, 1 H, CCl_3CH_2); 4.65 (*m*, partial overlap, H_a–C(6^I)); 4.64 (*dd*, $J = 7.7$, 11.4, partial overlap, 1 H, CCl_3CH_2); 4.60 (*d*, $J = 7.9$, H–C(1^I)); 4.52, 4.33 (2*d*, $J = 12.2$, PhCH_2); 4.49, 4.28 (2*d*, $J = 12.3$, PhCH_2); 4.48 (*ddd*, $J = 1.6$, 12.1, *ca.* 21.0, partial overlap, H_b–C(6^I)); 4.35 (*dq*, $J = 1.2$, 6.5, partial overlap, H–C(5^{II})); 4.13 (*dd*, $J = 7.9$, 9.4, H–C(2^I)); 3.99 (*dt*, $J = 4.8$, 10.8, H_a–C(1′)); 3.77–3.83 (*m*, H–C(3^I), H–C(2^{II})); 3.71 (*ddd*, $J = 4.9$, 6.1, 10.8, H_b–C(1′)); 3.50–3.65 (*m*, H–C(5^I), CH_2(2′), CH_2(3′), CH_2(4′), CH_2(5′)); 3.38 (br. *s*, H–C(4^{II})); 3.34 (*t*, $J = 5.0$, CH_2N_3); 2.08 (br. *dt*, $J = 3.8$, 12.9, H_{eq}–C(3^{II})); 1.83 (*dt*, $J = 2.4$, 12.9, H_{ax}–C(3^{II})); 1.13 (*d*, $J = 6.5$, Me(6^{II})). ^{13}C-NMR ($CDCl_3$, 150 MHz): 138.28; 138.03; 137.08; 128.38 (2 C); 128.19 (2 C); 128.16 (2 C); 127.74 (2 C); 127.69 (3 C); 127.52; 127.47; 127.17 (2 C); 101.58 (C(1^I)); 96.45 (C(1^{II})); 94.50 (*d*, $J = 10.7$, CCl_3); 79.93 (*d*, $J = 6.9$, C(3^I)); 77.68 (*d*, $J = 5.4$, CCl_3CH_2); 75.51 (C(4^{II})); 74.28 (*d*, $J = 5.6$, C(4^I)); 71.13 (C(2^I)); 70.93, 70.82, 70.67 (3 Ph*C*H_2); 70.55, 70.38, 70.21, 69.93 (C(2′), C(3′), C(4′), C(5′)); 70.25 (C(2^{II})); 70.19 (*d*, $J = 7.1$, partial overlap, C(6^I)); 68.38 (C(1′)); 66.06 (*d*, $J = 5.0$, partial overlap, C(5^I)); 66.04 (C(5^{II})); 50.52 (CH_2N_3); 27.32 (C(3^{II})); 16.33 (C(6^{II})). ^{31}P-NMR ($CDCl_3$): −8.96. ES-TOF-MS (pos.): 952.1, 954.1, 956.1 ($[M+Na]^+$). Anal. calc. for $C_{41}H_{51}Cl_3N_3O_{13}P$ (929.22): C 52.88, H 5.52, N 4.51; found: C 53.06, H 5.55, N 4.51.

*Data for 2-[2-(2-Azidoethoxy)ethoxy]ethyl 3-*O*-Benzyl-2-*O*-(2,4-di-*O*-benzyl-3,6-dideoxy-β-*L*-xylo-hexopyranosyl)-β-*D*-galactopyranoside Cyclic [P(S)]-4,6-(2,2,2-Trichloroethyl Phosphate)* (**14*β***): Nearly colorless oil. ^{1}H-NMR ($CDCl_3$, 600 MHz): 7.43–7.46 (*m*, 2 H); 7.20–7.33 (*m*, 13 H); 4.93 (*d*, $J = 3.2$, H–C(4^I)); 4.84 (*d*, $J = 7.6$, partial overlap, H–C(1^{II})); 4.83, 4.80 (2*d*, $J = 11.9$, PhCH_2); 4.77, 4.57 (2*d*, $J = 11.8$, PhCH_2); 4.69 (*dd*, $J = 7.6$, 11.4, 1 H, CCl_3CH_2); 4.64 (*dt*, $J = 2.8$, 12.2, H_a–C(6^I)); 4.61 (*dd*, $J = 7.6$, 11.4, partial overlap, 1 H, CCl_3CH_2); 4.59, 4.41 (2*d*, $J = 12.0$, PhCH_2); 4.47 (*ddd*, $J = 1.8$, 12.2, 20.8, partial overlap, H_b–C(6^I)); 4.45 (*d*, $J = 7.7$, partial overlap, H–C(1^I)); 4.14 (*dd*, $J = 7.7$, 9.3, H–C(2^I)); 3.94 (*ddd*, $J = 4.3$, 5.9, 10.5, H_a–C(1′)); 3.68 (*ddd*, $J = 4.2$, 6.0, 10.5, H_b–C(1′)); 3.50–3.66 (*m*, H–C(3^I), H–C(5^I), H–C(2^{II}), H–C(5^{II}), CH_2(2′), CH_2(3′), CH_2(4′), CH_2(5′)); 3.34 (br. *s*, H–C(4^{II})); 3.32 (*t*, $J = 5.1$, CH_2N_3); 2.36 (*ddd*, $J = 3.2$, 4.8, 14.0, H_{eq}–C(3^{II})); 1.46 (*ddd*, $J = 2.7$, 11.5, 14.0, H_{ax}–C(3^{II})); 1.23 (*d*, $J = 6.4$, Me(6^{II})). ^{13}C-NMR ($CDCl_3$, 150 MHz): 139.12; 138.51; 137.79; 128.18 (2 C); 128.17 (2 C); 128.16 (2 C); 127.91 (2 C); 127.56 (2 C); 127.52 (2 C); 127.50; 127.40; 127.33; 103.91 (C(1^{II})); 103.33 (C(1^I)); 94.58 (*d*, $J = 10.6$, CCl_3); 77.62 (*d*, $J = 5.3$, CCl_3CH_2); 76.90 (*d*, $J = 7.0$, C(3^I)); 75.91 (*d*, $J = 5.3$, C(4^I)); 75.34 (C(4^{II})); 74.43 (C(2^I)); 73.85 (C(2^{II})); 73.33 (C(5^{II})); 72.80, 72.24, 71.08 (3 Ph*C*H_2); 70.55, 70.33, 69.86 (C(3′), C(4′), C(5′)); 70.18 (C(2′)); 70.01 (*d*, $J = 6.0$, C(6^I)); 69.01 (C(1′)); 66.18 (*d*, $J = 7.0$, C(5^I)); 50.53 (CH_2N_3); 32.97 (C(3^{II})); 16.72 (C(6^{II})). ^{31}P-NMR ($CDCl_3$): −8.56. ES-TOF-MS (pos.): 952.2, 954.2, 956.2 ($[M+Na]^+$).

*2-[2-(2-Aminoethoxy)ethoxy]ethyl 2-*O*-(3,6-Dideoxy-α-*L*-xylo-hexopyranosyl)-β-*D*-galactopyranoside Cyclic 4,6-(Potassium Phosphate)* (**15**). A mixture of Pd/C (1.2 g), **13*α*** (1.200 g, 1.291 mmol), ^{i}PrOH (60 ml) and potassium phosphate buffer (pH 7, 0.1M; 60 ml) was shaken under H_2 (65 psi) until TLC indicated complete conversion (30–40 h). The catalyst was filtered off and washed with H_2O, and the combined filtrates were evaporated. The residue was treated with 95% aq. EtOH (30 ml), and the insoluble salts were filtered off and washed with 95% aq. EtOH (3×15 ml). The combined filtrate was evaporated and the residue subjected to CC (^{i}PrOH/H_2O 3:1) to give, after freeze-drying, **15** (460 mg, 66%). White amorphous solid. ^{1}H-NMR (D_2O, 600 MHz): 5.14 (*d*, $J = 3.7$, H–C(1^{II})); 4.61 (*d*, $J = 7.8$, H–C(1^I)); 4.56 (*d*, $J = 3.3$, H–C(4^I)); 4.39 (br. *d*, $J = 12.6$, H_a–C(6^I)); 4.22–4.30 (*m*, H–C(5^{II}), H_b–C(6^I)); 4.03–4.08 (*m*, H_a–C(1′)); 3.96–4.01 (*m*, H–C(2^{II})); 3.93 (*dt*, $J = 3.2$, 9.6, H–C(3^I)); 3.79–3.85 (*m*, H_b–C(1′), H–C(4^{II})); 3.65–3.77 (*m*, H–C(2^I), H–C(5^I), CH_2(2′), CH_2(3′), CH_2(4′), CH_2(5′)); 3.16–3.20 (*m*, CH_2NH_2); 1.91–1.98 (*m*, CH_2(3^{II})); 1.13 (*d*, $J = 6.6$, Me(6^{II})). ^{13}C-NMR (D_2O, 150 MHz): 101.44 (C(1^I)); 98.82 (C(1^{II})); 76.52 (*d*, $J = 4.7$, C(4^I)); 75.53 (C(2^I)); 72.24 (*d*, $J = 7.4$,

C(3^{I})); 69.89, 69.77, 69.54, 66.57 (C(2′), C(3′), C(4′), C(5′)); 69.05 (C(1′)); 68.61 (C(4^{II})); 68.36 (*d*, *J* = 5.5, C(6^{I})); 67.25 (*d*, *J* = 4.5, C(5^{I})); 66.62 (C(5^{II})); 63.42 (C(2^{II})); 39.26 (CH_2NH_2); 32.86 (C(3^{II})); 15.68 (C(6^{II})). ^{31}P-NMR (D_2O): −3.96. ES-TOF-MS (pos.): 504.2 ($[M-K+2H]^+$); 526.2 ($[M-K+Na+H]^+$). ES-TOF-MS (neg.): 502.2 ($[M-K]^-$).

*2-{2-{2-[(2-Ethoxy-3,4-dioxocyclobut-1-en-1-yl)amino]ethoxy}ethoxy}ethyl 2-*O*-(3,6-Dideoxy-α-*L*-xylo-hexopyranosyl)-β-*D*-galactopyranoside Cyclic 4,6-(Potassium phosphate)* (**2**). *Drierite* (287 mg, 10–20 mesh) was added to a stirred mixture of **15** (287 mg, 0.530 mmol) and abs. EtOH (5.3 ml). After 60 min, diethyl squarate (= 3,4-diethoxycyclobut-3-ene-1,2-dione; 98 µl, 0.663 mmol) and $KHCO_3$ (265 mg, 2.652 mmol) were added, and stirring was continued at r.t. until TLC indicated disappearance of **15**. The mixture was filtered, the solid washed with abs. EtOH (3 × 5 ml), the combined filtrate evaporated, and the residue subjected to CC ($MeCN/H_2O$ 85 : 15) to give, after freeze-drying, **2** (324 mg, 92%). White amorphous solid. ^{1}H-NMR (D_2O, 600 MHz): 5.18 (*m*, H−C(1^{II})); 4.75, 4.72 (2*q*, *J* = 7.1, partially overlapping, MeCH_2O); 4.62, 4.61 (2*d*, *J* = 8.0, partially overlapping, H−C(1^{I})); 4.58 (br. *d*, *J* = 3.4, H−C(4^{I})); 4.42 (br. *dt*, *J* = 1.7, 11.4, H_a−C(6^{I})); 4.29 (*m*, H−C(5^{II}), H_b−C(6^{I})); 3.99–4.60 (*m*, H−C(2^{II}), H_a−C(1′)); 3.96 (*dt*, *J* = 3.4, 9.6, H−C(3^{I})); 3.83 (br. *s*, partial overlap, H−C(4^{II})); 3.78–3.84 (*m*, H_b−C(1′), CH_aNH); 3.77 (*m*, H−C(5^{I})); 3.72 (*dd*, *J* = 8.0, 9.6, partial overlap, H−C(2^{I})); 3.64–3.72 (*m*, CH_bNH, CH_2(2′), CH_2(3′), CH_2(4′), CH_2(5′)); 1.92–2.02 (*m*, CH_2(3^{II})); 1.45, 1.43 (2*t*, *J* = 7.3, partially overlapping, *Me*CH_2O); 1.13 (*d*, *J* = 7.1, Me(6^{II})). ^{13}C-NMR (D_2O, 150 MHz): 189.45, 183.91, 183.88, 177.95, 177.71, 174.40, 174.36 (4 cyclobutene C); 101.94 (C(1^{I})); 99.20 (C(1^{II})); 77.0 (*d*, *J* = 5.0, C(4^{I})); 75.73, 75.68 (C(2^{I})); 72.85 (*d*, *J* = 7.2, C(3^{I})); 71.27, 71.21 (Me*C*H_2); 70.45, 70.43, 70.29, 70.26, 70.08 (C(2′), C(3′), C(4′), C(5′)); 69.38 (C(1′)); 69.14 (C(4^{II})); 68.81 (*d*, *J* = 5.6, C(6^{I})); 67.80 (*d*, *J* = 4.7, C(5^{I})); 67.05 (C(5^{II})); 63.91 (C(2^{II})); 44.60, 44.45 (*C*H_2NH); 33.38 (C(3^{II})); 16.17 (C(6^{II})); 15.76, 15.70 (*Me*CH_2O). ^{31}P-NMR (D_2O): −3.93. ES-TOF-MS (pos.): 650.2 ($[M-K+Na+H]^+$). ES-TOF-MS (neg.): 626.2 ($[M-K]^-$).

REFERENCES

[1] '*Vibrio Cholerae* and Cholera. Molecular to Global Perspectives.', Eds. I. K. Wachsmuth, P. A. Blake, and Ø. Olsvik, ASM Press, Washington, D. C., 1994.

[2] M. J. Albert, *J. Clin. Microbiol.* **1994**, *32*, 2345.

[3] P. Berche, C. Poyart, E. Abachin, H. Lelievre, J. Vandepitte, A. Dodin, J. M. Fournier, *J. Infect. Dis.* **1994**, *170*, 701; L. E. Comstock, J. A. Johnson, J. M. Michalski, J. G. Morris, J. B. Kaper, *Mol. Microbiol.* **1996**, *19*, 815; S. Dumontier, P. Berche, *FEMS Microbiol. Lett.* **1998**, *164*, 91.

[4] M. K. Waldor, R. Colwell, J. J. Mekalanos, *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 11388.

[5] A. Weintraub, G. Widmalm, P. E. Jansson, M. Jansson, K. Hultenby, M. J. Albert, *Microb. Pathog.* **1994**, *16*, 235; A. D. Cox, J. R. Brisson, V. Varma, M. B. Perry, *Carbohydr. Res.* **1996**, *290*, 43; A. D. Cox, M. B. Perry, *Carbohydr. Res.* **1996**, *290*, 59.

[6] G. Jonson, J. Osek, A. M. Svennerholm, J. Holmgren, *Infect. Immun.* **1996**, *64*, 3778.

[7] C. T. Bishop, H. J. Jennings, in 'The Polysaccharides', Ed. G. O. Aspinall, Academic Press, New York, 1982, Vol. 1, p. 291; F. A. Wyle, M. S. Artenstein, D. L. Brandt, D. L. Tramont, D. L. Kasper, P. Altieri, S. L. Berman, J. P. Lowenthal, *J. Infect. Dis.* **1972**, *126*, 514; E. C. Gotschlich, I. Goldschneider, M. L. Lepow, R. Gold, in 'Antibodies in Human Diagnosis and Therapy', Eds. E. Haber and R. M. Krause, Raven Press, New York, 1977, p. 391.

[8] W. F. Goebel, O. T. Avery, *J. Exp. Med.* **1929**, *50*, 521; O. T. Avery, W. F. Goebel, *J. Exp. Med.* **1929**, *50*, 533; O. T. Avery, W. F. Goebel, *J. Exp. Med.* **1931**, *54*, 437.

[9] 'Contributions to Microbiology and Immunology, Conjugate Vaccines', Eds. J. M. Cruse and R. E. Lewis Jr., Karger, Basel, 1989, Vol. 10; 'Neoglycoconjugates: Preparation and Application', Eds. Y. C. Lee and R. T. Lee, Academic Press, New York, 1994; C. Jones, *An. Acad. Bras. Cienc.* **2005**, *77*, 293.

[10] F. Reichel, P. R. Ashton, G.-J. Boons, *Chem. Commun.* **1997**, *21*, 2087; S. F. Slovin, G. Ragupathi, C. Musselli, K. Olkiewicz, D. Verbel, S. D. Kuduk, J. B. Schwarz, D. Sames, S. Danishefsky, P. O. Livingston, H. I. Scher, *J. Clin. Oncol.* **2003**, *21*, 4292; V. Pozsgay, B. Coxon, C. P. J. Glaudemans, R. Schneerson, J. B. Robbins, *Synlett* **2003**, *6*, 743; R. B. Hossany, M. A. Johnson, A. A. Eniade, B.

M. Pinto, *Bioorg. Med. Chem.* **2004**, *12*, 3743; R. K. Taylor, T. J. Kirn, N. Bose, E. Stonehouse, S. A. Tripathi, P. Kovac, W. F. Wade, *Chem. Biodiv.* **2004**, *1*, 1036.

[11] V. Verez-Bencomo, V. Fernández-Santana, E. Hardy, M. E. Toledo, M. C. Rodríguez, L. Heynngnezz, A. Rodriguez, A. Baly, L. Herrera, M. Izquierdo, A. Villar, Y. Valdés, K. Cosme, M. L. Deler, M. Montane, E. Garcia, A. Ramos, A. Aguilar, E. Medina, G. Toraño, I. Sosa, I. Hernandez, R. Martínez, A. Muzachio, A. Carmenates, L. Costa, F. Cardoso, C. Campa, M. Diaz, R. Roy, *Science (Washington, D.C.)* **2004**, *305*, 522.

[12] M. K. Waldor, J. J. Mekalanos, *J. Infect. Dis.* **1994**, *170*, 278; T. S. Coster, K. P. Killeen, M. K. Waldor, D. T. Beattie, D. R. Spriggs, J. R. Kenner, A. Trofa, J. C. Sadoff, J. J. Mekalanos, D. N. Taylor, *The Lancet* **1995**, *345*, 949; C. O. Tacket, G. Losonsky, J. P. Nataro, L. Comstock, J. Michalski, R. Edelman, J. B. Kaper, M. M. Levine, *J. Infect. Dis.* **1995**, *172*, 883.

[13] M. Jertborn, A. M. Svennerholm, J. Holmgren, *Vaccine* **1996**, *14*, 1459.

[14] Z. Kossaczka, J. Shiloach, V. Johnson, D. N. Taylor, R. A. Finkelstein, J. B. Robbins, S. C. Szu, *Infect. Immun.* **2000**, *68*, 5037.

[15] A. Boutonnier, S. Villeneuve, F. Nato, B. Dassy, J.-M. Fournier, *Infect. Immun.* **2001**, *69*, 3488.

[16] R. K. Taylor, T. J. Kirn, N. Bose, E. Stonehouse, S. A. Tripathi, P. Kovac, W. F. Wade, *Chem. Biodiv.* **2004**, *1*, 1036.

[17] S. Oscarson, U. Tedebark, D. Turek, *Carbohydr. Res.* **1997**, *299*, 159.

[18] A. D. McNaught, *Carbohydr. Res.* **1997**, *297*, 1.

[19] L. F. Tietze, M. Arlt, M. Beller, K.-H. Glüsenkamp, E. Jähde, M. F. Rajewsky, *Chem. Ber.* **1991**, *124*, 1215; L. F. Tietze, C. Schröter, S. Gabius, U. Brinck, A. Goerlach-Graw, H.-J. Gabius, *Bioconjugate Chem.* **1991**, *2*, 148.

[20] P.-H. Amvam-Zollo, P. Sinaÿ, *Carbohydr. Res.* **1986**, *150*, 199.

[21] D. W. White, G. K. McEwen, R. D. Bertrand, J. G. Verkade, *J. Chem. Soc. B* **1971**, 1454.

[22] T. D. Inch, G. J. Lewis, *J. Chem. Soc., Chem. Commun.* **1973**, 310.

[23] J. R. Cox Jr., J. O. B. Ramsay, *Chem. Rev.* **1964**, *64*, 317.

[24] J. G. Moffatt, H. G. Khorana, *J. Am. Chem. Soc.* **1957**, *79*, 1194.

[25] L. Lebeau, P. Oudet, C. Mioskowski, *Helv. Chim. Acta* **1991**, *74*, 1697.

[26] P. C. N. Rensen, S. H. van Leeuwen, L. A. J. M. Sliedregt, T. J. C. van Berkel, E. A. L. Biessen, *J. Med. Chem.* **2004**, *47*, 5798.

[27] P. Kovac, N. F. Whittaker, C. P. J. Glaudemans, *J. Carbohydr. Chem.* **1985**, *4*, 243.

[28] P. Kovac, C. P. J. Glaudemans, *Carbohydr. Res.* **1985**, *142*, 158.

[29] C. L. Penney, B. Belleau, *Can. J. Chem.* **1978**, *56*, 2396; A. R. Hill Jr., L. D. Nord, L. E. Orgel, R. K. Robins, *J. Mol. Evol.* **1988**, *28*, 170.

[30] T. W. Greene, P. G. M. Wuts, 'Protective Groups in Organic Synthesis', John Wiley & Sons, Inc., 1999.

[31] B. Ruttens, P. Kovac, *Synthesis* **2004**, *15*, 2505.

[32] A. Demchenko, T. Stauch, G.-J. Boons, *Synlett* **1997**, 818.

[33] G. H. Veeneman, S. H. Van Leeuwen, H. Zuurmond, J. H. Van Boom, *J. Carbohydr. Chem.* **1990**, *9*, 783; K. Zegelaar-Jaarsveld, S. C. Van der Plas, G. A. Van der Marel, J. H. Van Boom, *J. Carbohydr. Chem.* **1996**, *15*, 665.

[34] A. Paquet, *Int. J. Peptide Protein Res.* **1992**, *39*, 82.

[35] R. L. Augustine, 'Catalytic Hydrogenation. Techniques and Applications in Organic Synthesis', Marcel Dekker, Inc., New York, 1965, p. 45.

[36] J. March, 'Advanced Organic Chemistry. Reactions, Mechanisms, and Structure', John Wiley & Sons, New York, 4th edn., 1992, p. 415.

[37] C. W. Jung, J. D. Fellmann, P. E. Garrou, *Organometallics* **1983**, *2*, 1042.

[38] S. Dziadek, Ph.D. Thesis, Universität Mainz, Germany, 2005; S. Dziadek, D. Kowalczyk, H. Kunz, *Angew. Chem., Int. Ed.*, in press.

Received October 20, 2005