Oligosaccharide Synthesis on a Light-Sensitive Solid Support. I. The Polymer and Synthesis of Isomaltose (6-*O*-α-D-Glucopyranosyl-D-glucose)[1]

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Abstract: A light-sensitive polymer was synthesized by attaching 6-nitrovanillin, through an ether linkage, to a chloromethylated styrene–divinylbenzene copolymer. The aldehyde functions on the polymer were reduced by sodium borohydride to alcohols, HO–[PNV], which were utilized for condensation. The polymer was filtered and analyzed after every synthetic step. 6-*O*-*p*-Nitrobenzoyl-2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl bromide was allowed to react with the resin in benzene with pyridine as a base to give 6-*O*-*p*-nitrobenzoyl-2,3,4-tri-*O*-benzyl-D-glucopyranosyl-*O*-[PNV]. The *p*-nitrobenzoate was removed by sodium ethoxide in ethanol–dioxane, leaving the 6-OH free for a similar condensation with another molecule of the same bromide. Following this reaction the *p*-nitrobenzoyl group was removed, resulting in a resin to which substituted di- and monosaccharide units were attached. Irradiation (>320 nm) of the polymer, suspended in dioxane, released the saccharide, leaving a resin showing typical aldehyde absorption at 1720 cm^{-1}. The soluble products were reduced (Pd) to give isomaltose and glucose, which were separated chromatographically and identified.

The chemical synthesis of oligosaccharides is of fundamental importance for biological and chemical applications in many fields of contemporary interest including immunology, enzymology, and pharmacology. It is also of great importance in structure determination of oligo- and polysaccharides. The synthesis of oligosaccharides involves, however, complicated processes which make it difficult to tackle a variety of problems relating to these substances, including those of medicinal relevance. In many cases it is difficult, or impossible, to synthesize conventionally a desired structure. In many more cases the yields are low and the isolation of products becomes tedious.

The synthesis of oligosaccharides on a solid support, in general,[2] is a simple technique having the advantage that after every condensation or deblocking step the resin simply has to be washed to remove impurities before proceeding with the next step. There is also the possibility of using a great excess of soluble reagents in order to push the reaction closer to completion. In this case, the excess reagents can be recovered, if desired. One drawback to this method, however, is that mistakes in sequence occur due to incomplete reactions. Furthermore, oligosaccharide purification can only be carried out after detachment of the product from the polymeric carrier. In addition, one has to anticipate the possible formation of both α- and β-anomers.

Initial work in solid phase synthesis of oligosaccharides was carried out by two groups. Fréchet and Schuerch[3,4] have prepared substituted di- and trisaccharides attached to a polymer *via* a glycoside linkage. Following ozonolysis the saccharide was released from the resin as the 2-ethanal glycoside that could be reduced to the 2-hydroxyethyl glycoside. The method did not lead, however, to free oligosaccharides. Guthrie, Jenkins, and Stehlicek[5] have synthesized a soluble polymer to which a 1,2-ortho ester or 1-bromo derivative of glucose is bound. Such a polymeric reagent could, hopefully, serve in oligosaccharide synthesis.

In view of this situation, we were encouraged to try a new approach: the synthesis of oligosaccharides on a light-sensitive solid support. The synthetic route is similar to that of Fréchet and Schuerch,[3] except that we hoped to achieve a smooth release of the saccharide from the polymer as a reducing sugar by means of a photochemical reaction. The possibility of designing a resin for this purpose results from our study on 2-nitrobenzyl glycosides. These can be photolyzed in very high yields to the parent reducing sugars under conditions that do not affect *O*-benzyl groups.[6] Thus, quantitative release of D-glucose was observed following the irradiation of 2-nitrobenzyl β-D-glucopyranoside or 6-nitroveratryl β-D-glucopyranoside (Scheme I). After presenting this work,[1] we learned of another approach to oligosaccharide synthesis on a solid support.[7]

Results and Discussion

An aldehydo polymer (**1**) was synthesized by attaching 6-nitrovanillin[8] through an ether linkage to a chloromethylated styrene–divinylbenzene copolymer.[9] In addition to the binding of 6-nitrovanillin, some

(1) A preliminary account of this work has appeared: U. Zehavi and A. Patchornik, Abstracts, 164th National Meeting of the American Chemical Society, New York, N. Y., Aug 1972, No. CARB-27; Abstracts, 42nd Meeting of the Israel Chemical Society, Rehovot, Israel, Dec 1972, p 61.

(2) J. M. Stewart and J. D. Young, "Solid Phase Peptide Synthesis," W. H. Freeman, San Francisco, Calif., 1969.

(3) J. M. Fréchet and C. Schuerch, *J. Amer. Chem. Soc.*, **93**, 492 (1971).

(4) J. M. Frechet and C. Schuerch, *Carbohyd. Res.*, **22**, 399 (1972).

(5) R. D. Guthrie, A. D. Jenkins, and J. Stehlicek, *J. Chem. Soc. C*, 2690 (1971).

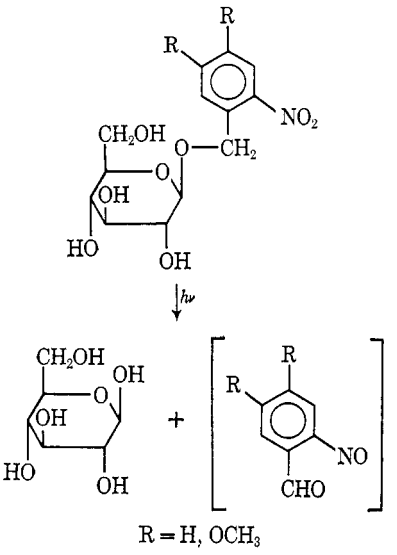
(6) U. Zehavi, B. Amit, and A. Patchornik, *J. Org. Chem.*, 37, 2281 (1972); U. Zehavi and A. Patchornik, *ibid.*, 37, 2285 (1972).

(7) In a personal communication by Professor L. Anderson, he mentioned initial attempts towards an oligosaccharide synthesis on a solid support employing a similar sequence to ours, but using an *S*-glycoside as the temporary hook to the polymer. Recently, the scheme was described for a model reaction sequence in solution: P. J. Pfäffli, S. H. Hixson, and L. Anderson, *Carbohyd. Res.*, **23**, 195 (1972).

(8) M. Julia, P. Manoury, and C. Voillaume, *Bull. Soc. Chim. Fr.*, **47**, 1417 (1965).

(9) R. B. Merrifield, *J. Amer. Chem. Soc.*, **85**, 2149 (1963).

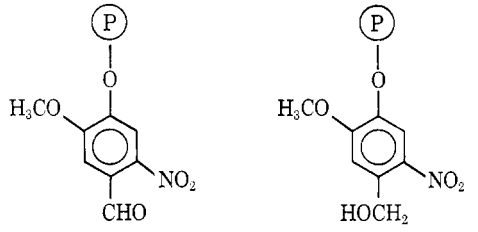
Scheme I



R = H, OCH_3

triethylamine, the base used in the reaction, was also bound as triethylammonium chloride. The aldehyde functions on the polymer were reduced to alcohols (**2**) by sodium borohydride which could be condensed with a sugar bromide. The attachment of 6-nitrovanillin to the polymer, the reduction step, and further synthetic steps were followed by ir spectroscopy and analysis. The data were in accord with the proposed structures. The substituted 6-nitroveratryl glycoside prepared thereby, as well as compound **2** itself, is light sensitive, as can be expected from the behavior of their low molecular weight analogs.[6]

Scheme II



(P) = styrene–divinylbenzene copolymer (CH_2)

Thus, 6-*O*-*p*-nitrobenzoyl-2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl bromide[10] reacts with the resin (**2**) in benzene, with pyridine as a base, to give a polymer to which 6-*O*-*p*-nitrobenzoyl-2,3,4-tri-*O*-benzyl-D-glucopyranosyl units are attached (**3**). The *p*-nitrobenzoate was removed by sodium ethoxide in ethanol–dioxane, leaving the 6-OH free (compound **4**) for a similar condensation with another molecule of the same bromide (elongation), yielding compound **5**. In principle, applying to compound **5** another cycle of deacylation and elongation would have led to a trisaccharide derivative. In the present work we describe only the deacylation of compound **5** to give the isomaltose derivative **6**.

Irradiation of polymer **6** suspended in dioxane at wavelengths longer than 320 nm released a mixture of benzylated saccharides and left an aldehydo polymer (**7**). The benzylated saccharides were reduced with palladium to give a mixture of D-glucose and isomaltose (6-*O*-α-D-glucopyranosyl-D-glucose). Hence, the synthesis of oligosaccharides on a light-sensitive solid support is feasible and the method has led to an α-glucoside (compare ref 10, 3, and 4).

The glucose and isomaltose obtained by synthesis were separated by preparative paper chromatography, and the former was estimated by its oxidation with glucose oxidase[11] and by the phenol–sulfuric acid test.[12] The synthetic isomaltose migrated on paper chromatography with an R_f value identical with that of an authentic sample of isomaltose, although the R_f is similar to that of gentibiose (6-*O*-β-D-glucopyranosyl-D-glucose). It could, however, be differentiated from gentibiose by glc of its trimethylsilyl derivative, by its susceptibility to α-glucosidase but not to β-glucosidase (emulsin),[13] and by its optical rotation (108°). The optical rotation reported for isomaltose is +119–+122° (ref 14) and for gentiobiose is +10.5° (ref 15).

The isolation of a mixture of glucose and isomaltose at the end of the synthesis, as well as the spectrophotometric determination of *p*-nitrobenzoate released upon deacylation of compounds **3** and **5**, indicated that the two condensation steps were incomplete. The photochemical cleavage of 2-nitrobenzyl glycosides in solution proceeds in very high yields.[6] The photolysis of resin-bound saccharide derivatives proceeds likewise, in analytical scale, in very high yields, although after much longer irradiation times. Irradiation of resin-bound saccharide derivatives in preparative scale is, apparently, not as effective and the next step of catalytic hydrogenolysis did not approach completion. Spots corresponding to partially benzylated saccharides can normally be observed on paper chromatograms.

A plausible mechanism of the photochemical reaction was already discussed by us to some extent.[6] It involves an intramolecular oxidation–reduction formation of a hemiacetal which splits to the reducing sugar and the nitroso aldehyde.

The main obstacle in the synthesis remains the incomplete condensation steps. In a paper that appeared after the completion of this work,[4] a few possibilities to eliminate this problem were described. These and others are presently being studied. Another obstacle, the partial hydrogenolysis of benzyl ethers, might be overcome by using different procedures for their removal or might be avoided altogether by using blocking groups other than benzyl ethers. One possibility might be 2-nitrobenzyl ethers, which should be cleaved during the irradiation of the polymer.

It is hoped that after improvements, refinement, and widening of the scope, oligosaccharide synthesis on a light-sensitive solid support will become a method of choice.

Experimental Section

Optical rotations were determined with a Bendix polarimeter. Ir spectra were measured with a Perkin-Elmer Model 237 spectro-

(10) T. Ishikawa and H. G. Fletcher, Jr., *J. Org. Chem.*, **34**, 563 (1969).

(11) E. Raabo and T. C. Terkildsen, *Scand. J. Clin. Lab. Invest.*, **12**, 402 (1966).

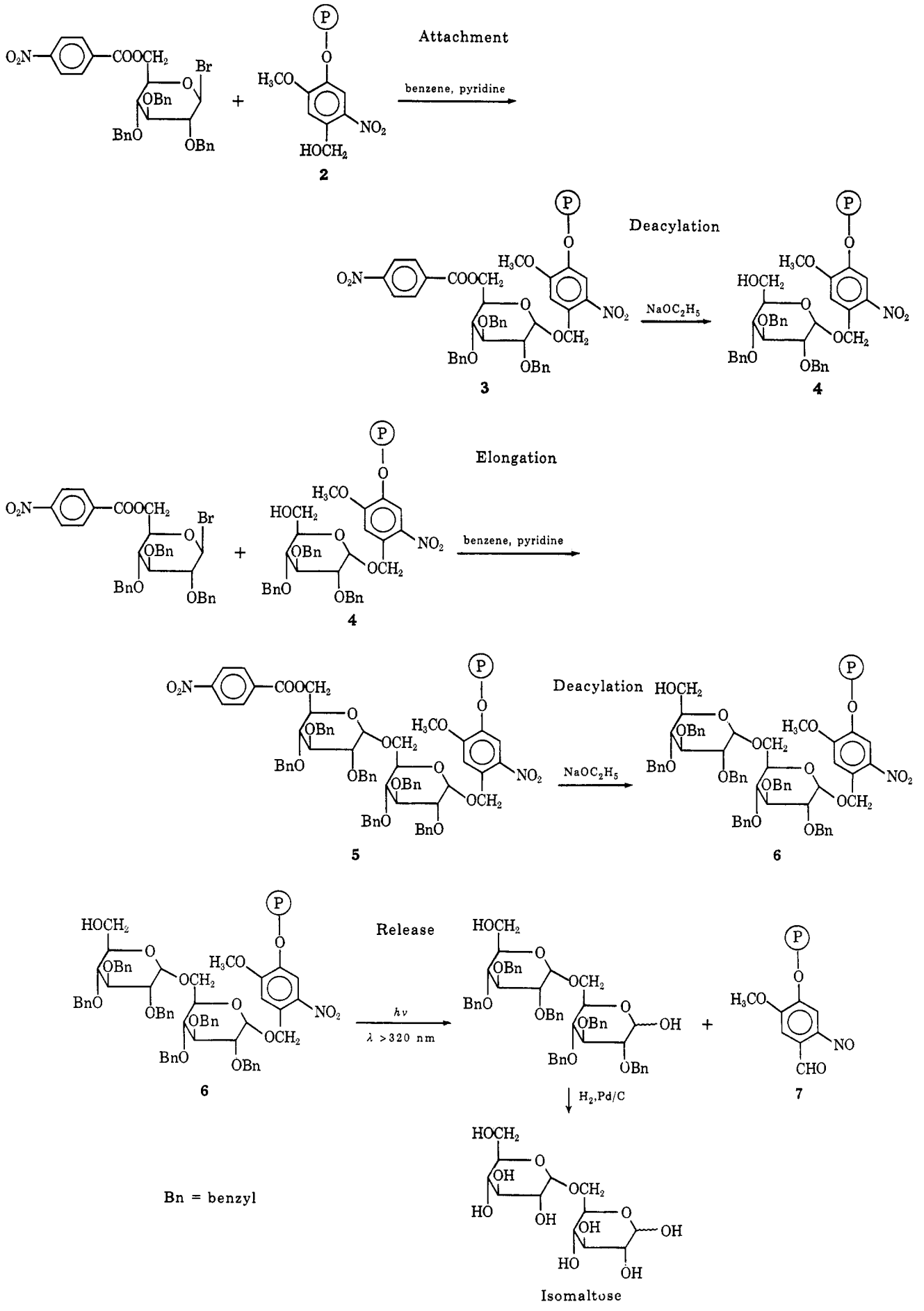
(12) M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Anal. Chem.*, **28**, 350 (1956).

(13) U. Zehavi and A. Patchornik, to be published.

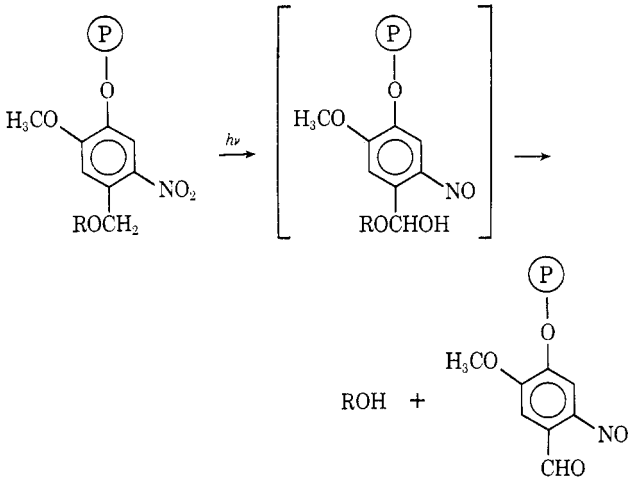
(14) R. W. Bailey, "Oligosaccharides," Pergamon, Oxford, 1965, p 67.

(15) A. Thompson and M. L. Wolfrom, *J. Amer. Chem. Soc.*, **75**, 3605 (1953).

Scheme III


Attachment
benzene, pyridine
2
Deacylation
NaOC2H5
3
4
Elongation
benzene, pyridine
4
5
Deacylation
NaOC2H5
6
Release
hν
λ >320 nm
6
H2,Pd/C
7
Isomaltose
Bn = benzyl

Scheme IV



R = H or substituted saccharide

photometer using KBr disks. Uv spectra were taken on a Cary Model 14 spectrophotometer. Colorimetric measurements were performed on a Klett–Summerson colorimeter equipped with filter no. 50. Descending paper chromatography was performed on Schleicher and Schull 2040a paper for qualitative work and on Whatman no. 3 MM paper, previously washed with water and methanol, for preparative work. The paper was developed with descending *n*-butyl alcohol–acetic acid–water (25:6:25, v/v/v, upper phase) (I) or with descending ethyl acetate–pyridine–water (2:1:2, v/v/v, upper phase) (II). The sugars were detected by silver nitrate.[16] The gas chromatograph Packard Model 7821 was used in conjunction with the Model 811 hydrogen flame ionization detector and 2 m × 4 mm silanized coiled glass column. The column packing used was 1% QS-1 on Gas Chrom Q of 80–100 or 100–120 mesh (Applied Science Laboratories; ref 17). Standards and samples were dissolved in "Sil-Prep" (Applied Science Laboratories) 30 min before injection. Photolysis was carried out in a RPR-100 apparatus (Rayonet, The Southern Co., Middletown, Conn.) with 320-nm lamps. α-Glucosidase (from yeast) and glucose oxidase (fungal) were purchased from Sigma Chemical Co. Peroxidase was a product of Boeringer and Sohne and β-glucosidase (emulsin) was obtained from Worthington Corp. Isomaltose (Sigma) and gentibiose (Calbiochem) served as standards after being purified by preparative paper chromatography (System II).

6-Nitrovanillin. This compound was prepared as described in the literature[8] except that the benzyl ether was cleaved by hydrobromic acid. Thus, 4-*O*-benzyl-6-nitrovanillin (8.0 g) was dissolved in acetic acid (50 ml) at 85°. A 33% solution of hydrobromic acid in acetic acid (18 ml) was added and the solution was kept, with a condenser and a calcium chloride seal, for 10 min in a 85° bath. The reaction mixture was then cooled to room temperature and the product precipitated. It was collected by filtration, washed with boiling water, dissolved in boiling ethanol, treated with some active charcoal, and filtered, and the resulting solution was left for crystallization: yield, 3.0 g (55% pure by tlc), mp 208° (lit.[8] 208°).

The Resin. Chloromethylated (6.60% Cl) 2% cross-linked styrene–divinylbenzene copolymer[9] (3.3 g, 200–400 mesh) and 6-nitrovanillin (1.77 g) were suspended in dioxane (40 ml). Triethylamine (0.9 ml) was added and the reaction was stirred magnetically for 24 hr in a 85° bath under reflux and with a calcium chloride seal. Triethylamine (0.9 ml) was added once again and the reaction was continued, under the same conditions, for an additional 3 days. The brown resin (**1**) was collected on a sintered glass filter and washed with dioxane and methanol: yield, 4.14 g (Thr 4.32 g). The ir spectrum of the polymer (**1**) had absorption bands (CH) characteristic of the styrene–divinylbenzene copolymer moiety at 3025, 2915, 1495, 1455, and 1390 cm^{-1} and bands due to the nitrovanillin moiety at 1680 (C=O), 1570 and 1330 cm^{-1} (NO_2).

Anal. Found: N, 2.06; Cl, 2.03.

(16) N. Sharon and R. W. Jeanloz, *J. Biol. Chem.*, **235**, 1 (1960).

(17) E. C. Horning, W. J. A. Vanden Heuvel, and B. G. Creech, *Methods Biochem. Anal.*, **11**, 69 (1963).

The aldehydo polymer (**1**) (3.0 g) was suspended in dimethylformamide (200 ml). Sodium borohydride (1 g) was added and the mixture stirred for 90 min. The reaction was then stopped by the addition of 30% acetic acid (60 ml) and the resin was subsequently washed with dimethylformamide, water, dioxane, ethanol containing 1.5% of triethylamine, ethanol–dioxane, and methanol: yield, 3.0 g of brown resin (**2**, HO– [PNV]). The resin had lost the carbonyl absorption at 1680 cm^{-1} present in compound **1**.

Anal. Found N, 1.96; Cl, 0.194; OMe, 2.48.

According to the methoxy analysis the resin had 0.8 mmol/g of bound nitroveratrol.

Irradiation for 18 hr of the resin (10 mg) suspended in dioxane (4 ml) did not release any significant amount of chromophore into the solution (measured at 258 nm), while the resulting yellow resin had acquired a new absorption at 1720 cm^{-1} (C=O).

Anal. Found: N, 2.17.

6-*O*-*p*-Nitrobenzoyl-2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl Bromide.[10] The bromo derivative was either isolated in its crystalline form or the methylene chloride solution[10] was diluted and washed with water, saturated sodium bicarbonate solution, and water, dried over sodium sulfate, evaporated under vacuum, and dissolved in benzene.

6-*O*-*p*-Nitrobenzoyl-2,3,4-tri-*O*-benzyl-D-glucopyranosyl-*O*-[PNV] (3). Resin **2** (2.0 g) was suspended, with stirring, in benzene (30 ml) containing 6-*O*-*p*-nitrobenzoyl-2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl bromide (prepared from 1,6-di-*O*-*p*-nitrobenzoyl-2,3,4-tri-*O*-benzyl-β-D-glucopyranoside,[10] 3 g, 2.5 molar equiv). Pyridine (0.36 ml) in benzene (5 ml) was added and the stirring was continued for 40 hr in a 80° bath and under calcium chloride seal. The product was collected by filtration on a sintered glass filter, and was washed with benzene, dioxane, and methanol: yield, 2.62 g (Thr 2.93 g). Compound **3** had a strong absorption at 1730 cm^{-1} (C=O). The amount of saccharide attached to the resin was 0.34 mmol/g (62%), as determined by transesterification, and 0.38 mmol/g (69%), determined by photolysis (Thr 0.55 mmol/g).

Anal. Found: N, 2.02.

Estimation of the Amount of Saccharide Attached to the Polymer (Compounds 3 and 5). a. Estimation as *p*-Nitrobenzoate Following Transesterification. The resin (2–3 mg) was suspended, by stirring, in a solution (1 ml) composed of dioxane and 0.2 *M* sodium ethoxide in ethanol (2:1). The stirring was continued overnight in a closed tube. The suspension was then filtered through a glass fiber filter (Whatman GFC), and diluted tenfold with the same solution and the absorbance at 272 nm was determined. *p*-Nitrobenzoic acid has λ_{max} 272 nm (ϵ 0.97 × 10^4) in this solution.

b. Estimation Following Photolysis. The resin (2–4 mg) was suspended, by stirring, in dioxane (1 ml) in a closed Pyrex test tube and irradiated for 12 hr. The suspension was then filtered, the solution was diluted 20-fold, and the absorbance at 258 nm was determined. 1,6-Di-*O*-*p*-nitrobenzoyl-2,3,4-tri-*O*-benzyl-β-D-glucopyranoside absorbs at 258 nm (ϵ 2.42 × 10^4 in dioxane). It is assumed that the released saccharide has half this extinction.

2,3,4-Tri-*O*-benzyl-D-glucopyranosyl-*O*-[PNV] (4). Compound **3** (1.0 g) was mixed in a solution (40 ml) composed of dioxane and 0.2 *M* sodium ethoxide in ethanol (2:1) for 18 hr in a closed erlenmeyer flask. The product (**4**) was then collected by filtration on a sintered glass filter, and washed with dioxane and methanol: yield, 0.84 g (Thr 0.94 g). The carbonyl absorption present in compound **3** (1730 cm^{-1}) is absent in compound **4**. Assuming quantitative transesterification of compound **3** and taking into account the theoretical decrease in weight of resin, the calculated amount of saccharide attached to resin **4** is 0.38 mmol/g.

Anal. Found: N, 1.63.

6-*O*-(6-*O*-*p*-Nitrobenzoyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-D-glucopyranosyl-*O*-[PNV] (5). Compound **4** (0.5 g) was suspended, by stirring, in benzene (10 ml) containing 6-*O*-*p*-nitrobenzoyl-2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl bromide that was prepared from 1,6-di-*O*-*p*-nitrobenzoyl-2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (1.5 g, 5 molar equiv). Pyridine (0.2 ml) was added and the stirring was continued for 48 hr in a 65° bath and under a calcium chloride seal. The product was collected by filtration on a sintered glass filter and washed with benzene, dioxane, and methanol: yield, 0.65 g (Thr 0.61 g). The ir spectrum showed a carbonyl absorption at 1730 cm^{-1}. The amount of saccharide attached to the resin was 0.29 mmol/g (93%) as determined by transesterification and 0.27 mmol/g (88%) as determined by photolysis (Thr 0.3 mmol/g, arbitrarily assuming the formation of the disaccharide derivative only).

Anal. Found: N, 2.16.

6-*O*-(2,3,4-Tri-*O*-benzyl-α-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-D-glucopyranosyl-*O*-[PNV] (6) was prepared from compound **5** (0.197 g) as described for the preparation of compound **4**: yield, 0.160 g (Thr, 0.187 g). The carbonyl absorption at 1730 cm^{-1} is not present in this compound. Assuming quantitative transesterification of compound **5** and taking in account the theoretical decrease in weight of resin, the calculated amount of saccharide attached to resin **6** is 0.32 mmol/g.

Anal. Found: N, 1.58; OCH_3, 2.36.

Release of Saccharide. Resin **6** (0.2 g) was suspended, by stirring, in dioxane (250 ml) and irradiated for 32 hr. The resin (**7**) was filtered off (it had an absorption at 1720 cm^{-1}) and the solution was evaporated *in vacuo* to a third of its original volume. Water (100 ml), acetic acid (1 drop), and a small amount of 10% palladium on charcoal were added and the solution was hydrogenated for 18 hr at room temperature and at 46 psi. The catalyst was then filtered off and the solution evaporated *in vacuo*. The residue was dissolved in water (10 ml) and was checked (40–100-μl samples) by paper chromatography (in the two solvent systems indicated) and was shown to contain a material migrating like isomaltose, a material migrating like glucose, and a few additional spots, probably corresponding to partially benzylated products. A portion of the aqueous solution (1.0 ml) was freeze dried and separated by preparative paper chromatography (system II). The products were eluted from the paper as follows. The bands containing glucose and isomaltose were cut into slices and placed into the outer part of a 5-ml disposable syringe that had been plugged with some washed cotton. The paper was then wet with water and the syringe centrifuged inside a 12-ml conical tube. The last step was repeated several times using a total of about 2.5 ml of water. Finally the yield of products was determined by the phenol–sulfuric acid test: glucose, 0.058 mmol/g (17.7%); isomaltose, 0.041 mmol/g (12.5%). Yields are based on the saccharide content of compound **6**. The yield of free disaccharide, isomaltose, remains similar (10.8%) also when calculated on the basis of the monosaccharide derivative **4**. Isomaltose was obtained on freeze drying. The trimethylsilyl derivative of the synthetic isomaltose was compared to trimethylsilylated isomaltose and gentibiose by glc (22 psi, 178°) and was shown to contain the peaks corresponding to isomaltose at retention time 13.7 and 18.5 min. No evidence for gentibiose (10% contamination should have been detected), retention time 16.8 and 18.5 min, was obtained. The synthetic isomaltose had $[\alpha]^{23}D$ + 108° (*c* 0.14, water) and its purity was evaluated by a method developed for the determination of glucose, isomaltose, and gentibiose in admixture.[13] A solution of the synthetic isomaltose (120 μg/ml, by phenol–sulfuric acid test minus a small amount of contaminating glucose determined with glucose oxidase) was digested by α-glucosidase and the glucose obtained was determined with glucose oxidase (97 μg/ml). When the same was repeated with β-glucosidase, no evidence for digestion was obtained.

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Synthesis and Some Pharmacological Properties of [1-Deamino,9-thioglycine]oxytocin[1,2]

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Abstract: [1-Deamino,9-thioglycine]oxytocin, an analog of deamino-oxytocin in which the C-terminal carboxamide function has been formally replaced by a thiocarboxamide group, has been synthesized by coupling deaminotocinoic acid (the cyclic disulfide of β-mercaptopropionyltyrosylisoleucylglutaminylasparaginylcysteine) with prolylleucylthioglycinamide. The latter peptide was elaborated from thioglycinamide prepared by the treatment of Z-Gly-NH_2 with P_2S_5, followed by removal of the protecting group. The analog was found to possess 46 ± 5 units/mg of oxytocic activity and 14.9 ± 0.3 units/mg of avian vasodepressor activity, or approximately 6 and 1.5%, respectively, of the corresponding activities of crystalline deamino-oxytocin.

During the systematic assessment of the importance of the various functional groups of oxytocin (Figure 1) to its biological activity, it was determined that the C-terminal amide is a structural feature important to the biological activity of the hormone. In these studies the C-terminal $CONH_2$ group of oxytocin, or its highly potent analog deamino-oxytocin, was formally replaced by COOH,[3,4] $CONHCH_3$,[5] $CON(CH_3)_2$,[5] and H.[6] For each of the resulting analogs, the biological activities were found to be drastically lower than those of the parent compounds. In the studies which left the glycine residue intact, all the structural modifications introduced were in the noncarbonyl portion of the amide. In this paper we wish to report an extension of this study, wherein the carbonyl moiety of the C-terminal amide of deamino-oxytocin has been formally replaced by a thiocarbonyl group, thus forming an analog bearing a thioglycinamide residue. The synthesis of this compound, [1-deamino,9-thioglycine]oxytocin ([1-deamino,9-Glyt]-oxytocin),[2] was undertaken not only as a part of this structure–activity study, but also as a test of the feasibility of using the thioamide functional group in the context of polypeptide synthetic chemistry.

Ried and coworkers[7] have reported the preparation of several thioamide containing dipeptides, which have

(1) This work was supported in part by Grant No. HL-11680 from the National Heart and Lung Institute, U. S. Public Health Service.

(2) The symbol Glyt is used to indicate the thiocarbonyl analog of the glycine residue. This symbol is consistent with the Recommendations (1971) of the IUPAC–IUB Commission on Biochemical Nomenclature, *J. Biol. Chem.*, **247**, 977 (1972), whose symbols for the other amino acid residues were followed. The optically active amino acid residues are of the L configuration.

(3) B. M. Ferrier and V. du Vigneaud, *J. Med. Chem.*, **9**, 55 (1966); H. Takashima and V. du Vigneaud, *J. Amer. Chem. Soc.*, **92**, 2501 (1970).

(4) A. S. Dutta, N. Anand, and K. Kar, *Indian J. Chem.*, **4**, 488 (1966).

(5) H. Takashima, W. Fraefel, and V. du Vigneaud, *J. Amer. Chem. Soc.*, **91**, 6182 (1969).

(6) L. A. Branda and V. du Vigneaud, *J. Med. Chem.*, **9**, 169 (1966).

(7) W. Ried and E. Schmidt, *Justus Liebigs Ann. Chem.*, **695**, 217 (1966); W. Ried and W. von der Emden, *ibid.*, **642**, 128 (1961).