Transglycosylation of N-*Arylglycosylamines.*

By R. Bognár and P. Nánási.

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N-*p*-Sulphamylphenyl- and *N*-*m*- and *N*-*p*-nitrophenyl-D-glucosylamine have been prepared by the interaction of various *N*-aryl-D-glucosylamines with the appropriate amines. A simple method is described for such transglycosylations, which proved to be reversible in certain circumstances. Mechanisms are discussed.

In a preliminary report Bognár and Nánási (*Nature*, 1953, **171**, 475) stated that "transglycosylation" of *N*-arylglycosylamines occurs with remarkable ease. The process is now found to be fairly general.

The term "transglycosylation" denotes migration or transfer of the glycosyl part of an *O*-glycosyl or *N*-glycosyl derivative to some other hydroxy- or amino-compound. These reactions are of interest both chemically and biochemically. According to recent investigations some enzymes appear to catalyse such processes. *E.g.*, Kalckar, MacNuff, and Hott-Jorgensen (*Biochem. J.*, 1952, **50**, 397) report that the extract of *Lactobacillus helveticus* contains a *trans*-*N*-glycosidase which catalytically promotes the reaction, adenine + hypoxanthine deoxyriboside ⟶ hypoxanthine + adenine deoxyriboside; and *Aspergillus orizae* yields a *trans*-*O*-glycosidase which promotes transfer of the glucosyl group of an *O* : 4-bonded disaccharide to the 6-position of another saccharide, thus converting maltose into a mixture of *iso*maltose and 4- and 6-*O*-*iso*maltosyl-D-glucose (Pazur and Rench, *J. Biol. Chem.*, 1952, **196**, 265). Reversibility of the reaction has recently been reported for *iso*maltose (Pazur, *Biochim. Biophys. Acta*, 1954, **13**, 158). Among the rare reports of transglycosylation of *O*-glycosides by purely chemical means, Pigman and Laffre (*J. Amer. Chem. Soc.*, 1951, **73**, 4994) prepared *n*-butyl α-D-glucoside from methyl α-D-glucoside by some hours' boiling with butanolic hydrochloric acid (they term the process transglycosidation).

The first example of transglycosylation of *N*-arylglucosylamines was given in 1936 by Kuhn and Dansi (*Ber.*, 1936, **69**, 1745) who in this way prepared *N*-nitroxylyl- from *N*-*p*-tolyl-D-glucosylamine in less than 10% yield. They reported that the reverse reaction was not realisable. Inonue and Onodera (*J. Agr. Chem. Soc. Japan*, 1947, **22**, 119) describe the preparation of *N*-*m*- and *N*-*p*-nitrophenyl-D-glucosylamine from *N*-*p*-tolyl-D-glucosylamine by the same method. The preparation of *N*-phenyl- from *N*-*p*-tolyl-D-glucosyl-

amine, likewise reported there, gave " white tabular crystals, m. p. 120—122° " (*Chem. Abs.*, 1951, **45**, 9481), which appears to be at variance with the statement by Honeyman *et al.* (*J.*, 1950, 967) that *N*-phenyl-D-glucosylamine is not crystalline.

Kuhn and Dansi's method consists in refluxing the glycosylamine in more than 100 parts of absolute alcohol for 8 hours with 4·5 mols. of the amine, and separation of the products by chromatography. Apparently no catalyst was used. We now report a study of the conditions and mechanism of the reaction and a simple general preparative method.

The reaction is dependent on pH. Transglycosylation involves proton-catalysis, while in absence of hydrogen ions there is often no reaction; 0·01—0·1 mol. of hydrogen chloride proved to be a reliable catalyst which will effect transglycosylation in 10—20% (by wt.) methanol or ethanol solution of the starting materials at room temperature in a few minutes. No more heating than is necessary for dissolution (maximum, 5 minutes' boiling) is advisable. Transglycosylation is an equilibrium reaction and can be made reversible under certain conditions: for preparative purposes the reverse reaction may require a different solvent or solvent mixture. The yields are generally good but are determined largely by the relative solubilities. Under proper conditions good yields are obtainable even with 1 mol. of amine per mol. of glycosylamine; an excess of up to 0·5 mol. of amine generally suffices to give a tolerable yield from an appropriately chosen solvent or solvent mixture; we never employed more than 1 mol. excess; the excess of amine then does not interfere with isolation of the products, generally remaining in the mother-liquor.

That the reaction is transglycosylation rather than hydrolysis followed by redistributive reglycosylation is shown by the fact that it occurs readily under completely anhydrous conditions with dry hydrogen chloride, such conditions in most cases affording the best yields. A water content not exceeding 5% generally does not influence the rate of reaction or the yield; in special cases presence of water is even advantageous, by leading to a less soluble hydrate or by influencing the solubility in the " mixed " solvent. More than 5% of water is however detrimental. In the presence of water distributive reglycosylation is of course a possible reaction mechanism. Nevertheless the view that hydrolysis is not involved is supported by the observation that *N*-*p*-sulphamylphenyl-D-glucosylamine is formed from *N*-*p*-nitrophenyl-, *N*-*p*-tolyl-, or *N*-(4-carboxy-3-hydroxyphenyl)-D-glucosylamine 5—10 times faster than by reaction of glucose with sulphanilamide in the presence of the appropriate second amine under similar conditions.

Finally, transglycosylation may be extended from glucose to other aldoses and oligosaccharides, and even to *O*-acetyl-*N*-arylglycosylamines (see following paper).

Experimental

N-p-*Sulphamylphenyl*-D-*glucosylamine*.—(i) *From* N-*phenyl*-D-*glucosylamine*. Sulphanilamide (5·0 g., 1 mol.) was dissolved in warm absolute alcohol (60 ml.) containing hydrogen chloride (0·045 g.), and finely ground, dry *N*-phenyl-D-glucosylamine (7·5 g., 1 mol.) was added. Precipitation began while the solution was still warm and was complete in about 1·5 min. The crystals, when washed with small amounts of absolute ethanol, were pure *N*-*p*-sulphamylphenyl-D-glucosylamine (6·7 g., 69%), m. p. 202—204° (decomp.), $[\alpha]_D^{22}$ −115° (*c*, 1·2 in pyridine), −122° (*c*, 1·2 in H_2O) (Found: N, 8·4; S, 9·7, 9·7. Calc. for $C_{12}H_{18}O_7N_2S$: N, 8·4; S, 9·6%). Kuhn and Birkofer (*Ber.*, 1938, **71**, 621) report m. p. 195° and $[\alpha]_D$ −123° in H_2O.

(ii) *From* N-m-*nitrophenyl*-D-*glucosylamine*. Sulphanilamide (2·5 g., 1·1 mol.) was dissolved in absolute ethanol (50 ml.) containing hydrogen chloride (0·015 g.). *N*-*m*-Nitrophenyl-D-glucosylamine (4·0 g., 1 mol.) was added and the mixture boiled for 2 min. Precipitation began during the boiling and the mixture soon set to a mass of crystals. Filtration and washing yielded pure *N*-*p*-sulphamylphenyl-D-glucosylamine (3·8 g., 85%), m. p. and mixed m. p. 203—204°, $[\alpha]_D^{22}$ −113·2° (*c*, 0·9 in pyridine) (Found: N, 8·4, 8·4; S, 9·8, 9·8%).

A 60% yield was obtained from 96% alcohol containing a small amount of concentrated hydrochloric acid. There was no reaction in absolute alcohol in absence of hydrogen chloride.

More than 0·2 mol. of acid gave inferior yields, and uniform products were not obtained if 0·5 or 2 mols. were used. Yields were best with 0·01—0·1 mol. of acid.

(iii) *From* N-p-*nitrophenyl*-D-*glucosylamine.* (*a*) Sulphanilamide (3·6 g., 1 mol.) was dissolved in absolute ethanol containing concentrated hydrochloric acid (0·3 ml.), and *N*-*p*-nitrophenyl-D-glucosylamine (6·0 g., 1 mol.) was added to the hot solution. It dissolved in a few seconds; after approx. 30 seconds heavy precipitation occurred {6·5 g., 97%; m. p. 202°, $[\alpha]_D^{22}$ −116·2° (*c*, 1·2 in pyridine)}. Recrystallisation from alcohol (1 g. in 10 ml.) yielded the sulphamyl derivative (0·75 g.), m. p. and mixed m. p. 204° (Found: N, 8·3; S, 9·6, 9·7%). Addition of water precipitated 70% of the calculated amount of *p*-nitroaniline from the mother-liquor.

Reaction in 96% ethanol in the presence of concentrated hydrochloric acid gave a 72% yield.

Hydrolysis of the pure product by dilute hydrochloric acid gave 78% of sulphanilamide, m. p. 165°.

(*b*) Sulphanilamide (0·86 g., 1 mol.) was dissolved in water (0·3 ml.) and absolute ethanol (20 ml.) containing 1 drop of concentrated hydrochloric acid. *N*-*p*-Nitrophenyl-D-glucosylamine (1·5 g., 1 mol.) was added to the hot solution, dissolving in a few seconds. The solution was heated on the water-bath. Crystals separated without inoculation within 1·5 min. (yield, 1·4 g., 85%; m. p. 202°).

(*c*) Glucose (0·9 g., 1 mol.) was dissolved in water (0·3 ml.); *p*-nitroaniline (0·7 g., 1 mol.) and sulphanilamide (0·86 g.) were added in absolute ethanol (20 ml.) containing 1 drop of concentrated hydrochloric acid. On the water-bath a clear solution was obtained in a few seconds, but precipitation began only after repeated inoculation after 10 min. and required about 30 min. for completion (yield, 1·25 g., 76%).

(iv) *From* N-p-*bromophenyl*-D-*glucosylamine.* Sulphanilamide (3·6 g., 1 mol.) was dissolved in absolute ethanol (62 ml.) containing hydrogen chloride (0·015 g.). *N*-*p*-Bromophenyl-D-glucosylamine (6·8 g., 1 mol.) was added to the hot solution. Crystallisation began in 30 seconds. The crude product {6·0 g., 84%; m. p. 202° (decomp.), $[\alpha]_D^{25}$ −112·0° (*c*, 1·7 in pyridine)} gave, on recrystallisation from 10 parts of ethanol, a 75—80% yield of the pure sulphamyl derivative, m. p. 204°, containing no bromine (Found: N, 8·3; S, 9·7, 9·8%). Hydrolysis as above gave 80% of sulphanilamide, m. p. 164°.

Reaction in presence of small quantities of concentrated hydrochloric acid gave 75% yields.

(v) *From* N-(4-*carboxy*-3-*hydroxyphenyl*)-D-*glucosylamine.* This glucosylamine (5·0 g., 1 mol.) was added to a hot solution of sulphanilamide (2·5 g., 1 mol.) in 99% methanol (70 ml.) containing concentrated hydrochloric acid (0·3 ml.). The solution became solid in 2 min. {yield, 4·9 g., 92%; m. p. 197°, $[\alpha]_D^{21}$ −112·0° (*c*, 1·2 in pyridine)}. One recrystallisation afforded a pure product, m. p. and mixed m. p. 204° (Found: N, 8·7; S, 9·7, 9·8%), hydrolysed to sulphanilamide (78% yield). In 96% ethanol, with 2 mols. of sulphanilamide and concentrated hydrochloric acid or ammonium chloride as catalyst, this reaction gave 89% or 83% yield, respectively; under these conditions (NH_4Cl) use of 1 mol. of sulphanilamide gave a 71% yield.

Reaction of 1 mol. each of sulphanilamide, 4-aminosalicylic acid, and glucose in 96% ethanol with ammonium chloride as catalyst was much slower. Precipitation began after 5 min. (instead of 1 min.) and required a further 10 min. for completion (instead of a further 1 min.). The quantity and the quality of the product were as in the previous experiment.

The transglycosylation was much slower in absence of a catalyst.

(vi) *From* N-p-*tolyl*-D-*glucosylamine.* (*a*) Sulphanilamide (0·86 g., 1 mol.) was dissolved in absolute ethanol (20 ml.) and water (0·3 ml.); concentrated hydrochloric acid (1 drop) and *N*-*p*-tolyl-D-glucosylamine (1·35 g., 1 mol.) were added to the hot solution. Crystallisation set in from the hot solution after 3 min. and was complete in a further 5—6 min. {yield 0·75 g., 45%; m. p. 202° (decomp.), $[\alpha]_D^{22}$ −116° (*c*, 1·2 in pyridine)} (Found: N, 8·3%). Hydrolysis of the product gave 76% of sulphanilamide, m. p. 164°.

(*b*) Sulphanilamide (1 mol.), D-glucose (1 mol.), and *p*-toluidine (1 mol.) under the same conditions gave a precipitate on the water-bath only after 25 min., in spite of repeated inoculation; crystallisation was complete in about 70 min. The yield was 0·65 g. (40%) of a product, m. p. 202°, $[\alpha]_D^{22}$ −114·2° (*c*, 1·2 in pyridine).

N-p-*Nitrophenyl*-D-*glucosylamine.*—(i) *From* N-*phenyl*-D-*glucosylamine.* To *N*-phenyl-D-glucosylamine (4·0 g., 1 mol.) in a mixture of water (9 ml.) and methanol (24 ml.) was added *p*-nitroaniline (2·2 g., 1 mol.) in hot methanol (12 ml.) containing concentrated hydrochloric acid (0·1 ml.). The whole was boiled for 5 min. Crystallisation began from the cooled solution

immediately upon scratching and was complete in a short time. The drained and washed product (1·88—2·02 g., 40—43%), when hydrolysed by dilute hydrochloric acid, gave 90% of *p*-nitroaniline, m. p. 147°. The glycosylamine was recrystallised from aqueous ethanol and washed with ether; it then had m. p. 184° (decomp.), $[\alpha]_D^{22}$ −193° (*c*, 1·4 in pyridine) (Found: N, 8·3; H_2O, 9·4. Calc. for $C_{12}H_{16}O_7N_2,2H_2O$: N, 8·3; H_2O, 10·7%). Weygand (*loc. cit.*) gives m. p. 184°, $[\alpha]_D^{20}$ −192° (*c*, 1·0 in pyridine).

The reaction also occurs in absolute ethanol in the presence of small amounts of hydrogen chloride, but the product is precipitated much more slowly (ice-cooling is necessary).

(ii) *From* N-o-*nitrophenyl*-D-*glucosylamine*. *N*-*o*-Nitrophenyl-D-glucosylamine (1·0 g., 1 mol.) and *p*-nitroaniline (0·5 g., 1 mol.) were boiled in methanol (12 ml.) and water (2 ml.) containing concentrated hydrogen chloride (0·05 ml.) for 10 min. and then kept for a day at 0°. The crude precipitate (0·8 g.; N, 8·55%) was recrystallised from ethanol (0·5 g. yielded 0·35 g.; overall yield of pure product 56%). The pure product had m. p. 184°, $[\alpha]_D^{22}$ −198° (*c*, 1·2 in pyridine). Hydrolysis of 0·2 g. as above gave 79% of *p*-nitroaniline, m. p. and mixed m. p. 147—148°.

(iii) *From* N-m-*nitrophenyl*-D-*glucosylamine*. Dry *N*-*m*-nitrophenyl-D-glucosylamine (4·0 g., 1 mol.) was dissolved in absolute methanol (30 ml.). *p*-Nitroaniline (2·0 g., 1·1 mol.) in warm absolute methanol (20 ml.) was added and then absolute methanol (3 ml.) containing hydrogen chloride (0·066 g.). The mixture was boiled for 5 min. Crystallisation began on cooling. Hydrolysis of the crude product yielded pure *p*-nitroaniline, m. p. 147°. The overall yield of pure glucosylamine, recrystallised from alcohol, was 65%, the product having m. p. 183°, $[\alpha]_D^{22}$ −200° (*c*, 1·2 in pyridine) (Found: N, 9·1. Calc. for $C_{12}H_{16}O_7N_2$: N, 9·3%). Hydrolysis gave 95% of *p*-nitroaniline.

Reaction does not occur in absence of hydrogen chloride. Crystallisation begins at once upon addition of one drop of concentrated hydrochloric acid.

(iv) *From* N-p-*sulphamylphenyl*-D-*glucosylamine*. This glucosylamine (10·0 g., 1 mol.) in hot methanol (60 ml.) and water (20 ml.) was treated with *p*-nitroaniline (8·0 g., 2 mols.) in methanol (40 ml.) containing concentrated hydrochloric acid (0·3 ml.). The mixture was refluxed for 10 min. Crystallisation began immediately on cooling. The crude product {7·7 g., 85%; m. p. 175°, $[\alpha]_D^{22}$ −180° in pyridine} was filtered off after 2 hr. and yielded on hydrolysis *p*-nitroaniline (0·14 g. from 0·4 g.), m. p. 148°. The crude product, when crystallised from absolute ethanol (overall yield 70%), had m. p. and mixed m. p. 182°, $[\alpha]_D^{22}$ −197·8° (Found: N, 9·4%).

(v) *From* N-p-*bromophenyl*-D-*glucosylamine*. This glucosylamine (6·0 g., 1 mol.) was dissolved in methanol (36 ml.) and water (12 ml.). A hot solution of *p*-nitroaniline (3·0 g., 1·15 mol.) in methanol (24 ml.) containing concentrated hydrochloric acid (0·2 ml.) was added. The whole was boiled for 5 min. Crystallisation began immediately upon cooling. The crude product (3·8 g., 67%) crystallised from aqueous ethanol. The pure product contained no bromine and had m. p. and mixed m. p. 181°, $[\alpha]_D^{22}$ −196° (*c*, 1·2 in pyridine) (Found: N, 8·3%). Hydrolysis gave 85% of *p*-nitroaniline.

(vi) *From* N-(4-*carboxy*-3-*hydroxyphenyl*)-D-*glucosylamine*. This amine (5·0 g.) and *p*-nitroaniline (3·0 g., 1·5 mols.) were dissolved in cold methanol (60 ml.). Concentrated hydrochloric acid (0·3 ml.) was added. Crystallisation set in after about 2 min. The crude product (4·8 g., 100%) was fairly uniform, having $[\alpha]_D^{22}$ −186·0° in pyridine (Found: N, 8·4%). Recrystallisation from ethanol and successive washing with water, methanol, and ether gave 75% of the pure product, m. p. and mixed m. p. 181°, $[\alpha]_D^{22}$ −198·5° (*c*, 1·2 in pyridine). Hydrolysis gave results as above.

Reaction does not occur in absence of acid. With 2 mols. of *p*-nitroaniline yields better than 90% were obtained.

N-m-*Nitrophenyl*-D-*glucosylamine*.—(i) *From* N-p-*nitrophenyl*-D-*glucosylamine*. Dry *N*-*p*-nitrophenyl-D-glucosylamine (4·0 g., 1 mol.) was dissolved in warm ethanol (20 ml.). *m*-Nitroaniline (3·0 g., 1·5 mol.) was added in alcohol (10 ml.) containing hydrogen chloride (0·0035 g.). The mixture was boiled for 5 min. and then cooled. The crystalline precipitate was filtered off and crystallised from absolute alcohol (yield, 60%; hydrolysis gave *m*-nitroaniline, m. p. 114°) and had m. p. and mixed m. p. 177—178°, $[\alpha]_D^{22}$ −167° (*c*, 1·2 in pyridine) (Found: N, 9·5%). Weygand (*loc. cit.*) gives m. p. 178°, $[\alpha]_D^{20}$ −171° in pyridine.

(ii) *From* N-p-*sulphamylphenyl*-D-*glucosylamine*. The sulphamyl derivative (6·0 g., 1 mol.) and *m*-nitroaniline (3·2 g., 1·5 mols.) were dissolved in aqueous ethanol (17 : 3) (30 ml.). Concentrated hydrochloric acid (1 ml.) was added and the whole boiled for 10 min., then kept at 0°. The precipitated product was filtered off, the mother-liquor was concentrated, and the new

crop separated. The precipitates were united and extracted with 6—7 parts of hot methanol; the methanolic extract was concentrated to small volume. The crystals obtained on cooling (1·8 g., 33%; m. p. 174—177°) crystallised from a small amount of ethanol and then had m. p. 176—177° (yield 21%), $[\alpha]_D^{22}$ −166·1° (*c*, 1·2 in pyridine) (Found : N, 9·4%; S, nil.). Hydrolysis gave 83% of *m*-nitroaniline, m. p. 114°.

INSTITUTE OF ORGANIC CHEMISTRY, KOSSUTH UNIVERSITY,
DEBRECEN, HUNGARY.

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