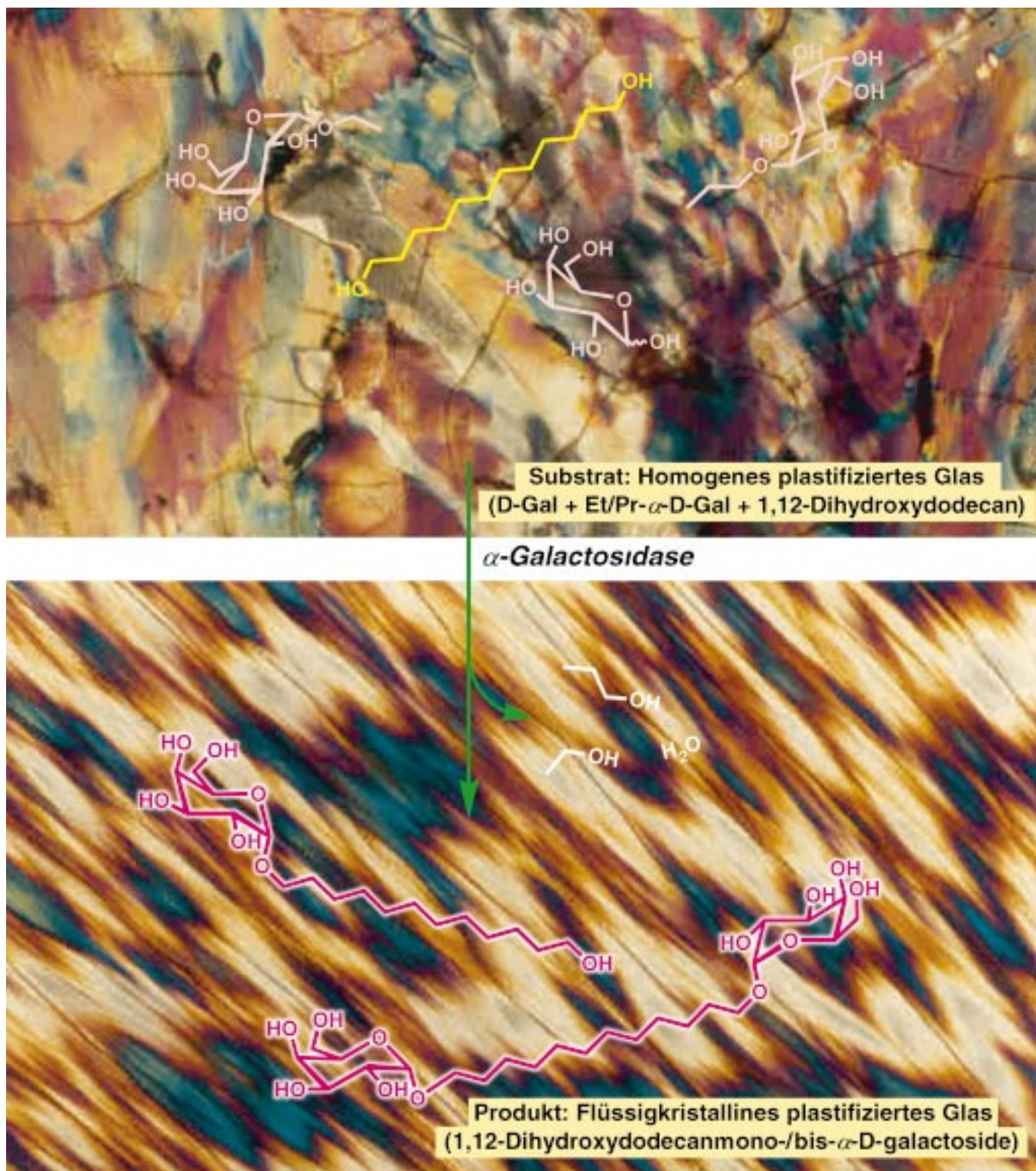


Monosaccharid-Alkylglycosid-Gläser



Dotieren von Monosacchariden mit Alkylglycosiden führt zu Gläsern mit verringerten Glasübergangstemperaturen und enorm hohen Hydrophobien. Die Gläser können durch Alkohole verflüssigt (plastifiziert) werden und sind in der Lage, eine Vielfalt hydrophober Verbindungen

unter Bildung homogener oder heterogener Flüssigphasen zu lösen. Glycosidasn sind in diesen Medien aktiviert und katalysieren in guten Ausbeuten die O-Glycosylierung einer großen Auswahl von Acceptoren. Mehr dazu auf den folgenden Seiten.

Monosaccharide–Alkyl Glycoside Glass Phases: Plasticization with Hydrophilic and Hydrophobic Molecules

Iqbal Gill* and Rao Valivety

The ability of carbohydrates to form amorphous glassy solids is a well-known phenomenon, which has assumed great importance in the fields of cryobiology, room-temperature stabilization of biologicals, microbiology, and food technology.^[1] In particular, the ability to engineer the glass transition temperature (T_g) of carbohydrate glasses and the facile encapsulation and stabilization of proteins, nucleic acids, lipids, and volatiles into glass matrices have found uses in areas as diverse as the production of shelf-stable enzymes, therapeutics, biomembranes, and other biologicals,^[2, 3] the encapsulation of flavours and aromas, and the production of low moisture foods.^[1]

Although such glasses are efficient matrices for hydrophilic substances, problems arise with the incorporation of hydrophobic molecules, which invariably lead to the formation of biphasic sugar–dopant glasses. As part of our efforts to develop new reaction media for the glycosidase-mediated synthesis of O-glycosides, we undertook a study of mixtures of monosaccharides, alkyl glycosides, and various dopants. We now report that admixtures of lower alkyl glycosides with their parent monosaccharides form glasses akin to those of pure sugars. The T_g values of the glasses can be lowered by the inclusion of water and/or hydrophilic or hydrophobic molecules to form monophasic liquids (plasticized glasses) that are stable to phase separation. To our knowledge, this is the first report of sugar–alkyl glycoside glasses, and of the solubilization of hydrophobic species, including alkyl and arylalkyl alcohols, phenols, terpenols, and lipids, therein.

Starting with glucose, galactose, mannose, or 2-acetamido-2-deoxyglucose, mixtures containing 72–87% (all percentages quoted are the weight to weight ratio (w:w)) of ethyl, propyl, butyl, and/or but-3'-enyl glycoside and 13–28% of monosaccharide were synthesized by the addition of excess alcohol to a supersaturated solution of the sugar containing the requisite enzyme. Thus, a glass with a T_g value of 23–26°C and composed of D-glucose (11%), ethyl β -D-glucopyranoside (39%), propyl β -D-glucopyranoside (45%), water (3%), and propan-1-ol (2%) was obtained by the almond β -D-glucosidase mediated condensation of a solution of D-glucose with ethanol:propan-1-ol (1:1), followed by evaporation.

As with conventional sugar glasses, the addition of water or lower alcohols resulted in the dramatic depression of the T_g value (Figure 1).^[1] More importantly, we found that the glass

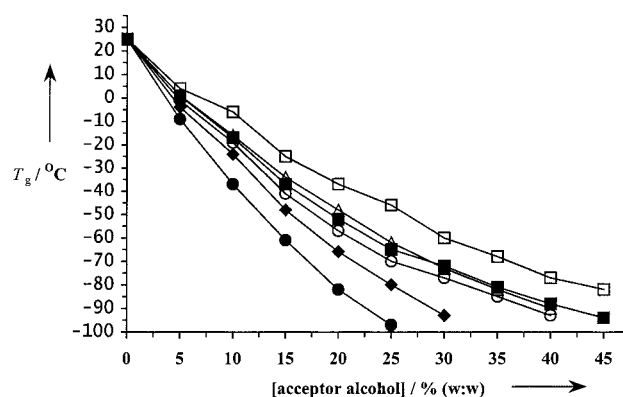


Figure 1. Plasticization of a D-glucose:ethyl β -D-glucoside:propyl β -D-glucoside:water:propanol glass (11:39:45:3:2) by various compounds. $\square$ = Benzyl alcohol, $\triangle$ = pent-4-en-1-ol, $\blacksquare$ = 2-hydroxyethyl methacrylate, $\circ$ = glycidol, $\blacklozenge$ = ethanol, and $\bullet$ = water.

could also be plasticized by other alcohols such as glycidol, 2-hydroxyethyl methacrylate (HEMA), pent-4-en-1-ol, and benzyl alcohol (Figure 1), with the depression in the T_g value being more pronounced for the more hydrophilic plasticizers. Further experiments showed that 41–68% of these plasticizers could be doped into the glasses without phase separation (Table 1). The resultant plasticized glasses were viscous, free-flowing isotropic liquids that were stable to phase separation and/or crystallization for over one week under ambient conditions, and displayed T_g values well below room temperature.

Differential scanning calorimetry (DSC) and microscopic studies of the system benzyl alcohol:(glucose:ethyl β -D-glucoside:propyl β -D-glucoside (12:41:47)):buffer uncovered six distinct regions in the phase diagram (Figure 2): (A) *monophasic glass*: a stable/metastable isotropic plasticized glass region for benzyl alcohol concentrations up to 53% and buffer contents up to 72%, with T_g values extending from below -70°C up to 44°C ; (B) *microemulsion-glass*: a solution of alkyl glucoside in buffer-saturated benzyl alcohol, dispersed as a microemulsion in a plasticized glass phase; (C) *macroemulsion-glass*: a two-phase system of benzyl alcohol:buffer:alkyl glucoside solution together with a plasticized glass phase; (D) *monophasic solution*: a saturated solution of alkyl glucoside in buffer-saturated benzyl alcohol; (E) *biphasic solution*: a biphasic system of alkyl glucoside in water-saturated benzyl alcohol, together with benzyl alcohol in a buffer/alkyl glucoside solution; (F) *monophasic solution*: a homogeneous, isotropic ternary solution of alkyl glucoside and benzyl alcohol in buffer.

Encouraged by these results, we examined the plasticization of sugar–alkyl glycoside glasses of α - and β -D-glucose and galactose, β -D-mannose, and *N*-acetyl- β -D-glucosamine by a variety of dopants including long-chain alcohols, phenols, amino acid derivatives, hydroxyacids, glycerolipids, sphingolipids, and cardenolides (Table 1). The T_g values of the anhydrous glasses ranged over 38–50°C, with the inclusion of 5–8% of water:propanol (w:w) depressing these to 18–25°C. Importantly, these glasses could be plasticized with 13–35% of hydrophobic dopants before phase separation was observed. Even hydrophobic compounds such as digi-

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Table 1. Plasticization of monosaccharide – alkyl glycoside glasses by various dopants (plasticizers).^[a]

Composition of pure glass phase ^[b] [%]	H ₂ O [%]	PrOH [%]	T _g ^[c] [°C]	Dopant	[Dop] _{sat} ^[d] [%]	T _g ^[e] [°C]
D-Glc:Et-β-D-Glcp:Pr-β-D-Glcp (12:41:47)	3	2	25 (44)	glycidol	68	< –100
	3	2	25 (44)	2-hydroxyethyl methacrylate	61	< –100
	3	2	25 (44)	pent-4-en-1-ol	56	< –100
	3	2	25 (44)	benzyl alcohol	41	–82
	3	2	25 (44)	hydroquinone	18	–28
	3	2	25 (44)	(R)-rhododendrol	32	–46
	3	2	25 (44)	(S)-N-Aloc-Ser-OMe	35	–50
	3	2	25 (44)	(R,S)-1-O-oleoylglycerol	19	–43
	3	2	25 (44)	sphingosine	23	–49
	3	2	25 (44)	digitoxigenin	11	–23
	5	10	–24 (44)	(R,S)-1-O-oleoylglycerol	28	–61
	5	10	–24 (44)	digitoxigenin	17	–60
D-Glc:Et-α-D-Glcp:Pr-α-D-Glcp (19:37:44)	3	1	27 (47)	geraniol	29	–77
D-Gal:Et-α-D-Galp:Pr-α-D-Galp (18:39:43)	4	1	22 (41)	nerol	24	–74
D-Gal:Et-β-D-Galp:Pr-β-D-Galp (16:36:48)	5	2	25 (50)	ricinoleic acid	14	–28
D-Man:Et-β-D-Manp:Pr-β-D-Manp (17:46:37)	6	2	18 (38)	vanillin	13	–45
D-GlcNAc:Et-β-D-GlcNAcp:Pr-β-D-GlcNAcp (15:59:26)	5	5	28 (54)	α-ionone	9	–37

[a] All percentages quoted are the weight to weight ratio. [b] Composition of the anhydrous sugar – alkyl glycoside glass. [c] T_g value of the anhydrous sugar – alkyl glycoside glass (in parenthesis) and the given monosaccharide:alkyl glycoside:water:propanol glass (without parenthesis). [d] Maximum amount of dopant possible without phase separation. [e] T_g value of the sugar:alkyl glycoside:water:propanol:dopant composition. The plasticized glasses were formed by heating the mixtures to above the T_g value of the pure sugar glasses, followed by cooling to room temperature.

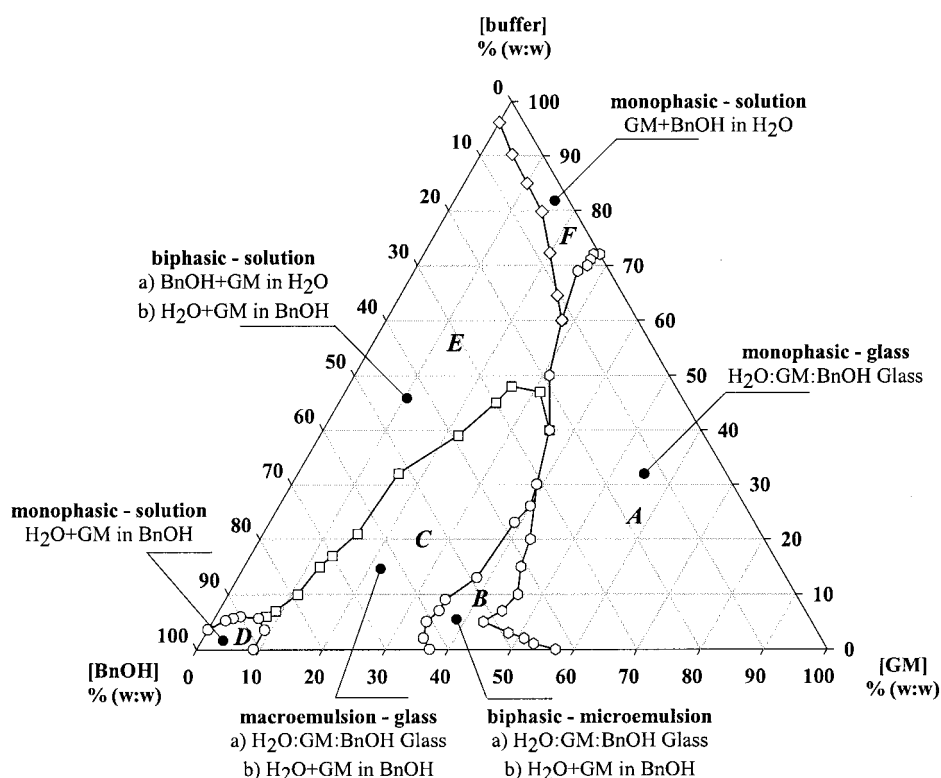


Figure 2. Ternary phase diagram for a system containing benzyl alcohol/glucose:(ethyl β-D-glucoside: (propyl β-D-glucoside (12:41:47))), and acetate buffer (50 mM, pH 6). The compositions were formed by heating the sugar – glucoside glass to 40 °C, followed by dilution with benzyl alcohol and/or buffer. The mixtures were allowed to equilibrate at room temperature for 24 h prior to examination by DSC and microscopy. Gm = glucose-glucoside mixture

toxigenin and ricinoleic acid could be incorporated at levels of 11–14% into the glasses, contrasting with their very low solubilities in pure monosaccharide glasses or aqueous solutions. Furthermore, the judicious elevation of the water:propanol contents of the glasses enabled significant increases in dopant concentrations, and also allowed the effective

solubilization of highly hydrophobic and nonhydroxylated molecules, such as α- and β-ionone (Table 1).

On examination of various amino acid, peptide, aromatic, lipid, and terpenoid acceptors, we discovered that above their saturating concentrations the plasticizers gave rise to a variety of microheterogeneous systems. In the simplest cases, these consisted of micro- and macroemulsions (higher alkyl alcohols, arylalkyl alcohols, ricinoleic acid) or solid suspensions (hydroquinones, cardenolides) of the excess dopant dispersed in the dopant-saturated plasticized glass (Figure 3b). However, in the cases of diols, diamides, and peptides, two-phase plasticized glass – eutectoid systems were readily formed (Figure 3c). Meanwhile, bicontinuous plasticized glass – liquid crystal phases were observed for glycerolipid and sphingolipid dopants (Figure 3d).

The results demonstrate the existence of a novel class of glasses derived from mixtures of monosaccharides and their alkyl glycosides, which are capable of solubilizing a variety of hydrophilic and hydrophobic compounds to form isotropic liquids. Preliminary results indicate the possibility of engineering the T_g values and plasticization profiles of the glasses by varying the alkyl glycosides used for glass formation, the ratios of the glass-forming components, and the amounts of modifiers such

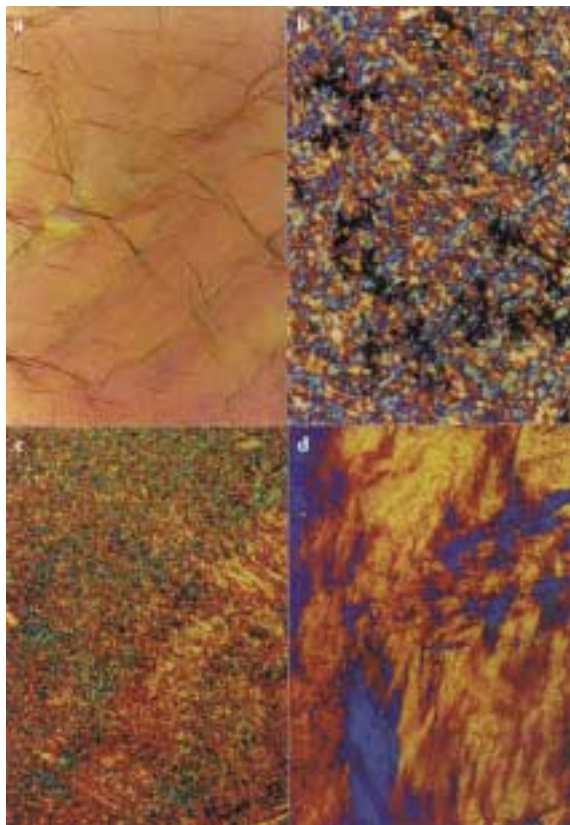


Figure 3. Examples of homogeneous and heterogeneous plasticized glass phases. a) Homogeneous glass: benzyl alcohol:(β -D-Glc:Et- β -D-Glcp:Pr- β -D-Glcp (12:41:47)):buffer:ethanol (4:4:1:1), -60°C . b) Heterogeneous suspension of a solid acceptor in a plasticized glass: hydroquinone:(β -D-Glc:Et- β -D-Glcp:Pr- β -D-Glcp (11:42)):buffer:ethanol (40:45:10:5), 30°C . c) Liquid eutectoid in plasticized glass: (*S,S*)-*N*¹-Aloc:serinylalanine methyl ester:(β -Gal:Et- β -D-Gal:Pr- β -D-Gal (16:36:48)):buffer:ethanol (25:55:10:10), 30°C . d) Liquid crystal in plasticized glass: (*R,S*)-1-oleoylglycerol:(β -Gal:Et- β -D-Galp:Pr- β -D-Galp (16:36:48)):buffer:ethanol (60:25:5:10), 30°C .

as water and lower alcohols. In addition to applications in the encapsulation/stabilization of hydrophobic bioactives and biological samples, such plasticized glasses are promising media for glycosidase-catalyzed glycosylations.

Experimental Section

Representative synthesis of glass-forming 12:41:47 β -D-Glc:Et- β -D-Glcp:Pr- β -D-Glcp mix. β -D-Glucose (100 g) was dissolved in hot acetate buffer (40 mL, 15 mM, pH 6, containing 5 mM of calcium and magnesium acetates), and the solution was cooled to 65°C . Ethanol (20 mL), then almond β -D-glucosidase (0.5 g in 5 mL of buffer containing 10 mM of 1,4-dithiothreitol), were added to the solution at 65°C , and ethanol:1-propanol (2:3, 500 mL) was added over 4 h. Further portions of the enzyme (1×0.5 g in 5 mL of buffer) and alcohol mixture (500 mL, over 6 h) were added, and reaction continued for 52 h. Filtration through silica gel, then rotary evaporation at 60°C gave the product as a pale yellow glass containing 3% of water and 2% of propanol (117 g, 88% of β -D-glucosides, $T_g = 23$ – 26°C). The other monosaccharide–glycoside glasses were synthesized in a similar manner, as described in the Supporting Information.

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- [1] a) E. Y. Shalaev, F. Franks, *J. Chem. Soc. Faraday Trans.* **1995**, *91*, 1511–1517; b) Y. Roos, *Carbohydr. Res.* **1993**, *238*, 39–48; c) Y. Roos, M. Karel, *J. Food Sci.* **1991**, *56*, 1676–1681; d) L. Finegold, F. Franks,

R. H. M. Hatley, *J. Chem. Soc. Faraday Trans. 1* **1989**, *85*, 2945–2951; e) F. Franks, *Pure Appl. Chem.* **1993**, *12*, 2527–2537.

- [2] a) F. Franks, *Biotechnology* **1994**, *12*, 253–256; b) T. Moreira, J. Pendas, A. Gutierrez, R. Pomes, J. Duque, F. Franks, *Cryo-Lett.* **1998**, *19*, 115–122; c) S. P. Duddu, P. R. DalMonte, *Pharm. Res.* **1997**, *14*, 591–595; d) W. Q. Sun, A. C. Leopold, L. M. Crowe, J. H. Crowe, *Biophys. J.* **1996**, *70*, 1769–1776; e) K. L. Koster, Y. P. Lei, M. Anderson, S. Martin, G. Bryant, *Biophys. J.* **2000**, *78*, 1932–1946.
- [3] a) J. H. Crowe, A. E. Oliver, F. A. Hoekstra, L. M. Crowe, *Cryobiology* **1997**, *35*, 20–30; b) F. A. Hoekstra, W. F. Wolters, J. Buitinik, E. A. Golovina, J. H. Crowe, L. M. Crowe, *Comp. Biochem. Physiol.* **1997**, *117*, 335–341.

Enzymatic Glycosylation in Plasticized Glass Phases: A Novel and Efficient Route to O-Glycosides**

Iqbal Gill* and Rao Valivety

Glycosidation is widely encountered in nature where it serves to modulate the biological activity, solubility, transport, bioavailability, and chemical/biological stability of aglycones.^[1,2] Major efforts have been invested into developing strategies for glycosidation,^[3] with enzymatic methods attracting especial interest, by virtue of their mildness, high selectivity, and acceptance of unprotected sugars as substrates.^[3b,4] The ability of glycosidases to use nonactivated sugars, their broad specificity for aglycones, and their wide availability, has made these enzymes attractive for synthetic applications.^[4] Thus, glycosidases have been used for the glycosylation of sugars and aza-sugars,^[5] aliphatic and aromatic alcohols,^[6,7] peptides,^[8] glycerides and sphingolipids,^[9] terpenoids,^[10] phenolics,^[11] alkaloids,^[12] and antibiotics.^[13]

In the preceding communication we disclosed the formation of plasticized glasses by mixtures of monosaccharides, alkyl glycosides and various hydrophilic and hydrophobic compounds. We now describe the application of these liquids as media for glycosidase-catalyzed reactions. Plasticized glasses support high concentrations of both acceptor and sugar donor and enable the synthesis of a variety of glycosides (Scheme 1) in good yields and with unprecedented productivities.

Despite extensive synthetic applications of glycosidases, the difficulties encountered in accommodating solvent requirements of the substrates, thermodynamic considerations, and prerequisites for efficient biocatalyst function have limited

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