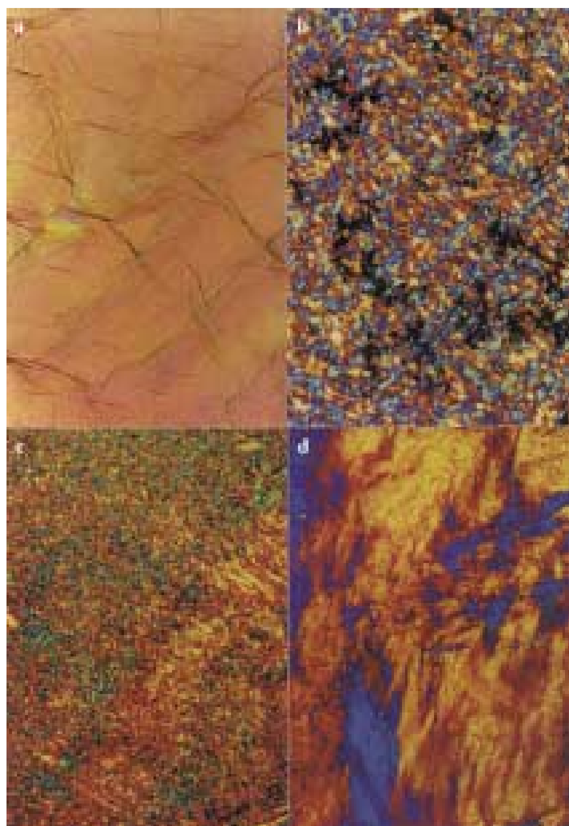

Figure 3. Examples of homogeneous and heterogeneous plasticized glass phases. a) Homogeneous glass: benzyl alcohol:(D-Glc:Et-β-D-Glcp:Pr-β-D-Glcp (12:41:47)):buffer:ethanol (4:4:1:1), −60°C. b) Heterogeneous suspension of a solid acceptor in a plasticized glass: hydroquinone:(D-Glc:Et-β-D-Glcp:Pr-β-D-Glcp (11:42)):buffer:ethanol (40:45:10:5), 30°C. c) Liquid eutectoid in plasticized glass: (*S,S*)-$N^1$-Aloc:serinylalanine methyl ester:(D-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:(D-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 D-Glc:Et-β-D-Glcp:Pr-β-D-Glcp mix. 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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## 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 preceeding 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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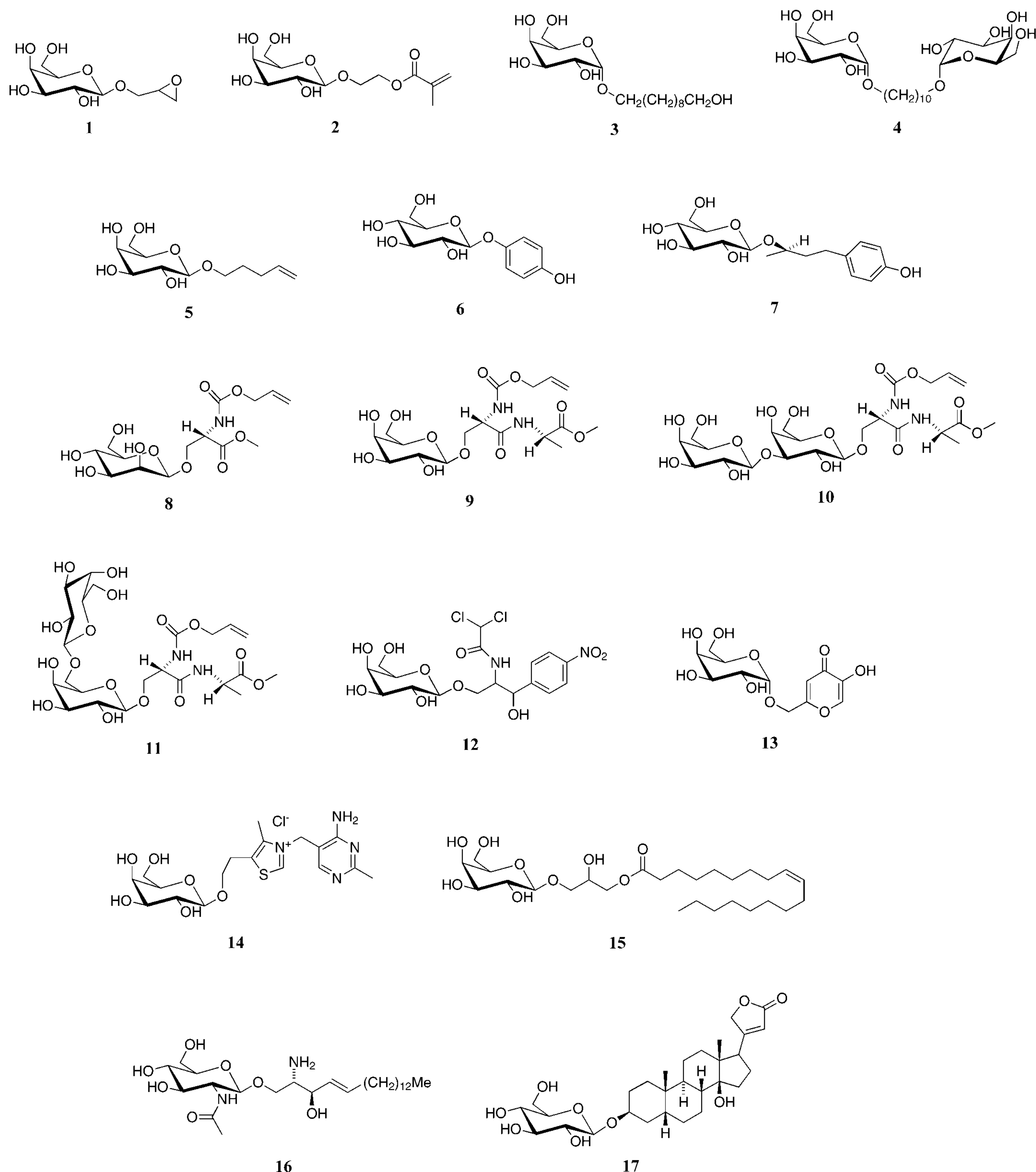


Scheme 1.

the synthetic efficiencies, notably with hydrophobic acceptors. The problems are: (1) The native enzymes perform poorly in aqueous or organic media with water contents below 5–10% (all percentages are weight to weight ratios (w/w) unless otherwise stated); (2) the thermodynamic considerations require the water content to be minimized for maximal yields; (3) it is difficult to satisfy the divergent solvent requirements of hydrophilic sugar donors and hydrophobic acceptors; and (4) glycosidases do not tolerate high levels of solvents such as DMF, DMSO, and pyridine, which have been employed for overcoming solubility issues in other hydrolase-catalyzed syntheses.[4d]

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To circumvent these issues, we examined whether sugar/alkyl glycoside/dopant plasticized glasses could be applied as reaction media. First, we examined the performance of almond $\beta$-glucosidase in liquids formed by plasticizing a 12/41/47 D-glucose/ethyl $\beta$-D-glucoside/propyl $\beta$-D-glucoside glass with glycidol, 2-hydroxyethyl methacrylate (HEMA), pent-4-en-1-ol, or benzyl alcohol. In all cases, the enzyme was found to readily catalyze glycosylation, providing that the initial water content was approximately 10% or higher. In Figure 1, the benzyl $\beta$-D-glucoside formation profiles are compared for a plasticized glass, the devitrified glass (obtained by freeze–thaw cycling), a biphasic system, and a solution in acetonitrile. The best performance was attained with the plasticized glass, which gave a final yield of 67%, corresponding to a benzyl $\beta$-D-glucoside concentration of 1.3 M (0.35 g per gram of reaction mixture). This compares very well with the yields of 53% (0.11 M) and 12% (0.024 M) and productivities of 0.03 and 0.007 g per gram of reaction mixture that were observed for the biphasic and acetonitrile media, respectively.

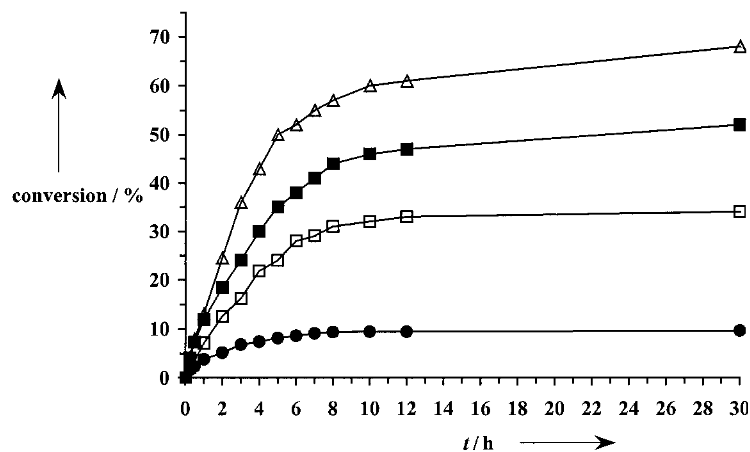


Figure 1. Almond $\beta$-glucosidase mediated formation of benzyl $\beta$-d-glucopyranoside in conventional media and plasticized glass phases. △ = Plasticized glass ($T_g < -50$ °C), with 40% glucose–glucoside (1.91 M), 40% benzyl alcohol (3.74 M), 10% acetate buffer (50 mM, pH 6, with 10 mM of calcium and magnesium acetates and DTT), and 10% ethanol. ■ = Biphasic medium, with 10% (v/v) glucose–glucoside solution (2.0 M in buffer, 0.2 M sugar) and 90% (v/v) benzyl alcohol (8.33 M). □ = Devitrified glass, consisting of glucose–glycoside saturated buffer, a benzyl alcohol rich phase, and solid glucose–glycoside. ● = Organic solvent medium, with 10% (v/v) aqueous glucose–glucoside solution (2.0 M), and 90% (v/v) of acetonitrile containing 1.1 M benzyl alcohol. A 12/41/47 mix of D-Glc/Et-$\beta$-D-Glcp/Pr-$\beta$-D-Glcp was used as the sugar donor. All reactions were run at 60 °C using enzyme (100 mg mL$^{-1}$ in buffer) at 100 U g$^{-1}$. Sealed, stirred vials were used for the conventional reactions, and static vials, closed for 2 h and open thereafter, for the glass-phase reactions.

In addition to almond $\beta$-glucosidase, $\beta$-galactosidases from *K. fragilis*, *E. coli*, *A. niger*, and *A. oryzae*, and the $\alpha$-galactosidases of *A. niger* and *C. arabica*, also catalyzed the glycosylation of glycidol, HEMA, pent-4-en-1-ol, and long-chain diols capable of forming plasticized glasses, providing that sufficient water (8–13%) was present to activate the biocatalyst (Table 1). Plasticized glasses doped with 20–60% of the acceptors furnished yields of 63–71%, corresponding to productivities of 0.31–0.65 g per gram of reaction mixture. Homogeneous liquids were not essential, with reactions also readily proceeding in micro- and macroemulsions, which comprised of excess acceptor dispersed in acceptor-saturated plasticized glass, albeit with lower rates and yields.

Analysis of reaction mixtures during the course of benzyl $\beta$-D-glucoside formation in a system with starting composition D-Glc (4.8), Et-$\beta$-D-Glcp (16.4), Pr-$\beta$-D-Glcp (18.8), BnOH (40), EtOH (10), and $H_2O$ (10%) indicated that most of the conversion occurred by transglycosylation rather than by reverse hydrolysis. Thus, after 4 h, the conversion of 41% of glucose/glycoside substrates into benzyl $\beta$-D-glucoside was largely at the expense of Et-$\beta$-D-Glcp and Pr-$\beta$-D-Glcp, the amounts of which decreased by 42 and 51% respectively, while the amount of glucose decreased by only 9%. Analysis of rate data indicated that the rate of transglycosylation exceeded that of reverse hydrolysis by a factor of 6.7–9.1. After 42 h, 67% of the substrates had been transformed into benzyl $\beta$-D-glucoside, with net corresponding decreases of 23, 81, and 93% in D-Glc, Et-$\beta$-D-Glcp, and Pr-$\beta$-D-Glcp, respectively. The final, dry reaction mixture had the composition D-Glc (4.7), Et-$\beta$-D-Glcp (5.6), Pr-$\beta$-D-Glcp (3.3), BnOH (35.1), Bn-$\beta$-D-Glcp (46.2), and oligosaccharides (5.1%).

We then examined systems where the acceptor was solid and only partially soluble in the glass phase. A 40/45/10/5 mix of hydroquinone/glucose–alkyl glucoside/buffer/ethanol, which contained the majority of the acceptor as crystalline solid, was used as the test system. Despite the heterogeneous medium, $\beta$-D-glucosidases from *P. dulcis* and *T. reesei* and bovine liver $\beta$-D-glucuronidase mediated glycosylation to afford hydroquinone $\beta$-D-glucoside with respective yields of 26, 19, and 65% and productivities of 0.18, 0.13, and 0.46 gram per gram of reaction mixture. Encouraged by these results, we attempted the glycosylation of other partially miscible/soluble acceptors which furnished heterogeneous mixtures with sugar–glycoside glasses (Table 1). As before, upon including approximately 5–10% of water and by operating at 40–60 °C above $T_g$ in order to ensure a low-viscosity plasticized glass, biocatalysis proceeded smoothly to furnish the required glycosides in good yields. Yields of 39–58% were obtained, with resulting productivities of 0.25–0.49 g per gram of reaction mixture, the latter exceeding those of conventional systems described to date by one order of magnitude or more.

In conclusion, glycosidases are catalytically active in plasticized glasses and mediate the glycosylation of a variety of acceptors. The high substrate concentrations enabled in these media can result in synthesis rates of up to one magnitude higher than for dilute systems, and can furnish product concentrations of 0.13–0.65 g per gram of reaction mixture.

## *Experimental Section*

Representative synthesis of a glycoside in a plasticized glass phase. **15**: A D-Gal/Et-$\beta$-D-Galp/Pr-$\beta$-D-Galp mixture (16/36/48, 0.42 g) was mixed with metaphosphate buffer (40 µL, 50 mM, pH 7, with 10 mM each of calcium and magnesium acetates) and ethanol (0.21 mL) in a vial. The vial was then sealed and the mixture heated until a homogeneous liquid was formed. After cooling, (*R*,*S*)-1-*O*-oleoylglycerol (1.0 g) was added, the mix incu-

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Table 1. Examples of glycosidation catalysed by glycosidases in plasticized glass phases.

| Product | Reaction mixture components (concentration [%]) | Scale [mmol] | System[a] ($T_t$ [°C]) | Enzyme | $T$ [°C] | $t$ [h] | Yield[b] [%] | [g g$^{-1}$] |
|---|---|---|---|---|---|---|---|---|
| **1** | D-Gal (8), Et-$\beta$-D-Gal (18) | 47.8 | type I | *K. fragilis* $\beta$-D-galactosidase | 40 | 26 | 71 (64)[c] | 0.48 |
| | Pr-$\beta$-D-Gal (24), $H_2O$ (10) | 4.78 | $T_g < -80$ | *E. coli* $\beta$-D-galactosidase | 40 | 26 | 67 | 0.44 |
| | (*R*,*S*)-glycidol (40) | | | | | | | |
| **2** | D-Gal (7), Et-$\beta$-D-Gal (16) | 17.5 | type I | *E. coli* $\beta$-D-galactosidase | 35 | 40 | 67 (58)[d] | 0.50 |
| | Pr-$\beta$-D-Gal (22), $H_2O$ (10), HEMA (45) | 17.5 | $T_g < -80$ | *A. niger* $\beta$-D-galactosidase | 35 | 40 | 43 | 0.32 |
| **3** | D-Gal (3), Et-$\alpha$-D-Gal (7) | 9.50 | type I | *A. niger* $\alpha$-D-galactosidase | 40 | 26 | 74 (71)[e] | 0.31 |
| | Pr-$\alpha$-D-Gal (10), $H_2O$ (10), EtOH (10) | 0.95 | $T_g = -34$ | *C. arabica* $\alpha$-D-galactosidase | 40 | 50 | 31 | 0.13 |
| | 1,10-dihydroxydecane (60) | | | | | | | |
| **4** | D-Gal (9), Et-$\alpha$-D-Gal (22) | 17.2 | type I | *A. niger* $\alpha$-D-galactosidase | 40 | 30 | 63 (58)[f] | 0.65 |
| | Pr-$\alpha$-D-Gal (29), $H_2O$ (10), EtOH (10) | | $T_g = -34$ | | | | | |
| | 1,10-dihydroxydecane (20) | | | | | | | |
| **5** | D-Gal (6), Pr-$\beta$-D-Gal (34) | 18.6 | type I | *A. oryzae* $\beta$-D-galactosidase | 40 | 50 | 64 (60)[g] | 0.47 |
| | $H_2O$ (10), EtOH (10), pent-4-en-1-ol (40) | 9.30 | $T_g = -63$ | *E. coli* $\beta$-D-galactosidase | 40 | 50 | 39 | 0.30 |
| **6** | D-Glc (5), Et-$\beta$-D-Glc (19) | 21.1 | type II | bovine $\beta$-D-glucuronidase | 40 | 30 | 65 (59)[h] | 0.46 |
| | Pr-$\beta$-D-Glc (21), $H_2O$ (10), EtOH (5) | 21.1 | $T_g = -47$ | almond $\beta$-D-glucosidase | 60 | 36 | 26 | 0.18 |
| | hydroquinone (40) | 21.1 | | *T. reesei* $\beta$-D-glucosidase | 40 | 62 | 19 | 0.13 |
| 7 | D-Glc (4), Et-$\beta$-D-Glc (14), | 6.64 | type II | almond $\beta$-D-glucosidase | 70 | 20 | 58 (54)[i] | 0.49 |
| | Pr-$\beta$-D-Glc (17), $H_2O$ (10), EtOH (5) | 1.66 | $T_g = -61$ | bovine $\beta$-D-glucuronidase | 35 | 52 | 38 | 0.31 |
| | (*R*)-rhododendrol (50) | | | | | | | |
| 8 | D-Man (4), Et-$\beta$-D-Man (9) | 3.03 | type III | *H. aspersa* $\beta$-D-mannosidase | 40 | 60 | 52 (48)[j] | 0.26 |
| | Pr-$\beta$-D-Man (7), $H_2O$ (10), EtOH (10) | | $T_e = 27$ | | | | | |
| | (*S*)-*N*-Aloc-Ser-OMe (60) | | $T_g = -68$ | | | | | |
| **9** | D-Gal (3), Et-$\beta$-D-Gal (7) | 6.37 | type IV | *E. Coli* $\beta$-D-galactosidase | 45 | 44 | 45 (41)[k] | 0.25 |
| | Pr-$\beta$-D-Gal (10), $H_2O$ (10), EtOH (10) | 1.90 | $T_e = 21$ | *K. fragilis* $\beta$-D-galactosidase | 40 | 52 | 28 | 0.15 |
| | (*S*,*S*)-$N^1$-Aloc-Ser-Ala-OMe (60) | 1.90 | $T_g = -64$ | *A. oryzae* $\beta$-D-galactosidase | 40 | 52 | 37 | 0.21 |
| **10** | D-Gal (9), Et-$\beta$-D-Gal (20) | 7.28 | type V | *E. Coli* $\beta$-D-galactosidase | 45 | 38 | 46 (41)[l] | 0.31 |
| **11** | Pr-$\beta$-D-Gal (26), $H_2O$ (10), EtOH (10) | | $T_e = 16$ | | | | 24 (21)[l] | 0.16 |
| | (*S*,*S*)-$N^1$-Aloc-Ser-Ala-OMe (25) | | $T_g = -57$ | | | | | |
| **12** | D-Gal (5), Et-$\beta$-D-Gal (11) | 7.20 | type II | *A. oryzae* $\beta$-D-galactosidase | 35 | 36 | 41 (37)[m] | 0.35 |
| | Pr-$\beta$-D-Gal (14), $H_2O$ (10), EtOH (5) | 1.44 | $T_g = -73$ | *E. coli* $\beta$-D-galactosidase | 35 | 36 | 26 | 0.21 |
| | chloramphenicol (50), *n*PrOH (5) | 1.44 | | *K. fragilis* $\beta$-D-galactosidase | 40 | 36 | 32 | 0.28 |
| **13** | D-Glc (5), Et-$\alpha$-D-Glc (11) | 3.60 | type II | *S. cerevisiae* $\alpha$-D-glucosidase | 40 | 60 | 43 (38)[n] | 0.29 |
| | Pr-$\alpha$-D-Glc (14), $H_2O$ (10), EtOH (5) | | $T_g = -59$ | | | | | |
| | kojic acid (50%), *n*PrOH (5) | | | | | | | |
| **14** | D-Gal (5), Et-$\beta$-D-Gal (11) | 7.15 | type II | *A. oryzae* $\beta$-D-galactosidase | 40 | 48 | 45 (41)[o] | 0.38 |
| | Pr-$\beta$-D-Gal (14), $H_2O$ (10), EtOH (10) | 1.43 | $T_g = -62$ | *K. fragilis* $\beta$-D-galactosidase | 40 | 48 | 27 | 0.21 |
| | thiamine chloride (50) | | | | | | | |
| 15 | D-Gal (4), Et-$\beta$-D-Gal (9) | 1.98 | type VI | *E. coli* $\beta$-D-galactosidase | 40 | 36 | 42 (38)[p] | 0.31 |
| | Pr-$\beta$-D-Gal (12), $H_2O$ (5), EtOH (10) | | $T_g = -51$ | *A. oryzae* $\beta$-D-galactosidase | 40 | 36 | 21 | 0.14 |
| | (*R*,*S*)-1-*O*-oleoylglycerol (60) | | | *K. fragilis* $\beta$-D-galactosidase | 40 | 36 | 37 | 0.28 |
| **16** | D-GlcNAc (4), Et-$\beta$-D-GlcNAc (8) | 1.23 | type VI | *A. oryzae* $\beta$-D-*N*-acetylhexosaminidase | 42 | 35 | 48 (44)[q] | 0.36 |
| | Pr-$\beta$-D-GlcNAc (18), $H_2O$ (10), EtOH (5) | | $T_g = -62$ | | | | | |
| | sphingosine (50), *n*PrOH (5) | | | | | | | |
| **17** | D-Glc (3), Et-$\beta$-D-Glc (8) | 1.34 | type II | bovine $\beta$-D-glucuronidase | 40 | 43 | 39 (36)[r] | 0.28 |
| | Pr-$\beta$-D-Glc (9), $H_2O$ (10), EtOH (10) | 0.54 | $T_g = -75$ | almond $\beta$-D-glucosidase | 60 | 46 | 21 | 0.16 |
| | digitoxigenin (50), *n*PrOH (10) | 0.54 | | *T. reesei* $\beta$-D-glucosidase | 40 | 69 | 35 | 0.24 |

[a] Phase types determined by microscopy and differential scanning calorimetry (DSC): I, homogeneous plasticized glass; II, solid acceptor suspended in plasticized glass; III, microemulsion of acceptor in plasticized glass; IV, plasticized glass in liquid eutectoid of acceptor; V, liquid eutectoid of acceptor in plasticized glass; VI, liquid crystal of acceptor in plasticized glass. $T_t$ = transition temperature, $T_g$ = glass transition temperature, $T_e$ = eutectic temperature. [b] The first figure is the conversion as determined by HPLC analysis, the figure in parentheses is the isolated yield (based on the limiting substrate), and the third figure is the quantity obtained, in grams, per gram of reaction mixture; Higher glycosides were also formed. [c] 10% of diglycosides formed. [d] 8% of diglycosides and 5% of triglycosides formed. [e] 11% of **4** formed. [f] 13% of tri- and tetraglycosides formed. [g] 11% of di- and triglycosides formed. [h] 9% of di- and triglycosides formed. [i] 15% of diglycosides formed. [j] 14% of diglycosides formed. [k] 16% of a mixture of **10** and **11** formed. [l] 11% of triglycosides formed. [m] 23% of diglycosides formed. [n] 16% of di- and triglycosides formed. [o] 19% of diglycosides formed. [p] 10% of 2-O-glycosides and 7% of diglycosides formed. [q] 18% of diglycosides and 12% of triglycosides formed. [r] 24% of di- and triglycosides formed. $T_e$, eutectic temperature.

bated at 40°C, and *E. coli* $\beta$-D-galactosidase (5 mg in 40 μL of 100 mM buffer containing 10 mM DTT) added with thorough mixing. The vial was sealed, placed in a heating block (40°C) for 3 h, and then opened, and the reaction was allowed to continue for 33 h. The mix was extracted with methanol (3 × 3 mL), and the extract was concentrated by rotary evaporation, then purified by medium pressure chromatography to give the product as a pale yellow oil (0.40 g, 38% yield, 95% purity). This and the other glycosides were fully characterized as detailed in the Supporting Information.

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# $(CN_3H_6)_4[Zn_3(SeO_3)_5]$: The First Organically Templated Selenite**

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An astonishing variety of inorganic networks templated by organic species have been reported over the last 10 years.[1] A great deal of attention has been paid to the structure-directing role of the organic species,[2] and the structural effect of variously coordinated cations, for example distorted octahedral vanadium[3] and pyramidal tin(II).[4] Less exploratory work has been carried out on the "anionic" part of the inorganic network, and most groups reported so far (phosphate,[5] germanate,[6] etc.) invariably adopt tetrahedral coordination. The possibilities of incorporating the pyramidal $[HPO_3]^{2-}$ hydrogen phosphite group into extended structures templated by *inorganic*, alkaline earth cations was explored a few years ago.[7] Herein we report the synthesis, crystal structure, and some properties of $(CN_3H_6)_4[Zn_3(SeO_3)_5]$, the first organically templated phase to contain the pyramidal selenite $[SeO_3]^{2-}$ ion.

$(CN_3H_6)_4[Zn_3(SeO_3)_5]$, which is built up from 13 framework atoms, consists of layers of distorted $ZnO_4$ tetrahedra and $SeO_3$ groups, sharing vertices. The two distinct zinc atoms both form four Zn-O-Se bonds to selenium atom neighbors resulting in average Zn1–O and Zn2–O bond lengths of 1.950(4) and 1.972(4) Å, respectively. Zn2 has twofold rotational symmetry. The three crystallographically distinct selenium(IV) atoms adopt their characteristic pyramidal coordination, with the lone pair of electrons presumably directed towards the fourth tetrahedral vertex. Average Se–O bond lengths of 1.679(4), 1.682(4), and 1.691(4) Å result for Se1, Se2, and Se3, respectively, in good agreement with previous studies.[8] The terminal Se1–O7 and Se3–O8 bonds are short ($d = 1.648(4)$ and 1.648(5) Å, respectively), indicating that the oxygen atoms are not protonated.[8] Se3 occupies a crystallographic mirror plane. The average Zn-O-Se bond angle of the six bridging O atoms is 124.9° (spread of values: 119.3(2)–127.7(2)°).

The connectivity of the $ZnO_4$ and $SeO_3$ units in $(CN_3H_6)_4[Zn_3(SeO_3)_5]$ results in infinite, anionic layers of stoichiometry $[Zn_3(SeO_3)_5]^{4-}$ which propagate normal to [010]. A novel grouping of three adjacent $ZnO_4$ tetrahedra

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