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# Solid-phase Extraction on C18 Silica as a Purification Strategy in the Solution Synthesis of a 1-Thio-β-D-galactopyranoside Library

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**Abstract**—A novel strategy for the purification of carbohydrate-based chemical libraries synthesized in solution was developed. Purification of reaction products was accomplished by means of solid-phase extraction enabled by protecting the 2-, 3-, 4-, and 6-hydroxyl groups of a galactose derivative as their hydrophobic *O*-laurates. The presence of multiple *O*-laurates allowed adsorption of reaction products onto C18 silica while reagents and by-products were washed away with MeOH. Products were quantitatively eluted with pentane. Purification of products using solid-phase extraction offers the combined advantages of solution synthesis (normal solution reactivity and ease of reaction monitoring) with those of solid-phase synthesis (facile product isolation permitting the use of large excesses of reagents). To demonstrate the utility of the hydrophobic recovery-procedure, tetra-*O*-lauroyl-β-D-galactopyranose-1-thiol was subjected to high-yielding reactions with a panel of Michael-acceptors and an α-chloro ketone. The resulting ketone adducts were then either reduced to the alcohols or reductively aminated with a selection of amino acids to give 30 different 1-thio-β-D-galactosides as mixtures of four diastereomers after removal of protecting groups. At each step, the product was separated from the reagents and their by-products by simple adsorption onto C18 silica, washing with MeOH and elution of product with pentane. After completion of the combinatorial chemistry sequence, the *O*-laurates were cleaved by methanolysis and the product methyl laurate in turn removed from the desired water-soluble products by C18 adsorption. Individual library members were thus conveniently produced on 10–30 mg scales at purity levels of >90%. One of the 1-thio-β-D-galactosides thus produced was found to be a competitive inhibitor of the β-galactosidase from *E. coli* with $K_i$ value of 1.7 μM. 

## Introduction

The design and synthesis of potent inhibitors of oligosaccharide-binding proteins is a challenging task, complicated by the fact that most of the natural carbohydrate ligands bind with low affinity ($K_d$ normally in the 0.1–1 mM range). The multistep synthesis of oligosaccharide analogues, essentially a medicinal chemistry approach, is one strategy to enhancing the affinity of the natural ligand. While several examples exist where significant enhancements have been produced[1] by this approach, sometimes guided by the availability of protein crystal data and molecular modeling,[2] the process is nevertheless extremely laborious and often fails.

Application of combinatorial chemistry to the enhancement of ligand affinity is an increasingly attractive strategy whose main advantage is the ability to produce a large number of active-site probes rapidly. There are several reports on the production of oligosaccharide libraries,[3–5] including one wherein the affinity of the a disaccharide for a lectin was enhanced as a result of appending functionality on the parent (or related) disaccharide bound by a lectin.[5] This particularly impressive

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work produced over 3000 disaccharide derivatives which were screened to yield the most active compound.

The binding of an oligosaccharide by the combining site of a protein, whether a lectin, selectin, antibody, toxin, or enzyme, is schematically represented in Figure 1(A). A key feature of the binding is that only a portion of the oligosaccharide is usually in intimate contact with the protein, the average combining site recognizing approximately 2.5 sugar residues.[6,7] Of these, often only one is completely buried in the protein,[8] the so called 'immunodominant sugar' for antibodies or 'key sugar' for other proteins. The key-monosaccharide usually binds only weakly and 10–100 mM concentrations can be required to inhibit the binding of the natural oligosaccharide. As shown in Figure 1(A), additional binding energy is derived because the remaining sugar residues on the oligosaccharide provide additional interaction with the protein combining site.

The most followed strategy for enhancing the affinity of an oligosaccharide ligand has therefore been to synthesize molecules containing all of the sugars that make contact with the protein and then to alter these oligosaccharides, either through step-wise synthesis or combinatorial techniques, to yield additional interactions. We present here an alternative approach, where only the key sugar unit, despite being weakly inhibitory on its own, is retained in the ligands in order to retain the carbohydrate-binding specificity of the natural structure. The approach is then to increase the affinity of this key sugar by the addition of additional molecular structure that is unrelated to the sugars of the natural oligosaccharide ligand. The resulting type of molecule, which is particularly amenable to synthesis using combinatorial chemistry approaches, is schematically shown in Figure 1(B) where the new molecular features are introduced on a scaffold at the reducing end of the key sugar, though it could in principle be introduced at any position not making critical interactions with the protein.

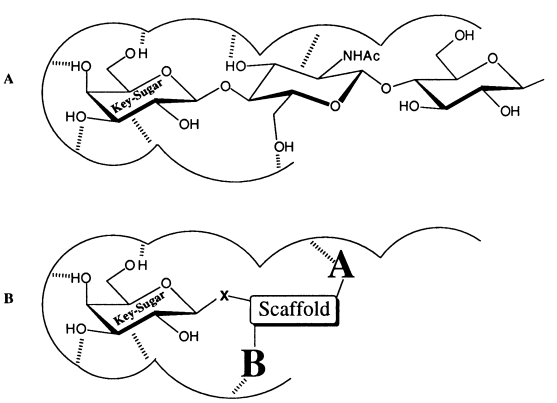



**Figure 1.** Schematic of the binding of an oligosaccharide by a protein. (**A**) The binding of the natural oligosaccharide showing a buried key sugar residue and additional contacts with adjacent sugar units. (**B**) A molecule containing the key sugar residues and making additional non-carbohydrate interactions with the proteins.

The main strategic issues to be addressed in initiating this project were:

(a) **What types of structures should be considered as scaffolds?** Since proteins binding terminal sugars, as shown schematically in Figure 1(A), normally encounter a second sugar structure as the aglycone they should be easily able to sterically accommodate cyclic scaffolds at this site. Scaffolds chosen were therefore 5-, 6-, and 7-membered rings.

(b) **What types of molecular features are desirable for A and B (Fig. 1(B))?** This would also dictate the types of chemical reactions to be used for their preparation. In situations where little is known about the target protein, a wide variety of structures should be examined and there should be no bias based on the structure of the natural ligand in the selection of the groups A and B (Figure 1(B)). Typical structures that might make beneficial interactions with the protein combining site or residues adjacent to it, include diverse polar-neutral, hydrophobic, aromatic, cationic, or anionic groups. The chemistry should therefore be flexible in order to allow a large variety of such structures to be covered by A and B.

(c) **How can the intermediates and final products be efficiently purified?** Combinatorial chemistry is usually performed on a relatively small scale with multiple reactions run in parallel. The challenge of rapid product purification has been addressed in several ways, especially using solid-phase organic synthesis. Although solid-phase synthesis[9] allows for the very convenient removal of excess reagents by simple filtration, it suffers from disadvantages relative to conventional organic synthesis in solution. Reaction monitoring is difficult, often anomalous reaction rates are encountered, and insoluble reagents or catalysts can usually not be used.

Recently, reports on solution-phase syntheses of chemical libraries have appeared. Some reports[10,11] relied on conventional silica gel column chromatography for purification, while others presented innovative procedures for the purification of products formed in solution. Immobilization of a substrate onto a precipitable polymer enabled purification of products by simple filtration.[12] This method allowed the use of large excesses of reagents as long as they were not precipitated.

Solution synthesis of a carboxamide library has been reported, where purification relied on extraction of reagents into acidic or alkaline aqueous phases leaving the neutral reaction products in the organic phase.[13] Such an approach does allow simple TLC monitoring of reaction progress but requires that reagent excess and by-products be either basic or acidic. A tetrasubstituted ethylene library was synthesized in solution and the products covalently captured on a resin.[14] This approach preserved the attractive features of performing the reactions in solution while still taking advantage of the easy purification of a product attached to a resin. However, if a second synthetic step is desired after resin capture, it has to be performed on the resin. Similar approaches, which capture excess reagents instead of products, on a resin, have also been reported.[15–17] Thus, a second step could in this case be performed in solution. Finally, an elegant protocol, based on labeling products with a perfluorinated alkylsilyl group, was recently introduced for solution synthesis of chemical libraries.[18,19] Three-phase liquid extraction with water, dichloromethane, and perfluorohexanes, allowed selective extraction of the labeled product into the perfluorohexanes. The method was recently extended to solid-phase, where fluorous reversed phase silica was used to extract perfluorinated alkylsilylated products from reaction mixtures.[20] Washing of the fluorous reversed phase silica with acetonitrile removed reagents, whereafter perfluorinated alkylsilylated products were eluted with hexane.

In the present work, we develop a novel approach to purifying carbohydrate-based libraries synthesized in solution by hydrophobic tagging of the carbohydrate starting material to facilitate solid-phase extraction on C18 silica. Hydrophobic tagging is a well-established technique to enhance the chromatographic properties of molecules, including carbohydrates,[3,21] but has not yet been used to enable solid-phase extraction for purification of combinatorial libraries. Since the primary characteristics of carbohydrates is their poly-hydroxylation, we chose this feature to distinguish carbohydrate-containing molecules from the organic reagents normally used in combinatorial chemistry (to generate the scaffold and groups A and B, Fig. 1(B)). This was achieved by protecting the sugar OH-groups with long-chain hydrophobic C-18 *O*-laurates which permitted the quantitative adsorption of reaction products derived from the starting sugar onto reverse-phase C18 silica gel from which organic reagents and by products were completely eluted by washing with methanol (Fig. 2). Lauroylated carbohydrate-derived products were then isolated by elution with pentane. The entire recovery procedure including solvent exchanges and evaporation takes 10–15 min per sample, and the reactions are easily monitored by TLC.

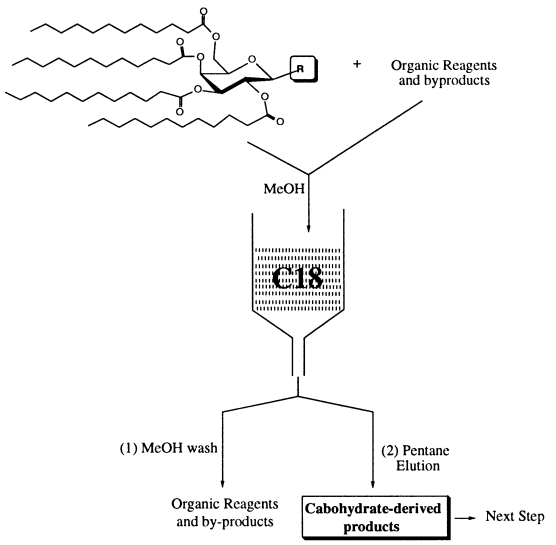
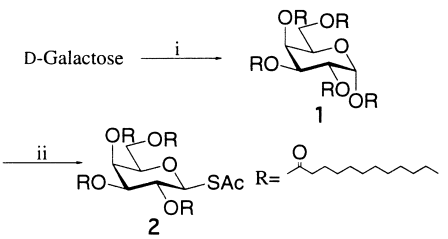


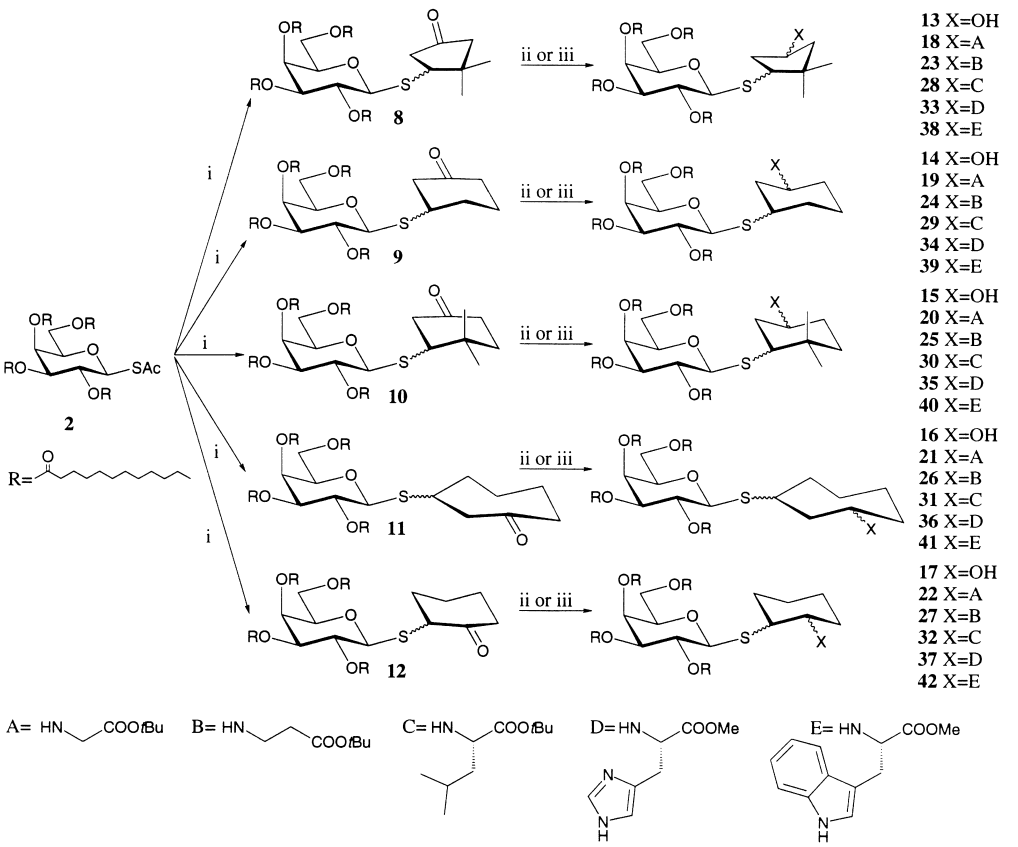

**Figure 2.** Solid-phase extraction of *O*-lauroylated sugars onto C18 reverse-phase silica.

In order to validate this method for the purification of carbohydrate libraries, we selected the construction of a small library terminating with the potential key sugar β-D-Gal, thus yielding potential ligands for β-Gal-binding lectins and β-galactosidase enzymes. A 1-thio-β-D-Gal derivative (**2**), tagged with *O*-laurates, was synthesized (Scheme 1) and used, after de-*S*-acetylation, in addition reactions to four Michael acceptors (**3**–**6**) and one substitution of an α-halo-ketone (**7**). The anomeric oxygen was replaced by the more nucleophilic sulfur in **2** to permit these rapid and high-yielding reactions. The five carbonyl compounds thus formed (**8**–**12**, Scheme 2) were subsequently reduced to the corresponding alcohol or reductively aminated with glycine, β-alanine, L-leucine, L-histidine, and L-tryptophan derivatives to produce a 1-thio-β-D-Gal based library of thirty (5×6) compounds produced as mixtures of four diastereomers each (i.e. 120 different 1-thio-β-D-galactosides, Fig. 4).

This approach to a small β-D-Gal library was chosen for several reasons. First, Michael additions and halide




**Scheme 1.** Reagents: (i) Lauroyl chloride, pyridine, DMAP, pentane, 86%; (ii) AcSH, $CF_3SO_3SiMe_3$, $CH_2Cl_2$, 77%.




**Scheme 2.** Reagents: (i) **3**–**7**, $CH_2Cl_2$, $Et_2NH$, 68–94%; (ii) $NaBH_4$, $(CH_3)_2CHOH$, THF, 71–98%; (iii) Glycine *t*-butyl ester hydrochloride, β-alanine *t*-butyl ester hydrochloride, L-leucine *t*-buthyl ester hydrochloride, L-histidine methyl ester hydrochloride, or L-tryptophan methyl ester hydrochloride, $NaCNBH_3$, MeCN, THF, 73–99%.

substitution by thiols, as well as reductions and reductive aminations of ketones, are general and high-yielding reactions. Second, a large number of Michael acceptors, α-halo-carbonyl compounds and amines (including amino acids) are commercially available, which ensures rapid access to large and diverse libraries.

## Results and Discussion

The 1-thio-β-D-Gal derivative **2**, tagged with hydrophobic *O*-laurates to enable solid-phase extraction onto C18 silica, was prepared in two steps from D-Gal (Scheme 1). Acylation with lauroyl chloride, pyridine, and DMAP, gave the per-*O*-lauroylated derivative **1** in 86% yield. Treatment of **1** with thioacetic acid and catalytic trimethylsilyl trifluoromethanesulfonate in dichloromethane furnished the key β-thioacetate **2** (77%). Formation of the corresponding α-thioacetate could not be detected, which was in contrast to when boron trifluoride etherate was used as a catalyst.

**Solid-phase extractions.** Reaction mixtures containing tetra-*O*-lauroylated products (i.e. **8**–**42**) were concentrated, the residue was re-dissolved in methanol and applied onto the C18 silica. Reagents were eluted by washing with methanol and the desired products were eluted with pentane (Fig. 2). In all cases described in this paper, no lauroylated products were present in the MeOH washing according to TLC and no reagents could be detected in the pentane eluate by $^1H$ NMR, MALDI-TOF MS, or TLC. This purification protocol has some similarities to that of solid-phase synthesis, with the difference being that the product is attached to a solid-phase via a *noncovalent* and *reversible* hydrophobic interaction in our protocol. Solid-phase extraction on C18 silica had the advantages in that purification is rapid and efficient and that large excesses of reagents can be used to drive reactions to completion. The advantages over solid-phase synthesis using this approach is that it allows simple monitoring of each reaction by TLC and characterization of each intermediate and final product by their MALDI-TOF MS

and $^1$H NMR spectra without the need for a cleavage step. The solution-phase strategy allowed us to routinely work on a scale providing 10–30 mg of final compound for each individual library member. The length of the fatty acid ester protecting group was found to be critical for successful solid-phase extraction, since the tetra-*O*-octanoylated 1-thio-β-D-gal derivative, corresponding to **2**, did not quantitatively adsorb on C18 silica when dissolved in methanol. Furthermore, C18 silica from several suppliers were evaluated in this procedure. Waters Prep C18 was found to produce the most consistent results and at the same time was the easiest to handle due to its large particle size.

### Michael additions and α-chloro ketone substitutions

Our first attempts to use the 1-thio-galactose derivative **2** in Michael additions involved selective removal of the anomeric thioacetate[22] to give the corresponding thiol, followed by Michael addition catalysed by tetrabutylammonium fluoride,[23] sodium methoxide, or triethylamine. These approaches gave good yields of Michael adducts in trial reactions with acrolein and 2-cyclohexen-1-one (**4**), but both the de-*S*-acetylation and Michael additions were always accompanied by an interfering formation of the disulfide dimer Gal-*S*-*S*-Gal. However, the disulfide formation could be suppressed by performing the de-*S*-acetylation and Michael addition or α-halo-carbonyl substitution in one pot. This was accomplished using a modification of the procedure of Bennett et al.,[24] which involved treatment of a mixture of **2** and the Michael acceptor or α-halo-carbonyl compound with diethylamine or piperidine in dichloromethane. Reproducibility and yields were consistently better using dichloromethane as compared to other solvents (DMF, THF, toluene, EtOAc, or MeCN).

With an efficient one-pot procedure for the activation of **2** and Michael addition/α-halo-carbonyl substitution in hand, 35 different commercially available Michael acceptors and α-halo-carbonyl compounds were evaluated (Fig. 3). These experiments revealed that Michael acceptors carrying bulky substituents on the γ-carbon or having tri- or tetra-substitution of the α,β-double bond were less efficient in Michael additions with **2** (Fig. 3(B)). Purification reactions mixtures using solid-phase extraction on C18 silica proved to be efficient. Not even the more hydrophobic electrophiles (e.g. *trans*-4-phenyl-3-buten-2-one, 3-nonen-2-one, 2-cyclohepten-1-one, or *trans*-1-(4,5-dimethoxy-2-methylphenyl)-2-buten-2-one in Figure 3(A)) adsorbed to the C18 silica during the MeOH wash, while tetra-*O*-lauroylated products efficiently adsorbed to the C18 silica during the MeOH wash and were recovered in high yields after elution with pentane. This indicates that the presence of multiple *O*-laurates is crucial for adsorbtion to the C18 silica.

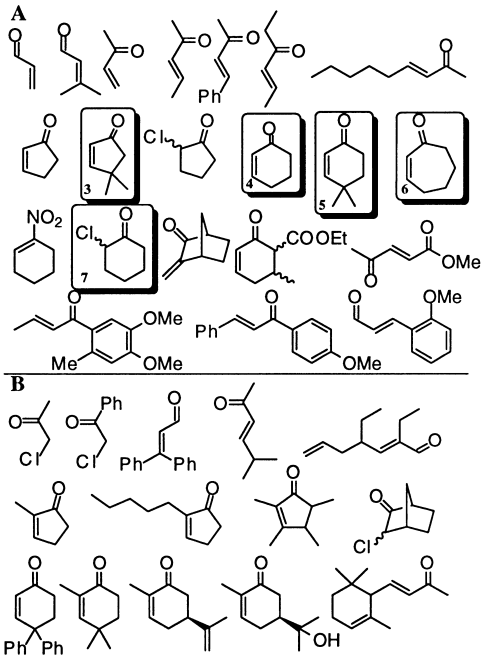



**Figure 3.** Commercial Michael acceptors and α-halo ketones used in the present study. (**A**) Compounds that gave high yields and rapid reactions with a 1-thio-Gal nucleophile. (**B**) Compounds of low reactivity.

Of the 21 alkylating agents of suitable reactivity (Fig. 3(A)), we chose four commercial Michael acceptors (**3**–**6**) and one α-halo-carbonyl (**7**) compound to demonstrate the ease of synthesis of a β-Gal-based library. Cyclic electrophiles were chosen for the reasons discussed above.

Reactions between **2** and the electrophiles **3**–**7** were performed in a parallel fashion, providing five, physically separated, ketones (**8**–**12**) (Scheme 2). As expected, none of the electrophiles **3**–**7** showed any stereoselectivity in reaction with compound **2** and diastereomers were formed in near equivalent amounts. Solid-phase extraction on C18 silica of the ketones (**8**–**12**) worked very well on a smaller scale ($<0.5$ g) providing products in high yields and purities. However, column chromatography on silica can be used as an alternative more cost-efficient purification method when reactions are performed on larger scale (1–3 g). On the other hand, further derivatization of the ketones **8**–**12**, as described below, involved 60 reactions (30 parallel ketone derivatizations on 70–200 mg scales (**13**–**42**) followed by 30 deblocking reactions (**43**–**72**)) for which solid-phase extraction was essential for the synthesis to be completed within a reasonable time. The final galactose-based library was then obtained by splitting each of the ketones (**8**–**12**) in six batches, each of which was further reacted in a parallel fashion.

**Reduction of ketones to alcohols.** The ketones **8**–**12** were reduced to the corresponding alcohols (**13**–**17**) using sodium borohydride in isopropanol/tetrahydrofuran (Scheme 2). The purity of the alcohols **13**–**17** after solid-phase extraction on C18 silica were >90% as assessed by $^1$H NMR, MALDI-TOF MS, and TLC. The reductions proceeded with significant stereoselectivity and two out of the four possible diastereomers usually dominated in the product mixture. In the final step, the *O*-laurates were removed with methanolic sodium methoxide and the methyl laurate formed was separated from the product by simply passing the mixture through C18 silica with 80% methanol, conditions under which the *O*-laurates quantitatively remained bound to the C18 silica. The five alcohols **43**–**47** (Fig. 4) were obtained in purities of >90% and yields of 78–99%.

**Reductive aminations of ketones with amino acid esters.** Reductive aminations of the ketones **8**–**12** with the unsubstituted amino acids (glycine and β-alanine *t*-butyl ester hydrochlorides), using sodium cyanoborohydride[25] in acetonitrile/THF, were high-yielding and produced an even distribution of all four possible diastereomers of the *N*-alkylated glycine (**18**–**22**) and β-alanine (**23**–**27**) derivatives (Scheme 2). Compounds **18**–**27** were >90% pure according to $^1$H NMR, MALDI-TOF MS, and TLC after solid-phase extraction on C18 silica. The use of solvents other than acetonitrile (methanol, DMF, neat THF, dichloromethane, ethyl acetate) resulted in lower yields and sometimes uneven distribution of diastereomers. Furthermore, reductive aminations of the ketones **8**–**12** with α-substituted L-amino acids (L-leucine as the *t*-butyl ester hydrochloride and L-histidine and L-tryptophan as methyl ester hydrochlorides) under these conditions also worked well, yielding the desired *N*-alkylated L-leucine (**28**–**32**), L-histidine (**33**–**37**), and L-tryptophan (**38**–**42**) derivatives (Scheme 2). However, varying amounts (10–50%) of the corresponding alcohols (**13**–**17**) were formed as the only side-product in these reactions. Presumably, formation of the intermediate iminium ion is disfavoured by α-substituents on the amino acids and competing reduction of the ketone to the corresponding alcohol occurs to a significant extent. Addition of acids,[26–29] in order to catalyse iminium ion formation, or the use of sodium triacetoxyborohydride[30,31] as reducing agent, did not decrease the formation of the corresponding alcohols. However, in cases where a mixture of a *N*-alkylated amino acid and an alcohol was obtained, the alcohol was readily

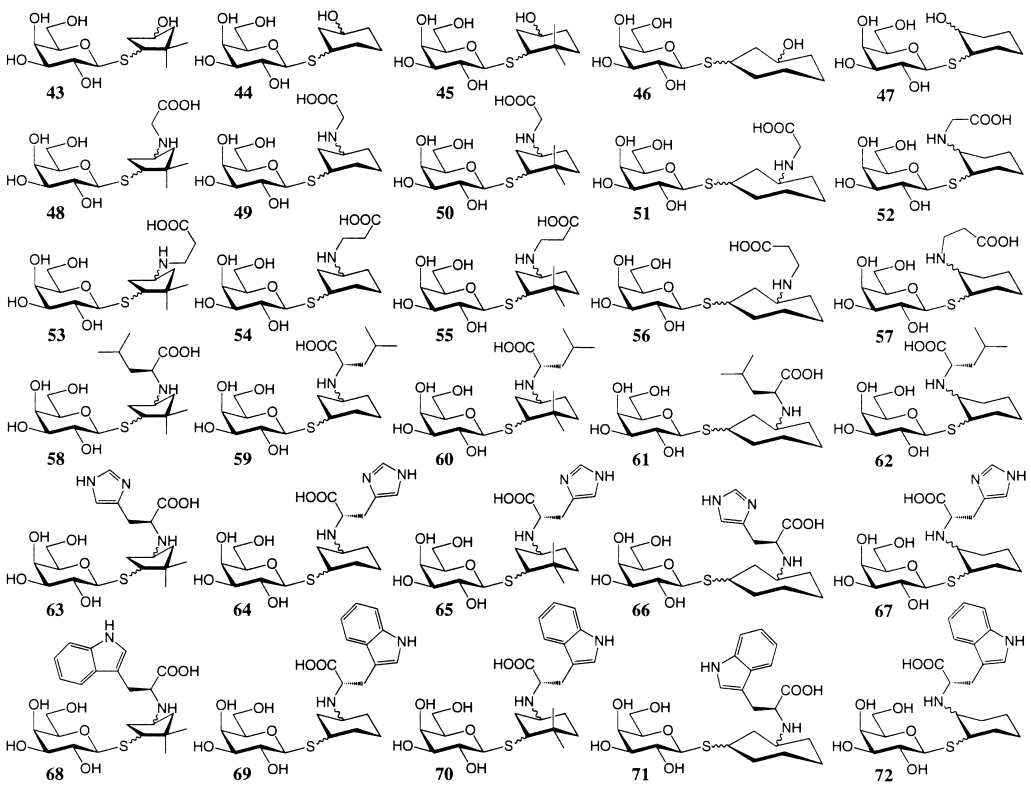



**Figure 4.** The final 1-thio-β-D-galatoside library, consisting of 30 physically seperated compounds as mixtures of four diastereomers each.

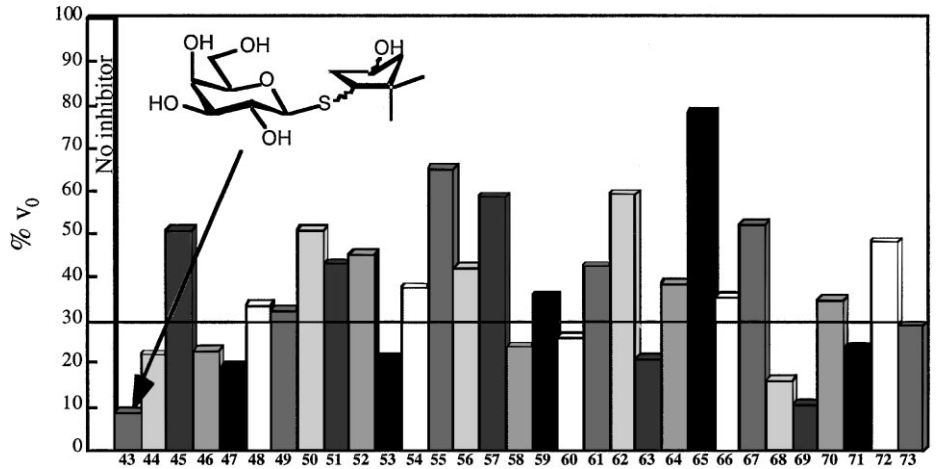



**Figure 5.** Evaluation of **43**–**73** as inhibitors of β-galactosidase from *E. coli*. The horizontal line shows the relative initial rate of hydrolysis in the presence of the reference inhibitor cyclohexyl 1-thio-β-D-galactoside (**73**).

separated from the *N*-alkylated amino acid on a silica Sep-Pak cartridge after removal protecting groups (*O*-laurates and methyl or *t*-butyl esters) as described below.

*t*-Butyl esters were removed from the protected *N*-alkylated glycine (**18**–**22**), β-alanine (**23**–**27**) and L-leucine (**28**–**32**) derivatives using trifluoroacetic acid in dichloromethane, followed by removal of *O*-laurates using methanolic sodium methoxide. The residual mixtures of *N*-alkylated amino acid (**48**–**62**), methyl laurate and, in some cases, the corresponding alcohol (**43**–**47**), were applied onto silica normal-phase Sep-Pak cartridges. The cartridges were washed with dichloromethane: methanol 9:1 (to remove methyl laurate and the corresponding alcohol) and the final *N*-alkylated glycine (**48**–**52**), β-alanine (**53**–**57**), and L-leucine (**58**–**62**) derivatives (Figure 4) were eluted with dichloromethane:methanol: water (65:35:5) in 31–99% yields and purities of >90%.

*O*-Laurates were removed from the protected *N*-alkylated L-histidine (**33**–**37**) and L-tryptophan (**38**–**42**) derivatives using methanolic sodium methoxide. The methyl laurate formed was thereafter removed by passing the residue through C18 silica using 80% methanol as a solvent. The eluate contained the desired product while methyl laurate was adsorbed onto the C18 silica. Methyl esters were then hydrolysed using aqueous LiOH and the resulting *N*-alkylated amino acids (**63**–**72**) were separated from traces of their corresponding alcohols (**43**–**47**) on a silica Sep-Pak cartridges, as described above for **48**–**62**. The final *N*-alkylated L-histidine (**63**–**67**) and L-tryptophan (**68**–**72**) derivatives (Fig. 4) were obtained in 45–93% yields and purities of >90%.

**Inhibition of β-galactosidase from *E. coli*.** The 5×6 1-thio-β-D-Gal library thus prepared is shown in Figure 4. The potential biological activity of this library was evaluated, for demonstration purposes, against the β-galactosidase from *Escherichia coli*, known to be inhibited by 1-thio-β-D-galactosides.[32] Cyclohexyl 1-thio-β-D-galactoside **73**[33] was chosen as a reference inhibitor, since it would reflect the effect of an anomeric sulfur atom and a cycloalkane aglycon have on inhibitory power. In order to rapidly obtain information of the inhibitory power of the library members, an initial screen was performed by briefly incubating the enzyme with *p*-nitrophenyl β-D-galactoside in the presence of the inhibitors **43**–**73**. The optical density at 405 nm was used as an approximation of the relative initial rates of hydrolysis (Fig. 5). The initial rate of hydrolysis in the presence of the reference inhibitor **73** was 28% of the rate in the absence of an inhibitor, which is indicated with a horizontal line in Figure 5. The important effect of the aglycon on the inhibitory power individual library members was clearly apparent. Eleven inhibitors were better and 19 were worse than **73**. Overall trends that could be observed were that alcohols and *N*-alkylated tryptophan derivatives, which are among the more hydrophobic library members, were good inhibitors. Furthermore, 1-thio-β-D-galactosides carrying a gem-dimethylated cyclopentane aglycon (**43**, **53**, **58**, **63**, and **68**) were generally good inhibitors.

Each of the library reactions used produced mixtures of diastereomers and in order to identify the active constituent(s) the compounds would clearly have to be resolved. The diastereomeric mixture of **13** could indeed be partially resolved by chromatography on silica gel and, on removal of the *O*-laurates, yielded two major isomers **43A** and **43B** (~90%) together with a mixture of the two minor diastereomers (**43CD**). Their structures were confirmed with high-resolution mass spectroscopy and

preliminary 2-D ROESY NMR experiments established that the aglycons of **43A** and **43B** had *cis* configuration (i.e. (1*S*,4*S*)-4-hydroxy-2,2-dimethyl-cyclopent-1-yl 1-thio-β-D-galactopyranoside and (1*R*,4*R*)-4-hydroxy-2,2-dimethyl-cyclopent-1-yl 1-thio-β-D-galactopyranoside). Inhibitor evaluation of these compounds revealed that **43A** and **43B** were good inhibitors, while **43CD** inhibited the enzyme poorly (Fig. 6). This indicated that the two major diastereomers, **43A** and **43B**, were responsible for the strong inhibitory activity of the mixture of four diastereomers (**43**).

Compounds **43A** and **43B** were shown to be competitive inhibitors and the inhibition constants determined to be 3.1 and 1.7 μM, respectively. Determination of the inhibition constant of **73** (14 μM), showed that relatively simple changes of the aglycon of a 1-thio-β-D-galactoside inhibitor (**43B** versus **73**) had significant effects on the inhibition constant (1.7 versus 14 μM; $\Delta\Delta G \approx 5.2$ kJ/mol). The results parallel the well-known observation that hydrophobic aglycons (alkyl or aryl aglycons) significantly enhance the binding of glycosides to many lectins and enzymes, including β-galactosidase from *E. coli*,[32] and the relatively strong inhibition of **43B** may be attributed to the hydrophobic nature of its aglycon. However, the fact that the different stereoisomers of **43** displayed different inhibitor potencies and that **43B** was almost one order of magnitude better than cyclohexyl 1-thio-β-D-galactopyranoside **73**, suggests a more specific hydrophobic interaction where the size and shape of the aglycon is important. Furthermore, it can not be excluded that the aglycon hydroxyl group of **43B** is involved in a hydrogen bond to the enzyme.

## Conclusions

Solid-phase extraction on C18 silica, relying on the presence of multiple *O*-laurates, was developed as a novel and efficient isolation tool in the synthesis of carbohydrate libraries. The protocol combines several advantages of solution synthesis (ease of reaction monitoring and intermediate characterization) with those of solid-phase synthesis (rapid purification, use of excess reagents to 'drive' reactions). To demonstrate the utility

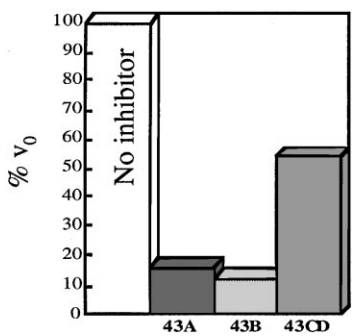



**Figure 6.** The relative initial rate of hydrolysis of *p*-nitrophenyl β-D-galactopyranoside by β-galactosidase from *E. coli.* in the presence of **43A**, **43B**, and **43CD**.

**Table 1.** Inhibition constants against β-galactosidase from *E. coli*

| Compd | $K_i$/μM |
|---|---|
| **43A** | 3.1 |
| **43B** | 1.7 |
| **73** | 14 |

**Table 2.** MALDI mass spectrometry data for lauroylated library intermediates

| Compd | Formula | M calcd | $(M+X)^+$ found[a] |
|---|---|---|---|
| **8** | $C_{61}H_{110}O_{10}S$ | 1035.59 | 1060.6 |
| **9** | $C_{60}H_{108}O_{10}S$ | 1021.57 | 1045.9 |
| **10** | $C_{62}H_{112}O_{10}S$ | 1050.57 | 1074.3 |
| **11** | $C_{61}H_{110}O_{10}S$ | 1035.59 | 1058.9 |
| **12** | $C_{60}H_{108}O_{10}S$ | 1021.57 | 1045.3 |
| **13** | $C_{61}H_{112}O_{10}S$ | 1037.61 | 1062.3 |
| **14** | $C_{60}H_{110}O_{10}S$ | 1023.59 | 1044.7 |
| **15** | $C_{62}H_{114}O_{10}S$ | 1052.63 | 1077.8 |
| **16** | $C_{61}H_{112}O_{10}S$ | 1037.61 | 1060.6 |
| **17** | $C_{60}H_{110}O_{10}S$ | 1023.59 | 1047.0 |
| **18** | $C_{67}H_{123}NO_{11}S$ | 1150.77 | 1115.1,[b] 1170.2 |
| **19** | $C_{66}H_{121}NO_{11}S$ | 1136.75 | 1083.7[b] |
| **20** | $C_{468}H_{125}NO_{11}S$ | 1164.80 | 1112.6[b] |
| **21** | $C_{67}H_{123}NO_{11}S$ | 1150.77 | 1096.9,[b] 1153.2 |
| **22** | $C_{66}H_{121}NO_{11}S$ | 1136.75 | 1139.0 |
| **23** | $C_{68}H_{125}NO_{11}S$ | 1164.80 | 1116.8[b] |
| **24** | $C_{67}H_{123}NO_{11}S$ | 1150.77 | 1095.8[b] |
| **25** | $C_{69}H_{127}NO_{11}S$ | 1178.83 | 1182.0 |
| **26** | $C_{68}H_{125}NO_{11}S$ | 1164.80 | 1109.1,[b] 1165.8 |
| **27** | $C_{67}H_{123}NO_{11}S$ | 1150.77 | 1192.8 |
| **28** | $C_{71}H_{131}NO_{11}S$ | 1206.88 | 1150.5[b] |
| **29** | $C_{70}H_{129}NO_{11}S$ | 1192.86 | 1193.8 |
| **30** | $C_{72}H_{133}NO_{11}S$ | 1219.90 | 1167.6,[b] 1225.7 |
| **31** | $C_{71}H_{131}NO_{11}S$ | 1206.88 | 1151.5,[b] 1229.4 |
| **32** | $C_{70}H_{129}NO_{11}S$ | 1192.86 | 1137.2,[b] 1192.8 |
| **33** | $C_{68}H_{121}N_3O_{11}S$ | 1188.78 | 1188.4 |
| **34** | $C_{67}H_{119}N_3O_{11}S$ | 1174.76 | 1172.0 |
| **35** | $C_{69}H_{123}N_3O_{11}S$ | 1201.75 | 1201.0 |
| **36** | $C_{68}H_{121}N_3O_{11}S$ | 1188.78 | 1188.3 |
| **37** | $C_{67}H_{119}N_3O_{11}S$ | 1174.76 | 1173.9 |
| **38** | $C_{73}H_{124}N_2O_{11}S$ | 1237.85 | 1239.7 |
| **39** | $C_{72}H_{122}N_2O_{11}S$ | 1223.83 | 1223.0 |
| **40** | $C_{74}H_{126}N_2O_{11}S$ | 1251.83 | 1252.7 |
| **41** | $C_{73}H_{124N_2}O_{11}S$ | 1237.85 | 1238.6 |
| **42** | $C_{72}H_{122}N_2O_{11}S$ | 1223.83 | 1223.9 |

[a]X = H, Na, or K.
[b]Loss of $^t$Bu due to fragmentation.

**Table 3.** MALDI mass and $^1$H NMR spectroscopy data for the final β-D-galactoside library

| Compd | Formula | M calcd | $(M+X)^+$ found[a] | δ/ppm[b] |
|---|---|---|---|---|
| **43** | $C_{13}H_{24}O_6S$ | 308.40 | 332.1 | 4.305, 4.310, 4.315, 4.34 |
| **44** | $C_{12}H_{22}O_6S$ | 294.36 | 317.3 | 4.38, 4.417, 4.422 |
| **45** | $C_{14}H_{26}O_6S$ | 322.42 | 346.6 | 4.32, 4.33, 4.34 |
| **46** | $C_{13}H_{24}O_6S$ | 308.40 | 332.1 | 4.381, 4.389, 4.394 |
| **47** | $C_{12}H_{22}O_6S$ | 294.36 | 318.8 | 4.34, 4.39, 4.43, 4.55 |
| **48** | $C_{15}H_{27}NO_7S$ | 365.44 | 368.0 | 4.30, 4.320, 4.325, 4.330 |
| **49** | $C_{14}H_{25}NO_7S$ | 351.42 | 375.5 | 4.36, 4.40, 4.44, 4.46 |
| **50** | $C_{16}H_{29}NO_7S$ | 379.47 | 380.6, 403.5 | 4.31, 4.34, 4.36, 4.38 |
| **51** | $C_{15}H_{27}NO_7S$ | 365.44 | 367.4, 389.9 | 4.38, 4.42, 4.45, 4.46 |
| **52** | $C_{14}H_{25}NO_7S$ | 351.42 | 353.5, 376.5 | 4.47, 4.48, 4.50, 4.58 |
| **53** | $C_{16}H_{29}NO_7S$ | 379.44 | 383.2 | 4.310, 4.315, 4.33, 4.34 |
| **54** | $C_{15}H_{27}NO_7S$ | 365.45 | 367.0, 389.9 | 4.36, 4.43, 4.44, 4.46 |
| **55** | $C_{17}H_{31}NO_7S$ | 393.50 | 399.3, 419.5 | 4.32, 4.34, 4.35 |
| **56** | $C_{16}H_{29}NO_7S$ | 379.45 | 381.7, 403.5 | 4.40, 4.46 |
| **57** | $C_{15}H_{27}NO_7S$ | 365.45 | 367.4, 389.9 | 4.34, 4.42, 4.44, 4.47 |
| **58** | $C_{19}H_{35}NO_7S$ | 421.55 | 422.3 | 4.315, 4.320 |
| **59** | $C_{18}H_{33}NO_7S$ | 407.53 | 408.4 | 4.33, 4.40, 4.42, 4.46 |
| **60** | $C_{20}H_{37}NO_7S$ | 436.58 | 438.0, 461.4 | 4.34, 4.38 |
| **61** | $C_{19}H_{35}NO_7S$ | 421.55 | 421.7, 448.0 | 4.36, 4.43, 4.44 |
| **62** | $C_{18}H_{33}NO_7S$ | 407.53 | 410.9, 435.5 | 4.37, 4.41, 4.44, 4.49 |
| **63** | $C_{19}H_{31}N_3O_7S$ | 445.53 | 447.6 | 4.30, 4.305, 4.32, 4.33 |
| **64** | $C_{18}H_{29}N_3O_7S$ | 431.50 | 433.2 | 4.35, 4.39, 4.425, 4.43 |
| **65** | $C_{20}H_{33}N_3O_7S$ | 459.56 | 462.2 | 4.34, 4.357, 4.364 |
| **66** | $C_{19}H_{31}N_3O_7S$ | 445.53 | 448.0 | 4.32, 4.41, 4.44, 4.50 |
| **67** | $C_{18}H_{29}N_3O_7S$ | 431.50 | 433.6 | 4.28, 4.40, 4.45, 4.52 |
| *68* | $C_{24}H_{34}N_2O_7S$ | 494.60 | 495.6 | 4.13,4.20, 4.22, 4.26 |
| **69** | $C_{23}H_{32}N_2O_7S$ | 480.59 | 483.9 | 4.24, 4.33, 4.35, 4.36 |
| **70** | $C_{25}H_{36}N_2O_7S$ | 508.63 | 512.1 | 4.21, 4.26, 4.30 |
| **71** | $C_{24}H_{34}N_2O_7S$ | 494.60 | 495.9 | 4.19, 4.29, 4.37 |
| **72** | $C_{23}H_{32}N_2O_7S$ | 480.59 | 481.9, 505.3 | 4.09, 4.24, 4.40, 4.46 |

[a]X = H or Na.

[b]Anomeric protons. $J = 9.5–10$ Hz. The observation of less than four anomeric signals is a consequence of overlapping signals.

of this solid-phase extraction protocol, high-yielding procedures for Michael additions and α-halo-carbonyl substitutions with a 1-thio-β-D-galactoside derivative, as well as for reductive aminations with amino acids, were employed in a practical solution-phase synthesis of a small 1-thio-β-D-galactoside library containing 10–30 mg of each individual library compound. The 1-thio-β-D-galactoside library was screened for inhibitors against β-galactosidase from *E. coli*, which showed that the nature of the aglycon strongly influenced the inhibitory power of individual library members. Despite the limited size of this demonstration library, an inhibitor (**43B**) almost one order of magnitude better than the reference compound cyclohexyl 1-thio-β-D-galactopyranoside **73**, was identified. These results show that solid-phase extraction on C18 silica is an efficient isolation tool in the synthesis of carbohydrate libraries and combinatorial addition of diverse noncarbohydrate structures to a natural carbohydrate ligand is a feasible route towards the discovery of potent inhibitors of carbohydrate binding proteins.

## Experimental

**General.** $^1$H NMR spectra were recorded with either a Bruker AM-360 or a Varian UNITY 500 spectrometer. MALDI-TOF mass spectra were recorded with a Kratos Kompact MALDI instrument. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Solid-phase extraction were performed with Prep C18, 55–105 μm, 125Å (Waters Corp., Milford, USA). Reactions were monitored by TLC on Silica Gel $FG_{254}$ (E. Merck, Darmstadt, Germany). $^1$H NMR assignments were derived from COSY experiments. Microtiter plate absorbances were read on a Thermomax microplate reader (Molecular Devices). β-Galactosidase from *E. coli* was from SIGMA (G6512). The buffer used for enzyme assays was 0.15 M PBS and 0.02 mM $MgCl_2$ at pH 7.2.

**Solid-phase extraction of lauroylated compounds.** Following Michael addition, α-chloro substitution, reduction, or reductive amination, the reaction mixture was

concentrated, re-dissolved in methanol, and applied onto C18 silica (1 g per 20 mg lauroylated carbohydrate), which had been pre-conditioned with pentane (50 mL) and MeOH (50 mL). In some cases , when the lauroylated product was poorly soluble in MeOH, the residue was sonicated in MeOH to a milky suspension before application onto the C18 silica. The C18 silica was washed with methanol (10 mL/g C18 silica), whereafter the product was eluted with pentane (10 mL/g C18 silica). No remaining reagents could in any case be detected by TLC, $^1$H NMR, or MALDI-TOF mass spectroscopy.

**1,2,3,4,6-Pent$\alpha$-*O*-lauroyl-$\alpha$-D-galactopyranose (1).** To a suspension of D-galactose (5.0 g, 27.8 mmol), pyridine (60 mL), and 4-dimethylaminopyridine (cat.) in pentane (195 mL) under argon atmosphere, was added lauroyl chloride (60 mL, 252 mmol) at −78 °C. The mixture was allowed to reach ambient temperature. The white slurry slowly dissolved and a fine precipitate of pyridinium hydrochloride formed. After 40 h, the pyridinium hydrochloride was filtered off and the pentane solution was concentrated. Column chromatography ($SiO_2$, pentane:EtOAc 20:19:1 gradient) gave **1** (26.1 g, 86%), $[\alpha]_d^{25}$ +39° (*c* 0.9, $CHCl_3$). $^1$H NMR data ($CDCl_3$) δ 6.39 (d, 1H, *J* = 2.4 Hz, H-1), 5.51 (br s, 1H, H-4), 5.35 (m, 2H, H-2 and H-3), 4.32 (br t, 1H, *J* = 6.6 Hz, H-5), 4.08 (d, 2H, *J* = 6.6 Hz, H-6a and H-6b), 2.39, 2.38, 2.30, 2.26 (4 t, 2H each, *J* = 7.5 Hz, -$CH_2$CO-), 2.21 (m, 2H, -$CH_2$CO-), 0.88 (t, 15 H, *J* = 7.0 Hz, -$CH_3$). Anal. calcd for $C_{66}H_{122}O_{11}$: C, 72.2; H, 11.3. Found: C, 72.6; H, 11.5.

**1-*S*-Acetyl-2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranose (2).** To **1** (20.0 g, 18.2 mmol) and thioacetic acid (5.0 mL, 1.9 equiv) in dry dichloromethane (300 mL) under argon, was added trimethylsilyl trifluoromethanesulfonate (5.0 mL, 0.5 equiv) at 0 °C. The cold-bath was immediately removed and after 48 h the mixture was diluted with dichloromethane, washed with satd sodium hydrogencarbonate, dried ($Na_2SO_4$), and concentrated. Column chromatography ($SiO_2$, pentane:EtOAc, 20:1) gave **2** (13.7 g, 77%), $[\alpha]_D^{25}$ +21° (*c* 1, $CHCl_3$). $^1$H NMR data ($CDCl_3$) δ 5.47 (d, 1H, *J* = 3.4 Hz, H-4), 5.32 (t, 1H, *J* = 10.0 Hz, H-2), 5.25 (d, 1H, *J* = 10.0 Hz, H-1), 5.12 (dd, 1H, *J* = 3.4 and 10.0 Hz, H-3), 4.08 (m, 3H, H-5, H-6a and H-6b), 2.14–2.43 (m, 8H, -$CH_2$CO-), 2.37 (s, 3H, -SAc), 0.88 (t, 15 H, *J* = 7.0 Hz, -$CH_3$). Anal. calcd for $C_{56}H_{102}O_{10}S$: C, 69.5; H, 10.6; S, 3.3. Found: C, 69.4; H, 10.8; S, 3.5.

**Typical procedure for small scale (< 0.5 g) Michael addition/$\alpha$-halo-carbonyl substitution; cycloheptan-3-on-1-yl 2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranoside (11).** To compounds **2** (210 mg, 0.22 mmol) and **6** (48 mg, 0.43 mmol) in dry $CH_2Cl_2$ (1.8 mL) under argon, was added $Et_2NH$ (0.45 mL). After 2 h, the mixture was concentrated and purified according to the solid-phase extraction protocol to give **11** (203 mg, 90%).

**Typical procedure for larger scale (> 1 g) Michael addition/$\alpha$-halo-carbonyl substitution; 2,2-dimethylcyclohexan-5-on-1-yl 2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranoside (10).** To compounds **2** (1.02 g, 1.05 mmol) and **5** (400 mg, 3.16 mmol) in dry $CH_2Cl_2$ (3 mL) under argon, was added $Et_2NH$ (2 mL). After 3 h, the mixture was concentrated and purified by column chromatography ($SiO_2$, pentane:EtOAc, 7:1) to give **10** (758 mg, 75%). In parallel reactions were obtained 2,2-dimethylcyclopentan-4-on-1-yl 2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranoside **8** (71%), cyclohexan-3-on-1-yl 2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranoside **9** (88%), cycloheptan-3-on-1-yl 2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranoside **11** (79%), and cyclohexan-2-on-1-yl 2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranoside **12** (94%).

**Typical procedure for reduction; 2,2-dimethyl-4-hydroxycyclopent-1-yl 2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranoside 13.** To **8** (80 mg, 77 μmol) in dry tetrahydrofuran (2.0 mL) and isopropanol (0.5 mL) under argon atmosphere, was added $NaBH_4$ (30 μmol). After 25 min, AcOH (30 μL) was added and the mixture was concentrated and purified according to the solid-phase extraction protocol to give **13** (65 mg, 81%). In parallel reactions were obtained 3-hydroxy-cyclohex-1-yl 2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranoside **14** (86%), 2,2-dimethyl-5-hydroxy-cyclohex-1-yl 2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranoside **15** (71%), 3-hydroxy-cyclohept-1-yl 2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranoside **16** (72%), and 2-hydroxy-cyclohex-1-yl 2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranoside **17** (98%).

**Typical procedure for reductive amination with amino acids; $N^{\alpha}$-[3-(2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranosyl)-cyclohept-1-yl]-L-tryptophan methyl ester (41).** To the ketone **11** (115 mg, 111 μmol) and L-tryptophan hydrochloride methyl ester (283 mg, 1.11 mmol) in dry MeCN (2.6 mL) and THF (0.9 mL), was added $NaCNBH_3$ (0.1 mmol). After 73 h, the mixture was concentrated and purified according to the solid-phase extraction protocol to give **41** (129 mg, 95%). (Yields in reductive aminations are calculated based on mass recovery and products contain varying amounts (0 to∼50%) of contaminating alcohols **13**–**17** as described under Results and Discussion.) In parallel reactions were obtained $N^{\alpha}$-[4,4-dimethyl-3-(2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranosyl)-cyclopent-1-yl]-glycine *t*-butyl ester **18** (98%), $N^{\alpha}$-[3-(2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-glycine *t*-butyl ester **19** (90%), $N^{\alpha}$-[4,4-dimethyl-3-(2,3,4,6-tetra-*O*-

lauroyl-1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-glycine *t*-butyl ester **20** (94%), $N^{\alpha}$-[3-(2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranosyl)-cyclohept-1-yl]-glycine *t*-butyl ester **21** (87%), $N^{\alpha}$-[2-(2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-glycine *t*-butyl ester **22** (93%), $N^{\beta}$-[4,4-dimethyl-3-(2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranosyl)-cyclopent-1-yl]-β-alanine *t*-butyl ester **23** (97%), $N^{\beta}$-[3-(2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-β-alanine *t*-butyl ester **24** (79%), $N^{\beta}$-[4,4-dimethyl-3-(2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-β-alanine *t*-butyl ester **25** (82%), $N^{\beta}$-[3-(2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranosyl)-cyclohept-1-yl]-β-alanine *t*-butyl ester **26** (77%), $N^{\beta}$-[2-(2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-β-alanine *t*-butyl ester **27** (73%), $N^{\alpha}$-[4,4-dimethyl-3-(2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranosyl)-cyclopent-1-yl]-L-leucine *t*-butyl ester **28** (79%), $N^{\alpha}$-[3-(2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-L-leucine *t*-butyl ester **29** (85%), $N^{\alpha}$-[4,4-dimethyl-3-(2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-L-leucine *t*-butyl ester **30** (95%), $N^{\alpha}$-[3-(2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranosyl)-cyclohept-1-yl]-L-leucine *t*-butyl ester **31** (99%), $N^{\alpha}$-[2-(2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-L-leucine *t*-butyl ester **32** (93%), $N^{\alpha}$-[4,4-dimethyl-3-(2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranosyl)-cyclopent-1-yl]-L-histidine methyl ester **33** (82%), $N^{\alpha}$-[3-(2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-L-histidine methyl ester **34** (92%), $N^{\alpha}$-[4,4-di-methyl-3-(2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-L-histidine methyl ester **35** (87%), $N^{\alpha}$-[3-(2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranosyl)-cyclohept-1-yl]-L-histidine methyl ester **36** (87%), $N^{\alpha}$-[2-(2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-L-histidine methyl ester **37** (quant), $N^{\alpha}$-[4,4-dimethyl-3-(2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranosyl)-cyclopent-1-yl]-L-tryptophan methyl ester **38** (95%), $N^{\alpha}$-[3-(2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-L-tryptophan methyl ester **39** (94%), $N^{\alpha}$-[4,4-dimethyl-3-(2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-L-tryptophan methyl ester **40** (95%), and $N^{\alpha}$-[2-(2,3,4,6-tetra-*O*-lauroyl-1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-L-tryptophan methyl ester **42** (92%).

**Typical procedure for deblocking of alcohols; 2-hydroxy-cyclohex-1-yl 1-thio-β-D-galactopyranoside (47).** To compound **17** (77 mg, 75 μmol) in dry methanol (5.5 mL) and dichloromethane (0.85 mL) under argon atmosphere, was added methanolic sodium methoxide (1 M, 38 μL). After 24 h, the mixture was neutralized with Amberlite IRC-50S ($H^+$) resin, filtered and concentrated. The residue was dissolved in water and applied onto a column of C18 silica (Waters Prep C18, 125 Å, 7 g). The C18 silica was washed with water (50 mL), whereafter the final de-protected alcohol was eluted with 70% methanol (50 mL) and concentrated to give a quantitative yield of **47**. In parallel reactions were obtained 2,2-dimethyl-4-hydroxy-cyclopent-1-yl 1-thio-β-D-galactopyranoside **43** (78%), 3-hydroxy-cyclohex-1-yl 1-thio-β-D-galactopyranoside **44** (quant), 2,2-dimethyl-5-hydroxy-cyclohex-1-yl 1-thio-β-D-galactopyranoside **45** (quant), 3-hydroxy-cyclohept-1-yl 1-thio-β-D-galactopyranoside **46** (95%).

**Typical procedure for deblocking of *N*-alkylated amino acid *t*-butyl esters; $N^{\alpha}$-[3-(1-thio-β-D-galactopyranosyl)-cyclohept-1-yl]-L-leucine (61).** Compound **31** (119 mg, 98.4 μmol) was treated with trifluoroacetic acid (3.5 mL) in dry dichloromethane (3.5 mL) for 330 min. *n*-Propyl acetate (7 mL) and toluene (20 mL) were added and the mixture was concentrated, then co-concentrated twice with toluene. To the residue in dry methanol (7 mL) and dichloromethane (1.2 mL) under argon atmosphere, was added methanolic sodium methoxide (1 M, 197 μL). After 20 h, the mixture was neutralized with Amberlite IRC-50S ($H^+$) resin, filtered and concentrated. The residue was dissolved in dichloromethane:methanol (9:1) and applied onto a Waters Sep-Pak Plus Longbody $SiO_2$ cartridge. The cartridge was washed with dichloromethane:methanol (9:1, 20 mL) to remove methyl laurate and traces of the corresponding alcohol (**46**), whereafter the **61** was eluted with dichloromethane:methanol:water (65:35:5, 20 mL) and concentrated. The residue was dissolved in water and applied onto a column of C18 silica (Waters Prep C18, 125 Å, 7 g). The C18 silica was washed with water (50 mL), whereafter elution with methanol (50 mL) and concentration gave **61** (37.7 mg, 91%). In parallel reactions were obtained $N^{\alpha}$-[4,4-dimethyl-3-(1-thio-β-D-galactopyranosyl)-cyclopent-1-yl]-glycine **48** (56%), $N^{\alpha}$-[3-(1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-glycine **49** (85%), $N^{\alpha}$-[4,4-dimethyl-3-(1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-glycine **50** (91%), $N^{\alpha}$-[3-(1-thio-β-D-galactopyranosyl)-cyclohept-1-yl]-glycine **51** (79%), $N^{\alpha}$-[2-(1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-glycine **52** (85%), $N^{\beta}$-[4,4-dimethyl-3-(1-thio-β-D-galactopyranosyl)-cyclopent-1-yl]-β-alanine **53** (32%), $N^{\beta}$-[3-(1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-β-alanine **54** (65%), $N^{\beta}$-[4,4-dimethyl-3-(1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-β-alanine **55** (99%), $N^{\beta}$-[3-(1-thio-β-D-galactopyranosyl)-cyclohept-1-yl]-β-alanine **56** (99%), $N^{\beta}$[2-(1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-β-alanine **57** (98%), $N^{\alpha}$-[4,4-dimethyl-3-(1-thio-β-D-galactopyranosyl)-cyclopent-1-yl]-L-leucine **58** (39%), $N^{\alpha}$-[3-(1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-L-leucine **59** (55%), $N^{\alpha}$-[4,4-dimethyl-3-(1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-L-leucine **60** (31%), and $N^{\alpha}$-[2-(1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-L-leucine **62** (60%).

**Typical procedure for deblocking of *N*-alkylated amino acid methyl esters; $N^{\alpha}$-[3-(1-thio-β-D-galactopyranosyl)-cyclohept-1-yl]-L-histidine (66).** To compound **36** (100 mg, 84.5 μmol) in dry methanol (6.2 mL) and dichloromethane (0.9 mL) under argon atmosphere, was added methanolic sodium methoxide (1 M, 43 μL). After 2 h, the mixture was neutralized with Amberlite IRC-50S ($H^+$) resin, filtered and concentrated. The residue was dissolved in 70% methanol and applied onto a column of C18 silica (Waters Prep C18, 125 Å, 7 g), whereafter the product was eluted with 70% methanol (50 mL) and concentrated. To the residue in water (3.1 mL) was then added aqueous lithium hydroxide (1 M, 0.3 mL). After 80 min, the mixture was neutralized with Amberlite IRC-50S ($H^+$) resin, filtered and concentrated. The residue was dissolved in dichloromethane:methanol (9:1) and applied onto a Waters Sep-Pak Plus Longbody $SiO_2$ cartridge. The cartridge was washed with dichloromethane:methanol (9:1, 10 mL) to remove traces of the corresponding alcohol (**46**), whereafter **66** was eluted with dichloromethane: methanol:water (65:35:5, 20 mL) and concentrated. The residue was dissolved in water and applied onto a column of C18 silica (Waters Prep C18, 125 Å, 7 g). The C18 silica was washed with water (50 mL), whereafter elution with 70% methanol (50 mL) and concentration gave **66** (30 mg, 80%). In parallel reactions were obtained $N^{\alpha}$-[4,4-dimethyl-3-(1-thio-β-D-galactopyranosyl)-cyclopent-1-yl]-L-histidine **63** (68%), $N^{\alpha}$-[3-(1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-L-histidine **64** (50%), $N^{\alpha}$-[4,4-dimethyl-3-(1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-L-histidine **65** (56%), $N^{\alpha}$-[2-(1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-L-histidine **67** (45%), $N^{\alpha}$-[4,4-dimethyl-3-(1-thio-β-D-galactopyranosyl)-cyclopent-1-yl]-L-tryptophan **68** (72%), $N^{\alpha}$-[3-(1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-L-tryptophan **69** (56%), $N^{\alpha}$-[4,4-dimethyl-3-(1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-L-tryptophan **70** (48%), $N^{\alpha}$-[3-(1-thio-β-D-galactopyranosyl)-cyclohept-1-yl]-L-tryptophan **71** (93%), and $N^{\alpha}$-[2-(1-thio-β-D-galactopyranosyl)-cyclohex-1-yl]-L-tryptophan **72** (76%).

**Screening for inhibitors of β-galactosidase from *E. coli*.** To solutions of compounds **43**–**73** (3 mM) and *p*-nitrophenyl β-D-galactoside (2 mM, $K_m = 0.04$ mM) in microtiter plate wells (50 μL/well) was added β-galactosidase (5 u/mL, 50 μL/well). Aqueous sodium hydroxide (1 M, 200 μL/well) was added after 8 min and the optical density at 405 nm was used as an approximate value related to the initial rates of hydrolysis. Each experiment was done in triplicate.

**Separation of diastereomers of 4-hydroxy-2,2-dimethylcyclopent-1-yl 1-thio-β-D-galactopyranoside (43).** Column chromatography ($SiO_2$, toluene:EtOAc, 19:1) of the diastereomeric mixture of **13** gave a faster moving fraction containing a mixture of the two major diastereomers (**13AB**, 27.1 mg) and a slower moving fraction ($^{13}$CD, 7.6 mg) containing a mixture of the two minor diastereomers. The two major diastereomers (**13AB**) were further separated using column chromatography ($SiO_2$, $CH_2Cl_2$:$Et_2O$, 19:1) to give pure diastereomers **13A** (13.5 mg) and **13B** (5.1 mg). To **13A** (13.5 mg, 13.0 μmol), **13B** (5.1 mg, 4.9 μmol), and $^{13}$CD (7.6 mg, 7.3 μmol) in dry methanol (1.0, 0.4, and 0.6 mL respectively) and dichloromethane (130, 49, and 73 μL respectively) under argon atmosphere, was added methanolic sodium methoxide (7.1, 2.7, and 4.0 μL respectively, 1 M). After 195 min, the mixtures were neutralized with AcOH and concentrated. The residues were dissolved in 70% methanol and applied onto Sep-Pak C18 cartridges, whereafter the products were eluted with 70% methanol (10 mL) and concentrated. The residues were dissolved in water and applied onto Sep-Pak C18 cartridges. The cartridges were washed with water (50 mL), whereafter elution with methanol (50 mL) gave the two major diastereomers **43A** (2.4 mg, 60% from **13A**) and **43B** (0.7 mg, 31% from **13B**), as well as a mixture of the two minor diastereomers **43CD** (1.2 mg, 53% from $^{13}$CD). $^1$H NMR data for **43A** (500 MHz, $CD_3OD$, aglycon signals are indicated with *) δ 4.30 (d, 1H, *J* 9.5 Hz, H-1), 4.20 (dddd, 1H, *J* = 3.5, 6.3, 7.3, and 8.4 Hz, H-4*), 3.87 (dd, 1H, *J* = 1.0 and 3.3 Hz, H-4), 3.72 (dd, 1H, *J* = 6.7 and 11.3 Hz, H-6), 3.68 (dd, 1H, *J* = 5.5 and 11.3 Hz, H-6), 3.495 (t, 1H, *J* = 9.3 Hz, H-2), 3.485 (ddd, 1H, *J* = 1.0, 5.5, and 6.7 Hz, H-5), 3.42 (dd, 1H, *J* = 3.3 and 9.3 Hz, H-3), 2.94 (dd, 1H, *J* = 7.4 and 11.8 Hz, H-1*), 2.59 (ddd, 1H, *J* = 7.3, 7.4, and 13.5 Hz, H-5a*), 1.81 (dd, 1H, *J* = 8.4 and 13.7 Hz, H-3a*), 1.79 (ddd, 1H, *J* = 6.3, 11.8, and 13.5 Hz, H-5b*), 1.55 (dd, 1H, *J* = 3.5 and 13.7 Hz, H-3b*), 1.09 and 1.07 (2 s, 3H each, Me*). $^1$H NMR data for **43B** (500 MHz, $CD_3OD$) δ 4.29 (d, 1H, *J* = 9.5 Hz, H-1), 4.20 (dddd, 1H, *J* = 3.5, 6.3, 7.3, and 8.2 Hz, H-4*), 3.87 (dd, 1H, *J* = 1.0 and 3.3 Hz, H-4), 3.71 (dd, 1H, *J* = 6.7 and 11.3 Hz, H-6), 3.68 (dd, 1H, *J* = 5.5 and 11.3 Hz, H-6), 3.50 (t, 1H, *J* = 9.3 Hz, H-2), 3.48 (ddd, 1H, *J* = 1.0, 5.5, and 6.7 Hz, H-5), 3.43 (dd, 1H, *J* = 3.3 and 9.3 Hz, H-3), 3.02 (dd, 1H, *J* = 7.4 and 11.8 Hz, H-1*), 2.61 (ddd, 1H, *J* = 7.3, 7.4, and 13.3 Hz, H-5a*), 1.83 (dd, 1H, *J* = 8.2 and 13.6 Hz, H-3a*), 1.78 (ddd, 1H, *J* = 6.3, 11.8, and 13.3 Hz, H-5b*), 1.57 (dd, 1H, *J* = 3.5 and 13.6 Hz, H-3b*), 1.070 and 1.065 (2 s, 3H each, Me*). $^1$H NMR data for **43CD** (360 MHz, $CD_3OD$) δ 4.35 and 4.31 (2 d, 1H each, *J* = 9.4 Hz, H-1), 4.29 and 4.26 (2 m, 1H each, H-4*), 3.90 and 3.88 (dd, 1H, *J* = 1.1 and 3.3 Hz, H-4), 3.74 and 3.73 (2 dd, 1H each, *J* = 6.2 and 11.1 Hz, H-6), 3.70 (dd, 2H, *J* = 5.9 and 11.1 Hz, H-6), 3.52 and 3.50 (2 t, 1H each, *J* = 9.4 Hz, H-2), 3.51 and 3.49 (2 br t, 1H each, H-5), 3.45 and 3.44 (2 dd, 1H each, *J* = 3.3 and 9.3 Hz, H-3), 3.34 and 3.25 (2 dd, 1H each, *J* = 8.3 and 11.5 Hz, H-1*), 2.20 to 2.06 (m, 4H, H-5a* and H-5b*),

2.01 and 2.00 (2 dd, 1H each, $J=7.0$ and 13.7 Hz, H-3a*), 1.48 and 1.46 (2 dd, 1H, $J=3.5$ and 13.6 Hz, H-3b*), 1.17 and 1.15 (2 s, 3H each, Me*), 0.90 (s, 6H, 2 Me*). $m/z$ calcd for $C_{13}H_{25}O_6S$ $(M+H^+)$, 309.1372, found 309.1367.

**Inhibition constants for 43A, 43B, and 73.** Initial rates of hydrolysis were measured at different concentrations of *p*-nitrophenyl β-D-galactoside (0.125–4 mM) and at the same time varying the inhibitor concentration (0.0016–0.1 mM) for each substrate concentration. The data were fitted to the Michaelis–Menten equation (according to the Levenberg–Marquardt algorithm) using the program Kaleidagraph (Abelbeck Software Inc.), which gave the apparent $K_m$ values. Secondary plots of the apparent $K_m$ values against [I] provided the $K_i$ values (Table 1).

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