Pergamon

Tetrahedron 54 (1998) 14925–14946

TETRAHEDRON

Regioselective Lipase-catalysed acylation of 4,6-*O*-benzylidene-α- and -β-D-pyranoside derivatives displaying a range of anomeric substituents

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Received 17 July 1998; revised 24 September 1998; accepted 8 October 1998

Abstract : The application of Lipase enzymes to effect regioselective C-3-*O*-acylation of 4,6-*O*-benzylidene-β-D-gluco- and -galactopyranosides displaying a range of anomeric substituents, and C-2-*O*-acylation of phenyl 4,6-*O*-benzylidene-α-D-glucopyranoside and ethyl 4,6-*O*-benzylidene-1-thio-α-D-glucopyranoside is reported. In particular this method has allowed introduction of a variety of acyl protecting groups at the C-3 hydroxyl group of ethyl 4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside **11**.

INTRODUCTION

It is now accepted that the total synthesis of oligosaccharides,[1] as well as their analogues,[2] is crucial if a full understanding of their biological roles is to be obtained.[3] Although traditional oligosaccharide syntheses are generally complex, multi-step procedures, in recent years, elegance and imagination have been introduced into new synthetic strategies and this has allowed for far superior methods of oligosaccharide assembly.[4] Indeed, a whole range of novel glycosyl donors, which can be activated by orthogonal reagents, are now available for oligosaccharide synthesis.[5]

However, certain oligosaccharide targets are still difficult to prepare efficiently and the development of selective procedures which are effective for all monosaccharides is still a major challenge to the synthetic

0040-4020/98/$ - see front matter
PII: S0040-4020(98)00935-1

chemist. For example, glucopyranosides which possess two pairs of *trans*-diequatorial hydroxyl groups are particularly difficult to differentiate (Figure 1). Thus, despite the ubiquitous nature of glucopyranoside units within important oligosaccharides, the selective activation of the C-3 hydroxyl group remains a difficult challenge which still merits attention. This is in contrast to results obtained with isomeric monosaccharide counterparts, for which largely successful strategies have now been established.[6]

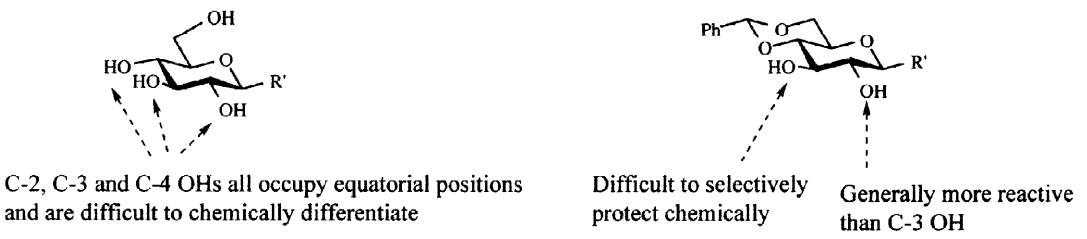


Figure 1 : Difficulty in obtaining regioselective protection with glucopyranosides.

ENZYME MEDIATED ACYLATION REACTIONS

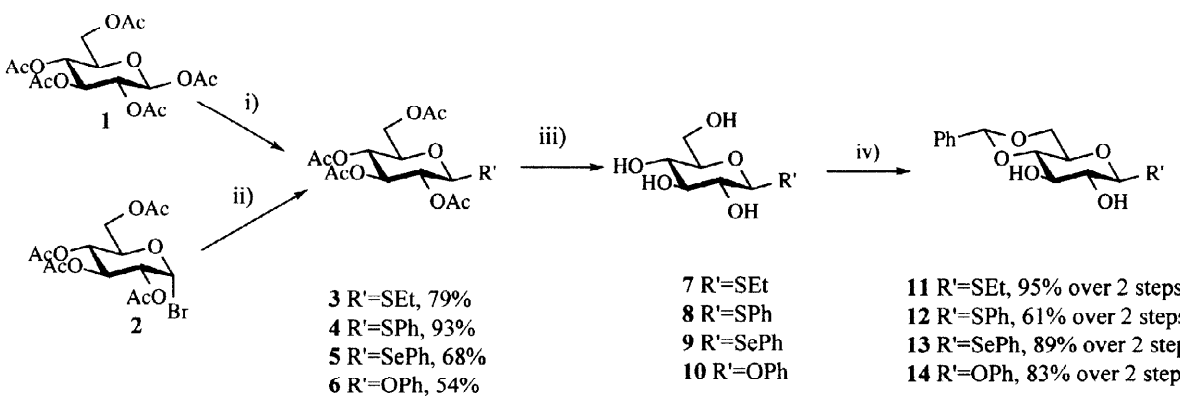
In recent years some interesting investigations have highlighted the potential of using commercially available enzymes to effect regioselective and regiospecific acylation of particular hydroxyl groups decorating furanose and pyranose rings.[7] Detailed studies have explored the effect of varying solvent, acylating agent and enzyme class on the efficiency of these transformations. For example, comprehensive accounts are available which review the ability of different enzymes to selectively protect the primary hydroxyl group or specific secondary hydroxyl groups of a range of pyranoside and furanoside derivatives.[7] Indeed, at the outset of this work, the regioselective mono-*O*-acylation of methyl 4,6-*O*-benzylidene-α- and β-D-glucopyranosides using either *Pseudomonas fluorescens* lipase (PFL),[8] or *Pseudomonas cepacia* lipase (Lipase P),[9] had been reported to proceed in a highly regioselective manner. Interestingly, whilst the α-anomer allowed access to the mono-*O*-acylated C-2 derivative, the β-anomer allowed entry to the isomeric mono-*O*-acylated C-3 derivative. However, the application of this procedure to more synthetically useful derivatives containing a wider range of anomeric heteroatom substituents had received only little attention.[10] Limited results involving non-alkyl substituted derivatives had also been described, for example, a range of aryl rhamnosides have been shown to act as excellent substrates for PFL resulting in regiospecific entry to the corresponding C-4 acetylated derivatives.[7] Such studies have neatly demonstrated that lipase mediated acylation may be incorporated as a

key step at a late stage of a synthetic pathway. Indeed, preliminary results using disaccharides as substrates have indicated that regioselective acylation of the C-6' hydroxyl group can be achieved under carefully controlled conditions.[11]

RESULTS AND DISCUSSION

Considering these interesting developments, we decided to commence a research programme to establish the suitability of a range of heteroatom containing gluco- and galactopyranoside monomers for enzyme mediated acylation reactions. If this strategy could be selectively developed, efficient entry to a range of intermediates suitable for one-pot oligosaccharide synthesis would become highly feasible. Thus a variety of enzymes were screened to determine whether regioselective acylation of 4,6-*O*-benzylidene-α- and β-D-pyranoside substrates displaying a range of anomeric substituents could be achieved. In this paper we will describe in full our recent results in this area.[12]

The benzylidene protected monomer building blocks required for our study were easily prepared in multi-gram quantities using the synthetic strategies outlined for the glucopyranoside substrates in Scheme 1.[13] Thus access to a preponderance of the β-anomer of each benzylidene protected substrate was achieved in excellent yield using this three step protocol.



i) For entry to **3**, **4** and **6**, R'H, $BF_3.OEt_2$, DCM, 0°C to room temp.; ii) For entry to **5**, PhSeSePh, $NaBH_4$, DCM, EtOH, 0°C to room temp.; iii) NaOMe, MeOH; iv) $PhCH(OMe)_2$, CSA. DMF

Scheme 1 : Preparation of benzylidene protected starting materials **11-14**.

Preparation of the thioethyl benzylidene protected galactopyranoside analogue **15** proved equally facile following an analogous procedure to that for the glucopyranoside derivative **11**. This procedure therefore involved formation of the analogous tetraacetate **16** and tetraol **17** from commercially available β-D-galactose pentaacetate. The overall yield of preparation of benzylidene **15** was lower than that for the corresponding glucopyranoside substrate **11**, due to the formation of small quantities of both the 3,4-benzylidene and 2,3-benzylidene protected regioisomers. These undesired regioisomers were readily removed by flash column chromatography on silica gel.

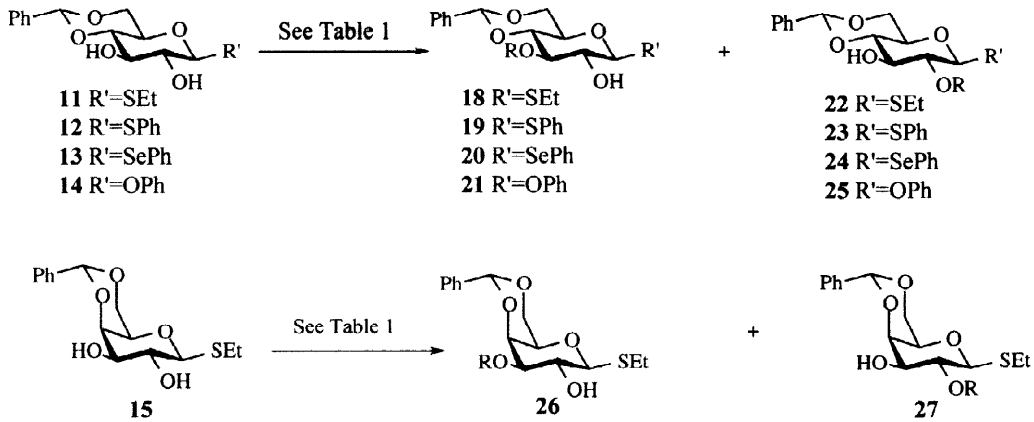
The suitability of the five derivatives **11**, **12**, **13**, **14** and **15** to act as substrates for a range of commercially available enzymes, to allow regioselective synthesis of orthogonally protected acceptors, was next investigated (Scheme 2). The results obtained to date using these five derivatives with three different enzymes are shown in Table 1.

For small scale reactions (approximately 100 mg of starting material), weights of enzyme equal to the weight of the substrates were typically required for the reaction to reach completion. In all cases the progress of the reaction was monitored by tlc and for certain substrates, small volumes of THF were required to enhance the solubility of the substrate in the acylating agent.

When mixtures of the C-2- and C-3-*O*-acylated regioisomers resulted, complete separation of the regioisomers by flash column chromatography was not always possible. The ratio of regioisomers resulting from such transformations was therefore determined by close examination of the ^{1}H nmr spectrum of the mixture of regioisomers. As expected, for the C-3-*O*-acylated regioisomer, the C-3 proton occurred at lower field than the C-2 proton. Further assignment of the ^{1}H nmr spectra of the regioisomers was possible from 2D experiments.

Our results highlighted in Table 1 illustrate that it was possible to attain some level of regioselectivity with all of the derivatives examined in this research programme. For example, although the selenium containing derivative **13** proved a poor substrate for PFL, better regioselectivities were obtained with Lipase P and *candida cylindracea* Lipase (CCL). Excellent results were obtained for the sulfur-containing derivatives **11** and **12**, with derivative **11** proving an excellent substrate for both PFL and Lipase P. Moreover, for substrate **11**, opposite regioselectivity was obtained with CCL, compared to PFL and Lipase P. This is in agreement with the analogous CCL catalysed reaction of the corresponding methyl pyranoside derivative,[8a] but in contrast to results obtained with substrates **12** and **13**. Although the rate of reaction of the isomeric galactose substrate **15**

was poor with all three enzymes studied, the regioselectivities of the reactions were notably high, in parallel with the corresponding methyl pyranoside substrate.[8b]



Substrate	Acylating agent : THF (v : v)	Enzyme[a]	Reaction conditions	Conversion (%)	Ratio[b,c]
11	Vinyl acetate (100 : 0)	PFL	45°C, 7.5 h	86	**18a : 22a** > 99 : < 1[d]
11	Vinyl acetate (100 : 0)	Lipase P	45°C, 7 h	94	**18a : 22a** > 99 : < 1[d]
11	Vinyl acetate (100 : 0)	CCL	45°C, 53 h	89	**18a : 22a** 36 : 64
12	Vinyl acetate (60: 40)	PFL	45°C, 42 h	79	**19a : 23a** 86 : 14
12	Vinyl acetate (60 : 40)	Lipase P	45°C, 65 h	87	**19a : 23a** 82 : 18
12	Vinyl acetate (60 : 40)	CCL	45°C, 48 h	17	**19a : 23a** 82 : 18
13	Vinyl acetate (100 : 0)	PFL	55°C, 50 h	78	**20a : 24a** 53 : 47
13	Vinyl acetate (100 : 0)	Lipase P	55°C, 64 h	98	**20a : 24a** 65 : 35
13	Vinyl acetate (100 : 0)	CCL	55°C, 47 h	25	**20a : 24a** 66 : 33
14	Vinyl acetate (60 : 40)	PFL	45°C, 72 h	71	**21a : 25a** 73 : 27
14	Vinyl acetate (60 : 40)	Lipase P	45°C, 72 h	79	**21a : 25a** 70 : 30
14	Vinyl acetate (60 : 40)	CCL	45°C, 72 h	0	-
15	Vinyl acetate (60 : 40)	PFL	45°C, 47.5 h	10	**26a : 27a** > 99 : < 1[d]
15	Vinyl acetate (60 : 40)	Lipase P	45°C, 62 h	3	**26a : 27a** > 99 : < 1[d]
15	Vinyl acetate (60 : 40)	CCL	45°C, 62 h	0	-

[a] PFL = *Pseudomonas Fluorescens* Lipase; Lipase P is supplied adsorbed into Celite®; CCL = *Candida cylindracea* Lipase; [b] Ratios determined by ^{1}H nmr analysis of crude mixtures, by integration of well defined benzylidene PhC*H* proton; [c] The terms **a,b,c,d** used in Tables 1, 2 and 3 are to distinguish derivatives displaying different acyl (R) groups; [d] No other isomer detected by ^{1}H nmr analysis.

Scheme 2, Table 1 : Initial enzyme investigations.

The full potential of this reaction to regioselectively introduce a range of protecting groups to the C-3 hydroxyl group of good substrates identified in these preliminary investigations was next examined, using PFL and Lipase P, as indicated in Table 2. In some cases, when reactions were performed on a larger scale than previously investigated, greater quantities of enzyme were required. For example, when attempting the PFL mediated regioselective protection of glucopyranoside **11** with vinyl pivaloate on a 4.5 g scale, up to six mass equivalents of the enzyme were required, despite the enzyme being added in small portions throughout the reaction to minimise the effect of enzyme denaturing. Attempts to buffer the enzyme with phosphate buffer (pH 7.2) led to partial improvements in the method but did not lead to reproducibly improved results.

Substrate	Acylating agent : THF (v : v)	Enzyme	Reaction conditions	Conversion (%)	Ratio[a,b]
11	Vinyl formate (60 : 40)	PFL	45°C, 48 h	97	**18b : 22b** 90 : 10
11	Vinyl methacrolate (40 : 60)	PFL	45-55°C, 156 h	89	**18c : 22c** 90 : 10
11	Vinyl pivaloate (60 : 40)	PFL	60°C, 144 h	100	**18d: 22d** > 99 : < 1[c]
11	Vinyl pivaloate (60 : 40)	Lipase P	60°C, 144 h	82	**18d: 22d** > 99 : < 1[c]
12	Vinyl formate (60 : 40)	PFL	45°C, 27 h	83	**19b : 23b** 53 : 47
12	Vinyl pivaloate (60 : 40)	PFL	60°C, 23 h	0	-
14	Vinyl pivaloate (60 : 40)	PFL	60°C, 192 h	0	-
14	Vinyl pivaloate (60 : 40)	Lipase P	60°C, 192 h	0	-

[a] Ratios determined by ^{1}H nmr analysis of crude mixtures, by integration of well defined benzylidene PhC*H* proton; [b] The terms **a,b,c,d** used in Tables 1, 2 and 3 are to distinguish derivatives displaying different acyl (R) groups ; [c] No other isomer detected by ^{1}H nmr analysis.

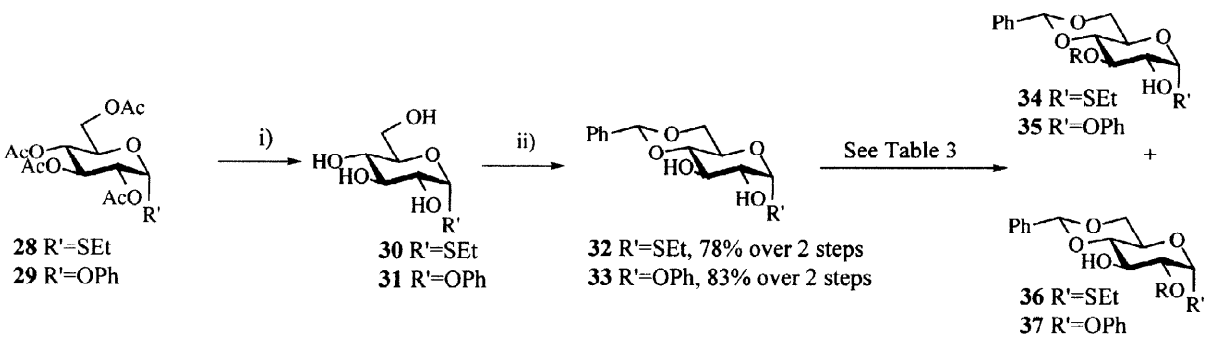
Table 2 : Further enzyme investigations.

These results confirm that PFL is an excellent enzyme for the regioselective incorporation of a range of acyl groups to the C-3 hydroxyl group of glucopyranoside derivative **11**. Although initial results with glucopyranoside **12** demonstrated some regioselectivity, these results could not be extended to encompass a wider range of acylating agents. Furthermore, substrate **14** proved unsuitable for further regioselective modifications under the conditions investigated.

Investigation of regioselectivity of enzyme reactions using 4,6-O-benzylidene-α-D-pyranoside derivatives.

In two cases we also investigated the enzyme mediated acylation reactions of the corresponding benzylidene protected α-anomers to determine the effect of the anomeric configuration on the yield and regioselectivity of the reaction. These substrates were prepared in the same manner as outlined above, using the small amounts of the α-anomer by-products **28** and **29** obtained from the initial Lewis acid catalysed reactions, as starting materials (Scheme 3).

Again, two regioisomers could result from the acylation reaction of each diol **32** and **33** under the reaction conditions investigated. It is already well established that benzylidene protected methyl pyranosides exhibit different selectivities in their enzyme mediated reactions, depending upon the configuration of the anomeric position.[8] This was further verified by the results obtained from these investigations.



i) NaOMe, MeOH; ii) $PhCH(OMe)_2$, CSA, DMF.

Substrate	Acylating agent : THF (v : v)	Enzyme	Reaction conditions	Conversion (%)	Ratio[a,b]
32	Vinyl acetate (50 : 50)	PFL	50°C, 15 h	26[c]	**36a : 34a** > 99 : < 1[d]
32	Vinyl pivaloate (50 : 50)	PFL	60°C, 50 h	73	**36b : 34b** > 99 : < 1[d]
33	Vinyl acetate (60 : 40)	PFL	45°C, 144 h	68	**37a : 35a** > 99 : < 1[d]
33	Vinyl acetate (60 : 40)	Lipase P	45°C, 144 h	65	**37a : 35a** > 99 : < 1[d]
33	Vinyl pivaloate (60 : 40)	PFL	60°C, 192 h	76	**37b : 35b** > 99 : < 1[d]
33	Vinyl pivaloate (60 : 40)	Lipase P	60°C, 192 h	46[c]	**37b : 35b** > 99 : < 1[d]

[a] Ratios determined by ^1H nmr analysis of crude mixtures, by integration of well defined benzylidene PhC*H* proton; [b] The terms **a,b,c,d** used in Tables 1, 2 and 3 are to distinguish derivatives displaying different acyl (R) groups; [c] Although ^1H nmr analysis of the crude reaction mixtures indicated the reactions to have neared completion, yields of isolated products were as indicated; [d] No other isomer detected by ^1H nmr analysis.

Scheme 3, Table 3 : Further enzyme investigations with α-anomers.

For example, the α-thioethyl derivative **32** showed excellent regioselectivity in the PFL mediated reaction both with vinyl acetate and vinyl pivaloate, affording the C-2 regioisomers **36a** and **36b** respectively. For both acylation reactions, only one regioisomer could be detected by ^{1}H nmr analysis of the crude reaction mixtures. Moreover, the results obtained with diol **32** were particularly striking. The corresponding β-phenylpyranoside **14** had afforded only modest levels of regioselectivity in the PFL or Lipase P mediated reaction with vinyl acetate, and had remained unchanged upon treatment with vinyl pivaloate. However, the corresponding α-anomer **33** allowed regiospecific entry to the C-2 acylated derivatives **36a** and **36b** in good yields. Recent discussions on the active site of PFL may explain these results.[14]

CONCLUSION

Various levels of regioselective acylation have been attained for the enzyme mediated reactions of α- and β-D-pyranoside derivatives displaying different heteroatomic anomeric groups. Excellent results were obtained with the sulfur-containing derivatives **11, 12** and **32** and with α-phenylpyranoside **33**. Following recent reports[15] which have also illustrated that solvents may greatly effect the regioselectivity of enzymatic acylation reactions, the scope for synthesising a range of differentially protected acceptors for incorporation into oligosaccharide synthesis strategies is immense.

ACKNOWLEDGEMENTS

We are grateful to the BBSRC and Dextra Laboratories for a CASE award (to J.J.G.). Financial support from the Royal Society is also gratefully acknowledged.

EXPERIMENTAL

General Methods.

All reactions were monitored by thin layer chromatography (TLC) on Merck Kieselgel 60 F_{254} 0.2 mm aluminum-backed plates. Visulization of the TLC plates was achieved using 254 nm UV light and by spray-head development using an ethanol-sulfuric acid reagent (25 : 1 EtOH : H_2SO_4). Flash column chromatography was performed using Merck silica gel 60 (70-230 mesh). Anhydrous solvents were purified and dried as follows: dichloromethane was distilled from calcium hydride; ethanol was distilled from iodine and magnesium filings; dimethylformamide was dried over 4 Å molecular sieves for 24 hours. When necessary, reactions were carried out under an inert atmosphere of argon or nitrogen.

Melting point analyses were carried out using an Electrothermal digital melting point apparatus and are uncorrected. Optical rotation measurements were carried out using a Perkin-Elmer 341 polarimeter and elemental analyses were furnished by Medac Ltd., Brunel Science Centre, Surrey. ^1H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz respectively on a Jeol AX400 spectrometer using $CHCl_3$ as an internal standard unless otherwise stated. Mass spectral analyses were obtained in the chemical ionisation mode using a Fisons VG Autospec. Infra red spectra were recorded using a Perkin-Elmer Paragon 1000 FTIR spectrophotometer.

PFL was purchased from Biocatalysts Ltd., Mid Glamorgan or from Amano, Milton Keynes. Lipase P and CCL were kindly donated by Dextra Laboratories.

General method for deacetylation of derivatives 3, 4, 5, 6, 16, 28 and 29.

To a solution of *tetraacetate* in the minimum amount of anhydrous dichloromethane was added methanol. After 20 minutes of stirring, a solution of sodium methoxide (catalytic) in methanol was added and the reaction was stirred over night, after which time no starting material was evident by tlc analysis. A suspension of Amberlite IR 120 (H^+) resin was added to neutralize the reaction. Following 10 minutes of stirring, the suspension was filtered and the solvent removed *in vacuo* to afford the deacetylated products as yellow gums/foams.

Phenyl 1-seleno-β-D-glucopyranoside 9. By following the general procedure outlined above, *tetraacetate* **5** (8.47 g, 17.37 mmol) in anhydrous dichloromethane (30 cm^3) was treated with methanol (200 cm^3) and then sodium methoxide (1.2 cm^3, 0.6 mmol, 0.5 M). Quenching of the reaction mixture and work-up as outlined above afforded *tetraol* **9** (5.53 g, 100 %) as a viscose oil. υ_{max} / cm^{-1} 3416 vs (O-H), 3066 s and 3056 (C-H), 2988 s and 2881 s and 2810 s (C-H), 2737 s (C-H), 1578 w and 1477 s and 1437 s (C=C), 1415 w and 1392 w and 1359 w (C-H), 1038 s (C-O), 741 w and 692 w (Ar); δ_H (400 MHz; CD_3OD) 3.23-3.38 (4H, m, H-2, H-4, H-5, H-3), 3.67 (1H, dd, *J* 5.5, 12.0, H-6_a), 3.83 (1H, d, *J* 12.0, H-6_b), 4.83 (1H, d, *J* 10.0, H-1), 7.26-7.68 (5H, m, ArH); δ_C (100 MHz; CD_3OD) 62.82 (C-6), 71.25, 74.40, 79.50, 83.10 (C-2, C-3, C-4, C-5), 85.99 (C-1), 128.66, 129.76, 129.92, 135.30 (Ar{C-1, C-2, C-6, C-3, C-5, C-4); m/z (CI) 338 ($M+NH_4$, 57 %), 313 (47), 233 (18), 180 (53), 157 (72), 78 (100). (Found [$M+NH_4$] 338.0558. $C_{12}H_{20}NO_5Se$ requires 338.0506).

Phenyl-1-*O*-α-D-glucopyranoside 31. By following the general procedure outlined above, *tetraacetate* **29** (1.192 g, 2.81 mmol) in anhydrous dichloromethane (10 cm^3) was treated with methanol (20 cm^3) and then sodium methoxide (0.2 cm^3, 0.1 mmol, 0.5 M). Quenching of the reaction mixture and work-up as outlined above afforded *tetraol* **31** (0.691 g, 96 %) as a yellow solid. υ_{max} / cm^{-1} 3473 br (O-H), 3421 br (O-H), 3300 br (O-H), 2925 s (C-H), 2900 s (C-H), 1601 s (C=C), 1590 s (C=C), 1497 vs (C-H), 1366 s (C-H), 1229 s (C-H), 1109 vs (C-O), 1035 vs (C-O), 1012 s (C-O), 756 s and 691 s (Ar); δ_H (400 MHz; CD_3OD) 3.49 (1H, app. t, *J* 9.5, 9.0, H-4), 3.63 (1H, dd, *J* 2.0, 9.5, H-2), 3.71-3.81 (3H, m, H-5, H-6_{ab}), 3.92 (1H, app. t, *J* 9.0, 9.5, H-3), 5.53 (1H, d , *J* 2.5, H-1), 7.04-7.35 (5H, m, ArH); δ_C (100 MHz; CD_3OD), 62.53 (C-6), 71.69, 73.54, 74.54, 75.17 (C-2, C-3, C-4, C-5), 99.53 (C-1), 118.40 (Ar{C-2, C-6}), 123.59 (Ar{C-4), 130.63 (Ar{C-3, C-5), 158.87 (Ar{C-1}); m/z (CI) 274 ($M+NH_4$, 8 %), 180 (15), 145 (7), 127 (4), 94 (100), 66 (8), 55 (6), 39 (6). (Found [$M+NH_4$] 274.1290. $C_{12}H_{20}NO_6$ requires 274.1290).

General method for the formation of benzylidene protected products 11, 12, 13, 14, 15, 32 and 33

To a solution of the *tetraol* in anhydrous DMF (80 cm^3) was added (+/-)-10-camphor sulfonic acid (0.2 equiv) and benzaldehyde dimethyl acetal (1.1 to 1.2 equiv) and the resultant mixture was placed on a rotary evaporator (45-50 °C, ≤20 mmHg). When tlc analysis showed the reaction to be complete,

triethylamine (TEA, 1 equiv) was added and the solution was concentrated *in vacuo* using toluene to azeotropically remove residual DMF. The resultant brown oil was dissolved in the minimum quantity of a 1 % TEA solution in dichloromethane, preadsorbed onto silica gel and submitted to flash column chromatography using gradient elution as detailed below.

Phenyl 4,6-*O*-benzylidene-1-seleno-β-D-glucopyranoside 13. By following the general procedure outlined above, *tetraol* **9** (5.42 g, 17.0 mmol) in anhydrous DMF (80 cm^3) was treated with (+/-)-10-camphor sulfonic acid (0.79 g, 3.39 mmol, 0.2 equiv) and benzaldehyde dimethyl acetal (2.80 cm^3, 18.65 mmol, 1.1 equiv). The reaction mixture was quenched and worked up as outlined above and the residue submitted to flash column chromatography (8 : 1 toluene : ethyl acetate then ethyl acetate with 1 % TEA as co-solvent). Concentration of the pure fractions *in vacuo* afforded *diol* **13** (6.13 g, 89 %) as an orange solid. m.p. 162.0-164.0 °C, [Lit.[16] (propan-2-ol) 150-151 °C]; $[\alpha]^{28}_D$ -44.9 (*c* 0.74 in DCM), [Lit.[17] $[\alpha]^{21}_D$ -43.3 (*c* 0.67 in DCM)]; υ_{max} / cm^{-1} 3456 br and 3269 br (O-H), 3071 s and 3028 s (C-H), 2970 vs and 2946 s (C-H), 2877 vs and 2845 s (C-H), 1463 s and 1455 s and 1440 w (C=C), 1377 s (C-H), 1107 s and 1085 s and 1058 s (O-H), 1040 w and 1006 s (C-O) 741 w and 691 w (Ar); δ_H (400 MHz; CD_3OD) 3.29-3.48 (3H, m, H-2, H-4, H-5), 3.62 (1H, app. t, *J* 9.0, 8.5, H-3), 3.72 (1H, t, *J* 10.0, H-6_a), 4.24 (1H, dd, *J* 4.5, 10.0, H-6_b), 4.89 (1H, d, *J* 10.0, H-1), 5.54 (1H, s, Ph-C*H*), 7.28-7.66 (10H, m, ArH); δ_C (100 MHz; CD_3OD) 69.77 (C-6), 76.32 (C-3), 73.03, 75.35, 82.19, (C-5, C-4, C-2), 86.28 (C-1), 103.02 (PhCH), 127.73, 129.23, 129.28, 130.12, 130.21, 136.23, 139.28 (ArSe, Ar{C-1, C-2, C-3, C-4, C-5, C-6}); m/z (CI) 409 (M+H, 6 %), 303 (5), 251 (100), 235 (35), 129 (37), 105 (53), 78 (30). (Found [M+H] 409.0537. $C_{19}H_{21}O_5Se$ requires 409.0554).

Phenyl 4,6-*O*-benzylidene-1-*O*-β-D-glucopyranoside 14. By following the general procedure outlined above, *tetraol* **10** (4.00 g, 15.63 mmol) in anhydrous DMF (45 cm^3) was treated with (+/-)-10-camphor sulfonic acid (0.73 g, 3.10 mmol, 0.2 equiv) and benzaldehyde dimethyl acetal (2.85 cm^3, 18.98 mmol, 1.2 equiv). The reaction mixture was quenched and worked up as outlined above and the residue recrystallized (propan-2-ol, methanol and dichloromethane) to afford *diol* **14** (2.51 g, 47 %) as a white crystalline solid. The remaining crude material was dissolved in the minimum amount of 1 % TEA solution in warm acetone, preadsorbed onto silica gel and submitted to flash column chromatography (2 : 1 : 1 hexane : dichloromethane :

ethyl acetate with 1 % TEA as co-solvent). Concentration of the pure fractions *in vacuo* afforded *diol* **14** (1.94 g, 36 %) as a white solid (total yield of *diol* **14** 4.45 g, 83 %). m.p. 179-181 °C, [Lit.[18] (ethanol) 194.5-195 °C]; $[\alpha]^{27}_D$ -36.8 (*c* 0.25 in acetone), [Lit.[18] $[\alpha]^{25}_D$ -56.5 (*c* 2 in acetone)]; υ_{max} / cm^{-1} 3386 br and 3328 br (O-H), 3070 w and 3040 w (C-H), 2929 s and 2867 vs (C-H), 1599 s (C=C), 1493 vs (C=C), 1452 s (C-H), 1389 s and 1372 s (C-H), 1227 vs (C-O), 1084 br (C-O), 1030 s and 1016 s (C-O), 751 s and 697 s (Ar); δ_H (400 MHz; CD_3OD) 3.59-3.68 (3H, m, H-2, H-5, H-4), 3.79-3.86 (2H, m, H-6_a, H-3), 4.36 (1H, m, H-6_b), 5.06 (1H, d, *J* 7.5, H-1), 5.62 (1H, s, Ph-C*H*), 7.05-7.68 (10H, m, ArH); δ_C (100 MHz; CD_3OD) 67.37 (C-5), 69.48 (C-6), 74.14 (C-3), 75.33 (C-4), 81.62 (C-2), 102.54, 102.76 (C-1, PhCH), 117.72, 123.67, 127.21, 128.95, 129.90, 130.29, (Ar, PhO{C-1, C-2, C-3, C-4, C-5, C-6}), 138.28 (Ar{C-1}), 158.32 (PhO{C-1}); m/z (CI) 345 (M+H, 34 %), 251 (M-OPh, 46), 183 (7), 179 (6), 149 (12), 127 (11), 105 (77), 94 (100), 91 (19), 77 (29), 66 (18), 65 (16), 55 (17), 39 (17). (Found [M+H] 345.1335. $C_{19}H_{21}O_6$ requires 345.1338).

Ethyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside 15. By following the general procedure outlined above, *tetraol* **17** (2.58 g, 11.5 mmol) in anhydrous DMF (35 cm^3) was treated with (+/-)-10-camphor sulfonic acid (0.54 g, 2.30 mmol, 0.2 equiv) and benzaldehyde dimethyl acetal (1.90 cm^3, 12.6 mmol, 1.1 equiv). The reaction mixture was quenched and worked up as outlined above and the residue submitted to flash column chromatography using gradient elution (2 : 1 toluene : ethyl acetate then ethyl acetate with 1 % TEA as co-solvent). Combining the pure fractions and concentration *in vacuo* afforded *diol* **15** (1.04 g, 29 %) as a white solid. m.p.157.6-158.6 °C, [Lit.[19] m.p. 154-156 °C]; $[\alpha]^{27}_D$ -60.6 (*c* 0.7 in $CHCl_3$), [Lit.[19] $[\alpha]^{27}_D$ -55 (*c* and solvent not specified)]; υ_{max} / cm^{-1} 3386 br (O-H), 2977 w (C-H), 2923 s (C-H), 2882 s (C-H), 2875 vs (C-H), 1496 w (C=C), 1450 s (C-H), 1404 s (C-H), 1365 s (C-H), 1248 s (C-O), 1169 s (C-O), 1100 s, 1075 s, 1058 s, 1027 s and 1000 s (C-O), 733 s and 697 s (Ar), 650 w (C-S); δ_H (400 MHz; $CDCl_3$) 1.34 (3H, t, *J* 7.5, SCH_2CH_3), 2.76-2.87 (2H, m, SCH_2CH_3), 3.52 (1H, m, H-5), 3.70 (1H, dd, *J* 3.5, 9.0, H-3), 3.80 (1H, t, *J* 9.0, H-2), 4.04 (1H, dd, *J* 2.0, 12.5, H-6_a), 4.25 (1H, d, *J* 2.5, H-4), 4.36-4.32 (2H, m, H-1, H-6_b), 5.54 (1H, s, Ph-C*H*), 7.36-7.49 (5H, m, ArH); δ_C (100 MHz; $CDCl_3$) 15.20 (SCH_2CH_3), 23.38 (SCH_2CH_3), 69.24 (C-6), 69.61 (C-2), 70.01 (C-5), 73.80 (C-3), 75.59 (C-4), 85.24 (C-1), 101.36 (PhCH), 126.37, 128.22, (Ar{C-2, C-6, C-3, C-5}), 129.21 (Ar{C-4}), 137.58 (Ar{C-1}); m/z (CI) 313 (M+H, 26 %), 295 (7), 251 (M-SEt, 100), 207 (49), 105 (25), 85 (10). (Found [M+H] 313.1109. $C_{15}H_{21}O_5S$ requires 313.1109).

Phenyl 4,6-*O*-benzylidene-1-*O*-α-D-glucopyranoside 33. By following the general procedure outlined above, *tetraol* **31** (0.619 g, 2.39 mmol) in anhydrous DMF (15 cm^3) was treated with (+/-)-10-camphor sulfonic acid (0.11 g, 0.47 mmol, 0.2 equiv) and benzaldehyde dimethyl acetal (0.43 cm^3, 2.86 mmol, 1.2 equiv). The reaction mixture was quenched and worked up as outlined above and the residue submitted to flash column chromatography (1 : 1 : 1 hexane : dichloromethane : ethyl acetate with 1% TEA as co-solvent). Combining the pure fractions and concentration *in vacuo* afforded *diol* **33** (0.70 g, 83 %) as a yellow solid. m.p. 194-196 °C, [Lit.[20] (Ethanol-KOH) 206 °C]; $[\alpha]^{27}_D$ +202.7 (*c* 0.23 in acetone), [Lit.[20] $[\alpha]^{14}_D$ +174.2 (*c* 1 in acetone)]; υ_{max} / cm^{-1} 3379 br (O-H), 3065 w (C-H), 2936 s (C-H), 2866 w (C-H), 1598 s (C=C), 1495 s (C=C), 1451 w (C-H), 1371 s (C-H), 1231 s (C-O), 1085 vs and 1074 vs and 1031 s (C-O), 747 s and 694 s (Ar); δ_H (400 MHz; C_3D_6O) 3.57 (1H, app. t, *J* 9.5, 9.0, H-4), 3.69-3.73 (1H, m, H-2), 3.76 (1H, t, *J* 10.5, H-6_a), 3.86-3.92 (1H, m, H-5), 4.04-4.15 (2H, m, H-3, H-6_b) 4.31 (1H, d, *J* 8.0, C-2-O*H*), 4.60 (1H, d, *J* 4.0, C-3-O*H*), 5.59 (1H, d, *J* 4.0, H-1), 5.62 (1H, s, Ph-C*H*), 7.02-7.51 (10H, m, ArH); δ_C (100 MHz; C_3D_6O); 64.92 (C-5), 69.35 (C-6), 71.69 (C-3), 73.78 (C-2), 82.48 (C-4), 99.27 (C-1), 102.29 (Ph*C*H), 117.80, (PhO{ C-2, C-6}) 123.14 (PhO{C-4}), 127.29 (Ar{C-3, C-5}), 128.73 (Ar{C-2, C-6}), 129.52 (Ar{C-4}), 130.29, (PhO{C-3, C-5}), 139.20 (Ar{C-1}), 158.05 (PhO{C-1); m/z (CI) 345 (M+H, 80 %), 251 (M-OPh, 100), 179 (8), 149 (9), 140 (9), 139 (8), 127 (12), 105 (42), 94 (53), 91 (18), 77 (18), 65 (11), 55 (8), 39 (12). (Found [M+H] 345.1338. $C_{19}H_{21}O_6$ requires 345.1338).

General Method for Enzyme Acetylation Reactions.

To a solution of the *diol* in vinyl acetate at 45 °C was added small portions of enzyme and the reaction was monitored to completion by tlc analysis. After this time the enzyme was removed by filtering through Celite® and the residue washed with ethyl acetate. The filtrate was washed with distilled water then extracted with ethyl acetate (3 x 20 cm^3), dried with $MgSO_4$, filtered and the solvent removed *in vacuo* to afford a crude reaction mixture which was analysed by ^1H nmr spectroscopy to obtain isomeric ratios of products present in the crude mixture. When possible, separation of the regioisomers was obtained by column chromatography on silica gel.

Ethyl 3-*O*-acetyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside 18a *Pseudomonas fluorescens* lipase (PFL) (500 mg) was added in portions to diol **11** (509 mg, 1.63 mmol) in vinyl acetate (15 cm^3) at 45 °C over 7.5 hr following the procedure outlined above. Purification of the reaction mixture by column chromatography on silica gel (4 : 1 toluene : ethyl acetate with 1% TEA as co-solvent) afforded *acetate* **18a** (497 mg, 86 %) as a white solid. m.p. 117.3-118.8 °C, [Lit.[10] m.p 118-120°C]; $[\alpha]^{22}_D$ -69.3 (*c* 1.0 in $CHCl_3$), [Lit.[10] $[\alpha]^{25}_D$ -67.30 (*c* 1.03 in $CHCl_3$]; υ_{max} / cm^{-1} 3372 br (O-H), 2984 s (C-H), 2826 s (C-H), 1714 vs (C=O), 1446 s (C-H), 1374 vs (C-H), 1267 vs (C-O), 1082 s (C-O), 1024 s (C-O); δ_H (400 MHz; $CDCl_3$) 1.25 (3H, t, *J* 7.5, $SCH_2C\underline{H}_3$), 2.06 (3H, s, $C\underline{H}_3COO$), 2.67-2.70 (2H, m, $SC\underline{H}_2CH_3$), 3.45-3.52 (2H, m, H-2, H-5), 3.58 (1H, app. t, *J* 9.0, 9.5, H-4), 3.69 (1H, app. t, *J* 10.0, 10.5, H-6_a), 4.27 (1H, dd, *J* 5.0, 10.5, H-6_b), 4.43 (1H, d, *J* 10.0, H-1), 5.16 (1H, app. t, *J* 9.0, 9.5, H-3) 5.42 (1H, s, Ph-C*H*), 7.27-7.38 (5H, m, ArH); δ_C (100 MHz; $CDCl_3$) 15.25 ($SCH_2\underline{C}H_3$), 21.01 ($\underline{C}H_3COO$), 24.92 ($S\underline{C}H_2CH_3$), 68.50 (C-6), 70.48 (C-5), 72.09 (C-2), 74.74 (C-3), 78.32 (C-4), 87.12 (C-1), 101.42 (Ph$\underline{C}$H), 126.11, 128.21, 129.07, 136.86 (Ar{C-1, C-2, C-3, C-4, C-5, C-6}), 171.01 ($CH_3\underline{C}O_2$); m/z (CI) 355 (M+H, 24 %), 293 (23), 249 (39), 187 (32), 105 (100), 43 (59). (Found [M+H] 355.1199. $C_{17}H_{23}O_6$ requires 355.1215).

Phenyl 3-*O*-acetyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside 19a. *Pseudomonas fluorescens* lipase (PFL) (301 mg) was added to *diol* **12** (151 mg, 0.42 mmol) in tetrahydrofuran : vinyl acetate (2 : 3, 8.3 cm^3) at 45 °C over 42 hours following the general procedure outlined above. ^1H nmr analysis of the crude reaction mixture demonstrated it to contain a mixture of *diol* **12**, *phenyl 3-O-acetyl-4,6-O-benzylidene-1-thio-β-D-glucopyranoside* **19a** and *phenyl 2-O-acetyl-4,6-O-benzylidene-1-thio-β-D-glucopyranoside* **23a** in a ratio of 12 : 76 : 12; For *acetate* **19a** δ_H (400 MHz; $CDCl_3$) 2.02 (3H, s, $C\underline{H}_3COO$), 3.41-3.53 (3H, m, H-5, H-4, H-2), 3.68 (1H, app. t, *J* 9.5, 10.5, H-6_a), 4.29 (1H, dd, *J* 5.0, 10.5, H-6_b), 4.58 (1H, d, *J* 9.5, H-1), 5.15 (1H, t, *J* 9.0, H-3) 5.40 (1H, s, Ph-C*H*), 7.24-7.47 (5H, m, ArH).

Phenyl 3-*O*-acetyl-4,6-*O*-benzylidene-1-seleno-β-D-glucopyranoside 20a. *Pseudomonas fluorescens* lipase (PFL) (300 mg) was added to *diol* **13** (149 mg, 0.365 mmol) in vinyl acetate (15 cm^3) at 55 °C over 50 hours following the general procedure outlined above. The crude mixture was submitted to flash column chromatography on silica gel using gradient elution (6 : 1 hexane : ethyl acetate then ethyl acetate with

1 % TEA as co-solvent). Evaporation of the pure fractions afforded *acetate* **20a** (6 mg, 4 %) as a white solid plus a mixture (50:50) of regioisomeric *acetates* **20a** and **24a** (122 mg, 74%). For the mixture of regioisomers υ_{max} / cm^{-1} 3372 br (O-H), 2962 w (C-H), 2935 w (C-H), 2874 w (C-H), 1714 s (C=O), 1438 w (C-H), 1366 w (C-O), 1262 s (C-O), 1089 s and 1020 s (C-O), 744 w and 695 w (Ar); for *acetate* **20a**, $[\alpha]^{27}_D$ -18.7 (*c* 0.15 in $CHCl_3$); δ_H (400 MHz; $CDCl_3$) 2.11 (3H, s, C*H*$_3$COO) 3.51-3.58 (3H, m, H-2, H-4, H-5), 3.76 (1H, app. t, *J* 10.5, 9.5, H-6_a), 4.40 (1H, m, H-6_b), 4.87 (1H, d, *J* 10.0, H-1), 5.20 (1H, t, *J* 9.0, H-3), 5.48 (1H, s, Ph-C*H*), 7.31-7.66 (10H, m, ArH); δ_C (100 MHz; $CDCl_3$) 20.98 (CH_3COO), 68.51 (C-6), 71.89, 72.24, 74.60, 78.20 (C-5, C-2, C-3, C-4), 85.43 (C-1), 101.50 (PhCH), 126.12, 128.24, 128.73, 129.13, 129.26, 131.49, 135.41, 136.80 (Ar ArSe{C-1, C-2, C-3, C-4, C-5, C-6}), 171.07 (CH_3CO_2); m/z (CI) 451 (M+H, 18 %), 390 (5), 344 (36), 293 (71), 250 (36), 233 (55), 187 (30), 148 (44), 127 (51), 105 (100), 78 (82), 43 (72). (Found [M+H] 451.0649. $C_{21}H_{23}O_6Se$ requires 451.0659).

Phenyl 3-*O*-acetyl-4,6-*O*-benzylidene-1-*O*-β-D-glucopyranoside 21a. *Pseudomonas fluorescens* lipase (PFL) (212 mg) was added to *diol* **14** (105 mg, 0.30 mmol) in tetrahydrofuran : vinyl acetate (2 : 3, 15 cm^3) at 45 °C over 72 hours following the general procedure outlined above. The crude mixture was dissolved in minimum 1 % TEA solution in dichloromethane, preadsorbed onto silica gel and submitted to flash column chromatography on silica gel using gradient elution (3 : 1 toluene : ethyl acetate then 1 : 1 toluene : ethyl acetate with 1 % TEA as co-solvent). Combining the pure fractions and removal of the solvent *in vacuo* afforded *acetates* **21a and 25b (21a : 25a** 2.7 : 1, **84** mg, 71 %) as a mixture of inseparable regioisomers. For the mixture of regioisomers. υ_{max} / cm^{-1} 3424 br (O-H), 3057 w, 3042 w, 2930 w and 2880 s (C-H), 1745 vs (C=O), 1600 s (C=C), 1590 w (C=C), 1497 s (C=C), 1451 w (C-H), 1391 s and 1373 s (C-H), 1233 vs (C-O), 1099 vs and 1081 vs (C-O), 1032 s and 1012 s (C-O), 754 s and 697 s (Ar). For **21a** δ_H (400 MHz; $CDCl_3$) 2.16 (3H, s, C*H*$_3$COO), 3.55-3.91 (4H, m, H-2, H-5, H-4, H-6_a), 4.36-4.43 (1H, m, H-6_b), 5.11 (1H, d, *J* 7.5, H-1), 5.31 (1H, t, *J* 9.5, H-3), 5.52 (1H, s, Ph-C*H*), 6.99-7.51 (10H, m, ArH). For **25a** δ_H (400 MHz; $CDCl_3$) 2.14 (3H, s, C*H*$_3$COO), 3.55-3.91 (4H, m, H-5, H-4, H-3, H-6_a), 4.36-4.43 (1H, m, H-6_b), 5.13 (1H, d, *J* 8.0, H-1), 5.22 (1H, app. t, *J* 8.0, 9.0, H-2), 5.57 (1H, s, Ph-C*H*), 6.99-7.51 (10H, m, ArH); m/z (CI) 387 (M+H, 21 %), 293 (M-OPh, 97), 233 (14), 187 (30), 149 (38), 127 (25), 105 (23), 94 (28), 92 (68), 91 (81), 77 (19), 58 (21), 43 (100), 39 (14). (Found [M+H] 387.1444. $C_{21}H_{22}O_7$ requires 387.1443).

Ethyl 3-*O*-acetyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside 26a. *Pseudomonas fluorescens* lipase (PFL) (204 mg) was added to *diol* **15** (101 mg, 0.32 mmol) in tetrahydrofuran : vinyl acetate (2 : 3, 8 cm^3) at 45 °C over 47.5 hours following the general procedure outlined above. The crude mixture was dissolved in minimum 1 % TEA solution in dichloromethane, preadsorbed onto silica gel and submitted to flash column chromatography on silica gel using gradient elution (3 : 1 toluene : ethyl acetate then 1 : 1 toluene : ethyl acetate with 1 % TEA as co-solvent). Combining the pure fractions and removal of the solvent *in vacuo* afforded *acetate* **26a** (11 mg, 10 %) as a white solid. $[\alpha]^{27}_D$ -6.0 (*c* 0.05 in $CHCl_3$); υ_{max} / cm^{-1} 3443 br (O-H), 2924 s (C-H), 2853 s (C-H), 1735 w (C=O), 1459 w (C-H), 1375 w (C-O), 1263 w (C-O), 1168 w (C-H), 1089 w, 1044 w and 1023 w (C-O), 737 w and 694 w (Ar), 668 w (C-S); δ_H (400 MHz; $CDCl_3$) 1.27 (3H, t, *J* 7.5, $SCH_2C\underline{H_3}$), 2.07 (3H, s, $C\underline{H_3}COO$), 2.70-2.77 (2H, m, $SC\underline{H_2}CH_3$), 3.49 (1H, s, H-5), 3.93-4.00 (2H, m, H-2, H-6_b), 4.24 (1H, app. d, *J* 12.5, H-4), 4.36-4.34 (2H, m, H-1, H-6_a), 4.80 (1H, dd, *J* 3.5, 10.0, H-3), 5.42 (1H, s, Ph-C*H*), 7.28-7.41 (5H, m, ArH); δ_C (100 MHz; $CDCl_3$) 15.33 ($SCH_2\underline{C}H_3$), 21.09 ($\underline{C}H_3COO$), 23.28 ($S\underline{C}H_2CH_3$), 69.20, 69.92, 73.78, 74.93, 77.64 (C-2, C-3, C-4, C-5, C-6), 85.88 (C-1), 101.10 (Ph$\underline{C}$H), 126.28, 128.20, (Ar{C-2, C-6, C-3, C-5}), 129.08, 130.87 (Ar{C-4, C-1}); m/z (CI) 355 (M+H, 9 %), 293 (100), 249 (16), 203 (16), 187 (44), 149 (59), 127 (25), 105 (43), 81 (19), 43 (63). (Found [M+H] 355.1212. $C_{17}H_{23}O_6$ requires 355.1215).

Ethyl 2-*O*-acetyl-4,6-*O*-benzylidene-1-thio-α-D-glucopyranoside 36a. *Pseudomonas fluorescens* lipase (PFL) (190 mg) was added to diol **32** (150 mg, 0.48 mmol) in tetrahydrofuran : vinyl acetate (1 : 1, 3 cm^3) at 50 °C over 15 hr following the procedure outlined above. Crude ^1H nmr showed only **36a** (45 mg, 26 %) as a white solid. m.p.117.8-118.8 °C, $[\alpha]^{22}_D$ +153.8 (*c* 0.55 in $CHCl_3$); υ_{max} / cm^{-1} 3437 br (O-H), 3064 w, 3034 w, 2958 w, 2927 s, 2904 w, and 2857 w (C-H), 1751 vs (C=O), 1449 w (C-H), 1451 w (C-H), 1375 s (C-H), 1256 vs (C-O), 1102 s, 1073 vs and 1029 s (C-O), 749 w and 695 s (Ar), 651 w (C-S); δ_H (400 MHz; $CDCl_3$) 1.18 (3H, t, *J* 7.5, $SCH_2C\underline{H_3}$), 2.06 (3H, s, $C\underline{H_3}COO$), 2.43-2.58 (3H, m, $SC\underline{H_2}CH_3$, C-3-O*H*), 3.49 (1H, t, *J* 9.0, H-4), 3.70 (1H, m, H-6_b), 4.00 (1H, t, *J* 9.5, H-3), 4.15-4.22 (2H, m, H-5, H-6_a), 4.83 (1H, app. dd, *J* 5.5, 10.0, 6.0, 10.0, H-2), 5.47 (1H, s, Ph-C*H*), 5.60 (1H, d, *J* 6.0, H-1) 7.29-7.44 (5H, m, ArH); δ_C (100 MHz; $CDCl_3$) 14.83 ($SCH_2\underline{C}H_3$), 20.92 ($\underline{C}H_3COO$), 24.40 ($S\underline{C}H_2CH_3$), 62.40 (C-5), 68.64 (C-6), 69.08 (C-3), 73.50 (C-2), 81.22 (C-4), 82.50 (C-1), 102.03 (Ph$\underline{C}$H), 126.30, 128.33, (Ar{C-2, C-3, C-5, C-6}), 129.32 (Ar{C-4}), 136.89 (Ar{C-1}), 170.36 ($CH_3\underline{C}O_2$); m/z (CI) 355 (M+H, 15 %), 293 (75), 275 (15), 249 (18), 233

(37), 187 (40), 169 (33), 149 (100), 127 (30), 105 (63), 91 (33), 77 (20), 43 (88). (Found [M+H] 355.1226. $C_{17}H_{23}O_6$ requires 355.1215).

Phenyl 2-*O*-acetyl-4,6-*O*-benzylidene-1-*O*-α-D-glucopyranoside 37a. *Pseudomonas fluorescens* lipase (PFL) (497 mg) was added to *diol* **33** (98 mg, 0.28 mmol) in tetrahydrofuran : vinyl acetate (2 : 3, 5 cm^3) at 45 °C over 144 hours following the general procedure outlined above. The crude mixture was dissolved in minimum 1 % TEA solution in dichloromethane, preadsorbed onto silica gel and submitted to flash column chromatography (1 : 2 hexane : diethyl ether with 1 % TEA as co-solvent). Concentration of the pure fractions afforded *acetate* **37a** (74 mg, 68 %) as a yellow solid. m.p. 151-152 °C; $[\alpha]^{27}_D$ +181.14 (*c* 0.98 in $CHCl_3$); υ_{max} / cm^{-1} 3515 br and 3427 br (O-H), 3062 w and 3029 w (C-H), 2941 w, 2925 w and 2870 w (C-H), 1752 vs and 1726 vs (C=O), 1597 s and 1491 s (C=C), 1449 s (C-H), 1375 vs (C-H), 1253 vs and 1218 vs (C-O), 1116 vs (C-H), 1072 vs, 1028 vs, 1007 s (C-O), 755 s and 696 vs (Ar); δ_H (400 MHz; $CDCl_3$) 2.07 (3H, s, C*H*$_3$COO), 3.59 (1H, t, *J* 9.5, H-4), 3.69 (1H, app. t, *J* 10.5, 10.0, H-6_a), 3.93-3.99 (1H, m, H-5), 4.15 (1H, dd, *J* 5.0, 10.5, H-6_b), 4.35 (1H, t, *J* 9.5, H-3), 4.86 (1H, dd, *J* 3.5, 9.5, H-2), 5.50 (1H, s, Ph-C*H*), 5.69 (1H, s, *J* 3.5, H-1), 6.97-7.43 (10H, m, ArH); δ_C (100 MHz; $CDCl_3$) 20.23 (*C*H$_3$COO), 63.94 (C-5), 68.47 (C-3), 68.65 (C-6), 73.83 (C-2), 81.79 (C-4), 96.04 (C-1), 101.95 (Ph*C*H), 117.49, 123.16, 126.84, 128.31, 129.15, 130.00, (Ar PhO{C-2, C-3, C-4, C-5, C-6}), 138.47 (Ar{C-1}), 157.25 (PhO{C-1}), 170.42 (CH$_3$*C*OO); m/z (CI) 387 (M+H, 71 %), 293 (M-OPh, 100), 281 (15), 275 (17), 233 (13), 187 (45), 149 (41), 127 (13), 105 (46), 94 (21), 91 (12), 77 (11), 43 (13), 39 (10). (Found [M+H] 387.1438. $C_{21}H_{22}O_7$ requires 387.1443).

General Method for Enzyme Acylation Reactions.

To a solution of the *diol* in a mixture of the acylating agent and THF (3 : 2) at the temperature stated in each method was added small portions of enzyme and the reaction was monitored to completion by tlc analysis. After this time the enzyme was removed by filtering through Celite® and the residue washed with ethyl acetate. The filtrate was washed with distilled water then extracted with ethyl acetate (3 x 20 cm^3), dried with $MgSO_4$, filtered and the solvent removed *in vacuo* to afford a crude reaction mixture which was analysed by ^{1}H nmr spectroscopy to obtain isomeric ratios of products present in the crude mixture. When possible, separation of the regioisomers was achieved by column chromatography on silica gel.

Ethyl 3-*O*-formyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside 18b. *Pseudomonas fluorescens* lipase (PFL) (100 mg) was added to *diol* **11** (101 mg, 0.32 mmol) in tetrahydrofuran : vinyl formate (2 : 3, 5 cm^3) at 45 °C over 2 days following the procedure outlined above. ^1H nmr analysis of the crude reaction mixture demonstrated it to contain 90 % *3-O-formate* **18b** and 10 % *2-O-formate* **22b** and < 1% di-*O*-formate. For **18b**, δ_H (400 MHz; $CDCl_3$) 1.34 (3H, t, *J* 7.5, $SCH_2C\underline{H}_3$), 2.75-2.79 (2H, m, $SC\underline{H}_2CH_3$), 3.59-3.72 (3H, m, H-4, H-2, H-5), 3.78 (1H, app. t, *J* 10.0, 10.5, H-6_a), 4.36 (1H, dd, *J* 5.0, 10.5, H-6_b), 4.51 (1H, d, *J* 10.0, H-1), 5.23 (1H, t, *J* 9.0, H-3), 5.51 (1H, s, PhC*H*), 7.34-7.49 (5H, m, ArH), 8.21 (1H, s, *H*CO).

Ethyl 3-*O*-methacryl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside 18c. *Pseudomonas fluorescens* lipase (PFL) (100 mg) was added to *diol* **11** (100 mg 0.32 mmol) in tetrahydrofuran : vinyl methacrolate (3 : 2, 5 cm^3) at 45-55 °C over 6.5 days following the general procedure outlined above. ^1H nmr analysis of the crude reaction mixture demonstrated it to contain a mixture of regioisomers (109 mg, 89 %) (>90 % 3-*O*-methacrylate **18c** and <10 % 2-*O*-methacrylate **22c**) which decomposed within days at room temperature. For the major regioisomer, *methacrylate* **18c**, δ_H (400 MHz; $CDCl_3$) 1.33 (3H, t, *J* 7.5, $SCH_2C\underline{H}_3$), 1.98 (3H, s, $CH_2=C(C\underline{H}_3)CO$) 2.75-2.80 (2H, m, $SC\underline{H}_2CH_3$), 3.55-381 (4H, m, H-5, H-2, H-4, H-6_a), 4.36 (1H, dd, *J* 5.0, 10.5, H-6_b), 4.54 (1H, d, *J* 10.0, H-1), 5.28 (1H, app. t, *J* 9.0, 9.5, H-3) 5.51 (1H, s, Ph-C*H*), 5.62 (1H, s, *H*CH=C(Me)CO), 6.18 (1H, s, HC*H*=C(Me)CO), 7.26-7.43 (5H, m, ArH).

Ethyl 3-*O*-pivaloyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside 18d. *Pseudomonas fluorescens* lipase (PFL) (250 mg) was added to *diol* **11** (100 mg, 0.32 mmol) in tetrahydrofuran : vinyl pivaloate (2 : 3, 5 cm^3) at 60 °C over 6 days following the general procedure outlined above. Purification of the crude mixture by column chromatography (10 : 1 toluene : ethyl acetate with 1 % TEA as co-solvent) on silica gel afforded *pivaloate* **18d** (132 mg, 100 %) as a brown viscose oil/foam. $[\alpha]^{20}_D$ -66.9 (*c* 1.02 in $CHCl_3$); υ_{max} / cm^{-1} 3492 br (O-H), 3067 w and 3037 w (C-H), 2981 s (C-H), 1737 vs and 1715 vs (C=O), 1481 s and 1397 s (C-H), 1282 s and 1079 s and 1019 s (C-O), 751 s and 698 s (Ar), 653 w (S-C); δ_H (400 MHz; $CDCl_3$) 1.16 (9H, s, $(C\underline{H}_3)_3CO$), 1.25 (3H, t, *J* 7.5, $SCH_2C\underline{H}_3$), 2.68-2.71 (2H, m, $SC\underline{H}_2CH_3$), 2.73 (1H, d, *J* 2.0, OH), 3.45-353 (2H, m, H-2, H-5), 3.61 (1H, t, *J* 9.5, H-4), 3.70 (1H, t, *J* 10.5, H-6_a), 4.29 (1H, dd, *J* 5.0, 10.5, H-6_b), 4.44 (1H, d, *J* 10.0, H-1), 5.12 (1H, t, *J* 9.0, H-3) 5.45 (1H, s, Ph-C*H*), 7.26-7.35 (5H, m, ArH); δ_C (100

MHz; $CDCl_3$); 15.25 ($SCH_2\underline{C}H_3$), 25.00 ($S\underline{C}H_2CH_3$), 27.08 ($\underline{C}H_3)_3C$) 38.97 ($(CH_3)_3\underline{C}CO$) 68.56 (C-6), 70.64 (C-5), 72.32 (C-2), 74.87 (C-3), 78.43 (C-4), 87.23 (C-1), 101.07 (Ph$\underline{C}$H), 125.85, 128.18, 128.34, 128.93, 129.02, 136.92 (Ar{C-1, C-2, C-3, C-4, C-5, C-6}), 178.78 $(CH_3)_3\underline{C}CO$); m/z (CI) 415 ($M+NH_4$, 24 %), 397 (80), 335 (72), 291 (100), 229 (64), 199 (48), 149 (61), 105 (63), 85 (48), 57 (53). (Found [M+H] 397.1705. $C_{20}H_{29}SO_6$ requires 397.1684).

Phenyl 3-*O*-formyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside 19b. *Pseudomonas fluorescens* lipase (PFL) (255 mg) was added to *diol* **12** (155 mg, 0.43 mmol) in tetrahydrofuran : vinyl formate (2 : 3, 8 cm^3) at 45 °C over 27 hours following the general procedure outlined above. ^1H nmr analysis of the crude mixture demonstrated it to contain a mixture of *phenyl 3-O-formyl-4,6-O-benzylidene-1-thio-β-D-glucopyranoside* **19b** : *phenyl 2-O-formyl-4,6-O-benzylidene-1-thio-β-D-glucopyranoside* **23b** : *phenyl 4,6-O-benzylidene-1-thio-β-D-glucopyranoside* **12** in a ratio of 45 : 41 : 13; For *formate* **19b** δ_H (400 MHz; $CDCl_3$) 3.48-3.63 (3H, m, H-2, H-4, H-5), 3.77 (1H, app. t, *J* 9.5, 10.5, H-6_a), 4.36-4.40 (1H, m, H-6_b), 4.63 (1H, d, *J* 9.5, H-1), 5.19 (1H, t, *J* 9.0, H-3) 5.47 (1H, s, Ph-C$\underline{H}$), 7.32-7.54 (5H, m, ArH), 8.15 (1H, s, $\underline{H}$CO).

Ethyl 2-*O*-pivaloyl-4,6-*O*-benzylidene-1-thio-α-D-glucopyranoside 37a. *Pseudomonas fluorescens* lipase (PFL) (600 mg) was added to *diol* **32** (200 mg, 0.64 mmol) in tetrahydrofuran : vinyl pivaloate (1 : 1, 3 cm^3) at 60 °C over 50 hours following the general procedure outlined above, with the addition of 3-4 drops of pH 7.2 phosphate buffer. Purification of the crude mixture by column chromatography (dichloromethane then dichloromethane : ethyl acetate 20 : 1 with 1 % TEA as co-solvent) on silica gel afforded *pivaloate* **37a** (186 mg, 73 %) as an oil which partially crystallizes with time. m.p. 110.7-111.3 °C; $[\alpha]^{22}_D$ +192.2 (*c* 0.5 in $CHCl_3$); υ_{max} / cm^{-1} 3470 vs (O-H), 3041 w and 3034 w (C-H), 2971 s, 2932 s, 2904 s and 2869 s (C-H), 1727 vs (C=O), 1481 s, 1464 s, 1396 s and 1373 s (C-H), 1277 s, 1151 s, 1099 s (C-O), 754 s and 699 s (Ar), 652 w (S-C); δ_H (400 MHz; $CDCl_3$) 1.13-1.16 (12H, m, ($C\underline{H}_3)_3CO$ and $SCH_2C\underline{H}_3$), 2.37-2.52 (2H, m, $SC\underline{H}_2CH_3$), 3.46 (1H, t, *J* 9.0, H-4), 3.68 (1H, m, H-6_b), 3.97 (1H, t, *J* 9.5, H-3), 4.13-4.20 (2H, m, H-6_a, H-5), 4.81 (1H, dd, *J* 6.0, 10.0, H-2), 5.44 (1H, s, Ph-C$\underline{H}$), 5.56 (1H, d, *J* 6.0, H-1) 7.28-7.43 (5H, m, ArH); δ_C (100 MHz; $CDCl_3$); 14.85 ($SCH_2\underline{C}H_3$), 24.11 ($S\underline{C}H_2CH_3$), 26.92 ($\underline{C}H_3)_3C$) 38.66 ($(CH_3)_3\underline{C}CO$) 62.40 (C-5), 68.55 (C-6), 69.13 (C-3), 73.03, (C-2), 81.15 (C-4), 82.45 (C-1), 101.83 (Ph$\underline{C}$H),

126.25, 128.22, 129.15, 136.94 (Ar{C-1, C-2, C-3, C-4, C-5, C-6}), 177.69 $(CH_3)_3\underline{C}CO$); m/z (CI) 397 (M+H, 22 %), 335 (100), 294 (88), 291 (26), 234 (12), 229 (22), 211 (16), 149 (70), 127 (9), 105 (54), 91 (25), 85 (75), 57 (89), 41 (23). (Found [M+H] 397.1688. $C_{20}H_{29}SO_6$ requires 397.1684).

Phenyl 2-*O*-pivaloyl-4,6-*O*-benzylidene-1-*O*-α-D-glucopyranoside 37b. *Pseudomonas fluorescens* lipase (PFL) (302 mg) was added to *diol* **33** (52 mg, 0.15 mmol) in tetrahydrofuran : vinyl pivaloate (2 : 3, 5 cm^3) at 60 °C over 192 hours following the general procedure outlined above. The crude material was submitted to flash column chromatography, (hexane : diethyl ether 2 : 1.5 then 1 : 2 hexane : diethyl ether with 1 % TEA as co-solvent) and concentration of the pure fractions afforded *pivaloate* **37b** (49 mg, 76 %) as a yellow solid. m.p. 126.8-128.2 °C; $[\alpha]^{20}_D$ +178.6 (*c* 0.35 in $CHCl_3$); υ_{max} / cm^{-1} 3554 br (O-H), 3061 w (C-H), 2975 s, 2934 s, 2921 s, 2873 s, and 2856 w, (C-H), 1733 vs (C=O), 1594 s, 1498 s and 1488 s (C=C), 1450 s (C-H), 1398 w and 1375 s (C-H), 1281 s and 1219 vs (C-O), 1145 vs and 1105 vs (C-H), 1053 s, 1032 vs, 992 vs (C-O), 749 s and 700 s (Ar); δ_H (400 MHz; $CDCl_3$) 1.14 (9H, s, $(C\underline{H_3})_3CCOO$), 3.58 (1H, app. t, *J* 9.0, 9.5, H-4), 3.69 (1H, t, *J* 10.5, H-6_a), 3.94-4.00 (1H, m, H-5), 4.15-4.19 (1H, app. dd, *J* 4.5, 9.5, H-6_b), 4.34 (1H, t, *J* 9.5, H-3), 4.80-4.83 (1H, app. dd, *J* 3.0, 9.5, H-2), 5.50 (1H, s, Ph-C*H*), 5.64 (1H, s, *J* 3.0, H-1), 6.95-7.42 (10H, m, ArH); δ_C (100 MHz; C_3D_6O) 27.29 ($(\underline{C}H_3)_3CCOO$), 39.31 ($(CH_3)_3\underline{C}COO$), 64.40 (C-5), 69.00, (C-3), 69.06 (C-6), 74.18 (C-2), 82.19 (C-4), 96.69 (C-1), 102.36 (Ph$\underline{C}$H), 117.83, 123.59, 127.29, 128.75, 129.59, 130.47 (Ar, PhO{C-2, C-3, C-4, C-5, C-6}), 138.92 (Ar{C-1}), 157.90 (PhO{C-1}); m/z (CI) 429 (M+H, 37 %), 335 (M-OPh, 100), 327 (17), 317 (7), 229 (21), 211 (11), 162 (7), 149 (28), 129 (9), 105 (37), 94 (20), 91 (12), 77 (8), 66 (8), 65 (8), 57 (27), 43 (8), 39 (6). (Found [M+H] 429.1924. $C_{23}H_{28}O_6$ requires 429.1913).

REFERENCES

1. For recent reviews in this area see a) G. -J. Boons, *Contemp. Org. Synth.*, 1996, **3**, 173; b) G. -J. Boons, *Tetrahedron*, 1996, **52**, 1095; c) S. J. Danishefsky, J. Y. Roberge, *Pure and Applied Chem.*, 1995, **67**, 1647.
2. For example see a) T. Suami, S. Ogawa, *Adv. Carbohydr. Chem. Biochem.*, 1990, **48**, 21; b) M. Hoffmann, F. Burkhart, G. Hessler, H. Kessler, *Helv. Chim. Acta.*, 1996, **79**, 1519.
3. For example see a) J. McAuliffe, O. Hindsgaul, *Chem. and Ind.*, 1997, 153; b) A. Varki, *Glycobiology*, 1993, **3**, 97.
4. a) G.-J. Boons, P. Grice, R. Leslie, S.V. Ley, L.L. Yeung, *Tetrahedron Lett.*, 1993, **34**, 8523; b) S.V. Ley, H.W.M. Priepke, *Angew. Chem., Int. Ed. Engl.*, 1994, **333**, 2292; c) P. Grice, S.V. Ley, J. Pietruszka, H.W.M. Priepke, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 197; d) P. Grice, S.V. Ley, J. Pietruszka, H.W.M. Priepke, E.P.E. Walther, *Synlett*, 1995, 781; e) M.-K. Cheung, N.L. Douglas, B. Hinzen, S.V. Ley, X. Pannecoucke, *Synlett*, 1997, 257; f) G.H. Veeneman, J.H. van Boom, *Tetrahedron Lett.*, 1990, **31**, 275; g) G.-J. Boons, R. Geurtsen, D. Holmes, *Tetrahedron Lett.*, 1995, **36**, 6325.
5. F. Barresi, O. Hindsgaul, *J. Carbohydr. Chem.*, 1995, **14**, 1043.
6. a) P.M. Collins, R.J. Ferrier, *Monosaccharides : Their Chemistry and their Roles in Natural Products*, Wiley, 1995, Ch. 5; b) A.H. Haines, *Adv. Carbohydr. Chem. Biochem.*, 1976, **33**, 11.
7. N.B. Bashir, S.J. Phythian, A.J. Reason, S.M. Roberts, *J. Chem. Soc., Perkin Trans I.*, 1995, 2203.
8. a) M.J. Chinn, G. Iacazio, D.G. Spackman, N.J. Turner, S.M. Roberts, *J. Chem. Soc., Perkin Trans. I.*, 1992, 661; b) G. Iacazio, S.M. Roberts, *J. Chem. Soc., Perkin Trans. I.*, 1993, 1099.
9. L. Panza, S. Brasca, S. Riva, G. Russo, *Tetrahedron: Asymm.*, 1993, **4**, 931.
10. I. Matsuo, M. Isomura, R. Walton, K. Ajisaka, *Tetrahedron Lett.*, 1996, **37**, 8795.
11. S. Riva, J. Chopineau, A.P.G. Kieboom, A.M. Klibanov, *J. Am. Chem. Soc.*, 1988, **110**, 584.
12. For our preliminary communication in this area see J.J. Gridley, A.J. Hacking, H.M.I. Osborn, D.G. Spackman, *Synlett*, 1997, 1397.
13. H.G. Fletcher, *Methods in Carbohydrate Chem.*, 1963, **2**, 307.
14. D.A. Leigh, J.P. Smart, A.M. Truscello, *Carbohydr.Res.*, 1995, **276**, 417.
15. D.A. Macmanus, E.N. Vulfson, *Enzyme Microb. Technol.*, 1997, **20**, 225.
16. H-G. Korth, R. Sustmann, J. Dupuis, B. Giese, *J. Chem. Soc., Perkin Trans. II*, 1986, 1453.

17. S. Mehta, B.M. Pinto, *J. Org. Chem.*, 1993, **58**, 3269.

18. C. McCloskey, *J .Org. Chem.*, 1945, **10**, 184, 187.

19. B. Bochkov, *Bull. Acad. Sci. USSR Div. Chem .Sci.*, 1975, **24**, 554, 557.

20. K. Aritomi, *Chem. Pharm. Bull.*, 1970, **18**, 677, 684.