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Regioselective Enzymatic Deacetylation of Sucrose Octaacetate in Organic Solvents.

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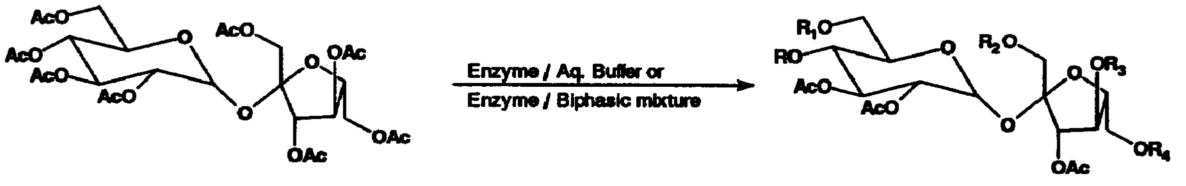
Abstract: The first examples of deacetylation of sucrose octaacetate using proteases and lipases in organic solvents are reported. The regiochemistry of the product sucrose hepta-, hexa- and pentaacetates was established by perdeuteroacetylation and ^{1}H-NMR. Preparation of key intermediates in the commercial synthesis of the high intensity sweetener, sucralose was achieved.

Selective functionalization in complex molecules is a challenge routinely faced in organic synthesis. Such transformations can be very difficult in carbohydrate chemistry where manipulation of identical or nearly identical hydroxy groups often requires protection/deprotection schemes to achieve the desired transformation.[2] One solution to this challenge has been provided by Klibanov's pioneering work using enzymes in organic solvents [3] and examples of regioselective enzymatic acylation of unprotected mono- and disaccharides and enzymatic deacylation of peracylated sugars have been described.[4,5]

Enzymatic deacylation of sucrose peresters has produced partially deacylated compounds useful as emulsifiers, food preservatives, fat substitutes, and tobacco enhancers.[6] Deacetylation of sucrose octaacetate (SOA) **1** by lipases or proteases in buffer or biphasic media has been used to prepare partially deacetylated analogues including 2,3,4,6,1',3',6' -heptaacetyl sucrose **2**,[7] 2,3,4,3',4' -pentaacetyl sucrose (4-PAS) **3** and 2,3,6,3',4'-pentaacetyl sucrose (6-PAS) **4**, all intermediates in the synthesis of high intensity, non-nutritive sweeteners.[8] Exclusive deacetylation in the fructose ring of **1** using wheat germ lipase was reported to produce 2,3,4,6,1',3' -hexaacetyl sucrose **6** and 2,3,4,6,3' -pentaacetyl sucrose **5** (Scheme I).[9] In view of the well known advantages of conducting enzymatic reactions in organic solvents [10] coupled with our interest in alternative approaches to **3** and **4**, we have investigated the reaction of **1** with lipases and proteases in organic solvents.

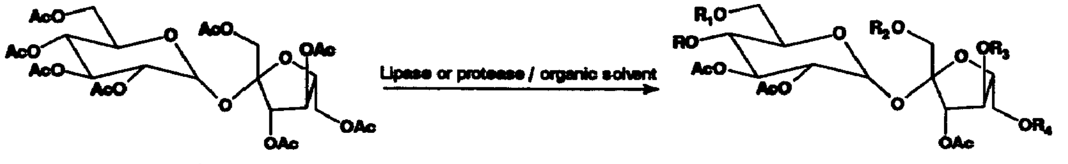


1

2 R, R_1, R_2, R_4 = Ac; R_3 =H
3 R, R_3 = Ac; R_1, R_2, R_4 =H
4 R_1, R_3 = Ac; R, R_2, R_4 = H
5 R, R_1 = Ac; R_2, R_3, R_4 = H
6 R, R_1, R_2 = Ac; R_3, R_4 = H

Scheme I

Routinely, commercial enzymes were precipitated from a pH adjusted buffer followed by vacuum drying and controlled rehydration by storage at 5° for 24 hours over a saturated K_2CO_3 solution prior to use.[3,11] After evaluating the activity of several enzymes in solvent screens we found ethers and hydrocarbons to be the most useful. This, coupled with the solubility characteristics of SOA and the products, prompted use of diisopropyl ether in our initital investigations.[12] In this manner, a number of proteases and lipases were found to deacetylate 1 yielding sucrose heptaacetates as shown (Scheme II).[13-14] The structures of all products were determined using Rathbone's O-perdeuteroacetylation technique.[15] In the ^{1}H-NMR a comparison of the relative intensity of the acetate peaks in 1 vs a pure deacetylated analogue permitted unambiguous regiochemical assignments while an estimate of the overall deacetylation pattern was possible for a given mixture of sucrose esters. From low field to high field, the order of acetate resonances for 1 in a 1:1 mixture of benzene-d$_6$/pyridine-d$_5$ is 3'<2<6<6'<1'<3<4<4'.



1

7 R, R$_1$, R$_3$, R$_4$ = Ac; R$_2$ = H
8 R, R$_1$, R$_2$, R$_3$ = Ac; R$_4$ = H
9 R = H; R$_1$, R$_2$, R$_3$, R$_4$ = Ac
10 R, R$_2$, R$_3$, R$_4$ = Ac; R$_1$ = H
11 R, R$_1$, R$_3$ = Ac; R$_2$, R$_4$ = H

Scheme II

Lipase AY 30 from *Candida cylindracea* yielded a 3:1 mixture of 2 and 2,3,4,6,3',4',6' -heptaacetyl sucrose 7 whereas lipase type II from porcine pancreas cleanly deacetylated 1 to yield 2. Predominant deacetylation at the 6'-position was achieved using SP-435 lipase from *Candida antarctica* to yield 8 while lipase AP 12 from *Aspergillus niger* effected deacetylation in the pyranose ring yielding a 1:1 crude mixture of 2,3,6,1',3',4',6' -heptaacetyl sucrose 9 and 2,3,4,1',3',4',6' -heptaacetyl sucrose 10. Interestingly, upon chromatographic purification (silica gel, hexane/EtOAc, 2/3) only 9 was obtained. We suspect that silica gel catalyzed rearrangement of 10 to 9 has occurred analogous to the known rearrangement of 3 to 4.[8b,8c,8e,16]

Virtually no regioselectivity was observed using the serine protease, protease N from *Bacillus subtilis* which afforded essentially an equimolar mixture of 2, 7 and 8. In contrast, both proleather, a serine protease from *Bacillus subtilis* and alcalase, a commercial form of subtilisin Carlsberg from *Bacillus licheniformis* predominately deacetylated the 1'-position to produce 7 together with minor amounts of 8 and, in the case of proleather, 2. Having demonstrated that this group of enzymes had complementary regioselectivity for all three key positions, we set out to evaluate this methodology for the synthesis of 3 and/or 4. In principle, this could be achieved either with a single enzyme which would deacetylate all three positions or by using a combination of enzymes in a "stepwise" manner to selectively unblock each position sequentially. The latter case would require a judicious choice of both the enzymes and the order of use to achieve this goal.

Longer treatment of 1 with either proleather or alcalase produced 7 together with essentially one major sucrose hexaacetate identified as 2,3,4,6,3',4' -hexaacetyl sucrose 11 after chromatographic purification (silica gel, hexane/EtOAc, 1/4), perdeuteroacetylation and ^{1}H-NMR. We conclude that both proteases deacetylate 1 to produce 7 which is then further deacetylated to yield 11. With both of the key furanose positions now "unblocked" we turned our attention to the one remaining position requisite for the synthesis of 3 or 4, namely that in the pyranose ring.

A mixture comprised of unreacted 1, two heptaacetates 7 and 8, essentially one hexaacetate 11 and two pentaacetates was obtained when 1 was treated with alcalase or proleather for four to five days. After chromatographic purification (silica gel, hexane/EtOAc, 2/3) 7 and 8 were isolated as a 7/1 mixture. Continued elution with hexane/EtOAc (1/4) afforded pure 11 with the pentaacetate mixture obtained by elution with EtOAc. HPLC coinjection of this mixture with authentic samples of 3 and 4 strongly supported the tentative structure assignments. The pentaacetate mixture was rechromatographed (silica gel, hexane/EtOAc, 1/9) to yield a single compound the structure of which was confirmed to be 4 by ^{1}H-NMR after perdeuteroacetylation (Figure 1). Again, silica gel apparently catalyzed the rearrangement of the acetate on the 4-position to the 6-position. Alternatively, treatment of 11 with lipase AP 12 produced the same pentaacetate mixture which, after chromatographic purification as before, yielded only 4.

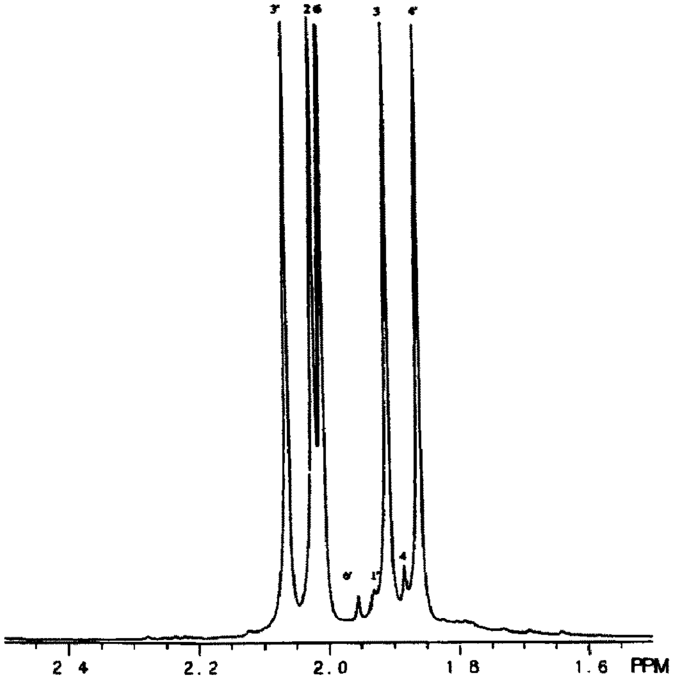


Figure 1 Acetate Region of 4

In conclusion we have shown that 1 can be regioselectively deacetylated in organic solvents using both lipases and proteases. Key intermediates in the synthesis of sucralose such as 3 and 4 can be prepared from 1 using either a single enzyme or a combination of enzymes in a "stepwise" manner. Further studies to expand the scope and applications of this work are in progress.

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13. General screening procedure: A scintillation vial was charged successively with 100 mg of powdered enzyme, 1.0 ml of 20 mmol SOA dissolved in diisopropyl ether. Following a 5 second sonication, the vial was incubated in a controlled-temperature shaker @ 45° C and 300 rpm for 24 hours. An 0.2 ml aliquot of the reaction mixture was withdrawn and centrifuged. The supernatant was collected, evaporated under a nitrogen stream and reconstituted in 0.2 ml of MeCN with 0.01 ml of this solution analyzed by HPLC.
14. For preparative reactions: The hydrolytic enzyme, 1.50 g was added to a solution of 0.203 g of SOA in 15 ml of diisopropyl ether. The suspension was sonicated for 5 seconds and then shaken @ 300 rpm and 45° C in a controlled-temperature rotary shaker for a specified time. The enzyme was removed by filtration and washed with MeCN. The combined filtrate/washes were evaporated and the residue chromatographed on silica gel as described in the text. Perdeuteroacetylation was accomplished using excess d-$_6$ acetic anhydride in pyridine-d$_5$ @ 45° C for 12-24 hours. Excess solvent and reagent were removed on a rotary evaporator and the residue analyzed by ^{1}H-NMR.
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