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PREPARATION OF 2,3,6,3',4'-PENTA-O-ACETYL SUCROSE, THE PRECURSOR OF SUCRALOSE, BY ENZYMATIC METHODS

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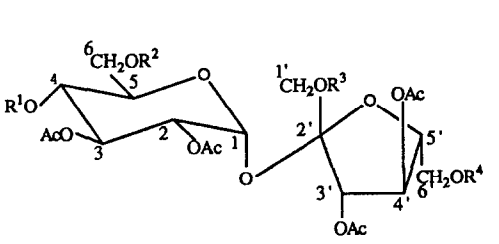
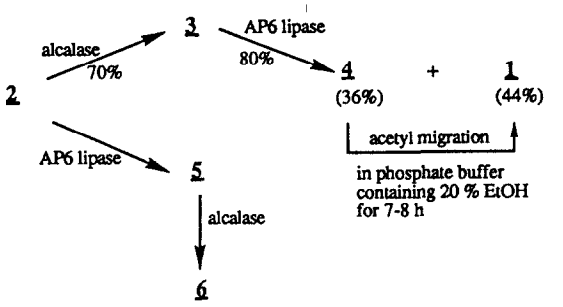
Summary : 2,3,6,3',4'-penta-O-acetyl sucrose, the precursor of sucralose, can be prepared from the sequential hydrolysis of sucrose octaacetate by alcalase and AP-6 lipase.

In 1976, Hough and Phadnis reported the synthesis of 4-chloro-4-deoxy-α-D-galactopyranosyl 1,6-dichloro-1,6-dideoxy-ß-D-fructofuranoside, called sucralose, and found it to possess sweetness several hundred times that of sucrose[1,2]. Because of non-nutrition, non-carcinogen and resistance to be hydrolyzed by α-galactosidase and ß-fructofuranosidase, the compound is quite safe for human use. The compound had been synthesized from 2,3,6,3',4'-pentaacetyl sucrose (**1**) by replacing hydroxyl groups with chlorine, especially the inversion of chirality at C-4 (gluco ---> galacto)[3]. Compound **1** was obtained from sucrose which was tritylated, acetylated, then detritylated and C_4 ---> C_6 acetyl migration[4,5], but the whole procedure was tedious and inefficient. A strategy that has more applicability for the production of **1** is to selectively remove the acetyl groups from sucrose octaacetate (**2**). Selective removal of acetyl groups from **2** for the preparation of partially acetylated sucrose has been studied in chemical[6-13] and enzymatic[14-16] ways. Enzymatic methods

are generally more selective than chemical methods. Recently, a British group has been developed a enzymatic method for preparing **1** directly by the subtilisin-catalyzed hydrolysis of **2**[17]. However, the yield was extremely low (about 1.6 %) and quite impractical. Here, we report that **2** prepared from the sequential hydrolysis of **1** by alcalase and lipase AP-6 with 55% of a total yield was shown in Scheme 1.



	R^1	R^2	R^3	R^4
1	H	Ac	H	H
2	Ac	Ac	Ac	Ac
3	Ac	Ac	H	H
4	Ac	H	H	H
5	H	Ac	Ac	Ac
6	H	Ac	H	Ac



Scheme 1.

In order to prepare **1** efficiently and practically, compound **2** was first hydrolyzed by alcalase (subtilisin Carlsberg from Novo, Denmark) to produce 2,3,4,6,3',4'-hexa-O-acetyl sucrose (**3**) and then **3** was further hydrolyzed by AP-6 lipase (Aspergillus

nigar from Amano, Japan) to form 2,3,4,3',4'-penta-O-acetyl sucrose (**4**) and **1**. Compound **4** could be converted to **1** by C_4 ---> C_6 acetyl migration in the phosphate buffer (0.2 M, pH 7.0) containing 20 % ethanol. The structure of **1** and **4** were determined by ^1H-^1H and ^1H-^{13}C 2D-COSY NMR spectra[18]. However, if compound **2** was first hydrolyzed by AP-6 lipase to produce 2,3,6,1',3',4',6'-hepta-O-acetyl sucrose (**5**) and then further hydrolyzed by alcalase, the major product was found to be 2,3,6,3',4',6'-hexa-O-acetyl sucrose (**6**) with little sucrose penta-acetate. It evidently showed that sucrose hexaacetates were not good substrates for subtilisin. That is reason why **2** could not be obtained directly from the subtilisin-catalyzed hydrolysis of **1** with substantial yield[17]. The method we developed has much higher yield and more practical than that of the British group.

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18. N.m.r. data of **1** (acetonitrile-d_3): ^{1}H, δ 1.97-2.10 (m, 15 H, 5 Ac), 3.20-3.3.30 (t, 1 H, OH-6'), 3.30-3.45 (m, 2 H, OH-1',4), 3.50 (d, 1 H, H-1'), 3.52-3.72 (m, 3 H, H-4,6',6'), 3.90-4.12 (m, 2 H, H-5,5'), 4.18-4.38 (m, 2 H, H-6,6), 4.65-4.75 (dd, 1 H, $J_{1,2}$ 3.7, $J_{2,3}$ 10.3 Hz, H-2), 5.15-5.28 (dd, 1 H, $J_{3,4}$ 9.7 Hz, H-3), 5.30-5.42 (dd, 1 H, $J_{3',4'} = J_{4',5'} \approx$ 7.8 Hz, H-4'), 5.52 (d, 1 H, H-3'), 5.55 (d, 1 H, H-1'); ^{13}C, δ 20.40-20.52 (5 C, -CO$\underline{C}$H$_3$), 62.12 ($C_{6'}$), 63.10 (2C, $C_{6,1'}$), 68.41 (C_4), 70.70 (C_2), 71.03 (C_5), 72.10 (C_3), 74.77 ($C_{4'}$), 75.98 ($C_{3'}$), 81.71 ($C_{5'}$), 90.26 (C_1), 105.19 ($C_{2'}$), 170.28-171.77 (5 C, -$\underline{C}$OCH$_3$).
N.m.r. data of **4** ($CDCl_3$): ^{1}H, δ 1.97-2.15 (m, 15 H, 5 Ac), 3.18 (t, 1 H, OH-6'), 3.37-3.80 (m, 6 H, H-1',1',6,6,6',6'), 3.95-4.00 (m, 1 H, H-5'), 4.07-4.14 (m, 1 H, H-5), 4.76-4.83 (dd, 1 H, $J_{1,2}$ 3.7, $J_{2,3}$ 10.3 Hz, H-2), 4.85-4.96 (dd, 1 H, $J_{3,4} = J_{4,5} = 7.8$ Hz, H-4), 5.34-5.47 (m, 3 H, H-3,3',4'), 5.64 (d, 1 H, H-1); ^{13}C, δ 20.71-20.81 (5 C, -CO$\underline{C}$H$_3$), 62.89 ($C_{6'}$), 63.19 ($C_{1'}$), 63.99 (C_6), 68.89 (C_4), 70.30 (C_2), 70.84 (C_5), 71.96 (C_3), 74.90 ($C_{4'}$), 76.50 ($C_{3'}$), 78.80 ($C_{5'}$), 90.15 (C_1), 105.34 ($C_{2'}$), 170.21-171.56 (5 C, -$\underline{C}$OCH$_3$).