Tetrahedron Letters, Vol.31, No.22, pp 3093-3096, 1990
Printed in Great Britain

0040-4039/90 $3.00 + .00
Pergamon Press plc

ENZYME-CATALYZED ACYLATION OF CASTANOSPERMINE AND 1-DEOXYNOJIRIMYCIN

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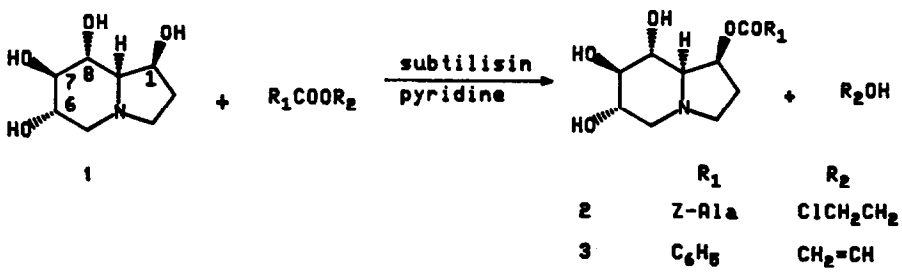
Abstract: Several esters of the alkaloids deoxynojirimycin and castanospermine have been synthesized via subtilisin-catalyzed regioselective acylation in pyridine.

The α-glucosidase-I inhibitors - castanospermine (**1**) and 1-deoxynojirimycin (**4**) - affect the processing of glycoproteins and therefore have a number of interesting biological activities.[1] Recent data suggest that these alkaloids have potential anti-HIV activity and may be useful in the treatment of acquired immunodeficiency syndrome (AIDS)[2]. It has been reported that several hydrophobic analogs (esters, N-alkyl derivatives) of **1**[3] and **4**[4] are more active than **1** and **4** themselves in inhibiting HIV replication.

The regioselective synthesis of monoesters of **1** and **4** by standard techniques of organic chemistry represents a laborious task since it usually requires several protection and deprotection steps[5]. The problem of regioselective acylation of these aminosugars can be, in principle, solved by using enzymatic catalysis in organic solvents[6].

We have recently developed a procedure which leads to the synthesis of several esters of **1**[7]. In this paper we are extending the proposed methodology to the synthesis of **2** and **3** and esters of **4**.

Since both **1** and **4** are poorly soluble in hydrophobic organic solvents we used pyridine as a reaction medium and subtilisin Carlsberg as a catalyst. A typical experimental procedure is illustrated by the synthesis of **2** (Scheme 1).



1

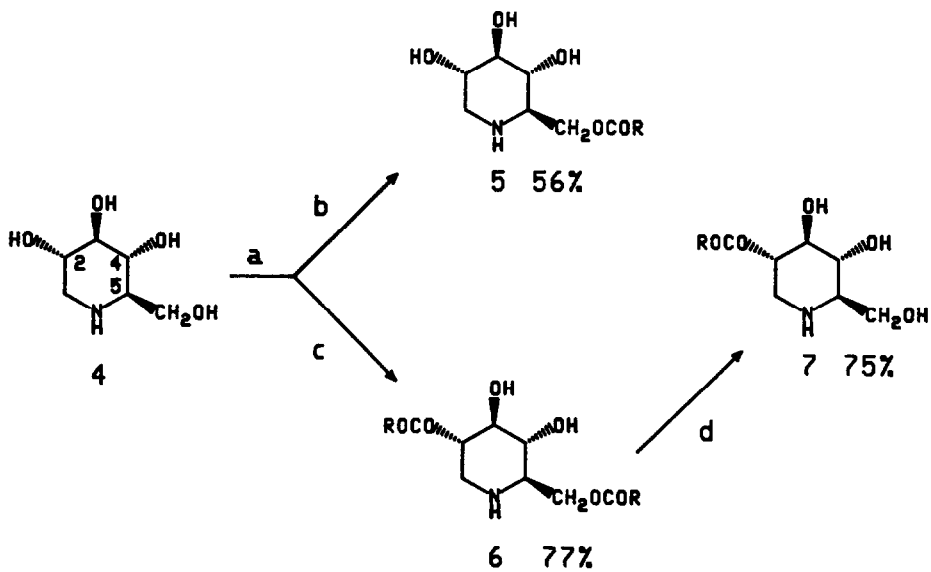
	R_1	R_2
2	Z-Ala	$ClCH_2CH_2$
3	C_6H_5	$CH_2=CH$

Scheme 1

We dissolved 1.57 mmol of castanospermine and 2.36 mmol of CBZ-L-Ala-OCH_2CH_2Cl in 26 ml of anhydrous pyridine followed by addition of 130 mg of subtilisin. The suspension was shaken at 45°C and 260 rpm for six days. The enzyme was removed by filtration, the pyridine was evaporated, and the product purified by radial silica-gel chromatography (8% $EtOH/CH_2Cl_2$). This procedure resulted in 1.01 mmol (64% yield) of 2.[8]

It is important to note that subtilisin has broad substrate specificity in pyridine. It catalyzes the reaction of 1 with such an "unnatural" substrate as vinyl benzoate to give 3 in 65% yield. On the other hand the regioselectivity of subtilisin in pyridine remains remarkably high. Out of four secondary hydroxyl groups, subtilisin acylates only at the C-1 position.

Unlike castanospermine, 1-deoxynojirimycin has primary and secondary OH groups and also a potentially reactive amino function. If vinyl benzoate is used in excess (6 eq) over 4 the reaction results in two products: 6-O-benzoyl-4 (24%) and 2,6-di-O-benzoyl-4 (36%). A similar product distribution is observed when 2,2,2-trichloroethylbutyrate (TCEB) is used as an acylating agent under these conditions (Scheme 2).



Scheme 2

(a) subtilisin 5mg/ml, 45°C, pyridine (b) 1.5 eq of TCEB, 6 days (c) 6 eq of TCEB, 6 days (d) enzymes (see text), 0.1M phosphate (pH 6.0),1-6 hr, r.t.

As expected, the product ratio is influenced by the excess of the acylating agent. When a small excess (1.5 eq) of acylating agent is used (b) the reaction predominantly gives monoester. When a large excess (6 eq) of TCEB is used (c) a nearly complete conversion of **4** takes place with a monoester/diester ratio 0.2. It is important to note that even under these conditions the secondary amino group as well as C-3 and C-4 hydroxyl groups of **4** remain untouched. Thus, subtilisin-catalyzed acylation of **4** in pyridine provides a convenient and direct approach to both **5** and **6**.

Several hydrolases exhibit a predominant preference toward a primary hydroxy group in deacylation reaction.[7,9] This approach has been employed for the synthesis of **7** in water (**d**). Lipase from *Candida* sp. and porcine pancreatic lipase, as well as subtilisin and porcine liver esterase effectively remove a butanoyl group from the C-6 position of **4** and thus allow for the preparative synthesis of **7** in 75% isolated yield.

Acknowledgment. We are grateful to Dr. M. Whalon and Mr. G. Ruba for their assistance with analytical data, and to Dr. P. Anzeveno for synthesizing 1-deoxynojirimycin.

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10. Selected analytical data. 6-O-butanoyl-2, crystalline solid, m.p. 99-101°C, ^{1}H NMR (DMSO-d_6, 300 MHz) δ 0.88 [3H,t,$\underline{CH_3}$], 1.51 [2H,m,OC(O)$CH_2\underline{CH_2}CH_3$], 2.20 [1H,dd,H1a,J= 11.9, 10.4Hz], 2.28 [2H,t,OC(O)$\underline{CH_2}CH_2CH_3$], 2.44 [1H,m,H5], 2.87 [1H,dd,H1e,J= 11.9, 5.0Hz], 2.90-3.00 [2H, m, H3,H4], 3.15 [1H,m,H2], 3.88 [1H,dd,H6,J= 7.3, 10.9Hz], 4.25[1H,dd,H6'J= 2.5, 10.9 Hz. MS: m/e (relative intensity): 234 (100), 216 (69), 198 (4), 174 (5), 146 (65), 128 (10), 89 (9), 71 (4). Anal calcd for $C_{10}H_{19}O_5N$: C,51.47;H, 8.21; N, 6.01. Found C, 51.39; H, 8.30; N, 5.73. 2,6-di-O-butanoyl-2, m.p. 93-94°C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 0.86 [6H,t,$\underline{CH_3}$], 1.52 [4H,m,OC(O)$CH_2\underline{CH_2}CH_3$], 2.22 2.32 [6H; (4H, OC(O)$\underline{CH_2}CH_2CH_3$), H1a, H5], 2.94 [1H,t,H4,J= 9.6, 9.6Hz], 2.94 [1H,dd,H1e,J= 11.7, 5.0Hz,], 3.20 [1H,t,H3,J= 9.6, 9.6Hz], 3.90 [1H,dd,H6,J= 7.0, 11.0Hz] 4.26 [1H,dd,H6',J= 2.4, 11.0Hz], 4.47 [1H,m,H2]. MS: m/e (relative intensity): 304 (62), 286 (22),244 (5),234 (15),216 (100),198 (7),174(2),146 (12),128 (9),96 (3),89 (25),71 (8). Anal Calcd for $C_{14}H_{25}O_6N$: C, 55.41; H, 8.31; N, 4.62. Found C,54.82; H, 8.46; N 4.47 2-O-butanoyl-2,m.p. 139-140°C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 0.88 [3H,-t,$\underline{CH_3}$], 1.53 [2H,m,OC(O)$CH_2\underline{CH_2}CH_3$], 2.22-2.30 [4H; (2H, OC(O)$\underline{CH_2}CH_2CH_3$), H1a, H5], 2.94 [1H,dd,H4,J= 9.0, 9.0Hz], 2.98 [1H,t,H1e,J= 11.5, 5.2Hz], 3.20 [1H,t,H3 J= 9.0, 9.0Hz], 3.30 [1H,dd,H6, J= 7.1, 10.5Hz], 3.65 [1H,dd,H6', J= 2.9 10.5Hz], 4.45 [1H,m,H2]. MS: m/e (relative intensity): 234 (82), 216 (58), 202 (5), 186 (2), 164 (8), 146 (100), 128 (16), 89 (15), 71 (5). Anal calcd for $C_{10}H_{19}O_5N$: C, 51.47; H,8.21;N, 6.01. Found C, 51.18; H, 8.19; N, 5.77.

(Received in USA 7 March 1990)