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Enzymatic Synthesis of Amino Acid Ester of Butyl α-D-Glucopyranoside

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Abstract: The regiospecific enzymatic esterification of butyl α-D-glucopyranoside with trichloro 2,2,2 ethyl N-tBoc 4-amino butyrate was achieved using Lipozyme® avoiding time consuming protection-deprotection steps. The tBoc group was subsequently removed using trifluoroacetic acid, leading to the formation of butyl 6-0-(4-amino butyroyl)-α-D-glucopyranoside.

The development of glycolipid as drug carriers towards the central nervous system was investigated. The butyl α-D-glucopyranoside[1] **1**, was chosen as the starting glycolipid as it is freely soluble in water and many other organic solvents. The 4-amino butyric acid (GABA) was selected as a model amino acid, as it does not cross the blood-brain barrier to any significant extent[2]. The chemical synthesis of a GABA derivative of octyl β-D-glucopyranoside (a more lipophilic alkyl glucopyranoside than **1**) via an ester bond, should involve a time-consuming protection-deprotection procedure resulting in the formation of octyl 6-0-(4-amino butyroyl)β-D-glucopyranoside.

In order to achieve the same regioselectivity in a limited number of steps, the synthesis of butyl 6-0-(4-amino butyroyl) α-D-glucopyranoside **5** was performed using Lipozyme® (lipase of *Mucor miehei* adsorbed on anionic resin) according to the following reaction scheme:

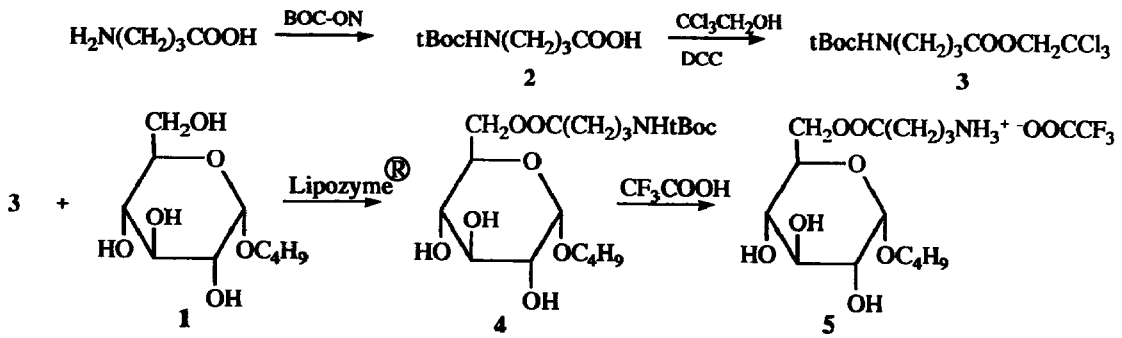

Figure 1: Chemoenzymatic synthesis of **5**

GABA was first protected with BOC-ON[3] (2-tert-butyloxy carbonyloxyimino-2 phenylacetonitrile) leading to the N-tBoc GABA **2**, the carboxylic function of which was subsequently activated with tricholoro 2,2,2 ethanol[4] using dicyclohexylcarbodiimide (DCC). The activated protected amino acid **3** has a lower m.p. than the unprotected mother compound and thus allows the enzymatic esterification of **1** in molten **3**, with Lipozyme® in a solvent free-process as previously reported[5].

The enzymatic reaction product was characterized as butyl 6-0-(N-tBoc 4-amino butyroyl)α-D-glucopyranoside **4** as previously described[6-7]. The final step consisting of the selective removal of the tBoc moiety, was achieved with trifluoroacetic acid[8] affording **5** in quantitative yields[9].

The overall yield of this chemoenzymatic synthesis was **60%** within a four step process. The regiospecificity that could be achieved with enzymatic methods led to the desired structure **5** within a shorter time-consuming procedure than a pure chemical route. This strategy can be applied to a wide variety of tBoc protected amino acids; this procedure with quantitative and easy removal of this protecting group is compatible with the ester formation.

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7. A typical procedure is as follows :
   0.5g (2.11mmol) of **1** was solubilized under magnetic stirring in molten **3**, at 45°C in a sealed vial. 0.1g of Lipozyme® was added and the reaction progress followed by t.l.c. analysis on silica gel (CH_2Cl_2/CH_3OH : 9/1) by spraying anthrone solution at 0.2% in H_2SO_4 and heating at 110°C for 10 min.. The enzyme was filtered off after 144h and **4** was obtained as a colorless syrup in 70% yield, after purification on silica gel in the same solvent system. A single spot was obtained on tlc plates corresponding to the 6-O-amino acid ester derivative **4** as determined by Yoshimoto's rules[6]. $[\alpha]_D$+53(C 0.92, chloroform). Infra-red analysis (NaCl plates)3389, 2943, 1725 cm^{-1}. Superscripts ' and " are referring to H and C of the aglycone and the amino acid moiety respectively. RMN ^1H ($CDCl_3$) : 0.91 (t, J = 4.7 Hz, 3H) H_4' ; 1.32 (m, 2H) H_3' ; 1.40 (s, 9H) H_7" ; 1.53 (m, 2H) H_2' ; 1.78 (m, 2H) H_3" ; 2.37 (m, 2H) H_2" ; 3.07 (m, 2H) H_4" ; 3.80 (m, 12H) H_1', H_{2-6}, OH, NH ; 4.81 (d, J = 3.7 Hz, 1H) H_1, ppm. RMN ^{13}C ($CDCl_3$) : 13.87 (C_4') ; 19.32 (C_3') ; 25.29 (C_3") ; 28.44 (C_7") ; 31.36 (C_2', C_2") ; 39.80 (C_4") ; 63.57 (C_6) ; 68.20 (C_1') ; 69.69 (C_4) ; 70.08 (C_5) ; 72.11 (C_2) ; 74.39 (C_3) ; 79.48 (C_6") ; 98.38 (C_1) ; 156.26 (C_5") ; 173.62 (C_1"), ppm. Anal.Calc.for $C_{19}H_{35}O_9N$ 0.5 H_2O C 53.14 H 8.62 N 3.56 Exp. C 53.22 H 8.25 N 3.51. Mass spectrum (DCI, NH_3) : *m/z*, 439 (M^++18), 422(MH^+)
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9. A typical procedure is as follows :
   0.2g(0.475mmol) of **4** was dissolved in 3ml of CH_2Cl_2 with stirring under nitrogen atmosphere at 0°C followed by a dropwise addition of 3ml of trifluoroacetic acid. The solvent was removed after 30 min. under reduced pressure yielding 0.2g (0.460mmol) of **5** after recristallization in ether as a hygroscopic colorless syrup (97% yield).$[\alpha]_D$+45(C 1, methanol).Infra red analysis (NaCl plates) : 3250, 2924, 1739, 1677, 1197, 1135 cm^{-1}. RMN ^1H (CD_3OD) : 0.95 (t, J = 7.1 Hz, 3H) H_4' ; 1.45 (m, 2H) H_3' ; 1.62 (m, 2H) H_2' ; 1.99 (td, J = 7.7 Hz, 2H) H_3" ; 2.51 (t, J = 7.1 Hz, 2H) H_2" ; 2.99 (t, J = 7.1 Hz, 2H) H_4" ; 3.50 (m, 6H) H_1', H_{2-5} ; 4.24 (dd, J = 10.5 Hz, J = 7.0 Hz, 1H) H_6 ; 4.40 (dd, J = 2.3 Hz, J = 10.5 Hz, 1H) H_6 ; 4.74 (d, J = 3.7 Hz, 1H) H_1, ppm. RMN ^{13}C (CD_3OD) : 14.27 (C_4') ; 20.50 (C_3') ; 23.76 (C_3") ; 31.57 (C_2") ; 32.75 (C_2') ; 40.06 (C_4") ; 65.17 (C_6) ; 69.06 (C_1') ; 71.08 (C_4) ; 71.99 (C_5) ; 73.52 (C_2) ; 75.02 (C_3) ; 100.24 (C_1) ; 112.30 , 118.10 , 123.90 , 129.70 (q, J = 291.9 Hz, CF_3) ; 166.46 , 167.16 , 167.86 , 168.56 (q, J = 35.2 Hz, $CF_3\underline{C}OO^-$) ; 173.95 (C_1"), ppm. Anal.Calc.for $C_{16}H_{28}F_3NO_9$, 2H_2O C 40.76 H 5.95 N 2.97. Exp. C 40.91 H 5.98 N 3.39.

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