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Chemoenzymatic polymer-supported liquid phase synthesis of glucose γ-aminobutyric ester

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Abstract—A concise, polymer-supported solution synthesis of 6-*O*-(γ-aminobutyryl)-D-glucose has been achieved. Glucose was attached to polyethylene glycol monomethyl ether (MPEG) through an α,α′-dioxyxylyl diether linker, and, subsequently, the HO-6 hydroxyl of the glucose was regioselectively esterified with γ-aminobutyric acid by a lipase-catalyzed reaction.

The presence of the blood–brain barrier (BBB) hinders pharmaceutical delivery to the brain, making difficult the treatment of brain disorders.[1,2] We have recently initiated[3] studies on a new approach to deliver neuroamines and neuropeptides into the central nervous system by linking the neuroactive molecule to a glucose moiety so that the resulting glycoconjugate may cross the BBB using the glucose carrier GLUT-1. This protein is located in the plama membrane of the endothelial cells composing the BBB and facilitates the passage of glucose,[4] the main energy source of the brain. We have found that glucosyl derivatives substituted at the C-6 position of the sugar show high affinities for GLUT-1 when compared to C-1, C-3 and C-4 substituted compounds.[5] For a closer understanding of the role of glycoconjugate structure in substrate recognition by GLUT-1, it is desirable to develop a simple and selective method to synthesize glucose derivatives substituted at C-6 position. Solid-phase synthesis of organic compounds has been shown to be an effective tool for obtaining a large number of compounds. However, the use of insoluble supports creates heterogeneous reaction conditions that can produce a non-linear kinetic behavior, solvation problems, etc.[6] These problems can be specially important in the case where enzymes are used as catalyst. Because of that we have chosen a soluble polymer as support. Polyethylene glycol monomethyl ether (MPEG) has been intensively used in polymer-supported solution synthesis because of its unique solubility properties.[7,8] Here we present a new approach to the synthesis of γ-aminobutyric acid ester of glucose using MPEG as polymeric support. The γ-aminobutyric acid (GABA) was selected as a model amino acid as it is a constituent of the mammalian brain[9] that does not cross the BBB to any significant extent.

To attach the glucose residue to the polymer we used an α,α′-dioxyxylyl diether, as the group -$CH_2C_6H_4CH_2$-, as linker that can be removed at the end of the synthesis by hydrogenolysis (Scheme 1). Thus, the reaction of glucosyl trichloroacetimidate **1** with **2**[10] in the presence of TMSOTf at 0°C gave the glycoside **3**. After work-up the polymer was recovered by precipitation after addition of Et_2O. Treatment of **3** with NaOMe led to the debenzoylated glucopyranoside derivative, which was purified by precipitation with Et_2O followed by crystallization from EtOH. The ^{1}H NMR spectrum showed the compound to be the glycoside with β-configuration **4**.[†]

Prior to the esterification of **4** with GABA, we studied the reaction on benzyl β-D-glucopyranoside **5** as a model of the glucosylated linker. In a first attempt, the esterification of **5** with the *N*-Cbz protected GABA **6** was tried under Mitsunobu conditions. The reaction in the presence of Ph_3P/DEAD gave mainly the diester **8** derived from the double displacement, together with the desired ester **7** in low yield. In a next stage, we explored

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[†] Selected ^{1}H NMR data for compound **4** ($CDCl_3$, 300 MHz): δ 7.31 (m, 4H, aromatic), 4.85 (d, 1H, J=11.6 Hz, GlcOC*H*Ph), 4.55 (d, 1H, J=11.6 Hz, GlcOC*H*Ph), 4.50 (s, 2H, PhCH_2OPEG), 4.37 (d, 1H, J=7.6 Hz, H-1 Glc), 3.60 (s, 3H, CH_3OPEG).

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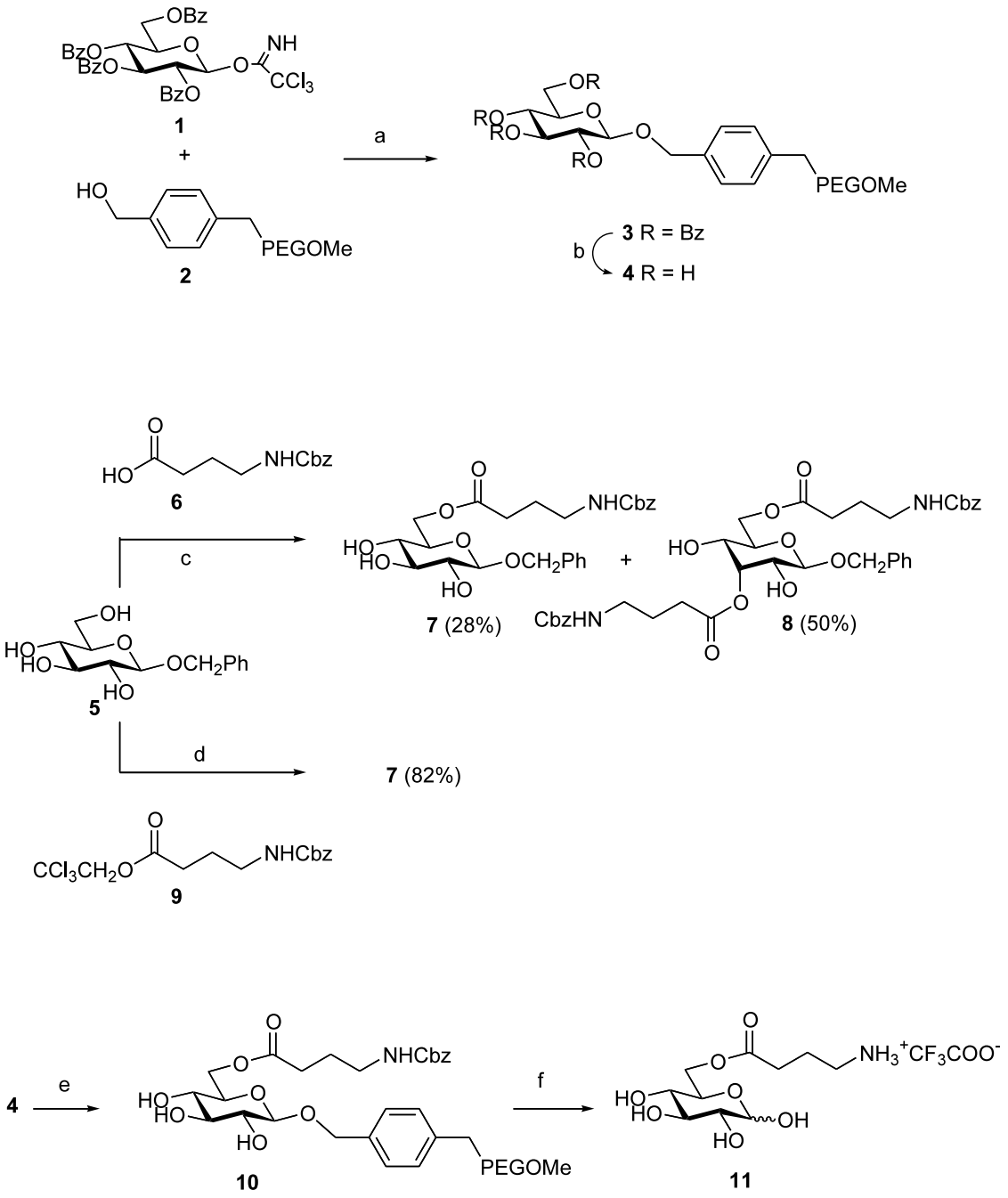

Scheme 1. *Reagents and conditions*: (a) **1** (0.7 mmol), **2** (0.5 mmol), TMSOTf (0.7 mmol), CH_2Cl_2, 0°C, 24 h, 95%. (b) NaOMe, MeOH, rt, 3 h, 89%. (c) **6** (1.7 equiv.), Ph_3P (1.7 equiv.), DEAD (1.7 equiv.), THF, 0°C. (d) **9** (3 equiv.), Novozym 435 (1 g/mmol), 4 Å molecular sieve, CH_3CN, 45°C, 29 h. (e) **9** (3.6 equiv.), Novozym 435 (1.5 g/mmol), 4 Å molecular sieve, toluene, 45°C, 72 h, 84%. (f) H_2, Pd/C, TFA, MeOH, rt, 24 h, 76%.

enzymatic reactions since it is known that highly regioselective acylations of the primary hydroxy group of monosaccharides are achieved using lipases.[11,12] Three commercially available lipases were examined: Novozym 435 (immobilized *Candida antartica* lipase, Novo-Nordisk), PPL (porcine pancratin lipase, Sigma), and PSL (*Pseudomonas cepacia* lipase, Amano). Trichloroethyl ester of *N*-Cbz protected GABA **9** was used as the acyl donor and, due to solubility limitations, the reactions were tried in polar solvents such as pyridine, acetonitrile, and THF. The best results were obtained using Novozym 435 in acetonitrile, affording exclusively acylation in position 6, in 82% yield (Scheme 1).

The enzymatic acylation was then carried out on the glucosyl polymer **4**. To force the reaction toward completion, toluene was used as solvent since more efficient acylations were obtained in the more hydrophobic solvents in agreement with previous observations.[13] This change of solvents was possible due to the solubilizing power of the polymer. Under these conditions, and

following precipitation with ether and crystallization from EtOH, the GABA ester derivative **10** was obtained in 84% yield. The ^{1}H NMR spectrum of **10** showed the signals of H-6 of the glucose moiety shifted at δ 4.30 indicating acylation at position 6.

Hydrogenolysis of **10** in the presence of TfOH led to the concomitant cleavage of the linker and the Cbz group, giving, after Bio-gel P2 column chromatography, the target **11** as its trifluoroacetate salt.‡

By the synthesis of the glucose GABA ester **11**, we have shown the feasibility of this approach, which combines the selectivity control of enzymes and the easy work-up of reactions using PEG as support. Considering that the lipase used is immobilized on a resin, a similar synthetic scheme using solid-phase methodology would not be possible.

The use of this synthetic scheme should allow an easy access to a variety of glucosyl derivatives substituted at C-6 of great utility in our studies on the transport of glycoconjugates through the BBB.

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‡ Preparation of **11**: To a solution of **10** (0.17 g, 0.03 mmol) in MeOH (1.5 mL) was added 10% Pd/C (0.09 g) and TfOH (0.0087 mL), and the reaction was stirred under a H_2 atmosphere for 24 h. The mixture was filtered through Celite and concentrated. The residue was dissolved in water and washed with CH_2Cl_2. The aqueous solution was concentrated and the residue purified through a Bio-gel P2 column chromatography (water as eluent), to give **11** (76%) as a syrup. $[\alpha]_D$ +13 (*c* 0.9, water). ^{1}H NMR (300 MHz, D_2O): δ 5.09 (d, <1H, J=3.7 Hz, H-1α), 4.53 (d, <1H, J=7.9 Hz, H-1β), 4.36–4.14 (m, 2H, H-6), 3.90 (m, <1H, H-5), 2.93 (m, 2H, CH_2NH_3), 2.42 (m, 2H, CH_2CO), 1.85 (m, 2H, $OCCH_2CH_2CH_2N$).