

BRIGITTE SCHWARZ<sup>1</sup>  
RENATE LÖPPERT<sup>2</sup>  
WERNER PRAZNIK<sup>2</sup>  
FRANK MICHAEL UNGER<sup>1,2</sup>  
HANSPETER KÄHLIG<sup>3</sup>  
HELMUT VIERNSTEIN<sup>1</sup>

<sup>1</sup>Institute of Pharmaceutical  
Technology and  
Biopharmaceutics, University of  
Vienna, Vienna, Austria

<sup>2</sup>Institute of Chemistry,  
University of Natural Resources  
and Applied Life Sciences,  
Vienna, Austria

<sup>3</sup>Institute of Organic Chemistry,  
University of Vienna, Vienna,  
Austria

SCIENTIFIC PAPER

661.728:615

## APPROACHES TO NEW DERIVATIVES OF CELLULOSE AS DESIGNED PHARMACEUTICAL EXCIPIENTS

*Recently, our group initiated a synthetic program directed at new derivatives of cellulose intended as novel pharmaceutical excipients. With several notable exceptions, the attempted regioselective introduction of chemical functionality into natural cellulose by direct chemical modification will result in heterogeneous products that are difficult to characterize and the preparation of which is insufficiently reproducible. Approaches to the chemical polymerization of appropriate glucose monomers are available, leading to a degree of polymerization in the order of 100. However, the nature of these processes does not readily lend itself to the preparation of products comprising regularly arranged protecting groups in defined positions. We have chosen a mixed organic chemical-enzyme catalyzed approach based on a procedure of Kobayashi, Shoda, Donnelly and Church. Fluoride derivatives of cellobiose may be polymerized, under catalysis by cellobiose hydrolase, to form cellulose oligosaccharides of different chain lengths. We describe the chemical syntheses of cellobiose fluoride derivatives comprising protecting groups in defined positions of the reducing or nonreducing glucose moieties of cellobiose. Such derivatives may be polymerized to afford cellulose derivatives with protecting groups on alternate glucose units. The processing of these protected cellulose derivatives to afford novel biomimetic polymers will be described.*

Cellulose is an abundant, natural polysaccharide made up of  $\beta$ -1,4-glycosidically linked glucose units. Cellulose itself and the corresponding neutral, acidic and basic derivatives are widely used as pharmaceutical excipients [1]. Thus, microcrystalline cellulose [2], obtained by the partial de-polymerization of cellulose, is used as a tablet binder and diluent in wet granulation or direct compression, and as a tablet disintegrant, anti-adherent or capsule diluent. Powdered cellulose [3] is available in several types and is used as a binder and disintegrant. Hydroxyethyl [4] and hydroxypropyl celluloses [5] are manufactured by reacting alkali cellulose with ethylene or propylene oxides and serve as film formers, binders, thickeners, stabilizers and dispersants. Carboxymethylcellulose [6] is a typical acidic cellulose derivative. Produced by the action of alkalinized cellulose on sodium monochloroacetate, it is used as emulsifier, gel-former or binding agent. Among cellulose derivatives with basic properties, chitosan [7,8,9] has been most extensively explored. Chitosan is a  $\beta$ -1,4-linked polysaccharide composed of 2-amino-2-deoxy-D-glucose units. It is prepared by the alkaline de-N-acetylation of chitin, a  $\beta$ -1,4-linked polysaccharide of 2-acetamido-2-deoxy-D-glucose units which occurs naturally as the main structural constituent of the shells of insects and crustaceans. Chitin is produced in large amounts as a waste product in the canning of crab and shrimp meat. Experimental applications of chitosan in pharmaceutical technology include the preparation of controlled release solid forms

as in microspherules or microcapsules, controlled release implant systems, formulations to improve the solubility of drug substances, or the oral/nasal application of polar drug substances such as peptides or proteins. To expand the range of basic cellulose derivatives available as experimental excipients, our groups have recently initiated a program of combined organic-chemical and enzyme-catalyzed syntheses of cellulose derivatives containing azido-, amino- or acetamido functionality regularly arranged in different positions. This program is based on three premises, as follows. i. Azidodeoxy derivatives of cellobiose may be produced by chemical synthesis. ii. The azido function serves for the introduction of nitrogen, functions as a protecting group for nitrogen, and, being electrically neutral, is compatible with enzyme-catalyzed reactions. iii. The cellobiose analogs, in the form of their  $\beta$ -fluorides, are polymerizable by the reverse action of cellulase from *Trichoderma viride* [10]. Presently, the synthesis of cellulose-type, large oligosaccharides containing the azido function at C-6 of every other glucose residue is described (Figure 1).

### MATERIALS AND METHODS

The  $\beta$ -glycosyl fluoride of 6-azido-6-deoxy-cellobiose was synthesized from cellobiose in twelve steps, as follows. Briefly, cellobiose was converted into the octa-O-acetyl derivative by treatment with acetic anhydride in pyridine. The peracetylated disaccharide derivative was treated with hydrogen bromide in glacial acetic acid to afford the acetobromo derivative, from which the  $\beta$ -allyl glycoside was prepared by a Helferich-type glycosylation protocol (Figure 2).

Following sodium methoxide-catalyzed methanolysis, the 4',6'-hydroxyl functions were regioselectively protected by the action of benzaldehyde-in the

Author address: B. Schwarz, Institute of Pharmaceutical Technology and Biopharmaceutics, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria

Paper received and accepted: November 14, 2003

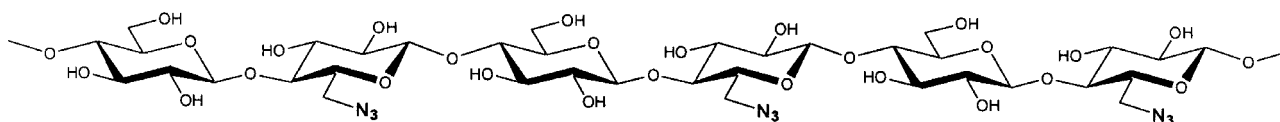


Figure 1.

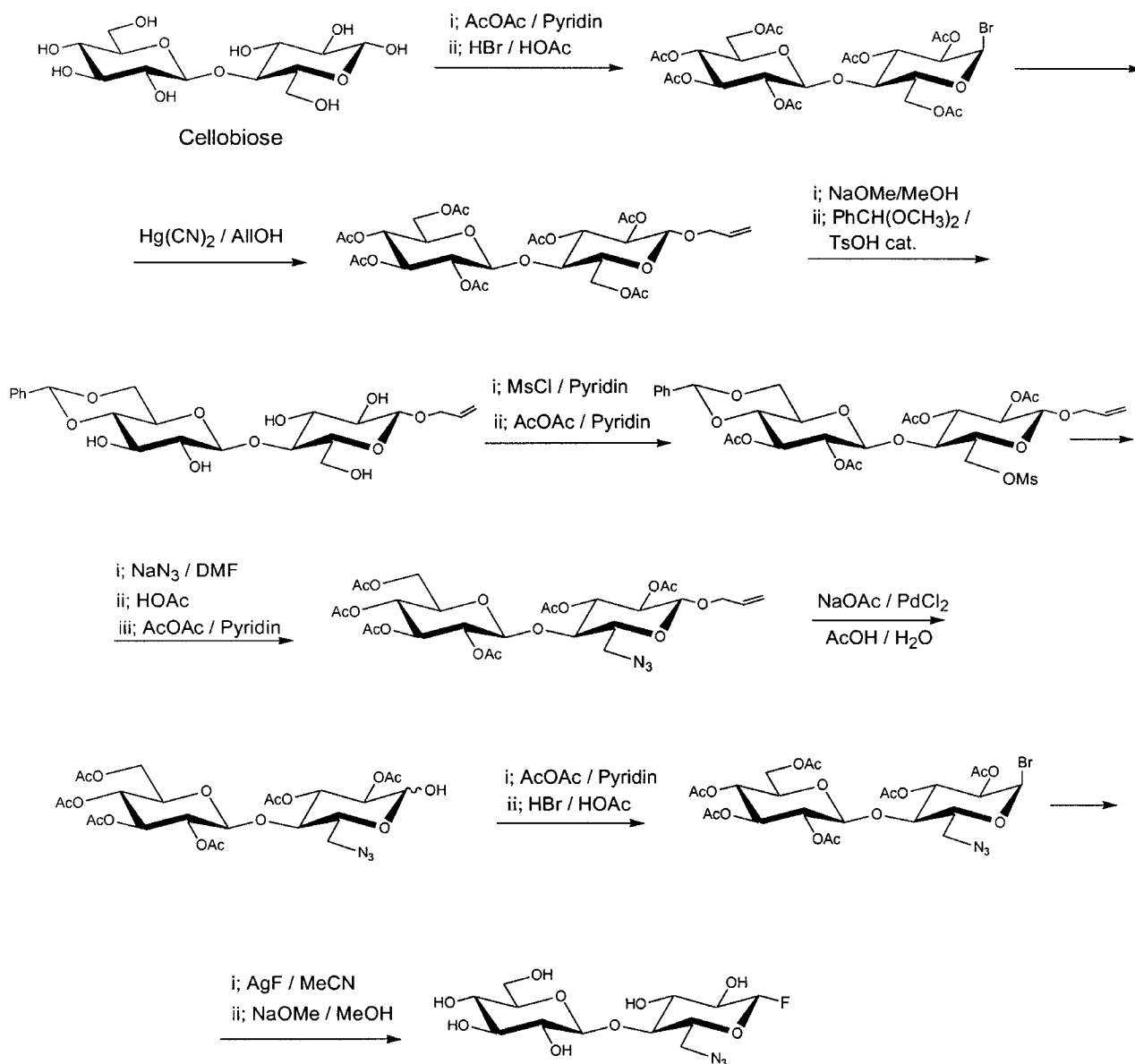


Figure 2. Scheme 1. Synthesis of a 6-Azido-6-deoxy-cellobiosyl fluoride

presence of zinc chloride. The resulting disaccharide acetal contains only one free primary hydroxyl function at C-6. Selective 6-O-mesylation followed by per-O-acetylation and treatment with sodium azide in dimethyl formamide afforded the 6-azido-6-deoxy cellobiose glycoside. Following a change from acetal to ester protection, the anomeric allyl group was removed by the action of palladium chloride and sodium acetate in acetic acid-water and the free glucose acetylated and treated with hydrogen bromide in glacial acetic acid to

afford the glycosyl bromide derivative [10]. Treatment with silver fluoride followed by sodium methoxide-catalyzed methanolysis finally afforded the  $\beta$ -glycosyl fluoride of 6-azido-6-deoxy-cellobiose.

The enzyme-catalyzed polymerization of the azido cellobiose analogue was performed essentially as described for the fluoride of unmodified cellobiose by Kobayashi, et al. [11,12,13] (Scheme 2), (Figure 3).

Briefly, the substrate monomer was dissolved in 0.05 M acetate buffer, pH 5.0; to this solution was added

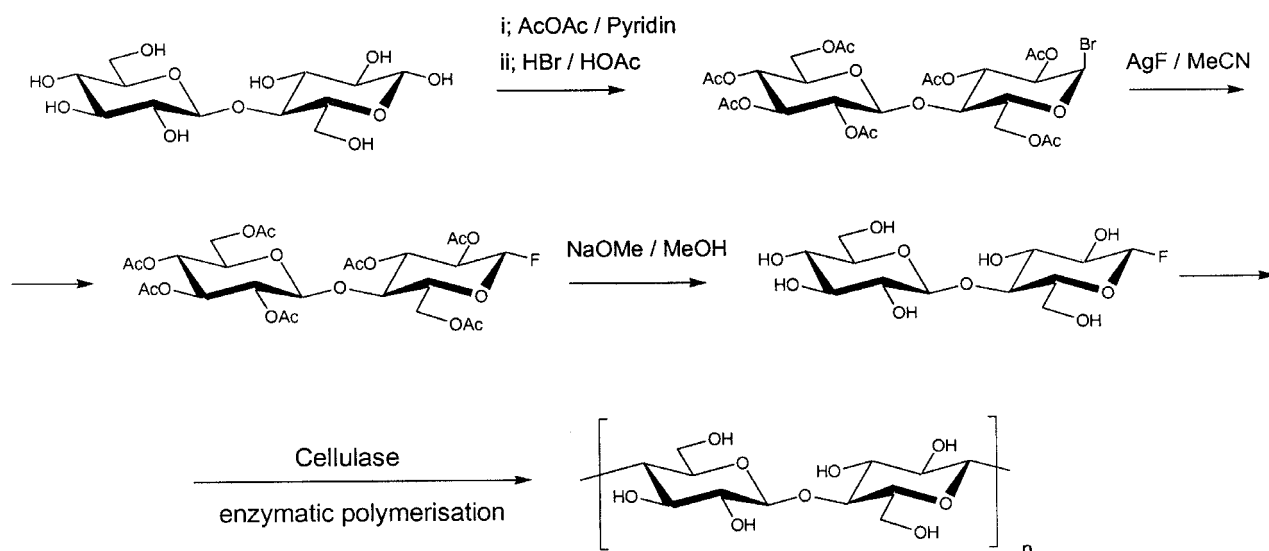


Figure 3. Scheme 2. Enzymatic synthesis of Cellulose

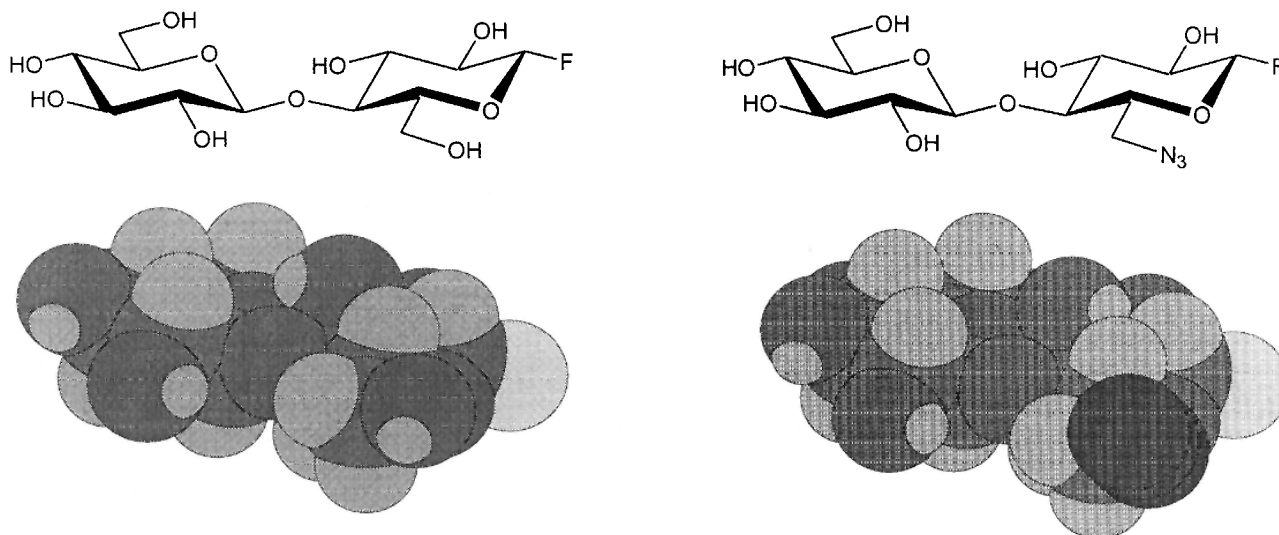


Figure 4. Molecular shapes of cellobiosyl fluoride and its 6-azido-6-deoxy analog

first acetonitrile, then a solution of crude cellulase from *Trichoderma viride* in the same acetate buffer. The mixture was incubated at 30° for 12 h. Subsequently, the enzyme was inactivated by heating the mixture to 100° C for 10 min.

## RESULTS AND DISCUSSION

Thin-layer chromatographic analysis indicates the formation, from the 6-azido-6-deoxy-cellobiose derivative, of a series of oligomers analogous to the products formed from the cellobiosyl fluoride. In contrast to the cellulose oligosaccharides, the products derived from 6-azido-6-deoxy-cellobiose were found to stain with ninhydrin following treatment of the thin-layer chromatography plates with hydrogen sulfide. As expected, the artificial azido oligosaccharides are less

polar than their cellulose counterparts, and their solution behaviour in the acetonitrile-acetate buffer mixtures differs from that of the unmodified cellulose derivatives. In view of the previous findings by Kobayashi, et al., who described the formation of alternately 6-O-methylated cellulose oligomers from appropriately modified cellobiosyl fluoride, the reactivity of the 6-azido-6-deoxy-cellobiosyl fluoride in the reverse cellulase reaction is not surprising. As shown in the Figure, the molecular shapes of cellobiosyl fluoride and its 6-azido-6-deoxy analog are very similar (Figure 4).

In view of the small amounts presently available, the tasks at hand include i. Scale up of the synthesis of azidocellobiose oligomers to obtain quantities sufficient for spectroscopic characterization and chemical modification. ii. Improvements of the organic-chemical

steps to access pilot quantities suitable for formulation experiments. iii. Syntheses of azido-cellobiose derivatives with azido substitution in alternative positions to explore the feasibility of additional artificial basic cellulose derivatives. The results of these efforts as well as the details of the chemical synthesis will be reported elsewhere.

#### ACKNOWLEDGMENTS

The authors thank Professor Horst Kunz of the University of Mainz, Germany, for suggesting the method of Reference 10 for de-O-allylation in the presence of an azido function. Dr. Alexander G. Hedenetz has kindly provided help with IR and NMR spectra.

#### REFERENCES

[1] The Pharmaceutical Society of Great Britain: Handbook of Pharmaceutical Excipients, A joint publication of the American Pharmaceutical Association and the

Pharmaceutical Society of Great Britain. The Pharmaceutical Press, Washington, D.C., 1986.

- [2] Ref. 1, p. 53.  
 [3] Ref. 1, p. 56.  
 [4] Ref. 1, p. 131.  
 [5] Ref. 1, p. 134.  
 [6] Ref. 1, p. 45.  
 [7] L. Illum, *Pharm. Res.*, **9** (1998) 1326-1331.  
 [8] F.L. Mi, H.W. Sung, S.S. Shyu, *Carbohydr. Polym.*, **48** (2002) 61  
 [9] A. Bernkop-Schnürch and G. Walker, *Critical Reviews in Therapeutic Drug Carrier Systems*, **18** (5) (2001) 459-501.  
 [10] T. Ogawa, S. Nakabayashi, and T. Kitajima, *Carbohydr. Res.*, **114** (1983) 225-236.  
 [11] S. Kobayashi, K. Kashiwa, T. Kawasaki, and S. Shoda, *J. Am.Chem.Soc.*, **113** (1991) 3079-3084.  
 [12] J.H. Lee, R.M. Brown, Jr., S. Kuga, S. Shoda, and S. Kobayashi, *I. Proc. Natl. Acad. Sci. USA*, **91** (1994) 7425-7429.  
 [13] E. Okamoto, T. Kiyosada, S. Shoda, and S. Kobayashi, *Cellulose* **4** (1997) 161-172.

#### IZVOD

#### POSTUPAK ZA DOBIJANJE NOVIH DERIVATA CELULOZE NAMENJENIH ZA KORIŠĆENJE U FARMACIJI

(Naučni rad)

Brigitte Schwarz<sup>1</sup>, Renate Löppert<sup>2</sup>, Werner Praznik<sup>2</sup>, Frank Michael Unger<sup>1,2</sup>, Hanspeter Kählig<sup>3</sup>, Helmut Viernstein<sup>1</sup>

<sup>1</sup>Institute of Pharmaceutical Technology and Biopharmaceutics, University of Vienna, Vienna, Austria

<sup>2</sup>Institute of Chemistry, University of Natural Resources and Applied Life Sciences, Vienna, Austria

<sup>3</sup>Institute of Organic Chemistry, University of Vienna, Vienna, Austria

Nedavno je naša grupa započela jedan program sinteze novih derivata celuloze namenjenih za farmaceutske svrhe. Sem u nekoliko izuzetaka, regioselektivno uvođenje hemijske funkcionalnosti u prirodnu celulozu pomoću direktne hemijske modifikacije rezultira u stvaranju heterogenih produkata koje je teško karakterisati i čije nastajanje je nedovoljno reproduktivno. Hemijska polimerizacija pogodnih glukoznih monomera koji dostiže stepen polimerizacije reda veličine 100, predstavlja alternativni pristup ispitivanom. Međutim, priroda ovih procesa ne vodi odmah ka stvaranju produkata sa zaštitnim grupama postavljenim u željenim definisanim pozicijama. Mi smo izabrali kombinovani pristup hemijsko-enzimske organske katalize baziran na proceduri koju su razvili Kobayashi, Shoda, Donnelly i Church. Fluoridni derivati celobioze se mogu polimerizovati pomoću katalitičkog dejstva celobiozo-hidrolaze, u cilju stvaranja oligosaharida celuloze različite dužine lanca. U radu je opisana hemijska sinteza fluoridnih derivata celobioze uključujući i zaštitu grupa u definisanim pozicijama redukujućih ili neredukujućih krajeva celobiose. Takvi derivati se dalje mogu polimerizovati da bi dali celulozne derivate sa zaštitnim grupama na alternativnim jedinicama glukoze. Dalje je opisano procesovanje ovih zaštićenih derivata celuloze u cilju dobijanja biomimetičnih polimera.

Ključne reči: Farmaceutski derivati  
 • Derivati celuloze • Organska hemijsko enzimsko kataliza • Biomimetični polimeri •  
 Key words: Pharmaceutical derivatives • Cellulose derivatives • Enzyme catalysis • Biomimetic polymers •