have been detected by Kroto et al. as molecules in interstellar space.[17] The formation of cyclic perchlorinated compounds such as **6**–**8** provides new and valuable clues to the formation of fullerenes. The isolation and characterization of capture products within the C plasma, such as the hitherto unknown **8** and components seen preferentially at low $(CN)_2$ concentrations, are the subject of further investigations.

Experimental Procedure

The fullerene reactor used was modified for the admission of $(CN)_2$ or Cl_2 by the addition of a gas inlet tube with a flat nozzle (Fig. 1). After it was synthesized [18], cyanogen was condensed in an autoclave, the outlet of which (needle valve) was connected to the gas inlet tube of the reactor. The following operating conditions were used: a voltage of 30 V DC, a current of 40 A, and a reactor pressure of 140 mbar He. After ignition of the electric arc, $(CN)_2$ or Cl_2 was fed into the reactor. The supply was quantitatively regulated so that the pressure in the reaction vessel remained constant. The cool surfaces of the reactor interior became coated with a yellow film in addition to soot. After completion of the reaction, the crude product was extracted with toluene. In each case the yield corresponded to 6–7% of the vaporized graphite.

Received: March 30, 1993
Revised version: June 4, 1993 [Z 5961 IE]
German version: *Angew. Chem.* **1993**, *105*, 1390

[1] W. Krätschmer, L. Lamb, K. Fostiropoulos, D. R. Huffman, *Nature* **1990**, *347*, 354.
[2] H. R. Karfunkel, T. Dressler, A. Hirsch, *J. Comput. Aided. Mol. Des.* **1992**, *6*, 521.
[3] H. W. Roesky, H. Hofmann, *Chem. Ztg.* **1984**, *108*, 231.
[4] A. Koch, K. C. Khemani, F. Wudl, *J. Org. Chem.* **1991**, *56*, 4543.
[5] MS (FD): *m/z* 124 (C_8N_2 (**1**)), 148 ($C_{10}N_2$ (**2**)), 172 ($C_{12}N_2$ (**3**)), 196 ($C_{14}N_2$ (**4**)), 220 ($C_{16}N_2$ (**5**)); UV/VIS (*n*-hexane): λ_{max}[nm] = 215, 225, 236, 248 (sh), 261, 284, 306, 322, 327, 338, 344, 363, 371, 399, 408; FT-IR (KBr): ν [cm^{-1}] = 2245 (sh, CN), 2237 (C≡N), 2187 (C≡C), 2120 (C≡C); ^{13}C NMR (62.9 MHz, $CDCl_3$, 25 °C, TMS): δ = 104.28, 104.10, 66.21, 65.64, 64.56, 63.27, 61.92, 51.99, 51.19 (quart. C atoms, spin-echo, coupled spectrum).
[6] Spectroscopic data for C_8N_2 **1**: UV/VIS (*n*-hexane): λ_{max} [nm] = 206 (sh), 215, 225, 236, 248 (sh), 261; FT-IR (NaCl): ν [cm^{-1}] = 2247 (C≡N), 2187 (C≡C), 2120 (C≡C); MS (FD) *m/z* 124 ($M^{\oplus}$, 100%); ^{13}C NMR (62.9 MHz, $CDCl_3$, 25 °C, TMS): δ = 104.10, 65.64, 63.27, 51.99.
[7] a) J. R. Heath, Q. Zhang, S. C. O'Brien, R. F. Curl, H. W. Kroto, R. E. Smalley, *J. Am. Chem. Soc.* **1987**, *109*, 359; b) A. A. Rohlfing, *J. Chem. Phys.* **1990**, *93*, 7851; c) M. Broyer, A. Goeres, M. Pellarin, E. Sedlmayer, J. L. Vialle, L. Wöste, *Chem. Phys. Lett.* **1992**, *198*, 128.
[8] a) A. Goeres, E. Sedlmayr, *Chem. Phys. Lett.* **1991**, *184*, 310; b) J. R. Chelikowsky, *Phys. Rev. Lett.* **1991**, *67*, 2970; c) C. Z. Wang, C. H. Xu, C. T. Chan, K. M. Ho, *J. Phys. Chem.* **1992**, *96*, 3563; d) J. R. Chelikowsky, *Phys. Rev. B.* **1992**, *45*, 12062; e) R. Kerner, K. A. Penson, K. H. Bennemann, *Europhys. Lett.* **1992**, *19*(5), 363.
[9] P. Gerhardt, K. H. Homann, *J. Phys. Chem.* **1990**, *94*, 5381.
[10] R. A. Albers, K. H. Homann, *Z. Phys. Chem. (Munich)* **1968**, *58*, 220.
[11] a) C. S. Yannoni, P. P. Bernier, D. S. Bethune, G. Meijer, J. R. Salem, *J. Am. Chem. Soc.* **1991**, *113*, 3190; b) J. M. Hawkins, A. Meyer, S. Loren, R. Nunlist, *J. Am. Chem. Soc.* **1991**, *113*, 9394; c) T. W. Ebbesen, J. Tabuchi, K. Tanigaki, *Chem. Phys. Lett.* **1992**, *191*, 336.
[12] The exact determination of the yield is difficult, since the dicyanopolyynes are transformed into a black voluminous soot within a split-second by the effect of pressure or upon heating. The yields given should be interpreted as a lower limit. It has also been shown that polyynes decompose into primary soot even in the solid state.
[13] W. Mack, *Tetrahedron Lett.* **1966**, *25*, 2875.
[14] The peaks in the mass spectra (FD, EI) of the fraction containing the higher capture products are attributed to $C_{14}Cl_8$, $C_{16}Cl_{10}$, $C_{20}Cl_{10}$ and show the isotopic pattern corresponding to the degree of chlorination.
[15] a) R. Eastmond, T. R. Johnson, D. R. M. Walton, *Tetrahedron* **1972**, *28*, 4601; b) R. Eastmond, D. R. M. Walton, *ibid.* **1972**, *28*, 4591; c) T. R. Johnson, D. R. M. Walton, *ibid.* **1972**, *28*, 5221.
[16] F. Diederich, Y. Rubin, *Angew. Chem.* **1992**, *104*, 1123; *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 1101.
[17] J. P. Hare, H. W. Kroto, *Acc. Chem. Res.* **1992**, *25*, 106.
[18] D. J. Park, A. G. Stern, R. L. Willer, *Synth. Commun.* **1990**, *20*, 2901.

Continuous Catalytic Synthesis of *N*-Acetyllactosamine**

By *Guido F. Herrmann, Udo Kragl,* and *Christian Wandrey**

Oligosaccharides are attracting much interest in immunological and pharmacological research because of their significance as fundamental structures of glycoproteins and glycolipids.[1] As well as the chemical syntheses of O-glycosides, syntheses employing enzymes have been established for a decade.[2] Access to large quantities of target compounds by means of both methods has been very limited.[3] The use of enzymes, in homogeneous solution, for reactions in an enzyme membrane reactor has proved to be a valuable tool in organic synthesis.[4] The enzyme-catalyzed synthesis of *N*-acetylneuraminic acid in an enzyme membrane reactor showed that large quantities of this compound could be produced in this way.[5]

We report here the enzyme-catalyzed continuous synthesis of *N*-acetyllactosamine (**3**, LacNAc) in an enzyme membrane reactor. Like *N*-acetylneuraminic acid, **3** is a structural component of many biologically active oligosaccharides.[1] Alongside the chemical syntheses of **3**[6] and as a part of higher oligosaccharides,[7] several authors used a galactosyltransferase (E.C. 2.4.1.38)[8] for the synthesis of **3**. The hitherto limited availability of this enzyme, its high price,[9] and its instability hampered its use. An alternative biocatalyst is a β-galactosidase (E.C. 3.2.1.23),[10] in particular the use of the β-galactosidase from *Bacillus circulans* resulted in high regioisomeric purity of the synthesized **3**.[10a]

Figure 1 shows the reaction scheme of the continuous synthesis described here. Starting with lactose **1**, transgalactosylation results in the transfer of the galactose moiety of **1** to *N*-acetylglucosamine **2** to afford **3** (Fig. 1a).

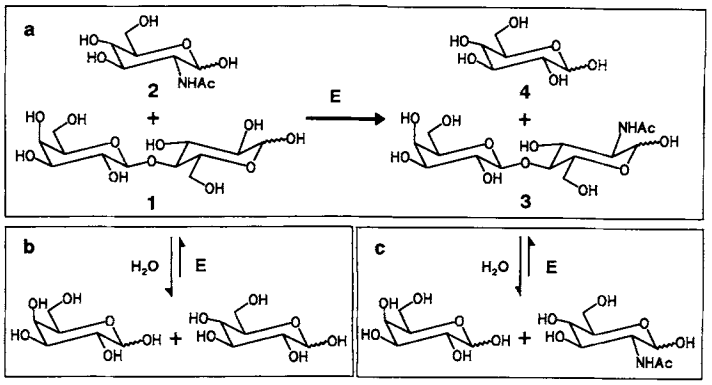

Fig. 1. Enzyme-catalyzed synthesis of *N*-acetyllactosamine **3**; the enzyme E is β-galactosidase a) Transgalactosylation, b) hydrolysis of **1** (side reaction), c) hydrolysis of the product (secondary hydrolysis).

The galactosyl donor **1** is hydrolyzed to galactose and glucose in a side reaction (Fig. 1b). The desired product **3** is also a substrate for the β-galactosidase and is hydrolyzed

[*] Prof. Dr. C. Wandrey, G. F. Herrmann, Dr. U. Kragl
Forschungszentrum Jülich, Institut für Biotechnologie
D-52425 Jülich (FRG)
Telefax: Int. code +(2461)61-3870

[**] We would like to thank the company Daiwa Kasei K. K., Osaka (Japan) for supplying β-galactosidase.

again in a subsequent reaction by the enzyme (secondary hydrolysis, Fig. 1c).

Experiments to investigate the stability of the enzyme showed that the β-galactosidase is a very stable enzyme,[11] and because of its low price and availability, it is a useful enzyme for synthesis.[12] The ratio of transgalactosylation to hydrolysis of **1**, that is, the selectivity[13] of the enzymatic reaction, could be optimized for the continuous process by changing the concentration ratio of **1** and **2**.

By selecting a suitable residence time τ for the reaction mixture in the reactor, the secondary hydrolysis of compound **3** could be minimized. Figure 2 shows the concentration-time curve for this continuous enzyme-catalyzed synthesis of **3** in an enzyme membrane reactor over 100 hours.

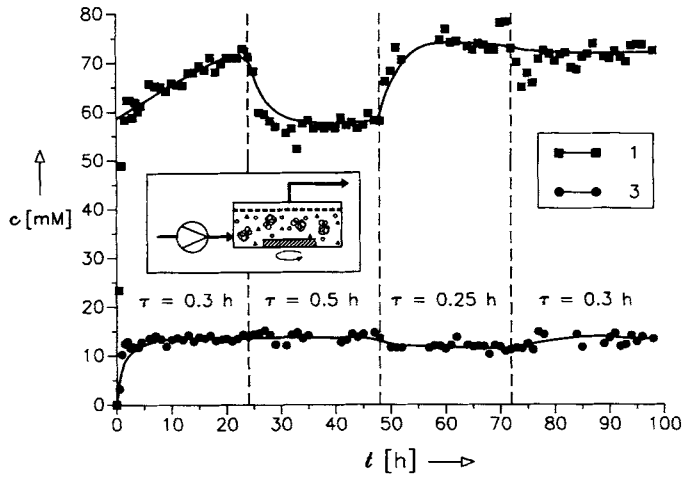

Fig. 2. Concentration-time curve for the continuous synthesis of *N*-acetyllactosamine **3** in an enzyme membrane reactor; starting concentrations: 120 mM lactose **1**, 300 mM *N*-acetylglucosamine **2**, 100 mM KH_2PO_4, 2 mM $MgCl_2 \cdot 6H_2O$, 5 mM dithiothreitol; pH 6.8; 25 °C; 3 mg mL^{-1} β-galactosidase; residence time: $\tau = 0.25$ h to $\tau = 0.5$ h.

During the course of the reaction there was no noticeable deactivation of the enzyme. The residence time was varied between $\tau = 0.25$ h and $\tau = 0.5$ h. With increasing residence time the concentration and therefore also the yield of **3** increased slightly.[14] The conversion of **1** increased from 0.37 to 0.51. The selectivity decreased with increasing conversion from 0.26 ($\tau = 0.25$ h) to 0.22 ($\tau = 0.5$ h). The space-time yield was 442 g L^{-1} d^{-1} at a residence time of $\tau = 0.25$ h and decreased to 261 g L^{-1} d^{-1} at a residence time of $\tau = 0.5$ h. After 100 h, 11.3 g of **3** was produced. The space-time yield was increased by a factor of 130 relative to the synthesis of **3** by using galactosyltransferase by Wong et al.[8a] Since reactions in a membrane reactor can be scaled up linearly,[4, 15] this method opens up an economic and a simple way of producing large amounts of **3**.[16] The process described here is the first continuous synthesis of a disaccharide that employs homogeneous catalysis. As a result of the broad spectrum of substrates for β-galactosidase from *B. ciruculans*,[17] the synthesis of derivatives of **3** with this advantageous technique is also possible.

Experimental Procedure

The enzyme membrane reactor (volume 10 mL; Bioengineering, Wald, Switzerland) fitted with an ultrafiltration membrane (YM-3, Amicon, Witten) preceded by a sterile filter (0.2 μm, Sartorius, Göttingen) was sterilized in an autoclave for 0.3 h at 120 °C (the experimental procedure corresponds to that described in [4] and [5]). Subsequently, bovine serum albumin (10 mg) and β-galactosidase (30 mg, 150 units) from *Bacillus circulans* (Daiwa Kasei K. K., Osaka, Japan) were fed into the reactor using buffer solution (100 mM KH_2PO_4, 2 mM $MgCl_2 \cdot 6H_2O$, 5 mM dithiothreitol, pH 6.8). A sterile solution (2.6 L) of the substrates (120 mM lactose **1**, 300 mM *N*-acetylglucosamine **2** in buffer solution) was then pumped through the reactor with residence times between $\tau = 0.25$ h and 0.5 h. Samples were taken regularly from the reactor outlet and analyzed by chromatography (HPLC: column ET 250/8/4 Nucleosil 5 NH_2 (Macherey-Nagel, Düren), 250 mm × 4 mm; eluent 75/25 (v/v) acetonitrile/water; flow-rate 1 mL min^{-1}; RI detection; capacity factors *k'*: *N*-acetylglucosamine 0.84, galactose 1.26, **3** 2.09, lactose 3.09). To isolate the product **3**, the solution (containing 11.3 g of **3**) was concentrated to 0.68 L. The product was characterized by chromatography of a small portion of the solution (0.02 L) on 2/1 (w/w) activated charcoal (Darco, 20–40 mesh)/celite AFA (38 cm × 3.5 cm, eluent: H_2O with 0%–10% (v/v) ethanol, 0.5 bar). The fractions of **3** were collected and lyophilized. Yield 0.19 g. Analysis by gas chromatography (column: OV1 (Machery-Nagel, Düren); 25 m × 0.25 mm; He; temperature 275 °C, silylation according to [18] showed 4.7% *N*-acetylallolactosamine (Galβ(1,6)GlcNAc) as a by-product. Capacity factors *k'*: **3** 5.00/5.35, allo-**3** 3.65/4.24. Correct elemental analysis.

$[\alpha]_{20.5\,°C}^{D} = 25.38$ ($c = 0.1$ in H_2O). The ^{1}H NMR spectrum (500 MHz, D_2O, internal standard [D$_4$]DSS (DSS = 3-trimethylsilyl-1-propanesulfonic acid), $\delta = 2.04$ (3H, s, NHAc, GlcNAc), 4.47 (1H, d, $J_{1,2} = 7.5$ Hz, HI, Gal), 5.2 (1H, s, H1, GlcNAc)) and the 50 MHz ^{13}C NMR spectrum for **3** are consistent with published values [8a, 10d].

Received: April 14, 1993 [Z 5946 IE]
German version: *Angew. Chem.* **1993**, *105*, 1399

[1] a) L. A. Lasky, *Science* **1992**, *258*, 964–969; b) A. Kobata, *Eur. J. Biochem.* **1992**, *209*, 483–501; c) T. Feizi, *Nature* **1985**, *314*, 53–57.
[2] a) Y. Ichikawa, G. C. Look, C.-H. Wong, *Anal. Biochem.* **1992**, *202*, 215–238, and references therein; b) H. Waldmann, *Nachr. Chem. Tech. Lab.* **1992**, *40*, 828–834, and references therein.
[3] S. Bormann, *Chem. Eng. News* **1992**, *70* (49), 25–28.
[4] a) U. Kragl, D. Vasic-Racki, C. Wandrey, *Chem. Ing. Tech.* **1992**, *64* (6), 499–509, and references therein; b) G. Herrmann, A. Schwarz, C. Wandrey, M.-R. Kula, G. Knaup, K. H. Drauz, H. Berndt, *Biotechnol. Appl. Biochem.* **1991**, *13*, 346–353.
[5] U. Kragl, D. Gygax, O. Ghisalba, C. Wandrey, *Angew. Chem.* **1991**, *103*, 854–855; *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 827–828.
[6] a) R. Kuhn, W. Kirschenloher, *Liebigs Ann. Chem.* **1956**, *600*, 135–143; b) E. Lattová, L. Petrus, *Carbohydr. Res.* **1992**, *235*, 289–293.
[7] a) A. Kameyama, H. Ishida, M. Kiso, A. Hasegawa, *Carbohydr. Res.* **1991**, 209, c1–c4; b) K. C. Nicolaou, C. W. Hummel, Y. Iwabuchi, *J. Am. Chem. Soc.* **1992**, *114*, 3126–3128; c) A. Toepfer, R. R. Schmidt, *Tetrahedron Lett.* **1992**, *33*, 5161–5164; d) Y. Matsuzaki, Y. Ito, Y. Nakahara, T. Ogawa, *ibid.* **1993**, *34*, 1061–1064.
[8] a) C.-H. Wong, S. L. Haynie, G. M. Whitesides, *J. Org. Chem.* **1982**, *47*, 5416–5418; b) H. Yuasa, O. Hindsgaul, M. M. Palcic, *J. Am. Chem. Soc.* **1992**, *114*, 5891–5892; c) J. Thiem, T. Wiemann, *Angew. Chem.* **1990**, *102*, 78–80; *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 827–828.
[9] 25 units of the galactosyltransferase from bovine milk is available for $ 637 (Sigma Chemie).
[10] a) K. Sakai, R. Katsumi, H. Ohi, T. Usui, Y. Ishido, *J. Carbohydr. Chem.* **1992**, *11*, 553–565; b) L. Hedbys. E. Johansson, K. Mosbach, P.-O. Larsson, A. Gunnarsson, S. Svensson, H. Lönn, *Glycoconjugate J.* **1989**, *6*, 161–168; c) F. Zilliken, P. N. Smith, C. S. Rose, P. György, *J. Biol. Chem.* **1955**, *217*, 79–82; d) K. Ajisaka, H. Fujimoto, H. Nishida, *Carbohydr. Res.* **1988**, *180*, 35–42; e) K. G. I. Nilsson, *Trends Biotechnol.* **1988**, *6*, 256–264, and references cited therein.
[11] Half-life of the enzyme: $t_{1/2} = 262$ h at pH 7 and 22 °C (McIlvaine buffer, 5 mM dithiothreitol, 2 mM $MgCl_2 \cdot 6H_2O$).
[12] β-galactosidase from *B. circulans* is available for $ 350 per kg (5×10^6 units) (Daiwa Kasei, Osaka Japan).
[13] Selectivity is defined as the quotient of product concentration (LacNAc **3**) and concentration of converted substrate (lactose **1**).
[14] At $\tau = 0.5$ h the concentration of **3** is 5.4 g L^{-1} (13.85 mmol L^{-1}). This corresponds to a yield of 11.5%. Concentration of allo-LacNAc is 0.25 g L^{-1}.
[15] A 0.2 L enzyme membrane reactor was used to produce 3.5 kg *N*-acetylneuraminic acid (unpublished results).
[16] 1 g β-galactosidase from *B. circulans* affords 376 g of **3** by the technique described here.
[17] G. F. Herrmann, Y. Ichikawa, C. Wandrey, F. C. A. Gaeta, J. C. Paulson, C.-H. Wong, *Tetrahedron Lett.* **1993**, *34*, 3091–3094.
[18] C. C. Sweeley, R. Bentley, W. W. Wels, *J. Am. Chem. Soc.* **1963**, 85, 2497–2507.