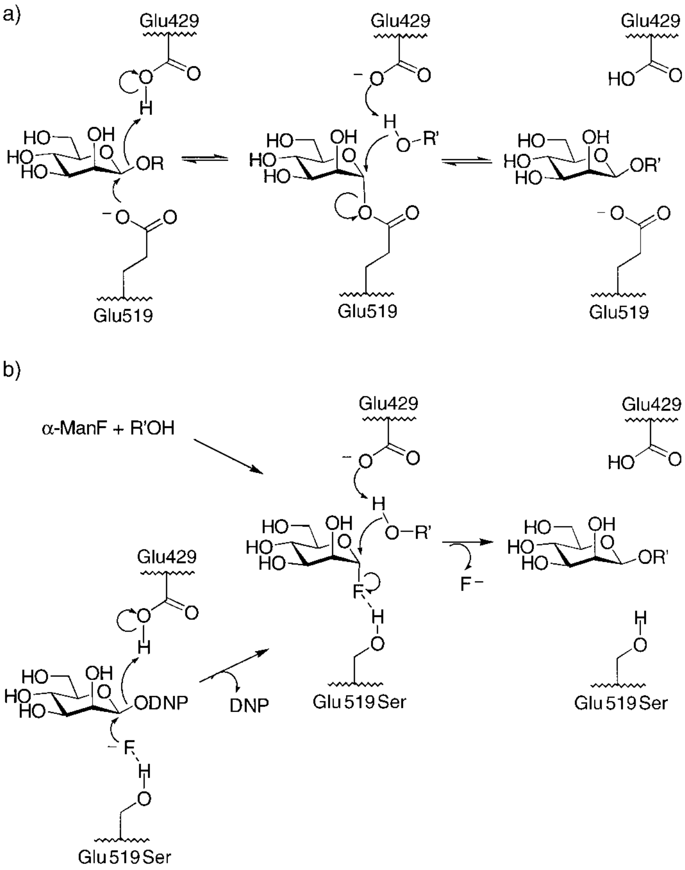
istry (Ed.: S. Hanessian), Marcel Dekker, New York, **1997**, Chap. 20; i) O. J. Plante, R. B. Andrade, P. H. Seeberger, *Org. Lett.* **1999**, *1*, 211–214; j) T. Zhu, G.-J. Boons, *Angew. Chem.* **1999**, *111*, 3704–3707; *Angew. Chem. Int. Ed.* **1999**, *38*, 3495–3497; k) G. X. Chang, T. L. Lowary, *Org. Lett.* **2000**, *2*, 1505–1508; l) for an orthogonal two-stage activation strategy employing glycosyl fluorides and sulfides, see: K. C. Nicolaou, R. E. Dolle, D. P. Papahatjis, J. L. Randall, *J. Am. Chem. Soc.* **1984**, *106*, 4189–4192.
[4] a) B. A. Garcia, J. L. Poole, D. Y. Gin, *J. Am. Chem. Soc.* **1997**, *119*, 7597–7598; b) B. A. Garcia, D. Y. Gin, *J. Am. Chem. Soc.* **2000**, *122*, 4269–4279; c) B. A. Garcia, D. Y. Gin, *Org. Lett.* **2000**, *2*, 2135–2138.
[5] Selected examples of previous methods for dehydrative glycosylation: a) E. Fischer, *Ber. Dtsch. Chem. Ges.* **1893**, *26*, 2400–2412; b) S. Koto, N. Morishima, S. Zen, *Bull. Chem. Soc. Jpn.* **1982**, *55*, 1543–1547; c) S. Suda, T. Mukaiyama, *Chem. Lett.* **1991**, 431–434; d) J. Inanaga, Y. Yokoyama, T. Hanamoto, *J. Chem. Soc. Chem. Commun.* **1993**, 1090–1091; e) H. Susaki, *Chem. Pharm. Bull.* **1994**, *42*, 1917–1918; f) W. R. Roush, X.-F. Lin, *J. Am. Chem. Soc.* **1995**, *117*, 2236–2250, and references therein; g) H. Uchiro, T. Mukaiyama, *Chem. Lett.* **1996**, 79–80, and references therein.
[6] These advantages are also present in the chemoselective iterative oxidative glycosylation with glycals, a strategy which has been widely successful in the synthesis of complex oligosaccharides: R. W. Friesen, S. J. Danishefsky, *J. Am. Chem. Soc.* **1989**, *111*, 6656–6660; see also reference [1c].
[7] I. Kitagawa, N. I. Baek, K. Kawashima, Y. Yokokawa, M. Yoshikawa, K. Ohashi, H. Shibuya, *Chem. Pharm. Bull.* **1996**, *44*, 1680–1692.
[8] Derived from acetate hydrolysis of **12**α.
[9] Excess **8** (0.8 equiv) could be recovered in pure form.

β-Mannosynthase: Synthesis of β-Mannosides with a Mutant β-Mannosidase**

Oyekanmi Nashiru, David L. Zechel, Dominik Stoll, Taraneh Mohammadzadeh, R. Antony J. Warren, and Stephen G. Withers*

The β-D-mannopyranoside linkage is found in a number of biological structures, most notably in the core trisaccharide of N-linked glycoproteins,[1] as well as within the antigenic polysaccharides of yeasts,[2] *Salmonella*,[3] and glycolipids.[4] This is arguably the most difficult glycosidic bond to synthesize chemically and has inspired numerous approaches.[5] Despite these successes, each method requires a number of protection and activation steps prior to glycosylation, and few methods can achieve complete anomeric stereoselectivity.[5f,h]

Enzymatic synthesis of β-mannosides avoids both of these problems.[6] Successful transfer of β-mannosyl residues to a variety of acceptor sugars has been achieved with retaining β-glycosidases,[7] which function through the mechanism shown in Scheme 1a (R' = glycoside acceptor). However, the yields of these transglycosylation reactions are inherently modest as the product formed is a substrate for the glycosidase used, resulting in hydrolysis. Further, the approach requires the preparation of a β-mannoside as a glycosyl donor, thereby minimizing advantages. Substantially better yields have been obtained with recombinant yeast and *Salmonella* β-mannosyltransferases (60–90%),[8] but these enzymes are limited by their requirement for a complex acceptor. In contrast to the above, glycosynthases[9] are new enzymatic catalysts for oligosaccharide synthesis that are derived by mutating the catalytic nucleophiles of retaining glycosidases.[10] Here we report the successful construction of a mannosynthase from a retaining β-mannosidase and its use in the synthesis of β-mannosides with the readily accessible donor, α-D-mannosyl fluoride (Scheme 1b, upper pathway).



Scheme 1. a) Hydrolysis ($R' = H$) and transglycosylation (R' = glycoside acceptor) mechanisms of the retaining *Cellulomonas fimi* β-mannosidase (Man2a). b) Mechanism of the Man2a Glu519Ser mutant acting as a mannosynthase (upper pathway) and catalyzing the in situ generation of α-mannosyl fluoride (α-ManF; lower pathway). R'OH = glycoside acceptor. DNP = 2,4-dinitrophenyl.

---

[*] Prof. S. G. Withers, D. L. Zechel, T. Mohammadzadeh
Protein Engineering Network of Centres of Excellence
Department of Chemistry
University of British Columbia
2036 Main Mall, Vancouver, BC, V6T 1Z1 (Canada)
Fax: (+1) 604-822-2847
E-mail: withers@chem.ubc.ca

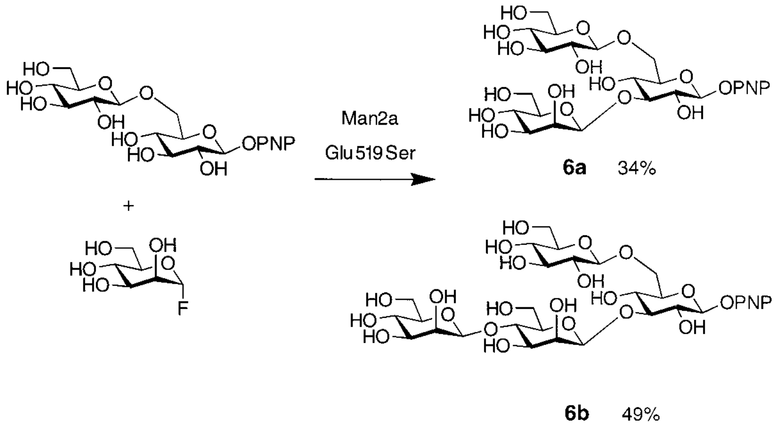
Dr. O. Nashiru, Dr. D. Stoll, Prof. R. A. J. Warren
Department of Microbiology
University of British Columbia (Canada)

[**] We thank the Protein Engineering Network of Centres of Excellence of Canada and the Natural Sciences and Engineering Research Council of Canada for support. D.L.Z. thanks the Izaak Walton Killam Foundation for a fellowship.

Supporting information for this article is available on the WWW under http://www.angewandte.com or from the author.

The retaining *β*-mannosidase Man2a was cloned from *Cellulomonas fimi* and overexpressed in *E. coli.* to yield a 95 kDa monomeric enzyme belonging to family 2 of the glycosyl hydrolases.[11] The active site nucleophile was identified as Glu519 by trapping the covalent mannosyl–enzyme intermediate with the mechanism-based inactivator 2-deoxy-2-fluoro *β*-D-mannosyl fluoride, followed by proteolysis and sequencing of the labeled peptide by ESI-MS/MS.[12, 13] Mutation of the nucleophile to alanine (Glu519Ala) produced an enzyme that was inactive as a hydrolase. However, Man2a Glu519Ala also proved to be a poor mannosynthase, from which only an 8% yield of di- and trisaccharides was obtained upon the reaction of *α*-D-mannosyl fluoride (*α*-ManF) with *para*-nitrophenyl *β*-D-mannopyranoside (PNP *β*-mannoside).

Based upon recent successes with a family 1 glycosynthase in which a much more effective catalyst was obtained by replacing the catalytic nucleophile with serine,[9c] the Glu519Ser mutant of Man2a was made. This mutant proved to be a much more effective glycosynthase affording a 99% overall yield (determined by HPLC) of oligosaccharides from the reaction of *α*-ManF with PNP *β*-D-cellobioside (Table 1). A number of other sugars also acted as viable acceptors, with oligosaccharide yields typically in excess of 70% (Table 1). Benzyl *β*-D-*N*-acetylglucosaminide, PNP *β*-D-*N*-acetylglucosaminide, or chitobiose would not function as an acceptors.[14] Reaction times varied from 24 hours for disaccharide acceptors to several days for the poorer monosaccharide acceptors. HPLC analyses of the reaction mixtures and ^{1}H/^{13}C NMR spectroscopic analyses of the per-O-acetylated products indicated that the oligosaccharide products contained a mixture of *β*(1,3) and *β*(1,4) linkages (Table 1).[15] A notable example was the mixture derived from PNP *β*-gentiobioside, which afforded branched tri- and tetrasaccharide products **6a** and **6b**, respectively, in which mannose residues are *β*(1,3) linked to the PNP-bearing glucose residues (Scheme 2).[16]



Scheme 2. Branched oligosaccharide products derived from the reaction of PNP *β*-gentiobioside with *α*-mannosyl fluoride in the presence of Man2a Glu519Ser. PNP = *para*-nitrophenyl.

Table 1. Product yields for the reaction of *α*-mannosyl fluoride with various acceptors, catalyzed by Man2a Glu519Ser.

Acceptor	Yield of oligosaccharide [%][a]					
	di	tri	tetra	penta	hexa	Total
PNP *β*-mannoside	21 **1a** *β*(1,4)	32 **1b** (5) *β*(1,4),*β*(1,4) **1c** (2) *β*(1,4),*β*(1,3)	19 **1d** (5) all *β*(1,4) **1e** (1) *β*(1,4),*β*(1,4),*β*(1,3)	2	–	74
2,4-DNP 2-deoxy-2-fluoro-*β*-mannoside	7	39 **2a** (1) *β*(1,4),*β*(1,4) **2b** (1) *β*(1,4),*β*(1,3)	15	8	1	70
PNP *β*-glucoside	22 **3a** *β*(1,3)	36 **3b** (1) *β*(1,4),*β*(1,4) **3c** (15) *β*(1,4),*β*(1,3)	13 **3d** *β*(1,4),*β*(1,4),*β*(1,3)	–	–	71
PNP *β*-xylobioside	8 **4a** (1) *β*(1,4) **4b** (1) *β*(1,3)	64 **4c** (1) *β*(1,4),*β*(1,4) **4d** (2) *β*(1,4),*β*(1,3)	19 **4e** (1) all *β*(1,4) **4f** (1) *β*(1,4),*β*(1,4),*β*(1,3)	3	–	94
PNP *β*-cellobioside	–	27 **5a** (8) *β*(1,3) **5b** (5) *β*(1,4)	56 **5c** (4) *β*(1,4),*β*(1,4) **5d** (1) *β*(1,4),*β*(1,3)	5 **5e** all *β*(1,4)	1	99
PNP *β*-gentiobioside	–	34 **6a** *β*(1,3)	49 **6b** *β*(1,4),*β*(1,3)	8	0.5	91

[a] Yields were determined by HPLC and are based on remaining acceptor. The relative ratios of products are indicated in parentheses. The sequence of linkages formed in each product is indicated. Spectroscopic data are provided in the Supporting Information.

Kinetic parameters for transglycosylation by the Man2a Glu519Ala and Ser mutants were determined by use of a fluoride electrode to monitor fluoride release during the coupling of α-ManF with PNP β-cellobioside. The reaction was first studied in the presence of a fixed concentration of acceptor. In this way apparent k_{cat} values of 12 min^{-1} (at 29 mM PNP β-cellobioside) and 0.33 min^{-1} (at 20 mM PNP β-cellobioside) were obtained for the Glu519Ser and Glu519Ala mutants, respectively. The apparent K_m values for α-ManF determined under the same conditions were very similar: 0.70 mM for Glu519Ser and 0.57 mM for Glu519Ala. The reaction was then studied in the presence of a fixed, saturating concentration of α-ManF (50 mM), but saturation with PNP β-cellobioside could not be obtained with either mutant. Thus, the apparent k_{cat}/K_m values of 0.35 min^{-1}mM^{-1} and 0.013 min^{-1}mM^{-1} for Glu519Ser and Glu519Ala, respectively, were determined from the slope of the plot of rate versus PNP β-cellobioside concentration. This corresponds to a 27-fold transglycosylation rate enhancement for the Glu519Ser mutant relative to the Ala mutant and is remarkably similar to the 24-fold increase obtained previously with the family 1 glycosynthase, Abg Glu358Ser.[9c] The mechanistic origins of this rate increase are uncertain, but may arise from a stabilizing interaction between the departing fluoride ion and the serine side-chain hydroxyl in the transglycosylation transition state (Scheme 1b). Transglycosylation rates were also studied as a function of pH, and the Man2a Glu519Ser mutant was found to be most active between pH 5 and 6 (data not shown). This is fortunate because α-ManF undergoes rapid spontaneous hydrolysis above pH 7, presumably through the base-catalyzed formation of a 1,2 epoxide.[17]

A characteristic feature of nucleophile mutants of retaining glycosidases is their ability to recover glycosidic bond cleaving activity when provided with anionic nucleophiles such as acetate, formate, and azide.[18] To our surprise, fluoride ions (2 M KF) also rescued significant bond-cleaving activity when 2′,4′-dinitrophenyl β-D-mannopyranoside (2,4-DNPMan) was used as a substrate for both the Man2a Glu519Ala (k_{cat} = 21 min^{-1}, K_m = 0.13 mM) and Glu519Ser (k_{cat} = 14 min^{-1}, K_m = 0.036 mM) mutants. Despite the poor nucleophilicity of fluoride ions in aqueous solution, this chemical rescue corresponds to at least a 10^4-fold increase in rate, since the nucleophile mutants are essentially inactive in the absence of fluoride ions.[19] This appears to be an exceedingly rare form of chemical rescue and to our knowledge is the first example of the fluoride ion acting as a substitute nucleophile in a mutant enzyme system.[20] The reaction of a fluoride ion with 2,4-DNPMan will result in the in situ generation of α-ManF, which may then be used as a mannosyl donor to a second molecule of 2,4-DNPMan acting as an acceptor (Scheme 1b, lower pathway).[21] Accordingly, thin layer chromatographic and ^{1}H NMR spectroscopic analyses of a preparative rescue reaction carried out with Man2a Glu519Ser confirmed the formation of the donor, α-ManF.

The existing glycosynthase methodology has been successfully extended to include the enzymatic synthesis of β-mannoside linkages. In what may be a general feature of these catalysts,[9c] the serine nucleophile mutant of Man2a proved to be a dramatically better glycosynthase than the corresponding alanine mutant. Finally, we report here the first instance of the fluoride ion acting as a nucleophile in the chemical rescue of mutant-enzyme activity.

Experimental Section

Mutagenesis, expression, and purification of Man2a Glu519Ser: The plasmid pETMan2aGlu519Ser carrying the Glu519Ser mutation was introduced by modification of a "megaprimer" procedure (details provided in the Supporting Information).[22] Recombinant Man2A Glu519Ser was purified from isopropyl-β-D-thiogalactopyranoside (IPTG)-induced cultures of *E. coli* BL21(DE3) electrocompetent cells carrying the plasmid pETMan2aGlu519Ser. Harvested cells were twice passed through a French press at 4 °C, then centrifuged at 15000 g for 30 min. Man2a Glu519Ser was isolated from the supernatant by Ni^{2+}-chelation chromatography (His-bind resin, Novagen). The yield of Man2a Glu519Ser was 350 mg L^{-1} culture.

Rescue and transglycosylation kinetics: Mutant Man2a concentrations were determined by absorbance at 280 nm using the extinction coefficient $E_{280}^{0.1\%} = 2.2\,\text{cm}^{-1}$, estimated from the corresponding amino acid sequences.[23] Rescue kinetics with the substrate 2,4-DNPMan[24] were performed at 25 °C in 100 mM citrate (pH 6.0) supplemented with bovine serum albumin (BSA; 1 mg mL^{-1}) and 2 M potassium fluoride (750 µL total volume). Reactions were initiated with the addition of Man2a Glu519Ser or Glu519Ala (5–10 µL, 0.1–0.4 µM final concentrations) and the resulting liberation of 2,4-dinitrophenol was monitored spectrometrically at 400 nm ($\Delta\varepsilon = 10900\,\text{M}^{-1}\text{cm}^{-1}$). Observed rates were corrected for the spontaneous hydrolysis of 2,4-DNPMan. Transglycosylation kinetics were performed at 25 °C in 100 mM citrate and 150 mM NaCl (pH 6.0; 400 µL total volume) using α-ManF as a donor and PNP β-cellobioside as an acceptor. Transglycosylation was initiated with the addition of Man2a Glu519Ser or Glu519Ala (20–50 µL, 5.5 to 70 µM final concentrations). The reactions were then stirred while monitoring fluoride release (until 5–20% donor had been consumed) using an Orion fluoride electrode (model 96–09BN) interfaced with Logger-Pro software.[25] For pH studies, transglycosylation rates were measured using 5.5 µM Man2a Glu519Ser, 6 mM α-ManF, 5 mM PNP β-cellobioside, and 1 mg mL^{-1} of BSA in the following buffers: 100 mM citrate/150 mM NaCl (pH 5.0–6.5), 100 mM NaPi/150 mM NaCl (pH 6.5–8.0), and 100 mM 2-hydroxy-3-[(2-hydroxy-1,1-dimethylethyl)amino]1-propanesulfonic acid (AMPs0)/150 mM NaCl (pH 8.0–9.0). Kinetic parameters for rescue and transglycosylation kinetics were calculated from the initial rates using GraFit.[26]

Oligosaccharide synthesis, purification, and characterization: In a typical reaction, acceptor glycoside (20–40 mg) was dissolved in 150 mM citrate buffer (2 to 3 mL, pH 6.0) to afford a 30–50 mM solution. α-ManF (1.5–3 equiv) was added as a concentrated solution, followed by Man2a Glu519Ser (1–2 mg mL^{-1}). The reaction mixture was maintained at room temperature and monitored by thin layer chromatography (Kieselgel 60 F_{254} (Merck), ethyl acetate/methanol/water (7/2/1)). Chromatographic plates were visualized under UV light and by exposure to 10% ammonium molybdate in 2 M H_2SO_4, followed by charring. Reaction times ranged from one to five days. Upon completion of the reaction the products were purified by preparative HPLC with UV detection.[9c] Masses of product fractions were confirmed by ESI-MS. Product fractions were evaporated, dried, and acetylated with acetic anhydride/pyridine (1/2), then purified by flash chromatography on silica gel (230–400 mesh) prior to NMR spectroscopic analysis.

Received: August 7, 2000 [Z15596]

---

[1] R. A. Dwek, *Chem. Rev.* **1996**, *96*, 683–720.

[2] a) D. Vinogradov, B. O. Petersen, J. O. Duus, *Carbohydr. Res.* **2000**, *325*, 216–221; b) P.-A. Trinel, Y. Plancke, P. Gerold, T. Jouault, F. Delplace, R. T. Schwarz, G. Strecker, D. Poulain, *J. Biol. Chem.* **1999**, *274*, 30520–30526; c) J. P. Martinez, M. L. Gil, J. L. Lopez-Ribot, W. L. Chaffin, *Clin. Microbiol. Rev.* **1998**, *11*, 121–141.

[3] B. Lindberg, K. Leontein, U. Lindquist, S. B. Svenson, G. Wrangsell, A. Dell, M. Rogers, *Carbohydr. Res.* **1988**, *174*, 313–322.

[4] a) M. W. Riggs, M. R. McNeil, L. E. Perryman, A. L. Stone, M. S. Scherman, R. M. O'Connor, *Infect. Immun.* **1999**, *67*, 1317–1322; b) M. Sugita, Y. Sanai, S. Itonori, T. Hori, *Biochim. Biophys. Acta* **1988**, *962*, 159–165.

[5] a) G. Ekborg, B. Lindberg, J. Lonngren, *Acta. Chem. Scand.* **1972**, *26*, 3287; b) H. Paulsen, R. Lebuhn, O. Lockhoff, *Carbohydr. Res.* **1982**, *103*, C7-C11; c) J. Alais, S. David, *Carbohydr. Res.* **1990**, *201*, 69–77; d) W. Gunther, H. Kunz, *Carbohydr. Res.* **1992**, *228*, 217–241; e) F. W. Lichtenthaler, U. Klares, M. Lergenmuller, S. Schwidetzky, *Synthesis* **1992**, 179–184; f) F. Barresi, O. Hindsgaul, *Can. J. Chem.* **1994**, *72*, 1447–1465; g) T. Ziegler, G. Lemanski, *Angew. Chem.* **1998**, *110*, 3367–3369; *Angew. Chem. Int. Ed.* **1998**, *37*, 3129–3132; h) D. Crich, S. Sun, *Tetrahedron* **1998**, *54*, 8321–8348; for reviews, see: i) F. Barresi, O. Hindsgaul in *Modern Methods in Carbohydrate Synthesis* (Eds.: S. H. Khan, R. A. O'Neill), Harwood Academic, Amsterdam, **1996**, pp. 251–276; j) J. J. Gridley, H. M. I. Osborn, *J. Chem. Soc. Perkin Trans. 1* **2000**, 1471–1492.

[6] D. H. G. Crout, G. Vic, *Curr. Opin. Chem. Biol.* **1998**, *2*, 98–111.

[7] a) M. Scigelova, S. Singh, D. H. G. Crout, *J. Chem. Soc. Perkin Trans. 1* **1999**, 777–782; b) N. Taubken, J. Thiem, *Glycoconjugate J.* **1998**, *15*, 757–767; c) H. Prade, L. F. Mackenzie, S. G. Withers, *Carbohydr. Res.* **1998**, *305*, 371–381; d) T. Usui, M. Suzuki, T. Sato, H. Kawagishi, K. Adachi, H. Sano, *Glycoconjugate J.* **1994**, *11*, 105–110; e) S. Kyosaka, S. Murata, Y. Tsuda, M. Tanaka, *Chem. Pharm. Bull.* **1986**, *34*, 5140–5143.

[8] a) G. M. Watt, L. Revers, M. C. Webberley, I. B. H. Wilson, S. L. Flitsch, *Angew. Chem.* **1997**, *109*, 2445–2447; *Angew. Chem. Int. Ed.* **1997**, *36*, 2354–2356; b) G. M. Watt, L. Revers, M. C. Webberley, I. B. H. Wilson, S. L. Flitsch, *Carbohydr. Res.* **1998**, *305*, 533–541; c) L. Revers, R. M. Bill, I. B. H. Wilson, G. M. Watt, S. L. Flitsch, *Biochim. Biophys. Acta* **1999**, *1428*, 88–98; d) Y. Zhao, J. B. Biggins, J. S. Thorson, *J. Am. Chem. Soc.* **1998**, *120*, 12986–12987; e) Y. Zhao, J. S. Thorson, *Carbohydr. Res.* **1999**, *319*, 184–191.

[9] a) S. Fort, V. Boyer, L. Greffe, G. J. Davies, O. Moroz, L. Christiansen, M. Schülein, S. Cottaz, H. Driguez, *J. Am. Chem. Soc.* **2000**, *122*, 5429–5437; b) A. Trincone, G. Perugino, M. Rossi, M. Moracci, *Biorg. Med. Chem. Lett.* **2000**, *10*, 365–368; c) C. Mayer, D. L. Zechel, S. P. Reid, R. A. J. Warren, S. G. Withers, *FEBS Lett.* **2000**, *466*, 40–44; d) C. Malet, A. Planas, *FEBS Lett.* **1998**, *440*, 208–212; e) L. F. Mackenzie, Q. Wang, R. A. J. Warren, S. G. Withers, *J. Am. Chem. Soc.* **1998**, *120*, 5583–5584.

[10] D. L. Zechel, S. G. Withers, *Acc. Chem. Res.* **2000**, *33*, 11–18.

[11] D. Stoll, H. Stalbrand, R. A. J. Warren, *Appl. Environ. Microbiol.* **1999**, *65*, 2598–2605.

[12] S. G. Withers, R. Aebersold, *Protein Sci.* **1995**, *4*, 361–372.

[13] D. Stoll, S. He, S. G. Withers, R. A. J. Warren, *Biochem. J.* **2000**, *351*, 833–838.

[14] More active mannosynthase mutants may prove successful in mannosylating derivatives of GlcNAc. Indeed, it proved possible to synthesize *N*-acetyl lactosamine in good yield from α-galactosyl fluoride and benzyl β-GlcNAc using the more active *Agrobacterium* sp. Glu358Ser glycosynthase.[9c]

[15] The mannoside linkage formed was confirmed to be the β anomer by the 160 Hz J(C,H) coupling constant, which was measured for C-1 of the mannose residue in the disaccharide products **1a** and **3a** (see: K. Bock, C. Pedersen, *J. Chem. Soc. Perkin Trans. 2* **1974**, 293–297).

[16] Branched oligosaccharide products synthesized by glycosynthases have been reported previously.[9a,b]

[17] B. Capon, *Chem. Rev.* **1969**, *69*, 407–498.

[18] H. D. Ly, S. G. Withers, *Annu. Rev. Biochem.* **1999**, *68*, 487–522.

[19] Equivalent concentrations of KCl did not rescue glycosidic bond cleaving activity.

[20] A 10^3-fold enhancement of activity for a mutant glutamate dehydrogenase in the presence of fluoride ions has been reported recently, but is believed to arise from the fluoride ions fulfilling an electrostatic role. See: B. M. Hayden, J. L. E. Dean, S. R. Martin, P. C. Engel, *Biochem. J.* **1999**, *340*, 555–560.

[21] The glycosynthase-catalyzed in situ generation of 1-*O*-formyl α-glycosides from activated β-glycosides and formate has been reported (J.-L. Viladot, E. de Ramon, O. Durany, A. Planas, *Biochemistry* **1998**, *37*, 11332–11342; M. Moracci, A. Trincone, G. Perugino, M. Ciaramella, M. Rossi, *Biochemistry* **1998**, *37*, 17262–17270) and successfully used as a method for oligosaccharide synthesis.[9b]

[22] A. Aiyar, J. Leis, *BioTechniques* **1993**, *11*, 404.

[23] S. C. Gill, P. H. von Hippel, *Anal. Biochem.* **1989**, *182*, 319–326.

[24] 2,4-DNPMan was synthesized in three steps by the method of Garegg et al. (P. J. Garegg, T. Iversen, T. Norberg, *Carbohydr. Res.* **1979**, *73*, 313–314).

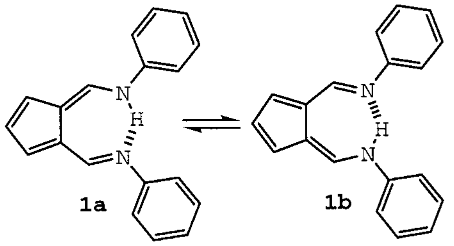
[25] Logger Pro Version 1.1, Tufts University and Vernier Software, **1999**.

[26] R. J. Leatherbarrow, GraFit 3.09b, Erithacus Software Ltd., **1996**.

6-Aminofulvene-1-aldimine: A Model Molecule for the Study of Intramolecular Hydrogen Bonds**

Rosa María Claramunt,* Dionísia Sanz, Sergio Hugo Alarcón, Marta Pérez Torralba, José Elguero, Concepción Foces-Foces, Mariusz Pietrzak, Uwe Langer, and Hans-Heinrich Limbach

The search for new systems that sustain phenomena such as intramolecular hydrogen bonds (IMHB),[1, 2] proton transfer along hydrogen bonds,[3, 4] and coupling constants through hydrogen bonds,[5, 6] led us to select 6-aminofulvene-1-aldimines. These compounds were reported in the 1970s by Müller-Westerhoff and Ammon, who determined the X-ray structure of **1** and its NMR spectroscopic properties in solution.[8, 9]



---

[*] Prof. Dr R. M. Claramunt, Dr. D. Sanz, Dr. S. H. Alarcón, M. Pérez Torralba
Departamento de Química Orgánica y Biología
Facultad de Ciencias, UNED
Senda del Rey 9, 28040 Madrid (Spain)
Fax: (+34) 91-398-6697
E-mail: rclaramunt@ccia.uned.es

Prof. Dr. J. Elguero
Centro de Química Orgánica Manuel Lora-Tamayo, CSIC
Juan de la Cierva 3, 28006 Madrid (Spain)

Prof. Dr. C. Foces-Foces
Departamento de Cristalografía
Instituto de Química Física "Rocasolano", CSIC
Serrano 119, 28006 Madrid (Spain)

M. Pietrzak, U. Langer, Prof. Dr. H.-H. Limbach
Fachbereich Biologie, Chemie, und Pharmazie
Freie Universität Berlin
Takustrasse 3, 14195 Berlin (Germany)

[**] We are indebted to DGES/MEC (PB96-0001-C03) and Comunidad de Madrid (grant no. 07N/0001/1999) of Spain for financial support and to Dr. Ibon Alkorta for carrying out the B3LYP/6-31G calculations.