Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 157–160

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

ENZYMATIC α(2-3)SIALYLATION OF NON-NATURAL TYPE-I (LEWIS^c) DISACCHARIDES WITH RECOMBINANT SIALYL-TRANSFERASE

Gabi Baisch, Reinhold Öhrlein*, Markus Streiff

NOVARTIS PHARMA AG, Schwarzwaldallee 211, CH-4002 Basle, Switzerland

Received 13 October 1997; accepted 26 November 1997

ABSTRACT

Recombinant α(2-3)sialyl-transferase from rat liver is used to sialylate a series of type-I (Lewis^c) disaccharides on a preparative scale. The enzyme tolerates a broad array of N-acetyl replacements of the N-glucosamine subunit ranging from small and large lipophilic groups to charged and heterocyclic amides.

Recent research in the carbohydrate area has revealed the important biological roles of cell-surface carbohydrates[1,2]. Besides many other adhesive interactions, oligosaccharides are e.g. involved in inflammatory responses[3] and host-graft rejections[4] to mention just a few. Therefore, there is an ongoing challenge for ever more efficient methods[5] to synthesize various glycoconjugates for drug discovery[6].

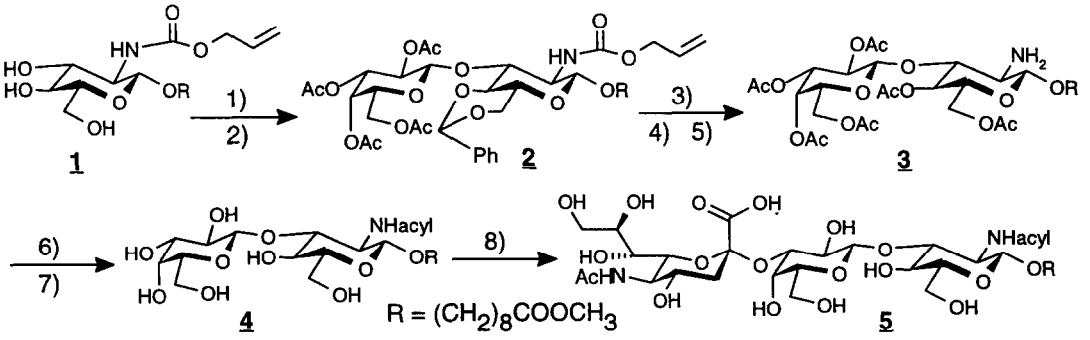
An alternative to the reliable but cumbersome chemical synthesis of carbohydrates[7] is a combined chemo-enzymatic approach[8]. We could recently show that glycosyl-transferases, which form oligosaccharides *in vivo* by sequential transfer of a monosaccharide from a nucleotide-activated donor to a growing oligosaccharide chain[9], can be used to prepare a wide range of non-natural oligosaccharides highly regio- and stereospecifically in a very efficient manner[10,11,12]. Here we wish to report our findings concerning an even more extended use of recombinant α(2-3)-(N)-sialyl-transferase (sia-T)[13] (EMBL accession no. M97754)[11] for the synthesis of non-natural oligosaccharides.

A series of type-I disaccharides is first synthesized enzymatically[14] or chemically (see scheme). The common precursor **1** is obtained from 9-hydroxy nonanoic acid methylester following a standard glycosylation protocol[15]. It is then 4,6-benzylidenated and subsequently galactosylated at the 3-OH group with commercial α-galactosyl bromide in the presence of mercury cyanide[16] to give disaccharide **2**. This compound is treated first

E-mail: REINHOLD.OEHRLEIN@pharma.novartis.com.

0960-894X/98/$19.00
PII: S0960-894X(97)10201-3

with trifluoro acetic acid to remove the benzylidene group and then reacetylated followed by a Pd^0-catalyzed deprotection of the amino group[15] to yield derivative **3**. The amine is acylated either with acyl chlorides or carbonic acids according known protocols[10]. A final deprotection step gives the type-I disaccharides **4**. In some cases (indicated in the table as alternative route) step 3) (see scheme) is immediately followed by step 7) and then step 5). The resulting O-deacetylated derivative of disaccharide **3** is finally N-acylated as described for the protected compound to give type-I sugars **4**. These disaccharides are incubated with recombinant sia-T and cytidine-monophosphosialic acid (CMP-sia) (see exp. procedure)[11,17].



Scheme: 1) $PhCH(OMe)_2$, CSA, 95%; 2) α-perAcgal-Br, $Hg(CN)_2$, 64%; 3) TFA, 100%; 4) Ac_2O, pyr, 76%; 5) $Pd(PPh_3)_4$, diethyl malonate or thiosalicylic acid, 89%; 6) acylate; 7) MeONa, MeOH; 8) Sia-T, CMP-sia (yields see table).

Although it was shown in a previous study[18] that the native rat liver enzyme tolerated minor replacements of the N-acetyl group, like an azido- or amino-group, the recombinant enzyme exhibits an unexpected broad substrate tolerance. Selected examples of a great number of type-I sugars **4**, which have been α-sialylated at the 3-OH group of the terminal galactose unit, are listed in the table. Likewise as the parent N-acetyl compound (entry 1) small and large lipophilic N-acyl replacements (entries 2 - 5, 10) are accepted by the sia-T. Even polar (entry 6) or positively and negatively charged substituents (entries 7, 8) are tolerated. The natural N-acetyl group can even be replaced by a sulfonamide (entry 9) or heterocyclic amides (entries 11, 12), which closely resemble nucleotides.

The structure of all new compounds has been proven by ^1H, ^{13}C and MS-spectra. The NMR-data are in good agreement with those of the parent compound (entry 1, compare also [19]). Selected shifts of some significant reporter groups are included in the table. The β(1-3)linkage of the galactose unit is nicely indicated by a large downfield shift of C-3 of the N-acyl glucosamine moiety. The attachment of sialic acid to the 3-OH of the galactose moiety is confirmed by a downfield shift of the galactose C-3 and also nicely by the H-3.

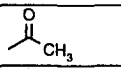
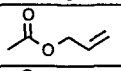
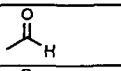
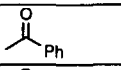
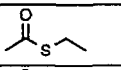
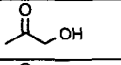
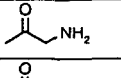
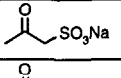
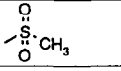
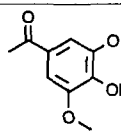
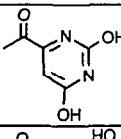
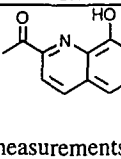
entry	acyl	% (mg)	GlcNacyl: H-1c); C-1, C-2, C-3	Gal: H-1c); H-3e); C-1, C-3	Sia: H-2e f); Ac, C-2, C-3	C-others: $\underline{OCH_3}$, NHR
1a)		70 (81.2)	4.39; 102.40; 56.08; 85.09	4.27; 3.96; 105.49; 77.72	2.73; 22.74; 101.17; 41.00	51.99; 23.44
2		71 (36.0)	4.62; 102.91; 57.74; 83.91	4.30; 3.94; 104.54; 77.40	2.75; 22.68; 101.23; 41.77	51.98; 117.43
3a)		55 (10.0)	4.44; 102.15; 55.32; 84.08 d)	4.30; 3.93; 105.19; 77.57	2.77; 22.61; 101.17; 42.22	51.98; 164.71
4		72 (33.0)	4.60; 102.67; 56.65; 83.83	4.35; 3.94; 104.59; 77.50	2.71; 22.72; 101.36; 41.39	51.97; 128.58
5b)		69 (25.8)	4.45; 102.58; 57.80; 83.10	4.36; 3.99; 104.25; 77.76	2.73; 22.67; 101.24; 41.72	51.98; 16.35
6a)		79 (14.3)	4.50; 102.22; 55.63; 84.49	4.28; 3.96; 105.35; 77.56	2.80; 22.61; 100.94; 42.32	51.98; 62.82
7b)		59 (12.4)	4.40; 102.22; 55.76; 85.53	4.25; 3.95; 105.79; 77.77	2.82; 22.58; 100.95; 42.42	51.98; 45.70
8		53 (13.0)	4.65; 102.09; 57.98; 82.56	4.51; 4.07; 103.81; 77.10	2.78; 22.84; 100.98; 41.65	g); 64.38
9		94 (28.1)	4.40; 103.01; 59.77; 84.37	4.28; 3.95; 104.89; 77.36	2.79; 22.76; 100.18; 41.84	51.97; 42.47
10		60 (31.3)	4.74; 102.76; 56.70; 83.39	4.39; 4.03; 104.31; 77.53	2.70; 22.68; 101.37; 41.40	51.95; 106.36
11		73 (55.2)	4.52; 102.14; 56.67; 84.17	4.31; 4.00; 105.35; 77.51	2.73; 22.78; 101.13; 41.62	52.02; 148.07
12		82 (38.9)	4.66; 102.80; 56.38; 83.43	4.44; 4.13; 103.95; 77.60	2.67; 22.66; 101.20; 41.46	51.96; 112.92

Table: all measurements in D_2O-CD_3OD (ref. D_2O: 4.80 ppm and $\underline{C}D_3OD$: 49.00 ppm); a) starting material from enzymatic synthesis[14]; b) alternative route, see text; c) all dubletts J ~ 8 Hz; d) main isomer; e) dd J ~ 9 and 3.5 Hz; f) dd J ~11.5 and ~3 Hz; g) free acid.

In conclusion our findings render recombinant sia-T a versatile and stereochemical reliable tool for the glycobiologist. The biocatalyst does not only accept a wide range of type-II disaccharides, as previously shown by us[11], but also tolerates a broad range of type-I sugars, as shown in the present study, to give a large number of sialyl Lewis^a precursors.

Representative experimental procedure: To a mixture of 800 µl of bidistilled water, 1600 µl of Na-cacodylate buffer (0.05 M, pH = 6.5) and 1600 µl of a 0.06 M $MnCl_2$-solution are added 12.0 mg (21.0 µmol) of disaccharide **4** (entry 6), 18.3 mg (29.0 µmol contains ~ 70%) CMP-sia[20] and 2.0 mg of bovine serum albumine (Boehringer). This mixture is incubated at 37°C in a plastic tube with 100 µl (670 mU) sia-T and 2 µl (34 U) of calf intestine alkaline phosphatase (Boehringer no. 108146, 7500 U/ 498 µl). After a TLC (CH_2Cl_2 - Methanol - water mixtures) shows the consumption of the starting acceptor **4**, the turbid solution is centrifuged and the clear supernatant passed over a C-18 reversed- phase column[17], washed with water, eluted with methanol and finally purified over silica-gel (CH_2Cl_2 - Methanol - water mixtures). Lyophilization from dioxane-water yields 14.3 mg (79%) of compound **5** (entry 6) as a white powder which is pure according its MS-, ^{1}H- and ^{13}C NMR-analysis. In order to increase the solubility of some acceptor substrates (entries 4, 11, 12) up to 8.5% of DMSO (vol/vol) can be added to the incubation mixture.

References:

1) R. A. Dwek, Chem. Rev. **1996**, *96*, 683.
2) A. Varki, Glycobiology **1993**, *3*, 97.
3) P. R. Crocker, T. Feizi, Curr. Opin. Struct. Biol. **1996**, *6*, 679.
4) F. A. Neethling, E. Koren, Y. Ye, S. V. Richards, M. Kujundzic, R. Oriol, D. K. C. Cooper, Transplantation **1994**, *57*, 959.
5) J. C. McAuliffe, O. Hindsgaul, Chem. & Ind. **1997**, *3*, 170.
6) M. J. Sofia, DDT **1996**, *1*, 27.
7) F. Barresi, O. Hindsgaul, J. Carbohydr. Chem. **1995**, *14*, 1043.
8) H. J. M. Gijsen, L. Qiao, W. Fitz, C.-H. Wong, Chem. Rev. **1996**, *96*, 443.
9) S. C. Crawley, M. M. Palcic, Modern Methods Carbohydr. Synth. **1996**, 492.
10) G. Baisch, R. Öhrlein, B. Ernst, Bioorg. Med. Chem. Lett. **1996**, *6*, 749.
11) G. Baisch, R. Öhrlein, M. Streiff, B. Ernst, Bioorg. Med. Chem. Lett. **1996**, *6*, 755.
12) G. Baisch, R. Öhrlein, A. Katopodis, B. Ernst, Bioorg. Med. Chem. Lett. **1996**, *6*, 759.
13) D. X. Wen, B. D. Livingston, K. F. Medziharadsky, S. Kelm, A. L. Burlingame, J. C. Paulson, J. Biol. Chem. **1992**, *267*, 21011.
14) G. Baisch, R. Öhrlein, M. Streiff, F. Kolbinger, manuscript submitted to Bioorg. Med. Chem. Lett. **1997**.
15) P. Boullanger, J. Banoub, G. Descotes, Can. J. Chem. **1987**, *65*, 1343.
16) R. M. Ratcliffe, D. A. Baker, R. U. Lemieux, Carbohydr. Res. **1981**, *93*, 35.
17) M. M. Palcic, Methods Enzymol. **1994**, *230*, 313.
18) K. B. Wlasichuk, M. A. Kashem, P. V. Nikrad, P. Bird, C. Jiang, A. P. Venot, J. Biol. Chem. **1993**, *268*, 13971.
19) A. Lubineau, C. Augé, P. François, Carbohydr. Res. **1992**, *228*, 137.
20) CMP-sia was gratefully supplied by Prof. U. Kragl (KFZ Jülich, Germany): M. Kittelmann, T. Klein, U. Kragl, C. Wandrey, O. Ghisalba, Appl. Microbiol. Biotechnol. **1995**, *44*, 59.