Oligosaccharide Synthesis

DOI: 10.1002/ange.200600433

Orthogonal Glycosylation Reactions on Solid Phase and Synthesis of a Library Consisting of a Complete Set of Fucosyl Galactose Isomers**

Osamu Kanie, Isao Ohtsuka, Takuro Ako, Shusaku Daikoku, Yoshimi Kanie, and Rumiko Kato*

The development of efficient methods for accessing synthetic oligosaccharides with diverse structures is essential to our understanding of the involvement of such molecules in biological phenomena.[1] Recent efforts directed toward the synthesis of complex glycoconjugates have made it possible to control the stereo- and regiospecificity of glycosylation reactions in solution- and solid-phase organic synthesis.[2–10] Current syntheses, however, can not be applied directly to the combinatorial chemistry of carbohydrates, for which the synthetic protocols must be extremely simple.[11] Various approaches to the creation of an oligosaccharide library have been described,[11–14] however, there are no reports of successful multiple glycosylation reactions that occur at every hydroxy-group position of a monosaccharide acceptor in a position-specific manner. We describe herein the synthesis of a complete disaccharide library with an L-fucosyl-D-galactose (Fuc-Gal) sequence with three parameters, namely, the anomeric configuration of the Fuc and Gal residues and the linkage position (Table 1). The library was synthesized on the basis of an orthogonal glycosylation strategy. The potential of this approach for solid-phase oligosaccharide synthesis is also illustrated with a focus on overall efficiency. The particular Fuc-Gal sequence used was chosen because αFuc(1→2)βGal is an O-blood-type epitope and also relates to the Le^b antigen, which has been reported to act as a ligand of *Helicobacter pylori*. Thus, a library may provide important information for the potential treatment of ulcers.

In planning the synthesis of an oligosaccharide library, we focused on the efficiency of the overall process. It was necessary to minimize the number of 1) types of protecting groups used and 2) reaction steps in the synthesis. To fulfill the first criterion, a benzyl group was chosen as the sole protecting group, with the exception of protection, when needed, at the connecting position to the resin.[15] To satisfy the second criterion, we chose to combine the use of an orthogonal glycosylation strategy[16] with solid-phase chemistry.[17–19] In the orthogonal glycosylation method, reaction starts from the nonreducing terminus. In this way, the formation of the deletion sequence is suppressed without the need for capping reactions, as has been described for glycal chemistry, because the by-products can not serve as glycosylating agents in the next cycle.[5] At the final stage of glycosylation, a hydrophobic tag was introduced to facilitate rapid isolation of the final product(s) after the deprotection reactions.[20–22]

Table 1: Anomeric configurations and linkage positions of library compounds **1**–**16**.

Fuc		Gal	Compound
Anomeric configuration	Linkage position	Anomeric configuration	
α	2	α	**1**
β	2	α	**2**
α	2	β	**3**[a]
β	2	β	**4**
α	3	α	**5**
β	3	α	**6**
α	3	β	**7**
β	3	β	**8**
α	4	α	**9**
β	4	α	**10**
α	4	β	**11**
β	4	β	**12**
α	6	α	**13**
β	6	α	**14**
α	6	β	**15**
β	6	β	**16**

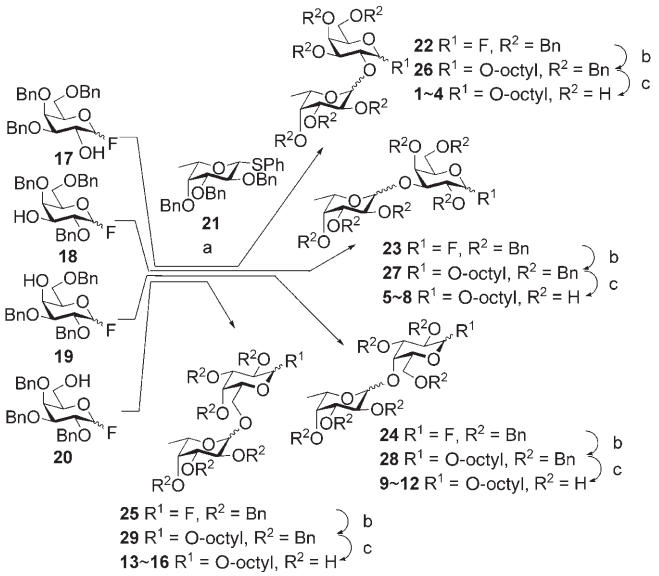
[a] Synthesis was reported in reference [21].

In our strategy, the position of the interglycosidic linkage is fixed and the anomeric configuration is randomized to avoid formation of a highly complex mixture that would make isolation extremely difficult.[11] The glycosyl fluorides **17**–**20** were synthesized from the corresponding phenylthioglycosides of galactopyranose[23] by protection of a hydroxy group with a chloroacetyl group (ClAc), conversion of the phenylthio group into a fluoride substituent by treatment with *N*,*N*-diethylaminosulfur trifluoride, and removal of the ClAc group.[24] The synthesis of a disaccharide library was carried out with **17**–**20**, which contain orthogonal leaving groups, and a fucosyl donor in the form of the phenylthioglycoside **21** (Scheme 1). The phenylthio group was activated chemoselectively over glycosyl fluoride with dimethyl(methylthio)sulfonium trifluoromethanesulfonate (DMTST)[25,26] to yield disaccharides in generally good yields (TLC analysis). Individual anomers in the disaccharide mixtures **22**–**25** were not isolated at this stage, to avoid the unnecessary loss of some anomers. The disaccharide fractions were collected as mixtures by gel-permeation chromatography. The mixtures were then used as the glycosyl donors and treated with *n*-octanol in the presence of hafnocene bis(trifluoromethanesulfonate) $[Cp_2Hf(OTf)_2]$.[27–30] An *n*-octyl group was introduced at the reducing-end anomeric position to facilitate the removal of

[*] Dr. O. Kanie, Dr. I. Ohtsuka, T. Ako, S. Daikoku, Dr. Y. Kanie, R. Kato
Mitsubishi Kagaku Institute of Life Sciences (MITILS)
11 Minamiooya, Machida-shi, Tokyo 194-8511 (Japan)
Fax: (+81) 42-724-6317
E-mail: kokanee@libra.ls.m-kagaku.co.jp

[**] Financial support provided by Mitsubishi Chemical Co. (MCC) and the Key Technology Research Promotion Program of the New Energy and Industrial Development Organization (NEDO), Ministry of Economy, Trade, and Industry (METI) of Japan is gratefully acknowledged.

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



Scheme 1. a) DMTST, MS-AW300, $C_2H_4Cl_2/CH_3CN$ (1:1), 0°C, 4 h; b) *n*-octanol, $[Cp_2Hf(OTf)_2]$, MS-AW300, $C_2H_4Cl_2/CH_3CN$ (1:1), −20°C, 4 h; c) H_2, Pd/C, MeOH. Bn = benzyl.

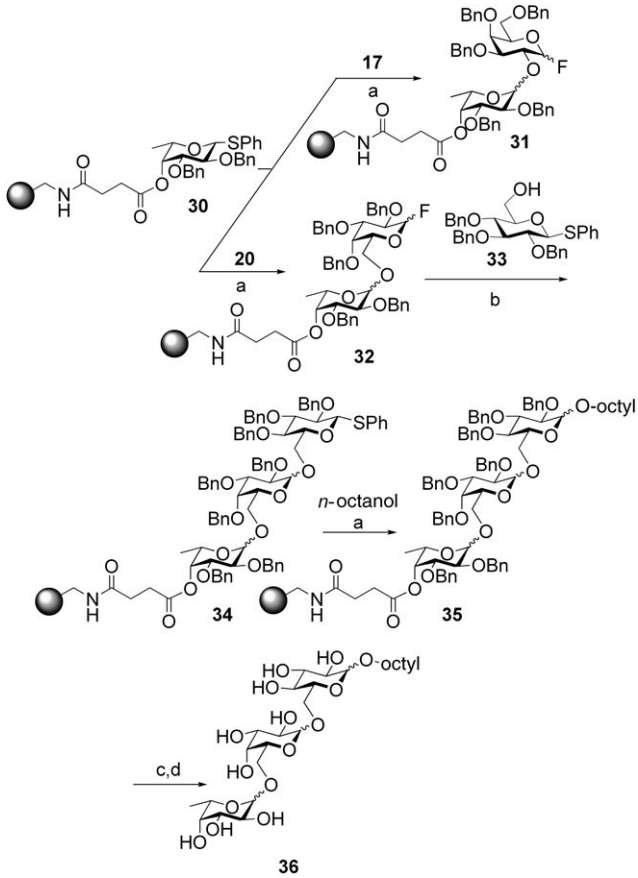
hydrophilic by-products after complete deprotection. This approach is considered particularly useful in solid-phase synthesis.[20] The octyl glycosides **26**–**29** were subjected to hydrogenation and purified roughly by using a C18 cartridge column (Sep-Pak). Ideally, only compounds with an octyl group were collected at this stage (compounds with 2-, 3-, 4-, and 6-linked Fuc-Gal were isolated in 58, 49, 65, and 68% yield, respectively, over three steps). Finally, anomeric mixtures were separated by HPLC with a C18 column to give the individual anomers **1**–**16**. Chemical shifts and coupling constants for the anomeric positions, retention times, and compound ratios are summarized in Table 2.[24,31]

Such a library might be used to obtain information regarding the specificities of various enzymes, especially in connection with methods of structural elucidation.[32] A preliminary assay of a library of 16 compounds with an α(1→2)-"specific" fucosidase from *Corynebacterium*[33,34] revealed that several compounds: **1** (0.5), **3** (4.0), **5** (1.0), **7** (1.2) and **15** (0.7), were accepted as substrates. (The numbers in parentheses indicate the hydrolysis rate relative to that of αFuc(1→2)βGal(1→4)Glc.) Compound **1** with an α-Gal moiety was found unexpectedly to undergo very slow hydrolysis, as well as α(1→3)-linked **5** and **7**, and α(1→6)-linked **15**. Although no information regarding the structure of this particular enzyme is available, it is possible that the 2-OH group of Gal in **5** and **7** may occupy the same space as the 3-OH group in **3** and the reference compound. The presence of these groups may not be essential, as PNP-fucoside (*p*-nitrophenyl α-fucoside) is a better substrate, but they do not

Table 2: Properties of compounds **1**–**16**.

	Fuc		Gal		t_R[b] [min]	Ratio of anomers[c]
	δ [ppm][a]	J [Hz][a]	δ [ppm][a]	J [Hz][a]		
1	5.05	3.6	5.02	3.5	73.3	12.0
2	4.45	7.9	5.08	3.7	46.4	1.0
3[21]	5.26	3.6	4.62	7.9	93.3	37.0
4	4.67	7.8	4.54	7.3	49.0	21.0
5	5.13	4.0	4.94	3.7	87.4	1.0
6	4.67	7.8	4.97	3.8	140	1.0
7	5.15	3.9	4.43	7.4	74.2	1.7
8	4.49	7.8	4.42	8.0	123	1.6
9	5.12	4.0	4.94	3.1	62.4	1.0
10	4.33	7.8	4.95	3.2	49.8	3.1
11	5.14	3.9	4.41	7.9	54.4	7.8
12	4.31	7.7	4.41	7.9	36.9	4.2
13	4.94	3.3	4.90	3.6	39.7	1.0
14	4.38	7.9	4.91	3.7	78.7	1.9
15	4.93	3.9	4.38	7.9	46.9	6.4
16	4.39	7.9	4.39	7.9	82.7	15.0

[a] NMR spectra (500 MHz) were recorded in D_2O at 298 K (DOH: δ = 4.768 ppm). [b] Retention time was measured by LC–MS; for conditions and chromatograms, see the Supporting Information. [c] Ratios of anomers were estimated from the LC–MS spectra on the basis of the assumption that in each case both anomers were ionized similarly. The ratio of the major isomer to the minor isomer of the same sequence is given.



Scheme 2. a) DMTST, $C_2H_4Cl_2/CH_3CN$ (1:1), −30→0°C (2.5°C h^{-1}); b) $[Cp_2Hf(OTf)_2]$, $C_2H_4Cl_2/CH_3CN$ (1:1), −15→0°C (1.25°C h^{-1}); c) NaOMe/MeOH/$C_2H_4Cl_2$; d) H_2, $Pd(OH)_2$/C, MeOH/EtOAc (1:1).

interfere with binding or catalysis. Interference by aglycons in α-linked Gal moieties with the surface of the protein may be responsible for the difference in the observed activity.

Having confirmed the eligibility of the orthogonal-glycosylation protocol in a library synthesis, we next investigated a combined method with solid-phase chemistry. Studies with potential orthogonal glycosyl donors attached to a solid support have been reported, but only single glycosylation reactions were carried out.[17–19] We conducted solid-phase reactions in which the glycosyl donor was linked to a bead through a succinyl ester (see **30**; Scheme 2).[24] Glycosylation with the acceptors **17** and **20**, which contain a secondary and a primary hydroxy group, respectively, proceeded smoothly to give the resin-bound disaccharides **31** and **32**, each of which consists of a mixture of anomers.[35] The excellent performance of the reactions was confirmed by the formation of disaccharides (determined by ESI MS) upon the treatment of a few beads with methanolic NaOMe (Figure 1 A).[36] The disaccharide fluoride **32** thus obtained was subjected to a further glycosylation reaction with **33**, which has a hydroxy group at C6 and a phenylthio group at C1, in the presence of $[Cp_2Hf(OTf)_2]$, to give **34** (Figure 1 B). Compound **34** underwent glycosylation with *n*-octanol readily with DMTST as a promoter. (For MS analysis of the trisaccharide, see Figure 1 C.) After hydrogenolysis, the anomeric mixture **36** was obtained in 33 % yield (five steps) by using a Sep-Pak column, which removed all accumulated by-products (Figure 1 D). The incorporation of a hydrophobic tag in the final step on the solid support is important, as then only the compounds with that tag are isolated. The yield of the glycosylation reaction in this solid-phase synthesis (glycosylation at the primary hydroxy group) was 72 %, which is comparable to that of the solution reaction (76 %).[37]

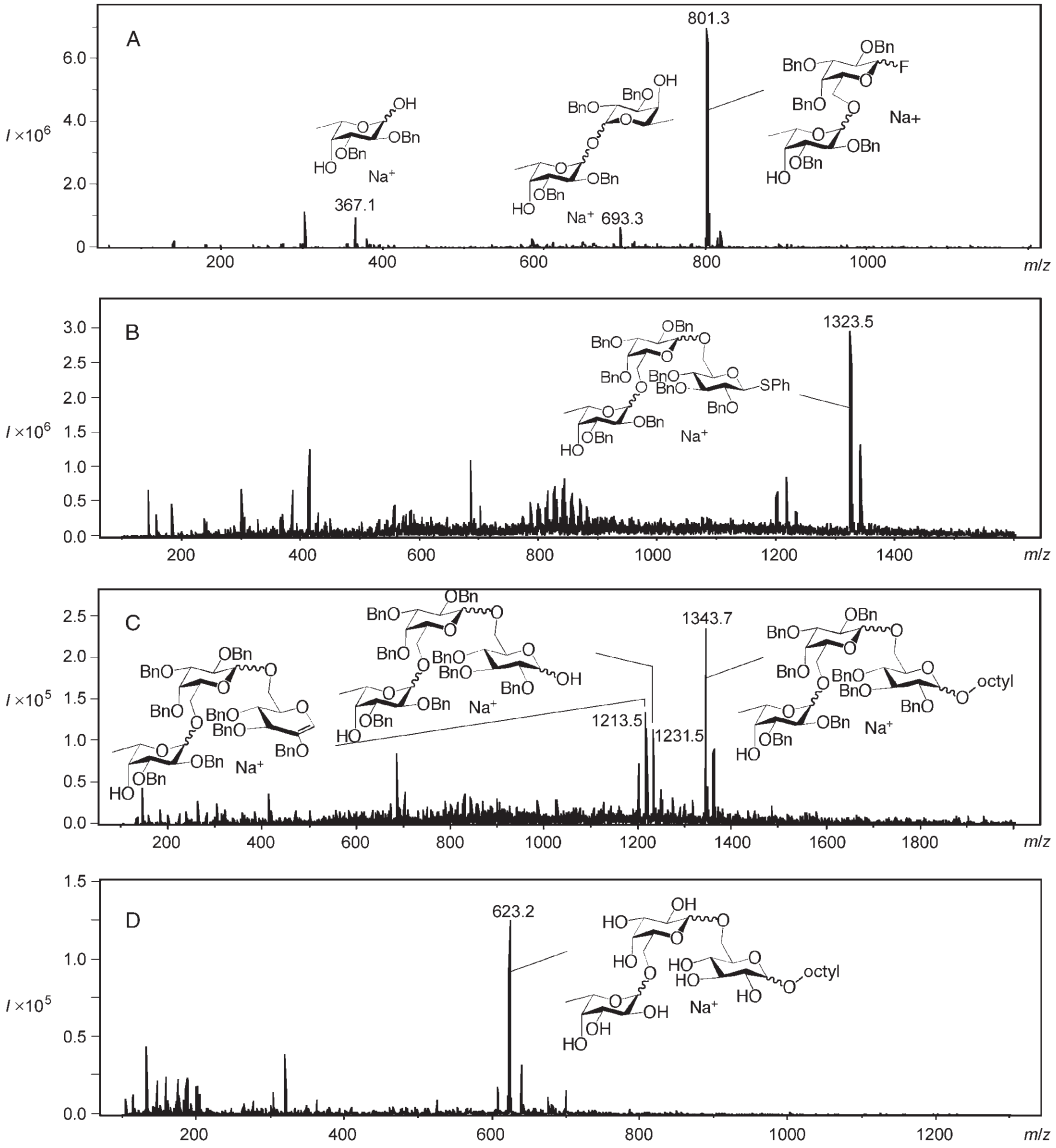


Figure 1. Mass spectra of glycosylation products. Spectra of the A) disaccharide, B) trisaccharide, C) octyl trisaccharide, and D) deprotected octyl trisaccharide **36** after purification by using a Sep-Pak C18 cartridge column.

In summary, we synthesized a library consisting of a complete set of structural isomers with Fuc-Gal sequences as octyl glycosides. Furthermore, in an assay of the library compounds, unexpected substrate specificity of fucosidase was discovered. The successful extension of an orthogonal-glycosylation protocol to solid-phase synthesis and the demonstration of a result comparable to that observed for the solution reaction suggest the potential usefulness of the present method in future combinatorial oligosaccharide synthesis.

Received: February 1, 2006
Published online: April 28, 2006

Keywords: chemoselectivity · compound libraries · glycosylation · oligosaccharides · solid-phase synthesis

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[35] Solid-phase reactions were carried out in the absence of molecular sieves to avoid problems such as clogging and/or resin breakdown.
[36] The yield of the solid-phase reaction was not determined at each step. Instead, the overall yield was calculated after deprotection and Sep-Pak purification. Experimental details and the structural assignments of all anomers will be reported elsewhere.
[37] The calculation was based on the assumption of a quantitative yield for the hydrogenolysis and 90% yield for the cleavage reaction. Calculations were based on the data obtained for O6-linked saccharides.

