Bioorganic & Medicinal Chemistry Letters 19 (2009) 6679–6681

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Use of ionically tagged glycosyl donors in the synthesis of oligosaccharide libraries

Eun Ju Kim [a,*], Gary R. Gray [b]

[a] Department of Science Education-Chemistry Major, Daegu University, Gyeongbuk 712-714, South Korea
[b] Department of Chemistry, University of Minnesota, Minneapolis, MN 55455, USA

ARTICLE INFO

Article history:
Received 25 June 2009
Revised 26 September 2009
Accepted 1 October 2009
Available online 4 October 2009

Keywords:
Oligosaccharide synthesis
A masked/caged ion-tag
Glycosyl donors
Reductive-cleavage method
Random glycosyl strategy

ABSTRACT

We have developed a new procedure based on the random glycosyl reaction of a partially benzoylated glycosyl acceptor with a glycosyl donor containing a 4,6-*O*-(4-methoxycarbonylbenzylidene) protecting group as a masked/caged ion-tag. Glycosylated products are ionically tagged by saponification of the methyl ester and the use of this anion-tag greatly simplifies the separation of the desired oligosaccharides from unreacted or excess glycosyl acceptors as well as from over-glycosylated oligosaccharides. In addition, the use of partially benzoylated acceptors greatly improves their solubility in dichloromethane increasing the yield of product formation and, also, of altering the distribution of positional isomers in favor of products derived by reaction of the donors at hydroxyl groups which otherwise would be considerably less reactive. Using this new approach in random glycosyl reactions, several oligosaccharide libraries were readily prepared in overall yields of 60–70% and the individual positional isomers present in the libraries were identified using the 'reductive-cleavage' method.

Oligosaccharides are thought to play essential roles in coding for the vast amount of information required in various biological recognition processes. For example, inside a cell, oligosaccharides promote the folding of proteins[1] and O-GlcNAc modification of nuclearcytoplasmic proteins initiates a dynamic signaling mechanism in concert with phosphorylation.[2] On the cell surface, they are the key surface molecules that communicate with neighboring molecules, constituting a dense information storage system.[3] This 'carbohydrate code' serves as recognition elements and thus also induces immunological responses to both bacterial and viral infections.[4,5] Abnormalities in oligosaccharide display are closely associated with several human diseases such as immune dysfunction[6] and cancer.[7] Therefore, gaining insight into how carbohydrates function as recognition signals and developing efficient ways to prevent undesirable interactions between carbohydrates and their protein signals are very important. Toward this end, the synthesis of oligosaccharides and the screening of their biological activities are often the first steps to be accomplished. However, the discrete synthesis and biological screening of individual oligosaccharides is extremely laborious. While combinatorial chemistry is well suited to the generation of a large number of chemicals (libraries) in a short time and has already seen successful application to the preparation of peptides,[8] oligonucleotides,[9] and small organic molecules,[10] the application of such technology to the synthesis of oligosaccharide libraries is much more challenging due to the inherent chemical complexity of carbohydrates.

Spurred by the recent explosion of interest in glycobiology, tremendous progress has been made in the synthesis of oligosaccharides through the development of new glycosyl donors[11] and advanced protecting group chemistry.[12] However, little work has been reported on the development of technology for the combinatorial synthesis of oligosaccharides. Kahne and co-workers used a protocol of splitting and mixing beads during the synthesis in combination with the use of chemical tagging at each combinatorial step.[13] By screening the library prepared in this combinatorial fashion against a flower lectin, they discovered new carbohydrate ligands with a higher affinity to the protein than the natural ligand.[13] On the other hand, a straightforward oligosaccharide library synthesis was reported by Kanie et al., which involves an orthogonal glycosylation method on a polymeric support.[14] These methods use the solid-phase technique which presents several limitations such as decreased glycosylation reaction rates compared to solution methods and incomplete coupling. Also, inconvenient monitoring of reaction progress and product identification in each step of multistep oligosaccharide synthesis would greatly reduce the efficiency of this technique because it is customary to cleave the products from the polymer to check the reaction of each step. Different from these solid support methods, Hindsgaul et al. developed a solution method called 'random glycosylation'.[15] In the random glycosylation approach, a fully protected glycosyl donor is reacted with an excess of an unprotected acceptor under

* Corresponding author. Tel.: +82 10 7127 4089; fax: +82 53 850 6989.
E-mail address: eunkim@daegu.ac.kr (E.J. Kim).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.10.002

conditions where all possible positional isomers are hopefully produced. This procedure suffers, however, from the low yield of product formation, due to limited solubility of the unprotected glycosyl acceptor, as well as the extensive purification required for product isolation. In particular, this approach requires removal of excess oligosaccharide acceptor as well as over-glycosylated oligosaccharides if a pure mixture of oligosaccharides of defined size is to be obtained.

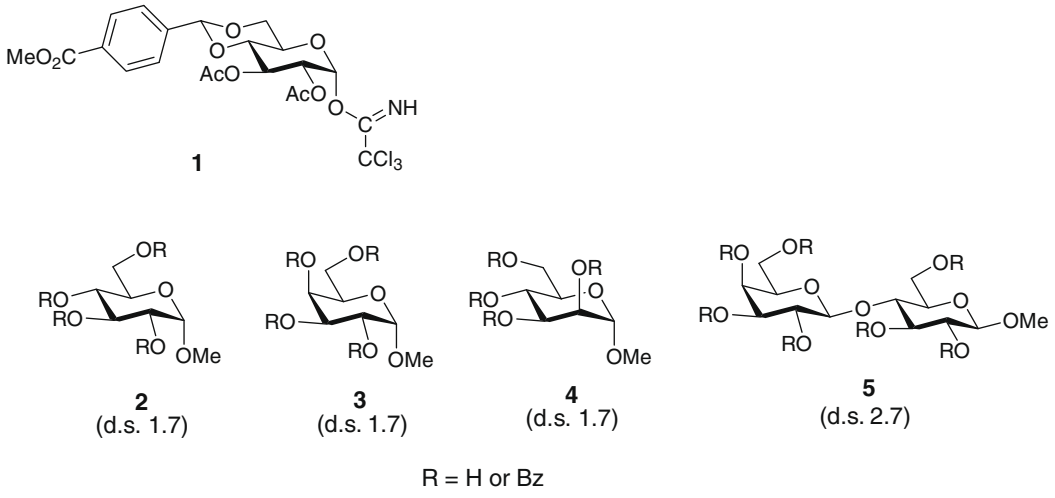
In order to overcome the difficulties inherent in the random glycosylation approach, we have developed a new procedure based on the reaction of a partially benzoylated glycosyl acceptor with a glycosyl donor containing a masked/caged ion-tag. Thus, a glycosyl donor (**1**) containing a 4,6-*O*-(4-methoxycarbonyl-benzylidene) protecting group was reacted with a slight excess (20%) of the partially and randomly benzoylated glycosyl acceptors **2–5** [degree of substitution (ds) 1.7 for **2–4** and 2.7 for **5**] in dichloromethane in the presence of boron trifluoride etherate. Upon completion, the reactions were neutralized and evaporated to dryness. After de-O-acylation and saponification of the methyl ester, the crude products were applied to a DEAE-Sephadex A-25 column. Elution with water to remove neutral material (unreacted glycosyl acceptor) followed by elution with a 0.0–0.15 M gradient of NH_4HCO_3 afforded a mixture of the negatively charged disaccharides (from **2** to **4**) and trisaccharides (from **5**) as a single peak as detected by the phenol–sulfuric acid assay.[16] After lyophilization to remove NH_4HCO_3 and debenzylidenation, the crude disaccharide and trisaccharide libraries were subjected to gel-permeation chromatography on a column of Bio-Gel P-2 in water to remove any monomers arising from the unreacted glycosyl donor. Fractions containing the respective disaccharides (from **2** to **4**) and trisaccharides (from **5**) were lyophilized to yield the pure oligosaccharide libraries in overall yields of 60–70%.

Table 1
Mole fractions of products formed by the reaction of glycosyl donor **1** with partially benzoylated acceptors **2–5**

Glycosyl	Positional isomer (mole fraction)							
Acceptor	2	3	4	6	2′	3′	4′	6′
2 (ds 1.7)	0.173	0.725	0.068	0.034	—	—	—	—
3 (ds 1.7)	0.591	0.219	0.014	0.176	—	—	—	–
4 (ds 1.7)	0.031	0.528	0.282	0.159	—	—	—	—
5 (ds 2.7)	0.336	0.014	—	0.051	0.171	0.040	0.268	0.120

tant mixtures of methylated and partially methylated 1,5-anhydro-D-glucitol, -D-mannitol, and -D-galactitol derivatives were acetylated and analyzed by gas–liquid chromatography combined with chemical ionization (NH_3) and electron ionization mass spectrometry and the peaks were identified by comparison of their mass spectra with those of authentic standards.[20] Integration of the peaks and correction for molar response by the effective carbon-response method[21] gave the mole fraction of each product in each of the libraries. From these values, the mole fractions of the individual positional oligosaccharide isomers in each library were determined (Table 1).

As is evident in Table 1, all possible positional isomers were formed for each of the glycosyl acceptors. As expected, however, the positional isomers were not formed in equimolar proportions. In particular, products arising by reaction of the glycosyl donor at the 6-positions were formed in lower proportions as a consequence of prior benzoylation of the more reactive primary hydroxyl groups of the acceptors. Although prior benzoylation alters the distribution of products, it has the advantage of rendering the glycosyl acceptor soluble in dichloromethane, thus decreasing the amount of acceptor required and increasing the glycosylation yield in comparison to



Glycosyl donor having a masked/caged ion-tag and partially benzoylated glycosyl acceptors

Analysis of the pure libraries by ^{1}H NMR spectroscopy revealed that the newly formed glycosidic linkages were β due to the neighboring group participation of the acetamido group at C2 as expected, but this analysis did not reveal the content of the libraries with regard to the positional isomers that were formed. However, analysis of the individual libraries by the reductive-cleavage method[17] readily established the mole fraction of each of the constituent positional isomers. In this method, the oligosaccharides were methylated fully[18] and all glycosidic linkages were reductively cleaved by treatment with triethylsilane and trimethylsilyl trifluoromethanesulfonate in dichloromethane.[19] The resulting reactions employing underivatized acceptors in DMF.[15] The use of dichloromethane as a solvent is also advantageous in comparison to DMF because of its ease of removal. Should greater proportions of products arising by glycosylation at the more reactive hydroxyl groups be desired, the ionically tagged glycosyl donor could always be reacted with an excess of the unprotected acceptor as described by Hindsgaul et al.[15]

In summary, the use of ionically masked/caged glycosyl donors in random glycosylation reactions greatly simplifies the separation of the desired oligosaccharides from unreacted or excess glycosyl acceptors as well as from oligosaccharides arising as a consequence

of reaction of the donors with the acceptors at two or more sites (over glycosylation). Furthermore, the use of partially benzoylated acceptors has the advantage of rendering them soluble in dichloromethane and, also, of altering the distribution of positional isomers in favor of products derived by reaction of the donors at hydroxyl groups which otherwise would be considerably less reactive. This novel strategy using glycosyl donors having a masked/caged ion-tag and partially protected glycosyl acceptors in random glycosyl reactions will greatly facilitate the generation of oligosaccharide libraries.

Acknowledgements

This work was supported by NIH Grant GM 34710.

Supplementary data

Supplementary data (figures, schemes, spectral data, and detailed experimental procedures) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.10.002.

References and notes

1. Helenius, A.; Aebi, M. *Science* **2001**, *291*, 2364.
2. For a review, see: Love, D. C.; Hanover, J. A. *Sci. STKE* **2005**, *312*, re13.
3. Gabius, H.-J.; Siebert, H.-C.; André, S.; Jiménez-Barbero, J.; Rüdiger, H. *ChemBioChem* **2004**, *5*, 740.
4. Cundell, D. R.; Tuomanen, E. *Microb. Pathog.* **1994**, *17*, 361.
5. (a) Varki, A. *Glycobiology* **1993**, *3*, 97; (b) Paulson, J. C. *Trends Biochem. Sci.* **1989**, *14*, 272; (c) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683.
6. Dube, D. H.; Bertozzi, C. R. *Nat. Rev. Drug Disc.* **2005**, *4*, 477.
7. Fuster, M. M.; Esko, J. D. *Nat. Rev. Cancer* **2005**, *5*, 526.
8. Gallop, M. A.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gordon, E. M. *J. Med. Chem.* **1994**, *37*, 1233.
9. Gordon, E. M.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gallop, M. A. *J. Med. Chem.* **1994**, *37*, 1385.
10. (a) Thompson, L. A.; Ellman, J. A. *Chem. Rev.* **1996**, *96*, 555; (b) Balkenhohl, F.; Bussche-Hunnefeld, C.; Lansky, A.; Zechel, C. *Angew. Chem., Int. Ed.* **1996**, *35*, 2288.
11. (a) Tsuchiya, T. *Adv. Carbohydr. Chem. Biochem.* **1990**, *48*, 91; (b) Matsumoto, T.; Katsuki, M.; Suzuki, M. *Tetrahedron Lett.* **1988**, *29*, 6935; (c) Takahashi, Y.; Ogawa, T. *Carbohydr. Res.* **1987**, *164*, 277; (d) Grundler, G.; Schmidt, R. R. *Carbohydr. Res.* **1985**, *135*, 203; (e) Marino-Albernas, J.-R.; Harris, S. L.; Verma, V.; Pinto, B. M. *Carbohydr. Res.* **1993**, *245*, 245; (f) Ding, Y.; Liu, Y. *Carbohydr. Res.* **1991**, *209*, 306; (g) Matsuzaki, Y.; Nunomura, S.; Ito, Y.; Sugimoto, M.; Nakahara, Y.; Ogawa, T. *Carbohydr. Res.* **1993**, *242*, C1; (h) Fügedi, P.; Garegg, P. J. *Carbohydr. Res.* **1986**, *149*, C9.
12. For a review, see: Schelhaas, M.; Waldmann, H. *Angew. Chem., Int. Ed.* **1996**, *35*, 2056.
13. Liang, R.; Yan, L.; Loebach, J.; Ge, M.; Uozumi, Y.; Sekanina, K.; Horan, N.; Gildersleeve, J.; Thompson, C.; Smith, A.; Biswas, K.; Still, W. C.; Kahne, D. *Science* **1996**, *274*, 1520.
14. Ako, T.; Daikoku, S.; Ohtsuka, I.; Kato, R.; Kanie, O. *Chem. Asian J.* **2006**, *1*, 798.
15. (a) Kanie, O.; Barresi, F.; Ding, Y.; Labbe, J.; Otter, A.; Forsberg, L. S.; Ernst, B.; Hindsgaul, O. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2720; (b) Ding, Y.; Kanie, O.; Labbe, J.; Palcic, M. M.; Ernst, B.; Hindsgaul, O. *Adv. Exp. Med. Biol.* **1995**, *376*, 261; (c) Ding, Y.; Labbe, J.; Kanie, O.; Hindsgaul, O. *Bioorg. Med. Chem.* **1996**, *4*, 683; (d) Hindsgaul, O. *Glycoimmunology* **1998**, *2*, 219.
16. Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. *Anal. Chem.* **1956**, *28*, 350.
17. (a) Rolf, D.; Gray, G. R. *J. Am. Chem. Soc.* **1982**, *104*, 3539; (b) Rolf, D.; Bennek, J. A.; Gray, G. R. *J. Carbohydr. Chem.* **1983**, *2*, 373; (c) Rolf, D.; Gray, G. R. *Carbohydr. Res.* **1984**, *131*, 17.
18. Ciucanu, I.; Kerek, F. *Carbohydr. Res.* **1984**, *131*, 209.
19. Rolf, D.; Bennek, J. A.; Gray, G. R. *Carbohydr. Res.* **1985**, *137*, 183.
20. (a) Elvebak, L. E., II; Gray, G. R. *Carbohydr. Res.* **1995**, *274*, 85; (b) Elvebak, L. E., II; Cha, H. J.; McNally, P.; Gray, G. R. *Carbohydr. Res.* **1995**, *274*, 71; (c) Elvebak, L. E., II; Abbott, C.; Wall, S.; Gray, G. R. *Carbohydr. Res.* **1995**, *269*, 1.
21. (a) Sweet, D. P.; Shapiro, R. H.; Albersheim, P. *Carbohydr. Res.* **1975**, *40*, 217; (b) Bowie, J. U.; Trescony, P. V.; Gray, G. R. *Carbohydr. Res.* **1984**, *125*, 301.