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From Glycals to Glycopeptides: A Convergent and Stereoselective Total Synthesis of a High Mannose N-Linked Glycopeptide**

Zhi-Guang Wang, XuFang Zhang, David Live, and Samuel J. Danishefsky*

Given the elaborate machinery required for the biosynthesis of glycoproteins in cells, it seems likely that such systems perform significant biological functions.[1, 2] Indeed, protein glycosylation has been implicated in mediating protein folding,[3] in protecting against proteolysis,[4] in cellular differentiation,[5] and in cell–cell communication.[6] Major breakthroughs in the detection, purification, sequencing, and spectroscopic analysis of glycans have enabled a growing appreciation of the role of glycobiology in vital life processes.[7] Chemical synthesis[8–10] can play an important role in our understanding of glycobiology by providing access to well-selected, homogeneous, but realistically complex, probe structures for elucidating the relationship of glycoarchitecture and function.[11, 12]

Broadly speaking, glycoproteins are of two major types. In one motif, the terminal galNAc hexose of the saccharide domain is joined to the polypeptide through an α-*O*-glycosidic linkage to the hydroxyl group of a serine (or threonine).[13] The target systems which prompted the research described herein are N-linked glycoproteins, wherein the two domains are joined through a β-N linkage of an asparagine group to a glcNAc unit at the reducing end of the oligosaccharide.[14]

Specifically, we focused on a target where the consensus core high mannose pentamer sequence (see below) would be joined to the peptide domain through a carboxyl group of an Asp side chain (**1**, Scheme 3). Our goals in reaching **1** by chemical synthesis included a concise and efficient assembly of the required oligosaccharide.[15] Clearly, global deprotection of diversely protected functionalities would eventually be required. To this set of specifications we added another, namely, that the fashioning of the asparagine linkage be conducted in a maximally convergent sense with high stereocontrol by joining a fully mature high mannose saccharide to a fully mature peptide. In this way we hoped to pave the way for

[*] Prof. S. J. Danishefsky, Dr. Z.-G. Wang, X. Zhang
Laboratory for Bioorganic Chemistry
Sloan-Kettering Institute for Cancer Research
1275 York Avenue, New York, N.Y. 10021 (USA)
Fax: (+1) 212-772-8691
E-mail: s-danishefsky@ski.mskcc.org

Dr. D. Live
Deptartment of Biochemistry
Molecular Biol. and Biophysics University of Minnesota Minneapolis, MN 55455 (USA)

Prof. S. J. Danishefsky
Department of Chemistry
Columbia University, Havemeyer Hall
New York, N.Y. 10027 (USA)
E-mail: dshefsky@chem.columbia.edu

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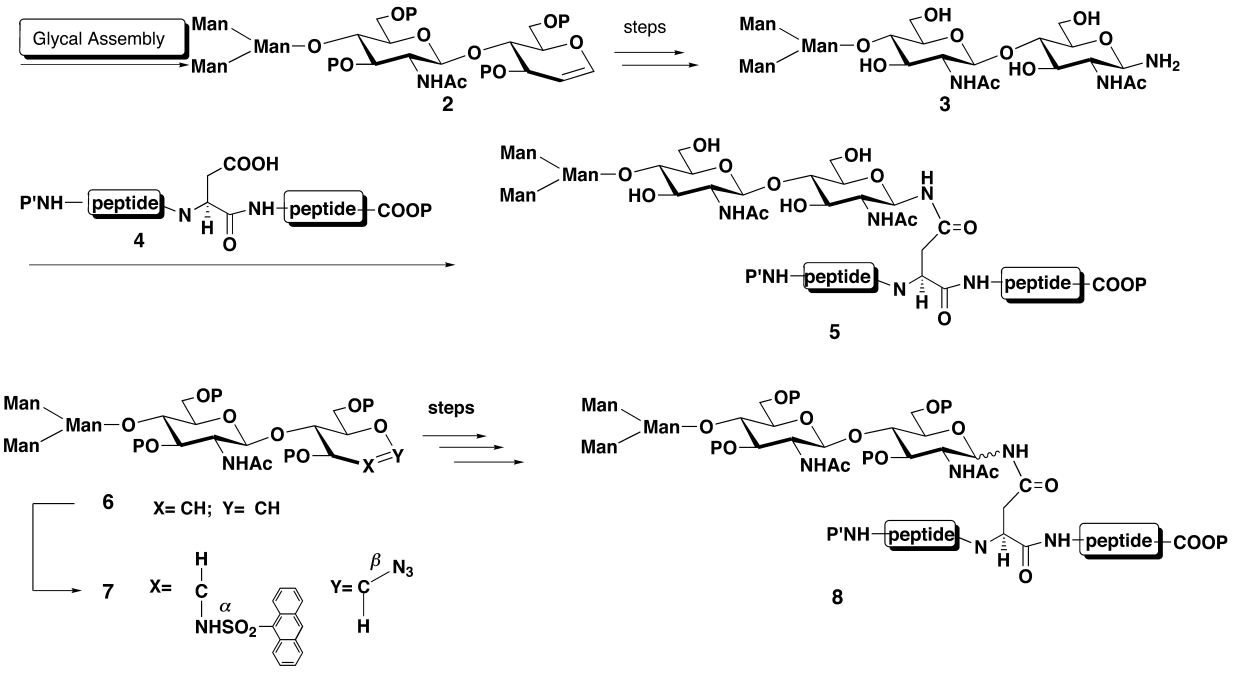
addition of fully synthetic high mannose core structures to preselected aspartate-presenting polypeptides.

Toward this quest, the progression shown in Scheme 1 presented itself. The pertinent high mannose core oligosaccharide would be synthesized as a terminal glycal, thereby taking advantage of the economies of glycal assembly.[16] The double bond would be transformed to the signature chitobiose subunit bearing the β-anomeric amine (**3**). The fully synthetically derived pentamer ensemble would be joined, in a maximally convergent acylation reaction, to the aspartate-presenting peptide, which contains an interior Asp residue and is equipped with a differentiated ω-carboxyl group (**4**). The two central challenges we faced were the conversion of **2** $\rightarrow$ **3** and the acylation of the latter with **4**, all with tight stereochemical control, en route to **5**.

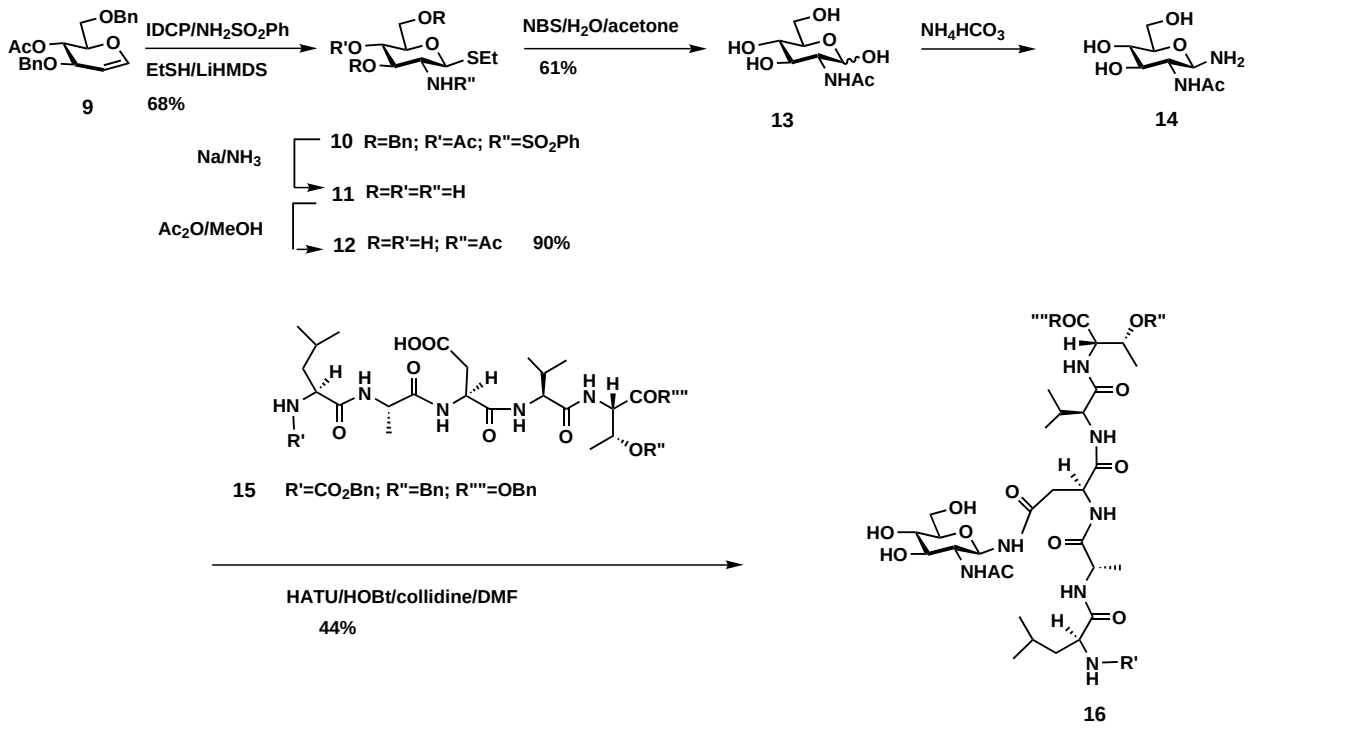
In a previously reported first generation protocol[17] a fully protected glycal was converted after a complex sequence into a terminal 1-β-azido glcNAc residue (**6** and **7**). However, following reduction of the azido linkage and acylation of the resulting amine with an Asp-containing pentapeptide, the product glycopeptide was obtained as an approximate 1:1 mixture of anomers. During the reduction/acylation sequence, the β anomeric stereochemistry of the azide had been badly compromised en route to the glycopeptide (**8**). A productive solution to the problem of convergence and high stereochemical maintenance in a total synthesis setting is described herein.

As a model for what had to be accomplished, we started with the simple differentiated glycal **9** (Scheme 2). Following implementation of a standard iodosulfonimidation/ethane thiolate rearrangement sequence,[18] **10** was in hand. Conversion of **10** $\rightarrow$ **11** was conducted as shown. The 2-α-amino group of the latter was selectively acetylated with acetic anhydride in methanol, containing no added base or acylation catalyst, to give **12**. A key step in the model series involved oxidative hydrolytic cleavage of the anomeric β-thioethyl group through the use of *N*-bromosuccinimide (NBS)[19] to produce the model free reducing sugar **13**. Treatment of **13** with ammonium bicarbonate afforded β-anomeric glycosylamine **14**.[20, 21] The latter condensed with Asp-containing peptide **15**, under the conditions indicated, to afford **16**. Remarkably, as was implicit in earlier work of Cohen-Anisfeld and Lansbury,[21, 22] the reaction is highly selective for producing the β-glycosylasparagine-linked glycopeptide. In fact, we did not detect any α-glycoside or aspartoylation through the α-carboxyl group.

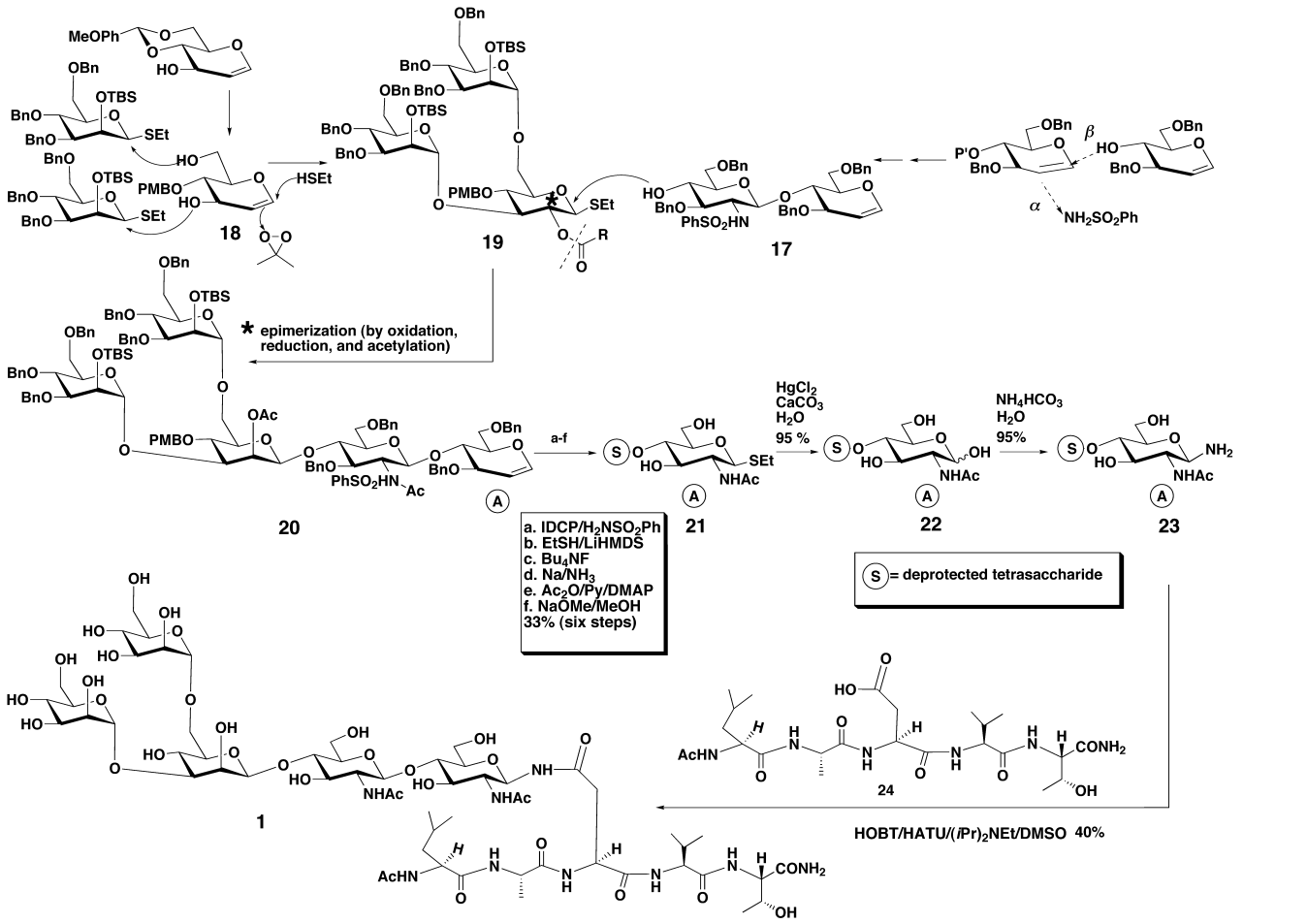
The issue to be faced now was whether the methodology used for the simple model could be transferred to a strategically protected high mannose glycal, itself obtained by total synthesis. The logic of the glycal assembly used here had already been charted, but the implementation was now modified and optimized. The "pre-chitobiose glycal" segment **17** was fashioned by the joining of glucal building blocks through azaglycosidation (Scheme 3).[18] A dimannosylated glucosyl donor (**19**) was derived from glycal **18**, itself obtained from the corresponding 4,6-*p*-methoxybenzylidene glycal derivative. Coupling **19** (R = phenyl) with **17** produced the expected β-glucosyl attachment smoothly and stereospecifically. Epimerization at C2[17] (see asterisk) was accomplished by an oxidation/reduction sequence, thereby leading to the protected high mannose glycal (**20**). In the first stage (steps a, b) of converting **20** into **23**, the terminal glycal was used to introduce the 1β-thioethyl (and 2-α-sulfonamido) functions. In step c the silyl protecting groups were removed and in step d both C2 α-amino groups of the sulfonamidochitobiose were exposed. In this case, as opposed to the monosaccharide in Scheme 1, we resorted to peracetylation (all hydroxyl and the two amino groups) for purification. Concurrent deacetylation of all of the ester linkages led to compound **21**. The anomeric thioethyl function suffered cleavage under



Scheme 1. The current and past synthetic strategy from glycal to glycopeptide; P, P′: protecting groups.



Scheme 2. Bn: benzyl; IDCP: bisdicollidine iodonium perchloride; LiHMDS = lithium hexamethyldisilazanide; HATU: *N*-[(dimethylamino)-1*H*-1,2,3-triazole[4,5-*b*]-pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate; HOBT: 1-hydroxy-1*H*-benzotriazole hydrate; collidine = 2,4,6-trimethylpyridine.



Scheme 3. Synthesis of the high mannose glycopeptide; Py: pyridine; DMAP: 4-dimethylaminopyridine.

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mediation by mercury(II) catalysis (anomers **22**).[23] Once again, aminolysis, following the precedents of Cohen-Anisfeld and Lansbury,[20, 21] led to the pure β-amino anomer **23**. The latter underwent acylation with peptide construct **24**,[24] as shown, to provide the desired homogeneous target **1** (40% after purification by HPLC). The structure assignment of **1** is fully consistent with mass spectral analysis[25] (calcd: m/z 1449 $[M^+]$; found: m/z 1472 $[M+Na^+]$).

Furthermore, the ^{1}H NMR spectrum of **1** measured at 800 MHz (Figure 1) is fully supportive of the stereochemical assignment of the five anomeric linkages, including the β-Asn configuration ($\delta = 5.0$, $J = 10$ Hz). The eight NH signals of secondary amides within the glycopeptide were also found and assigned (see inserts). A full assessment of the spectroscopically derived conformation of this highly organized glycopeptide will be published separately.

With proof of principle demonstrated, focus is already directed to new avenues. These include building longer peptide constructs so that the effects of glycosylation on conformation can be probed in detail along a peptide chain. Also underway are experiments where the oligosaccharides entering into the glycopolypeptides present determinants of established biological function. The concise, *totally synthetic* routes to homogeneous glycopeptides demonstrated here will prove to be valuable in furthering progress in glycobiology.

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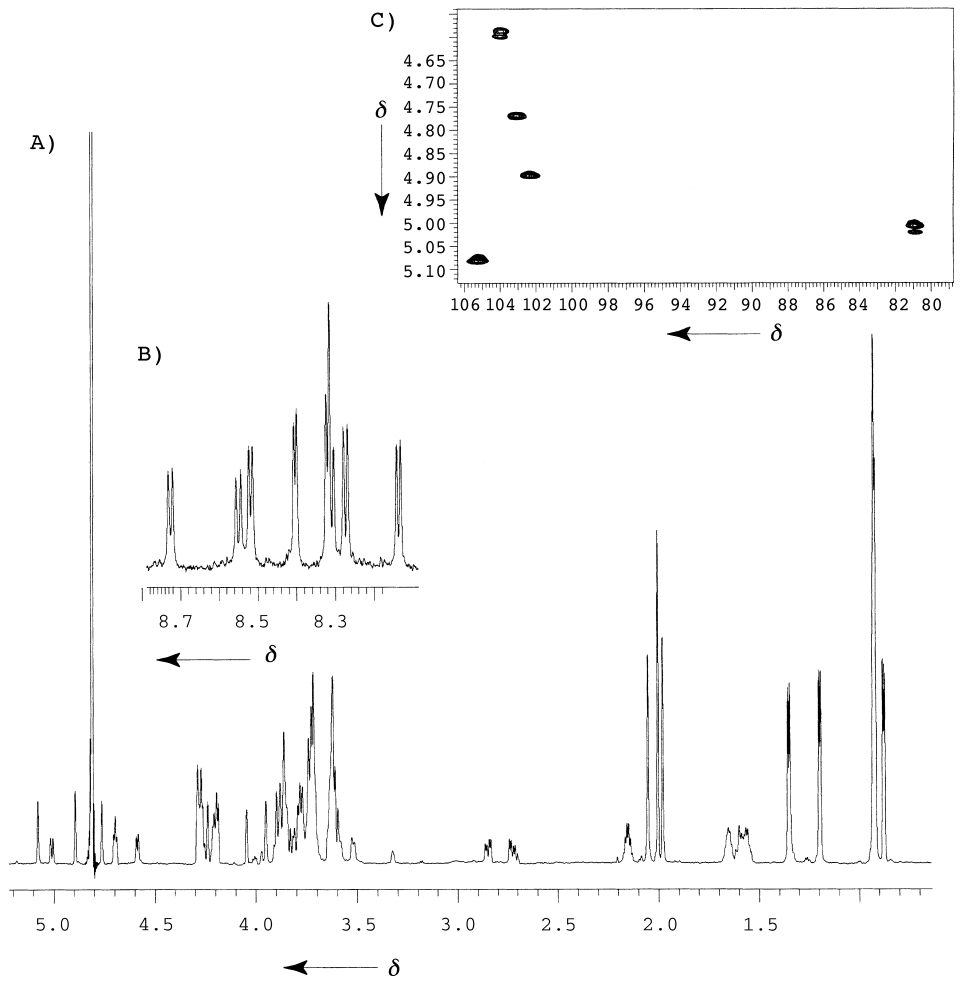

Figure 1. A) ^{1}H NMR (800 MHz) spectrum of **1** in D_2O at 20 °C and pH 3.7 (phosphate buffer). B) Section of the ^{1}H spectrum of **1** in H_2O at 5 °C and pH 3.5 (phosphate buffer) showing the secondary NH signals of amides from the peptide backbone, side chain, and GlcNAc sites. The protons of the terminal carboxamide (not shown) are observed slightly upfield of this region. C) The anomeric region of the ^{1}H-^{13}C HMQC spectrum at 800 MHz of **1** in D_2O at 20 °C and pH 3.7 (phosphate buffer). The GlcNAc anomeric sites at $\delta = 4.58$ and 5.01(^{1}H) are clearly distinguished by their larger ^{1}H couplings.

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[22] Since our yield is far from quantitative, we cannot rule out the possibility of aspartimide formation, or even products arising from N-acylation by the α-carboxy group of the Asn residue via such an intermediate. This same issue, which can be raised with respect to the previous cases,[21] requires further detailed study.
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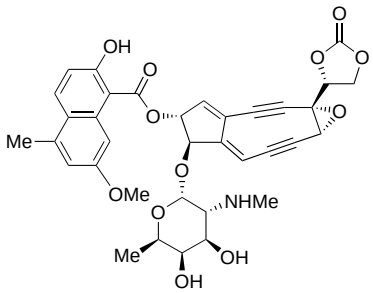
Chemical Synthesis and DNA Photocleavage of the Intercalator–Carbohydrate Hybrid Moiety of the Neocarzinostatin Chromophore**

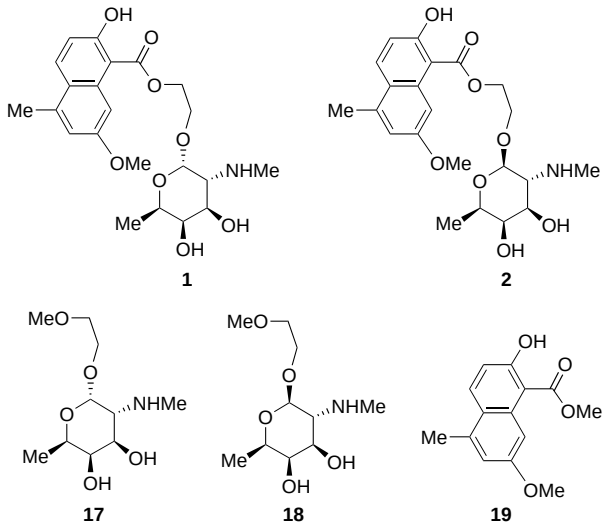
Kazunobu Toshima,* Shigeki Takai, Yutaka Maeda, Ryusuke Takano, and Shuichi Matsumura

Dedicated to Professor Kuniaki Tatsuta on the occasion of his 60th birthday

The development of photochemical DNA-cleaving agents, which effectively cleave DNA by irradiation at a specific wavelength under mild conditions and without any additives such as metals and reducing agents, is very interesting from a chemical and biological standpoint and offers considerable potential in medicine.[1] Indeed, photodynamic therapy using a photosensitizing drug has recently emerged as a promising modality against cancer and allied diseases.[2] Sugiura et al.[3] first demonstrated the light-induced DNA cleavage by the antitumor enediyne antibiotic neocarzinostatin[4] and Hirama et al.[5] reported the photoinduced cycloaromatization of the neocarzinostatin chromophore (Scheme 1), which is responsible for the DNA-cleavage activity of neocarzinostatin. In this context, we anticipated that if the intercalator–carbohydrate hybrid moiety of the neocarzinostatin chromophore, without its enediyne moiety, interacts with DNA and if the C=O bond in the hybrid generates a photoexcited $^3(n\text{-}\pi^*)$ radical-like[5] state by photoirradiation, then the intercalator–carbohydrate hybrid moiety of the enediyne-free neocarzinostatin chromophore could be capable of DNA cleavage. Herein, we report the chemical synthesis and DNA-photocleavage properties of the intercalator–carbohydrate hybrids[6, 7] **1** and **2**, which correspond to the intercalator and the carbohydrate moieties of the enediyne antibiotic, neocarzinostatin (Scheme 2).



Scheme 1. Neocarzinostatin chromophore.



Scheme 2. The intercalator–carbohydrate hybrids and their components.

To confirm our hypothesis, we designed and synthesized **1** and **2**, in which the aromatic and sugar moiety of the neocarzinostatin chromophore were linked by only an ethylene glycol unit to each other. Compounds **1** and **2** are the anomers of each other. Their synthesis began with the

[*] Prof. Dr. K. Toshima, S. Takai, Y. Maeda, R. Takano, Prof. Dr. S. Matsumura
Department of Applied Chemistry
Faculty of Science and Technology
Keio University
3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223-8522 (Japan)
Fax: (+81) 45-566-1576
E-mail: toshima@applc.keio.ac.jp

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