[9] We are also investigating a different approach to the total synthesis of **1** based on an intramolecular [4+2] cycloaddition: see a) Y. Kita, R. Okunaka, T. Honda, M. Shindo, O. Tamura, *Tetrahedron Lett*. **1989**, *30*, 3995 – 3998; b) Y. Kita, R. Okunaka, T. Honda, M. Kondo, O. Tamura, Y. Tamura, *Chem. Pharm. Bull.* **1991**, *39*, 2106 – 2114; c) S. Akai, K. Iio, Y. Takeda, H. Ueno, Y. Kita, *Synlett* **1997**, 310 – 312; d) Y. Kita, S. Akai, H. Fujioka, *Yuki Gosei Kagaku Kyokaishi* **1998**, *56*, 963 – 974. A similar intramolecular strategy was reported separately: see e) M. Toyota, S. Terashima, *Terahedron Lett.* **1989**, *30*, 829 – 832.

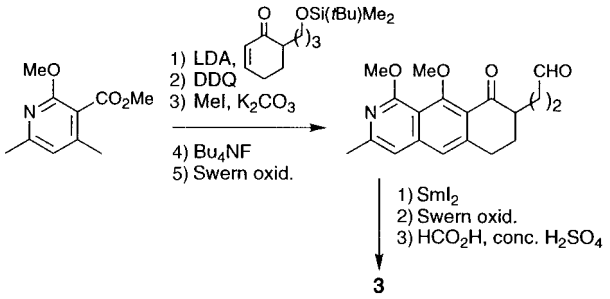
[10] For a study on construction of optically active quaternary carbon in connection with **1**: see W. Trypke, A. Steigel, M. Braun, *Synlett* **1992**, 827 – 829.

[11] a) Y. Tamura, M. Sasho, K. Nakagawa, T. Tsugoshi, Y. Kita, *J. Org. Chem*. **1984**, *49*, 473 – 478; b) Y. Tamura, F. Fukata, M. Sasho. T. Tsugoshi, Y. Kita, *J. Org. Chem.* **1985**, *50*, 2273 – 2277; c) Y. Kita, K. Iio, A. Okajima, Y. Takeda, K. Kawaguchi, B. A. Whelan, S. Akai, *Synlett* **1998**, 292 – 294.

[12] Application of this method to the total syntheses of *peri*-hydroxy polyaromatic compounds: see reviews, a) Y. Tamura, Y. Kita, *Yuki Gosei Kagaku Kyokaishi* **1988**, *46*, 205 – 217 [*Chem. Abstr.* **1988**, *109*, 129465d]; b) Y. Kita, Y. Takeda, *Kagaku to Kogyo (Osaka)* **1997**, *71*, 298 – 309 [*Chem. Abstr*. **1997**, *127*, 190540n]; c) M. Kirihara, Y. Kita, *Heterocycles* **1997**, *46*, 705 – 726. See also other examples done by other groups, d) F. Matsuda, M. Kawasaki, M. Ohsaki, K. Yamada, S. Terashima, *Tetrahedron* **1988**, *44*, 5745 – 5759; e) J.-F. Lavallée, R. Rej, M. Courchesne, D. Nguyen, G. Attardo, *Tetrahedron Lett*. **1993**, *34*, 3519 – 3522; f) T. Matsumoto, H. Yamaguchi, K. Suzuki, *Synlett* **1996**, 433 – 434; g) M. D. Shair, T. Y. Yoon, K. K. Mosny, T. C. Chou, S. J. Danishefsky, *J. Am. Chem. Soc.* **1996**, *118*, 9509 – 9525.

[13] Y. Kita, S. Kitagaki, R. Imai, S. Okamoto, S. Mihara, Y. Yoshida, S. Akai, H. Fujioka, *Tetrahedron Lett.* **1996**, *37*, 1817 – 1820. See also, Y. Kita, S. Kitagaki, Y. Yoshida, S. Mihara, D.-F. Fang, M. Kondo, S. Okamoto, R. Imai, S. Akai, H. Fujioka, *J. Org. Chem.* **1997**, *62*, 4991 – 4997; Y. Kita, S. Kitagaki, Y. Yoshida, S. Mihara, D.-F. Fang, H. Fujioka, *Tetrahedron Lett*. **1997**, *38*, 1061 – 1064.

[14] The enone **3** was obtained from methyl 2-methoxy-4,6-dimethylpyridine-3-carboxylate as per Scheme 5. The details will be presented in the forthcoming full paper.



Scheme 5. Synthesis of **3**.

[15] E. J. Corey, R. K. Bakshi, S. Shibata, C.-P. Chen, V. K. Singh, *J. Am. Chem. Soc.* **1987**, *109*, 7925 – 7926.

[16] Optical purities of the intermediates (**5**, **6**, and **9**) were determined by HPLC using Daicel CHIRALCEL OD (*n*-hexane-*i*PrOH), and those of **2**, **7**, **8**, (*R*)- and (*S*)-**14**, and (*S*)-**15** by Daicel CHIRALPAK AD (*n*-hexane-*i*PrOH).

[17] Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC-102892 (for (−)-**2**), CCDC-102893 (for (+)-**7**), and CCDC-102894 {for methyl 2-methoxy-2-[3,4,6-trimethoxy-2-(methoxycarbonyl)phenyl]acetate, a precursor of **13**}. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

[18] We have recently elucidated that the dienophiles having sulfinyl group [X = S(O)Ph] are much more reactive for the anionic [4 + 2] cycloaddition to homophthalic anhydrides than the well known halogen-substituted ones.[11c]

[19] J. M. Blatchly, J. F. W. McOmie, J. B. Searle, *J. Chem. Soc. C* **1969**, 1350 – 1353.

[20] Y. Kita, S. Akai, N. Ajimura, M. Yoshigi, T. Tsugoshi, H. Yasuda, Y. Tamura, *J. Org. Chem*. **1986**, *51*, 4150 – 4158.

[21] Y. Kita, H. Ueno, S. Kitagaki, K. Kobayashi, K. Iio, S. Akai, *J. Chem. Soc. Chem. Commun.* **1994**, 701 – 702.

[22] Extensive research towards complete isomerization of the (*E*,*Z*)-**16** to its (*E*,*E*)-isomer by using a catalytic amount of I_2 in the dark under various conditions (in $CDCl_3$, $CHCl_3$, or CH_2Cl_2 at room temperature for two days to two weeks) as well as direct demethylation – isomerization by the combined use of I_2 and BBr_3 did not lead to the complete isomerization and, in some cases, caused gradual decomposition. Since **16** is sensitive to visible light leading to decomposition and diene-isomerization, the subsequent steps after chain elongation, that is, demethylation, autooxidation, and HPLC separation, were done immediately. The purified sample (**1**) readily isomerizes in organic solvents containing strong acids such as CF_3COOH.

Regioselective Lactonization of α-(2 → 8)-Trisialic Acid**

Mou-Chi Cheng, Chun-Hung Lin,* Kay-Hooi Khoo, and Shih-Hsiung Wu*

The polymer of α-(2,8)-linked *N*-acetylneuraminic acids (poly(2,8-NeuAc)) is mainly distributed in mammalian cells and bacteria, and associated with many different biological functions.[1] It has been reported that δ-lactonization, the condensation of the carboxyl group at C-2 of one residue with the hydroxyl group at C-9 of an adjacent residue, is observed in α-2,8-linked polysialic acids at low pH.[2] Likewise, in gangliosides (glycosphingolipids containing one to three sialic acid moieties), δ-lactone is also formed under acidic conditions.[3] The δ-lactones of gangliosides have been suggested to be the true immunogens in the preparation of anti-ganglioside antibodies.[4] Since polysialic acid is a sugar polymer with highly negative charges, lactonization, which reduces the number of carboxylate groups, would influence the charge density. As a consequence, it was proposed that lactone formation may represent an on/off signal of a physiological function.[5]

Here we report the regioselective lactonization of the α-2,8-linked trisialic acid. There are two lactonized sites in the sialic acid trimer, one at the reducing end and the other at the

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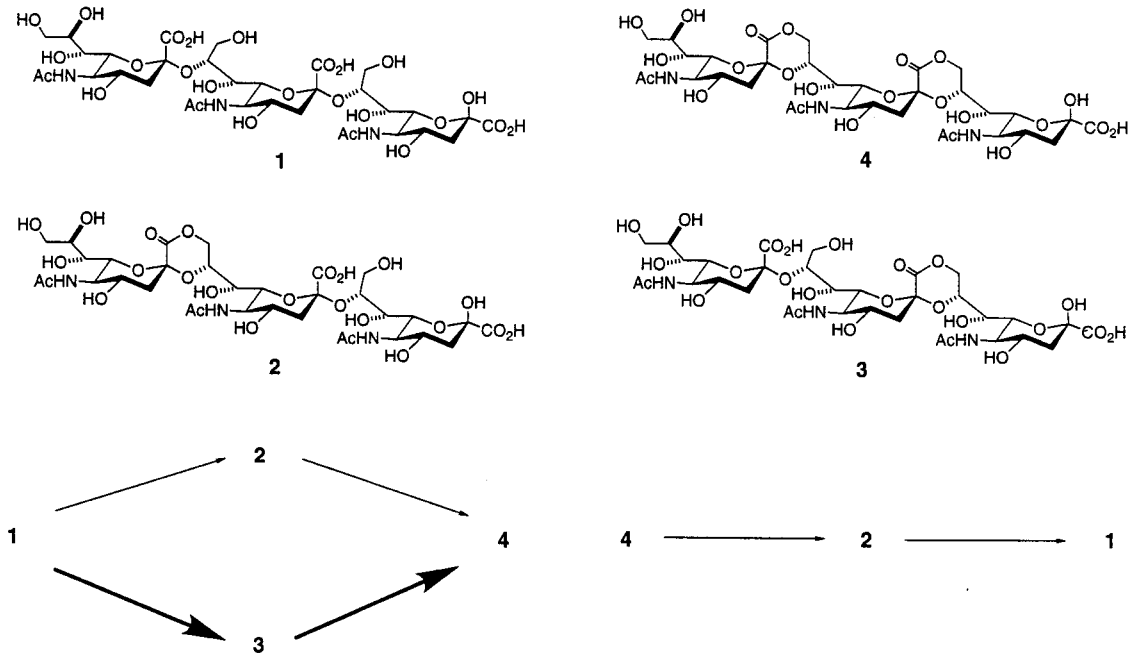

Scheme 1. Structures of α-2,8-linked sialic acid trimer **1**, 1-monolactone trimer **2**, 2-monolactone trimer **3**, and dilactone trimer **4** as well as the reaction pathway of lactonization (bottom left) and hydrolysis (bottom right).

nonreducing end (Scheme 1). Two different conditions were found under which each of the monolactones was obtained with high regioselectivity. This is the first report that oligosialic acid can undergo regioselective lactonization and delactonization.

The trimer was dissolved in glacial acetic acid at room temperature, and the progress of the lactonization was followed by capillary electrophoresis (CE). As shown in Figure 1, there were four major peaks in the CE spectra corresponding to two monolactones, one dilactone, and the unchanged trimer. Peak A should correspond to the dilactone **4** since it has the lowest charge density. Peak D was identified as belonging to the unchanged trimer **1** by co-injection with an authentic sample. The remaining peaks B and C were assigned to the two monolactonized trimers, which could be identified and distinguished by enzymatic hydrolysis with neuraminidase. Since this enzyme is an exo-glycosidase, it recognizes and releases the sialic acid molecule at the nonreducing terminal, and does not attack the lactonized sialic acid. Peak C, which gradually decreased in intensity during the course of the neuraminidase hydrolysis, is due to the 2-monolactone **3**; the unchanged peak B was assigned to 1-monolactone **2**. Figure 2 shows the time course of enzymatic hydrolysis of the lactonized trimers. The intensities of peaks A and B did not change in the presence of neuraminidase since **2** and **4**, with a lactone ring at the nonreducing end, are resistant to enzymatic hydrolysis. 2-Monolactone **3** (peak C) was hydrolyzed enzymatically into the lactonized dimer (peak a) and sialic acid (peak b), whereas trimer **1** (peak D) was cleaved into the dimer (peak c) and sialic acid. According to Figure 1, lactonization of **1** preferentially occurs first at the reducing terminal under the acidic conditions, so that 2-monolactone **3** is the major product. The mass spectrometry analysis also supported our conclusion that monolactonized

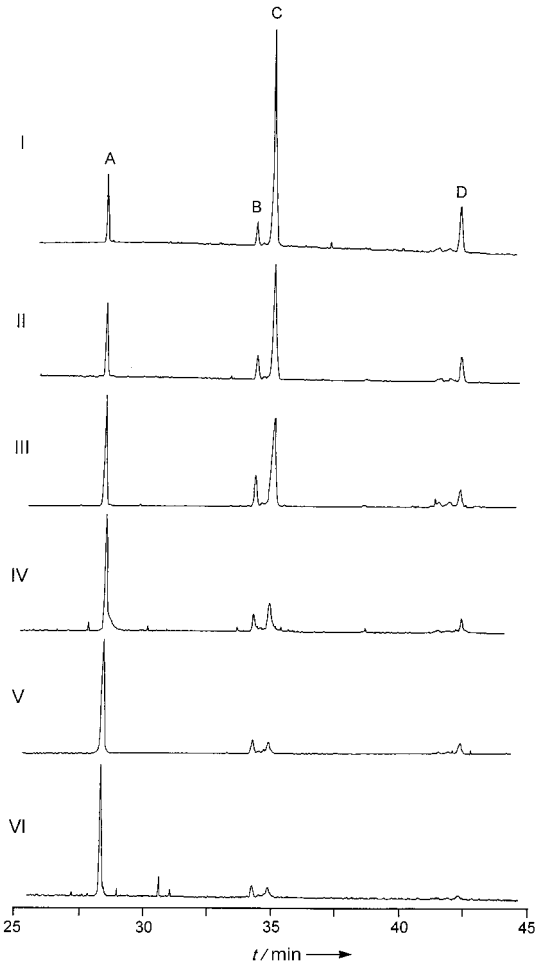

Figure 1. CE spectra of the lactonization of **1** in glacial acetic acid after different reaction times (I: 10 min; II: 20 min; III: 30 min; IV: 1 h; V: 4 h; VI: 8 h).

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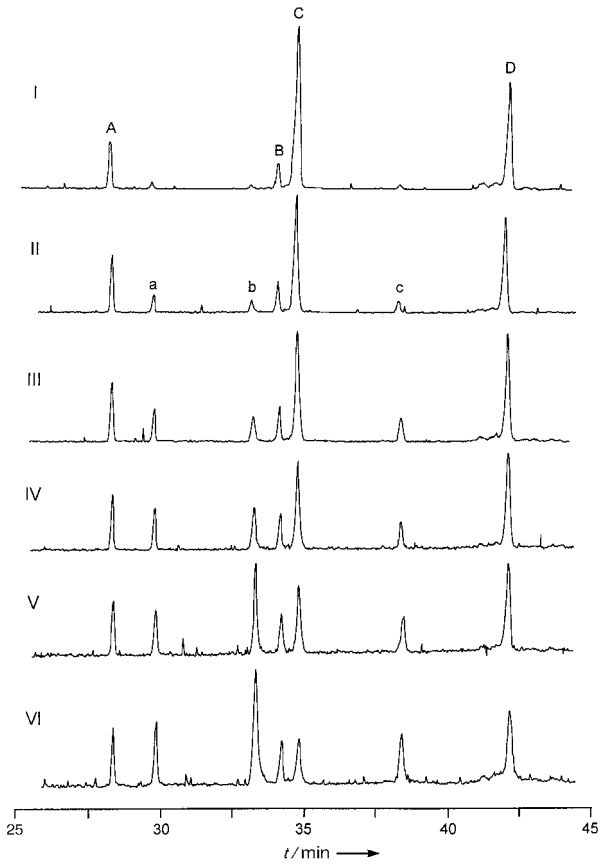

Figure 2. The product mixture obtained from the lactonization of **1** in glacial acetic acid for 10 min was hydrolyzed by neuraminidase and analyzed by CE after different times (I: 0 h; II: 1 h; III: 2 h; IV: 4 h; V: 8 h; VI: 20 h). Peaks A – D were assigned to **4**, **2**, **3**, and **1**, respectively. Peaks a – c are due to lactone dimer, sialic acid monomer, and sialic acid dimer, respectively.

trimers are the major products together with the dilactone and non-lactonized trimers upon treatment with glacial acetic acid for 10 min (Figure 3).

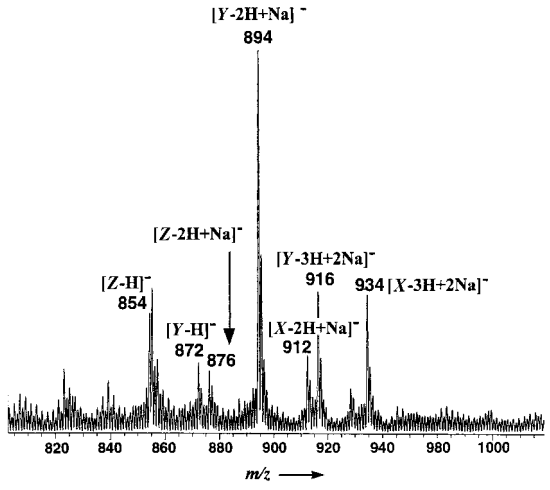

Figure 3. Negative-mode FAB mass spectrum of the sample obtained from the lactonization of α-2,8-linked sialic acid trimer in glacial acetic acid for 10 min. The major signals were assigned and identified as belonging to the molecular ions of the lactonized products and unchanged starting material. *X*: molecular ion of **1** ($C_{33}H_{53}O_{25}N_3$); *Y*: molecular ion of **2** and **3** ($C_{33}H_{51}O_{24}N_3$); *Z*: molecular ion of **4** ($C_{33}H_{49}O_{23}N_3$).

Compound **4** was chemically hydrolyzed in an aqueous solution of 0.1N $(NH_4)_2CO_3$. The CE analysis indicated that **4** was first hydrolyzed to give **2** exclusively and then further hydrolyzed to generate **1** (Figure 4). A co-injection of samples

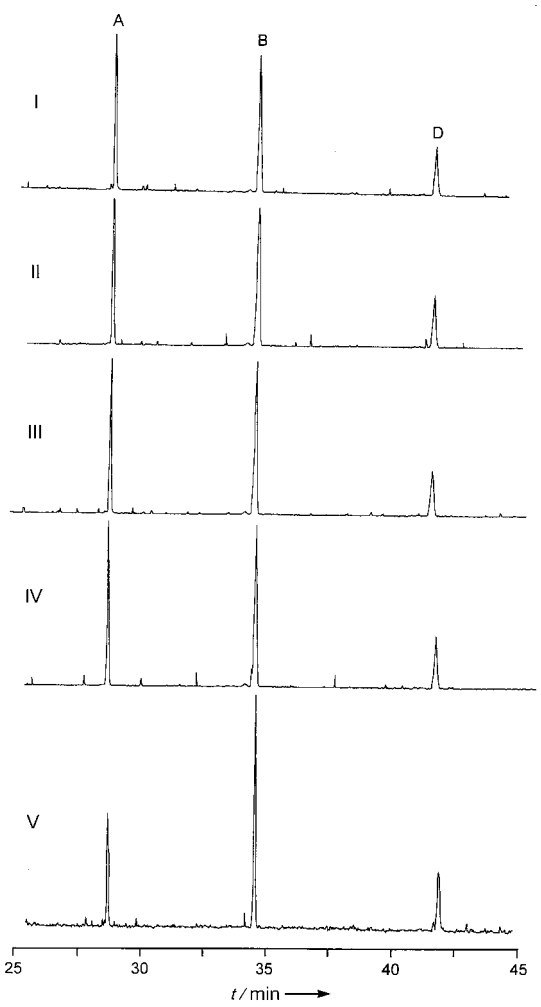

Figure 4. CE spectra of the hydrolysis of **4** under basic conditions after different times (I: 5 min; II: 10 min; III: 20 min; IV: 40 min; V: 80 min).

from both studies (esterification and hydrolysis) verified that peak B in Figure 4 is indeed due to the 1-monolactone **2** and different from the 2-monolactone **3** preferably generated by acidic lactonization (Figure 1).[6] The assignment of the three peaks in Figure 4 was corroborated by FAB-MS analysis, which afforded the expected molecular ions.

The preferential generation of **3** instead of **2** under the acidic conditions and the exclusive formation of **2** in the alkaline hydrolysis of dilactone trimer **4** is explained by structual differences in each component of the sialic acid trimer. The glycerol side chain at the nonreducing terminal is more flexible. It can interact with the carboxyl group at C-2, so that the formation of **2** is more difficult under acidic conditions. Likewise, similar interaction with the lactone ring at the nonreducing end may prevent its breakdown. The interaction of the sugar side chain with the functional group at the anomeric position has been suggested for a KDO-type molecule (KDO = 2-keto-3-deoxyoctulosonate)[7] that is

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closely related to sialic acid. On the other hand, such an interaction is not possible for the side chains of the other two sugar units since the rigidity is created by the glycosidic linkage fixed at C-8. Other possible explanations cannot be simply ruled out. For example, the different pK_a values of these carboxyl groups[8] may result in such regioselectivity. However, our speculation waits for the isolation and purification of both monolactone trimers for further NMR studies;[6] computer modeling is currently in progress.

In conclusion, two different methods are presented to obtain the two possible monolactones of an α-2,8-linked trisialic acid with regioselectivity. The neuraminidase hydrolysis demonstrates a novel way to distinguish both regioisomers from each other. The methods developed here can be further extended and applied to prepare other lactonized oligomers for the investigation of their unknown biological functions.

Experimental Section

Reagents: *N*-Acetylneuraminic acid trimer ([→8Neu5Acα2→]$_3$) was obtained from NGK Biochemical Ltd. (Handa, Japan) with the help of Prof. Yasuo Inoue. Neuraminidase from *Anthrobacter ureafaciens* was purchased from Sigma (St. Louis, USA). All other reagents for reactions and high-performance capillary electrophoresis (HPCE) were of the highest grade commercially available.

Lactonization of the α-2,8-linked tri-*N*-acetylneuraminic acid: Free trimers of [α2→8] *N*-acetylneuraminic acid (25 μg) were incubated in glacial acetic acid (1 mL) at room temperature. The reaction mixtures were frozen with liquid nitrogen and then dried immediately by SpeedVac (Savant, USA) to remove acetic acid. Dried samples were dissolved in doubly distilled water, and an aliquot (5 μL) of the mixture was analyzed by HPCE.

Preparation of the dilactone **4**: free trimers of [α2→8] *N*-acetylneuraminic acid (25 μg) were left in glacial acetic acid (1 mL) at room temperature for 8 h, frozen with liquid nitrogen, and then dried immediately by SpeedVac (Savant, USA) to remove acetic acid.

Hydrolysis of **4**: A sample of **4** (50 μg) was dissolved in 0.1N $(NH_4)_2CO_3$ (500 μL) at 37 °C. After 20, 40, and 80 min an aliquot was removed, frozen with liquid nitrogen, and then dried by SpeedVac (Savant, USA). Dried samples were dissolved in doubly distilled water, and an aliquot (5 μL) of the mixture was analyzed by HPCE.

Chromatographic analysis: Capillary electrophoreses (CE) were performed on a Beckman capillary electrophoresis system (P/ACE 2100) with a fused silica capillary (118 cm×75 μm (inner diameter)) at 20 kV and 25 °C. Phosphate buffer (50 mM, pH 8.0) was used as the running buffer. The UV absorption at 200 nm was monitored. Samples were injected into the capillary under a high pressure of nitrogen (1.3 bar) for 3 s. The capillary was regenerated by washing with doubly distilled water for 3 min and then 0.1N NaOH for 5 min.

Neuraminidase hydrolysis: Partially lactonized samples (10 μg) in 100 mM ammonium acetate buffer (pH 5) were digested with neuraminidase (1 mU) from *Anthrobacter ureafaciens* in 20-μL CE vials at room temperature. The progress of hydrolysis was monitored by HPCE at regular time intervals.

Fast atom bombardment (FAB) mass spectrometry: Negative-mode FAB mass spectra of the samples were obtained on an Autospec OA-TOF mass spectrometer (Micromass, UK) fitted with a cesium ion gun operated at 26 kV. Samples were dissolved in Milli Q water for loading on to the probe tip coated with monothioglycerol as matrix.

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[1] a) F. A. Troy II, *Glycobiology* **1992**, *2*, 5 – 23; b) U. Rutishauser, A. Acheson, A. K. Hall, D. M. Mann, J. Sunshine, *Science* **1988**, *240*, 53 – 57; c) K. Kitajima, S. Inoue, Y. Inoue, F. A. Troy II, *J. Biol. Chem.* **1988**. *263*, 18269 – 18276; d) C. Zuber, P. M. Lackie, W. A. Caterall, J. Roth, *J. Biol. Chem.* **1992**, *267*, 9965 – 9971; e) S. Inoue, M. Iwasaki, *Biochem. Biophys. Res. Commun.* **1978,** *83*, 1018 – 1023; f) J. B. Robbin, G. H. McCracken, Jr., E. C. Gotschlich, F. Ørskov, I. Ørskov, L. A. Hanson, *N. Engl. J. Med*. **1974,** *290*, 1216 – 1220; g) M. S. Schiffer, E. Oliverira, M. P. Glode, G . H. McCracken, Jr., L. M. Sarff, J. B. Robbin, *Pediatr. Res.* **1976,** *10*, 82 – 87.

[2] a) M. R. Lifely, A. S. Gilbert, C. Moreno, *Carbohydr. Res.* **1981,** *94*, 193 – 203. b) M. R. Lifely, A. S. Gilbert, C. Moreno, *Carbohydr. Res.* **1984,** *134*, 229 – 243.

[3] a) L. Riboni, S. Sonnino, D. Acquotti, A. Malesci, R. Ghidoni, H. Egge, S. Mingrino, G. Tettamanti, *J. Biol. Chem.* **1986**, *261*, 8514 – 8519; b) G. A. Nores, T. Dohi, M. Taniguchi, S. Hakomori, *J. Immunol.* **1989**, *139*, 3171 – 3176; c) R. Bassi, L. Riboni, S. Sonnino, *Carbohydr. Res.* **1989**, *193*, 141 – 146.

[4] S. Ando, R. K. Yu, J. N. Scarsdale, S. Kusunoki, J. H. Prestegard, *J. Biol. Chem.* **1989,** *264*, 3478 – 3483.

[5] a) R. K. Yu, T. A. W. Koerner, S. Ando, H. C. Yohe, J. H. Prestegard, *J. Biochem.* **1985**, *98*, 1367 – 1373; b) B. Maggio, T. Agria, R. K. Yu, *Biochemistry* **1990,** *29*, 8729 – 8734.

[6] Based on the results of 2D NMR experiments, the partial assignment of compound **2** is given in the following. The detailed and complete NMR characterizations are currently in progress and will be published in due course. ^{1}H NMR (500 MHz, HOD): $\delta = 1.694$ (1H, t, $J_{3a\text{-}3e} = J_{3e\text{-}4} = 12$ Hz, H-3a[II]), 1.740 (1H, t, $J_{3a\text{-}3e} = J_{3e\text{-}4} = 12$ Hz, H-3a[III]), 1.830 (1H, t, $J_{3a\text{-}3e} = J_{3e\text{-}4} = 13$ Hz, H-3a[I]), 2.065 (6H, s, 2acetyl), 2.088 (3H, s, acetyl), 2.229 (1H, dd, $J_{3e\text{-}3a} = 12$, $J_{3e\text{-}4} = 4.5$ Hz, H-3e[I]), 2.254 (1H, dd, $J_{3e\text{-}3a} = 12$, $J_{3e\text{-}4} = 4.5$ Hz, H-3e[III]), 2.814 (1H, dd, $J_{3e\text{-}3a} = 12$, $J_{3e\text{-}4} = 4.5$ Hz, H-3e[II]), 3.625 (1H, m, H-4[II]), 3.651 (1H, m, H-4[III]), 3.802 (1H, m, H-5[III]), 3.895 (1H, m, H-5[I]), 3.973 (1H, m, H-5[II]), 4.022 (1H, m, H-4[I]). [I] is defined as the sugar unit located at the reducing terminal of **2**, and [III] is at the nonreducing end. For this assignment, see also T. Ercégovic, G. Magnusson, *J. Org. Chem.* **1996**, *61*, 179 – 184; ref. [4].

[7] a) C.-H. Lin, B. W. Murray, I. R. Ollmann, C.-H. Wong, *Biochemistry* **1997**, *36*, 780 – 785. b) T. Sugai, C.-H. Lin, G.-J. Shen, C.-H. Wong, *Bioorg. Med. Chem.* **1995**, *3*, 313 – 320.

[8] A. E. Manzi, H. H. Higa, S. Diaz, A. Varki, *J. Biol. Chem.* **1994**, *269*, 23617 – 23624.

Convergent Route to Organometallic Dendrimers Composed of Platinum – Acetylide Units**

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There is increasing interest in the development of new strategies to synthesize well-defined nanosize macromolecules with specific functions. Dendrimers have a regularly branched architecture and have large, spherical dimensions to meet the requirements for new materials.[1] One method for the functionalization of dendrimers is the incorporation of

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