2 represents a plot of ion intensity of $(DME)_nCH_3OH_2^+$ as a function of n for a variety of electron energies. For energies above 20 eV, a prominant magic number at $n = 2$ is exhibited. As shown at the bottom of Figure 2, we believe this is due to hydrogen bonding of two DME molecules directly to the hydroxy hydrogens of the protonated methanol. This type of "magic number" stability within hydrogen-bonded ion clusters has previously been demonstrated by Stace and Moore.[10]

It is interesting to now note that while the $CH_3OCH_2^+$ cation is extremely intense in the monomer mass spectrum of DME (~34% of all ion intensity), the same *cluster* cation $(DME)_nCH_3OCH_2^+$ is now substantially reduced in intensity (Figure 1). We postulate that this lowered intensity of the fragment cation is due to the $CH_3OCH_2^+$ being consumed in an ion-molecule reaction within the cluster. One likely candidate is the ion-molecule reaction of the $CH_3OCH_2^+$ cation with a neutral DME (within the bulk cluster) to form a trimethyloxonium cation intermediate by loss of formaldehyde.

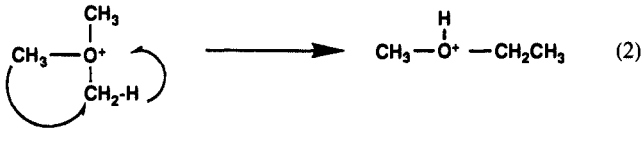
$$CH_3OCH_2^+ + (CH_3)_2O \rightarrow (CH_3)_3O^+ + CH_2O \quad (1a)$$

This type of ion-molecule reaction has been previously observed by Harrison and Young through the use of a tandem mass spectrometer.[11] This newly formed trimethyloxonium cation may then undergo a rearrangement to form a protonated methanol cation and ethylene.

$$(CH_3)_3O^+ \rightarrow CH_3OH_2^+ + C_2H_4 \quad (1b)$$

This mechanism is similar to that observed for the decomposition of $(CH_3)_2O$ over zeolite catalysts. The most commonly accepted mechanism for such a decomposition involves just such a trimethyloxonium intermediate.[12] This intermediate is believed to undergo, within the zeolite, a Stevens-type rearrangement giving methyl ethyl ether (methoxyethane), which then generates the olefin products via elimination. van Hooff et al.[13] also observed that conversion of DME over a zeolite catalyst gave comparable amounts of ethylene and propene as primary olefins, and once again, methoxyethane was believed to play a role as an intermediate.

We speculate that the DME cluster reactions leading to the formation of protonated methanol involve an intermediate similar to that found to occur on the zeolite catalysts. That is, following formation of the trimethyloxonium ion within the DME cluster (reaction 1a), excess energy derived from the ionization/reaction processes can drive a simple rearrangement reaction (reaction 1b) to form the products of protonated methanol and ethylene. For the case of DME clusters, we postulate that the internally generated trimethyloxonium ion internally isomerizes to protonated methoxyethane (reaction 2), where it then forms protonated



(2)

methanol via elimination of ethylene. This analogous process has previously been reported for the collisional activation of the monomer $(CH_3)_3O^+$ ion.[14] However, recent additional work appears to be at variance with that original result.[15] This variance could be due to the thermodynamic instability of the bare $(CH_3)_3O^+$, in that unimolecular dissociation can now effectively compete with the rearrangement reaction (2). However, within the solvating environs of a cluster, this unstable intermediate may be stabilized on a long enough time scale to now allow it to undergo this rearrangement reaction.

To gain insight into this mechanism, we have measured the appearance potentials of the relevant cluster ions observed in this experiment. We observe that the $(CH_3)_3O^+$ cation and the $M_n(CH_3OH_2)^+$ cations all have the same appearance potential (12.5-12.7 eV).[16] This result is consistent with the conjecture that the $M_n(CH_3OH_2)^+$ ions are produced via a $(CH_3)_3O^+$ intermediate.

As a further probe, we generated mixed clusters of DME-H_2O, via bubbling gas through a reservoir containing water at room temperature, and observed that the ions corresponding to the formula $(DME)_nCH_3OH_2^+$ decreased by a factor of 3 in intensity, compared to a pure DME expansion.[17] This effect is consistent with the proposed mechanism (2) in that a water molecule would presumably strongly hydrogen bond directly to the oxygen end of the trimethyloxonium cation. This additional water molecule then sterically hinders any possible rearrangement, thereby quenching the reaction. Just such a mechanism has recently been suggested by Tzeng et al.[18] to explain the quenching of an acetone dehydration reaction in mixed clusters of acetone and water.

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(10) Stace, A. J.; Moore, C. *J. Phys. Chem.* **1982**, *86*, 3681.
(11) Harrison, A. G.; Young, A. B. *Int. J. Mass. Spectrom. Ion Processes* **1989**, *94*, 321.
(12) van der Berg, M. P.; Wolthuizen, J. P.; van Hooff, J. H. C. *Proc. Conf. Zeolites, 5th* 1980; p 649. Olah, G. A. *Pure Appl. Chem.* **1981**, *53*, 201.
(13) van Hooff, J. H. C.; van der Berg, J. P.; Wolthuizen, J. P.; Volmer, A. *Proc. Int. Zeolite Conf., 6th* **1983**, 489.
(14) Sigsby, M. L.; Day, R. J.; Cooks, R. G. *Org. Mass Spectrom.* **1979**, *14*, 274.
(15) Farcasiu, D.; Pancirov, R. G. *Int. J. Mass Spectrom. Ion Processes* **1986**, *74*, 207.
(16) Coolbaugh, M. T.; Vaidyanathan, G.; Peifer, W. R.; Garvey, J. F., in preparation.
(17) This result also suggests that the protonated methanol ion is not produced via a reaction between the DME cluster and a water impurity. In particular, within the sensitivity of our apparatus, we do not observe ions of the formula $M_n(CH_3OD_2)^+$ or $M_n(CH_3OHD)^+$ when we generate mixed clusters of DME-D_2O, which would be the obvious ion products if such a side reaction were occurring.
(18) Tzeng, W. B.; Wei, S.; Castleman, A. W., Jr. *J. Am. Chem. Soc.* **1989**, *111*, 6035.

Total Synthesis of the Tumor-Associated Le^X Family of Glycosphingolipids†

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Glycosphingolipids carrying the Lewis antigen X (Le^X) determinant [Gal-β-1→4-(Fuc-α-1→3)-GlcNAc] are known to accumulate in a wide variety of human cancers[1] and have been

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(1) (a) Holmes, E. H.; Hakomori, S.; Ostrander, G. K. *J. Biol. Chem.* **1987**, *262*, 15649. (b) Holmes, E. H.; Ostrander, G. K.; Hakomori, S. *J. Biol. Chem.* **1985**, *260*, 7619. (c) Fukushi, Y.; Hakomori, S.; Nudelman, E.; Cochran, N. *J. Biol. Chem.* **1984**, *259*, 4681. (d) Kannagi, R.; Nudelman, E.; Levery, S. B. *J. Biol. Chem.* **1982**, *257*, 14865. (e) Brockhaus, M.; Magnani, J. L.; Herlyn, M.; Blaszczyk, M.; Steplewski, Z.; Koprowski, H.; Ginsburg, V. *Arch. Biochem. Biophys.* **1982**, *217*, 647. (f) Hakomori, S.; Nudelman, E.; Kannagi, R.; Levery, S. B. *Biochem. Biophys. Res. Commun.* **1982**, *109*, 36. (g) Kannagi, R.; Nudelman, E.; Levery, S. B.; Hakomori, S. *J. Biol. Chem.* **1982**, *157*, 14874. (h) Yang, H.; Hakomori, S. *J. Biol. Chem.* **1971**, *246*, 1192. (i) Hakomori, S.; Jeanloz, R. W. *J. Biol. Chem.* **1964**, *239*, 3606.

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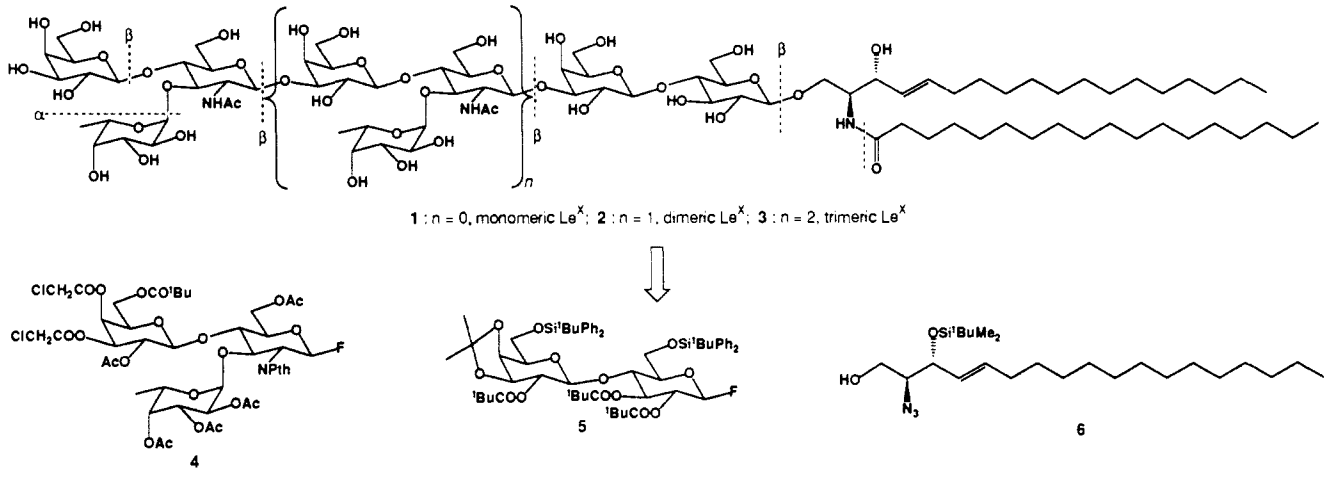
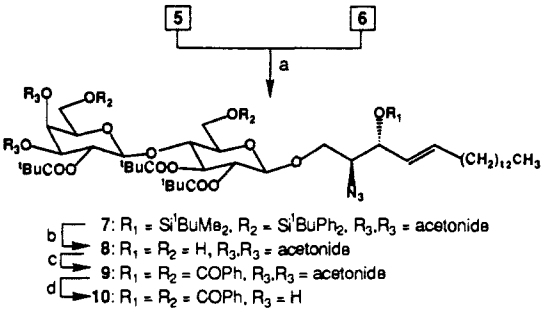
Scheme I. Structures and Retrosynthetic Disconnections of the Le^X Family of Glycosphingolipids (**1–3**)



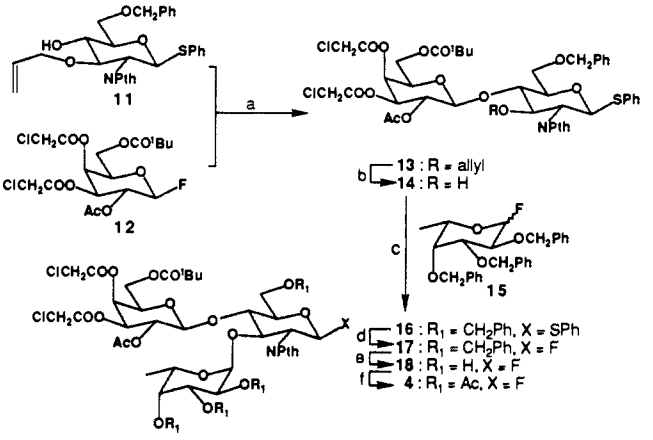
Scheme II. Synthesis of Key Intermediate **10**[a]



[a] Reagents and conditions: (a) 2.0 equiv of AgOTf, 2.0 equiv of $SnCl_2$, 1.0 equiv of 2,6-lutidine, 4-Å molecular sieves, CH_2Cl_2, 0 °C, 4 h, 84%; (b) 5.0 equiv of nBu_4NF, THF, 0–25 °C, 3 h, 98%; (c) 4.0 equiv of PhCOCN, 5.0 equiv of Et_3N, DMF, 25 °C, 0.5 h, 94%; (d) CF_3COOH:THF:H_2O, 10:5:1, 0 °C, 1.5 h, 95%.

identified as embryonic antigens maximally expressed at the morula stage.[2] Significantly these substances are conspicuously absent in normal liver, colonic mucosa, and human granulocytes,[3] an observation that led to their recognition as tumor-associated antigens.[4] The extreme scarcity of these tumor cell marker compounds coupled with their potential usefulness in diagnostics and immunotherapy prompted us to undertake their synthesis. In this communication we report the total synthesis of all three members (monomeric, dimeric, and trimeric Le^X) of this class of compounds by an efficient and stereospecific route based on our previously reported two-stage activation procedure[5] for oligosaccharide construction. The Le^X family of glycosphingolipids represents some of the most complex oligosaccharides ever to be targeted for synthesis.[6] The successful syntheses described in this paper demonstrate the capabilities of the two-stage activation method.[7]

Scheme III. Synthesis of Le^X Trisaccharide Intermediate **4**[a]



[a] Reagents and conditions: (a) 2.5 equiv of $AgClO_4$, 2.5 equiv of $SnCl_2$, 4-Å molecular sieves, CH_2Cl_2, 0 °C, 3 h, 72%; (b) 0.05 equiv of $H_2Ru(PPh_3)_4$, EtOH, 95 °C, 1.5 h, then 0.1 equiv of TsOH, MeOH, 25 °C, 2.5 h, 86%; (c) 1.5 equiv of 2,3,4-tri-*O*-benzyl-L-fucosyl fluoride, 2.5 equiv of $AgClO_4$, 2.5 equiv of $SnCl_2$, 4-Å molecular sieves, Et_2O, −30 °C, 3 h, 87%; (d) 3.0 equiv of DAST, 1.4 equiv of NBS, CH_2Cl_2, −30–0 °C, 4 h, 87%; (e) H_2, $Pd(OH)_2$–C, EtOH:EtOAc, 2:1, 3 h; (f) 0.05 equiv of DMAP. Ac_2O:2,6-lutidine, 2:1, 25 °C, 13 h, 84% overall.

Scheme I designates (dotted lines) the strategic retrosynthetic disconnections utilized to design a general and flexible route to this family of compounds which eventually delivered all three members (**1–3**) in their naturally occurring enantiomeric form. Careful selection of protecting groups allowed not only for high selectivity in the sequence but also for exclusive formation of the desired stereochemistry of all glycoside bonds. This analysis led to the utilization of compounds **4–6** as common intermediates for the construction of all three Le^X antigens. Below we describe the synthesis of trimeric Le^X (**3**); the contructions of monomeric (**1**) and dimeric (**2**) Le^X are included in the supplementary material.

Scheme II summarizes the synthesis of the lactosyl ceramide segment **10** from the previously reported sphingosine equivalent **6**[7a] and lactosyl fluoride **5**.[8] Thus, coupling of **5** and **6** in the presence of AgOTf–$SnCl_2$ led efficiently (84%) and stereospecifically to glycoside **7**[9] (β-glycoside bond, C-2 group directing

(2) (a) Hakomori, S.; Nudelman, E.; Levery, S. B.; Solter, D.; Knowles, B. B. *Biochem. Biophys. Res. Commun.* **1981**, *100*, 1578. (b) Gooi, H. C.; Feizi, T.; Kapadia, A.; Knowles, B. B.; Solter, D.; Evans, J. M. *Nature (London)* **1981**, *292*, 156. (c) Solter, D.; Knowles, B. B. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *25*, 5565.

(3) (a) Holmes, E. H.; Ostrander, G. K.; Clausen, H.; Gream, N. *J. Biol. Chem.* **1987**, *262*, 11331. (b) Hakomori, S.; Nudelman, E.; Levery, S. B.; Kannagi, R. *J. Biol. Chem.* **1984**, *259*, 4672.

(4) Hakomori, S. *Sci. Am.* **1986**, *154*, 44.

(5) Nicolaou, K. C.; Dolle, R. E.; Papahatjis, D. P.; Randall, J. L. *J. Am. Chem. Soc.* **1984**, *106*, 4159.

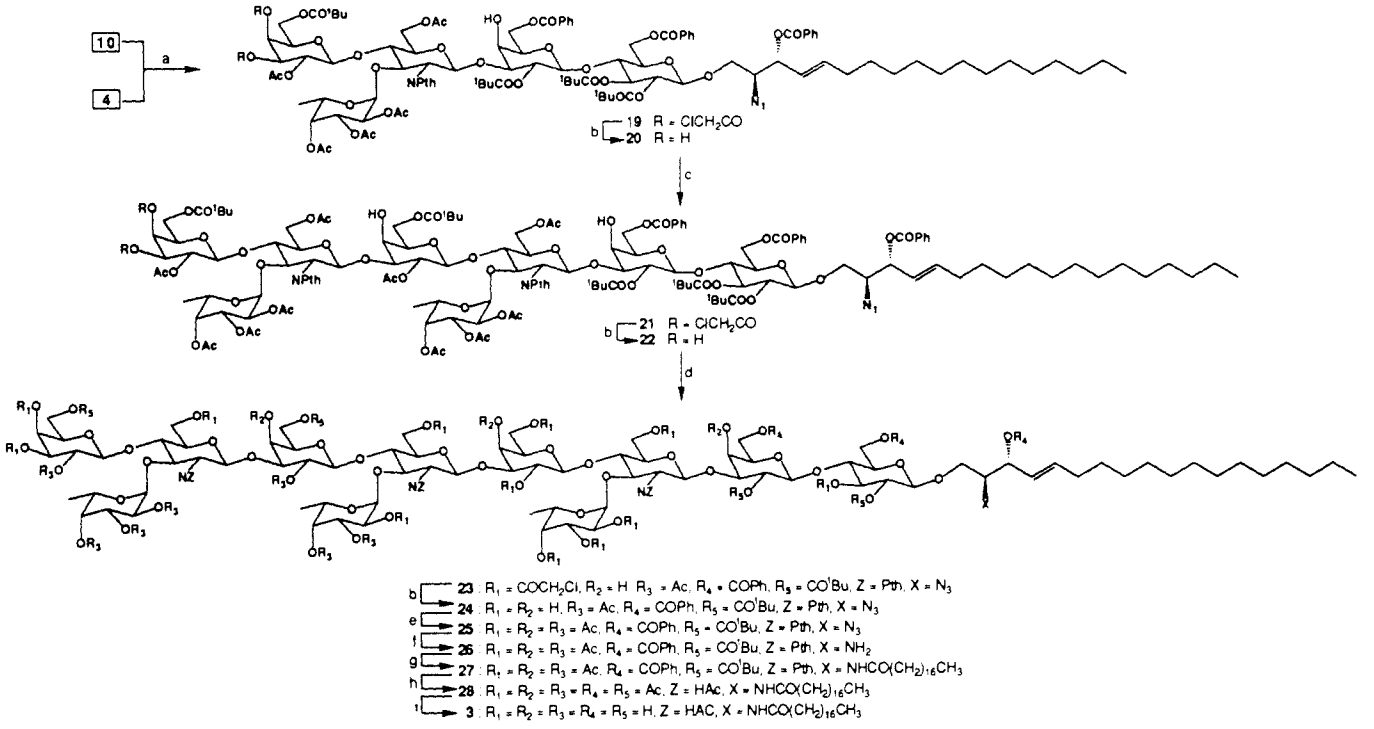
(6) For previous synthetic work toward the Le^X family of antigens, see the following. (a) Dimeric Le^X: Sato, S.; Yukishige, I.; Ogawa, T. *Tetrahedron Lett.* **1988**, *29*, 5267. (b) Monomeric Le^X: Sato, S.; Yukishige, I.; Nukada, T.; Nakahara, Y.; Ogawa, T. *Carbohydr. Res.* **1987**, *167*, 197. (c) Le^X fragments: Nilsson, M.; Norberg, T. *Carbohydr. Res.* **1988**, *183*, 71. Lönn, H. *Carbohydr. Res.* **1985**, *139*, 115. Hindsgaul, O.; Norberg, T.; LePendu, J.; Lemieux, R. U. *Carbohydr. Res.* **1982**, *109*, 109. Jacquinet, J. C.; Sinay, P. *J. Chem. Soc., Perkin Trans. 1* **1979**, 314.

(7) For previous applications of the two-stage activation procedure in complex oligosaccharide synthesis, see: (a) Nicolaou, K. C.; Caulfield, T.; Kataoka, H.; Kumazawa, T. *J. Am. Chem. Soc.* **1988**, *110*, 7910. (b) Nicolaou, K. C.; Randall, J. L.; Furst, G. T. *J. Am. Chem. Soc.* **1985**, *107*, 5556. (c) Dolle, R. E.; Nicolaou, K. C. *J. Am. Chem. Soc.* **1985**, *107*, 1695.

(8) See supplementary material for synthesis.

(9) All new compounds exhibited satisfactory spectral and analytical and/or exact mass data. Yields refer to spectroscopically and chromatographically homogeneous materials.

Scheme IV. Synthesis of Trimeric Le^X (3)[a]



[a] Reagents and conditions: (a) 7 equiv of **10**, 2.0 equiv of AgOTf, 2.0 equiv of $HfCp_2Cl_2$, 4-Å molecular sieves, CH_2Cl_2, −25 °C, 14 h, 91%; (b) 5.0 equiv of thiourea, 5.0 equiv of 2,6-lutidine, MeOH:CH_2Cl_2, 1:1, 40 °C, 8 h; (c) 2.5 equiv of **4**, 8.0 equiv of AgOTf, 8.0 equiv of $HfCp_2Cl_2$, 4-Å molecular sieves, CH_2Cl_2, 0 °C, 5 h, 84%; (d) 4.0 equiv of **4**, 12 equiv of AgOTf, 12 equiv of $HfCp_2Cl_2$, 4-Å molecular sieves, CH_2Cl_2, 0 °C, 5 h, 79%; (e) 12 equiv of Ac_2O, 10 equiv of Et_3N, 0.1 equiv of DMAP, CH_2Cl_2, 0 °C, 2 h, 99%; (f) 5.0 equiv of $Sn(SPh)_2$, 5.0 equiv of PhSH, 5.0 equiv of Et_3N, 25 °C, 0.2 h, 89%; (g) 5.0 equiv of BOPCl, 5.0 equiv of octadecanoic acid, 5.0 equiv of Et_3N, 25 °C, 0.5 h, 85%; (h) (1)10 equiv of NaOMe, MeOH, 60 °C, 68 h, (2) CH_3NHNH_2:EtOH, 1:1, 70 °C, 52 h, (3) 0.1 equiv of DMAP, Ac_2O:pyridine, 2:1, 25 °C, 14 h, 68% overall; (i) 1.0 equiv of NaOMe, MeOH, 25 °C, 12 h, 96%.

effect), which was converted sequentially to intermediates **8**, **9**, and **10** by functional-group manipulation as shown (88% overall yield).

Scheme III outlines the construction of the Le^X trisaccharide intermediate **4** from the readily available glucosamine derivative **11**[8] and galactosyl fluoride intermediate **12**.[8] Thus, coupling of **11** with **12** under the standard conditions[10] gave stereospecifically, as expected, the β-glycoside **13** in 72% yield. Selective removal of the allyl group from **13** then produced **14** (86%), which was coupled with 2,3,4-tri-*O*-benzyl-L-fucosyl fluoride (**15**),[8] to afford the trisaccharide derivative **16** in 87% yield and with the newly generated glycoside bond of the desired α-configuration. Conversion of the thioglycoside **16** to the β-glycosyl fluoride **17** using NBS–DAST[5] (88%) followed by protecting-group exchange as summarized in Scheme III furnished the requisite trisaccharide fragment **4** in 84% overall yield. With both synthons **4** and **10** readily available, the undecasaccharide framework of trimeric Le^X (**3**) was rapidly assembled as shown in Scheme IV. Thus, the coupling of **4** with **10** under the influence of AgOTf–$HfCp_2Cl_2$[11] occurred regiospecifically at the more reactive 3-position and stereospecifically in the desired β sence to provide the pentasaccharide **19** in 91% yield. The monochloroacetate groups were selectively removed from **19** by the action of thiourea leading to **20** (93%). Reiteration of the coupling and deprotection procedures led to octasaccharide **22** (84% overall) via its bis(monochloroacetyl) derivative **21** and thence to undecasaccharide **24** (74% overall) via its bis(monochloroacetyl) derivative **23** as indicated in Scheme IV. Acetylation of the liberated hydroxy groups in **24** led to compound **25** (99%), which was functionalized at the azido site by reduction with $[Et_3NH][Sn(SPh)_3]$,[12] producing amine **26**, followed by acylation with octadecanoic acid (BOPCl, Et_3N), affording amide **27** in 76% overall yield from **25**.

Generation of trimeric Le^X (**3**) in 60% overall yield from **27** proceeded as follows: (i) NaOMe-induced ester cleavage; (ii) $MeNHNH_2$-induced removal of the phthalimido groups; (iii) peracetylation (to facilitate chromatographic purification) giving **28**; and finally, (iv) selective ester cleavage as in i leading to the final target **3**. Synthetic Le^X (**3**), pure by TLC and HPLC, was characterized by ^1H and ^{13}C NMR,[13] FAB mass spectra, and $[\alpha]_D$ (see supplementary material).

The described total synthesis of trimeric Le^X (**3**) demonstrates the practicality of our two-stage activation procedure[5] for complex oligosaccharide synthesis and renders this important glycosphingolipid and its relatives **1** and **2** available in pure form for further biological investigations. Thus, immunizations and the development of antibodies against pure forms of these tumor-associated antigens for diagnostic and therapeutic applications may become possible.

Acknowledgment. We express our many thanks to Drs. S. Hakomori and T. Ogawa for ^1H NMR spectra of authentic Le^X compounds **1**–**3**. Our thanks are also due to Drs. G. Furst and J. Dykins of the University of Pennsylvania for NMR and mass spectroscopic assistance. This work was financially supported by the National Institutes of Health; Merck, Sharp and Dohme, USA; and Nippon Zeon Co., Japan.

Supplementary Material Available: Schemes with reagents and conditions for the synthesis of compounds **5**, **11**, **12**, **15**, **2**, and **1** and listing of selected R_f, $[\alpha]$, ^1H NMR, ^{13}C NMR, and mass spectral data for compounds **4**, **10**, **20**, **21**, **23**, **28**, **3**, **2**, and **1** (14 pages). Ordering information is given on any current masthead page.

(10) Mukaiyama, T.; Muria, Y.; Shode, S. *Chem. Lett.* **1981**, 431.
(11) Suzuki, K.; Maeta, H.; Matsumoto, T.; Tsuchihashi, G. *Tetrahedron Lett.* **1988**, *29*, 3571.
(12) Batra, M.; Urpi, F.; Villarassa, J. *Tetrahedron Lett.* **1987**, *28*, 5941.

(13) We thank Professors S. Hakomori and T. Ogawa for ^1H NMR spectra of authentic samples of the Le^X family of antigens.