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# A Practical Total Synthesis of Globo-H for Use in Anticancer Vaccines

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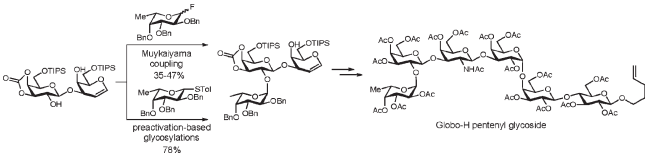


An improved synthesis of the hexasaccharide MBr1 antigen (globo-H) is reported. Enhanced efficiency in the synthesis was necessary for the scale-up production of globo-H, in order to advance globo-H-based anticancer vaccines to clinical trials. The key features of the improved synthesis include preactivation-based glycosylations and a revised iodosulfonimidation/rearrangement.

The glycosphingolipid globo-H (**1**, Figure 1), first isolated by Hakomori and colleagues from the breast cancer cell line MCF-7,[1] is recognized by the monoclonal antibody MBr1.[2]

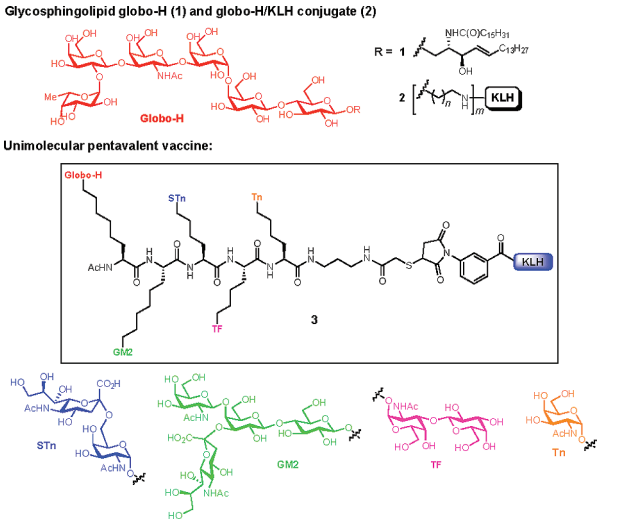


**FIGURE 1.** Glycosphingolipid globo-H (**1**) and representative globo-H containing anticancer vaccines.

This human breast-cancer-associated antigen was found to be distinctively overexpressed on the surfaces of a variety of other epithelial cancer cells such as prostate, ovary, lung, colon, and small cell lung cancers.[3] For this reason, the globo-H antigen has played an important role in our ongoing carbohydrate-based cancer vaccine program.[4] Of particular interest to our laboratory was a recent report, indicating that globo-H and SSEA3—the pentasaccharide precursor of globo-H—are also overexpressed in *breast cancer stem cells*. Interestingly, when co-administered with an immunological adjuvant (α-galactosylceramide), a globo-H-based vaccine induces antibodies against both globo-H and SSEA3.[5] In the light of the fact that cancer stem cells are often responsible for relapse and metastasis of cancerous tissues,[6] it is envisioned that globo-H-based therapy might form the basis of an important new direction in cancer treatment. Indeed, the globo-H vaccine, **2**, synthesized in our laboratories as a glycoconjugate appended to immunogenic carrier protein, has shown promise as a potential breast and prostate cancer vaccine, in preclinical, and even clinical settings.[4a−e] More recently, we also disclosed the synthesis and preclinical evaluation of the unimolecular pentavalent vaccine **3**,[4f,g] displaying globo-H and other antigens known to be overexpressed on prostate and breast cancer cell surfaces. The thought was to construct a single entity antigen system which reflects the actual degree of carbohydrate heterogeneity associated with most cancers.[7] Nonetheless, the accessibility

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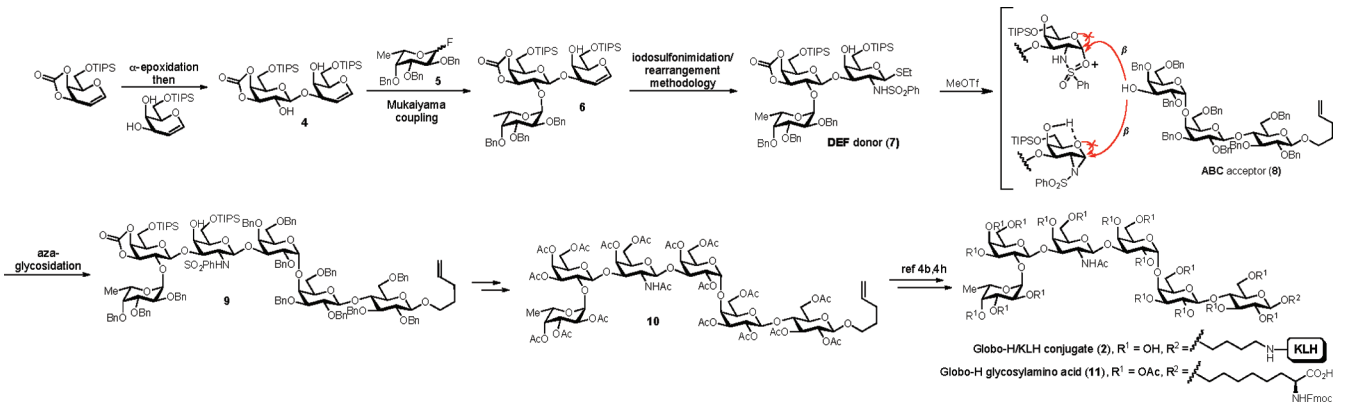


**FIGURE 2.** Our previous syntheses of globo-H KLH conjugate (**2**) and glycosylamino acid (**11**).

of such complex carbohydrate antigens remained as an important question. Globo-H isolated from human cancer tissue collections is typically limited to submilligram levels, which impedes broader immunological studies, including more advanced clinical trials. For this reason, globo-H has been an important synthetic target for our laboratory and others, including Schmidt,[8] Boons,[9] Wong,[10] Seeberger,[11] and Huang.[12] As part of our anticancer vaccine program to advance construct **2** to phase II/III clinical trials and **3** to phase I clinical trials for breast and prostate cancer, we required a scale-up synthesis of the globo-H antigen based on our previously established routes. Our modified route would need to overcome the yield and efficiency issues encountered in our small-scale synthesis (vide infra). We describe herein the development of an improved synthesis of globo-H, which provides ready access to large quantities of antigen.

Our earlier total synthesis of globo-H utilized a glycal building block assembly strategy for the rapid construction of the complex hexasaccharide.[4b,13] Key transformations include (i) $\beta$-glycosylation leading to **4** via DMDO-mediated α-epoxidation,[14] (ii) Mukaiyama[15]−Nicolaou[16] coupling using fucosyl donor **5** to obtain trisaccharide glycal **6**, (iii) iodosulfonimidation/rearrangement methodology[17] to yield DEF donor **7**, (iv) $Cp_2Zr(OTf)_2$-mediated construction of ABC acceptor **8**,[4b] and finally (v) $\beta$-selective [3 + 3] ABC + DEF coupling to furnish **9** via sulfonamide participation (Figure 2). Although this concise and stereoselective strategy allows rapid access to globo-H, some steps, particularly those leading to DEF donor **7**, still require further optimization if we are to secure the globo-H antigen in amounts required for our ongoing clinical trials with globo-H, both in monovalent and multivalent settings.

In this regard, we report herein our efforts to seek a much improved synthesis of the DEF donor **7** and ABC acceptor **8**. As described in our earlier report,[4b,13] DEF trisaccharide glycal **6** was obtained from the coupling of disaccharide glycal **4** and fucosyl donor **5**[18] using Mukaiyama[15]−Nicolaou[16] conditions, that is, $SnCl_2/AgClO_4$ (Table 1, entry 1). The fucosylation had earlier occurred in moderate regioselectivity mainly at the equatorial (35−47%) rather than the axial alcohol (~8%) of **4**. In the case of AB + C coupling using fluorogalactosyl donor **14**[19] and AB acceptor **12** under the same conditions, ABC trisaccharide **13** was generated in 42% yield with modest anomeric selectivity ($\alpha/\beta$ = 3:1) (Table 1, entry 3). However, the yield and selectivity of the AB + C coupling could be improved markedly (80%, $\alpha/\beta$ = 10:1) when the strongly fluorophilic $Cp_2Zr(OTf)_2$ was employed as the promoter (Table 1, entry 4).

Alternatively, the glycosylation employing preactivated glycosyl donor[20] using the promoter *p*-TolSOTf[21] has proven useful for the construction of stereoselective α- or $\beta$-linkages. In particular, Huang and co-workers have shown a successful application of this protocol—using thiofucosyl donor **15** and thiogalactosyl donor **16**—to their fascinating multicomponent one-pot strategy for the synthesis of globo-H.[12] In our system, glycosylation leading to trisaccharide glycal **6** requires the fucosyl donor to distinguish between the equatorial C2′ and axial C4 hydroxyl groups of the DE acceptor **4** with high α-selectivity. Gratifyingly, fucosylation of **4** using thiofucosyl donor **15** under slightly modified conditions based on Huang's protocol[12] afforded the desired α-trisaccharide **6** in high yields (78%, entry 2), with no indication of the presence of C4 fucosylated isomer. In a similar manner, coupling of thiogalactosyl donor **16** and AB acceptor **12** proceeded stereoselectively to furnish α-trisaccharide **13** in 76% yield (Table 1, entry 5). The [2 + 1]

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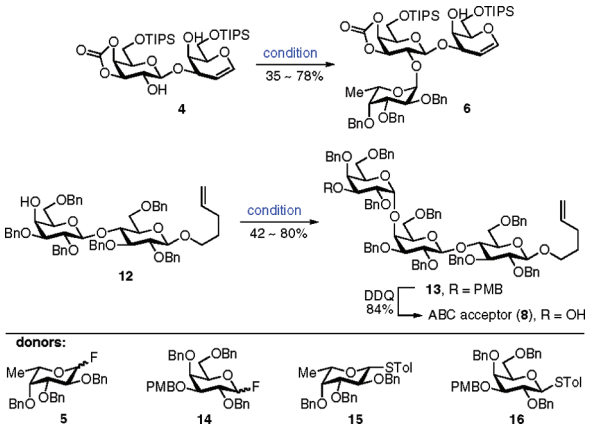
**TABLE 1. Coupling Conditions Used To Construct the Trisaccharide 6 and Trisaccharisde 13**




| entry | donor | acceptor | condition | *t* (h) | product (yield) |
|---|---|---|---|---|---|
| 1 | **5** | **4** | $SnCl_2$, $AgClO_4$, ether, 2,6-di-*tert*-butylpyridine | 35 | **6** (35−47%)[a] |
| 2 | **15** | **4** | TolSCl, AgOTf, $CH_2Cl_2/Et_2O$ (2:1), 2,6-di-*tert*-butylpyridine | 4 | **6** (78%)[b] |
| 3 | **14** | **12** | $SnCl_2$, $AgClO_4$, ether, 2,6-di-*tert*-butylpyridine | 35 | **13** (42%) |
| 4 | **14** | **12** | $Cp_2Zr(OTf)_2$, toluene/THF (5:1), dark, 2,6-di-*tert*-butylpyridine | 72 | **13** (80%) |
| 5 | **16** | **12** | TolSCl, AgOTf, $CH_2Cl_2/Et_2O$ (2:1), 2,6-di-*tert*-butylpyridine | 4 | **13** (76%) |

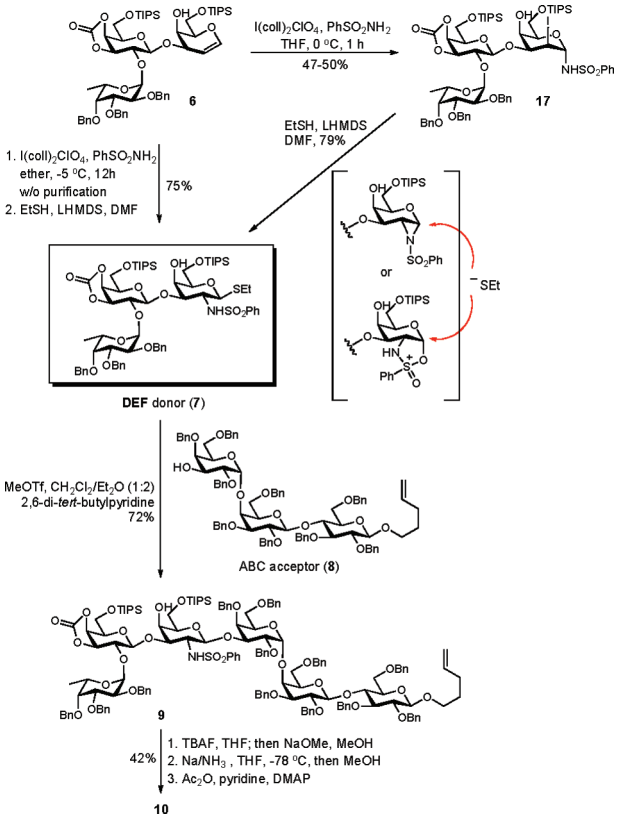
[a]Approximately 8% of monofucosylated product at C4 of the galactose was also observed. [b]Trace amounts of the difucosylated product were detected by NMR.

couplings using thioglycosyl donors and the promoter *p*-TolSOTf gave superior yields with shorter reaction times than the reactions performed under Mukaiyama conditions. Importantly, the reactions deliver more consistent results, particularly in our gram-scale synthesis of the target trisaccharides **6** and **13**.

We next turned our attention to the transformation of trisaccharide glycal **6** to DEF thiodonor **7** bearing a 2-α-phenylsulfonamido function (Scheme 1). According to precedent established in our laboratories,[17] iodosulfonimidation of glycal **6** using I(*sym*-coll)$_2$ClO$_4$ and benzenesulfonamide followed by treatment with lithium ethanethiolate afforded DEF thiodonor **7** in 37−40% yields over two steps.[13] The somewhat modest overall conversion is most likely due to the formation of unidentified impurities associated with the initial iodosulfonimidation step. We found, however, that by tuning reaction conditions, such as temperature and solvent system, we were able to effectively suppress the formation of byproducts to negligible amounts. Specifically, when we substituted THF solvent with diethyl ether and carefully maintained the reaction temperature at −5 °C for 12 h, we were able to obtain *trans*-diaxial iodosulfonamide **17** as almost the exclusive product. Without purification, the somewhat labile **17** was immediately subjected to the next step employing excess lithium ethanethiolate to furnish the desired DEF thiodonor **7** in 75% yield. The DEF thiodonor **7** was then treated with MeOTf[22] in the presence of the PMB-deprotected ABC acceptor **8** to efficiently afford hexasaccharide **9** in 72% yield. Global deprotection of **9** followed by peracetylation proceeded smoothly to afford globo-H pentenyl glycoside **10**.

In summary, we have successfully optimized synthetic steps to DEF donor **7**. The DEF donor **7** serves as a key strategic intermediate for the [3 + 3] ABC + DEF coupling via β-selective sulfonamidogalactosylations leading to the fully elaborated hexasaccharide skeleton of globo-H. The improved synthesis reported herein allows facile production

**SCHEME 1. Synthesis of globo-H pentenyl glycoside 10 via DEF donor 7**

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of globo-H up to gram-scale with quite consistent yields. Advanced clinical results using synthetic vaccines containing globo-H, such as **2** and **3**, will be reported in due course.

## Experimental Section

**Ethyl 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)-3,4-carbonyl-6-*O*-tri-isopropylsilyl-β-D-galactopyranosyl-(1→3)-6-*O*-triisopropylsilyl-2-deoxy-2-phenylsulfonylamino-1-thio-β-D-galactopyranoside (7).** A solution of thiofucosyl donor **15** (2.38 g, 4.41 mmol) and freshly activated 4 Å molecular sieves (2 g) in $CH_2Cl_2/Et_2O$ (50 mL/25 mL) was stirred at rt for 1 h, then cooled to −78 °C. To the cooled solution were added AgOTf (3.4 g, 13.22 mmol) and 2,6-di-*tert*-butylpyridine (2.92 mL, 13.92 mmol). After 10 min, freshly distilled *p*-TolSCl (619 μL, 4.41 mmol) was added. After 40 min, the characteristic yellow color of *p*-TolSCl in the reaction solution disappeared, indicating depletion of *p*-TolSCl. A solution of acceptor **4** (2.85 g, 4.41 mmol) in $CH_2Cl_2/Et_2O$ (6 mL/3 mL) was added dropwise via a syringe. The reaction mixture was warmed to rt under stirring over 3 h and stirred for an additional 1 h at rt. The mixture was filtered over Celite and further washed with $CH_2Cl_2$. After evaporation of solvent in vacuo, the mixture was diluted with ethyl acetate then washed with a saturated aqueous solution of $NaHCO_3$, water, and brine and dried over $MgSO_4$. After removal of the solvent, the crude was purified via silica gel flash chromatography using 10−15% EtOAc/hexane to give 3.66 g (78%) of α-trisaccharide **6**. A mixture of trisaccharide glycal **6** (1 g, 0.94 mmol) and benzenesulfonamide (887 mg, 5.64 mmol) was azeotroped with anhydrous benzene once and further dried on high vacuum for 1 h. It was dissolved in freshly distilled $Et_2O$ (40 mL), and freshly activated 4 Å molecular sieves (1 g) were added. The resulting mixture was stirred at rt for 1 h, then cooled to −5 °C. To the solution was added $I(sym\text{-}coll)_2ClO_4$ (925 mg, 1.974 mmol), and the mixture was further stirred at −5 °C for 12 h. After filtration on Celite pad, the crude was washed with saturated $Na_2S_2O_3$ solution, saturated $CuSO_4$, and brine and dried over $MgSO_4$. After concentration, the crude material, containing iodosulfonamide **17**, was immediately subjected to the next step without purification. To a solution of EtSH (695 μL, 9.4 mmol) in DMF (5 mL) was added lithium bis(trimethylsilyl)amide (LHMDS, 1.0 M in THF, 4.7 mL, 4.7 mmol) at −45 °C. After 15 min of stirring, the solution was transferred dropwise via a cannula to a flask containing iodosulfonamide **17** in DMF (25 mL) at −45 °C. The reaction mixture was allowed to warm to rt and stirred for a total of 3 h. After dilution with saturated $NH_4Cl$, the crude was extracted four times with ethyl acetate. The combined extracts were washed with water and brine and dried over $MgSO_4$. Concentration and purification by silica gel chromatography (15−25% EtOAc in hexane) afforded 903 mg (75% over two steps) of DEF donor **7**.

**4-Pentenyl 2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (8).** A solution of thiogalactosyl donor **16** (3.97 g, 5.86 mmol) and freshly activated 4 Å molecular sieves (4 g) in $CH_2Cl_2/Et_2O$ (100 mL/50 mL) was stirred at rt for 1 h, then cooled to −78 °C. To the cooled solution were added AgOTf (4.52 g, 17.6 mmol) and 2,6-di-*tert*-butylpyridine (3.9 mL, 17.6 mmol). After 10 min, freshly distilled *p*-TolSCl (824 μL, 5.86 mmol) was added. After 10 min, a solution of acceptor **12** (4.65 g, 4.88 mmol) in $CH_2Cl_2/Et_2O$ (10 mL/5 mL) was added dropwise. The reaction mixture was warmed to rt under stirring over 3 h and then stirred for an additional 1 h at rt. After filtration and usual workup as described previously, the crude was purified via silica gel flash chromatography using 15% EtOAc/hexane to give 5.55 g (76%) of α-trisaccharide **13**. This PMB-protected **13** (5.25 g, 3.49 mmol) in $CH_2Cl_2$ (130 mL) at 0 °C was treated with phosphate buffer (20 mL, pH 7.2) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (1.19 g, 5.24 mmol, 1.5 equiv) and stirred at 0 °C for 5 h. The reaction mixture was diluted with saturated $NaHCO_3$ and further stirred at rt for 1 h. The mixture was extracted twice with $CH_2Cl_2$, then washed with brine, dried over $MgSO_4$, and concentrated to dryness. The crude material was purified by flash column chromatography using 16% EtOAc/hexane to give 4.06 g (84%) of ABC acceptor **8**.

**4-Pentenyl 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)-3,4-carbonyl-6-*O*-triisopropylsilyl-β-D-galactopyranosyl-(1→3)-6-*O*-triisopropylsilyl-2-deoxy-2-phenylsulfonylamino-β-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (9).** The DEF donor **7** (2.13 g, 1.66 mmol) and ABC acceptor **8** (1.15 g, 0.831 mmol) were combined, azeotroped twice with anhydrous benzene, and placed under high vacuum for 5 h. The mixture was then dissolved in $CH_2Cl_2$ (20 mL) and $Et_2O$ (40 mL), treated with freshly activated 4 Å molecular sieves (2 g) and 2,6-di-*tert*-butylpyridine (734 μL, 3.32 mmol), then cooled to −78 °C. Methyl triflate (4.0 equiv, 365 μL) was added in one portion, and the reaction was allowed to warm to rt slowly overnight. The reaction was quenched by the addition of $Et_3N$ (5 mL) and filtered through Celite with $Et_2O$. The filtrate was washed with saturated $NaHCO_3$ and brine and dried over $MgSO_4$. Concentration and purification by silica gel chromatography (15−20% EtOAc/hexane) afforded 1.551 g (72%) of hexasaccharide **9**.

**4-Pentenyl 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-*O*-triacetyl-2-acetylamino-2-deoxy-β-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (10).** TBAF (1.0 M in THF, 37.34 mL, 60 equiv) was added to a solution of the hexasaccharide **9** (1.62 g, 0.622 mmol) and acetic acid (2.14 mL, 60 equiv) in THF (35 mL). The reaction was stirred at rt for 3 days, poured into ice water, and extracted with EtOAc. The organic extracts were washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated. This desilylated intermediate was purified through a short plug of silica gel with EtOAc. The resulting triol was dissolved in anhydrous MeOH (20 mL), and sodium methoxide was added (0.75 mL of a 25% solution in MeOH). The reaction was stirred at rt for 18 h, neutralized with Dowex-$H^+$, filtered with MeOH washings, and concentrated. THF (5 mL) and condensed liquid $NH_3$ (~70 mL) were added at −78 °C to the resulting white solid. Sodium (~1.2 g) was added, and the resulting blue solution was stirred at −78 °C for 2 h. The reaction was quenched with anhydrous MeOH (20 mL), brought to rt, and concentrated under a stream of dry $N_2$ to a volume of ~15 mL. The reaction was neutralized with Dowex-$H^+$, filtered with MeOH washing, and concentrated to a white solid. The white solid was dissolved in pyridine (10 mL) and $CH_2Cl_2$ (10 mL) and cooled to 0 °C. A few crystals of DMAP were added followed by acetic anhydride (10 mL). The ice bath was removed and the reaction stirred at rt overnight. The mixture was diluted with EtOAc and washed with water, saturated $NaHCO_3$, and brine and dried over $MgSO_4$. Concentration followed by purification by flash column chromatography using 60% EtOAc/$CH_2Cl_2$ to give **10** as a white solid (470 mg, 42%).

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**Supporting Information Available:** NMR spectra for **6**, **7**, **8**, **9**, and **10**. This material is available free of charge via the Internet at http://pubs.acs.org.