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Solid-Phase Synthesis of Complex Oligosaccharides Using a Novel Capping Reagent

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Solid-phase-supported oligosaccharide synthesis of a core *N*-glycan tetrasaccharide and of a trisaccharide containing the Galili antigen is reported. The synthesis is based on a hydroxymethylbenzyl benzoate spacer−linker system attached to the Merrifield resin, *O*-Fmoc-protected *O*-glycosyl trichloroacetimidates as glycosyl donors, and benzoyl isocyanate as a capping reagent for low-reactivity hydroxy groups. In this way, the target molecules could be efficiently obtained with little byproduct formation, and hence final purification was convenient.

Introduction

Oligosaccharides play an important role in various biological processes.[1−3] Therefore, many innovative methods for their synthesis have been recently developed.[4] Hence, efforts have also been devoted to increase the efficiency of polymer-support-based approaches,[5] which have become standard for the synthesis of oligopeptides[6] and oligonucleotides.[7] Progress in this challenging task would provide several advantages over solution-phase-based oligosaccharide synthesis: (i) standardized building blocks will become available, (ii) use of excess building blocks and/or reagents will increase the reaction yields and thus the overall efficiency, (iii) synthesis will be automated and thus become much faster, and (iv) much less sophisticated purification steps will be required.[8] However, with the existing glycosylation procedures, completion can hardly be reached for all glycosylation steps. Therefore, similar to solid-phase synthesis of peptides[9] and oligonucleotides[10] for unreactive hydroxy groups as glycosyl acceptors, a capping step in the synthetic cycle is required in order to reduce the accumulation of product mixtures. Thus, particularly $n-1$ side product formation will be reduced, hence leading to greatly simplified purification of the target molecules. Obviously, such capping methods can also be combined with monitoring of desired products or undesired byproducts or with tagging of the target molecule.

Application of the common capping concept to polymer-supported oligosaccharide synthesis has so far received little attention. Seeberger and co-workers[11a] successfully utilized the capping and tagging (cap−tag) concept for the automated synthesis of (1−6)-linked trisaccharides. For the capping (and tagging) of the primary hydroxy groups, the sterically demanding α-azidoisobutyric or the (heptadecafluorodecyl)diisopropylsilyl groups were employed. Furthermore, Ito and co-workers[11b] successfully synthesized a tetrasaccharide employing an attractive capping procedure based on the use of chloroacetyl moieties. We would like to introduce in this paper a novel capping reagent that fulfills the following demands: (i) the capping reagent must readily react with all types of hydroxy groups; (ii) the linkage formed between the capping reagent and the hydroxy group must lead to nonbasic/nonnucleophilic and nonacidic caps that are inert to all reactions all along the oligosaccharide construction; (iii) the caps must be compatible with the

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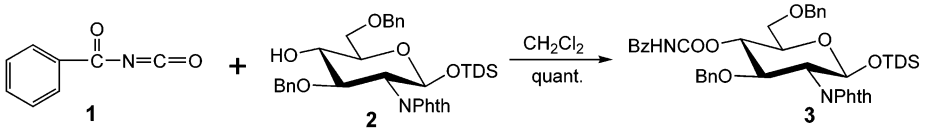
SCHEME 1. Capping Reaction with Benzoyl Isocyanate



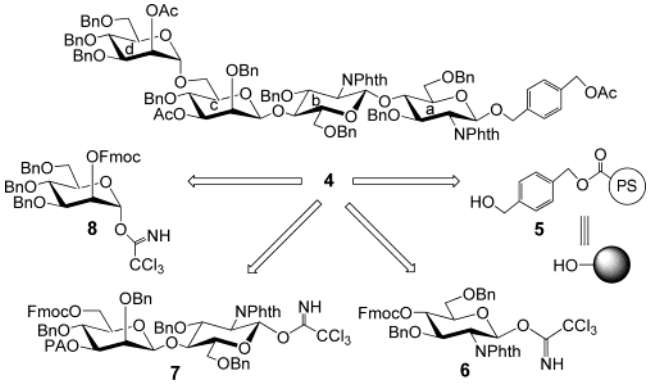
permanent and temporary protecting groups used in the synthesis. Hence, these caps have to be orthogonal to the temporary protecting group pattern.

Results and Discussion

The outlined prerequisites for a capping reagent led us, for instance, to investigate *O*-benzyl,[12] *O*-(4-methoxybenzyl),[13] and *O*-phthalimidomethyl trichloroacetimidates;[14] however, incomplete reaction with hydroxy groups of low reactivity was observed. Reaction, for instance, with benzoyl chloride in the presence of a base affected the stability of Fmoc groups. Therefore, highly reactive isocyanates should be a reasonable choice. Trichloroacetyl isocyanate[15] could satisfy all the requirements for a good capping reagent, yet the *N*-trichloroacetylcarbamoyl cap is not stable under the weakly basic conditions essential for the cleavage such as phenoxyacetyl (PA) protecting groups. However, benzoyl isocyanate[16] (Scheme 1, **1**) satisfied all conditions for a good capping reagent: (i) **1** reacts readily and quantitatively with sterically less accessible hydroxy groups under neutral conditions, as shown for the reaction with 4-*O*-unprotected glucosamine derivative **2** that gave 4-*O*-(*N*-benzoylcarbamoyl)-protected **3** within 5 min in quantitative yield. (ii) The *N*-benzoylurethane moiety formed is nonacidic/nonnucleophilic and nonbasic, and (iii) it is not affected by the cleavage of Fmoc and PA temporary protecting groups under weakly basic conditions. The versatility of this capping reagent is demonstrated in the successful solid-phase oligosaccharide synthesis (SPOS) of a tetrasaccharide of the glycan core of *N*-glycopeptides and of a trisaccharide containing the Galili antigen.

SPOS of a Tetrasaccharide of the *N*-Glycan Core. Recently, we introduced a highly efficient solid-phase synthesis of a branched *N*-glycan hexasaccharide containing the core structure.[8] The novel linker and the temporary protecting group pattern containing different types of ester linkages permitted highly chemoselective reactions and release of the product from the polymer support with a benzyl aglycon moiety that can be removed by hydrogenolysis. *O*-Fmoc-protected *O*-glycosyl trichloroacetimidates[17] as glycosyl donors permitted also the desired stereocontrol at the anomeric center.[4] This concept was also applied to the synthesis of core *N*-glycan tetrasaccharide **4** (Scheme 2)[18] in combination with **1** as a capping reagent. Hence, the retrosynthetic analysis leads to spacer−linker−polymer support **5** containing the previously employed (4-hydroxymethyl-phenyl)methanol unit[19] and *O*-glycosyl trichloroacetimidates **6**−**8** as glycosyl donors. Because of the lack of a highly β-selective mannosyl donor,[20,21] the required Manβ(1−4)GlcN disaccharide donor **7** had to be prepared independently.[8,20] Building blocks **6** and **8** are readily available following previously reported procedures.[17d,22]

SCHEME 2. Retrosynthesis of Target Core *N*-Glycan Tetrasaccharide 4



For the synthesis of spacer−linker−polymer support **5**, (4-hydroxymethyl-phenyl)methanol (Scheme 3, **9**) was monotritylated to give **10**,[17e] which on reaction with Merrifield resin containing benzoyl chloride groups **11**[17e] in pyridine in the presence of 4-(dimethylamino)pyridine (DMAP) gave ester **12**; unreactive acid chloride groups were quenched by treatment with methanol. Detritylation of **12** with trifluoroacetic acid (TFA) in dichloromethane afforded polymer **5**. The loading could be easily determined by the amount of trityl cation released: 0.146 mmol/g of dry resin led to good glycosylation results; loadings below 0.1 or above 0.2 mmol/g of dry resin diminished the overall yields of the oligosaccharide synthesis.

The synthesis of **7** is based on a recently reported direct β-selective mannosylation procedure[20] with **13**[20h] as a donor having 2,3-*O*-alkyl-4,6-*O*-benzylidene protection and **14**[20h] as an acceptor, furnishing the desired β-disaccharide **15** in good yield (β:α = 4:1, 71%) (Scheme 4). For the introduction of the temporary protecting groups, the allyl protecting group was first removed under Pd

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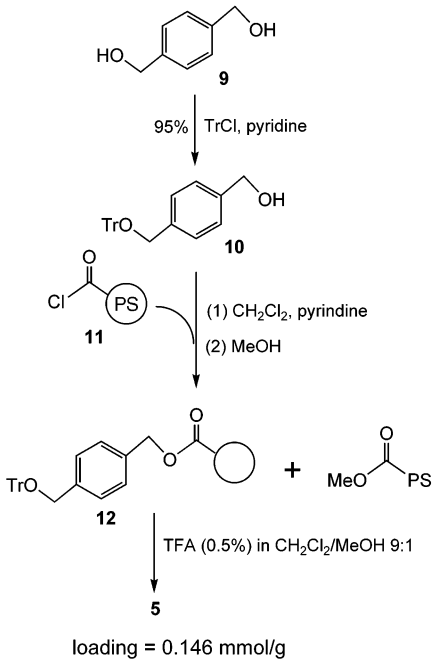
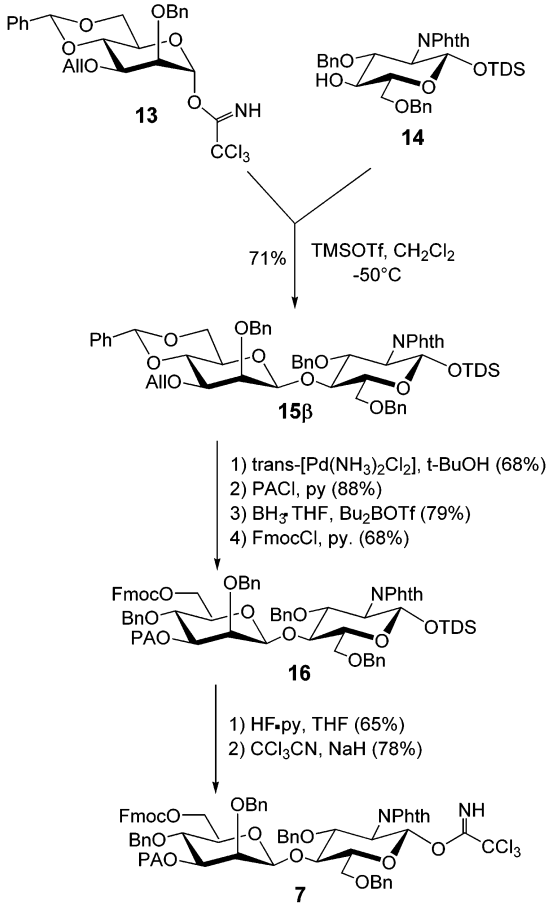
SCHEME 3. Preparation of Spacer−Linker and Attachment to Resin



SCHEME 4. Synthesis of the Required Man*β*(1→4)GlcN Disaccharide Donor 7



catalysis with *tert*-butyl alcohol as the nucleophile[23] and then phenoxyacetylation was performed. Reductive opening of the 4,6-*O*-benzylidene group with borane in the presence of dibutylborane triflate[24] afforded the desired 6-*O*-unprotected intermediate, which on treatment with FmocCl in pyridine gave disaccharide **16** with the two orthogonal temporary protecting groups. Removal of the *O*-silyl group with HF·pyridine and then reaction with trichloroacetonitrile in the presence of sodium hydride as a base furnished the desired glycosyl donor **7** without affecting the temporary protecting groups; even the base-sensitive Fmoc group was retained.

With the required building blocks at our disposal, the multistep synthesis of tetrasaccharide **4** was initiated (Scheme 5). Linker-loaded polystyrene resin **5** was glycosylated with 3 equiv of *O*-glucosaminyl trichloroacetimidate **6** under trimethylsilyl triflate (TMSOTf) activation (0.3 equiv) to furnish the corresponding support-bound monosaccharide **17** (Scheme 5).

The glycosylation also in the next steps was carried out twice at −40 °C to ensure high-yielding reaction. The Fmoc protecting group was selectively removed by treatment of **17** with triethylamine in dichloromethane affording monosaccharide acceptor resin **18**. Next, an elongation of the immobilized monosaccharide **18** was performed by reaction with 3 equiv of Man*β*(1→4)GlcN disaccharide donor **7** upon activation with TMSOTf (0.35 equiv), thus leading to resin-bound trisaccharide **19**; repetition of the glycosylation reaction twice ensures a high yield. Because the C-2 phthalimido protecting group reduces the nucleophilicity of the 4-hydroxy group of glucosamine, a capping reaction was performed to block unconsumed acceptor resin **18**. Hence, reaction with highly reactive benzoyl isocyanate in dichloromethane at room temperature afforded 4-*O*-protected resin **20** without affecting the Fmoc protecting group. Removal of the Fmoc group by treatment of resin **19** with Et_3N followed by washing with CH_2Cl_2/THF (1:1) and then glycosylation with donor **8** in CH_2Cl_2 at −20 °C in the presence of TMSOTf (0.3 equiv) as a catalyst led to the desired tetrasaccharide resin **21**. The supernatant of an analytical cleavage reaction from 2 to 4 mg of resin **21** can be analyzed spectroscopically by MALDI-TOF MS, thus proving the success of the capping strategy (Figure 1 in Supporting Information). Two signals corresponding to the MNa^+ ion of **22** and **23** as the only detectable peaks [**23** (MNa^+: 1879.9); **22** (MNa^+: 631.1, 778.2)] are obtained. Preparative cleavage under the conditions of analytical cleavage and then *O*-acetylation with acetic anhydride furnished after HPLC pure target molecule **4** as ascertained by NMR data (^{1}H NMR: δ 4.72 (d, $J_{1b,2b}$ = 7.4 Hz), 4.67 (s, $J_{1c,2c}$ < 1.0 Hz), 4.79 (s, $J_{1d,2d}$ < 1.0 Hz)). The total yield was 39% over eight steps, which is an average yield of 86% per step. In comparison with previous results[8a] capping does, as expected, lead to the same overall yield; however, the number of byproducts is reduced.

SPOS of a Trisaccharide Containing the Galili Antigen. A natural IgG antibody, directed against the Galα(1−3)Gal moiety, the Galili antigen, was found to be present in large amounts in human serum,[25] which greatly affects organ transplantation. Typically, this disaccharide is β(1−4)-linked to glucosamine; therefore,

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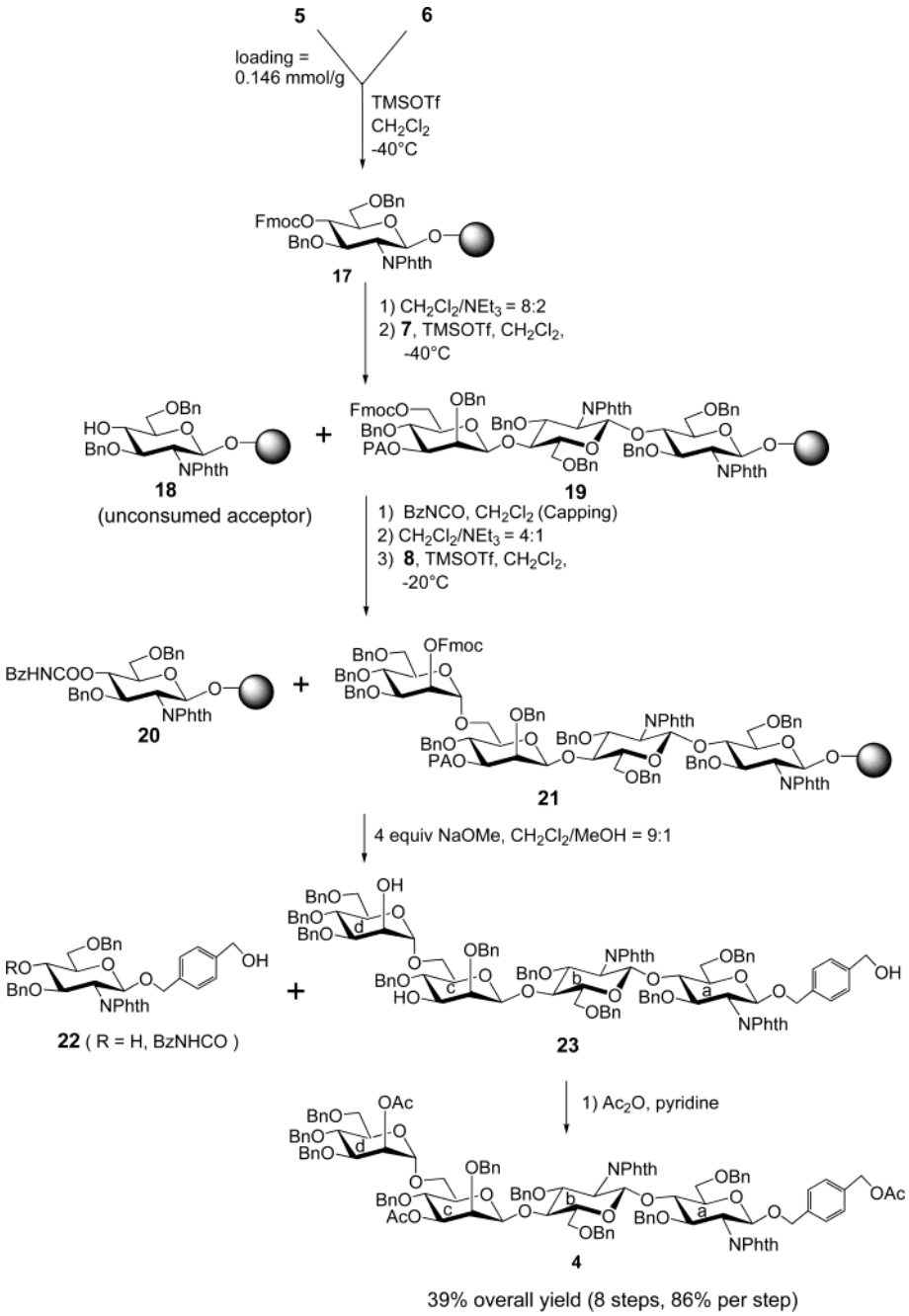
SCHEME 5. Solid-Phase Synthesis of Tetrasaccharide 4 with Benzoyl Isocyanate 1 as a Capping Reagent

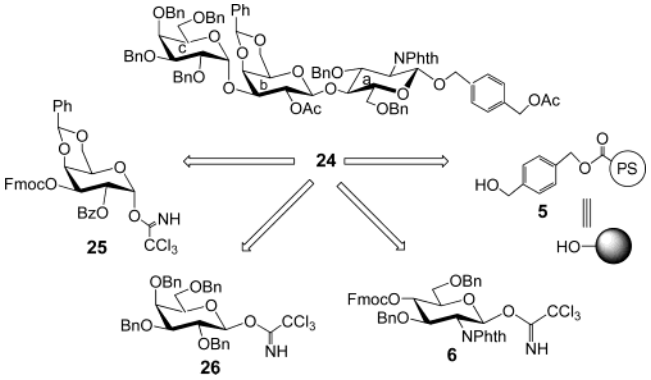


trisaccharide **24** (Scheme 6) seemed to be another interesting target molecule for the investigation of the utility of capping reagent **1** in SPOS.

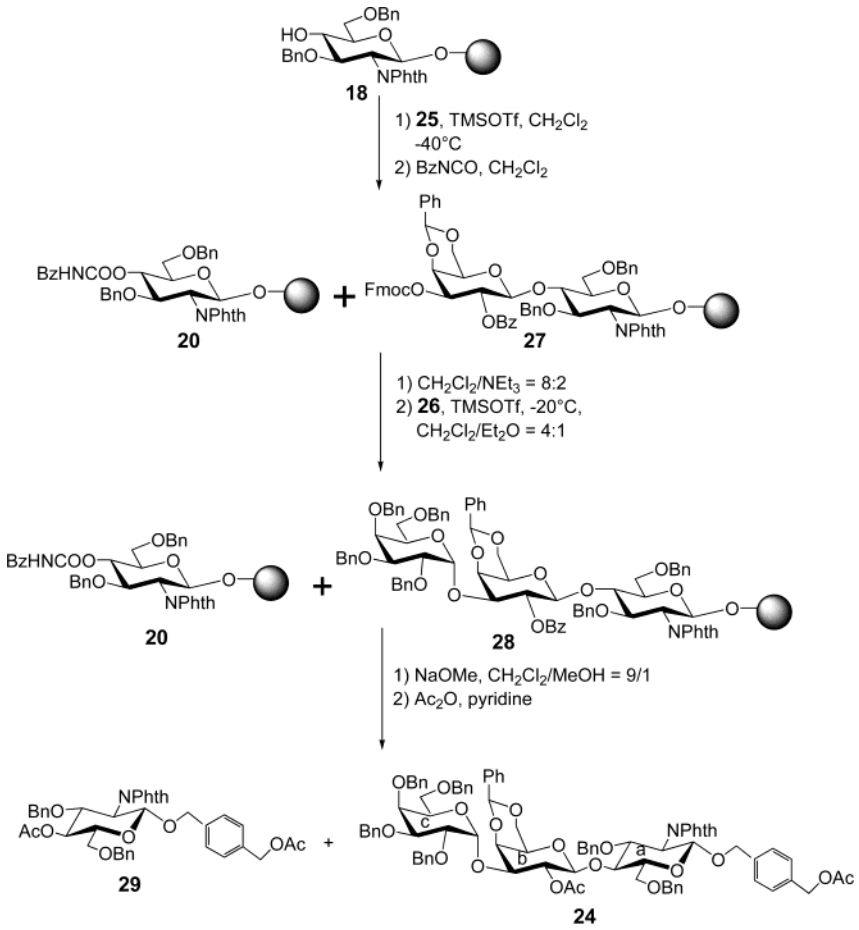
The retrosynthetic analysis follows the concept for the synthesis of target molecule **4**, thus leading to spacer−linker−polymer support **5** and to known glycosyl donors **6**, **25**, and **26**.[17d,22] Thus, resin **18** (Schemes 5 and 7) was common to both syntheses. Glycosylation of **18** with 3 equiv of *O*-galactosyl trichloroacetimidate **25** in the presence of TMSOTf (0.35 equiv) at −40 °C furnished the corresponding support-bound disaccharide **27** (Scheme 7), and repetition of the glycosylation reaction twice ensures a high yield; capping reaction with **1** was carried out in CH_2Cl_2 at room temperature, affording resin **20**.

Removal of the Fmoc group by treatment of resin **27** with Et_3N and then washing with CH_2Cl_2/THF (1:1) and

SCHEME 6. Retrosynthesis of Target Trisaccharide 24 Containing the Galα(1−3)Gal Galili Antigen



SCHEME 7. Solid-Phase Synthesis of Trisaccharide 24 with Benzoyl Isocyanate 1 as a Capping Reagent



glycosylation with donor **26** in a mixture solvent of Et_2O/CH_2Cl_2 (1:4) at −20 °C in the presence of TMSOTf (0.3 equiv) as a catalyst led to the desired trisaccharide resin **28**. Therefore, preparative cleavage of the product from the resin **28** and an O-acetylation step led, after HPLC, to pure target molecule **24** (the α/β ratio of the galactosylation is 8:1) as ascertained by NMR data (^{1}H NMR: δ 4.54 (d, $J_{1b,2b}$ = 8.2 Hz, 1b−H), 5.03 (d, $J_{1c,2c}$ = 3.2 Hz, 1c−H), 5.11 (d, $J_{1a,2a}$ = 8.3 Hz, 1a−H)). The total yield was 25% over eight steps, which is an average yield of 80% per step. MALDI-TOF-MS analysis of the final product showed two signals corresponding to the MNa^+ ion of **24** and **29** as practically the only detectable peaks [**24** (MNa^+: 1489.7); **29** (MNa^+: 716.3)] (Figure 2 in Supporting Information).

Conclusion

In summary, efficient solid-phase synthesis of a core *N*-glycan tetrasaccharide and of a trisaccharide containing the Galili antigen with benzoyl isocyanate as a capping reagent could be performed. This capping reagent fulfills the demands, i.e., complete reaction with unconsumed hydroxy groups in difficult glycosylation reactions, inertness to all reactions along the construction of the oligosaccharide on solid support, and compatibility with the protecting groups and the linker system employed. Thus, accumulation of products stemming from incomplete capping is avoided, which makes purification of final products convenient. Application of this novel methodology to the solid-phase synthesis of more complex and branched oligosaccharides is currently under way.

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Supporting Information Available: Spectral data for all new compounds and Experimental Section. This material is available free of charge via the Internet at http://pubs.acs.org.

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