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A new glycosylation method. Part 2: Study of carbohydrate elongation onto the gold nanoparticles in a colloidal phase[☆]

Hiroki Shimizu,[a,*] Masahiro Sakamoto,[b] Noriko Nagahori[b] and Shin-Ichiro Nishimura[a,b,*]

[a]*Drug-Seeds Discovery Research Laboratory, Hokkaido Center, National Institute of Advanced Industrial Science and Technology (AIST), Sapporo 062-8517, Hokkaido, Japan*

[b]*Division of Advanced Chemical Biology, Graduate School of Advanced Life Science, Frontier Research Center for Post-Genomic Science and Technology, Hokkaido University, Sapporo 011-0021, Hokkaido, Japan*

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Abstract—A new reaction for carbohydrate elongation for synthesis of oligosaccharide using gold colloidal nanoparticles (GCNPs) has been developed. The gold core in this colloidal phase synthesis was prepared by a reduction of tetrachloroauric acid with 30,31-dithia-3,6,9,12,15,18,43,46,49,52,55,58-dodecaoxa-1,60-hexacontanediol. The presented alkanethionyl oligomeric ethylene glycol worked as a stabilizer of GCNPs and as a linker in chemical elongations of carbohydrates. This colloidal phase synthesis has several advantages such as (1) remnants of reagents and glycosyl donors in each reaction could be easily removed by ultrafiltration or gel filtration column chromatography, (2) further purifications are not required, and (3) the reactions can be monitored by MALDI-TOF MS directly without any pretreatment. In fact, we have successfully synthesized lactose derivative on GCNPs and will report these results in this paper.
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1. Introduction

Since the great advent of solid-phase peptide synthesis by Merrifield,[1] preparation of diverse peptides in large amounts became easier, and this established a universal method that has deeply contributed to the revelation of the biological roles of peptides.[2] Although carbohydrates have been recognized as key compounds in a wide range of biological functions, many of their molecular based ‘cause and effect’ roles remain in a black box. To open this box, we believe that establishing methods to prepare a variety of oligosaccharides in large amounts is important. We have, therefore, already reported on the development of an automated oligosaccharide synthesizer[3] and new synthesis approaches using microwave irradiation for glycopeptide[2,4] and oligosaccharides.[5]

Polymer or solid supported oligosaccharide syntheses are, of course, typical methods used to make oligosaccharide synthesis easy.[6] Although there are many reports that have shown many advantages of these methods, it is still hard to say that universal oligosaccharide synthesis is finally established. The difficulty in applying polymer or solid supported syntheses to carbohydrate chemistry is that creating carbohydrate diversity, namely glycosylation, requires regioselectivity among hydroxyl groups and stereoselectivity on an anomeric position. On the other hand, polymer or solid supported syntheses have several disadvantages, for example, monitoring the reactions is difficult, the reactivity often decreases compared to that in solution phase, and so on.

Recently, gold colloidal nanoparticles (GCNPs), the first related chemical research reported by Faraday,[7] became widely used in chemical and biological research.[8] Biologically, for example, they have been used as biosensing materials for proteins and biological ligands,[9–11] and also applied in a carbohydrate mediated interaction research field, namely carbohydrate–proteins (i.e., lectin)[12,13] and even in weak carbohydrate–carbohydrate binding systems.[14] Meanwhile in chemical applications, GCNPs have been used in the synthesis of monolayer-protected cluster molecules,[15] NMR study,[16] direct observation of ligand–protein binding by time-of-flight mass spectrometry (MALDI-TOF MS),[17,18] enzymatic oligosaccharide synthesis,[18] peptide synthesis,[19] and so on.

Among these reports, we have especially focused on the use of GCNPs in mass spectrometry. Recent reports have shown that the self-assembled monolayer (SAM) of a thiol compound chemisorbed onto a gold surface could be ionized directly with cleavage of the Au–S bond by laser irradiation.[17] This means that if chemical reactions are carried out on the

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* Corresponding authors. Tel.: +81 11 857 8497; fax: +81 11 857 8435 (H.S.); tel.: +81 11 857 8472; fax: +81 11 706 9042 (S.-I.N.); e-mail addresses: hiroki.shimizu@aist.go.jp; shin@glyco.sci.hokudai.ac.jp; tiger.nishimura@aist.go.jp

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compound conjugated through Au–S bond to GCNPs, we can monitor the reactions by MALDI-TOF MS. In addition, if we can avoid reduction of the chemical reactivity by carrying out reactions in the colloidal phase, we can overcome some of the disadvantages in polymer or solid supported syntheses as mentioned above.

Here we will report some of our efforts to open the door to new oligosaccharide synthesis methods. We have chosen a lactose derivative as a target of the demonstration of this colloidal phase synthesis, because it required two sets of alternating steps between the glycosylation and selective deprotection and because the use of lactose on GCNPs has already been reported.[14]

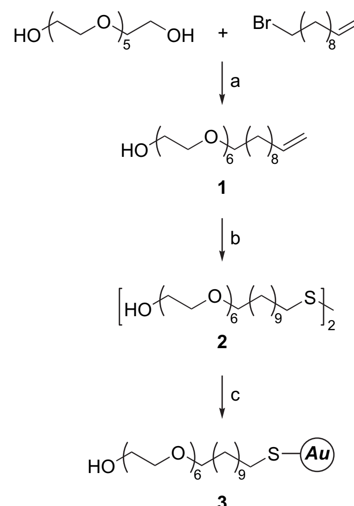
2. Results and discussion

2.1. Strategy

Figure 1 shows our strategy for this research. The linker between the core gold and sugar functioned also as a stabilizer of the GCNPs. Glycosylation and selective deprotection of hydroxyl protecting groups were performed in order on these GCNPs. In each step, reagents could be easily washed out by ultrafiltration. The reactions could be detected directly by MALDI-TOF MS.

2.2. Preparation of the GCNPs

First, as in Scheme 1, we synthesized a long alkanethiol chain having a hydrophilic oligomeric ethylene glycol, which functioned as a stabilizer of GCNPs and also as a linker to connect synthesized oligosaccharides and GCNPs.[18] Yamada et al. showed peptide syntheses on gold nanoparticles with a simple linker such as undecyl alkane and got good yields,[19] but in our study, glycosylation required dissolving in organic solvents and deprotection to yield oligosaccharides would proceed in water or alcohols. In addition, for our further aim of using them for biological research, i.e. binding assay, it was necessary to dissolve in water. So we had decided to introduce ethylene glycols as part of the linkers. Glycosylation did not occur onto the GCNPs with undecyl di(ethylene glycol) as a linker, and the GCNPs with undecyl tetra(ethylene glycol) were precipitated when Lewis acid (TMSOTf or BF_3OEt_2) was added to a solution of the GCNPs in organic solvents, e.g. acetonitrile, dichloromethane. Finally hexa(ethylene glycol) was used in our study for carbohydrate elongation and deprotection.



Scheme 1. Preparation of gold nanoparticles, **3**. Reagents and conditions: (a) NaH, 100 °C, 18 h (67%); (b) (1) AcSH, AIBN, toluene, 70 °C, 20 h; (2) NaOMe, MeOH, rt, 18 h (83%); (c) $KAuCl_4$, $NaBH_4$, $MeOH/H_2O$ (3/2), rt, 2 h.

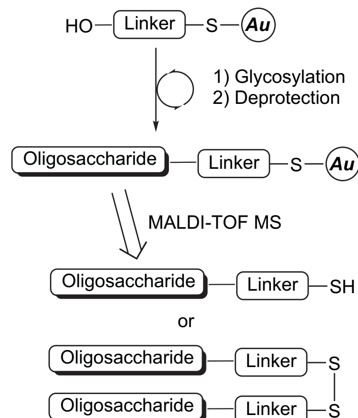


Figure 1. Overview of synthesis on gold nanoparticles and detection by MALDI-TOF MS.

Compound **1**[20] was prepared by a coupling of hexaethylene glycol and 11-bromo-1-undecene with sodium hydride in 67% yield. Radical reaction with thioacetic acid followed by saponification gave disulfide **2** in 83% yield. Reduction of tetrachloroauric acid with sodium borohydride in a mixed solvent of methanol and water was performed in the presence of **2**.[16] Depending on the mixed solvent ratio, we got two differently characterized GCNPs (Fig. 2). When the reaction was carried out in 15/1 MeOH/water, GCNPs turned red, transmission electron microscopy (TEM) showed that the majority of GCNPs were well-separated as single particles with diameters ranging from 5 to 8 nm (Fig. 3), and disulfide **2** was mainly observed by MALDI-TOF MS analysis. On the other hand, when the reaction was carried out in 3/2 MeOH/water, the GCNPs' color became brown, the size of the GCNPs was less than 5 nm, and the monosulfide, which was a thiol of undecyl hexa(ethylene glycol), was mainly observed by MALDI-TOF MS analysis. In this study, we have used the latter GCNPs as they make MS analysis and scientific discussion easier.

2.3. Preparation of monosaccharide derivatives

As donors of glycosylations onto GCNPs, we have designed the glucose derivative **7** and galactose derivative **10** (Scheme 2). After levulinoylation of **4**,[21] deprotection of the allyl group on the anomer gave hemiacetal **6**. Treatment with trichloroacetonitrile and DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) afforded the trichloroacetoimidate donor **7** in 87% yield. Similarly, for the galactosyl moiety, levulinoylation and deprotection of the *p*-methoxyphenyl group followed by treatment with trichloroacetonitrile and DBU gave the glycosyl donor **10**.

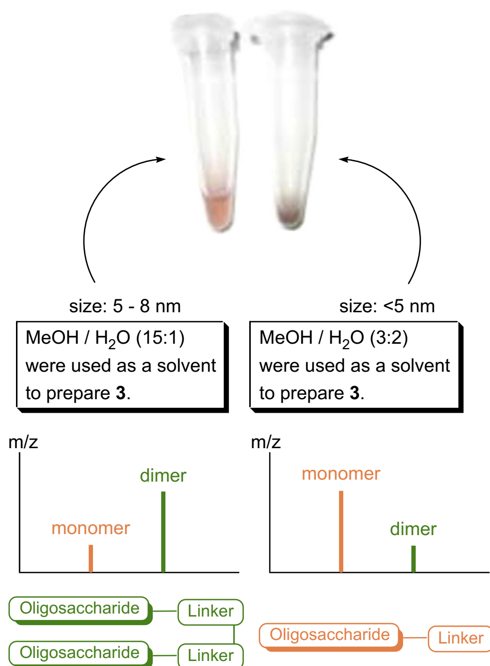


Figure 2. Physical characteristics of gold nanoparticles. When the gold nanoparticle was prepared in MeOH/H_2O (15/1), particle size was 5–8 nm and was observed mainly as a dimer of oligosaccharide by MALDI-TOF MS, while the gold nanoparticle was prepared in MeOH/H_2O (3/2), particle size was smaller than 5 nm and was observed mainly as a monomer of oligosaccharide by MALDI-TOF MS.

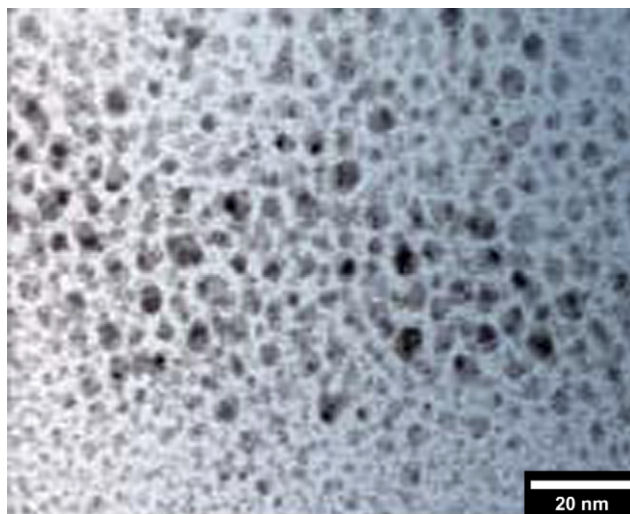
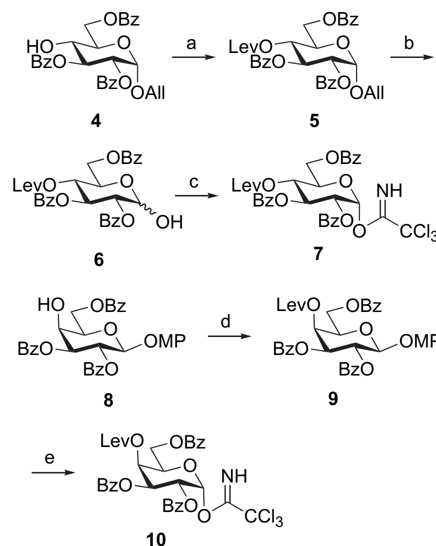


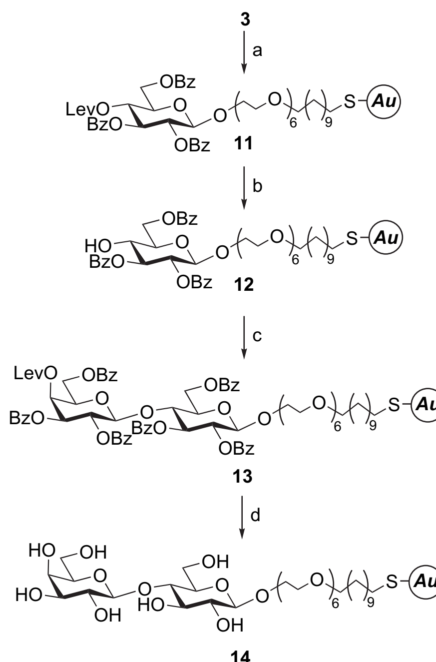
Figure 3. TEM image of GCNP prepared in MeOH/water (15/1).

2.4. Carbohydrate elongation onto GCNP 3

The first glycosylation onto GCNPs **3** was performed with the glycosyl donor **7** and the promoter trimethylsilyl trifluoromethanesulfonate (Scheme 3). MALDI-TOF MS for glycosylated GCNPs gave ion signals, some of which were at *m/z* 491 and at *m/z* 1063 for the sodium adducts of the linker thiol itself and the glycosylated linker, respectively. The ratio of their intensity was around 3:1 (Fig. 4a). Although it was hard to discuss the glycosylation yield based on these intensities because their ionization efficiency would be different and the ion signal at *m/z* 491 might contain the fragment ion of **11**, this result of only 25% of hydroxyl groups being glycosylated might be reasonable. In the case of the size of GCNPs assumed as 4 nm, the number of linkers onto these GCNPs could be calculated as 326 molecules.[22] The surface area of one GCNP (ϕ=4 nm) was around 50 nm^2, thus occupation area for one linker onto GCNPs was around 0.15 nm^2. This indicated that the linker existed every 0.4 nm. If the carbohydrate elongation could not occur to the linker existing side by side with the already glycosylated linker due to the



Scheme 2. Preparation of monosaccharide synthetic moieties. Reagents and conditions: (a) LevOH, DCC, DMAP, CH_2Cl_2, 0 °C–rt, 15 h (99%); (b) Ir{(COD)[PCH_3Ph_2]$_2$}PF_6, THF, rt, 2 h, then 1 M HCl, AcOH, 70 °C, 2 h (93%); (c) CCl_3CN, DBU, CH_2Cl_2, rt, 2 h (87%); (d) LevOH, DCC, DMAP, CH_2Cl_2, 0 °C–rt, 15 h (89%); (e) (1) CAN, toluene/CH_3CN/H_2O (4/3/3), 0 °C–rt, 4.5 h; (2) CCl_3CN, DBU, CH_2Cl_2, rt, 1.5 h (80%).



Scheme 3. Carbohydrate elongation onto gold nanoparticle. Reagents and conditions: (a) (1) **7**, TMSOTf, CH_2Cl_2, CH_3CN, 0 °C–rt, 3 h; (2) Ac_2O, Py, rt, 12 h; (b) $H_2NNH_2 \cdot H_2O$, AcOH, THF, MeOH, 0 °C–rt, 3 h; (c) **10**, TMSOTf, CH_3CN, 0 °C–rt, 3 h; (d) 0.5 M NaOMe in MeOH, rt, 12 h.

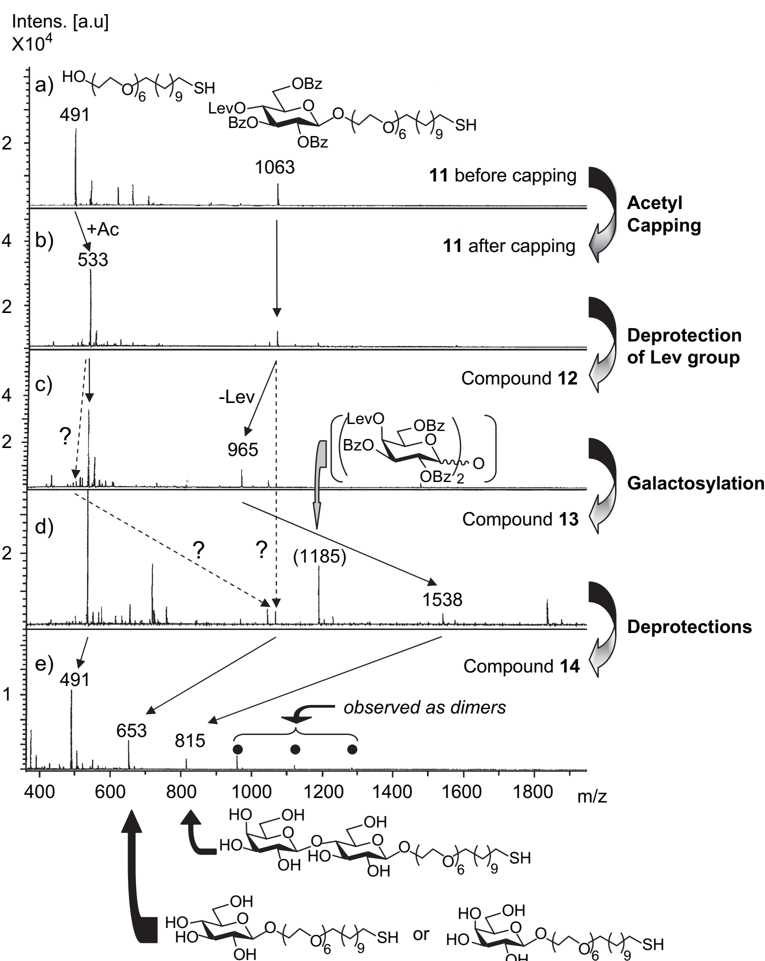


Figure 4. Tendency for MALDI-TOF MS along chemical reactions onto GCNPs: (a) **11** before acetyl capping; (b) **11** after acetyl capping; (c) **12** obtained by deprotection of the levulinoyl group of **11**; (d) **13** obtained by galactosylation of **12**; (e) **14** after deprotection of **13**.

steric hindrance of benzoyl protected carbohydrates, theoretically only 25% of the linkers could be glycosylated.

Acetyl capping for these non-reacted hydroxyl groups would be necessary to avoid unfavorable byproducts in further synthesis steps and to reveal whether the fragment ion's signals have been obtained at *m/z* 491 or not. Although small signals of disulfides were observed in Figure 4b at *m/z* 1041 and at *m/z* 1572 (not marked) as the sodium adducts of the dimer of the acetylated linkers and the conjugated compound with glycosylated and acetylated linkers, respectively, the sodium adducts of monosulfides, the acetylated linker thiol and the glycosylated linker have been observed at *m/z* 533 and at *m/z* 1063, respectively. The ratio of their intensities was around 4.5:1, and we could not find the linker thiol itself at *m/z* 491. Selective deprotection of the levulinoyl group in **11** proceeded well, which was proved by MALDI-TOF MS of **12** as shown in Figure 4c. The second glycosylation, introducing the galactose moiety, which resulted in the supposed lactose derivative also succeeded, but we should point out a few things at this stage. The resulting disaccharide would be a lactose due to the use of regioselective hydroxyl protected monosaccharides and well-known neighboring effect for glycosylation, but unfortunately only mass spectral data were available. MALDI-TOF MS for **13**, as shown in Figure 4d, revealed that the second glycosylation proceeded very well as there was no signal at *m/z* 965. Meanwhile, on the other hand, we could see signals of a monosaccharide with the linker having a levulinoyl group at *m/z* 1063, although we have determined that there was no levulinoylated monosaccharide in the previous step as shown in Figure 4c. It might be that either the levulinoylated monosaccharide was not detected in Figure 4c due to a lesser ionization efficiency than other compounds, or some of the acetyl capped linker had been deacylated in the delevulinoylation stage and subsequently galactosylated. We also observed disaccharide at *m/z* 1185 in Figure 4d. This might be the result of donor byproduct in the galactosylation step as 1–1 linked trehalose-type, which indicated that washing of the gold nanoparticle **13** was not enough although it was washed carefully several times. To confirm which compounds were actually synthesized onto the GCNPs, deprotection of hydroxyl acetate was carried out against **13** and the MALDI-TOF MS result is shown in Figures 4e and 5. We have observed not only the designed supposed lactose derivatives **14**, but also oligosaccharide moieties, namely monosulfides and disulfides of disaccharide (supposed lactose), monosaccharides (glucose or galactose), and the original linker itself. (N.B. In this discussion, we have not considered the low *m/z* area (< around 700) in MALDI-TOF MS, unless particular assignable numbers were seen.) In addition as shown in Figure 5, all the disulfide signals

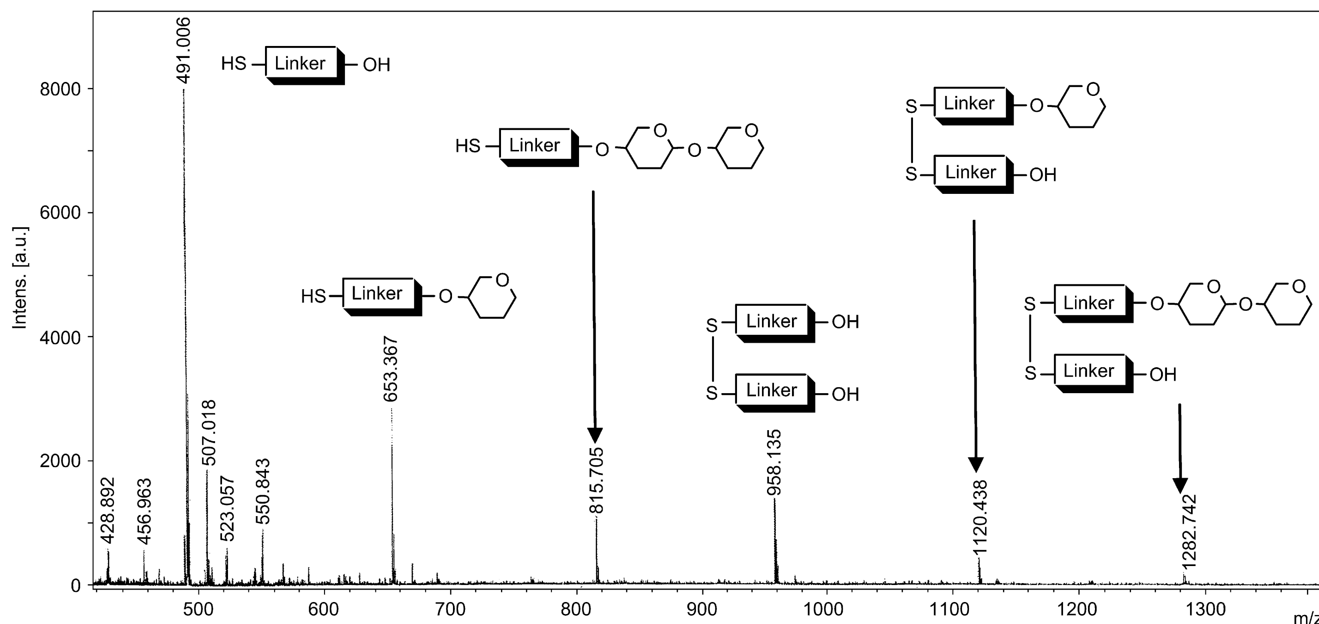


Figure 5. Details of Figure 4e, which is the MALDI-TOF MS of GCNPs **14**. Mass signals were assigned.

came from the combination of the linker with an oligosaccharide and the one without. This result supported the assumption that the carbohydrate elongation could not occur to the linker existing side by side with the already glycosylated linker on the GCNPs, because it is natural to think that coupling the closest linkers with each other to form the disulfides in the ionization stage for the MALDI-TOF MS was favored.

3. Conclusions

In conclusion, we have succeeded in demonstrating carbohydrate elongations on GCNPs, which has some advantages over solid-phase syntheses, namely the direct monitoring of the chemical reaction by MALDI-TOF MS and the fact that the resulting GCNPs can be applied directly to biological carbohydrate research. There was good reactivity onto GCNPs as we have discussed for the galactosylation in Figure 4d, but it is hard to conclude at this point that the glycosylation reactivity of the colloidal phase is better than that of solid phase. Although we successfully obtained these fruitful results to create a new approach for oligosaccharide synthesis, many issues remain to be solved including determination of the suitable density of glycosylation points (linkers) onto GCNPs for the synthesis of oligosaccharides, establishment of a quantitative monitoring system by MALDI-MS, application of large scale synthesis with introduction of a cleavage point to yield the oligosaccharide itself, preparation of more complicated oligosaccharides, and so on. We will report these in due course.

4. Experimental

4.1. General

All reagents were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan), Tokyo Kasei Kogyo Co. (Tokyo, Japan) or Aldrich Chemical Co. (Milwaukee, WI, USA) and used as purchased without further purification. Reactions in the solution phase were monitored by TLC, which was performed with 0.25 mm precoated silica gel $60F_{254}$ on glass from Merck (Darmstadt, Germany). Compounds were detected by dipping the TLC plates in an ethanolic solution of sulfuric acid (5% v/v) and heating. Silica gel N60 (40–50 nm) from Kanto Chemical (Tokyo, Japan) was used for silica gel chromatography. Melting points were determined on an ASONE ATM-01 and are uncorrected. Optical rotations were recorded on a Perkin Elmer Polarimeter 343 at room temperature (approximately 18–23 °C). All NMR data are reported in parts per million downfield shift from tetramethylsilane. ^{1}H NMR spectra were routinely recorded at 400 MHz on a BRUKER AVANCE 400 spectrometer at 300 K and chemical shifts are expressed relative to that of the residual proton in the NMR solvents (δ 7.26 ppm for C*H*Cl_3). ^{13}C NMR spectra were routinely recorded at 100 MHz on a BRUKER AVANCE 400 spectrometer at 300 K and chemical shifts are expressed relative to that of the deuterated solvents (δ 77.0 ppm for *C*DCl_3). High-resolution FAB-mass data were recorded by the in-house mass spectrometry service at the Center for Instrumental Analysis, Hokkaido University. MALDI-TOF MS analyses were performed in reflector mode with the Ultraflex instrument (Bruker, Germany) with 2,5-dihydroxybenzoic acid (DHB). Ions generated by a pulsed UV laser beam (nitrogen laser, λ=337 nm, 5 Hz) were accelerated to a kinetic energy of 23.5 kV. Ultrafiltration was performed by Centriplus YM-50 (MWCO=50,000) and prewashed once with proper solvents.

4.2. Preparation of the GCNPs

4.2.1. 3,6,9,12,15,18-Hexaoxa-28-nonacosen-1-ol (1).[20] To stirred hexaethylene glycol (11.5 mL, 46.0 mmol) were added sodium hydride (460 mg, 60% oil dispersion, 11.5 mmol) and 11-bromo-1-undecene (2.50 mL, 11.5 mmol) successively. The reaction mixture was stirred overnight at 100 °C, cooled to room temperature, and poured

into water. The aqueous layer was extracted four times with chloroform. Combined extracts were dried over $MgSO_4$ and concentrated in vacuo. The resulting residue was purified by column chromatography [silica gel: EtOAc/EtOH/water (25/2/1)] to give **1** (3.37 g, 67%) as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.75 (1H, ddt, J=17.1, 10.2, 6.8 Hz, CH_2=C*H*CH_2–), 4.94 (1H, dd, J=17.1, 2.0 Hz, CH_2=$CHCH_2$–), 4.87 (1H, dd, J=10.2, 2.0 Hz, CH_2=$CHCH_2$–), 3.68–3.51 (24H, m, –O(CH_2CH_2O)$_6$H), 3.39 (2H, t, J=6.8 Hz, –CH_2O(CH_2CH_2O)$_6$H), 2.89 (1H, br s, –O*H*), 1.99 (2H, q, J=6.8 Hz, CH_2=CHCH_2–), 1.52 (2H, qui, J=6.8 Hz, C_9H_{17}C$H_2$$CH_2$O–), 1.34–1.18 (12H, m, $C_3H_5C_6H_{12}C_2H_4O$–).

4.2.2. 30,31-Dithia-3,6,9,12,15,18,43,46,49,52,55,58-dodecaoxa-1,60-hexacontanediol (2). To a stirred solution of **1** (2.78 g, 6.40 mmol) in toluene (20 mL) were added thioacetic acid (4.76 mL, 64.0 mmol) and AIBN (2,2′-azobisisobutyronitrile) (525 mg, 3.20 mmol) successively. The reaction mixture was stirred for 20 h at 70 °C, cooled to room temperature, and concentrated in vacuo. The resulting residue was roughly purified by column chromatography [silica gel: EtOAc/EtOH/water (25/2/1)]. To the product in methanol (10 mL) was added sodium methoxide (173 mg, 3.20 mmol). The reaction mixture was stirred overnight at room temperature, neutralized with Dowex, and concentrated in vacuo. The resulting residue was purified by column chromatography [silica gel: EtOAc/EtOH/water (10/2/1)] to give **2** (2.49 g, 83%) as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.74–3.56 (48H, m, –O(CH_2CH_2O)$_6$H×2), 3.44 (4H, t, J=7.0 Hz, –CH_2O(CH_2CH_2O)$_6$H×2), 2.71 (2H, br s, –O(CH_2CH_2O)$_6$*H*×2), 2.68 (4H, t, J=7.3 Hz, –SCH_2–×2), 1.67 (4H, qui, J=7.3 Hz, –SCH_2CH_2–×2), 1.57 (4H, qui, J=7.0 Hz, C_9H_{17}C$H_2$$CH_2$O–×2), 1.39–1.27 (28H, m, –$SC_2H_4C_7H_{14}$–×2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 72.6, 71.5, 70.63, 70.59, 70.5, 70.43, 70.35, 70.1, 61.8 (–*C*H_2OH), 39.2 (–S*C*H_2–), 29.65, 29.58, 29.54, 29.50, 29.2, 28.5, 26.1; FAB HRMS *m*/*z* for $C_{46}H_{95}O_{14}S_2$ (M+H$^+$) calcd 935.6163, found 935.6171.

4.3. Preparation of monosaccharide derivatives

4.3.1. Allyl 2,3,6-tri-*O*-benzoyl-4-*O*-levulinoyl-α-D-glucopyranoside (5). To a stirred solution of allyl 2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside (**4**)[21] (1.97 g, 3.69 mmol), DMAP (4-(dimethylamino)pyridine) (90.2 mg, 738 μmol), and DCC (*N*,*N*′-dicyclohexylcarbodiimide) (914 mg, 4.43 mmol) in dichloromethane (10 mL) was added levulinic acid (454 μL, 4.43 mmol) dropwise at 0 °C and the reaction mixture was stirred for 15 h as it was allowed to slowly warm to room temperature. The mixture was then diluted with chloroform, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by column chromatography [silica gel: *n*-hexane/EtOAc (1/1)] to give **5** (2.32 g, 99%) as a colorless oil; $[\alpha]_D^{20}$ +150.7 (*c* 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11–7.35 (15H, m, Aromatic-*H*), 6.03 (1H, t, J=10.0 Hz, H-3), 5.84 (1H, dddd, J=17.2, 10.4, 6.1, 5.2 Hz, CH_2=C*H*CH_2–), 5.44 (1H, t, J=10.0 Hz, H-4), 5.33 (1H, d, J=3.7 Hz, H-1), 5.27 (1H, dq, J=17.2, 1.5 Hz, CH_2=$CHCH_2$–), 5.25 (1H, dd, J=10.0, 3.7 Hz, H-2), 5.13 (1H, dq, J=10.4, 1.5 Hz, CH_2=$CHCH_2$–), 4.59 (1H, dd, J=12.2, 2.3 Hz, H-6), 4.47 (1H, dd, J=12.2, 5.0 Hz, H-6), 4.34 (1H, ddd, J=10.0, 5.0, 2.3 Hz, H-5), 4.25 (1H, ddt, J=13.1, 5.2, 1.5 Hz, CH_2=CHCH_2–), 4.07 (1H, ddt, J=13.1, 6.1, 1.5 Hz, CH_2=CHCH_2–), 2.66–2.37 (4H, m, H_3CCOC*H*$_2$C*H*$_2$CO–O–), 2.00 (3H, s, *H*$_3$CCOCH_2CH_2COO–); ^{13}C NMR (100 MHz, $CDCl_3$): δ 205.8 (H_3C*C*OCH_2CH_2COO–), 171.5 ($H_3CCOCH_2CH_2$*C*OO–), 166.2 (C_6H_5*C*O–), 165.79 (C_6H_5*C*O–), 165.77 (C_6H_5*C*O–), 118.0 (*C*H_2=$CHCH_2$–), 95.2 (C-1), 71.8 (C-2), 70.6 (C-3), 69.0 (CH_2=CH*C*H_2–), 68.9 (C-4), 67.9 (C-5), 62.6 (C-6), 37.9 (H_3CCO*C*H_2CH_2COO–), 29.4 (H_3CCOCH_2*C*H_2COO–), 27.9 (H_3*C*COCH_2CH_2COO–); FAB HRMS *m*/*z* for $C_{35}H_{35}O_{11}$ (M+H$^+$) calcd 631.2179, found 631.2191.

4.3.2. 2,3,6-Tri-*O*-benzoyl-4-*O*-levulinoyl-α,β-D-glucopyranose (6). A solution of Ir{(COD)[PCH_3Ph_2]$_2$}PF_6 (156 mg, 184 μmol) in THF (3 mL) was stirred under hydrogen atmosphere for 5 min and the reaction mixture turned clear. After degassing and changing to nitrogen atmosphere, to the mixture was added **5** (2.32 g, 3.68 mmol) in THF (10 mL). The reaction mixture was stirred for 2 h at room temperature, and then were added acetic acid (10 mL) and 1 M HCl (2 mL). The reaction mixture was then stirred for 2 h at 70 °C, cooled to room temperature, and concentrated in vacuo with toluene. The residue was dissolved in EtOAc, washed with satd $NaHCO_3$ aq twice and brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by column chromatography [silica gel: *n*-hexane/EtOAc (2/1, 1/1)] to give **6** (2.02 g, 93%, α/β=ca. 5/1 by ^{1}H NMR) as a yellow oil; $[\alpha]_D^{20}$ +108.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13–7.34 (15H, m, α- and β-Aromatic-*H*), 6.07 (1H, t, J=10.0 Hz, α-H-3), 5.77 (1H, t, J=9.7 Hz, β-H-3), 5.70 (1H, t, J=3.7 Hz, α-H-1), 5.49 (1H, t, J=10.0 Hz, α-H-4), 5.44 (1H, t, J=9.7 Hz, β-H-4), 5.29 (1H, dd, J=9.7, 8.0 Hz, β-H-2), 5.23 (1H, dd, J=10.0, 3.7 Hz, α-H-2), 5.00 (1H, t, J=8.0 Hz, β-H-1), 4.64 (1H, dd, J=12.3, 2.3 Hz, α-H-6), 4.64 (1H, dd, J=12.3, 2.3 Hz, β-H-6), 4.54 (1H, ddd, J=10.0, 4.9, 2.3 Hz, α-H-5), 4.47 (1H, dd, J=12.3, 4.9 Hz, β-H-6), 4.43 (1H, dd, J=12.3, 4.9 Hz, α-H-6), 4.20 (1H, d, J=8.0 Hz, β-O*H*), 4.40 (1H, ddd, J=9.7, 4.9, 2.3 Hz, β-H-5), 3.84 (1H, d, J=3.7 Hz, α-O*H*), 2.64–2.34 (4H×2, m, α- and β-H_3CCOC*H*$_2$C*H*$_2$–), 1.98 (3H, s, α-*H*$_3$CCO–), 1.97 (3H, s, β-*H*$_3$CCO–); ^{13}C NMR (100 MHz, $CDCl_3$): δ 206.0 (α-H_3C*C*O–), 205.9 (β-H_3C*C*O–), 171.5 (α-$H_3CCOCH_2CH_2$*C*OO–), 171.3 (β-$H_3CCOCH_2CH_2$*C*OO–), 166.7 (β-C_6H_5*C*O–), 166.4 (α-C_6H_5*C*O–), 166.3 (β-C_6H_5*C*O–), 165.9 (α- and β-C_6H_5*C*O–), 165.8 (α-C_6H_5*C*O–), 96.0 (β-C-1), 90.4 (α-C-1), 74.2 (β-C-2), 72.5 (β-C-3 or β-C-5), 72.4 (β-C-3 or β-C-5), 72.2 (α-C-2), 70.3 (α-C-3), 68.8 (α- and β-C-4), 67.7 (α-C-5), 62.7 (β-C-6), 62.5 (α-C-6), 37.9 (α- and β-H_3CCO*C*H_2CH_2–), 29.4 (α- and β-H_3CCOCH_2*C*H_2COO–), 27.9 (α- and β-H_3*C*CO–); FAB HRMS *m*/*z* for $C_{32}H_{31}O_{11}$ (M+H$^+$) calcd 591.1866, found 591.1876.

4.3.3. 2,3,6-Tri-*O*-benzoyl-4-*O*-levulinoyl-α-D-glucopyranosyl 2,2,2-trichloroacetimidate (7). To a stirred solution of **6** (258 mg, 437 μmol) in dichloromethane (4 mL) were added trichloroacetonitrile (438 μL, 3.45 mmol) and DBU (13 μL, 87.4 μmol). The reaction mixture was stirred for 2 h at room temperature and directly purified by column chromatography [silica gel: *n*-hexane/EtOAc (5/2, 1/1)] to give **7** (281 mg, 87%) as a yellow oil; $[\alpha]_D^{20}$ +9.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.61 (1H, s, N*H*),

8.09–7.34 (15H, m, Aromatic-*H*), 6.78 (1H, d, J=3.7 Hz, H-1), 6.11 (1H, t, J=10.0 Hz, H-3), 5.57 (1H, t, J=10.0 Hz, H-4), 5.53 (1H, dd, J=10.0, 3.7 Hz, H-2), 4.65–4.61 (1H, m, H-6), 4.54–4.48 (2H, m, H-5 and H-6), 2.66–2.38 (4H, m, $H_3CCOCH_2CH_2$–), 1.99 (3H, s, H_3CCO–); ^{13}C NMR (100 MHz, $CDCl_3$): δ 205.6 (H_3CCO–), 171.5 ($H_3CCOCH_2CH_2COO$–), 166.1 (C_6H_5CO–), 165.7 (C_6H_5CO–), 165.4 (C_6H_5CO–), 160.5 (–OC(=NH)CCl_3), 93.1 (C-1), 90.7 (–OC(=NH)CCl_3), 70.64 (C-2 or C-5), 70.63 (C-2 or C-5), 70.3 (C-3), 68.0 (C-4), 62.1 (C-6), 37.9 ($H_3CCOCH_2CH_2$–), 29.4 ($H_3CCOCH_2CH_2$–), 27.9 (H_3CCO–); FAB HRMS *m/z* for $C_{34}H_{30}Cl_3NNaO_{11}$ ($M+Na^+$) calcd 756.0782, found 756.0771.

4.3.4. *p*-Methoxyphenyl 2,3,6-tri-*O*-benzoyl-4-*O*-levulinoyl-β-D-galactopyranoside (9). To a solution of *p*-methoxyphenyl 2,3,6-tri-*O*-benzoyl-β-D-galactopyranoside (**8**)[23] (1.83 g, 3.06 mmol), DMAP (74.8 mg, 612 μmol), and DCC (758 mg, 3.67 mmol) in dichloromethane (10 mL) was added levulinic acid (376 μL, 3.67 mmol) dropwise at 0 °C. The reaction mixture was stirred for 15 h as it was allowed to slowly warm to room temperature. The reaction mixture was then diluted with chloroform, washed with satd $NaHCO_3$ aq and brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by column chromatography [silica gel: *n*-hexane/EtOAc (1/1)] to give **9** (2.13 g, 89%) as a white powder; $[\alpha]_D^{20}$ +47.2 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.06–7.37 (15H, m, Aromatic-*H*), 6.94 (2H, d, J=9.1 Hz, Aromatic-*H*), 6.66 (2H, d, J=9.1 Hz, Aromatic-*H*), 5.93 (1H, dd, J=10.4, 8.0 Hz, H-2), 5.79 (1H, d, J=3.4 Hz, H-4), 5.52 (1H, dd, J=10.4, 3.4 Hz, H-3), 5.17 (1H, d, J=8.0 Hz, H-1), 4.64 (1H, dd, J=11.4, 7.6 Hz, H-6), 4.49 (1H, dd, J=11.4, 5.7 Hz, H-6), 4.32 (1H, dd, J=7.6, 5.7 Hz, H-5), 3.71 (3H, s, CH_3O–), 2.80–2.69 (4H, m, $H_3CCOCH_2CH_2$–), 2.12 (3H, s, H_3CCO–); ^{13}C NMR (100 MHz, $CDCl_3$): δ 205.9 (H_3CCO–), 171.8 ($H_3CCOCH_2CH_2COO$–), 166.0 (C_6H_5CO–), 165.6 (C_6H_5CO–), 165.3 (C_6H_5CO–), 155.8 (Aromatic-*C*), 151.1 (Aromatic-*C*), 119.0 (Aromatic-*C*), 114.5 (Aromatic-*C*), 101.2 (C-1), 71.7 (C-3), 71.5 (C-5), 69.5 (C-2), 67.6 (C-4), 62.0 (C-6), 55.6 (CH_3O–), 37.9 ($H_3CCOCH_2CH_2$–), 29.7 ($H_3CCOCH_2CH_2$–), 27.9 (H_3CCO–); FAB HRMS *m/z* for $C_{39}H_{37}O_{12}$ ($M+H^+$) calcd 697.2285, found 697.2292.

4.3.5. 2,3-Di-*O*-benzoyl-4-*O*-levulinoyl-α-D-galactopyranosyl 2,2,2-trichloroacetimidate (10). To a solution of **9** (1.90 g, 2.73 mmol) in toluene/CH_3CN/water (20 mL, 4/3/3 v/v/v) was added CAN (cerium(IV) ammonium nitrate) (13.1 g, 24.0 mmol) at 0 °C and the reaction mixture was stirred for 4.5 h as it was allowed to slowly warm to room temperature. The reaction mixture was then diluted with EtOAc, washed with water, satd $NaHCO_3$ aq twice and brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was roughly purified by column chromatography [silica gel: toluene/EtOAc (4/1, 1/1)] to give crude supposed hemiacetal. The product (1.20 g) was dissolved in dichloromethane, and to the solution were added trichloroacetonitrile (2.80 mL, 20.3 mmol) and DBU (62 μL, 406 μmol). The reaction mixture was stirred for 1.5 h at room temperature and directly purified by column chromatography [silica gel: toluene/EtOAc (5/2)] to give **10** (1.35 g, 80%) as a yellow oil; $[\alpha]_D^{20}$ +74.1 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.60 (1H, s, N*H*), 8.01–7.34 (15H, m, Aromatic-*H*), 6.83 (1H, d, J=3.6 Hz, H-1), 5.96 (1H, dd, J=9.5, 3.2 Hz, H-3), 5.90 (1H, dd, J=3.2, 1.0 Hz, H-4), 5.83 (1H, dd, J=9.5, 3.6 Hz, H-2), 4.75 (1H, ddd, J=6.9, 6.3, 1.0 Hz, H-5), 4.54 (1H, dd, J=11.4, 6.9 Hz, H-6), 4.42 (1H, dd, J=11.4, 6.3 Hz, H-6), 2.77–2.69 (4H, m, $H_3CCOCH_2CH_2$–), 2.10 (3H, s, H_3CCO–); ^{13}C NMR (100 MHz, $CDCl_3$): δ 205.7 (H_3CCO–), 171.8 ($H_3CCOCH_2CH_2COO$–), 165.9 (C_6H_5CO–), 165.6 (C_6H_5CO–), 165.5 (C_6H_5CO–), 160.6 (–OC(=NH)CCl_3), 93.8 (C-1), 90.8 (–OC(=NH)CCl_3), 69.6 (C-5), 68.2 (C-3), 68.1 (C-4), 67.7 (C-2), 61.9 (C-6), 37.8 ($H_3CCOCH_2CH_2$–), 29.7 ($H_3CCOCH_2CH_2$–), 27.8 (H_3CCO–); FAB HRMS *m/z* for $C_{34}H_{30}Cl_3NNaO_{11}$ ($M+Na^+$) calcd 756.0782, found 756.0797.

4.4. Carbohydrate elongation onto GCNP 3

4.4.1. Gold nanoparticle (3). To a solution of **2** in water (2.00 mL, 10 mM) were added MeOH (3 mL), potassium tetrachloroaurate(III) in water (35 μL, 270 μM), and sodium borohydride in water (200 μL, 100 μM) with vigorous stirring. The reaction mixture was stirred vigorously for 2 h at room temperature and passed through the centrifugal UF unit (3000*g*, 20 min) with methanol four times to yield supposed **3**.

4.4.2. Glycosylations and deprotections onto gold nanoparticle (Scheme 3). After co-evaporation with toluene, to **3** (2.79 μmol of calculated amount based on hydroxyl groups) in dichloromethane (3 mL) and acetonitrile (1 mL) were added **7** (42.5 mg, 57.8 μmol) and TMSOTf (10 μL, 55.3 μmol) at 0 °C under nitrogen atmosphere. The reaction mixture was stirred for 3 h as it was allowed to slowly warm to room temperature, diluted with acetonitrile, and passed through the centrifugal UF unit (3000*g*, 20 min) with acetonitrile four times to yield supposed **11**.

For acetyl capping of the remaining hydroxyl groups, to the product were added acetic anhydride (1 mL) and pyridine (2 mL). The reaction mixture was stirred for 12 h at room temperature, concentrated in vacuo, dissolved in mixed solvent, acetonitrile/methanol (1/1 v/v), and passed through the centrifugal UF unit (3000*g*, 20 min) with acetonitrile/methanol (1/1) four times.

To a solution of acetyl capped **11** in THF/methanol (2.5 mL, 1/1 v/v) were added acetic acid (40 μL) and hydrazine monohydrate (20 μL) at 0 °C. The reaction mixture was stirred for 3 h as it was allowed to slowly warm to room temperature, added acetic acid (1 mL), concentrated in vacuo, dissolved in mixed solvent, acetonitrile/methanol (1/1 v/v), and passed through the centrifugal UF unit (3000*g*, 20 min) with acetonitrile/methanol (1/1) four times to yield supposed **12**.

After co-evaporation of **12** with toluene, to **12** in acetonitrile (2 mL) were added **10** (48.5 mg, 66.0 μmol) and TMSOTf (10 μL, 55.3 μmol) at 0 °C under nitrogen atmosphere. The reaction mixture was stirred for 3 h as it was allowed to slowly warm to room temperature, neutralized with triethylamine (100 μL), diluted with acetonitrile, and passed through the centrifugal UF unit (3000*g*, 20 min) with acetonitrile four times to yield supposed **13**.

A solution of **13** in 0.5 M sodium methoxide in methanol (1.5 mL) was stirred for 12 h at room temperature. To the reaction mixture was added acetic acid (1 mL) and passed through the centrifugal UF unit (3000*g* 20 min) with methanol four times to yield supposed **14**.

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