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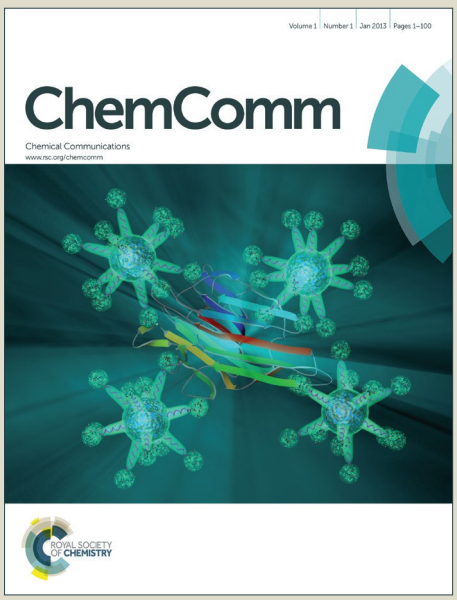

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N-Oxyamide-linked glycoglycerolipid coated AuNP for receptor-targeting imaging and drug delivery

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Synthesis of a series of new *N*-oxyamide-linked glycoglycerolipids and their assembly with gold nanoparticles for the receptor-targeting imaging and drug delivery are reported.

Glycolipids are known to play crucial roles in a variety of important biological processes such as cell-cell interactions, viral and bacterial infections, activation and modulation of the immune system, signal transduction and cell proliferation.[1] Glycolipids are composed of one or several monosaccharide residues linked by a glycosidic bond to a hydrophobic moiety, such as an acylglycerol (termed glycoglycerolipids – GGLs) or a ceramide (termed glycosphingolipids – GSLs). While the design of glycolipid mimics has been a useful strategy in drug discovery,[2-4] we[5] and others[6-8] have also demonstrated the interesting biological activities of synthetic glycolipids. Modifications have been made on the substitution and variation of the glycosyl moiety, the configuration and nature of the anomeric bond, the polar moieties of the ceramide or glycerol, and the length and location of the lipid chains.

Recently, we have been interested in the *N*-oxyamide-modified compounds because of their improved metabolic stability than the amide-linked counterparts,[9] and their interesting secondary structures produced by intramolecular H-bonding in *N*-oxyamide-linked peptides.[10] We have developed a methodology for the synthesis of *N*-oxyamide-linked glucoglycerolipids, by replacing the ester group in GGLs by an *N*-oxyamide, as new analogues of both GGL and GSL.[11] With continuing interest in *N*-oxyamide-modified biomolecules,[12-20] we report here the synthesis of a new series of galactoglycerolipids and glucoglycerolipids. Considering their amphiphilic properties and cellular targeting ability, the synthesized glycoglycerolipids have been used to self-assemble with a gold nanoparticle (AuNP). The produced AuNPs coated with galactoglycerolipids have been proven effective for receptor-targeting hepatocellular imaging and drug delivery (Fig. 1).

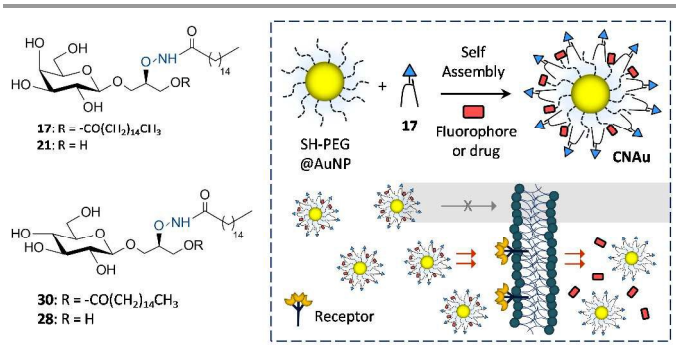

Fig. 1 Structure of the *N*-oxyamide-linked glycoglycerolipids used for bio-tests and schematic illustration of the self-assembly of a SH-PEG@AuNP with **17** for the receptor-targeting fluorophore/drug delivery.

The galactoglycerolipids were prepared from D-galactose pentaacetate and (*S*)-1,2-di-*O*-benzyl-glycerol **1** according to our previous strategy.[11] The D-galactose pentaacetate was converted to galactosyl bromide, which was then treated with benzylated glycerol **1** catalysed by $HgBr_2$ and $Hg(CN)_2$ in acetonitrile to give the β-D-galactopyranoside **2** in 75% yield (Scheme 1). Hydrogenolysis followed by a selective silylation gave alcohol **4**. Then, a Mitsunobu reaction with PhthN-OH smoothly produced the key *N*-phthaloyl oxyamine **5** in 97% yield. Subsequently, desilylation was carried out with catalytic AcCl in MeOH (instead of TBAF which led to partial deacetylation products). Then, a coupling with palmitic acid gave galactolipid **7**, and the phthaloyl group was removed by a controlled hydrazinolysis (1.1 equiv. at 0 °C) to avoid deacetylation. Acylation of oxyamine **8** with different fatty acids produced the *N*-oxyamide-linked galactolipids **9-13** in good yields. The NH signals appeared in the range of 8.81-8.87 ppm on the ^1H NMR spactra in $CDCl_3$ for these compounds.

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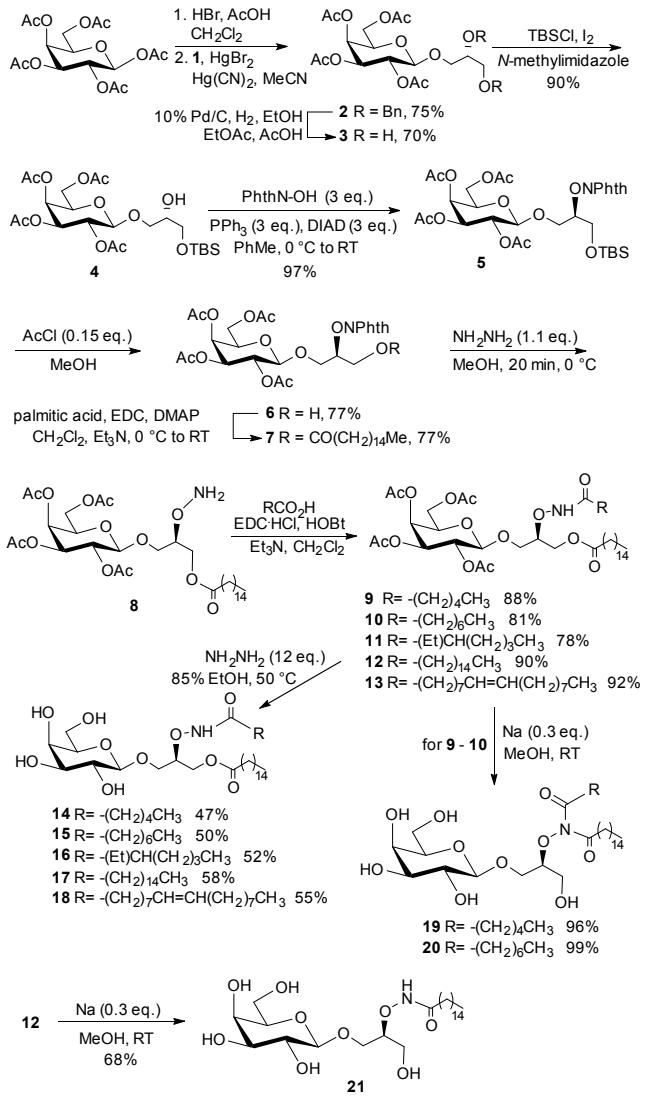
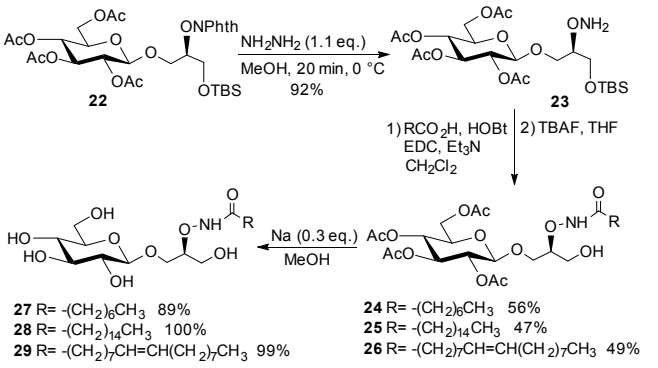


Scheme 1 Synthesis of galactoglycerolipids.

To obtain the deprotected galactoglycerolipids, we have tried different deacetylation conditions. The acetyl groups can be removed with 12 equivalents of hydrazine at 50 °C in EtOH, leading to the desired galactolipids **14-18**. Surprisingly, deacetylation of compounds **9** and **10** under Zemplan condition (Na/MeOH) gave the intramolecular transacylation products **19** and **20** in excellent yields, but this condition cleaved all the ester bonds of compound **12**, affording galactolipid **21**.

Using the similar protocol, we also prepared glucoglycerolipids with a single lipid chain by hydrazinolysis of a previously prepared *O*-phthalimido glucoglycerol **22**.[11] A subsequent coupling with fatty acids followed by removal of TBS and Zemplan deacetylation afforded the glucoglycerolipids **27**-**29** (Scheme 2).



Scheme 2 Synthesis of glucoglycerolipids.

With the *N*-oxyamide-linked glycoglycerolipids in hand, we tested their ability to self-assemble with an SH-PEG coated gold nanoparticle (AuNP),[21] producing a new class of glycoglycerolipid-based functional AuNPs. The glyconanoparticle formation was probably because of the insertion of amphiphilic glycolipids to the SH-PEG coated core-shell AuNP.[21] Galactolipid **17** was used because of its suitable lipid chain length and the targeting ability of galactose for hepatocellular receptors.[22-26] Shown in Fig. 2a are the scanning electron microscopic images of the AuNP and its ensemble with **17** (**CNAu**). Both AuNP and **CNAu** were observed to be well dispersed, whereas the addition of a galactose-selective peanut agglutinin (PNA) caused aggregation of **CNAu**. This is in agreement with previous reports on the aggregation of glyco-AuNPs caused by sugar-lectin recognition.[27-30] **CNAu** was determined to have good stability by a long-term incubation in a serum system (Fig. S1).

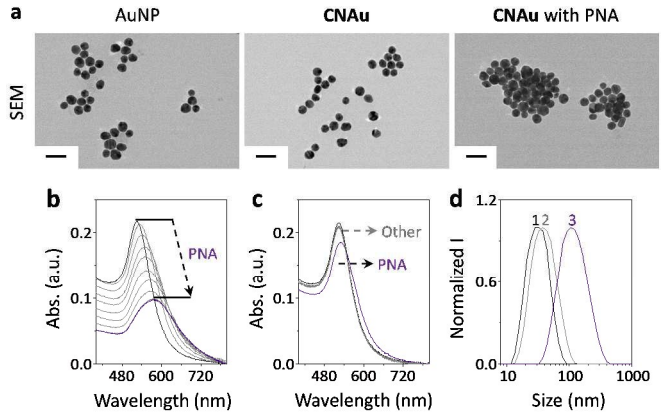


Fig. 2 (a) Scanning electron microscopic (SEM) images of AuNP, **CNAu** and **CNAu** with PNA (0.3 μM) (Scale bar: 50 μm). UV-vis absorption spectra of **CNAu** in the presence of (b) increasing PNA (0-0.3 μM) and (c) PNA or other proteins including the mannose-selective lens culinaris lectin, GalNAc-selective soybean agglutinin, GlcNAc-selective wheat germ agglutinin, bovine serum albumin and pepsin (0.05 μM each). (d) Dynamic light scattering of (1) AuNP, (2) **CNAu** and (3) **CNAu** with PNA (0.3 μM).

The aggregation was also corroborated by dynamic light scattering; the size of **CNAu** drastically increased in the presence of PNA (Fig. 2d and Fig. S2). A large difference in Zeta potential was observed between AuNP and **CNAu** (Fig. S3), suggesting the presence of **17** on the gold nanoparticles. The loading concentration of galactoglycerolipid **17** was determined to be 21.6 μg/mg AuNP by an an-

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throne/sulfuric acid method (Fig. S4). UV-vis spectroscopy suggests that the absorption band of **CNAu** gradually red-shifted (which is a signature of AuNP aggregation)[27-30] in the presence of increasing PNA (Fig. 2b), but not other unselective proteins (Fig. 2c). These data suggest the biospecificity of the **CNAu**. To test the generality of the glyco-AuNP formation with *N*-oxyamide-linked glycoglycerolipids, the mono-lipid galactoglycerolipid **21**, mono-lipid glucoglycerolipid **28** and a previously synthesized di-lipid glucoglycerolipid **30**[11] (Fig. 1) were used. Likewise, we observed that the absorption band of the formed glyco-AuNPs red-shifted with a selective lectin in a concentration-dependent manner (Fig. S5). The limit of detection of the di-lipid AuNPs was found to be lower than the mono-lipid counterparts (Fig. S1).

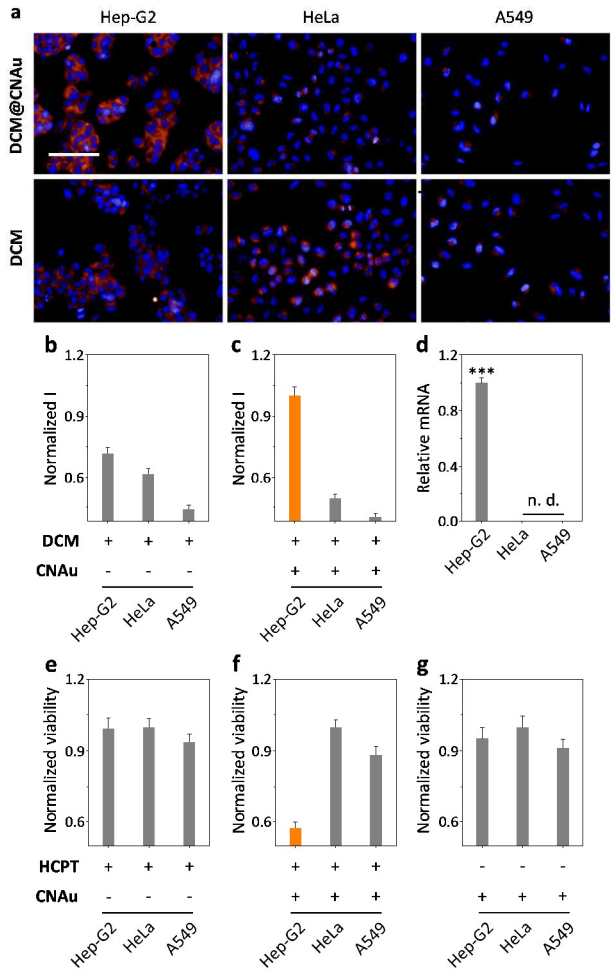

Fig. 3 (a) Fluorescence imaging of Hep-G2 (human liver cancer), HeLa (human cervix cancer) and A549 (human lung cancer) cells with **DCM** (1 μM) and **DCM@CNAu** (1/0.1 μM) (Scale bar: 100 μm; cell nucleus were stained by Hoechst 33342). Fluorescence quantification of different cells treated with (b) **DCM** and (c) **DCM@CNAu**. (d) Relative mRNA level of ASGPr of different cells determined by real-time quantitative polymerase chain reaction (***$P < 0.001$). Normalized viability of different cells treated with (e) **HCPT** alone (1 μM), (f) **HCPT@CNAu** (1/0.1 μM) and (g) **CNAu** alone (0.1 μM).

Next, we tested the ability of **CNAu** for hepatocellular imaging and drug delivery considering the selective recognition between galactose and the asialoglycoprotein receptor (ASGPr) on hepatocytes.[21-25] A red-emitting dicyanomethylene (**DCM**, Fig. S6)[31] was used to load **CNAu** for the fluorescence imaging of Hep-G2 (human liver cancer cell line) using HeLa (human cervix cancer cell line) and A549 (human lung cancer cell line) as control. We observed that, while **DCM** alone (Fig. 3a and 3b) and **DCM@AuNP** (i.e. **DCM** loaded with bare SH-PEG coated AuNP, Fig. S6) produced a similar level of fluorescence after incubation with different cells, association of **DCM** with **CNAu** (**DCM@CNAu**) largely enhanced the fluorescence imaging of the resulting material for Hep-G2 (Fig. 3a and 3c). This result is in agreement with the ASGPr expression level of the cells as determined by real-time quantitative polymerase chain reaction (Fig. 3d). Then, we used an anticancer drug, hydrocamptothecin (**HCPT**), to test the receptor-targeting drug delivery ability of **CNAu**. Interestingly, while a short-term (15 min) incubation of **HCPT** (Fig. 3e) or **CNAu** (Fig. 3g) alone did not induce cell death, the association of the drug with glyconanoparticle led to an evident suppression of the cell viability of Hep-G2, but not that of HeLa and A549 (Fig. 3f). We also determined that the receptor-targeting **HCPT** delivery was both concentration- and time-dependent (Fig. S7). These data suggest the promise of galactoglycerolipid-coated AuNPs for target-specific theranostics.

In summary, we have synthesized a series of *N*-oxyamide-linked glycoglycerolipids, which could be used to form a new type of glyco-AuNP capable of receptor-targeting cell imaging and drug delivery. This study provides an insight into the construction of glycomaterials for target-specific disease theranostics.[32]

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