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Oxime Ligation on the Surface of Mesoporous Silica Nanoparticles

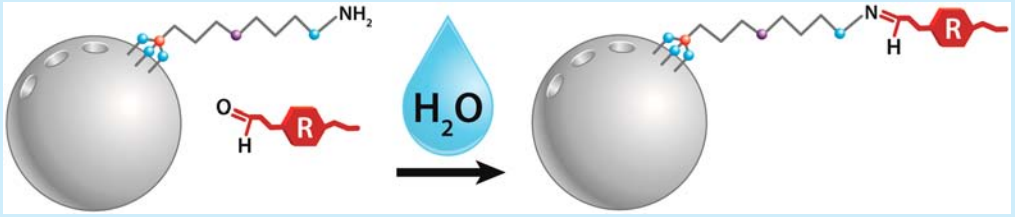
Daniel P. Ferris,[†,∥] Paul R. McGonigal,[†,∥] Leah S. Witus,[†,∥] Takatoshi Kawaji,[†,‡] Mohammed M. Algaradah,[†,§] Ahmed R. Alnajadah,[†,§] Majed S. Nassar,[†,§] and J. Fraser Stoddart*[,†]

[†]Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208-3113, United States

[‡]Department of Materials Science, National Institute of Technology, Wakayama College, Nada, Gobo, Wakayama 644-0023, Japan

[§]Joint Center of Excellence in Integrated Nanosystems, King Abdulaziz City for Science & Technology, Riyadh 11442, Kingdom of Saudi Arabia

Supporting Information



ABSTRACT: A versatile surface-functionalization strategy applicable to mesoporous silica nanoparticles, which could potentially serve as drug delivery vehicles, is described that makes use of alkoxyamine tethers on the surface of the nanoparticles. A wide variety of carbonyl compounds can be attached readily to these tethers under the mild conditions of oxime ether formation, simply by incubating the chemically modified mesoporous silica nanoparticles with aldehydes or ketones in water.

The development of drug delivery vehicles promises to improve the therapeutic efficacy of traditional chemotherapies in a number of ways. Carrier vehicles can concentrate pharmaceuticals within diseased tissues,[1] improve the pharmacokinetic properties[2] of drug candidates, and have the potential to achieve targeted drug delivery,[3] thereby reducing the exposure of healthy cells to harmful chemotherapeutics. Over the past decade, mesoporous silica nanoparticles[4] (MSNPs) have emerged as a particularly promising drug delivery platform on account of (i) their high surface areas and/or large interior volumes,[4] suitable for loading an array of therapeutic agents, (ii) their ease of modification,[5] which enhances their versatility, as well as (iii) their biocompatibility,[6] even at high doses of silica nanoparticles. Previously, we and other groups have explored novel (supra)molecular designs that exhibit stimulus-responsive mechanized release of payloads[7] and/or the formation of MSNP-targeting agent conjugates for selective recognition[8] by cells. To improve the performance of these "smart" MSNP-based materials for the widest number of applications possible, methods for the surface modification of MSNPs must be versatile yet simple. For applications in vivo, the milieu of biochemical reactions must not interfere with the surface attachment chemistry, resulting in unintended coupling reactions or leading to defective materials.

A wide variety of targeting groups are currently under investigation for pharmaceutical and drug delivery applications,[9] with the majority of them being based upon endogenous biomolecules, such as antibodies or folate. The introduction of site-specific attachment groups into these biomolecules can involve an intensive remodeling phase, involving chemical modification with functional handles (e.g., biotin), purification by substrate recognition chromatography, and then cross-coupling to produce the desired functional biomolecule conjugate. As the number of "state-of-the art" targeting molecules expands, highly modular surface attachment chemistries are required for their rapid integration with nanoparticle drug delivery vehicles such as MSNPs. In attempts to address this challenge, research into a single, but highly versatile surface chemistry for interfacial functionalization is desirable. Since most targeting groups are biomolecular in nature, we sought to create a method for surface attachment that uses mild, aqueous reaction conditions and functional groups that can be incorporated readily into biomolecules in a site-specific manner.

Herein, we report the development of a multipurpose, surface-modification chemistry for MSNPs using alkoxyamine tethers. This particular brand of reactive end group was selected because it is well-known to condense with carbonyl groups under mild conditions to form oxime ethers,[10] which are stable for days in a physiological setting. Besides the high-yielding and mild nature of this ligation, it also takes advantage of extremely convenient carbonyl coupling partners, namely aldehydes or ketones. For biomedical applications, carbonyl groups can be introduced into biomolecules using a variety of genetic, chemical, and enzymatic methods,[11] which are comparable with traditional biomolecule modification techniques. Aldehydes and ketones are also of significant interest for use in biological systems because they are not commonly found in

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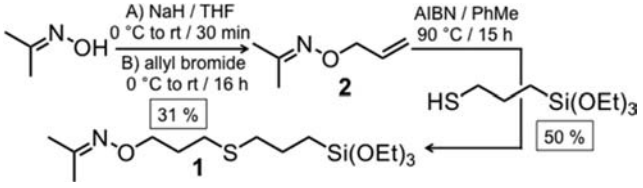
naturally occurring biopolymers but are often present in small-molecule drugs or metabolites, making the chemistry unresponsive to cellular machinery but reactive toward critical small molecules. Another aspect of the oxime ether tether we exploit here is its reversible formation. The dynamic nature of the oxime bond has enabled us to characterize the surface exchange between small molecules of various structural complexity with MSNPs and may facilitate future applications in the realm of mixed-monolayer nanoparticles. In this work, we have (i) developed an alkoxyamine silyl ether tether to decorate the surface of MSNPs, (ii) attached a variety of aldehyde- and ketone-containing small molecules, and (iii) quantified their attachment by exploiting their dynamic covalent chemistry in combination with ^{1}H NMR spectroscopy.

Nanoparticles (**MCM-41**) were prepared according to established literature procedures[12] using cetyltrimethylammonium bromide as a template for pore formation. Extraction using HCl in MeOH was performed twice to ensure removal of the template from the nanopores. The **MCM-41** nanoparticles were washed thoroughly in neutral solution prior to surface modification. Transmission electron microscopy (TEM), which was used to characterize (Supporting Information, Figure S1)[13] the nanoparticles at this stage, revealed that they are approximately 150 nm in diameter with regular, tessellated channels.

To install a reactive alkoxyamine tether for oxime ligation to aldehydes and ketones, a bifunctional linker **1** was designed. The linker consists of a triethoxysilane end group for attachment to the MSNPs, connected by a short dialkylthioether chain to a protected alkoxyamine. Acetone was selected as the protecting group because it could be removed to yield the free alkoxyamine under mild conditions, employing a dynamic covalent chemistry exchange protocol mediated by an excess of hydroxylamine. The linker **1** was prepared (Scheme 1) in two steps from the commercially available acetone oxime.

Scheme 1. Synthesis of Linker 1

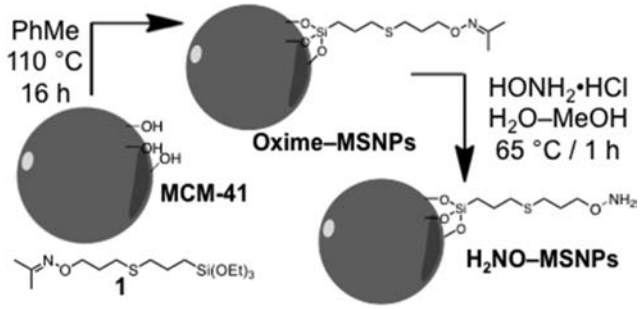


The synthetic sequence involved allylation to form acetone *O*-allyl oxime **2**[14] prior to thiol−ene coupling with 3-mercaptopropyl triethoxysilane in the presence of a radical initiator to form the linker **1**.[15]

The **MCM-41** nanoparticles were modified (Scheme 2) with linker **1** by stirring them together in PhMe at 110 °C for 16 h. Subsequently, the excess of **1** was removed by washing the nanoparticles in PhMe five times. The solvent was then exchanged by suspending the nanoparticles in MeOH, centrifuging, decanting the supernatant, and then repeating the procedure a total of four times. The resulting **Oxime-MSNPs**[16] were analyzed by TEM (Figure 1a−c), dynamic light scattering (Supporting Information, Table S2),[13] and scanning electron microscopy (Supporting Information, Figure S1)[13] in order to confirm that the integrity of the mesoporous silica scaffold is not adversely affected by the deposition of **1**.

To activate the **Oxime-MSNPs** for oxime ligation with an aldehyde or a ketone, the acetone protecting group was

Scheme 2. Chemical Modification of MCM-41



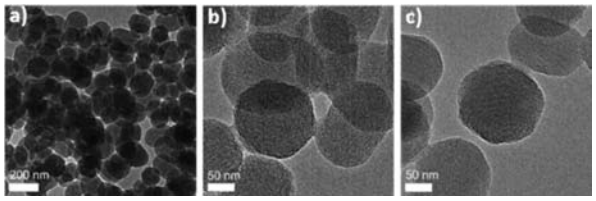


Figure 1. TEM images of **Oxime-MSNPs**: (a) 150,000× magnification; (b,c) 500,000× magnification of nanoparticles at two different locations on the dried nanoparticle aggregate.

removed, resulting in nanoparticles with free alkoxyamine tethers, namely **H_2NO-MSNPs**.[17] This deprotection was achieved by suspending the nanoparticles in a solution of hydroxylamine hydrochloride in aqueous acetate buffer and heating to 65 °C for 1 h. The resulting nanoparticles were washed by resuspending them in solvent, centrifuging, and decanting the solvent four times using a sequence of water−MeOH, MeOH, and then CH_2Cl_2. Experiments indicated (Supporting Information, Table S3)[13] that the proportion of acetone end groups that are deprotected to reveal the free hydroxylamine end group can be enhanced by repeating this sequence. For simplicity, however, subsequent substrate screening was performed using the **H_2NO−MSNPs** obtained after just one round of deprotection.

With the reactive **H_2NO−MSNPs** in hand, a variety of aldehyde- or ketone-containing compounds could be ligated to the nanoparticles using a mild and straightforward protocol. The ligation was achieved simply by suspending the nanoparticles in an aqueous solution of a carbonyl substrate and incubating at room temperature for 16 h. The functionalized nanoparticles were then rinsed twice with water, MeOH, and then CH_2Cl_2 in order to ensure that the excess of reagent was removed. One common challenge in the chemical functionalization of nanoparticles is quantifying the extent of attachment. In this system, we were able to develop a method to approximate the amount of carbonyl substrate ligated to the nanoparticle surface by exploiting the dynamic nature of the oxime bonds. After the carbonyl derivatives were tethered covalently to the nanoparticles, they could be cleaved off and into solution through exchange[18] with an excess of a competing carbonyl compound, e.g., acetone. ^{1}H NMR spectroscopy was then used to measure the amount of substrate in solution with maleic acid as an internal standard. A representative ^{1}H NMR spectrum that confirms the successful ligation and subsequent cleavage of salicylaldehyde is shown in Figure 2.

This dynamic exchange, followed by analysis employing ^{1}H NMR spectroscopy,[18] was used to assess (Table 1) the generality of the carbonyl ligation to the **H_2NO−MSNPs**. A

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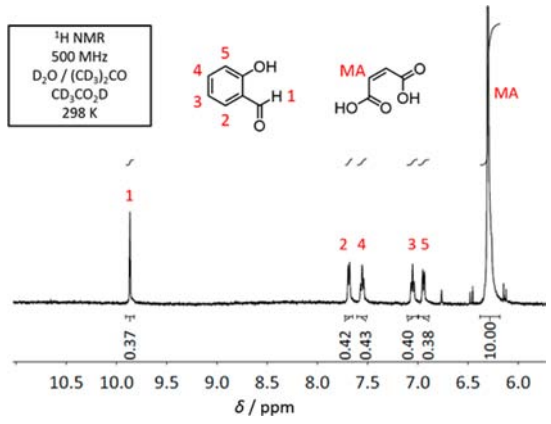

Figure 2. ^{1}H NMR spectrum (500 MHz, 298 K) of the supernatant obtained after treating salicylaldehyde-functionalized nanoparticles with 90:9:1 D_2O–$(CD_3)_2O$–D_3CO_2D containing maleic acid (8.6 mM) as internal standard.

Table 1. Ligation of Carbonyl Substrates

entry	substrate	mass of MSNPs (mg)	aldehyde cleaved[a] (nmol)	ratio (nmol/mg particle)
1	salicylaldehyde	83.7	342	4.1
2	3-formylpyridine	83.9	263	3.1
3	4′-hydroxy-acetophenone	82.2	140	1.7
4	pyruvic acid	79.8	215	2.7
5	cyclopentanone	87.9	1551	17.6
6	glucose	75.3	0	0
7	dimethylformamide	82.8	0	0
8	streptomycin sulfate	83.6	148	1.7

[a]Determined by integration of ^{1}H NMR peak areas relative to a maleic acid internal standard following oxime exchange.[13]

small selection of substrates was chosen to span a variety of different carbonyl derivatives. Aryl aldehydes (entries 1 and 2), aryl ketones (entry 3), and alkyl derivatives (entries 4 and 5) were all found to be compatible with the method without any need to alter the simple ligation conditions. Compounds that lack a ketone or aldehyde functional group were tested (entries 6 and 7) as negative controls to check for nonspecific interactions.

Neither dimethylformamide (DMF) nor glucose were observed to bind to the **H_2NO–MSNPs**, indicating that, in these cases at least, there are not any strong nonspecific interactions in the absence of a covalent attachment. This observation confirms the specificity of the surface functionalization and the validity of the method of quantification. It is particularly relevant to drug delivery and the use of biomolecules that the amide of DMF and the aldehyde of glucose's open-chain isomer do not appear to interfere with the ligation chemistry. Also of note, we screened a biologically relevant and rather more complicated formyl-containing compound, streptomycin sulfate, an antibiotic used to treat tuberculosis. Although we envision that the oxime surface chemistry will find use in the attachment of targeting groups, this example demonstrates that it can also be used to attach covalently any molecule containing an accessible and reactive carbonyl group to MSNPs.

Mesoporous silica nanoparticles provide a promising platform to entrap small molecules in their interior nanopores, as long as robust chemistry is available for the introduction of additional functionality to their exterior. We have developed an oxime-based surface modification chemistry and demonstrated its utility in attaching ketone- and aldehyde-containing substrates, as well as a structurally complex antibiotic. The chemistry is performed under mild aqueous conditions. At the same time, oxime formation[10] is selective for aldehydes and ketones, making it orthogonal to the majority of the bonding motifs present in biological molecules. The chemistry that we have developed offers a versatile platform that could enable the production of mixed-monolayer mesoporous silica nanoparticles.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, NMR spectroscopic characterization of small-molecule building blocks, and SEM, TEM, TGA, and IR characterization of nanoparticles. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: stoddart@northwestern.edu

Author Contributions

‖These authors contributed equally.

Notes

The authors declare no competing financial interest.

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(13) See the Supporting Information.
(14) **2**: A solution of acetone oxime (20.0 g, 274 mmol) in anhydrous THF (40 mL) was added carefully to a stirred suspension of NaH (329 mmol) in anhydrous THF (560 mL) at 0 °C. The mixture was allowed to warm to rt and stirred for 30 min before cooling to 0 °C again. Allyl bromide (47.4 mL, 548 mmol) was added slowly over 5 min before allowing the reaction mixture to warm to rt and stirring for 16 h. The crude reaction mixture was filtered through a pad of Celite to remove the insoluble inorganic salt, and the filtrate was concentrated at 35 °C and 200 mbar on a rotary evaporator, taking care to minimize evaporation of the volatile product. The residue was purified by column chromatography (220 g SiO_2, pentane as eluent) to give acetone *O*-allyl oxime **2** (9.5 g, 84.0 mmol, 31%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 5.99 (ddt, J = 17.3, 10.5, 5.6 Hz, 1H), 5.33−5.25 (m, 1H), 5.21−5.17 (m, 1H), 4.53 (dt, J = 5.6, 1.4 Hz, 2H), 1.88 (s, 6H). Spectroscopic data were consistent with those reported previously in Tarasova, O. A.; Shmidt, E. Y.; Sinegovskaya, L. M.; Petrova, O. V.; Sobenina, L. N.; Mikhaleva, A. I.; Brandsma, L.; Trofimov, B. A. *Russ. J. Org. Chem.* **1999**, *35*, 1581.
(15) **1**: Azobisisobutyronitrile (821 mg, 5 mmol) was added to a solution of **2** (2.04 g, 18.0 mmol) and 3-mercaptopropyl triethoxysilane (2.38 g, 10.0 mmol) in anhydrous PhMe (90 mL), and the resulting solution was heated to 90 °C for 15 h. The crude reaction was concentrated under reduced pressure, and the residue was purified by column chromatography (80 g SiO_2, hexanes−EtOAc), which was conducted as rapidly as possible to minimize product loss through condensation with the SiO_2 stationary phase. The title compound **1** (1.77 g, 9.0 mmol, 50%) was isolated as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 4.11 (t, J = 6.2 Hz, 2H), 3.84 (q, J = 7.0 Hz, 6H), 2.63−2.54 (m, 4H), 1.94 (tt, J = 7.4, 6.2 Hz, 2H), 1.89 (s, 3H), 1.87 (s, 3H), 1.77−1.68 (m, 2H), 1.25 (t, J = 7.0 Hz, 9H), 0.80−0.73 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 154.88, 71.89, 58.52, 35.26, 29.55, 28.62, 23.30, 22.04, 18.45, 15.69, 10.02. HRESI-MS m/z = 374.181 $[M+ Na]^+$ (calculated for $C_{15}H_{33}NO_4SiNa$ = 374.179).
(16) **Oxime−MSNPs**: The linker **1** (75 μL) was added to a rapidly stirred suspension of **MCM-41** (250 mg) in PhMe (25 mL) by slow injection using a 200 μL micropipet (Eppendorf). The mixture was heated to 110 °C for 16 h. The **Oxime-MSNPs** were cooled to rt and isolated by centrifugation. The nanoparticles were washed five times by suspension in fresh PhMe, centrifugation (5 min, 5000 rpm), and decanting of the supernatant. Residual PhMe was then removed by washing several times with MeOH. The nanoparticles were suspended in MeOH, centrifuged (15 min, 12000 rpm), and the supernatant decanted. This process was repeated three times with MeOH to ensure exchange from PhMe to MeOH. The **Oxime-MSNPs** were analyzed by DLS (Supporting Information, Table S2), SEM (Supporting Information, Figure S4), and TEM (Supporting Information, Figure S5) to ensure nanoparticle structure remained unchanged.
(17) **H_2NO−MSNPs**: The **Oxime−MSNPs** (2.0 g) were sonicated in MeOH (100 mL) and aqueous acetate buffer (100 mL) until a suspension was obtained. Hydroxylamine hydrochloride (2.0 g) was added, and the mixture was stirred at 65 °C for 1 h. After cooling to rt, the suspension was centrifuged and the supernatant was decanted. The resulting **H_2NO−MSNPs** were washed by resuspending in solvent, centrifuging, and decanting the solvent four times using, in sequence, H_2O−MeOH (2 × 100 mL), MeOH (100 mL), and CH_2Cl_2 (100 mL). After drying under vacuum overnight, **H_2NO−MSNPs** (1.8 g) were isolated and characterized using electron microscopy. Experiments monitoring the attachment of salicylaldehyde indicated (Supporting Information, Table S3) that the proportion of acetone protecting group that is deprotected to reveal the free hydroxylamine can be enhanced by repeating this deprotection and washing sequence. In this study, substrate screening was performed using the **H_2NO−MSNPs** obtained after one round of deprotection.
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