Magnetic Field-Induced Cyclizations of Amino-Esters and –Carbonates Bound to Iron Oxide Nanoparticles

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ABSTRACT

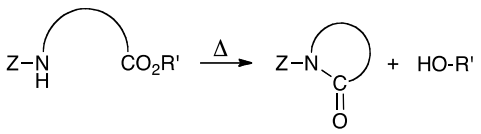
A panel of amino-esters and amino-carbonates was prepared and evaluated for their ability to undergo thermally-induced intramolecular cyclization to form the corresponding lactams and oxazolidinones. We found that *gem*-dimethylated carbonate **16**, the precursor to a 7-membered oxazolidinone, had ideal properties for use as a linker between drug and NP. To generate the heat necessary for intramolecular cyclization, we have devised a method to covalently load the amino-linkers onto Fe_3O_4 nanoparticles.

Keywords: iron oxide, nanoparticles, intramolecular cyclization, AMF, controlled release

1 INTRODUCTION

Intramolecular cyclizations, such as an intramolecular nucleophilic displacement of a pendant leaving group, lactonization, lactamization, or an intramolecular Diels-Alder cycloaddition, are often thermally induced [1, 2]. In cyclizations involving nucleophilic addition to a carbonyl of a carboxylic acid derivative, formation of the cyclic product is accompanied by release of another molecule; for example, an alcohol (e.g., HO-R', Figure 1) is displaced on lactamization. Thus, cyclization processes potentially can be harnessed as a means for release of small molecules, such as drugs or fluorophores [3]. We describe here the intramolecular cyclizations of amino-functionalized linkers as a method for controlled substrate release. Specifically, we have engineered the amino-linkers for attachment to iron oxide nanoparticles (NPs) to achieve control over cyclization and accompanying substrate release using the heat afforded by the NPs on exposure to an alternating magnetic field (AMF).

Figure 1



Lactamization, a representative heat induced intramolecular cyclization (Z = nanoparticle-based heat source).

Iron oxide, in particular Fe_3O_4, possesses many qualities that make it a great choice for drug delivery. Fe_3O_4 NPs are biocompatible [4], have low cytotoxicity [5], and provide multiple means for surface modification. Fe_3O_4 NPs are set apart from other NPs due to paramagnetic (or superparamagnetic) qualities [6]. The magnetic properties of Fe_3O_4 NPs have been used for a variety of applications including use as a heat source to induce local hyperthermia for treatment of cancer [7]. Even at low concentrations, AMF-induced Fe_3O_4 NPs can heat tissue to temperatures as high as 45 °C and cause cell death [8]. Furthermore, when Fe_3O_4 NPs are fitted with drugs, either through ionic association or by entrapment in a polymer gel coating containing the drug, the NPs can be guided to tumor regions using a magnet, as first demonstrated by Meyers in 1963 [9]. Through more advanced methods, Fe_3O_4 NPs also can be extensively functionalized with complex delivery mechanisms and be targeted to cancer-specific receptors [10].

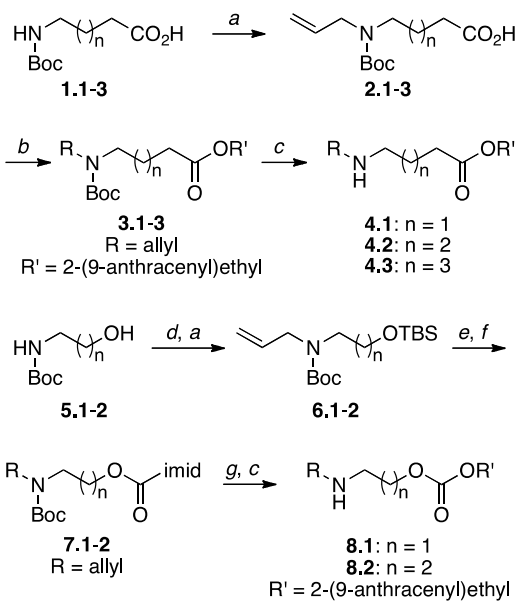
Generally, the AMF-induced heat is used to overcome ionic and/or hydrogen bonding interactions so that a bound drug releases, or to cause an encompassing polymer shell to either contract and force out a drug [11] or to expand and allow a drug to escape from the polymer matrix [12]. Despite the advantages of an external AMF trigger, present magnetic NP drug delivery systems have the problem of premature drug release (i.e., cargo leakage). In these instances, drugs may be slowly released prior to application of the magnetic field. This is largely due to the inability of the drugs in these systems to be covalently bound to the NPs.

To address this limitation, we have examined a panel of amino-esters and amino-carbonates for their ability to cyclize on heating. We sought to develop an optimal combination of amine-nucleophile and carbonyl-electrophile that would be unreactive at physiological temperature but undergo intramolecular cyclization at temperatures exceeding 37 °C. These properties would circumvent the need to mask the amine-nucleophile at the stage of injection into a patient, thus obviating the requirement for an internal unmasking mechanism (i.e., amine deprotection step). However, by engineering thermal stability into a linker, we also increase the thermal requirement for its cyclization to occur. Consequently, we envisioned attachment of the linker to iron oxide NPs as a means to achieve the high local temperature needed to induce efficient intramolecular cyclization. To develop a linker having the desired cyclization and release properties, we modified commercially available ω-amino acids to create a panel of amino-linkers for study.

2 RESULTS AND DISCUSSION

Treatment of the Boc-protected ω-amino acids **1.1-3** (Scheme 1) with excess NaH and allyl bromide afforded the *N*-allylated products **2.1-3**. We installed the allyl group as a progenitor of a terminal trialkoxysilane moiety necessary for ultimate covalent attachment of the amino-linker onto iron oxide NPs. Next, we esterified the panel with 2-(9-anthracenyl)-ethanol using standard carbodiimide conditions to yield the Boc-protected amino esters **3.1-3**. The Boc protection group was readily cleaved using trifluoroacetic acid (TFA) to provide the panel of amino esters **3.1-3** on base work-up. In similar fashion, carbonate esters were synthesized from ω-amino alcohols by first Boc-protecting the amine to afford **5.1-2** (Scheme 1) followed by alcohol silylation [13] and *N*-allylation using the established method. Alcohol deprotection (nBu_4NF) and esterification using an acyl imidazole approach [14] gave the corresponding carbonates **8.1-2** on TFA-mediated Boc-deprotection.

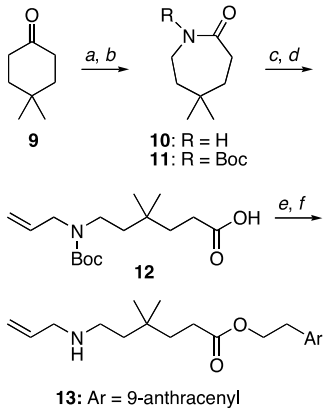
Scheme 1[a]



[a]Conditons: *a*. allyl bromide, NaH, THF, 0 °C to rt, 87%; *b*. 2-(9-anthracenyl)ethanol (**17**) (or the ethanamine), DIC, cat. DMAP, CH_2Cl_2, 12h, 59%; *c*. TFA, CH_2Cl_2, 0 °C, 1h, 100%; *d*. TBSCl, Et_3N, imidazole, CH_2Cl_2, 0 °C to rt, 98%; *e*. TBAF, THF, 0 °C to rt, 95%; *f*. $(imid)_2C=O$, $(i\text{-}Pr)_2NEt$, DCM, 0 °C to rt, 95%; *g*. **17**, KOH, toluene, 60 °C, 45%.

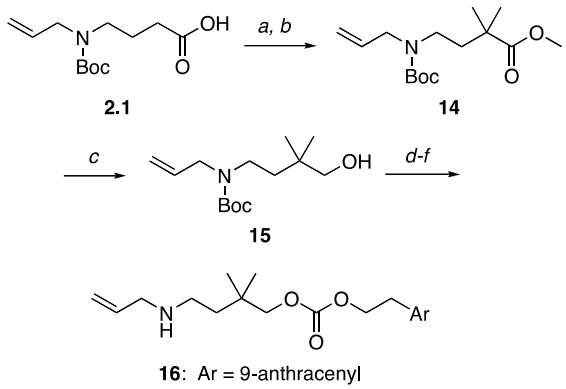
As first observed in 1915 [15], geminal dimethylation of an intervening carbon center can be used to reduce the temperature required for an intramolecular cyclization. This structural feature compresses the aliphatic C-C bond angles of the *gem*-dimethylated carbon from 115.3° to 109.5°. We hypothesized that the enhanced cyclization rate, known as the Thorpe-Ingold effect, may be used to improve the rates of cyclization for larger-ring amino-linkers. Consequently, we synthesized two additional linkers, *gem*-dimethyl amino-ester **13** (Scheme 2) and *gem*-dimethyl amino-carbonate **16** (Scheme 3), both precursors of seven-membered heterocyclic rings.

Scheme 2[a]



[a]Conditions: *a.* H_2NOSO_3H, HCO_2H, reflux, 73%; *b.* Boc_2O, DMAP, THF, reflux, 4h, 68%; *c.* LiOH, H_2O, THF, 60 °C, 96%; *d*. allyl bromide, NaH, THF, 0 °C to rt, 68%; *e*. **11**, DIC, cat. DMAP, CH_2Cl_2, 12h, 87%; *f.* TFA, CH_2Cl_2, 0 °C, 1h.

Scheme 3[a]



[a]Conditions: *a.*MeOH, DIC, cat. DMAP, rt, 80%; *b.*LHMDS, MeI, THF, -78 °C to rt, 67%; *c*. $LiBH_4$, THF, 0 °C to rt, 73%; *d*. $(imid)_2C=O$, $(i\text{-}Pr)_2NEt$, CH_2Cl_2, 0 °C to rt, 92%; *e*. 2-(9-anthracenyl)ethanol, NaH, THF, – 5 °C to rt, 48%; *f*. TFA, CH_2Cl_2, 0 °C, 1h.

Commercial 4,4-dimethyl-2-cyclohexen-1-one was reduced (H_2, Pd/C) to ketone 9 (Scheme 2) and then transformed via a Beckmann rearrangement in a one-pot synthesis using the method developed by Olah and Fung [16] to provide lactam 10. The lactam was Boc-protected to give imide 11. Hydrolysis (LiOH) followed by *N*-allylation and esterification as described above gave *gem*-dimethyl amino-ester 13 in good overall yield.

To prepare *gem*-dimethyl amino-carbonate **16** we pursued a bis-α-alkylation approach (Scheme 3). The methyl ester of previously prepared amino acid **2.1** was doubly methylated by reaction with excess LiHMDS and MeI [3]. Subsequent reduction of the ester moiety of **14** using $LiBH_4$ afforded alcohol **15**. Transformation to the target *gem*-dimethyl amino-carbonate **16** then was accomplished using the acyl imidazole approach as previously described followed by Boc-deprotection.

2.1 Choice of Drug Surrogate

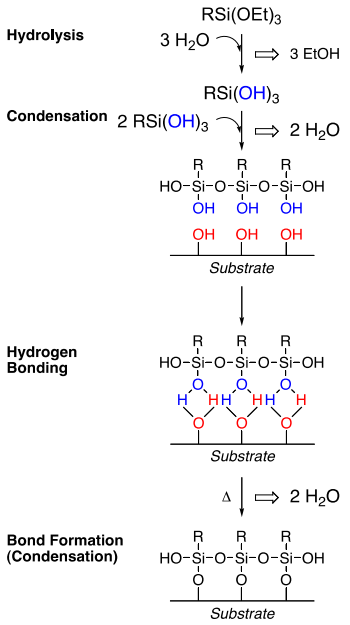
We selected 9-anthracene ethanol to serve as a drug surrogate in the linkers due to its ultraviolet (UV) and fluorescent (FL) properties. Anthracene is a highly UV active molecule that emits a blue fluorescent signal (λ 400-500 nm) under UV light, allowing for easy detection at low concentrations. The combination of the UV and FL attributes enables quantitative study of the linkers both on and off of iron oxide NPs. Initially, we aimed to use commercially available 9-anthracene methanol to prepare the amino-ester and amino-carbonate subtrates. Despite the ease of forming an anthracene ester using 9-anthracene methanol, we found that this particular ester was readily cleaved in the TFA-mediated Boc-deprotection steps. Homologation to the 9-anthracene ethanol [17] successfully prevented the undesired loss of drug surrogate on acid-mediated Boc-deprotection.

2.2 Hydrosilylation and NP Loading

Using a representative allyl amine, we followed the procedure described by Sabourault *et.al.* [18] to introduce a terminal triethoxysilane. PtO_2 catalyzed hydro-silylation of the allyl group yielded a triethoxysilane capable of bonding to the surface hydroxyl groups of the iron oxide NPs (Figure 2). Uniformity of the linker loading is crucial for delivery applications. As the loading density increases, the thermal energy provided by the NP is distributed over a larger area, thus reducing the efficacy of intramolecular cyclization and drug release. We found that ethanol, though commonly used as a solvent for loading alkoxysilanes onto NPs, caused inconsistent loading. To overcome this problem we adopted a procedure outlined by Galeotti [19] that utilized chloroform as the solvent.

As depicted in Figure 2, the process of forming a covalent bond between the trialkoxysilane and the iron oxide surface involves three-steps. First, the alkoxysilane linker is hydrolyzed *in situ* to a silanol that then hydrogen bonds to NP hydroxyl groups. The hydrogen bound linker remains bound throughout numerous washes, but may release from the NPs if exposed to organic solvent (e.g., MeCN) for an extended period. Finally, to effect covalent attachment, the hydrogen bound NP-linker complex must be heated at temperatures ≥ 100 °C; we found that application of a vacuum at ambient temperature is not sufficient.

Figure 2



Alkoxysilane methodology for bonding to NPs.

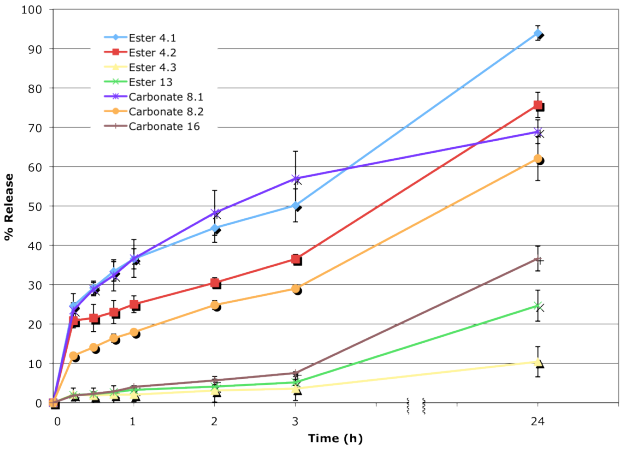
The high thermal requirement for covalent bond formation necessitated that the linkers be loaded onto iron oxide NPs as their Boc-protected counterparts. We were concerned that subsequent Boc-deprotection using TFA might cause hydrolysis of the key siloxane linkages. To test the stability of the Fe-O-Si bond, we synthesized *tert*-butyl allyl(methyl)carbamate, then hydrosilylated the alkene and loaded the resultant triethoxysilane substrate onto Fe_3O_4 NPs (20-30 nm average diameter, US Research Nanomaterials, Inc). Thermogravimetric analysis showed a loading density of 10.8 molecules/nm^2. The loaded NPs then were exposed to a solution of 1:1 TFA:CH_2Cl_2 for 1 h at room temperature before basification (aq. $NaHCO_3$). IR analyses confirmed the removal of Boc and TGA analysis showed that the loading density had reduced to 5.5 molecules/nm^2. To ascertain if the Boc-deprotected amines were functional, the amino-NPs were treated with a solution of fluorescein isothiocyanate (FITC). The resultant FITC-NPs were examined by FL spectrometry after ample washes to ensure removal of any unbound FITC. By comparison to control (i.e., unfunctionalized) NPs, we were gratified to observe that thiourea formation (FITC attachment) had indeed occurred.

2.3 Intramolecular Cyclizations

With a NP loading process in hand, we next analyzed the release rates of 9-anthracene ethanol (**17**, Scheme 4) from the synthesized panel of linkers. The cyclization rate of each linker was determined by warming at 55 °C in MeOH under dilute conditions (0.01 *M*). The release rate of anthracene **17**, an indication of the propensity for cyclization, was measured using normal phase HPLC. As

expected, the 5-membered lactam and oxazolidinone formed at rates significantly faster than the corresponding 6- and 7-membered lactams and oxazolidinones (Figure 3). However, the high performance of the carbonate substrates was pleasantly surprising, in particular the cyclization of the *gem*-substrate **16**. Whereas the dilute conditions were used to reduce the incidence of intermolecular reactions, these cannot be ruled out as a source of **17** especially with the linkers that lead to 7-membered rings. The results of the heat-induced cyclizations are depicted in Figure 3.

Figure 3



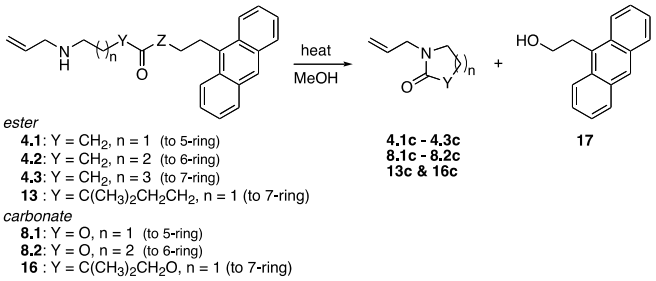
Heat-induced release of alcohol **17**. Shown are the standard deviations from the mean (n = 3).

The results from Figure 3 clearly show the effect of ring size on cyclization rates. **4.1c** and **8.1c** (5-membered rings) were formed too rapidly to withstand extended exposure at 37 °C. **4.2c** also was too prone to cyclize for the intended application. The effect of *gem*-dimethylation can be seen by comparing the cyclization of **4.3** to that of **13**. We noted a >10% increase in the release of **17** through *gem*-dimethylation. **16** provided the most promising results with only 5.7% ± 0.7 release at 37 °C and nearly 40% release at 55 °C over 24 h.

3 CONCLUSION

We have investigated the intramolecular cyclizations of a panel of amino-esters and amino-carbonates for their propensity to release 9-anthracene ethanol serving as a drug surrogate. The results indicate that the carbonate linkage is ideal for tethering a substrate when the intramolecular cyclization yields a 7-membered ring aided by *gem*-dimethylation. This particular combination of structural features ensures little reaction at physiological temperature yet leads to excellent substrate release on warming. Attachment of the optimal linkers to iron oxide nanoparticles for thermal triggering using an applied AMF is ongoing.

Scheme 4



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