

(51) International Patent Classification:
A61K 47/48 (2006.01)

(21) International Application Number:

PCT/US2015/044080

(22) International Filing Date:

6 August 2015 (06.08.2015)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/034,059

6 August 2014 (06.08.2014)

US

(71) Applicant: **UNIVERSITY OF LOUISVILLE RE-
SEARCH FOUNDATION, INC.** [US/US]; 300 East Mar-
ket Street, Suite 300, Louisville, Kentucky 40202 (US).

(72) Inventors; and

(71) Applicants : **NANTZ, Michael, H.** [US/US]; 300 East
Market Street, Suite 300, Louisville, Kentucky 40202
(US). **KNIPP, Ralph** [US/US]; 300 East Market Street,
Suite 300, Louisville, Kentucky 40202 (US).(74) Agents: **MICKELSON, John, W.** et al.; Viksnins Harris
& Padys PLLP, 7900 International Drive, Suite 670,
Bloomington, Minnesota 55425 (US).(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,
BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,
DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR,
KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG,
MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM,
PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC,
SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN,
TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ,
TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU,
TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE,
DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU,
LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK,

[Continued on next page]

(54) Title: NANOPARTICLES FOR DRUG DELIVERY

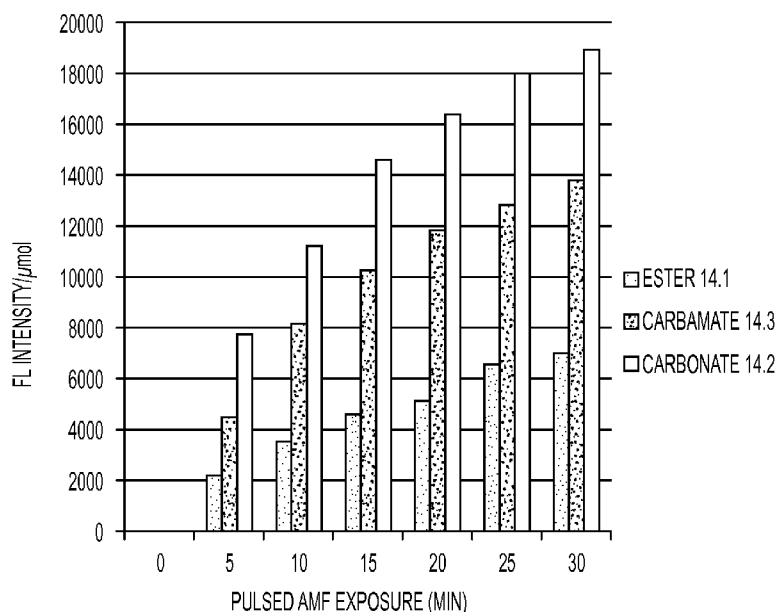


FIG. 17

[Continued on next page]



SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG). **Published:**

— with international search report (Art. 21(3))

(57) Abstract: The invention provides therapeutic magnetic nanoparticles containing a therapeutic agent connected to a magnetic nanoparticle core through a stable functional group and a linker that can be induced to release the therapeutic agent from the core, through hydrolysis of the functional group. Also provided are methods for making nanoparticles, and methods for using nanoparticles.

NANOPARTICLES FOR DRUG DELIVERY

Related Application

This application claims priority to U.S. Provisional Patent Application No. 62/034,059 filed
5 August, 6 2014, the entirety of which is incorporated herein by reference.

Background

An important attribute of a drug delivery system is its ability to allow for spatial and temporal regulated drug release, thereby minimizing side effects and improving therapeutic
10 efficacy of conventional pharmaceuticals. Iron oxide nanoparticles (NPs), specifically Fe_3O_4 nanoparticles, possess many appropriate qualities that make them a viable choice for drug delivery. Fe_3O_4 NPs are biocompatible (Kievit, F. M., et al., *Accounts of Chemical Research* **2011**, 44 (10), 853-862), have low cytotoxicity (Bulte, J. W. M., et al., *NMR in Biomedicine* **2004**, 17, 484-499), and provide multiple means for surface modification. Though these attributes are needed in a drug
15 delivery vehicle, there are multiple different NPs that possess similar qualities including gold and silica. Fe_3O_4 is set apart from these NPs due to its paramagnetic or superparamagnetic (SPM) qualities (Yang, C., et al., *Chemical Communications* **2011**, 47, 5130-5141). The SPM properties of Fe_3O_4 NPs have been used for a variety of applications. A basic utilization of SPM capability is to induce non-invasive hyperthermia within cancer cells. Alternating electromagnetic field (AMF)-
20 induced Fe_3O_4 NPs heat body tissue to temperatures as high as 45 °C, and this causes cell death. In addition, when functionalized either by ionic interactions or through entrapment via a polymer gel coating, drugs can be guided to tumor regions through the use of a magnet, as first demonstrated by Meyers in 1963 (Meyers, P.H., et al., *American Journal of Roentgenology, Radium Therapy, and Nuclear Medicine* **1963**, 90, 1068-1077). Through more advanced methods, Fe_3O_4 NPs are now
25 extensively functionalized with complex delivery mechanisms and can be directed by taking advantage of tumor folate receptors (Kim, J., et al., *Advanced Materials* **2008**, 20, 478-483, Zhang, Z., et al., *Biomaterials* **2007**, 28 (10), 1889-1899, Zhang, L., et al., *International Journal of Pharmaceutics* **2004**, 287 (1-2), 155-162). Finally, iron oxide also can be used as a magnetic resonance imaging contrast agent, so delivery systems based on this material can be visualized
30 (Lee, J. E.; et al., *Journal of the American Chemical Society* **2010**, 132, 552-557).

Some of the most common methods of functionalization or attachment of drug payloads to Fe_3O_4 NPs involve the use of ionic attraction (Nantz, M. H., et al., *PCT Int. Appl.* **2011**, WO 2011049972 A1 20110428), the addition of a mesoporous silica shell around the Fe_3O_4 NPs

followed by further functionalization of the silica (Meng, H., et al., A. E., *ACS Nano* **2010**, *4* (8), 4539-4550, Lin, Meng M., et al., *Nano Reviews* **2010**, *1*, 4883) or the use of a polymer coating around the Fe₃O₄ NPs (Yu, M. K., et al., *Angewandte Chemie International Edition* **2008**, *47* (29), 5362-5365, Rahimi, M., et al., *Nanomedicine: Nanotechnology, Biology, and Medicine* **2010**, *6*, 672-680). Once the NPs reach target (e.g., cancerous) tissue, a release mechanism is initiated so that the drug payloads are available only to the target tissue. One of the most common release methods involves use of a pH sensitive trigger, such as when using a hydrazone linkage (Aryal, S., et al., *Journal of Materials Chemistry* **2009**, *19*, 7879-7884). For example, when the loaded NP enters into a tumor, the reduced pH of the tumor can hydrolyze the hydrazone linkage to unmask the drug (a carbonyl-based drug). Another method of release is photochemical. By adding a photolabile group into a linker, usually an aromatic ring with a nitro-group ortho to a leaving group, the drug can be released upon exposure to a specific wavelength of light (Choi, S. K., et al., *Bioorganic & Medicinal Chemistry* **2012**, *20*, 1281-1290).

Another method to release the drug involves the use of an alternating electromagnetic field (AMF). An AMF, similar to an AC current, switches the poles of the magnetic current at a quick pace, and this causes resident iron oxide NPs to heat as they struggle to stay aligned with the applied magnetic field (Carrey, J., et al., *Journal of Applied Physics* **2011**, *109*, 083921). AMF-mediated drug delivery has a distinct advantage over the pH sensitive linker approach in that drug release relies on a controllable *external* stimulus whereas the acid labile linker requires a stimulus within the patient that cannot be easily controlled. If the tumor is not sufficiently acidic, then the linker-bound drug will not be released. In the same way, if certain healthy cells happen to be overly acidic, then the drug is released and can exert its pharmacological effect on healthy cells. In contrast, AMF exposure allows for the controlled release in a specific region and at a specific time without the need for precise, and often unpredictable, internal conditions. Thus AMF-mediated delivery systems offer the advantages of spatial and temporal control.

Despite the advantages in controlled release using an AMF trigger, many present NP drug delivery systems have a problem of premature drug release (i.e., leakage). In these instances, drugs are slowly released prior to application of the external stimulus. This is largely due to the inability of the drugs in these delivery systems to be covalently retained until the stimulus is applied. For example, AMF-induced NP heating commonly is used to reduce ionic interactions and/or hydrogen bonding interactions (Biswas, S. Functionalized Nanoparticles for AMF-Induced Gene and Drug Delivery. University of Louisville, Louisville, KY, 2011), or to cause a polymer shell to squeeze out the drug payload (Liu, T.-Y., et al., *Langmuir* **2008**, *24*, 13306-13311) or to expand and allow

the drug payload to diffuse away (Liu, J., et al., *Journal of Physical Chemistry C* **2010**, *114*, 7673-7679). In these cases, the ambient heat or biological milieu of a living system can reduce the ionic/hydrogen bonding interactions between NP and drug, or cause polymer contractions or expansions. Premature drug release occurs since the drug is not covalently attached to the NP carrier.

Patent application publication WO2014/124329 describes therapeutic magnetic nanoparticles which include a therapeutic agent connected to a magnetic nanoparticle through a linker and functional group. These nanoparticles include a linker that will undergo an intramolecular cyclization with the functional group thereby releasing the therapeutic agent from the magnetic nanoparticle core.

There is a need for alternative drug delivery systems with reduced premature release of its drug payload and that can optionally target the drug spatially, temporally, or both spatially and temporally.

Summary of Invention and Certain Embodiments

It has been discovered that therapeutic magnetic nanoparticles that connect a therapeutic agent to a magnetic nanoparticle core through a stable functional group and a linker can be induced to release the therapeutic agent from the core (e.g., through hydrolysis of the functional group). The release of the therapeutic agent can occur without the linker and functional group undergoing intramolecular cyclization.

It has also been discovered that therapeutic magnetic nanoparticles that connect a therapeutic agent to a magnetic nanoparticle core through a linker and functional group which include a linker comprising a protected amine moiety at a specified distance from the functional group can undergo deprotection of the protected amine followed by intramolecular cyclization with the functional group to release the therapeutic agent.

Accordingly, one embodiment provides a therapeutic magnetic nanoparticle, or a salt thereof, comprising a magnetic nanoparticle covalently bonded to one or more -L-D groups wherein D is a residue of a therapeutic agent (e.g., a residue of a therapeutic agent, a residue of a prodrug of a therapeutic agent or a residue of a functional group derivative of a therapeutic agent) and L is a linker, wherein:

L is -L¹-L²-;

each L^1 is covalently bonded to the magnetic nanoparticle and is independently a branched or unbranched chain or cyclic group or a combination of chain and cyclic groups that comprises 1-200 atoms; and

each L^2 is independently a group capable of releasing a therapeutic agent; and

5 wherein L is not capable of undergoing intramolecular cyclization to release the therapeutic agent.

One embodiment provides a therapeutic magnetic nanoparticle, or a salt thereof, comprising a magnetic nanoparticle covalently bonded to one or more -L-D groups wherein;

D is a residue of a therapeutic agent (e.g., a residue of a therapeutic agent, a residue of a prodrug of a therapeutic agent or a residue of a functional group derivative of a therapeutic agent);

10 L is a linker $-L^1-L^2-$;

each L^1 is covalently bonded to the magnetic nanoparticle and is independently a branched or unbranched chain or cyclic group or a combination of chain and cyclic groups that comprises 1-250 atoms; and

each L^2 is independently a group capable of releasing a therapeutic agent; and

15 wherein L is not capable of undergoing intramolecular cyclization to release the therapeutic agent.

One embodiment provides a therapeutic magnetic nanoparticle, or a salt thereof, comprising a magnetic nanoparticle covalently bonded to one or more -L-D groups wherein D is a residue of a therapeutic agent (e.g., a residue of a therapeutic agent, a residue of a prodrug of a therapeutic agent or a residue of a functional group derivative of a therapeutic agent) and L is a linker, wherein:

20 L is $-L^1-L^2-$;

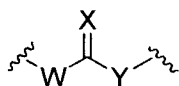
each L^1 is covalently bonded to the magnetic nanoparticle (e.g., the magnetic nanoparticle and/or the coating of a coated magnetic nanoparticle) and is independently a branched or unbranched chain or cyclic group or a combination of chain and cyclic groups that comprises 1-50 atoms; and

25 each L^2 is independently a group capable of releasing a therapeutic agent.

In certain embodiments L^2 can be hydrolyzed to release the therapeutic agent.

In certain embodiments L^2 can release the therapeutic agent by application of an alternating electromagnetic field to the magnetic nanoparticle.

In certain embodiments L^2 is:



W is absent, -O-, -S- or $-NR^b-$;

X is O, S or NR^c ;

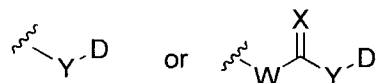
Y is absent, -O-, -S- or -NR^d;

R^b is H, (C₁-C₆)alkyl, (C₂-C₆)alkenyl or (C₂-C₆)alkynyl;

R^c is H, (C₁-C₆)alkyl, (C₂-C₆)alkenyl or (C₂-C₆)alkynyl;

R^d is H, (C₁-C₆)alkyl, (C₂-C₆)alkenyl or (C₂-C₆)alkynyl; and

5 wherein D together with Y of L² or D together with W-C(=X)-Y of L² is the moiety:



wherein the moiety is a residue of a therapeutic agent.

In certain embodiments the atoms of L¹ are selected from carbon, oxygen, nitrogen, sulfur and silicon.

10 In certain embodiments L¹ does not include an -NH-, -NH₂ or -NHR group wherein R is (C₁-C₆)alkyl.

In certain embodiments L¹ does not include a hydrazone group.

In certain embodiments L¹ does not include an oxime ether group.

In certain embodiments L¹ comprises 2-20 atoms.

15 In certain embodiments L¹ comprises 2-10 atoms.

In certain embodiments L¹ comprises 4-50 atoms.

In certain embodiments L¹ comprises 4-40 atoms.

In certain embodiments L¹ comprises 4-30 atoms.

In certain embodiments L¹ comprises 4-20 atoms.

20 In certain embodiments L¹ comprises 4-15 atoms.

In certain embodiments L¹ comprises 7-20 atoms.

In certain embodiments L¹ comprises 7-15 atoms.

In certain embodiments L¹ comprises 6-15 atoms.

In certain embodiments L¹ comprises 12-150 atoms.

25 In certain embodiments L¹ comprises 12-120 atoms.

In certain embodiments L¹ comprises 12-90 atoms.

In certain embodiments L¹ comprises 12-60 atoms.

In certain embodiments L¹ comprises 12-45 atoms.

In certain embodiments L¹ comprises 21-150 atoms.

30 In certain embodiments L¹ comprises 21-90 atoms.

In certain embodiments L¹ includes 1-200 atoms.

In certain embodiments L¹ includes 1-100atoms.

In certain embodiments L^1 includes 21-60 atoms.

In certain embodiments L^1 includes 2-20 atoms.

In certain embodiments L^1 includes 2-10 atoms.

In certain embodiments L^1 includes 4-50 atoms.

5 In certain embodiments L^1 includes 4-40 atoms.

In certain embodiments L^1 includes 4-30 atoms.

In certain embodiments L^1 includes 4-20 atoms.

In certain embodiments L^1 includes 4-15 atoms.

In certain embodiments L^1 includes 7-20 atoms.

10 In certain embodiments L^1 includes 7-15 atoms.

In certain embodiments L^1 includes 6-15 atoms.

In certain embodiments L^1 includes 12-150 atoms.

In certain embodiments L^1 includes 12-120 atoms.

In certain embodiments L^1 includes 12-90 atoms.

15 In certain embodiments L^1 includes 12-60 atoms.

In certain embodiments L^1 includes 12-45 atoms.

In certain embodiments L^1 includes 21-150 atoms.

In certain embodiments L^1 includes 21-90 atoms.

In certain embodiments L^1 includes 21-60 atoms.

20 In certain embodiments L^1 comprises a branched or unbranched chain.

In certain embodiments L^1 comprises a branched or unbranched chain comprising carbon atoms.

In certain embodiments L^1 is (C_1-C_{15}) alkylene, (C_1-C_{15}) heteroalkylene, (C_2-C_{15}) alkenylene or (C_2-C_{15}) alkynylene, wherein any (C_1-C_{15}) alkylene, (C_1-C_{15}) heteroalkylene, (C_2-C_{15}) alkenylene or (C_2-C_{15}) alkynylene of L^1 is optionally substituted with one or more halogen.

25 In certain embodiments L^1 is (C_1-C_{15}) alkylene, (C_2-C_{15}) alkenylene or (C_2-C_{15}) alkynylene, wherein any (C_1-C_{15}) alkylene, (C_2-C_{15}) alkenylene or (C_2-C_{15}) alkynylene of L^1 is optionally substituted with one or more halogen.

In certain embodiments L^1 comprises (C_1-C_{15}) alkylene, (C_1-C_{15}) heteroalkylene, (C_2-C_{15}) alkenylene or (C_2-C_{15}) alkynylene, wherein any (C_1-C_{15}) alkylene, (C_1-C_{15}) heteroalkylene, (C_2-C_{15}) alkenylene or (C_2-C_{15}) alkynylene of L^1 is optionally substituted with one or more halogen.

30

In certain embodiments L^1 comprises (C_1-C_{15}) alkylene, (C_2-C_{15}) alkenylene or (C_2-C_{15}) alkynylene, wherein any (C_1-C_{15}) alkylene, (C_2-C_{15}) alkenylene or (C_2-C_{15}) alkynylene of L^1 is optionally substituted with one or more halogen.

In certain embodiments L^1 is covalently bonded to the magnetic nanoparticle through a
5 silicon or sulfur atom.

In certain embodiments the magnetic nanoparticle further comprises a coating.

In certain embodiments the coating comprises gold.

In certain embodiments the coating comprises silica.

In certain embodiments the magnetic nanoparticle comprises iron.

10 In certain embodiments the magnetic nanoparticle comprises iron oxide.

In certain embodiments the magnetic nanoparticle is an iron oxide nanoparticle coated with silica.

In certain embodiments a magnetic nanoparticle is an iron oxide nanoparticle or a coated iron oxide nanoparticle.

15 In certain embodiments the magnetic nanoparticle is an iron alloy.

In certain embodiments the magnetic nanoparticle is an iron oxide alloy.

In certain embodiments the magnetic nanoparticle is a coated iron oxide nanoparticle.

In certain embodiments the magnetic nanoparticle is an iron oxide nanoparticle.

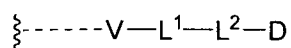
In certain embodiments the coated iron oxide nanoparticle is an iron oxide nanoparticle
20 coated with silica.

In certain embodiments the coated iron oxide nanoparticle is an iron oxide nanoparticle coated with gold.

In certain embodiments the magnetic nanoparticle is an iron oxide nanoparticle coated with silica.

25 In certain embodiments the magnetic nanoparticle is an iron oxide nanoparticle coated with gold.

Certain embodiments provide a therapeutic magnetic nanoparticle, or a salt thereof comprising a magnetic nanoparticle covalently bonded to one or more -L-D groups wherein each -L-D independently has the following formula I:

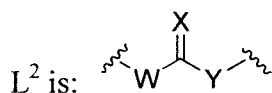


I

wherein:

V is -OSi(G)₂-, and the dashed line represents a covalent bond between the oxygen atom of -OSi(G)₂- and the magnetic nanoparticle; or V is -S-, and the dashed line represents a covalent bond between -S- and the magnetic nanoparticle;

L¹ is (C₁-C₁₅)alkylene, (C₁-C₁₅)heteroalkylene, (C₂-C₁₅)alkenylene or (C₂-C₁₅)alkynylene, wherein any (C₁-C₁₅)alkylene, (C₁-C₁₅)heteroalkylene, (C₂-C₁₅)alkenylene or (C₂-C₁₅)alkynylene of L¹ is optionally substituted with one or more halogen;



W is absent, -O-, -S- or -NR^b-;

X is O, S or NR^c;

Y is absent, -O-, -S- or -NR^d-;

each G is independently -OR^{a1}, -OR^{a2} or (C₁-C₆)alkyl;

R^{a1} is a covalent bond between the oxygen atom of -OR^{a1} and the magnetic nanoparticle;

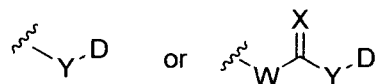
each R^{a2} is independently H or (C₁-C₆)alkyl; or two -OR^{a2} groups of two adjacent L-D groups together form -O-;

R^b is H, (C₁-C₆)alkyl, (C₂-C₆)alkenyl or (C₂-C₆)alkynyl;

R^c is H, (C₁-C₆)alkyl, (C₂-C₆)alkenyl or (C₂-C₆)alkynyl;

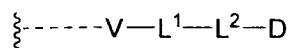
R^d is H, (C₁-C₆)alkyl, (C₂-C₆)alkenyl or (C₂-C₆)alkynyl; and

wherein D (a) together with Y of L² or (b) together with W-C(=Z)-Y of L² is the moiety:



wherein the moiety is a residue of a therapeutic agent.

Certain embodiments provide a therapeutic magnetic nanoparticle, or a salt thereof comprising a magnetic nanoparticle covalently bonded to one or more -L-D groups wherein each -L-D independently has the following formula I:



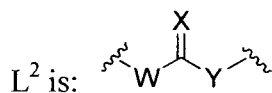
I

wherein:

V is -OSi(G)₂-, and the dashed line represents a covalent bond between the oxygen atom of -OSi(G)₂- and the magnetic nanoparticle; or V is -S-, and the dashed line represents a covalent bond between -S- and the magnetic nanoparticle;

L¹ is (C₁-C₁₅)alkylene, (C₁-C₁₅)heteroalkylene, (C₂-C₁₅)alkenylene or (C₂-C₁₅)alkynylene, wherein any (C₁-C₁₅)alkylene, (C₁-C₁₅)heteroalkylene, (C₂-C₁₅)alkenylene or (C₂-C₁₅)alkynylene

of L^1 is optionally substituted with one or more halogen and wherein the (C_1-C_{15}) heteroalkylene does not include nitrogen;



W is absent, -O-, -S- or -NR^b-;

X is O, S or NR^c;

Y is absent, -O-, -S- or -NR^d-;

each G is independently -OR^{a1}, -OR^{a2} or (C_1-C_6) alkyl;

R^{a1} is a covalent bond between the oxygen atom of -OR^{a1} and the magnetic nanoparticle;

each R^{a2} is independently H or (C_1-C_6) alkyl; or two -OR^{a2} groups of two adjacent L-D

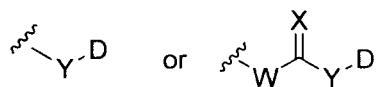
groups together form -O-;

R^b is H, (C_1-C_6) alkyl, (C_2-C_6) alkenyl or (C_2-C_6) alkynyl;

R^c is H, (C_1-C_6) alkyl, (C_2-C_6) alkenyl or (C_2-C_6) alkynyl;

R^d is H, (C_1-C_6) alkyl, (C_2-C_6) alkenyl or (C_2-C_6) alkynyl; and

wherein D (a) together with Y of L^2 or (b) together with W-C(=Z)-Y of L^2 is the moiety:



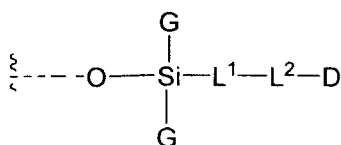
wherein the moiety is a residue of a therapeutic agent.

In certain embodiments V is ---OSi(G)₂-, the magnetic nanoparticle is optionally coated with silica, and the dashed line represents a covalent bond between the oxygen atom of -OSi(G)₂- and the magnetic nanoparticle optionally coated with silica; or V is ---S-, the magnetic nanoparticle is magnetic nanoparticle coated with gold, and the dashed line represents a covalent bond between -S- and the magnetic nanoparticle coated with gold.

In certain embodiments V is ---OSi(G)₂-, the magnetic nanoparticle is coated with silica, and the dashed line represents a covalent bond between the oxygen atom of -OSi(G)₂- and the magnetic nanoparticle coated with silica.

In certain embodiments V is ---OSi(G)₂-, the magnetic nanoparticle is an iron oxide nanoparticle coated with silica, and the dashed line represents a covalent bond between the oxygen atom of -OSi(G)₂- and the iron oxide nanoparticle coated with silica.

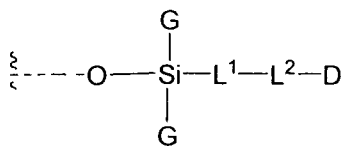
In certain embodiments -L-D has the following formula Ia:



Ia

wherein the dashed bond represents a covalent bond to the magnetic nanoparticle.

In certain embodiments the therapeutic magnetic nanoparticle is further coated with silica and -L-D has the following formula Ia:



Ia

wherein the dashed bond represents a covalent bond to the magnetic nanoparticle further coated with silica.

In certain embodiments each G is -OR^{a1} .

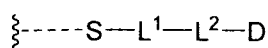
10 In certain embodiments each G is -OR^{a2} , wherein each -OR^{a2} together with another -OR^{a2} group on an adjacent L-D group forms an -O-.

In certain embodiments each G is -OR^{a1} or -OR^{a2} , wherein each -OR^{a2} together with another -OR^{a2} group on an adjacent L-D group form an -O-.

15 In certain embodiments V is ---S-, the magnetic nanoparticle is coated in gold, and the dashed line represents a covalent bond between -S- and the magnetic nanoparticle coated in gold.

In certain embodiments V is ---S-, the magnetic nanoparticle is coated in gold, and the dashed line represents a covalent bond between -S- and a gold atom of the magnetic nanoparticle coated in gold.

In certain embodiments -L-D has the following formula Ib:



Ib

wherein the dashed bonds represent a covalent bond to the magnetic nanoparticle.

In certain embodiments L^1 is $(\text{C}_2\text{-C}_{12})$ alkylene optionally substituted with one or more halogen.

25 In certain embodiments L^1 is $(\text{C}_2\text{-C}_{12})$ alkylene.

In certain embodiments L^1 is $(\text{C}_2\text{-C}_8)$ alkylene optionally substituted with one or more halogen.

In certain embodiments L^1 is $(\text{C}_2\text{-C}_6)$ alkylene.

In certain embodiments L^1 is $\text{-(CH}_2\text{)}_6\text{-}$, $\text{-(CH}_2\text{)}_3\text{-}$ or $\text{-(CH}_2\text{)}_4\text{-}$.

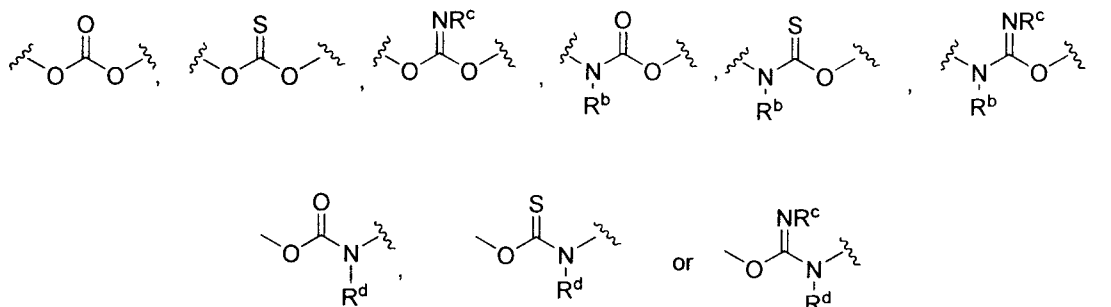
30 In certain embodiments L^1 is $\text{-(CH}_2\text{)}_3\text{-}$ or $\text{-(CH}_2\text{)}_6\text{-}$.

In certain embodiments W is -O- or $\text{-NR}^{\text{b}}\text{-}$.

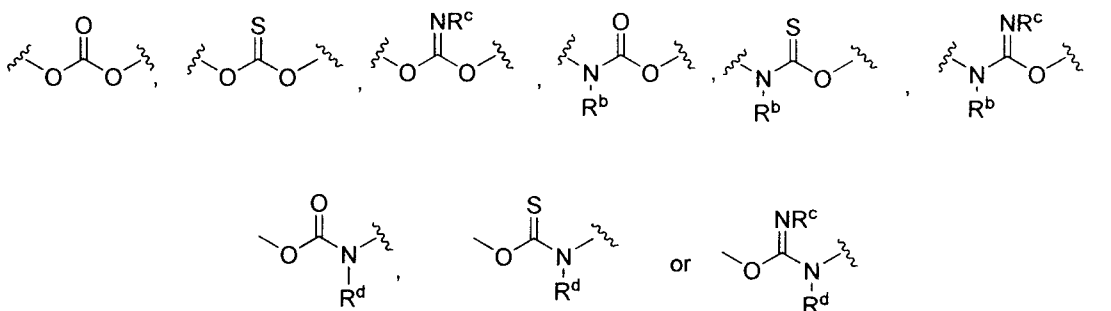
In certain embodiments X is O.

In certain embodiments Y is -O- or -NR^d-.

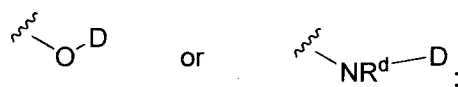
In certain embodiments L² is:



5 In certain embodiments L² is:



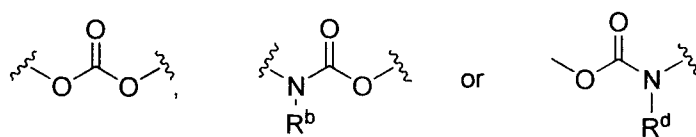
wherein D together with the -O- or -N(R^d)- of L² to which it is attached is the moiety:



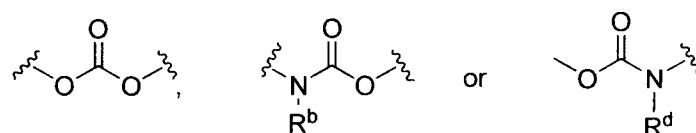
wherein the moiety is a residue of a therapeutic agent.

10 In certain embodiments R^b, R^c and R^d are each H.

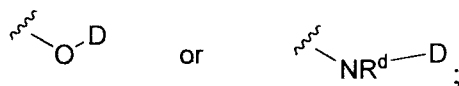
In certain embodiments L² is:



In certain embodiments L² is:

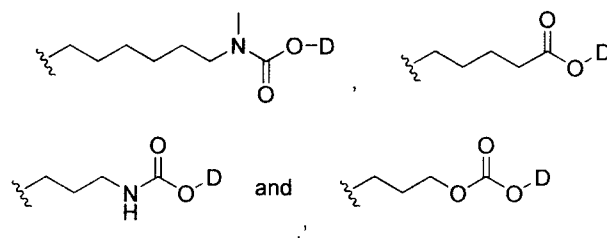


15 wherein D together with the -O- or -N(R^d)- of L² to which it is attached is the moiety:

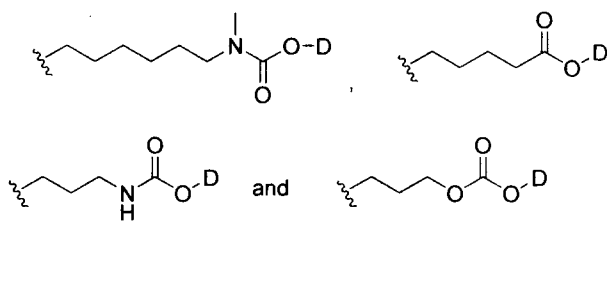


wherein the moiety is a residue of a therapeutic agent.

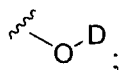
In certain embodiments -L¹-L²-D is selected from;



5 In certain embodiments -L¹-L²-D is selected from;



wherein D together with the -O- to which it is attached is the moiety:



wherein the moiety is a residue of a therapeutic agent.

10 It is to be understood that two or more of the embodiments discussed herein above and below may be combined.

In certain embodiments the therapeutic nanoparticle further comprises a targeting element.

One embodiment provides a pharmaceutical composition comprising a therapeutic magnetic nanoparticle or a pharmaceutically acceptable salt thereof (e.g., a magnetic nanoparticle covalently
15 bonded to one or more -L-D groups or a pharmaceutically acceptable salt thereof as described herein) and a pharmaceutically acceptable carrier.

One embodiment provides a method for administering a therapeutic agent to an animal (e.g., a mammal such as a human) comprising administering a therapeutic magnetic nanoparticle or a pharmaceutically acceptable salt thereof (e.g., a magnetic nanoparticle covalently bonded to one or
20 more -L-D groups, or a pharmaceutically acceptable salt thereof as described herein), to the animal.

One embodiment provides a method for treating cancer, a bacterial infection, a fungal infection, a parasitic infection or an antiviral infection in an animal (e.g., a mammal such as a human) in need thereof comprising administering an effective amount of a therapeutic magnetic

nanoparticle or a pharmaceutically acceptable salt thereof (e.g., a magnetic nanoparticle covalently bonded to one or more -L-D groups, or a pharmaceutically acceptable salt thereof as described herein), comprising providing conditions to release the therapeutic agent (D) from the therapeutic magnetic nanoparticle wherein the therapeutic agent is effective to treat cancer, a bacterial
5 infection, a fungal infection, a parasitic infection or an antiviral infection.

One embodiment provides a method for treating cancer, a bacterial infection, a fungal infection, a parasitic infection or an antiviral infection in an animal (e.g., a mammal such as a human) comprising treating the animal with a therapeutic magnetic nanoparticle or a pharmaceutically acceptable salt thereof (e.g., a magnetic nanoparticle covalently bonded to one or
10 more -L-D groups, or a pharmaceutically acceptable salt thereof as described herein), to the animal wherein the therapeutic agent (D) is effective to treat cancer, a bacterial infection, a fungal infection, a parasitic infection or an antiviral infection..

One embodiment provides a method for treating cancer, a bacterial infection, a fungal infection, a parasitic infection or an antiviral infection in an animal (e.g., a mammal such as a
15 human) in need thereof, comprising treating the animal with an effective amount of a therapeutic magnetic nanoparticle or a pharmaceutically acceptable salt thereof (e.g., a magnetic nanoparticle covalently bonded to one or more -L-D groups, or a pharmaceutically acceptable salt thereof as described herein), to the animal.

Certain embodiments provide magnetically targeting the therapeutic magnetic nanoparticle
20 to a specific location in the animal (e.g., a mammal such as a human).

Certain embodiments provide delivering a source of heat to the therapeutic magnetic nanoparticle to induce deprotection of a protected amine in the linker and cyclization of the linker thereby releasing the therapeutic agent from the therapeutic magnetic nanoparticle.

Certain embodiments provide applying an alternating electromagnetic field to the
25 therapeutic magnetic nanoparticle to induce deprotection of a protected amine in the linker and cyclization of the linker thereby releasing the therapeutic agent from the therapeutic nanoparticle (e.g., a mammal).

Certain embodiments provide for further treating the animal (e.g., a mammal) with one or more additional therapeutic agents.

30 In certain embodiments the additional therapeutic agent is iron oxide nanoparticle.

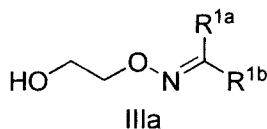
One embodiment provides a therapeutic magnetic nanoparticle or a pharmaceutically acceptable salt thereof (e.g., a magnetic nanoparticle covalently bonded to one or more -L-D

groups, or a pharmaceutically acceptable salt thereof as described herein), for use in medical therapy.

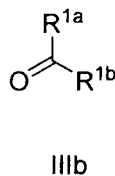
One embodiment provides the use of a therapeutic magnetic or a pharmaceutically acceptable salt thereof (e.g., a magnetic nanoparticle covalently bonded to one or more -L-D groups, or a pharmaceutically acceptable salt thereof as described herein), to prepare a medicament for treating cancer, a bacterial infection, a fungal infection, a parasitic infection or an antiviral infection in an animal (e.g., a mammal such as a human).

One embodiment provides a therapeutic magnetic nanoparticle or a pharmaceutically acceptable salt thereof (e.g., a magnetic nanoparticle covalently bonded to one or more -L-D groups, or a pharmaceutically acceptable salt thereof as described herein), for the therapeutic or prophylactic treatment of cancer, a bacterial infection, a fungal infection, a parasitic infection or an antiviral infection.

As described in Example 1 and 2 a ketone or aldehyde of a therapeutic agent has been converted to a prodrug of the therapeutic agent as shown in formula IIIa which prodrug is attached to the linker. Accordingly, one embodiment provides a therapeutic agent which is a prodrug of the therapeutic agent and is represented by formula IIIa:

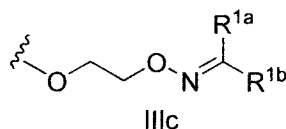


wherein R^{1a} and R^{1b} together with the remainder of formula IIIa are the prodrug of the therapeutic agent. It is to be understood that the prodrug of formula IIIa represents a therapeutic agent of formula IIIb (wherein R^{1a} and R^{1b} and the carbonyl to which they are attached represent a therapeutic agent):



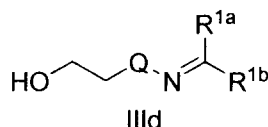
wherein the ketone or aldehyde of the therapeutic agent of formula IIIb has been condensed with the aminooxy moiety of HO-(CH₂)₂-O-NH₂ to arrive at the prodrug of the therapeutic agent of formula IIIa.

In one embodiment a residue of a therapeutic agent (which is a residue of a prodrug of a therapeutic agent) (D or -Z-D¹) is represented by formula IIIc:

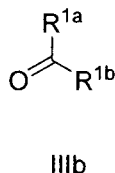


wherein R^{1a} and R^{1b} together with the remainder of formula IIIc are the residue of the therapeutic agent (D or -Z-D¹).

One embodiment provides a therapeutic agent which is a prodrug of the therapeutic agent
5 and is represented by formula IIIId:

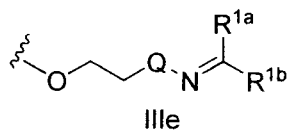


wherein Q is -O- or -NH- and R^{1a} and R^{1b} together with the remainder of formula IIIId are the prodrug of the therapeutic agent. It is to be understood that the prodrug of formula IIIId represents a therapeutic agent of formula IIIb (wherein R^{1a} and R^{1b} and the carbonyl to which they
10 are attached represent a therapeutic agent):



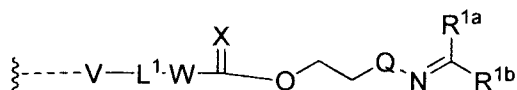
wherein the ketone or aldehyde of the therapeutic agent of formula IIIb has been condensed with the moiety of HO-(CH₂)₂-Q-NH₂ to arrive at the prodrug of the therapeutic agent of formula IIIId.

In one embodiment a residue of a therapeutic agent (which is a residue of a prodrug of a
15 therapeutic agent) (D or -Z-D¹) is represented by formula IIIe:



wherein Q is -O- or -NH- and R^{1a} and R^{1b} together with the remainder of formula IIIe are the residue of the therapeutic agent (D or -Z-D¹).

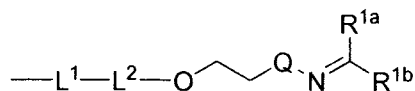
In one embodiment -L-D is represented by the formula:



20

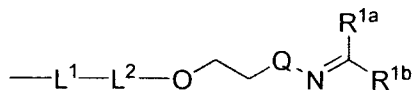
wherein V, L¹, W and X have the values described herein and Q is -O- or -NH- and R^{1a} and R^{1b} together with -O-(CH₂)₂-Q-N=C are the residue of the prodrug of the therapeutic agent.

In one embodiment -L-D is represented by the formula:



wherein L^1 , L^2 have the values described herein and Q is -O- or -NH- and R^{1a} and R^{1b} together with -O-(CH₂)₂-Q-N=C are the residue of the prodrug of the therapeutic agent.

In one embodiment -L-D is represented by the formula:



wherein L^1 has the values described herein, L^2 has the values described herein provided Y is absent and Q is -O- or -NH- and R^{1a} and R^{1b} together with -O-(CH₂)₂-Q-N=C are the residue of the prodrug of the therapeutic agent.

Brief Description of the Figures

Figure 1 illustrates the DLS of SiO₂@Fe₃O₄ NPs in Millipore water.

Figure 2 illustrates the ζ -Potential (-46.58 ± 0.47 mV) and mobility (-3.64 ± 0.04) of SiO₂@Fe₃O₄ NPs in Millipore water.

Figure 3 illustrates the size distribution (24.1 ± 0.6 nm) of SiO₂@Fe₃O₄ NPs as determined by TEM measurements.

Figure 4 illustrates SQUID measurement showing the superparamagnetism of SiO₂@Fe₃O₄ NPs.

Figure 5 illustrates the DLS of AO@SiO₂@Fe₃O₄ NPs in Millipore water.

Figure 6 illustrates the ζ -Potential (-44.56 ± 2.18 mV) and mobility (-3.48 ± 0.17) of AO@SiO₂@Fe₃O₄ NPs in Millipore water.

Figure 7 illustrates the Size distribution (30.4 ± 0.8 nm) of AO@SiO₂@Fe₃O₄ NPs as determined by TEM measurements.

Figures 8A and 8B illustrates the FT-IR of SiO₂@Fe₃O₄ NPs.

Figures 9A and 9B illustrates the FT-IR of AO@SiO₂@Fe₃O₄ NPs (8).

Figure 10 illustrates the TGA of SiO₂@Fe₃O₄ NPs ($\square$) and 8 AO@SiO₂@Fe₃O₄ NPs ($\bullet$).

Figure 11 illustrates the AMF-induced heating/cooling of 2:1 PBS:acetonitrile (bottom line), SiO₂@Fe₃O₄ NPs (middle line), AMF 5 min on/5 min off sequence (top line).

Figure 12 shows the MALDI-TOF of the radical carbocation (Macha, S. F.; et al., *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 731-737) of S5 (bottom) and 11 (top) after AMF-induced hydrolysis and release from FL@SiO₂@Fe₃O₄ NPs (9).

Figure 13 illustrates the AMF-triggered hydrolysis of pendant functionality (FL = anthracene fluorophore (•); carbamate moiety (darker shaded box in nanoparticle on left); terminal amine residue (lighter shaded box in nanoparticle on right)).

Figure 14A and 14B illustrate TEM images of (14A) $\text{SiO}_2@\text{Fe}_3\text{O}_4$ NPs used for synthesis of **5** (Scheme 1) and **8** (Scheme 2) and (14B) aminooxy-coated $\text{AO}@\text{SiO}_2@\text{Fe}_3\text{O}_4$ NPs (**8**).

Figure 15 illustrates AMF-induced release of anthracene fluorophore from NPs **5** and **6**. NPs (7 mg) suspended in 2:1 PBS:acetonitrile (0.75 mL) at pH 7.4 were irradiated with an AMF (501.6 amps, 204 kHz) for 5-minute bursts followed by 5-minute intervals. Vertical axis shows fluorescence intensity normalized to μmol of bound anthracene fluorophore as determined by thermogravimetric analysis.

Figure 16 AMF-induced release of **11** from $\text{FL}@\text{SiO}_2@\text{Fe}_3\text{O}_4$ NPs in PBS:acetonitrile were irradiated with an AMF (501.6 amps, 204 kHz) for 5-minute bursts followed by 5-minute intervals; release of **11** from $\text{FL}@\text{SiO}_2@\text{Fe}_3\text{O}_4$ NPs in PBS:acetonitrile were incubated at 37 °C for the indicated time. Vertical axis shows the percent of **11** released as determined by fluorescent measurements; n = 3.

Figure 17 illustrates the Pulsed AMF-induced hydrolysis of an ester (bar on left), carbamate (bar in the middle) and carbonate (bar on the right) bound to Fe_3O_4 NPs in 2:1 PBS:MeCN. AMF was set at 200 amps and 204 kHz.

Figure 18 illustrates the hydrolysis of an ester, carbamate and carbonate bound to Fe_3O_4 NPs without AMF. Functionalized NPs were incubated at 37 °C in 2:1 PBS:MeCN for 20 h.

Figure 19 illustrates the suppression of hydrolysis at 37 °C by restricting access to Fe_3O_4 core in 2:1 PBS:MeCN.

Figure 20 illustrates a coated magnetic nanoparticle. Figure 20A illustrates a fully coated magnetic nanoparticle; Figure 20B illustrates a partially coated magnetic nanoparticle; and Figure 20C illustrates a partially coated magnetic nanoparticle wherein the coating is non-contiguous (for example spotted).

Figure 21 illustrates the hydrolysis and condensation loading of alkoxysilane.

Figure 22 illustrates the AMF-induced heating/cooling of 2:1 PBS:acetonitrile (0.75 mL) (bottom line), $\text{SiO}_2@\text{Fe}_3\text{O}_4$ NPs (7 mg) in 2:1 PBS:acetonitrile (0.75 mL) (middle line), AMF 2 min on/off sequence (top line). AMF conditions: 500 amps, 204 kHz.

Figure 23 illustrates the AMF-induced release, as determined by fluorescence measurements, of **11** from $\text{FL}@\text{SiO}_2@\text{Fe}_3\text{O}_4$ NPs in 2:1 PBS:acetonitrile at AMF amperages of

100.7 (n=1), 300.2 (n=1) or 501.6 (n=3). The control, incubated at 37 °C for the indicated time, received no AMF pulses.

Figure 24 illustrates AMF-induced release of **11** from FL@SiO₂@Fe₃O₄ NPs during 2 minute (n=1) or 5 minute (n=3) AMF pulses at 501.6 amps versus incubation at 37 °C.

Detailed Description

Described herein are therapeutic magnetic nanoparticle drug delivery systems that are designed to reduce the problem of payload leakage. Magnetic nanoparticles can be covalently attached to a molecular linker wherein the linker is also covalently bound to a therapeutic agent (e.g., drug) through a functional group such as but not limited to an ester, carbonate or carbamate functional group. It has been discovered that therapeutic magnetic nanoparticles that link a therapeutic agent to a magnetic nanoparticle through a linker and a stable functional group can be induced to release (e.g., hydrolysis of the functional group) the therapeutic agent. In one embodiment the heat generated by the magnetic nanoparticle on AMF exposure induces separation of the therapeutic agent from the linker (e.g., through breaking the bond(s) of the functional group that connects the linker to the therapeutic agent). In one embodiment the functional group is hydrolyzed (e.g., by water or hydroxide ion) thereby releasing the therapeutic agent from the linker. This release mechanism provides a platform for the purposes of drug delivery with both spatial and temporal control.

Also described herein is an alternative therapeutic magnetic nanoparticle drug delivery system that is designed to reduce the problem of payload leakage. Magnetic nanoparticles can be covalently attached to a molecular linker containing a protected amine wherein the linker is also covalently bound to a therapeutic agent (e.g., drug) through a functional group such as through an ester, carbonate or carbamate functional group. By placing the protected amine at a specified distance from the ester, carbonate or carbamate carbonyl functional group, the protected amine can undergo deprotection followed by intramolecular cyclization, thereby releasing the bound therapeutic agent. In one embodiment the heat generated by the magnetic nanoparticle on AMF exposure induces the deprotection and thereby the subsequent intramolecular cyclization. This release mechanism also provides a platform for the purposes of drug delivery with both spatial and temporal control.

It is possible to target the therapeutic magnetic nanoparticles to a specific location in a patient's body, e.g., by magnetically guiding the nanoparticles to the target tissue and/or by

conjugating appropriate targeting elements (*e.g.*, an antibody fragment, a small molecule ligand of a cellular receptor) to the therapeutic nanoparticle.

In certain embodiments, the nanoparticles can be magnetically guided to the desired location in the body of the patient. This delivery system provides a method for delivering therapeutic agents including agents that are toxic when administered systemically by allowing for targeting of the drug to a specific location. Thus, this system is particularly useful for delivering drugs that are beneficially delivered to a specific location at a high concentration, *e.g.*, anticancer, antibiotic, antifungal, antiparasitic, and antiviral drugs. An advantage of this delivery system is the delivery of a therapeutic agent to a specific location and the release of the therapeutic agent at a specific time through the selective heating of the magnetic nanoparticle by exposure to an AMF.

The following definitions are used, unless otherwise described.

Magnetic nanoparticle.

Magnetic nanoparticles include any nanoparticles that possess paramagnetic or superparamagnetic (SPM) properties such as those paramagnetic or SPM properties of nanoparticles that comprise iron (iron nanoparticles) which for example include nanoparticles that comprise iron oxide (*e.g.*, iron oxide nanoparticles). The desirable paramagnetic or superparamagnetic (SPM) properties include properties that make the magnetic nanoparticle responsive to a magnetic field (*e.g.*, the magnetic nanoparticles will heat when exposed to an AMF). Thus, magnetic nanoparticles include iron nanoparticles such as nanoparticles comprising iron oxide (*e.g.*, Fe_3O_4 , the partially oxidized preparations $\text{Fe}_2\text{O}_3/\text{Fe}_3\text{O}_4$ or the fully oxidized Fe_2O_3). Magnetic nanoparticles also include metal alloys that possess the desired paramagnetic or superparamagnetic (SPM) properties such as those paramagnetic and SPM properties of iron nanoparticles (*e.g.*, iron oxide nanoparticles). Accordingly the term “magnetic nanoparticle” includes nanoparticle alloys, that possess magnetic properties such as but not limited to alloys of iron oxide (for a discussion on magnetic nanoparticle alloys see: Tang, Q., et al., Using Thermal Energy Produced by Irradiation of Mn-Zn Ferrite magnetic Nanoparticles (MZF-NPs) for Heat-Inducible Gene Expression. *Biomaterials* **2008**, 29, 2673-2679 which reference is incorporated herein in its entirety). It is to be understood that the amount of magnetic material (such as iron) in a magnetic nanoparticle can vary as long as the nanoparticle possesses the desired magnetic properties. Magnetic nanoparticles also include magnetic nanoparticles that are coated (*e.g.*, coated magnetic nanoparticles) by another substance or material such as but not limited to gold, graphene or silica. As used herein the term “coated magnetic nanoparticle” includes magnetic nanoparticles wherein the surface of the magnetic nanoparticle is coated (*e.g.*, fully or partially) by the substance

or material. It is to be understood the surface of the coated magnetic nanoparticle may be fully coated or partially coated and that when the coated magnetic nanoparticle is partially coated the coating may or may not be contiguous and the coating may be of any shape (e.g., spotted). In one embodiment the surface is at least 1%, at least 10%, at least 20%, at least 40%, at least 60%, at least 80%, at least 90% or completely covered by the substance or material. In one embodiment the core of a coated magnetic nanoparticle is magnetic but the coating may not be magnetic. In one embodiment the magnetic nanoparticle is coated with two or more different coatings. The size of the magnetic nanoparticle can vary. In one embodiment the size of the magnetic nanoparticle is about 1-750 nM in diameter. In one embodiment the size of the magnetic nanoparticle is about 1-500 nM in diameter. In one embodiment the size of the magnetic nanoparticle is about 1-250 nM in diameter. In one embodiment the size of the magnetic nanoparticle is about 1-150 nM in diameter. In one embodiment the size of the magnetic nanoparticle is about 1-50 nM in diameter. In one embodiment the size of the magnetic nanoparticle is about 5-750 nM in diameter. In one embodiment the size of the magnetic nanoparticle is about 5-500 nM in diameter. In one embodiment the size of the magnetic nanoparticle is about 5-250 nM in diameter. In one embodiment the size of the magnetic nanoparticle is about 5-150 nM in diameter. In one embodiment the size of the magnetic nanoparticle is about 5-50 nM in diameter.

Linker.

As described herein, the magnetic nanoparticles can be connected to a therapeutic agent through a linker. The linker can be (a) covalently bonded to the magnetic nanoparticle by at least one atom of the linker and (b) covalently bonded to a therapeutic agent at another atom of the linker. Thus, the linker can be covalently bonded to the magnetic nanoparticle or if the magnetic nanoparticle is coated it can be covalently bonded to the coating. It is also to be understood that if a magnetic nanoparticle is coated some of the linkers can be covalently bonded to the coating and some of the linkers can be covalently bonded to the magnetic nanoparticle. For example, the linker can be covalently bonded to the iron oxide magnetic nanoparticle through a silicon atom of the linker. The linker can also be bonded to a coated magnetic nanoparticle, such as a silica coated magnetic nanoparticle through a silicon atom of the linker. The linker can also be bonded to a coated magnetic nanoparticle, such as a gold-coated magnetic nanoparticle. For example, a sulfur atom of a linker can be covalently bonded to a gold atom of a gold-coated magnetic nanoparticle. The linker can be bonded to the magnetic nanoparticle wherein the magnetic nanoparticle is further

coated; thus the magnetic nanoparticle is coated but the linker is bonded to the magnetic nanoparticle and not the coating.

In one embodiment the linker can be covalently bound to the therapeutic agent via a functional group (e.g., ester, amide, carbonate, carbamate, urea, thioester, thioamide, thiocarbonate, thiocarbamate, thiourea) that allows the therapeutic agent to be separated from linker when a bond connecting the functional group to therapeutic agent is broken (e.g., through the hydrolysis of the functional group). In one embodiment the linker is not capable of undergoing intramolecular cyclization. In one embodiment the hydrolysis is facilitated by heat. In one embodiment the hydrolysis is facilitated by AMF.

In one embodiment the linker can be covalently bound to the therapeutic agent via a functional group (e.g., ester, amide, carbonate, carbamate, urea, thioester, thioamide, thiocarbonate, thiocarbamate, thiourea) that allows the therapeutic agent to be cleaved from the functional group when the linker containing a protected amine undergoes deprotection and intramolecular cyclization as described herein (a protected amine is deprotected to provide a nucleophilic amine that undergoes intramolecular cyclization). The therapeutic agent is generally connected to a carbonyl or thiocarbonyl moiety of the functional group via a labile bond. Thus, when the linker undergoes intramolecular cyclization the bond connecting the therapeutic agent to the carbonyl or thiocarbonyl moiety (for example a bond such as an oxygen, nitrogen or sulfur bonded to either the carbonyl or thiocarbonyl) of the functional group is broken thereby releasing the therapeutic agent from the linker. In one embodiment, the linker, upon heating (e.g., upon AMF irradiation of the attached magnetic NP), undergoes amine deprotection and intramolecular cyclization thereby releasing the therapeutic agent from the linker. The intramolecular cyclization generally occurs through reaction of an amine nitrogen within the linker and the carbon of the carbonyl carbon or thiocarbonyl carbon of the functional group that connects the therapeutic agent to the linker.

The linkers described herein can vary in length and composition and be branched or non-branched. In one embodiment the linker is a chain (non-cyclic) that is branched or non-branched.

In one embodiment the linker comprises about 4-50 atoms in the linker. In one embodiment the linker comprises about 4-40 atoms in the linker. In one embodiment the linker comprises about 4-30 atoms in the linker. In one embodiment the linker comprises about 4-20 atoms in the linker.

In one embodiment the linker comprises about 4-15 atoms in the linker. In one embodiment the linker comprises about 7-50 atoms in the linker. In one embodiment the linker comprises about 7-40 atoms in the linker. In one embodiment the linker comprises about 7-30 atoms in the linker. In one embodiment the linker comprises about 7-20 atoms in the linker. In one embodiment the linker

comprises about 7-15 atoms in the linker. In one embodiment the linker comprises about 6-15 atoms in the linker. In one embodiment the linker comprises about 7-14 atoms in the linker. In one embodiment the linker comprises about 8-14 atoms in the linker. In one embodiment the linker comprises about 9-13 atoms in the linker. In one embodiment any of the above the atoms are
5 independently selected from carbon, nitrogen, oxygen, sulfur and silicon. In one embodiment any of the above the atoms are independently selected from carbon, nitrogen, oxygen, sulfur and silicon provided the linker contains at least one protected amine and one group selected from (C=O) and (C=S). In one embodiment no oxygen, nitrogen, silicon or sulfur are directed bonded (e.g., adjacent) to another oxygen, nitrogen, silicon or sulfur. In one embodiment no oxygen, nitrogen or
10 sulfur are directed bonded (e.g., adjacent) to another oxygen, nitrogen or sulfur. In one embodiment the linker is covalently attached to the magnetic nanoparticle or the coated magnetic nanoparticle by a silicon or sulfur atom. It is to be understood that the atoms that make up the linker include hydrogen atoms to fulfill the valency requirements of any carbon, nitrogen, oxygen, sulfur and silicon atom of the linker. Thus, any of the atoms of the linker are independently
15 selected from carbon, nitrogen, oxygen, sulfur, silicon and hydrogen. In one embodiment the linker comprises about 12-150 atoms in the linker. In one embodiment the linker comprises about 12-120 atoms in the linker. In one embodiment the linker comprises about 12-90 atoms in the linker. In one embodiment the linker comprises about 12-60 atoms in the linker. In one embodiment the linker comprises about 12-45 atoms in the linker. In one embodiment the linker comprises about
20 21-150 atoms in the linker. In one embodiment the linker comprises about 21-120 atoms in the linker. In one embodiment the linker comprises about 21-90 atoms in the linker. In one embodiment the linker comprises about 21-60 atoms in the linker. In one embodiment the linker comprises about 14-45 atoms in the linker. In one embodiment the linker comprises about 18-45 atoms in the linker. In one embodiment the linker comprises about 21-42 atoms in the linker. In
25 one embodiment the linker comprises about 24-42 atoms in the linker. In one embodiment the linker comprises about 27-39 atoms in the linker. In one embodiment, for any of the above embodiments the linker is the atom range specified.

It is to be understood that the magnetic nanoparticle may be bonded with multiple linker groups and that some of these groups are adjacent (e.g., in close proximity) to one another. In such
30 situations it is possible that certain groups of the adjacent linkers may interact (e.g., be bonded to each other). One example of this would include linkers which comprise a silicon atom wherein the silicon atoms on adjacent linkers can be connected to one another via a bridging oxygen atom (e.g., -O-).

Therapeutic agent

The term “therapeutic agent” includes agents that are useful for the treatment of a disease or a physiological condition in an animal (e.g., a mammal such as a human) and thus includes known drugs. Thus, the term “therapeutic agent” includes but is not limited to known drugs and/or drugs that have been approved for sale in the United States. For example, therapeutic agents include but are not limited to chemotherapeutic agents, antibiotic agents, antifungal agents, antiparasitic agents and antiviral agents. The term “therapeutic agent” also includes “prodrugs” of such therapeutic agents or drugs. The term “therapeutic agent” also includes functional group derivatives of such therapeutic agents or drugs. Such functional group derivatives include for example, but are not be limited to, alcohols of the corresponding ketone of a therapeutic agent. Accordingly, the term “therapeutic agent” includes a therapeutic agent, a prodrug of a therapeutic agent and a functional group derivative of therapeutic agent. It is to be understood that the bond between the therapeutic agent and the linker can be at any suitable atom of the therapeutic agent such as (a) the therapeutic agent itself, (b) the prodrug portion of the prodrug of a therapeutic agent or (c) the functional group derivative portion of the functional group derivative of a therapeutic agent.

The therapeutic agent can be connected to the linker described herein by the removal of a hydrogen atom from the therapeutic agent (e.g., a residue of a therapeutic agent) which provides the open valency to be connected to the linker. In one embodiment the term $-Z-D^1$ of formula I can be a residue of a therapeutic agent and the corresponding group $H-Z-D^1$ can be the corresponding therapeutic agent. In one embodiment the term D of formula I can be a residue of a therapeutic agent and the corresponding group $H-D$ can be the corresponding therapeutic agent. In one embodiment the term D of formula I can be a residue of a prodrug of a therapeutic agent. Thus, one embodiment provides therapeutic agents comprising one or more hydroxyl ($-OH$), thiol ($-SH$) or amine (e.g., primary ($-NH_2$) or secondary ($-NH-$, $-NH(C_1-C_6)alkyl$), groups which groups can be connected to the linker as described herein.

In one embodiment D together with Y of L^2 or D together with $W-C(=X)-Y$ of L^2 is the

moiety: $\text{---}Y-D$ or $\text{---}W-C(=X)-Y-D$ wherein the moiety is a residue of a therapeutic agent. In one embodiment the corresponding therapeutic agent of the residue of the therapeutic agent described in

the preceding embodiment is a therapeutic agent of the formula $H-Y-D$ or $H-W-C(=X)-Y-D$.

In one embodiment the therapeutic agent is a therapeutic agent (e.g., a drug) or a prodrug of the therapeutic agent.

In one embodiment the therapeutic agent is a therapeutic agent (e.g., drug) and not a prodrug and not a functional group derivative of the therapeutic agent.

- 5 In one embodiment the therapeutic agent is selected from Cladribine, Azacitidine, Abraxane, Adcetris, Doxorubicin, Afinitor, Vinblastine, Amifostine, Amifostine, Arabinosylcytosine, Cytarabine, Pamidronic Acid, Nelarabine, Bicalutamide, Blemycin, Bortezomib, Cabazitaxel, Irinotecan, Camptothecin, Capecitabine, Temsirolimus, Daunorubicin, Cortisone, Decitabine, Dasatinib, Dexamethasone, Prednisolone, Dexamethasone Acetate,
- 10 Mitoxantrone, Docetaxel, Hydroxycarbamide, Methylprednisolone, Epirubicin, Curcumin, Estramustine, Eribulin, Etoposide, Everolimus, Raloxifene, Fulvestrant, Floxuridine, Fludarabine, Fluoxymesterone, Gemcitabine, Goserelin, Topotecan, Hydrocortisone, Hydrocortone Phosphate, Idarubicin, Ixabepilone, Vincristine, Leuprolide (Leuprorelin), Megestrol, Vinorelbine, Nelarabine, Pentostatin, Octreotide, Paclitaxel, Streptozotocin, Teniposide, Valrubicin, Vorinostat, Zoledronic
- 15 Acid Cladribine, Azacitidine, Mecaptopurine, Tioguanine, Actinomycin D, Doxorubicin, Anagrelide, Pemetrexed, Vinblastine, Melphalan, Methotrexate, Amifostine, Aminoglutethimide, Arabinosylcytosine, Cytarabine, Pamidronic Acid, Nelarabine, Axitinib, Bleomycin, Bosutinib, Folinic Acid (Na or Ca), Leucovorin, Vandetanib, Lenalidomide, Daunorubicin, Crizotinib, Dacarbazine, Decitabine, Dasatinib, Mitoxantrone, Eribulin, Erlotinib, Fludarabine, Pralatrexate,
- 20 Gefitinib, Gemcitabine, Imatinib, Goserelin, Idarubicin, Lapatinib, Vincristine, Leuprolide, Procarbazine, Methotrexate, Mitomycin, Vinorelbine, Nelarabine, Nilotinib, Pentostatin, Octreotide, Pazopanib, Sunitinib, Abraxane, Actinomycin D, Doxorubicin, Afinitor, Exemestane, Carfilzomib, Daunorubicin, Cortisone, Prednisolone, Prednisone, Dexamethasone Acetate, Docetaxel, Methylprednisolone, Epirubicin, Curcumin, Everolimus, Fluoxymesterone, Hydrocortisone,
- 25 Hydrocortone Phosphate, Idarubicin, Ixabepilone, Vincristine, Megestrol, Valrubicin, Mesna, 13-cis-Retinoic Acid, Isotretinoin, Alitretinoin, Melphalan, Tretinoin, Methotrexate, Anastrozole, Bendamustine, Bexarotene, Carmustine, Lomustine, Chlorambucil and Ibritumomab Tiuxetan.

In one embodiment the therapeutic agent is a chemotherapeutic agent, an antibiotic agent, an antifungal agent, an antiparasitic agent or an antiviral agent or a prodrug thereof.

- 30 In one embodiment the therapeutic agent is a chemotherapeutic agent, an antibiotic agent, an antifungal agent, an antiparasitic agent or an antiviral agent.

In one embodiment the therapeutic agent has at least one amine (e.g., -NH₂ or -NH(C₁-C₆)alkyl), hydroxy or a thiol group.

In one embodiment the therapeutic agent has at least one hydroxy (-OH), thiol (-SH) or amine (e.g., primary (-NH₂) or secondary (-NH-, -NH(C₁-C₆)alkyl)) group.

In one embodiment the therapeutic agent has at least one hydroxy (-OH), thiol (-SH) or amine (e.g., primary (-NH₂) or secondary (-NH-)) group.

5 In one embodiment the therapeutic agent has at least one hydroxy or thiol group.

In one embodiment the therapeutic agent has at least one hydroxy group.

In one embodiment the therapeutic agent has at least one amine (e.g., -NH₂ or -NH(C₁-C₆)alkyl), hydroxy (-OH) or a thiol group and is attached to the linker through the amine (e.g., -NH₂ or -NH(C₁-C₆)alkyl), hydroxy (-OH) or a thiol group of the therapeutic agent.

10 In one embodiment the therapeutic agent has at least one hydroxyl (-OH), thiol (-SH) or amine (e.g., primary (-NH₂) or secondary (-NH-, -NH(C₁-C₆)alkyl)) group and is attached to the linker through the hydroxyl (-OH), thiol (-SH) or amine (e.g., primary (-NH₂) or secondary (-NH-, -NH(C₁-C₆)alkyl)) group of the therapeutic agent.

15 In one embodiment the therapeutic agent has at least one hydroxy (-OH), thiol (-SH) or amine (e.g., primary (-NH₂) or secondary (-NH-)) group and is attached to the linker through the hydroxyl (-OH), thiol (-SH) or amine (e.g., primary (-NH₂) or secondary (-NH-)) group of the therapeutic agent.

In one embodiment the therapeutic agent has at least one hydroxy or thiol (-SH) group and is attached to the linker through the hydroxy (-OH) or thiol (-SH) group of the therapeutic agent.

20 In one embodiment the therapeutic agent has at least one hydroxy group (-OH) and is attached to the linker through the hydroxy (-OH) group of the therapeutic agent.

In one embodiment the therapeutic agent has at least one amine (e.g., primary (-NH₂) or secondary (-NH-)) and is attached to the linker through the amine (e.g., primary (-NH₂) or secondary (-NH-)) group of the therapeutic agent.

25 In one embodiment the therapeutic agent has at least one thiol (-SH) group and is attached to the linker through the thiol (-SH) group of the therapeutic agent.

In one embodiment the therapeutic agent has at least one ketone or aldehyde.

30 In one embodiment the therapeutic agent has at least one ketone or aldehyde and is attached to the linker through the ketone or aldehyde group (or the corresponding imine) of the therapeutic agent.

In one embodiment the therapeutic agent is selected from Cladribine, Azacitidine, Abiraterone, Adcetris, Doxorubicin, Afatinib, Vinorelbine, Amifostine, Arabinoside, Cytarabine, Pamidronic Acid, Nelarabine, Bicalutamide, Blemycin,

Bortezomib, Cabazitaxel, Irinotecan, Camptothecin, Capecitabine, Temsirolimus, Daunorubicin, Cortisone, Decitabine, Dasatinib, Dexamethasone, Prednisolone, Dexamethasone Acetate, Mitoxantrone, Docetaxel, Hydroxycarbamide, Methylprednisolone, Epirubicin, Curcumin, Estramustine, Eribulin, Etoposide, Everolimus, Raloxifene, Fulvestrant, Floxuridine, Fludarabine, 5 Fluoxymesterone, Gemcitabine, Goserelin, Topotecan, Hydrocortisone, Hydrocortone Phosphate, Idarubicin, Ixabepilone, Vincristine, Leuprolide (Leuprorelin), Megestrol, Vinorelbine, Nelarabine, Pentostatin, Octreotide, Paclitaxel, Streptozotocin, Teniposide, Valrubicin, Vorinostat and Zoledronic Acid.

In one embodiment the therapeutic agent is selected from Cladribine, Azacitidine, 10 Mecaptopurine, Tioguanine, Actinomycin D, Doxorubicin, Anagrelide, Pemetrexed, Vinblastine, Melphalan, Methotrexate, Amifostine, Aminoglutethimide, Arabinosylcytosine, Cytarabine, Pamidronic Acid, Nelarabine, Axitinib, Bleomycin, Bosutinib, Folinic Acid (Na or Ca), Leucovorin, Vandetanib, Lenalidomide, Daunorubicin, Crizotinib, Dacarbazine, Decitabine, Dasatinib, Mitoxantrone, Eribulin, Erlotinib, Fludarabine, Pralatrexate, Gefitinib, Gemcitabine, 15 Imatinib, Goserelin, Idarubicin, Lapatinib, Vincristine, Leuprolide, Procarbazine, Methotrexate, Mitomycin, Vinorelbine, Nelarabine, Nilotinib, Pentostatin, Octreotide, Pazopanib and Sunitinib.

In one embodiment the therapeutic agent is selected from Abraxane, Actinomycin D, Doxorubicin, Afinitor, Exemestane, Carfilzomib, Daunorubicin, Cortisone, Prednisolone, Prednisone, Dexamethasone Acetate, Docetaxel, Methylprednisolone, Epirubicin, Curcumin, 20 Everolimus, Fluoxymesterone, Hydrocortisone, Hydrocortone Phosphate, Idarubicin, Ixabepilone, Vincristine, Megestrol, Valrubicin and Mesna.

In one embodiment the therapeutic agent is selected from 13-cis-Retinoic Acid, Isotretinoin, Alitretinoin, Melphalan, Tretinoin, Methotrexate, Bendamustine, Bexarotene, Chlorambucil, and Ibritumomab Tiuxetan.

25 In one embodiment the therapeutic agent is selected from Carmustine, Lomustine, Chlorambucil and Bendamustine.

Targeting elements (*e.g.*, an antibody fragment, a small molecule ligand of a cellular receptor) can be attached to the therapeutic nanoparticle at any suitable location including the magnetic nanoparticle (directly to nanoparticle or coating of nanoparticle), linker or therapeutic 30 agent by any suitable means.

“Prodrug” of a therapeutic agent refers to a labile functional group which separates from the active compound during metabolism, systemically, inside a cell, by hydrolysis, enzymatic cleavage, or by some other process (Bundgaard, Hans, “Design and Application of Prodrugs” in A Textbook of

Drug Design and Development (1991), P. Krogsgaard-Larsen and H. Bundgaard, Eds. Harwood Academic Publishers, pp. 113-191). Enzymes which are capable of an enzymatic activation mechanism with the prodrug compounds of the invention include, but are not limited to, amidases, esterases, microbial enzymes, phospholipases, cholinesterases, and phosphatases. Prodrug moieties
5 can serve to enhance solubility, absorption and lipophilicity to optimize drug delivery, bioavailability and efficacy. A prodrug may include an active metabolite of drug itself.

"Alkyl" is a straight or branched saturated hydrocarbon. For example, an alkyl group can have 1 to 8 carbon atoms (i.e., (C₁-C₈)alkyl) or 1 to 6 carbon atoms (i.e., (C₁-C₆)alkyl) or 1 to 4 carbon atoms. "Alkylene" refers to an alkyl group having two monovalent radical centers derived
10 by the removal of two hydrogen atoms from the same or two different carbon atoms of the alkyl.

"Alkenyl" is a straight or branched hydrocarbon with at least one (e.g., one or more) carbon-carbon double bond. For example, an alkenyl group can have 2 to 8 carbon atoms (i.e., C₂-C₈ alkenyl), or 2 to 6 carbon atoms (i.e., C₂-C₆ alkenyl). "Alkenylene" refers to an alkenyl group having two monovalent radical centers derived by the removal of two hydrogen atoms from the
15 same or two different carbon atoms of the alkenyl.

"Alkynyl" is a straight or branched hydrocarbon with at least one (e.g., one or more) carbon-carbon, triple bond. For example, an alkynyl group can have 2 to 8 carbon atoms (i.e., C₂-C₈ alkyne), or 2 to 6 carbon atoms (i.e., C₂-C₆ alkynyl). "Alkynylene" refers to an alkynyl group having two monovalent radical centers derived by the removal of two hydrogen atoms from the
20 same or two different carbon atoms of the alkyne.

The term "chain" includes any branched or unbranched arrangement of atoms that are bonded together but are not cyclic. Thus chains include by way of example but are not limited to alkyl, alkenyl, alkynyl and heteroalkyl groups.

The term "halo" or "halogen" as used herein refers to fluoro, chloro, bromo and iodo.

25 The term "carbocycle" or "carbocyclyl" refers to a single saturated (i.e., cycloalkyl) or a single partially unsaturated (e.g., cycloalkenyl, cycloalkadienyl, etc.) all carbon ring having 3 to 7 carbon atoms (i.e. (C₃-C₇)carbocycle). "Carbocyclene" refers to an carbocycle group having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of the carbocycle.

30 "Phenylene" refers to a phenyl group having two monovalent radical centers derived by the removal of two hydrogen atoms from two different carbon atoms of the phenyl.

The term "heteroalkyl" as used herein refers to an alkyl as defined herein, wherein one or more of the carbon atoms of the alkyl are replaced by an O, S, or NR_q, (or if the carbon atom being

replaced is a terminal carbon with an OH, SH or NR_{q2}) wherein each R_q is independently H or $(\text{C}_1\text{-C}_6)\text{alkyl}$. "Heteroalkylene" refers to a heteroalkyl group having two monovalent radical centers derived by the removal of two hydrogen atoms from a same or two different carbon atoms or an OH, SH or NHR_q of the heteroalkyl.

5 The term "heterocyclyl" or "heterocycle" as used herein refers to a single saturated or partially unsaturated ring that has at least one atom other than carbon in the ring, wherein the atom is selected from the group consisting of oxygen, nitrogen and sulfur. Thus, the term includes 3, 4, 5, 6, 7 or 8-membered single saturated or partially unsaturated rings from about 1 to 7 carbon atoms and from about 1 to 4 heteroatoms selected from the group consisting of oxygen, nitrogen and
10 sulfur in the ring. The ring may be substituted with one or more (e.g., 1, 2 or 3) oxo groups and the sulfur and nitrogen atoms may also be present in their oxidized forms. Such rings include but are not limited to azetidiny, tetrahydrofuranyl or piperidiny.

 The term "cyclic group" includes any arrangement of atoms that are bonded together that form a cyclic structure. Thus cyclic groups include by way of example but are not limited to
15 carbocycle, phenyl and heterocycle.

 Silica (silicon dioxide (SiO_2)) includes all forms of silica such as amorphous silica, silica gel, mesoporous silica and fumed silica.

 Specific values listed below for radicals, substituents, and ranges are for illustration only; they do not exclude other defined values or other values within defined ranges for the radicals and
20 substituents. It is to be understood that two or more values may be combined.

 A specific group of compounds of formula I are compounds wherein V is $\text{---OSi(G)}_2\text{---}$, the magnetic nanoparticle is optionally coated with silica, and the dashed line represents a covalent bond between the oxygen atom of $\text{---OSi(G)}_2\text{---}$ and the magnetic nanoparticle optionally coated with silica; or V is ---S--- , the magnetic nanoparticle is magnetic nanoparticle coated with gold, and the
25 dashed line represents a covalent bond between ---S--- and the magnetic nanoparticle coated with gold.

 A specific group of compounds of formula I are compounds wherein V is $\text{---OSi(G)}_2\text{---}$, the magnetic nanoparticle is coated with silica, and the dashed line represents a covalent bond between the oxygen atom of $\text{---OSi(G)}_2\text{---}$ and the magnetic nanoparticle coated with silica.

 A specific group of compounds of formula I are compounds wherein V is $\text{---OSi(G)}_2\text{---}$, the
30 magnetic nanoparticle is an iron oxide nanoparticle coated with silica, and the dashed line represents a covalent bond between the oxygen atom of $\text{---OSi(G)}_2\text{---}$ and the iron oxide nanoparticle coated with silica.

A specific group of compounds of formula I are compounds wherein V is $\text{---OSi(G)}_2\text{--}$, the magnetic nanoparticle is an iron oxide nanoparticle, and the dashed line represents a covalent bond between the oxygen atom of $\text{---OSi(G)}_2\text{--}$ and the iron oxide nanoparticle.

5 A specific group of compounds of formula I are compounds wherein V is $\text{---OSi(G)}_2\text{--}$, the magnetic nanoparticle is an iron oxide nanoparticle optionally coated with silica, and the dashed line represents a covalent bond between the oxygen atom of $\text{---OSi(G)}_2\text{--}$ and the iron oxide nanoparticle optionally coated with silica.

10 A specific group of compounds of formula I are compounds wherein V is $\text{---OSi(G)}_2\text{--}$, the magnetic nanoparticle is an iron oxide nanoparticle coated with silica, and the dashed line represents a covalent bond between the oxygen atom of $\text{---OSi(G)}_2\text{--}$ and the iron oxide nanoparticle coated in silica.

One embodiment provides a therapeutic magnetic nanoparticle linked to a residue of the therapeutic agent with a linker wherein linker includes a protected amine as described in the embodiments E1-E59 below.

15 E1. A therapeutic magnetic nanoparticle, or a salt thereof, comprising a magnetic nanoparticle covalently bonded to one or more ---L-D groups wherein D is a residue of a therapeutic agent and L is a linker that comprises a protected amine wherein the protected amine when deprotected provides an amine which amine is capable of undergoing an intramolecular cyclization.

20 E2. The therapeutic magnetic nanoparticle of E1, wherein the linker capable of undergoing an intramolecular cyclization is suitable to release a therapeutic agent from the linker upon intramolecular cyclization.

E3. The therapeutic magnetic nanoparticle of E1 or E2, wherein the linker capable of undergoing an intramolecular cyclization can form a 3-8 membered heterocyclic ring upon cyclization.

25 E4. The therapeutic magnetic nanoparticle of E3, wherein the 3-8 membered ring comprises a group selected from an amide, carbamate, urea, carbamothioate, thioamide, thiocarbamate, thiourea and carbamodithioate.

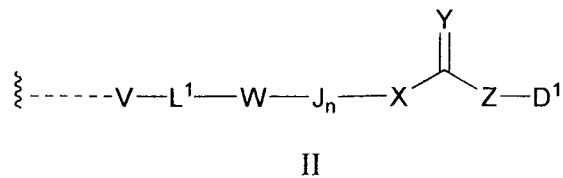
E5. The therapeutic magnetic nanoparticle of any one of E1-E4, wherein the magnetic nanoparticle further comprises a coating.

30 E6. The therapeutic of magnetic nanoparticle of any one of E1-E5, wherein the protected amine is an amide, carbamate or urea.

E7. The therapeutic magnetic nanoparticle of any one of E1-E6, wherein the protected amine is an amide, urea or carbamate and the amine of the protected amine is a primary amine or secondary amine.

- E8. The therapeutic magnetic nanoparticle of any one of E1-E7, wherein the protected amine is an amide, urea or carbamate and the amine of the protected amine is a primary amine or secondary amine.
- E9. The magnetic nanoparticle of E5, wherein the coating is gold.
- 5 E10. The magnetic nanoparticle of E5, wherein the coating is silica.
- E11. The therapeutic magnetic nanoparticle of any one of E1-E10, wherein the magnetic nanoparticle comprises iron.
- E12. The therapeutic magnetic nanoparticle of any one of E1-E11, wherein the magnetic nanoparticle is an iron oxide nanoparticle coated with silica.
- 10 E13. The therapeutic magnetic nanoparticle of any one E1-E12, wherein the deprotection of the protected amine can be induced by heating the magnetic nanoparticle.
- E14. The therapeutic magnetic nanoparticle of any one of E1-E12, wherein the deprotection of the protected amine occurs prior to the heating the magnetic nanoparticle.
- E15. The therapeutic magnetic nanoparticle of any one of E1-E14, wherein the cyclization can be
15 induced by heating the magnetic nanoparticle.
- E16. The therapeutic magnetic nanoparticle of any one of E1-E12, wherein deprotection of the protected amine can be induced by application of an alternating electromagnetic field to the magnetic nanoparticle.
- E17. The therapeutic magnetic nanoparticle of any one of E1-E12, wherein the deprotection of the
20 protected amine occurs prior to the application of an alternating electromagnetic field to the magnetic nanoparticle.
- E18. The therapeutic magnetic nanoparticle of any one of E1-E17, wherein the cyclization can be induced by application of an alternating electromagnetic field to the magnetic nanoparticle.
- E19. The therapeutic magnetic nanoparticle of any one of E1-E18, wherein the linker comprises
25 about 4-50 atoms.
- E20. The therapeutic magnetic nanoparticle of any one of E1-E18, wherein the linker comprises about 4-20 atoms.
- E21. The therapeutic magnetic nanoparticle of E19 or E20, wherein the atoms are independently selected from silicon, carbon, nitrogen, oxygen and sulfur.
- 30 E22. The therapeutic magnetic nanoparticle of any one of E1-E21, wherein the linker is covalently bonded to the magnetic nanoparticle through a silicon or sulfur atom.
- E23. The therapeutic magnetic nanoparticle of any one of E1-E21, wherein the linker does not include a t-butoxycarbonyl (BOC) protected amine.

E24. The therapeutic magnetic nanoparticle, or a salt thereof, of any one of E1-E23, wherein each -L-D independently has the following formula I:



5 wherein

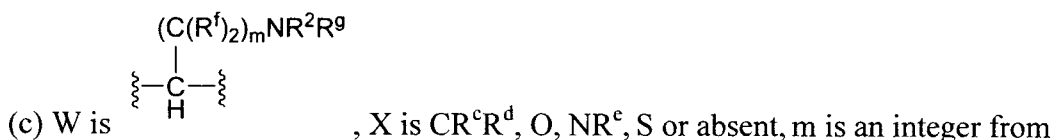
V is $-\text{OSi}(\text{G})_2-$, and the dashed line represents a covalent bond between the oxygen atom of $-\text{OSi}(\text{G})_2-$ and the magnetic nanoparticle; or V is $-\text{S}-$, and the dashed line represents a covalent bond between $-\text{S}-$ and the magnetic nanoparticle;

L¹ is (C₁-C₆)alkylene, (C₁-C₆)heteroalkylene, (C₂-C₆)alkenylene, (C₂-C₆)alkynylene, phenylene or (C₃-C₇)carbocyclene, wherein (C₁-C₆)alkylene, (C₁-C₆)heteroalkylene, (C₂-C₆)alkenylene, (C₂-C₆)alkynylene, phenylene or (C₃-C₇)carbocyclene are optionally substituted with one or more halogen;

each J is $C(R^b)_2$ wherein one $C(R^b)_2$ of J may be replaced by -O-, -S- or $-N(R^c)-$;

(a) W is $\mathbb{NR}^2$, X is $\mathbb{CR}^c \mathbb{R}^d$, and n is an integer from 0-5; or

15 (b) W is NR², X is O, NR^e or S, and n is an integer from 1-5; or



0-5 and n is an integer from 0-5, wherein the sum of m and n is 0-5;

Y is O or S;

Z-D¹ is a residue of a therapeutic agent wherein Z is O, NR^h or S;

20 each G is independently -OR^{a1}, -OR^{a2} or (C₁-C₆)alkyl;

R^{al} is a covalent bond between the oxygen atom of $-OR^{al}$ and the magnetic nanoparticle;

each R^{a2} is independently H or (C₁-C₆)alkyl; or two -OR^{a2} groups of two adjacent L-D groups together form -O-;

each R^b is independently selected from H and (C₁-C₃)alkyl; or two R^b groups together with
25 the carbon to which they are attached form a (C₃-C₇)carbocycle;

each R^c is independently selected from H and (C₁-C₆)alkyl, and each R^d is independently selected from H and (C₁-C₆)alkyl; or an R^c group and an R^d group together with the carbon to which they are attached form a (C₃-C₇)carbocycle;

each R^e is independently selected from H and (C₁-C₆)alkyl;

each R^f is independently selected from H and (C₁-C₆)alkyl; or two R^f groups together with the carbon to which they are attached form a (C₃-C₇)carbocycle;

R^g is selected from H, (C₁-C₆)alkyl and R^2 ;

R^h is selected from H and (C₁-C₆)alkyl;

5 each R^2 is independently selected from -C(=O) R^{2a} , -C(=O)OR^{2b}, -C(=O)N(R^{2c})₂, C(=S) R^{2a} , -C(=S)OR^{2b} or -C(=S)N(R^{2c})₂;

each R^{2a} is independently selected from H, (C₁-C₁₀)alkyl, aryl or (C₃-C₇)carbocycle wherein (C₁-C₁₀)alkyl, aryl or (C₃-C₇)carbocycle is optionally substituted with one or more halogen;

10 each R^{2b} is independently selected from H, (C₁-C₁₀)alkyl, aryl or (C₃-C₇)carbocycle wherein (C₁-C₁₀)alkyl, aryl or (C₃-C₇)carbocycle is optionally substituted with one or more halogen;

each R^{2c} is independently selected from H, (C₁-C₁₀)alkyl, aryl or (C₃-C₇)carbocycle wherein (C₁-C₁₀)alkyl, aryl or (C₃-C₇)carbocycle is optionally substituted with one or more halogen; or two R^{2c} groups together with the nitrogen to which they are attached for a 3-7 membered heterocycle.

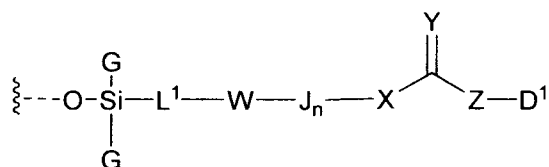
15 E25. The therapeutic magnetic nanoparticle of E24, wherein V is -OSi(G)₂-, the magnetic nanoparticle is optionally coated with silica, and the dashed line represents a covalent bond between the oxygen atom of -OSi(G)₂- and the magnetic nanoparticle optionally coated with silica; or V is -S-, the magnetic nanoparticle is magnetic nanoparticle coated with gold, and the dashed line represents a covalent bond between -S- and the magnetic nanoparticle coated with gold.

20 E26. The therapeutic magnetic nanoparticle of E24, wherein V is -OSi(G)₂-, the magnetic nanoparticle is coated with silica, and the dashed line represents a covalent bond between the oxygen atom of -OSi(G)₂- and the magnetic nanoparticle coated with silica.

E27. The therapeutic magnetic nanoparticle of any one of E24-E26, wherein the magnetic nanoparticle comprises iron.

25 E28. The therapeutic magnetic nanoparticle of E24, wherein V is -OSi(G)₂-, the magnetic nanoparticle is an iron oxide nanoparticle coated with silica, and the dashed line represents a covalent bond between the oxygen atom of -OSi(G)₂- and the iron oxide nanoparticle coated with silica.

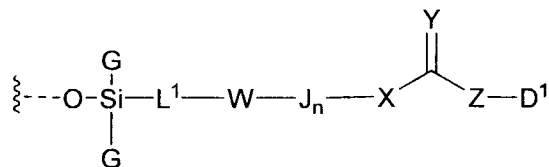
E29. The therapeutic magnetic nanoparticle of E24, wherein -L-D has the following formula IIa:



IIa

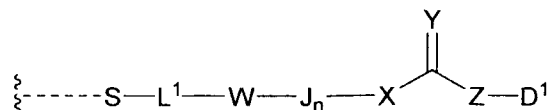
wherein the dashed bond represents a covalent bond to the magnetic nanoparticle.

E30. The therapeutic magnetic nanoparticle of E24, wherein the magnetic nanoparticle is further coated with silica and wherein -L-D has the following formula IIa:



IIa

- 5 wherein the dashed bond represents a covalent bond to the magnetic nanoparticle further coated with silica.
- E31. The therapeutic magnetic nanoparticle of E29 or E30, wherein the magnetic nanoparticle is an iron oxide nanoparticle.
- E32. The therapeutic magnetic nanoparticle of any one of E24-E31, wherein each G is $-\text{OR}^{\text{a1}}$.
- 10 E33. The therapeutic magnetic nanoparticle any one of E24-E31, wherein each G is $-\text{OR}^{\text{a2}}$, wherein each $-\text{OR}^{\text{a2}}$ together with another $-\text{OR}^{\text{a2}}$ group on an adjacent L-D group forms an $-\text{O}-$.
- E34. The therapeutic magnetic nanoparticle any one of E24-E31, wherein each G is $-\text{OR}^{\text{a1}}$ or $-\text{OR}^{\text{a2}}$, wherein each $-\text{OR}^{\text{a2}}$ together with another $-\text{OR}^{\text{a2}}$ group on an adjacent L-D group form an $-\text{O}-$.
- 15 E35. The therapeutic magnetic nanoparticle of E24, wherein V is $-\text{S}-$, the magnetic nanoparticle is coated in gold, and the dashed line represents a covalent bond between $-\text{S}-$ and the magnetic nanoparticle coated in gold.
- E36. The therapeutic magnetic nanoparticle of E35, wherein the dashed line represents a covalent bond between $-\text{S}-$ and a gold atom of the magnetic nanoparticle coated in gold.
- 20 E37. The therapeutic magnetic nanoparticle of E35 or E36, wherein the magnetic nanoparticle is an iron oxide nanoparticle.
- E38. The therapeutic magnetic nanoparticle of E24 or any one of E35-E37, wherein -L-D has the following formula IIb:



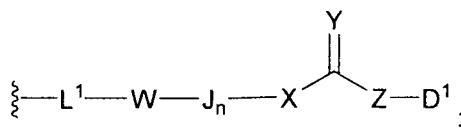
IIb

- 25 wherein the dashed bonds represent a covalent bond to the magnetic nanoparticle.

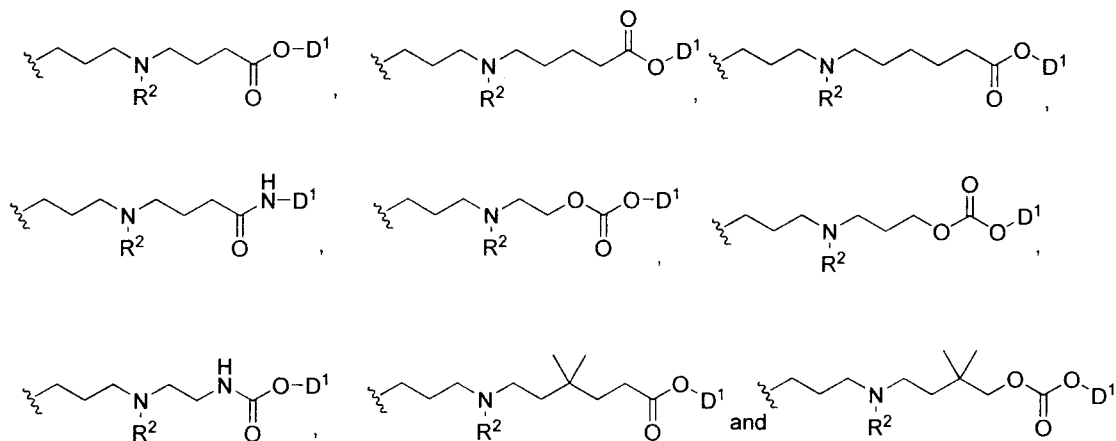
E39. The therapeutic magnetic nanoparticle of any one of E24-E38, wherein L^1 is $(\text{C}_1\text{-C}_6)$ alkylene optionally substituted with one or more halogen.

E40. The therapeutic magnetic nanoparticle of any one of E24-E38, wherein L^1 is $(\text{C}_1\text{-C}_6)$ alkylene.

- E41. The therapeutic magnetic nanoparticle of any one of E24-E38, wherein L^1 is (C_2-C_4) alkylene optionally substituted with one or more halogen.
- E42. The therapeutic magnetic nanoparticle of any one of E24-E38, wherein L^1 is (C_2-C_4) alkylene.
- E43. The therapeutic magnetic nanoparticle of any one of E24-E38, wherein L^1 is
- 5 $-(CH_2)_2-$, $-(CH_2)_3-$, or $-(CH_2)_4-$.
- E44. The therapeutic magnetic nanoparticle of any one of E24-E38, wherein L^1 is $-(CH_2)_3-$.
- E45. The therapeutic magnetic nanoparticle of any one of E24-E44, wherein:
- (a) W is $-NR^2-$, X is CR^cR^d , and n is an integer from 0-5; or
- 10 (b) W is $-NR^2-$, X is O, NR^e or S, and n is an integer from 1-5.
- E46. The therapeutic magnetic nanoparticle of any one of E24-E44, wherein:
- (a) W is $-NR^2-$, X is CR^cR^d , and n is an integer from 0-5; or
- (b) W is $-NR^2-$, X is O, and n is an integer from 1-5.
- E47. The therapeutic magnetic nanoparticle of any one of E24-E44, wherein W is $-NR^2-$, X is
- 15 CR^cR^d and n is an integer from 0-5.
- E48. The therapeutic magnetic nanoparticle of any one of E24-E47, wherein R^c and R^d are each independently selected from H and methyl.
- E49. The therapeutic magnetic nanoparticle of any one of E24-E47, wherein R^c and R^d are each H.
- E50. The therapeutic magnetic nanoparticle of any one of E24-E47, wherein R^c and R^d are each
- 20 methyl.
- E51. The therapeutic magnetic nanoparticle of any one of E24-E44, wherein W is $-NR^2-$, X is O, and n is an integer from 1-5.
- E52. The therapeutic magnetic nanoparticle of any one of E24-E51, wherein n is 2, 3 or 4.
- 25 E53. The therapeutic magnetic nanoparticle of any one of E24-E52, wherein each J is $C(R^b)_2$.
- E54. The therapeutic magnetic nanoparticle of any one of E24-E53, wherein each R^b is independently H or methyl.
- E55. The therapeutic magnetic nanoparticle of any one of E24-E54, wherein each R^b is H.
- E56. The therapeutic magnetic nanoparticle of any one of E24-E52, wherein J_n is
- 30 $-(CH_2)_2-$, $-(CH_2)_3-$, $-(CH_2)_4-$ or $-CH_2CH_2C(Me)_2CH_2-$.
- E57. The therapeutic magnetic nanoparticle of any one of E24-E56, wherein Y is O.
- E58. The therapeutic magnetic nanoparticle of E24, wherein the portion of formula I as shown in the formula below:



is selected from;



E59. The therapeutic magnetic nanoparticle of any one of E24-E58 wherein R² is not

5 -C(=O)OC(CH₃)₃.

E60. The therapeutic magnetic nanoparticle of any one of E24-E58 wherein aryl is phenyl.

Salts

10 In cases where compounds are sufficiently basic or acidic, a salt of a therapeutic magnetic nanoparticle as a pharmaceutically acceptable acid or base salt may be appropriate. Examples of pharmaceutically acceptable salts are organic acid addition salts formed with acids which form a physiological acceptable anion, for example, tosylate, methanesulfonate, acetate, citrate, malonate, tartarate, succinate, benzoate, ascorbate, α -ketoglutarate, and α -glycerophosphate. Suitable inorganic salts may also be formed, including hydrochloride, sulfate, nitrate, bicarbonate, and

15 carbonate salts.

Pharmaceutically acceptable salts may be obtained using standard procedures well known in the art, for example by reacting a sufficiently basic compound such as an amine with a suitable acid affording a physiologically acceptable salt. Alkali metal (for example, sodium, potassium or lithium) or alkaline earth metal (for example calcium) salts of carboxylic acids can also be made.

20

Administration

The method of administering the therapeutic magnetic nanoparticle to the desired area for treatment and the dosage may depend upon, but is not limited to, the type and location of the disease material. The size range of the nanoparticles may allow for microfiltration for sterilization.

Some methods of administration include intravascular injection, intravenous injection, intraperitoneal injection, subcutaneous injection, and intramuscular injection. The nanoparticles may be formulated in an injectable format (*e.g.*, suspension, emulsion) in a medium such as, for example, water, saline, Ringer's solution, dextrose, dimethylsulfoxide, albumin solution, and oils.

5 The nanoparticles may also be administered to the patient through topical application via a salve or lotion, transdermally through a patch, orally ingested as a pill or capsule or suspended in a liquid or rectally inserted in suppository form. Nanoparticles may also be suspended in an aerosol or pre-aerosol formulation suitable for inhalation via the mouth or nose. Once administered to the patient, delivery of the nanoparticles to the target site may be assisted by an applied static magnetic field
10 due to the magnetic nature of the nanoparticles. Assisted delivery may depend on the location of the targeted tissue. The nanoparticles may also be delivered to the patient using other methods. For example, the nanoparticles may be administered to the patient orally, or may be administered rectally. It is to be understood the therapeutic magnetic nanoparticles described herein may also be useful in diagnostics as well as studies in cells, tissues and animals.

15

The invention will now be illustrated by the following non-limiting Examples.

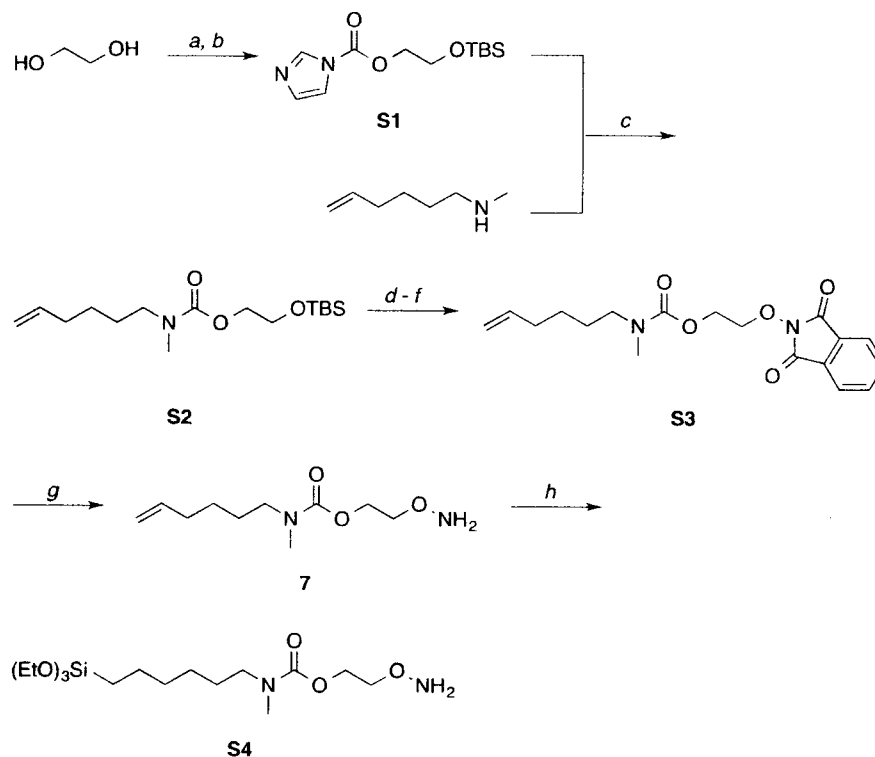
Example 1.

General.

20 Reagent grade solvents were used for extraction and flash chromatography. Acetonitrile for carbamate synthesis was dried by distillation from CaH_2 . All other commercial reagents were used as received without additional purification. The progress of reactions was checked by thin-layer chromatography (TLC, silica gel 60 Å F-254 plates). The plates were visualized first with UV illumination followed by staining using iodine, *p*-anisaldehyde, phosphomolybdic acid hydrate, or
25 ninhydrin. Column chromatography was performed using silica gel (230-400 mesh). NMR spectra were obtained using a Varian/Agilent 400-MR NMR spectrometer equipped with a 5mm z-axis gradient AutoX probe operating at the nominal ^1H frequency of 399.66 MHz and ^{13}C frequency of 100.49 MHz. All spectra are reported in parts per million (ppm) relative to the residual solvent peak in ^1H NMR and the deuterated solvent peak in ^{13}C NMR. High-resolution mass spectra were
30 obtained using a Finnigan LTQ-FT spectrometer (Thermo Electron Corp) in positive detection mode. Fluorescent measurements were taken on a Molecular Devices SpectraMax M5 in fluorescent mode at an excitation of 366 nm and emission wavelength of 408 nm. The fluorescent measurements were taken using a quartz semi-micro VWR Spectrosil spectrophotometer cell with a

10 mm light path. Transmission electron microscopy (TEM) was used to analyze size distribution and morphology of fabricated nanoparticles, including uniformity and thickness of silica coating. For this, ethanol-based nanoparticle dispersions were prepared and drop-casted on commercially available 300 mesh TEM support Cu grids coated with ultra thin carbon films. After ethanol
 5 evaporation, samples were transferred to and analyzed using a field emission gun FEI Tecnai F20 transmission electron microscope operating at the accelerating voltage of 200 kV. DLS and ζ -potential measurements were taken using a Brookhaven Instruments 90Plus Particle Size Analyzer. All measurements were taken using aqueous colloids of the nanoparticles in Millipore water. The alternating magnetic field (AMF) was generated with a Ambrell EasyHeat L1 set at 501.6 amps and
 10 204 kHz using a 5-turn coil.

Synthesis of Linker S4.



a) TBSCl, Et_3N , CH_2Cl_2 , 0°C ; b) 1,1'-carbonyldiimidazole, $(i\text{-Pr})_2\text{Net}$, CH_2Cl_2 , 0°C ; c) DBU,
 15 MeCN, rt, 79% over 3 steps; d) TBAF, THF, 0°C ; e) MsCl, Et_3N , CH_2Cl_2 , 0°C ; f) *N*-hydroxyphthalimide, K_2CO_3 , DMSO, 75°C , 71% over 3 steps; g) $\text{N}_2\text{H}_4\cdot\text{H}_2\text{O}$, CH_2Cl_2 , 0°C , 96%,
 h) $(\text{EtO})_3\text{SiH}$, cat. PtO_2 , 90°C , 48 h.

A solution of TBSCl (2.34 g, 15.5 mmol) in dry CH_2Cl_2 (20 mL) was added dropwise over 1
 20 h to a solution of ethylene glycol (8 mL, 143 mmol) and Et_3N (2.80 mL, 19.9 mmol) in dry CH_2Cl_2

(25 mL) at 0 °C and was stirred overnight. The solvent was removed *in vacuo* and the remaining oil was extracted with hexanes (4x) and the combined extractions were washed twice with sat. NH₄Cl, once with brine and was dried over Na₂SO₄, filtered and concentrated *in vacuo* to afford the mono-TBS protected diol 2-((tert-butyldimethylsilyl)oxy)ethan-1-ol (2.52 g, 92%) as a colorless oil and was used without further purification. *R_f* 0.59 (1:3, EtOAc:hexanes).

1,1'-Carbonyldiimidazole (3.47 g, 21.4 mmol) was added to a solution of the mono-TBS protected diol (2.52 g, 14.3 mmol) and *N,N*-diisopropylethylamine (3.70 mL, 21.4 mmol) in dry CH₂Cl₂ (48 mL) at 0 °C and was stirred for 6 h. The reaction was washed twice with water and the combined aqueous phases were extracted once with CH₂Cl₂. The combined organic layers were then washed twice with sat. NH₄Cl, once with brine and were dried over Na₂SO₄, filtered and concentrated *in vacuo* to afford crude **S1** as a colorless oil and was used without further purification. *R_f* 0.36 (1:3, EtOAc:hexanes); ¹H NMR (400 MHz, CDCl₃) δ 0.06 (s, 6H), 0.88, (s, 9H), 3.93 (t, *J* = 4.8 Hz, 2H), 4.71 (t, *J* = 4.8 Hz, 2H), 7.06 (s, 1H), 7.42 (s, 1H), 8.13 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ -5.2, 18.4, 25.9, 61.0, 69.4, 117.3, 130.9, 137.3, 149.0.

The carbamate was synthesized as described in the literature (Heller, S. T.; et al., *Angew. Chem., Int. Ed.* **2012**, 51, 8304-8308). DBU (2.13 mL, 14.3 mmol) was added to a solution of crude **S1** (14.3 mmol) in dry MeCN (70 mL) and was stirred for 10 min, then *N*-methyl-1-aminohex-5-ene (1.60 g, 14.3 mmol) was added and the reaction was stirred overnight. The reaction was then washed twice with sat. NH₄Cl and the combined aqueous layers were extracted three times with EtOAc. The combined organic phases were washed with brine, dried over Na₂SO₄, filtered and concentrated *in vacuo*. The crude material was purified by column chromatography (SiO₂, 1:3, EtOAc:hexanes) to give a colorless oil **S2** (3.56 g, 79% over 3 steps). *R_f* 0.40 (1:3, EtOAc:hexanes); ¹H NMR (400 MHz, CDCl₃) δ 0.06 (s, 6H), 0.89 (s, 9H), 1.38 (quin, *J* = 7.4 Hz, 2H), 1.52, (quin, *J* = 7.6 Hz, 2H), 2.07 (q, *J* = 7.2 Hz, 2H), 2.88 (s, 3H), 3.25 (t, *J* = 7.2 Hz, 2H), 3.80 (t, *J* = 5.0 Hz, 2H), 4.13 (t, *J* = 5.0 Hz, 2H), 4.93-5.02 (m, 2H), 5.73-5.84 (m, 1H); ¹³C NMR (100 MHz, CDCl₃) δ -5.1, 18.5, 26.1, 27.6, 33.6, 34.1, 49.0, 62.0, 66.8, 114.9, 138.7, 156.7; FT-ICR-MS calcd for C₁₆H₃₄NO₃Si⁺ [M+H]⁺ *m/z* 316.2302, found 316.2307.

TBAF (1 *M* in THF, 10.6 mL, 10.6 mmol) was added dropwise to a solution of **S2** (2.58 g, 8.17 mmol) in dry THF (16 mL) at 0 °C and was stirred overnight. The reaction solution was washed twice with sat. NaHCO₃ and the combined aqueous layers were extracted three times with Et₂O. The combined organic phases were washed with brine, dried over Na₂SO₄, filtered and concentrated *in vacuo* to afford crude 2-hydroxyethyl *N*-(hex-5-en-1-yl)-*N*-methylcarbamate as a yellow oil and was used without further purification. *R_f* 0.12 (1:3, EtOAc:hexanes); ¹H NMR (400

MHz, CDCl₃) δ 1.40 (quin, J = 7.8 Hz, 2H), 1.55 (quin, J = 7.6 Hz, 2H), 2.08 (q, J = 6.8 Hz, 2H), 2.90 (s, 3H), 3.26 (t, J = 7.2 Hz, 2H), 3.78-3.82 (q, J = 4.4 Hz, 2H), 4.23 (t, J = 4.4 Hz, 2H), 4.94-5.02 (m, 2H), 5.74-5.84 (m, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 25.9, 27.4, 33.6, 34.7, 48.9, 62.6, 67.7, 115.0, 138.6, 157.4.

5 Methanesulfonyl chloride (728 μ L, 9.40 mmol) was added dropwise to a solution of the crude alcohol (8.17 mmol) and triethylamine (1.72 mL, 12.3 mmol) in CH₂Cl₂ (27 mL) at 0 °C and stirred overnight. The reaction was then washed twice with sat. NH₄Cl and the combined aqueous layers were extracted twice with CH₂Cl₂. The combined organic phases were washed with brine, dried over Na₂SO₄, filtered and concentrated *in vacuo* to give crude 2-(methanesulfonyloxy)ethyl *N*-(hex-10 5-en-yl)-*N*-methylcarbamate as a light yellow oil (2.15 g). R_f 0.36 (major product) & 0.68 (1:1, EtOAc:hexanes); ¹H NMR (400 MHz, CDCl₃, R_f 0.36) δ 1.37 (quin, J = 7.6 Hz, 2H), 1.54 (quin, J = 7.6 Hz, 2H), 2.07 (q, J = 7.2 Hz, 2H), 2.89 (s, 3H), 3.01 (s, 3H), 3.26 (t, J = 7.2 Hz, 2H), 4.32-4.34 (m, 2H), 4.39-4.41 (m, 2H), 4.93-5.02 (m, 2H), 5.74-5.81 (m, 1H); ¹³C NMR (100 MHz, CDCl₃, R_f 0.36) δ 26.1, 27.0, 33.5, 34.1, 37.9, 49.2, 62.8, 68.1, 114.9, 138.6, 155.9.

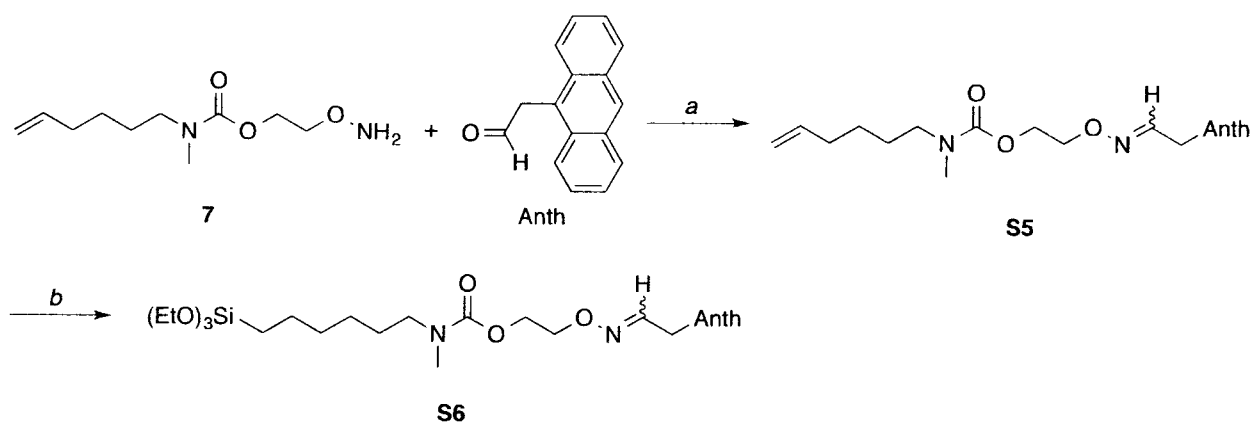
15 K₂CO₃ (1.17 g, 8.47 mmol) was added to a solution of the combined mesyl carbamate products (R_f 0.36 & 0.68) (2.15 g, 7.70 mmol) in DMSO (26 mL), followed by the addition of *N*-hydroxyphthalimide (2.39 g, 14.6 mmol) and the dark red solution was heated at 75 °C overnight. The yellow solution was cooled to rt and slowly quenched with water and extracted four times with EtOAc. The combined organic phase was washed three times with water, once with brine, dried 20 over Na₂SO₄, filtered and concentrated *in vacuo*. The crude material was purified by column chromatography (SiO₂, 1:1, EtOAc:hexanes) to give a yellow oil **S3** (2.02 g, 71% over 3 steps). R_f 0.55 (1:1, EtOAc:hexanes); ¹H NMR (400 MHz, CDCl₃) δ 1.35 (quin, J = 7.6 Hz, 2H), 1.52 (quin, J = 7.6 Hz, 2H), 2.05 (q, J = 7.2 Hz, 2H), 2.85 (br s, 3H), 3.24 (t, J = 7.2 Hz, 2H), 4.41 (s, 4H), 4.91-5.00 (m, 2H), 5.72-5.82 (m, 1H), 7.72-7.75 (m, 2H), 7.80-7.82 (m, 2H); ¹³C NMR (100 MHz, 25 CDCl₃) δ 26.1, 27.2, 33.5, 34.6, 49.1, 62.8, 76.7, 114.8, 123.7, 129.2, 134.7, 138.7, 156.1, 163.5; FT-ICR-MS calcd for C₁₈H₂₃N₂O₅⁺ [M+H]⁺ m/z 347.1601, found 347.1606.

Hydrazine monohydrate (34 μ L, 0.70 mmol) was added to a solution of **S3** (258 mg, 0.74 mmol) in CH₂Cl₂ (4 mL) at 0 °C and was stirred for 1 h. The solids were then filtered off and the filter cake was washed with ample CH₂Cl₂. The filtrate was then concentrated *in vacuo* to afford 7 30 (154 mg, 96%) as a colorless oil without further purification. R_f 0.24 (1:1, EtOAc:hexanes); ¹H NMR (400 MHz, CDCl₃) δ 1.36 (quin, J = 7.6 Hz, 2H), 1.52 (quin, J = 7.6 Hz, 2H), 2.05 (q, J = 7.2 Hz, 2H), 2.86 (s, 3H), 3.24 (t, J = 7.2 Hz, 2H), 3.80 (t, J = 3.6 Hz, 2H), 4.26 (t, J = 4.4 Hz, 2H), 4.91-5.00 (m, 2H), 5.42 (br s, 2H), 5.71-5.82 (m, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 26.0, 27.3,

33.5, 34.6, 48.8, 62.9, 74.3, 114.8, 138.7, 156.7; FT-ICR-MS calcd for $C_{10}H_{21}N_2O_3^+$ $[M+H]^+$ m/z 217.1547, found 217.1548.

Following a related literature example (Sabourault, N.; et al., *Org. Lett.* **2002**, 4, 2117-2119), catalytic PtO_2 and triethoxysilane (131 μ L, 0.71 mmol) was added to **7** (153 mg, 0.71 mmol) in a pressure tube. The tube was flushed to N_2 , sealed and heated to 90 °C for 48 h. The reaction solution was cooled to rt and filtered through Celite into a flask flushed with N_2 and the filter cake was washed with ample dry CH_2Cl_2 . The filtrate was concentrated *in vacuo* to afford crude **S4** as a light brown oil which was used without further characterization or purification due to its sensitivity to moisture.

Synthesis of Linker S6.



a) CH_2Cl_2 , rt, 1 h, 96%; b) $(EtO)_3SiH$, cat. PtO_2 , 90 °C, 48 h.

2-(Anthracen-9-yl)acetaldehyde (175 mg, 0.79 mmol; (Jiang, H.; et al., *Angew. Chem. Int. Ed.* **2012**, 51, 10271-10274)) was added to a solution of **7** (156 mg, 0.72 mmol) in CH_2Cl_2 (4 mL) and stirred for 1 h. The reaction solution was concentrated *in vacuo*. The crude material was purified by column chromatography (SiO_2 , 0:1 to 3:17, EtOAc: CH_2Cl_2 gradient) to give a yellow oil **S5** (291 mg, 96%). R_f 0.55 (1:19, EtOAc: CH_2Cl_2); 1H NMR (400 MHz, $CDCl_3$) δ 0.78 (br s, 0.25H), 0.87 (br s, 0.25H), 1.26 (br s, 1.44H), 1.35-1.41 (m, 2H), 1.55-1.63 (m, 2H), 1.92-2.07 (m, 2H), 2.78 (br s, 0.68H), 2.95 (s, 1.91H), 3.14 (br s, 0.46H), 3.22 (br s, 0.46H), 3.30-3.34 (m, 1.33H), 4.26 (dd, J = 16.8, 5.4 Hz, 1.78H), 4.49-4.52 (m, 3.48H), 4.68 (d, J = 4.4 Hz, 1.31H), 4.88-5.02 (m, 2H), 5.69-5.79 (m, 1H), 6.74 (t, J = 5.0 Hz, 0.60H), 7.47-7.57 (m, 4.48H), 8.01-8.05 (m, 2H), 8.19 (d, J = 8.4 Hz, 1H), 8.30 (d, J = 8.8 Hz, 1H), 8.43 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.3, 26.6, 28.7, 33.6, 34.8, 48.8, 64.1, 72.9, 114.9, 124.2, 125.3, 126.3, 127.1, 128.7, 129.4, 130.3, 131.7, 138.7, 150.3, 156.6; FT-ICR-MS calcd for $C_{26}H_{31}N_2O_3^+$ $[M+H]^+$ m/z 419.2329, found

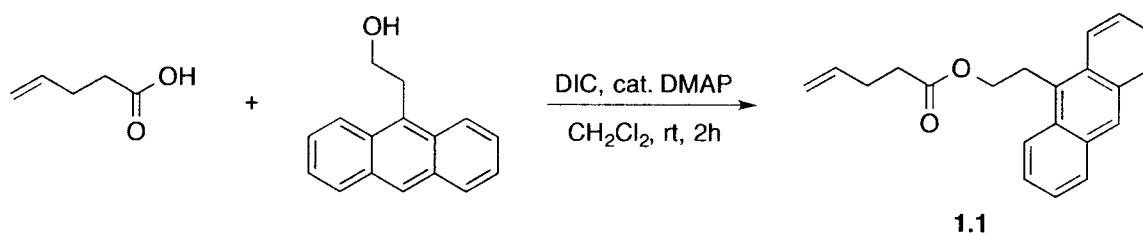
419.2333.

Linker **S6** was synthesized using the procedure described for the synthesis of linker **S4**.

Synthesis of linker precursor 12.3.

5

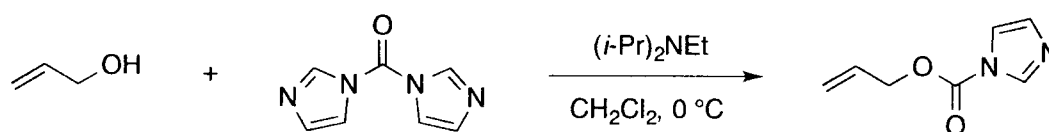
2-(Anthracen-9-yl)ethyl pent-4-enoate



4-Pentenoic acid (159 μL , 1.56 mmol) and 2-(anthracen-9-yl)ethanol (281 mg, 1.26 mmol) were dissolved in dry CH_2Cl_2 (15 mL) with stirring. DIC (305 μL , 1.95 mmol) was added to the reaction solution followed by cat. DMAP. After 2 h, the white solids were filtered out and the filter cake was washed with CH_2Cl_2 . The filtrate was condensed *in vacuo* and the crude material was purified by column chromatography (SiO_2 , 1:1 hexanes: CH_2Cl_2) to give **12.1** (359 mg, 93%) as a yellow oil. R_f 0.36 (1:1 hexanes: CH_2Cl_2); ^1H NMR (400 MHz, CDCl_3) δ 2.35-2.46 (m, 4H), 3.99 (t, $J = 8.0$ Hz, 2H), 4.50 (t, $J = 7.8$ Hz, 2H), 5.00-5.08 (m, 2H), 5.79-5.88 (m, 1H), 7.46-7.50 (m, 2H), 7.54-7.58 (m, 2H), 8.02 (d, $J = 8.4$ Hz, 2H), 8.38 (d, $J = 8.8$ Hz, 2H), 8.40 (s, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 27.5, 29.0, 33.8, 64.3, 115.7, 124.3, 125.1, 126.2, 127.0, 129.2, 129.4, 130.5, 131.7, 136.8, 173.4.

Allyl 1*H*-imidazole-1-carboxylate

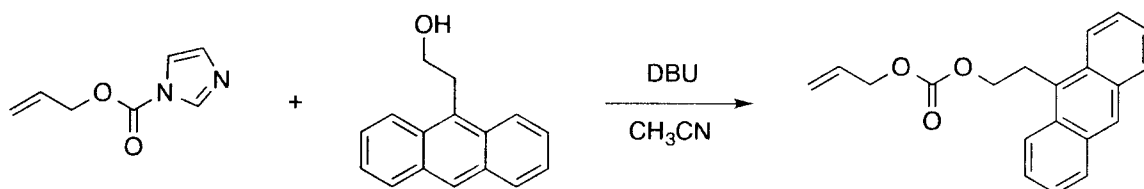
20



N,N-Diisopropylethylamine (5.37 mL, 30.8 mmol) was added to a solution of allyl alcohol (1.21 g, 20.8 mmol) in dry CH_2Cl_2 (69 mL). The solution was cooled to 0 $^\circ\text{C}$ and 1,1'-carbonyldiimidazole (5.07 g, 31.2 mmol) was added and the reaction was stirred overnight. The reaction was washed with water (2 x 40 mL), brine (40 mL) and dried over Na_2SO_4 , filtered and concentrated *in vacuo*. The crude material was purified by column chromatography (SiO_2 , EtOAc) to give the acyl imidazole intermediate (2.24 g, 71%) as a light yellow oil. R_f 0.51 (EtOAc); ^1H NMR (400 MHz, CDCl_3) δ 4.87 (d, $J = 5.6$ Hz, 2H), 5.37 (d, $J = 10.0$ Hz, 1H), 5.44 (d, $J = 16.8$

Hz, 1H), 5.95-6.03 (m, 1H), 7.05 (s, 1H), 7.42 (s, 1H), 8.13 (s, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 68.8, 117.3, 120.6, 130.6, 130.9, 137.3, 148.6.

Allyl (2-(anthracen-9-yl)ethyl) carbonate

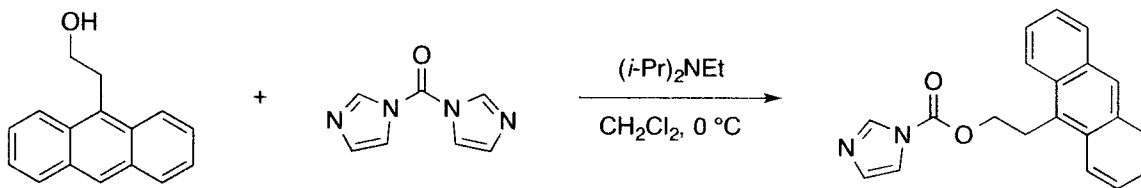


1.2

5

1,8-Diazabicyclo[5.4.0]undec-7-ene (1.68 mL, 11.2 mmol) was added to a solution of the acyl imidazole intermediate (1.71 g, 11.2 mmol) in dry CH_3CN (56 mL). The reaction solution was stirred for 10 minutes before adding 2-(anthracen-9-yl)ethanol (2.50 g, 11.2 mmol). The reaction
 10 was stirred overnight before quenching with sat. NH_4Cl (40 mL). The aqueous phase was extracted with EtOAc (2 x 40 mL) and the combined organic phases were washed with brine, dried over Na_2SO_4 , filtered and concentrated *in vacuo*. The crude material was purified by column chromatography (SiO_2 , 3:1, CH_2Cl_2 :hexanes) to give **1.2** (2.33 g, 68%) as yellow crystals. R_f 0.63 (3:1, CH_2Cl_2 :hexanes); ^1H NMR (400 MHz, CDCl_3) δ 4.06 (t, J = 8.0 Hz, 2H), 4.52 (t, J = 8.4 Hz, 2H), 4.69 (d, J = 5.6 Hz, 2H), 5.31 (dd, J = 10.8, 1.8 Hz, 1H), 5.40 (dd, J = 17.6, 1.2 Hz, 1H), 5.93-
 15 6.03 (m, 1H), 7.47-7.50 (m, 2H), 7.55-7.59 (m, 2H), 8.02 (d, J = 8.4 Hz, 2H), 8.35 (d, J = 8.8 Hz, 2H), 8.40 (s, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 27.6, 67.4, 68.7, 119.1, 124.1, 125.2, 126.4, 127.2, 128.3, 129.5, 130.5, 131.7, 131.8, 155.3.

2-(Anthracen-9-yl)ethyl 1*H*-imidazole-1-carboxylate

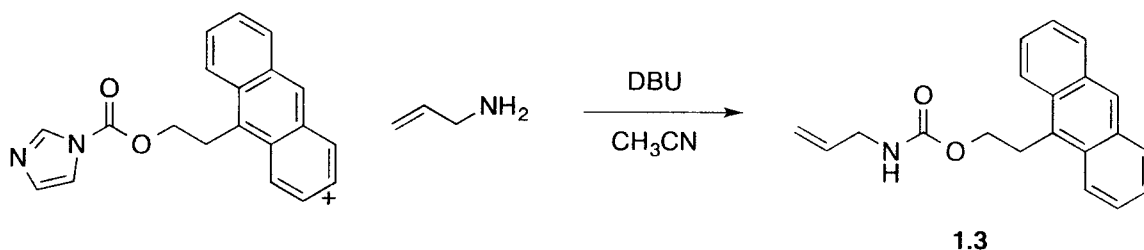


20

N,N-Diisopropylethylamine (408 μL , 2.34 mmol) was added to a solution of 2-(anthracen-9-yl)ethanol (346 mg, 1.55 mmol) in dry CH_2Cl_2 (8 mL), the solution was cooled to 0 °C and 1,1'-carbonyldiimidazole (380 mg, 2.34 mmol) was added. After stirring overnight, the reaction solution was washed with water (2 x 5 mL), brine, dried over Na_2SO_4 , filtered and concentrated *in vacuo*. The crude material was purified by column chromatography (SiO_2 , CH_2Cl_2 to 3:2, EtOAc: CH_2Cl_2 gradient) to give the acyl imidazole intermediate (436 mg, 89%) as a pale yellow
 25

solid. R_f 0.23 (1:19, EtOAc:CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 4.16 (t, J = 7.4 Hz, 2H), 4.80 (t, J = 7.6 Hz, 2H), 7.02 (s, 1H), 7.31 (s, 1H), 7.50 (t, J = 7.6 Hz, 2H), 7.58 (t, J = 7.4 Hz, 2H), 8.00 (s, 1H), 8.04 (d, J = 8.0 Hz, 2H), 8.33 (d, J = 8.8 Hz, 2H), 8.44 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 27.0, 68.0, 117.3, 123.8, 125.3, 126.6, 127.5, 127.9, 129.7, 130.5, 130.8, 131.7, 137.3, 149.0.

2-(Anthracen-9-yl)ethyl allylcarbamate



1,8-Diazabicyclo[5.4.0]undec-7-ene (206 μ L, 1.38 mmol) was added to a solution of the acyl imidazole intermediate (436 mg, 1.38 mmol) in dry CH₃CN (7 mL). The reaction solution was stirred for 10 minutes before adding allylamine (114 μ L, 1.51 mmol). The reaction was stirred overnight before quenching with sat. NH₄Cl (40 mL). The aqueous phase was extracted with EtOAc (2 x 5 mL) and the combined organic phases were washed with brine, dried over Na₂SO₄, filtered and concentrated *in vacuo*. The crude material was purified by column chromatography (SiO₂, CH₂Cl₂ to 2:3, EtOAc:CH₂Cl₂ gradient) to give **1.3** (356 mg, 85%) as pale yellow crystals. R_f 0.72 (1:19, EtOAc:CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 3.85 (br s, 2H), 4.00 (t, J = 7.8 Hz, 2H), 4.47 (t, J = 7.6 Hz, 2H), 4.74 (br s, 1H), 5.13-5.22 (m, 2H), 5.84-5.90 (m, 1H), 7.45-7.49 (m, 2H), 7.53-7.56 (m, 2H), 8.01 (d, J = 8.4 Hz, 2H), 8.36 (d, J = 8.8 Hz, 2H), 8.39 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 28.1, 43.7, 64.9, 116.3, 124.4, 125.1, 126.2, 126.9, 129.4, 130.6, 131.7, 134.7, 156.7.

Synthesis of SiO₂@Fe₃O₄ NPs.

The Fe₃O₄ NPs were coated with a thin silica shell using a modified procedure described in the literature (Pinho, S.; et al., *ACS Nano* **2010**, *4*, 5339-5349). EMG 304 ferrofluid (1 mL, 233 mg NPs, 8.55 x 10¹⁶ NPs) was added to Millipore water (98 mL) and was then added to a solution of EtOH (312 mL) and NH₄OH (6.2 mL, 28-30%) with rapid mechanical stirring. Tetraethyl orthosilicate (TEOS) (2.13 mL) was then added to the colloidal suspension and was stirred for 12 h. An aliquot was removed to allow for characterization. The NPs were magnetically separated and the remaining colloidal supernatant was centrifuged at 13,200 RPM for 20 min. The NPs were then washed 5x with EtOH with magnetic separation and centrifugation after each wash. The excess

EtOH was then removed by rotary evaporation and the NPs were dried under vacuum for 3 h. The resulting $\text{SiO}_2@\text{Fe}_3\text{O}_4$ NPs were characterized by IR, TGA, DLS (Figure 1), ζ -potential (Figure 2), SQUID (Figure 4) and TEM (Figure 3).

5 SQUID measurements.

The magnetic properties of the MNP were measured using a Quantum Design MPMS-5S superconducting quantum interference device (SQUID) magnetometer (in the temperature range from 2 to 400 K). The samples were loaded in a gel capsule and secured inside a standard drinking straw with small holes punched in both the straw and capsule for equalization of pressure and
10 temperature. $M(H)$ data was taken by starting the sample at saturation, 5 T, and then cycling to -5 T and back to 5 T in different step ranges to see the details of the hysteresis. The data was taken using DC Magnetization as to disturb the MNP as little as possible during the measurement (Figure 4).

15 Synthesis of $\text{AO}@\text{SiO}_2@\text{Fe}_3\text{O}_4$ and $\text{FL}@\text{SiO}_2@\text{Fe}_3\text{O}_4$ NPs.

The suspension of $\text{SiO}_2@\text{Fe}_3\text{O}_4$ NPs was placed under mild vacuum and heated to 60 °C for 8 h. This process was used to remove the catalytic ammonia thus increasing the chances of obtaining a thin organic monolayer on the surface of the NPs. The removal of the ammonia decreased the pH of the solution from 11.7 to 8.8. Then, EtOH (5 mL) was added to **S4** or **S6** (1.454 mmol) and the
20 solution was added to the NP suspension with rapid mechanical stirring. The mixture was stirred for 20 h. The majority of the $\text{AO}@\text{SiO}_2@\text{Fe}_3\text{O}_4$ or $\text{FL}@\text{SiO}_2@\text{Fe}_3\text{O}_4$ NPs were then magnetically separated and the remaining colloidal supernatant was centrifuged at 13,200 RPM for 20 min. The NPs were then washed 5x with EtOH with magnetic separation and centrifugation after each wash. The excess EtOH was then removed by rotary evaporation and the NPs were dried under vacuum
25 for 3 h. The resulting NPs were characterized by IR, TGA, DLS (Figure 5), ζ -potential (Figure 6) and TEM (Figure 7).

Assay of Percent FL Released.

To determine the percent of **11** released from $\text{FL}@\text{SiO}_2@\text{Fe}_3\text{O}_4$ NPs, the residual amount of
30 **S6** remaining on the $\text{FL}@\text{SiO}_2@\text{Fe}_3\text{O}_4$ NPs post AMF exposure was determined as follows. After recording the fluorescence intensity of the last (eighth) AMF pulse, the NPs were magnetically separated and the AMF supernatant was removed. The isolated NPs then were washed 5x with MeCN followed by magnetic separation to remove any non-covalently bound **11**. To the washed

NPs was added 1 mL of 5% HF in EtOH. The suspension was stirred until all NPs had dissolved resulting in a light yellow solution. The acidic solution was added to a separatory funnel and then basified using sat. aq. NaHCO₃. The basic solution was extracted 3 x with Et₂O and the combined extracts were concentrated by rotary evaporation and dried under vacuum. The resulting residue was dissolved in a 2:1 mixture of PBS:MeCN (0.75 mL) at pH 7.4 and the fluorescence was measured. This measured fluorescence intensity was then added to the total fluorescence intensity measured in the supernatant after the final AMF pulse, and the combined intensity was set to 100%. The results from all three experiments were normalized by dividing the fluorescent intensity by the milligrams of FL@SiO₂@Fe₃O₄ NPs used for the experiment.

FT-IR Spectra.

FT-IR measurements were taken on a Perkin-Elmer Spectrum 100 FT-IR with a universal ATR attachment. All IR spectra underwent ATR correction using Perkin-Elmer software (Figures 8A, 8B, 9A and 9B).

TGA.

TGA measurements were made on a TA Instruments Hi-Res TGA 2950 Thermogravimetric Analyzer using a Pt basket and maintaining a flow of N₂ gas through the oven (Figure 10). Each TGA experiment was run from 35 °C to 800 °C at a ramp rate of 20 °C/min.

AMF heating of bulk solution.

Temperature measurements were taken with a Neoptix Nomad fitted with a fiber optic temperature sensor. All data was recorded using Neoptix NeoLink software. AMF conditions were set to 501.6 amps and a frequency of 204 kHz. Each AMF pulse lasted for 5 min followed by 5 min of without an AMF for a combined total of 40 min of AMF exposure (Figure 11). All samples were started at room temperature and were allowed to cool at room temperature; all heating was induced using only an AMF. The control was 0.75 mL of 2:1 PBS:acetonitrile without SiO₂@Fe₃O₄ NPs, which gave a ΔT of 4.8 ± 0.1 °C with a 5 min AMF pulse. The ΔT of a 5 min AMF pulse with 7.0 mg of SiO₂@Fe₃O₄ NPs in 0.75 mL 2:1 PBS:acetonitrile was 16.4 ± 0.3 °C.

MALDI-TOF results.

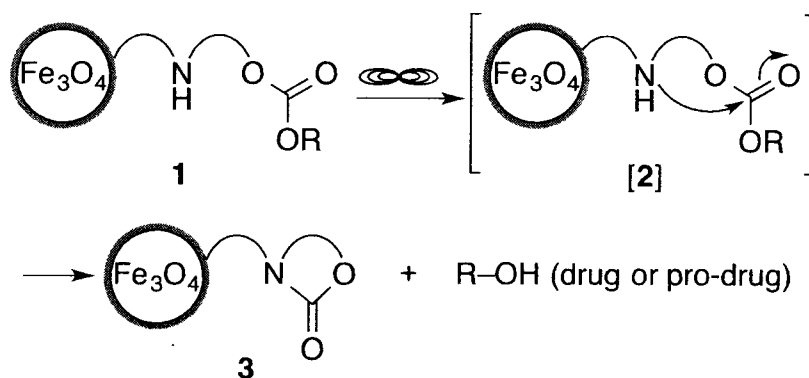
MALDI-TOF analysis was done on a Voyager DE-Pro MALDI-TOF instrument (PE

Biosystems). Spectra were acquired in positive reflectron mode and calibration was achieved by using known peaks from the 2,5-dihydroxybenzoic acid (2,5-DHB) matrix (Figure 12). Data was analyzed with mMASS data analysis software.

5 Results and Discussion.

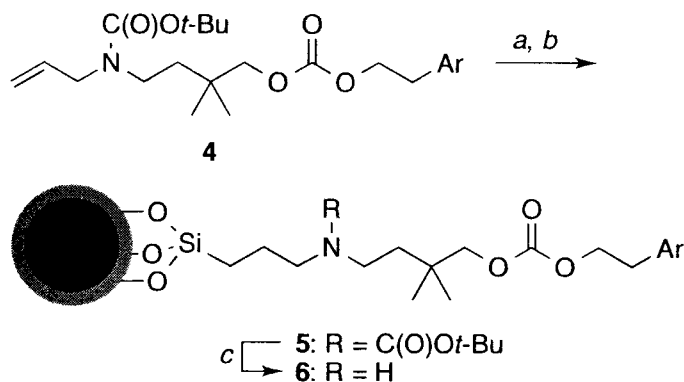
It has been discovered that AMF can cause the rapid hydrolysis of an otherwise robust chemical linkage, an *N,N*-disubstituted carbamate. As depicted graphically in Figure 13, a core-shell Fe_3O_4 - SiO_2 NP construct fitted with short chains that terminate with a carbamate-bound fluorophore was engineered. Pulsed AMF irradiation of an aqueous, 37 °C suspension of these NPs buffered at pH 7.4 results in carbamate hydrolysis to release the fluorophore at a rate considerably faster than the limited release observed without irradiation.

Scheme 1 illustrates covalently tethering a drug to shell-core SiO_2 - Fe_3O_4 NPs via carbonate functionality using a linking chain that contains a secondary amine, such as shown by NP assembly **1**. Application of an AMF to raise the surrounding temperature resulted in an intramolecular cyclization via reaction of the amine with the carbonate moiety, as in WO2014/124329. The resultant formation of oxazolidinone **3** would be accompanied by release of the bound substrate R-OH.



Scheme 1. AMF-mediated intramolecular cyclization results in release of ROH.

Studies were conducted to evaluate the thermal responsiveness of a panel of homologous ester and carbonate linkers (Knipp, R. J.; Estrada, R.; Sethu, P.; Nantz, M. H. *Tetrahedron* **2014**, 70, 3422-3429). One study involved carbonate **4** (Scheme 2).



Scheme 2. Synthesis of NP-carbonate linker conjugates with anthracenyl fluorophore (Ar = 9-anthracenyl). Conditions: *a.* $(\text{EtO})_3\text{SiH}$, cat. PtO_2 , 90 °C; *b.* $\text{SiO}_2@\text{Fe}_3\text{O}_4$, EtOH, H_2O ; *c.* i. $\text{CF}_3\text{CO}_2\text{H} : \text{CH}_2\text{Cl}_2$, 1:1, 0 °C, 1 h; ii. $\text{Et}_3\text{N} : \text{CH}_2\text{Cl}_2$, 1:1, rt.

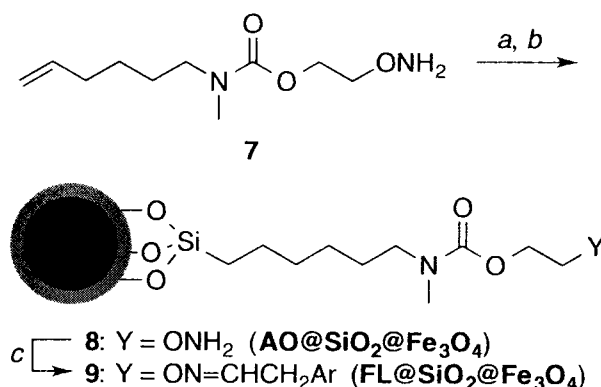
A shell-core $\text{SiO}_2@\text{Fe}_3\text{O}_4$ NPs was prepared using a modified version of the Stöber process as reported (Pinho, S.; et al., *ACS Nano* **2010**, *4*, 5339-5349). Commercial Fe_3O_4 NPs (EMG 304, FerroTec) with a reported average diameter of 10 nm were silylated to form monodispersed superparamagnetic $\text{SiO}_2@\text{Fe}_3\text{O}_4$ NPs with a particle size distribution of 24 ± 6 nm and a silica shell thickness of 6-7 nm, as determined by transmission electron microscopy (Figure 14A). Residual ammonia from the shell application procedure was removed prior to NP functionalization by placing the ethanol/water suspension of $\text{SiO}_2@\text{Fe}_3\text{O}_4$ NPs under mild vacuum and then heating at 60 °C for 8h. Removal of the ammonia in this way decreased the pH of the suspension from 11.7 to 8.8. The resultant $\text{SiO}_2@\text{Fe}_3\text{O}_4$ NPs then were functionalized by adding an ethanolic solution of the triethoxysilane-derived linker obtained from PtO_2 -catalyzed (Sabourault, N. et al., *Org. Lett.* **2002**, *4*, 2117-2119) hydrosilylation of alkene **4** (Knipp, R. J.; Estrada, R.; Sethu, P.; Nantz, M. H. *Tetrahedron* **2014**, *70*, 3422-3429) with $(\text{EtO})_3\text{SiH}$ to obtain Boc-protected NPs **5** (Scheme 2). The Boc group was cleaved by treatment with trifluoroacetic acid followed by neutralization with triethylamine to unmask the nucleophilic amine needed for the AMF-induced intramolecular cyclization. NPs **6** then were screened in a pulsed AMF experiment for release of the bound anthracene fluorophore.

A suspension of the free-amine NPs **6** in a 2:1 mixture of PBS:acetonitrile (pH adjusted to 7.4) at room temperature was sequentially pulsed with an AMF for six 5- minute bursts with a delay time of 5 minutes between pulses. Magnetic-assisted sedimentation of the NPs allowed facile measurement of supernatant fluorescence, imparted by the fluorophore released on intramolecular cyclization, at the end of each pulse (Figure 15). The data clearly show an AMF-induced release of

fluorophore, confirmed by MALDI-TOF analysis as 2-(anthracen-9-yl)ethan-1-ol, presumably as a consequence of amine attack onto the proximal carbonate moiety. To test the proposed mechanism of substrate release, the Boc-protected NPs **5**, which would be incapable of undergoing the postulated intramolecular cyclization, under identical conditions was examined. Fluorophore release from **5** also occurred in response to AMF irradiation, albeit at a rate slower than the free amine formulation (Figure 15). This result suggests carbonate cleavage by a route other than oxazolidinone formation, and points toward enhanced hydrolysis as a function of the energy deposited by AMF irradiation. Significant, rapid heating within nanometers of an iron oxide NP surface is known to occur on application of an AMF (Riedinger, A.; et al., *T. Nano Lett.* **2013**, *13*, 2399-2406). Another possible mechanism for release of the fluorophore would involve removal of the Boc group followed by intramolecular cyclization. Pulsed AMF irradiation at this concentration of NPs did not significantly increase the temperature of the suspension, as confirmed by measurements taken using a fiber optic temperature sensor ($+16.4 \pm 0.3$ °C from start of AMF pulse to end of AMF pulse, $+4$ °C from start of first pulse to start of sixth pulse). On examination of the stability of NPs **5** under the same conditions but without AMF irradiation, it was noted the gradual release of 2-(anthracen-9-yl)ethan-1-ol over an hour at 37 °C to ~50% of the cumulative level achieved by the six 5-minute bursts of AMF irradiation. This finding is noteworthy because dialkylcarbonates are stable to such mild conditions and generally require highly basic conditions for their hydrolysis (e.g., 5M NaOH, MeOH, rt, 3d (Zhang, J.; et al., *Macromolecules* **2013**, *46*, 2535-2543). Exposure of carbonate **4** (Scheme 1) to SiO₂@Fe₃O₄ NPs in 2:1 phosphate buffer solution (PBS):acetonitrile at 37 °C resulted in no reaction, even after 24 h exposure. It is possible that the close proximity and the tethered relationship of the carbonate linkage in **5** to the iron oxide core and silica shell enhance, possibly through Lewis acid or hydrogen bonding interactions, respectively, the rate of hydrolysis of an otherwise robust chemical linkage.

Based on this study additional NPs for an AMF-responsive delivery system that resists hydrolysis at 37 °C and pH 7.4 were examined. One study modified the linker by removing the nucleophilic element and used an *N*-methyl carbamate linkage to replace the carbonate moiety. Even unsubstituted carbamates are extremely resistant to hydrolysis and typically require treatment with base at elevated temperatures for cleavage (e.g., KOH, diglyme, 200 °C) (Melamed, J. Y.; et al., *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4700-4703). A strategy for convenient attachment of substrate to the simplified carbamate-linked NPs, namely oximation was used. Click chemistry reaction of NP-bound aminooxy groups with aldehydes to form oxime ethers can be used as a convenient means for surface functionalization (Biswas, S.; et al., *Biomaterials* **2011**, *32*, 2683-

2688; Beaudette, T. T et al., *J. Am. Chem. Soc.* **2009**, *131*, 10360-10361; Kolb, H. C.; et al., *Angew. Chem. Int. Ed.* **2001**, *40*, 2004-2021). Importantly, any newly formed oxime ether linkage would be sufficiently resistant to hydrolysis and thus decrease the risk of a non-AMF induced release (Kalia, J.; Raines, R. *Angew. Chem. Int. Ed.* **2008**, *47*, 7523-7526). The combination of these structural alterations is embodied by NP assembly **9** (Scheme 3) in which the anthracene fluorophore probe has been introduced by oximation using (9-anthracenyl)-acetaldehyde (**10**) (Jiang, H.; et al., *Angew. Chem. Int. Ed.* **2012**, *51*, 10271-10274).

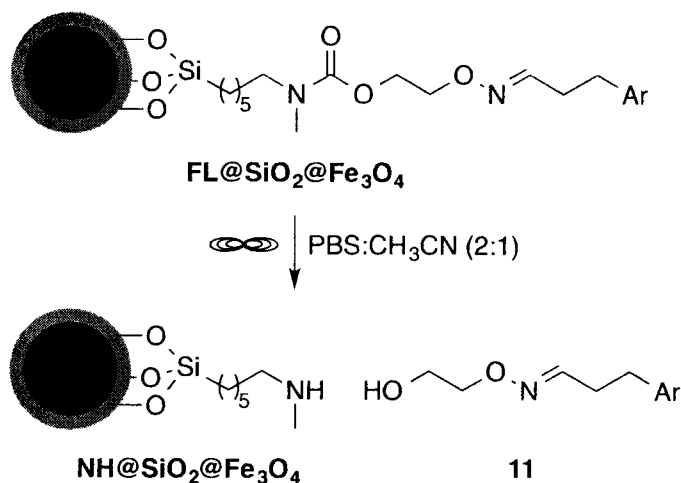


Scheme 3. Synthesis and reaction of aminooxy-coated SiO₂@Fe₃O₄ NPs (Ar = 9-anthracenyl). Conditions: *a.* (EtO)₃SiH, cat. PtO₂, 90 °C, 48 h; *b.* SiO₂@Fe₃O₄, EtOH, H₂O; *c.* (9-anthracenyl)-CH₂CHO (**10**), EtOH, H₂O, rt, 8 h.

Aminooxy *N*-methyl-carbamate **7** (Scheme 3) was loaded onto SiO₂@Fe₃O₄ NPs using the established methodology (Sabourault, N.; et al., *Org. Lett.* **2002**, *4*, 2117-2119) to obtain aminooxy NPs **8** (AO@SiO₂@Fe₃O₄). This functionalization increased the shell thickness by ~3 nm to afford AO@SiO₂@Fe₃O₄ NPs with a diameter of 30 ± 8 nm as determined by TEM (Figure 14B). The hydrodynamic diameter of aqueous AO@SiO₂@Fe₃O₄ NPs was measured at 96 nm with a polydispersity of 0.16, roughly similar to the hydrodynamic diameter of the starting SiO₂@Fe₃O₄ NPs at 103 nm with a polydispersity of 0.17. Little change was observed in ζ-potential, where both the SiO₂@Fe₃O₄ and AO@SiO₂@Fe₃O₄ NPs registered *ca.* -45 mV as aqueous suspensions. Simple mixing of AO@SiO₂@Fe₃O₄ with anthracene aldehyde **10** in aqueous ethanol gave NPs **9** (FL@SiO₂@Fe₃O₄). In an alternate approach, the oximation step was conducted first by reacting anthracene aldehyde **10** with **7** (Scheme 3) followed by hydrosilylation of the oxime ether adduct and then loading onto SiO₂@Fe₃O₄ NPs to obtain **9**.

With the FL@SiO₂@Fe₃O₄ NPs in hand, the AMF-induced hydrolysis of the carbamate

linkage was studied by measuring the release of anthracene oxime ether fragment **11** (Scheme 4).

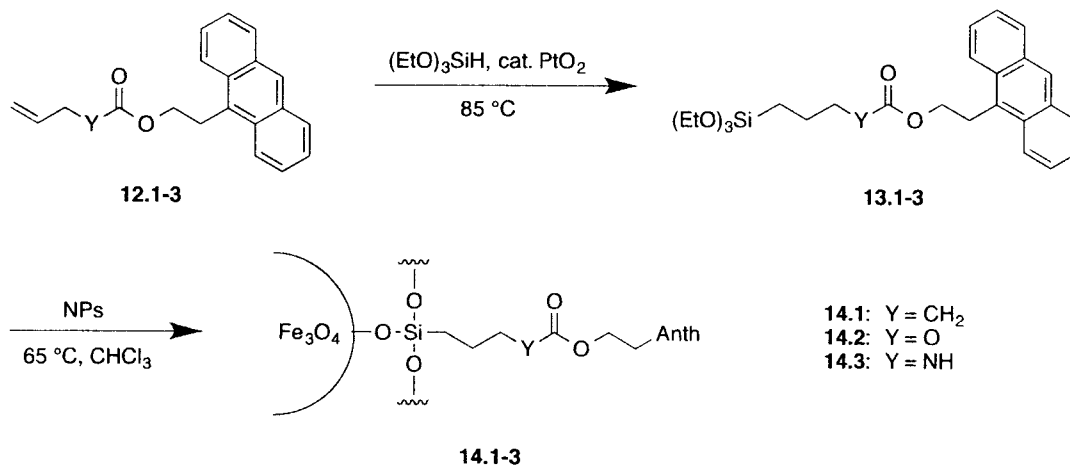


Scheme 4. AMF-induced carbamate hydrolysis.

5 The AMF experiments were conducted as described above (2:1 PBS:acetonitrile solution, pH 7.4). As can be seen in Figure 16, AMF irradiation stimulated a rapid release of substrate from FL@SiO₂@Fe₃O₄. MALDI-TOF analysis confirmed that the oxime ether-anthracene fragment **11** was liberated from the NP formulation on AMF irradiation. Based on fluorescent measurements, 40 minutes of AMF exposure resulted in 67 ± 3% of payload release; longer exposure times did not
 10 release additional **11**. The switch from carbonate to carbamate dramatically reduced non-AMF release of substrate (Figure 16) — less than 10% of the payload was released at 37 °C after 40 minutes. The rapid release of the fluorophore fragment on AMF application is quite remarkable in that *N,N*-dialkyl carbamates are not expected to cleave under these mild conditions (e.g., a previous kinetic study estimates a typical half-life of >550 years at 25 °C and pH 7) (Dittert, L. W.; Higuchi,
 15 T. *J. Pharm. Sci.* **1963**, 52, 852-857).

Uncoated Fe₃O₄ NPs were prepared that incorporated ester, carbonate and carbamate linkers. Fe₃O₄ NPs were loaded with the alkoxysilane linker following a literature procedure (Galeotti, F.; et al., *J. Colloid Interface Sci.* **2011**, 360, 540-547 and shown in Scheme 5. The alkoxysilane (10 mmol/g NPs) were added to a suspension of Fe₃O₄ NPs in CHCl₃. The mixture
 20 was heated to 65 °C for 48 h with mechanical stirring. Upon completion, the functionalized NPs were magnetically separated and the supernatant was decanted. The resulting NPs were washed with CHCl₃ (5x) followed by magnetic separation and decantation. After washing, the NPs were heated to 100 °C for 24 h to induce the final condensation and form a covalent bond between the linker and the iron NP. The loading of the functionalized NPs was determined by
 25 thermogravimetric analysis. All fluorescence measurements were taken at an excitation of 360 nm

and an emission wavelength of 413 nm in a quartz cuvette. Figure 17 shows the pulsed AMF-hydrolysis testing while Figure 18 show hydrolysis without AMF.



Scheme 5. Hydrosilylation and Fe₃O₄ NP loading with triethoxysilane-ester, -carbonate and -carbamate linkers.

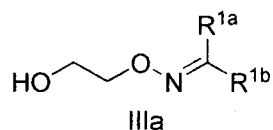
Fe₃O₄ NPs with different coatings were prepared to test the effect of restricting excess to the Fe₃O₄ core (Figure 19). Excess polysaccharide, D-lactose or dextran (6 kD), was added to **3.1** (10 mg) in DMSO (1 mL) and the suspension was mixed for 17 h. The NPs were magnetically separated and the supernatant was decanted. The resulting NPs were washed once with DMSO and twice with MeOH before being dried under vacuum.

Unfunctionalized Fe₃O₄ NPs were coated with a silica shell using the Stöber process (Hui, C.; et al., *Nanoscale* **2011**, 3, 701-705). Briefly, Fe₃O₄ NPs (115 mg) were suspended in Millipore water with sonication, then NH₄OH (25%, 4.4 mL, 28.6 mmol) and EtOH (180 mL) were added to the suspension. With rapid mechanical stirring, tetraethyl orthosilicate (TEOS) (1.76 mL, 7.94 mmol) was added dropwise at rt and reaction was stirred overnight. The SiO₂@Fe₃O₄ NPs were magnetically separated and the supernatant was decanted. The remaining NPs were washed with EtOH (4x) and were dried under vacuum. Figure 19 shows the hydrolysis of the carbonate linker with these various coatings.

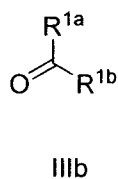
Therapeutic magnetic nanoparticles with therapeutic agents comprising a ketone or aldehyde group can be readily prepared according Scheme 3 above using chemical steps analogous to those described herein.

The ketone or aldehyde of the therapeutic agent has been converted to a prodrug of the therapeutic agent as shown in formula IIIa which prodrug is attached to the linker. Accordingly, one embodiment provides a therapeutic agent which is a prodrug of the therapeutic agent and is

represented by formula IIIa:

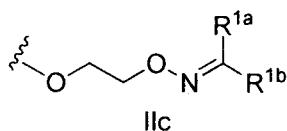


wherein R^{1a} and R^{1b} together with the remainder of formula IIIa are the prodrug of the therapeutic agent. It is to be understood that the prodrug of formula IIIa represents a therapeutic agent of formula IIIb (wherein R^{1a} and R^{1b} and the carbonyl to which they are attached represent a therapeutic agent):



wherein the ketone or aldehyde of the therapeutic agent of formula IIIb has been condensed with the aminooxy moiety of HO-(CH₂)₂-O-NH₂ to arrive at the prodrug of the therapeutic agent of formula IIIa.

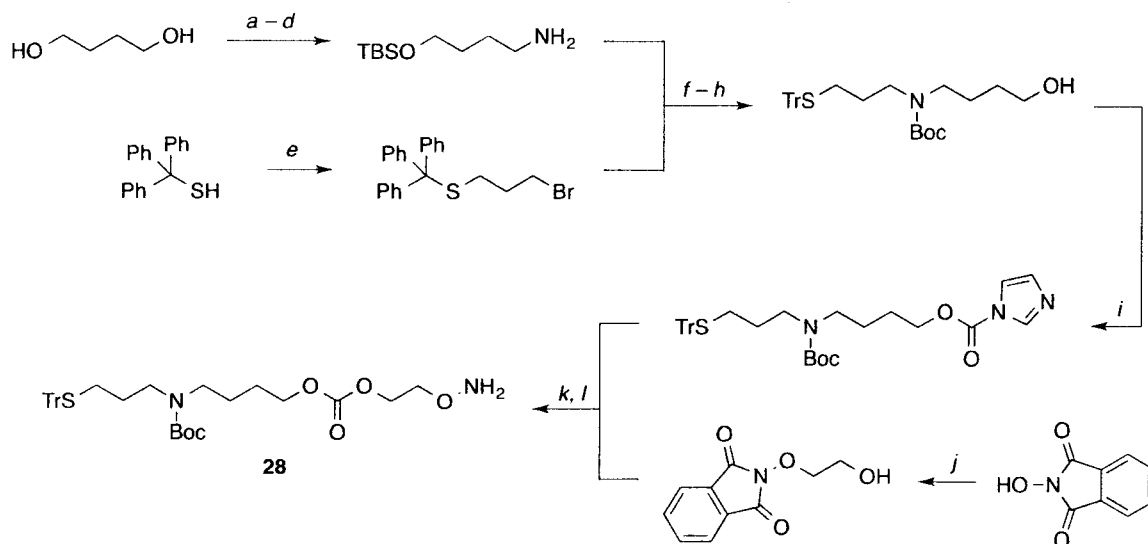
In one embodiment a residue of a therapeutic agent (D or -Z-D¹) is represented by formula IIIc:



wherein R^{1a} and R^{1b} together with the remainder of formula IIIc are the residue of the therapeutic agent (D or -Z-D¹).

Example 2. Preparation of Gold NP Linker

Scheme 6



Scheme 6. Synthesis of carbonate linker **28** for attachment to Au@Fe₃O₄ NPs. Conditions: *a.*

TBSCl, Et₃N, CH₂Cl₂, 0 °C, 17 h; *b.* MsCl, Et₃N, CH₂Cl₂, 0 °C, 1.5 h; *c.* phthalimide, K₂CO₃,

5 DMSO, 75 °C, 18 h; *d.* N₂H₄•H₂O, 1:2 EtOH:CH₂Cl₂, 20 h; *e.* 1,2-dibromoethane, K₂CO₃, THF,

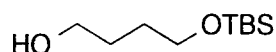
reflux, 24 h, 98%; *f.* MeCN, 55 °C, 24 h, 82%; *g.* Boc₂O, Et₃N, CH₂Cl₂, 0 °C, 2 h; *h.* 1 M TBAF in

THF, THF, 0 °C, 20 h, 62%; *i.* 1,1-carbonyldiimidazole, (*i*-Pr)NEt, CH₂Cl₂, 0 °C, *j.* 2-

bromoethanol, AcONa, DMSO, 70 °C, 67%; *k.* DBU, MeCN, 18 h, 55%; *l.* N₂H₄•H₂O, CH₂Cl₂, 0 °C, 92%.

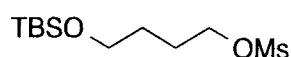
10

Synthesis of Thiol Linker for Loading onto Au@Fe₃O₄ NPs



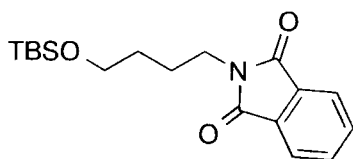
4-((*tert*-Butyldimethylsilyl)oxy)butan-1-ol. A solution of TBSCl (5.20 g, 34.5 mmol) in dry CH₂Cl₂ (60 mL) was added dropwise over 1 h to a solution of 1,4-butanediol (14.7 mL, 166 mmol) and Et₃N (6.99 mL, 49.8 mmol) in dry CH₂Cl₂ (50 mL) at 0 °C and was stirred overnight. The solvent was removed *in vacuo* and the remaining oil was extracted with hexanes (4x) and the combined extractions were washed twice with sat. NH₄Cl, once with brine and was dried over Na₂SO₄, filtered and concentrated *in vacuo* to afford crude 4-((*tert*-butyldimethylsilyl)oxy)butan-1-

20 EtOAc:hexanes).

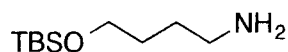


4-((*tert*-Butyldimethylsilyl)oxy)butyl methanesulfonate. Methanesulfonyl chloride (2.87 mL, 37.0 mmol) was added to a solution of crude 4-((*tert*-butyldimethylsilyl)oxy)butan-1-ol (6.58 g, 32.2 mmol) and Et₃N (6.79 mL, 48.3 mmol) in dry CH₂Cl₂ (107 mL) at 0 °C and was stirred for 2

h. The reaction solution was washed twice with sat. NH_4Cl and the combined aqueous layers were extracted twice with CH_2Cl_2 . The combined organic phases were washed once with brine and was dried over Na_2SO_4 , filtered and concentrated *in vacuo* to afford 4-((*tert*-butyldimethylsilyl)oxy)butyl methanesulfonate (9.03 g, 99%) as an orange oil and was used without further purification. R_f 0.52 (1:3, EtOAc:hexanes); ^1H NMR (400 MHz, CDCl_3) δ 0.04 (s, 6H), 0.88 (s, 9H), 1.62 (quin, $J = 6.0$ Hz, 2H), 1.83 (quin, $J = 7.2$ Hz, 2H), 2.99 (s, 3H), 3.64 (t, $J = 6.0$ Hz, 2H), 4.26 (t, $J = 6.6$ Hz, 2H); ^{13}C NMR (100 MHz, CDCl_3) δ -5.2, 18.5, 26.1, 28.8, 37.6, 62.4, 70.3.

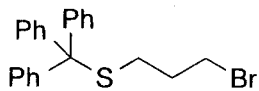


2-(4-((*tert*-Butyldimethylsilyl)oxy)butyl)-2,3-dihydro-1*H*-isoindole-1,3-dione. Phthalimide (8.55 g, 58.1 mmol) was added to a solution of 4-((*tert*-butyldimethylsilyl)oxy)butyl methanesulfonate (8.64 g, 30.6 mmol) and K_2CO_3 (5.07 g, 36.7 mmol) in DMSO (180 mL) and the solution was heated to 75 °C for 17 h. The reaction was then cooled to rt and was quenched with water. The aqueous solution was extracted with EtOAc (4x) and the combined organic phases were washed with water (3x). The organic phase was then washed with brine, dried over Na_2SO_4 , filtered and concentrated *in vacuo* to afford 2-(4-((*tert*-butyldimethylsilyl)oxy)butyl)-2,3-dihydro-1*H*-isoindole-1,3-dione (9.27 g, 91%) as white crystals and was used without further purification. R_f 0.59 (1:3, EtOAc:hexanes); ^1H NMR (400 MHz, CDCl_3) δ 0.03 (s, 6H), 0.87 (s, 9H), 1.55 (quin, $J = 6.4$ Hz, 2H), 1.74 (quin, $J = 7.4$ Hz, 2H), 3.63 (t, $J = 6.4$ Hz, 2H), 3.70 (t, $J = 7.2$ Hz, 2H), 7.65 (dd, $J = 2.4, 2.8$ Hz, 2H), 7.83 (dd, $J = 1.6, 3.4$ Hz, 2H); ^{13}C NMR (100 MHz, CDCl_3) δ -5.1, 18.5, 25.3, 26.1, 30.2, 38.1, 62.7, 123.4, 132.4, 134.0, 168.6.



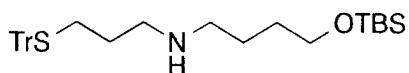
(4-Aminobutoxy)(*tert*-butyl)dimethylsilane. Hydrazine monohydrate (6.74 mL, 139 mmol) was added to a solution of crude 2-(4-((*tert*-butyldimethylsilyl)oxy)butyl)-2,3-dihydro-1*H*-isoindole-1,3-dione (9.27 g, 27.8 mmol) in CH_2Cl_2 (140 mL) at 0 °C and was stirred overnight, allowing the reaction to come to rt. The white precipitate was filtered and the filter cake was washed with ample CH_2Cl_2 . The crude solution was concentrated *in vacuo* to afford (4-aminobutoxy)(*tert*-butyl)dimethylsilane (3.99 g, 71%) as a light yellow oil and was used without further purification. R_f 0.26 (1:9, MeOH: CH_2Cl_2 with 1% NH_4OH); ^1H NMR (400 MHz, CDCl_3) δ 0.03 (s, 6H), 0.87 (s,

9H), 1.44-1.55 (m, 4H), 1.61 (br s, 2H), 2.68 (t, $J = 6.8$ Hz, 2H), 3.60 (t, $J = 6.2$ Hz); ^{13}C NMR (100 MHz, CDCl_3) δ -5.1, 18.5, 26.1, 30.4, 30.5, 42.3, 63.3.



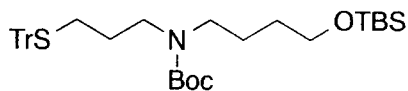
(((3-Bromopropyl)sulfanyl)diphenylmethyl)benzene. K_2CO_3 (2.28 g, 16.5 mmol) followed by

- 5 1,3-dibromopropane (7.61 mL, 75 mmol) was added to a solution of triphenylmethanethiol (4.22 g, 15.3 mmol) in dry THF (75 mL) under N_2 . The reaction was refluxed for 24 h before cooling to rt. The reaction solution was washed twice with water, extracted twice with Et_2O , washed with brine, dried over Na_2SO_4 , filtered and concentrated *in vacuo*. The excess 1,3-dibromopropane was distilled off to afford (((3-bromopropyl)sulfanyl)diphenylmethyl)benzene (5.94 g, 98%) as white
- 10 crystals. R_f 0.54 (1:3, CH_2Cl_2 :hexanes); ^1H NMR (400 MHz, CDCl_3) δ 1.81 (quin, $J = 6.8$ Hz, 2H), 2.32 (t, $J = 6.8$ Hz, 2H), 3.32 (t, $J = 6.8$ Hz, 2H), 7.19-7.30 (m, 9H), 7.40-7.44 (m, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 30.5, 31.8, 32.5, 66.9, 126.9, 128.1, 129.8, 144.9.



12,12,13,13-Tetramethyl-1,1,1-triphenyl-11-oxa-2-thia-6-aza-12-silatetradecane. (((3-

- 15 bromopropyl)sulfanyl)diphenylmethyl)benzene (800 mg, 2.01 mmol) was added to a solution of (4-aminobutoxy)(*tert*-butyl)dimethylsilane (1.02 g, 5.03 mmol) in MeCN (20 mL) and the reaction was heated to 55 °C for 24 h. After cooling to rt, the reaction was quenched with sat. NaHCO_3 and extracted three times with EtOAc. The combined organic phases were washed with brine, dried over Na_2SO_4 , filtered and concentrated *in vacuo*. The crude material was purified by column
- 20 chromatography (SiO_2 , 0:1 to 1:4, MeOH: CH_2Cl_2 with 1% NH_4OH gradient) to give 12,12,13,13-tetramethyl-1,1,1-triphenyl-11-oxa-2-thia-6-aza-12-silatetradecane (852 mg, 82%) as an orange oil. R_f 0.33 (1:4, MeOH: CH_2Cl_2 with 1% NH_4OH); ^1H NMR (400 MHz, CDCl_3) δ 0.04 (s, 6H), 0.88 (s, 9H), 1.51-1.52 (m, 4H), 1.60 (quin, $J = 7.4$ Hz, 2H), 2.17 (t, $J = 7.0$ Hz, 2H), 2.57 (t, $J = 7.2$ Hz, 4H), 3.59 (t, $J = 6.0$ Hz, 2H), 7.17-7.28 (m, 9H), 7.39-7.41 (m, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ
- 25 -5.1, 18.6, 25.9, 26.2, 28.5, 29.9, 30.7, 48.6, 49.5, 63.2, 66.8, 126.8, 128.1, 129.8, 145.1.



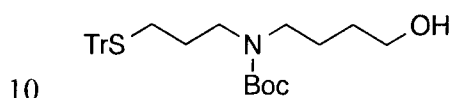
***tert*-Butyl *N*-(4((*tert*-butyldimethylsilyloxy)butyl)-*N*-(3-((triphenylmethyl)sufanyl)**

- propyl)carbamate.** Boc_2O (348 mg, 1.60 mmol) was added to a solution of 12,12,13,13-tetramethyl-1,1,1-triphenyl-11-oxa-2-thia-6-aza-12-silatetradecane (754 mg, 1.45 mmol) and Et_3N
- 30 (224 μL , 1.60 mmol) in CH_2Cl_2 (5 mL) at 0 °C. After 3 h, the reaction solution was washed twice

with sat. NH_4Cl and the aqueous phase was extracted twice with CH_2Cl_2 . The combined organic phases were washed with brine, dried over Na_2SO_4 , filtered and concentrated *in vacuo* to afford crude *tert*-butyl *N*-(4((*tert*-butyldimethylsilyl)oxy)butyl)-*N*-(3-

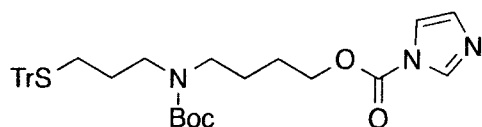
((triphenylmethyl)sufanyl)propyl)carbamate as an orange oil and was used without further

- 5 purification. R_f 0.81 (1:19, EtOAc: CH_2Cl_2); ^1H NMR (400 MHz, CDCl_3) δ 0.03 (s, 6H), 0.88 (s, 9H), 1.39 (br s, 4H), 1.45 (br s, 2H), 1.52 (s, 9H), 2.13 (t, $J = 7.4$ Hz, 2H), 3.05 (br s, 4H), 3.58 (t, $J = 6.0$ Hz, 2H), 7.18-7.29 (m, 9H), 7.39-7.41 (m, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ -5.1, 18.5, 25.9, 26.2, 27.6, 28.6, 29.6, 30.3, 46.5, 47.1, 63.1, 66.8, 79.3, 126.8, 128.1, 129.8, 145.1, 155.6.



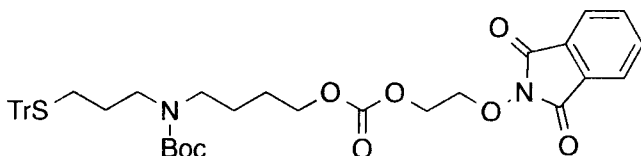
***tert*-Butyl *N*-(4-hydroxybutyl)-*N*-(3-((triphenylmethyl)sulfanyl)propyl)carbamate.** TBAF (1.89 mL of 1 *M* solution in THF, 1.89 mmol) was added to a solution of crude *tert*-butyl *N*-(4((*tert*-butyldimethylsilyl)oxy)butyl)-*N*-(3-((triphenylmethyl)sufanyl) propyl)carbamate (1.45 mmol) in dry THF (3 mL) at 0 °C and the reaction was stirred overnight, allowing the reaction to warm to rt.

- 15 Upon completion, the reaction was washed twice with sat. NaHCO_3 and the combined aqueous phases were extracted three times with Et_2O . The combined organic layers were washed with brine, dried over Na_2SO_4 , filtered and concentrated *in vacuo*. The crude material was purified by column chromatography (SiO_2 , 3:7, EtOAc: CH_2Cl_2) to give *tert*-butyl *N*-(4-hydroxybutyl)-*N*-(3-((triphenylmethyl)sulfanyl)propyl)carbamate (453 mg, 62% over 2 steps) as a light yellow oil. R_f
- 20 0.52 (3:7, EtOAc: CH_2Cl_2); ^1H NMR (400 MHz, CDCl_3) δ 1.40 (s, 9H), 1.47-1.51 (m, 4H), 1.58 (quin, $J = 7.6$ Hz, 2H), 3.08 (br s, 4H), 3.63 (t, $J = 6.0$ Hz, 2H), 7.19-7.30 (m, 9H), 7.41-7.43 (m, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 24.8, 28.2, 28.6, 29.5, 29.7, 46.6, 46.9, 62.6, 66.8, 79.5, 126.8, 128.0, 129.7, 145.0, 155.7.



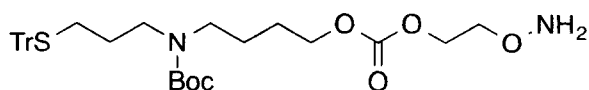
- 25 **4-(((*tert*-Butoxy)carbonyl)((3-((triphenylmethyl)sulfanyl)propyl))amino) butyl 1*H*-imidazole-1-carboxylate.** 1,1'-Carbonyldiimidazole (214 mg, 1.32 mmol) was added to a solution of *tert*-butyl *N*-(4-hydroxybutyl)-*N*-(3-((triphenylmethyl)sulfanyl)propyl) carbamate (444 mg, 0.88 mmol) and (*i*-Pr) $_2\text{NEt}$ (228 μL , 1.32 mmol) in dry CH_2Cl_2 (3 mL) at 0 °C and was stirred overnight. The reaction solution was washed twice with water and extracted once with CH_2Cl_2 . The combined
- 30 organic layers were washed twice with sat. NH_4Cl , once with brine, were dried over Na_2SO_4 ,

filtered and concentrated *in vacuo* to afford crude 4-(((*tert*-butoxy)carbonyl)((3-
 ((triphenylmethyl)sulfanyl)propyl)) amino) butyl 1*H*-imidazole-1-carboxylate (517 mg, 98%) as a
 light yellow oil and was used without further purification. R_f 0.50 (3:7, EtOAc:CH₂Cl₂); ¹H NMR
 (400 MHz, CDCl₃) δ 1.42 (s, 9H), 1.59 (br s, 4H), 1.76 (quin, J = 6.8 Hz, 2H), 2.18 (t, J = 7.4 Hz,
 2H), 3.11 (br s, 4H), 4.42 (t, J = 6.6 Hz, 2H), 7.08 (s, 1H), 7.20-7.31 (m, 9H), 7.42-7.44 (m, 7H),
 8.15 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 24.6, 25.9, 28.2, 28.5, 29.4, 46.5 (2 C's), 66.8, 68.1,
 79.6, 117.2, 126.7, 128.0, 129.7, 130.8, 137.2, 144.9, 148.8, 155.5.



2-(2-(((4-(((*tert*-Butoxy)carbonyl)((3-((triphenylmethyl)sulfanyl)propyl))amino)

- butoxy)carbonyl)oxy)ethoxy)-2,3-dihydro-1*H*-isoindole-1,3-dione.** DBU (129 μ L, 0.86 mmol)
 was added to a solution of crude *tert*-butyl *N*-(4-hydroxybutyl)-*N*-(3-
 ((triphenylmethyl)sulfanyl)propyl)carbamate (517 mg, 0.86 mmol) in dry MeCN (4 mL). After
 stirring for 10 min, 2-(2-hydroxyethoxy)-2,3-dihydro-1*H*-isoindole-1,3-dione (178 mg, 0.86 mmol)
 was added and the reaction was stirred overnight. Upon completion, the reaction was washed twice
 with sat. NH₄Cl and the combined aqueous layers were extracted three times with EtOAc. The
 combined organic layers were washed with brine, dried over Na₂SO₄, filtered and concentrated *in*
vacuo. The crude material was purified by column chromatography (SiO₂, 3:17, EtOAc:CH₂Cl₂) to
 give 2-(2-(((4-(((*tert*-butoxy)carbonyl)((3-((triphenylmethyl)sulfanyl)
 propyl))amino)butoxy)carbonyl)oxy) ethoxy)-2,3-dihydro-1*H*-isoindole-1,3-dione (350 mg, 55%
 over 2 steps) as a colorless oil. R_f 0.75 (3:17, EtOAc:CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 1.40
 (s, 9H), 1.46-1.64 (m, 6H), 2.15 (t, J = 7.4 Hz, 2H), 3.06 (br s, 4H), 4.14 (t, J = 6.4 Hz, 2H), 4.43-
 4.48 (m, 4H), 7.18-7.29 (m, 9H), 7.39-7.42 (m, 6H), 7.73-7.76 (m, 2H), 7.81-7.85 (m, 2H); ¹³C
 NMR (100 MHz, CDCl₃) δ 24.6, 26.1, 28.2, 28.6, 29.5, 46.5, 46.6, 65.2, 68.1, 75.6, 79.5, 123.8,
 126.8, 128.0, 129.0, 129.7, 134.7, 145.0, 155.1, 155.5, 163.5.



28

***N*-(4-(((2-(aminooxy)ethoxy)carbonyl)oxy)butyl)-*N*-(((*tert*-butoxy)carbonyl)-3-**

((triphenylmethyl)sulfanyl)propan-1-amine. Hydrazine monohydrate (51 μ L, 1.04 mmol) was
 added to a solution of 2-(2-(((4-(((*tert*-butoxy)carbonyl)((3-
 ((triphenylmethyl)sulfanyl)propyl))amino)butoxy)carbonyl) oxy)ethoxy)-2,3-dihydro-1*H*-

isoindole-1,3-dione (154 mg, 0.21 mmol) in CH₂Cl₂ at 0 °C and stirred for 2 h. When complete, the white precipitate was removed by filtration and the filter cake was washed with ample CH₂Cl₂. The filtrate was concentrated *in vacuo* to afford **28** (127 mg, 100%) as a colorless oil and did not require further purification. *R_f* 0.52 (1:3, EtOAc:CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 1.38 (s, 9H), 1.48-1.62 (m, 6H), 2.14 (t, *J* = 7.4 Hz, 2H), 3.05 (br s, 4H), 3.85 (t, *J* = 4.6 Hz, 2H), 4.12 (t, *J* = 6.4 Hz, 2H), 4.33 (t, *J* = 4.4 Hz, 2H), 5.51 (br s, 2H), 7.18-7.21 (m, 3H), 7.25-7.29 (m, 6H), 7.39-7.41 (m, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 24.7, 26.2, 27.5, 28.6, 29.5, 46.7 (2 C's), 65.5, 66.8, 68.0, 73.4, 79.5, 126.8, 128.0, 129.7, 145.0, 155.5, 155.6.

All publications, patents and patent applications cited herein are incorporated herein by reference. While in the foregoing specification this invention has been described in relation to certain embodiments thereof, and many details have been set forth for purposes of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that certain of the details described herein may be varied considerably without departing from the basic principles of the invention.

The use of the terms “a” and “an” and “the” and similar referents in the context of describing the invention are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “having,” “including,” and “containing” are to be construed as open-ended terms (*i.e.*, meaning “including, but not limited to”) unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (*e.g.*, “such as”) provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

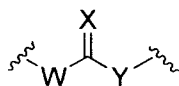
Embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention

includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

Claims

What is claimed is:

1. A therapeutic magnetic nanoparticle, or a salt thereof, comprising a magnetic nanoparticle covalently bonded to one or more -L-D groups wherein;
 D is a residue of a therapeutic agent;
 L is a linker -L¹-L²-;
 each L¹ is covalently bonded to the magnetic nanoparticle and is independently a branched or unbranched chain or cyclic group or a combination of chain and cyclic groups that comprises 1-200 atoms; and
 each L² is independently a group capable of releasing a therapeutic agent; and
 wherein L is not capable of undergoing intramolecular cyclization to release the therapeutic agent.
2. The therapeutic magnetic nanoparticle of claim 1, or a salt thereof, comprising a magnetic nanoparticle covalently bonded to one or more -L-D groups wherein D is a residue of a therapeutic agent and L is a linker, wherein:
 L is -L¹-L²-;
 each L¹ is covalently bonded to the magnetic nanoparticle and is independently a branched or unbranched chain or cyclic group or a combination of chain and cyclic groups that comprises 1-50 atoms; and
 each L² is independently a group capable of releasing a therapeutic agent.
3. The therapeutic magnetic nanoparticle of claim 1 or claim 2, wherein L² can be hydrolyzed to release the therapeutic agent.
4. The therapeutic magnetic nanoparticle of any one of claims 1-3, wherein L² can release the therapeutic agent by application of an alternating electromagnetic field to the magnetic nanoparticle.
5. The therapeutic magnetic nanoparticle of any one of claims 1-4, wherein L² is:



W is absent, -O-, -S- or -NR^b-;

X is O, S or NR^c;

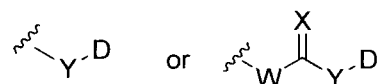
Y is absent, -O-, -S- or -NR^d-;

R^b is H, (C₁-C₆)alkyl, (C₂-C₆)alkenyl or (C₂-C₆)alkynyl;

R^c is H, (C₁-C₆)alkyl, (C₂-C₆)alkenyl or (C₂-C₆)alkynyl;

5 R^d is H, (C₁-C₆)alkyl, (C₂-C₆)alkenyl or (C₂-C₆)alkynyl; and

wherein D together with Y of L² or D together with W-C(=X)-Y of L² is the moiety:



wherein the moiety is a residue of a therapeutic agent.

10 6. The therapeutic magnetic nanoparticle of any one of claims 1-5, wherein the atoms that comprise L¹ are selected from carbon, oxygen, nitrogen, sulfur and silicon.

7. The therapeutic magnetic nanoparticle of any one of claims 1-5, wherein the atoms that comprise L¹ are selected from hydrogen, carbon, oxygen, nitrogen, sulfur and silicon.

15

8. The therapeutic magnetic nanoparticle of any one of claims 1-7, wherein the atoms of L¹ are selected from hydrogen, carbon, oxygen, nitrogen, sulfur and silicon and wherein L¹ does not include an -NH-, -NH₂ or -NHR group wherein R is (C₁-C₆)alkyl.

20 9. The therapeutic magnetic nanoparticle of any one of claims 1-8, wherein L¹ does not include an -NH-, -NH₂ or -NHR group wherein R is (C₁-C₆)alkyl.

10. The therapeutic magnetic nanoparticle of any one of claims 1-9, wherein L¹ does not include a hydrazone group.

25

11. The therapeutic magnetic nanoparticle of any one of claims 1-10, wherein L¹ does not include an oxime ether group.

12. The therapeutic magnetic nanoparticle of any one of claims 1 or 3-11, wherein L¹ comprises
30 2-60 atoms.

13. The therapeutic magnetic nanoparticle of any one of claims 1-11, wherein L^1 comprises 2-30 atoms.
14. The therapeutic magnetic nanoparticle of any one of claims 1-13, wherein L^1 comprises a
5 branched or unbranched chain.
15. The therapeutic magnetic nanoparticle of any one of claims 1-13, wherein L^1 comprises a branched or unbranched chain comprising hydrogen, carbon, oxygen, sulfur or silicon atoms but not nitrogen atoms.
- 10 16. The therapeutic magnetic nanoparticle of any one of claims 1-15, wherein L^1 comprises a (C_1-C_{15}) alkylene, (C_1-C_{15}) heteroalkylene, (C_2-C_{15}) alkenylene, (C_2-C_{15}) alkynylene, wherein any (C_1-C_{15}) alkylene, (C_1-C_{15}) heteroalkylene, (C_2-C_{15}) alkenylene or (C_2-C_{15}) alkynylene of L^1 is optionally substituted with one or more halogen.
- 15 17. The therapeutic magnetic nanoparticle of any one of claims 1-15, wherein L^1 comprises a (C_1-C_{15}) alkylene, (C_2-C_{15}) alkenylene, or (C_2-C_{15}) alkynylene, wherein any (C_1-C_{15}) alkylene, (C_2-C_{15}) alkenylene or (C_2-C_{15}) alkynylene of L^1 is optionally substituted with one or more halogen.
- 20 18. The therapeutic magnetic nanoparticle of any one of claims 1-17, wherein L^1 is covalently bonded to the magnetic nanoparticle through a silicon or sulfur atom.
19. The therapeutic magnetic nanoparticle of any one of claims 1-18, wherein the magnetic nanoparticle further comprises a coating.
- 25 20. The therapeutic magnetic nanoparticle of claim 19, wherein the coating comprises gold.
21. The therapeutic magnetic nanoparticle of claim 19, wherein the coating comprises silica.
- 30 22. The therapeutic magnetic nanoparticle of any one of claims 1-21, wherein the magnetic nanoparticle comprises iron.

24. The therapeutic magnetic nanoparticle, or a salt thereof, of any one of claims 1-23, wherein each -L-D independently has the following formula I:



L¹ is (C₁-C₁₅)alkylene, (C₁-C₁₅)heteroalkylene, (C₂-C₁₅)alkenylene or (C₂-C₁₅)alkynylene, wherein any (C₁-C₁₅)alkylene, (C₁-C₁₅)heteroalkylene, (C₂-C₁₅)alkenylene or (C₂-C₁₅)alkynylene of L¹ is optionally substituted with one or more halogen and wherein the (C₁-C₁₅)heteroalkylene does not include nitrogen;

25. The therapeutic magnetic nanoparticle of claim 24, wherein V is -OSi(G)₂-, the magnetic nanoparticle is optionally coated with silica, and the dashed line represents a covalent bond

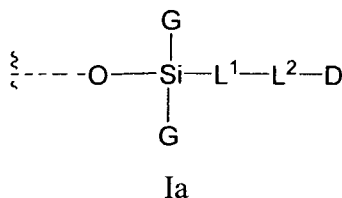
between the oxygen atom of $-\text{OSi}(\text{G})_2-$ and the magnetic nanoparticle optionally coated with silica; or V is $-\text{S}-$, the magnetic nanoparticle is magnetic nanoparticle coated with gold, and the dashed line represents a covalent bond between $-\text{S}-$ and the magnetic nanoparticle coated with gold.

5 26. The therapeutic magnetic nanoparticle of claim 24, wherein V is $-\text{OSi}(\text{G})_2-$, the magnetic nanoparticle is coated with silica, and the dashed line represents a covalent bond between the oxygen atom of $-\text{OSi}(\text{G})_2-$ and the magnetic nanoparticle coated with silica.

10 27. The therapeutic magnetic nanoparticle of any one of claims 24-26, wherein the magnetic nanoparticle comprises iron.

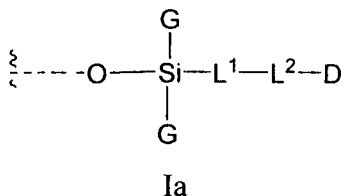
15 28. The therapeutic magnetic nanoparticle of claim 24, wherein V is $-\text{OSi}(\text{G})_2-$, the magnetic nanoparticle is an iron oxide nanoparticle coated with silica, and the dashed line represents a covalent bond between the oxygen atom of $-\text{OSi}(\text{G})_2-$ and the iron oxide nanoparticle coated with silica.

29. The therapeutic magnetic nanoparticle of claim 24, wherein $-\text{L}-\text{D}$ has the following formula Ia:



20 wherein the dashed bond represents a covalent bond to the magnetic nanoparticle.

30. The therapeutic magnetic nanoparticle of claim 24, wherein the magnetic nanoparticle is further coated with silica and wherein $-\text{L}-\text{D}$ has the following formula Ia:



25 wherein the dashed bond represents a covalent bond to the magnetic nanoparticle further coated with silica.

31. The therapeutic magnetic nanoparticle of claim 29 or claim 30, wherein the magnetic nanoparticle is an iron oxide nanoparticle.

32. The therapeutic magnetic nanoparticle of any one of claims 24-31, wherein each G is -OR^{a1}.

33. The therapeutic magnetic nanoparticle any one of claims 24-31, wherein each G is -OR^{a2}, wherein each -OR^{a2} together with another -OR^{a2} group on an adjacent L-D group forms an -O-.

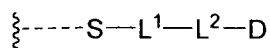
34. The therapeutic magnetic nanoparticle any one of claims 24-31, wherein each G is -OR^{a1} or -OR^{a2}, wherein each -OR^{a2} together with another -OR^{a2} group on an adjacent L-D group form an -O-.

35. The therapeutic magnetic nanoparticle of claim 24, wherein V is -S-, the magnetic nanoparticle is coated in gold, and the dashed line represents a covalent bond between -S- and the magnetic nanoparticle coated in gold.

36. The therapeutic magnetic nanoparticle of claim 35, wherein the dashed line represents a covalent bond between -S- and a gold atom of the magnetic nanoparticle coated in gold.

37. The therapeutic magnetic nanoparticle of claim 35 or claim 36, wherein the magnetic nanoparticle is an iron oxide nanoparticle.

38. The therapeutic magnetic nanoparticle of claim 24 or claims 35-37, wherein -L-D has the following formula Ib:



Ib

wherein the dashed bonds represent a covalent bond to the magnetic nanoparticle.

39. The therapeutic magnetic nanoparticle of any one of claims 24-38, wherein L¹ is (C₁-C₁₅)alkylene, (C₁-C₁₅)heteroalkylene, (C₂-C₁₅)alkenylene or (C₂-C₁₅)alkynylene, wherein any (C₁-C₁₅)alkylene, (C₁-C₁₅)heteroalkylene, (C₂-C₁₅)alkenylene or (C₂-C₁₅)alkynylene of L¹ is optionally substituted with one or more halogen and wherein the (C₁-C₁₅)heteroalkylene

40. The therapeutic magnetic nanoparticle of any one of claims 24-38, wherein L^1 is (C₁-C₁₅)alkylene, (C₂-C₁₅)alkenylene or (C₂-C₁₅)alkynylene, wherein any (C₁-C₁₅)alkylene, (C₂-C₁₅)alkenylene or (C₂-C₁₅)alkynylene of L^1 is optionally substituted with one or more halogen.
- 5
41. The therapeutic magnetic nanoparticle of any one of claims 24-38, wherein L^1 is (C₂-C₁₂)alkylene optionally substituted with one or more halogen.
42. The therapeutic magnetic nanoparticle of any one of claims 24-38, wherein L^1 is (C₂-C₁₂)alkylene.
- 10
43. The therapeutic magnetic nanoparticle of any one of claims 24-38, wherein L^1 is (C₂-C₈)alkylene optionally substituted with one or more halogen.
44. The therapeutic magnetic nanoparticle of any one of claims 24-38, wherein L^1 is (C₂-C₆)alkylene.
- 15
45. The therapeutic magnetic nanoparticle of any one of claims 24-38, wherein L^1 is -(CH₂)₆-, -(CH₂)₃-, or -(CH₂)₄-.
- 20
46. The therapeutic magnetic nanoparticle of any one of claims 24-38, wherein L^1 is -(CH₂)₃- or -(CH₂)₆-.
47. The therapeutic magnetic nanoparticle of any one of claims 1-23, wherein L^1 comprises a (C₂-C₁₂)alkylene optionally substituted with one or more halogen.
- 25
48. The therapeutic magnetic nanoparticle of any one of claims 1-23, wherein L^1 comprises a (C₂-C₁₂)alkylene.
49. The therapeutic magnetic nanoparticle of any one of claims 1-23, wherein L^1 comprises a (C₂-C₈)alkylene optionally substituted with one or more halogen.
- 30

50. The therapeutic magnetic nanoparticle of any one of claims 1-23, wherein L^1 comprises a (C_2-C_6) alkylene.

51. The therapeutic magnetic nanoparticle of any one of claims 1-23, wherein L^1 comprises a -
5 $(CH_2)_6$ -, $-(CH_2)_3$ -, or $-(CH_2)_4$ -.

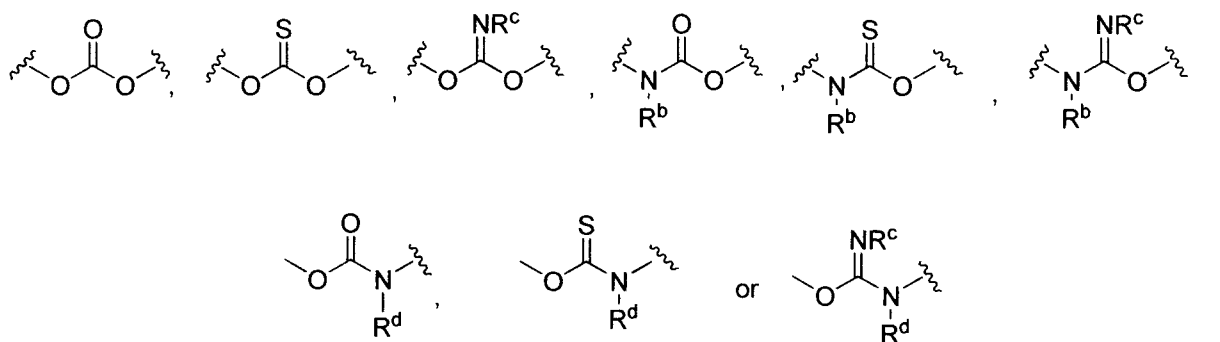
52. The therapeutic magnetic nanoparticle of any one of claims 1-23, wherein L^1 comprises a -
 $(CH_2)_3$ - or $-(CH_2)_6$ -.

10 53. The therapeutic magnetic nanoparticle of any one of claims 5-52, wherein W is -O- or $-NR^b$ -.

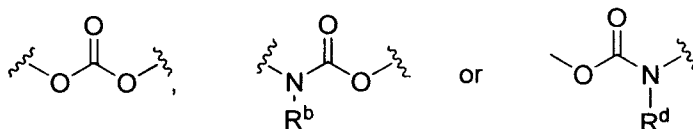
54. The therapeutic magnetic nanoparticle of any one of claims 5-53, wherein X is O.

15 55. The therapeutic magnetic nanoparticle of any one of claims 5-54, wherein Y is -O- or $-NR^d$.

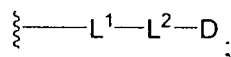
56. The therapeutic magnetic nanoparticle of any one of claims 5-52, wherein L^2 is:



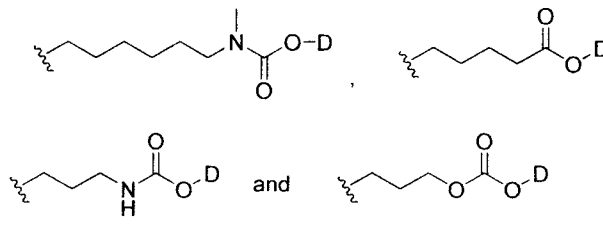
57. The therapeutic magnetic nanoparticle of any one of claims 5-52, wherein L^2 is:



58. The therapeutic magnetic nanoparticle of any one of claims 24-57, wherein the portion of formula I as shown in the formula below:

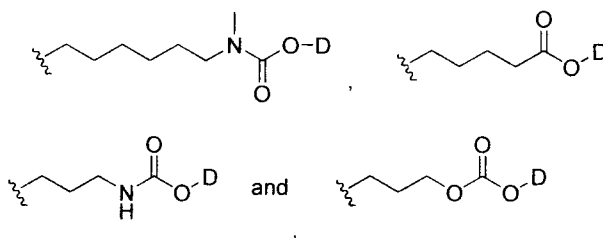


is selected from;



5

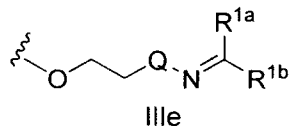
59. The therapeutic magnetic nanoparticle of any one of claims 1-23, wherein -L-D comprises a group selected from;



10

60. The therapeutic magnetic nanoparticle of claim 58, wherein the group covalently bonded to the magnetic nanoparticle through a silicon or sulfur.

61. The therapeutic magnetic nanoparticle of any one of claims 1-47, wherein the residue of a therapeutic is a residue of a prodrug of a therapeutic agent represented by formula IIIe:



wherein Q is -O- or -NH- and R^{1a} and R^{1b} together with the remainder of formula IIIe are the residue of the prodrug of the therapeutic agent.

62. The therapeutic magnetic nanoparticle of any one of claims 1-61, wherein the residue of a therapeutic agent is a residue of a chemotherapeutic agent, an antibiotic agent, an antifungal agent, an antiparasitic agent or an antiviral agent or a prodrug thereof.
- 5 63. The therapeutic magnetic nanoparticle of any one of claims 1-61, wherein the residue of a therapeutic agent is a residue of a chemotherapeutic agent, an antibiotic agent, an antifungal agent, an antiparasitic agent or an antiviral agent.
64. The therapeutic magnetic nanoparticle of any one of claims 1-61, wherein the residue of a
10 therapeutic agent or prodrug thereof is a residue of Cladribine, Azacitidine, Abraxane, Adcetris, Doxorubicin, Afinitor, Vinblastine, Amifostine, Amifostine, Arabinosylcytosine, Cytarabine, Pamidronic Acid, Nelarabine, Bicalutamide, Bleomycin, Bortezomib, Cabazitaxel, Irinotecan, Camptothecin, Capecitabine, Temsirolimus, Daunorubicin, Cortisone, Decitabine, Dasatinib, Dexamethasone, Prednisolone, Dexamethasone Acetate, Mitoxantrone, Docetaxel,
15 Hydroxycarbamide, Methylprednisolone, Epirubicin, Curcumin, Estramustine, Eribulin, Etoposide, Everolimus, Raloxifene, Fulvestrant, Floxuridine, Fludarabine, Fluoxymesterone, Gemcitabine, Goserelin, Topotecan, Hydrocortisone, Hydrocortone Phosphate, Idarubicin, Ixabepilone, Vincristine, Leuprolide (Leuprorelin), Megestrol, Vinorelbine, Nelarabine, Pentostatin, Octreotide, Paclitaxel, Streptozotocin, Teniposide, Valrubicin, Vorinostat, Zoledronic Acid Cladribine,
20 Azacitidine, Mecaptopurine, Tioguanine, Actinomycin D, Doxorubicin, Anagrelide, Pemetrexed, Vinblastine, Melphalan, Methotrexate, Amifostine, Aminoglutethimide, Arabinosylcytosine, Cytarabine, Pamidronic Acid, Nelarabine, Axitinib, Bleomycin, Bosutinib, Folinic Acid (Na or Ca), Leucovorin, Vandetanib, Lenalidomide, Daunorubicin, Crizotinib, Dacarbazine, Decitabine, Dasatinib, Mitoxantrone, Eribulin, Erlotinib, Fludarabine, Pralatrexate, Gefitinib, Gemcitabine,
25 Imatinib, Goserelin, Idarubicin, Lapatinib, Vincristine, Leuprolide, Procarbazine, Methotrexate, Mitomycin, Vinorelbine, Nelarabine, Nilotinib, Pentostatin, Octreotide, Pazopanib, Sunitinib, Abraxane, Actinomycin D, Doxorubicin, Afinitor, Exemestane, Carfilzomib, Daunorubicin, Cortisone, Prednisolone, Prednisone, Dexamethasone Acetate, Docetaxel, Methylprednisolone, Epirubicin, Curcumin, Everolimus, Fluoxymesterone, Hydrocortisone, Hydrocortone Phosphate,
30 Idarubicin, Ixabepilone, Vincristine, Megestrol, Valrubicin, Mesna, 13-cis-Retinoic Acid, Isotretinoin, Alitretinoin, Melphalan, Tretinoin, Methotrexate, Anastrozole, Bendamustine, Bexarotene, Carmustine, Lomustine, Chlorambucil and Ibritumomab Tiuxetan.

65. The therapeutic magnetic nanoparticle of any one of claims 1-64, wherein the therapeutic nanoparticle further comprises a targeting element.

66. A pharmaceutical composition comprising a therapeutic magnetic nanoparticle as described
5 in any one of claims 1-64, or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier.

67. A method for administering a therapeutic agent to an animal comprising administering the therapeutic magnetic nanoparticle as described in any one or claims 1-64, or a pharmaceutically
10 acceptable salt thereof, to the animal.

68. A method for treating cancer, a bacterial infection, a fungal infection, a parasitic infection or an antiviral infection in an animal in need thereof that has been administered an effective amount of a therapeutic magnetic nanoparticle as described in any one of claims 1-51, or a pharmaceutically
15 acceptable salt thereof, comprising providing conditions to release the therapeutic agent from the therapeutic magnetic nanoparticle.

69. The method of claim 67 or claim 68, further comprising magnetically targeting the therapeutic magnetic nanoparticle to a specific location in the animal.
20

70. The method of any one of claims 67-69, further comprising delivering a source of heat to the therapeutic magnetic nanoparticle to hydrolyze the group capable of releasing a therapeutic agent thereby releasing the therapeutic agent from the therapeutic magnetic nanoparticle.

25 71. The method of any one of claims 67-69, further comprising applying an alternating electromagnetic field to the therapeutic magnetic nanoparticle to hydrolyze the group capable of releasing thereby releasing the therapeutic agent from the therapeutic nanoparticle.

72. The method of any one of claims 67-71, further comprising treating the animal with one or
30 more additional therapeutic agents.

73. The method of claim 72, wherein the additional therapeutic agents are selected from chemotherapeutic agents, antibiotic agents, antifungal agents, antiparasitic agents and antiviral agents.

5 74. The method of claim 72 wherein one of the additional therapeutic agent is iron oxide nanoparticles.

75. A therapeutic magnetic nanoparticle, or a pharmaceutically acceptable salt thereof as described in any one of claims 1-65 for use in medical therapy.

10

76. The use of a therapeutic magnetic nanoparticle, or a pharmaceutically acceptable salt thereof as described in any one of claims 1-65 to prepare a medicament for treating cancer, a bacterial infection, a fungal infection, a parasitic infection or an antiviral infection in an animal.

15 77. A therapeutic magnetic nanoparticle, or a pharmaceutically acceptable salt thereof as described in any one of claims 1-65 for the therapeutic or prophylactic treatment of cancer, a bacterial infection, a fungal infection, a parasitic infection or an antiviral infection.

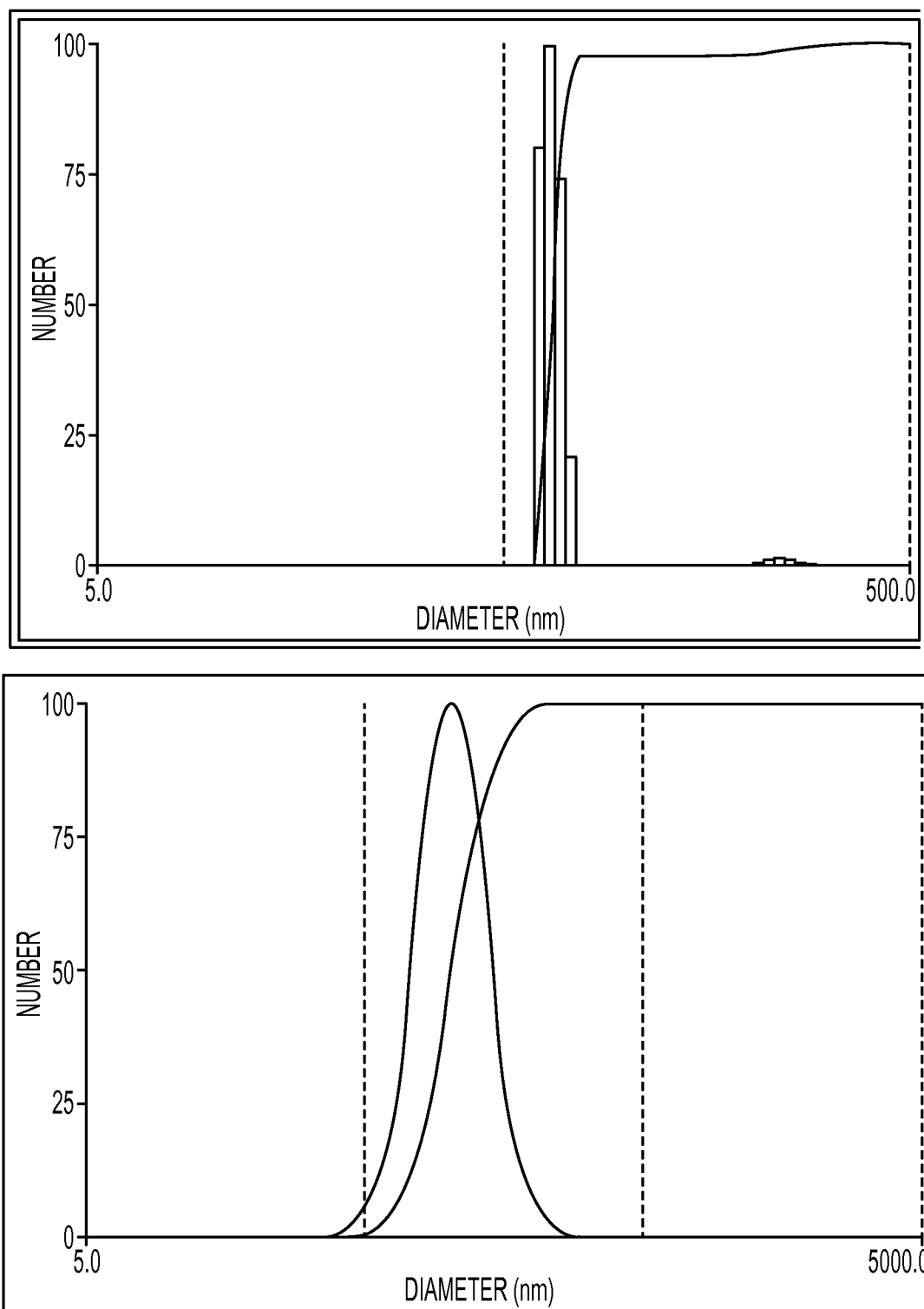


FIG. 1

SiO₂@Fe₃O₄NPs_H₂O

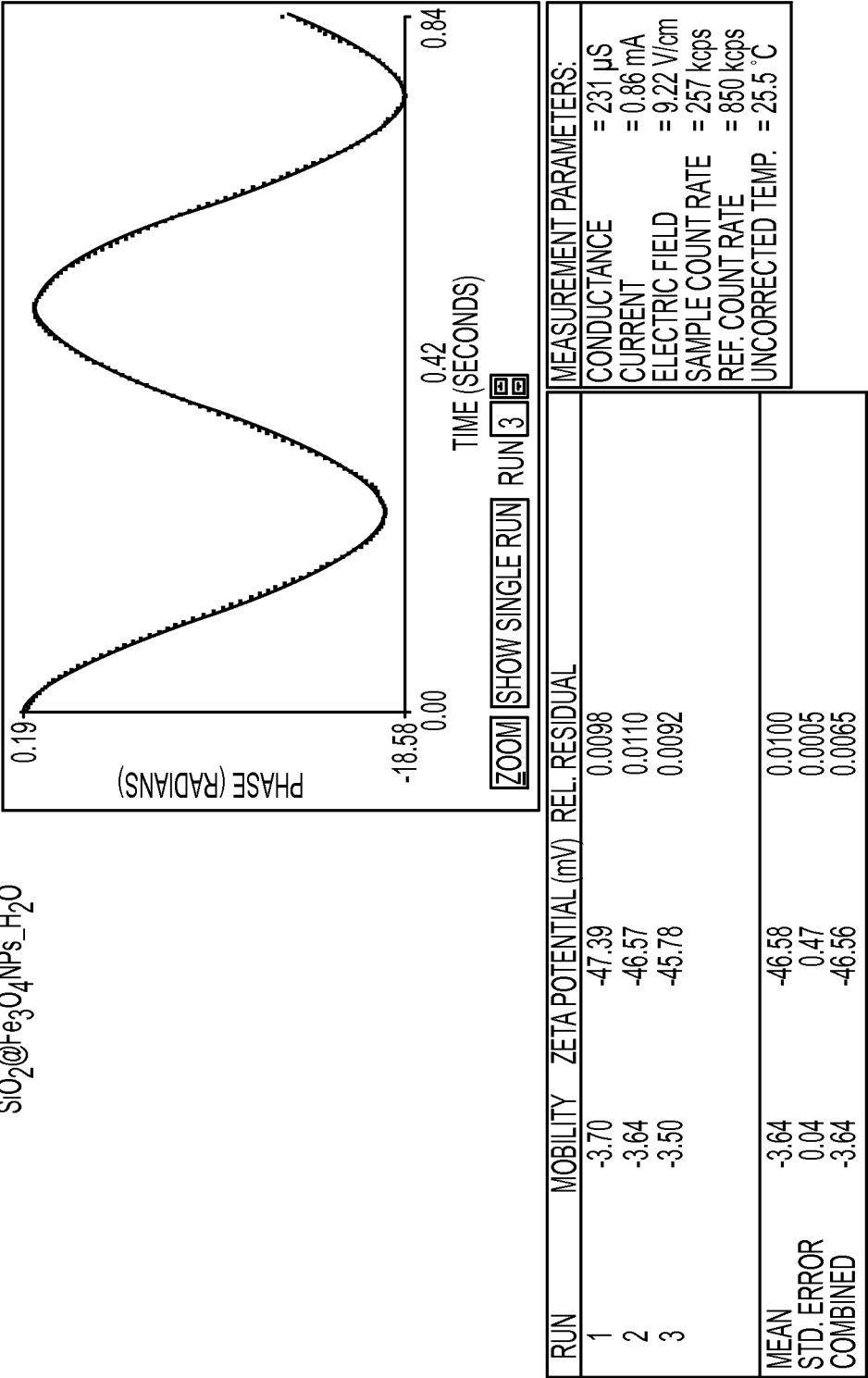


FIG. 2

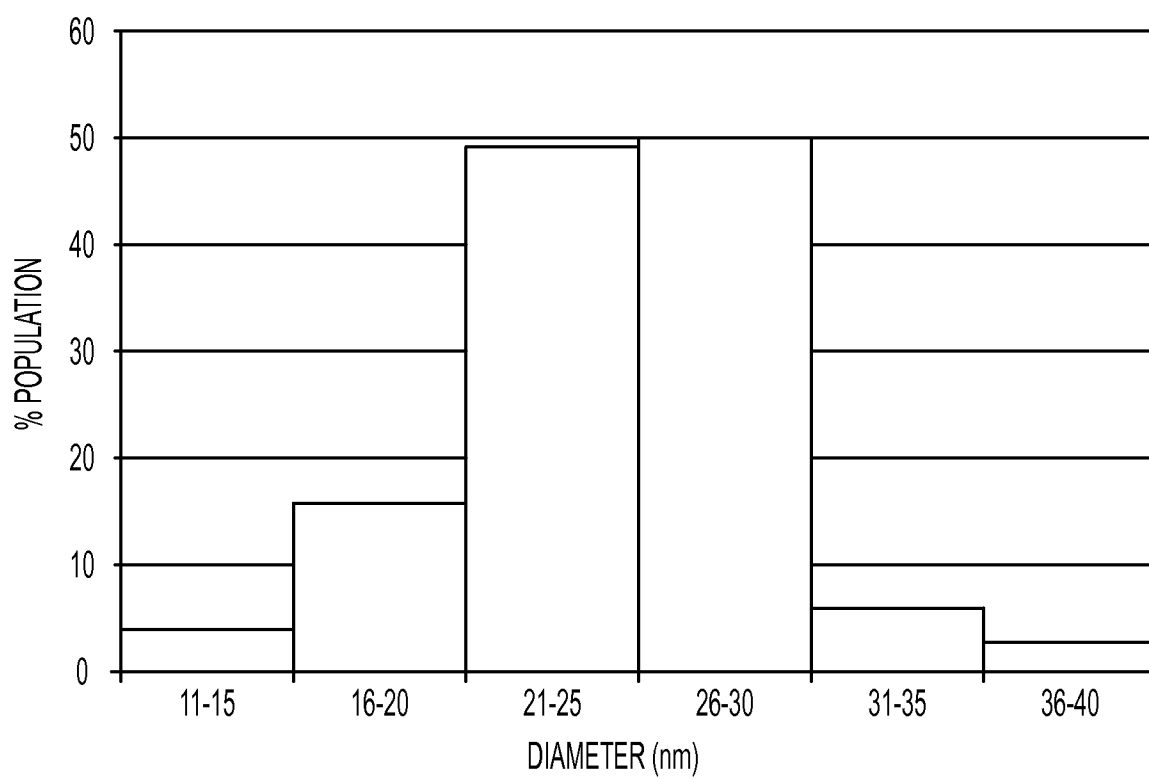


FIG. 3

4/24

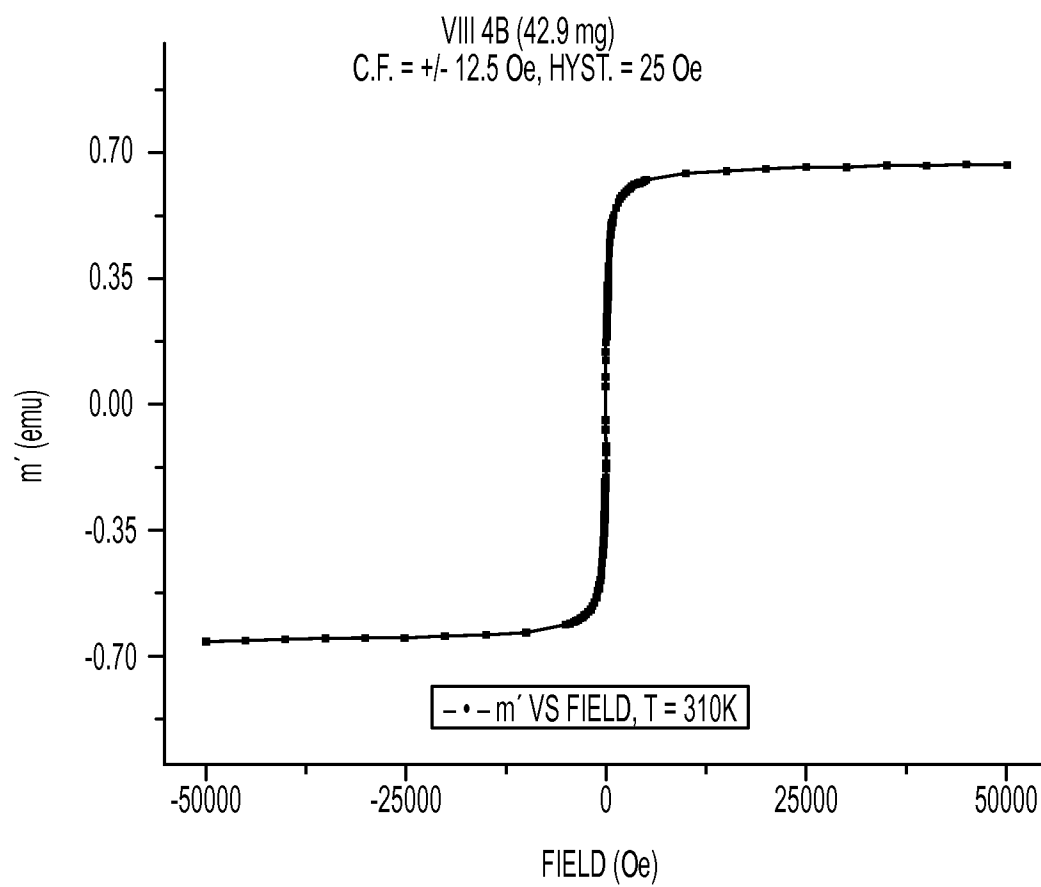


FIG. 4

5/24

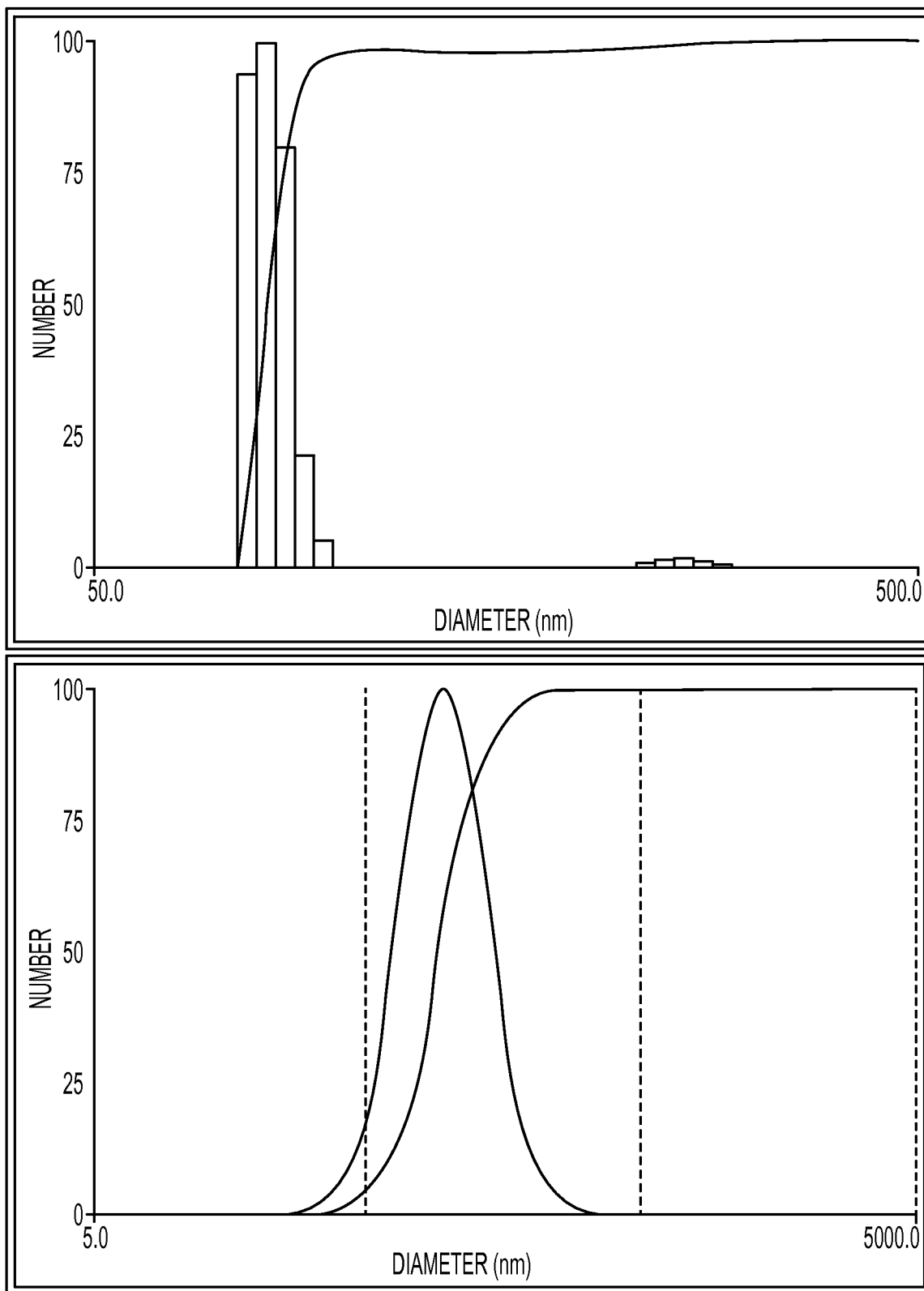


FIG. 5

AO@SiO₂@Fe₃NPs·H₂O

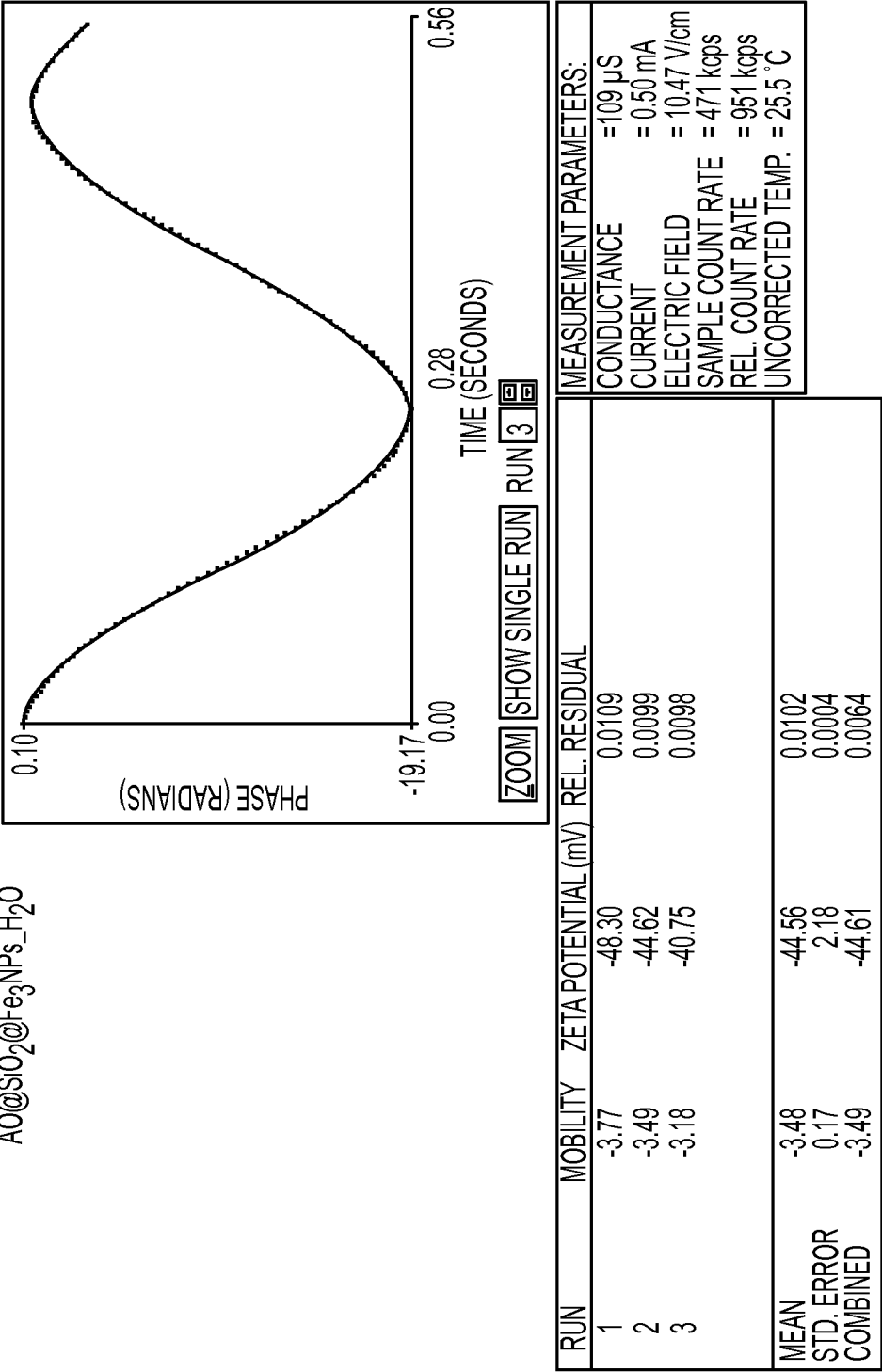


FIG. 6

7/24

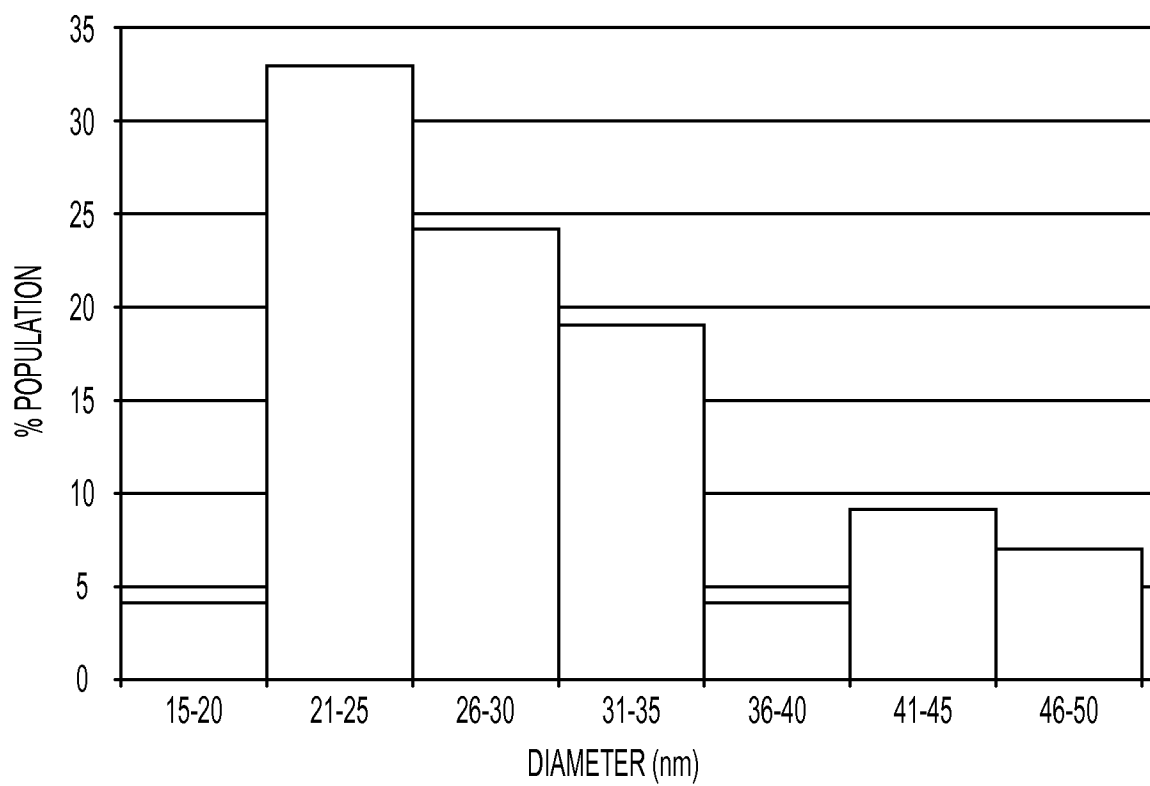


FIG. 7

8/24

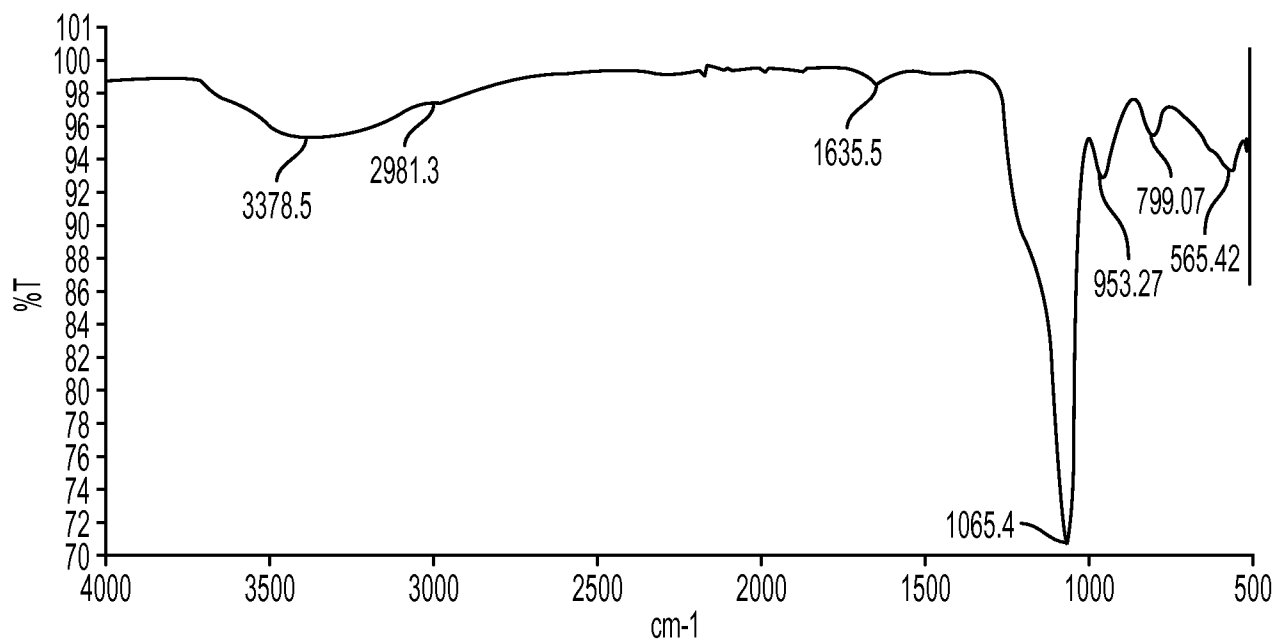


FIG. 8A

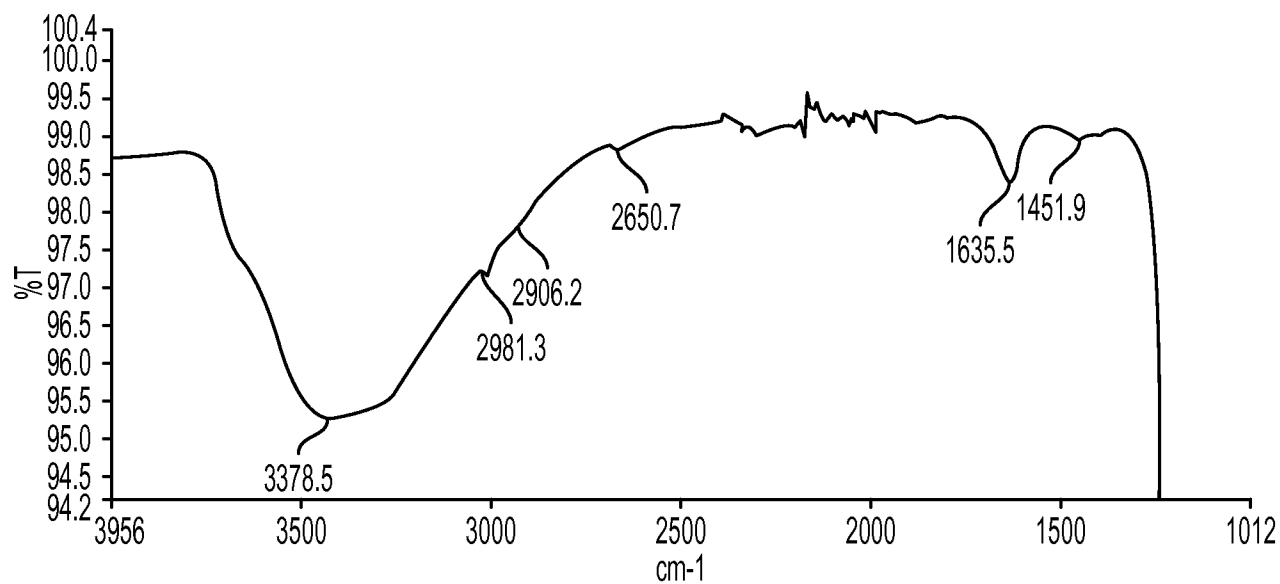


FIG. 8B

9/24

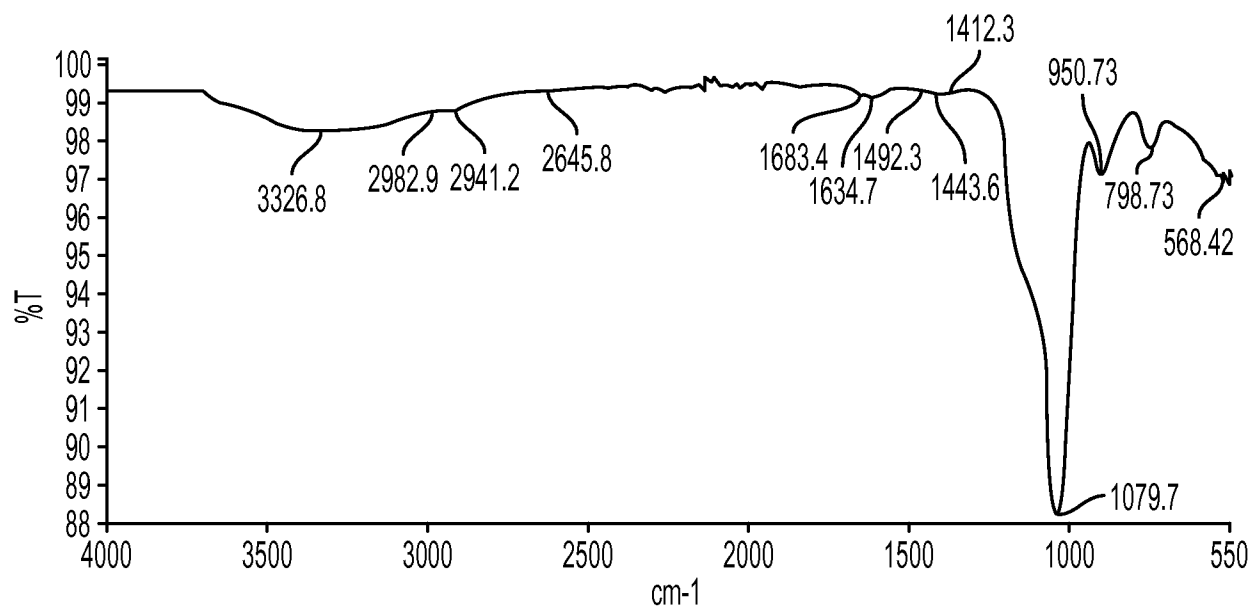


FIG. 9A

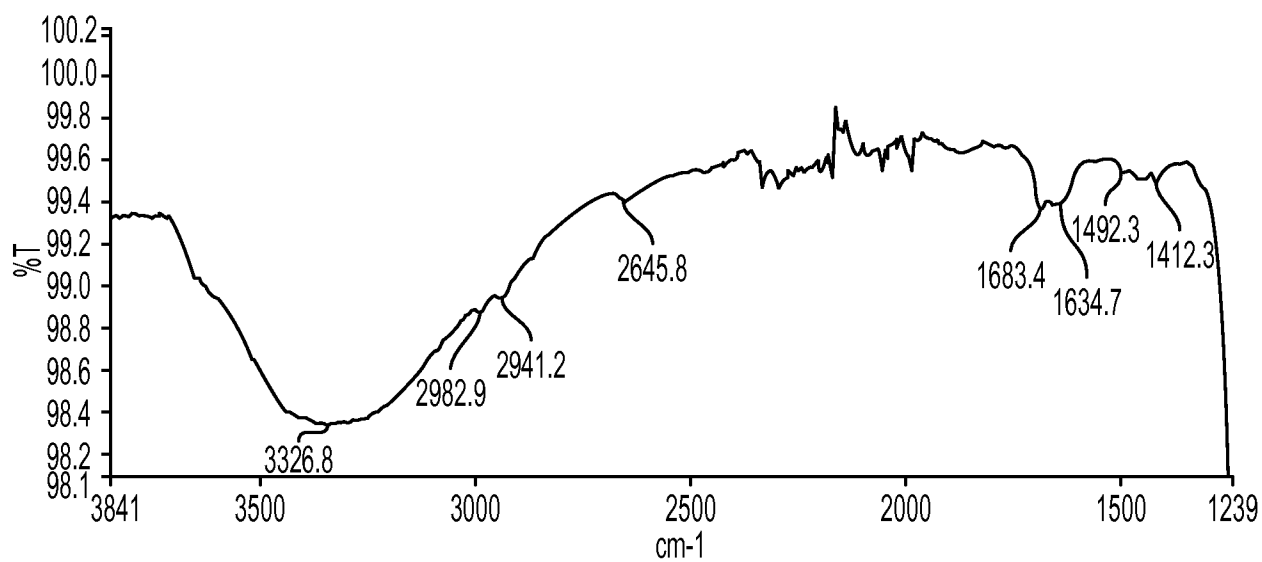


FIG. 9B

10/24

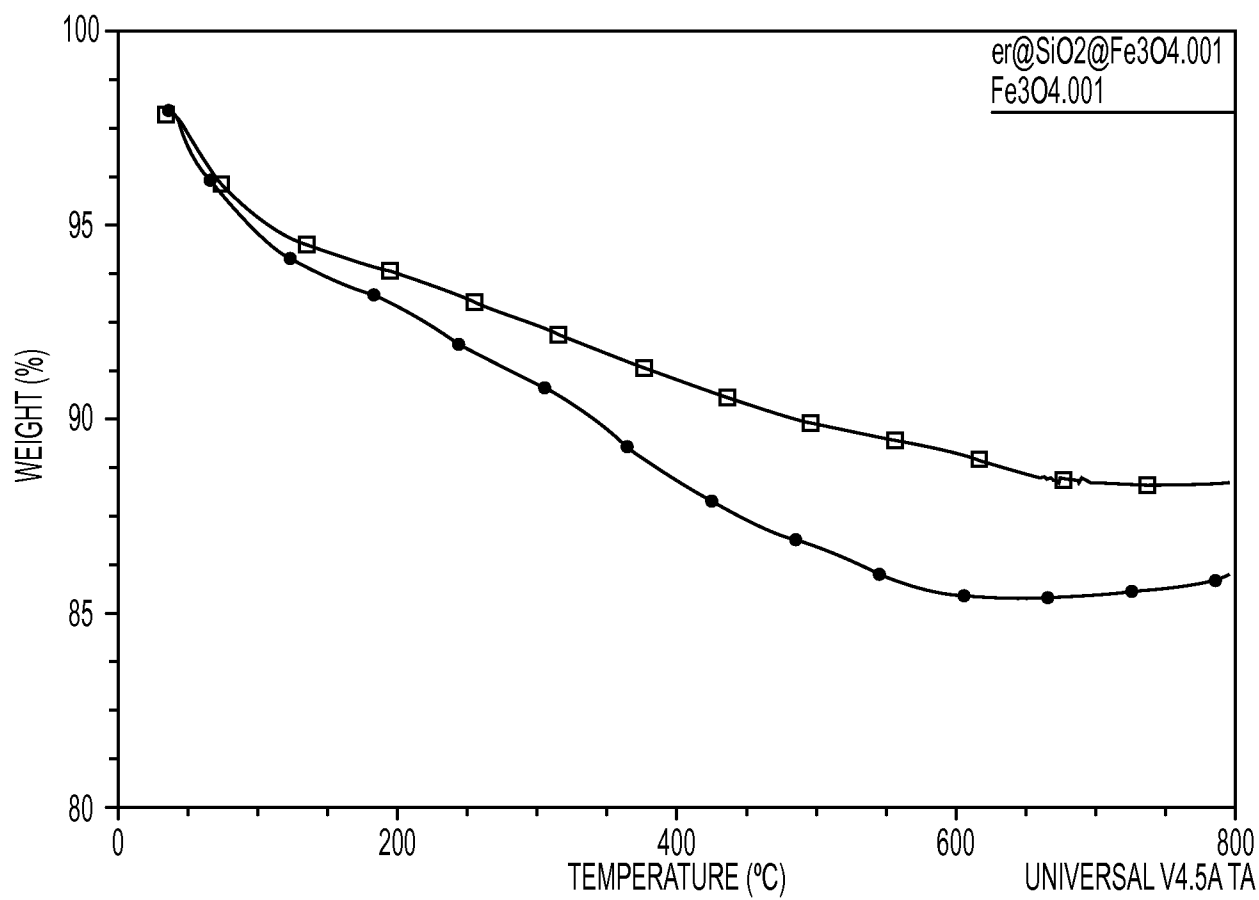


FIG. 10

11/24

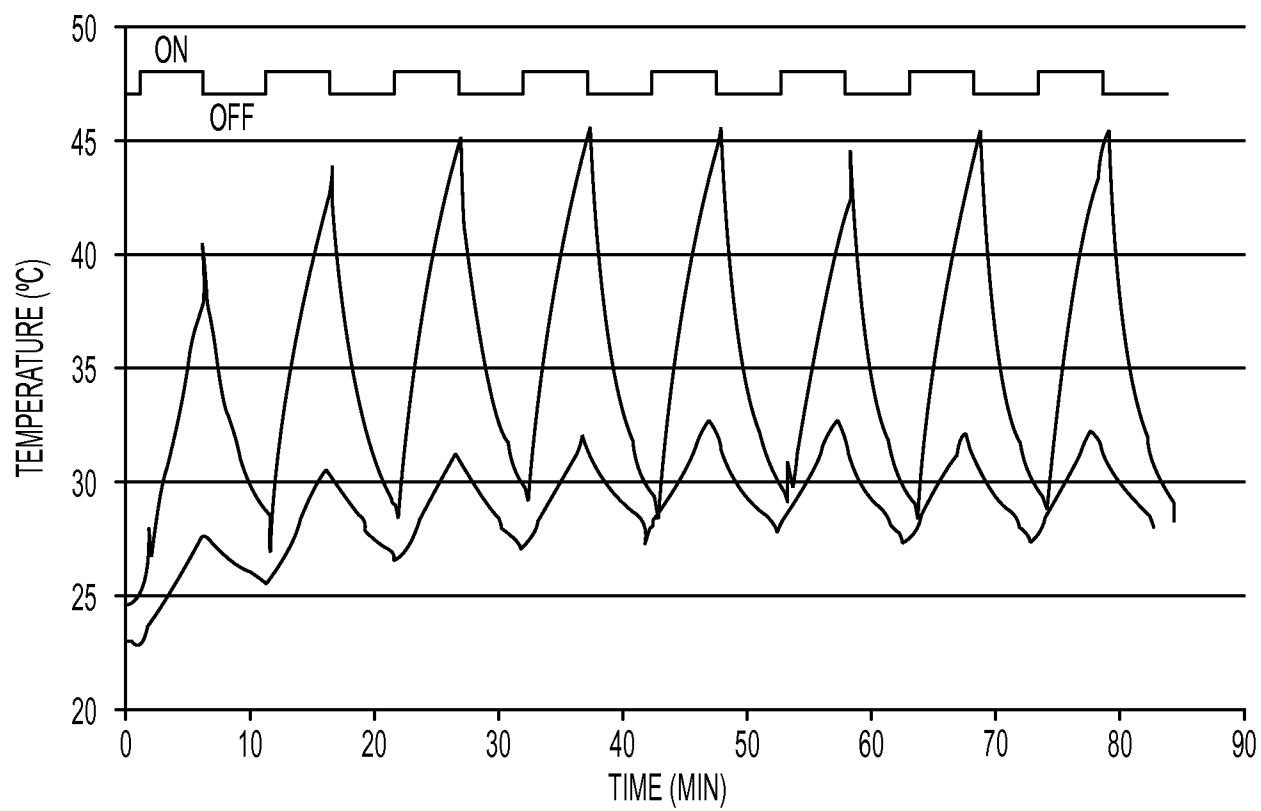


FIG. 11

12/24

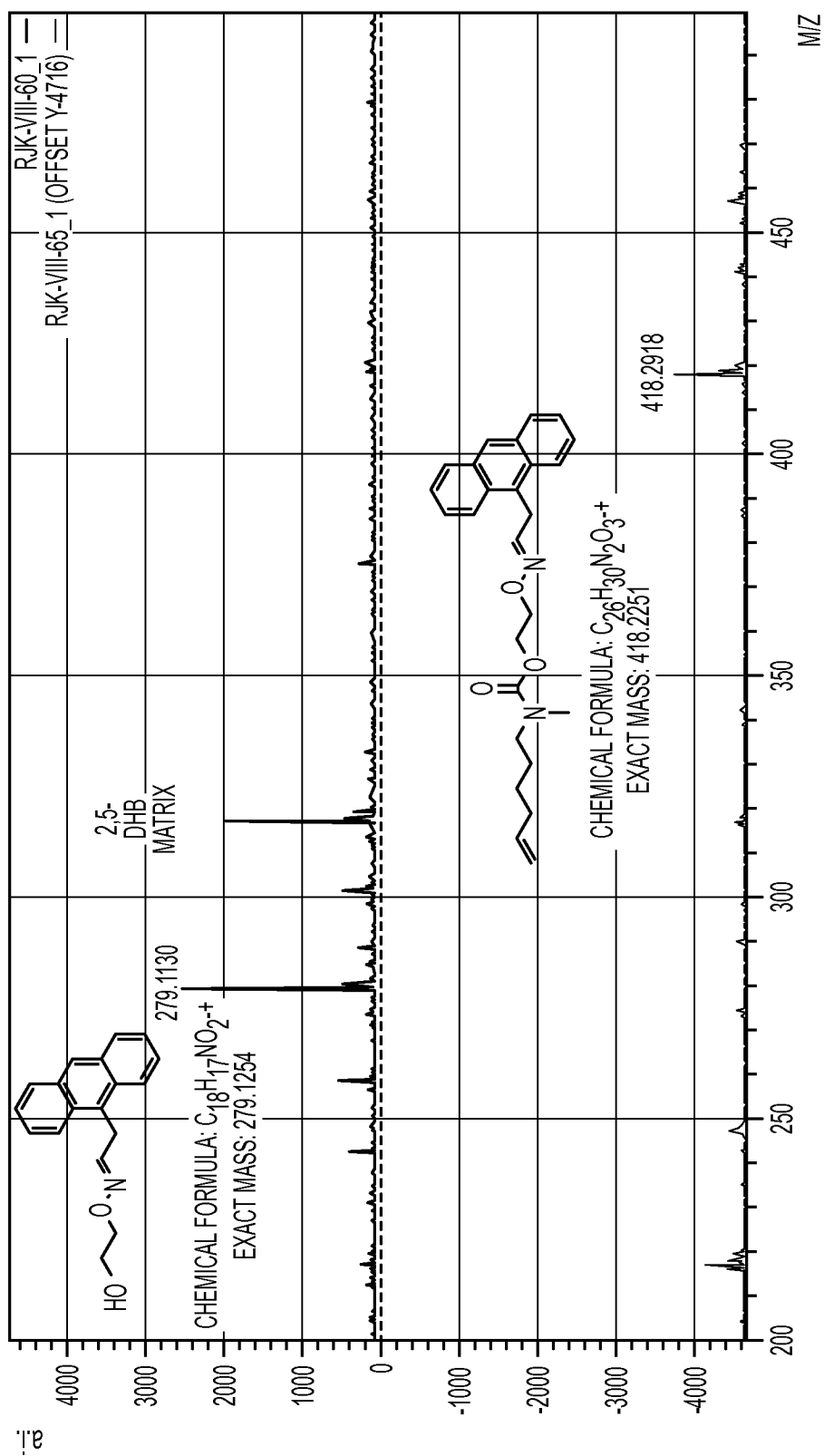


FIG. 12

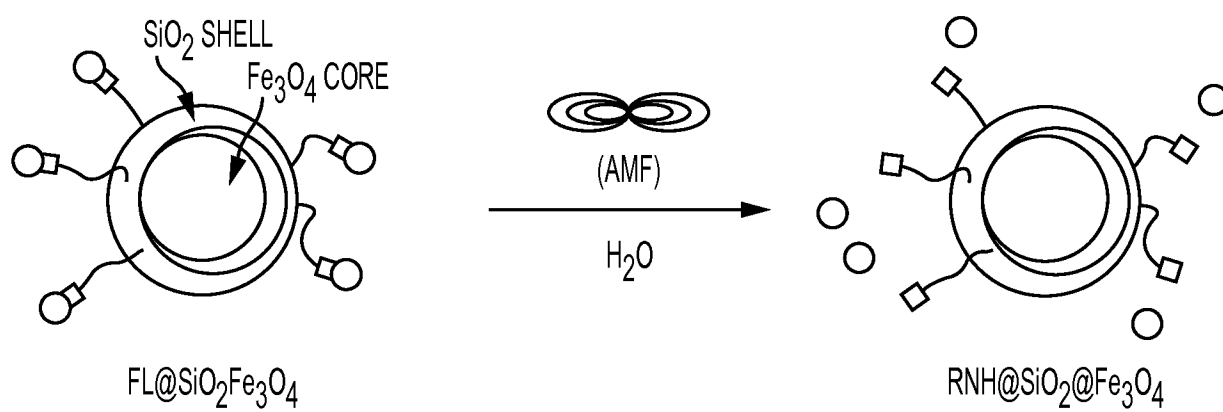


FIG. 13

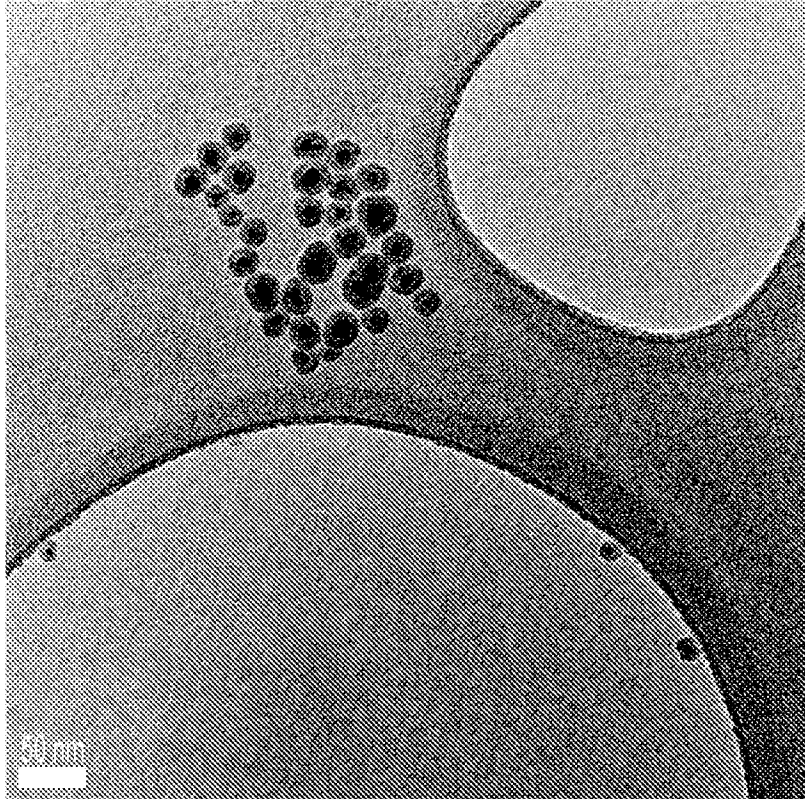


FIG. 14A

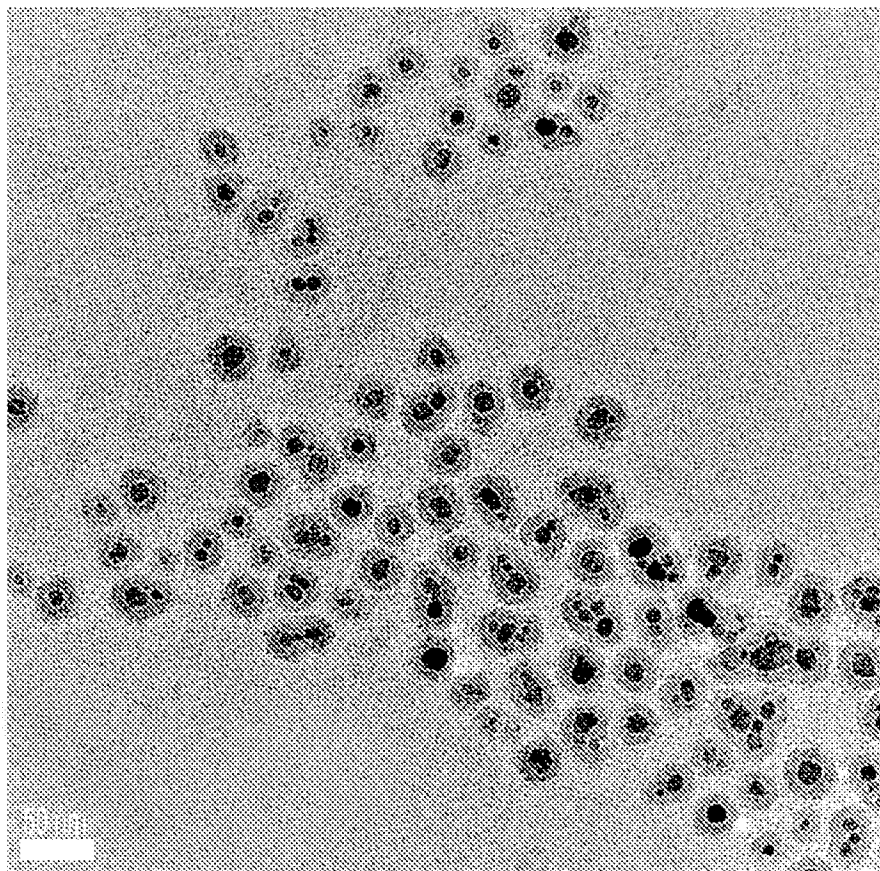


FIG. 14B

15/24

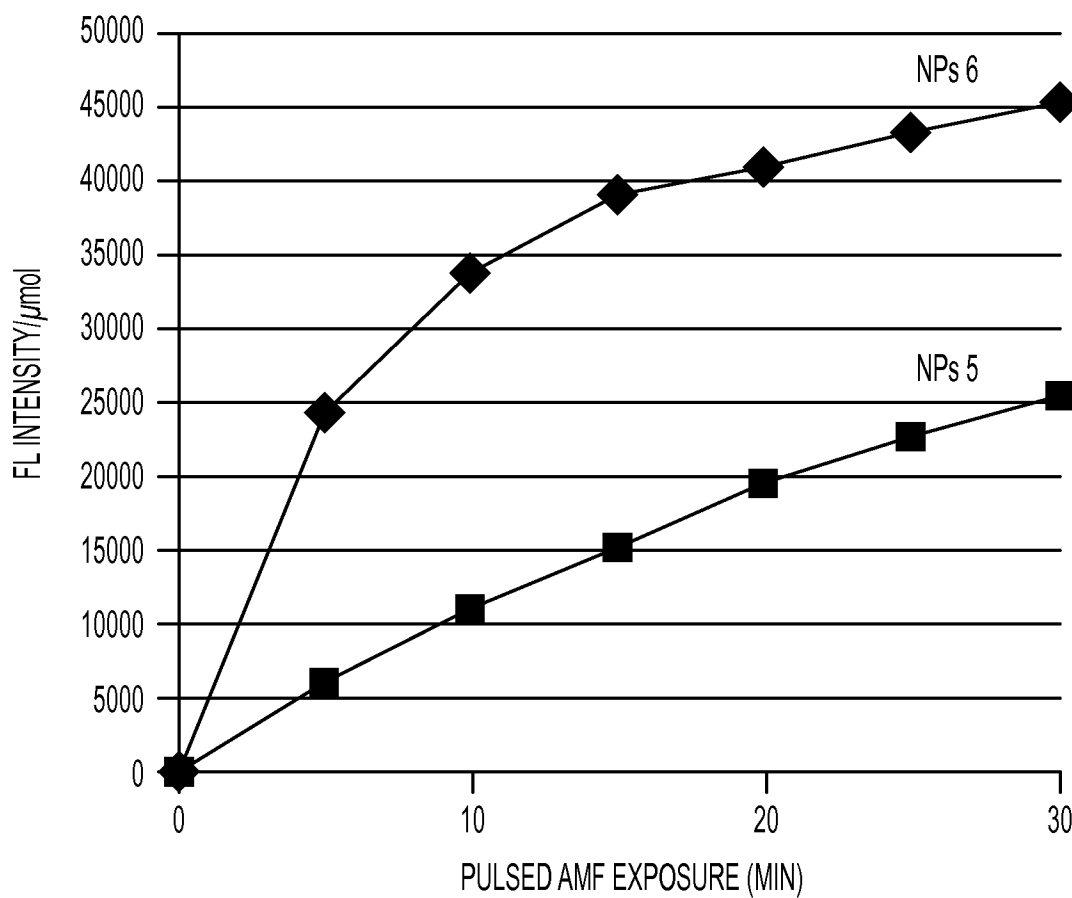


FIG. 15

16/24

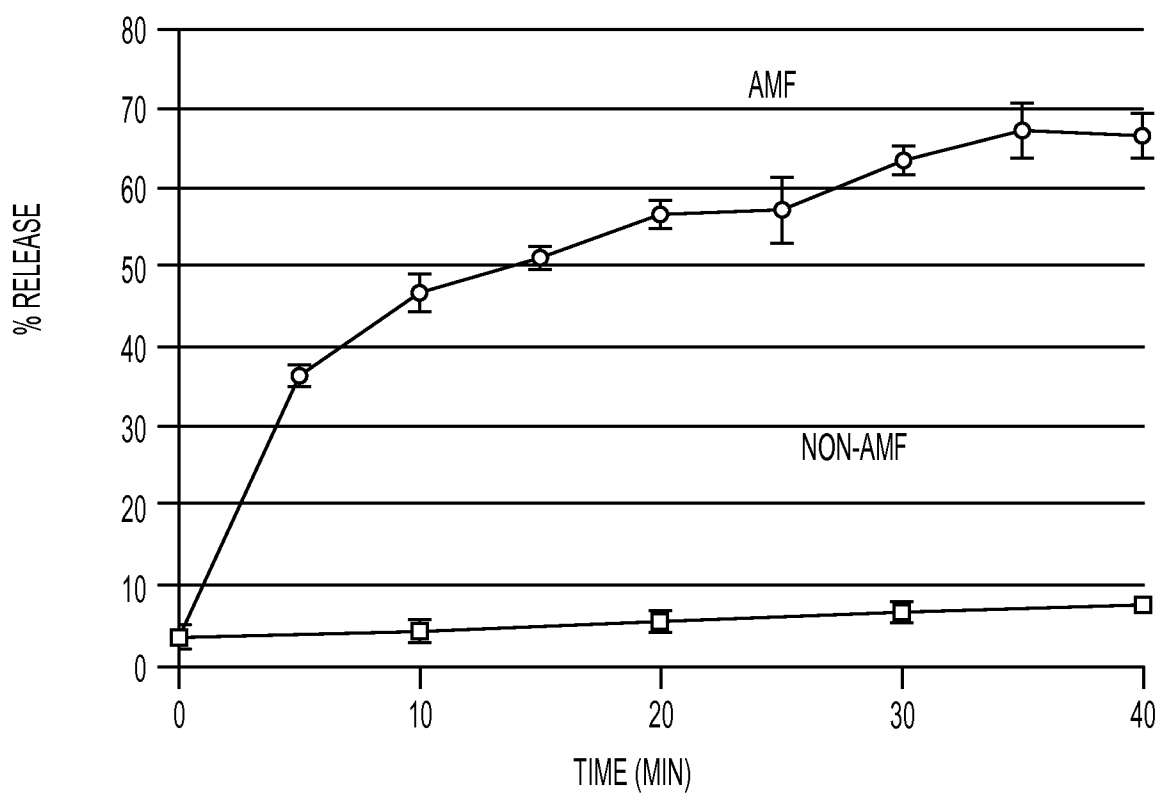


FIG. 16

17/24

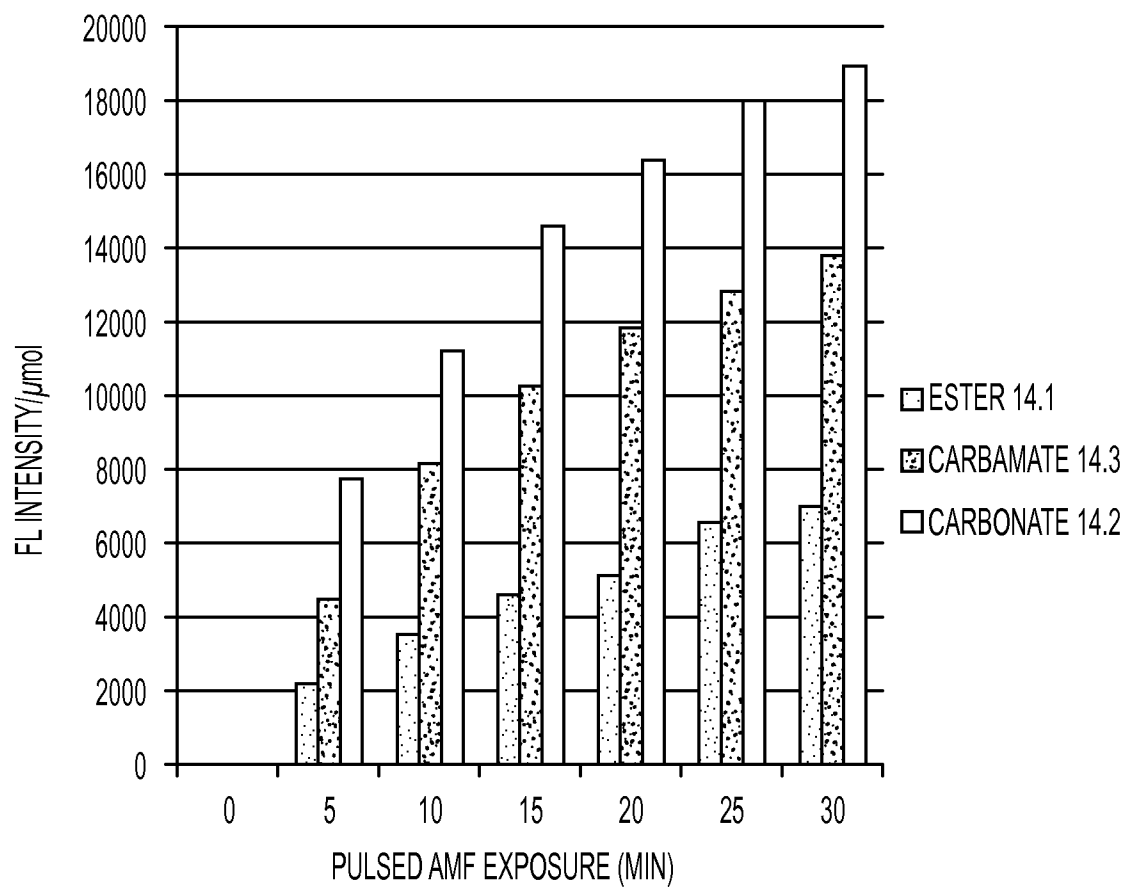


FIG. 17

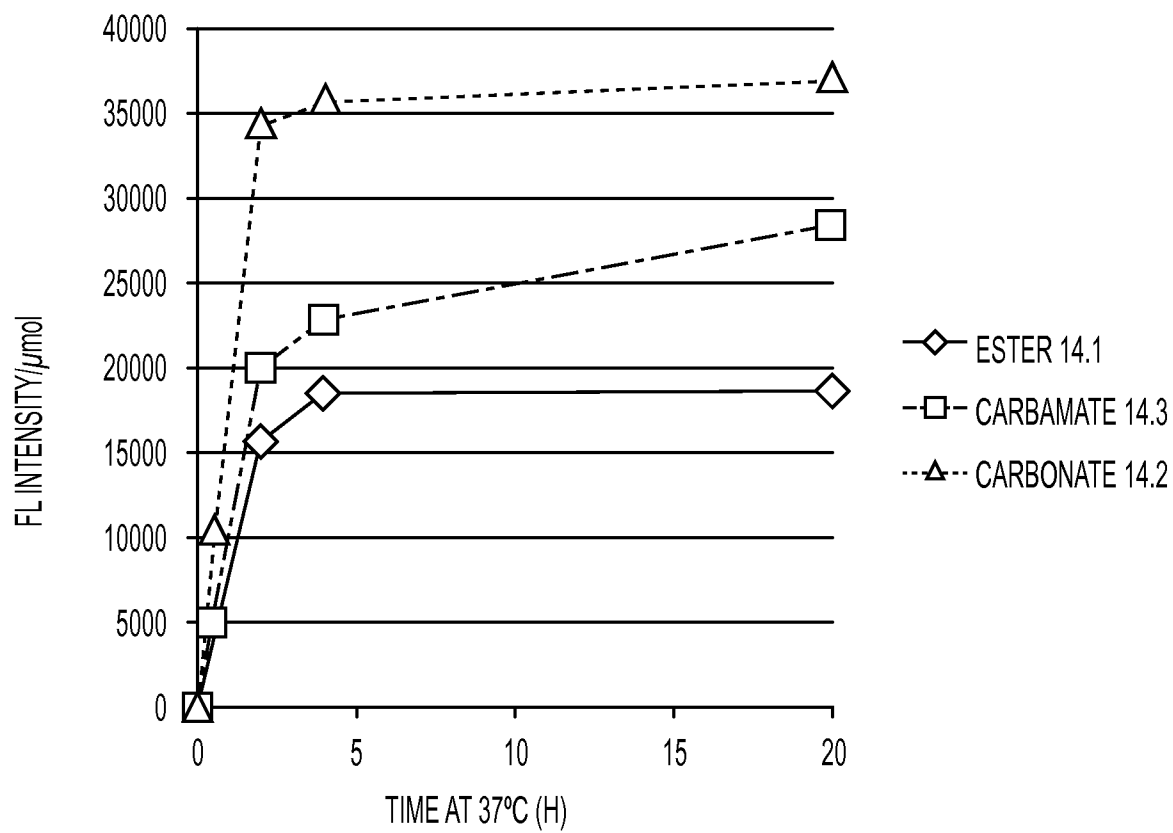


FIG. 18

19/24

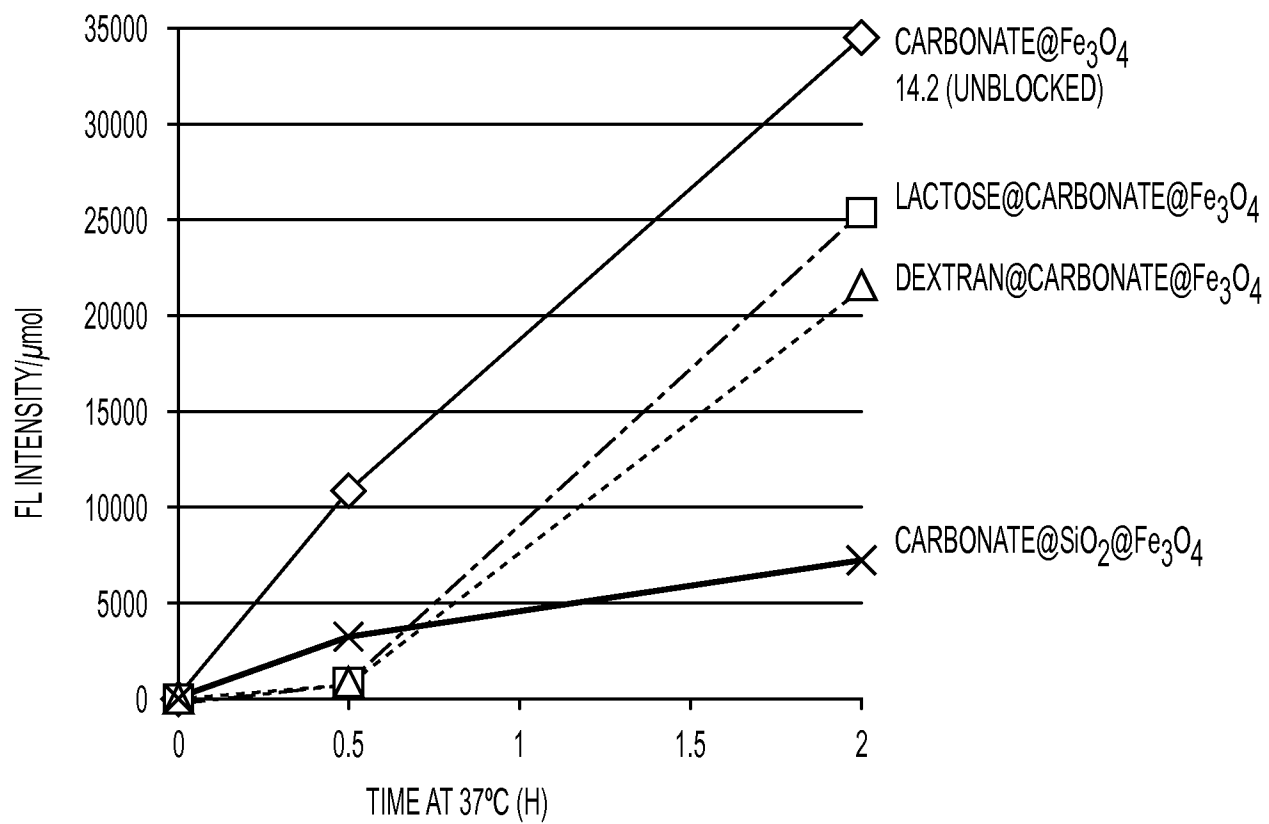


FIG. 19

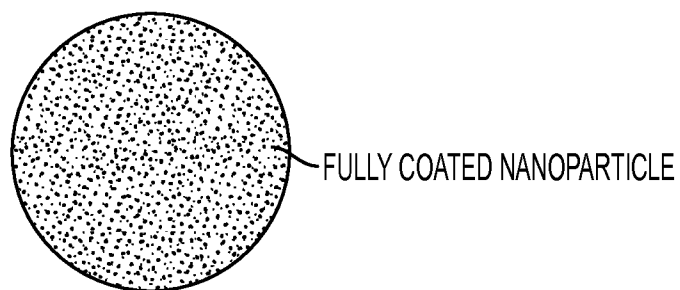


FIG. 20A

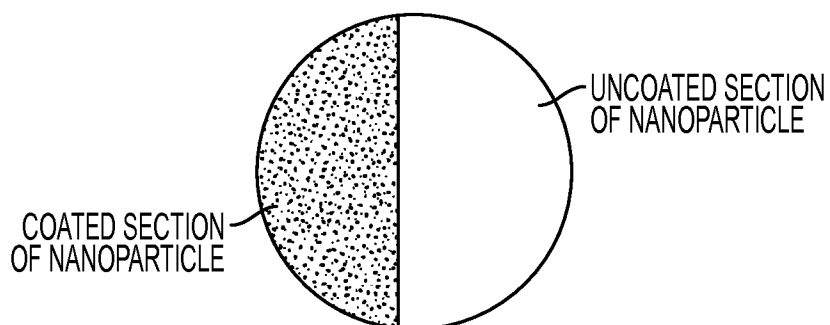


FIG. 20B

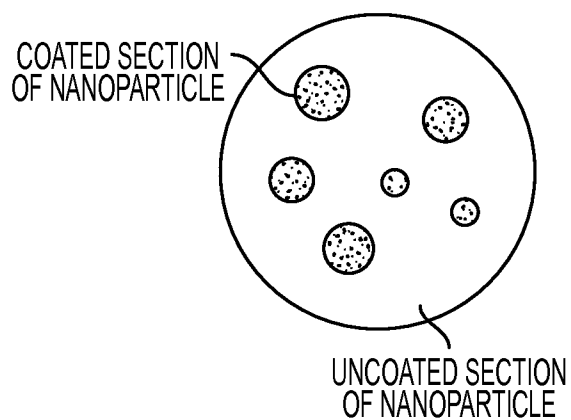


FIG. 20C

21/24

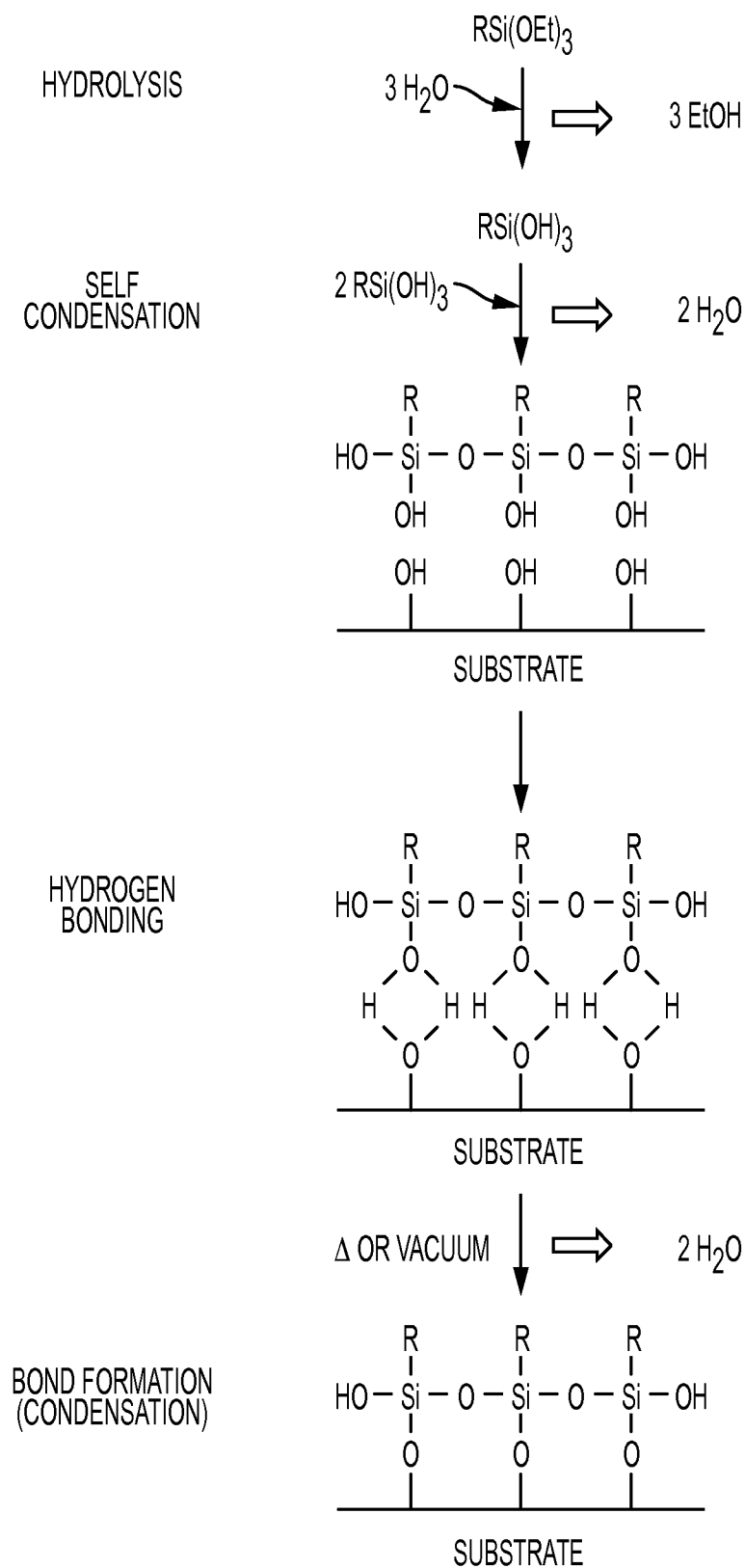


FIG. 21

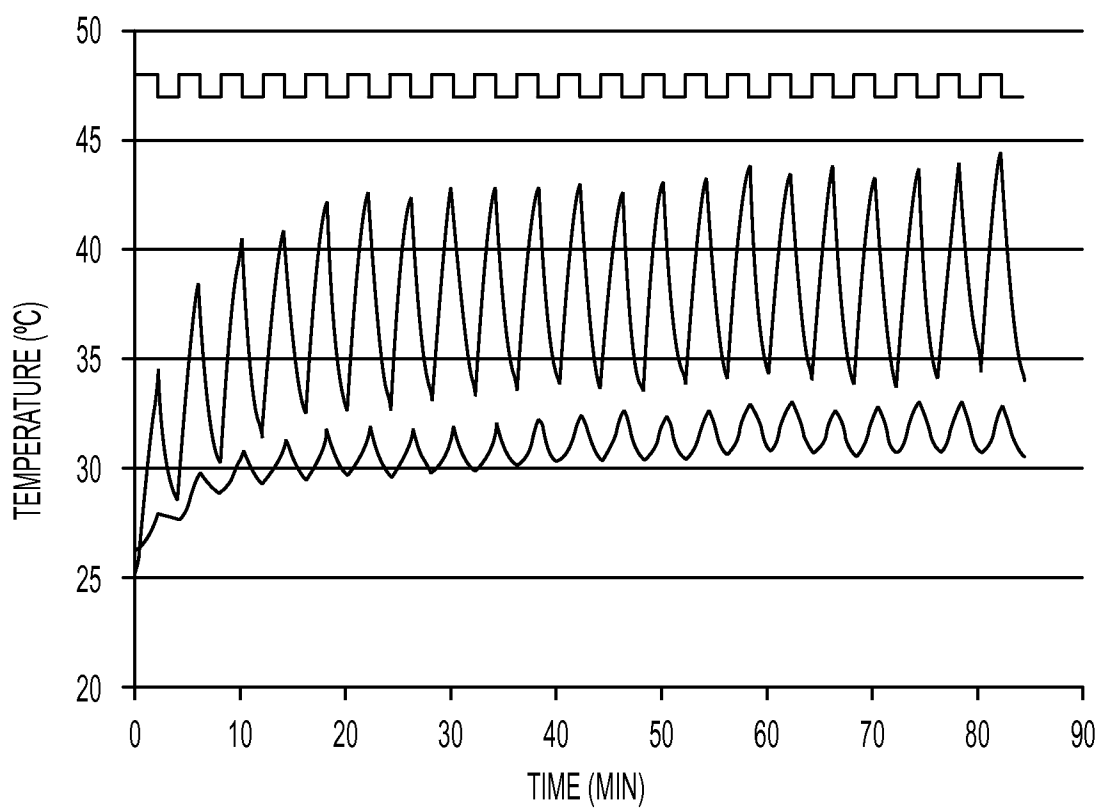


FIG. 22

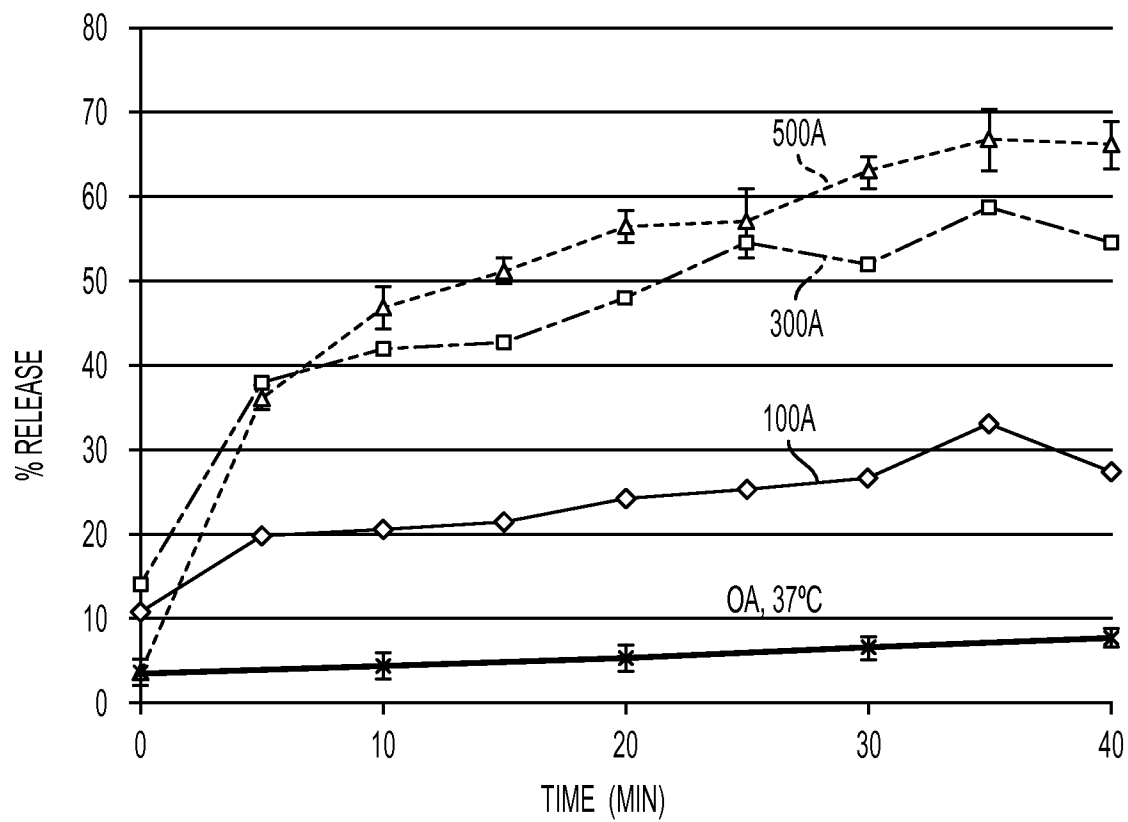


FIG. 23

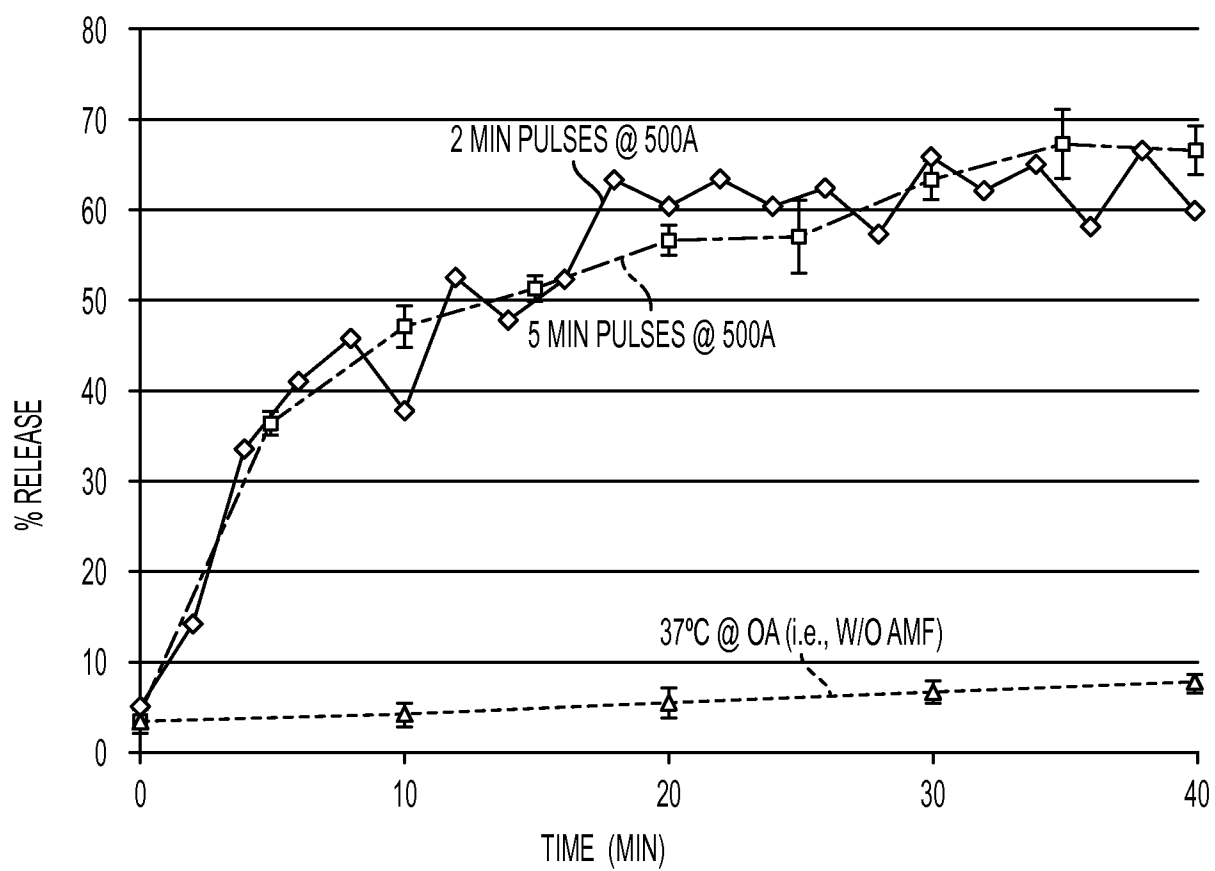


FIG. 24

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 15/44080

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 47/48 (2015.01)

CPC - A61K41/0028; A61K47/48861; B82Y5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

CPC: A61K41/0028; A61K47/48861; B82Y5/00

IPC: A61K 47/48 (2015.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 977/773; 977/838; 977/906 (See Search Words Below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PATBASE: Full-text = AU BE BR CA CH CN DE DK EP ES FI FR GB IN JP KR SE TH TW US WO

Google: Scholar/Patents: Magnetic nanoparticles linker therapeutic agent hydrolysis covalent bond intramolecular cyclization oxime amide drug delivery

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2012/0302516 A1 (NANTZ et al.) 29 November 2012 (29.11.2012) para [0004];[0057];[0058];[0097]; Scheme D	1-3
A	US 2011/0130616 A1 (SEENEY et al.) 02 June 2011 (02.06.2011) para [0005]-[0066]	1-3
A	US 2013/0302408 A1 (WEAVER) 14 November 2013 (14.11.2013) Entire Document	1-3

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

30 September 2015 (30.09.2015)

Date of mailing of the international search report

03 NOV 2015

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 15/44080

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 4-77
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.