Molecular Probes

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X-ray Photolysis To Release Ligands from Caged Reagents by an Intramolecular Antenna Sensitive to Magnetic Resonance Imaging**

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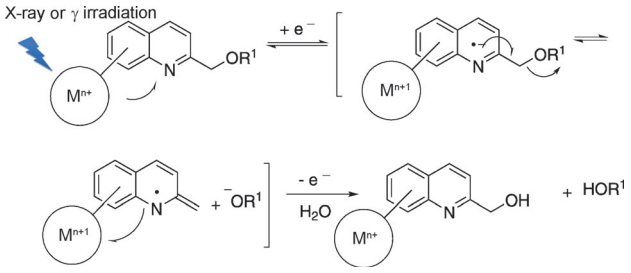
Light is important tool for the external control of biological processes in life sciences. It is used directly for the optical stimulation of neurons[1,2] for photolysis of caged compounds,[3] actuators,[4] and switches.[5] The principal problem that remains is the poor penetration of light in tissues, limited to depths of around 100 μm even with near-IR wavelengths.[6] Although the use of fiberoptics,[7] implantable photoelectrodes, laser-coupled microelectrode arrays,[7,8] and micromechanical systems[9] improves the situation, release of biologically active ligands with high spatiotemporal control in otherwise inaccessible materials remains a challenge.[10]

The use of X-rays for the activation of molecular devices has the potential to perform radiolysis in vivo.[11] Hard X-rays used in radiography are known to penetrate soft tissues to depths of centimeters and this has been exploited previously.[12] X-ray excitation can activate photosensitizers tethered to lanthanum fluoride nanoparticles and generate toxic singlet oxygen.[13a] Also the photothermal effect of X-ray irradiation of noble-metal nanostructures may be used for cancer treatment.[13b,c] More recently radiation-sensitive diselenide-derived block copolymer aggregates were prepared that load and release drugs in aqueous solution.[14] However, the photoactivation of organic probes by X-rays with subsequent release of covalently linked molecules has not been reported because organic reagents are poorly absorbing at these wavelengths.

We speculated, that photoactivated organic probes may undergo one-electron reduction in the presence of Auger electrons, and the radical anion intermediate may follow reaction paths similar to those of photoexcited intermediates.[15] Auger electrons are generated by the interaction of X-ray or γ irradiation with heavy metals. The one-electron reduction of hydroxymethyl quinolines would afford a radical anion intermediate that may undergo fragmentation as depicted in Scheme 1.[16] If the reaction proceeds in protic solvent such as water the fragmentation products are quenched after electron transfer rendering the process irreversible.



Scheme 1. Auger electron-mediated electron transfer (ET) leading to fragmentation of hydroxymethyl quinolines.

Here, we report the proof-of-principle of this concept and show that quinolene-derived probes are activated through interactions of X-ray or γ irradiation with gadolinium (III) complexes, which act as intramolecular antennae and convert part of the energy to fragmentation processes.[17] For this the Gd^{III} complex is coupled to well-characterized aminoquinoline-based photoprotecting groups,[18] normally cleaved by UV light and only weakly by shorter wavelengths than 300 nm. Photolabile groups which are photolytically activated by wavelengths in the 300–400 nm range are routinely used in cell biology and neuroscience to release ligands with high spatio-temporal resolution.[3a–d] In this case the activity of the biomolecule is masked by the photosensitive group and the light activation restores the activity by a process called "uncaging" or "photorelease".

As photoremoveable protecting groups 7-bromo-8-hydroxy-2-hydroxymethylquinoline (BHQ) and 7-(*N*,*N*-dimethylamino)-2-hydroxymethylquinoline (DMAQ) were selected (Figure 1).[18a–c,e] These groups absorb efficiently in the near-UV region at a wavelength of maximum absorbtion (λ_{max}) of 369 nm for BHQ with an absorbance, $\varepsilon_{\lambda_{max}}$(BHQ), of $2600\,M^{-1}cm^{-1}$ [tris(hydroxymethyl)aminomethane hydrochlo-

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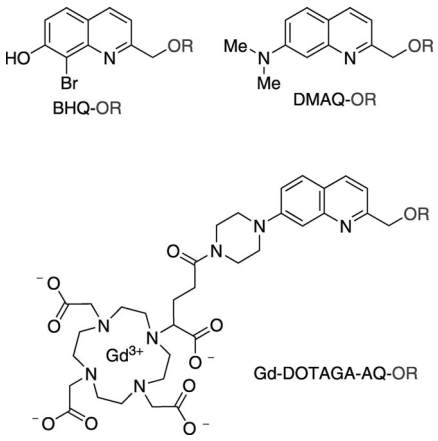
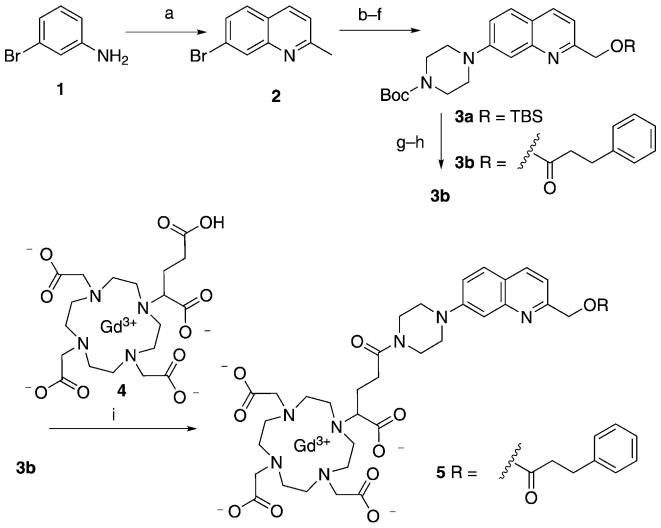


Figure 1. Quinolin-based light-activated protecting groups.



Scheme 2. Synthesis of the Gd-DOTAGA-AQ dihydrocinnamate **5** and the truncated control substrate **3b**. a) Paraldehyde (3 equiv), HCl(cc), RT 1 h then 80 °C, 3 h, (28 %). b) SeO_2 (1.3 equiv), dioxane, 80 °C, 3 h (95 %). c) $NaBH_4$ (1.1 equiv), EtOH, RT, 1 h (78 %). d) *Tert*-butyldimethylsilyl chloride (TBS-Cl, 1.2 equiv), imidazoline (1.2 equiv), *N*,*N*-dimethylformamid (DMF), RT, 3 h (64 %). e) Piperazine (5 equiv), tris(dibenzylideneacetone)dipalladium (Pd_2dba_3, 10 mol %), P*t*Bu_3 (40 mol %) NaO*t*Bu (1.2 equiv), toluene, 110 °C, 16 h (68 %). f) Boc_2O (1.1 equiv, boc is *tert*-butoxycarbonyl), DMAP (0.2 equiv), CH_2Cl_2, RT, 2 h (90 %). g) TBAF (5 equiv), THF, RT, 5 h (89 %). h) $Ph(CH_2)_2COOH$ (1.2 equiv), oxalyl chloride (1.5 equiv), DMF (cat), ether, 0 °C to RT, then NEt_3 (1.2 equiv), dimethylaminopyridine (DMAP, 0.2 equiv), CH_2Cl_2, RT, 3 h (80 %). i) HCl 6 N, dioxane, RT, 10 min, then Gd^{III}-DOTAGA (1.5 equiv), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI·HCl, 3.0 equiv), hydroxybenzotriazole (HOBt, 0.6 equiv), DMF/H_2O of 2:1, RT, 3 h (44 %).

ride (TRIS) buffer, pH 7.4, 298 K] and a λ_{max} value of 368 nm for DMAQ with an $\varepsilon_{\lambda_{max}}$(DMAQ) value of 4600 $M^{-1}cm^{-1}$ (KMOPS buffer, pH 7.2, 298 K). These groups are versatile and have been used with molecules coupled to carboxylate, phosphate, and diol units. For our purpose, the modified aminoquinoline was tethered to Gd^{III}-1,4,7,10-tetraazacyclododecane-1-glutaric acid-4,7,10-triacetic acid [Gd^{III}-DOTAGA)][19] (Figure 1) prepared previously for magnetic resonance imaging (MRI) applications.[20] The DOTAGA analogue has a glutarate arm to bind a substrate without lowering the stability of the complex or the exchange rate of water on the Gd^{3+} ion. Macrocyclic Gd^{III} complexes have high thermodynamic and kinetic stabilities relative to linear Gd^{III} complexes that renders them compatible with in vivo applications.[21] Notably, gadolinium derivatives are also used as X-ray contrast agents.[22]

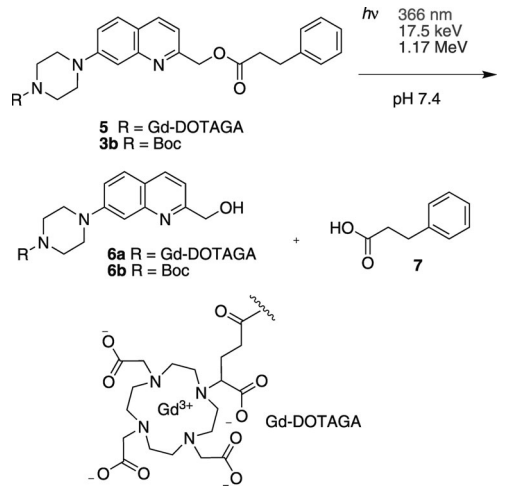
For the fragmentation studies dihydrocinnamate was selected as model leaving group because of its great hydrolytic stability relative to that of the acetate ester under physiological conditions, and also because both photolysis products can easily be monitored by HPLC.

The synthesis of the Gd-DOTAGA-AQ-caged dihydrocinnamate **5** was realized from 7-bromoquinaldine (**2**) which was prepared in quantities of grams by the Doebner–Miller synthesis from 3-bromoaniline (Scheme 2) under modified Dore's conditions.[18a] The bromoquinaldine **2** was transformed to the corresponding hydroxymethylene compound by an oxidation–reduction sequence, and the free alcohol was secured as *tert*-butyldimethylsilyl (TBS) ether. The piperazine group was introduced under conditions of Buchwald–Hartwig amination (tris(dibenzylideneacetone)dipalladium (Pd_2dba_3), P(*t*$Bu)_3$, NaO*t*Bu, toluene, 110 °C, 68 %),[23] and the secondary amine was converted to the *tert*-butoxycarbonyl (Boc) derivative **3a** using a standard protocol. The introduction of the dihydrocinnamate model substrate was realized after cleveage of the TBS protecting group in the presence of excess tetrabutylammonium fluoride (TBAF, 1 M in THF) and the dihydrocinnamate was introduced after activation of oxalyl chloride (80 %). The assembly of **3a** and Gd^{III}-DOTAGA acid **4** were realized in the presence of hydroxybenzotriazole (HOBt) hydrate and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI·HCl) coupling agents (Scheme 2).

The photolysis and radiolysis of compounds **3b** and **5** were performed in TRIS buffer under UV, X-ray, or γ irradiation conditions, as illustrated in Scheme 3. For near-UV irradiation of **3b** and **5** a 366 nm lamp (8 W; Carl Roth) was used, X-rays of 17.5 keV were produced by an X-ray generator (Diffractis 583 Enraf Nonius), and γ radiolysis experiments at 1.17 MeV were performed on a panoramic ^{60}Co source (IL60PL Cis-Bio International). The dose rates of 21 and 28 Gy min^{-1}, respectively, were determined with a Fricke dosimeter.

The photolysis of **3b** and **5** by irradiation at 366 nm (TRIS 20 mM, NaCl 100 mM, pH 7.4) was monitored by disappearance of the starting materials and the appearance of the uncaged hydroxy compounds **6a** and **6b** (see Table 1), and by dihydrocinnamate **7** through liquid-chromatography-coupled mass spectrometry (LC-MS) analysis. The structure of the products **6a** and **6b** were also proven by comparison of the HPLC-MS data with samples prepared by chemical synthesis.

Compound **3b** undergoes photolysis at 366 nm with a quantum sensitivity (εQ_u) of 213, which is comparable to the εQ_u value of 211 of the parent DMAQ-OAc compound.[18a] The presence of the Gd tether reduces, however, the sensitivity to photolysis of the quinoline group as Gd^{III}-



Scheme 3. Photo- and radiolysis of caged dihydrocinnamates **6a** and **6b**.

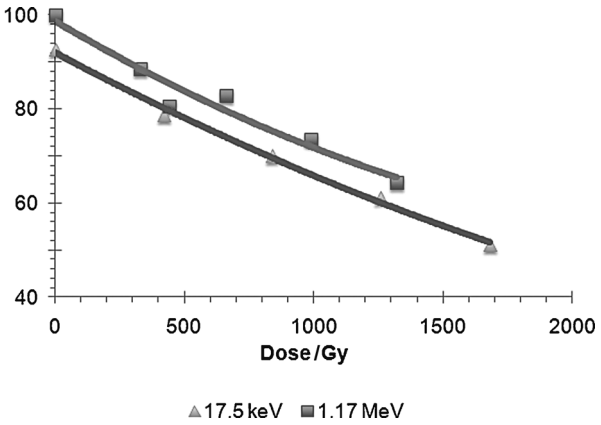


Figure 2. Progressive radiolysis of 0.4 mM of **5**. The remaining fraction (in %) is plotted against the cumulative dose of X-rays of 17.5 keV delivered at 21 Gray min^{-1} (full triangles) and the γ photons of 1.17 MeV at 28 Gray min^{-1} (full squares). Experimental details are given in the Supporting Information.

Table 1: Photolysis of compounds **3b** and **5**.

	λ_{max} [a] [nm]	$\varepsilon_{\lambda_{max}}$ [b] [M^{-1} cm^{-1}]	ε_{366} [b] [M^{-1} cm^{-1}]	Q_u [c]	εQ_u [d] [M^{-1} cm^{-1}]
5	340	3100	2100	0.020	42
3b	350	7320	5750	0.037	213

General conditions: TRIS 20 mM, NaCl 100 mM pH 7.4. [a] Wavelength at maximum absorption. [b] Absorbance at the given wavelength. [c] Uncaging quantum efficiency. [d] Sensitivity upon irradiation at 366 nm.

DOTAGA-senzitized **5** undergoes photolysis with a εQ_u value of 42. Yet, the high aqueous solubility of compound **5** represents a major advantage over other caged compounds that exhibit often poor solubility which reduces their efficiency in biological studies.

Progressive radiolysis of the Gd antenna-sensitized compound **5** at X-ray irradiation of 17.5 keV showed clean fragmentation with the appearance of the radioproducts **6a** and dihydrocinnamate **7** (Scheme 3 and Figure 2). When **3b** was irradiated under identical conditions, **6b** and **7** appeared solely as the products of hydrolysis at a conversion rate of less than 1% per hour, and this observation was identical to that of a control experiment in the dark. Likewise, when compound **5** was submitted to radiolysis at γ irradiation of 1.17 MeV, fragmentation was observed and **6a** and dihydrocinnamic acid **7** (Scheme 3 and Figure 2) were produced. No radiolysis was observed, however, when compound **3b** was irradiated without the Gd-DOTAGA sensitizer. Figure 2 shows the fractional loss of **5** plotted against the dose received by continuous irradiation at a dose rate of 21 Gray min^{-1} at 17.5 keV (full triangles) and at 28 Gray min^{-1} at 1.17 MeV radiation (full squares). Uncaging profiles of compound **5** with X-ray and γ irradiations are very similar with calculated G values of -93 nmol J^{-1}, where the G value denotes the number of moles of species produced/consumed per Joule of absorbed energy. In these experiments the release of dihydrocinnamic acid **7** by X-ray or γ irradiation occurred only if the caged compound was labeled by the Gd^{III} complex. This observation suggests that the Gd^{III} complex plays the role of a sensitizer.

Under conditions of X-ray or γ irradition in the presence of oxygen, radiolysis of water induces the formation of several species and mostly of HO$^{\bullet}$ or $O_2^{\bullet-}$ radicals. Hydroxyl radicals are very strong oxidants. Therefore, to exclude their role in the fragmentation process of compound **5**, we performed the same experiment in phosphate buffer because TRIS buffer is known to scavenge most of these radicals. The identical G values obtained (data not shown) suggest that radiolytic fragmentation is not dependent on the presence of hydroxyl radicals in the solution, and fragmentation is attributed to the direct interaction of radiation with compound **5**.

MRI characteristics of compound **5** were evaluated by in vitro r_1 and r_2 relaxivity measurements, where r_1 is the longitudinal and r_2 is the transversal relaxivity parameter. The obtained r_1 value of 7.4 mM^{-1} s^{-1} and r_2 value of 9.6 mM^{-1} s^{-1} of **5** at 300 MHz agrees with a low molecular weight and a low rotational correlation time τ_R relative to the values (r_1 = 3.9 mM^{-1} s^{-1} and r_2 = 4.5 mM^{-1} s^{-1} at 300 MHz, 300 K) of DOTAREM® (Gd^{III}-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid, Gd-DOTA). Likewise, the radioproduct **6a** shows relatively low relaxivity values r_1 of 5.7 mM^{-1} s^{-1} and r_2 of 7.4 mM^{-1} s^{-1} that agree with a lower rotational correlation time of **6a** relative to **5**.

In conclusion, a first energy dispersive X-ray-activated probe (EDiXA) irradiated by X-rays or γ photons has been prepared to address the problem of controlled photorelease of biologically active ligands deep within the tissues of the body. We have demonstrated the release of dihydrocinnamate in aqueous solution at physiological pH. Because caged compounds are used principally as research tools in molecular biology, cell physiology, and neuroscience, X-ray cages may extend the range to in vivo experiments. This method has the potential to improve photodynamic therapies by extending the depth of penetration of the radiation and by expanding the range of molecules that are released; particularly it has the potential application in chemotherapy of delivering toxic drugs to their sites of action as nontoxic caged precursors.

However, the efficiency of Gd-DOTAGA-AQ is not high enough for direct long-term application in vivo. The lethal dosage (LD_{50}) of X-ray or γ irradiation is at approximately 5 Gray for mice, suggesting that an improvement of sensitivity of three orders is needed for nonintrusive use. Given the apparent dependence of this mechanism on the energy dose alone, an improvement may best be achieved by generating multisite probes.

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[1] a) G. Nagel, T. Szellas, W. Huhn, S. Kateriya, N. Adeishvili, P. Berthold, D. Ollig, P. Hegemann, E. Bamberg, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 13940 – 13945; b) L. A. Gunaydin, O. Yizhar, A. Berndt, V. S. Sohal, K. Deisseroth, P. Hegemann, *Nat. Neurosci.* **2010**, *13*, 387 – 392.

[2] a) Special issue in *J. Neural Eng.* **2010**, *7*, 040201 (Eds.: S. Shoham, K. Deisseroth); b) E. Papagiakoumou, F. Anselmi, A. Begue, V. de Sars, J. Gluckstad, E. Y. Isacoff, V. Emiliani, *Nat. Methods* **2010**, *7*, 848 – 854.

[3] a) G. Mayer, A. Heckel, *Angew. Chem.* **2006**, *118*, 5020 – 5042; *Angew. Chem. Int. Ed.* **2006**, *45*, 4900 – 4921; b) A. Specht, F. Bolze, Z. Omran, J.-F. Nicoud, M. Goeldner, *HFSP J.* **2009**, *3*, 255 – 264; c) H.-M. Lee, D. R. Larson, D. S. Lawrence, *ACS Chem. Biol.* **2009**, *4*, 409 – 427; d) G. C. R. Ellis-Davies, *Nat. Methods* **2007**, *4*, 619 – 628; e) D. Warther, S. Gug, A. Specht, F. Bolze, J. F. Nicoud, A. Mourot, M. Goeldner, *Bioorg. Med. Chem.* **2010**, *18*, 7753 – 7758.

[4] a) A. R. Dunn, I. J. Dmochowski, J. R. Winkler, H. B. Gray, *J. Am. Chem. Soc.* **2003**, *125*, 12450 – 124561; b) E. Beaumont, J.-C. Lambry, C. Gautier, A.-C. Robin, S. Gmouh, V. Berka, A.-L. Tsai, M. Blanchard-Desce, A. Slama-Schwok, *J. Am. Chem. Soc.* **2007**, *129*, 2178 – 2186.

[5] a) L. Luo, E. M. Callaway, K. Svoboda, *Neuron* **2008**, *57*, 634 – 660; b) F. Zhang, A. M. Aravanis, A. Adamantidis, L. de Lecea, K. Deisseroth, *Nat. Rev. Neurosci.* **2007**, *8*, 577 – 581; c) C. Allen, *Nat. Neurosci.* **2004**, *7*, 1291; d) G. A. Woolley, *Acc. Chem. Res.* **2005**, *38*, 486 – 493; e) M. Erdélyi, M. Varedian, C. Sköld, I. B. Niklason, J. Nurbo, Å. Persson, A. Gogoll, *Org. Biomol. Chem.* **2008**, *6*, 4356 – 4373; f) R. Numano, S. Szobota, A. Y. Lau, P. Gorostiza, M. Volgraf, B. Roux, D. Trauner, E. Y. Isacoff, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 6814 – 6819; g) M. A. Priestman, D. S. Lawrence, *Biochim. Biophys. Acta Proteins Proteomics* **2010**, *1804*, 547 – 558; h) S. Szobota, E. Y. Isacoff, *Annu. Rev. Biophys.* **2010**, *39*, 329 – 348.

[6] a) E. Beaurepaire, M. Oheim, J. Mertz, *Opt. Commun.* **2001**, *188*, 25 – 29; b) M. Oheim, E. Beaurepaire, E. Chaigneau, J. Mertz, S. Charpak, *J. Neurosci. Methods* **2001**, *111*, 29 – 37; c) M. Oheim, E. Beaurepaire, E. Chaigneau, J. Mertz, S. Charpak, *J. Neurosci. Methods* **2001**, *112*, 205 – 205.

[7] A. M. Aravanis, L. P. Wang, F. Zhang, L. A. Meltzer, M. Z. Mogri, M. B. Schneider, K. Deisseroth, *J. Neural Eng.* **2007**, *4*, S143 – S156.

[8] a) Y. X. Zhao, P. Larimer, R. T. Pressler, B. W. Strowbridge, C. Burda, *Angew. Chem.* **2009**, *121*, 2443 – 2446; *Angew. Chem. Int. Ed.* **2009**, *48*, 2407 – 2410; *Angew. Chem.* **2009**, *121*, 2443 – 2446; b) D. Ghezzi, A. Menegon, A. Pedrocchi, F. Valtorta, G. Ferrigno, *J. Neurosci. Methods* **2008**, *175*, 70 – 78.

[9] a) N. M. Elman, Y. Patta, A. W. Scott, B. Masi, H. L. H. Duc, M. J. Cima, *Clin. Pharm. Ther.* **2009**, *85*, 544 – 547; b) Y. F. Liu, W. Chen, S. P. Wang, A. G. Joly, *Appl. Phys. Lett.* **2008**, *92*, 043901; c) J. F. Lovell, T. W. B. Liu, J. Chen, G. Zheng, *Chem. Rev.* **2010**, *110*, 2839 – 2857.

[10] a) P. Rai, S. Mallidi, X. Zheng, R. Rahmanzadeh, Y. Mir, S. Elrington, A. Khurshid, T. Hasan, *Adv. Drug Delivery Rev.* **2010**, *62*, 1094 – 1124; b) J. P. Celli, B. Q. Spring, I. Rizvi, C. L. Evans, K. S. Samkoe, S. Verma, B. W. Pogue, T. Hasan, *Chem. Rev.* **2010**, *110*, 2795 – 2838.

[11] R. R. Anderson, J. A. Parrish, *Science* **1983**, *220*, 524 – 527.

[12] a) E. F. G. Dickson, R. L. Goyan, R. H. Pottier, *Cell. Mol. Biol.* **2002**, *48*, 939 – 954; b) J. D. Spikes, *Photochem. Photobiol.* **1991**, *54*, 1079 – 1092.

[13] a) P. K. Jain, X. H. Huang, I. H. El-Sayed, M. A. El-Sayed, *Acc. Chem. Res.* **2008**, *41*, 1578 – 1586; b) S. Lal, S. E. Clare, N. J. Halas, *Acc. Chem. Res.* **2008**, *41*, 1842 – 1851; c) S. T. Wang, K. J. Chen, T. H. Wu, H. Wang, W. Y. Lin, M. Ohashi, P. Y. Chiou, H. R. Tseng, *Angew. Chem.* **2010**, *122*, 3865 – 3869; *Angew. Chem. Int. Ed.* **2010**, *49*, 3777 – 3781.

[14] N. Ma, H. Xu, L. An, J. Li, Z. Sun, X Zhang, *Langmuir* **2011**, *27*, 5874—5878.

[15] A. Houmam, *Chem. Rev.* **2008**, *108*, 2180 – 2237.

[16] P. I. Dalko, *Tetrahedron* **1995**, *51*, 7579 – 7653.

[17] M. Petit, G. Bort, D. Ogden, C. Sicard, P. I. Dalko, EU Patent Appl. EP 10290323.4, **2010**.

[18] a) M. J. Davis, C. H. Kragor, K. G. Reddie, H. C. Wilson, Y. Zhu, T. M. Dore, *J. Org. Chem.* **2009**, *74*, 1721 – 1729; b) O. D. Fedoryak, T. M. Dore, *Org. Lett.* **2002**, *4*, 3419 – 3422; c) Y. Zhu, C. M. Pavlos, J. P. Toscano, T. M. Dore, *J. Am. Chem. Soc.* **2006**, *128*, 4267 – 4276; d) Y. M. Li, J. Shi, R. Cai, X. Y. Chen, Q. X. Guo, L. Liu, *Tetrahedron Lett.* **2010**, *51*, 1609 – 1612; e) M. C. Pirrung, T. M. Dore, Y. Zhu, V. S. Rana, *Chem. Commun.* **2010**, *46*, 5313 – 5315.

[19] a) K. P. Eisenwiener, P. Powell, H. R. Mäcke, *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2133 – 2135; b) S. G. Levy, V. Jacques, K. L. Zhou, S. Kalogeropoulos, K. Schumacher, J. C. Amedio, J. E. Scherer, S. R. Witowski, R. Lombardy, K. Koppetsch, *Org. Process Res. Dev.* **2009**, *13*, 535 – 542.

[20] P. Caravan, J. J. Ellison, T. J. McMurry, R. B. Lauffer, *Chem. Rev.* **1999**, *99*, 2293 – 2352.

[21] J. M. Idée, M. Port, C. Medina, E. Lancelot, E. Fayoux, S. Ballet, C. Corot, *Toxicology* **2008**, *248*, 77 – 88.

[22] a) A. Y. Louie, *Chem. Rev.* **2010**, *110*, 3146 – 3195; b) S. B. Yu, A. D. Watson, *Chem. Rev.* **1999**, *99*, 2353 – 2377.

[23] a) B. P. Fors, N. R. Davis, S. L. Buchwald, *J. Am. Chem. Soc.* **2009**, *131*, 5766 – 5768; b) G. D. Vo, J. F. Hartwig, *J. Am. Chem. Soc.* **2009**, *131*, 11049 – 11061.

