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Nitrocatechols as Tractable Surface Release Systems

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We report the development of a molecular surface modification platform based on nitrocatechol derivatives that allows for small molecule functionalization of TiO_2 under mild aqueous conditions and efficient release triggered by light therefore uncaging a small molecule cargo on demand. Surface modifications by using molecular approaches have found applications in a wide variety of fields, as they allow combining the bulk properties of a material with the molecular features of the coating, which can additionally be tailored by synthetic chemistry.[1] A central challenge remains the nature of the molecular anchor that links coating and surface. In this respect, catechols display unique properties owing to their ability to strongly bind to metal oxides in aqueous media. Originally found in mussel adhesive proteins,[2] dihydroxyphenylalanine (DOPA) or dopamine-derived catechols were successfully used in surface modifications despite their shortcomings of weak binding and sensitivity to oxidation. We have introduced catechols with electron-withdrawing substituents based on the iron chelator anachelin that result in stable coatings with non-polymeric architectures.[3] A second-generation design introduced nitrocatechols such as nitrodopamine owing to the ease of preparation, improved adhesion properties, and stability towards oxidation.[4] All these anchors have found widespread applications,[1,5] for example, we have shown that such catechols can be used to functionalize surfaces with complex antibiotics by an operationally simple dip-and-rinse procedure to generate antimicrobial surfaces.[6]

In addition to immobilization, the release of small molecules from a modified surface triggered by an external stimulus would be highly desirable. Potential applications would range from drug delivery, small molecule microarrays to selective probes in chemical biology. In particular in the latter area, such an approach would combine the well-known strategy of temporarily disabling the biological activity of a small molecule (caging) with the possibility of controlled release on demand. This would allow for both high spatial control (through immobilization) and temporal control (through caging) of the biological activity of a small molecule, two key elements in chemical biology approaches.[7,8] Herein, we report on the development of such a molecular platform that allows for the spatiotemporal control of small molecule release. In particular, we propose a bio-inspired approach that leverages the presence of a catechol (easily tunable by a variety of parameters such as pH, ion strength, or temperature) with the versatility of the nitrophenyl system (Figure 1).

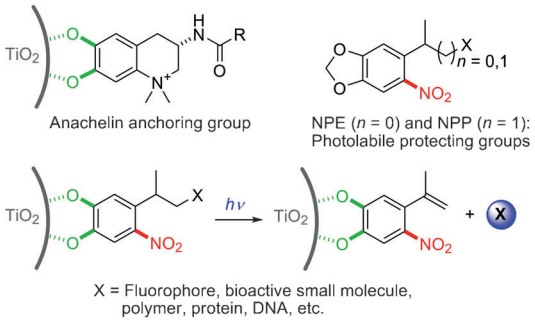
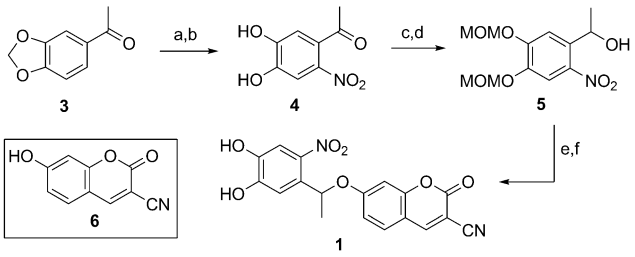


Figure 1. Concept of the light induced surface release of carrier molecules for the photolabile NPP protecting group.

Among the most commonly used photocleavable protecting groups are several nitrophenyl derivatives, including (2-nitrophenyl)ethyl (NPE) and (2-nitrophenyl)propyl (NPP) derivatives.[9,10] However, upon inspection of the literature, we were surprised to find that no free nitrocatechols have been utilized as photocleavable groups and the surface functionalization and release properties of such systems have not been investigated.[11] We have targeted both NPE and NPP systems **1** and **2**, respectively, and demonstrate in this study that controlled bonding and release is readily achieved for the latter derivatives.

The first target compound constituted the 2-nitrophenyl ethyl derivative **1**. Its synthesis started with the preparation of nitrocatechol **4** by nitration of commercially available 3,4-(methylenedioxy)acetophenone (**3**) using HNO_3 in acetic acid (Scheme 1). Treatment with $AlCl_3$ at low temperature and sub-



Scheme 1. Preparation of NPE conjugate **1** for surface modification. Reagents and conditions: a) HNO_3, AcOH, 0 °C→RT, 2.5 h, 59%; b) $AlCl_3$, DCE, −5 °C, 1 h, then 48% HBr, RT, 24 h, 84%; c) MOMCl, K_2CO_3, MeCN, 0 °C→RT, 3 h, 87%; d) $NaBH_4$, MeOH, 0 °C→RT, 3.5 h, 99%; e) **6**, PPh_3, DIAD, THF, 0 °C→RT, overnight, 41%; f) TFA, H_2O. DCE = 1,2-dichloroethane, DIAD = diisopropyl diazodicarboxylate, MOM = methoxymethyl, TFA = trifluoroacetic acid, THF = tetrahydrofuran.

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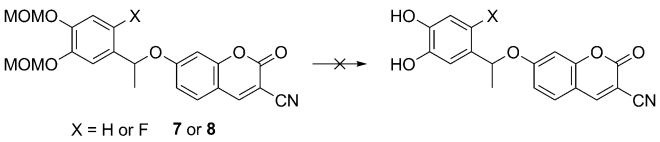
sequent hydrolysis with concentrated HBr gave nitrocatechol **4** in good yield. Catechol **4** was then further protected as the MOM ether and the keto group was reduced using $NaBH_4$ leading to alcohol **5**. In the context of this synthesis, it was found necessary to protect the catecholate OH groups, to prevent attachment of these compounds to silica gel during chromatographic purification. The alcohol **5** was then attached to the fluorophore[12] **6** through Mitsunobu reaction in moderate yields. When alcohol **5** was coupled, reactions were performed under exclusion of light to prevent decomposition of the formed product. During isolation of the photolabile compound from the reaction mixtures, it became already apparent that the uncaging process could be induced, when TLC plates were exposed to UV light at 366 nm (see video and Supporting Information). The MOM protecting group could be finally removed using aqueous TFA, leading to the NPE derivative **1**.

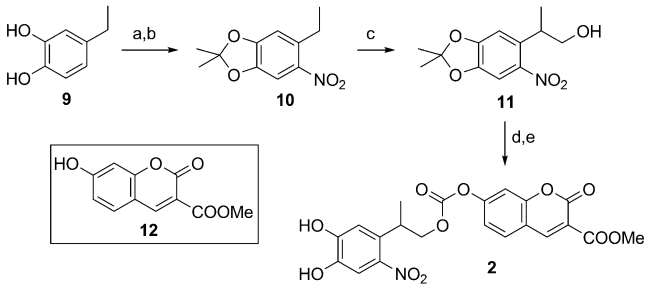
With the NPE derivative in hand, we sought to prepare the related unsubstituted compound **7** (X = H) as well as the fluorinated compound **8** (X = F, for detailed synthesis see Supporting Information) to investigate the role of the nitro substituent on the cleavage mechanism (Scheme 2). There are at least two



Scheme 2. Deprotection studies of fluorinated and unsubstituted catechols.

fundamentally different pathways for cleavage of such catechols from titania possible, either via photocatalytic oxidation to the quinone[7] or via nitroaryl mediated bond cleavage.[8,9] To investigate these two options and to delineate the role of the nitro substituent, we have prepared control compounds **7** and **8** lacking this substituent, where only photocatalytic oxidation to the quinone would be possible. We were surprised to discover that the deprotection under a variety of conditions of these compounds proved to be impossible, as only decomposition of the starting material and the free fluorophore could be detected. This could be explained by the formation of reactive quinone methides by cleavage of the fluorophore at the benzylic position.[13]

To circumvent the decomposition via the quinone methide pathway, we wanted to investigate the corresponding homologated propyl substituted nitrophenols **2**. Therefore, acetonide **10** was successfully prepared from catechol **9** by using 2,2-dimethoxypropane and catalytic amounts of TsOH in benzene at reflux (Scheme 3). Subsequent nitration of the acetonide with half-concentrated HNO_3 afforded **10** after 1.5 hours in very good yield. To our delight, these strongly acidic, aqueous conditions did not affect the acetonide protection group. A crystal structure of **10** could be obtained, which confirmed the correct installment of the nitro group (see Supporting Information).[14] The reverse synthetic order, nitration followed by protection, failed as no acetonide formation could be detected. The crucial elongation reaction of **10** with paraformaldehyde and Triton B gave the desired product **11**,[15] although only moderate conversion was achieved (with 40% starting material **10** recovered). With the catechol protected as the acetonide, carbonate formation using triphosgene and triethylamine in THF led to the desired chloroformate in quantitative yield within 25 minutes at 0 °C.[16] To prevent decomposition, the chloroformate



Scheme 3. Preparation of NPP conjugate **2** for surface modification. Reagents and conditions: a) 2,2-dimethoxypropane, cat. TsOH, benzene, reflux, overnight, b) HNO_3/H_2O (1:1), 0 °C→RT, 1.5 h, 86% (over 2 steps); c) CH_2O, Triton B in MeOH, 85 °C, 65 h, 80% (brsm); d) i) triphosgene, Et_3N, THF; 0 °C, 25 min, ii) **3b**, pyridine, CH_2Cl_2, 0 °C→RT, 1.5 h; e) TFA, H_2O, RT, overnight, 68% (over 2 steps). brsm = based on recovery of starting material, Ts = 4-toluenesulfonyl.

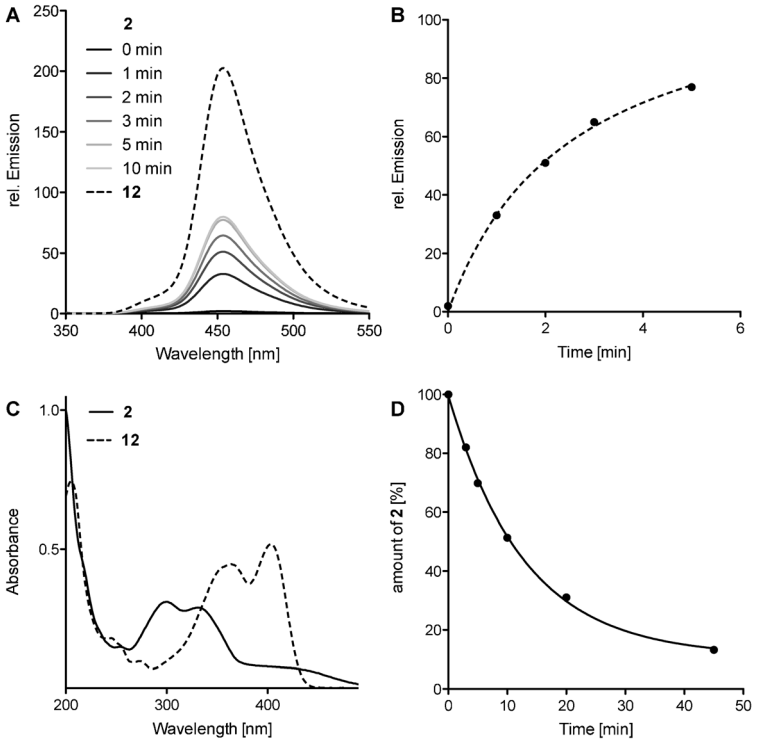


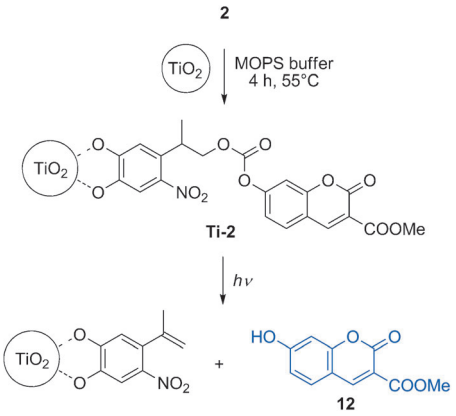
Figure 2. A) Fluorescence spectra of **12** and **2** after certain irradiation times. B) Fluorescence emission intensity at 454 nm depending on the irradiation time at 366 nm of **2** as a $5 \cdot 10^{-5}$ M solution in MOPS buffer. C) UV spectra of **2** and **12**. D) Decay of **2** upon UV irradiation at 366 nm, determined by HPLC-MS analysis.

was coupled immediately to coumarin **12** to afford the desired carbonate, which was deprotected to give the target catechol **2** using neat TFA. In total the preparation of **2** was achieved in five steps using only two purification steps rendering **2** an easily accessible and attractive candidate for further investigations. In particular, we expect that the alcohol **11** could serve as an ideal starting point for the attachment of various cargo compounds by coupling chemistry.

The stability of the different caged compounds was then investigated using a variety of methods and assays. Gratifyingly, the NPP linker **2** was fully stable to hydrolysis in aqueous medium (MOPS buffer) at pH 5.5 in the dark for at least 72 hours. This is contrast to the ethyl derivative **1**, which displays only limited stability. In order to determine the half-life time of **2** in aqueous solution (MOPS buffer) under near UV-irradiation, photocleavage of catechol **2** was investigated by detection of the evolving fluorescence during release of coumarin **12**.[17] As expected, a rapid increase in fluorescence was detected during the first minutes of irradiation and the fluorescence maximum was identified at 454 nm (Figure 2A). However, after approximately 15 minutes, the detected fluorescence intensity was decreasing, thus indicating that photobleaching of coumarin **12** occurred, which could be confirmed by control experiments (Figure 2B, and Supporting Information). Quantification by HPLC-MS determined the half-life time of **2** to be around 12 minutes at a concentration of 5×10^{-5} M in buffer (Figure 2D). This is an attractively short period for cleavage, since the light intensity of a simple laboratory UV-lamp is rather low ($\approx$20 mW cm^{-2}).

To evaluate the surface release properties, TiO_2 particles (1.0–2.0 μm) were functionalized with nitrocatechol **2** in MOPS buffer at 55 °C according to previously developed conditions (Scheme 4).[4,18] After the incubation, the functionalized particles **Ti-2** were washed three times with CH_3CN, and HPLC analysis of the washing solutions determined only small amounts of fluorophore **12** and no free **2** present.

The release of the fluorescent cargo from the functionalized beads was investigated next. The functionalized TiO_2 beads **Ti-2** were suspended in MOPS buffer and the mixture was irradiated. Fluorescence became immediately visible (Figure 3B). Aliquots were taken after certain irradiation time intervals and analyzed by HPLC at 366 nm. The obtained data demonstrates that no catechol **2** is present and the amount of **12** was increasing over the irradiation time (Figure 3C), providing evidence that photocleavage of surface-adsorbed nitrocatechol **2** and concomitant release of coumarin **12** has occurred. In this reaction setup, a half-life time of approximately 19 minutes was determined for the release of **12**. Qualitatively, these observations could be corroborated by the immobilization of **2** on silica (TLC plate) and controlled release under UV light (Figure 3A, see Supporting Information for videos). In this simple application, a printed foil was used as template to generate the desired surface pattern, which could be unmasked by release of the fluorophore through irradiation.



Scheme 4. Immobilization of nitrocatechol **2** on TiO_2 microparticles to deliver **Ti-2** and release of the cargo upon irradiation.

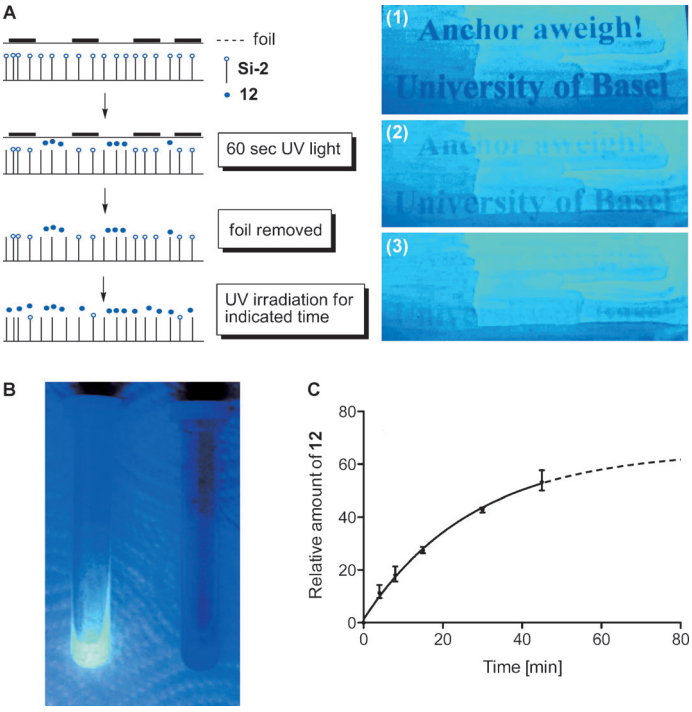


Figure 3. A) TLC plate coated with nitrocatechol **2** (**Si-2**) and irradiated for 60 sec at 366 nm under a printed foil, after 10 sec exposure to UV light without foil (1), after 60 sec (2), and after 180 sec (3) UV irradiation at 366 nm (see videos and the Supporting Information). B) **Ti-2** (left) and negative control (uncoated TiO_2 particles, right) in MOPS buffer upon UV irradiation. C) Release kinetics of **12** from **Ti-2** upon UV irradiation at 366 nm in MOPS buffer.

In conclusion, we report in this Communication the development of a bio-inspired surface modification platform based on nitrocatechols that allows for the controlled release of small molecules under an external stimulus. Salient features of this method involve 1) ease of functionalization of TiO_2 particles under an operationally simple dip-and-rinse procedure, 2) stability of the resulting functionalized particles to repeated washing, and 3) rapid release of the small molecule cargo under an

external stimulus, that is, UV light. We think that this method displays advantages with regard to controlled release. This surface modification platform might find applications in drug delivery, as caged probes in chemical biology or for direct assays on chip. The utilization of this platform for the immobilization and release of biologically active small molecules is currently under investigation in our laboratories.

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Keywords: adhesion · biomimetic synthesis · photochemistry · release on demand · synthesis design

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[14] CCDC 881462 (**10**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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