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Micellar Brønsted Acid-Mediated Synthesis of DNA-Tagged Heterocycles

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ABSTRACT: The translation of well-established molecular biology methods such as genetic coding, selection and DNA sequencing to combinatorial organic chemistry and compound identification has made extremely large compound collections, termed DNA-encoded libraries, accessible for drug screening. However, the reactivity of the DNA imposes limitations on the choice of chemical methods for encoded library synthesis. For example, strongly acidic reaction conditions must be avoided because they damage the DNA by depurination, i. e. the cleavage of purine bases from the oligomer. Application of micellar catalysis holds much promise for encoded chemistry. Aqueous micellar dispersions enabled compound synthesis under often appealingly mild conditions. Amphiphilic block copolymers covalently functionalized with sulfonic acid moieties in the lipophilic portion assemble in water and locate the Brønsted catalyst in micelles. These acid nanoreactors enabled the reaction of DNA-conjugated aldehydes to diverse substituted tetrahydroquinolines and aminoimidazopyridines by Povarov, and Groebke-Blackburn-Bienaymé reactions, respectively, and the cleavage of tBoc protective groups from amines. The polymer micelle design was successfully translated to the Cu/Bipyridine/TEMPO system mediating the oxidation of DNA-coupled alcohols to the corresponding aldehydes. These results suggest a potentially broad applicability of polymer micelles for encoded chemistry.

INTRODUCTION

The last decade has witnessed the rise of DNA-encoded small molecules libraries (DELs) to a widely used small molecule screening technology in academia and industry.[1-6] Encoded libraries are synthesized through split-and-pool combinatorial routines encompassing alternated chemical compound synthesis and DNA-tagging steps that endow the compound with genetic information (Figure 1a).[7-11] The technology benefited from technical advances in molecular biology, i.e. robust enzymatic concatenation of DNA barcode fragments,[7-11] detection of minute DNA amounts by polymerase chain reaction, and advanced DNA sequencing platforms to read and quantitate genetic information. These genetic techniques allow chemists to synthesize unprecedented numbers of compounds as pools, and to identify bioactive compounds from these pools by selection experiments on proteins and DNA barcode sequencing (Figure 1b).[1-6]

While DNA tagging enables efficient handling of large compound numbers for target-based screening, it imposes formidable challenges to synthesis methodology development.[12,13] To be encodable, chemistry must be compatible with water as (co-)solvent, must afford the target compound with high yields despite high starting material dilution, should display a broad scope of ideally readily available starting materials, must be operationally simple to be compatible with multi-well plate reactor formats, and the genetic information must be preserved under the reaction conditions. The latter point requires pH values of above 3, and avoidance of harsh reaction conditions, particularly in combination with oxidizing agents and many Lewis acids. DEL synthesis methods focused mostly on building block-appending reactions, such as carbonyl chemistry,

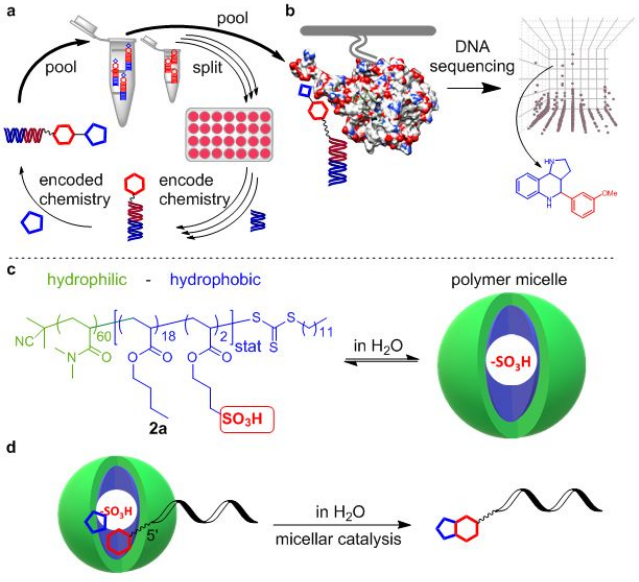

Figure 1. The technology of DNA-encoded libraries (DELs). (a) Schematic presentation of combinatorial DNA-encoded chemistry. (b) Compounds binding to a target protein of interest are identified by selection of DELs and DNA barcode sequencing. (c) Structure of sulfonic acid-substituted block copolymer **2a** that localizes sulfonic acid moieties in polymer micelles. (d) Application of **2a** as micelle-based reaction system for DNA-compatible synthesis of DNA-tagged target molecules.

nucleophilic substitution reactions, and Cu- and Pd- mediated cross coupling reactions.[14] Broadening the scope of encoded chemistry has therefore been cited as particularly important to advance the technology of DNA-encoded libraries.[15,16] Lately, modern photo redox chemistries have greatly expanded the

choice of building block-appending reactions for encoded chemistry.[17] Heterocyclic structures, i.e. small ring systems containing heteroatoms, are well represented in the chemical space of bioactive molecules.[18-24] A recent analysis of FDA-approved drugs found that with 59 % the majority of them contain a nitrogen heterocycle, underpinning the importance of heterocyclic chemistry in drug research.[25] Research efforts towards encoded heterocyclic chemistry have resulted to date in only a few synthesis methods. These are cycloadditions such as the (hetero) Diels-Alder and 3+2 cycloaddition reactions,[26] and condensation and substitution reactions of functionalized starting materials.[14] Brønsted acids are essential catalysts in organic chemistry, enabling a plethora of reactions, among them many that give heterocycles from simple starting materials.[27] However, the application of protic acid catalysis in encoded compound synthesis entails the risk of DNA depurination, i.e. the loss of purine bases from the oligomer by purine N7 protonation and subsequent cleavage of the acetal-like ribosidic linkage (see **DNA-1** incubated at different pH values in Figure S1, supplementary information).[28] Protic acid catalysis for encoded compound synthesis is currently restricted to barcoding with more stable peptide nucleic acids or dedicated DNA-barcoding strategies.[29-31]

Compartmentation of reaction media is an intriguing principle in chemistry with many applications. It can be effected by amphiphilic, soap-like molecules, among them block copolymers.[32-37] Above a certain concentration called critical micelle concentration (CMC) amphiphiles associate in water to nanometer-sized micelles that are characterized by a lipophilic core and a hydrophilic corona (Figure 1c).[38,39] These oil-in-water (o/w) micelles can serve as microheterogeneous systems to solubilize hydrophobic chemicals in water. Micellar dispersions have repeatedly been shown to accelerate reaction rates by concentrating reactants in nanometer-sized, confined reaction vessels and/or by altering their reactivity in the micellar environment, often resulting in appealingly mild reaction conditions.[34,40,41] Second-generation micellar nanoreactor designs linked catalysts covalently to the lipophilic portion of the amphiphile preventing catalyst partitioning between phases.[42-45] A few times, micellar systems have been used to react DNA to target conjugates. For instance, amino-linker modified DNA was coupled to water-insoluble carboxylic acids by a means of a micellar system, and micelles with a corona composed of DNA strands have been designed for DNA-templated chemistry.[46,47] A micellar catalyst system composed of a lipidated dopamine oxidizing DNAzyme and a lapidated dopamine binding DNA aptamer was shown to accelerate dopamine oxidation by concentrating the substrate close to the catalytic site.[48] We hypothesized that a micellar reaction system designed to direct a catalyst into the lipophilic core and to minimize its phase distribution might enable Brønsted acid catalysis for the selective reaction of DNA-conjugated starting materials. It benefits from the inaccessibility of the catalytic site to water-soluble DNA and from the typically low catalyst concentrations employed in micellar catalysis. To this end, we explored the sulfonic acid-substituted, amphiphilic copolymer **2a** (Figure 1c, Figures S2-S5). In water it forms already at low micromolar concentrations micellar nanoreactors containing sulfonic acid moieties (pKa ≈ -2.5) covalently connected to the lipophilic polymer block. Here, we show that up to millimolar concentrations of copolymer **2a** were well tolerated by DNA oligonucleotides, and enabled the reaction of DNA-conjugated aldehydes to diverse substituted tetrahydroquinolines by Povarov reaction (Figure 2) without detectable depurination. The scope of micellar Brønsted acid catalysis was subsequently extended to the synthesis of aminoimidazopyridines by Groebke-Blackburn-Bienaymé (GBB) reaction and the removal of *t*Boc-protective groups from amines. Finally, we could translate the copolymer micelle design to a Cu(I)/TEMPO catalyst for the selective oxidation of DNA-coupled alcohols to the corresponding aldehydes.

RESULTS AND DISCUSSION

Investigation of the Povarov reaction to DNA-tetrahydroquinoline conjugates under homogeneous reaction conditions. Tetrahydroquinolines are frequently occurring scaffolds among bioactive molecules. They can be found as central structures in natural products, a very recently disclosed inhibitor of CRISPR-Cas9, and clinical candidates (**4**-**6**, Figure 2a).[49-51] The Povarov reaction, a formal aza-Diels-Alder reaction, affords in one pot diverse tetrahydroquinolines **11** from readily available aldehydes **7**, anilines **8**, and electron-rich olefins **10** (Figure 2b).[52-64]

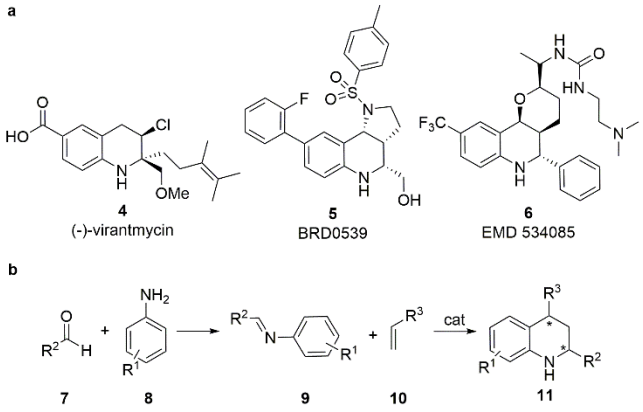

Figure 2. The tetrahydroquinoline is a frequently occurring heterocyclic core structure in bioactive compounds. (a) Natural product virantmycin **4**; inhibitor of CRISPR-Cas9 **5**, and clinical candidate **6**. (b) The Povarov three-component reaction gives straightforward excess to the characteristic tetrahydroquinoline core **11** displayed by compounds **5** and **6**.

Two three-dimensionality inducing sp^3-C-C bonds are formed in the course of tetrahydroquinoline synthesis. This, together with the broad reactant scope makes the reaction highly attractive for screening library synthesis.[50,58] We investigated a translation of published, homogenous reaction conditions for the Povarov reaction to a DNA-tagged format. The DNA-aldehyde conjugate **3a** (Figure S6), serving as a model substrate for a DNA-encoded compound, was reacted with aniline **8a**, and the olefin **10a** screening different equivalents of reactants, co-solvent systems and protic acid catalysts.[55] Acid concentrations in a DNA-compatible range failed to yield

the target DNA tetrahydroquinoline conjugate **DNA-12a** in detectable amounts (Figure 3, Table S3, Figures S7, S8). The Lewis acids Yb(III), Sc(III), Ceric ammonium nitrate (CAN), and Ce(III) were previously shown to catalyse the Povarov reaction, but failed under several tested reaction conditions to yield the DNA-coupled heterocycle, too (Table S3, Figures S9-12).[59-64] Thus, none of the evaluated homogeneous reactions based on Brønsted acid or Lewis acid catalysis met under the evaluated reaction conditions the requirement for encodable chemistry to afford the target product at high yields and preserve DNA integrity at the same time. These results prompted us to explore whether we could harness the aforementioned attractive features of micellar catalysis to enable the Povarov reaction on DNA-linked substrates.

Design of a micelle-based acid catalyst. We aimed at amphiphiles that form oil-in-water micelles, locate a catalyst in the micellar environment, and associate at very low concentrations to minimize the fraction of catalysts free to interact with DNA in the water phase. Block copolymer **2a** meets all of these demands. It is composed of hydrophilic poly(*N*,*N*-dimethylacrylamide) and lipophilic poly(*n*-butyl acrylate), the latter was statistically copolymerized with a sulfonic acid-substituted monomer.[4a] Above the CMC that was determined to be approximately 1 µM, block copolymer **2a** formed micellar aggregates in aqueous medium. Based on the block copolymer structure it can be assumed that the sulfonic acid moieties are located in the hydrophobic core and the interface to the hydrophilic shell (Figure 1c). According to DLS measurements block copolymer **2a** formed micelles with a diameter of circa 20 nm in water (Figure S2). Electron microscopic images confirmed the DLS measurements and revealed formation of uniform, spherical structures (Figure 5a, Figure S5).

Development of the micelle-mediated Povarov reaction. Polymer **2a** was incubated at different concentrations with the DNA-aldehyde conjugate **3a** for 18 h at room temperature. The DNA tolerated concentrations of up to 0.5 mM and up to 200 equivalents of the polymer (Figure 1d and Table S4, Figure S13). Some DNA degradation by depurination (approx. 30 %) occurred only at 2 mM polymer concentration and a very high, 500fold excess of the polymer versus DNA. Having established a concentration range at which DNA can be incubated with the acid catalyst, we systematically explored a micelle-mediated variant of the Povarov reaction with the 14mer DNA-aldehyde conjugate **DNA-3a**, aniline **8a**, and olefin **10a** to the target tetrahydroquinoline conjugate **DNA-12a** (Figure 3). A first series of experiments investigated the effect of copolymer concentration on product formation. Low, sub-CMC concentrations failed to yield the target compound. To our delight, at higher copolymer concentrations of 0.5 mM and 50-100fold excess versus DNA the aldehyde **DNA-3a** was almost completely converted to the desired tetrahydroquinoline **DNA-12a** without detectable depurination. HPLC analysis showed formation of a late eluting major product with a mass matching the target **DNA-12a**, accompanied by a minor side-product eluting slightly before the main peak, which was later confirmed to be caused by oxidative heterocycle aromatization (Figure 3, Scheme S6, Table S6, Figures S16-S18).[63] DNA conjugates are routinely analysed by HPLC and mass spectrometry. More complex reactions call for comparison of exemplary "on-DNA" reactions with authentic samples for confirmation. We isolated product **DNA-12a**, and co-injected it with an authentic sample **ref-DNA-12a** into an HPLC-column to confirm the micellar Povarov reaction (Figure 3, Figure S19). Likewise, a DNA-conjugated quinoline reference molecule **ref-DNA-13a** matched the retention time of the side-product, supporting the hypothesized heterocycle aromatization as side reaction during micellar catalysis (Figure 3, Figure S19).[65] Analysis of the reaction kinetics in a range between 25-60 °C revealed a clear temperature dependency of the micellar reaction. **DNA-3a** was almost quantitatively converted to the tetrahydroquinoline **DNA-12a**, at 40 °C after 6 h, at 50 °C after 4 h, and at 60 °C after 2 h (Figure 4, Table S7, Figures S20-23).

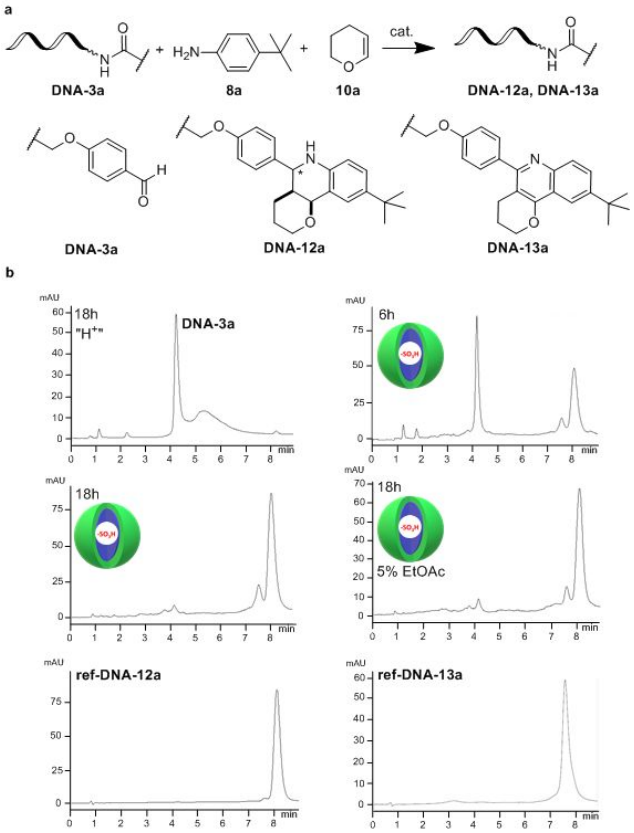

Figure 3. Comparison of homogeneous and micellar acid-mediated reactions of DNA-aldehyde **DNA-3a** to DNA-tetrahydroquinoline **DNA-12a**. (a) Scheme of the Povarov reaction yielding **DNA-12a** and an oxidized side product **DNA-13a** under the conditions of micellar catalysis. (b) HPLC traces of an attempted homogeneous Povarov reaction (upper left-hand trace), the Povarov reaction mediated by block copolymer **2a** at 25 °C after 6 and 18 h (upper right-hand and middle left-hand traces), the micelle-mediated reaction upon addition of 5 % of ethyl acetate as co-solvent (middle left-hand trace), and reference molecules **ref-DNA-12a** and **ref-DNA-13a** (lower traces).

Prolonging reaction times at elevated temperatures led to notable DNA degradation and increased formation of the oxidized side product (Figure S22). Electron microscopic images were taken of different reagent mixtures to gain insight into the micelle-mediated reaction. Addition of either **DNA-3a** or olefin **10a** to copolymer **2a** did not change the overall

morphology of the micelles (Figure 5b,c, Figure S5). However, addition of **DNA-3a**, and both aniline **8a** and olefin **10a** at concentrations optimized for Povarov reaction to block copolymer **2a** had a pronounced impact on micelle shape. Several micelles aggregated to large conglomerates, and a substantial fraction of micelles was transformed from a spherical to a rod-like shape (Figure 5d, Figure S5).

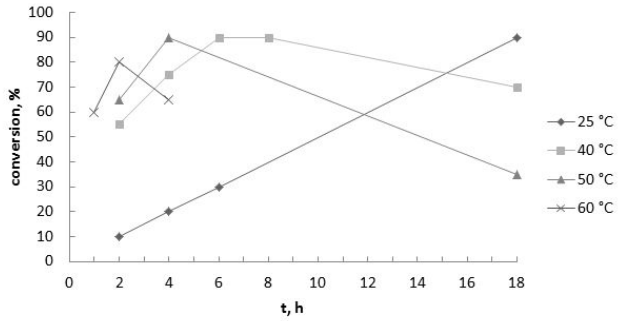

Figure 4. Kinetics of the block copolymer **2a**-mediated reaction of aldehyde **DNA-3a** to tetrahydroquinoline **DNA-12a** as depicted in Figure 3. Longer reaction times at elevated temperature decreased conversion to target **DNA-12a** due to DNA degradation. The conversions were estimated by HPLC analysis of the crude reaction mixture, missing percentage to 100 %: either **DNA-3a** or degraded DNA, and side products.

DLS measurements of this complex reactant mixture indicated swelling of the micelles from a diameter of approx. 20 nm to approx. 100 nm and supported the EM images (Figure S1). Thus, both EM images and DLS measurements are in line with our hypothesis that micelles composed of block copolymer **2a** accommodate organic reactants concentrating them in a confined nanoreactor for subsequent reaction. Water-insoluble starting materials often require co-solvents or biphasic solvent systems to obtain synthetically useful concentrations. Ethyl acetate, CH_2Cl_2, 1,2-dichlorethane, THF, and hexafluoroisopropanol, added at 5 vol % to the micellar dispersion, were compatible with the reaction, whereas addition of MeOH, DMSO, and DMF led to increased side product formation (Figure 3, Table S9, Figure S25). The robustness of a catalyst impacts its potential for application. Polymer synthesis is inherently less controllable than small organic molecule synthesis. Differences in the *dp* (degree of polymerization) may occur and lead to batch-to-batch differences in the polymer size. Fortunately, the micellar Povarov reaction could be reproduced faithfully with two batches of block copolymer **2a** that differed in the *dp* (Table S10, Figure S26). We then explored the requirements of the micellar reaction. Exclusion of any single component of the reaction precluded tetrahydroquinoline formation (Figure S27). Two additional polymers were designed to study the impact of catalyst design on the Povarov reaction: block copolymer **2b** without covalently linked sulfonic acid served as a control for catalyst immobilization and block copolymer **2c** directed the sulfonic acid into the hydrophilic corona. As expected, block copolymer **2b** failed to mediate synthesis of **DNA-12a** due to the absence of a catalytically active moiety. Addition of methane sulfonic acid or the more lipophilic *para*-toluenesulfonic acid to a dispersion of block copolymer **2b** following a well-established concept in micellar catalysis failed to yield the product (Figure S28).[34,40,41] These experiments underpinned the need for immobilization of the catalytic moiety in the micelle. Next, we investigated the placement of the catalyst moiety. Locating the catalyst group in the hydrophilic part of the amphiphile has been reported for interfacial catalyst application and was realized with block copolymer **2c**.[66] Two batches of this block copolymer with slightly differing *dp* were compared head-to-head with block copolymer **2a**. Both block copolymers **2a** and **2c** showed similar DNA-degrading activity at room temperature (Tables S4,S5, Figures S13,S14). Block copolymer **2c** mediated the reaction of **DNA-3a** with aniline **8a** and olefin **10a** with comparable, if slightly less product formation rates at room temperature and 40 °C, respectively (Figures S29, S30). However, at higher temperatures the catalytic behaviour of block copolymer **2c** differed notably from block copolymer **2a** (Figure 6, Table S11, Figures S31, S32). In contrast to catalyst **2a** which showed a clear temperature-dependent catalytic activity, block copolymer **2c** yielded reproducibly less **DNA-12a** at 50 °C than at 40 °C at any time point.

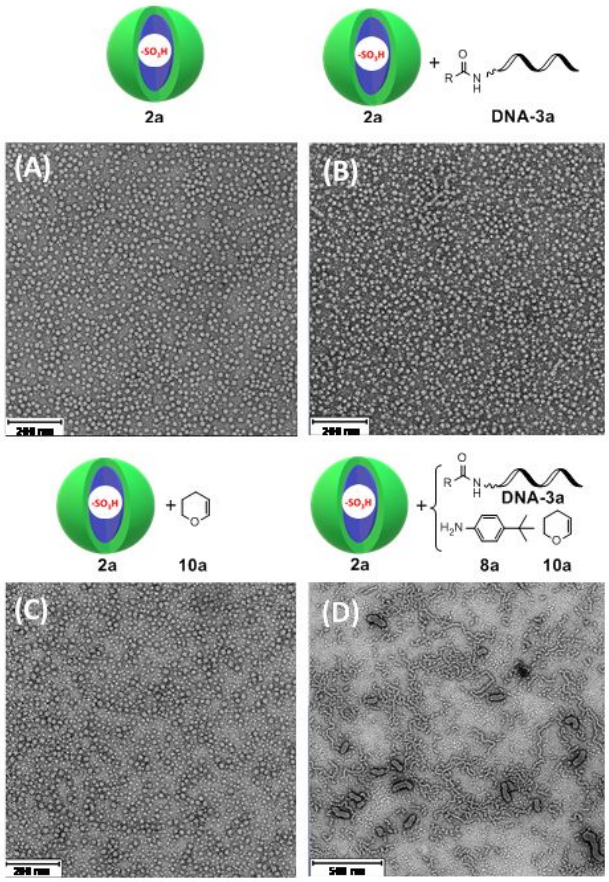

Figure 5. Structural characterization of polymer **2a** and reactant mixtures by transmission electron microscopy (TEM). (a) TEM image of polymer **2a** from an aqueous 0.25 mM polymer solution. (b) TEM image of DNA conjugate **DNA-3a** and 50 equivalents of polymer **2a**. (c) TEM image of polymer **2a** and 160 equivalents of 3,4-dihydropyran **10a**. (d) TEM image of **DNA-3a**, 50 equivalents of block copolymer **2a**, 8000 equivalents of aniline **8a**, and 8000 equivalents of 3,4-dihydropyran **10a**. Enlarged and further images are shown in Figure S5.

Strikingly, neither batch of block copolymer **2c** mediated the Povarov reaction to **DNA-12a** at 60 °C, rather we found significant DNA degradation after 4 h reaction time (one batch tested). The reason for this behaviour is probably related to the fact that polymer micelles tend to be less aggregated at higher

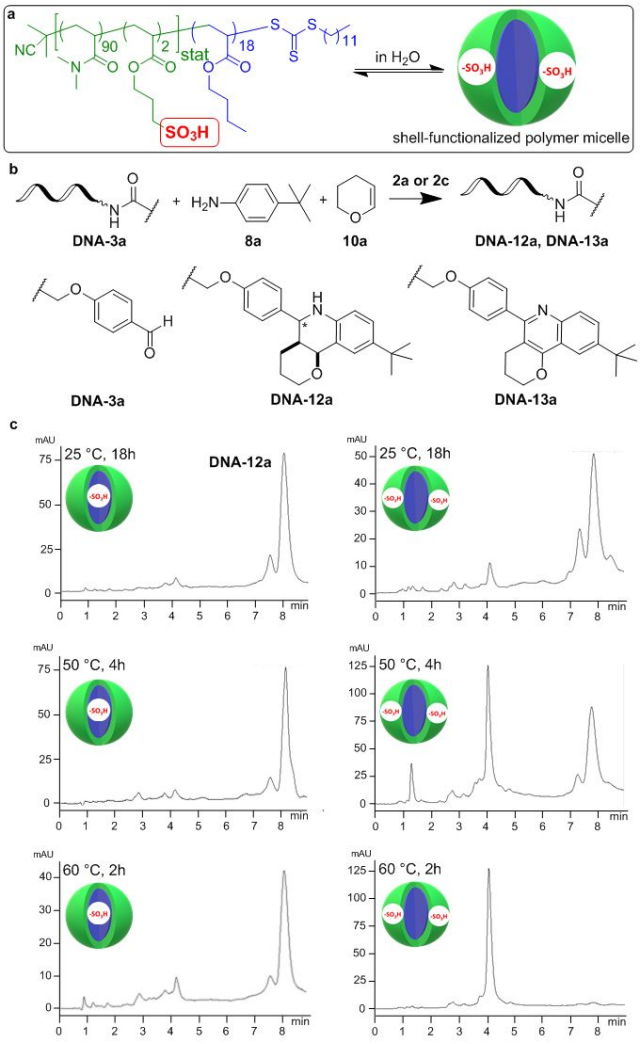

Figure 6. Comparison of the catalytic activity of block copolymers **2a** and **2c**. (a) Structure of block copolymer **2c**. (b) Reaction scheme of the micelle-mediated Povarov reaction to **DNA-12a/13a**. (c) Head-to-head comparison of reactions mediated by block copolymers **2a** and **2c**. Block copolymer **2a** is more efficacious than **2c** at elevated temperatures. In striking contrast to catalyst **2a** block copolymer **2c** even failed to mediate the Povarov reaction at 60 °C.

temperatures due to increasing dynamics of the polymer chain.[67]At temperatures of 50 °C and 60 °C, block copolymer **2c** assembled the reactants and the acid catalyst less efficiently or not at all in the manner required for product formation. Finally, a 69mer single stranded DNA was incubated with the micellar catalyst **2a** under the conditions used in the reaction to compounds **DNA-12**, precipitated, and amplified by PCR. The yield of the PCR product was comparable to a control experiment without catalyst **2a** (Figure S33).

Scope of the micelle-mediated Povarov reaction. The micellar Povarov reaction was established with a 5'-C(6)-aminolinker-conjugated aldehyde **DNA-3a** (Figure 3). This aldehyde was coupled via a longer and more hydrophilic 5'-terminal PEG(4)-aminolinker, and via a more lipophilic 5'-C(12)-aminolinker to the DNA oligomer. Neither linker had an impact on the reaction result (Table S12, Figure S34). A single-stranded 21mer DNA aldehyde conjugate gave the target DNA-tetrahydroquinoline conjugate as well (Table S13, Figure S35). The scope of the three-component reaction was then explored with a range of reagents (Figure 7a). One intriguing aspect of this reaction is the possibility to synthesize different heterocyclic cores through the choice of olefin **10** (Figure 7b). The 3,4-dihydropyran furnished a tricyclic 6-6-6 ring system **DNA-12**, while *N*-Boc-protected pyrroline **10b** and 2,3-dihydrofuran **10c** gave 6-6-5 ring systems **DNA-14** and **-15** (Figure S36, S37). Finally, ethyl vinyl ether **10d** gave the 6-6 bicycle quinoline **DNA-16** after heterocycle oxidation and elimination of the ethyloxy-substituent (Figure 7b and Figures S38, S39).[59-61] Next, we tested the aniline scope with 20 diverse substituted anilines **8a-t** that were reacted with **DNA-3a** and either 3,4-dihydropyran **10a** or *N*-Boc-protected pyrroline **10b** (23 anilines, **8a-z**). Ten of these reactions are shown in Figure 7c, the remainder can be found in the extended Tables S14 and S15, and in Figures S40-S44. No product was formed with 2,6-disubstituted aniline **8b**, which would only allow for addition of an electron-rich olefin **10** to an imine **9** (Figure 2b). Aniline **8c** gave the target product **DNA-12c** with a 45 % conversion and **DNA-14c** with more than 90 % conversion as determined by HPLC analysis of the crude reaction mixtures. *Ortho*-substituted anilines such as **8d/m**, rather poorly represented in literature on the Povarov reaction, gave the products **DNA-12d/m** at conversions of 90 % and 55 %, respectively. However, the analogous tetrahydroquinolines **DNA-14d/m** were obtained both with >90 % conversions. *Meta*-substituted anilines **8e-g** which can furnish two regioisomers were tested as well. These starting materials gave high conversions to both target heterocycles **DNA-12** and **DNA-14** in most cases. A notable exception was the highly polar sulfonamide **8s** that reacted poorly to **DNA-12s** but afforded **DNA-14s** with an excellent conversion. Finally, *para*-substituted anilines **8h-j** gave mixed results in the reac tion to tetrahydroquinoline **DNA-12**. For instance, *para*-alkyl, and -phenyl substituents were well tolerated, also 4-aminobenzoic acid **8i** gave rise to the target conjugate **DNA-12i** at more than 90 % conversion, whereas *para*-bromoaniline **8j** gave **DNA-12j** only with a conversion of 35 %. The micelle-mediated Povarov reaction with *N*-Boc-pyrroline **10b** and all *para*-substituted anilines allowed for synthesis of tetrahydroquinolines **DNA-14** at conversions exceeding 90 %. Overall, the micelle-mediated Povarov reaction to DNA-tetrahydroquinolines **DNA-14** was higher yielding than the micelle-mediated reaction to DNA-tetrahydroquinolines **DNA-12**. Out of 20 anilines that were tested, 7 anilines afforded the tetrahydroquinoline conjugates **DNA-12** at conversions of 90 %, whereas 21 out of 23 anilines gave rise to tetrahydroquinolines **DNA-14** with full consumption of the aldehyde. The different lipophilicity of olefins **10a** and **10b** could plausibly explain the differential reactivity of these two olefins in a micellar environment. Higher effective concentrations of the more lipophilic *N*-Boc-protected

pyrroline **10b** might be reached in the micelle core. The significant difference in reactivity between olefins **10a** and **10b** in the micelle-mediated Povarov reaction prompted us to compare the kinetics of both reactions. Indeed, the reaction to **DNA-14a** proceeded much faster than to **DNA-12a** (Table S16, Figure S45). Aldehyde

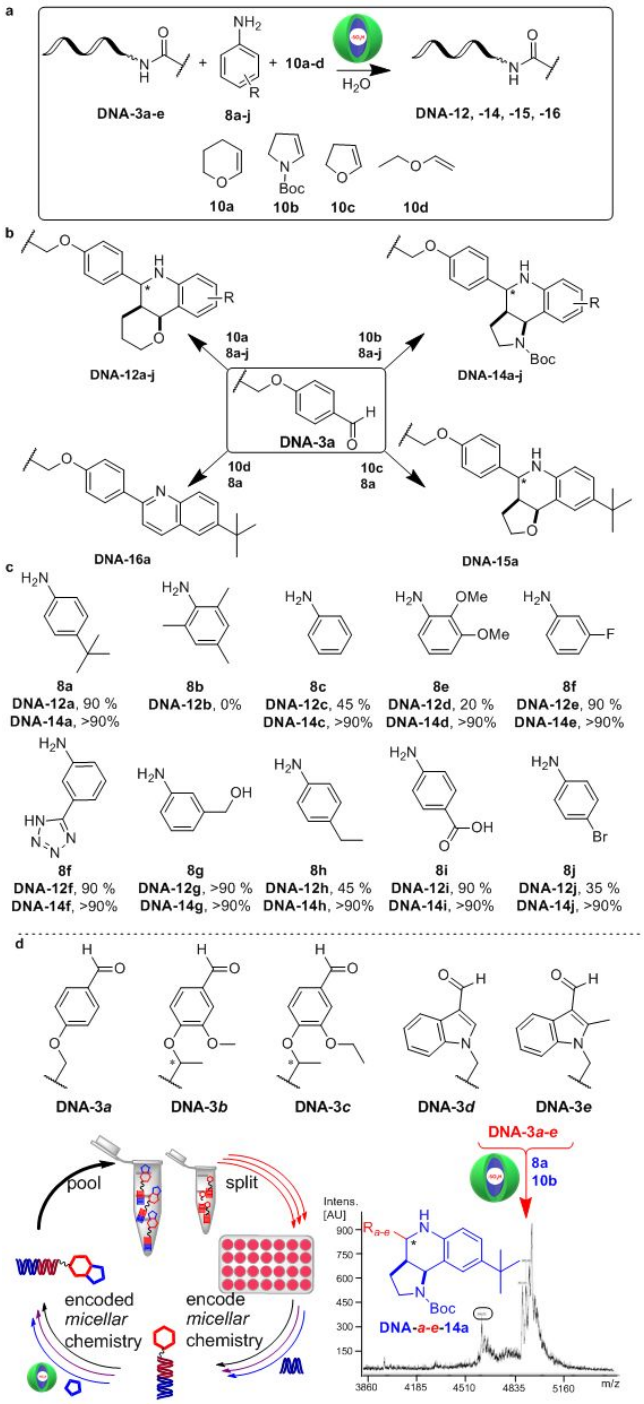

Figure 7. Scope of the micellar Povarov reaction to DNA-tetrahydroquinoline conjugates. (a) Reaction scheme. (b) Different core heterocycles are accessible depending on the olefin **10**. (c) A broad scope of anilines **8** furnished the target heterocycles. The conversion rates were estimated by HPLC. (d) Simulating an encoded library synthesis scenario, a pool of DNA-aldehyde conjugates was converted to tetrahydroquinolines by micellar catalysis with high yields. Only *ortho*-substituted aldehyde **DNA-3e** gave partial conversion to the product (boxed peak in the MALDI spectrum).

DNA-3a was almost completely converted to tetrahydroquinoline **DNA-14a** after 2 h at room temperature, and after 1 h at 40 °C. Of note, the reactions to **DNA-14** did not produce oxidized side products. The micellar reaction system shall be applied in combinatorial encoded library synthesis. In this application it must provide target products with high yields from complex mixtures of starting materials. We simulated a library synthesis with eight carboxaldehydes **DNA-3a-h** representing different substitution patterns (*ortho*, *meta*, *para*, electron-rich and -poor, heterocyclic) that were mixed to two partly redundant pools of five compounds each. Both pools were reacted with aniline **8a** and pyrroline **10b**. Mass spectrometric analysis of the product mixtures showed full conversion of five DNA-conjugated aldehydes. The *ortho*-substituted aldehyde **3e** gave ca. 30% conversion and in case of two aldehydes **3g-h** we found reduced conversion to products due to partial aldehyde oxidation in aqueous solution (Figures 7d, S46-S54).

Expanding the scope of micellar acid catalysis: Gröbke-Blackburn-Bienaymé reaction and *t*Boc protective group removal. Similar to the Povarov reaction, the Gröbke-Blackburn-Bienaymé (GBB) reaction is catalyzed either by Brønsted or Lewis acids.[68,69] A micellar variant of the GBB reaction gave access to DNA-coupled 3-aminoimidazo[1,2-*a*]pyridines **DNA-19** from aldehyde **DNA-3a**, 2-aminopyridines **17**, and isocyanides **18** (Figure 8a). First efforts towards DNA-tagged 3-aminoimidazo[1,2-*a*]pyridines **DNA-19** involved reaction conditions that were optimized for the synthesis of DNA-tetrahydroquinoline conjugates. However, under these conditions only 30 % of **DNA-3a** was converted to the product **DNA-19a** (Table S17, Figure S55). Prolonged reaction times increased formation of **DNA-19a** to 55 % after 36 h, and 75 % after 54 h. At 40 °C the starting material was almost quantitatively converted to the target heterocycle (Table S17, Figure S56). Ethanol, acetonitrile, and ethyl acetate added at 5 vol% to the reaction mixture did not have any impact on the formation of **DNA-19a** (Table S18, Figure S57). The micellar GBB reaction was sensitive to positioning of the catalyst within the micelle, as the block copolymer **2c**-mediated reaction gave the target 3-aminoimidazo[1,2-*a*]pyridine conjugate (Figure S58) with only 40 % conversion. A small scope of the GBB reaction was explored with different 2-aminopyridines **17** and isocyanides **18** (Table S19, Figure S59). **DNA-3a** was reacted with 2-aminopyridine **17b** and cyclohexyl isocyanide **18a** to target **DNA-19b** with 60 % conversion, the 5-ethyl substituted 2-aminopyridine **17c** gave **DNA-19c** with a similar 70 % conversion, while the functionalized 5-(*N*-*t*Boc-aminomethyl)-2-aminopyridine gave only 30 % of **DNA-19d**. **DNA-3a** was then reacted with either 2-amino-5-methylpyridine **17a** and *tert*-butyl isocyanide **18b**, or 2-amino-5-methylpyridine **17a** and benzyl isocyanide **18c**. The two isocyanides gave DNA-3-aminoimidazo[1,2-*a*]pyridine conjugates **DNA-19e/f** with 50 % and 70 % conversions, respectively (Table S19, Figure S59). Last, benzyl isocyanide **18c** was successfully reacted with **DNA-3a** and 2-aminopyridine **17b** or 2-amino-5-

ethylpyridine **17c**, furnishing **DNA-19g/h** with 90 % and 50 % conversion, respectively. Thus, block copolymer **2a** facilitated the synthesis of diverse DNA-tagged 3-aminoimidazo[1,2-*a*]pyridines by Gröbke-Blackburn-Bienaymé reaction.

In light of the ready availability of *t*Boc-protected building blocks, removal of this protective group is an important operation in encoded library synthesis. In the literature, this protective group is removed by heating encoded libraries at pH 9.4 for prolonged time.[14] A micellar dispersion of block copolymer **2a** could remove the *t*Boc protective group from the *t*Boc-glycine conjugate **DNA-20**. However, the reaction was accompanied by severe DNA damage. A systematic investigation in micellar deprotection of the *t*Boc-glycine conjugate **DNA-20** could not arrive at conditions that separated removal of the protective group from depurination in a satisfactory manner (Figure 8b, Table S20, Figures S61-S64). It has been reported that Mg(II) salts can decrease the rate of depurination in acidic buffers.[70] Addition of increasing amounts of $MgCl_2$ to the micellar dispersion of block copolymer **2a** and *t*Boc-glycine conjugate **DNA-20** indeed supressed depurination to an acceptable level of ca. 10 % while yielding 40 % of the deprotected glycine conjugate (Table S21, Figures S65-S67). These results were reproduced with the *t*Boc-protected tetrahydroquinoline conjugate **DNA-14a** (Figure S69). Removal of the *t*Boc group from **DNA-14a** was followed by amide coupling as would be done in an obvious library synthesis scenario leading to compounds that are closely related to the chemotype of the CRISPR-Cas9 inhibitor **5** (Figure S70).

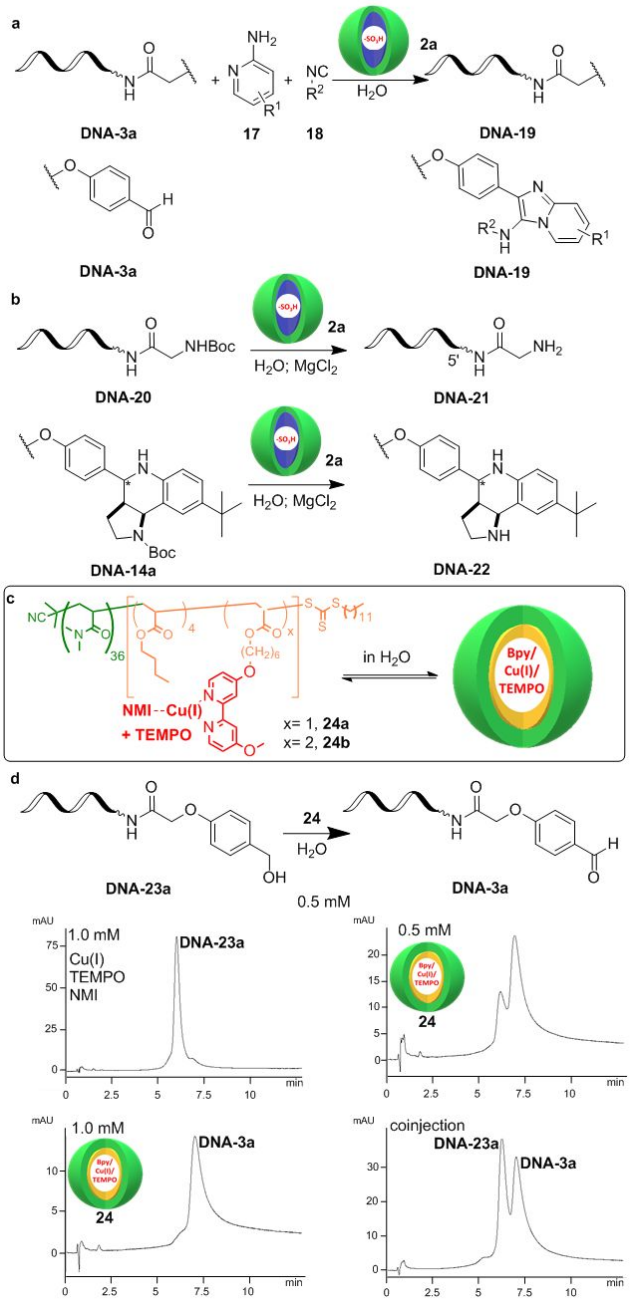

Figure 8. Expanding the scope of micellar catalysis. (a) Block copolymer **2a**-mediated Gröbke-Blackburn-Bienaymé reaction to 3-aminoimidazo[1,2-*a*]pyridines **DNA-19**. (b) Removal of a *t*Boc protective group required addition of $MgCl_2$ to protect DNA against depurination. (c) A block copolymer-based micellar system for the Cu(I)/bipyridine/TEMPO catalyst. (d) Comparison of the homogeneous Cu(I)/bipyridine/TEMPO system with the micelle-based variant for the oxidation of a DNA-tagged benzyl alcohol to the corresponding benzaldehyde.

Oxidation of DNA-coupled benzyl alcohol by a micellar Cu/bipyridine/TEMPO catalyst system. Functional group transformations are rarely described in encoded chemistry.[11,14,71,72] The selective transformation of alcohols to the corresponding aldehydes and ketones represents a key transformation in modern organic chemistry that has yet to be demonstrated on DNA-coupled substrates. Oxidation reactions may damage DNA by 8-oxoA or 8-oxoG formation. These DNA lesions cause increased depurination rates and misreading of the code by DNA polymerases.[73] Alcohols are conveniently oxidized by a catalyst system consisting of copper ions, the stable radical TEMPO and atmospheric oxygen.[74] We attempted to oxidize a DNA-coupled benzyl alcohol under the published homogeneous reaction conditions but obtained only starting materials under conditions that did not damage DNA. Recently, we have developed a micellar catalytic approach for the Cu(I)/TEMPO based aerobic oxidation of alcohols in aqueous medium.[42] Two amphiphilic block copolymers **24a**,**b** functionalized with either one or two bipyridine ligands in the lipophilic portion were designed to immobilize copper(I) in the micelle (Figures 8c, S71-S75). In analogy to a homogeneous catalyst system,[74] the oxidation of DNA-benzyl alcohol **23** was investigated in the presence of micelles formed by block copolymers **24a**,**b**, CuBr, and TEMPO as the N-oxyl source. At lower catalyst loadings (5-10 equivalents versus DNA) and in contrast to the homogeneous conditions (Figure S77) the micellar system showed a successful time- and micelle concentration-dependent oxidation of both DNA-coupled benzyl alcohols and an aliphatic alcohol to the corresponding aldehydes with no detectable formation of 8-oxopurines or over-oxidation to the carboxylic (Figure 8d, Table S24, Figures S78-S82). Indeed, we were able to synthesize a DNA tetrahydroquinoline conjugate from a DNA-conjugated alcohol by two consecutive micelle-mediated reactions: the product of micellar alcohol oxidation was isolated and reacted to the heterocycle by micellar Povarov reaction (Figure S79).

CONCLUSION

Reactions mediated by low pH are challenging to translate to a DNA-tagged format due to the risk of DNA degradation by depurination. In this study, we explored sulfonic acid catalysts based on amphiphilic block copolymers (**2a**, Figure 1c) for the reaction of DNA-coupled starting materials. The block copolymers aggregated in water at low, micromolar concentration and localized a sulfonic acid in nanometer-sized oil-in-water micelles. These acidic nanoreactors facilitated three reactions to DNA-tagged target molecules: Povarov and GBB reactions to diverse substituted heterocycles and the removal of a *t*Boc protective group from amines. Both heterocycle-forming three-component reactions showed a broad scope of simple, readily available starting materials and proceeded without detectable DNA degradation. A micelle-mediated high-yielding synthesis of pools of DNA-conjugated tetrahydroquinolines suggested the utility of our catalytic system for encoded library synthesis. The block copolymer catalyst design was subsequently extended to a Cu(I)/bipyridine/TEMPO reagent. It allowed for the oxidation of both a DNA-coupled benzylic and aliphatic alcohol to the corresponding aldehyde without detectable oxidative DNA damage such as 8-oxoG formation. The two micellar systems demonstrated in this study hint at a potentially broad scope of designed micellar catalysts for DNA-encoded compound synthesis. Future research directions in this field could comprise investigations in the compatibility of block copolymer-based catalysis with different biomacromolecules; further micelle-based catalysts for synthesis of DNA-tagged target molecules; and the use of micellar catalysis in different DEL formats - DNA-recorded as well as DNA-templated chemistry - for screening library synthesis.

EXPERIMENTAL

Block copolymer 2a-mediated Povarov reaction. To an aqueous solution of DNA-aldehyde conjugate **DNA-3** (500 pmol) were added an aniline **8** (4 µmol, 8000 eq.), dissolved in 2.5 µL of ethyl acetate, an olefine **10** (4 µmol, 8000 eq.), and block copolymer **2a** (25 nmol, 50 eq.) dissolved in 16 µL of distilled water. The reaction mixture was filled with water to a volume of 50 µL giving a final concentration of 0.5 mM of block copolymer **2a**. The reaction mixture was shaken at 25 °C for 18 h. After that, 70 µL of water was added and the reaction mixture was thoroughly extracted with ethyl acetate (at least 6 x 400 µL). The aqueous solution was evaporated, the residue was re-dissolved in 40 µL of water for analysis by RP-HPLC and by MALDI-MS.

Block copolymer 2a-mediated Gröbke-Blackburn-Bienaymé reaction. The protocol for the GBB reaction included the following changes compared to the Povarov reaction: A 2-aminopyridine **17** (4 µmol, 8000 eq.) was dissolved in 2.5 µL of ethanol, the isocyanide **18** (4 µmol, 8000 eq.) was directly pipetted into the reaction mixture, and the reaction mixtures were shaken at 40 °C for 54 h. The crude reactions were extracted seven times with each 500 µL of ethyl acetate prior solvent evaporation and compound analysis.

Block copolymer 2a-mediated cleavage of *t*Boc protective group from amines. To an aqueous solution of DNA-*t*Boc protected conjugate **DNA-21** or **DNA-14a** (500 pmol) were added $MgCl_2$ (0.5 µmol, 1000 eq.), dissolved in 1 µL of water and copolymer **2a** (12.5 nmol, 25 eq.) dissolved in 8 µL of water. The reaction mixture was filled with distilled water to a volume of 50 µL giving a final concentration of 0.25 mM of copolymer **2a**. The reaction mixture was shaken at 50 °C for 4 h. After that, 70 µL of water was added and the reaction mixture was extracted with ethyl acetate. The aqueous solution was evaporated, the residue was re-dissolved in 40 µL of water, and the DNA-*t*Boc deprotected conjugates **DNA-20** and **DNA-22a** were analyzed by RP-HPLC and MALDI-MS.

Block copolymer 24-mediated oxidation of DNA-alcohol to DNA-aldehyde conjugates. To a solution of block copolymer **24b** (500 nmol) in 190 µL of dry acetonitrile was added CuBr (1 µmol, 2.0 eq.) dissolved in 10 µL of dry acetonitrile. The reaction mixture was stirred at room temperature under argon atmosphere for 30 minutes. The solvent was evaporated *in vacuo*, and the micelle catalyst **24** was dissolved in 500 µL of distilled water. To a solution of micelle catalyst **24** (40 nmol, 10 eq.) in distilled water were added *N*-methylimidazole (80 nmol, 20 eq.), dissolved in 1 µL of distilled water, and TEMPO (40 nmol, 10 eq.), dissolved in 1 µL of distilled water. The reagent mixture was incubated at room temperature for 5 minutes and DNA-alcohol conjugate **DNA-23** (4 nmol) was added. The reaction mixture was filled with distilled water to a volume of 40 µL giving a final concentration of 1.0 mM of catalyst **24**. The reaction mixture was shaken at room temperature for 22 hours. After that, 70 µL of water was added and the reaction mixture was thoroughly extracted with ethyl acetate. The aqueous solution was evaporated, and the residue was re-dissolved in 100 µL of water for analysis by RP-HPLC and by MALDI-MS.

ASSOCIATED CONTENT

Supporting Information contains protocols for the synthesis of DNA conjugates, HPLC traces and MALDI MS spectra of DNA conjugates; synthesis and characterization of reference molecules and amphiphilic block copolymers; and detailed synthesis protocols for the micelle-mediated reactions. This material is available free of charge via the Internet at http://pubs.acs.org.

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ABBREVIATIONS

CMC, critical micellar concentration; DEL, DNA-encoded library; *dp*, degree of polymerization; GBB, Gröbke-Blackburn-Bienaymé; TEM, transmission electron microscopy.

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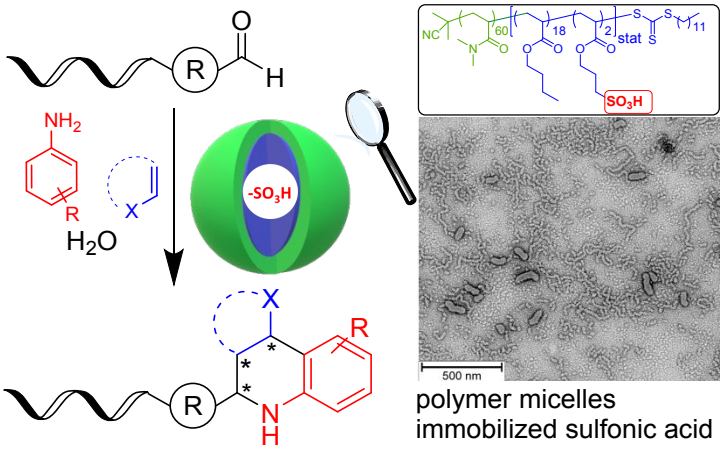
SYNOPSIS TOC



polymer micelles
immobilized sulfonic acid