A biomolecule-compatible visible-light-induced azide reduction from a DNA-encoded reaction-discovery system

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Using a system that accelerates the serendipitous discovery of new reactions by evaluating hundreds of DNA-encoded substrate combinations in a single experiment, we explored a broad range of reaction conditions for new bond-forming reactions. We discovered reactivity that led to a biomolecule-compatible, Ru(II)-catalysed azide-reduction reaction induced by visible light. In contrast to current azide-reduction methods, this reaction is highly chemoselective and is compatible with alcohols, phenols, acids, alkenes, alykes, aldehydes, alkyl halides, alkyl mesylates and disulfides. The remarkable functional group compatibility and mild conditions of the reaction enabled the azide reduction of nucleic acid and oligosaccharide substrates, with no detectable occurrence of side reactions. The reaction was also performed in the presence of a protein enzyme without the loss of enzymatic activity, in contrast to two commonly used azide-reduction methods. The visible-light dependence of this reaction provides a means of photocuncaging functional groups, such as amines and carboxylates, on biological macromolecules without using ultraviolet irradiation.

Results and discussion

Choice of substrates and reaction conditions. The second-generation DNA-encoded reaction-discovery system requires a pool of $n \times m$ DNA-linked substrate pairs assembled from two separate subpools of $n$ and $m$ substrates (Fig. 1a). We selected 30 substrates (two groups of 15) that comprised 28 distinct pairwise substrate combinations. Each reaction-discovery experiment required only 0.5 pmol of the substrate pool, so this quantity of material was sufficient to assess the reactivity of these azide-reduction reactions that involve metal hydrides or catalytic hydrogenation cannot be used on substrates with acidic groups, such as alcohols and acids, or with unsaturated groups, such as alkenes, alykes and carbonyls. Similarly, the Staudinger reduction is not compatible with disulfides and alkyl halides and can require elevated temperatures and lengthy reaction times. The limitations of the existing azide-reduction methods may explain the near absence of examples of azide reduction in biological macromolecules, despite the common use of both azides and amines as chemical handles in proteins, nucleic acids and oligosaccharides. In addition to being highly tolerant of a variety of acidic and basic functional groups, a reaction compatible with biological macromolecules must also operate efficiently in neutral aqueous environments. The azide reduction discovered and developed in this work exhibits these key features of a reaction compatible with biological macromolecules.
substrate combinations under \( \approx 2,000 \) different reaction conditions, which collectively represent the evaluation of \( \approx 450,000 \) potential reactions. See the Supplementary Information for full details of the procedures used to assemble and characterize the substrate pool.

We performed control experiments to validate the system using the known Cu(I)-catalysed cycloaddition between alkynes and azides\(^{19}\). We confirmed the ability of the system to detect alkyne–azide coupling under these conditions in the DNA-encoded library format and verified the formation of the expected triazole products using DNA-linked substrates (Fig. 1b,c).

Next we determined regions of reaction-condition space compatible with the reaction-discovery system. As the second-generation system does not rely on DNA hybridization to organize substrates into pairwise combinations, compatible reaction conditions include any that do not degrade nucleic acids rapidly. Reaction conditions that led to the chemical modification or partial degradation of DNA were not necessarily excluded, given the high sensitivity of PCR amplification and the tolerance of PCR to some types of chemical modifications; indeed, conditions observed to degrade DNA were shown to be compatible with our earlier reaction-discovery system\(^1,3\).

Conditions that preserved the ability of the remaining DNA to be detected readily after 25 cycles of PCR were considered potentially compatible reaction conditions. These conditions included the presence of transition metals (Fe(II), Fe(III), Co(II), Ni(II), Ni(III), Cu(II), Cu(II), Ru(II), Ru(II), Ru(III), Rh(III), Rh(II), Pd(0), Pd(II), La(III) and 10 mM CuCl\(_{9/1}\)CH\(_3\)CN/H\(_2\)O, 25 °C, 10 minutes. The green/red ratio of each spot on the DNA microarray (left) is plotted on the right. Error bars represent the standard deviation of signals from three microarray replicates. c. The reaction products of DNA-linked alkynes and non-DNA-linked azides are consistent with the anticipated triazole products. Conversion efficiencies, estimated by LC-MS analysis, are shown in parentheses.

Figure 1 | DNA-encoded reaction-discovery system and validation experiments. a. Selection and analysis method for the detection of bond-forming reactions between DNA-linked small-molecule substrates. b. Detection of the known Cu(I)-catalysed cycloaddition\(^{19}\) between alkynes and azides. Conditions: 10 mM CuCl in 9/1 CH\(_3\)CN/H\(_2\)O, 25 °C, 10 minutes. The green/red ratio of each spot on the DNA microarray (left) is plotted on the right. Error bars represent the standard deviation of signals from three microarray replicates. c. The reaction products of DNA-linked alkynes and non-DNA-linked azides are consistent with the anticipated triazole products. Conversion efficiencies, estimated by LC-MS analysis, are shown in parentheses.
esters and electron-rich \( \text{indole} \), \( \text{imidazole} \), \( \text{benzyl alcohol} \) and \( \text{imidazole} \) (green circles in Fig. 2c).

When the corresponding reactions were attempted in \( \text{alkene} \), \( \text{malonic ester} \) and \( \text{ethanol} \) in \( \text{aqueous} \) and \( \text{organic solvents} \), bisindole formation was observed (Fig. 2b).

In each case, the formation of a coupling product was observed (Fig. 2). For example, when reacting \( \text{aryl azide} \) and \( \text{nitrile} \), the bisindole adduct was observed (Fig. 2b).

2.4 Azide-coupling and reduction reactions induced by visible light.

We first studied the reaction between \( \text{DNA-linked aryl azides} \) and \( \text{azides} \). Under conditions similar to those used in the \( \text{DNA-encoded} \) system (5 mM \( \text{Ru(bpy)}_3\text{Cl}_2 \) in \( \text{200 \mu l} \) of \( \text{water} \)) and \( \text{acetonitrile (1/9)} \) with \( \text{30 mM Na}_2\text{CO}_3 \) placed 20 cm from a 26 W CFL bulb), DNA-linked aryl azide 21 reacted with acetonitrile to generate a product consistent, by liquid chromatography–mass spectroscopy (LC-MS) analysis, with the imidate adduct 22 (Fig. 2d).

No significant product formation was observed in the absence of the ruthenium complex or in the absence of a CFL light source.

As bond formation between \( \text{aryl azides} \) and \( \text{nitriles} \) induced by visible-light catalysis was unprecedented, we developed a mechanistic hypothesis to explain the above observations (Fig. 3a). Ground-state \( \text{Ru(bpy)}_3\text{Cl}_2 \) is known to absorb visible light (\( \lambda_{\text{max}} = 452 \text{ nm} \)) to generate the excited triplet \( \text{Ru(bpy)}_3^* \) (Fig. 3a)

When the excited electron donor is present, 24 can be quenched reductively to give 25 (25,30,31).

We speculated that 25 may reduce azide 26 through a one-electron transfer to generate the azide radical anion 27. After extrusion by nitrogen gas and protonation, the resulting aminyl radical 28 may undergo a radical addition to give the nitrile 24,35.

With this mechanistic hypothesis in mind, we studied these reactions further in a non-DNA-linked format on a 0.1 mmol scale (Table 1). Although, for reasons unknown, the conditions that generated the coupling product of the DNA-linked azide and nitrile did not lead to significant coupling product formation with azide 4 in the flask, the addition of the commonly used sacrificial electron donor disopropylethylamine to the reaction conditions resulted in moderate yields (~60%) of the azide-reduction product 31 after 96 hours (Table 1, entry 1). The amine can form through formal hydrogen abstraction by the proposed aminyl radical intermediate (Fig. 3a)33,34, although future studies are needed to probe these and other mechanistic possibilities.

We speculated that this reaction may offer significant advantages over existing azide-reduction methods and sought to further develop the transformation.

Optimization of an azide-reduction reaction induced by visible light.

Although the initial azide-reduction reaction proceeded slowly and in moderate yields, the addition of formic acid or the Hantzsch
ester (diethyl 1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate) as a hydrogen donor to supplement or replace tertiary amines as hydrogen donors decreased the reaction time to 12 hours and increased yields to \( \approx 90\% \) (Table 1, entries 2 and 3). Without the ruthenium complex or the CFL light source, no azide reduction was observed under these conditions (Table 1, entries 4 and 5). Further optimization revealed that the tertiary ammonium salt provided a neutral reaction media and, when combined with the Hantzsch ester, improved the reaction efficiency to 92–96\% yields in 2 hours in a variety of solvents (dimethylformamide (DMF), acetonitrile or dichloromethane; Table 1, entries 6–8). The use of acetate rather than formate as

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**Figure 2 | Selection results from four reaction conditions.** **a–d.** The scatter plots show green/red fluorescence ratios of substrate combinations in the DNA-encoded library. The schemes show the outcomes of reactions in a flask. **a.** In the presence of CAN, coupling was observed between malonic esters and electron-rich \( \pi \)-systems. Conditions: 10 mM CAN in MeOH, 25 °C, 2 h. **b.** CuCl\(_2\)-induced coupling of indoles and pyruvates, consistent with coupling of the Friedel–Crafts type. Conditions: 1 mM CuCl\(_2\) in 9/1 CH\(_3\)CN/H\(_2\)O, 25 °C, 16 h. Et = ethyl, cat. = catalytic. **c.** Phenyliodine bis(trifluoroacetate) (PIFA)-generated positives consistent with imidazole–indole and imidazole–benzyl alcohol coupling, but in a non-DNA-linked format only substrate oxidation was observed. Conditions: 10 mM PIFA in CF\(_3\)CH\(_2\)OH, 25 °C, 2 h. **d.** Ru(bpy)\(_3\)Cl\(_2\) under visible-light irradiation resulted in several positives including azide–nitrile coupling. Conditions: 10 mM Ru(bpy)\(_3\)Cl\(_2\) in 100 mM aqueous sodium carbonate, pH 9.5, 20 cm from a 26 W CFL bulb, 25 °C, 1 h. For (a), (b) and (c), isolated yields are shown in parentheses; for (d), the conversion efficiency by LC-MS analysis is shown in parentheses. Error bars represent the standard deviation of signals from three microarray replicates.
Compatibility of the azide-reduction reaction with DNA, oligosaccharide and protein. Previous reports demonstrated the use of Ru(bpy)$_3$Cl$_2$ to mediate reactions on protein substrates in crude cell extracts$^{38}$, and related complexes were used as cell-permeable agents to image DNA in living eukaryotic and prokaryotic cells$^{39}$. These observations, coupled with the excellent specificity of the azide-reduction reaction, encouraged us to develop an aqueous version to explore the possibility that this azide reduction might be applicable to biological molecules.

We chose to test the azide-reduction reaction with DNA as a model substrate to study its compatibility and specificity. We found that the reaction proceeded efficiently with DNA, with yields ranging from 92% to 99% (Table 1, entry 9). As little as 1 mol% Ru(bpy)$_3$Cl$_2$ was sufficient to afford the reduction product in 95% yield in 2 hours (Table 1, entry 10).

Next we evaluated the functional group compatibility of the optimized azide reduction under the conditions shown in Table 1, entry 8 (5 mol% Ru(bpy)$_3$Cl$_2$, 10 equiv. i-Pr$_2$NEt/DMF, 1.5 equiv. Hantzsch ester in CH$_3$CN, hv, 25 °C). We observed that a variety of substrates that contained protic functional groups, including free indoles, acids and alcohols, were compatible with the reaction (Fig. 3b, substrates 32–35). In addition, functional groups sensitive to hydrogenation, including amines, alkynes and aryl halides, were not affected by these reaction conditions (Fig. 3b, substrates 36–39). Similarly, functional groups sensitive to nucleophiles, including alkyl halides, alkyl mesylates and aldehydes, also emerged intact under the reaction conditions (Fig. 3b, substrates 40–42). An alkyl azide was also reduced in 72% yield in 24 hours under modified conditions (Fig. 3b, substrate 43).

Encouraged by the remarkable chemoselectivity of this reaction, we sought to develop an aqueous version to explore the possibility that this azide reduction might be applicable to biological molecules. Under the optimized conditions above (Table 1, entry 8), but in CH$_3$CN/H$_2$O (1/1), the efficiency of the reaction was reduced drastically (Table 1, entry 11). Efficient product formation was restored with the removal of formic acid, which presumably increased the availability of an unprotonated tertiary amine as a competent electron donor (Table 1, entry 12). When the water-soluble reducing agent nicotinamide adenine dinucleotide (NADH) was used instead of the Hantzsch ester there was only a modest reduction in reaction efficiency (Table 1, entry 13). Although effective, we speculated that the basic conditions above may compromise the reaction’s compatibility with biological macromolecules. We discovered, however, that sodium ascorbate can act as both an electron donor and a hydrogen donor$^{37}$ without significantly lowering yields or lengthening reaction times (Table 1, entry 14). In the presence of sodium ascorbate the reduction proceeded efficiently in buffers of different acidities (pH 4.0, pH 7.4 and pH 9.5; see Supplementary Fig. S3.1).

### Table 1 | Optimization of the azide-reduction reaction.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reaction conditions</th>
<th>[Ru]$_{10}$ (mol%)</th>
<th>Solvent</th>
<th>Time (h)</th>
<th>Yield (%)</th>
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<tr>
<td>1</td>
<td>2 equiv. i-Pr$_2$NEt, hv</td>
<td>5</td>
<td>CH$_3$CN</td>
<td>96</td>
<td>60</td>
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<tr>
<td>2</td>
<td>10 equiv. i-Pr$_2$NEt/HCOOH, hv</td>
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<td>CH$_3$CN</td>
<td>12</td>
<td>90</td>
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<tr>
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<td>2 equiv. i-Pr$_2$NEt, 1.5 equiv. Hantzsch ester, hv</td>
<td>5</td>
<td>CH$_3$CN</td>
<td>12</td>
<td>89</td>
</tr>
<tr>
<td>4</td>
<td>10 equiv. i-Pr$_2$NEt/HCOOH, no light</td>
<td>5</td>
<td>CH$_3$CN</td>
<td>48</td>
<td>&lt;5</td>
</tr>
<tr>
<td>5</td>
<td>10 equiv. i-Pr$_2$NEt/HCHO, hv</td>
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<td>CH$_3$CN</td>
<td>1,680</td>
<td>&lt;5</td>
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<tr>
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<td>92</td>
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<td>DMF</td>
<td>2</td>
<td>92</td>
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<td>CH$_3$Cl$_2$</td>
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<td>90</td>
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*Reported yields above 5% are isolated yields.

$^{a}$ Denotes light from a 26 W CFL bulb at 20 cm. Reactions were performed at 25 °C.
functional group tolerance and mild reaction conditions of the azide reduction, raised the possibility that this reaction could be performed on biomolecules rich in functional groups without inducing any side reactions. We tested this possibility by performing the reduction on, or in the presence of, nucleic acids, a protein and an oligosaccharide and characterizing these macromolecules after each reaction.

We prepared DNA oligonucleotide 44, which contains both an aryl azide and a disulfide, and treated 0.5 mM of this substrate with 1 mM Ru(bpy)2Cl2 and 50 mM of sodium ascorbate in aqueous 200 mM tris(hydroxymethyl)aminomethane (Tris), pH 7.4, positioned 20 cm from a 26 W CFL bulb (standard aqueous reduction conditions). Azide reduction proceeded cleanly in 10 minutes without disulfide-bond reduction (equation (1) in Fig. 4a).

Figure 4 | Compatibility of the azide-reduction reaction with biological molecules. a, Azide- and disulfide-linked DNA oligonucleotides (0.5 μM each) were reduced to the corresponding amines under the aqueous reaction conditions shown without inducing side reactions, such as disulfide reduction. In contrast, TCEP reduced both the azide and disulfide groups. Reaction conversions were calculated by LC-MS analysis. Conversion efficiencies are shown in parentheses. b, A functional group-rich azide analogue of the disaccharide naringin (100 μM) was reduced cleanly to give the corresponding amine. Conversion was calculated by HPLC analysis. c, Ru(bpy)2Cl2-mediated reduction, induced by visible light, of the naringin azide analogue (100 μM) in the presence of the protein enzyme RNase A (10 μM in the reaction, 0.83 μM when assayed) did not alter the enzyme’s covalent structure or activity. In contrast, thiol- or phosphine-induced azide reduction both resulted in loss of RNase A activity. Conditions are as follows. Control: 200 mM Tris, pH 7.4, 20 cm from 26 W CFL, 6 h, 25 °C. [Ru] + ascorbate: 1 mM [Ru], 50 mM ascorbate, 200 mM Tris, pH 7.4, 20 cm from 26 W CFL, 6 h, 25 °C. TCEP: 100 mM TCEP in 1 M phosphate, pH 8.0, 6 h, 25 °C. DTT: 500 mM DTT in 1 M phosphate, pH 8.0, 6 h, 25 °C. Error bars represent the standard deviation of results from three independent activity assays. d, Photouncaging, triggered by visible light, of the carboxylic acid functional group in oligonucleotide substrates. Conversion was calculated by LC-MS analysis.
In the absence of light, no azide reduction was observed after 15 hours, and exposure to light after 15 hours of darkness resulted in complete azide reduction in 10 minutes. In comparison, TCEP, a common Staudinger reaction agent, reduced both the azide and disulfide non-selectively (equation (2) in Fig. 4a). Likewise, DNA oligonucleotide 47, which contains an alkyl azide and a disulfide, underwent selective azide reduction in 2 hours when treated with 5 mM Ru(bpy)3Cl2 and 100 mM of sodium ascorbate (equation (3) in Fig. 4a).

To test the compatibility of the azide-reduction reaction with oligosaccharide substrates, we prepared an azide-containing variant of naringin, a flavanone disaccharide (Fig. 4b). When 100 mM of 49 was treated with standard aqueous reduction conditions, we observed complete reduction in 30 minutes with no other transformations detected by high-performance liquid chromatography (HPLC). 

Finally, we performed the azide reduction in the presence of a protein enzyme. We treated a solution that contained 100 mM of naringin azide (49) and 10 mM of RNase A with standard aqueous reduction conditions. After 6 hours, azide 49 was reduced completely, as detected by HPLC (Fig. 4c). We isolated the RNase A from the completed reaction by size-exclusion chromatography. No new species consistent with the covalent modification of RNase A were observed by electron spray ionization (ESI)-MS of the macromolecular fraction. Likewise, enzymatic assay of RNase A activity, which requires intact disulfide bonds40, revealed a ≤10% loss of catalytic activity from the azide-reduction reaction developed in this work, although thiol-based reduction and the Staudinger azide reduction both resulted in the loss of 85–100% of enzyme activity (Fig. 4c).

Photouncaging of carboxylic acids triggered by visible light. We hypothesized that the reaction described above may also enable azide-protected groups beyond amines to be uncaged efficiently on functional group-rich biomolecules under mild conditions with visible light. Current photouncaging41,42 reactions typically require ultraviolet light that can induce undesired side reactions, including protein degradation, photobleaching, inhibition of photosynthesis and nucleic acid damage43. We prepared a DNA oligonucleotide-linked carboxylic acid caged as a 4-azidobenzyl ester44,45 (52) and treated it with standard aqueous reduction conditions. Photouncaging of the free carboxylic acid through azide reduction and 1,6-elimination was complete within 10 minutes without any detected by-products by LC-MS (Fig. 4d). As a deprotection strategy, this reaction proceeded rapidly under very mild conditions and is orthogonal to most carboxyl-deprotection strategies used currently, such as acid or base hydrolysis45.

Conclusion

Using a second-generation, DNA-encoded reaction-discovery system, we explored a broad range of transition metals and reaction conditions to identify combinations of substrates and reaction conditions that result in bond formation between substrates. From the reactivity revealed by the resulting hits we developed a Ru(ii)-catalysed azide-reduction reaction induced by visible light that is efficient and can be conducted in organic or aqueous solvents, open to the air, at room temperature and under neutral conditions. This reaction exhibited remarkable chemoselectivity, in contrast to existing azide-reduction methods, and is compatible with alcohols, phenols, acids, amines, alkenes, aldehydes, alkyl halides, mesylates and disulfides. The unusual functional group compatibility and mild conditions required for the reaction enabled azide reduction to be performed on oligonucleotide and oligosaccharide substrates, and in the presence of a protein enzyme, without compromising the structure or the enzymatic activity of the biomolecules. This reaction can also be used to photouncage groups, such as amines and carboxylates, on biomolecules without the need of ultraviolet light.

Methods

See the Supplementary Information for additional experimental details.

General reaction-discovery procedure. Each reaction-discovery experiment was performed in 200 μl total volume that contained 0.5 pmol of the total substrate pool, which corresponded to 2.2 fmol of each unique substrate combination. After exposure to the reaction conditions shown in Figs 1b and 2, each solution was precipitated with ethanol to recover DNA-linked species. The pellet was dissolved in 30 μl of water, and 150 μl of 0.1 M TCEP in 1.0 M aqueous sodium phosphate, pH 8.0, was added to effect disulfide cleavage. After 30 minutes at 25 °C, this solution was combined with streptavidin-linked magnetic particles (14 μl, which corresponded to a 20 pmol biotin-binding capacity, Roche Biosciences) suspended in 300 μl of 10 mM Tris-Cl, 0.1 M NaCl, 1 mM EDTA, pH 7.5. After incubation for 15 minutes at 25 °C the supernatant was removed and the particles captured by a magnetic separator, then rinsed once with 200 μl of 10 mM Tris-Cl, 1 M NaCl, 1 mM EDTA, pH 7.5, and once with 200 μl H2O. The particles were suspended in 40 μl deionized water and incubated at 70 °C for 5 minutes to elute the captured DNA45. The supernatant was collected and the elution step repeated once. The combined supernatants were used directly in PCR reactions for microarray analysis, as described previously46 and detailed in the Supplementary Information.

Representative small-molecule azide reductions. For aryl azides in organic solvent, to a solution of Ru(bpy)3Cl2 (7.5 mg, 0.01 mmol, 0.05 equiv.), Hantzsch ester (76 mg, 0.03 mmol, 1.5 equiv.), i-Pr2NEt (350 μl, 2.0 mmol, 10 equiv.) and HCOOH (57 μl, 2.0 mmol, 10 equiv.) in 1 ml CH3Cl, was added azide 4 (43.6 mg, 0.2 mmol, 1 equiv.) to stir at 25 °C. The dark-orange solution (stirred at 25 °C for 2 hours) was treated with standard aqueous reduction conditions to 26 W CFL bulb until thin-layer chromatography (TLC) indicated the complete consumption of azide 4 (usually ≤2 hours). The reaction mixture was subjected directly to silica-gel chromatography. Isolated yields for other aryl azides are shown in Fig. 3b. For aryl azides in mixed aqueous–organic solvent, to a solution of Ru(bpy)3Cl2 (3.8 mg, 0.005 mmol, 0.05 equiv.) and azide 4 (21.8 mg, 0.01 mmol, 0.1 equiv.) in 1 ml DMF/H2O (1/1 ratio) was added sodium ascorbate (19 mg, 0.1 mmol, 10 equiv.) in two equal portions (the second portion was added after 24 hours). The orange solution was stirred at 25 °C at a distance of 20 cm from a 26 W CFL bulb until TLC indicated the complete consumption of azide 4 (≏48 hours). The resulting reaction mixture was diluted with ethyl acetate, washed with water and brine, dried over Na2SO4, concentrated in vacuo and subjected to silica-gel chromatography. For alkyl azides, to a solution of Ru(bpy)3Cl2 (15 mg, 0.02 mmol), Hantzsch ester (102 mg, 0.04 mmol) and i-Pr2NEt (105 μl, 0.6 mmol) in 2 ml DMF/McOH (3/1 ratio) was added alkyl azide 43 (37.2 mg, 0.2 mmol). The dark-orange suspension was stirred at 25 °C at a distance of 20 cm from a 26 W CFL bulb until TLC indicated the complete consumption of azide 43 (≏24 hours). To the reaction mixture was added di-i-butyl dicarbonate (67 mg, 0.4 mmol) and triethylamine (56 mg, 0.6 mmol) and this mixture was stirred for 12 hours. The resulting solution was diluted with ethyl acetate, washed with water and brine, dried over Na2SO4, concentrated in vacuo and subjected to silica-gel chromatography.

Representative DNA-linked azide reduction. To a solution of 1 mM Ru(bpy)3Cl2 and 50 mM sodium ascorbate in 200 μl of aqueous 200 mM Tris-Cl, pH 7.4, was added DNA-linked azide to give a final concentration of 0.5 μM. After incubation at 25 °C at a distance of 20 cm from a 26 W CFL bulb for 10 minutes, the reaction was quenched by precipitation with ethanol. The pellet containing DNA-linked small molecule was dissolved in 20 μl of 10 mM phosphate buffer, pH 8, and subjected to LC-MS analysis.

Naringin-azole reduction in the presence of RNase A. To a solution of 1 mM Ru(bpy)3Cl2 and 50 mM sodium ascorbate in 200 μl of aqueous 200 mM Tris-Cl, pH 7.4, was added naringin azide and RNase A to final concentrations of 100 μM and 10 μM, respectively. After incubation at 25 °C at a distance of 20 cm from a 26 W CFL bulb for 6 hours, RNase A was collected through size-exclusion chromatography (Centri-Sep column, Princeton Separations) and characterized by MS. After sample dilution 12-fold, RNase A enzyme activity was assayed by following cytidine 2′,3′-cyclic phosphate hydrolysis spectrophotometrically as described previously46. The small-molecule fraction from polyethersulfone membrane ultrafiltration (Corning Life Sciences) was analysed directly by reverse-phase HPLC to assess the naringin-azole reduction.

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References


