A EUROPEAN JOURNAL

CHEMBIOCHEM

OF CHEMICAL BIOLOGY

Chemistry & Life Sciences

Reprint

A Journal of

ChemPubSoc Europe

WILEY-VCH

www.chembiochem.org

ChemPubSoc Europe

DOI: 10.1002/cbic.201800028

CHEMBIOCHEM Full Papers

VIP *Very Important Paper*

SPECIAL ISSUE

The ULTIMATE Reagent: A Universal Photocleavable and Clickable Reagent for the Regiospecific and Reversible End Labeling of Any Nucleic Acid

Piyush K. Jain and Simon H. Friedman*[a]

There is a need for methods to chemically incorporate photocleavable labels into synthetic and biologically sourced nucleic acids in a chemically defined and reversible manner. We have previously demonstrated that the light-cleaved diazo di-methoxy nitro phenyl ethyl (diazo-DMNPE) group has a remarkable regiospecificity for modifying terminally phosphorylated siRNA. Building on this observation, we have identified conditions under which a diazo-DMNPE reagent that we designed (diazo-DMNPE-azide or DDA) is able to singly modify any nucleic acid (RNA, DNA, single-stranded, double-stranded, 3′ or 5′ phosphate). It can then be modified with any clickable reagent to incorporate arbitrary labels such as fluorophores into the nucleic acid. Finally, native nucleic acid can be regenerated directly through photolysis of the reagent. Use of the described approach should allow for the tagging of any nucleic acid, from any source—natural or unnatural—while allowing for the light-induced regeneration of native nucleic acid.

Introduction

The convenient introduction of labels into nucleic acids is a central technique for a wide range of applications, including detection, capture, quantification, and delivery.[1] Stimuli-responsive nucleic acid tags in particular, provide control and reversibility for understanding and manipulating biology.[2] In an early example of this, Okamoto and co-workers used Bhc-diazo to post-synthetically modify the backbone of mRNA to control its activity with light.[3] Although nucleic acids are routinely modified during solid-phase synthesis, only a few existing methods allow modification of nucleic acids post-synthetically.[1g,h,4] Furthermore, few of these post-synthetic methods enable site-specific modifications or allow removal of tags with an external stimulus.[1e,o,5]

For existing techniques, there is no easily applicable and general way to specifically, and importantly, reversibly, introduce chemical labels into naturally occurring nucleic acids or synthetic nucleic acids once solid phase synthesis is completed. In this work, we describe a new method of efficiently end-labeling any nucleic acid, which introduces an azide-containing group that can then be linked with any clickable label. In addition, this group can be cleanly removed through photolysis, yielding native nucleic acid.

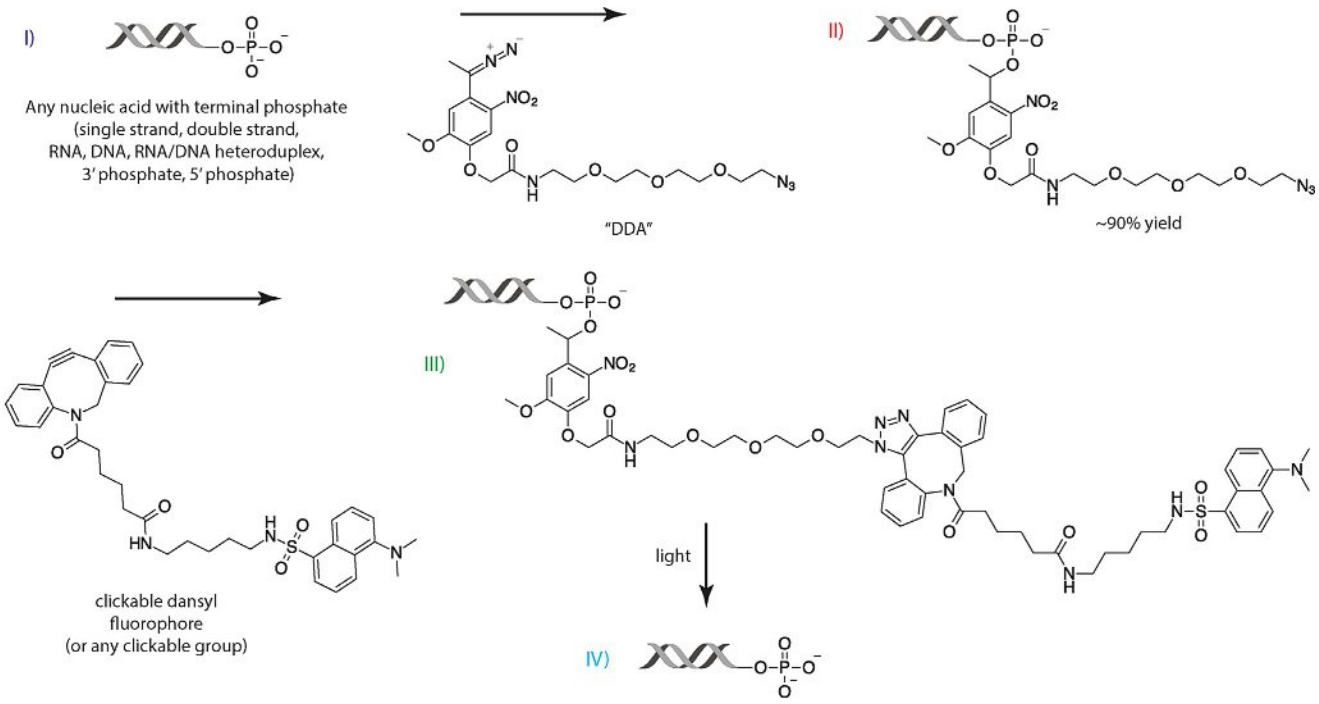
This approach is based on our earlier observation that the diazo form of the dimethyl nitro phenyl ethyl group (diazo-DMNPE) has remarkable regiospecificity in siRNA for terminal phosphates over internal phosphates or nucleobases.[6] We observed this during experiments to modify siRNA and dsRNA with photocleavable groups to bring RNA interference under light control. We demonstrated that diazo-DMNPE incorporated very poorly into siRNA without terminal phosphates but would install four DMNPE groups into siRNA with four terminal phosphates. This was further confirmed by using model nucleotides and MS/MS analysis of the products.[6a] These showed conclusively that the diazo-DMNPE group reacted rapidly with terminal phosphates and could be cleanly installed into an siRNA wherever a terminal phosphate was present.

We recently created a version of DMNPE with a PEG linker terminated with an azide group (diazo-DMNPE-azide (DDA)) to allow insulin to be linked to an insoluble resin and then released with light (from what we termed a photoactivated depot (PAD)).[7] We subsequently realized that this reagent can also be used to label nucleic acids by exploiting the regiospecificity of DMNPE that we previously discovered. This reagent can be used to modify any nucleic acid that contains a terminal phosphate. It can then be treated with any clickable label. And finally, the native nucleic acid can be released from the label by using light. This reaction cycle is summarized in Scheme 1. In the work described in this article, we identified universal conditions under which this reagent could singly modify any nucleic acid, not just siRNA. Specifically, we demonstrated the installation of a single DDA into a wide range of nucleic acids, including double-stranded (ds)RNA, dsDNA, single-stranded (ss)RNA, ssDNA, and RNA/DNA heteroduplex. We showed this worked efficiently to allow both 3′ and 5′ terminal phosphate modification. In addition, we demonstrated

[a] *P. K. Jain, S. H. Friedman*
University of Missouri–Kansas City, Department of Pharmaceutical Sciences
2464 Charlotte Street, Kansas City, MO 64108 (USA)
E-mail: friedmans@umkc.edu

Supporting information and the ORCID identification numbers for the authors of this article can be found under https://doi.org/10.1002/cbic.201800028: complete materials and methods, including NMR, HPLC and MS characterization of all intermediates; HPLC/MS characterization of all 18 nucleic acid species and detailed results of optimization experiments.

This article is part of a Special Issue on the Optical Control of Biological Processes.



Scheme 1. Complete reaction cycle of nucleic acid with DDA: alkylation, click reaction, photolysis. Phosphorylated nucleic acid **I** reacts with DDA to form adduct **II**. This adduct is reacted with a clickable group to form **III**. This product can then be photolyzed to reform the starting nucleic acid (**IV**).

that the DDA-modified nucleic acids could be linked to reactive labeling moieties by a click reaction. Finally, we showed that the original native nucleic acid could be regenerated through photolysis of the linker between the reagent and the phosphate group. This reagent exhibited most of the needed properties of a universal cleavable nucleic acid labeling reagent; therefore, we named it the Universal Light-cleaved TerminI-Modifying And TaggablE (ULTIMATE) reagent.

Results and Discussion

DDA was prepared as previously described.[7] Briefly, the precursor carboxylic acid was condensed with an amino-PEG-azide.[8] This was then converted to the hydrazone by using hydrazine, and finally to the diazo form by oxidation with MnO_2. Our aim was to find conditions that allowed labeling of any type of nucleic acid, so we examined and optimized conditions by using a specific 20-mer sequence that contained an equal mixture of all four bases randomly distributed. We examined this sequence in the form of duplexes (RNA/RNA, RNA/DNA, DNA/RNA, DNA/DNA) and single strands (RNA, DNA). With each of the species, we created three subspecies that incorporated a single phosphate on the 5′ or the 3′ terminus or incorporated no terminal phosphate. This gave a total of 18 different nucleic acid species examined. The purpose of examining a non-phophorylated species was to confirm our previous observation that the terminal phosphate was required for reaction in all examined species.

Modification was performed by reaction of DDA with the individual nucleic acid species. We found that previously described conditions that worked effectively with siRNA did not work for all nucleic acid species, particularly single-stranded species. Specifically, we observed overreaction with single-stranded species under our previous conditions. We have previously shown that higher modifications were due to reactions at nucleobase sites. This makes sense, as the nucleobases are more exposed in single strands than double strands. Our aim was to have a single set of conditions that could be universally applied to all nucleic acids, so we optimized conditions, examining the level of modification in screening experiments by using mass spectrometry (MS) signals. Although direct MS signals cannot be used to determine absolute levels of different species, they are effective in examining relative changes in the synthesis of different species. The ultimate yields of reactions with different nucleic acid species were determined by HPLC and MS analyses.

The factors optimized include the buffer constituents, pH, presence and concentration of $MgCl_2$, the presence and concentration of DMSO, and the molar ratio of DDA to nucleic acid species. These optimization experiments are detailed in the Supporting Information (Figure S5–S19). The results from representative studies are shown in Figure 1. We examined the effect of $MgCl_2$ concentration on the levels of modification of 3′-phosphorylated ssDNA (Figure 1 A). We found that low $MgCl_2$ levels allowed multiple modifications in single-stranded species, and higher $MgCl_2$ levels suppressed the modifications. The optimal concentration of $MgCl_2$ (10 mM) was incorporated into the final reaction conditions. We also examined the effect of DDA stoichiometry on the levels of modification under early, unoptimized conditions (Figure 1 B).

Through this process of optimization, we identified conditions that resulted in 80–97% yields of singly modified nucleic

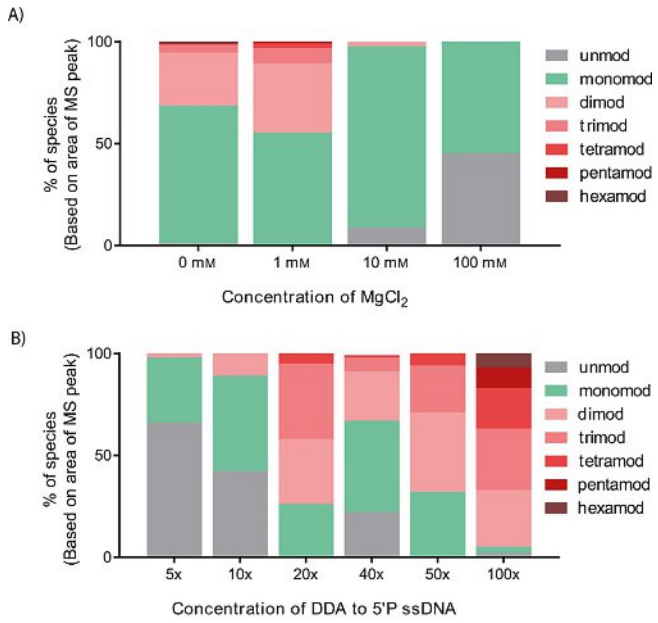


Figure 1. Example optimization experiments. A) Effect of changing concentrations of $MgCl_2$ during reaction of 10 mM DDA with ssDNA containing 3′-phosphate oligonucleotide (250 μM). Solution conditions were: 10 mM Tris·HCl, 1 mM EDTA, pH 7.6, 25% DMSO. 10 mM $MgCl_2$ was found to be optimal for single modification. B) Different levels of modifications observed by MS for various concentrations of DDA reacted with 25 μM of ssDNA oligonucleotide containing 5′-phosphate in 25% DMSO/water, reacted for 24 h.

acid, independent of the nucleic acid species. The final conditions were 10 mM Tris·HCl, pH 6, 1 mM EDTA, 10 mM $MgCl_2$, 200 μM nucleic acid, 20% *v/v* DMSO and DDA (3 mM with single-stranded nucleic acids, 5.3 mM with double-stranded). Using the optimized conditions, we analyzed the reaction of all 18 species with DDA in greater detail by using HPLC and ESI-MS, as previously described.[9] Two representative reactions are depicted in Figure 2, specifically 5′-phosphorylated ssDNA, and 3′-phosphorylated dsRNA. For both of these examples, reaction of the identical non-phosphorylated species is also shown to illustrate the requirement of a terminal phosphate for reaction. These results are typical of all 18 nucleic acid species, which are detailed in the Supporting Information. For example, in Figure 2A (the reaction of 5′-phosphorylated ssDNA), we observed a single major HPLC peak of the starting material that had a molecular weight consistent with the unmodified single strand (blue chromatogram). Upon reaction with DDA, we observed near-quantitative consumption of the starting material peak and conversion to a tight cluster of two peaks of nearly identical height (red chromatogram). These both gave masses that were consistent with the mass of the singly modified product. We interpreted these two peaks as being the diastereomers formed upon alkylation of the terminal phosphate. We have previously observed similar diastereomers in examining the reaction of DMNPE with model nucleotides.[6a]

In Figure 2B, the paired, non-phosphorylated ssDNA species showed no consumption of the starting material and no significant formation of singly modified species. This is because the terminal phosphate group is required for modification. This identical pattern of reactivity is illustrated in Figure 2C and D, which details the reaction of dsRNA with and without a 3′-terminal phosphate. Indeed, all 18 species examined showed the same pattern, summarized in Table 1. They showed the reaction of DDA with nucleic acids to form exclusively a singly modified species, but only when there was a terminal phosphate present. This product was formed in 80–97% yield, with higher yields found with duplex reactants. In the absence of this terminal phosphate, we observed few to no modifications; this is consistent with the terminal phosphate being required

Table 1. Summary of DDA reactions with 18 nucleic acid species: ssDNA, ssRNA, RNA/RNA, DNA/DNA, RNA/DNA, and DNA/RNA—all with a 5′- or 3′-terminal phosphate or no modification.[a]

Nucleic acid	Location of terminal phosphate	Major species obs. by MS	Mass of major species		Proportion of total [% by HPLC]
			Calcd	Obs.	
ssRNA	5′	RNA + DDA	6900.3	6903.0	88.8
	3′	RNA + DDA	6900.3	6903.0	83.5
	none	RNA	6366.8	6369.0	96.5
ssDNA	5′	DNA + DDA	6651.5	6653.0	80.6
	3′	DNA + DDA	6651.5	6653.0	79.7
	none	DNA	6118.0	6119.0	100.0
RNA/RNA	5′	**RNA** + DDA/RNA	6900.3/6367.8	6903.0/6370.0	93.5
	3′	**RNA** + DDA/RNA	6900.3/6367.8	6903.0/6369.0	96.3
	none	RNA/RNA	6367.8/6367.8	6369.0	96.6
RNA/DNA	5′	**RNA** + DDA/DNA	6900.3/6118.0	6903.0/6120.0	92.1
	3′	**RNA** + DDA/DNA	6900.3/6118.0	6904.0/6120.0	96.7
	none	RNA/DNA	6367.8/6118.0	6370.0/6119.0	100.0
DNA/RNA	5′	**DNA** + DDA/RNA	6651.5/6367.8	6653.0/6369.0	95.0
	3′	**DNA** + DDA/RNA	6651.5/6367.8	6653.0/6369.1	92.1
	none	DNA/RNA	6118.0/6366.8	6119.0/6370.0	100.0
DNA/DNA	5′	**DNA** + DDA/DNA	6651.5/6118.0	6653.0/6120.0	95.2
	3′	**DNA** + DDA/DNA	6651.5/6118.0	6653.0/6119.0	97.4
	none	DNA/DNA	6118.0/6118.0	6120.0	100.

[a] All sequences are 5′-ACTGA TACGT GTGCA CTCAG-3′ except for RNA strands, which contain U instead of T. The terminal phosphate is on either the 5′- or 3′-end of this strand. In double-stranded species, the second strand is the exact complement, 5′-CTGAG TGCAC ACGTA TCAGT-3′, except for RNA strands that contain U instead of T. Bold font indicates the strand that contains the terminal phosphates in duplexes. DDA = diazo-DMNPE-azide.

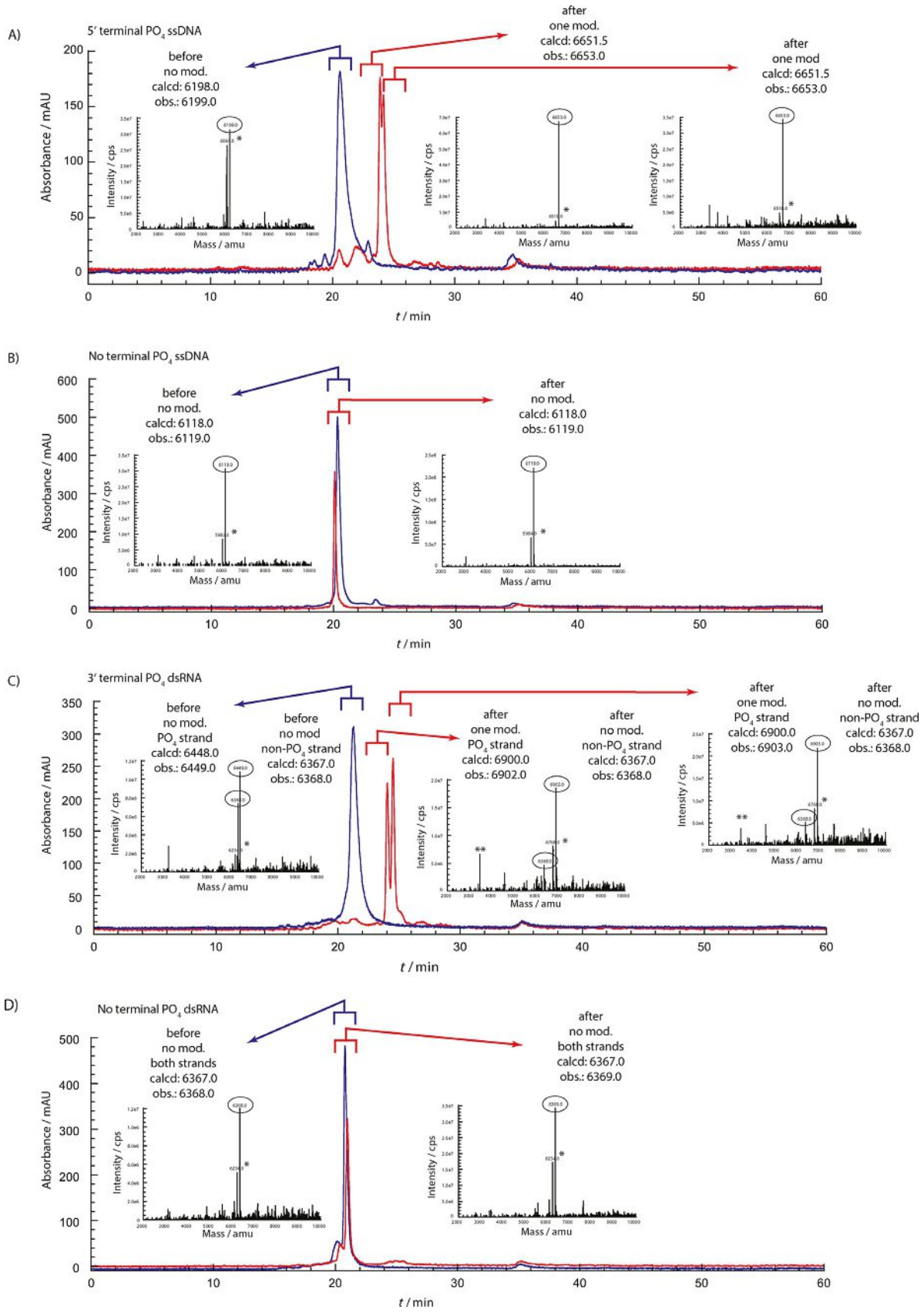


Figure 2. Examples of specific reactions of DDA with nucleic acid terminal phosphates. Reaction of ssDNA A) with and B) without a 5′-terminal phosphate. Reaction of dsRNA C) with and D) without a 3′-terminal phosphate. Blue traces show the nucleic acid before reaction, and red traces show it after reaction. Insets show the mass spectra of each of the indicated regions. *: commonly observed −135 depurination peaks. As described in the text, double peaks are likely due to diastereoisomers that form during the modification reaction. All HPLC was monitored at 260 nm.

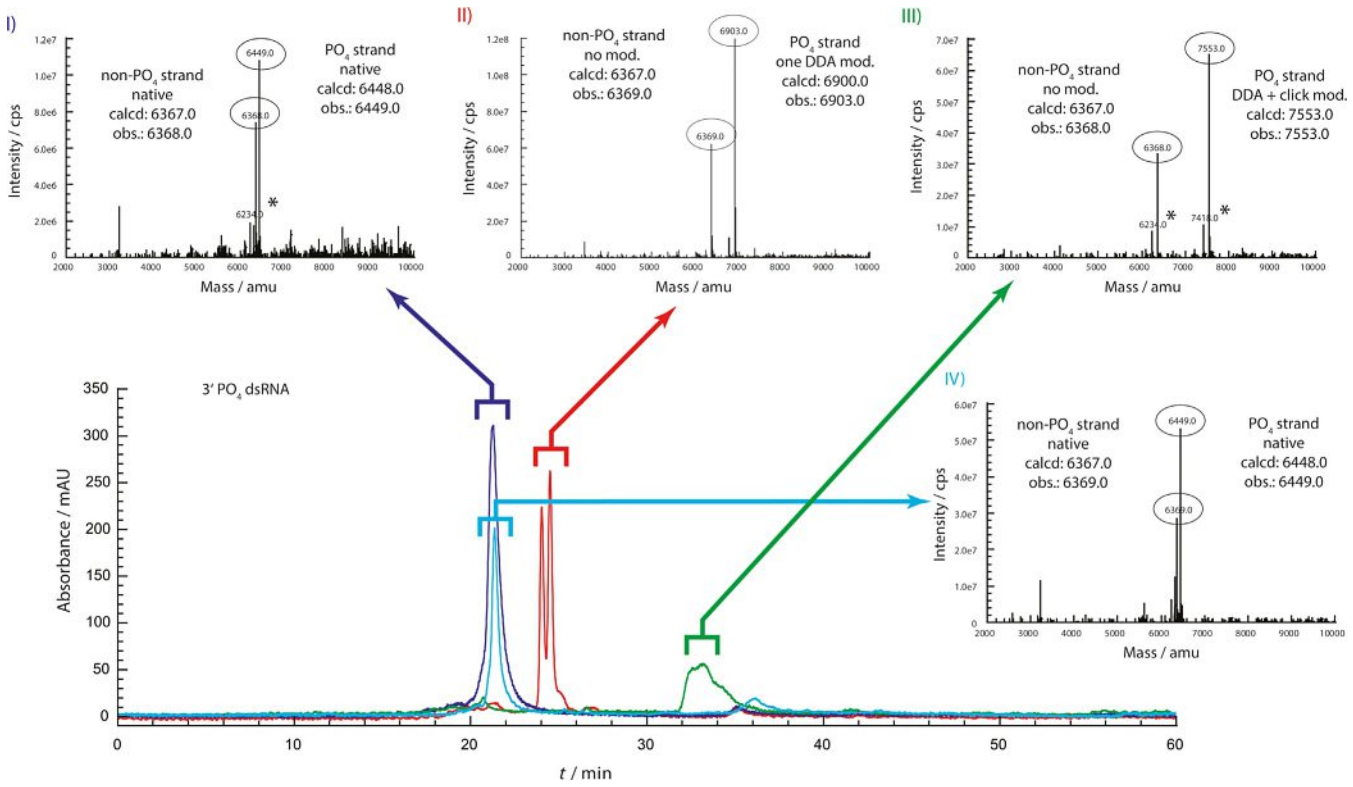


Figure 3. Complete reaction cycle of 3′-phosphorylated dsRNA. RNA **I** reacted with DDA to form **II**. A dansyl derivative was clicked onto the DDA-modified RNA to form **III**. Photolysis regenerated native RNA **IV**. *: commonly observed −135 depurination peaks. All HPLC was monitored at 260 nm.

for reaction. This was also illustrated in the MS spectra of the terminally phosphorylated duplexes, which typically showed each of the two strands as separate masses. In these, upon reaction, only the phosphorylated strand showed modification by DDA.

In addition, we took one of the nucleic acid species, 3′-phosphorylated dsRNA, through a complete cycle of 1) DDA modification, 2) click reaction with a fluorophore, and 3) photolysis, regenerating native dsRNA, to demonstrate the efficiency of these transformations. These results are shown in Figure 3. Again, we saw a single peak on the HPLC trace of the starting nucleic acid. Upon reaction, we saw the doublet of singly modified diastereomers and no other products. This species was then reacted with a clickable fluorophore (dansylcadaverine-modified DBCO). The DBCO moiety is a strained cyclooctyne that allows for copper-free click reactions.[10] The product gave a broad peak on HPLC with a mass that was consistent with the expected click product, indicating the fluorophore had attached to the oligonucleotide. Finally, upon irradiation of this species with a 365 nm light source, the peak for the modified nucleic acid was consumed, and a peak with a retention time (and molecular weight) of the starting material was quantitatively generated.

Conclusion

In this work, we have demonstrated a new method for incorporating any label into a defined terminal position in any nucleic acid. Although methods exist for specifically labeling nucleic acids, they typically depend on solid phase synthesis, so there is no way of doing this labeling on naturally occurring nucleic acids (e.g., pools of isolated mRNA, miRNA etc.) With our approach, even naturally occurring nucleic acids can potentially be primed, through phosphorylation with polynucleotide kinase, for example. In labeling applications, the label itself has the potential to interfere with or confound the nature of the action of the nucleic acid of interest. With our approach, after the label has been specifically incorporated, it can be easily removed by using light, generating native nucleic acid. As such, it should enable a wide range of studies with nucleic acids, especially in which pools of natural nucleic acids would benefit from visualization and isolation.

Experimental Section

Synthesis of dansylcadaverine-DBCO: DBCO acid (1 equiv, 31.8 μmol, 10.6 mg), dansylcadaverine (2 equiv, 63.6 μmol, 21.3 mg), and 1-hydroxybenzotrizole hydrate (2 equiv, 63.6 μmol, 9.7 mg) were dissolved in DMF (97.1 μL). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.7 equiv, 54.1 μmol, 10.4 mg) was then added to the reaction mixture, which was stirred for 4 days. DBCO-DC amide product was purified by acid/base extraction. The product was purified by partitioning the reaction mixture between EtOAc (10 mL) and saturated NaCl (10 mL). The EtOAc layer was washed twice with saturated NaCl solution, and the combined aqueous layers were washed once with EtOAc. The combined organic layers were washed with saturated sodium bicarbonate solution, HCl (1 N), and again with saturated sodium bicarbonate solution. The organic layer was then dried with mag-

nesium sulfate and evaporated to give an off-white solid (yield: 17.5 mg, 84.6%); TLC (EtOAc/MeOH, 75:25, *v/v*): $R_f = 0.55$; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 8.56$ (d, $J = 8.4$ Hz, 1H), 8.42 (d, $J = 8.4$ Hz, 1H), 8.22 (d, $J = 7.2$ Hz, 1H), 7.64–7.54 (m, 3H), 7.49–7.47 (m, 4H), 7.37–7.30 (m, 3H), 7.28–7.26 (m, 2H), 6.75–6.71 (m, 1H), 5.11 (d, $J =$ 14 Hz, 1H), 3.62 (d, $J = 13.6$ Hz, 1H), 2.94–2.83 (m, 2H), 2.87 (s, 6H), 2.26–2.20 (m, 2H), 1.87–1.79 (m, 4H), 1.39–1.16 ppm (m, 10H); ^{13}C NMR (100 MHz, [D$_6$]acetone): $\delta = 172.8$, 172.4, 153.2, 152.8, 149.6, 137.4, 133.4, 130.7, 130.6, 130.5, 130.3, 129.7, 129.6, 128.8, 128.8, 128.6, 128.4, 127.7, 126.0, 124.2, 124.0, 123.0, 120.5, 116.0, 115.3, 109.0, 55.8, 45.6, 43.7, 43.6, 39.2, 36.3, 35.1, 25.8, 29.8, 25.5, 24.4 ppm; UV/Vis (DMSO): λ_{max} (ε): 291, 310 nm; MS (*m/z*): calcd for $C_{38}H_{42}N_4O_4S$: 651.3 $[M+H]^+$; found, 651.2; reversed-phase HPLC-MS (flow rate: 0.4 mL min^{-1}, runtime: 30 min, injection volume: 25 μL); solvent A (0.1% formic acid in H_2O), solvent B (0.1% formic acid in acetonitrile (ACN)), gradient: 0% B to 50% B over 5 min, then 50% B to 100% B over 22 min; isocratic: 100% B for 2 min; gradient: 100% B to 0% B over 1 min; C_8 Hypersil column (5 μm, 100× 4.6 mm, Varian): $t_R = 17.64$ min. Reversed-phase HPLC-MS (flow rate: 0.4 mL min^{-1}, runtime: 30 min, injection volume: 25 μL), solvent A (0.1% trifluoroacetic acid in H_2O), solvent B (0.1% trifluoroacetic acid in ACN), gradient: 0% B to 100% B over 20 min; isocratic: 100% B for 6 min; gradient: 100% B to 0% B over 2 min; isocratic: 0% B for 2 min; C_8 Hypersil column (5 μm, 100×4.6 mm, Varian): $t_R = 14.66$ min; ESI-MS (*m/z*): calcd for $C_{38}H_{42}N_4O_4S$: 651.3 $[M+H]^+$; found, 651.5.

Annealing of strands: Annealing of duplexes was performed by heating both strands at 400 μM in RNase free water at 85 °C for 15 min, followed by slow cooling for about 2 h.

Oligonucleotide caging: Oligonucleotides were caged according to this optimized protocol. Briefly, Tris·HCl buffer (2 μL, 100 mM, pH 6.0), containing EDTA (10 mM) and MgCl (100 mM) was added to both single-stranded oligonucleotides and annealed double-stranded oligonucleotides (400 μM, 10 μL) and mixed by shaking. For single-stranded oligonucleotides, DMNPE-diazo (15 mM) in DMSO (4 μL) was added to each sample, whereas for double-stranded oligonucleotides, DMNPE-diazo (26.5 mM) in DMSO (4 μL) was added. Each sample was allowed to react for 1 h by shaking gently, followed by ethanol precipitation.

Precipitation of nucleic acids: Briefly, caging reactions were terminated by addition of ammonium acetate (9 M) to the mixture to achieve a final concentration of 3 M ammonium acetate. Samples were vortexed for 15 min. Glycogen (2 μL, 20 mg mL^{-1}) and three volumes of ethanol were added, and the mixture was stored at −20 °C overnight. The mixture was then centrifuged at 17400 *g* and 0 °C for 30 min and washed twice with 75% ethanol. The pellet was air-dried and re-dissolved in RNase-free water (100 μL). An aliquot (10 μL) of this sample was used to prepare samples for MS analysis

ESI-MS infusion analysis of caged oligonucleotides: ESI-MS analysis of all oligonucleotides was performed according to protocols established earlier in our laboratory with slight modifications. Briefly, caged dsRNA or siRNA solution stock (10 μL, ≈35 μM) was diluted by addition of RNase free water (25 μL), then triethylamine (TEA, 1 μL) and ACN (35 μL) were added to the solution to achieve a final concentration of 5 μM in a mixture of H_2O/ACN/TEA (35:35:1). Samples were analyzed by mass spec (2000 QTrap, Applied Biosciences) by infusing (10–20 μL min^{-1}) in negative-ion mode with an enhanced multiple charge (EMC) scan type. Finally, EMC spectra were deconvoluted by using Bayesian protein reconstruct tool in bioanalyst software (AB Sciex).

HPLC analysis of samples: Caged oligonucleotides were purified by reversed-phase HPLC on a Microsorb-MV 100-5 C8 column (250×4.6 mm, Varian). Solvent A was triethylammonium acetate buffer (0.1 M, pH 7.0), and solvent B was ACN (50%) in solvent A. HPLC-grade water (Fisher) was used to prepare the buffer. The flow rate was 1.0 mL min^{-1}. The gradient was 0% B to 40% B over 45 min, then an increase to 100% B at 50 min, maintaining at 100% B until reaching 60 min. Injections (50 μL, ≈50 μM) of DMNPE-azide caged oligonucleotide samples were used for purification. Collected fractions were further dried by Speed-Vac to completely evaporate the buffer. Samples were reconstituted in RNase-free water and prepared for ESI-MS according to the procedure described earlier. Percentage yields of each species were determined by calculating the ratio of area of representative peak to the summation of areas represented by all the species.

Click reaction: Caged oligonucleotide (2.5 nmol, 40 μL) in RNase-free water was treated with DBCO-DC (20 nmol, 40 μL) in DMSO with a final total volume of 80 μL 50% DMSO/water. The solution was stirred at 37 °C overnight. After the reaction clicked, ssRNA was precipitated following standard ammonium acetate/glycogen/ethanol precipitation. Finally, ssRNA was dissolved in 40 μL of RNase-free water, and ESI-MS was performed on 10 μL of this sample by following general ESI-MS sample preparation. A majority of the desired product was formed, as confirmed by ESI-MS analysis

Photolysis: Precipitated clicked oligonucleotide (≈200 μM in RNase-free water, 10 μL) was placed in a flat-bottom glass vial insert (100 μL, i.d.: 3.4 mm, o.d.: 4.5 mm, height: 30.5 mm; Agilent). It was then irradiated from the bottom for 10 min by using a Nichia 200 mW 365 nm LED source at a distance of ≈1 mm. After the irradiation, the sample was prepared for ESI-MS analysis as described earlier.

Acknowledgements

This work was supported by a National Science Foundation grant (Chemistry of Life Processes 1052871). We would like to thank Prof. William Gutheil for guidance with the mass spectrometry.

Conflict of Interest

The authors declare no conflict of interest.

Keywords: click chemistry · DNA · end-labeling · nucleic acids · photocleavage · RNA

[1] a) B. A. Armitage, *Curr. Opin. Chem. Biol.* **2011**, *15*, 806–812; b) A. H. El-Sagheer, T. Brown, *Acc. Chem. Res.* **2012**, *45*, 1258–1267; c) A. F. Kolb, C. J. Coates, J. M. Kaminski, J. B. Summers, A. D. Miller, D. J. Segal, *Trends Biotechnol.* **2005**, *23*, 399–406; d) A. R. Kore, I. Charles, *Curr. Org. Chem.* **2013**, *17*, 2164–2191; e) G. Leriche, L. Chisholm, A. Wagner, *Bioorg. Med. Chem.* **2012**, *20*, 571–582; f) F. Li, J. Dong, X. Hu, W. Gong, J. Li, J. Shen, H. Tian, J. Wang, *Angew. Chem. Int. Ed.* **2015**, *54*, 4597–4602; *Angew. Chem.* **2015**, *127*, 4680–4685; g) E. Paredes, S. R. Das, *ChemBioChem* **2011**, *12*, 125–131; h) E. Paredes, M. Evans, S. R. Das, *Methods* **2011**, *54*, 251–259; i) J. Qi, M. S. Han, Y. C. Chang, C. H. Tung, *Bioconjugate Chem.* **2011**, *22*, 1758–1762; j) A. Raulf, C. K. Spahn, P. J. M. Zessin, K. Finan, S. Bernhardt, A. Heckel, M. Heilemann, *RSC Adv.* **2014**, *4*, 30462–30466; k) A. Samanta, A. Krause, A. Jäschke, *Chem. Commun.* **2014**, *50*, 1313–1316; l) D. Schulz, A. Rentmeister, *ChemBioChem* **2014**, *15*, 2342–2347; m) E. M. Sletten, C. R. Bertozzi, *Angew. Chem. Int. Ed.*

2009, *48*, 6974 – 6998; *Angew. Chem.* **2009**, *121*, 7108 – 7133; n) G. J. Smith, T. R. Sosnick, N. F. Scherer, T. Pan, *RNA* **2005**, *11*, 234 – 239; o) S. Solomatin, D. Herschlag, *Methods Enzymol.* **2009**, *469*, 47 – 68.

[2] a) D. Zhang, C. Y. Zhou, K. N. Busby, S. C. Alexander, N. K. Devaraj, *Angew. Chem. Int. Ed.* **2018**, *57*, 2822 – 2826; *Angew. Chem.* **2018**, *130*, 2872 – 2876; b) B. K. Ruble, S. B. Yeldell, I. J. Dmochowski, *J. Inorg. Biochem.* **2015**, *150*, 182 – 188.

[3] H. Ando, T. Furuta, R. Y. Tsien, H. Okamoto, *Nat. Genet.* **2001**, *28*, 317 – 325.

[4] a) P. S. Song, C. N. Ou, *Ann. N. Y. Acad. Sci.* **1980**, *346*, 355 – 367; b) J. Temsamani, S. Agrawal, *Mol. Biotechnol.* **1996**, *5*, 223 – 232; c) M. L. Fontanel, H. Bazin, R. Teoule, *Anal. Biochem.* **1993**, *214*, 338 – 340.

[5] E. Hilario, *Mol. Biotechnol.* **2004**, *28*, 77 – 80.

[6] a) S. Shah, P. K. Jain, A. Kala, D. Karunakaran, S. H. Friedman, *Nucleic Acids Res.* **2009**, *37*, 4508 – 4517; b) S. Shah, S. Rangarajan, S. H. Friedman, *Angew. Chem. Int. Ed.* **2005**, *44*, 1328 – 1332; *Angew. Chem.* **2005**, *117*, 1352 – 1356.

[7] P. K. Jain, D. Karunakaran, S. H. Friedman, *Angew. Chem. Int. Ed.* **2013**, *52*, 1404; *Angew. Chem.* **2013**, *125*, 1444.

[8] C. P. Holmes, *J. Org. Chem.* **1997**, *62*, 2370 – 2380.

[9] S. Shah, S. H. Friedman, *Nat. Protoc.* **2008**, *3*, 351 – 356.

[10] a) N. J. Agard, J. A. Prescher, C. R. Bertozzi, *J. Am. Chem. Soc.* **2004**, *126*, 15046 – 15047; b) M. F. Debets, S. S. van Berkel, J. Dommerholt, A. T. Dirks, F. P. Rutjes, F. L. van Delft, *Acc. Chem. Res.* **2011**, *44*, 805 – 815; c) M. F. Debets, S. S. van Berkel, S. Schoffelen, F. P. Rutjes, J. C. van Hest, F. L. van Delft, *Chem. Commun.* **2010**, *46*, 97 – 99; d) I. S. Marks, J. S. Kang, B. T. Jones, K. J. Landmark, A. J. Cleland, T. A. Taton, *Bioconjugate Chem.* **2011**, *22*, 1259 – 1263.

Manuscript received: January 13, 2018

Accepted manuscript online: March 7, 2018

Version of record online: April 27, 2018