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Selective Labeling of Living Cells by a Photo-Triggered Click Reaction

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Abstract: Phototriggering of the metal-free azide to acetylene cycloaddition reaction was achieved by masking the triple bond of dibenzocyclooctynes as cyclopropenone. Such masked cyclooctynes do not react with azides in the dark. Irradiation of cyclopropenones results in the efficient ($\Phi_{355} = 0.33$) and clean regeneration of the corresponding dibenzocyclooctynes, which then undergo facile catalyst-free cycloadditions with azides to give corresponding triazoles under ambient conditions. *In situ* light activation of a cyclopropenone linked to biotin made it possible to label living cells expressing glycoproteins containing *N*-azidoacetyl-sialic acid. The cyclopropenone-based phototriggered click chemistry offers exciting opportunities to label living organisms in a temporally and spatially controlled manner and may facilitate the preparation of microarrays.

Introduction

The bioorthogonal chemical reporter strategy is emerging as a versatile method for labeling of biomolecules such as nucleic acids, lipids, proteins, and carbohydrates.[1,2] In this approach, a unique chemical functionality is incorporated into a targeted biomolecule, preferably by the biosynthetic machinery of the cell, followed by a specific chemical reaction of the functional group with an appropriate probe. In particular, the azide is an attractive chemical reporter because of its small size, diverse mode of reactivity, and bio-orthogonality. Azides can be incorporated into biomolecules using a variety of strategies such as post-synthetic modification,[3] *in vitro* enzymatic transfer,[4] the use of covalent inhibitors,[5] and metabolic labeling by feeding cells a biosynthetic precursor modified with an azido function.[1]

The most commonly employed bioorthogonal reactions with azides include the Staudinger ligation with phosphines,[6] copper(I)-catalyzed cycloaddition with terminal alkynes,[7] and strain-promoted cycloaddition with cyclooctynes.[8,9] The latter type of reaction, which was coined copper-free click chemistry, does not require a cytotoxic metal catalyst thereby offering a unique opportunity for labeling living cells. The attraction of this type of technology was elegantly demonstrated by a study of the Bertozzi laboratory in which glycans of the developing zebrafish were imaged using a difluorinated cyclooctyne derivative.[10] We have recently demonstrated that derivatives of 4-dibenzocyclooctynol (**1a**,**b**; DIBO, Scheme 1) react exceptionally fast in the absence of a Cu^I catalyst with azido-containing saccharides and amino acids and can be employed for visualizing glycoconjugates of living cells that are metabolically labeled with azido-containing monosaccharides.[9]

The utility of azide-based bioorthogonal reporter strategy can be further extended by the development of a photochemically triggered click reaction as this approach provides opportunities for the spatial and temporal control of the labeling of the target substrates. In fact, photochemical release or generation of an active molecule is a widely employed strategy to deliver bioactive compounds to addressable target sites in a time-controlled manner.[11] To achieve this goal, we have explored photochemical generation of reactive dibenzocyclooctynes. It

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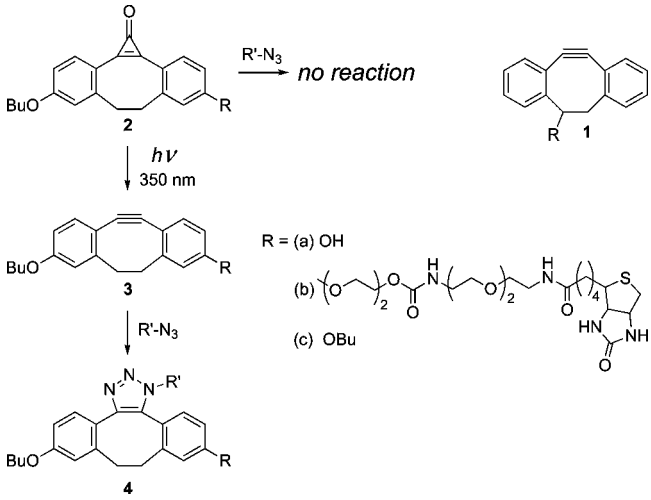
Scheme 1. Photochemical Initiation of the Copper-Free Acetylene−Azide Cycloaddition

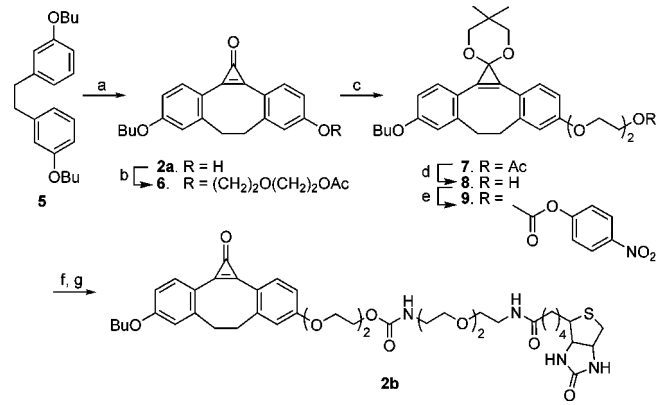


is known that single[12,13] or two-photon[14] excitation of cyclopropenones results in the formation of corresponding acetylenes. Photochemical decarbonylation of thermally stable diaryl-substituted cyclopropenones is especially efficient ($\Phi = 0.2 - 1.0$) and produces alkynes in a quantitative yield.[13] This reaction is also extremely fast and is complete within few hundred picoseconds after excitation.[15] We have already employed cyclopropenone moiety in the development of photoswitchable enediynes.[16] Here we report a novel phototriggered click strategy for metal-free ligation of azides (Scheme 1). Cyclopropenones, such as **2**, do not react with azides under ambient conditions in the dark but efficiently produce reactive dibenzocyclooctynes **3** upon irradiation. The latter type of compound could be employed for labeling of living cells modified with azido-containing cell surface saccharides.

Results and Discussion

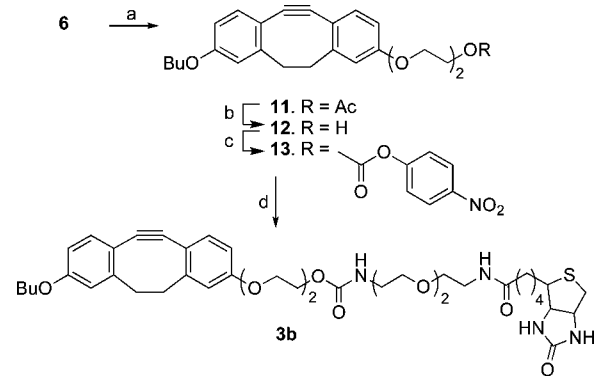
Synthesis of Cyclopropenones 3a−c and Acetylene 3b. Friedel−Crafts alkylation of appropriate substrates with trichlorocyclopropenium cation followed by a controlled hydrolysis of the resulting dichlorocyclopropene offers a convenient synthesis of aromatic cyclopropenones.[13] Thus, the target cyclopropenone **2a** was obtained by treatment of 3,3′-bisbutoxybibenzyl (**5**) with tetrachlorocyclopropene in the presence of aluminum chloride followed by *in situ* hydrolysis of the intermediate dichlorocyclopropene (Scheme 2). In addition to **2a**, a small amount of a *bis*-butoxy analog (**2c**) was isolated.

Scheme 2. Synthesis of Cyclopropenones[a]



[a] Reagents and conditions: (a) $AlCl_3$, tetrachlorocyclopropene, CH_2Cl_2; (b) $HO(CH_2)_2O(CH_2)_2OAc$, PPh_3, DEAD, THF; (c) neopentyl glycol, $BF_4O(C_2H_5)_3$, Et_3N, CH_2Cl_2; (d) NaOH, MeOH; (e) *p*-nitrophenyl chloroformate, pyridine; (f) *N*-biotinyl-3,6-dioxaoctane-1,8-diamine, Et_3N, DMF; (g) Amberyst-15, acetone.

Scheme 3. Preparation of Biotinylated Dibenzocyclooctyne **3b**[a]



[a] Reagents and conditions: (a) 350 nm irradiation, MeOH-THF; (b) NaOH, MeOH; (c) *p*-nitrophenyl chloroformate, pyridine; (d) *N*-biotinyl-3,6-dioxaoctane-1,8-diamine, Et_3N, DMF.

Biotinylated cyclopropenone **2b** was prepared to explore the utility of the phototriggered click chemistry for the light controlled labeling of living cells (Scheme 2). Thus, cyclopropenone **2a** was coupled with diethylene glycol acetate under Mitsunobu conditions to give **6** in 92% yield. The carbonyl moiety of cyclopropenone **6** was protected as a neopentyl glycol acetal by treatment with neopentyl glycol in the presence of $BF_4O(C_2H_5)_3$ and the acetyl ester of the resulting compound **7** was saponified with sodium hydroxide in methanol to produce **8**. Treatment of **8** with 4-nitrophenyl chloroformate gave activated intermediate **9**, which was immediately reacted with *N*-biotinyl-3,6-dioxaoctane-1,8-diamine to provide carbamate **10**. Finally, the acetal-protecting group of **10** was removed to give the required cyclopropenone-biotin conjugate **2b** by the treatment with Amberlyst 15 in acetone. The performance of the phototriggered click reagent **2b** was compared to the known labeling reagent **1b**[9] and to biotinylated dibenzocyclooctyne **3b** (Scheme 3) prepared by an independent route. For this purpose, cyclopropenone **6** was converted into dibenzocyclooctyne **11** by preparative photolysis, which was modified with a biotin moiety to give compound **3b** by a similar procedure employed for the conversion of acetal **7** into compound **10**.

The UV spectra of methanol solutions of cyclopropenones **2a**-**c** showed two close-lying intense bands ($\lambda_{max} = 331$ and 347 nm, $\log\varepsilon \approx 4.5$, Figure 1). Irradiation of **2a**−**c** with 350

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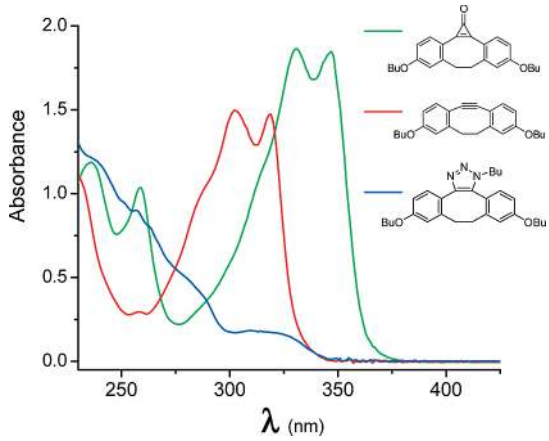

Figure 1. Spectra of 5×10^{-5} M methanol solutions of cyclopropenone **2c** (green), acetylene **3c** (red), and triazole **4c** (blue) (R = Bu).

nm light resulted in efficient ($\Phi_{355} = 0.33$) decarbonylation of the starting material, which could be observed by bleaching of the 331−347 nm bands, and the quantitative formation of acetylenes **3a**-**c**. The thermal stability of the cyclopropenone **2c** in aqueous solution was tested by incubating 1 mM solutions of **2c** at 60 °C. After 12 h at this temperature, negligible loss of starting material was observed in aqueous solution (ca. 3%) and methanol (ca. 4%). It should be noted that thermal decomposition of cyclopropenones in nucleophilic solvents results in ring-opening and the formation of acrylic acid derivatives rather than decarbonylation and thus will not produce an alkyne.[13] Incubation of methanolic solutions of cyclopropenone **2a**−**c** and benzyl- or phenyl azide in the dark for several days did not result in the detectable changes in UV absorbance and HPLC analysis of the mixture showed only the presence of starting materials. Upon irradiation of the solutions, however, the azides rapidly reacted with photogenerated cycloalkyne **3a**−**c** to produce the corresponding triazoles **4a**−**c** in quantitative yields. It is important to note that photoproducts **3a**−**c** and **4a**−**c** have virtually no absorbance above 340 nm (Figure 1), thus allowing for selective irradiation of cyclopropenones **2a**−**c** in their presence and for the convenient monitoring of the reaction progress.

Kinetics of the Cycloaddition Reaction. The rate measurements of cycloaddition of acetylenes **3c** and **1a** were conducted by UV spectroscopy at 25 ± 0.1 °C. A calculated amount of 0.25 M solutions of an azide required to achieve desired azide concentration ($6 \times 10^{-4} - 1.5 \times 10^{-2}$ M) was added to a thermally equilibrated 6×10^{-5} M solution of acetylene in MeOH. Reactions were monitored by following the decay of the characteristic absorbance of acetylenes ca. 317 nm (Figure 1). Consumption of starting material followed a first order equation and the pseudofirst order rate constants were obtained by least-squares fitting of the data to a single exponential equation. The rate dependence as a function of the concentration of azide was linear. Least-squares fitting of the data to a linear equation produced bimolecular rate constants summarized in Table 1. It was found that this method gives more accurate rate constants compared to the use of NMR.[8,9] In this respect, the UV spectroscopic method can be performed under pseudo first order conditions over a wide range of reagent concentrations making the analysis of second-order kinetic curves more reliable. Interestingly, the rate constants for cycloaddition of acetylene **3c** with benzyl azide were very similar to that of dibenzocyclooctynol (**1a**),[9] and thus, the aromatic alkoxy-substitutents of **3a**−**c** do not appear to influence the rate constants.

Table 1. Bimolecular Rate Constants for the Reaction of Acetylenes with Various Azides

acetylene	azide	rate ($M^{-1}\,s^{-1}$)
1a	Benzyl azide[a]	$(5.67 \pm 0.27) \times 10^{-2}$
3c	Benzyl azide[a]	$(7.63 \pm 0.11) \times 10^{-2}$
3c	*n*-Butyl azide[a]	$(5.86 \pm 0.17) \times 10^{-2}$
3c	1-Phenyl-2-azidopropane[a]	$(3.43 \pm 0.03) \times 10^{-2}$
3c	Phenyl azide[a]	$(1.63 \pm 0.06) \times 10^{-2}$
3c	*N*-azidoacetylmannosamine[a]	$(4.41 \pm 0.34) \times 10^{-2}$[c]
12	*N*-azidoacetylmannosamine[b]	$(3.90 \pm 0.32) \times 10^{-2}$

[a] In methanol at 25 ± 0.1 °C. [b] In aqueous solution at 25 ± 0.1 °C. [c] Evaluated from a rate measured at a single azide concentration.

Biological Evaluation. Having established that light activation of cyclopropenones results in the clean formation of the corresponding dibenzocyclooctynes, which can undergo metal-free cycloadditions with azides to give corresponding triazoles, attention was focused on labeling living cells modified with azido moieties. Thus, Jurkat cells were cultured in the presence of 25 μM of peracetylated *N*-azidoacetylmannosamine (Ac_4ManNAz) for 3 days to metabolically introduce *N*-azidoacetyl-sialic acid (SiaNAz) moieties into glycoproteins and glycolipids.[17] As a negative control, Jurkat cells were employed that were grown in the presence of peracetylated *N*-acetylmannosamine (Ac_4ManNAc). The cells were exposed to 30 μM of compound **1b**, **2b**, and **3b** for 1 h at room temperature. In addition, cells and cyclopropenone **2b** were exposed to light (350 nm) for 1 min to form *in situ* cyclooctyne **3b** and then incubated for 1 h at room temperature. Next, the cells were washed and stained with avidin-fluorescein isothiocyanate (FITC) for 15 min at 4 °C. The efficiency of the two-step cell surface labeling was determined by measuring the fluorescence intensity of the cell lysates. Cyclooctynes **1b** and **3b** exhibited strong labeling of the cells (Figure 2a). Furthermore, *in situ* activation of **2b** to give **3b** resulted in equally efficient cell labeling. As expected, low fluorescence intensities were measured when cells were exposed to cyclopropenone **2b** in the dark demonstrating that this compound can be selectively activated by a short irradiation with 350 nm light. Similar staining patterns were obtained when the living cells were analyzed by flow cytometry (Figure S1, Supporting Information).[18]

Some background labeling was observed when the control cells (labeled with Ac_4ManNAc) were exposed to **2b** or **3b** and then treated with avidin-FITC (Figure 2a). To exclude the possibility that the background labeling is due to unwanted side reactions of the compounds with protein, the cell lysates were analyzed by Western blotting using an antibiotin antibody conjugated to HRP (Figure 2b). Gratifyingly, the control cells gave negligible staining, demonstrating that background staining

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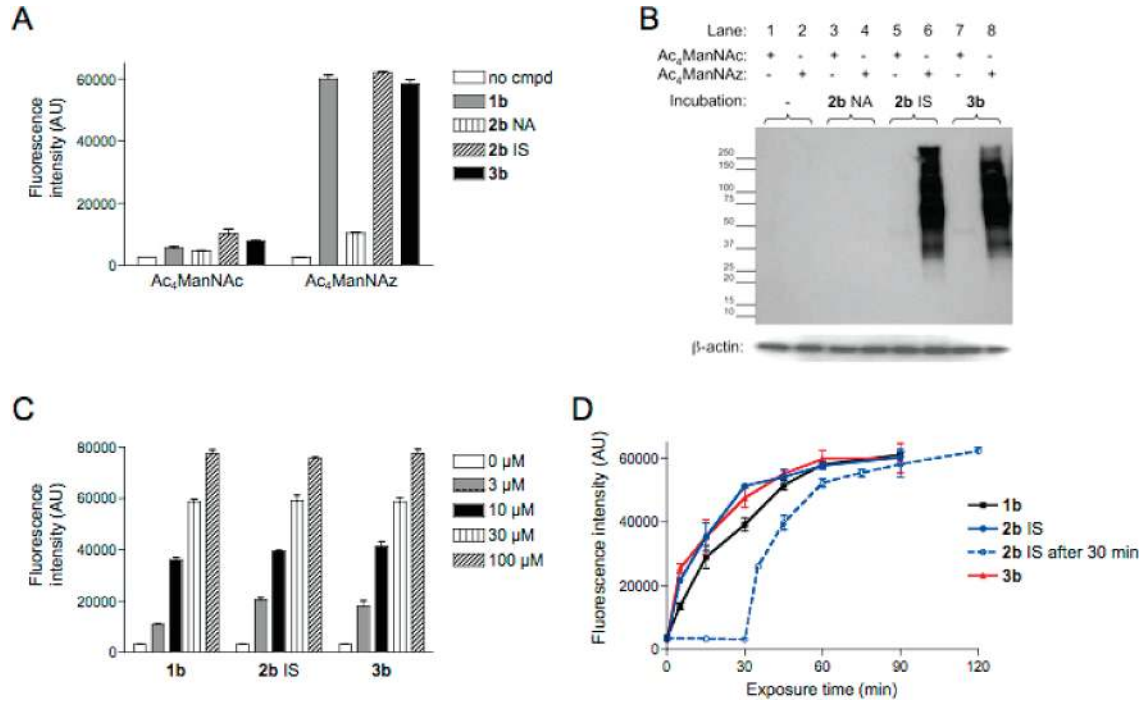

Figure 2. Cell surface labeling with compounds **1b**, **2b**, and **3b**. Jurkat cells grown for 3 days in the presence of Ac_4ManNAc (25 μM) (A, B) or Ac_4ManNAz (25 μM) (A-D) were incubated at room temperature with compounds **1b**, **2b**, and **3b** at 30 μM for 1 h (A, B), 0−100 μM for 1 h (C), or 30 μM for 0−90 min (D). Compound **2b** was assessed without activation (**2b** NA), after immediate light activation *in situ* (1 min at 350 nm; **2b** IS), and after delayed light activation for 30 min *in situ* (**2b** IS after 30 min). Next, either cells were incubated with avidin-FITC for 15 min at 4 °C, after which cell lysates were assessed for fluorescence intensity (A, C, D) or cell lysates (15 μg total protein per lane) were resolved by SDS-PAGE and the blot was probed with an antibiotin antibody conjugated to HRP. Total protein loading was confirmed by Coomassie staining (Figure S3, Supporting Information).[18] AU indicates arbitrary fluorescence units.

is not due to chemical reactions of the compounds with protein and probably arises from noncovalent interactions with the cell membrane. As expected, similar patterns of staining were observed for cells labeled with Ac_4ManNAz and then exposed to **3b** or *in situ* activated **2b**.

The concentration-dependency of the cell surface labeling was examined by incubating cells with various concentrations of **1b**, *in situ* activated **2b**, and **3b**, followed by staining with avidin-FTIC (Figure 2c). The cells displaying azido moieties showed a dose-dependent increase in fluorescence intensity. Reliable fluorescent labeling was achieved at a concentration of 3 μM, however, optimal results were obtained at concentrations ranging from 10 to 100 μM. Interestingly, at low concentration, **3b** gave a somewhat higher fluorescent reading than **1b**.

A time course experiment demonstrated that labeling with **1b** and **3b** (30 μm) at 25 °C reaches an apparent plateau after an incubation time of approximately 45 min, which gradually increased after prolonged exposure (Figure 2d). A similar experiment at a lower temperature (4 °C) also showed an initial fast- followed by a slow and gradual increase in fluorescent intensity; however, the responses were somewhat lower compared to the reaction at 25 °C (Figure S2, Supporting Information).[18]

Light activation of cyclopropenone **2b** provides an attractive opportunity for labeling cells in a temporal controlled manner. To establish a proof of principle for such labeling, a time course experiment was performed whereby cells were first incubated in the presence of **2b** for 30 min in the dark, and then exposed to UV light to form *in situ* alkyne **3b**, which was allowed to react with cell surface azide moieties for different periods of time. Importantly, an identical pattern of labeling was observed compared to cells immediately exposed to UV light, however, with a 30 min delay (Figure 2d).

The heat-sensitivity of the cyclopropenone extrusion reaction was examined by exposing cells labeled with Ac_4ManNAz to **2b** at 37 °C in the dark, and no significant increase in fluorescence was observed compared to exposure at room temperature (Figure S4, Supporting Information).[18] To ensure that *in situ* activation of **2b** had no effect on cell viability and morphology, cells were assessed for the ability to exclude trypan blue and fortunately no changes were observed compared to cells that were not exposed to **2b** both with and without UV light activation (Figure S5, Supporting Information).[18] Cell viability was also examined after incubation with **2b** with and without light activation followed by reincubation for 5 and 24 h (Figure S6, Supporting Information).[18] In each case, there was no significant difference in the ability of the cells to reduce MTT to its insoluble formazan salt as compared to control cells.

Finally, attention was focused on visualizing azido-containing glycoconjugates of cells by confocal microscopy. Thus, adherent Chinese hamster ovary (CHO) cells were cultured in the presence of Ac_4ManNAz (100 μM) for three days. The resulting cell surface azido moieties were reacted with *in situ* generated **3b** (30 μM) and then visualized with avidin-Alexa fluor 488. As expected, staining was only observed at the cell surface (Figure 3) and showed similar cell surface labeling as obtained by staining with **1b**.[9] Cells cultured in the presence of Ac_4ManNAc (100 μM) exhibited very low fluorescence staining. As expected, cells metaboli-

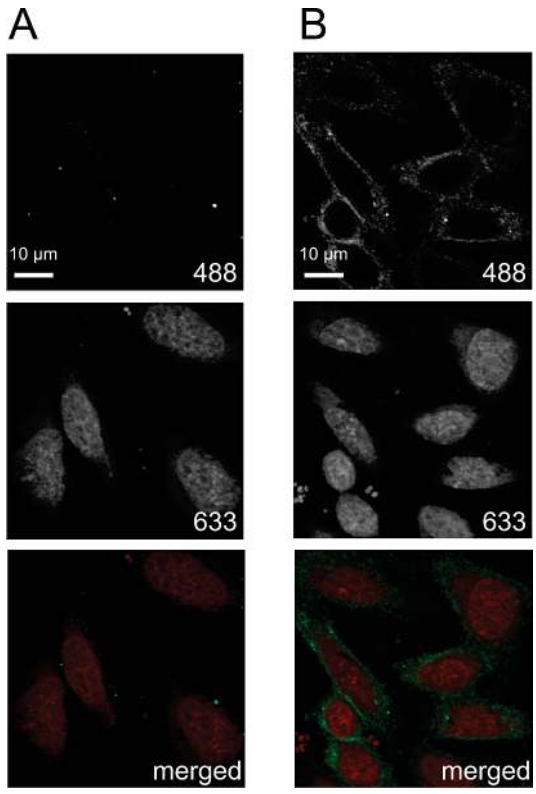

Figure 3. Fluorescence images of cells labeled with compound 2b and avidin-Alexa fluor 488. CHO cells grown for 3 days in the presence of Ac_4ManNAc (100 μM; A) or Ac_4ManNAz (100 μM; B) were given compound **2b** (30 μM), subjected to 1 min UV light for *in situ* activation (**2b** IS), and further incubated for 1 h at room temperature. Next, cells were incubated with avidin-Alexa Fluor 488 for 15 min at 4 °C and, after washing, fixing, and staining for the nucleus with the far-red-fluorescent dye TO-PRO-3 iodide, imaged. Merged indicate that the images of cells labeled with Alexa Fluor (488 nm) and TO-PRO iodide (633 nm) are merged and shown in green and red, respectively.

cally labeled with Ac_4ManNAz and exposed to **2b** in the dark showed also negligible staining (data not shown).

Conclusions

It has been shown that light activation of cyclopropenones **2a**−**c** results in the clean formation of the corresponding dibenzocyclooctynes **3a**−**c**, which can undergo fast and catalyst-free cycloadditions with azides to give corresponding triazoles. *In-situ* light activation of **2b** made it possible to efficiently label living cells expressing glycoproteins containing *N*-azidoacetyl-sialic acid. It is to be expected that the properties of compounds such as **2b** will make it possible to label living organisms in a temporally and/or spatially controlled manner.

It has already been demonstrated that glycoconjugates of model organisms, such as zebrafish, can be metabolically labeled with azido-containing sugars and such an approach has been employed to demonstrate tissue specific expression of glycoconjugates.[10] It is to be expected that the use of compounds such as **2b** will make it possible to visualize azido-labeled biomolecules in model organisms or tissues in a more controlled and reliable manner. In this respect, differences in staining intensities and patterns may arise when classical metal-free click reagents[19] such as **1b** are employed due to possible concentration gradients. On the other hand, the use of a phototriggered click reaction will make it possible to achieve a homogeneous concentration of reagent before initiating the click reaction. The phototriggered click reagent reported here has a much higher quantum yield than the previously described photoactivated Diels−Alder reaction and hence will exhibit much less light-induced toxicity.

It is to be expected that compounds such as **2b** can be activated in a spatial controlled manner, however, the resulting alkyne (**3b**) is a stable derivative, which may diffuse to surrounding space thereby reducing the resolution of labeling. Although future studies will need to establish the spatial resolution of the phototriggered click reaction, it is to be expected that it can selectively label organs or tissues of model organisms. Such an approach provides a unique opportunity for biotinylation of glycoconjugates of specific organs or tissues, which can then be isolated for glycomics or glycoproteomics studies. Wong and co-workers have already reported a combined use of metabolic labeling, Cu-mediated click reactions and glycoconjugate isolation for glycomics.[20] However, such an approach cannot be employed for living organisms.

It is to be expected that other fields of science such as the fabrication of microarrays and the preparation of multifunctional materials, may benefit from phototriggered click chemistry. In this respect, Cu-mediated click reactions have been used for the fabrication of saccharide microarrays by offering a convening approach to immobilize azide-modified saccharides to an alkyne-modified surface.[21] It is to be expected that surface modification with compounds **2a** will offer exciting opportunities for spatially controlled ligand immobilization using light activation followed by copper-free ligation. Furthermore, metal-free click reactions have been applied in material chemistry,[22] and the obvious advantage of such a synthetic approach is that it offers a reliable approach for macromolecule modification without the need of using toxic reagents. Therefore, it is to be expected that the combined use of traditional- and photoactivated metal-free click reactions will offer an attractive approach for multifunctionalization of polymers and macromolecules.[23]

Experimental Section

General Synthetic Procedures and Materials. All NMR spectra were recorded in $CDCl_3$ and referenced to TMS unless otherwise noted. Melting points are uncorrected. Purification of products by column chromatography was performed using 40−63 μm silica gel. Tetrahydrofuran was distilled from sodium/benzophenone ketyl; ether and hexanes were distilled from sodium. Other reagents were obtained from Aldrich or VWR and used as received unless otherwise noted. 11,12-didehydro-5,6-dihydro-dibenzo[a,e]cyclooctен-5-ol (**1a**) and 11,12-didehydro-5,6-dihydrodibenzo [a,e]cyclooctен-5-yl ester of 19-[(3a*S*,4*S*,6aR)-hexahydro-2-oxo-1*H*-thieno[3,4-d]imidazol-4-yl]-15-oxo-5,8,11-trioxa-2,14-diazanonadecanoic acid (**1b**) were prepared as reported previously.[9]

1,2-Bis(3-butoxyphenyl)ethane (5). BBr_3 (11.3 g, 45 mmol) was added to a solution of 1,2-bis(3-methoxyphenyl)ethane[24] (11.56 g,

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47.8 mmol) in CH_2Cl_2 at −78 °C. The reaction mixture was slowly warmed to r.t., and stirred overnight. The reaction mixture was quenched with water, diluted with CH_2Cl_2, and extracted with 2 M solution of NaOH (3 × 100 mL). The aqueous layer was slowly acidified at 0 °C with concentrated HCl to c.a. pH = 1, the gray precipitate was filtered, washed with water, dried in the air at r.t., and then under vacuum at 85 °C over 5 h to provide 10.3 g of crude 1,2-*bis*(3-hydroxyphenyl)ethane as a gray solid. A suspension of crude 1,2-bis(3-hydroxyphenyl)ethane (10.3 g), BuBr (6.50 g, 143.4 mmol), and K_2CO_3 (20.08 g, 143.4 mmol) in DMF (70 mL) was stirred overnight at 75 °C, cooled to r.t., diluted with hexanes (∼150 mL) and water (∼250 mL). The organic layer was separated, washed with water, brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was separated by chromatography (Hex: EtOAc 40:1) to provide 1,2-bis(3-butoxyphenyl)ethane (11.22 g, 72%,) as a slightly yellow oil that slowly crystallizes on standing. ^{1}H NMR: δ 7.18 (dt, J = 8.8, 1.2 Hz, 2 H), 6.77 (d, J = 8.0 Hz, 2 H), 6.75−6.70 (m, 4 H), 3.93 (t, J = 6.4 Hz, 4 H), 2.88 (s, 4 H), 1.75 (5, J = 6.4 Hz, 4 H), 1.48 (six, J = 7.2 Hz, 4 H), 0.98 (t, J = 6.8 Hz, 6 H), 1.60−1.55 (m, 4 H), 0.87 (s, 9 H), 0.03 (s, 6 H); ^{13}C NMR: 159.4, 143.6, 129.5, 120.9, 115.0, 112.1, 67.8, 38.1, 31.6, 19.5, 14.1; MS calcd for $C_{22}H_{30}O_2$ (M^+) 326.2246, EI-HRMS found 326.2280.

4-Butoxy-9-hydroxy-6,7-dihydro-1H-dibenzo[a,e]cyclopropa[c]-[8]annulen-1-one (2a) and 4,9-Dibutoxy-6,7-dihydro-1H-dibenzo[a,e]-cyclopropa[c][8]annulen-1-one (2c). Tetrachlorocyclopropene was added to a suspension of $AlCl_3$ (2.45 g, 13.76 mmol) in CH_2Cl_2 (200 mL), the reaction mixture was stirred for 10 min at r.t., and then cooled to −78 °C. A solution of **5** (4.48 g, 13.76 mmol) in CH_2Cl_2 (∼10 mL) was added dropwise, and the reaction mixture was stirred for ∼2 h. at −78 °C, slowly warmed to r.t., and stirred for an extra hour at r.t. The reaction was quenched by 5% aqueous HCl solution, the organic layer was separated, washed with water, dried over anhydrous $MgSO_4$, and concentrated. The residue was separated by chromatography (CH_2Cl_2: MeOH 20: 1) to provide **2a** (0.997 g, 23%) as a yellow powder and **2c** (0.628 g, 12%) as a white powder.

2a: ^{1}H NMR (DMSO): δ 10.41 (s, 1 H), 7.73 (d, J = 8.4 Hz, 1 H), 7.66 (d, J = 8.4 Hz, 1 H), 7.05 (d, J = 2.4 Hz, 1 H), 6.97 (dd, J = 8.8, 2.4 Hz, 1 H), 6.86 (d, J = 2.4 Hz, 1 H), 6.80 (dd, J = 8.4, 2.4 Hz, 1 H), 4.05 (t, J = 6.4 Hz, 2 H), 3.42−3.35 (m, 1 H) 2.45−2.35 (m, 3 H), 1.69 (p, J = 7.2 Hz, 2 H), 1.41 (sxt, J = 7.6 Hz, 2 H), 0.91 (t, J = 7.2 Hz, 3 H), 1.60−1.55 (m, 4 H), 0.87 (s, 9 H), 0.03 (s, 6 H); ^{13}C NMR: 158.9, 155.42, 155.19, 155.07, 127.1, 126.9, 117.5, 116.96, 116.72, 116.1, 113.3, 112.1, 110.79, 110.34, 68.1, 36.8, 36.7, 31.5, 19.5, 14.1. MS calcd for $C_{21}H_{21}O_3$ (MH^+) 321.1491, APCI-HRMS found 321.1482.

2c: H^1 NMR: δ 7.73 (d, J = 9.6 Hz, 2 H), 6.69 (m, 4 H), 4.04 (t, J = 6.0 Hz, 4 H), 3.33 (d, J = 10.4 Hz, 2 H), 2.63 (d, J = 10.4 Hz, 2 H), 1.80 (p, J = 6.0 Hz, 4 H), 1.52 (s, J = 7.6 Hz, 4 H), 1.00 (t, J = 7.6 Hz, 6 H); ^{13}C NMR: 162.3, 154.0, 148.0, 142.3, 136.0, 116.5, 112.5, 68.2, 37.4, 31.4, 19.42, 14.03.

2-[2-(9-Butoxy-6,7-dihydro-1H-dibenzo[a,e]cyclopropa[c][8]an-nulen-1-one)ethoxy]ethyl Acetate (6). A solution of DEAD (0.635 g, 3.75 mmol) in THF (5 mL) was added to a suspension of **2a** (0.75 g, 2.34 mmol), PPh_3 (0.983 g, 3.75 mmol), and 2-(2-hydroxyethoxy)ethyl acetate (0.44 g, 3.0 mmol) in THF (100 mL), and the reaction mixture was stirred for 30 min. Solvents were removed *in vacuo*, and the residue purified by silica gel chromatography (Hex:EtOAc 2:1 → Hex:EtOAc:CH_2Cl_2 4:3:1 → Hex: EtOAc:(CH_2Cl_2+5% of MeOH) 5:5:4) to give **6** (0.971 g, 92%) as a slightly yellow oil that crystallizes on standing. ^{1}H NMR: δ 7.93 (d, J = 8.4 Hz, 2 H), 6.94−6.86 (m, 4 H), 4.27 (t, J = 4.4 Hz, 2 H), 4.22 (t, J = 4.4 Hz, 2 H), 4.04 (t, J = 6.0 Hz, 2 H), 3.90 (t, J = 4.4 Hz, 2 H), 3.72 (t, J = 4.4 Hz, 2 H), 3.33 (d, J = 10.4 Hz, 2 H), 2.62 (d, J = 11.2 Hz, 2 H), 2.09 (s, 3 H), 1.80 (p, J = 7.2 Hz, 2 H), 1.52 (sxt, J = 7.6 Hz, 2 H), 1.00 (t, J = 7.2 Hz, 3 H); ^{13}C NMR: 171.3, 162.1, 161.5, 153.5, 147.81, 147.78, 142.5, 135.8, 135.7, 116.7, 116.4, 116.36, 116.13, 112.32, 112.30, 69.43, 69.39, 68.0, 67.6, 63.5, 37.2, 31.1, 21.0, 19.2, 13.8.

2-{2-[(9-Butoxy-5′,5′-dimethyl-6,7-dihydrospiro[dibenzo[a,e]cy-clopropa[c][8]annulene-1,2′-[1,3]dioxan]-4-yl)oxy]ethoxy}ethyl Ac-etate (7). $BF_4O(C_2H_5)_3$ (0.45 g, 2.38 mmol) was added to a solution of cyclopropenone **6** (0.97 g, 2.16 mmol) in CH_2Cl_2 (5 mL), and the resulting solution was stirred for 20 min at r.t. A solution of neopentyl glycol (0.27 g, 2.59 mmol) and Et_3N (0.33 g, 3.24 mmol) in CH_2Cl_2 (1.5 mL) was added, the reaction mixture was stirred for 20 min, and the solvents were then removed under reduced pressure. The residue was purified by silica gel column chromatography (Hex:EtOAc 5:1 + 1.5% of Et_3N → Hex:EtOAc 1:1 + 1.5% of Et_3N → Hex:EtOAc:CH_2Cl_2 5:5:4 + 5% of MeOH and 1.5% of Et_3N) to provide **7** (0.593 g, 96% calculated on consumed substrate) as an oil, and unreacted cyclopropenone **6** (0.431 g, 0.96 mmol). ^{1}H NMR: δ 7.65 (dd, J = 8.4, 2.4 Hz, 2 H), 6.92−6.82 (m, 4 H), 4.26 (t, J = 4.4 Hz, 2 H), 4.18 (t, J = 4.4 Hz, 2 H), 4.00 (t, J = 6.4 Hz, 2 H), 3.9a (m, 4 H), 3.88 (t, J = 4.4 Hz, 2 H), 3.78 (t, J = 4.4 Hz, 2 H), 3.24 (d, J = 10.4 Hz, 2 H), 2.41 (d, J = 11.2 Hz, 2 H), 2.08 (s, 3 H), 1.79 (p, J = 7.2 Hz, 2 H), 1.51 (sxt, J = 7.6 Hz, 2 H), 1.21 (s, 3 H), 1.19 (s, 3 H), 0.99 (t, J = 7.2 Hz, 3 H); ^{13}C NMR: 171.1, 159.6, 159.0, 147.1, 131.5, 131.4, 124.2, 123.4, 119.5, 118.9, 116.05, 115.94, 111.97, 111.92, 83.9, 79.2, 69.6, 69.4, 63.5, 36.9, 31.3, 30.6, 22.62, 22.59, 21.0, 19.2, 13.9.

2-{2-[(9-Butoxy-5′,5′-dimethyl-6,7-dihydrospiro[dibenzo[a,e]cy-clopropa[c] (8)annulene-1,2′-[1,3]dioxan]-4-yl)oxy]ethoxy}etha-nol (8). A solution of NaOH (1.2 mL, 1.2 mmol, 1 M aqueous solution) was added to **7** (0.593 g, 1.11 mmol) in a mixture of MeOH and THF (13 mL, 10/3, v/v) at r.t., and the reaction mixture was stirred for 30 min. The reaction mixture was partially concentrated under reduced pressure, diluted with EtOAc (∼25 mL) and washed with water (∼10 mL). The organic layer was separated, washed with brine, and dried ($MgSO_4$), filtered and the filtrate concentrated under reduced pressure. The residue was purified by silica gel column chromatography (Hex:EtOAc:CH_2Cl_2 3:2:1 + 1.5% of Et_3N) to provide **8** (0.493 g, 81%) as an oil that crystallized on standing. ^{1}H NMR: δ 7.65 (dd, J = 8.4, 2.4 Hz, 2 H), 6.92−6.82 (m, 4 H), 4.18 (t, J = 4.4 Hz, 2 H), 4.04 (t, J = 6.4 Hz, 2 H), 3.92 (m, 4 H), 3.88 (t, J = 4.4 Hz, 2 H), 3.77 (t, J = 4.4 Hz, 2 H), 3.68 (t, J = 4.4 Hz, 2 H), 3.24 (d, J = 10.8 Hz, 2 H), 2.41 (d, J = 10.8 Hz, 2 H), 1.76 (p, J = 7.2 Hz, 2 H), 1.50 (sxt, J = 7.6 Hz, 2 H), 1.21 (s, 3 H), 1.19 (s, 3 H), 0.99 (t, J = 7.2 Hz, 3 H); ^{13}C NMR: 159.8, 159.2, 147.4, 131.84, 131.75, 131.67, 131.57, 124.4, 123.6, 119.8, 119.1, 116.3, 116.2, 112.2, 84.1, 79.4, 72.8, 69.8, 68.0, 76.7, 62.0, 37.1, 31.5, 30.8, 22.9, 19.4, 14.2.

2-{2-[(9-Butoxy-5′,5′-dimethyl-6,7-dihydrospiro[dibenzo[a,e]cy-clopropa[c] (8)annulene-1,2′-[1,3]dioxan]-4-yl)oxy]ethoxy}ethyl 4-Ni-trophenyl Carbonate (9). A solution of alcohol **8** (0.439 g, 0.89 mmol) and pyridine (0.25 g, 3.21 mmol) in CH_2Cl_2 (5 mL) was added to a solution of 4-nitrophenyl chloroformate (0.30 g, 1.49 mmol) in CH_2Cl_2 (25 mL) at r.t., and the reaction mixture was stirred for 20 min. Solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (Hex:EtOAc 4:1 + 1.5% of Et_3N) to provide **9** (0.317 g, 80%) and starting material **8** (0.113 g, 0.23 mmol). ^{1}H NMR: δ 8.25 (d, J = 8.8 Hz, 2 H) 7.65 (dd, J = 8.4, 2.0 Hz, 2 H), 7.35, (d, J = 9.2, 2 H), 6.92−6.82 (m, 4 H), 4.43 (t, J = 4.4 Hz, 2 H), 4.19 (t, J = 6.4 Hz, 2 H), 3.98 (t, J = 4.4 Hz, 2 H), 3.92 (m, 7 H), 3.22 (d, J = 10.8 Hz, 2 H), 2.43 (d, J = 10.8 Hz, 2 H), 1.75 (p, J = 7.2 Hz, 2 H), 1.51 (sxt, J = 7.6 Hz, 2 H), 1.21 (s, 3 H), 1.19 (s, 3 H), 0.98 (t, J = 7.2 Hz, 3 H); ^{13}C NMR: 159.9, 159.2, 155.7, 152.7, 150.0 147.4, 145.6, 131.77, 131.63, 125.5, 124.6, 123.4, 122.0, 119.8, 119.1, 116.25, 116.19, 112.2, 112, 15, 84.1, 79.4, 70.0, 69.1, 68.4, 70.0, 67.8, 37.1, 31.5, 30.8, 22.87, 22.79, 19.5, 14.1.

2-{2-[(9-Butoxy-1-oxo-6,7-dihydro-1H-dibenzo[a,e]cyclopropa[c] [8]annulen-4-yl)oxy] ethoxy}ethyl {2-[2-(2-{[5-(2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanoyl]amino} ethoxy)ethoxy]ethyl}carbamate (2b). A solution of cyclopropenone acetal **9** (0.21 g, 0.312 mmol) in DMF (2 mL) was added to a solution of Et_3N (0.18 g, 1.75 mmol) and *N*-biotinyl-3,6-dioxaoctane-1,8-diamine[9] (0.13 g, 0.35 mmol) in DMF (35 mL) at r.t. The reaction mixture was stirred for 18 h and then most of the solvent was evaporated under reduced pressure. The residue was passed through a short column of silica gel (CH_2Cl_2:MeOH 25:1 + 1.5% of Et_3N) to provide crude **10** (0.275 g) that was used in the next step without further purification. A suspension of crude cyclopropenone acetal **10** (0.199 g) and Amberlyst 15 (0.10 g) in Me_2CO (10 mL) was stirred for 60 min at r.t. Solids were removed by filtration, the solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH, 10:1) to provide cyclopropenone **2b** (17 mg) as an amorphous solid. ^{1}H NMR: δ 7.65 (dd, J = 8.4, 3.0 Hz, 2 H), 6.93−6.87 (m, 4 H), 6.66 (s, b, 1 H), 6.25, (s, b, 1 H) 5.61 (m, b, 1 H) 5.39 (s, b, 1 H) 4.48 (m, b, 1 H), 4.30−4.24 (m, 4 H), 4.21 (t, J = 5.0 Hz, 2 H), 4.05 (t, J = 7.5 Hz, 2 H), 3.88 (t, J = 5.5 Hz, 2 H), 3.78 (m, 2 H), 3.60 (s, 4 H), 3.44 (q, J = 6.5 Hz, 2 H), 3.40−3.30 (m, 4 H), 3.18−3.1 (m, 3 H), 2.27 (dd, J = 16.0, 6.0 Hz, 1 H), 2.73 (d, J = 16.0 Hz, 1 H), 2.62 (d, J = 14.0 Hz, 2 H), 2.20 (t, J = 9.0 Hz, 2 H), 2.19−2.02 (m, 4 H), 1.81 (p, J = 8.5 Hz, 2 H), 1.74−1.60 (m, 4 H), 1.51 (six, J = 9.0 Hz, 2 H), 1.46−1.4 (m, 2 H), 1.36 (t, J = 9 Hz, 2 H), 1.00 (t, J = 9.5 Hz, 3 H); ^{13}C NMR: 173.4, 163.8, 162.2, 161.5, 156.5, 153.8, 147.86, 147.83, 142.5, 141.9, 135.85, 135.76, 116.71, 116.4, 116.28, 116.14, 112.39, 112.34, 70.13, 70.07, 69.99, 69.88, 69.4, 68.0, 67.7, 63.9, 62.8, 60.2, 55.5, 45.8, 40.8, 40.5, 39.1, 37.20, 37.15, 35.8, 31.1, 28.13, 28.07, 25.5, 19.2, 13.8, 8.6; MS calcd for $C_{41}H_{56}N_4O_9S$ (M^+-CO+Na) 803.3666, ESI-HRMS found 803.3677.

2-{2-[(9-Butoxy-5,6-didehydro-11,12-dihydrodibenzo[a,e][8]annulen-2-yl)oxy]ethoxy} Ethanol (12). A solution of cyclopropenone **6** (0.54 g, 1.35 mmol) in MeOH:THF (1:1, v:v, 60 mL) was irradiated with 350 nm lamps for 20 min. The solution was concentrated under reduced pressure to 10 mL, and 1 M aqueous NaOH solution (1.68 mL, 1.68 mmol) was added to the mixture and stirring was continued for 30 min. Ethyl acetate was added, and the organic layer was separated, washed with water, brine, dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (EtOAc: Hex 1:1.5) to provide **12** (0.375 g, 73%) as an amorphous white solid. ^{1}H NMR: δ 7.20 (dd, J = 8.4, 0.8 Hz, 2 H), 6.87 (dd, J = 11.2 Hz, 2.0, 2 H), 6.75 (td, J = 8.0, 2.4 Hz, 2 H), 4.15 (t, J = 4.4 Hz, 2 H), 3.97 (t, J = 6.0 Hz, 2 H), 3.87 (t, J = 4.4 Hz, 2 H), 3.76 (s, b, 2 H), 3.68 (d, J = 4.4 Hz, 2 H), 3.17 (d, J = 11.2 Hz, 2 H), 2.43 (d, J = 10.4 Hz, 2 H), 1.77 (p, J = 7.2 Hz, 2 H), 1.50 (six, J = 7.2 Hz, 4 H), 0.98 (t, J = 7.2 Hz, 6 H); ^{13}C NMR: 158.9, 158.3, 155.1, 126.99, 126.84, 117.05, 116.93, 116.10, 112.08, 112.05, 110.91, 110.39, 72.8, 69.8, 68.0, 67.7, 62.0, 36.94, 36.77, 31.5, 19.5, 14.1, 14.01. MS calcd for $C_{24}H_{28}O_4$ (M^+) 380.1988, EI-HRMS found 380.1982.

2-{2-[(9-Butoxy-5,6-didehydro-11,12-dihydrodibenzo[a,e][8]annulen-2-yl)oxy]ethoxy} Ethyl 3-Nitrophenyl Carbonate (13). A solution of pyridine (0.20 g, 2.60 mmol) in CH_2Cl_2 (~1 mL) was added to **12** (0.24 g, 0.63 mmol) and 4-nitrophenyl chloroformate (0.20 g, 1.00 mmol) in CH_2Cl_2 (5 mL), and the reaction mixture was stirred for 3 h. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (Hex:EtOAc 4:1) to provide **13** (0.34 g, 99%) as an oil. ^{1}H NMR: δ 8.25 (d, J = 8.8 Hz, 2 H), 7.36 (d, J = 9.2 Hz, 2 H), 7.19 (d, J = 8.8 Hz, 2 H), 6.89 (dd, J = 14.0, 2.4 Hz, 2 H), 6.79−6.75 (m, 2 H), 4.47 (t, J = 4.4 Hz, 2 H), 4.18 (t, J = 4.4 Hz, 2 H), 3.97 (t, J = 6.6 Hz, 2 H), 3.92−3.88 (m, 4 H), 3.17 (d, J = 10.8 Hz, 2 H), 2.42 (d, J = 10.8 Hz, 2 H), 1.77 (p, J = 7.2 Hz, 2 H), 1.49 (six, J = 7.2 Hz, 4 H), 0.98 (t, J = 7.2 Hz, 6 H); ^{13}C NMR: 158.9, 158.3, 155.7, 155.13, 155.08, 152.7, 145.6, 127.0, 126.9, 112.1, 121.9, 117.0, 116.97, 116.94, 112.15, 112.11, 112.00, 111.0, 110.3, 70.1, 69.1, 68.5, 68.0, 67.8, 36.9, 36.7, 31.5, 19.5, 14.2, 14.0.

2-{2-[(9-Butoxy-5,6-didehydro-11,12-dihydrodibenzo[a,e][8]annulen-2-yl)oxy]ethoxy}Ethyl{2-[2-(2-{[5-(2-Oxohexahydro-1H-thieno-[3,4-d]imidazol-4-yl)pentanoyl]amino}ethoxy)ethoxy]ethyl}carbamate (3b). A solution of carbonate **13** (0.15 g, 0.28 mmol) in DMF (2 mL) was added to a solution of Et_3N (0.5 g, 4.95 mmol) and *N*-biotinyl-3,6-dioxaoctane-1,8-diamine[9] (0.01 g, 0.28 mmol) in DMF (10 mL) The reaction mixture was stirred for 18 h at ambient temperature and then the solvents were evaporated under reduced pressure, and the residue purified by silica gel chromatography (CH_2Cl_2:MeOH 30:1) to provide **3b** (0.164 g, 75%). ^{1}H NMR: 7.19 (d, J = 8.4 Hz, 2H), 6.88 (dd, J = 9.5, 2.5 Hz, 2H), 6.76 (td, J = 8.2, 2.5, 2H), 6.74 - 6.65 (m, 1H), 6.54 (s, b, 1H), 5.74 (s, b, 1H), 5.60 (s, b 1 H), 4.49 - 4.43 (m, 1H), 4.29−4.22 (m, 3H), 4.16 - 4.10 (m, 2H), 3.97 (t, J = 6.5, 2H), 3.87 - 3.81 (m, 2H), 3.76 (m, 2H), 3.59−3.48 (m, 10H), 3.42 (m, 2H), 3.37 - 3.12 (m, 2H), 3.21 - 3.09 (m, 4H), 2.86 (dd, J = 12.6, 4.7 Hz, 1H), 2.72 (d, J = 12.7, 1H), 2.42 (d, J = 10.9, 2H), 2.21 (t, J = 7.4, 4H), 1.81−1.56 (m, 6H), 1.48 (six, J = 7.4 Hz, 2H), 1.44−1.36 (m, 2H), 1.32 (t, J = 7.4 Hz, 1H), 0.98 (t, J = 7.4, 3H); ^{13}C NMR: 173.4, 164.1, 158.7, 158.1, 156.5, 154.8, 126.66, 126.63, 116.80, 116.72, 116.59, 115.8, 111.91, 111.83, 110.67, 110.14, 70.09, 70.04, 69.95, 69.90, 69.80, 69.54, 67.78, 67.52, 63.88, 61.80, 60.2, 55.6, 45.6, 40.8, 40.5, 39.1, 36.63, 36.61, 35.9, 31.3, 28.22, 28.08, 25.6, 19.2, 13.8, 8.5. MS calcd for $C_{41}H_{56}N_4O_9S$ (M^+ + Na) 803.3666, ESI-HRMS found 803.3672.

General Procedure for Preparative Photolyses of Cyclopropenones 2: 3,9-Dibutoxy-5,6-didehydro-11,12-dihydrodibenzo[a,e]-[8]annulen-2-yl (3c). A solution of cyclopropenone **2c** (0.20 g, 0.532 mmol) in MeOH (20 mL, 2.72×10^{-2}M) was irradiated (4 × 350 nm) for 20 min at r.t. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (Hex:EtOAc 1:20) to provide **3c** (0.160 g, 86%) as a slightly yellow oil. ^{1}H NMR: 7.19 (d, J = 8.4 Hz, 2 H), 6.87 (d, J = 2.4 Hz, 2 H), 6.75 (dd, J = 8.4, 2.4 Hz, 2 H), 3.97 (t, J = 6.4 Hz, 4 H), 3.18 (d, J = 11.2 Hz, 2 H), 2.44 (d, J = 11.2 Hz, 2 H), 1.77 (p, J = 7.2 Hz, 4 H), 1.52 (six, J = 7.2 Hz, 4 H), 0.98 (t, J = 7.2 Hz, 6 H); ^{13}C NMR: 158.9, 155.1, 126.9, 116.9, 116.2, 112.0, 110.6, 68.0, 36.9, 31.5, 19.5, 14.1.

General Procedure for the Preparation of Triazoles 4. A solution of **3c** (0.5 mmol) and appropriate organic azide (0.75 mmol) in MeOH was stirred for 18 h at r.t. The solvent was evaporated under reduced pressure, and the excess of azide was removed by silica gel column chromatography.

1-Phenyl-6,11-dibutoxy-8,9-dihydro-1H-dibenzo[3,4:7,8]cycloocta[1,2-d][1,2,3]triazoles (4a, R = Ph). ^{1}H NMR: δ 7.53 (d, J = 8.8 Hz, 1 H), 7.39 (s, 5 H), 6.85 (d, J = 2.4 Hz, 1 H), 6.79 (dd, J = 8.4, 2.4 Hz, 1 H), 6.74 (d, J = 2.4 Hz, 1 H), 6.62 (d, J = 8.8 Hz, 1 H), 6.51 (dd, J = 8.4, 2.8 Hz, 1 H), 3.94 (t, J = 6.4 Hz, 2 H), 3.89 (t, J = 6.4 Hz, 2 H), 3.50−3.30 (m, 2 H), 3.17−2.92 (m, 2 H), 1.78−1.68 (m, 4 H), 1.46 (sep, J = 7.2 Hz, 4 H), 0.96 (t, J = 7.2 Hz, 3 H), 0.95 (t, J = 7.2 Hz, 3 H); ^{13}C NMR: 159.9, 159.2, 147.0, 142.5, 139.7, 137.0, 133.6, 133.0, 131.8, 129.5, 128.8, 124.8, 122.5, 118.8, 116.5, 115.8, 112.8, 112.6, 67.81, 67.77, 36.2, 34.2, 31.5, 19.47, 19.45, 14.10, 14.07.

6,11-Dibutoxy-1-butyl-8,9-dihydro-1H-dibenzo[3,4:7,8]cycloocta[1,2-d][1,2,3]triazole (4a, R = *n*-Bu). ^{1}H NMR: δ 7.43 (d, J = 8.4 Hz, 1 H), 7.06 (d, J = 8.4 Hz, 1 H), 6.87 (d, J = 2.4 Hz, 1 H), 6.78 (dd, J = 8.4, 2.4 Hz, 1 H), 6.75 (dd, J = 8.4, 2.4 Hz, 1 H) 6.67 (d, J = 2.4 Hz, 1 H), 4.42−4.24 (m, 2 H), 3.96 (t, J = 6.4 Hz, 2 H), 3.93 (t, J = 6.8 Hz, 2 H), 3.40−3.32 (m, 1 H), 3.14−2.98 (m, 2 H), 2.88−2.78 (m, 1 H), 1.86−1.68 (m, 6 H), 1.54−1.41 (m, 4 H), 1.34−1.18 (m, 2 H), 0.98 (t, J = 7.6 Hz, 3 H), 0.95 (t, J = 7.2 Hz, 3 H), 0.85 (t, J = 7.2 Hz, 3 H); ^{13}C NMR: 160.1, 158.9, 146.6, 143.3, 139.2, 133.6, 133.2, 130.2, 122.8, 118.9, 116.6,

115.9, 112.9, 112.5, 67.9, 67.7, 48.2, 36.9, 33.4, 32.3, 31.55, 31.50, 19.8, 19.5, 14.1, 13.7.

General Procedures for Biological Experiments. Synthetic compounds **1b**, **2b**, and **3b** were reconstituted in DMF and stored at −80 °C. Final concentrations of DMF never exceeded 0.56% to avoid toxic effects. The *in situ* photoactivation of biotinylated cyclopropenone **2b** was performed using a mini-Rayonet photoreactor equipped with two 350 nm fluorescent tubes (4W). The irradiated cell suspensions were kept in plastic vials, which served as an additional short band-path filter. The vial wall absorbs ca. 60% of light at 350 nm, 70% at 300 nm, and is virtually not transparent below 275 nm.

Cell Culture Conditions. Human Jurkat cells (Clone E6−1; ATCC) were cultured in RPMI 1640 medium (ATCC) with L-glutamine (2 mM), adjusted to contain sodium bicarbonate (1.5 g/L), glucose (4.5 g/L), HEPES (10 mM), and sodium pyruvate (1 mM) and supplemented with penicillin (100 u/ml)/streptomycin (100 μg/mL; Mediatech) and fetal bovine serum (FBS, 10%; Hyclone). Chinese hamster ovary (CHO) cells (Clone K1; ATCC) were cultured in Kaighn's modification of Ham's F-12 medium (F-12K) with L-glutamine (2 mM), adjusted to contain sodium bicarbonate (1.5 g L^{-1}) and supplemented with penicillin (100 μg mL^{-1})/streptomycin (100 μg mL^{-1}) and FBS (10%). Cells were maintained in a humid 5% CO_2 atmosphere at 37 °C.

Cell Surface Azide Labeling. Jurkat cells were seeded at a density of 75,000 cells mL^{-1} in a total volume of 40 mL culture medium in the presence of peracetylated *N*-azidoacetylmannosamine (Ac_4ManNaz; 25 μM final concentration) and grown for 3 days, leading to the metabolic incorporation of the corresponding *N*-azidoacetyl sialic acid (SiaNAz) into their cell surface glycoproteins. Control cells were grown in the presence of peracetylated *N*-acetylmannosamine (Ac_4ManNac; 25 μM final concentration) for 3 days. Similarly, CHO cells were grown for 3 days in the presence of Ac_4ManNaz (100 μM final concentration) or Ac_4ManNac (100 μM final concentration).

Click Chemistry and Detection by Fluorescence Intensity. Jurkat cells bearing azides and control cells were washed with labeling buffer (DPBS, pH 7.4 containing 1% FBS and 1% BSA), transferred to round-bottom tubes (1×10^6 cells/sample) and incubated with the biotinylated compounds **1b**, **2b**, or **3b** (0−100 μM) in labeling buffer for 0−90 min at r.t. To activate **2b** *in situ*, the cell suspension was subjected to UV light (350 nm) for 1 min immediately after adding the compound to the cells, unless stated otherwise. The cells were washed three times with cold labeling buffer and then incubated with avidin conjugated with fluorescein (0.5 μg/mL; Molecular Probes) for 15 min at 4 °C. Following three washes, cells were either lysed in passive lysis buffer (Promega) and cell lysates were analyzed for fluorescence intensity (485 ex/520 em) using a microplate reader (BMG Labtech) or live cells were assessed by flow cytometry using the FACSCalibur flow cytometer (Becton Dickinson Immunocytometry Systems) and data analysis was performed with FlowJo software (Tree Star, Inc.). Data points were collected in triplicate and are representative of three separate experiments. Fluorescence of Jurkat cell lysates was expressed as fluorescence (arbitrary units; AU) per 800 000 cells.

Measurement of Cytotoxicity. Cell viability and cell morphology were assessed by exclusion of trypan blue and microscopic evaluation immediately after photoactivation or after reincubation of the labeled cells in cell culture medium for 5 or 24 h. After the reincubation, viability was measured by quantifying the cellular ability to reduce the water-soluble tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) to its insoluble formazan salt.[25] Data points were collected in triplicate and expressed as normalized values for control cells (100%).

Western Blot Analysis. Jurkat cells were harvested by centrifugation (5 min at 500× *g*) and resuspended as 5×10^6 cells/mL. The cell suspensions (200 μL per sample) were incubated with biotin-conjugated alkynes **1b**, **2b**, and **3b** (30 μM) or without compound as control for 1 h. To activate **2b** *in situ*, immediately after adding the compound to the cells, the cell suspension was subjected to UV light (350 nm) for 1 min. The cells were washed (4 × 10 min) with cold DPBS, pH 7.4 containing FBS (1%) and lysed in passive lysis buffer. The cell lysates were clarified by centrifugation at 22 000× *g* for 15 min and the total protein content of the clear supernatants was assessed using the bicinchonic acid assay (BCA; Pierce Biotechnology). Cell lysate samples (20 μg protein) in SDS-PAGE sample buffer containing 2-mercaptoethanol were boiled for 5 min, resolved on a 4−20% Tris-HCl gel (Bio-Rad) and transferred to nitrocellulose membrane. Next the membrane was blocked in blocking buffer (nonfat dry milk (5%; Bio-Rad) in PBST (PBS containing 0.1% Tween-20 and 0.1% Triton X-100)) for 2 h at r.t. The blocked membrane was incubated for 1 h at r.t. with an antibiotin antibody conjugated to horseradish peroxidase (HRP) (1:100 000; Jackson ImmunoResearch Lab, Inc.) in blocking buffer and washed with PBST (4 × 10 min). Final detection of HRP activity was performed using ECL Plus chemiluminescent substrate (Amersham), exposure to film (Kodak) and development using a digital X-ray imaging machine (Kodak). Next the blot was stripped and reprobed for loading control (β-actin) as described above. Coomassie staining was used to confirm total protein loading.

Detection of Cell Labeling by Fluorescence Microscopy. CHO cells bearing azides and untreated control cells were transferred to glass coverslips and cultured for 36 h in their original medium. Live CHO cells were treated with the biotinylated compound **2b** (30 μM) in labeling buffer (DPBS, supplemented with FBS (1%)) for 1 h at r.t. To activate **2b** *in situ*, immediately after adding the compound to the cells, the cells were subjected to UV light (350 nm) for 1 min. Next, the cells were incubated with avidin conjugated with Alexa Fluor 488 (Molecular Probes) for 15 min at 4 °C. Cells were washes 3 times with labeling buffer and fixed with formaldehyde (3.7% in PBS). The nucleus was labeled with the far red-fluorescent TO-PRO-3 dye (Molecular Probes). The cells were mounted with PermaFluor (Thermo Electron Corporation) before imaging. Initial analysis was performed on a Zeiss Axioplan2 fluorescent microscope. Confocal images were acquired using a 60× (NA1.42) oil objective. Stacks of optical sections were collected in the *z* dimensions. The step size, based on the calculated optimum for each objective, was between 0.25 and 0.5 μm. Subsequently, each stack was collapsed into a single image (*z*-projection). Analysis was performed offline using ImageJ 1.39f software (National Institutes of Health, USA) and Adobe Photoshop CS3 Extended Version 10.0 (Adobe Systems Incorporated), whereby all images were treated equally.

Statistical Analysis. Statistical significance between groups was determined by two-tailed, unpaired Student's *t* test. Differences were considered significant when $P < 0.05$.

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Supporting Information Available: Figures S1−S6 and copies of NMR spectra of newly synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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