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A Route to Cyclooct-2-ynol and Its Functionalization by Mitsunobu Chemistry

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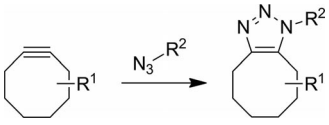
A new silver-free synthesis for cyclooctynol is introduced. The obtained alcohol was further functionalized by a Mitsunobu reaction to give an assortment of imide and phenol derivatives. It was also possible to further functionalize the cyclooctyne by treatment with a fluorescein dye. As an interesting application, we used a cyclooctyne-maleimide conjugate as an azide-thiol crosslinker.

Introduction

Since Sharpless[1] and Meldal[2] introduced the concept, the copper-catalyzed azide–alkyne cycloaddition has fast become a powerful tool in material science,[3] organic synthesis, and chemical biology.[3a,4] A major drawback, however, arises when it is applied to living organisms, as the required copper salts are often cytotoxic. A copper-free variant that overcomes this problem is the strain-promoted 1,3-dipolar cycloaddition (SPAAC) of cyclooctynes with azides (see Scheme 1). This variation, first introduced by Bertozzi[5] and based on the pioneering work of Wittig and Krebs,[6] has now become one of the most important tools in the field of bioconjugation,[7] as shown by the fact that various biomolecules or whole cells have been functionalized by the use of cyclooctynes.[5,8] Besides the biological applications, cyclooctynes have also found applications in material science[9] when the absence of copper is required.



Scheme 1. Strain-promoted 1,3-dipolar cycloaddition.

Since its introduction in 2004, the cyclooctyne family has steadily grown, and various systems presently exist. The first employed systems were alkynes with a propargylic ether group (i.e., **1**), which are normally synthesized by a silver-mediated ring-opening reaction (see Figure 1).[5] Further variants include systems that contain one (i.e., **2**)[10] or two (i.e., **3**)[11] fluorine atoms in the propargylic position and an annelated benzene[12] (i.e., **4** and **5**) or cyclopropane ring[13] (i.e., **6**). With these modifications, the reaction rate can be tuned by either electronic effects or ring strain, thus, enabling a reaction rate that is comparable to the copper-catalyzed variant. The substituents can also influence the solubility, which is especially important in the biological context in which reactions are carried out in water or buffer solutions.

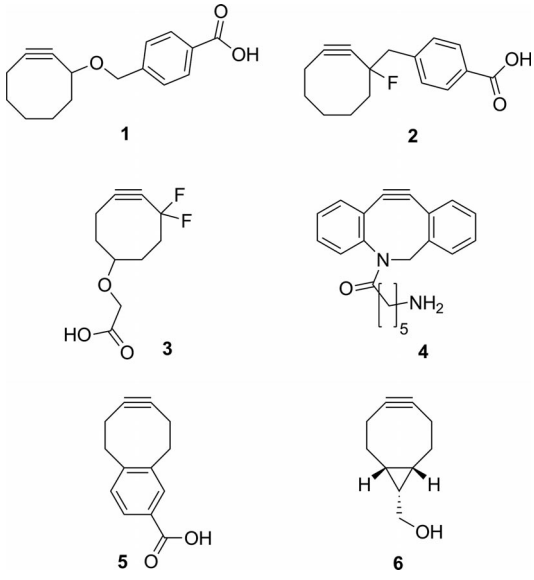


Figure 1. Different generations of cyclooctynes.

During our studies in the field of cyclooctyne chemistry, we regularly encountered problems with the silver-promoted ring-opening of 8,8-dibromobicyclooctane **7** in the reaction sequence towards cyclooctynol and its derivatives.[14] Consequently, we tried to establish a silver-free route to afford cyclooctynol **13**. Although the cyclooctynol derivatives are slow to react in a SPAAC reaction [1.3×10^{-3} M s^{-1} vs. 310×10^{-3} M s^{-1} for dibenzoazacyclooct-

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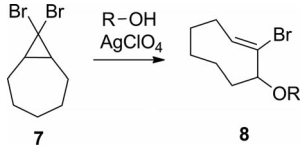
yne (DIBAC[15])], they are still used.[16] Furthermore, we designed a new synthetic pathway that allows for new substituents in the propargylic position.

Results and Discussion

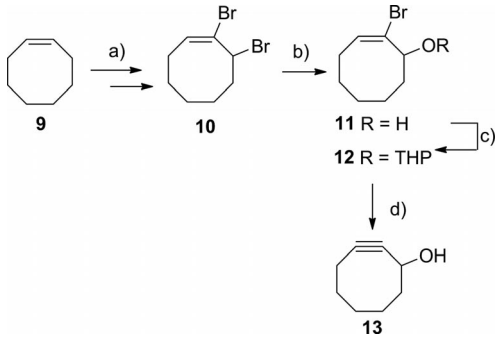
We were interested in a silver-free synthesis of cyclooct-2-ynol and its derivatives because known procedures require the use of an excess amount of silver in the form of expensive silver perchlorate. Furthermore, we experienced problems obtaining the desired (*Z*)-bromocyclooctenes **8** in many experiments that used silver salts (see Scheme 2). We were able to obtain solely the (*Z*) isomer from our very first experiment, however, the reproduction failed. Thus, we carried out the reaction in the dark under various reaction conditions:
(1) The solvent was either pure methanol or a methanolic solution in toluene (9:5 v/v).
(2) Either 2 or 3 equiv. of the silver salt were used with regard to dibromide **7**.
(3) The reaction temperature was set at either 0 °C or room temp.
(4) The mixture was stirred for a reaction time of 30 min or up to 3 h.
(5) The scale of the reaction was varied with regard to dibromide **7** (between 0.100 and 5.00 g).
(6) Different batches of the silver salt were employed.



Scheme 2. Key step in the silver-promoted synthesis of cyclooctynes.

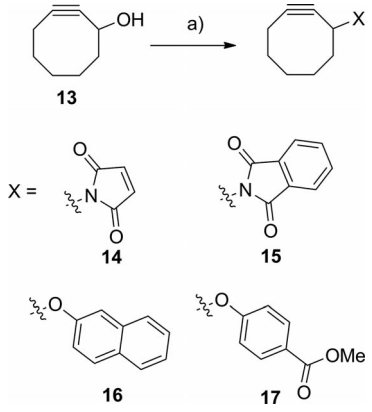
In every case, mixtures of (*E*) and (*Z*) isomers were formed, and these were not suitable for further transformations. When the mixture was treated with NaH to allow for an elimination reaction, the reactant mixture was isolated again without the formation of the alkyne. When lithium diisopropylamide (LDA) was used as the base, only decomposition took place, and no reaction products could be identified.

To avoid this problem, we established a route to cyclooct-2-ynol (**13**) by starting from cyclooctene (**9**; see Scheme 3). The first steps comprised a Wohl–Ziegler bromination followed by a sequence of bromination and elimination reactions as described in the literature.[17] Next, the hydrolysis of dibromocyclooctene **10** to give the corresponding alcohol **11** was carried out by using a DMSO/water mixture.[18] Alcohol **11** was then protected by treatment with dihydropyran to obtain the THP-protected alcohol **12**. After the protection step, the vinylic (*E*)-bromide **12** could undergo an elimination reaction by treatment with LDA to yield the corresponding cyclooctyne. When an equimolar amount of bromide **12** and LDA was used, only decomposition of the reactant was observed, and no reaction products were identified. Because of this, the amount of LDA was decreased to 0.5 equiv. After the elimination reaction, a mixture was obtained of the reactant and product, which could not be separated by flash column chromatography. This mixture then underwent a deprotection step, and after the removal of the THP protecting group, the resulting alcohols **11** and **13** could be separated to yield the desired cyclooct-2-ynol (**13**).



Scheme 3. Reagents and conditions for the cyclooctynol synthesis: (a) *N*-bromosuccinimide (NBS), azobis(isobutyronitrile) (AIBN), CCl_4, 2 h, reflux, 51%; then (1) Br_2, dichloromethane (DCM), –40 °C, 1 h; (2) KO*t*Bu, tetrahydrofuran (THF), –50 °C, 3 h, 52%, see Banert et al.[17]; (b) $CuSO_4 \cdot 5H_2O$, dimethyl sulfoxide (DMSO), H_2O, reflux, 2 h, 51%; (c) Dihydropyran (DHP), Py·TosOH, DCM, 3 h, 99% (THP = tetrahydropyran); d) (1) LDA, THF, –78 °C to r.t., 12 h; (2) TosOH, MeOH, 2 h, room temp., 36% over two steps.

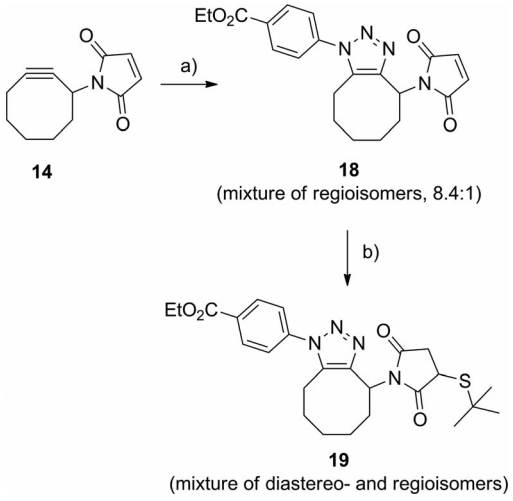
With the cyclooct-2-ynol (**13**) in hand, we decided to convert the alcohol moiety into imide and phenol functional groups by employing a Mitsunobu reaction (see Scheme 4).[19] By treating the alcohol with the corresponding nucleophiles in the presence of diisopropyl azodicarboxylate (DIAD) and PPh_3 in THF as the solvent, the Mitsunobu reaction was carried out. As a result, we obtained different cyclooctynes with imide (i.e., **14** and **15**), naphthol (i.e., **16**), and phenol (i.e., **17**) substitutents.



Scheme 4. Reagents and conditions for Mitsunobu reactions: (a) PPh_3, DIAD, phenol or imide derivative, THF, 12 h, room temp., 44% (**14**), 38% (**15**), 65% (**16**), and 55% yield (**17**).

The maleimide functionalized cyclooctyne **14** proved especially interesting because of its potential use as a crosslinker that can undergo reactions with azides and thi-

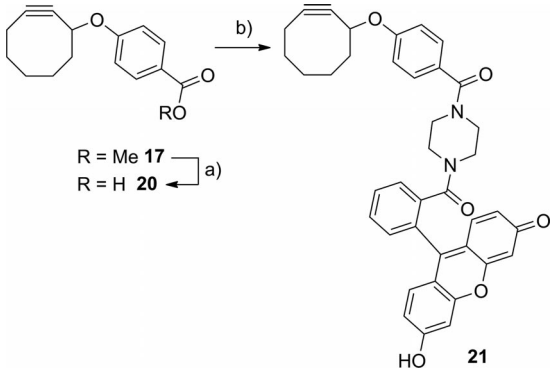
ols. To determine whether the maleimide conjugate was reactive towards azides and thiols, we carried out a click reaction followed by a subsequent reaction between a thiol and the maleimide. As an azide we chose ethyl 4-azidobenzoate. The cycloaddition afforded **18** as a mixture of regioisomers (8.4:1) that was then used in a further reaction with *tert*-butylthiol. The addition of the thiol to maleimide **18** worked out well, and we could, therefore, prove that the new cyclooctyne-maleimide **14** could be used as a crosslinker between azides and thiols (see Scheme 5). Because cyclooctynol **13** was racemic, we obtained a mixture of diastereomers when the thiol was treated with the maleimide.



Scheme 5. Reagents and conditions: a) 4-azidobenzoate, THF, 12 h, room temp., 28%; b) *tert*-butylthiol, DMSO, 12 h, room temp., 98%.

We also attempted the use of thiols as nucleophiles in the Mitsunobu reaction of alcohol **13**. However, only an addition reaction between the thiol and alkyne was observed instead of the desired coupling product (not shown).[20]

As cyclooctyne-dye conjugates are constantly used in cell labeling, we also wanted to link our cyclooctynol derivative to a dye molecule. Ester **17** underwent a saponification reaction, and the resulting acid was linked to an amine-functionalized fluorescein[21] by using standard peptide coupling reagents (see Scheme 6).



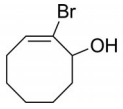
Scheme 6. Attachment of fluorescein-piperazine amide to acid **20**. Reagents and conditions: (a) NaOH, MeOH/THF/water, 50 °C, 3 h, 65%; b) *N*,*N*′-diisopropylcarbodiimide (DIC), 1-hydroxybenzotriazole (HOBt), *N*,*N*-dimethylformamide (DMF), room temp., 12 h 43%.

Conclusions

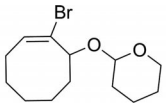
We introduced a new synthesis of cyclooct-2-ynol that does not require expensive silver salts in the process. Furthermore, under Mitsunobu conditions, we were able to substitute the alcohol group of cyclooct-2-ynol with different functionalities such as imides and phenols. We were also able to synthesize dye-functionalized cyclooctynes by using this method.

Experimental Section

General Methods: All organic chemicals were purchased from ThermoFisher, ABCR, Alfa Aesar, or Sigma Aldrich. The NMR spectroscopic data were recorded with a Bruker Avance 300 (300 MHz) or Bruker AM 400 (400 MHz) spectrometer, and $CDCl_3$ or [D_6]DMSO was used as the solvent. Mass spectrometry, FAB-MS and EI-MS, was performed with a Finnigan MAT 90 mass spectrometer. Infrared spectroscopy was carried out with a Bruker IFS 88 device.

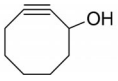


(*E*)-2-Bromocyclooct-2-enol (11): (*E*)-1,8-Dibromocyclooct-1-ene (30.0 g, 0.112 mol, 1.00 equiv.) was dissolved in water (40 mL) and DMSO (100 mL). Copper sulfate pentahydrate (37.0 g, 0.149 mol, 1.33 equiv.) was added, and the mixture was heated to 100 °C for 2 h. After this time, the reaction mixture was cooled to room temperature, and then water (100 mL) and ethyl acetate (100 mL) were added. The organic phase was separated and washed with water (5× 100 mL). After drying the organic phase with Na_2SO_4, the solvent was removed, and the crude product was purified using flash chromatography (silica gel, 20 × 8 cm; cyclohexane/ethyl acetate, 10:1) to afford **11** (11.7 g, 51% yield) as a colorless liquid; R_f = 0.20 [cyclohexane (CH)/diethyl ether (EE), 10:1]. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.18 (ddd, $^3J_{H,H}$ = 8.8 Hz, $^3J_{H,H}$ = 8.8 Hz, $^4J_{H,H}$ = 0.8 Hz, 1 H, CH), 4.65 (dd, $^3J_{H,H}$ = 11.1 Hz, $^3J_{H,H}$ = 4.8 Hz, 1 H, C*H*OH), 2.27–2.10 (m, 2 H, CH_2), 1.93 (br. s, 1 H, OH), 1.84–1.55 (m, 5 H, CH_2), 1.44–1.27 (m, 3 H, CH_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 132.1 (+, *C*H=CBr), 129.7 (C_{quat}, CBr), 68.8 (+, COH), 36.6 (–, CH_2), 30.3 (–, CH_2), 28.9 (–, CH_2), 26.8 (–, CH_2), 23.9 (–, CH_2) ppm. IR [attenuated total reflectance (ATR) platinum diamond): $\tilde{\nu}$ = 3383 (m), 2927 (s), 2851 (m), 1636 (w), 1450 (w), 1063 (m) cm^{-1}. MS (EI, 70 eV): *m*/*z* (%) = 204/206 (3/3) [M]$^+$. HRMS (EI): calcd. for $C_8H_{13}OBr$ 204.0150; found 204.0147.

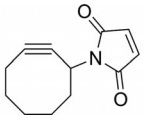


(*E*)-2-[(2-Bromocyclooct-2-en-1-yl)oxy]tetrahydro-2*H*-pyran (12): Alcohol **11** (0.410 g, 2.00 mmol, 1.00 equiv.), 3,4-dihydropyran (0.252 g, 3.00 mmol, 1.50 equiv.), and pyridinium tosylate (0.050 g, 0.200 mmol, 0.100 equiv.) were stirred in DCM (16 mL) for 2 h.

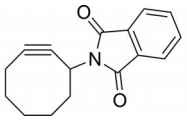
The solvent was removed, and the crude product was purified by flash chromatography (silica gel, 20 × 2 cm; cyclohexane/ethyl acetate, 10:1) to afford a mixture of diastereomers of **12** (0.573 g, 99% yield) as a colorless liquid; R_f = 0.48 (CH/EE, 10:1). ^{1}H NMR: (400 MHz, $CDCl_3$): δ = 6.29 (dd, $^3J_{H,H}$ = 8.8 Hz, $^3J_{H,H}$ = 8.8 Hz, 0.67 CH=CBr), 6.17 (dd, $^3J_{H,H}$ = 8.9 Hz, $^3J_{H,H}$ = 8.9 Hz, 0.06 H, CH=CBr), 6.13 (dd, $^3J_{H,H}$ = 8.8 Hz, $^3J_{H,H}$ = 8.8 Hz, 0.28 H, CH=CBr), 4.86–4.78 (m, 1 H, OCHO), 4.66 (dd, $^3J_{H,H}$ = 7.9 Hz, $^3J_{H,H}$ = 7.9 Hz, 0.37 H, CHO), 4.56 (dd, $^3J_{H,H}$ = 4.7 Hz, $^3J_{H,H}$ = 2.9 Hz, 0.66 H, CHO), 3.94–3.85 (m, 1 H, OCH_2), 3.57–3.45 (m, 1 H, OCH_2), 2.29–2.09 (m, 2 H, CH_2), 1.93–1.83 (m, 1 H, CH_2), 1.83–1.59 (m, 7 H, CH_2), 1.59–1.47 (m, 3 H, CH_2), 1.42–1.28 (m, 3 H, CH_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 135.0 (+, *C*H=CBr), 131.9 (+, *C*H=CBr), 127.6 (C_{quat}, CBr), 127.5 (C_{quat}, CBr), 97.1 (+, OCHO), 96.3 (+, OCHO), 72.5 (+, CHO), 70.9 (+, CHO), 62.9 (–, OCH_2), 62.1 (–, OCH_2), 33.7 (–, CH_2), 33.4 (–, CH_2), 30.6 (–, CH_2), 30.5 (–, CH_2), 30.4 (–, CH_2), 30.2 (–, CH_2), 29.1 (–, CH_2), 28.9 (–, CH_2), 27.0 (–, CH_2), 26.9 (–, CH_2), 25.5 (–, CH_2), 25.5 (–, CH_2), 23.7 (–, CH_2), 23.5 (–, CH_2), 19.6 (–, CH_2), 18.9 (–, CH_2) ppm. IR (ATR platinum diamond): $\tilde{\nu}$ = 3442 (w), 2928 (s), 2852 (m), 1636 (w), 1452 (m) cm^{-1}. MS (EI, 70 eV): *m*/*z* (%) = 209 (7) $[M - Br]^+$, 85 (100) $[C_5H_9O]^+$. HRMS (EI): calcd. for $C_{13}H_{21}BrO_2$ 288.0725; found 288.0723.



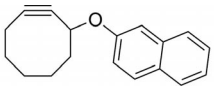
Cyclooct-2-ynol (13): A solution of LDA (5.72 mmol, 1.00 equiv.) in THF (8 mL) was cooled to –78 °C, and a solution of protected alcohol **12** (3.31 g, 11.5 mmol, 2.00 equiv.) in dry THF (10 mL) was added dropwise under argon. The cooling bath was removed, and the mixture was stirred overnight. Water (20 mL) was then added, and the mixture was extracted with ethyl acetate (3 × 15 mL). The organic phase was separated and dried with sodium sulfate, and the solvent was removed. The residue was purified by flash chromatography (silica gel; cyclohexane/ethyl acetate, 10:1; R_f = 0.48) to afford a mixture of the protected alkynol and **12**. This mixture was dissolved in methanol (30 mL), and *p*-toluenesulfonic acid (0.336 g, 1.77 mmol) was added. The mixture was stirred for 3 h. Water (50 mL) was added, and the aqueous phase was extracted with ethyl acetate (3 × 20 mL). The organic phase was dried with sodium sulfate, and the crude product was purified by flash chromatography (silica gel, 40 × 2 cm; cyclohexane/ethyl acetate, 10:1) to afford **13** (0.336 g, 36% yield over two steps with regard to 1.00 equiv. of LDA) as a colorless oil; R_f = 0.10 (CH/EE, 10:1). ^{1}H NMR: (400 MHz, $CDCl_3$): δ = 4.47–4.39 (m, 1 H, C*H*OH), 2.37 (br. s, 1 H, OH), 2.28–2.04 (m, 2 H, CH_2), 1.97–1.80 (m, 3 H, CH_2), 1.78–1.70 (m, 1 H, CH_2), 1.70–1.53 (m, 2 H, CH_2), 1.54–1.27 (m, 2 H, CH_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 99.7 (C_{quat}, C-alkyne), 94.1 (C_{quat}, C-alkyne), 64.6 (+, COH), 45.2 (–, CH_2), 34.4 (–, CH_2), 29.7 (–, CH_2), 25.9 (–, CH_2), 20.7 (–, CH_2) ppm. IR (ATR platinum diamond): $\tilde{\nu}$ = 3386 (m), 2927 (s), 2852 (m), 2211 (w), 1707 (w), 1449 (m) cm^{-1}. MS (EI, 70 eV): *m*/*z* (%) = 125 (3) $[M + H]^+$, 124 (3) $[M]^+$, 95 (100) $[C_7H_{11}]^+$. HRMS (EI): calcd. for $C_8H_{12}O$ 124.0888; found 124.0890.



1-(Cyclooct-2-yn-1-yl)-1*H*-pyrrole-2,5-dione (14): To a solution of alcohol **13** (0.160 g, 1.30 mmol, 1.00 equiv.) in dry THF (15 mL) under argon were added maleimide (0.164 g, 1.69 mmol, 1.30 equiv.) and triphenylphosphine (0.440 g, 1.69 mmol, 1.30 equiv.), and the solution was cooled to 0 °C. Then, DIAD (0.328 g, 1.62 mmol, 1.25 equiv.) was added, and the solution was warmed to room temperature overnight. The solvent was removed, and the crude product was purified by flash chromatography (silica gel, 20 × 2 cm; cyclohexane/ethyl acetate, 100:1) to afford **14** (0.116 g, 44% yield) as a white solid; m.p. 85.7 °C. R_f = 0.23 (CH/EE, 20:1). ^{1}H NMR: (300 MHz, $CDCl_3$): δ = 6.65 (s, 2 H, C*H*=C*H*), 4.99–4.86 (m, 1 H, CHN), 2.44–2.21 (m, 2 H, CH_2), 2.21–1.94 (m, 5 H, CH_2), 1.82–1.62 (m, 1 H, CH_2), 1.53–1.36 (m, 1 H, CH_2), 1.34–1.37 (m, 1 H, CH_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.7 (C_{quat}, C=O), 134.1 (+, CH), 100.6 (C_{quat}, C-alkyne), 88.1 (C_{quat}, C-alkyne), 44.0 (+, CHN), 40.3 (–, CH_2), 34.4 (–, CH_2), 29.1 (–, CH_2), 28.4 (–, CH_2), 20.6 (–, CH_2) ppm. IR (ATR platinum diamond): $\tilde{\nu}$ = 2926 (w), 2852 (w), 1700 (s), 1439 (w), 1387 (w), 1360 (w), 1170 (w), 1138 (w) cm^{-1}. MS (EI, 70 eV): *m*/*z* (%) = 203 (68) $[M]^+$, 175 (100) $[C_{11}H_{13}NO]^+$. HRMS (EI): calcd. for $C_{12}H_{13}NO_2$ 203.0946; found 203.0947.

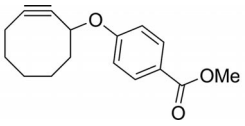


2-(Cyclooct-2-yn-1-yl)isoindoline-1,3-dione (15): To a solution of alcohol **13** (0.100 g, 0.810 mmol, 1.00 equiv.) in dry THF (8 mL) under argon were added phthalimide (0.155 g, 1.05 mmol, 1.30 equiv.) and triphenylphosphine (0.276 g, 1.05 mmol, 1.30 equiv.), and the solution was cooled to 0 °C. Then, DIAD (0.205 g, 1.02 mmol, 1.25 equiv.) was added, and the solution was warmed to room temperature overnight. The solvent was removed, and the crude product was purified by flash chromatography (silica gel, 20 × 2 cm; cyclohexane/ethyl acetate, 50:1) to afford **15** (0.077 g, 38% yield) as a white solid; m.p. 179.4 °C. R_f = 0.41 (CH/EE, 10:1). ^{1}H NMR: (400 MHz, $CDCl_3$): δ = 7.84–7.78 (m, 2 H, H-Ar), 7.72–7.67 (m, 2 H, H-Ar), 5.18–5.10 (m, 1 H, CHN), 2.46–2.31 (m, 2 H, CH_2), 2.23–2.00 (m, 5 H, CH_2), 1.83–1.66 (m, 1 H, CH_2), 1.56–1.46 (m, 1 H, CH_2), 1.38–1.24 (m, 1 H, CH_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.3 (C_{quat}, CO), 133.9 (+, C-Ar), 131.9 (C_{quat}, C-Ar), 123.2 (+, C-Ar), 100.4 (C_{quat}, C-alkyne), 88.4 (C_{quat}, C-alkyne), 44.1 (+, CHO), 40.2 (–, CH_2), 34.5 (–, CH_2), 29.2 (–, CH_2), 28.5 (–, CH_2), 20.7 (–, CH_2) ppm. IR (ATR platinum diamond): $\tilde{\nu}$ = 3464 (w), 2929 (w), 2851 (w), 2214 (w), 1775 (w), 1708 (s), 1367 (m), 1329 (m) cm^{-1}. MS (EI, 70 eV): *m*/*z* (%) = 254 (67) $[M + H]^+$, 253 (65) $[M]^+$, 225 $[M - CO]^+$, 77 (100). HRMS (EI): calcd. for $C_{16}H_{15}NO_2$ 253.1103; found 253.1100.

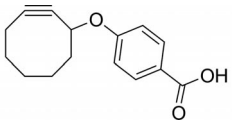


2-(Cyclooct-2-yn-1-yloxy)naphthalene (16): To a solution of alcohol **13** (0.080 g, 0.650 mmol, 1.0 equiv.) in dry THF (8 mL) under argon were added 2-naphthol (0.121 g, 0.850 mmol, 1.30 equiv.) and triphenylphosphine (0.220 g, 0.840 mmol, 1.30 equiv.), and the solution was cooled to 0 °C. Then, DIAD (0.205 g, 1.02 mmol, 1.57 equiv.) was added, and the solution was warmed to room temperature overnight. The solvent was removed, and the crude product was purified by flash chromatography (silica gel, 20 × 2 cm; cyclohexane/ethyl acetate, 100:1) to afford **13** (0.103 g, 65% yield) as a light yellow oil; R_f = 0.77 (CH/EE, 20:1). ^{1}H NMR: (400 MHz, $CDCl_3$): δ = 7.73–7.59 (m, 3 H, H-Ar), 7.40–7.31 (m, 1 H, H-Ar), 7.28–7.20 (m, 1 H, H-Ar), 7.17–7.12 (m, 1 H, H-Ar), 7.10–7.04 (m, 1 H, H-Ar), 4.88–4.81 (m, 1 H, CHO), 2.36–2.04 (m, 4 H, CH_2), 1.95–1.76 (m, 3 H, CH_2), 1.74–1.51 (m, 3 H, CH_2) ppm. ^{13}C NMR

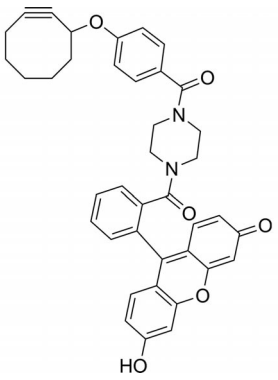
(100 MHz, $CDCl_3$): δ = 155.5 (C_{quat}, C-O/C-Ar), 134.4 (C_{quat}, C-Ar), 129.3 (+, C-Ar), 129.1 (C_{quat}, C-Ar), 127.5 (+, C-Ar), 127.0 (+, C-Ar), 126.2 (+, C-Ar), 123.7 (+, C-Ar), 119.1 (+, C-Ar), 108.2 (+, C-Ar), 101.5 (C_{quat}, C-alkyne), 91.8 (C_{quat}, C-alkyne), 69.9 (+, CHO), 42.3 (–, CH_2), 34.1 (–, CH_2), 29.7 (–, CH_2), 26.2 (–, CH_2), 20.7 (–, CH_2) ppm. IR (ATR platinum diamond): $\tilde{\nu}$ = 3396 (w), 3056 (w), 2924 (m), 2849 (m), 2212 (w), 1628 (m), 1598 (m), 1509 (m), 1466 (m), 1447 (m) cm^{-1}. MS (EI, 70 eV): *m*/*z* (%) = 250 (8) $[M]^+$, 84 (100). HRMS (EI): calcd. for $C_{18}H_{18}O$ 250.1358; found 250.1354.



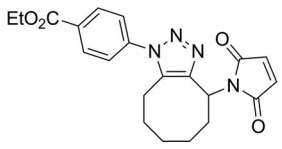
Methyl 4-(Cyclooct-2-yn-1-yloxy)benzoate (17): To a solution of alcohol **13** (0.100 g, 0.810 mmol, 1.00 equiv.) in dry THF (8 mL) under argon were added methyl 4-hydroxybenzoate (0.160 g, 1.05 mmol, 1.30 equiv.) and triphenylphosphine (0.276 g, 1.05 mmol, 1.30 equiv.), and the solution was cooled to 0 °C. Then, DIAD (0.205 g, 1.02 mmol, 1.26 equiv.) was added, and the solution was warmed to room temperature overnight. The solvent was removed, and the crude product was purified by flash chromatography (silica gel, 20 × 2 cm; cyclohexane/ethyl acetate, 100:1) to afford **17** (0.114 g, 55% yield) as a white solid; m.p. 49.8 °C. R_f = 0.38 (CH/EE, 20:1). ^1H NMR (400 MHz, $CDCl_3$): δ = 8.00–7.95 (m, 2 H, H-Ar), 6.99–6.90 (m, 2 H, H-Ar), 4.86–4.80 (m, 1 H, CHO), 3.87 (s, 3 H, CH_3), 2.33–2.15 (m, 4 H, CH_2), 1.98–1.83 (m, 3 H, CH_2), 1.81–1.56 (m, 3 H, CH_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.9 (C_{quat}, C=O), 161.5 (C_{quat}, C-O/C-Ar), 131.5 (+, C-Ar), 122.8 (C_{quat}, C-Ar), 114.9 (+, C-Ar), 102.1 (C_{quat}, C-alkyne), 91.0 (C_{quat}, C-alkyne), 70.1 (+, CHO), 51.9 (+, CH_3), 42.2 (–, CH_2), 34.1 (–, CH_2), 29.7 (–, CH_2), 26.1 (–, CH_2), 20.7 (–, CH_2) ppm. IR (ATR platinum diamond): $\tilde{\nu}$ = 2923 (w), 2849 (w), 1717 (m), 1603 (m), 1579 (w), 1506 (m), 1437 (m) cm^{-1}. MS (EI, 70 eV): *m*/*z* (%) = 258 (17) $[M]^+$, 243 (17) $[M – Me]^+$, 199 (100) $[C_{14}H_{15}O]^+$. HRMS (EI): calcd. for $C_{16}H_{18}O_3$ 258.1256; found 258.1254.



(Cyclooct-2-yn-1-yloxy)benzoic Acid (20): Ester **17** (0.089 g, 0.345 mmol, 1.00 equiv.) was dissolved in THF (3 mL) and methanol (3 mL). Then, sodium hydroxide (0.200 g, 5.00 mmol, 14.5 equiv.) that was dissolved in water (2 mL) was added, and the mixture was stirred at 50 °C for 3 h. The mixture was then diluted with water (5 mL), and the aqueous phase was extracted with ethyl acetate (2 × 10 mL). The organic phase was discarded, and the cooled aqueous phase was acidified with concentrated HCl to pH = 2. Then, the aqueous phase was extracted with ethyl acetate (2 × 10 mL). The organic phase was then dried with sodium sulfate. Removal of the solvent yielded **20** (0.055 g, 65% yield) as a white solid; m.p. 161.9 °C. ^1H NMR: (400 MHz, $[D_6]$DMSO): δ = 12.63 (s, 1 H, OH), 7.90–7.85 (m, 2 H, H-Ar), 7.01–6.96 (m, 2 H, H-Ar), 5.09–5.03 (m, 1 H, CHO), 2.29–2.07 (m, 4 H, CH_2), 1.89–1.74 (m, 3 H, CH_2), 1.72–1.51 (m, 3 H, CH_2) ppm. ^{13}C NMR (100 MHz, $[D_6]$DMSO): δ = 167.0 (C_{quat}, C=O), 160.8 (C_{quat}, C-O, C-Ar), 131.2 (+, C-Ar), 123.3 (C_{quat}, C-Ar), 115.0 (+, C-Ar), 101.8 (C_{quat}, C-alkyne), 91.5 (C_{quat}, C-alkyne), 69.5 (+, CHO), 41.7 (–, CH_2), 33.8 (–, CH_2), 29.2 (–, CH_2), 25.7 (–, CH_2), 19.9 (–, CH_2) ppm. IR (ATR platinum diamond): $\tilde{\nu}$ = 2929 (w), 2212 (w), 2058 (w), 1658 (s), 1599 (s), 1574 (m), 1449 (w), 1424 (m), 1354 (m), 1305 (w), 1244 (s) cm^{-1}. MS (FAB): *m*/*z* = 245 $[M + H]^+$, 137 $[C_7H_5O_3]^+$. HRMS (FAB): calcd. for $C_{15}H_{17}O_3$ 245.1178; found 245.1176.

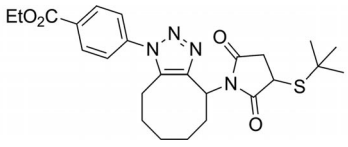


9-(2-{4-[4-(Cyclooct-2-yn-1-yloxy)benzoyl]piperazine-1-carbonyl}-phenyl)-6-hydroxy-3*H*-xanthen-3-one (21): Acid **20** (0.045 g, 0.184 mmol, 1.00 equiv.), *N*,*N*-diisopropylethylamine (DIPEA, 0.038 mL, 0.029 g, 0.223 mmol, 1.21 equiv.), HOBt (0.042 g, 0.274 mmol, 1.49 equiv.), and fluorescein-piperazine amide (0.110 g, 0.275 mmol, 1.49 equiv.) were dissolved in dry DMF (5 mL) under argon. DIC (0.042 mL, 0.034 g, 0.271 mmol, 1.47 equiv.) was added, and the solution was stirred at room temperature for 24 h. The solvent was removed, and the crude product was purified by flash chromatography (silica gel, 20 × 2 cm; DCM/MeOH, 40:1–10:1) to afford **21** (0.050 g, 43% yield) as an orange solid; m.p. 244 °C (decomposition). R_f = 0.28 (DCM/MeOH, 20:1). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.93 (br. s, 1 H, OH), 7.62–7.50 (m, 2 H, H-Ar), 7.47–7.38 (m, 1 H, H-Ar), 7.35–7.28 (m, 1 H, H-Ar), 7.25–7.18 (m, 2 H, H-Ar), 7.03–6.94 (m, 2 H, H-Ar), 6.87–6.80 (m, 2 H, H-Ar), 6.75–6.66 (m, 4 H, H-Ar), 4.74–4.66 (m, 1 H, CHO), 3.53–3.16 (m, 8 H, piperazine-H), 2.23–2.04 (m, 4 H, CH_2), 1.89–1.76 (m, 3 H, CH_2), 1.70–1.46 (m, 3 H, CH_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 175.2 (C_{quat}, C=O), 170.5 (C_{quat}, C=O), 167.7 (C_{quat}, C-Ar), 159.2 (C_{quat}, C-Ar), 157.4 (C_{quat}, C-Ar), 151.8 (C_{quat}, C-Ar), 134.9 (C_{quat}, C-Ar), 131.6 (C_{quat}, C-Ar), 130.8 (+, C-Ar), 130.6 (+, C-Ar), 129.9 (+, C-Ar), 129.6 (+, C-Ar), 129.0 (+, C-Ar), 127.3 (+, C-Ar), 126.7 (C_{quat}, C-Ar), 121.9 (+, C-Ar), 115.2 (+, C-Ar), 115.1 (C_{quat}, C-Ar), 103.9 (+, C-Ar), 102.0 (C_{quat}, C-alkyne), 91.1 (C_{quat}, C-alkyne), 70.0 (+, C-O), 47.4 (–, CH_2-piperazine), 42.1 (–, CH_2), 41.9 (–, CH_2-piperazine), 34.0 (–, CH_2), 29.6 (–, CH_2), 26.0 (–, CH_2), 20.6 (–, CH_2) ppm. IR (ATR platinum diamond): $\tilde{\nu}$ = 2921 (w), 2848 (w), 1632 (m), 1590 (s), 1504 (m), 1454 (m), 1417 (m), 1378 (m), 1237 (s), 1202 (m), 1172 (m) cm^{-1}. MS (FAB): *m*/*z* = 627 $[M + H]^+$. HRMS (FAB): calcd. for $C_{39}H_{35}N_2O_6$ 627.2495; found 627.2494.



Ethyl 4-[4-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-4,5,6,7,8,9-hexahydro-1*H*-cycloocta[*d*]-[1,2,3]triazol-1-yl]benzoate and Regioisomer (18): Alkyne **14** (0.050 g, 0.250 mmol, 1.00 equiv.) was dissolved in THF (10 mL). Ethyl 4-azidobenzoate (0.070 g, 0.370 mmol, 1.48 equiv.) was added, and the solution was stirred at room temperature for 12 h. The solvent was removed, and the crude product was purified by flash chromatography (silica gel, 20 × 2 cm; cyclo-

hexane/ethyl acetate, 3:1) to afford **18** (0.027 g, 28% yield) as a highly viscous oil; R_f = 0.23 (EE/CH, 3:1). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.17 [d, ^3J = 8.4 Hz, 2H (major isomer), H-Ar], 8.02 [d, ^3J = 8.4 Hz, 2H (minor isomer), H-Ar], 7.67 [d, ^3J = 8.4 Hz, 2H (major isomer), H-Ar], 7.59 [d, ^3J = 8.4 Hz, 2H (minor isomer), H-Ar], 7.06 [s, 2H (major isomer), H-olefin], 6.69 [s, 2H (minor isomer), H-olefin], 5.36 (dd, ^3J = 12.5 Hz, ^3J = 3.8 Hz, 1 H, CH), 4.36 (q, ^3J = 7.0 Hz, 2 H, CH_2CH$_3$), 3.16–2.99 (m, 1 H, CH$_2$), 2.87–2.68 (m, 1 H, CH$_2$), 1.98–1.86 (m, 1 H, CH$_2$), 1.86–1.50 (m, 4 H, CH$_2$), 1.58–1.42 (m, 2 H, CH$_2$), 1.35 (t, ^3J = 7.1 Hz, 3 H, CH$_2$CH_3) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 170.6 (C$_{quat}$, *C*O$_2$Et), 169.1 (C$_{quat}$, *C*O$_2$Et), 164.8 (C$_{quat}$, C-imid), 164.8 (C$_{quat,}$ C-imid), 144.7 (C$_{quat}$, C-Ar), 142.6 (C$_{quat}$, C-Ar), 139.6 (C$_{quat}$, C-Ar), 139.1 (C$_{quat}$, C-Ar), 134.7 (+, C-olefin), 134.0 (C$_{quat}$, C-Ar), 133.9 (+, C-olefin), 132.2 (C$_{quat}$, C-Ar), 130.8 (C$_{quat}$, C-Ar), 130.6 (+, C-Ar), 130.3 (+, C-Ar), 125.9 (+, C-Ar), 125.8 (+, C-Ar), 61.3 (–, CO$_2$*C*H$_2$CH$_3$), 61.2 (–, CO$_2$*C*H$_2$CH$_3$), 46.6 (+, CH), 44.1 (+, CH), 29.5 (–, CH$_2$), 29.4 (–, CH$_2$), 26.3 (–, CH$_2$), 25.6 (–, CH$_2$), 24.6 (–, CH$_2$), 24.4 (–, CH$_2$), 24.3 (–, CH$_2$), 23.9 (–, CH$_2$), 20.9 (–, CH$_2$), 14.1 (+, CH$_3$) ppm. IR (ATR platinum diamond): $\tilde{\nu}$ = 2925 (w), 2854 (w), 1702 (m), 1606 (m), 1513 (w), 1475 (w), 1445 (w), 1387 (m), 1354 (m), 1272 (m), 149 (m), 1100 (m), 1019 (m), 1003 (m) cm^{-1}. MS (EI, 70 eV): *m*/*z* (%) = 395 (100) [M + H]$^+$, 270 (35) [C$_{17}$H$_{20}$NO$_2$]$^+$. HRMS (EI): calcd. for C$_{21}$H$_{23}$O$_4$N$_4$ 395.1714; found 395.1712.



Ethyl 4-{4-[3-(*tert*-Butylthio)-2,5-dioxopyrrolidin-1-yl]-4,5,6,7,8,9-hexahydro-1*H*-cycloocta[*d*][1,2,3]triazol-1-yl}benzoate (19): Triazole **18** (0.020 g, 0.051 mmol, 1.00 equiv.) was dissolved in DMSO (2 mL) and *tert*-butylthiol (0.006 g, 0.066 mmol, 1.30 equiv.), and the resulting mixture was stirred for 12 h. After this time, the excess amount of thiol was removed under high vacuum. The DMSO solution was then diluted with water (5 mL), and the aqueous phase was extracted with ethyl acetate (2 × 2 mL). The organic phase was washed thoroughly with water (10 × 2 mL). The organic phase was dried with sodium sulfate, and the solvent was removed to yield pure product **19** (0.024 g, 98% yield, complex mixture of regioisomers and diastereomers) as a highly viscous oil. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.24–8.12 (m, 2 H, H-Ar), 7.72–7.56 (m, 2 H, H-Ar), 5.47–5.26 (m, 1 H, CHN), 4.37 (q, ^3J = 7.1 Hz, 2 H, CO$_2$CH_2), 4.15–4.04 (m, 1 H, CHS), 3.39–3.27 (m, 1 H, NCOCH$_2$), 3.23–3.04 (m, 1 H, NCOCH$_2$), 2.94–2.68 (m, 2 H, CH$_2$), 2.65–2.54 (m, 1 H, CH$_2$), 1.93–1.46 (m, 7 H, CH$_2$), 1.46–1.28 (m, 11 H, CH$_3$), 1.28–1.12 (m, 1 H, CH$_3$) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 176.2 (C$_{quat}$, *C*O$_2$Et), 176.1 (C$_{quat}$, *C*O$_2$Et), 174.5 (C$_{quat}$, C-imid), 164.8 (C$_{quat}$, C-imid), 141.8 (C$_{quat}$, C-Ar), 141.8 (C$_{quat}$, C-Ar), 139.07 (C$_{quat}$, C-Ar), 134.5 (C$_{quat}$, C-Ar), 130.8 (C$_{quat}$, C-Ar), 130.7 (+, C-Ar), 125.77 (+, C-Ar), 61.2 (–, CO$_2$*C*H$_2$), 47.6 (+, CHN), 44.4 (C$_{quat}$, CS), 44.4 (C$_{quat}$, CS), 38.9 (–, *C*H$_2$CHS), 38.8 (+, CHS), 31.0 [+, CH$_3$(*tert*-butyl)], 31.0 [+, CH$_3$(*tert*-butyl)], 28.0 (–, CH$_2$), 28.0 (–, CH$_2$), 25.6 (–, CH$_2$), 25.6 (–, CH$_2$), 24.4 (–, CH$_2$), 24.3 (–, CH$_2$), 24.3 (–, CH$_2$), 20.8 (–, CH$_2$), 14.1 [+, CH$_3$(ester)] ppm. IR (ATR platinum diamond): $\tilde{\nu}$ = 2924 (vw), 2862 (vw), 1776 (vw), 1702 (w), 1607 (vw), 1513 (vw) cm^{-1}. MS (EI, 70 eV): *m*/*z* (%) = 484 (33) [M]$^+$, 456 (57) [M – N$_2$]$^+$, 270 (100) [C$_{17}$H$_{20}$NO$_2$]$^+$. HRMS (EI): calcd. for C$_{25}$H$_{32}$O$_4$N$_4$S 484.2139; found 484.2138.

Supporting Information (see footnote on the first page of this article): Copies of the ^{1}H and ^{13}C NMR spectra of all key intermediates and final products.

Acknowledgments

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