HETEROCYCLES, Vol. 101, No. 1, 2020, pp. 263 - 272. © 2020 The Japan Institute of Heterocyclic Chemistry
Received, 4th June, 2019, Accepted, 16th July, 2019, Published online, 16th August, 2019
DOI: 10.3987/COM-19-S(F)21

SYNTHESIS OF OXYGEN-HETEROCYCLES HAVING LINKER COMPONENTS FOR TRAPPING CYSTEINE DERIVATIVES

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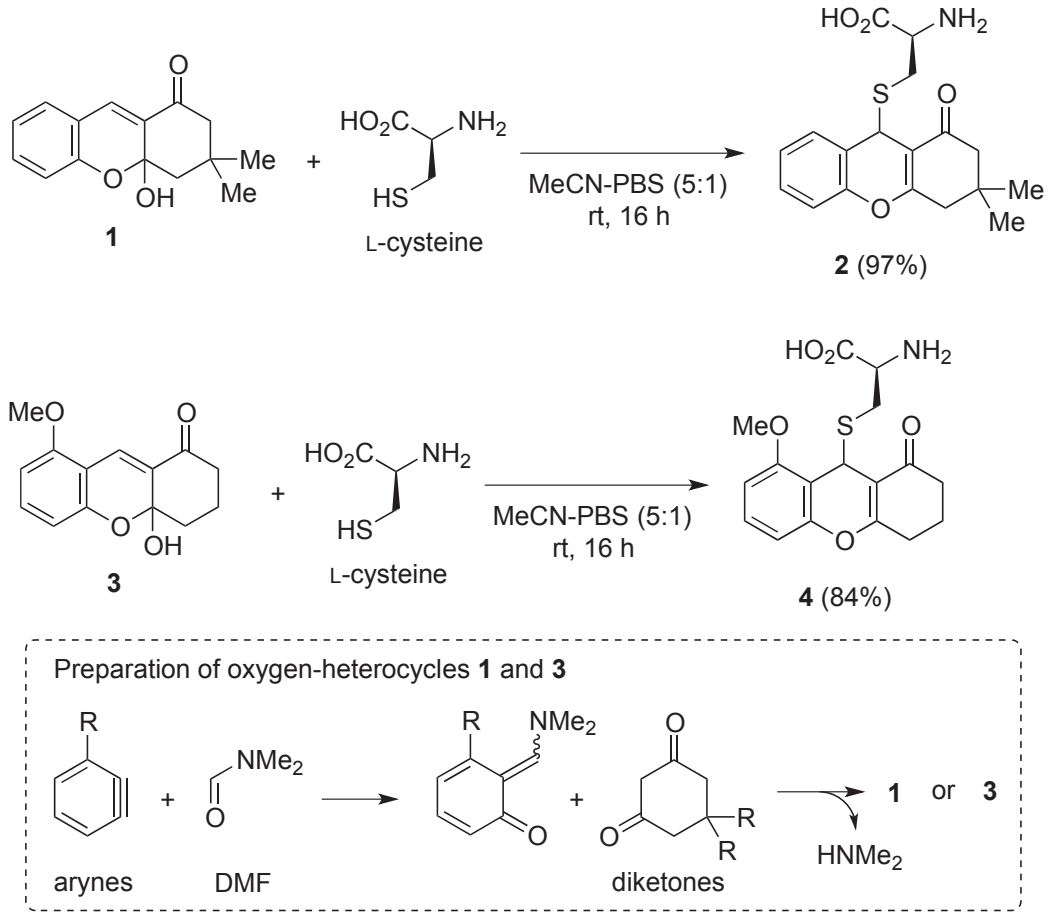
Abstract – Tricyclic oxygen-heterocycles **10**, **13a**, **13b** and **18** having a linker component were synthesized for the site-specific modification of proteins and peptides. The linker components were initially introduced by Sonogashira-Hagihara cross coupling of 5-bromo-2-hydroxybenzaldehyde **5** and a variety of alkynes. Next, the desired oxygen-heterocycles **10**, **13a**, **13b** and **18** were synthesized by the condensation reaction of coupling products with cyclohexane-1,3-dione in the presence of *N*,*N*-diisopropylethylamine. Finally, the trapping ability of these oxygen-heterocycles was demonstrated by the representative reaction of oxygen-heterocycle **10** with glutathione **19** as a nucleophile having a thiol group.

INTRODUCTION

The site-specific modification of proteins and peptides has attracted extensive attention in the biochemical or biomedical chemistry.[1] Among twenty α-amino acids incorporated into proteins, lysine having the amino group as a reactive residue has been most extensively investigated as the targeting α-amino acid for the protein/peptide modification, although a variety of methods for trapping other α-amino acids have been developed.[1–4] Cysteine is a relatively rare α-amino acid in natural proteins. Therefore, the cysteine-selective trapping methods are of particular importance for the site-specific modification in the bioconjugation chemistry;[5] thus, new methods for trapping a thiol group of cysteine have continued to increase.[5–10]

Recently, we reported that tricyclic oxygen-heterocycles **1** and **3** have the thiol-selective reactivity (Scheme 1).[11,12] These oxygen-heterocycles reacted well with a thiol group of L-cysteine, homocysteine, captopril and glutathione under the mild and aqueous reaction conditions.[12] Therefore, our laboratory is interested in the further functionalization of these oxygen-heterocycles (Figure 1). In this paper, we report the synthetic study for preparing the oxygen-heterocycles having a linker component.



Scheme 1. Trapping reaction of L-cysteine with oxygen-heterocycles **1** and **3**

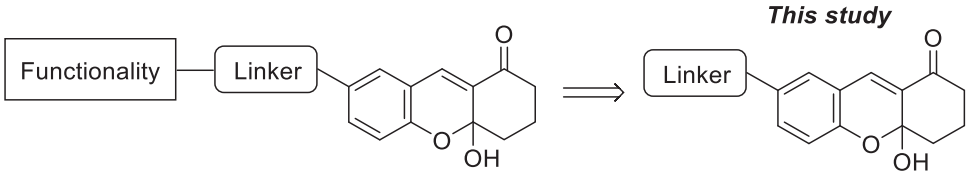
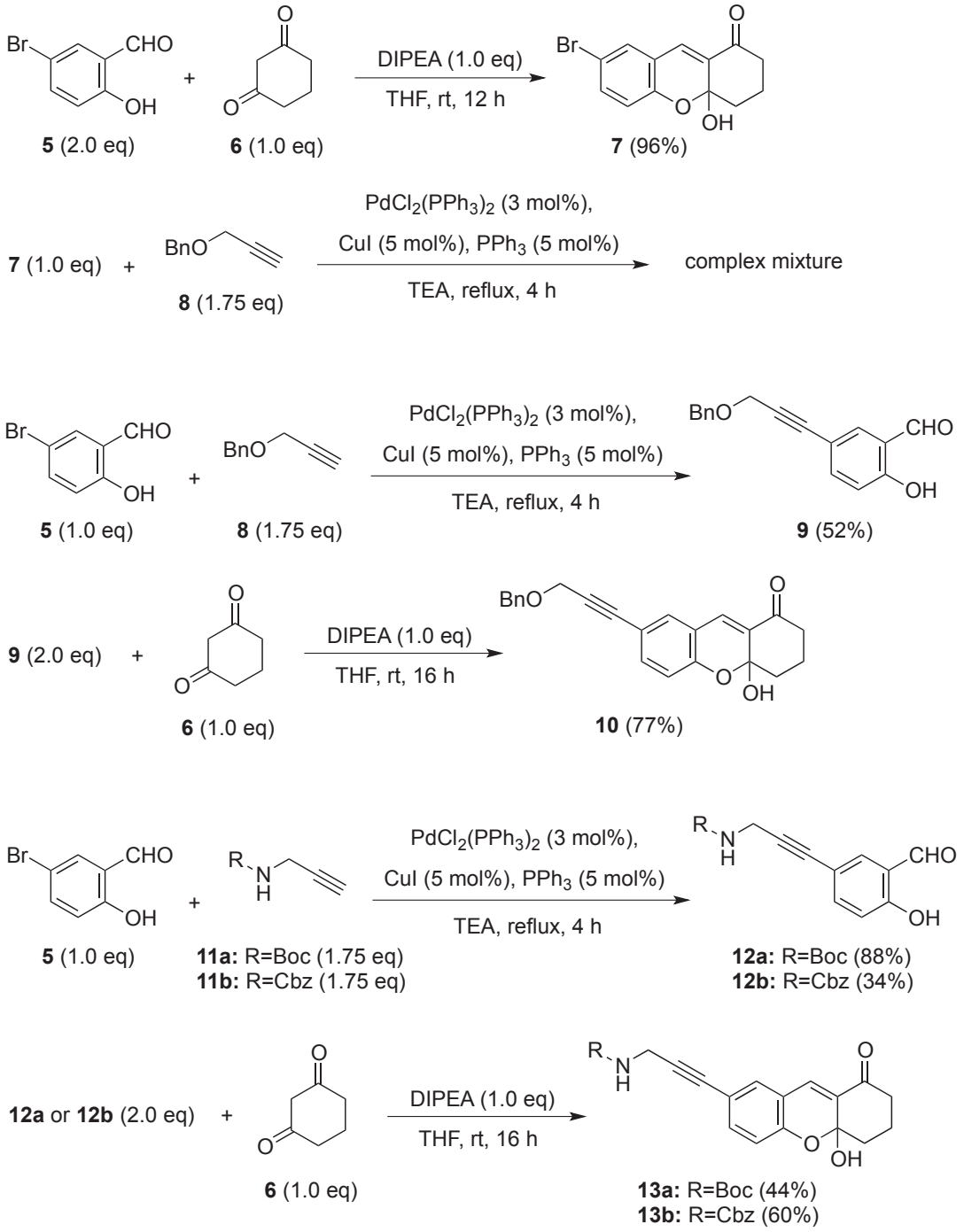


Figure 1. Oxygen-heterocycles having a linker component

RESULTS AND DISCUSSION

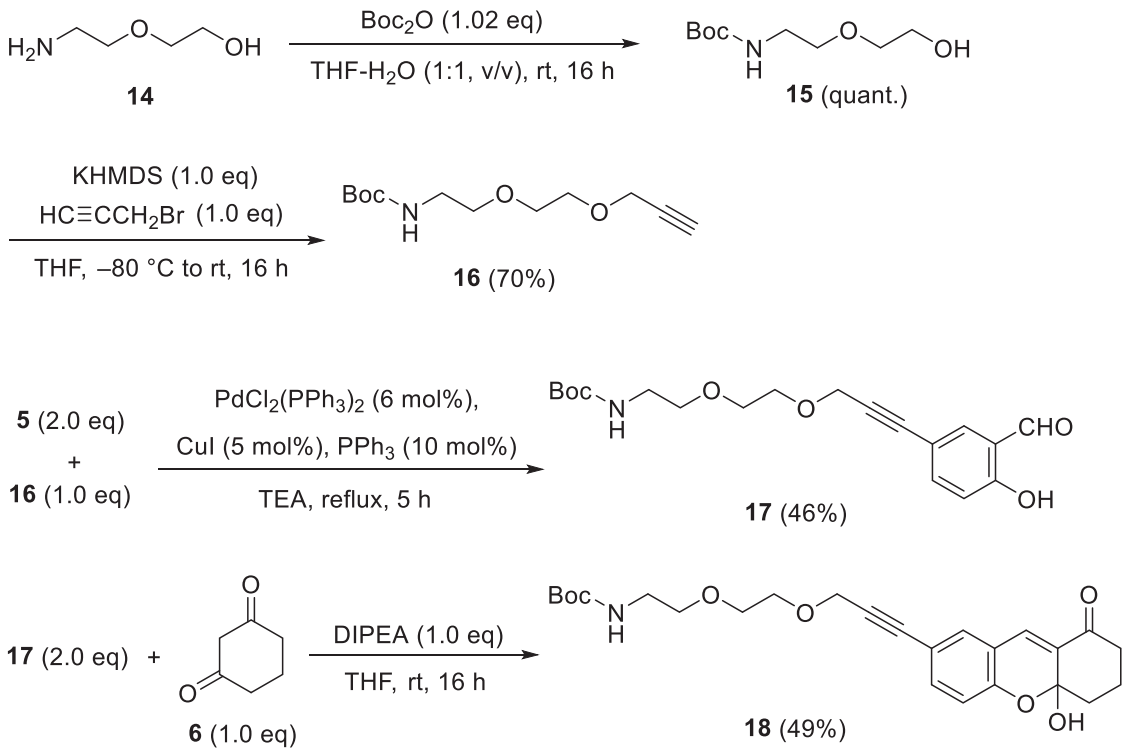
Tricyclic oxygen-heterocycles **1** and **3** can be prepared by our one-pot three-components coupling method using arynes, *N*,*N*-dimethylformamide (DMF) and cyclic 1,3-diketones (Scheme 1).[11,13] However, it is difficult to prepare the functionalized oxygen-heterocycles by the use of our aryne-based method owing to the low availability of functionalized aryne precursors. Moreover, the use of unsymmetrically substituted arynes is constrained by the low regioselectivity leading to the formation of two regioisomers. Therefore, we first investigated another synthetic method using salicylaldehyde derivative **5** and cyclic 1,3-diketone **6** (Scheme 2). In the presence of *N*,*N*-diisopropylethylamine (DIPEA), treatment of 5-bromo-2-hydroxybenzaldehyde **5** (2.0 equiv.) with cyclohexane-1,3-dione **6** (1.0 equiv.) in THF at

room temperature gave the desired oxygen-heterocycle **7** in 96% yield. It is important to note that the oxygen-heterocycle **7** can react with cyclohexane-1,3-dione **6**.[11,13] To avoid this side reaction, the excess amount of 5-bromo-2-hydroxybenzaldehyde **5** (2.0 equiv.) was used, leading to the selective formation of oxygen-heterocycle **7**. Next, Sonogashira-Hagihara cross coupling reaction between oxygen-heterocycle **7** and alkyne **8** was examined under several different reaction conditions. However, the desired coupling product could not be obtained.



Scheme 2. Synthesis of oxygen-heterocycles **10**, **13a** and **13b** having a linker component

The introduction of linker components was achieved by Sonogashira-Hagihara cross coupling of 5-bromo-2-hydroxybenzaldehyde **5** with alkynes (Scheme 2). When the catalytic amounts of $PdCl_2(PPh_3)_2$, CuI and PPh_3 were employed in triethylamine (TEA) under the reflux conditions, the desired coupling product **9** was obtained in 52% yield. The corresponding oxygen-heterocycle **10** was prepared in 77% yield by the condensation reaction of the coupling product **9** with cyclohexane-1,3-dione **6**. Next, alkynes **11a** and **11b** having the protected amino group were used as linker components. The coupling reaction of **5** with alkynes **11a** and **11b** also proceeded to give the products **12a** and **12b**, although the chemical efficiency of the coupling reaction using alkyne **11b** having the Cbz-protected amino group was low. Similarly, the oxygen-heterocycles **13a** and **13b** were synthesized by the condensation reaction, although these chemical yields were moderate due to the further reaction of **13a** and **13b** with cyclohexane-1,3-dione **6**.[11,13]

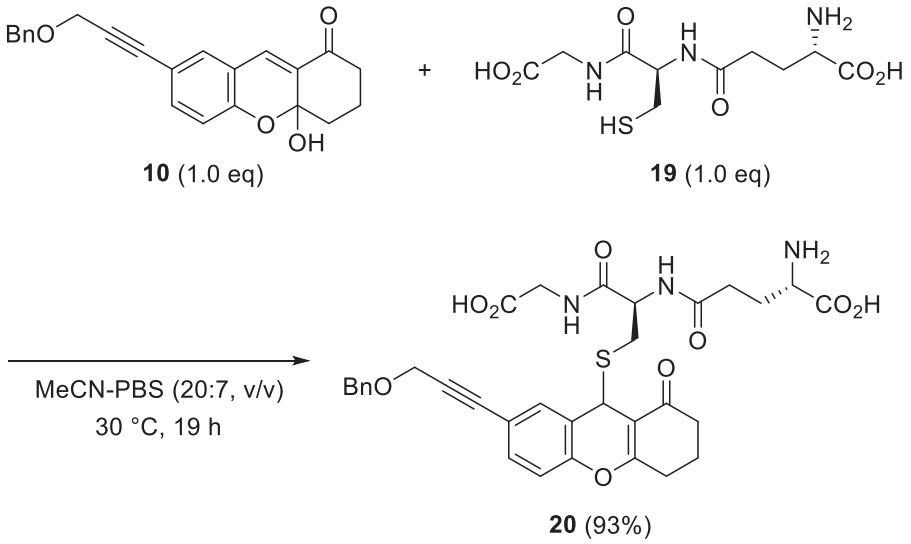


Scheme 3. Synthesis of oxygen-heterocycle **18** having a hydrophilic linker

Next, the introduction of a hydrophilic linker was examined (Scheme 3). The amino group of 2-(2-aminoethoxy)ethan-1-ol **14** was protected with di-*tert*-butyl dicarbonate to afford the *N*-Boc derivative **15**. Subsequent *O*-propargylation of **15** using propargyl bromide and KHMDS gave the alkyne **16**. The coupling reaction of 5-bromo-2-hydroxybenzaldehyde **5** with alkyne **16** led to the coupling product **17** in 46% yield, which was converted into the oxygen-heterocycle **18** having a hydrophilic linker

in 49% yield under the condensation conditions. Unfortunately, the deprotection of Boc group in **18** cannot be achieved at the present stage.

Finally, we tested the trapping ability of oxygen-heterocycle **10** as a model substrate having a linker component (Scheme 4). The reactivity of **10** was confirmed by employing glutathione **19** as a nucleophile having a thiol group of cysteine. In our previous study, we found that the combinations of MeCN and phosphate-buffered saline (PBS) is the best solvent for trapping cysteine and related thiols with oxygen heterocycles;[12] thus, the reaction of **10** with glutathione **19** was carried in MeCN-PBS (20:7, v/v) as aqueous solvent at 30 °C. As expected, the desired adduct **20** was obtained in 93% yield *via* the S_N2'–type mechanism.



Scheme 4. Reactivity of oxygen-heterocycle **10** toward glutathione **19**

EXPERIMENTAL

Melting points were taken on a Yanaco MP-J3 micro melting point apparatus and are uncorrected. Optical rotation was recorded on a JASCO P-2100 polarimeter. Infrared spectra were measured on a JASCO FT/IR-4100 Fourier-transfer infrared spectrometer. ^{1}H NMR spectra were measured on a JEOL ECX-400 PSK (400 MHz) with $CDCl_3$ as an internal standard (7.26 ppm) or CD_3OD as an internal standard (3.30 ppm). ^{13}C NMR spectra were measured on a JEOL ECX-400 PSK (101 MHz) with $CDCl_3$ as an internal standard (77.0 ppm) or CD_3OD as an internal standard (49.0 ppm). Mass spectra (ESI-MS) were obtained by Thermo Fisher Scientific Exactive LC/MS spectrometer. For flash silica gel column chromatography, SiliCycle Inc. SiliaFlash F60 was used.

***tert*-Butyl (2-(2-hydroxyethoxy)ethyl)carbamate (15).** To a solution of 2-(2-aminoethoxy)ethan-1-ol **14**

(2.0 mL, 20 mmol) in THF–H_2O (100 mL, 1:1 v/v) was added di-*tert*-butyl dicarbonate (4.80 g, 22 mmol) under open system at room temperature. After being stirred at room temperature for 16 h, the reaction mixture was concentrated under reduced pressure. Purification of the residue by flash silica gel column chromatography (acetone:hexanes = 1:2) afforded the product **15** (4.07 g, almost quantitative yield); colorless oil; IR (KBr) 3343 (br), 2976, 1691, 1525, 1366 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.99 (1H, br s), 3.73 (2H, m), 3.58–3.53 (4H, m), 3.32 (2H, br m), 2.36 (1H, br t, *J* = 5.0 Hz), 1.44 (9H, s); ^{13}C NMR ($CDCl_3$) δ 156.1, 79.4, 72.2, 70.3, 61.7, 40.4, 28.4; HRMS (ESI^+) Calcd for $C_9H_{19}NO_4Na$ ($M+Na^+$): 228.1206. Found: 228.1206.

***tert*-Butyl (2-(2-(prop-2-yn-1-yloxy)ethoxy)ethyl)carbamate (16).** To a solution of **15** (410 mg, 2.0 mmol) in freshly distilled THF (20 mL) was added KHMDS in toluene (0.50 mol/L, 4.2 mL, 2.1 mmol) under argon atmosphere at –80 °C. After being stirred at 0 °C for 0.5 h, propargyl bromide (0.20 mL, 2.2 mmol) was added to the reaction mixture. After being stirred from at 0 °C to at room temperature for 16 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution, diluted with saturated aqueous NaCl solution and extracted with $CHCl_3$. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. Purification of the residue by flash silica gel column chromatography (acetone:hexanes = 1:5–1:2) afforded the product **16** (340 mg, 70%); colorless oil; IR (KBr) 3295 (br), 2977, 2115, 1710, 1514, 1251 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.97 (1H, br s), 4.20 (2H, d, *J* = 2.3 Hz), 3.69 (2H, br dd, *J* = 5.5, 3.0 Hz), 3.64 (2H, br dd, *J* = 5.5, 3.0 Hz), 3.54 (2H, br m), 3.32 (2H, br m), 2.44 (1H, t, *J* = 2.3 Hz), 1.43 (9H, s); ^{13}C NMR ($CDCl_3$) δ 156.0, 79.5, 79.2, 74.6, 70.3, 70.1, 69.4, 58.4, 40.3, 28.4; HRMS (ESI^+) Calcd for $C_{12}H_{21}NO_4Na$ ($M+Na^+$): 266.1363. Found: 266.1365.

General Procedure for Cross Coupling Reaction Leading to Products 9, 12a, 12b and 17. To a solution of 5-bromosalicylaldehyde **5** (239 mg, 1.2 mmol), $PdCl_2(PPh_3)_2$ (25 mg, 0.036 mmol), PPh_3 (16 mg, 0.060 mmol) and CuI (12 mg, 0.060 mmol) in triethylamine (12 mL) was added terminal alkynes **8**, **11a**, **11b** (2.1 mmol) or **16** (0.6 mmol) under argon atmosphere at room temperature. After being stirred at 90 °C for 4–5 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution, diluted with saturated aqueous NaCl solution and extracted with $CHCl_3$. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. Purification of the residue by flash silica gel column chromatography (acetone:hexanes = 1:5–1:2) afforded the products **9**, **12a**, **12b** and **17**.

5-(3-(Benzyloxy)prop-1-yn-1-yl)-2-hydroxybenzaldehyde (9): colorless crystals; mp 47–49 °C (hexanes); IR (KBr) 3033, 2852, 2224, 1658, 1483, 1287 cm^{-1}; ^1H NMR ($CDCl_3$) δ 11.12 (1H, s), 9.86 (1H, s), 7.68 (1H, d, *J* = 1.8 Hz), 7.60 (1H, dd, *J* = 8.7, 1.8 Hz), 7.41–7.30 (5H, m), 6.96 (1H, d, *J* = 8.7 Hz), 4.67 (2H, s), 4.39 (2H, s); ^{13}C NMR ($CDCl_3$) δ 196.1, 161.5, 140.0, 137.3, 137.2, 128.5, 128.1, 128.0, 120.4, 118.1, 114.5, 84.6 (2C), 71.8, 57.8; HRMS (ESI^+) calcd for $C_{17}H_{14}O_3Na$ ($M+Na^+$):

289.0835. Found: 289.0836.

***tert*-Butyl (3-(3-formyl-4-hydroxyphenyl)prop-2-yn-1-yl)carbamate (12a):** a white solid; IR (KBr) 3338 (br), 2980, 2229, 1685, 1656, 1484, 1288 cm^{-1}; ^1H NMR ($CDCl_3$) δ 11.09 (1H, s), 9.85 (1H, s), 7.64 (1H, d, *J* = 1.8 Hz), 7.55 (1H, dd, *J* = 8.7, 1.8 Hz), 6.94 (1H, d, *J* = 8.2 Hz), 4.77 (1H, br s), 4.14 (2H, br d, *J* = 5.0 Hz), 1.47 (9H, s); ^{13}C NMR ($CDCl_3$) δ 196.0, 161.4, 155.3, 139.9, 137.0, 120.4, 118.1, 114.6, 85.0, 81.3, 80.1, 31.1, 28.4; HRMS (ESI^+) Calcd for $C_{15}H_{17}NO_4Na$ ($M+Na^+$): 298.1050. Found: 298.1048.

Benzyl (3-(3-formyl-4-hydroxyphenyl)prop-2-yn-1-yl)carbamate (12b): colorless crystals; mp 101–103 °C (CH_2Cl_2–hexanes); IR (KBr) 3311 (br), 2231, 1684, 1527, 1256 cm^{-1}; ^1H NMR ($CDCl_3$) δ 11.09 (1H, s), 9.85 (1H, s), 7.64 (1H, br s), 7.54 (1H, br d, *J* = 8.7 Hz), 7.38–7.32 (5H, m), 6.94 (1H, d, *J* = 8.7 Hz), 5.15 (2H, s), 4.99 (1H, br s), 4.22 (2H, d, *J* = 5.5 Hz); ^{13}C NMR ($CDCl_3$) δ 196.0, 161.5, 155.9, 139.9, 137.1, 136.2, 128.6, 128.3, 128.2, 120.4, 118.1, 114.4, 84.5, 81.6, 67.2, 31.6; HRMS (ESI^+) Calcd for $C_{18}H_{15}NO_4Na$ ($M+Na^+$): 322.0893. Found: 322.0895.

***tert*-Butyl (2-(2-((3-(3-formyl-4-hydroxyphenyl)prop-2-yn-1-yl)oxy)ethoxy)ethyl)carbamate (17):** colorless oil; IR (KBr) 3357 (br), 2976, 2869, 2225, 1710, 1658, 1516, 1484, 1287 cm^{-1}; ^1H NMR ($CDCl_3$) δ 11.10 (1H, s), 9.85 (1H, s), 7.67 (1H, d, *J* = 1.8 Hz), 7.57 (1H, dd, *J* = 8.7, 1.8 Hz), 6.94 (1H, d, *J* = 8.7 Hz), 4.98 (1H, br s), 4.41 (2H, s), 3.74 (2H, m), 3.67 (2H, m), 3.55 (2H, m), 3.33 (2H, br m), 1.43 (9H, s); ^{13}C NMR ($CDCl_3$) δ 196.0, 161.5, 155.9, 139.9, 137.1, 120.4, 118.1, 114.4, 84.6, 84.5, 79.2, 70.3, 70.1, 69.1, 59.1, 40.3, 28.4; HRMS (ESI^+) Calcd for $C_{19}H_{25}NO_6Na$ ($M+Na^+$): 386.1574. Found: 386.1574.

General Procedure for Condensation Reaction Leading to Oxygen-Heterocycle 7, 10, 13a, 13b and 18. To a solution of salicylaldehyde derivatives **5**, **9**, **12a**, **12b** and **17** (1.0 mmol) in THF (10 mL) were added cyclohexane-1,3-dione **6** (56 mg, 0.50 mmol) and *N*,*N*-diisopropylethylamine (87 μL, 0.50 mmol) under argon atmosphere at room temperature. After being stirred at room temperature for 12–16 h, the reaction mixture was concentrated under reduced pressure. Purification of the residue by flash silica gel column chromatography (AcOEt:hexanes = 1:6–1:1) afforded the product **7**, **10**, **13a**, **13b** and **18**.

7-Bromo-4a-hydroxy-2,3,4,4a-tetrahydro-1*H*-xanthen-1-one (7): a light yellow solid; mp 175–179 °C (red discolorination) and 250 °C (decomp) (acetone–benzene); IR (KBr) 3308 (br), 2921, 1582, 1476, 1440 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.49 (1H, d, *J* = 2.3 Hz), 7.45 (1H, s), 7.44 (1H, dd, *J* = 8.7, 2.3 Hz), 6.94 (1H, d, *J* = 8.7 Hz), 2.82 (1H, s), 2.70 (1H, dquin, *J* = 17.4, 2.3 Hz), 2.50–2.33 (2H, m), 2.26–2.08 (2H, m), 2.06–1.98 (1H, m); ^{13}C NMR ($CDCl_3$) δ 197.2, 151.6, 134.8, 131.9, 131.3, 128.9, 121.5, 119.1, 114.3, 96.7, 38.9, 36.0, 18.1; HRMS (ESI^+) Calcd for $C_{13}H_{11}{}^{79}BrO_3Na$ ($M+Na^+$): 316.9784. Found: 316.9781; Calcd for $C_{13}H_{11}{}^{81}BrO_3Na$ ($M+Na^+$): 318.9765. Found: 318.9767.

7-(3-(Benzyloxy)prop-1-yn-1-yl)-4a-hydroxy-2,3,4,4a-tetrahydro-1*H*-xanthen-1-one (10): pale yellow

crystals; mp 124–125 °C (CH_2Cl_2–hexanes); IR (KBr) 3399 (br), 3085, 2845, 1732, 1678, 1654, 1614, 1441, 1414, 1248 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.49–7.30 (8H, m), 7.00 (1H, d, *J* = 8.7 Hz), 4.67 (2H, s), 4.39 (2H, s), 2.89 (1H, br s), 2.70 (1H, dquin, *J* = 17.9, 2.3 Hz), 2.50–2.28 (2H, m), 2.26–2.08 (2H, m), 2.05–2.00 (1H, m); ^{13}C NMR ($CDCl_3$) δ 197.2, 152.6, 137.4, 135.6, 133.1, 131.1, 129.4, 128.5, 128.1, 127.9, 119.7, 117.5, 116.7, 96.9, 85.3, 84.7, 71.8, 57.9, 38.9, 35.9, 18.1; HRMS (ESI^+) Calcd for $C_{23}H_{20}O_4Na$ ($M+Na^+$): 383.1254. Found: 383.1253.

***tert*-Butyl (3-(4a-hydroxy-1-oxo-2,3,4,4a-tetrahydro-1*H*-xanthen-7-yl)prop-2-yn-1-yl)carbamate (13a):** colorless crystals; mp 180–182 °C (CH_2Cl_2–hexanes); IR (KBr) 3338 (br), 2977, 2237, 1686, 1612, 1561, 1514, 1251 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.47 (1H, s), 7.42 (1H, d, *J* = 1.8 Hz), 7.40 (1H, dd, *J* = 8.2, 2.0 Hz), 6.98 (1H, d, *J* = 8.7 Hz), 4.76 (1H, br s), 4.14 (2H, br d, *J* = 4.6 Hz), 2.94 (1H, br s), 2.69 (1H, dquin, *J* = 17.9, 2.2 Hz), 2.50–2.33 (2H, m), 2.26–2.08 (2H, m), 2.04–1.99 (1H, m), 1.47 (9H, s); ^{13}C NMR ($CDCl_3$) δ 197.2, 173.3, 152.5, 135.4, 133.0, 131.1, 129.4, 119.7, 117.5, 116.8, 96.9, 85.0, 81.9, 80.0 (br), 38.9, 36.0, 31.2 (br), 28.4, 18.1; HRMS (ESI^+) Calcd for $C_{21}H_{23}NO_5Na$ ($M+Na^+$): 392.1468. Found: 392.1463.

Benzyl (3-(4a-hydroxy-1-oxo-2,3,4,4a-tetrahydro-1*H*-xanthen-7-yl)prop-2-yn-1-yl)carbamate (13b): a orange solid; IR (KBr) 3220 (br), 2952, 2248, 1403, 1638, 1492, 1232 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.46 (1H, s), 7.41–7.30 (7H, m), 6.98 (1H, d, *J* = 8.2 Hz), 5.14 (2H, s), 5.01 (1H, br s), 4.21 (2H, br d, *J* = 4.1 Hz), 2.71 (1H, br s), 2.69 (1H, br d, *J* = 17.9 Hz), 2.50–2.30 (2H, m), 2.26–2.05 (2H, m), 2.04–1.95 (1H, m); HRMS (ESI^+) Calcd for $C_{24}H_{21}NO_5Na$ ($M+Na^+$): 426.1312. Found: 426.1308.

***tert*-Butyl (2-(2-((3-(4a-hydroxy-1-oxo-2,3,4,4a-tetrahydro-1*H*-xanthen-7-yl)prop-2-yn-1-yl)oxy)-ethoxy)ethyl)carbamate (18):** colorless oil; IR (KBr) 3349 (br), 2935, 2223, 1712, 1686, 1613, 1252 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.45 (2H, m), 7.41 (1H, dd, *J* = 8.2, 2.0 Hz), 6.98 (1H, d, *J* = 8.2 Hz), 5.00 (1H, br s), 4.41 (2H, s), 3.74 (2H, m), 3.65 (2H, m), 3.54 (2H, m), 3.35 (1H, br m), 3.31 (2H, br q, *J* = 5.0 Hz), 2.67 (1H, dqn, *J* = 17.4, 2.0 Hz), 2.50–2.32 (2H, m), 2.25–2.05 (2H, m), 2.03–1.98 (1H, m), 1.43 (9H, s); ^{13}C NMR ($CDCl_3$) δ 197.3, 156.0, 152.7, 135.4, 133.1, 131.1, 129.3, 119.7, 117.5, 116.5, 96.9, 85.3, 84.4, 79.2, 70.3, 70.1, 69.1, 59.2, 40.3, 38.9, 35.9, 28.4, 18.1; HRMS (ESI^+) Calcd for $C_{25}H_{31}NO_7Na$ ($M+Na^+$): 480.1993. Found: 480.1989.

***N*^{5}-((2R)-3,-((7-(3-(Benzyloxy)prop-1-yn-1-yl)-1-oxo-2,3,4,9-tetrahydro-1*H*-xanthen-9-yl)thio)-1-((carboxymethyl)amino)-1-oxopropan-2-yl)-*L*-glutamine (20).** To a solution of **10** (22 mg, 0.061 mmol) in MeCN (1.2 mL) was added a solution of glutathione **19** (18.7 mg, 0.061 mmol) in phosphate-buffered saline (PBS, 0.42 mL) under argon atmosphere at room temperature. After being stirred at 30 °C for 19 h, silica gel (0.3 g) was added to the reaction mixture, which was concentrated under reduced pressure. Purification of the residue by flash silica gel column chromatography

(AcOEt:MeOH = 1:0–0:1) afforded the adduct **20** (37 mg, 93%); a white solid; $[\alpha]_D^{26}$ –23.3 (*c* 0.505, MeOH); IR (KBr) 3288 (br), 3065, 2940, 2223, 1658 cm^{-1}; The presence of diastereoisomers and rotermers precluded a comprehensive assignment of all proton and carbon resonances. ^1H NMR (CD_3OD) δ 7.54 (1H, br s), 7.38–7.27 (6H, m), 7.08 (1H, br m), 5.06 (1/2H, s), 5.02 (1/2H, s), 4.64 (2H, br s), 4.58 (1/2H, br m), 4.43 (1/2H, br m), 4.40 (2/2H, s), 4.39 (2/2H, s), 3.87–3.83 (2H, br m), 3.65–3.60 (1H, br m), 2.93–2.42 (8H, br m), 2.15–2.05 (4H, br m); ^{13}C NMR (CD_3OD) δ 199.3, 199.2, 175.2, 175.1, 173.8 (2C), 173.6 (2C), 172.8, 172.7, 170.8, 170.7, 151.9, 151.6, 139.0 (br), 134.3, 133.9, 133.0 (br), 129.5, 129.2, 128.9, 124.6, 124.2, 121.3, 121.2, 117.9 (2C), 112.7, 112.5, 86.5, 86.2, 72.8, 58.6, 55.4, 54.7, 42.6, 37.8, 36.5, 36.0, 32.9, 32.8, 28.6, 27.8, 27.7, 21.3, 21.2; HRMS (ESI^-) Calcd for $C_{33}H_{34}N_3O_9S$ ($M–H^-$): 648.2021. Found: 648.2022.

ACKNOWLEDGEMENTS

This work was partially supported by JSPS KAKENHI Grant-in-Aid for Scientific Research (C) Grant Number 15K07879 (to E.Y.).

REFERENCES

1. (a) W.-Y. Hu, F. Berti, and R. Adamo, *Chem. Soc. Rev.*, 2016, **45**, 1691; (b) P. Akkapeddi, S.-A. Azizi, A. M. Freedy, P. M. S. D. Cal, P. M. P. Gois, and G. J. L. Bernardes, *Chem. Sci.*, 2016, **7**, 2954; (c) O. Koniev and A. Wagner, *Chem. Soc. Rev.*, 2015, **44**, 5495; (d) P. Agarwal and C. R. Bertozzi, *Bioconjugate Chem.*, 2015, **26**, 176.
2. Y. Seki, T. Ishiyama, D. Sasaki, J. Abe, Y. Sohma, K. Oisaki, and M. Kanai, *J. Am. Chem. Soc.*, 2016, **138**, 10798.
3. H. Ban, J. Gavrilyuk, and C. F. Barbas III, *J. Am. Chem. Soc.*, 2010, **132**, 1523.
4. Y. Takaoka, H. Tsutsumi, N. Kasagi, E. Nakata, and I. Hamachi, *J. Am. Chem. Soc.*, 2006, **128**, 3273.
5. C.-X. Yin, K.-M. Xiong, F.-J. Huo, J. C. Salamanca, and R. M. Strongin, *Angew. Chem. Int. Ed.*, 2017, **56**, 13188.
6. Y. Fujita, Y. Murakami, A. Noda, and S. Miyoshi, *Bioconjugate Chem.*, 2017, **28**, 642.
7. S. P. Brown and A. B. Smith III, *J. Am. Chem. Soc.*, 2015, **137**, 4034.
8. F. J. Lopez-Jaramillo, M. Ortega-Muñoz, A. Megia-Fernandez, F. Hernandez-Mateo, and F. Santoyo-Gonzalez, *Bioconjugate Chem.*, 2012, **23**, 846.
9. C. Chatterjee and T. W. Muir, *J. Biol. Chem.*, 2010, **285**, 11045.
10. R. S. wanwick, A. M. Daines, L.-H. Tey, S. L. Flitsch, and R. K. Allemann, *ChemBioChem*, 2005, **6**, 1338.

11. E. Yoshioka, M. Nishimura, T. Nakazawa, S. Kohtani, and H. Miyabe, *J. Org. Chem.*, 2015, **80**, 8464.
12. E. Yoshioka, Y. Goto, I. Minato, and H. Miyabe, *Synthesis*, 2017, **49**, 4887.
13. E. Yoshioka, S. Kohtani, and H. Miyabe, *Angew. Chem. Int. Ed.*, 2011, **50**, 6638.