JOC The Journal of Organic Chemistry

Note

pubs.acs.org/joc

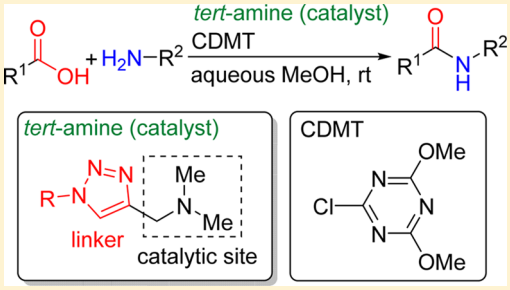
Role of Linkers in Tertiary Amines That Mediate or Catalyze 1,3,5-Triazine-Based Amide-Forming Reactions

Masanori Kitamura, Fumitaka Kawasaki, Kouichi Ogawa, Shuichi Nakanishi, Hiroyuki Tanaka, Kohei Yamada, and Munetaka Kunishima*

Faculty of Pharmaceutical Sciences, Institute of Medical, Pharmaceutical, and Health Sciences, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan

Supporting Information

ABSTRACT: We studied 1,3,5-triazine-based amide-forming reactions that are mediated or catalyzed by various *tert*-amines. The representative *tert*-amine was trimethylamine, which has amido, 1,2,3-triazolyl, aryl, and alkyl linkers. It was found that electron-deficient aryl and heteroaryl linkers, particularly 1,2,3-triazolyl linkers, are superior. On the basis of our findings, we synthesized ligand catalysts, including a 1,2,3-triazolyl linker that connects a protein ligand to a trimethylamine moiety, and found that fluorescent-labeling of a targeting protein using the ligand catalysts proceeded in good yields.



Amides and esters are two of the most fundamental and significant functional groups in chemistry, biochemistry, and materials science. Therefore, many dehydrocondensing reagents for synthesizing amide and ester bonds from carboxylic acids and amines or alcohols have been developed over the past century.[1−3] We have developed a dehydrocondensing reagent, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM), which selectively forms an amide in alcoholic or aqueous media.[4−7] As shown in Scheme 1a, it has also been found that tertiary amines (*tert*-amines, **1**) catalyze similar dehydrocondensing reactions.[8−11] Triazinylammonium salt (**2**), generated in situ from the *tert*-amines and 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT), reacts with a carboxylic acid (**3**) to form an activated acyloxytriazine (**4**), which then undergoes attack by an amine (**5**) to give a corresponding amide (**6**). The *tert*-amine (**1**) is regenerated by a proton capture agent (PCA).

On the basis of this catalytic condensing reaction, we have exploited artificial enzymes by introducing a cyclodextrin or a crown ether to *tert*-amines as substrate recognition sites.[8,9] In addition, *tert*-amines connected to ligands or drugs with specific affinity for target proteins, i.e., a ligand catalyst, enable a modular method for affinity labeling (MoAL method), as shown in Scheme 1b.[10,11] A triazinylammonium salt, formed from a ligand catalyst (**7**) and 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT), reacts with a protein to give a complex (**8**). Because of a linker of suitable length, the triazinylammonium moiety in **8** can reach a carboxy group of aspartic acid or glutamic acid at the protein surface to form an activated ester (**9**). Reaction of **9** with a primary amino group of a labeling module affords a labeled protein (**10**). Consequently, it is necessary to use appropriate linkers between *tert*-amines and ligand moieties for successful labeling of various targeting proteins.

To date, we have employed *N*,*N*-dimethylglycine ester as a linker and a *tert*-amine moiety (Figure 1a) for the amide-forming reaction for the following three reasons. (1) Introduction of the *N*,*N*-dimethylaminomethyl group to functional molecules via the ester bond is convenient. (2) The electron-withdrawing β-carbonyl group decreases the pK_a of the conjugate acid of the *N*,*N*-dimethylaminomethyl group; thus, it should be present in a reactive nonprotonated form in a protic solvent. The resulting nonprotonated *N*,*N*-dimethylaminomethyl group reacts smoothly with CDMT to form the dehydrocondensing reagent **2** and shows good catalytic activity.[12] (3) We have reported that the structure of *tert*-amines affects the reactivity toward CDMT and proposed the *gauche* β-alkyl group effect; i.e., the existence of a β-alkyl group in a *gauche* relationship against the electron pair of the amine nitrogen decreases the reactivity of *tert*-amines.[13] The *N*,*N*-dimethylaminomethyl group does not have the *gauche* β-alkyl group effect.

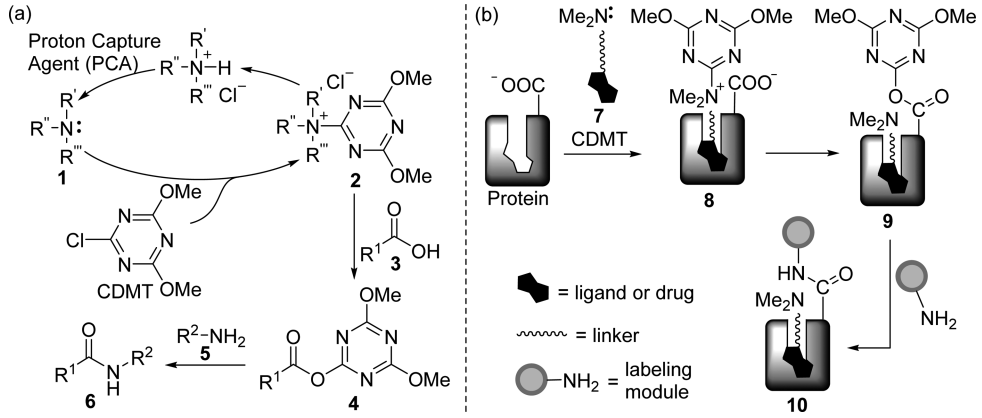
However, the ester bonds might be hydrolyzed during a dehydrocondensing reaction in an aqueous solvent or storage under atmospheric conditions. Thus, an alternative method for tethering between a *N*,*N*-dimethylaminomethyl group and various functional molecules is required.

Herein, we report on the synthesis of various *tert*-amines (**1** in Figure 1b) that have amido, 1,2,3-triazolyl, aryl, and alkyl linkers, and their reactivity for amide-forming reactions. Trimethylamine was chosen as the representative *tert*-amine moiety because there is no influence of the *gauche* β-alkyl group effect, which changes the reactivity of the *tert*-amines. Thus, the observed result will indicate the role of the linkers.

Received: February 24, 2014
Published: March 20, 2014

dx.doi.org/10.1021/jo500376m | J. Org. Chem. 2014, 79, 3709−3714

Scheme 1. Reaction Mechanisms for (a) the Catalytic Amide-Forming Reaction and (b) Modular Method for Affinity Labeling (MoAL)



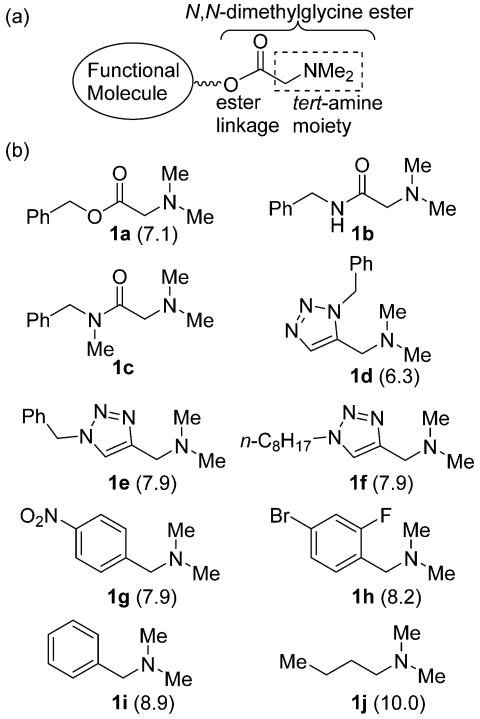
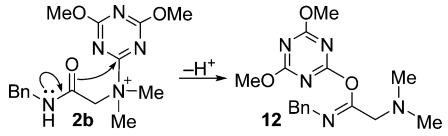


Figure 1. (a) Conventional *tert*-amines. (b) Structure of *tert*-amines used in this study. The pK_a values of conjugate acids of *tert*-amines shown in parentheses were determined by potentiometric pH titration experiments or were obtained from the literature; see the Supporting Information.

Furthermore, on the basis of our findings, ligand catalysts were synthesized and examined for fluorescent-labeling of a targeting protein.

To examine the reactivity of *tert*-amines (**1**) for the amide-forming reaction, a carboxylic acid (**3a**) and amines (**5a**, **5b**) were used as model reactants under stoichiometric conditions because these compounds have primary aliphatic substituents and the phenyl group allows their detection by UV absorption. The amide (**6a**) from **3a** and **5a** was obtained in 75% yield using **1a** and CDMT in methanol at rt (Table 1, entry 1). When we examined *tert*-amine **1b** containing a secondary amide, which is more stable for hydrolysis than the ester, the reaction gave rather disappointing results in terms of product yields (entries 2 and 3, 16% yield for **6a** and 15% yield for **6b**). The lower yields using **1b** are possibly due to the migration of the 1,3,5-triazinyl group to carbonyl oxygen in the intermediate **2b** to form an imidate ester **12**[14] (Scheme 2). In fact, **1c**, which

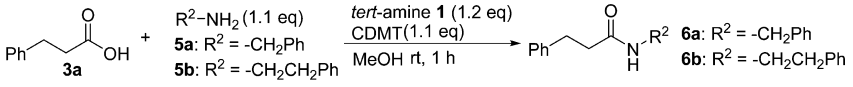
Scheme 2. Plausible Byproduct Formation from 2b



consists of a *tert*-amide moiety, and, therefore, does not undergo such migration, improved the product yield (entry 4).

We then investigated *tert*-amines having a hydrolytically stable and electron-deficient[15] 1,2,3-triazolyl group (**1d**–**1f**), which are readily synthesized by 1,2,3-triazole forming reactions between azides and alkynes, the so-called click chemistry.[16−22] The electron-deficient 1,2,3-triazolyl group effectively decreases the pK_a of the conjugate acid of *N*,*N*-dimethylaminomethyl moieties, as shown in Figure 1b. We have previously reported that weakly basic *tert*-amines afford higher yields of the amide **6**

Table 1. Dehydrocondensing Reactions Mediated by *tert*-Amines 1

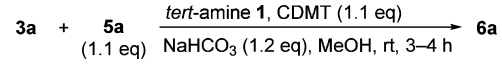


entry	5	6	1	yield (%)[a]	entry	5	6	1	yield (%)[a]	entry	5	6	1	yield (%)[a]
1	**5a**	**6a**	**1a**	75	5	**5a**	**6a**	**1d**	84	9	**5a**	**6a**	**1h**	88
2	**5a**	**6a**	**1b**	16	6	**5a**	**6a**	**1e**	84	10	**5a**	**6a**	**1i**	76
3	**5b**	**6b**	**1b**	15	7	**5a**	**6a**	**1f**	95	11	**5a**	**6a**	**1j**	74
4	**5a**	**6a**	**1c**	60	8	**5a**	**6a**	**1g**	88					

[a]Yields based on ^{1}H NMR analysis.

dx.doi.org/10.1021/jo500376m | *J. Org. Chem.* 2014, 79, 3709−3714

Table 2. Catalytic Dehydrocondensing Reactions of Carboxylic Acid (3a) and Amine (5a) in MeOH



entry	1 (eq)	yield (%)[a]	entry	1 (eq)	yield (%)[a]	entry	1 (eq)	yield (%)[a]
1	**1a** (0.2)	77	6	**1f** (0.2)	93	11	**1h** (0.05)	78
2	**1c** (0.2)	50	7	**1f** (0.05)	85	12	**1i** (0.2)	84
3	**1d** (0.2)	86	8	**1g** (0.2)	89	13	**1i** (0.05)	66
4	**1e** (0.2)	81	9	**1g** (0.05)	74	14	**1j** (0.2)	76
5	**1e** (0.05)	83	10	**1h** (0.2)	91	15	**1j** (0.05)	48

[a]Yields based on ^{1}H NMR analysis.

compared with strongly basic *tert*-amines in protic solvents because weakly basic *tert*-amines are present in a reactive nonprotonated form toward CDMT.[12] It is considered that a greater proportion of a nonprotonated *N*,*N*-dimethylaminomethyl moiety by the electron-deficient 1,2,3-triazolyl group results in a smooth formation of the dehydrocondensing reagent **2** in situ, and consequently, **1d**–**1f** furnished the product **6a** in good yields (entries 5–7 in Table 1). Similarly, aryl linkers with electron-withdrawing groups expand the amount of nonprotonated form and the yield gradually decreased with increased pK_a of the conjugate acids of **1g**–**1j**, as shown in entries 8–11 in Table 1.

To perform the catalytic dehydrocondensing reaction using *tert*-amines, it is necessary to add proton capture agents (PCAs) in the reaction mixture to regenerate nonprotonated *tert*-amines (Scheme 1a). Sodium hydrogen carbonate and *N*,*N*-diethylaniline, which are weakly basic, were chosen as the PCAs in protic solvents for minimal levels of byproduct formation (Table S2 in the Supporting Information).[12] The results of the catalytic reactions using various *tert*-amines **1** in absolute methanol are shown in Table 2. Although *tert*-amines that have ester, amido, and alkyl linkers (**1a**, **1c**, and **1j**) produced **6a** in moderate yields (entries 1, 2, and 14), the catalysts with 1,2,3-triazolyl (**1d**–**1f**) and aryl linkers (**1g**–**1i**) provided **6a** in good yields (entries 3, 4, 6, 8, 10, and 12). In contrast to the cases of **1g**–**1j** (entries 9, 11, 13, and 15), the reaction using **1e** and **1f** (entries 5 and 7) can be conducted with a lower catalyst amount (0.05 equiv) without any decrease in reactivity.

One of the characteristics of the triazine-based amide-forming reaction is that the reaction can be conducted in aqueous solutions that can dissolve polar substrates. In 50% aqueous methanol, it is considered that salts, such as ammonium and carboxylate ions, in the reaction mixture are more stabilized by solvation than in methanol. Therefore, it is anticipated that influence of the pK_a of the conjugated acids of **1** would be increased in the catalytic amide-forming reaction. Indeed except for **1a**, a linear relationship between the yields and pK_a is observed, as shown in Figure 2. Similar to the catalytic reaction in absolute methanol mentioned above, *tert*-amines with 1,2,3-triazolyl linkers (**1d**–**1f**) produced the product **6a** in good yields in 50% aqueous methanol. The lower yield of **1a** could be partly explained by the hydrolysis of the ester, resulting in the loss of catalyst.

With information regarding the 1,2,3-triazolyl linkers in hand, we further examined the MoAL method (Scheme 1b) for labeling of avidin, which has high affinity for biotin. We first synthesized ligand catalysts in which *tert*-amines are connected to a biotin moiety via the 1,2,3-triazolyl linker (synthetic schemes for **7a**, **7b** are shown in Schemes S2, S3 in the Supporting Information). To investigate whether the 1,2,3-triazolyl linker is effective for the MoAL method, ligand catalysts **7c** and **7d**,[11]

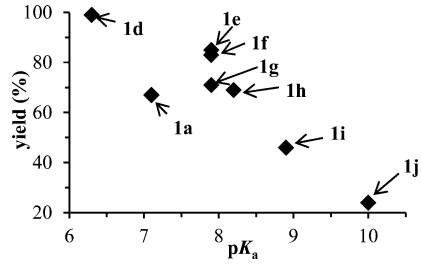
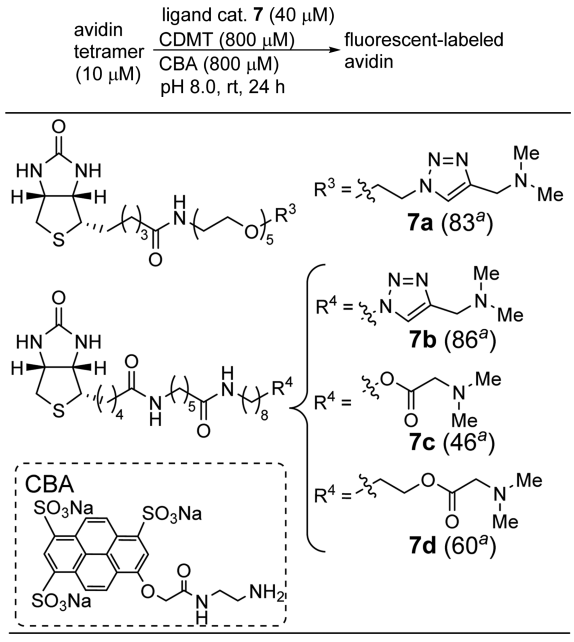


Figure 2. Catalytic dehydrocondensing reactions of **3a** and **5a** in 50% aqueous MeOH. The details of the results are shown in Table S3 in the Supporting Information.

which have similar linker length and an ester group, were also examined.[23] Avidin was treated with the ligand catalysts, CDMT, and Cascade Blue ethylenediamine (CBA), which is a fluorescent primary amine, at rt for 24 h. The results are summarized in Table 3. Compared with ligand catalysts **7c** and

Table 3. Avidin Labeling Study Using Ligand Catalysts That Have 1,2,3-Triazolyl or Ester Linkers[a]



[a]The labeling yields (%) of avidin.

7d, **7a** and **7b** effectively labeled avidin, indicating that the 1,2,3-triazolyl linker is more efficient for the MoAL method than the ester linker.

In summary, we have studied the triazine-based dehydrocondensing reaction in protic solvents using trimethylamines

dx.doi.org/10.1021/jo500376m | J. Org. Chem. 2014, 79, 3709–3714

that have amido, 1,2,3-triazolyl, aryl, and alkyl linkers. It was found that the 1,2,3-triazolyl and substituted aryl linkers were superior for the amide-forming reactions. In the case of the 1,2,3-triazolyl linkers, various functionalities were readily introduced to the *N*,*N*-dimethylaminomethyl group because of the availability of click chemistry, and thus, the ligand catalysts were conveniently prepared. In addition, the electron-deficient 1,2,3-triazolyl group increases the reactivity of the ligand catalysts in the MoAL method, which resulted in better yields of the fluorescent-labeled avidin compared with the yields afforded by the ester linker. We are currently investigating the application of catalysts with 1,2,3-triazolyl linkers for various dehydrocondensing reactions.

EXPERIMENTAL SECTION

General Experimental Methods. 2-Chloro-4,6-dimethoxy-1,3,5-triazine (CDMT),[24] *N*,*N*-dimethyl-4-nitrobenzylamine (**1g**),[25] ligand catalysts (**7c**, **7d**),[11] and Cascade Blue ethylenediamine[26] were prepared by procedures reported in the literature. *N*,*N*-Dimethylbenzylamine (**1i**), *N*,*N*-dimethylbutylamine (**1j**), dry dichloromethane (CH_2Cl_2), dry acetonitrile (CH_3CN), dry dimethylsulfoxide (DMSO), and other chemicals and solvents were obtained from commercial sources and used without further purification. ^{1}H NMR spectra were recorded on 270, 400, and 600 MHz spectrometers using $CDCl_3$ or CD_3OD. Chemical shifts (δ) were determined relative to an internal reference of tetramethylsilane for ^{1}H NMR and solvent peaks for ^{13}C NMR. Analytical TLC was performed using a Merck silica gel 60 F254 TLC plate. Silica gel column chromatography was performed using Kanto Silica Gel 60 Spherical (63−210 μm) and Merck Silica Gel 60 (<63 μm, 7729).

*2-(Dimethylamino)acetic Acid Benzyl Ester (**1a**).* To a stirred solution of *N*,*N*-dimethylglycine hydrochloride (2.09 g, 15.0 mmol), benzylalcohol (1.55 mL, 15.0 mmol), and *N*-methylmorpholine (NMM, 1.65 mL, 15.0 mmol) in CH_2Cl_2 (44 mL) were added 4-dimethylaminopyridine (DMAP, 0.92 g, 7.5 mmol) and *N*,*N*′-dicyclohexylcarbodiimide (DCC, 4.64 g, 22.5 mmol) at 0 °C under a N_2 atmosphere. After warming to rt, the mixture was stirred overnight. The reaction mixture was filtrated on Celite and concentrated. The concentrate was dissolved in CH_2Cl_2, washed with brine (2 times), dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (hexane/EtOAc = 1:1) to give **1a** (2.52 g, 87% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ 7.37−7.33 (m, 5H), 5.18 (s, 2H), 3.23 (s, 2H), 2.36 (s, 6H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.30, 135.59, 128.40, 128.21, 128.14, 66.12, 60.21, 45.10 ppm; IR (neat) 2944, 2871, 2823, 2773, 1749, 1456, 1284, 1234, 1191, 1149, 1063, 858, 739, 698 cm^{-1}; LRMS (ESI-hexapole) *m*/*z* 194 $[M + H]^+$, 216 $[M + Na]^+$; HRMS (DART-TOF) *m*/*z* $[M + H]^+$ Calcd for $C_{11}H_{16}NO_2$ 194.1181; found 194.1171.

*N-Benzyl-2-(dimethylamino)acetamide (**1b**).* **1b** was prepared in a similar manner as that for **1a** except for a dehydrocondensing reagent. *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) was used as a dehydrocondensing reagent instead of DCC. The crude product was purified by column chromatography on silica gel (MeOH/$CHCl_3$ = 1:5) to give **1b** (1.32 g, 98% yield) as a pale yellow oil. ^{1}H NMR (270 MHz, $CDCl_3$/TMS) δ 7.47 (brs, 1H), 7.38−7.24 (m, 5H), 4.48 (d, *J* = 6.1 Hz, 2H), 3.01 (s, 2H), 2.28 (s, 6H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.55, 138.44, 128.67, 127.72, 127.39, 63.13, 46.04, 42.93 ppm; IR ($CHCl_3$) 3363, 2950, 2852, 2833, 2787, 1668, 1603, 1537, 1508, 1456, 1419, 1269, 1149, 930, 733, 727 cm^{-1}; LRMS (ESI-hexapole) *m*/*z* 193 $[M + H]^+$, 215 $[M + Na]^+$; HRMS (DART-TOF) *m*/*z* $[M + H]^+$ Calcd for $C_{11}H_{17}N_2O$ 193.1341; found 193.1346.

*N-Benzyl-N-methyl-2-(dimethylamino)acetamide (**1c**).* **1c** was prepared in a similar manner as that for **1b**. The crude product was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 7:3) to give **1c** (3.00 g, 97% yield) as a pale yellow oil. Two sets of NMR signals are attributable to the presence of amide *cis* and *trans* isomers. ^{1}H NMR (270 MHz, $CDCl_3$/TMS) δ 7.39−7.17 (m, 5H), 4.70 and 4.59 (s, 2H), 3.17 and 3.16 (s, 2H), 2.98 and 2.91 (s, 3H), 2.34 and 2.30 (s, 6H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.70, 169.29, 136.80, 136.38, 128.26, 127.97, 127.46, 126.89, 126.72, 126.00, 61.74, 61.46, 52.29, 50.23, 45.13, 45.10, 33.80, 33.06 ppm; IR ($CHCl_3$) 2943, 2862, 2823, 2773, 1647, 1496, 1454, 1404, 1263, 1172, 1120, 1038, 862, 737, 700 cm^{-1}; HRMS (DART-TOF) *m*/*z* $[M + H]^+$ Calcd for $C_{12}H_{19}N_2O$ 207.1497; found 207.1497.

*N-(1-Benzyl-1H-1,2,3-triazol-5-ylmethyl)-N,N-dimethylamine (**1d**).*[27] To a stirred THF (10 mL) solution of benzyl azide (173 mg, 1.30 mmol) and *N*,*N*-dimethylpropargylamine (0.167 mL, 1.56 mmol) was added pentamethylcyclopentadienylbis(triphenylphosphine)-ruthenium(II) chloride ($Cp^*RuCl(PPh_3)_2$, 20.6 mg, 0.0259 mmol), and the mixture was refluxed for 4 h. The reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 95:5) to give **1d** (231 mg, 82% yield) as a colorless oil.

*N-(1-Benzyl-1H-1,2,3-triazol-4-ylmethyl)-N,N-dimethylamine (**1e**).*[28] Benzylbromide (1.80 mL, 15.1 mmol) was added dropwise to a DMSO (33 mL) solution of sodium azide (1.07 g, 16.5 mmol), and the mixture was stirred for 1.5 h at rt. After addition of water (50 mL), the mixture was extracted with diethyl ether. The combined organic layer was washed with water and brine and then dried over $MgSO_4$ and filtrated. Concentration of the organic layer under reduced pressure afforded benzyl azide (1.87 g) as a colorless oil. To a stirred solution of benzyl azide (1.87 g), *N*,*N*-dimethylpropargylamine (2.26 mL, 21.0 mmol) in *t*-BuOH (10 mL) and water (5 mL) were added sodium ascorbate (277 mg, 1.40 mmol) and $CuSO_4{\cdot}5H_2O$ (175 mg, 0.70 mmol) at rt. After stirring for 2 h, the reaction mixture was extracted with CH_2Cl_2. The combined organic layer was washed with saturated aqueous Na_2CO_3, water, and brine. The extract was dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (1% Et_3N in CH_2Cl_2) to give **1e** (2.58 g, 79% yield for two steps) as a colorless solid. mp 41−42 °C.

*N-(1-Octyl-1H-1,2,3-triazol-4-ylmethyl)-N,N-dimethylamine (**1f**).* **1f** was prepared in a similar manner as that for **1e**. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate = 1:1 to 1% Et_3N in ethyl acetate) to give **1f** (1.94 g, 72% yield for two steps) as a colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$/TMS) δ 7.47 (s, 1H), 4.33 (t, *J* = 7.3 Hz, 2H), 3.61 (s, 2H), 2.28 (s, 6H), 1.89 (tt, *J* = 7.3, 7.3 Hz, 2H), 1.31−1.26 (m, 10H), 0.87 (t, *J* = 6.7 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ = 145.11, 121.98, 54.38, 50.17, 45.09, 31.57, 30.18, 28.92, 28.82, 26.34, 22.47 ppm; IR (neat) 2927, 2856, 2817, 2767, 1458, 1377, 1331, 1263, 1219, 1174, 1144, 1097, 1045, 1016, 850, 814, 787 cm^{-1}; LRMS (ESI-hexapole) *m*/*z* 239 $[M + H]^+$; HRMS (DART-TOF) *m*/*z* $[M + H]^+$ Calcd for $C_{13}H_{27}N_4$ 239.2236; found 239.2226.

*N,N-Dimethyl-4-bromo-2-fluorobenzylamine (**1h**).* To a stirred solution of 4-bromo-2-fluorobenzylbromide (1.00 g, 3.73 mmol) and potassium carbonate (3.10 g, 22.4 mmol) in CH_3CN (19 mL) was added dimethylamine (11.09 M solution in water, 1.01 mL, 11.2 mmol) at 0 °C. After stirring at rt for 10 min, the reaction mixture was concentrated and water was added, and the resulting mixture was extracted with ethyl acetate. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated to obtain 0.795 g of 4-bromo-2-fluoro-*N*,*N*-dimethylbenzylamine (**1h**) as a colorless oil in quantitative yield. A portion of the product was filtered over silica gel using ethyl acetate as the eluent, and it was purified with recycling preparative HPLC ($CHCl_3$) for potentiometric pH titrations. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ 7.28−7.21 (m, 3H), 3.44 (s, 2H), 2.25 (s, 6H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.12 (d, J_{C-F} = 251.1 Hz), 132.58 (d, J_{C-F} = 5.8 Hz), 127.22 (d, J_{C-F} = 3.8 Hz), 124.71 (d, J_{C-F} = 14.4 Hz), 120.97 (d, J_{C-F} = 9.6 Hz), 118.93 (d, J_{C-F} = 26.0 Hz), 56.14, 45.20 ppm; IR (neat) 2976, 2945, 2860, 2819, 2771, 1605, 1577, 1483, 1456, 1404, 1367, 1267, 1219, 1174, 1149, 1109, 1068, 1025, 879, 852, 816 cm^{-1}; Anal. Calcd for $C_9H_{11}BrFN$: C, 46.58; H, 4.78; N, 6.03. Found: C, 46.48; H, 4.64; N, 6.00; LRMS (ESI-hexapole) *m*/*z* 232, 234 $[M + H]^+$.

Potentiometric pH Titrations. The preparation of the test solutions and the method used for calibration of the electrode system

dx.doi.org/10.1021/jo500376m | J. Org. Chem. 2014, 79, 3709−3714

(Potentiometric Automatic Titrator 794 Basic Titrino, Metrohm) with a Combined LL pH glass electrode (Metrohm) have been described in ref 29. All of the test solutions (50 mL) were maintained under a nitrogen atmosphere. The potentiometric pH titrations were performed with I = 0.1 ($NaNO_3$) at 25.0 °C (0.1 M aqueous NaOH was used as the base). The deprotonation constants were determined using the "BEST" software program.[30] The K_W (equivalent to $a_{H^+}a_{OH^-}$), K_W (equivalent to $[H^+][OH^-]$), and f_{H^+} values used at 25 °C were $10^{-14.00}$, $10^{-13.79}$, and 0.825, respectively. The corresponding mixed constants K_2 (= [HO^--bound species]a_{H^+}/[H_2O-bound species]), were derived using $[H^+] = a_{H^+}/f_{H^+}$.

General Procedure for the Amide-Forming Reaction. To a stirred solution of 3-phenylpropionic acid (38 mg, 0.25 mmol), benzylamine (30 μL, 0.28 mmol), and *tert*-amine **1** (0.30 mmol) in MeOH (1.0 mL) was added a MeOH solution of CDMT (48 mg, 0.28 mmol) at rt. After stirring at rt, the reaction mixture was quenched with 1 M aqueous solution of $KHSO_4$ (3 mL) and was then concentrated under reduced pressure. The residue was extracted with Et_2O, and the organic phase was washed sequentially with water, 1 M aqueous HCl, water, 1 M aqueous NaOH, water, saturated aqueous $NaHCO_3$, and brine. The extract was dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. The yields of **6a** and **6b**[7] were determined using ^{1}H NMR measurements of the crude product, which contained imidazole or (*E*)-*N*,*N*-dimethylcinnamamide as an internal standard.

In the case of entry 2 in Table 1, an unstable imidate ester **12**, which could be analyzed only by ^{1}H NMR and mass spectroscopy, was obtained (13.7 mg, 15% yield based on CDMT). ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ 7.37−7.19 (m, 5H), 5.29 (s, 2H), 3.99 (s, 6H), 3.91 (s, 2H), 2.29 (s, 6H) ppm; LRMS (ESI-hexapole) *m*/*z* 332 $[M + H]^+$.

*N-(17-Azido-3,6,9,12,15-pentaoxaheptadecyl)biotinamide (**13**).* To a solution of 17-azido-3,6,9,12,15-pentaoxa-1-heptadecylamine[31] (180 mg, 0.588 mmol) and (+)-biotin (144 mg, 0.588 mmol) in MeOH (5 mL) was added DMT-MM[4−7] (244 mg, 0.881 mmol) at rt. After stirring overnight at rt, the solvent was removed under vacuum. The crude mixture was purified by silica gel column chromatography ($CHCl_3$/MeOH = 9:1) to give the desired compound as colorless crystals (179 mg, 57% yield). mp 96−97 °C. ^{1}H NMR (400 MHz, methanol-d_4/TMS) δ 8.03 (s, 1H), 4.49 (dd, *J* = 7.8, 4.6 Hz, 1H), 4.30 (dd, *J* = 7.8, 4.6 Hz, 1H), 3.81−3.64 (m, 18H), 3.54 (t, *J* = 4.8 Hz, 2H), 3.37−3.30 (brs, 4H), 3.23−3.18 (m, 1H), 2.93 (dd, *J* = 12.6, 4.8 Hz, 1H), 2.70 (d, *J* = 12.8 Hz, 1H), 2.22 (t, *J* = 7.1 Hz, 2H), 1.76−1.61 (m, 4H), 1.48−1.40 (m, 2H) ppm; ^{13}C NMR (100 MHz, methanol-d_4) δ 176.08, 166.06, 71.62, 71.55, 71.51, 71.23, 71.13, 70.56, 63.33, 61.59, 57.00, 51.76, 41.05, 40.34, 36.71, 29.77, 29.49, 26.84 ppm; IR ($CHCl_3$) 2931, 2102, 1702, 1648, 1452, 1107 cm^{-1}; LRMS (ESI-hexapole) 533 $[M + H]^+$; HRMS (ESI-TOF) *m*/*z* $[M + Na]^+$ Calcd for $C_{22}H_{40}N_6NaO_7S$ 555.2577; found 555.2554.

*Ligand Catalyst (**7a**).* To a solution of **13** (80 mg, 0.15 mmol), *N*,*N*-dimethylpropargylamine (19 mg, 0.23 mmol), and sodium ascorbate (3.0 mg, 0.015 mmol) in *t*-BuOH/H_2O (2:1, 2 mL) was added $CuSO_4·5H_2O$ (1.9 mg, 0.0076 mmol) at rt. After stirring for 20 h at rt, the solvent was removed under vacuum. The crude mixture was purified by silica gel column chromatography ($CHCl_3$/MeOH = 85:15 containing 1% Et_3N) to give 65 mg of the desired compound as colorless crystals (71% yield). mp 116−117 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ 7.70 (s, 1H), 6.89 (brs, 1H), 6.29 (brs, 1H), 5.39 (brs, 1H), 4.55 (t, *J* = 5.3 Hz, 2H), 4.51 (dd, *J* = 7.1, 4.8 Hz, 1H), 4.32 (dd, *J* = 7.1, 4.4 Hz, 1H), 3.88 (t, *J* = 5.0 Hz, 2H), 3.63 (t, *J* = 5.0 Hz, 18H), 3.56 (t, *J* = 5.0 Hz, 2H), 3.46−3.41 (m, 2H), 3.15 (dd, *J* = 11.9, 7.3 Hz, 1H), 2.91 (dd, *J* = 12.8, 5.0 Hz, 1H), 2.74 (d, *J* = 12.8 Hz, 1H), 2.29 (s, 6H), 2.23 (t, *J* = 7.3 Hz, 2H), 1.80−1.61 (m, 4H), 1.48−1.40 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.32, 163.93, 144.63, 123.79, 70.55, 70.53, 70.51, 70.46, 70.44, 70.38, 70.07, 69.96, 69.54, 61.75, 60.16, 55.59, 54.26, 50.21, 45.03, 40.55, 39.14, 35.92, 28.19, 28.10, 25.60 ppm; IR ($CHCl_3$) 2931, 1701, 1658, 1458, 1103 cm^{-1}; LRMS (ESI-hexapole) 616 $[M + H]^+$; HRMS (FAB) calcd for $C_{27}H_{50}N_7O_7S$ $[M + H]^+$ 616.3492; found 616.3495.

*N-[5-(8-Azidooct-1-ylaminocarbonyl)pent-1-yl]biotinamide (**14**).* This compound was synthesized from 6-(biotinylamino)hexanoic acid and 8-azidooct-1-ylamine[32] in a similar manner as that for **13**. The crude product was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 12:1) to give **14** (23 mg, 30% yield). ^{1}H NMR (400 MHz, methanol-d_4/TMS) δ 7.94 (brs, 1H) 4.49 (dd, *J* = 7.8, 4.5 Hz, 1H), 4.30 (dd, *J* = 7.8, 4.5 Hz, 1H), 3.29−3.13 (m, 7H), 2.92 (dd, *J* = 12.8, 5.0 Hz, 1H), 2.70 (d, *J* = 12.8 Hz, 1H), 2.19 (t, *J* = 7.1 Hz, 2H), 2.17 (t, *J* = 7.6 Hz, 2H), 1.76−1.34 (m, 24H) ppm; ^{13}C NMR (100 MHz, methanol-d_4) δ 176.02, 175.97, 166.13, 63.39, 61.63, 57.02, 52.45, 41.05, 40.34, 40.21, 37.01, 36.82, 30.39, 30.27, 26.76, 30.20, 30.14, 29.90, 29.80, 29.50, 27.90, 27.77, 27.57, 26.94 ppm; HRMS (FAB) calcd for $C_{24}H_{44}N_7O_3S$ $[M + H]^+$ 510.3221; found 510.3222.

*Ligand Catalyst (**7b**).* This compound was synthesized from **14** and *N*,*N*-dimethylpropargylamine in a similar manner as that for **7a**. The crude product was purified by preparative thin layer column chromatography ($CHCl_3$/MeOH = 10:1 containing 0.5% Et_3N) to give **7b** (18 mg, 73% yield). ^{1}H NMR (400 MHz, methanol-d_4/TMS) δ 7.97 (s, 1H) 4.48 (dd, *J* = 7.8, 4.5 Hz, 1H), 4.41 (t, *J* = 7.1 Hz, 2H), 4.30 (dd, *J* = 7.8, 4.5 Hz, 1H), 3.81 (s, 2H), 3.21 (dd, *J* = 9.7, 5.0 Hz, 1H), 3.15 (t, *J* = 7.8 Hz, 2H), 3.14 (t, *J* = 7.8 Hz, 2H), 2.92 (dd, *J* = 12.8, 5.0 Hz, 1H), 2.70 (d, *J* = 12.8 Hz, 1H), 2.40 (s, 6H), 2.18 (t, *J* = 7.8 Hz, 2H), 2.17 (t, *J* = 7.8 Hz, 2H), 1.77−1.26 (m, 24H) ppm; ^{13}C NMR (100 MHz, methanol-d_4) δ 175.97, 175.94, 166.10, 142.73, 125.92, 63.37, 61.61, 57.02, 53.82, 51.39, 44.36, 41.06, 40.30, 40.19, 36.99, 36.82, 31.24, 30.35, 30.16, 30.13, 29.97, 29.80, 29.50, 27.83, 27.55, 27.39, 26.94, 26.74 ppm; IR (KBr) 3430, 3310, 2927, 2854, 1698, 1633, 1558, 1471, 1458, 1261, 1149, 1054, 1037 cm^{-1}; HRMS (FAB) calcd for $C_{29}H_{53}N_8O_3S$ $[M + H]^+$ 593.3961; found 593.3962.

General Procedure for Affinity Labeling of Avidin. Avidin (0.75 nmol) in a phosphate buffer (10 μL, 50 mM, pH 8.0) and ligand catalyst (3.0 nmol) in a phosphate buffer (5 μL, 50 mM, pH 8.0) were mixed in a microtube and left to stand at rt for 30 min. To this solution, Cascade Blue ethylenediamine (60 nmol) in a phosphate buffer (20 μL, 50 mM, pH 8.0), CDMT (60 nmol) in a mixture (10 μL) of a phosphate buffer (50 mM, pH 8.0) and MeOH (5% v/v), and a phosphate buffer (30 μL, 50 mM, pH 8.0) were added. The resulting solution was shaken using a vortex mixer. The final concentration of each component in the reaction mixture was as follows: avidin, 10 μM; ligand catalyst, 40 μM; Cascade Blue ethylenediamine, 800 μM; CDMT, 800 μM. The reaction mixture was then left to stand at rt for 24 h. It was then subjected to gel chromatography at rt (support: Sephadex G-50 medium; column size: 350 × 7 mm; elution: 50 mM phosphate buffer at pH 8.0), and avidin-containing fractions were collected. The yields of labeling of avidin were determined by UV absorption spectra of the collected fractions.[10,11]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Potentiometric pH titration experiments for **1** (Figures S1 and S2, Table S1, and Scheme S1); Schemes S2 and S3; Tables S2 and S3; and ^{1}H and ^{13}C NMR spectra for **1a**−**1h**, **7a**, **7b**, **13**, and **14**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: kunisima@p.kanazawa-u.ac.jp.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by JSPS KAKENHI Grant (Nos. 23590004 and 25460013).

■ REFERENCES

(1) Montalbetti, C. A. G. N.; Falque, V. *Tetrahedron* **2005**, *61*, 10827−10852.
(2) El-Faham, A.; Albericio, F. *Chem. Rev.* **2011**, *111*, 6557−6602.

(3) Pattabiraman, V. R.; Bode, J. W. *Nature* **2011**, *480*, 471−479.
(4) Kitamura, M.; Kunishima; M. 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride. In *e-EROS (Encyclopedia of Reagents for Organic Synthesis)* [Online]; Crich, D., Charette, A. B., Fuchs, P. L., Eds.; John Wiley & Sons, Ltd: West Sussex, U.K, 2013. http://onlinelibrary.wiley.com/o/eros/articles/rn01530/frame.html (accessed April 22, 2013).
(5) Kunishima, M.; Kawachi, C.; Iwasaki, F.; Terao, K.; Tani, S. *Tetrahedron Lett.* **1999**, *40*, 5327−5330.
(6) Kunishima, M.; Kawachi, C.; Morita, J.; Terao, K.; Iwasaki, F.; Tani, S. *Tetrahedron* **1999**, *55*, 13159−13170.
(7) Kunishima, M.; Kawachi, C.; Hioki, K.; Terao, K.; Tani, S. *Tetrahedron* **2001**, *57*, 1551−1558.
(8) Kunishima, M.; Yoshimura, K.; Morigaki, H.; Kawamata, R.; Terao, K.; Tani, S. *J. Am. Chem. Soc.* **2001**, *123*, 10760−10761.
(9) Kunishima, M.; Hioki, K.; Moriya, T.; Morita, J.; Ikuta, T.; Tani, S. *Angew. Chem., Int. Ed.* **2006**, *45*, 1252−1255.
(10) Kunishima, M.; Nakanishi, S.; Nishida, J.; Tanaka, H.; Morisaki, D.; Hioki, K.; Nomoto, H. *Chem. Commun.* **2009**, 5597−5599.
(11) Nakanishi, S.; Tanaka, H.; Hioki, K.; Yamada, K.; Kunishima, M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7050−7053.
(12) Kunishima, M.; Kitamura, M.; Tanaka, H.; Nakakura, I.; Moriya, T.; Hioki, K. *Chem. Pharm. Bull.* **2013**, *61*, 882−886.
(13) Kunishima, M.; Ujigawa, T.; Nagaoka, Y.; Kawachi, C.; Hioki, K.; Shiro, M. *Chem.—Eur. J.* **2012**, *18*, 15856−15867.
(14) The imidate ester **12** was unstable and only characterized by ^{1}H NMR and mass spectroscopy (see the Experimental Section).
(15) Potratz, S.; Michra, A.; Bäuerle, P. *Beilstein J. Org. Chem.* **2012**, *8*, 683−692.
(16) Finn, M. G.; Fokin, V. V. Copper-Catalyzed Azide-Alkyne Cycloaddition (CuAAC). In *Catalysis without Precious Metals*; Bullock, R. M., Ed.; Wiley-VCH: Weinheim, 2010; pp 33−54.
(17) Finn, M. G.; Fokin, V. V. *Chem. Soc. Rev.* **2010**, *39*, 1231−1232 and there are many reviews in this issue.
(18) Majumdar, K. C.; Ray, K. *Synthesis* **2011**, 3767−3783.
(19) Agalave, S. G.; Maujan, S. R.; Pore, V. S. *Chem.—Asian J.* **2011**, *6*, 2696−2718.
(20) Tsukada, Y.; Yamada, K.; Kunishima, M. *Tetrahedron Lett.* **2011**, *52*, 3358−3360.
(21) Li, X. *Chem.—Asian J.* **2011**, *6*, 2606−2616.
(22) Valverde, I. E.; Mindt, T. L. *Chimia* **2013**, *67*, 262−266.
(23) We reexamined the labeling study of avidin using **7c** and **7d** because reaction conditions used in this paper are different from those of ref 11.
(24) Cronin, J. S.; Ginah, F. O.; Murray, A. R.; Copp, J. D. *Synth. Commun.* **1996**, *26*, 3491−3494.
(25) Murugesan, D.; Mital, A.; Kaiser, M.; Shackleford, D. M.; Morizzi, J.; Katneni, K.; Campbell, M.; Hudson, A.; Charman, S. A.; Yeates, C.; Gilbert, I. H. *J. Med. Chem.* **2013**, *56*, 2975−2990.
(26) Whitaker, J. E.; Haugland, R. P.; Moore, P. L.; Hewitt, P. C.; Reese, M.; Haugland, R. P. *Anal. Biochem.* **1991**, *198*, 119−130.
(27) Lamberti, M.; Fortman, G. C.; Poater, A.; Broggi, J.; Slawin, A. M. Z.; Cavallo, L.; Nolan, S. P. *Organometallics* **2012**, *31*, 756−767.
(28) Özçubukçu, S.; Ozkal, E.; Jimeno, C.; Pericàs, A. *Org. Lett.* **2009**, *11*, 4680−4683.
(29) Kimura, E.; Shiota, T.; Koike, T.; Shiro, M.; Kodama, M. *J. Am. Chem. Soc.* **1990**, *112*, 5805−5811.
(30) Martell, A. E.; Motekaitis, R. J. *Determination and Use of Stability Constants*, 2nd ed.; VCH: New York, 1992.
(31) Risseeuw, M. D. P.; De Clercq, D. J. H.; Lievens, S.; Hillaert, U.; Sinnaeve, D.; den Broeck, F. V.; Martins, J. C.; Tavernier, J.; Calenbergh, S. V. *ChemMedChem* **2013**, *8*, 521−526.
(32) Hur, G. H.; Meier, J. L.; Baskin, J.; Codelli, J. A.; Bertozzi, C. R.; Marahiel, M. A.; Burkart, M. D. *Chem. Biol.* **2009**, *16*, 372−381.