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Acceleration of Cu(I)-mediated Huisgen 1,3-dipolar cycloaddition by histidine derivatives

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Abstract—Acceleration of Cu(I)-mediated Huisgen 1,3-dipolar cycloaddition (Sharpless 'click reaction') by non-basic histidine derivatives was found. An efficient 'self-activating' click reaction between the azide- and acetylene-containing peptides on the solid-phase has also been achieved by introducing the N^{im}-benzylhistidine residue on the reacting peptides.

The synthetic utility of Huisgen 1,3-dipolar cycloaddition between azides and alkynes[1] was significantly improved since Sharpless and co-workers have found that Cu(I) salts accelerate the reaction.[2,3] Thus, Cu(I)-catalyzed azide/alkyne condensation, now known as Sharpless 'click reaction', has been widely applied to bioorganic and medicinal research fields, such as preparation of bioconjugates[4] or microarrays.[5] The reaction provides 1,2,3-triazole derivatives regioselectively under the mild conditions, even in aqueous media, at room temperature, and within a reasonable reaction time.

In general, CuI-mediated Huisgen reaction proceeds more rapidly in the presence of excess bases to give the triazoles in much better yields than in the absence of bases.[3] This is because the bases prevent degradation of Cu(I) from oxidation or disproportionation by coordinating to the copper ion,[6] while they accelerate the formation of the copper acetylide intermediates,[7] from which the cycloaddition with azides proceeds. In most cases of the CuI-catalyzed Huisgen cycloaddition, either diisopropylethylamine or Et_3N has been mostly used as the base additives in organic solvents, while tris(triazolyl)amine is the powerful activator being extensively applied to bioconjugate purposes.[4] Although the base-free conditions have recently been disclosed in aqueous media, that is, by using the copper nanoparticle in PBS buffer solution,[8] the use of base additives is still critical, especially for the reactions performed in organic solvents as well as on the solid-phase,[9] owing to very slow formation of copper acetylide without the bases.[7] Expanding the entries of the non-basic additives, which strongly accelerate the reaction, or more preferably, the additive-free conditions in organic media, is highly important for the CuI-mediated Huisgen cycloadditions with broad applications, especially to the base-sensitive reaction systems. In this Letter, we report the histidine derivatives as the new non-basic additives of Sharpless click reaction and an application to the 'self-activating' solid-phase reaction between the azide- and acetylene-containing peptides under the additive-free conditions.

During our optimization of the Cu(I)-mediated Huisgen cycloaddition between N^{α}-Boc-ornithine derived azide **1** and *N*-Boc-4-ethynylaniline **2** using 1.0 equiv of CuI and non-basic additives, we found that the histidine derivatives significantly accelerated the reaction similar to diisopropylethylamine or Et_3N (Table 1). Thus, while the reaction in DMF for 3 days in the absence of additives provided triazole **3** only in 20% yield (entry 1), the use of histidine, as well as Et_3N or diisopropylethylamine, successfully gave **3** in quantitative yield for 10 min (entries 2–4). The same acceleration effects were also observed for N^{im}-benzylhistidine **5** and N^{α}-Boc-N^{im}-benzylhistidine **6**, and cycloadduct **3** was produced rapidly and quantitatively (entries 5 and 6). To our surprise, imidazole or *N*-methylimidazole, the side chain substituents of histidine, did not act as an activator, which indicates that both imidazole and amino acid moieties in histidines are necessary for the acceleration of Cu(I)-mediated Huisgen cycloaddition (entries 7 and 8).

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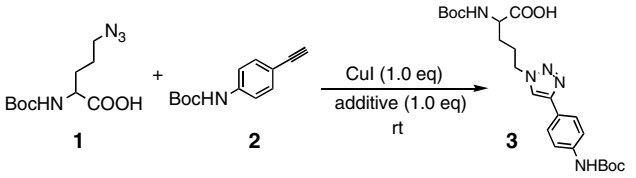
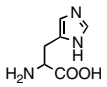
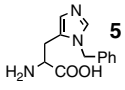
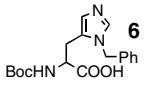
Table 1. Effects of additives on Cu(I)-mediated Huisgen 1,3-dipolar cycloaddition[a]



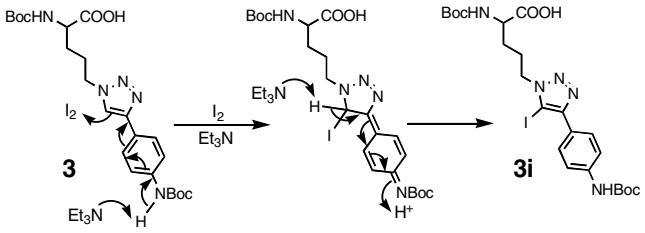
Entry	Additive	Solvent	Reaction time	Yield (%)
1	None	DMF	3 d	20%
2	Et_3N	DMF	10 min	Quant
3	DIPEA	DMF	10 min	Quant
4	 **4**	DMF	10 min	Quant
5	 **5**	DMF	10 min	Quant
6	 **6**	DMF	10 min	Quant
7		DMF	3 d	None
8		DMF	3 d	None
9	**4**	H_2O/CH_3CN (1:2)	3 d	None
10	**5**	H_2O/CH_3CN (1:2)	3 d	Quant
11	Et_3N	H_2O/CH_3CN (1:2)	3 d	99[b]

[a] The reaction was conducted in the presence of 1.0 equiv of CuI at room temperature.

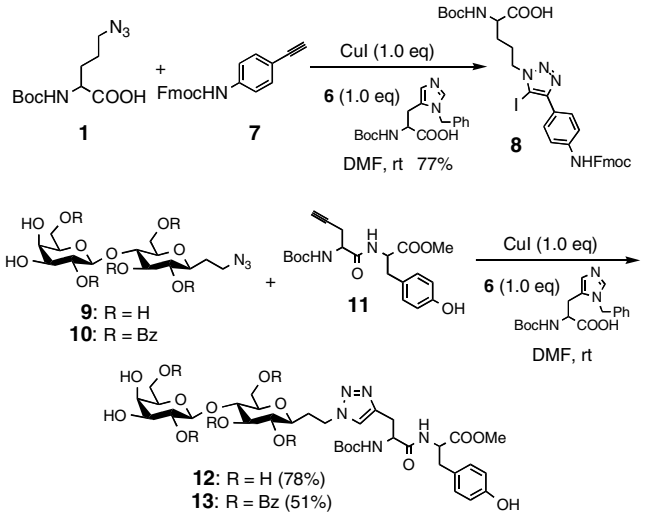
[b] 60% of **3** and 39% of the iodinated compound **3i** were obtained; see Scheme 1.

In aqueous media, N^{im}-benzylhistidine **5** was found to be a stronger activator than simple histidine **4**; triazole **3** was obtained in quantitative yield for 3 days in the presence of **5** in a mixed solvent of H_2O and MeCN (1:2), while the starting materials were completely recovered by using histidine (entries 9 and 10). Interestingly, under the same aqueous conditions of entries 9 and 10, the Et_3N additive provided the click products in high yield as a mixture of **3** and its iodinated compound **3i** (Scheme 1, **3**:**3i** = 60:39). Although the iodination on the triazole ring of the initial click products during the prolonged reaction could also be observed for other examples in Scheme 2 and Table 2 (vide infra), the basic nature of the Et_3N might facilitate the iodination of **3** via the mechanism shown in Scheme 1.

A few examples of histidines-accelerated Sharpless click reaction were shown in Scheme 2. Thus, the reaction of azide **1** with *N*-Fmoc-4-ethynylaniline **7** in the presence of 1.0 equiv of CuI and N^{α}-Boc-N^{im}-benzylhistidine **6** for 15 h provided triazole **8** in 77% yield. The Fmoc-protected **7** showed the retarded reactivity compared



Scheme 1.

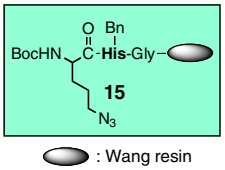
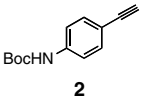
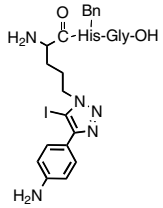
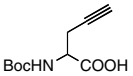
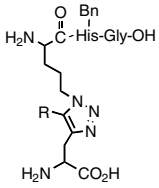
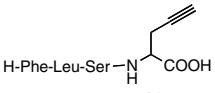
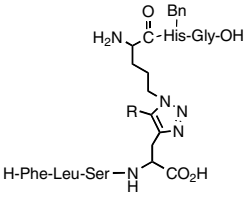
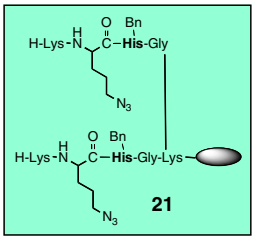
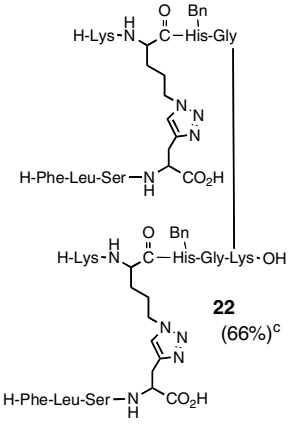
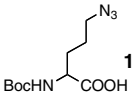
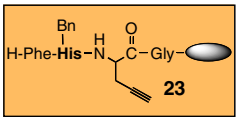
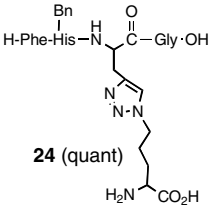


Scheme 2.

with that of Boc-protected **2**, and the prolonged reaction resulted in the incorporation of the iodide at the triazole ring. Nevertheless, the use of non-basic histidine derivative **6** as an additive did not affect the Fmoc protecting group, while the Et_3N additive caused the base-induced Fmoc-deprotection under the identical reaction conditions. Furthermore, the reactions of lactose derivative **9** and its benzoyl-protected congener **10** with Boc-Pra-Tyr-OMe **11** (Pra = propargyl glycine) similarly provided the coupling products **12** and **13** in 78% and 51% yields, respectively.[5a,10]

It is well known that histidine strongly coordinates to the copper ions, which has been extensively investigated by ESI-MS analysis.[11] More recently, the accumulating experimental evidence shows that the β-amyloid peptides Aβs, in particular, Aβ-42 has a very high affinity to copper ions via histidine residues, in association with the pathophysiology of Alzheimer's disease.[12] The acceleration of click reaction by histidine derivatives found in Table 1, therefore would be due to the strong chelating effects of histidines to the copper ion, and thus stabilized Cu(I) complex is responsible for rapid and high yielding cycloaddition reaction. Our ESI-MS analysis of **4**–**6** and dipeptide **14** in the presence of CuI in DMF exclusively detected the molecular ion peak corresponding to $[M\text{-}H+Cu]^+$ (Scheme 3), while those of serine derivatives, as the examples that did not accelerate the parent click reaction, exhibited the molecular ions of $[M+H]^+$ and $[M+Na]^+$ as main peaks and copper ion adduct ions

Table 2. 'Self-activated' Sharpless click reaction of peptide derivatives[a]

Entry	Azide	Acetylene	Products and yields (%)
1		 **2**	 **16** (quant)
2	**15**	 **17**	 **18a**: R = H (57%) **18b**: R = I (43%)
3	**15**	 **19**	 **20a**: R = H (18%) **20b**: R = I (70%)
4[b]		**19**	 **22** (66%)[c]
5			 **24** (quant)

[a] Otherwise noted, the reaction was performed in the presence of 3.0 equiv of CuI and 3.0 equiv of **2**, **17**, **19**, and **1** in DMF for 12 h at room temperature. After the reactions, the resins were treated with TFA/TES/H_2O (31:1:1) and the resulting peptides were precipitated from ether and analyzed by HPLC.

[b] 6.0 equiv of CuI and **19** were used.

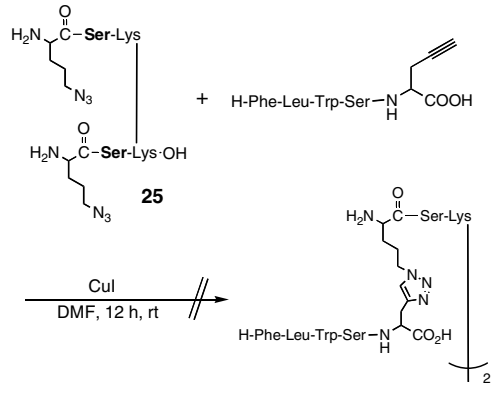
[c] Mono-click products were obtained as the main other products.

with only weakly detectable signal. These results support the chelation effects of histidines as the outcome of the reaction acceleration.

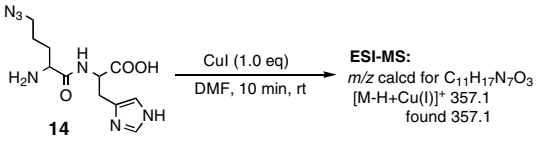
The truly advantageous application of histidine activators comes when the peptide fragments are jointed by click reaction under the additive-free conditions, namely, the histidine residue is purposely introduced into either azide- or acetylene-containing peptide sequences, so that the peptide conjugates would be produced without the base additives. Since it is known that the peptides usually coordinate to the copper ion, the peptides are one of the challenging substrates for Sharpless click reaction. In order to circumvent the problems, an excess amount of strongly basic additives, such as diisopropylethylamine has always being utilized. When the histidine of strong Cu(I)-chelator, being embodied in the peptides can bring the copper ion to the acetylene and/or azide reactive sites, an efficient 'self-activating' click reaction of even complex peptides on the solid-phase is envisioned.

We therefore prepared the N^{im}-benzylhistidine containing peptides, Boc-Orn(N_3)-His(Bn)-Gly-OR **15**, its dimeric peptide **21**, and H-Phe-His(Bn)-Pra-Gly-OR **23** on the solid-supports (R = polystyrene-based Wang resin), and investigated the additive-free click reaction (Table 2). The reaction was performed in the presence of 3.0 equiv of acetylenes or azides for 12 h at room temperature. The products were released from the resin by TFA treatments and analyzed by HPLC after the removal of the residual copper ion by the scavenger resin (ArgoPoreTM-NH_2) followed by precipitation from ether.

Gratefully, the reaction of Boc-Orn(N_3)-His(Bn)-Gly-OR **15** with *N*-Boc-4-ethynylaniline **2** provided the corresponding triazole **16** quantitatively (entry 1).[13] Similarly, the reaction with Boc-protected propargyl glycine **17** gave the mixture of triazole **18a** and its iodinated derivative **18b** in quantitative yield (**18a**:**18b** = 57:43) (entry 2). It is worthy to note that even heptapeptides **20a**,**b** and heptadecyl peptide **22** were successfully obtained in 88% and 66% yields by the reaction of **15** or its dimeric **21** with tetrapeptide **19** (entries 3 and 4). The reverse usage of histidine, that is, N^{im}-benzylhistidine at the acetylene site on the solid-supports **23**, was also possible and triazole **24** was produced quantitatively by the reaction with azide **1** under the identical conditions performed in entries 1–4 (entry 5).[14]



Scheme 4.



Scheme 3.

Peptide-based 'self-activated' click reactions could also be performed in the solution-phase (in DMF) using the sequences of the peptides and the substrates similar to those in Table 2. When 1.0 equiv of CuI was used, the reactions smoothly provided the corresponding cycloadducts within 30 min based on the ESI-TOF-MS analysis of the reaction mixtures, while the peptides lacking the histidine residue, such as the corresponding serine derivative **25**, did not provide any click products in the presence of only CuI (Scheme 4). For the solution-phase reactions, however, the presence of the copper ions made the isolation of the products very difficult, and exact yields could not be calculated.

In summary, we have found that the non-basic histidine derivatives strongly accelerate the Cu(I)-mediated Huisgen reaction,[15,16] as the same acceleration rate as those obtained by Et_3N or diisopropylethylamine. The rate enhancement of the reaction might possibly be due to the strong coordination of copper ion to the histidines, which stabilizes the reactive Cu(I) species. The histidine-activated click reaction was applied to the self-activating peptides couplings on the solid-phase under the additive-free conditions. Further applications to the peptides-based cycloadditions, bioconjugations, and combinatorial library synthesis based on the current findings are now in progress in our laboratory.

Acknowledgement

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References and notes

1. Huisgen, R. In *1,3-Dipolar Cycloadditional Chemistry*; Padwa, A., Ed.; Wiley: New York, 1984.
2. (a) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004–2021; (b) Kolb, H. C.; Sharpless, K. B. *Drug Discovery Today* **2003**, *8*, 1128–1137.
3. Bock, V. D.; Hiemstra, H.; van Maarseveen, J. H. *Eur. J. Org. Chem.* **2006**, 51–68.
4. (a) Wang, Q.; Chan, T. R.; Hilgraf, R.; Fokin, V. V.; Sharpless, K. B.; Finn, M. G. *J. Am. Chem. Soc.* **2003**, *125*, 3192–3193; (b) Speers, A. E.; Adam, G. C.; Cravatt, B. F. *J. Am. Chem. Soc.* **2003**, *125*, 4686–4687; (c) Link, A.

J.; Tirrell, D. A. *J. Am. Chem. Soc.* **2003**, *125*, 11164–11165; (d) Deiters, A. D.; Cropp, T. A.; Mukherji, M.; Chin, J. M.; Anderson, J. C.; Schultz, P. G. *J. Am. Chem. Soc.* **2003**, *125*, 11782–11783; (e) Speers, A. E.; Cravatt, B. F. *Chem. Biol.* **2004**, *11*, 535–546; (f) Zhan, W.-h.; Barnhill, H. N.; Sivakumar, K.; Tian, H.; Wang, Q. *Tetrahedron Lett.* **2005**, *46*, 1691–1695; (g) Yamaguchi, M.; Kojima, K.; Hayashi, N.; Kakizaki, I.; Kon, A.; Takagaki, K. *Tetrahedron Lett.* **2006**, *47*, 7455–7458; (h) Lin, P.-C.; Ueng, S.-H.; Yu, S.-C.; Jan, M.-D.; Adak, A. K.; Yu, C.-C.; Lin, C.-C. *Org. Lett.* **2007**, *9*, 2131–2134; (i) van Kasteren, S. I.; Kramer, H. B.; Jensen, H. H.; Campbell, S. J.; Kirkpatrick, J.; Oldham, N. J.; Anthony, D. C.; Davis, B. G. *Nature* **2007**, *446*, 1105–1109.

5. (a) Fazio, F.; Bryan, M. C.; Blixt, O.; Paulson, J. C.; Wong, C.-H. *J. Am. Chem. Soc.* **2002**, *124*, 14397–14402; (b) Nyffeler, P. T.; Liang, C.-H.; Koeller, K. M.; Wong, C.-H. *J. Am. Chem. Soc.* **2002**, *124*, 10773–10778; (c) Bryan, M. C.; Fazio, F.; Lee, H.-K.; Huang, C.-Y.; Chang, A.; Best, M. D.; Calarese, D. A.; Blixt, O.; Paulson, J. C.; Burton, D. R.; Wilson, I. A.; Wong, C.-H. *J. Am. Chem. Soc.* **2004**, *126*, 8640–8641; (d) Calarese, D. A.; Lee, H.-K.; Huang, C.-Y.; Best, M. D.; Astronomo, R. D.; Stanfield, R. L.; Katinger, H.; Burton, D. R.; Wong, C.-H.; Wilson, I. A. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 13372–13377.
6. Kolthoff, I. M.; Coetzeem, J. *J. Am. Chem. Soc.* **1957**, *79*, 1852.
7. Himo, F.; Lovell, T.; Hilgraf, R.; Rostovtsev, V. V.; Noodleman, L.; sharpless, K. B.; Fokin, V. V. *J. Am. Chem. Soc.* **2005**, *127*, 210–216.
8. (a) Pachon, L. D.; van Maarseveen, J. H.; Rothenberg, G. *Adv. Synth. Catal.* **2005**, *347*, 811–815; (b) Wan, Q.; Chen, J.; Chen, G.; Danishefsky, S. J. *J. Org. Chem.* **2006**, *71*, 8244–8249.
9. (a) Tornoe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057–3064; (b) Franke, R.; Doll, C.; Eichler, J. *Tetrahedron Lett.* **2005**, *46*, 4479–4482.
10. Similar examples, see: Kuijpers, B. H. M.; Groothuys, S.; Keereweer, A. B. R.; Quaedflieg, P. J. L. M.; Blaauw, R. H.; Delft, F. L.; Rutjes, F. P. J. T. *Org. Lett.* **2004**, *6*, 3123–3126.
11. (a) Lei, Q. P.; Amster, I. J. *J. Am. Soc. Mass. Spectrom.* **1996**, *7*, 722–730; (b) Gatlin, C. L.; Turecek, F. *J. Mass. Spectrom.* **2000**, *35*, 172–177; (c) Mendola, D. L.; Mineo, P.; Rizzarelli, E.; Scamporrino, E.; Vecchio, G.; Vitalini, D. *J. Supramol. Chem.* **2001**, *1*, 147–151; (d) Munakata, M.; Sakamoto, O.; Kitamura, T.; Ishitobi, M.; Yokoyama, H.; Haginoya, K.; Togashi, N.; Tamura, H.; Higano, S.; Takahashi, S.; Ohura, T.; Kobayashi, Y.; Onuma, A.; Iinuma, K. *Brain Dev.* **2005**, *27*, 297–300; (e) Rimola, A.; Rodriguez-Santiego, L.; Sodupe, M. *J. Phys. Chem. B* **2006**, *110*, 24189–24199; (f) Himes, R. A.; Park, G. Y.; Barry, A. N.; Blackburn, N. J.; Karlin, K. D. *J. Am. Chem. Soc.* **2007**, *129*, 5352–5353.
12. (a) Finefrock, A. E.; Bush, A. I.; Doraiswamy, P. M. *J. Am. Geriatr. Soc.* **2003**, *51*, 1143–1148; (b) Raffa, D. F.; Gomez-Balderas, R.; Brunelle, P.; Rickard, G. A.; Rauk, A. *J. Biol. Inorg. Chem.* **2005**, *10*, 887–902.
13. Use of 1.0 equiv of acetylene **2** also gave the click products in 81% (78% of **16** and 3% of non-iodinated product). However, when the equiv of CuI was decreased down to 1.0, the starting materials were completely recovered.
14. Substrate dependency for the incorporation of the iodide on the triazole rings cannot be explained from the experimental data in Table 2. The acceleration effects of other Cu(I) salts on the peptide-based click reaction have not been examined.
15. *Representative procedure in solution phase*: To a solution of N^{α}-Boc-ornithine derived azide **1** (10 mg, 46 μmol) and *N*-Boc-4-ethynylaniline **2** (7.3 mg, 46 μmol) in dry DMF (1.0 mL) were added N^{α}-Boc-N^{im}-benzylhistidine **6** (16 mg, 46 μmol) and CuI (8.8 mg, 46 μmol) at room temperature. After the reaction mixture was stirred for 10 min under argon atmosphere at room temperature, the mixture was concentrated and purified by column chromatography on silica gel (gradually from 0.9% to 33% methanol in chloroform) to give the triazole **3** as a white powder (22 mg, 100%). Data for **3**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.33 (s, 9H), 1.43 (s, 9H), 1.60 (br ds, 1H), 1.76 (br ds, 1H), 1.94 (br ds, 2H), 4.04 (br ds, 1H), 4.37 (t, 2H, $J = 6.7$ Hz), 7.38 (d, 2H, $J = 8.5$ Hz), 7.60 (d, 2H, $J = 8.5$ Hz), 8.14 (s, 1H); ESI-MS *m*/*z* calcd for $C_{23}H_{33}N_5O_6$ $[M+H]^+$ 476.2, found 476.2. Data for **3i**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.32 (s, 9H), 1.43 (s, 9H), 1.59–1.61 (m, 1H), 1.73–1.78 (m, 1H), 1.90–1.93 (m, 2H), 3.92 (br ds, 1H), 4.42 (t, 2H, $J = 6.9$ Hz), 7.42 (d, 2H, $J = 8.6$ Hz), 7.65 (d, 2H, $J = 8.8$ Hz); ESI-MS *m*/*z* calcd for $C_{23}H_{33}IN_5O_6$ $[M+H]^+$ 602.1, found 602.1.
16. *Representative procedure on solid-phase*: To a suspended solution of azide-containing peptide on Wang resin **15** (25 mg, 14 μmol) and acetylene **2** (8.9 mg, 41 μmol) in dry DMF (1.0 mL) was added CuI (7.8 mg, 41 μmol) at room temperature. After the mixture was shaken at room temperature for 12 h, the resin was washed by DMF (×5) and diethylether (×3), and then dried in vacuo. The resulting resin was treated with 2.0 mL of TFA/TES/H_2O (31:1:1) and the mixture was shaken for 30 min at room temperature. After the mixture was filtered and the filtrate was concentrated in vacuo, the click peptide **16** was precipitated from diethylether. HPLC and ESI-MS analyses confirmed **16** to be produced quantitatively. HPLC conditions: column, $5C_{18}$-AR300 (Nacalai Tesque, 4.6×250 mm); mobile phase, MeCN (0.5% TFA)/H_2O (0.5% TFA) 0/100 for 30 min, and then 30/100 to 40/100 with linear gradient over 10 min; flow rate, 1.0 mL/min; detection, UV (220 nm). Data for **16**: ESI-MS *m*/*z* calcd for $C_{28}H_{32}IN_9O_4$ $[M+H]^+$ 686.2, found 686.3. Data for **18a**: ESI-MS *m*/*z* calcd for $C_{25}H_{33}N_9O_6$ $[M+H]^+$ 556.3, found 556.3. Data for **18b**: ESI-MS *m*/*z* calcd for $C_{25}H_{32}IN_9O_6$ $[M+H]^+$ 682.2, found 682.2. Data for **20a**: ESI-MS *m*/*z* calcd for $C_{43}H_{58}N_{12}O_{10}$ $[M+H]^+$ 903.5, found 903.5. Data for **20b**: ESI-MS *m*/*z* calcd for $C_{43}H_{57}IN_{12}O_{10}$ $[M+H]^+$ 1029.3, found 1029.4. Data for **22**: ESI-MS *m*/*z* calcd for $C_{104}H_{150}N_{30}O_{22}$ $[M+3H]^{3+}$ 724.7, found 724.7. Data for **24**: ESI-MS *m*/*z* calcd for $C_{34}H_{42}N_{10}O_7$ $[M+H]^+$ 703.3, found 703.3.