Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 2433–2436

Synthesis of a bis-azido analogue of acromelic acid for radioisotope-free photoaffinity labeling and biochemical studies

Pi Sun, Guang Xing Wang,* Kyoji Furuta and Masaaki Suzuki

Regeneration and Advanced Medical Science, Graduate School of Medicine, Gifu University, Gifu 501-1193, Japan

Received 14 December 2005; revised 12 January 2006; accepted 24 January 2006
Available online 15 February 2006

Abstract—A novel acromelic acid analogue containing a phenyl group possessing two different types of azido functional groups, of which one is the aromatic N_3 acting as a photoaffinity group to bind to a target protein by photoirradiation and the other is alkyl N_3 group which survives photolysis acting as a detecting group through the Staudinger–Bertozzi reaction to identify the ligated product, was designed and synthesized as a radioisotope-free biochemical probe potentially for studies on kainoid receptors.

Acromelic acid (**1**) isolated from a toadstool, *citocybe acromelalga*,[1] is a potent neuroexcitatory amino acid which belongs to a class of so-called kainoids bearing a pyrrolidine dicarboxylic acid structure represented by kainic acid (**2**).[2] These compounds possess a structure similar to that of glutamic acid, a major excitatory neurotransmitter in the human central nervous system. Therefore, they can be looked as conformationally constrained glutamic acid analogues and are believed to exert their biological activities through glutamate receptors[3] that are classified as ionotropic and metabotropic receptors comprising of three and eight subtypes, respectively.[4] By binding and acting at the subclass of kainate receptor and AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptor, the kainoids have been shown to display powerful neuroexcitatory activity in the mammalian central nervous system.[3] Like kainic acid (**2**), acromelic acid (**1**) can also strongly depolarize the neurons, but its in vivo behavioral and pathological effects are reportedly different from those of kainic acid,[5] suggesting the existence of distinct types of kainoid receptors. Therefore, the actual receptor for acromelic acid and its signaling pathway are yet to be determined. During our efforts to elucidate the molecular mechanism behind the neuro-toxicity of acromelic acid and associated receptor functions, we have designed and synthesized an acromelic acid analogue (GIF-0448, **3**) possessing an azido group as photoaffinity group and ^{125}I as a radioactive detecting group as a bifunctional probe for the object.[6] The compound is successfully utilized as a substitute of acromelic acid in the biochemistry study of receptor signaling analysis, but the radio property of this compound is obviously inconvenient and unhealthy for the treatment and preparation. Recently, we have developed a novel method for radioisotope-free photoaffinity labeling, in which a bifunctional ligand is connected to a target protein by activation of a photoreactive group, such as an aromatic azido group, and identification of the ligated product is achieved by anchoring of a detectable tag through the Staudinger–Bertozzi reaction with an alkyl azido moiety that survives photolysis. The chemical ground of this method was also confirmed using model compounds with the bifunctional group under photoirradiation in the presence of trapping agents for reactive intermediates and the method was demonstrated by specific labeling of the catalytic portion of human HMG-CoA reductase.[7] In this letter, we wish to adopt this idea and report the design and synthesis of an acromelic acid analogue (**4**) related to compound (**3**) possessing a phenyl group functionalized with two different azido groups as the radioisotopic-free probe for the acromelic acid receptor signaling analysis (see Fig. 1).

Retrosynthetically, compound **4** could be obtained by deprotection of *N*-Boc and mild hydrolysis of bis-methyl ester of compound **5** under mild condition without destroying the azido group.[6] The alkyl N_3-group could be introduced by the substitution of the corresponding mesylate **6**, which could be prepared from the alcohol

Keywords: Acromelic acid; Radioisotopic free; Molecular probe; Bis-azodo analogue; Staudinger–Bertozzi reaction; Photoaffinity.
* Corresponding author. Tel.: +86 13524617726; e-mail: wgxwys@126.com

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.01.083

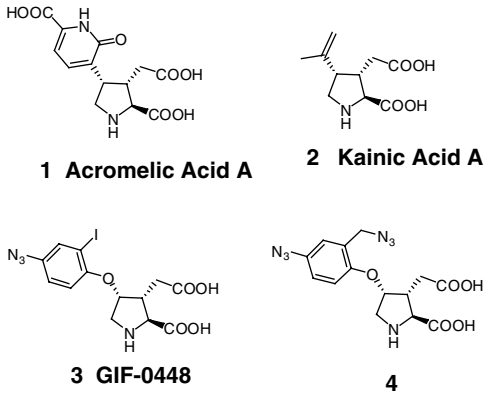


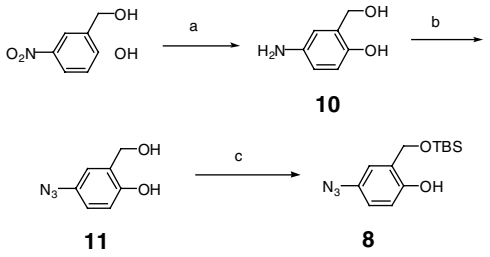
Figure 1.

7. Like the step in the preparation of compound **3**, the compound **7** could be prepared by incorporating the azidophenol **8**, prepared from 2-hydroxymethyl-4-nitrophenol, to the pyrrolidine derivatives **9** by Mitsunobu condition (see Scheme 1).

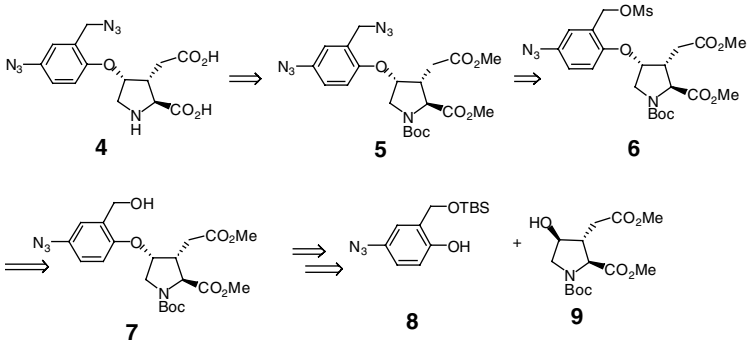
The pyrrolidine derivative **9** was synthesized from commercially available *trans*-4-hydroxyproline following the reported procedure.[6,8] The azido phenol **8** was prepared from 2-hydroxymethyl-4-nitrophenol as follows. Hydrogenation of nitro group in 2-hydroxymethyl-4-nitrophenol under the atmosphere of hydrogen catalyzed by 10% Pd/C afforded the aminophenol **10**. Without purification, compound **10** was treated with $NaNO_2$ in 2 N HCl at 0 °C and then exchanged with NaN_3 to give the azido phenol **11** in 50% yield over 3 steps. Selective protection of the primary hydroxy group as TBS ether was achieved by using TBSCl and Et_3N in DMF (Scheme 2).

With both intermediates in hand, the synthesis of compound **5** was started. Though phenol is a well-known substrate for the Mitsunobu reaction, coupling azido-containing phenol seemed to be uneasy task because the azido group could be reduced with Ph_3P. We performed this reaction by changing the addition sequence of reagents in order to avoid this reduction, but the reaction still gave a complex mixture and after removal of TBS ether using TBAF compound **7** could be obtained but only in 13% yield. Therefore, an improved procedure for preparing compound **7** starting from the beginning was envisaged (Scheme 3). TBS protection of the primary OH of 2-hydroxymethyl-4-nitrophenol afforded compound **12** smoothly. Coupling **12** with compound **9** under Mitsunobu condition gave compound **13** in 69% yield. The stereochemistry of compound **13** was deduced by the general mechanism of Mitsunobu reaction, which usually reverses the stereochemistry of hydroxy group. Also the ^1H NMR spectrum of **13** represented the characteristic pattern of 3,4-*cis*-configuration as judged by accumulated data of analogs.[6,9] The nitro group in compound **13** was then converted to the amino group by hydrogenation catalyzed by 10% Pd/C to give compound **14**. Removal of TBS group in **14** using TBAF in THF afforded compound **15** in high yield. With both amino and hydroxy groups in the body, compound **15** is much soluble in the 3 N HOAc which is beneficial to the next azidation step. Thus, dissolving compound **15** in 3 N HOAc and treatment with $NaNO_2$ and then NaN_3 provided the compound **7** in 86% combined yield.[10] Mesylation of the hydroxy group using MsCl in the presence of Et_3N gave almost quantitative yield of the mesylate **6**, which was displaced by another azido group using NaN_3 in DMSO at 40 °C to provide the bis-azido compound **5**. Removal of the *N*-Boc group of **5** with trifluoroacetic acid and hydrolysis of methyl esters by treatment with lithium hydroxide in methanol–water afforded the desired compound **4**[11] as a pale yellow amorphous solid in 94% yield, after ion-exchange chromatography and lyophilization (Scheme 3).

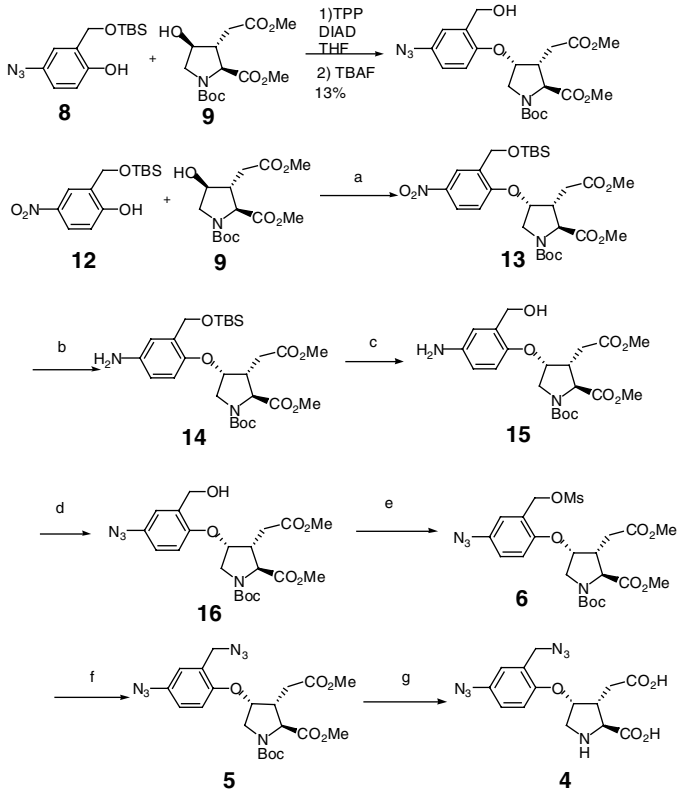
The complete preservation the biological property of acromelic acid A (**1**) by GIF-0448 (**3**) is quite intriguing



Scheme 2. Reagents and conditions: (a) H_2, Pd/C, EtOAc, 6 h: (b) 1—$NaNO_2$, 2 N HCl, 0 °C, 5 min; 2—NaN_3, 1 h, 50% for 3 steps; (c) TBSCI, DMF, Et_3N, rt, 5 h, 55%.



Scheme 1. Retrosynthetic analysis.



Scheme 3. Reagents and conditions: (a) TPP, DIAD, THF, rt overnight, 69%; (b) H_2, Pd/C, EtOH, rt, 90%; (c) TBAF, THF, rt, 86%; (d) $NaNO_2$, 3 N HOAc, 0 °C, 5 min, then NaN_3, 1 h, 86%; (e) MsCl, Et_3N, CH_2Cl_2, 0 °C, 3 h, 99%; (f) NaN_3, DMSO, 50 °C, overnight, 76%; (g) 1—LiOH, MeOH/H_2O, rt, 6 h; 2—TFA, CH_2Cl_2, rt, 3 h; 3—ion-exchange resin, 94%.

because they have the substantial differences in structure.[6] Particularly interesting, lots of analogues of GIF-0448 (**3**) with different substitute patterns in the phenyl ring have shown almost the same biological activity as GIF-0448 (**3**).[12] It seems reasonable to assume that the phenyloxy substitute at C-4 of the pyrrolidine rings plays a crucial role for binding and biological activity. As one of the close and similar analogues of GIF-0448 (**3**), compound **4** should exhibit the same biological activity as GIF-0448 (**3**).[13]

In conclusion, we elaborated a simple acromelic acid analogue **4** bearing a 4-phenyloxy group possessing two different types of azido groups as a photoisotopic-free biochemical probe for acromelic acid. The compound is more stable and safer and would be usable not only as a photoaffinity labeling probe, but also as a biochemical tool for acromelic acid receptor signaling analysis.[13,14] The simplicity of the synthesis would allow diverse structural modification for further investigations on kainoid activities. Designs along this way are performed in this laboratory and will be reported in due course.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Creative Scientific Research 'In vivo Molecular Science for Discovery of New Biofunctions and Pharmaceutical Drugs' No. 13NP0401, from the Ministry of Education, Culture, Sports, Science and Technology, Japan. G.X.W. thanks the JSPS Postdoctoral Fellowship for Foreign Researchers.

References and notes

1. (a) Konno, K.; Shirahama, H.; Matsumoto, T. *Tetrahedron Lett.* **1983**, *24*, 939; (b) Konno, K.; Hashimoto, K.; Ohfune, Y.; Shirahama, H.; Matsumoto, T. *J. Am. Chem. Soc.* **1988**, *110*, 4807.
2. For reviews, see: (a) Shinozaki, H. In *Kainic Acid as a Tool in Neurobiology*; McGeer, E. G., Olney, J. W., McGeer, P. L., Eds.; Raven: New York, 1978; pp 17–35; (b) Hashimoto, K.; Shirahama, H. *Trends Org. Chem.* **1991**, *2*, 1; (c) Parsons, A. F. *Tetrahedron* **1996**, *52*, 4149; (d) Bleakman, D.; Lodge, D. *Neuropharmacology* **1998**, *37*, 1187.
3. (a) Tsai, C.; Schneider, J. A.; Lehmann, J. *Neurosci. Lett.* **1988**, *92*, 298; (b) Hashimoto, K.; Ohfune, Y.; Shirahama, H. *Tetrahedron Lett.* **1995**, *36*, 6235; (c) Cantrell, B. E.; Zimmerman, D. M.; Monn, J. A.; Kamboj, R. K.; Hoo, K. H.; Tizzano, J. P.; Pullar, I. A.; Farrell, L. N.; Bleakman, D. *J. Med. Chem.* **1996**, *39*, 3617; (d) Hollmann, M.; Heinemann, S. *Annu. Rev. Neurosci.* **1994**, *17*, 31; (e) Chevliakov, M. V.; Montgomery, J. *J. Am. Chem. Soc.* **1999**, *121*, 11139.
4. For recent reviews, see: (a) Conn, P. J.; Pin, J. P. *Annu. Rev. Pharmacol. Toxicol.* **1997**, *37*, 205; (b) Moloney, M. G. *Nat. Prod. Rep.* **1998**, *15*, 205; (c) Dingledine, R.; Borges, K.; Bowie, D.; Traynelis, S. F. *Pharmacol. Rev.* **1999**, *51*, 7; (d) Brauner-Osborne, H.; Egebjerg, J.; Nielsen, E. O.; Madsen, U.; Krogsgaard-Larsen, P. *J. Med. Chem.* **2000**, *43*, 2609; (e) Schoepp, D. D. *J. Pharmacol. Exp. Ther.* **2001**, *299*, 12.

5. (a) Shinozaki, H.; Ishida, M.; Gotoh, Y.; Kwak, S. *Brain Res.* **1989**, *503*, 330; (b) Kwak, S.; Aizawa, H.; Ishida, M.; Shinozaki, H. *Life Sci.* **1991**, *49*, PL91; (c) Tsuji, K.; Nakamura, Y.; Ogata, T.; Mitani, A.; Kataoka, K.; Shibata, T.; Ishida, M.; Shinozaki, H. *Neuroscience* **1995**, *68*, 585.
6. Furuta, K.; Wang, G. X.; Minami, T.; Nishizawa, M.; Ito, S.; Suzuki, M. *Tetrahedron Lett.* **2004**, *45*, 3933.
7. Hosoya, T.; Hiramatsu, T.; Ikemoto, T.; Nakanishi, M.; Aoyama, H.; Hosoya, A.; Iwata, T.; Maruyama, K.; Endo, M.; Suzuki, M. *Org. Biomol. Chem.* **2004**, *2*, 637.
8. Baldwin, J. E.; Bamford, S. J.; Fryer, A. M.; Rudolph, M. P. W.; Wood, M. E. *Tetrahedron* **1997**, *53*, 5233.
9. Baldwin, J. E.; Fryer, A. M.; Pritchard, G. J. *J. Org. Chem.* **2001**, *66*, 2597.
10. Azidation using compound **14** as starting material afforded the corresponding product in low yield because compound **14** is insoluble in the reaction media.
11. Physical data: compound **13**: ^{1}H NMR ($CDCl_3$, 400 MHz, δppm): 8.24(d, J = 2.0 Hz, 1H), 7.98(dd, J = 8.8/2.0 Hz, 1H), 6.67(d, J = 8.8 Hz, 1H), 4.98(t, J = 3.6 Hz, 1H), 4.55(s, 2H), 3.95(d, J = 10.0 Hz, 1H), 2.75 ∼ 2.81(m, 1H), 2.65–2.69(m, 1H), 2.5–2.6(m, 1H), 1.41(s, 9H), 0.9(s, 9H), 0.1(s, 6H). Compound **14**: ^{1}H NMR ($CDCl_3$, 400 MHz, δppm): 6.85(d, J = 8.8 Hz, 1H), 6.54(d, J = 8.8 Hz, 1H), 6.52(d, J = 8.8 Hz, 1H), 4.81(t, J = 3.6 Hz, 1H), 4.64(d, J = 14 Hz, 1H), 4.55(d, J = 14 Hz, 1H), 4.10(d, J = 9.2 Hz, 1H), 3.76(s, 3H), 3.76(s, 3H), 3.64(s, 3H), 3.56–3.61(m, 2H), 2.86–2.89(m, 2H), 2.59–2.66(m, 1H), 1.41(s, 9H). Compound **15**: ^{1}HNMR ($CDCl_3$, 400 MHz, δ ppm): 6.72(s, 1H), 6.58(d, J = 8.8 Hz, 1H), 6.25(d, J = 8.8 Hz, 1H), 4.76(t, J = 3.2 Hz, 1H), 4.45(s, 2H), 4.07(d, J = 9.6 Hz, 1H), 3.73(s, 3H), 3.63(s, 3H), 3.38(br s, 2H), 2.80–2.90(m, 2H), 2.68(d, J = 12.6 Hz, 1H), 1.38(s, 9H). Compound **16**: ^{1}HNMR ($CDCl_3$, 400 MHz, δ ppm): 7.26(d, J = 0.8 Hz, 1H), 6.88(d, J = 8.8 Hz, 1H), 6.78(d, J = 8.8 Hz, 1H), 4.94(t, J = 3.6 Hz, 1H), 4.60(s, 2H), 4.11(d, J = 9.2 Hz, 1H), 3.83(d, J = 12.8 Hz, 1H), 3.79(s, 3H), 3.68 (d, J = 12.8 Hz, 1H), 3.66(s, 3H), 2.85–2.92(m, 2H), 2.69(dd, 18.9/3.4 Hz, 1H), 1.41(s, 9H). Compound **6**: ^{1}HNMR ($CDCl_3$, 400 MHz, δ ppm): 7.05(d, J = 2.4 Hz, 1H), 7.01(dd, J = 8.8/2.4 Hz, 1H), 6.83(d, J = 8.8 Hz, 1H), 5.18(s, 2H), 5.03(t, J = 3.2 Hz, 1H), 4.18(d, J = 9.6 Hz, 1H), 3.82(d, J = 12.8 Hz, 1H), 3.78(s, 3H), 3.69–3.72(m, 1H), 3.64(s, 3H), 2.96–3.01(m, 2H), 2.93(s, 3H), 2.69(dd, J = 18.9/3.4 Hz, 1H), 1.41(s, 9H). Compound **5**: ^{1}HNMR ($CDCl_3$, 400 MHz, δppm): 6.97(d, J = 8.8 Hz, 1H), 6.95(d, J = 2.4 Hz, 1H), 6.82(d, J = 8.8 Hz, 1H), 5.00(t, J = 3.2 Hz, 1H), 4.26(s, 2H), 4.13(d, J = 9.2 Hz, 1H), 3.82(d, J = 12.8 Hz, 1H), 3.78(s, 3H), 3.67–3.69(m, 1H), 3.65(s, 3H), 2.88–2.96(m, 2H), 2.67(dd, J = 18.6/3.4 Hz, 1H), 1.4(s, 9H). Compound **4**: pale yellow amorphous powder. ^{1}H NMR (D_2O, 400 MHz, δ ppm): 7.13(s, 1H), 7.09(d, J = 8.8 Hz, 1H), 7.03(d,J = 8.8 Hz, 1H), 5.09(t, J = 3.4 Hz, 1H), 4.61(d, J = 14.0 Hz, 1H), 4.39(d, J = 14.0 Hz, 1H), 3.54(dd, J = 12.6/4.8 Hz, 1H), 3.44(d, J = 10.4 Hz, 1H), 2.96(d, J = 13.2 Hz, 1H), 2.6–2.67(m, 2H), 2.52–2.58(m, 1H). MALDI-TOF-MS (m/z) $[M+Na]^+$ calcd for $C_{14}H_{15}NaN_7O_5$ 384.1032. Found: 384.1029.
12. The analogues of GIF-0448 with non-substituted phenyloxy group at 4-position also showed almost the same biological activity as acromelic acid A, unpublished results.
13. Incorporation of a fluorescence detector and biological assays, such as the measurements of electrophysiological responses and intracellular Ca^{2+} increase in neurons, is currently underway and detailed descriptions will be reported separately.
14. The compound can be handled for routine purpose of biochemical experiments without special care under laboratory conditions and kept for months unchanged in the refrigerator unless exposed to UV light.