

## Design of dantrolene-derived probes for radioisotope-free photoaffinity labeling of proteins involved in the physiological $\text{Ca}^{2+}$ release from sarcoplasmic reticulum of skeletal muscle

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Received 21 December 2004; accepted 13 January 2005

**Abstract**—Bifunctional dantrolene derivatives have been synthesized as probes for radioisotope-free photoaffinity labeling with the aim of elucidating the molecular mechanism of skeletal muscle contraction. GIF-0430 and GIF-0665 are aromatic azido-functionalized derivatives that were designed to selectively inhibit physiological  $\text{Ca}^{2+}$  release (PCR) from sarcoplasmic reticulum (SR) in mouse skeletal muscle without a strong effect on  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release (CICR). These photoaffinity probes consist of either an azidomethyl or an ethynyl group, respectively, which could function as a tag for introduction of an optional detectable marker unit by an appropriate chemoselective ligation method after the photo-cross-linking operation. Actually, the former probe worked to photolabel its target proteins specifically as confirmed by subsequent fluorescent visualization.

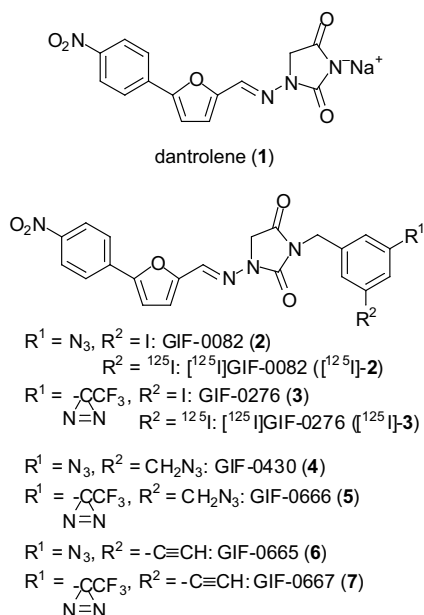
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Dantrolene (**1**) is a muscle relaxant known as an efficient drug used clinically for the treatment of malignant hyperthermia (MH),<sup>1,2</sup> a genetic disease triggered by excessive release of  $\text{Ca}^{2+}$  from the sarcoplasmic reticulum (SR), the intracellular  $\text{Ca}^{2+}$  store, into the cytoplasm of skeletal muscle cells. This unusual  $\text{Ca}^{2+}$  release occasionally occurs when skeletal-type ryanodine receptor (RyR1), a  $\text{Ca}^{2+}$ -releasing channel on the SR membrane, has been activated by inhalational anesthetics. Compound **1** is considered to relieve MH by suppressing this abnormal  $\text{Ca}^{2+}$  release.<sup>1c,3</sup> It has also been widely used as an analytical tool to regulate intracellular  $\text{Ca}^{2+}$  concentration, particularly in the study of the excitation–contraction (E–C) coupling of skeletal muscle.<sup>4</sup> In skeletal muscle, physiological  $\text{Ca}^{2+}$  release (PCR) from SR is controlled by RyR1, whose function is regulated by a signal from the dihydropyridine recep-

tor (DHPR), a voltage sensor in the cell membrane.<sup>5</sup> Although there is an established consensus that DHPR and RyR1 locate face-to-face together in the triad junction, the question of whether the signal from DHPR is transmitted to RyR1 directly or indirectly via undetermined molecules, is still controversial.<sup>4,6</sup> Additionally, RyR1 mediates  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release (CICR) in addition to working as a PCR channel in skeletal muscle, although its physiological significance is yet to be solved.<sup>4,5b,c,6</sup>

In order to elucidate the molecular mechanism involved in the  $\text{Ca}^{2+}$ -related processes of skeletal muscle cells more precisely, we have developed several efficient chemical probes based on the structural modification of **1**, a non-selective inhibitor for both PCR and CICR.<sup>7–9</sup> Of these, the radioisotope (RI)-labeled probes, [<sup>125</sup>I]GIF-0082 ([<sup>125</sup>I]-**2**) and [<sup>125</sup>I]GIF-0276 ([<sup>125</sup>I]-**3**), whose structures include azido- and trifluoromethyl-diaziriny-benzylated units, respectively, were designed to capture and identify the target proteins of **1** using a photoaffinity labeling method.<sup>9a,10,11</sup>

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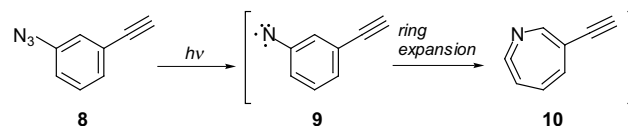


The corresponding non-radiolabeled compounds, GIF-0082 (2) and GIF-0276 (3), showed a highly selective inhibitory effect on PCR without affecting CICR.<sup>9a</sup> Actually, we have succeeded in the photolabeling of target proteins using [ $^{125}I$ ]-2 and the characterization of the primary structure of one target protein purified from an independent muscle preparation using molecular weight stratification by SDS-PAGE analysis of photolabeled mixtures.<sup>12</sup>

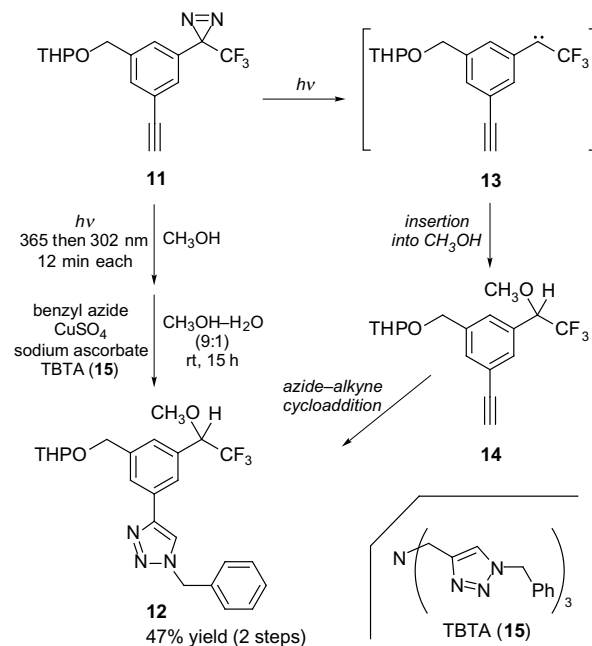
In the course of this study, using RI-labeled molecular probes as described above, we learned several disadvantages in the RI-photoaffinity labeling method, including: (1) the inaccessibility of photolabeled proteins to direct analysis, (2) the requirement of special care and facility to avoid the radiation exposure, and (3) the inherent instability of RI probes, for example, half-life decay time of  $^{125}I$ -radionuclide is 60 days. In this context, we recently developed an efficient method of RI-free photoaffinity labeling using a probe with compact bifunctional unit consisting of a photoreactive function and an aliphatic azido group.<sup>13</sup> We chemically showed that the latter is relatively photo-stable and, therefore, can be utilized as a tag to introduce detectable markers such as fluorescent or biotin units after the photo-cross-linking operation, using azide-specific bioconjugation reactions such as Staudinger–Bertozzi ligation<sup>14</sup> and Huisgen 1,3-dipolar cycloaddition.<sup>15</sup> This method is more advantageous than the preceding ones<sup>16</sup> because it only requires the introduction of an additional azidomethyl group to the original ligand, thereby allowing more flexibility in designing probe candidates without much influence on its intrinsic biological activity and selectivity. Indeed, the utility of this method was successfully demonstrated by the efficient fluorescent detection of photolabeled HMG-CoA reductase using a bifunctional cerivastatin derivative.<sup>13</sup> Described herein is the synthesis of bifunctional dantrolene derivatives suitable for elucidating proteins involved in the PCR process by the RI-free photoaffinity labeling method.

Regarding the design of RI-free photoaffinity probes based on the dantrolene structure, we conceived to convert the efficient radiolabeled probes, [ $^{125}I$ ]-2 and [ $^{125}I$ ]-3,<sup>9a</sup> to the corresponding RI-free structures. Thus, we designed GIF-0430 (4) and GIF-0666 (5), where the  $^{125}I$  group of [ $^{125}I$ ]-2 and [ $^{125}I$ ]-3 is switched to an azidomethyl group as a latent detectable group. We envisaged that biological activity and target specificity would be maintained because the azidomethyl group does not differ considerably from an iodo group in terms of its size and the polarity.<sup>13</sup>

We also planned to prepare GIF-0665 (6) and GIF-0667 (7) with an ethynyl group instead of an azidomethyl group. The ethynyl function was designed to be used for Huisgen 1,3-dipolar cycloaddition<sup>15</sup> with an azido group, generally described as ‘click chemistry’,<sup>17</sup> a convenient and efficient bioconjugation technique, which has been developed recently. Indeed, the azido function in the ethynyl-substituted phenyl azide **8** undergoes photoreaction cleanly to give didehydroazepine **10** via a phenylnitrene species **9** (Scheme 1).<sup>18</sup> Furthermore, we confirmed that a trifluoromethyldiazirine group, instead of the azido function, also works well in the photo-reactive function in the presence of an ethynyl group (Scheme 2). Thus, photoirradiation of methanol solution of **11** by UV light with a wavelength of 365 nm



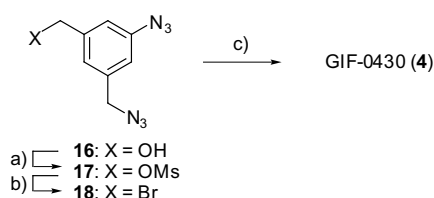
Scheme 1. Photoreaction of 1-azido-3-ethynylbenzene (**8**).<sup>18</sup>



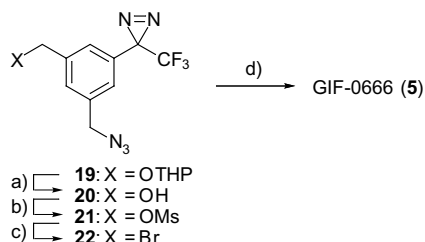
Scheme 2. Photoreaction of ethynyl-functionalized phenyltrifluoromethyldiazirine **11** in methanol followed by cycloaddition with benzyl azide (THP = tetrahydropyran-2-yl).

(UVP, UVL-56, 6W) for 12 min and then with a wavelength of 302 nm (UVP, UVM-57, 6W) also for 12 min,<sup>19</sup> followed by successive treatment of the mixture with benzyl azide in the presence of CuSO<sub>4</sub>, sodium ascorbate, and the triazole ligand TBTA (**15**)<sup>20</sup> to promote the azide-alkyne [3+2] cycloaddition, afforded the desired triazole **12** at a 47% overall yield. This result clearly shows that the carbene species **13** was generated by the photoreaction of **11**, which underwent an insertion reaction with methanol to give **14**.<sup>21</sup>

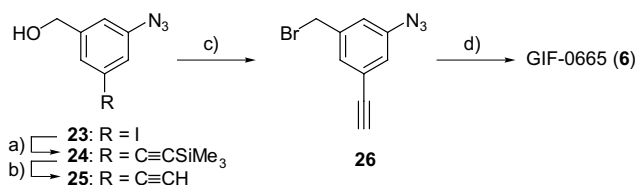
All of the newly designed compounds, **4–7**, were prepared by coupling the corresponding bifunctional benzyl bromides with **1** (Schemes 3–6).<sup>9a</sup> Thus, starting from the diazido-functionalized alcohol **16**,<sup>13</sup> its hydroxy group was successively mesylated and brominated to afford benzyl bromide **18**, which was coupled with **1** to give **4** (Scheme 3).<sup>22</sup> Similarly, the corresponding trifluoromethyldiaziriny derivative **5** was derived from the bifunctional unit **19**,<sup>13</sup> whose THP group was removed, followed by bromination of the resulting hydroxy group to afford **22**, which was then linked with **1** to furnish **5** (Scheme 4). On the other hand, the terminal acetylenic



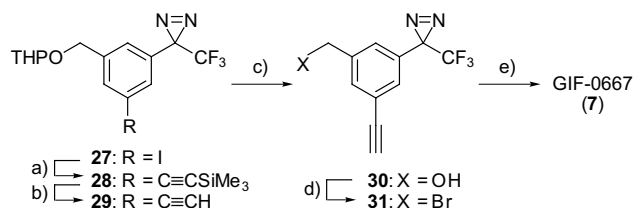
**Scheme 3.** Synthesis of GIF-0430 (**4**). Reagents and conditions: (a) MsCl, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 3.5 h, 94%; (b) KBr, DMF, rt, 13 h, 99%; (c) **1**, DMF, rt, 22 h, 79%.



**Scheme 4.** Synthesis of GIF-0666 (**5**). Reagents and conditions: (a) pyridinium *p*-toluenesulfonate, EtOH, 55 °C, 12 h, 94%; (b) MsCl, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 1.5 h, 99%; (c) KBr, DMF, 0 °C to rt, 13 h, 95%; (d) **1**, DMF, rt, 17 h, 78%.



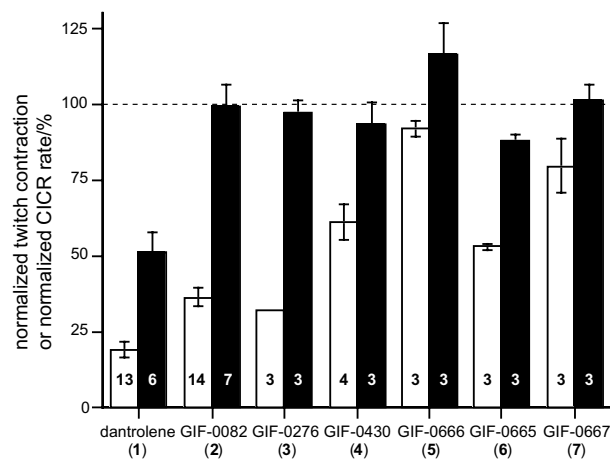
**Scheme 5.** Synthesis of GIF-0665 (**6**). Reagents and conditions: (a) Me<sub>3</sub>SiC≡CH, Pd<sub>2</sub>(dba)<sub>3</sub>, (*n*-Bu)<sub>4</sub>NOAc, DMF, rt, 0.5 h, 64%; (b) KOH, MeOH, 0 °C to rt, 0.5 h, 88%; (c) CBr<sub>4</sub>, PPh<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, rt, 0.5 h, 88%; (d) **1**, DMF, 0 °C to rt, 2 h, 61%.



**Scheme 6.** Synthesis of GIF-0667 (**7**). Reagents and conditions: (a) Me<sub>3</sub>SiC≡CH, (Ph<sub>3</sub>P)<sub>2</sub>PdCl<sub>2</sub>, CuI, Et<sub>3</sub>NH, rt, 13 h, 77%; (b) KOH, MeOH, 0 °C to rt, 0.5 h, 91%; (c) pyridinium *p*-toluenesulfonate, EtOH, 50 °C, 19 h, 98%; (d) CBr<sub>4</sub>, PPh<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, rt, 2.5 h, 82%; (e) **1**, DMF, 0 °C to rt, 2 h, 85%.

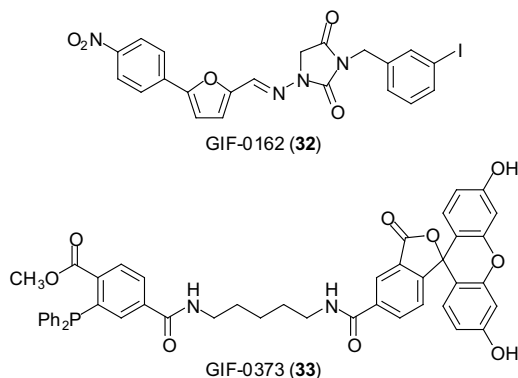
compounds, **6** and **7**, were synthesized based on the use of Sonogashira-type coupling reactions.<sup>23</sup> Thus, the former was synthesized starting from **23**,<sup>9a</sup> which was coupled with trimethylsilylacetylene to provide **24** (Scheme 5). Here, the Pd-catalyzed reaction under Cu(I) salt-free conditions<sup>23b</sup> was preferable to avoid the side reaction of silylacetylene with the azido group. Subsequent deprotection of the trimethylsilyl group of **24** followed by bromination gave **26**, which was coupled with **1** to afford **6**. Similarly, **7** was prepared from the iodide **27**<sup>13</sup> in a similar five step sequence (Scheme 6).

We evaluated the effects of newly synthesized dantrolene derivatives **4–7** on two kinds of Ca<sup>2+</sup> release, PCR and CICR. The effects on PCR were determined by comparing the twitch tension of intact mouse skeletal muscle at room temperature before and 15 min after treatment with the derivatives.<sup>8,9,24</sup> Effects on CICR were evaluated by measuring the rates of Ca<sup>2+</sup> release induced by 1 μM Ca<sup>2+</sup> in saponin-treated skinned muscle fibers of mouse skeletal muscle under the same condition in our previous reports.<sup>8,9,24</sup> The final concentration of each derivative in both experiments was fixed at 50 μM to compare the efficacy between the derivatives. The results are shown in Figure 1 with the comparison of **1** (20 μM),

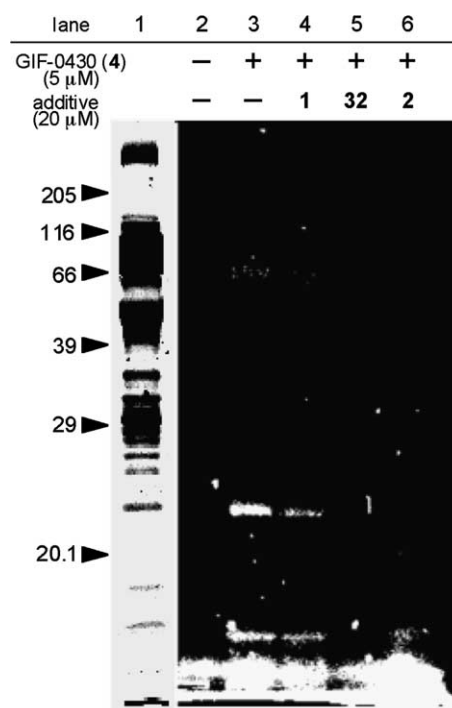


**Figure 1.** Effects of dantrolene (**1**) (20 μM) and its derivatives **2–7** (50 μM) on twitch contraction (open column) and CICR (filled column) of mouse skeletal muscle. The magnitude of twitch contraction and the rates of Ca<sup>2+</sup> release in the presence of **1** or its derivatives were normalized to the control value (100%) obtained in the absence of these compounds. The data for compounds **1–3** are cited from Ref. 9a. The numbers of experiments (*n*) are indicated in the column.

2, and 3.<sup>9a</sup> As shown, azido-functionalized probes **4** and **6** had a selective inhibitory effect on PCR without strongly affecting CICR. Although their effects were slightly decreased compared to those of **2** and **3**, both compounds certainly inhibited PCR to a similar extent, thereby indicating that these probes are potentially useful in selective photolabeling of target molecules involved in PCR. In contrast, the inhibitory effect of trifluoromethyldiazirine compounds **5** and **7** on PCR decreased considerably compared with **4** and **6**. Thus, we can conclude that the introduction of an azido group as a photoreactive unit is a better choice than a trifluoromethyldiazirine group when designing dantrolene-derived RI-free photoaffinity probes.



With promising RI-free photoaffinity probes in hand, we conducted actual photolabeling experiment using the most promising diazido-functionalized probe **4** in terms of inhibitory effect and selectivity, according to the protocol in our previous report.<sup>13</sup> In Figure 2 is shown the SDS-PAGE analysis of terminal cisternae (TC) fraction of rabbit skeletal muscle<sup>25</sup> (1.0 mg/mL, 20 µg total proteins) photolabeled with **4** (5 µM) in the absence or presence of **1**, its derivative, GIF-0162 (**32**)<sup>26</sup> or **2** (20 µM), followed by Staudinger–Bertozzi ligation with GIF-0373 (**33**) (500 µM, incubated overnight), a fluorescein-anchored triarylphosphine derivative. Photo-irradiation was performed at room temperature for 180 s using a portable UV lamp (UVP, UVG-54, 254 nm, 6 W, from a distance of 1 cm). Consequently, two sharp fluorescent bands, which exhibited apparent low  $M_r$  values (<29,000), were observed (lane 3).<sup>27</sup> Since **33** alone did not react with any protein (lane 2), these bands can be considered as candidate target proteins of **4**. Furthermore, these clearly visualized protein bands were indistinct or almost invisible when the photolabeling was performed under the coexistence of an excess amount of the PCR inhibitor, **1**, **32**,<sup>26</sup> or **2** (lanes 4–6, respectively), where the replacement of **4** with such a compound would have taken place. This fact indicates that there exists a specific interaction between **4** and the labeled proteins. Unexpectedly, the RyR1, which exhibits higher  $M_r$  value (>350,000), was not captured by **4**. This is in marked disagreement with previous reports using tritium labeled azido congener of **1**.<sup>11</sup> Although the reason for the discrepancy is presently unclear, it may be attributed to the lack of an inhibitory effect of **4** on the CICR mechanism as shown in Figure 1.<sup>28</sup>



**Figure 2.** SDS-PAGE analysis of rabbit TC fraction (1.0 mg/mL, 20 µg total proteins) photolabeled with 5 µM of GIF-0430 (**4**) in the absence or presence of dantrolene (**1**), its derivative, GIF-0162 (**32**) or GIF-0082 (**2**) (20 µM), followed by Staudinger–Bertozzi ligation with GIF-0373 (**33**) (500 µM) overnight. The photograph was taken with a monochrome CCD camera (ATTO, Printgraph AE-6914) and visualized on a UV transilluminator (UVP, TLW-20, 365 nm, 8 W × 4). For comparison, Coomassie brilliant blue (CBB)-stained proteins contained in the TC fraction are shown in lane 1. The arrowheads and the attached numbers ( $M_r \times 10^3$ ) indicate apparent values of the fluorescent high molecular weight marker (Sigma).

In summary, we designed and synthesized novel bifunctional dantrolene derivatives, which exhibit an selective inhibitory effect on the PCR process involved in the contraction of skeletal muscle, to extend the utility of the newly devised RI-free photoaffinity labeling method.<sup>13</sup> The actual photolabeling experiment using the most promising probe served to detect candidate target proteins of the dantrolene derivative. The direct determination of their structures and subsequent functional studies will be provided in later reports.

### Acknowledgements

We are grateful to Mr. Kin-ichi Oyama of the Nagoya University Chemical Instrument Center for performing the mass spectra analysis. This work was supported in part by a Grant-in-Aid for Creative Scientific Research (No. 13NP0401) of the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan.

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19. (a) From  $^{19}\text{F}$  NMR study in  $\text{CD}_3\text{OD}$  ( $\text{CF}_3\text{COOH}$ ,  $\delta$  0 ppm), the initial irradiation of **11** ( $\delta$  10.8 ppm) for 10 min with 365 nm UV affords a mixture of  $\text{CD}_3\text{OD}$  adduct **14** ( $\delta$  –0.5 ppm) and a linear diazoisomer of **11** ( $\delta$  18.7 ppm) in ca. 2:1 ratio. Due to its UV absorption character, the latter was smoothly converted to **14** by exposure to UV at a wavelength of 302 nm for 10 min. See Brunner, J.; Senn, H.; Richards, F. M. *J. Biol. Chem.* **1980**, 255, 3313; (b) Nassal, M. *Liebigs Ann. Chem.* **1983**, 1510, See also Ref. 13.
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21. The compound **14** was able to isolate in 63% yield.
22. All new compounds were characterized by spectroscopic means. GIF-0430 (**4**): Yellow solid; TLC  $R_f$  = 0.53 (*n*-hexane/EtOAc = 1:2);  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  4.48 (s, 2H), 4.49 (s, 2H), 4.68 (s, 2H), 7.07 (br s, 2H), 7.08 (d, 1H,  $J$  = 3.6), 7.14 (br s, 1H), 7.47 (d, 1H,  $J$  = 3.6 Hz), 7.86 (s, 1H), 7.99–8.03 (AA'BB', 2H), 8.29–8.34 (AA'BB', 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6 + \text{CDCl}_3$ )  $\delta$  41.3, 48.4, 53.0, 112.1, 115.7, 117.8, 118.0, 123.9, 124.3 (2C), 124.4 (2C), 133.2, 135.0, 138.0, 138.4, 139.9, 146.1, 150.6, 152.2, 152.5, 167.0; IR (KBr,  $\text{cm}^{-1}$ ) 853, 1150, 1250, 1329, 1447, 1512, 1597, 1717, 2109; UV (EtOH, nm)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 294 (4.16), 309 (4.16), 382 (4.50); HRMS ( $\text{FAB}^+$ )  $m/z$  Calcd for  $\text{C}_{22}\text{H}_{17}\text{N}_{10}\text{O}_5$ : 501.1383. Found: 501.1388 [ $M+\text{H}$ ] $^+$ . GIF-0666 (**5**): Yellow solid; TLC  $R_f$  = 0.59 (*n*-hexane/EtOAc = 1:2);  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  4.48 (s, 2H), 4.54 (s, 2H), 4.71 (s, 2H), 7.08 (d,  $J$  = 3.6 Hz, 1H), 7.23–7.27 (m, 2H), 7.47–7.49 (m, 2H), 7.87 (s, 1H), 8.00–8.04 (AA'BB', 2H), 8.32–8.34 (AA'BB', 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6 + \text{CDCl}_3$ )  $\delta$  27.8 ( $^2J(^{19}\text{F}-^{13}\text{C})$  = 40.1 Hz), 41.1, 48.1, 52.7, 111.9, 115.5, 121.6 ( $^1J(^{19}\text{F}-^{13}\text{C})$  = 275.3 Hz), 124.1 (2C), 124.3 (2C), 125.2, 125.5, 128.5, 129.2, 133.3, 135.0, 137.4, 137.7, 146.2, 150.6, 152.3, 152.5, 167.0;  $^{19}\text{F}$  NMR (372 MHz,  $\text{DMSO}-d_6$ )  $\delta$  10.6 (s, 3F); IR (KBr,  $\text{cm}^{-1}$ ) 603, 656, 694, 704, 749, 752, 764, 795, 855, 922, 970, 994, 1028, 1111, 1150, 1179, 1202, 1246, 1269, 1337, 1374, 1412, 1441, 1480, 1512, 1599, 1721, 1779, 2107; UV (EtOH, nm)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 309 (4.15), 320 (4.13), 381 (4.49); HRMS ( $\text{FAB}^+$ )  $m/z$  Calcd for  $\text{C}_{24}\text{H}_{17}\text{F}_3\text{N}_9\text{O}_5$ : 568.1305. Found: 568.1301 [ $M+\text{H}$ ] $^+$ .

GIF-0665 (**6**). Yellow solid; TLC  $R_f$  = 0.56 (*n*-hexane/EtOAc = 1:2);  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  4.33 (s, 1H), 4.49 (s, 2H), 4.66 (s, 2H), 7.08 (d,  $J$  = 3.6 Hz, 1H), 7.12–7.15 (m, 2H), 7.25 (br s, 1H), 7.48 (d,  $J$  = 3.6 Hz, 1H), 7.86 (s, 1H), 8.00–8.04 (AA'BB', 2H), 8.31–8.34 (AA'BB', 2H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$  +  $\text{CDCl}_3$ )  $\delta$  40.8, 48.2, 81.2, 81.9, 111.9, 115.5, 119.0, 120.9, 123.4, 124.1 (2C), 124.3 (2C), 127.4, 133.3, 135.0, 138.3, 140.0, 146.2, 150.6, 152.3, 152.5, 167.0; IR (KBr,  $\text{cm}^{-1}$ ) 690, 735, 752, 803, 852, 924, 972, 1140, 1210, 1240, 1300, 1337, 1416, 1439, 1522, 1601, 1723, 1790, 2107; UV (EtOH, nm)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 295 (4.23), 309 (4.26), 383 (4.55); HRMS (FAB $^+$ )  $m/z$  Calcd for  $\text{C}_{23}\text{H}_{16}\text{N}_7\text{O}_5$ : 470.1213. Found: 470.1198  $[\text{M}+\text{H}]^+$ . GIF-0667 (**7**): Yellow solid; TLC  $R_f$  = 0.60 (*n*-hexane/EtOAc = 1:2);  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  4.41 (s, 1H), 4.47 (s, 2H), 4.70 (s, 2H), 7.08 (d,  $J$  = 3.6 Hz, 1H), 7.31 (br s, 2H), 7.48 (d,  $J$  = 3.6 Hz, 1H), 7.59 (br s, 1H), 7.86 (s, 1H), 8.00–8.04 (AA'BB', 2H), 8.30–8.34 (AA'BB', 2H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$  +  $\text{CDCl}_3$ )  $\delta$  27.5 ( $^2J(^{19}\text{F}-^{13}\text{C})$  = 40.1 Hz), 40.9, 48.1, 81.4, 81.7, 111.7, 115.4, 121.4 ( $^1J(^{19}\text{F}-^{13}\text{C})$  = 274.5 Hz), 123.2, 124.0 (2C), 124.3 (2C), 125.5, 126.2, 128.6, 132.5, 133.3, 135.0, 137.6, 146.1, 150.5, 152.3, 152.4, 166.9;  $^{19}\text{F}$  NMR (372 MHz, DMSO- $d_6$ )  $\delta$  10.7 (s, 3F); IR (KBr,  $\text{cm}^{-1}$ ) 690, 735, 752, 803, 852, 924, 972, 1140, 1210, 1240, 1300, 1337, 1416, 1439, 1522, 1601, 1723, 1790, 2107; UV

(EtOH, nm)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 309 (4.14), 320 (4.11), 382 (4.47); HRMS (FAB $^+$ )  $m/z$  Calcd for  $\text{C}_{25}\text{H}_{16}\text{F}_3\text{N}_6\text{O}_5$ : 537.1134. Found: 537.1152  $[\text{M}+\text{H}]^+$ .

23. (a) Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett.* **1975**, 4467; (b) Urgaonkar, S.; Verkade, J. G. *J. Org. Chem.* **2004**, 69, 5752.
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25. TC fraction was isolated from back and leg muscles from Japanese white rabbit (2–2.5 kg, male) by sucrose density gradient centrifugation according to the reported protocol Saito, A.; Seiler, S.; Chu, A.; Fleischer, S. *J. Cell Biol.* **1984**, 99, 875.
26. GIF-0162 (**32**) is a selective inhibitor for PCR without an azido function. Normalized values on twitch contraction and CICR rate of **32** (50  $\mu\text{M}$ ) were  $36.3 \pm 10.2\%$  ( $n = 3$ ) and  $90.5 \pm 4.2\%$  ( $n = 4$ ), respectively.
27. These photolabeled proteins by **4** were in the same molecular mass size with those labeled by  $^{125}\text{I}$ -**2**. The detailed analyses to compare them are in progress.
28. We prepared the phenyl ring-modified azido congener of **1** and found that it strongly inhibits not only the PCR but also the CICR, as is the case with **1** (unpublished data). This implicates that such an azido congener is functionally different from the derivatives described in the present study.