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Synthesis of a novel bioactive photoaffinity probe based on a leaf-movement factor with potential high binding affinity to its receptor molecule

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Abstract—Nyctinastic leaf-movement is induced by the binding of the leaf-movement factor with a motor cell located in the pulvini of the plant. Some receptors of the leaf-movement factor should be involved in this biological event. We developed a novel photoaffinity probe (**1**) for the detection of receptor molecules. Probe compound **1** bears a photolabeling group on the 2′-position of the glycon moiety, which is near the potential binding site of the molecule with its receptor. This molecular design would be effective for the improvement of photolabeling yield.

Most leguminous plants close their leaves in the evening, as if to sleep, and open them early in the morning according to the circadian rhythm controlled by the biological clock. Extensive studies on nyctinastic plants led to the isolation of a variety of leaf-closing and leaf-opening substances. We found that the biological clock regulates the balance of concentration between leaf-opening and -closing substances in the plant body during the day.[1] We also revealed that some receptors for this leaf-movement factor are located on motor cells,[2–4] which plays a key role in nyctinastic leaf-movement.[5] Recently, we succeeded in synthesizing biologically active photoaffinity probe (**2**) to look for the native factor receptor based on potassium lespedezate (**3**),[6] which is a leaf-opening substance of *Cassia mimosoides*.[7] However, probe **2** was designed to bear a photoaffinity group on the 6′-position of the sugar moiety: thus the aglycon moiety, which is important for bioactivity and expected to be a binding site with its receptor, is far from the photoaffinity group (Fig. 1).

In this paper, we report a novel photoaffinity probe (**1**) which bears a photolabeling group on the 2′-position of the sugar moiety and is expected to have high photolabeling yield in the binding examination with its recep-

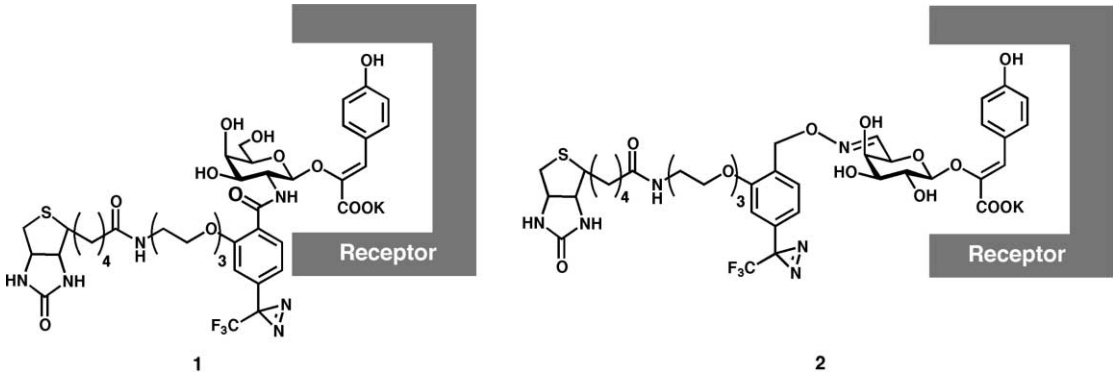

Figure 1. Molecular design of the photoaffinity probe (**1**).

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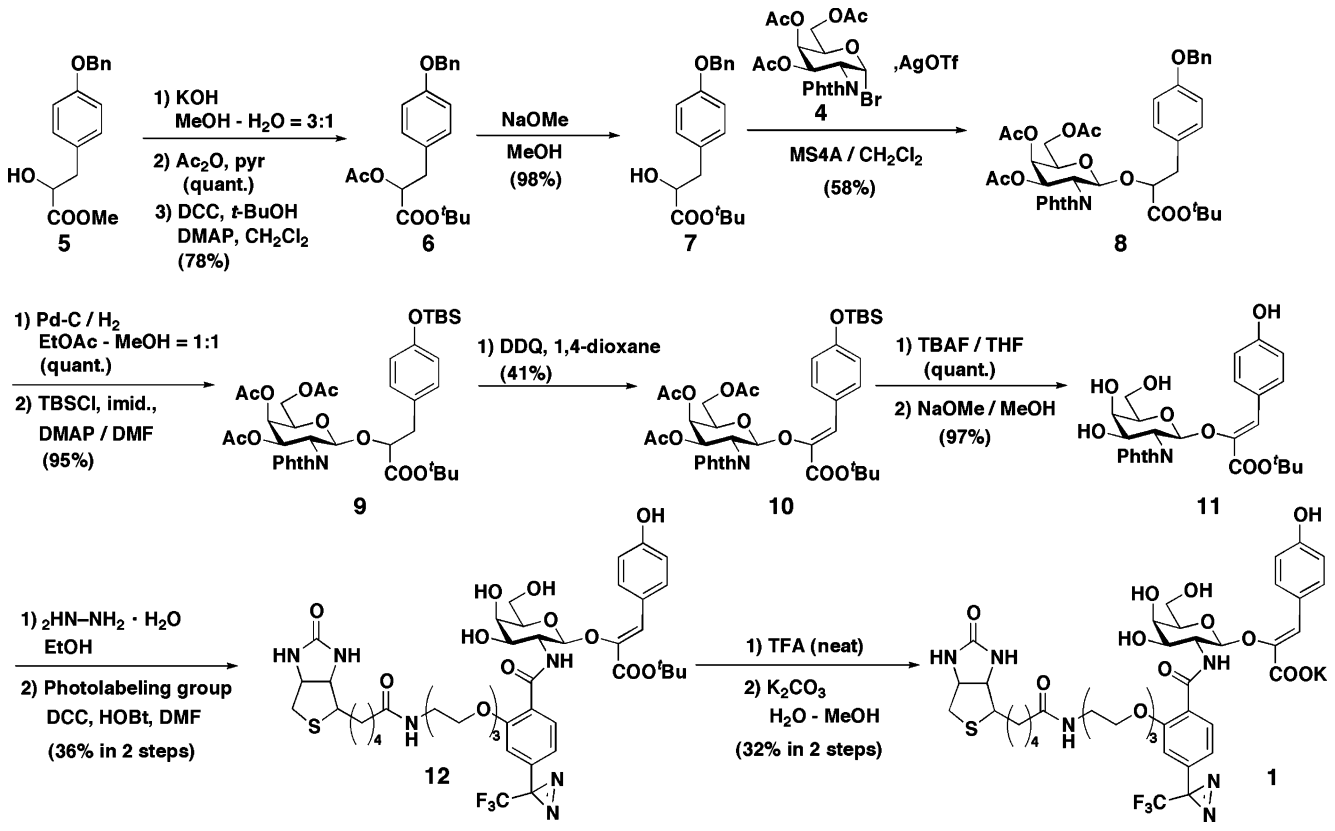
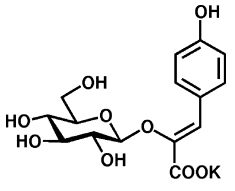


Scheme 1. Synthetic route of photoaffinity probe (**1**).

tor. Probe **1** was designed according to the molecular design of **2**. However, we used galactosamine which has an amino group on the 2′-position to introduce the photolabeling unit. Amide linkage was used to connect the photolabeling unit with the glycon part. This molecular design would be effective for the improvement of labeling yield because the photolabeling group on the 2′-position is extruded to the direction of aglycon which is expected to be a binding site with the receptor. This change in molecular design requires revision of the synthetic route that was used in **2**.



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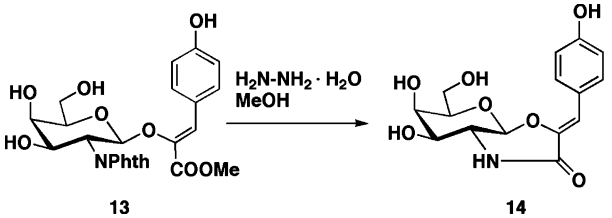
The synthetic route of probe **1** is summarized in Scheme 1. According to the method by Lee,[8] we synthesized 3,4,6-tri-*O*-acetyl-2-deoxy-2-phtalimido-α,β-D-galacto-pyranosyl bromide (**4**). Compound **4** was coupled with **7** by using AgOTf. After conversion of the benzyl group in resulting **8** to a TBS group, DDQ oxidation was carried out to give **10**. Compound **10** was treated with TBAF, and then sodium methoxide to give **11**. Compound **11** was deprotected with hydrazine monohydrate, and then coupled with the photolabeling unit bearing trifluoromethydiazirine and biotin, which is separately prepared according to the method by Hatanaka.[9] Free amine, which was obtained in the deprotection of **11**, was used in the coupling reaction without purification. *t*-Butyl ester in **11** was essential for the synthesis of **12**. When we used the methyl ester (**13**) instead of **11**, we obtained lactam (**14**)[10] [$\delta_{H\text{-}2'}$ 3.63 ppm] quantitatively in the following deprotection of the amino group on the 2′-position (Scheme 2). The *t*-butyl group would prevent the formation of lactam by its steric hindrance.

However, deprotection of the *t*-butyl ester in **12** raises a serious problem. We examined the reaction conditions on the deprotection of **12** thoroughly. But the use of several weak acids,[11] such as formic acid, acetic acid, TFA, and benzoic acid gave a complex mixture of decomposed products or ended in no reaction. The resulting complex mixture mainly contained the frag-

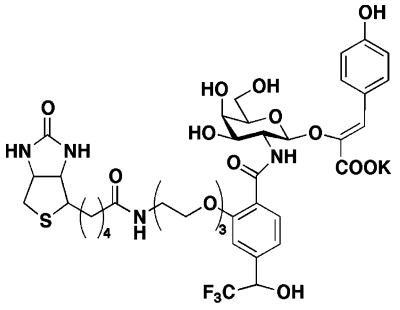


Scheme 2. The formation of lactam in the deprotection of phtalimido group.

ments obtained by the dissociation of the glycosidic bond or the amido bond. After many trials, we found that the treatment of **12** by neat TFA within 30 s at rt provided good results. The main product was a free carboxylate form of **1** (82%) with a small amount of the recovered starting material (9%). After subsequent treatment with sodium carbonate, photoaffinity probe **1**[12] was obtained.

Probe **1** was effective for the leaf-opening of *C. mimosoides* at 8×10^{-5} M. Thus, the bioactivity of **1** was one-eightieth as strong as that of the native factor. The decrease in bioactivity compared with probe **3** could be due to the steric hindrance in the binding with the receptor molecule (Fig. 1).

Photodegradation of probe **1** was also examined. UV light (λ 365 nm) was irradiated to an aqueous solution of 0.33 mM **1** for 60 min. The reaction was monitored by the decrease in the peak intensity at 360 nm in UV spectrum of the reaction mixture, that corresponds to the absorption of the trifluoromethyldiazirine group. The reaction mixture was then dried up and analyzed by using negative-mode FAB MS to give the peak at m/z 915 which corresponds to [**15**-H]$^-$ ion. This result showed that probe **1** could react with the receptor molecule by the radiation of UV light for 15 min.



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The molecular design of photolabeling probes involves an ambivalent issue: the nearer the large photolabeling group is placed to the potential binding site of the probe molecule, the weaker the bioactivity of the photoaffinity probe becomes. In past photolabeling studies, molecular design of the probe requires the cost of either high binding affinity or biological activity because these two factors are never compatible. This is the most important problem in the photolabeling studies using probe compound. However no example has been reported to deal with this issue to evaluate the effectives of these two factors.

We resolved this issue by using two photoaffinity probes designed on different concepts. Now, we synthesized two types of photoaffinity probes designed on different concepts: probe **1** was designed for high labeling yield with the receptor molecule, whereas probe **2** was designed for high affinity with the receptor by reducing the steric hindrance by large photolabeling group. The detection of receptor molecules using these two probes is now in progress.

Acknowledgements

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10. **14** ^{1}H NMR (400 MHz, CD_3OD, rt): 7.64 (2H, d, $J=8.8$ Hz), 6.74 (2H, d, $J=8.8$ Hz), 6.71 (1H, s), 4.86 (1H, d, $J=7.1$ Hz), 3.91 (1H, dd, $J=8.3$, 12.5 Hz), 3.87 (1H, dd, $J=0.7$, 2.7 Hz), 3.81 (1H, dd, $J=4.6$, 12.5 Hz), 3.80 (1H, dd, $J=0.7$, 4.6 Hz), 3.66 (1H, dd, $J=2.9$, 10.7 Hz), 3.63 (1H, dd, $J=7.1$, 10.7 Hz) ppm.; FAB MS (positive) m/z 324 $[M+H]^+$: IR (film) ν: 1670, 1606, 1512, 1441 cm^{-1}.
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12. **1**: ^{1}H NMR (270 MHz, CD_3OD, rt): 7.88 (1H, d, $J=8.2$ Hz), 7.65 (2H, d, $J=8.6$ Hz), 6.96 (1H, d, $J=8.2$ Hz), 6.80 (2H, s), 6.66 (2H, d, $J=8.6$ Hz), 5.32 (1H, d, $J=8.6$ Hz), 4.43 (2H, m), 4.25 (3H, m), 3.92–3.78 (5H, m), 3.73–3.45 (11H, m), 2.90 (1H, dd, $J=4.9$, 12.9 Hz), 2.67 (1H, d, $J=12.9$ Hz), 2.18 (2H, t, $J=7.5$ Hz), 1.66–1.41 (6H, m) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, rt): 172.3, 165.8, 165.8, 162.8, 157.2, 156.5, 131.6, 131.4, 131.0, 128.4, 125.9, 125.4, 123.1, 120.4, 118.9, 115.0, 111.3, 99.5, 77.6, 75.8, 75.5, 73.2, 69.8, 69.6, 69.2, 68.6, 67.2, 61.1, 60.1, 59.2, 55.4, 53.8, 38.4, 35.1, 29.0, 28.2, 28.0, 25.3 ppm.; HR-FAB MS (negative): $[M-K]^-$. Found m/z 925.2899, $C_{40}H_{48}O_{14}N_6F_3S$ requires m/z 925.2901; IR (film) ν: 1693 1651, 1608, 1549, 1512 cm^{-1}; $[\alpha]_D^{24}$ −21.2° (c 0.5, MeOH).