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Fluorescence study on the nyctinasty of *Phyllanthus urinaria* L. using novel fluorescence-labeled probe compounds

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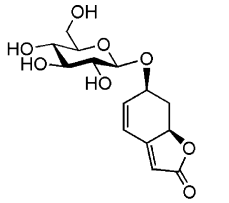
Abstract—We report the synthesis of fluorescence-labeled probes based on phyllanthurinolactone **1**, which is a leaf-closing substance of *Phyllanthus urinaria* L. The fluorescence study using biologically active probe **2** and inactive probes (*epi*-**2** and **31**) revealed that the target cell for **1** is a motor cell and suggested that some receptors, which recognize the aglycon of **1** exist on the plasma membrane of the motor cell, as with leaf-opening substances. Moreover, binding of probe **2** was specific to the plant motor cell contained in the plants belonging to the genus *Phyllanthus*. These results showed that the binding of probe **2** with a motor cell is specific to the plant genus and suggested that the genus-specific receptor for the leaf-closing substance would be involved in nyctinasty.

1. Introduction

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 a biological clock.[1] Nyctinastic leaf-movement is induced by the swelling and shrinking of motor cells in the pulvini, a small organ located in the joint of the leaf to the stem. Motor cells play a key role in plant leaf-movement. The flux of potassium ions across the plasma membranes of the motor cells is followed by massive water flux, which results in swelling and shrinking of these cells.[2] We have revealed that nyctinasty is controlled by a pair of leaf-movement factors: leaf-opening and leaf-closing substances.[3] It is already clarified by using fluorescence-labeled leaf-opening substances that the target cell of the leaf-opening substance is a motor cell.[4] On the other hand, no attempt has been carried out to clarify the target cell of the leaf-closing substances because the structure of most leaf-closing substances is too simple[3] to develop a molecular probe, such as a fluorescence-labeled one. This is because large fluorescence dye would cause serious decrease in the bioactivity of the synthetic probe.

Phyllanthurinolactone **1** is a glycoside-type leaf-closing substance of *Phyllanthus urinaria* L.[5] (Fig. 1). The structure of **1** is comparatively large enough for the structure modification



Phyllanthurinolactone (1)

Figure 1.

that is essential for a synthetic probe. Syntheses of **1** and its analogs were completed by Mori and Audran[6] and our group.[7] The structure–activity relationship studies using them showed that the stereochemistry in aglycon of natural product **1** is important for its bioactivity and the structure modification in the sugar moiety has no effect on the bioactivity. Recently, we developed the fluorescence-labeled probe **2** based on the result of structure–activity relationship studies, and revealed that probe **2** binds to a motor cell specifically.[8] However, in order to prove the existence of a receptor on a motor cell, an appropriate control experiment using biologically inactive probe compounds such as C-4 *epi*-**2** and unsaturated derivative **31** that cannot bind to the target cell is essential. In this paper, we carried out the direct observation of the target cell for **1** using biologically active and inactive fluorescence-labeled probes and revealed that the receptor of **1** exists in a motor cell. Additionally, we found by using the biologically active probe **2** that genus-specific receptors of leaf-closing substances are involved in nyctinasty.

Keywords: Nyctinasty; Leaf-closing substance; Fluorescence; Probe compound; Motor cell; Mitsunobu reaction; Glycosidation.
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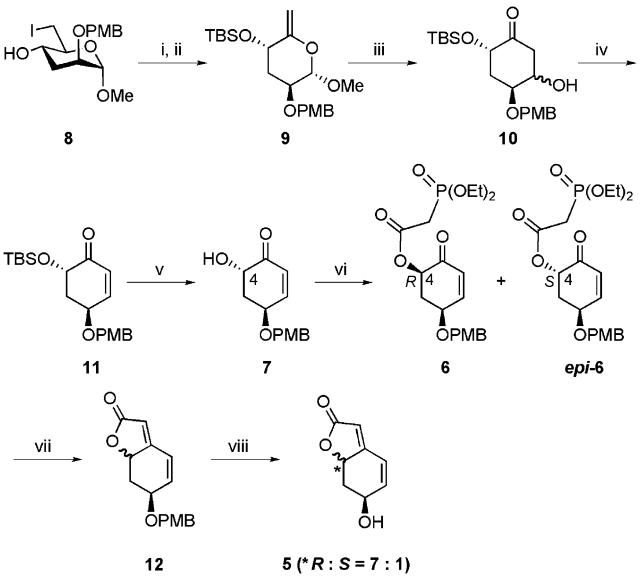
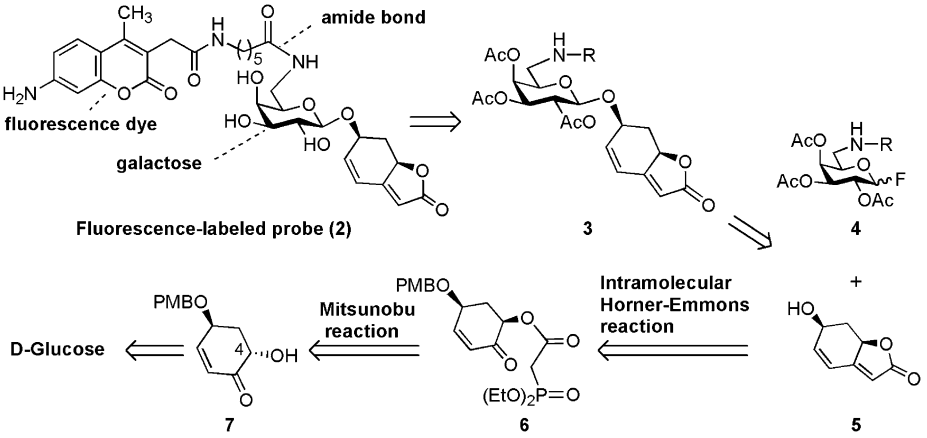
2. Results and discussion

2.1. The design and synthetic plan of fluorescence-labeled probe compounds

Based on the previously obtained result[6,7] that the structure modification of the sugar moiety did not affect the bioactivity, the big fluorescence dye was introduced into the hydroxy group at the C6′ position of the sugar moiety of **1**. Since **1** is easily hydrolyzed by β-glucosidase, the sugar moiety was changed from D-glucose into D-galactose.[9] Moreover, in order to increase the resistance against the esterase involved in a plant body, an amide bond was selected instead of ester bond connecting the fluorescence dye with **1**. The retrosynthesis of **2** is shown in Scheme 1. The fluorescence-labeled probe **2** could be synthesized from the intermediate **3**, which was bisected into the sugar moiety **4** and aglycon **5**. The lactone in aglycon **5** could be constructed by the intramolecular Horner–Emmons reaction[10] of phosphonoacetate **6**, which could be synthesized from the ketoalcohol **7** via Mitsunobu inversion[11] of the C4 stereocenter using phosphonoacetic acid. The ketoalcohol **7** could be synthesized from D-glucose.



Scheme 2. (i) TBSOTf, lutidine, CH_2Cl_2, 99%; (ii) *t*-BuOK, THF, 95%; (iii) cat. $Hg(OCOCF_3)_2$, acetone/H_2O; (vi) MsCl, Et_3N, CH_2Cl_2, 70% (two steps); (v) TBAF, AcOH, THF, quant.; (vi) see Table 1; (vii) *t*-BuOK, THF, 0 °C, 63%; (viii) DDQ, CH_2Cl_2/H_2O, 71%.



Scheme 1.

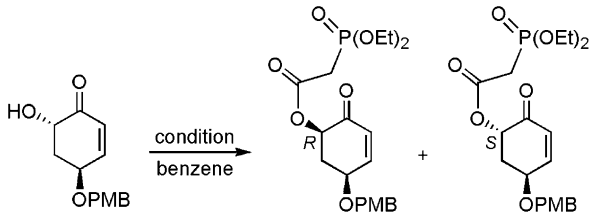
2.2. Synthesis of fluorescence-labeled probe compounds

The synthesis of aglycon is shown in Scheme 2.[8] Intermediate **8**, prepared according to the previous paper,[7] was protected by the TBS group, and then subjected to elimination of iodide to afford the enol ether **9**. A catalytic amount of $Hg(OCOCF_3)_2$-mediated Ferrier's carbocyclization[12] of **9** and following treatment of the resulting alcohol **10** with mesyl chloride and triethylamine, that is, a one-pot reaction involving mesylation and β-elimination, afforded the cyclohexanone derivative **11** in 70% overall yield in two steps. Deprotection of the TBS group gave the alcohol **7**. We considered that epimerization of the C4 stereocenter and introduction of diethyl phosphonoacetate could be carried out simultaneously using the Mitsunobu reaction[11] (Table 1). At first, the reaction did not proceed under the standard Mitsunobu reaction condition (entry 1). Tributylphosphine gave many products (entry 2). The phosphonoacetate **6** was obtained as a mixture of C4 stereoisomers (*R*:*S* = 5:1) in moderate yield by using excess amount of the Mitsunobu reagents (entry 3). However, the purification of **6** was very difficult because of a large amount of triphenylphosphine oxide, which was produced as a byproduct. Therefore, we examined phosphines **14** and **15**, which are easily removed by acid treatment (entry 4 and 5).[13] Phosphine **14** gave lower yield and stereoselectivity (*R*:*S* = 3:1). However, phosphine **15** afforded phosphonoacetate **6** in moderate yield with higher stereoselectivity (*R*:*S* = 10:1) than triphenylphosphine. Additionally, the phosphine oxide from **15** was easily removed by simple acid treatment.

Intramolecular Horner–Emmons reaction of the resulting **6** using *t*-BuOK as the base afforded lactone **12**. Finally, deprotection of the PMB group by DDQ gave the aglycon **5** as a 7:1 diastereomeric mixture.

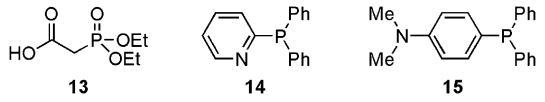
Next, we examined the conditions of the glycosidation reaction. In our previous synthesis, β-D-galactopyranosyl bromide was used for glycosidation according to the method reported by Mori and Audran.[6] In this method, the desired

Table 1. Mitsunobu reaction of alcohol **7**



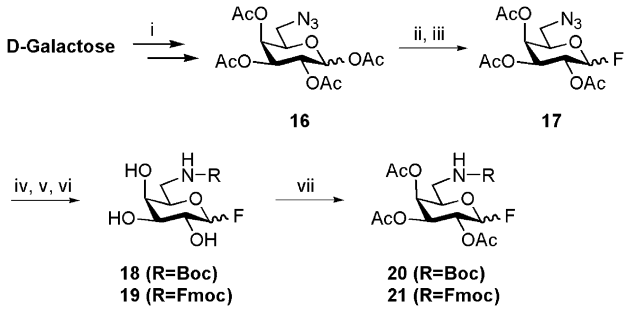
7 **6** ***epi*-6**

Entry	Phosphine (equiv)	DEAD (equiv)	**13** (equiv)	Temp (°C)	Yield % (**6**+*epi*-**6**)	Ratio (**6**:*epi*-**6**)
1	PPh_3 (2.0)	2.0	2.0	rt	0	—
2	PBu_3 (2.0)	2.0	2.0	rt	Messy	—
3	PPh_3 (8.0)	8.0	8.0	0	42	5:1
4	**14** (2.0)	8.0	8.0	0	16	3:1
5	**15** (2.0)	8.0	8.0	0	40	10:1



13 **14** **15**

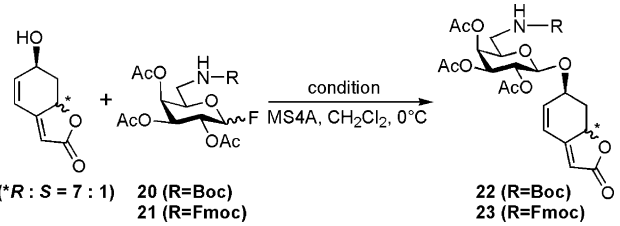
coupling product was obtained in low yield (28% yield) with a large amount of acetyl aglycon. Therefore, we examined the use of glycosyl fluoride as a glycosyl donor. The synthesis of β-D-galactopyranosyl fluoride is shown in Scheme 3. Acetate **16**, prepared from D-galactose according to the procedure in Ref. 14, was treated with hydrazine monohydrate and fluorination of the resulting hydroxy group with DAST afforded fluoride **17**. Removal of acetates and reduction of azide were followed by protection of amine with a Boc or Fmoc group to give the triols **18** and **19**. Finally, acetylation of the triols **18** and **19** yielded the 1-fluorosugars **20** and **21**.



Scheme 3. (i) See Ref. 14; (ii) H_2NNH_2/AcOH, DMF, 0 °C, 74%; (iii) DAST, CH_2Cl_2, 92%; (iv) MeONa, MeOH, 0 °C; (v) H_2, Pd/C, MeOH; (vi) $(Boc)_2O$, pyridine or FmocCl, DIPEA, THF, 0 °C; (vii) Ac_2O pyridine, 65% (four steps) (R = Boc), 28% (four steps) (R = Fmoc).

Glycosidation using β-D-galactopyranosyl fluoride is summarized in Table 2. In the case of *N*-Boc protected galactopyranosyl fluoride **20**, both Suzuki[15] and Mukaiyama methods[16] gave only a trace amount of glycoside **22** (entry 1 and 2). Under these conditions, *N*-Boc protected sugar was decomposed and the aglycon **5** was recovered. On the other hand, glycosidation of *N*-Fmoc protected galactopyranosyl fluoride **21** via the Suzuki method gave glycoside **23** in moderate yield (entry 3). Using 1.6 equiv of the sugar **21**, the yield was improved to 52% (entry 4). In this reaction condition, aglycon **5** was recovered in 15% yield with no production of acetyl aglycon.

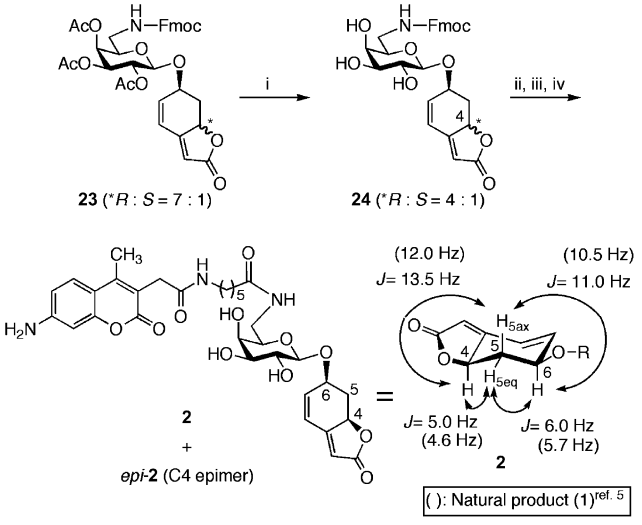
Table 2. Glycosidation



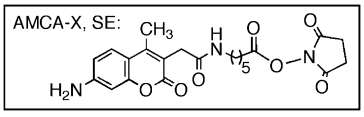
5 (*R : S = 7 : 1) **20** (R=Boc) **21** (R=Fmoc) **22** (R=Boc) **23** (R=Fmoc)

Entry	**20** or **21** (equiv)	Condition (equiv)	**22** or **23**	**5** (%)
1	**20** (2.0)	Cp_2HfCl_2 (3.0), $AgClO_4$ (3.0)	**22** (trace)	32
2	**20** (2.0)	$SnCl_2$ (5.0), $AgClO_4$ (5.0)	**22** (trace)	45
3	**21** (1.2)	Cp_2HfCl_2 (2.0), $AgClO_4$ (2.0)	**23** (26%)	40
4	**21** (1.6)	Cp_2HfCl_2 (2.0), $AgClO_4$ (2.0)	**23** (52%)	15

The synthesis of fluorescence-labeled probe **2** was then examined (Scheme 4). Acetyl groups of the glycoside **23** were removed with KCN[6,17] to give the triol **24** as a 4:1 mixture of C4 stereoisomers. At this stage, further epimerization occurred. Because the C4 proton in **24** could be easily abstracted by KCN, the resulting stable furan-type intermediate gave a mixture of **24** and its epimer by protonation. Deprotection of the Fmoc group and introduction of AMCA-X gave fluorescence-labeled probe **2** as a 4:1 diastereomeric mixture. Diastereomerically pure **2** and its epimer *epi*-**2** were obtained after purification by HPLC (COSMOSIL 5C18-AR, 20% CH_3CN aq, 260 nm). The desired **2** was confirmed by coupling constants in the ^1H NMR spectra (Scheme 4). The relation between H4 and H6 was determined to be *syn* by the coupling constants between H4 and H5ax, H4 and H5eq, H5ax and H6, and H5eq and H6.



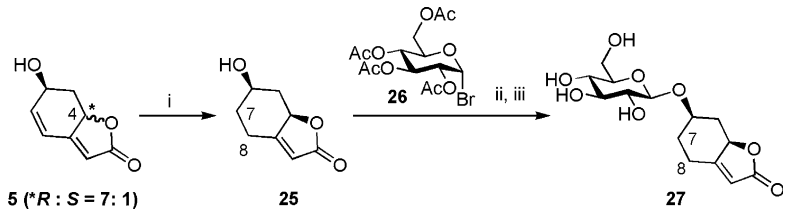
Scheme 4. (i) KCN, MeOH, 0 °C, 47%; (ii) piperidine, DMF, 0 °C; (iii) AMCA-X, SE, DMF, 0 °C, quant. (two steps); (iv) HPLC separation.



To prove the existence of a receptor, it is essential to demonstrate that biologically inactive probe compounds cannot

bind to the target cell for active compounds. Structure modification in the aglycon moiety of **2** reduces its bioactivity. Thus, we planned synthesis of fluorescence-labeled compound **31**, which has the reduced C7–C8 double bond, as a biologically inactive probe for the control fluorescence experiment.

Synthesis of 7,8-dihydro analog **27** is shown in Scheme 5. The hydrogenation of aglycon **5** using the Lindlar catalyst gave 7,8-dihydro aglycon **25**. At this stage, the reduced aglycon and its C4 epimer were easily separated by silica gel column chromatography. Glycosidation of 7,8-dihydro aglycon **25** and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide



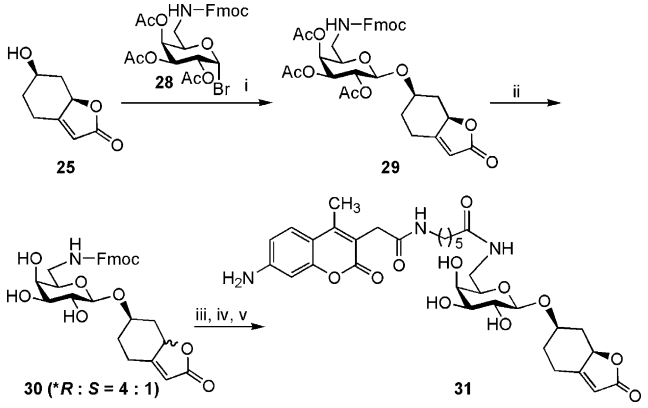
5 (*R : S = 7: 1) 25 27

Scheme 5. (i) H_2, Lindlar catalyst, AcOEt, 68%; (ii) **26**, AgOTf, Ag_2CO_3, MS4Å, CH_2Cl_2, 20%; (iii) KCN, MeOH, 31%.

26 was carried out with Ag_2CO_3 and AgOTf to afford the glycoside and subsequent deprotection of acetyl groups yielded 7,8-dihydro phyllanthurinolactone **27**.

The 7,8-dihydro analog **27** showed no leaf-closing activity against *P. urinaria* L. even at 1×10^{-4} M. From this result, we judged that the fluorescence-labeled compound with 7,8-dihydro aglycon could be used as a biologically inactive probe.

Synthesis of the biologically inactive probe **31** is shown in Scheme 6. Glycosidation of 7,8-dihydro aglycon **25** and glucopyranosyl bromide **28** via Koenigs–Knorr reaction afforded glycoside **29**. Acetyl groups of glycoside **29** were removed with KCN to give triol **30** as a 4:1 mixture of C4 stereoisomers. At this stage, epimerization of the C4 stereocenter was observed as in the case of **23**, which is the intermediate of the biologically active probe **2**. Deprotection of the Fmoc group and introduction of AMCA-X gave fluorescence-labeled probe **31** as a 4:1 diastereomeric mixture. Diastereomerically pure **31** was obtained after purification by HPLC (COSMOSIL 5C18-AR, 40% CH_3CN aq, 260 nm).



Scheme 6. (i) **28**, AgOTf, Ag_2CO_3, MS4Å, CH_2Cl_2, 48%; (ii) KCN, MeOH 61%; (iii) piperidine, DMF, 0 °C; (iv) AMCA-X, SE, DMF, 0 °C, 62% (two steps); (v) HPLC separation.

2.3. Bioassay of fluorescence-labeled probes

With the fluorescence-labeled probes **2**, *epi*-**2** and **31** were now available. Then, we examined their bioactivities. The young leaves detached from the stem of the plant *P. urinaria* with a sharp razor blade were used for bioassay. One leaf was placed in H_2O (ca. 300 μl) using a 5-ml glass tube in the biotron kept at 32 °C and allowed to stand overnight. The leaves, which opened again in the morning were used for the bioassay. The test solution was carefully poured into test tubes with a microsyringe at around 10:00 a.m. The bioactive fraction was judged by the leaf-closing activity before the leaf-closing of the plant leaf in the blank solution. Probe **2** was effective for the leaf-closing of *P. urinaria* at 1×10^{-5} M, that is, one-hundredth as effective as natural product **1**. On the other hand, *epi*-**2**, which is the C4 epimer of **2**, and 7,8-dihydro probe **31** were biologically inactive ($>1\times10^{-4}$ M).

2.4. Fluorescence study using fluorescence-labeled probes

We used fluorescence-labeled probes to seek the target cell for **1**. For this purpose, the binding experiment using a plant section was carried out. The leaf of *P. urinaria* opening in the daytime was cut into an appropriate size and fixed in agar. The agar was sliced perpendicular to the petiole by a microslicer (Dousaka EM Co., Ltd) to a thickness of 30 μm and the sections containing the pulvini were floated on distilled water. The sections were immersed in a solution containing 1×10^{-4} M of the probes (**2**, *epi*-**2**, and **31**), and allowed to stand for staining under shaded condition at room temperature for 6 h. After staining, the stained section was incubated for 15 min in equilibrium buffer (Slow Fade™ Gold Antifade Reagent, Molecular Probes Inc.) to remove excess fluorescence probes. Then, the stained section was placed on a slide glass and covered by a cover glass after adding a drop of antifade reagent (Slow Fade™ Gold Antifade Reagent, Molecular Probes Inc.). The observation of these sections was carried out by a fluorescence microscope (ECLIPSE E800, Nicon Co., Ltd) with an appropriate filter (B-2A, Nicon Co., Ltd; excitation wavelength 450–490 nm). At this time, the use of an antifade reagent was essential to prevent photobleaching (fading of fluorescence). Figure 2 shows photographs of plant pulvini, which contains a motor cell, under a fluorescence microscope. No stain was observed in the control section, which was treated with an aqueous solution containing no fluorescence-labeled probe (upper center). Red stains seen in the fluorescence images are due to the porphyrin in the plant tissue. The staining pattern for the fluorescence of the biologically active probe **2** was observed on the surface of the motor cell (upper right). We also carried out fluorescence studies of the interaction

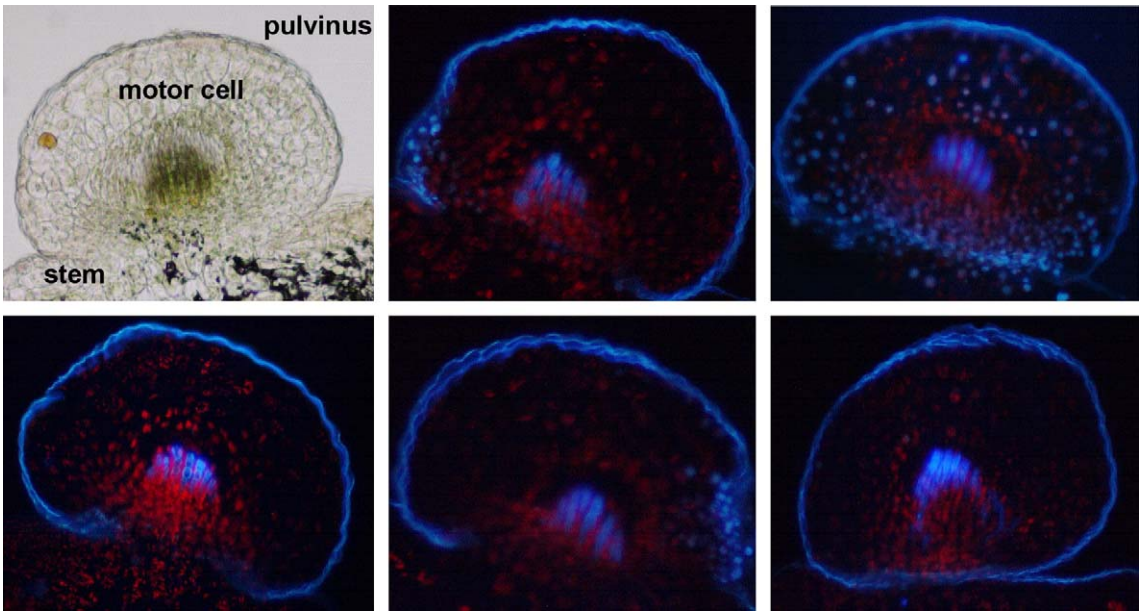


Figure 2. Fluorescence study using plant pulvini containing motor cell with fluorescence-labeled probes; upper left: Nomarskii image of plant section, upper center: fluorescence image of control section, upper right: fluorescence image of a section treated with **2**, lower left: fluorescence image of a section treated with *epi*-**2**, lower center: fluorescence image of a section treated with **31**, lower right: fluorescence image of a section treated with **2** in the presence of 100 molar excess of phyllanthurinolactone **1**.

between biologically inactive probes (*epi*-**2** and **31**) and the plant motor cell. Stains were not observed in the section treated with *epi*-**2** and **31** (lower left and lower center). Thus, it was proved that biologically inactive probe compounds cannot bind to the plant motor cell. Also, binding of probe **2** was inhibited by the coexistence of 100-fold concentration of the natural product **1**. When the section was treated by 1×10^{-4} M of **2** together with 1×10^{-2} M of **1**, no staining was observed in the plant section (lower right). Results of the structure–activity relationship were consistent with that of binding experiments. Also, it was clearly shown that the binding of biologically active fluorescence-labeled probe **2** with a motor cell is due to the specific binding of the aglycon moiety, which is the active site of this molecule, and is not a nonspecific binding due to the hydrophobicity of the fluorescence dye group (AMCA group). These results strongly suggested that some receptor for **1** exists in the motor cell, which plays a central role in the plant leaf-movement,[18] as with leaf-opening substances.[4] Along with the previous result,[6,7] some properties were revealed in a receptor molecule of **1**, that is, this receptor recognizes the precise structure of the aglycon moiety, whereas it does not recognize the sugar moiety at all.

2.5. Specific binding ability of fluorescence-labeled probe compound to *P. urinaria* L.

From our previous studies, it was revealed that each nyctinastic plant has a different leaf-movement factor whose bioactivity is specific to the plant genus.[19] Recently, we proved that the leaf-opening substances do not bind with motor cells of plants belonging to other genus by using the fluorescence-labeled probes based on the leaf-opening substances of *Cassia mimosoides* L.[4] and *Albizzia julibrissin* Durazz.[20] Thus, fluorescence-labeled probe **2** is expected to show specific leaf-closing activity to genus *Phyllanthus*, and not to be effective for other plants as well as leaf-opening substances. We examined the genus-specificity and the bioactivity of probe **2**. Probe **2** did not show leaf-closing activity against the leaves of *C. mimosoides*, *Mimosa pudica*, and *Leucaena leucocephala* at 1×10^{-4} M. From these results, the binding of probe **2** is expected to be specific to the section of plants belonging to the genus *Phyllanthus* and no binding would be observed in the experiment using the section of other plants. Then, we used probe **2** for the binding experiment with the sections of *M. pudica*, *C. mimosoides*, and *L. leucocephala*. The binding experiments were carried out according to the same method used in the case of *P. urinaria*; however, these sections gave no staining pattern resulting from **2** (Fig. 3). These results showed that the binding of probe **2** with a motor cell is specific to the genus *Phyllanthus* and suggested that a genus-specific receptor molecule for the leaf-movement factor, which is located on a motor cell would be involved in nyctinasty.

3. Conclusion

The binding of probes can be strongly correlated with leaf-closing activity. Biologically inactive *epi*-**2** and **31** did not bind to a motor cell at all. Additionally, the staining pattern resulting from the binding of probe **2** disappeared by the coexistence of an excess amount of the natural product **1**. These results strongly suggested that some receptors for **1**, which specifically recognize the stereochemistry of aglycon of **1** would be involved in the leaf-closing movement of *P. urinaria*. Moreover, since probe **2** did not show leaf-closing activity against plants belonging to other genus, it was revealed that the binding of a leaf-closing substance with a motor cell is specific to the plant genus as well as the leaf-opening substance and suggested that the genus-specific receptor for the leaf-closing substance in a motor cell would be involved in nyctinasty. In conclusion, we have succeeded in visualization of the target cell of the leaf-closing substance in the plant body by using the biologically active probe **2**

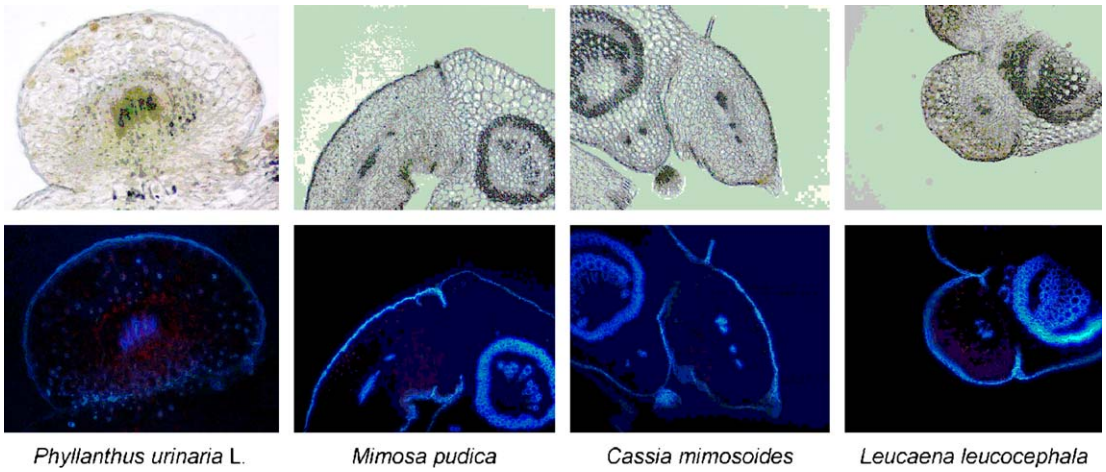


Figure 3. Photographs of plant sections in the binding experiments, which show specific binding of probe **2** with the motor cells of *Phyllanthus* plants (upper: Nomarski image of the plant section, lower: fluorescent image of the plant section treated with probe **2**.

and inactive probes *epi*-**2** and **31**, and revealed that the binding between a leaf-closing substance and its receptor in a motor cell is genus-specific. Some receptor for **1** would be involved in the leaf-closing movement in *P. urinaria* L. To reveal a receptor protein of **1**, the synthesis of photoaffinity labeling probes based on **1** is now in progress.

4. Experimental

4.1. General procedures

NMR spectra were recorded on a Jeol JNM-A600 spectrometer [^{1}H (600 MHz) and ^{13}C (150 MHz)], Jeol JNM-A400 [^{1}H (400 MHz) and ^{13}C (100 MHz)], JNM AL300 [^{1}H (300 MHz) and ^{13}C (75 MHz)], a Jeol JNM-EX 270 spectrometer [^{1}H (270 MHz) and ^{13}C (67.5 MHz)] using TMS in $CDCl_3$, CD_2HOD in CD_3OD (^{1}H; 3.33 ppm, ^{13}C; 49.8 ppm), or *t*-BuOH (^{1}H; 1.23 ppm, ^{13}C; 32.1 ppm) in D_2O as internal standards at various temperatures. The FABMS and HR-FABMS spectra were recorded on a Jeol JMS-700 or JMS-SX102 spectrometer, using glycerol or *m*-nitrobenzylalcohol as a matrix. The ESI-HRMS spectra were recorded on a Bruker APEX-III spectrometer. The IR spectra were recorded on a JASCO FT/IR-410 spectrometer. The specific rotations were measured by JASCO DIP-360 polarimeter. The HPLC purification was carried out with a Shimadzu LC-6A pump equipped with SPD-6A detector using COSMOCIL 5C_{18}-AR column (ϕ20×250 mm) (Nakalai Tesque Co., Ltd). The solvents used for HPLC were available from Kanto Chemical Co., and were filtered through a Toyo Roshi membrane filter (cellulose acetate of 0.45 mm pore size, 47 mm d.) before use. Silica gel column chromatography was performed on silica gel 60 K070 (Katayama Chemical Co., Ltd) or silica gel 60N (Kanto Chemical Co., Ltd). Reversed-phase open-column chromatography was performed on Cosmosil 75C_{18}-OPN (Nakalai Tesque Co., Ltd). TLC was performed on silica gel F_{254} (0.25 or 0.5 mm, MERCK) or RP-18F_{254S} (0.25 mm, MERCK).

4.1.1. *tert*-Butyl-[6-methoxy-5-(4-methoxy-benzyloxy)-2-methylene-tetrahydro-pyran-3-yloxy]-dimethyl-silane 9. To a solution of **8** (86.9 mg, 213 μmol) in CH_2Cl_2 (2.4 ml) was slowly added TBSOTf (72.4 μl, 320 μmol) at 0 °C under Ar atmosphere. After stirring for 30 min at 0 °C, to the reaction mixture was added H_2O (2 ml). The aqueous layer was extracted with $CHCl_3$. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 2:1) to afford a TBS ether (110 mg, 99%) as a colorless oil: $[\alpha]_D^{21}$ +75.5 (*c* 1.00, MeOH). IR (film) ν 2856, 2835, 1612, 1514 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$, rt) δ 7.25 (2H, d, *J*=8.6 Hz), 6.90 (2H, d, *J*=8.6 Hz), 4.60 (1H, s), 4.55 (2H, s), 3.82 (3H, s), 3.62 (1H, ddd, *J*=4.0, 8.5, 11.0 Hz), 3.50 (1H, dd, *J*=2.2, 10.1 Hz), 3.45 (1H, d, *J*=2.2 Hz), 3.40 (3H, s), 3.20 (1H, dd, *J*=8.5, 10.1 Hz), 1.90 (1H, ddd, *J*=3.6, 4.0, 13.2 Hz), 1.65 (1H, ddd, *J*=3.0, 11.0, 13.2 Hz), 0.95 (9H, s), 0.15 (3H, s), 0.12 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 159.2, 130.1, 129.1, 113.8, 98.5, 74.6, 73.2, 70.7, 67.6, 55.2, 54.9, 33.3, 25.7, 7.7, −4.1, −4.6. ESI-HRMS (positive-ion) calcd for $C_{21}H_{35}IO_5SiNa$: $(M+Na)^+$ 545.1196; found: 545.1191.

To a solution of the above TBS ether (55.9 mg, 107 μmol) in THF (1.0 ml) was added *t*-BuOK (39.3 mg, 321 μmol) at room temperature under Ar atmosphere. After stirring for 15 h, to the reaction mixture was added 1 N HCl (2 ml). The aqueous layer was extracted with AcOEt. The combined organic layer was washed with satd $NaHCO_3$ aq and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 2:1) to afford **9** (70.8 mg, 99%) as a colorless oil: $[\alpha]_D^{22}$ +11.8 (*c* 0.42, MeOH). IR (film) ν 2832, 2858, 2837, 1660, 1612 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$, rt) δ 7.25 (2H, d, *J*=8.6 Hz), 6.90 (2H, d, *J*=8.6 Hz), 4.68 (1H, s), 4.70 (1H, d, *J*=1.5 Hz), 4.62 (1H, d, *J*=11.2 Hz), 4.59 (1H, d, *J*=11.2 Hz), 4.55 (1H, d, *J*=1.5 Hz), 4.59 (1H, d, *J*=11.2 Hz), 4.41 (1H, m), 3.82 (3H, s), 3.62 (1H, s), 3.45 (3H, s), 2.05 (1H, ddd, *J*=2.9, 4.0, 13.2 Hz), 1.85 (1H, ddd, *J*=2.9, 11.4, 13.2 Hz), 0.95 (9H, s), 0.15 (3H, s), 0.12 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 159.2, 159.0, 130.0, 129.3, 113.9, 100.4, 94.0, 74.5, 70.9, 64.2, 55.2, 55.0, 34.0, 25.7, 25.6, 25.6, −4.9, −5.0. ESI-HRMS (positive-ion) calcd for $C_{21}H_{34}O_5SiNa$: $(M+Na)^+$ 417.2073; found: 417.2068.

4.1.2. 2-(*tert*-Butyl-dimethyl-silanyloxy)-5-hydroxy-4-(4-methoxy-benzyloxy)-cyclohexanone 10. To a solution of **9** (42.4 mg, 108 μmol) in acetone/H_2O (3:1, 600 μl) was slowly added $Hg(OCOCF_3)_2$ (2.3 mg, 5.4 μmol) at 0 °C under Ar atmosphere. After stirring for 9 h at 0 °C, the reaction mixture was diluted with AcOEt and washed with 10% KI aq, 20% $Na_2S_2O_3$ aq, and brine. The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:1) to afford **10** (28.8 mg, 70%) as a colorless oil: $[\alpha]_D^{19}$ +27.0 (*c* 1.00, MeOH). IR (film) ν 3422, 2856, 1719 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) δ 7.25 (2H, d, *J*=8.6 Hz), 6.90 (2H, d, *J*=8.6 Hz), 4.65 (1H, d, *J*=11.5 Hz), 4.57 (1H, d, *J*=11.5 Hz), 4.27 (1H, ddd, *J*=1.0, 4.8, 7.7 Hz), 4.09 (1H, m), 3.90 (3H, s), 2.76 (1H, dd, *J*=4.3, 13.8 Hz), 2.65 (1H, dd, *J*=7.0, 13.8 Hz), 2.25 (1H, m), 1.95 (1H, m), 0.95 (9H, s), 0.15 (3H, s), 0.12 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 129.4, 114.0, 73.6, 71.9, 71.3, 55.3, 43.3, 35.0, 25.7, 25.7, 18.2, −4.8, −5.3. ESI-HRMS (positive-ion) calcd for $C_{20}H_{32}O_5SiNa$: $(M+Na)^+$ 403.1917; found: 403.1911.

4.1.3. 6-(*tert*-Butyl-dimethyl-silanyloxy)-4-(4-methoxy-benzyloxy)-cyclohex-2-enone 11. To a solution of **10** (5.8 mg, 15.3 μmol) and Et_3N (21.3 μl, 153 μmol) in CH_2Cl_2 (1.0 ml) was slowly added MsCl (3.3 μl, 42.8 μmol) at 0 °C under Ar atmosphere. After stirring for 1 h at room temperature, the reaction mixture was diluted with $CHCl_3$ and washed with 1 N H_2SO_4, satd $NaHCO_3$ aq, and brine. The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 2:1) to afford **11** (5.5 mg, quant.) as a colorless oil: $[\alpha]_D^{19}$ −127.5 (*c* 1.00, MeOH). IR (film) ν 3483, 2941, 2116, 1719 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) δ 7.25 (2H, d, *J*=8.6 Hz), 6.90 (2H, dd, *J*=2.0, 8.6 Hz), 6.89 (1H, d, *J*=10.3 Hz), 5.97 (1H, d, *J*=10.3 Hz), 4.65 (1H, d, *J*=11.5 Hz), 4.57 (1H, d, *J*=11.5 Hz), 2.25 (2H, m), 0.95 (9H, s), 0.15 (3H, s), 0.12 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 197.5, 159.4, 147.8, 129.8, 129.4, 128.1, 113.9, 71.2, 71.0, 70.2, 55.2, 37.6, 25.7, −4.7, −5.4. ESI-HRMS (positive-ion) calcd for $C_{20}H_{30}O_4SiNa$: $(M+Na)^+$ 385.1811; found: 385.1806.

4.1.4. 6-Hydroxy-4-(4-methoxy-benzyloxy)-cyclohex-2-enone 7. To a solution of **11** (10.1 mg, 27.9 μmol) and AcOH (4.8 μl, 83.7 μmol) in THF (500 μl) was slowly added TBAF (1 M in THF, 83.7 μl, 83.7 μmol) at 0 °C under Ar atmosphere. After stirring for 46 h at room temperature, the reaction mixture was diluted with AcOEt and washed with H_2O and brine. The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:1) to afford **7** (6.9 mg, quant.) as a colorless oil: $[\alpha]_D^{19}$ −173.1 (*c* 1.00, MeOH). IR (film) ν 3449, 2837, 1693, 1612, 1514 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) δ 7.25 (2H, d, *J*=8.6 Hz), 6.90 (2H, d, *J*=8.6 Hz), 6.89 (1H, dd, *J*=5.5, 12.3 Hz), 4.53 (1H, d, *J*=11.3 Hz), 4.34 (1H, ddd, *J*=2.0, 4.0, 5.1 Hz), 3.90 (3H, s), 2.63 (1H, ddd, *J*=2.0, 5.5, 13.5 Hz), 1.98 (1H, ddd, *J*=4.0, 12.3, 13.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 200.3, 159.4, 146.7, 129.6, 129.4, 127.8, 113.9, 71.5, 69.7, 68.9, 55.2, 35.0. ESI-HRMS (positive-ion) calcd for $C_{14}H_{16}O_4Na$: $(M+Na)^+$ 271.0946; found: 271.0941.

4.1.5. (Diethoxy-phosphoryl)-acetic acid 5-(4-methoxy-benzyloxy)-2-oxo-cyclohex-3-enyl ester 6. To a solution of **7** (67.2 mg, 271 μmol) in benzene (20 ml) were slowly added DEAD (40% toluene solution, 1.0 ml, 2.17 mmol), **15** (662 mg, 2.17 mmol), and **13** (425 mg, 2.17 mmol) at 0 °C under Ar atmosphere. After stirring for 1.5 h at room temperature, the reaction mixture was diluted with AcOEt and washed with 0.5 N HCl, satd $NaHCO_3$ aq, and brine. The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:5) to afford **6** (46.3 mg, 40%. *R*:*S* = 10:1) as a colorless oil: $[\alpha]_D^{20}$ −6.8 (*c* 1.00, $CHCl_3$). IR (film) ν 1747, 1703, 1612, 1514, 1250 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$, rt) δ 7.28 (2H, d, *J*=8.2 Hz), 6.97 (1H, d, *J*=10.6 Hz), 6.91 (2H, d, *J*=8.2 Hz), 6.06 (1H, d, *J*=10.6 Hz), 5.31 (1H, dd, *J*=5.0, 13.7 Hz), 4.62 (1H, d, *J*=11.5 Hz), 4.56 (1H, d, *J*=11.5 Hz), 4.43 (1H, m), 4.21 (2H, q, *J*=6.9 Hz), 4.18 (2H, q, *J*=6.9 Hz), 3.82 (3H, s), 3.14 (1H, d, *J*=5.0 Hz), 3.06 (1H, d, *J*=5.0 Hz), 2.75–2.67 (1H, m), 2.17 (1H, dt, *J*=8.6, 11.2 Hz), 1.36 (6H, t, *J*=6.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 192.2, 164.9, 159.4, 151.8, 129.4, 129.0, 127.4, 113.6, 72.3, 72.2, 70.9, 62.9, 55.3, 36.0, 34.0. ESI-HRMS (positive-ion) calcd for $C_{20}H_{28}O_8P$: $(M+H)^+$ 427.1522; found: 427.1540.

4.1.6. 6-(4-Methoxy-benzyloxy)-7,7a-dihydro-6*H*-benzofuran-2-one 12. To a solution of **6** (4.7 mg, 11.0 μmol) in THF (500 μl) was added *t*-BuOK (1.2 mg, 11.0 μmol) at 0 °C under Ar atmosphere. After stirring for 30 min at 0 °C, the reaction mixture was diluted with AcOEt and washed with 1 N HCl, satd $NaHCO_3$ aq, and brine. The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:5) to afford **12** (1.9 mg, 63%. *R*:*S* = 10:1) as a colorless oil: $[\alpha]_D^{24}$ −1.1 (*c* 1.00, $CHCl_3$). IR (film) ν 1741, 1641, 1612, 1585, 1514 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$, rt) δ 7.28 (2H, d, *J*=8.6 Hz), 6.90 (2H, d, *J*=8.6 Hz), 6.57 (1H, dd, *J*=2.3, 9.9 Hz), 6.34 (1H, d, *J*=9.9 Hz), 5.80 (1H, s), 4.82 (1H, dd, *J*=5.0, 13.5 Hz), 4.61 (1H, d, *J*=11.5 Hz), 4.55 (1H, d, *J*=11.5 Hz), 4.35–4.31 (1H, m), 3.82 (3H, s), 2.94 (1H, dt, *J*=5.0, 10.9 Hz), 1.71 (1H, dt, *J*=10.9, 13.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 173.1, 162.8, 159.4, 141.5, 129.4, 129.3, 120.2, 114.0, 111.4, 78.0, 72.5, 70.8, 55.3, 37.0. ESI-HRMS (positive-ion) calcd for $C_{16}H_{17}O_4$: $(M+H)^+$ 273.1127; found: 273.1147.

4.1.7. Aglycon 5. To a solution of **12** (10.1 mg, 37.1 μmol) in CH_2Cl_2 (500 μl) were added H_2O (25 μl) and DDQ (12.6 mg, 55.7 μmol) at 0 °C under Ar atmosphere. After stirring for 4.5 h at 0 °C, to the reaction mixture was added satd $NaHCO_3$ aq. The aqueous layer was extracted with AcOEt. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:5) to afford **5** (4.0 mg, 71%) as a white powder: $[\alpha]_D^{17}$ −13.6 (*c* 0.70, $CHCl_3$). IR (film) ν 3441, 1732, 1639, 1105 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) δ 5.76 (1H, s), 6.57 (1H, dd, *J*=1.8, 9.9 Hz), 6.36 (1H, d, *J*=9.9 Hz), 5.81 (1H, s), 4.90 (1H, dd, *J*=4.4, 13.2 Hz), 4.70–4.58 (1H, m), 3.40–3.50 (1H, br s, OH), 2.93 (1H, td, *J*=5.2, 10.8 Hz), 1.68 (1H, q, *J*=11.0 Hz). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 173.8, 163.5, 144.1, 119.6, 111.0,

78.3, 66.5, 39.7. ESI-HRMS (positive-ion) calcd for $C_8H_8O_3Na$: $(M+Na)^+$ 175.0371; found: 175.0365.

4.1.8. Acetic acid 4,5-diacetoxy-6-azidomethyl-2-fluoro-tetrahydro-pyran-3-yl ester 17. To a solution of **16** (378 mg, 1.01 mmol) in DMF (10 ml) was slowly added H_2NNH_2/AcOH (1:1, 41 μl) at 0 °C under Ar atmosphere. After stirring for 1.5 h at 0 °C, the reaction mixture was diluted with $CHCl_3$ and washed with 1 N HCl, satd $NaHCO_3$ aq, and brine. The organic layer was dried over Na_2SO_4, and concentrated in vacuo to afford a crude mixture of anomeric galactopyranoses (268 mg) as a colorless oil. To a solution of the mixture (248 mg) in CH_2Cl_2 (7.5 ml) was added DAST (98.2 μl, 750 μmol) at 0 °°C. After stirring for 30 min at 0 °C, the reaction mixture was diluted with $CHCl_3$ and washed with H_2O. The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:1) to afford **17** (250 mg, 80% in two steps) as a white powder. α-*anomer*: $[\alpha]_D^{20}$ +90.3 (*c* 1.00, MeOH). IR (film) *ν* 2941, 2116, 1718 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) *δ* 5.80 (1H, dd, *J*=2.2, 53.3 Hz), 5.50 (1H, d, *J*=2.5 Hz), 5.35 (1H, dd, *J*=2.5, 11.0 Hz), 5.20 (1H, ddd, *J*=2.2, 11.0, 23.4 Hz), 4.30 (1H, dd, *J*=5.1, 7.7 Hz), 3.50 (1H, dd, *J*=7.7, 12.9 Hz), 3.25 (1H, d, *J*=5.1, 12.9 Hz), 2.20 (3H, s), 2.18 (1H, s), 2.00 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$, rt) *δ* 171.5, 171.2, 171.1, 106.5, 103.4, 70.6, 70.6, 68.4, 67.9, 67.6, 67.4, 50.6, 20.8, 20.7. ESI-HRMS (positive-ion) calcd for $C_{12}H_{16}FN_3O_7Na$: $(M+Na)^+$ 356.0870; found: 356.0864. β-*anomer*: $[\alpha]_D^{20}$ +28.6 (*c* 1.00, MeOH). IR (film) *ν* 2837, 2108, 1751 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) *δ* 5.50 (1H, d, *J*=3.3, Hz), 5.33 (1H, ddd, *J*=6.9, 9.9, 10.6 Hz), 5.30 (1H, dd, *J*=6.9, 52.5 Hz), 5.06 (1H, dd, *J*=3.3, 9.9 Hz), 3.95 (1H, dd, *J*=4.8, 7.6 Hz), 3.60 (1H, dd, *J*=7.6, 12.8 Hz), 3.32 (1H, d, *J*=4.8, 12.8 Hz), 2.20 (3H, s), 2.10 (3H, s), 2.00 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$, rt) *δ* 171.2, 171.1, 170.5, 109.1, 106.2, 73.0, 72.9, 70.3, 70.1, 69.2, 68.9, 67.5, 50.5, 20.7, 20.6, 20.6. ESI-HRMS (positive-ion) calcd for $C_{12}H_{16}FN_3O_7Na$: $(M+Na)^+$ 356.0870; found: 356.0864.

4.1.9. (6-Fluoro-3,4,5-trihydroxy-tetrahydro-pyran-2-yl-methyl)-carbamic acid 9*H*-fluoren-9-ylmethyl ester 19. To a solution of **17** (59.0 mg, 177 μmol) in MeOH (1.8 ml) was slowly added MeONa (31.6 mg, 585 μmol) at 0 °C under Ar atmosphere. After stirring for 30 min at 0 °C, Amberlite IR-120B (H^+) was added to this solution for neutralization. After filtration, the filtrate was concentrated in vacuo to afford a crude mixture. To a solution of the mixture in MeOH (1.5 ml) was added palladium on activated carbon (17.4 mg). Hydrogen was admitted via a balloon and the reaction mixture was stirred for 1.5 h and the catalyst removed by filtration. The filtrate was concentrated in vacuo to afford a crude mixture. To a solution of the mixture and diidopropylethylamine (30.8 μl, 177 μmol) in THF (8.0 ml) was added FmocCl (45.8 mg, 177 μmol) at room temperature. After stirring for 43 h, the reaction mixture was concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH=5:1) to afford **19** (22.7 mg, 32% in three steps) as a white powder. α-*anomer*: $[\alpha]_D^{23}$ +88.4 (*c* 0.10, MeOH). IR (film) *ν* 3436, 1697 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, rt) *δ* 7.85 (2H, d, *J*=7.8 Hz), 7.68 (2H, d, *J*=7.3 Hz), 7.40 (4H, m), 5.50 (1H, dd, *J*=2.2, 55.5 Hz), 4.30 (2H, d, *J*=7.0 Hz), 4.21 (1H, t, *J*=7.0 Hz), 3.82 (1H, t, *J*=6.3 Hz), 3.70–3.50 (2H, m), 3.50 (1H, dd, *J*=7.7, 12.9 Hz), 3.20 (2H, m). ^{13}C NMR (75 MHz, DMSO-d_6, rt) *δ* 156.3, 143.9, 140.8, 127.7, 127.1, 125.2, 120.1, 109.7, 106.8, 71.6, 68.9, 68.7, 65.4, 46.7, 41.1. ESI-HRMS (positive-ion) calcd for $C_{21}H_{22}FNO_6Na$: $(M+Na)^+$ 426.1329; found: 426.1323. β-*anomer*: $[\alpha]_D^{22}$ −43.0 (*c* 0.10, MeOH). IR (film) *ν* 3165, 1643 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, rt) *δ* 7.85 (2H, d, *J*=7.8 Hz), 7.68 (2H, d, *J*=7.3 Hz), 7.40 (4H, m), 4.90 (1H, dd, *J*=6.9, 54.7 Hz), 4.30 (2H, d, *J*=7.0 Hz), 4.21 (1H, t, *J*=7.0 Hz), 3.60–3.10 (6H, m). ^{13}C NMR (75 MHz, DMSO-d_6, rt) *δ* 158.5, 145.5, 142.5, 129.5, 128.9, 126.8, 121.7, 113.1, 110.4, 80.0, 74.2, 74.2, 73.1, 73.0, 71.7, 71.4, 69.2, 66.7, 47.7, 41.9. ESI-HRMS (positive-ion) calcd for $C_{21}H_{22}FNO_6Na$: $(M+Na)^+$ 426.1329: found: 426.1323.

4.1.10. Acetic acid 4,5-diacetoxy-6-(*tert*-butoxycarbonyl-amino-methyl)-2-fluoro-tetrahydro-pyran-3-yl ester 20. To a solution of **17** (24.0 mg, 72.1 μmol) in MeOH (700 μl) was slowly added MeONa (12.9 mg, 238 μmol) at 0 °C under Ar atmosphere. After stirring for 30 min at 0 °C, Amberlite IR-120B (H^+) was added to this solution for neutralization. After filtration, the filtrate was concentrated in vacuo to afford a crude mixture. To a solution of the mixture in MeOH (700 μl) was added palladium on activated carbon (8.7 mg). Hydrogen was admitted via a balloon and the reaction mixture was stirred for 30 min and the catalyst removed by filtration. The filtrate was concentrated in vacuo to afford a crude mixture. To a solution of the mixture and triethylamine (10.1 μl, 71.9 μmol) in MeOH (1.0 ml) was added Boc_2O (16.6 μl, 71.9 μmol) at 0 °C. After stirring for 2 h at 0 °C, the reaction mixture was concentrated in vacuo to afford a crude mixture. To a solution of the mixture in pyridine (1.0 ml) was added Ac_2O (0.5 ml) at room temperature. After stirring for 7 h, the reaction mixture was diluted with AcOEt and washed with 1 N HCl, satd $NaHCO_3$ aq, and brine. The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:1) to afford **20** (19.1 mg, 65% in four steps) as a colorless oil: $[\alpha]_D^{18}$ +51.4 (*c* 0.40, $CHCl_3$). IR (film) *ν* 2108, 1751, 1371, 1221, 1067, 1020 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) *δ* 5.78 (1H, dd, *J*=2.2, 53.4 Hz), 5.43 (1H, d, *J*=2.7 Hz), 5.36 (1H, dd, *J*=2.7, 11.0 Hz), 5.19 (1H, ddd, *J*=2.2, 11.0, 23.5 Hz), 4.20 (1H, t, *J*=6.6 Hz), 3.28 (2H, m), 2.19 (3H, s), 2.15 (3H, s), 2.02 (3H, s), 1.45 (9H, s). ^{13}C NMR (75 MHz, $CDCl_3$, rt) *δ* 170.3, 169.9, 155.6, 104.3 (d, J_{C-F}=113 Hz), 80.0, 70.1, 67.9, 67.7, 67.4, 67.1, 39.9, 28.3, 20.6. ESI-HRMS (positive-ion) calcd for $C_{17}H_{26}FNO_9Na$: $(M+Na)^+$ 430.1489; found: 430.1484.

4.1.11. Acetic acid 3,5-diacetoxy-2-[(9*H*-fluoren-9-yl-methoxycarbonylamino)-methyl]-6-fluoro-tetrahydro-pyran-4-yl ester 21. To a solution of **19** (22.7 mg, 56.3 μmol) in pyridine (1.0 ml) was added Ac_2O (0.5 ml) at room temperature. After stirring for 7 h, the reaction mixture was diluted with AcOEt and washed with 1 N HCl, satd $NaHCO_3$ aq. and brine. The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:1) to afford **21** (25.9 mg, 87%) as a colorless oil. α-*anomer*: $[\alpha]_D^{20}$ +39.5

(*c* 0.10, MeOH). IR (film) ν 3366, 2961, 1701 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) δ 7.78 (2H, d, *J*=7.3 Hz), 7.58 (2H, d, *J*=7.3 Hz), 7.35 (4H, m), 5.80 (1H, dd, *J*=2.5, 53.9 Hz), 5.50 (1H, d, *J*=2.5 Hz), 5.35 (1H, dd, *J*=2.5, 11.0 Hz), 5.18 (1H, ddd, *J*=2.5, 11.0, 23.5 Hz), 4.43 (2H, d, *J*=7.0 Hz), 4.23 (2H, m), 3.34 (2H, t, *J*=6.6 Hz), 2.20 (3H, s), 2.15 (1H, s), 2.03 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 170.3, 169.8, 156.2, 143.7, 141.3, 127.7, 127.1, 125.0, 120.0, 105.7, 102.7, 69.9, 68.1, 67.6, 67.3, 67.0, 47.1, 40.5, 20.6. ESI-HRMS (positive-ion) calcd for $C_{27}H_{28}FNO_9Na$: $(M+Na)^+$ 552.1646; found: 552.1639. β-*anomer*: $[\alpha]_D^{17}$ +7.4 (*c* 0.49, MeOH). IR (film) ν 3437, 2963, 1637 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) δ 7.78 (2H, d, *J*=7.3 Hz), 7.58 (2H, d, *J*=7.3 Hz), 7.35 (4H, m), 5.40–5.13 (4H, m), 4.43 (2H, d, *J*=6.6 Hz), 4.21 (1H, t, *J*=6.6 Hz), 3.88 (1H, t, *J*=6.6 Hz), 3.39 (2H, t, *J*=6.6 Hz), 2.20 (3H, s), 2.15 (1H, s), 2.03 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 172.0, 171.4, 171.1, 158.8, 145.4, 145.2, 142.6, 128.8, 128.2, 126.3, 126.2, 121.0, 109.9, 107.1, 79.5, 73.2, 73.1, 71.8, 71.7, 70.6, 70.3, 68.4, 67.9, 48.2, 41.2, 20.6, 20.5. ESI-HRMS (positive-ion) calcd for $C_{27}H_{28}FNO_9Na$: $(M+Na)^+$ 552.1646: found: 552.1639.

4.1.12. Acetic acid 3,5-diacetoxy-2-[(9*H*-fluoren-9-ylmethoxycarbonylamino)-methyl]-6-(2-oxo-2,6,7,7a-tetrahydro-benzofuran-6-yloxy)-tetrahydro-pyran-4-yl ester 23. A mixture of **5** (6.6 mg, 43.4 μmol), Cp_2HfCl_2 (32.9 mg, 86.8 μmol), $AgClO_4$ (18.0 mg, 86.8 μmol) and dried molecular sieves 4Å (5.0 mg) in anhydrous CH_2Cl_2 (500 μl) was stirred at 0 °C under Ar atmosphere for 1 h. Then a solution of **4** (36.0 mg, 68.1 μmol) in anhydrous CH_2Cl_2 (300 μl) was added to the stirred mixture. After stirring for 6 h, the reaction mixture was diluted with $CHCl_3$ and filtered through Celite. The filtrate was washed with brine. The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (toluene/acetone = 1:3) to afford **23** (15.0 mg, 52%, *R*:*S* = 7:1) as a colorless oil: $[\alpha]_D^{18}$ +12.6 (*c* 0.10, $CHCl_3$). IR (film) ν 1747, 1221, 1155, 1070, 760 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$, rt) δ 7.77 (2H, d, *J*=7.4 Hz), 7.59 (2H, d, *J*=7.4 Hz), 7.41 (2H, t, *J*=7.4 Hz), 7.33 (2H, t, *J*=7.4 Hz), 6.59 (1H, d, *J*=10.0 Hz), 6.20 (1H, d, *J*=10.0 Hz), 5.84 (1H, s), 5.35 (1H, d, *J*=2.0 Hz), 5.21 (1H, dd, *J*=7.8, 10.0 Hz), 5.12 (1H, t, *J*=6.0 Hz), 5.03 (1H, dd, *J*=2.0, 10.0 Hz), 4.83 (1H, dd, *J*=4.5, 12.0 Hz), 4.62 (1H, d, *J*=7.8 Hz), 4.59–4.54 (1H, br s), 4.44 (2H, d, *J*=6.6 Hz), 4.22 (1H, t, *J*=6.6 Hz), 3.78 (1H, t, *J*=6.8 Hz), 3.46 (1H, td, *J*=6.8, 14.0 Hz), 3.15 (1H, td, *J*=6.8, 14.0 Hz), 2.94 (1H, td, *J*=4.5, 12.0 Hz), 2.20 (3H, s), 2.07 (3H, s), 2.02 (3H, s), 1.81 (1H, q, *J*=12.0 Hz). ^{13}C NMR (150 MHz, $CDCl_3$, rt) δ 172.9, 171.0, 170.0, 169.3, 162.0, 156.4, 143.7, 141.3, 139.6, 127.8, 127.1, 125.0, 121.1, 120.0, 115.5, 100.8, 77.9, 74.1, 71.7, 70.8, 69.0, 67.8, 67.0, 47.2, 40.4, 38.3, 20.8, 20.6. ESI-HRMS (positive-ion) calcd for $C_{35}H_{35}NO_{12}Na$: $(M+Na)^+$ 684.2057; found: 684.2051.

4.1.13. [3,4,5-Trihydroxy-6-(2-oxo-2,6,7,7a-tetrahydro-benzofuran-6-yloxy)-tetrahydro-pyran-2-ylmethyl]-carbamic acid 9*H*-fluoren-9-ylmethyl ester 24. To a solution of **23** (1.8 mg, 2.9 μmol) in MeOH (200 μl) was added KCN (6 mM in MeOH, 200 μl, 1.2 μmol) at 0 °C under Ar atmosphere. After stirring for 5 h at 0 °C, the reaction mixture was concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 1:1) to afford **24** (0.7 mg, 47%, *R*:*S*=4:1) as a colorless oil: $[\alpha]_D^{20}$ −7.1 (*c* 0.10, MeOH). IR (film) ν 3533, 3308, 1719, 1686, 1638, 1535, 1448, 1263, 1078 cm^{-1}. ^1H NMR (300 MHz, CD_3OD, rt) δ 7.77 (2H, d, *J*=7.3 Hz), 7.66 (2H, t, *J*=7.3 Hz), 7.37 (2H, t, *J*=7.3 Hz), 7.30 (2H, t, *J*=7.3 Hz), 6.63 (1H, dd, *J*=2.0, 10.1 Hz), 6.46 (1H, d, *J*=10.1 Hz), 5.83 (1H, s), 4.80 (1H, dd, *J*=4.5, 13.4 Hz), 4.57–4.62 (1H, m), 4.44 (1H, dd, *J*=6.5, 10.5 Hz), 4.39 (1H, dd, *J*=6.5, 10.5 Hz), 4.37 (1H, d, *J*=7.0 Hz), 4.38 (2H, dd, *J*=6.4, 10.4 Hz), 4.22 (1H, t, *J*=6.4 Hz), 3.70 (1H, d, *J*=2.2 Hz), 3.63 (1H, s), 3.52–3.44 (4H, m), 2.94 (1H, dt, *J*=4.5, 11.4 Hz), 1.64 (1H, dt, *J*=10.6, 13.2 Hz). ^{13}C NMR (125 MHz, CD_3OD, rt) δ 175.8, 165.9, 159.1, 145.4, 145.3, 143.2, 142.7, 128.8, 128.2, 128.1, 121.1, 120.9, 120.9, 111.7, 104.8, 79.9, 75.3, 74.9, 74.8, 72.2, 70.5, 67.6, 49.6, 42.5, 40.1. ESI-HRMS (positive-ion) calcd for $C_{29}H_{29}NO_9Na$: $(M+Na)^+$ 558.1740; found: 558.1735.

4.1.14. Biologically active fluorescence-labeled probe 2. To a solution of **24** (1.6 mg, 3.0 μmol) in DMF (300 μl) was added piperidine (1.0 μl, 10 μmol) at 0 °C under Ar atmosphere. After stirring for 4 h at 0 °C, piperidine was removed in vacuo. To the reaction mixture were added DMF (200 μl) and AMCA-X, SE (1.4 mg, 3.3 μmol) at room temperature under Ar atmosphere. After stirring for 2 h, the reaction mixture was concentrated in vacuo. The residue was purified by ODS TLC (RP-18W, H_2O/MeOH = 1:1) and HPLC with COSMOSIL $5C_{18}$-AR column (ϕ20.0× 250 mm, H_2O/CH_3CN = 1:1) to afford **2** (0.9 mg, 48%) and *epi*-**2** (0.2 mg, 11%). Compound **2**: white powder. $[\alpha]_D^{18}$ +28.0 (*c* 1.00, MeOH). IR (film) ν 3292, 2856, 1734 cm^{-1}. ^1H NMR (500 MHz, CD_3OD, rt) δ 7.48 (1H, d, *J*=8.5 Hz), 6.64 (1H, dd, *J*=2.5, 8.5 Hz), 6.63 (1H, dd, *J*=3.0, 10.0 Hz), 6.50 (1H, d, *J*=2.5 Hz), 6.47 (1H, d, *J*=10.0 Hz), 5.80 (1H, s), 5.00 (1H, ddd, *J*=2.0, 5.0, 13.0 Hz), 4.69 (1H, m), 4.40 (1H, d, *J*=7.5 Hz), 3.73 (1H, dd, *J*=1.0, 3.0 Hz), 3.60 (1H, ddd, *J*=1.0, 5.5, 8.0 Hz), 3.54 (2H, s), 3.50 (1H, dd, *J*=2.5, 7.0 Hz), 3.46 (1H, dd, *J*=7.5, 10.0 Hz), 3.45 (1H, dd, *J*=5.5, 13.5 Hz), 3.36 (1H, dd, *J*=8.0, 13.5 Hz), 3.18 (2H, dt, *J*=2.5, 7.0 Hz), 2.98 (1H, ddd, *J*=5.0, 6.0, 10.5 Hz), 2.38 (3H, s), 2.22 (2H, t, *J*=7.5 Hz), 1.69 (1H, ddd, *J*=10.5, 11.0, 13.0 Hz), 1.64 (2H, m), 1.52 (2H, m), 1.36 (2H, m). ^{13}C NMR (125 MHz, CD_3OD, rt) δ 176.7, 175.8, 172.9, 165.9, 164.8, 155.9, 153.9, 152.9, 143.7, 127.4, 121.1, 114.5, 113.2, 111.8, 111.7, 105.0, 100.5, 79.9, 75.7, 74.8, 74.4, 72.2, 70.6, 41.3, 40.3, 40.2, 37.0, 35.2, 30.0, 27.5, 26.6, 15.4. ESI-HRMS (positive-ion) calcd for $C_{32}H_{39}N_3O_{11}Na$: $(M+Na)^+$ 664.2478; found: 664.2478. Compound *epi*-**2**: white powder. $[\alpha]_D^{17}$ −37.3 (*c* 0.07, MeOH). IR (film) ν 3348, 2926, 1741, 1639, 1601, 1556, 1051 cm^{-1}. ^1H NMR (300 MHz, CD_3OD, rt) δ 7.49 (1H, d, *J*=7.5 Hz), 6.71 (1H, d, *J*= 8.0 Hz), 6.65 (1H, dd, *J*=2.0, 7.5 Hz), 6.48 (1H, d, *J*=2.0 Hz), 6.45 (1H, dd, *J*=4.0, 8.0 Hz), 5.85 (1H, d, *J*=1.5 Hz), 5.38 (1H, ddd, *J*=1.5, 5.5, 11.5 Hz), 4.65 (1H, m), 4.37 (1H, d, *J*=6.0 Hz), 3.62 (1H, dd, *J*=1.0, 4.0, 6.5 Hz), 3.52 (2H, s), 3.50 (1H, dd, *J*=6.5, 8.5 Hz), 3.48 (1H, dd, *J*=4.0, 12.0 Hz), 3.45 (1H, dd, *J*=3.0, 8.5 Hz), 3.38 (1H, dd, *J*=6.5, 12.0 Hz), 3.17 (1H, t, *J*=6.0 Hz), 2.82 (1H, ddd, *J*=2.0, 5.5, 11.5 Hz), 2.37 (3H, s), 2.22 (2H, t, *J*=7.5 Hz), 1.73 (1H, dt, *J*=3.0, 11.5 Hz), 1.62 (2H,

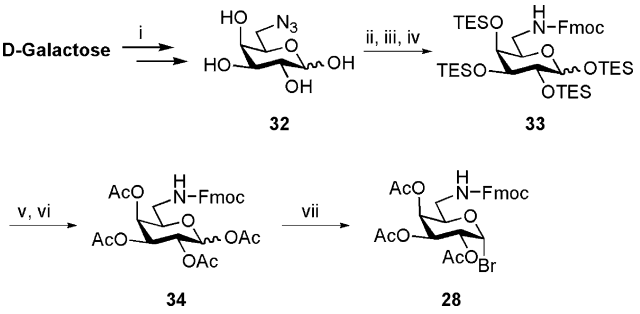
m), 1.51 (2H, m), 1.35 (2H, m). ESI-HRMS (positive-ion) calcd for $C_{32}H_{39}N_3O_{11}Na$: $(M+Na)^+$ 664.2478: found: 664.2478.

4.1.15. 7,8-Dihydro aglycon 25. To a solution of **5** (6.2 mg, 41.0 μmol) in AcOEt (800 μl) was added Lindlar catalyst (8.2 mg). Hydrogen was admitted via a balloon and the reaction mixture was stirred for 15 min and the catalyst removed by filtration. The filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 20:1) to afford **25** (4.3 mg, 68%) as a colorless oil: $[\alpha]_D^{24}$ −52.1 (*c* 0.20, $CHCl_3$). IR (film) ν 3422, 1732, 1647, 1005 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) δ 5.76 (1H, s), 4.73 (1H, dd, *J*=5.7, 10.8 Hz), 3.92 (1H, tt, *J*=3.0, 10.8 Hz), 2.84–2.83 (1H, m), 2.79–2.71 (1H, m), 2.33 (1H, ddd, *J*=1.8, 5.7, 14.1 Hz), 2.23–2.19 (1H, m), 1.49–1.36 (1H, m), 1.36 (1H, dd, *J*=11.4, 14.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 173.2, 169.9, 113.4, 79.5, 66.9, 42.1, 34.7, 24.2. ESI-HRMS (positive-ion) calcd for $C_8H_{10}O_3Na$: $(M+Na)^+$ 177.0528; found: 177.0521.

4.1.16. 7,8-Dihydro phyllanthurinolactone 27. A mixture of **25** (8.6 mg, 56.0 μmol), AgOTf (7.2 mg, 27.9 μmol), Ag_2CO_3 (32.3 mg, 117 μmol) and dried molecular sieves 4A (17.7 mg) in anhydrous CH_2Cl_2 (200 μl) was stirred at 0 °C under Ar atmosphere for 1 h. Then a solution of **26** (22.7 mg, 55.0 μmol) in anhydrous CH_2Cl_2 (500 μl) was added to the stirred mixture. After stirring for 2 h, the reaction mixture was diluted with $CHCl_3$ and filtered through Celite. The filtrate was washed with satd $NaHCO_3$ aq and brine. The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:3) to afford a glycoside (5.4 mg, 20%) as a colorless oil: $[\alpha]_D^{16}$ −15.3 (*c* 0.10, $CHCl_3$). IR (film) ν 1751, 1225, 1038 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) δ 5.79 (1H, s), 5.21 (1H, t, *J*=9.6 Hz), 5.08 (1H, t, *J*=9.6 Hz), 4.95 (1H, dd, *J*=8.1, 9.6 Hz), 4.74 (1H, dd, *J*=6.3, 12.3 Hz), 4.65 (1H, d, *J*=8.1 Hz), 4.27 (1H, dd, *J*=4.8, 12.0 Hz), 4.14 (1H, dd, *J*=2.7, 12.0 Hz), 3.88 (1H, tt, *J*=3.6, 11.1 Hz), 3.71 (1H, ddd, *J*=2.7, 4.8, 9.6 Hz), 2.93–2.83 (2H, m), 2.36–2.19 (2H, m), 2.15 (3H, s), 2.09 (3H, s), 2.03 (3H, s) 2.01 (3H, s), 1.53–1.38 (2H, m). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 172.9, 170.6, 170.3, 169.3, 169.1, 169.0, 113.7, 99.8, 79.2, 74.5, 72.7, 71.9, 71.3, 68.4, 62.0, 40.3, 31.6, 23.9, 20.7, 20.6. ESI-HRMS (positive-ion) calcd for $C_{22}H_{28}O_{12}Na$: $(M+Na)^+$ 507.1478; found: 507.1470.

To a solution of the above glycoside (5.4 mg, 11 μmol) in MeOH (600 μl) was added KCN (8 mM in MeOH, 100 μl, 800 nmol) at room temperature under Ar atmosphere. After stirring for 6 h, the reaction mixture was concentrated in vacuo. The residue was purified by ODS TLC (RP-18W, H_2O/MeOH = 1:1) to afford **27** (1.1 mg, 31%) as a colorless oil: $[\alpha]_D^{18}$ −26.8 (*c* 0.05, MeOH). IR (film) ν 3393, 1738, 1072 cm^{-1}. ^1H NMR (600 MHz, CD_3OD, 40 °C) δ 5.79 (1H, s), 4.91 (1H, ddd, *J*= 1.2, 6.2, 11.8 Hz), 4.41 (1H, d, *J*= 7.9 Hz), 4.05 (1H, tt, *J*= 4.0, 11.2 Hz), 3.87 (1H, dd, *J*= 2.1, 11.8 Hz), 3.66 (1H, dd, *J*=5.6, 11.8 Hz), 3.36–2.26 (3H, m), 3.14 (1H, dd, *J*=7.9, 9.1 Hz), 2.90–2.84 (2H, m), 2.42–2.34 (2H, m), 1.44–1.38 (1H, m), 1.35 (1H, q, *J*=11.8 Hz). ^{13}C NMR (150 MHz, CD_3OD, rt) δ 176.0, 173.9, 113.4, 103.2, 81.6, 78.1, 78.0, 75.0, 74.6, 71.6, 62.8, 42.0, 32.7, 24.8. ESI-HRMS (positive-ion) calcd for $C_{14}H_{20}O_8Na$: $(M+Na)^+$ 339.1056; found: 339.1049.

4.1.17. Acetic acid 3,5-diacetoxy-2-bromo-6-[(9*H*-fluoren-9-ylmethoxycarbonyl amino)-methyl]-tetrahydropyran-4-yl ester 28. Synthesis of **33**, **34**, and **28** is shown in Scheme 7.



Scheme 7. (i) See Ref. 15; (ii) TESOTf, 2,6-lutidine, DMF, 92%; (iii) H_2/Pd–C, CH_2Cl_2; (iv) FmocCl, $NaHCO_3$, H_2O, 1,4-dioxane, 80% (two steps); (v) TFA, THF, H_2O; (vi) Ac_2O, pyridine, 80% (two steps); (vii) $BiBr_3$, TMSBr, CH_2Cl_2.

4.1.17.1. Synthesis of 33. To a solution of **32** (340 mg, 1.66 mmol) and 2,6-lutidine (1.86 ml, 15.9 mmol) in DMF (16 ml) was slowly added TESOTf (1.79 ml, 8.00 mmol) at 0 °C under Ar atmosphere. After stirring for 1 h at 0 °C, to the reaction mixture was added H_2O (2 ml). The aqueous layer was extracted with AcOEt. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 9:1) to afford TES ether (1.03 g, 92%, α:β = 1:1) as a colorless oil: $[\alpha]_D^{24}$ +15.3 (*c* 0.50, $CHCl_3$). IR (film) ν 2878, 2102, 1458, 1414, 1283, 1240 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) δ 5.10 (1H, d, *J*=2.7 Hz, α), 4.47 (1H, d, *J*=6.5 Hz, β), 3.97 (1H, dd, *J*=2.0, 9.4 Hz, α), 3.96–3.91 (1H, m, α), 3.88 (1H, dd, *J*=2.7, 9.4 Hz, β), 3.82 (1H, br s, α), 3.77 (1H, br s, β), 3.63 (1H, dd, *J*=6.5, 9.0 Hz, β), 3.49 (1H, dd, *J*=7.8, 12.4 Hz, α), 3.46–3.34 (4H, m, β), 3.20 (1H, dd, *J*=5.3, 12.4 Hz, α), 1.01–0.92 (72H, m), 0.72–0.62 (48H, m). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 103.2, 94.3, 87.3, 84.3, 78.9, 77.2, 73.9, 72.1, 70.9, 70.1, 54.2, 51.6, 7.14, 7.03, 6.99, 6.90, 6.82, 6.78, 6.72, 6.66, 5.29, 5.15, 5.12, 4.97, 4.92, 4.77, 4.67. ESI-HRMS (positive-ion) calcd for $C_{30}H_{67}N_3O_5Si_4Na$: $(M+Na)^+$ 684.4050; found: 684.4052.

To a solution of the above TES ether (63.7 mg, 94.1 μmol) in CH_2Cl_2 (2 ml) was added palladium on activated carbon (31.7 mg). Hydrogen was admitted via a balloon and the reaction mixture was stirred for 30 min and the catalyst removed by filtration. The filtrate was concentrated in vacuo to afford a crude mixture. To a solution of the mixture and 10% $NaHCO_3$ aq (118 μl, 141 μmol) in 1,4-dioxane (1.5 ml) was added FmocCl (36.5 mg, 141 μmol) at 0 °C. After stirring for 12 h, to the reaction mixture was added H_2O (2 ml). The aqueous layer was extracted with AcOEt. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 9:1) to afford **33** (65.8 mg, 81%, α:β = 3:2) as a colorless oil: $[\alpha]_D^{19}$ +41.2 (*c* 0.50, $CHCl_3$). IR (film) ν 2955,

2912, 2878, 1724, 1514, 1458, 1414, 1240, 1140, 1105, 1055 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) δ 7.76 (4H, d, J=7.4 Hz), 7.59 (4H, d, J=7.4 Hz), 7.40 (4H, t, J=7.4 Hz), 7.30 (4H, t, J=7.4 Hz), 5.11 (1H, d, J=2.5 Hz, α), 5.06 (1H, br d, α), 4.45 (1H, d, J=7.5 Hz, β), 4.41 (1H, dd, J=6.9, 10.4 Hz, α), 4.37 (2H, d, J=6.9 Hz, β), 4.30 (1H, dd, J=6.9, 10.4 Hz, α), 4.21 (2H, t, J=6.9 Hz), 3.98 (1H, dd, J=1.8, 9.7 Hz, α), 4.00–3.88 (1H, m, β), 3.90 (1H, dd, J=2.5, 9.7 Hz, α), 3.85 (1H, br s, α), 3.75 (1H, br s, β), 3.64 (1H, dd, J=7.5, 8.7 Hz, β), 3.54–3.47 (2H, m), 3.40 (1H, br d, β), 3.29–3.22 (2H, m), 0.99–0.93 (72H, m), 0.71–0.56 (48H, m). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 156.5, 144.0, 141.3, 128.7, 127.0, 125.1, 120.0, 99.2, 94.4, 77.2, 75.7, 74.1, 73.9, 73.8, 70.9, 70.6, 70.2, 66.9, 47.3, 42.5, 7.20, 7.07, 6.99, 6.87, 6.73, 6.61, 5.82, 5.43, 5.34, 5.25, 5.18, 4.78. ESI-HRMS (positive-ion) calcd for $C_{45}H_{79}NO_7Si_4Na$: $(M+Na)^+$ 880.4826; found: 880.4828.

4.1.17.2. Synthesis of 34. To a solution of **33** (65.8 mg, 76.6 μmol) in THF/H_2O (2:1, 900 μl) was slowly added TFA (1.5 ml) at 0 °C. After stirring for 1 h at room temperature, the reaction mixture was concentrated in vacuo to afford a crude mixture. To a solution of the mixture in pyridine (1.0 ml) was added Ac_2O (1.0 ml) at room temperature. After stirring for 3 h, the reaction mixture was concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 2:1) to afford **34** (36.6 mg, 84%, α:β = 1:2) as a colorless oil: $[\alpha]_D^{19}$ +35.7 (c 0.50, $CHCl_3$). IR (film) ν 3382, 3020, 1751, 1526, 1450, 1369, 1221, 1074 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) δ 7.77 (4H, d, J=7.2 Hz), 7.58 (4H, d, J=7.2 Hz), 7.41 (4H, t, J=7.2 Hz), 7.32 (4H, t, J=7.2 Hz), 6.37 (1H, br s, α), 5.70 (1H, d, J=8.4 Hz, β), 5.48 (1H, br s, α), 5.40 (1H, d, J=2.7 Hz, β), 5.34 (1H, dd, J=8.4, 9.8 Hz, β), 5.37–5.31 (2H, m, α), 5.08 (1H, dd, J=2.7, 9.8 Hz, β), 5.10–5.06 (1H, m, α), 4.40 (2H, d, J=6.9 Hz, α), 4.38 (2H, dd, J=6.9 Hz, β), 4.22 (2H, t, J=6.9 Hz), 3.89 (1H, t, J=6.8 Hz, β), 3.40–3.27 (4H, m), 2.19 (6H, s), 2.13 (6H, s), 2.05 (6H, s), 2.03 (3H, s, α), 2.01 (3H, s, β). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 170.5, 170.0, 169.9, 169.8, 169.4, 168.9, 156.2, 143.8, 141.3, 127.7, 127.0, 125.0, 120.0, 92.2, 89.6, 77.2, 72.8, 70.8, 69.8, 68.4, 68.0, 67.6, 67.4, 67.0, 66.5, 47.1, 40.5, 20.9, 20.8, 20.6, 20.5. ESI-HRMS (positive-ion) calcd for $C_{29}H_{31}NO_{11}Na$: $(M+Na)^+$ 592.1789; found: 592.1790.

4.1.17.3. Synthesis of 28. To a solution of **34** (27.2 mg, 47.8 μmol) in CH_2Cl_2 (500 μl) were added $BiBr_3$ (0.5 mg, 1.20 μmol) and TMSBr (24.7 μl, 191 μmol) at 0 °C under Ar atmosphere. After stirring for 1 h at 0 °C, to the reaction mixture was added satd $NaHCO_3$ aq. The aqueous layer was extracted with $CHCl_3$. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:1) to afford **28** (24.8 mg, 88%) as an orange oil: $[\alpha]_D^{19}$ +137.8 (c 0.50, $CHCl_3$). IR (film) ν 3379, 3067, 2950, 1749, 1526, 1450, 1371, 1223, 1078 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) δ 7.76 (2H, d, J=7.4 Hz), 7.57 (2H, d, J=7.4 Hz), 7.40 (2H, t, J=7.4 Hz), 7.31 (2H, t, J=7.4 Hz), 6.70 (1H, d, J=3.9 Hz), 5.49 (1H, d, J=2.8 Hz), 5.40 (1H, dd, J=2.8, 10.6 Hz), 5.05 (1H, dd, J=3.9, 10.6 Hz), 4.46–4.36 (2H, m), 4.33 (1H, t, J=6.8 Hz), 4.22 (1H, t, J=6.9 Hz), 3.46–3.27 (2H, m), 2.16 (3H, s), 2.11 (3H, s), 2.02 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 170.2, 170.1, 169.7, 156.2, 143.9, 141.3, 127.7, 127.1, 125.0, 120.0, 88.2, 72.2, 68.1, 67.9, 37.7, 67.0, 47.1, 40.2, 20.7, 20.6. ESI-HRMS (positive-ion) calcd for $C_{27}H_{28}BrNO_9Na$: $(M+Na)^+$ 612.0840; found: 612.0842.

4.1.18. Acetic acid 4,5-diacetoxy-2-[(9*H*-fluoren-9-yl-methoxycarbonylamino)-methyl]-6-(2-oxo-2,4,5,6,7,7a-hexahydro-benzofuran-6-yloxy)-tetrahydro-pyran-3-yl ester 29. A mixture of **25** (4.9 mg, 32.0 μmol), AgOTf (4.1 mg, 16.0 μmol), Ag_2CO_3 (18.4 mg, 67.0 μmol) and dried molecular sieves 4Å (15.2 mg) in anhydrous CH_2Cl_2 (200 μl) was stirred at 0 °C under Ar atmosphere for 1 h. Then a solution of **28** (26.5 mg, 44.9 μmol) in anhydrous CH_2Cl_2 (200 μl) was added to the stirred mixture. After stirring for 1 h, the reaction mixture was diluted with $CHCl_3$ and filtered through Celite. The filtrate was washed with satd $NaHCO_3$ aq and brine. The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:5) to afford **29** (10.1 mg, 48%) as a colorless oil: $[\alpha]_D^{20}$ −2.25 (c 0.20, $CHCl_3$). IR (film) ν 2920, 2851, 1720, 1649, 1529, 1450, 1369, 1225 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) δ 7.75 (2H, d, J=7.5 Hz), 7.57 (2H, d, J=7.5 Hz), 7.39 (2H, t, J=7.5 Hz), 7.31 (2H, t, J=7.5 Hz), 5.76 (1H, s), 5.32 (1H, d, J=3.0 Hz), 5.15 (1H, t, J=8.1 Hz), 5.00 (1H, dd, J=3.0, 8.1 Hz), 4.69 (1H, dd, J=5.9, 11.3 Hz), 4.54 (1H, d, J=8.1 Hz), 4.40 (2H, d, J=6.6 Hz), 4.20 (1H, t, J=6.6 Hz), 3.84–3.65 (2H, m), 3.47 (1H, td, J=6.9, 13.7 Hz), 3.09 (1H, td, J=6.9, 13.7 Hz), 2.89–2.80 (2H, m), 2.32–2.10 (2H, m), 2.18 (3H, s), 2.02 (3H, s), 1.99 (3H, m), 1.45–1.22 (2H, m). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 172.9, 171.1, 170.0, 169.2, 169.1, 156.4, 143.7, 141.3, 127.7, 127.1, 125.0, 120.0, 113.6, 100.3, 79.2, 74.3, 71.5, 70.8, 69.1, 67.8, 67.0, 47.1, 40.4, 40.3, 31.4, 23.8, 20.7, 20.6, 20.6. ESI-HRMS (positive-ion) calcd for $C_{35}H_{37}N_1O_{12}Na$: $(M+Na)^+$ 686.2208; found: 686.2207.

4.1.19. [3,4,5-Trihydroxy-6-(2-oxo-2,4,5,6,7,7a-hexahydro-benzofuran-6-yloxy)-tetrahydro-pyran-2-ylmethyl]-carbamic acid 9*H*-fluoren-9-ylmethyl ester 30. To a solution of **29** (5.1 mg, 7.7 μmol) in MeOH (150 μl) was added KCN (8 mM in MeOH, 100 μl, 800 nmol) at room temperature under Ar atmosphere. After stirring for 4.5 h, the reaction mixture was concentrated in vacuo. The residue was purified by ODS TLC (RP-18W, H_2O/MeOH = 1:4) to afford **30** (1.7 mg, 41%) as a colorless oil: $[\alpha]_D^{18}$ +21.4 (c 0.20, MeOH). IR (film) ν 3358, 2926, 2361, 1705, 1533, 1448, 1261, 1069 cm^{-1}. ^1H NMR (600 MHz, CD_3OD, rt) δ 7.78 (2H, d, J=7.5 Hz), 7.65 (2H, d, J=7.5 Hz), 7.38 (2H, t, J=7.5 Hz), 7.33 (2H, t, J=7.5 Hz), 5.78 (1H, s), 4.70 (1H, dd, J=6.0, 11.3 Hz), 4.41 (2H, d, J=6.6 Hz), 4.31 (1H, d, J=7.0 Hz), 4.21 (1H, t, J=6.6 Hz), 3.94 (1H, tt, J=3.6, 11.2 Hz), 3.69 (1H, d, J=2.4 Hz), 3.52–3.49 (1H, m), 3.45–3.37 (4H, m), 3.32–3.27 (2H, m), 2.86–2.80 (2H, m), 2.33–2.28 (2H, m), 1.40–1.28 (2H, m). ^{13}C NMR (150 MHz, CD_3OD, rt) δ 175.9, 173.7, 159.2, 145.4, 145.3, 142.7, 128.8, 128.2, 126.2, 126.1, 121.4, 120.9, 114.2, 113.4, 112.6, 104.0, 81.5, 75.0, 74.8, 74.7, 72.3, 70.4, 67.7, 49.6, 42.4, 42.0, 32.8, 24.8. ESI-HRMS (positive-ion) calcd for $C_{29}H_{31}N_1O_9Na$: $(M+Na)^+$ 560.1891; found: 560.1892.

4.1.20. Biologically inactive fluorescence-labeled probe 31. To a solution of **30** (3.5 mg, 6.5 μmol) in DMF

(720 μl) was added piperidine (4.8 μl, 49 mol) at 0 °C under Ar atmosphere. After stirring for 2 h at 0 °C, piperidine was removed in vacuo. To the reaction mixture was added AMCA-X, SE (3.1 mg, 7.2 μmol) at room temperature under Ar atmosphere. After stirring for 2 h, the reaction mixture was concentrated in vacuo. The residue was purified by ODS TLC (RP-18W, H_2O/MeOH = 1:1) and HPLC with COSMOSIL 5C_{18}-AR column (ϕ20.0×250 mm, H_2O/CH_3CN = 6:4) to afford **31** (1.3 mg, 62% in two steps) as a colorless oil: $[\alpha]_D^{21}$ +6.6 (*c* 0.05, MeOH). IR (film) ν 3348, 2924, 1734, 1652, 1558, 1057 cm^{-1}. ^1H NMR (600 MHz, CD_3OD, 40 °C) δ 7.48 (1H, d, *J*=8.8 Hz), 6.65 (1H, dd, *J*=2.2, 8.8 Hz), 6.51 (1H, d, *J*=2.2 Hz), 5.74 (1H, s), 4.77 (1H, dd, *J*=5.5, 11.1 Hz), 4.33 (1H, d, *J*=7.6 Hz), 3.99 (1H, tt, *J*=4.0, 11.5 Hz), 3.72 (1H, d, *J*=1.4 Hz), 3.58 (1H, dd, *J*=5.4, 7.2 Hz), 3.52 (2H, s), 3.49–3.45 (3H, m), 3.35 (1H, dd, *J*=7.8, 13.7 Hz), 3.18 (2H, t, *J*=7.5 Hz), 2.88–2.84 (2H, m), 2.37 (3H, s), 2.41–2.32 (2H, m), 2.21 (2H, t, *J*=7.5 Hz), 1.62 (2H, quintet, *J*=7.5 Hz), 1.52 (2H, quintet, *J*=7.5 Hz), 1.42 (1H, dd, *J*=5.0, 11.7 Hz), 1.36 (2H, t, *J*=7.5 Hz), 1.34 (1H, m). ^{13}C NMR (150 MHz, CD_3OD, 40 °C) δ 176.7, 175.9, 173.8, 173.0, 164.8, 155.9, 154.0, 152.9, 127.4, 114.4, 113.4, 113.2, 111.8, 104.1, 100.5, 81.6, 75.4, 74.7, 74.3, 72.3, 70.5, 42.0, 41.3, 40.3, 37.0, 35.2, 32.9, 30.0, 27.5, 26.7, 24.3, 15.4. ESI-HRMS (positive-ion) calcd for $C_{32}H_{41}N_3O_{11}Na$: $(M+Na)^+$ 666.2633; found: 666.2635.

4.1.21. Bioassay. The young leaves detached from the stem of the plant *P. urinaria* L., which was grown in the biotron of Tohoku University, with a sharp razor blade were used for bioassay. One leaf was placed in H_2O (ca. 300 μL) using a 5-ml glass tube in the greenhouse kept at 32 °C and allowed to stand overnight. The leaves, which opened again in the morning were used for the bioassay. Each test solution was carefully poured into test tubes with a microsyringe around 10:00 a.m. The bioactive fraction was judged by the leaf-closing activity before the leaf-closing of the plant leaf in the blank solution containing no sample. Other nyctinastic plants, *M. pudica*, *C. mimosoides*, and *L. leucocephala*, used in bioassay were also grown in the biotron of Tohoku University.

4.1.22. Fluorescence study using a fluorescence microscope. The leaf of *P. urinaria* L. opening in the morning was cut in an appropriate size and fixed in agar. The agar was sliced perpendicular to the petiole by a microslicer (DSK-1000, Dousaka EM Co., Ltd) to a thickness of 30 μm and the sections containing the pulvini were floated on distilled water. The sections were immersed in a solution containing various concentration of fluorescence-labeled probe compound, and allowed to stand for staining under shielded condition at room temperature for 6 h. After staining, the sections were washed by being incubated with equilibration buffer (Slow Fade™ Gold Antifade Reagent, Molecular Probes Inc.) for 15 min. This section was placed on a slide glass and covered by a cover glass after adding a drop of antifade reagent (Slow Fade™ Gold Antifade Reagent, Molecular Probes Inc.). The observation of these sections was carried out by using ECLIPSE E800 microscope (Nicon) equipped with VFM fluorescence instrument. B-2A filter (Nicon Co., Ltd; excitation wavelength 450–490 nm) was used against AMCA. The plant sections of other nyctinastic plants, *M. pudica*, *C. mimosoides*, and *L. leucocephala* were prepared and treated with fluorescence-labeled probe compound in the same procedure.

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