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FUNCTIONAL 1,3-BENZODIOXOLES MAKING A FLUORESCENT RESPONSE TO LOCAL TRANSFORMATION IN CHEMO-REACTIVE PENDANT GROUPS

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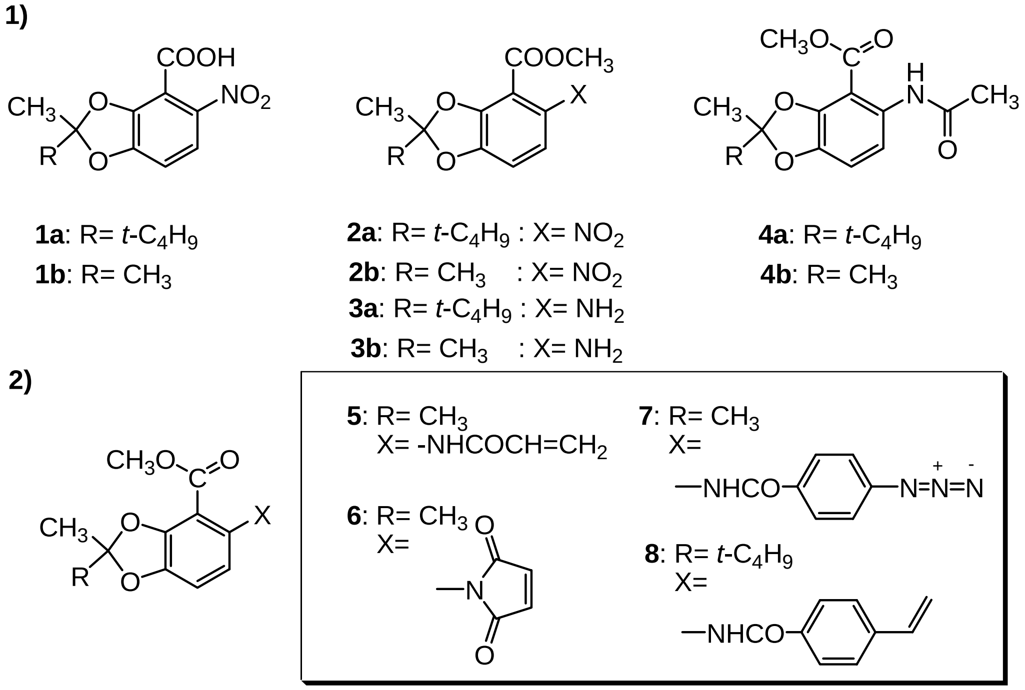
Abstract – From bi-functional methyl 5-nitro-1,3-benzodioxole-4-carboxylates designed as artificial β-amino acid homologues, a series of chemo-functional 5-amide derivatives (acrylamide, maleimide, 4-azidobenzamide and 4-vinylbenzamide) were prepared. These functional compounds were non-fluorescent while they all gave fluorescent products in the corresponding thiol coupling, photo-activated transformation, hydrogenation, and polymerization reations.

INTRODUCTION

1,3-Benzodioxoles occur widely in plant products, some of which are known to possess potent anti-oxidant and antibacterial activities.[1] Judging from the natural abundance, they may have high potential in applications to chemicals, medicines, and others.[2] Although the 1,3-benzodioxole skeleton itself is achiral, we have previously reported a concept of asymmetric 1,3-benzodioxoles.[3] This is based on the unique structure of 2-substituted 1,3-benzodioxoles which afford prochiral positions in the aromatic ring. Thus, aromatic substitution reactions (C-4 and C-5 positions) give the corresponding asymmetric 1,3-benzodioxoles having a stereogenic centre at the C-2 position. The 1,3-benzodioxole skeleton is fluorescent, although the emission and excitation wavelengths vary depending on the aromatic substituting groups.[4] The chiral and fluorescent properties are of major advantage for functional 1,3-benzodixoles in applications for analytical purposes.

In our preceding study,[4] we extended the asymmetric 1,3-benzodioxole to bi-functional 5-nitro-1,3-benzodioxole-4-carboxylates, such as compound (**1a**) and its optically pure forms [(*S*)- and

(*R*)-**1a**]. We expect that the designed bi-functional compounds, possessing carboxyl and nitro (amino) groups as well as the side group at the C-2 position, constitute a novel class of β-amino acid homologues. In addition to their potential as analytical and biological reagents, the bi-functional skeleton may serve as a functional linker useful for synthetic purposes. In the present study, we prepared a series of chemo-functional amide derivatives (**5**-**8**) (acrylamide, maleimide, 4-azidobenzamide, and 4-vinylbenzamide). We describe in this paper that these chemo-functional groups quench fluorescence, whereas they trigger the fluorescence upon the corresponding chemical transformation. The observed property bestows a major advantage upon these 1,3-benzodioxole-based β-amino acid homologues toward practical applications.



Scheme 1 1,3-Benzodioxole-based β-amino acid homologues (**1a**) and (**1b**) and chemo-functional 1,3-benzodioxoles (**5**-**8**) examined in this study.

RESULTS AND DISCUSSION

In a preceding study,[4] we have observed that 5-nitro compounds (**1a**) and (**2a)** are non-fluorescent, while 5-amino and 5-acetamido derivatives (**3a**) and (**4a**) emit fluorescence. The fluorescence quantum yield was close (90-95%) to that of quinine sulfate, which enables us sub-pico molar detection with FL-spectroscopy.[5] ^{1}H-NMR spectral and X-Ray diffraction studies[6] have indicated that the 5-acetamido compound (**4a**) takes a coplanar conformation at the amide linkage. The existence of an intra-molecular hydrogen bond is predicted between 5-NH and 4-C=O positions. These results support that these β-amino acid homologues will be conveniently applied for assembly of various kinds of fluorescent compounds. To verify this assumption, we prepared the chemo-functional 5-amido compounds (**5**-**8**).

The amide compounds (**5-8**) were prepared *via* condensation of the 5-amino derivatives (**3a**) and (**3b**) with the corresponding carbonyl reagents (Scheme 1). Compounds (**5**) and (**6**) have α,β-unsaturated carbonyl groups (acrylamide and maleimide) which will react with –SH compounds[7] to afford the corresponding 1,4-adducts. The 4-azidophenyl group in **7** is photo-reactive,[8] while the terminal –C=C– bond in **5** and **8** can be used for radical polymerisation and co-polymerisation. Most of these amide derivatives were prepared from the 2,2-dimethyl compound (**1b**) except for **8**. For evaluating fundamental utility, the use of achiral compound (**1b**) seems appropriate despite that chiral products derived from **1a** may be of higher value with the respect of biological recognition and transformation. For these functional amide derivatives, the 4-carboxyl group was protected persistently with methyl ester.

Judging from fluorescent spectroscopy, compounds (**4a**) and (**4b**) have no difference in the fluorescent and conformational properties each other. Evidently, the C-2 alkyl groups have no substantial effect on these properties. Although the derived functional amides (**5-8**) except for the tertiary amide derivative (**6**) are considered to hold the similar conformational property, they emitted little fluorescence (< 10 % intensity to the fluorescence of **4a** and **4b** in MeOH). Obviously, these chemo-functional groups quench the 1,3-benzodioxole-based fluorescence. Maleimide is known to quench diverse fluorogens,[9-11] and this property is of marked advantage for analytical applications. This is because they usually turn to be fluorescent upon reactions with –SH compounds. Indeed, a solution of **5** in CH_3CN began to emit fluorescence upon a reaction with *N*-acetyl-L-cysteine. From the reaction mixture, a fluorescent adduct (**9**) (FAB-MS: $[M+H]^+ = 441$) was isolated as a major product (TLC detection) (Figure 2). In a similar way, **6** afforded a fluorescent 1,4-adduct (**10**) (FAB-MS: $[M+H]^+ = 467$).

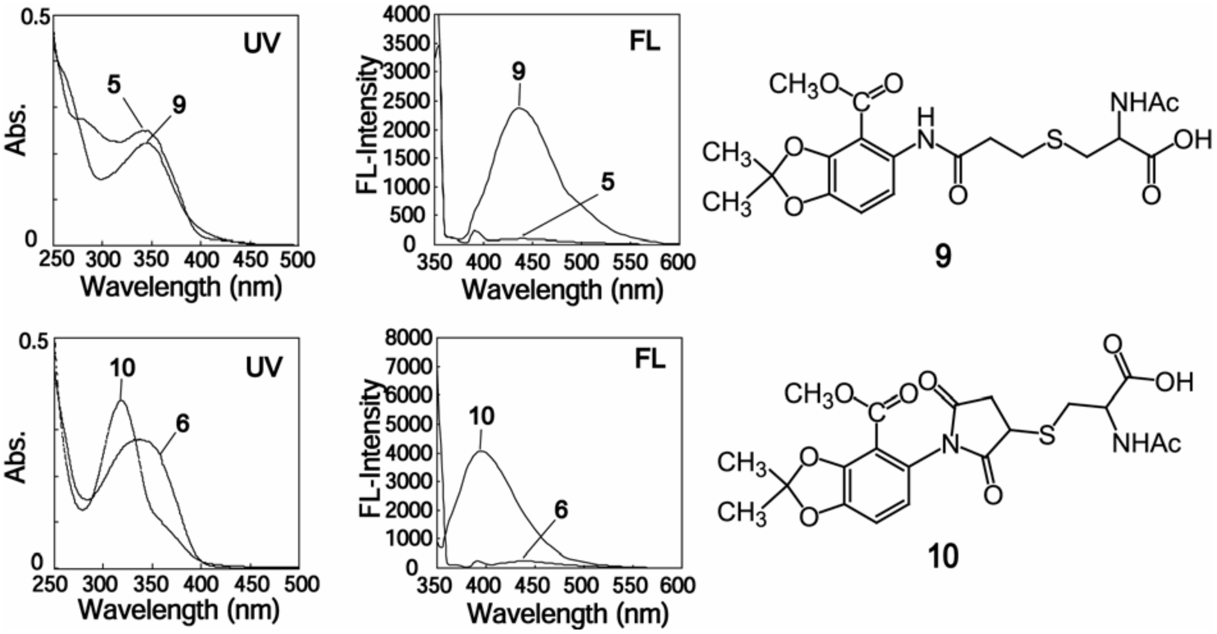


Figure 2 UV and FL-changes of **5** and **6** upon the treatment with *N*-acetyl-L-cysteine and the major structures of L-cysteine adducts (**9** and **10**).

As can be seen in Figure 2, **10** emitted fluorescence in a shorter wavelength region (Ex. 320 nm, Em. 400 nm) than **9** (Ex. 350 nm, Em. 445 nm). A similar blue shift was observed also in their UV spectra. This shift is considered to reflect the coplanar and the non-coplanar conformations of **9** and **10**, respectively, at the 5-amide moiety relative to the aromatic face. It is also notable that the UV spectroscopy gives a marked difference between **6** and **10**. In literatures,[7,11] it is generally accepted that the maleimide group quenches fluorescence as the result of extending the π-electron conjugation of the linked fluorophore. The reaction with a –SH compound destabilises the π-bonding orbital to the level allowing radiating relaxation during π* to π transition. The blue shift in the UV of **10** accords with this de-conjugation mechanism. In the case of **9**, which has shown no blue shift in the UV, the fluorescence recovery may be due to an alternative mechanism such as a loss of rotating –C=C- bond at the terminal.

When a human hair was treated with 5-amino (**3b**) or 5-acrylamide compound (**5**), the hair was stained in a different way each other (Figure 3). The 5-amino compound stained the hair rod non-selectively, while the 5-acylamide compound stained a part of the surface. We assume that **5** stained cuticle proteins on the hair surface through coupling with L-Cys (SH) residues.

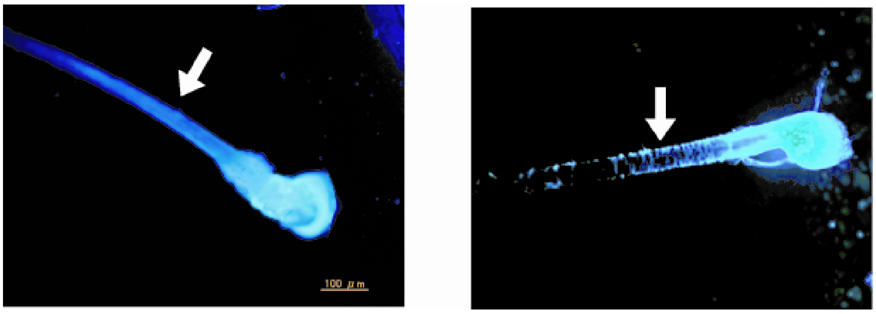


Figure 3 Fluorescence staining of human hairs with **3b** (left) and **5** (right) [5% solution of each 1,3-benzodioxole in ethanol was applied to a human hair immersed in water.]

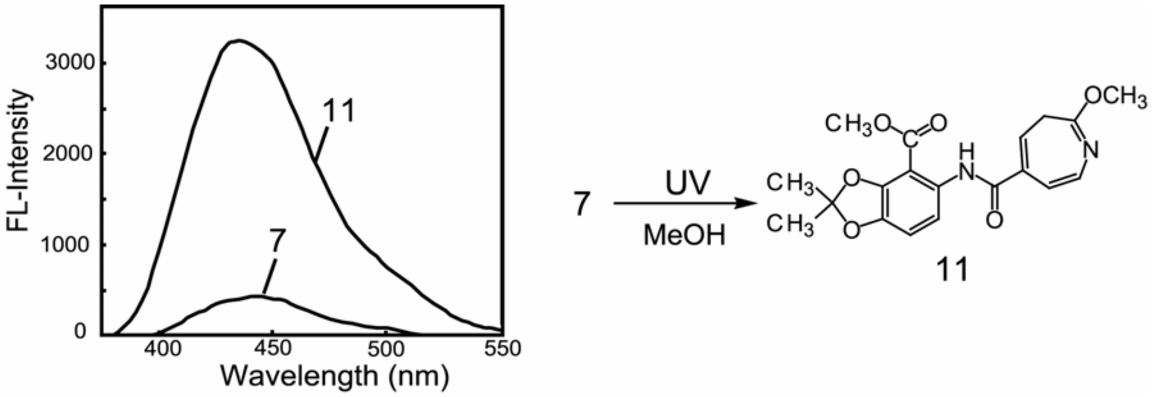


Figure 4 FL-change of **7** under photo-irradiation (left, Ex. 350 nm) and the structure of a major fluorescent product (**11**) (right) [For spectroscopic data of **11**, see Experimental]

It is reported that the 4-azidophenyl group introduced in **7** affords 2-aminoazepines in photo-activating reactions with amines.[12] When the little fluorescent (**7**) in methanol was photo-irradiated (Xe-lamp, 400W), the solution began to emit blue fluorescence. A major fluorescent product was isolated with silica gel chromatography and identified to be 2-methoxyazepine derivative (**11**) (FAB-MS: $[M]^+ = 372$) (Figure 4). This means that the 4-azidobenzamide derivative (**7**) provides one of the very few skeletons affording photo-reactive FL-labelling reagents.[13]

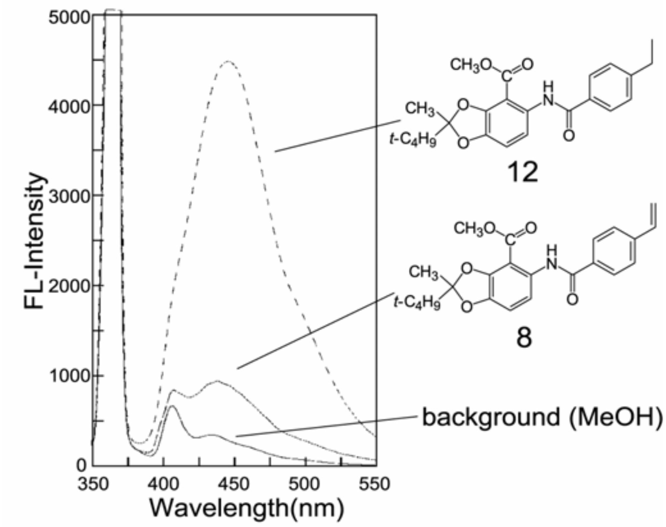
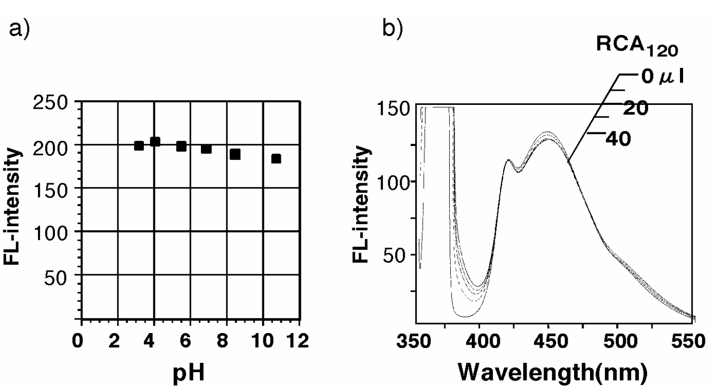


Figure 5 Fluorescence recovery of styrene derivative (**8**) by catalytic hydrogenation (Ex. 360 nm, in MeOH)



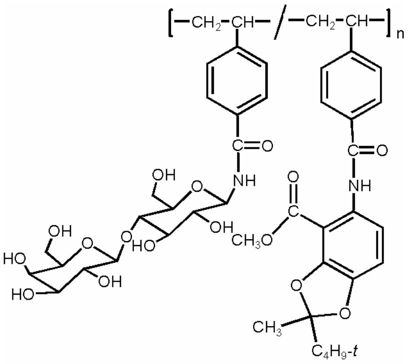


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Figure 6 Effects of (a) pH and (b) lactose binding protein [RCA_{120} in phosphate buffer (pH 7.0)] on the fluorescence of lactose-conjugated copolymer (**14**).

In the case of 4-vinylbenzamide derivative (**8**), the fluorescence was recovered by catalytic hydrogenation to afford a fluorescent 5-(4-ethylbenzoyl)amide derivative (**12**) (Figure 5). Similarly to the case of **5** having a terminal–C=C- bond, the 4-vinyl group plays a determining role in quenching and triggering the fluorescence. The styrene compound (**8**) is potentially applicable to polymer reactions to afford fluorescent polymers and copolymers. To verify this expectation, compound (**8**) was co-polymerized with a carbohydrate vinyl-monomer (**13**) (1-*N*-*p*-vinylbenzamido-β-lactoside)[14] in a molar ratio (**8**/**13**) of 2.5:100. The reaction afforded a lactose-embedded copolymer (**14**) ($Mn = 2 \times 10^4$), which was soluble in water and fluorescent (Ex. 360 nm, Em. 450 nm). Figure 5 shows that the fluorescence was stable in a wide pH range even in the presence of a carbohydrate binding protein (RCA_{120}:β-galactopyranoside, binding constant (*Ka*) = 2.0 x10^5 M^{-1}). The stable fluorescence seems characteristic of the 1,3-benzodioxoles bearing no ionic auxo-chrome, and this property will have practical value when applied to quantitative analysis and fluorescent microscopy.

SUMMARY

We have demonstrated that the bi-functional 1,3-benzodixoles (**1a**) and (**1b**) can afford various types of chemo-functional amides, which include acrylamide, maleimide, 4-azidobenzamdie, and 4-vinylbenzamide derivatives. Although these amides are non-fluorescent, they were found to afford fluorescent products upon the corresponding reaction and transformation. This means that these chemo-functional groups serve as fluorescence triggers. This property will be useful for determining chemical or biological reactants responsible for the conversion, nevertheless, little is known to the underlying mechanism. Empirically, the rotating –C=C- and –N=N-N- bonds at the terminal seems to have a quenching effect on the 1,3-benzodioxole fluorophore.

In the present study, the 4-carboxyl position was protected in a form of methyl ester. The fluorescence of 1,3-benzodioxoles is little affected by the minor structural changes at the 4-carbonyl position such as 4-carboxylic acid, 4-carboxy ester, and 4-carbamide. This fact allows us to expect that various types of bio- and chemo-functional group can be introduced without affecting the fluorescent property. Along with our continuous studies on the design and biological applications of artificial glycoconjugates,[15] we are trying to establish a fluorescent glycosylation methodology. For this purpose, we are applying the present skeleton to the assembly of fluorescent glycosyl donors. The study along this line will be reported elsewhere.

EXPERIMENTAL

Materials and Methods.

^{1}H NMR spectra were recorded on Varian INOVA-500 (500 MHz) and Varian Mercury-300 (300 MHz) at ambient temperature in $CDCl_3$ or D_2O using TMS as an internal standard. FT-IR (JASCO FT-IR-230), UV (JASCO V-530), FL (JASCO FP777), and MS spectra (JAOL JMS-700) were recorded in conventional ways at room temperature. Nitronium tetrafluoroborate (0.5 M solution in sulfolane) was purchased from Aldrich Chemical Company Inc. Hexafluoropropene-diethylamine complex (HFPDA) was purchased from Tokyo Kasei Kogyo (Japan). 2-*tert*-Butyl-2-methyl-5-nitro-1,3-benzodioxole-4-carboxylic acid (**1a**),[4] methyl 2-*tert*-butyl-2-methyl-5-nitro-1,3-benzodioxole-4-carboxylate (**2a**),[4] methyl

5-acetamido-2-*tert*-butyl-2-methyl-1,3-benzodioxole-4-carboxylate (**4a**),[4] and 2,2-dimethyl-1,3-benzodioxole carboxylic asid[16] were prepared by reported methods from 3-methylcatechol. 4-*O*-(β-D-Galactopyranosyl)-*N*-(4-vinylbenzoyl)-β-D-glucopylanosylamine (**13**)[11] was prepared by reported ways. The other chemicals and solvents were commercially available and used without further purification.

2,2-Dimethyl-5-nitro-1,3-benzodioxole-4-carboxylic acid (1b)

A solution of 2,2-dimethyl-1,3-benzodioxole-4-carboxylic acid (5.0 g, 26 mmol) in CH_3CN (100 mL) was stirred at 0 °C under nitrogen atmosphere for 15 min. NO_2BF_4 (0.5 M in sulfolane (61.8 mL), 30.9 mmol) was added and the solution was stirred at the same temperature for 1 h. The mixture was poured into water (200 mL), and extracted with chloroform. The combined extracts were washed with water and dried over $MgSO_4$. After filtrating and evaporating, the residue was purified by silica gel column chromatography (chloroform-methanol-acetic acid, 100: 2: 3) to afford 5-nitro compound (**1b**) (3.38 g, 55 %) as a pale yellow crystal togather with 6-nitro compound (1.44 g, 23 %) as a pale yellow crystal.

5-Nitro compound (**1b**): mp 192-193 °C; ^{1}H NMR (500 MHz) δ ppm: 1.79 (6H, s, 2Me), 6.82 (1H, d, *J*= 8.5 Hz, H-7), 7.71 (1H, d, *J*= 8.5 Hz, H-6); Anal. Calcd for $C_{10}H_9NO_6$: C, 50.22; H, 3.79; N, 5.86. Found: C, 50.22; H, 4.03; N, 5.62; HR-MS (FAB): calcd for $C_{10}H_{10}NO_6$ $[M+H]^+$: 240.0509; found 240.0520

6-Nitro compound: mp 253-254 °C; ^{1}H NMR (500 MHz) δ ppm: 1.818 (6H, s, 2Me), 7.707 (1H, d, *J*= 2.5 Hz, H-7), 8.443 (1H, d, *J*= 2.5 Hz, H-6): HR-MS(FAB): calcd for $C_{10}H_{10}NO_6$ $[M+H]^+$: 240.0509; found 240.0512

Methyl 2,2-dimethyl-5-nitro-1,3-benzodioxole-4-carboxylate (2b)

To the solution of compound (**1b**) (336 mg, 1.40 mmol) in CH_2Cl_2 (10 mL) was added HFPDA (600 mg, 1.40 mmol) and stirred at rt for 1 h. The solution was cooled to 0 ˚C, and treated with a mixture of triethylamine (1.08 mL, 7.8 mmol) and MeOH (211 μL, 5.2 mmol). The mixture was washed with 0.5 N HCl and water, and dried over $MgSO_4$. After filtrating and evaporating, the residue was purified by silica gel column chromatography (toluene-ethyl acetate, 10: 1 (v/v)) to afford methyl ester (**2b**) (267 mg, 75 %) as a yellow solid. mp 63 °C; ^{1}H-NMR (500 MHz) δ ppm: 1.783 (6H, s, 2Me), 3.964 (3H, s, COO$\underline{Me}$), 6.808 (1H, d, *J* = 8.5 Hz, H-7), 7.741 (1H, d, *J* = 8.5 Hz, H-6). HR-MS(FAB): calcd for $C_{11}H_{12}NO_6$ $[M+H]^+$: 254.0664; found 254.0655

Methyl 5-acetamido-2,2-dimethyl-1,3-benzodioxole-4-carboxylate (4b)

Compound (**2b**) (50 mg, 0.197 mmol) was hydrogenated in methanol (8 mL) in the presence of 20 %

$Pd(OH)_2/C$ (5 mg) at rt under atmospheric pressure for 3 h. The reaction mixture was filtrated and treated with acetic anhydride (0.5 mL, 5.28 mmol) at rt for 15 min. After evaporating, the residue was purified by silica gel column chromatography (toluene-ethyl acetate, 10:1 (v/v)) to afford compound (**4b**) (60.5 mg, 98%) as a pale yellow solid. mp 163°C; ^1H NMR (500 MHz) δ ppm: 1.699 (6H, s, 2Me), 2.188 (3H, s, Ac), 3.929 (3H, s, COOMe), 6.848 (1H, d, *J*= 8.5 Hz, H-7), 8.058 (1H, d, *J*= 9.0 Hz, H-6), 10.633 (1H, br s, -NH-); HR-MS(FAB): calcd for $C_{13}H_{15}NO_5$ $[M]^+$: 265.0950; found 265.0959

Methyl 5-acrylamido-2,2-dimethyl-1,3-benzodioxole-4-carboxylate (5)

Compound (**2b**) (50 mg, 0.197 mmol) was hydrogenated in methanol (8 mL) in the presence of 20 % $Pd(OH)_2/C$ at rt under atmospheric pressure for 3 h. After fitrating and evaporating, the residue and triethylamine (123 µL, 0.883 mmol) in CH_2Cl_2 (5 mL) was stirred for 15 min at 0 °C. Acryloyl chloride (24 µL, 0.29 mmol) was added, and the solution was stirred for 1 h, washed with *sat.* $NaHCO_3$ and water, and then dried over $MgSO_4$. The filtrate was concentrated and purified by silica gal column chromatography (toluene-ethyl acetate,10:1 (v/v)) to afford compound (**5**) (42.0 mg, 76.8 %) as a pale yellow solid. mp 118 °C; ^1H NMR (500 MHz) δ ppm: 1.703 (6H, s, 2Me), 3.934 (3H, s, -COOMe), 5.745 (1H, dd, *J*= 1.5 and 10.0 Hz), 6.274 (1H, dd, *J*= 10.0 and 17.0 Hz), 6.376 (1H, dd, *J*= 1.0 and 17.0 Hz), 6.877 (1H, d, *J*= 8.5 Hz, H-7), 8.217 (1H, d, *J*= 8.5 Hz, H-6), 10.960 (1H, bread s, -NH-); Anal. Calcd for $C_{14}H_{15}NO_5$: C, 60.64; H, 5.45; N, 5.05. Found: C, 60.95; H, 5.64; N, 4.88; HR-MS(FAB): calcd for $C_{14}H_{15}NO_5$ $[M]^+$: 277.0950; found 277.0953

Methyl 5-maleimide-2,2-dimethyl-1,3-benzodioxole-4-carboxylate (6)

Compound (**2b**) (33 mg, 0.130 mmol) was hydrogenated in methanol (5 mL) in the presence of 20 % $Pd(OH)_2/C$ at rt under atmospheric pressure for 3 h. After filtrating and evaporating, the residue was dissolved in CH_2Cl_2 (5 mL), treated with maleic anhydride (26 mg 0.265 mmol) for 2 h and then concentrated. The residue was dissolved in acetic anhydride (2 mL) and a small amount of sodium acetate was added. After 10 h, the mixture was quenched with methanol (20 mL) for 30 min and concentrated. The residue was purified by silica gel column chromatography (toluene-ethyl acetate,30:1 (v/v)) to afford compound (**6**) (25 mg, 63 %) as a yellow solid. mp 159 °C; ^1H NMR (500 MHz) δ ppm: 1.742 (6H, s, 2Me), 3.778 (3H, s, -COOMe), 6.692 (1H, d, *J*= 8.0 Hz, H-7), 6.848 (2H, s, maleimide), 6.883 (1H, d, *J*= 8.0 Hz, H-6) HR-MS(FAB): calcd for $C_{15}H_{13}NO_6$ $[M]^+$: 303.0743; found 303.0770

1,4-Addition reaction of 5 and 6 with *N*-acetyl-L-cysteine

To the solution of **5** (5.7 mg, 0.021 mmol) in CH_3CN (2 mL) was added *N*-acetyl-L-cysteine (6.9 mg, 0.042 mmol). The mixture was stirred at rt for 3 h, and an aliquot of the reaction mixture was diluted with CH_3CN and subjected to fluorescence and UV measurements (see Figures in the main text). The remaining mixture was purified by preparative TLC (chloroform-methanol-acetic acid, 100: 3: 5) to give fluorescent compound (**9**) (1.5 mg, 16 %). HR-MS(FAB): calcd for $C_{19}H_{25}N_2O_8S$ $[M+H]^+$: 441.1332; found: 441.1296

N-Acetyl-L-cysteine adduct of compound (**6**) was prepared by the same procedure. (2.5 mg, 29 %); HR-MS(FAB): calcd for $C_{20}H_{23}N_2O_9S$ $[M+H]^+$: 467.1124; found: 467.1122

Methyl 5-(*p*-azidobenzamido)-2,2-dimethyl-1,3-benzodioxole-4-carboxylate (7)

Compound (**2b**) (50 mg, 0.197 mmol) was hydrogenated in methanol (8 mL) in the presence of 20 % $Pd(OH)_2/C$ (5 mg) at rt under atmospheric pressure for 3 h. After filtrating and evaporating, the residue was dissolved in DMF (5 mL). 4-Azidobenzoic acid (38.5 mg, 0.236 mmol), HATU (89.8 mg, 0.236 mg) and diisopropylamine (120 μL, 0.708 mmol) were added, and the solution was stirred under nitrogen atmosphere for 8 h. The mixture was diluted with ethyl acetate (50 mL), washed with 0.5 N HCl, *sat.* $NaHCO_3$ and water, and then dried over $MgSO_4$. The filtrate was concentrated and purified by silica gel column chromatography (toluene-ethyl acetate, 10:1 (v/v)) to afford compound (**7**) (52.7 mg, 70.0 %) as a pale yellow solid. mp 128 °C; ^1H NMR (500 MHz) δ ppm: 1.740 (6H, s, 2Me), 3.983 (3H, s, -COOMe), 6.941 (1H, d, *J*= 8.5 Hz, H-7), 7.164 (2H, d, *J*= 9.0 Hz, Ar-H), 8.040 (2H, d, *J*= 9.0 Hz, Ar-H), 8.344 (1H, d, *J*= 9.0 Hz, H-6), 11.730 (1H, br s, -NH-); Anal. Calcd for $C_{18}H_{16}NO_5$: C, 58.69; H, 4.38; N, 15.21. Found: C, 59.94; H, 4.94; N, 14.39; IR (KBr): 3297 (-NHCO-), 2121 (azido), 1679 (CO)

Photoreaction of 7

A solution of compound (**7**) (10 mg, 0.0261mmol) in MeOH (4 mL) was stirred under a UV lamp (254 nm) for 10 h. The solution was concentrated and purified by silica gel column chromatography (toluene-ethyl acetate, 15:1 (v/v)) to afford compound (**11**) (6 mg, 59 %). ^1H NMR (500 MHz) δ ppm: 1.723 (6H, s, 2Me), 2.820 (2H, d, *J*=7.0 Hz), 3.776 (3H, s, OMe), 3.965 (3H, s, -COOMe), 6.290 (1H, t, *J*=7.0 Hz), 6.518 (1H, dd, *J*= 1.0 and 8.5 Hz), 6.892 (1H, d, *J*= 9.0 Hz, H-7), 7.214 (1H, d, J= 8.5 Hz), 8.217 (1H, d, *J*= 8.5 Hz, H-6), 11.187 (1H, br s, -NH-); HR-MS(FAB): calcd for $C_{19}H_{20}N_2O_6$ $[M]^+$ 372.1321; found: 372.1320

Methyl 5-*p*-vinylbenzamido-2-*tert*-butyl-2-methyl-1,3-benzodioxole-4-carboxylate (8)

Compound (**2a**) (130 mg, 0.440 mmol) was hydrogenated in methanol (10 mL) in the presence of 20 % $Pd(OH)_2/C$ (5 mg) for 2 h. After filtrating, *p*-vinylbenzoyl chloride (146.7 mg, 0.880 mmol) and triethylamine (960 μL, 6.9 mmol) in CH_2Cl_2 (5 mL) were added and the solution was stirred for 3 h. After concentrating, the residue was purified by silica gel column chromatography (toluene-ethyl acetate, 7:1

(v/v)) to afford **8** (87 mg, 50 %) as yellow syrup. ^{1}H-NMR (500 MHz) δ ppm: δ 1.078 (9H, s, *t*-Bu), 1.596 (3H, s, Me), 3.953 (3H, s, COOMe), 5.363 (1H, dd, *J* = 0.5 and 11.0 Hz), 5.858 (1H, dd, *J* = 1.0 Hz and 17.5 Hz), 6.767 (1H, dd, *J* = 11.0 and 17.5 Hz), 6.905 (1H, d, *J* = 8.5 Hz, H-7), 7.531 (2H, d, *J* = 8.0 Hz, ArH), 7.980 (2H, d, *J*= 8.5 Hz, ArH), 8.305 (1H, d, *J* = 8.5 Hz, H-6), 11.674 (1H, br s, -NH-); HR-MS(FAB): calcd for $C_{23}H_{25}NO_5$ $[M]^+$: 395.1733; found 395.1720

Methyl 5-(4-ethylbenzoyl)amido-2-*tert*-butyl-2-methyl-1,3-benzodioxole-4-carboxylate (12)

Compound (**8**) (50 mg, 0.126 mmol) was hydrogenated in methanol (8 mL) in the presence pf 20 % $Pd(OH)_2/C$ (5 mg) at rt under atmospheric pressure for 3 h. After fitrating and evaporating, the residue was purified by silica gel column chromatography (toluene-ethyl acetate,10:1 (v/v)) to afford **12** (50 mg, >99 %) as colorless syrup. ^{1}H NMR (300 MHz) δ ppm: 1.078 (9H, s, *t*-Bu), 1.270 (3H, t, *J*= 7.5 Hz, $-CH_2C\underline{H}_3$), 1.594 (3H, s, Me), 2.719 (2H, dd, *J*= 7.5 and 15.3 Hz, $-C\underline{H}_2CH_3$), 3.952 (3H, s, -COOMe), 6.901 (1H, d, *J*= 8.7 Hz, H-7), 7.328 (2H, d, *J*= 8.4 Hz, ArH), 7.933 (2H, d, *J*= 8.4 Hz, ArH), 8.298 (1H, d, *J*= 8.7 Hz, H-6), 11.606 (1H, br s, -NH-); HR-MS(FAB): calcd for $C_{23}H_{27}NO_5$ $[M]^+$: 397.1889; found 397.1880

Fluorescence-labeled polymer (14)

A solution of 4-*O*-(β-D-galactopyranosyl)-*N*-(4-vinylbenzoyl)-β-D-glucopylanosylamine (**13**) (40 mg, 0.084 mmol), compound (**8**) (0.837 mg, 2.1 μmol) and α,α'-azobisisobutyronitrile (0.137 mg, 0.84 μmol) in DMSO (0.3 mL) was degassed under reduced pressure. The mixture was frozen and degassed repeatedly under reduced pressure. The polymerization tube was sealed under reduced pressure, and incubated at 60 °C for 7 h. The reaction mixture was dialyzed in water (Mw 3500 cut off) for 3 days, and lyophilized to give white polymer (**14**) (27.4 mg, 67 %). Mn 2.1×10^4 (SEC analysis, pullulan standard). ^{1}H NMR (D_2O, 500 MHz, 60 °C) δ ppm: 1.40 (br, main chain $-CH_2-CH-$), 5.05 (br s, H-1), 3.44-3.92 (br m, other proton from sugar), 6.50 and 7.48 (br, ArH). Fluorescence (0.0001 mg/mL, H_2O): Ex. 360nm, Em. 450 nm

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