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Design and synthesis of a photocleavable biotin-linker for the photoisolation of ligand–receptor complexes based on the photolysis of 8-quinolinyl sulfonates in aqueous solution

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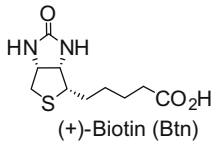
ABSTRACT

The ability of avidin (Avn) to form strong complex with biotin (Btn) is frequently used in the detection and isolation of biomolecules in biochemical, analytical, and medicinal research. The fact that the binding is nealy irreversible, however, constitutes a drawback in term of the isolation and purification of intact biomolecules. We recently found that 8-quinolinyl esters of aromatic or aliphatic sulfonic acids undergo photolysis when irradiated at 300–330 nm in aqueous solution at neutral pH. In this work, a biotin–dopamine (BD) conjugate containing a photocleavable 8-quinolinyl benzenesulfonate (QB) linker, BDQB, was designed and synthesized for use in the efficient recovery of dopamine–protein (e.g., antibody) complexes from an Avn–Btn system. The complexation of BDQB with a primary anti-dopamine antibody (anti-dopamine IgG_1 from mouse) on an Avn-coated plate was confirmed by an enzyme-linked immunosorbent assay (ELISA) utilizing a secondary antibody (anti-IgG_1 antibody) conjugated with horseradish peroxidase (HRP). Upon the photoirradiation (at 313 nm) of the BDQB–IgG_1 complex, the release of dopamine–IgG_1 complex was confirmed by ELISA. Characterization of the resulting photoreleased dopamine–anti-dopamine IgG_1 complex was performed by SDS–PAGE and Western blot.

1. Introduction

Biotin (Btn) is a water soluble vitamin found in egg yolk, beef liver, and milk concentrates.[1] Biotin forms stable complexes with avidin (Avn, M_r = 67,000), a protein contained by egg white,[2] neutravidin (Nevn, M_r = 60,000), a deglycosylated form of avidin, and streptavidin (Stvn, M_r = 66,000–75,000), isolated from the bacterium *Streptomyces avidinii*. Biotin forms extraordinary stable complexes (the dissociation constant, K_d, for the Avn–Btn complex is ca. 10^{-15} M), mainly due to van der Waals and hydrophobic interactions at the binding site of avidin.[3] The half-time for dissociation of the Avn–Btn complex has been estimated to be 130–200 days. As a result, this complexation is considered to be essentially irreversible.



(+)-Biotin (Btn)

Very stable Avn–Btn systems are in widespread use in the areas of biological chemistry, cell biology, analytical chemistry, medicinal chemistry, and therapeutic applications, mainly for the isolation of specific receptors (affinity chromatography), the localization of target molecules (affinity cytochemistry, cell cytometry, and blotting technology), and medical diagnostics (e.g., immunoassay, histopathology, and gene probes).[2] However, the separation of biotinylated ligands or ligand–receptor complexes from the avidin matrix requires harsh denaturing conditions such as treatment with detergent, high or low pH, high salt concentrations and other similar conditions.[4]

Past efforts to address and solve this problem can be classified into following three categories. The first involves the development of Btn derivatives which have a lower affinity for Avn (or Stvn) than that of Btn,[5–7] such as a $N^{\alpha,B1}$-iminobiotinyl-linker (dethiobiotin–X(spacer) linker)[5,6] and a $N^{\alpha,B1}$-iminobiotinyl-linker (iminobiotin).[7]

The second approach is the development of an Avn mutant having a reduced affinity for Btn,[8] which requires tedious biological manipulation.

The third approach is the design and synthesis of Btn linkers that contain chemically cleavable groups.[9–16] This category includes biotinylated derivatives containing the disulfide linker,[9,10] the silyl-containing Btn linker,[11] and the acid-labile Btn linker.[12] The

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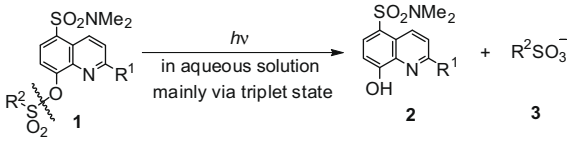
cleavage of these three biotin-linkers requires excessive amounts of reagents such as dithiothreitol (DTT),[9,10] F^-,[11] and acid (H^+),[12] respectively, which might induce conformational changes and loss of function of the target molecules.

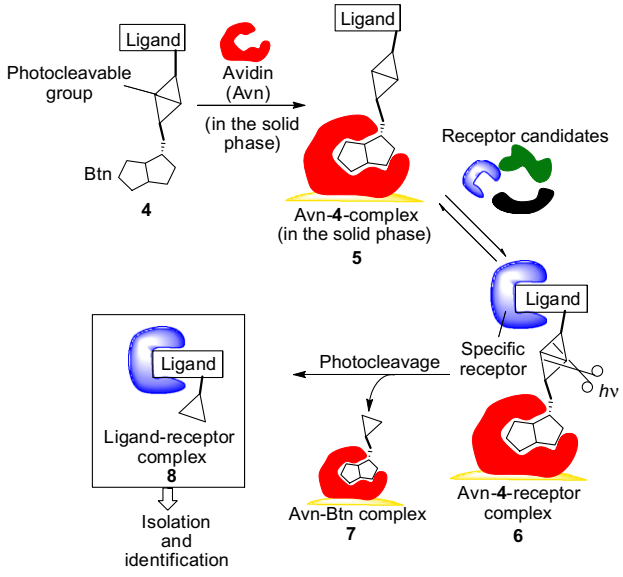
Meanwhile, light-induced cleavage reactions of chemically masked groups have been a subject of considerable interest for some time.[13] Such procedures assume that photochemically cleavable biotin linkers would afford clean and useful methodologies for the isolation of intact ligand–receptor complexes.[14] In this context, photocleavable biotin linkers having a 2-nitrobenzyl moiety were developed previously.[15–17] As of this writing, however, biological applications have been limited.

During our efforts to develop new fluorescent sensors for zinc(II) ions,[18] we recently discovered that sulfonates of 5-*N*,*N*-dimethylaminosulfonyl-8-hydroxyquinoline (general structure: **1**) undergo photolysis upon UV irradiation (at 300–330 nm) in CH_3CN/H_2O (10 mM HEPES with I = 0.1 ($NaNO_3$)) to yield the corresponding alcohols **2** and sulfonates **3** ($R^2SO_3^-$), as shown in Scheme 1.[19] Our recent studies on mechanistic aspects of this reaction indicated that this photolysis proceeds mainly via excited triplet state, in which the S–O bond of **1** is homolytically cleaved[20].

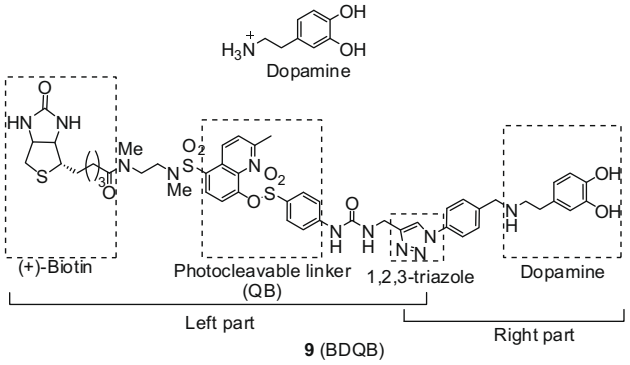
This finding prompted us to prepare a new photocleavable biotin-linker. As summarized in Scheme 2, we synthesized a biotinylated ligand that contains a photocleavable 8-quinolinyl sulfonate linker **4**. It is likely that **4** would form an Avn–**4** complex **5** in the solid phase. A mixture of specific receptor candidates is added to obtain ternary complexes **6**, which are composed of Avn, **4**, and the ligand–specific receptor. We hypothesized that ligand–receptor complexes **8** could be isolated upon the photoirradiation of **6** in aqueous solution, leaving Avn-Btn complex **7**, without the need for any other reagents.



Scheme 1.



Scheme 2.



Scheme 3.

To demonstrate our methodology, we synthesized a dopamine derivative that contains a photocleavable Btn linker **9** (a biotin–dopamine (BD) conjugate containing a photocleavable 8-quinolinyl benzenesulfonate (QB) linker, BDQB) (Scheme 3). Dopamine is an important neurotransmitter found in the brain.[21] Several dopamine receptors (D_1 to D_5 receptors) have been identified[21] and are recognized as targets of therapeutic agents. In addition, antibodies against dopamine and dopamine receptors are commercially available. In this manuscript, we describe the synthesis of **9** and related model compounds, the complexation of **9** with an anti-dopamine antibody, and the results of an enzyme-linked immunosorbent assay (ELISA). The photorelease of the dopamine–anti-dopamine antibody complex (corresponding to **8** in Scheme 2), as verified by ELISA, sodium dodecylsulfonate–polyacrylamide gel electrophoresis (SDS–PAGE), and Western blot, will be described.

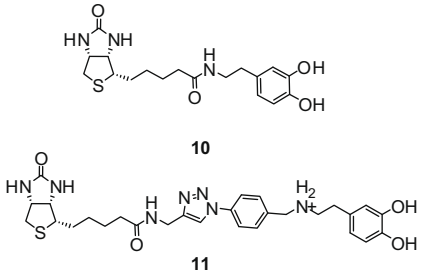
2. Results and discussion

2.1. Synthesis of reference compounds 10 and 11 and examination of their binding to anti-dopamine antibody (IgG_1)

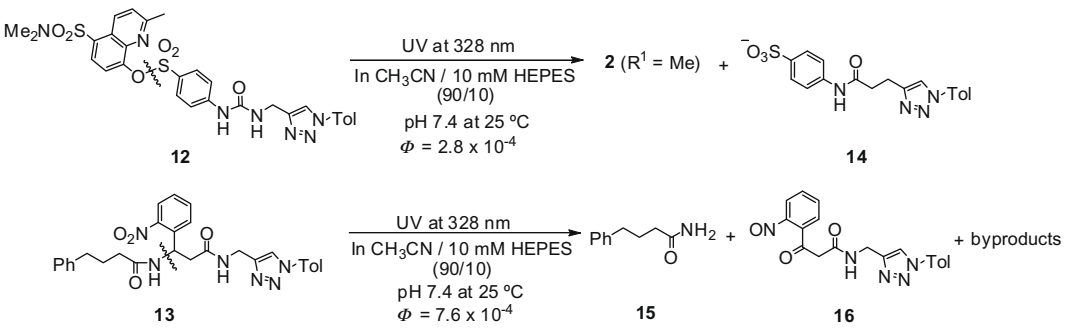
Prior to the synthesis of **9**, reference compounds **10**[22] and **11** (for their synthesis, see the Supplementary data) were synthesized (Scheme 4). Using ELISA with an anti-dopamine antibody (IgG_1), it was found that **10** is not recognized by IgG_1, while **11** is able to readily bind to IgG_1, suggesting that the ammonium cation form of dopamine is important for its binding to IgG_1, as previously suggested for dopamine–dopamine-receptor interactions.[23]

2.2. Comparison of the photoreactivity of 12 and the reference compound 13

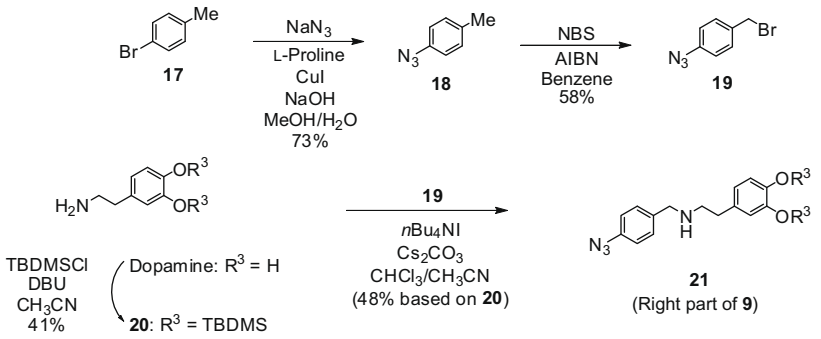
The photoreactivity (photoirradiation at 328 nm) in CH_3CN/10 mM HEPES (pH 7.4 with I = 0.1 ($NaNO_3$) (9/1)) of **12** and refer-



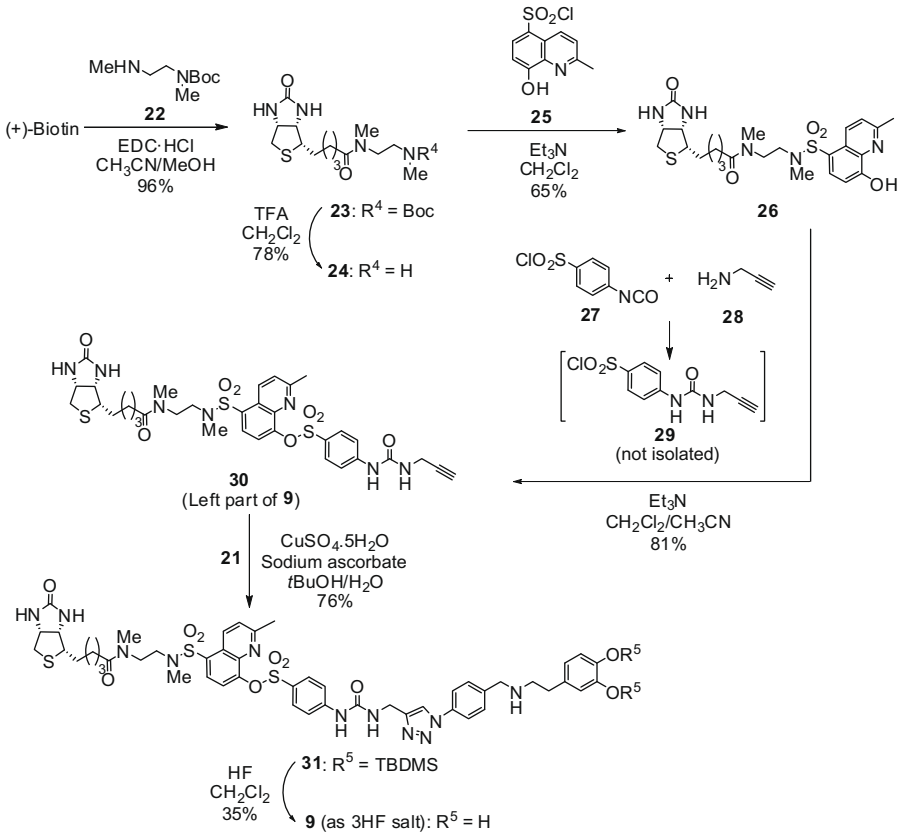
Scheme 4.



Scheme 5.



Scheme 6.



Scheme 7.

ence compound **13**, which contains commercially available *o*-nitrophenyl-β-alanine as a photocleavable group (Scheme 5),[24] was almost identical, as shown in Figure S1 in the Supplementary data. It should be noted that the molar absorption coefficient of **12** at 328 nm ($\varepsilon_{328} = 1.1 \times 10^3\ M^{-1}\ cm^{-1}$) is ca. three times as great as that of **13** ($\varepsilon_{328} = 0.4 \times 10^3\ M^{-1}\ cm^{-1}$) and the quantum yields for the photolysis of **12** ($\Phi = 2.8 \times 10^{-4}$) is ca. one-third that of **13** ($\Phi = 7.6 \times 10^{-4}$), resulting in a similar photolysis profile. More importantly, the photolysis of **12** yielded **2** and **14** with almost no detectable byproducts (Figs. S2 and S3 in the Supplementary data), while the photoirradiation of **13** afforded several byproducts as well as **15** and **16** (data not shown).

2.3. Synthesis of 9

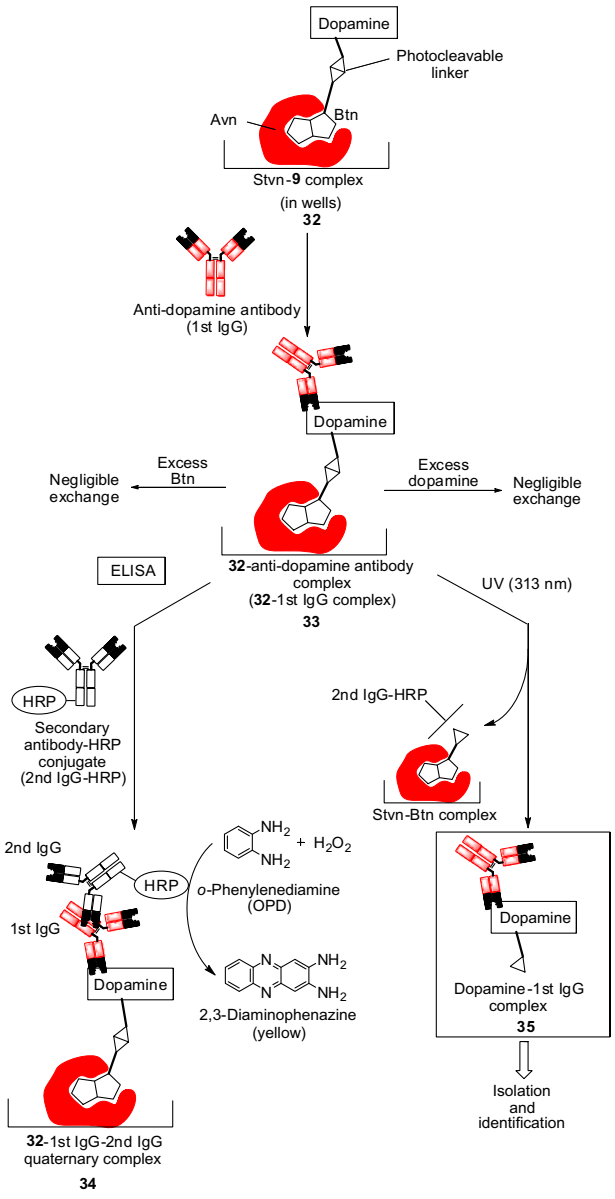
The convergent synthesis of **9** is shown in Schemes 6 and 7. For the dopamine part (the right part of **9** indicated in Scheme 3), the CuI-catalyzed coupling reaction of **17** with sodium azide in the presence of proline gave **18**,[25] which was then brominated to give **19**.[26] The two hydroxyl groups of dopamine were protected with *t*-butyldimethylsilyl (TBDMS) groups to give **20**.[27] The monoalkylation of **20** with **19** in the presence of nBu$_4$NI and Cs_2CO_3 gave **21**, the right part of **9**, in 48% yield based on the used **20**.

For the synthesis of the left part of **9** (Scheme 7), (+)-biotin was reacted with *N*-Boc-*N*,*N*′-dimethylethylenediamine **22**[28] to give **23**, and the Boc group was deprotected by treatment with trifluoroacetic acid (TFA) to afford **24**. Upon treatment of **24** with **25**,[18a,29] **26** was obtained and the reaction of **26** with **29** (prepared from **27** and **28** and used without purification) yielded **30**.

A Huisgen 1,3-dipolar [3+2] cycloaddition of the terminal alkyne group of **30** with **21** in the presence of a catalytic amount of Cu^{2+} and sodium ascorbate according to procedures of click chemistry developed by Hartmuth and Sharpless and co-workers[30] gave **31** in moderate yields. Finally, deprotection of the TBDMS groups of **31** by treatment with HF gave **9** as the 3HF salt.

2.4. Verification of the complexation of 9 with anti-dopamine antibody (IgG_1) by enzyme-linked immunosorbent assay (ELISA)

The complexation of **9** with a primary anti-dopamine antibody (mouse IgG_1) was verified by means of an enzyme-linked immunosorbent assay (ELISA) (Scheme 8).[31] A primary anti-dopamine antibody (IgG_1, 1st IgG) was added to a complex of Stvn and **9** in wells of micro 96-wells plates (**32**→**33** in Scheme 8). After washing with phosphate buffered saline containing 0.05% (v/v) Tween 20 (PBST), the **32**–IgG_1 complex **33** was treated with an anti-IgG_1 antibody conjugated with horseradish peroxidase (2nd IgG–HRP) to give **34**, as indicated in the left half of Scheme 8. After washing, *o*-phenylenediamine (OPD) and H_2O_2 were added as substrates of HRP, and the increase in UV absorption at 450 nm (OD_{450}) for 2,3-diaminophenazine formed by the HRP-catalyzed oxidative dimerization of OPD[32] was detected. Figure 1 shows that the OD_{450} values increased with increasing amounts of **9**, strongly suggesting the formation of the quaternary complex **34** (Stvn–**9**–1st IgG–2nd IgG–HRP). Addition of an excess amount of Btn or dopamine to **33** (and successive washing) had negligible effect on the results of ELISA (data are not shown), indicating that the exchange reaction of **33** with Btn and dopamine hardly occurred.



Scheme 8.

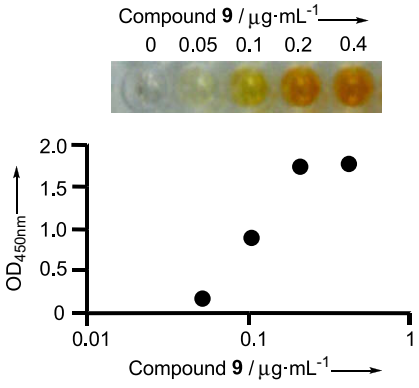


Figure 1. Results of ELISA of **9** with anti-dopamine antibody (1st IgG) and 2nd IgG–HRP.

2.5. Evaluation of the complexation characteristics of 9 with neutravidin (Nevn) and an anti-dopamine antibody (IgG_1) using a 27-MHz quartz-crystal microbalance

The complexation constant (K_{s1}) for **9** with Nevn was determined by means of a 27-MHz quarts-crystal microbalance

(QCM).[33] The log K_{s1} value for Nevn and **9** was determined to be 8.2 ± 0.2 (K_d = 6 ± 3 nM) by analysis of typical titration curves shown in Figure 2A. The log K_s value for Nevn and **30**, which lacks the dopamine part, was determined to be 8.1 ± 0.2, indicating that these modified biotin derivatives have a lower affinity than that of biotin itself.[34] In addition, IgG_1 was added to the Nevn–**9** complex to obtain the titration curves displayed in Figure 2B, from which the log K_{s2} value for the complexation of the Nevn–**9** complex with IgG_1 was estimated to be 7.5 ± 0.2 (K_d = 20 ~ 50 nM).

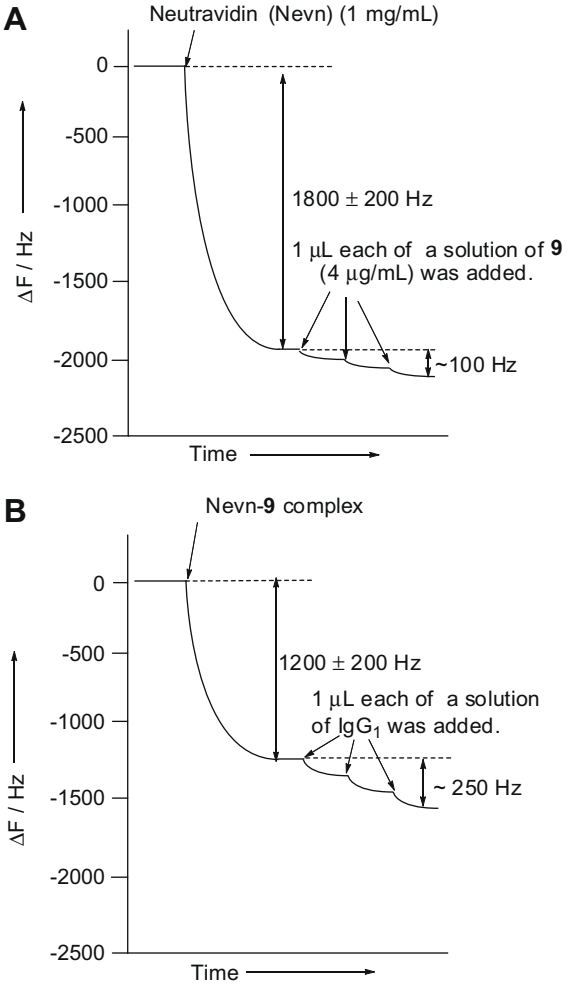


Figure 2. Results of 27-MHz QCM of **9**, Nevn, and IgG_1. Time course of frequency change of QCM. (A) A solution of **9** was added to Nevn; (B) a solution of IgG_1 was added to Nevn–**9** complex.

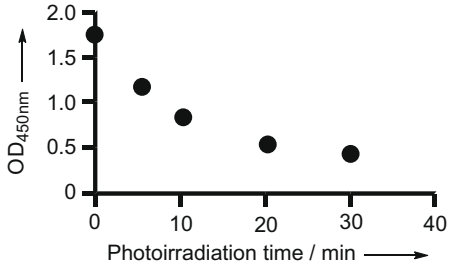


Figure 3. The results of ELISA of **9**–anti-dopamine antibody complex after photoirradiation at 313 nm.

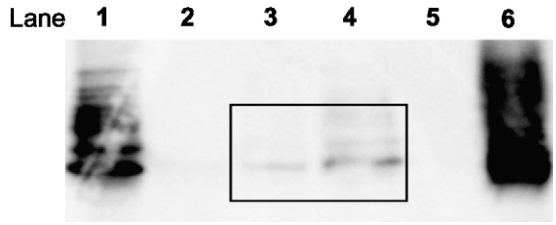


Figure 4. Results of Western blot of **9**–IgG_1 complex after photoreaction. Lane 1: The sample obtained by treatment of **9**–IgG_1 with SDS without photoirradiation. Lane 2, 3, 4: photoproducts after photoirradiation (313 nm) for 0 min, 5 min and 30 min. Lane 5: Blank. Lane 6: Anti-dopamine antibody commercially purchased.

2.6. Photolysis of 9–IgG_1 complex in streptavidin (Stvn)-coated well, as followed by ELISA and detection of a dopamine–IgG_1 complex by Western blot

The photocleavage of the **9**–IgG_1 complex in Stvn-coated wells was carried out by photoirradiation at 313 nm, as displayed in the right half of Scheme 8. After irradiation of the ternary complex **33** (**33**→**35** in Scheme 8), an ELISA of the photoreaction mixtures was carried out. As shown in Figure 3, OD_{450} decreases with increasing photoirradiation time, suggesting that the dopamine–IgG_1 complex is photochemically released from the wells.

The photoreaction product **35** (Scheme 8) was analyzed by Western blot. In this experiment, avidin beads were used instead of 96-wells for the solid phase. Lane 1 of Figure 4 shows the sample obtained by the treatment of the **9**–IgG_1 complex on avidin beads with SDS before photoirradiation. Lanes 2–4 show that the amount of photoreleased IgG_1–dopamine complex is dependent on the photoirradiation time (lane 2, 0 min; lane 3, 5 min; lane 4, 30 min), which shows good agreement with the results of ELISA (Fig. 3).

3. Conclusion

In this study, we have reported on the design and synthesis of a new biotinylated dopamine containing a photolabile 8-quinolinyl benzenesulfonate moiety, **9** (BDQB). This photocleavable biotin-linker can be easily incorporated into small molecules using a Huisgen 1,3-dipolar [3+2] cycloaddition. We examined the complexation of **9** with avidins and an anti-dopamine antibody (IgG_1) and the photorelease of dopamine–IgG_1 complexes by ELISA, which was also confirmed by Western blot. These methods may provide effective strategies for the recovery of intact ligand–receptors complexes under mild conditions, without the need for damaging chemical reagents. Moreover, the photolysis of **9** proceeds cleanly to give the corresponding quinolinols and sulfonates, which would be advantage over the previous chemically cleavable biotin linkers. We presume that this method should have merits in cases where ligands and receptors are bound by covalent bonds. Efforts to improve the efficiency of the photoreaction are currently underway.

4. Experimental

4.1. Materials

All reagents and solvents purchased were of the highest commercial quality and were used without further purification. Anhydrous acetonitrile (CH_3CN), THF, dichloromethane (CH_2Cl_2) were obtained by distillation from calcium hydride. All aqueous solutions were prepared using deionized and distilled water. The Good's buffer reagents (Dojindo) were commercially obtained: HEPES (*N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid,

pK_a = 7.5). Melting points were determined on a Yanaco Melting Point Apparatus and are uncorrected. UV spectra were recorded on a JASCO UV/vis spectrophotometer V-550 at 25 ± 0.1 °C. IR spectra were recorded on a Horiba FTIR-710 spectrophotometer at room temperature. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a JEOL Lambda 400 spectrometer. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a JEOL Always 300 spectrometer. Elemental analyses were performed on a Perkin–Elmer CHN 2400 analyzer. Thin-layer (TLC) and silica gel column chromatographies were performed using a Merck 5554 (silica gel) TLC plates and Fuji Silysia Chemical FL-100D, respectively.

4.2. Synthesis

4.2.1. Synthesis of 2-[3,4-bis(*tert*-butyldimethylsilyloxy) phenyl]ethylamine (20)[22]

Dopamine hydrochloride (1 g, 5.27 mmol) was added to *t*-butyldimethylsilyl chloride (1.75 g, 11.6 mmol) in CH_3CN (20 mL) at 0 °C, and the reaction mixture was stirred for 10 min at 0 °C. After the dropwise addition of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (3.21 g, 21.08 mmol), the resulting solution was stirred at 0 °C for 4 h and then at room temperature for 20 h. The reaction mixture was concentrated under reduced pressure and the resulting residue was purified by silica gel column chromatography (hexane/$CHCl_3$/MeOH) to yield **20** as a brown amorphous solid (833 mg, 41% yield). IR (KBr): 3436, 2930, 2891, 2859, 1513, 1472, 1254, 842, 781 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 0.18 (s, 12H), 0.96 (s, 9H), 0.97 (s, 9H), 2.97 (t, *J* = 8.8 Hz, 2H), 3.18 (t, *J* = 8.6 Hz, 2H), 6.67 (m, 2H), 6.75 ppm (d, *J* = 8.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = −4.0, −4.1, 18.4, 18.4, 25.9, 33.3, 121.3, 121.6, 121.6, 129.2, 146.1, 147.0 ppm.

4.2.2. Synthesis of *N*-(4-azidobenzyl)-*N*-(2-[3,4-bis(*tert*-butyldimethylsilyloxy)phenyl]ethyl)amine (21)

To a mixture of tetrabutylammonium iodide (118 mg, 0.32 mmol), Cs_2CO_3 (609 mg, 1.87 mmol), and **20** (242 mg, 0.63 mmol) in $CHCl_3$/CH_3CN (7/3), 4-azidobenzylbromide **19**[26] (45 mg, 0.21 mmol) was added and the resulting solution was stirred for 1.5 h at room temperature. After removing the insoluble salts by filtration, water was added to the filtrate and extracted with $CHCl_3$. After drying the combined organic layer over anhydrous Na_2SO_4, the solvent was filtered and concentrated under reduced pressure. The remaining residue was purified by silica gel column chromatography (hexane/$CHCl_3$/MeOH) to yield **21** as a brown amorphous solid (52 mg, 48% yield based on the used **19**). IR (KBr): 2954, 2929, 2889, 2857, 1577, 1507, 1471, 1254, 839, 781 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 0.17 (s, 6H), 0.18 (s, 6H), 0.97 (s, 9H), 0.98 (s, 9H), 2.69 (t, *J* = 6.4 Hz, 2H), 2.82 (t, *J* = 6.6 Hz, 2H), 3.75 (s, 2H), 6.62 (dd, *J* = 2.0, 8.0 Hz, 1H), 6.66 (d, *J* = 2.0 Hz, 1H), 6.73 (d, *J* = 8.0 Hz, 1H), 6.96 (d, *J* = 8.1 Hz, 2H), 7.25 ppm (d, *J* = 8.4 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ = −4.1, 18.4, 25.9, 35.4, 50.5, 53.2, 118.9, 120.9, 121.5, 129.5, 132.9, 137.0, 138.6, 145.2, 146.6 ppm; HRMS (EI): *m*/*z*: calcd for $C_{27}H_{44}N_4O_2Si_2$: 512.3003 [M$^+$]; found: 512.3000.

4.2.3. Synthesis of *N*-(*tert*-Butoxycarbonyl)-*N*′-[2-(D-biotinyl amino)-ethyl]-*N*,*N*′-dimethylamine (23)

A solution of D-biotin (508 mg, 2.08 mmol), *N*-Boc-*N*,*N*′-dimethylamine **22**[28] (475 mg, 2.50 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) (518 mg, 2.70 mmol) in a 1/3 mixture of MeOH and CH_3CN (20 mL) was stirred for 5 h at room temperature and the reaction mixture was concentrated under reduced pressure. The residue was resuspended in MeOH and filtered through a Celite (No. 545). The filtrate was concentrated under reduced pressure and the resulting residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH) to yield **23** as a pale yellow amorphous solid (828 mg, 96% yield). IR (KBr): 3284, 2972, 2930, 2866, 1697, 1458, 1397, 686 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 1.47 (br, 11H), 1.62–1.76 (m, 4H), 2.32–2.35 (m, 2H), 2.73 (m, 1H), 2.88–3.02 (m, 7H), 3.14–3.21 (m, 1H), 3.34–3.53 (m, 4H), 4.31–4.35 (m, 1H), 4.53–4.59 ppm (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ = 24.7, 28.2, 28.3, 28.4, 31.9, 33.0, 33.6, 34.7, 40.6, 47.3, 51.6, 55.4, 60.1, 61.8, 61.8, 61.9, 70.5, 163.5 ppm.

4.2.4. Synthesis of *N*-[2-(D-Biotinylamino)-ethyl]-*N*,*N*′-dimethylamine (24)

TFA (5.5 g, 47.7 mmol) was added dropwise over 5 min to a solution of **23** (661 mg, 1.59 mmol) in CH_2Cl_2 (7.5 mL) at 0 °C and the resulting solution was then stirred for 3 h at room temperature. After concentrating the reaction mixture under reduced pressure, the resulting residue was azetroped with toluene to remove TFA. The residue was dissolved in $CHCl_3$ and the solution was washed with 10% aqueous NaOH. After drying the organic layers over anhydrous Na_2SO_4, the solvent was filtered and concentrated under reduced pressure to yield **24** as a colorless solid (468 mg, 94% yield). IR (KBr): 3286, 2934, 2859, 1696, 1624, 1464, 1331, 685 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 1.44–1.51 (m, 2H), 1.64–1.80 (m, 4H), 2.34–2.46 (m, 5H), 2.72–3.04 (m, 7H), 3.15–3.18 (m, 1H), 3.38–3.53 (m, 2H), 4.30–4.33 (m, 1H), 4.48–4.53 ppm (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ = 24.8, 25.4, 28.2, 28.3, 32.5, 32.9, 33.4, 35.9, 36.2, 36.5, 40.5, 47.3, 49.3, 49.6, 49.7, 55.5, 55.6, 60.1, 61.8, 163.9, 173.3, 173.4 ppm; HRMS (FAB): *m*/*z*: calcd for $C_{14}H_{26}N_4O_2S$: 315.1855 [M+H$^+$]; found: 318.1852.

4.2.5. Synthesis of 8-hydoxy-5-{*N*-[2-(D-biotinylamino)-ethyl]-(*N*,*N*′-dimethyl)sulfonamide}-2-methylquinoline (26)

Distilled Et_3N (134 mg, 1.32 mmol) was added to a solution of **24** in CH_2Cl_2 (26 mL) at 0 °C under an argon atmosphere, to which a solution of **25** (170 mg, 0.66 mmol)[18a,29] in CH_2Cl_2 (5 mL) was added dropwise over 30 min. The reaction mixture was stirred at 0 °C for 30 min and at room temperature for 3 h. The solvent was removed under reduced pressure and the remaining residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH) to yield **26** as a yellow amorphous solid (196 mg, 56% yield). IR (KBr): 3293, 2929, 2859, 1699, 1631, 1462, 1326, 1149, 715 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ = 1.43–1.47 (m, 2H), 1.59–1.80 (m, 2H), 2.23 (t, *J* = 7.4 Hz, 2H), 2.71–3.02 (m, 11H), 3.27 (d, *J* = 5.8 Hz, 2H), 3.48–3.60 (m, 2H), 4.32–4.36 (m, 1H), 4.48–4.52 (m, 1H), 7.15 (d, *J* = 8.3 Hz, 1H), 7.49 (d, *J* = 8.8 Hz, 1H), 8.04 (d, *J* = 8.3 Hz, 1H), 8.87 ppm (d, *J* = 9.0 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD): δ = 24.8, 25.8, 26.2, 29.5, 29.8, 33.3, 33.9, 34.1, 34.6, 35.9, 36.5, 41.1, 46.2, 48.2, 56.9, 61.6, 63.2, 63.3, 110.0, 123.7, 124.3, 124.9, 125.4, 125.6, 132.7, 132.8, 134.9, 135.1, 139.3, 158.7, 158.9, 159.7, 159.8, 166.0, 175.5, 175.6 ppm; HRMS (FAB): *m*/*z*: calcd for $C_{24}H_{33}N_5O_5S_2$: 536.2002 [M+H$^+$]; found: 536.1998.

4.2.6. Synthesis of 2-methylquinoline-8-[*p*-(*N*′-2-propylureido) benzenesulfonyloxy]-5-{*N*,*N*′-dimethyl-*N*′-[2-(D-biotinylamino) ethyl]}sulfonamide (30)

A solution of propargylamine **28** (105 mg, 1.91 mmol) in CH_2Cl_2 (3 mL) was added dropwise over 1 h to a solution of 4-(chlorosulfonyl)phenyl isocyanate **27** (403 mg, 1.85 mmol) in CH_2Cl_2 (8 mL). After stirring the resulting solution was stirred for 7 h at room temperature, the solvent was removed under reduced pressure. The remaining precipitate was resuspended in $CHCl_3$, filtered, and the filtrate concentrated under reduced pressure to yield **29** as a colorless solid (314 mg), which was used without further purification for the synthesis of **30**. ^{1}H NMR of **29** (300 MHz, $CDCl_3$, TMS):

δ = 2.30 (t, J = 2.5 Hz, 1H), 4.10 (d, J = 2.5 Hz, 2H), 6.97 (s, 1H), 7.62 (d, J = 9.0 Hz, 2H), 7.94 ppm (d, J = 9.0 Hz, 2H).

A solution of crude **29** (68 mg) in CH_3CN (7 mL) was added dropwise to a mixture of **26** (115 mg, 0.21 mmol) and Et_3N (34 μL, 0.25 mmol) in CH_2Cl_2 (3 mL) at 0 °C. The solution was stirred at 0 °C for 30 min and at room temperature for 3 h. Water (1 mL) was added to the reaction mixture and the resulting solution stirred for further 30 min. The reaction mixture was poured into aq K_2CO_3, and extracted with CH_2Cl_2. The combined organic layer was dried over K_2CO_3 and filtered, and then the filtrate was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH) to yield **30** as a yellow solid (132 mg, 81% yield). Mp 120 °C; IR (KBr): 3285, 2928, 2857, 1696, 1591, 1462, 1408, 1370, 1326, 1224, 1193, 1172, 715, 648 cm^{-1}; ^1H NMR (300 MHz, CD_3OD, TSP): δ = 2.12–2.46 (m, 6H), 2.81 (t, J = 6.9 Hz, 2H), 3.27–3.34 (m, 4H), 3.39 (d, J = 12.9 Hz, 1H), 3.58–3.66 (m, 7H), 3.87–3.94 (m, 1H), 4.01 (q, J = 1.7 Hz, 2H), 4.21–4.29 (m, 2H), 4.66 (d, J = 2.7 Hz, 2H), 5.02 (m, 1H), 5.18 (m, 1H), 8.15–8.23 (m, 3H), 8.39–8.56 (m, 3H), 8.82 (d, J = 8.1 Hz, 1H), 9.50 ppm (d, J = 9.0 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ = 25.0, 25.9, 29.5, 29.8, 30.1, 33.9, 34.8, 36.2, 36.4, 41.1, 46.0, 48.3, 57.0, 61.6, 63.2, 72.2, 81.3, 118.4, 122.7, 125.4, 125.4, 128.5, 129.6, 131.4, 134.7, 134.7, 142.4, 147.2, 149.8, 156.5, 162.2, 166.1, 175.8 ppm; HRMS (ESI): m/z: calcd for $C_{34}H_{41}N_7O_8S_3$: 772.2251 [M+H$^+$]; found: 772.2251.

4.2.7. Synthesis of 1-(4-{2-[3,4-bis(*tert*-butylsilyloxy)phenyl] ethylaminomethyl}phenyl)-4-{2-methylquinoline-8-[*p*-(*N*′-2-propynylureido)benzensulfonyloxy]}-5-{*N*,*N*′-dimethyl-*N*-[2-(D-biotinylamino)]sulfamoyl}-1,2,3-triazole (31)

Sodium ascorbate (0.034 mmol, 34 μL of a 1 M solution in water) and $CuSO_4{\cdot}5H_2O$ (3.4 μmol, 34 μL of 100 mM solution in water) were added to a suspension of **30** (132 mg, 0.17 mmol) and **21** (94 mg, 0.18 mmol) in a 1/1 mixture of water and *tert*-BuOH (12 mL). After the reaction mixture was stirred for 29 h at room temperature, the solution was diluted with water and extracted with $CHCl_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The remaining residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH) to yield **31** as a yellow amorphous solid (162 mg, 76% yield). IR (KBr): 3329, 2929, 2857, 1697, 1592, 1541, 1463, 1409, 1362, 1254, 1225, 1193, 1171, 840, 784, 714 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, TMS): δ = 0.17 (s, 6H), 0.18 (s, 6H), 0.97 (s, 9H), 0.97 (s, 9H), 1.33–1.43 (m, 2H), 1.51–1.57 (m, 2H), 1.59–1.62 (m, 2H), 1.91–2.05 (m, 4H), 2.60 (s, 3H), 2.73 (s, 3H), 2.79 (d, J = 11.4 Hz, 1H), 2.88 (s, 3H), 2.90–2.95 (m, 1H), 3.02–3.05 (m, 2H), 3.13–3.15 (m, 1H), 3.24–3.27 (m, 2H), 3.37 (t, J = 4.8 Hz, 2H), 3.49 (s, 2H), 3.51 (t, J = 4.8 Hz, 2H), 4.34–4.37 (m, 1H), 4.52–4.54 (m, 1H), 6.06 (s, 1H), 6.54 (s, 1H), 6.65 (d, J = 6.0 Hz, 1H), 6.68 (s, 1H), 6.74 (d, J = 6.0 Hz, 1H), 7.29 (d, J = 6.6 Hz, 1H), 7.39–7.60 (m, 8H), 7.72 (d, J = 6.3 Hz, 1H), 7.96 (s, 1H), 8.05 (d, J = 6.3 Hz, 1H), 8.72 ppm (d, J = 6.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = −4.2, −4.1, 18.3, 18.3, 24.3, 25.1, 25.9, 27.7, 27.9, 32.4, 33.8, 34.4, 35.0, 35.3, 35.7, 40.5, 45.0, 47.1, 50.7, 53.0, 55.4, 60.2, 61.7, 77.2, 117.1, 120.4, 120.9, 120.9, 121.4, 124.1, 124.1, 126.5, 128.1, 129.3, 130.1, 130.1, 130.1, 132.7, 132.7, 133.1, 135.5, 141.2, 145.1, 145.9, 146.0, 146.6, 148.6, 155.1, 160.6, 164.2, 173.2, 173.4 ppm; HRMS (ESI): m/z: calcd for $C_{61}H_{85}N_{11}O_{10}S_3Si_2$: 1284.5254 [M+H$^+$]; found: 1284.5254.

4.2.8. Synthesis of 1-{4-[2-(3,4-dihydroxyphenyl)ethylamino methyl]phenyl}-4-{2-methylquinoline-8-[*p*-(*N*′-2-propyny lureido)benzensulfonyloxy]}-5-{*N*,*N*′-dimethyl-*N*-[2-(D-biotinyl amino)]sulfamoyl}-1,2,3-triazole (9)

HF (15 drops) was added to a solution of **31** (43 mg, 0.033 mmol) in CH_3CN (6 mL), the solution stirred for 30 min at room temperature. After removing the solvent under reduced pressure, the remaining residue was azetroped with CH_3CN twice or three times to remove HF to give a colorless amorphous solid. THF was added to the amorphous solid and the insoluble solid was collected by filtration and washed with THF to yield **9** as a colorless solid (13 mg, 35% yield). Mp 150–152 °C; IR (KBr): 3390, 2932, 2859, 1687, 1593, 1522, 1459, 1408, 1365, 1327, 1225, 1193, 1171, 795, 714 cm^{-1}; ^1H NMR (400 MHz, CD_3OD, TSP): δ = 2.02–2.54 (m, 6H), 2.82 (t, J = 5.5 Hz, 2H), 3.31 (s, 3H), 3.42 (d, J = 9.5 Hz, 1H), 3.56–3.60 (m, 2H), 3.59 (s, 3H), 3.63 (s, 3H), 3.86–3.90 (m, 1H), 3.92 (t, J = 5.9 Hz, 2H), 4.05–4.10 (m, 2H), 4.20–4.31 (m, 2H), 4.97 (s, 2H), 5.05 (q, J = 2.6 Hz, 1H), 5.20 (q, J = 3.2 Hz, 1H), 5.26 (s, 2H), 7.30 (dd, J = 1.5, 4.6 Hz, 1H), 7.41 (d, J = 1.5 Hz, 1H), 7.46 (d, J = 6.1 Hz, 1H), 8.19–8.23 (m, 3H), 8.39 (d, J = 6.0 Hz, 2H), 8.45 (d, J = 6.6 Hz, 2H), 8.53 (dd, J = 4.2, 2.0 Hz, 1H), 8.68 (d, J = 6.2 Hz, 2H), 8.86 (d, J = 6.2 Hz, 1H), 9.20 (s, 1H), 9.53 ppm (d, J = 6.6 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ = 25.0, 25.9, 29.8, 33.6, 33.9, 34.7, 36.1, 36.1, 36.3, 41.1, 46.0, 46.1, 49.5, 49.7, 49.9, 50.4, 52.0, 56.9, 57.0, 61.6, 63.3, 116.7, 116.8, 118.3, 118.4, 120.9, 121.9, 122.3, 122.7, 125.3, 125.4, 128.3, 128.4, 129.6, 129.6, 129.7, 131.5, 132.3, 134.7, 138.7, 142.4, 145.5, 146.7, 147.3, 148.0, 156.9, 162.2, 175.8; HRMS (FAB): m/z: calcd for $C_{49}H_{57}N_{11}O_{10}S_3$: 1056.3530 [M+H$^+$]; found: 1056.3530; elemental Anal. Calcd for $C_{49}H_{60}F_3N_{11}O_{10}S_3$ (1116.26): C, 52.72; H, 5.42; N, 13.42. Found: C, 53.00; H, 5.17; N, 13.42.

4.3. ELISA for 9, 10 and 11 with an anti-dopamine antibody

We used a monoclonal anti-dopamine antibody (IgG_1 from mice, Abcam) as a primary antibody and HRP (horseradish peroxidase)-conjugated anti-IgG_1 antibody (IgG from rabbit, MP Biomedicals) as a secondary antibody. A streptavidin immobilized 96-well plate (purchased from Nunc Co. Ltd) was washed three times with phosphate buffered saline containing 0.05% (v/v) Tween 20 (PBST) prior to use. The biotinylated dopamine **9** diluted with PBST was added at 100 μL/well, followed by incubation at room temperature for 1 h. After washing, the **9** in each well was sequentially incubated at room temperature for 1 h with anti-dopamine antibody (1:2500 dilution) and the secondary antibody–HRP conjugate (1:1000 dilution) and the plates were incubated for 1 h at room temperature. After washing, *o*-phenylenediamine and H_2O_2 was added to each well. After incubation at room temperature for 15 min, the absorbance at 450 nm was measured on a Bio-Rad model 550 microplate reader (Bio-Rad, Hemel, Hempstead, UK).

4.4. Determination of K_s values for 9 with Nevn and anti-dopamine IgG_1 by 27-MHz quartz-crystal microbalance (QCM) analysis

QCM experiments were performed on an Affinix-Q4 apparatus (Initium Inc., Japan). The clean Au (4.9 mm^2) electrode equipped on quartz crystal was incubated with an aqueous solution of 3,3′-dithiodipropionic acid (3 mM) at room temperature for 45 min. The surfaces were activated by treatment with a mixture of EDC·HCl (0.26 M) and *N*-hydroxysuccinimide (0.44 M) for 45 min. The chip was allowed to stand to reach equilibrium at 25 °C in 500 μL phosphate buffered saline (PBS). Then, 5 μL of Nevn solution (1 mg/mL) was injected and the frequency change of quartz oscillator was recorded for specific time points. After adding 1 μL of an aqueous solution of ethanolamine (1 M) to block the remaining activated groups, the PBS was replaced with PBST. For the immobilization of Nevn–**9**, 1 μL of an aqueous solution of **9** (4 μg/mL) was injected at several time points and the change in frequency was recorded. Based on the fact that a frequency decrease (ΔF) of 1 Hz corresponds to a mass increase of 30 pg on the electrode (0.049 cm^2), K_{s1} values were determined from the

relationship between the decrease in frequency and the time for equilibration.

4.5. Photoreaction for the release of dopamine–IgG complex

Streptavidin coated wells were treated with **9** and an anti-dopamine antibody as described above and photoirradiated at 313 nm utilizing a USHIO Optical Module X equipped with a super high pressure UV lamp (USHIO), UV transmitting and a visible absorbing filter U-330 (Kenko, Co. Ltd, Japan), and an optical filter HQBP313-UV (Asahi Spectra, Co. Ltd, Japan). After irradiation for given periods, the wells were washed with PBST (100 μL) and the secondary antibody (diluted 1:1000 with PBST) was added to the wells. After incubation at room temperature and washing, *o*-phenylenediamine and H_2O_2 were added and the absorbance was measured at 450 nm on a Bio-Rad model 550 microplate reader (Bio-Rad, Hemel, Hempstead, UK).

4.6. Western blot

Avidin–agarose beads (purchased from Sigma–Aldrich) were reacted with **9** in PBST at room temperature for 2 h, washed three times with PBST, and then reacted with the anti-dopamine antibody at room temperature for 1 h. After washing the resulting avidin beads three times with PBST, the beads were resuspended in 0.5 mM Tris–HCl buffer, and then transferred to 24-well plate. These suspensions were photoirradiated at 313 nm as described above to release dopamine-antibody complexes. The resulting mixture was filtered through DISMIC-13HP PTFE (0.45 μm, ADVANTEC) to remove the beads, which were washed with 0.5 mM Tris–HCl buffer, and the combined filtrate was freeze-dried. The resulting materials were dissolved in a mixture of 10% SDS (8 μL), glycerol (4 μL), and water (8 μL), loaded on a 15% SDS–polyacrylamide gel for electrophoresis (20 μL/lane), and then transferred to a PVDF membrane (Immobilon-P Transfer Membrane, Millipore) under wet conditions. The membrane was blocked with 3% bovine serum albumin (BSA, Sigma) and incubated with HRP–conjugated anti-IgG_1 antibody diluted with PBST for 1 h at room temperature. The membrane was rinsed with TBST (5 min × 3) and visualized using ECL Plus Western blotting detection reagents on a LAS 3000 lumino-image analyzer (FUJIFILM).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.03.031.

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34. Recently, we have synthesized biotin–dopamine conjugates having longer photocleavable linkers than **9**. Preliminary experiments have indicated that its affinity with Nevn and its photoreactivity were almost identical to that of **9**. Detail will be described elsewhere.