Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron
Tetrahedron 63 (2007) 3465–3470

Synthesis and fluorescence properties of dimethylaminonaphthalene–deoxyuridine conjugates as polarity-sensitive probes

Akimitsu Okamoto,[a,b,*] Kazuki Tainaka,[a] Tomo Unzai[b] and Isao Saito[c,*]

[a]*Frontier Research System, RIKEN (The Institute of Physical and Chemical Research), Wako, Saitama 351-0198, Japan*
[b]*Department of Synthetic Chemistry and Biological Chemistry, Faculty of Engineering, Kyoto University, Kyoto 615-8510, Japan*
[c]*NEWCAT Institute, School of Engineering, Nihon University, and SORST, Japan Science and Technology Agency, Tamura, Koriyama 963-8642, Japan*

Received 29 June 2006; revised 25 September 2006; accepted 25 September 2006
Available online 2 February 2007

Abstract—The design of probes for monitoring various structures and dynamics of DNA and its surroundings is an important step in understanding biological events accompanying interbiomolecular interaction. We have developed novel fluorescent nucleosides in which the uracil base and the fluorophore are tethered by rigid linkers. They show unique absorption and fluorescence emission spectra. Nucleoside **2** is a fluorophore with high CT character and the fluorescence is very sensitive to solvent polarity. Nucleoside **3** shows absorption and emission maxima with longer wavelength due to extension of the DAN-conjugate system. These fluorophore–deoxyuridine conjugates with unique fluorescence properties would work as reporter probes sensitive to the change in microenvironment around specific sites of DNA.

1. Introduction

The development of photodevices for monitoring microenvironmental change around nucleic acids is a very important research target for understanding biological events accompanying interbiomolecular interactions such as replication, transcription, expression activation, and inactivation.[1] In particular, monitoring the change of local microenvironments such as dielectric properties in DNA and its binding proteins is highly important for understanding interbiomolecular interactions. A high-level calculation of the local dielectric environment of DNA strongly suggests that the inside of DNA grooves exhibits a much lower dielectric constant compared with that of water.[2] An ideal probe for monitoring various structures and dynamics of DNA and its surroundings should be sensitive to its local microenvironments and should be incorporated site-specifically throughout any DNA sequence of interest.

Therefore, we need to develop a reporter device with high sensitivity to the dielectric environment, and connect it to the target sequences of nucleic acids. For example, an artificial nucleobase with strong fluorescence, 2-aminopurine, is often used as a reporter for monitoring DNA conformational transition.[3] Benzopyridopyrimidine derivatives[4] and benzodeazapurine derivatives[5] show strong fluorescence emission only when they form a base pair with a target base. Fluorescent nucleobases in which the chromophores are directly linked with riboses, such as pyrene riboside,[6] coumarin riboside,[7] and Nile red riboside,[8] have also been developed. The nucleosides labeled with pyrenecarboxyamide, which show higher fluorescence intensity with higher polarity,[9] were applied to the sequence-selective typing of the complementary sequences.[10] The function of these fluorophores will be useful for the typing of single-nucleotide polymorphisms. 6-Dimethylamino-2-acylnaphthalene (DAN) is also a promising reporter device for the monitoring of dielectric change that has been extensively employed as a fluorescence probe for studying the microenvironments of various chemical and biological systems.[11] Nucleosides labeled with DAN have also been developed. Using DAN-labeled DNA probes, the dielectric constants of the microenvironment around the grooves of DNA duplexes were measured,[12] and we have estimated the dielectric constant around DNA polymerase-binding DNA using a DNA probe containing DAN–nucleoside conjugate **1** (Fig. 1).[13]

Fixation of the location of the fluorophore against DNA is one of the very important points of molecular design for monitoring the local environment around DNA. If we use a flexible linker between the fluorophore and DNA, data on the microenvironment obtained from fluorescence would be very ambiguous. Thus, we should design fluorophore–DNA

* Corresponding authors. Tel.: +81 48 467 9238; fax: +81 48 467 9205; e-mail addresses: aki-okamoto@riken.jp; saito@mech.ce.nihon-u.ac.jp

0040–4020/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2006.09.113

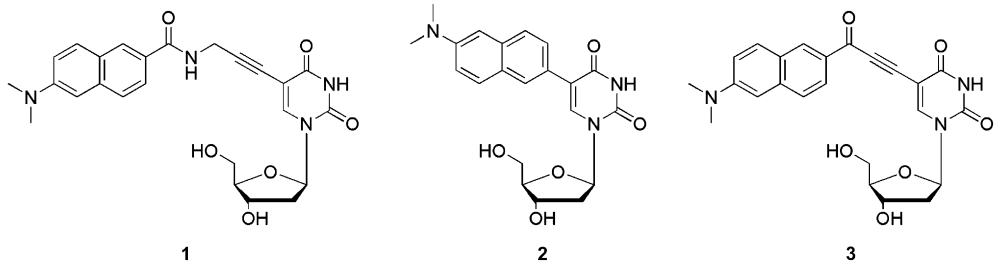


Figure 1. Novel fluorophore–deoxyuridine conjugates.

conjugates using rigid linkers for analyzing local microenvironmental changes around DNA.

In this paper, the synthesis and fluorescence properties of new DAN–deoxyuridine conjugates are reported. We designed DAN–deoxyuridine conjugates in which chromophores and 2′-deoxyuridine were covalently linked by rigid linkers. These nucleosides were synthesized efficiently via the palladium-mediated coupling of 5-iodo-2′-deoxyuridine and chromophores. Their fluorescence emission wavelength changed sensitively depending on solvent polarity.

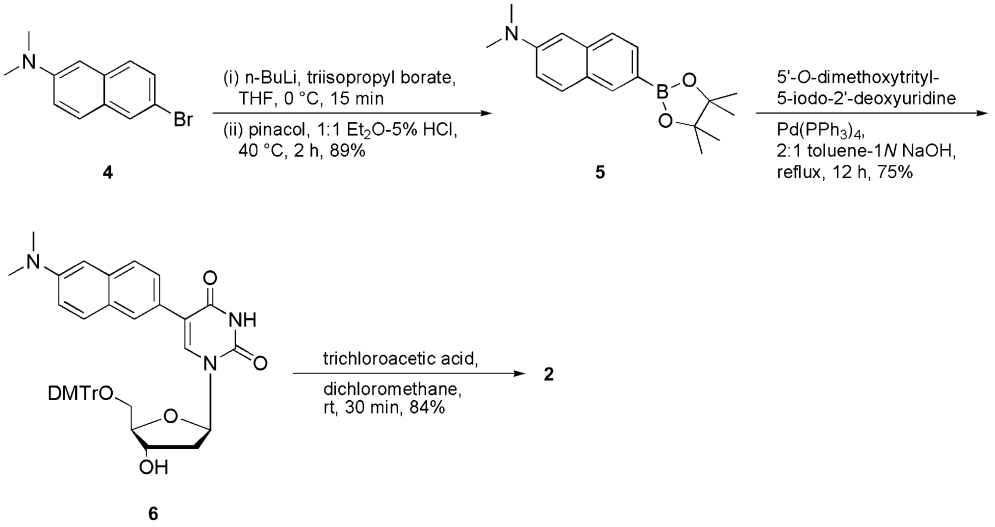
2. Results and discussion

DAN–deoxyuridine conjugates **2** and **3** have been designed (Fig. 1). Compound **2** is a conjugate in which dimethylaminonaphthalene and uracil base are directly tethered. The synthesis of **2** started with 2-dimethylamino-6-bromonaphthalene (**4**) (Scheme 1).[14] We converted **4** into the borate ester **5** (89%) and then **5** was coupled with 5′-protected 5-iodo-2′-deoxyuridine by a Suzuki coupling to give **6** (75%). Finally, acid hydrolysis of **6** provided the fluorescent nucleoside **2** (84%).

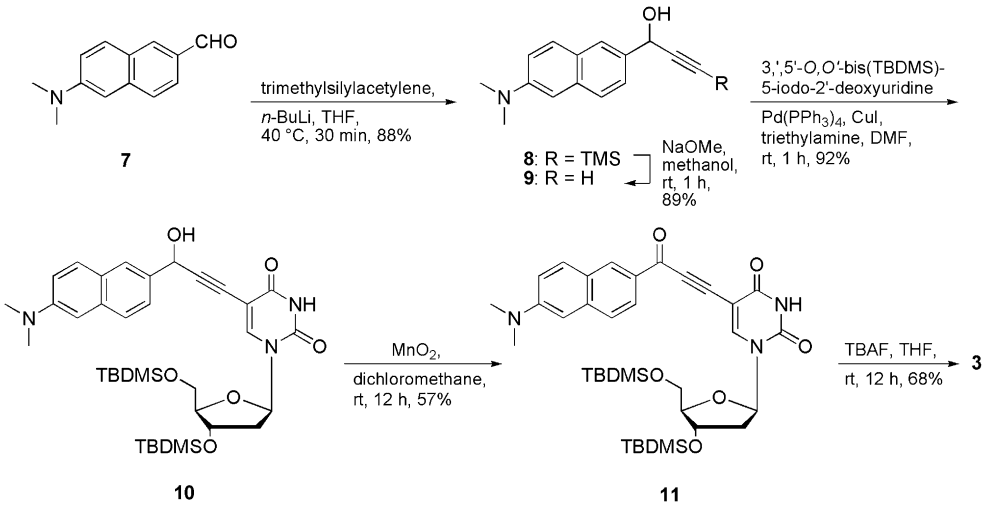
For the synthesis of **3**, 6-dimethylaminonaphthalene-2-carboxaldehyde (**7**) was used as the starting material (Scheme 2). Addition of trimethylsilyl acetylene to **7** efficiently gave **8** (88%). After deprotection of the acetylene (89%), **9** was coupled with 3′,5′-protected 5-iodo-2′-deoxyuridine[4a] through a Sonogashira coupling to give **10** (92%). The alcohol of the linker was oxidized to ketone **11** (57%), and then desilylation by TBAF provided the DAN-tethered nucleoside **3** (68%).

The absorption spectra of synthetic nucleosides **2** and **3** in ethyl acetate are shown with that of **1** in Figure 2. Their photophysical data are also summarized in Table 1. The absorption maxima for nucleosides **1**, **2**, and **3** were observed at 320, 330, and 410 nm, respectively. The absorption wavelength of **1** was much shorter than that reported for PRODAN, which is a representative DAN derivative and has absorption maxima at 342–370 nm.[11a,15] The large blue shift observed for **1** suggests that the conjugation between a naphthalene ring and a carbonyl group is weakened by the formation of an amide group. On the other hand, the absorption maximum wavelength of **2** was still shorter than that of PRODAN, but much longer than that observed for 2-dimethylaminonaphthalene (310 nm).[16] The red shift and signal broadening of the spectrum mean that the naphthalene ring conjugates well with the uracil base. Nucleoside **3** comprises the dimethylaminonaphthalene unit linked via a propynoyl group. The absorption spectrum of this nucleoside showed a broad signal at a wavelength above 400 nm, which is much longer than that observed for PRODAN.[17]

We next investigated the fluorescence properties of DAN–deoxyuridine conjugates. The nucleoside **1** showed a solvatofluorochromic character.[13] The emission maximum shifted from 406 nm for dioxane to 433 nm for dimethylsulfoxide



Scheme 1. Synthesis of a fluorescent nucleoside **2**.



Scheme 2. Synthesis of a fluorescent nucleoside **3**.

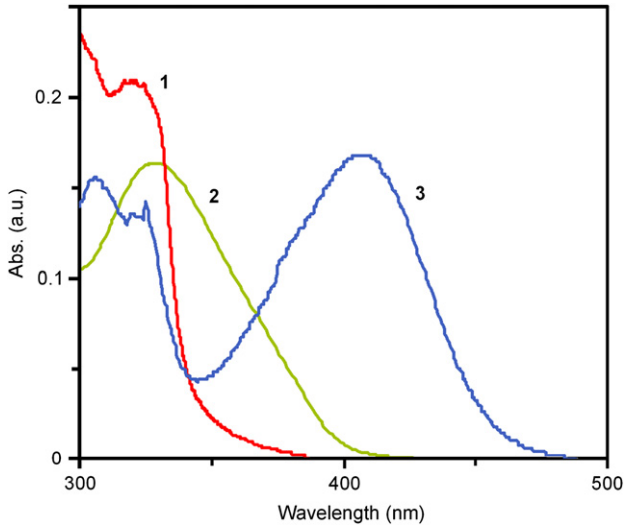


Figure 2. Absorption spectra of fluorescent nucleosides, **1**–**3**. The spectra were measured at 25 °C using 1 μM solution in ethyl acetate.

Table 1. Photophysical data of fluorescent nucleosides, **1**–**3**[a]

Nucleosides	Solvents	$\lambda_{ab.max}$ (nm)	ε	$\lambda_{em.max}$ (nm)	Φ[b]	E_T (kJ mol^{-1})[c]
1	Dioxane	325	1580	406	0.257	36
	EtOAc	325	2090	415	0.208	38
	CH_2Cl_2	332	1420	421	0.126	41
	DMSO	332	1640	433	0.124	45
2	Dioxane	333	1640	461	0.115	36
	EtOAc	331	1630	478	0.067	38
	CH_2Cl_2	341	1150	507	0.032	41
	CH_3CN	325	1540	527	0.003	46
3	EtOAc	405	1660	530	0.033	38
	CH_2Cl_2	410	2110	532	0.041	41
	DMSO	426	2010	535	0.136	45
	CH_3CN	411	1780	541	0.010	46

[a] A solution of 1 μM nucleoside in each solvent was measured at 25 °C.
[b] Quantum yields were calculated according to Ref. 20.
[c] See Ref. 19.

(Table 1). Nucleoside **2** exhibited a higher solvatofluorochromicity (Fig. 3). The emission wavelength of the fluorescence spectra changed significantly depending on the solvent polarity. The fluorescence quantum yields markedly decreased with increase in solvent polarity. The fluorescence in acetonitrile was faint (Φ=0.003). These properties indicate that **2** has a strong CT character. The conjugation between the dimethylaminonaphthalene as an electron donor and the uracil base as an electron acceptor[18] plays an important role in the large dipole change during excitation. In contrast, the solvatofluorochromism observed for nucleoside **3** was very small (Fig. 4). The excitation and emission spectra showed some dependence on solvent polarity, but the shift of spectra was not so large compared with those of **1** and **2**. The fluorescence emission maximum wavelength of **3** was longest among three DAN–deoxyuridine conjugates. The conjugation with a propynoyl group strongly affects the large red shift of the emission maxima and the decrease in sensitivity to solvents.

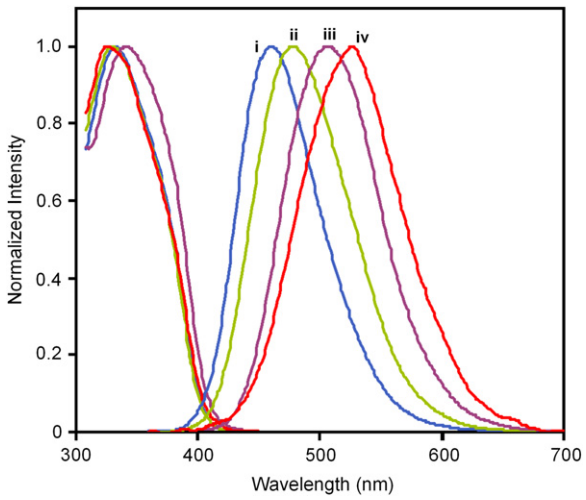


Figure 3. Fluorescence excitation and emission spectra of **2**. All spectra were measured at 25 °C using 1 μM solution in ethyl acetate. Emission spectra were measured with excitation at $\lambda_{ex.max}$ in each solvent. Excitation spectra were measured for $\lambda_{em.max}$ in each solvent. (i) Dioxane (blue), (ii) ethyl acetate (green), (iii) dichloromethane (purple), (iv) acetonitrile (red).

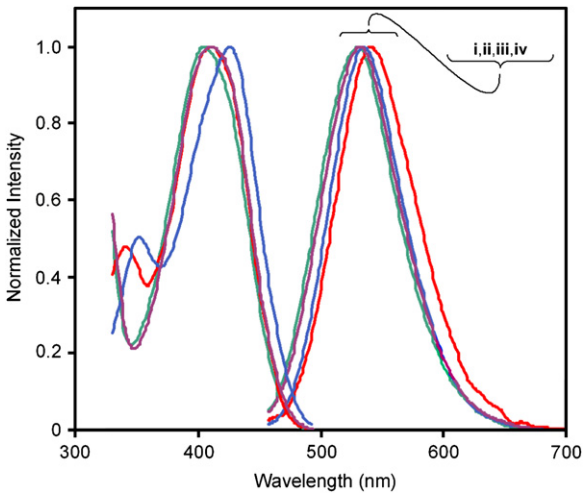


Figure 4. Fluorescence excitation and emission spectra of **3**. All spectra were measured at 25 °C using 1 μM solution in ethyl acetate. Emission spectra were measured with excitation at $\lambda_{ex.max}$ in each solvent. Excitation spectra were measured for $\lambda_{em.max}$ in each solvent. (i) Ethyl acetate (green), (ii) dichloromethane (brown), (iii) dimethylsulfoxide (blue), (iv) acetonitrile (red).

The relationship between solvent polarity and emission wavelength of DAN–deoxyuridine conjugates was analyzed. The emission maxima in various solvents were plotted versus the solvent $E_T(30)$ values, a solvent polarity parameter,[19] based on the data summarized in Table 1 (Fig. 5). The change of the emission wavelength of the DAN–deoxyuridine conjugates was proportional to the solvent polarity, indicating their potential use as CT fluorescent probes. Among the three DAN–deoxyuridine conjugates, **2** showed the largest slope, revealing that **2** has the highest CT property, i.e., the fluorescence from **2** is most sensitive to solvent polarity.

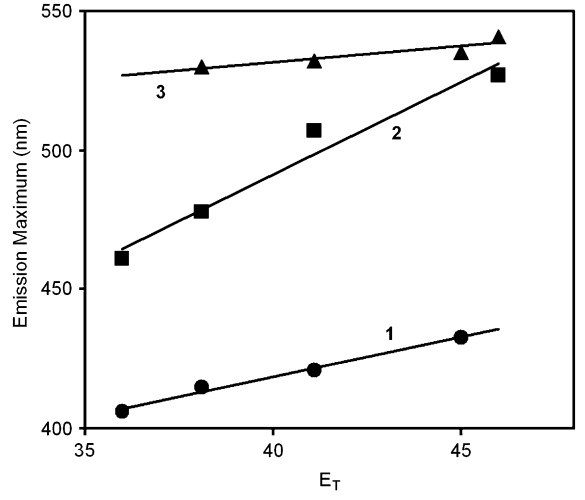


Figure 5. Plot of emission maxima of fluorescent nucleosides, **1**–**3**, as a function of $E_T(30)$ at 25 °C.

3. Conclusion

We have developed novel fluorescent nucleosides **2** and **3**, in which the uracil base and the fluorophore are tethered by rigid linkers. They showed unique absorption and fluorescence emission spectra. Nucleoside **2** is a fluorophore with high CT character, and the fluorescence was very sensitive to solvent polarity. Nucleoside **2** may be ineffective for micropolarity analysis in polar environments, because the fluorescence quantum yields are not large in polar solvents. However, the large emission shifts might make **2** a very effective probe for the analysis of relatively non-polar regions. Nucleoside **3** showed absorption and emission maxima at longer wavelength due to extension of the DAN-conjugate system. Although **3** may not be suitable for microenvironment analysis using the spectrum shift because the solvatofluorochromicity of **3** is small, it can be expected to work as a fluorophore with long wavelength in which the fluorescence intensity changes according to the change in micropolarity. These fluorescent nucleosides might be useful as probes sensitive to changes in the microenvironment around specific sites of DNA upon incorporation.

4. Experimental

4.1. General

^{1}H NMR spectra were measured with Varian Mercury 400 (400 MHz) spectrometer. ^{13}C NMR spectra were measured with JEOL JNM α-500 (125 MHz) spectrometer. Coupling constants (*J* value) are reported in Hertz. The chemical shifts are expressed in ppm downfield from tetramethylsilane, using residual chloroform (δ=7.24 in ^{1}H NMR, δ=77.0 in ^{13}C NMR), methanol (δ=3.30 in ^{1}H NMR, δ=49.1 in ^{13}C NMR), and DMSO (δ=2.49 in ^{1}H NMR, δ=39.5 in ^{13}C NMR) as internal standards. FAB mass spectra were recorded on JEOL JMS DX-300 spectrometer or JEOL JMS SX-102A spectrometer.

4.2. Synthesis of 5-(6-dimethylamino-2-naphthyl)-2′-deoxyuridine (2)

4.2.1. 6-Dimethylamino-2-naphthaleneboronic acid, pinacol ester (5). To a solution of **4** (125 mg, 0.50 mmol) in THF (2 mL) was slowly added 1.6 M *n*-BuLi (343 μL, 0.55 mmol) at −78 °C under nitrogen. After the mixture was stirred for 30 min, triisopropylborate (0.23 mL, 1.00 mmol) was added to the solution at −78 °C. The reaction mixture was stirred at 0 °C for 15 min. To the resulting pale yellow solution was slowly added a solution of pinacol (236 mg, 2.00 mmol) in diethyl ether (5 mL) and 5% aq HCl (5 mL). After being stirred for 2 h at 40 °C, the mixture was extracted with ether and dried over $MgSO_4$. The crude product was purified by silica gel chromatography (hexane–ethyl acetate=25:1) to yield **5** (132 mg, 0.45 mmol, 89%) as a purple solid; ^{1}H NMR ($CDCl_3$) δ 8.21 (s, 1H), 7.74 (d, 1H, *J*=8.8 Hz), 7.72 (d, 1H, *J*=7.5 Hz), 7.62 (d, 1H, *J*=8.4 Hz), 7.13 (dd, 1H, *J*=2.0, 9.2 Hz), 6.88 (s, 1H), 3.07 (s, 6H), 1.37 (s, 12H); MS (FAB) *m/z* 297 [M^+]; HRMS (FAB) calcd for $C_{18}H_{24}O_2NB$ [M^+] 297.1900, found 297.1901.

4.2.2. 5-(6-Dimethylamino-2-naphthyl)-5′*O*-dimethoxytrityl-2′-deoxyuridine (6). To a solution of **5** (119 mg, 0.40 mmol), dimethoxytrityl-protected 5-iodo-2′-deoxyuridine (263 mg, 0.40 mmol), and tetrakis(triphenylphosphine)palladium(0) (46 mg, 10 mol %) in toluene (10 mL)

was added 1 N NaOH (5 mL) under nitrogen. The mixture was refluxed for 12 h. The resulting solution was neutralized with 5% aq HCl and extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by silica gel chromatography (chloroform–methanol=40:1) to yield **6** (211 mg, 0.30 mmol, 75%) as a pale yellow solid; ^{1}H NMR ($CDCl_3$) δ 9.00 (br s, 1H), 7.76 (s, 1H), 7.63 (s, 1H), 7.41 (d, 1H, J=8.6 Hz), 7.35–7.34 (m, 2H), 7.28 (d, 1H, J=1.6 Hz), 7.24 (d, 1H, J=9.2 Hz), 7.19–7.12 (m, 7H), 7.00 (dd, 1H, J=2.4, 9.0 Hz), 6.80 (d, 1H, J=2.4 Hz), 6.61–6.58 (m, 4H), 6.42 (dd, 1H, J=5.7, 7.5 Hz), 4.52–4.49 (m, 1H), 4.12–4.10 (m, 1H), 3.62 (s, 6H), 3.38 (dd, 1H, J=3.5, 10.4 Hz), 3.30 (dd, 1H, J=3.7, 10.4 Hz), 3.02 (s, 6H), 2.54 (ddd, 1H, J=2.4, 5.5, 13.5 Hz), 2.36–2.29 (m, 1H); MS (FAB) m/z 699 [M$^+$]; HRMS (FAB) calcd for $C_{42}H_{41}O_7N_3$ [M$^+$] 699.2945, found 699.2975.

4.2.3. 5-(6-Dimethylamino-2-naphthyl)-2′-deoxyuridine (2). To a solution of **6** (70 mg, 0.10 mmol) in dichloromethane (1 mL) was added 3% trichloroacetic acid in dichloromethane (2 mL) and the mixture was stirred for 30 min at room temperature. The resulting mixture was evaporated and purified by silica gel chromatography (chloroform–methanol=10:1) to yield **2** (33 mg, 0.08 mmol, 84%) as a yellow solid; ^{1}H NMR (CD_3OD) δ 8.29 (s, 1H), 7.76 (d, 1H, J=1.5 Hz), 7.70 (d, 1H, J=9.2 Hz), 7.63 (d, 1H, J=8.5 Hz), 7.50 (dd, 1H, J=1.8, 8.9 Hz), 7.20 (dd, 1H, J= 2.4, 8.9 Hz), 6.94 (d, 1H, J=2.4 Hz), 6.37 (t, 1H, J=6.7 Hz), 4.45 (dd, 1H, J=5.2, 8.5 Hz), 3.95 (dd, 1H, J=3.1, 6.4 Hz), 3.82 (dd, 1H, J=3.1, 12.2 Hz), 3.73 (dd, 1H, J=3.4, 11.9 Hz), 3.02 (s, 6H), 2.54 (dd, 2H, J=5.2, 6.7 Hz); MS (FAB) m/z 397 [M$^+$]; HRMS (FAB) calcd for $C_{21}H_{23}O_5N_3$ [M$^+$] 397.1638, found 397.1627.

4.3. Synthesis of 5-[3-(6-dimethylamino-2-naphthyl)-3-oxo-2-propynyl]-2′-deoxyuridine (3)

4.3.1. 6-Dimethylamino-2-(3-trimethylsilyl-1-hydroxy-2-propynyl)naphthalene (8). To a solution of trimethylsilyl acetylene (0.242 mL, 1.71 mmol) in THF (2 mL) was added 1.6 M *n*-BuLi (1.03 mL, 1.65 mmol) at −78 °C under nitrogen. The mixture was stirred at room temperature for 30 min. To the reaction mixture was added **7** (273 mg, 1.37 mmol) in THF (3 mL) at −78 °C, and stirred at 0 °C for 30 min. After the reaction was quenched with aq NH_4Cl at 0 °C, the mixture was evaporated under reduced pressure and extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by silica gel chromatography (hexane–ethyl acetate=20–8:1) to yield **8** (341 mg, 1.15 mmol, 88%) as a yellow solid; ^{1}H NMR ($CDCl_3$) δ 7.80 (s, 1H), 7.69–7.64 (m, 2H), 7.52 (dd, 1H, J=1.7, 8.5 Hz), 6.91 (s, 1H), 3.03 (s, 6H), 2.33 (br s, 1H), 0.21 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 148.9, 134.8, 133.9, 129.0, 126.8, 125.4, 125.1, 116.6, 106.4, 105.3, 91.4, 65.3, 40.9, −0.1; MS (FAB) m/z 297 [M$^+$]; HRMS (FAB) calcd for $C_{18}H_{23}ONSi$ [M$^+$] 297.1549, found 297.1550.

4.3.2. 6-Dimethylamino-2-(1-hydroxy-2-propynyl)naphthalene (9). To a solution of **8** (1.33 g, 4.47 mmol) in methanol (50 mL) was added sodium methoxide (241 mg, 4.47 mmol) under nitrogen, and the reaction mixture was stirred at room temperature for 1 h. The resulting mixture was neutralized with 1 M HCl and extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by silica gel chromatography (hexane–ethyl acetate=10–1:1) to yield **9** (890 mg, 3.96 mmol, 89%) as a yellow solid; ^{1}H NMR ($CDCl_3$) δ 7.84 (s, 1H), 7.73–7.67 (m, 2H), 7.54 (dd, 1H, J=1.7, 8.5 Hz), 7.18 (dd, 1H, J=2.5, 9.1 Hz), 6.92 (s, 1H), 5.57 (s, 1H), 3.06 (s, 6H), 2.71 (d, 1H, J=2.2 Hz), 2.25 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 149.0, 134.9, 133.5, 129.0, 126.9, 126.3, 125.3, 124.9, 116.7, 106.3, 83.9, 74.6, 64.7, 40.8; MS (FAB) m/z 225 [M$^+$]; HRMS (FAB) calcd for $C_{15}H_{15}ON$ [M$^+$] 225.1154, found 225.1150.

4.3.3. 5-[3-(6-Dimethylamino-2-naphthyl)-3-hydroxy-2-propynyl]-3′*O*,5′*O*-bis(*tert*-butyldimethylsilyl)-2′-deoxyuridine (10). To a solution of *tert*-butyldimethylsilyl-protected 5-iodo-2′-deoxyuridine (367 mg, 0.630 mmol), tetrakis(triphenylphosphine)palladium(0) (133 mg, 0.115 mmol, 20 mol %), and copper(I) iodide (44 mg, 0.23 mmol, 40 mol %) in DMF (0.5 mL) were added **9** in DMF (278 mg, 1.15 mmol in 3.5 mL) and triethylamine (0.165 mL, 1.15 mmol) under nitrogen. The mixture was stirred at room temperature for 1 h. The resulting mixture was evaporated in vacuo and diluted with ethyl acetate. The solution was washed with saturated EDTA solution and 5% sodium bisulfite solution, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (toluene–ethyl acetate=4:1) to yield a diastereomeric mixture of **10** (393 mg, 0.58 mmol, 92%) as a yellow solid; ^{1}H NMR ($CDCl_3$) δ 8.59 (br s, 1H), 7.98 (d, 1H, J=3.3 Hz), 7.88 (s, 1H), 7.71 (d, 1H, J=9.0 Hz), 7.64 (d, 1H, J= 9.6 Hz), 7.56 (dd, 1H, J=1.6, 8.5 Hz), 7.15 (dd, 1H, J=2.5, 9.1 Hz), 6.90 (d, 1H, J=2.0 Hz), 6.27 (dd, 1H, J=5.8, 7.6 Hz), 5.73 (s, 1H), 4.39–4.37 (m, 1H), 3.97–3.96 (m, 1H), 3.85 (ddd, 1H, J=2.6, 5.4, 11.4 Hz), 3.74–3.71 (m, 1H), 3.04 (s, 6H), 2.69 (br s, 1H), 2.30 (ddd, 1H, J=2.5, 5.8, 13.1 Hz), 2.04–1.96 (m, 1H), 0.89–0.83 (m, 18H), 0.08–0.02 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 161.2, 149.0, 142.5, 134.9, 133.7, 129.1, 126.8, 126.42, 126.43, 125.52, 125.46, 125.12, 125.07, 116.6, 106.3, 99.6, 94.2, 88.4, 85.91, 85.88, 77.6, 77.5, 72.4, 65.3, 63.0, 42.0, 40.8, 25.9, 25.7, 18.3, 18.0, −4.8, −4.7, −5.4, −5.6; MS (FAB) m/z 679 [M$^+$]; HRMS (FAB) calcd for $C_{36}H_{53}O_6N_3Si_2$ [M$^+$] 679.3473, found 679.3475.

4.3.4. 5-[3-(6-Dimethylamino-2-naphthyl)-3-oxo-2-propynyl]-3′*O*,5′*O*-bis(*tert*-butyldimethylsilyl)-2′-deoxyuridine (11). To a solution of **10** (138 mg, 0.20 mmol) in dichloromethane (8 mL) was added activated manganese dioxide (878 mg, 10.1 mmol) under nitrogen. The mixture was vigorously stirred at room temperature for 12 h. To the resulting mixture were added diethyl ether (16 mL) and Florisil (150–250 μm, 60–100 mesh, 140 mg). The suspension was stirred at room temperature for 15 min, filtered through Celite, and extracted with chloroform. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and evaporated reduced pressure. The crude product was purified by silica gel column chromatography (hexane–ethyl

acetate=3–2:1) to yield **11** (78 mg, 0.12 mmol, 57%) as an orange solid; ^{1}H NMR ($CDCl_3$) δ 8.82 (d, 1H, J=8.4 Hz), 8.52 (br s, 1H), 8.36 (s, 1H), 8.09 (dd, 1H, J=1.7, 8.7 Hz), 7.89 (d, 1H, J=9.2 Hz), 7.62 (d, 1H, J=8.8 Hz), 7.13 (dd, 1H, J=2.5, 9.1 Hz), 6.85 (d, 1H, J=2.4 Hz), 6.30 (dd, 1H, J=6.0, 7.2 Hz), 4.44–4.43 (m, 1H), 4.05–4.04 (m, 1H), 3.92 (dd, 1H, J=2.7, 11.4 Hz), 3.79 (dd, 1H, J=2.3, 11.4 Hz), 3.10 (s, 6H), 2.41 (ddd, 1H, J=2.7, 5.9, 13.2 Hz), 2.11–2.05 (m, 1H), 0.99–0.85 (m, 18H), 0.24–0.07 (m, 12H); MS (FAB) m/z 678 [M^+]; HRMS (FAB) calcd for $C_{36}H_{51}O_6N_3Si_2$ [M^+] 677.3316, found 677.3320.

4.3.5. 5-[3-(6-Dimethylamino-2-naphthyl)-3-oxo-2-propynyl]-2′-deoxyuridine (3). To a solution of **11** (35 mg, 0.05 mmol) in THF (0.5 mL) was added 1 M solution of TBAF (0.1 mL, 0.1 mmol) and the mixture was stirred at room temperature for 12 h. The mixture was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (chloroform–methanol= 20:1) to yield **3** (16 mg, 0.04 mmol, 68%) as an orange solid; ^{1}H NMR (DMSO-d_6) δ 11.9 (br s, 1H), 8.75 (s, 1H), 8.73 (s, 1H), 7.96–7.91 (m, 2H), 7.71 (d, 1H, J=8.8 Hz), 7.31 (dd, 1H, J=2.3, 9.1 Hz), 6.98 (d, 1H, J=2.2 Hz), 6.12 (t, 1H, J=6.2 Hz), 5.29–5.25 (m, 2H), 4.29–4.28 (m, 1H), 3.85–3.84 (m, 1H), 3.75–3.70 (m, 1H), 3.66–3.61 (m, 1H), 3.09 (s, 6H), 2.29–2.17 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 175.6, 161.7, 150.6, 149.3, 148.1, 137.9, 132.8, 131.0, 129.9, 126.1, 124.3, 123.8, 116.6, 104.7, 95.2, 91.3, 87.8, 87.4, 85.5, 69.5, 60.6, 40.4, 39.8; MS (FAB) m/z 450 [$(M+H)^+$]; HRMS (FAB) calcd for $C_{24}H_{24}O_6N_3$ [$(M+H)^+$] 450.1665, found 450.1665.

References and notes

1. (a) Ranasinghe, R. T.; Brown, T. *Chem. Commun.* **2005**, 5487–5502; (b) Rist, M. J.; Marino, J. P. *Curr. Org. Chem.* **2002**, *6*, 775–793.
2. (a) Lamm, G.; Pack, G. R. *J. Phys. Chem. B* **1997**, *101*, 959–965; (b) Young, M. A.; Jayaram, B.; Beveridge, D. L. *J. Phys. Chem. B* **1998**, *102*, 7666–7669.
3. (a) Rachofsky, E. L.; Osman, R.; Ross, J. B. A. *Biochemistry* **2001**, *40*, 946–956; (b) Frey, M. W.; Sowers, L. C.; Millar, D. P.; Benkovic, S. J. *Biochemistry* **1995**, *34*, 9185–9192; (c) Menger, M.; Tuschl, T.; Eckstein, F.; Porschke, D. *Biochemistry* **1996**, *35*, 14710–14716; (d) Beechem, J. M.; Otto, M. R.; Bloom, L. B.; Eritja, R.; Reha-Krantz, L. J.; Goodman, M. F. *Biochemistry* **1998**, *37*, 10144–10155; (e) Holz, B.; Klimasauskas, S.; Serva, S.; Weinhold, E. *Nucleic Acids Res.* **1998**, *26*, 1076–1083.
4. (a) Okamoto, A.; Tainaka, K.; Saito, I. *J. Am. Chem. Soc.* **2003**, *125*, 4972–4973; (b) Okamoto, A.; Tainaka, K.; Saito, I. *Tetrahedron Lett.* **2003**, *44*, 6871–6874.
5. (a) Okamoto, A.; Tainaka, K.; Fukuta, T.; Saito, I. *J. Am. Chem. Soc.* **2003**, *125*, 9296–9297; (b) Okamoto, A.; Tanaka, K.; Fukuta, T.; Saito, I. *ChemBioChem* **2004**, *5*, 958–963.
6. (a) Strässler, C.; Davis, N. E.; Kool, E. T. *Helv. Chim. Acta* **1999**, *82*, 2160–2171; (b) Kool, E. T. *Acc. Chem. Res.* **2002**, *35*, 936–943.
7. Brauns, E. B.; Madaras, M. L.; Coleman, R. S.; Murphy, C. J.; Berg, M. *J. Am. Chem. Soc.* **1999**, *121*, 11644–11649.
8. Okamoto, A.; Tainaka, K.; Fujiwara, Y. *J. Org. Chem.* **2006**, *71*, 3592–3598.
9. (a) Kalyanasundaram, K.; Thomas, J. K. *J. Phys. Chem.* **1977**, *81*, 2176–2180; (b) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515–1566.
10. (a) Okamoto, A.; Kanatani, K.; Saito, I. *J. Am. Chem. Soc.* **2004**, *126*, 4820–4827; (b) Saito, Y.; Miyauchi, Y.; Okamoto, A.; Saito, I. *Chem. Commun.* **2004**, 1704–1705; (c) Saito, Y.; Miyauchi, Y.; Okamoto, A.; Saito, I. *Tetrahedron Lett.* **2004**, *45*, 7827–7831; (d) Dohno, C.; Saito, I. *ChemBioChem* **2005**, *6*, 1075–1081; (e) Saito, Y.; Hanawa, K.; Motegi, K.; Omoto, K.; Okamoto, A.; Saito, I. *Tetrahedron Lett.* **2005**, *46*, 7605–7608; (f) Okamoto, A.; Tainaka, K.; Ochi, Y.; Kanatani, K.; Saito, I. *Mol. BioSyst.* **2006**, *2*, 122–127.
11. (a) Weber, G.; Farris, F. J. *Biochemistry* **1979**, *18*, 3075–3078; (b) MacGregor, R. B.; Weber, G. *Nature* **1986**, *319*, 70–73; (c) Parasassi, T.; Conti, F.; Gratton, E. *Cell. Mol. Biol.* **1986**, *32*, 103–108.
12. (a) Kimura, T.; Kawai, K.; Majima, T. *Org. Lett.* **2005**, *7*, 5829–5832; (b) Kimura, T.; Kawai, K.; Majima, T. *Chem. Commun.* **2006**, 1542–1544.
13. Okamoto, A.; Tainaka, K.; Saito, I. *Bioconjugate Chem.* **2005**, *16*, 1105–1111.
14. Umezawa, H.; Tsuji, K.; Anwar, D. X.; Okada, S.; Oikawa, H.; Matsuda, H.; Nakanishi, H. *MCLC S&T, Sect. B: Nonlinear Optics* **2000**, *24*, 73–78.
15. Ilich, P.; Prendergast, F. G. *J. Phys. Chem.* **1989**, *93*, 4441–4447.
16. Stephen, R. M.; Desmond, V. O.; David, P. *J. Chem. Soc., Faraday Trans. 2* **1983**, *79*, 1563–1584.
17. Prendergast, F. G.; Meyer, M.; Carlson, G. L.; Iida, S.; Potter, J. D. *J. Biol. Chem.* **1983**, *258*, 7541–7544.
18. (a) Kerr, C. E.; Mitchell, C. D.; Headrick, J.; Eaton, B. E.; Netzel, T. L. *J. Phys. Chem. B* **2000**, *104*, 2166–2175; (b) Amann, N.; Pandurski, E.; Fiebig, T.; Wagenknecht, H.-A. *Chem.—Eur. J.* **2002**, *8*, 4877–4883.
19. Reichardt, C. *Chem. Rev.* **1994**, *94*, 2319–2358.
20. Morris, J. V.; Mahaney, M. A.; Huber, J. R. *J. Phys. Chem.* **1976**, *80*, 969–974.