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Synthesis of bicyclic dioxetanes tethering a fluororescer through an ω-carbamoyl-substituted linker and their high-performance chemiluminescence in an aqueous system

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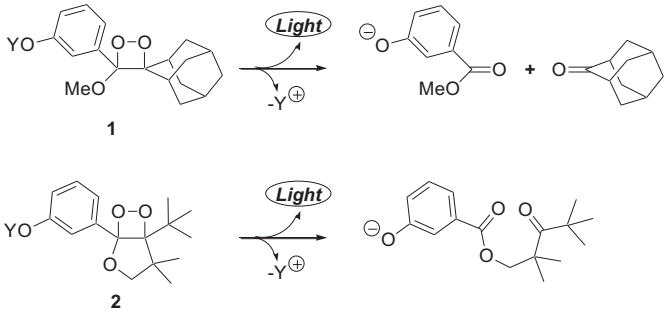
ABSTRACT

Bicyclic dioxetanes **3** and **4** tethering a fluorescein or 4-(benzothiazol-2-yl)-3-hydroxyphenyl moiety through a linker were synthesized by the use of dihydrofuran-intermediate **5** or its advanced intermediate **6**. These dioxetanes underwent base-induced decomposition to effectively give light due to intramolecular energy-transfer from an excited oxidobenzoate to a tethered fluorophore. Although the chemiluminescence efficiency Φ^{CL} values for **3** and **4** were only ca. 2-fold greater than that for parent **2** in a TBAF/acetonitrile system, these values were 30–550-fold greater than that for **2** in a NaOH/H_2O system. Such marked increase of Φ^{CL} was hardly observed by the simple addition of **25** or **26** as a model of a tethered fluorescer.

1. Introduction

The intramolecular charge-transfer-induced decomposition (CTID) of oxidophenyl-substituted dioxetanes has received considerable attention due to interest in the mechanisms of bioluminescence and chemiluminescence and because of possible applications in modern biological and clinical analyses using chemiluminescence.[1–4] Typical examples of such CTID-active dioxetanes are adamantylidene-substituted dioxetane **1** and bicyclic dioxetane **2** (Scheme 1).[2,5,6] Although these dioxetanes effectively emit light in an aprotic polar solvent, they give light in quite poor yield in an aqueous medium. This significant defect has been considerably improved through the addition of a fluorescer such as fluorescein and/or a surfactant such as quaternary ammonium or phosphonium salt for practical use in an aqueous system.[7,8]

This situation prompted us to realize new CTID-active dioxetanes tethering a fluorescer that show highly effective chemiluminescence without any additives in an aqueous system.[9,10] Since it is more appropriate than **1** to structural modification for the present purpose, dioxetane **2** was selected as a basic skeleton.[11,12] To modify the structure of **2** with a minimal decrease in thermal stability and minimal change in the structure around the dioxetane ring, we planned to functionalize a methyl of the *tert*-butyl group in **2**. The resulting dioxetanes **3** and **4** tethered a fluorescein or 4-(benzothiazol-2-yl)-3-hydroxyphenyl moiety as a highly efficient fluorescer (Fig. 1).



Scheme 1. Base-induced chemiluminescent decomposition of 3-oxyphenyl-substituted dioxetanes **1** and **2**.

We report here that **3** and **4** were effectively synthesized by using dihydrofuran-intermediate **5** bearing an ω-carboxy-substituted linker,[13,14] which was prepared from the key building block **7** or by using advanced intermediate **6** bearing an *N*-hydroxysuccinimide (HOSu) ester moiety (Fig. 1).[15] We also report that dioxetanes **3** and **4** gave light far more efficiently than the parent **2a** in an aqueous medium.

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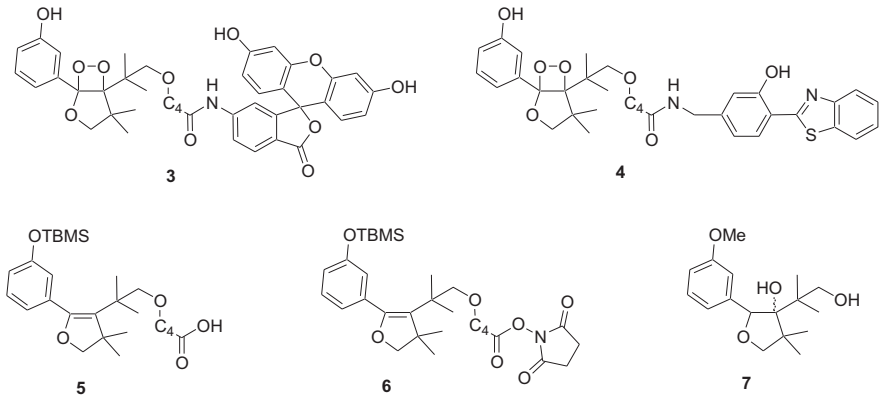
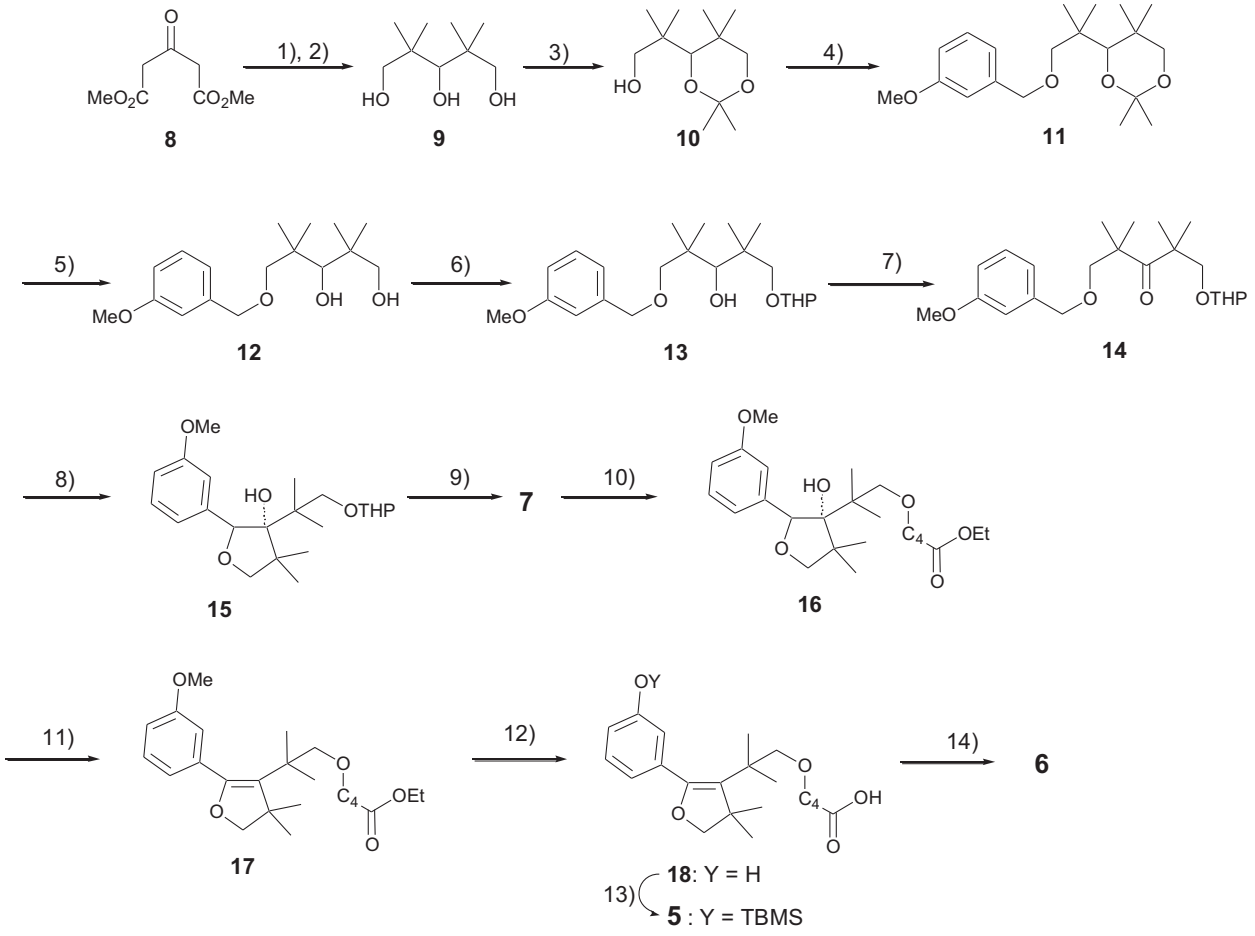


Fig. 1. Bicyclic dioxetanes **3** and **4** tethering a fluorescer and their synthetic intermediates **5**–**7**.

2. Results and discussion

2.1. Synthesis of bicyclic dioxetanes tethering a fluorescer through a linker

First, we synthesized a key building block **7** for the preparation of dihydrofuran-intermediate **5** starting from 2,2,4,4-tetramethylpentane-1,3,5-triol (**9**). The triol **9** was prepared from dimethyl 3-oxopentanedioate **8** through the introduction of four methyl groups followed by reduction with $LiAlH_4$ (Scheme 2). Two hydroxy groups in triol **9** were protected as cyclic acetal **10**, which was in turn subjected to Williamson synthesis with 3-methoxybenzyl chloride to selectively give benzyl ether **11**. Compound **11** was then deprotected to give diol **12**, in which only the primary OH was protected to give tetrahydropyranyl (THP) ether **13**. The remaining secondary OH in **13** was oxidized with PCC to give



Reagents: 1) NaH/MeI, 2) $LiAlH_4$; 3) $Me_2C(OMe)_2$/PPTS; 4) 3-$MeOC_6H_4CH_2Cl$/NaH; 5) H_2O/MeOH/1N HCl; 6) DHP/PPTS; 7) PCC; 8) LDA; 9) MeOH/1N HCl; 10) $Br(CH_2)_4CO_2Et$/ NaH; 11) $SOCl_2$/pyridine; 12) MeSNa/DMF/Δ; 13) t-$BuMe_2SiCl$/DMAP; 14) di(N-hydroxysuccimidyl) carbonate

Scheme 2. Synthetic pathway starting from dimethyl 3-oxopentanedioate **8** to intermediate **5** and advanced intermediate **6** through key building block **7**.

1-benzyloxypentan-3-one **14**. LDA-mediated cyclization[16] of **14** effectively took place at low temperature to give 3-hydroxytetrahydrofuran **15** bearing a THP-oxy group, deprotection of which quantitatively gave the desired key building block **7** as a mixture of stereoisomers.

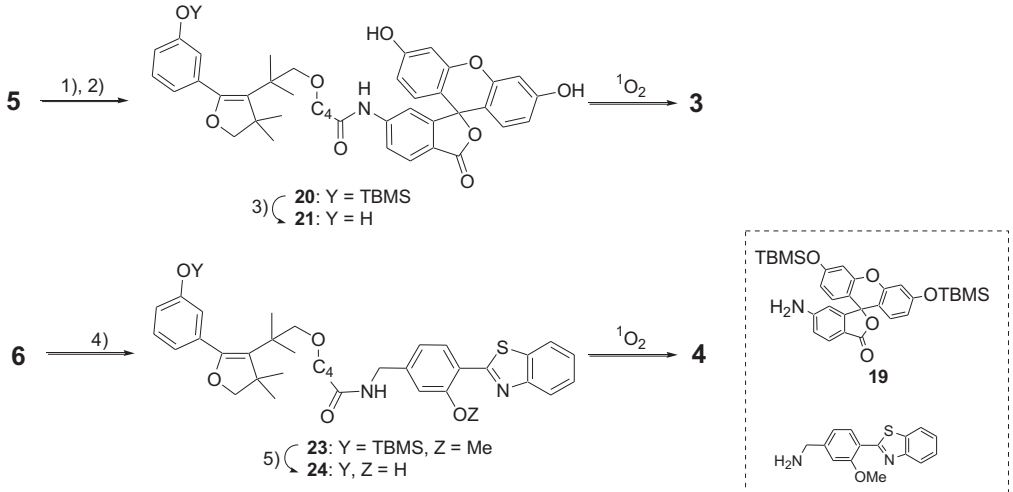
Key building block **7** was condensed with ethyl 5-bromopentanoate to selectively give ester **16**. The hydroxytetrahydrofuran **16** was dehydrated with $SOCl_2$/pyridine to give the corresponding dihydrofuran **17** (Scheme 2). When **17** was treated with MeSNa in hot DMF, both demethylation and saponification took place to give 5-(3-hydroxyphenyl)-2,3-dihydrofuran **18** bearing an ω-carboxy-substituted linker. Then, a phenolic hydroxyl group in **18** was protected with *tert*-butyldimethylsilyl (TBMS) chloride to give the desired dihydrofuran-intermediate **5**. Condensation of **5** with bis(*N*-hydroxysuccinimidyl) carbonate gave HOSu ester **6** as an advanced intermediate.

Intermediate **5** was transformed to its acid chloride in situ, which was coupled with TBMS-protected 5-aminofluorescein **19** in pyridine to give dihydrofuran **20** bearing a fluorescein moiety (Scheme 3). Deprotection of a siloxy group in **20** with tetrabutylammonium fluoride (TBAF) gave precursor **21** of dioxetane tethering a fluorescein. On the other hand, condensation of **6** with 4-(benzothiazol-2-yl)-3-methoxybenzylamine **22** gave dihydrofuran **23** tethering a 4-(benzothiazol-2-yl)-3-methoxyphenyl moiety through an amide linkage. Amide **23** was further treated with hot MeSNa in DMF gave precursor **24** leading to dioxetane **4**.

Finally, dihydrofurans **21** and **24** were individually irradiated together with a catalytic amount of tetraphenylporphin (TPP) in CH_2Cl_2 with a Na-lamp under an oxygen atmosphere at 0 °C. Thus, 1,2-addition of singlet oxygen to **21** and **24** smoothly took place to selectively give the corresponding dioxetanes **3** and **4**. The structures of these dioxetanes were determined by ^{1}H NMR, ^{13}C NMR, IR, and HRMass spectral analyses.

2.2. Chemiluminescent decomposition of bicyclic dioxetanes tethering a fluorescein or 4-(benzothiazol-2-yl)-3-hydroxyphenyl moiety through a linker

First of all, we investigated CTID of dioxetanes **3** and **4** in a TBAF/acetonitrile system, since this triggering system has been often used to evaluate chemiluminescence properties of CTID-active dioxetanes.[3–6] When **3** was treated with a large excess of TBAF in acetonitrile at 25 °C, **3** decomposed according to pseudo-first-order kinetics to effectively give light with λ_{max}^{CL} = 535 nm (Fig. 2A), rate of CTID k^{CTID}=4.7×10^{-3} s^{-1} and chemiluminescence efficiency Φ^{CL}=0.19,[17,18] the value of which was 1.7 times larger than that for **2a** (Table 1, entries 1 and 2). This chemiluminescence spectrum coincided with fluorescence spectrum of acetamidofluorescein **25**



Reagents: 1) $SOCl_2/CH_2Cl_2$, 2) **19**/pyridine; 3) TBAF/THF; 4) **22**/Et_3N; 5) MeSNa/DMF/Δ

Scheme 3. Synthesis of dioxetanes **3** and **4** through precursors **21** and **24**.

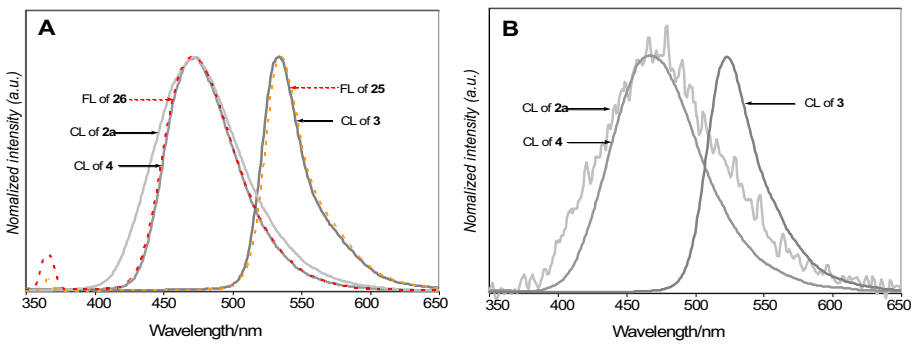


Fig. 2. (A) Chemiluminescence (CL) spectra of dioxetanes **2a**, **3**, and **4**, and fluorescence (FL) spectra of **25** and **26** in TBAF/acetonitrile. (B) Chemiluminescence (CL) spectra of dioxetanes **2a**, **3**, and **4** in NaOH/H_2O.

Table 1
Base-induced chemiluminescent decomposition of dioxetane **2a** and dioxetanes **3** and **4** tethering a fluorescer[a]

Entry	Dioxetane	System[b]	Additive	λ_{max}^{CL}/nm	Φ^{CL}[c]	k^{CTID}/s^{-1}	Relative Φ^{CL}[d]
1[e]	**2a**	A	—	467	0.11	2.8×10^{-2}	1
2	**3**	A	—	535	0.19	4.7×10^{-3}	1.7
3	**4**	A	—	469	0.21	2.6×10^{-2}	1.9
4[e]	**2a**	B[e]	—	467	1.1×10^{-5}	8.6×10^{-4}	1
5	**2a**	B	**25**	542	1.3×10^{-4}	8.7×10^{-3}	12
6	**2a**	B	**26**	481	3.2×10^{-5}	8.4×10^{-3}	3
7	**3**	B	—	525	6.0×10^{-3}	1.3×10^{-3}	550
8	**4**	B	—	468	3.7×10^{-4}	1.2×10^{-3}	30
9	**3**	B	**27**	535	4.4×10^{-3}	3.6×10^{-4}	400
10	**4**	B	**27**	473	2.6×10^{-2}	1.1×10^{-3}	2400

[a] All reactions were carried out at 25 °C.
[b] A: TBAF/acetonitrile system, B: NaOH/H_2O system.
[c] Based on a value reported for the chemiluminescent decomposition of 3-adamantylidene-4-(3-*tert*-butyldimethylsiloxy)phenyl-4-methoxy-1,2-dioxetane **1b** in TBAF/DMSO.[18]
[d] Values for entries 2 and 3 were based on the Φ^{CL} in entry 1, while values for entries 5–10 were based on the Φ^{CL} in entry 4.
[e] Ref. 6a.

(Fig. 3) as a model of a tethered fluorescein moiety, which showed maximum wavelength λ_{max}^{fl} = 535 nm, but was significantly different from that for **2a** (λ_{max}^{CL} = 467 nm, Table 1, entry 1) in a TBAF/actonitrile system (Fig. 2A). These results strongly suggested that CTID of **3** showed chemiluminescence due to energy-transfer from initially formed excited oxidobenzoate to fluorescein moiety.[19] Similarly to the case of **3**, dioxetane **4** underwent CTID on treatment with TBAF/acetonitrile to give light. As shown in Table 1 (entry 3), λ_{max}^{CL} and k^{CTID} for **4** were only a little different from those for **2**, though Φ^{CL} was 1.9 times increased. The chemiluminescence spectrum of **4** was somewhat narrower than that for **2a** but coincided with fluorescence spectrum (λ_{max}^{fl} = 469 nm) of *N*-[4-(benzothiazol-2-yl)-3-hydroxybenzyl]acetamide **26** (Fig. 3) as a model of a tethered fluorescer in **4** in TBAF/acetonitrile (Fig. 2A). From these results, we can see that energy-transfer most likely occurred for **4** similarly to the case of **3** (Scheme 4).

Singlet-chemiexcitation efficiency $\Phi_S=\Phi^{CL}\times\Phi^{fl}$ (Φ^{fl}: fluorescence efficiency of emitter) has been estimated to be 0.46 for **2a** in TBAF/acetonitrile.[6a] Here, the Φ_S for **2a** is presumably maintained even for both **3** and **4**. On the other hand, Φ^{fl}s were estimated to be 0.52 for **25** and 0.48 for **26** in TBAF/acetonitrile. These values are also expected to be not so much different from those for fluorescein moiety of **3** and benzothizolylphenol moiety of **4**, respectively. Thus, we can estimate formally that singlet-chemiexcitation energy generated from the dioxetane moiety transferred to the tethered fluorescer in efficiency of 80% for **3** and 95% for **4**.

The results described above encouraged us to investigate chemiluminescent decomposition of **3** and **4** in an aqueous system. When **3** was treated with 0.1 M NaOH/H_2O, **3** decomposed with the accompanying emission of yellow light with λ_{max}^{CL} = 525 nm, $\Phi^{CL}=6.0\times10^{-3}$ and $k^{CTID}=1.3\times10^{-3}\ s^{-1}$ [Fig. 2B, Table 1 (entry 7)]. This Φ^{CL} value was 550-fold greater than that for **2a**. Similarly, **4** decomposed to give light with λ_{max}^{CL} = 468 nm, $\Phi^{CL}=3.7\times10^{-4}$ and $k^{CTID}=1.2\times10^{-3}\ s^{-1}$ [Fig. 2B, Table 1 (entry 8)]. In this case, Φ^{CL} value was 30-fold greater than that for **2a**.[6a]

Such marked enhancement of Φ^{CL} was not observed when the chemiluminescent decomposition of **2a** was carried out by simply adding fluorescer **25** or **26** in NaOH/H_2O system. When **2a** (1.0×10^{-4} M, 1 mL) was treated with NaOH/H_2O (0.1 M, 2 mL) including **25** (1.0×10^{-3} M) at 25 °C,[20] **2a** decomposed with the accompanying emission of yellow light (λ_{max}^{CL} = 542 nm), but not blue light (λ_{max}^{CL} = 467 nm), the spectrum of which is shown in Fig. 2B. Table 1 (entry 5) shows that the value of Φ^{CL} for **2a** increased

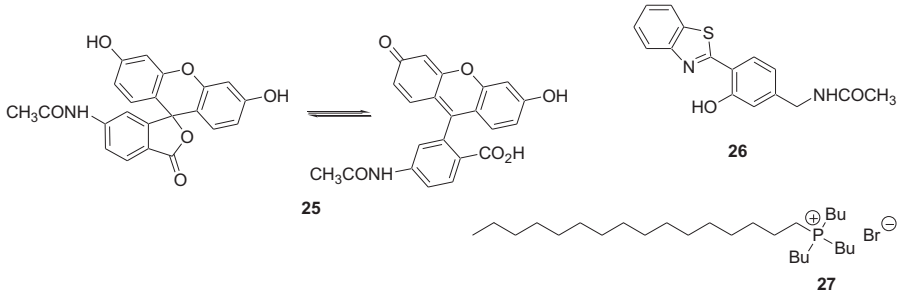
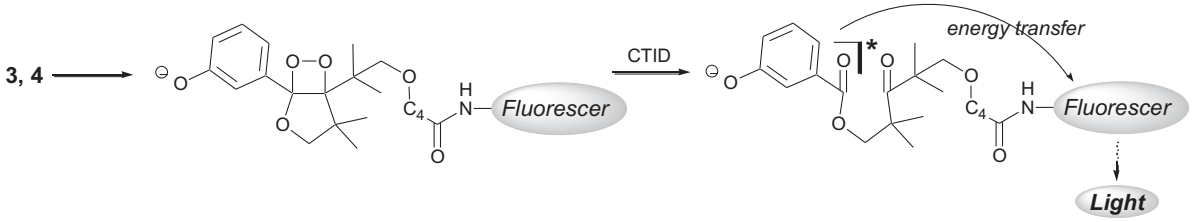


Fig. 3. Fluorescers **25** and **26** and surfactant **27**.



Scheme 4. Chemiluminescence based on energy-transfer for dioxetanes **3** and **4**.

12 times by the addition of **25**, though it was only 1/50 of that for **3**. On the other hand, the CTID of **2a** in the presence of **26** gave light only three times more than the case of **2a** without any additive fluorescer (Table 1, entry 6.

The results described above showed that Φ^{CL} was markedly increased by tethering a fluorescer to dioxetane **2** skeleton in NaOH/H_2O system. However, the magnitude of enhancement of Φ^{CL} for **4** was only 1/16 of that for **3**, though model **26** was yet an effective fluorescer like as **25** in NaOH/H_2O: Φ^{fl}=0.73 for **25**, and 0.36 for **26**. This suggested that the energy-transfer did not operate well for the CTID of **4** in an aqueous system differently from a non-aqueous system such as TBAF/acetonitrile.

Although it was unclear the reason why the fluorescer moiety did not act well in **4** in an aqueous system, a hydrophobic circumstance appeared to be favorable to the energy-transfer chemiluminescence for the CTID of **4**. Thus, we finally attempted to use a surfactant with expectation that it should provide more or less a hydrophobic microenvironment in an aqueous system. A surfactant selected as a representative was tributylhexadecylphosphonium bromide **27** (Fig. 3), since it was used for a chemiluminescent clinical analysis using dioxetane **2c** (phosphate form).[8] When **4** was treated with NaOH/H_2O including an equimolar amount of **27** (Fig. 3), emission of light markedly increased. As shown in Table 1 (entry 10), the value of Φ^{CL} became 2400-fold greater than that for innocent **2**. On the other hand, **27** rather did not act to increase Φ^{CL} for CTID of **3** in NaOH/H_2O system (Table 1, entry 9).[21]

3. Conclusion

Bicyclic dioxetanes **3** and **4** tethering a fluorescein or 4-(benzothiazol-2-yl)-3-hydroxyphenyl moiety were synthesized through the preparation of dihydrofuran-intermediate **5** and its advanced intermediate **6**. These dioxetanes underwent base-induced decomposition to effectively give light due to intramolecular energy-transfer from an excited oxidobenzoate to a fluorescein or 4-(benzothiazol-2-yl)-3-hydroxyphenyl moiety. Although the values of chemiluminescence efficiency (Φ^{CL}) for **3** and **4** were only slightly larger than that for parent **2a** in a TBAF/acetonitrile system, these values were 30–550-fold greater than that for **2a** in a NaOH/H_2O system. A comparison of the chemiluminescent decomposition of **3** and **4** to that of parent **2a** with additive model fluorescer **25** or **26** in an aqueous system showed that tethering a fluorophore was 10–50 times more effective to increase Φ^{CL} than a simple combination of dioxetane and fluorescer in a NaOH/H_2O system.

4. Experimental

4.1. General

Melting points were uncorrected. IR spectra were taken on an FT/IR infrared spectrometer. ^1H and ^{13}C NMR spectra were recorded on a 400 MHz and 500 MHz spectrometers. Mass spectra were obtained by using double-focusing mass spectrometers and an ESI-TOF mass spectrometer. Column chromatography was carried out using SiO_2 or NH–SiO_2.

4.2. Data for compounds

*4.2.1. 2,2,4,4-Tetramethylpentane-1,3,5-triol (**9**).* A solution of dimethyl 2,2,4,4-tetramethyl-3-oxopentanedioate (40.1 g, 0.174 mol), prepared from dimethyl 3-oxopentanedioate (**8**), in dry THF (40 mL) was added dropwise to a suspension of $LiAlH_4$ (10.2 g, 0.269 mol) in dry THF (110 mL) under a N_2 atmosphere at 0 °C and stirred overnight. After the usual workup, the crude product was chromatographed on SiO_2 and eluted with AcOEt/hexane (3:1) to give triol **9** in 53% yield (16.2 g). Colorless needles; mp 61.0–61.5 °C (from hexane); ^1H NMR (400 MHz, $CDCl_3$): δ_H 1.01 (s, 6H), 1.09 (s, 6H), 2.11 (br s, 3H), 3.50 (q_{AB}, J=10.7 Hz, 4H), 3.64 (s, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 20.3, 24.7, 40.3, 75.4, 85.7 ppm; IR (KBr): $\tilde{\nu}$ 3305, 2988, 2960, 2875, 1043 cm^{-1}; HRMS (ESI): 199.1266, calcd for $C_9H_{20}O_3Na$ $[M+Na]^+$ 199.1310.

*4.2.2. 4-(2-Hydroxy-1,1-dimethylethyl)-2,2,5,5-tetramethyl-1,3-dioxane (**10**).* 2,2-Di-methoxypropane (7.10 mL, 56.6 mmol) and pyridinium *p*-toluenesulfonate (PPTS) (1.41 g, 5.61 mmol) were added to a solution of triol **9** (9.76 g, 55.4 mmol) in CH_2Cl_2 (60 mL) under N_2 atmosphere at room temperature and stirred overnight. The reaction mixture was poured into aq $NaHCO_3$ and was extracted with CH_2Cl_2. The organic layer was washed three times with aq NaCl, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on SiO_2 and eluted with AcOEt/hexane (1:3) to give 1,3-dioxane **10** in 97% yield (11.6 g). Colorless oil; ^1H NMR (400 MHz, $CDCl_3$): δ_H 0.89 (s, 3H), 1.02 (s, 3H), 1.02 (s, 3H), 1.21 (s, 3H), 1.42 (s, 6H), 3.01 (br s, 1H), 3.11 (d, J=11.1 Hz, 1H), 3.33 (d, J=11.1 Hz, 1H), 3.50–3.57 (m, 2H), 3.59 (s, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 18.7, 20.3, 21.2, 24.1, 24.5, 29.0, 35.4, 40.2, 73.0, 74.4, 83.2, 98.5 ppm. IR (liquid film): $\tilde{\nu}$ 3451, 2991, 2958, 2873, 1201, 1091, 738 cm^{-1}. HRMS (ESI): 239.1589, calcd for $C_{12}H_{24}O_3Na$ $[M+Na]^+$ 239.1623.

*4.2.3. 4-[2-(3-Methoxybenzyloxy)-1,1-dimethylethyl]-2,2,5,5-tetramethyl-1,3-dioxane (**11**).* A solution of 1,3-dioxane **10** (11.6 g, 53.6 mmol) in dry THF/DMF (4:3, 70 mL) was added to a suspension of NaH (60% in oil, 2.45 g, 61.3 mmol) in dry THF (80 mL) under a N_2 atmosphere at 0 °C and then stirred at room temperature for 1 h. To the solution, 3-methoxybenzyl chloride (8.0 mL, 53.4 mmol) was added at 0 °C and stirred at room temperature overnight. The reaction mixture was quenched with aq NH_4Cl, and extracted with AcOEt. The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on SiO_2 and eluted with AcOEt/hexane (1:20→1:9) to give benzyl ether **11** in 91.7% yield (16.6 g). Colorless oil; ^1H NMR (400 MHz, $CDCl_3$): δ_H 0.88 (s, 3H), 0.95 (s, 3H), 1.05 (s, 3H), 1.14 (s, 3H), 1.34 (s, 3H), 1.37 (s, 3H), 3.01 (d, J=8.4 Hz, 1H), 3.08 (d, J=11.4 Hz, 1H), 3.37 (d, J=8.4 Hz, 1H), 3.51 (d, J=11.4 Hz, 1H), 3.65 (s, 1H), 3.81 (s, 3H), 4.45 (q_{AB}, J=12.5 Hz, 2H), 6.82 (d with fine coupling, J=8.2 Hz, 1H), 6.88–6.92 (m, 2H), 7.25 (t, J=8.2 Hz, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 19.0, 20.9, 21.9, 23.4, 24.2, 29.1, 35.3, 40.5, 55.1, 72.9, 74.7, 78.4, 78.5, 98.5, 112.7 (×2), 119.5, 129.2, 140.6, 159.6 ppm; IR (liquid film): $\tilde{\nu}$ 2991, 2941, 2871, 1602, 1092 cm^{-1}; Mass (*m*/*z*, %): 336 (M^+, 15), 222 (72), 143 (14), 137 (39), 121 (100). HRMS (ESI): 359.2181, calcd for $C_{20}H_{32}O_4Na$ $[M+Na]^+$ 359.2198.

*4.2.4. 5-(3-Methoxybenzyloxy)-2,2,4,4-tetramethylpentane-1,3-diol (**12**).* Benzyl ether **11** (15.8 g, 47.0 mmol) was heated in refluxing dioxane (160 mL) and 1 N HCl (40 mL) for 3 h. The reaction mixture was poured into aq $NaHCO_3$ and extracted with AcOEt. The organic layer was washed with aq NaCl, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on SiO_2 and eluted with AcOEt/hexane (1:9→1:1) to give benzyloxypentanediol **12** in 86.1% yield (12.0 g). Colorless oil; ^1H NMR (400 MHz, $CDCl_3$): δ_H 0.98 (s, 3H), 1.02 (s, 3H), 1.05 (s, 3H), 1.11 (s, 3H), 3.33 (s, 2H), 3.37 (d with fine coupling, J=10.7 Hz, 1H), 3.48 (dd, J=10.7 and 2.2 Hz, 1H), 3.58 (d, J=2.2 Hz, 1H), 3.61 (br s, 1H), 3.81 (s, 3H), 4.26 (br s, 1H), 4.49 (s, 2H), 6.83–6.90 (m, 3H), 7.24–7.30 (m, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 20.1, 21.1, 24.7, 25.0, 40.4, 40.4, 55.1, 73.5, 75.4, 83.0, 85.3, 112.9, 113.3, 119.8, 129.5, 139.0, 159.7 ppm; IR (liquid film): $\tilde{\nu}$ 3412, 2959, 2914, 2874, 1602, 1267, 1080 cm^{-1}; Mass (*m*/*z*, %): 296 (M^+, 31), 138 (100), 121 (92), 97 (14), 91 (10); HRMS (ESI): 319.1856, calcd for $C_{17}H_{28}O_4Na$ $[M+Na]^+$ 319.1885.

*4.2.5. 1-(3-Methoxybenzyloxy)-2,2,4,4-tetramethyl-5-(tetrahydro-2H-pyran-2-yloxy)-pentan-3-ol (**13**).* PPTS (540 mg, 2.15 mmol) and 3,4-dihydro-2*H*-pyran (4.8 mL, 51.0 mmol) were added to a solution of benzyloxypentanediol **12** (12.0 g, 40.5 mmol) in dry CH_2Cl_2 (120 mL) under a N_2 atmosphere at room temperature and stirred at room temperature for 3 h. The reaction mixture was poured into satd aq $NaHCO_3$ and extracted with AcOEt. The organic layer was washed with aq NaCl, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on SiO_2 and eluted with AcOEt/hexane (1:9) to give THP-ether **13** in 92.1% yield (14.2 g). Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.02 (s, 1.5H), 1.04 (s, 1.5H), 1.04 (s, 1.5H), 1.05 (s, 1.5H), 1.06 (s, 1.5H), 1.09 (s, 1.5H), 1.10 (s, 1.5H), 1.11 (s, 1.5H), 1.47–1.85 (m, 6H), 3.14–3.70 (m, 7H), 3.76–3.86 (m, 1H), 3.81 (s, 3H), 4.45–4.53 (m, 2H), 4.54–4.59 (m, 1H), 6.82 (d with fine coupling, J=8.2 Hz, 1H), 6.88–6.92 (m, 2H), 7.25 (t, J=8.2 Hz, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 19.3, 19.6, 21.6, 21.9, 21.9, 22.0, 24.4, 24.7, 24.9, 25.0, 25.3, 25.4, 30.5, 30.6, 40.4, 40.5, 40.8 (×2), 55.1 (×2), 61.9, 62.4, 73.1 (×2), 77.7, 78.2, 80.4, 80.5, 80.6, 81.1, 99.0, 99.3, 112.7 (×2), 112.9, 113.0, 119.6 (×2), 129.3, 129.3, 140.1, 140.2, 159.6 (×2) ppm; IR (liquid film): $\tilde{\nu}$ 3500, 2942, 2871, 1602, 1266, 1119, 1034 cm^{-1}; Mass (*m*/*z*, %): 380 (M^+, trace), 295 (3), 138 (31), 136 (22), 121 (100), 109 (17); HRMS (ESI): 403.2434, calcd for $C_{22}H_{36}O_5Na$ $[M+Na]^+$ 403.2460.

*4.2.6. 1-(3-Methoxybenzyloxy)-2,2,4,4-tetramethyl-5-(tetrahydro-2H-pyran-2-yloxy)-pentan-3-one (**14**).* Alcohol **13** (7.90 g, 20.8 mmol) in CH_2Cl_2 (20 mL) was added to a suspension of PCC (6.90 g, 32.0 mmol), pyridine (2.7 mL, 33.4 mmol), and Celite (15.7 g) in CH_2Cl_2 (60 mL) at room temperature and refluxed for 4 h. After the usual workup, the crude product was chromatographed on SiO_2 and eluted with AcOEt/hexane (1:16→1:2) to give ketone **14** in 85.9% yield (6.76 g). Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.23 (s, 3H), 1.28 (s, 6H), 1.32 (s, 3H), 1.42–1.80 (m, 6H), 3.46 (d, J=9.2 Hz, 1H), 3.43–3.54 (m, 1H), 3.51 (q_{AB}, J=8.8 Hz, 2H), 3.72 (d, J=9.2 Hz, 1H), 3.77–3.84 (m, 1H), 3.80 (s, 3H), 4.47 (s, 2H), 4.55 (s with fine coupling, 1H), 6.80 (d with fine coupling, J=8.1 Hz, 1H), 6.85–6.89 (m, 2H), 7.23 (t, J=8.1 Hz, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 19.3, 23.3, 23.5, 23.6, 23.7, 25.5, 30.5, 50.1, 50.3, 55.1, 61.8, 73.0, 76.0, 78.3, 98.9, 112.5, 112.9, 119.5, 129.1, 140.1, 159.5, 215.9 ppm; IR (liquid film): $\tilde{\nu}$ 2940, 2870, 1686, 1602, 1266 cm^{-1}; Mass (*m*/*z*, %): 378 (M^+, 2), 294 (12), 138 (42), 137 (13), 121 (100); HRMS (ESI): 401.2279, calcd for $C_{22}H_{34}O_5Na$ $[M+Na]^+$ 401.2304.

*4.2.7. 3-[1,1-Dimethyl-2-(tetrahydro-2H-pyran-2-yloxy)ethyl]-3-hydroxy-2-(3-methoxyphenyl)-4,4-dimethyltetrahydrofuran (**15**).* Ketone **14** (12.6 g, 33.2 mmol) in dry THF (50 mL) was added to a solution of LDA, prepared from BuLi (1.61 M solution, 48.0 mL, 77.3 mmol) and diisopropylamine (12 mL), in THF (80 mL) at −78 °C and stirred for 5 h. The reaction mixture was poured into aq NH_4Cl, and extracted with AcOEt. The organic layer was washed with aq NaCl, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The crude hydroxytetrahydrofuran **15** (3.20 g, an oil) as a stereoisomeric mixture was used for the next reaction without purification.

*4.2.8. 3-Hydroxy-3-(2-hydroxy-1,1-dimethylethyl)-2-(3-methoxyphenyl)-4,4-dimethyl-2,3,4,5-tetrahydrofuran (**7**).* HCl (1 N, 5 mL) was added to a solution of tetrahydrofuran **15** (12.9 g) in MeOH (120 mL) at room temperature and stirred overnight. The reaction mixture was poured into aq $NaHCO_3$ and extracted with AcOEt. The organic layer was washed with aq NaCl, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on SiO_2 and eluted with AcOEt/hexane (1:4) to give hydroxytetrahydrofuran **7** in 97.4% yield (2.33 g) (a mixture of *trans*-3-hydroxy form and *cis*-3-hydroxy form, trans/cis=95:5). Colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 0.78 (br s, 2.85H), 0.93 (s, 0.15H), 1.02 (s, 2.85H), 1.20 (s, 0.15H), 1.22 (s, 0.15H), 1.25 (s, 2.85H), 1.37 (s, 2.85H), 1.39 (s, 0.15H), 2.22 (br s, 1H), 3.21 (dd, J=11.0 and 5.1 Hz, 1H), 3.33 (d, J=5.1 Hz, 0.1H), 3.45–3.65 (m, 0.95H), 3.50 (d, J=7.2 Hz, 0.05H), 3.70 (d, J=8.1 Hz, 0.95H), 3.81 (s, 2.85H), 3.82 (s, 0.15H), 3.89 (d, J=8.1 Hz, 0.95H), 4.09 (d, J=7.2 Hz, 0.05H), 4.57 (br s, 0.95H), 5.04 (s, 0.95H), 5.27 (s, 0.05H), 6.81 (d with fine coupling, J=8.0 Hz, 0.95H), 6.86 (d with fine coupling, J=8.2 Hz, 0.05H), 7.04–7.10 (m, 0.1H), 7.13 (s, 0.95H), 7.16 (d, J=7.8 Hz, 0.95H), 7.20–7.25 (m, 0.05H), 7.22 (dd, J=8.0 and 7.8 Hz, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 20.3, 22.7 (×2), 22.8, 23.2, 23.2, 25.4, 26.6, 40.6, 41.4, 47.9, 48.3, 55.2 (×2), 72.0, 73.1, 80.4, 81.3, 84.0, 86.7, 89.6, 93.7, 113.0, 113.6, 114.5, 115.2, 121.3, 122.0, 128.6, 128.9, 141.4, 142.2, 159.0, 159.2 ppm; IR (liquid film): $\tilde{\nu}$ 3295, 2938, 2877, 1607, 1284, 1043 cm^{-1}; Mass (*m*/*z*, %): 294 (M^+, 20), 276 (33), 236 (45), 190 (33), 159 (20), 136 (100), 126 (68); HRMS (ESI): 377.1703, calcd for $C_{17}H_{26}O_4Na$ $[M+Na]^+$ 317.1729.

*4.2.9. 3-(7-Ethoxycarbonyl-1,1-dimethyl-3-oxaheptyl)-3-hydroxy-2-(3-methoxyphenyl)-4,4-dimethyl-2,3,4,5-tetrahydrofuran (**16**).* 3-Hydroxy-3-(2-hydroxy-1,1-dimethylethyl)tetrahydrofuran **7** (1.00 g, 3.40 mmol) was added to a suspension of NaH (60% in oil, 255 mg, 6.38 mmol) in dry DMF (7 mL) under a N_2 atmosphere at 0 °C and stirred at room temperature for 30 min. To the solution, ethyl 5-bromopentanoate (0.81 mL, 7.16 mmol) was added at 0 °C and stirred at room temperature for 2 h. The reaction mixture was poured into aq NH_4Cl and extracted with AcOEt. The organic layer was washed with aq NaCl, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on SiO_2 and eluted with AcOEt/hexane (1:6) to give ester **16** in 94.7% yield (1.36 g) as a mixture of stereoisomers (cis/trans=93:7), from which a small amount of pure isomers were isolated.

*3-r-(7-Ethoxycarbonyl-1,1-dimethyl-3-oxaheptyl)-3-hydroxy-2-trans-(3-methoxyphenyl)-4,4-dimethyl-2,3,4,5-tetrahydrofuran (**16**-trans)*: Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.80 (br s, 3H), 1.06 (s, 3H), 1.19 (s, 3H), 1.26 (t, J=7.1 Hz, 3H), 1.35 (s, 3H), 1.55–1.73 (m, 4H), 2.32 (t, J=7.1 Hz, 2H), 2.80 (d, J=9.3 Hz, 1H), 3.10–3.30 (m, 3H), 3.68 (d, J=7.9 Hz, 1H), 3.80 (s, 3H), 3.87 (d, J=7.9 Hz, 1H), 4.13 (q, J=7.1 Hz, 2H), 4.89 (br s, 1H), 5.04 (s, 1H), 6.80 (d with fine coupling, J=7.8 Hz, 1H), 7.10–7.17 (m, 2H), 7.21 (t, J=7.8 Hz, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 14.1, 21.6, 23.4 (br×3), 25.3, 28.8, 33.7, 41.5, 47.7, 55.1, 60.1, 70.6, 80.1, 81.7, 88.4, 92.3 (br), 112.8, 114.0 (br), 120.8 (br), 128.4, 142.2, 159.0, 173.2 ppm; IR (liquid film): $\tilde{\nu}$ 3447, 2936, 2873, 1734, 1603, 1093 cm^{-1}; Mass (*m*/*z*, %): 422 (M^+, 8), 258 (22), 245 (100), 243 (53), 135 (64), 129 (60); HRMS (ESI): 445.2562, calcd for $C_{24}H_{38}O_6Na$ $[M+Na]^+$ 445.2566. *3-r-(7-Ethoxycarbonyl-1,1-dimethyl-3-oxaheptyl)-3-hydroxy-2-cis-(3-methoxyphenyl)-4,4-dimethyl-2,3,4,5-tetrahydrofuran (**16**-cis)*: Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.01 (s, 3H), 1.16 (s, 3H), 1.21 (s, 3H), 1.26 (t, J=7.2 Hz, 3H), 1.37 (s, 3H), 1.30–1.40 (m, 2H), 1.45–1.55 (m, 2H), 2.23 (t, J=7.3 Hz, 2H), 2.54 (dt, J=9.3 and 6.4 Hz, 1H), 2.82 (d, J=9.3 Hz, 1H), 2.94 (dt, J=9.3 and 6.4 Hz, 1H), 3.08 (d, J=9.3 Hz, 1H), 3.44 (d, J=7.1 Hz, 1H), 3.81 (s, 3H), 4.08–4.15 (m, 3H), 4.87 (s, 1H), 5.23 (s, 1H), 6.83 (d with fine coupling, J=8.3 Hz, 1H), 7.10 (d with fine coupling, J=7.6 Hz, 1H), 7.16 (s with fine coupling, 1H), 7.21 (dd with fine coupling, J=8.3 and 7.6 Hz, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 14.3, 20.5, 21.6, 23.7 (×2), 26.9, 28.7, 33.9, 40.4, 48.4, 55.2, 60.2, 70.5, 81.0, 81.7, 83.9, 86.4, 113.5, 115.5, 122.5, 128.3, 142.4, 159.0, 173.1 ppm; IR (liquid film): $\tilde{\nu}$ 3403, 2963, 2873, 1734, 1599, 1372, 1094 cm^{-1}; Mass (*m*/*z*, %): 422 (M^+, 21), 245 (24), 147 (28), 140 (32), 136 (92), 129 (100); HRMS (ESI): 445.2563, calcd for $C_{24}H_{38}O_6Na$ $[M+Na]^+$ 445.2566.

*4.2.10. 4-(7-Ethoxycarbonyl-1,1-dimethyl-3-oxaheptyl)-5-(3-methoxyphenyl)-3,3-dimethyl-2,3-dihydrofuran (**17**).* $SOCl_2$ (0.53 mL, 7.27 mmol) was added to a solution of hydroxytetrahydrofuran (**16**) (2.55 g, 6.02 mmol) and pyridine (5.0 mL, 61.8 mmol) in dry CH_2Cl_2

(30 mL) under a N_2 atmosphere at 0 °C and stirred for 3 h. The reaction mixture was poured into satd aq $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was washed twice with satd aq NaCl, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on SiO_2 and eluted with AcOEt/hexane (1:6) to give dihydrofuran **17** in 85.7% yield (2.09 g). Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.04 (s, 6H), 1.25 (t, J=7.2 Hz, 3H), 1.31 (s, 6H), 1.51–1.59 (m, 2H), 1.63–1.72 (m, 2H), 2.31 (t, J=7.3 Hz, 2H), 3.10 (s, 2H), 3.24 (t, J=6.2 Hz, 2H), 3.80 (s, 3H), 3.87 (s, 2H), 4.12 (q, J=7.2 Hz, 2H), 6.83–6.87 (m, 2H), 6.90 (d with fine coupling, J=7.5 Hz, 1H), 7.20–7.25 (m, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 14.3, 21.9, 27.3 (×2), 27.4 (×2), 29.1, 34.1, 37.0, 47.0, 55.2, 60.1, 70.4, 79.5, 83.0, 113.9, 115.1, 122.3, 122.3, 128.7, 137.0, 150.9, 158.9, 173.5 ppm; IR (liquid film): $\tilde{\nu}$ 2956, 2866, 1735, 1596, 1048 cm^{-1}; Mass (m/z, %): 404 (M^+, 2), 259 (12), 258 (19), 246 (20), 245 (100), 243 (43), 135 (20). HRMS (ESI): 427.2420, calcd for $C_{24}H_{36}O_5Na$ $[M+Na]^+$ 427.2460.

*4.2.11. 4-(7-Carboxy-1,1-dimethyl-3-oxaheptyl)-5-(3-hydroxyphenyl)-3,3-dimethyl-2,3-dihydrofuran (**18**).* CH_3SNa (95%, 988 mg, 13.5 mmol) was added to a solution of dihydrofuran (**17**) (1.19 g, 2.94 mmol) in dry DMF (10 mL) under a N_2 atmosphere at room temperature and stirred at 140 °C for 2 h. The reaction mixture was poured into diluted aq HCl and extracted with AcOEt. The organic layer was washed three times with satd aq NaCl, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on SiO_2 and eluted with AcOEt/hexane (1:1) to give carboxylic acid **18** in 92.7% yield (1.09 g). Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.03 (s, 6H), 1.30 (s, 6H), 1.54–1.62 (m, 2H), 1.68–1.77 (m, 2H), 2.41 (t, J=7.2 Hz, 2H), 3.10 (s, 2H), 3.26 (t, J=6.0 Hz, 2H), 3.86 (s, 2H), 6.77 (d with fine coupling, J=8.1 Hz, 1H), 6.83–6.87 (m, 2H), 7.16 (t with fine coupling, J=8.1 Hz, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 21.9, 27.3(×2), 27.5 (×2), 28.9 33.7, 37.1, 47.0, 70.5, 79.5, 82.9, 115.3, 116.9, 122.0, 122.1, 128.9, 136.9, 150.7, 155.2, 178.8 ppm; IR (liquid film): $\tilde{\nu}$ 3376, 2957, 2869, 1709, 1595, 1047 cm^{-1}; Mass (m/z, %): 362 (M^+, 3), 244 (22), 232 (28), 231 (100), 230 (12), 229 (46), 121 (37), 55 (10); HRMS (ESI): 385.1954, calcd for $C_{21}H_{30}O_5Na$ $[M+Na]^+$ 385.1991.

*4.2.12. 5-(3-tert-Butyldimethylsilyloxyphenyl)-4-(7-carboxy-1,1-dimethyl-3-oxaheptyl)-3,3-dimethyl-2,3-dihydrofuran (**5**).* Imidazole (1.53 g, 22.5 mmol) and TBMSCl (97%, 3.17 g, 21.0 mmol) were added to a solution of carboxylic acid **18** (2.70 g, 7.45 mmol) in dry DMF (6.8 mL) under a N_2 atmosphere at room temperature and stirred for 2 h. The reaction mixture was poured into satd aq NH_4Cl and extracted with AcOEt. The organic layer was washed three times with satd aq NaCl, dried over anhydrous $MgSO_4$, and concentrated in vacuo. K_2CO_3 (484 mg, 3.50 mmol) in H_2O (10 mL) was added to a solution of the residue in CH_3OH (40 mL) at room temperature and stirred for 1 h. The reaction mixture was poured into satd aq NH_4Cl and extracted with AcOEt. The organic layer was washed twice with satd aq NaCl, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on SiO_2 and eluted with AcOEt/hexane (1:5) to give dihydrofuran **5** in 92.6% yield (3.29 g). Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.18 (s, 6H), 0.98 (s, 9H), 1.04 (s, 6H), 1.30 (s, 6H), 1.54–1.62 (m, 2H), 1.65–1.75 (m, 2H), 2.38 (t, J=7.5 Hz, 2H), 3.10 (s, 2H), 3.25 (t, J=6.1 Hz, 2H), 3.86 (s, 2H), 6.76–6.81 (m, 2H), 6.90 (d with fine coupling, J=7.6 Hz, 1H), 7.14–7.19 (m, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ_C −4.4 (×2), 18.1, 21.6, 25.7 (×3), 27.2 (×2), 27.4 (×2), 28.9, 33.8, 37.0, 47.0, 70.4, 79.6, 83.0, 119.8, 121.7, 122.4, 123.0, 128.7, 137.1, 151.0, 155.0, 179.6 ppm; IR (liquid film): $\tilde{\nu}$ 2956, 2930, 2860, 1711, 1595, 1262, 1118 cm^{-1}; Mass (m/z, %): 476 (M^+, trace), 358 (35), 343 (100), 309 (13), 231 (10); HRMS (ESI): 499.2815, calcd for $C_{27}H_{44}O_5SiNa$ $[M+Na]^+$ 499.2856.

*4.2.13. N-Hydroxysuccinimide ester of 5-(3-tert-butyldimethylsilyloxyphenyl)-4-(7-carboxy-1,1-dimethyl-3-oxaheptyl)-3,3-dimethyl-2,3-dihydrofuran (**6**).* Di(*N*-succinimidyl) carbonate (760 mg, 2.97 mmol) and Et_3N (0.64 mL, 4.59 mmol) were added to a solution of carboxylic acid **5** (445 mg, 0.932 mmol) in dry CH_3CN (8 mL) under a nitrogen atmosphere at room temperature and stirred for 1 h. The reaction mixture was concentrated in vacuo. The residue was chromatographed on silica gel and eluted with ether/hexane (2:1) to give 1.30 g of *N*-hydroxysuccinimide ester **6** as a colorless oil in 99.8% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.18 (s, 6H), 0.98 (s, 9H), 1.04 (s, 6H), 1.31 (s, 6H), 1.59–1.67 (m, 2H), 1.76–1.85 (m, 2H), 2.63 (t, J=7.5 Hz, 2H), 2.83 (br s, 4H), 3.10 (s, 2H), 3.26 (t, J=6.1 Hz, 2H), 3.86 (s, 2H), 6.75–6.81 (m, 2H), 6.90 (d with fine coupling, J=7.3 Hz, 1H), 7.14–7.20 (m, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ_C −4.4 (×2), 18.1, 21.6, 25.5 (×2), 25.6 (×3), 27.2 (×2), 27.4 (×2), 28.6, 30.6, 36.9, 47.0, 70.0, 79.6, 83.0, 119.8, 121.6, 122.3, 123.0, 128.7, 137.1, 151.0, 155.0, 168.5, 169.1 (×2) ppm. IR (liquid film): $\tilde{\nu}$ 2956, 2930, 2860, 1816, 1746, 1480, 1206, 1068, 840 cm^{-1}; Mass (m/z, %): 573 (M^+, trace), 359 (12), 358 (33), 345 (19), 344 (37), 343 (100), 309 (10), 231 (10), 99 (23), 75 (14), 73 (17), 57 (17), 56 (26), 55 (11). HRMS (ESI): 596.2974, calcd for $C_{31}H_{47}NO_7SiNa$ $[M+Na]^+$ 596.3020.

*4.2.14. 5-[3-(tert-Butyldimethylsiloxy)phenyl]-4-[7-(fluorescein-5-yl)carbamoyl-1,1-dimethyl-3-oxaheptyl]-3,3-dimethyl-2,3-dihydrofuran (**20**).* $SOCl_2$ (0.02 mL, 0.27 mmol) was added to a solution of carboxylic acid **5** (99.7 mg, 0.209 mmol) in dry CH_2Cl_2 (1 mL) at room temperature under a N_2 atmosphere and stirred for 3 h. The solution was concentrated, and then the residue was dissolved in dry THF (1 mL) and stirred at room temperature under a N_2 atmosphere. To the solution [3′,6′-bis(*tert*-butyldimethylsiloxy)-5-aminofluorescein (**19**) (120 mg, 0.208 mmol) and pyridine (0.02 mL, 0.247 mmol) were added and stirred for 24 h. The reaction mixture was poured into satd aq NH_4Cl and extracted with AcOEt. The organic layer was washed three times with satd aq NaCl, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on SiO_2 and eluted with AcOEt/hexane (1:1) to give dihydrofuran (**20**) in 79.5% yield (111 mg). Amorphous orange solid; ^{1}H NMR (500 MHz, CD_3OD): δ_H 0.19 (s, 6H), 0.98 (s, 9H), 1.05 (s, 6H), 1.31 (s, 6H), 1.61–1.69 (m, 2H), 1.76–1.85 (m, 2H), 2.46 (t, J=7.3 Hz, 2H), 3.14 (s, 2H), 3.28–3.35 (m, 2H), 3.84 (s, 2H), 6.51–6.58 (m, 4H), 6.60–6.70 (m, 4H), 6.75 (s with fine coupling, 1H), 6.81 (d with fine coupling, J=8.2 Hz, 1H), 6.89 (d with fine coupling, J=7.6 Hz, 1H), 7.14 (d, J=8.2 Hz, 1H), 7.20 (dd, J=8.2 and 7.6 Hz, 1H), 7.83–7.90 (m, 1H), 8.29 (br s, 1H) ppm; ^{13}C NMR (125 MHz, CD_3OD): δ_C −3.8, −3.6, 19.3, 24.0, 26.5 (×3), 28.0 (×2), 28.3 (×2), 30.6, 38.1, 38.4, 48.3, 49.6, 71.9, 81.0, 84.3, 103.9 (×2), 111.8 (×2), 113.9 (×2), 116.3, 121.3, 123.2, 124.2, 124.7, 126.0, 128.3, 129.4, 130.2, 130.4 (×2), 138.8, 142.1, 149.0, 152.5, 154.4 (×2), 156.6, 161.7 (br×2), 171.6, 175.0 ppm; IR (KBr): $\tilde{\nu}$ 3338, 2956, 2930, 2860, 1738, 1609, 1259, 1179, 1114 cm^{-1}; HRMS (ESI): 828.3565, calcd for $C_{47}H_{55}NO_9SiNa$ $[M+Na]^+$ 828.3544.

*4.2.15. 4-[7-(Fluorescein-5-yl)carbamoyl-1,1-dimethyl-3-oxahept-1-yl]-5-(3-hydroxyphenyl)-3,3-dimethyl-2,3-dihydrofuran (**21**).* Tetrabutylammonium fluoride (1 M in THF, 2.08 mL, 2.08 mmol) was added to a solution of dihydrofuran **20** (508 mg, 0.630 mmol) in dry THF (4 mL) at 0 °C under a N_2 atmosphere and stirred for 0.5 h. The reaction mixture was poured into diluted aq HCl and extracted with AcOEt. The organic layer was washed three times with satd aq NaCl, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on SiO_2 and eluted with AcOEt/hexane (9:1) to give phenolic dihydrofuran **21** in 81.7% yield (356 mg). Amorphous orange solid; ^{1}H NMR (500 MHz, CD_3OD): δ_H 1.06 (s, 6H), 1.30 (s, 6H), 1.60–1.70 (m, 2H), 1.76–1.84 (m, 2H), 2.46 (t, J=7.3 Hz, 2H), 3.13 (s, 2H), 3.27–3.32 (m, 2H), 3.82 (s, 2H), 6.53 (dd, J=8.7 and 2.3 Hz, 2H), 6.62 (d, J=8.7 Hz, 2H), 6.66

(d, J=2.3 Hz, 2H), 6.72–6.73 (m, 1H), 6.75 (dd, J=7.8 and 1.8 Hz, 2H), 7.11–7.16 (m, 2H), 7.85 (dd, J=8.2 and 1.8 Hz, 1H), 8.31 (d, J=1.4 Hz, 1H) ppm; ^{13}C NMR (125 MHz, CD_3OD): δ_C 24.0, 28.0 (×2), 28.2 (×2), 30.5, 38.0, 38.3, 48.3, 50.1, 71.8, 80.9, 84.2, 103.8 (×2), 111.8 (×2), 114.0 (×2), 116.4 (×2), 118.3, 122.7, 123.9, 126.0, 128.3, 129.3, 130.1, 130.5 (×2), 138.5, 142.0, 148.9 (br), 152.7, 154.4 (×2), 158.1, 161.7 (×2), 171.6, 175.2 ppm; IR (KBr): $\tilde{\nu}$ 3393, 2957, 2869, 1735, 1607, 1313, 1179, 1115 cm^{-1}; HRMS (ESI): 692.2869, calcd for $C_{41}H_{42}NO_9$ $[M+H]^+$ 692.2860, 714.2687, calcd for $C_{41}H_{41}NO_9Na$ $[M+Na]^+$ 714.2679.

*4.2.16. 4-{7-[N-(4-Benzothiazol-2-yl)-3-hydroxyphenyl]methylcarbamoyl]-1,1-dimethyl-3-oxahept-1-yl}-5-(3-tert-butyldimethylsilyloxyphenyl)-3,3-dimethyl-2,3-dihydrofuran (**23**).* 2-(4-Aminomethyl-2-methoxyphenyl)benzothiazole (320 mg, 1.18 mmol) and Et_3N (0.20 mL, 1.43 mmol) were added to a solution of *N*-hydroxysuccinimide ester **6** (602 mg, 1.05 mmol) in CH_3CN (6 mL) under a nitrogen atmosphere at room temperature and stirred for 5 h. The reaction mixture was concentrated in vacuo. The residue was chromatographed on silica gel and eluted with AcOEt/hexane (1:2) to give amide **23** as a colorless oil in 86.3% yield (661 mg); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.17 (s, 6H), 0.97 (s, 9H), 1.03 (s, 6H), 1.29 (s, 6H), 1.55–1.64 (m, 2H), 1.71–1.80 (m, 2H), 2.30 (t, J=7.4 Hz, 2H), 3.10 (s, 2H), 3.27 (t, J=6.1 Hz, 2H), 3.84 (s, 2H), 4.02 (s, 3H), 4.49 (d, J=5.9 Hz, 2H), 5.95 (br s, 1H), 6.74–6.79 (m, 1H), 6.77 (s, 1H), 6.89 (d with fine coupling, J=7.7 Hz, 1H), 6.98 (s, 1H), 7.00 (d, J=7.9 Hz, 1H), 7.15 (t, J=7.7 Hz, 1H), 7.37 (dd with fine coupling, J=8.2 and 7.2 Hz, 1H), 7.49 (dd with fine coupling, J=8.2 and 7.2 Hz, 1H), 7.92 (d with fine coupling, J=7.9 Hz, 1H), 8.07 (d with fine coupling, J=8.2 Hz, 1H), 8.47 (d with fine coupling, J=7.9 Hz, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ_C −4.4 (×2), 18.1, 22.9, 25.6 (×3), 27.2 (×2), 27.4 (×2), 29.0, 36.4, 37.0, 43.3, 47.0, 55.7, 70.7, 79.7, 83.0, 111.0, 119.8, 120.2, 121.2, 121.4, 121.7, 122.3, 122.7, 123.0, 124.6, 125.9, 128.7, 129.7, 136.0, 137.1, 142.8, 151.0, 152.1, 155.0, 157.3, 162.8, 172.9 ppm; IR (liquid film): $\tilde{\nu}$ 3299, 2955, 2930, 2859, 1649, 1577, 1463, 1419, 1264, 1125, 940 cm^{-1}; Mass (*m*/*z*, %): 728 (M^+, 1), 346 (31), 345 (100), 254 (10), 235 (13); HRMS (ESI): 729.3747, calcd for $C_{42}H_{57}N_2O_5SSi$ $[M+Na]^+$ 729.3757.

*4.2.17. 4-{7-[N-(4-Benzothiazol-2-yl)-3-hydroxyphenyl]methylcarbamoyl]-1,1-dimethyl-3-oxahept-1-yl}-5-(3-hydroxyphenyl)-3,3-dimethyl-2,3-dihydrofuran (**24**).* CH_3SNa (95%, 211 mg, 2.86 mmol) was added to a solution of amide **23** (493 mg, 0.676 mmol) in dry DMF (5 mL) under a nitrogen atmosphere at room temperature and stirred at 140 °C for 1 h. The reaction mixture was poured into satd aq NH_4Cl and extracted with AcOEt. The organic layer was washed three times with satd aq NaCl, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel and eluted with AcOEt/hexane (2:1) to give amide **24** in 94.8% yield (385 mg). Colorless granules mp 132.5–133.0 °C (from AcOEt/hexane); ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 1.01 (s, 6H), 1.29 (s, 6H), 1.57–1.64 (m, 2H), 1.77–1.90 (m, 2H), 2.35 (t, J=7.2 Hz, 2H), 3.10 (s, 2H), 3.29 (t, J=5.7 Hz, 2H), 3.85 (s, 2H), 4.50 (d, J=5.8 Hz, 2H), 5.91 (br s, 1H), 6.77–6.83 (m, 1H), 6.83 (d with fine coupling, J=8.2 Hz, 1H), 6.90 (dd, J=7.9 and 1.5 Hz, 1H), 7.00 (s with fine coupling, 1H), 7.03 (s with fine coupling, 1H), 7.16 (t, J=7.9 Hz, 1H), 7.42 (t with fine coupling, J=7.9 Hz, 1H), 7.51 (t with fine coupling, J=7.9 Hz, 1H), 7.66 (d, J=8.2 Hz, 1H), 7.91 (d, J=7.9 Hz, 1H), 7.99 (d, J=7.9 Hz, 1H), 8.32 (s, 1H), 12.60 (br s, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 22.9, 27.2 (×2), 27.7 (×2), 29.2, 36.1, 37.1, 43.3, 46.9, 70.3, 79.3, 82.9, 115.4, 115.8, 116.4, 117.3, 118.9, 121.1, 121.4, 121.4, 122.0, 125.5, 126.6, 128.7, 128.8, 132.5, 136.9, 143.2, 151.2, 151.6, 156.3, 157.9, 168.9, 173.9 ppm. IR (KBr): $\tilde{\nu}$3316, 2955, 2929, 2865, 1634, 1578, 1481, 1440, 1215, 759 cm^{-1}; Mass (*m*/*z*, %): 600 (M^+, 1), 255 (13), 244 (15), 232 (17), 231 (100); HRMS (ESI): 623.2517, calcd for $C_{35}H_{40}N_2O_5SNa$ $[M+Na]^+$ 623.2556.

*4.2.18. 5-[7-(Fluorescein-5-yl)carbamoyl-1,1-dimethyl-3-oxaheptyl]-1-(3-hydroxyphenyl)-4,4-dimethyl-2,6,7-trioxabicyclo[3.2.0]heptane (**3**).* Dihydrofuran **21** (100 mg, 0.145 mmol) was irradiated in the presence of TPP (1.0 mg) externally with a 940 W Na-lamp in CH_2Cl_2 (10 ml) and CH_3OH (10 drops) under an O_2 atmosphere at 0 °C for 1 h. The photolysate was concentrated in vacuo. The residue was chromatographed on SiO_2 and eluted with CH_2Cl_2/ether to give dioxetane **3** in 84.6% yield (88.8 mg). Amorphous orange solid; ^{1}H NMR (500 MHz, CD_3OD): δ_H 0.87 (s, 3H), 1.13 (s, 3H), 1.17 (s, 3H), 1.39 (s, 3H), 1.56–1.66 (m, 2H), 1.72–1.84 (m, 2H), 2.45 (t, J=7.3 Hz, 2H), 3.25 (d, J=9.2 Hz, 1H), 3.33–3.40 (m, 3H), 3.38 (d, J=9.2 Hz, 1H), 3.79 (d, J=8.0 Hz, 1H), 4.47 (d, J=8.0 Hz, 1H), 6.53 (dd, J=8.7 and 2.3 Hz, 2H), 6.63 (d, J=8.7 Hz, 2H), 6.60 (d, J=2.3 Hz, 2H), 6.80–6.85 (m, 1H), 7.05 (s, 1H), 7.06 (d, J=7.9 Hz, 1H), 7.14 (d, J=8.2 Hz, 1H), 7.22 (dd, J=8.2 and 7.9 Hz, 1H), 7.86 (dd, J=8.2 and 1.8 Hz, 1H), 8.31 (d, J=1.8 Hz, 1H) ppm; ^{13}C NMR (125 MHz, CD_3OD): δ_C 18.5, 21.6, 23.3, 24.0 (×2), 25.3, 30.3, 38.0, 42.5, 46.9, 72.1, 77.7, 81.4, 103.8 (×2), 106.6, 111.8 (×2), 114.0 (br, ×2), 116.4 (br), 116.8, 117.8, 118.5, 121.0, 126.0, 128.4, 129.4, 130.3, 130.5 (×2), 138.9, 142.1, 149.0 (br), 154.5 (×2), 158.5, 161.7 (br×2), 171.6, 175.1 ppm; IR (KBr): $\tilde{\nu}$ 3353, 2925, 2871, 1735, 1671, 1606, 1312, 1114 cm^{-1}; HRMS (ESI): 746.2553, calcd for $C_{41}H_{41}NO_{11}Na$ $[M+Na]^+$ 746.2577.

*4.2.19. 5-{7-[N-(4-Benzothiazol-2-yl)-3-hydroxyphenyl]methylcarbamoyl]-1,1-dimethyl-3-oxahept-1-yl}-1-(3-hydroxyphenyl)-4,4-dimethyl-2,6,7-trioxabicyclo[3.2.0]heptane (**4**).* A solution of dihydrofuran **24** (150 mg, 0.250 mmol) and TPP (3 mg) in CH_2Cl_2 (10 ml) was irradiated externally with 940 W Na-lamp under an oxygen atmosphere at 0 °C for 1 h. The photolysate was concentrated in vacuo. The residue was chromatographed on silica gel and eluted with CH_2Cl_2 and ether/CH_2Cl_2 (1:1) to give dioxetane **4** in quantitative yield. Compound **4**: Colorless amorphous solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.87 (s, 3H), 1.04 (s, 3H), 1.12 (s, 3H), 1.35 (s, 3H), 1.50–1.82 (m, 4H), 2.27–2.43 (m, 2H), 3.23 (d, J=9.3 Hz, 1H), 3.29–3.42 (m, 2H), 3.57 (d, J=9.3 Hz, 1H), 3.80 (d, J=8.0 Hz, 1H), 4.45–4.55 (m, 2H), 4.54 (d, J=8.0 Hz, 1H), 6.35 (t, J=5.6 Hz, 1H), 6.89 (d with fine coupling, J=8.1 Hz, 1H), 6.96 (d with fine coupling, J=8.1 Hz, 1H), 7.06–7.10 (m, 2H), 7.22–7.28 (m, 2H), 7.41(dd with fine coupling, J=7.9 and 7.3 Hz, 1H), 7.51 (dd with fine coupling, J=8.1 and 7.3 Hz, 1H), 7.67 (d, J=8.1 Hz, 1H), 7.90 (d with fine coupling, J=7.9 Hz, 1H), 7.99 (d with fine coupling, J=8.1 Hz, 1H), 12.72 (br s, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 18.0, 20.9, 22.4, 23.5, 24.6, 28.2, 36.0, 40.8, 43.2, 45.5, 71.5, 76.9, 80.0, 105.6, 115.7, 115.8, 116.6, 116.8, 116.9, 119.2, 119.9, 121.4, 122.0, 125.5, 126.6, 128.7, 129.2, 132.5, 136.9, 143.8, 151.6, 156.4, 157.7, 169.0, 174.1 ppm. IR (KBr): $\tilde{\nu}$ 3342, 2948, 2872, 1632, 1583, 1481, 1316, 1216, 759 cm^{-1}; Mass (*m*/*z*, %): 632 (M^+, 89), 482 (12), 356 (22), 355 (10), 340 (12), 339 (58), 326 (11), 278 (35). 257 (17), 256 (30), 255 (100), 241 (12), 240 (14), 228 (11), 227 (18), 221 (26), 121 (26), 100 (45), 94 (55), 83 (24), 71 (10), 56 (11); HRMS (ESI): 655.2434, calcd for $C_{35}H_{40}N_2O_7SNa$ $[M+Na]^+$ 655.2454.

4.3. Measurement of chemiluminescence and time-course of the base-induced decomposition of dioxetanes: general procedure

Chemiluminescence was measured using a JASCO FP-750 and/or FP-6500 spectrometer, and a Hamamatsu Photonics PMA-11 multichannel detector.

4.3.1. TBAF/acetonitrile system. A freshly prepared solution (2.00 mL) of TBAF (1.0×10^{-2} mol/L) in acetonitrile was transferred to a quartz cell (10×10×50 mm), which was placed in a spectrometer that was thermostated with stirring at 25 °C. After 3–5 min, a solution of dioxetane **3** or **4** in acetonitrile (1.0×10^{-4} mol/L, 1.00 mL) was added by means of a syringe, and measurement was

started immediately. The time-course of the intensity of light emission was recorded and processed according to first-order kinetics. The total light emission was estimated by comparing it with that of an adamantylidene dioxetane, the chemiluminescent efficiency Φ^{CL} of which has been reported to be 0.29 and which was used here as a standard.[17,18]

4.3.2. NaOH/H_2O system. A freshly prepared solution (2.00 mL) of NaOH (0.1 M) in H_2O was transferred to a quartz cell (10×10×50 mm), which was placed in a spectrometer that was thermostated with stirring at 25 °C. After 3–5 min, a solution of dioxetane **3** or **4** in H_2O containing acetonitrile (9:1) (1.0×10^{-4} mol/L, 1.00 mL) was added by means of a syringe, and measurement was started immediately. The time-course of the intensity of light emission was recorded and processed according to first-order kinetics. The total light emission was estimated as in the case of solvent-promoted decomposition described above.

4.3.3. NaOH/H_2O system including a fluorescer. Except for the use of NaOH in H_2O (0.1 M) including acetamidofluorescein **25** (1.0×10^{-3} M) or *N*-[4-(benzothiazol-2-yl)-3-hydroxybenzyl]acetamide **26** (1.0×10^{-3} M) in place of NaOH in H_2O (0.1 M) without any additive, the chemiluminescent reaction was carried out as in the NaOH/H_2O system described above.

4.3.4. NaOH/H_2O system including a surfactant. Except for the use of NaOH in H_2O (0.1 M) including tributylhexadecylphosphonium bromide **27** (1.0×10^{-3} M) in place of NaOH in H_2O (0.1 M) without any additive, the chemiluminescent reaction was carried out as in the NaOH/H_2O system described in Section 4.3.2.

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Supplementary data

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9. It has been reported in a patent 10 that a dioxetane tethering a fluorescein through an ω,ω′-dioxyalkylene linker instead of a methoxy group in **1** shows enhanced chemiluminescence.
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11. Although the methoxy group of parent dioxetane **1** can easily be modified to a substituted alkoxy, aryloxy or alkylsulfanyl group, such modification directly affects chemiluminescence properties. 10,12 On the other hand, structural modification of the adamantylidene would not easily be attained: even if such modification is attained, the thus-synthesized analogs of **1** decompose to two molecules, i.e., an oxidobenzoate in the excited state and a modified adamantanone, which would rapidly separate from each other.
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13. We attempted to use ω-oxypentanoic acid unit as a representative of linker, since ω-oxy- or ω-amino-substituted C_5 or C_6 carboxylic acid has been often used as a linker for fluorescent probes. 14
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15. Advanced intermediate **6** could lead, under mild reaction conditions, to dioxetanes tethering a various functional auxiliary or junctioning a biomacromolecule or a modified solid surface. Key building block **7** was also useful to synthesize various analogs of dioxetane **2** functionalized on a methyl of a *tert*-butyl group, since a primary OH group in **7** was far more reactive than another OH group for nucleophilic displacements. The results will be reported elsewhere in near future.
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17. Φ^{CL} was estimated based on the value 0.29 for the chemiluminescent decomposition of 3-adamantylidene-4-methoxy-4-(3-oxidophenyl)-1,2-dioxetane in a TBAF/DMSO system. 18
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20. Effect of concentration of fluorescer **25** or **26** on Φ^{CL} was preliminarily examined in the range of 10^{-5}–10^{-2} M and 10^{-3} M of the fluorescer (**25**(**26**)/**2a**≈20) was found to be the most effective to improve Φ^{CL} for CTID of **2a** in NaOH/H_2O system. On the other hand, in a TBAF/acetonitrile system, even 10^{-4} M of the fluorescer (**25**/**2a**≈200) was insufficient for the intermolecular energy-transfer chemiluminescence.
21. Surfactant **27** was initially expected not to improve Φ^{fl} of a fluorescein moiety, since Φ^{fl} of acetamidofluorescein **25** as a model fluorophore in **3** was higher in an aqueous medium rather than in an aprotic medium: 0.73 in NaOH/H_2O versus 0.52 in TBAF/acetonitrile.