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Synthesis and enhanced chemiluminescence of new cyclomaltooligosaccharide (cyclodextrin)-bound 6-phenylimidazo[1,2-*a*]pyrazin-3(7*H*)-one

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Abstract

In order to provide chemiluminescent substrates that have high light-emitting efficiency in aqueous solution, the structural design on 6-phenylimidazo[1,2-*a*]pyrazin-3(7*H*)-one compounds was studied in the covalent attachment of a light-producing chromophore to a cyclomaltooligosaccharide (cyclodextrin). The synthesis of cyclodextrin-bound 6-phenylimidazo[1,2-*a*]pyrazin-3(7*H*)-one compounds was achieved by the formation of an amido bond between a 6-phenylimidazo[1,2-*a*]pyrazin-3(7*H*)-one and a mono-6-amino-6-deoxycyclodextrin. The properties of oxygen-induced chemiluminescence of the synthesized cyclodextrin-bound light-emitting chromophores were investigated. The light-emitting efficiency in pH 8.3 phosphate buffer was remarkably dependent on the kind of bound cyclodextrin and the binding site between the chromophore and cyclodextrin. The light-emitting efficiency of a cyclodextrin-bound compound in which cyclomaltoheptaose (β-cyclodextrin) had been covalently attached to the 2-position of the imidazo[1,2-*a*]pyrazin-3(7*H*)-one ring system showed an up to 11-fold enhancement over that of a non-cyclodextrin chromophore, whereas attachment to cyclomaltohexaose (α-cyclodextrin) resulted in no enhancement. Moreover, this study indicated that the strategy that involves covalently attaching a light-producing chromophore onto a cyclodextrin for the enhancement of chemiluminescence is more efficient than the use of an aqueous solution containing very large amounts of cyclodextrin.

Keywords: Cyclomaltooligosaccharide; Cyclodextrin; Cyclodextrin-bound light-producing compound; Chemiluminescence; Enhanced chemiluminescence; MCLA

1. Introduction

The chemiluminescent reaction of Cypridina luciferin without enzyme in dimethyl sulfoxide and in

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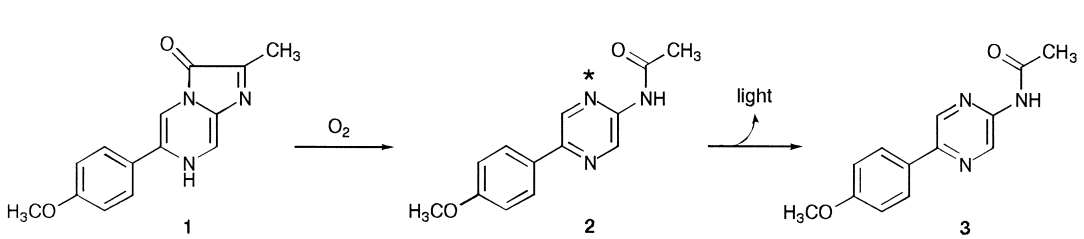
the presence of oxygen was first reported in 1966 by Johnson et al. [1]. Diethylene glycol dimethyl ether, containing a trace amount of acetate buffer (pH 5.6) was later reported as an efficient solvent by Goto et al. [2]. In this solvent system the quantum efficiency of Cypridina chemiluminescence is about 10% that of Cypridina bioluminescence, which was reported as ca. 0.28 [3], whereas the quantum efficiency in dimethyl sulfoxide was reported to be below 0.0005 [1]. Thereafter, it has been shown that several synthesized analogues of Cypridina luciferin, such as 2-methyl-6-indolyl-, 2-methyl-6-phenyl-, and 2-methyl-6-(*p*-methoxyphenyl)-imidazo[1,2-*a*]pyrazin-3(7*H*)-one (MCLA) (**1**), generate light and the emitter **3** via formation of a singlet-excited amide **2** by oxidation with triplet oxygen in buffer solutions [4,5], as shown in Scheme 1, and with superoxide anion in aqueous solvents [6–8]. However, the chemiluminescent quantum efficiency of Cypridina luciferin and its analogues in an aqueous solvent has been less than 1% that of Cypridina bioluminescence.

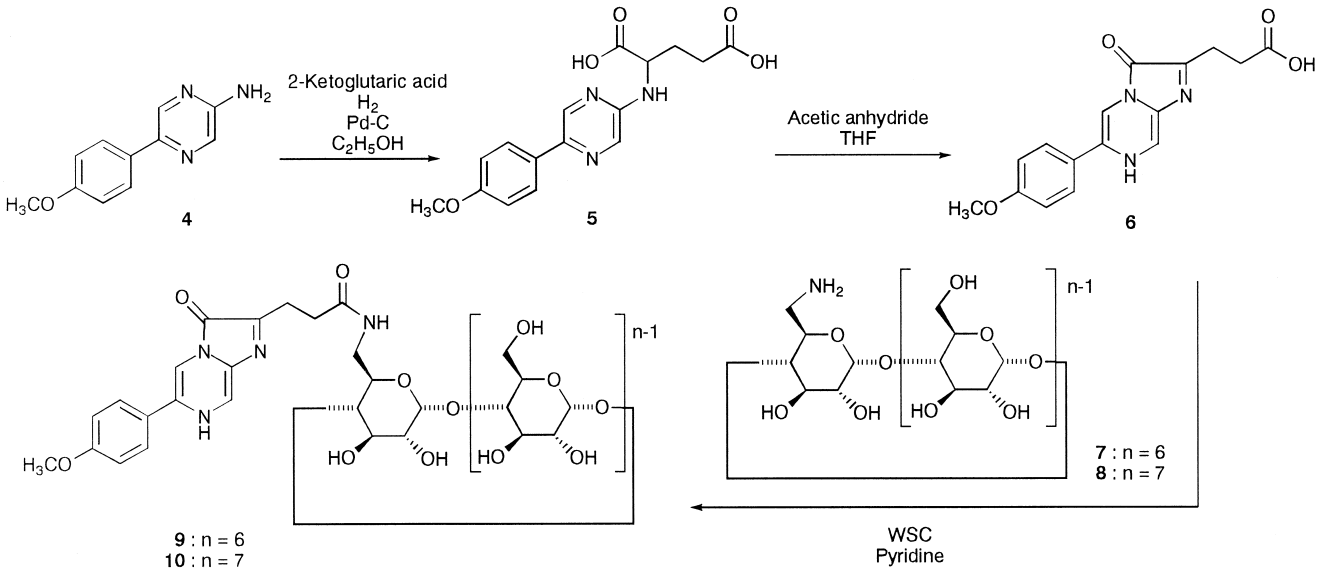
Chemiluminescence offers several potential advantages for analytical applications. Many studies on the enhancement of chemiluminescence for analysis have dealt with the regulated medium, such as using a micellar surfactant or cyclomaltooligosaccharide (cyclodextrin). It has been shown that cyclodextrin enhances the chemiluminescent intensity of luminescent compounds, such as lucigenin [9], peroxyoxalate [10], isoluminol [11], and Cypridina luciferin analogues [12] in aqueous solvents. Cypridina luciferin analogue MCLA (**1**) (1.7 mM) in 0.01 M aqueous solutions of cyclomaltoheptaose (*β*-cyclodextrin) or heptakis(2,6-di-*O*-methyl)-*β*-cyclodextrin showed chemiluminescence with 0.0034 or 0.010 quantum efficiency, respectively. The study showed that the cavity of *β*-cyclodextrin may be most closely fitted to MCLA, but that very large amounts of cyclodextrin are needed for the aqueous chemiluminescent system. For analysis by chemiluminescence, the analytical system should not affect the subject except to promote luminescence. It is noteworthy to construct a novel chemiluminescent system that promotes greater chemiluminescent activity. In this paper, we report the first example of the synthesis of light-producing compounds, in which MCLA, a chemiluminescent chromophore, is covalently bound to one cyclodextrin molecule, and show that the chemiluminescent intensity of the cyclodextrin-bound luminescent chromophore is effectively enhanced in an aqueous solvent.

2. Results and discussion

The synthetic route used is shown in Scheme 2. The coupling of amino pyrazine **4** [8] and *α*-ketoglutaric acid gave amino diacide **5** with a 30% yield. Compound **5** was treated with acetic anhydride in tetrahydrofuran (THF) at room temperature to give the yellow cyclized compound **6** in a 30% yield. Then, **6** and mono-6-amino-6-deoxycyclomaltohexaose (mono-6-amino-6-deoxy-*α*-cyclodextrin) (**7**) [13] or mono-6-amino-6-deoxy-*β*-cyclodextrin (**8**) [13] were coupled in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (WSC) to afford cyclodextrin-bound luminescent compounds **9** and **10** in 47% and 48% yields, respectively. Cyclodextrin-bound luminescent compound **14**, which possesses a different binding mode, was prepared by the route shown in Scheme 3. Compound **12** was prepared in a 72% yield by the condensation of compound **11** (which was obtained by the treatment of compound **4** with pyridine hydrochloride at 210 °C) with sodium hydride and ethyl 4-bromobutyrate in *N*,*N*-dimethylformamide (Me_2NCHO). A mixture of compound **12** and pyruvic aldehyde was heated in a water–1,4-dioxane mixture in the presence of HCl to afford compound **13** in 34% yield. Condensation of compound **13** with **8** was carried out by treatment



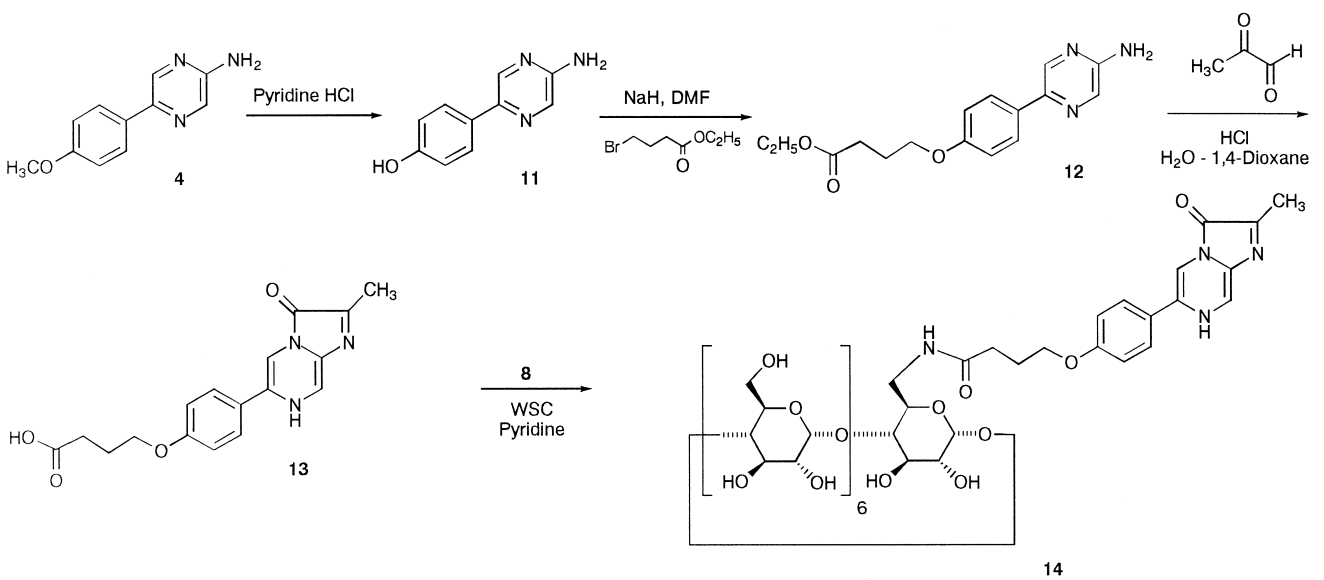
Scheme 1.



Scheme 2.

with WSC in pyridine similar to the procedure used for the preparation of **10**.

The chemiluminescent reaction consists of many reaction steps such as binding with an oxygen molecule, generation of intermediate(s) having high energy, formation of an excited singlet state, and emission of light, and efficiencies in these reaction steps determine the quantum yield of chemiluminescence. Efficiency in binding with an oxygen molecule is influenced by the molecular state of the light-producing chromophore. Electronic spectra (Fig. 1) of **9**, **10** and **14** show the molecular states of the chromophores in these compounds in 30 mM phosphate buffer (pH 8.3). The binding with β-cyclodextrin in **10** and **14** influences the spectra in comparison to **1** as shown in Fig. 1, whereas **9** is similar in shape and intensity to **1**. These results show that the molecular conditions of the light-producing chromophores in **10**



Scheme 3.

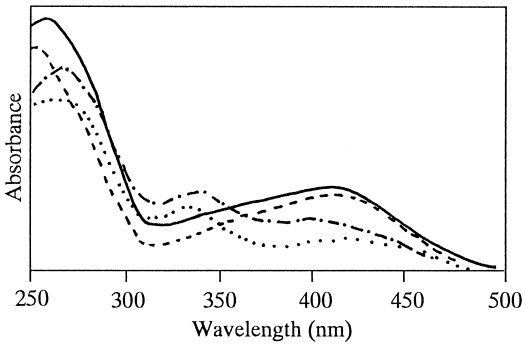


Fig. 1. Electronic spectra of **1**, **9**, **10** and **14** in 30 mM phospate buffer (pH 8.3). (————) **1**; (---) **9**; (· · · · ·) **10**; (-·-·-·) **14**. Concentration of luminescent compound is 10 μM.

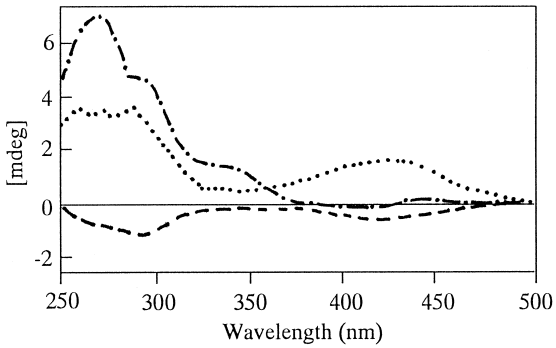


Fig. 2. Induced circular dichroism spectra of **9**, **10** and **14** in 30 mM phosphate buffer (pH 8.3). (——) **9**; (· · · · ·) **10**; (-·-·-·) **14**. Concentration of luminescent compound is 10 μM.

and **14** are different from those of **1** and **9** and that reactivities to oxygen are not the same. Only when the cyclodextrin forms the inclusion complex with an achiral chromophore does it show circular dichroism in the absorption area of the chromophore. The magnitude of intensity and pattern of the induced circular dichroism (ICD) spectrum indicates the inclusion mode. Fig. 2 shows ICD spectra of **9**, **10** and **14** in 30 mM phosphate buffer (pH 8.3) without oxygen. The ICD spectrum of **10** exhibits positive bands below 500 nm, whereas **9** shows too small negative bands in a similar region. The structural features of the cyclodextrin compounds **9**, **10** and **14** could be reflected in the ICD spectra, and the light-emitting chromophore in **10**, which exhibits strong positive bands, must be included deeply in the β-cyclodextrin cavity. Since compound **9** exhibits a very weak band, the light-emitting chromophore cannot be deeply included in the cavity. The cavity of α-cyclodextrin is not sufficiently deep to include the light-emitting chromophore in contrast with that of β-cyclodextrin, and the imidazopyrazinone moiety of the light-producing chromophore may not have hydrophobic character for the inclusion. In comparison with the case of **10**, the ICD spectrum of **14** exhibits positive bands only below 380 nm. This observation suggests that the moiety of the light-emitting chromophore in **14** is included in the β-cyclodextrin cavity differently with **10**. It is possible that the light-emitting chromophore of **14** could not be sufficiently included into the cavity of cyclodextrin in contrast to **10**, because the imidazopyrazinone ring that is located at the tip of the chromophore of **14** seems to be more hydrophilic than the anisole group.

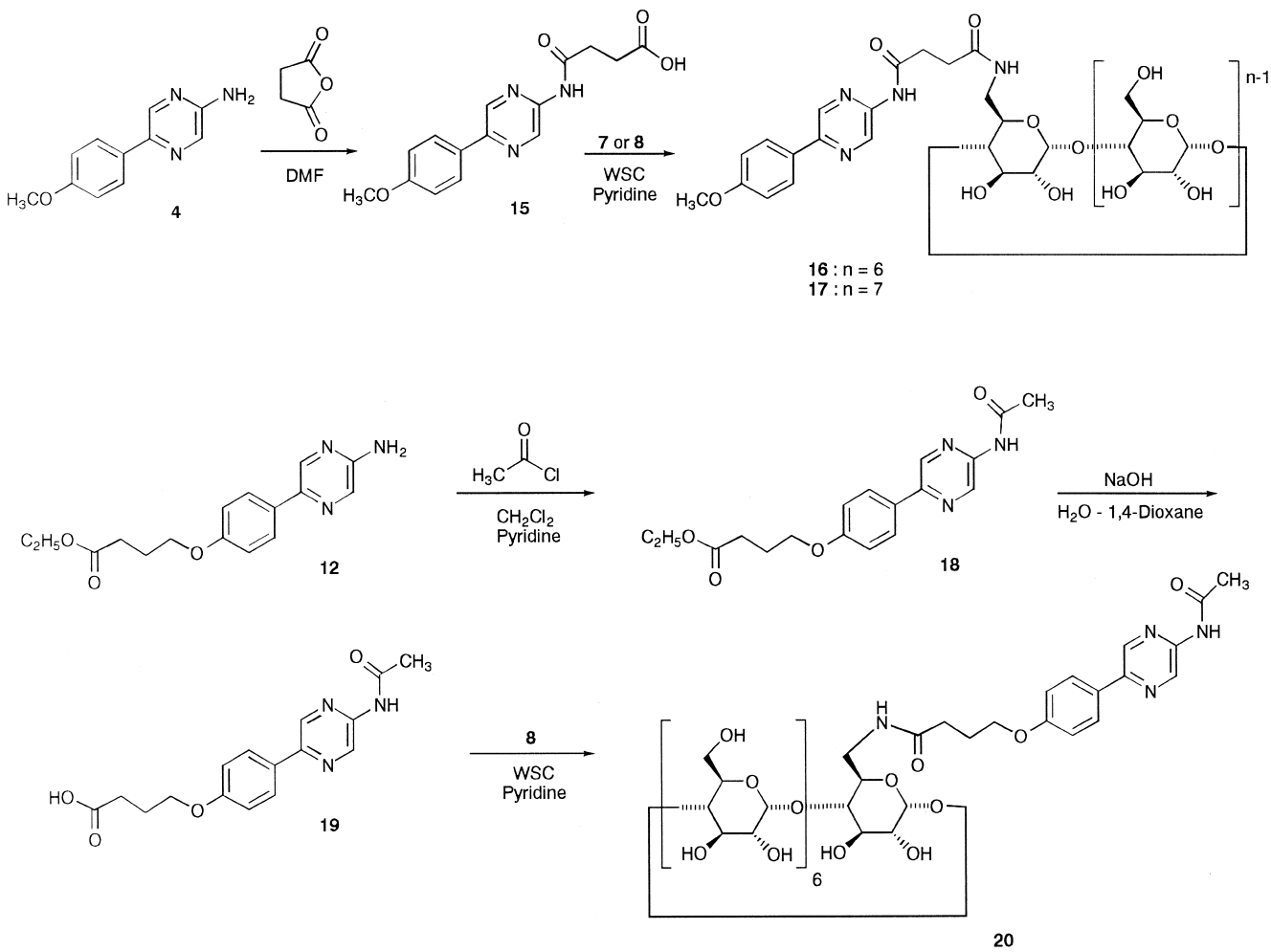
Table 1
Chemiluminescent properties of MCLA and cyclodextrin-bound MCLA[a] in phosphate buffer[b,c]

No. of compound	Φ_{CL} ($\times 10^{-2}$)	$t_{1/2}$ (min)	Φ_R ($\times 10^{-2}$)	Φ_F ($\times 10^{-2}$)	Φ_S ($\times 10^{-2}$)
1 (MCLA)	0.17	80	27	5	13
9	0.21	170	39	13	4
10	1.9	32	49	17	23
14	0.51	85	41	23	5
1 + β-cyclodextrin[d]	0.29	80	31	27	3
1 + β-cyclodextrin[e]	1.1	45	53	22	9

[a]Concentration of compounds is 10 μM.
[b]30 mM phosphate buffer (pH 8.3) at 25 °C.
[c]Abbreviations include the following:
[d]Concentration of β-cyclodextrin is 1 mM.
[e]Concentration of β-cyclodextrin is 10 mM.
Φ_{CL} = Quantum efficiency of chemiluminescence.
$t_{1/2}$ = Half time of chemiluminescence.
Φ_R = Formation yield of the corresponding amide.
Φ_F = Quantum efficiency of fluorescence of the corresponding amide.
Φ_S = Quantum efficiency of excited singlet state formation calculated from the equation $\Phi_{CL} = \Phi_R \times \Phi_S \times \Phi_F$.

Because intermediate(s) having high energy such as dioxetane and singlet excited state molecule are too unstable and short-lived to be detected with conventional apparatus, the influence of these short-lived molecule(s) by bound cyclodextrin cannot be directly analyzed in particular. Generally, one of the chemiluminescent properties, the chemiluminescent quantum efficiency (Φ_{CL}), is given by the following equation; $\Phi_{CL} = \Phi_R \times \Phi_S \times \Phi_F$ (Φ_R: formation yield of emitter, Φ_S: quantum efficiency of excited singlet state formation of the emitter, Φ_F: quantum efficiency of fluorescence of the emitter). Φ_{CL} and Φ_R are easily determined by experiments. Veritable quantum efficiency of excited-singlet-state formation and quantum efficiency of fluorescence, however, cannot be determined, because the same singlet excited-state molecule(s) as the singlet excited-state intermediate(s) formed in the course of a chemiluminescent reaction cannot be duplicated for analysis of these efficiencies. In the present case, it was observed that the fluorescent spectrum of the emitter is not superimposable on the corresponding chemiluminescent spectrum.

The chemiluminescent quantum efficiency and chemiluminescent half life of **9**, **10** and **14** were tested in 30 mM phosphate buffer (pH 8.3) in the presence of oxygen. The results are summarized in Table 1, in comparison with MCLA (**1**). The chemiluminescent quantum efficiency of β-cyclodextrin-bound compound **10** was about 11 times greater than that of MCLA, whereas the quantum efficiency of α-cyclodextrin-bound compound **9** was practically the same as that of MCLA. A decrease in chemiluminescent half-life was shown in the chemiluminescence of **10**. These observations suggest, in the case of β-cyclodextrin, that an intramolecular inclusion of the chemiluminescent emitter by the cyclodextrin cavity has occurred, thus causing the enhancement of



Scheme 4.

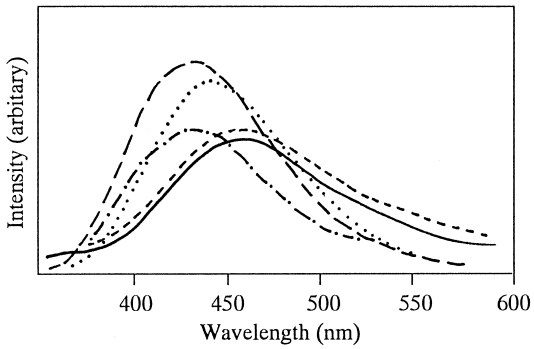


Fig. 3. Chemiluminescent spectra of **1**, **9**, **10** and **14** in 30 mM phosphate buffer (pH 8.3). (————) **1**; (---) **9**; (· · · · ·) **10**; (-·-·-·) **14**; (— — — — —) **1** + β-cyclodextrin (10 mM). Concentration of luminescent compound is 10 μM.

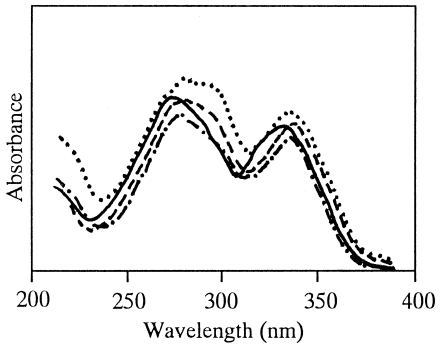


Fig. 5. Electronic spectra of **3**, **16**, **17** and **20** in 30 mM phospate buffer (pH 8.3). (————) **3**; (---) **16**; (· · · · ·) **17**; (-·-·-·) **20**. Concentration of luminescent compound is 10 μM.

luminescence due to the hydrophobic environment; in the case of α-cyclodextrin, the cavity size is probably insufficient to accommodate the luminescent emitter(s). It is noted that the quantum efficiency of **10** was greater than that of MCLA in using cyclodextrin solutions reported by Toya [12]. The quantum efficiency of another β-cyclodextrin-bound compound **14** was only three times that of MCLA. This observation indicates that the binding site of the chemiluminescent emitter(s) to cyclodextrin is important for effective enhancement. Binding a β-cyclodextrin to MCLA has some effect on the chemiluminescent spectra as shown in Fig. 3. The luminescent maxima of **10** and **14** are 440 and 428 nm, respectively, which are shorter than that of **1**, whereas α-cyclodextrin-bound compound **9** shows no effect. The shift in luminescent maximum is similarly observed with MCLA in β-cyclodextrin solution. That β-cyclodextrin in **10** and **14** has a larger effect than α-cyclodextrin supports the theory that singlet excited emitters of **10** and **14** are included into β-cyclodextrin as discussed in the section on the chemiluminescent quantum yield.

Amido compounds **16**, **17** and **20** (see Scheme 4) are products of the chemiluminescent reactions of **9**, **10** and **14**, respectively, and are considered to be the light emitters according to the comparison of their chemiluminescent spectra and the fluorescent spectra (Fig. 4) of the corresponding amides. They were synthesized from the amino compounds **4** or **12** as shown in Scheme 4, for several analyses. As shown in Table 1, the cyclodextrin-bound compound **10** afforded 1.8 times higher formation yield of the amide **17** than **1**. In the case of using β-cyclodextrin (10 mM) solution, the formation yield of **3**, which was generated from **1** by the chemiluminescent reaction, was twice as high as that of the non-β-cyclodextrin similar to the case of **10**. These results indicate that using covalent and noncovalent cyclodextrin

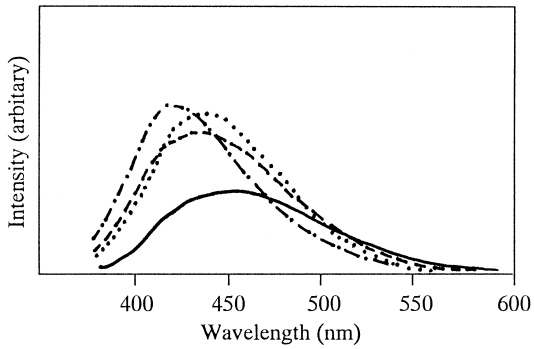


Fig. 4. Fluorescent spectra of **3**, **16**, **17** and **20** in 30 mM phosphate buffer (pH 8.3) (————) **3**; (---) **16**; (· · · · ·) **17**; (-·-·-·) **20**. Concentration of luminescent compound is 10 μM.

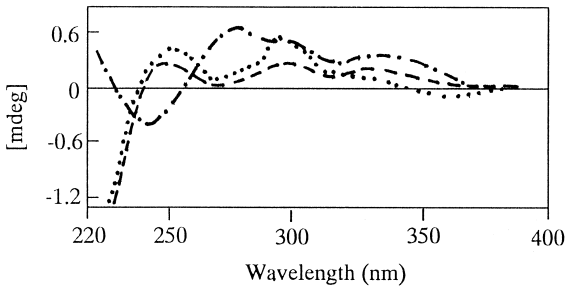


Fig. 6. Induced circular dichroism spectra of **16**, **17** and **20** in 30 mM phosphate buffer (pH 8.3). (---) **16**; (· · · · ·) **17**; (-·-·-·) **20**. Concentration of luminescent compound is 10 μM.

is one of the methods for the effective oxidation of MCLA. Fluorescent quantum efficiencies of the amides were measured under the same conditions as the chemiluminescent reaction, and the results are shown in Table 1. The cyclodextrin-bound amido compounds **16**, **17** and **20** gave higher fluorescent quantum efficiencies than the amide **3**. The binding of cyclodextrin to the amide **3** has an effect on its fluorescent spectrum, electronic spectrum (Fig. 5), and ICD spectrum (Fig. 6). The fluorescent maxima of **16**, **17** and **20** are shorter than that of **3** and show large hypsochromic effects. Bathochromic shifts in the electronic spectra are observed with **16**, **17** and **20**. The ICD spectra of **16**, **17** and **20** show strong positive bands in contrast with the ICD spectra of **9**, **10** and **14**. The influence on the fluorescent quantum efficiencies and these spectra suggests that all the chromophores of amides **16**, **17** and **20** are strongly included in the cyclodextrin cavity, because the amido molecules are more hydrophobic than the imidazopyrazinone molecules. Quantum efficiency in the singlet excited-state formation of **17** was increased over that of **3**, whereas those of **16** and **20** had an opposite effect. The β-cyclodextrin-bound compound **10** has an ability for increasing all the efficiencies in chemiluminescence.

In summary, this study shows the first example of cyclodextrin-bound (covalently bound) chemiluminescent compounds whose luminescence is enhanced by the intramolecular inclusion into β-cyclodextrin in an aqueous solvent. This strategy to increase the chemiluminescent quantum efficiency is more efficient than the use of ordinary cyclodextrin solutions alone in typical host–guest interactions.

3. Experimental

General methods.—HPLC analysis and isolation were carried out using a JASCO Gulliver HPLC system with a MD-910 three-dimensional UV–vis detector. A Fuji Silysia Chromatorex-ODS DU0005MT column (4.6 mm × 150 mm) was used for the analysis. Analytical TLC was performed on Merck Kieselgel $60F_{254}$ precoated glass plates of 0.25 mm layer thickness. All melting point (mp) values were measured with a Yanagimoto Seisakusho apparatus and are uncorrected. IR spectra were taken with a Shimadzu IR-470 infrared spectrometer, and UV spectra were obtained with a Shimadzu UV-3100 spectrometer. ^{1}H NMR spectra were determined on a JEOL JNM-A500 spectrometer at 20 °C using tetramethylsilane or *tert*-butanol as an internal reference. Chemical shift values are reported in δ (ppm) relative to an internal standard of Me_4Si, and coupling constants (J) are in Hz. FAB mass spectra (positive-ion mode) or SIMS spectra (positive-ion mode) were measured with a JEOL DX-303 or HITACHI M-80LCAPI instrument using glycerol as a matrix. Elemental analyses were measured with a Yanaco CHN-Corder MT-3 instrument. Chemiluminescent intensity time curves were obtained using an Aloka Luminescence Reader BLR-3, and chemiluminescent spectra and fluorescent spectra were obtained using a Hitachi F-2000 spectrofluorometer. ICD spectra were obtained using a JASCO J-720M spectrometer. All chemicals not otherwise mentioned were purchased from Tokyo Kasei Kogyo (Tokyo, Japan) or Aldrich Chemical Co. (Milwaukee, WI, USA) in chemically pure grade and were used as such. Mono-6-amino-6-deoxy-α-cyclodextrin [13], mono-6-amino-6-deoxy-β-cyclodextrin [13], **1** [8], **3** [8], and **4** [8] were prepared according to the published method.

2 - [5 - (4 - Methoxyphenyl) - 2 - pyrazinyl]aminopentanedioic acid (**5**).—Pd–C (5%, 2.0 g) was added to a solution of **4** (5.0 g, 25 mmol) and α-ketoglutaric acid (7.3 g, 50 mmol) in ethanol (100 mL), and the mixture was stirred under a hydrogen atmosphere at 55 °C for 5 h. The reaction mixture was filtered through Celite, and the filtrate was evaporated under reduced pressure to dryness. The product was crystallized from methanol to afford **5** (2.5 g, 30%): mp 190–191 °C; UV (MeOH) λ_{max} nm (ε) 354 (7580), 285 (26,700), and 221 (6720); IR (KBr): ν 3370, 1740, 1600 and 1500 cm^{-1}; ^{1}H NMR (CD_3OD): δ 2.0–2.6 (m, 4 H, CH_2CH_2), 3.77 (s, 3 H, OCH_3), 4.55 (m, 1 H, HOOCC*H*N), 6.92 (d, 2 H, J 9.0 Hz, ArH), 7.70 (d, 2 H, J 9.0 Hz, ArH), 8.00 (s, 1 H, CH pyrazine), and 8.30 (s, 1 H, CH pyrazine); Anal. Calcd for $C_{16}H_{17}N_3O_5$: C, 58.00; H, 5.17; N, 12.68. Found: C, 58.33; H, 5.26; N, 12.32. SIMS: *m/z* 332 [M + 1].

3 - [6 - (4 - Methoxyphenyl)imidazo[1, 2 - a]pyrazin - 3(7H) - one - 2 - yl]propionic acid (**6**).—Acetic anhydride (4.5 mL of 1.0 M in tetrahydrofuran) was added to a solution of **5** (1.0 g, 3.0 mmol) in tetrahydrofuran (60 mL) at room temperature, and the reaction mixture was stirred at room temperature for 3 days under argon. The reaction mixture was filtered, and the resulting crystals were washed with ethyl acetate and dried under reduced pressure to give cyclized compound **6** (0.28 g, 30% yield): mp 213–214 °C;

UV (MeOH) λ_{max} nm (ε) 431 (6900), 356 (4810), and 264 (18,800); IR (KBr): ν 3450, 1690, 1610, 1560, and 1510 cm^{-1}; ^1H NMR (CD_3OD): δ 2.83 (t, 2 H, *J* 7.3 Hz, CH_2), 3.11 (t, 2 H, *J* 7.3 Hz, CH_2), 3.87 (s, 3 H, OCH_3), 7.07 (d, 2 H, *J* 8.6 Hz, ArH), 7.62 (d, 2 H, *J* 8.6 Hz, ArH), 7.83 (s, 1 H, CH pyrazine), and 7.90 (brs, 1 H, CH pyrazine); Anal. Calcd for $C_{16}H_{15}N_3O_4$: C, 61.34; H, 4.83; N, 13.41. Found: C, 61.52; H, 5.44; N, 13.26. SIMS: *m/z* 314 [M + 1].

6^I-Deoxy-6^I-[[6-(4-methoxypheny)imidazo[1,2-a]pyrazin-3(7H)-one-2-yl]propionyl]amino-cyclomalthohexaose (**9**).—Pyridine (4.0 mL) was added dropwise to a mixture of **6** (0.10 g, 0.32 mmol), WSC (0.49 g, 2.6 mmol), and 6^I-amino-6^I-deoxycyclomaltohexaose (0.62 g, 0.64 mmol) at about −100 °C under argon gas, and the solution was deoxygenated by bubbling argon gas into the mixture. The mixture was stirred at room temperature for 2 h and evaporated under reduced pressure to dryness. The residue was dissolved with water and filtered through a membrane filter. The filtrate was subjected to high-performance liquid chromatography (HPLC) on an ODS column (Fuji Silysia Chromatorex-ODS DU0005MT column, 20 mm × 250 mm) using acetonitrile–water mixture as eluent to afford **9** (0.19 g, 47% yield); yellow powder, mp 189 °C (dec, from acetonitrile–water); UV (0.03 M phosphate buffer, pH 8.3, with little oxygen) λ_{max} nm (ε) 410 (6000) and 262 (24,300); IR (KBr): ν 3400 and 1630 cm^{-1}; ^1H NMR (0.02 M TFA–D_2O): δ 2.73 (m, 1 H, CH_2CH_2), 2.83 (m, 1 H, CH_2CH_2), 3.05–3.30 (m, 2 H, CH_2CH_2), 3.05–3.30 (m, 2 H, H α-cyclodextrin), 2.7–5.2 (m, H α-cyclodextrin), 3.86 (s, 3 H, OCH_3), 4.86 (d, 1 H, *J* 3.0 Hz, H-1 α-cyclodextrin), 4.99 (d, 1 H, *J* 3.0 Hz, H-1 α-cyclodextrin), 7.05 (d, 2 H, *J* 8.5 Hz, ArH), 7.63 (d, 2 H, *J* 8.5 Hz, ArH), 7.95 (s, 1 H, CH pyrazine), and 8.46 (s, 1 H, CH pyrazine); Anal. Calcd for $C_{52}H_{74}N_4O_{32} \cdot 4H_2O$: C, 46.64; H, 6.17; N, 4.18. Found: C, 46.01; H, 6.52; N, 4.06. FABMS *m/z* 1267 [M + 1].

6^I-Deoxy-6^I-[[6-(4-methoxypheny)imidazo[1,2-a]pyrazin-3(7H)-one-2-yl]propionyl]amino-cyclomaltoheptaose (**10**).—This compound was prepared from 0.10 g (0.32 mmol) of **6**, WSC (0.50 g, 2.6 mmol), and 6^I-amino-6^I-deoxy-cyclomaltoheptaose (0.72 g, 0.63 mmol) as described for the synthesis of **9**, giving 0.092 g (48% yield); yellow powder, mp 175 °C (dec, from acetonitrile–water); UV (0.03 M phosphate buffer, pH 8.3, with little oxygen) λ_{max} nm (ε) 418 (2700), 340 (5200), and 268 (14,100); IR (KBr): ν 3400 and 1640 cm^{-1}; ^1H NMR (0.02 M TFA–D_2O): δ 2.6–4.2 (m, H β-cyclodextrin, CH_2CH_2, and OCH_3), 4.8–5.1 (m, H-1 β-cyclodextrin), 7.26 (d, 2 H, *J* 8.0 Hz, ArH), 7.41 (s, 1 H, CH pyrazine), 7.60 (d, 2 H, *J* 8.0 Hz, ArH), and 8.05 (s, 1 H, CH pyrazine); Anal. Calcd for $C_{58}H_{84}N_4O_{37} \cdot 5H_2O$: C, 45.85; H, 6.24; N, 3.69. Found: C, 45.35; H, 6.33; N, 3.12. FABMS: *m/z* 1429 [M + 1].

2-Amino-5-(4-hydroxyphenyl)pyrazine (**11**).—A mixture of **2** (2.5 g, 12 mmol) and pyridine hydrochloride (12.5 g, 0.11 mol) was heated at 210 °C for 30 min. After cooling, the mixture was dissolved in ethyl acetate, neutralized with 10% $NaHCO_3$, and extracted with ethyl acetate three times. The extracts were washed with saturated NaCl, dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue was crystallized from a mixture of ethyl acetate and hexane to give a powder (1.7 g, 74% yield); mp 210–211 °C; UV (MeOH) λ_{max} nm (ε) 352 (6040), and 280 (20,800); IR (KBr): ν 3450, 3300, 3200, 1625, 1605, 1595, 1510, 1485, 1440, and 1390 cm^{-1}; ^1H NMR (CD_3OD): δ 6.85 (d, 2 H, *J* 8.0 Hz, ArH), 7.65 (d, 2 H, *J* 8.0 Hz, ArH), 7.95 (s, 1 H, CH pyrazine), and 8.25 (s, 1 H, CH pyrazine); Anal. Calcd for $C_{10}H_9N_3O$: C, 64.16; H, 4.85; N, 22.45. Found: C, 64.13; H, 5.01; N, 22.72.

Ethyl 4-(2-amino-5-pyrazinyl)phenoxybutyrate (**12**).—Sodium hydride (0.27 g, 60% NaH, 6.8 mmol) was added to a solution of **11** (1.0 g, 5.3 mmol) in Me_2NCHO (20 mL), and the mixture was stirred at 5 °C for 30 min. To the mixture was added ethyl 4-bromobutyrate (1.9 mL, 13 mmol), and the mixture was stirred at 20 °C for 12 h. The reaction mixture was dissolved in a mixture of dichloromethane and water and extracted with dichloromethane three times. The extracts were evaporated to dryness. The residue was chromatographed on a silica gel column (ethyl acetate–hexane) to give **12** (1.2 g, 75%); mp 96–97 °C; UV (MeOH) λ_{max} nm (ε) 351 (7280), and 281 (25,200); IR (KBr): ν 3400, 3300, 3200, 1715, 1640, 1505, and 1470 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.26 (t, 3 H, *J* 7.3 Hz, CH_3CH_2O), 2.14 (m, 2 H, $CH_2CH_2CH_2O$), 2.54 (t, 2 H, *J* 7.3 Hz, $CH_2CH_2CH_2O$), 4.06 (t, 2 H, *J* 6.1 Hz, $CH_2CH_2CH_2O$), 4.15 (q, 2 H, *J* 7.3 Hz, CH_3CH_2O), 4.56 (brs, 2 H, NH_2), 6.96 (d, 2 H, *J* 8.6 Hz, ArH), 7.79 (d, 2 H, *J* 8.6 Hz, ArH), 8.04 (d, 1 H, *J* 1.2 Hz, CH pyrazine), and 8.39 (d, 1 H, *J* 1.2 Hz, CH pyrazine); Anal. Calcd for $C_{16}H_{19}N_3O_3$: C, 63.77; H, 6.36; N, 13.94. Found: C, 64.21; H, 6.72; N, 13.76. SIMS: *m/z* 302 [M + 1].

4-[4-[2-Methylimidazo[1,2-a]pyrazin-3(7H)-one-6-yl]phenoxy]butyric acid (**13**).—A mixture of **12** (1.0

g, 3.3 mmol), pyruvic aldehyde (0.76 mL, 5.0 mmol), 5 N HCl (6.6 mL), 1,4-dioxane (20 mL), and water (10 mL) was heated at 100 °C for 1 h. After cooling, the mixture was evaporated to dryness. The residue was chromatographed on an ODS column (Fuji Silysia Chromatorex-ODS DM1020T, acetonitrile–water) to give **13** (0.37 g, 34%); mp 205 °C (dec); UV (MeOH) λ_{max} nm (ε) 430 (4640), 355 (3300), and 263 (12,100); IR (KBr): ν 3450, 1720, and 1570 cm^{-1}; ^1H NMR (CD_3OD): δ 2.10 (m, 2 H, $HOOCH_2CH_2CH_2O$), 2.45 (s, 3 H, CH_3), 2.51 (t, 2 H, *J* 7.3 Hz, $HOOCH_2CH_2CH_2O$), 4.10 (t, 2 H, *J* 6.1 Hz, $HOOCH_2CH_2CH_2O$), 7.08 (d, 2 H, *J* 7.9 Hz, ArH), 7.62 (d, 2 H, *J* 7.9 Hz, ArH), 7.63 (brs, 1 H, CH pyrazine), 7.91 (brs, 1 H, CH pyrazine); Anal. Calcd for $C_{17}H_{17}N_3O_4$: C, 62.38; H, 5.23; N, 12.84. Found: C, 62.81; H, 5.66; N, 12.44. SIMS: *m/z* 328 [M + 1].

*6^I-Deoxy-6^I-[[4-[2-methylimidazo[1,2-a]pyrazin-3(7*H*)-one-6-yl]phenoxy]butyryl]amino-cyclomaltoheptaose* (**14**).—This compound was prepared from 0.10 g (0.31 mmol) of **13**, WSC (0.50 g, 2.6 mmol), and 6^I-amino-6^I-deoxy-cyclomaltoheptaose (0.72 g, 0.63 mmol) as described for the synthesis of **9**, giving 0.15 g (33% yield); mp 205 °C (decomposition); UV (0.03 M phosphate buffer, pH 8.3, with little oxygen) λ_{max} nm (ε) 273 (16,400), 345 (6300), and 396 (4000); IR (KBr): 3400, and 1640 cm^{-1}; ^1H NMR (D_2O): δ 2.0–5.3 (m, H β-cyclodextrin, $CH_2CH_2CH_2O$, and CH_3), 7.17 (d, 2 H, *J* 8.5 Hz, ArH), 8.02 (d, 2 H, *J* 8.5 Hz, ArH), 8.21 (s, 1 H, CH pyrazine), and 8.73 (s, 1 H, CH pyrazine); Anal. Calcd for $C_{59}H_{86}N_{37}O_4 \cdot 5H_2O$: C, 46.21; H, 6.31; N, 3.65. Found: C, 46.00; H, 6.80; N, 3.21. FABMS: *m/z* 1443 [M + 1].

[5-(4-Methoxyphenyl)-2-pyrazinyl]succinamic acid (**15**).—A mixture of **4** (0.50 g, 2.5 mmol), succinic anhydride (1.2 g, 12 mmol), and Me_2NCHO (10 mL) was heated at 120 °C for 3 h. The Me_2NCHO was removed under reduced pressure and the resulting solids were dissolved in ethyl acetate and the product was crystallized to give compound **15** (0.61 g, 81% yield); mp 238–239 °C; UV (MeOH) λ_{max} nm (ε) 334 (15,200), 291 (18,850), and 279 (19,900); IR (KBr): ν 3450, 3200, 3100, 1690, 1605, 1560, 1495, 1475, 1440, 1415, 1360, 1310, 1255, and 1170 cm^{-1}; ^1H NMR (Me_2SO-d_6): δ 2.52 (t, 2 H, *J* 6.7 Hz, CH_2), 2.67 (t, 2 H, *J* 6.7 Hz, CH_2), 3.82 (s, 3 H, OCH_3), 7.06 (d, 2 H, *J* 9.0 Hz, ArH), 8.04 (d, 2 H, *J* 9.0 Hz), 8.92 (s, 1 H, CH pyrazine), 9.31 (s, 1 H, CH pyrazine), and 10.88 (s, 1 H, COOH); Anal. Calcd for $C_{15}H_{15}N_3O_4$: C, 59.80; H, 5.02; N, 13.95. Found: C, 59.92; H, 5.26; N, 13.90. SIMS *m/z* 302 [M + 1].

6^I - Deoxy - 6^I - N′ - [5 - (4 - methoxyphenyl) - 2 - pyrazinyl]succinamidocyclomaltohexaose (**16**).—Pyridine (4.0 mL) was added to a mixture of **15** (0.10 g, 0.33 mmol), WSC (0.51 g, 2.7 mmol), and 6^I-amino-6^I-deoxycyclomlatohexaose **7** (0.65 g, 0.67 mmol) at room temperature, and the mixture was stirred at room temperature for 3.5 h. The pyridine was evaporated to dryness under reduced pressure. The residue was dissolved with water and filtered through a membrane filter. The filtrate was chromatographed on an ODS column (Fuji Silysia Chromatorex-ODS DM1020T column, 15 mm × 150 mm) using acetonitrile–water mixtures as eluent to afford **16** (0.25 g, 56% yield); mp 293 °C (dec, from acetonitrile–water); UV (0.03 M phosphate buffer, pH 8.3) λ_{max} nm (ε) 334 (12,200), 291 (13,200), and 279 (14,300); IR (KBr): ν 3400, 2950, 1640, 1545, 1500, 1470, 1415, 1355, 1250, and 1150 cm^{-1}; ^1H NMR (CD_3OD): δ 2.5–2.9 (m, 4 H, CH_2CH_2), 3.0–4.0 (m, H α-cyclodextrin), 3.85 (s, 3 H, OCH_3), 4.8–5.0 (m, H-1 α-cyclodextrin), 7.04 (d, 2 H, *J* 9.0 Hz, ArH), 7.94 (d, 2 H, *J* 9.0 Hz, ArH), 8.75 (s, 1 H, CH pyrazine), and 9.35 (s, 1 H, CH pyrazine); Anal. Calcd for $C_{51}H_{74}N_4O_{32} \cdot 5H_2O$: C, 45.54; H, 6.29; N, 4.16. Found: C, 45.46; H, 6.53; N, 4.01. FABMS *m/z* 1255 [M + 1].

6^I - Deoxy - 6^I - N′ - [5 - (4 - methoxyphenyl) - 2 - pyrazinyl]succinamido - cyclomaltohepttaose (**17**).—This compound was prepared from 0.10 g (0.33 mmol) of **15**, WSC (0.51 g, 2.7 mmol), and 6^I-amino-6^I-deoxy-cyclomaltoheptaose **8** (0.75 g, 0.66 mmol) as described for the synthesis of **16**, giving 0.22 g (0.43% yield); mp 305 °C (dec, from acetonitrile–water); UV (0.03 M phosphate buffer, pH 8.3) λ_{max} nm (ε) 333 (13,000), 294 (15,300), and 280 (15,900); IR (KBr): ν 3400, 2950, 1650, 1540, 1350, 1160, and 1030 cm^{-1}; ^1H NMR (CD_3OD): δ 2.5–2.9 (m, 4 H, CH_2CH_2), 3.1–4.0 (m, β-cyclodextrin and OCH_3), 4.8–5.0 (m, H 1 β-cyclodextrin), 7.06 (d, 2 H, *J* 8.5 Hz, ArH), 7.94 (d, 2 H, *J* 8.5 Hz, ArH), 8.76 (s, 1 H, CH pyrazine), and 9.35 (s, 1 H, CH pyrazine); Anal. Calcd for $C_{57}H_{84}N_4O_{37} \cdot 7H_2O$: C, 44.36; H, 6.40; N, 3.63. Found: C, 44.10; H, 6.56; N, 3.36. FABMS: *m/z* 1417 [M + 1].

4 - [4 - (2 - acetamido - 5 - pyrazinyl)phenoxy]butyric acid (**19**).—Acetyl chloride (0.16 mL, 2.3 mmol) was added to a solution of **12** (0.45 g, 1.5 mmol) in a mixture of dichloromethane (4.5 mL) and pyridine (4.5 mL) at 0 °C, and the mixture was stirred at 20 °C for 1 h. The mixture was cooled to 0 °C and neutral-

ized with 10% $NaHCO_3$. The mixture was extracted with dichloromethane three times, and the organic extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was dissolved in a mixture of 1,4-dioxane (20 mL) and water (6.7 mL), and to the solution was added 1 N NaOH (1.8 mL, 1.8 mmol) at 0 °C. The mixture was stirred at 20 °C for 8 h. The reaction mixture was dissolved in a mixture of ethyl acetate and water (pH 3) and extracted with ethyl acetate three times. The extracts were washed with saturated NaCl solution, dried over anhydrous Na_2SO_4, and evaporated to dryness. The product was crystallized from a mixture of methanol and ethyl acetate to give **19** (0.29 g, 62% yield); mp 240 °C decomposition; UV (MeOH) λ_{max} nm (ε) 334 (15,500), 291 (19,600), and 279 (20,300); IR (KBr): ν 3450, 3050, 1695, 1605, 1560, 1470, 1425, 1355, 1295, 1250, 1185, and 1160 cm^{-1}; ^1H NMR (Me_2SO-d_6): δ 1.97 (m, 2 H, $HOOCCH_2CH_2CH_2O$), 2.15 (s, 3 H, $NHCOCH_3$), 2.40 (t, 2 H, *J* 7.3 Hz, $HOOCCH_2CH_2CH_2O$), 4.05 (t, 2 H, *J* 6.7 Hz, $HOOCCH_2CH_2CH_2O$), 7.05 (d, 2 H, *J* 9.0 Hz, ArH), 8.02 (d, 2 H, *J* 9.0 Hz, ArH), 8.92 (s, 1 H, CH pyrazine), 9.31 (s, 1 H, CH pyrazine), and 10.78 (s, 1 H, COOH); Anal. Calcd for $C_{16}H_{17}N_3O_4$: C, 60.94; H, 5.43; N, 13.33. Found: C, 60.68; H, 5.53; N, 13.19. SIMS: *m/z* 316 [M + 1].

6^I-Deoxy-6^I-[4-[4-(2-acetamido-5-pyrazinyl)phenoxy]butyryl]aminocyclomaltoheptaose (**20**).—This compound was prepared from 0.050 g (0.16 mmol) of **19**, WSC (0.24 g, 1.3 mmol), and 6^I-amino-6^I-deoxycyclomaltoheptaose **8** (0.36 g, 0.32 mmol) as described for the synthesis of **16**, giving 0.20 g (0.83% yield); mp 287 °C decomposition (from water); UV (0.03 M phosphate buffer, pH 8.3) λ_{max} nm (ε) 333 (11,100), 293(12,300), and 280 (13,200); IR (KBr): ν 3400, 2950, 1640, 1540, 1155, and 1030 cm^{-1}; ^1H NMR (D_2O): δ 2.02 (m, 1 H, $COCH_2CH_2CH_2O$), 2.25 (m, 1 H, $COCH_2CH_2CH_2O$), 2.27 (s, 3 H, $NCOCH_3$), 2.41 (m, 2H, $COCH_2CH_2CH_2O$), 2.8–4.2 (m, H β-cyclodextrin), 4.9–5.2 (m, H 1 β-cyclodextrin), 7.03 (d, 2 H, *J* 8.5 Hz, ArH), 7.96 (d, 2 H, *J* 8.5 Hz, ArH), 8.72 (s, 1 H, CH pyrazine),and 9.27 (s, 1 H, CH pyrazine); Anal. Calcd for $C_{58}H_{86}N_4O_{37} \cdot 5H_2O$: C, 45.79; H, 6.36; N, 3.68. Found: C, 45.63; H, 6.59; N, 3.30. FABMS *m/z* 1431 [M + 1].

Procedure for chemiluminescence measurement.—Chemiluminescent intensity time curves were obtained as follows: 20 μL of 0.5 mM **1** dissolved in methanol or 0.5 mM **9**, **10** or **14** dissolved in distilled water was added to 0.98 mL of 30 mM phosphate buffer (pH 8.3), 30 mM phosphate buffer (pH 8.3) containing β-cyclodextrin (10 mM) or Me_2NCHO at 25 °C. The reaction mixture was placed in the photometer, and the chemiluminescent intensity time curve was obtained at 25 °C. The chemiluminescent quantum efficiencies were determined on the basis of Toya's report [12]. The fluorescent quantum efficiencies of the corresponding amides were determined on the basis of quinine sulfate. Chemiluminescent spectra were obtained as follows: The luminescent solution above was placed in the spectrofluorometer, and the spectrum was obtained without emission light. Yields of the amido compounds generated by the chemiluminescent reaction were obtained follows: an HPLC system was employed for analyzing the luminescence-spent products and the yields of the amides were determined by comparison with the corresponding synthesized amides. Elution conditions for amide **16**: solvent, 20:80 acetonitrile–water; flow rate, 0.8 mL/min; for amide **17**: solvent, 15:85 acetonitrile–water; flow rate, 0.8 mL/min; and for amide **20**: solvent, 10:90 acetonitrile–water; flow rate, 0.8 mL/min.

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