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Artificial-signaling receptors for biologically important chemical species

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Contents

Abstract

In this article, I describe the performance of artificial-signaling receptors that have been synthesized. The receptors were designed to enable molecular recognition information to be signaled as changes in the optical properties of the receptors. The contents are divided into two parts. The first deals with spiropyran derivatives as artificial-signaling receptors. This new type of artificial receptor is conceptually different from the signaling receptors so far synthesized such as the crown ether dyes and functionally referred to as self-indicating receptors. The second part is concerned with artificial-signaling acetylcholine receptors, in which the presence of acetylcholine induces a large fluorescence enhancement of the receptors in protic media. The artificial acetylcholine receptors may be applied to histochemistry for acetylcholine.

Keywords: Artificial-signaling receptors

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1. Introduction

A receptor is generally defined as a complex molecule or molecular assembly that, upon recognition of a specific substrate, undergoes a structural change that usually

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induces a series of functions (allosteric effect, signal transduction), which eventually results in a physiological response [1].

One aspect of bioorganic chemistry is the development of simple and artificial models of biological functions. The two definitive and essential vital functions are heredity and metabolism. The former results from duplication of DNA, and the latter from the catalytic action of an enzyme. Both phenomena involve molecular recognition at the initial stage of the process. During the last two decades, artificial models developed in molecular recognition chemistry have demonstrated the importance of complementarity in size, shape, and functional groups at the molecular level for selective host–guest binding [2]. Recent investigations in the field reveal a shift of attention from static phenomena to dynamic ones, i.e., from simple synthetic hosts to artificial receptors (Fig. 1).

The final aim of our research is to create "intelligent" supramolecules and supramolecular systems, in which several conjugated functions are induced by molecular recognition and the whole process is completely regulated at our will. In 1990, we introduced conceptually new artificial receptors [3], in which recognition of alkali-metal cations induces a configurational change in the receptor frameworks accompanying signaling (coloration). Our initial goal in this area is to develop such specific receptors for each group of key molecules. In this article, I describe a series of artificial-signaling receptors for biologically important chemical species [4].

2. Spiropyran derivatives as artificial-signaling receptors

2.1. Principle of information-signaling

Spiropyran derivatives are an important class of photo- and thermochromic compounds which can be converted to the corresponding zwitterionic merocyanine isomers [5]. The isomerization is unique in terms of accompanying large changes in structural and electric characteristics of the molecules. When the factors affecting this equilibrium are heat and light, the functions produced by the isomerization are called thermochromism and photochromism, respectively. When the factor is a

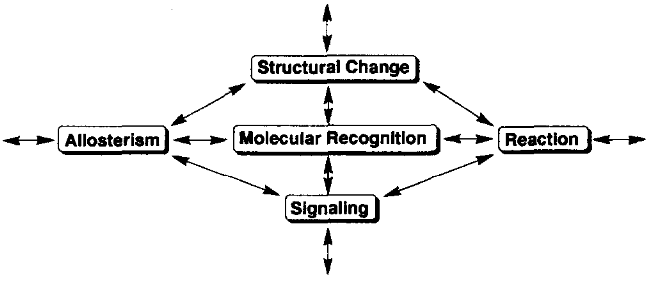


Fig. 1. From static molecular recognition to dynamic molecular recognition.

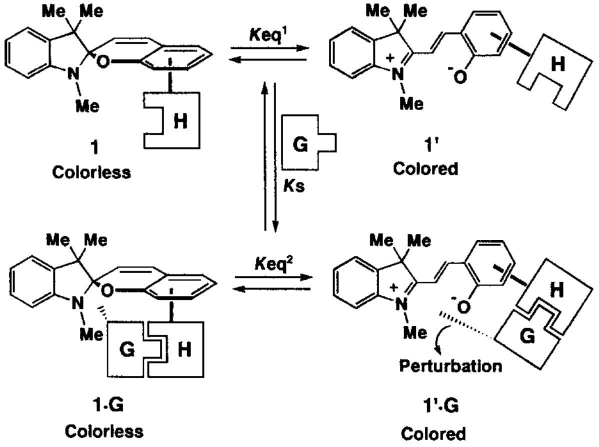


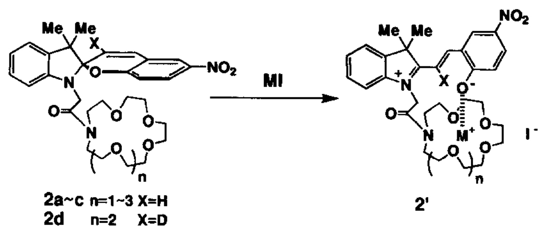
Fig. 2. Equilibrium between spiropyrans possessing a molecular recognition site and merocyanines.

chemical species, especially a specific substrate, new functions of the spiropyrans appear, i.e., structural change and signaling based on molecular recognition.

Fig. 2 shows the equilibrium between spiropyrans possessing a molecular recognition site and merocyanines. We expected that the equilibrium would be perturbed by recognition of the guest molecule. Thus, when the equilibrium constant $K_{eq}{}^2$ is greater than $K_{eq}{}^1$, color will be developed, and when $K_{eq}{}^2$ is smaller than $K_{eq}{}^1$, the color will disappear. The coloration results from the structural change in the spiropyrans. Hence these artificial receptors have two conjugated functions, structural change and signaling based on molecular recognition. Thus we call the spiropyrans multifunctional artificial receptors [6].

2.2. Crowned spirobenzopyrans as alkali-metal cation receptors

Our own approach began with the crown ether chemistry because crown ethers act almost exclusively on ionic substrates, and the selectivity for metal ions has well been documented [7]. We hoped that the strong interaction between the complexed cations and *p*-nitrophenolate oxy-anion of the merocyanine form could be responsible for the isomerization from the spiropyran to merocyanine (i.e., $K_{eq}{}^1 < K_{eq}{}^2$ in Fig. 2). Thus, we synthesized spirobenzopyrans possessing a monoaza-crown ring as a recognition site (**2**) (Scheme 1) [3]. The crowned spirobenzopyrans **2** showed no absorption bands above 450 nm in nonpolar solvents (CH_3CN, $CHCl_3$, acetone, etc.), indicating the closed spiropyran forms. However, a 5-fold molar quantity of LiI was added to the acetonitrile solutions (0.2 mM) of **2a** and **2b**, new absorption bands appeared (**2a**: $\lambda_{max} = 530$ nm, $\varepsilon = 4700$; **2b**: $\lambda_{max} = 530$ nm, $\varepsilon = 10000$). Only negligible changes were observed upon addition of NaI, KI, RbI, and CsI (Fig. 3(a)). While **2c** revealed a small but significant selective coloration with NaI, cation-induced hypsochromic band shifts of Li^+, Na^+, K^+, Rb^+ and Cs^+, which decreased in that order, were explained satisfactorily by the electrostatic interactions between



Scheme 1.

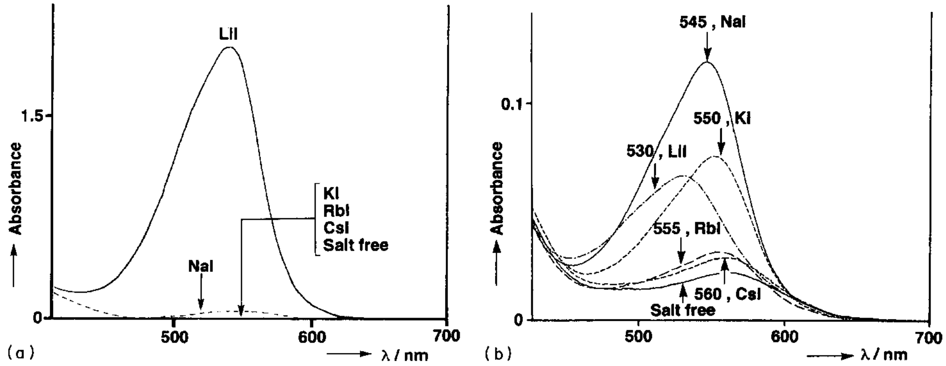


Fig. 3. Electronic absorption spectra of the acetonitrile solutions of (a) **2b** (0.2 mM) and (b) **2c** (0.2 mM) in the presence of alkali-metal iodides (1.0 mM). The spectra of **2a** were similar to those of **2b**.

the complexed cations and the *p*-nitrophenolate dipole of the merocyanine (Fig. 3(b)). Conversely, the hypsochromic band shifts demonstrated that the open colored merocyanine exists mainly as a charge-separated zwitterionic form and not a neutral keto form (vide infra) [8].

The emerging absorption bands were shown to be due unambiguously to the merocyanine structures **2a**′ and **2b**′ by NMR investigations. To a CD_3CN solution (0.8 ml) of **2b** (50 μmol) was added LiI (250 μmol), and ^1H NMR spectra were measured as a function of time in the dark. Almost immediately after the addition of LiI, the signals of only **2b** were detected, but the downfield shifts and broadening of the signals in the aza-crown ring were observed. These results indicated that lithium cations were bound to the macrocycle of **2b**. With time, however, new resonances began to appear, and after 48 h, equilibrium was reached. The new resonances were assigned as those of a merocyanine **2b**′ on the basis of various NMR techniques. The determination of the trans-olefinic protons of **2b**′ ($H^{a\prime}$ and $H^{b\prime}$ in Fig. 4: $J = 15.3$ Hz) was made by comparison of its NMR spectrum with that of **2d**′. The conversion of **2b** to **2b**′ in the stationary state was found to be about 50% under the conditions employed using the integrations of the spectrum. Large downfield shifts were observed for *gem*-dimethyl protons (**2b**, 1.23 and 1.28 ppm; **2b**′, 1.86 ppm). These shifts might be attributed to the increased diamagnetic anisotropy of **2b**′ compared with that of **2b**, in other words, **2b**′ has a longer conjugation system.

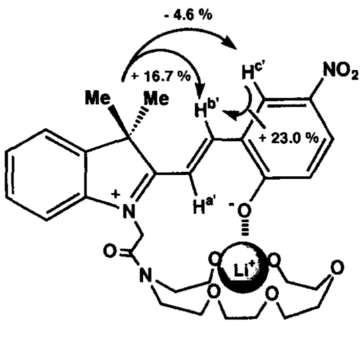
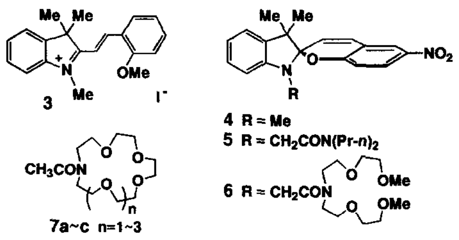


Fig. 4. NOEs for **2b**′.

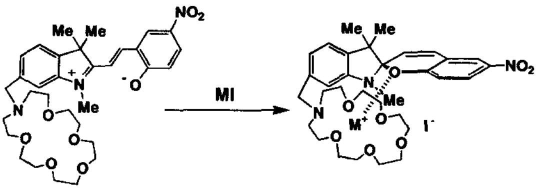
In the $^{13}C\{H\}$ NMR spectrum, two lines at 182 and 186 ppm were assigned to the immonium and amide carbons of **2b**′. This result reinforced previous speculation (vide supra): the open colored merocyanine structure existed in a charge-separated zwitterionic form not a neutral keto form, since the immonium carbon of **3**, which was a simple model compound of the former, appeared at 184 ppm (Scheme 2). The resonances of **2b**′ disappeared after irradiation with strong visible light. No NMR evidence was obtained for the open colored merocyanine under identical experimental conditions in the presence of any other alkali-metal iodides, in agreement with the UV spectra.

Subsequently, NOE experiments were carried out to determine the conformation of **2b**′. Irradiation of *gem*-dimethyl protons resulted in significant changes in the intensities of $H^{b'}$ and $H^{c'}$, but no change was observed for $H^{a'}$. Further, irradiation of $H^{c'}$ resulted in a positive NOE for $H^{b'}$. These results suggest that the conformation of **2b**′ is that depicted in Fig. 4 and that neither the 3*H*-indolium nor *p*-nitrophenolate units rotate around the olefinic double bond on the NMR time scale.

In order to search for the main factors contributing to the coloration of the spirobenzopyrans (i.e., isomerization to the merocyanines), the following experiments were carried out. (1) The absorption spectra of **4** and **5** were not at all affected by the alkali-metal iodides, and **6**, the corresponding acyclic analog of **2a**, showed much less Li^+ selectivity than **2a**. Furthermore, little change in the spectrum of **4** occurred even in the presence of 1 equiv of *N*-acetylmonoaza-18-crown-6 (**7b**) and the alkali-metal iodides. (2) The position of the complexed cations in the molecule was also



Scheme 2.



8 8'

Scheme 3.

found to be important for the isomerization. Spirobenzopyran **8** was designed to recognize cations in which the complexed cations could interact with the ether oxygen of the closed spiropyran and not the phenolate oxygen of the open merocyanine: i.e., ${K_{eq}}^1 > {K_{eq}}^2$ in Fig. 2 (Scheme 3). Indeed, isomerization of **8** to the open colored merocyanine was most strongly suppressed by the presence of a potassium cation, which was most strongly recognized by the crown ring (Fig. 5). (3) It was found, however, that the thermodynamic stabilities of *N*-acetylmonoaza-crown ether **7**, which were thought to be "isolated" recognition sites of **2**, for alkali-metal cations were not compatible with color selectivities of **2** possessing a corresponding crown ether. For example, **2b** showed color selectivity for Li^+, but **7b** revealed the highest stability constant for Na^+ [9]. (4) Under identical experimental conditions, the degree of isomerization of the spironaphthoxazin counterparts **9** was too small to obtain any NMR evidence for the open merocyanine forms, although color selectivities of **9** were similar to those of **2** bearing the same size of crown ring (Li^+ for **9b**, $\varepsilon = 700$; Na^+ for **9c**, $\varepsilon = 40$). This observation is not surprising in view of the fact that the open colored forms of spironaphthoxazins are well known to be in their neutral keto forms, so that stabilization of the colored forms by an alkali-metal cation may not be effective (Scheme 4). These results (1–4) showed that isomerization of the spirobenzopyrans to the merocyanines was governed not only by recognition of alkali-metal cations with the crown rings at the suitable positions but also by the electrical properties of both the complexed cations and the merocyanine dipoles [10].

We developed a new class of artificial-signaling receptors, crowned spirobenzopyrans. Next, we endeavored to design and synthesize more advanced crowned

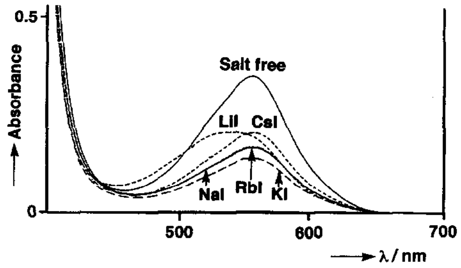
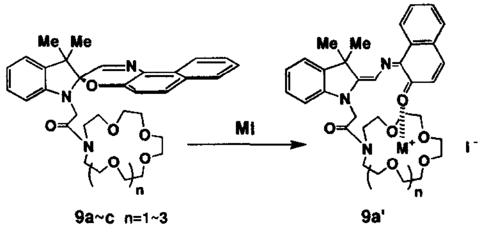


Fig. 5. Electronic absorption spectra of the acetonitrile solutions of **8** (1.7 mM) in the presence of alkali-metal iodides (8.5 mM).

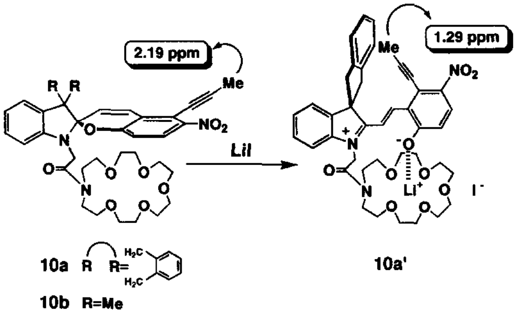


Scheme 4.

spirobenzopyrans in which molecular recognition induces a structural change in the molecule accompanying coloration that results in the proximity of two remote sites in the molecules [11]. Transmission of recognition-information to other sites in the molecules is crucial in many biological systems, such as enzyme and nervous systems, so that mimicking the process using simple and artificial molecules may be a worthwhile subject in its own right.

Our strategy utilizes the fact that very large structural change results from the isomerization of the spirobenzopyrans. We expected that the isomerization of a rationally designed new spirobenzopyran (**10a**) possessing a monoaza-crown ether, propynyl, and indane groups might have propynyl-Me groups approaching the π-electrons of the indane-benzene ring, and any change in the microscopic environment of the Me groups could be easily detected by NMR (Scheme 5). This new type of receptor is conceptually different from the artificial allosteric receptors so far synthesized, because in the latter cases transmission of recognition-information to the second recognition site is carried out through only a conformational change in the molecules [12].

The receptor **10a** showed no absorption bands above 400 nm in nonhydroxylic solvents, indicating the closed spiropyran form. When a 5-fold molar quantity of LiI (1.25 mM) was added to the $CHCl_3$ solution of **10a** (0.25 mM), however, new absorption bands appeared ($\lambda_{max} = 562$ nm, $\varepsilon = 2270$), while only negligible or no changes were observed upon addition of other alkali-metal iodides. In the ^1H NMR spectrum, the propynyl-Me protons of **10a** (20 μmol in 0.8 ml $CDCl_3$) appeared at



Scheme 5.

2.19 ppm, a normal position. After having added LiI (300 μmol) to the solution, initially, only the downfield shifts and broadening of the signals in the aza-crown ring were observed, which indicated that the lithium cations were bound to the macrocycle. With the passage of time, however, new resonances began to appear. The new resonances were assigned as those of a merocyanine (**10a′**), and it is noteworthy that the Me protons of **10a′** were largely shifted upfield by 0.90 ppm, denoting that the Me groups of **10a′** were placed on the center of the indane-benzene ring, as expected (Scheme 5). This upfield shift was shown to be unambiguously due to the diamagnetic anisotropy of the indane ring by comparison of its NMR spectrum with that of **10b**, in which the Me groups of the open colored merocyanine (**10b′**) appeared at 2.35 ppm.

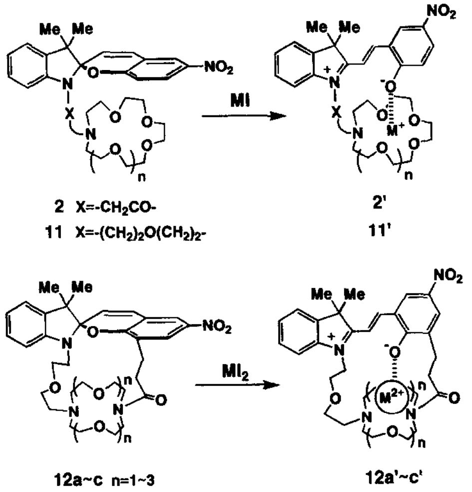
The present work demonstrated that recognition of lithium cations causes the spirobenzopyran to isomerize to the merocyanine, which results in the proximity of the two remote sites in the molecules. In future investigations, the design of molecules which possess reacting groups and/or a second recognition site in the transmitted parts promises further development for the multifunctional artificial receptors.

In this section, various crowned spirobenzopyrans as artificial-signaling receptors for alkali-metal cations are presented. This type of new chromoionophore is conceptually different from the crown ether dyes so far synthesized, because in the latter cases the absorption bands of chromophores are merely shifted by the complexation of cations. Our chromoionophores are thus functionally referred to as self-indicating ionophores [13].

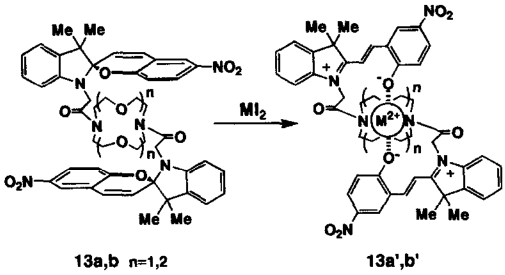
2.3. Cryptand spirobenzopyrans as alkaline-earth metal cation receptors

In the previous section, I demonstrated that the spirobenzopyrans bearing a short linkage **2** showed color selectivities for Li^+ ($n=2$) and Na^+ ($n=3$). Selective coloration, however, for larger alkali-metal cations could not be obtained even in the cases of the $n>3$ crowned spirobenzopyrans. While the spirobenzopyrans **11**, in which the spirobenzopyran moiety was much further separated from the crown ether units by alkyl chains than **2**, showed a small selective coloration for larger alkali-metal cations, such as K^+ and Cs^+, the molar absorptivities in the presence of alkali-metal iodides were considerably smaller when compared to those of **2** [10]. We thought that the low coloring efficiency might result from the enthalpic and entropic disadvantage, the reduced probability of the existence of the complexed cations in the neighborhood of the phenolate oxygen of the merocyanines, and from the weak electrostatic interaction between the complexed large univalent cations and the *p*-nitrophenolate dipole of the merocyanines. Taking into account the above points, we developed cryptand and bibrachial lariat type crowned spirobenzopyrans **12** and **13**, respectively (Scheme 6 and 7) [14].

The cryptand spirobenzopyrans **12** showed no absorption bands above 400 nm in nonhydroxylic solvents, indicating the closed spiropyran forms. Indeed, this interpretation was verified by NMR studies (vide infra). The absorption spectra were scarcely affected upon addition of any alkali-metal iodides in CH_3CN. In the ^1H NMR spectra of **12b** in CD_3CN, however, downfield shifts (for aromatic and crown



Scheme 6.



Scheme 7.

ring protons), splitting (crown ring and alkyl spacer protons) and sharpening (aromatic protons) of the signals in the spiropyran form were observed after the addition of KI. This result indicated that the alkali-metal cations were bound to the macrocycle of **12**, and that the colorless form was attributed to the weak electrostatic interaction between the complexed univalent cations and the *p*-nitrophenolate dipole of the merocyanines. On the other hand, addition of alkaline-earth metal iodides to these CH_3CN solutions gave rise to changes in their spectra. Thus, **12a** and **12b** gave the most intense coloration for Ca^{2+} and Sr^{2+}, respectively (Fig. 6). Titration experiments demonstrated that almost 1 equiv of SrI_2 is enough to obtain the maximum coloration of **12b**.

Coloration of bibrachial lariat type crowned spirobenzopyrans **13** was examined. The new crowned spirobenzopyrans were designed to recognize divalent cations in which the complexed cations could interact with the two phenolate oxygens of the open merocyanines **13′** at the up and down areas perpendicular to the crown rings (Scheme 7). Although the molar absorptivities of **13** in the presence of 1 equiv of

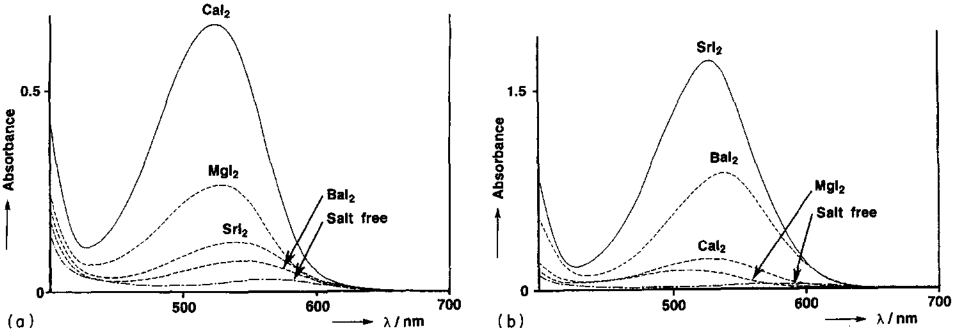


Fig. 6. Electronic absorption spectra of the acetonitrile solutions of (a) **12a** (0.1 mM) and (b) **12b** (0.1 mM) in the presence of alkaline-earth metal iodides (0.1 mM).

alkaline-earth metal iodides in CH_3CN were rather smaller when compared to those of **12**, **13a** (0.1 mM) and **13b** (0.1 mM) revealed the highest coloration for Mg^{2+} ($\lambda_{max} = 513$ nm, $\varepsilon = 700$) and Ca^{2+} ($\lambda_{max} = 524$ nm, $\varepsilon = 3500$), respectively. These ions were smaller than those for **12** possessing the same type of crown ether. This observation was satisfactorily explained by CPK molecular model examinations, which indicated that the radius of the cavity of (*N,N'*-diacetyl)diaza-crown ethers (in **13**) was smaller than that of the corresponding (*N*-monoacetyl)diaza-crown ethers (in **12**). As expected, little change in the spectra of **2** and **11** occurred under the same conditions.

The isomerization of the cryptand spirobenzopyran **12b** to the open colored merocyanine **12b'** in the presence of SrI_2 has been studied by ^1H NMR spectroscopy at 500 MHz. In several deuterated solvents ($CDCl_3$, CD_3CN, DMSO-d^6, etc.), the ^1H NMR signals of **12b** were considerably broadened at room temperature. High-temperature NMR (85 °C) in DMSO-d^6 succeeded in resolving the identifiable species. (In contrast, the spectra of **12a** and **12c** at room temperature in $CDCl_3$ were well-resolved.) To a CD_3CN solution (0.7 ml) of **12b** (20 μmol) was added SrI_2 (40 μmol), and ^1H NMR spectra were measured as a function of time in the dark. After 30 min, new resonances were already detected, which were assigned to a merocyanine (**12b'**), and the remaining signals of the spiropyran form (**12b**) sharpened and shifted. Equilibrium was reached after 3 h (>80% conversion). These observations suggested the fast and strong binding of Sr^{2+} by **12b**, and the slow isomerization of the resulting complex (**12b**·Sr^{2+}) to the merocyanine form (**12b'**).

The fast and strong binding of Sr^{2+} by **12b** was corroborated on the basis of FAB mass experiments. Before addition of SrI_2, ion peaks for $(M+H)^+$ and $(M+Na)^+$ were detected; after the addition, these signals decreased and finally disappeared, while peaks for $(M-H+Sr)^+$ and $(M+Sr+I)^+$ appeared and increased.

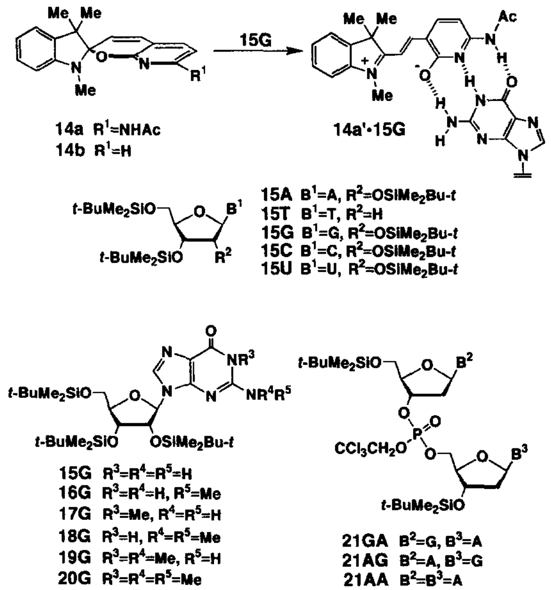
We developed new crowned spirobenzopyrans, cryptand-type spirobenzopyrans. In the spirobenzopyrans, coloration was efficiently induced in the presence of 1 equiv of alkaline-earth metal iodides. The cryptand spirobenzopyrans represent highly sensitive and selective artificial signaling-receptors for alkaline-earth metal cations.

2.4. Spiropyridopyrans as nucleobase receptors

The recognition and selective binding of nucleotide and oligonucleotide bases play critical roles in entire living systems. Complementary hydrogen bonds arise in a very specific fashion between the purine and pyrimidine bases of the two strands of double-helix DNA in order to define the duplex structures and to regulate the information-transfer functions [15]. The specific binding for nucleotide bases, other than the base pairing of oligonucleotides, is also seen in many natural enzymes, such as a nuclease. In this connection, the artificial receptors that recognize and bind to specific nucleoside bases are attracting much attention [16]. Especially, investigation of the chemical recognition of guanine [17] has been stimulated by recent discoveries of the role of GTP-binding regulatory proteins (G-proteins) functioning as cell-surface receptors [18] and of the cap-binding protein (CBP) that recognizes the m^7Gppp-cap structure at the 5′ end of most eukaryotic mRNAs [19]. In this section, I present the design and selective coloration of spiropyridopyrans for guanine nucleoside and oligonucleotide derivatives [20].

The design of the spiropyridopyran **14a** was based on the triple hydrogen bond complementarity between the acetamidopyridopyran unit of **14a** or the acetamidopyridone anion unit of the open merocyanine form **14a**′ and guanine. We expected that equilibrium between the colorless spiropyridopyran and the colored merocyanine would be shifted to the latter by recognition of guanine (i.e., $K_{eq}^1 < K_{eq}^2$ in Fig. 2) because of highly polar hydrogen bonds (Scheme 8).

The spiropyridopyran **14a** showed only weak absorption bands above 350 nm in



Scheme 8.

nonpolar solvents, indicating that **14a** exists mainly as the closed spiropyran form. In CH_2Cl_2, however, addition of 2′,3,′5′-tris-*O*-(*tert*-butyldimetylsilyl)guanosine (**15G**, 10 equiv) to **14a** produced changes in the absorption spectra, and strong absorption bands appeared ($\lambda_{max} = 550$ nm, $\varepsilon = 48000$). On the other hand, only negligible changes were observed upon addition of other nucleoside derivatives (**15A**, **15T**, **15C**, **15U**) (Fig. 7(a)). The increasing absorption bands were attributed to the increasing proportion of the merocyanine form to that of the spiropyran form by recognition of **15G**. Indeed, ca. 10, 43, and 56% of the spiropyran (12.5 mM in $CDCl_3$) exists as the merocyanine form in the presence of **15G**, 0, 6.3, and 12.5 mM, respectively, as judged by integrations of the *N*-Me protons (**14a** and **14a·15G**, 2.77 ppm; **14a′** and **14a′·15G**, 3.67 ppm) in the ^1H NMR spectra. The complexation was also shown on the basis of the following NMR experiments. To a $CDCl_3$ solution (0.8 ml) of **14a** (20 μmol) was added **15G** (20 μmol), and ^1H NMR spectra were measured. The NH protons on both **14a** and **15G** were shifted downfield by 1.83 (**14a**–NH), 1.25 (**15G**–NH_2), and 0.82 (**15G**–NH) ppm, reflecting the formation of **14a·15G**, and new broad peaks, which might be assigned to NH protons of **14a′·15G**, appeared. As expected, the presence of the cytidine derivative interfered with this selective coloration of **14a** for the guanosine derivative because of the competitive formation of the Watson–Crick G–C base pairs. Thus, addition of **15C** (3.0 equiv to **15G**) to the colored solution caused dramatic fading of the color, but other nucleoside derivatives (**15A**, **15T**, and **15U**) had little influence (Fig. 7(b)).

Spiropyridopyran **14b**, which was expected to bind guanine via two hydrogen bonds, revealed less selective coloration for **15G** when compared with that of **14a**, indicating that the triple hydrogen bond complementarity between **14a** and **15G** was important for the coloration (Fig. 7(c)). This interpretation was reinforced by the coloration of **14a** for several *N*-methylated guanosine derivatives. Thus, the coloration for the mono-methylated guanosine derivative **16G**, which still had the possibility of a triple hydrogen bond with **14a**, was almost similar to that for **15G**. However, much less coloration was observed for **17G**, which was a regio-isomer of **16G** but was not expected to form the triple hydrogen-bonded complex with **14a**. Further, little change in the spectra occurred upon addition of **18G**, **19G**, and **20G** (Fig. 7(d)). Recent investigations have revealed that small nuclear RNAs (snRNA), the 5′-termination of which had *N*-methylated guanine bases, played a very crucial role in the splicing of eukaryotic pre-mRNAs [21]. Our spiropyridopyran could distinguish guanosine derivatives from *N*-methylated ones on the basis of coloration.

Subsequently, the color selectivities of **14a** for dideoxynucleotide derivatives (**21GA**, **21AG**, and **21AA**) were examined. As expected, while **14a** showed almost similar absorption bands for **21GA** and **21AG**, indicating that the position of guanine bases in the oligonucleotides had little influence on it, no change in the spectra of **14a** occurred in the presence of **21AA** (Fig. 7(e)). This result suggested that such artificial receptors might operate well for larger oligodeoxynucleotides.

In this section, I described a new class of spiropyrans, spiropyridopyrans, as multifunctional artificial receptors for guanosine derivatives, namely, "recognition–structural change–signaling (coloration)" receptors. In the coloration, triple hydrogen bond complementarity between **14a** and guanine was found to be crucial. This selective coloration was also observed for dinucleotide derivatives. We are currently

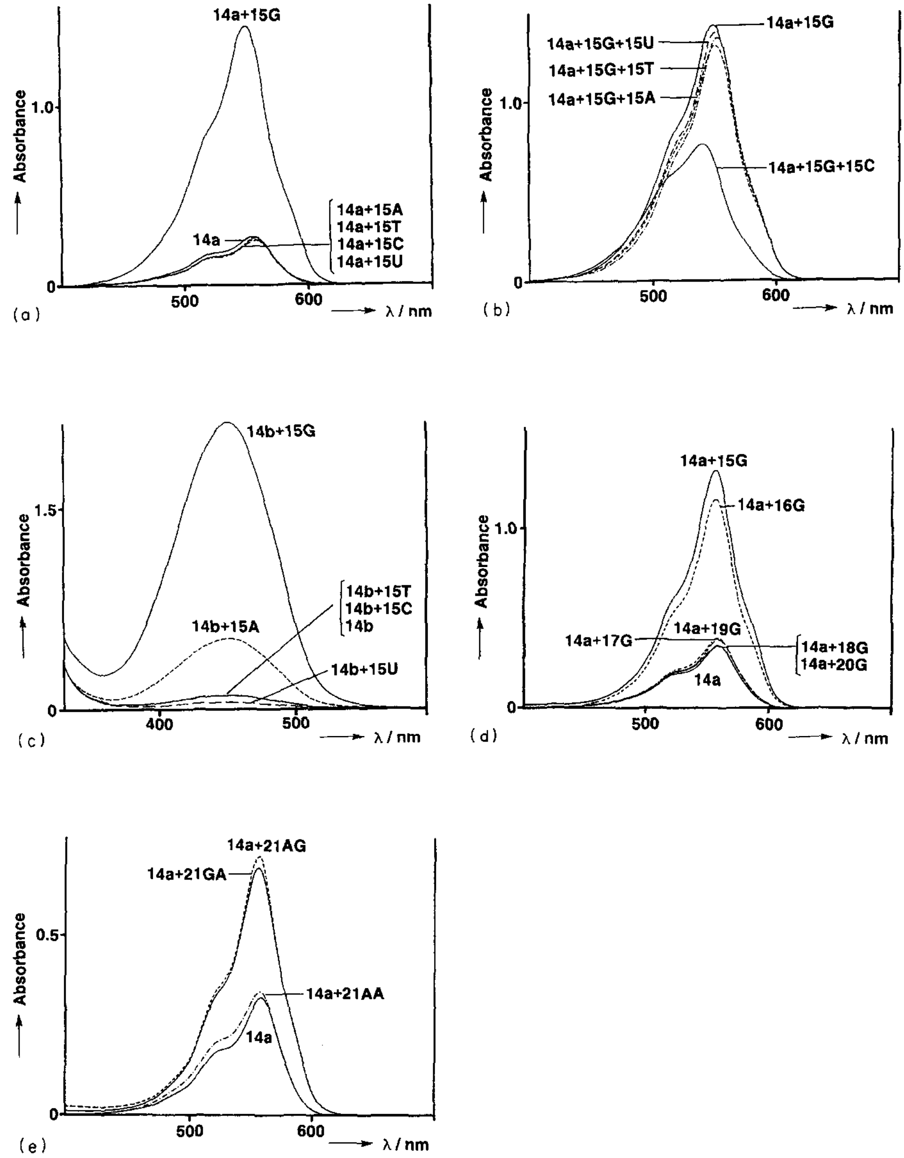
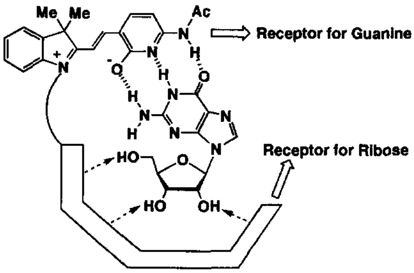


Fig. 7. Electronic absorption spectra of the CH_2Cl_2 solutions of (a, d, and e) **14a** (0.03 mM) and (c) **14b** (0.03 mM) in the presence of (a and c) **15A**–**15U** (0.3 mM), (d) **15G**–**20G** (0.3 mM), and (e) **21GA**–**21AA** (0.15 mM). In part b, electronic absorption spectra of **14a** (0.03 mM)+**15G** (0.3 mM) were measured in the presence of other nucleoside derivatives (0.9 mM).

investigating a ribose recognition site as well as connecting it to the spiropyridopyran in order to construct an advanced receptor such as **22** (Scheme 9), which is expected to bind native guanosine [22].



22

Scheme 9.

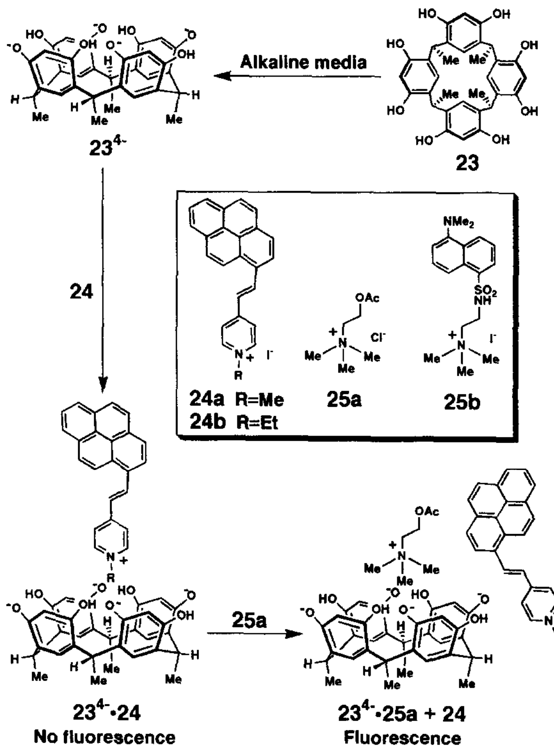
3. Artificial-signaling acetylcholine receptors

3.1. Principle and intermolecular systems

The nervous system is very complex and involves many different kinds of specific interaction with different neurotransmitters. Because of this complexity, there is great interest in the development of molecular sensors that change optical properties in response to the presence of specific neurotransmitters [4]. In this section, I present artificial-signaling acetylcholine receptors [23], in which the presence of acetylcholine (not other neurotransmitters) induces a large fluorescence enhancement in protic media (H_2O, MeOH, EtOH, etc.) [24]. The inherent significance of these receptors for biological applications lies in the fact that acetylcholine is one of the most abundant neurotransmitters in nerve cells, and that no reliable methods are currently available for the chemical transformation of acetylcholine to its fluorescent derivatives in the presence of other neurotransmitters [25].

Resorcinol/acetaldehyde cyclic tetramer **23** has been studied by numerous workers, and recently also with the use of NMR spectroscopy and X-ray crystallography. The tetramer is well known to form tetraphenolate $\mathbf{23}^{4-}$ in alkaline media. The tetraphenolate adopts a bowl-shaped conformation with four intramolecular $OH\cdots{}^{-}O$ hydrogen bonds, and can incorporate acetylcholine (**25a**) into the cavity by the electrostatic and/or cation–π interactions [26]. Pyrene-modified *N*-alkylpyridinium cations (**24**) were also bound to the receptor (the association constants were similar to that obtained for the acetylcholine analogue [27], as judged by fluorescence spectroscopy: 23 (**24a**), 5.3 (**24b**), 7.6 (**25b**) $\times 10^4$ M^{-1} in 0.01 M KOH/MeOH) and that orange fluorescence of the pyridinium dyes was strongly quenched by the complexation (Scheme 10). This type of fluorescence quenching may be explained by electron transfer from $\mathbf{23}^{4-}$ to the excited **24** (PET mechanism) [28]. The fluorescence regeneration was observed by the addition of acetylcholine (0.2 mM) to the nonfluorescent solution of $\mathbf{23}^{4-}\cdot\mathbf{24}$ (**23** 0.2 mM and **24a** 0.1 mM in 0.01 M KOH/MeOH; the excitation wavelength was 430 nm). On the other hand, the fluorescence spectra were not affected at all by the addition of any of the other low molecular weight neurotransmitters (0.2 mM): adrenaline, noradrenaline, dopamine, tyramine, serotonin, tryptamine, histamine, glutamic acid, aspartic acid, GABA, glycine, taurine, proline. The regenerated fluorescence emission was attributed to an increasing proportion of the free-pyridinium dye to that of the complexed one by the addition of acetylcholine. Indeed, this interpretation was verified by the following NMR studies. To a CD_3OD solution (0.7 ml) of **24b** (2.0 μmol) and NaOD (15 μmol) was added **23** (2.0 μmol), and the ^1H NMR spectra were measured. The pyridinium-Et protons were largely shifted upfield by 1.94 (CH_3) and 3.19 (CH_2) ppm, reflecting the formation of $\mathbf{23}^{4-}\cdot$**24b**. Further, other protons of **24b** remained almost unchanged. This observation indicated that the complexation mode was that depicted in Scheme 10. Subsequent addition of acetylcholine (2.0 μmol) to the solution resulted in a fair relaxation of the upfield shifts, while the acetylcholine signals were shifted upfield when compared to those of acetylcholine alone. These results indicated that a considerable amount of the complexed **24b** was displaced by acetylcholine under these conditions, as expected. The high selectivity observed here is due to the strong interaction between **23** and the quaternary ammonium moiety of acetylcholine: there are no such structures in other neurotransmitters.

The pyridinium dyes were easily prepared from commercially available *N*-alkyl-4-methylpyridinium iodide and pyrene-1-carboxaldehyde. Thus, present molecular-



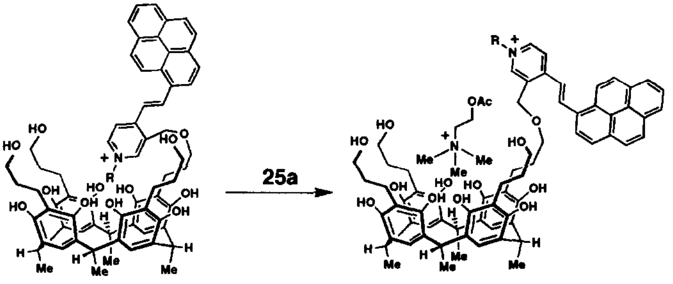
Scheme 10.

sensing systems (**23**+**24**) enabled acetylcholine to be detected nondestructively without any sophisticated chemical manipulation and without any interference from other neurotransmitters.

3.2. Intramolecular systems

It is preferable, however, that such detection be carried out in neutral media. Unfortunately, in neutral protic solvents, the interaction of **23** with **24** was too weak to obtain substantial changes in their fluorescence spectra [29]. We thought that this difficulty could be overcome by attaching the pyridinium dyes to the wall of **23**: intramolecular interaction and the resulting intramolecular quenching of the fluorescence would be more efficient (Scheme 11). Thus, we synthesized fluorescence probe modified artificial acetylcholine receptors **26**. The solubility of **26** is poor in neutral water, so that EtOH was used for the measurements.

The receptors **26** thus prepared showed weak fluorescence in neutral EtOH, indicating that the intramolecular quenching occurred efficiently. The addition of acetylcholine to the solution produced a strong fluorescence emission (Fig. 8). This spectral behavior illustrated that acetylcholine was accommodated into the cavity, so that the pyrene-modified pyridinium moiety was released from the cavity into the bulk EtOH, which resulted in the fluorescence enhancement. No other neurotransmitters induced such enhancement. The conformational change in the receptors was confirmed from ^{1}H NMR spectra. In DMSO-d^6, in which solvent the CH–π interaction is not effective because of the competitive interaction with the solvent, *N*-Me protons appeared at 4.30 ppm, a normal position, suggesting that in this solvent the Me group was free from the recognition site. In contrast, in CD$_3$OD, *N*-Me protons appeared at 2.21 ppm, and signals for other resonances became rather unsymmetrical compared to those in DMSO-d^6. These observations indicated that the CH–π interaction occurred efficiently intramolecularly, and that the conformation of the receptor is such as is depicted in Scheme 11. After having added acetylcholine to the CD$_3$OD solution, the *N*-Me protons were shifted downfield by 0.25 ppm, as for



26a R=Me
26b R=Et
Weak fluorescence

26•25a
Strong fluorescence

Scheme 11.

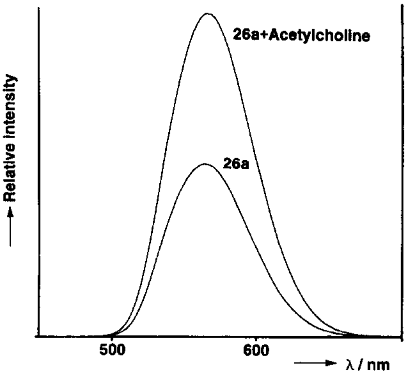


Fig. 8. Fluorescence emission spectra of the EtOH solutions of **26a** (0.3 mM) in the presence of acetylcholine (30 mM). The exitation wavelength is 430 nm.

the intermolecular processes previously described. These results showed that the expected change of the conformation of the receptor occurred in neutral protic solvents on recognition of acetylcholine.

In summary, we have developed artificial-signaling acetylcholine receptors. In the cases of the intermolecular (the easily prepared pyridinium dyes **24** and the receptor **23**) and intramolecular (the fluorescence probe-modified receptors **26**) systems, selective off/on signaling in alkaline media and in neutral protic media were observed, respectively. The present results suggest that our methods may be applied to histochemistry of acetylcholine. The design of more efficient and selective receptors is now under way.

4. Conclusions and outlook

This account emphasized the performance of the artificial-signaling receptors we have synthesized. The receptors were designed to enable molecular recognition information to be signaled as changes of the optical properties of the receptors. The signaling process also synchronized with structural (configurational or conformational) changes of the receptors, and we can add further functions to the receptors such as transmission of recognition information. Now, the aim of molecular recognition chemistry is no longer to discover whether something can be bound or not. We must keep in mind that molecular recognition is no more than an initial process in the functions of naturally occurring receptors. The time for requiring molecular recognition chemistry to move to the next stage may have arrived: from synthesis of host structures to host functions i.e., real artificial receptors. Although the work described here was not purposely directed toward analytical application, some of the systems mentioned seem to have great potential as molecular sensors. Larger and more complex biologically essential substrates such as peptides, oligonucleotides and carbohydrates will be future targets for our receptors.

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