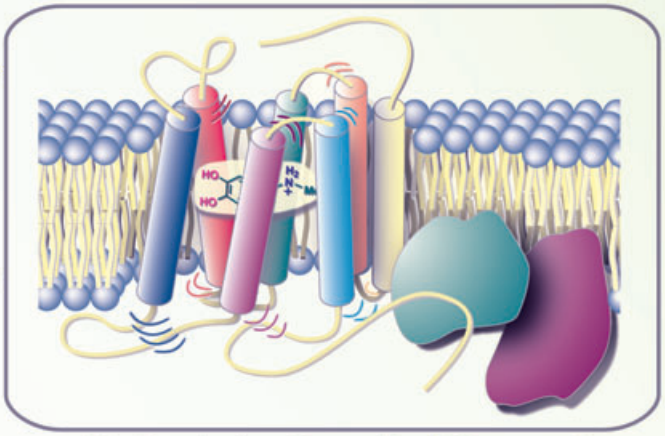
# Adrenaline Recognition by Hydrindacene-Based Receptor

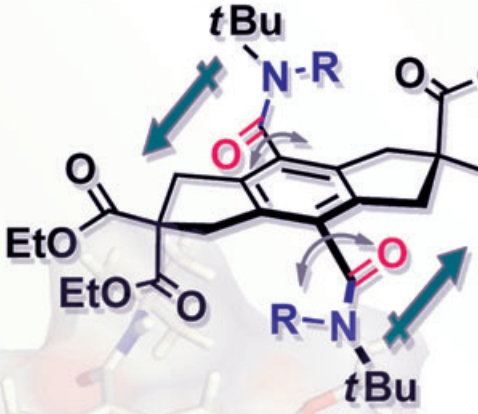


G-Protein Coupled Receptor

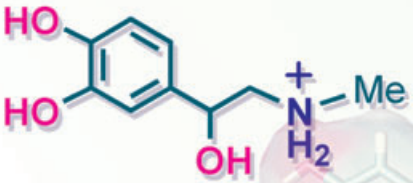
Multipoint Recognition

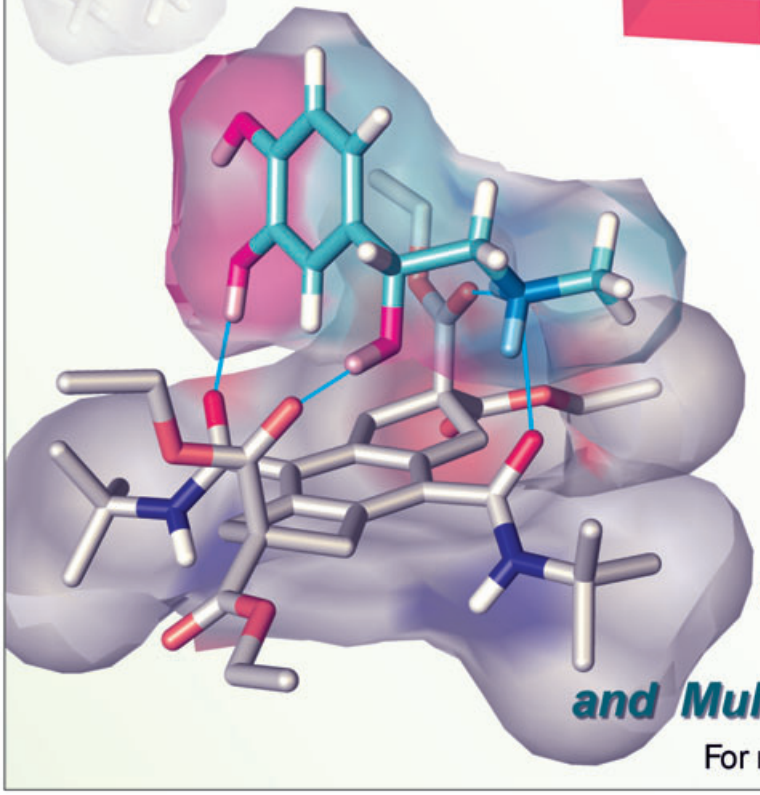
Dynamic Structural Change

Enzyme Activation




Inspired by GPCR




## Conformational Switching by Dipole Reversal and Multipoint Hydrogen Bonding

For more information see the following pages.

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# Multipoint Recognition of Catecholamines by Hydrindacene-Based Receptors Accompanied by the Complexation-Induced Conformational Switching

**Hidetoshi Kawai,* Ryo Katoono, Kenshu Fujiwara, Takashi Tsuji,* and Takanori Suzuki[a]**

**Abstract:** The molecular recognition of catecholamines by hydrindacene-based receptors **1** and **2**, as well as the durene-based receptor **3**, and the guest-induced conformational changes are reported. These receptors selectively bind adrenaline and dopamine salts through the guests' ammonium group and 3-hydroxyl group on the aromatic ring. In the case of adrenaline, an additional hydrogen bond with a benzylic hydroxyl group is formed. In 2% $CD_3CN/CDCl_3$, the association constants are of the order of $10^4\,M^{-1}$, which is much larger than with guests without the 3-hydroxyl groups ($10^3\,M^{-1}$). The two amide groups of receptor **1** can rotate freely around the $C_{aromatic}$–$C_{amide}$ bond, whereas the *tert*-amide in **2** changes between two stable conformations at a slow enough rate to allow detection by $^1H$ NMR spectroscopy. In the absence of a guest molecule, the *syn*-conformer is less stable than the *anti*-conformer. On complex formation with adrenaline, the *syn*-conformer becomes dominant due to an intramolecular dipole-reversal effect in addition to multipoint hydrogen bonding.

**Keywords:** adrenalines · amides · atropisomerism · dopamines · molecular recognition · receptors

## Introduction

The design of novel artificial receptors for the molecular recognition of biogenic compounds is an important area of supramolecular chemistry.[1] Mimicking the binding processes observed in nature[2] has recently attracted considerable interest. Dynamic molecular recognition, in which the guest determines the conformation of the receptor,[3] is of particular importance in terms of allosteric binding, regulation, or feedback.[4] To date, there are several examples of dynamic, molecular recognition systems exhibiting complexation-induced atropisomerism or conformational changes.[5,6]

Catecholamines, such as adrenaline and dopamine, are important hormones and neurotransmitters. They are involved in vital signal-transduction processes, which themselves consist of multipoint molecular recognition by G-protein-coupled receptors (GPCRs),[7] the subsequent dynamic structural transformation of receptors, and the resultant activation or inactivation of enzymes, such as adenylate cyclase or phospholipase. Hence, the mechanistic understanding and realization of signal-transduction processes by using artificial receptors has attracted great interest in the field of supramolecular chemistry. In the past decade, numerous studies have been conducted on the construction of artificial receptors toward catecholamines.[8,9] However, as reported by Schrader,[8] there are a limited number of examples that exhibit functional selectivity towards catechol amino alcohols. Furthermore, to the best of our knowledge, no precedents have realized a conformational change of the receptor upon binding with catecholamines, despite it being of importance in the signal-transduction processes of natural adrenergic receptors.[7]

These mechanistic aspects of GPCRs,[7] such as adrenergic receptors, led us to study the dynamic molecular recognition and functional selectivity of catecholamines on the hydrindacene-based receptor **1**, since we recently found the positive homotropic allosteric binding of catechol derivatives to **1**.[10] This study could provide a basis for the development of

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Supporting information for this article is available on the WWW under http://www.chemeurj.org/ or from the author: Synthetic details of receptor **3**; Experimental details of 2D-EXSY NMR, Job plots, NMR titrations, and molecular modeling; Complexation-induced shifts (CISs) data of various guests.



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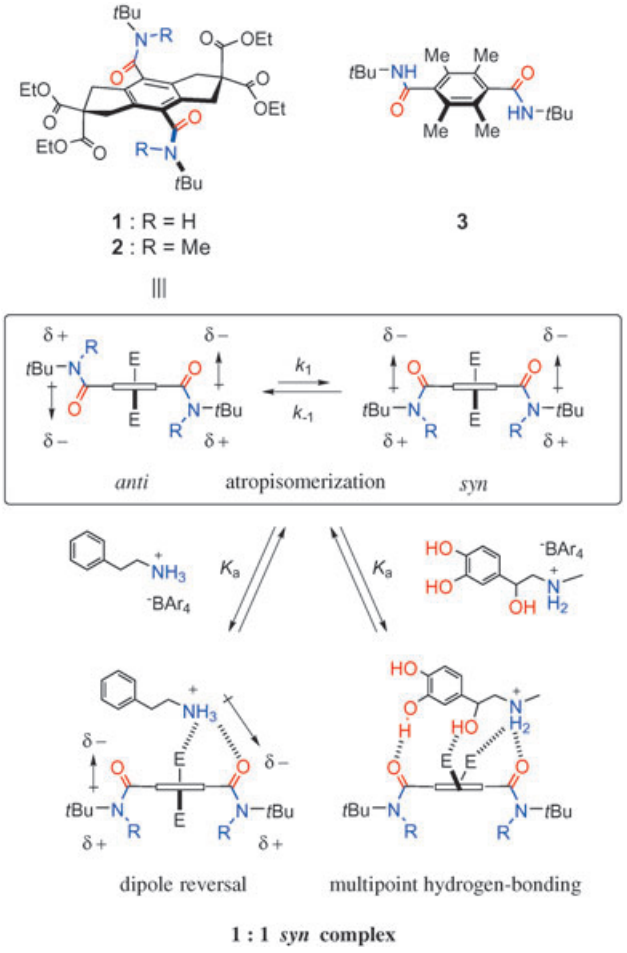
artificial signal-transduction systems, as well as insights into local structural changes of natural receptors on the binding of catecholamines. The preferential conformation of **1** has the two amide groups twisted about the plane of the hydrindacene platform. In a guest-free state, the *syn*- and *anti*-conformers of this twisted state are easily interconverted by rotation around the $C_{aromatic}$–$C_{amide}$ bonds (Scheme 1). Although the *anti*-conformation is preferred in terms of polarization of the amide groups, upon complex formation with catecholamines a dynamic structural change to the *syn*-conformer may be induced.

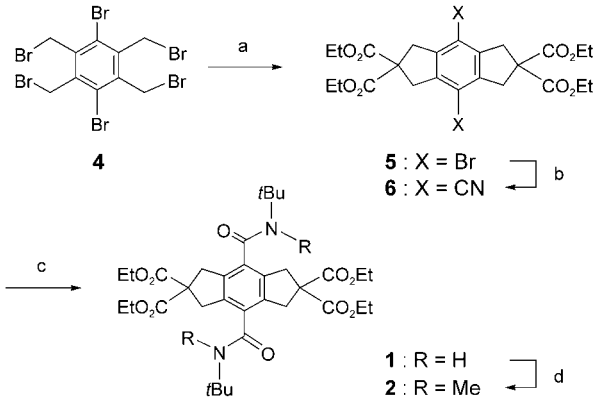



Scheme 1. The atropisomeric interconversion of receptors **1**, **2**, and **3**, and a schematic representation showing plausible mechanisms of shifts in equilibrium to the *syn*-conformation upon complexation with catecholamines.

Here we report the complex formation properties of the hydrindacene-based receptors **1** and **2**, as well as the durene-based receptor **3**, with catecholamines, such as adrenaline and dopamine, in 2% or 10% $CD_3CN/CDCl_3$. The multipoint hydrogen bonding to catecholamines makes these receptors functionally selective. Moreover, the *syn*-preference of the complex is caused not only by multipoint hydrogen bonding, but also by the reversed dipole moment of one receptor amide group upon complex formation with the guest ammonium group. This last result might indicate that this induced-fit effect on complexation of charged molecules could be a useful aid in designing artificial allosteric systems that exhibit selective molecular recognition.

## Results and Discussion

**Design and preparation of receptors**: Receptor **1** was designed to incorporate the hydrindacene (1,2,3,5,6,7-hexahydro-*s*-indacene) skeleton as the platform. It consists of a rigid aromatic ring and two flexible five-membered alicyclic rings puckered into an envelope conformation. This receptor is provided with two secondary amide groups and four ester groups as hydrogen-bonding sites to the periphery. The former are the main moieties involved in the binding and recognition of catecholamines. Receptor **1** was readily prepared in three steps from 1,4-dibromo-2,3,5,6-tetrakis(bromomethyl)benzene **4**[11] (Scheme 2).




Scheme 2. Preparation of receptors **1** and **2**; reagents and conditions: a) $CH_2(CO_2Et)_2$, EtONa, EtOH, reflux (64%); b) CuCN, HMPA, 150°C (60%); c) *t*BuOH, $H_2SO_4$, $Ac_2O$, AcOH, 65°C (90%); d) NaH, MeI, DMF, 25°C (97%).

Treatment of **4** with the sodium enolate of diethyl malonate in EtOH gave tetraester **5**. Cyanation of the aromatic rings of **5**, and the subsequent Ritter reaction of **6** gave the secondary amide **1**. To further examine the role of each binding site, receptors **2** and **3** were also designed here. Tertiary amide **2**, without an acidic NH, was prepared by methylation of **1**. The durene-type receptor **3**, without any ester-binding sites, was prepared by condensation of the corresponding acid chloride **7**[12] with *t*BuNH$_2$.[13] Catecholamine salts and their analogues were prepared as tetraarylborate salts by metathesis of the commercially available hydrochloride salts with sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate,[14] which are soluble in 2% $CD_3CN/CDCl_3$.

**Structure and conformational dynamics of receptors**: For each receptor, **1**–**3**, there are two rotational isomers that can be interconverted by rotation of the two amide groups

through their $C_{aromatic}$–$C_{amide}$ bonds (Scheme 1). In $CDCl_3$, the $^1H$ NMR signals of the secondary amides **1** and **3** exhibited higher symmetry ($D_{2h}$) than expected due to rotational freedom around the $C_{aromatic}$–$C_{amide}$ bonds (Figure 1).

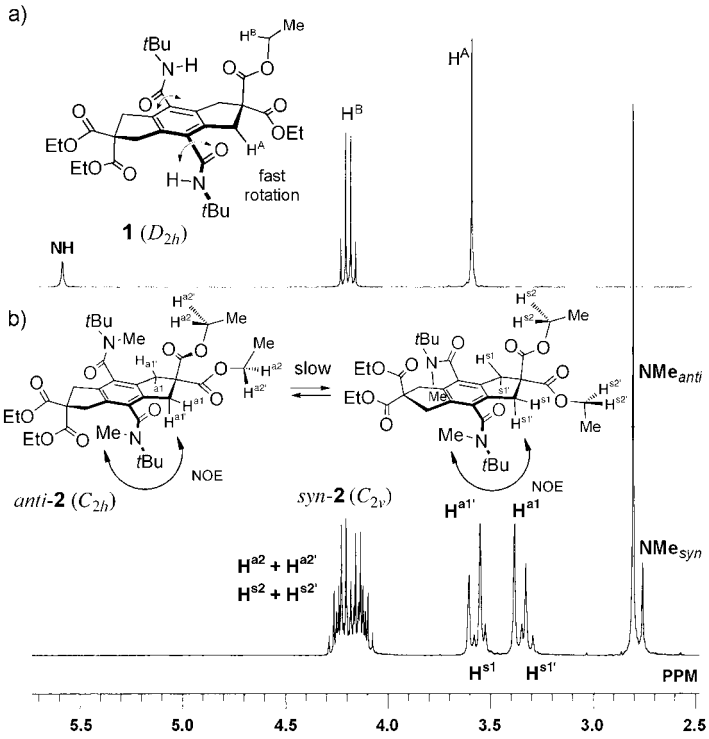



Figure 1. $^1H$ NMR spectra (300 MHz) of receptors a) **1** and b) **2** in $CDCl_3$ at 298 K.

By contrast, the spectrum of tertiary amide **2** exhibited two set of less-symmetric resonances with a ratio of 85:15 in $CDCl_3$ at 303 K. These were assigned as the $C_{2h}$-symmetric *anti*- and $C_{2v}$-symmetric *syn*-conformers.[15] The methylene protons of the ethyl ester groups for the major conformer seem to appear as two sets of a doublet of quartets signal; therefore the preferential conformer is *anti*-geometry. This assignment was supported by computational calculations for receptors **1** and **2**. Monte-Carlo conformer searches using MacroModel 6.5 (Amber*, GB/SA solvation model for $CHCl_3$) showed that the *anti*-conformer is more stable than the *syn* and the calculated energy difference (5.4 kJ mol$^{-1}$ for **1** and 4.7 kJ mol$^{-1}$ for **2**) is similar to the experimental value (4.4 kJ mol$^{-1}$ for **2**). This preference for the *anti*- over the *syn*-conformation in receptors **1**–**3** is in accord with the generally observed offset effect of dipole moments in nonpolar solvents.[16,17] The atropisomers of **2** could not be separated by column chromatography. Furthermore, X-ray structural analysis of a single crystal of **2** revealed that all the molecules adopt the *anti*-conformation, with the two amide groups[17] oriented in opposite directions (Figure 2). They are significantly twisted about the $C_{aromatic}$–$C_{amide}$ bonds (63.2 and 77.7° for two crystallographically independent molecules of **2**, respectively). This is also true of the crystal structure of **1** (49.5°). Additionally, once crystals of **2** are dissolved in $CDCl_3$, the above-mentioned spectrum containing two isomers was recovered.

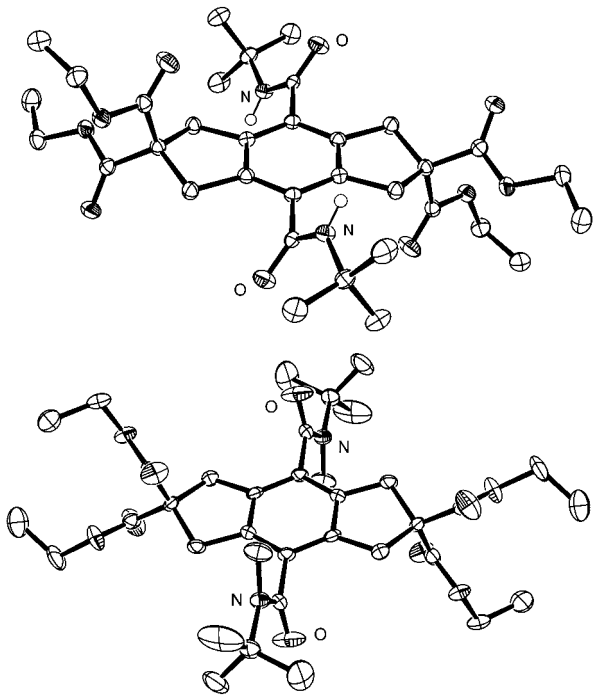


Figure 2. X-ray crystal structures of **1** (top) and **2** (bottom: one of two crystallographically independent molecules). ORTEP representations with 50% probability. Hydrogen atoms except for amide NH groups are omitted for clarity.

Rotation of the amide groups around the $C_{aromatic}$–$C_{amide}$ bonds in **2** was studied by $^1H$ NMR spectroscopy in terms of a nondegenerate four-site exchange process.[6f,18,19] In variable-temperature (VT) NMR experiments on **2**, the coalescence of the NMe proton signals occurred at 103 °C in $C_6D_5Cl$, corresponding to barriers of $\Delta G_1 = 82.1$ and $\Delta G_{-1} = 80.2$ kJ mol$^{-1}$ at 376 K (*anti*:*syn* = 70:30, at 298 K in $C_6D_5Cl$) for $C_{aromatic}$–$C_{amide}$ rotation. The rate of the rotation in **2** could be independently determined by $^1H$ NMR 2D-exchange spectroscopy (2D-EXSY) experiments.[18] Cross peaks were observed between the NMe signal assigned to both isomers, indicating that the two sets of resonances are those of interconvertible conformers (Figure 3).

The rate of rotation was determined by measuring the ratios between the heights of the diagonal and cross peaks for each isomer at several mixing times (500–800 ms). The rate of the rotation ($k = k_1 + k_{-1}$) at 303 K was thus determined to be $0.117 \pm 0.002$ s$^{-1}$, which corresponds to barriers of $\Delta G_1 = 84.4 \pm 0.1$ and $\Delta G_{-1} = 80.1 \pm 0.1$ kJ mol$^{-1}$ at 303 K in $CDCl_3$. These values are in good agreement with those derived from VT NMR experiments. The rotation around the $C_{aromatic}$–$C_{amide}$ bonds[19] in **2** is much slower than in **1** and **3**, due to the increased steric hindrance of the tertiary amide group and the five-membered rings of the hydrindacene skeleton.

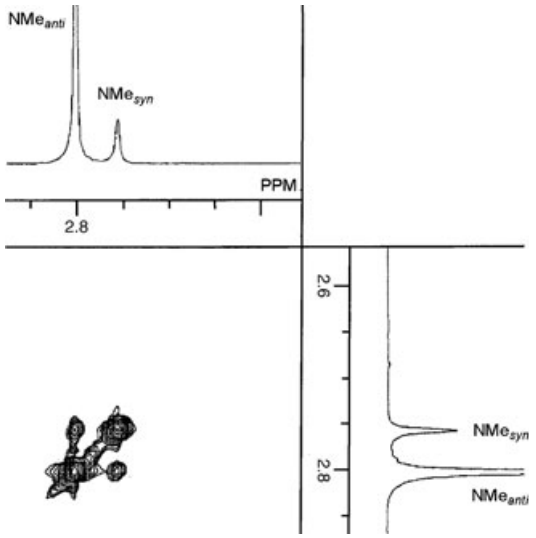



Figure 3. The NMe region of the $^1$H NMR 2D-EXSY spectrum (600 MHz) of **2** in $CDCl_3$ at 303 K with $t_m = 800$ ms.

**Binding studies of receptors with adrenaline**: Binding behavior of **1** with an adrenaline salt was initially investigated by $^1$H NMR spectroscopy in 2% and 10% $CD_3CN/CDCl_3$ (v/v). Under these conditions, no indication of self-association of **1** was observed. Mixing receptor **1** with the adrenaline salt resulted in significant changes in the chemical shifts of adrenaline protons and moderate shifts of the receptor protons (Figure 4). The preservation of the symmetry of the receptor resonances on complex formation indicates this complexation is at the fast-exchange limit. The 1:1 stoichiometry of receptor **1**/adrenaline complexes was shown by a Job plot,[20] which indicates that this complexation is clearly different from that of the previously observed 1:2 complexation of *anti*-conformation **1** with catechols.[10] This difference in the binding of adrenaline, as opposed to benzenediols, with **1** was also indicated by a surprisingly small shift of the amide NH protons of receptor **1** on binding. The binding constants ($K_a = \sim 50\,000\ M^{-1}$ and $2600\ M^{-1}$ in 2% and 10% $CD_3CN/CDCl_3$, respectively) were determined by NMR titration experiments, whereby the titration isotherms were analyzed by nonlinear-regression methods (Figure 5, Table 1).[21]

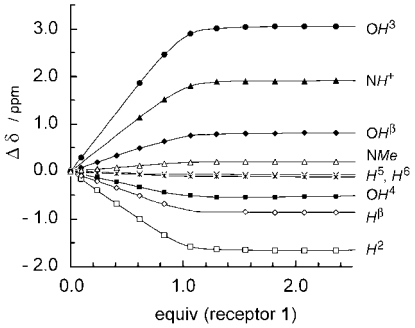



Figure 5. NMR titration curve showing the complexation-induced shifts (CISs) of adrenaline salt (O$H^3$ (●), O$H^4$ (■), O$H^\beta$ (◆), N$H^+$ (▲), $H^2$ (□), $H^5$ (×), $H^6$ (*), $H^\beta$ (◇) and N$Me$ (△)) upon addition of receptor **1**. [adrenaline salt] = 2.0 mM in 2% $CDCl_3/CD_3CN$ (v/v) at 298 K.

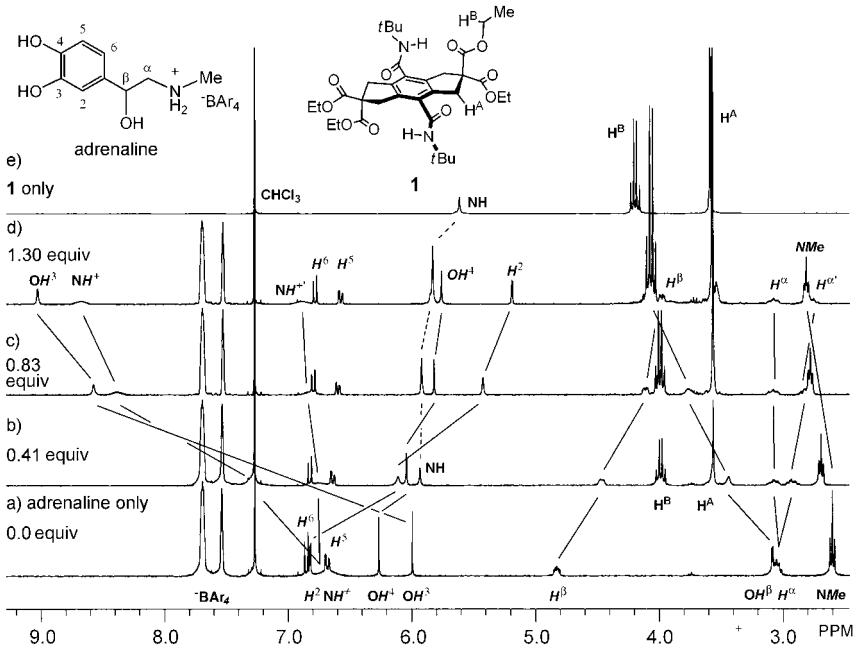



Figure 4. Complexation of receptor **1** with adrenaline salt. $^1$H NMR spectra (300 MHz, 2% $CDCl_3/CD_3CN$ (v/v)) from a titration experiment of adrenaline salt with **1**, the equivalents of which are indicated on the right of the spectra. a) Adrenaline salt. b) 0.41 equiv, c) 0.83 equiv, and d) 1.30 equiv of receptor **1** was added. e) Receptor **1**.

The complexation-induced chemical shifts (CISs) derived from NMR titration of the adrenaline salt with **1** supply a great deal of information about their binding (Figure 5, Table S1[13]). The guest binding based on multipoint hydrogen bonding was evidenced by the significant downfield shifts of one of the ammonium protons (N$H^+$), the phenolic OH at C3 (O$H^3$), and the benzylic OH (O$H^\beta$). Conversely, the phenolic OH at C4 (O$H^4$) was shifted upfield, indicating that this group does not participate in hydrogen bonding upon complexation. It is noteworthy that the aliphatic CH protons ($H^\alpha$ and $H^\beta$) and only one of the aromatic protons at C2 ($H^2$) were shifted upfield by −0.29, −0.88, and −1.58 ppm, respectively. This suggests that these protons

Table 1. Binding constants of complexes of receptor **1**, **2** and **3** with various guest molecules. Calculated from NMR titrations in 2% and 10% $CD_3CN/CDCl_3$ (v/v) at 298 K.

| | Guest molecules[a,b] | Solvent (v/v) $CD_3CN/CDCl_3$ | $K_a$ ($K_{a1}$[c]) [$M^{-1}$] | $\Delta G$ [kJ mol$^{-1}$] |
|---|---|---|---|---|
| **1** | adrenaline | 2:98 | ~50000±7%[c] | −26.8±0.2 |
| **1** | dopamine | 2:98 | ~50000±18%[c] | −26.8±0.5 |
| **1** | phenylephrine | 2:98 | 42000±8%[c] | −26.4±0.2 |
| **1** | tyramine | 2:98 | 2600±37%[c] | −19.4±0.9 |
| **1** | halostachine | 2:98 | 2200±21%[c] | −19.1±0.5 |
| **1** | phenethylamine | 2:98 | 2800±11%[c] | −19.7±0.3 |
| **1** | $PhCH_2CH_2N^+Me_3$ | 2:98 | 98±47%[d] | −11.4±1.3 |
| **1** | adrenaline | 10:90 | 2600±17%[d] | −19.5±0.4 |
| **2** | adrenaline | 10:90 | 4900±21%[d] | −21.0±0.5 |
| **3** | adrenaline | 10:90 | 480±24%[d] | −15.3±0.6 |
| **1** | dopamine | 10:90 | 1200±10%[d] | −17.7±0.2 |
| **3** | dopamine | 10:90 | 500±18%[d] | −15.4±0.4 |

[a] As $HBAr_4$ salts. [b] [Guest] = 2.0 mM. [c] Macroscopic binding constants analyzed as complexation with 2:1 stoichiometry of receptor-guest. All binding constants of the second step ($K_{a2}$) are smaller than 5 $M^{-1}$ and therefore omitted from the table for clarity. See Supporting Information. [d] Analyzed as complexation with 1:1 stoichiometry.

are magnetically shielded by the π cloud of the hydrindacene skeleton.

The Monte-Carlo simulations (MacroModel 6.5, Amber*, GB/SA solvation model for $CHCl_3$, 5000 steps) for the complex formation between **1** and adrenaline salt in chloroform suggest the energy-minimum structure shown in Figure 6.

The adrenaline molecule is located above the hydrindacene aromatic plane in accordance with the observed upfield shifts of $H^2$, $H^\alpha$, and $H^\beta$ signals in the $^1$H NMR spectrum. Each of the two ammonium protons, $NH_2$, of adrenaline is hydrogen bonded to the amide carbonyl and ester carbonyl of the receptor, respectively. The phenolic OH at C3 ($OH^3$) is bound to another amide carbonyl, thus conferring the *syn* orientation on the receptor amide. The phenolic OH at C4 ($OH^4$) does not form any hydrogen bonds, whereas there is an additional hydrogen bond between the benzylic OH ($OH^\beta$) and the ester carbonyl. In total, three out of four functional groups of adrenaline are involved upon complexation with **1**, forming four hydrogen bonds. Due to the fast equilibration between free and complexed **1**, as well as the fast rotation of the two amide groups, the CISs for the protons of receptor **1** are relatively small and do not unfortunately provide significant supporting information about the proposed geometry of the complex.

To obtain more detailed information about the geometry of the complex, titration experiments for **2** and **3** in 10% $CD_3CN/CDCl_3$ were performed ($K_a$ = 4900 and 480 $M^{-1}$ for **2** and **3**; Table 1). Similar CISs for adrenaline protons were observed upon complexation of the tertiary amide **2** with adrenaline. This indicates that **2** also complexes with adrenaline in an identical binding manner, confirming that the amide NH part of **1** does not participate in complex formation with adrenaline. A weaker complexation with **3** indicates that the ester groups of **1** and **2** substantially stabilize the complex by forming hydrogen bonds with adrenaline.

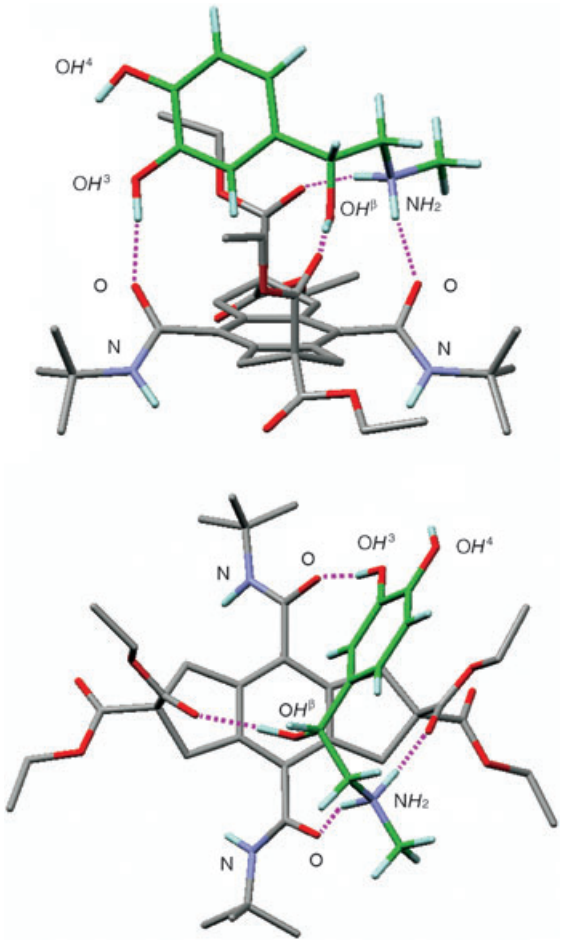
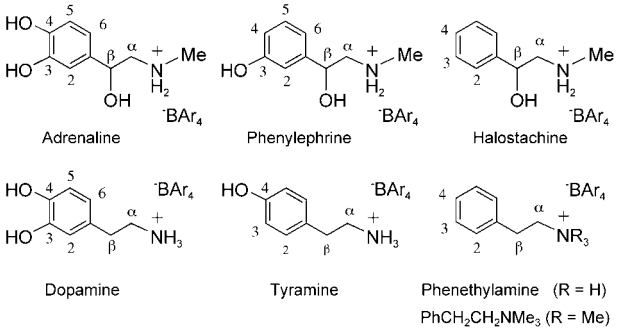



Figure 6. Energy-minimized geometry for the complex of **1** with adrenaline salt according to Monte-Carlo simulations in $CHCl_3$. Top: side view. Bottom: top view.

**Guest selectivity**: Molecular recognition based on multipoint hydrogen bonding enables the receptors to be guest selective towards various catecholamine analogues. To evaluate the contribution of each hydrogen bond to the binding strengths, we systematically examined the properties of **1** when complexed with various truncated guests (Scheme 3 and Table 1; see also Table S1: CIS data[13]).




Scheme 3. Guest molecules for binding experiments with **1**, **2**, and **3**. Ar = 3,5-$(CF_3)_2C_6H_3$.

The binding constant of **1** with dopamine in 10% $CD_3CN/CDCl_3$ ($K_a = 1200\,M^{-1}$) is reduced to one-half of that with adrenaline ($K_a = 2600\,M^{-1}$). Since the lack of an *N*-methyl group cannot account for such a drastic change, this result demonstrates the important contribution of the benzylic OH of adrenaline in hydrogen bonding with the ester group of **1**. In fact, both guests bind with a similar strength to the receptor that is without the ester groups, **3** ($K_a = 480\,M^{-1}$ for adrenaline and $500\,M^{-1}$ for dopamine in 10% $CD_3CN/CDCl_3$). Complex formation between **1** and halostachine is much weaker than with catechol derivatives in 2% $CD_3CN/CDCl_3$ ($K_a = 2200\,M^{-1}$), which indicates that the phenolic OH of adrenaline is important in obtaining the large binding constant. However, the phenolic OH at C4 ($OH^4$) is not important as phenylephrine binds **1** ($K_a = 42\,000\,M^{-1}$) as strongly as adrenaline. Similarly, comparisons of the binding constants of **1** with tyramine ($K_a = 2600\,M^{-1}$), phenethylamine ($K_a = 2800\,M^{-1}$), and dopamine ($K_a = \sim 50\,000\,M^{-1}$) clearly show that removal of the phenolic OH at C4 has a minimal effect on the binding strength; by contrast, the lack of OH at C3 ($OH^3$) leads to a marked drop of the binding constant. These results strongly support the validity of the complex geometry postulated in Figure 6. At the same time, the hydrindacenes **1** and **2** are proven to be functionally selective receptors for the catecholamines as a result of multipoint hydrogen bonding.

**Complexation-induced conformational changes of receptor 2**: Complexation of **2** with adrenaline induced switching of conformational preference from the *anti*- to the *syn*-conformer (Scheme 1). Upon titration of **2** with adrenaline, the proportion of the *anti*- and *syn*-conformers (85:15) gradually changed to favor *syn*, which provides the suitable orientation of the two carbonyl sites for binding, and only the *syn*-rotamer was eventually observed after addition of 1.2 equivalents of adrenaline to **2** (Figure 7I).

These results clearly indicate that the orientation of the amide groups of **2** is switched from the inherently more stable *anti*- to the *syn*-conformation upon binding with adrenaline acting as an effector molecule. This process of induced structural changes partly resembles complex formation between adrenaline and natural adrenergic receptors,[7] and might provide a unique prototype as an artificial signal-transduction system.[5,6]

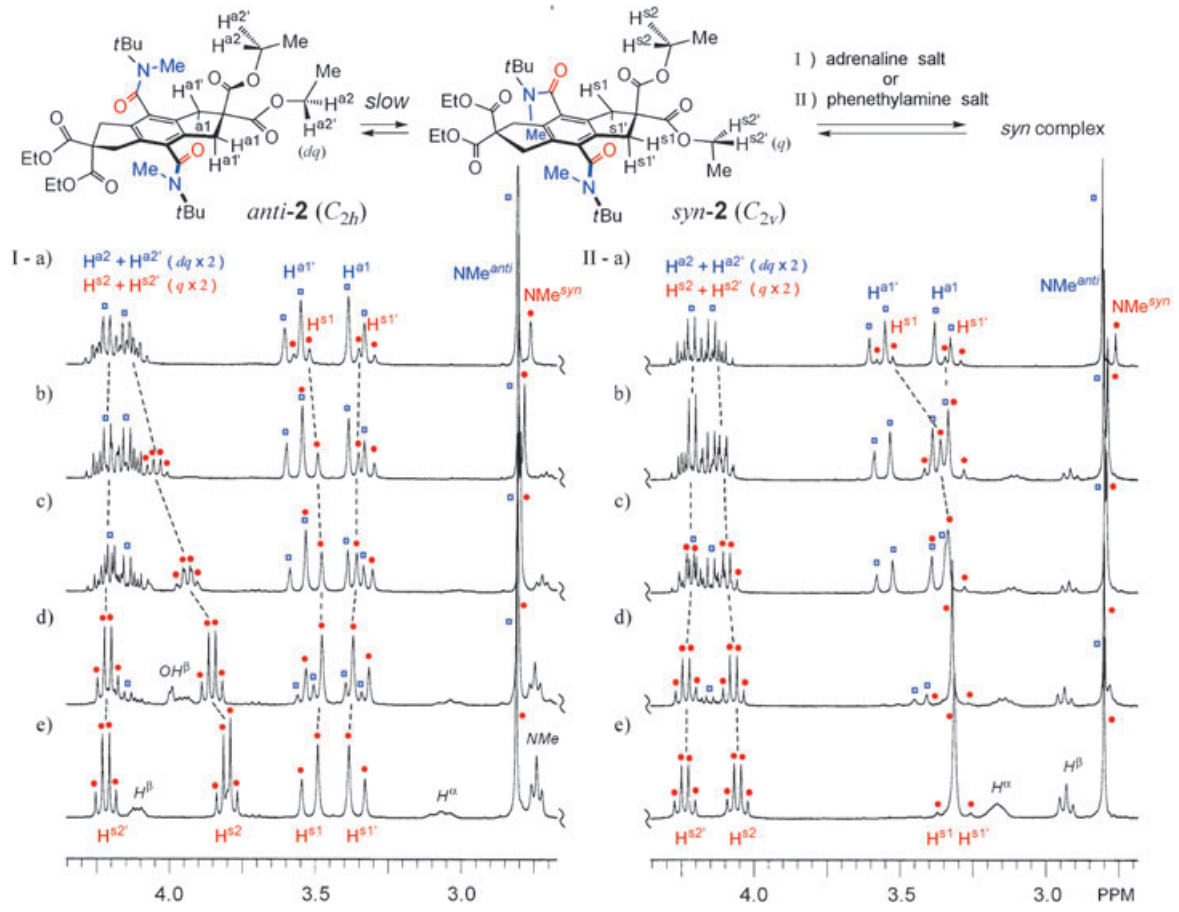
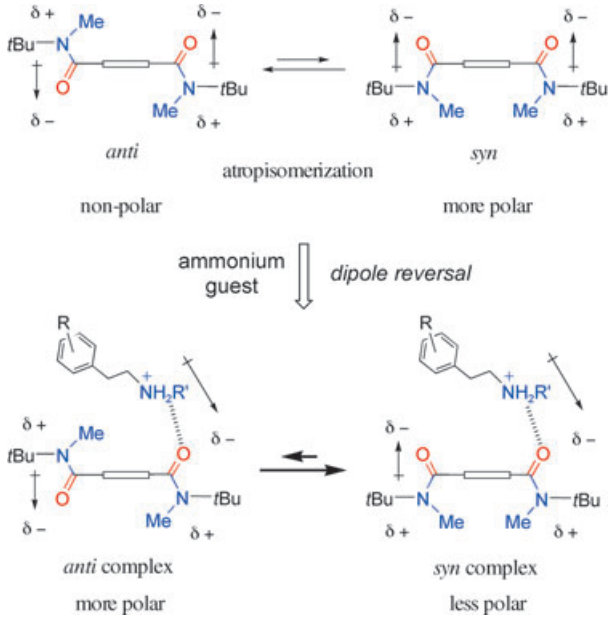



Figure 7. $^1H$ NMR spectra (300 MHz) of mixtures of receptor **2** (*anti*- **2** (□), *syn*-**2** (●)) and I) adrenaline salt or II) phenethylamine salt in $CDCl_3$ at 298 K. I: a) Receptor **2**, b) 0.12 equiv, c) 0.32 equiv, d) 0.66 equiv, and e) 1.20 equiv of adrenaline salt were added. II: a) Receptor **2**, b) 0.35 equiv, c) 0.45 equiv, d) 0.75 equiv, and e) 1.37 equiv of phenethylamine salt were added. The assignments of guest protons correspond to the lettering shown in Scheme 3. Methylene protons of the ester groups for the major conformer of **2** in the presence of guest appear as two sets of quartet signal ($H^{s2}$ and $H^{s2'}$), which is in accordance with the assumption that *syn*-conformer is involved for complex formation.

To obtain more information on this switching process, the complexes of **2** with other catecholamines were examined. One might expect that multipoint hydrogen bonding of the ammonium NH proton ($NH^+$) and phenolic OH proton at C3 ($OH^3$) with the two amide carbonyl groups are solely responsible for the conformational switching. Surprisingly, even the complexes with tyramine or phenethylamine that are without those OH protons caused a similar conformational switch (Figure 7 II). Since a bifurcated hydrogen bond formation of the ammonium $NH_3$ groups with the two amide carbonyl groups of **2** is unlikely, these results clearly show that conformational switching is not only caused by multiple hydrogen bonds, but also by a dipole-reversal effect (Scheme 4). Rotation of the amide group on one side in the *anti*-conformer can partially offset the reversed dipole generated by hydrogen bonding between the ammonium group




Scheme 4. The atropisomeric interconversion of receptors **2**, and a plausible mechanism of the equilibrium shift to *syn*-conformation upon complexation.

and the amide group on the other side. This mechanism was supported on a computational calculation. A conformational search for the complex formation between **2** with phenethylammonium salt in $CHCl_3$ showed that complexation with the *syn*-confomer is more stable than that with the *anti*-conformer ($-18.5\,kJ\,mol^{-1}$), as opposed to guest free state, in which the *syn*-confomer is more unstable ($+4.4\,kJ\,mol^{-1}$).

Hence, the conformational switching properties in this system are induced mainly by the ammonium group, which can make a stronger hydrogen bond with a carbonyl group. This is true even in the case of catecholamines that have both an ammonium group and phenolic OH protons, although the effect of multiple hydrogen bonding is also important. During the complexation of **2** with adrenaline or dopamine, such conformational switching accompanied by binding of the guest ammonium group has the advantage of pre-organizing the amide group for recognition of the phenolic OH proton at C3; the successive binding of the OH proton at C3 no longer accompanies enthalpy loss caused by the rotation of the amide groups on forming the *syn*-conformation. Thus, this induced-fit mechanism, based on complexation-induced dipole reversal, can serve as an important means to effect the construction of an artificial allosteric system as well as the selective molecular recognition of highly functionalized biomolecules, such as catecholamines.

## Conclusion

This work has revealed the molecular recognition properties of hydrindacene-based receptors **1** and **2**, as well as their guest-induced conformational changes on binding catecholamines. These receptors selectively bind adrenaline and dopamine, which have an ammonium group and a phenolic hydroxyl group at C3 on the aromatic ring. Additional binding of the ester groups with the benzylic hydroxyl group and the ammonium group further stabilizes complexes with adrenaline. In 2% $CD_3CN/CDCl_3$, the association constants are in the range of $10^4\,M^{-1}$, and are therefore much larger than those with guests without hydroxyl groups at C3 ($10^3\,M^{-1}$).

The two amide groups of receptor **1** rotate freely around the $C_{aromatic}$–$C_{amide}$ bond. However, receptor **2** changes between the two stable conformations at a rate that is slow enough to permit $^1H$ NMR detection. The *syn*-conformer is less stable than the *anti*-conformer in the absence of a guest. When **2** forms a complex with adrenaline, the *syn*-conformer becomes dominant due to intramolecular dipole-reversal effect in addition to multipoint hydrogen bonding. These results could make important contributions to the design of artificial signal-transduction systems, and also provide useful information in the understanding of local structural changes in the binding of catecholamines to natural receptors.[7] We are now planning to use these hydrindacene-based receptors to construct an artificial signal-transduction system with allosteric control. Preliminary studies show that the positive homotropic allosteric binding of **1** with benzenediols[10] is effectively inhibited by the addition of adrenaline, implying that adrenaline functions as an allosteric inhibitor. These results will be reported in due course.

## Experimental Section

**General**: $^1H$ and $^{13}C$ NMR spectra were recorded on a JEOL ECP-300 ($^1H$/300 MHz, $^{13}C$/75 MHz) spectrometer in $CDCl_3$ at 25 °C unless otherwise indicated. ROESY and 2D-EXSY spectra were recorded on a JEOL α600 spectrometer. IR spectra were taken on a Hitachi Model 215 grating spectrometer. Mass spectra were recorded on a JEOL JMS-01SG-2 (FD) spectrometer. Column and thin-layer chromatography (TLC) were performed on silica gel 60 (Merck) of particle size 63–200 and 5–20 μm, respectively. Elemental analyses were taken on a Yanako MT-6 CHN corder at the Center for Instrumental Analysis of Hokkaido University. 1,4-Dibromo-2,3,5,6-tetrakis(bromomethyl)benzene (**4**)[11] and

2,3,5,6-tetramethylterephthaloyl dichloride (**7**)[12] were prepared following the known procedures. All commercially available compounds were used without further purification unless otherwise indicated.

**Tetraethyl 4,8-dibromo-1,2,3,5,6,7-hexahydro-*s*-indacene-2,2,6,6-tetracarboxylate (5)**: An EtONa solution was prepared by dissolving Na metal (14.2 g, 617 mmol) in absolute EtOH (1.0 L). Diethyl malonate (49.1 g, 306 mmol) and then compound **4**[11] (62.3 g, 103 mmol) were added. The reaction mixture was heated under reflux with stirring for 7 h under Ar, after which the solvent was removed under reduced pressure. The residue was neutralized with HCl (0.5 M, 400 mL) and extracted with $CHCl_3$ (200 mL×3). The extracts were combined, dried over $MgSO_4$, and filtered, and the filtrate was concentrated under reduced pressure. The residue was recrystallized from $CHCl_3$/EtOH (1:1) to give pure **5** (39.7 g, 64%) as colorless crystals. M.p. 203.0–204.0 °C; $^1$H NMR: $\delta$ = 4.23 (q, $J$ = 7.3 Hz, 8H), 3.64 (s, 8H), 1.27 ppm (t, $J$ = 7.3 Hz, 12H); $^{13}$C NMR: $\delta$ = 170.97, 140.60, 114.78, 62.03, 58.53, 42.44, 14.00 ppm; IR (KBr): $\tilde{\nu}$ = 2984, 1736, 1446, 1282, 1246, 1160, 1072, 862, 802 cm$^{-1}$; MS (FD): *m/z* (%): 602 (48) [$M^+$], 604 (100) [$M^+$+2], 606 (56) [$M^+$+4]; elemental analysis calcd (%) for $C_{24}H_{28}O_8Br_2$: C 47.70, H 4.67; found: C 47.29, H 4.63.

**Tetraethyl 4,8-dicyano-1,2,3,5,6,7-hexahydro-*s*-indacene-2,2,6,6-tetracarboxylate (6)**: A mixture of **5** (7.50 g, 12.4 mmol), CuCN (3.57 g, 39.8 mmol), and hexamethylphosphoric triamide (HMPA, 10 mL) was heated with stirring at 150 °C for 8 h under Ar, and then poured into aqueous $FeCl_3$ solution (10.8 g, 40.1 mmol in $H_2O$ 50 mL) to decompose the complex. The resulting solid was separated from the liquid layer by filtration and washed successively with water, aqueous $NaHSO_4$, and water. The residue was dissolved in $CHCl_3$ and washed with aqueous $FeCl_3$ solution, water, and brine, and was then dried over $MgSO_4$. The brown solid obtained by evaporation of the solvent was subjected to chromatography on silica gel eluting with $CHCl_3$. The resulting yellow solid (4.38 g) was recrystallized from $CHCl_3$/EtOH (1:2) to give pure **6** (3.77 g, 60%) as colorless crystals. M.p. 196.5–198.0 °C; $^1$H NMR: $\delta$ = 4.24 (q, $J$ = 7.2 Hz, 8H), 3.75 (s, 8H), 1.28 ppm (t, $J$ = 7.2 Hz, 12H); $^{13}$C NMR: $\delta$ = 170.12, 144.31, 114.63, 109.06, 62.47, 59.61, 39.87, 13.97 ppm; IR (KBr): $\tilde{\nu}$ = 2984, 2228, 1732, 1304, 1250, 1192, 1074, 858 cm$^{-1}$; MS (FD): *m/z* (%): 496 (100) [$M^+$]; elemental analysis calcd (%) for $C_{26}H_{28}N_2O_8$: C 62.90, H 5.68, N 5.64; found: C 62.63, H 5.55, N 5.58.

**Tetraethyl 4,8-bis(*t*-butylcarbamoyl)-1,2,3,5,6,7-hexahydro-*s*-indacene-2,2,6,6-tetracarboxylate (1)**: Concentrated $H_2SO_4$ (54 μL, 1.0 mmol) was added to a mixture of **6** (200 mg, 0.40 mmol), *t*BuOH (386 μL, 4.0 mmol), $Ac_2O$ (380 μL, 4.0 mmol), and AcOH (5.0 mL). After stirring at 65 °C for 20 h, the reaction mixture was poured slowly into 5% aqueous $NaHCO_3$. The resulting suspension was extracted with EtOAc. The organic layer was washed with 5% aqueous $NaHCO_3$, water, and brine, dried over $MgSO_4$, and then filtered. The white solid was obtained by evaporation of the solvent was subjected to chromatography on silica gel eluting with EtOAc/hexane (4:6). The resulting white solid was recrystallized from EtOH to give pure **1** (233 mg, 90%) as colorless crystals. M.p. 242.0–242.5 °C; $^1$H NMR: $\delta$ = 5.56 (s, 2H), 4.20 (q, $J$ = 7.2 Hz, 8H), 3.59 (s, 8H), 1.48 (s, 18H), 1.25 ppm (t, $J$ = 7.2 Hz, 12H); $^{13}$C NMR: $\delta$ = 171.20, 166.76, 137.54, 131.35, 61.94, 60.52, 52.06, 39.14, 28.96, 14.01 ppm; IR (KBr): $\tilde{\nu}$ = 3384, 2988, 1732, 1668, 1534, 1276, 1078, 860 cm$^{-1}$; MS (FD): *m/z* (%): 644 (100) [$M^+$]; elemental analysis calcd (%) for $C_{34}H_{48}N_2O_{10}$: C 63.34, H 7.50, N 4.34; found: C 63.17, H 7.62, N 4.33.

**Tetraethyl 4,8-bis(*t*-butylmethylaminocarbonyl)-1,2,3,5,6,7-hexahydro-*s*-indacene-2,2,6,6-tetracarboxylate (2)**: 60% NaH in oil (ca. 70 mg, 1.75 mmol) was added to a solution of **1** (200 mg, 0.31 mmol) in DMF (4.0 mL), while stirring at 25 °C. After the evolution of hydrogen gas had ceased, MeI (0.6 mL, 9.3 mmol) was added to the suspension and the reaction mixture was stirred for 12 h. It was then poured into EtOAc (30 mL) and aqueous HCl (1 M, 30 mL). The aqueous layer was extracted with EtOAc. The combined organic layers were washed with aqueous HCl (1 M), water, and brine, then dried over $MgSO_4$, and filtered. The white solid obtained by evaporation of the solvent was passed through a short silica-gel column eluting with EtOAc/$CHCl_3$ (2:8) to afford **2** as a white solid (202 mg, 97%). The analytical sample was obtained by recrystalization from EtOH. M.p. 158.0–158.5 °C; $^1$H NMR: $\delta$ = 4.29–4.08 (m, 8H; *anti* and *syn*), 3.58 (d, $J$ = 15.9 Hz, 4H; *anti*), 3.55 (d, $J$ = 15.9 Hz, 4H; *syn*), 3.36 (d, $J$ = 15.9 Hz, 4H; *anti*), 3.32 (d, $J$ = 15.9 Hz, 4H; *syn*), 2.81 (s, 3H; *anti*), 2.76 (s, 3H; *syn*), 1.54 (s, 18H; *anti*), 1.53 (s, 18H; *syn*), 1.28–1.20 ppm (m, 12H; *anti* and *syn*); $^{13}$C NMR: $\delta$ = 171.24, 169.78, 136.08, 131.83, 61.90 (*anti*), 61.69 (*syn*), 60.29, 56.97, 38.49 (*anti*), 38.30 (*syn*), 33.01, 28.03, 14.00 ppm; IR (KBr): $\tilde{\nu}$ = 2980, 1734, 1642, 1482, 1370, 1280, 1240, 1188, 1078, 860 cm$^{-1}$; MS (FD): *m/z* (%): 672 (100) [$M^+$]; elemental analysis calcd (%) for $C_{36}H_{52}N_2O_{10}$: C 64.27, H 7.79, N, 4.16; found: C 64.12, H 7.66, N 4.19.

**Preparation of catecholamine HBAr$_4$ Salts**: These salts were prepared by modified metathesis[22] of appropriate hydrochloride salts with sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate[14] under argon in the dark in freshly purified $CHCl_3$, and subsequent removal of resulted NaCl by filtration and concentration. $^1$H NMR data are as follows.

**DL-Adrenaline salt**: $^1$H NMR (2% $CD_3CN/CDCl_3$): $\delta$ = 7.69 (brs, 8H), 7.54 (brs, 4H), 6.90–6.50 (brs, 2H), 6.85 (d, $J$ = 8.2 Hz, 1H), 6.82 (d, $J$ = 2.2 Hz, 1H), 6.69 (dd, $J$ = 8.2, 2.2 Hz, 1H), 6.28 (s, 1H), 6.00 (s, 1H), 4.86–4.80 (m, 1H), 3.08 (d, $J$ = 3.1 Hz, 1H), 3.09–3.01 (m, 2H), 2.60 ppm (t, $J$ = 5.8 Hz, 3H).

**Dopamine salt**: $^1$H NMR (2% $CD_3CN/CDCl_3$): $\delta$ = 7.70 (brs, 8H), 7.54 (brs, 4H), 6.82 (d, $J$ = 8.0 Hz, 1H), 6.67 (d, $J$ = 2.2 Hz, 1H), 6.54 (dd, $J$ = 8.0, 2.2 Hz, 1H), 6.30–6.04 (brs, 4H), 6.04–5.90 (brs, 1H), 3.08 (t, $J$ = 6.6 Hz, 2H), 2.79 ppm (t, $J$ = 6.6 Hz, 2H).

**DL-Phenylephrine salt**: $^1$H NMR (2% $CD_3CN/CDCl_3$): $\delta$ = 7.69 (brs, 8H), 7.54 (brs, 4H), 7.23 (t, $J$ = 8.0 Hz, 1H), 6.84–6.79 (m, 3H), 6.82–6.62 (brs, 2H), 6.03 (s, 1H), 4.92–4.87 (m, 1H), 3.30 (brs, 1H), 3.15–3.03 (m, 2H), 2.62 ppm (s, 3H).

**Tyramine salt**: $^1$H NMR (2% $CD_3CN/CDCl_3$): $\delta$ = 7.70 (brs, 8H), 7.54 (brs, 4H), 7.00 (d, $J$ = 8.5 Hz, 2H), 6.82 (d, $J$ = 8.5 Hz, 2H), 6.17 (brs, 3H), 5.89 (s, 1H), 3.09 (t, $J$ = 6.7 Hz, 2H), 2.83 ppm (t, $J$ = 6.7 Hz, 2H).

**DL-Halostachine salt**: $^1$H NMR (2% $CD_3CN/CDCl_3$): $\delta$ = 7.70 (brs, 8H), 7.54 (brs, 4H), 7.42–7.36 (m, 3H), 7.33–7.28 (m, 2H), 7.10–6.84 (brs, 2H), 6.10–5.80 (brs, 1H), 4.96 (dd, $J$ = 8.5, 4.4 Hz, 1H), 3.38 (brs, 1H), 3.12 (dd, $J$ = 12.6, 4.4 Hz, 1H), 3.06 (dd, $J$ = 12.6, 8.5 Hz, 1H), 2.64 ppm (s, 3H).

**Phenethylamine salt**: $^1$H NMR (2% $CD_3CN/CDCl_3$): $\delta$ = 7.70 (brs, 8H), 7.54 (brs, 4H), 7.39–7.30 (m, 3H), 7.18–7.14 (m, 2H), 6.30–5.90 (brs, 3H), 3.15 (t, $J$ = 7.0 Hz, 2H), 2.92 ppm (t, $J$ = 7.0 Hz, 2H).

**Phenethyltrimethyl ammonium salt**: $^1$H NMR (2% $CD_3CN/CDCl_3$): $\delta$ = 7.69 (brs, 8H), 7.54 (brs, 4H), 7.38–7.32 (m, 3H), 7.18–7.14 (m, 2H), 3.43–3.37 (m, 2H), 3.04–2.98 (m, 2H), 2.98 ppm (s, 9H).

**X-ray analyses**

*Crystal data for **1***: Single-crystalline sample was obtained by recrystallizing from EtOH. $C_{34}H_{48}N_2O_{10}$, $M_r$ = 644.75, colorless block, 0.5×0.4×0.4 mm$^3$, monoclinic $P2_1/c$, $a$ = 8.287(3), $b$ = 20.037(8), $c$ = 10.151(4) Å, $\beta$ = 95.856(6)°, $V$ = 1676(1) Å$^3$, $\rho_{calcd}$($Z$ = 2) = 1.277 g cm$^{-1}$. A total of 3779 unique data ($2\theta_{max}$ = 55°) were measured at $T$ = 153 K by a Rigaku Mercury CCD apparatus ($Mo_{K\alpha}$ radiation, $\lambda$ = 0.71069 Å). Numerical absorption correction was applied ($\mu$ = 0.94 cm$^{-1}$). The structure was solved by the direct method (SIR92) and refined by the full-matrix least-squares method on $F$ with anisotropic temperature factors for non-hydrogen atoms. All the hydrogen atoms were located in the D map and refined with isotropic temperature factors. The final $R$ and $Rw$ values are 0.037 and 0.048 for 2539 reflections with $I > 3\sigma(I)$ and 208 parameters. Estimated standard deviations are 0.002 Å for bond lengths and 0.1° for bond angles, respectively.

*Crystal data for **2***: Single-crystalline sample was obtained by recrystallizing from EtOH. $C_{36}H_{52}N_2O_{10}$, $M_r$ = 672.81, colorless block, 0.25×0.25×0.25 mm$^3$, monoclinic $P\bar{1}$, $a$ = 11.387(8), $b$ = 12.905(9), $c$ = 13.76(1) Å, $\alpha$ = 102.286(8), $\beta$ = 107.869(9), $\gamma$ = 98.866(9)°, $V$ = 1827(2) Å$^3$, $\rho_{calcd}$($Z$ = 2) = 1.223 g cm$^{-1}$. A total of 7887 unique data ($2\theta_{max}$ = 55°) were measured at $T$ = 153 K by a Rigaku Mercury CCD apparatus ($Mo_{K\alpha}$ radiation, $\lambda$ = 0.71069 Å). Numerical absorption correction was applied ($\mu$ = 0.89 cm$^{-1}$). The structure was solved by the direct method (SIR92) and refined by the full-matrix least-squares method on $F$ with anisotropic temperature factors for non-hydrogen atoms. All the hydrogen atoms are located at the calculated positions. The final $R$ and $Rw$ values are 0.078 and 0.089 for 3104 reflections with $I > 3\sigma(I)$ and 433 parameters. Estimated stan-

dard deviations are 0.006–0.010 Å for bond lengths and 0.4–0.6° for bond angles, respectively.

CCDC-228165 (**1**) and 242814 (**2**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

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