# Fluorescent Molecular Cages with Sucrose and Cyclotriveratrylene Units for the Selective Recognition of Choline and Acetylcholine 

Łukasz Szyszka,* Marcin Górecki, Piotr Cmoch, and Sławomir Jarosz*



Cite This: J. Org. Chem. 2021, 86, 5129-5141


Read Online

| ACCESS | Lull Metrics 8 More | (ras Aricie Recommendations | - Supporting liformation |
| :---: | :---: | :---: | :---: |


#### Abstract

The synthesis of four fluorescent diastereoisomeric molecular cages containing cyclotriveratrylene and sucrose moieties connected via the naphthalene linkers is reported. These diastereoisomers were found to be selective and efficient receptors for acetylcholine and choline. Compound $P$-5a has a better affinity for choline over acetylcholine, while cage $\mathbf{M}$-5a exhibits a higher association constant for acetylcholine over choline. The highest selectivity value was observed for compound $\boldsymbol{M}-5 \mathbf{a}\left(K_{\mathrm{ACh}} / K_{\mathrm{Ch}}=3.1\right)$. Cages $\boldsymbol{P}-\mathbf{5 a}, P-5 b, M-5 \mathrm{a}$, and $\boldsymbol{M}-5 \mathbf{b}$ were fully characterized by the advanced NMR techniques, and ECD spectroscopy was supported by DFT calculations. The binding constants $K_{\mathrm{a}}$ of these receptors were determined by fluorescence titration experiments in acetonitrile. 


## INTRODUCTION

Molecular cages with fluorescent properties able to selectively recognize various biologically essential compounds have been recently extensively studied. ${ }^{1}$ Fluorescence imaging techniques are attractive and powerful tools for the nondestructive visualization of biological processes with high spatial resolution. ${ }^{2}$

The main advantages of fluorescence recognition studies are high sensitivity, fast response time, and technical simplicity, which makes this technique a useful tool for analytical detections and optical imaging. ${ }^{3}$ In the last decades, several macrocycle derivatives containing fluorophores with different geometries and cavities capable of encapsulating guest molecules have been reported. ${ }^{4}$

The synthesis of molecular cages is particularly attractive due to its selective recognition properties. ${ }^{5}$ These receptors can find application as catalysts, ${ }^{6}$ separators, ${ }^{7}$ sensors, ${ }^{8}$ porous materials, ${ }^{9}$ polymers, ${ }^{10}$ transporters, ${ }^{11}$ or drug delivery systems. ${ }^{12}$ Among them, cryptophanes and hemicryptophanes are of particular interest as they are able to recognize small organic compounds. ${ }^{13}$ This class of receptors is based on the rigid and bowl-shaped $\mathrm{C}_{3}$-symmetrical cyclotriveratrylene (CTV) unit. ${ }^{14}$

The significant contribution to the synthesis of the CTVbased cages was made by Martinez group. ${ }^{15}$ They obtained a wide range of molecular cages in which the CTV moiety is triply connected with tris(2-aminoethyl)amine or $1,3,5$-tris(bromomethyl)benzene via different linkers. These synthetic receptors can selectively recognize carbohydrates, ${ }^{16}$ zwitterions, ${ }^{17}$ or neurotransmitters. ${ }^{18}$
Although chiral receptors are important in selective recognition, only a few examples of such derivatives have been reported so far due to the difficulties in their syntheses. ${ }^{19}$

Preparation of chiral macrocyclic receptors usually requires a multistep procedure, and the final yield is generally low for both steric and entropic reasons. The vast majority of these compounds are prepared as racemic mixtures. ${ }^{15}$
Acetylcholine (ACh) and choline (Ch), structurally related biologically important compounds, are the subject of interest for many years. ${ }^{20}$ Acetylcholine plays a crucial role in the human central nervous system, in particular, in memory processes and transmission of the nervous impulse. This neurotransmitter, released at nerve-muscle synapse, is hydrolyzed to acetic acid and choline by acetylcholinesterase to prevent its high concentrations in the synaptic cleft. ${ }^{21}$ Several diseases are connected with cholinergic failures, such as Parkinson's disease, Alzheimer's disease, Schizophrenia, or other mental diseases. ${ }^{22}$ Choline ( Ch ) is an essential nutrient and has a critical role in neurotransmitter function because of its impact on acetylcholine synthesis and dopaminergic function. ${ }^{23}$ Thus, the selective differentiation of both compounds could provide the understanding of the mechanism of the transmission of nervous signals.

In the last decade, several fluorescent receptors able to recognize ACh and Ch have been reported., ${ }^{20 a 24}$ Martinez et al. obtained three fluorescent hemicryptophanes containing naphthalene ${ }^{25}$ or phenylacetylene ${ }^{26}$ linkers, which can efficiently distinguish ACh over Ch. In another paper, they

[^0]
reported a fluorescent heteroditopic host with the naphthalene units and a Zn (II) complex for the selective recognition of choline phosphate. ${ }^{27} \mathrm{Wu}$ et al. reported a self-assembled triple anion helicate acting as a fluorescence displacement sensor, able to differentiate effectively choline, acetylcholine, glycine betaine, and L-carnitine. ${ }^{28}$ In contrast to Martinez's hemicrypotophanes, this supramolecular host system displays high selectivity toward Ch over ACh. Sarmentero and Ballester developed a fluorescent hybrid cavitand-resorcin[4]arene receptor with the pH -modulated binding properties toward choline. ${ }^{29}$ Moreover, this receptor is able to form thermodynamically stable complexes with complementary ammonium cations in protic solvents.

For many years, our group is involved in the synthesis of macrocyclic derivatives with sucrose scaffold able to recognize chiral and achiral guests. ${ }^{30}$ We have prepared a vast array of chiral receptors based on this disaccharide that could effectively complex ammonium salts, ${ }^{31}$ amino acid esters, ${ }^{32}$ or simple anions. ${ }^{33}$

Our current studies are concentrated on chiral molecular cages bearing CTV and sucrose scaffolds connected via different linkers. In 2019, we presented, for the first time, the water-soluble chiral molecular cages consisting of cyclotriveratrylene and sucrose units. ${ }^{34}$ Recently, we demonstrated an efficient, short, and high-yield route to four diastereoisomeric molecular cages $\boldsymbol{P} \mathbf{- 1} \mathbf{1}, \mathbf{M} \mathbf{- 1} \mathbf{a}, \boldsymbol{P}-\mathbf{1 b}$, and $\boldsymbol{M}-\mathbf{1 b}$ connected via the $p$-phenylene linkers (Figure 1). ${ }^{35}$ These compounds, unfortunately, are not able to recognize choline or acetylcholine.

Herein, we report the synthesis of fluorescent chiral CTV-sucrose-based cages with the naphthalene linkers and disclose their recognition properties toward choline and acetylcholine.
We decided to combine (i) a CTV unit as a binding center for an ammonium part of neurotransmitters, (ii) a sucrose unit as a chiral scaffold, which provide a unique shape of the cavity,



Figure 1. Structures of four molecular cages $\boldsymbol{P}-\mathbf{1 a}, \mathbf{M - 1 a}, P-1 \mathbf{b}$, and $\mathbf{M - 1 b}$ based on CTV and sucrose moieties connected via $p$-phenylene linkers.
and (iii) the naphthalene linkers as fluorophores, which will ensure the fluorescence properties, rigid cavity, and additional $\pi$-system for supporting the recognition.

## RESULTS AND DISCUSSION

The synthesis of the CTV-sucrose-based cages was initiated from commercial sucrose, which was transformed into triol 2 in a three-step route, consisting of selective protection of secondary hydroxyl groups, according to our previously reported procedure. ${ }^{34}$ Alkylation of this triol with an excess of 2,6-bis(bromomethyl)naphthalene at room temperature gave tribromide 3 in $24 \%$ yield (Scheme 1). When this

Scheme 1. Synthesis of Sucrose Tribromide 3

reaction was carried out at reflux, the decomposition of the main product 3 was observed. Racemic cyclotriguaiacylene (4) was synthesized according to the previously reported literature procedure. ${ }^{34}$

The $\mathrm{Cs}_{2} \mathrm{CO}_{3}$-catalyzed macrocyclization of sucrose tribromide 3 with racemic cyclotriguaiacylene (4) in acetonitrile at very low concentration ( $c=0.001 \mathrm{M}$ ) at reflux provided four diastereoisomeric molecular cages: $P$-5a, $\mathbf{M - 5 a}, P-5 b$, and $M$ $\mathbf{5 b}$ in $13,13,6$, and $7 \%$ yield, respectively (total yield of macrocyclization reaction: $39 \%$, ratio 2:2:1:1) (Scheme 2). These diastereoisomers were successfully separated by preparative HPLC using as the eluent, a mixture of three solvents: hexanes/dichloromethane/ethyl acetate in a ratio 50:50:10 v/v.
The structures of these cages were fully characterized by the advanced NMR techniques ( ${ }^{1} \mathrm{H}-{ }^{1} \mathrm{H}$ COSY, TOCSY, ROESY, and ${ }^{1} \mathrm{H}-{ }^{13} \mathrm{C}$ HSQC, HMBC, HSQC-TOCSY), as well as ECD spectroscopy and ESI-HRMS.

NMR Spectroscopy Results. The identification of all four separated isomers was more complex comparing to compounds described earlier. ${ }^{35}$ Each of these structures contains three naphthalene rings, the CTV scaffold, and five benzyl groups, which significantly complicate the identification process, due to big crowding of the ${ }^{1} \mathrm{H} /{ }^{13} \mathrm{C}$ chemical shifts in the range typical for aromatic rings. Careful analysis of all NMR spectra supported by additional results obtained from the ECD measurements allowed us to determine unambiguously the structures of each isomer. Correct assignments of the proper structures for each compound based only on the NMR data could be misleading since such simplified analysis would give ambiguous results in proper structure determination. For example, the correct structure of isomer $\boldsymbol{P}-5$ a, in which the C $1^{\prime}$ atom of fructose is connected with ring C of the CTV unit and the C-6' atom with ring B, while the CTV scaffold has a $P$ stereodescriptor, cannot be assigned only from the NMR data. The set of the ${ }^{1} \mathrm{H} /{ }^{13} \mathrm{C}$ chemical shifts and ROESY effects observed in the CTV part may also suggest the structure $\mathbf{M - 5 b}$

Scheme 2. Syntheses of Four Diastereoisomeric Molecular Cages P-5a, M-5a, P-5b, and M-5b






for this cage. The unambiguous assignment can be done only when the NMR spectra are supported with the ECD experiments (for more information, see the next part of this article).

Based on correct assignments of the ${ }^{1} \mathrm{H} /{ }^{13} \mathrm{C}$ signals in the NMR spectra for each individual isomer, some interesting remarks could be drawn. In the case of cages $P-5$ a and $M-5$ a,
the value of ${ }^{3} \mathrm{~J}(\mathrm{H}-\mathrm{H})$ for the $\mathrm{H}-1$ anomeric proton of glucose is $c a .3 .3 \mathrm{~Hz}$, whereas for counter pair $\mathbf{P}-\mathbf{5 b}$ and $\mathbf{M}-\mathbf{5 b}$ is $c a .3 .8$ Hz . It could be concluded that a change of direction in the CTV cap can cause an appropriate effect in the $\mathrm{H}-1 / \mathrm{H}-2$ position of protons related to a change of the dihedral angle between them. Comparison of the ${ }^{1} \mathrm{H}$ and especially ${ }^{13} \mathrm{C}$ chemical shifts for both nontwisted ( $\boldsymbol{P}-\mathbf{5 a} / \mathbf{M}-\mathbf{5 a}$ ) and twisted ( $\boldsymbol{P}-\mathbf{5 b} / \mathbf{M}-\mathbf{5 b}$ ) molecules indicates the relatively good compatibility of these data in the sucrose part for both pairs (Table S1). The analysis of the NMR data, in particular, ${ }^{13} \mathrm{C}$ NMR chemical shifts, shows that, in the formation process of $P-5$ and M-5 derivatives, much more significant changes are observed for the fructose ring. This is manifesting, depending on the form of the cage, in strong shielding/deshielding effects at the $\mathrm{C}-1^{\prime}$ and $\mathrm{C}-6^{\prime}$ nuclei. In the case of cages $P-5 a$ and $M-5 a$, the combination of sucrose and CTV fragments is connected with strong shielding increase by $c a .5 \mathrm{ppm}$ of the $\mathrm{C}-1^{\prime}$ nucleus, as compared to shielding in cages $\boldsymbol{P}-\mathbf{5 b}$ and $\mathbf{M - 5 b}$ (Table 1). The opposite effect, however less pronounced, is noticed for the C$6^{\prime}$ nuclei. It suggests that its chemical shifts depend more on the position of the C-6' methylene group in a specific product, which is clearly evident in ${ }^{1} \mathrm{H}$ chemical shifts for the $\mathrm{H}-\mathrm{6}^{\prime}$ protons. In the $\mathbf{M}-\mathbf{5 b}$ structure, a very strong shielding effect for the C-6' methylene protons is observed ( $\delta=2.25$ and 2.95 ppm ), as compared to other isomers (Table 1). The difference between positions of these diastereoisomeric protons is bigger for twisted structures $\mathbf{P}-\mathbf{5 b} / \mathbf{M}-\mathbf{5 b}$ (ca. $0.5-0.7 \mathrm{ppm}$ ) than for nontwisted $P$-5a/M-5a (ca. $0-0.2 \mathrm{ppm}$ ). This observation is probably strongly connected with other arrangement of the fructose fragment in $\mathbf{5 a} / \mathbf{5 b}$ isomers.

Another conclusion, which may be drawn from the NMR data is related to the signals of the benzyl groups. In the ${ }^{1} \mathrm{H}$ NMR spectra of $\mathbf{P - 5 b}$ and $\mathbf{M - 5 b}$, the chemical shifts of such groups at the C-4' atom are significantly different, as compared to $P-5 a$ and $M-5 a$ isomers. This is especially visible for structure $P-5 \mathbf{b}$, where the signals of the methylene protons of the benzyl group appear at $\delta=3.51$ and 3.70 ppm (Table 1). In this case, also, phenyl ring protons of the benzyl group are in the special isolated range ( $\delta=6.30-6.60 \mathrm{ppm}$ ). For isomer $M$ $\mathbf{5 b}$, the above mentioned phenomena also exist, but the results are less highlighted. A similar trend is typical for phenyl ring protons of the benzyl group attached to the C-4 atom in compound M-5a. Signals of these protons $(\delta=5.92-6.50$ $\mathrm{ppm})$ are separated and more shielded than most aromatic protons of this cage. The position of the methoxy groups in the CTV part is also different for all isomers. The ${ }^{1} \mathrm{H}$ chemical shifts are in the typical range ( $\delta \mathrm{ca} .3 .0-4.0 \mathrm{ppm}$ ), but the structure of the cage determines the values of their shifts. The biggest difference between ${ }^{1} \mathrm{H}$ chemical shifts for these

Table 1. Comparison of the Selected ${ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ NMR Chemical Shifts $\delta$ ( ppm ) of $P-5 \mathrm{a}, \mathrm{P}-5 \mathrm{~b}, M-5 \mathrm{a}$, and $M$ - 5 b Cages

| atom's number ${ }^{\text {a }}$ | ${ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ NMR chemical shifts $\delta(\mathrm{ppm})$ |  |  |  |
| :---: | :---: | :---: | :---: | :---: |
|  | $P-5 \mathrm{a}$ | $P-5 b$ | M-5a | M-5b |
| H-1 | 5.19 | 5.55 | 5.60 | 5.54 |
| H-6'a/H-6'b | 3.36/3.54 | 2.84/3.37 | 3.46/3.49 | 2.25/2.95 |
| $\mathrm{C} 4{ }^{\prime}-\mathrm{OCH}_{2} \mathrm{Ph}$ | 4.40/4.48 | 3.51/3.70 | 4.37/4.42 | 4.09/3.93 |
| $\mathrm{C} 4^{\prime}-\mathrm{OCH}_{2}-\underline{\mathrm{H}-\mathrm{Ph}}$ | 7.19-7.29 | 6.58/6.41/6.30 | 7.16-7.27 | 7.07/7.06/6.96 |
| H-26/H-26'/H-26" $\left(\mathrm{OCH}_{3}\right)$ | 3.40/3.55/3.21 | 3.60/3.44/3.73 | 3.92/3.22/3.48 | 3.02/4.03/3.19 |
| C-1 ${ }^{\prime}$ | 69.9 | 74.8 | 69.1 | 74.8 |
| C-6' | 73.0 | 70.9 | 73.1 | 72.7 |

[^1]methoxy groups is noted for compound $\mathbf{M}-5 \mathbf{b}$ (ca. 1.0 ppm , Table 1). Moreover, due to the specific through-space interactions of the methoxy groups and aromatic protons from the CTV fragment, the proper assignment of the ${ }^{1} \mathrm{H} /{ }^{13} \mathrm{C}$ chemical shifts and thus structure correctness can be verified. All these above-mentioned remarks can be used in the future to find a relation of the NMR data and spatial arrangements of atoms defining the specific structure.

ECD Spectroscopy Results. For more detailed structural information that would allow more deeply to explore the stereochemistry of these diastereoisomeric molecular cages, we turned our attention to the electronic circular dichroism (ECD) spectroscopy, which is one of the most suitable spectroscopic tools for this purpose. It is based on the study of interactions of circularly polarized light in the UV-vis region for exploring the 3D environment of chiral nonracemic compounds and allows to monitor even the smallest subtle changes in their structures. The successful combination of the ECD spectroscopy with quantum chemical calculations expands significantly the range of applicability of this spectroscopy. ${ }^{34-36}$

Thus, the UV and ECD spectra of four diastereoisomeric molecular cages $P-5 \mathrm{Fa}, \mathbf{M}-\mathbf{5}, \mathbf{P}-\mathbf{5 b}$, and $\mathbf{M}-5 \mathbf{b}$ were recorded in $\mathrm{CH}_{3} \mathrm{CN}$ to assign their absolute stereochemistry (Figure 2).


Figure 2. (a) ECD and (b) UV spectra of $P-5 a, M-5 a, P-5 b$, and $M$ $\mathbf{5 b}$ measured in $\mathrm{CH}_{3} \mathrm{CN}$ at room temperature.

The UV spectra of these compounds are almost identical and showed a manifold of bands at 280 and 225 nm associated with the CTV and naphthalene chromophores.

In the ECD spectra, there are a few very intense bands centered at around 235,225 , and 210 nm and rather intense ones at lower energy wavelengths at about 282 and 255 nm . In the case of molecular cage $\mathbf{M - 5 b}$, being eluted as the fourth compound in the elution order under our conditions (see the Experimental Section part), the ECD curve showed some aberrations in the range $220-250 \mathrm{~nm}$. Remarkably, the two
curves, i.e., $\mathbf{P}-\mathbf{5 a}$ and $\mathbf{M - 5 a}$ are associated with almost perfect mirror image of the ECD pattern. This is clearly evidenced by crossing exactly at zero values. In contrast, for $P-5 \mathbf{b}$ and $\mathbf{M - 5 b}$, the mirror image correlation was not perfect, as evidenced in Figure 2a by different absolute values of the ECD intensities in the range $220-250 \mathrm{~nm}$, and by the fact that these two spectra do not cross precisely at zero values. Nevertheless, all spectra show characteristic features related to ${ }^{1} L_{b}$ and ${ }^{1} L_{a}$ transitions of aromatic chromophores. According to Collet et al., the signs of the ${ }^{1} L_{a}$ bands can be used to assign the absolute configuration of the CTV unit: $M$-configuration is determined for molecules, which exhibit in their ECD spectra a sequence of signs negative/positive from low to high energy within this region, so analogously for $P$-configuration sequence is opposite. ${ }^{37}$ Thus, ad hoc for the first and second eluted peaks with positive/ negative sequence of signs, the configuration was immediately assigned as $P-5 \mathbf{a} / P-5 \mathbf{b}$, while for the third and fourth the opposite sequence indicates $\mathbf{M - 5 a} / \mathbf{M}-\mathbf{5 b}$ configuration.

DFT Calculation Results. To support this assignment, the quantum chemical calculations were carried out. First, the conformational search was done at the molecular mechanics level using a simplified structure in which benzyl groups (Bn) in the sucrose moiety were substituted with the hydrogen atom to facilitate the further computational predictions of ECD spectra. This approach preserves the main conformational landscapes of the investigated compounds and does not have any impact on the final stereochemical assignment. Then, the lowest energy structures within $3 \mathrm{kcal} / \mathrm{mol}$ were submitted for DFT optimization using Gaussian16 program ${ }^{38}$ at the B3LYP/ 6-31G(d) level of theory applying PCM for $\mathrm{CH}_{3} \mathrm{CN}$. In this way, for each compound, two conformers were identified for ECD calculations. They mainly fluctuate in the rotation around the $\mathrm{C}-\mathrm{O}$ bond(s) linking the CTV unit with naphthalene linker(s), while the rest of the molecule is well-kept. The lowest energy structures are presented in Figure 3. For TDDFT simulations, the following functional/basis-set combination was used: B3LYP/SVP with the polarizable continuum model (PCM) for $\mathrm{CH}_{3} \mathrm{CN}$.

This level of approximation was indicated as one of the most successful in recent studies for investigating their ECD properties of systems with CTV moiety. ${ }^{34,35,366, c}$

The simulated spectra are consistent with experimental ones (Figure 4); however, some minor inconsistencies are found in the range of ${ }^{1} \mathrm{~L}_{\mathrm{b}}$ transitions. This is a well-known issue in TDDFT calculations of the ECD spectra since this band is simulated without taking into account a vibronic effect. ${ }^{39}$ Consequently, here, this subregion is excluded from our discussion.

The distinction of diastereoisomeric molecular cages was made by in-depth analysis of their chiroptical properties. Although the shapes of the ECD bands for two pairs of diastereoisomers are in line for $P-5 a$ and $M-5 a$, the relative intensity of bands in the range $220-250 \mathrm{~nm}$ is higher in respect to the second pair $\boldsymbol{P}-\mathbf{5 b}$ and $\boldsymbol{M}-\mathbf{5 b}$. The same observation can be found from TDDFT-calculated ECD spectra, which provides further strong evidence on the correctness of this stereochemical assignment.

Recognition Studies. Then, we investigated the recognition properties of $P-5 \mathrm{a}, \mathrm{P}-\mathbf{5 b}, \mathbf{M}-5 \mathrm{a}$, and $\mathbf{M - 5 b}$ cages toward biologically interesting compounds, acetylcholine (ACh) and choline (Ch). The binding properties were determined by the fluorescence titration evaluating the emission spectra after the progressive addition of ACh or Ch solution to the host

$P-5 a$


P-5b



Figure 3. Lowest-energy conformers calculated at the B3LYP/6$31 \mathrm{G}(\mathrm{d}) / \mathrm{PCM} / \mathrm{CH}_{3} \mathrm{CN}$ level of theory. Note: the hydrogen atoms are omitted for the sake of clarity.


Figure 4. Comparison of calculated ECD spectra at the B3LYP/SVP/ PCM $\left(\mathrm{CH}_{3} \mathrm{CN}\right)$ level of (a) P-5a, M-5a, and (b) P-5b, M-5b with experimental ones measured in $\mathrm{CH}_{3} \mathrm{CN}$ at room temperature. Note: all spectra are red-shifted by 5 nm and simulated using 0.15 eV Gaussian band-widths.
solution in the same solvent. We decided to choose this method due to the fast response time, high sensitivity, and the presence of the naphthalene linkers, which ensure fluorescence properties. The titration experiments were performed in
acetonitrile, and iodide was chosen as a guest counter-ion because of good solubility.

Fluorescence emission spectra of the hosts strongly differ after the addition of appropriate equivalents of guests. Indeed, an increase of fluorescence is observed for $P-5$ a and $M-5$ a hosts, whereas receptors $\boldsymbol{P}-\mathbf{5 b}$ and $\mathbf{M - 5 b}$ display a decrease of the fluorescence intensity upon the addition of acetylcholine or choline (Figure 5 and Figures S3, S5, S9, and S11). This opposite behavior might suggest the differences in formation of the host-guest complexes and the influence of chiral twisted/ non-twisted structure of each diastereoisomer. The fluorescence enhancement might be assigned to the formation of rigid host-guest complex structures of nontwisted $P$-5a and $M-5$ a cages stabilized by intermolecular hydrogen bonds. ${ }^{26 a}$ Moreover, the twisted structures of compounds $\mathbf{P - 5 b}$ and $\mathbf{M - 5 b}$ ensure the different size and shape of the cavity than nontwisted $P-5 a$ and $M-5 a$, which might also explain this binding differences.

The addition of acetylcholine resulted in a significant increase of the fluorescence of the $P-5 a$ and $M-5 a$ host at $c a$. 330 nm (Figure 5a,c). The binding constant $\left(K_{\mathrm{a}}\right)$ for $\boldsymbol{P}$ - 5 a was $2.2 \times 10^{3} \mathrm{M}^{-1}$, whereas for $M-5$ a was $5.6 \times 10^{3} \mathrm{M}^{-1}$. In the case of hosts $\boldsymbol{P}-\mathbf{5 b}$ and $\mathbf{M - 5 b}$, the $K_{\mathrm{a}}$ values were $2.4 \times 10^{3}$ and $0.6 \times 10^{3} \mathrm{M}^{-1}$, respectively (Table 2). These results show that compound $\mathrm{M}-5 \mathrm{a}$ is the most efficient host for ACh.

During our recognition studies of choline, the remarkable increase of the fluorescence intensity was observed for $P$-5a and $\boldsymbol{M}$-5a cages at $c a .330 \mathrm{~nm}$ (Figure $5 \mathbf{b}, \mathrm{~d}$ ). In the case of $\boldsymbol{P}$ $\mathbf{5 b}$ and $\mathbf{M - 5 b}$, quenching of fluorescence intensity was observed (Figures S9 and S11). The most significant value of binding constant $K_{\mathrm{a}}\left(3.8 \times 10^{3} \mathrm{M}^{-1}\right)$ was achieved by receptor $\boldsymbol{P}-\mathbf{5 a}$.

Lower binding constants were obtained for $\mathbf{P}-\mathbf{5 b}$ and $\mathbf{M - 5 a}$ cages, $2.6 \times 10^{3}$ and $1.8 \times 10^{3} \mathrm{M}^{-1}$, respectively. The lowest $K_{\mathrm{a}}$ value was obtained for the $\mathbf{M}-\mathbf{5 b}$ host $\left(0.5 \times 10^{3} \mathrm{M}^{-1}\right)$ (Table 2). Comparing the recognition selectivity of ACh and Ch by these hosts, we can notice that compound $P-5$ a is the most efficient sensor for choline ( $K_{\mathrm{Ch}} / K_{\mathrm{ACh}}=1.7$ ), while $M$-5a is a more suitable receptor for acetylcholine $\left(K_{A C h} / K_{\mathrm{Ch}}=3.1\right)$. The M-5a host could efficiently distinguish acetylcholine over choline. This selectivity is meaningful since both guests participate in the metabolic pathway. In the case of compounds $\boldsymbol{P}-\mathbf{5 b}$ and $\mathbf{M - 5 b}$, no binding selectivity was observed. These differences in recognition of both guests could be rationalized by the structure of the molecular cages. As we can conclude from the DFT calculated structures, the chiral sucrose platform provides the unique shapes of the cavities of these receptors, which might allow to distinguish acetylcholine over choline and vice versa.

To supply the fluorescence recognition studies and get more information about the binding sites, the ${ }^{1} \mathrm{H}$ NMR titration experiments of $\boldsymbol{P}-\mathbf{5 a}$ and $\mathbf{M - 5 a}$ cages with ACh and Ch were carried out (Figures S13, S16, S19, and S21). For this purpose, appropriate amounts of ACh or Ch solutions in $\mathrm{CD}_{3} \mathrm{CN}$ / $\mathrm{CDCl}_{3}$ (80:20) were gradually added to the host solution in the mixture of the same solvent. The ${ }^{1} \mathrm{H}$ NMR studies show changes in the chemical shifts of both host and guest protons, which is in line with fast host-guest exchange on the NMR time scale. In all cases, the signals from $\left(\mathrm{CH}_{3}\right)_{3} \mathrm{~N}^{+}$and methylene protons are shifted downfield with increasing amount of ACh or Ch guests (Figures S15 and S18). This could be explained by increasing the ratio of the unbounded guest. ${ }^{26}$ Compared to the spectra of pure ACh or Ch , the


Figure 5. Fluorescent titration of hosts: (a) P-5a with ACh, (b) P-5a with Ch, (c) M-5a with ACh, and (d) $M-5 a$ with $\mathrm{Ch}^{\text {in }} \mathrm{CH}_{3} \mathrm{CN}$ at 298 K excited at 280 nm (counter-ion $\mathrm{I}^{-}$).

Table 2. Comparison of Binding Constants $K_{a}\left(\mathbf{M}^{-1}\right)$ of $P$ $5 \mathrm{a}, \mathrm{P}-5 \mathrm{~b}, \mathrm{M}-5 \mathrm{a}$, and $M-5 \mathrm{~b}$ Hosts with ACh and Ch

| Guest | Host | $K_{\mathrm{a}}\left(\mathrm{M}^{-1}\right)^{a}$ | $K_{A C h} / K_{\text {Ch }}$ |
| :---: | :---: | :---: | :---: |
|  | $P$-5a | $2.2 \times 10^{3} \pm 1.6 \%$ | 0.6 |
|  | $P$-5b | $2.4 \times 10^{3} \pm 3.5 \%$ | 0.9 |
|  | M-5a | $5.6 \times 10^{3} \pm 1.7 \%$ | 3.1 |
|  | M-5b | $0.6 \times 10^{3} \pm 0.9 \%$ | 1.2 |
| Guest | Host | $K_{\mathrm{a}}\left(\mathrm{M}^{-1}\right)^{a}$ | $K_{\text {Ch }} / K_{\text {ACh }}$ |
|  | $P$-5a | $3.8 \times 10^{3} \pm 0.9 \%$ | 1.7 |
|  | $P$-5b | $2.6 \times 10^{3} \pm 3.8 \%$ | 1.1 |
|  | M-5a | $1.8 \times 10^{3} \pm 1.4 \%$ | 0.3 |
|  | M-5b | $0.5 \times 10^{3} \pm 1.1 \%$ | 0.8 |

${ }^{a}$ Association constants $K$ a were determined by fitting fluorescence titration curves $\left(\mathrm{CH}_{3} \mathrm{CN}, 298 \mathrm{~K}\right)$ using Bindfit program. ${ }^{40}$
signals of both guests are shifted upfield during the titration studies, which confirms the encapsulation of both guests in the host cavities. There are a few reports about binding the $\left(\mathrm{CH}_{3}\right)_{3} \mathrm{~N}^{+}$part of ACh and Ch inside the electron-rich CTV cavity., ${ }^{25,26 a, 27}$ In the case of $\mathbf{M - 5 a}$, the gradual addition of ACh provided downfield chemical shifts of the CTV aromatic protons and upfield shifts of the protons from naphthalene ring linkers (Figure S14). On the other hand, the recognition studies of Ch also show downfield shifts of the CTV aromatic
protons, but upfield chemical shifts of naphthalene ring linkers were less significant (Figure S17).

The additional interactions of ACh ester group with the host may explain the higher binding constant and selectivity for ACh by $\boldsymbol{M}-5$ a cage. While the ${ }^{1} \mathrm{H}$ NMR titration studies of $\boldsymbol{P}$ 5a cage with Ch show evident changes in the chemical shifts of aromatic part of CTV unit, as well as naphthalene rings, the binding studies with ACh demonstrate only slight changes of such chemical shifts (Figures S20 and S22). In this case, most likely, the shape of the cavity may cause the preference for Ch binding.

All these results indicate the formation of the corresponding host-guest complexes. Both guests are bind inside the electron-rich cavities, which is reflected in chemical shift changes observed during ${ }^{1} \mathrm{H}$ NMR titration experiments, as well as in the changes of the fluorescence intensity. The observed differences in ACh and Ch recognition by these diastereoisomeric cages are, most likely, caused by original shapes of their cavities, created by various connections of both CTV and sucrose scaffolds. To further supply the binding properties of these cages and the stoichiometry of the complexation, ESI-MS measurements were carried out. Both hosts, $P$-5a and $M-5$ a, form noncovalent complexes with choline cations $[\mathrm{M}+\mathrm{Ch}]^{+}$with an $m / z$ value of 1761.81 (Figures S77 and S79), as well as with acetylcholine cations [M $+\mathrm{ACh}]^{+}$with an $m / z$ of value 1803.82 (Figures S78 and S80) in acetonitrile. These results show that adducts formed between cages $\mathbf{P}-\mathbf{5 a}$ or $\mathbf{M}-5$ a with Ach or Ch are relatively stable proving a $1: 1$ stoichiometry ratio. Adducts containing
two guest molecules $[\mathrm{M}+2 \mathrm{ACh}]^{2+}$ or $[\mathrm{M}+2 \mathrm{Ch}]^{2+}$ were not detected.

Next, the DFT calculations of the host-guest inclusion complexes were performed to investigate further the selectivity of cage $\mathbf{M - 5 a}$ toward ACh over Ch. The optimized structures of both complexes show that ACh , as well as Ch , is partially encapsulated in the M-5a host cavity (Figure 6). These results


Figure 6. DFT-calculated structures of encapsulated complexes (a) M-5a $\subset A C h$ and (b) M-5a CCh.
are consistent with previously described CTV-based hemicryptophanes capable of binding $\mathrm{ACh} .^{41}$ In the case of $M$ $5 a \subset A C h$ complex, the ammonium unit is situated below the bowl-shaped CTV moiety, while the ester function is located between naphthalene linkers. Several $\mathrm{CH}-\pi$ interactions between the $\left(\mathrm{CH}_{3}\right)_{3} \mathrm{~N}^{+}$part of ACh and phenyl rings of the CTV unit or naphthalene rings with distances ranging from 2.8 to $3.1 \AA$ and from 2.5 to $3.0 \AA$, respectively, are observed. Moreover, cation $-\pi$ interactions between positively charged nitrogen from ACh and CTV's phenyl or naphthyl centroids occur with distances from 4.8 and 4.9 or 4.1 to $4.4 \AA$, respectively. Additionally, the interactions between (i) $\mathrm{C}=\mathrm{O}$ or -O- from ACh and $\mathrm{CH}_{3}$ from the methoxy group ( $2.8 \AA$ or $2.7 \AA$ ), (ii) $\mathrm{C}(\mathrm{O}) \mathrm{CH}_{3}$ from the ACh and naphthalene centroid (3.4 Å), and (iii) both $\mathrm{CH}_{2}$ from ACh and naphthalene centroids (distances from 2.4 to $3.4 \AA$ ) can be found. In the case of $\mathbf{M}-\mathbf{5 a} \subset \mathbf{C h}$, complex similar cation $-\pi$, as well as $\mathrm{CH}-\pi$, interactions between the $\left(\mathrm{CH}_{3}\right)_{3} \mathrm{~N}^{+}$part of choline and aromatic rings of the CTV unit or naphthalene linkers are observed, but with greater distances. Indeed, the distance between positively charged nitrogen from choline and phenyl or naphthyl centroids from the host ranging from 5.1 to 5.6 or 4.2 to $4.5 \AA$, respectively. These results show that the ammonium part of Ch is bound weaker than ACh by the CTV unit. The $\mathrm{CH}-\pi$ interactions between $\left(\mathrm{CH}_{3}\right)_{3} \mathrm{~N}^{+}$and phenyl or naphthyl centroids in $\mathbf{M}-\mathbf{5 a C C h}$ are in distance ranging from 3.0 to 3.7 or 2.5 to $3.1 \AA$, respectively. Both $\mathrm{CH}_{2}$ groups from choline are also in distance from 3.1 to $3.5 \AA$ in relation to naphthyl centroids. These DFT studies of both complexes give an insight in the binding details of the $\mathbf{M}$-5a cage and support its selectivity toward ACh. The additional interactions of the ester group with $\mathbf{M}$-5a cage and shorter distances between the CTV moiety and ammonium part of ACh could be responsible for this selectivity compared to Ch .

## - CONCLUSIONS

In summary, we described the synthesis of four fluorescent diastereoisomeric molecular cages based on CTV and sucrose
units connected via the naphthalene linkers. These compounds can act as efficient fluorogenic sensors for the detection of acetylcholine or choline. Application of the sucrose platform to the host structure is, as we assume, responsible for the unique shape of the cavity resulting in the selective recognition of these biologically important guests. Cage $\mathbf{M}$-5a displays the strongest binding with acetylcholine, while cage $P$-5a mostly prefers choline. Both guests acetylcholine and choline could be, therefore, selectively recognized by these molecular cages using fluorescence spectroscopy.

## EXPERIMENTAL SECTION

General Methods. All reagent-grade chemicals and solvents were received from commercial suppliers. TLC was performed on Merck silica gel $60 \mathrm{~F}_{254}$ plates. Compounds were purified using an automatic flash chromatography system Knauer with UV and ELSD detection and Grace Resolv or Reveleris cartridges. Preparative HPLC was conducted on a Shimadzu SPD-6a spectrometer using a UV detector ( 254 nm ) with a Vathsil 100 column ( $250 \mathrm{~mm} \times 10 \mathrm{~mm}$, particle size: $5 \mu \mathrm{~m}$ ) and a $5 \mathrm{~mL} / \mathrm{min}$ flow rate. The NMR spectra were recorded with a Varian VNMRS 600 MHz (at 600 MHz and 150 MHz for ${ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ NMR spectra, respectively) spectrometer for solutions in $\mathrm{CDCl}_{3}$ and TMS as internal standards. All significant resonances were assigned by COSY $\left({ }^{1} \mathrm{H}-{ }^{1} \mathrm{H}\right)$, ROESY $\left({ }^{1} \mathrm{H}-{ }^{1} \mathrm{H}\right)$, TOCSY $\left({ }^{1} \mathrm{H}-{ }^{1} \mathrm{H}\right)$, HSQC $\left({ }^{1} \mathrm{H}-{ }^{13} \mathrm{C}\right)$, and $\mathrm{HMBC}\left({ }^{1} \mathrm{H}-{ }^{13} \mathrm{C}\right)$ correlations. Mass spectra were measured using a Synapt G2-S HDMS (Waters Inc.) mass spectrometer equipped with an electrospray ion source and $q$-TOF type mass analyzer or using an AutoSpec Premier (Waters Inc.) double-focusing magnetic sector mass spectrometer with an EBE geometry equipped with an EI (electron impact) ion source. Optical rotations were measured with a Jasco P 2000 apparatus in $\mathrm{CHCl}_{3}$ or $\mathrm{CH}_{2} \mathrm{Cl}_{2}$ with a sodium lamp at room temperature. Elemental analyses were obtained with a Perkin-Elmer 2400 CHN analyzer. The ECD and UV spectra were recorded in a $\mathrm{CH}_{3} \mathrm{CN}$ on a Jasco J-715 spectropolarimeter. The fluorescence titration experiments were performed using a Shimadzu RF-6000 fluorescence spectrometer.

2,6-Bis(bromomethyl)naphthalene was synthesized according to the literature procedure. ${ }^{42}$ All reactions were carried out under an argon atmosphere. Organic solutions were dried over anhydrous $\mathrm{Na}_{2} \mathrm{SO}_{4}$.

Synthesis of 2,3,3',4,4'-Penta-O-benzyl-1',6,6'-tri-O-[5-( bromo-methyl)-naphthyl]-sucrose (3).


3
Sodium hydride ( $453.6 \mathrm{mg}, 18.9 \mathrm{mmol}, 60 \%$ dispersion in mineral oil) was added portionwise to a solution of triol $2(1 \mathrm{~g}, 1.26 \mathrm{mmol})$ in dry THF ( 20 mL ). After stirring for 20 min . at room temperature, 2,6bis(bromomethyl)naphthalene $(2.37 \mathrm{~g}, 7.56 \mathrm{mmol})$ was added in one portion, and the mixture was stirred at room temperature overnight. The reaction was quenched by careful addition of methanol ( 2 mL ), and the mixture was poured onto dichloromethane $(20 \mathrm{~mL})$ and water $(20 \mathrm{~mL})$. The layers were separated, the aqueous one was extracted with dichloromethane $(3 \times 20 \mathrm{~mL})$, combined organic phases were washed with brine $(60 \mathrm{~mL})$, dried over $\mathrm{Na}_{2} \mathrm{SO}_{4}$, and concentrated, and the residue was purified by flash chromatography (hexanes/ethyl acetate $=$ from 100:0 to $75: 25$ ) to afford $3(446 \mathrm{mg}$, $0.3 \mathrm{mmol}, 24 \%$ ) as a yellowish oil. $[\alpha]_{D}^{25}+34.3\left(c 0.5, \mathrm{CHCl}_{3}\right) .{ }^{1} \mathrm{H}$ NMR ( $600 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ): $\delta=7.76(\mathrm{~s}, 1 \mathrm{H}, \mathrm{H}-\mathrm{Naphth}), 7.65-7.74$ (m, 9H, $9 \times$ H-Naphth), 7.62 (d, $J=7.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-$ Naphth), 7.62 (s,

1H, H-Naphth), 7.43 (dd, $J=18.2 \mathrm{~Hz}, J=1.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-\mathrm{Naphth}$ ), 7.43 (t, 1H, H-Naphth), 7.35-7.41 (m, 4H, $4 \times$ H-Naphth), 7.11$7.24(\mathrm{~m}, 23 \mathrm{H}, 23 \times \mathrm{H}-\mathrm{Ph}), 7.01-7.04(\mathrm{~m}, 2 \mathrm{H}, 2 \times \mathrm{H}-\mathrm{Ph}), 5.78(\mathrm{~d}$, $\left.J_{1,2}=3.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-1\right), 4.83(\mathrm{~d}, J=10.8 \mathrm{~Hz}, 1 \mathrm{H}$, benzylic H), $4.80(\mathrm{~d}$, $J=11.0 \mathrm{~Hz}, 1 \mathrm{H}$, benzylic H), $4.64\left(\mathrm{~s}, 2 \mathrm{H}, 2 \times \mathrm{C} \underline{H}_{2}-\mathrm{Br}\right), 4.61(\mathrm{~s}, 2 \mathrm{H}$, $\left.2 \times \mathrm{CH}_{2}-\mathrm{Br}\right), 4.70\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-7^{\prime \prime} \mathrm{a}\right), 4.67\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{H}-7^{\prime} \mathrm{a}\right.$, benzylic H$)$, $4.66(\mathrm{~m}, 1 \mathrm{H}$, benzylic H$), 4.64\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}^{\prime} 7^{\prime} \mathrm{b}\right), 4.60(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-7 \mathrm{a})$, $4.58\left(\mathrm{~s}, 2 \mathrm{H}, 2 \times \mathrm{CH}_{2}-\mathrm{Br}\right), 4.57\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-7^{\prime \prime} \mathrm{b}\right), 4.56(\mathrm{~d}, \mathrm{~J}=12.4 \mathrm{~Hz}$, 1 H , benzylic H), $4.52(\mathrm{~d}, J=11.1 \mathrm{~Hz}, 1 \mathrm{H}$, benzylic H), $4.50(\mathrm{~d}, J=$ $11.0 \mathrm{~Hz}, 1 \mathrm{H}$, benzylic H), 4.46 (m, 1H, H-3'), 4.43 (m, 1H, H-7b), (4.41-4.47 (m, 3H, $3 \times$ benzylic H), $4.24\left(\mathrm{dd}, J_{4^{\prime}, 3^{\prime}}=7.5 \mathrm{~Hz}, J_{4^{\prime}, 5^{\prime}}=\right.$ 7.5 Hz 1H, H-4'), 4.14-4.17 (m, 1H, H-5'), 4.09 (m, 1H, H-5), 3.92 $\left(\mathrm{dd}, J_{3,4}=9.3 \mathrm{~Hz}, J_{3,2}=9.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3\right), 3.72-3.80\left(\mathrm{~m}, 3 \mathrm{H}, \mathrm{H}^{\prime} 1^{\prime} \mathrm{a}\right.$, H-6'a, H-6'b), 3.62 (dd, $\left.J_{4,5}=9.8 \mathrm{~Hz}, J_{4,3}=9,4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-4\right), 3.59(\mathrm{~d}$, $\left.J_{1^{\prime} b, 1^{\prime} \mathrm{a}}=11.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-1^{\prime} \mathrm{b}\right), 3.48\left(\mathrm{dd}, J_{6 \mathrm{~b}, 5}=3.5 \mathrm{~Hz}, J_{6 \mathrm{~b}, 6 \mathrm{a}}=10.3 \mathrm{~Hz}\right.$ $1 \mathrm{H}, \mathrm{H}-6 \mathrm{a}), 3.46\left(\mathrm{dd}, J_{2,1}=3.5 \mathrm{~Hz}, J_{2,3}=9.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2\right), 3.39(\mathrm{dd}$, $\left.J_{6 \mathrm{~b}, 5}=1.7 \mathrm{~Hz}, J_{6 \mathrm{~b}, 6 \mathrm{a}}=10.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-6 \mathrm{~b}\right) \mathrm{ppm} .{ }^{13} \mathrm{C}\left\{{ }^{1} \mathrm{H}\right\}$ NMR $(150$ $\left.\mathrm{MHz}, \mathrm{CDCl}_{3}\right): \delta=139.0,138.6,138.4,138.2,138.2\left(\mathrm{C}_{\text {quat }} 5 \times \mathrm{C}\right.$ $\mathrm{Ph}), 136.7,136.5,136.4$ (C-8, C-8', C-8" $)$, 135.3, 135.2, 135.1 (C-13, C-13', C-13"'), 133.1, 133.0, 132.9, 132.8, 132.8, 132.7 (C-10, C-10', C-10" $\left., \mathrm{C}-15, \mathrm{C}-15^{\prime}, \mathrm{C}-15^{\prime \prime}\right), 125.9-128.9$ (m, 43C, $25 \times \mathrm{C}-\mathrm{Ph}, 18 \times$ C-Naphth), 104.7 (C-2'), 90.0 (C-1), 84.0 (C-3'), 82.3 (C-4'), 82.1 (C-3), 80.0 (C-2), 79.7 (C-5'), 77.8 (C-4), $75.6\left(\mathrm{C} 3-\mathrm{OCH}_{2} \mathrm{Ph}\right)$, 74.9 ( $\mathrm{C} 4-\mathrm{OCH}_{2} \mathrm{Ph}$ ), 73.5 (C-7"), 73.4 (C-7), 73.3 (C-7'), 73.2 $\left(\mathrm{C}^{\prime}-\mathrm{O}_{\underline{C}} \mathrm{H}_{2} \mathrm{Ph}\right), 72.7\left(\mathrm{C}^{\prime}-\mathrm{OC}_{2} \mathrm{Ph}\right), 72.3\left(\mathrm{C} 2-\mathrm{O} \underline{C H}_{2} \mathrm{Ph}\right), 71.6$ (C-1'), 71.6 (C-6'), 70.7 (C-5), 68.8 (C-6), 34.2, 34.2, 34.2 (C-18, C$\left.18^{\prime}, \mathrm{C}-18^{\prime \prime}\right)$ ppm. MS (ESI-TOF) $m / z:[\mathrm{M}+\mathrm{Na}]^{+}$calcd for $\mathrm{C}_{83} \mathrm{H}_{79} \mathrm{O}_{11} \mathrm{Br}_{3} \mathrm{Na}$ 1511.31; found 1511.35. Anal. calcd for $\mathrm{C}_{83} \mathrm{H}_{79} \mathrm{O}_{11} \mathrm{Br}_{3}$ : C, 66.81; H, 5.34; found: C, $66.69 ; \mathrm{H}, 5.54$.

Syntheses of CTV-Sucrose-Based Cages P-5a, M-5a, P-5b, and $M-5 b$. To a solution of $( \pm) 4(24.5 \mathrm{mg}, 0.06 \mathrm{mmol})$ in dry acetonitrile $(40 \mathrm{~mL}), \mathrm{Cs}_{2} \mathrm{CO}_{3}(175 \mathrm{mg}, 0.54 \mathrm{mmol})$ was added and the mixture was stirred at room temperature for 30 min . The solution of compound 3 ( $89 \mathrm{mg}, 0.06 \mathrm{mmol}$ ) in dry acetonitrile ( 20 mL ) was added dropwise by a syringe pump within 4 h , and the mixture was stirred at reflux for additional 48 h . After cooling to room temperature, the mixture was filtered through Celite and the solvent was removed under vacuum. The residue was dissolved in $\mathrm{CH}_{2} \mathrm{Cl}_{2}(20$ mL ) and washed with water $(20 \mathrm{~mL})$. The aqueous phase was extracted with $\mathrm{CH}_{2} \mathrm{Cl}_{2}(3 \times 20 \mathrm{~mL})$, and the combined organic solutions were washed with brine $(30 \mathrm{~mL})$, dried, and concentrated. The resulting residue was purified by preparative HPLC (hexanes/ dichloromethane/ethyl acetate $=50: 50: 10)$ to afford pure compounds $\boldsymbol{P}$-5a ( $13 \mathrm{mg}, 0.0078 \mathrm{mmol}, 13 \%$, colorless solid), $\boldsymbol{P}-\mathbf{5 b}$ ( $6 \mathrm{mg}, 0.0036$ $\mathrm{mmol}, 6 \%$, white solid), M-5a ( $13 \mathrm{mg}, 0.0078 \mathrm{mmol}, 13 \%$, colorless solid), and $\mathbf{M - 5 b}$ ( $7 \mathrm{mg}, 0.0042 \mathrm{mmol}, 7 \%$, colorless solid). Total yield: $39 \%$.

## Characterization of Compound P-5a.


$[\alpha]_{D}^{25}+129.5\left(c \quad 0.22, \mathrm{CH}_{2} \mathrm{Cl}_{2}\right) \cdot{ }^{1} \mathrm{H} \operatorname{NMR}\left(600 \mathrm{MHz}, \mathrm{CDCl}_{3}\right): \delta=$ 7.77 ( $\mathrm{s}, 1 \mathrm{H}, \mathrm{H}-14^{\prime \prime}$ ), 7.65 (d, $\left.J_{11^{\prime \prime}, 12^{\prime \prime}}=8.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-11^{\prime \prime}\right)$, $7.64(\mathrm{~s}$, $\left.1 \mathrm{H}, \mathrm{H}-9^{\prime \prime}\right), 7.58\left(\mathrm{~d}, J_{16^{\prime \prime}, 17^{\prime \prime}}=8.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-16^{\prime \prime}\right), 7.51\left(\mathrm{~d}, J_{11,12}=8.1\right.$ $\mathrm{Hz}, 1 \mathrm{H}, \mathrm{H}-11), 7.51$ (s, 1H, H-9'), 7.47 (s, 1H, H-9), 7.46 (s, 1H, H-
14), $7.44\left(\mathrm{~d}, J_{11^{\prime}, 12^{\prime}}=8.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-11^{\prime}\right), 7.39\left(\mathrm{~d}, J_{16,17}=8.4 \mathrm{~Hz}, 1 \mathrm{H}\right.$, $\mathrm{H}-16$ ), 7.36 ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{H}-17^{\prime \prime}$ ), 7.35 (m, 1H, H-12"), 7.34-7.36 (m, $2 \mathrm{H}, 2 \times \mathrm{H}-\mathrm{Ph}), 7.21-7.29(\mathrm{~m}, 15 \mathrm{H}, 15 \times \mathrm{H}-\mathrm{Ph}), 7.16-7.19(\mathrm{~m}, 4 \mathrm{H}$, $4 \times \mathrm{H}-\mathrm{Ph}), 7.16(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-12), 7.11\left(\mathrm{~d}, 1 \mathrm{H}, \mathrm{H}-12^{\prime}\right), 7.03(\mathrm{~d}, 1 \mathrm{H}, \mathrm{H}-$ 17), $7.00-7.02$ (m, $4 \mathrm{H}, 4 \times \mathrm{H}-\mathrm{Ph}), 6.99$ (s, $\left.1 \mathrm{H}, \mathrm{H}-25^{\prime \prime}\right), 6.95$ ( $\mathrm{s}, 1 \mathrm{H}$, $\left.\mathrm{H}-25^{\prime}\right), 6.94\left(\mathrm{~s}, 1 \mathrm{H}, \mathrm{H}-14^{\prime}\right), 6.88\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-17^{\prime}\right), 6.86(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-$ 25), $6.68\left(\mathrm{~d}, J_{16,17}=7.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-16^{\prime}\right), 6.67\left(\mathrm{~s}, 1 \mathrm{H}, \mathrm{H}-21^{\prime}\right), 6.64(\mathrm{~s}$, $1 \mathrm{H}, \mathrm{H}-21), 6.61\left(\mathrm{~s}, 1 \mathrm{H}, \mathrm{H}-21^{\prime \prime}\right), 5.36\left(\mathrm{~d}, J_{18^{\prime \prime} \mathrm{a}, 18^{\prime \prime} \mathrm{b}}=13.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-\right.$ $18^{\prime \prime}$ a), 5.26 ( $\mathrm{d}, J_{18^{\prime \prime}, 18^{\prime \prime} \mathrm{b}}=12.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-18^{\prime} \mathrm{a}$ ), 5.24 ( $\mathrm{d}, J_{18 \mathrm{a}, 18 \mathrm{~b}}=10.8$ $\mathrm{Hz}, 1 \mathrm{H}, \mathrm{H}-18 \mathrm{a}), 5.19$ (d, $\left.J_{1,2}=3.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-1\right), 5.16(\mathrm{~d}, 1 \mathrm{H}, \mathrm{H}-$ $\left.18^{\prime \prime} \mathrm{b}\right), 5.15$ (d, $\left.1 \mathrm{H}, \mathrm{H}-18^{\prime} \mathrm{b}\right), 4.81$ (d, $\left.1 \mathrm{H}, \mathrm{H}-18 \mathrm{~b}\right), 4.76$ (d, $J_{7^{\prime \prime} \mathrm{a}, 7^{\prime \prime} \mathrm{b}}=$ $\left.12.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-7^{\prime \prime} \mathrm{a}\right), 4.76\left(\mathrm{~d}, J=11.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}^{\prime}-\mathrm{OCH}_{2} \mathrm{Ph}\right), 4.70$ (m, 3H, H-23a, H-23'a, C4-OCH $2_{2} \mathrm{Ph}$ ), 4.68 (m, 2H, H-23" a, C3$\left.\mathrm{OC} \underline{H}_{2} \mathrm{Ph}\right), 4.63\left(\mathrm{~d}, J=11.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}^{\prime}-\mathrm{OCH}_{2} \mathrm{Ph}\right), 4.49(\mathrm{~m}, 1 \mathrm{H}$, $\left.\mathrm{C} 3-\mathrm{OC} \underline{H}_{2} \mathrm{Ph}\right), 4.48\left(\mathrm{~m}, 3 \mathrm{H}, 2 \times \mathrm{C} 2-\mathrm{OCH}_{2} \mathrm{Ph}, \mathrm{C}^{\prime}-\mathrm{OC} \underline{H}_{2} \mathrm{Ph}\right) 4.46$ $\left(\mathrm{m}, 1 \mathrm{H}, \mathrm{H}^{\prime} 7^{\prime} \mathrm{a}\right), 4.45(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-7 \mathrm{a}), 4.41\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C} 4-\mathrm{OCH}_{2} \mathrm{Ph}\right)$, $4.40\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{C} 4^{\prime}-\mathrm{OC}_{2} \mathrm{Ph}\right), 4.38\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-3^{\prime}\right), 4.30\left(\mathrm{~d}, J_{7^{\prime} \mathrm{b}, 7^{\prime} \mathrm{a}}=\right.$ $13.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-7^{\prime} \mathrm{b}$ ), 4.27 (d, 1H, H-7"b), 4.15 (d, $J_{7 \mathrm{~b}, 7 \mathrm{a}}=12.4 \mathrm{~Hz}$, $1 \mathrm{H}, \mathrm{H}-7 \mathrm{~b}), 4.07\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-5^{\prime}\right), 3.83\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-4^{\prime}\right), 3.81(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-$ 5), $3.79(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-3), 3.65\left(\mathrm{~d}, J_{1^{\prime} \mathrm{a}, 1^{\prime} \mathrm{b}}=11.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-1^{\prime} \mathrm{a}\right), 3.56(\mathrm{~m}$, $1 \mathrm{H}, \mathrm{H}-4), 3.55\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{H}-26^{\prime}\right), 3.54\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-6^{\prime} \mathrm{a}\right), 3.53(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-$ 6a), 3.51 ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{H}-23 \mathrm{~b}$ ), 3.48 ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{H}-23^{\prime \prime} \mathrm{b}$ ), 3.47 ( $\mathrm{m}, 1 \mathrm{H}, \mathrm{H}-$ $23^{\prime} \mathrm{b}$ ), 3.45 (d, 1H, H-1'b), 3.40 ( s, 3H, H-26), 3.36 (dd, J $\mathrm{J}^{\prime} \mathrm{b}, 5^{\prime}=8.00$ $\left.\mathrm{Hz}, J_{6^{\prime} \mathrm{b}, 6^{\prime} \mathrm{a}}=11.9 \mathrm{~Hz} 1 \mathrm{H}, \mathrm{H}-6^{\prime} \mathrm{b}\right), 3.30\left(\mathrm{dd}, J_{2,3}=9.7 \mathrm{~Hz}, J_{2,1}=3.4 \mathrm{~Hz}\right.$, $1 \mathrm{H}, \mathrm{H}-2), 3.21\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{H}-26^{\prime \prime}\right), 3.17$ (dd, $J_{6 \mathrm{~b}, 5}=1.4 \mathrm{~Hz}, J_{6 \mathrm{~b}, 6 \mathrm{a}}=10.8$ $\mathrm{Hz}, 1 \mathrm{H}, \mathrm{H}-6 \mathrm{~b}$ ) ppm. ${ }^{13} \mathrm{C}\left\{{ }^{1} \mathrm{H}\right\}$ NMR $\left(150 \mathrm{MHz}, \mathrm{CDCl}_{3}\right): \delta=149.1$ (C-20'), 148.8 (C-20"), 148.5 (C-20), 147.0 (C-19"), 146.1 (C-19), 145.9 (C-19'), 138.8, 138.6, 138.3, 138.2, $138.2\left(\mathrm{C}_{\text {quat }} 5 \times \mathrm{C}-\mathrm{Ph}\right)$, 136.9 (C-8'), 135.9 (C-8), 135.8 (C-8"), 135.3 (C-13"), 134.2 (C13), 133.7 (C-22'), 133.3 (C-13'), 133.3 (C-22"), 132.8 (C-10/C15), 132.7 (C-22), 132.6 (C-15"), 132.6 (C-10"), 132.6 (C-10'), 132.5 (C-15'), 132.0 (C-24"), 132.0 (C-10/C-15), 131.7 (C-24'), 131.6 (C-24), 128.1 (C-11"), 127.9 (C-16"), 127.8 (C-11), 127.7 (C$\left.16^{\prime}\right)$, 127.7 (C-16), 127.2-128.3 (m, $25 \times \mathrm{C}-\mathrm{Ph}$ ), 126.8 (C-14'), 126.3 (C-14), 126.3 (C-17"), 126.2 (C-9"), 126.2 (C-17), 126.1 (C9), 126.1 (C-11'), 125.6 (C-17'), 125.6 (C-12'), 125.4 (C-14"), 125.4 (C-12), 124.8 (C-12" $), 124.5$ (C-9'), 118.6 (C-25'), 117.6 (C$\left.25^{\prime \prime}\right), 116.3$ (C-25), 113.4 (C-21"), 113.4 (C-21'), 113.0 (C-21), 104.1 (C-2'), 90.7 (C-1), 84.1 (C-3'), 82.4 (C-4'), 81.6 (C-3), 80.9 (C-5'), 79.9 (C-2), 77.5 (C-4), $75.4\left(\mathrm{C} 3-\mathrm{OCH}_{2} \mathrm{Ph}\right), 74.7$ (C4$\left.\mathrm{OCH}_{2} \mathrm{Ph}\right), 73.1(\mathrm{C}-7), 73.0\left(\mathrm{C}-7^{\prime \prime}\right), 73.0\left(\mathrm{C}-6^{\prime}\right), 72.9$ ( $\mathrm{C}^{\prime}-$ $\left.\mathrm{OCH}_{2} \mathrm{Ph}\right), 72.7\left(\mathrm{C}-18^{\prime \prime}\right), 72.6\left(\mathrm{C}-18^{\prime}\right), 72.5\left(\mathrm{C} 2-\mathrm{O} \underline{C H}_{2} \mathrm{Ph}\right), 72.5$ ( $\left.\mathrm{C}-7^{\prime}\right), 72.4\left(\mathrm{C} 4^{\prime}-\mathrm{OCH}_{2} \mathrm{Ph}\right), 71.4(\mathrm{C}-18), 70.6(\mathrm{C}-5), 69.9\left(\mathrm{C}-1^{\prime}\right)$, 68.1 (C-6), 55.9 (C-26'), 55.5 (C-26), 55.5 (C-26"), 36.4 (C-23), 36.4 (C-23'), 36.2 (C-23") ppm. HRMS (ESI-TOF) m/z: $[\mathrm{M}+\mathrm{Na}]^{+}$ calcd for $\mathrm{C}_{107} \mathrm{H}_{100} \mathrm{O}_{17} \mathrm{Na}$ 1679.6858; found 1679.6844. Anal. calcd for $\mathrm{C}_{107} \mathrm{H}_{100} \mathrm{O}_{17}+\mathrm{H}_{2} \mathrm{O}: \mathrm{C}, 76.68 ; \mathrm{H}, 6.13$; found: C, $76.55 ; \mathrm{H}, 6.25$.

Characterization of Compound P-5b.

$[\alpha]_{D}^{25}+103.2\left(c 0.22, \mathrm{CH}_{2} \mathrm{Cl}_{2}\right) \cdot{ }^{1} \mathrm{H} \operatorname{NMR}\left(600 \mathrm{MHz}, \mathrm{CDCl}_{3}\right): \delta=$ 7.65 (d, $\left.J_{11,12}=8.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-11\right), 7.62(\mathrm{~s}, 1 \mathrm{H}, \mathrm{H}-9), 7.57(\mathrm{~s}, 1 \mathrm{H}, \mathrm{H}-$ 14), 7.56 (s, 1H, H-14"), 7.55 (m, 2H, H-11', H-16), 7.52 (d, $J_{16^{\prime \prime}, 17^{\prime \prime}}$ $\left.=8.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-16^{\prime \prime}\right), 7.49\left(\mathrm{~s}, 1 \mathrm{H}, \mathrm{H}-9^{\prime \prime}\right), 7.47\left(\mathrm{~s}, 1 \mathrm{H}, \mathrm{H}-9^{\prime}\right), 7.38$ (m, 1H, H-17), 7.32 (m, 1H, H-12'), 7.27 (m, 1H, H-12), 7.26-7.40
$(\mathrm{m}, 16 \mathrm{H}, 16 \times \mathrm{H}-\mathrm{Ph}), 7.23\left(\mathrm{~d}, J_{11^{\prime \prime}, 12^{\prime \prime}}=8.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-11^{\prime \prime}\right), 7.09(\mathrm{~s}$, $1 \mathrm{H}, \mathrm{H}-25^{\prime}$ ), 7.07 ( $\mathrm{s}, 1 \mathrm{H}, \mathrm{H}-25^{\prime \prime}$ ), 7.06 ( $\mathrm{s}, 1 \mathrm{H}, \mathrm{H}-14^{\prime}$ ), $7.02-7.05$ (m, $\left.5 \mathrm{H}, \mathrm{H}-17^{\prime \prime}, 4 \times \mathrm{H}-\mathrm{Ph}\right), 6.91\left(\mathrm{~d}, J_{17^{\prime}, 16^{\prime}}=8.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-17^{\prime}\right), 6.88(\mathrm{~s}$, $1 \mathrm{H}, \mathrm{H}-25), 6.87\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-12^{\prime \prime}\right), 6.86\left(\mathrm{~s}, 1 \mathrm{H}, \mathrm{H}-21^{\prime \prime}\right), 6.81(\mathrm{~s}, 1 \mathrm{H}, \mathrm{H}-$ $\left.21^{\prime}\right), 6.79\left(\mathrm{~d}, J_{16^{\prime}, 17^{\prime}}=8.4 \mathrm{~Hz} 1 \mathrm{H}, \mathrm{H}-16^{\prime}\right), 6.70(\mathrm{~s}, 1 \mathrm{H}, \mathrm{H}-21), 6.58(\mathrm{t}$, $J=7.6 \mathrm{~Hz}, 2 \mathrm{H}, 2 \times \mathrm{H}-\mathrm{Ph}), 6.41(\mathrm{~d}, J=7.4 \mathrm{~Hz}, 2 \mathrm{H}, 2 \times \mathrm{H}-\mathrm{Ph}), 6.30$ $(\mathrm{t}, J=7.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-\mathrm{Ph}), 5.55\left(\mathrm{~d}, J_{1,2}=3.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-1\right), 5.46$ (d, $\left.J_{18^{\prime}, 18^{\prime} \mathrm{b}}=13.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-18^{\prime} \mathrm{a}\right), 5.35\left(\mathrm{~d}, 1 \mathrm{H}, \mathrm{H}-18^{\prime} \mathrm{b}\right), 5.27$ (d, $\left.J_{18^{\prime \prime}, 18^{\prime \prime} \mathrm{b}}=12.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-18^{\prime \prime} \mathrm{a}\right), 5.09\left(\mathrm{~d}, J_{18 \mathrm{a}, 18 \mathrm{~b}}=11.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-\right.$ 18a), 4.94 (d, $\left.1 \mathrm{H}, \mathrm{H}-18^{\prime \prime} \mathrm{b}\right), 4.93\left(\mathrm{~d}, J=11.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C} 3-\mathrm{OC} \underline{H}_{2} \mathrm{Ph}\right)$, 4.89 (d, $\left.J=11.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C} 4-\mathrm{OCH}_{2} \mathrm{Ph}\right), 4.87(\mathrm{~d}, 1 \mathrm{H}, \mathrm{H}-18 \mathrm{~b}), 4.79$ (m, 1H, C3-OCH ${ }_{2} \mathrm{Ph}$ ), $4.78(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-7 \mathrm{a}), 4.77(\mathrm{~m}, 3 \mathrm{H}, \mathrm{H}-23 \mathrm{a}, \mathrm{H}-$ $23^{\prime} \mathrm{a}, \mathrm{H}-23^{\prime \prime} \mathrm{a}$ ), 4.74 (d, $\left.J=12.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C} 2-\mathrm{OCH}_{2} \mathrm{Ph}\right), 4.67(\mathrm{~d}, J=$ $\left.11.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C} 2-\mathrm{OC} \underline{H}_{2} \mathrm{Ph}\right), 4.66$ ( $\mathrm{m}, 2 \mathrm{H}, \mathrm{H}-7^{\prime \prime} \mathrm{a}, \mathrm{C} 4-\mathrm{OC} \underline{H}_{2} \mathrm{Ph}$ ), $4.58\left(\mathrm{~d}, J_{7^{\prime \prime}, 7^{\prime \prime} \mathrm{a}}=12.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-7^{\prime \prime} \mathrm{b}\right), 4.46\left(\mathrm{~d}, J=11.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C} 3^{\prime}-\right.$ $\left.\mathrm{OCH}_{2} \mathrm{Ph}\right), 4.24\left(\mathrm{~d}, J_{3^{\prime}, 4^{\prime}}=8.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3^{\prime}\right), 4.23\left(\mathrm{~d}, J_{7 \mathrm{~b}, 7 \mathrm{a}}=11.9 \mathrm{~Hz}\right.$, $1 \mathrm{H}, \mathrm{H}-7 \mathrm{~b}), 4.21\left(\mathrm{~d}, J_{7^{\prime} \mathrm{a}, 7^{\prime} \mathrm{b}}=12.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-7^{\prime} \mathrm{a}\right), 4.19(\mathrm{~d}, J=11.4$ $\left.\mathrm{Hz}, 1 \mathrm{H}, \mathrm{C}^{\prime}-\mathrm{OCH}_{2} \mathrm{Ph}\right), 4.13(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-5), 3.95\left(\mathrm{~d}, 1 \mathrm{H}, \mathrm{H}-7^{\prime} \mathrm{b}\right)$, 3.92 (dd, $\left.J_{3,2}=9.1 \mathrm{~Hz}, J_{3,4}=9.3,1 \mathrm{H}, \mathrm{H}-3\right), 3.73\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{H}-26^{\prime \prime}\right), 3.71$ $\left(\mathrm{d}, J_{1^{\prime}, 1^{\prime} \mathrm{b}}=11.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-1^{\prime} \mathrm{a}\right), 3.70\left(\mathrm{~d}, J=11.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C} 4^{\prime}-\right.$ $\left.\mathrm{OC} \underline{H}_{2} \mathrm{Ph}\right) 3.61(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-2), 3.60(\mathrm{~s}, 3 \mathrm{H}, \mathrm{H}-26), 3.59(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-$ $\left.23^{\prime \prime} \mathrm{b}\right), 3.58\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-\mathrm{1}^{\prime} \mathrm{b}\right), 3.57\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{H}-4, \mathrm{H}-23^{\prime} \mathrm{b}\right), 3.55(\mathrm{~m}, 1 \mathrm{H}$, H-23b), 3.53 (m, 1H, H-4'), 3.51 (d, J $=10.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C} 4^{\prime}-$ $\mathrm{OC} \underline{H}_{2} \mathrm{Ph}$ ), $3.44\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{H}-26^{\prime}\right), 3.37\left(\mathrm{dd}, J_{6^{\prime} \mathrm{a}, 6^{\prime} \mathrm{b}}=10.9 \mathrm{~Hz}, J_{6^{\prime} \mathrm{a}, 5^{\prime}}=\right.$ $\left.11.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}^{\prime} 6^{\prime} \mathrm{a}\right), 3.32\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-5^{\prime}\right), 3.31(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-6 \mathrm{a}), 3.26$ $\left(\mathrm{dd}, J_{6 \mathrm{~b}, 5}=4.0 \mathrm{~Hz}, J_{6 \mathrm{~b}, 6 \mathrm{a}}=10.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-6 \mathrm{~b}\right), 2.84\left(\mathrm{dd}, J_{6^{\prime}, 5^{\prime}}=1.9\right.$ $\mathrm{Hz}, 1 \mathrm{H}, \mathrm{H}-6^{\prime} \mathrm{b}$ ) ppm. ${ }^{13} \mathrm{C}\left\{{ }^{1} \mathrm{H}\right\}$ NMR ( $150 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ): $\delta=149.7$ (C-20"), 149.1 (C-20'), 148.4 (C-20), 147.6 (C-19') 147.3 (C-19), 145.3 (C-19"), 139.0, 138.8, 138.4, 137.8, $137.8\left(\mathrm{C}_{\text {quat }} 5 \times \mathrm{C}-\mathrm{Ph}\right)$, 136.5 (C-8"), 136.1 (C-8'), 135.3 (C-8), 135.1 (C-13), 134.7 (C$13^{\prime \prime}$ ), 134.0 (C-22"), 133.6 (C-22'), 133.5 (C-13'), 132.8 (C-22), 132.8 (C-10" $/ \mathrm{C}-15^{\prime \prime}$ ), 132.8 (C-15), 132.6 (C-10), 132.5 (C-24'), 132.4 (C-10'), 132.3 (C-10" $/ \mathrm{C}-15^{\prime \prime}$ ), 132.3 (C-24"), 132.2 (C-15'), 131.6 (C-24), 128.2 (C-16), 127.8 (C-11), 127.7 (C-16"), 127.6 (C$\left.16^{\prime}, \mathrm{C}-11^{\prime}\right), 127.4-128.4$ (m, $\left.22 \times \mathrm{C}-\mathrm{Ph}\right), 127.0(\mathrm{C}-9), 126.8,126.7$ $(3 \times \mathrm{C}-\mathrm{Ph}), 126.5(\mathrm{C}-17), 126.3$ (C-14", C-14'), 126.3 (C-17'), 126.0 (C-9'), 125.9 (C-14), 125.8 (C-12'), 125.5 (C-9"), 125.4 (C$\left.12^{\prime \prime}, \mathrm{C}-17^{\prime \prime}\right)$, 125.3 (C-11", C-12), 120.0 (C-25"), 118.5 (C-25'), 116.4 (C-25), 114.4 (C-21'), 113.9 (C-21"), 113.5 (C-21), 104.0 (C$\left.2^{\prime}\right), 88.7$ (C-1), 82.5 (C-3'), 82.3 (C-3), 81.3 (C-4'), 79.3 (C-2), 79.2 (C-5'), 78.0 (C-4), 75.4 (C3-OCH ${ }_{2} \mathrm{Ph}$ ), 74.8 (C-1'), 74.7 (C4$\mathrm{OCH}_{2} \mathrm{Ph}$ ), 74.4 ( $\mathrm{C}-7^{\prime \prime}$ ), 73.9 ( $\mathrm{C}-18^{\prime \prime}$ ), $73.0(\mathrm{C}-7)$, 72.6 (C-7'), 72.3 $\left(\mathrm{C}-18^{\prime}\right), 72.2\left(\mathrm{C} 2-\mathrm{OCH}_{2} \mathrm{Ph}\right), 72.2\left(\mathrm{C}^{\prime}-\mathrm{O}_{\mathrm{C}} \mathrm{H}_{2} \mathrm{Ph}\right), 71.4\left(\mathrm{C}^{\prime}-\right.$ $\left.\mathrm{OCH}_{2} \mathrm{Ph}\right), 71.3$ (C-18), $70.9\left(\mathrm{C}-6^{\prime}\right), 69.9(\mathrm{C}-5), 67.6(\mathrm{C}-6), 56.3$ (C$26^{\prime \prime}$ ), 56.2 (C-26'), 56.0 (C-26), 36.6 (C-23'), 36.5 (C-23"), 36.3 (C23) ppm. HRMS (ESI-TOF) $m / z:[\mathrm{M}+\mathrm{Na}]^{+}$calcd for $\mathrm{C}_{107} \mathrm{H}_{100} \mathrm{O}_{17} \mathrm{Na}$ 1679.6858; found 1679.6836. Anal. calcd for $\mathrm{C}_{107} \mathrm{H}_{100} \mathrm{O}_{17}+\mathrm{H}_{2} \mathrm{O}: \mathrm{C}, 76.68 ; \mathrm{H}, 6.13$; found: C, $76.23 ; \mathrm{H}, 6.25$.

Characterization of Compound $\mathrm{M}-5 \mathrm{a}$.

$[\alpha]_{D}^{25}-90.5\left(c 0.22, \mathrm{CH}_{2} \mathrm{Cl}_{2}\right) .{ }^{1} \mathrm{H} \operatorname{NMR}\left(600 \mathrm{MHz}, \mathrm{CDCl}_{3}\right): \delta=$ $7.83\left(\mathrm{~s}, 1 \mathrm{H}, \mathrm{H}-9^{\prime \prime}\right), 7.79\left(\mathrm{~d}, J_{16^{\prime \prime}, 17^{\prime \prime}}=8.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-16^{\prime \prime}\right), 7.76$ (d, $\left.J_{11^{\prime \prime}, 12^{\prime \prime}}=8.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-11^{\prime \prime}\right), 7.73$ (s, 1H, H-14"), 7.48 (d, 1H, H-
$17^{\prime \prime}$ ), 7.45 (s, 1H, H-14), 7.44 (d, 1H, H-11'), 7.40 (s, 1H, H-9'), $7.38\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-12^{\prime \prime}\right), 7.36(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-9), 7.30(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-11), 7.29-$ $7.40(\mathrm{~m}, 5 \mathrm{H}, 5 \times \mathrm{H}-\mathrm{Ph}), 7.23(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-16), 7.21(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-12)$, $7.17\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-12^{\prime}\right), 7.16-7.27(\mathrm{~m}, 13 \mathrm{H}, 13 \times \mathrm{H}-\mathrm{Ph}), 7.12(\mathrm{~s}, 1 \mathrm{H}$, H-25'), 7.09 ( $\mathrm{s}, 1 \mathrm{H}, \mathrm{H}-25$ ), 7.02 (m, 1H, H-17), 7.01 (m, 1H, H-17'), $6.99(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-21), 6.98\left(\mathrm{~d}, J_{16^{\prime}, 17^{\prime}}=8.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-16^{\prime}\right), 6.98-7.04$ (m, 2H, $2 \times \mathrm{H}-\mathrm{Ph}$ ), 6.93 ( $\mathrm{s}, 1 \mathrm{H}, \mathrm{H}-21^{\prime \prime}$ ), 6.84 ( $\mathrm{s}, 1 \mathrm{H}, \mathrm{H}-14^{\prime}$ ), 6.76 ( s , $\left.1 \mathrm{H}, \mathrm{H}-25^{\prime \prime}\right), 6.64\left(\mathrm{~s}, 1 \mathrm{H}, \mathrm{H}-21^{\prime}\right), 6.50(\mathrm{~d}, J=7.5 \mathrm{~Hz}, 2 \mathrm{H}, 2 \times \mathrm{H}-\mathrm{Ph})$, $6.39(\mathrm{t}, J=7.6 \mathrm{~Hz}, 2 \mathrm{H}, 2 \times \mathrm{H}-\mathrm{Ph}), 5.92(\mathrm{t}, J=7.3 \mathrm{~Hz}, 1 \mathrm{H}, 1 \times \mathrm{H}-\mathrm{Ph})$, $5.60\left(\mathrm{~d}, J_{1,2}=3.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-1\right), 5.40\left(\mathrm{~d}, J_{18 \mathrm{a}, 18 \mathrm{~b}}=13.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-\right.$ 18a), 5.31 (d, 1H, H-18b), 5.21 (d, $J_{18^{\prime \prime}, 18^{\prime \prime} \mathrm{b}}=11.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-18^{\prime \prime} \mathrm{a}$ ), $5.20\left(\mathrm{~d}, J_{18^{\prime} \mathrm{a}, 18^{\prime} \mathrm{b}}=11.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-18^{\prime} \mathrm{a}\right), 5.07\left(\mathrm{~d}, J_{7^{\prime \prime} \mathrm{a}, 7^{\prime \prime} \mathrm{b}}=12.5 \mathrm{~Hz}\right.$, $1 \mathrm{H}, \mathrm{H}-7{ }^{\prime \prime} \mathrm{a}$ ), 4.97 (d, 1H, H-18"b), 4.79 (m, 1H, H-23a), 4.76 (m, $\left.2 \mathrm{H}, 2 \times \mathrm{C}^{\prime}-\mathrm{OCH}_{2} \mathrm{Ph}\right), 4.74\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{H}-23^{\prime} \mathrm{a}, \mathrm{H}-23^{\prime \prime} \mathrm{a}\right), 4.64$ (d, $J_{7 \mathrm{a}, 7 \mathrm{~b}}$ $=12.7 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-7 \mathrm{a}), 4.60\left(\mathrm{~d}, \mathrm{~J}=11.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C} 2-\mathrm{OC} \underline{H}_{2} \mathrm{Ph}\right), 4.55$ (d, $\left.1 \mathrm{H}, \mathrm{C} 2-\mathrm{OC} \underline{H}_{2} \mathrm{Ph}\right), 4.54\left(\mathrm{~d}, J=10.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C} 3-\mathrm{OC} \underline{H}_{2} \mathrm{Ph}\right), 4.50$ (d, $\left.J=10.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C} 4-\mathrm{OC} \underline{H}_{2} \mathrm{Ph}\right), 4.50\left(\mathrm{~d}, 1 \mathrm{H}, \mathrm{H}-7^{\prime \prime} \mathrm{b}\right), 4.42(\mathrm{~d}, J=$ $11.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C} 4^{\prime}-\mathrm{OCH}_{2} \mathrm{Ph}$ ), 4.40 (d, $\left.1 \mathrm{H}, \mathrm{H}-18^{\prime} \mathrm{b}\right), 4.37$ (d, 1 H , $\left.\mathrm{C} 4^{\prime}-\mathrm{OC} \underline{H}_{2} \mathrm{Ph}\right), 4.35\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-3^{\prime}\right), 4.34\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{H}-7^{\prime} \mathrm{a}, \mathrm{H}-7^{\prime} \mathrm{b}\right)$, 4.15 (d, 1H, C3-OC $\underline{H}_{2} \mathrm{Ph}$ ), 4.04 (m, 2H, H-7b, C4-OCH ${ }_{2} \mathrm{Ph}$ ), 4.03 (m, 1H, H-4'), $3.92\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-5^{\prime}\right), 3.92$ ( $\left.\mathrm{s}, 3 \mathrm{H}, \mathrm{H}-26\right), 3.90(\mathrm{~m}, 1 \mathrm{H}$, H-5), 3.72 (m, 2H, H-1'a, H-3), 3.66 (d, $J_{1^{\prime} b, 1^{\prime}{ }^{\prime}}=12.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-$ $\left.1^{\prime} \mathrm{b}\right), 3.61\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{H}-23 \mathrm{~b}, \mathrm{H}-23^{\prime} \mathrm{b}\right), 3.51$ (d, $J_{23^{\prime \prime} \mathrm{b}, 23^{\prime \prime} \mathrm{a}}=13.5 \mathrm{~Hz}, 1 \mathrm{H}$, H-23"b), 3.49 (m, 1H, H-6'a), 3.48 ( s, 3H, H-26"), 3.46 (m, 1H, H$6^{\prime} \mathrm{b}$ ), 3.41 ( m, 1H, H-4), 3.39 (m, 1H, H-6a), 3.38 (m, 1H, H-2), 3.22 (s, 3H, H-26'), 3.06 (dd, $\left.J_{6 \mathrm{~b}, 5}=1.2 \mathrm{~Hz}, J_{6 \mathrm{~b}, 6 \mathrm{a}}=10.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-6 \mathrm{~b}\right)$ ppm. ${ }^{13} \mathrm{C}\left\{{ }^{1} \mathrm{H}\right\}$ NMR ( $\left.150 \mathrm{MHz}, \mathrm{CDCl}_{3}\right): \delta=150.3\left(\mathrm{C}-20^{\prime}\right), 148.6$ (C-20), 148.0 (C-20"), 147.3 (C-19"), 146.8 (C-19'), 145.8 (C-19), 138.7, 138.6, 138.1, 138.0, $137.8\left(\mathrm{C}_{\text {quat }} 5 \times \mathrm{C}-\mathrm{Ph}\right), 136.3\left(\mathrm{C}-8^{\prime}\right)$, 135.9 (C-8"), 135.4 (C-8), 134.9 (C-13"), 134.7 (C-22'), 134.3 (C13), 133.6 (C-13'), 133.2 (C-22), 132.8, 132.8, 132.6, 132.5, 132.5 ( $\mathrm{C}_{\text {quat }} 5 \times$ C-Naphth $) 132.4$ (C-24"), 132.1 ( $\mathrm{C}_{\text {quat }}$ C-Naphth), 132.1 (C-24'), 131.9 (C-24), 131.8 (C-22"), 128.4 (C-11"), 128.2 (C-14'), 128.0 (C-16"), 128.0 (C-16), 127.9 (C-16'), 127.8 (C-11), 127.4 (C$\left.9^{\prime \prime}\right), 127.3-128.3(\mathrm{~m}, 25 \times \mathrm{C}-\mathrm{Ph}), 127.2\left(\mathrm{C}-17^{\prime \prime}\right), 127.2$ (C-11'), 126.7 (C-9), 126.6 (C-14), 126.6 (C-12'), 126.3 (C-17), 126.3 (C$\left.14^{\prime \prime}\right), 125.9$ (C-12), 125.5 (C-12"), 125.0 (C-17'), 124.4 (C-9'), 121.9 (C-25'), 116.7 (C-25), 115.0 (C-21"), 113.8 (C-21), 113.5 (C$\left.21^{\prime}\right), 113.3$ (C-25"), 104.6 (C-2'), 91.4 (C-1), 84.9 (C-3'), 83.1 (C$\left.4^{\prime}\right), 81.4$ (C-3), 80.8 (C-5'), 79.7 (C-2), 77.7 (C-4), 76.2 (C-18'), $75.1\left(\mathrm{C} 3-\mathrm{OCH}_{2} \mathrm{Ph}\right), 74.3\left(\mathrm{C} 4-\mathrm{O} \underline{C H}_{2} \mathrm{Ph}\right), 73.9\left(\mathrm{C}-7^{\prime \prime}\right), 73.9(\mathrm{C}-7)$, $73.3\left(\mathrm{C}^{\prime}-\mathrm{OC}_{2} \mathrm{Ph}\right), 73.1\left(\mathrm{C}-6^{\prime}\right), 72.7\left(\mathrm{C}^{\prime}-\mathrm{OCH}_{2} \mathrm{Ph}\right), 72.5(\mathrm{C} 2-$ $\left.\mathrm{O}_{\mathrm{C}}^{\mathrm{H}} \mathrm{H}_{2} \mathrm{Ph}\right), 72.3$ (C-7'), 71.3 (C-18"), 70.4 (C-5), 70.2 (C-18), 69.1 (C-1'), 67.9 (C-6), 56.3 (C-26), 55.7 (C-26"), 55.5 (C-26'), 36.5 (C23'), 36.4 (C-23"), 36.2 (C-23) ppm. HRMS (ESI-TOF) m/z: [M + $\mathrm{Na}]^{+}$calcd for $\mathrm{C}_{107} \mathrm{H}_{100} \mathrm{O}_{17} \mathrm{Na} 1679.6858$; found 1679.6866. Anal. calcd for $\mathrm{C}_{107} \mathrm{H}_{100} \mathrm{O}_{17}+\mathrm{H}_{2} \mathrm{O}: \mathrm{C}, 76.68$; $\mathrm{H}, 6.13$; found: $\mathrm{C}, 76.40 ; \mathrm{H}$, 6.28 .

Characterization of Compound M-5b.

$[\alpha]_{D}^{25}-82.1\left(c 0.21, \mathrm{CH}_{2} \mathrm{Cl}_{2}\right) .{ }^{1} \mathrm{H} \operatorname{NMR}\left(600 \mathrm{MHz}, \mathrm{CDCl}_{3}\right): \delta=$ $7.78(\mathrm{~s}, 1 \mathrm{H}, \mathrm{H}-9), 7.70\left(\mathrm{~s}, 1 \mathrm{H}, \mathrm{H}-14^{\prime \prime}\right), 7.69\left(\mathrm{~d}, J_{16,17}=8.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-\right.$ 16), 7.67 (s, 1H, H-11), 7.62 ( s, 1H, H-9"), 7.58 (s, 1H, H-14), 7.52
(d, 1H, H-17), 7.49 (d, $\left.J_{11^{\prime \prime}, 12^{\prime \prime}}=8.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-11^{\prime \prime}\right), 7.49\left(\mathrm{~d}, J_{16^{\prime \prime}, 17^{\prime \prime}}=\right.$ $\left.8.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-16^{\prime \prime}\right), 7.34$ ( $\mathrm{s}, 1 \mathrm{H}, \mathrm{H}-25^{\prime \prime}$ ), 7.32 (m, 1H, H-12"), 7.247.42 (m, 20H, $20 \times \mathrm{H}-\mathrm{Ph}$ ), 7.21 (d, 1H, H-17"), 7.19 (s, 1H, H-21'), 7.18 (d, 1H, H-11'), 7.16 (s, 1H, H-9'), $7.14(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-12), 7.13$ ( s , $\left.1 \mathrm{H}, \mathrm{H}-25^{\prime}\right), 7.12$ (d, $\left.J_{12^{\prime}, 11^{\prime}}=8.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-12^{\prime}\right), 7.04-7.09$ (m, 4H, $4 \times \mathrm{H}-\mathrm{Ph}), 6.96(\mathrm{t}, J=6.9 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-\mathrm{Ph}), 6.55\left(\mathrm{~s}, 1 \mathrm{H}, \mathrm{H}-14^{\prime}\right), 6.54$ ( s, 1H, H-21), 6.49 (d, $\left.J_{17^{\prime}, 16^{\prime}}=8.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-17^{\prime}\right), 6.42(\mathrm{~s}, 1 \mathrm{H}, \mathrm{H}-$ 25), 6.33 ( $\left.\mathrm{s}, 1 \mathrm{H}, \mathrm{H}-21^{\prime \prime}\right), 6.14$ (d, 1H, H-16'), 5.64 (d, $J_{18^{\prime \prime} \mathrm{a}, 18^{\prime \prime} \mathrm{b}}=14.7$ $\left.\mathrm{Hz}, 1 \mathrm{H}, \mathrm{H}-18^{\prime \prime} \mathrm{a}\right), 5.54$ (d, $\left.J_{1,2}=3.8 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-1\right), 5.45(\mathrm{~d}, 1 \mathrm{H}, \mathrm{H}-$ $\left.18^{\prime \prime} \mathrm{b}\right), 5.35\left(\mathrm{~d}, J_{18^{\prime} \mathrm{a}, 18^{\prime} \mathrm{b}}=11.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-18^{\prime} \mathrm{a}\right), 5.00\left(\mathrm{~d}, 1 \mathrm{H}, \mathrm{H}-18^{\prime} \mathrm{b}\right)$, $4.94(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-7 \mathrm{a}), 4.93\left(\mathrm{~m}, 2 \mathrm{H}, \mathrm{C} 3-\mathrm{OC} \underline{H}_{2} \mathrm{Ph}, \mathrm{C} 4-\mathrm{OC}_{2} \mathrm{Ph}\right), 4.87$ (d, $\left.J_{18 \mathrm{a}, 18 \mathrm{~b}}=9.3 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-18 \mathrm{a}\right), 4.83(\mathrm{~d}, J=11.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C} 3-$ $\left.\mathrm{OCH}_{2} \mathrm{Ph}\right), 4.79\left(\mathrm{~d}, J_{23^{\prime} \mathrm{a}, 23^{\prime} \mathrm{b}}=13.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-23^{\prime} \mathrm{a}\right), 4.77(\mathrm{~d}, J=11.2$ $\left.\mathrm{Hz}, 1 \mathrm{H}, \mathrm{C} 4-\mathrm{OCH}{ }_{2} \mathrm{Ph}\right), 4.74\left(\mathrm{~d}, J_{23 \mathrm{a}, 23 \mathrm{~b}}=13.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-23 \mathrm{a}\right), 4.71$ $\left(\mathrm{d}, 1 \mathrm{H}, \mathrm{H}-23^{\prime \prime} \mathrm{a}\right), 4.70\left(\mathrm{~d}, J=13.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C}^{\prime}-\mathrm{OCH}_{2} \mathrm{Ph}\right), 4.69(\mathrm{~m}$, $2 \mathrm{H}, \mathrm{C} 2-\mathrm{OCH}_{2} \mathrm{Ph}$ ), 4.67 (d, 1H, H-7"a), 4.59 (d, $J_{7^{\prime \prime} \mathrm{b}, 7^{\prime \prime} \mathrm{a}}=11.9 \mathrm{~Hz}$, $\left.1 \mathrm{H}, \mathrm{H}-7^{\prime \prime} \mathrm{b}\right), 4.45\left(\mathrm{~d}, \mathrm{~J}=11.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C} 3^{\prime}-\mathrm{OC} \underline{H}_{2} \mathrm{Ph}\right), 4.29\left(\mathrm{~d}, \mathrm{~J}_{3^{\prime}, 4^{\prime}}=\right.$ $\left.8.1 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-3^{\prime}\right), 4.23\left(\mathrm{~d}, J_{7 \mathrm{~b}, 7 \mathrm{a}}=12.0 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-7 \mathrm{~b}\right), 4.19(\mathrm{~m}, 1 \mathrm{H}$, $\mathrm{H}-5), 4.09\left(\mathrm{~d}, J=11.2 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{C} 4^{\prime}-\mathrm{OCH}_{2} \mathrm{Ph}\right), 4.03\left(\mathrm{~s}, 3 \mathrm{H}, \mathrm{H}-26^{\prime}\right)$, 3.96 (m, 1H, H-7'a), 3.94 (m, 1H, H-18b), 3.93 (m, 2H, H-3, C4' $\left.\mathrm{OC} \underline{H}_{2} \mathrm{Ph}\right), 3.80\left(\mathrm{~d}, J_{1^{\prime}, 1^{\prime} \mathrm{b}}=11.5 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-1^{\prime} \mathrm{a}\right), 3.76\left(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-5^{\prime}\right)$, 3.73 (m, 1H, H-7'b), $3.70(\mathrm{~m}, 1 \mathrm{H}, \mathrm{H}-4), 3.67\left(\mathrm{~d}, 1 \mathrm{H}, \mathrm{H}-23^{\prime} \mathrm{b}\right), 3.64$ (dd, $\left.J_{2,3}=9.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-2\right), 3.57(\mathrm{~d}, 1 \mathrm{H}, \mathrm{H}-23 \mathrm{~b}), 3.46$ (dd, $J_{6 \mathrm{a}, 5}=2.7$ $\left.\mathrm{Hz}, J_{6 \mathrm{a}, 6 \mathrm{~b}}=10.4 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-6 \mathrm{a}\right), 3.43\left(\mathrm{~d}, J_{1^{\prime} \mathrm{b}, 1^{\prime} \mathrm{a}}=11.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-1^{\prime} \mathrm{b}\right)$, $3.37\left(\mathrm{~d}, J_{23^{\prime \prime}, 23^{\prime \prime} \mathrm{a}}=13.6 \mathrm{~Hz}, 1 \mathrm{H}, \mathrm{H}-23^{\prime \prime} \mathrm{b}\right), 3.20\left(\mathrm{dd}, J_{6 \mathrm{~b}, 5}=2.2 \mathrm{~Hz}, 1 \mathrm{H}\right.$, H-6b), 3.19 (s, $\left.3 \mathrm{H}, \mathrm{H}-26^{\prime \prime}\right), 3.06\left(\mathrm{dd}, J_{4^{\prime}, 3^{\prime}}=9.1 \mathrm{~Hz}, J_{4^{\prime}, 5^{\prime}}=9.2 \mathrm{~Hz}\right.$, $\left.1 \mathrm{H}, \mathrm{H}-4^{\prime}\right), 3.02$ ( $\mathrm{s}, 3 \mathrm{H}, \mathrm{H}-26$ ), 2.95 (dd, $J_{6^{\prime} \mathrm{a}, 5^{\prime}}=8.7 \mathrm{~Hz}, J_{6^{\prime} \mathrm{a}, 6^{\prime} \mathrm{b}}=11.9$ $\left.\mathrm{Hz}, 1 \mathrm{H}, \mathrm{H}-6^{\prime} \mathrm{a}\right), 2.25\left(\mathrm{~d}, 1 \mathrm{H}, \mathrm{H}-6^{\prime} \mathrm{b}\right) \mathrm{ppm} .{ }^{13} \mathrm{C}\left\{{ }^{1} \mathrm{H}\right\}$ NMR ( 150 MHz , $\left.\mathrm{CDCl}_{3}\right): \delta=151.1\left(\mathrm{C}-20^{\prime \prime}\right), 148.0\left(\mathrm{C}-20^{\prime}\right), 147.6(\mathrm{C}-20), 147.1$ (C19), 146.9 (C-19'), 144.4 (C-19"), 139.1, 139.1, 138.3, 138.2, 138.1 $\left(\mathrm{C}_{\text {quat, }} 5 \times \mathrm{C}-\mathrm{Ph}\right), 136.6\left(\mathrm{C}-8^{\prime \prime}\right), 136.5\left(\mathrm{C}-8^{\prime}\right), 135.6$ (C-22"), 135.4 (C-8), 135.2 (C-13"), 134.2 (C-13), 133.0 (C-22'), 132.8 (C-15), 132.8 (C-10"), 132.8 (C-10), 132.5 (C-15"), 132.4 (C-24'), 132.2 (C-10'), 131.6 (C-24" ), 131.4 (C-24), 131.1 (C-15'), 131.1 (C-13'), 130.9 (C-22), 129.9 (C-14'), 128.8 (C-11"), 128.5 (C-9), 128.2 (C16), 128.2 (C-17), 128.1 (C-14), 128.0 (C-12'), 128.0 (C-11), 127.6 (C-16"), 127.2 (C-12), 127.2-128.3 (m, $25 \times \mathrm{C}-\mathrm{Ph}), 126.9$ (C-16'), 126.4 (C-11'), 125.3 (C-17"), 125.1 (C-9" ), 124.9 (C-17'), 124.1 (C$\left.14^{\prime \prime}\right), 124.1$ (C-25"), 123.7 (C-12"), 123.1 (C-9'), 113.8 (C-25'), 113.7 (C-21'), 112.9 (C-21"), 112.4 (C-25), 111.8 (C-21), 103.0 (C$2^{\prime}$ ), 88.9 (C-1), 83.4 (C-3'), 82.4 (C-3), 81.8 (C-4'), 79.5 (C-2), 77.9 (C-4), 77.6 ( $\left.\mathrm{C}-5^{\prime}\right), 77.0\left(\mathrm{C}-18^{\prime}\right), 75.4\left(\mathrm{C} 3-\mathrm{OCH}_{2} \mathrm{Ph}\right), 74.8\left(\mathrm{C}-1^{\prime}\right)$, 74.6 ( $\mathrm{C} 4-\mathrm{OCH}_{2} \mathrm{Ph}$ ), 74.2 (C-7"), 72.8 (C-7), 72.7 (C-6'), 72.6 $\left(\mathrm{C} 2-\mathrm{OCH}_{2} \mathrm{Ph}\right), 72.5\left(\mathrm{C}^{\prime}-\mathrm{OCH}_{2} \mathrm{Ph}\right), 72.4\left(\mathrm{C}^{\prime}-\mathrm{O} \underline{C H}_{2} \mathrm{Ph}\right), 71.6$ (C-7'), 70.6 (C-18), 69.4 (C-5), 69.4 (C-18"), 65.9 (C-6), 56.4 (C$\left.26^{\prime}\right), 55.4$ (C-26" ), 54.5 (C-26), 36.6 (C-23), 36.2 (C-23"), 36.0 (C23') ppm. HRMS (ESI-TOF) $m / z:[\mathrm{M}+\mathrm{Na}]^{+}$calcd for $\mathrm{C}_{107} \mathrm{H}_{100} \mathrm{O}_{17} \mathrm{Na}$ 1679.6858; found 1679.6860. Anal. calcd for $\mathrm{C}_{107} \mathrm{H}_{100} \mathrm{O}_{17}+\mathrm{H}_{2} \mathrm{O}: \mathrm{C}, 76.68 ; \mathrm{H}, 6.13$; found: C, $76.62 ; \mathrm{H}, 6.22$.

ECD Spectra. ECD spectra were measured in acetonitrile at room temperature. All spectra were collected between $180-400 \mathrm{~nm}$ at room temperature using solutions at concentrations $2.5 \times 10^{-5} \mathrm{M}$ in quartz cells with path length 0.2 or 0.5 cm . All spectra were recorded using a $100 \mathrm{~nm} / \mathrm{min}$ scanning speed, a step size of 0.2 nm , a bandwidth of 1 nm , a response time of 0.5 s , and an accumulation of 3 scans. The spectra were background corrected using acetonitrile.

Computational Details. Conformational search was carried out at the molecular mechanics level using a simplified structure in which all benzyl groups (Bn) in a sucrose moiety were exchanged by a hydrogen atom to save computational time and facilitate the computational predictions of ECD spectra for all investigated diastereoisomers. Next, the lowest energy structures (\#10) within 3 $\mathrm{kcal} / \mathrm{mol}$ were submitted for DFT optimization using Gaussian 16 program ${ }^{38}$ at the B3LYP/6-31G(d) level of theory applying PCM for $\mathrm{CH}_{3} \mathrm{CN}$. In each case, structures were confirmed to contain no imaginary frequencies. Finally, for the most abundant structures (\#2), TDDFT calculations were carried out using the B3LYP and CAMB3LYP functionals with SVP basis set using the PCM model for $\mathrm{CH}_{3} \mathrm{CN}$. Since they gave fully coherent results, we are only presenting results from the B3LYP functional and SVP basis set. Other basis sets,
i.e., TZVP, $6-311+G(d, p)$, were also checked for improving consistency of the obtained results. The UV and ECD spectra are simulated by overlapping Gaussian functions for 350 transitions. An optimum Gaussian band-shape and UV-correction were selected according to the similarity analysis with experimental data in $\mathrm{CH}_{3} \mathrm{CN}$ performed using SpecDis. ${ }^{43}$ The $\mathbf{M - 5 a \subset A C h}$ and $\boldsymbol{M - 5 a \subset C h ~}$ encapsulated complexes were optimized at the B3LYP/6-31G(d) level of theory using Gaussian 16 program. ${ }^{38}$

Fluorescence Titration Experiments. The stock solutions of the hosts $\boldsymbol{P}$-5a, $\boldsymbol{P}-\mathbf{5 b}, \mathbf{M}-5$ a, and $\mathbf{M}-\mathbf{5 b}$ were prepared at concentrations $c a$. 0.001 M in acetonitrile. A volume of 2.5 mL of acetonitrile was taken to the quartz cuvette, and the appropriate amount of host stock solution was added to obtain concentrations between 2.26 and $2.44 \times$ $10^{-5} \mathrm{M}$. The guest solutions were prepared by dissolving the required amount of acetylcholine iodide or choline iodide in a host stock solution to provide constant host concentration during the titration studies. Portions of the guest solution were gradually added to the cuvette containing appropriate host solution, mixed, and incubated for 30 s before irradiation at 280 nm at $25^{\circ} \mathrm{C}$. The corresponding emission spectra during titration were recorded. The measured emission spectra for the host during the titration studies were plotted as a function of the guest/host ratio using nonlinear regression via Bindfit program. ${ }^{40}$ The value of association constant $K_{\mathrm{a}}$ was calculated by nonlinear least-squares using as input parameters $1: 1$ binding model and the Nelder-Mead method.
${ }^{1} H$ NMR Titration Experiments. The ${ }^{1} \mathrm{H}$ NMR titration experiments were conducted by measuring the ${ }^{1} \mathrm{H}$ NMR spectra at 400 MHz with a Bruker Avance II apparatus at 303 K . The solutions of the hosts $P$-5a and M-5a were prepared at concentrations $3.73 \times 10^{-3}$ and $1.78 \times 10^{-3} \mathrm{M}$, respectively, in the mixture of solvents $\mathrm{CD}_{3} \mathrm{CN} /$ $\mathrm{CDCl}_{3}=80: 20$. The guest solutions were prepared by dissolving the required amount of acetylcholine iodide or choline iodide salts in a host stock solution to ensure constant host concentration during the experiment. Next, to the NMR tube containing appropriate host solution, portions of the guest solution were gradually added and the ${ }^{1} \mathrm{H}$ NMR spectrum was recorded.

## ASSOCIATED CONTENT

## s) Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.1c00019.

Table with comparison of ${ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ NMR signals of $\boldsymbol{P}$ $\mathbf{5 a}, \mathbf{M}-\mathbf{5 a}, P-5 b$, and $\mathbf{M - 5 b}$; fluorescence titration data; Cartesian coordinates of calculated structures; and copies of NMR spectra (PDF)

## AUTHOR INFORMATION

## Corresponding Authors

Sławomir Jarosz - Institute of Organic Chemistry, Warsaw 01-224, Poland; © orcid.org/0000-0002-9212-6203; Email: slawomir.jarosz@icho.edu.pl
Łukasz Szyszka - Institute of Organic Chemistry, Warsaw 01224, Poland; © orcid.org/0000-0002-7739-9713; Email: lukasz.szyszka@icho.edu.pl

## Authors

Marcin Górecki - Institute of Organic Chemistry, Warsaw 01224, Poland; © orcid.org/0000-0001-7472-3875
Piotr Cmoch - Institute of Organic Chemistry, Warsaw 01224, Poland; © orcid.org/0000-0002-8413-9290

Complete contact information is available at:
https://pubs.acs.org/10.1021/acs.joc.1c00019

## Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

This work was financed by the Polish National Science Centre (grant UMO-2016/21/B/ST5/03382). We would like to thank the Wroclaw Centre for Networking and Supercomputing (WCSS) and PLGrid platform for the computational support. We thank Mr. Wiktor Ignacak for preparative HPLC equipment support. Dr. Shashuk's Group (Institute of Physical Chemistry, PAS) is acknowledgment for fluorescence spectrometer support.

## REFERENCES

(1) (a) Santos-Figueroa, L. E.; Moragues, M. E.; Climent, E.; Agostini, A.; Martínez-Máñez, R.; Sancenón, F. Chromogenic and fluorogenic chemosensors and reagents for anions. A comprehensive review of the years 2010-2011. Chem. Soc. Rev. 2013, 42, 34893613. (b) Pinalli, R.; Pedrini, A.; Dalcanale, E. Biochemical sensing with macrocyclic receptors. Chem. Soc. Rev. 2018, 47, 7006-7026. (c) Wu, D.; Sedgwick, A. C.; Gunnlaugsson, T.; Akkaya, E. U.; Yoon, J.; James, T. D. Fluorescent chemosensors: the past, present and future. Chem. Soc. Rev. 2017, 46, 7105-7123.
(2) Park, S.-H.; Kwon, N.; Lee, J.-H.; Yoon, J.; Shin, I. Synthetic ratiometric fluorescent probes for detection of ions. Chem. Soc. Rev. 2020, 49, 143-179.
(3) (a) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. Signaling Recognition Events with Fluorescent Sensors and Switches. Chem. Rev. 1997, 97, 1515-1566. (b) Czarnik, A. W. Fluorescent Chemosensors for Ion and Molecule Recognition; American Chemical Society: Washington, DC, 1992. (c) Chen, X.; Tian, X.; Shin, I.; Yoon, J. Fluorescent and luminescent probes for detection of reactive oxygen and nitrogen species. Chem. Soc. Rev. 2011, 40, 4783-4804.
(4) For example: (a) Lai, W.-F.; Rogach, A. L.; Wong, W.-T. Chemistry and engineering of cyclodextrins for molecular imaging. Chem. Soc. Rev. 2017, 46, 6379-6419. (b) Li, J.; Yim, D.; Jang, W.-D.; Yoon, J. Recent progress in the design and applications of fluorescence probes containing crown ethers. Chem. Soc. Rev. 2017, 46, 2437-2458. (c) Li, Y.; Dong, Y.; Cheng, L.; Qin, C.; Nian, H.; Zhang, H.; Yu, Y.; Cao, L. Aggregation-Induced Emission and LightHarvesting Function of Tetraphenylethene-Based Tetracationic Dicyclophane. J. Am. Chem. Soc. 2019, 141, 8412-8415. (d) Han, X.-N.; Han, Y.; Chen, C.-F. Pagoda[4]arene and i-Pagoda[4]arene. J. Am. Chem. Soc. 2020, 142, 8262-8269.
(5) (a) Kolesnichenko, I. V.; Anslyn, E. V. Practical applications of supramolecular chemistry. Chem. Soc. Rev. 2017, 46, 2385-2390. (b) Liu, Z.; Nalluri, S. K. M.; Stoddart, J. F. Surveying macrocyclic chemistry: from flexible crown ethers to rigid cyclophanes. Chem. Soc. Rev. 2017, 46, 2459-2478. (c) You, L.; Zha, D.; Anslyn, E. V. Recent Advances in Supramolecular Analytical Chemistry Using Optical Sensing. Chem. Rev. 2015, 115, 7840-7892.
(6) (a) Brevé, T. G.; Filius, M.; Araman, C.; van der Helm, M. P.; Hagedoorn, P.-L.; Joo, C.; van Kasteren, S. I.; Eelkema, R. Conditional Copper-Catalyzed Azide-Alkyne Cycloaddition by Catalyst Encapsulation. Angew. Chem., Int. Ed. 2020, 59, 93409344. (b) de Simone, N. A.; Meninno, S.; Talotta, C.; Gaeta, C.; Neri, P.; Lattanzi, A. Solvent-Free Enantioselective Michael Reactions Catalyzed by a Calixarene-Based Primary Amine Thiourea. J. Org. Chem. 2018, 83, 10318-10325. (c) Sashuk, V.; Butkiewicz, H.; Fiałkowski, M.; Danylyuk, O. Triggering autocatalytic reaction by host-guest interactions. Chem. Commun. 2016, 52, 4191-4194. (d) Tang, B.; Zhao, J.; Xu, J.-F.; Zhang, X. Cucurbit[n]urils for Supramolecular Catalysis. Chem.-Eur. J. 2020, 26, 15446-15460.
(7) (a) Hua, B.; Shao, L.; Zhang, Z.; Liu, J.; Huang, F. Cooperative Silver Ion-Pair Recognition by Peralkylated Pillar[5]arenes. J. Am. Chem. Soc. 2019, 141, 15008-15012. (b) Moosa, B.; Alimi, L. O.; Shkurenko, A.; Fakim, A.; Bhatt, P. M.; Zhang, G.; Eddaoudi, M.; Khashab, N. M. A Polymorphic Azobenzene Cage for Energy-Efficient
and Highly Selective p-Xylene Separation. Angew. Chem., Int. Ed. 2020, 59, 21367-21371.
(8) (a) Sun, X.; Wang, Y.; Lei, Y. Fluorescence based explosive detection: from mechanisms to sensory materials. Chem. Soc. Rev. 2015, 44, 8019-8061. (b) Liu, Y.; Song, Y.; Chen, Y.; Li, X.-Q.; Ding, F.; Zhong, R.-Q. Biquinolino-Modified $\beta$-Cyclodextrin Dimers and Their Metal Complexes as Efficient Fluorescent Sensors for the Molecular Recognition of Steroids. Chem.-Eur. J. 2004, 10, 36853696.
(9) (a) Huang, Y.; Gao, R.-H.; Liu, M.; Chen, L.-X.; Ni, X.-L.; Xiao, X.; Cong, H.; Zhu, Q.-J.; Chen, K.; Tao, Z. Cucurbit[n]uril-Based Supramolecular Frameworks Assembled through Outer-Surface Interactions. Angew. Chem., Int. Ed. 2021, DOI: 10.1002/ anie.202002666. (b) Mastalerz, M. Permanent Porous Materials from Discrete Organic Molecules-Towards Ultra-High Surface Areas. Chem.-Eur. J. 2012, 18, 10082-10091. (c) Schaub, T. A.; Prantl, E. A.; Kohn, J.; Bursch, M.; Marshall, C. R.; Leonhardt, E. J.; Lovell, T. C.; Zakharov, L. N.; Brozek, C. K.; Waldvogel, S. R.; Grimme, S.; Jasti, R. Exploration of the Solid-State Sorption Properties of Shape-Persistent Macrocyclic Nanocarbons as Bulk Materials and Small Aggregates. J. Am. Chem. Soc. 2020, 142, 87638775.
(10) (a) Kim, H.-J.; Whang, D. R.; Gierschner, J.; Park, S. Y. Highly Enhanced Fluorescence of Supramolecular Polymers Based on a Cyanostilbene Derivative and Cucurbit[8]uril in Aqueous Solution. Angew. Chem., Int. Ed. 2016, 55, 15915-15919. (b) Liang, T.; Collin, D.; Galerne, M.; Fuks, G.; Vargas Jentzsch, A.; Maaloum, M.; Carvalho, A.; Giuseppone, N.; Moulin, E. Covalently Trapped Triarylamine-Based Supramolecular Polymers. Chem.-Eur. J. 2019, 25, 14341-14348. (c) Zeng, R.; Gong, Z.; Yan, Q. Chalcogen Bonding Supramolecular Polymers. J. Org. Chem. 2020, 85, 83978404.
(11) (a) Davis, J. T.; Gale, P. A.; Quesada, R. Advances in anion transport and supramolecular medicinal chemistry. Chem. Soc. Rev. 2020, 49, 6056-6086. (b) Grauwels, G.; Valkenier, H.; Davis, A. P.; Jabin, I.; Bartik, K. Repositioning Chloride Transmembrane Transporters: Transport of Organic Ion Pairs. Angew. Chem., Int. Ed. 2019, 58, 6921-6925. (c) Tapia, L.; Pérez, Y.; Bolte, M.; Casas, J.; Solà, J.; Quesada, R.; Alfonso, I. pH-Dependent Chloride Transport by Pseudopeptidic Cages for the Selective Killing of Cancer Cells in Acidic Microenvironments. Angew. Chem., Int. Ed. 2019, 58, 1246512468.
(12) (a) Dasgupta, S.; Mukherjee, P. S. Carboxylatopillar[n]arenes: a versatile class of water soluble synthetic receptors. Org. Biomol. Chem. 2017, 15, 762-772. (b) Hu, X.-Y.; Jia, K.; Cao, Y.; Li, Y.; Qin, S.; Zhou, F.; Lin, C.; Zhang, D.; Wang, L. Dual Photo- and pHResponsive Supramolecular Nanocarriers Based on Water-Soluble Pillar[6]arene and Different Azobenzene Derivatives for Intracellular Anticancer Drug Delivery. Chem.-Eur. J. 2015, 21, 1208-1220. (c) Xia, D.; Li, Y.; Jie, K.; Shi, B.; Yao, Y. A Water-Soluble Cyclotriveratrylene-Based Supra-amphiphile: Synthesis, pH-Responsive Self-Assembly in Water, and Its Application in Controlled Drug Release. Org. Lett. 2016, 18, 2910-2913.
(13) (a) He, Q.; Vargas-Zúñiga, G. I.; Kim, S. H.; Kim, S. K.; Sessler, J. L. Macrocycles as Ion Pair Receptors. Chem. Rev. 2019, 119, 97539835. (b) Brotin, T.; Dutasta, J.-P. Cryptophanes and Their Complexes-Present and Future. Chem. Rev. 2009, 109, 88-130.
(14) Hardie, M. J. Recent advances in the chemistry of cyclotriveratrylene. Chem. Soc. Rev. 2010, 39, 516-527.
(15) Zhang, D.; Martinez, A.; Dutasta, J.-P. Emergence of Hemicryptophanes: From Synthesis to Applications for Recognition, Molecular Machines, and Supramolecular Catalysis. Chem. Rev. 2017, 117, 4900-4942.
(16) Long, A.; Perraud, O.; Albalat, M.; Robert, V.; Dutasta, J.-P.; Martinez, A. Helical Chirality Induces a Substrate-Selectivity Switch in Carbohydrates Recognitions. J. Org. Chem. 2018, 83, 6301-6306.
(17) Perraud, O.; Robert, V.; Martinez, A.; Dutasta, J.-P. A Designed Cavity for Zwitterionic Species: Selective Recognition of Taurine in Aqueous Media. Chem.-Eur. J. 2011, 17, 13405-13408.
(18) Yang, J.; Chatelet, B.; Dufaud, V.; Hérault, D.; Jean, M.; Vanthuyne, N.; Mulatier, J.-C.; Pitrat, D.; Guy, L.; Dutasta, J.-P.; Martinez, A. Enantio- and Substrate-Selective Recognition of Chiral Neurotransmitters with $\mathrm{C}_{3}$-Symmetric Switchable Receptors. Org. Lett. 2020, 22, 891-895.
(19) (a) Taratula, O.; Kim, M. P.; Bai, Y.; Philbin, J. P.; Riggle, B. A.; Haase, D. N.; Dmochowski, I. J. Synthesis of Enantiopure, Trisubstituted Cryptophane-A Derivatives. Org. Lett. 2012, 14, 3580-3583. (b) Cochrane, J. R.; Schmitt, A.; Wille, U.; Hutton, C. A. Synthesis of cyclic peptide hemicryptophanes: enantioselective recognition of a chiral zwitterionic guest. Chem. Commun. 2013, 49, 8504-8506.
(20) (a) Pradhan, T.; Jung, H. S.; Jang, J. H.; Kim, T. W.; Kang, C.; Kim, J. S. Chemical sensing of neurotransmitters. Chem. Soc. Rev. 2014, 43, 4684-4713. (b) Park, Y. S.; Kim, Y.; Paek, K. Specific Encapsulation of Acetylcholine Chloride by a Self-Assembled Molecular Capsule with Sulfonamido Moiety. Org. Lett. 2019, 21, 8300-8303. (c) Ballester, P.; Shivanyuk, A.; Far, A. R.; Rebek, J. A Synthetic Receptor for Choline and Carnitine. J. Am. Chem. Soc. 2002, 124, 14014-14016.
(21) (a) Tsai, T.-H. Separation methods used in the determination of choline and acetylcholine. J. Chromatogr. B: Biomed. Sci. Appl. 2000, 747, 111-122. (b) Hasselmo, M. E.; Sarter, M. Modes and models of forebrain cholinergic neuromodulation of cognition. Neuropsychopharmacology 2011, 36, 52-73.
(22) (a) White, K. E.; Cummings, J. L. Schizophrenia and Alzheimer's disease: clinical and pathophysiologic analogies. Compr. Psychiatry 1996, 37, 188-195. (b) Doody, R. S. Current treatments for Alzheimer's disease: cholinesterase inhibitors. J. Clin. Psychiatry 2003, 64, 11-17. (c) Higley, M. J.; Picciotto, M. R. Neuromodulation by acetylcholine: examples from schizophrenia and depression. Curr. Opin. Neurobiol. 2014, 29, 88-95.
(23) Zeisel, S. H.; da Costa, K.-A. Choline: An Essential Nutrient for Public Health. Nutr. Rev. 2009, 67, 615-623.
(24) (a) Korbakov, N.; Timmerman, P.; Lidich, N.; Urbach, B.; Sa'ar, A.; Yitzchaik, S. Acetylcholine Detection at Micromolar Concentrations with the Use of an Artificial Receptor-Based Fluorescence Switch. Langmuir 2008, 24, 2580-2587. (b) Liu, Y.; Perez, L.; Mettry, M.; Gill, A. D.; Byers, S. R.; Easley, C. J.; Bardeen, C. J.; Zhong, W.; Hooley, R. J. Site selective reading of epigenetic markers by a dual-mode synthetic receptor array. Chem. Sci. 2017, 8, 3960-3970. (c) Erieau-Peyrard, L.; Coiffier, C.; Bordat, P.; Bégué, D.; Chierici, S.; Pinet, S.; Gosse, I.; Baraille, I.; Brown, R. Selective, direct detection of acetylcholine in PBS solution, with self-assembled fluorescent nano-particles: experiment and modelling. Phys. Chem. Chem. Phys. 2015, 17, 4168-4174. (d) Dumartin, M.-L.; Givelet, C.; Meyrand, P.; Bibal, B.; Gosse, I. A fluorescent cyclotriveratrylene: synthesis, emission properties and acetylcholine recognition in water. Org. Biomol. Chem. 2009, 7, 2725-2728.
(25) Long, A.; Fantozzi, N.; Pinet, S.; Genin, E.; Pétuya, R.; Bégué, D.; Robert, V.; Dutasta, J.-P.; Gosse, I.; Martinez, A. Selective recognition of acetylcholine over choline by a fluorescent cage. Org. Biomol. Chem. 2019, 17, 5253-5257.
(26) (a) Long, A.; Antonetti, E.; Insuasty, A.; Pinet, S.; Gosse, I.; Robert, V.; Dutasta, J.-P.; Martinez, A. Hemicryptophanes with Improved Fluorescent Properties for the Selective Recognition of Acetylcholine over Choline. J. Org. Chem. 2020, 85, 6400-6407. (b) Fantozzi, N.; Pétuya, R.; Insuasty, A.; Long, A.; Lefevre, S.; Schmitt, A.; Robert, V.; Dutasta, J.-P.; Baraille, I.; Guy, L.; Genin, E.; Bégué, D.; Martinez, A.; Pinet, S.; Gosse, I. A new fluorescent hemicryptophane for acetylcholine recognition with an unusual recognition mode. New J. Chem. 2020, 44, 11853-11860.
(27) Zhang, D.; Gao, G.; Guy, L.; Robert, V.; Dutasta, J.-P.; Martinez, A. A fluorescent heteroditopic hemicryptophane cage for the selective recognition of choline phosphate. Chem. Commun. 2015, 51, 2679-2682.
(28) Jia, C.; Zuo, W.; Yang, D.; Chen, Y.; Cao, L.; Custelcean, R.; Hostaš, J.; Hobza, P.; Glaser, R.; Wang, Y.-Y.; Yang, X.-J.; Wu, B.

Selective binding of choline by a phosphate-coordination-based triple helicate featuring an aromatic box. Nat. Commun. 2017, 8, 938.
(29) Ballester, P.; Sarmentero, M. A. Hybrid Cavitand-Resorcin[4]arene Receptor for the Selective Binding of Choline and Related Compounds in Protic Media. Org. Lett. 2006, 8, 3477-3480.
(30) Jarosz, S.; Sokołowska, P.; Szyszka, Ł. Synthesis of fine chemicals with high added value from sucrose: Towards sucrosebased macrocycles. Tetrahedron Lett. 2020, 61, 151888.
(31) (a) Potopnyk, M. A.; Jarosz, S. Synthesis and Complexation Properties of "Unsymmetrical" Sucrose-Based Receptors. Eur. J. Org. Chem. 2013, 5117-5126. (b) Lewandowski, B.; Jarosz, S. Chiral recognition of $\alpha$-phenylethylamine by sucrose-based macrocyclic receptors. Chem. Commun. 2008, 6399-6401.
(32) Potopnyk, M. A.; Lewandowski, B.; Jarosz, S. Novel sucrosebased macrocyclic receptors for enantioselective recognition of chiral ammonium cations. Tetrahedron: Asymmetry 2012, 23, 1474-1479.
(33) (a) Łęczycka-Wilk, K.; Dąbrowa, K.; Cmoch, P.; Jarosz, S. Chloride-Templated Macrocyclization and Anion-Binding Properties of C2-Symmetric Macrocyclic Ureas from Sucrose. Org. Lett. 2017, 19, 4596-4599. (b) Łęczycka-Wilk, K.; Ulatowski, F.; Cmoch, P.; Jarosz, S. "Choose-a-size" control in the synthesis of sucrose based urea and thiourea macrocycles. Org. Biomol. Chem. 2018, 16, 60636069.
(34) Szyszka, Ł.; Cmoch, P.; Butkiewicz, A.; Potopnyk, M. A.; Jarosz, S. Synthesis of Cyclotriveratrylene-Sucrose-Based Capsules. Org. Lett. 2019, 21, 6523-6528.
(35) Szyszka, Ł.; Cmoch, P.; Górecki, M.; Ceborska, M.; Potopnyk, M. A.; Jarosz, S. Chiral Molecular Cages Based on Cyclotriveratrylene and Sucrose Units Connected with p-Phenylene Linkers. Eur. J. Org. Chem. 2021, 897-906.
(36) (a) Górecki, M. A configurational and conformational study of (-)-Oseltamivir using a multi-chiroptical approach. Org. Biomol. Chem. 2015, 13, 2999-3010. (b) Long, A.; Colomban, C.; Jean, M.; Albalat, M.; Vanthuyne, N.; Giorgi, M.; Di Bari, L.; Górecki, M.; Dutasta, J.-P.; Martinez, A. Enantiopure $\mathrm{C}_{1}$-Cyclotriveratrylene with a Reversed Spatial Arrangement of the Substituents. Org. Lett. 2019, 21, 160-165. (c) Long, A.; Jean, M.; Albalat, M.; Vanthuyne, N.; Giorgi, M.; Górecki, M.; Dutasta, J.-P.; Martinez, A. Synthesis, resolution, and chiroptical properties of hemicryptophane cage controlling the chirality of propeller arrangement of a $\mathrm{C}_{3}$ triamide unit. Chirality 2019, 31, 910-916.
(37) Canceill, J.; Collet, A.; Gabard, J.; Gottarelli, G.; Spada, G. P. Exciton approach to the optical activity of $\mathrm{C}_{3}$-cyclotriveratrylene derivatives. J. Am. Chem. Soc. 1985, 107, 1299-1308.
(38) Frisch, M.J.; Trucks, G.W.; Schlegel, H.B.; Scuseria, G.E.; Robb, M.A.; Cheeseman, J.R.; Scalmani, G.; Barone, V.; Petersson, G.A.; Nakatsuji, H.; Li, X.; Caricato, M.; Marenich, A.V.; Bloino, J.; Janesko, B.G.; Gomperts, R.; Mennucci, B.; Hratchian, H.P.; Ortiz, J. V.; Izmaylov, A. F.; Sonnenberg, J. L.; Williams-Young, D.; Ding, F.; Lipparini, F.; Egidi, F.; Goings, J.; Peng, B.; Petrone, A.; Henderson, T.; Ranasinghe, D.; Zakrzewski, V.G.; Gao, J.; Rega, N.; Zheng, G.; Liang, W.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Throssell, K.; Montgomery, J.A., Jr.; Peralta, J.E.; Ogliaro, F.; Bearpark, M.J.; Heyd, J.J.; Brothers, E.N.; Kudin, K.N.; Staroverov, V.N.; Keith, T.A.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.P.; Burant, J.C.; Iyengar, S.S.; Tomasi, J.; Cossi, M.; Millam, J.M.; Klene, M.; Adamo, C.; Cammi, R.; Ochterski, J.W.; Martin, R.L.; Morokuma, K.; Farkas, O.; Foresman, J.B.; Fox, D.J. Gaussian 16, Revision B.01; Gaussian, Inc.: Wallingford CT, 2016.
(39) (a) Pescitelli, G.; Barone, V.; Di Bari, L.; Rizzo, A.; Santoro, F. Vibronic Coupling Dominates the Electronic Circular Dichroism of the Benzene Chromophore ${ }^{1} \mathrm{~L}_{\mathrm{b}}$ band. J. Org. Chem. 2013, 78, 73987405. (b) Del Bello, F.; Bonifazi, A.; Giorgioni, G.; Piergentili, A.; Sabbieti, M. G.; Agas, D.; Dell'Aera, M.; Matucci, R.; Górecki, M.; Pescitelli, G.; Vistoli, G.; Quaglia, W. Novel Potent Muscarinic Receptor Antagonists: Investigation on the Nature of Lipophilic Substituents in the 5-and/or 6-Positions of the 1,4-Dioxane Nucleus. J. Med. Chem. 2020, 63, 5763-5782.
(40) Brynn Hibbert, D.; Thordarson, P. The death of the Job plot, transparency, open science and online tools, uncertainty estimation methods and other developments in supramolecular chemistry data analysis. Chem. Commun. 2016, 52, 12792-12805.
(41) Makita, Y.; Katayama, N.; Lee, H.-H.; Abe, T.; Sogawa, K.; Nomoto, A.; Fujiwara, S.-I.; Ogawa, A. A tri-aromatic amide hemicryptophane host: synthesis and acetylcholine binding. Tetrahedron Lett. 2016, 57, 5112-5115.
(42) Łukasik, B.; Milczarek, J.; Pawlowska, R.; Żurawiński, R.; Chworos, A. Facile synthesis of fluorescent distyrylnaphthalene derivatives for bioapplications. New J. Chem. 2017, 41, 6977-6980.
(43) Bruhn, T.; Schaumlöffel, A.; Hemberger, Y.; Pescitelli, G. SpecDis Version 1.70, 2017, Brerlin, Germany; https://specdissoftware.jimdo.com.


[^0]:    Received: January 4, 2021
    Published: March 12, 2021

[^1]:    ${ }^{a}$ For numbering of atom, see Experimental Section.

