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Molecular Tubes for Lipid Sensing: Tube Conformations Control Analyte Selectivity and Fluorescent Response

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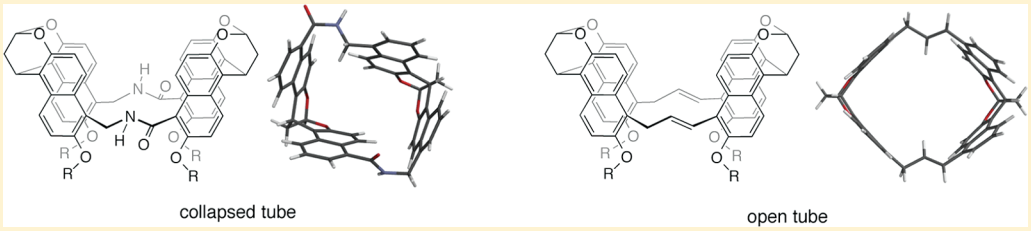
Supporting Information



ABSTRACT: Two fluorescent sensors for lipids have been prepared and tested for detection of a number of hydrophobic compounds of varying shape and size. The data suggest that the two sensors have a different mode of fluorescent response. Yet, the two sensors are only different in the bridging group—one having a flexible amide and one having a rigid allyl bridge. The fluorescence data are explained based on a difference in conformation of the two sensors in aqueous solution.

INTRODUCTION

As part of an ongoing program focused on the construction of fluorescent sensors for bioactive compounds,[1] we have been investigating receptors and sensors for lipids.[2] There are quite a number of bioactive lipids for which sensors might be useful.[3] Many current approaches to lipid sensors have focused mainly on recognition of the headgroup of the lipid.[4] Few sensors are designed to recognize the aliphatic portion of the lipid.[5] However, the aliphatic portion of the lipid serves to anchor the headgroup into a membrane, where it is biologically active. Thus, to extract the lipid and modulate its activity, one must bind to the aliphatic portion as well as the headgroup. This has spurred our interest in recognition of the aliphatic portion of the lipid. For many years, cyclodextrins have been found to bind simple lipids in water via hydrophobic interactions.[6] Indeed, cyclodextrin derivatives have been used to extract and repartition lipids from cellular membranes.[7] However the cyclodextrin cavity is difficult to manipulate as would be required for truly selective lipid recognition. More recently various deep cavity calixarenes have been employed in lipid recognition, including aqueous lipid recognition.[8] Lipid recognition and sensing has many potential biochemical and biomedical applications, yet selectivity between various lipids remains a daunting problem. Thus, we recently prepared a molecular tube,[2] which demonstrated shape-selective[9] binding of straight chain lipids. Herein we describe a second, related tube-like fluorescent sensor, and by comparison to the original sensor, draw conclusions about their distinct mechanisms of fluorescent activation.

DESIGN CONSIDERATIONS

We[10] and others[11] have been interested in extended calixarene motifs which use a naphthalene core termed calixnapthalenes.[12] We were particularly interested in these compounds because various substituted naphthalenes are known to be environment-sensitive fluorophores which could enable these hosts to be used as fluorescent sensors. We initially envisioned construction of molecular tubes via calixnaphthalenes such as compound **2** (Figure 1). Unfortunately, electrophilic reactions of naphthalene rings are typically directed to the 1,8 positions; thus, these positions must be blocked to produce the desired 3,6-linked receptors such as in compound **2**.[10] Synthetically, this was an inconvenient procedure at best, thus alternate methods for constructing such molecular tubes were explored.

It has been known for some time that the reaction of 2-naphthols with malonaldehyde diacetal produces a naphthalene dimer via reaction at the 1-position and subsequent formation of the internal acetal[13] (top, Figure 2). This dimer forms a very rigid cleft which would lend itself to construction of receptors and sensors. We reasoned that linking two such clefts would produce two different macrocycles, which we termed syn and anti. The syn structure had the tube-like cavity we sought, though the anti isomer appeared to be much more flexible and had a more extended conformation. CPK models indicated that, for the syn isomer, the use of a three atom linker would provide a cavity which would be well matched to the volume occupied by an unbranched alkyl chain in the extended conformation (right, Figure 2). Thus, construction of tubes from naphthol dimers with three atom linkers was examined.

Based initially on synthetic accessibility, we examined amide-linked structures such as compound **3**. (Figure 3). This macrocycle has two major low energy conformations. One conformation was deemed the "open" conformer in which the

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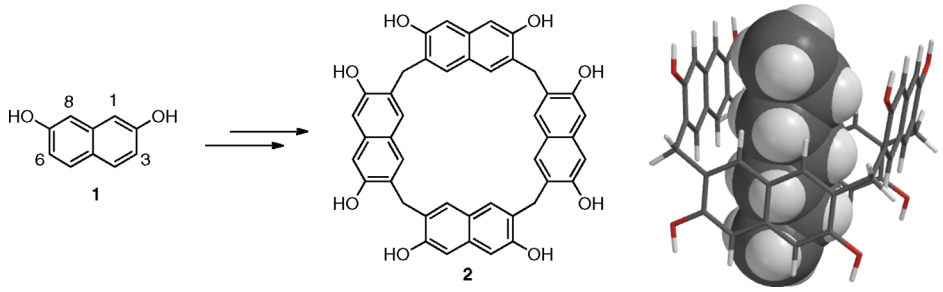


Figure 1. Design of 3,6-linked calix[4]naphthalene. (Right) Model of a 1,3-alternate conformation of compound **2** with an alkane guest.

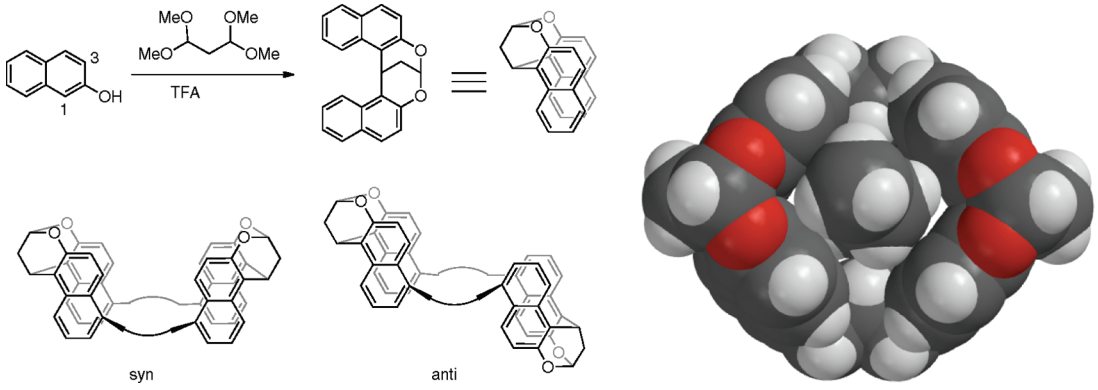


Figure 2. Two ways of connecting naphthalene dimers (syn and anti). (Right) CPK model of an alkane guest in a syn receptor (with a simple three carbon linker).

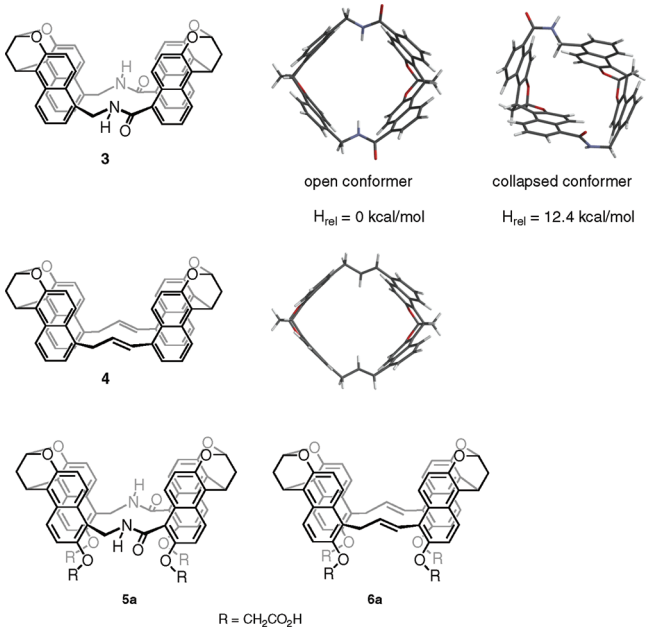


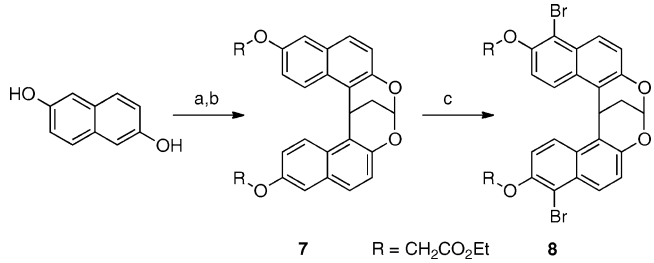
Figure 3. Accessible conformations of two potential tube structures. Relative energies of the open and collapsed conformers of compound **3** were determined by molecular mechanics (MMFF94).

amide carbonyl groups point outside the cavity and the amide hydrogen atoms point into the cavity. This arrangement produces an open tube with a cavity diameter (C to C distance) of 7.3 Å, which is suitable for binding a straight chain alkane in its fully extended conformation. The other major conformation was deemed the "closed" conformer in which the cavity is collapsed, producing only a shallow cavity. The collapse arises from one amide switching to a *cis* rotamer and the adjacent methylene rotating such that the hydrogens point into the cavity, and the other amide maintaining the *trans* geometry but with the carbonyl rotating such that the oxygen points into the tube. The preference for amides to adopt a *trans* geometry ensures that for compound **3**, the open geometry is preferred in the gas phase. In this case, the open conformation is favored over the closed by 12.4 kcal/mol based on molecular mechanics calculations. To probe the issues associated with multiple conformations, we also explored allyl linked tubes such as compound **4**. Since the double bonds are rigidly *trans*, only the open geometry is accessible (Figure 3). To provide adequate water solubility, four acetate groups were incorporated in the tube design. Thus, compounds **5a** and **6a** were the initial targets for exploring this class of tube-like receptors.

SYNTHESIS

We envisioned the allyl-linked tube **6a** being prepared via a Suzuki cross coupling reaction. The first coupling partner was prepared as shown in Scheme 1. 2,6-Dihydroxynaphthalene was

Scheme 1. [a]



[a](a) NaH, ethyl bromoacetate, DMF, 31%; (b) malonaldehyde bis-dimethyl acetal, TFA, DCM, 82%; (c) Br_2, $MeNO_2$, 95%.

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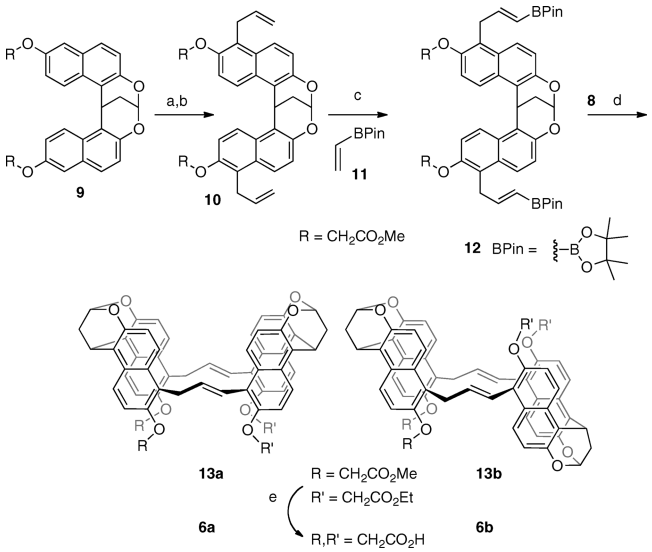
alkyated with ethyl bromoacetate and reacted with malonaldehyde diacetal to give **7**. Bromination gave compound **8** as the only regioisomer.

The other coupling partner was prepared by bromination of bis-methyl ester **9**[2] followed by Stille coupling with allyl tributyltin (Scheme 2). The methyl ester was used in this case

Scheme 2. [a]



[a](a) Br_2, DCM, $MeNO_2$, 99%; (b) allyl tributyl tin, CsF, $PdCl_2(PPh_3)_2$, DMF, 72%; (c) Neolyst 1, vinyl pinacol-boronate (**14**), DCM, 70% (d) Pd_2dba_3, S-Phos, CsF, THF, 38% (1:1 syn/anti mixture); (e) NaOH, MeOH, THF, H_2O, 99%.

in order to differentiate the two halves of the final construct for characterization purposes. Olefin metathesis[14] with vinyl boronate ester **11** gave the bis-allyl boronate **12**. After substantial optimization, Suzuki coupling[15] of **12** with **8** produced the diastereomeric tubes **13a** and **13b** in a 1:1 ratio and a 38% overall yield. The diastereomers were separated and then saponified to produce the final water-soluble sensor **6a** along with its anti isomer **6b**. Sensor **5a** and its corresponding anti isomer were prepared as previously described.[2]

For sensor **5a**, assignment of the two isomers as syn and anti was carried out by 2D NMR. As shown in Figure 4, NOESY contact between the chemically distinct naphthyl groups supported assignment of isomer **5a** as the syn compound and contacts between the naphthyl hydrogens and the opposing ester methylene protons supported assignment of **5b** as the anti isomer.

For sensor **6a**, assignment of the two isomers as syn and anti was carried out by one-dimensional NOE studies using the pair of precursor diesters **13a** and **13b**. Surprisingly, no NOE contacts were observed between the two chemically distinct naphthyl groups in **13a** as observed in the amide linked construct **5a**. This result was the first evidence that compound **5a** was more flexible than compound **6a**; apparently the rigidity of compound **13a** precluded an observable NOE signal. However, NOE contacts between the methylene of one of the acetate groups and *both* the methyl and ethyl groups supported assignment of isomer **13a** as the syn compound (Figure 4). Contacts between the naphthyl hydrogens and the ester methylene protons supported assignment of **13b** as the anti isomer.

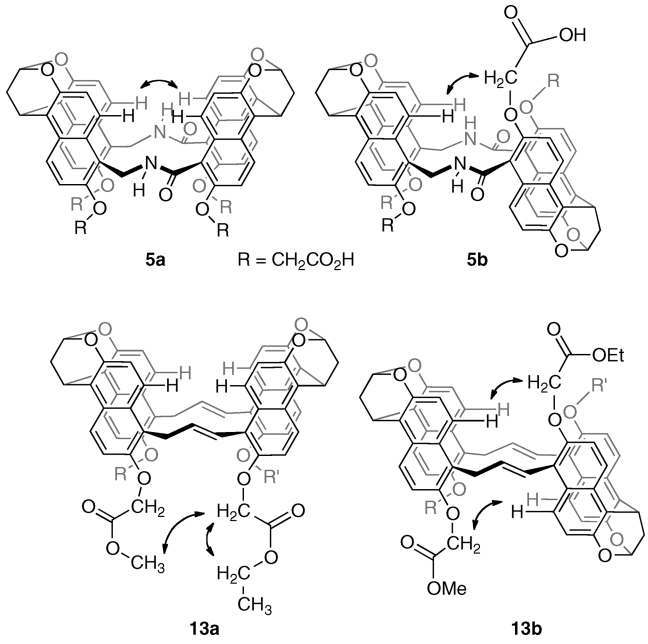


Figure 4. NOE contacts used to assign syn and anti structures for compounds **5** and **13**.

RESULTS AND DISCUSSION

The recognition properties of the diastereomeric sensors were then probed spectroscopically in water at a pH of 8.4 to maintain solubility of all species. Various lipids were tested with a range of size and hydrophobicity. Because these sensors are tubes, any length of lipid can theoretically fit through the cavity, however very long lipids are known to form micelles in aqueous solution[16] which would greatly complicate the analysis of the sensors. So for the purpose of this study, the lipids were restricted to those with chains of twelve carbons or fewer. Over the range of concentrations used, all of the sensors exhibited Beer's law behavior suggesting that the sensors themselves did not aggregate. Although no change in UV absorption was observed upon addition of lipids, all sensors showed significant changes in fluorescence upon addition of hydrophobic guests (Figure 5).

Table 1 lists results for both isomers of sensors **5** and **6** including binding constants and maximum fluorescence change (I_{sat}/I_0) which is the fluorescence at saturation taken from the theoretical fit (I_{sat}) divided by the initial fluorescence with no guest (I_0). The fluorescent response of sensors **5a** and **5b** have been reported.[2] Briefly, we found that extended sensor **5b** gave a fluorescence decrease when titrated with lipids wherein the more hydrophobic lipids had higher association constants in all cases. The binding properties of sensor **5a** were more complex. Lipids which were short (less than eight carbons) or branched did not bind as well as the corresponding straight-chain analyte of similar hydrophobicity. In addition, the short or branched lipids gave a fluorescence increase rather than a decrease. Thus sensor **5a** was unusually selective for long straight-chained analytes both in terms of binding constant and fluorescent response. This selectivity was not observed in the anti isomer **5b** which matched our expectation that only isomer **5a** had the tube-like structure we desired. Finally, we demonstrated that the macrocyclic nature of the sensors is essential by examining the saponified derivative of compound **7** and showed that it gave no fluorescent response to anionic guests.[17]

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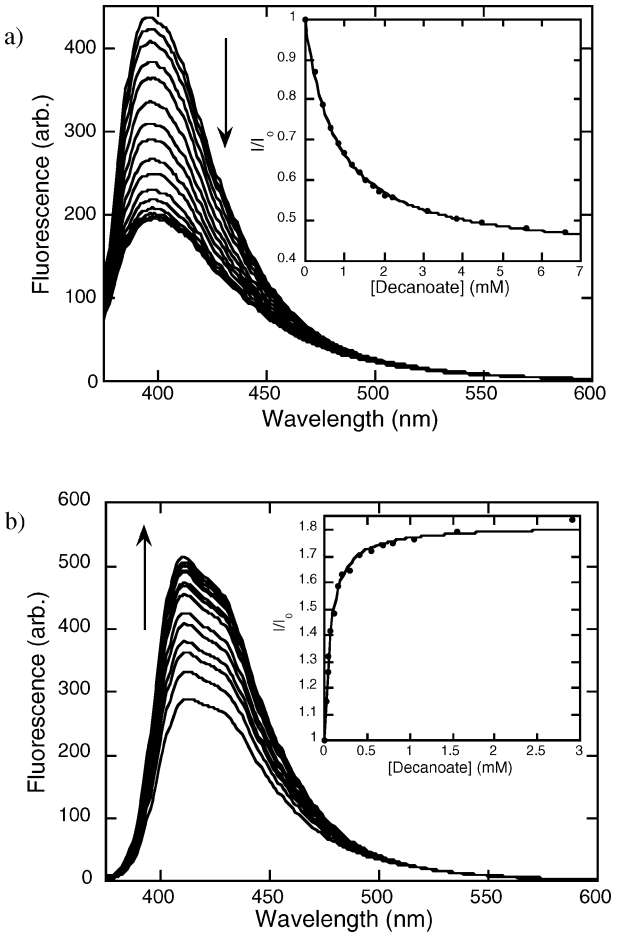

Figure 5. Fluorescence titration of (a) compound **5a** with decanoic acid in buffer and (b) compound **6a** with decanoic acid in buffer (20 mM HEPES, pH = 8.4, [sensor] = 10^{-5} M, λ_{ex} = 365 nm). (Inset) Fit of the titration data to a single site binding isotherm at λ_{em} = 396 nm for **5a** and 410 nm for **6a**.

While the binding properties of sensor **5a** could be explained by its shape, the difference in fluorescent response to various analytes was challenging to describe mechanistically. One potential explanation for the unusually selective fluorescent response was that **5a** normally exists in its collapsed form (see Figure 3) in aqueous solution. Analytes which are short or branched can only bind to the shallow cleft of the collapsed conformer. This type of binding expels water from the cavity resulting in a mild fluorescence increase which is commonly observed in environment sensitive fluorophores. However, analytes which can thread through the cavity cause the tube to transition to its open form and the fluorescence decreases, though the mechanism for this quenching is unclear.

This hypothesis was supported by NMR binding studies. Sensor **5a** bound to various simple aliphatic acids as evidenced by shifts in the aromatic region of the NMR spectra (Figure 6).

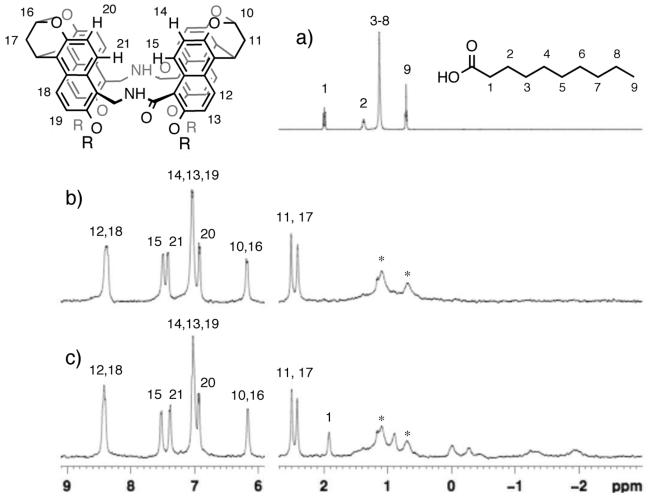

Figure 6. NMR (D_2O with 40 mM Na_2CO_3) of (a) decanoic acid; (b) **5a**; (c) **5a** with decanoic acid. Resonances marked with an asterisk (*) are due to impurities in the sensor sample.

The largest changes in the sensor aromatic region are the hydrogens labeled 15 and 21. This result supports a conformational change since those are the hydrogens most affected by such a change. Also, as seen in Figure 6c, bound lipid exhibits broad peaks between zero and −2 ppm, indicating that the lipid is located inside the tube. The broadness of the resonances indicate that the bound guest is exchanging with free guest on the NMR time scale even when bound, making assignment of individual peaks in the bound lipid impossible. The latter result is distinct from that observed with lipid

Table 1. Association Constants and Fluorescence Changes of Sensors 5 and 6 with Lipid Guests[a]

		sensor **5a**		sensor **5b**		sensor **6a**		sensor **6b**	
entry	lipid	K_a (M^{-1})	I_{sat}/I_0[b]	K_a (M^{-1})	I_{sat}/I_0[b]	K_a (M^{-1})	I_{sat}/I_0[b]	K_a (M^{-1})	I_{sat}/I_0[b]
1	Butyric acid	17	1.25	18	0.92				
2	Hexanoic acid	100	1.14	72	0.32	800	1.54		
3	Octanoic acid	250	0.39	190	0.25	17000	1.78	5400	1.37
4	Decanoic acid	3500	0.48	3800	0.32	180000	1.97	120000	1.46
5	Dodecanoic acid	18000	0.47	27000	0.31	520000	1.79	320000	1.79
6	*trans*-3-Octenoic acid	92	0.61	110	0.26	1300	0.61	7200	0.61
7	4-Methyl-octanoic acid	71	1.05	200	0.32	5200	2.02	5800	1.44
8	8-Methyl-nonanoic acid	1600	0.57	690	0.25	93000	2.07	13000	1.61
9	Cyclohexane carboxylic acid	470	7.1	100	0.80			157	1.11
10	1-Heptanol	4900	0.28	6700	0.19	430000	1.1	230000	1.47
11	*cis*-4-Hepten-1-ol	1200	1.32	5200	0.58	39000	0.64	28000	1.22
12	Heptylamine	16000	0.42	16000	0.11	980000	0.63	3100000	1.25

[a]Measured by titration of the sensor with the indicated lipid under the conditions listed in Figure 5. Error in K are ±10%. [b]I_{sat} is the fluorescence intensity at saturation taken from the fit of the titration data; I_0 is initial fluorescence intensity.

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binding in closed capsules in which the bound alkane resonances are often sharp, presumably because the lipid is not exchangeable on the NMR time scale.[5c] Importantly, small guests or guests with branching, such as 4-methyl-octanoic acid did not exhibit any resonances below zero ppm when added to a solution of sensor **5a** which further supports the notion that such short or branched lipids do not thread into the cavity.

To further explore our hypothesis, tubes **6a** and **6b** were prepared and tested via fluorescence titrations with lipids. As seen in Table 1, the binding constants of these two sensors mirrored those of **5a** and **5b** except that all association constants were higher for sensor **6a** and **6b** owing to the more hydrophobic and rigidly open cavity. For sensor **6a**, very similar trends in association constant were observed compared to sensor **5a**, with more hydrophobic guests binding better. Indeed the binding of straight-chain alcohols and amines to **6a** is very strong (entry 10 and 12). The binding selectivity for long straight chain alkanes was still quite evident from the data: both branching and alkenes drastically decreased affinity (compare entries 3, 6, and 7). Chains with branching at the end bind well, presumably because the branch can thread all the way through the tube (entry 8).

Although sensor **6a** mirrored **5a** in binding affinity, the fluorescent response of the two sensors was quite different. Because the allyl linkers of **6a** enforced an open cavity, we expected that all guest binding would produce a fluorescence increase based on the environment sensitive nature of the fluorophores. Indeed, addition of hydrophobic analytes to both sensors **6a** and **6b** gave fluorescence increases upon binding of nearly every analyte. This data validates our hypothesis on the mechanism of fluorescence changes observed in **5a**. Interesting, three analytes did quench the fluorescence of sensor **6a**: the two alkene-bearing lipids (entries 6 and 11) and the amine (entry 12). Amines are known to act as fluorescence quenchers due to photoinduced electron transfer (PET) mechanisms, so this result is not surprising. The origin of the quenching affect of the alkene guests is less clear, though it should be noted that octenoic acid also quenches the less rigid tube **6b**, thus the quenching appears to be related to analyte rather than a specific interaction with the sensor. Indeed there is some evidence that alkenes can quench fluorescence.[18] Overall, the binding data supported our hypothesis in which a conformational transition gives rise to a distinct fluorescence sensing mechanism in sensor **5a**, while sensor **6a** does not possess such a conformational transition.

CONCLUSIONS

The pair of molecular tubes **5a** and **6a** represent competent sensors for lipids, giving shape-selective sensing of straight chain alkanes in water. In particular, the syn amide-linked isomer **5a** reports on the presence of hydrophobic compounds with shape selective recognition by fluorescent quenching for straight-chain lipids and fluorescent enhancement for other lipids. Our hypothesis regarding this selective fluorescent response stems from the two potential conformations of the receptor. Our calculations suggest that the collapsed structure is disfavored, however, in water the hydrophobic effect may override the gas phase tendency of the tube to remain in the open conformation, and allow the collapsed structure to dominate. Then, guests which bind by threading through the tube return the structure to its open conformation, producing a fluorescence decrease. Guests which cannot thread through the tube, either due to branching, *cis*-alkenes, or even small size, give a small increase in fluorescence as expected for a sensor which is constructed from an environment sensitive fluorophore. To our knowledge, this type of spectroscopic selection between simple lipids based only on the shape of their hydrophobic surface is unprecedented. Our hypothesis was supported by the binding affinity and fluorescent responses of the rigid allyl-linked sensor **6a** because this sensor is held rigidly open. Indeed all guests bound sensor **6a** with much higher affinity since guest binding did not have to overcome the energetic penalty of forcing the tube to adopt an open conformation. Furthermore, most alkane guests give a fluorescence increase as expected as a consequence of ejecting water from the center of the open tube. This sensor not only validates our hypothesis, it also stands as a competent "turn-on" sensor for lipids, selective for straight chain alkanes.

EXPERIMENTAL SECTION

Compound 7. NaH (3.56 g, 60% in mineral oil, 89 mmol) was added to a solution of 2,6-dihydroxynaphthalene (13.4 g, 90 mmol) in DMF (400 mL). After stirring for 45 min, ethyl bromoacetate (10.0 mL, 90 mmol) was added via syringe, and the resulting mixture was stirred for 16 h. The solvent was removed *in vacuo*, and the residue was partitioned between EtOAc and 10% HCl. After collecting the organic layer, the aqueous phase was extracted with additional EtOAc (2 × 200 mL). The combined organic layers were dried over Na_2SO_4 and filtered. After removal of the solvents *in vacuo*, purification by chromatography (1−3% Et_2O/DCM) gave the monoalkylated product (6.78 g, 31%) as a white solid (m.p.: 133−134 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.61 (d, 1H, J = 8.6 Hz), 7.59 (d, 1H, J = 8.9 Hz), 7.19 (dd, 1H, J = 9.0, 2.6 Hz), (m, 2H, J = 9.3 Hz), 7.03 (d, 1H, J = 2.6 Hz), 5.013 (s, 1H), 4.71 (s, 2H), 4.30 (q, 2H, J = 7.1 Hz), 1.31 (t, 3H, J = 7.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 169.1, 154.3, 152.2, 130.4, 129.4, 128.6, 128.1, 119.2, 118.3, 109.7, 107.5, 65.6, 61.5, 14.2. IR (neat, cm^{-1}): 3383, 2981, 1732, 1604, 1442, 1387, 1206, 1160, 1074. HRMS for M^+ calcd for $C_{14}H_{14}O_4$ 246.0892, found 246.0891.

The monoalkylated dihydroxynaphthalene (14.94 g, 60.7 mmol) was dissolved in nitromethane (150 mL), DCM (50 mL) and TFA (50 mL). 1,1,3,3-Tetramethoxypropane (6.0 mL, 36.4 mmol) was added via syringe pump over 1 h while stirring. Stirring was continued for an additional 3 h, after which the reaction was quenched with excess saturated aqueous Na_2CO_3. The organic layer was extracted with DCM, dried with $MgSO_4$, and the solvent was evaporated *in vacuo*. The crude product was purified by filtering through a plug of silica (1 EtOAc: 1 DCM) followed by recrystallization from EtOAc to give compound **7** (13.2 g, 82%) as a white solid (m.p.: 155−156 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.45 (d, 2H, J = 9.3 Hz), 7.44 (d, 2H, J = 8.9 Hz), 7.29 (dd, 2H, J = 9.3, 2.7 Hz), 7.11 (d, 2H, J = 8.9 Hz), 6.98 (d, 2H, J = 2.7 Hz), 6.23 (d, 1H, J = 1.2 Hz), 5.23 (s, 1H), 4.65 (s, 4H), 4.26 (t, 4H, J = 7.2 Hz), 2.38 (t, 2H, J = 2.5 Hz) 1.27 (t, 6H, J = 7.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.7, 154.7, 149.6, 131.1, 127.8, 127.4, 125.1, 119.6, 119.5, 118.9, 109.2, 92.1, 66.0, 52.6, 27.3, 23.5. IR (neat, cm^{-1}): 2981, 1756, 1605, 1389, 1202, 1082, 1013. HRMS for M^+ calcd for $C_{31}H_{28}O_8$ 528.1784, found 528.1785.

Compound 8. Compound **7** (2.87 g, 5.4 mmol) was dissolved in DCM (50 mL) and nitromethane (50 mL). The solution was cooled in an ice bath. HOAc (0.05 mL, 0.87 mmol) and Br_2 (0.65 mL, 12.6 mmol) were added. The solution was stirred for one hour and warmed to room temperature for an additional 2 h. The reaction was quenched with excess saturated aqueous $Na_2S_2O_3$. The organic layer was extracted with DCM, dried with Na_2SO_4 and the solvent was removed. The crude product was purified by chromatography (DCM) followed by treatment with activated carbon to give compound **8** (3.52 g, 95%) as a white solid (m.p.: 175−178 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.42 (d, 2H, J = 9.3 Hz), 8.08 (d, 2H, J = 9.3 Hz), 7.26 (d, 4H, J = 9.2 Hz), 6.28 (d, 1H, J = 1.1 Hz), 5.25 (s, 1H), 4.77 (s, 4H), 4.28 (q, 4H, J = 7.1 Hz), 2.43 (t, 2H, J = 2.5 Hz), 1.29 (t, 6H, J = 7.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 168.7, 151.0, 149.8, 129.4, 128.2, 127.3, 123.1, 120.5, 118.3, 115.9, 112.1, 91.4, 67.3, 61.5, 26.8, 23.2, 14.2. IR (neat,

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cm^{-1}): 1756, 1599, 1214, 1108, 1085. HRMS for M + H^+ calcd for $C_{31}H_{27}O_8Br_2$ 685.0073, found 685.0064.

Compound 10. Compound **9** (1.2 g, 2.4 mmol) was dissolved in DCM (25 mL) and $MeNO_2$ (25 mL) and chilled to 0 °C, followed by addition of acetic acid (22 μL) and bromine (0.28 mL, 5.5 mmol). The resulting solution was stirred 1 h at 0 °C, and then 2 h at room temperature before quenching with saturated $Na_2S_2O_3$ aqueous solution. After extraction 3 times with CH_2Cl_2, the combined organic layers was dried over $MgSO_4$, filtered and evaporated to dryness. The gray solid (1.75 g, 98%) was used without further purification. The obtained dibromide (400 mg, 0.607 mmol), $PdCl_2(PPh_3)_2$ (34.4 mg, 0.048 mmol), CsF(233.2 mg, 1.52 mmol), PPh_3 (64.3 mg, 0.243 mmol) were dissolved in DMF. Allyltributylstannane (0.63 mL, 2.13 mmol) was added via syringe. The resulting mixture was stirred 3 days at 85 °C. The DMF was removed *in vacuo*, the residue was flushed through a plug of silica gel with EtOAc. The filtrate was concentrated, then purified by silica column chromatography (30−50% Et_2O/Hexane) to give compound **10** (255 mg, 72%) as a white solid (m.p.: 144−145 °C). ^1H NMR (300mHz, $CDCl_3$) δ: 8.45 (d, 2H, J = 9.4 Hz), 7.76 (d, 2H, J = 9.3 Hz), 7.23 (d, 2H, J = 9.3 Hz), 7.17 (d, 4H, J = 9.2 Hz), 6.26 (s, 1H), 6.03−5.90 (m, 2H), 5.29 (s, 1H), 4.96 (dd, 2H, J = 3.4, 1.6 Hz), 4.92 (dd, 2H, J = 12.3, 1.6 Hz), 4.73 (s, 4H), 3.85 (d, 4H, J = 5.7 Hz), 3.80 (s, 6H), 2.41 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 169.8, 155.2, 151.6, 151.5, 148.8, 129.5, 127.5, 124.5, 122.8, 122.7, 119.1, 118.7, 116.8, 91.3, 82.9, 67.3, 52.1, 31.4, 27.0, 24.7, 23.0. IR (neat, cm^{-1}): 2954, 1759, 1600, 1519, 1403, 1210, 1107, 1059. HRMS for M^+ calcd for $C_{35}H_{32}O_8$ 580.2097, found 580.2098. Elemental analysis calcd %C 72.40, %H 5.56. Found %C 72.48, %H 5.50.

Compound 12. Compound **10** (698 mg, 1.2 mmol), vinyl pinacolboronate (1.25 g, 8.1 mmol), and Neolyst 1 (50 mg, 0.05 mmol) were placed in a 100 mL round-bottom flask under N_2, dissolved in 50 mL DCM, and brought to reflux. Additional quantities of Neolyst 1 (49 mg, 0.05 mmol) and vinyl pinacolboronate (0.91 g, 5.9 mmol) were dissolved in 5 mL DCM each and added to the reaction via syringe pump over several hours. The solvent was removed after 24 h and the crude product was purified by flash chromatography on silica gel (1−7% EtOAc/DCM) followed by precipitation from PhH with hexanes to give compound **12** (704 mg, 70%) as a white solid (m.p.: 204−205 °C). ^1H NMR (300 MHz, $CDCl_3$) δ: 8.43 (d, 2H, J = 9.4 Hz), 7.68 (d, 2H, J = 9.3 Hz), 7.21 (d, 2H, J = 9.4 Hz), 7.15 (d, 2H, J = 9.2 Hz), 6.73 (dt, 2H, J = 17.9, 5.6 Hz), 6.25 (s, 1H), 5.28 (s, 1H), 5.27 (d, 2H, J = 2.7 Hz), 4.71 (s, 4H), 3.95 (t, 4H, J = 2.3 Hz), 3.80 (2, 6H), 2.42 (s, 2H) 1.17 (s, 12H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 169.8, 155.2, 151.6, 151.5, 148.8, 129.5, 127.5, 124.5, 122.8, 122.7, 119.1, 118.7, 116.8, 91.3, 82.9, 67.3, 52.1, 31.4, 27.0, 24.7, 23.0. IR (neat, cm^{-1}): 2977, 1761, 1633, 1361. HRMS for M^+ calcd for $C_{47}H_{54}B_2O_{12}$ 832.3801, found 832.3802.

Compound 13. Bromide **8** (44.1 mg, 0.064 mmol), boronate ester **12** (53.9 mg, 0.065), CsF (43.9 mg, 0.289 mmol), Pd_2dba_3 (3.7 mg, 0.004 mmol) and S-Phos (7.1 mg, 0.017 mmol) where placed in a 100 mL round-bottom flask with a reflux condenser. The system was evacuated under vacuum and refilled with N_2 (×3). Freshly distilled THF 50 (mL) was added. The system was evacuated and refilled with N_2 (×3). This mixture was then refluxed over 48 h. The solvent was removed and the crude product was purified by flash chromatography on SiO_2 (0−5% Et_2O/DCM) to afford compound **13b** (anti, 14 mg, 19%) as a white solid (m.p.: 235 °C dec.) and compound **13a** (syn, 14 mg, 19%) as a white solid (m.p.: 195 °C dec.).

13a: ^1H NMR (500mHz, CD_2Cl_2) δ: 8.23, (d, 2H, J = 9.4 Hz), 8.09 (d, 2H, J = 9.3 Hz), 7.77 (t, 4H, J = 8.2 Hz), 7.10 (d, 2H, J = 9.2 Hz), 7.07 (d, 2H, J = 9.3 Hz), 6.96−6.91 (m, 4H,), 6.88 (d, 2H, J = 9.2 Hz), 6.20 (s, 1H), 6.17 (s, 1H), 5.43−5.37 (m, 2H), 5.27 (s, 1H), 5.22 (s, 1H), 4.78−4.47 (m, 8H), 4.20−4.13 (m, 6H), 3.84 (dd, 2H, J = 15.7, 4.7), 3.79 (s, 6H), 2.60 (s, 1H), 2.52 (s, 1H), 1.20, (t, 2H, J = 7.2 Hz). ^{13}C NMR (75 MHz, CD_2Cl_2) δ: 170.3, 169.3, 151.5, 150.5, 149.2, 149.1, 136.1, 128.4, 128.3, 127.7, 127.5, 125.1, 124.5, 124.1, 123.3, 123.1, 122.8, 122.6, 119.3, 119.2, 118.8, 118.5, 115.8, 116.3, 91.1, 90.9, 68.0, 67.3, 61.1, 52.1, 29.6, 26.0, 25.6, 22.4, 14.0. IR (Neat, cm^{-1}): 2976, 1756, 1598, 1401, 1207, 1109, 1062. IR (neat, cm^{-1}): 2976, 1755, 1598, 1518, 1401, 1207, 1109, 1062. HRMS for $(M + H)^+$ calcd for $C_{66}H_{57}O_{16}$ 1105.3647, found 1105.3643.

13b: ^1H NMR (300mHz, CD_2Cl_2) δ: 8.23, (d, 2H, J = 9.4 Hz), 8.07 (d, 2H, J = 9.3 Hz), 7.77 (d, 4H, J = 8.2 Hz), 7.07 (d, 2H, J = 9.3 Hz), 7.00−6.90 (m, 4H,), 6.84 (d, 2H, J = 9.2 Hz), 6.20 (s, 2H), 5.43−5.36 (m, 2H), 5.23 (s, 1H), 4.65 (d, 4H, J = 9.2 Hz), 4.57 (s, 4H), 4.37 (dd, 2H, J = 14.1, 4.1), 4.19−4.03 (m, 4H), 3.75 (s, 6H), 3.63 (dd, 2H, J = 14.4, 9.4), 2.60 (s, 1H), 2.49 (s, 1H), 1.07, (t, 2H, J = 11.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 169.7, 169.0, 150.6, 150.5, 149.0, 149.0, 136.8, 129.0, 128.5, 127.3, 126.9, 125.0, 124.2, 123.7, 123.6, 122.9, 122.8, 118.9, 118.9, 118.7, 118.1, 114.1, 113.7, 91.2, 91.0, 67.1, 67.0, 60.9, 52.1, 29.2, 26.1, 25.6, 22.5, 22.4, 13.3. IR (Neat, cm^{-1}): 2975, 1758, 1598, 1400, 1209, 1108, 1062. HRMS for $(M + H)^+$ calcd for $C_{66}H_{57}O_{16}$ 1105.3647, found 1105.3625.

Sensor 6a. Compound **13a** (3.24 mg, 0.003 mmol) was placed in a 4 mL vial with a stir bar and fitted with a septum screw cap. The vial was purged with N_2. THF (1.3 mL), CH_3OH (0.65 mL), and NaOH (0.65 mL 6M) were added and the contents were stirred and sparged with N_2 for 15 min and then stirred thereafter for an additional 75 min. The vial was cooled in a methanol/ice bath, and quenched with chilled aqueous TFA (0.65 mL, 6M). The mixture was transferred to a separatory funnel containing chilled water. The aqueous phase was made slightly acidic with aqueous TFA (6M) and extracted into DCM/THF (×3). The extracts were rinsed with water (×2), then brine and dried over Na_2SO_4. The solvent was removed *in vacuo* at room temperature. The crude material obtained was triturated (×2) with a 1:1 mixture of DCM and pentane to afford sensor **6a** (2.9 mg, 97%) as a white solid (m.p.: 182 °C dec.). ^1H NMR (300 mHz, $CDCl_3$, CD_3OD) δ: 8.27, (d, 2H, J = 9.4 Hz), 8.12 (d, 2H, J = Hz), 7.76 (d, 2H, J = Hz), 7.73 (d, 2H, J = Hz), 7.11 (d, 2H, J = Hz), 7.06 (d, 2H, J = Hz), 6.92 (m, 6H), 6.24 (s, 1H), 6.20 (s, 1H), 5.50−4.41 (m, 2H), 5.30 (s, 1H), 5.25 (s, 1H), 4.73−4.55 (m, 8H), 4.16 (dd, 2H, J = 15.4, 8.9), 2.65 (s, 1H), 2.57 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$, CD_3OD) δ: 174.2, 173.7, 151.2, 150.3, 149.2, 149.0, 136.6, 128.6, 128.4, 127.3, 126.8, 124.7, 123.5, 123.4, 123.2, 123.1, 122.8, 122.5, 119.6, 119.4, 119.3, 118.7, 114.7, 112.7, 91.4, 91.3, 67.7, 66.8, 29.3, 26.1, 25.7, 22.6, 22.6. IR (neat, cm^{-1}): 2928, 1731, 1598, 1517, 1401, 1215, 1061. HRMS for $(M + H)^+$ calcd for $C_{60}H_{45}O_{16}$ 1021.2708, found 1021.2711.

Sensor 6b. Compound **13b** (2.66 mg, 0.002 mmol) was placed in a 4 mL vial with a stir bar and fitted with a septum screw cap. The vial was purged with N_2. THF (1.3 mL), CH_3OH (0.65 mL), and NaOH (0.65 mL 6M) were added and the contents were stirred and sparged with N_2 for 15 min and then stirred thereafter for an additional 75 min. The vial was cooled in a methanol/ice bath, and quenched with chilled aqueous TFA (0.65 mL, 6 M). The mixture was transferred to a separatory funnel containing chilled water. The aqueous phase was made slightly acidic with aqueous TFA (6 M) and extracted into DCM/THF (×3). The extracts were rinsed with water (×2), then brine and dried over Na_2SO_4. The solvent was removed *in vacuo* at room temperature. The crude material obtained was triturated (×2) with a 1:1 mixture of DCM and pentane to afford sensor **6b** (2.4 mg, 98%) as a white solid (m.p.: 192 °C dec.). ^1H NMR (300 mHz, $CDCl_3$, CD_3OD) δ: 8.22 (d, 2H, J = 9.4 Hz), 8.06 (d, 2H, J = 9.4 Hz), 7.80 (d, 2H, J = 9.3 Hz), 7.75 (d, 2H, J = 9.3 Hz), 7.02 (m, 6H), 6.95 (d, 2H, J = 9.3 Hz), 6.88 (d, 2H, J = 9.2 Hz), 6.16 (s, 2H), 5.46 (m, 2H), 5.25 (s, 2H), 4.65 (s, 2H), 4.63 (s, 2H), 4.52 (s, 2H), 4.51 (s, 2H), 4.39 (dd, 2H, J = 15.0, 3.8 Hz), 3.72(dd, 2H, J = 14.8, 8.8 Hz), 2.62 (s, 2H), 2.52 (s, 2H) ^{13}C NMR (75 MHz, $CDCl_3$, CD_3OD) δ: 171.9, 171.9 151.0, 150.9, 149.2, 137.3, 129.3, 128.9, 127.6, 127.6, 126.3, 125.2, 124.1, 124.0, 123.6, 123.1, 123.0, 122.8, 119.2, 119.2, 119.0, 118.3, 115.0, 114.4, 91.4, 91.2, 67.4, 66.9, 29.3, 26.3, 25.8, 22.5 IR (neat, cm^{-1}): 2920, 1732, 1595, 1515, 1459, 1402, 1211, 1105, 1059. HRMS for $(M + H)^+$ calcd for $C_{60}H_{45}O_{16}$ 1021.2708, found 1021.2726.

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■ ASSOCIATED CONTENT

Supporting Information

NMR spectra of new compounds and additional fluorescence data on sensors **5a**, **5b**, **6a**, and **6b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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