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Exploring the effects of cooperative interactions on affinity using a pinwheel sensor system

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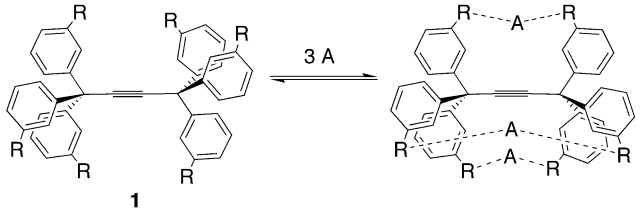
Abstract—A series of three pinwheel sensors were constructed with 1, 2, and 3 binding sites. Binding of Zn^{+2} and Cd^{+2} was monitored by fluorescence over a range of temperatures. The data demonstrate that cooperative interactions generally increase the effective affinity of the sensor. This effect is more pronounced in systems which have lower inherent affinity for the analyte.

1. Introduction

Fluorescent chemical sensors are becoming an increasingly valuable tool for the detection of many analytes in a wide range of applications.[1] As part of an ongoing project geared toward creating more effective chemical sensors, we have been exploring a novel class of cooperative receptors based on bistrityl acetylene compounds, termed pinwheel receptors (Scheme 1).[2] The recognition elements appended to the trityl skeleton bind the analyte across the receptor framework producing three identical binding pockets. Cooperative recognition is seen because binding of the first analyte preorganizes the symmetrical receptor into a conformation in which the second and third analyte can bind more strongly. This mechanism of cooperativity is conceptually similar to the restricted rotational freedom employed by Rebek's cooperative biphenyl bis-crown ether[3] and Shinkai's cooperative porphyrin sandwich complexes.[4]

In fact, several artificial cooperative receptors have been developed,[5] though few have been applied toward chemical sensor applications.[6] From a design perspective, this method of recognizing a guest is attractive. Many elegant (non-cooperative) receptor designs have been developed over the years in which two or more recognition elements are displayed in a convergent fashion for binding of a guest. Strong binding is most often observed when the recognition elements are held rigidly apart such that there is little entropic loss to the receptor upon binding the guest.[7] The challenge of receptor design becomes one of creating the optimal spacing and orientation of the recognition elements in a rigid fashion.[8] For receptors of the class depicted in Scheme 1, there is a range of distances which the recognition elements can span owing to free rotation about the acetylenic axis. Binding of the first analyte rigidly sets the correct distance between the remaining pairs of binding groups. Thus, the entropic loss upon binding the analyte is effectively averaged over three consecutive binding events, diminishing the overall entropic penalty to binding. This receptor design should be general for a range of analytes of varying size within the outer limits of receptor framework. The spacing between the recognition elements need only be roughly adjusted to the size of the desired guest. Upon binding of the first guest, the receptor is then fully organized to match the size of the guest.

We have previously argued that the type of cooperative recognition described above increases the affinity and selectivity of a sensor for its analyte relative to a similar non-cooperative sensor.[2a] These points were initially addressed using sensors for metal ions[2b] and dicarboxylates.[9] In this report, we explore these issues using a series of



R=Recognition element; A=Analyte

Scheme 1.

Keywords: Sensor; Allosteric; Cooperative.

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metal ion sensors with varying numbers of binding sites. Comparison of the recognition properties of sensors with varying degrees of cooperativity provides insight into the advantages and disadvantages of the cooperative recognition method.

2. Results

The sensors used for this study are shown in Figure 1. Compounds **2a–c** are based on a bis-acetylene bis-trityl system for ease of synthesis.[10] The amino-methoxyquinaldine groups would serve not only as recognition elements, but also as the fluorescent readout mechanism. Two recognition elements (R) should bind a metal ion between them, across the bisacetylene axis in a tetrahedral fashion (as per Fig. 1). The benzylic amine will participate in photoelectron transfer (PET)[1b] quenching of the fluorophore in the unbound state. Upon metal chelation the amine will no longer be able to quench the fluorophore and a significant increase in fluorescence is anticipated. Receptor **2a**, with one set of binding groups, was designed to be a non-cooperative receptor while receptors **2b** and **2c** were designed to be two-fold and three-fold cooperative receptors, respectively. Assuming that the binding pockets in the three molecules are similar, this series of sensors should provide information on the effect of cooperativity on the recognition properties of the system.

2.1. Synthesis

The synthesis of receptors **2a–c** is shown in Scheme 2. The fluorophore portion was prepared by bromination of methoxyquinaldine followed by substitution with methylamine to give **5**. Trityl chlorides **6a–c** were reacted with ethynyl Grignard to yield alkynes **7a–c**. Dimerization of the alkynes was effected by copper chloride and *N*-methyl pyrrolidine or TMEDA under aerobic conditions. Treatment of butadiyne **8a–c** with excess titanium tetrachloride and α,α-dichloromethyl methyl ether regioselectively formylated only the anisole rings, yielding aldehydes **9a–c**. Reductive amination of the aldehydes with amine **5** using triacetoxy sodium borohydride yielded sensors **2a–c**.

2.2. Metal ion binding studies

2.2.1. UV/vis studies. Metal ion binding was first studied by titrating sensors **2a–c** with metal ions and following changes in the UV/vis absorptions. All three sensors appeared to bind many metals tightly including Ni^{+2}, Cd^{+2}, Zn^{+2}, Mn^{+2}, Hg^{+2}, and Ag^{+1}. Figure 2 shows a representative example of the titrations of sensors **2a–c** with $Cd(ClO_4)_2$ in acetonitrile. All metals produced a red-shift in the major UV band centered at 325 nm. With the exception of extinction coefficient, which varied as per the number of chromophores per molecule, all three sensors had similar UV behavior indicating that the binding pockets are similar between the various sensors.

Unfortunately, all of the binding constants were too high to derive useful data from the UV/vis titrations. In fact, titration curves based on Figure 2 showed near linear responses which saturated at approximately the stoichiometric point for each sensor (1, 2, and 3 equiv of Cd^{+2} for sensors **2a**, **2b**, and **2c**, respectively). These results verify the expected stoichiometry of the sensors.

2.2.2. Fluorescence studies. The fluorescence titrations of the sensors were much more informative as the concentration of sensor required for such analysis was significantly lower (0.3 μM). Of all of the metals tested, only zinc and cadmium produced well behaved fluorescence modulation

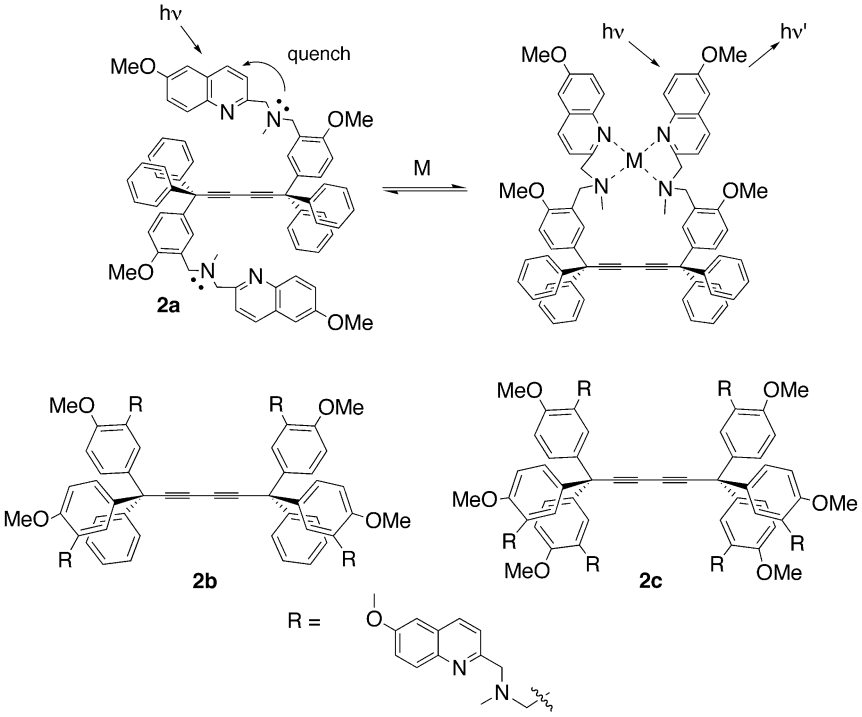
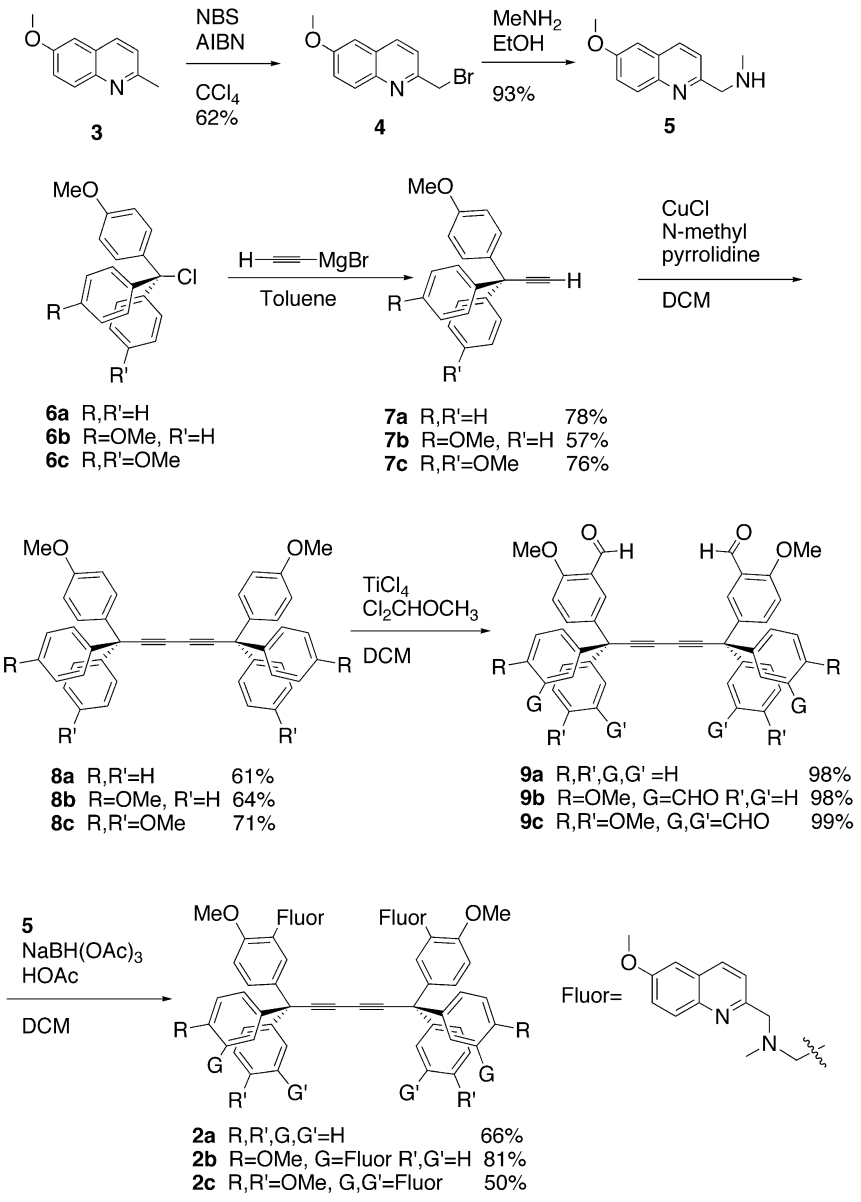


Figure 1. A series of three bis-trityl receptors.



Scheme 2.

when added to the sensor. Figure 3 shows a representative example in which **2a** is titrated with Zn^{+2} in acetonitrile. As anticipated, the sensor has little native fluorescence due to the PET quenching of the benzylic amines. Binding of the metal ions produced a substantial increase in fluorescence. Generally, cadmium produced a 3–4 fold increase in emission at 393 nm (excited at 336 nm) and zinc produced a 7–9 fold increase in emission at the same wavelength. It is likely that quenching metal ions such as silver did not give a good response with this sensor because they quench the fluorophore as they bind, retaining an 'off' state of the sensor.

The binding isotherms for all three sensors with zinc are shown in Figure 4. While the fluorescence experiment allows the concentration of host to be very low, the apparent dissociation constants of the receptors are still close to the concentration of host. This implies that the concentration of added guest does not really approximate the concentration of free guest in solution very well. Since the binding isotherms used to calculate the dissociation constants rely on free guest concentrations, the data were corrected using the known concentration of sensor so that the *x*-axis reflects the equilibrium concentration of free zinc in solution. Using this correction, more accurate dissociation constants and Hill coefficients could be obtained. Therefore, this correction was applied to all titration data.

Binding constants for the three sensors with Zn^{+2} and Cd^{+2} were determined by fitting the experimental data to the Hill equation (Table 1). For the purpose of internal consistency, sensor **2a** was fitted to the Hill equation and in all cases gave a Hill coefficient of very near 1.0, which is consistent with a non-cooperative sensor.

The UV/vis titration data was used to determine the stoichiometry of the sensors. In order to verify this in the fluorescence mode, job plots were prepared. As expected,

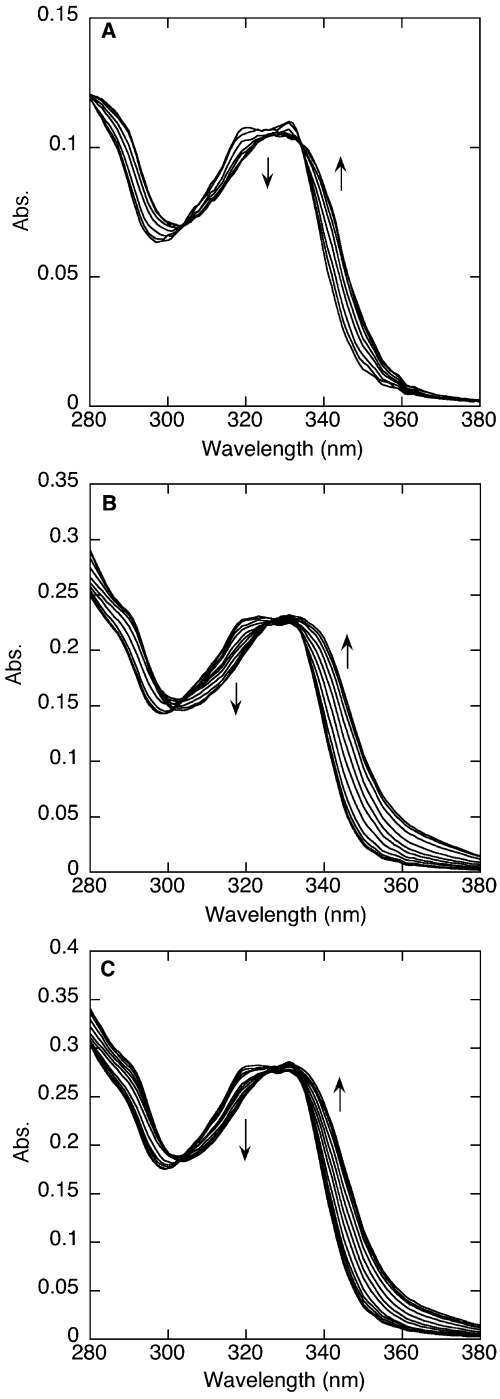


Figure 2. UV/Vis titration of sensors **2a–c** ([**2**] = 10 μM) with $Cd(ClO_4)_2$ in CH_3CN with 0.5 mM $NaClO_4$ as a supporting electrolyte. (A) Sensor **2a**. (B) Sensor **2b**. (C) Sensor **2c**.

each sensor gave a maximum change in fluorescence close to its stoichiometric point for both metals tested. The Job plot for the three sensors and Cd^{+2} are overlaid in Figure 5. Sensor **2a** had maximal change in fluorescence at 48 mol% fraction of Cd^{+2}, sensor **2b** at 67% and sensor **2c** at 73%. Interestingly, sensors **2b** and **2c** show suppressed changes in fluorescence at low mole fraction of metal, in keeping with the cooperative nature of these receptors.

2.2.3. Variable temperature studies. Fluorescence titrations were then conducted with receptors **2a–c** and

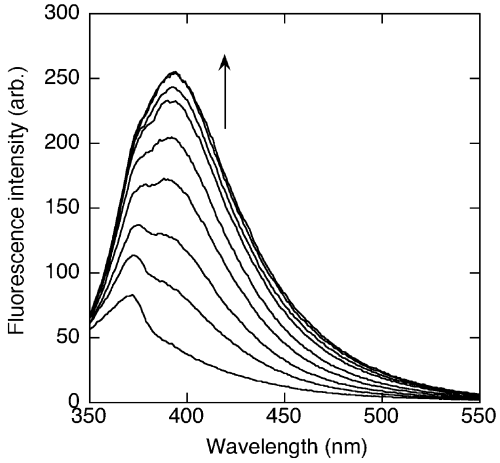


Figure 3. Fluorescence titration of compound **2a** with $Zn(ClO_4)_2$ at 25 °C. [**2a**] = 0.3 μM in CH_3CN with 0.50 mM $NaClO_4$ as a supporting electrolyte. λ_{ex} = 336 nm.

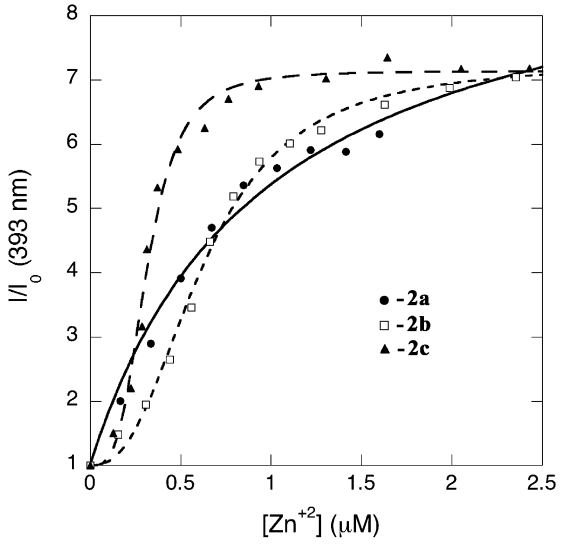


Figure 4. Binding isotherms for receptors **2a–c** with $Zn(ClO_4)_2$ at 393 nm in acetonitrile with 0.50 mM $NaClO_4$ as a supporting electrolyte. Concentration of Zn^{+2} is given as the equilibrium concentration of free zinc in solution. (A) Receptor **2a**. The best fit line (solid) is to a single site isotherm. (B) Receptor **2b**. The best fit line (short dash) is to a Hill equation. (C) Receptor **2c**. The best fit line (long dash) is to a Hill equation.

$Zn(ClO_4)_2$ at different temperatures to investigate the effect of cooperativity on apparent K_d. Figure 6 summarizes these experiments. Generally, the K_d of the non-cooperative sensor increased with increasing temperature, while the K_d of the cooperative sensors was relatively unaffected.

3. Discussion

Shinkai has described a detailed study of five receptors based on his cooperative cerium sandwich complexes with 1, 2, 3, and 4 sites.[11] While the Hill coefficient correlated nicely with the number of sites, the binding pockets communicated with each other electronically such that it was not possible to compare association constants in a meaningful way. In this study, we attempted to make comparisons of dissociation constants between sensors with

Table 1. Hill coefficients and dissociation constants for sensors **2a–c** under conditions listed in Figure 4

Sensor	Analyte	Hill coeff.	K_d (μM)[a]	I/I_0 max.[b]
2a	Zn^{+2}	0.97	0.85	9.2
2b	Zn^{+2}	1.9	0.54	7.3
2c	Zn^{+2}	2.9	0.33	7.2
2a	Cd^{+2}	0.99	0.42	4.8
2b	Cd^{+2}	2.0	0.26	4.7
2c	Cd^{+2}	2.9	0.26	7.6

[a] Error in K_d is approximately ±15%.
[b] The maximum fluorescence change of each sensor at saturation.

different numbers of binding pockets. It has been shown previously for a related pinwheel sensor, that two sets of binding groups on one trityl unit cannot interact to bind a metal, therefore binding must occur across the acetylene axis as shown in Figure 1. In the case of sensors **2a–c**, the similarity of the three sets of UV titration data indicates that the binding sites are similar between the three sensors. This result is supported by NMR titrations of the three sensors with metal ions (data not shown) in which similar behavior is observed between all three sensors. Therefore, to a first approximation, the binding pocket in **2a** appears to be equivalent to those of **2b** and **2c**.

To compare affinity between sensors, binding constants were determined in terms of apparent dissociation constants (K_d). This value is the half saturation value of the sensor, a value that is commonly used in biological systems when the number of binding sites is unknown.[12] In this case, it allows direct comparison of affinity between sensors with different numbers of sites, since the actual association constants of the three sensors have different units. This type of analysis is also convenient in cases where the Hill coefficient is non-integral implying a mixture of species of varying analyte occupancy. Ultimately, this value is the most useful measure of binding as it describes the concentration of analyte that the sensor is capable of recognizing.

Visual inspection of the binding isotherms in Figure 4 supports our assertion that cooperativity enhances binding. The cooperative sensors have steeper binding isotherms and saturate much more quickly giving an overall lower apparent K_d (higher binding) than the non-cooperative sensor. Moreover, the 3-fold cooperative system (**2c**) has a lower apparent K_d than the 2-fold cooperative sensor (**2b**). Similarly, the effective range of analyte over which the sensor is useful is larger for the non-cooperative system (**2a**) than **2b** which is again larger than the **2c**. Thus, the advantages and disadvantages of cooperativity are immediately evident.

As expected, the hill coefficients for sensors **2b** and **2c** correlated to the number of binding sites indicating that the multi-site sensors are binding in a cooperative mode. It is evident from Figure 4 and Table 1 that for zinc, the cooperative sensors bind more tightly than the non-cooperative sensors. However, the differences in this case (ca. 2.5-fold decrease in K_d for **2c** vs. **2a**) are small compared to what we have observed previously (50-fold).[2a] We believe that the difference arises from the high inherent affinity of **2** for metal ions. The contribution of cooperativity arises from the freezing out of rotational entropy upon binding of the first metal ion. The higher affinity of the second metal ion can be viewed in terms of free energy of association in which the total free energy is a combination of the inherent free energy of the recognition elements for the metal plus the free energy of cooperativity[12] (which stems from preorganization of the sensor upon the first binding event). Therefore, it stands to reason that in this system where the inherent affinity of the sensor for zinc is quite high (large free energy of association) the added free energy of cooperativity makes less of an impact in terms of higher affinity compared to a system with lower inherent affinity for the analyte. This assertion is qualitatively supported by the cadmium binding data (Table 1) in which **2a** binds Cd^{+2} even more tightly than Zn^{+2} and

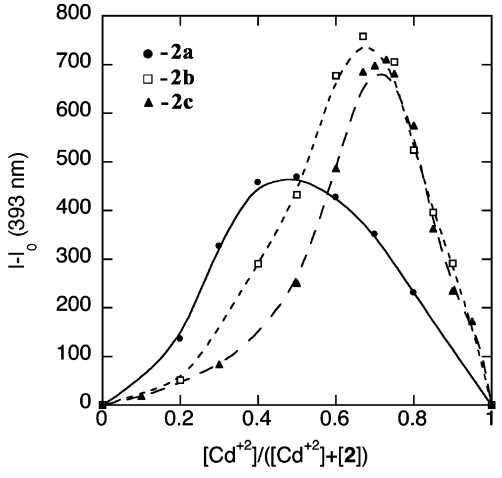


Figure 5. Job plot for receptors **2a–c** with $Cd(ClO_4)_2$. $[Cd^{2+}]+[host]=20$ μM in CH_3CN with 0.50 mM $NaClO_4$ as a supporting electrolyte. The lines are merely illustrative and do not represent a fit to the data.

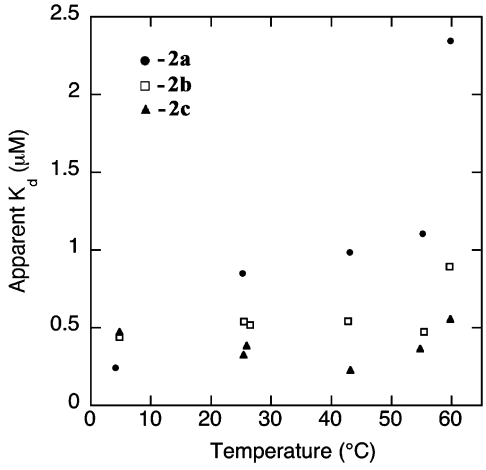


Figure 6. Apparent K_d of sensors **2a–c** with $Zn(ClO_4)_2$ as a function of temperature.

the ratio of K_d for **2c** and **2a** is smaller. Thus, it is reasonable to expect that cooperativity will have the biggest impact on association in systems with relatively low inherent free energy of association.

To explore these effects further, we examined the effect of temperature on K_d. The plot of K_d versus temperature (Fig. 6) has some scatter due to the error inherent in calculation of K_d, however, the trends are very informative. The non-cooperative sensor demonstrated the greatest variability in K_d over the relatively small range of temperatures explored. The increase in K_d (decrease in affinity) as temperature increased is in agreement with expectation since the organization of the sensor to bind the metal is entropically disfavored. At higher temperatures, binding affinity should be decreased. Interestingly, the cooperative sensors showed little variation in apparent K_d over the temperature range. Again this is consistent with the picture of a sensor architecture in which the entropic loss in organizing the system for binding is averaged over multiple binding events. Thus the binding of the cooperative receptors is expected to be dominated by the enthalpic contribution of the metal–ligand bonds. It is evident that as the binding affinity of the non-cooperative system decreases (at higher temperature), the effect of cooperativity is more pronounced. Thus the cooperative sensor **2c** has a five-fold greater effective binding constant for zinc than **2a** at the higher temperatures.

4. Conclusions

A series of three sensors for metal ions has been synthesized which vary by the number of binding sites. The sensors incorporate an amino-methoxyquinaldine moiety which functions not only as a recognition unit but also as a PET based fluorescent readout. Fluorescence studies were performed at various temperatures and association was measured in terms of apparent K_d. Binding data for zinc and cadmium ions indicated that cooperative interactions produced an increase in affinity by lowering the entropic penalty for organizing the receptor for tight binding. The extent of the increase in affinity depended on the inherent strength of the recognition elements for the analyte. Taken together, these studies highlight the advantages of cooperative recognition.

5. Experimental

5.1. General procedures

NMR spectra were recorded on a Bruker WP-200, AC-200, DPX-300, AMX-360, DRX-400 in $CDCl_3$ with residual protonated solvent or tetramethylsilane (TMS) as an internal reference unless otherwise noted. Fluorescence titrations were conducted on a Shimadzhu 5301PC spectrofluorimeter using 0.5 or 1.0 mL sample volumes with 0.3 μM sensor and 0.5 mM $NaClO_4$ as a supporting electrolyte. UV/vis titrations were conducted on a Cary 1E spectrophotometer using 1.0 mL sample volumes and 10 μM sensor and 0.5 mM $NaClO_4$ as a supporting electrolyte. Dissociation constants and Hill coefficients were determined by fitting the experimentally determined binding isotherms to the Hill equation using Kaleidagraph or by the standard linearization techniques for cooperative systems.[13]

5.2. Synthesis

5.2.1. 2-Bromomethyl-6-methoxy-quinoline 4. A mixture of 6-methoxyquinaldine (820 mg, 4.7 mmol) and NBS (838 mg, 4.7 mmol) was refluxed in CCl_4 (100 mL). AIBN (40 mg, 0.24 mmol) was added to the solution. The reaction was refluxed for an additional 3 h then cooled to 10 °C. The reaction mixture was filtered and the filtrate was concentrated in vacuo. The resultant residue was purified via chromatography (SiO_2 1:1 DCM–hexanes) to give compound **4** as a pink solid (732 mg, 62%). Mp=98–100 °C dec. ^{1}H NMR (300 MHz, $CDCl_3$): 8.05 (d, J=8.5 Hz, 1H), 7.96 (d, J=9.2 Hz, 1H), 7.52 (d, J=8.5 Hz, 1H), 7.37 (dd, J=9.2, 2.8 Hz, 1H), 7.12 (d, J=2.8 Hz, 1H), 4.69 (s, 2H), 3.93 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): 159.2, 155.3, 144.5, 136.9, 131.5, 129.2, 123.5, 122.3, 105.7, 55.9, 34.8. FTIR (neat): 2938, 1625, 1599, 1503, 1482, 1380, 1254, 1225, 1163, 1117, 1030 cm^{-1}. HRMS (*m*/*z*): calculated for $C_{11}H_{11}BrNO$ $[M+H]^+$: 252.0019, found: 252.0017.

5.2.2. (6-Methoxy-quinolin-2-yl-methyl)-methylamine 5. Bromide **4** (362 mg, 1.4 mmol) was dissolved in THF (2.0 mL) and added via syringe to a solution of $MeNH_2$ in EtOH (24.2 g, 5.7 mmol $MeNH_2$/g solution) over 1 hr at room temperature. It was found that using the commercially available solutions of methylamine (40% in water or 1 M in THF) gave poorer results. The reaction was stirred for an additional 2 h. The solvent was removed and DCM (50 mL) was added to the resultant solid. Aqueous NaOH (2.0 M) was added via syringe to the stirred DCM suspension until all solids were dissolved (ca. 800 μL). The organic layer was separated and dried with $MgSO_4$. The solvent was removed and the remaining oil purified by chromatography (SiO_2, 15% TEA in EtOAc) to give compound **5** as a yellow oil (262 mg, 93%). ^{1}H NMR (360 MHz, $CDCl_3$): 7.99 (d, J=8.5 Hz, 1H), 7.95 (d, J=9.3 Hz, 1H), 7.39 (d, J=8.5 Hz, 1H), 7.34 (dd, J=9.2, 2.8 Hz, 1H), 7.05 (d, J=2.8 Hz, 1H), 4.01 (s, 2H), 3.90 (s, 3H), 2.53 (s, 3H), 2.26 (br, 1H). ^{13}C NMR (90 MHz, $CDCl_3$): 157.6, 157.4, 143.8, 135.1, 130.3, 128.1, 121.9, 120.8, 105.1, 57.7, 55.4, 36.3. FTIR (neat): 3321, 2937, 2837, 1622, 1601, 1568, 1501, 1461, 1380, 1233, 1163, 1111, 1030 cm^{-1}. HRMS (*m*/*z*): calculated for $C_{12}H_{15}N_2O$ $[M+H]^+$: 203.1179, found: 203.1189.

5.2.3. 4-Methoxytrityl alkyne 7a. Chloride **6a** (2.5 g, 8.1 mmol) was dissolved in argon-sparged toluene (300 mL) and cooled to −78 °C. To this was added ethynylmagnesium bromide (32 mL, 0.5 M in THF) via syringe over 10 min. The reaction was allowed to warm to room temperature and was stirred an additional 3 h. The reaction was quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was dried with $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified via chromatography (SiO_2, 1:5 DCM–hexanes) to give compound **7a** as a white solid (1.889 g, 78%). Mp=100–103 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 7.29–7.15 (m, 10H), 7.17–7.14 (m, 2H), 6.82–6.79 (m, 2H), 3.77 (s, 3H), 2.67 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 158.4, 145.0,

136.9, 130.1, 129.0, 127.9, 126.8, 113.3, 90.0, 73.2, 55.2, 54.8. FTIR (neat): 3301, 3054, 2986, 2306, 1606, 1509, 1490, 1445, 1421, 1264, 1180 cm^{-1}. HRMS (*m/z*): calculated for $C_{23}H_{19}O$ $[M+H]^+$: 299.1430, found: 299.1424.

5.2.4. 1,4-Bis-(4-methoxytrityl)-butadiyne 8a. Alkyne **7a** (1.02 g, 3.40 mmol) and CuCl (3.36 g, 34.0 mmol) were added to DCM (35 mL) at room temperature. *N*-methyl pyrrolidine (7.0 mL, 68 mmol) was added drop wise to the reaction. O_2 was bubbled through the reaction via a dispersion tube for 8 h. The reaction was filtered through a SiO_2 plug with EtOAc to remove the copper salts. The filtrate was concentrated and the residue purified via chromatography (SiO_2, 15% v/v DCM in hexanes) to give compound **8a** as an off white solid (626.7 mg, 61%). Mp= 187–188 °C. ^1H NMR (360 MHz, CD_2Cl_2): 7.32–7.23 (m, 20H), 7.15–7.13 (m, 4H), 6.86–6.82 (m, 4H), 3.78 (s, 6H). ^{13}C NMR (75 MHz, CD_2Cl_2): 159.1, 145.0, 136.7, 130.5, 129.3, 128.5, 127.4, 113.8, 84.9, 70.0, 56.0, 55.6. FTIR (neat): 3057, 2954, 2835, 2037, 1954, 1901, 1605, 1582, 1508, 1489, 1462, 1445, 1298, 1251, 1178 cm^{-1}. HRMS (*m/z*): calculated for $C_{44}H_{35}O_2$ $[M]^+$: 594.2553, found: 594.2564.

5.2.5. 1,4-Bis-(4-methoxytrityl-3-carboxaldehyde)-butadiyne 9a. Bis-trityl **8a** (280 mg, 0.47 mmol) and α,α-dichloromethyl methyl ether (460 μL, 5.0 mmol) were dissolved in DCM (60 mL) and the resulting solution cooled to −5 °C. $TiCl_4$ (660 μL, 6.0 mmol) was added drop wise via syringe over 2 min. The reaction was allowed to warm to room temperature and was stirred an additional 1 hr. The reaction was poured into aqueous HCl (100 mL, 2.0 M) and stirred for 2 min. The aqueous suspension was extracted with EtOAc (3×100 mL). The organic extracts were combined, dried with $MgSO_4$ and concentrated. The resultant residue was purified via column chromatography (SiO_2, 1:1 DCM–EtOAc) to give compound **9a** as an off white amorphous solid (300 mg, 98%). ^1H NMR (300 MHz, CD_2Cl_2): 10.4 (s, 2H), 7.56 (dd, *J*=8.7, 2.7 Hz, 2H), 7.51 (d, *J*=2.5 Hz, 2H), 7.35–7.20 (m, 20H), 7.01 (d, *J*=8.7 Hz, 2H), 3.93 (s, 6H). ^{13}C NMR (75 MHz, CD_2Cl_2): 189.4, 161.4, 144.2, 137.1, 136.8, 129.2, 128.7, 128.6, 127.7, 124.5, 112.3, 84.6, 70.4, 56.3, 56.0. FTIR (neat): 3058, 2942, 2861, 1684, 1604, 1579, 1490, 1446, 1417, 1393, 1282, 1254, 1205, 1180, 1110, 1025 cm^{-1}. HRMS (*m/z*): calculated for $C_{46}H_{35}O_4$ $[M+H]^+$: 651.2530, found: 651.2555.

5.2.6. 1,1,4,4-Tetraphenyl-1,4-bis(2-methoxy-benz-4-yl-(6-methoxyquinolin-2-ylmethyl) methylamino)-butadiyne 2a. Aldehyde **9a** (60 mg, 0.077 mmol) and amine **5** (60 mg, 0.30 mmol) were dissolved in DCM (3.0 mL). To this was added HOAc (14 μL, 0.22 mmol) and $NaBH(OAc)_3$ (61 mg, 0.30 mmol). The reaction was allowed to stir for 10 h. Aqueous NaOH (1 M, 2 mL) was added and the reaction was stirred for 5 min. The organic layer was separated and dried with $MgSO_4$. The solvent was removed and the resultant residue was purified via chromatography twice (SiO_2, 2% TEA/EtOAc then 2% MeOH(NH_3)/DCM) to give compound **2a** as an amorphous yellow solid (53 mg, 66%). ^1H NMR (300 MHz, CD_3CN/CD_2Cl_2): 7.82 (d, *J*=8.5 Hz, 2H), 7.78 (d, *J*=9.2 Hz, 2H), 7.45 (d, *J*=2.5 Hz, 2H), 7.32 (d, *J*=8.5 Hz, 2H), 7.30–7.20 (m, 22H), 6.98 (d, *J*=2.7 Hz, 2H), 6.97 (dd, *J*=8.6, 2.5 Hz, 2H), 6.78 (d, *J*=8.7 Hz, 2H), 3.83 (s, 6H), 3.73 (s, 6H), 3.69 (s, 4H), 3.55 (s, 4H), 2.14 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): 158.3, 157.2, 156.6, 144.8, 143.3, 136.1, 135.1, 131.1, 130.2, 129.0, 128.4, 128.2, 128.0, 126.9, 126.6, 121.5, 121.3, 109.6, 105.2, 84.5, 69.8, 64.1, 55.7, 55.5, 55.3, 42.5. FTIR (neat): 2939, 2836, 1623, 1601, 1558, 1498, 1456, 1377, 1310, 1234, 1161, 1110, 1031 cm^{-1}. HRMS (*m/z*): calculated for $C_{70}H_{64}N_4O_4$ $[M+2H]^{2+}$: 512.2458, found: 512.2439.

5.2.7. 4,4′-Dimethoxytrityl-alkyne 7b. Chloride **6b** (2.5 g, 7.4 mmol) was dissolved in argon sparged toluene (300 mL) and cooled to −78 °C. To this was added ethynylmagnesium bromide (20 mL, 0.5 M in THF) via syringe over 10 min. The reaction was allowed to warm to room temperature and was stirred an additional 3 h. The reaction was quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was dried with $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified via chromatography (SiO_2, 1:4 DCM–hexanes) to give compound **7b** as a white solid (1.400 g, 57%). Mp=107–108 °C. ^1H NMR (300 MHz, $CDCl_3$): 7.35–7.25 (m, 5H), 7.22–7.19 (m, 4H), 6.87–6.84 (m, 4H), 3.83 (s, 6H), 2.72 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 158.3, 145.3, 137.2, 130.1, 128.9, 128.0, 126.8, 113.3, 90.2, 73.0, 55.2, 54.1. FTIR (neat): 3053, 2986, 2305, 1607, 1509, 1421, 1422, 1265, 1179, 1034 cm^{-1}. HRMS (*m/z*): calculated for $C_{23}H_{21}O_2$ $[M+H]^+$: 329.1536, found: 329.1515.

5.2.8. 1,4-Bis-(4,4′-dimethoxytrityl)-butadiyne 8b. Alkyne **7b** (691 mg, 2.1 mmol) and CuCl (2.0 g, 21 mmol) were added to DCM (20 mL) at room temperature. *N*-methyl pyrrolidine (4.4 mL, 42 mmol) was added drop wise to the reaction. O_2 was bubbled through the reaction via a dispersion tube for 8 h. The reaction was poured into 100 mL dilute aqueous HCl and extracted with DCM. The organic layer was dried with $MgSO_4$, concentrated in vacuo and the residue purified via chromatography (SiO_2, 20% v/v DCM in hexanes) to give compound **8b** as an off white solid (420 mg, 64%). Mp=172–173 °C. ^1H NMR (360 MHz, CD_2Cl_2): 7.35–7.23 (m, 10H), 7.18–7.16 (m, 4H), 6.86–6.84 (m, 4H), 3.80 (s, 6H). ^{13}C NMR (75 MHz, CD_2Cl_2): 159.1, 145.4, 137.1, 130.5, 129.3, 128.5, 127.3, 113.8, 85.2, 69.9, 55.6, 55.4. FTIR (neat): 3055, 2955, 2836, 2042, 1960, 1894, 1734, 1606, 1583, 1507, 1464, 1444, 1415, 1300, 1251, 1177, 1033 cm^{-1}. HRMS (*m/z*): calculated for $C_{46}H_{39}O_4$ $[M+H]^+$: 655.2843, found: 655.2831.

5.2.9. 1,4-Bis-(4,4′-dimethoxytrityl-3,3′-di-carboxaldehyde)-butadiyne 9b. Bis-trityl **8b** (420 mg, 0.64 mmol) and α,α-dichloromethyl methyl ether (574 μL, 6.4 mmol) were dissolved in DCM (60 mL) and the resulting solution cooled to −5 °C. $TiCl_4$ (840 μL, 7.6 mmol) was added drop wise via syringe over 2 min. The reaction was allowed to warm to room temperature and was stirred an additional 20 min. The reaction was poured into aqueous HCl (100 mL, 2.0 M) and stirred for 2 min. The aqueous suspension was extracted with EtOAc (3×100 mL). The organic extracts were combined, dried with $MgSO_4$ and concentrated. The resultant residue was purified via chromatography (SiO_2, 1:1 DCM–EtOAc) to give compound **9b** as a pink

amorphous solid (505 mg, 98%). ^{1}H NMR (300 MHz, CD_2Cl_2): 10.45 (s, 4H), 7.61 (dd, J=8.6, 2.7 Hz, 4H), 7.58 (d, J=2.3 Hz, 4H), 7.45–7.25 (m, 10H), 7.08 (d, 8.6 Hz, 4H), 4.00 (s, 12H). ^{13}C NMR (75 MHz, CD_2Cl_2): 189.4, 161.5, 143.8, 136.7, 129.9, 129.1, 128.8, 128.5, 127.9, 124.6, 112.4, 84.5, 70.1, 56.3, 55.3. FTIR (neat): 3057, 2943, 2864, 1683, 1604, 1578, 1491, 1463, 1418, 1393, 1282, 1255, 1205, 1180, 1112, 1023 cm^{-1}. HRMS (*m/z*): calculated for $C_{50}H_{39}O_8$ $[M+H]^+$: 767.2640, found: 767.2612.

5.2.10. 1,4-Diphenyl-1,1,4,4-tetra(2-methoxy-benz-4-yl-(6-methoxyquinolin-2-ylmethyl)-methylamino)-butadiyne 2b. Aldehyde **9b** (17 mg, 0.026 mmol), amine **5** (43 mg, 0.21 mmol) were dissolved in DCM (2.0 mL). To this was added HOAc (10 μL, 0.17 mmol) and $NaBH(OAc)_3$ (45 mg, 0.21 mmol). The reaction was allowed to stir for 10 h. Aqueous NaOH (1 M, 2 mL) was added and the reaction was stirred for 5 min. The organic layer was separated and dried with $MgSO_4$. The solvent was removed and the resultant residue was purified via chromatography twice (SiO_2, 2% TEA/EtOAc then 2% MeOH(NH_3)/DCM) to give compound **2b** as an amorphous yellow solid (30 mg, 81%). ^{1}H NMR (300 MHz, $CDCl_3$): 7.86 (d, J=9.2 Hz, 4H), 7.73 (d, J=8.6 Hz, 4H), 7.44 (d, J=2.2 Hz, 4H), 7.40 (d, J=7.8 Hz, 4H), 7.35–7.15 (m, 14H), 7.06, (dd, J=8.4, 2.4 Hz, 4H), 6.83 (d, J=2.7 Hz, 4H), 6.66 (d, J=8.7 Hz, 4H), 3.83 (s, 12H), 3.75 (s, 8H), 3.69 (s, 12H), 3.56 (s, 8H), 2.13 (s, 12H). ^{13}C NMR (75 MHz, $CDCl_3$): 158.2, 157.2, 156.5, 145.2, 143.3, 136.5, 135.1, 131.0, 130.2, 129.0, 128.4, 128.2, 127.9, 126.7, 126.5, 121.5, 121.2, 109.6, 105.2, 84.8, 69.7, 64.0, 55.4, 55.3, 55.3, 55.2, 42.5. FTIR (neat): 2940, 2836, 1623, 1601, 1497, 1456, 1378, 1310, 1235, 1161, 1110, 1031 cm^{-1}. HRMS (*m/z*): calculated for $C_{98}H_{96}N_8O_2$ $[M+2H]^{2+}$: 756.3670, found: 756.3661.

5.2.11. 4,4′,4″-Trimethoxytrityl alkyne 7c. Chloride **6c** (2.66 g, 7.14 mmol) was dissolved in argon sparged toluene (200 mL) and cooled to −78 °C. To this was added ethynylmagnesium bromide (30 mL, 0.5 M in ether) via syringe over 10 min. The reaction was allowed to warm to room temperature and was stirred an additional 3 h. The reaction was quenched with saturated aqueous NH_4Cl and extracted with EtOAC. The organic layer was dried with $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified via chromatography (SiO_2, 40% DCM/hexanes) to give compound **6c** as a white solid (1.97 g, 76%). Mp=126–128 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 7.23–7.20 (m, 6H), 6.87–6.84 (m, 6H), 3.82 (s, 9H), 2.71 (s, 1H). ^{13}C NMR (75 MHz, CD_2Cl_2): 158.3, 137.5, 130.0, 113.2, 90.3, 72.7, 55.2, 53.4. FTIR (neat): 3305, 3154, 2958, 2838, 2253, 1794, 1606, 1583, 1507, 1464, 1381, 1297, 1250, 1178, 1095 cm^{-1}. HRMS (*m/z*): calculated for $C_{24}H_{22}O_3$ $[M]^+$: 358.1564, found: 358.1560.

5.2.12. 1,4-Bis-(4,4′,4″-trimethoxytrityl)-butadiyne 8c. Alkyne **7c** (500 mg, 1.39 mmol) and CuCl (3.45 g, 34.8 mmol) were suspended in DCM (5.0 mL). To this was added TMEDA (5.85 mL, 38.7 mmol) via syringe over 10 min. The reaction was stirred for 2 days while being vented to the atmosphere via an 18 gauge needle. The reaction was quenched with saturated aqueous NH_4Cl and then extracted with DCM. The organic layer dried with $MgSO_4$ and the solvent removed. The residue was purified via chromatography (SiO_2, gradient elution from 30% DCM/hexanes to 100% DCM then to 100% EtOAc) to give compound **8c** as an off white solid (355 mg, 71%). Mp=265–266 °C. ^{1}H NMR (360 MHz, $CDCl_3$): 7.14–7.12 (m, 12H), 6.83–6.80 (m, 12H), 3.79 (s, 18H). ^{13}C NMR (50 MHz, $CDCl_3$): 158.4, 137.2, 130.1, 113.3, 84.6, 69.4, 55.2, 54.3. FTIR (KBr): 2932, 2836, 2055, 2896, 1742, 1606, 1582, 1502, 1461, 1414, 1300, 1246, 1176, 1114, 1031 cm^{-1}. HRMS (*m/z*): calculated for $C_{48}H_{42}O_6$ $[M]^+$: 714.2976, found: 715.2971.

5.2.13. 1,4-Bis-(4,4′,4″-trimethoxytrityl 3,3′,3″-tricarboxaldehyde)-butadiyne 9c. Bis-trityl **8c** (243 mg, 0.339 mmol) and α,α-dichloromethyl methyl ether (570 μL, 6.1 mmol) were dissolved in DCM (30 mL) and the resulting solution cooled to 0 °C. $TiCl_4$ (872 μL, 7.7 mmol) was added drop wise via syringe over 2 min. The reaction was allowed to warm to room temperature and was stirred an additional 2 hr. The reaction was poured into aqueous HCl (100 mL, 2.0 M) and stirred for 2 min. The aqueous suspension was extracted with EtOAc (3× 100 mL). The organic extracts were combined, dried with $MgSO_4$ and concentrated. The resultant residue was purified via column chromatography (SiO_2, 1:1 DCM–EtOAc) to give compound **9c** as an off white amorphous solid (300 mg, 99%). ^{1}H NMR (300 MHz, $CDCl_3$): 10.39 (s, 6H), 7.55 (d, J=2.6 Hz, 6H), 7.49 (dd, J=8.7, 2.6 Hz, 6H), 6.98 (d, J=8.8 Hz, 6H), 3.93 (s, 18H). ^{13}C NMR (75 MHz, $CDCl_3$): 189.4, 161.1, 136.4, 136.0, 128.3, 124.2, 112.0, 83.9, 70.5, 55.8, 54.1. FTIR (neat): 3157, 2944, 2868, 2041, 1682, 1603, 1494, 1462, 1418, 1393, 1281, 1258, 1181, 1113, 1023 cm^{-1}. HRMS (*m/z*): calculated for $C_{54}H_{43}O_{12}$ $[M+H]^+$: 883.2749, found: 883.2716.

5.2.14. 1,1,1,4,4,4-Hexa-(2-methoxybenz-4-yl-(6-methoxyquinolin-2-yl-methyl) methylamino)-butadiyne 2c. Aldehyde **9c** (30 mg, 0.033 mmol), amine **5** (81 mg, 0.40 mmol) were dissolved in DCM (2.0 mL). To this was added HOAc (23 μL, 0.40 mmol) and $NaBH(OAc)_3$ (85 mg, 0.40 mmol). The reaction was allowed to stir for 10 h. Aqueous NaOH (1 M, 2 mL) was added and the reaction was stirred for 5 min. The organic layer was separated and dried with $MgSO_4$. The solvent was removed and the resultant residue was purified via chromatography twice on using (SiO_2, 2% TEA/EtOAc then 2% MeOH(NH_3)/DCM) to give compound **2c** as an amorphous yellow solid (33 mg, 50%). ^{1}H NMR (360 MHz, $CDCl_3$): 7.84 (d, J=9.2 Hz, 6H), 7.67 (d, J=8.4 Hz, 6H), 7.47 (d, J=2.3 Hz, 6H), 7.37 (d, J=8.8 Hz, 6H), 7.24 (dd, J=9.2, 2.8 Hz, 6H), 7.10 (dd, J=8.3, 2.2 Hz, 6H), 6.77 (d, J=2.5 Hz, 6H), 6.60 (d, J=8.7 Hz, 6H), 3.81 (s, 18H), 3.69 (s, 12H), 3.63 (s, 18H), 3.52 (s, 12H), 2.09 (s, 18H). ^{13}C NMR (90 MHz, $CDCl_3$): 158.3, 157.2, 156.5, 143.3, 137.1, 135.0, 131.0, 130.3, 128.4, 128.1, 126.5, 121.4, 121.2, 109.7, 105.4, 85.2, 69.7, 64.0, 55.4, 55.3, 55.2, 54.7, 42.5. FTIR (neat): 2935, 2836, 2049, 1681, 1624, 1601, 1499, 1462, 1378, 1310, 1235, 1162, 1110, 1031 cm^{-1}. HRMS (*m/z*): calculated for $C_{126}H_{128}N_{12}O_{12}$ $[M+2H]^{2+}$: 1000.4882, found: 1000.4865.

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