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NMR Method for Simultaneous Host–Guest Binding Constant Measurement

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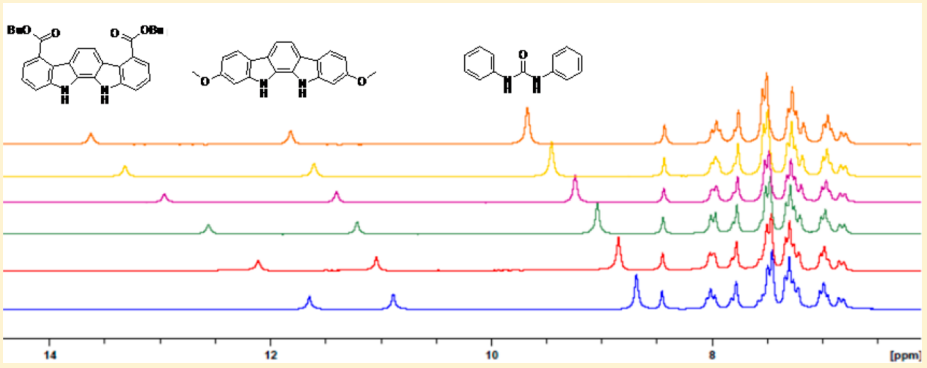
Supporting Information



ABSTRACT: An NMR-based relative binding affinity measurement method has been developed in which differences in the binding affinities of different hosts toward a particular guest ($\Delta\log K_{ass}$ values) are measured in the same solution. As an advancement, the method allows the simultaneous determination of several $\Delta\log K_{ass}$ values in a single run. As a proof of principle, the method was used to measure binding affinity differences of a number of indolocarbazole- and urea-based synthetic receptors toward acetate ion in DMSO-d_6/H_2O (99.5%:0.5% m/m). As a result, a binding affinity scale containing 33 receptors and spanning 2.32 log units with excellent self-consistency (consistency standard deviation = 0.01 log unit) was created. Together with the very good agreement of the results with those obtained by UV–vis spectrophotometry, this demonstrates the high accuracy of the method and the fact that the NMR and UV–vis techniques can be used interchangeably (in spite of the very different concentrations used in these techniques). Additionally, it was found for symmetrical receptor molecules from the same compound family that there is a correlation between the acetate binding affinity of a receptor and the ^{15}N chemical shift of the nitrogen atoms of its binding centers.

INTRODUCTION

Molecular recognition, one of the core processes in supramolecular chemistry, involves binding of a complementary guest to a host via non-covalent interactions. It is the prerequisite for self-organization, self-assembly, transport, and supramolecular catalysis.[1,2] The binding affinity of a host toward particular guest under equilibrium conditions can be quantified by the binding (or association or stability) constant (K_{ass}) (eq 1). Binding constants are key characteristics of supermolecules, and when determined for a series of supermolecules, they can be useful for predicting the properties of new molecular assemblies.[3,4] Differences in binding strength (K_{ass} ratios) for binding of different guests by the same host characterize the selectivity of the interaction, which is very important in molecular recognition studies and is a key parameter used in the design of synthetic molecular receptors. Without question, the accurate and reliable determination of binding constants plays an important role in supramolecular studies.

The binding constant K_{ass} expressing the affinity of a host H toward a guest G is in the case of 1:1 stoichiometry defined by eqs 1 and 2:

$$\mathrm{H} + \mathrm{G} \overset{K_{ass}}{\rightleftarrows} \mathrm{HG} \tag{1}$$

$$K_{ass} = \frac{a_{HG}}{a_H a_G} \tag{2}$$

wherein a_{HG}, a_H, and a_G are the activities of the species in the solution. Binding constants are generally measured directly according to eq 2.[5] The consistency of the measurements is mostly evaluated by repeatability (standard deviation of measurements repeated within the same day).[6] Possible systematic effects, which often are the main source of measurement error, are left out of consideration. Systematic effects in the case of anion binding by synthetic receptors may be caused, for example, by ion pairing[7] and homoconjugation

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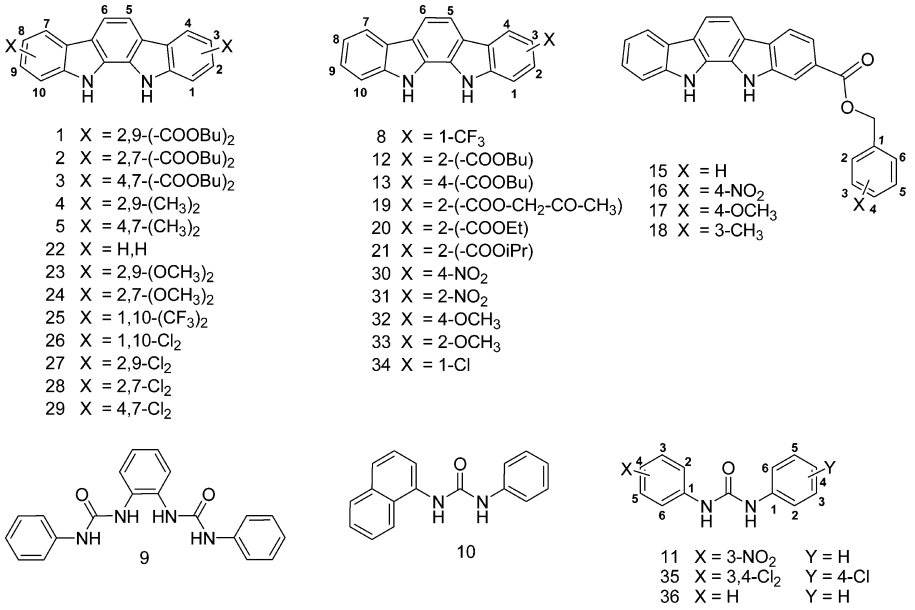
Scheme 1. Structures of the Molecular Receptors



(association of an acid and its anion).[8] Both of these can significantly decrease the activity of the free anion, leading to biased results. As another example, even low levels of water in organic solvents (often used as media) decrease the effective activity of both hydrogen-bond (HB) donors and acceptors by selective solvation. Systematic effects such as those described here introduce bias by shifting all of the results in a series in the same direction, while at the same time the agreement between the individual results can be good, leaving the wrong impression of highly accurate data.

In a previous work,[9] a UV−vis spectrophotometric relative binding affinity measurement method was reported. It is based on measuring the *relative* binding affinity of two hosts H_1 and H_2 toward the same guest when all of the species are dissolved in the same solvent, as described by eq 3:

$$H_1 + H_2G \overset{\Delta\log K_{ass}}{\rightleftharpoons} H_1G + H_2 \tag{3}$$

The relative binding affinity is expressed by $\Delta\log K_{ass}$, which is defined in eq 4:

$$\begin{aligned}\Delta\log K_{ass} &= \log K_{ass}(H_1G) - \log K_{ass}(H_2G)\\ &= \log \frac{a_{H_1G}a_{H_2}}{a_{H_2G}a_{H_1}}\end{aligned} \tag{4}$$

From eqs 3 and 4 it can be seen that the need to determine the activity of the guest is eliminated. This means that the possible side processes involving the guest (e.g., ion pairing and homoconjugation) influence binding to both hosts simultaneously, cancel out, and thus do not affect the measurement result. The activities of the free and bound hosts enter eq 4 as ratios. Thus, possible factors affecting the hosts also largely cancel (e.g., the composition of the solvent is automatically identical for both hosts). A reasonable assumption to make is that the ratios of activity coefficients of $\gamma(H_x)/\gamma(H_xG)$ are similar for the two host molecules.[6,8] Consequently, the activities in eq 4 can be replaced with the equilibrium concentrations:

$$\Delta\log K_{ass} = \log \frac{[H_1G][H_2]}{[H_2G][H_1]} \tag{5}$$

Because many sources of error cancel with relative binding affinity measurements, it is possible to obtain highly accurate results. The proposed method is analogous by nature to relative acidity and basicity measurement methods, which have been used for the determination of pK_a values in nonaqueous media[6,8] and in competition experiments.[10]

In ref 9, the usability and high accuracy of the obtained data were successfully demonstrated on a series of synthetic anion receptors. Besides its numerous merits as a method for studying host−guest binding, UV−vis spectroscopy also has important limitations: the presence of a chromophore is needed in the host molecule, there should be sufficient spectral change upon host−guest binding, and the solvent should be transparent over the spectral range used. Furthermore, the measurement and (especially) calculation procedures are rather complex.[9]

The omnipresence and ease of use of NMR spectroscopy in organic synthesis have contributed to making NMR analysis one of the most common methods used to study binding affinities.[11−14] Besides enabling the measurement of binding constants, it also gives important additional information on the possible side processes (e.g., possible deprotonation, association, etc.). If the complexation reaction is fast (which is usually the case), then the association or dissociation degree can be easily monitored from the change in chemical shift.

In this work, we have developed a relative binding affinity measurement method based on NMR spectroscopy. As a proof of concept, this method was applied to the measurement of binding constants between a series of synthetic chelating anion-binding receptor building blocks (different ureas and indolocarbazoles; see Scheme 1) as HB donors and acetate anions as HB acceptors. The obtained data were compared with the results obtained by UV−vis spectrophotometry.

Anion recognition and detection is important in medicine, chemical industry, and the environment. The design of synthetic anion receptors has become a prominent research field.[15−21] Therefore, the development of sensitive and selective anion receptors that are able to function in detection,

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Table 1. Scale of Relative Binding Affinities for Acetate in DMSO-d_6/H_2O (99.5%:0.5% m/m)[a]

No	Receptor molecule	logK_{ass}	u_c[b]	u_c[c]	ΔlogK_{ass}
35	3,4,4-Cl_3-diphenylurea	4.01	0.01	0.05	
11	1-(3-NO_2-phenyl)-3-phenylurea	3.78	0.01	0.05	
30	4-NO_2-indolocarbazole	3.76	0.01	0.05	
29	4,7-Cl_2-indolocarbazole	3.72	0.01	0.05	
1	2,9-(BuOCO)$_2$-indolocarbazole	3.70	0.01	0.05	
2	2,7-(BuOCO)$_2$-indolocarbazole	3.67	0.01	0.04	
3	4,7-(BuOCO)$_2$-indolocarbazole	3.65	0.01	0.05	
9	O-Phenylenediaminourea	3.58	0.01	0.05	
31	2-NO_2-indolocarbazole	3.57	0.01	0.05	
28	2,7-Cl_2-indolocarbazole	3.55	0.01	0.05	
16	4-NO_2-C_6H_4-CH_2-OCO-indolocarbazole	3.47	0.01	0.05	
19	CH_3-CO-CH_2-OCO-Indolocarbazole	3.46	0.01	0.05	
20	2-EtO-CO-Indolocarbazole	3.46	0.01	0.05	
21	$(CH_3)_2$CH-OCO-Indolocarbazole	3.45	0.01	0.05	
17	4-MeO-C_6H_4-OCO-indolocarbazole	3.44	0.01	0.05	
18	3-CH_3-C_6H_4-CH_2-OCO-indolocarbazole	3.43	0.01	0.04	
15	Ph-CH_2-OCO-Indolocarbazole	3.43	0.01	0.04	
12	2-BuOCO-indolocarbazole	3.42	0.01	0.05	
27	2,9-Cl_2-indolocarbazole	3.40	0.01	0.05	
13	4-BuOCO-indolocarbazole	3.39	0.01	0.04	
36	1,3-diphenylurea	3.20	0.01	0.04	
5	4,7-$(CH_3)_2$-indolocarbazole	3.17	0.01	0.05	
33	2-MeO-indolocarbazole	3.15	0.01	0.05	
32	4-MeO-indolocarbazole	3.14	0.01	0.05	
24	2,7-$(MeO)_2$-indolocarbazole	3.14	0.01	0.04	
23	2,9-$(MeO)_2$-indolocarbazole	3.14	0.01	0.05	
22	Indolocarbazole	3.14	0.01	0.05	
4	2,9-$(CH_3)_2$-indolocarbazole	3.11	0.01	0.04	
34	1-Cl-indolocarbazole	2.76	0.01	0.05	
10	1-Naphthalen-1-yl-3-phenyl-urea	2.72	0.03	0.05	
8	1-CF_3-indolocarbazole	2.50	0.01	0.04	
26	1,10-Cl_2-indolocarbazole	2.15	0.01	0.04	
25	1,10-$(CF_3)_2$-indolocarbazole	1.69	0.02	0.05	

[a]Solvent: DMSO-d_6/H_2O (99.5%:0.5% m/m). In all cases, the binding stoichiometry was 1:1. ΔlogK_{ass} values in rectangles were determined using the UV−vis method. [b]Standard uncertainties for comparison of logK_{ass} values on the scale. [c]Standard uncertainties for comparison of logK_{ass} values with those from other research groups.

extraction, or transport is of high interest. Derivatives of urea[22,23] and indolocarbazole[24] are among the most frequently used building blocks in anion receptor design.

RESULTS

^{1}H NMR-Based Relative Measurements Study. Altogether 78 relative acetate binding measurements between 33 receptors were carried out in DMSO-d_6/H_2O (99.5%:0.5% m/m) using the NMR-based relative binding measurement method. For comparison and validation of the NMR method, 17 relative binding measurements between 11 receptors were carried out in DMSO/H_2O (99.5%:0.5% m/m) using the previously reported UV−vis spectrophotometric method. The resulting scale of relative binding affinities for acetate ranging over 2.32 log units and incorporating measurements from both methods is presented in Table 1. Each arrow in the scale corresponds to the difference in absolute binding affinity between two receptor molecules on the logarithmic scale expressed as ΔlogK_{ass} values. Each additional measurement contributes to circular validation[25] of the whole scale. The absolute logK_{ass} values of the receptors on the scale were found by minimizing the sum of the squares of the differences between the directly measured ΔlogK_{ass} values and the assigned logK_{ass} values, which is denoted as SS in the following equation:[8]

$$SS = \sum_{i=1}^{n_m} \{\Delta \log K_{ass}^{i} - [\log K_{ass}(H_yG) - \log K_{ass}(H_xG)]\}^2 \quad (6)$$

Every ΔlogK_{ass}^{i} value is the directly measured relative binding strength of the hosts H_y and H_x. The absolute logK_{ass} values for all of the compounds were found by anchoring the scale by the least-squares procedure to the logK_{ass} values of indolocarbazole (**22**), 1,10-dichloroindolocarbazole (**26**), and 4-nitroindolocarbazole (**30**) (see Table 2). The consistency of the assigned absolute logK_{ass} values with the measured ΔlogK_{ass} values can be evaluated by the consistency standard deviation of the scale (s),[8] which is found according to the following equation:

$$s = \sqrt{\frac{SS}{n_m - n_c}} \quad (7)$$

where n_m = 95 is the number of ΔlogK_{ass} measurements and n_c = 33 is the number of absolute logK_{ass} values that were determined. For the current scale, which includes both the NMR and UV−vis results, s = 0.01 log units. The separate s values for the NMR and UV−vis results are 0.01 and 0.02, respectively. These s values indicate high consistency of the results and good agreement between the NMR and UV−vis data, enabling us to put all of the results on the same scale. This also suggests that there is no practical difference in the choice of method, in spite of the very different concentrations used in NMR and UV−vis measurements. The values in blue boxes on the scale in Table 1 were measured via UV−vis spectrophotometry. The high consistency of the results enabled differentiation between receptors with a binding strength difference of less than 0.05 logK_{ass} units. This is significantly lower than is possible in the case of absolute measurements, especially if they are performed in different laboratories.

The logK_{ass} differences of the anchor compounds obtained from the relative measurements are also well-consistent with the differences of their absolute logK_{ass} values.

Absolute Binding Measurements. The absolute logK_{ass} values were obtained for the receptors 4-nitroindolocarbazole (**30**), indolocarbazole (**22**), and 1,10-dichloroindolocarbazole (**26**). For each of them, the logK_{ass} value was measured in at least two different days. The values of logK_{ass} for **22** and **26** were obtained using both UV−vis and NMR methods. Several independent data sets were obtained on each day, and for each

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Table 2. Results of Measurements of Absolute logK_{ass} Values

receptor	method	absolute logK_{ass} [a]	s [b]	n [b]	CI (95%) [b]	absolute logK_{ass} from scale [c]	difference [c]
30	UV−vis	3.75	0.04	5	0.05	3.76	0.005
22	UV−vis	3.15	0.01	5	0.01	3.1	−0.002
26	UV−vis	2.09	0.05	4	0.09	2.15	0.057
22	NMR	3.17	0.25	4	0.41	3.14	−0.020
26	NMR	2.19	0.06	3	0.14	2.15	−0.040

[a]Values of logK_{ass} were obtained as averages of independent measurement runs. [b]s is the standard deviation, n the number of measurement runs, and CI (95%) the confidence interval of the mean value at 95% probability. [c]Difference between the logK_{ass} value obtained for the same receptor molecule from the scale by the least-squares procedure and the directly determined absolute logK_{ass} value.

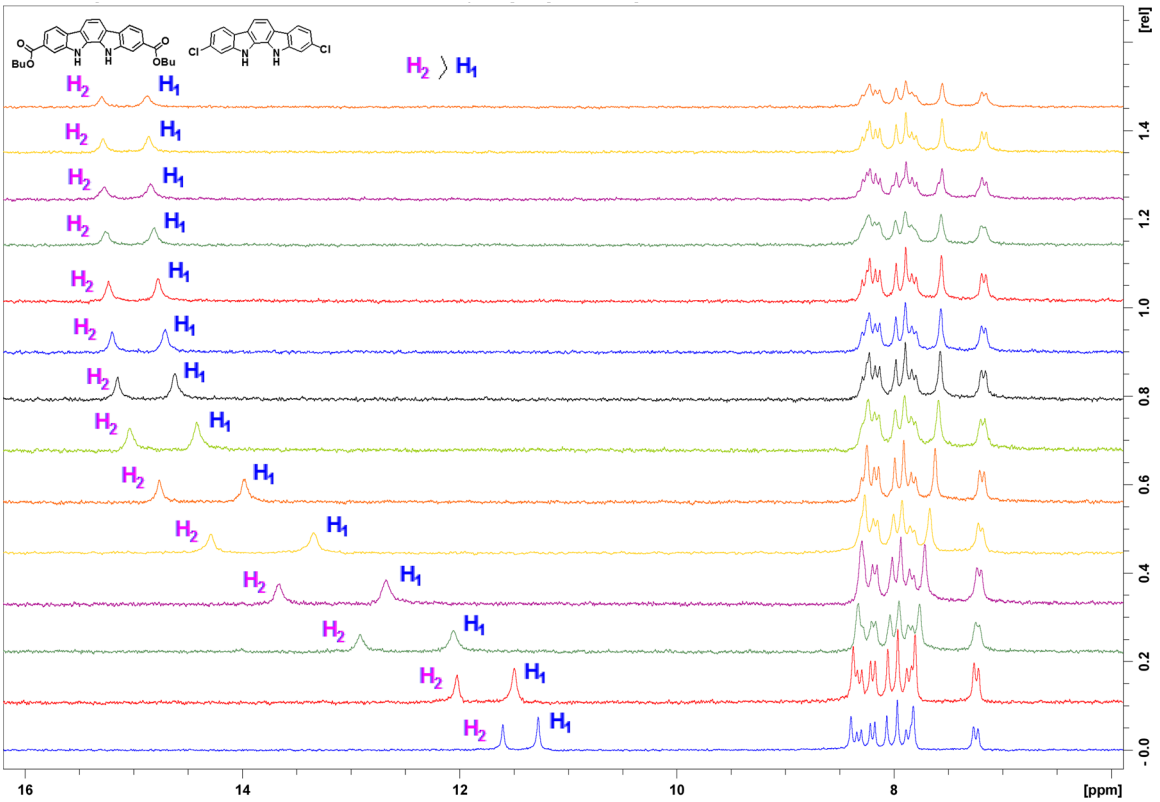

Figure 1. ^{1}H NMR spectra for measuring the relative binding affinity of receptors **1** and **27** for acetate in DMSO-d_6/H_2O (99.5%:0.5% m/m). The titration proceeds from bottom to top. The bottom spectrum corresponds to solution without added titrant.

of the data sets three independent calculation procedures were applied.[9] The results of the measurements are presented in Table 2 and display very good agreement between the UV−vis and NMR results.

The binding scale for acetate was anchored to the three independently measured logK_{ass} values for receptors **30**, **22**, and **26**. The small absolute values of the differences between the values of logK_{ass} from the scale and the directly determined values (last column of Table 2) demonstrate the good consistency between the absolute and the relative measurement results and offer evidence for absence of artificial expansion or contraction of the scale.

An absolute binding constant measurement for receptor **3** was also attempted, but high scatter of the parallel measurement results was observed. The reason probably is that NMR spectroscopy is not suitable for accurate measurements of high absolute binding constant values: because of the high concentrations used in NMR measurements, almost all of the anions added to the solution at any titration point are bound, and finding the concentration of free anions is difficult. At the same time, reproducible results were obtained with relative measurements in the region of logK_{ass} = 3.6−4.0, implying that in contrast to the absolute binding constant measurements by NMR spectroscopy, the relative method described here enables the measurement of high binding affinities.

DISCUSSION

Advantages of Relative Binding Measurement Using NMR Spectroscopy. Measurement of binding constants via NMR spectroscopy is performed by monitoring the changes in the chemical shift of one or more proton signals in the ^{1}H NMR spectra of the receptor molecule at different guest concentrations. For a 1:1 binding equilibrium under the conditions of fast exchange, the chemical shift of the signal is linearly dependent on the degree of host−guest association, α (see eq 8),[26] which can be found directly from the titration spectra of a mixture containing both hosts. As outlined above, applying the relative binding affinity measurement method eliminates the need to determine the activity of the guest. This becomes particularly useful if binding constants with high

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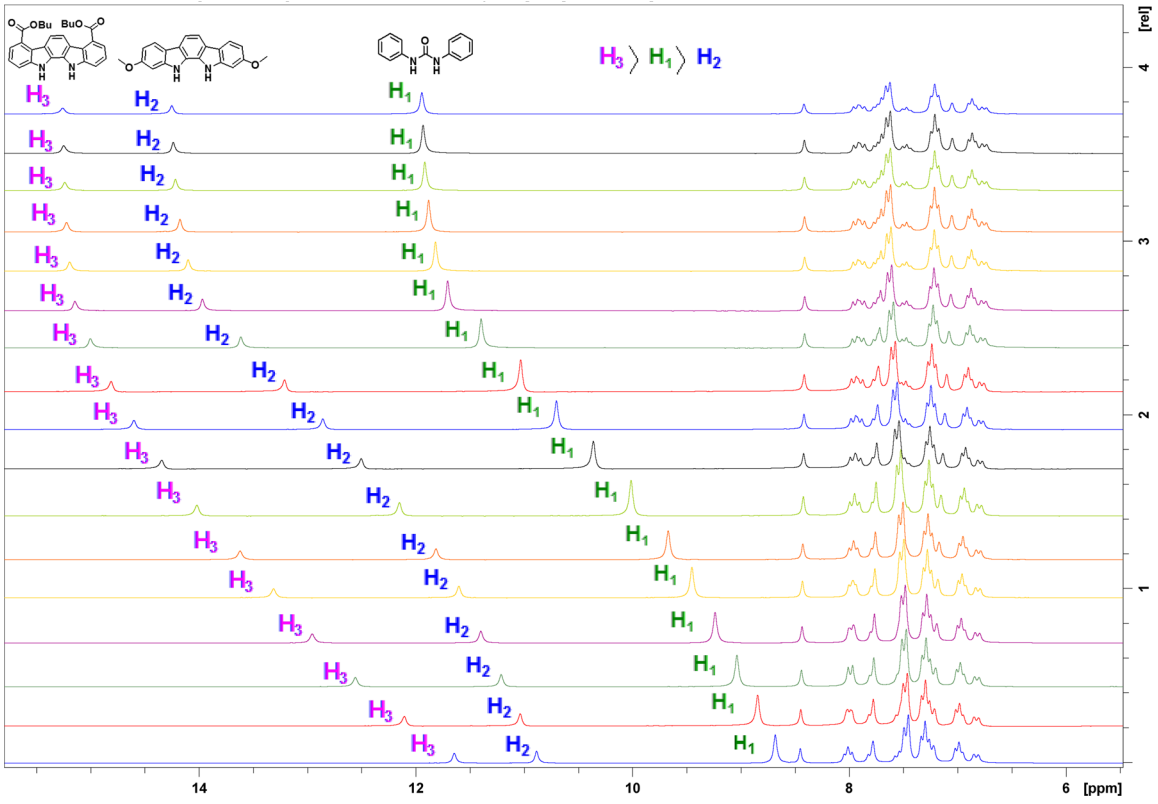

Figure 2. ^{1}H NMR spectra for measuring the relative binding affinities between receptors **3**, **23**, and **36** toward acetate in DMSO-d_6/H_2O (99.5%:0.5% m/m). The titration proceeds from bottom to top.

affinity are determined with the NMR approach. Because of the high concentrations used in NMR analysis, it can be difficult to determine the small content of the unbound ligand in solution when absolute measurements are made, but with the relative measurement this is not necessary. Therefore, if the ladder approach described in this work is used, then high binding affinities can be measured using NMR spectroscopy without problems.

In UV−vis spectrophotometry measurements it is also necessary to titrate solutions containing only one receptor molecule. The degrees of association are found from the titration spectra of the mixture via complex multiregression analysis using also the spectral data from the titrations of the two pure receptors. Furthermore, in the NMR method the binding process for each host toward the selected guest can be followed directly from the ^{1}H NMR spectra, which is not possible in the case of the UV−vis method. This enables the difference in binding affinities toward a particular guest to be estimated already during the titration. Possible side processes, such as deprotonation or additional unwanted association processes, can be better observed with NMR than with UV−vis. Under the conditions of fast exchange, the change in the chemical shift is observed, not the change in signal intensity.[5] Chemical shifts can be measured much more accurately than signal intensities, and there is no necessity to correct for the dilution during the NMR titration. Figure 1 demonstrates the NMR measurement of the relative binding affinity of two receptors toward acetate. The difference in the chemical shifts of the NH protons of the free receptor and the receptor−anion complex of compounds **1** and **27** are $\Delta\delta$ = 3.69 and 3.59 ppm, respectively. During the addition of acetate, the NH proton chemical shift of receptor **1** moves downfield faster (complex formation is faster) in comparison with receptor **27**. Calculation results confirmed that receptor **1** has a higher binding affinity toward acetate than receptor **27**.

UV−vis spectrophotometry is limited to measuring the difference in the binding affinities of only two receptors toward a selected anion. NMR spectroscopy does not have this limitation, as differences in the binding affinities of more than two receptors can be determined simultaneously within a single measurement series. This capability enables the same amount of data to be obtained with NMR spectroscopy around three times faster than is possible with UV−vis spectrophotometry. Figure 2 shows ^{1}H NMR spectra for the measurement of the affinities of receptors **3**, **23**, and **36** toward acetate. The chemical shift of the NH protons of each receptor molecule is observed, and the degrees of association for all of the receptors are found. The relative binding constants ($\Delta\log K_{ass}$ values) between receptors H_1 and H_2, H_2 and H_3, and H_1 and H_3 can be calculated according to eq 9. During the titration, receptor **3** showed a larger change in the NH chemical shift ($\Delta\delta$ = 3.61 ppm) than receptor **36** ($\Delta\delta$ = 3.26 ppm) and receptor **23** ($\Delta\delta$ = 3.36 ppm). The differences in binding affinity between receptors **3** and **36**, **36** and **23**, and **3** and **23** are accordingly 0.45, 0.05, and 0.50 $\log K_{ass}$ units. The higher the binding affinity of a receptor, the larger is the proportion of anion-bound receptor molecules in solution and the faster its NH proton signal shifts downfield (deshielding), which a general feature in NMR spectra upon HB formation.[26] The aromatic protons show only negligible shielding shifts upon binding ($\Delta\delta$ = 0.02 ppm) because they are distant from the binding center.

Substituent and Solvent Effects on the Binding Affinity. As observed previously,[9] electron-withdrawing groups (EWGs) (e.g., $-NO_2$, −COOBu, −Cl) increase the HB

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donicity of the receptor molecule, leading to an increase in the binding affinities toward anions. In broad terms, one nitro group is as powerful as two chloro or two ester groups, but the effect of the substituents depends also on their positions. When bulky groups (concrete data are available for −Cl and $-CF_3$) are located next to the binding site, as in the case of 1-substitution or 1,10-disubstitution, the anion complexation is hindered. This effect is further enhanced by the negative charge of the substituent, leading to charge−charge repulsion.

It is of interest to compare the present results in DMSO-d_6 containing 0.5% water with those obtained earlier by us in acetonitrile (AN) containing 0.5% water.[9] Acetonitrile has very low HB-donating and -accepting abilities, and therefore, binding of anions to receptor molecules in acetonitrile is stronger than in more competitive solvents such as DMSO. When the main solvent is changed from AN to DMSO, the binding affinity decreases by more than 1 logK_{ass} unit (Table 3).

Table 3. Comparison of Binding Constant Values Measured in DMSO and AN (Both Containing 0.5% Water)

	logK_{ass}		
receptor	DMSO	AN	difference
1,3-diphenylurea	3.20	4.28	1.08
3,4,4′-trichlorodiphenylurea	4.01	5.20	1.19
indolocarbazole	3.14	4.46	1.32
2,7-dimethoxyindolocarbazole	3.14	4.46	1.32
2-methoxyindolocarbazole	3.15	4.50	1.35
1-chloroindolocarbazole	2.76	4.24	1.48
4-nitroindolocarbazole	3.76	5.24	1.48
4,7-dichloroindolocarbazole	3.72	5.20	1.49
2,7-dichloroindolocarbazole	3.55	5.05	1.51
2-nitroindolocarbazole	3.57	5.09	1.52
2,9-dichloroindolocarbazole	3.39	4.95	1.56
1,10-bis(trifluoromethyl)indolocarbazole	1.69	3.36	1.67
1,10-dichloroindolocarbazole	2.15	3.84	1.69

There are also changes in binding affinity order. It can be seen that the binding affinities of diphenylureas decrease by slightly more than 1 order of magnitude and that this is not strongly dependent on their substitution. The decrease in binding affinity in the case of indolocarbazoles is in the range of 1.3−1.7 logK_{ass} units, and the change is clearly dependent on the substitution: indolocarbazole and methoxyindolocarbazoles display changes of around 1.3 logK_{ass} units. Indolocarbazoles with EWGs (chloro, nitro) display a decrease of around 1.5 logK_{ass} units in binding affinity. If the EWGs are at positions 1 and 10, then the change amounts to around 1.7 logK_{ass} units. In the latter case, there seems to be a synergy between the action of the EWGs and the steric effect. Three factors are at work. First, the anion is repelled by the negative charge of the EWGs and by the steric hindrance, preventing it from assuming a suitable conformation. Second, the solvent molecule is neutral and is not repelled by the negative charge. Third, the hydrogen atoms of the NH groups carry strong positive partial charge and are thus in principle strong HB donors, and DMSO is a much stronger HB acceptor than AN. Therefore, in DMSO the solvent molecules compete more efficiently with acetate anions than in AN.

^{15}N NMR Chemical Shifts. It is of interest to compare the trends in the ^{15}N chemical shifts with the trends in logK_{ass} values when the substitution pattern changes. Figure 3 displays the relationship between these two parameters in the case of symmetrically substituted indolocarbazoles. There is a strong correlation between these two parameters (R^2 = 0.977) if the 1,10-disubstituted compounds (**25** and **26**) are not included. These two compounds have significantly lower binding affinities toward acetate than could be predicted from the ^{15}N chemical shifts. The obvious reason is that binding of acetate to these compounds is sterically hindered, while the ^{15}N chemical shifts are not influenced by steric effects.

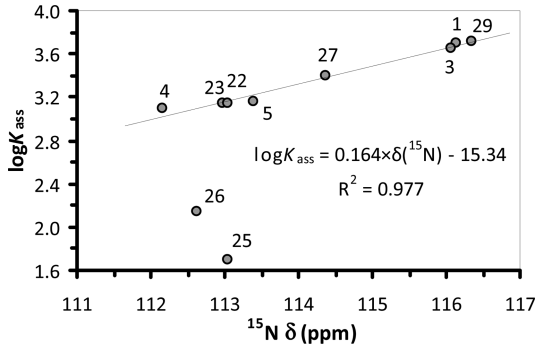

Figure 3. Relationship between the acetate binding constants (logK_{ass}) of the symmetrical indolocarbazole receptors and the ^{15}N chemical shifts of the ring nitrogen atoms.

For the unsymmetrically substituted indolocarbazoles, there are two different chemical shifts in the ^{15}N NMR spectrum. We attempted to correlate either of them individually or their average with the value of logK_{ass}. Worse correlations were observed in all of these cases (the highest was R^2 = 0.938), indicating in this case a more complex pattern of interrelation among the charge distribution in the receptor, the shielding of the nitrogen atoms, and the binding strength.

CONCLUSION

The applicability of an NMR method for relative binding affinity measurement has been demonstrated, and its advantages have been outlined: it removes the necessity to determine the activity of the guest, substantially simplifies the calculation model for relative binding affinity measurement, enables estimation of difference in binding affinity during the titration experiment, and enables determination of more than two relative binding affinities simultaneously in a single experiment. The good agreement between the measurement results obtained using NMR spectroscopy and UV−vis spectrophotometry shows that it makes no difference which experimental technique is used. This is a valuable conclusion because the concentration ranges used in UV−vis and NMR methods usually differ by around 2 orders of magnitude.

EXPERIMENTAL SECTION

Instruments. NMR measurements were carried out on a 200 MHz NMR spectrometer, and UV−vis spectrophotometric measurements were carried out on a double-beam spectrophotometer. The water content of the DMSO solvent was checked with a coulometric Karl Fischer titrator. Melting points were determined in open capillaries and are uncorrected. All of the receptor molecules were characterized on a 400 MHz NMR spectrometer. High-resolution mass spectra were obtained on an FT-ICR mass spectrometer with a 7 T magnet using negative ion electrospray ionization. The ionization chamber temperature was set to 40 °C, the spray needle voltage to −3500 V, the shield voltage to −300 V, the nebulizing gas (N_2) pressure to 25 psi, and the drying gas (N_2) pressure to 10 psi at 200 °C. The ion capillary voltage was optimized for every compound infused. The mass axis was calibrated in negative mode daily prior to infusion experiments using

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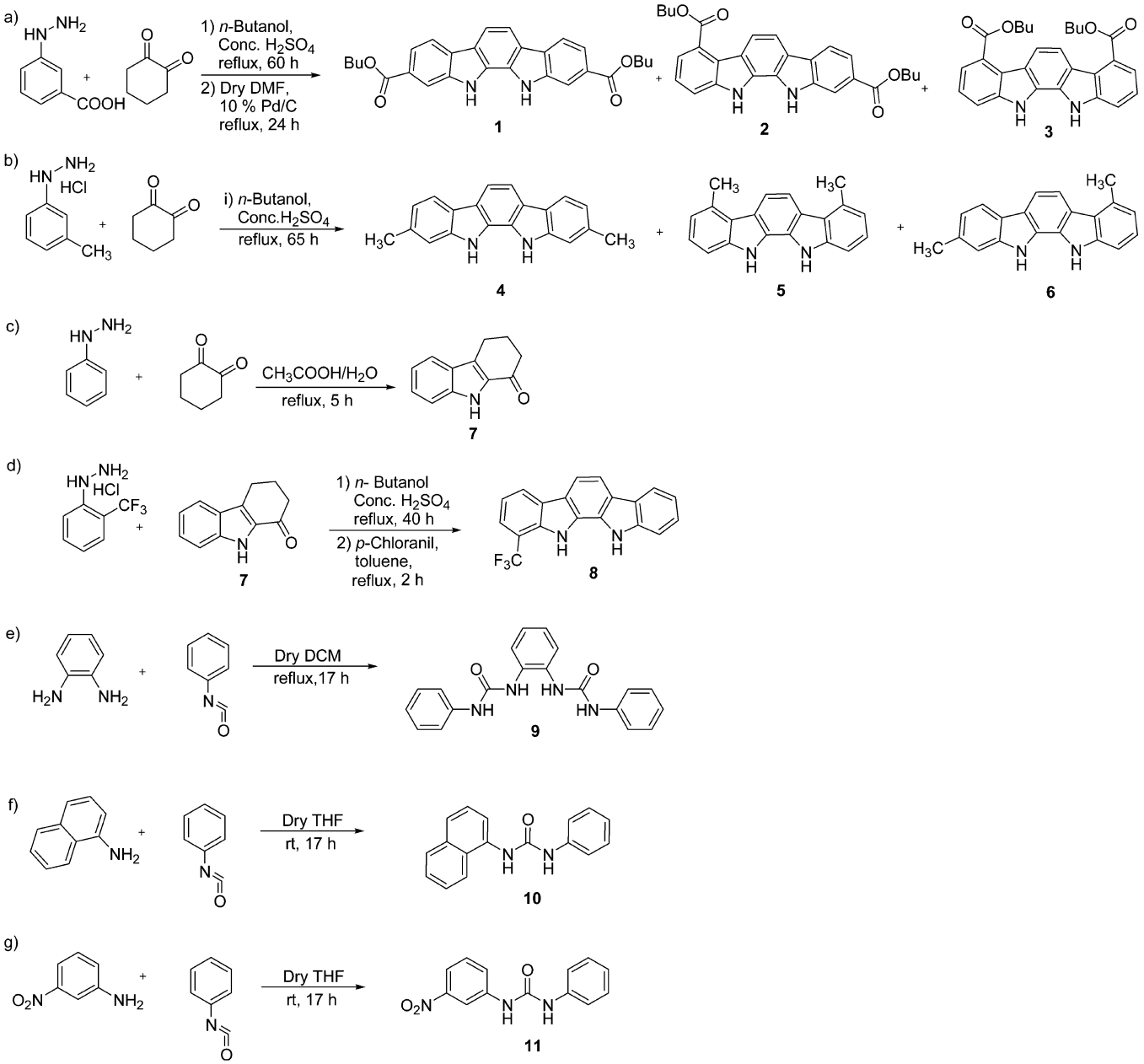
Scheme 2. Synthesis of Different Substituted Indolocarbazole- and Urea-Based Receptors



an in-house calibration mixture containing perfluorinated Brønsted superacids $C_{12}F_{10}NO_4S_2H$ (anion m/z 475.91145) $C_8F_{18}NO_4S_2H$ (anion m/z 579.89868), $C_{12}F_{26}NO_4S_2H$ (anion m/z 779.88591), and $C_{18}F_{37}O_6S_3H_2$ (anion m/z 1111.83499). In addition, two ions formed during ionization, $C_8F_{17}NO_2H^-$ (m/z 497.94620) and $C_4F_9NO_2S^-$ (m/z 296.95115), were used for calibration. Purification of the compounds was performed by flash chromatography on silica gel (pore size 60 Å, 230−400 mesh). Analytical thin-layer chromatography (TLC) was conducted on TLC plates (silica gel 60 with fluorescent UV_{254} marker on aluminum sheets). The samples for HRMS were dissolved in DMSO so that the concentration of the stock solution was 1.0 mg/mL. A 1 μL aliquot was dissolved in 1 mL of methanol, so the concentration of the infusion solution was roughly 1 μg/mL.

Solvents and Chemicals. The solvent for binding measurements (DMSO with 0.5% water) was prepared using anhydrous 99.9% DMSO (for UV−vis measurements) or 99.8% DMSO-d_6 (for NMR measurements) and water from a Milli-Q Advantage A10 system. The final water content of the solvent was checked by Karl Fischer titration and was always between 0.45% and 0.55%. Titrant solutions were prepared from tetrabutylammonium acetate (TBAA) (99%). The solvent for the synthesis, THF (Romil, 99.9%, according to Karl Fischer titration, water content less than 5 ppm), was dried by means of continuous circulation through a column filled with alumina and was delivered inside a glovebox. DCM, DMF, and acetone were dried as described in ref 27.

Receptor Molecules. The following commercially available receptor molecules were used: 3,4,4′-trichlorodiphenylurea (**35**) and 1,3-diphenylurea (**36**). Receptors **22**−**34** were the same as in ref 9. All of the remaining receptors were synthesized in this study.

Synthesis of Indolocarbazoles and Ureas. A series of mono- or disubstituted indolocarbazole and urea-type anion receptor molecules (compounds **1**−**6** and **8**−**21**) were prepared as illustrated in Schemes 2 and 3. The general synthetic strategy was as follows. The reaction of 3-hydrazinobenzoic acid with cyclohexane-1,2-dione under reflux in concentrated H_2SO_4 and subsequent dehydrogenation with 10% Pd/C yielded compounds **1**, **2**, and **3** (Scheme 2).[9] A similar reaction with 3-methylphenylhydrazine yielded compounds **4**, **5**, and **6** (in this case, dehydrogenation proceeded without the dehydrogenation catalyst).

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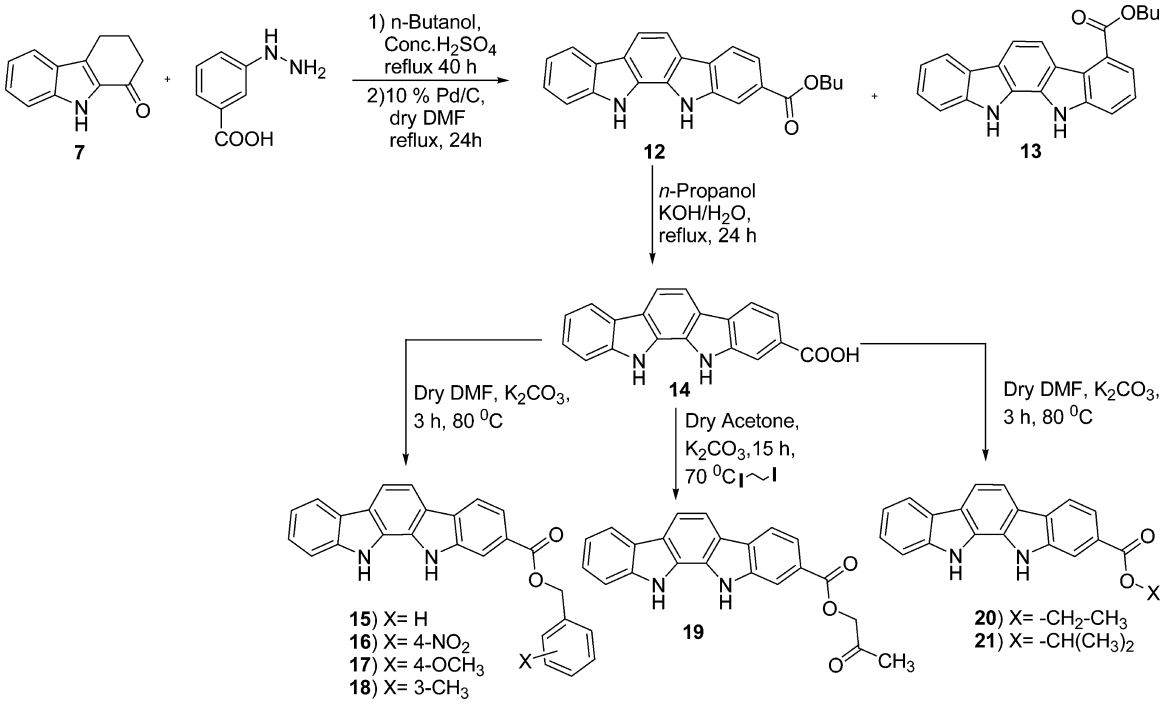
Scheme 3. Synthesis of Ester Derivatives of Indolocarbazoles



Intermediate **7** was prepared by refluxing phenylhydrazine and cyclohexane-1,2-dione in 1:1 (v/v) aqueous acetic acid. Compound **8** was prepared by refluxing **7** and 2-(trifluoromethyl)phenylhydrazine hydrochloride in concentrated H_2SO_4 and *n*-butanol and then dehydrogenating with *p*-chloranil (Scheme 2). The synthesis of indolocarbazoles substituted with a single ester group began from 3-hydrazinobenzoic acid and intermediate **7**, which were refluxed in *n*-butanol and concentrated H_2SO_4; subsequent dehydrogenation using 10% Pd/C afforded compounds **12** and **13** (Scheme 3). Compound **12** was converted to compound **14** by hydrolysis, and a series of ester derivatives of indolocarbazole were prepared from **14**. Compound **14** reacted with different substituted benzyl halides and alkyl halides in DMF in the presence of K_2CO_3 as described in Scheme 3, leading to compounds **15**−**18**, **20**, and **21**.[28] Compound **19** was prepared from **14** using 1,2-diiodoethane in dry acetone (Scheme 3). Finally, compounds **9**−**11** were prepared from the reactions of benzene-1,2-diamine,[29] 1-aminonaphthalene,[30] and 3-nitroaniline,[31] respectively, with phenyl isocyanide in dry DCM or THF.

Preparation of Compounds 1−3. Cyclohexane-1,2-dione (0.13 g, 1.18 mmol) and 3-hydrazinobenzoic acid (0.40 g, 2.63 mmol) were suspended in *n*-butanol (24 mL), and then concentrated H_2SO_4 (0.23 mL) was added dropwise via syringe. The mixture was heated to reflux for 60 h and then concentrated under reduced pressure. The thick oily mass was then dissolved in dry DMF (10 mL), and 10% Pd/C (0.03 g, 10% by weight) was added. The mixture was heated to reflux under an atmosphere of N_2 for 24 h and then filtered to remove the 10% Pd/C, which was washed with hot DMF (3 × 3 mL). H_2O (50 mL) was added to the combined filtrates to precipitate the crude product, which was collected by filtration and washed with H_2O (2 × 20 mL). The mixture of structural isomers was a light-yellow solid, which was washed in diethyl ether (30 mL). One isomer, dibutyl indolo[2,3-*a*]carbazole-4,7-dicarboxylate (**3**) (light-green solid, 0.10 g, 0.21 mmol, 18.5% yield), was soluble in diethyl ether and remained in the filtrate. The other two isomers were insoluble and remained in the solid fraction, which was mixed with hot ethyl acetate (30 mL) and filtered. Dibutyl indolo[2,3-*a*]carbazole-2,7-dicarboxylate (**2**) (light-yellow solid, 0.060 g, 0.13 mmol, 11.1% yield), dissolved in ethyl acetate and was obtained from the filtrate. Dibutyl indolo[2,3-*a*]carbazole-2,9-dicarboxylate (**1**) (light-yellow solid, 0.070 g, 0.15 mmol, 12.9% yield) did not dissolve and was obtained as the solid residue.

Data for **1**: Mp: decomposed above 350 °C. R_f = 0.68 (50% THF in hexane). ^{1}H NMR (400.1 MHz, DMSO-d_6, +25 °C): δ 11.56 (bs, 2H, N*H*-11,12); 8.37 (dd, $^4J_{HH}$ = 1.5 Hz, $^5J_{HH}$ = 0.8 Hz, 2H, C*H*-1,10); 8.29 (dm, $^3J_{HH}$ = 8.2 Hz, 2H, C*H*-4,7); 8.04 (bs, 2H, C*H*-5,6); 7.84 (ddd, $^3J_{HH}$ = 8.2 Hz, $^4J_{HH}$ = 1.5 Hz, $^6J_{HH}$ = 0.4 Hz, 2H, C*H*-3,8); 4.35 (t, $^3J_{HH}$ = 6.6 Hz, 4H, 2 × OC*H*$_2$); 1.76 (m, 4H, 2 × OCH$_2$C*H*$_2$); 1.49 (m, 4H, 2 × C*H*$_2$CH$_3$); 0.99 (t, $^3J_{HH}$ = 7.4 Hz, 6H, 2 × C*H*$_3$). ^{13}C NMR (100.6 MHz, DMSO-d_6, +25 °C): δ 166.5 (*C*OO); 138.5 (*C*-10′,12′); 127.2 (*C*-4′,7′ and *C*-11′,11″); 125.9 (*C*-2,9); 120.3 (*C*-5′,6′); 119.7 (*C*H-3,8 and *C*H-4,7); 113.3 (*C*H-1,10); 112.8 (*C*H-5,6); 64.2 (O*C*H$_2$); 30.4 (OC*H*$_2$CH$_2$); 18.8 (*C*H$_2$CH$_3$); 13.6 (*C*H$_3$). ^{15}N NMR (40.6 MHz, DMSO-d_6, +25 °C): δ 116.14 (*N*H-11,12). IR (ATR-FT-IRS) $\tilde{\nu}$: 3355, 2958, 1705, 1668 cm^{-1}. ESI-ICR (*m*/*z*): solvent ∼0.1% DMSO/MeOH, calcd for $C_{28}H_{27}N_2O_4$ $[M - H]^-$ 455.19763, found 455.19742.

Data for **2**: Mp: decomposed above 350 °C. R_f = 0.55 (50% THF in hexane). ^{1}H NMR (400.1 MHz, DMSO-d_6, +25 °C): δ 11.77 (bs, 1H, N*H*-11); 11.34 (bs, 1H, N*H*-12); 8.53 (dd, $^3J_{HH}$ = 8.7 Hz, $^5J_{HH}$ = 0.8 Hz, 1H, C*H*-6); 8.39 (dd, $^4J_{HH}$ = 1.5 Hz, $^5J_{HH}$ = 0.6 Hz, 1H, C*H*-1); 8.27 (ddd, $^3J_{HH}$ = 8.2 Hz, $^5J_{HH}$ = 0.6 Hz, $^5J_{HH}$ = 0.5 Hz, 1H, C*H*-4); 8.00 (dd, $^3J_{HH}$ = 8.1 Hz, $^4J_{HH}$ = 1.1 Hz, 1H, C*H*-10); 7.96 (dd, $^3J_{HH}$ = 8.7 Hz, $^5J_{HH}$ = 0.4 Hz, 1H, C*H*-5); 7.84 (dd, $^3J_{HH}$ = 8.2 Hz, $^4J_{HH}$ = 1.5 Hz, 1H, C*H*-3); 7.79 (dd, $^3J_{HH}$ = 7.5 Hz, $^4J_{HH}$ = 1.1 Hz, 1H, C*H*-8); 7.52 (dd, $^3J_{HH}$ = 8.1 Hz, $^3J_{HH}$ = 7.5 Hz, 1H, C*H*-9); 4.46 (t, $^3J_{HH}$ = 6.6 Hz, 2H, 7-COOC*H*$_2$); 4.34 (t, $^3J_{HH}$ = 6.6 Hz, 2H, 2-COOC*H*$_2$); 1.80 (m, 2H, 7-COOCH$_2$C*H*$_2$); 1.75 (m, 2H, 2-COOCH$_2$C*H*$_2$); 1.49 (m, 2H, 7-COO(CH$_2$)$_2$C*H*$_2$); 1.48 (m, 2H, 2-COO(CH$_2$)$_2$C*H*$_2$); 0.98 (m, 6H, 2 × C*H*$_3$). ^{13}C NMR (100.6 MHz, DMSO-d_6, +25 °C): δ 167.5 (7-*C*OO); 166.5 (2-*C*OO); 140.0 (*C*-10′); 138.5 (*C*-12′); 127.16 (*C*-4′ or *C*-11″); 127.11 (*C*-4′ or *C*-11″); 126.7 (*C*-11′); 125.8 (*C*-2); 124.6 (*C*-7); 123.9 (*C*H-9); 122.0 (*C*H-8); 121.4 (*C*-7′); 119.72 (*C*H-3 or *C*H-4); 119.69 (*C*H-3 or *C*H-4); 119.6 (*C*-5′); 119.4 (*C*-6′); 116.6 (*C*H-6); 116.2 (*C*H-10); 113.3 (*C*H-1); 111.8 (*C*H-5); 64.4 (7-COO*C*H$_2$); 64.2 (2-COO*C*H$_2$); 30.38 (2-COOCH$_2$*C*H$_2$ or 7-COOCH$_2$*C*H$_2$); 30.32 (2-COOCH$_2$*C*H$_2$ or 7-COOCH$_2$*C*H$_2$); 18.8 (2-COO(CH$_2$)$_2$*C*H$_2$ or 7-COO(CH$_2$)$_2$*C*H$_2$); 13.6 (2-COO-(CH$_2$)$_3$*C*H$_3$ and 7-COO-(CH$_2$)$_3$*C*H$_3$). ^{15}N NMR (40.6 MHz, DMSO-d_6, +25 °C): δ 117.05, 115.23 (*N*H-11,12). IR (ATR-FT-IRS) $\tilde{\nu}$: 3334, 2957, 1714, 1657, 1256 cm^{-1}. ESI-ICR (*m*/*z*): solvent

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~0.1% DMSO/MeOH, calcd for $C_{28}H_{27}N_2O_4$ $[M - H]^-$ 455.19763, found 455.19750.

Data for **3**: Mp: 155 °C. R_f = 0.29 (50% THF in hexane). ^{1}H NMR (400.1 MHz, DMSO-d_6, +25 °C): δ 11.53 (bs, 2H, N*H*-11,12); 8.43 (bs, 2H, C*H*-5,6); 8.01 (dd, $^3J_{HH}$ = 8.1 Hz, $^4J_{HH}$ = 1.1 Hz, 2H, C*H*-1,10); 7.78 (dd, $^3J_{HH}$ = 7.5 Hz, $^4J_{HH}$ = 1.1 Hz, 2H, C*H*-3,8); 7.52 (dd, $^3J_{HH}$ = 8.1 Hz, $^3J_{HH}$ = 7.5 Hz, 2H, C*H*-2,9); 4.47 (t, $^3J_{HH}$ = 6.6 Hz, 4H, 2 × OCH_2); 1.81 (m, 4H, 2 × OCH$_2$CH_2); 1.50 (m, 4H, 2 × CH_2CH$_3$); 0.98 (t, $^3J_{HH}$ = 7.4 Hz, 6H, 2 × CH_3). ^{13}C NMR (100.6 MHz, DMSO-d_6, +25 °C): δ: 167.5 (*C*OO); 140.0 (*C*-10′,12′); 126.6 (*C*-11′,11″); 124.6 (*C*-4,7); 123.9 (*C*H-2,9); 121.9 (*C*H-3,8); 121.4 (*C*-4′,7′); 118.9 (*C*-5′,6′); 116.1 (*C*H-1,10); 115.6 (*C*H-5,6); 64.4 (O*C*H$_2$); 30.3 (OCH$_2$*C*H$_2$); 18.8 (*C*H$_2$CH$_3$); 13.6 (*C*H$_3$). ^{15}N NMR (40.6 MHz, DMSO-d_6, +25 °C): δ 116.06 (*N*H-11,12). IR (ATR-FT-IRS) $\tilde{\nu}$: 3321, 2960, 1718, 1662 cm^{-1}. ESI-ICR (m/z): solvent ~0.1% DMSO/MeOH, calcd for $C_{28}H_{27}N_2O_4$ $[M - H]^-$ 455.19763, found 455.19745.

Preparation of Compounds 4–6. Cyclohexane-1,2-dione (0.60 g, 5.35 mmol) and *m*-tolylhydrazine hydrochloride (2.1 g, 13.36 mmol) were suspended in *n*-butanol (24 mL), and then concentrated H_2SO_4 (0.6 mL) was added dropwise via syringe. The mixture was heated to reflux for 65 h. After disappearance of the starting material (as monitored by TLC), the reaction mixture was cooled to room temperature. The formed precipitate was filtered, and 2,9-dimethylindolo[2,3-*a*]carbazole (**4**) (0.55 g, 1.93 mmol, 36.2% yield) was isolated on the filter as a brown solid. The filtrate was concentrated under reduced pressure, and the crude product obtained from the filtrate was purified by column chromatography (silica 230–400 mesh), eluting with 6–7% ethyl acetate in hexane, to afford a 3:84:13 mixture of structural isomers **4**, 4,7-dimethylindolo[2,3-*a*]carbazole (**5**), and 2,7-dimethylindolo[2,3-*a*]carbazole (**6**) (0.52 g, 1.83 mmol, 34.2% yield) as a brown solid.

Data for **4**: Mp: decomposed above 350 °C. R_f = 0.29 (30% ethyl acetate in hexane). ^{1}H NMR (400.1 MHz, DMSO-d_6, +25 °C): δ 10.88 (bs, 2H, N*H*-11,12); 7.99 (dm, $^3J_{HH}$ = 7.9 Hz, 2H, C*H*-4,7); 7.81 (bs, 2H, C*H*-5,6); 7.45 (ddq, $^4J_{HH}$ = 1.5 Hz, $^5J_{HH}$ = 0.7 Hz, $^4J_{HH}$ = 0.7 Hz, 2H, C*H*-1,10); 7.01 (ddq, $^3J_{HH}$ = 7.9 Hz, $^4J_{HH}$ = 1.5 Hz, $^4J_{HH}$ = 0.6 Hz, 2H, C*H*-3,8); 2.49 (m, 6H, CH_3-2,9). ^{13}C NMR (100.6 MHz, DMSO-d_6, +25 °C): δ 139.4 (*C*-10′,12′); 133.7 (*C*-2,9); 125.5 (*C*-11′,11″); 121.6 (*C*-4′,7′); 120.3 (*C*H-3,8); 119.8 (*C*-5′,6′); 119.3 (*C*H-4,7); 111.4 (*C*H-1,10); 111.2 (*C*H-5,6); 21.6 (*C*H$_3$-2,9). ^{15}N NMR (40.6 MHz, DMSO-d_6, +25 °C): δ 112.16 (*N*H-11,12). IR (ATR-FT-IRS) $\tilde{\nu}$: 3186, 3023, 1399, 1043 cm^{-1}. ESI-ICR (m/z): solvent ~0.1% DMSO/MeOH, calcd for $C_{20}H_{15}N_2$ $[M - H]^-$ 283.12407, found 283.12403.

Data for **4**, **5**, and **6** (3:84:13 mixture): Mp: decomposed above 314.6–316.0 °C. R_f = 0.29 (30% ethyl acetate in hexane). IR (ATR-FT-IRS) $\tilde{\nu}$: 3392, 3047, 1650, 1608 cm^{-1}. ESI-ICR (m/z): solvent ~0.1% DMSO/MeOH, calcd for $C_{20}H_{15}N_2$ $[M - H]^-$ 283.12407, found 283.12381.

Data for **5**: ^{1}H NMR (400.1 MHz, DMSO-d_6, +25 °C): δ 11.05 (bs, 2H, N*H*-11,12); 7.97 (bs, 2H, C*H*-5,6); 7.54 (ddq, $^3J_{HH}$ = 8.1 Hz, $^4J_{HH}$ = 1.0 Hz, $^6J_{HH}$ = 0.6 Hz, 2H, C*H*-1,10); 7.29 (ddq, $^3J_{HH}$ = 8.1 Hz, $^3J_{HH}$ = 7.2 Hz, $^5J_{HH}$ = 0.4 Hz, 2H, C*H*-2,9); 7.00 (ddq, $^3J_{HH}$ = 7.2 Hz, $^4J_{HH}$ = 1.0 Hz, $^4J_{HH}$ = 0.9 Hz, 2H, C*H*-3,8); 2.88 (ddd, $^4J_{HH}$ = 0.9 Hz, $^6J_{HH}$ = 0.6 Hz, $^5J_{HH}$ = 0.4 Hz, 6H, CH_3-4,7). ^{13}C NMR (100.6 MHz, DMSO-d_6, +25 °C): δ 139.0 (*C*-10′,12′); 131.6 (*C*-4,7); 125.4 (*C*-11′,11″); 124.3 (*C*H-2,9); 122.2 (*C*-4′,7′); 120.3 (*C*H-3,8); 120.0 (*C*-5′,6′); 113.6 (*C*H-5,6); 109.1 (*C*H-1,10); 20.6 (*C*H$_3$-4,7). ^{15}N NMR (40.6 MHz, DMSO-d_6, +25 °C): δ 113.39 (*N*H-11,12).

Data for **6**: ^{1}H NMR (400.1 MHz, DMSO-d_6, +25 °C): δ 11.05 (bs, 1H, N*H*-11); 10.86 (bs, 1H, N*H*-12); 8.01 (dddq, $^3J_{HH}$ = 7.9 Hz, $^5J_{HH}$ = 0.6 Hz, $^5J_{HH}$ = 0.6 Hz, $^5J_{HH}$ = 0.3 Hz, 1H, C*H*-4); 7.91 (dd, $^3J_{HH}$ = 8.2 Hz, $^5J_{HH}$ = 0.6 Hz, 1H, C*H*-6); 7.86 (dd, $^3J_{HH}$ = 8.3 Hz, $^5J_{HH}$ = 0.5 Hz, 1H, C*H*-5); 7.51 (ddq, $^3J_{HH}$ = 8.0 Hz, $^4J_{HH}$ = 0.9 Hz, $^6J_{HH}$ = 0.6 Hz, 1H, C*H*-10); 7.49 (ddq, $^4J_{HH}$ = 1.5 Hz, $^4J_{HH}$ = 0.8 Hz, $^5J_{HH}$ = 0.6 Hz, 1H, C*H*-1); 7.27 (ddq, $^3J_{HH}$ = 8.0 Hz, $^3J_{HH}$ = 7.1 Hz, $^5J_{HH}$ = 0.3 Hz, 1H, C*H*-9); 7.03 (ddq, $^3J_{HH}$ = 7.9 Hz, $^4J_{HH}$ = 1.5 Hz, $^4J_{HH}$ = 0.6, 1H, C*H*-3); 6.98 (ddq, $^3J_{HH}$ = 7.1 Hz, $^4J_{HH}$ = 0.9 Hz, $^4J_{HH}$ = 0.9 Hz, 1H, C*H*-8); 2.86 (ddd, $^4J_{HH}$ = 0.9 Hz, $^6J_{HH}$ = 0.6 Hz, $^5J_{HH}$ = 0.3 Hz, 3H, CH_3-7); 2.51 (ddd, $^4J_{HH}$ = 0.8 Hz, $^4J_{HH}$ = 0.6 Hz, $^5J_{HH}$ = 0.3 Hz, 3H, CH_3-2). ^{13}C NMR (100.6 MHz, DMSO-d_6, +25 °C): δ 139.5 (*C*-12′); 138.9 (*C*-10′); 134.0 (*C*-2); 131.6 (*C*-7); 125.6 (*C*-11′); 125.4 (*C*-11″); 124.3 (*C*H-9); 122.4 (*C*-7′); 121.5 (*C*-4′); 120.5 (*C*H-3); 120.4 (*C*H-8); 120.3 (*C*-6′); 119.7; (*C*-5′); 119.5 (*C*H-4); 113.6 (*C*H-6); 111.6 (*C*H-1); 111.4 (*C*H-5); 109.2 (*C*H-10); 21.7 (*C*H$_3$-2); 20.7 (*C*H$_3$-7). ^{15}N NMR (40.6 MHz, DMSO-d_6, +25 °C): δ 113.47 (*N*H-11); 111.85 (*N*H-12).

Preparation of Compound 7. Cyclohexane-1,2-dione (3.00 g, 26.78 mmol) and phenylhydrazine (3.01 g, 26.78 mmol) were dissolved in a 1:1 (v/v) mixture of acetic acid (40 mL) and water (40 mL), and the reaction mixture was stirred at reflux temperature for 5 h. After disappearance of the starting material (as monitored by TLC), the reaction mixture was quenched in aqueous $NaHCO_3$ (1 M, 1 L). The formed precipitate was filtered and washed with water. The dried crude solid was washed with pentane to obtain pure compound **7** (3.53 g, 71.4% yield) as a brown solid.[9]

Preparation of Compound 8. Compound **7** (0.10 g, 0.53 mmol) and 2-(trifluoromethyl)phenylhydrazine hydrochloride (0.22 g, 1.07 mmol) were dissolved in *n*-butanol (10 mL), and the mixture was stirred at room temperature for 15 min. Then concentrated H_2SO_4 (0.01 mL) was added dropwise. The mixture was stirred at reflux temperature for 60 h. After disappearance of the starting material (as monitored by TLC), the reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue was dissolved in ethyl acetate (25 mL), and the solution washed with water (50 mL). The ethyl acetate layer was evaporated under reduced pressure to obtain the 5,6-dihydro intermediate as a thick oil. It was dissolved in toluene (10 mL), and *p*-chloranil (0.10 g, 1.05 mmol) was added. The reaction mixture was stirred for 2 h at reflux temperature. After disappearance of the intermediate (as monitored by TLC), the reaction mixture was concentrated under reduced pressure. The concentrated mass was dissolved in ethyl acetate (25 mL), and the solution was washed with saturated $NaHSO_4$ (50 mL) and water (2 × 50 mL). The ethyl acetate solution was dried over anhydrous $MgSO_4$ for 5 min and filtered. The filtrate was evaporated under reduced pressure to obtain the crude product, which was purified by column chromatography (silica 230–400 mesh), eluting with 1–2% ethyl acetate in hexane, to afford 1-(trifluoromethyl)indolo[2,3-*a*]carbazole (**8**) (0.090 g, 0.27 mmol, 51.7% yield) as a gray solid.

Data for **8**: Mp: 198.7 °C. R_f = 0.36 (10% ethyl acetate in hexane). ^{1}H NMR (400.1 MHz, DMSO-d_6, +25 °C): δ 11.44 (bs, 1H, N*H*-12); 11.09 (bs, 1H, N*H*-11); 8.45 (dm, $^3J_{HH}$ = 7.8 Hz, 1H, C*H*-4); 8.16 (dddd, $^3J_{HH}$ = 7.8 Hz, $^4J_{HH}$ = 1.2 Hz, $^5J_{HH}$ = 0.7 Hz, $^5J_{HH}$ = 0.6 Hz, 1H, C*H*-7); 7.97 (dd, $^3J_{HH}$ = 8.3 Hz, $^5J_{HH}$ = 0.4 Hz, 1H, C*H*-5); 7.95 (dd, $^3J_{HH}$ = 8.3 Hz, $^5J_{HH}$ = 0.4 Hz, 1H, C*H*-6); 7.71 (ddd, $^3J_{HH}$ = 8.2 Hz, $^4J_{HH}$ = 1.0 Hz, $^5J_{HH}$ = 0.7 Hz, 1H, C*H*-10); 7.70 (ddq, $^3J_{HH}$ = 7.6 Hz, $^4J_{HH}$ = 1.1 Hz, $^4J_{HF}$ = 0.8 Hz, 1H, C*H*-2); 7.41 (ddd, $^3J_{HH}$ = 8.2 Hz, $^3J_{HH}$ = 7.1 Hz, $^4J_{HH}$ = 1.2 Hz, 1H, C*H*-9); 7.36 (ddq, $^3J_{HH}$ = 7.8 Hz, $^3J_{HH}$ = 7.6 Hz, $^5J_{HF}$ = 0.9 Hz, 1H, C*H*-3); 7.22 (ddd, $^3J_{HH}$ = 7.8 Hz, $^3J_{HH}$ = 7.1 Hz, $^4J_{HH}$ = 1.0 Hz, 1H, C*H*-8). ^{13}C NMR (100.6 MHz, DMSO-d_6, +25 °C): δ 138.7 (*C*-10′); 133.9 (q, $^3J_{CF}$ = 2.0 Hz, *C*-12′); 126.0 (*C*-11″); 125.7 (*C*-4′); 125.3 (*C*-11′); 125.0 (q, $^1J_{CF}$ = 271.5 Hz, *C*F$_3$-1); 124.9 (*C*H-9); 124.2 (*C*H-4); 123.4 (*C*-7′); 121.4 (q, $^3J_{CF}$ = 4.6 Hz, *C*H-2); 120.8 (*C*-6′); 119.8 (*C*H-7); 119.03 (*C*H-8); 119.01 (*C*-5′); 118.5 (*C*H-3); 112.7 (*C*H-5); 111.57 (q, $^2J_{CF}$ = 32.2 Hz, *C*-1); 111.55 (*C*H-10); 111.4 (*C*H-6). ^{15}N NMR (40.6 MHz, DMSO-d_6, +25 °C): δ 112.96 (q, $^4J_{NF}$ = 0.8 Hz, *N*H-12); 113.77 (*N*H-11). ^{19}F NMR (188.3 MHz, DMSO-d_6, +25 °C): δ −59.71 (C*F*$_3$). IR (ATR-FT-IRS) $\tilde{\nu}$: 3436, 3384, 1308, 1105 cm^{-1}. ESI-ICR (m/z): solvent ~0.1% DMSO/MeOH, calcd for $C_{19}H_{10}F_3N_2$ $[M - H]^-$ 323.08015, found 323.08015.

Preparation of Compound 9. Benzene-1,2-diamine (0.68 g, 6.28 mmol) was dissolved in dry DCM (75 mL), and then phenyl isocyanate was added dropwise (1.64 g, 13.83 mmol). The reaction mixture was heated to reflux under under an atmosphere of N_2 for 17 h. After disappearance of the starting material (as monitored by TLC), the formed precipitate was filtered and washed with diethyl ether to

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obtain pure compound **9** (2.20 g, 11.89 mmol, 96.3% yield) as a white solid.

Data for **9**: Mp: 240.5 °C. R_f = 0.37 (5% methanol in DCM). ^{1}H NMR (400.1 MHz, DMSO-d_6, +25 °C): δ 9.05 (bs, 2H, $C_6H_5N\underline{H}$); 8.05 (bs, 2H, $C_6H_4N\underline{H}$); 7.60 (AA′ of AA′XX′, 2H, C$\underline{H}$-3,6); 7.47 (m, 4H, C$\underline{H}$-2′,6′); 7.27 (m, 4H, C$\underline{H}$-3′,5′); 7.09 (XX′ of AA′XX′, 2H, C$\underline{H}$-4,5); 6.96 (m, 2H, C$\underline{H}$-4′). ^{13}C NMR (100.6 MHz, DMSO-d_6, +25 °C): δ 153.2 (NH$\underline{C}$ONH); 139.8 ($\underline{C}$-1′); 131.3 ($\underline{C}$-1,2); 128.7 ($\underline{C}$H-3′,5′); 124.0 ($\underline{C}$H-3,6); 123.9 ($\underline{C}$H-4,5); 121.7 ($\underline{C}$H-4′); 118.1 ($\underline{C}$H-2′,6′). ^{15}N NMR (40.6 MHz, DMSO-d_6, +25 °C): δ 108.58 ($C_6H_5\underline{N}H$); 99.81 ($C_6H_4\underline{N}H$). IR (ATR-FT-IRS) $\tilde{\nu}$: 3277, 3056, 1600, 1305 cm^{-1}. ESI-ICR (m/z): solvent ∼0.1% DMSO/MeOH, calcd for $C_{20}H_{17}O_2N_4$ [M − H]$^-$ 345.13570, found 345.13542.

Preparation of Compound 10. 1-Aminonaphthalene (0.20 g, 1.39 mmol) was dissolved in dry THF (20 mL), and then phenyl isocyanate was added dropwise via syringe (0.21 g, 1.81 mmol). The reaction mixture was stirred at room temperature under an atmosphere of N_2 for 17 h. A precipitate was formed, and the progress of the reaction was monitored by TLC. After disappearance of the starting material, the reaction mixture was cooled to 0 °C and filtered. The crude solid product was washed with diethyl ether to obtain pure compound **10** (0.30 g, 1.14 mmol, 82.0% yield) as a white solid.

Data for **10**: Mp: 211.0 °C. R_f = 0.42 (30% THF in hexane). ^{1}H NMR (400.1 MHz, DMSO-d_6, +25 °C), δ 9.04 (bs, 1H, Ph-N$\underline{H}$); 8.76 (bs, 1H, Naph-N$\underline{H}$); 8.13 (dm, $^3J_{HH}$ = 8.3 Hz, 1H, C$\underline{H}$-8); 8.02 (dd, $^3J_{HH}$ = 7.6 Hz, $^4J_{HH}$ = 1.2 Hz, 1H, C$\underline{H}$-2); 7.93 (dm, $^3J_{HH}$ = 8.0 Hz, 1H, C$\underline{H}$-5); 7.64 (dm, $^3J_{HH}$ = 8.3 Hz, 1H, C$\underline{H}$-4); 7.60 (ddd, $^3J_{HH}$ = 8.3 Hz, $^3J_{HH}$ = 6.8 Hz, $^4J_{HH}$ = 1.6 Hz, 1H, C$\underline{H}$-7); 7.55 (ddd, $^3J_{HH}$ = 8.0 Hz, $^3J_{HH}$ = 6.8 Hz, $^4J_{HH}$ = 1.4 Hz, 1H, C$\underline{H}$-6); 7.51 (m, 2H, C$\underline{H}$-2′,6′); 7.48 (dd, $^3J_{HH}$ = 8.3 Hz, $^3J_{HH}$ = 7.6 Hz, 1H, C$\underline{H}$-3); 7.31 (m, 2H, C$\underline{H}$-3′,5′); 6.99 (m, 1H, C$\underline{H}$-4′). ^{13}C NMR (100.6 MHz, DMSO-d_6, +25 °C): δ 152.9 (NH$\underline{C}$ONH); 139.8 ($\underline{C}$-1′); 134.3 ($\underline{C}$-8a); 133.7 ($\underline{C}$-4a); 128.8 ($\underline{C}$H-3′,5′); 128.4 ($\underline{C}$H-5); 125.9 ($\underline{C}$-1); 125.84 ($\underline{C}$H-6); 125.83 ($\underline{C}$H-3); 125.7 ($\underline{C}$H-7); 122.9 ($\underline{C}$H-4); 121.8 ($\underline{C}$-4′); 121.3 ($\underline{C}$H-8); 118.1 ($\underline{C}$H-2′,6′); 117.4 ($\underline{C}$H-2). ^{15}N NMR (40.6 MHz, DMSO-d_6, +25 °C): δ 108.89 (Ph-$\underline{N}$H); 101.83 (Naph-$\underline{N}$H). IR (ATR-FT-IRS) $\tilde{\nu}$: 3277, 3046, 1639, 1550 cm^{-1}. ESI-ICR (m/z): solvent ∼0.1% DMSO/MeOH, calcd for $C_{17}H_{13}N_2O$ [M − H]$^-$ 261.10333, found 261.10347.

Preparation of Compound 11. 3-Nitroaniline (0.20 g, 1.44 mmol) was dissolved in dry THF (20 mL), and then phenyl isocyanate was added dropwise (0.26 g, 2.17 mmol). The reaction mixture was stirred at room temperature under an atmosphere of N_2 for 17 h. After disappearance of the starting material (as monitored by TLC), the formed precipitate was filtered and washed with diethyl ether to obtain pure compound **11** (0.35 g, 1.36 mmol, 94.1% yield) as a white solid.

Data for **11**: Mp: 209.3 °C. R_f = 0.74 (5% methanol in DCM). ^{1}H NMR (400.1 MHz, DMSO-d_6, +25 °C): δ 9.20 (bs, 1H, 3-$NO_2C_6H_4N\underline{H}$); 8.83 (bs, 1H, Ph-N$\underline{H}$); 8.56 (ddd, $^4J_{HH}$ = 2.3 Hz, $^4J_{HH}$ = 2.2 Hz, $^5J_{HH}$ = 0.4 Hz, 1H, C$\underline{H}$-2); 7.82 (ddd, $^3J_{HH}$ = 8.2 Hz, $^4J_{HH}$ = 2.3 Hz, $^4J_{HH}$ = 1.0 Hz, 1H, C$\underline{H}$-4); 7.71 (ddd, $^3J_{HH}$ = 8.2 Hz, $^4J_{HH}$ = 2.2 Hz, $^4J_{HH}$ = 1.0 Hz, 1H, C$\underline{H}$-6); 7.56 (ddd, $^3J_{HH}$ = 8.2 Hz, $^3J_{HH}$ = 8.2 Hz, $^5J_{HH}$ = 0.4 Hz, 1H, C$\underline{H}$-5); 7.48 (m, 2H, C$\underline{H}$-2′,6′); 7.30 (m, 2H, C$\underline{H}$-3′,5′); 7.00 (m, 1H, C$\underline{H}$-4′). ^{13}C NMR (100.6 MHz, DMSO-d_6, +25 °C): δ 152.4 (NH$\underline{C}$ONH); 148.1 ($\underline{C}$-3); 141.0 ($\underline{C}$-1); 139.2 ($\underline{C}$-1′); 130.0 ($\underline{C}$H-5); 128.8 ($\underline{C}$H-3′,5′); 124.3 ($\underline{C}$H-6); 122.3 ($\underline{C}$-4′); 118.6 ($\underline{C}$H-2′,6′); 116.2 ($\underline{C}$H-4); 112.1 ($\underline{C}$H-2). ^{15}N NMR (40.6 MHz, DMSO-d_6, +25 °C): δ 371.8 ($\underline{N}O_2$); 109.66 (2 × $\underline{N}$H). IR (ATR-FT-IRS) $\tilde{\nu}$: 3309, 3269, 1638, 1523 cm^{-1}. ESI-ICR (m/z): solvent ∼0.1% DMSO/MeOH, calcd for $C_{13}H_{10}N_3O_3$ [M − H]$^-$ 256.07276, found 256.07289.

Preparation of Compounds 12 and 13. Compound **7** (6.00 g, 32.43 mmol) and 3-hydrazinobenzoic acid (5.40 g, 35.52 mmol) were dissolved in *n*-butanol (250 mL). The mixture was stirred at room temperature for 15 min, and then concentrated H_2SO_4 (3.00 mL) was added dropwise. The mixture was stirred at reflux temperature for 40 h until disappearance of the starting material (as monitored by TLC). The mixture was then cooled to room temperature and kept there for 15 h. A yellow precipitate formed. The precipitate was filtered. The filtrate was concentrated under reduced pressure to obtain a thick oily liquid. The crude product was purified by column chromatography (silica 230−400 mesh, 1% methanol in DCM) to get butyl indolo[2,3-*a*]carbazole-4-carboxylate (**13**) (3.2 g, 8.98 mmol, 27.7% yield) as a yellow solid. The insoluble precipitate was dissolved in dry DMF (50 mL), and Pd/C (0.3 g, ∼10 wt %) was added. The reaction mixture was heated to reflux under an atmosphere of N_2 for 24 h and then filtered. The Pd/C remained on the filter and was washed with DMF (3 × 10 mL). The filtrate was diluted with H_2O (200 mL), and a precipitate was formed. This was collected by filtration, washed with H_2O (200 mL), and dried under vacuum to yield butyl indolo[2,3-*a*]carbazole-2-carboxylate (**12**) (4.20 g, 11.79 mmol, 36.4% yield) as an off-white solid.

Data for **12**: Mp: 364.4 °C. R_f = 0.64 (50% THF in hexane). ^{1}H NMR (400.1 MHz, DMSO-d_6, +25 °C): δ 11.32 (bs, 2H, N$\underline{H}$-11,12); 8.36 (dd, $^4J_{HH}$ = 1.5 Hz, $^5J_{HH}$ = 0.7 Hz, 1H, C$\underline{H}$-1); 8.25 (ddd, $^3J_{HH}$ = 8.2 Hz, $^5J_{HH}$ = 0.7 Hz, $^5J_{HH}$ = 0.6 Hz, 1H, C$\underline{H}$-4); 8.19 (dddd, $^3J_{HH}$ = 7.8 Hz, $^4J_{HH}$ = 1.2 Hz, $^5J_{HH}$ = 0.8 Hz, $^5J_{HH}$ = 0.6 Hz, 1H, C$\underline{H}$-7); 7.97 (bs, 2H, C$\underline{H}$-5,6); 7.83 (dd, $^3J_{HH}$ = 8.2 Hz, $^4J_{HH}$ = 1.5 Hz, 1H, C$\underline{H}$-3); 7.70 (ddd, $^3J_{HH}$ = 8.1 Hz, $^4J_{HH}$ = 1.0 Hz, $^5J_{HH}$ = 0.8 Hz, 1H, C$\underline{H}$-10); 7.41 (ddd, $^3J_{HH}$ = 8.1 Hz, $^3J_{HH}$ = 7.1 Hz, $^4J_{HH}$ = 1.2 Hz, 1H, C$\underline{H}$-9); 7.22 (ddd, $^3J_{HH}$ = 7.8 Hz, $^3J_{HH}$ = 7.1 Hz, $^4J_{HH}$ = 1.0 Hz, 1H, C$\underline{H}$-8); 4.34 (t, $^3J_{HH}$ = 6.5 Hz, 2H, OC$\underline{H}_2$); 1.76 (m, 2H, OCH$_2$C$\underline{H}_2$); 1.49 (m, 2H, C$\underline{H}_2$CH$_3$); 0.98 (t, $^3J_{HH}$ = 7.3 Hz, 3H, C$\underline{H}_3$). ^{13}C NMR (100.6 MHz, DMSO-d_6, +25 °C): δ 166.6 ($\underline{C}$OO); 139.2 ($\underline{C}$-10′); 138.4 ($\underline{C}$-12′); 127.5 ($\underline{C}$-4′); 127.5 ($\underline{C}$-11″); 125.5 ($\underline{C}$-2); 125.4 ($\underline{C}$-11′); 124.9 ($\underline{C}$H-9); 123.6 ($\underline{C}$-7′); 121.0 ($\underline{C}$-6′); 119.9 ($\underline{C}$H-7); 119.7 ($\underline{C}$H-3); 119.5 ($\underline{C}$H-4); 119.3 ($\underline{C}$-5′); 119.1 ($\underline{C}$H-8); 113.1 ($\underline{C}$H-1); 112.4 ($\underline{C}$H-5 or $\underline{C}$H-6); 112.1 ($\underline{C}$H-5 or $\underline{C}$H-6); 111.7 ($\underline{C}$H-10); 64.2 (O$\underline{C}$H$_2$); 30.4 (OCH$_2\underline{C}$H$_2$); 18.8 ($\underline{C}$H$_2$CH$_3$); 13.7 ($\underline{C}$H$_3$). ^{15}N NMR (40.6 MHz, DMSO-d_6, +25 °C): δ 114.97 ($\underline{N}$H-12); 114.25 ($\underline{N}$H-11). IR (ATR-FT-IRS) $\tilde{\nu}$: 3351, 2957, 1666, 1613 cm^{-1}. ESI-ICR (m/z): solvent ∼0.1% DMSO/MeOH, calcd for $C_{23}H_{19}O_2N_2$ [M − H]$^-$ 355.14520, found 355.14511.

Data for **13**: Mp: 169.6 °C. R_f = 0.42 (50% THF in hexane). ^{1}H NMR (400.1 MHz, DMSO-d_6, +25 °C): δ 11.49 (bs, 1H, N$\underline{H}$-12); 11.08 (bs, 1H, N$\underline{H}$-11); 8.48 (dd, $^3J_{HH}$ = 8.7 Hz, $^5J_{HH}$ = 0.5 Hz, 1H, C$\underline{H}$-5); 8.16 (dddd, $^3J_{HH}$ = 7.8 Hz, $^4J_{HH}$ = 1.2 Hz, $^5J_{HH}$ = 0.8 Hz, $^5J_{HH}$ = 0.6 Hz, 1H, C$\underline{H}$-7); 7.98 (dd, $^3J_{HH}$ = 8.1 Hz, $^4J_{HH}$ = 1.1 Hz, 1H, C$\underline{H}$-1); 7.90 (dd, $^3J_{HH}$ = 8.7 Hz, $^5J_{HH}$ = 0.5 Hz, 1H, C$\underline{H}$-6); 7.77 (dd, $^3J_{HH}$ = 7.5 Hz, $^4J_{HH}$ = 1.1 Hz, 1H, C$\underline{H}$-3); 7.71 (ddd, $^3J_{HH}$ = 8.1 Hz, $^4J_{HH}$ = 1.0 Hz, $^5J_{HH}$ = 0.8 Hz, 1H, C$\underline{H}$-10); 7.49 (dd, $^3J_{HH}$ = 8.1 Hz, $^3J_{HH}$ = 7.5 Hz, 1H, C$\underline{H}$-2); 7.41 (ddd, $^3J_{HH}$ = 8.1 Hz, $^3J_{HH}$ = 7.1 Hz, $^4J_{HH}$ = 1.2 Hz, 1H, C$\underline{H}$-9); 7.22 (ddd, $^3J_{HH}$ = 7.8 Hz, $^3J_{HH}$ = 7.1 Hz, $^4J_{HH}$ = 1.0 Hz, 1H, C$\underline{H}$-8); 4.46 (t, $^3J_{HH}$ = 6.6 Hz, 2H, OC$\underline{H}_2$); 1.81 (m, 2H, OCH$_2$C$\underline{H}_2$); 1.50 (m, 2H, C$\underline{H}_2$CH$_3$); 0.98 (t, $^3J_{HH}$ = 7.4 Hz, 3H, C$\underline{H}_3$). ^{13}C NMR (100.6 MHz, DMSO-d_6, +25 °C): δ 166.6 ($\underline{C}$OO); 139.8 ($\underline{C}$-12′); 139.2 ($\underline{C}$-10′); 127.0 ($\underline{C}$-11″); 125.2 ($\underline{C}$-11′); 124.8 ($\underline{C}$H-9); 124.4 ($\underline{C}$-4); 123.53 ($\underline{C}$H-2); 123.49 ($\underline{C}$-7′); 121.71 ($\underline{C}$H-3); 121.67 ($\underline{C}$-4′); 120.4 ($\underline{C}$-6′); 119.9 ($\underline{C}$H-7); 119.0 ($\underline{C}$H-8); 118.5 ($\underline{C}$-5′); 116.0 ($\underline{C}$H-1); 115.9 ($\underline{C}$H-5); 111.7 ($\underline{C}$H-10); 111.4 ($\underline{C}$H-6); 64.4 (O$\underline{C}$H$_2$); 30.3 (OCH$_2\underline{C}$H$_2$); 18.8 ($\underline{C}$H$_2$CH$_3$); 13.6 ($\underline{C}$H$_3$). ^{15}N NMR (40.6 MHz, DMSO-d_6, +25 °C): δ 115.79 ($\underline{N}$H-12); 113.28 ($\underline{N}$H-11). IR (ATR-FT-IRS) $\tilde{\nu}$: 3344, 2928, 1662, 1609 cm^{-1}. ESI-ICR (m/z): solvent ∼0.1% DMSO/MeOH, calcd for $C_{23}H_{19}O_2N_2$ [M − H]$^-$ 355.14520, found 355.14509.

Preparation of Compound 14. Compound **12** (4.20 g, 11.79 mmol) was suspended in 2-propanol (100 mL), and then a solution of KOH (32.0 g, 570.4 mmol) in H_2O (120 mL) was added. The mixture was heated under reflux temperature for 40 h until disappearance of the starting material (as monitored by TLC). The reaction mixture was then cooled to room temperature, and the aqueous layer pH was adjusted to 7 using aqueous HCl (1 M). A precipitate was formed and was extracted into ethyl acetate (200 mL). The ethyl acetate solution was dried over anhydrous $MgSO_4$ for 5 min and filtered. The filtrate was evaporated under reduced pressure to obtain pure indolo[2,3-*a*]carbazole-2-carboxylic acid (**14**) (3.20 g, 10.66 mmol, 90.4% yield) as an off-white solid.

Data for **14:** Mp: decomposed above 350 °C. R_f = 0.13 (50% THF in hexane). ^{1}H NMR (400.1 MHz, DMSO-d_6, +25 °C): δ 12.72 (bs,

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1H, COO*H*); 11.27 (bs, 1H, N*H*-12); 11.26 (bs, 1H, N*H*-11); 8.34 (dd, $^4J_{HH}$ = 1.5 Hz, $^5J_{HH}$ = 0.7 Hz, 1H, C*H*-1); 8.24 (ddd, $^3J_{HH}$ = 8.2 Hz, $^5J_{HH}$ = 0.7 Hz, $^5J_{HH}$ = 0.5 Hz, 1H, C*H*-4); 8.18 (dddd, $^3J_{HH}$ = 7.8 Hz, $^4J_{HH}$ = 1.2 Hz, $^5J_{HH}$ = 0.9 Hz, $^5J_{HH}$ = 0.6 Hz, 1H, C*H*-7); 7.97 (bs, 2H, C*H*-5,6); 7.82 (dd, $^3J_{HH}$ = 8.2 Hz, $^4J_{HH}$ = 1.5 Hz, 1H, C*H*-3); 7.70 (ddd, $^3J_{HH}$ = 8.1 Hz, $^4J_{HH}$ = 1.0 Hz, $^5J_{HH}$ = 0.9 Hz, 1H, C*H*-10); 7.41 (ddd, $^3J_{HH}$ = 8.1 Hz, $^3J_{HH}$ = 7.1 Hz, $^4J_{HH}$ = 1.2 Hz, 1H, C*H*-9); 7.22 (ddd, $^3J_{HH}$ = 7.8 Hz, $^3J_{HH}$ = 7.1 Hz, $^4J_{HH}$ = 1.0 Hz, 1H, C*H*-8). ^{13}C NMR (100.6 MHz, DMSO-d_6, +25 °C): δ 168.1 (*C*OO); 139.1 (*C*-10′); 138.3 (*C*-12′); 127.3 (*C*-11″); 127.2 (*C*-4′); 126.5 (*C*-2); 125.4 (*C*-11′); 124.8 (*C*H-9); 123.6 (*C*-7′); 120.9 (*C*-6′); 119.9 (*C*H-3); 119.8 (*C*H-7); 119.4 (*C*-5′); 119.3 (*C*H-4); 119.0 (*C*H-8); 113.3 (*C*H-1); 112.2 (*C*H-5 or *C*H-6); 112.0 (*C*H-5 or *C*H-6); 111.7 (*C*H-10). ^{15}N NMR (40.6 MHz, DMSO-d_6, +25 °C): δ: 114.78 (*N*H-12); 114.14 (*N*H-11). IR (ATR-FT-IRS) $\tilde{\nu}$: 3410, 2951, 2831, 1679 cm^{-1}. ESI-ICR (m/z): solvent ∼0.1% DMSO/MeOH, calcd for $C_{19}H_{11}O_2N_2$ [M − H]$^-$ 299.08260, found 299.08281.

General Procedure for Compounds 15−18, 20, and 21. Compound **14** (0.16 or 0.32 mmol) and K_2CO_3 (2 equiv) were dissolved in dry DMF (2−3 mL), and then the respective reactant (1.3 equiv) was added dropwise. The reactants were the following: bromomethylbenzene, 1-bromomethyl-4-nitrobenzene, 1-chloromethyl-4-methoxybenzene, 1-chloromethyl-3-methylbenzene, iodoethane, and 2-iodopropane. The reaction mixture was stirred for 3 h at 80 °C under an atmosphere of N_2. After disappearance of the starting material (as monitored by TLC), the reaction mixture was cooled to room temperature and quenched in water, and the product was extracted with ethyl acetate. The ethyl acetate solution was washed with water and saturated aqueous NaCl solution, dried over $MaSO_4$, filtered, and concentrated under reduced pressure. The crude product was washed with diethyl ether to get the pure compound **15−18**, **20**, or **21** as a solid.

Data for **15**: Yield: 60.0 mg, 0.15 mmol, 92.3%. Light-yellow solid. Mp: decomposed above 350 °C. R_f = 0.59 (50% THF in hexane). ^{1}H NMR (400.1 MHz, DMSO-d_6, +25 °C): δ 11.36 (bs, 2H, N*H*-11,12); 8.41 (dd, $^4J_{HH}$ = 1.5 Hz, $^5J_{HH}$ = 0.6 Hz, 1H, C*H*-1); 8.27 (dd, $^3J_{HH}$ = 8.2 Hz, $^5J_{HH}$ = 0.6 Hz, 1H, C*H*-4); 8.18 (ddd, $^3J_{HH}$ = 7.8 Hz, $^4J_{HH}$ = 1.2 Hz, $^5J_{HH}$ = 0.7 Hz, 1H, C*H*-7); 7.98 (bs, 2H, C*H*-5 or C*H*-6); 7.87 (dd, $^3J_{HH}$ = 8.2 Hz, $^4J_{HH}$ = 1.5 Hz, 1H, C*H*-3); 7.70 (ddd, $^3J_{HH}$ = 8.1 Hz, $^4J_{HH}$ = 1.0 Hz, $^5J_{HH}$ = 0.7 Hz, 1H, C*H*-10); 7.54 (m, 2H, C*H*-2,6$_{Ph}$); 7.45 (m, 2H, C*H*-3,5$_{Ph}$); 7.41 (ddd, $^3J_{HH}$ = 8.1 Hz, $^3J_{HH}$ = 7.1 Hz, $^4J_{HH}$ = 1.2 Hz, 1H, C*H*-9); 7.38 (m, 1H, C*H*-4$_{Ph}$); 7.22 (ddd, $^3J_{HH}$ = 7.8 Hz, $^3J_{HH}$ = 7.1 Hz, $^4J_{HH}$ = 1.0 Hz, 1H, C*H*-8); 5.43 (s, 2H, C*H*$_2$). ^{13}C NMR (100.6 MHz, DMSO-d_6, +25 °C): δ 166.4 (*C*OO); 139.1 (*C*-10′); 138.3 (*C*-12′); 136.5 (*C*-1$_{Ph}$); 128.5 (*C*-3,5$_{Ph}$); 128.1 (*C*H-4$_{Ph}$); 128.0 (*C*H-2,6$_{Ph}$); 127.6 (*C*-4′); 127.5 (*C*-11″); 125.3 (*C*-11′); 125.1 (*C*-2); 124.9 (*C*H-9); 123.5 (*C*-7′); 121.0 (*C*-6′); 119.9 (*C*H-7); 119.7 (*C*H-3); 119.5 (*C*H-4); 119.3 (*C*-5′); 119.0 (*C*H-8); 113.3 (*C*H-1); 112.4 (*C*H-5 or *C*H-6); 112.1 (*C*H-5 or *C*H-6); 111.7 (*C*H-10); 66.0 (*C*H$_2$). ^{15}N NMR (40.6 MHz, DMSO-d_6, +25 °C): δ 115.14 (*N*H-12); 114.45 (*N*H-11). IR (ATR-FT-IRS) $\tilde{\nu}$: 3387, 3343, 1675, 1282 cm^{-1}. ESI-ICR (m/z): solvent ∼0.1% DMSO/MeOH, calcd for $C_{26}H_{17}N_2O_2$ [M − H]$^-$ 389.12955, found 389.12955.

Data for **16**: Yield: 59.7 mg, 0.13 mmol, 59.7%. Yellow solid. Mp: decomposed above 350 °C. R_f = 0.46 (50% THF in hexane). ^{1}H NMR (400.1 MHz, DMSO-d_6, +25 °C): δ 11.34 (bs, 1H, N*H*-11); 11.32 (bs, 1H, N*H*-12); 8.44 (dd, $^4J_{HH}$ = 1.5 Hz, $^5J_{HH}$ = 0.6 Hz, 1H, C*H*-1); 8.30 (XX′ of AA′XX′, 2H, C*H*-3,5$_{Ph}$); 8.29 (ddd, $^3J_{HH}$ = 8.2 Hz, $^5J_{HH}$ = 0.6 Hz, $^5J_{HH}$ = 0.6 Hz, 1H, C*H*-4); 8.18 (dddd, $^3J_{HH}$ = 7.8 Hz, $^4J_{HH}$ = 1.2 Hz, $^5J_{HH}$ = 0.8 Hz, $^5J_{HH}$ = 0.6 Hz, 1H, C*H*-7); 7.98 (bs, 2H, C*H*-5,6); 7.90 (dd, $^3J_{HH}$ = 8.2 Hz, $^4J_{HH}$ = 1.5 Hz, 1H, C*H*-3); 7.81 (AA′ of AA′XX′, 2H, C*H*-2,6$_{Ph}$); 7.71 (ddd, $^3J_{HH}$ = 8.1 Hz, $^4J_{HH}$ = 1.0 Hz, $^5J_{HH}$ = 0.8 Hz, 1H, C*H*-10); 7.42 (ddd, $^3J_{HH}$ = 8.1 Hz, $^3J_{HH}$ = 7.1 Hz, $^4J_{HH}$ = 1.2 Hz, 1H, C*H*-9); 7.23 (ddd, $^3J_{HH}$ = 7.8 Hz, $^3J_{HH}$ = 7.1 Hz, $^4J_{HH}$ = 1.0 Hz, 1H, C*H*-8); 5.57 (s, 2H, C*H*$_2$). ^{13}C NMR (100.6 MHz, DMSO-d_6, +25 °C): δ 166.2 (*C*OO); 147.1 (*C*-4$_{Ph}$); 144.3 (*C*-1$_{Ph}$); 139.1 (*C*-10′); 138.3 (*C*-12′); 128.6 (*C*H-2,6$_{Ph}$); 127.8 (*C*-4′); 127.6 (*C*-11″); 125.3 (*C*-11′); 124.9 (*C*H-9); 124.7 (*C*-2); 123.7 (*C*-3,5$_{Ph}$); 123.5 (*C*-7′); 121.1 (*C*-6′); 119.9 (*C*H-7); 119.8 (*C*H-3); 119.6 (*C*H-4); 119.3 (*C*-5′); 119.1 (*C*H-8); 113.4 (*C*H-1); 112.4 (*C*H-5 or *C*H-6); 112.1 (*C*H-5 or *C*H-6); 111.7 (*C*H-10); 64.8 (*C*H$_2$). ^{15}N NMR (40.6 MHz, DMSO-d_6, +25 °C): δ 115.14 (*N*H-12); 114.45 (*N*H-11). IR (ATR-FT-IRS) $\tilde{\nu}$: 3384, 3349, 1678, 1277 cm^{-1}. ESI-ICR (m/z): solvent ∼0.1% DMSO/MeOH, calcd for $C_{26}H_{16}N_3O_4$ [M − H]$^-$ 434.11463, found 434.11457.

Data for **17**: Yield: 120.0 mg, 0.28 mmol, 85.7%. Light-yellow solid. Mp: decomposed above 350 °C. R_f = 0.53 (50% THF in hexane). ^{1}H NMR (400.1 MHz, DMSO-d_6, +25 °C): δ 11.34 (bs, 1H, N*H*-11); 11.28 (bs, 1H, N*H*-12); 8.37 (dd, $^4J_{HH}$ = 1.5 Hz, $^5J_{HH}$ = 0.6 Hz, 1H, C*H*-1); 8.26 (ddd, $^3J_{HH}$ = 8.2 Hz, $^5J_{HH}$ = 0.7 Hz, $^5J_{HH}$ = 0.6 Hz, 1H, C*H*-4); 8.18 (dddd, $^3J_{HH}$ = 7.9 Hz, $^4J_{HH}$ = 1.2 Hz, $^5J_{HH}$ = 0.7 Hz, $^5J_{HH}$ = 0.6 Hz, 1H, C*H*-7); 7.97 (bs, 2H, C*H*-5,6); 7.83 (dd, $^3J_{HH}$ = 8.2 Hz, $^4J_{HH}$ = 1.5 Hz, 1H, C*H*-3); 7.70 (ddd, $^3J_{HH}$ = 8.1 Hz, $^4J_{HH}$ = 1.0 Hz, $^5J_{HH}$ = 0.7 Hz, 1H, C*H*-10); 7.48 (AA′ of AA′XX′, 2H, C*H*-2,6$_{Ph}$); 7.41 (ddd, $^3J_{HH}$ = 8.1 Hz, $^3J_{HH}$ = 7.1 Hz, $^4J_{HH}$ = 1.2 Hz, 1H, C*H*-9); 7.22 (ddd, $^3J_{HH}$ = 7.9 Hz, $^3J_{HH}$ = 7.1 Hz, $^4J_{HH}$ = 1.0 Hz, 1H, C*H*-8); 6.99 (XX′ of AA′XX′, 2H, C*H*-3,5$_{Ph}$); 5.34 (s, 2H, C*H*$_2$); 3.78 (s, 3H, OC*H*$_3$). ^{13}C NMR (100.6 MHz, DMSO-d_6, +25 °C): δ 166.4 (*C*OO); 159.2 (*C*H-4$_{Ph}$); 139.1 (*C*-10′); 138.3 (*C*-12′); 130.0 (*C*H-2,6$_{Ph}$); 128.3 (*C*-1$_{Ph}$); 127.5 (*C*-4′); 127.5 (*C*-11″); 125.3 (*C*-2); 125.3 (*C*-11′); 124.9 (*C*H-9); 123.5 (*C*-7′); 121.0 (*C*-6′); 119.9 (*C*H-7); 119.7 (*C*H-3); 119.5 (*C*H-4); 119.3 (*C*-5′); 119.1 (*C*H-8); 113.9 (*C*-3,5$_{Ph}$); 113.3 (*C*H-1); 112.4 (*C*H-5 or *C*H-6); 112.1 (*C*H-5 or *C*H-6); 111.7 (*C*H-10); 65.9 (*C*H$_2$); 55.1 (O*C*H$_3$). ^{15}N NMR (40.6 MHz, DMSO-d_6, +25 °C): δ 114.98 (*N*H-12); 114.37 (*N*H-11). IR (ATR-FT-IRS) $\tilde{\nu}$: 3372, 3336, 1669, 1245 cm^{-1}. ESI-ICR (m/z): solvent ∼0.1% DMSO/MeOH, calcd for $C_{27}H_{19}N_2O_3$ [M − H]$^-$ 419.14012, found 419.14024.

Data for **18**: Yield: 100.0 mg, 0.24 mmol, 74.6%. Light-yellow solid. Mp: decomposed above 324.5−326.5 °C. R_f = 0.55 (50% THF in hexane). ^{1}H NMR (400.1 MHz, DMSO-d_6, +25 °C): δ 11.38 (bs, 1H, N*H*-11); 11.34 (bs, 1H, N*H*-12); 8.40 (dd, $^4J_{HH}$ = 1.5 Hz, $^5J_{HH}$ = 0.6 Hz, 1H, C*H*-1); 8.27 (ddd, $^3J_{HH}$ = 8.2 Hz, $^5J_{HH}$ = 0.6 Hz, $^5J_{HH}$ = 0.6 Hz, 1H, C*H*-4); 8.18 (dddd, $^3J_{HH}$ = 7.8 Hz, $^4J_{HH}$ = 1.2 Hz, $^5J_{HH}$ = 0.7 Hz, $^5J_{HH}$ = 0.6 Hz, 1H, C*H*-7); 7.97 (bs, 2H, C*H*-5,6); 7.86 (dd, $^3J_{HH}$ = 8.2 Hz, $^4J_{HH}$ = 1.5 Hz, 1H, C*H*-3); 7.70 (ddd, $^3J_{HH}$ = 8.1 Hz, $^4J_{HH}$ = 1.0 Hz, $^5J_{HH}$ = 0.7 Hz, 1H, C*H*-10); 7.41 (ddd, $^3J_{HH}$ = 8.1 Hz, $^3J_{HH}$ = 7.1 Hz, $^4J_{HH}$ = 1.2 Hz, 1H, C*H*-9); 7.34 (m, 1H, C*H*-2$_{Ph}$); 7.33 (m, 1H, C*H*-5$_{Ph}$); 7.32 (m, 1H, C*H*-6$_{Ph}$); 7.22 (ddd, $^3J_{HH}$ = 7.8 Hz, $^3J_{HH}$ = 7.1 Hz, $^4J_{HH}$ = 1.0 Hz, 1H, C*H*-8); 7.19 (m, 1H, C*H*-4$_{Ph}$); 5.38 (s, 2H, C*H*$_2$); 2.35 (dd, $^4J_{HH}$ = 1.0 Hz, $^4J_{HH}$ = 0.6 Hz, 3H, C*H*$_3$). ^{13}C NMR (100.6 MHz, DMSO-d_6, +25 °C): δ 166.4 (*C*OO); 139.2 (*C*-10′); 138.3 (*C*-12′); 137.7 (*C*-3$_{Ph}$); 136.3 (*C*-1$_{Ph}$); 128.7 (*C*H-4$_{Ph}$); 128.6 (*C*H-2$_{Ph}$); 128.4 (*C*H-5$_{Ph}$); 127.6 (*C*-4′); 127.5 (*C*-11″); 125.4 (*C*-11′); 125.15 (*C*-2); 125.12 (*C*H-6$_{Ph}$); 124.9 (*C*H-9); 123.5 (*C*-7′); 121.0 (*C*-6′); 119.9 (*C*H-7); 119.7 (*C*H-3); 119.5 (*C*H-4); 119.3 (*C*-5′); 119.0 (*C*H-8); 113.3 (*C*H-1); 112.4 (*C*H-5 or *C*H-6); 112.1 (*C*H-5 or *C*H-6); 111.7 (*C*H-10); 66.0 (*C*H$_2$); 21.0 (*C*H$_3$). ^{15}N NMR (40.6 MHz, DMSO-d_6, +25 °C): δ 115.17 (*N*H-12); 114.49 (*N*H-11). IR (ATR-FT-IRS) $\tilde{\nu}$: 3377, 3335, 1671, 1282 cm^{-1}. ESI-ICR (m/z): solvent ∼0.1% DMSO/MeOH, calcd for $C_{27}H_{19}N_2O_2$ [M − H]$^-$ 403.14520, found 403.14556.

Data for **20**: Yield: 40.0 mg, 0.12 mmol, 73.2%. Light-yellow solid. Mp: decomposed above 350 °C. R_f = 0.58 (50% THF in hexane). ^{1}H NMR (400.1 MHz, DMSO-d_6, +25 °C): δ 11.33 (bs, 1H, N*H*-11); 11.29 (bs, 1H, N*H*-12); 8.37 (dd, $^4J_{HH}$ = 1.5 Hz, $^5J_{HH}$ = 0.7 Hz, 1H, C*H*-1); 8.26 (ddd, $^3J_{HH}$ = 8.2 Hz, $^5J_{HH}$ = 0.7 Hz, $^5J_{HH}$ = 0.6 Hz, 1H, C*H*-4); 8.18 (dddd, $^3J_{HH}$ = 7.8 Hz, $^4J_{HH}$ = 1.2 Hz, $^5J_{HH}$ = 0.8 Hz, $^5J_{HH}$ = 0.6 Hz, 1H, C*H*-7); 7.97 (bs, 2H, C*H*-5,6); 7.83 (dd, $^3J_{HH}$ = 8.2 Hz, $^4J_{HH}$ = 1.5 Hz, 1H, C*H*-3); 7.70 (ddd, $^3J_{HH}$ = 8.1 Hz, $^4J_{HH}$ = 1.0 Hz, $^5J_{HH}$ = 0.8 Hz, 1H, C*H*-10); 7.42 (ddd, $^3J_{HH}$ = 8.1 Hz, $^3J_{HH}$ = 7.1 Hz, $^4J_{HH}$ = 1.2 Hz, 1H, C*H*-9); 7.22 (ddd, $^3J_{HH}$ = 7.8 Hz, $^3J_{HH}$ = 7.1 Hz, $^4J_{HH}$ = 1.0 Hz, 1H, C*H*-8); 4.38 (q, $^3J_{HH}$ = 7.1 Hz, 2H, C*H*$_2$); 1.39 (t, $^3J_{HH}$ = 7.1 Hz, 3H, C*H*$_3$). ^{13}C NMR (100.6 MHz, DMSO-d_6, +25 °C): δ 166.5 (*C*OO); 139.1 (*C*-10′); 138.3 (*C*-12′); 127.4 (*C*-4′,11″); 125.5 (*C*-2); 125.4 (*C*-11′); 124.8 (*C*H-9); 123.6 (*C*-7′); 121.0 (*C*-6′); 119.9 (*C*H-7); 119.7 (*C*H-3); 119.4 (*C*H-4); 119.3 (*C*-5′); 119.0 (*C*H-8); 113.1 (*C*H-1); 112.3 (*C*H-5 or *C*H-6); 112.0 (*C*H-5 or *C*H-6); 111.7 (*C*H-10); 60.5 (*C*H$_2$); 14.3 (*C*H$_3$). ^{15}N NMR (40.6 MHz, DMSO-d_6,

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+25 °C): δ 114.96 (NH-12); 114.38 (NH-11). IR (ATR-FT-IRS) $\tilde{\nu}$: 3357, 2995, 1674, 1282 cm^{-1}. ESI-ICR (*m*/*z*): solvent ∼0.1% DMSO/MeOH, calcd for $C_{21}H_{15}N_2O_2$ $[M - H]^-$ 327.11390, found 327.11377.

Data for **21**: Yield: 80.0 mg, 0.23 mmol, 70.1%. Off-white solid. Mp: decomposed above 350 °C. R_f = 0.64 (50% THF in hexane). ^{1}H NMR (400.1 MHz, DMSO-d_6, +25 °C): δ 11.35 (bs, 1H, NH-11); 11.29 (bs, 1H, NH-12); 8.35 (dd, $^4J_{HH}$ = 1.5 Hz, $^5J_{HH}$ = 0.7 Hz, 1H, CH-1); 8.25 (ddd, $^3J_{HH}$ = 8.2 Hz, $^5J_{HH}$ = 0.7 Hz, $^5J_{HH}$ = 0.6 Hz, 1H, CH-4); 8.18 (dddd, $^3J_{HH}$ = 7.8 Hz, $^4J_{HH}$ = 1.2 Hz, $^5J_{HH}$ = 0.8 Hz, $^5J_{HH}$ = 0.6 Hz, 1H, CH-7); 7.97 (bs, 2H, CH-5,6); 7.82 (dd, $^3J_{HH}$ = 8.2 Hz, $^4J_{HH}$ = 1.5 Hz, 1H, CH-3); 7.70 (ddd, $^3J_{HH}$ = 8.1 Hz, $^4J_{HH}$ = 1.0 Hz, $^5J_{HH}$ = 0.8 Hz, 1H, CH-10); 7.41 (ddd, $^3J_{HH}$ = 8.1 Hz, $^3J_{HH}$ = 7.1 Hz, $^4J_{HH}$ = 1.2 Hz, 1H, CH-9); 7.22 (ddd, $^3J_{HH}$ = 7.8 Hz, $^3J_{HH}$ = 7.1 Hz, $^4J_{HH}$ = 1.0 Hz, 1H, CH-8); 5.21 (sept, $^3J_{HH}$ = 6.2 Hz, 1H, OCH); 1.39 (d, $^3J_{HH}$ = 6.2 Hz, 6H, 2 × CH_3). ^{13}C NMR (100.6 MHz, DMSO-d_6, +25 °C): δ 166.0 (COO); 139.2 (C-10′); 138.3 (C-12′); 127.4 (C-4′); 127.4 (C-11″); 125.9 (C-2); 125.4 (C-11′); 124.9 (CH-9); 123.6 (C-7′); 121.0 (C-6′); 119.9 (CH-7); 119.7 (CH-3); 119.39 (CH-4); 119.35 (C-5′); 119.0 (CH-8); 113.1 (CH-1); 112.3 (CH-5 or CH-6); 112.0 (CH-5 or CH-6); 111.7 (CH-10); 67.7 (OCH); 21.8 (CH_3). ^{15}N NMR (40.6 MHz, DMSO-d_6, +25 °C): δ 114.88 (NH-12); 114.38 (NH-11). IR (ATR-FT-IRS) $\tilde{\nu}$: 3367, 3068, 1672, 1653 cm^{-1}. ESI-ICR (*m*/*z*): solvent ∼0.1% DMSO/MeOH, calcd for $C_{22}H_{17}N_2O_2$ $[M - H]^-$ 341.12955, found 341.12948.

Preparation of Compound 19. Compound **14** (0.050 g, 0.16 mmol) and K_2CO_3 (0.060 g, 0.43 mmol) were dissolved in dry acetone (3 mL) under nitrogen, and then 1,2-diiodoethane (0.032 g, 0.11 mmol) was added to the solution. The mixture was heated to reflux for 15 h until disappearance of the starting material (as monitored by TLC). Then the reaction mixture was quenched in water and extracted with ethyl acetate. The ethyl acetate solution was washed with water and a saturated aqueous solution of NaCl, dried over $MgSO_4$, and filtered. The ethyl acetate layer was concentrated under reduced pressure. The crude product was purified by column chromatography, eluting with 1−2% methanol in DCM, to afford compound **19** (29 mg, 0.08 mmol, 48.9% yield) as a yellow solid.

Data for **19**: Mp: decomposed above 300 °C. R_f = 0.5 (5% methanol in DCM). ^{1}H NMR (400.1 MHz, DMSO-d_6, +25 °C): δ 11.32 (bs, 1H, NH-11); 11.31 (bs, 1H, NH-12); 8.41 (dd, $^4J_{HH}$ = 1.5 Hz, $^5J_{HH}$ = 0.7 Hz, 1H, CH-1); 8.29 (ddd, $^3J_{HH}$ = 8.2 Hz, $^5J_{HH}$ = 0.7 Hz, $^5J_{HH}$ = 0.6 Hz, 1H, CH-4); 8.19 (dddd, $^3J_{HH}$ = 7.8 Hz, $^4J_{HH}$ = 1.2 Hz, $^5J_{HH}$ = 0.7 Hz, $^5J_{HH}$ = 0.6 Hz, 1H, CH-7); 7.99 (bs, 2H, CH-5,6); 7.86 (dd, $^3J_{HH}$ = 8.2 Hz, $^4J_{HH}$ = 1.5 Hz, 1H, CH-3); 7.71 (ddd, $^3J_{HH}$ = 8.1 Hz, $^4J_{HH}$ = 1.0 Hz, $^5J_{HH}$ = 0.7 Hz, 1H, CH-10); 7.42 (ddd, $^3J_{HH}$ = 8.1 Hz, $^3J_{HH}$ = 7.1 Hz, $^4J_{HH}$ = 1.2 Hz, 1H, CH-9); 7.23 (ddd, $^3J_{HH}$ = 7.8 Hz, $^3J_{HH}$ = 7.1 Hz, $^4J_{HH}$ = 1.0 Hz, 1H, CH-8); 5.07 (s, 2H, CH_2); 2.21 (s, 3H, CH_3). ^{13}C NMR (100.6 MHz, DMSO-d_6, +25 °C): δ 202.1 (CO); 165.9 (COO); 139.1 (C-10′); 138.3 (C-12′); 127.7 (C-4′); 127.6 (C-11″); 125.4 (C-11′); 124.9 (CH-9); 124.5 (C-2); 123.5 (C-7′); 121.1 (C-6′); 119.9 (CH-7); 119.8 (CH-3); 119.6 (CH-4); 119.3 (C-5′); 119.1 (CH-8); 113.4 (CH-1); 112.4 (CH-5 or CH-6); 112.1 (CH-5 or CH-6); 111.7 (CH-10); 68.7 (CH_2); 26.0 (CH_3). ^{15}N NMR (40.6 MHz, DMSO-d_6, +25 °C): δ 115.11 (NH-12); 114.36 (NH-11). IR (ATR-FT-IRS) $\tilde{\nu}$: 3344, 2919, 2850, 1675 cm^{-1}. ESI-ICR (*m*/*z*): solvent 80% ACN/20% H_2O (0.1% formic acid), calcd for $C_{22}H_{15}N_2O_3$ $[M - H]^-$ 355.10882, found 355.10898.

^{15}N NMR Measurements. The ^{15}N NMR spectra were recorded at 40.6 MHz (^{15}N) on a 400 MHz NMR instrument. ^{15}N NMR chemical shifts were directly obtained from ^{15}N INEPT or indirectly from ^{1}H-detected ^{1}H−^{15}N gs-HMBC or ^{1}H−^{15}N gs-HSQC spectra. The ^{15}N chemical shifts were referenced externally to the signal of neat nitromethane (381.7 ppm).[32]

Measurements of Relative Binding Constants. NMR measurements were carried out with the 200 MHz instrument. Some measurements were also repeated on the 400 MHz instrument. The results did not differ. All of the solutions were prepared in 95.5% DMSO-d_6/0.5% water (m/m). The concentrations of receptor molecules during the titration experiments were in the range of 0.006−0.009 M. The concentrations of the concentrated and dilute solutions of the anion titrant (TBAA) were in the range of 0.17−0.44 and 0.65−1.41 M, respectively. In the course of titration, the dilute titrant was used to obtain degrees of association at different levels of anion concentration. Near the end point of the titration, the concentrated titrant was added, so that virtually all of the receptor molecules in solution were converted to receptor−anion complexes (8−10 equiv of titrant was eventually added). The medium used was not buffered because the introduction of additional anionic species would interfere with the binding process. In each measurement series, approximately 14−18 spectra were recorded. Most of the spectra from the titration experiments are provided in the Supporting Information. From the spectra, the degrees of complexation for both receptor−anion complexes at a particular titration step were found using the following equation:

$$\alpha = \frac{[H_xG]}{[H_x] + [H_xG]} = \frac{\delta - \delta_{H_x}}{\delta_{H_xG} - \delta_{H_x}} \quad (8)$$

The δ values refer to the chemical shifts of the NH protons. δ denotes the chemical shift at the titration step, and δ_{H_x} and δ_{H_xG} are the chemical shifts of the free receptor molecule and the receptor−anion complex, respectively. Replacing the equilibrium concentrations in eq 5 with the degrees of association for H_1 and H_2 (α_1 and α_2, respectively) gives the following equation for $\Delta\log K_{ass}$:

$$\Delta\log K_{ass} = \log \frac{\alpha_1(1 - \alpha_2)}{(1 - \alpha_1)\alpha_2} \quad (9)$$

The 1:1 binding ratios for the indolocarbazoles and substituted ureas were confirmed in ref 9, and that of compound **9** was confirmed in ref 29.

Compound **5** was obtained as a mixture that also contained **4** and **6** (altogether 16% yield). Nevertheless, the $\Delta\log K_{ass}$ measurements involving **5** were not disturbed by the impurities because the signals in the NMR spectra were sufficiently separated (see pp 305 and 310 in the SI). As further evidence that these measurements were reliable, the within-series standard deviations $s(\Delta\log K_{ass})$ were in the range of 0.001−0.008 log units and the circular validation showed good consistency with other overlapping measurements.

The experimental setup and UV−vis spectrophotometric relative binding affinity measurement method were the same as used in the previous work.[9]

Measurements of Absolute Binding Constants. The working conditions and solvents used the NMR measurements of absolute binding constants were the same as in measurements of relative binding constants. The concentrations of the receptor molecules also were similar to the ones used in the measurements of relative binding constants. The concentrations of TBAA in the dilute and concentrated solutions were in the ranges of 0.15−0.5 and 0.6−1.4 M, respectively. Over the course of the titration, 12−17 spectra were recorded. The spectrum of the free receptor was obtained before the first addition of titrant. The spectrum of the receptor−anion complex was obtained at the end of titration by adding a large excess of titrant. From the weighing data the exact amounts of added titrant were found.

The UV measurements of absolute $\log K_{ass}$ values were performed as in ref 9, except that the concentrations used were different: 0.185−0.220 and 0.01−0.18 M for the concentrated and dilute titrant solutions, respectively.

■ ASSOCIATED CONTENT

Supporting Information

Relative binding measurement spectra (^{1}H NMR and UV−vis spectrophotometric), additional compound characterization data (NMR, IR, and HRMS) of the synthesized compounds, and an example calculation file of relative binding measurements and uncertainty estimation procedures (in .xlsx format). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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