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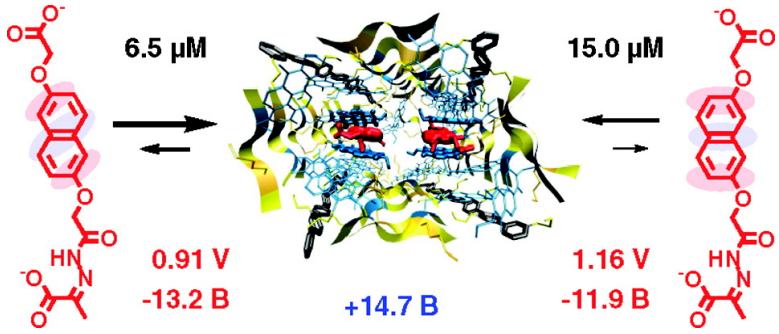

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Screening of π-Basic Naphthalene and Anthracene Amplifiers for π-Acidic Synthetic Pore Sensors

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Abstract: Synthetic ion channels and pores attract current attention as multicomponent sensors in complex matrixes. This application requires the availability of reactive signal amplifiers that covalently capture analytes and drag them into the pore. π-Basic 1,5-dialkoxynaphthalenes (1,5-DAN) are attractive amplifiers because aromatic electron donor−acceptor (AEDA) interactions account for their recognition within π-acidic naphthalenediimide (NDI) rich synthetic pores. Focusing on amplifier design, we report here the synthesis of a complete collection of DAN and dialkoxyanthracene amplifiers, determine their oxidation potentials by cyclic voltammetry, and calculate their quadrupole moments. Blockage experiments reveal that subtle structural changes in regioisomeric DAN amplifiers can be registered within NDI pores. Frontier orbital overlap in AEDA complexes, oxidation potentials, and, to a lesser extent, quadrupole moments are shown to contribute to isomer recognition by synthetic pores. Particularly important with regard to practical applications of synthetic pores as multianalyte sensors, we further demonstrate that application of the lessons learned with DAN regioisomers to the expansion to dialkoxyanthracenes provides access to privileged amplifiers with submicromolar activity.

Introduction

As far as practical applications are concerned, the grand vision of synthetic ion channels and pores[1] as sensors[2] has failed to deliver for decades. The problem is the persistent incompatibility with multianalyte sensing in complex matrixes.[3,4] This problem was solved last year with the introduction of reactive signal amplifiers, molecules that can capture otherwise elusive analytes after enzymatic signal generation and drag them into the pore for signal transduction.[3] 1,5-Dialkoxynaphthalene (1,5-DAN) hydrazides are excellent amplifiers because they can capture analytes containing ketones and aldehydes as hydrazones and can use aromatic electron donor−acceptor (AEDA)[5] interactions to be recognized by synthetic pores with π-acidic naphthalenediimides (NDIs)[3,5−8] (Figures 1 and 2). With effective concentrations for pore inactivation down to the low micromolar range, signal amplification by up to 4 orders of magnitude can be achieved with this approach.[3,7,8] Focusing on amplifier design, the objective of this study was to explore the structural variability of DAN amplifiers to identify, on the one hand, the minimal structural changes detectable by synthetic pores and, on the other hand, to find the best amplifiers. We report that a process as complex as isomer recognition by synthetic pores is clearly influenced by the basic principles of AEDA interactions such as frontier orbital overlap, redox potentials, and, less pronounced, quadrupole moments. Moreover, we demonstrate

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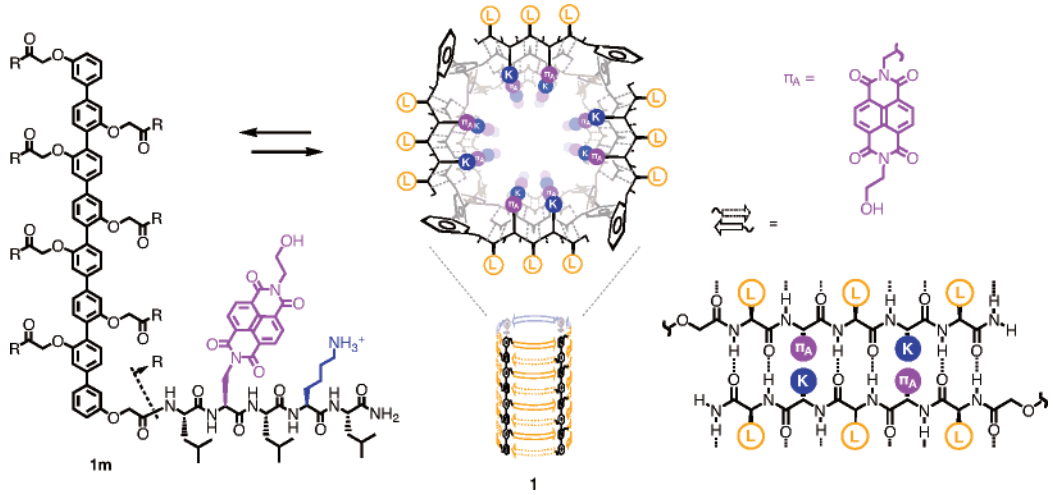

Figure 1. Self-assembly of pore **1** from monomers **1m**. All suprastructures are speculative representations drawn with the only intention to illustrate the design. They are, however, consistent with experimental results and molecular dynamics simulations. For details on pore **1**, please see ref 8.

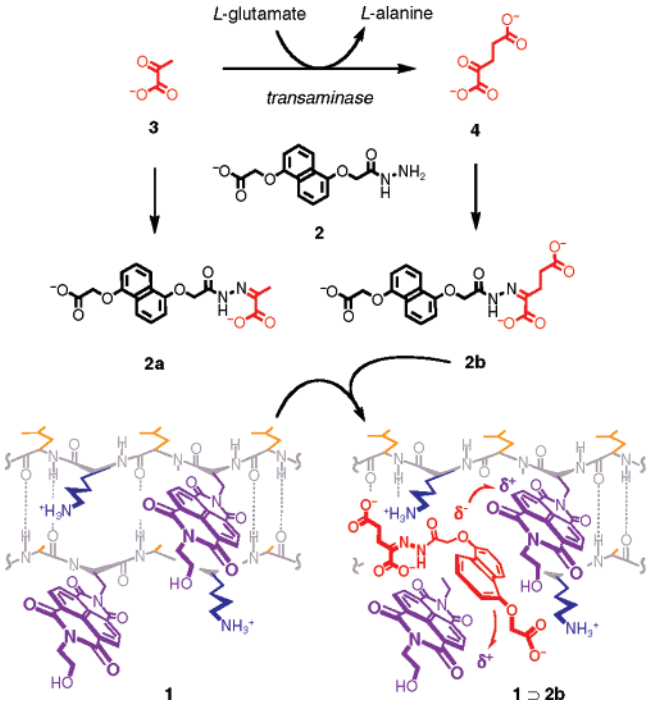

Figure 2. Umami sensing with pore **1** as example to study selective signal amplification. The analyte is detected by incubation with cosubstrate **3** and transaminase for signal generation, hydrazide **2** for signal amplification, and pore **1** for signal transduction. As shown in the close-up structure of pore **1**, selective amplification of **2b** in notional AEDA complex **1** ⊃ **2b** is crucial to avoid interference from **2a** (for data, models, and comments on **1** ⊃ **2b**, see ref 8).

that expansion from naphthalene regioisomers to dialkoxyanthracene (DAA) amplifiers provides access to privileged amplifiers with submicromolar sensitivity.

Pore **1**,[7,8] a rigid-rod β-barrel, is designed to self-assemble from four monomers **1m** (Figure 1). Rigid-rod β-barrels are barrel-stave supramolecules composed of *p*-octiphenyl staves that are brought together by β-sheet hoops.[9] In pore **1**, artificial NDI residues (π_A) are introduced at the inner surface. They are positioned too far apart for mutual π-stacking but just right to π-clamp DAN blocker such as **2b** and form AEDA complexes such as **1** ⊃ **2b** (Figure 2).[7,8] These adhesive π-clamps, operating on AEDA interactions, were introduced to expand the recognition motifs within synthetic pores beyond multipoint ion pairing. Their design combines two classical motifs from biosupramolecular chemistry. Bifunctional intercalation has been studied in many variations as a particularly productive mode of action of bifunctional acyclic receptors.[10,11] Most related to this study are tryptophan clamps on one face of synthetic β-hairpins.[11] The second design motif (i.e., the AEDA complexes formed between DANs and NDIs) has been studied extensively for other purposes including the creation of supramolecular functional π-stack architectures (single- and double-stranded foldamers, rotaxanes, catenanes, ion channels, gels).[5,6] In this context, systematic studies on regioisomer recognition are rare. Pioneering examples focus on the intercalation of DAN regioisomers into NDI gels[6] and anthraquinone isomers into π-stacked rosettes.[12] Regioisomer recognition by synthetic pores has not been reported previously.

In pore **1**, lysines (K) are placed next to the internal NDI clamps to assist π-clamping by ion pairing.[7,8,11] This ion pairing was expected to contribute significantly to selectivity. π-Clamping of hydrazones **2a** and **2b**, for instance, is the same for both and serves exclusively to increase efficiency by orders of magnitude (tetraanionic alkyl analogues of **2b** without DAN do not block pore **1** at all).[7] However, ion pairing of the anions at both sides of the AEDA complex with the proximal lysine residues is different for hydrazones **2a** and **2b** and contributes

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to the discrimination between the two.[3,8] The different positioning of the negative charges and the different donor twist in AEDA complexes achievable with different DAN isomers (below) was expected to influence multi-ion pairing and thus not only blockage efficiency but also selectivity. Evaluation of this aspect of regioisomer recognition was one specific objective of this study.

Hydrazones **2a** and **2b** are ideal model compounds to study selective amplification because of their relevance for practical applications such as lactate, citrate, or umami sensing with synthetic pores.[3] According to current protocols, samples such as soy sauce or tomato juice are first filtered through a hydrazine resin to remove all aldehydes and ketones. Incubation of the sample with transaminase and pyruvate **3** to selectively convert the analyte glutamate is used for signal generation. Since pore **1** fails to respond to enzymatic signal generation, the product α-ketoglutarate **4** is reacted with DAN hydrazide **2**. With the analyte-amplifier conjugate **2b**, signal transduction by pore **1** is possible with high selectivity and without interference from the amplified cosubstrate **2a** because of the selectivity of ion-pair-assisted π-clamping within pore **1**. The sensitivity of synthetic pore sensors is given by the lowest IC_{50} of the system (here **2b**, IC_{50} = 2.7 μM; IC_{50} is the blocker concentration needed to reduce pore activity to 50%).[3] Their selectivity is given by the discrimination factor *D* between that IC_{50} and the most relevant interference (here $D = IC_{50}$ (**2a**)/IC_{50} (**2b**) = 5.3). $D \geq 3$ is sufficient for selective sensing with pores.

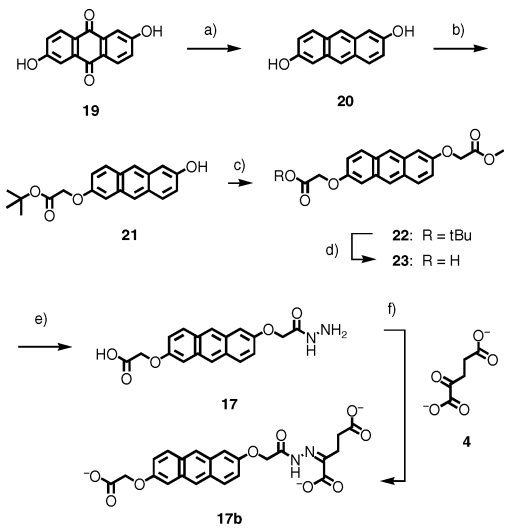
Results and Discussion

The synthesis of monomers **1m**, their self-assembly into pore **1**, and the characteristics of pore **1** have been reported previously.[7,8] The regioisomeric DAN amplifiers **5**−**16** (Table 1) were prepared from dihydroxynaphthalenes following the procedures developed for isomers **2** and **15** (Schemes S1 and S2). Pairs of DAN regioisomers such as 1,7-isomers **6** and **7** or 1,3-isomers **10** and **12** were separated by column chromatography or recrystallization. Incubation of the obtained collection of amplifiers **5**−**16** with pyruvate **3** and α-ketoglutarate **4** as model analytes gave the desired hydrazone blockers **5a**−**16a** and **5b**−**16b**, respectively (Scheme 1 and Figure 2).

DAA amplifier **17** and **18** were synthesized from 2,6-dihydroxyanthraquinone **19** (Scheme 1).[13] Conversion of the starting material into dihydroxyanthracene **20** was accomplished following previously published procedures.[14] Partial Williamson ether synthesis with *t*-butyl bromoacetate gave monoether **21**, and completion with methyl bromoacetate gave the asymmetric diether **22**. Acid-catalyzed hydrolysis of the *t*-butyl ester afforded DAA **23** with a free carboxylic acid and a methyl ester. Treatment of the latter with hydrazine afforded the desired hydrazide amplifier **17**. The dihydrazide DAA amplifier **18** was accessible in a single step from the symmetric side product obtained together with **21** (Scheme S3).[13]

The ability of DAN monohydrazones **5a**−**13a** and **5b**−**13b** to block pore **1** was determined first under standard conditions in egg yolk phosphatidylcholine large unilamellar vesicles loaded with 5(6)-carboxyfluorescein (CF).[3,4,7,8] In this assay, pore activity is observed as appearance of green fluorescence

Scheme 1. Synthesis (a−e) and Use (f) of DAA Amplifier **17**[a]



[a] (a) 1. Methyl-*p*-toluenesulfonate, Na_2CO_3, 2. $NaBH_4$, 3. BBr_3, see ref 14. (b) *t*-Butyl bromoacetate, K_2CO_3, acetone, reflux, overnight, 16% (partial conversion). (c) Methyl bromoacetate, K_2CO_3, acetone, reflux, overnight, 82%. (d) TFA, CH_2Cl_2, 2 h, rt, quant. (e) $H_2NNH_2 \cdot H_2O$, MeOH, reflux, overnight, quant. (f) DMSO, 2 h, 50 °C.

because the local dilution during CF efflux eliminates the self-quenching of CF. Pore blockage is observed as decreasing pore activity with increasing blocker concentration and quantified as IC_{50}. The IC_{50}'s of isomeric DAN amplifiers as pyruvate (**5a**−**13a**) and α-ketoglutarate (**5b**−**13b**) monohydrazones are summarized in Table 1, entries 1−10. The maximal isomer discrimination factors found for DAN amplifiers were $D = IC_{50}$ (**5b**)/IC_{50} (**13b**) = 3.3 for α-ketoglutarates and $D = IC_{50}$ (**5a**)/IC_{50} (**13a**) = 2.3 for pyruvates. Interestingly, the structural difference between the best (2,6) and the worst regioisomer (2,7) is very small. The found discrimination between these two very similar molecules was almost as good as the discrimination $D = 2.7-5.5$ between the doubly charged pyruvate and the triply charged α-ketoglutarate blockers **5a**−**13a** and **5b**−**13b**, respectively (Table 1, column 8). To elaborate on the origin of this substantial isomer recognition within pore **1**, the oxidation potentials of the DAN and DAA diester derivatives of **2** and **5**−**18** were measured by cyclic voltammetry (Tables 1, column 9, and S1).[13] Moreover, the global quadrupole moments Q_{zz} of the dimethoxy DAN and DAA analogues of **2** and **5**−**18** were computed using the MP2/6-311G**//PBE1PBE/6-311++G** method (Table 1, column 10).[13,15] Additionally, the molecular orbitals of DAN and DAA isomers and of the NDI clamps were computed (Figures 3, S1, and S2).

Comparison of the obtained results did not reveal simple correlations in most cases (Table 1 and Figure 3). As an exception, the agreement of the poor recognition of 2,7-isomers **5** with high oxidation potential and small quadrupole moment was excellent, indicating that weak AEDA interactions with the NDI within pore **1** account for poor blockage (Table 1, entry 1). The HOMO of the 2,7-isomer **5** overlapped favorably with a LUMO of the NDI acceptor **1** when the long axis of the DAN naphthalene was oriented collinear to the N,N-axis of the NDI (Figure 3). This collinear AEDA complex for 2,7-isomers was

(13) See Supporting Information.
(14) Méry, S.; Haristoy, D.; Nicoud, J. F.; Guillon, D.; Monobe, H.; Shimizu, Y. *J. Mater. Chem.* **2003**, *13*, 1622−1630.
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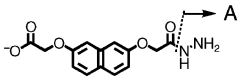
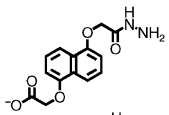
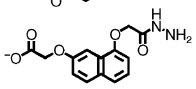
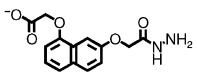
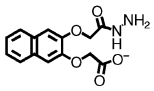
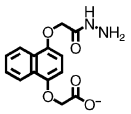
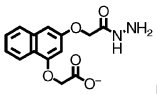
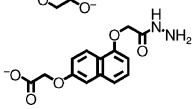
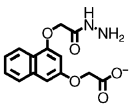
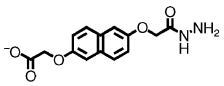
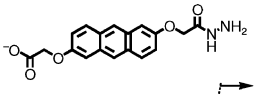
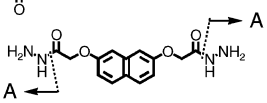
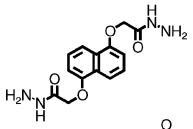
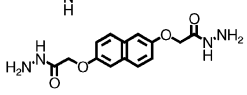
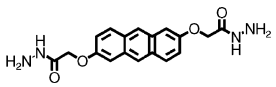
Table 1. DAN and DAA Amplifiers for Pore **1**[a]

Entry	Amplifier	Isomer[a]	A =	IC_{50} (μM)[b]	A =	IC_{50} (μM)[c]	D[d]	$E_{1/2}$ (V)[e]	Q_{zz} (B)[f]
1		**5** (2,7)	**5a**	15.0 ± 0.9	**5b**	4.4 ± 0.7	3.4	1.16	-11.9
2		**2** (1,5)	**2a**	14.4 ± 0.9[g]	**2b**	2.7± 0.2[g]	5.3	0.92[h]	-18.1
3		**6** (7,1)	**6a**	13.0 ± 0.5	**6b**	2.4 ± 0.4	5.4	0.92	-17.8
4		**7** (1,7)	**7a**	12.0 ± 0.6	**7b**	2.5 ± 0.2	4.8	0.92	-17.8
5		**8** (2,3)	**8a**	9.9 ± 1.1	**8b**	3.7 ± 0.8	2.7	1.23[i]	-13.2
6		**9** (1,4)	**9a**	9.7 ± 1.0	**9b**	3.0 ± 0.3	3.2	0.80	-16.4
7		**10** (1,3)	**10a**	8.8 ± 0.4	**10b**	3.0 ± 0.5	2.9	0.92	-15.4
8		**11** (1,6)	**11a**	7.2 ± 0.2	**11b**	1.9 ± 0.2	3.8	0.92[h]	-16.6
9		**12** (3,1)	**12a**	6.6 ± 0.7	**12b**	1.8 ± 0.1	3.7	0.93	-15.4
10		**13** (2,6)	**13a**	6.5 ± 1.4	**13b**	1.3 ± 0.1	5.0	0.91	-13.2
11		**17** (2,7)	**17a**	0.75 ± 0.25	**17b**	0.32 ± 0.05	2.3	0.71	-15.7
12		**14** (2,7)	**14a**	3.2 ± 0.2	**14b**	0.31 ± 0.03	10.3	1.16	-11.9
13		**15** (1,5)	**15a**	4.5 ± 0.3[g]	**15b**	0.24 ± 0.04[g]	18.9	0.92[h]	-18.1
14		**16** (2,6)	**16a**	7.1 ± 0.7	**16b**	0.50 ± 0.06	14.2	0.91	-13.2
15		**18** (2,7)	**18a**	0.49 ± 0.05	**18b**	0.15 ± 0.01	3.3	0.71	-15.7

[a] Compare Figure 1 for pore structures. Substitution locants are given in parentheses. [b] Concentration of blockers **2a** and **5a**−**18a** required for 50% pore blockage. [c] Same for blockers **2b** and **5b**−**18b**. [d] Discrimination factor $D = IC_{50}$ (**a**)/IC_{50} (**b**). [e] Oxidation potentials of DANs and DAAs determined by cyclic voltammetry in acetonitrile against Fc/Fc^+; synthetic diester isomers such as **22** (Table 1) or dimethoxy isomers such as **13c** (Table S1) were used for measurements, as specified in the Supporting Information. [f] Computed global quadrupole moments Q_{ZZ} of the corresponding dimethoxy DANs in Buckinghams (NDIs in pore **1**: $Q_{zz} = +14.9$ B).[13,15] [g] From refs 3 and 7. [h] Extrapolated from literature data (Table S1).[13] [i] Literature values are lower (Table S1).[13]

unfavorable because one of the two substituents collided with the β-sheet of the surrounding β-barrel walls (Figure 2).

More favorable substituent orientation in the same collinear AEDA complex with 2,3-isomers **8** could explain slightly improved recognition despite unfavorable oxidation potential (Figure 3 and Table 1, entry 5). Similar arguments could be found for other isomers with average blockage efficiency. The superb recognition of 2,6-isomers **13** did not coincide with

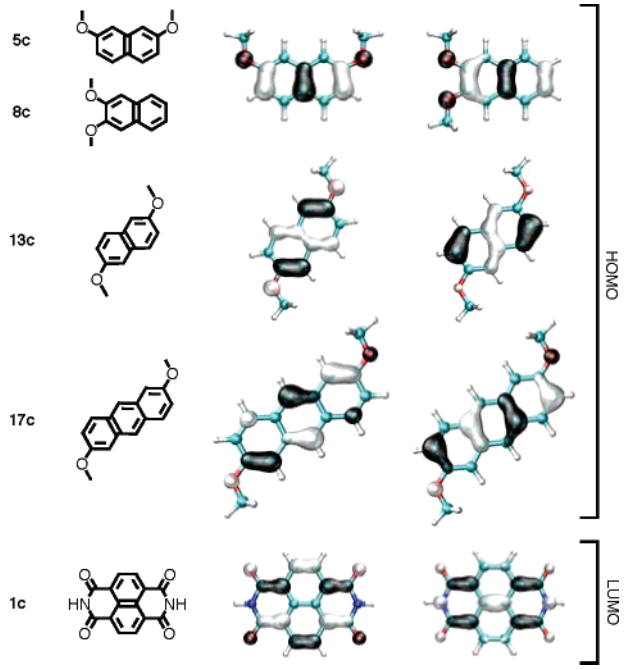


Figure 3. Selection from quasi-degenerate HOMOs of DAN and DAA isomers oriented for best fit with selected quasi-degenerate LUMOs of NDIs in notional AEDA complexes (compare Figures S1 and S2; **5c** on the top left is oriented to match the **1c** on the bottom right).[13]

exceptional $E_{1/2}$ or Q_{ZZ} but with favorable interactions of two quasi-degenerate HOMOs with LUMOs of the NDIs in a twisted AEDA complex with an angle of ~45° between the donor and acceptor naphthalenediimides (Figure 3 and Table 1, entry 10). Interestingly, 2,6-isomers were also best in otherwise unrelated DAN-NDI gels.[6] Completely different trends found with dihydrazone blockers suggested dominant interference from analyte termini and demonstrated that all possible contributions from AEDA interactions to isomer recognition should not be overinterpreted.

2,7-DAA isomer **17** was selected for expansion to dialkoxyanthracene amplifiers because the HOMO orbitals were similar to those of the ideal 2,6-DAN amplifier **13**, producing a favorably twisted AEDA complex with even better HOMO−LUMO overlap (~45°, Figure 3). Two hundred millivolts below that of DAN **13**, the oxidation potential of DAA **17** was excellent for AEDA interactions (Table 1, entry 11). The recognition of DAA monohydrazones **17a** and **17b** was correspondingly better than that of DAN monohydrazones **5a**−**13a** and **5b**−**13b** and, remarkably, also of DAN dihydrazones **14a**−**16a** (Table 1, entry 11). The maximized ion pairing in DAA dihydrazones **18a** and **18b** provided access to efficiencies as low as IC_{50} = 150 nM (Table 1, entry 15).

Conclusions

This study focuses on scope and limitation of the development of efficient amplifiers for pore sensors. Pores that are equipped with internal, π-acidic π-clamps to capture π-basic amplifiers by AEDA interactions are used as example. The results from systematic screening of regioisomeric DAN amplifiers demonstrate that synthetic pores that operate by ion-pair-assisted π-clamping are capable of regioisomer recognition, responding to structural differences as subtle as the one between 2,6- and 2,7-DAN isomers. The finding that the fundamental principles of AEDA interactions, from redox potential to frontier orbital overlap, contribute substantially to this high selectivity underscores that the creation of new recognition motifs for synthetic pores is significant because it leads to new functions. Best results obtained for the 2,6-isomer suggest that ideal recognition occurs in a twisted AEDA complex, where the face-to-face naphthalene planes of amplifier and π-clamp are twisted by ca. 45° for optimal overlap of the HOMO and LUMO orbitals of the DAN donor and NDI acceptors, respectively. The application of these insights from isomer recognition with naphthalene amplifiers to the design of anthracene amplifiers resulted in the discovery of the most efficient amplifier known today. These findings demonstrate that the rational design of privileged amplifiers is possible, a conclusion that is of general interest because it opens attractive perspectives for the development of hypersensitive and highly selective pore sensors and beyond.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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