# ChemComm

## Accepted Manuscript






ROYAL SOCIETY OF CHEMISTRY

rsc.li/chemcomm

View Article Online
DOI: 10.1039/C6CC09478C

Journal Name

COMMUNICATION

# Three in One: Prototropy-free Highly Stable AADD-type Self-Complementary Quadruple Hydrogen-Bonded Molecular Duplexes with Built-in Fluorophore†‡

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Sanjeev Kheria,[a] Suresh Rayavarapu,[a] Amol S. Kotmale,[b] and Gangadhar J. Sanjayan*[a]

**This communication reports an effective approach for addressing the prototropy-related problems in heterocycle-based AADD-type self–assembling systems by freezing their hydrogen-bonding codes, by utilizing intramolecular bifurcated hydrogen bonding interactions. Using this strategy, we have also developed a hydroquinone-conjugated AADD-type self-assembling system adorned with three valuable features such as prototropy-free dimerization yielding single duplex, high duplex stability and a built-in fluorophore, which would augment its application potential. The rational approach used herein to arrest prototropic shift may also find application elsewhere, wherein proton shift-mediated structural changes become a detrimental factor.**

Quadruple hydrogen bonding systems have triggered wide research interest in the design and developmemt of well-defined supramolecular architectures[1] due to their high strength, specificity, directionality and reversibility. Various heterocycle-based multiple hydrogen-bonding modules were developed and have shown good application potential.[2] These systems, most often urea derivatives, are inspired by Nature's use of DNA base pairs such as purines and pyrimidines. In particular, Meijer's ureidopyrimidone[3] (UPy) system, due to its easy synthetic accessibility and high dimerization constant ($K_{dim}$ > $10^7$ $M^{-1}$), has found extensive application in various fields such as supramolecular chemistry, material science, and catalysis (Fig. 1a).[4] A closely-related deazapterin-based self-assembling system also shows high duplex stability (Fig. 1b).[5]

It is noteworthy that heterocycle-based **AADD**-type self-assembling systems are associated with prototropy-related issues and as a result, the protamers (*keto* and *enol* forms) generate different structures – both in solution- and solid-state.[3a] Continuous research in this area by various groups[6] has led to the development of different types of the hydrogen-bonding motifs devoid of tautomerization which will be promising candidates for diverse applications.

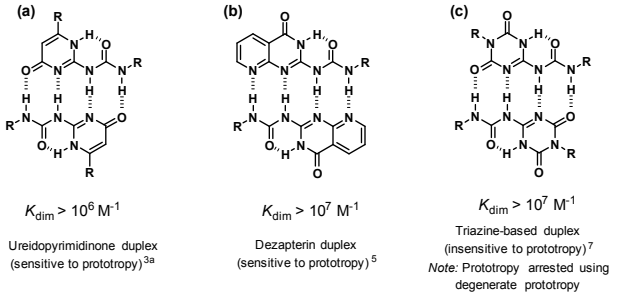



Fig 1. **AADD**-type self-assembling systems forming highly stable duplexes.

Recently, our group has reported a new class of triazine-based highly stable **AADD**-type self-assembling systems devoid of prototropy - without compromising duplex stability (Fig. 1c).[7] These systems have high dimerization constant in apolar solvent ($K_{dim}$ > $10^7$ $M^{-1}$ in $CDCl_3$).

For the development of self-complementary systems featuring high $K_{dim}$, but without prototropy-related issues, herein we propose an effective strategy to address the prototropy issues associated with heterocycle-based **AADD**-type quadruple hydrogen-bonding modules by freezing their H-bonding codes, by exclusively using intramolecular bifurcated hydrogen bonding interactions (Fig. 2, *vide supra*). It was envisaged that the "enol prototropy", usually associated with heterocycle-based quadruple hydrogen-bonding systems, will not occur in the designed systems, primarily because of the H-bonding "locking" mechanism, which would effectively prevent the crucial proton shift leading to the enol form. As a proof-of-principle, we have developed **1**, wherein the methoxy group acts as a H-bonding acceptor, in conjunction with the urea carbonyl group (another H-bonding acceptor), to arrest the labile pyrimidine ring N-H (H-bonding donor) from shifting its position. Thus, proton shift is effectively arrested leading to the formation of a single set of molecular duplex. Using the same principle, quinoline derivative **2** was also developed,

[a] Organic Chemistry Division, CSIR-National Chemical Laboratory (CSIR-NCL), Dr. Homi Bhabha Road, Pune-411008, India. E-mail: gj.sanjayan@ncl.res.in; Fax: +91-20-25902629; Tel: +91-20-25902082.
[b] Central NMR Facility, CSIR-National Chemical Laboratory (CSIR-NCL), Dr. Homi Bhabha Road, Pune-411008, India

†Dedicated to mark the 60th birth anniversary of Prof. Uday Maitra

‡Electronic Supplementary Information (ESI) available: Details on experimental procedure, characterization data of all compounds. See DOI: 10.1039/x0xx00000x

View Article Online
DOI: 10.1039/C6CC09478C





wherein the quinoline ring nitrogen participates in bifurcated H-bonding. The hydroquinone derivative **3** is a close analog of **1**, except that it is adorned with fluorescent properties.

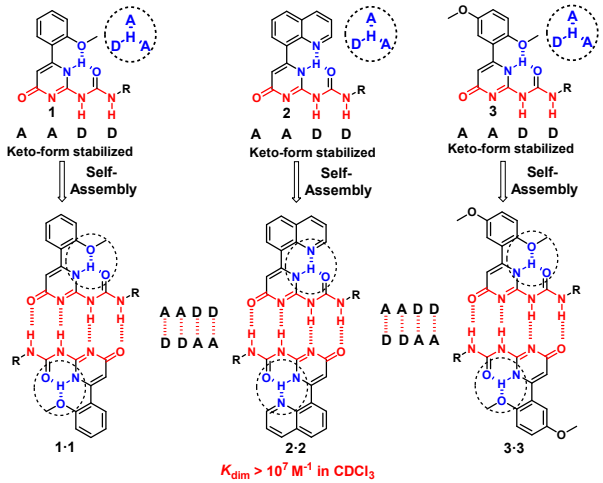
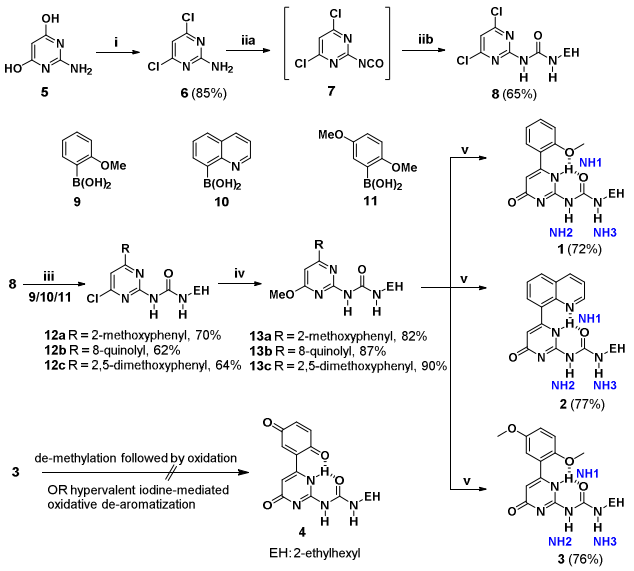


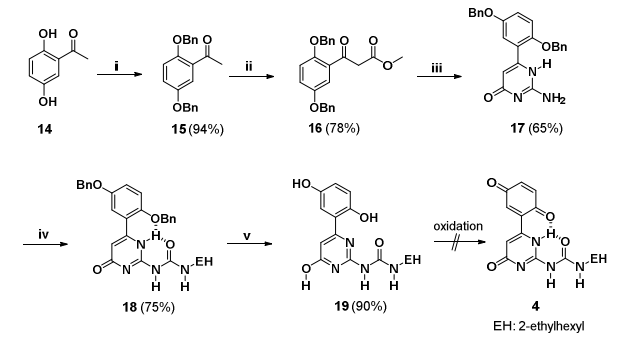

**Fig 2.** Design strategy to arrest prototropy using bifurcated hydrogen-bonding.

The urea intermediate **8** was synthesized by a three-step process (Scheme 1). The dichloro amino pyrimidine **6**, obtained by reacting 2-amino-4,6-dihydroxypyrimidine **5** with $POCl_3$ and $Et_3N$ under reflux condition, was converted to its isocyanate **7**[9], by treatment with oxalyl chloride, in refluxing benzene. The highly reactive and unstable isocyanate **7** was treated with 2-ethylhexylamine at room temperature to afford the urea intermediate **8**, which could be coupled with various boronic acids under standard Suzuki coupling conditions to furnish **12a**-**c**. Direct conversion of **12a**-**c** to **1**-**3**, by hydrolysis of chloro substituent did not yield a tangible result. Therefore, we adopted an alternative two-step strategy in which the chloro of **12a**-**c** was methoxylated followed by demethylation under HBr/acetic acid condition, resulting in good yields of **1**-**3**.

We also had an interest to synthesize a potential redox-active quinone analog **4** (Scheme 1), starting from **3**, by demethylation followed by oxidation or hypervalent iodine-mediated oxidative de-aromatization.[10] However, all our efforts in this direction failed, since intractable mixture of products were obtained. Therefore, we sought an alternate strategy to synthesize **4**, starting from **14** (Scheme 2). Following literature procedures, **14** was bis-benzylated to yield **15**, followed by its reaction with dimethyl carbonate to obtain the active methylene compound **16**, which reacted with guanidine carbonate to furnish **17**. The amino pyrimidine **17** could be efficiently converted to its benzyl protected urea analog **18** by reacting with 2-ethylhexyl isocyanate. Hydrogenation of **18** readily afforded its free hydroquinone derivative **19**. Quite unfortunately, all our extensive efforts to convert **19** to its oxidized form **4**, failed, under a variety of oxidation conditions (PIFA/CAN/Oxone/*m*CPBA, etc) owing to the formation of intractable mixture of products. Compound **19** may assume **DADD**-type H-bonding code and not **AADD**-type H-bonding code, possibly because the free phenol would stabilize the pyrimidine ring in its aromatized form, owing to protonation of the pyrimidine nitrogen. However, this opportunity does not exist in its oxidized form **4**.




**Scheme 1** Synthesis of compounds **1**, **2** and **3**. Reagents and conditions: (i) $POCl_3$, $Et_3N$, $CH_3CN$, reflux, 1h; (ii) a) $(COCl)_2$, $C_6H_6$, reflux, 2h, b) 2-ethylhexylamine, $C_6H_6$, RT, 18h; (iii) $Pd(PPh_3)_4$, $Na_2CO_3$, DME/$H_2O$ (3:1), 110°C, 12h; (iv) $K_2CO_3$, $CH_3OH$, reflux, 6h; (v) HBr/AcOH, reflux, 1h.




**Scheme 2** Attempted synthesis of **4**. Reagents and conditions: (i) BnBr, $K_2CO_3$, acetone, reflux, 24h; (ii) $(CH_3O)_2CO$, NaH, 75°C, 24h; (iii) guanidine carbonate, $C_2H_5OH$, reflux, 20h; (iv) 2-ethylhexyl isocyanate, pyridine, 100°C, 10h; (v) Pd/C, THF, $H_2$, balloon, 24h.

Although self-assembling systems **1** and **3** are close analogs, **3** exhibited fluorescence owing to the presence of the hydroquinone moiety.[8] Therefore, **3** was taken up as a representative case for extensive structural studies. Further, compounds **1** and **3** showed similar $^1$H NMR chemical shift values for their urea NHs, suggesting similar H-bonding arrangements in the duplex. Full characterization data of **1** is provided (ESI,‡ page S14-S16).

The duplex formation by **2** and **3** were investigated, as representative examples, by $^1$H NMR experiments. $^1$H and $^{13}$C NMR spectra of **2** and **3** in $CDCl_3$ clearly showed a *single set* of well-resolved signals suggesting the effectiveness of our design strategy (Fig. 3, *vide infra*).

View Article Online
DOI: 10.1039/C6CC09478C




The significant downfield shift of NHs of **2** (11.86, 10.00 ppm) and **3** (11.87, 10.03 ppm), were indicative of strong intermolecular hydrogen bonding interactions involving the urea NH protons. Furthermore, the far downfield shift of NH signals of **2** and **3** at 15.14 and 13.97 ppm, respectively, clearly suggested the strong involvement of intramolecular bifurcated H-bonding (involving two acceptors).

The dimerization of **2** and **3** were investigated by $^{1}$H NMR dilution experiments (ESI,‡ Fig. S23 and S24 respectively). When a $CDCl_3$ solution of **2** and **3** were diluted from 100 mM to 10 μM, no detectable changes were observed in the chemical shift values of all the NH protons. This showed that dimerization of **2** and **3** persisted at a lower concentration in $CDCl_3$ with high $K_{dim}$ value.

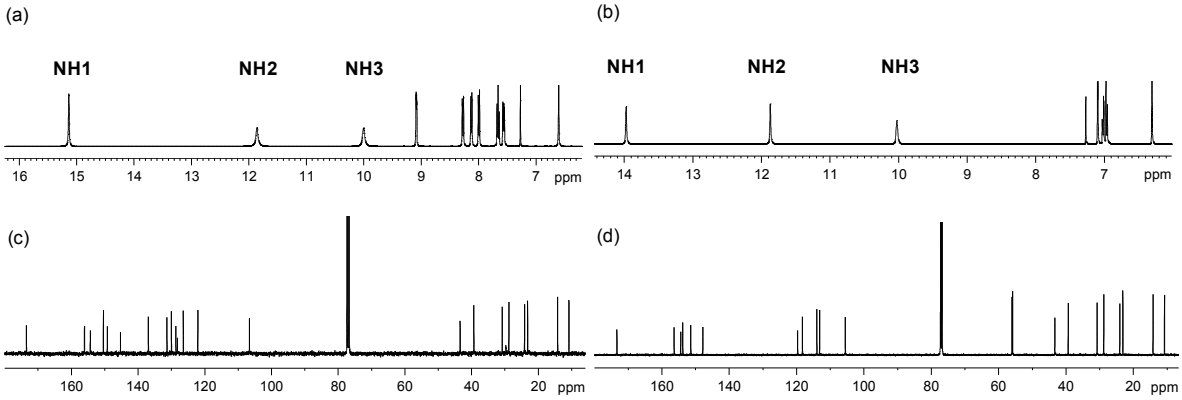



**Fig. 3.** $^{1}$H excerpts [(a), (b) (500 MHz)] and $^{13}$C [(c), (d) (125 MHz) spectra of **2** and **3**, respectively, in $CDCl_3$ showing *single set* of well-resolved signals.

Conservatively assuming more than 95% dimer formation at the lowest concentration studied (10 μM), the $K_{dim}$ of compounds **2** and **3** were estimated to be at a lower limit of 1.9 x $10^{7}$ $M^{-1}$ in $CDCl_3$.

The dimerization of **2** and **3** in various DMSO-$d_6$/$CDCl_3$ mixtures were studied in order to further support self-assembly by increasing the polarity of the solvent. The $K_{dim}$ values of **2**·**2** and **3**·**3** were determined quantitatively in the range from 5% to 20% DMSO-$d_6$/$CDCl_3$ (v/v) mixtures by $^{1}$H NMR dilution experiments. In 5% and 10% DMSO-$d_6$/$CDCl_3$ mixtures, we observed negligible chemical shift changes of urea protons. Therefore, $K_{dim}$ values were estimated to be at a lower limit of $10^{5}$ $M^{-1}$ for **2** and **3** under this condition. These findings proved that the duplexes are quite stable in mixture of solvents even though DMSO is a strongly competitive solvent for hydrogen bonded complexes. Nonlinear regression analysis[11] of the chemical shift gave the dimerization constant $K_{dim}$ value of 5.08 x 10 $M^{-1}$ for **2** and 2.57 x 10 $M^{-1}$ for **3** in 20% DMSO-$d_6$/$CDCl_3$, respectively.

Variable temperature $^{1}$H NMR studies (ranging from 223-323K) further provided additional evidence for the stability of molecular duplexes. Signals of NH2 (-1.00 ppb/K for **2**, -0.60 ppb/K for **3** and -0.66 ppb/K for **18**) and NH3 (-2.10 ppb/K for **2**, -2.10 ppb/K for **3** and -2.20 ppb/K for **18**) showed temperature coefficients suggestive of their strong involvement in intermolecular hydrogen bonds when compared to NH1 which is involved in intramolecular S(6)-type hydrogen bonds (-0.20 ppb/K for **2**, -1.40 ppb/K for **3** and -1.10 ppb/K for **18**). These values also indicate the high stability of the molecular duplexes (ESI,‡ Fig. S1, S3 and S5 respectively).

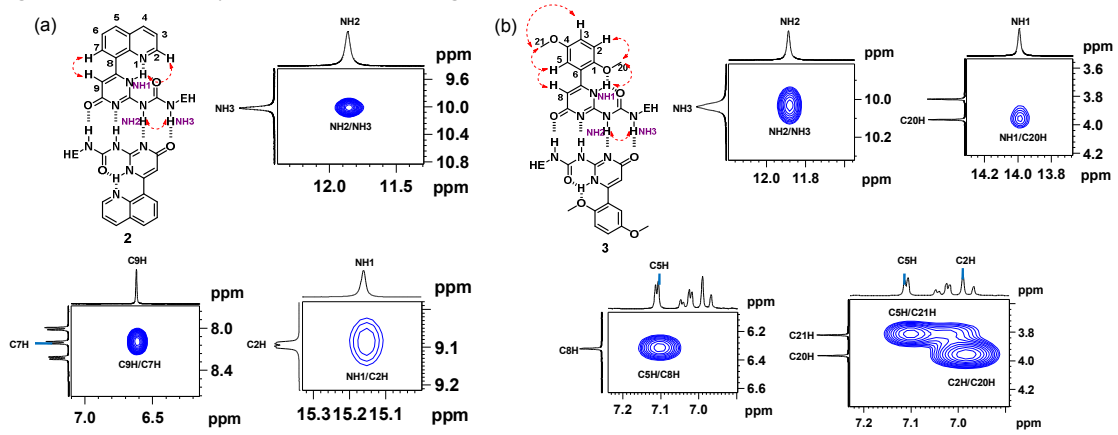



**Fig. 4.** Characteristic NOE excerpts of homodimer of **2** (a) and **3** (b) ($CDCl_3$, 400 MHz, 298 K). *Note:* EH = 2-ethylhexyl

The self-assembling system **3** exhibited fluorescence, owing to the presence of the hydroquinone moiety.[8] Being fluorescent, the duplex stability of **3**·**3** was also investigated by fluorescence spectroscopic methods, in addition to NMR

View Article Online
DOI: 10.1039/C6CC09478C




methods (*vide supra*). The dimerization constant of **3** was determined by using a fluorescence spectroscopic method, as reported previously by the Meijer group.[3b] Concentration-dependent changes in the intensities of **3** at 400 nm appeared in the range of $10^{-9}$ M to $10^{-6}$ M in chloroform. Nonlinear regression analysis[11a] of the fluorescence data gave a dimerization constant $K_{dim}$ value of (2.02 ± 2.42) x $10^{8}$ $M^{-1}$ for **3** (ESI,‡ Fig. S31 and S32).

Despite several efforts, compounds **1**, **2** and **3** were highly resistant to crystal formation. Therefore, their duplex formation was further investigated *via* solution-state NMR and HRMS-ESI studies. Two-dimensional NOESY NMR studies in $CDCl_3$ provided the most diagnostic evidence for the formation of homodimer structures of **2** and **3** in solution. The characteristic NOE correlation between NH2 and NH3 proton of **2** in $CDCl_3$ suggested a linear arrangement. The NOEs between C9H and C7H and NH1 and C2H in **2** confirmed the **AADD**-type self-complementary dimer formation (Fig. 4a, *vide supra*). The compound **3** exhibited similar characteristic NOEs (NH2/NH3, NH1/C20H, C5H/C8H, C5H/C21H and C2H/C20H; Fig. 4b, *vide supra*). In addition, HRMS-ESI mass spectra showed molecular ion peaks (787.4402 for $[\mathbf{2}\cdot\mathbf{2} + H]^+$, calcd 787.4393) and (805.4607 for $[\mathbf{3}\cdot\mathbf{3} + H]^+$, calcd 805.4587), corresponding to the presence of the duplex (ESI,‡ page S23 and S31 respectively).

In summary, the tricky issue of prototropy in heterocycle-based **AADD**-type self-assembling systems was effectively solved by an effective method of freezing their hydrogen bonding codes, by using intramolecular bifurcated hydrogen bonding interactions. It is noteworthy that this elegant strategy may also find application elsewhere, wherein proton shift-mediated structural changes become a detrimental factor. Based on the results obtained from extensive NMR studies, we could unambiguously confirm that the modified ureidopyrimidone-based **AADD**-type self-complementary quadruple hydrogen-bonded systems show high $K_{dim}$ value ($K_{dim} > 10^{7}$ $M^{-1}$ in $CDCl_3$), without competition from undesired tautomers. Advantageously, using this strategy, we could also design a novel **AADD**-type quadruple H-bonding system endowed with a built-in fluorophore, which could augment its application potential in supramolecular chemistry.

S.K, S.R and A.S.K are thankful to CSIR, New Delhi for research fellowships. GJS thanks the SSB-000726 project for financial support.

## Notes and references

1 (a) L. Yang, X. Tan, Z. Wang and X. Zhang, *Chem. Rev.*, 2015, **115**, 7196; (b) T. Aida, E. W. Meijer and S. I. Stupp, *Science*, 2012, **335**, 813; (c) J. D. Fox and S. J. Rowan, *Macromolecules*, 2009, **42**, 6823; (d) M. Fathalla, C. M. Lawrence, N. Zhang, J. L. Sessler and J. Jayawickramarajah, *Chem. Soc. Rev.*, 2009, **38**, 1608; (e) J.-M. Lehn, *Chem. Soc. Rev.*, 2007, **36**, 151; (f) A. J. Wilson, *Soft Matter*, 2007, **3**, 409; (g) P. Y. W. Dankers and E. W. Meijer, *Bull. Chem. Soc. Jpn.*, 2007, **80**, 2047; (h) L. J. Prins, D. N. Reinhoudt and P. Timmerman, *Angew. Chem. Int. Ed.*, 2001, **40**, 2382; (i) C. Schmuck and W. Wienand, *Angew. Chem. Int. Ed.*, 2001, **40**, 4363.

2 (a) Y. Hisamatsu, S. Banerjee, M. Avinash, T. Govindaraju and C. Schmuck, *Angew. Chem. Int. Ed.*, 2013, **52**, 12550; (b) M. Pichlmaier, R. F. Winter, M. Zabel, S. Zalis, *J. Am. Chem. Soc.,* 2009, **131**, 4892; (c) A. M. Kushner, V. Gabuchian, E. G. Johnson and Z. Guan, *J. Am. Chem. Soc.*, 2007, **129**, 14110; (d) P. Y. W. Dankers, M. C. Harmsen, L. A. Brouwer, M. J. Van Luyn and E. W. Meijer, *Nat. Mater.*, 2005, **4**, 568; (e) M. Ikeda, T. Nobori, M. Schmutz and J.-M. Lehn, *Chem. Eur. J.*, 2005, **11**, 662.

3 (a) F. H. Beijer, R. P. Sijbesma, H. Kooijman, A. L. Spek and E. W. Meijer, *J. Am. Chem. Soc.*, 1998, **120**, 6761; (b) S. H. M. Söntjens, R. P. Sijbesma, M. H. P. van Genderen and E. W. Meijer, *J. Am. Chem. Soc.*, 2000, **122**, 7487.

4 (a) A. Micoli, M. Nieuwenhuizen, M. Koenigs, M. Quintana, R. Sijbesma and M. Prato, *Chem. Eur. J.*, 2015, **21**, 14179; (b) S. Wang, H. Guo, X. Wang, Q. Wang, J. Li and X. Wang, *Langmuir*, 2014, **30**, 12923; (c) J.-S. Chen, P.-W. Zhou, G.-Y. Li, T.-S. Chu and G.-Z. He, *J. Phys. Chem. B*, 2013, **117**, 5212; (d) L. Shi, X. Wang, C. A. Sandoval, M. Li, Q. Qi, Z. Li and K. Ding, *Angew. Chem. Int. Ed.*, 2006, **45**, 4108; (e) O. A. Scherman, G. Ligthart, H. Ohkawa, R. P. Sijbesma and E. W. Meijer, *Proc. Natl. Acad. Sci. U.S.A.*, 2006, **103**, 11850; (f) H. Sun, J. Steeb and A. E. Kaifer, *J. Am. Chem. Soc.*, 2006, **128**, 2820; (g) L. Sánchez, N. Martín and D. M. Guldi, *Angew. Chem. Int. Ed.*, 2005, **44**, 5374; (h) M. T. Rispens, L. Sanchez, J. Knol and J. C. Hummelen, *Chem. Commun.*, 2001, 161; (i) J. J. Gonzalez, S. Gonzalez, E. M. Priego, C. Luo, D. M. Guldi, J. de Mendoza and N. Martin, *Chem. Commun.*, 2001, 163.

5 P. S. Corbin and S. C. Zimmerman, *J. Am. Chem. Soc.*, 1998, **120**, 9710.

6 (a) B. P. Mudraboyina and J. A. Wisner, *Chem. Eur. J.*, 2012, **18**, 14157; (b) X. Li, Y. Fang, P. Deng, J. Hu, T. Li, W. Feng and L. Yuan, *Org. Lett.*, 2011, **13**, 4628; (c) W.-J. Chu, Y. Yang and C.-F. Chen, *Org. Lett.*, 2010, **12**, 3156; (d) J. S. Nowick, *Acc. Chem. Res.*, 2008, **41**, 1319; (e) J. Zhu, J.-B. Lin, Y.-X. Xu, X.-B. Shao, X.-K. Jiang and Z.-T. Li, *J. Am. Chem. Soc.*, 2006, **128**, 12307; (f) V. G. H. Lafitte, A. E. Aliev, P. N. Horton, M. B. Hursthouse, K. Bala, P. Golding and H. C. Hailes, *J. Am. Chem. Soc.* 2006**, 128,** 6544; (g) Y. Yan, H. Zeng and B. Gong, *J. Am. Chem. Soc.*, 1999, **121**, 5607.

7 S. Kheria, S. Rayavarapu, A. S. Kotmale, R. G. Gonnade and G. J. Sanjayan, *Chem. Eur. J.*, 2017, **23**, 783.

8 Hydroquinone-conjugated systems often show fluorescence; see: (a) B. Gui, X. Meng, Y. Chen, J. Tian, G. Liu, C. Shen, M. Zeller, D. Yuan and C. Wang, *Chem. Mater.*, 2015, **27**, 6426; (b) V. K. Praveen, C. Ranjith, E. Bandini, A. Ajayghosh and N. Armaroli, *Chem. Soc. Rev.*, 2014, **43**, 4222; (c) P. Jonkheijm, N. Stutzmann, Z. Chen, D. M. de Leeuw, F. Wurthner, E. W. Meijer and A. P. H. J. Schenning, *J. Am. Chem. Soc.,* 2006, **128**, 9535.

9 I. V. Boldyrev, I. F. Vladimirtsev, E. A. Romanenko, N. G. Korzhenevskaya, E. V. Titov and V. M. Cherkasov, *Chem. Heterocycl. Compd.*, 1977, **13**, 1006.

10 (a) T. Yakura, Y. Yamauchi, Y. Tian and M. Omoto, *Chem. Pharm. Bull.* 2008, **56**, 1632; (b) K. I. Shivakumar and G. J. Sanjayan, *Synthesis,* 2013, **45**, 896.

11 (a) C. S. Wilcox, *Frontiers in supramolecular organic chemistry and photochemistry*, H.-J. Schneider and H. Dürr, VCH, New York, 1991, pp 123; (b) K. A. Connors, *Binding constants: the measurement of molecular complex stability*, Wiley-Interscience, New York 1987.