Convenient synthesis of multivalent zinc(II)–dipicolylamine complexes for molecular recognition

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ABSTRACT

A pair of novel dipicolylamine ligands bearing isothiocyanate groups were used as conjugation reagents to prepare multivalent molecules with anionic recognition capability. The isothiocyanates were reacted with two classes of dendritic scaffolds bearing primary amines, squaraine rotaxanes, and PAMAM dendrimers, and the products were converted into water soluble zinc(II) coordination complexes. The multivalent squaraine rotaxanes exhibit high fluorescence quantum yields in water and are very well suited for biological imaging applications.

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There is a need for molecular probes that can selectively target phosphorylated biomolecules in various types of biomedical samples. Supramolecular chemists have explored a wide array of organic and inorganic structures.[1] For operation in aqueous solution, metal cation coordination complexes are especially effective because they have the ability to form strong coordination bonds with the phosphate oxygens. One of the most prominent families of synthetic phosphate receptors uses zinc(II)–dipicolylamine (Zn(II)–DPA) coordination complexes as the molecular recognition units.[2] We have contributed to this topic by demonstrating that Zn(II)–DPA complexes can selectively target membrane surfaces that are rich in anionic phospholipids.[2c] This discovery has high biomedical relevance since several cell systems associated with disease, such as bacterial cells and apoptotic mammalian cells, have anionic surfaces. We have developed a number of fluorescent Zn(II)–DPA probes and used them to image bacterial infection and cell death in vitro and in vivo.[3]

To date, our probe designs have incorporated one or two Zn(II)–DPA recognition units, and we have observed a general trend that the divalent systems exhibit a higher and more selective affinity for anionic membranes over near-neutral membranes.[3] These results have motivated us to investigate higher order multivalent systems. To access these compounds, we needed to develop convenient synthetic methods for attaching multiple copies of dipicolylamine (DPA) or bis(dipicolylamine) (BDPA) ligands to a molecular scaffold. There are several literature reports of nanoparticles that

are coated with Zn(II)–DPA complexes,[4,5] but we wanted to prepare smaller structures using organic scaffolds bearing primary amines.[6] Thus, a logical conjugation choice was reaction of the primary amines with isothiocyanates to form the corresponding thioureas.[7] This reaction typically proceeds under convenient conditions with good yields and does not require a catalyst or coupling agent. Additionally, isothiocyanates are easily prepared and can be stored for extended periods of time. These reasons lead us to synthesize DPA isothiocyanate 1 and BDPA isothiocyanate 2 (Chart 1) and react them with dendritic structures having multiple branches terminating in primary amines.

We chose to modify two classes of dendritic scaffolds; squaraine rotaxanes, and PAMAM dendrimers. Squaraine rotaxanes, which were developed in our laboratory,[8] are interlocked molecules each comprising a highly fluorescent squaraine dye encapsulated inside a tetralactam macrocycle. The steric protection provided by the surrounding macrocycle inhibits decomposition
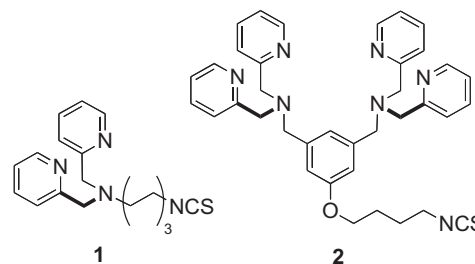

Chart 1.

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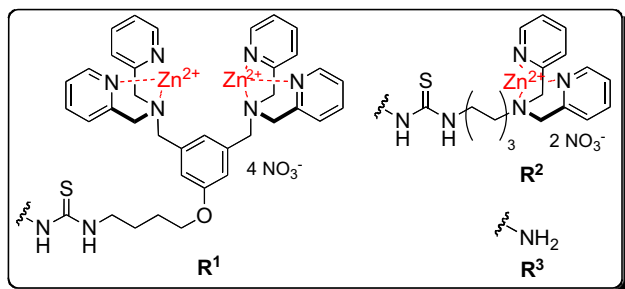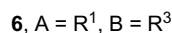 E-mail address: smith.115@nd.edu (B.D. Smith).

3, X = R[1]

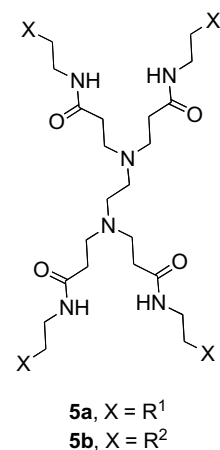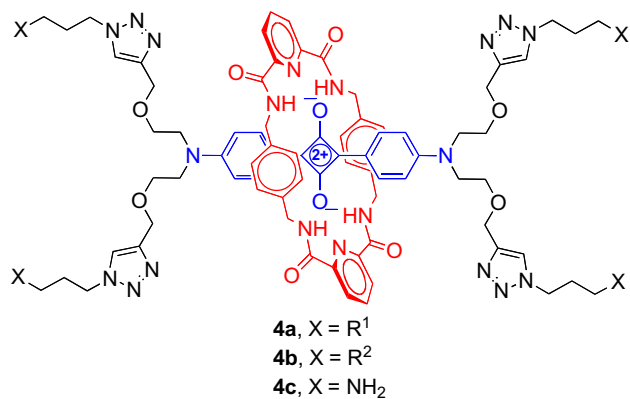

4a, X = R[1]
4b, X = R[2]
4c, X = NH₂

5a, X = R[1]
5b, X = R[2]

6, A = R[1], B = R[3]

Chart 2.

Scheme 1. Reagents and conditions: (i) 2-chloromethyl pyridine, Na₂CO₃, MeCN, reflux, 16 h, 80%; (ii) CH₂Cl₂, TFA, rt, 16 h, quant.; (iii) CS₂, NEt₃, THF, 0 °C, 30 min followed by H₂O₂, THF, 0 °C, 40% (30% for **10**).

Scheme 2. Reagents and conditions: (i) N₃(CH₂)₃NHBoc, CuSO₄, sodium ascorbate, CHCl₃, H₂O, rt, 16 h, 90%; (ii) TFA, CH₂Cl₂, 0 °C to rt, 16 h, quant.; (iii) **2**, DMF, rt, 16 h, 40%; (iv) Zn(NO₃)₂, MeOH, 16 h, quant.
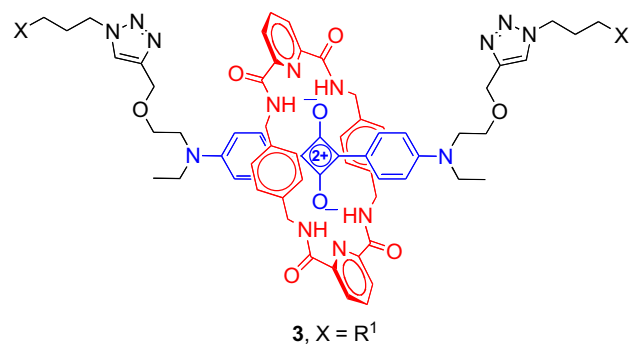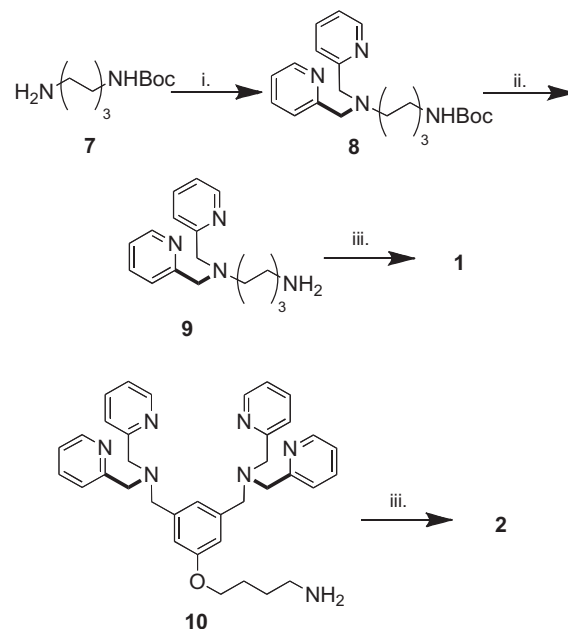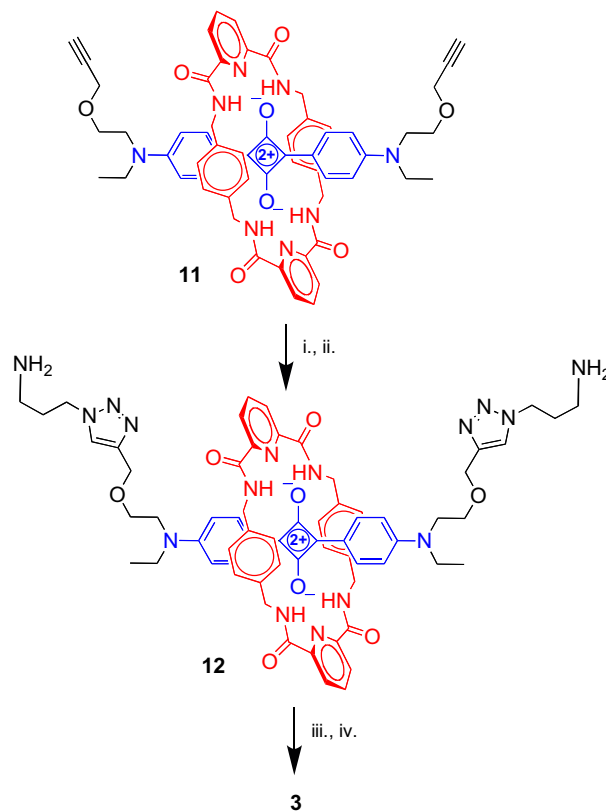
of the squaraine by nucleophilic attack.[9] Squaraine rotaxanes emit a strong and narrow fluorescence in the deep red/near-infrared region, and their high stabilities make them very well suited for animal imaging studies as tracer dyes or as targeted probes for

molecular imaging.[10] We chose to make multivalent versions, **3**, **4a**, and **4b** for eventual study as fluorescence imaging probes. In addition, isothiocyanate reagents **1** and **2** were then used to convert PAMAM dendrimers into multivalent recognition mole-
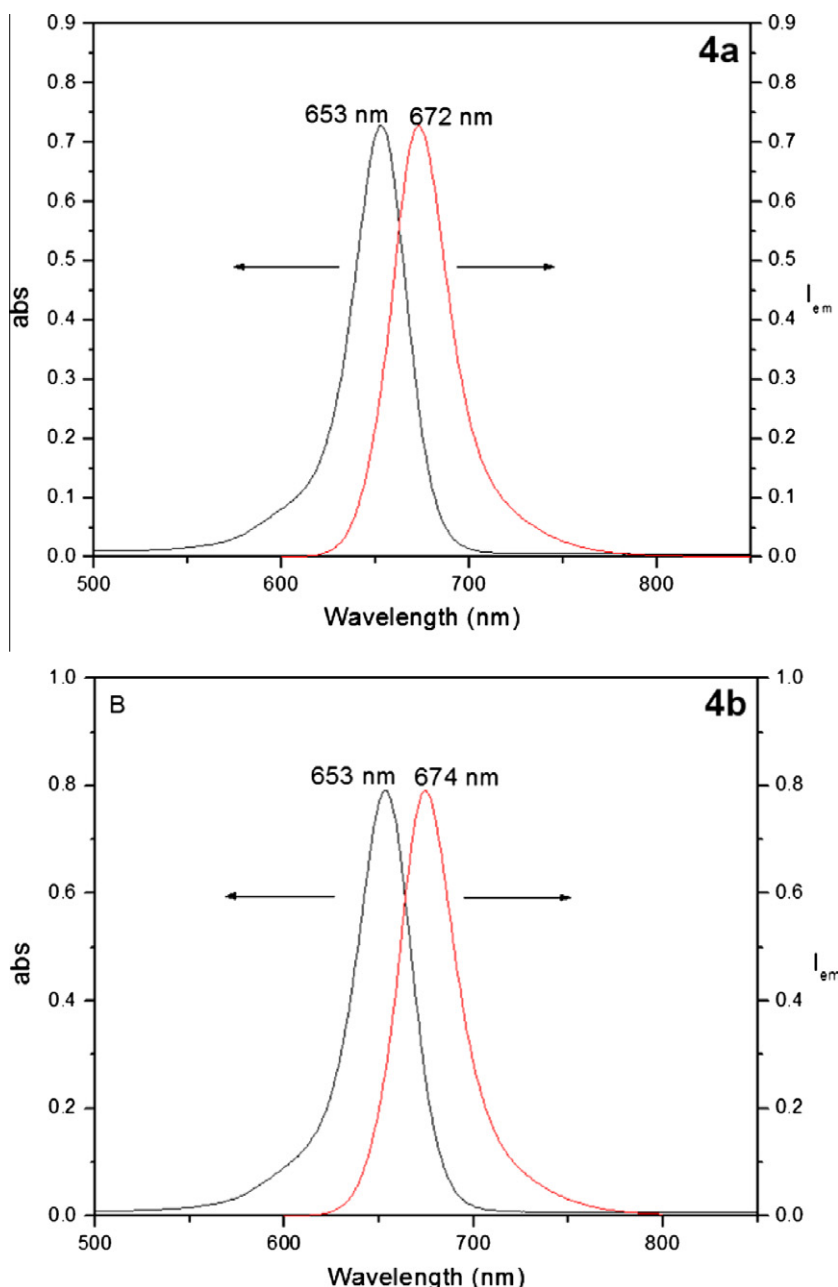
Figure 1. Absorption and emission spectra for squaraine rotaxanes **4a** (upper panel) and **4b** (lower panel).

cules.[11] Specifically, we modified commercially available G0 and G4 PAMAM dendrimers and prepared the multivalent Zn(II)–DPA complexes **5a**, **5b**, **6** (Chart 2).

A number of methods exist for preparing isothiocyanates. Primary amines can be reacted with carbon disulfide (CS_2) in ammonium hydroxide solution and the resulting ammonium dithiocarbonate salts are treated with lead nitrate or tosyl chloride to give the corresponding isothiocyanates.[12] Another method involves the thermal fragmentation of 1,4,2-oxathiazoles.[13] In our hands, the reaction of primary amines with CS_2 and subsequent treatment with hydrogen peroxide proved to be a convenient method.[14] Preparation of isothiocyanate (**1**) required the synthesis of precursor DPA-hexylamine, **9**. Thus, monoprotected 1,6-bisaminohexane was refluxed in acetonitrile with 2-(chloromethyl)pyridine in the presence of Na_2CO_3 to furnish the N-protected DPA derivative **8** in good yield which was quantitatively deprotected using TFA in CH_2Cl_2 to give **9**. Treatment of **9** with CS_2 followed by H_2O_2 afforded the desired isothiocyanate **1**

in moderate yield. Isothiocyanate **2** was prepared in a similar fashion (Scheme 1) starting with the known BDPA-butylamine **10**.[15]

With isothiocyanates **1** and **2** in hand, we prepared squaraine rotaxanes **3**, **4a**, and **4b** bearing multiple copies of DPA or BDPA ligands. The bis(amine) **12** was prepared by conducting a copper-catalyzed cycloaddition reaction using the known bis-alkyne **11** and N-tert-butoxycarbonyl-3-azidopropylamine and then removing the Boc protecting groups using TFA in CH_2Cl_2.[16] Reaction of **12** and **2** in the presence of NEt_3 in DMF, followed by complexation with $Zn(NO_3)_2$ in methanol gave the targeted squaraine rotaxane **3**. Squaraine rotaxanes **4a** and **4b** were prepared in a similar fashion by treating the known tetra(amine) **4c** (Chart 2) with **1** or **2** followed by complexation with $Zn(NO_3)_2$ (Scheme 2).[17] Squaraine rotaxanes **3**, **4a**, and **4b** exhibited high water solubility and excellent solution phase stability even when exposed to light. The spectral plots in Figure 1 show the characteristic narrow intense absorption and emission bands, with no evidence for intermolecular aggregation. The photophysical properties listed in

Table 1
Photophysical parameters for multivalent squaraine rotaxanes in water

Compound	λ_{abs} (nm)	λ_{em} (nm)	$\log \varepsilon$	Φ_f
3	653	672	5.16	0.30
4a	653	672	5.16	0.30
4b	653	674	5.20	0.25

Table 1 include the impressive high quantum yields in water of 0.25–0.30. Taken together, the chemical and photophysical properties of squaraine rotaxanes **3**, **4a**, and **4b** indicate that they are well suited for both in vitro and in vivo biological imaging applications.[18]

We also modified commercially available PAMAM G0 (4 primary amines) and PAMAM G4 (64 primary amines) dendrimers. Reaction of PAMAM G0 with **1** or **2** in DMF overnight furnished the corresponding thioureas in good yields (78% and 75%, respectively) and complexation of these products with $Zn(NO_3)_2$ in methanol afforded **5a** and **5b** as water soluble compounds. The reaction of PAMAM G4 with **2** was much slower, and ^1H NMR of product **6** indicated that only ∼50% of the peripheral amines had reacted after stirring in DMF for one week. This sluggish reactivity is attributed to steric crowding at the surface of the dendrimer. Thus, there appears to be a steric limit to the number of Zn(II)–BDPA units that can be appended to the surface of high generation polyamine dendrimers. This finding is not unexpected, and is not a major drawback since partially coated high generation dendrimers are known to exhibit powerful multivalent molecular recognition effects.[19,20] Indeed, preliminary membrane recognition experiments that added the above multivalent Zn(II)–DPA compounds to dispersions of anionic liposomes composed of 1:1 phosphatidylcholine/phosphatidylserine induced crosslinking and precipitation of the liposomes, with the higher order multivalent systems producing the most liposome precipitation. Conversely, no liposome precipitation was observed in control experiments using liposomes composed of entirely zwitterionic phosphatidylcholine. In due course we will describe the impressive ability of these multivalent Zn(II)–DPA complexes to target the anionic membrane surfaces of apoptotic mammalian cells and bacteria.

In summary, we prepared novel dipicolylamine isothiocyanates **1** and **2** and showed that they are convenient conjugation reagents for attaching Zn(II)–DPA molecular recognition units to dendritic scaffolds terminating in primary amines. The multivalent squaraine rotaxanes **3**, **4a**, and **4b** exhibit high fluorescence quantum yields in water and are very well suited for biological imaging applications. We anticipate that compounds **1** and **2** will be broadly useful for conjugation to a wide range of amine-containing molecules, including peptides and proteins.

Acknowledgments

Supplementary data

Supplementary data (synthetic procedures and spectral data) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.11.103.

References and notes

1. For reviews of phosphate recognition see: (a) Kubik, S.; Reyheller, C.; Stuwe, S. *J. Inclusion Phenom. Macrocyclic* **2005**, *52*, 137–187; (b) Hirsch, A. K. H.; Fischer, F. R.; Diederich, F. *Angew. Chem., Int. Ed.* **2007**, *46*, 338–352; (c) Blondeau, P.; Segura, M.; Perez-Fernandez, R.; de Mendoza, J. *Chem. Soc. Rev.* **2007**, *36*, 198–210; (d) Kim, S. K.; Lee, D. H.; Hong, J.-I.; Yoon, J. *Acc. Chem. Res.* **2009**, *42*, 23–31; (e) Bazziacalupi, C.; Bencini, A.; Lippolis, V. *Chem. Soc. Rev.* **2010**, *39*, 3709–3728; (f) Hargrove, A. E.; Nieto, S.; Zhang, T.; Sessler, J. L.; Anslyn, E. V. *Chem. Rev.* **2011**, *111*, 6603–6782.
2. For reviews on Zn(II)–DPA complexes as phosphate receptors see: (a) Sakamoto, T.; Ojida, A.; Hamachi, I. *Chem. Commun.* **2009**, *2*, 141; (b) Kruppa, M.; König, B. *Chem. Rev.* **2006**, *106*, 3520; (c) O'Neil, E. J.; Smith, B. D. *Coord. Chem. Rev.* **2006**, *250*, 3068; (d) Kim, S. K.; Lee, D. H.; Hong, J.; Yoon, J. *Acc. Chem. Res.* **2009**, *42*, 23; (e) Zhou, Y.; Xu, Z.; Yoon, J. *Chem. Soc. Rev.* **2011**, *40*, 2222; (f) Ngo, H. T.; Liu, X.; Jolliffe, K. A. *Chem. Soc. Rev.* **2012**, *41*, 4928.
3. (a) Smith, B. A.; Akers, W. J.; Leevy, W. M.; Lampkins, A. J.; Xiao, S.; Wolter, W.; Suckow, M. A.; Achilefu, S.; Smith, B. D. *J. Am. Chem. Soc.* **2010**, *132*, 67–69; (b) Smith, B. A.; Gammon, S. T.; Xiao, S.; Wang, W.; Chapman, S.; Ryan McDermott, R.; Suckow, M. A.; Johnson, J. R.; Piwnica-Worms, D.; Gokel, G. W.; Smith, B. D.; Leevy, W. M. *Mol. Pharm.* **2011**, *8*, 583–590; (c) Lee, J.-J.; White, A. G.; Baumes, J. M.; Smith, B. D. *Chem. Commun.* **2010**, *46*, 1068–1069; (d) Smith, B. A.; Xiao, S.; Wolter, W.; Wheeler, J.; Suckow, M. A.; Smith, B. D. *Apoptosis* **2011**, *16*, 722–731; (e) White, A. G.; Gray, B. D.; Pak, K. Y.; Smith, B. D. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2833–2836.
4. (a) Yamaguchi, S.; Yoshimura, I.; Kohira, T.; Tamaru, S.; Hamachi, I. *J. Am. Chem. Soc.* **2005**, *127*, 11835–11841; (b) Zhang, S.; Wang, J.; Han, L.; Li, C.; Wang, W.; Yuan, Z. *Sens. Actuators, B* **2010**, *147*, 687–690; (c) Oh, D. J.; Kim, K. M.; Ahn, K. H. *Chem. Asian J.* **2011**, *6*, 2034–2039; (d) Moro, A. J.; Schmidt, J.; Doussineau, T.; Lapresta-Fernandez, A.; Wegener, J.; Mohr, G. J. *Chem. Commun.* **2011**, *47*, 6066–6068; (e) Liu, D. J.; Credo, G. M.; Su, X.; Wu, K.; Lim, H. C.; Elibol, O. H.; Bashir, R.; Varma, M. *Chem. Commun.* **2011**, *47*, 8310–8312; (f) Liu, G.; Choi, K. Y.; Bhirde, A.; Swierczewska, M.; Yin, J.; Lee, S. W.; Park, J. H.; Hong, J.-I.; Xie, J.; Niu, G.; Kiesewetter, D. O.; Lee, S.; Chen, X. *Angew. Chem., Int. Ed.* **2012**, *51*, 445–449.
5. Leevy, W. M.; Lambert, T. N.; Johnson, J. R.; Morris, J.; Smith, B. D. *Chem. Commun.* **2008**, 2331–2333.
6. For other ways of converting amines into DPA ligands, see: (a) DiVittorio, K. M.; Johnson, J. R.; Johansson, E.; Reynolds, A. J.; Jolliffe, K. A.; Smith, B. D. *Org. Biomol. Chem.* **2006**, *4*, 1966–1976; (b) Carolan, J. V.; Butler, S. J.; Jolliffe, K. A. *J. Org. Chem.* **2009**, *74*, 2992–2996.
7. Hermanson, G. T. *Bioconjugate Techniques*, 2nd ed.; Academic Press: New York, 2008. p 170.
8. Gassensmith, J. J.; Baumes, J. M.; Smith, B. D. *Chem. Commun.* **2009**, 6329–6338.
9. Arunkumar, E.; Forbes, C. C.; Noll, B. C.; Smith, B. D. *J. Am. Chem. Soc.* **2005**, *127*, 3288–3289.
10. (a) Johnson, J. R.; Fu, N.; Arunkumar, E.; Leevy, W. M.; Gammon, S. T.; Piwnica-Worms, D.; Smith, B. D. *Angew. Chem., Int. Ed.* **2007**, *46*, 5528–5531; (b) Smith, B. A.; Xie, B.-W.; van Beek, E. R.; Ivo Que, I.; Blankevoort, V.; Xiao, S.; Cole, E. L.; Hoehn, M.; Kaijzel, E. L.; Löwik, C. W. G. M.; Smith, B. D. *ACS Chem. Neurosci.* **2012**, *3*, 530–537; (c) Cole, E. L.; Arunkumar, E.; Xiao, S.; Smith, B. A.; Smith, B. D. *Org. Biomol. Chem.* **2012**, *10*, 5769–5773.
11. For examples of other multivalent systems based on the PAMAM scaffold see: (a) Yordanov, A. T.; Kobayashi, H.; English, S. J.; Rejinders, K.; Milenic, D.; Krishna, M. C.; Mitchell, J. B.; Brechbiel, M. W. *J. Mater. Chem.* **2003**, *13*, 1523–1525; (b) Roy, R.; Baek, M.-G. *Methods Enzymol.* **2003**, *362*, 240–249; (c) Howell, B. A.; Fan, D. *Proc. R. Soc. A* **2010**, *466*, 1515–1526; (d) Myung, J. H.; Gajja, K. A.; Saric, J.; Eddington, D. T.; Hong, S. *Angew. Chem., Int. Ed.* **2011**, *50*, 1–5.
12. Wong, R.; Dolma, S. J. *J. Org. Chem.* **2007**, *72*, 3969–3971.
13. Kim, N. J.; Jung, K. S.; Lee, H. J.; Son, J. S. *Tetrahedron Lett.* **1997**, *38*, 1597–1598; (b) O'Reilly, R. J.; Radom, L. *Org. Lett.* **2009**, *11*, 1325–1328.
14. Li, G.; Tajima, H.; Ohtani, T. *J. Org. Chem.* **1997**, *62*, 4539–4540.
15. Lakshmi, C.; Hanshaw, R. G.; Smith, B. D. *Tetrahedron* **2004**, *60*, 11307–11315.
16. Gassensmith, J. J.; Arunkumar, E.; Lorna Barr, L.; Baumes, J. M.; Noll, B. C.; Smith, B. D. *J. Am. Chem. Soc.* **2007**, *129*, 15054–15060.
17. Xiao, S.; Fu, N.; Peckham, K.; Smith, B. D. *Org. Lett.* **2010**, *12*, 140–143.
18. For recent reviews of fluorescent sensors for biological applications see: (a) Lavis, L. D.; Raines, R. T. *ACS Chem. Biol.* **2008**, *3*, 142–155; (b) Panigrahi, M.; Dash, S.; Patel, S.; Mishra, B. K. *Tetrahedron* **2012**, *68*, 781–805.
19. Incomplete derivatization of terminal amines in high generation PAMAM dendrimers is precedented; see Ref. 11a.
20. For an example of enhanced multivalent recognition using partially coated dendrimers, see Ref. 11d.