Tetrahedron Letters 53 (2012) 5110–5113

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Synthesis of a new tripodal chemosensor based on 2,4,6-triethyl-1,3,5-trimethylbencene scaffolding bearing thiourea and fluorescein for the chromo-fluorogenic detection of anions

María E. Moragues, Luis E. Santos-Figueroa, Tatiana Ábalos, Félix Sancenón *, Ramón Martínez-Máñez *

Centro de Reconocimiento Molecular y Desarrollo Tecnológico (IDM), Unidad Mixta Universidad Politécnica de Valencia-Universidad de Valencia, Camino de Vera s/n, 46022, Valencia, Spain
CIBER de Bioingeniería, Biomaterailes y Nanomedicina (CIBER-BBN), Spain

ARTICLE INFO

Article history:
Received 22 May 2012
Revised 9 July 2012
Accepted 10 July 2012
Available online 15 July 2012

Keywords:
Chromogenic chemosensors
Anion recognition
Tripodal receptor
Prearranged scaffolds

ABSTRACT

A tripodal receptor containing thiourea as binding site and fluorescein as signalling subunit has been designed, synthesized and used for the colorimetric detection of basic anions in DMSO solutions.

The design of abiotic anion receptors able to display sensing features has attracted increasing attention due to the key roles played by these negatively charged species in chemical, biological and environmental processes.[1] Host molecules generally comprise two subunits, that is, the 'binding site' (properly the host, responsible of coordination event) and the 'signalling subunit' (in charge of the transduction event) that are usually attached forming a superstructure.[2] In these signalling systems optical outputs are especially attractive with respect to other possible transductions of the signal, because detection uses cheap, easy-to-handle and widely extended instrumentation. Apart from fluorescence systems, colorimetric recognition has become more and more popular because it additionally offers the opportunity to develop signalling systems for the 'naked eye' detection of target species.

During the last years, a large number of receptors using groups, such as amide,[3] urea,[4] thiourea,[5] sulfonamide,[6] pyrrole[7] and indole,[8] which give hydrogen bonding interactions with anions, have been reported and have been adequately coupled with different signalling reporters. Moreover this approach is usually related with the design and covalent link of the binding site and the signalling subunit in a pre-determined fashion. Anion binding hosts may also be designed on the basis of their flexibility or degree of preorganization. These concepts have been crystallized in many cases via the design of receptors showing multiple coordination sites in a pre-defined fashion. For instance, some remarkable examples involve the development of anion receptors based on 1,3,5–2,4,6 functionalized benzene scaffoldings with 1,3,5-positions directed to one face of the ring in order to prepare trifurcate anion receptors containing three arms with coordinating groups in cooperative mode.[9]

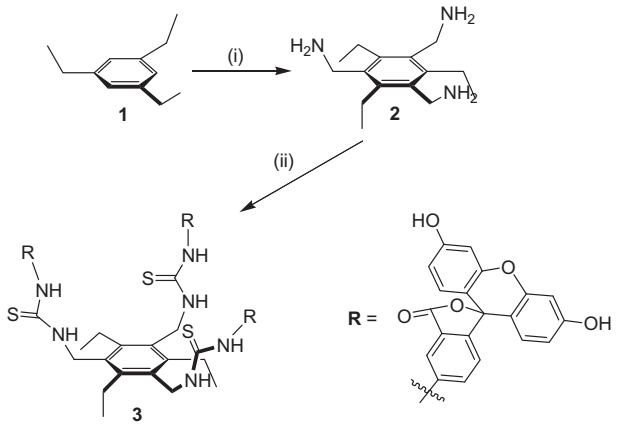
Following our interest in the development of anion chemosensors,[10] we report herein the synthesis of an acyclic tripodal receptor based on a 1,3,5-trisubstituted-2,4,6-triethylbenzene scaffold. The host molecule **3** contains thiourea binding sites and fluorescein groups as signalling subunits. The chromogenic and fluorogenic behaviour of receptor **3** was tested against certain selected anions in organic and organic–water mixed solutions.

The synthesis of tripodal 1,3,5-trimethylamino-2,4,6-triethylbenzene (**2**) has been published elsewere.[11] Reaction of **2** with fluorescein isothiocyanate and catalytic amounts of triethylamine in refluxing CH_2Cl_2 afforded receptor **3** (see Scheme 1) in moderate yields as an orange solid. The ^1H NMR spectra of tripodal receptor **3** were characterized by the presence of two signals at 1.15 and 2.95 ppm attributed to the three ethyl moieties and one singlet at 4.67 ppm ascribable to the six methylene protons that linked the three thiourea moieties with the benzene ring. The aromatic signals of the three fluorescein moieties appeared in the 5.12–8.5 ppm interval, whereas the thiourea protons appeared at 8.1 and 8.25 ppm as broad singlets.[12]

The UV–visible response of receptor **3** in the presence of selected anions was tested in DMSO. DMSO solution of receptor

* Corresponding authors. Tel.: +34 963877343; fax: +34 963879349.
E-mail addresses: fsanceno@upvnet.upv.es (F. Sancenón), rmaez@qim.upv.es (R. Martínez-Máñez).

0040-4039/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tetlet.2012.07.039



Scheme 1. Synthesis of receptor **3**. Reagents and conditions: (i) (1) paraformaldehyde–$ZnBr_2$–HBr/AcOH–90 °C; (2) potassium phthalimide–dry DMSO–90 °C; (3) hydrazine hydrate–EtOH/toluene (2:1) –reflux[11]; (ii) fluorescein isothiocyanate–CH_2Cl_2–triethylamine–reflux.

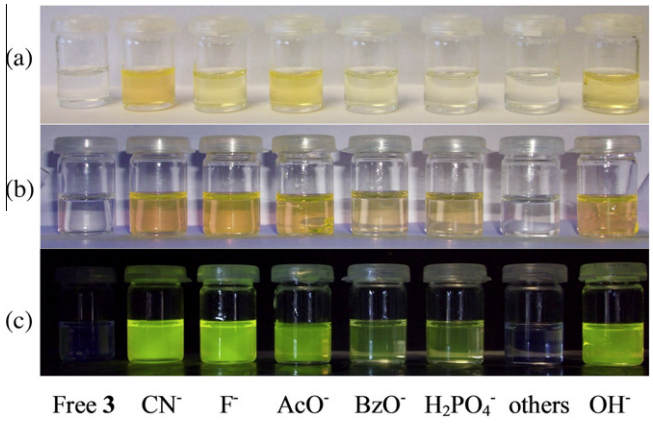


Figure 2. Colour change observed for chemosensor **3** (2.70×10^{-5} mol dm^{-3}) in DMSO upon addition of: (a) 4 equiv and (b) 10 equiv of various anions as tetrabutylammonium salts at room temperature; (c) Fluorescence emission of DMSO solutions of receptor **3** with 10 equiv of anions when irradiating at 365 nm.

3 (5.0×10^{-5} mol dm^{-3}) showed very weak absorption bands (centred at 432, 457, 486 and 528 nm) in the visible zone (see Fig. 1). From previous studies on fluorescein derivatives,[13] the absence of colour suggested that the colourless cyclic lactone form of fluorescein is the predominant isomer in receptor **3** in DMSO solution.

In a further step, the UV–visible response of receptor **3** was tested against several anions (CN^-, F^-, Cl^-, Br^-, I^-, NO_3^-, $H_2PO_4^-$, HSO_4^-, ClO_4^-, AcO^-, BzO^- and Cit^{3-}). The response of **3** in the presence of 1 equiv of the selected anions can be seen in Figure 1. Addition of Cl^-, Br^-, I^-, NO_3^-, HSO_4^-, ClO_4^- and Cit^{3-} induced negligible changes in the UV–visible profiles, whereas $H_2PO_4^-$ and BzO^- promoted a small increase in the absorbance of the visible bands. However, the most remarkable changes were observed upon addition of CN^-, F^- and AcO^- for which the absorbances of the four visible bands were highly enhanced. These changes were reflected in colour modulations from colourless to bright orange (see Fig. 2a and b). These bands in the 400–550 nm range can be attributed to the presence of the open form of the fluorescein dye. A remarkable enhancement of the fluorescence was also observed (see Fig. 2c).

Titration profiles of receptor **3** upon addition of increasing quantities of CN^-, F^-, $H_2PO_4^-$, AcO^- and BzO^- anions were carried out. Figure 3 shows the visible spectral changes of receptor **3** in

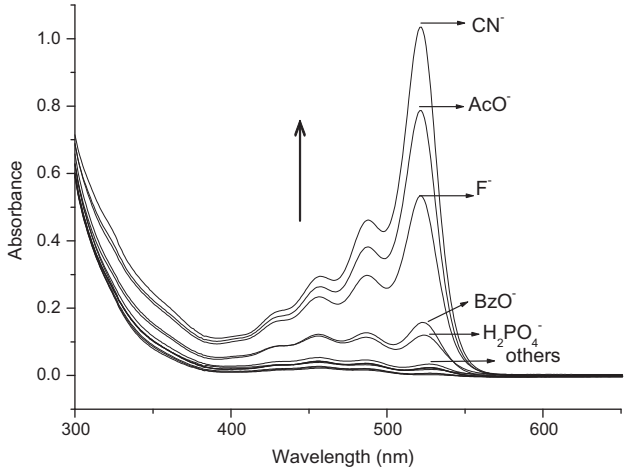


Figure 1. Changes in the UV–visible spectra of receptor **3** (5.0×10^{-5} mol dm^{-3}) in DMSO after addition of 1 equiv of selected anions.

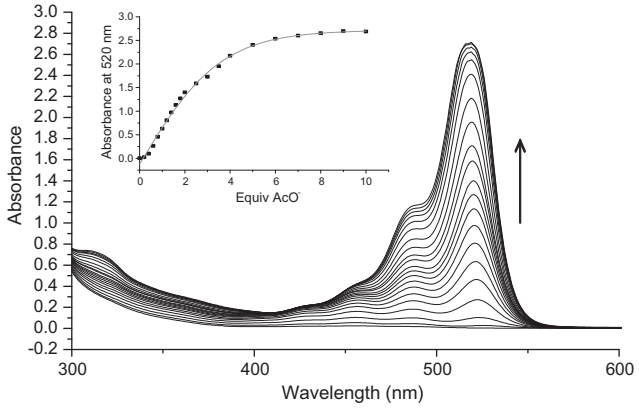


Figure 3. UV–visible spectral changes of receptor **3** (5.0×10^{-5} mol dm^{-3}) in DMSO upon addition of AcO^- (0.2–10 equiv). The inset shows the nonlinear curve fitting of the absorbance at 520 nm against the added AcO^-.

DMSO solution (5.0×10^{-5} mol dm^{-3}) upon addition of increasing quantities of AcO^- anion (from 0.2 to 10 equiv), as well as the nonlinear curve fitting of the absorbance band centred at ca. 520 nm. As it can be seen a clear and gradual absorbance enhancement of the visible band was observed.

The titration profiles of receptor **3** upon addition of CN^- and F^- anions induced similar absorption enhancements to those observed upon addition of AcO^- and allowed us to fit the experimental data to a two step mechanism (see also Table 1). The changes in the visible zone upon addition of $H_2PO_4^-$ and BzO^- anions were less pronounced (see Fig. 4 for BzO^- anion and also Table 1 for the stability constants) reflecting the lower interaction of these anions with **3** when compared with CN^-, F^- and AcO^-. The chromo-

Table 1
Stability constants for the interaction of receptor **3** with some anions in DMSO

Anion	Interaction	Log β[a]
OH^-	1:2	3.41 ± 0.01
CN^-	1:2	4.79 ± 0.01
F^-	1:2	3.67 ± 0.01
AcO^-	1:2	3.73 ± 0.01
BzO^-	1:2	0.43 ± 0.01
$H_2PO_4^-$	1:2	ND[b]

[a] Calculated using the programme HypSpec 1.1.18 for Eq. 3.
[b] The spectral changes were too small to calculate binding constant.

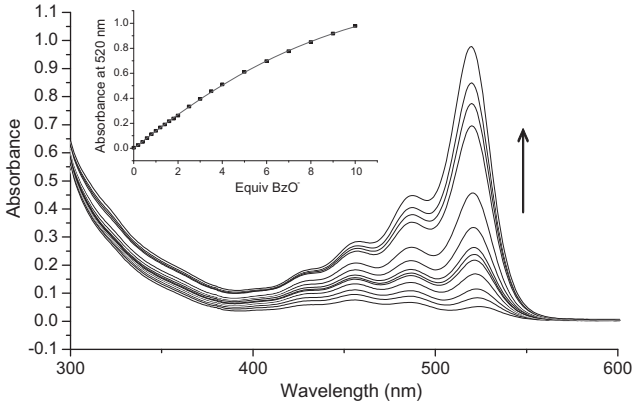


Figure 4. UV–visible spectral changes of **3** (5.0×10^{-5} mol dm^{-3}) in DMSO upon addition of BzO^- (0.2–10 equiv) anion. The inset shows the nonlinear curve fitting of the absorbance at 520 nm against the added BzO^-.

genic response of receptor **3** is clearly related with the basicity of the tested anions and it was clear from the results that an increase in the basicity of the anion ($CN^- \approx F^- > AcO^- > BzO^- > H_2PO_4^-$ was directly reflected in a larger enhancement of the intensity of the visible bands and also in an enhancement of the fluorescence. In fact the same colour modulation and fluorescence enhancement were observed upon titration of **3** in DMSO with tetrabutylammonium hydroxide.

The best fitting of the titration data using the program HypSpec was obtained when two consecutive processes were considered; that is, the formation of 1:1 hydrogen-bonding complexes between **3** and the corresponding anion Eq. 1 and a deprotonation reaction upon addition of excess of anion Eq. 2:

$$3 + A^- \leftrightarrows 3 - A^- \quad (1)$$

$$3 - A^- + A^- \leftrightarrows 3^- + HA_2^- \quad (2)$$

$$3 + 2A^- \leftrightarrows 3^- + HA_2^- \quad (3)$$

From the titration profiles the stability constants for the interaction of **3** with the anions F^-, CN^-, AcO^-, BzO^-, $H_2PO_4^-$ and OH^- were calculated (see Table 1).

Values of stability constants for the overall process in Eq. 3 followed the order $CN^- > Ac^- > F^- > OH^- > BzO^-$. Moreover it was found that the logarithms of the stability constants for the coordination process Eq. 1 were small (that is, −9.32, −3.21, −3.32, −0.27 and −2.34 for OH^-, CN^-, F^-, AcO^- and BzO^-, respectively). The low value found for coordination with OH^- (and its large value observed for deprotonation that is, 12.73) clearly indicated a favourable proton transfer reaction between **3** and OH^- anion whereas the other anions formed 1:1 hydrogen-bonding complexes (most likely with the thiourea binding sites) at low anion concentrations. Upon addition of increasing quantities of anions a deprotonation reaction occurs. The enhancement of the absorption and the fluorescence observed in the presence of basic anions suggested that the sensing mechanism would most likely involve the opening of the lactone ring in the fluorescein chromophore.

Additional studies were also carried out with DMSO–water 90:10 v/v solutions (pH 7.0) of receptor **3** (5.0×10^{-5} mol dm^{-3}) in order to test the possible application for anion sensing in an aqueous medium. Addition of CN^-, F^-, $H_2PO_4^-$, AcO^- and BzO^- anions to DMSO–water solutions of **3** resulted in same changes on the UV–visible spectrum than the observed when using DMSO alone. The only difference was related with the fact that more equivalents of the anions were necessary to obtain the same change in the absorbance. This is a clear consequence of the partial solvation of the anion in the presence of water that reduced their basicity.

In summary, we have synthesized a new tripodal chemosensor containing thiourea binding sites and fluorescein as signalling reporter for the colorimetric detection of basic anions in DMSO and mixed water–DMSO solutions. The colorimetric response observed is due to the opening of the lactone ring of fluorescein induced by the deprotonation of the hydroxyl moieties.

Acknowledgments

Financial support from the Spanish Government (project MAT2009-14564-C04-01) and the Generalitat Valenciana (project PROMETEO/2009/016) is gratefully acknowledged. M.E.M. thanks the Ministerio de Educación for a Doctoral FPU Fellowship.

References and notes

1. (a) Schmdtchen, F. P.; Berger, M. *Chem. Rev.* **1997**, *97*, 1609–1646; (b) Beer, P. D.; Gale, P. A. *Angew. Chem., Int. Ed.* **2001**, *40*, 486–516; (c) Wenzel, M.; Hiscock, J. R.; Gale, P. A. *Chem. Soc. Rev.* **2012**, *41*(1), 480–520; (d) Suksai, C.; Tuntulani, T. *Chem. Soc. Rev.* **2003**, *32*, 192–202; (e) Gunnlaugsson, T.; Glynn, M.; Tocci, G. M.; Kruger, P. E.; Pfeffer, F. M. *Coord. Chem. Rev.* **2006**, *250*, 3094–3117.
2. (a) Moragues, M. E.; Martínez-Máñez, R.; Sancenón, F. *Chem. Soc. Rev.* **2011**, *40*, 2593–2643; (b) Martínez-Máñez, R.; Sancenón, F. *Chem. Rev.* **2003**, *103*, 4419–4476; (c) Martínez-Máñez, R.; Sancenón, F. *Coord. Chem. Rev.* **2006**, *250*, 3081–3093; (d) Wiskur, S. L.; Aït-Haddou, H.; Lavigne, J. J.; Anslyn, E. V. *Acc. Chem. Res.* **2001**, *34*, 963–972; (e) Amendola, V.; Esteban-Gómez, D.; Fabbrizzi, L.; Lichelli, M. *Acc. Chem. Res.* **2006**, *39*, 343–348.
3. (a) Kang, J.; Jo, J. H.; In, S. *Tetrahedron Lett.* **2004**, *45*, 5225–5228; (b) Chmielewski, M.; Jurczak, J. *Tetrahedron Lett.* **2004**, *45*, 6007–6010; (c) Chmielewski, M.; Jurczak, J. *Tetrahedron Lett.* **2005**, *46*, 3085–3088; (d) Choi, K.; Hamilton, A. D. *Coord. Chem. Rev.* **2003**, *240*, 101–110; (e) Evans, L. S.; Gale, P. A. *Chem. Commun.* **2004**, 1286–1287.
4. (a) Cho, E. J.; Ryu, B. J.; Lee, Y. J.; Nam, K. C. *Org. Lett.* **2005**, *7*, 2607–2609; (b) Burns, D. H.; Calderon-Kawasaki, K.; Kularatne, S. *J. Org. Chem.* **2005**, *70*, 2803–2807; (c) Bao, X. P.; Zhang, H.; Zhang, Z.; Wu, L.; Li, Z. Y. *Inorg. Chem. Commun.* **2007**, *10*, 728–729; (d) Esteban-Gómez, D.; Fabbrizzi, L.; Licchelli, M.; Monzani, E. *Org. Biomol. Chem.* **2005**, *3*, 1495–1500; (e) Loeb, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 5030–5031.
5. (a) Kato, R.; Nishizawa, S.; Hayashita, T.; Teramae, N. *Tetrahedron Lett.* **2001**, *42*, 5053–5056; (b) Kondo, S. I.; Nagamine, M.; Yano, Y. *Tetrahedron Lett.* **2003**, *44*, 8801–8804; (c) Bao, X. P.; Wang, L.; Wu, L.; Li, Z. Y. *Supramol. Chem.* **2008**, *20*, 467–478; (d) Gunnlaugsson, T.; Davis, A. P.; O'Brien, J. E.; Glynn, M. *Org. Biomol. Chem.* **2005**, *3*, 48–56; (e) Jun, E. J.; Swamy, K. M. K.; Bang, H.; Kim, S.-J.; Yoon, J. *Tetrahedron Lett.* **2006**, *47*, 3103–3106.
6. (a) Starnes, S. D.; Arungundram, S.; Saunders, C. H. *Tetrahedron Lett.* **2002**, *43*, 7785–7788; (b) Chung, Y. M.; Raman, B.; Kim, D. S.; Ahn, K. H. *Chem. Commun.* **2006**, 186–188.
7. (a) Black, C. B.; Andrioletti, B.; Try, A. C.; Ruiperez, C.; Sessler, J. L. *J. Am. Chem. Soc.* **1999**, *121*, 10438–10439; (b) Sessler, J. L.; Maeda, H.; Mizuno, T.; Lynch, V. M.; Furuta, H. *Chem. Commun.* **2002**, 862–863; (c) Sessler, J. L.; Pantos, G. D.; Katayev, E.; Lynch, V. M. *Org. Lett.* **2003**, *5*, 4141–4144; (d) Gale, P. A.; Light, M. E.; McNally, B.; Navakhun, K.; Sliwinski, K. E.; Smith, B. D. *Chem. Commun.* **2005**, 3773–3775.
8. (a) Chang, K. J.; Moon, D.; Lah, M. S.; Jeong, K. S. *Angew. Chem., Int. Ed.* **2005**, *44*, 7926–7929; (b) Kim, N. K.; Chang, K. J.; Moon, D.; Lah, M. S.; Jeong, K. S. *Chem. Commun.* **2007**, 3401–3403; (c) Yu, J. O.; Browning, C. S.; Farrar, D. H. *Chem. Commun.* **2008**, 1020–1022.
9. (a) Metzger, A.; Anslyn, E. V. *Angew. Chem. Int. Ed.* **1998**, *37*, 649–652; (b) Wiskur, S. L.; Anslyn, E. V. *J. Am. Chem. Soc.* **2001**, *123*, 10109–10110; (c) Aït-Haddou, H.; Wiskur, S. L.; Lynch, V. M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2001**, *123*, 11296–11297.
10. For recent works on anion chemosensors see for example: (a) Climent, E.; Giménez, C.; Marcos, M. D.; Martínez-Máñez, R.; Sancenón, F.; Soto, J. *Chem. Commun.* **2011**, *47*, 6873–6875; (b) Calero, P.; Hecht, M.; Martínez-Máñez, R.; Sancenón, F.; Soto, J.; Vivancos, J. L.; Rurack, K. *Chem. Commun.* **2011**, *47*, 10599–10601; (c) Ábalos, T.; Jiménez, D.; Moragues, M.; Royo, S.; Martínez-Máñez, R.; Sancenón, F.; Soto, J.; Costero, A. M.; Parra, M.; Gil, S. *Dalton Trans.* **2010**, *39*, 3449–3459; (d) Climent, E.; Calero, P.; Marcos, M. D.; Martínez-Máñez, R.; Sancenón, F.; Soto, J. *Chem. Eur. J.* **2009**, *15*, 1816–1820; (e) Ábalos, T.; Royo, S.; Martínez-Máñez, R.; Sancenón, F.; Soto, J.; Costero, A. M.; Gil, S.; Parra, M. *New J. Chem.* **2009**, *33*, 1641–1645; (f) Comes, M.; Marcos, M. D.; Martínez-Máñez, R.; Sancenón, F.; Soto, J.; Villaescusa, L. A.; Amorós, P. *Chem. Commun.* **2008**, 3639–3641.
11. Vacca, A.; Nativi, C.; Cacciarini, M.; Pergoli, R.; Roelens, S. *J. Am. Chem. Soc.* **2004**, *126*, 16456–16464.
12. *Synthesis of compound* ***2***: In a first step, to a mixture of paraformaldehyde (16.7 g, 556.3 mmol) and triethylbenzene (**1**, 10 mL, 53.1 mmol) in HBr/AcOH (100 mL, 30 wt %) zinc bromide (19.7 g, 87.5 mmol) was slowly added at room temperature. The mixture was heated to 90 °C for 16.5 h, during which time white crystals were formed. The reaction was cooled to room temperature, and the white solid was filtered off, washed with water, and dried under vacuum

overnight to give *1,3,5-tris(bromomethyl)-2,4,6-triethylbenzene* (22.8 g, 51.7 mmol, 97%) as a white solid. In a second step, to a suspension of potassium phthalimide (8.4 g, 45.4 mmol) in dry DMSO (75 mL) *1,3,5-tris(bromomethyl)-2,4,6-triethylbenzene* (5.0 g, 11.3 mmol) was added at room temperature, under nitrogen atmosphere. The reaction mixture was heated to 84 °C for 8 h; the solution obtained was cooled to 0 °C, and the formation of a white solid was observed. After 1 h at room temperature, the solid was filtered off, dissolved in water (100 mL), and extracted with CH_2Cl_2 (2 × 100 mL). The combined organic layers were washed with water (2 × 50 mL), dried over Na_2SO_4, and concentrated to give *1,3,5-tris(phthalimidomethyl)-2,4,6-triethylbenzene* (4.88 g, 7.63 mmol, 67%) as white crystals. Then the mother liquor was poured into water (200 mL), and the white precipitate formed was filtered off. The solid was dissolved in CH_2Cl_2 (100 mL), washed with water (3 × 50 mL), and dried over Na_2SO_4. Evaporation of the organic solvent gave a crude (2.69 g) which was purified by flash column chromatography on silica gel (hexane/EtOAc, 1/1, v/v) to afford a second amount of *1,3,5-tris(phthalimidomethyl)-2,4,6-triethylbenzene* (1.45 g, 2.27 mmol, 20%). In the thrid step, to a suspension of *1,3,5-tris(phthalimidomethyl)-2,4,6-triethylbenzene* (3.2 g, 5.0 mmol) in EtOH/toluene 2:1 v/v (18 mL) hydrazine hydrate (0.98 mL, 30.8 mmol) was added at room temperature under nitrogen atmosphere. The reaction mixture was refluxed for 20 h, and during this time a white solid was formed. The reaction was cooled to room temperature, and the white solid was filtered off, dissolved in a 40% aqueous solution of KOH (120 mL), and extracted with $CHCl_3$ (3 × 150 mL). The combined organic layers were washed with water (3 × 150 mL) and dried over Na_2SO_4. Evaporation of the organic solvent gave **2** (0.973 g, 3.90 mmol, 78%) as a white solid. Mp 138–140 °C; ^{1}H NMR (0.1 mol dm^{-3} in $CDCl_3$, 200 MHz) δ: 3.87 (bs, 6H), 2.82 (q, 6H), 1.26 (bs, 6H), 1.23 (t, 9H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 140.3, 137.4, 39.6, 22.5, 16.8.

Synthesis of receptor 3: A mixture of **2** (26.8 mg, 0.106 mmol) and fluorescein isothiocyanate (125 mg, 0.32 mmol) was dissolved in dichloromethane (20 mL). Then, triethylamine (1.5 mL, 10.7 mmol) was added and the mixture refluxed for 24 h. After cooling to 25 °C, the solvent was removed in vacuo giving a residue, which was purified by silica column (increasing polarity from ethyl acetate/hexane, 10/1, v/v to ethyl acetate/hexane, 20/1, v/v) to yield receptor **3** as an orange solid (42 mg, 0.03 mmol, 28%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.2 (s, 6H), 8.25 (s, 3H), 8.10 (s, 3H), 7.75 (d, 3H), 7.21 (d, 3H), 7.14 (d, 3H), 6.62 (dd, 3H), 6.58 (dd, 3H), 5.24 (d, 3H), 5.12 (s, 3H), 4.67 (s, 6H), 2.95 (q, 6H), 1.15 (t, 9H). MS (FAB): m/z 1415 (M^+).

13. Huo, F.-J.; Yin, C.-X.; Yang, Y.-T.; Su, J.; Chao, J.-B.; Liu, D.-S. *Anal. Chem.* **2012**, *84*, 2219–2223.