Carbohydrate Research 344 (2009) 161-166

Contents lists available at ScienceDirect

Carbohydrate Research

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

# Note Efficient synthesis of a fluorescent tripod detection system for pesticides by microwave-assisted click chemistry

Isabelle Mallard-Favier, Philippe Blach, Francine Cazier, François Delattre\*

Laboratoire de Synthèse Organique et Environnement, E.A. 2599, Université du Littoral Côte d'Opale, 145 Av. M. Schumann, 59140 Dunkerque, France

#### ARTICLE INFO

Article history: Received 8 September 2008 Received in revised form 8 October 2008 Accepted 14 October 2008 Available online 1 November 2008

Keywords: Click chemistry Microwave Cyclodextrin Cyclomaltooligosaccharide Fluorescence Pesticide

# ABSTRACT

A new tripod molecule containing an aromatic core bearing three peracetylated cyclodextrins was synthesized via a microwave-assisted Huisgen 1,3-dipolar cycloaddition and was studied by fluorescence spectroscopy. The photoluminescent properties of complexation phenomena with different pesticides were evaluated in acetonitrile. Fluorescence titrations have been performed to calculate binding constants, sensitivity factors, and limit of detection of the resulting complexes. 2D NMR experiments confirmed the inclusion of pesticide in the hydrophobic cavity of the macrocycle and validated the supramolecular association responsible for the quenching of the fluorescence.

© 2008 Elsevier Ltd. All rights reserved.

#### 1. Introduction

Among the rapid and efficient methods widely used in organic chemistry, the elaboration of 1,2,3-triazole derivatives by Huisgen 1,3-dipolar cycloaddition has become a powerful design tool for organic synthetic materials, and with the introduction of the 'click' concept, by Sharpless and co-workers in 2001, it has emerged as the most popular reaction to date.<sup>1,2</sup> At the same time, the 1,2,3-triazole-based derivatives have received attention due to their properties that are widely compatible with molecules of biological interest such as antibacterial, antimicrobial, or antiallergic agents.<sup>3</sup> Curiously enough, they were only recently explored for their spectroscopic fluorescent properties.<sup>4</sup>

In a continuing program on the design and synthesis of chemosensors for recognition of environmentally important molecular species,<sup>5</sup> we have investigated the synthesis and emission properties of a new tripod fluorescent system, which bears a triazine core with cyclomaltooligosaccharide (cyclodextrin, CD) units as wellknown molecular receptors. While the design and synthesis of target-selective receptors with luminescent signaling systems have attained a significant position in supramolecular  $\beta$ -cyclodextrin chemistry for alcohol,<sup>6</sup> volatile organic compound, or metal-ion detection,<sup>7,8</sup> these systems are less frequently employed in the case of pesticides. While relatively few reports in the literature have been devoted to the spectrofluorimetric study of CD complexes with pesticides, none, to the best of our knowledge, was dedicated to the direct determination of a pesticide guest by a fluorescent macrocycle– $\beta$ -CD complex.<sup>9</sup> For this purpose, we decided to explore the variation of fluorescence emission intensity induced by the inclusion of a pesticide. While the synthesis of CD trimers is more difficult than the synthesis of the analogous CD dimers,<sup>10</sup> the central-substituted benzene architecture provides a tuneable luminescent and easy-to-modulate core to build a multiple cyclodextrin recognition system that should provide a higher binding affinity.<sup>11</sup> For the host moiety, we chose 6<sup>1</sup>-azido-6<sup>1</sup>-deoxy-cyclomaltoheptaose peracetate (1) (6-azido-6-deoxy-peracetylated- $\beta$ cyclodextrin) for its high solubility in organic solvents, which allowed us to carry out an investigation of pesticide complexation in acetonitrile.

Herein, we report the new fluorophore triazine tripod system obtained by the Cu(I)-catalyzed azide–alkyne cycloaddition reaction (CuAAC) between an excess of **1** and 1,3,5-tris(2-propynyloxy)benzene (**2**). Moreover, based on the fact that new organic synthetic strategies have been nowadays deeply affected by a portfolio of technical improvements such as microwave-assisted organic chemistry (MAOS), which are well known to achieve new chemical entities at a faster rate,<sup>12</sup> we obtained the target structure faster than is usually described for the classical 'click' reaction. To our knowledge, this is the first example of the combination of cyclodextrin synthesis with microwave-assisted click chemistry. The photoluminescent properties of the complexation phenomenon of this new fluorescent third cavity were also studied with four pesticides,  $\alpha$ -cypermethrin (an insecticide), pendimethalin





<sup>\*</sup> Corresponding author. Tel.: +33 328658246; fax: +33 328237605. *E-mail address*: delattre@univ-littoral.fr (F. Delattre).

<sup>0008-6215/\$ -</sup> see front matter @ 2008 Elsevier Ltd. All rights reserved. doi:10.1016/j.carres.2008.10.014



**Chart 1.** Structure of pesticides (A:  $\alpha$ -cypermethrin, B: pendimethalin, C: thiram, D: imidacloprid).

(a herbicide), thiram (an antimicrobial), and imidacloprid (an insecticide) (Chart 1). The inclusion of these latter guests was investigated by 2D NMR experiments to validate the supramolecular association.

## 2. Experimental

### 2.1. Apparatus

All chemicals were purchased from Acros and Sigma–Aldrich (USA), in the highest purity available. All solvents were used as supplied without further purification. The pesticides pendimetha-lin (97% purity),  $\alpha$ -cypermethrin (97% purity), thiram (97% purity), and imidacloprid (97% purity) were purchased from AccuStandard, Inc. Native  $\beta$ -cyclodextrin was purchased from Waters (Millipore Corp.).

<sup>1</sup>H, <sup>13</sup>C, 2D ROESY were recorded with a Bruker DPX 250 spectrometer working at 250 MHz and 63 MHz for <sup>1</sup>H and <sup>13</sup>C, respectively. CDCl<sub>3</sub> (99.9%) and CD<sub>3</sub>CN (99.9%) were purchased from Acros Chemicals. <sup>1</sup>H NMR data are reported as chemical shift, multiplicity (s, singlet; d, doublet; m, t, triplet; m multiplet). Fouriertransform infrared (FTIR) spectra were recorded on a Perkin–Elmer Spectrometrer BX in transmission mode between 400 and 4000 cm<sup>-1</sup>, using pellets made with dried KBr and sample. MAL-DI-TOFMS spectra were recorded on a Voyager-DE STR Applied Biosystems spectrometer with 2,5-DHB as the matrix. UV–vis spectra and fluorescence spectra were measured in a conventional quartz cell on a Perkin–Elmer Lambda 2S spectrometer and on a LS50B spectrometer, respectively. The fluorescence measurements required that the excitation and emission slits be set at 4 nm.

The host–guest binding constants  $K_b$  for the inclusion complexes of pesticides with fluorescent  $\beta$ -cyclodextrin **3** were calculated by the nonlinear Benesi–Hildebrand equation assuming a 1:1 inclusion model.<sup>13</sup>

#### 2.2. Preparation of 1,3,5-tris(2-propynyloxy)benzene (2)

To a stirred solution of propargyl bromide (80% toluene in weight, 8.50 g, 4.5 equiv) and  $K_2CO_3$  (8.87 g, 4.05 equiv) in DMF (20 mL) was added phloroglucinol (2 g, 1 equiv) in DMF (12 mL) over a period of 20 min. The mixture was stirred at room temperature for 4 days and filtered. The solvents were evaporated in vacuo. The resulting residue was dissolved in dichloromethane,

washed with water and brine, dried over  $Na_2SO_4$ , and concentrated. The crude product was then purified on silica gel (SiO<sub>2</sub>, 3:2 hexane–EtOAc,  $R_f$ : 0.81) to give a cream-colored solid: 3.23 g, 85%.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 250 MHz):  $\delta$  6.29 (s, 3H, Ar); 4.68 (d, 6H, J 2.1 Hz, OCH<sub>2</sub>); 2.46 (d, 3H, J 3 Hz, CH). <sup>13</sup>C NMR (63 MHz, CDCl<sub>3</sub>):  $\delta$  159.3, 95.4, 78.5, 75.8, 56.1. The NMR data were in agreement with those reported.<sup>14</sup>

# 2.3. 1,3,5-Tris{N<sup>1</sup>-tris[(6<sup>1</sup>-deoxycyclomaltoheptaos-6<sup>1</sup>-yl)-1,2,3-triazol-4-ylmethanoxy]}benzene peracetate (3)

1,3,5-Tris(2-propynyloxy)benzene (92 mg, 1 equiv),  $6^{l}$ -azido- $6^{l}$ -deoxy-cyclomaltoheptaose peracetate (1, 3.09 g, 4.5 equiv), Cul (219 mg, 3 equiv), and *N*-ethyldiisopropylamine (0.6 mL, 9 equiv) were dissolved in 10 mL of DMSO in an 80-mL glass vial equipped for mechanical stirring. The mixture was then irradiated for 1 h at 80 °C using an irradiation power of 210 W. After cooling, the mixture was dissolved in CH<sub>2</sub>Cl<sub>2</sub>, washed with 10% EDTA (to oxidize the Cu(I) ion into Cu(II) ion until the color of the washing reaction changed from blue to colorless), and then with water and brine. The organic extract was dried (Na<sub>2</sub>SO<sub>4</sub>), concentrated, and then purified by column chromatography (SiO<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>, *R*<sub>f</sub>: 0.61). A slightly brown compound (358 mg, 15%) was obtained upon recrystallization in EtOAc/hexane.

<sup>1</sup>H NMR (CD<sub>3</sub>CN, 250 MHz): *δ* 7.95 (s, 3H, CHN); 6.38 (s, 3H, Ar); 5.42–5.20 (m, 21H, H-1, H-5); 5.11–4.90 (m, 31H, CH<sub>2</sub>, H-1, H-6); 4.76–4.60 (m, 18, H2, H-6); 4.58–4.15 (m, 46H, CH<sub>2</sub>, H-3, H-5, H-6), 4.11–3.94 (m, 16H, H-2, H-5), 3.90–3.70 (m, 14H, H-4), 3.54–3.42 (7H, t, H-4); 2.32–2.18 (m, 117H, CH<sub>3</sub>CO). <sup>13</sup>C NMR (63 MHz, CD<sub>3</sub>CN): *δ* 170.1, 166.2, 144.4 (C4<sub>triaz</sub>), 131.6, 126.3 (C5<sub>triaz</sub>), 96.9 (C-1), 94.6 (C<sub>aro</sub>), 76.5 (C-4), 70.5–69.7 (C-2, C-3, C-5), 62.4 (C-6), 61.4 (C-6'), 59.8, 19.9. IR (KBr pellets, *ν* cm<sup>-1</sup>) 2958 (C–H), 1752 (C=O), 1618 (N=N). MALDI-TOFMS calcd for C<sub>261</sub>H<sub>339</sub>N<sub>9</sub>O<sub>165</sub>Na, *m/z* 6265.44; found *m/z* 6264.70. Anal. Calcd for C<sub>261</sub>H<sub>339</sub>N<sub>9</sub>O<sub>165</sub>: C, 50.22; H, 5.47; N, 2.02. Found: C, 49.13; H, 5.75; N, 1.99.

#### 3. Results and discussion

#### 3.1. Synthesis

The synthetic route to the tripod is depicted in Scheme 1.

The starting material mono  $6^{I}$ -azido- $6^{I}$ -deoxy- $\beta$ -cyclodextrin was prepared by total azidolysis under microwave conversion from mono 6<sup>1</sup>-deoxy-6<sup>1</sup>-O-(*p*-tolylsulfonyl)-β-cyclodextrin synthesized by the method of Defaye et al.<sup>15</sup> or Cazier et al.<sup>16</sup> Peracetylation with acetic anhydride in pyridine gave mono 6<sup>1</sup>-azido-6<sup>1</sup>-deoxycyclomaltoheptaose peracetate (1) in good yield.<sup>17</sup> O-Alkylation of phloroglucinol with propargyl bromide in the presence of potassium carbonate in DMF gave the desired compound 2 in 80% yield.<sup>14,18</sup> The final step consisted of 1,2,3-triazole ring formation between azide 1 and alkyne 2. Click chemistry, which has already been used in the synthesis of cyclodextrin derivatives, can be performed using a Cu(I) ion source like CuI, CuSO<sub>4</sub>, or copper turnings to catalyze the reaction.<sup>4b,19,20</sup> The click reaction was performed under microwave activation using CuI/DIEAP (N-ethyldiisopropylamine) as catalyst with a much shorter time compared to the 18 h of reaction time of the thermal method.<sup>21</sup> To confirm the product, <sup>1</sup>H spectra depicted in Figure 1 showed clearly 1,2,3-triazole ring formation as demonstrated new peaks at  $\delta$  7.95 assigned to the 1,2,3-triazole linker. In terms of geometry, examination of the <sup>13</sup>C NMR chemical shifts shows that carbons C-4 and C-5 of the triazole ring at  $\delta$  144.4 and 126.3, respectively, correspond to the formation of the 1,4 regioisomer.<sup>22</sup>



 $\label{eq:scheme1.} Synthesis of 1,3,5-tris[(N^1-tris[(6^1-deoxycyclomaltoheptaos-6^1-yl)-1,2,3-triazol-4-ylmethanoxy]] benzene peracetate (3).$ 

# 3.2. 2D NMR spectra

A 2D ROESY NMR experiment was carried out in deuterated acetonitrile to obtain the spacial information on the potential inclusion of the guest molecules in the hydrophobic moieties of the tripod detection system. The complexation of pendimethalin (**B**) with the macrocycle was chosen as an example of interactions between the two partners (Fig. 2).

As expected, the ROESY experiment shows spatial interactions between the acetyl protons of the peracetylated cyclodextrin and external protons of the cavity. Moreover, various dipolar correlations were observed between various protons of pendimethalin (**B**) and the inner protons (H-3 and H-5) of the CD unit, indicating the presence of a guest in the hydrophobic cavity of the macrocycle. To elucidate the contribution of each pesticide proton, the pendimethalin was investigated by <sup>1</sup>H COSY NMR spectroscopy.



Figure 1. <sup>1</sup>H NMR spectrum of 1,3,5-tris[N<sup>1</sup>-tris[(6<sup>1</sup>-deoxycyclomaltoheptaos-6<sup>1</sup>-yl)-1,2,3-triazol-4-ylmethanoxy]}benzene peracetate (3) in acetonitrile.



Figure 2. Partial NMR 2D ROESY spectra of 3 (mixing time: 700 ms) in acetonitrile.

This experiment shows that only the methyl aromatic groups ( $\delta$  2.17–2.20 and  $\delta$  2.31, respectively) were involved in the complexation, and no dipolar correlation was noted between the aromatic protons of pendimethalin and H-3, H-5 of  $\beta$ -cyclodextrin. These results suggest a non-axial accommodation of the phenyl moiety of the guest associated with a partial inclusion into the hydrophobic cavity through the secondary hydroxyl side of the cyclodextrins.

#### 3.3. Excitation and emission spectra

The excitation spectrum of **3** exhibited two absorption maxima at 227 nm and 326 nm. The fluorescence optimal emission was observed for an excitation at 227 nm, and showed characteristic emission peaks at 315 and 329 nm that would arise from an  $n-\pi^*$  transition of the heteroaromatic molecular structure. Therefore, the presence of oxygen did not influence the fluorescence emission of 1,3,5-tris[ $N^1$ -tris[( $6^1$ -deoxycyclomaltoheptaos- $6^1$ -yl)-1,2,3-triazol-4-ylmethanoxy]}benzene peracetate (**3**).

Figure 3 shows the fluorescence spectra of 3, at constant concentration, in acetonitrile with or without pendimethalin (B) as an example of pesticide recognition. The fluorescence intensity decreases with increasing pesticide concentration until the extinction, which confirms that the pollutant compound was included in the hydrophobic cavity of the macrocycle chemosensor. The absence of wavelength shift suggests that the environment around the fluorescent tripod has not undergone changes during the complexation phenomena and is consistent with the absence of correlations between the aromatic core of the trimer and the pesticide. Consequently, we assume that the quenching of fluorescence is only induced by the inclusion of the pesticide in the cyclodextrin cavity and is not due to  $\pi - \pi$  stacking interactions. Similar phenomena were observed for all guests used in this work with modulations in variations of fluorescence emission according to the nature of the guest. This guest-induced variation of fluorescence emission is coherent with the 2D NMR experiment, which has shown an inclusion of pendimethalin in the peracetylated cyclodextrin moieties of the triazole derivative.



**Figure 3.** Fluorescence spectra of **1**  $[1.64 \times 10^{-5} \text{ mol L}^{-1}, \lambda_{exc} = 227 \text{ nm}]$  in acetonitrile upon addition of pendimethalin **II** ((1) 0, (2)  $1.53 \times 10^{-6}$ , (3)  $3.07 \times 10^{-6}$ , (4)  $4.6 \times 10^{-6}$ , ..., (19)  $1.43 \times 10^{-4}$  M); inset: fluorescence emission at 329 nm (point) and calculated curves obtained from nonlinear Benesi–Hildebrand equation (line).

Binding constants $(K_{\rm b})$ limit of detection (LOD) and sensitivity factors of <b>3</b>	
binning constants (RB), mint of detection (EOD), and sensitivity factors of S	sitivity factors of <b>3</b>

	I	II	III	IV
$K_{\rm b}~(\times 10^3~{ m M}^{-1})$	202.9 ± 43	$143.4 \pm 25$	117.0 ± 16	19.7 ± 1.2
$\Delta I/I_0$	0.72	0.95	0.63	0.15
$LOD^{a} (\times 10^{-6} \text{ mol } L^{-1})$	0.8	1.5	2.1	4

<sup>a</sup> LOD: Limit of detection.

To classify compound **3** as the sensory system for detecting pesticide molecules, we have determined the binding constants, and have investigated the molecule-sensing ability for guest molecules as shown in Table 1.

The binding constants were estimated assuming a 1:1 hostguest complex formation, at different temperatures, by a nonlinear square fitting analysis from the plot of the guest-induced emission variation at 329 nm as a function of the guest concentration. Indeed, although the host-guest complexation seems to be 1:3, it is obvious that each cavity can include one guest. The inset in Figure 3 shows a good correlation between fluorescence titration and computer simulation for complex formation of 3 and pendimethalin, assuming departure of 1:1 complex formation. To show the sensing ability of cyclodextrin derivative **3**, the  $\Delta I/I_0$  value was used as a sensitivity parameter. Here  $\Delta I$  is  $I_0 - I$ , where  $I_0$  is the fluorescence intensity for the host alone, and *I* is the fluorescence intensity for a complex. This parameter is defined for [Guest] =  $10 \times [3]$  mol L<sup>-1</sup>, and is suitable for comparing the fluorescent chemosensors.<sup>13</sup> The results show very high binding constants compared to those reported in the literature for  $\alpha$ cypermethrin (**A**)  $(K_{\rm b} = 202,900),$ pendimethalin  $(\mathbf{B})$  $(K_{\rm b} = 143,400)$ , thiram (**C**)  $(K_{\rm b} = 117,000)$ , and imidacloprid (**D**)  $(K_{\rm b} = 19,700)$ <sup>23</sup> They are consistent with high sensitivity factors, and show that 1,3,5-tris $\{N^1$ -tris $[(6^1$ -deoxycyclomaltoheptaos- $6^1$ yl)-1,2,3-triazol-4-ylmethanoxy]}benzene peracetate (3) is useful in detecting various pesticides with remarkable capacities. Moreover, the LOD values throughout the  $\mu$ mol L<sup>-1</sup> are in the range of 0.8-4 ppm, allowing a good detection for an organic fluorescent chemosensor.

#### 4. Conclusion

In conclusion, the synthesis of a new peracetylated cyclodextrin trimer bearing three 1,2,3-triazole linkers was efficiently accomplished in shorter time by microwave-assisted Huisgen 1,3-dipolar cycloaddition. A fluorescence study revealed that the fluorescent tripod exhibits a very good variation of emission fluorescence when introducing pesticide guests inside the cyclodextrin cavity, allowing one to classify this host as a fluorescent chemosensor. In further work, we will concentrate our efforts on the improvement of new sensitive probes for studying the interaction of nonfluorescent pesticide guests with derivatives of our structural cyclodextrin design.

#### Acknowledgments

We thank the 'Centre Commun de Mesure de Spectrometry de masse' of the Université des Sciences et Technologies de Lille for Maldi-Tof spectra. The mass spectrometry facility used in this study was funded by the European Community (FEDER), the Region Nord-Pas de Calais (France), the CNRS, and the Université des Sciences et Technologies de Lille.

#### References

- 1. Huisgen, R.; Padwa, A. 1,3 Dipolar Cycloaddition, Wiley: New York, 1984.
- Kolb, H. C.; Finn, M. G.; Sharpless, B. Angew. Chem., Int. Ed. 2001, 40, 2004–2021.
- Genin, M. J.; Allwine, D. A.; Anderson, D. J.; Barbachyn, M. R.; Emmert, D. E.; Garmon, S. A.; Graber, D. R.; Grega, K. C.; Hester, J. B.; Hutchinson, D. K.; Morris, J.; Reischer, R. D.; Stper, D.; Yagi, B. H. *J. Med. Chem.* **2000**, 43, 953–970.
- (a) Içli, S.; Astley, S. Å.; Timur, C.; Anaç, Ö.; Sezer, Ö.; Dabak, K. J. Lumin 1999, 82, 41–48; (b) David, O.; Maisonneuve, S.; Fang, Q.; Xie, J. Tetrahedron Lett. 2007, 48, 6527–6530; (c) Xie, F.; Sivakumar, K.; Zeng, Q.; Bruckman, M. A.; Hodges, B.; Wang, Q. Tetrahedron 2008, 64, 2906–2914; (d) Jin, Y.; Zhu, J.; Zhang, Z.; Cheng, Z.; Zhang, W.; Zhu, X. Eur. Polym. J. 2008, 44, 1743–1751.
- Delattre, F.; Woisel, P.; Surpateanu, G.; Cazier, F.; Blach, P. Tetrahedron 2005, 61, 3939–3945.
- 6. Takenaka, Y.; Nakashima, H.; Yoshida, N. J. Mol. Struct. 2007, 871, 149–155.
  - Delattre, F.; Woisel, P.; Bria, M.; Surpateanu, G. Carbohydr. Res. 2005, 340, 1706– 1713.
  - 8. Maisonneuve, S.; Fang, Q.; Xie, J. Tetrahedron 2008, 64, 8716–8720.
  - (a) Coly, A.; Aaron, J. J. Anal. Chim. Acta 1998, 360, 129–141; (b) Pacioni, N. L.; Sueldo Ocello, V. N.; Lazzarotto, M.; Veglia, A. V. Anal. Chem. Acta 2008, 624, 133–140.
- 10. Leung, D. K.; Atkins, J. H.; Breslow, R. Tetrahedron Lett. 2001, 42, 6255-6258.
- 11. Faiz, J. A.; Spencer, N.; Pikramenou, Z. Org. Biomol. Chem. 2005, 3, 4239-4245.
- Bougrin, K.; Loupy, A.; Soufiaoui, M. J. Photochem. Photobiol. C 2005, 6(2-3), 139–167.
- Wang, Y.; Ikeda, T.; Ikeda, H.; Ueno, A.; Toda, F. Bull. Chem. Soc. Jpn. 1994, 67, 1598–1607.
- Berscheid, R.; Nieger, M.; Vögtle, F. J. Chem. Soc., Chem. Commun. 1991, 19, 1364–1366.
- 15. Defaye, J.; Crouzy, S.; Evrard, N.; Law, H. WO 9961483 (A1), 1999.

- Cazier, F.; Woisel, P.; Surpateanu, G. FR Patent 2873120 (A1), 2006.
   Zhang, L.-F.; Wong, Y.-C.; Chen, L.; Ching, C. B.; Ng, S.-C. Tetrahedron Lett. 1999, 40, 1815–1818.
- Nithyanandhan, J.; Jayaraman, N. J. Org. Chem. 2002, 67, 6282–6285.
   Wang, Y.; Xia, Y.; Tan, T. T. Y.; Ng, S.-C. Tetrahedron Lett. 2008, 49, 5190–5191.
- Liu, X-M.; Lee, H-T.; Reinhardt, R. A.; Marky, L. A.; Wang, D. J. Controlled Release 2007, 122, 54–62.
   Mourer, M.; Hapiot, F.; Monflier, E.; Menuel, S. Tetrahedron 2008, 64, 7159–
- 7163.
- Limapichat, W.; Basu, A. J. Colloid Interface Sci. 2008, 318, 140–144.
   Pacioni, N. L.; Veglia, A. V. Anal. Chim. Acta 2007, 583, 63–71.