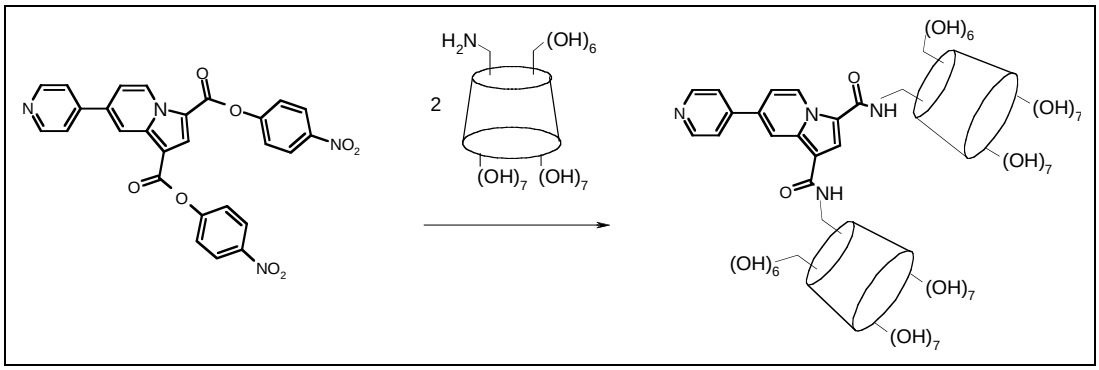
New Fluorescent Bis-β-Cyclodextrin-Indolizine Sensor. Synthesis and Sensing Ability.

Georgiana G. Surpateanu, David Landy*, Neculai C. Lungu, Sophie Fourmentin and Gheorghe Surpateanu

Laboratoire de Synthèse Organique et Environnement, EA 2599, Université du Littoral Côte d'Opale, 145 Av. M. Schumann, 59140 Dunkerque, FRANCE
*Corresponding author. Tel: 33 03 2865 8257; Fax: 33 03 2823 7605; e-mail: landy@univ-littoral.fr

Received April 18, 2006



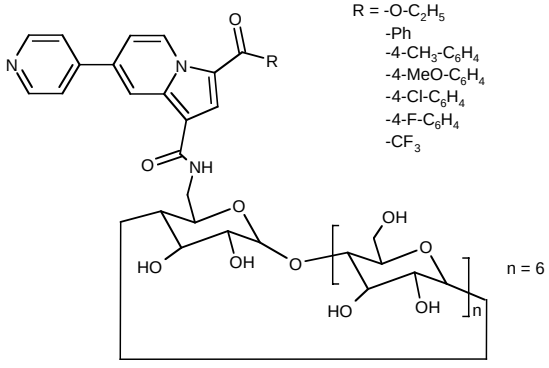
The synthesis of 1,3-[bis-*N*-6A-deoxy-β-cyclodextrin-6A-yl-aminocabonyl]-7-pyridin-4-yl indolizine is reported. The reaction proceeds by an amidation between 6-amino-β-cyclodextrin and 1,3-[bis-(-4-nitrophenoxycarbonyl)-7-[pyridine-4-yl)] and yields the first sensor having in its structure the fluorescent indolizine and two β-cyclodextrin fragments. The sensing ability towards phenol, *p*-cresol and adamantan-1-ol has been evaluated by fluorescence spectroscopy. The molecular modelling study realised by MM3 and AM1 methods shows that non cooperative conformations are favoured, thus explaining that inclusion ability is not increased by such dimer, and that sensitivity is not enhanced as compared to corresponding monomeric sensors.

J. Heterocyclic Chem., **44**, 783 (2007).

INTRODUCTION

Designing functionalisable β-cyclodextrin (β-CD), which can be used as building blocks for the synthesis of derivatives with different physical and chemical properties, is of great interest to chemists and biologists [1]. Inclusion in cyclodextrins exerts a profound effect on the physico-chemical properties of guest molecules as they are temporarily caged within the host cavity, giving rise to beneficial modifications of the guest molecule properties [2]. Therefore CDs are used as carriers for biologically active substances [3], enzyme models [4], sensors or solubilising agents for volatile organic compounds (VOC) [5], protecting agents for perfumes [6].

In previous papers [7], the synthesis of a new class of fluorescent sensor towards VOC with a pyridinoindolizine unit bonded to 6-amino-βCD was reported (Scheme 1). All the monomeric fluorescent β-CD depicted in Scheme 1 have been characterized as sensor for some VOC by fluorescence spectroscopies [8]. On the other hand, some bis-cyclodextrin sensors have appeared in the literature [9]. Thus, as a part of our ongoing research program in synthesizing a new range of fluorescent β-cyclodextrin sensors, we herein reports for the first time the synthesis of 1,3-[bis-*N*-6A-deoxy-β-cyclodextrin-6A-yl-aminocabonyl]-7-pyridin-4-yl indolizine, **8**. To our knowledge, it is the first sensor having in its structure two cyclodextrins connected to a fluorescent indolizine group.



RESULTS AND DISCUSSION

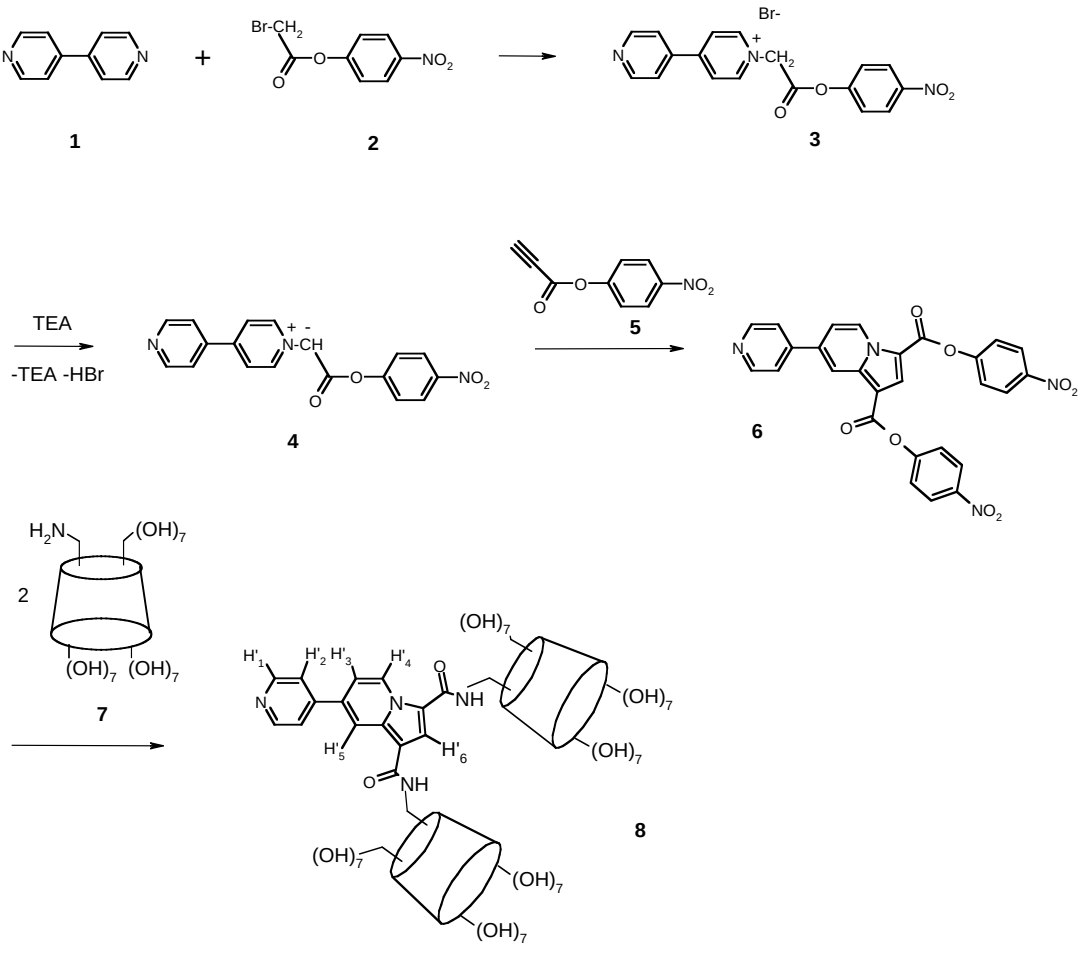
The synthesis of 1,3-[bis-*N*-6A-deoxy-β-cyclodextrin-6A-yl-aminocabonyl]-7-pyridin-4-yl indolizine **8** was conducted according to the reaction pathway depicted in Scheme 2.

First, the salt method has been used in order to obtain the bipyridine ylide **4** [10]. The 4,4'-bipyridine was reacted with *p*-nitro-2-bromoacetophenone **2**, in boiling dry acetone to furnish, after recrystallization, the 1-(4-nitrophenoxycarbonylmethyl)-[4,4']-bipyridinium bromide **3**. This salt in presence of the mild base triethylamine (TEA) gave *in situ* at 0-5°C in DMF the red-purple monosubstituted cycloimmonium ylide **4**. It should be noted that this reaction must be carried out without light in order to prevent cleavage of the N^+-C^- ylide bond [11]. The ester **5** has been obtained by a direct esterification of propynoic acid with 4-nitrophenol in presence of dicyclohexylcarbodiimide (DCC) [12]. The 6-deoxy-6-amino-β-cyclodextrin **7** was prepared according to Hamasaki method [13], *i.e.* by the transformation of cyclodextrin in its 6-tosyl derivative, then in its 6-azido and finally 6-amino derivative. Next, the ylide **4**, generated *in situ* in dry DMF, was reacted with propynoate **5** to generate the indolizine **6** by a (3+2) cyloaddition reaction. This transformation involves an intermediate aromatisation of the initial cycloadduct by losing two hydrogen atoms. In the last step of the synthesis, two amidations, between two molecules of 6-deoxy-6-aminoβ-cyclodextrin **7** and the fluorescent indolizine **6**, occur with relatively good yield (19%). The isolation and purification of the final fluorescent sensor **8** consists in precipitation in acetone, dissolution in water and successive chromatography on sephadex CM25 and G15 resins.

All three new reported compounds **3**, **6** and **8** have been characterized using FTIR, ESIM and ^{1}H-NMR. In particular, the purification of the final fluorescent β-cyclodextrin sensor **8** leads to a single peak on the ESIM spectra, corresponding to the $[M+23]^+$ adduct. The molecular structure of this sensor has been established principally by comparison of its ^{1}H NMR spectra with previously published NMR data for analogous compound having only one β-cyclodextrin fragment [8].

In order to evaluate the fluorescence sensing ability of the sensor **8**, the $\Delta I/I_0$ parameter was considered as sensitivity factor, where ΔI corresponds to $I-I_0$ (being respectively the fluorescence intensity of the sensor **8** alone and of the host/guest mixture). The studied guests were phenol, *p*-cresol and adamantan-1-ol. The first two

Scheme 2



guests are semi-volatile compounds, whereas the third one is known to fit tightly to the β-cyclodextrin cavity. The fluorescence spectra of the sensor in presence of an excess of each guest are described in Figure 1 (curves with smaller guest concentrations are not given for the sake of clarity).

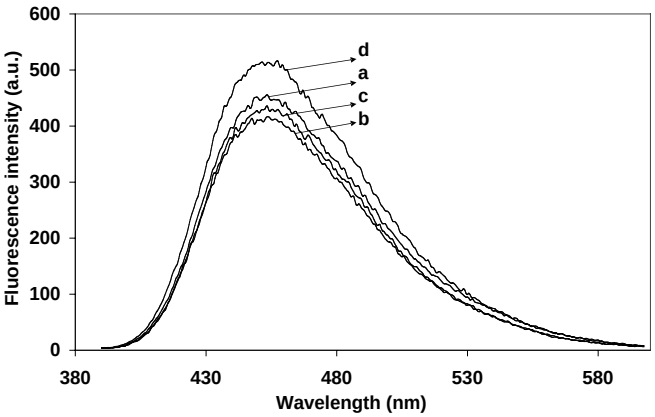


Figure 1. Fluorescence spectra of $3*10^{-6}$M sensor **8** alone (a), and of $3*10^{-6}$M sensor **8** in presence of 10^{-2}M phenol (b), $5*10^{-3}$M p-cresol (c) and $7.5*10^{-4}$M adamantan-1-ol (d).

The sensitivity factors as well as the corresponding excitation and emission wavelengths are presented in Table 1, and have been obtained for the conditions described in Figure 1 caption.

Table 1. Sensitivity factors (λ_{exc} = 380 nm ; λ_{em} =453 nm).

	phenol	*p*-cresol	adamantan-1-ol
$\Delta I/I_0$	-0.05	-0.09	+0.14

One can notice that adamantan-1-ol gives rise to an increased fluorescence, whereas phenol and *p*-cresol rather act as quenching agents since a decrease of the intensity is observed. No bathochromic or hypsochromic shift of the maximum of emission is observed for any of the substrate. In addition, as could be expected from the tighter fit of adamantan-1-ol, a greater sensitivity is observed for this guest. Nevertheless, keeping in mind that the sensor **8** concentration (3.10^{-6} M) is of the same order than for fluorescent indolizine sensor with only one cyclodextrin fragment [9], no evident influence of cooperative inclusion on the sensing ability is observed, since no increase of the sensitivity factors are observed for sensor **8**. In order to find an explanation for such experimental finding, we developed a theoretical molecular modelling study.

For this purpose, the sensor **8** was built starting from the data provided by the structural data base system of the Cambridge Crytallographic Data Center concerning the β-cyclodextrin. The structural manipulations were made using CAChe on a PC computer [14]. After a multiconformationnal search based on MM3 force field, the most stable structure has been optimised with the AM1 hamiltonian, in gaseous state and in water (MM3 energy = 782.1 kcal/mol; AM1 energy = -3058.2 kcal/mol; AM1/H_2O energy = -3143.0 kcal/mol). No significant structural change is observed between the methods, and a representative conformation is illustrated in Figure 2.

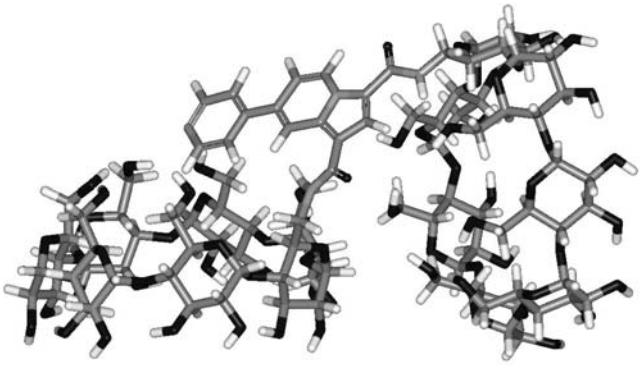


Figure 2. Predicted structure for the sensor **8**.

As can be seen, the bipyridine arm of the indolizine fragment covers up the primary face of one of the two cyclodextrin frames. Moreover, the two cavities are directed in such a way that cooperation could not occur. Indeed, the inclusion of guest such as phenol, *p*-cresol or adamantanol requires the face to face conformation of the dimer to allow the simultaneous interactions of the guest with both cavities. Such results could explain the relatively poor efficiency observed in fluorescence detection, the dimeric structure not being more sensitive than the monomeric sensors that we previously reported.

In conclusion, we reported for the first time the synthesis of a fluorescent indolizine sensor having in its structure two β-cyclodextrin fragments. The fluorescence behaviour of the compound **8** proves its sensing ability towards phenol, *p*-cresol and adamantan-1-ol. Nevertheless, this ability is not greater than for monomeric sensors, leading to think that cooperative inclusion is not effective. This assumption is confirmed by our molecular modelling study, since the multi-conformationnal search recommends the compound **8** as a partial capped cavity sensor.

EXPERIMENTAL

General. ^{1}H-NMR spectra were recorded with a Bruker AMX 250 spectrometer with tetramethylsilane as external standard. Mass spectra were measured using a Platform II Micromass Apparatus. IR-spectra were using a Perkin-Elmer instrument. Melting points were obtained with a Reichert Thermospan

apparatus and are uncorrected. Fluorescence spectra were recorded using a Perkin-Elmer LS50B spectrometer with a scan speed of 100nm/min and slit of 4nm.

1-(4-Nitrophenoxycarbonyl)-4-pyridin-4-yl-pyridinium bromide (3). The 4-4'-bipyridine **1** and 4-nitrophenyl bromoacetate **2** are commercially available. In a 100 mL round-bottomed flask, a solution of 2.6 g (1 mmol) ester 2 in dry acetone (15 mL) was added gradually to a solution of 1.56 g (1 mmol) bipyridine 1 also in dry acetone (50 mL). The reaction mixture was warmed under argon to reflux for 16 h. The crude product that precipitated was collected by filration and next washed with acetone to afford the solid salt **3**. The pure product, a little bit coloured was then obtained by recrystallisation in ethanol. Yield : 78%. ^{1}H NMR (DMSO-d_6/TMS): δ=9.19 (d, 2H, H_{ortho}/N+, J=7.1Hz); 8.82 (d, 2H, H_{ortho}/N, J=5.9Hz); 8.64 (d, 2H, H_{meta}/N+, J=7.1Hz); 8.13 (d, 2H, H_{meta}/N, J=5.9Hz); 8.10 (d, 2H, H_{ortho}/O, J=6.1Hz); 6.97 (d, 2H, H_{meta}/O, J=6.1Hz); 5.67 (s, 2H, CH_2/N+); IR (KBR) cm^{-1}: 2928; 1651; 1518; 1338; 1289; 1110; 817; MS (ES+, cone 10) m/Z (%) : 336 [M-Br]+ (96%); 376 [M-Br+K]+ (100%). *Anal.* Calcd for $C_{18}H_{14}BrN_3O_4$: C 51.92, H 3.36, N 10.09; found C 52.04, H 3.48, N 10.20.

3-(4-Nitrophenoxycarbonyl)-7-(pyridin-4-yl)-1-(4-nitrophenoxycarbonyl)-indolizine (6). In a 100 mL round-bottomed flask is dissolved 0.19 g (1 mmol) 4-nitrophenyl propynoate 5 in dry DMF (20 mL). Next, it is added under stirring 0.41 g (1 mmol) of solid salt **3**. To the stirred and cooled (0-5°C) reaction mixture, a solution of 0.2 mL (1.5 mmol) of triethylamine (TEA) in dry DMF (3 mL) is gradually added over a period of 15 minutes using a dropping funnel. Stirring is carried on in the dark, at room temperature for 8 hours. Distillation of DMF is then realised up to a volume of 7 mL. Finally, the obtained viscous final product was poured into methanol and collected by filtration. If necessary, the dissolution in DMF followed by precipitation in methanol may be repeated. Yield: 25%. ^{1}H NMR (DMSO-d_6/TMS): δ=9.58 (d, 1H, H'4, J=8.0Hz), 8.84 (d, 2H, H'1, J=7.1Hz), 8.51 (s, 1H, H'5), 8.45 (d, 2H, H'2, J=7.1Hz), 8.40 (dd, 4H, H_{ortho}/NO2, J=8.1Hz), 7.92 (s, 1H, H'6), 7.80 (d, 1H, H'3, J=8.1Hz), 7.70 (dd, 4H, H_{meta}/NO_2, J=8.1Hz); IR (KBR) cm^{-1}: 2930, 1715, 1580, 1521, 1345, 1179, 1106, 985, 863; MS (ES+, cone 40) m/Z (%): 525 [M+H]+ (100%), 547 [M+Na]+ (33%). *Anal.* calcd for $C_{27}H_{16}N_4O_2$: C 61.83, H 3.05, N 10.68; found C 61.71, H 3.20, N 11.02.

1,3-[Bis-*N*-6A-deoxy-β-cyclodextrin-6A-yl-aminocabonyl]-7-pyridin-4-yl indolizine (8). To a solution of 6-amino-β-cyclodextrin 7 (1 mmol) in dry DMF (20 mL) was added a solution of fluorescent indolizine **6** (0.5 mmol) also in dry DMF (10 mL). The stirred reaction mixture under nitrogen was maintained at 50°C over 20 hours. The liquid mixture was then poured in dry acetone (200 mL). The crude solid collected by filtration was dissolved in water (200 mL) in order to remove the unreacted starting material **6** by filtration. The aqueous solution, after evaporation to 7-10 mL, was passed through a CM-25 column with water as elutant. The fraction containing the fluorescent sensor **8** were combined and concentrated in vacuum. A final purification by sephadex G15 was applied to afford the sensor **8** in pure state as a fine powder. Yield 19%. ^{1}H NMR (DMSO-d_6/TMS): δ=9.51 (d, 1H, H'4, J=8.0Hz), 8.98 (s, 1H, H'5), 8.70 (d, 2H, H'1, J=6.1Hz), 8.05 (s, 2H, NH), 7.85 (s, 1H, H'6), 7.75 (d, 2H, H'2, J=6.1Hz), 7.45 (dd, 1H, H'3, J=2.5Hz and J=6.1Hz), 5.88-5.53 (m, 28H, -OH-2, -OH-3), 5.00-4.80 (m, 14H, H-1), 4.57-4.29 (m, 16H, -OCH2, -OH-6), 3.89-2.94 (m, 84H, H-2, H-4, H-3, H-5, H-6A,B); IR (KBR) cm^{-1}: 3377, 1651, 1542, 1457, 1419, 1156, 1080, 1020, 944, 756; MS (ES+, cone 60) m/Z (%) : 2535 [M+Na]+ (100%). *Anal.* calcd for $C_{99}H_{148}N_4O_{70}$: C 47.29, H 5.89, N 2.22; found C 47.46, H 6.09, N 2.17.

REFERENCES

[1] (a) A.R. Khan, P. Forgo, K.J. Stine and V.T. D'Souza, *Chem. Rev.*, **98(5)**, 1977 (1998). (b) A.V. Fokin and A.F. Kolomiyets, *J. Fluorine Chem.*, **40**, 247 (1988).

[2] M. Singh, R. Sharma,and U.C. Barnerjee, *Biotechnol. Adv.*, **20(5-6)**, 341 (2002).

[3] M.Adamczyk and J. Grote, *Bioorg. Med. Chem. Lett.*, **13**, 2327 (2003).

[4] K. Uekama, F. Hirayama and T. Irie, *Chem. Rev.*, **98(5)**, 2045 (1998).

[5] (a) F. Hamada, K. Hishikawa, Y. Higuchi, Y. Akagami and T. Osa, *J. Inclusion Phenom. Mol. Recognit. Chem*, **25(4)**, 283 (1996). (b) S. Fourmentin, P. Blach, D. Landy and G. Surpateanu, *Proceeding of the 12th International Cyclodextrin Symposium*, APGI, Paris, 621 (2004).

[6] (a) C.X. Wang and S.L. Chen, *J. Industrial Textiles*, **34(3)**, 157 (2005). (b) V. Sarveiya, J.F. Templeton and A.E. Benson, *J. Incl. Phenom. Macro. Chem.*, **49**, 275 (2004). (c) K. Hara, K. Minuki, K. Hara and H. Hashimoto, *J. Incl. Phenom. Macro. Chem.*, **44**, 241 (2002).

[7] (a) M. Becuwe, F. Delattre, G.G. Surpateanu, F. Cazier, P. Woisel, G. Garçon, P. Shirali and G. Surpateanu, *Heterocycl. Commun.*, **11(3-4)**, 355 (2005). (b) N.C. Lungu, A. Depret, F. Delattre, G.G. Surpateanu, F. Cazier, P. Woisel, P. Shirali and G. Surpateanu, *J. Fluorine Chem.*, **126**, 393 (2005). (c) F. Delattre, P. Woisel, G. Surpateanu, M. Bria, F. Cazier and P. Decock, *Tetrahedron*, **60(7)**, 1557 (2004). (d) F. Delattre, P. Woisel, G. Surpateanu, F. Cazier and P. Blach, *Tetrahedron*, **61**, 3939 (2005).

[8] (a) D. Landy, G.G. Surpateanu, S. Fourmentin, P. Blach, P. Decock, and G. Surpateanu, *Internet Electron. J. Mol. Des.*, **4**, 545 (2005). (b) F. Delattre, P. Woisel, M. Bria and G. Surpateanu, *Carbohydr. Res.*, **340**, 1706 (2005). (c) S. Fourmentin, G.G. Surpateanu, P. Blach, D. Landy, P. Decock and G. Surpateanu, *J. Incl. Phenom. Macro. Chem.*, accepted, ref. JIPH-S-05-00110.

[9] (a) M. Toda, N. Ogawa, H. Itoh and F. Hamada, *Anal. Chim. Acta*, **548**, 1 (2005). (b) M. Narita, S. Mima, N. Ogawa and F.Hamada, *Anal. Sci.*, **17**, 379 (2001). (c) M. Narita, S. Mima, N. Ogawa and F. Hamada, *Anal. Sci.*, **16**, 865 (2000). (d) Y. Liu, Y.-W. Yang, Y. Chen and F. Ding, *Bioorg. Med. Chem.*, **13**, 963 (2005).

[10] (a) F. Krönke, *Ber. Dtsch. Chem. Ges.*, **68**, 1177 (1935). (b) L. Depature and G.Surpateanu, *Heterocycles*, **57**, 2235 (2002).

[11] G. Surpateanu, J.P. Catteau, P. Karafiloglou and A. Lablache-Combier, *Tetrahedron*, **32**, 2647 (1976).

[12] E. Bialecka-Florjanczyk, *Synth. Commun.*, **30(24)**, 4417 (2000).

[13] A. Hamasaki, H. Ikeda, A. Nakamura, A. Ueno, F. Toda, I. Suzuki and T. Osa, *J. Am. Chem. Soc.*, **115(12)**, 5035 (1993).

[14] CAChe, Work System, 6.01. (2003).