Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 17 (2006) 1332–1336

Indicator displacement sensor for efficient determination of α-hydroxydicarboxylic acids and their chiral discrimination

Karol Kacprzak,* Jakub Grajewski and Jacek Gawronski

A. Mickiewicz University, ul. Grunwaldzka 6, 60-780 Poznań, Poland

Received 14 February 2006; accepted 31 March 2006

Abstract—An indicator displacement sensing ensemble, composed of bis-*Cinchona* alkaloid diimide triad (A) and bromophenol blue (I), was developed for easy and efficient assay of α-hydroxcarboxylic acids with a high affinity for tartrates, for which the detection limit of 0.015 mg/ml was achieved. This system also discriminates enantiomeric α-hydroxycarboxylic acids and can be used for quick quantitative determination of natural tartaric acid in wine.

1. Introduction

Artificial receptors and sensors have recently received wide attention as versatile and efficient tools of modern analytical chemistry, for a broad spectrum of applications, including clinical, food, and environmental analysis.[1] One of the most important and relatively new approaches in this field is indicator displacement assay (IDAs), which is based on competitive binding of an indicator (signalling unit) or an analyte to a receptor (host molecule).[2] The main advantages of IDAs include: (1) no need for covalent attachment of the signalling unit to the receptor, (2) easy replacement of the signalling unit, as well as tuning the performance and selectivity of the sensing system. IDAs have found many interesting applications, as demonstrated by Anslyn (e.g., tartrate,[3] inositol trisphosphate,[4] scotch whisky aging,[5] heparin,[6] and phosphate[7] determination) and other groups.[8] Recently Anslyn successfully adapted IDAs for the enantioselective analysis of α-hydroxycarboxylates.[9]

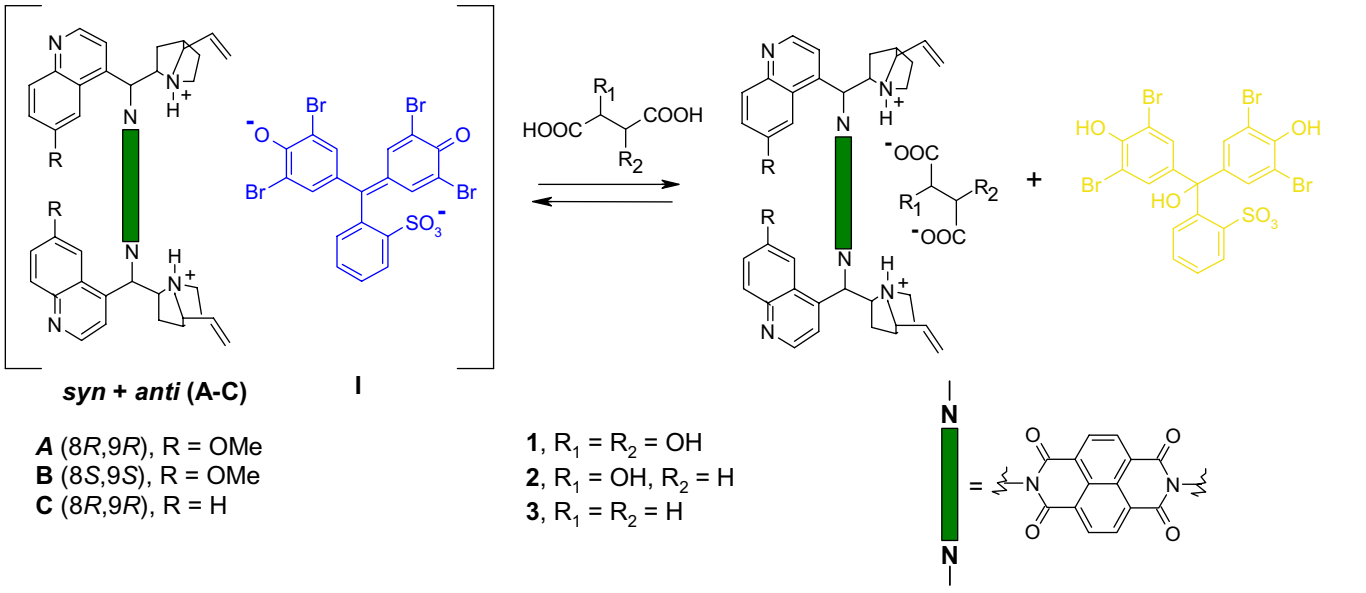
Over the course of our research on *Cinchona* alkaloid based receptors, we have found that readily accessible, chiral, C_2-symmetric aromatic diimides possessing two alkaloid moieties can recognize and discriminate a range of mono- and dicarboxylic acids.[10] Due to the hindered rotation along the C–N_{imide} bond, these receptors are present in solution as equimolar mixtures of *syn* and *anti* conformers[11] differing in their recognition selectivity. Whereas monocarboxylic acids exert an *anti* conformation of these receptors, 1,2-dicarboxylic acids induce a *syn*-conformation. The recognition process can be monitored either by ^{1}H NMR or by CD spectra. Association of *Cinchona* alkaloids with sulfophtalein dyes has been previously demonstrated by induced CD.[12] These observations provided the foundations of the development of a new indicator displacement system for easy spectrophotometric (colorimetric) assay of carboxylic acids and for their enantiomer discrimination.

2. Results and discussion

First we investigated the association of host **A** (9-epiquinidine configuration) with bromophenol blue **I**, a bulky, diprotic acid (pK_a 3.85). The ^{1}H NMR spectrum of the equimolar mixture of **A** and **I** in CD_3OD shows that ca. 60% of **A** is in a *syn* conformation, whereas the addition of 3 equiv of **I** to **A** resulted in an almost equal *syn* and *anti* conformer distribution. These results show that both acidic groups of the indicator (sulfonic and phenolic) are involved in the interaction with host **A**.[13] The association constant[14] of (*syn*+*anti*)-**A**+**I**, as determined by a UV–vis measurement using Scatchard's method, was found to be $2100 \pm 100\ mol^{-1}$. Further screening of the indicator displacement ensemble **A**+**I** using (*R*,*R*)-tartaric acid (*R*,*R*)-**1** showed that the highest response in the absorption spectra in methanol was observed for a 1:1 ratio of **A**+**I** in the complex. As expected, we observed a color change

* Corresponding author. Tel.: +48 61 8291367; fax: +48 61 8658008; e-mail: karol.kacprzak@gmail.com

0957-4166/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2006.03.037



Scheme 1. Recognition of dicarboxylic acids by the sensing ensembles (**A**–**C**)+**I**.

from blue (absorption maximum 597 nm) of the doubly deprotonated indicator to yellow (426 nm) for the liberated indicator (Scheme 1).

Having in hand three triads of different *Cinchona* alkaloid configurations (**A** = 9-epiquinidine, **B** = 9-epiquinine, and **C** = 9-epicinchonine) we screened experiments in order to test the selectivity and efficiency of the corresponding receptor-indicator ensembles toward binding guests **1**–**4**.

The best results (Fig. 1), in terms of selectivity (α-hydroxydicarboxylic vs monocarboxylic acids) and enantioselectivity, were obtained for epiquinidine-based triad **A** and all further measurements were carried out with this receptor. We found a very good selectivity of the **A**+**I** ensemble for α-hydroxydicarboxylic acids **1** and **2**, whereas simple dicarboxylic succinic **3** and monocarboxylic benzoic acid **4** produced a comparable analytical signal only in a large excess (Table 1). Detection limits for tartaric and malic acids are at the level of 0.02 mg/ml and their visual detection is presented in Figure 2.

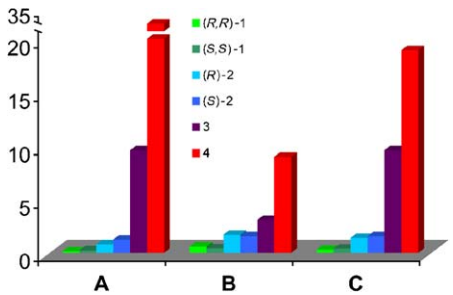


Figure 1. Molecular recognition of carboxylic acids by the three sensing ensembles composed of 1:1 bromophenol blue **I** and triad **A**, **B**, or **C** ($c = 10^{-4}$ mol dm^{-3}). *Y* axis values denote the number of equivalents of the guest **1**–**4** sufficient for 50% decrease of absorption of the sensing ensemble at 597 nm in methanol solution.

The higher selectivity toward binding 1,2-dicarboxylic acids over monocarboxylic (benzoic) acid by *Cinchona* receptors is the result of a good fit of the former in the binding pocket of the *syn* conformer of **A**–**C**, as previously observed.[10] However, the significant discrimination between α-hydroxydicarboxylic acids and succinic acid is unclear. This could be due to the lower acidity of **3** (pK_a of tartaric and malic acid is 3.04 and 3.46, for succinic acid 4.21) or due to the involvement of the hydroxy group(s) in the stabilization of the complex. Another interesting feature of the sensing ensembles presented (**A**–**C**)+**I** is a promising degree of enantiomer discrimination (Fig. 1). Two pairs of enantiomeric α-hydroxydicarboxylic acids, tartaric, and malic were studied and for both, $\Delta\Delta G_{R\backslash S} = -1.0$ kJ was calculated. It was found that 9-epiquinidine derived receptor **A** and indicator **I** ensemble prefers (Fig. 3a) natural tartaric and malic acids enantiomers (*R*,*R*)-**1** and (*S*)-**2**. 9-Epiquinine derived receptor **B** showed, as expected, opposite preference toward tartaric and malic acids (*S*,*S*)-**1** and (*R*)-**2**, however the degree of enantiodiscrimination was lower (Fig. 1).

Table 1. Detection limit and association constants of binding analytes **1**–**4** to **A**+**I** ensemble

Analyte	K_a[a] (M^{-1})	Detection limit	
		mg/ml[b]	equiv
***R*,*R*-1**	8300	0.019	0.125
***S*,*S*-1**	5500	0.019	0.125
***R*-2**	2600	0.017	0.125
***S*-2**	1700	0.017	0.125
3	220	0.12	1
4	100	0.49	4

[a] Calculated from at least two determinations by method given in Ref. 6 with ±10% accuracy.

[b] Lowest amount of guest (equivalents) producing detectable change (min 10%) of absorbance of **A**+**I** at 597 nm in methanol.

Figure 2. Recognition of (*R*,*R*)-tartaric (upper panel), (*S*)-malic (middle), and succinic acid (bottom) by **A**+**I** ensemble (vials on the left). Each vial contains: (from left to right) 1, 2, 4, 10, 20, and 50 equiv of the guest. (**A**+**I**, $c = 10^{-4}$ mol dm^{-3} in methanol).

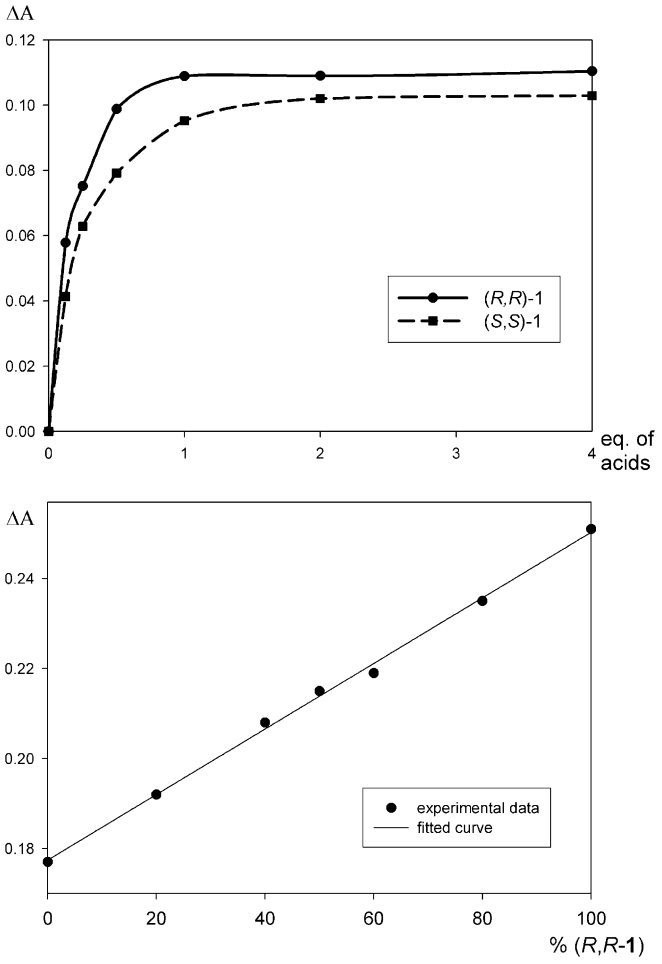


Figure 3. Displacement isotherms at 597 nm for the addition of (*R*,*R*)-**1** and (*S*,*S*)-**1** to **A**+**I**; (c **A**+**I** $= 1 \times 10^{-4}$ mol dm^{-3}) (upper panel); Change of absorbance of **A**+**I** at 599 nm as a function of the enantiomeric ratio of tartaric acid added (c **A**+**I** $= 1.17 \times 10^{-4}$ mol dm^{-3}) (bottom panel). All measurements in methanol.

We also analyzed the behavior of the sensing ensemble **A**+**I** as a function of enantiomeric composition of the guest. It was found that a change of absorbance at 599 nm increased linearly with lowering of the enantiomeric ratio of the stronger binding enantiomer (*R*,*R*)-**1** due to less competitive replacement (Fig. 3b). This is in agreement with the Anslyn's work and will be utilized further for developing a simple tool for the determination of ee of the guest.

Finally we studied the utility of our IDAs for the determination of natural tartaric acid in wine. Its content is an important parameter for wine quality and identity, and is usually determined by titration (sum of acids), or by HPLC.[15] Recently Anslyn developed IDAs for tartrates using another, less accessible synthetic receptor.[16] To achieve this goal, we modified the procedure by changing the solvent from methanol[17] to methanol–water mixture (1:1). In separate tests we have found that sugars (sacharose, glucose) and tartrates (sodium–potassium tartrate) were not active toward sensing ensemble **A**+**I**, even in a large excess. The calibration curve for tartaric acid is shown in Figure 4. We have found that 20 μl of a white wine sample (Mainzer Domherr Spätlese, 2003) was enough to locate the absorbance value in the most responsive

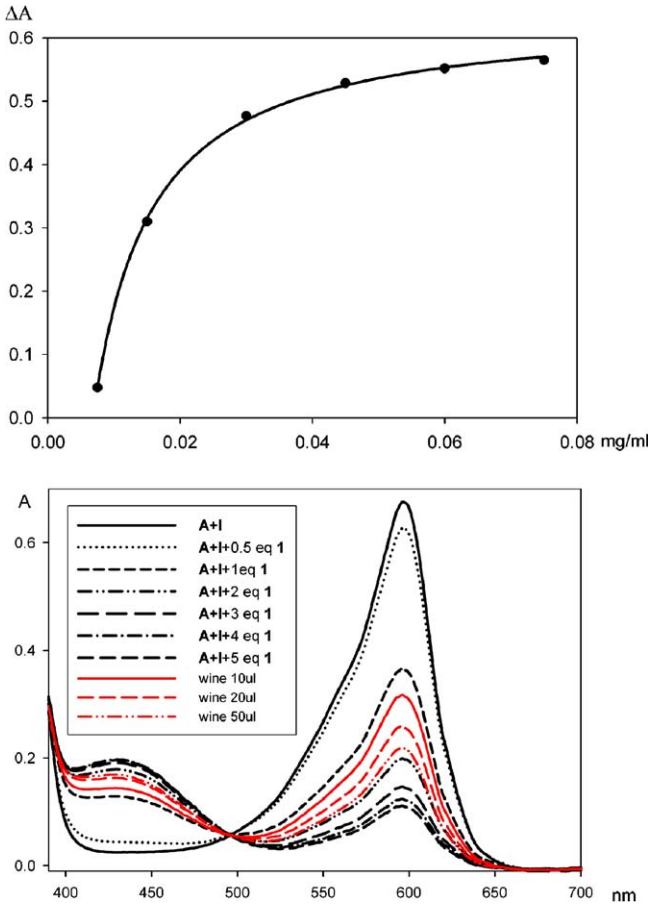


Figure 4. (Upper panel) Calibration curve for (*R*,*R*)-tartaric acid determination by **A**+**I** ensemble in water–methanol mixture at 597 nm. For details see text. (Lower panel) Effect of (*R*,*R*)-tartaric acid **1** and wine samples on the absorbance spectra of **A**+**I** and in methanol–water 1:1, c **AI** $= 1 \times 10^{-4}$ mmol.

region of the calibration curve (Fig. 4). In this way, we have determined the tartaric acid content in the wine at 2.24 mg/ml level, compared to 2.72 mg/ml determined independently by HPLC. The higher tartaric acid content as determined by the HPLC method, is the result of the use of water (pH 2) as a mobile phase, converting all dissociated tartrates to tartaric acid. We have not included the response of malic acid (1.94 mg/ml, as determined by HPLC) in our assay. Although its binding is weaker (about 25% that of tartaric acid), it contributes to the overall result of the assay. We have also found that this assay can be applied to red wine, as the absorption of red pigments (λ_{max} ca. 500 nm) lies well beyond the absorption band of the **A**+**I** ensemble. Further exploration of the presented IDAs to the tartaric acid content determination in wine is currently under study.

3. Conclusion

In conclusion, we demonstrated that easily accessible *Cinchona* alkaloid diimides and bromophenyl blue form a new sensitive indicator displacement ensemble for efficient spectrophotometric (or visual) recognition of α-hydroxydicarboxylic acids and their enantiomer discrimination. This ensemble is also of high potential for the ready determination of tartaric acid content in wine. As *Cinchona* alkaloid configuration can be easily modulated, the development of dedicated enantiospecific ensembles can be expected in the near future.

4. Experimental

UV–vis spectra were measured on a Jasco J-810 spectropolarimeter. ^{1}H NMR spectra were recorded on a Varian EM-360 or an AC-200 spectrometers (300 MHz) in $CDCl_3$, with TMS as an internal standard. Mass spectra were recorded on a AMD 604/402, IR spectra (KBr pellets) were recorded on a Brucker ITS 113v spectrometer. HPLC analyses were performed on a Waters HPLC instruments equipped with photodiode array detector, with the use of an Xterra RP18 column (5 μm, 25 cm, 4.6 mm). All reagents were obtained from commercial suppliers and used without further purification. Solvents (methanol, dichloromethane, and water) were of analytical or HPLC purity. Bromophenol blue **I** was crystallized from glacial acetic acid than dried under vacuum. *Cinchona* alkaloid-substituted 1,4,5,8-naphthalenetetracarboxydiimides (**A**–**C**) were prepared according to Ref. 11.

4.1. *N*,*N*′-Bis-[(8*R*,9*R*)-9-deoxy-9-epiquinidinyl]-1,4,5,8-naphthalenetetracarboxydiimide A

Yield 85%, ^{1}H NMR ($CDCl_3$) δ 8.84 (d, $J = 4.7$ Hz, 2H), 8.81 (s, 1H), 8.74 (d, $J = 7.7$ Hz, 1H), 8.54 (d, $J = 7.7$ Hz, 1H), 8.42 (s, 1H), 8.00 (m, 4H), 7.73 (d, $J = 2.5$ Hz, 2H), 7.32 (m, 2H), 6.87 (d, $J = 11.0$ Hz, 2H), 5.94 (m, 2H), 5.17 (dd, $J = 17$, 10.4 Hz, 4H), 4.53 (m, 2H), 3.95 (s, 3H), 3.89 (s, 3H), 3.18 (m, 2H), 2.90–2.65 (m, 6H), 2.25 (m, 2H), 1.85–1.57 (m, 8H), 1.21 (t, $J = 9.9$ Hz, 2H); HRMS (FAB) calcd for $C_{54}H_{51}N_6O_6$ 879.3870, found 879.3892, IR (KBr) ν (cm^{-1}) 2936, 1706, 1664, 1622, 1580, 1509, 1473, 1453, 1434, 1325, 1244, 1030, 855, 773.

4.1.1. Procedure for testing the recognition of carboxylic acids by sensing ensembles (A–C)+I. To a solution of (**A**–**C**)+**I** ensemble (1 ml, 1×10^{-4} mmol of both **A**, **B** or **C** and **I**) in methanol (containing 1% of dichloromethane), guest molecules (0.125, 0.25, 0.5, 1, 2, 4, 10, 20, and 50 equiv) were added by microsyringe as solutions (usually 20 mg/ml) in methanol.

4.1.2. Procedure for determination of carboxylic acids in wine by sensing ensemble A+I. *Stock solution of* **A**: 9 mg of **A** (0.010 mmol) was dissolved in 1 ml of dichloromethane and then diluted with methanol to 10 ml in volumetric flask.

Stock solution of **I**: 8.5 mg of **I** was dissolved in a 1:1 mixture water–methanol (10 ml).

Working solution of **A**+**I**: This was prepared by mixing 2.5 ml of the stock solution of **A** with 2.02 ml of the stock solution of **I** and dilution to 25 ml in a volumetric flask with 1:1 mixture water–methanol. One milliliter of such solution contains 1.024×10^{-4} mmol of **A**+**I**.

Assay: 1 ml of the **A**+**I** working solution was treated with an appropriate volume of wine sample (10, 20, or 50 μl) or a standard (*R*,*R*)-tartaric acid solution (15 mg/ml, in water) using microsyringe. After addition, the visible spectrum was measured in a 1 mm cuvette.

4.1.3. Curve fitting in Figures 3b and 4. Curve fitting was performed using SigmaPlot software, following data were obtained:

Figure 3b—linear regression, $f = 0.1774 + 0.0007x$, Rsqr = 0.998; Figure 4—cubic regression, $f = 0.6400 + (-0.0053/x) + (6.49645e - 6/x^2)$, Rsqr = 0.999.

4.1.4. HPLC determination of (*R*,*R*)-tartaric and (*S*)-malic acid in wine. HPLC assays were performed using modified procedure given in Ref. 2. Five microliters of sample of wine (Mainzer Domherr Spätlese, 2003) diluted with distilled water (1:1) and filtered was used.

Acknowledgments

We thank the Committee for Scientific Research (KBN) for financial support (Grant No. 7 T09A 040 25) and Mr. Piotr Filipiak for HPLC analysis.

References

1. (a) *Functional Synthetic Receptors*; Schrader, T., Hamilton, A. D., Eds.; Wiley-VCH, 2005; (b) Bell, T. W.; Hext, N. M. *Chem. Soc. Rev.* **2004**, *33*, 589; (c) Maier, N. M.; Franco, P.; Lindner, W. *J. Chromatogr., A* **2001**, *906*, 3.

2. (a) Wiskur, S. L.; Ait-Haddou, H.; Lavigne, J.; Anslyn, E. V. *Acc. Chem. Res.* **2001**, *34*, 963; (b) Wright, A. T.; Anslyn, E. V. *Chem. Soc. Rev.* **2006**, *35*, 14.
3. Lavigne, J.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **1999**, *38*, 3666.
4. Anslyn, E. V.; Niikura, K. *J. Org. Chem.* **2003**, *68*, 10156.
5. Wiskur, S. L.; Anslyn, E. V. *J. Am. Chem. Soc.* **2001**, *123*, 10109.
6. Zhong, Z.; Anslyn, E. V. *J. Am. Chem. Soc.* **2002**, *124*, 9014.
7. Tobey, S. L.; Anslyn, E. V. *Org. Lett.* **2003**, *5*, 2029.
8. (a) Buryak, A.; Severin, K. *J. Am. Chem. Soc.* **2005**, *127*, 3700; (b) Hortala, M. A.; Fabrizzi, L.; Marcotte, N.; Stomeo, F.; Taglietti, A. *J. Am. Chem. Soc.* **2003**, *125*, 20.
9. (a) Zhu, L.; Zhong, Z.; Aslyn, E. V. *J. Am. Chem. Soc.* **2005**, *127*, 4260; (b) Zhu, L.; Aslyn, E. V. *J. Am. Chem. Soc.* **2004**, *126*, 3676.
10. Kacprzak, K.; Gawroński, J. *Chem. Commun.* **2003**, 1532.
11. Gawroński, J.; Gawrońska, K.; Kacprzak, K. *Chirality* **2001**, *13*, 322.
12. Gawroński, J.; Gawrońska, K. *J. Chem. Res. (S)* **1984**, 304.
13. Thus, because of complete binding of indicator (pK_a 3.85) to *Cinchona* triad (pK_b 5.48 for quinine) non-specific color change due to pH change after acid addition is negligible.
14. Refers to the sum of *syn* and *anti* **A**+**I** complexes.
15. Kordis-Krapez, M.; Abram, V.; Kac, M.; Ferjancic, S. *Food Technol. Biotechnol.* **2001**, *39*, 93, and references cited therein.
16. Lavigne, J. J.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **1999**, *38*, 3666.
17. After addition of wine sample to methanolic solution of **A**+**I** the precipitation occurred.