# Defense Technical Information Center Compilation Part Notice

# ADP013598

TITLE: Molecularly Imprinted Polymers [MIPs] Against Uracils : Functional Monomer Design, Monomer-Template Interactions in Solution and MIP Performance in Chromatography

DISTRIBUTION: Approved for public release, distribution unlimited

## This paper is part of the following report:

TITLE: Materials Research Society Symposium Proceedings. Volume 723. Molecularly Imprinted Materials - Sensors and Other Devices. Symposia Held in San Francisco, California on April 2-5, 2002

To order the complete compilation report, use: ADA412559



The following component part numbers comprise the compilation report:
ADP013597 thru ADP013618

Mat. Res. Soc. Symp. Proc. Vol. 723 © 2002 Materials Research Society 

# Molecularly Imprinted Polymers (MIPs) Against Uracils : Functional Monomer Design, Monomer-Template Interactions In Solution And MIP Performance In Chromatography

Andrew J. Hall[1], Panagiotis Manesiotis, Jakob T. Mossing and Börje Sellergren
Institut für Anorganische Chemie und Analytische Chemie, Johannes Gutenberg-Universität, Duesbergweg 10-14, D-55099 Mainz, Germany.

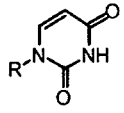
## ABSTRACT

The interaction of $N^1$-substituted uracils (cyclohexyl (**1**) and benzyl (**2**)) with three polymerisable recognition elements, the novel monomers 9-(3/4-vinylbenzyl)adenine (**3**) and 2,6-diamino-9-(3/4-vinylbenzyl)purine (**4**) and the previously synthesised monomer 2,6-bis(acrylamido)pyridine (**5**), has been studied *via* $^1$H NMR in deuterio-chloroform solution. MIPs against (**2**) have been prepared using each of the monomers and tested in the chromatographic mode. The effect of the number and type of hydrogen bonds formed between the templates and the functional monomers is reflected in the values of the apparent association constants obtained from the solution study and by the performance of the subsequently prepared MIPs in the chromatographic mode.

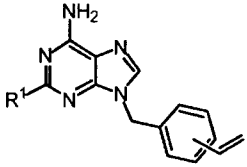
## INTRODUCTION

Interest in molecularly imprinted polymers (MIPs) has burgeoned in recent years [1]. The imprinting of nucleic acid bases and related compounds has attracted particular interest [2]. A survey of this literature shows that much MIP research has focused on the use of commercially available functional monomers, e.g. methacrylic acid, to create the binding sites in such non-covalent MIPs [3]. Among the exceptions is the use of 2,6-*bis*(acrylamido)pyridine (**5**) as a functional monomer for the imprinting of alloxan, where the selectivity of the MIP over the non-imprinted polymer (NIP) with respect to the recognition of thymine was also assessed [4]. Here, the ability of the monomer to form multiple hydrogen-bond interactions with substrate molecules was stressed. The same group has also recently reported the use of (**5**) as functional monomer in the imprinting of 5-fluorouracil [5].

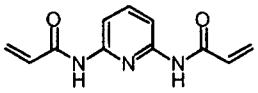
We now wish to report the preparation of the novel monomers 9-(3/4-vinylbenzyl) adenine (**3**) and 2,6-diamino-9-(3/4-vinylbenzyl)purine (**4**) and their ability to form hydrogen-bonded complexes with uracils. As a comparison, the previously reported functional monomer (**5**) has also been studied. Adenine is, of course, the base-pair partner of thymine in nucleic acids and so (**3**) could be expected to participate in hydrogen-bonding interactions with uracil molecules. The additional amino- function in (**4**) should lead to stronger association with uracils (**1**) and (**2**) by virtue of the potential extra hydrogen-bond interaction. The association of uracils with (**5**) has been studied to explore the difference between the amido- and amino-functionalities.



**(1)** R = cyclohexyl
**(2)** R = benzyl



**(3)** $R^1 = H$
**(4)** $R^1 = NH_2$



**(5)**

## EXPERIMENTAL

**(1)** was purchased from Sigma, while **(2)** and 1,3-(dibenzyl)uracil were prepared *via* a published procedure [6]. Uracil and thymine were purchased from Acros, while AZT was purchased from Sigma.

Novel monomer **(3)** was synthesised in 45% yield *via* the reaction of adenine and vinylbenzyl chloride (mixture of *meta-* and *para-* isomers) in the presence of potassium carbonate in dimethylformamide. Novel monomer **(4)** was obtained in 50% yield by modification of a published procedure for the synthesis of 9-benzyl-2,4-diaminopurine [7]. **(5)** was synthesised according to the method of Oikawa *et al.* [8].

$^1$H NMR titrations were performed by adding increasing amounts of monomer (0.5-10mM in $CDCl_3$) to solutions of either **(1)** or **(2)** (1mM in $CDCl_3$) and the spectra were recorded using a Bruker Advance DRX 400 spectrometer. The complex induced shift (CIS) of the imide proton of **(1)** or **(2)** was followed. Apparent association constants were extracted from the raw data by fitting to a 1:1 binding isotherm [9], using Microcal™ Origin.

Molecularly imprinted polymers (**$P_M$(x)**) were prepared by dissolving the template **(2)** (0.05mmol), the functional monomer **(x)** (0.1mmol), ethyleneglycol dimethacrylate (20mmol) and polymerisation initiator (ABDV) (1%w/w total monomer) in chloroform (5.6cm$^3$) in glass tubes, degassing the solutions with $N_2$ for 10 minutes, sealing the tubes and polymerising thermally at 40°C for 24 hours. The glass tubes were then broken and the polymers extracted with chloroform in a Soxhlet apparatus for 24 hours. The polymers were then crushed and sieved to obtain particles in the size range 25-50μm. After repeated sedimentation (methanol/water : 80/20) to remove fine particles, the polymers were slurry- packed (methanol/water : 80/20) into HPLC columns (125mm x 5mm, i.d.). Control, non-imprinted polymers (**$P_N$(x)**) were prepared in the same manner, but with the omission of **(2)**.

HPLC analyses were performed using an HP1050 system equipped with a DAD-UV detector. The mobile phase was acetonitrile (HPLC grade), the flow-rate 1ml/min, analyte injection volume was 20μl and the analyte concentrations were 0.01-0.5mM. Analyte detection was performed at 260nm. Analyte retentions are quoted as their capacity factors, $k' = (t-t_o)/t_o$, where t is the retention time for the analyte and $t_o$ is the retention time of the void volume marker (acetone).

**Table 1.** Apparent association constants ($K_{app}$) for the interaction of (**1**) and (**2**) with the respective functional monomers.

| Monomer | $K_{app}$ ($M^{-1}$) with (**1**) | $K_{app}$ ($M^{-1}$) with (**2**) |
|---|---|---|
| (**3**) | 63 ± 7 | 57 ± 8 |
| (**4**) | 282 ± 10 | 320 ± 16 |
| (**5**) | 567 ± 28 | 757 ± 28 |

## DISCUSSION

### $^1$H NMR Titrations

The extent of the association between the (**1**) and (**2**) and each of the functional monomers was followed *via* NMR titration by examining the change in chemical shift of the imide-proton of the respective uracil with varying concentrations of the respective functional monomers. A large downfield shift was observed in all three cases. The maximum observed $\Delta\delta$ (ppm) with (**1**)/(**2**) as guest (at the concentrations detailed in the Experimental section) were, for (**3**) = 2.23/2.24, for (**4**) = 3.95/4.33 and for (**5**) = 4.41/4.42. This is indicative of hydrogen-bonding interactions between the uracils and the monomers. The apparent association constants ($K_{app}$) extracted from the raw data, as described in the previous section, for the three monomers with each uracil are shown in Table 1.

The results of these experiments demonstrate the effects of using monomers capable of two-point and three-point binding, respectively. Gratifyingly, in keeping with our assumptions in the design of the monomers, (**4**) exhibited a much higher association constant with (**1**) and (**2**) than did (**3**), thus demonstrating the effect of the "extra" hydrogen-bonding interaction. While there is also the possibility that (**3**) might self-associate, which would also reduce its effectiveness in forming a complex with (**1**) or (**2**), previous studies have shown that in $CDCl_3$ such self-association may be considered negligible [10]. The use of monomer (**5**) illustrates the increase in association constant obtained when switching from amino- to amido- functionalities; the association constants obtained here are also consistent with previous studies [11]. Thus, of the three monomers studied, (**5**) exhibits the largest association constant with (**1**) and (**2**).

### Polymer Preparation and Chromatographic Evaluation

To test whether the above observations would be translated into the subsequent polymers, MIPs were prepared using each of the monomers using (**2**) as the template molecule (polymers $\mathbf{P_M(3)}$, $\mathbf{P_M(4)}$ and $\mathbf{P_M(5)}$, respectively). Control, non-imprinted polymers (NIPs) were prepared in the same manner, but with the omission of the template molecule (polymers $\mathbf{P_N(3)}$, $\mathbf{P_N(4)}$ and $\mathbf{P_N(5)}$, respectively). In all cases, the crosslinking monomer was ethyleneglycol dimethacrylate (EDMA) and the porogenic solvent was chloroform.

The recognition properties of the polymers were then evaluated in the chromatographic mode. In Table 2 are shown the imprinting factors ($\mathbf{IF} = k'_{MIP}/k'_{NIP}$) obtained for the different MIP/NIP combinations when injecting the template (**2**). In general agreement with the association constant data derived from the NMR titration experiments, it can be seen that $\mathbf{P_M(3)}$ and $\mathbf{P_N(3)}$ exhibit the same behaviour; (**2**) is equally weakly retained on both polymers and no

**Table 2.** Imprinting factors (IF) for (**2**) for polymers prepared against (**2**)with the respective functional monomers at various analyte loads.

| **Monomer used** | **IF** (0.01mM) | **IF** (0.05mM) | **IF** (0.1mM) |
|---|---|---|---|
| **(3)** | 0.99 | 0.97 | 0.96 |
| **(4)** | 2.02 | 1.80 | 1.68 |
| **(5)** | 4.64 | 3.50 | 3.07 |

imprinting effect is observed. For $\mathbf{P_M(4)}$ and $\mathbf{P_N(4)}$, we observe a difference in the retention behaviour of (**2**) on the respective polymers and a mild imprinting effect is seen. Finally, for the $\mathbf{P_M(5)}$ and $\mathbf{P_N(5)}$ polymer pair, a much larger imprinting effect is obtained, as the MIP is seen to recognise its template. We attribute this trend in the behaviour of the polymer pairs to the strength of the template-monomer complex in the pre-polymerisation solution, i.e. the stronger the association, the more complex is present and, subsequently, more and higher quality binding sites are obtained.

In Table 3 are shown the capacity factors obtained at different template concentrations and the capacity factors of molecules containing similar functionality to the template. Here we see the effect of changing either the peripheral substitution or hydrogen-bonding capabilities of the analyte.

For polymers prepared with (**3**) as the functional monomer, little or no change in the retention behaviour of the analytes is observed (on either the MIP or the NIP); this is consistent with the lack of imprinting effect observed for the template molecule (and the weak solution association exhibited by this monomer).

For polymers prepared with (**4**) as the functional monomer, little shape selectivity is observed for the template over different 1-substituted uracils or for unsubtituted uracils. However, the retention behaviour of 1,3-dibenzyl uracil, where a hydrogen-bonding site has been removed (compared to (**2**)), is markedly different.

Finally, the retention behaviour of the different analytes on the polymers prepared from (**5**) show the largest differences. Thus, we observe signs of shape selectivity on changing the

**Table 3.** Capacity factors (k') for different analytes on the respective imprinted and non-imprinted polymers.

| **Analyte** (concentration) | $\mathbf{P_M(3)}$ **k'** | $\mathbf{P_N(3)}$ **k'** | $\mathbf{P_M(4)}$ **k'** | $\mathbf{P_N(4)}$ **k'** | $\mathbf{P_M(5)}$ **k'** | $\mathbf{P_N(5)}$ **k'** |
|---|---|---|---|---|---|---|
| **2** (0.01mM) | 0.49 | 0.50 | 1.06 | 0.53 | 3.32 | 0.72 |
| **2** (0.05mM) | 0.48 | 0.49 | 0.95 | 0.53 | 2.42 | 0.69 |
| **2** (0.1mM) | 0.47 | 0.49 | 0.90 | 0.54 | 2.07 | 0.67 |
| **2** (0.5mM) | - | - | - | - | 1.16 | 0.46 |
| **1** (0.1mM) | 0.48 | 0.50 | 0.97 | 0.61 | 1.43 | 0.67 |
| 1,3-dibenzyluracil (0.1mM) | 0.31 | 0.31 | 0.31 | 0.23 | 0.27 | 0.30 |
| Uracil (0.1mM) | 0.38 | 0.67 | 0.77 | 0.49 | 0.97 | 0.82 |
| Thymine (0.1mM) | 0.41 | 0.56 | 0.88 | 0.56 | 1.16 | 0.96 |
| AZT (0.5mM) | 0.41 | 0.42 | 0.82 | 0.50 | 0.81 | 0.56 |

substituent at the 1-position of uracil ($k'_M$**(2)** versus $k'_M$**(1)** and $k'_M$**(AZT)**)). We also observe that removing or adding hydrogen-bonding sites to the analyte adversely affects the retention behaviour (with 1,3-dibenzyl uracil being extremely weakly retained).

## CONCLUSION

We have demonstrated that the strength of the interaction between the template and functional monomer in a solution mimicking the pre-polymerisation solution is indeed translated into the subsequently prepared MIPs. Novel monomers **(3)** and **(4)** are seen to perform less well than the previously reported monomer **(5)** and we are currently pursuing the synthesis and evaluation of improved functional monomers as part of a continuing programme of functional monomer design for use in molecular imprinting.

## ACKNOWLEDGEMENTS

The authors are grateful for financial support from the European Union under the framework of the Programme Training and Mobility of Researchers (TMR) (Project MICA, Contract Number : FMRX-CT98-0173).

## REFERENCES

1. B. Sellergren (Ed.), *Molecularly Imprinted Polymers : Man-Made Mimics of Antibodies and Their Applications in Analytical Chemistry* (Elsevier Science B.V. (NL), 2001); R.A. Bartsch and M. Maeda (Eds.), *Molecular and Ionic Recognition with Imprinted Polymers* (ACS Symposium Series 703, Washington, D.C., 1998).
2. K.J. Shea, D.A. Spivak, B.Sellergren, J. Am. Chem. Soc., **115**, 3368 (1993).
3. R. Arshady and K. Mosbach, Makromol. Chem., **182**, 687 (1981); B. Sellergren, M. Lepistö, K. Mosbach, Tetrahedron Lett., **25**, 5211 (1984).
4. K. Yano, K. Tanabe, T. Takeuchi, J. Matsui, K. Ikebukuro, I. Karube, Anal. Chim. Acta, **363**, 111-117 (1998).
5. A. Kugimiya, T. Mukawa & T. Takeuchi, *The Analyst*, **126** (6), 772-774 (2001).
6. N.G. Kundu, S. Sikdar, R.P. Hertzberg, S.A. Schmitz, S.G. Khatri, J. Chem. Soc., Perkin Trans. 1, *1985*, 1295.
7. J.A. Montgomery and K. Hewson, J. Am. Chem. Soc., **82**, 463 (1960).
8. E. Oikawa, K. Motomi, T. Aoki, J. Polym. Sci. : Part A : Polym. Chem., **31**, 457-465 (1993).
9. K.A. Connors, *Binding Constants : The Measurement of Molecular Complex Stability* (J.Wiley & Sons, New York, 1987).
10. G. Lancelot, J. Am. Chem. Soc., **99**, 7037 (1977).
11. L. Yu & H.-J. Schneider, Eur. J. Org. Chem., *1999*, 1619-1625.