Supramolecular Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713649759

Molecularly Imprinted Polymers Selective for β-Estradiol

Alexandre Rachkov[a]; Scott McNiven[a]; Soo-Hwan Cheong[a]; Anna El'skaya[b]; Kazuyoshi Yano[a]; Isao Karube[a]
[a] Research Center for Advanced Science and Technology, The University of Tokyo, Tokyo, Japan [b] Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, Kiev, Ukraine

To cite this Article Rachkov, Alexandre , McNiven, Scott , Cheong, Soo-Hwan , El'skaya, Anna , Yano, Kazuyoshi and Karube, Isao(1998) 'Molecularly Imprinted Polymers Selective for β-Estradiol', Supramolecular Chemistry, 9: 4, 317 — 323
To link to this Article: DOI: 10.1080/10610279808035001
URL: http://dx.doi.org/10.1080/10610279808035001

SUPRAMOLECULAR CHEMISTRY, Vol. 9, pp. 317–323
Reprints available directly from the publisher
Photocopying permitted by license only

Molecularly Imprinted Polymers Selective for β-Estradiol

ALEXANDRE RACHKOV[a], SCOTT McNIVEN[a], SOO-HWAN CHEONG[a], ANNA EL'SKAYA[b], KAZUYOSHI YANO[a] and ISAO KARUBE[a,*]

[a] *Research Center for Advanced Science and Technology, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153, Japan;*
[b] *Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, 150 Zabolotnogo Str., Kiev 252143, Ukraine*

(Received 19 November 1997; In final form 29 April 1998)

Polymers able to recognize the female steroid hormone, β-estradiol, have been synthesized using molecular imprinting based on non-covalent interactions. A chromatographic study of the polymers demonstrated a strong dependence of the selectivity and strength of their interaction with analytes on the solvents used both as porogen and chromatographic mobile phase. Hydrogen bonding between the OH-group at C-17 of β-estradiol and methacrylic acid (the functional monomer of the polymer) plays a major role in the process of molecular recognition but hydrophobic and dipole-dipole interactions also contribute.

Keywords: Molecular recognition, molecularly imprinted polymer, β-estradiol, solvent properties

INTRODUCTION

One of the most interesting areas in host-guest chemistry is the development of biomimetic recognition systems able to replace unstable biomolecules in numerous applications in medicine, biotechnology and environmental monitoring [1]. The self-assembled molecular imprinting approach involves host-guest complexes produced by relatively weak non-covalent interactions between the print molecule (template) and the functional monomer. These complexes are spontaneously established in the liquid phase and are then sterically fixed by polymerization with a high degree of cross-linking. After extraction of the print molecules from the polymer, unoccupied binding sites that are selective for the print molecule remain in the polymer matrix. The method results in polymers possessing a combination of high substrate specificity and good mechanical and chemical stability. Consequently, the high-performance liquid chromatographic (HPLC) separation of amino acids, sugars and many other biologically active compounds using molecularly imprinted polymers (MIPs) as stationary phases has been extensively studied [2–5].

In spite of the great importance of steroids to living organisms, there have been very few examples of their use as templates in molecular imprinting. Moreover, the abundance and availability of steroids that possess a similar core structure and a variety of functional groups in

*Corresponding author.

different arrangements allow the systematic investigation of the roles of these functional groups in the process of molecular recognition. Byström and coworkers [6] have synthesized MIPs for the regioselective reduction of steroid 3- and 17-ketones. MIPs selective for cortisol and corticosterone [7], and for cholesterol [8, 9] have been synthesized and analyzed in batch binding experiments. Another MIP imprinted against cholesterol was prepared as a membrane for use in a conductometric sensor [10].

In our previous chromatographic studies of MIPs imprinted against testosterone we found that these stationary phases bound β-estradiol quite strongly; presumably due to the similarity of the C- and D-rings of the compounds [11–12]. Thus we were prompted to synthesize and investigate the properties of MIPs for β-estradiol.

RESULTS AND DISCUSSION

Effect of Porogen

The success of molecular imprinting is usually associated with the use of apolar porogens such as chloroform or toluene [3,4]. This study demonstrates clearly an efficient imprinting process in polymers prepared using both acetonitrile (Tab. I) and a mixture AD (acetonitrile and 1,2-dichloroethane, 1:1 v/v) (Tab. II). Both solvents have a low propensity to form hydrogen bonds [13], and therefore do not hinder the formation of template-functional monomer complexes. A set of steroids of similar structure (Fig. 1) was used to evaluate the selectivity of the polymer-template interaction. Acetic acid and DMF were also chromatographed to assist in elucidating the nature of the interactions. Imprinting factors for the print molecule, β-estradiol, range from 4.7 to 8.2. Steroids, which do not possess an OH-group at C-3 or C-17 (Δ^4-androstene-3,17-dione, progesterone and testosterone propionate), have I values near 1.0, indicating a similar level of interaction with both imprinted- and non-imprinted polymers, as is true of acetic acid and DMF. This also indicates the imprinted- and non-imprinted polymers have a similar quantity of accessible functional groups. Steroids with structures more closely resembling the template: β-estradiol-derivatives (almost the entire molecule), testosterone (C- and D-rings) and estrone (A- and B-rings), had intermediate I values.

Analysis of the capacity factors shows that polymers prepared in the solvent mixture AD retain all analytes more strongly. For acetic acid and DMF, the k' values in the same mobile phase using I_{AD} and N_{AD} were 1.2–1.4 times those using I_A and N_A. Using N_{AD} in an acetonitrile mobile phase, the k' values for all steroids were about twice those using N_A. In the case of imprinted polymers (I_{AD} and I_A) this factor was about 1.5. Thus the use of the less polar porogen leads to the formation of polymers with increased non-specific hydrophobic interactions. This tendency is not be observed using the less polar mobile phase, the mixture AD, demonstrating the role of the mobile phase.

Effect of Chromatographic Mobile Phase

Independent of the porogen, use of the less polar mobile phase significantly reduced the k' values for all steroids on both imprinted- and non-imprinted polymers. The effect, however, was much less for β-estradiol and testosterone than for any of the other compounds. This is due to the weakening of hydrophobic interactions between analytes and polymers. Consequently, other steroids were barely retained on any of the polymers. Thus, the properties of the porogen influence the polymers' overall binding capacity, realization of which depends up on the composition of the chromatographic mobile phase.

Unlike the steroids, acetic acid and DMF showed higher k' values in the less polar mobile phase. To explain this result, the interactions not

TABLE I HPLC data for polymers I_A and N_A, prepared in acetonitrile

Mobile phase	Analyte	k'_n	k'_i	I	α
MeCN	β-Estradiol	**0.43**	**3.53**	**8.21**	**1**
	β-E Benzoate	0.37	0.90	2.43	3.92
	β-E Dansylate	0.34	0.76	2.24	4.64
	Estrone	0.17	0.42	2.47	8.40
	Testosterone	0.54	0.97	1.80	3.64
	Progesterone	0.26	0.36	1.38	9.81
	Androstene	0.23	0.30	1.30	11.77
	Testost. Propionate	0.17	0.24	1.41	14.71
	Diethylstilbestrol	0.09	0.13	1.44	27.15
	Acetic Acid	0.17	0.18	1.06	19.61
	DMF	0.46	0.55	1.20	6.42
Mixture AD (1:1 v/v mixture of MeCN and 1,2-dichloroethane)	β-Estradiol	**0.22**	**1.03**	**4.68**	**1**
	β-E Benzoate	0.06	0.15	2.50	6.87
	β-E Dansylate	0.03	0.09	3.00	11.44
	Estrone	0.03	0.09	3.00	11.44
	Testosterone	0.25	0.44	1.76	2.34
	Progesterone	0.06	0.06	1.00	17.17
	Androstene	0.06	0.09	1.50	11.44
	Testost. Propionate	0.03	0.03	1.00	34.33
	Diethylstilbestrol	0.03	0.03	1.00	34.33
	Acetic Acid	0.44	0.53	1.20	1.94
	DMF	0.63	0.77	1.22	1.33

TABLE II HPLC data for polymers I_{AD} and N_{AD}, prepared in a mixture AD

Mobile phase	Analyte	k'_n	k'_i	I	α
MeCN	β-Estradiol	**0.97**	**4.99**	**5.14**	**1**
	β-E Benzoate	0.84	1.38	1.64	3.62
	β-E Dansylate	0.83	1.16	1.40	4.30
	Estrone	0.38	0.63	1.66	7.92
	Testosterone	1.12	1.49	1.33	3.35
	Progesterone	0.53	0.56	1.05	8.91
	Androstene	0.44	0.47	1.07	10.62
	Testost. Propionate	0.41	0.41	1.00	12.17
	Diethylstilbestrol	0.21	0.25	1.19	19.96
	Acetic Acid	0.20	0.25	1.25	19.96
	DMF	0.65	0.69	1.06	7.23
Mixture AD (1:1 v/v mixture of MeCN and 1,2-dichloroethane)	β-Estradiol	**0.39**	**1.90**	**4.87**	**1**
	β-E Benzoate	0.13	0.17	1.31	11.18
	β-E Dansylate	0.07	0.09	1.29	21.11
	Estrone	0.09	0.13	1.44	14.62
	Testosterone	0.40	0.51	1.28	3.73
	Progesterone	0.06	0.06	1.00	31.67
	Androstene	0.09	0.06	0.67	31.67
	Testost. Propionate	0.03	0.00	–	–
	Diethylstilbestrol	0.06	0.06	1.00	31.67
	Acetic Acid	0.61	0.54	0.89	3.52
	DMF	0.86	0.84	0.98	2.26

only between analyte and polymer, but between analyte and solvent should be taken into account. Steroids, possessing a hydrophobic core, are better solvated by the less polar mobile phase and, consequently, are more weakly retained. For acetic acid and DMF, we observe the opposite situation; they interact more strongly with the polar functional monomer

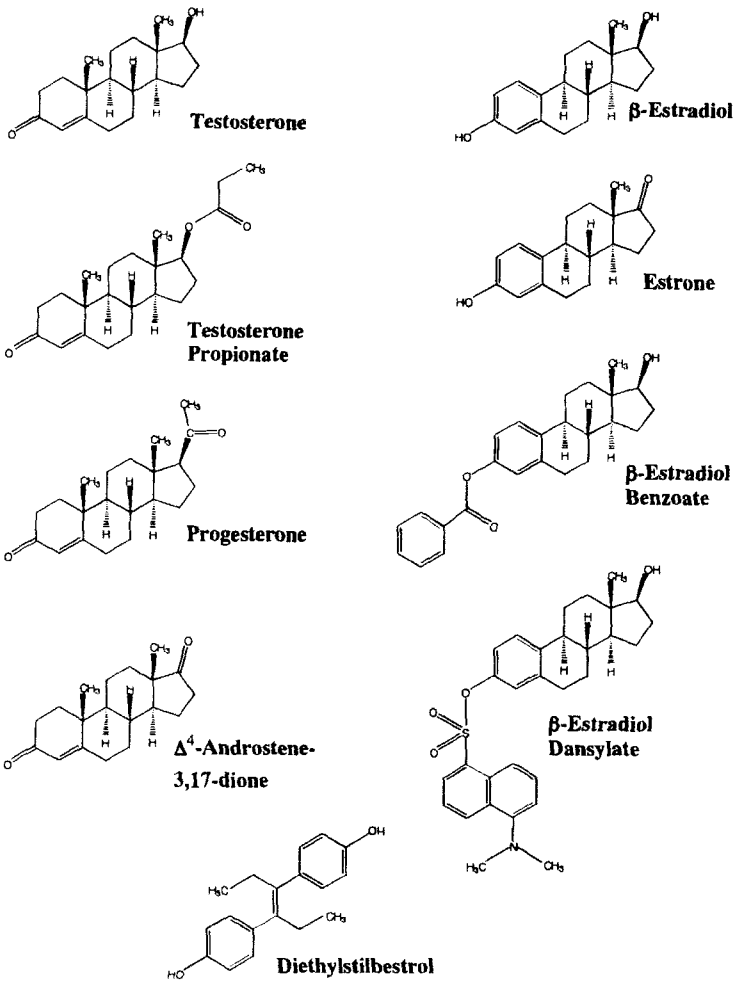

FIGURE 1 Structures of the steroids under study.

than with the less polar mobile phase, and are thus more strongly retained.

The effect of different mobile phases can, in some cases, be explained by their influence on the structure of MIPs. However, the polymers show similar swellability in both solvents. The volume increases of I_A and N_A were 1.14–1.18, and 1.32–1.39 for I_{AD} and N_{AD}, independent of the solvent. Thus the mobile phase plays a more active role by interacting with both the analyte and the polymer itself, including the EGDMA backbone and the binding sites.

Comparison of the k' values presented in Tables I and II leads to the simple conclusion that the use of less polar porogens tends to increase the overall binding of the template and its analogs while the α values demonstrate that, for any given porogen, the use of a less polar mobile phase tends to enhance the selectivity of the MIP. In other words, for the same analyte, the k' values depend on the polarity of the porogen while the α values depend on the polarity of a mobile phase. However, experiments using different mobile phases with similar polarity indicate the necessity to take into account other factors (Tab. III). The addition of 10% ethanol to acetonitrile only slightly changes the polarity of the mobile phase, but

TABLE III Dependence of HPLC data for polymer I_{AD} on the mobile phases

Analyte	MeCN		MeCN:EtOH 9:1		MeCN:CH_2Cl_2 9:1	
	k'	a	k'	a	k'	a
β-Estradiol	**4.99**	**1**	**0.65**	**1**	**2.94**	**1**
Testosterone	1.49	3.35	0.42	1.55	1.07	2.75
Estrone	0.63	7.92	0.22	2.95	0.33	8.91
Progesterone	0.56	8.91	0.26	2.50	0.35	8.40
Androstene	0.44	10.60	–	–	0.30	9.80
Testost. Propionate	0.41	12.17	0.19	3.42	0.08	36.75
Acetic Acid	0.25	19.96	0.03	21.67	0.08	36.75
DMF	0.69	7.23	0.22	2.95	0.67	4.39

produces a sharp decrease in the k' value of β-estradiol (7.7 times), and only 3.5, 2.2, and 2.2 times for testosterone, progesterone, and testosterone propionate, respectively.

Using a 9:1 v/v mixture of acetonitrile and methylene chloride, the polarity of which is also close to that of pure acetonitrile, leads to a 1.4–1.9 fold decrease in the k' values for all steroids. According to Snyder's classification of solvents [13], ethanol has a much higher propensity to engage in hydrogen bonding than the other solvents used in this study. Methylene chloride, however, possesses a large dipole moment. Thus, in pure acetonitrile, recognition of β-estradiol by the MIP is achieved mainly by hydrogen bonding, but also by dipole-dipole and hydrophobic interactions. Changing the mobile phase by mixing solvents with different properties is one way to regulate the contribution of these interactions to the overall recognition process.

Role of Steroid Functionalities

Analysis of the role of the different functional groups of steroids clearly indicates that only steroids possessing an OH-group at C-17 (β-estradiol and testosterone and, to a lesser extent, β-estradiol-derivatives) can interact efficiently with MAA-containing polymers. Comparison of the k' values of testosterone with those of testosterone propionate, Δ^4-androstene-3,17-dione and progesterone shows that any change of the C-17 substituent leads to dramatically decreased retention, particularly by the imprinted polymer.

The selectivity β-estradiol over testosterone is attributed to the relatively acidic OH-group at C-3. This functionality alone (in, for example, estrone) cannot provide strong binding, but together with the OH-group at C-17 this leads to a high level of selective recognition by the MIP. The role of the OH-group at C-3 becomes more evident in a less polar medium. Similarly, the k' values of acetic acid increase 3-fold in the solvent mixture AD. Decreasing k' values in the series β-estradiol > β-estradiol benzoate > β-estradiol dansylate demonstrate the influence of size of the substituent at C-3. Due to the bulk of the substituent, the molecules are simply less able to bind to the cavities designed for the parent compound.

One of the first synthetic compounds possessing β-estradiol-like activity was diethylstillbestrol. It comprises two OH-groups approximately the same distance apart as in β-estradiol. However, it was barely retained on any of our polymers using any mobile phase. The co-planar orientation of the phenolic OH-groups and the lack of a strongly binding aliphatic hydroxyl group prevent it from effective binding to the MIPs. The cross-reactivities of MIPs with compounds of related structure have been shown to resemble those obtained in studies with monoclonal antiobodies [7, 14, 15] and opioid receptors [14] and it seems that our polymers, which were designed to mimic β-estradiol receptors, possess greater selectivity than their natural counterparts.

Separation of Mixtures of Steroids

The usual goal of HPLC is the separation of a mixture of compounds. Under isocratic elution MIPs usually show a strong broadening of the peak corresponding to the template molecule [2, 16]. This phenomenon can be explained by the

heterogeneity of the recognition sites (as in polyclonal antibodies). Consequently, although the selectivity of MIPs is good, it is not so easy to achieve complete separation. In spite of this, the base line separation of equimolar mixtures of β-estradiol with diethylstilbestrol (a non-steroid possessing estrogenic activity), β-estradiol and testosterone propionate have been achieved (Figs. 2A, B). Estrone which, like β-estradiol, possesses the OH-group at C-3 is less easily resolved (Fig. 2C). The resolutions R_S of these mixtures were 1.02, 0.97, and 0.78, respectively. The lowest R_S value was obtained for mixture of β-estradiol and testosterone – *ca.* 0.58 (Figs. 2D). All of these results support our conclusion concerning the leading role of the OH-group at C-17 in the process of molecular recognition. Improving the resolution using gradient elution and screening other functional monomers is the aim of further investigations.

In conclusion, the present study demonstrates the successful synthesis of β-estradiol-specific MIPs using porogens of different polarity. The print molecule was rebound with high affinity and good selectivity. Analysis of the role of different functional groups of the steroid molecules leads to the conclusion that the OH-group at C-17 of β-estradiol dominates in its interaction with MAA-containing imprinted polymers. The use of different porogens and chromatographic mobile phases has confirmed the major role of hydrogen bonding and demonstrates the lesser, non-specific contribution of hydrophobic and dipole-dipole interactions to the process of molecular recognition.

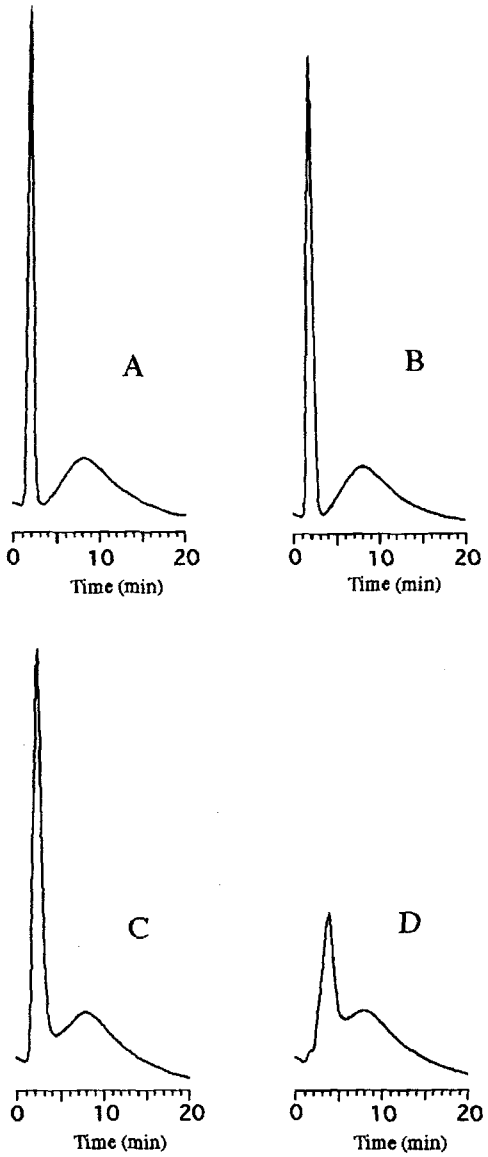

FIGURE 2 Separation of mixtures of β-estradiol and (A) diethylstilbestrol, (B) testosterone propionate, (C) estrone and (D) testosterone using I_A in the acetonitrile mobile phase. 10 μL of equimolar mixtures were analyzed at a flow rate of 1 mL min^{-1}, monitored at 220 nm.

MATERIALS AND METHODS

Materials

Methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA) and 2,2′-azobis(isobutyronitrile) were obtained from WAKO (Japan). β-Estradiol, testosterone, Δ^4-androstene-3,17-dione, progesterone, testosterone propionate, estrone, and diethylstilbestrol were obtained from Tokyo Chemical Industry Company (Japan). The benzoyl- and dansylderivatives of β-estradiol were synthesized using standard techniques [17]. All solvents used were of HPLC

grade. Chromatographic analyses were performed using Gilson system consisting of a 805 manometric module, two 305 modules, 306 HPLC pumps and 118 UV/VIS detector.

Polymerization

MIPs were polymerized at 40°C for 16 h using methacrylic acid as functional monomer at a template/monomer ratio of 1:8, ethylene glycol dimethacrylate as crosslinking agent and 2,2′-azobis(isobutyronitrile) as initiator. The imprinted- I_A and non-imprinted N_A polymers were synthesized in acetonitrile while for I_{AD} and N_{AD} mixture AD (acetonitrile and 1,2-dichloroethane, 1:1 v/v) was used. The mixture was transferred to a 50 mL glass vial, degassed under vacuum in an ultrasonic bath and purged with nitrogen for 3 min. The polymers obtained were ground in a mortar and sieved to collect the fraction 25–45 μm. The polymers were washed 5 to 6 times with ethanol for 24 h at 60°C until the template could not be detected in the supernatant. Finally, polymers were dried in a vacuum over for 16 h at 37°C. Non-imprinted polymers were synthesized under the same conditions except for the addition of template.

Chromatographic Evaluation

Polymers were slurry packed into 100 × 4.6 mm stainless steel columns for HPLC. 10 μL of 0.2 mM samples were analyzed at a flow rate of 1 mL min^{-1}, monitored at 235 nm, using ethyl acetate as a void marker. The separation of mixtures of steroids using the acetonitrile mobile phase was monitored at 220 nm. The capacity factor (k'), separation factor (α) and resolution (R_S) were calculated as usual [18]. α-values were calculated using pure samples. We define the imprinting factor (I) as $I = k'_i/k'_n$, where k'_i and k'_n refer to the imprinted- and non-imprinted polymers [11]. Thus the capacity factor is a measure of the affinity of the analyte for the polymer while imprint factor is a measure of the effect of the imprinting process.

Acknowledgments

This work is supported by the Grants-in-Aid from the Japanese Ministry of Education, Science, Sports and Culture. A. R., S. M. and S.-H. C. are grateful recipients of Japan Society for the Promotion of Science (JSPS) Fellowships.

References

[1] Kriz, D., Ramström, O. and Mosbach, K. (1997). *Anal. Chem.*, **69**, 345A.
[2] Wulff, G. (1995). *Angew. Chem. Int. Ed. Engl.*, **34**, 1812.
[3] Shea, K. J. (1994). *Trends Polym. Sci.*, **2**, 166.
[4] Mosbach, K. and Ramström, O. (1996). *Bio/Technology*, **14**, 163.
[5] Matsui, J., Kato, T., Takeuchi, T., Suzuki, M., Yokoyama, K., Tamiya, E. and Karube, I. (1993). *Anal. Chem.*, **65**, 2223.
[6] Byström, S. E., Boerje, A. and Akermark, B. (1993). *J. Am. Chem. Soc.*, **115**, 2081.
[7] Ramström, O., Ye, L. and Mosbach, K. (1996). *Chemistry and Biology*, **3**, 471.
[8] Whitcombe, M. J., Rodriguez, M. E., Villar, P. and Vulfson, E. N. (1995). *J. Am. Chem. Soc.*, **117**, 7105.
[9] Asanuma, H., Kakazu, M., Shibata, M., Hishiya, T. and Komiyama, M. (1997). *Chem. Commun.*, 1971.
[10] Piletsky, S. A., Parhometz, Yu. P., Lavryk, N. V., Panasyuk, T. L. and El'skaya, A. V. (1994). *Sensors & Actuators B*, **18–19**, 629.
[11] Cheong, S.-H., McNiven, S., Rachkov, A., Levi, R., Yano, K. and Karube, I. (1997). *Macromolecules*, **30**, 1317.
[12] Cheong, S.-H., Rachkov, A., Park, J. K., Yano, K. and Karube, I. (1998). *Journal of Polymer Science, Part A: Polymer Chemistry* (in press).
[13] Snyder, L. R. (1978). *J. Chromatogr.*, **16**, 223.
[14] Anderson, L. I., Müller, R., Vlatakis, G. and Mosbach, K. (1995). *Proc. Nat. Acad. Sci. USA*, **92**, 4788.
[15] Vlatakis, G., Andersson, L. I., Müller, R. and Mosbach, K. (1995). *Nature*, **361**, 645.
[16] Sellergren, B. and Shea, K. J. (1993). *J. Chromatogr. A*, **654**, 17.
[17] Forniss, B. S., Hannaford, A. J., Smith, P. W. G. and Tatchel, A. R. (1989). Vogel's Textbook of Practical Organic Chemistry, Longman, New York, p. 689, 886.
[18] Snyder, L. R. and Kirkland, J. J. (1974). Introduction to Modern Liquid Chromatography, John Wiley and Sons, New York, p. 16.