Bioorganic Chemistry **27,** 363–371 (1999)
Article ID bioo.1999.1136, available online at http://www.idealibrary.com on IDEAL®

Enantioselective Tröger's Base Synthetic Receptors

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Received August 25, 1998

Synthetic receptors selective for the enantiomers of Tröger's base (**1**), a compound containing chiral nitrogen atoms, have been prepared by the molecular imprinting of **1** in methacrylic acid–ethylene glycol dimethacrylate copolymers. Spectroscopic evaluation of the self-assembly phase, prior to polymerization, demonstrated the formation of template-functional monomer adducts of K_{diss} 0.7 ± 0.1 mM at 293 K in chloroform. The synthetic receptors demonstrated enantioselectivity when used as HPLC chiral stationary phases; enantioseparation factors (α) of up to 4.8 ± 0.2 are reported. Baseline resolution of racemic **1** was readily achieved. Furthermore, the influence of water on polymer–ligand selectivity was examined, which yielded insights into the molecular basis for ligand selectivity in these synthetic receptors.

INTRODUCTION

A growing awareness of the often profoundly different pharmacological activities of the enantiomers of chiral substances (*1*) has motivated recent FDA directives governing their use as pharmaceuticals (*2*). This has led to intense activity directed toward the development of new and improved means for the separation of optical isomers (*3*).

Molecular imprinting technology offers the possibility of producing tailor-made ligand-selective reversible recognition sites in synthetic polymers (*4–9*). The technique relies upon the selective coordination of functionalized monomers by a template which, after polymerization in the presence of an inert cross-linking agent and extraction of template, yields recognition sites complementary in shape and functionality to the template structure. These sites may be used to selectively rebind the template, *e.g.*, a single enantiomer, in the presence of structurally related structures, *e.g.*, its optical antipode. The versatility of the technique has led to many recent reports of it being used to prepare custom chiral stationary phases for chromatographic separations (*10*). To date a diverse array of chemical structures has been studied using this technique

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0045-2068/99 $30.00

and the resultant polymers have found application in a number of other application areas: synthetic enzymes (*11*), sensor recognition components (*12*), antibody substitutes in clinical diagnosis (*13–15*), and the study of molecular recognition phenomena (*16–18*).

The enantiomers of Tröger's base ([2,8-dimethyl-6*H*,12*H*-5,11-methanodibenzo [*b*,*f*][1,5]diazocine, **1**) (*19*), which contain chiral nitrogen atoms, are used for the resolution of racemates (*20*) (Scheme 1). The chiral resolving power of the enantiomers of **1**, arising from their molecular asymmetry, suggested that their use as templates in molecular imprinting protocols would yield highly enantioselective synthetic receptors, which could in turn be useful for the resolution of racemic **1** (Fig. 1).

MATERIALS AND METHODS

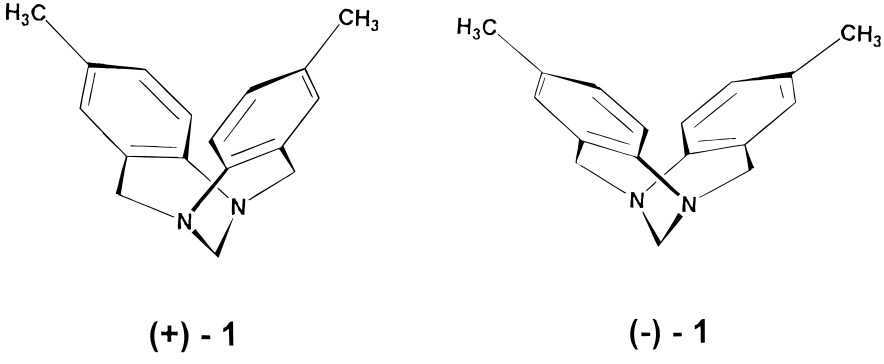
All chemicals and solvents were of analytical or HPLC grade. Monomers were redistilled prior to use. UV spectra were recorded on a Hitachi U-2001 double-beam instrument. HPLC was performed using a Perkin–Elmer Series 200 Ic pump and Applied Biosystems 785A programmable absorbance detector.

Spectrophotometric Evaluation of Tröger's Base-Functional Monomer Prepolymerization Mixtures

UV-spectrophotometric titration studies were performed according to a previously described method (*21*). A 0.315 mM solution of **1** in chloroform and a chloroform reference were each titrated with acetic acid, previously shown to be a suitable analogue for the functional monomer methacrylic acid. Analyses were performed in triplicate at 293 ± 1 K. A plot of the relative change in absorbance, ΔAbs (Abs/Abs_0), against molar ratio (mol **1**/mol acetic acid) yielded a saturation isotherm. A Hill-style binding plot was prepared from these data. The slope of this plot yielded an apparent dissociation constant for the complex, K_{diss}.

Polymer Preparation

In a typical polymer preparation Tröger's base (**1**, 0.58 mmol) was dissolved in a solution of methacrylic acid (**2**, 1.27 mmol) and ethylene glycol dimethacrylate (**3**,



SCHEME 1.

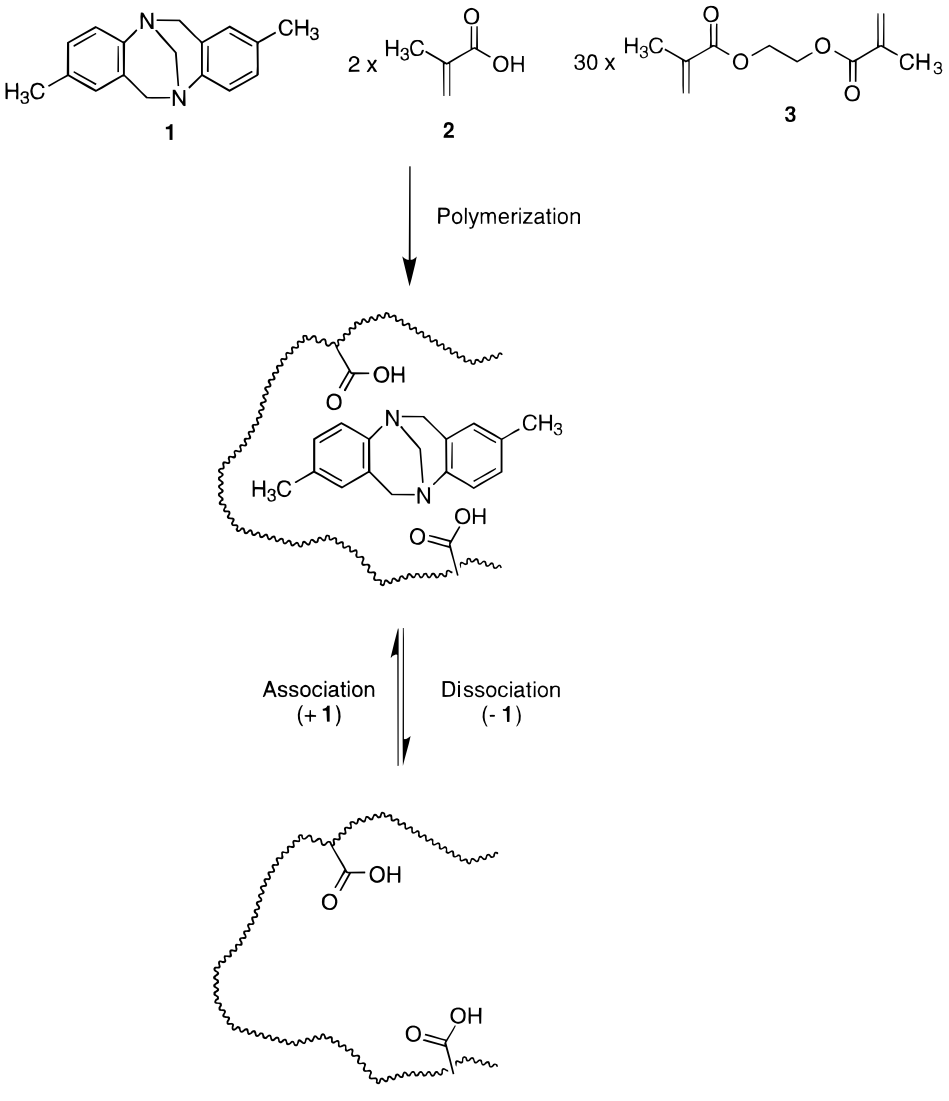


FIG. 1. A highly schematic representation of the molecular imprinting of **1**. The functional monomer methacrylic acid (**2**) is mixed with the template in the presence of a the cross-linking agent, ethylene glycol dimethacrylate (**3**). The complementarily interacting functionalities form predictable solution structures. Polymerization and removal of the template lead to the definition of a recognition site of steric and functional topography complementary to the template molecule.

17.37 mmol) in chloroform (5 ml) in a borosilicate glass reaction vial. The polymerization mixture was cooled on ice and sparged with dry nitrogen before the addition of azobisisobutyronitrile (70 mg). The reaction vial was then sealed and irradiated with 366-nm light for 21 h at 277 K. The bulk polymer product was ground in a mechanical mortar (Retsch, Germany), and the polymer particles were wet sieved (ethanol/water)

to yield a ≤25-μm fraction, which was sedimented from acetone (200 ml, 4 × 15 min) to remove fines. Polymer particles were then dried under vacuum after being washed with diethyl ether (20 ml). The nonimprinted reference polymer, **P(B)**, was prepared identically, except for the absence of template.

HPLC Evaluation of Polymer Recognition Characteristics

Polymer samples were packed in stainless steel HPLC columns (i.d. 4.6 mm, length 100 mm) with an air-driven fluid pump (Haskel Engineering, U.S.A.) at 340 bar. Initial chromatographic analyses were performed by isocratic elution with an optimized eluent system, acetonitrile/acetic acid (99.9%/0.1%). Due to the very long retention times obtained, shorter HPLC columns (i.d. 4.6 mm, length 50 mm) were used for the study of the influence of water on retention characteristics. A flow rate of 0.5 ml/min was employed for all systems studied and detection was carried out at 260 nm. Samples, 0.1 mg/ml, were injected in a volume of 20 μl. Capacity factors (k') were calculated from the retention volumes (V_R) and the void volume (V_0) using the equation $k' = (V_R - V_0)/V_0$. Acetone was used as the void marker. Separation factors (α) were calculated from the relationship $\alpha = k'_a/k'_b$, where k'_a is the capacity factor of the more retained enantiomer and k'_b that of the least retained. All chromatographic data reported represent the average of a minimum of five injections.

RESULTS AND DISCUSSION

Methacrylic acid (**2**) was selected as a suitable functional monomer on the basis of the salt bridge interactions it should form with the tertiary amines of the template (*21*,*22*). The formation of template-functional monomer-selective interactions in the polymerization mixture was verified using a UV-spectroscopic titration (*21*), with acetic acid being used as a functional monomer analogue. By monitoring spectral changes at 289 nm as a function of solution acetic acid concentration, a saturation isotherm plot was prepared (Fig. 2). These data were subjected to a Hill-type binding

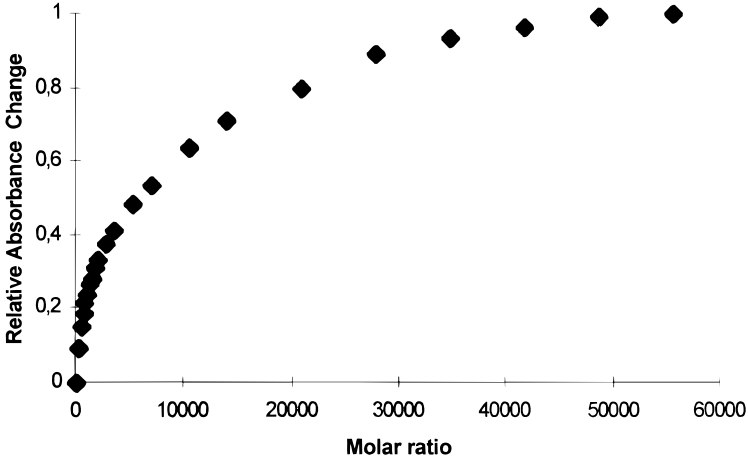


FIG. 2. Saturation isotherm obtained from the titration of **1** with acetic acid in chloroform at 293 ± 1 K.