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SELECTIVE BINDING TO POLYMERS *via* COVALENT BONDS. THE CONSTRUCTION OF CHIRAL CAVITIES AS SPECIFIC RECEPTOR SITES

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Abstract - Separations of substances on polymers by fast and reversible covalent interactions are discussed. For this type of chemoselective affinity chromatography suitable new binding groups were developed for diols, monoalcohols and amines. The selectivity of the separations can be improved by cooperative binding of substrates *via* two or more binding groups. Polymers with binding groups located in a definite spatial proximity and cooperativity in cavities of specific shape show high selectivity *e.g.* for the resolution of racemates. These cavities can be looked at as models for natural receptor sites.

INTRODUCTION

Selective binding of substances to polymeric sorbents can be based on different types of interactions. Most frequently non-covalent interactions are used for separations of substances e.g. in chromatography. In this case Van-der-Waals forces, hydrophobic interactions, hydrogen bonding, dipol-dipol-interactions, charge-transfer interactions and Coulomb interactions can play an important role (Ref. 3,4,5). Usually the selectivity in these separations is limited.

Extremely high selectivity is obtained in bioselective affinity chromatography (Ref. 6). These separations can be based e.g. on antigen — antibody reactions, enzyme — substrate (or inhibitor) binding, or hormone — hormone receptor binding. In practice one of the two components of these systems is immobilized by covalent coupling to a solid support. Subsequent separation is based on the ability of these ligands to bind selectively to the other component of this system. The type of interaction between ligand and substance is mostly of a rather complex nature, in which a highly cooperative combination of the above-mentioned noncovalent interactions being worked. In addition an exact steric fit of the two complementary compounds aids selectivity.

The kind of interaction in the so-called covalent affinity chromatography is more clearly defined. In this case the adsorbent of the column reacts chemically with the substance which is to be separated and is covalently bound to the polymer. After elution of all unbound components the compound is released from the polymer by splitting the covalent bond. This method has been used, for example, for the isolation of acetylcholinesterase by means of immobilized organophosphates forming phosphate ester bonds which can

afterwards be cleaved by potent nucleophiles (Ref. 7). Other examples are the thiol-disulfide interchange (Ref. 8), the formation of thiolester linkages (Ref. 9), the binding of tryptophan-containing peptides to sulfenyl-chloride-containing polymers (Ref. 10), and methionine-containing peptides to polymeric chloroacetamide (Ref. 11). It is also possible to use resins containing nucleophilic reactive ends in the purification of some plant extracts by selective removal of some undesirable components (Ref. 12) or to use resins containing semicarbazide moieties to trap aldehydic side products of reactions (Ref. 13).

In all these cases the separation is achieved by forming a covalent bond. This process is not reversible under the conditions used. It is necessary to cleave these covalent bonds in a second stage, in order to release the substances from the polymer. In contrast it was our aim to investigate reversible covalent interactions formed in a fast equilibrium and use these interactions for effective separation of substances.

SELECTIVE BINDING OF SUBSTANCES TO POLYMERS VIA ONE COVALENT BOND

1. Covalent binding giving stable compounds

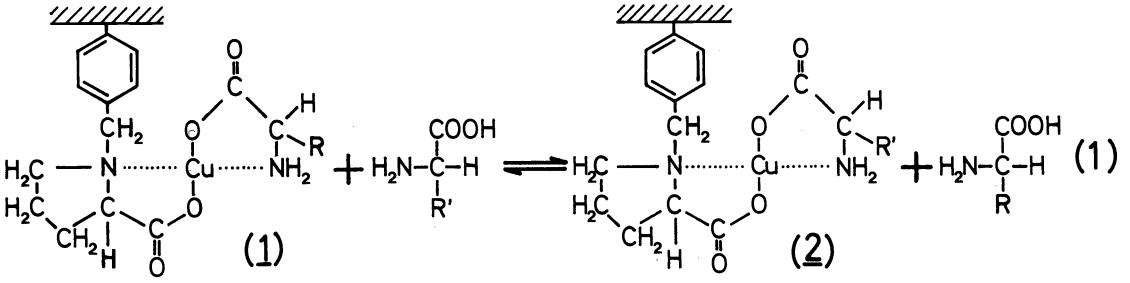
Covalent binding of substances to polymers usually leads to stable compounds. Apart from the above-mentioned covalent affinity chromatography, covalent binding to polymers is utilized in the case of polymeric reagents, anchoring agents, and polymeric protecting groups. Polymeric reagents (Ref. 14) usually contain an activated functional moiety covalently bound to the polymer that can be transferred to a low molecular weight reactant. Anchoring agents (Ref. 15) are used by covalently attaching a bifunctional compound to this agent and then performing a number of synthetic steps e.g. lengthening a peptide's chain. Eventually the final product is released from the polymer (Ref. 16). Closely related to this is the use of suitable polymers as protecting groups (Ref. 17,18). In this case after covalent attachment of the compound usually only one reaction step is performed.

2. Reversible binding to polymers in a fast equilibrium reaction

a. General remarks. Chromatographic separations of substances on polymers by fast and reversible covalent interactions may be regarded as particularly promising. With this method functional-group-specific separations of high selectivity are possible. Principally it should be possible to find a suitable binding group on polymers for each type of functional group. They should show selective and reversible binding only for substances containing this function. This type of chromatography therefore is chemoselective affinity chromatography.

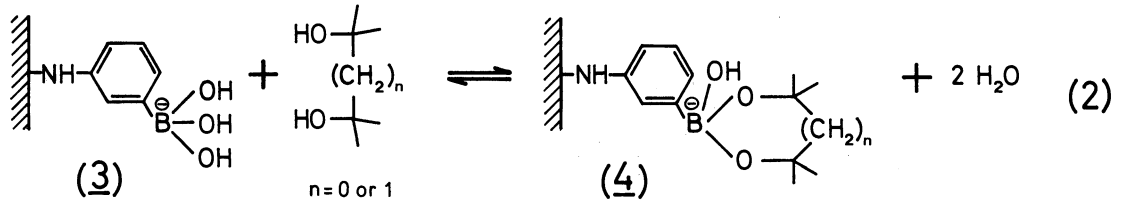
The main problem with covalent binding reactions is the usually high activation energy of this type of binding. Fast equilibria, necessary for chromatography, are difficult to obtain. There are only a few examples in literature of this type of separation. In some cases it was possible to increase the kinetics considerably by suitable neighbouring groups near to the binding site (Ref.2).

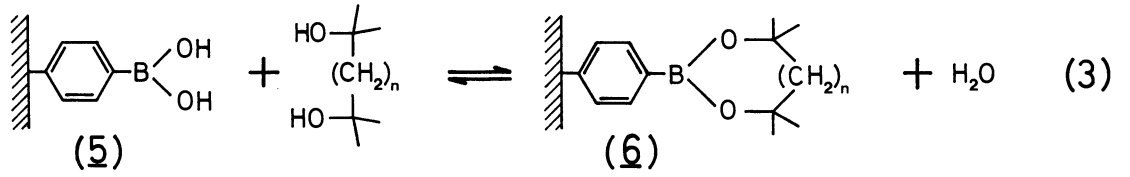
b. Literary examples of reversible binding. Ligand exchange chromatography may be regarded as reversible binding by covalent bonds. It has been used, for example, by Davankov and Rogozhin (Ref. 19) for racemic resolution of amino acids. An optically active amino acid is firmly attached to a polymer and the amino acid of the buffer system is bound via a copper chelate complex (1).



In the presence of a racemate of another amino acid, a fast ligand exchange occurs (Equ.(1)), whereby the complexes (2) of the two enantiomers have different stabilities thus allowing the separation of the racemate. Chelating polymers can also be used for selective and reversible binding of metal ions; this subject is discussed in other articles of this issue (Ref. 20,21,22).

Another important example is the use of boronate ligands attached to polymers for reversible binding of diols through the formation of cyclic boronic esters (Equ.(2)). This method was introduced by Gilham et. al. (Ref. 23).

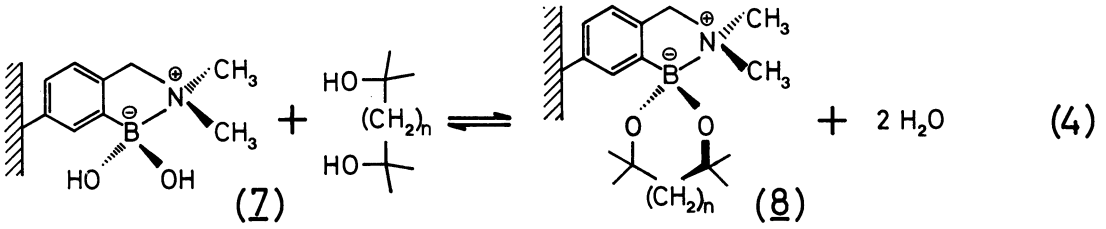




Basic esters (4) with tetrahedrally coordinated boron atoms are found in aqueous alkaline solutions (pH>8). At lower pH values only neutral esters can be formed (Equ.(3)), which hydrolyse in aqueous solutions. This type of binding takes place in organic solvents (Ref. 2,24). Chromatography on immobilized boronates in aqueous solutions today plays an important role in biochemistry (Ref.25,26). These resins are already commercially available from several companies.

c. New binding groups containing boronic acids. There are some disadvantages in the usual chromatography of diols at immobilized boronate ligands. The

chromatography in aqueous solutions has to be performed in alkaline medium affecting degradation of labile substances. In organic solvents esters of type (6) are formed without addition of bases, but the kinetics of the binding reaction (Equ.(3))are several orders of magnitude slower than in (Equ.(2))(Ref.2). Furthermore many substances which are to be chromatographed are only soluble in water.



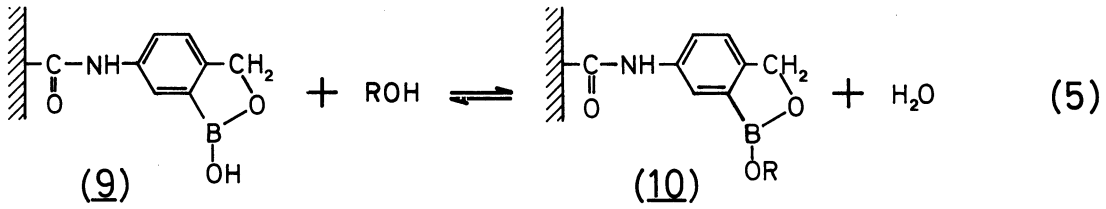
(4)

An attempt was therefore made to modify the binding sites to overcome these disadvantages. In this context it was found that polymers containing the 2-dimethylaminomethylbenzeneboronic acid moiety have favourable properties (Equ.(4)) (Ref.2,27). These polymers could be obtained by copolymerization of 5-vinyl-2-dimethylaminomethylbenzeneboronic acid -- a newly synthesized monomer (Ref.27) -- with suitable comonomers. In this polymer the attainment of equilibrium of the binding reaction was several orders of magnitude faster than in (Equ.(3)) (as measured with low-molecular-weight analogs (Ref.2)). Therefore it is possible to perform a fast chromatography in organic solvents with this polymer.

Furthermore the pK-values of the underlying 2-(dimethylaminomethyl)benzeneboronic acid show pK_1=5.2 and pK_2=11.8. A boronic acid with a pK=5.2 represents the most acidic boronic acid used for this purpose. Until now the most acidic boronic acid used in polymeric form was prepared by Yurkevich et.al. (Ref.28) and contains a 4-(trialkylammoniummethyl)benzene boronic acid. Boronic acids of this type show a pK_1=7.04.

2-(Dimethylaminomethyl)benzeneboronic acids (as well as (7) and (8)) exist in a form with a closed ring originating from a B-N-bond (Ref.29), which is frequently closed and formed. In principle esters of type (8) can therefore be classified as tetrahedral boronic esters.

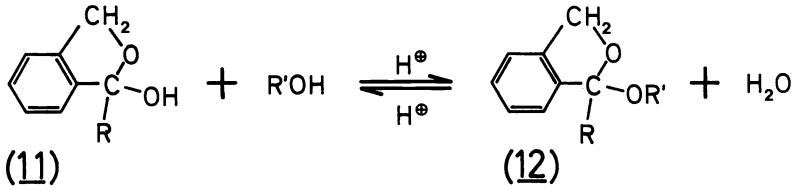
d. Binding groups for monoalcohols. A new binding group for monoalcohols was found in the boronphthalide system. Polymers with these groups were prepared



(5)

by copolymerization of esters of the newly synthesized 1,3-dihydro-1-hydroxy-6-methacryloylamino-(2,1)-benzoxaborol (Ref.30). The boronophthalide system binds fast and reversibly monoalcohols (Ref.2,30). In this case without a neighbouring group participation, a quick esterification as well as a hydrolysis took place. This binding is now studied with the polymer (9) (Equ. (5)).

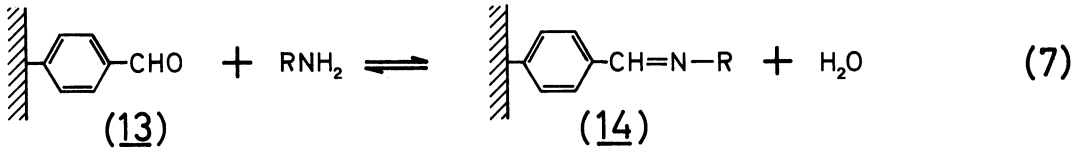
Another possibility for binding monoalcohols would be the formation of acetals with polymeric aldehydes. These investigations were first performed with low molecular weight model substances. It was found, that the formation and the hydrolysis of acetals is only fast enough under proton catalysis. With simple aldehydes, such as benzaldehyde or salicylaldehyde, only a small amount of acetal is formed under equilibrium conditions. Substances with the 1,3-dihydro-1-hydroxy-isobenzofurane moiety proved to be more favourable (Ref.31). This type of substance has been investigated before for other purposes (Ref. 32,33). The attainment of equilibrium according to (Equ.(6)) was very quick. With equimolar amounts of starting components ((11) and R'OH, or (12) and H_2O) 77% of (12) was obtained under equilibrium conditions (in $CDCl_3$/DMSO-d_6 10:1, R=H, R'=CH_3). Therefore polymers containing 11 should show favourable binding behaviour for monoalcohols.



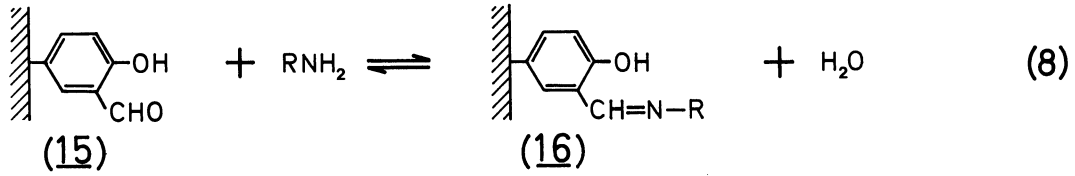
(11) (12) (6)

e. Binding groups for amines. Aldehydes or ketons may also be suitable binding groups for primary amines. In this case formation of the azomethine bond would be the binding reaction. Investigations with polymeric benzaldehyde (13) or salicylaldehyde (15) as well as investigations with low molecular weight model substances show (Ref.34), that the positions of the equilibrium of (Equ.(7)) and (8) are favourable but that the kinetics are too slow even with proton catalysis.

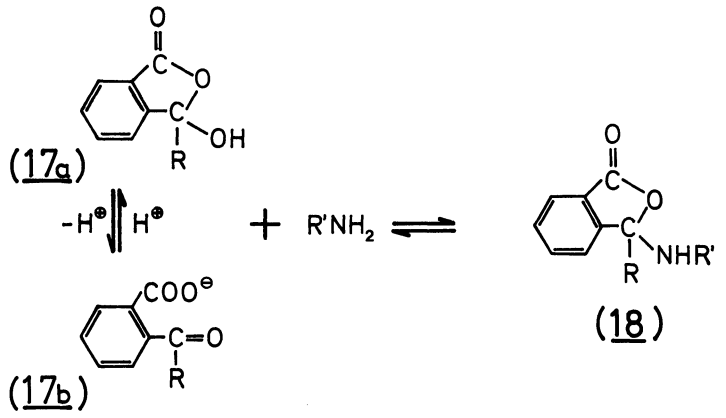
In order to accelerate the kinetics of both azomethine formation and hydrolysis a number of aldehydes and ketones with suitable neighbouring groups have been investigated (Ref.31). Promising properties in this respect showed derivatives of the 3-hydroxy-phthalide (17) whose reactivity towards alcohols,



(13) (14) (7)



(15) (16) (8)

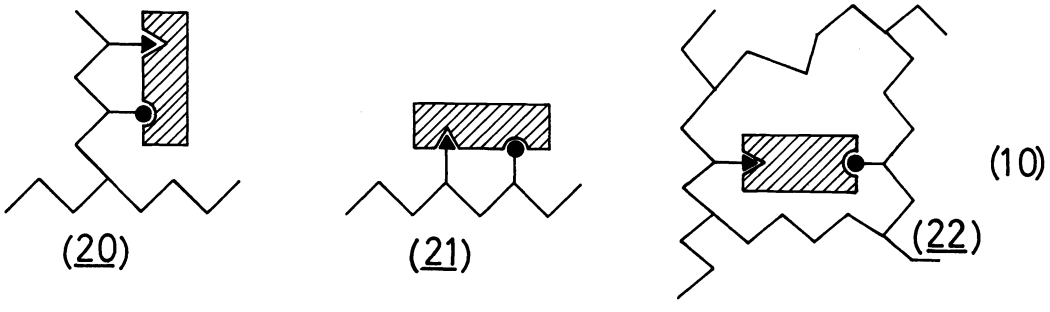


(9)

thiols, amines and amides has been investigated before (Ref.35). (17) mainly exists in the cyclic form (17a), but arranges itself to the open chain compound (17b) in presence of bases. The equilibrium (Equ.(9)) can be reached very quickly from both sides. In CD_3CN/H_2O 10:1 an equilibrium with 48% (18) (R=H; R'=Cyclohexyl) is formed with equimolar concentration of starting compounds (Ref.31). This type of substance therefore is a good candidate as a binding group for primary amines.

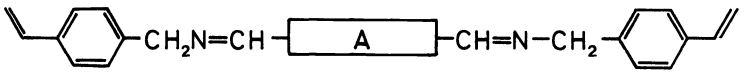
SELECTIVE BINDING VIA TWO BINDING GROUPS

Increased binding selectivity of polymers can be obtained by cooperative binding of substrates via two or more binding groups. In the case of two different binding groups such arrangements can be obtained principally in three different ways (see (10)). One possibility is to graft side chains with neighbouring binding groups onto a polymer (20), another to polymerize monomers already containing two binding groups within a definite distance from each other (21) (so-called continuate word arrangement (Ref.36)). Another procedure yields binding groups located at different points of the polymer chain (22), and fixed in a definite proximity by crosslinking (Ref. 37,38). In biochemistry this type of arrangement has been called discontinuate word by Schwyzer (Ref.36). With synthetic polymers this can be achieved by a special type of template polymerization, which will be discussed later in greater detail.



(10)

Thus in analogy to (22) two amino groups can be introduced respectively into a polymer at a defined distance by polymerizing bis(azomethines) (23) (Ref.39). It is also possible to obtain crosslinked polymers with two mercapto groups at a definite distance (Ref.40,41).



(23) A=Spacer

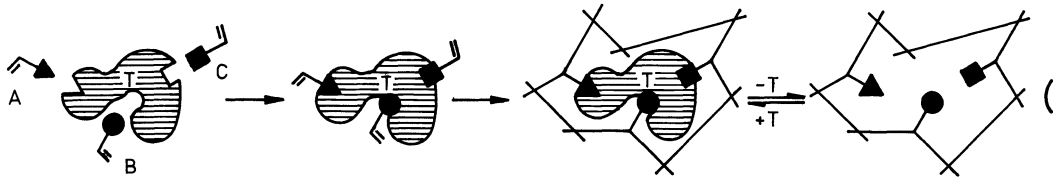
SELECTIVE BINDING INCLUDING STERIC EFFECTS

1. Examples of steric effects

Apart from the cooperativity of several binding groups the selectivity can be increased by additional steric effects. In particular separations using polymers containing cavities or pores of a distinct size or shape can lead to higher selectivity. In some cases separations result nearly exclusively from steric effects as in the case of size-exclusion gel filtration chromatography (Ref.42), osmosis and reverse osmosis (Ref.43), or separations by means of inclusion in crystal lattices (e.g. clathrates) (Ref.44). The steric fit also plays an important role in the selective embedding in cavities of cyclodextrins (Ref.45) or crown ethers and related compounds (Ref.46).

2. Binding in chiral cavities prepared by template polymerization

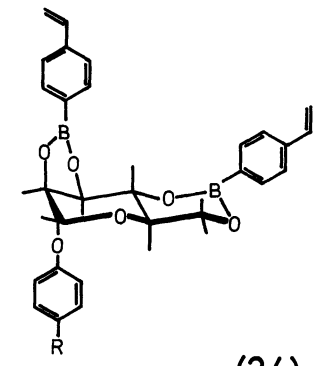
Polymers with binding groups located in a definite spatial proximity and cooperativity in cavities of specific shape should show high selectivity in binding. This arrangement is similar to those of natural receptor sites. To prepare such binding sites we used a new approach (Ref.37,38,47,48,49). The binding groups, which were to be introduced, were bound in the form of polymerizable vinyl derivatives to suitable template molecules (Equ.(11)).



(11)

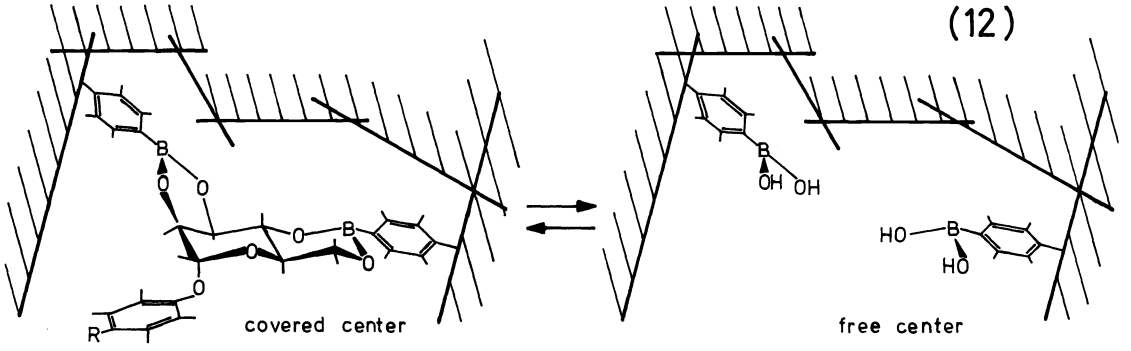
This monomer was then copolymerized under such conditions that highly crosslinked polymers with chains in a fixed arrangement were formed. After removal of the template, free cavities resulted with a shape and an arrangement of functional groups which correspond to those of the template. The functional groups on this polymer are located at quite different points of the polymer chain, they are held in spatial relationship by the crosslinking of the polymer.

For the optimization of this procedure we have chosen the monomer (24) (Ref.50,51,52). The template is a phenyl-α-D-mannopyranoside to which two 4-vinylbenzeneboronic acids are bound by two diester linkages. The boronic



R=H or NO_2 (24)

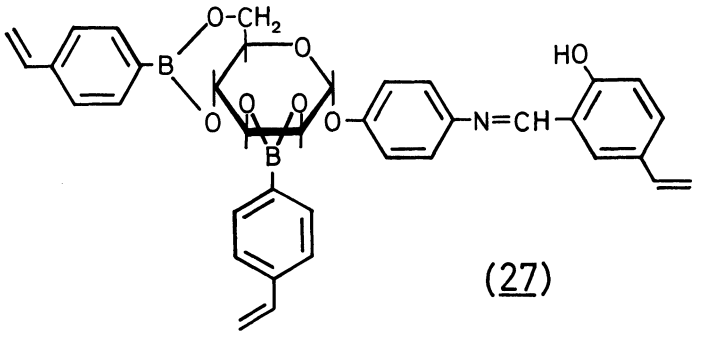
acid groups act as the binding groups. Since a chiral template was chosen, the accuracy of the steric arrangement in the cavity could be tested by the ability of the polymer to resolve the racemate of the template after cleavage of the original template. The monomer then was copolymerized with radical initiation in the presence of an inert solvent with a high amount of a bifunctional crosslinking agent. Under these conditions macroporous polymers were obtained which possess a permanent pore structure and a high inner surface area. Consequently a good



accessibility and a low swelling ability and therefore a limited mobility of the polymer chains can be expected.

From this type of polymer the templates could be split off at a degree of 40-80% with water or alcohol (see (12)). If this polymer is treated with the racemate of the template in a batch procedure under equilibrium conditions, preferably the enantiomer is taken which has been used for the preparation of the polymer. If the specificity is expressed by the separation factor α, which is the ratio of the distribution coefficients between solution and polymer of L and D-form, values in this and similar cases ranging from 1,20 to 3,64 were obtained depending on the equilibration condition and the polymer structure (Ref.50,52). The highest α-value obtained so far was 3,64. In this case in the simple batch procedure an enrichment of the L-form in the filtrate of 12,8% and of the D-form at the polymer of 40,4% was observed (Ref.52). Polymers of somewhat less specifity have been used as chromatographic sorbents for the chromatographic resolution of racemates (Ref.24). 80% of the first eluting peak could be obtained in optically pure form in a separation of the template racemate.

Other monomers with different templates have also been used in the same procedure such as (27) (Ref.53), or (28) (Ref.54), or (29) (Ref.34)



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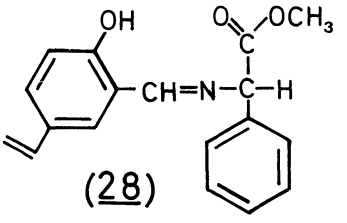

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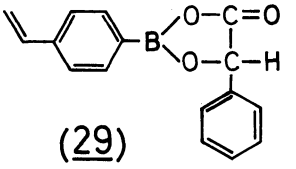

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