Macrocycles for Sensors

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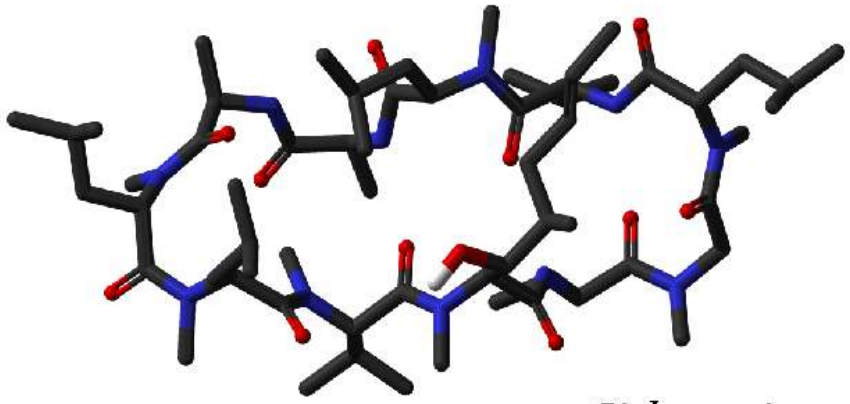
A molecule with a ring containing roughly 10 or more atoms is called a macrocycle.

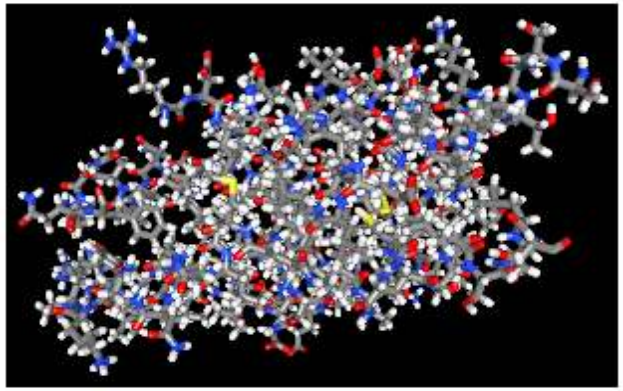
Compared to an open chain, the ring closure *restricts conformational freedom:* the macrocycle is preorganized, giving stronger and more specific association to molecules that are geometrically and electrically complementary to the macrocycle.



Ciclosporin

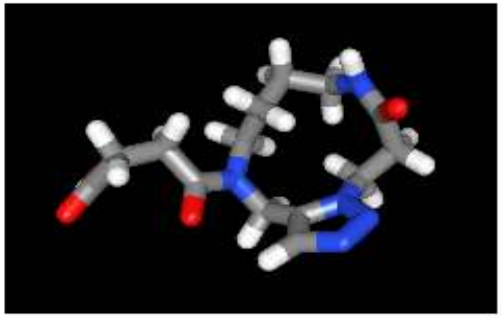
This property makes macrocycles useful as simple receptor molecules in sensors and for other applications.

This association between a macrocycle and a target molecule is non-covalent. It can be compared to the specific association between natural proteins (especially antibodies) and other molecules:



Protein:

+ Very good semi-rigid surface for association, giving stong and specific association
– Sensitive to harsh conditions
– Hard to engineer without destroying the folding
– Cost can be high



Synthetic macrocycle:

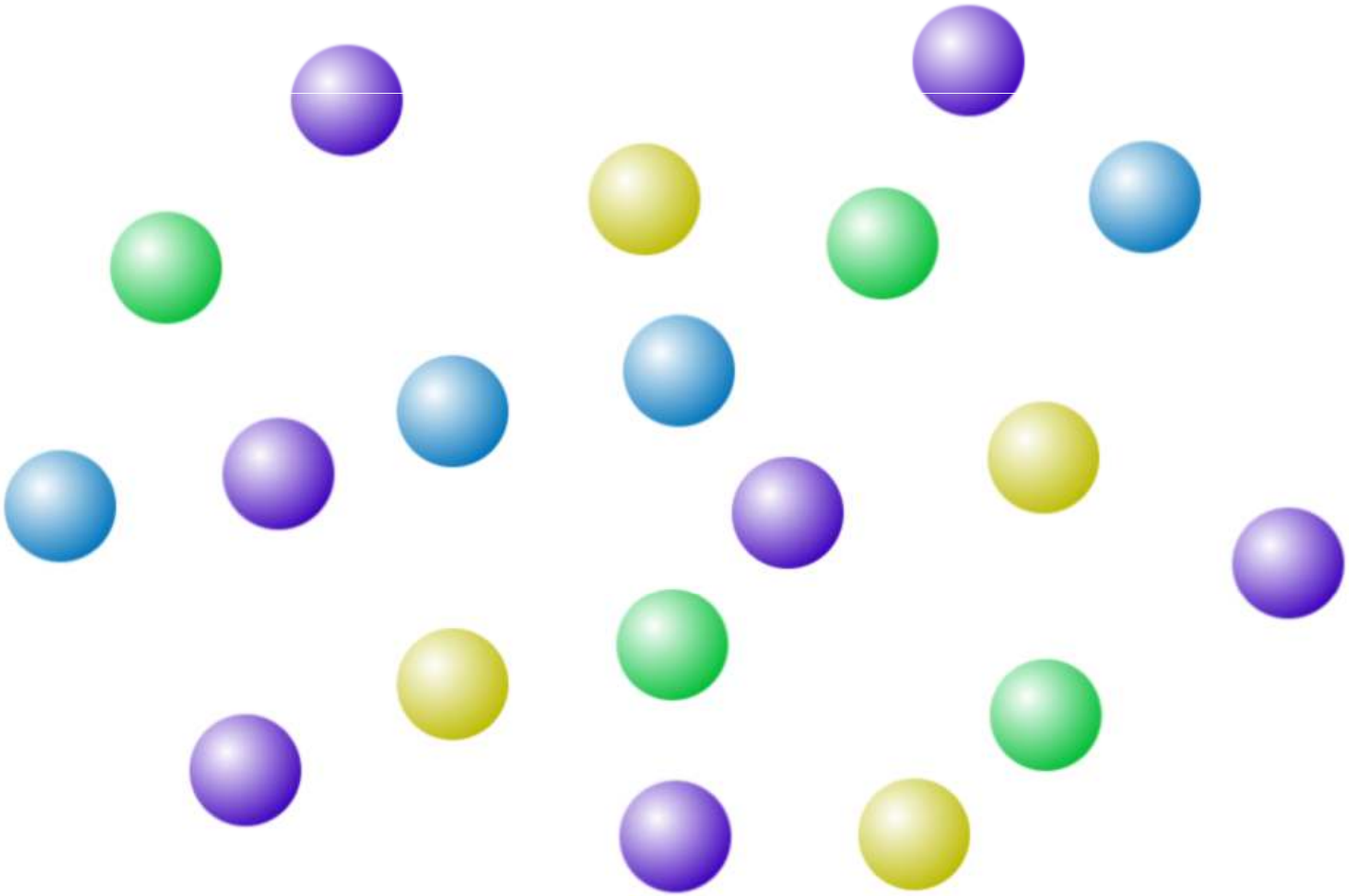
+ Easy to engineer and produce in bulk quantities
+ Chemically and thermally stable
– Not as strong and specific association as proteins

Different methods can be used to find a macrocycle that is capable of associating with a given target molecule:

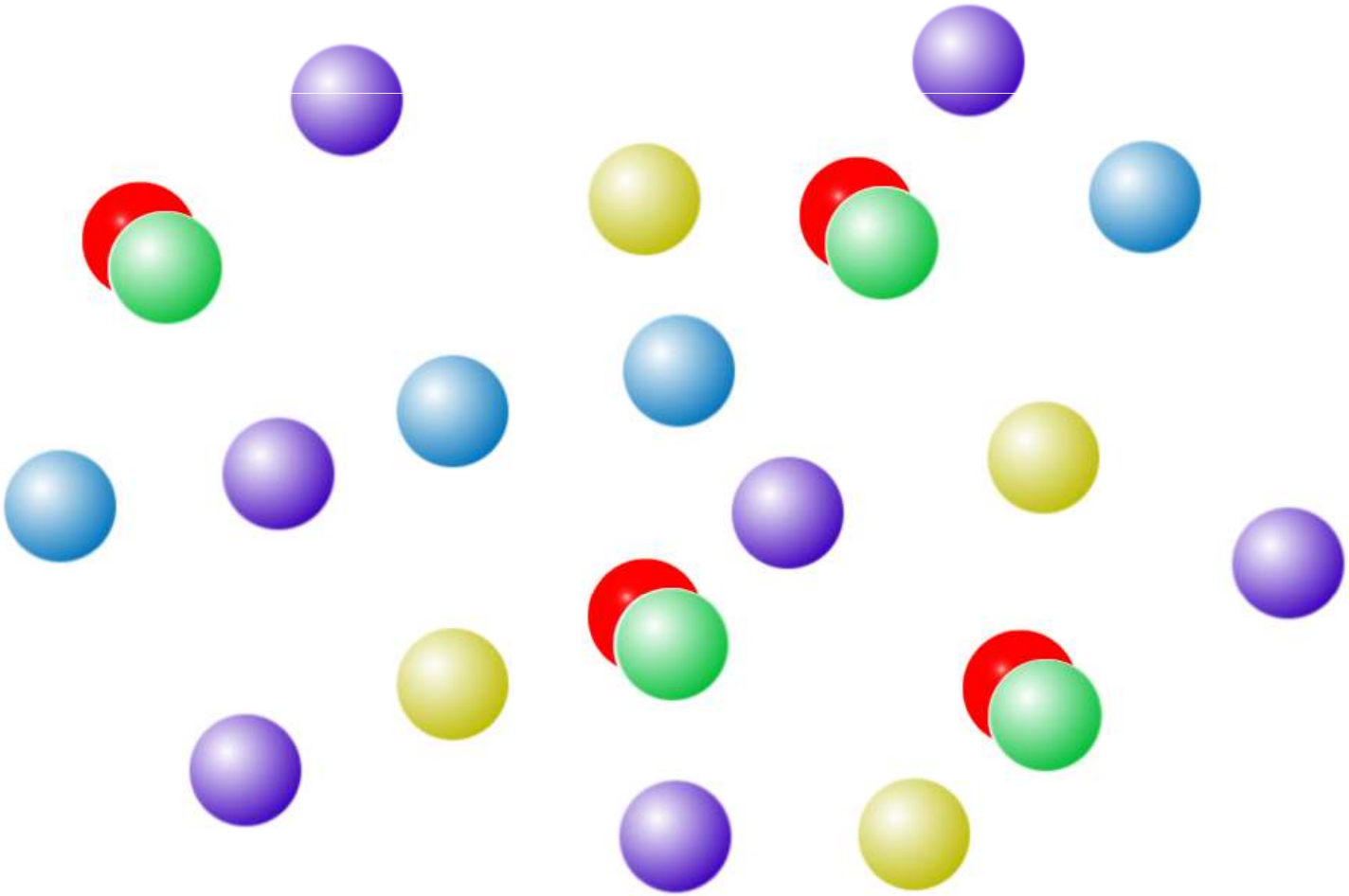
- Build a library of macrocycles, immobilized on polymeric beads: one macrocycle type per bead.
- Immobilize the target molecule onto a chromatographic column, and use it for affinity chromatography with a mixture of dissolved macrocycles.
- Use existing molecular docking software to find a suitable macrocycle from a virtual library of macrocycles. Docking software is used extensively in the drug industry and is readily available.

These methods will be briefly described.

Macrocycle library on beads:

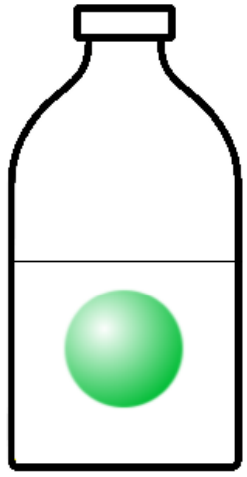


Macrocycle library on beads: immerse the beads in a solution of the target molecule (red)

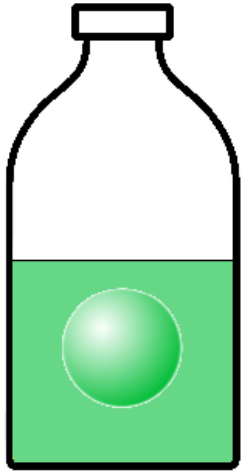


Macrocycle library on beads: Pick a bead with bound target molecules (chemical, enzymatic or instrumental methods can be used for this)

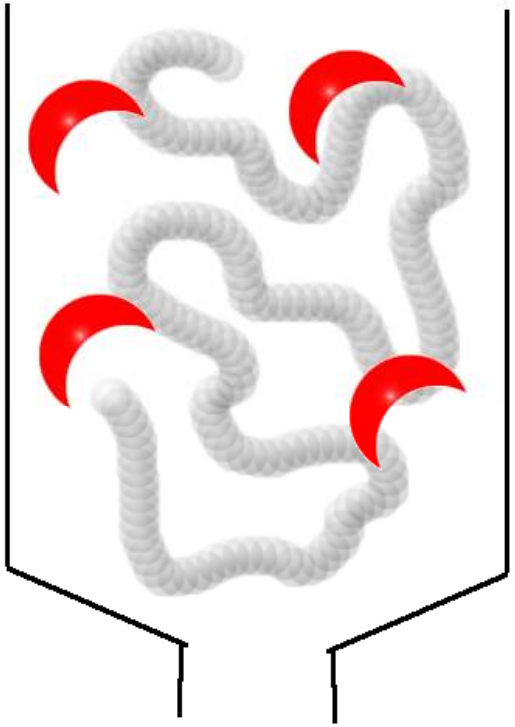
Macrocycle library on beads: Remove the target molecules and put the bead into a vial



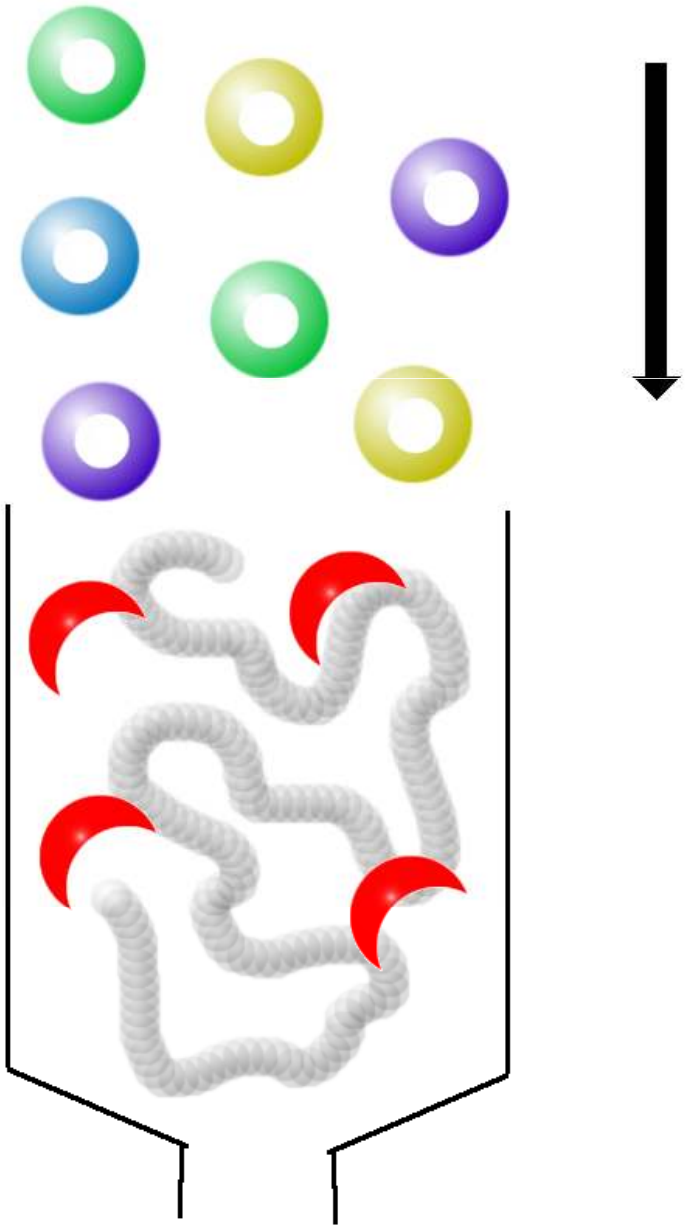
Macrocycle library on beads: Cleave the macrocycles from the bead and identify the macrocycle.



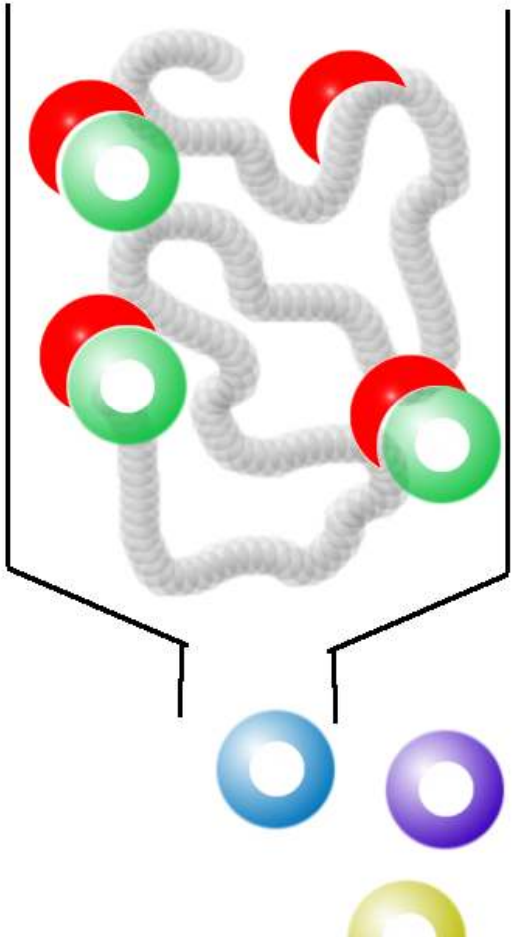
Affinity chromatography with immobilized target:



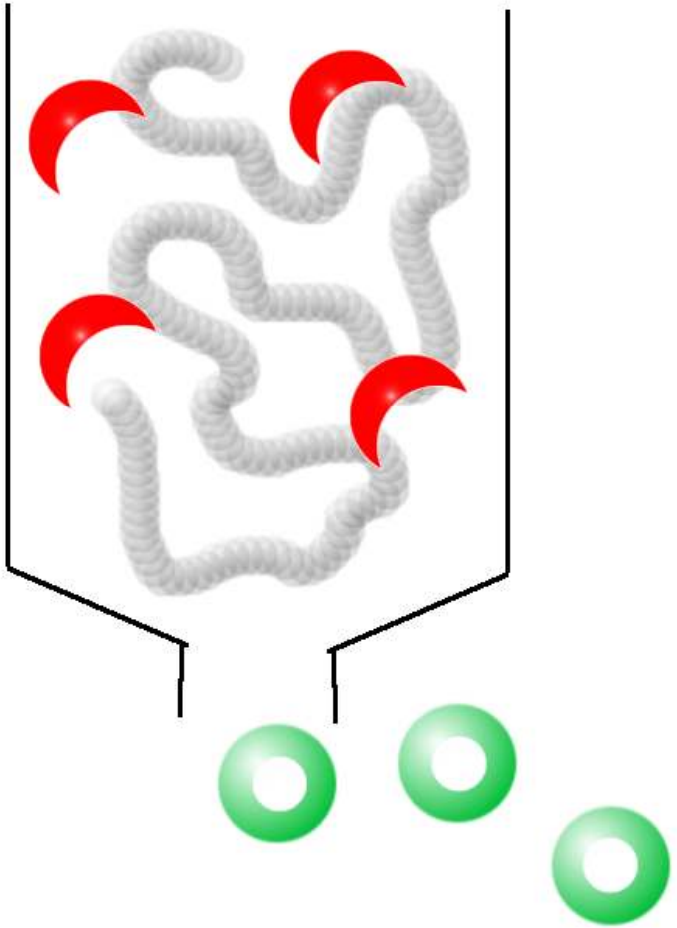
Affinity chromatography with immobilized target: add mixture of dissolved macrocycles



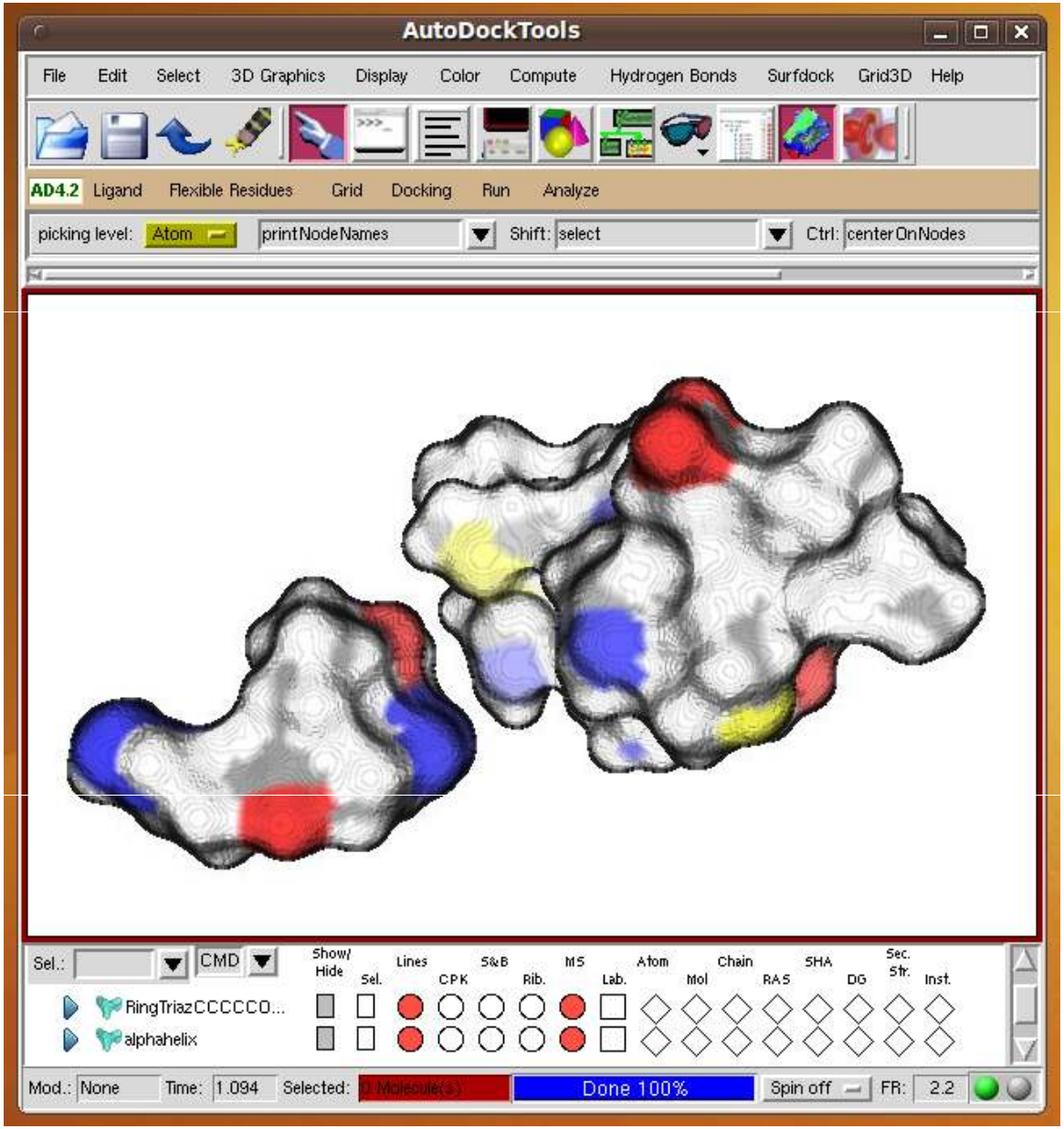
Affinity chromatography with immobilized target: elute non-bound macrocycles



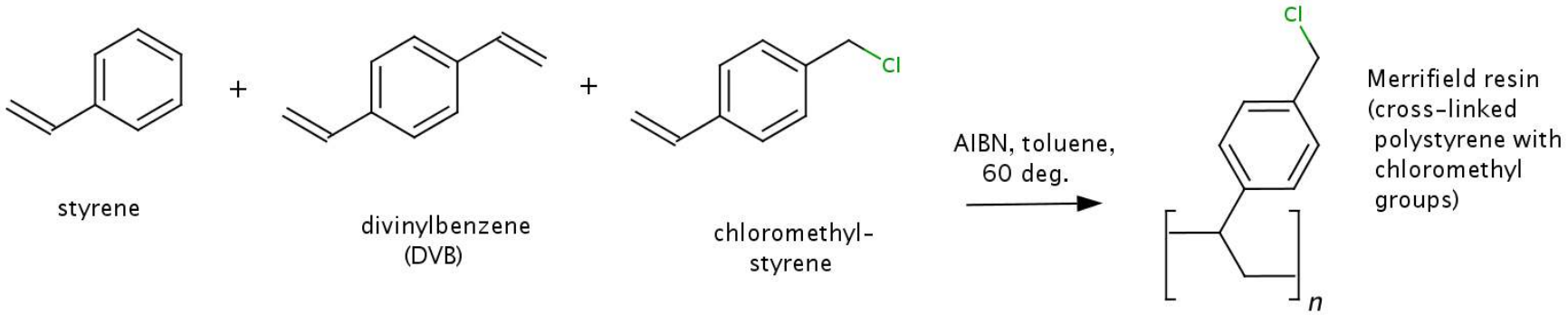
Affinity chromatography with immobilized target: elute bound macrocycles, identify macrocycles (LC-MS).



Use molecular docking software to find a suitable macrocycle from a virtual library. This is a screen-shot from AutoDock, a program freely available from the Scripps Research Institute (GNU license).

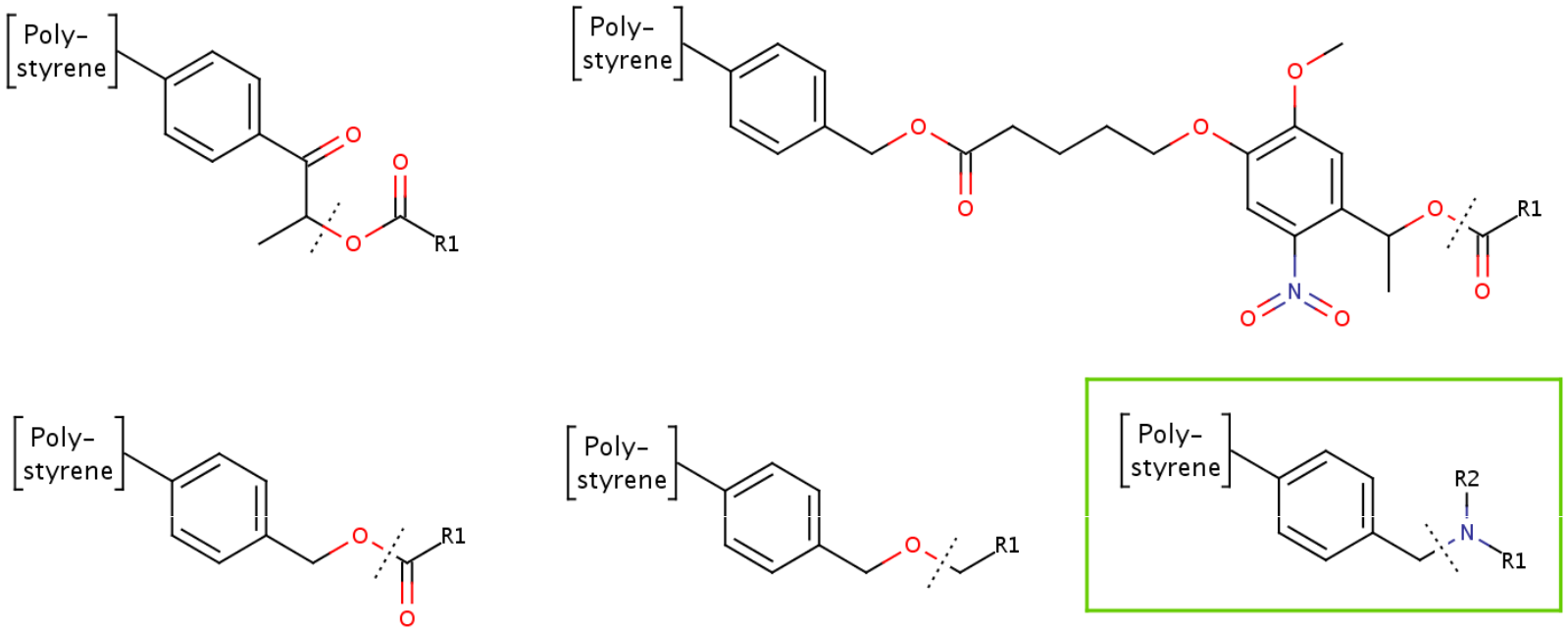


The macrocycles are synthesized on Merrifield solid support, in bead-form (commercial) or powder (synthesized in-house):



It is also anticipated that the macrocycles can be synthesized on any surface with immobilized halomethyl groups, or macrocycles in solution can be coupled to immobilized halomethyl groups.

A linker, connecting the macrocycle to the polymeric solid support is also needed. The linker should be chemically stable, but still cleavable under certain conditions. Several linkers were synthesized and tested:

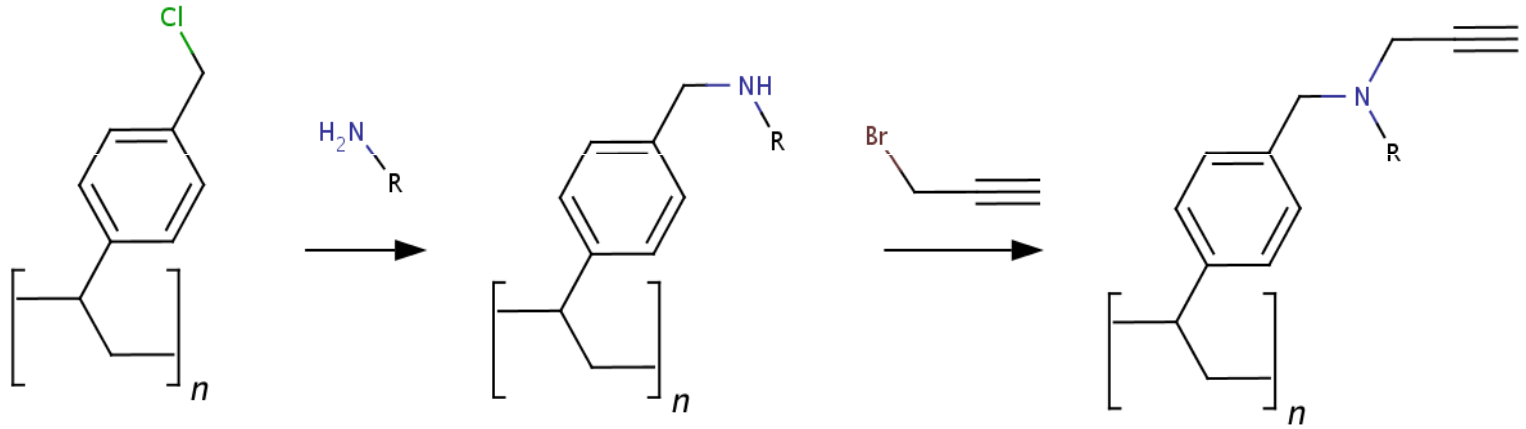


The benzylamine linker (in green frame) was chosen because of clean cleavage product and chemical stability.

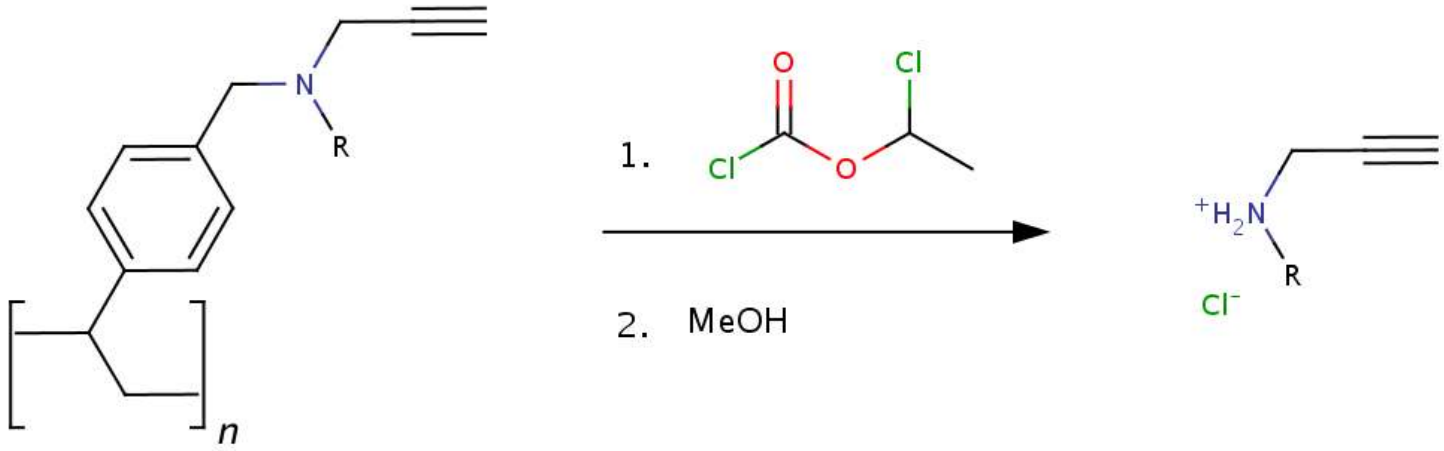
Tetrahedron Letters, **1997**, *38, No. 16*, 2915-2918

Because it must be possible to close the macrocycle while it is immobilized on the polymer, the first monomer must have a group for this purpose, with ring closure chemistry that is orthogonal to the monomer coupling chemistry. Presently, Cu-catalyzed Huisgen 1,3-dipolar cycloaddition between a propargyl group and an azide is used, but also other chemistries could be used, such as Sonogashira coupling.

The first monomer is built directly on the polymer support in the following way:

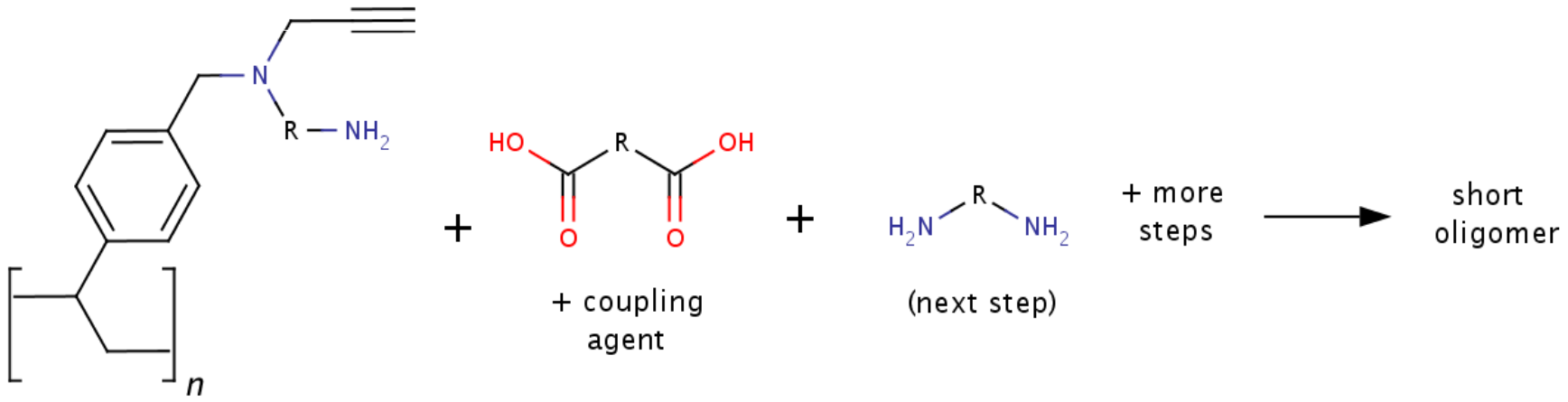


The linker is cleaved using 1-chloroethyl chloroformate (“Ace-Cl”):



The product is a secondary amine hydrochloride.

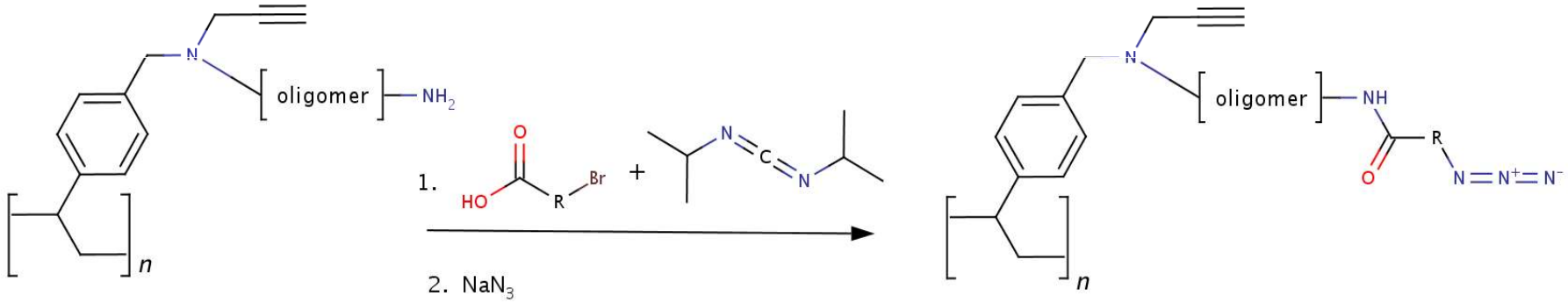
Protecting groups will incur extra cost. Because of this, diamins and diacids are used, when possible, instead of amino acids:



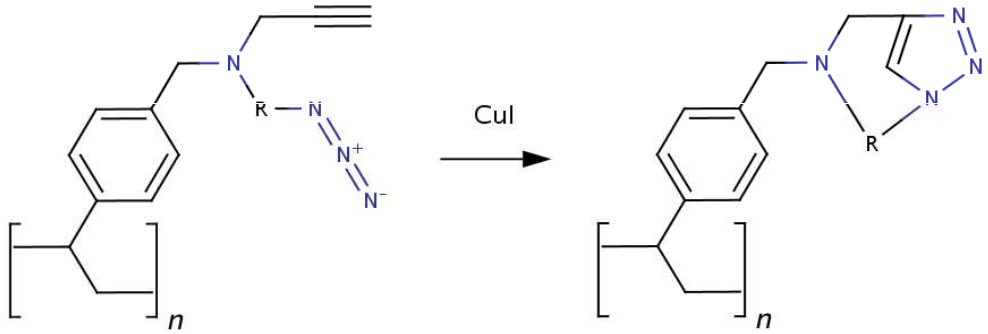
Such symmetrical monomers are also easier to synthesize.

Currently, amide compling chemistry is used, with a range of carboxylic acid activating agents such as $SOCl_2$ and HBTU, but later the coupling chemistry will be expanded to also include (non-benzylic) tertiary amines and ethers. Because the coupling takes place in basic conditions, side-chains will be protected by acid-labile groups.

To complete the cycle, the final monomer must contain an azide group. Because organic azides can be explosive, the final monomer is synthesized on the solid support, eliminating the need to handle free organic azide:



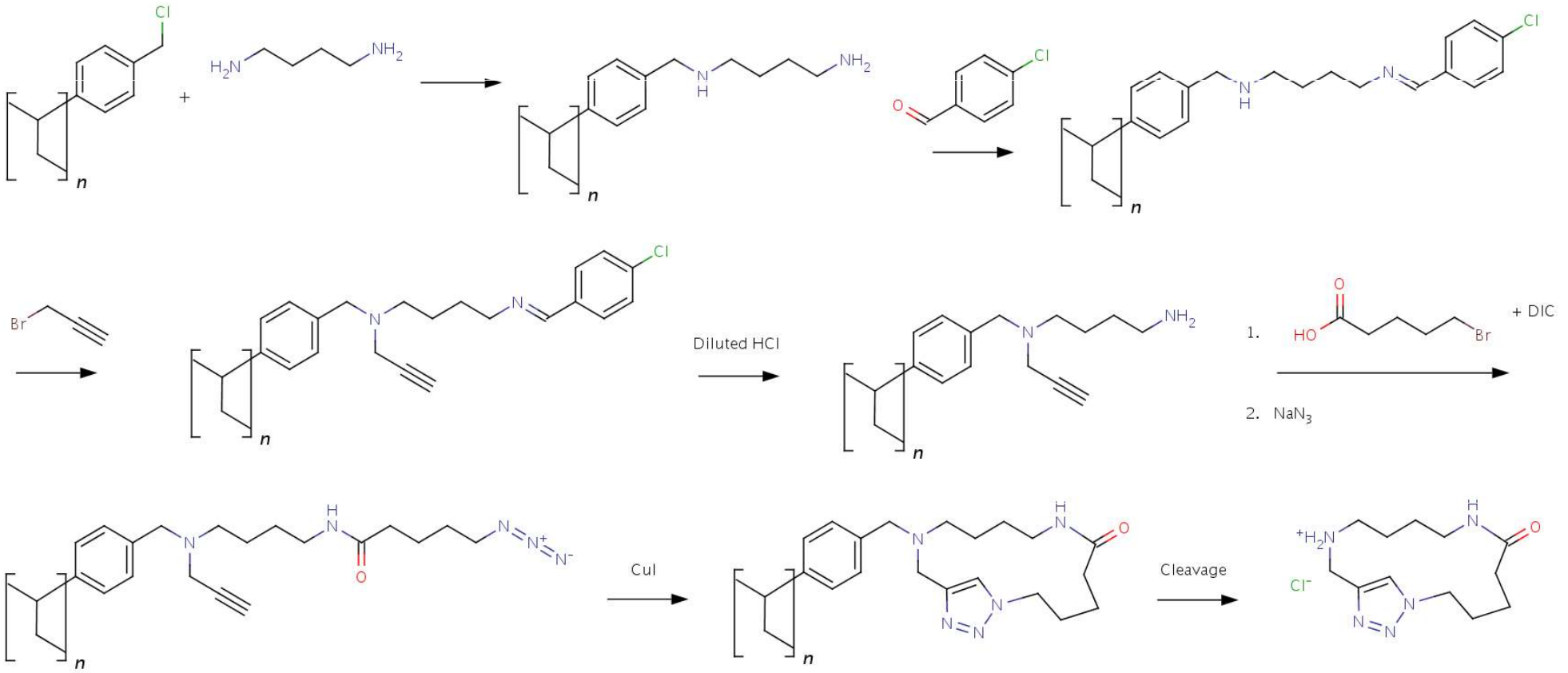
Finally, the ring is closed by a Cu-catalyzed Huisgen cycloaddition:



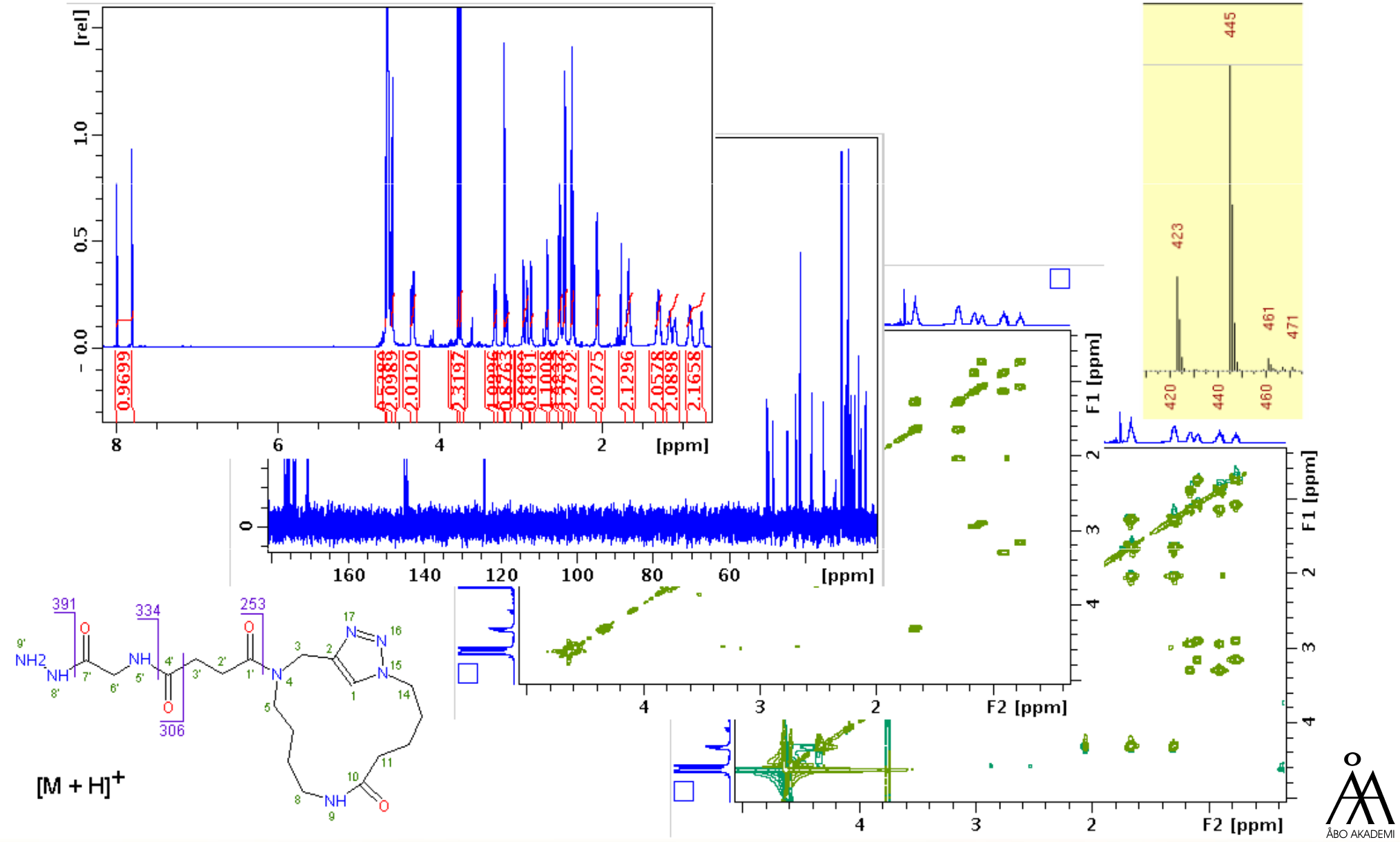
The five-membered triazole ring that is the result is aromatic and quite stable.

J. Org. Chem. **2002**, *67*, 3057-3064

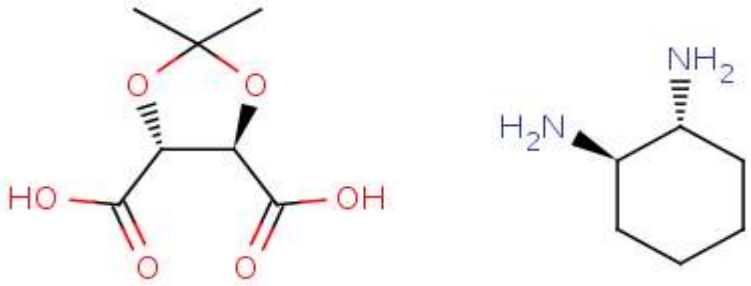
This is the complete synthesis path:



The macrocycle has been characterized by NMR and mass spectrometry.

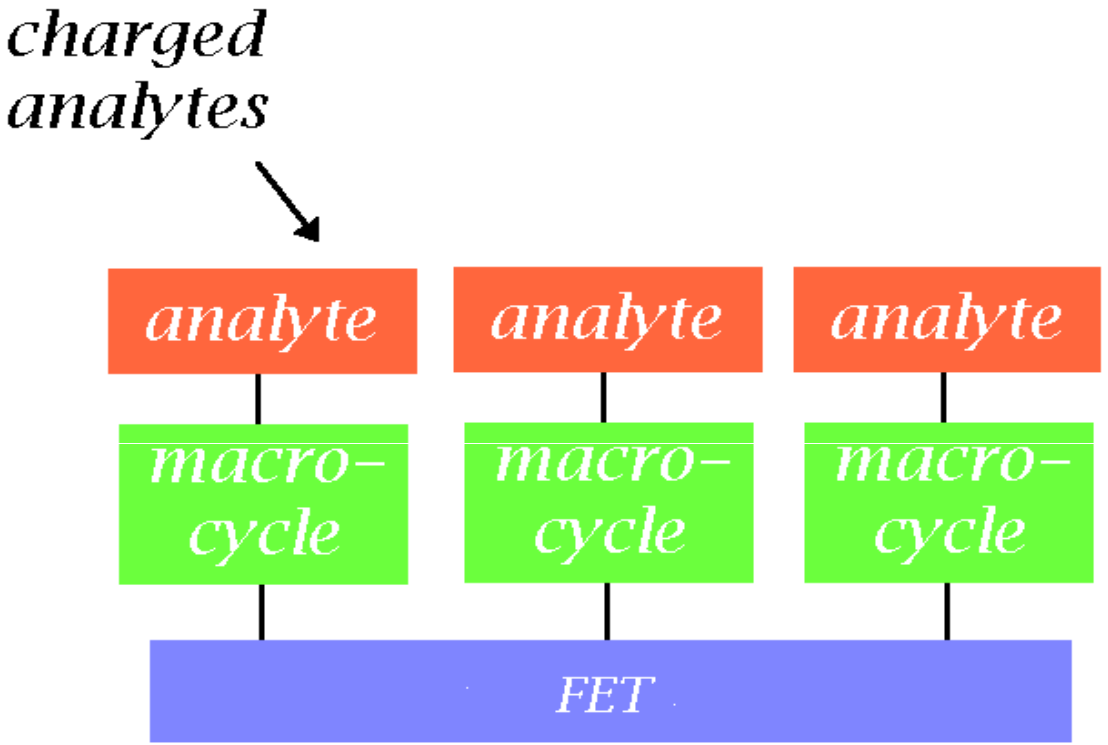


Chiral macrocycles are needed to be able to differentiate between analyte enantiomers. It is anticipated that a chiral monomer will induce chirality into the conformation of the macrocycle. Suitable monomers can be synthesized from tartaric acid, 1,2-diaminocyclohexane and cheap amino acids.



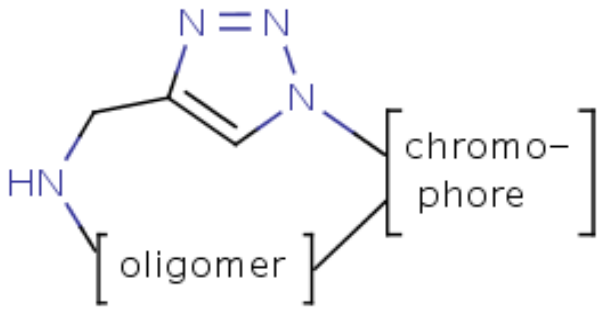
chiral monomers

Applications: there are a number of devices built around a field effect transistor, with single stranded DNA (”GenFETs”) or antibodies immobilized on the gate. They give a signal when carged analytes are captured onto the gate. Instead of DNA or antibodies, it might be possible to use macrocycles immobilized onto the gate:



Biosensors and Bioelectronics, **2007**, *22*, 3080-3085

By building a chromophore into the macrocycle, a directly visible signal indicating bound analyte might be possible:



Finally it can be noted that other applications are also possible:

- As preprocessing filters for sensors, to remove contaminants
- To concentrate samples for sensors
- For purification of synthesis mixtures, natural products or proteins
- Removal of toxins