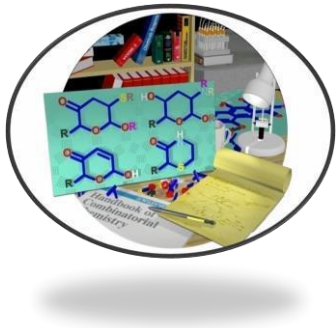
Lec8: Combinatorial Chemistry:

Drug design approach and HTS



Drug design approach:

1. Classical approach: make a change on an existing compound or synthesize a new structure and see what happens.

- Most drug compounds were synthesized in milligram quantities in a serial one-at-a-time fashion. After synthesis, the compound was sent to a biologist, who tested it in several in vitro assays and returned the results to the chemist.

Drug design approach:

1. Classical approach: Based on the assay results, the chemist would apply some structure activity relationship (SAR) or use chemical intuition to decide what changes to make in future versions of the molecule to improve activity.

Drug design approach:

1. Classical approach: Using this iterative process, a chemist would be able to synthesize only a handful of structures per week.

Since the yield of marketable drugs from compounds synthesized and tested is only about 1 in 10,000, the road to success has been a long and expensive one, taking 6 to 12 years and costing $500 to $800 million per drug.

2. Combinatorial chemistry approach:

In the mid- 1980s, classical approach to drug synthesis changed dramatically with the introduction of combinatorial chemistry.

The drug discovery process became a highly parallel one, in which hundreds or even thousands of structures could be synthesized at one time.

2. Combinatorial chemistry approach:

Interestingly, biologists had, for some time, been using high-throughput screening (HTS) to perform their in vitro assays, running assays in 96-well microtiter plates and even using laboratory robotics for pipetting and analysis.

2. Combinatorial chemistry approach:

- The term combinatorial chemistry was coined to refer to the parallel generation of all possible combinations of substituents or components in a synthetic experiment.

2. Combinatorial chemistry approach:

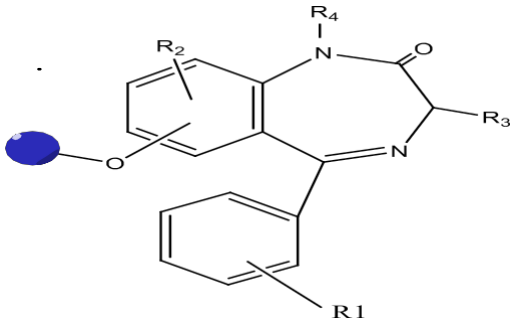
- Whereas the yield from a serial synthesis is a single compound, the yield from a combinatorial synthesis is a chemical library.

- Figure 1 shows two common types of chemical libraries:

1. **Generic library**, based on a single parent or scaffold structure and multiple substituents or residues.
2. **Mixture library**, containing a variety of structure types.

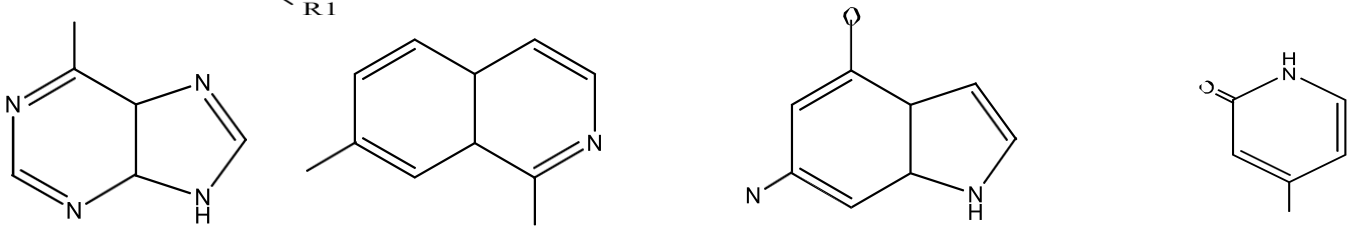
- The total number of structures in a library is either the product of the various numbers of substituents (for a generic library) or the total number of structures in a mixture.

A



RI= H , 4' -OH
R2= 5-Cl,4-COOH+ 5Cl
R3= CH3,CH(CH3)2, CH2Ph(4-OH),CH2Ph
R4= H, CH3, CH2CH3,CH2CH=CH2,CH2Ph

B



The size of the generic library is the product of the various numbers of **substituents** (here, 2x2x4x5 or 80) .
The size of the mixture library is simply the total number of **structures**.

Fig.1

This "rational" approach to drug design assumes that there is some understanding of the target receptor and that there is a lead molecule, commonly called the prototype molecule.

A classic example is the dihydrofolate reductase inhibitor methotrexate, which has been one of the prototypes that laboratories have used to synthesize and test new inhibitors.

The medicinal chemist can select subsets of substituents that vary in lipophilicity, steric bulk, induction, and resonance effects.

The goal of combinatorial chemistry is to be able to synthesize, purify, chemically analyze, and biologically test all the structures in the library, using as ***few*** synthetic experiments as possible.

3. Computer aided drug design:

Drug design increasingly is based on modern computational chemical techniques; it also uses sophisticated knowledge of disease mechanisms and receptor properties.

Combinatorial chemistry was first applied to the synthesis of peptides, since a convenient method for the automated synthesis of these compounds was already in wide spread use. In 1963, Merrifield introduced the efficient synthesis of peptides on a solid support or resin as shown in figure 2:

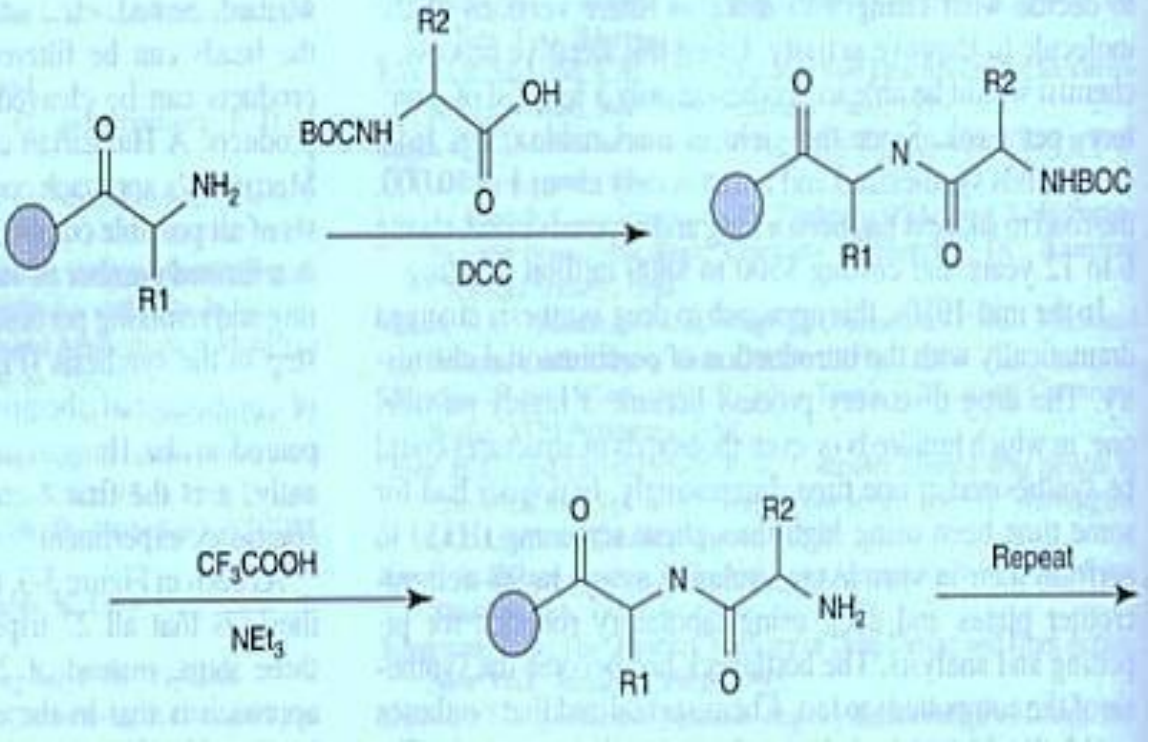


Figure 3–2 ■ Merrifield synthesis on a polymer bead support. The growing peptide chain is attached to a polymer support, usually in the form of small beads. The next amino acid (bearing R2) is attached, and its protecting group (BOC) is removed with acid/base treatment. *BOC*, butyloxycarbonyl; *DCC*, dicyclohexylcarbodiimide.

This made the rapid and automated synthesis of peptides possible.

A key feature of his approach is the attachment of a growing peptide chain to an inert polymer bead.

The beads can be immersed in solvents, washed, heated, etc...and when the synthesis is completed, the beads can be filtered from solution, and the reaction products can be cleaved from the polymer, yielding pure products.

A Hungarian chemist, Arpad Furka, realized that Merrifield's approach could be extended to allow the synthesis of all possible combinations of a given set of amino acids in a limited number of steps.

He accomplished this by splitting and remixing portions of the peptide-bound resin at each step in the synthesis (Fig: 3).

His description of the use of combinatorial chemistry to synthesize polypeptides appeared in the Hungarian patent literature in 1982. Apparently, it is the first literature reference to a combinatorial chemistry experiment.

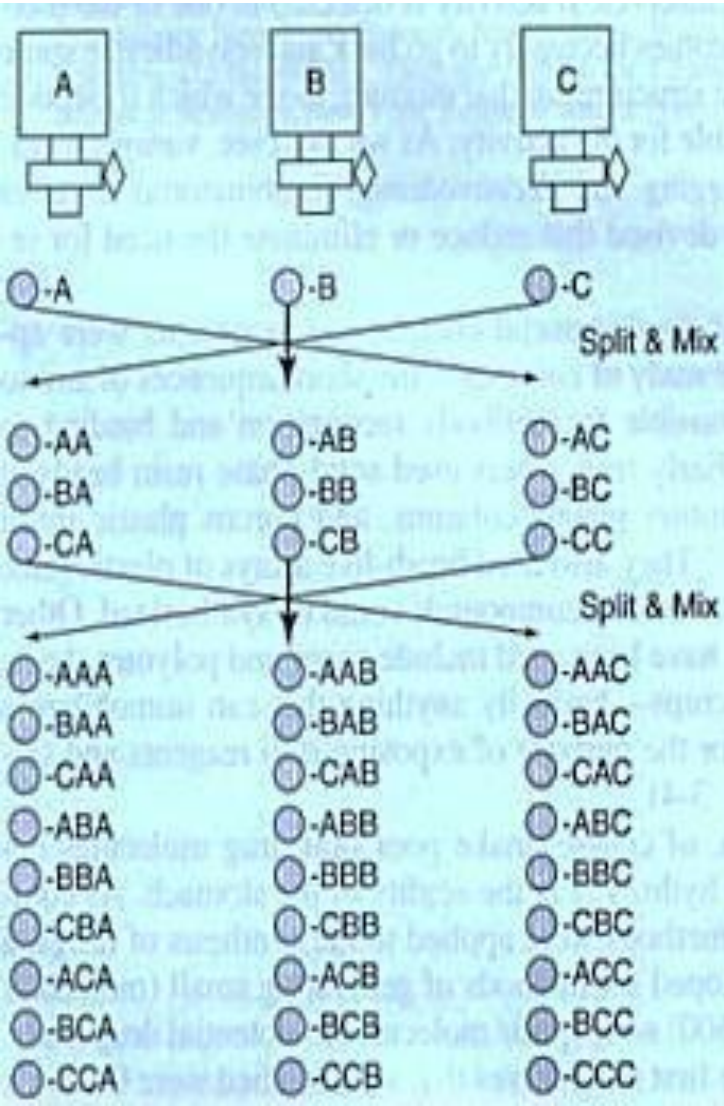
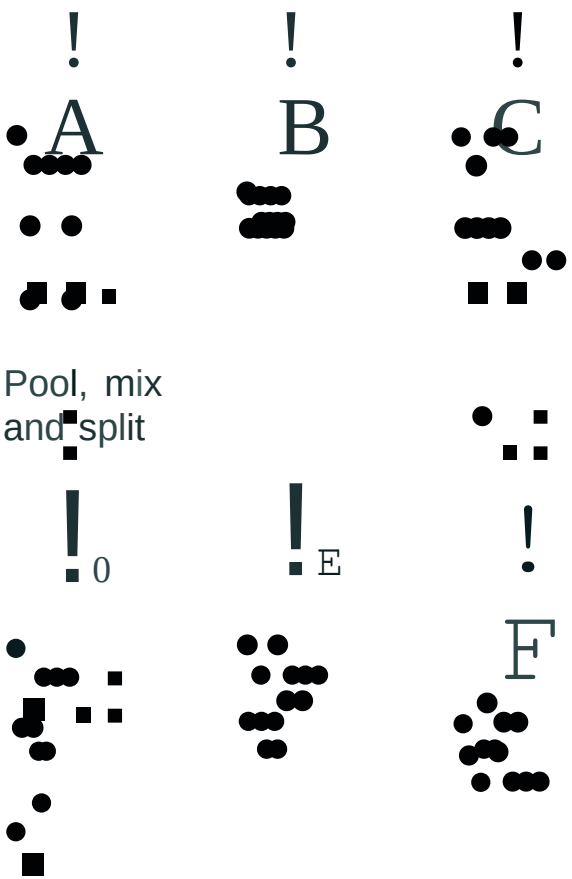


Figure 3–3 ▪ Split-and-mix synthesis of tripeptides. In the first step, all the beads in a given container have a single monopeptide. These are all mixed together, then split into three aliquots and re-treated, attaching a second peptide. After just one more step, all 27 possible combinations exist, spread among the three containers.

As seen in Figure 3, the advantage of split-and-mix synthesis is that all 27 tripeptides can be synthesized in just three steps, instead of 27 steps

a



- 3 x 3 x 3 = 27 compounds

b

		d	e	f	
	a	ad	ae	af	Microliter Plate
Library 1	b	bd	be	bf	3x3=9 compounds
	c	cd	ce	cf	

Divide into 3 duplicate plates

1

adg	aeg	afg	adh	aeh	alh	adi	aei	ali
bdg	beg	bfg	bdh	beh	blh	bdi	bei	bll
cdg	ceg	cfg	cdh	ceh	cfh	cdl	cei	cli

3 x 3 x 3 = 27

- The disadvantage of this approach:
- Is that at the end, one obtains three mixtures of beads with tripeptides attached, rather than the pure compounds themselves.
- If activity is detected in one of the mixtures, it becomes necessary to go back and resynthesize some or all of the structures in that mixture, to see which tripeptide is responsible for the activity.

- In a process called deconvolution, the synthesis is repeated in an iterative manner, producing smaller and sometimes overlapping mixtures.

- The screening is repeated until the active compounds are identified.

- Deconvolution: To make the results of a combinatorial experiment less complex, usually by backtracking and reanalyzing or resynthesizing a subset of the structures in the library.
- The goal of deconvolution is to determine which of a mixture of compounds is actually responsible for activity.

- Examine the following Table, this simplified outline shows how four steps will identify the three active components in a 20-compound investigation. (Keep in mind that the actual combinatorial process will produce hundreds or thousands of compounds for testing.)

- Assume that the project calls for synthesizing 20 compounds, A to T. Rather than carry out 20 distinct syntheses followed by 20 separate screening experiments, all of which can take weeks, the combinatorial syntheses are carried out and then screened as seen in this table.

Simplified deconvolution scheme for a 20-compound combinatorial chemistry screen

A	B^a	C	D	E	F	G	H^a	I	J	K	L	M	N^a	O	P	Q	R	S	T

Carry out the synthesis producing four five-component mixtures.
Screen the mixtures.

AB^aCDE	FGH^aIJ	$KLMN^aO$	PQRST

Retain only the three mixtures containing components.
Repeat the synthesis producing three-component mixtures and repeat the screening.

		JKL	MN^aO

Discard the inactive mixtures.
Repeat the synthesis producing overlapping two-component products and repeat the screening.

AB^a	B^aC	GH^a	H^aI	MN^a	N^aO

Only compounds B, H, and N need to be chemically characterized.

Instead of 20 synthesis and 20 assays, only 15 were required. Further, time-consuming purification of each mixture was not required. This process is very similar to that carried out by natural-product chemists.

The microbial, plant, or animal tissue is extracted with a variety of solvents, beginning with nonpolar hydrocarbons and ending with an alcohol or water, and the fractions are screened for activity. Only the active fractions are retained.

In either combinatorial synthesis or natural product isolation, once active compounds are identified, larger-scale, more focused synthesis can be done.

Early researchers used solid-phase resin beads in vials, microtiter plates, columns, and porous plastic mesh "tea bags".

They also used brush-like arrays of plastic pins, at the ends of which compounds could be synthesized.

Other media that have been used include paper and polymer sheets and glass chips—basically anything that can immobilize a structure for the purpose of exposing it to reagents and solvents (Fig.2-26).

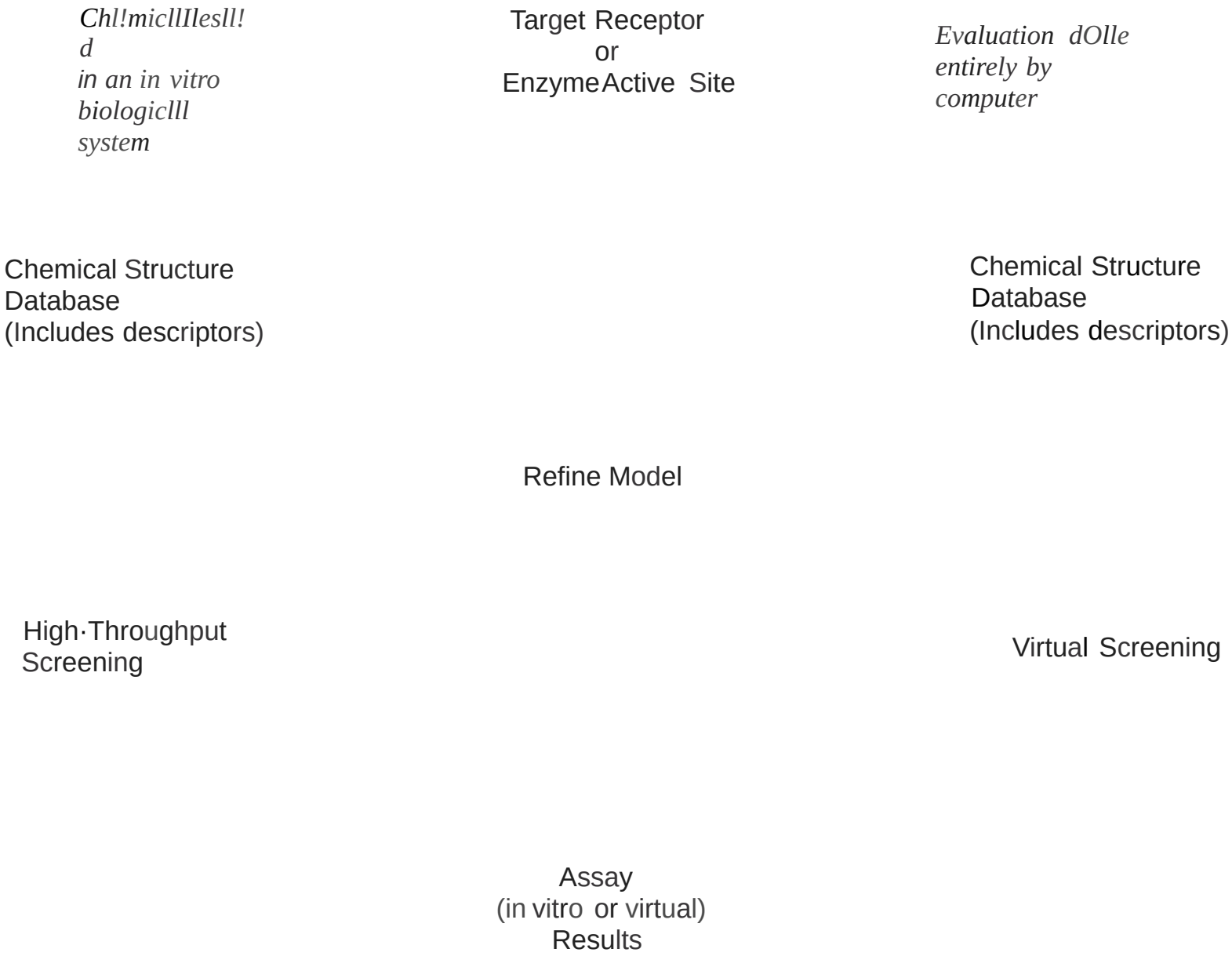


Figure 2.26 • Hiqh-throuqhput screening.

Peptides and Peptoids

- Peptides problem: Peptides, of course, make poor oral drug molecules because they hydrolyze in the acidity of the stomach.
- Among the first alternatives that were studied were Chiron's "peptoids"—molecules in which the variation occurs in (molecular weight <500) the attachment to the amide nitrogen (Fig.3-5)

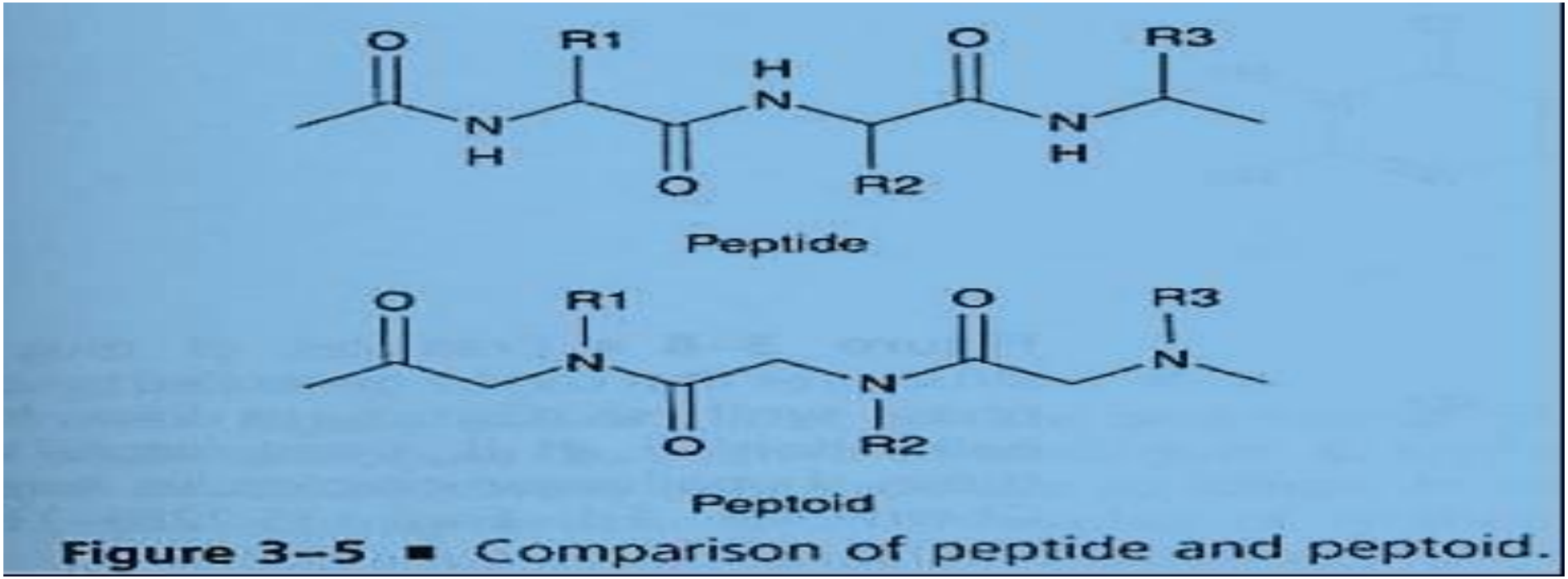


Figure 3–5 ■ Comparison of peptide and peptoid.

Although these structures could potentially place side chain functional groups in positions similar to those on the corresponding peptides, they differ significantly in that:

1. *They lack peptide hydrogen bonds.*
2. *They lack chiral centers.*
3. *They also show more rotational flexibility than the corresponding peptides, since the peptoid amide bonds show less double-bond character than those in peptides.*

Several potent peptoid ligands were found, including:

A nanomolar α-adrenergic inhibitor and a similarly active μ-opiate receptor ligand. Because of the ease of synthesis, other classes of linear chain molecules have been investigated. These include oligonucleotides (DNA and RNA), oligoureas, and carbohydrates.

References:

- Wilson and Gisvold Textbook of Organic Medicinal and Pharmaceutical Chemistry; Delgado JN, Remers WA, (Eds.); 12th ed., 2011.

- An introduction to Medicinal chemistry,3rd edition, Graham L.Patric