108. Variation and Selection

by **Gerhard Quinkert***

Institut für Organische Chemie der Universität Frankfurt am Main, Marie-Curie-Strasse 11, D-60439 Frankfurt am Main

Holger Bang

Institut für Pharmakologie der Universität Erlangen, Universitätsstrasse 22, D-91054 Erlangen

and **Dietmar Reichert**

ASTA Medica AG, Weismüllerstrasse 45, D-60314 Frankfurt am Main

Dedicated to Professor *Vladimir Prelog* on the occasion of his 90th birthday

(29.V.96)

Ninety years ago, chemical methods were used in solving biological problems. In our days, methods of evolutionary biology are going to be used in solving problems of synthetic chemistry.

1. Introduction. – *'The close connexion of chemistry with bology* [...] *should be re-established'*. *Emil Fischer* made this appeal on the occasion of his *Faraday* Lecture in 1907 [1]. It is only in our days, however, that it has been not only assented to, but also demonstrably adhered to[1]). In the course of time, the scientific world-view has come to perceive chemistry and physics as united in a continuous transition, through chemical physics and physical chemistry. From a different perspective, a similar, gradual blending of chemistry and biology, through biological chemistry and chemical biology[2]), has also become apparent. The difference in mentality, however, has probably never attained such a degree between physicists and chemists[3]) as that between chemists and biologists. In the latter case, *two different cultures* has even been spoken of [4]. It is impossible to evade the question of why the greater part of a century had to go by, before this cultural gap between chemists and biologists was to be perceived as disturbing. In answer, it is helpful to remember the development structural chemistry still had to undergo from a static understanding of molecules to a dynamic one, as well as to consider the twin realms of genotypes and phenotypes[4]) typical of viable systems as we know them.

1.1. *From a Static to a Dynamic View of Molecules.* Since the beginning of the 20th century, it has been possible to formulate questions relating to structural chemistry,

---

[1]) The Harvard *'Department of Chemistry'* voted last year to change its name to the *'Department of Chemistry and Chemical Biology'*. The *'Skaggs Institute of Chemical Biology'* is going to be established within the *Scripps Research Institute* [2].

[2]) *Chemical biology* and *biological chemistry* both dwell in the borderline region between the living and the non-living, the former more on the biological and the latter more on the chemical side of that borderline.

[3]) *Cf.* [3]: Chapt. 16.

[4]) The important distinction between genotype and phenotype was introduced by *W. Johannsen* in 1909 [5].

concerning the constitution and (relative or absolute) configuration of discrete molecules, in a clear and unambiguous way[5]). However, a similarly focused picture capable of explaining not only the conformations favored by a molecule, but also the functions performed by them, was only to come about some fifty years later. Only, in fact, when molecules were considered not merely statically as discontinuous structural singularities, but dynamically as (quasi-)continuous structural pluralities [12] [13].

1.2. *Phenotypes and Genotypes.* The regularities observed by *Gregor Mendel* [14] in his crossing experiments were evidence of the function of discrete molecules, later to be called genes. In their syntactic structure, these genes contain the specific information for all the processes which keeps the cell alive, and which are essential for the cell's duplication. These genes or their copies are passed on in every cell division. Genetics, after its molecular basis had become apparent, developed from a discipline of *general* biology into that of *chemical* biology. Both biologists and chemists are invited to cooperate in following *Emil Fischer*'s appeal.

The biologist is interested in genetic analysis, *i.e.*, in determination of all those genes involved in the emergence of a cell, a tisssue, or even a whole organism. He concentrates his attention on the relationship between the *genotype*, as the representative of a particular gene structure, and the *phenotype*, subject to the influence of both the internal and the external environment, as the representative of the totality of visible characters and typical functions. Because of environmental influences, there is no 1:1 correspondence between genotype and phenotype. Different living beings with the same genotype may develop into differing phenotypes, and different living beings with differing genotypes may develop into the same phenotype. It should be noted in passing that, as a rule, the unrestricted terms *genotype* and *phenotype* are used with the restricted meanings of *partial genotype* and *partial phenotype*, without the fact being especially stressed [15]. Such a differentiation in terminology corresponds with the shift in attention from the cell to cellular macromolecules, and here especially to nucleic acids and proteins.

The chemist is interested in molecular analysis, *i.e.*, determination of all those nucleic-acid sections that code for a particular protein. For him, therefore, double-stranded DNA or single-stranded RNA is the molecular genotype, expressing itself in the corresponding protein as the molecular phenotype[6]). The distinction between genotype and phenotype in living beings is what, in the end, makes clear the division of labor which must have taken place between nucleic acids and proteins during the course of evolution in biomacromolecules.

A deeper understanding of the role played by the genotype-phenotype dichotomy in *Darwin*ian evolution, in the spheres both of organisms and of biomacromolecules, prescribes a position in a *discontinuum* for the genotype. The discontinuum is an ideal world of memory, because the quantized genetic information can be effectively stored there [17]. Experience shows that the natural nucleic acid DNA, by virtue of variation of its four nucleobases, is excellently suited to discontinuous storage of genetic information and, by

---

[5]) The ideas of *van't Hoff* [6] *Le Bel* [7], based upon views of *Pasteur* [8], *Kekule* [9], and *Wislicenus* [10], were supported experimentally by *E. Fischer* [11].

[6]) Several possible relationships may be envisioned between the arrangement of exons in a gene and the structural domains of the protein it encodes (*cf.* [16]: Fig. 1 in Chapt. 6).

duplication, each time with a complementary single strand, to being read with suitable accuracy and copied in geometric progression.

Unlike the genotype, the phenotype should have a place in the *continuum* if possible. The continuum is an ideal world of function, as smooth structural adaptations to functional requirements are permitted there in a continuous manner [17]. The natural proteins fulfil the requirements made of their conformation-controlled function by virtue of a certain plasticity, or put another way, through *quasi*-continuous diversity of easily interconvertible conformational variants.

2. Diverse Scenarios. – Variation within a population (of living beings or of molecules) and the alteration of that population by selection must occur, if evolution is ever to take place. A whole range of diffent scenarios is conceivable, depending on the type of variants thrown up and the nature of the selection which takes place. We will confine ourselves here to scenarios which can be smoothly described in chemists' terminology. *Table 1* summarizes various cases which differ in variant composition (*stochastic* and/or *non-stochastic*), type of selection (*natural* or *non-natural*), and nature of reproduction (*self-* or *non-selfreplication*).

Table 1. *Various Scenarios with Selection in Action from the Level of Populations and Organisms to the Molecular Level*

Scenario	Variation	Selection	Replication
Living Systems			
natural life, undisturbed	stochastic	natural	self
recombinant DNA technology	non-stochastic	natural	self
breeding by artificial selection	stochastic	non-natural	self
non-natural life	stochastic	non-natural	self
Non-living Systems			
in vitro evolution	stochastic	non-natural	self
combinatorial compound libraries	(non-)stochastic	non-natural	non-self

2.1. *Living-System Scenarios.* Here, we immediately hit upon those molecules which act either as educts or as products in natural processes, and which, consequently, ought to be called natural-product molecules. As *Vladimir Prelog* has emphasized, natural products have a history to relate, having withstood the test of natural selection[7]), while *E. Zuckerkandl* and *L. Pauling* have even elevated these molecules to the rank of *documents of evolutionary history*[8]). In the past, however, chemists have mostly used the term 'natural products' to denote an arbitrarily accentuated minority of naturally occuring compounds of relatively low molecular mass, the biopolymeric nucleic acids and proteins being relegated more to the margins of interest[9]). In this essay, these latter compounds most certainly do count as natural products.

[7]) *V. Prelog* [18]: '*Ich bin überzeugt, dass sie* [die Naturstoffe] *immer eine Botschaft enthalten, und dass es unsere Aufgabe ist, diese zu entziffern*'.

[8]) *E. Zuckerkandl* and *L. Pauling* [19] have subdivided natural-product molecules, according to their utility as evolutionary historical pointers, into semantophoretic (nucleic acids and proteins), episemantophoretic (synthesized with the participation of enzymes), and asemantophoretic molecule classes (*cf.* [3]: Chapt. 16).

[9]) To be fair, there are exceptions, *i.e.*, [20].

2.1.1. *Natural Life. Darwin*ian evolution by natural selection belongs here: a process which has led to life, as we know it, in innumerable small steps and despite boundless numbers of formal, possible alternatives. The development of the living world, in a mere four billion years, acts subliminally on critical observes, making it difficult to accept chance as the sole driving force behind evolution and totally exclude deterministic elements. A mathematical model of directed evolution proposed by *Manfred Eigen* [21] makes allowance both for stochastic mutation in a continually altering population of variants and for deterministic selection of advantaged mutants. In this physically based model, natural selection is founded upon a system-inherent value control combined with automatic optimization. When applied to the self-reproducing nucleic acids, the model does not, however, set its sights on one single nucleic-acid sequence, but on the sequences of a hierarchically ordered distribution of variants. The genotypes among this variant distribution have emerged through mutagenic reproduction out of a precursor and – like species – have succeeded as *quasispecies* by selection.

Directed selection of a quasispecies may be illustrated geometrically using a polydimensional *variable space* [10]), with each molecule represented by a point, and each quasispecies by a set of points. The arrangement of these points in the variable space, together with the distances between them, correctly conveys the relationships between all of the sequences and, if one wishes, between participating conformers.

The dimensionality of the variable space is dependent both on the number of structural parameters to be taken into account and on a specific *fitness value* which can be assigned to each point, and which is itself dependent on actual external conditions. For just one structural parameter, a diagram with the abscissa representing the parameter scale and the ordinate the associated fitness value suffices. The correlation between the two quantities is given by a curve. For two structural parameters, a *Cartesian* coordinate system with a rugged mountain range (*fitness landscape* [11])) in the appropriate variable space is needed. The fitness landscape corresponds to a potential surface, a dynamic system being able to move along it. A spatial coordinate system is also adequate to deal with three structural parameters, as long as the fitness values for a particular point set are printed in different colors (*Fig. 1*).

The above-mentioned model may formally be extended into n dimensions, represented clearly in a hypercube as a higher-dimensional variable space. In it, the point set of

---

[10]) '*The facts in logical space are the world*', says *Ludwig Wittgenstein*, right at the Beginning of his *Tractatus Logico-Philosophicus* [22]. Points in space are loci of truths, irrespective of whether these are indeed existent or merely could exist. *Logical space* is hence a representational space for possible truths, just as *geometric-physical space* is a representational space for possible bodies or their models. Chemists' *structure space*, with its discrete molecular and supramolecular structures or their models, may be represented in polydimensional variable space. This polydimensional variable space has room for every structure conceivably possible according to the principles of chemical-structure theory [3], whether or not it has actually been made, or merely could be made. A survey of this polydimensional space reveals that the population density of actual known molecules and supermolecules, while very erratic, is relatively thin overall. Some regions are empty, while a few others appear as though so full as to be continuous. The concept of representing genotypes by a point space (*nucleotide space*) was introduced 1973 by *T. Rechenberg* [17]. *J. Maynard Smith* [23] had used the term *protein space* as early as 1970 with a similar meaning with regard to phenotypes.

[11]) A brief account of the history and importance of the landscape concept in evolutionary biology is given in [24]. The evolutionary concept of the *adaptive landscape* and an evolving system's working toward its point of maximum fitness is entertained in [25].

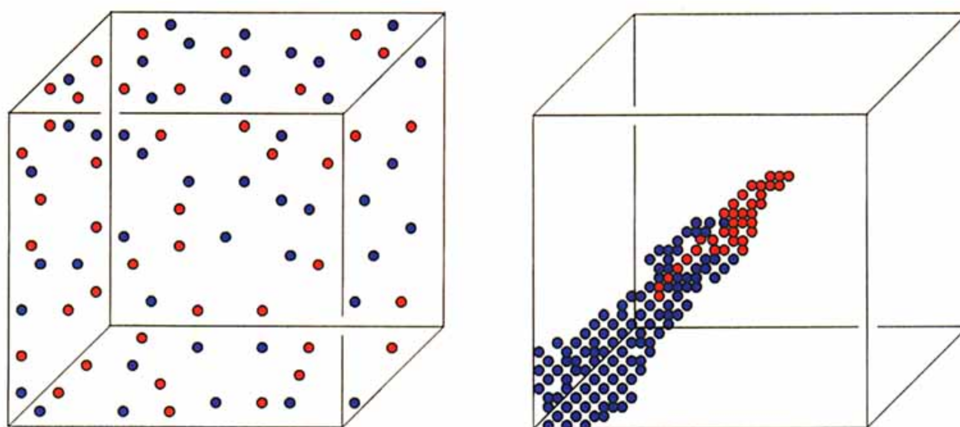

Fig. 1. *Schematic representation of stochastic distribution* (left) *and directed evolution* (right) *of a population characterized by lower* (blue) *and higher* (red) *fitness values*

the evolving quasispecies travels in the direction of increasing fitness values; any other movement has been selected against. The speed with which the point set progresses is primarily dependent on the local topography of the mountainous fitness landscape. Selection makes itself evident in the variable space as the favoring of a particular variant distribution in a mountainous region of the fitness landscape, and biological evolution as alteration of the variant distribution in the direction of maximum fitness values.

To understand the effects of natural selection on molecules, it is advisable to make use of members of a molecular class in which the splitting-up into genotype and phenotype has not yet been realized. This double role is one attribute of RNA molecules [26]. Among other things, RNA molecules contain the program required for replication, and are also capable of autocatalyzing this replication. Replication of the genomic RNA of the Q_β-coliphage [27] proceeds in this manner, with the involvement of a Q_β-RNA-dependent RNA polymerase (Q_β-replicase [28]). It is convenient to study, not least because Q_β-RNA exists as a single-stranded molecule. Experimental examination of replication requires the Q_β-RNA-template[12]), upon which enzymatic synthesis of the complementary single strand, from the four activated nucleotide components, takes place. The resulting synthesized strand similarly shows no tendency to form stable duplexes, which would be unsuited for replication [30]. Instead, it tends towards partial intracatenary base pairing and formation of domaines of irregular secondary structure. 75% of its nucleotide components are involved in a specific folding pattern with numerous hairpin loops [30]. This folding pattern is necessary for the Q_β-RNA to play its role in replication [31]: on the one hand, to hinder the 'deadly' duplex formation, and, on the other, to bind the replicase in a conformationally pre-organized sequence section, on which the enzyme can unleash its

[12]) With Q_β-RNA as the template, sequence-specific amplification by Q_β-replicase proceeds with impressive efficacy (more than 10^9-fold increase within 15 min). With suitable selection pressure, Q_β-replicase induces evolution of poorly replicating contaminant RNA into efficiently replicating mutants, which then are capable of acting as endogenic RNA-templates [29].

full catalytic effectiveness in RNA polymerization. The message of the RNA replication of the Q_β-coliphage is therefore: the *conformation* is the phenotype to be functionally evaluated and evolutionarily improved, the *sequence* is the genotype to be syntactically selected in a feedback loop[13]). Deeper insights into the nature of this enzymatic, template-controlled Q_β-RNA replication are furnished by *in vitro* evolutionary experiments (see *Sect. 2.2.1*)[14]).

The 'natural life' field of research also includes the study of the origins of life on Earth [37] [38]. Assuming that today's highly differentiated DNA-RNA-protein world was preceded by a simpler RNA world [26], and the latter by a still simpler pre-RNA world [38] [39], then the synthetic chemist is faced with three experimentally approachable problems. The first of these concerns the emergence, under prebiological conditions, of the principal biopolymers' elementary building blocks[15]) [37] [40] [41]. The second problem addresses, initially, the non-enzymatic oligomerization of these building blocks, presumably on a mineral surface as a template [42–44], to be followed later by ensuring the supply of the now essential oligomers by enzymatic synthesis. The third problem deals with the autocatalytic, template-directed replication[16]) of a rather primitive semantophoretic oligomer, easily formed under prebiological conditions, and its takeover by another, more advanced oligomer. Such a takeover, taking place at the heart of supramolecular oligomerization, has been believed to occur [46]: RNA oligonucleotides facilitate the synthesis of complementary PNA[17]) strands and *vice versa*. Is PNA, then, a candidate for non-enzymatic self-replication under potentially natural conditions? Because of its chemical properties, *Albert Eschenmoser*'s pyranosyl-RNA (p-RNA), a constitutional isomer of (furanosyl-) RNA has a much better chance of carrying through [48].

2.1.2. *Non-natural Life*. There is one big unsolved problem of synthetic chemistry which appears soluble in the foreseeable future. It is the discovery of molecular replicators other than the naturally occurring nucleic acids, with the capability to self-assemble, replicate autocatalytically, and, because of unavoidable errors in this latter process, exist as a greater or lesser number of variants, whose composition can be modified and narrowed down by selection pressure. Solving the problem requires finding experimental

[13]) In an RNA (or pre-RNA) world, the autocatalytic replication cycle relies on feedback control of replication by the genotypes's phenotypical information. In the DNA-RNA-protein world, the replicator expresses additional information by transcription and translation, whereby a second feedback loop coupling genotype and phenotype comes into play [32].

[14]) When particular nucleic acids differ from one another, whether in different development phases of a single living being, in variants within a population or in different branches of the phylogenetic tree of living entities, this will also, as a rule, apply for their expressed proteins as well. Consequently, suitable sections of semantophoretic molecules of every type have been used for ontogenetic and phylogenetic comparative study: polypeptides of the globin superfamily, for example, for better understanding of evolution at the molecular level [33], or ribosomal RNA sections for the elucidation of evolutionary relationships in bacteria [34]. With enzyme evolution, the episemantophoretic molecules affected by them have also altered. These have been exploited in the evolutionary perfecting of molecular anchors for the reinforcement of membranes, for example, setting out an organism-based chronology of cyclization products derived from squalene [35] [36].

[15]) Biological building blocks of elementary structure suggest an intrinsic capacity for constitutional self-assembly of a handful of the simplest chemical compounds under potentially natural conditions [41].

[16]) See the state-of-the-art report by *L. E. Orgel* and his school [45].

[17]) PNA: polyamide nucleic acid [47].

models where the spontaneous transition from inanimate into living matter might be observed in non-natural circumstances [49], allowing empirical determination of the requirements for that phenomenon which, as a whole, we call life – over and above the single instance known to us today. If we take biology to be the science concerned with *all* forms of life, and not just 'natural life', then it is clear that the synthetic chemist investigating in this field of non-natural life synthesis is standing foursquare in chemical biology's central sphere of interest.

2.2. *Non-living System Scenarios.* 2.2.1. *in vitro Evolution.* The first *in vitro* experiments in which evolution of RNA molecules by non-natural selection was observed were carried out thirty years ago in *S. Spiegelman*'s [50] laboratory. Q_β-RNA was put into a reaction vessel containing Q_β-replicase and the four activated ribonucleotide monomers. A sample was taken after *ca.* 30 min and transferred to a second vessel, also containing the polymerase and the four monomeric components. The process was repeated 73 times, after which selection was clearly observed to have taken place, as rapidly as conceivable, although not in a faultlessly reproducible manner. The principal product-component was much shorter than the original starting substance: 83% of its original genome had disappeared. Of the original phenotypic functions, the only ones remaining were those involved in replication.

Spiegelmann's pioneering experiments were carried forward by *M. Eigen* [51], *G. Joyce* [52], *J. Szostak* [53], *D. Ellington* [54], and *M. Famulok* [55], and have meanwhile developed into the dynamic research field of *in vitro* evolution. Primarily, this work centers around finding RNA molecules capable of performing certain specified functions: binding small molecules as ligands, for example, or themselves acting as ligands for polypeptides, or even as ribozymes in autocatalytic oligonucleotide replicator synthesis.

2.2.2. *Molecular Libraries by Combinatorial Chemistry.* The preparation and screening of compound libraries[18]) – populations of variants with a systematic variant distribution – is a topic currently attracting considerable attention among the scientific community[19]). As always when legitimate interest and fashionable methodology march hand-in-hand, catchwords and acronyms are springing up like mushrooms. Terms like *parallel synthesis* and *simultaneous synthesis* have been avoided here, since different authors use them differently, and sometimes mutually exclusively. Instead, molecular libraries have been characterized with the aid of two orthogonal criteria: a logistic criterium and one of reaction compartmentation.

Logistically, combinatorial libraries may be accessible by a singular library synthesis or by iterative procedure leading to a library and a number of sub-libraries. Compartmentally, a vast library of variants may be situated on resin beads commonly used in solid-phase synthesis or on an appropriately prepared reaction field. In such a way, each resin bead, in the former case, and each of the precisely subdivided squares of a reaction field, in the latter case, is loaded with many copies of one and the same variant.

---

[18]) In combinatorial chemistry, a *molecular library* means a systematic (*non-stochastic*) or random (*stochastic*) collection of molecules that differ in their arrangement of a given number of submolecular modules.

[19]) Articles on combinatorial chemistry are distributed all over the chemical literature. Some are given in [56]. In addition, *Bioorg. Med. Chem. Lett.* (**1993,** *3,* 387–475) and *Acc. Chem. Res.* (**1996,** *29,* 112–170) had a special issue, *ChemTracts, Org. Chem.* (**1995,** *8,* 1–25) a focus point on this topic.

The title page of the 15 February 1991 issue of *Science* shows how a molecular library may be prepared by a combinatorial procedure on a 'miniaturized chessboard'. The identity of individual variants can be deduced from the minute record of experimental procedure, by their position in the matrix field. Using this technique, scientists of the *Affymax Research Institute* [57] have prepared a total of 1024 peptides in a square of 1.28-cm side length, in ten synthetic operations in a synthesis field provided. The desired variation was achieved using a combination of efficient *solid-phase peptide synthesis* (on a glass sheet affording great numbers of linear linker molecules with a photolabile group protecting an amino functionality at the terminus) and *high-resolution photolithography* (used to deprotect specific amino groups in regions not shielded from light of a suitable wavelength by a stencil, thus freeing them for synthetic purposes).

The principle may be clarified by a simple example, in which the four (colored) amino acids *A*, *C*, *D*, and *E* are introduced in sequence to construct a distribution of 15 mono-, di-, tri- and tetrapeptides (*Fig. 2*).

In the first round of synthesis, columns C and D are covered with a stencil. The protecting groups of the linker molecules in columns A and B are removed photochemically, by exposure to light, and an amino-acid residue – *A* in this instance – added to the

	A	B	C	D
4	ACDE	ACD	CDE	CD
3	ACE	AC	CE	C
2	ADE	AD	DE	D
1	AE	A	E	

Fig. 2. *Schematic representation of a reaction field occupied by 15 variants of possible permutations*

liberated amino-groups. In the second round, rows 1 and 2 are covered with a stencil, so that rows 3 and 4 this time participate in the reaction, the cycle of photodeprotection and introduction of an amino-acid residue – *C* in this case – being repeated. In round three, rows 1 and 3 are covered up, so that amino-acid residue *D* can be introduced onto 2 and 4. Finally in round four, columns B and D are covered up and amino acid *E* added to A and C.

The *Affymax* binary synthesis procedure affords the complete set of possible permutations of different chain-length: a total of 15 variants in the above example; one variant with four components, four variants with three, six with two and four variants with only one component.

To construct a singular molecular library of linearly constituted variants with identical chain lengths, a different experimental strategy is used. *One-bead-one-variant-type* combinatorial libraries [58] are accessible by solid-phase synthesis on suitable polymer beads furnishing resin-bound variants, which may be screened directly using soluble receptors. Only those variants which reveal themselves by color change through complexation with the fluorescently labeled receptor, for example, have to be subjected to structure determination. Finally, larger quantities of selected variants must be resynthesized using conventional methods.

The advantage of the combinatorial synthesis technique lies in the very small number of synthetic steps needed to produce a great number of variants. This is made especially clear by comparing *combinatorial* strategies with their *divergent*, or even *linear*, counterparts. For the 27 triplet combinations resulting from three different repeating units in three synthetic rounds on 27 fractions, *linear synthesis strategy* requires $3 \times 27 = 81$ steps in total (*Fig. 3*).

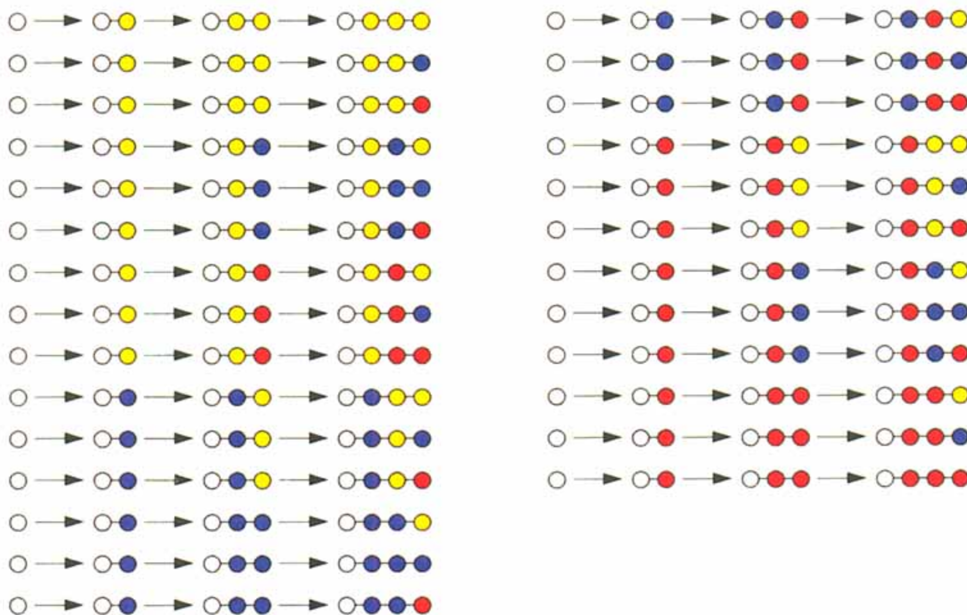

Fig. 3. *Schematic representation of the solid-phase synthesis on resin beads* (grey) *using three different* (colored) *building blocks: linear strategy*

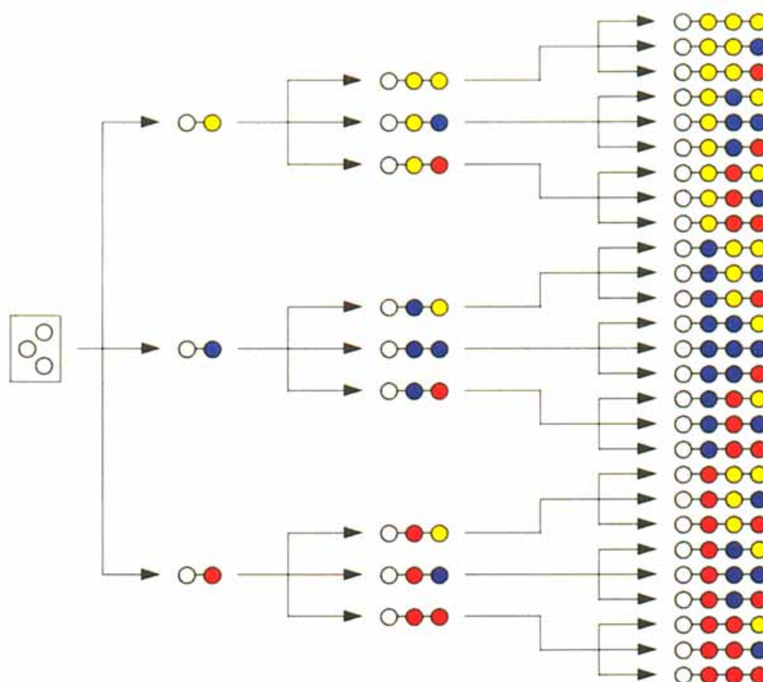

Fig. 4. *Schematic representation of the solid-phase synthesis on resin beads* (grey) *using three different* (colored) *building blocks: divergent strategy*

For the same 27 triplet combinations, *divergent synthesis strategy* needs 3 synthesis rounds on 3^n fractions ($n = 1$–3) $= 3^1 + 3^2 + 3^3 = 39$ steps (*Fig. 4*).

Using the *combinatorial synthesis strategy*, the 27 triplet combinations can be produced in only three synthesis rounds on three fractions; a total of $3 \times 3 = 9$ steps (*Fig. 5*). Unlike in linear and divergent synthesis strategies, however, the polymer-bound variants are not each available separately, but in a mixture with all the others. It is true that each polymer bead carries only one single variant type of all the possible ones, but for the time being one does not know which one.

A single polymer bead can comfortably accomodate *ca.* 10^{-10} mol. This is a sufficient quantity for direct sequencing in the case of peptides of proteinogenic amino acids. If non-proteinogenic amino acids are also involved, or if non-polypeptide or non-polynucleotide (polynucleotides may always be prepared in sufficient quantities by PCR) compounds are the ones of interest, then it is necessary to develop suitable custom-built analytical techniques. A code may be used, whereby every synthetic step undertaken is assigned a molecular reporter. To show how this *encoded combinatorial chemistry* [59] works in a particular case, an example from *Clark Still*'s laboratory [60], in which 6 synthesis rounds, each on 7 fractions, a total of $7 \times 6 = 42$ steps, were needed to make a complete molecular library of $7^6 = 117{,}649$ variants is given in *Fig. 6*.

Each of the six sequential synthesis rounds begins with the fractionation of a particular weight of *Merrifield* polymer beads (P), charged ester-fashion with a peptide fragment

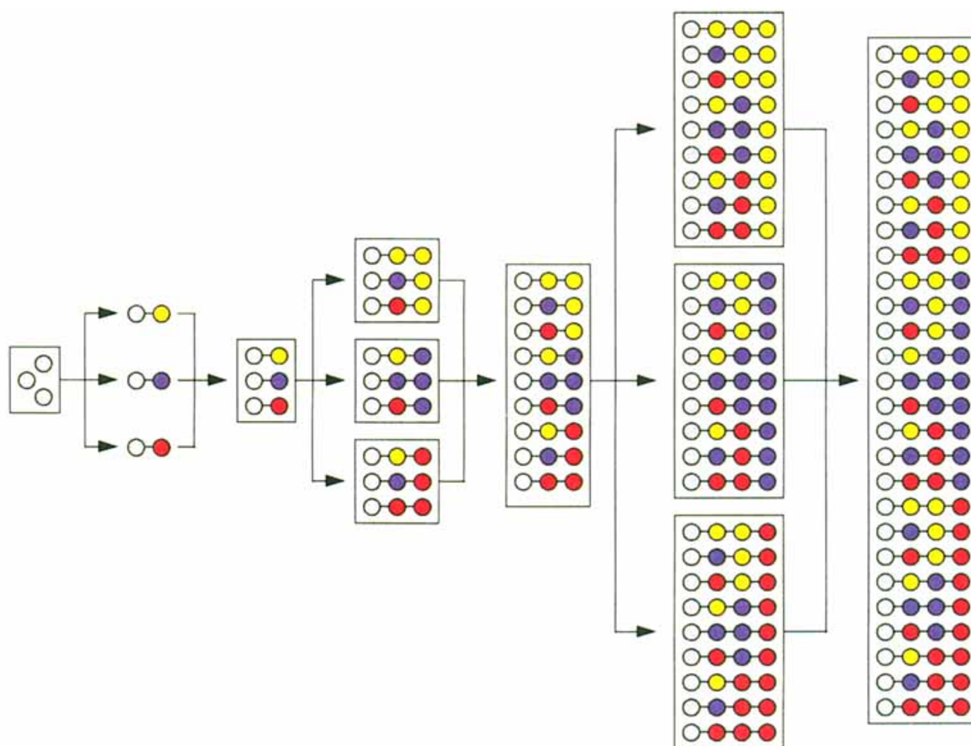

Fig. 5. *Schematic representation of the solid-phase synthesis on resin beads* (grey) *using three different* (colored) *building blocks: combinatorial strategy*

of constant sequence GGGGLDEE, and their subsequent division between seven (because of the seven amino acids, S, I, K, L, Q, E, and D used) reaction vessels. In each of these reaction vessels, a reporter molecule R1–R7 (R1.1–R1.7 for the first round, R6.1–R6.7 for the sixth and so on) is bound ester-fashion onto the polymer bead. Finally, the chain-extension takes place, with the assigned amino acid in each reaction vessel.

In this manner, each one of the total of 42 synthesis steps can be marked with a specific molecular reporter. After the resin beads from the seven vessels have been brought together again, they are once more divided between seven reaction vessels for carrying out the second round. This split-and-mix alternation amounts to a *combinatorial synthesis strategy*, giving access to 117,649 different peptides in this case. Selective removal of the reporters from a particular polymer bead and analytical decoding gives a record of the course of the synthesis on that particular bead, and hence the sequence of chain elongation. In other words, a singular molecular library is obtained in which each individual variant is already labeled. A *divergent synthetic strategy* would require 137,256 steps and a *linear strategy* 705,894, instead of the 42 steps needed for the *combinatorial strategy*.

The *Still coding technology* uses members of a homologous series with 3 to 12 CH_2 units. One end is blocked ether-fashion with one of three differently halogenated phenyl

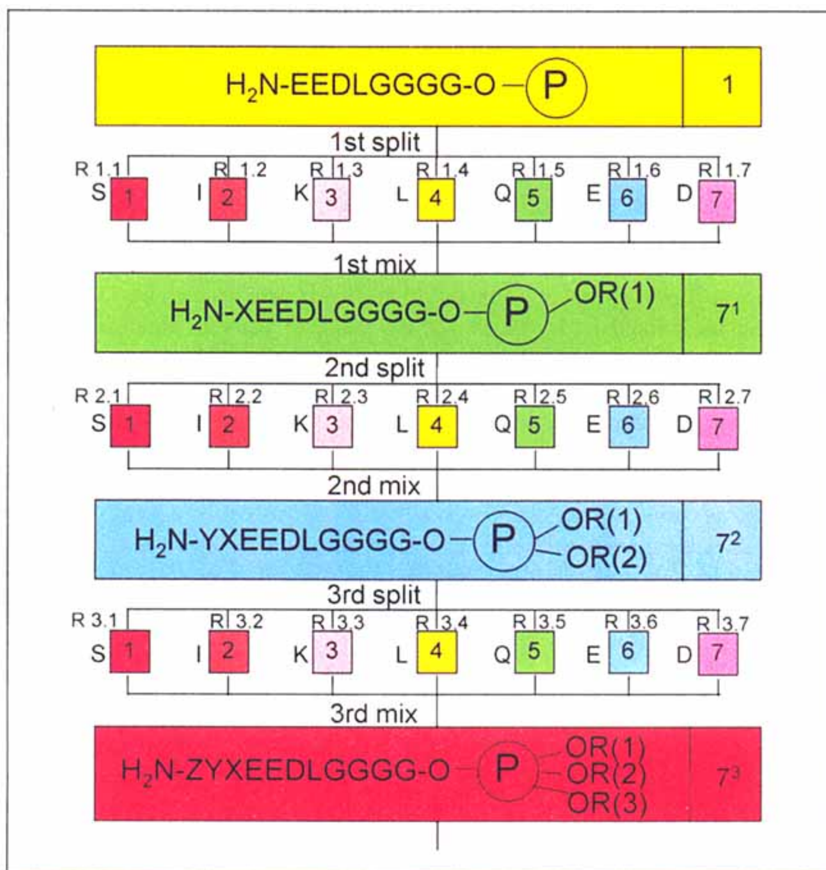

Fig. 6. *Schematic representation of three rounds of an encoded combinational synthesis following the split-and-mix-strategy*

residues. The other is joined *via* a –O–CO–O– bridge to a benzene derivative designed to bind ester-fashion to the resin bead on one hand, and to be photolytically removable on the other. The resulting alcohols may be identified in picomolar quantities using electron-capture GC.

Not only the synthesis, but also its detailed documentation, is carried out in combinatorial fashion. To document 42 synthesis steps, it is not necessary to use 42 molecular reporters, but a mere 18. 18 Reporters suffice for the registration of 42 single steps with 18 single combinations, 18 double combinations and 6 triple combinations.

TentaGel resin beads (*Rapp Polymers GmbH*), solvatable in both water and organic solvents and with an average diameter of 90 µm were used in the solid-phase synthesis [61]. These consist of a graft copolymer of linear polyethylene glycol and cross-linked polystyrene as matrix, and serve as the source of amino groups in a reaction medium, somewhere between Et_2O and THF. The amino groups are not situated in the interior of the beads, but are each on the end of a linker molecule, joined on with 11-*N*-Boc-pro-

tected 11-aminoundecanoic acid. 1 g resin contains *ca.* $2.8 \cdot 10^6$ beads. Each bead may comfortably be charged with *ca.* 100 pmol of its particular synthetic variant and *ca.* 1 pmol of each molecular reporter. To find out how many beads are needed to be statistically sure of obtaining all of the possible variants, it is necessary to conduct some very elaborate statistical calculations [62]. Using the idealized assumption that the chemical yield in every single one of the 42 synthesis steps is 100%, then for 117,649 variants, a six-fold quantity – *ca.* 10^6 spheres – will be sufficient.

The library of oligopeptide ligands obtained using this combinatorial synthesis strategy was studied using the sensitive ELISA procedure for specific complex formation with a monoclonal antibody receptor. The polymer beads where complex formation had occurred could be stained and picked out by hand. It proved possible to identify a consensus sequence with the terminal triad serine-isoleucine-leucine from the oligonucleotides on the polymer beads picked out.

Identification of specific sequences in an extensive library of ligands which are necessary for supramolecular interaction with a complexing partner is the first step on the road to a biologically active substance. Whether the particular ligand acts as an agonist or as an antagonist requires further clarification. Medicinal chemists' combinatorial synthesis, therefore, does not lead immediately to the preparation of new drugs. In making such vast numbers of non-natural variants available to us, though, screening has given a whole new and inexhaustible dimension to medicinal chemistry. This should greatly benefit the optimization of lead compounds.

3. In Search of an Anti-asthma Prototype Drug. – 3.1. *Preconditions.* To systematically attempt to find and develop a drug[20]) a *clinical feature* of a disease is needed to start with. Some *pharmacological observation* concerning the potential value of a given compound intended for use as a component in a medicine would certainly prove helpful. A *biological model* able to schematically describe the supramolecular cell chemistry involved is required. Last but not least, *several bioassays* have to be developed which, if properly designed, select from a spectrum of biofunctions and allow predictions about clinical outcomes.

To do a bioassay on a variant population of compounds, one needs these compounds and, therefore, a prior decision has to be made as to whether they are to be custom-built, aiming at predetermined target structures, one by one, or to be obtained through screening of a systematically or randomly approached compound library.

3.2. *Asthma as an Autoimmune Disease.* 3.2.1. *Clinical Feature.* Asthma is a complex disorder of the respiratory tracts with many variants. The most striking feature of the histopathology of asthma is the intense infiltration of the bronchial mucosa with eosinophils, macrophages, and lymphocytes. In fact, the disease has many of the features of a chronic, cell-mediated immune response causing hypersensitivity of the bronchial system. A good start towards gaining a better understanding of the pathophysiology of asthma might be obtained by focusing on its immunological basis [64].

3.2.2. *Pharmacological Observation.* The potential utility of cyclosporin A, a cyclopeptide built up of eleven amino-acid residues, for the treatment of asthma has been

---

[20]) In his *Nobel* Lecture, *J. Black* [63] gives a lucid account of how to discover a drug.

demonstrated. The improvement in airway parameters corresponded with an inhibition of *IL-2* synthesis or *IL-2*-dependent T-cell proliferation [65].

3.2.3. *Biological Model*[21]) (*Fig. 7*). Binding of antigens to *TCR* initiates a cascade of signal transduction events that ultimately leads to lymphokine gene expression and T cell proliferation. Stimulation of the *TCR* induces *PLC* activity. The activation of *PLCγ1* results in the hydrolysis of PIP_2, furnishing the second messengers IP_3 and *DAG*. The latter are responsible for the rapid and sustained increase in the levels of *TCR*-induced cytoplasmatic free Ca^{2+} ions and activation of *PKC*, respectively. The increased [Ca^{2+}] and activation of *PKC* have been causally linked to cellular responses in B and T cells. The best characterized of these responses is the transcriptional activation of the *IL-2* gene. Evidence supporting a role for the *PI* pathway [22]) in the induction of *IL-2* transcription includes

- the stimulatory effects of reagents that increase [Ca^{2+}] and activate *PKC*,
- the inhibitory effects of calcium chelators and *PKC* inhibitors,
- the ability of activated forms of *PKC* and *CN* to bypass cellular signals in inducing *IL-2* gene transcription.

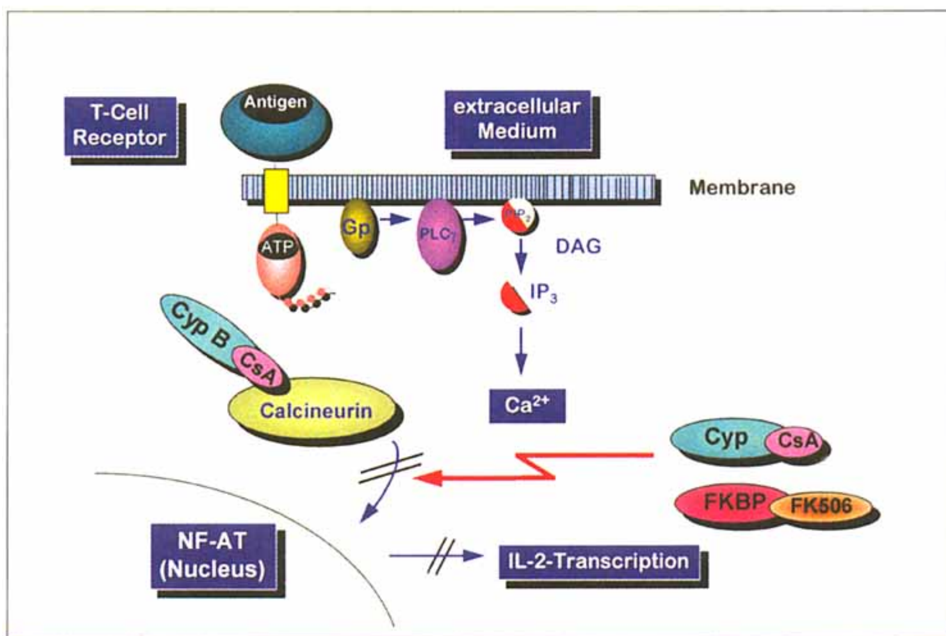

Fig. 7. *Schematic illustration of a whole cascade of signal transductions caused by antigen/T cell-receptor complexation. CN:* calcineurin; *CsA:* cyclosporin A; *Cyp:* cyclophilin (binding protein to *CsA*); *DAG:* 1,2-diacylglycerol; *FK506:* immunosuppressive, 23-membered macrolide; *FKBP:* binding protein to *FK506*; *IL 2:* interleukin 2; IP_3: inositol-1,4,5-triphosphate; *NF-AT:* nuclear factor of activation in T cells; *PI:* phosphatidylinositol; PIP_2: phosphatidylinositol-4,5-biphosphate; *PKC:* protein kinase C; *PLC:* phospholipase C; *TCR:* T-cell receptor.

---

[21]) *Cf.* [66] and literature cited therein.

[22]) *Cf.* [67]: Chapt. 12.

Rapid and sustained increase in [Ca^{2+}] resulting from *PLCγ1* activation is thought to influence calcium/calmodulin-dependent events. At least one critical event regulated by the increase in [Ca^{2+}] is the activation of the phosphatase *CN*. A function of *CN* has been most firmly established in the regulation of *IL-2* gene expression. The *CsA*-sensitive factor *NF-AT* resides as a preformed cytoplasmic protein which is translocated to the nucleus upon activation. Its cytoplasmatic form is a phosphoprotein, while the nuclear form is considerably dephosphorylated by *CN*. The naturally occuring immunosuppressive drugs *CsA* and *FK506* each bind to cytoplasmatic proteins, members of the immunophilins, forming immunophilin-ligand complexes (*CsA-Cyp* or *FK506-FKBP*) interacting with high affinity with *CN*. The binary complexes *CsA-Cyp* or *FK506-FKBP* block the enzymatic function of *CN*, essential for *IL-2* gene activation and T cell proliferation.

3.2.4. *Bioassays*. A *receptor binding assay* (essentially following a published procedure [68]) reveals whether substantial complexation of a fluorescently labeled immunophiline (cyclophilin B[23]) and FKBP, respectively) and a ligand (on the bead) actually did take place. An *enzyme inhibition assay* [69] discloses the ability of a particular ligand, individually resynthesized in solution, to inhibit the peptidylprolyl *cis/trans*-isomerization (*PPI*) activity of the cyclophilins. By a *whole cell assay* the resynthesized ligands' capacity to inhibit the proliferation of human peripheral blood lymphocytes is examined. Stimulation of the lymphocytes (isolated by density centrifugation) by the *OCT 3* antibody is measured (inspecting the incorporation of radioactive thymidine into proliferating cells,

Table 2. *Decoding Data Obtained from the 30 Darkest Beads*[a])

Bead No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Pos. 1:1–7	1	1	1	1	2	1	1	1	3	3	2	4	4	3	1
Pos. 2:8–14	8	8	8	8	8	9	8	10	11	8	8	11	X	X	11
Pos. 3:15–21	15	16	18	17	18	15	15	17	17	16	18	18	18	18	18
Bead No.	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Pos. 1:1–7	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1
Pos. 2:8–14	11	12	11	8	11	8	8	8	X	13	8	8	10	8	8
Pos. 3:15–21	17	16	16	17	18	16	18	17	16	16	16	16	16	16	17

[a]) X: Decoding uncertain.

Position 1	Position 2	Position 3
22 × monomer **1**	16 × monomer **8**	11 × monomer **16**
3 × monomer **2**	6 × monomer **11**	9 × monomer **18**
3 × monomer **3**	2 × monomer **10**	7 × monomer **17**
2 × monomer **4**	1 × monomer **9**	3 × monomer **15**
	1 × monomer **12**	
	1 × monomer **13**	

[23]) Cyclophilin A and cyclophilin B behave similarly.

with an uninhibited control signal). IC_{50} values are estimated from inhibition of proliferation observed at various ligand concentration.

3.2.5. *Preparation and Screening of a Small Molecular Library.* An encoded combinatorial library has been prepared on 90-μm *TentaGel S* NH_2 resin beads by the split and mix procedure in 21 steps of synthesis altogether: with building blocks of types **1–7** in the first, **8–14** in the second, and **15–21** in the third synthesis round. The resin beads in all fractions were encoded using a combination of nine distinct reporter molecules. The $7^3 = 343$ variants were screened on-bead by the above mentioned binding assay. To this, the library beads were incubated with PBS buffer containing physiological salt concentration and the fluorescein-conjugated immunophilin for 3 min. Subsequently, the beads were washed several times with PBS buffer and detergent (tween 20). The washed beads were resuspended, distributed among microscopic slides, and visualized using a microscope with a fluorescence illuminator. Highly fluorescent beads (30 out of *ca.* 1,000) were isolated. They were removed under a microscope with the aid of a syringe, their molecular reporters decoded by electron-capture GC as described in [60] to specify the selected ligands. Decoding revealed a *consensus sequence*: position 1 being occupied by monomer *rac*-**1** in 22, position 2 by monomer *rac*-**8** in 16, and position 3 by monomer **16** in 11 out of 30 resin beads (*Table 2* and *Fig. 8*).

The resynthesized trimer (with a C-terminal carbamoyl instead of a carboxy group), containing the residues of *rac*-**1**, *rac*-**8**, and **16** in positions 1, 2, and 3, showed a low

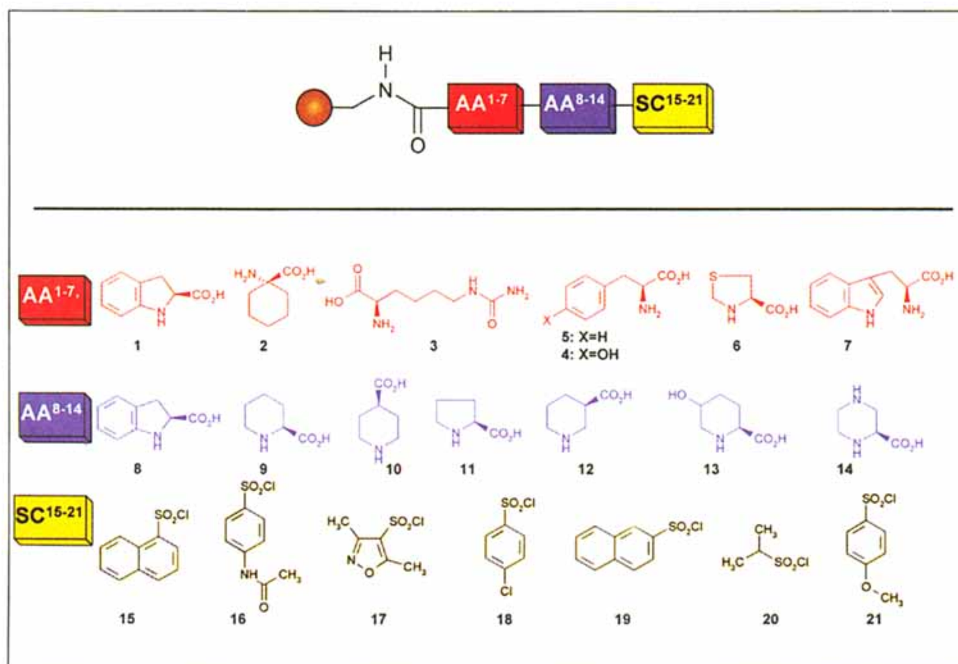

Fig. 8. *Building blocks for the preparation of 343 trimers by encoded combinatorial synthesis on resin beads*

potency to inhibit *PPI* activity. A 10 μM solution, however, inhibited the *IL-2*-dependent T cell proliferation as effectively as *CsA* (77%). Although the physiological function of cyclophilins is not well understood [70], there is no denying the fact that the rotamase activity of immunophilins is not an essential part of T cell proliferation [71]. Further studies have to reveal whether the newly found inhibitor of *IL-2* synthesis and T-cell proliferation is more specific than, say, *CsA*.

This work was supported by the *Bundesminister für Bildung, Wissenschaft, Forschung und Technologie* (Project No. 0311030). We thank Prof. *Axel Kleemann*, *ASTA Medica AG*, for his active interest in establishing and running the joint postdoctoral research program. We are most grateful to Prof. *Christian Noe*, Frankfurt University, who invited us to practice combinatorial chemistry in his *Institut für Pharmazeutische Chemie*. The experiments carried out by *Holger Deppe*, *Hans-Günther Schaible*, *Klaus Paulini*, and *Bertram Cezanne* will be published in detail in the near future.

REFERENCES

[1] E. Fischer, *J. Chem. Soc.* **1907**, 1749.
[2] S. L. Schreiber, K. C. Nicolaou, *Chem. Biol.* **1996**, *3*, 1.
[3] G. Quinkert, E. Egert, C. Griesinger, 'Aspects of Organic Chemistry: Structure', Verlag Helvetica Chimica Acta, Basel, 1996.
[4] A. Kornberg, *Biochemistry* **1987**, *26*, 6888; *Chemistry Biology* **1996**, *3*, 3.
[5] W. Johannsen, 'Elemente der exakten Erblichkeitslehre', Gustav Fischer, Jena, 1909.
[6] J. H. van't Hoff, 'Die Lagerung der Atome im Raume', 3rd edn., F. Vieweg und Sohn, Braunschweig, 1908.
[7] J. A. Le Bel, *Bl. Soc. Chim. Fr.* **1874**, *22*, 337.
[8] L. Pasteur, 'Leçons sur la dissymétrie moléculaire', Paris, 1861.
[9] A. v. Kekulé, *Z. Chem.* **1867**, *3*, 216.
[10] J. Wislicenus, 'Über die räumliche Anordnung der Atome in organischen Molekülen', 2. Aufl., Abhandl. der K. S. Ges. Wiss. 1889.
[11] E. Fischer, *Ber. Dtsch. Chem. Ges.* **1890**, *23*, 2114; *ibid.* **1890**, *24*, 2683; *ibid.* **1894**, *27*, 3189.
[12] D. H. R. Barton, in 'Perspectives in Organic Chemistry', Ed. A. Todd, Interscience Publ., New York, 1956, p. 68.
[13] V. Prelog, in 'Perspectives in Organic Chemistry', Ed. A. Todd, Interscience Publ., New York, 1956, p. 96.
[14] G. Mendel, 'Versuche über Pflanzenhybride', Verhandl. d. naturforsch. Vereins, Brünn, 1866.
[15] A. J. F. Griffiths, J. H. Miller, D. T. Suzuki, R. C. Lewontin, W. M. Gelbart, 'An Introduction to Genetic Analysis', 5th edn., Freeman and Comp., New York, 1993.
[16] W.-H. Li, D. Graur, 'Fundamentals of Molecular Evolution', Sinauer Assoc., Sunderland, Mass., 1991.
[17] I. Rechenberg, 'Evolutionsstrategie '94', Frommann-Holzboog, Stuttgart-Bad Cannstatt, 1994.
[18] V. Prelog, in 'Chemie und Gesellschaft', Ed. G. Boche, Wissenschaftliche Verlagsgesellschaft, Stuttgart, 1984, p. 57.
[19] E. Zuckerkandl, L. Pauling, *J. Theor. Biol.* **1965**, *8*, 357.
[20] R. H. Thomson, Ed., 'The Chemistry of Natural Products', Blackie, Glasgow, 1985.
[21] M. Eigen, *Naturwissenschaften* **1971**, *58*, 465; M. Eigen, P. Schuster, 'The Hypercycle', Springer-Verlag, Berlin, 1979; M. Eigen, J. McCaskill, P. Schuster, *Adv. Chem. Phys.* **1989**, *75*, 149; M. Eigen, R. Winkler-Oswatitsch, *Methods Enzymol.* **1990**, *183*, 505.
[22] L. Wittgenstein, 'Tractatus Logico-Philosophicus', 5th impress., Routledge + Kegan Paul, London, 1951.
[23] J. Maynard Smith, *Nature (London)* **1970**, *225*, 563.
[24] W. Fontana, FD.A.M. Konings, P. F. Stadler, P. Schuster, *Biopolymers* **1993**, *33*, 1389.
[25] J. Weiner, 'The Beak of the Finch – A Story of Evolution in Our Time', A. A. Knopf, New York, 1995.
[26] R. F. Gesteland, J. F. Atkins, Eds., 'The RNA World', Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1993.
[27] C. Weissmann, M. A. Billeter, H. M. Goodman, J. Hindley, H. Weber, *Ann. Rev. Biochem.* **1968**, *42*, 303.
[28] T. Blumenthal, G. G. Carmichael, *Ann. Rev. Biochem.* **1979**, *48*, 525.
[29] M. D. Moody, J. L. Burg, R. DiFrancesco, D. Loven, W. Stanik, J. Lin-Goerke, K. Mahdavi, Y. Wu, M. P. Farrell, *Biochemistry* **1994**, *33*, 13836.

[30] T. Nishihaea, D. R. Mills, F. R. Kramer, *J. Biochem.* **1983,** *93,* 669.
[31] C. Priano, F. R. Kramer, D. R. Mills, *Cold Spring Harbor Symp. Quant. Biol.* **1987,** *52,* 321.
[32] M. Eigen, C. K. Biebricher, M. Gebinoga, W. C. Gardiner, *Biochemistry* **1991,** *30,* 11005.
[33] R. C. Hardison, in 'Evolution at the Molecular Level', Eds. R. K. Selander, A. G. Clark, and T. S. Whittam, Sinauer Associates, Sunderland, MA, 1991, p. 272.
[34] C. R. Woese, in 'Evolution at the Molecular Level', Eds. K. Selander, A. G. Clark, and T. S. Whittam, Sinauer Associates, Sunderland, MA, 1991, p. 1.
[35] K. Bloch, 'Blondes in Venetian Paintings, the Nine- Banded Armadillo, and Other Essays in Biochemistry', Yale University Press, New Haven, 1994.
[36] G. Ourisson, Y. Nakatani, *Chem. Biol.* **1994,** *1,* 11.
[37] L. E. Orgel, 'The Origins of Life', Chapman & Hall, London, 1973; S. Miller, L. E. Orgel, 'The Origins of Life on the Earth', Prentice-Hall, Englewood Cliffs, 1974.
[38] A. Eschenmoser, *Origins Life Evol. Biosphere* **1994,** *24,* 389.
[39] L. E. Orgel, *J. Theor. Biol.* **1986,** *123,* 127; G. F. Joyce, A. W. Schwartz, S. L. Miller, L. E. Orgel, *Proc. Natl. Acad. Sci. U.S.A.* **1987,** *84,* 4398; G. F. Joyce, L. E. Orgel, in 'The RNA World', Eds. R. Gesteland and J. F. Atkins, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1993, p. 1.
[40] J. Oro, *Biochem. Biophys. Res. Commun.* **1960,** *2,* 407; J. P. Ferris, W. J. Hagan, *Tetrahedron* **1984,** *40,* 1093; S. L. Miller, *Cold Spring Harbor Symp. Quant. Biol.* **1987,** *52,* 17; J. P. Ferris, *ibid.* **1987,** *52,* 29; D. Müller, S. Pitsch, A. Kittaka, E. Wagner, C. E. Wintner, A. Eschenmoser, *Helv. Chim. Acta* **1990,** *73,* 1410.
[41] A. Eschenmoser, E. Loewenthal, *Chem. Soc. Rev.* **1992,** *21,* 1.
[42] S. Pitsch, E. Pombo-Villar, A. Eschenmoser, *Helv. Chim. Acta* **1994,** *77,* 2251.
[43] J. P. Ferris, A. R. Hill, R. Liu, L. E. Orgel, *Nature (London)* **1996,** *381,* 59.
[44] A. G. Cairns-Smith, Genetic Takeover, Cambridge University Press, Cambridge, 1982.
[45] L. E. Orgel, *Nature (London)* **1992,** *358,* 203; *Acc. Chem. Res.* **1995,** *28,* 109; G. F. Joyce, *Cold Spring Harbor Symp. Quant. Biol.* **1987,** *52,* 41.
[46] C. Böhler, P. E. Nielsen, L. E. Orgel, *Nature (London)* **1995,** *376,* 578.
[47] P. E. Nielsen, M. Egholm, R. H. Berg, O. Buchardt, *Science* **1991,** *254,* 1497.
[48] S. Pitsch, R. Krishnamurthy, M. Bolli, S. Wendeborn, A. Holzner, M. Minton, C. Lesueur, I. Schlönvogt, B. Jaun, A. Eschenmoser, *Helv. Chim. Acta* **1995,** *768,* 1621.
[49] A. Eschenmoser, M. V. Kısakürek, *Helv. Chim. Acta* **1996,** *79,* 1249.
[50] D. R. Mills, R. L. Peterson, S. Spiegelman, *Proc. Natl. Acad. Sci. U.S.A.* **1967,** *58,* 217; D. R. Mills, F. R. Kramer, S. Spiegelman, *Science* **1973,** *180,* 916.
[51] M. Eigen, *Cold Spring Harbor Symp. Quant. Biol.* **1987,** *52,* 307.
[52] G. F. Joyce, *Scient. Am.* **1992,** *267(6),* 48; A. A. Beaudry, G. F. Joyce, *Science* **1992,** *257,* 635; N. Lehman, G. F. Joyce, *Nature (London)* **1993,** *361,* 182; R. R. Breaker, G. F. Joyce, *Proc. Natl. Acad. Sci. U.S.A.* **1994,** *91,* 6093; Tsang, G. F. Joyce, *Biochemistry* **1994,** *33,* 5966.
[53] J. W. Szostak, *Trends Biochem. Sci.* **1992,** *267(6),* 48; D. P. Bartel, J. W. Szostak, *Science* **1993,** *261,* 1411; J. R. Lorsch, J. W. Szostak, *Nature (London)* **1994,** *371,* 31; M. Famulok, J. W. Szostak, *Angew. Chem. Int. Ed.* **1992,** *31,* 979.
[54] A. D. Ellington, *Methods Enzymol.* **1993,** *224,* 646; *Ber. Bunsenges. Phys. Chem.* **1994,** *98,* 1115.
[55] M. Famulok, *J. Am. Chem. Soc.* **1994,** *116,* 1698; P. Burgstaller, M. Famulok, *Angew. Chem. Int. Ed.* **1994,** *33,* 1084; M. G. Wallis, U. von Ahsen, R. Schroeder, M. Famulok, *Chem. Biol.* **1995** *2,* 543; P. Burgstaller, M. Kochoyan, M. Famulok, *Nucleic Acids Res.* **1995,** *23,* 4769; M. Famulok, A. Hüttenmhofer, *Biochemistry* **1996,** *35,* 4265; A. Geiger, P. Burgstaller, H. von der Eltz, A. Roeder, M. Famulok, *Nucleic Acid Res.* **1996,** *24,* 1029.
[56] M. C. Pirrung, *ChemTracts, Org. Chem.* **1993,** *6,* 88; M. A. Gallop, R. W. Barrett, W. J. Dower, S. P. A. Fodor, E. M. Gordon, *J. Med. Chem.* **1994,** *37,* 1233; E. M. Gordon, R. W. Barrett, W. J. Dower, S. P. A. Fodor, M. A. Gallop, *ibid.* **1994,** *37,* 1385; E. R. Felder, *Chimia* **1994,** *48,* 531; K. D. Janda, *Proc. Natl. Acad. Sci. U.S.A.* **1994,** *91,* 10779; K. D. Janda, *ibid.* **1994,** *91,* 10779; G. Quinkert, in 'High Tech – Das neue Gesicht der Arzneimittelforschung', Eds. H. J. Dengler and S. Meuer, Gustav Fischer Verlag, Stuttgart, 1995; G. Lowe, *Chem. Soc. Rev.* **1995,** *24,* 309; N. K. Terrett, M. Gardner, D. W. Gordon, R. J. Kobylecki, J. Steele, *Tetrahedron* **1995,** *51,* 8135; H. Han, M. M. Wolfe, S. Brenner, K. D. Janda, *Proc. Natl. Acad. Sci. U.S.A.* **1995,** *92,* 6419; V. Krchňák, M. Lebl, *Molecular Diversity* **1996,** *1,* 193.
[57] S. P. A. Fodor, J. Leighton Read, M. C. Pirrung, L. Stryer, A. T. Lu, D. Solas, *Science* **1991,** *251,* 767; C. P. Holmes, C. L. Adams, L. M. Kochersperger, R. B. Mortensen, L. A. Aldwin, *Biopolymers* **1995,** *37,* 199.
[58] M. Lebl, V. Krchnák, N. F. Sepetov, B. Seligmann, P. Strop, S. Felder, *Biopolymers* **1995,** *37,* 177.

[59] S. Brenner, R. Lerner, *Proc. Natl. Acad. Sci. U.S.A.* **1992,** *89,* 5381.
[60] M. J. H. Ohlmeyer, R. N. Swanson, L. W. Dillard, J. C. Reader, G. Asouline, R. Kobayashi, M. Wigler, W. C. Still, *Proc. Natl. Acad. Sci. U.S.A.* **1993,** *90,* 10922; W. C. Still, *Acc. Chem. Res.* **1996,** *29,* 155.
[61] E. Bayer, *Angew. Chem. Int. Ed.* **1991,** *30,* 113; J. S. Früchtel, G. Jung, *ibid.* **1996,** *35,* 17.
[62] P.-L. Zhao, R. Zambias, J. A. Bolognese, D. Boulton, K. Chapman, *Proc. Natl. Acad. Sci. U.S.A.* **1995,** *92,* 10212; P.-L. Zhao, R. B. Nachbar, J. A. Bolognese, K. Chapman, *J. Med. Chem.* **1996,** *39,* 350.
[63] J. Black, in 'Nobel Lectures Physiology or Medecine 1981–1990', Eds. T. Frängsmyr and J. Lindsten, World Scientific, Singapore, 1993, p. 418.
[64] C. J. Corrigan, A. B. Kay, *Immunol. Today* **1992,** *13,* 501; S. Holgate, *Thorax* **1993,** *48,* 103; D. S. Robinson, S. R. Durham, A. B. Kay, *ibid.* **1993,** *48,* 845; W. H. Parsons, N. H. Sigal, M. J. Wyvratt, *Ann. N. Y. Acad. Sci.* **1993,** *685,* 22.
[65] A. G. Alexander, N. C. Barnes, A. B. Kay, *Lancet* **1992,** *339,* 324; A. G. Alexander, C. J. Corrigan, P. Jardieu, N. C. Barnes, A. B. Kay, *Am. Rev. Respir. Dis* **1993,** A981.
[66] S. L. Schreiber, J. Liu, M. W. Albers, M. K. Rosen, R. F. Standaert, T. J. Wandless, P. K. Somers, *Tetrahedron* **1992,** *48,* 2545; M. K. Rosen, S. L. Schreiber, *Angew. Chem. Int. Ed.* **1992,** *31,* 401; G. R. Crabtree, N. A. Clipstone, *Annu. Rev. Biochem.* **1994,** *63,* 1045; J. P. Griffith, J. L. Kim, E. E. Kim, M. D. Sintchak, J. A. Thomson, M. J. Fitzgibbon, M. A. Fleming, P. R. Caron, K. Hsiao, M. A. Navia, *Cell* **1995,** *82,* 507; G. Fischer, *Angew. Chem. Int. Ed.* **1994,** *33,* 1415; *Nachr. Chem. Techn. Lab.* **1994,** *42,* 987.
[67] C. A. Janeway, P. Travers, 'Immunologie', Spektrum Akademischer Verlag, Heidelberg, 1995.
[68] H. Yu, J. K. Chen, S. Feng, D. C. Dalgarno, A. W. Brauer, S. L. Schreiber, *Cell* **1994,** *76,* 933.
[69] G. Fischer, H. Bang, C. Mech, *Biomed. Biochim. Acta* **1984,** *10,* 1101.
[70] A. Matouschek, S. Rospert, K. Schmid, B. S. Glick, G. Schatz, *Proc. Natl. Acad. Sci. U.S.A.* **1995,** *92,* 6319.
[71] S. L. Schreiber, *Science* **1991,** *251,* 283.