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UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION
INTERNATIONAL CENTRE FOR SCIENCE AND HIGH TECHNOLOGY, TRIESTE, ITALY

AMBASCIATA D'ITALIA BUDAPEST. HUNGARY

EÖTVÖS UNIVERSITY, BUDAPEST

ICS-UNIDO Workshop on

Trends and Applications of Combinatorial Chemistry and Combinatorial Technologies

Budapest, Hungary 15–18 October, 2001

Co-sponsors:
Bayer AG, Germany
Lab-Comp Kft, Hungary
Merck Kft., Hungary
Chinoin Rt., Hungary
Spectrum-3D, Hungary
Mettler Toledo Kft., Hungary
Reanal Rt., Hungary

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION
INTERNATIONAL CENTRE FOR SCIENCE AND HIGH TECHNOLOGY

supported by the Italian Embassy in Budapest

AMBASCIATA D'ITALIA BUDAPEST

SUNDAY

October 14, 2001

12:00 – 18:00 REGISTRATION

Eötvös University, Faculty of Science
Chemistry Building, Gate 1/A
Pázmány Péter sétány 1/A
Budapest, H-1117

18:00 DEPARTURE FOR HOTEL AURA (Symposium venue)

Methodology and Information Centre for In-service Teacher Traning of the Ministry of Education
Pilisborosjenô Fô út 1.
Pilisborosjenô, H-2097

19:00 HOTEL REGISTRATION
19:15 Dinner

MONDAY

October 15, 2001

7:30 – 8:30 Breakfast

8:30 – 9:00 **Stanislav MIERTUS** *(ICS-UNIDO, Trieste, Italy)*
ICS-UNIDO Programmes – An Introduction

9:00 – 10:45 **Giorgio FASSINA** *(Xeptagen SpA, Naples, Italy)*
Combinatorial Technologies – An Overview

10:45 – 11:00 Coffee Break

11:00 – 12:45 **Alexey, ELISEEV** *(State University of New York, Buffalo, NY, USA)*
Dynamic Combinatorial Libraries

12:45 – 13:15 Discussion

13:15 – 14:15 Lunch

14:15 – 16:15 **Claude MIRODATOS** *(CNRS, Villeurbanne, France)*
Combinatorial Optimization of Heterogenous Catalysis

16:15 – 16:30 Coffee Break

17:00 – **WELCOME RECEPTION**

In the Aula of the Methodology and Information Centre for In-service Teacher Training of the Ministry of Education,
Pilisborosjenô, Fô út 1.

TUESDAY

October 16, 2001

7:30 – 8:30 Breakfast

8:30 – 9:30 **Wolfgang BENDER** *(Bayer AG, Wuppertal, Germany)*
The Bayer Synthon Concept

9:30 – 10:30 **Ferenc HUDECZ** *(Hungarian Academy of Sciences, Budapest, Hungary)*
Application of MS for Library Characterization

10:30 – 10:45 Coffee Break

10:45 – 11:45 **Giorgio FASSINA** *(Xeptagen SpA, Naples, Italy)*
Biological Methods for Library Characterization and Screening

11:45 – 12:45 **István T. HORVÁTH** *(Eötvös University, Budapest, Hungary)*
Application of Fluorous Biphase Chemistry in Combinatorial Technology

12:45 – 13:15 Discussion

13:15 – 14:15 Lunch

14:15 – 15:15 **István GREINER** *(Richter Gedeon, Budapest, Hungary)*
Robotics & Lab Automation

15:15 – 16:15 **László KOVÁCS** *(InFarmatik, Budapest, Hungary)*
Combinatorial Process Research & Development

16:15 – 16:30 Coffee Break

16:30 – 18:30 **Wolfram ALTENHOFEN** *(Chemical Computing Group, Lörrach, Germany)*
QSAR Modelling to Library Design Strategies

18:30 – 19:30 Dinner

19:30 – **ROUND-TABLE DISCUSSION**

WEDNESDAY

October 17, 2001

7:30 – 8:30 Breakfast

8:30-9:30 **Menotti RUVO** *(Xeptagen SpA, Naples, Italy)*
Combinatorial Chemistry in Biotechnology - A Case Study

9:30-10:30 **Béla NOSZÁL** *(Semmelweis University, Budapest, Hungary)*
Combinatorial Phenomena in Biological Systems

10:30 – 10:45 Coffee Break

10:45-12:45 **Pierfausto SENECI** *(NAD AG, München, Germany)*
Molecular Diversity in Drug Discovery: A Critical Assessment

12:45 – 13:15 Discussion

13:15 – 14:15 Lunch

14:15 – 16:15 **Aubrey MENDONCA** *(Polymer Laboratories, Amherst, MA, USA)*
Solid Phase Synthesis – An Overview

16:15 – 16:30 Coffee Break

16:30 – 17:30 **Aubrey MENDONCA** *(Polymer Laboratories, Amherst, MA, USA)*
Solid Phase Synthesis – Recent Developments in Resin Technology

17:30 – 18:30 **Péter ARÁNYI** (Chinoin-Sanoffi, Budapest, Hungary)
Role of Combinatorial Chemistry in Original Drug Discovery

18:30 – 19:30 Dinner

19:30 – **ROUND-TABLE DISCUSSION**

THURSDAY

October 18, 2001

7:30 – 8:30 Breakfast

8:30 – 10:15 **Ian E. MAXWELL** *(Avantium Technologies BV, Amsterdam, The Netherlands)*
High Throughput Technologies: An Exciting New Development in Process Chemistry Research and Development

10:15 – 10:30 Coffee Break

10:30 – 12:30 **György KÉRI** *(Semmelweis University, Budapest, Hungary)*
Rational Drug Design and Signal Transduction Therapy
11:30 – 12:30
12:30 – 13:30 **György DORMÁN** *(ComGenex, Budapest, Hungary)*
Good Quality Libraries (Predicted and Measured Parameters)

13:30 – 14:15 Lunch

14:15 – 15:45 **COUNTRY REPORT**

15:45 – 16:00 Coffee Break

16:00 – 17:30 **FOLLOW-UP SESSION**

17:30 – 18:30 **Árpád FURKA** *(Eötvös University, Budapest, Hungary)*
Twenty Years in Combinatorial Chemistry

18:30 – **BANQUETTE**

Combinatorial Technologies – An Overview

Giorgio Fassina
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fassina@xeptagen.com

COMBINATORIAL TECHNOLOGIES – AN OVERVIEW

Giorgio Fassina
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fassina@xeptagen.com

The time and cost needed for the development of new drugs have increased steadily during the past three decades. Estimated costs for introducing a new drug in the market now reach around 200-300 millions USD, and this process takes around 10-12 years after discovery. This increase in time and cost is due mainly to the extensive clinical studies of new chemical entities required by competent regulatory agencies, such as the FDA, and to a lesser extend to the increased costs associated to research. The time and cost required for clinical and pre clinical evaluation of new drugs is not likely to decrease in the near future, and as a consequence, a key issue for pharmaceutical companies to stay in the market has been to increase the number of new drugs in the development pipeline. Drug discovery in the past has been based traditionally on the random screening of collection of chemically synthesized compounds or extracts derived from natural sources, such as microorganisms, bacteria, fungi, plants, of terrestrial or marine origin or by modifications of chemicals with known physiological activities. This approach has resulted in many important drugs, however the ratio of novel to previously discovered compounds has diminished with time. In addition, this process is very time consuming and expensive.
A limiting factor was linked to the restricted number of molecules available or extract samples to be screened, since the success rate in obtaining useful lead candidates depends directly from the number of samples tested. Chemical synthesis of new chemical entities often is a very laborious task, and additional time is required for purification and chemical characterization. The average cost of creating a new molecular entity in a pharmaceutical company is around 7500 USD/compound. Generation of natural extracts, while very often providing interesting new molecular structures endowed with biological properties, leads to mixtures of different compounds at different concentrations, thus making activity comparisons very difficult. In addition, once activity is found on a specific assay, the extract needs to be fractionated in order to identify the active component. Quite often, the chemical synthesis of natural compounds is extremely difficult, thus making the lead development in to a new drug a very complex task. While the pharmaceutical industry was demanding more rapid and cost effective approaches to lead discovery, the advent of new methodologies in molecular biology, biochemistry, and genetic, leading to the identification and production of an ever increasing number enzymes, proteins, receptors, involved in biological processes of pharmacological relevance, and good candidates for the development of screening assay, complicated even more this scenario. The introduction of combinatorial technologies provided an unlimited source of new compounds, capable to satisfy all these needs. This approach was so appealing and full of promises that many small companies started to flourish financed by capitals raised from private investors.

Combinatorial approaches were originally based on the premise that the probability of finding a molecule in a random screening process is proportional to the number of molecules subjected to the screening process. In its earliest expression, the primary objective of combinatorial chemistry focused on the simultaneous generation of large numbers of molecules and on the simultaneous screening of their activity. Following this approach, the success rate to identify new leads is greatly enhanced, while the time required is considerably reduced.
The development of new processes for the generation of collection of structurally related compounds (libraries) with the introduction of combinatorial approaches has revitalized

random screening as a paradigm for drug discovery and has raised enormous excitement about the possibility of finding new and valuable drugs in short times and at reasonable costs. However the advent of this new field in drug discovery did not obscure the importance of "classical" medicinal chemistry approaches, such as computer-aided rational drug design and QSAR, for example, but catalyzed instead their evolution to complement and integrate with combinatorial technologies.

The word "combinatorial" appeared in the scientific literature at the beginning of the '90, but the generation of the first combinatorial libraries can be dated back to the beginning of the '80. The first reports dealt with the simultaneous production of collection of chemically synthesized peptides, produced by solid phase methods on solid supports. Peptides were particularly suited for combinatorial synthesis given the will established synthetic protocols available, the great number of different molecules attainable, and the potential to generate leads of biological and pharmaceutical value. The use of peptide libraries was greatly accelerated by the introduction of biological methods for library preparation, by the use of the phage display technology, which provided interesting advantages over the synthetic counterpart. At the same time, the first papers on the generation of oligonucleotide libraries appeared in the literature, thus suggesting the possibility to extend the applicability of combinatorial approaches even to other classes of synthetic or natural oligomeric compounds, such as carbohydrates. A broad variety of new synthesis and screening methods are currently grouped under the term combinatorial. These methods include parallel chemical synthesis and testing of multiple individual compounds or compounds mixtures in solution, synthesis, and testing of compounds on solid supports, and biochemical or organism-based synthesis of biological oligomers coupled to selection and amplification strategies. Combinatorial technologies merged different disciplines such as solid phase and solution phase chemistry, analytical chemistry, molecular biology, molecular design, automation and miniaturization in an integrated platform technology. The philosophy of combinatorial technologies is to make rational the random approach to drug discovery. The main advantage of using combinatorial technologies is the speed in finding and optimizing useful leads. The disadvantage is that it is impossible to explore the entire chemical space in the combinatorial format, i.e. not all chemical structures can be produced by combinatorial approaches. Combinatorial technologies can now be considered an integrated approach for novel compounds discovery, where chemistry, molecular design and screening are the essential components. Molecular design methods have reached a satisfactory level in helping the design of rational libraries. To the same extent, hardware for libraries generation and screening has reached a good level of implementation. Existing screening assays still need implementation, to be more representative of the disease under investigation, and more sophisticated cell-based assays for screening replacing biochemical in vitro assays, by integrating modern molecular biology approaches to pharmacology, are needed. The bottleneck for the development of new drugs is not anymore related to the capacity to synthesize and screen large number of compounds, but to select out of a large number of leads only few candidates already endowed with the best characteristics for clinical development.

Development of assays, and/or computational methods to determine preliminary pharmacodynamic / pharmacocinetic / toxicology parameters applicable to large numbers of compounds represent an emerging research trend in CCCT

The different technologies and strategies used in the production of combinatorial libraries are now so well developed that is easy to plan synthetic schemes for the generation of a huge number of compounds. Since the rate at which compounds can be screened does constitute a limitation to the use of combinatorial technologies, it is important to be selective about the compounds, which are synthesized. Computational methods are very

valuable from this point of view to assist in the design of combinatorial libraries. The main requirement for lead generation is often to maximize the range of structural types within the library with the expectation that a broad range of activities will result. As a consequence, diversity analysis is an important aspect of library design. The diversity of libraries may be measured by the use of similarity or dissimilarity indexes, which make intermolecular comparisons possible. Measures of chemical similarity have been developed for similarity searching in chemical databases. The calculation of the similarity between two molecules involves the characterization of the molecules by using chemical/structural descriptors, and then the application of similarity coefficients to quantify the similarity.

In combinatorial chemistry, due to the high number of chemical manipulations required to synthesize libraries of compounds automation is unavoidable. Many research groups, both in academia and industrial settings are developing automated instruments specifically tailored to these needs, and this technology field is acquiring an extremely important role for the development of combinatorial technologies for the next millennium. On the other hand, the huge number of compounds produced simultaneously with these technologies requires automation also in purification protocols, quality assessment, sample dispensing and testing. In addition, the ever increasing number of compounds generated by combinatorial technologies pushes towards miniaturization of screening assays, in order to handle an increasing number of tests at the same time with little consumption of reagents. The rapidity of new chemical entity generation and screening allows validation of molecular targets associated to diseases in short time. This is a very important emerging trend in combinatorial technologies, since the advent of new methodologies in molecular biology, biochemistry, and genetic, leads to the identification of many factors which should be screened quickly in order to define their relevance to biomedical processes. With the increased speed at which new drug entities are now synthesized and evaluated for pharmacological activity, a need has arisen to provide fundamental metabolism data at the early stages of drug discovery. Strategies are being developed to permit drug metabolism data to be an important part of early drug discovery. Many important properties of drugs related to metabolism could be the deciding factor in whether or not a compound is selected for clinical development, and application of combinatorial approaches to such assessments is emerging as a new trend of application.

Many active compounds have been selected to date following combinatorial methodologies, and a considerable number of those have progresses in to clinical trials. However, combinatorial chemistry (CC) and related technologies for producing and screening large number of molecules find useful applications also in other industrial sectors not necessarily related to pharmaceutical industry. Emerging fields of application of combinatorial technologies are the diagnostic, the down-stream processing, the catalysis, and the new material sectors.

SUMMARY

Combinatorial approaches were originally based on the premise that the probability of finding a molecule in a random screening process is proportional to the number of molecules subjected to the screening process. In its earliest expression, the primary objective of combinatorial chemistry focused on the simultaneous generation of large numbers of molecules and on the simultaneous screening of their activity. Following this approach, the success rate to identify new leads is greatly enhanced, while the time required is considerably reduced.
The development of new processes for the generation of collection of structurally related compounds (libraries) with the introduction of combinatorial approaches has revitalized random screening as a paradigm for drug discovery and has raised enormous excitement about the possibility of finding new and valuable drugs in short times and at reasonable costs

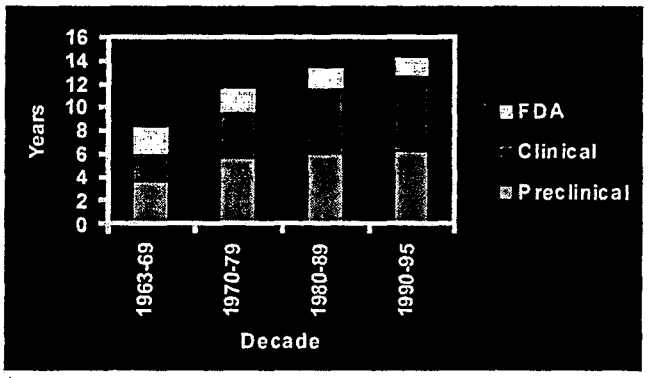
OLD WAY OF DRUG DISCOVERY

- Find "Lead Compound"
- Improve potency
- Improve selectivity
- Go to bioavailibity studies
- Go to short-term toxicology studies

INTRODUCTION

Drug discovery in the past has been based traditionally on the random screening of collection of chemically synthesized compounds or extracts derived from natural sources, such as microorganisms, bacteria, fungi, plants, of terrestrial or marine origin or by modifications of chemicals with known physiological activities. This approach has resulted in many important drugs, however the ratio of novel to previously discovered compounds has diminished with time. In addition, this process is very time consuming and expensive. A limiting factor was linked to the restricted number of molecules available or extract samples to be screened, since the success rate in obtaining useful lead candidates depends directly from the number of samples tested. Chemical synthesis of new chemical entities often is a very laborious task, and additional time is required for purification and chemical characterization. Generation of natural extracts, while very often providing interesting new molecular structures endowed with biological properties, leads to mixtures of different compounds at different concentrations, thus making activity comparisons very difficult. In addition, once activity is found on a specific assay, the extract needs to be fractionated in order to identify the active component. Quite often, the chemical synthesis of natural compounds is extremely difficult, thus making the lead development in to a new drug a very complex task. While the pharmaceutical industry was demanding more rapid and cost effective approaches to lead discovery, the advent of new methodologies in molecular biology, biochemistry, and genetic, leading to the identification and production of an ever increasing number enzymes, proteins, receptors, involved in biological processes of pharmacological relevance, and good candidates for the development of screening assay, complicated even more this scenario. The introduction of combinatorial technologies provided an unlimited source of new compounds, capable to satisfy all these needs.

TIME FOR DRUGS DEVELOPMENT



The time and cost needed for the development of new drugs have increased steadily during the past three decades (Figure 2). Estimated costs for introducing a new drug in the market now reach around 200-300 millions USD, and this process takes around 10-12 years after discovery. This increase in time and cost is due mainly to the extensive clinical studies of new chemical entities required by competent regulatory agencies, such as the FDA, and to a lesser extend to the increased costs associated to research. The time and cost required for clinical and preclinical evaluation of new drugs is not likely to decrease in the near future, and as a consequence, a key issue for pharmaceutical companies to stay in the market has been to increase the number of new drugs in the development pipeline.

FACTORS AFFECTING STRATEGY CHANGES IN DRUG DISCOVERY

1] BIOTECHNOLOGY (GENOMICS): provides molecular targets of therapeutic relevance (receptors, hormones, proteins).

2] COMBINATORIAL TECHNOLOGY: provide the possibility of generating huge collections of molecules which are simultaneously produced with a built-in decoding capability.

3] HIGH TRHOUGHPUT SCREENING (HTS): provides the possibility of handling many assays at the same time.

While the pharmaceutical industry was demanding more rapid and cost effective approaches to lead discovery, the advent of new methodologies in molecular biology, biochemistry, and genetic, leading to the identification and production of an ever increasing number enzymes, proteins, receptors, involved in biological processes of pharmacological relevance, and good candidates for the development of screening assay, complicated even more this scenario. The introduction of combinatorial technologies provided an unlimited source of new compounds, capable to satisfy all these needs.

SOURCES OF MOLECULAR DIVERSITY

- **Plants extracts**
- **Microbial extracts**
- **Collection of chemical compounds (synthetic)**
- **Oligonucleotide libraries (biological or synthetic)**
- **Oligosaccharide libraries**
- **Chemical compounds libraries (synthetic)**
- **Peptide libraries (biological or synthetic)**

LIBRARIES

Collection of structurally related compounds (peptides, oligonucleotides, oligosaccharides, organic molecules) obtainable by chemical or biological means simultaneously as a mixture and screened for activity as a mixture of compounds, without any isolation step. Identification of active compounds derives from the synthesis/production protocol used to generate the library. Great acceleration of leads identification since millions of different compounds can be screened simultaneously. Combinatorial approaches were originally based on the premise that the probability of finding a molecule in a random screening process is proportional to the number of molecules subjected to the screening process. In its earliest expression, the primary objective of combinatorial chemistry focused on the simultaneous generation of large numbers of molecules and on the simultaneous screening of their activity.

XEPA GEN

COMBINATORIAL CHEMISTRY

• ON SOLID PHASE

- **large excess of reagents allowed**
- **multistep synthesis allowed**
- **easy workup-isolation**
- **mix and split possible**

• IN SOLUTION

- **all organic reactions can be used**
- **no chemistry assessment**
- **no linker/cleavage chemistry**
- **unlimited product quantities**

The development of new processes for the generation of collection of structurally related compounds (libraries) with the introduction of combinatorial approaches has revitalized random screening as a paradigm for drug discovery and has raised enormous excitement about the possibility of finding new and valuable drugs in short times and at reasonable costs. However the advent of this new field in drug discovery did not obscure the importance of "classical" medicinal chemistry approaches, such as computer-aided rational drug design and QSAR for example, but catalyzed instead their evolution to complement and integrate with combinatorial technologies.

XEPA GEN

PEPTIDES CHEMICAL DIVERSITY

Given a linear amino acid sequence of n residues
X_1-X_2-X_3-X_4-.....-X_n
the total number of different peptides obtainable equals to:

$$y^n$$

n = peptide length
y = number of different amino acids used in the synthesis (usually 18)

n = 3	**5.832 peptides**
n = 4	**104.976 peptides**
n = 5	**1.889.568 peptides**
n = 6	**34.012.224 peptides**

The word "combinatorial" appeared in the scientific literature at the beginning of the '90, but the generation of the first combinatorial libraries can be dated back to the beginning of the '80. The first reports dealt with the simultaneous production of collection of chemically synthesized peptides, produced by solid phase methods on solid supports . Peptides are particularly suited for combinatorial synthesis given the well established synthetic protocols available, the great number of different molecules attainable, and the potential to generate leads of biological and pharmaceutical value.

COMBINATORIAL & HTS TECHNOLOGIES

STRATEGIES

Conventional

- One molecule at a time
- Make → Purity → Test
- Hundreds of molecules a month
- Slower lead generation
- High risk of failure

Combinatorial

- Many molecules at a time
- Make → Test → Purity
- Thousands of molecules a month
- Faster lead generation
- Low risk of failure

Synergy

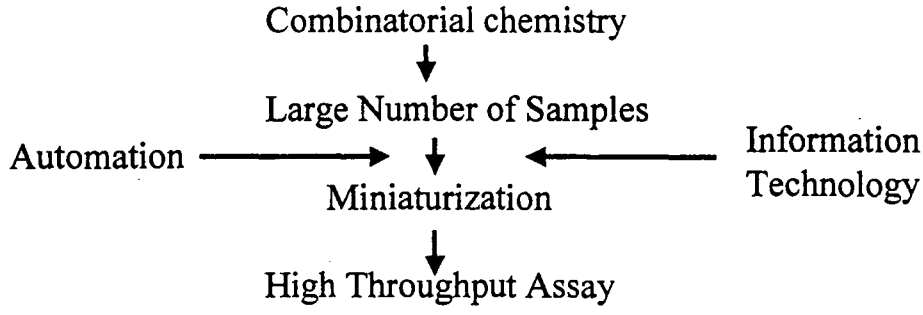
↓

LEAD IDENTIFICATION

•Leads identified by combinatorial approaches may be refined following classical medicinal chemistry.

•Conventional and combinatorial methodologies complement each other accelerating drug discovery.

HIGH THROUGHPUT SCREENING



• Combinatorial technologies merged different disciplines such as solid phase and solution phase chemistry, analytical chemistry, molecular biology, molecular design, automation and miniaturization in an integrated platform technology.

•Automation in Combinatorial Technologies speeds up lead identification

COMBINATORIAL DIVERSITY GENERATION

Oligomeric Space

Biological Oligomers

peptides
oligonucleotides
oligosaccharides

Chemical Oligomers

peptoides
PNA
vinylogous peptoids
tertiary amines
morpholinos
ethylene glycols
hidroxymethyl
pyrrolidinones
carbamates
pyrrolinones
β turn mimetics

Biological Oligomers

RNA
DNA
polysomes
modified DNA/RNA
random chemistry
oligomers

Biological Oligomers

Phage proteins
bacterial membrane proteins
peptide-plasmids

A broad variety of new synthesis and screening methods are currently grouped under the term combinatorial. These methods include parallel chemical synthesis and testing of multiple individual compounds or compounds mixtures in solution, synthesis, and testing of compounds on solid supports, and biochemical or organism-based synthesis of biological oligomers coupled to selection and amplification strategies. Combinatorial technologies merged different disciplines such as solid phase and solution phase chemistry, analytical chemistry, molecular biology, molecular design, automation and miniaturization in an integrated platform technology.

CHEMICAL DIVERSITY

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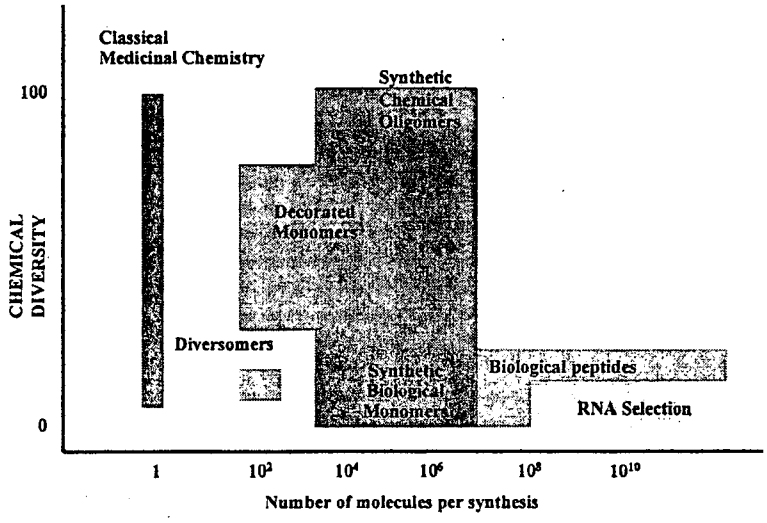
LIBRARIES: Chemical Diversity



The diversity of libraries may be measured by the use of similarity or dissimilarity indexes which make intermolecular comparisons possible. Measures of chemical similarity have been developed for similarity searching in chemical databases. The calculation of the similarity between two molecules involves the characterization of the molecules by using chemical/structural descriptors, and then the application of similarity coefficients to quantify the similarity.

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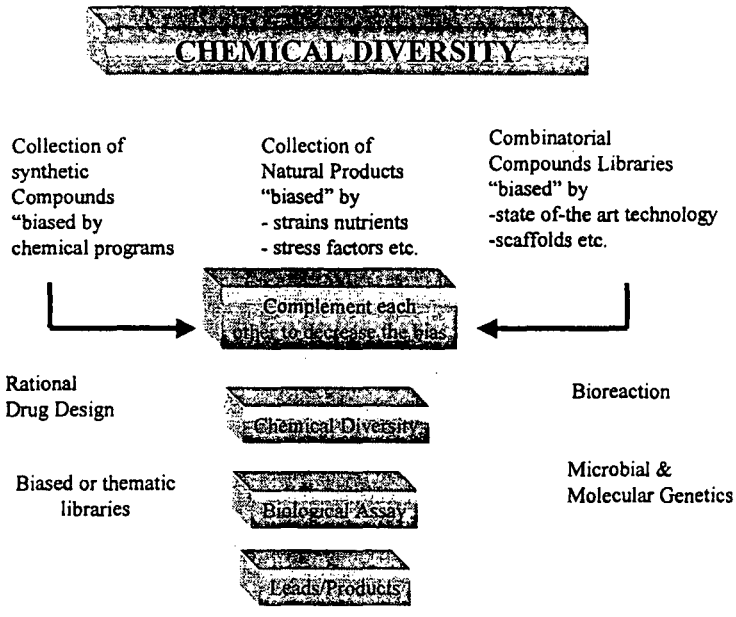

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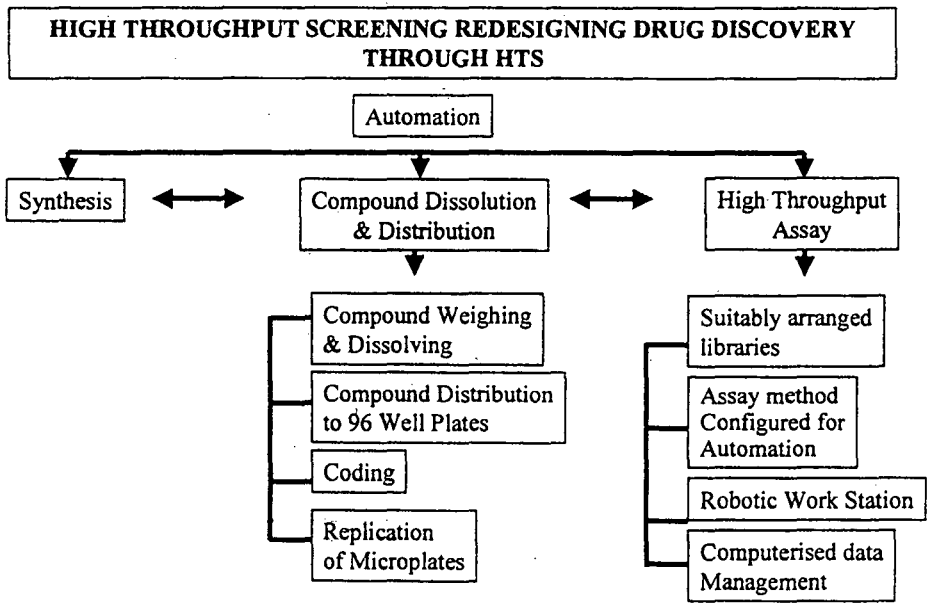
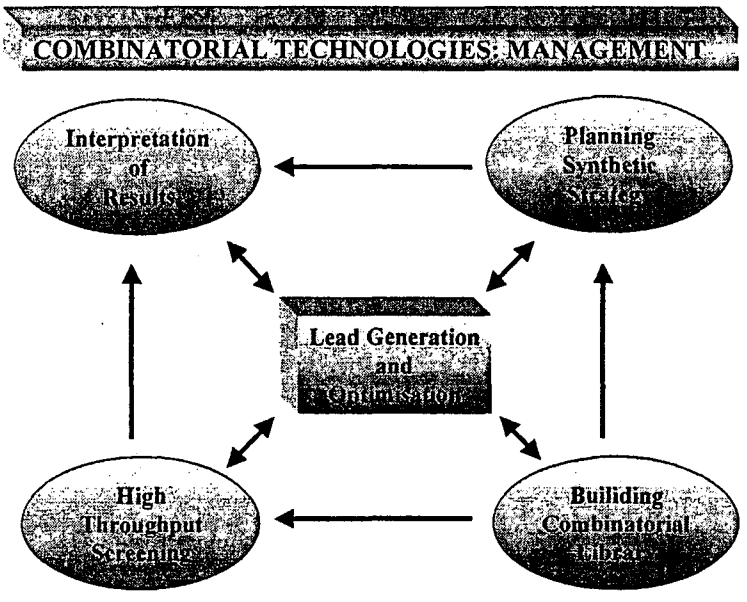


Due to the high number of chemical manipulations required to synthesize libraries of compounds automation is unavoidable.The huge number of compounds produced simultaneously with these technologies requires automation also in purification protocols, quality assessment, sample dispensing and testing. In addition, the ever increasing number of compounds generated by combinatorial technologies pushes towards miniaturization of screening assays, in order to handle an increasing number of tests at the same time with little consumption of reagents.



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Dynamic Combinatorial Chemistry

Alexey Eliseev
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DYNAMIC COMBINATORIAL CHEMISTRY

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The major effort of today's combinatorial chemistry is focused on the synthesis and screening of libraries of individual compounds. The alternative approach, use of mixtures (pools) of compounds, is significantly less labor and resource consuming, but requires elaborate analytical tools to identify effective components in complex mixtures.

This lecture will consider dynamic combinatorial chemistry (DCC), an approach to molecular diversity generation and screening that involves reorganization of pools of compounds, existing in a dynamic equilibrium, *via* their interactions with the target compound. Such reorganization results in the formation of amplified amounts of those components that form the strongest complexes with the target and thereby simplifies their isolation and identification. DCC offers a potentially new approach to drug discovery that combines library synthesis and screening in a single step and allows one to rapidly explore and customize pharmaceutical diversity space for a given target.

The following subjects will be considered in the presentation.

1) DCC as a general approach to synthesis and screening of combinatorial libraries: advantages and limitations as compared to parallel techniques.
A. Case studies of early examples of dynamic libraries. Bioactive peptides, cation receptors, inhibitors of carbonic anhydrase.
B. Mechanisms and quantitative assessment of amplification effect in dynamic libraries. Thermodynamic vs. kinetic effects.
C. Basic reactions used in DCC. Examples of imine exchange, transesterification, coordination chemistry,alkene metathesis.

2) DCC as emerging tool of drug discovery. Case study of neuraminidase inhibitors formed from *in vitro* virtual libraries.

3) Other applications of dynamic libraries.
A. Nucleic acid recognition.
B. Ion separation.

4) Methodological developments in DCC:
A. Dynamic deconvolution.
B. Multi-level dynamic libraries.
C. Analytical techniques: case study of regiochemical tagging.

Suggested Literature

1. A. Ganesan, *Angew. Chem. Int. Ed. Engl.* **37**, 2828-2831 (1998).
2. J. M. Lehn, *Chem. Eur. J.* **5**, 2455-2463 (1999).
3. J. M. Lehn, A. V. Eliseev, *Science* **291**, 2331-2332 (2001).

Combinatorial Optimization of Heterogenous Catalysis

Claude Mirodatos

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COMBINATORIAL APPROACHES FOR SPEEDING UP HETEROGENOUS CATALYST DISCOVERY AND OPTIMISATION: STRATEGIES AND PERSPECTIVES FOR ACADEMIC RESEARCH

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http://catalyse.univ-lyon1.fr

The application of combinatorial chemistry to heterogeneous catalysis is analysed in terms of current strategies and perspectives on the industrial and academic levels. Potential methodologies for academic research laboratories are proposed with emphasis on both theoretical and practical considerations.

As a case study, the European consortium "COMBICAT" "Catalyst Design and Optimisation by Fast Combinatorial Procedures" is presented focusing on the chosen strategy [1].

"COMBICAT" started on 01/01/00 is dedicated to the "Competitive and Sustainable Growth" EU programme. It mainly deals with the development of innovative combinatorial methods of fast preparation and high-speed testing of solid materials to be used as heterogeneous catalysts to reduce R&D time and costs. The new methods to be developed will be validated using a widespread of catalytic reaction categories of importance for European chemical industry.

In that consortium, 10 research partners (3 large companies, 2 SME, 4 research institutions, 1 university) from 6 European countries are grouped to fulfil the work program. The partners cover all point of views within the project: Research institutions with widespread basic knowledge on catalyst development, experienced SME´s as specialists for development of chemical research software and high-tech robotics hardware and large catalyst production companies as well as catalyst end users (engineering entities) of the European chemical industry.

Various aspects of the running research will be presented:
- analysis of the combinatorial approach to heterogeneous catalysis,
- strategies and technologies for secondary screening,
- preparation and testing of catalyst libraries : development of hard and software tools adapted to case studies
- strategies for a combinatorial approach of kinetic modelling, applied to transient operations.

As a general conclusion, once a certain level of confusion (in terms of concepts and strategies) is put aside, the combinatorial approach seems like a real opportunity to grasp in this initial phase of extension to the vast field of heterogeneous catalysis, and especially for academic research. The basic technical and theoretical tools of combinatorial catalysis already exist, and in the short term, advancements of varying degrees depending on the chemistry attempted can reasonably be envisioned. Though considerable human and material investments are necessary for the expansion of combinatorial catalysis, we must remember that this approach that combines *discovery and comprehension* is at the heart of the goals of research. In this way, it can only reinforce the creativity of our laboratories [2].

References :

[1] website: www.ec-combicat.org

[2] Combinatorial approaches to heterogeneous catalysis: strategies and perspectives for academic research, Arnold Holzwarth, Patricia Denton, Horst Zanthoff and Claude Mirodatos
Catalysis Today 2441 (2001) 1-10.

From Synthons to Bioactive Molecules: Efficient Strategies in Modern Lead Structure Research

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Major incurable diseases

Arteriosclerosis
Underlying disorder in cardiovascular disease; infarction/stroke as most common cause of death

Tumor diseases
Second most common cause of death

Alzheimer's disease/senile dementia
5-10% of the population over 65, high costs

Diabetes
Approx. 3 % of world population

Viral diseases
HIV, hepatitis, HCMV

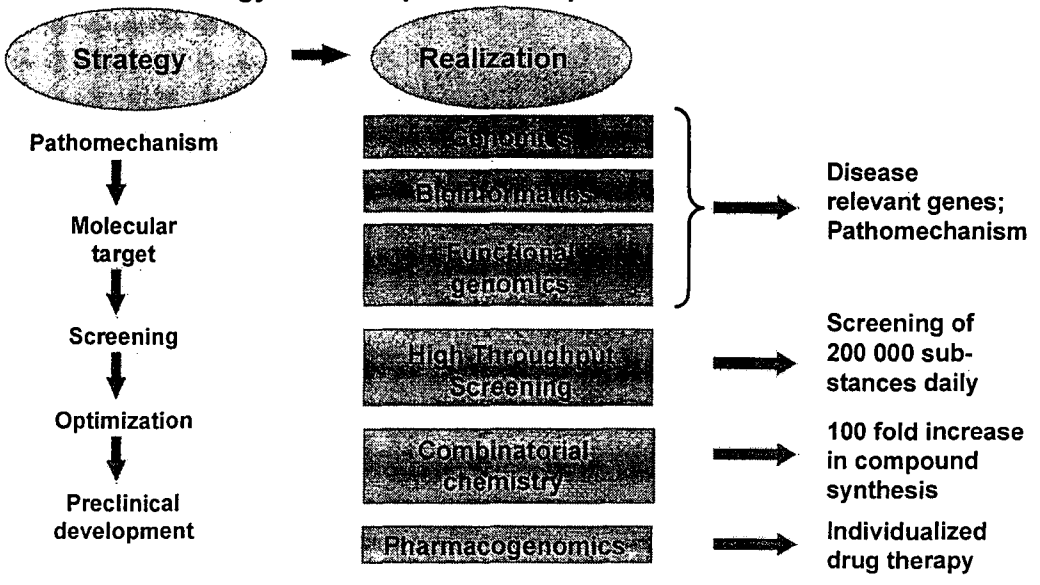
Rheumatic disorders
Leading cause of morbidity, health care expenditures and invalidity

Infectious diseases
Development of resistance

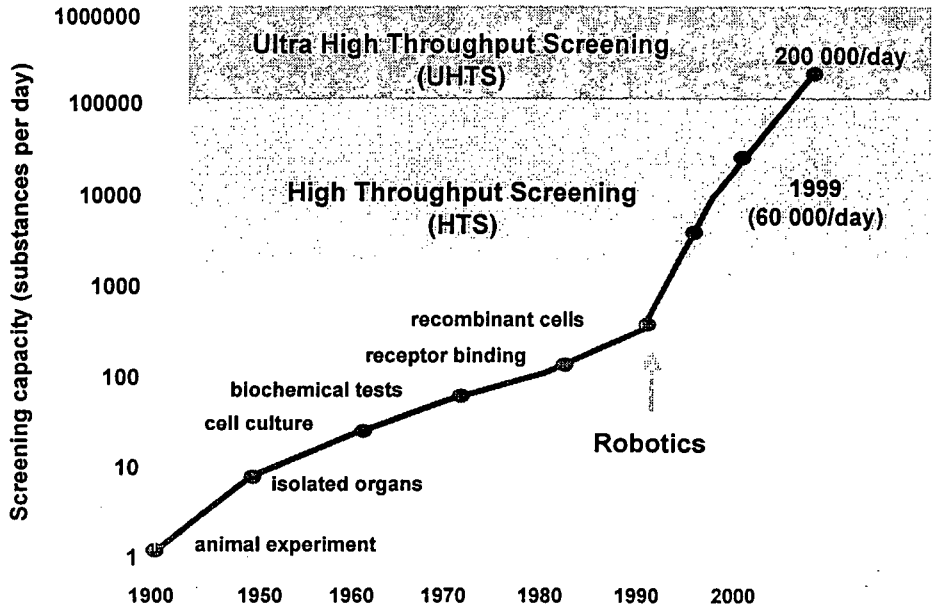
Allergies
Asthma, neurodermatitis

Discovery research is technology driven

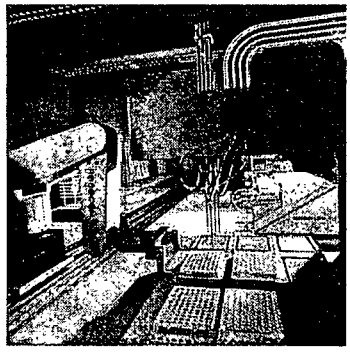
Technology leadership is most important factor of success

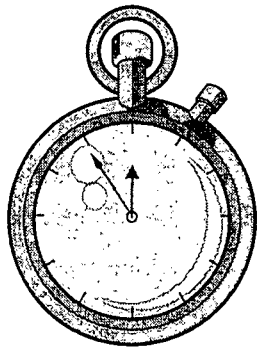


Explosion of Throughput in Drug Screening



Trends in "Actives" Research





the same commercially available "building blocks" and often create... very similar test compounds.

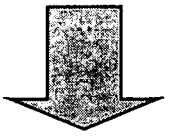
Test Compounds

and

Key Building Blocks

- From all corners of the company, hitherto never or seldom used

- For primary synthesis and resynthesis for the evaluation of potential hits



BAYER Synthons

- For the *de novo* creation of test compounds

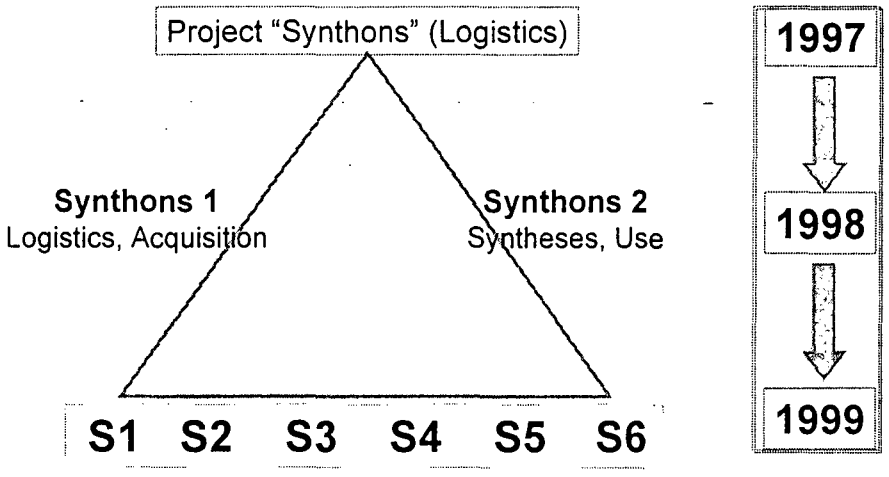
The BAYER Synthon Concept

What are BAYER Synthons?

All Compounds that are exclusively available to BAYER or that are not readily commercially available on grounds of cost or time.

These compounds must possess at least one derivatizable functional group or reactive centre.

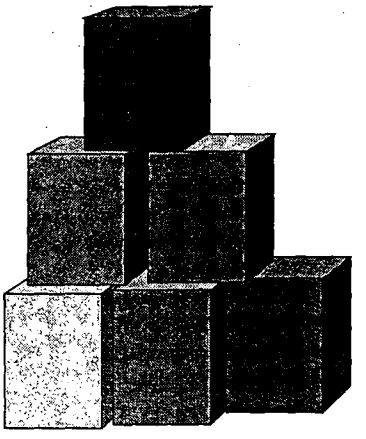
Chronology of the Synthon Concept



Main Projects 1999

- S1: Basic Project "Synthons"
- S2: Internal Synthon Syntheses
- S3: External Synthon Syntheses
- S4: Strategic Synthon Purchase
- S5: Molecular Diversity by
  Chemo- and Biocatalysis
- S6: Integrated Synthesis and Testing

Synthon Concept – Activities

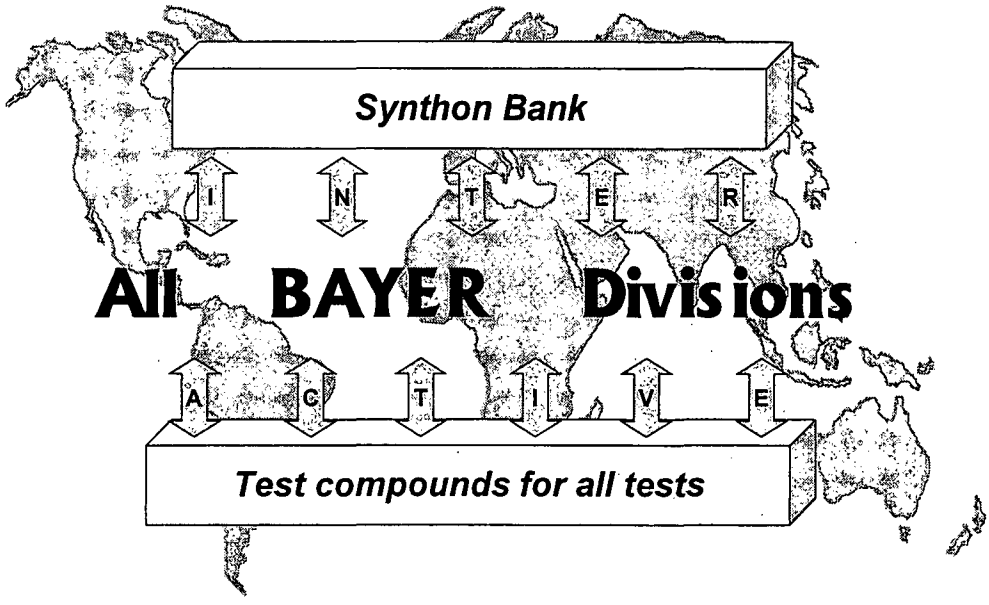


between all BAYER Divisions

- Strengthening of BAYER Life Science research through new exclusive synthons
- Preparation of a high diversity of test compounds through *online* microsyntheses with synthons

The Basis Project "Synthons"

- Interactive Depot Management
- Synthon Exchange
- Synthon Markets
- Coordination of internal and external Cooperation Networks
- Synthesis Planing
- Gap and Trend Analysis
- Biophore Models and Diversity



The Synthon Bank

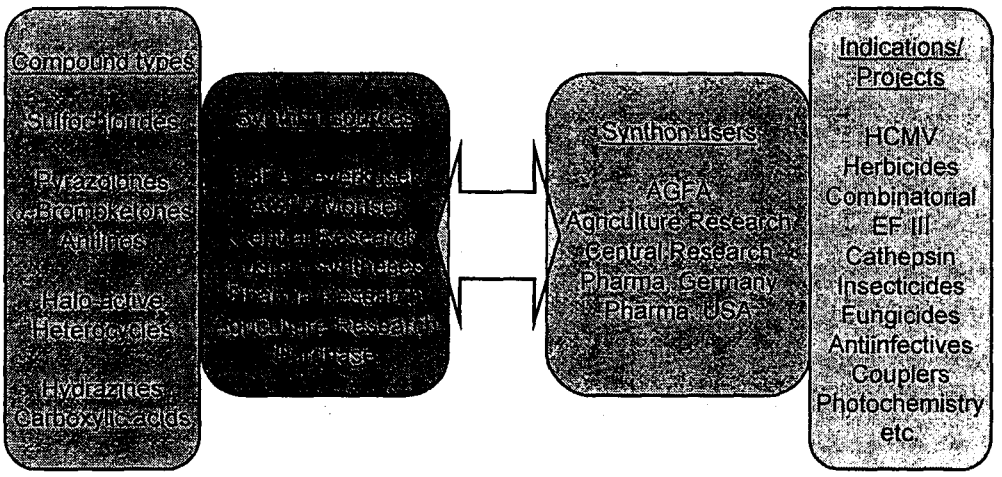
Internal Sources

- Old stocks ("Old Synthons")
- Process Development, Kilo Labs, Production
- Synthon labs from 1998
- All chemical laboratories within the Company

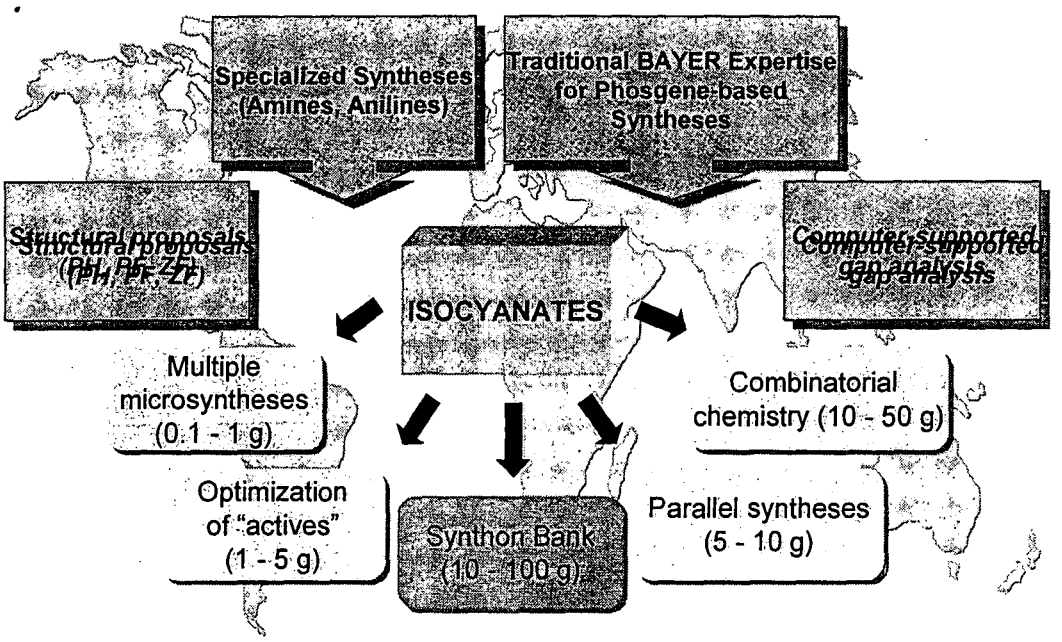
External Sources

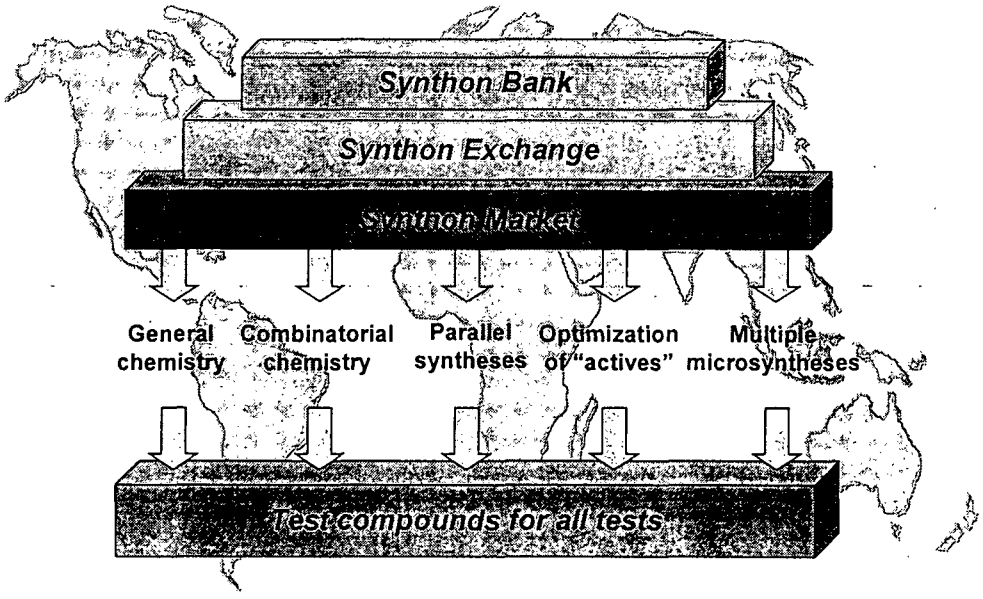
- Cooperation with Universities and their chemical collections
- Custom synthesis companies
- Specialty companies
- Selected big chemical companies

Synthon Exchange (Selected Examples)

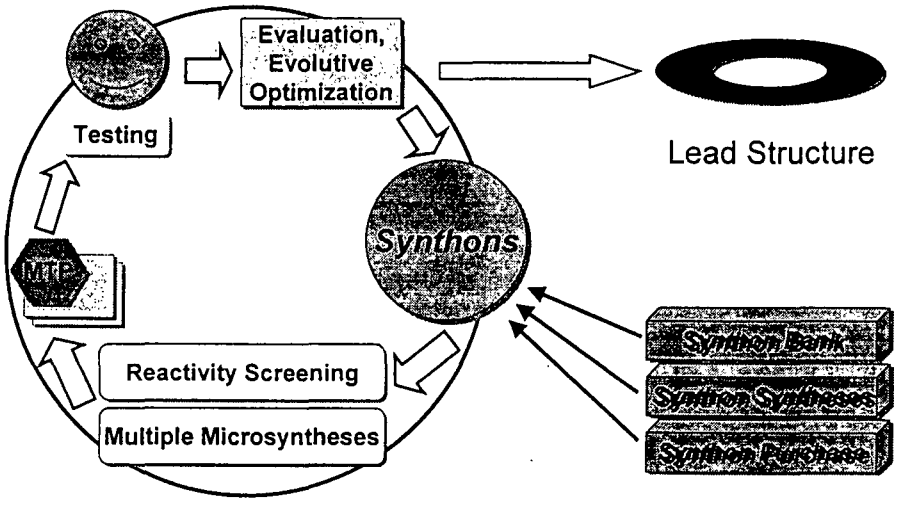


Synthon Market (Example)

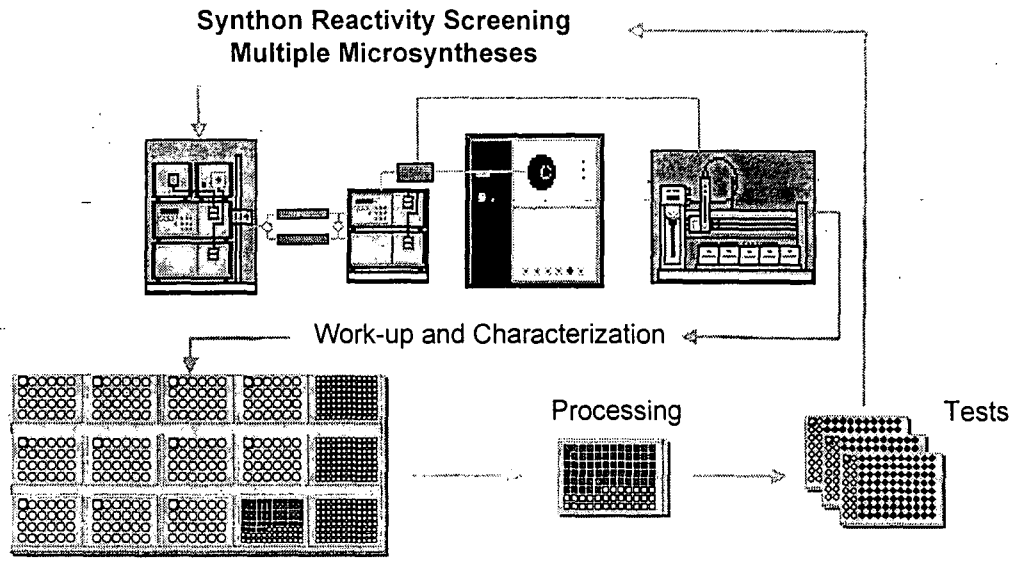


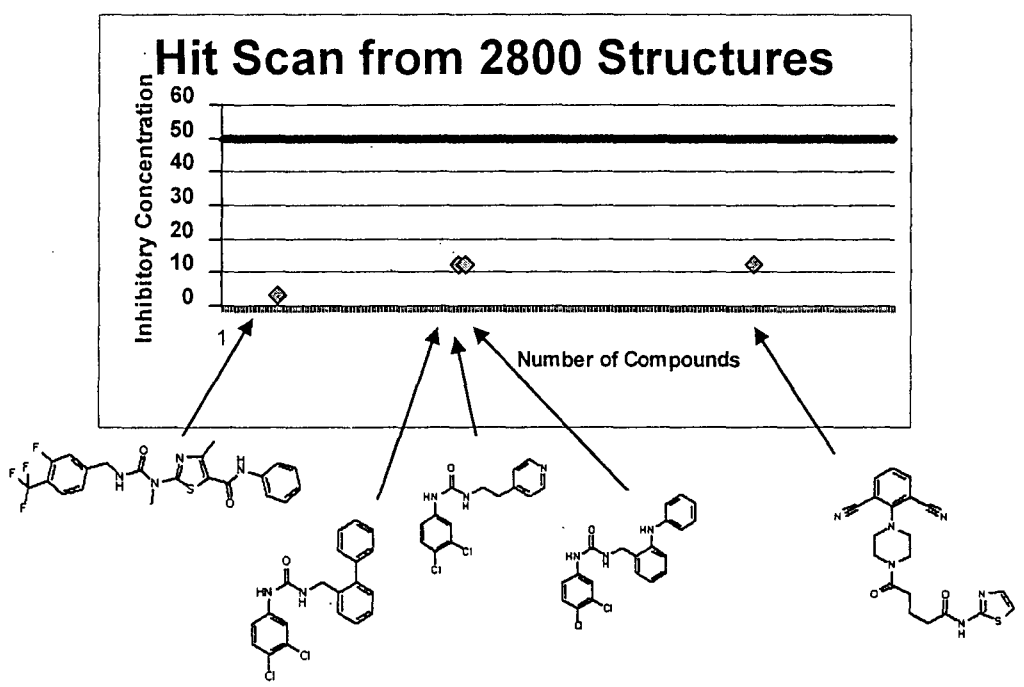


Exploratory Lead Structure Research with Synthons

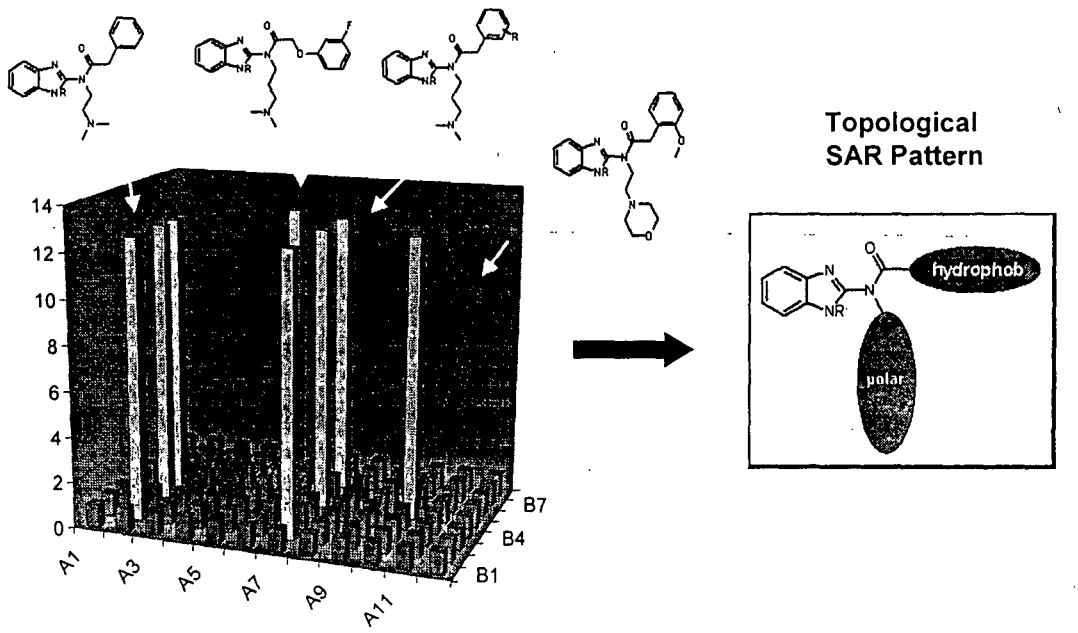


Integration of Synthesis, Purification and Testing

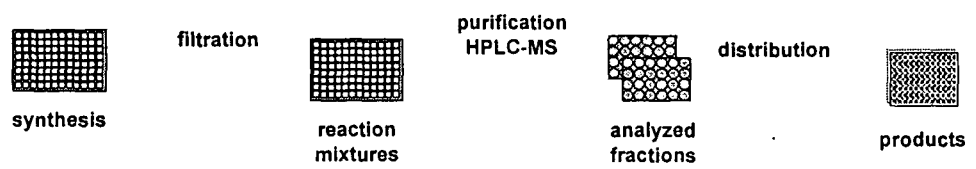




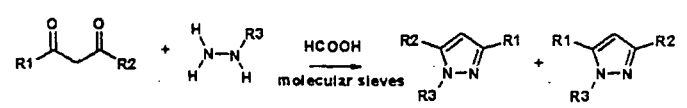
Activity Profile of a Hit Region



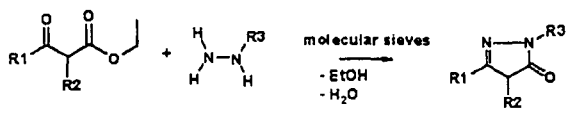
Synthon - Chemistry realized on Microtiter Plates



Examples



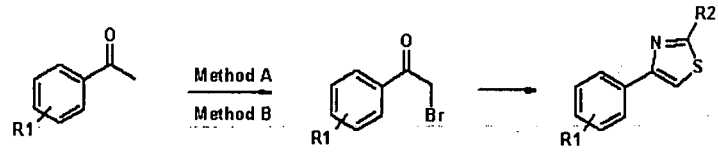
Pyrazoles



Pyrazolones

Synthon-Chemistry realized on Robot System

Hantzsch-Thiazole Synthesis



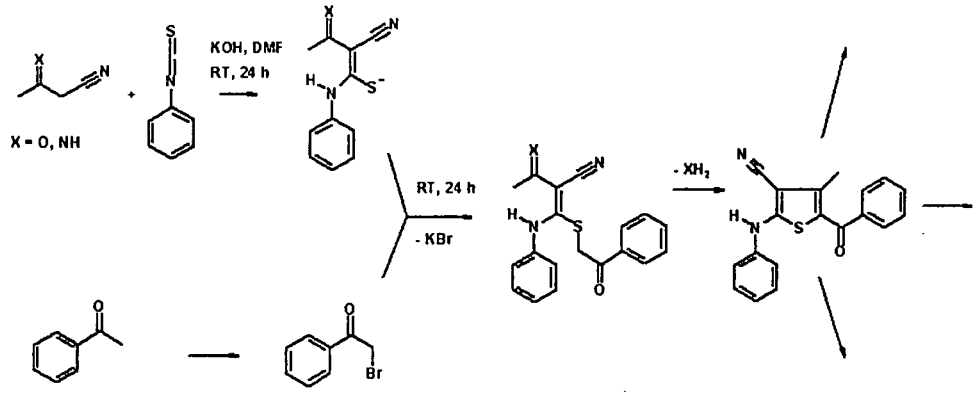
Associated difficulties (classic way / Method A):

- only a limited number of phenacyl bromides available
- synthesis requires use of bromine and glacial acetic acid

Polymer supported: Bromination with Br_3^- on cationic ion exchange resin (Method B)

- commercial reagent / use in excess / removal by filtration
- pure phenacyl bromides
- many acetophenones available

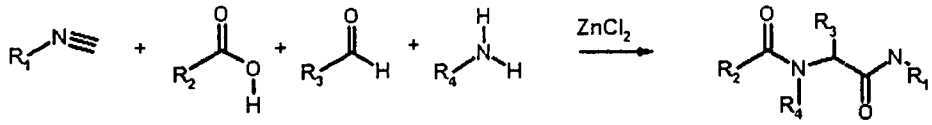
From Synthons to Polyfunctional Thiophenes



"Robot-Synthons"

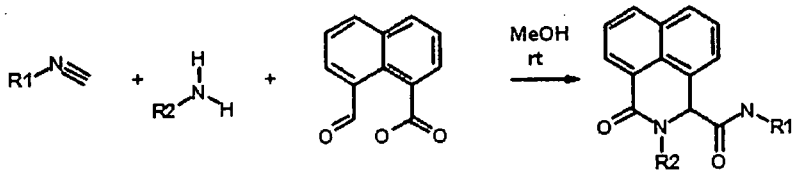
further reactions on MTP´s

UGI Reaction



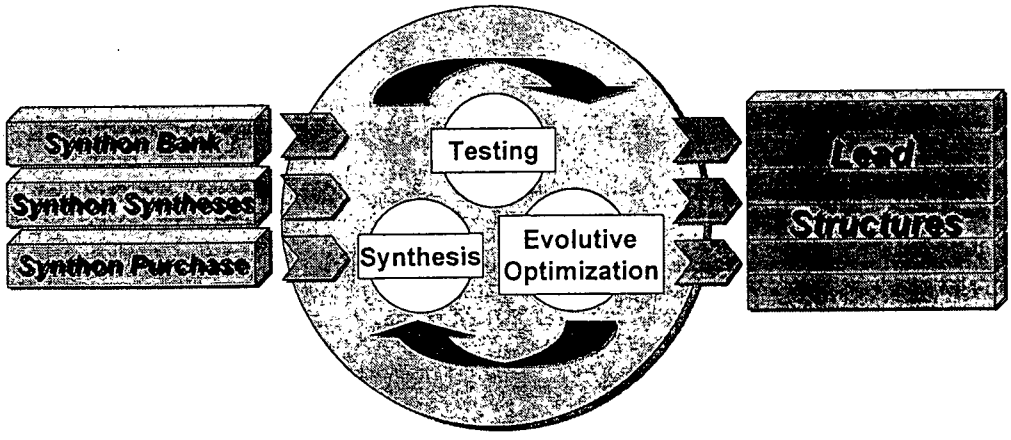
Cyclic UGI compounds

1,8-naphta-δ-lactames



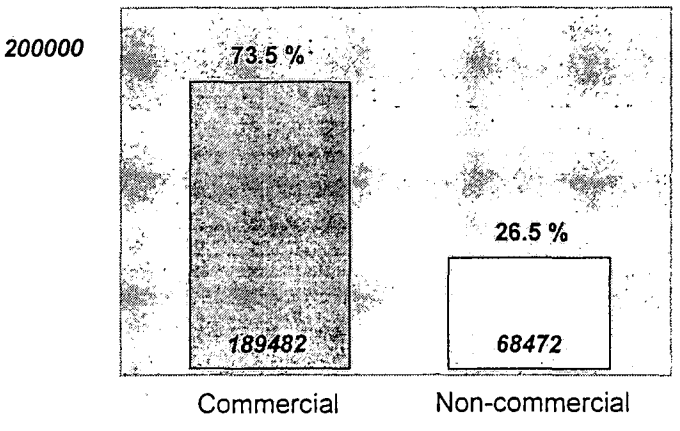
Reactions were performed in commercially available 96-2ml-MTPs in MeOH at room temperature

Pre-Exploratory Lead Structure Research with Synthons



Availability of Synthons and their Precursors

Reactive compounds in over 60 reactive compound classes
(March 1999, mol wt up to 1000 g/mol)



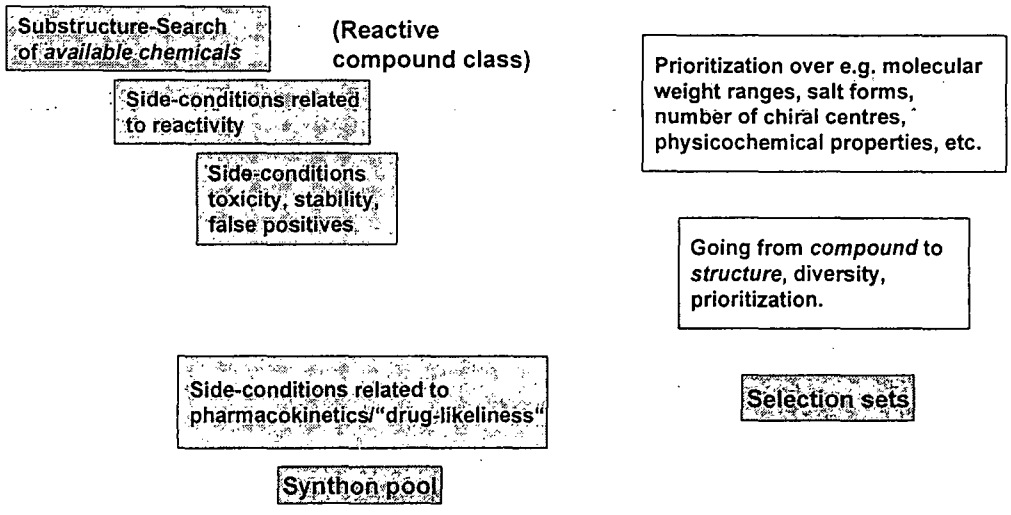
Verfügbare Chemikalien, 08.03.99
(≡ BAYER´s proprietary ACD)

Reactive Compound Classes

AAB	Boc-protected amino acids	EPO	epoxide
AAE	Amino acid esters	EST	ester (overlap with CAC)
AAF	Fmoc-protected amino acids	GRI	Grignards
AAL	amino alcohols	HAL	aliphatic halogenides
AAS	amino acids	HAR	arom-CH2-Hal
AAT	t-Butyl protected amino acids	HET	heterocycles with substitution groups
ACC	acid chlorides	HYA	hydroxyl amine like
ACE	acetylenes	HYD	hydrazine
ALC	alcohols	ISA	isatines
ALD	aldehydes	ISO	isocyanate
ALK	alkenes	ISN	isonitrils
AME	primary amide	IST	isothiocyanate
AMI	aromatic amidines	KES	beta-ketoester
AMP	primary amines	KET	ketons (no KES)
AMS	secondary amines	NAL	aliphatic nitro compnds.
ANH	anhydrides	NAR	aromatic nitro compnds.
ANI	anilines	NIT	nitril
AOH	aldehyde & alcohol on same phenyl ring	PHE	phenolic compnds.
APY	alpha-aminopyridine	PIT	phosphites
AZL	azoles	PMD	pyrimidine
BCA	bromoketones	POS	phosphonium salts
BOH	boronic acids	SAA	N-alkylated sulfonamides
CAA	carboxy aldehydes	SFA	sulfonamides
CAC	carboxylic acids	SFO	sulfones
CAD	dicarboxylic acids	SIL	silane
CAH	hydroxy carboxylic acids	STN	stannane
CFE	chloroformic ester	SUC	sulfonylhalides
CYC	carbamoyl chloride	THA	thioamide
DBA	1,2-diketons	THU	thiourea
DCA	1,3-diketons	TOL	thiol
DDA	1,4-diketons	URE	urea
DEP	dienophiles (1,3-dipol. Cycloadd., Diels-A.)	UTH	urethanes
ENA	enamines	XAR	aromatic halogenides

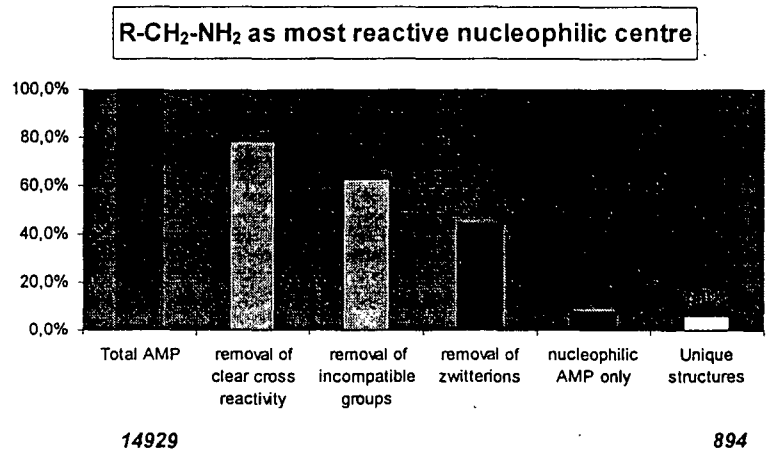
Availability of Synthons and their Precursors

Selection from all available chemicals



Availability of Suitable Synthons

Primary amines registered from commercial sources



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(≡ BAYER's proprietary ACD)

Drugs Based on Substituted Benzene Rings

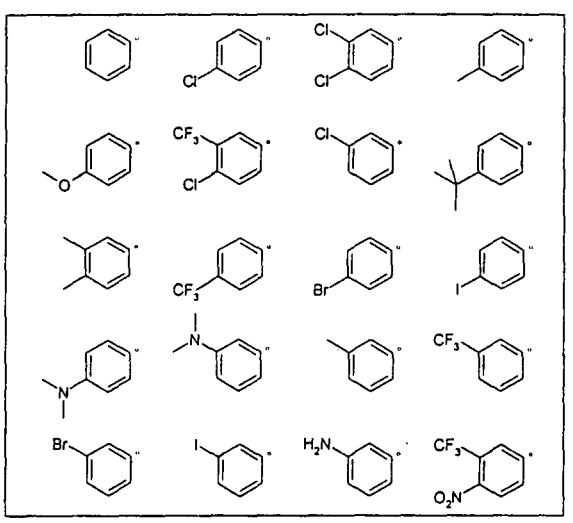
Arylethanol amines	beta-adrenergics
Aryloxypropanol amines	beta-blockers, non-tricyclic antidepressants
Arylsulfonic acid derivatives	antibacterials, diuretics, oral hypoglycemics, thromboxane antagonists.
Arylacetic acids, arylpropionic acids	NSAIDs*, anti-arrythmics
others	aspirin, verapamil

After: Daniel Lednicer, Strategies for Organic Drug Synthesis and Design, John Wiley & Sons, Inc, New York, 1998.

* Non-steroidal anti-inflammatory drugs.

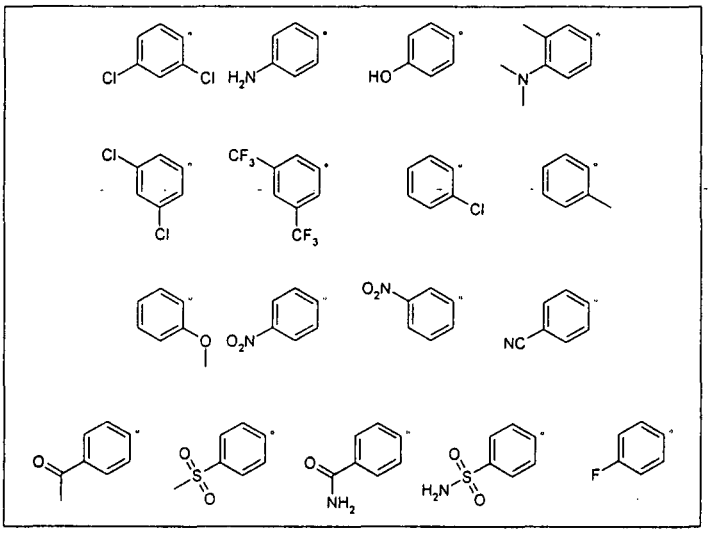
Topliss Tree for Substituents on a Phenyl Ring (1)

(J.G. Topliss, *J. Med. Chem.*, 1972, 15, 1006-1011)



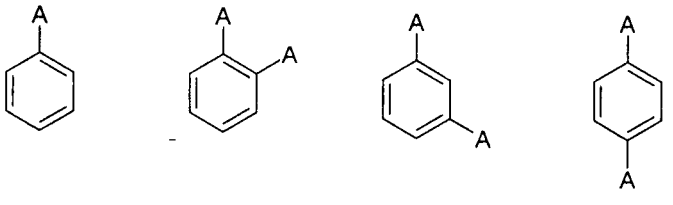
Topliss Tree for Substituents on a Phenyl Ring (2)

(J.G. Topliss, *J. Med. Chem.*, 1972, 15, 1006-1011)



Drugs Based on Substituted Benzene Rings

47 % of a collection of over 14000 clinical candidates and drugs contain a (substituted) phenyl ring

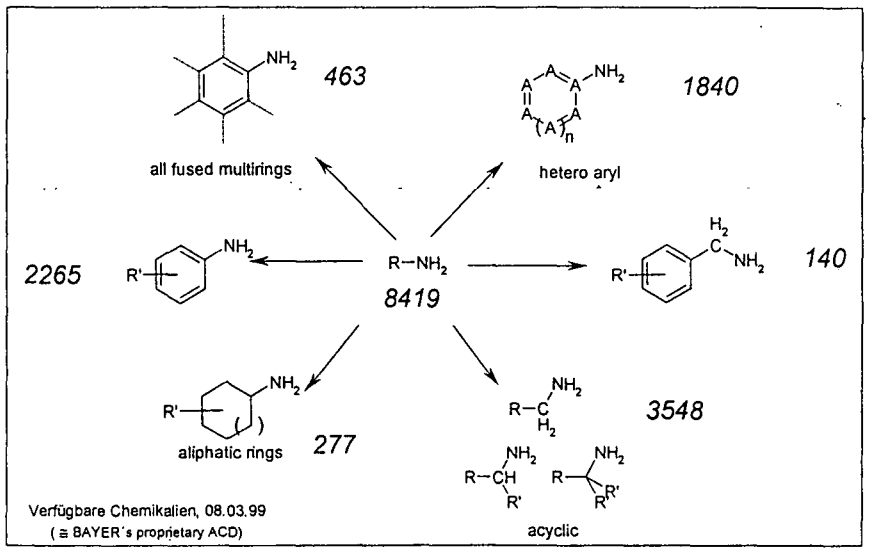


17.3 % **5.2 %** **3.5 %** **17.2 %**

Pharma projects, PJB, 20 Hill Rise, Richmond, Surrey, UK; Bayer Pharma Research, 1998

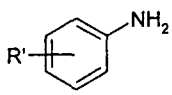
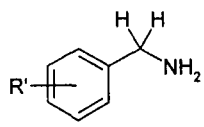
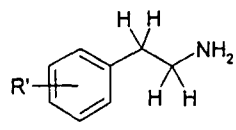
Primary Amines from Commercial Sources

Reduced to the synthon pool - absolute numbers



Availability of Suitable Synthons

Selected amines from commercial sources

	Registered compounds	Unique structures
	>> 1000	>> 1000
	158	133
	88	61

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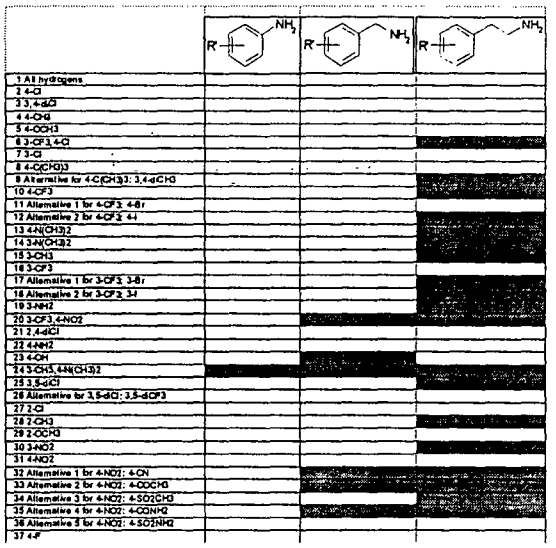
(≡ BAYER´s proprietary ACD)

Availability of Suitable Synthons

Substituents and gaps in the "commercial" Topliss tree



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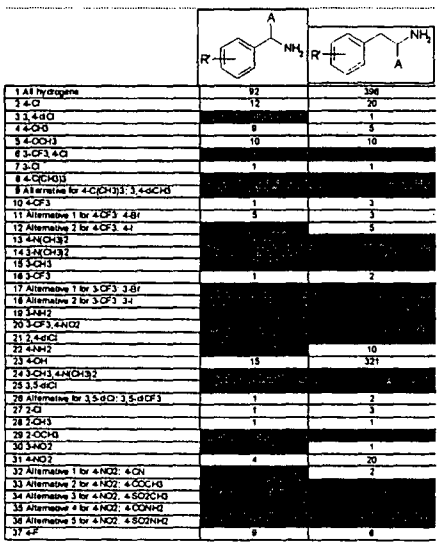
(≡ BAYER´s proprietary ACD)

Availability of Suitable Synthons

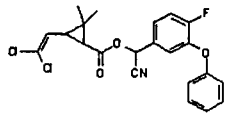
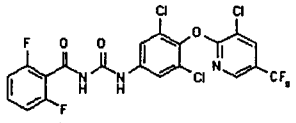
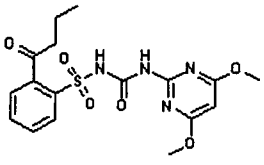
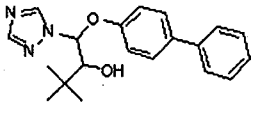
Substituents and gaps in the "commercial" Topliss tree



A is non-H

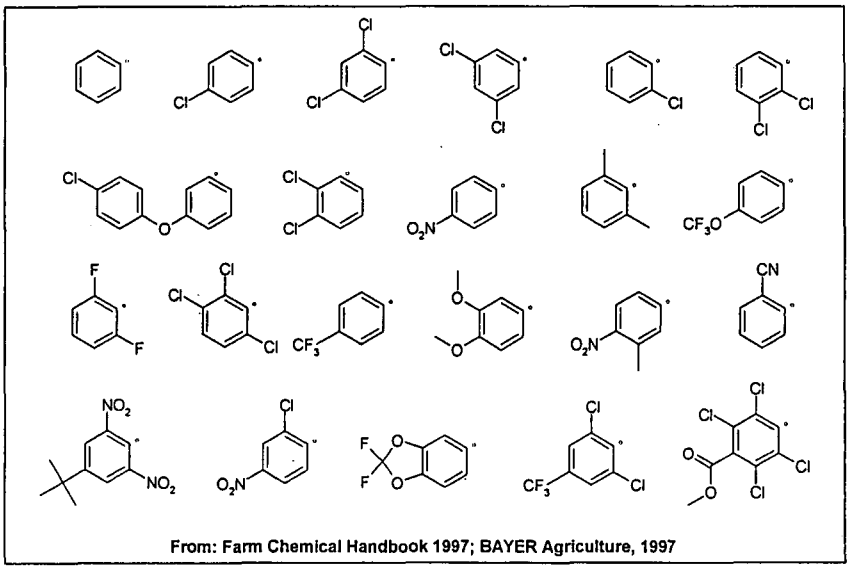
Verfügbare Chemikalien, 08.03.99
(≡ BAYER´s proprietary ACD)

Crop Protection Agents containing Substituted Benzene Rings

Pyrethroids	*insecticides*	
Benzoylureas	*insecticides*	
Sulfonylureas	*herbicides*	
Azoles	*fungicides*	
etc.		

Substituted Benzene Rings in Crop Protection Agents

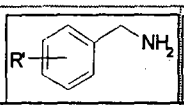
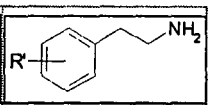
Some examples



From: Farm Chemical Handbook 1997; BAYER Agriculture, 1997

Availability of Suitable Synthons

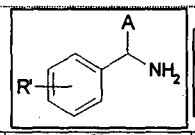
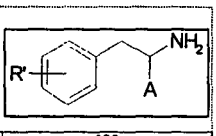
Typical substitution patterns in crop protection agents
(commercial availability)

		
1 phenyl		
2 4-Cl		
3 2,4-diCl		
4 3,5-diCl		
5 2-Cl		
6 diphenylether		
7 3,4-diCl		
8 4-NO2		
9 2,6-diCH3		
10 2,6-diF		
11 2-Cl,4-CF3		
12 4-tBu		
13 4-F		
14 2,4-diNO2		
15 2,6-diCl		
16 2,4,5-triCl		
17 2,3,6-triCl		
18 2,6-diNO2/4-CF3		

Verfügbare Chemikalien, 08.03.99
(≅ BAYER's proprietary ACD)

Availability of Suitable Synthons

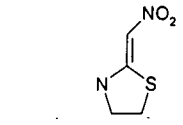
Typical substitution patterns in crop protection agents
(commercial availability)

		
1 phenyl	92	396
2 4-Cl	12	20
3 2,4-diCl		
4 3,5-diCl		
5 2-Cl	1	3
6 diphenylether		
7 3,4-diCl		1
8 4-NO2	4	20
9 2,6-diCH3		
10 2,6-diF	1	2
11 2-Cl,4-CF3		
12 4-tBu		
13 4-F	9	6
14 2,4-diNO2	1	2
15 2,6-diCl		
16 2,4,5-triCl		
17 2,3,6-triCl		
18 2,6-diNO2/4-CF3		

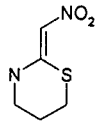
A is non-H

Verfügbare Chemikalien, 08.03.99

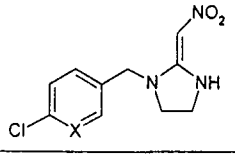
If the crucial Synthons had not been there....



Gompper, 1965. Not tested then. Not active.
Cysteamine easily available.
The homologue NH_2-$CH_2CH_2CH_2$-SH was not available...



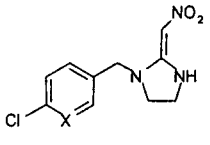
Feurer, 1970. Tested. Active as insecticide.
Without the homologue mentioned above, it would not have been found.



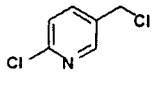
X = CH *Hoechst, 1974*: only weak activity found

Then.....

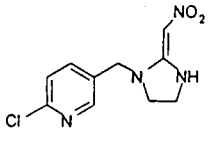
If the crucial Synthons had not been there....



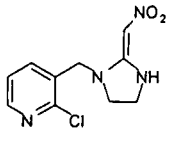
X = CH *Hoechst, 1974*: only weak activity found
X = N *Bayer, 1984:* highly active



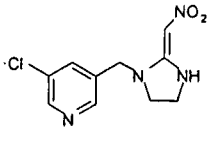
If this synthon had not been available, one would have given up on the class of chloronicotinyl insecticides.



Imidacloprid
(++++)

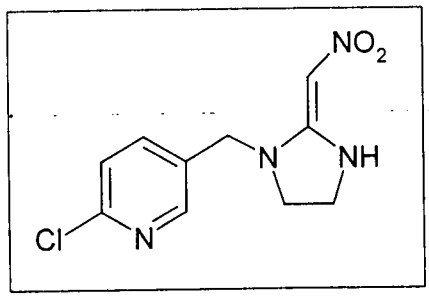


(+/-)



(+)

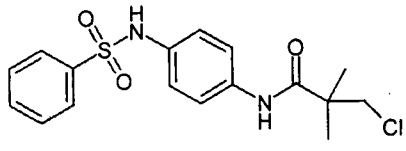
Imidacloprid



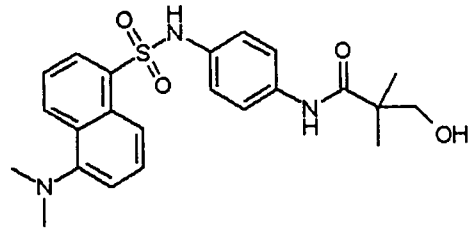
Advantage®

„Heroes of Chemistry Award", *American Chemical Society*, August 1999

Human Cytomegalovirus (HCMV)



An AGFA compound as hit!

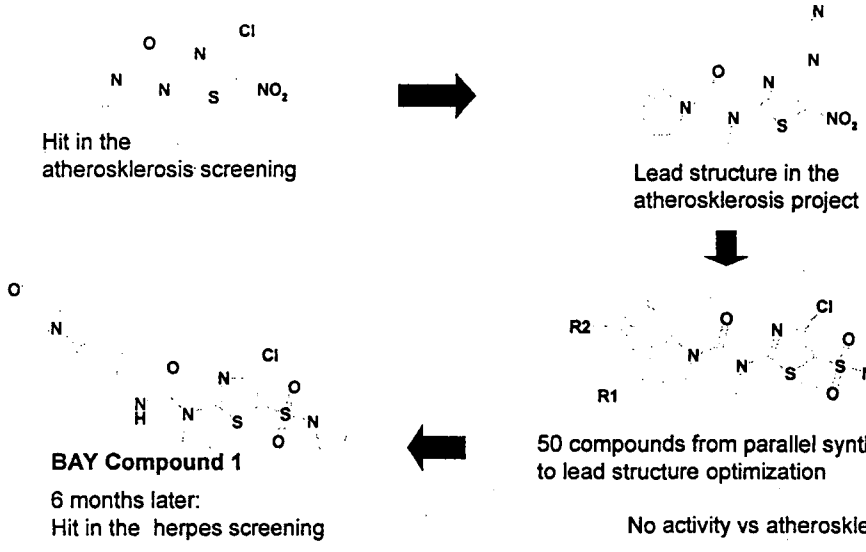


BAY 38-4766

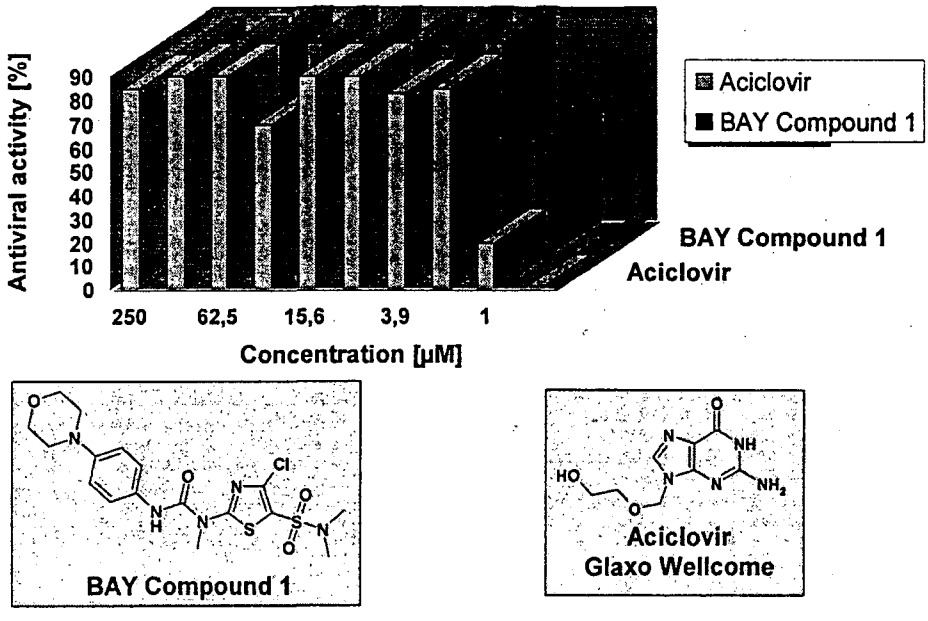
- Non-nucleosidic Inhibitor of HCMV
- New chemical class
- New mechanism of action

Patents: WO 9937608 A1, WO 9937609 A1, Published 29-JUL-99; Priority 23-JAN-98

History of the first Hit in HSV (BAY Compound 1)

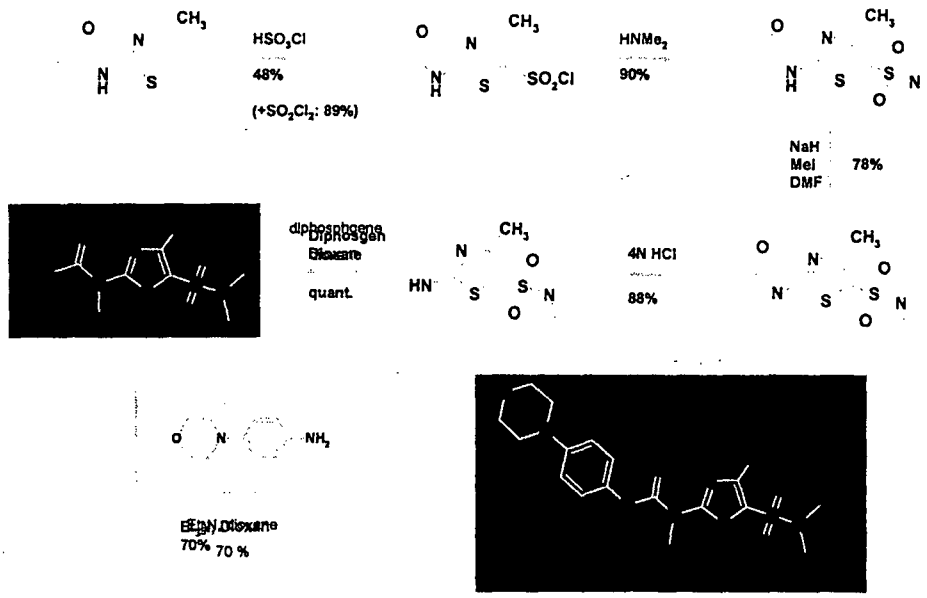


Antiviral Activity of BAY Compound 1 vs Aciclovir

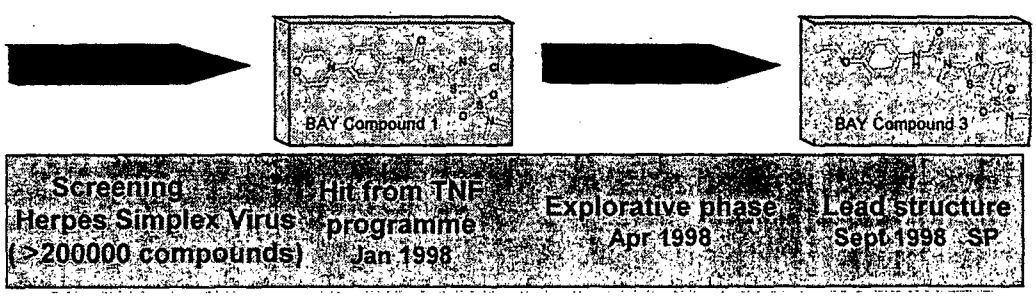


Synthesis of BAY Compound 2

Replacement of 4-Chloro- with 4-Methylthiazole

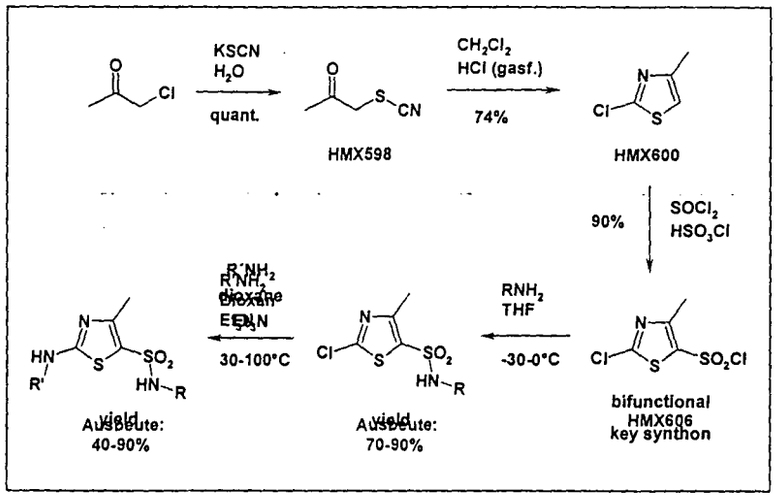


Intramural Synthon Capacity Shortens Important Learning Curves

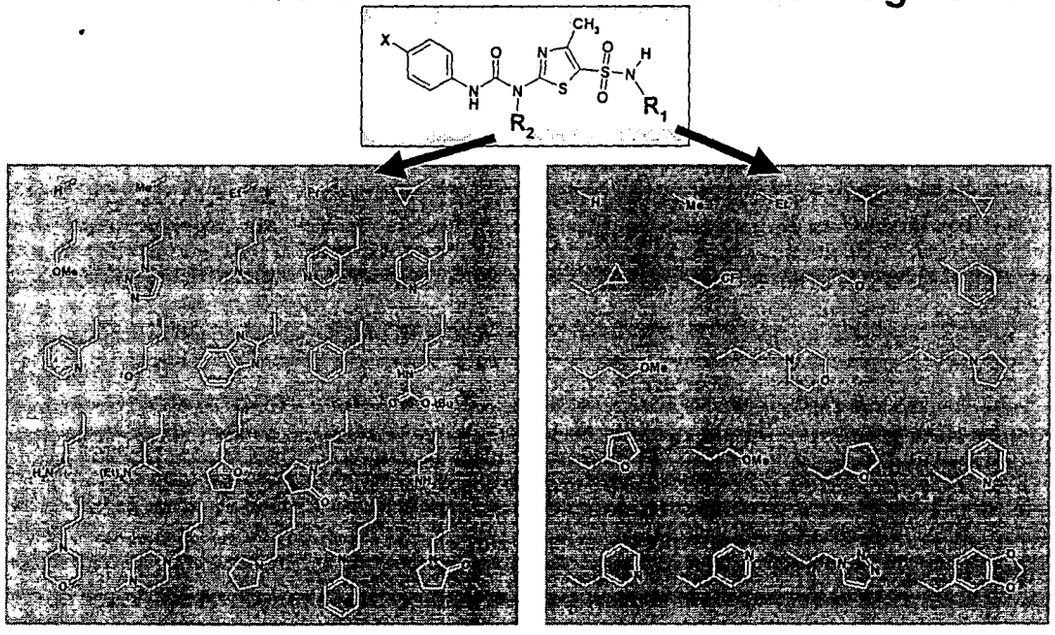


New synthons from the methylthiazole class led to active compounds with improved stability

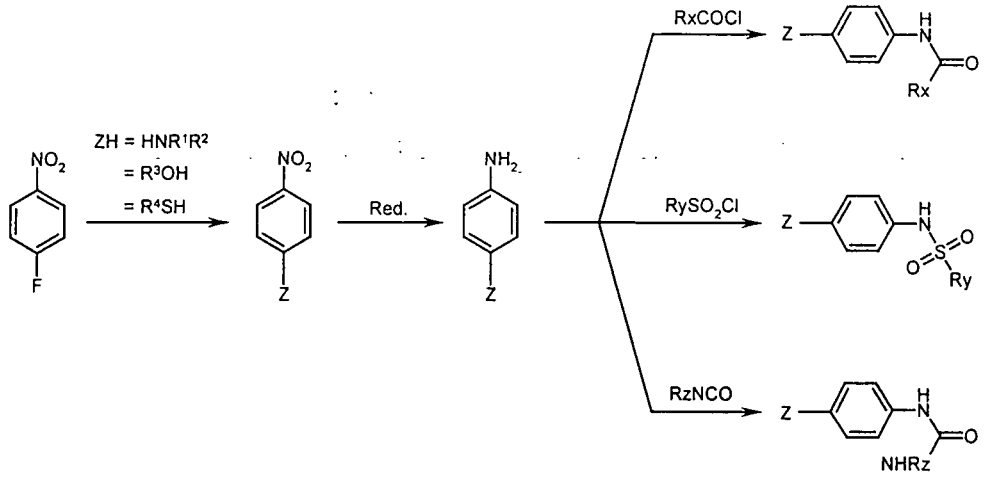
Synthesis of Thiazolesulfonamide Synthons



Variations at the Urea- and Sulfonamide-Fragments

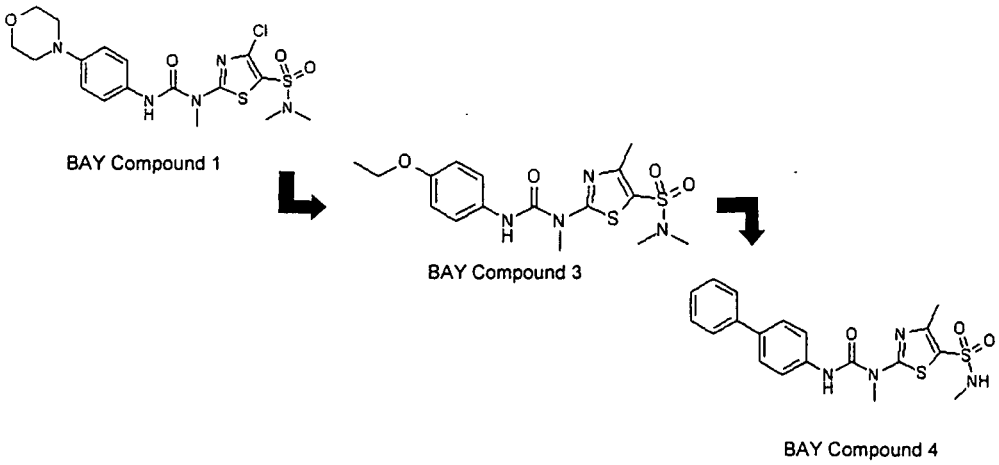


Multiple Step Cascade Reactions



Intramural Synthon Capacity

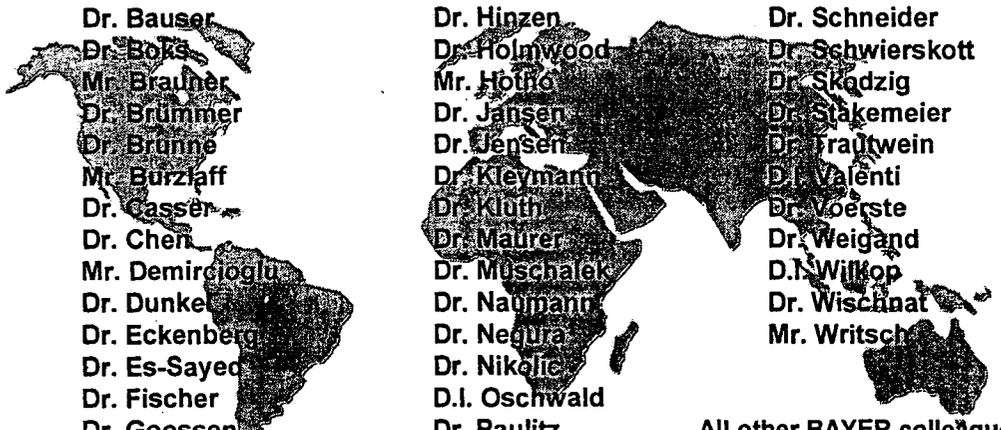
Leads to Fast Discovery of Clinical Candidates



Concluding Remarks

- Relying on fine-chemical wholesale dealers and other commercial sources is not enough...
- Knowledge and experience from the discovery research cycle should flow into the selection/prioritization of synthons.
- Simple, yet important gaps have been illustrated for primary amines only.
- Many other important gaps have not been revealed...
- Any synthon is welcome, but many diverse synthons are best for every case. Every synthon has a chance for success.

Acknowledgement



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Dr. Bauser
Dr. Boks
Mr. Brauner
Dr. Brümmer
Dr. Brunne
Mr. Burziaff
Dr. Casser
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Dr. Stakemeier
Dr. Trautwein
D.I. Valenti
Dr. Voerste
Dr. Weigand
D.I. Wilkop
Dr. Wischnat
Mr. Writsch

.... All other BAYER colleagues scattered around the globe who actively support the BAYER Synthon Projects and Combinatorial Chemistry

Biological Methods for Library Characterization and Screening

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BIOLOGICAL METHODS FOR LIBRARY CHARACTERIZATION AND SCREENING

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Biological methods for library preparation are mainly limited to peptide or oligonucleotide libraries. For peptide libraries, methods are based on the construction of a pool of clones each one expressing a different peptide on its surface. The peptides are fused to proteins normally expressed on the surface of the microorganism used. Phage display libraries are the most commonly used. Screening is accomplished by incubation of the target molecule, adsorbed to a solid support, with the phage population. Active phages will bind the target even after extensive washing steps. Target-bound phages are isolated and propagated by infection of E.coli and subjected to an additional round of adsorption to the immobilized target. This procedure increases both the number of active phages and the stringency of selection, since harsher condition may be employed in the washing steps to reduce the number of non-specifically bound phages. As for the case of synthetic libraries, iterative cycles of adsorption, washing, elution and propagation in E.coli are performed to enrich the phage population in the active or in few active sequences. Active phages may then be subjected to DNA sequencing in order to decode the active peptide sequence. In a very similar way, also oligonucleotide libraries can be screened for immobilized targets using the polymerase chain reaction (PCR) methodology to expand the number of active sequences after each selection cycles.

The construction of biological display libraries requires the introduction into a microorganism of the genetic information necessary for the peptide synthesis. For the construction of a random peptide display library it is necessary to synthesize pools of DNA fragments that are then inserted into specific vectors. The DNA fragments are chemically synthesized as a mixture of single-stranded degenerated oligonucleotides containing constant regions and one or more degenerated stretches of DNA. DNA consists of sequences of 4 different nucleotides and each trinucleotide codes for a corresponding amino acid. Because of the codon degeneracy, most of the amino acids are coded by more than one triplet. Since in fully degenerated oligonucleotides there is the possibility to introduce stop codons that will interrupt protein synthesis, the oligonucleotides are synthesized using different mixtures of nucleotides especially in the third position of each triplet. The DNA fragments to be cloned must be in a double-stranded form, at least at the end of each fragment. This is normally done by annealing short oligonucleotides to a complementary constant region inserted during the synthesis and by enzymatically completing the complementary DNA strand. After compatible ends are prepared by restriction enzyme digestion, the fragments are ligated into an appropriate vector and then introduced into the microorganism.

The ligand selection process is called Biopanning. The target molecule must be bound to a solid support, usually a microtiter plate or a small Petri dish. Less common alternative supports are magnetic particles, column with solid matrices, cells, mammalian organs. In a typical experiment, the number of phages that are incubated with the target corresponds to about 100 to 1000 times the complexity of the library. After the unbound clones are washed away, the bound ones are eluted by different methods, like low pH, high concentration of free target, direct infection of bacteria cells. The eluted phages are grown, purified and submitted to a new cycle of selection. Usually 3 to 4 rounds of selection are

sufficient, and the entire process can be completed in about a week. At the end, several clones are isolated and their DNA extracted and sequenced. The DNA portions coding for the peptides are translated into amino acids and the sequences compared. If a consensus sequence can be identified, the screening may have been successful. One or more peptides are chosen and chemically synthesized in order to verify their binding affinity, outside of the microorganism system.
Compared to chemical libraries, biological display libraries have several advantages and disadvantages. Some of the major advantages are the possibility to use a library for many different selection processes (even 100s), the easy propagation of the library and of the selected clones. The possibility to build larger size libraries is another advantage together with simple selection and sequencing procedures. On the contrary, a disadvantage is the fusion of peptides to a microorganism protein, and, therefore, the binding site can be extended to the fusion protein or the fusion protein may influence the peptide conformation.

Suggested readings

- Smith, G.P., Scott, J.K. (1993) Methods Enzymol. 217, 228.
- Lu, Z., Murray, K.S., Van Cleave, V., laVallie, E.R., Stahl, M.L., McCoy, J.M. (1995) Biotechnology 13, 366.
- Scott, J.K., Smith, G.P. (1990) Science 249, 386.
- Smith, G.P. (1991) Curr. Opin. in Biotecnol. 2, 668.
- Parmley, S.F., Smith, G.P. (1988) Gene 73, 305.
- Cwirla, S.E., Peters, E.A., Barrett, R.W., Dower, W.J. (1990) Proc. Natl. Acad. Sci. USA 87, 6378.
- McCafferty, J., Griffiths, A.D., Winter, G. Chiswell, D.J. (1990) Nature 348, 552.
- Markland, W. Roberts, B.L., Saxena, M.J. Guterman, S.K., Ladner, R.C. (1991) Gene 109, 13.
- Felici, F., Castagnoli L., Mustacchio, A., Jappelli, R., Cesareni, G. (1991) J. Mol. Biol. 222, 301.

Robotics & Laboratory Automation

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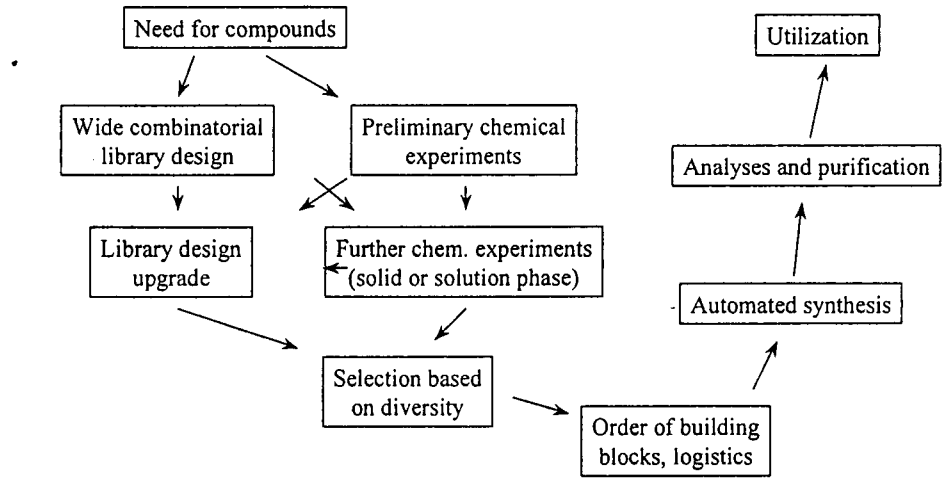
Introduction

- Combinatorial chemistry process
- Robotics and automation in general
- Automated processes
- Products show-case

Combinatorial chemistry process



Automation as such

- Repeated actions - reason to consider automatiom
- 3 shift - high time to count on automation
- Accuracy and reliability is extremely important - automation has to be implemented
- One of the most important part of automation is standardising relevant instruments to ensure proper transfer processes
- Solid IT background is a must
- Targeted softwares are necessary

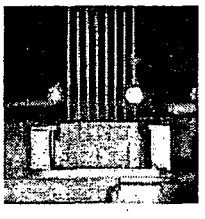
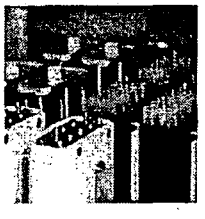
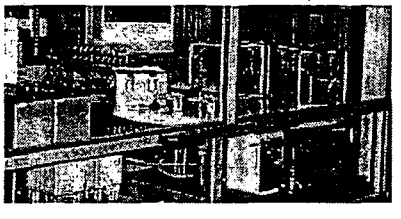
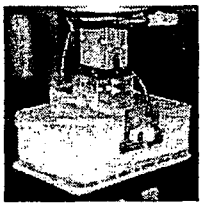
Processes can be automated like

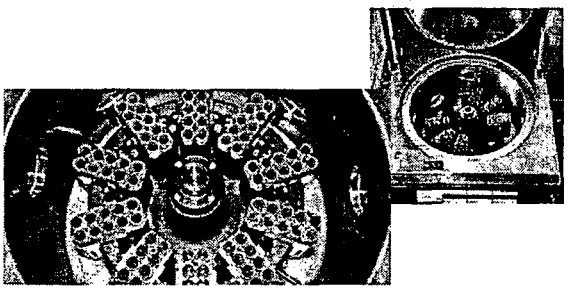
- Order of materials (e-business), dissolving them
- Dosing
- Reaction
- Downstream process (liq.-liq. extraction)
- Evaporation
- Analyses, purification, quality control
- Standard solution preparation (for biological testing)

Vendors

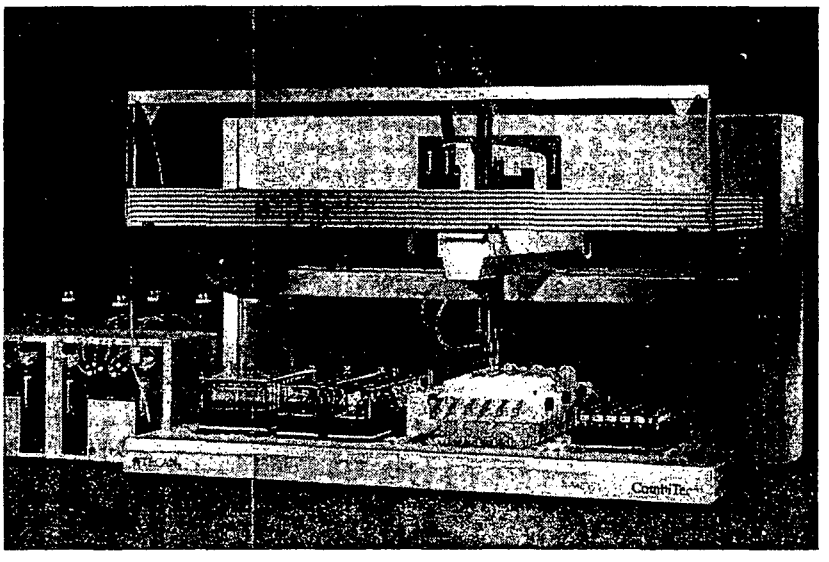
- Two main types of suppliers
  - Standardised equipment
  - Custom made systems (integrated equipment)
- It is not a marketing presentation!
- Quick shot about some product on the market
- Validity is limited (rapid development time)
- Experience is also limited due to limited access

accelab: arcosyn98



Tecan: Combitec



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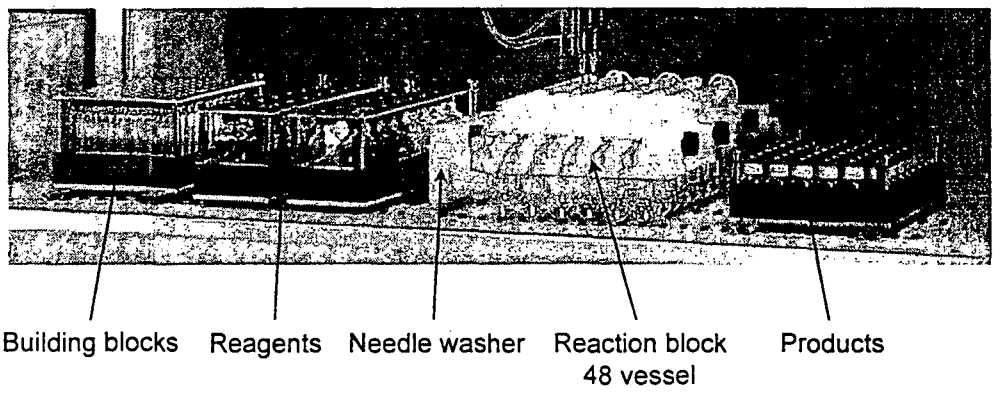

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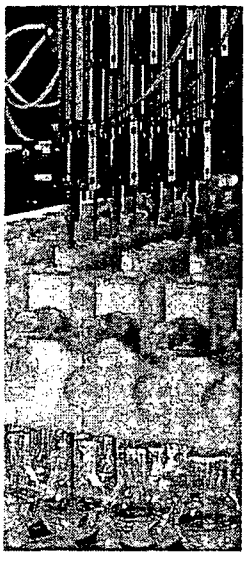
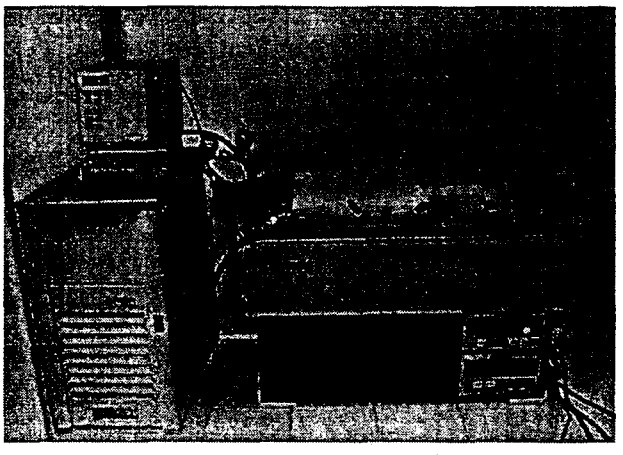
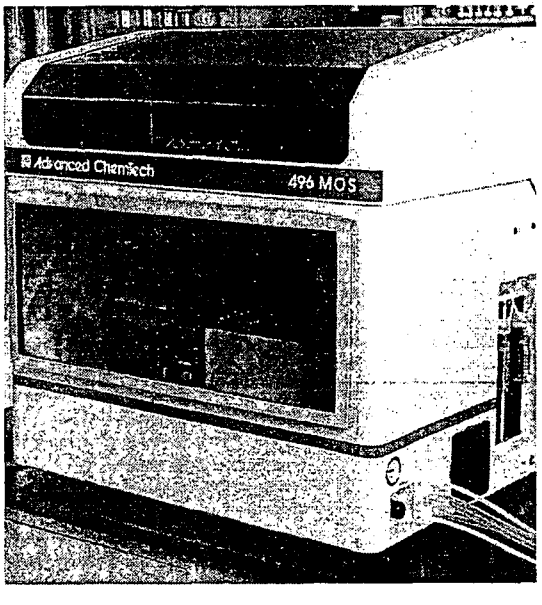


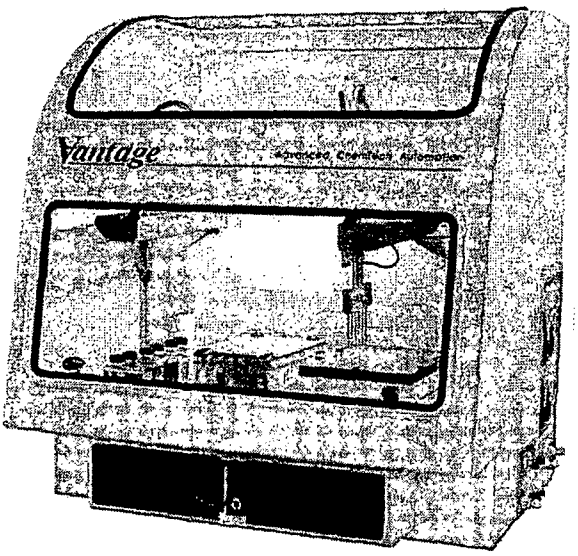


Advanced ChemTech: 496 MOS



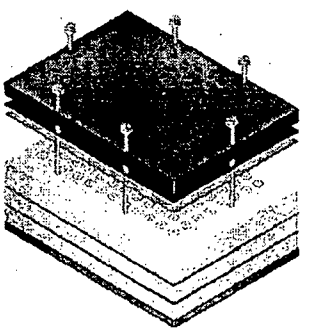
- 96 vessels
- bottom filtration
- one needle
- inert gas application
- shaker
- -70 - 150°C

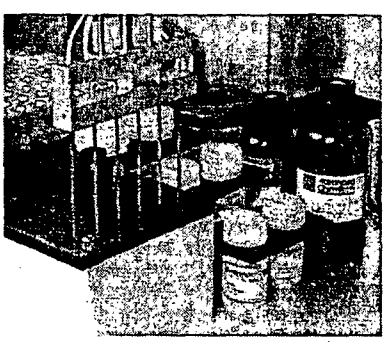
Advanced ChemTech: Vantage



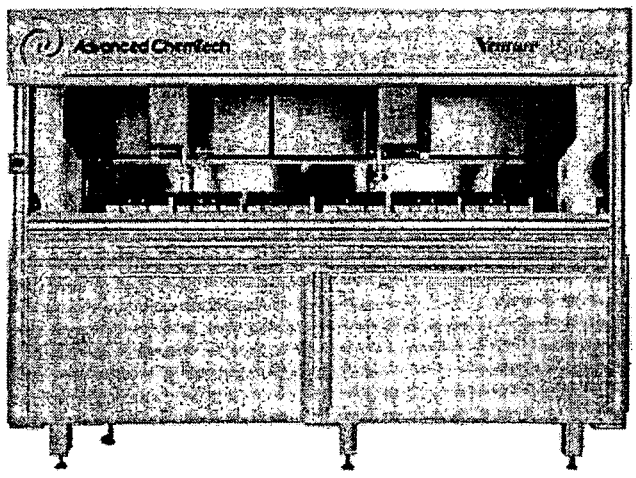
- 96 vessels
- bottom filtration
- 6+1 needle
- inert gas application
- shaker
- -70 - 150°C

Advanced ChemTech: Vantage

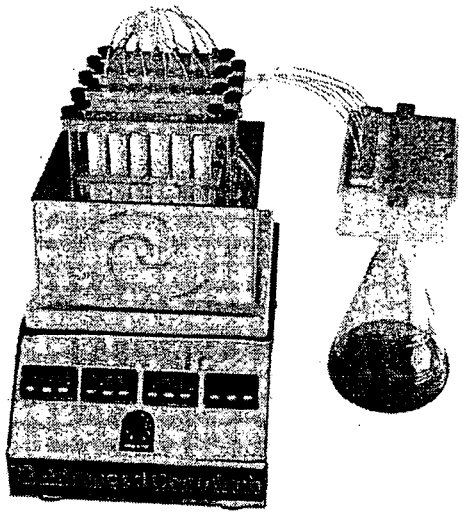


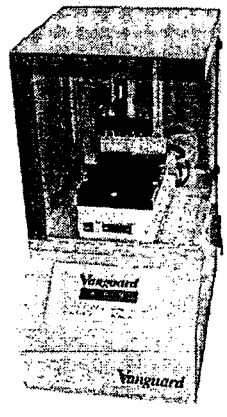


Advanced ChemTech: Venture

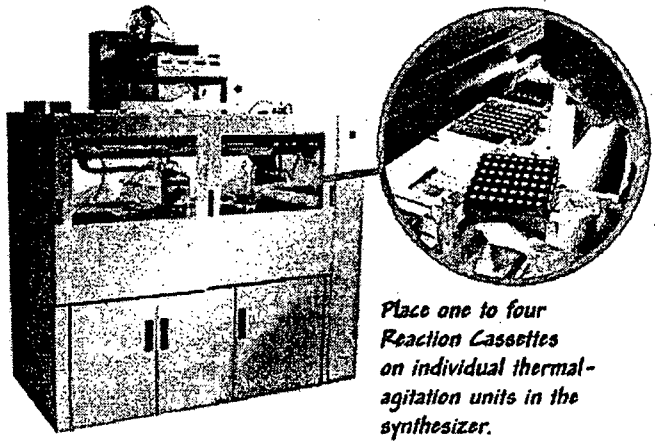


Advanced ChemTech: LabMate & Vanguard

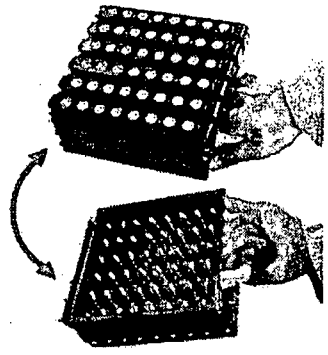




Argonaut Technologies: Trident

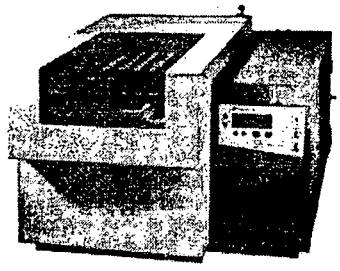


Place one to four Reaction Cassettes on individual thermal-agitation units in the synthesizer.



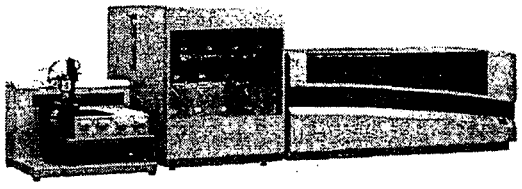
Each Reaction Cassette holds 48 visible glass reaction vessels.

Argonaut Technologies: Trident



- 48 vessels by cassettes (4 ml)
- top filtration (solution phase synthesis)
- Gilson dosing unit
- separate reaction unit is available
- -40 - 150°C

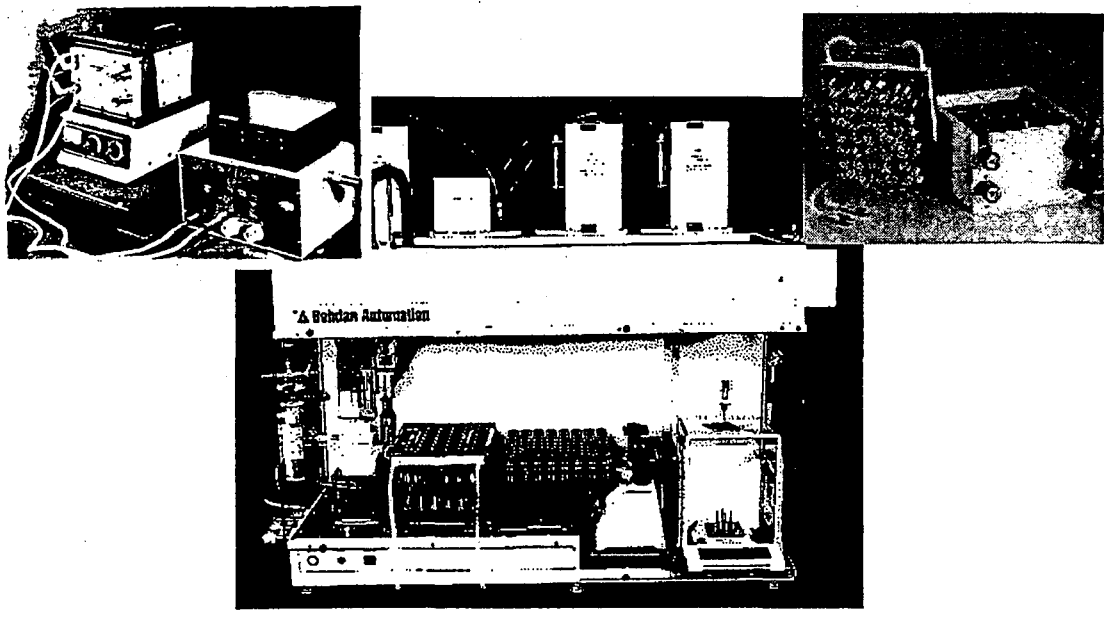
Argonaut Technologies: Nautilus



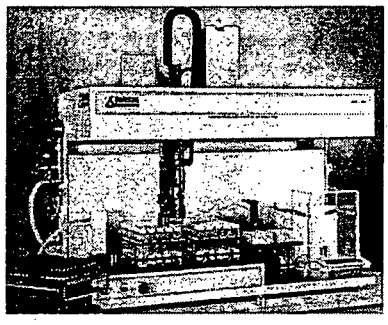
- 24 vessels
- top filtration

- Temperature control for each vessel (+/-10°C)
- for process optimisation
- -40 - 150°C

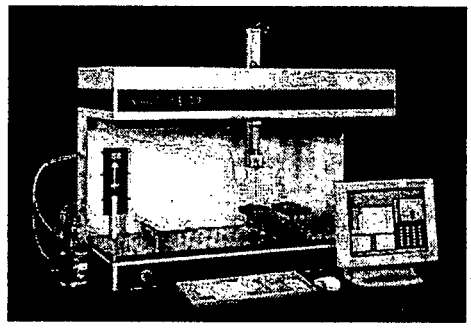
Bohdan: Full range of appliances



Bohdan: Full range of appliances

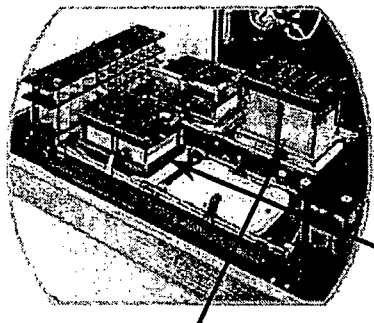


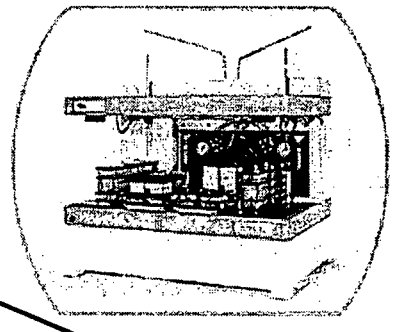
Reagent dissolving unit



Liquid-liquid extraction

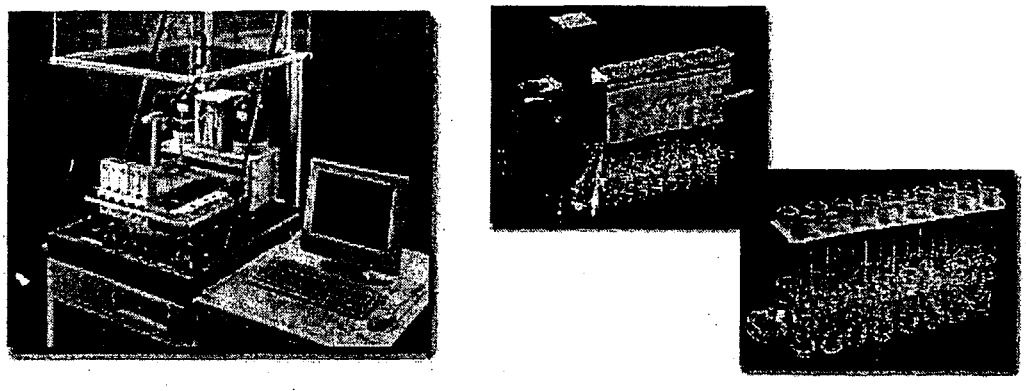
Charybdis: Gemini





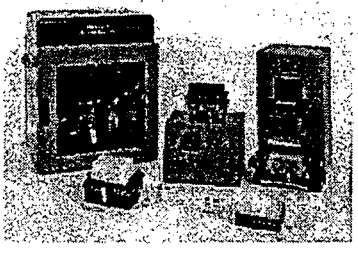
- 4 reaction blocks, max. 96 vessels (-40/150°C)
- or -80/180°C reaction block
- bottom filtration

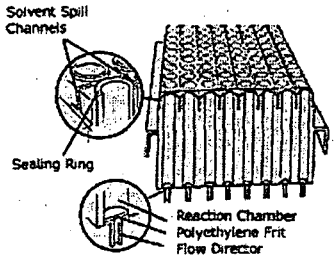
ChemSpeed: ASW2000



- 80 parallel reaction (-70/150°C) in glass vessels
- both solid and solution phase chemistry
- in situ cooling-heating-shaking

Robbins: FlexChem

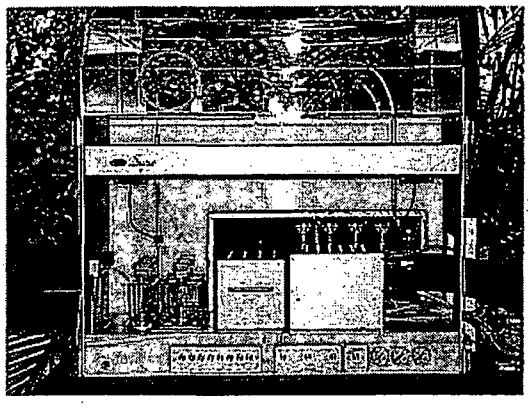




Cutaway View of a FlexChem Multiple Synthesis Reactor

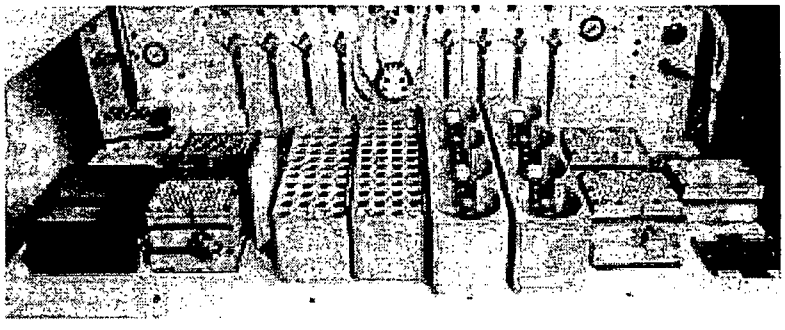
- 96 reaction vessels, separate cooling and heating
- bottom filtration

MultiSynTech: Syro



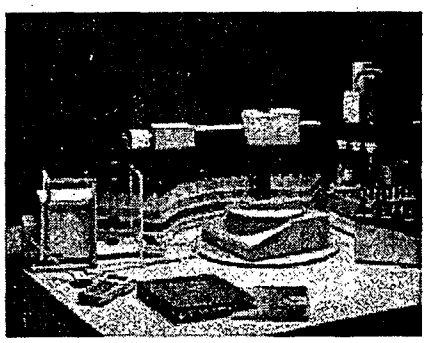
- special mixing (levitation)
- bottom filtration, two needles

Zinsser: Sophas M



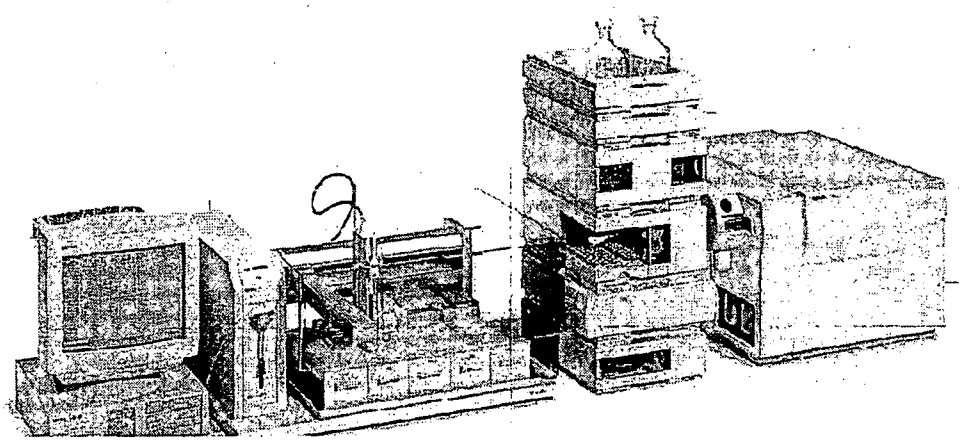
- max. 96 vessels
- top or bottom filtration
- 1-8 needles

Zymark: Solution Phase Synthesis System (SPSS)



- weighing, reagent adding
- reaction performing (-30/150)
- cap screwing (on and off)
- filtration
- liquid-liquid extraction

Agilent (earlier HP): HPLC/MS purification

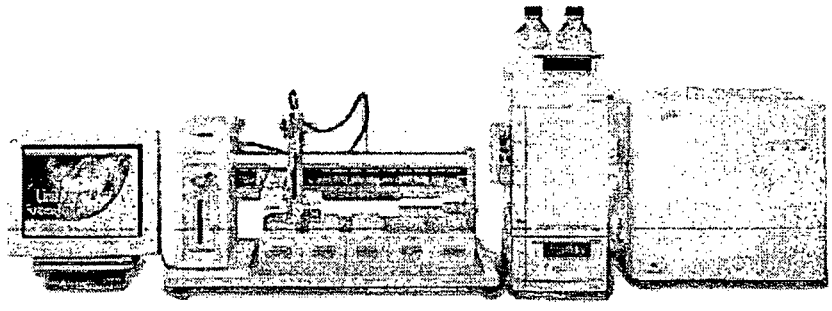


Biotage: Parallex

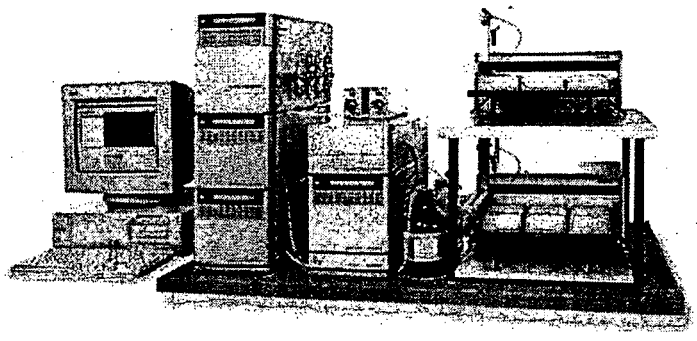
- 1-4 channel HPLC
- fully automated
- MS confirmation
- sample monitoring by software
- good throughput, quick purification

Gilson: HPLC/MS system

GILSON ThermoQuest

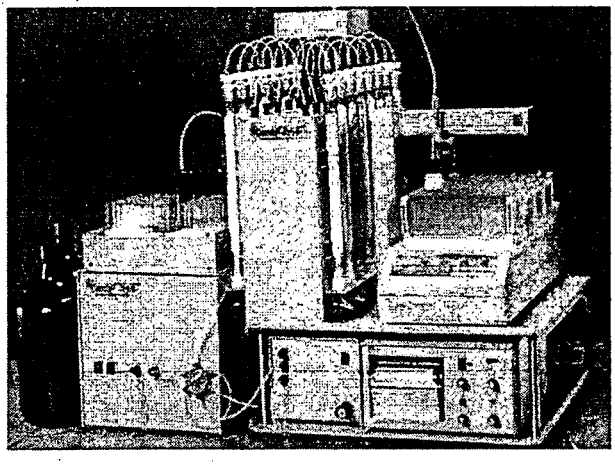


Merck: HPLC/MS system

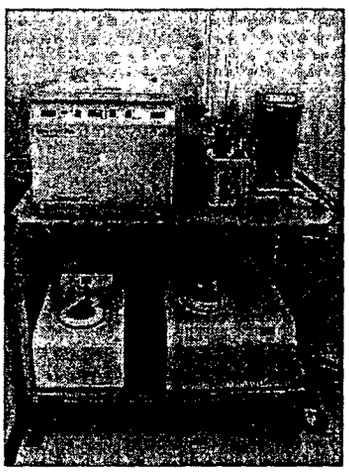


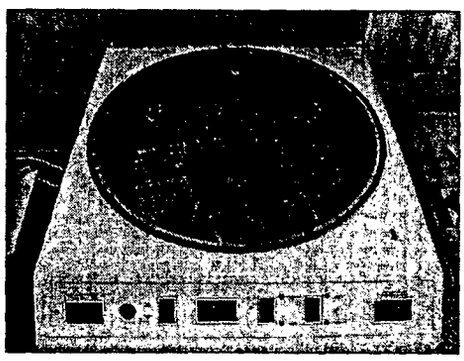
- Controlled both UV or MS detector

ISCO: LC purification

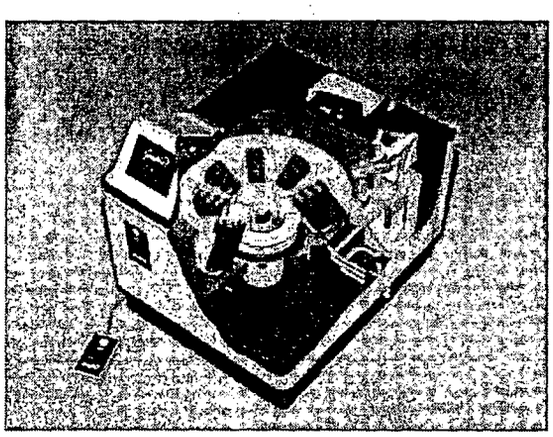


Savant: SpeedVac for evaporation



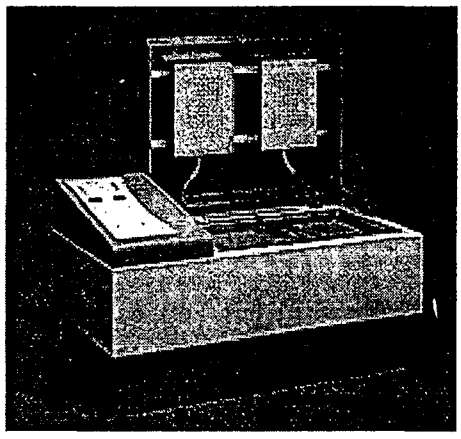


GeneVac: Open Access Evaporator



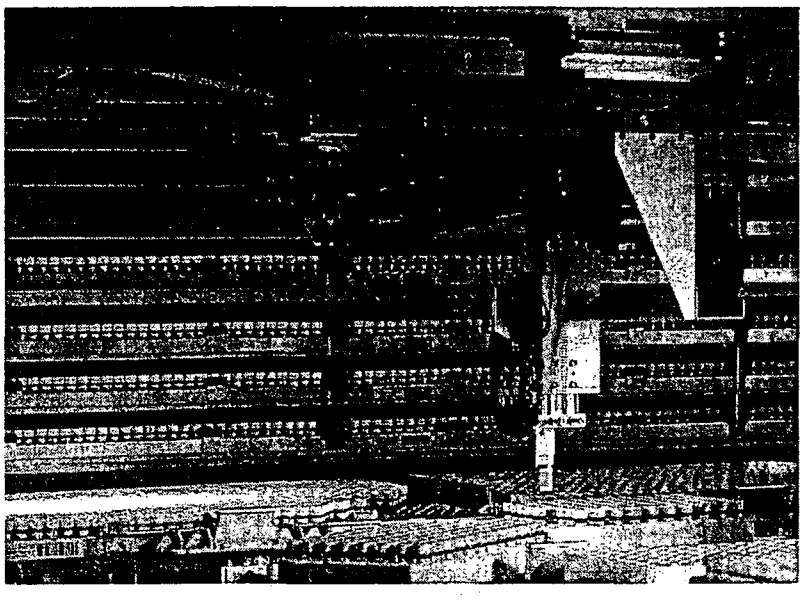
- Special auto-balanced rotor
- Build in cryopump and solvent recovery system

Zymark: TurboVap 96



Continuos nitrogen flow increases evaporation using special gas jets

Haystack (GSK)



Combinatorial Process Research & Development

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COMBINATORIAL PROCESS RESEARCH & DEVELOPMENT

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lackova@mail.datanet.hu

Introduction:
The accelerated drug discovery and increasing outsourcing have increased the importance of the Process Research & Development (P R&D) in the pharmaceutical industry. Beside the obvious direct benefit of reducing manufacturing cost of the drugs, other useful applications were find for P R&D. Since combichem provide methodology and tools: labware, automation, software, and complete instrumentation, the automated P R&D brought a lot of results quickly.

Discussion:
The lecture deals only with real combinatorial part of automated PR&D: process scouting and process optimization. In these stages vary large parameter (factorial) field should be mapped.
In order to be able to deal with this large factorial field one should combine the following feautres:

- Parallel synthesis reactors
- Liquid handlers
- Analysis
- Control software
- Design of experiments

Since temperature is a key factor in chemical reactions and properties beside the traditional isotherm block reactors, the manufacturers have developed machines with thermal zones or individual heating and cooling.
Integrated systems control the whole procedure from preaparation of reactions till collecting the data from the analyisis (mostly HPLC) detecors(s).
The control software is a key issue in these systems, since rational handling of limited resources might be a key issue in the success.
Design of experiments can substantially reduce the number of experiments, needed to find the optimum of a process.
The examples are collected to cover the whole range of the affected pharma and agro industry, from the discovery till the manufacturing of active substances. Different methods for optimum search are demonstarted.

Combinatorial Chemistry in Biotechnology - A Case Study

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COMBINATORIAL CHEMISTRY IN BIOTECHNOLOGY - A CASE STUDY

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Monoclonal antibodies are becoming an important class of therapeutic agents useful for the treatment of a vast array of diseases. Many monoclonals are waiting for FDA approval, and they represent almost 30 % of biotechnology derived drugs under development. Production of MAb's by hybridoma technology or transgenic animals can be easily scaled up, but still immunoglobulins purification from crude feedstocks poses several problems. Main difficulties are due to the low antibody concentration in cell culture supernatants or milk of transgenic animals and the high amounts of contaminating proteins. Purification by affinity chromatography of monoclonal antibodies for therapy is based on the use of protein A or protein G immobilized on appropriate supports [1], as a first step to capture and concentrate the immunoglobulin from diluted feedstocks. These two proteins, which bind to the constant portion of the immunoglobulins, and so can be used to purify the majority of antibodies, are obtained from microorganisms or genetically modified bacteria, trough complex and expensive procedures, requiring in addition time consuming analytical controls to check for the presence of contaminants such as viruses, pirogens, or DNA fragments, which may affect the safety of the purified MAb for clinical purposes. Given the importance of the application of MAb's for therapy, and given the role of the purification process in assuring the quality, consistency and safety of the products, it is clear that the availability of synthetic ligands able to mimic protein A or G in the purification of antibodies is of remarkable industrial importance, since may lead to less expensive production costs and reduced risks of contamination. A synthetic ligand [Protein A Mimetic, PAM], able to mimic protein A in the recognition of the immunoglobulin Fc portion, has been previously identified in our laboratory through the synthesis and screening of multimeric combinatorial peptide libraries [2]. Its applicability in affinity chromatography for the downstream processing of antibodies has been fully characterized, examining the specificity and selectivity for polyclonal and monoclonal IgG derived from different sources. Ligand specificity is broader than protein A, since IgG derived from human, cow, horse, pig, mouse, rat, rabbit, goat, and sheep sera [3], as well as IgY derived from egg yolk [4], are efficiently purified on PAM-affinity columns. Adsorbed antibodies are conveniently eluted by a buffer change to 0.1 M acetic acid or 0.1 M sodium bicarbonate pH 9 with full retention of immunological properties. Monoclonal antibodies deriving from cell culture supernatants or ascitic fluids are also conveniently purified on PAM-affinity columns, even from very diluted samples. The ligand is useful not only for IgG and IgY purification from different sources, but also for IgM [5], IgA [6], and IgE [7] isolation from sera or crude cell supernatants.

Affinity constant for PAM:IgG interaction is 0.3 μM, as determined by plasmon resonance experiments. Antibody purity after affinity purification is close to 95 %, as determined by densitometric scanning of SDS-PAGE gels of purified fractions, and maximal column capacity reachs 30 mg Ig/ml support under optimized conditions. Validation of antibody affinity purification processes for therapeutic use, a very complex, laborious, and costly procedure, is going to be simplified by the use of PAM, which could reduce considerably the presence of biological contaminants in the purified preparation, a very recurrent

problem when using recombinant or extractive biomolecules as affinity ligands. In vivo toxicity studies in mice indicate a ligand oral toxicity >2000 mg/kg, while intravenous toxicity is close to 150 mg/kg [**8**]. Additional studies have suggested that PAM, given its ability to interfere with Protein A/immunoglobulin interaction, may find applications also as a novel therapeutic agent.

Protein A is the bacterial receptor for IgG, and this protein binds to IgG in a site partially overlapping with that of immunoglobulin receptors (FcγR). In further studies, a PAM derivative stable to proteolysis, prepared by replacing the natural amino acids with the corresponding D analogues, has shown to inhibit IgG/ FcγR in vitro in a dose dependent manner. Inhibition of FcγR is important in a wide range of diseases, such as Systemic Lupus Erythematosus (SLE). Administration of this derivative to MRL/*lpr* mice, the animal model to study SLE, has resulted in a remarkable enhancement of the survival rate (80 %) compared to placebo treated animals (10 %) and the significant reduction of proteinuria, the typical clinical sign associated to SLE. Kidney histological examination of treated animals has confirmed the preservation of tissue integrity and a remarkable reduction of immune-complexes deposition [**8**]. These results have confirmed the role of Fcγ receptors in SLE pathogenesis opening new perspectives for the development of new drugs for treating autoimmune disorders.

Bibliography

1] Fuglistaller, P. (1989) Comparison of immunoglobulin binding capacities and ligand leakage using eight different protein A affinity chromatography matrices. *J. Immunol. Meth.* 124,171-177.

2] Fassina, G., Verdoliva, A., Odierna, M.R., Ruvo, M., and Cassani, G. Protein A mimetic peptide ligand for affinity purification of antibodies. *J. Mol. Recogn.* 9 (1996) 564-569.

3] Fassina, G., Verdoliva, A., Palombo, G., Ruvo, M., and Cassani, G., Immunoglobulin specificity of TG 19318: A novel synthetic ligand for antibody affinity purification. *J. Mol. Recogn.* 11 (1998) 128-133.

4] Verdoliva, A., Basile, G., and Fassina, G.; Affinity purification of immunoglobulins from chicken egg yolk using a new synthetic ligand. *J. Chromatogr. Biom. Appl.*, 749 (2000) 233-242.

5] Palombo, G., Verdoliva, A., and Fassina, G. Affinity purification of IgM using a novel synthetic ligand. J. Chromatogr. Biom. Appl. 715 (1998) 137-145.

6] Palombo, G., De Falco, S., Tortora, M., Cassani, G., and Fassina, G. A synthetic ligand for IgA affinity purification. *J. Molec. Recogn.* 11 (1998) 243-246.

7] Palombo, G., Rossi, M., Cassani, G., and Fassina,G. Affinity purification of mouse monoclonal IgE using a protein A mimetic ligand [TG 19318] immobilized on solid supports. *J. Molec. Recogn.*, 11 (1998) 247-249.

8] Marino, M., Ruvo, M., De Falco, S., and Fassina, G.; Prevention of Systemic Lupus Erythematosus in MRL/*lpr* mice by administration of an immunoglobulin binding peptide. *Nat. Biotechnology* 18 (2000) 735-739.

BIOTECH DRUGS APPROVED FOR SALE AND/OR CURRENTLY IN CLINICAL TRIALS

250 TOTAL DRUGS (100%)

STATUS	NUMBER OF DRUGS	PERCENTAGE OF TOTAL
DRUGS APPROVED	23	9
PLAs PENDING	13	5
PHASE III	53	21
PHASE II	72	29
PHASE I	106	42

27% OF TOTAL BIOTECHNOLOGY DRUGS ARE MONOCLONAL ANTIBODIES (68 DRUGS)

XCELLAGEN

MONOCLONAL ANTIBODIES FOR THERAPY

PRODUCTION

- HYBRIDOMA TECHNOLOGY
- TRANSGENIC ANIMALS

PURIFICATION

- IONIC EXCHANGE
- HYDROPHOBIC INTERACTION
- GEL FILTRATION
- AFFINITY CHROMATOGRAPHY

XCELLAGEN

PROTEIN A & G AFFINITY COLUMNS FOR mAb PURIFICATION: MAIN PROBLEMS

- BIOLOGICAL CONTAMINATION
- COLUMN LEAKAGE
- COST OF LIGAND
- SANITATION
- PROCESS VALIDATION
- IgG RESTRICTION

XEPTAGEN

PAM

IDENTIFICATION APPROACH

- GENERATION OF HIGHLY DIVERSIFIED COMBINATORIAL LIBRARIES "MULTIMERIC LIBRARIES"
- SCREENING ASSAY ON MICROTITER ELISA PLATES BY INHIBITION OF PROTEIN A /IgG INTERACTION

XEPTAGEN

LIBRARIES

CHEMICAL DIVERSITY. MULTIMERIC LIBRARIES

Given a tetrameric amino acid sequence composed of n residues

$$X_1 - X_2 - X_3 - X_4 - \ldots\ldots - B_n$$

$$K- X_1 - X_2 - X_3 - X_4 - \ldots\ldots - B_n$$

$$K- K- X_1 - X_2 - X_3 - X_4 - \ldots\ldots - B_n$$

$$X_1 - X_2 - X_3 - X_4 - \ldots\ldots - B_n$$

The total number of different peptides N_T equals to:

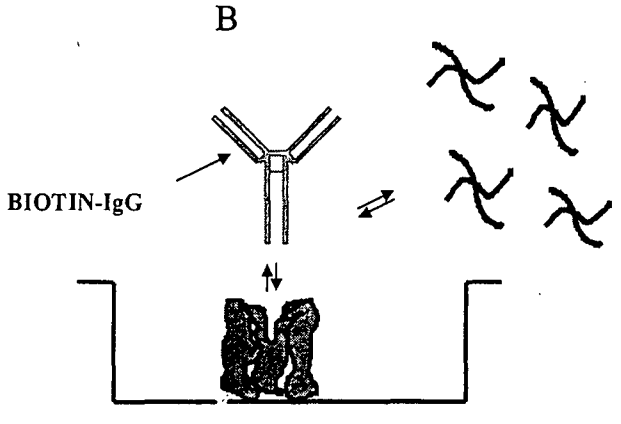
$$N_T = A^n$$

A: number of building blocks

The library has been prepared applying the Portioning-Mixing method to obtain homogeneous molecules

PAM IDENTIFICATION

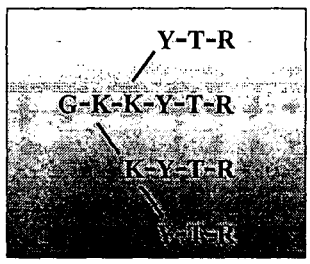
CHEMICAL LIBRARY SCREENING ASSAY

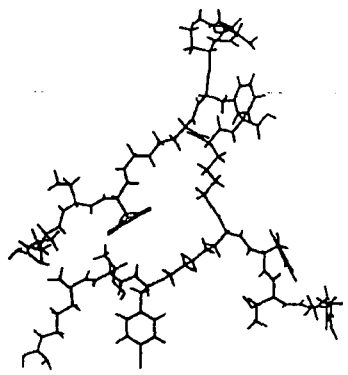


TETRAMERIC TRIPEPTIDE LIBRARY. USING 18 NATURAL AMINOACIDS (CYS TRP OMITTED)

Fassina, Verdoliva, Odierna, Ruvo, Cassani, (1996) *J. Mol. Rec.* 9:564-569

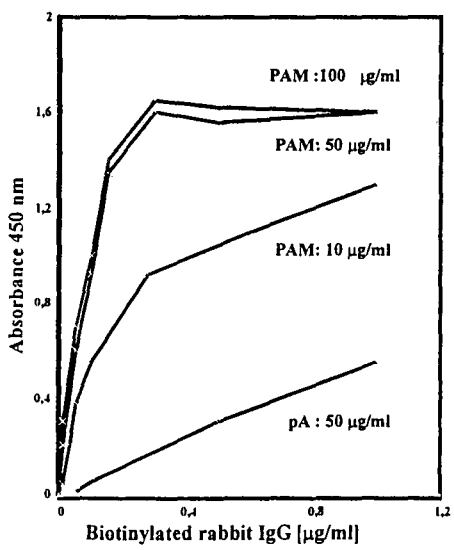
PAM
(PROTEIN A MIMETIC)

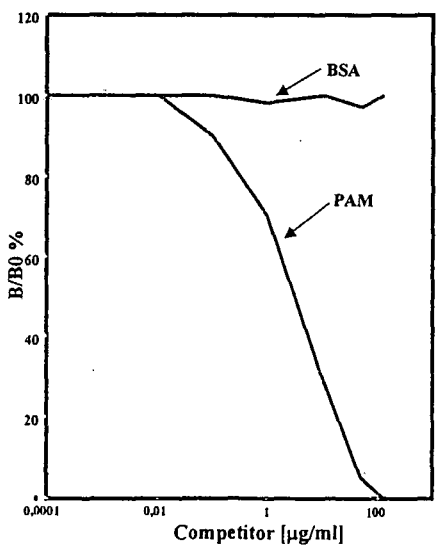




M.W.: 2141 amu
SOLUBILITY: >100 mg/ml (water)
A.A. COMPOSITION: G (1), K (3), Y (4), T (4), R (4)

BINDING OF BIOTINYLATED RABBIT ANTIBODIES TO IMMOBILIZED PAM



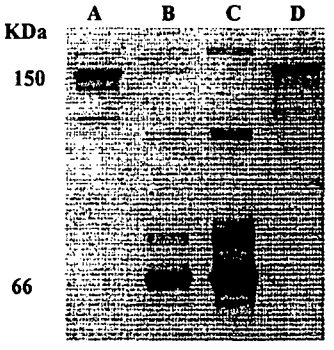


AFFINITY PURIFICATION OF mAb 7H3 (mouse IgG 1k) FROM CELL CULTURE SUPERNATANTS ON PAM-EMPHAZE

CHROMATOGRAPHIC CONDITIONS:

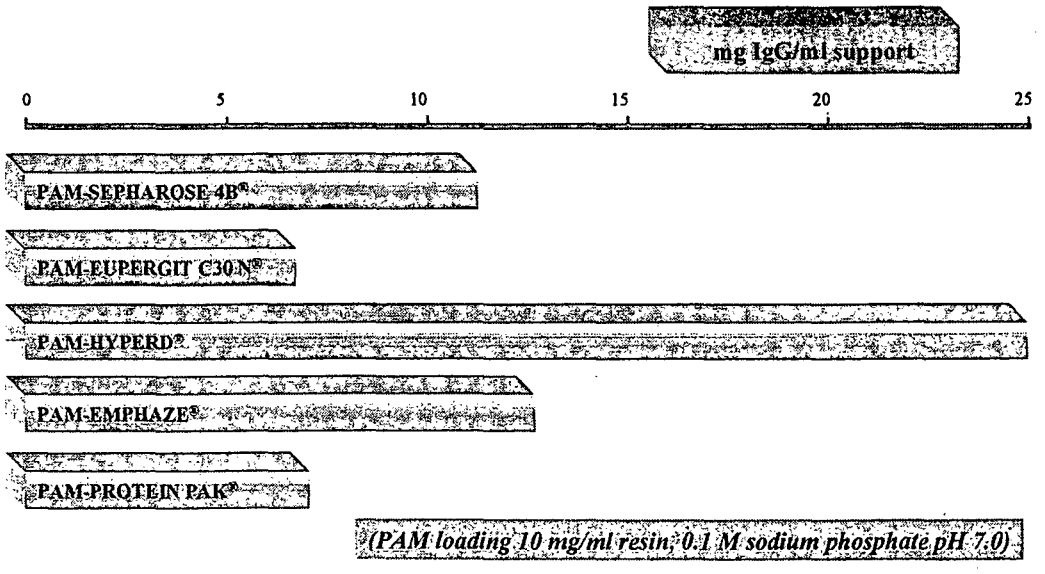
COLUMN VOLUME: 1 ml
PAM LOADING: 10 mg/ml
SAMPLE VOLUME: 40 ml
mAb CONCENTRATION: 100µg/ml
BINDING BUFFER: 50 mM BIS-TRIS pH 6.5
ELUTION BUFFER: 0.1 M ACETIC ACID
FLOW RATE: 1ml/min
mAb RECOVERY: 95%
mAb PURITY: 90%

SDS-PAGE ANALYSIS



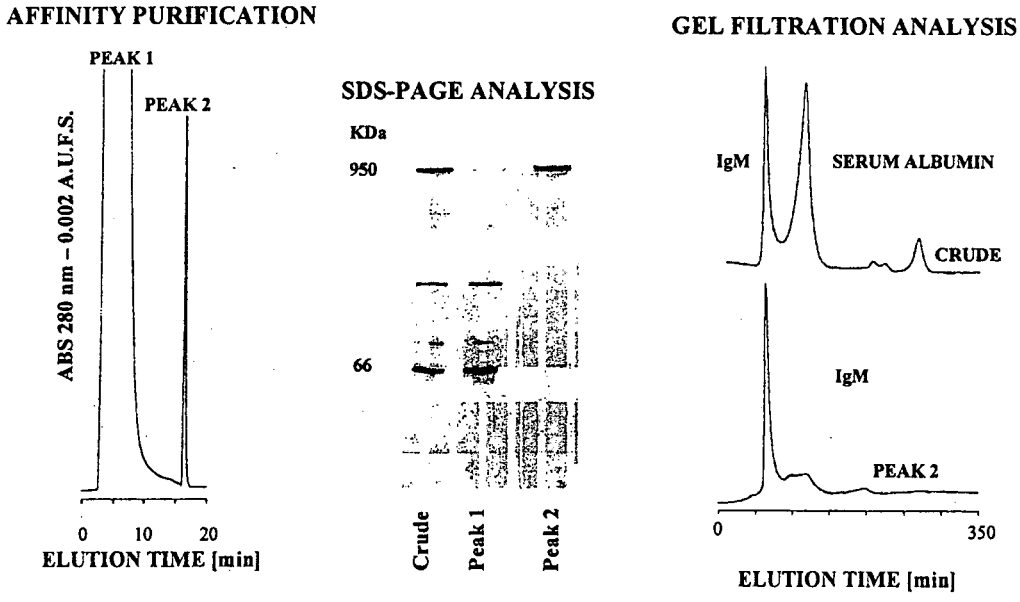
A. mAb 7H3 (protein A)
B. CELL CULTURE SN
C. UNBOUND
D. BOUND

RABBIT IgG BINDING CAPACITY OF PAM -AFFINITY COLUMN



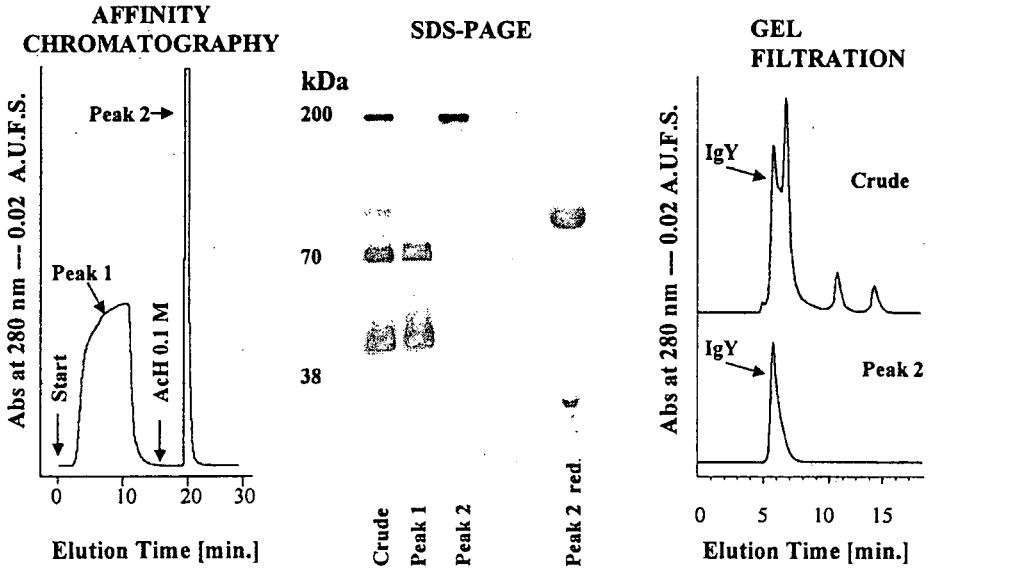
Fassina, G., Verdoliva, A., Palombo, G., Ruvo, M., and Cassani, G. *J. Mol. Recognit.* (1998) 11:128-133

PURIFICATION OF MURINE IgM mAb FROM ASCITIC FLUID ON PAM/CH-Sepharose-4B



Palombo, G., Verdoliva, A., and Fassina, G. (1998) *J. Chrom. B,* 715:137-145.

AFFINITY PURIFICATION OF IgY FROM CHICKEN EGG YOLK ON PAM-Emphaze

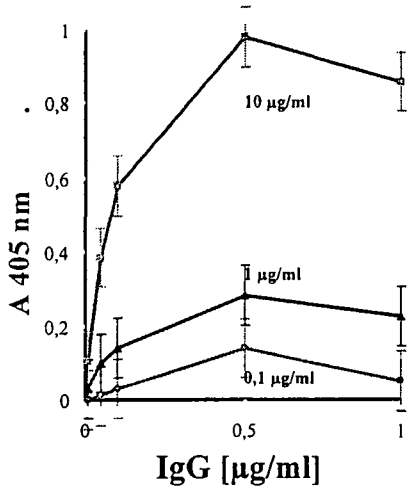


Verdoliva, A., Basile, G., and Fassina, G. (2000) J. Chrom. B, 749: 233-242

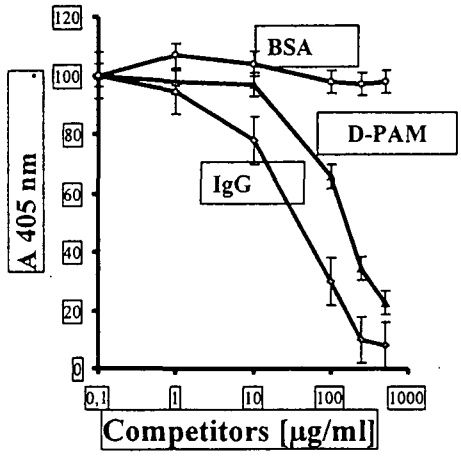
PAM CHROMATOGRAPHIC FEATURES

- SYNTHETIC PRODUCT, NO BIOLOGICAL CONTAMINANTS
- LOW PRODUCTION COST AT LARGE SCALE
- STABLE TO SANITISING AND DENATURING AGENTS (NaOH)
- CAPACITY UP TO 25 mg IgG/ml SUPPORT (HYPERD®, BIOSEPRA)
- EASY IMMOBILIZATION ON AFFINITY MATRICES
- IMMUNOGLOBULINS ADSORPTION AT pH 5.0-7.0
- IMMUNOGLOBULINS DESORPTION AT pH 3.0 OR pH 9.0
- USEFUL FOR IgG, IgM, IgA, IgE AND IgD PURIFICATION
- LOW TOXICITY (LD $_{50}$ >2000 mg/kg OS, 150 mg/kg i.v.)

Binding of Biotinylated hIgG to various amount of U937 FcγRs

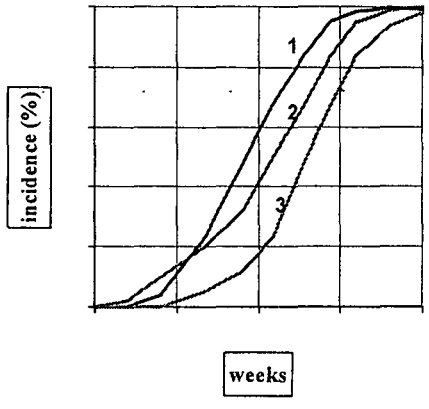


Inhibition of Biotinylated hIgG/U937 FcγRs Interaction



Lupus Erythematosus: experimental model

MRL/lpr mouse strain



1. Immune complexes deposition
2. Multiple autoantibodies anti-double stranded DNA and histon
3. Proteinuria

Molecular Diversity in Drug Discovery: A Critical Assessment

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MOLECULAR DIVERSITY IN DRUG DISCOVERY: A CRITICAL ASSESSMENT

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This Lecture will at first examine the phases of modern drug discovery and see where diversity [1,2] and combinatorial chemistry [3-6] are going to play a major role (Figure 1). Target identification and target validation are now crucial milestones, as the unraveling of the human genome is providing thousands of uncharacterized genes as potential targets for the cure of important diseases. Research laboratories able to identify and validate targets better and faster than competitors will be significantly advantaged, and combinatorial approaches and tools will provide relevant benefits at this stage [7]; nevertheless, the full potential of chemical diversity and combinatorial libraries is evident in the following three steps of the process (Figure 1).

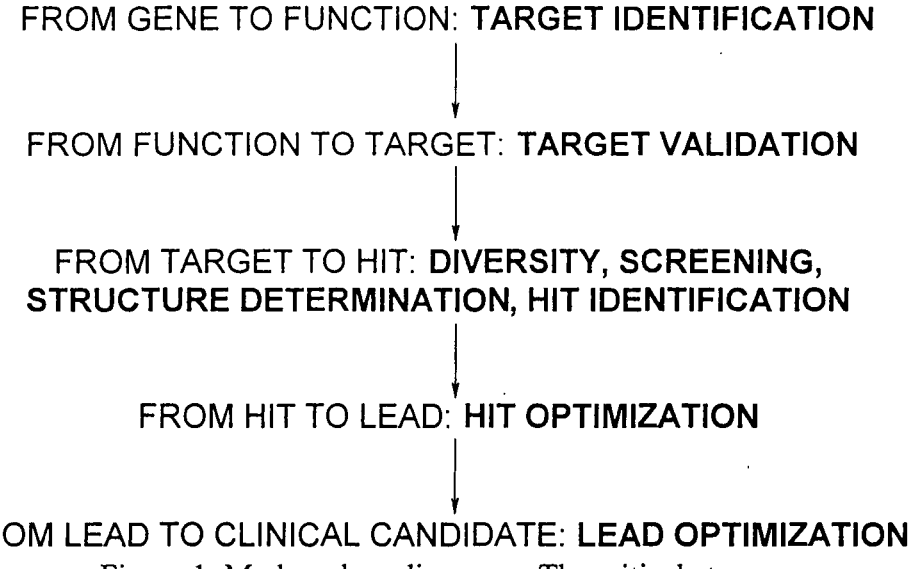

Figure 1. Modern drug discovery: The critical steps.

Traditionally the accent in Drug Discovery was put on the throughput, i.e. on the availability of large diversity collections (>>100K), of high-throughput robotics for the handling and the screening of the diversity, and of high-throughput analytical tools for the determination of the structure(s) and of the quality of active compounds. As for the collections, four major sources of compounds are available:

- Single compounds (externally acquired or in house prepared);
- Natural products from living organisms;
- Discrete libraries (parallel synthesis, individual compounds);
- Pool libraries (mix and split synthesis, mixtures).

Each source has its advantages and disadvantages, and will be thoroughly examined during the Lecture. Several key messages summarize the current tendencies related to chemical diversity and screening in hit identification:

-

- A collection must contain subsets from all diversity sources, and must evolve by acquisition/synthesis/isolation of novel, relevant individuals or libraries;
- Large pool primary libraries are becoming less popular;
- Medium-small, high quality, modular discrete libraries are increasingly popular;
- Libraries inspired by natural products' complex structures are increasingly popular, especially concerning the so-called chemical genetics approach [8,9].

The second part of this Lecture will present three recent examples referring to lead discovery and lead optimization. The first covers the synthesis of so called "activity profiling libraries", used to determine the nature of proteases in in vitro and in vivo assays and to validate their relevance as targets in Drug Discovery [10]. The second covers modular libraries in solution derived from a common chalcone library [11]. The third [12] reports a high quality solid phase pool library of complex, natural products-like compounds obtained from high quality and yield chemical transformations.

References

[1] L. Weber, *Curr. Opin. Chem. Biol.* **4**, 295-302 (2000).
[2] P. Willett, *Curr. Opin. Biotechnol.* **11**, 85-88 (2000).
[3] N. Terrett, *Combinatorial chemistry*, Oxford University Press, Oxford, 1998.
[4] *Combinatorial chemistry and molecular diversity in drug discovery*, Eds. E.M. Gordon and J.F. Kerwin, Wiley-Liss, New York, 1998.
[5] P. Seneci, *Solid-phase synthesis and combinatorial technologies*, Wiley, New York, 2000.
[6] F. Balkenhohl, C. von dem Bussche-Huennefeld, A. Lansky and C. Zechel, *Angew. Chem. Int. Ed. Engl.* **35**, 2288-2337 (1996)..
[7] D.S. Thorpe, *Combi. Chem. High Throughput Screening* **3**, 421-436 (2000).
[8] C.M. Crews and U. Splittgerber, *Trends Biol. Sci.* **24**, 317-320 (1999).
[9] B.R. Stockwell, *Trends Biotechnol.* **18**, 449-455 (2000).
[10] J.L. Harris, B.J. Backes, F. Leonetti, S. Mahrus, J.A. Ellman and C.S. Craik, *PNAs* **97**, 7754-7759 (2000).
[11] D.G. Powers, D.S. Casebier, D. Fokas, W.J. Ryan, J.R. Troth and D.L. Coffen, *Tetrahedron* **54**, 4085-4096 (1998).
[12] D.S. Tan, M.A. Foley, B.R. Stockwell, M.D. Shair and S.L. Schreiber, *J. Am. Chem. Soc.* **121**, 9073-9087 (1999).

Solid Phase Syntesis - An Overview

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Talk Overview

- Introduction and definitions of combinatorial chemistry
- Literature references
- Needs for solid phase chemistry
- Manufacture of the resin beads
- Linkers for solid phase chemistry
- Resins in solution phase chemistry
- Analog resins
- Suppliers for resins

Introductions and Definitions of Combinatorial Chemistry

Background reading

- Solid Phase Organic Synthesis: Anthony Czarnik Ed; Volume One; Wiley
- Combinatorial Chemistry: Nicholas Terret Ed.; Oxford Chemistry Masters
- Combinatorial Chemistry: A Practical Approach, Hicham Fenniri Ed.; Oxford University Press
- Solid-Phase Synthesis and Combinatorial Technologies Pierfausto Seneci Ed.; Wiley
- Organic Synthesis on Solid Phase: Supports, Linkers, Reactions Florencio Zaragoza Dorwald Ed.; Wiley-VCH

Background reading

- Solid Supported Combinatorial and Parallel Synthesis of Small Molecular Weight Compound Libraries Daniel Obrecht and Jose M. Villalgordo Eds. Pergamon (Tetrahedron Org. Chem. Ser. Vol 17)
- Combinatorial Chemistry and Technology Principles, Methods and Applications Stanislav Miertus and Giorgio Fassina Eds:Marcel Dekker
- Solid Phase Synthesis: Steven Kates and Fernando Albericio Eds. Marcel Deckker
- Combinatorial Chemistry and Molecular Diversity in Drug Discovery Eric M. Gordon and James F. Kerwin Jr. Eds: Wiley

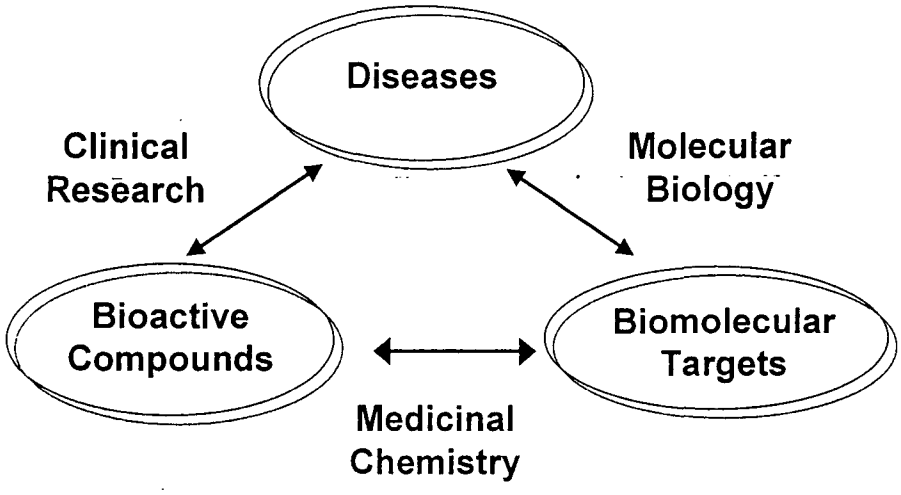
 5

Relevant websites

- **www.5z.com**
- **www.acs.org**
- **www.combichem.net**
- **www.biospace.com**
- **www.bioworld.com**
- **www.drugdiscoveryonline.com**
- **www.spoc.cc**

 6

Medicinal Chemistry Based Approach



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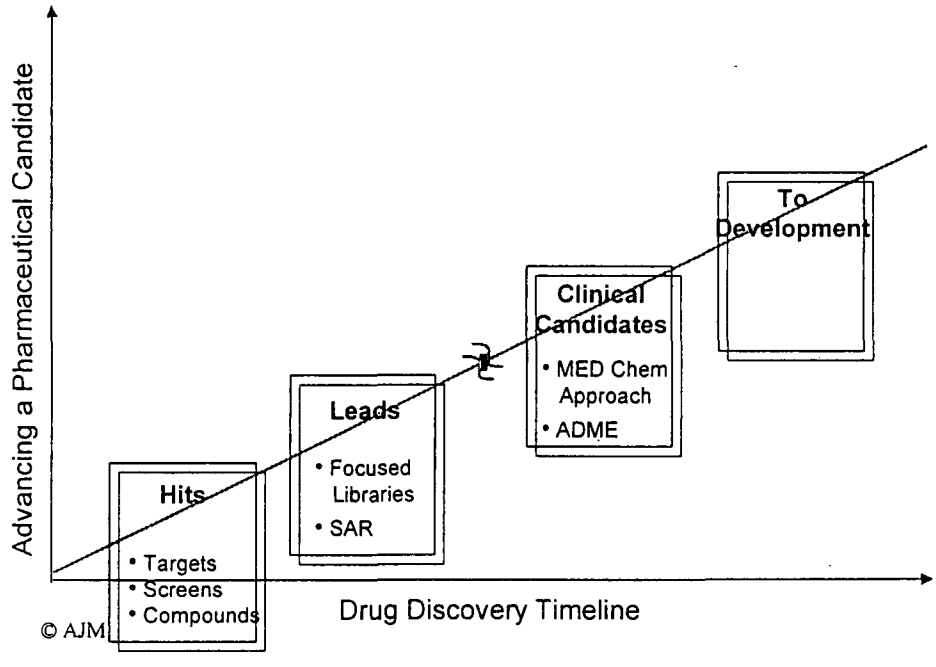
Value of a Compound



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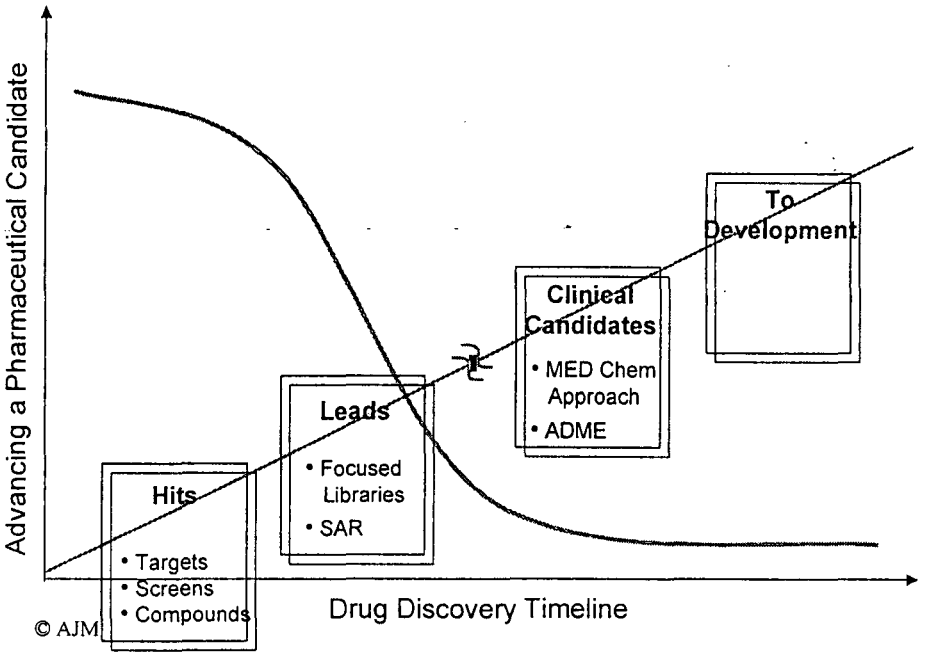
Need for Low Cost per Compound



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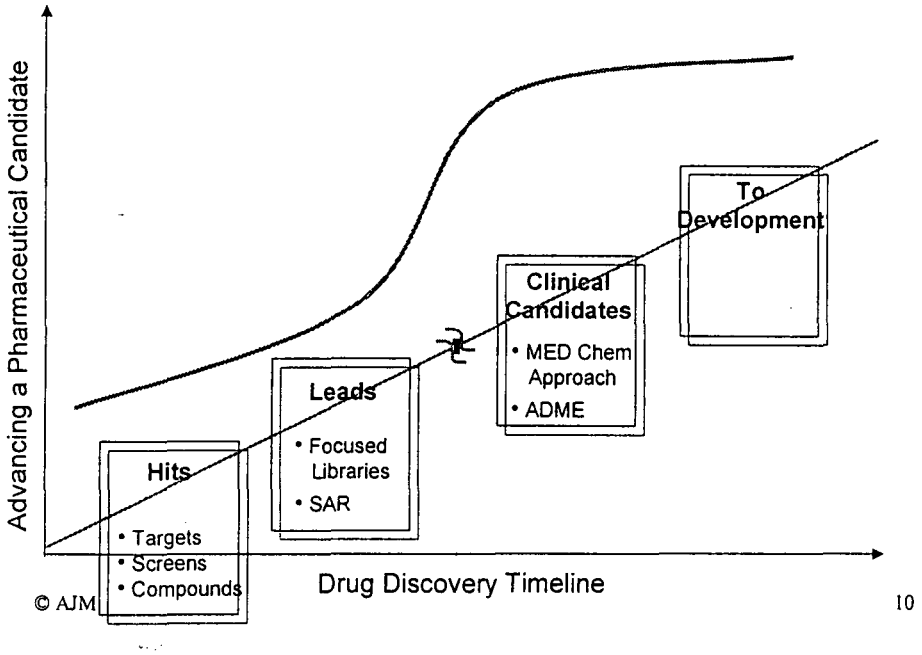
Quality of the Compounds



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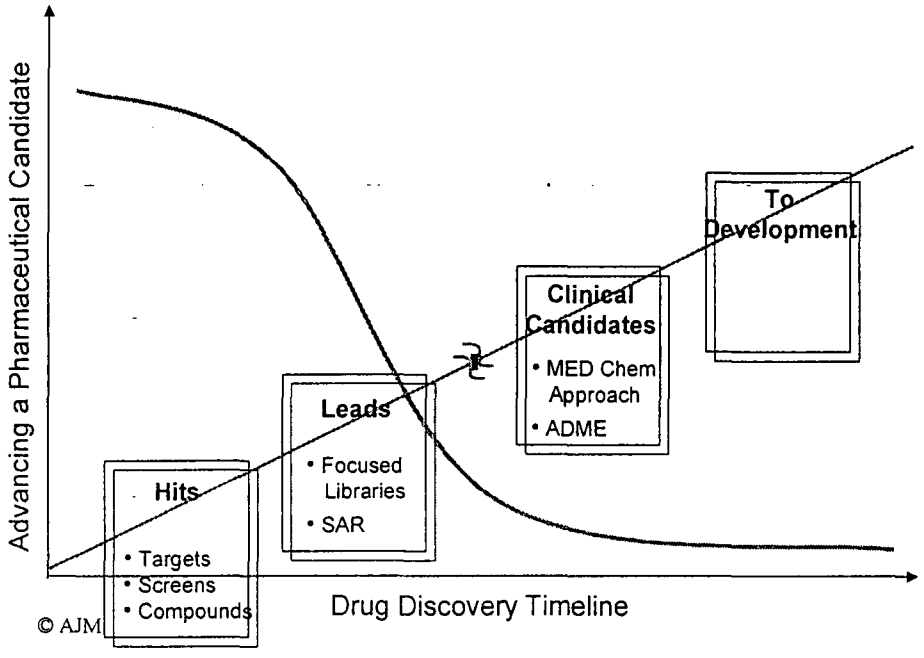
Need for High Throughput



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What is Combinatorial Chemistry?

It is a method which encompasses many strategies and processes for rapid synthesis of large or organized collection of compound called libraries

Libraries are intentionally created collections of differing molecules that may be screened for sets of pre-selected criteria

 12

Combinatorial Chemistry process

- ***Library Design*** (Medicinal Chemistry, Informatics, Computational Chemistry, Molecular Modeling)
- ***Building Block Selection*** (Inventory supply, Diversity measurement, Medicinal Chemistry)
- ***Chemical Rehearsal*** (Building Blocks, Synthesis technique, Automation, Linker technology, Bead handling, Informatics, Solid Phase chemistry)
- ***Library Synthesis*** (Analytical chemistry, Solid phase chemistry, Automation, Organic chemistry)

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Combinatorial Chemistry process

- ***Biological Screening*** (Protein expression, Assay development, Biochemistry, Automation-robotics, Informatics, Imaging Technology)
- ***Structure Elucidation*** (Microchemistry, Derivitisation, Analytical Chemistry)
- ***Hit Confirmation*** (Organic serial synthesis, Automated parallel synthesis, Individual assays)
- ***Interpretation*** (Informatics, Computational chemistry, Molecular modeling, Medicinal Chemistry)

 14

Kinds of Libraries: Discovery

- Lead Discovery:
  - Mainly used for broad screening
  - Large library size
  - Broad structural diversity
  - Many building blocks used
  - No specific structural goal
  - Undefined order of combination

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Kinds of Libraries: Focussed

- Lead Optimization:
  - Moderate Library size
  - Used for chemical analoging
  - Narrow structural diversity
  - Specific structural role
  - Specific retro building blocks
  - Specific order of combination

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Synthesis Techniques

- Solution phase
  - Parallel method
- Solid Phase
  - Split and Pool
  - Parallel Synthesis
  - Directed Sorting

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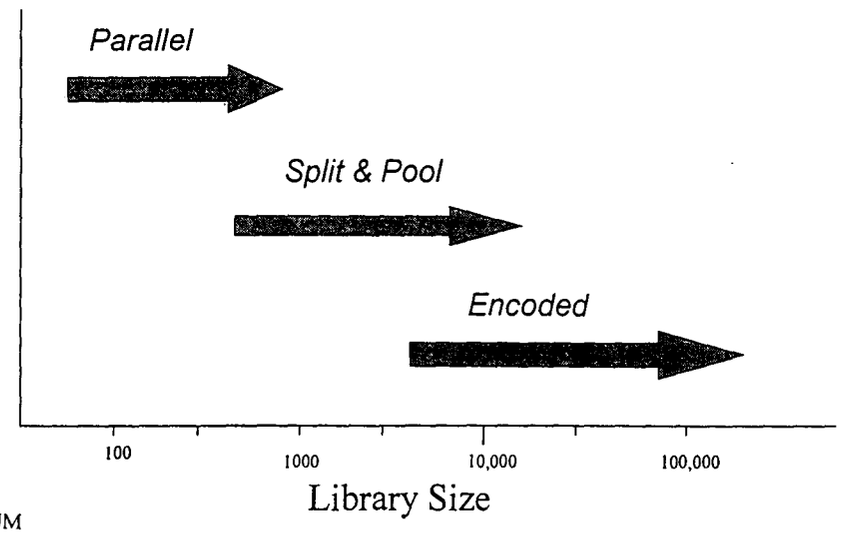
Synthesis formats



 18

Solution phase synthesis:Features

- Yields congeneric sets of compounds in SAR ordered arrays
- In principle, any chemistry and any reaction can be employed, including complex organometallic reagents, bio-catalysis, etc
- Minimal development effort is needed to "combinatorialize" a viable synthetic reaction
- Greater range of temperature accessible, e.g. -20° to + 150°
- Some degree of in-process control is possible (TLC, HPLC)
- Identity is keyed to a location (it is where you put it)

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Solution phase synthesis: Limitations

- Identity is keyed to a location (it stays where you put it)
- Cannot use mass action to drive reactions to completion, unless reagents are volatile
- Separation of products from co-products, reagents and inorganic salts is difficult
- Purity of products deteriorates rapidly in multi-step syntheses
- Massive investment in laboratory automation is needed for "big library" operation. e.g. Myriad system

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Solid Phase Synthesis: Features

- Ideally suited to split-and-pool amplification
- Excess reagents, co-products, salts are readily removed at each stage by simple washing
- Affords access to "big library" operations with modest capital investment
- Tagging techniques allow free movement/transfer of in-process materials without loss of identity

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Solid Phase Synthesis: Limitations

- Relatively small proportion of synthetic reactions adapted to solid phase methods
- Development of a "new" reaction can be long and complicated because tethering and cleavage strategies must be devised and perfected
- Library compounds tend to have "navels"
- Incompatible with solid reagents and catalysts (MnO_2, Pd/C)
- Resins and linkers can add substantial material costs
- Large scale re-synthesis of bioactive library members may not be straightforward

 22

Solid Phase Synthesis Reports

- Solid Phase Synthesis of Peptides: Merrifield R. B. *JACS* **85** 2149-54 (1963) (Nobel Prize 1984)
- First Report of Combinatorial Chemistry (Mimetope strategy) Geyson H. M. et al *PNAS* **91** 3998-4002 (1984)
- Split - and - Pool Method Furka A. et al *14th Congress Biochem Prague* 1988 pp 47, Int J Pept Protein Res. 1991, **37**, 487
- Multiple peptide synthesis using "Tea bags": Houghten R.A. *PNAS* **82** 5131-5135 (1985)

Literature Review

- **Molecular Diversity**
  obtained from natural product: Hylands P.J. and Nisbet L. *J. Ann. Rep. Med. Chem.* **26** 259-269 (1991)
  or from Peptides
  Dover W.J. and Fodor, S.P.A. *J. Ann. Rep. Med. Chem.* **26** 271-280 (1991)
- **Early reviews barely mentioned Organic Chemistry**:
  Pavia M.R.et al *Bioorg.Med.Chem.Ltr.* **3** 387-396 (1993)
  Moos W.H. et al *Ann. Rep. Med. Chem* **28** 315-324 (1993)
- **Recent reviews**: (Most referenced review of the literature)
  Peptide: Gallop M.A. et al *J. Med. Chem.* **37** 1233 (1994)
  Organic: Gordon E.M. et al *J. Med. Chem.* **37** 1385 (1994)

Reviews 1992-1996

- Jung G. *Angew. Chem. Intl.Ed Engl.* **31**, 367-486 (1992)
- Liskamp R.M.J. *Angew. Chem. Intl.Ed Engl.* **33**, 633-636 (1994)
- Rohr J. *Angew. Chem. Intl.Ed Engl.* **34**, 881-883 (1995)
- Baum R.M. *Chem Eng. News* Feb. 7, 20-26 (1994)
- Feldor, E. R. *Chimia* **48**, 531-541 (1994)
- Janda K.D.*PNAS* **91**, 10779-10785 (1994)
- Madden, D. et al *Perseptives Drug Discovery Design* **2**, 269-285 (1994)
- Desai M.C. et al *Drug Dev. Res.* **22**, 174 (1994)
- Scott J.K.et al *Curr.Opin. Biotechnol.* **5**, 40 (1994)
- Terret N.K. et al *Tetrahedron* **51**, 8135-8173 (1995)
- Thompson L.A. and Ellman J.A. *Chem Rev.* **96**, 555 (1996)

 26

Reviews: 1996-1997

- Ellman, J.A.. *Acc.Chem. Res.*, **29**, 132-143 (1996)
- Shuttleworth, S.J. et al *Synthesis-Stuttgart*, 1217-1239 (1997)
- Pirrung, M.C. *Chem. Rev.*, **97**, 473-488 (1997)
- Plunkett, M.J., and Ellman, J.A. *Sci. Amer.*, **267**, 68-73 (1997)
- Lebl, M., & Krchnßk, V. *Methods in Enzymology*, **289**, 336-392 (1997)
- Lam, K.S. et al *Chem. Rev.*, **97**, 411-448 (1997)
- Brown, R.D. *Perseptives. in Drug Discovery & Design*, **7-8**, 31-49 (1997)
- Albericio, F., & Carpino, L.A. Methods Enzymol., 289, 104-126 (1997)

 27

Reviews: 1998

- Blackburn, C. et al. *Drugs Future*, **22**, 1007-1025 (1998)
- Sofia, M.J. *Mol. Diversity*, **3**, 75-94 (1998)
- Li, J. et al *Drug Disc. Today*, **3**, 105-112 (1998)
- James, I.W. *Mol. Diversity*, **3**, 181-190 (1998)
- Seeberger, P.H., & Danishefsky, S.*J. Account. Chem. Res.*, **31**, 685-695 (1998)
- Blackburn, C. *Biopolymers (Pept. Sci.)*, **47**, 311-351 (1998)
- Dolle, R.E. *Mol. Diversity*, **3**, 199-233 (1998)
- Flynn, D.L., Devraj, R.V., & Parlow, J.J. *Curr. Opin. Drug. Disc. Develop.*, **1**, 41-50 (1998)

 28

Reviews: 1999

- Dolle, R.E., Nelson, K.H. *J. Comb. Chem.* **1**(4), 235-282 (1999)
- Booth, R.J., & Hodges, J.C. *Account. Chem. Res.*, **32**, 18-26 (1999)
- Drewry, D.H., Coe, D.M., & Poon, S. *Med. Res. Rev.*, **19**, 97-148 (1999)
- Dorner, B., Steinauer, R., & White, P. *Chimia*, **53**, 11-17 (1999)
- Furka, A., & Bennett, W.D. *Comb. Chem. High Throughput Screening*, **2**, 105-122 (1999)
- Liu, D.R., & Schultz, P.G. *Angew. Chem. Int. Ed.*, **38**, 36-54 (1999)

 29

Publications: 2000

- Clapman B., Sutherland A. J. *Tet. Lett.* **41**, 2253-2260 (2000)
- Drew M., Orton E., Krolikowski P. Salvino J.M., Kumar N.V. *J. Comb. Chem.* **2**, 8-9 (2000)
- Gerritz S.W., Sefler A.M. *J. Comb. Chem.*, **2**, 39-41 (2000)
- Hodges J.C., Harikrishnan L.S., Ault-Justus S., *J. Comb. Chem.*, **2**, 80-88 (2000)
- Hulme C., Ma L., Kumar N.V., Krolikowski P.H., Allen A.C., Labaudiniere R. *Tet. Lett.* **41**, 1509-1514 (2000)
- Karan C., Miller B.L. *Drug Disc. Today* **5**, 67-75 (2000)
- Leznoff C.C. *Can. J. Chem.*, **78**, 167-183 (2000)
- Li W.R., Hsu N.M., Chou H.H., Lin S.T., Lin Y.S. *Chem Commun.* 401-402 (2000)
- Munoz B., Chen C.X., McDonald I.A. *Biotech. Bioeng.* **71**, 78-84
- Nicolaou, K.C., Cao G.Q., Pfefferkorn J.A. *Angew.Chem. Int.Ed.* **39**, 734-743 (2000)

Reviews: 2000

- An, H., Cook P.D. *Chem. Rev.* **100**, 3311-3340 (Soln.)
- Baldino, C.M. *J. Comb. Chem.* **2**, 89-103 (Soln.)
- Bennett, W.M. *Comb.Chem. A Practical Approach*, Fenniri H. Oxford University Press pp 139-262 (SPS)
- Dolle, R.E. *J. Comb. Chem.* **2**, 383-433 (Overview)
- Hodges, J.C.*Synlett* 152-158 (Scavenger)
- Ley, S.V., Baxendale, I., et. al. *J.Chem.Soc. Perkin Trans. I*, 3815 (2000) (300 pgs, Solid Supported reagents and scavengers order from RSC ISBN 0854049440)
- Toy, P. H.; Reger, T. S.; Janda, K. D. *Aldrichimica Acta.*, **33**, 87 (2000)

Publications and Reviews: 2001

- Mendal, M. et al *J.Comb. Chem.* **3,** 28-44
- Henlin J. M. et al *J. Pept. Res.* **57,** 87-96
- Albericio F. *Biopolymers (Pept. Sci)* **55**, 123-139 (2000)
- Kassel D.B. *Chem. Rev.* **101,** 255-267 (analysis)
- Sankan S. *Angew. Chem. Intl. Ed.*, **40**, 312-329 (catalysis)
- Reetz M. T. *Angew. Chem. Intl. Ed.* **40**, 284-310 (catalysis)
- Yan B. *J. Comb. Chem.* **3**, 78-84 (analysis)
- Arya P. et al. *Chem. Eur. J.*, 7, 555-563 (carbohydrates)
- Domling A. et al. *Angew. Chem. Intl. Ed.* **39**, 3169-3210 (2000)
- Prestwich G.D. *Trends Biotech.*, **18**, 64-77 (2000)

Solid Phase Organic Synthesis

- First done to support the peptide field
  Boc chemistry (Merrifield : ABI instruments)
  Fmoc chemistry (Sheppard: Biosearch instruments)
- Main advantage was that excess reagent removed by washing steps
- Early papers in the 1970 did not support SPS for organic chemistry

- Early success with SPS for Organic chemistry:
  Leznoff C. *Acc. Chem. Res.* **11**, 327-333 (1978)
  Neckers D.C. *ChemTech* 108-116 (1978)
  Frechet J.M.J. *Tetrahedron* **37**, 663-683 (1981)

Solid Phase Organic Synthesis

- 1970-1980's: Period for the development of selective reagents and methods for solution phase organic synthesis
- Only organic and medicinal chemists involved in SPS were the peptide and oligonulceotide chemists
- Explosion of the present day started in 1992 with the Bunin/Ellman publication of benzodiazepines on solid phase (*JACS* **114**, 10997-10998) Followed by the Parke-Davis (Divosomer paper) *PNAS* **90**, 6909-13 (1993)
- Review of 1992-1996 papers
  Fruchtel, J.S. and Jung, G. *Angew. Chem. Intl.Ed Engl.* **35**, 17-42 (1996)

 34

Needs for Solid Phase Synthesis

Organic Synthesis

- Traditional medicinal chemist: 1-2 weeks for synthesis of a compound and then submits it to screening where it takes weeks for assays
- New Automation for synthesis : 5-10K compounds/week achievable
- Screening goals: 500K compounds/week

Needs for SPOC

- Resin beads: mainly been Polystyrene beads with a 1% crosslink. Europe used the 200-400 mesh (35-75mm), USA used the 100-200 mesh (75-150 mm) (StratoSpheres, NovaSyn)
- Solvents in which these beads swell the best to ensure maximum reaction
- Good loading on the resin beads and consistent from batch to batch
- Other resins which have been used are the PEG-PS grafted resins (TentaGel, ArgoGel, SPOCC, NovaGel, HypoGel and PEG-PS); Macroreticular resins (ArgoPore, TentaPore, ScavengePore); Methylacrylate resins (PEGA)
- Variety of linkers and scaffolds

The Market

- Applications
  - Peptide synthesis
  - Solid phase combinatorial chemistry
  - Solution phase combinatorial chemistry
  - Reagents on resins
- Particle size and formats
  - Microporus and Macroporus resins
  - 75-150 mm(100-200 mesh) and 150-300 mm (50-100 mesh)
- Base resins
  - Polystyrene based
  - Pegylated based resins

Resin Particle

- Microporous
  - Peptide synthesis
  - Combinatorial chemistry
  - Scavenger resins
  - Reagents on resin
- Macroporous
  - Scavenger resins
  - Reagents on resins
- Magnetic particles
  - Combinatorial chemistry

Solid Phase Syntesis – Resent Developments in Resin Technology

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mendonca@powersurfr.com

Uses of Resin

- Solid Phase synthesis
  - Peptide synthesis
  - Oligonucleotide synthesis
  - Small molecule organic synthesis
  - Material sciences
- Solution phase synthesis
  - Scavenger resins
  - Analog resins
  - Purification resins
- Purification and Analysis
  Ion exchange

 6

Manufacture of a Resin Bead

Reproducibility

"The key to reproducible synthesis is to use a resin that is manufactured reproducibly"

 8

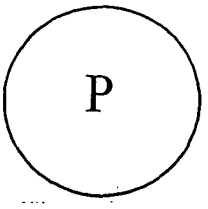
How does one choose a resin

- Pick a product catalog
- Pick the appropriate linker-resin
  - MERRIFIELD RESIN
    - 0.2-1.2 mmol/gm
    - 100-200 mesh (75-150 μm)
    - 1% DVB
    - 8.5 mL/gm in DCM

Introduction

- Resins which are base polymers with a linker attached serve to be an inert carrier of a synthetic substrate
- Linker is a functional moiety which allows the attachment and cleavage of the substrate under controlled conditions
- Linkers should be stable to a variety of reaction conditions; have points of diversity and have a functional group easily attached or cleaved

Original Criteria for the Solid Support



- Insoluble and yet permeable
- Inert and yet functional
- Useful and yet minimal side reactions

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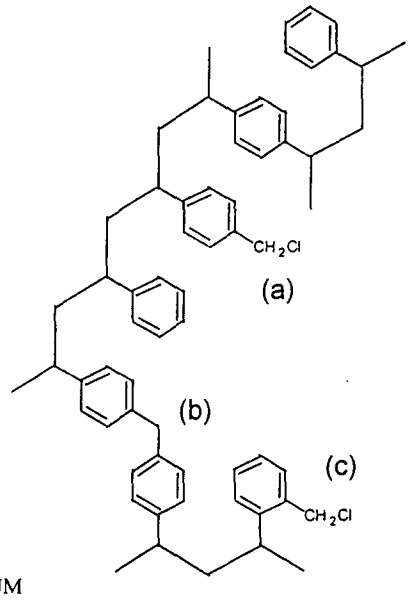
Chloromethylation of Polystyrene



Degree of functionalisation is dependent on reaction conditions and choice of catalyst.

(a) Chloromethylation giving desired product
(b) Undesired additional crosslinking
(c) Chloromethylation in hindered ring positions

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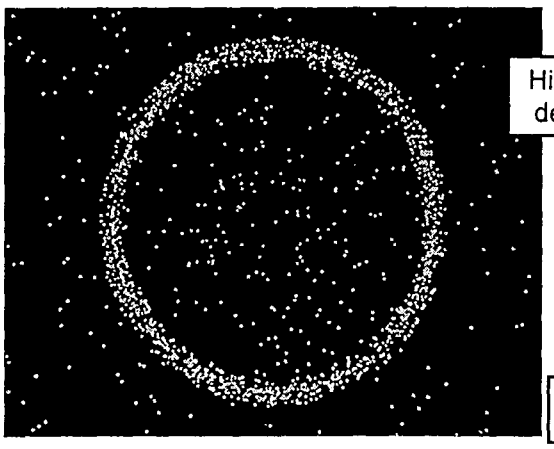
Chloromethylation : Problems

- Particles are no longer homogeneous ...



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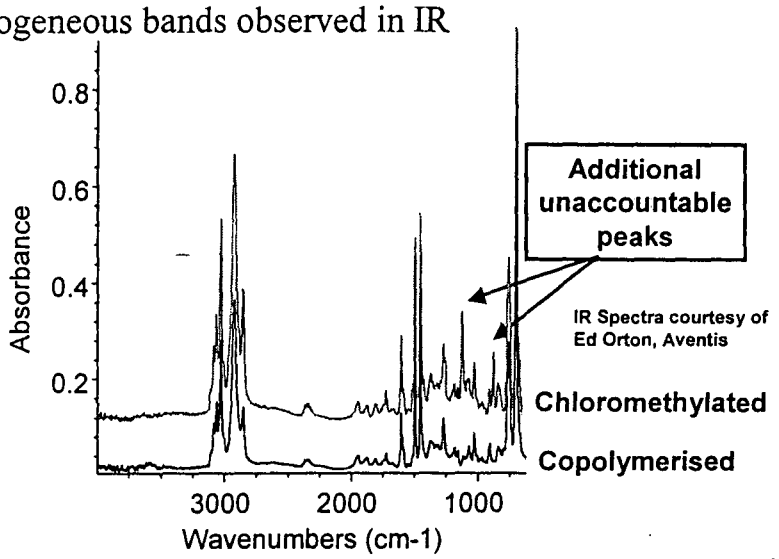
Chloromethylation : Problems

- Heterogeneous bands observed in IR



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Developing New Solid Supports : *Objectives*

- Maximizing the chemical properties of beads for improved synthesis
- Optimizing the physical properties of the beads for better performance and consistency
- Improving the loading capacity of beads to optimize yields
- Using the new resins for the synthesis of new molecules

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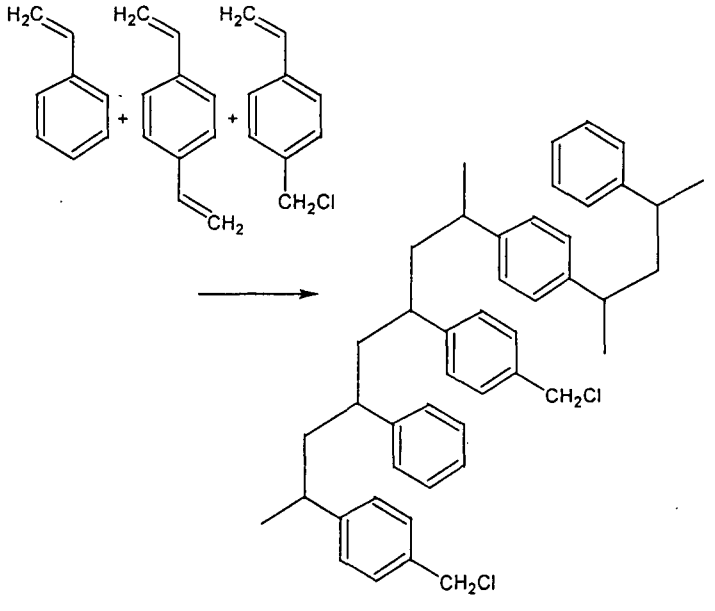
Optimizing Chemical Properties : Co-polymerization



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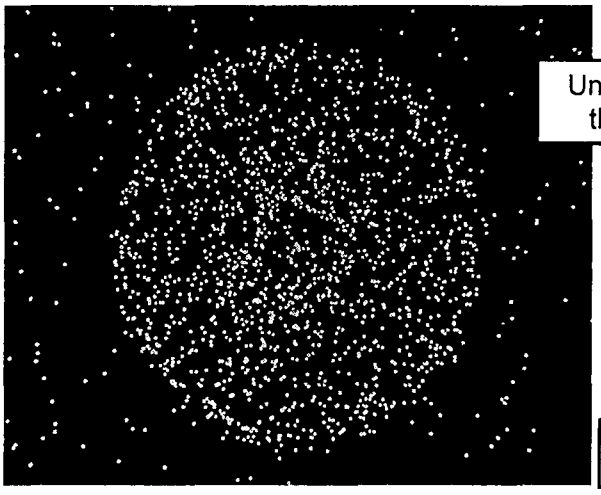
Copolymerisation : Advantages

- Particles are homogeneous



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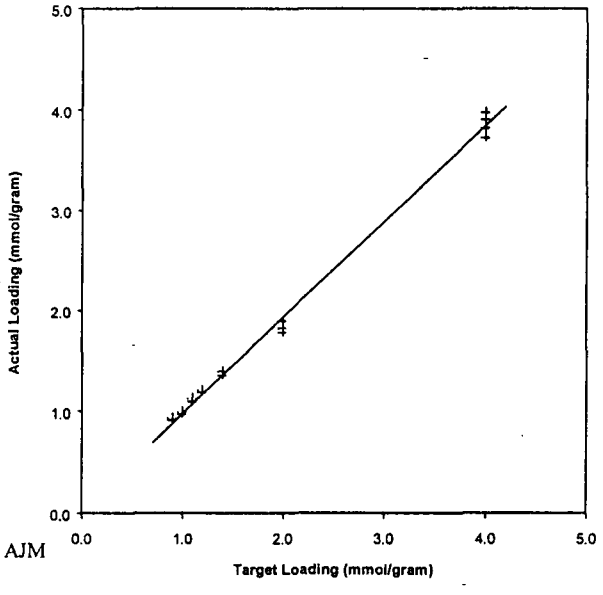
Optimizing Chemical Properties : Loading Reproducibility



Loading (mmol/g)	Mol%
0.0	0%
1.0	11%
2.0	23%
4.0	52%
6.5	98%

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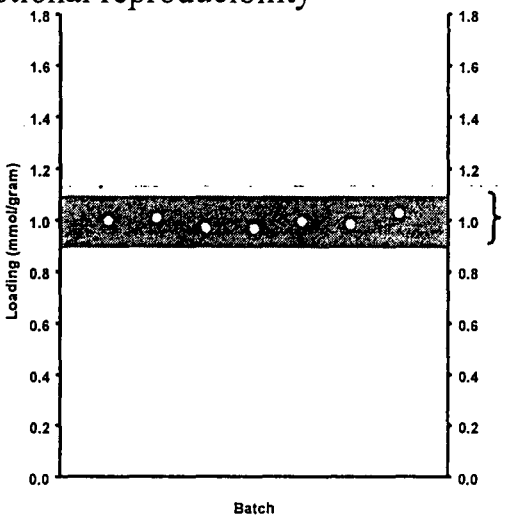
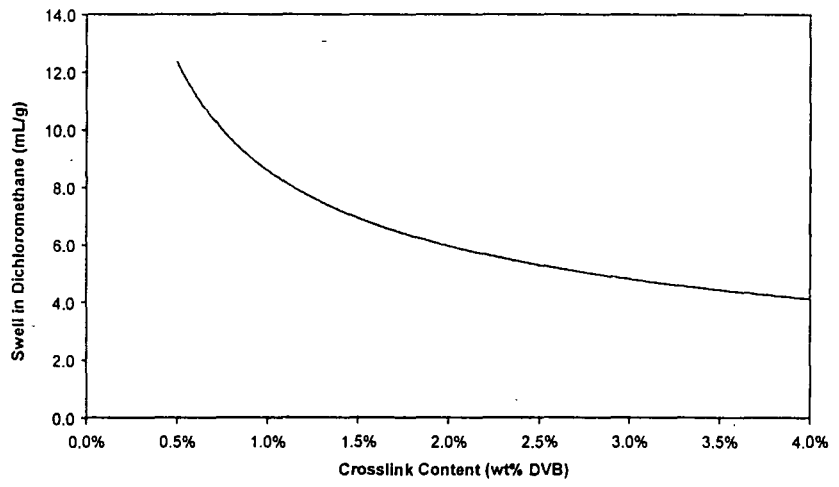
Copolymerisation : Advantages

- Exceptional reproducibility

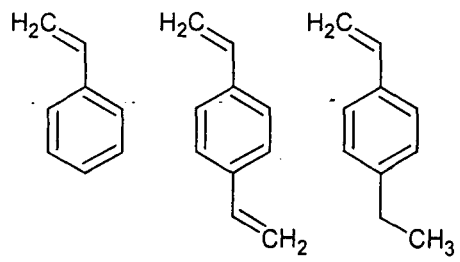


Narrow Specification

Optimizing Chemical Properties : Effect of Crosslinker

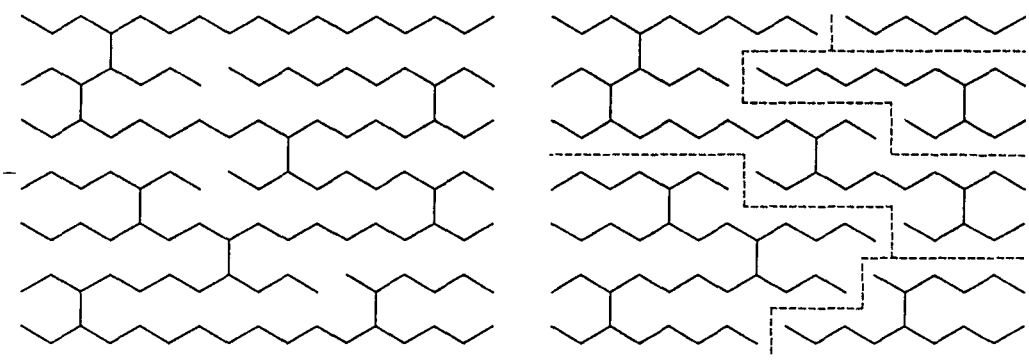


Reasons for Swell Variation : Monomer Purity



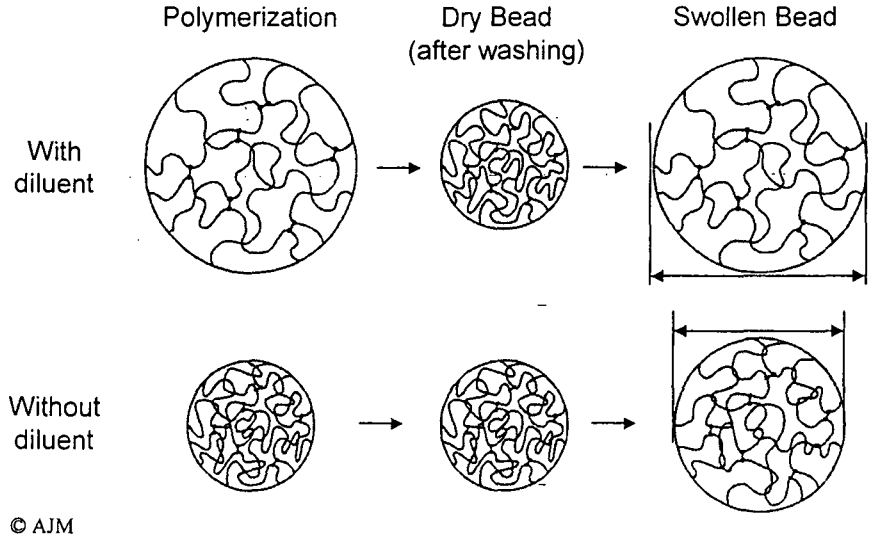
Technical grade divinylbenzene typically contains around 35% ethylvinylbenzene impurity.

Reasons for Swell Variation : Initiator Concentration



Higher levels of initiator creates shorter chains.

Reasons for Swell Variation : Effect of Diluent



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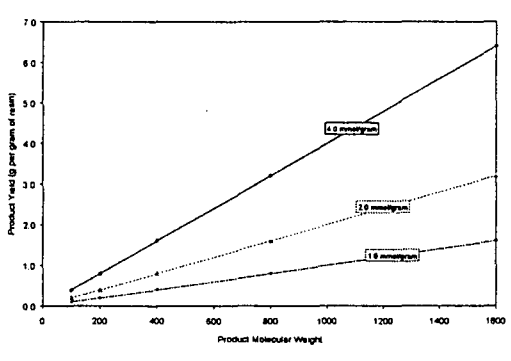
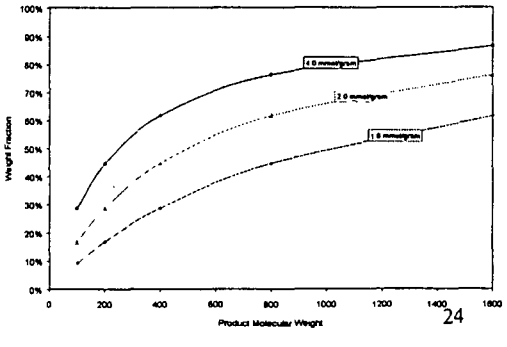
Optimizing Physical Properties : Effect On Yield



... increased loading gives increased yield.

Increased loading gives higher product-to-resin ratio ...



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Sizes: Resin Beads

- Commercial sources generally offer a size variation of the beads
- Size of >50 micron needed (avoid frit plugging)
- Smaller resin beads called fines major problem with leakage and plugging
- Most commonly used beads are 100-200 mesh (75-150 micron) in the USA and 200-400 mesh (75-38 micron)
- Large beads show slow reactivity and fragile

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Advantages and Disadvantages

- ***Advantages***
  All excess reagents and soluble by-products are removed with washing. Resin bound toxic materials handled safely. Excess reagents used to drive reactions to completion
- ***Disadvantages***
  Additional attachment and cleavage steps
  Swelling properties of the resin to be factored in.
  Batch to batch reproducibility of the loading and resin manufacture. Chemistry transfer from existing protocols.

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Base Polymer resins

- Polystyrene 0.5-2.0% cross linking with DVB (StratoSpheres)
- Polyethylene glycol polymerised onto 1% cross linked PS-DVB (TentaGel, ArgoGel, NovaGel)
- Polyethylene glycol grafted onto 1% cross linked PS (PEG-PS)
- Highly crosslinked polystyrene matrix Macroporous resin (ArgoPore, TentaPore)
- Bis -2-acrylamide-co-bisacrylamidopolyethylene glycol-co-monoacrylamido polyethylene glycol (PEGA)
- Dimethylacrylamide supported within the macropores of kieslguhr matrix (Pepsyn K)
- Dimethylacrylamide supported within the macropores of 50% cross linked polystyrene-DVB matrix (Polyhipe)

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Resin Loading Determination

- Loading values supplied is a measure of the amount of reactive groups present on the resin
- Normally published as mmol/gm
- Methods of loading determination used
  Quantitative cleavage of the chromophore e.g. Fmoc (301 nm) (amine, alcohol and acid resins)
  Elemental analysis (aldehydes, sulfonamides)
  Quantitative product obtained on cleavage after reaction
  ^{1}H NMR

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Swell Comparison : Other Resin Types

Solvent	Polarity	PEGA Resin 0.4 meq/g PEG crosslinked	CMS Resin 1.0 meq/g 1% crosslinked	Tentagel 0.3 meq/g 130µm
Toluene	2.4	12.0 mls/g	8.0 mls/g	5 mls/g
CH_2Cl_2	3.1	13.4 mls/g	8.0 mls/g	5 mls/g
THF	4.0	12.7 mls/g	8.5 mls/g	6 mls/g
$CHCl_3$	4.1	13.4 mls/g	8.5 mls/g	-
MeOH	5.1	13.4 mls/g	-	4 mls/g
DMF	6.4	11.7 mls/g	5.5 mls/g	5 mls/g
Water	10.2	15.5 mls/g	*2.0 mls/g*	4 mls/g

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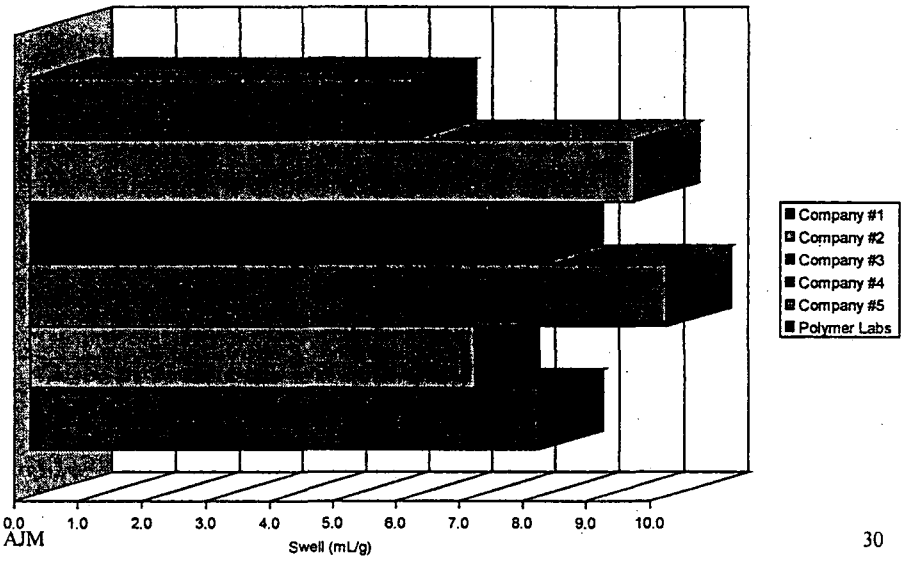
Swell Comparison : Various 100-200 mesh Resins



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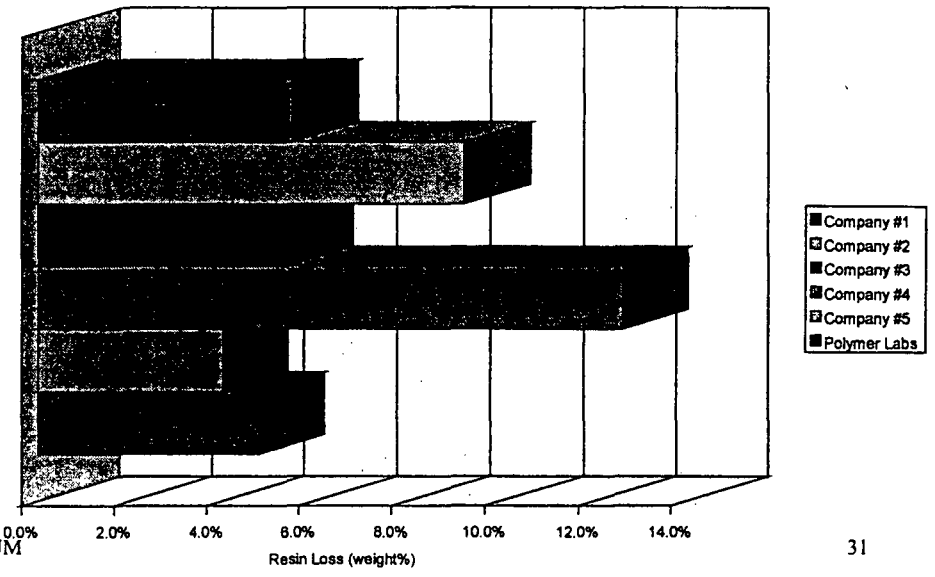
Resin Loss from Kans (100-200 mesh particle size)



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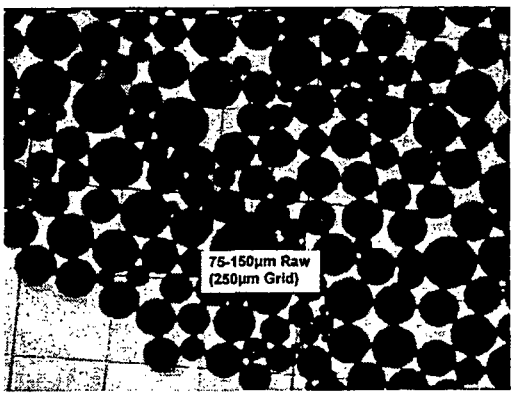
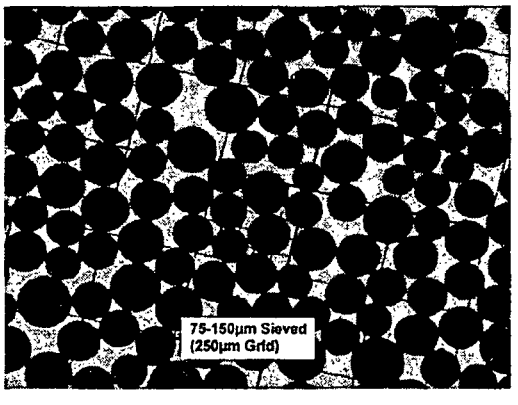
Optimizing Physical Properties : Effect of Particle Size

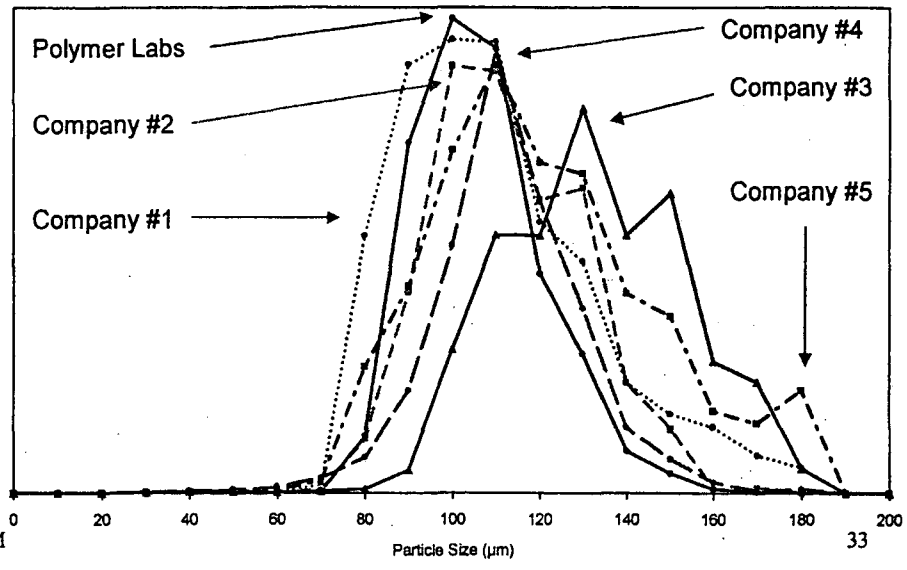


Raw distribution prior to sieving

Finished distribution after sieving

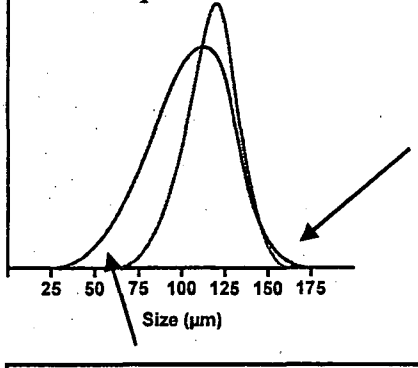


Particle Size Comparison : Various 100-200 mesh Resins



Sieving

- Finished particle size distribution



Removal of oversized particles and aggregates

Removal of undersized "fines" to prevent frit clogging

Linkers for Solid Phase Chemistry

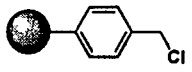
Linkers for SPOC

- They are functional groups attached to resin to enable the attachment and cleavage of a variety of substrates under specific conditions
- Should be stable to a variety of reaction conditions
- Sometimes referred to a polymer bound protecting group
- Linker substrate bond is cleaved and the reagents and by-products removed easily
- Classifications done various ways
  (1) functional groups (Carboxylic acid, Alcohol, Amine)
  (2) cleavage conditions (Acid, Nucleophilic attack, Safety catch, Traceless, Photolabile)

Overview

- Linkers used
  - Halomethyl
  - Hydroxy
  - Amino
  - Aldehyde
  - Carbonate
  - Silyl

Halomethyl Resins

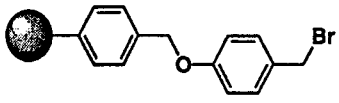


Merrifield Resin

Attachment: carboxylic acids, alcohols phenols, thiols, amines

Synthesis: acids, alcohols, esters, thioesters

Cleavage: TFMSA, H_2/Pd, DIBAL, MeONa, HF



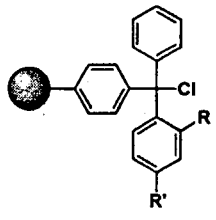
Bromo Wang Resin

Attachment: alkyl and aryl amines

Synthesis: anilides and sulfonamides

Cleavage: TFA, thionyl chloride/TFA

Halomethyl Resins



Trityl resins

R=H, Cl R^1=Me, OMe

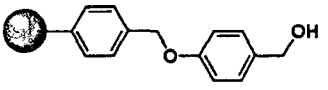
Attachment: alcohols, acids, phenols, thiols, amines

Synthesis: alcohols, acid, thiols, amines, esters,

Cleavage: 1-5%TFA, 30% HFIP (hexafluoroisopropanol)

2ClTrt resins is useful for the prevention of DKP formation in peptides
If OH is present has to be first converted to the reactive chloride

Hydroxy resins

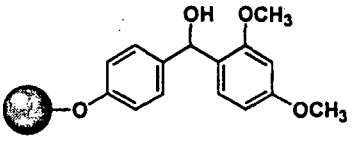


Wang Resin or HMP Resin

Attachment: alcohols, acids

Synthesis: alcohols, acid, amides

Cleavage: TFA, amine/$AlCl_3$, DIBAL



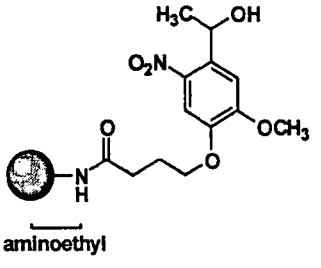
Rink Acid Resin

Attachment: alcohols, acids

Synthesis: alcohols, acids

Cleavage: 5%TFA, 10% AcOH

Hydroxy Resins



Hydroxyethyl Photolinker Resin

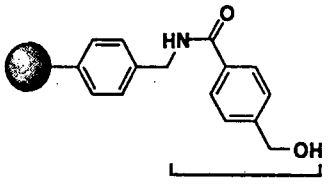
Attachment: acids

Synthesis: acids

Cleavage: light 365 nm

Linker stable to TFA and PIP conditions

Hydroxy Resin



Sheppard linker (hydroxymethylbenzoic acid)

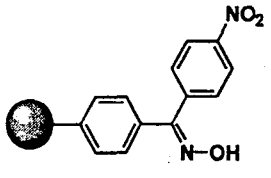
HMBA- AM Resin

Attachment: acids,

Synthesis: acids amides, alcohols, esters, hydrazides

Cleavage: Nucleophiles (NaOH; NH_3/MeOH; $NaBH_4$/EtOH; MeOH/TFE; NH_2NH_2/DMF

Hydroxy Resins

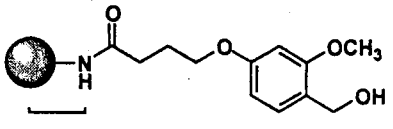


Oxime Resin

Attachment: protected peptides, acids

Synthesis: cyclic peptides, ureas, segment condensation

Cleavage: 25% TFA, hydrazide



BHA or MBHA resin

Rinker linker

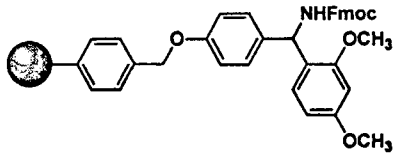
HMPB Resin

Attachment: alcohols, acids, phenols

Synthesis: alcohols, acid, phenols

Cleavage:1-5% TFA

Amino Resins



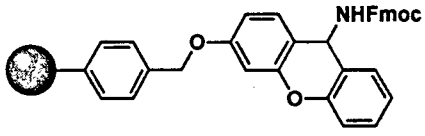
Rink Amide Resin

Attachment: acids

Synthesis: carboxamides

Cleavage: two step TFA,

AM/MBHA has a one step 95% TFA



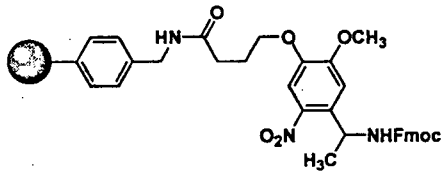
Sieber Resin

Attachment: acids

Synthesis: protected amides

Cleavage: 1% TFA

Amino Resins

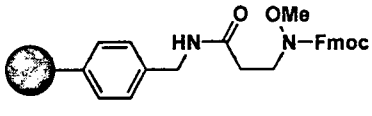


Fmoc-aminoethyl Photolinker AM Resin

Attachment: acids

Synthesis: protected amides

Cleavage: light 365 nm



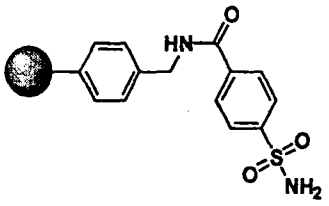
Weinreb AM Resin

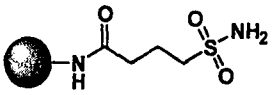
Attachment: acids

Synthesis: aldehydes and ketones

Cleavage: $LiALH_4$ or Grignard reagent

Amino Resins: Safety catch





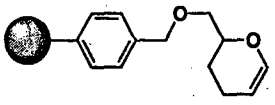
4-sulfamylbenzoyl AM resin 4-sulfamylbutyryl AM resin

Attachment: carboxylic acids

Synthesis: amides or carboxylic acids

Cleavage: activation of sulfonamide with diazomethane or bromoacetonitrile followed by Nu^- attack by amine or hydroxide

Aldehyde Resins

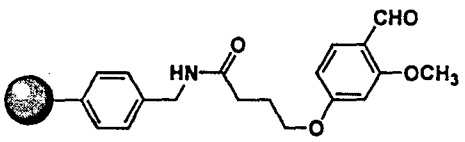


Attachment: primary/secondary alcohols

Synthesis: alcohols

Cleavage: 95% TFA/H2O or TFA/DCM/EtOH

DHP-AM Resin



Attachment: amines

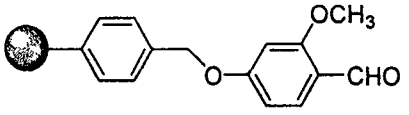
Synthesis: carboxamides or sulfonamides

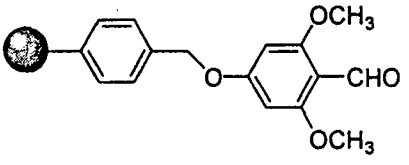
Cleavage: 25% or 5% TFA

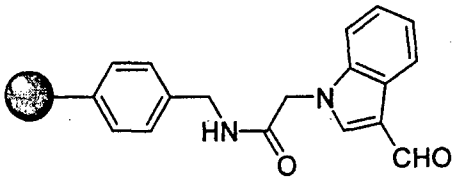
FMPB-AM Resin

4-(4-Formyl-3-methoxyphenoxyl)butyryl AM Resin

New Aldehyde resins







Attachment:Amines

Synthesis: Carboxamides, sulfonamides

Cleavage: TFA

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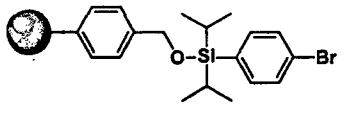
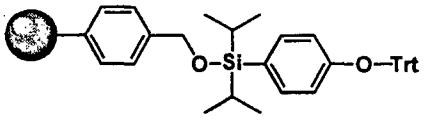
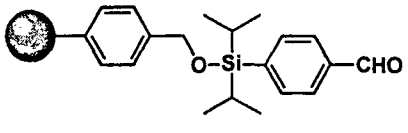
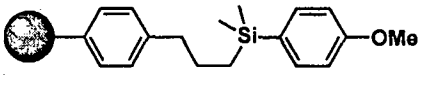
Silicon resins: Traceless linkers









Useful for the SPS synthesis of substituted arenes.
Cleavage and simultaneous desilylation effected with HF, TFA, or TBAF depending on nature of substituents

15

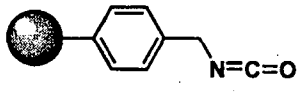
Resins for Solution Phase Synthesis

Use of Resins

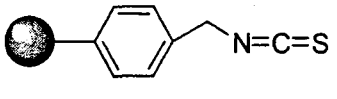
- Scavengers
  - Nucleophilic scavenger resins
  - Electrophile scavenger resins
- Reagents on resins
  - Coupling reagents
  - Oxidising reagents
  - Reducing reagents
  - Bases on resin
  - Miscellaneous resins

 17

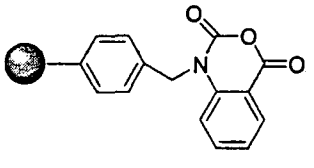
Electrophilic Scavenger Resins



Methylisocyanate Resin
Removal of Nucleophilic reagents like amines and hydrazines



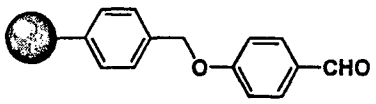
Methylisothiocyanate resin
Removal of amines and hydrazines



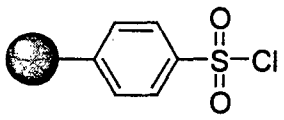
Isotoic Anhydride
Removal of primary and Secondary amines

 19

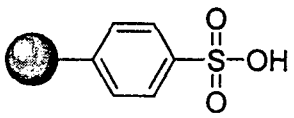
Electrophilic Scavenger Resins



Benzyloxybenzaldehyde Resin
Removal of hydrazines,
hydroxylamines and 1,2 aminothiols

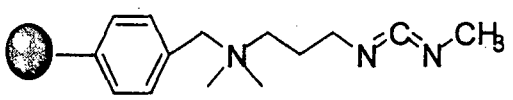


Sulfonyl chloride
Used for the imbolization
of alcohols

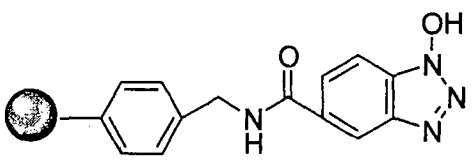


Sulfonic acid
Immobilize olefins and epoxides
Scavenge Nitrogen nucleophiles
EDC and activated esters

Coupling Reagents on Resin

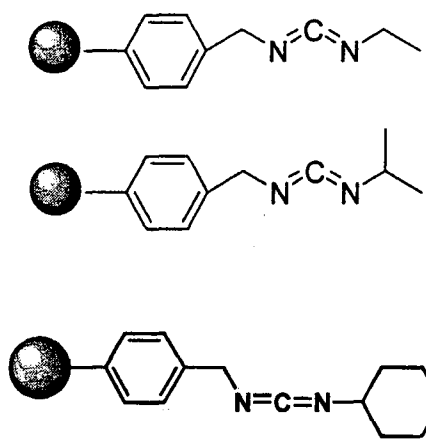


EDC on resin
Useful for coupling reactions



HOBt on Resin
Useful for amide and
Peptide synthesis

Coupling Reagents on Resin



Various carbodiimide Resin
Catalyst for the mediation solution phase coupling reactions.
Solid phase catalyst for acylation reactions
Urea byproduct remains on the resin

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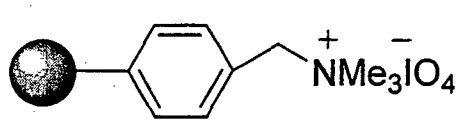
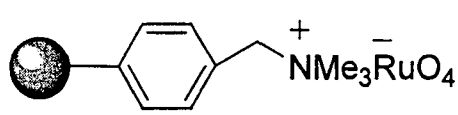
Polymer-bound oxidizing agents



Useful for conversions of Alkenes to aldehydes

Oxidation of sulfur compds



Useful for the oxidation of Primary alcohols

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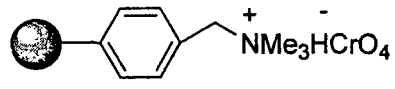
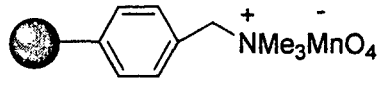
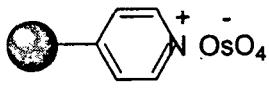
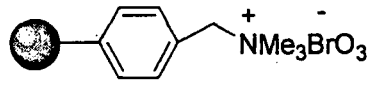
Polymer-bound oxidizing agents



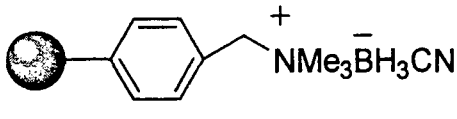




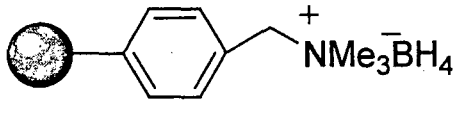


Mainly available as Amberlyst resins
Uses depend on chemistry

Polymer-bound reducing agents

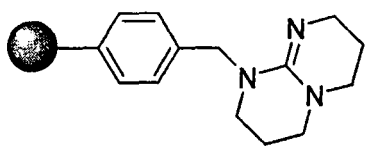


Cyano boroydride on resin
Useful for reductive aminations

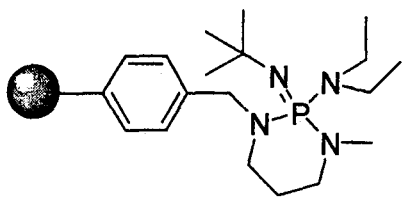


Borohydride on resin
Useful for reduction of aldehydes, ketones, imines and α,β unsaturated carbonyl compounds without reduction of the double bond

Supported Bases on Resin



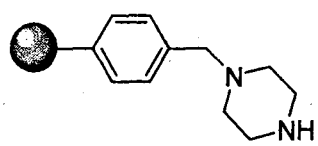
TBD on resin
Useful as an acylation catalyst



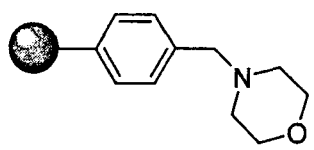
BEMP on resin
Strong base useful
For N-Alkylation

© AJM 26

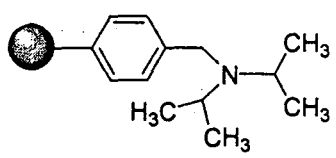
Supported Bases on Resin



Piperzine on resin
Useful as a Knovenagel catalyst



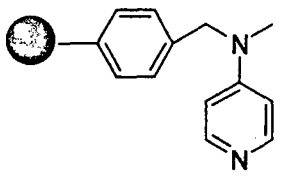
Morpholine on resin
Useful in the synthesis of Amides, sulfonamides
Used as proton sponge



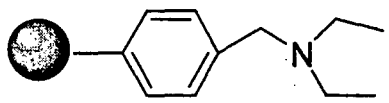
Diisopropyl amine on resin
Useful for the synthesis of Amides, sulfonamides and carbonates

© AJM 27

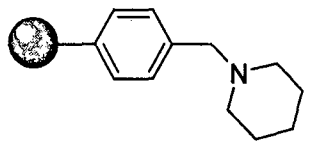
Supported Bases on Resin



DMAP on resin
Useful as an acylation catalyst

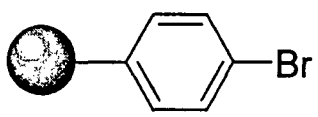


Diethylamine on resin
Useful as a proton sponge



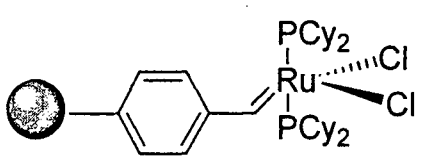
Piperdine on resin
Useful as tertiary base

Miscellaneous Reagents on Resin



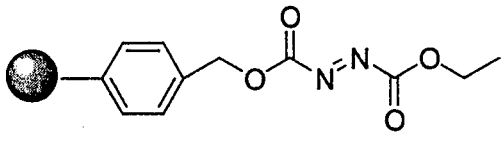
PBS Resin (p-bromostyrene)

Useful for preparation of supports and solid supported reagents via controlled lithiation or Pd mediated coupling

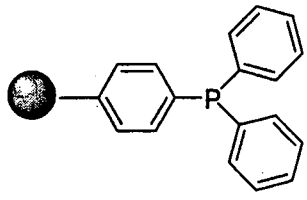


Polymer Supported Grubbs Catalyst
Useful for construction of macrocycles by ring closing alkene methasesis

Miscellaneous Reagents on Resin



Azodicarboxylate on resin
Useful for Mitsonubu reactions



Triphenyl phosphine on resin
Useful for Wittig, Mitsunobu, and halogenation reactions
Scavenger for alkyl halides and palladium

© AJM 30

Analog Resins

Analoging in Lead Optimization

- Well-defined lead structures
- Class(es) of analogs
- Straightforward, established chemistry
- 10's to low 100's of analogs
- Relatively high purity
- Fast turn-around

© AJM 32

Current Process

- Mostly solution chemistry
- One analog a time, or
- Small Multiples by parallel (array) synthesis
- Individual work-up and purification
- Slow and labor-intensive

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New Process

- Solid phase chemistry
- Clean reactions
- Parallel (array) synthesis
- Multiple 100's
- No/minimum purification/work-up
- Fast turn-around

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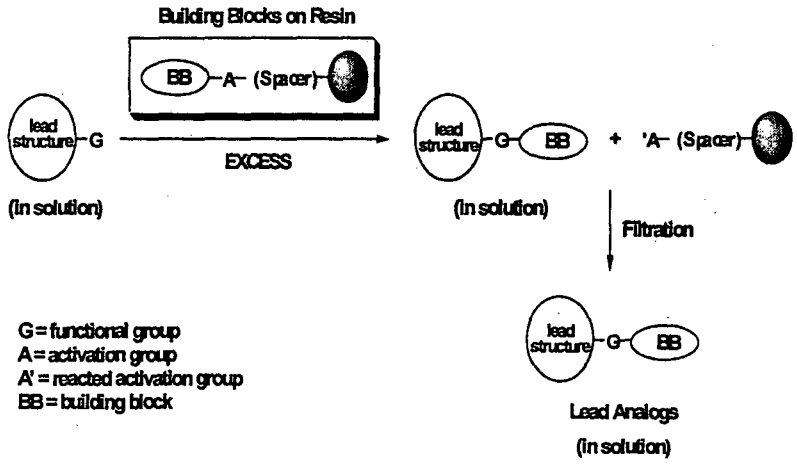
General Concept



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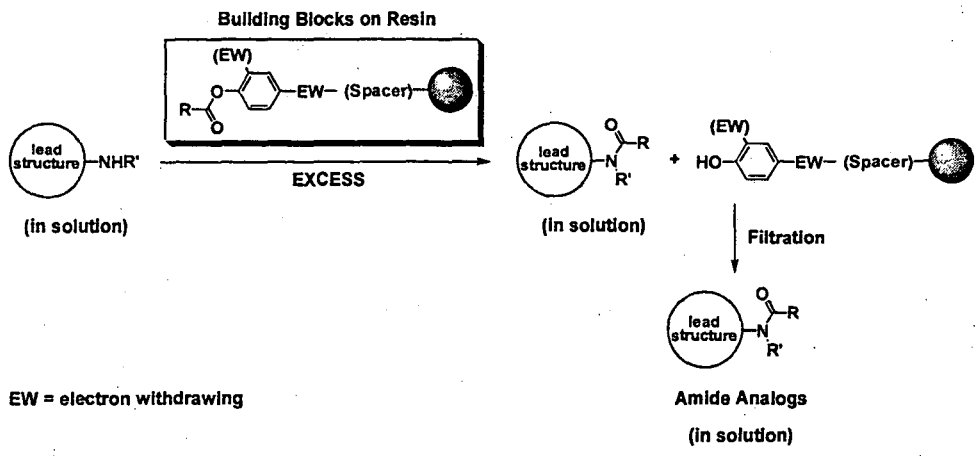
Functional Trapping: Acylation



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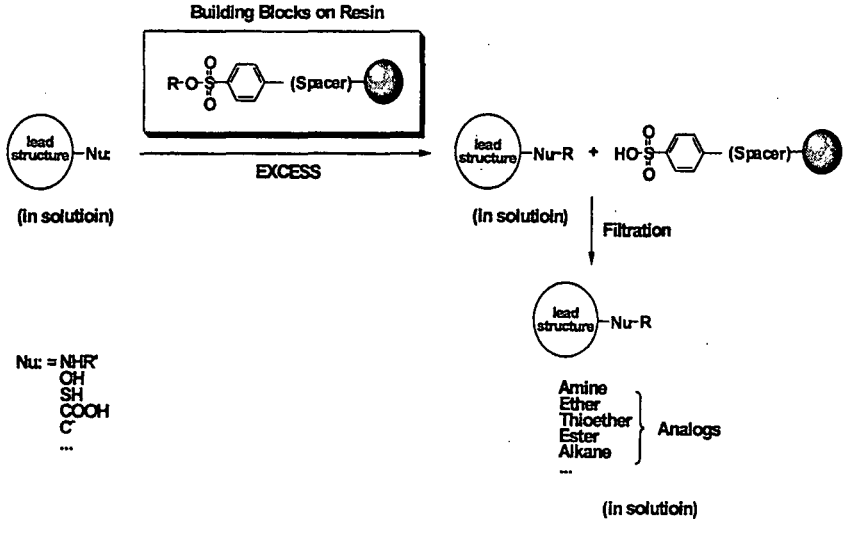
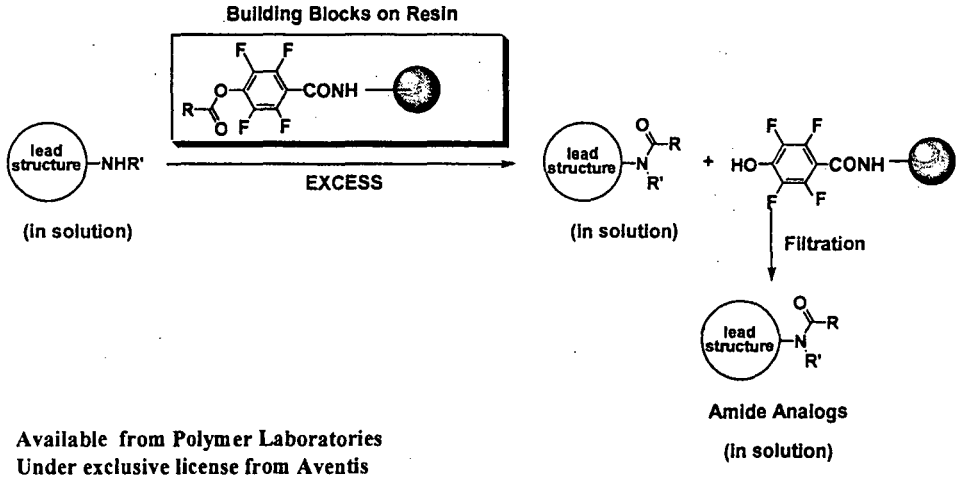
Functional Trapping: Alkylation



Tetrafluorophenol Resin



Available from Polymer Laboratories
Under exclusive license from Aventis

Objectives for analog resins

- Creation of Hit to Lead libraries
- Selected, pharmacologically interesting building blocks
- Suitably selected and tuned activation groups (reactivity/stability)
- Accompanying use of scavenging/work-up resins when required
- For Medicinal/organic/solution phase chemists
- Allows for the creation of new building blocks

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Resin suppliers

The Players

- Polymer Laboratories
- Rapp Polymere
- Aldrich/Sigma
- Novabiochem
- Senn Chemicals
- Bachem
- Advanced ChemTech
- Argonaut

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Talk Overview

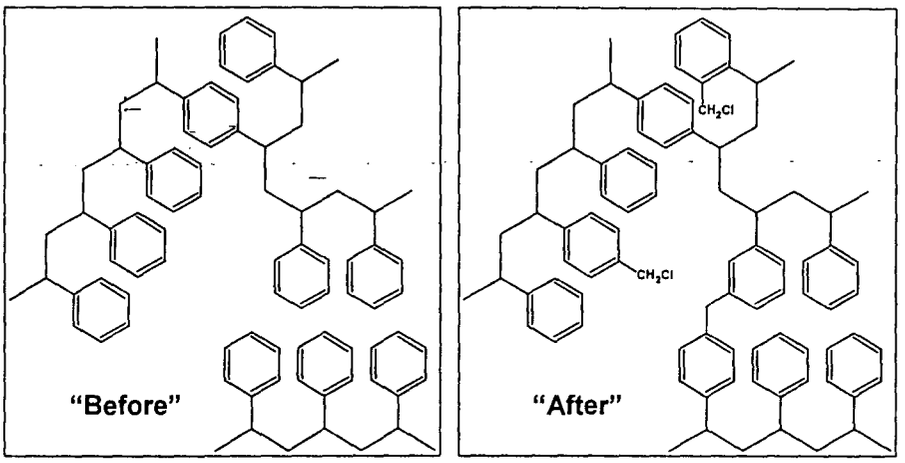
- Issues with present resin offerings
- Solid phase synthesis
  - Loadings
  - Resin reproducibility
- Scavenger resins
  - Costs
  - Speed
- New alternate supports
- New encoding techniques

Using Resins for Solid Phase Synthesis

Issues With Present Resin Offerings

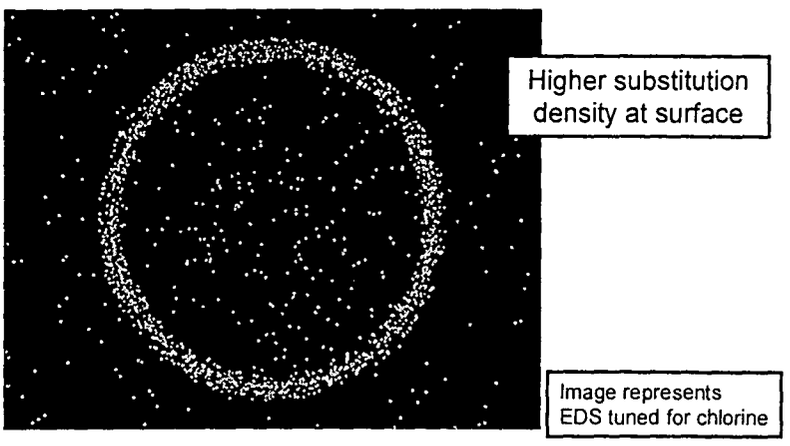
- Peptide Resins being used for small molecule chemistry
- Low loadings
  - Ideal for large peptide synthesis (high MW products)
  - Low yields of small molecule products (low MW products)
- Batch to batch reproducibility
- Wide loading range
- Resin fines
- Uniform bead size

Introduction of Functionality : Chloromethylation

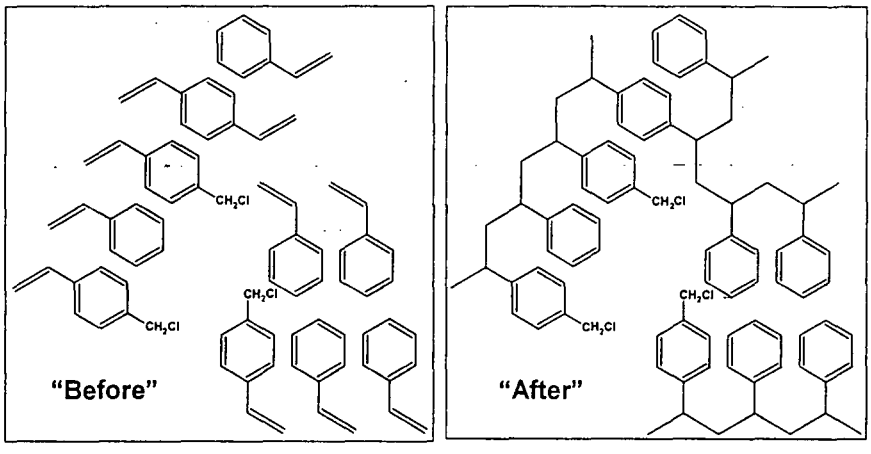


Chloromethylation : Problems

- Particles are no longer homogeneous ...



Introduction of Functionality : Copolymerisation



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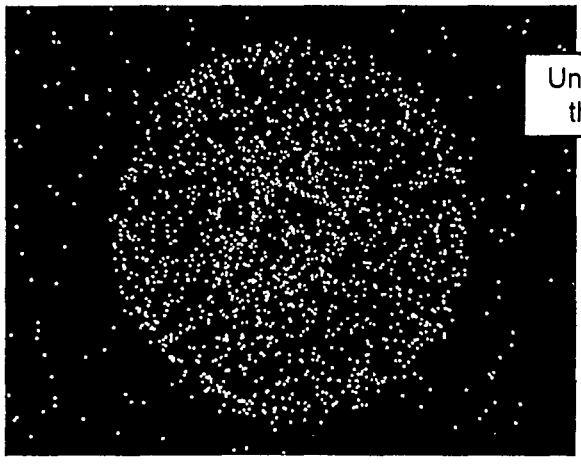
Copolymerisation : Advantages

- Particles are homogeneous



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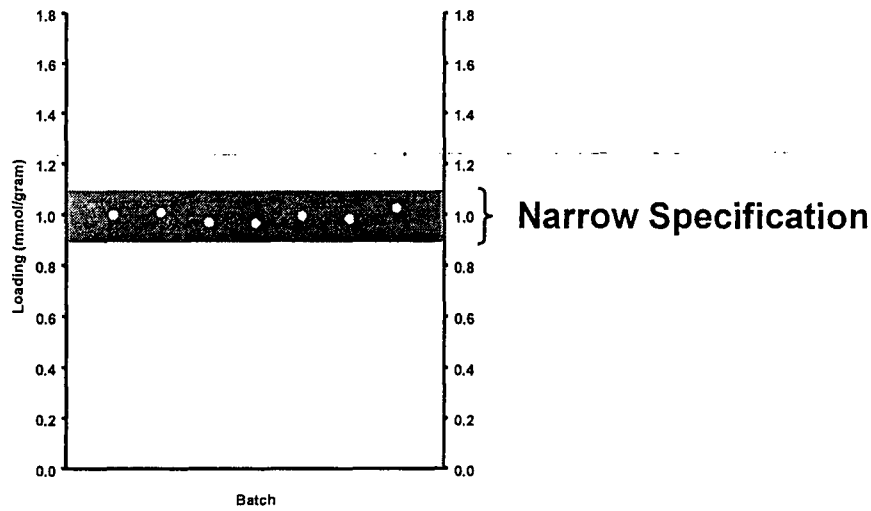
Copolymerisation : Advantages



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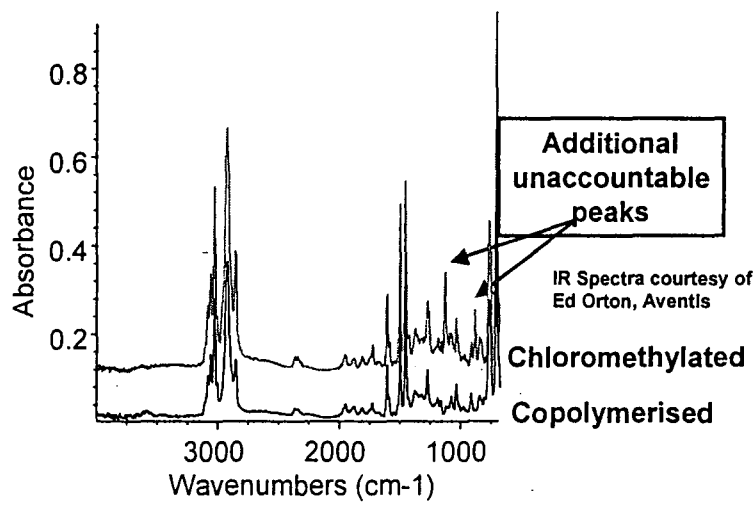
Chloromethylation : Problems

- Heterogeneous bands observed in IR



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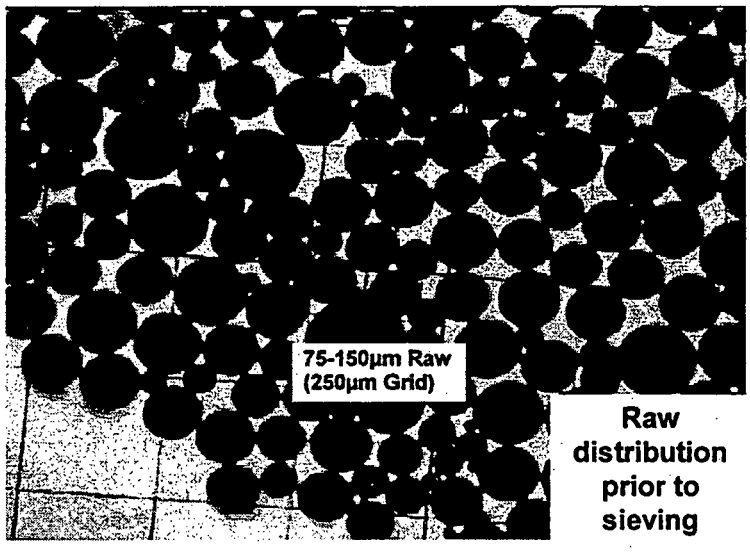
Raw Particle Size Distribution



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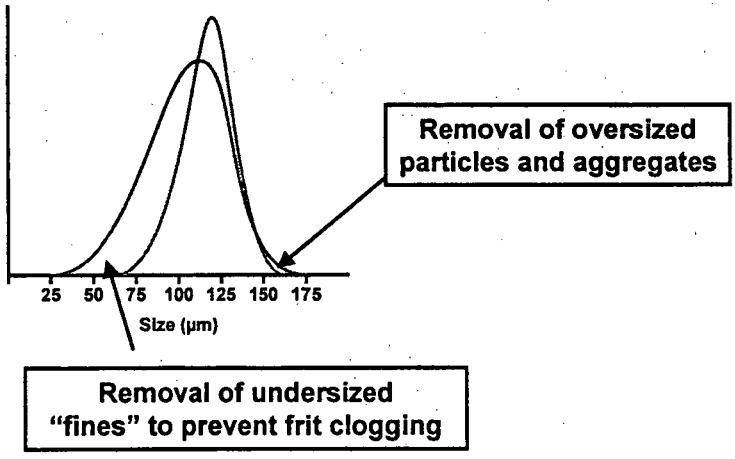
Sieving

- Finished particle size distribution



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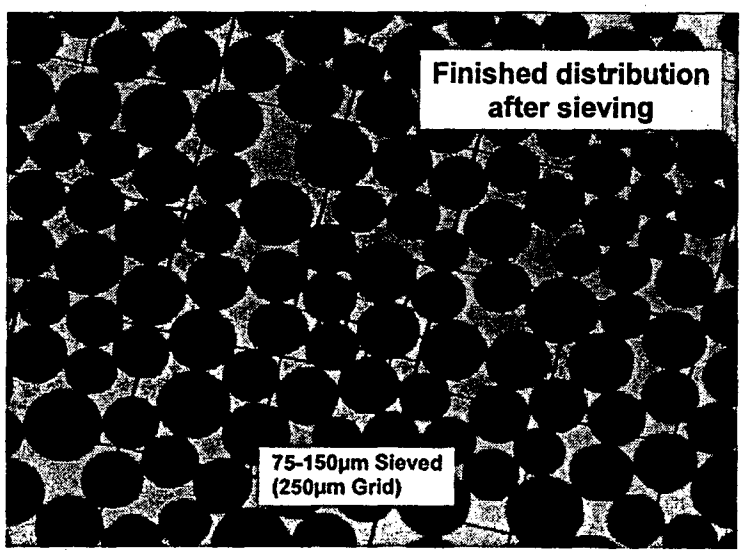
Final Particle Size Distribution



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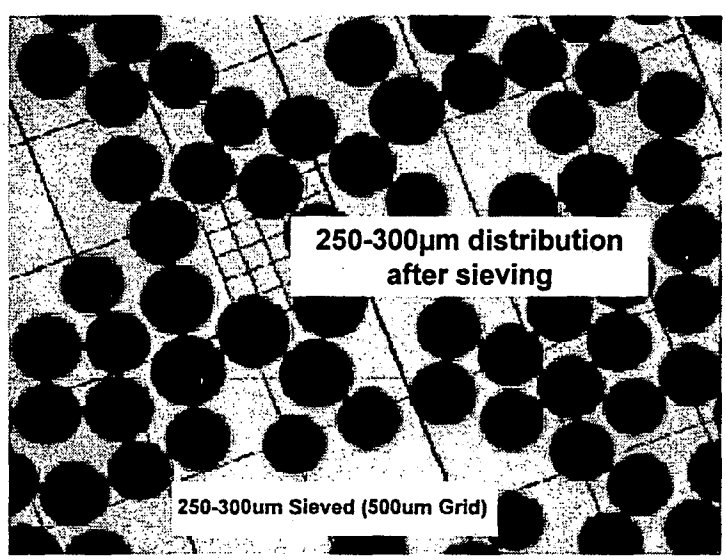
Narrow Particle Size Distribution



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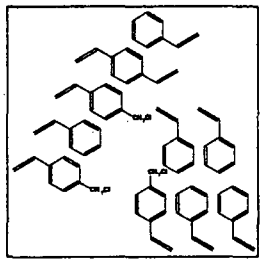
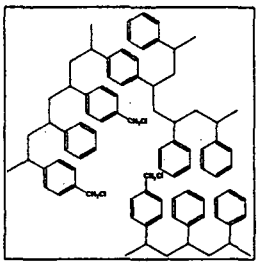
Introduction of Functionality : Copolymerisation



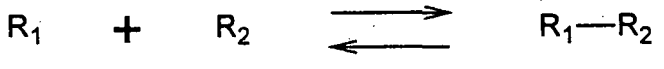


- This allows accurate control of the loading from batch to batch
- Narrow loading range +/- 10%
- Allows for high loading for the synthesis resins (2.0 mmol/gm) hence more compound off the bead
- Extensive washings resulting in no byproducts or side reactions

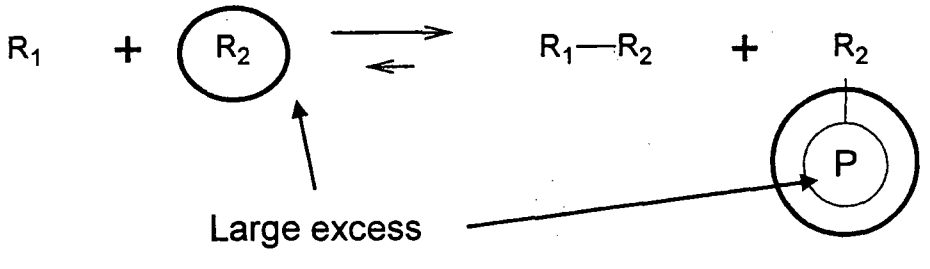
Using Resins for Solution Phase Synthesis

Application of Solid Phase Particles to Solution Synthesis

Solution phase synthesis



Use of scavenger resin



Practical Solution Phase Example

- Solution phase
  - 1M solution of reagent (#1) (e.g. 1.5mmol in 15mL)
  - 3x excess of reagent (#2)
  - React for several hours
  - Perform liquid-liquid extraction (3 – 4 times reaction volume, or 45 – 60 mL per wash)
  - Evaporate to dryness

Solid Phase Assisted Example

- Solution phase
  - 1M solution of reagent (#1) (e.g. 1.5mmol in 15mL)
  - 3x excess of reagent (#2)
  - React for several hours
  - *Remove excess reagent (#2) using scavenger resin (i.e. 3.0mmol to be removed)*
  - Evaporate to dryness

Solid Phase Assisted Example

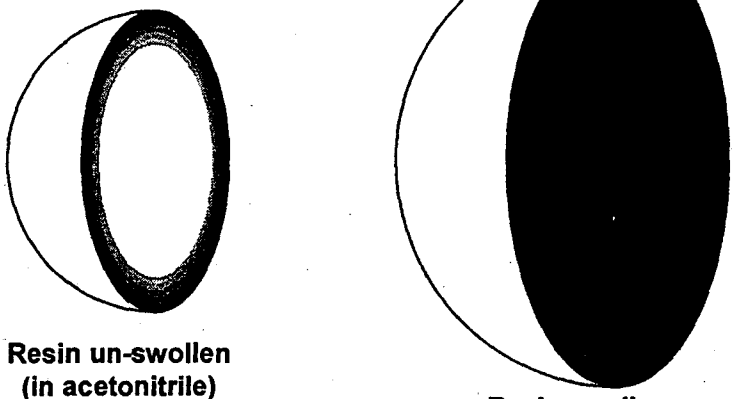
- Conventional low load resin
  - 1.0 mmol/g
  - 1% crosslinked
  - 75-150μm
  - ~ 8 mL/g
- Require 3x excess ...
  - 9.0 mmol resin functionality
  - 9.0 g resin
  - 72.0 mL gel volume !!!

- Superior high load resin
  - 4.0 mmol/g
  - 1% crosslinked
  - 150-300μm
  - ~ 8 mL/g
- Require 3x excess ...
  - 9.0 mmol resin functionality
  - 2.3 g resin
  - 18.4 mL gel volume

Development of a New Hybrid Macroporus Resin

Combining the advantages of HL synthesis resins and speed of chromatographic particles

Microporous Bead Permeation During Scavenging



Resin un-swollen (in acetonitrile)

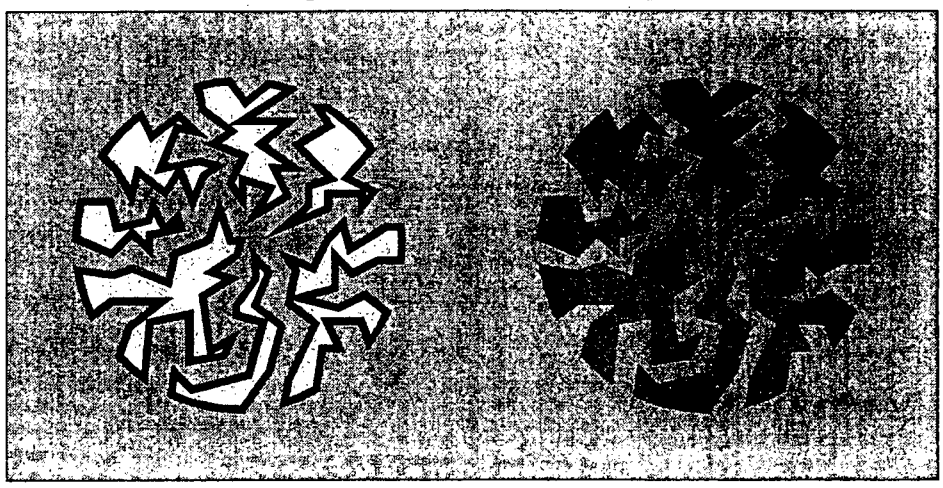
Resin swollen (in tetrahydrofuran)

Polystyrene/DVB : Microporous vs Macroporous

- Low crosslink content (< 12% DVB)
- Soft
- High swell (must be used in good solvents)
- Slow diffusion rates
- High loading
- Designed for solid phase synthesis

- High crosslink content (> 20% DVB)
- Rigid
- Low swell (can be used in poor solvents)
- Very high diffusion rates
- Low loading
- Designed for chromatography

Macroporous Bead Permeation During Scavenging ?



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Hybrid Particle Goals:

- Macroporous for rapid diffusion and solvent toleration
- Microporous for maximum capacity
- Easily handled particle size
- Try to mimimise costs

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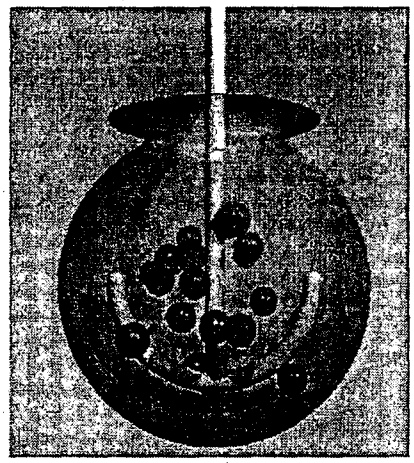
Hybrid Particle Parameters:

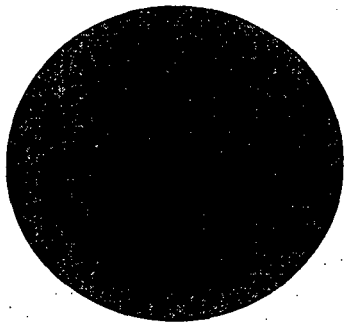
- Pore size
- Loading / crosslink content
- Particle size

Macroporous Particles : Method of Manufacture

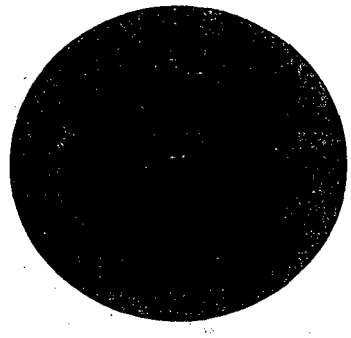
- Suspension Polymerisation



Macroporous Particle Formation

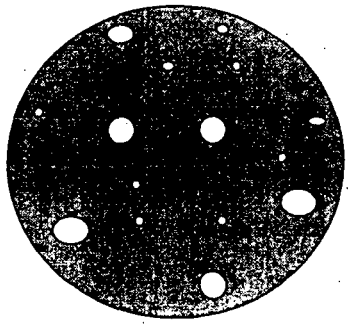


Monomer droplet contains : Monomer, crosslinker, porogen, initiator

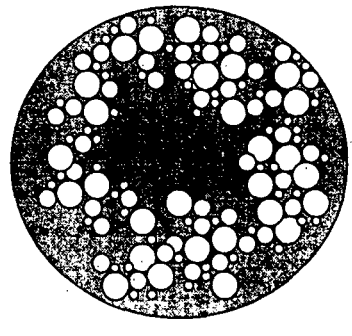


Polymerisation commences : Short polymer chains remain in solution

Macroporous Particle Formation

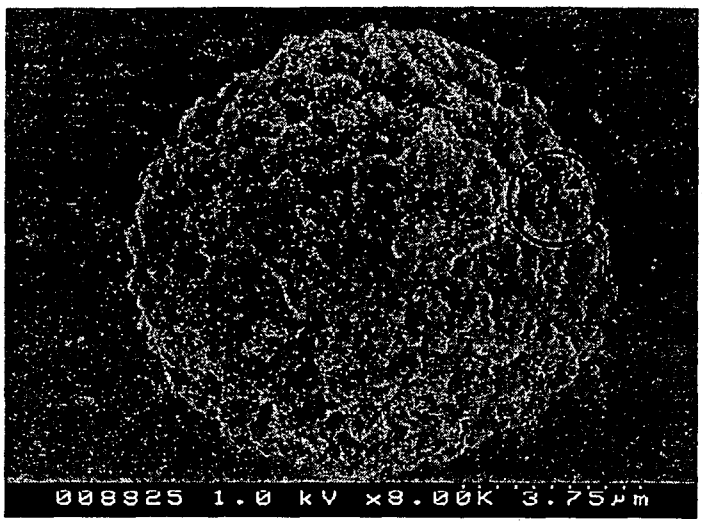


Polymerisation progresses : Longer polymer chains precipitate from solution



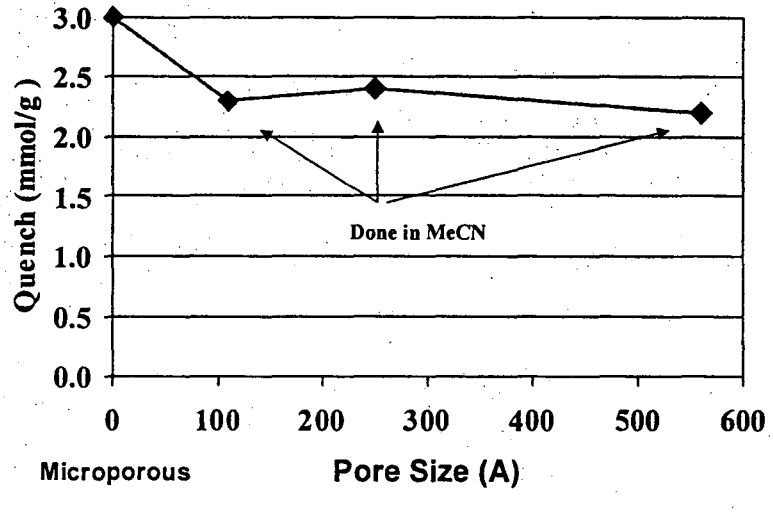
Polymerisation progresses further: Precipitated polymer particles adhere together

Macroporous Particle Image

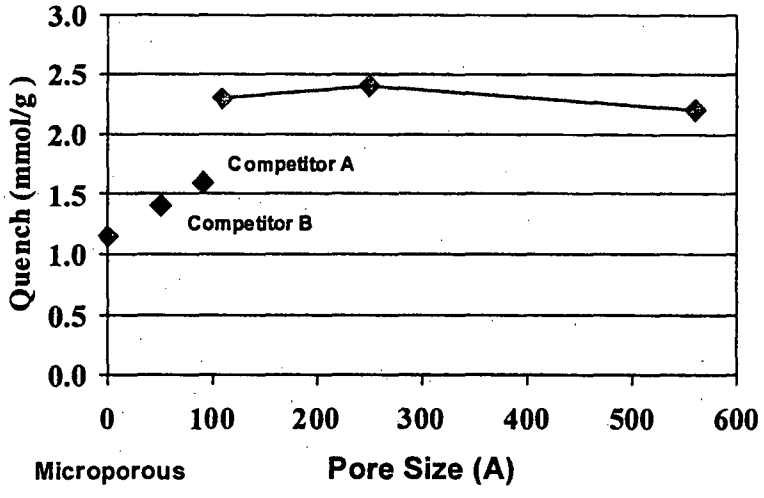


Extreme Limit: microspheres are visible, as are macropores

Macroporous Resin Quenching Activity



Macroporous Resin Quenching Activity



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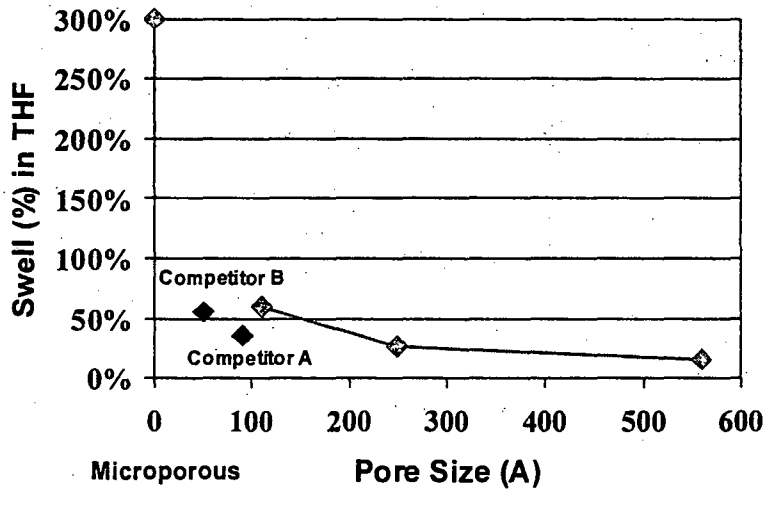
Macroporous Resin: "Swell"



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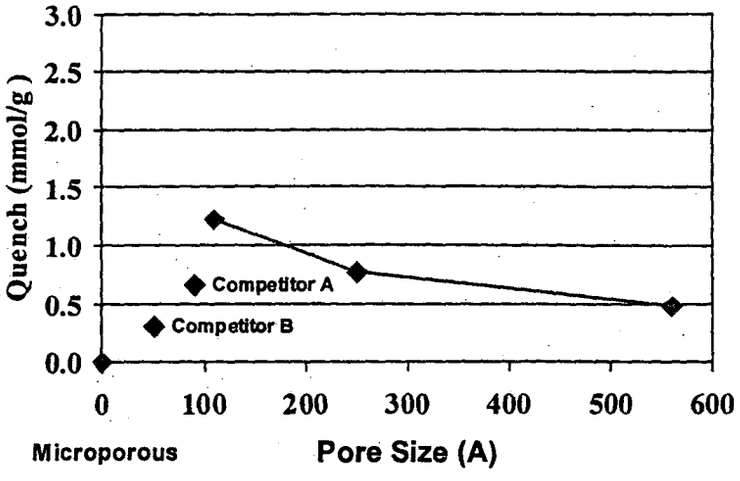
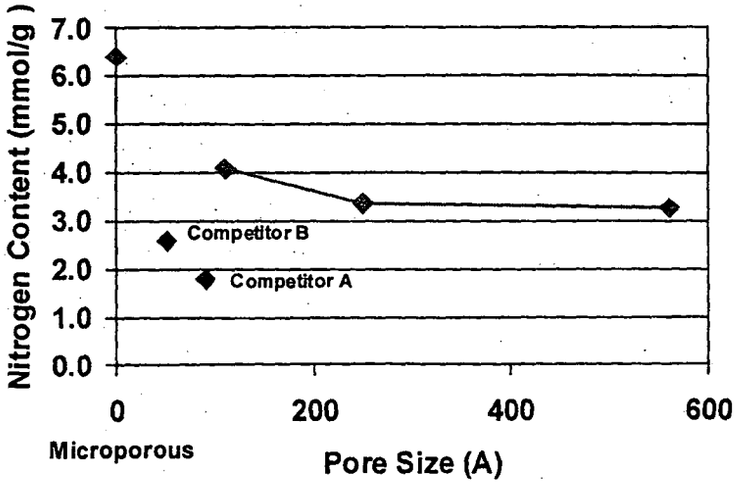
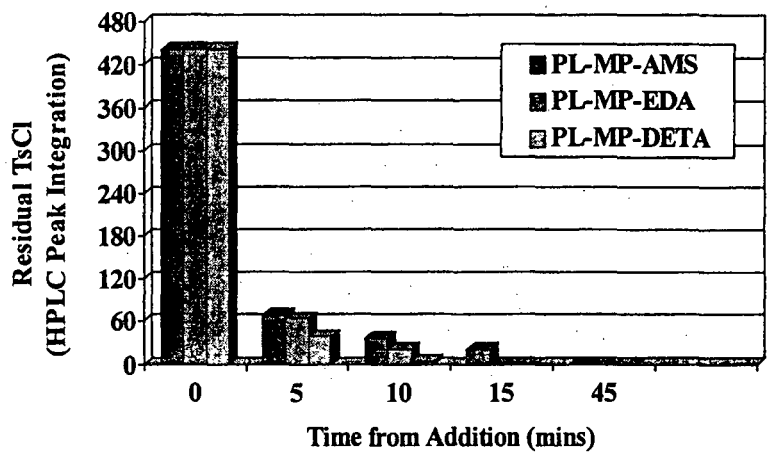
Macroporous Resin
Rapid Quenching Activity



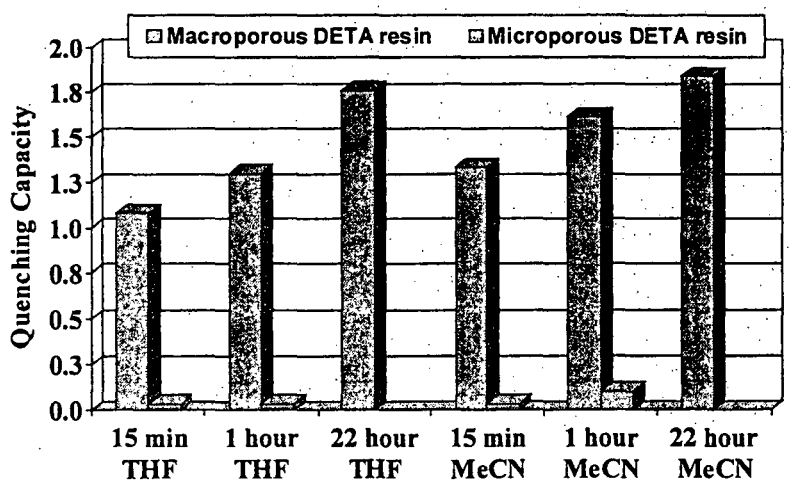
Macroporous Resin
Elemental Analysis Results



Macroporus Scavenging Results



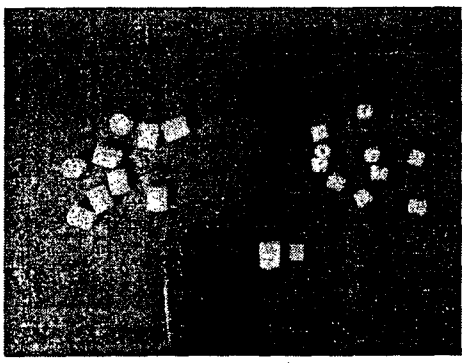
Scavenging Rate Comparison



Scavenger Resin Design : Conclusions

- Scavenger Resins are an important aid in solution phase synthesis
- Microporous Resins are
  - readily available
  - easy to use, but can be very slow to react
  - *more suited to bulk applications*
- Macroporous Resins are
  - more difficult to manufacture
  - react extremely rapidly and can be made in high load
  - *more suited to high throughput applications*

Plug Dimensions



- Plug weight: 170 mg
- Resin weight: 85 mg
- Loading/plug: 85μmol using 1.0 mmol/gm resin

- New format: Larger beads
- Increased loading on resin
- Better distribution

Types of resins used

- Synthesis resins (Wang, Amino-methyl, Rink, TentaGel, Oxime, ClTrt, Formyl)
- Scavenger resins (Aldehyde, Amino, Acid)
- Catalysts (Under development)

Advantages of Plug Use

- Ease of handling
- Use with multiple synthesis formats
- Amenable to encoding techniques
- Low void volume (lower reagent quantities)
- Low swelling
- Costs

Alternate Encoding Techniques

Improving detection and compound tracking

Existing Encoding Techniques

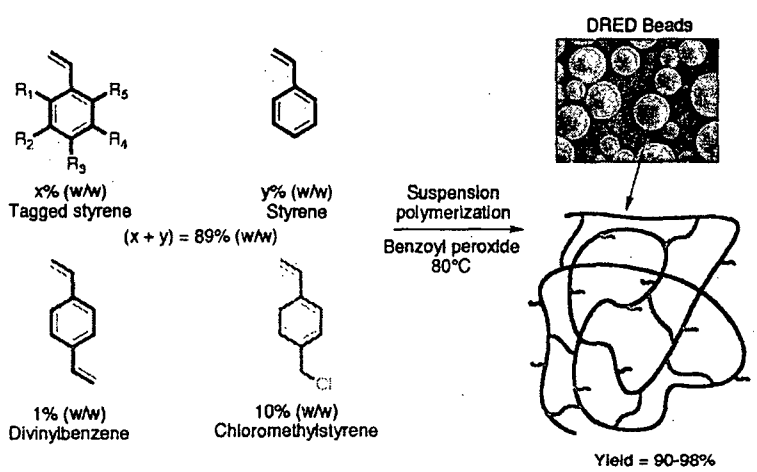
- Invasive labeling or encoding
  - Chemical tags
  - Radio labeling
  - Orthogonal labeling
- Non invasive labeling or encoding
  - Radio frequency (Rf) labeling

Use of Imaging Technology

- Chemically specific
- Sensitive
- High throughput and Real time monitoring
- Suitable for catalysts

- Non availability of easy to use experiments and instruments
- Bead size distribution and availability

DRED Bead Synthesis

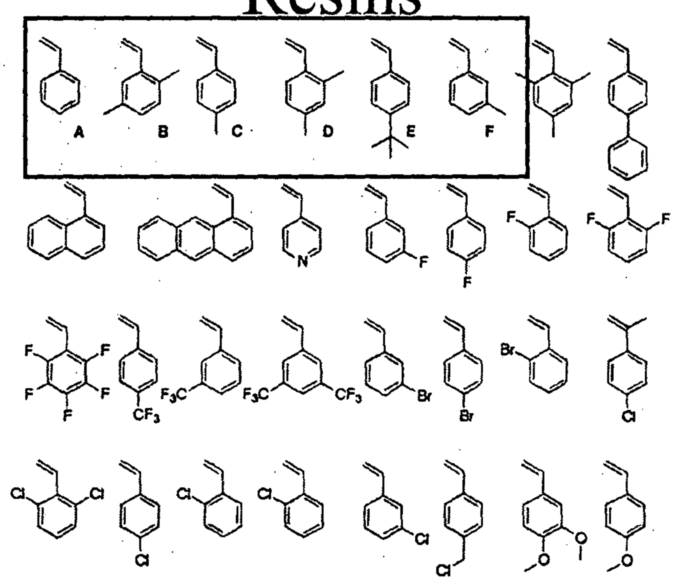


Fenniri, H., et al. *J. Am. Chem. Soc.* **2001**, *123*, 8151.

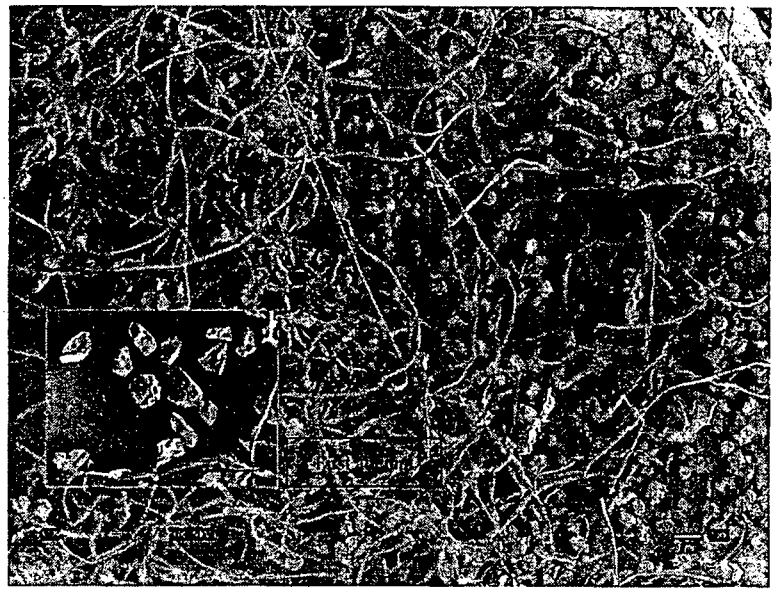
Co monomer Selection

- Commercial availability and costs
- Unique Raman and IR specifications
- Relative chemical inertness
- Amenable to suspension polymerization techniques

First Generation Merrifield Resins



Scanning Electron Microscopy



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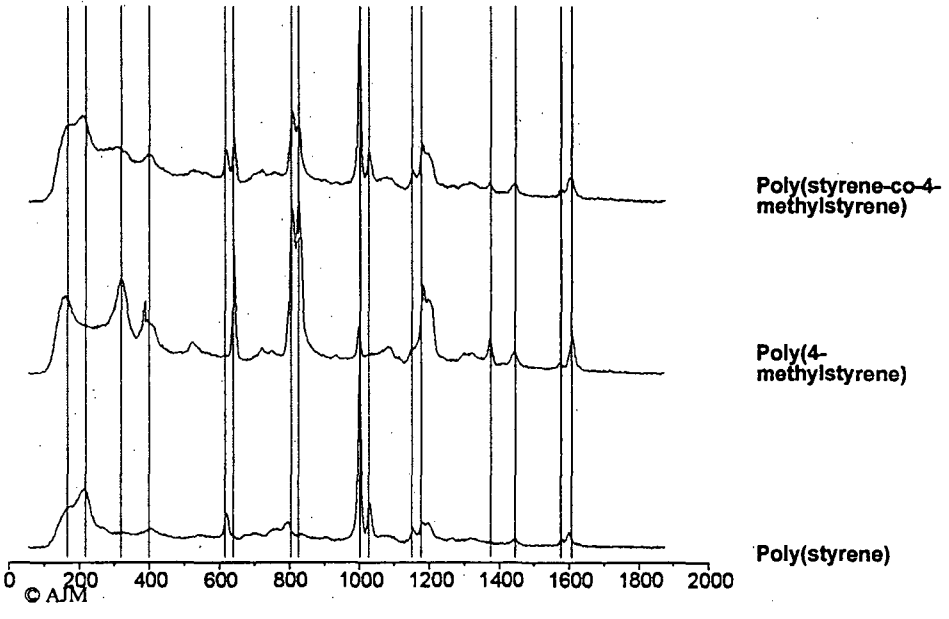
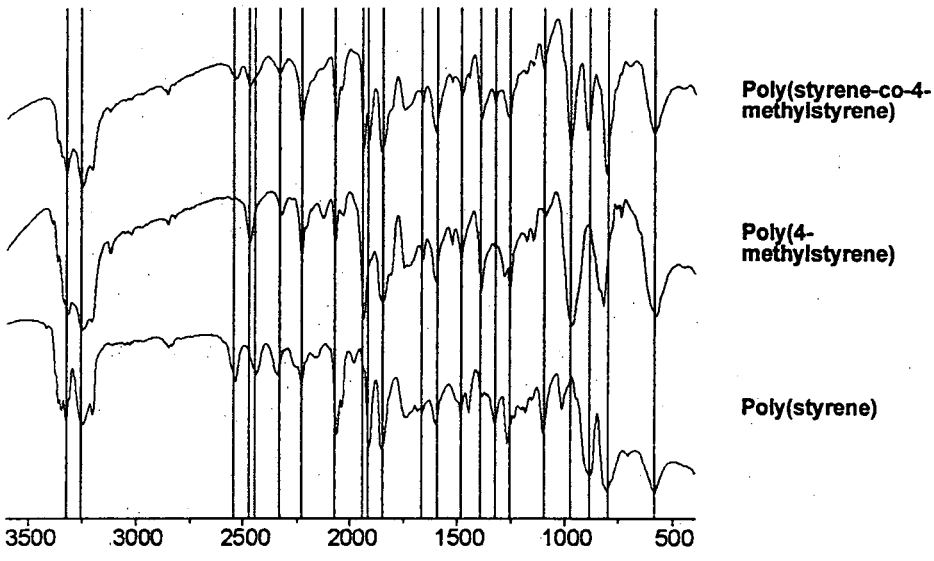
Raman Spectral Features



FT-IR Spectral Features



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Summary

- Co-polymerisation of monomers controls bead characteristics
- Development of high load base material for scavenger resins helps to decrease cost per compound in solution phase synthesis
- Development of hybrid macroporous resins help increase speed of scavenging action
- Development of plugs help in resin delivery
- Development of new encoding technique using IR and Raman spectral character of the monomers

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Role of Combinatorial Chemistry in Original Drug Discovery

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ROLE OF COMBINATORIAL CHEMISTRY IN ORIGINAL DRUG DISCOVERY

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Combinatorial synthetic methods became a routine in drug discovery during the nineties. Use of combinatorial libraries find two well discernible applications. In order to identify random hits, a diverse combinatorial library can be added to in-house existing compounds and tested in first screen assays. Later in the discovery process a focussed library is more useful to optimize the structure in order to get a lead. Several different technical solutions exist today. The most straightforward approach apparently is parallel synthesis of individual compounds. An aspect that should be considered while designing the basic scaffold (and set of substituents) is drug-likeness of the resulting compounds. Known toxicophores, mutagenic cores, alkylating, acylating or other highly reactive side chains should be avoided. Molecular weight of the compounds should remain below or in the vicinity of 500. Many published libraries are built around core structures of known drugs on the market or in development. Structures that are not stable in the biological milieu, or otherwise have poor bioavailability, such as peptides or alkyl esters are defavorized even if their chemistry is easy to master.

QSAR MODELING AND LIBRARY DESIGN STRATEGIES

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The session will be devided into an introduction to basic concepts of QSAR Modeling and Library Design and a hands-on tutorial which will allow participants to experience the basic steps from deriving a QSAR model to designing a focused library themselves.
In the theory section, a general overview on

- representation of chemical structures in the context of computer applications,
- deriving physico-chemical properties
- the theory of ligand-protein interactions
- building QSAR models
- strategies for library design
- will be presented.

During the tutorial, a methodology is presented that guides through the drug design cycle starting from the analysis of experimental HTS data, constructing a QSAR model and using the model to design a virtual focused combinatorial library for cyclic GMP Phospho-diesterase V inhibitors in an almost fully automated way.

The analysis of the experimental dataset is based on 2.5D descriptors. These descriptors are fast and easy to calculate since they rely on 2D information and still reflect 90 % of the information inherent in 3D structures. They were specifically designed to provide a tool for a rapid though stable initial approach to large datasets of unknown SAR. The descriptor values correspond to binned van-der-Waals surface areas. The binning procedure was based on logP, MR and partial charge (PEOE), supposed to be fundamental physico-chemical properties that cover most of the relevant property space in an intuitive and interpretable manner.

The QSAR model applies a non-linear probabilistic binary method rather than a linear regression based technique. The focused library design uses virtual enumeration with a binary QSAR model as product-based scoring agent for reagent selection.

The dataset consists of about 400 known cGMP Phosphodiesterase V inhibitors with activity data selected from the literature and a total of 1800 molecules. The initial QSAR model is about 20 times more potent in selecting active compounds over random picking. The building blocks (2 x 10 x 12 x 27 = 6500 potential products) used in the combinatorial design of a focused quinazoline library (1 x 3 x 3 x 5 = 45 products) reflect chemical intuition and input from the literature. Using the binary QSAR model as focusing agent the percentage of predicted active compounds increases from 5 % in the unfocused library to 75 % in the focused library. The resulting focused library preserves the essential SAR known from the literature.

``How to ?´´ in Computer Aided Library Design

- Understand the basic requirements for a small molecule to become a potent drug
- Linear and non-linear Filters
- A closer look at principles ruling the activity of a compound
- Teach the computer to handle chemical structures
- Find efficient methods to handle large amounts of data within reasonable time
- Correlate chemical structures with desired/undesired property, choose descriptors, build model, choose reagents

What turns a small molecule into a potent drug?

Resorption

Metabolism

Affinity

Distribution

Specificity

Toxicity

Excretion...

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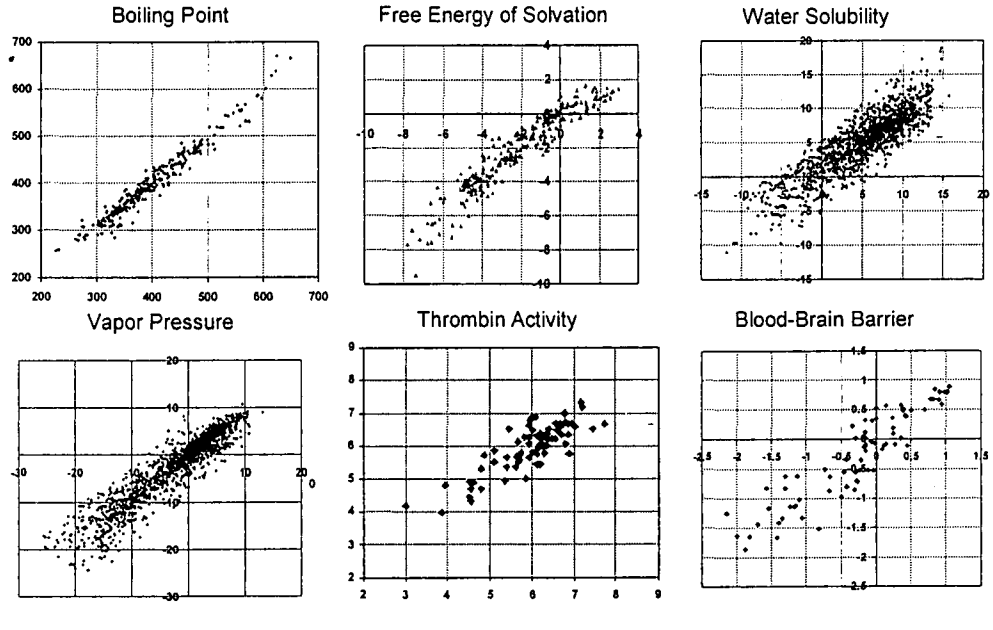
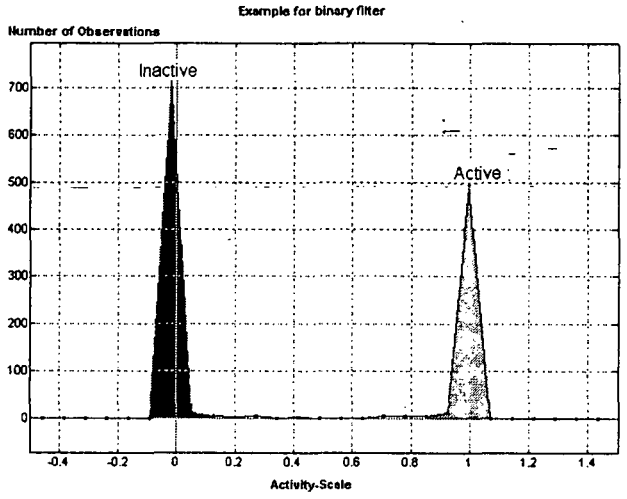
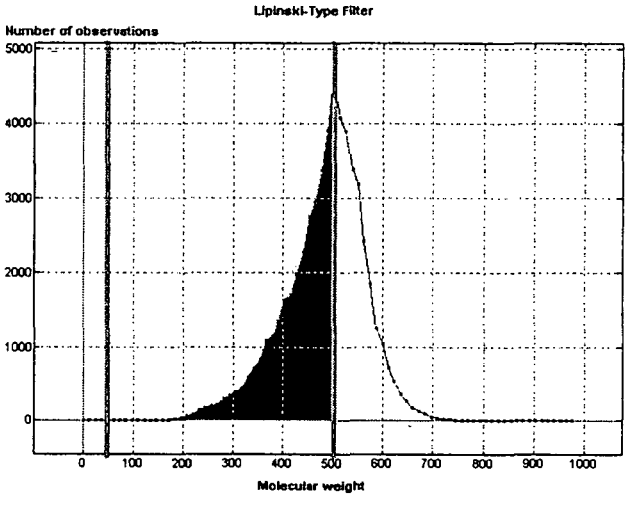
Examples for linear Filters



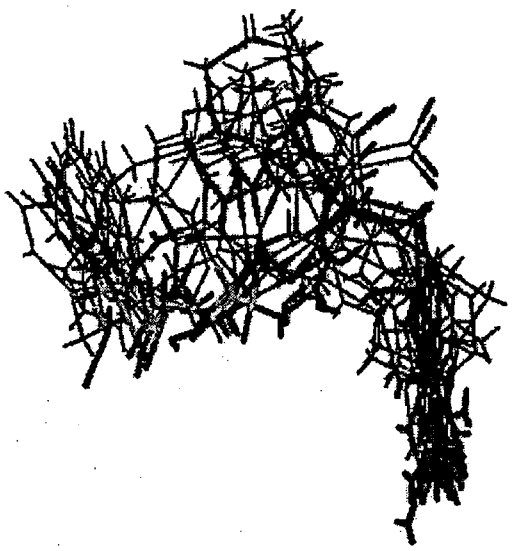
Example for non-linear Filter



Example for Lipinski-Type Property Filter



Pharmacophor Models

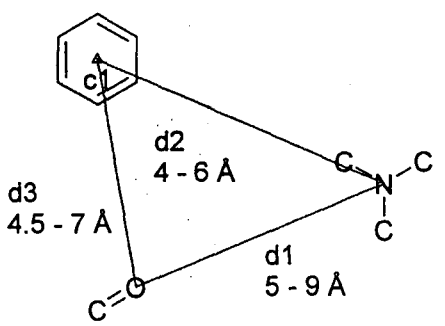


Thrombin Inhibitors from Superimposed Crystal Structures

Pharmacophore based 3D Filter

Functional Groups:

- H-Bond Donors
- H-Bond Acceptors
- Positively charged groups
- Negatively charged groups
- Hydrophobic groups



Constraints:

- Distances
- Angles
- Vectors
- Planes
- Excluded Volumes

Generation:

- Automatically or manually
- Broad range of activities and molecular features
- Bind to one site in identical binding mode
- Start with set of superimposed, diverse, rigid molecules
- Add more flexible molecules

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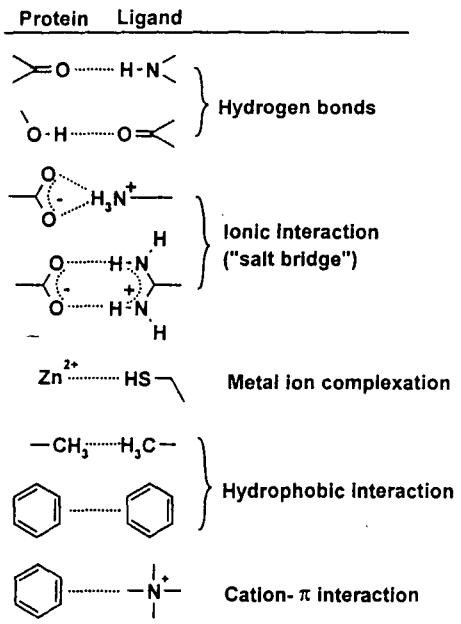
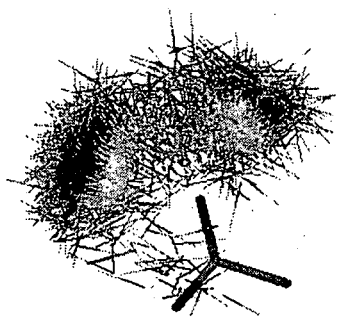
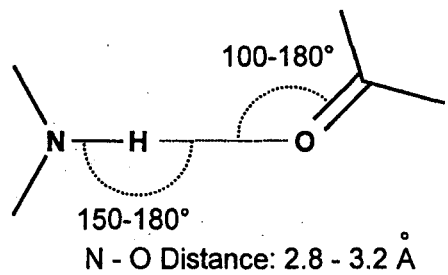
Noncovalent interactions in ligand-protein complexes

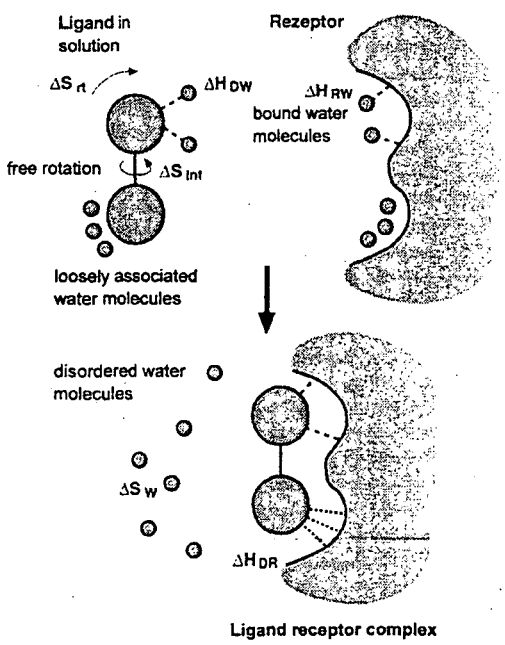


Hydrogen Bonds



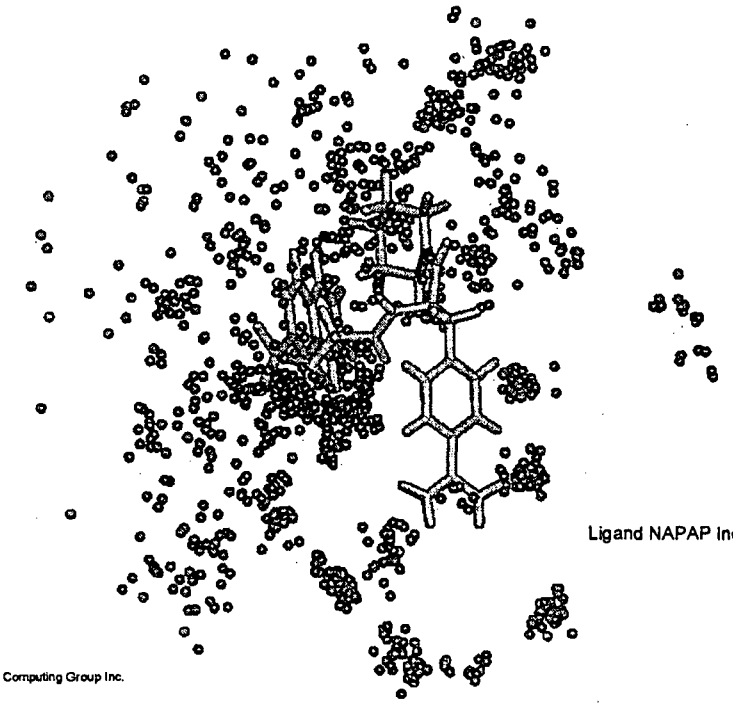


The Process of Ligand Binding



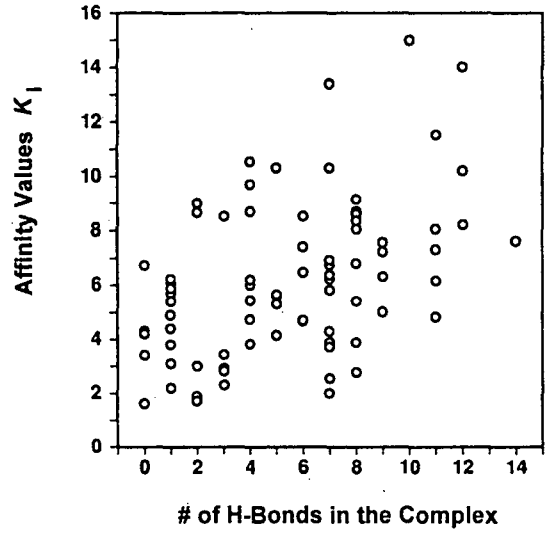
$$\Delta G = RT \ln K_i = \Delta H - T\Delta S$$

Distribution of Water Molecules in Unoccupied Active Sites of Thrombin



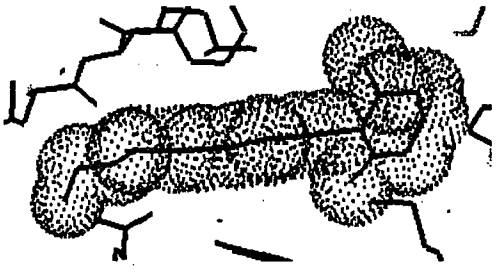
Ligand NAPAP Indicated in Yellow

Contribution of Hydrophilic Interactions to Ligand Affinity

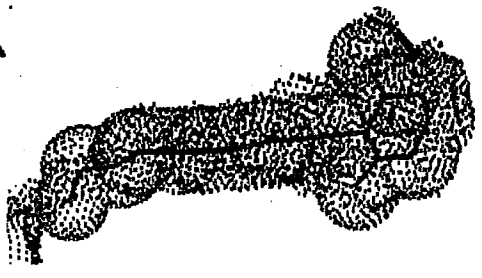


Contribution of Hydrophobic Interactions to Ligand Affinity

Retinol Binding Protein:
Residue side chains (magenta) of the protein
and vdW surface of the ligand Retinol (blue)



Retinol Binding Protein:
Connolly surface (magenta) of the protein
and vdW surface of the ligand Retinol (blue)



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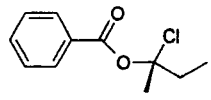
Structural Representations of Small Molecules

1D: c1ccccc1C(=O)O[C@](CC)(C)Cl

2D:



3D:

Textfile:

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ISIS

 30 30  0  0  0  0            1 V2000
   -0.0169    1.3921    0.0097 C   0  0  0
    1.1701    2.0948    0.0021 C   0  0  0
    2.3776    1.4193   -0.0126 C   0  0  0
    2.4036    0.0359   -0.0207 C   0  0  0
    1.2241   -0.6793   -0.0137 C   0  0  0
    0.0021   -0.0041    0.0020 C   0  0  0
   -1.2651   -0.7633    0.0096 C   0  0  0
   -1.2455   -1.9779    0.0020 O   0  0  0
   -2.4424   -0.1088    0.0247 O   0  0  0
   -3.6879   -0.8551    0.0322 C   0  0  2
   -4.8667    0.1202    0.0493 C   0  0  0
   -4.8118    1.0085   -1.1952 C   0  0  0
   -3.7428   -1.7434    1.2766 C   0  0  0
   -3.7667   -1.7284   -1.2217 C   0  0  0
   -0.9586    1.9205    0.0260 H   0  0  0
    1.1570    3.1747    0.0081 H   0  0  0
    .
    .
    .
  1  6  1  0
  1  2  2  0
  1 15  1  0
  2  3  1  0
  2 16  1  0
  3  4  2  0
  3 17  1  0
  4  5  1  0
  4 18  1  0
  5  6  2  0
  5 19  1  0
  6  7  1  0
  7  8  2  0
  7  9  1  0
  9 10  1  0
 10 11  1  0
 10 13  1  0
    .
    .
    .
M  END
$$$$
```

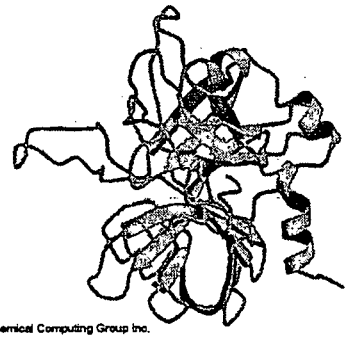
Structural Representations of Proteins

1D: EADCGLRPLFEKKSLEDKTERELLESYIDIVEGSDAEIGMSPWQVMLFRKSPQE
LLCGASLISDRWVLTAAHCLLYPPWDKNFTENDLLPDRETAASLLQAGYKGRVT
GWGNLKETWTANVGKGQPSVLQVVNLPIVERPVCKDSTRIR

"2D": IVEGSDAEIGMSPWQVMLFRKSPQELLCGASLISDRWVLTAAHCLLYPPWDKN
T SS TTT TTSSSSSSTTTTSSSSSSSS TTTSSSS 333TSS333

IGKHSRTRYERNIEKISMLEKIYIHPRYNWRENLDRDIALMKLKKPVAFSDYI
SST TT TTT SSSSSSSSS TT TTTT T SSSSSTT TT

3D:



Textfile:

```
ATOM      1  N   GLU L  1C      63.691  25.940  17.920  0.00
ATOM      2  CA  GLU L  1C      64.331  26.594  16.778  0.00
ATOM      3  C   GLU L  1C      63.325  27.181  15.823  0.00
ATOM      4  O   GLU L  1C      63.253  28.400  15.711  0.00
ATOM      5  CB  GLU L  1C      65.333  25.719  16.023  0.00
ATOM      6  N   ALA L  1B      62.540  26.309  15.144  0.00
ATOM      7  CA  ALA L  1B      61.528  26.767  14.192  0.00
ATOM      8  C   ALA L  1B      60.677  27.829  14.830  0.00
ATOM      9  O   ALA L  1B      60.267  27.670  15.965  0.00
ATOM     10  CB  ALA L  1B      60.675  25.608  13.772  0.00
ATOM     11  N   ASP L  1A      60.419  28.936  14.148  0.00
ATOM     12  CA  ASP L  1A      59.616  29.916  14.837  0.00
ATOM     13  C   ASP L  1A      58.180  29.473  14.803  0.00
ATOM     14  O   ASP L  1A      57.740  28.843  13.838  0.00
ATOM     15  CB  ASP L  1A      59.843  31.394  14.473  0.00
ATOM     16  CG  ASP L  1A      58.777  31.961  13.594  0.00
ATOM     17  OD1 ASP L  1A      57.587  32.151  14.206  0.00
ATOM     18  OD2 ASP L  1A      58.998  32.224  12.393  0.00
ATOM     19  N   CYS L  1       57.464  29.769  15.871  0.00
ATOM     20  CA  CYS L  1       56.104  29.368  15.999  0.00
ATOM     21  C   CYS L  1       55.465  30.177  17.051  0.00
ATOM     22  O   CYS L  1       56.146  30.757  17.853  0.00
ATOM     23  CB  CYS L  1       56.102  27.939  16.548  0.00
ATOM     24  SG  CYS L  1       56.982  27.807  18.150  0.00
ATOM     25  N   GLY L  2       54.159  30.186  17.080  0.00
ATOM     26  CA  GLY L  2       53.489  30.931  18.092  0.00
ATOM     27  C   GLY L  2       53.388  32.388  17.738  0.00
ATOM     28  O   GLY L  2       52.651  33.115  18.393  0.00
ATOM     29  N   LEU L  3       54.104  32.832  16.706  0.00
```

``How to ?´´ in Computer Aided Library Design

- Understand the basic requirements for a small molecule to become a potent drug
- Linear and non-linear Filters
- A closer look at principles ruling the activity of a compound
- Teach the computer to handle chemical structures
- Find efficient methods to handle large amounts of data within reasonable time
- Correlate chemical structures with desired/undesired property, choose descriptors, build model, choose reagents

Explicit Treatment of Atomic Properties vs *Atom Typing*

- *Ab initio* Methods
- Semi Empirical Methods
- Empirical Methods (Force Field Based Systems, differentiable)
  - Molecular Mechanics (static picture), Molecular Dynamics (somewhat dynamic picture)
- Knowledge (Rule) Based Systems (discrete sampling)

Force Fields

Necessary building blocks for a force field:

- **A list of atom types and atomic charges**
- **Atom typing rules**
- **Functional forms for the components (bonded, non bonded terms) of the energy expression**
- **Parameters for the function terms (rules for generating them if not explicitely given)**
- **An algorithm to calculate new atomic coordinates**

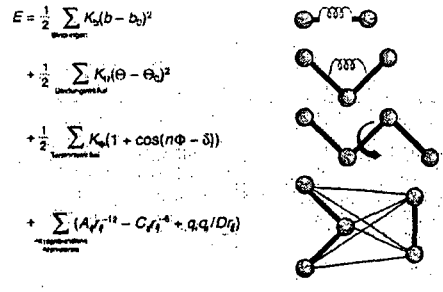
$$E = E_{\text{Bindungsstärke}} + E_{\text{Bindungswinkel}} + E_{\text{Torsion}} + E_{\text{nichtbindend}}$$

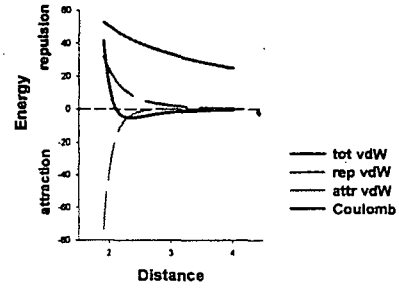
$$E = \frac{1}{2}\sum_{\text{Bindungen}} K_b(b - b_0)^2$$

$$+ \frac{1}{2}\sum_{\text{Bindungswinkel}} K_\theta(\theta - \theta_0)^2$$

$$+ \frac{1}{2}\sum_{\text{Torsionswinkel}} K_\Phi(1 + \cos(n\Phi - \delta))$$

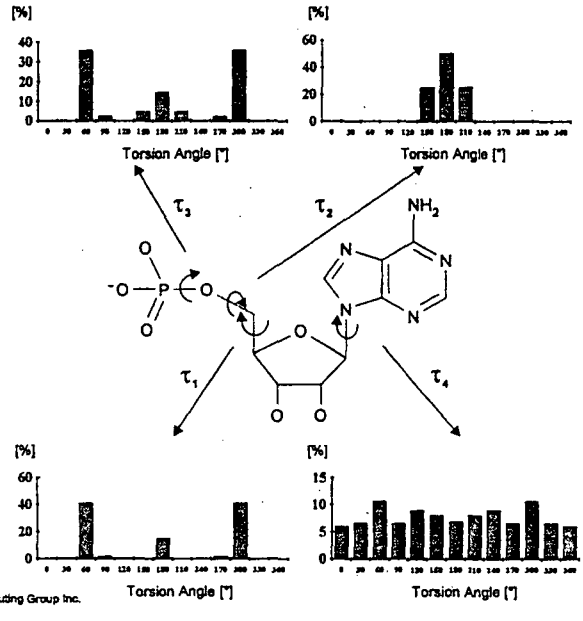
$$+ \sum_{\text{nichtbindende Atompaare}} (A_{ij}r_{ij}^{-12} - C_{ij}r_{ij}^{-6} + q_iq_j/Dr_{ij})$$





Rule Based Systems

Torsion Angle Pseudopotentials Derived from the CSDB



Molecular Descriptors

Used for QSAR, Diversity Analysis, Combinatorial Library Design

2D Descriptors

Use only the atom and connection information of the molecule

- Physical poperties (wight, logP, vdW voume or aea, plarizability)
- Charge dscriptors
- Atom counts (atom types, donors, acceptors)
- Bond counts
- Connectivity indices
- Graph distance matrix descriptors
- Pharmacophore atom types

3D Descriptors

Internal 3D descriptors use 3D coordinate information, but they are invariant to rotations and translations

External 3D descriptors require an absolute frame of reference

- Moments (Dipole, PMI)
- Areas, volumes and shape
- Energy descriptors

Fingerprints, Similarity and Dissimilarity

Fingerprints:

- **Set of features derived from the structure of a molecule**
- **Can be based on 2D or 3D features.**
- **Used as a "surrogate" for the chemical structure.**

Example:
Assume our universe to describe chemical structures has the following 8 features:

U = {Is-aromatic, has-ring, has-C, has-N, has-O, has-S, has-P, has-halogen}

All structures are then described by subsets of *U*. There are $2^8 = 256$ possible Fingerprints - or different "molecules".

Metrics:

- **Needed to compare (cluster) fingerprints / molecules**
- **A common metric is the Tanimoto coefficient**
  - **The number of common features divided by the number of total features in both fingerprints.**
  - **A number between 0 and 1, where 0 means maximally dissimilar and 1 means maximally similar**

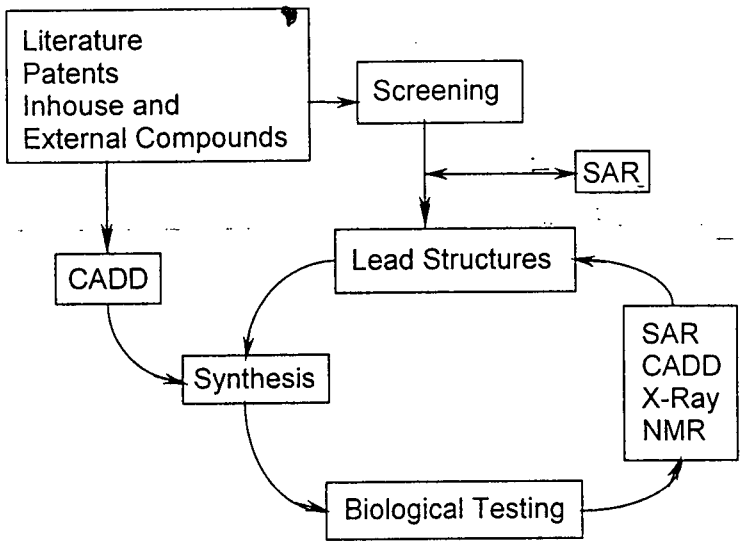
Library Enumeration Tools

- Explicit (explore all combinations)
- Virtual (explore representative subset)
- Prepare Reagents (R-group clipping)
- Apply filter
  - (Diversity, Activity, Cost, Availability, ADME/T, Lipinski-Rules)
- Select reagent subset

``How to ?´´ in Computer Aided Library Design

- Understand the basic requirements for a small molecule to become a potent drug
- Linear and non-linear Filters
- A closer look at principles ruling the activity of a compound
- Teach the computer to handle chemical structures
- Find efficient methods to handle large amounts of data within reasonable time
- Correlate chemical structures with desired/undesired property, choose descriptors, build model, choose reagents

The Drug Design Cycle



Probabilistic Modeling in High Throughput Discovery

Probability based QSAR in Drug Discovery

- Use joint probability (Y = active(0/1) D = drugable(0/1) S = structure)
- Decompose joint probability into measurable components:

$$\Pr(Y,D,S) = \Pr(D \mid Y,S)\ \Pr(Y \mid S)\ \Pr(S)$$

Drugable given active structure (approximated by "is drug-like" efforts)

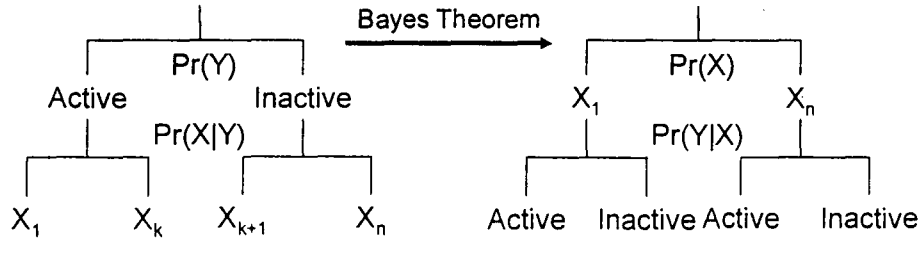
Activity assuming structure (probabilistic QSAR efforts)

- Product of probabilities balances competing goals
  - Classification alone (e.g., RP) is not enough: weighted outcomes needed
  - Methodology similar to "soft" classification problems or fuzzy logic
- Make predictions using computed model of Pr(A,B,C)

Pr(Y|X) via Binary QSAR

- If Y is "binary activity" and X is a descriptor vector then

$$\Pr(Y=1 \mid X=x) = \left[1 + \frac{\Pr(X=x \mid Y=0)\Pr(Y=0)}{\Pr(X=x \mid Y=1)\Pr(Y=1)}\right]^{-1}$$



Distribution Estimates

- Four distributions in formula are of two types
  - Pr(Y=0), Pr(Y=1) Prior probability of inactive/active
  - Pr(X=x|Y=0), Pr(X=x|Y=1) Probability of ligand assuming inactive/active
- Modeling assumption: independent ⇔ uncorrelated!
  - Decompose multi-dimensional distribution into a product

$$\Pr(X = x \mid Y = y) = \prod_i \Pr(X_i = x_i \mid Y = y)$$

  - Estimate $2n+2$ distributions instead of original four
- Binary QSAR Algorithm
  - Compute descriptor vectors d_i
  - De-correlate descriptors $x_i = Q(d_i - u)$
  - Estimate distributions from $\{x_i, y_i\}$ $\Pr(X = x \mid Y = y)$
  - Assemble $p(x)$ $\Pr(Y = 1 \mid X = x)$
  - Predict for new molecules $p(Q(\bar{d} - u))$

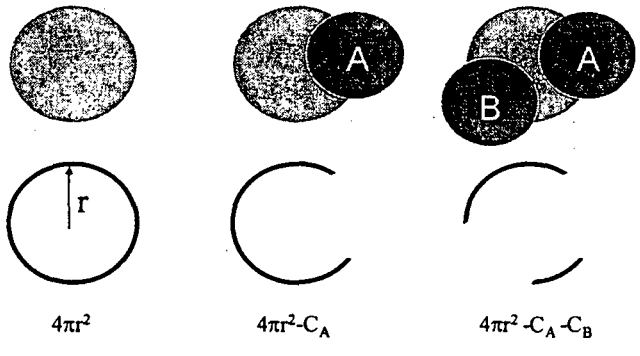
A Meaningful Subset of Descriptors

Fundamental Notions

- Use a meaningful set of descriptors for diversity and QSAR/QSPR
  - A meaningful chemistry space should not require customization
  - In QSAR/QSPR automatic variable selection can be dangerous
- Model 3D properties from 2D (connectivity) information
  - 3D information from 2D connectivity = 2½ D descriptors
  - HTS QSAR and large-scale diversity require fast calculation times
  - 2D topological descriptors too weak, 3D descriptors too expensive
  - Use approximate atomic surface areas as fundamental representation
- Intended applications
  - QSAR/QSPR models - linear and nonlinear - early and late in project
  - Chemistry space for library design
  - Diversity analysis, ADMET filter etc.

Exposed Van der Waals Surface Area (VSA)

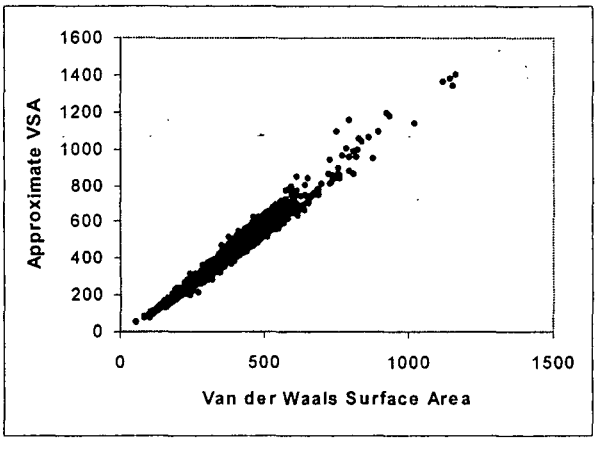
- Calculate exposed Van der Waals surface area for each atom by subtracting off surface area inside neighbors



- Correction factors to sphere formula depend on atomic radii and inter-atomic distances

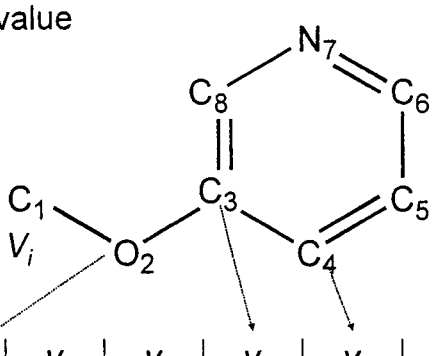
Quality of Approximate VSA Calculation

- Data set of 1,947 conformations
  - MOE 2D → 3D converter, MMFF94 force field, 0.01 RMS gradient
  - Molecular weights in [300,1600] range
- VDW Surface Area
  - 3D dot calculation
- Accuracy
  - $r = 0.9856$
  - $r^2 = 0.9666$
  - <10% error
  - Largest errors on steroids an other fused ring systems



Subdivision of VSA by Properties

- Given an atomic property value P_i for each atom i
  - O2 1.2
  - C3 4.5
  - C4 5.9
  - N7 0.2
- Bin P_i by ranges and sum V_i



V_i values:	V_7	V_2	V_1	V_6 + V_8	V_3	V_4 + V_5
P_i range:	[0,1)	[1,2)	[2,3)	[3,4	[4,5)	[5,6)
Descriptors:	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**

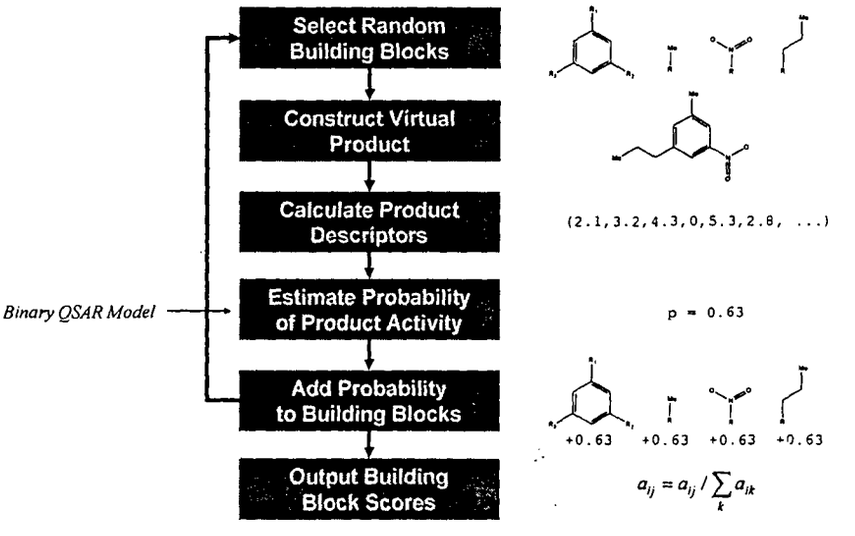
Encoding of Traditional Descriptors

- Traditional descriptors modeled with VSA descriptors
  - 1,932 small organic molecules with weights in (28,800)
  - SlogP_VSA, SMR_VSA and PEOE_VSA descriptors calculated
  - Principal components regression models for 64 traditional descriptors

chi0	0.99	chi0v_C	0.97	b_ar	0.89	b_1rotN	0.78
Kier1	0.99	KierA1	0.97	Kier2	0.89	b_double	0.77
vdw_area	0.99	a_hyd	0.96	vsa_pol	0.89	b_rotN	0.77
vdw_vol	0.99	a_nC	0.96	vsa_acc	0.88	a_ICM	0.73
vsa_hyd	0.99	a_nH	0.96	diameter	0.87	vsa_don	0.73
a_count	0.98	a_nO	0.95	VadjEq	0.87	KierFlex	0.69
a_heavy	0.98	b_heavy	0.95	a_nN	0.86	balabanJ	0.61
a_IC	0.98	chi1_C	0.95	KierA2	0.86	a_nP	0.60
apol	0.98	chi1v_C	0.95	radius	0.86	Kier3	0.57
b_count	0.98	SlogP	0.95	VdistMa	0.86	a_nCl	0.56
chi0v	0.98	a_acc	0.94	wienPath	0.85	KierA3	0.55
chi1	0.98	chi1v	0.94	wienPol	0.84	a_nS	0.53
SMR	0.98	Weight	0.93	VadjMa	0.82	b_1rotR	0.50
b_single	0.97	a_aro	0.91	VdistEq	0.82	density	0.49
bpol	0.97	a_don	0.91	vsa_oth	0.82	b_rotR	0.48
chi0_C	0.97	zagreb	0.91	a_nF	0.80	b_triple	0.46

Library Design Tools

Building Block Scoring Algorithm



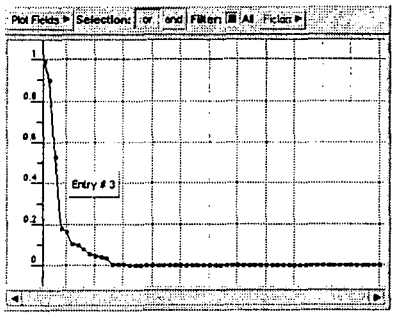
Quinazoline Library R-Group Scores

R1	R2	R3	R4
0.121 CH2-(2-thienyl)	0.191 1-imidazolyl	0.197 Me	0.960 H
0.120 benzyl	0.160 2-pyridyl	0.192 H	0.040 Me
0.109 CH2-benzyl	0.145 3-pyridyl	0.142 Cl	
0.083 phenyl	0.127 H	0.120 F	
0.081 CH2-(3-pyridyl)	0.111 4-pyridyl	0.112 CH3O	
0.062 CH2-(2-furanyl)	0.099 2-thienyl	0.093 Br	
0.061 2-pyridyl	0.076 2-furyl	0.065 CH3S	
0.041 3-(5-Me-isoxazolyl)	0.072 Cl	0.042 CH3SO2	
0.038 2-ClPh	0.016 4-morpholine	0.024 NO2	
0.037 3-ClPh	0.003 c-hexyl	0.013 NCC	
0.036 4-ClPh	0.001 4-Me-1-piperazinyl		
0.034 3-pyridyl			
0.032 1-pyrrolyl			
0.023 3-CH3OPh			
0.015 CH2CH2-2(3-Me-pyrrolyl)			
0.015 3-NO2Ph			
0.011 H			
0.008 CH2(CH2)4OH			
0.007 CH2(cPr)			
0.007 4-(CO2Me)Ph			
0.006 CH2-(c-hexyl)			
0.005 3-(CO2Me)Ph, c-pentyl, c-hexyl			
0.004 CH2-(2-THF)			
0.003 CH2(CH2)2OCOCH2CH3			

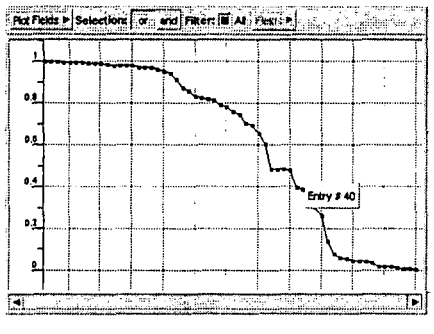
- Use median cutoff at each position (selected R-groups shown in blue)
- Retain only high-scoring R-groups that account for 50% of the probability

Enrichment of predicted active compounds

Random picked



Focused



COMBINATORIAL PHENOMENA IN BIOLOGICAL SYSTEMS

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Combinatorial chemistry (C.c.) is a recent branch of sciences, with several applications in drug research.

C.c. produces a wide variety of compounds, in order to provide the target moiety of the drug receptor with a large selection of possibly binding countermolecules.

The number of compounds formed can be expressed in terms of combinatorics, such as the number of combinations, variations, permutations, and numerous exponential formulas.

For example, if pentapeptide libraries are produced using 7 amino acids, the number of constitutionally distinct peptides is $\binom{7}{2} = \frac{7!}{5!\,2!} = 21$ (the number of combinations regardless the sequence). The possible, non-repeating sequences within a given set of five amino acids are 5! = 120, the number of permutations, which allows for 2520 pentapeptides of 5 different amino acids each. If repeating sequences are also permitted, the total number of pentapeptides with 7 building blocks is 7^5 = 16807. Such cornucopia of compounds represents a substantial chance of receptor binding.

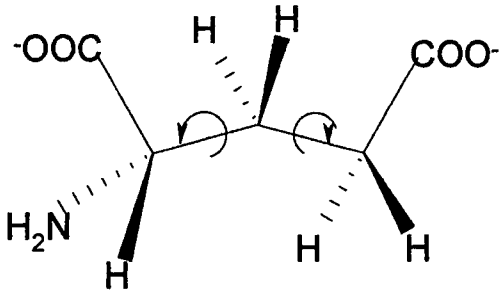
Several analogous combinatorial phenomena occur in biological systems.

Two of such combinatorial events are the protonation and conformation changes of biomolecules, in which a wide variety of distinct species are formed in a spontaneous manner. Prime examples are the neutrotransmitters

that constitute an extremely important group of versatile, multiconform biomolecules.

These compounds are typically of low molecular mass and relatively few atoms, but they usually bear several biological functions, due to their structural and coulombic chageability, and the concomitant set of distinct forms that can be counted by operations of combinatorics.

For example, glutamic acid, one of the 20 "classical" amino acids and a ubiquitous neurotransmitter on excitatory amino acid receptors, carries at least 6 biological functions, which can be assigned to its $F = 2^n . 3^m$ different solution forms, where n is the number of basic sites, and m is the number of rotational axes. For glutamic acid, n = 3, m = 2, and F = 72.



All the 72 forms of glutamic acid coexist in solution, providing the various receptors with a multitude of binding choices, being each of them is a particular microform of glutamic acid. The various microforms have different physico-chemical properties, with individual capabilities not only in receptor binding, but also in enzyme-catalysis, metabolism and membrane penetration. The significance, methods and results of combinatorial phenomena in biological systems will be further exemplified on N-acetylcysteine, the most widely used mucolytic agent[1], and amphetamine, a psychostimulant drug[2].

[1]Noszál, B., Visky, D., Kraszni, M.: *J. Med. Chem.* 2000, **43**, 2176-2182

[2]Noszál, B., Kraszni, M.: *J. Phys. Chem. B.* 2001, in press

Molecular Diversity in Drug Discovery: A Critical Assessment

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MOLECULAR DIVERSITY IN DRUG DISCOVERY: A CRITICAL ASSESSMENT

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This Lecture will at first examine the phases of modern drug discovery and see where diversity [1,2] and combinatorial chemistry [3-6] are going to play a major role (Figure 1). Target identification and target validation are now crucial milestones, as the unraveling of the human genome is providing thousands of uncharacterized genes as potential targets for the cure of important diseases. Research laboratories able to identify and validate targets better and faster than competitors will be significantly advantaged, and combinatorial approaches and tools will provide relevant benefits at this stage [7]; nevertheless, the full potential of chemical diversity and combinatorial libraries is evident in the following three steps of the process (Figure 1).

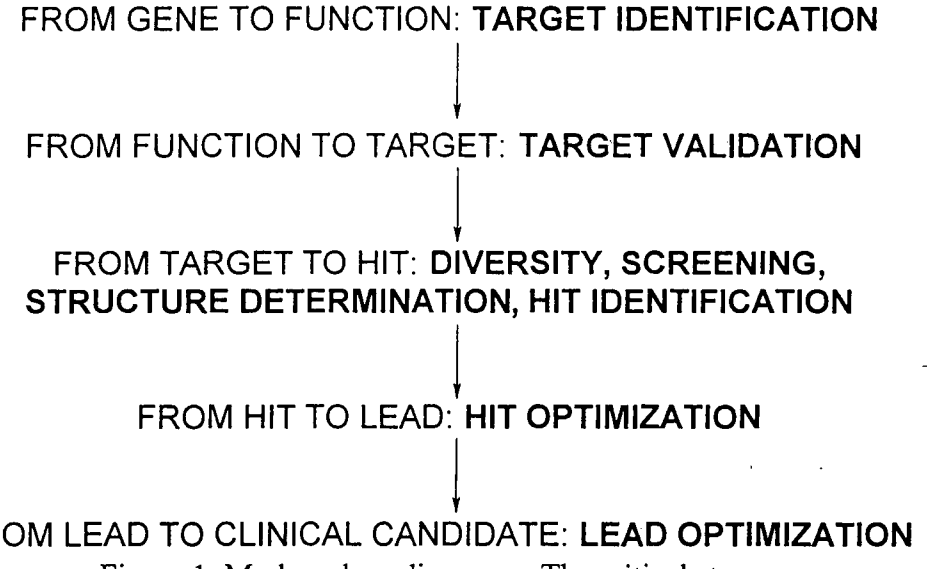

Figure 1. Modern drug discovery: The critical steps.

Traditionally the accent in Drug Discovery was put on the throughput, i.e. on the availability of large diversity collections (>>100K), of high-throughput robotics for the handling and the screening of the diversity, and of high-throughput analytical tools for the determination of the structure(s) and of the quality of active compounds. As for the collections, four major sources of compounds are available:

- Single compounds (externally acquired or in house prepared);
- Natural products from living organisms;
- Discrete libraries (parallel synthesis, individual compounds);
- Pool libraries (mix and split synthesis, mixtures).

Each source has its advantages and disadvantages, and will be thoroughly examined during the Lecture. Several key messages summarize the current tendencies related to chemical diversity and screening in hit identification:

-

- A collection must contain subsets from all diversity sources, and must evolve by acquisition/synthesis/isolation of novel, relevant individuals or libraries;
- Large pool primary libraries are becoming less popular;
- Medium-small, high quality, modular discrete libraries are increasingly popular;
- Libraries inspired by natural products' complex structures are increasingly popular, especially concerning the so-called chemical genetics approach [8,9].

The second part of this Lecture will present three recent examples referring to lead discovery and lead optimization. The first covers the synthesis of so called "activity profiling libraries", used to determine the nature of proteases in in vitro and in vivo assays and to validate their relevance as targets in Drug Discovery [10]. The second covers modular libraries in solution derived from a common chalcone library [11]. The third [12] reports a high quality solid phase pool library of complex, natural products-like compounds obtained from high quality and yield chemical transformations.

References

[1] L. Weber, *Curr. Opin. Chem. Biol.* **4**, 295-302 (2000).
[2] P. Willett, *Curr. Opin. Biotechnol.* **11**, 85-88 (2000).
[3] N. Terrett, *Combinatorial chemistry*, Oxford University Press, Oxford, 1998.
[4] *Combinatorial chemistry and molecular diversity in drug discovery*, Eds. E.M. Gordon and J.F. Kerwin, Wiley-Liss, New York, 1998.
[5] P. Seneci, *Solid-phase synthesis and combinatorial technologies*, Wiley, New York, 2000.
[6] F. Balkenhohl, C. von dem Bussche-Huennefeld, A. Lansky and C. Zechel, *Angew. Chem. Int. Ed. Engl.* **35**, 2288-2337 (1996)..
[7] D.S. Thorpe, *Combi. Chem. High Throughput Screening* **3**, 421-436 (2000).
[8] C.M. Crews and U. Splittgerber, *Trends Biol. Sci.* **24**, 317-320 (1999).
[9] B.R. Stockwell, *Trends Biotechnol.* **18**, 449-455 (2000).
[10] J.L. Harris, B.J. Backes, F. Leonetti, S. Mahrus, J.A. Ellman and C.S. Craik, *PNAs* **97**, 7754-7759 (2000).
[11] D.G. Powers, D.S. Casebier, D. Fokas, W.J. Ryan, J.R. Troth and D.L. Coffen, *Tetrahedron* **54**, 4085-4096 (1998).
[12] D.S. Tan, M.A. Foley, B.R. Stockwell, M.D. Shair and S.L. Schreiber, *J. Am. Chem. Soc.* **121**, 9073-9087 (1999).

Molecular Diversity in Drug Discovery: a critical assessment

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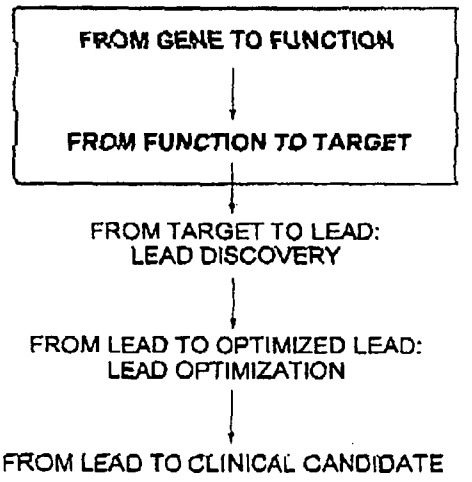
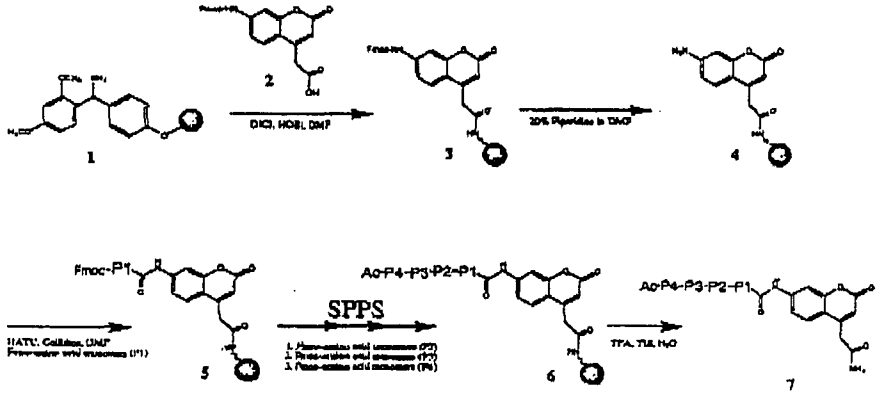
The Drug Discovery Process



Activity Profiling Libraries

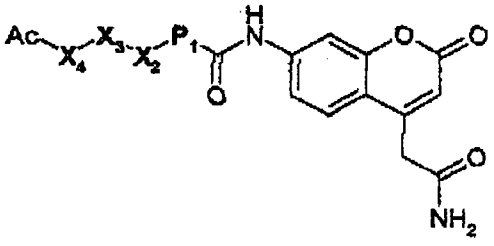
- Functional proteomics
- Targeted onto broad enzyme classes: e.g., proteases
- Reversible or irreversible probes
- This example: peptide libraries as reversible probes for serine and cysteine proteases

Activity Profiling Libraries



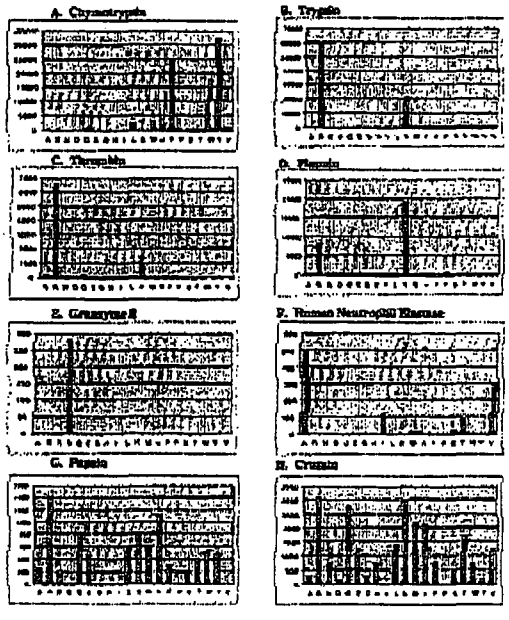
Harris, J.L et al., PNAs 97, 7754-7759 (2000)

Activity Profiling Libraries

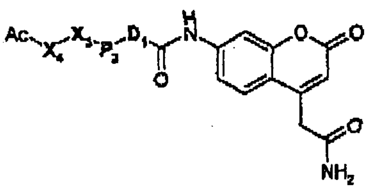


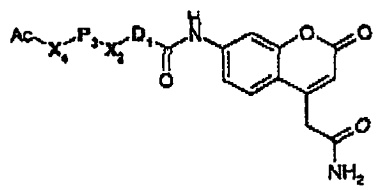
P_1-determined SP tetrapeptide library
P_1 = 20 determined AAs (-Cys, +Nle)
X_2-X_4 = 19 randomized AAs (-Cys)
20 pools, 137,180 compounds, 6,859 compounds/pool

ACTIVITY PROFILING

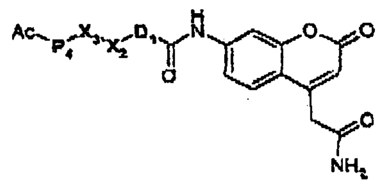


Activity Profiling Libraries

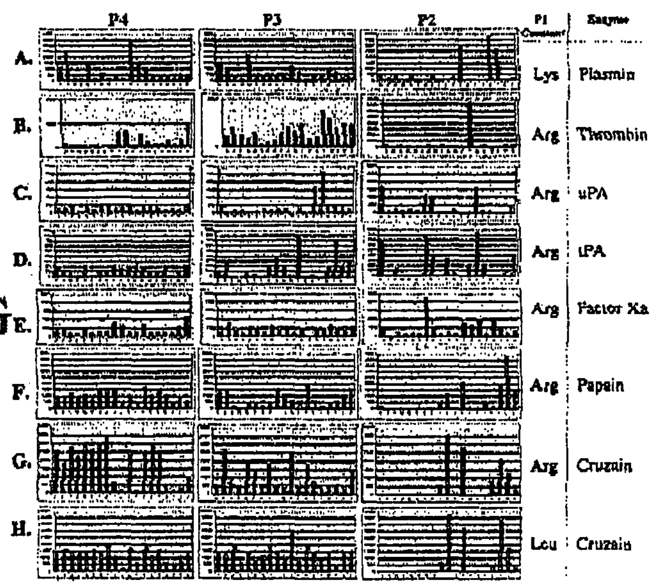




three P_2-determined SP tetrapeptide sublibraries
D_1 = 3 AAs (Lys, Leu and Arg)
P_2 = 19 determined AAs (-Cys)
X_2-X_4 = 19 randomized AAs (-Cys)
19 pools per sublibrary, 6,859 compounds per sublibrary,
361 compounds/pool



ACTIVITY PROFILING



The Drug Discovery Process

FROM GENE TO FUNCTION

↓

FROM FUNCTION TO TARGET

↓

FROM TARGET TO LEAD:
LEAD DISCOVERY

↓

FROM LEAD TO OPTIMIZED LEAD:
LEAD OPTIMIZATION

↓

FROM LEAD TO CLINICAL CANDIDATE

The golden circle - Lead Discovery

CD Sources – Single Compounds

- In house: historically built (large pharmas)
- CD depends on company history
- Ex novo: time- and money- requiring
- Very "diverse", if well planned: high CD embedded
- From any source (synthesis, acquisition, natural products, etc.)

CD Sources - SP pool libraries

- HT synthesis with minimal efforts
- No or little automation required
- Low embedded CD
- Problematic screening: false positives, false negatives, etc.
- Problematic structural characterization: throughput, missing compounds, etc.

CD Sources -Discrete libraries

- Extensive automation required
- Slower, more expensive
- Medium-Low embedded CD
- Screening and structural characterization: no problems

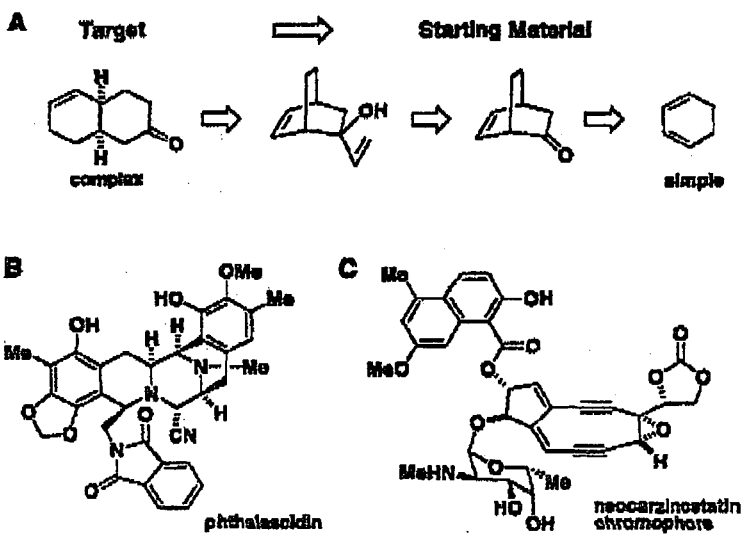
CD Sources - Natural products

- Troublesome isolation/purification
- "Deja vu" effect: novelty
- Particular skills required
- If novel sources are available, valuable and unpredictable CD is found
- If novel selection criteria are applied, valuable and unpredictable CD is found

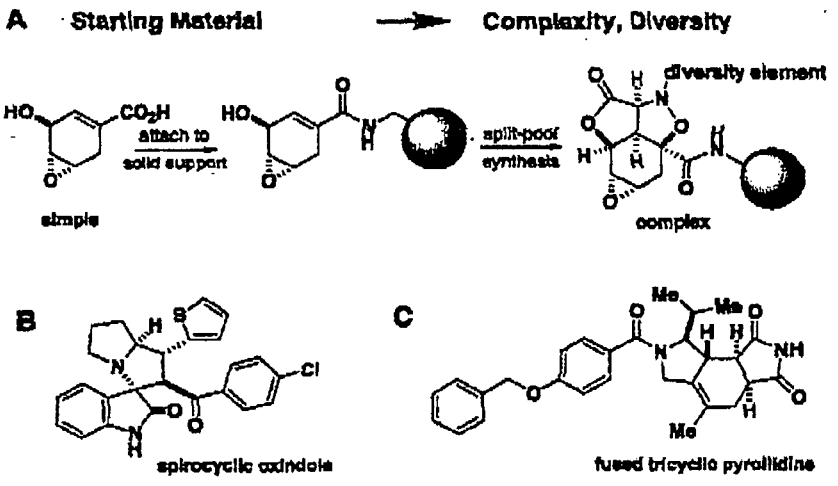
Trends:

- Multiple CD sources in an "evolutionary" screening collection
- Less large, primary libraries (only if expensive technology is available)
- More small, high quality, modular discrete libraries
- Back to novel natural products and NP-like compounds/libraries
- Meaningful screening assays with in vivo relevance: chemical genetics

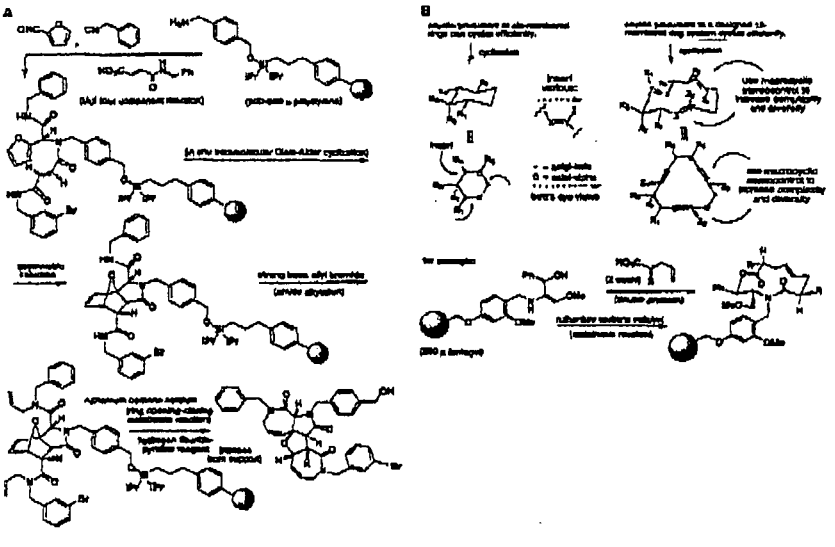
Target-Oriented Retrosynthesis



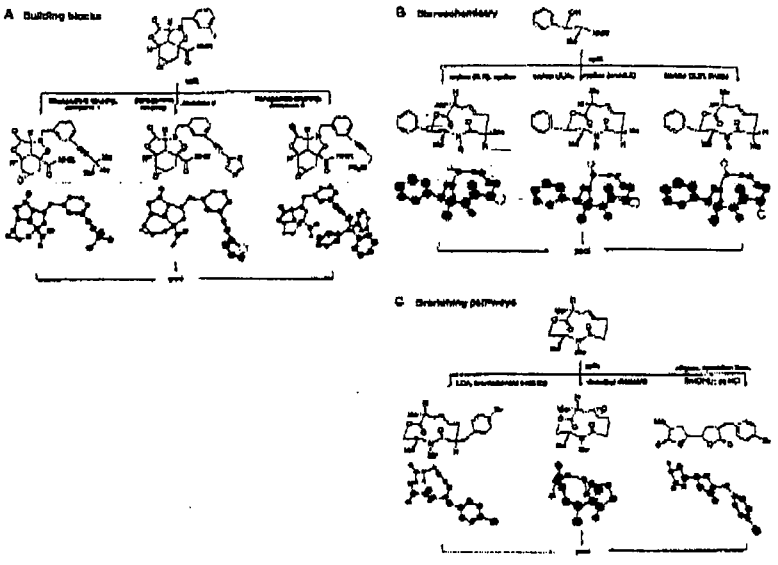
Diversity-OrientedRetrosynthesis



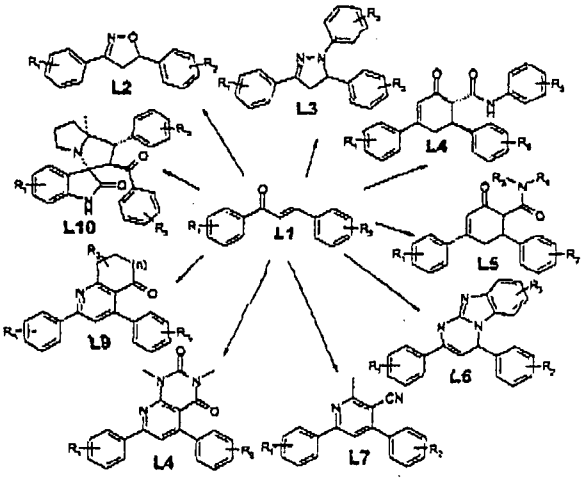
Diversity-OrientedRetrosynthesis



Diversity-OrientedRetrosynthesis

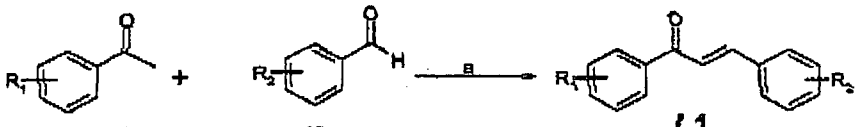


MODULAR DISCRETE LIBRARIES



Powers, D.G. et al., *Tetrahedron* 54, 4085-4096 (1998).

MODULAR DISCRETE LIBRARIES



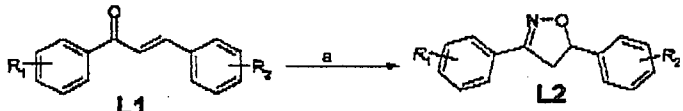
32
acetophenones, acetylfurans, acetylthiophenes, acetylpyrroles

40
benzaldehydes, furancarboxaldehydes, thiophenecarboxaldehydes, pyrrolecarboxaldehydes

L1
1280 discretes

a: NaOH, 4/1 EtOH/H_2O, rt, overnight.

MODULAR DISCRETE LIBRARIES



L1
1280 discretes

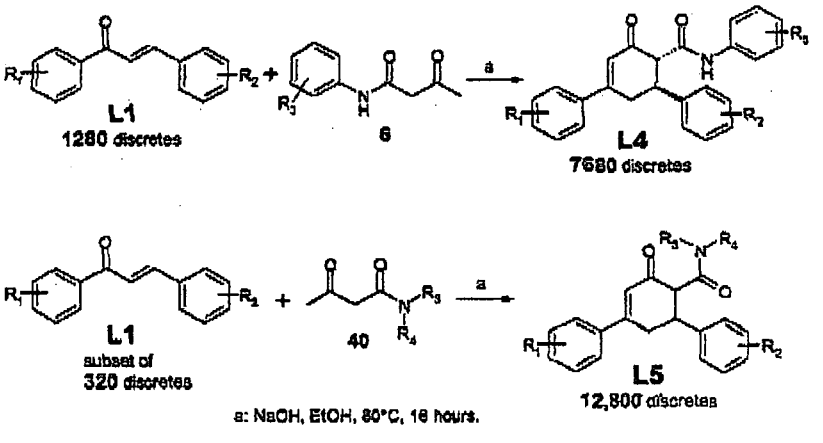
L2
1280 discretes

L1
1280 discretes

L3
7680 discretes

a: $H_2NOH.HCl$, NaOH, EtOH, 80°C, 12 hours;
b: $H_2NNHPhR_3$ (8), NaOH, 70°C, 8 hours.

MODULAR DISCRETE LIBRARIES



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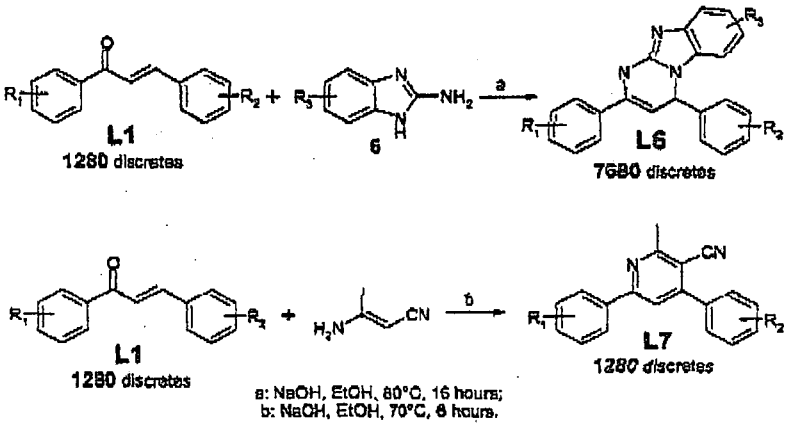

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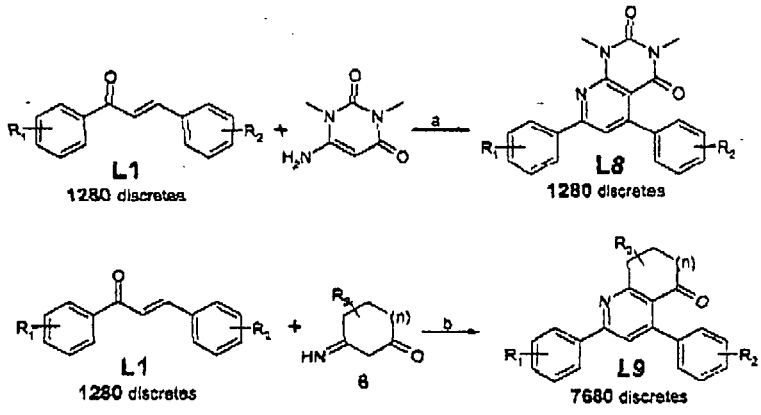
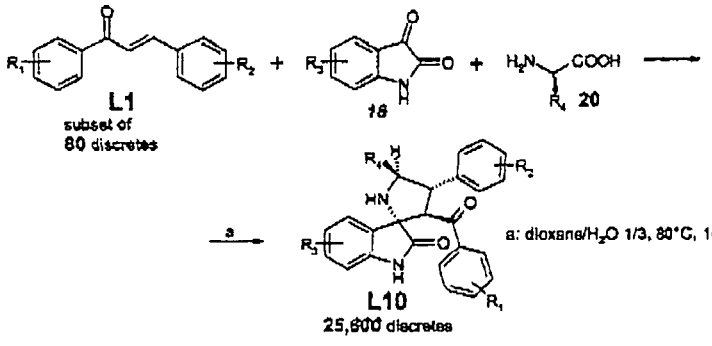


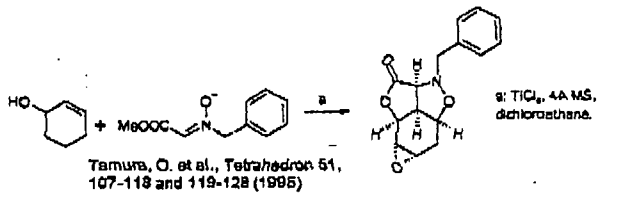
a: NaOH, EtOH, 80°C, 16 hours;
b: NaOH, EtOH, 80°C, 12 hours.

MODULAR DISCRETE LIBRARIES

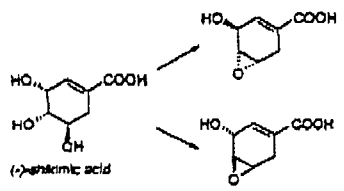


a: dioxane/H_2O 1/3, 80°C, 16 hours.

NP-LIKE, LARGE POOL LIBRARIES

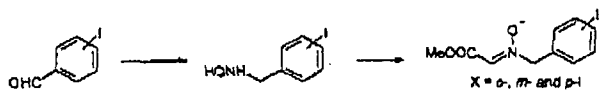


Tamura, O. et al., *Tetrahedron* 51, 107-118 and 119-128 (1995)



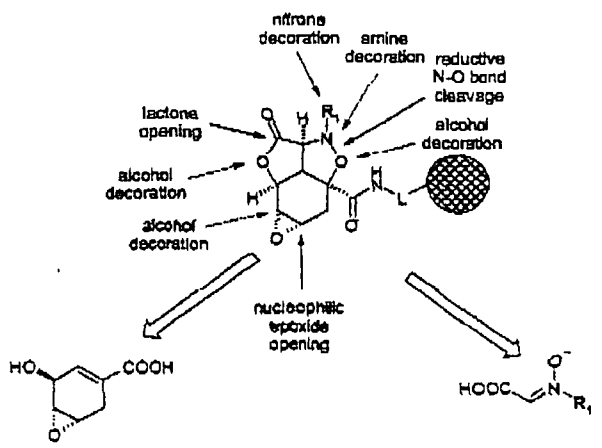
McGowan, D.A. and Berchtold, G.A., JOC 48, 2381-2383 (1981)

Wood, H.B. and Ganem, B.J., *JACS* 112, 8907-8909 (1990)



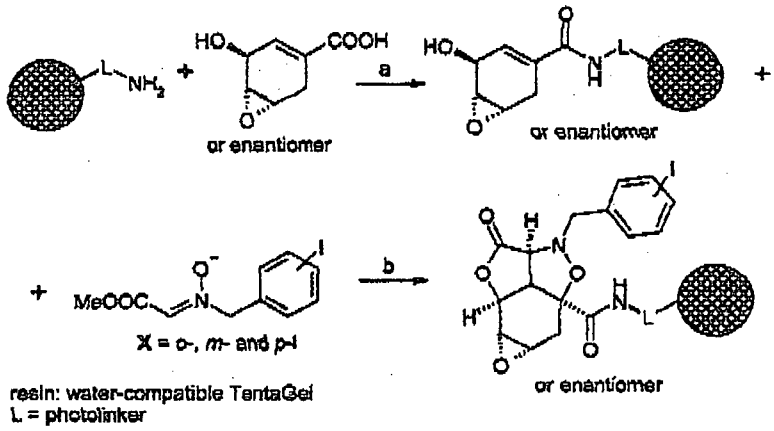
Keirs, D. and Overton, K., *Heterocycles* 28, 841-848 (1989)

NP-LIKE, LARGE POOL LIBRARIES



Tan, D.S. et al., *JACS* 121, 9073-9087 (1999)

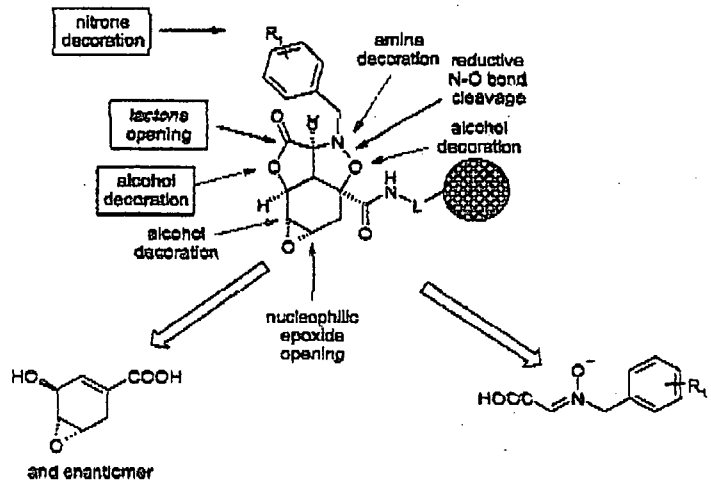
NP-LIKE, LARGE POOL LIBRARIES



resin: water-compatible TentaGel
L = photolinker

a: PyBOP, DIPEA, NMP, rt; b: HATU, DIPEA, DMAP, DCM, rt.

NP-LIKE, LARGE POOL LIBRARIES



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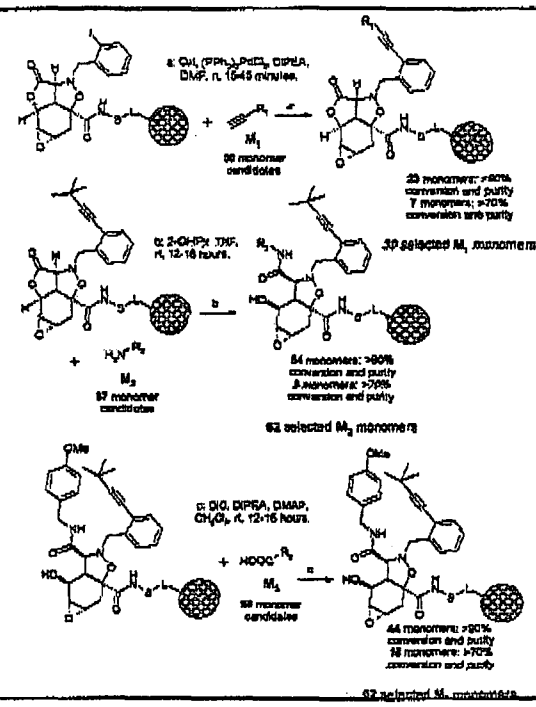

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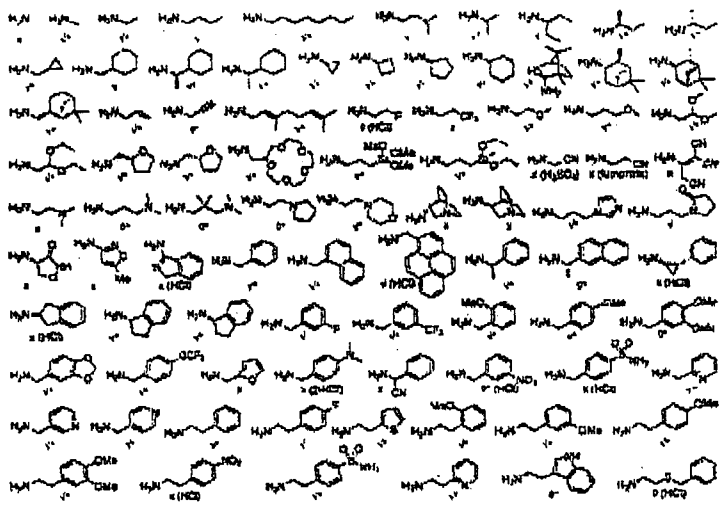

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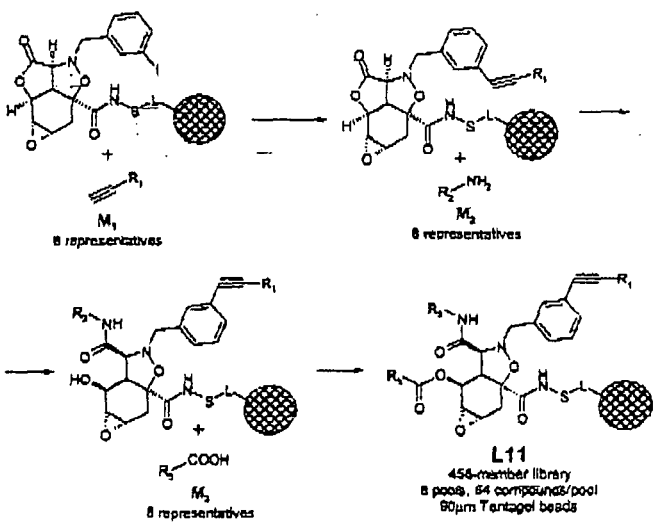
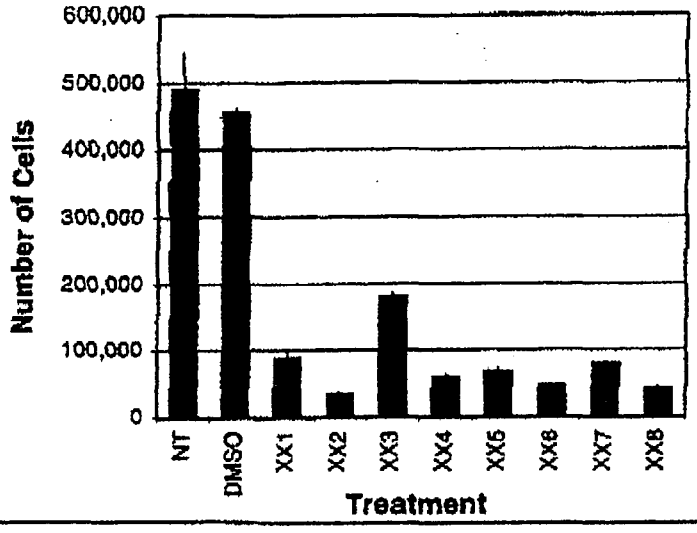


NP-LIKE, LARGE POOL LIBRARIES

- DECONVOLUTION OF L11:
- Activity on a mink lung cell proliferation assay (low micromolar)
- Difficult deconvolution (synergystic effects)
- Low quantity of supported compound per bead

NP-LIKE, LARGE POOL LIBRARIES



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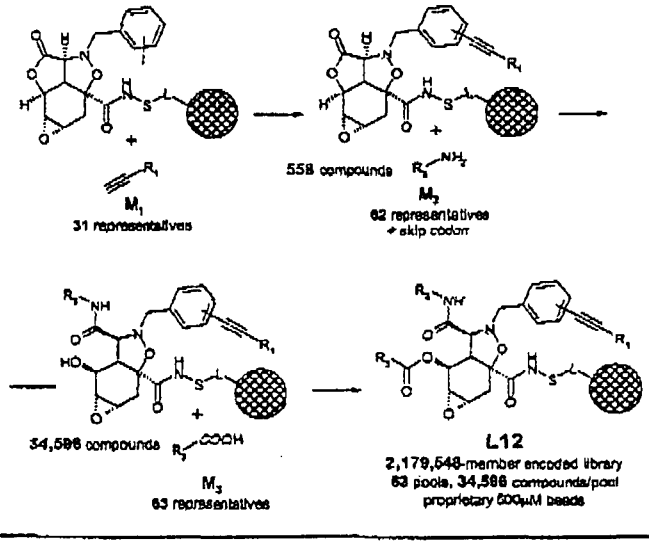
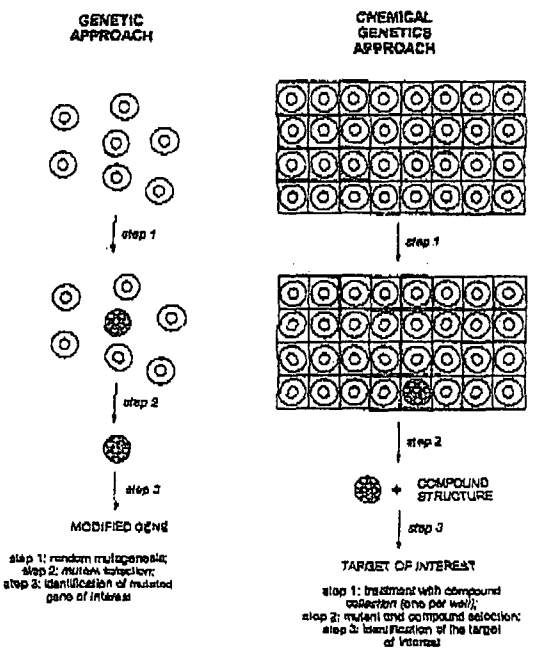


NP-LIKE, LARGE POOL LIBRARIES

- DECODING OF L12:
- Simple and reliable decoding
- Large quantity of supported compound per bead, sufficient for bead-based test and reconfirmation

Chemical genetics



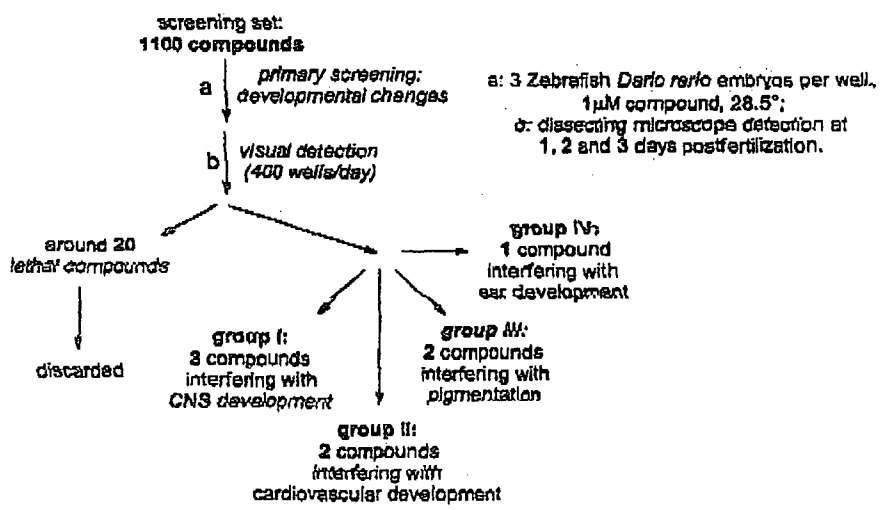
Chemical genetics

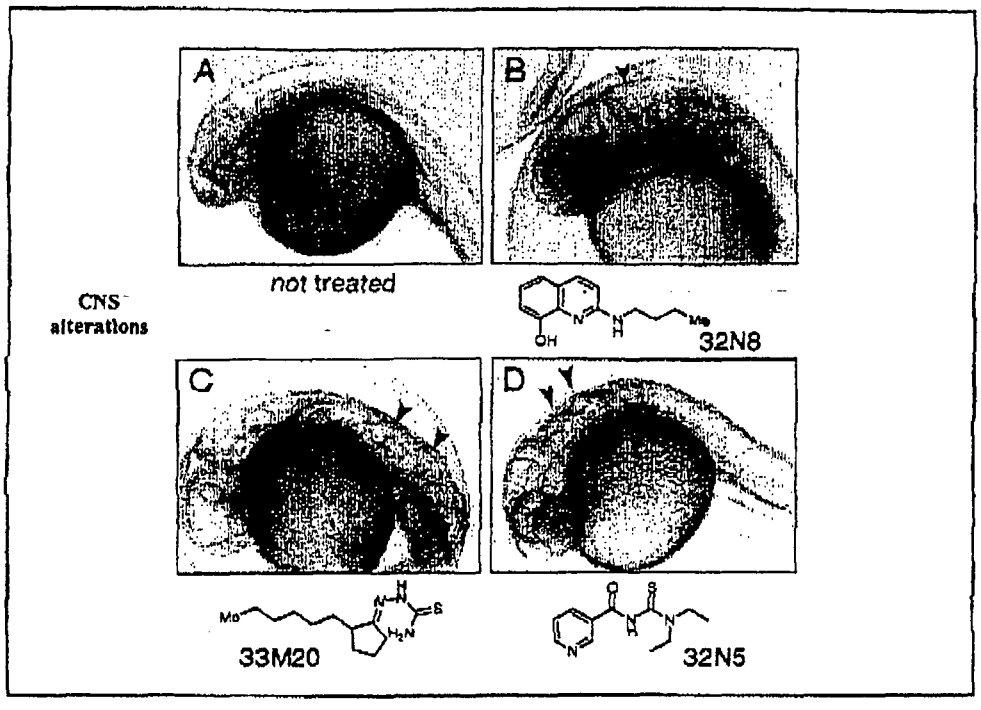
Proc. Natl. Acad. Sci. USA, Vol. 97, Issue 24, 12965-12969, November 21, 2000

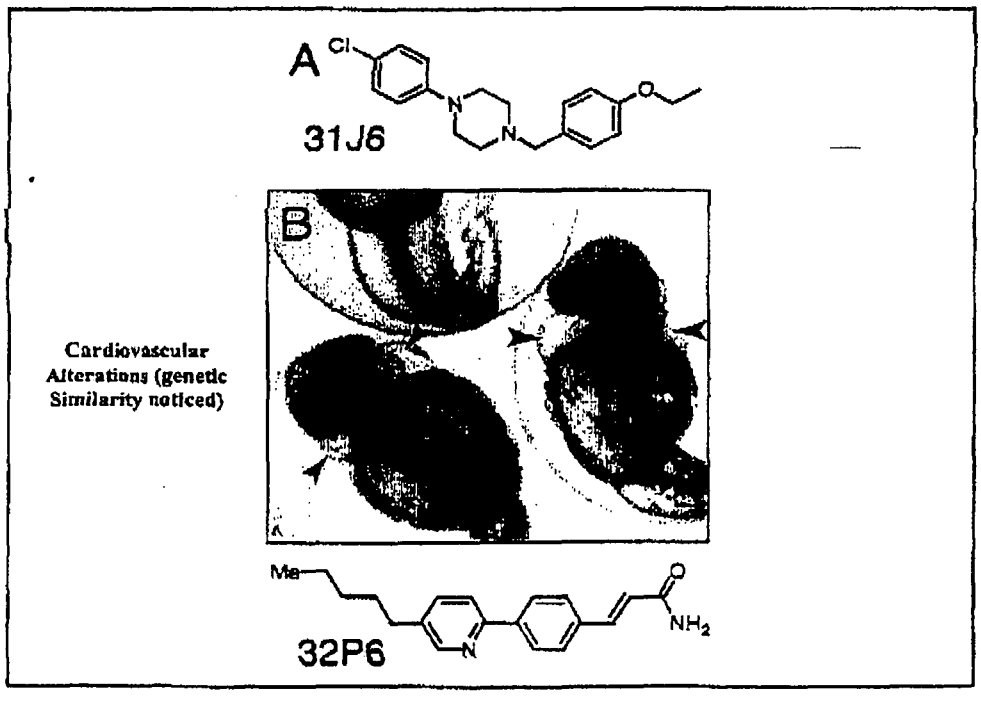
Small molecule developmental screens reveal the logic and timing of vertebrate development

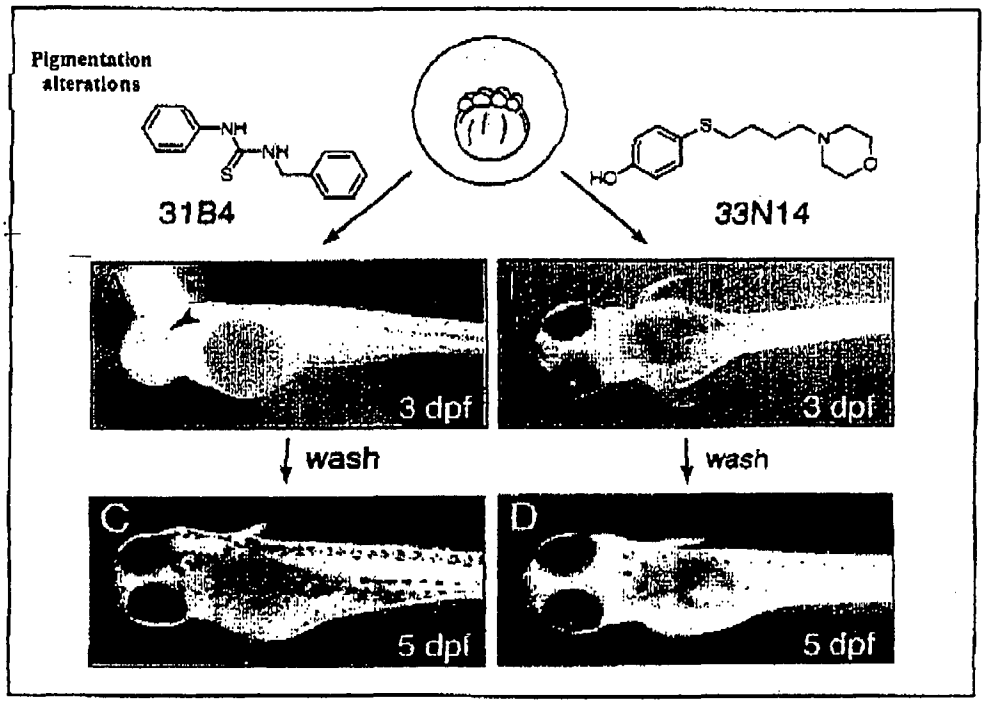
Randall T. Peterson*, Brian A. Link, John E. Dowling, and Stuart L. Schreiber*

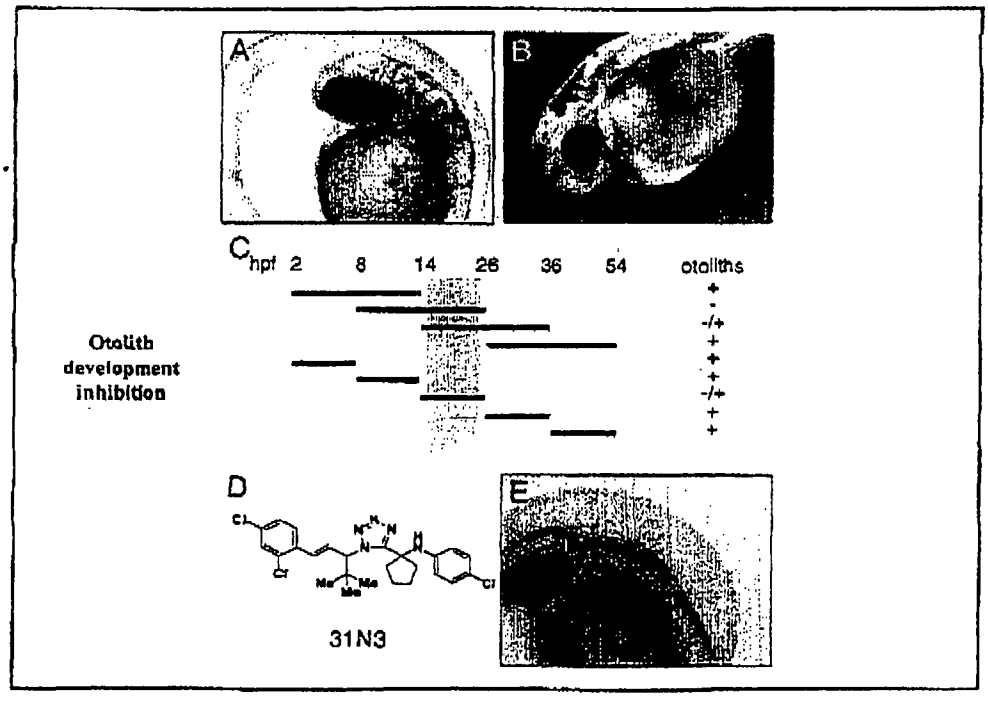
Chemical genetics




A
B
not treated
CNS alterations
32N8
C
D
33M20
32N5


A
31J6
B
Cardiovascular Alterations (genetic Similarity noticed)
32P6

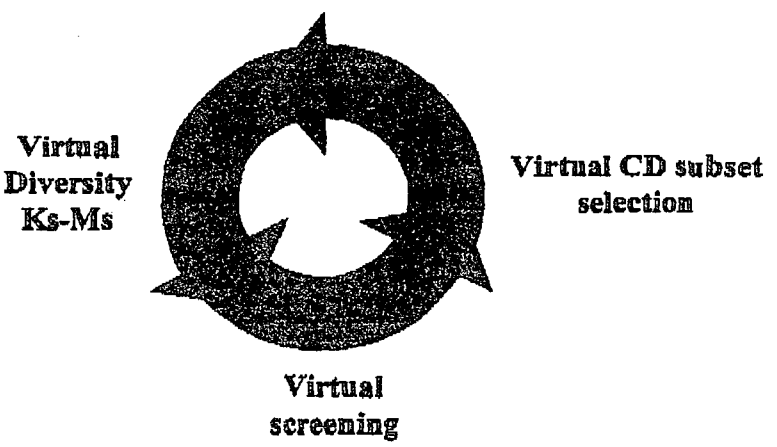

Pigmentation alterations
31B4
33N14
A
3 dpf
B
3 dpf
wash
wash
C
5 dpf
D
5 dpf


A
B
C
hpf 2 8 14 26 36 54 otoliths
Otolith development inhibition
D
31N3
E

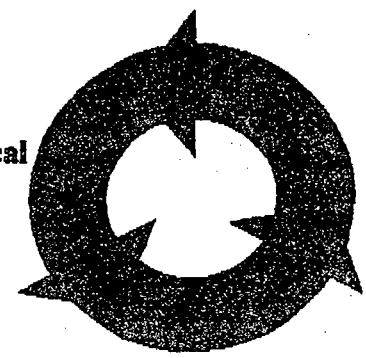
CD Sources - Virtual compounds

- Particular skills required
- Equipment (software, hardware)
- Risk taking (ruling out candidates), approximated
- Significant reduction of necessary CD
- Significant reduction of screening/structural characterization

The golden circle - Virtual Lead Discovery



The golden circle - Lead Discovery and Optimization



Rational Chemical Diversity 1K-10K

Low/medium-throughput str. characterization

Low/medium-throughput screening