# Programme

## Viernes / Friday 15

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<td>Max-Planck-Institut fur Kohlenforschung. Mülheim an der Ruhr, Germany</td>
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<td>➢ Directed Evolution of Enantioselective Enzymes</td>
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<td><strong>Barbara Imperiali</strong></td>
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<td>Department of Chemistry, Massachusetts Institute of Technology, USA</td>
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<td>The Skaggs Institute for Chemical Biology, The Scripps Research Institute, CA, USA</td>
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<td><strong>James E Audia</strong></td>
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<td>Department of Chemistry, University of Michigan, USA</td>
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<td><strong>Miguel A Yus</strong></td>
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<td>Dep Química Orgánica, Facultad de Ciencias, Universidad de Alicante, Alicante, Spain</td>
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<td>➢ New methodologies based on an arene-catalyzed lithiation</td>
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### Round table: High Throughput Synthesis

**Moderator/Chairperson:** Fernando Albericio  
*Barcelona Biomedical Research Institute, Univ of Barcelona, Spain*

- José Manuel Villalgordo, VillaPharma Research S.L.Spain (Moléculas pequeñas)
- Joaquín Pastor, Janssen-Cilag, Spain (Diversidad en síntesis)
- Rafael Ferrito, Lilly, S.A. Spain (Automatización en síntesis)
- Antoni Molins, Almirall-Prodesfarma, Soain (Quimioinformática)

### Session 3

**Moderator/Chairperson:** Rafael Ferrito  
*Lilly Research Laboratories, Alcobendas, Madrid, Spain*

#### 08:45

**Takashi Takahashi**  
Dept Applied Chemistry, Graduate School of Science and Engineering, Tokyo Institute of Technology, Tokyo, Japan

- **Solid- and Solution-Phase Synthesis of Natural Products Libraries**

#### 09:40

**Claudio Palomo Nicolau**  
Dpto Química Orgánica, Facultad de Química, Universidad del País Vasco. San Sebastián, Spain

- **Chiral Auxiliaries-Assisted Reactions: Inspiration for Developing Catalytic Enantioselective Processes**

### 10:35

**Café**

### 11:00

**C Oliver Kappe**  
Department of Chemistry, University of Graz, Austria

- **Microwaves in organic synthesis**

### 11:55

**Round table: Retos para la I+D en España: el caso de la Química Médica**

**Moderator/Chairperson:** José A Gutiérrez Fuentes  
*Fundación Lilly, Spain*

- Robert W Armstrong, Lilly Research Laboratories, Indianapolis, USA
- Miquel A Pericás, Instituto Catalán de Investigación Química, Tarragona, Spain
- Carlos Martínez Alonso, Consejo Superior de Investigaciones Científicas, Spain
- Jorge Martín Juárez, Crystal Pharma/Ragactives, S.A. Valladolid, Spain
- Nazario Martín, Real Sociedad Española de Química, Spain

### 13:00

**Closure Lecture**

**Moderator/Chairperson:** Julio Alvarez Builla  
Javier de Mendoza Institute of Chemical Research of Catalonia (ICIQ), Tarragona, Spain

- **Self-assembly: a bio-inspired approach to chemical complexity**

### 13:50

**Closure / Farewell**

Julio Alvarez Builla, Fernando Albericio, Jesús Ezquerra, José A Gutiérrez Fuentes
Inventing and developing a new drug is a long, complex, costly and risky process that has few peers in the industry world. Historically, as its today, creation of a new drug rides much—although not only—over the wave of new synthetic technologies. The new synthetic methods, by which scientists can create increasingly complex molecules, are often in the basis of the new, and more efficient molecular entities recently developed. In addition, present miniaturization and automation of testing techniques is producing a parallel effort in improvement of synthetic efficiency. Moreover, our increasingly sophisticated chemical tools are opening ways to study complex biological—and hence pharmacological—processes in a molecular way. All that approaches are expanding our knowledge in medicinal chemistry, and in the end are allowing the development of new and more efficient therapies, which serve all human beings.

The seventh Lilly Scientific Symposium “New Frontiers in Organic Synthesis” had tried to mix scientists with different views and cultures in their approach to creation of new molecules. From the use of enzymes and antibodies as catalysts, the use of chemical tools to study complex biological systems, to the use of new technologies as ionic liquids or synthesis with microwaves. From the use of new approaches to the synthesis of natural products, or the use of parallel synthesis to develop product libraries, to the use of new lithiation methods or new chiral auxiliaries. From the use of self-assembly to develop complex chemicals, to new concepts on Pharma productivity. In all those lectures always an equilibrium have been present between two philosophies: one takes in nature its inspiration, while the other uses new tools which technology is putting in our hands, and in our labs, to improve our synthetic efficiency. We hope this intimate mixture, had created an inspiring atmosphere useful to all participants in the Symposium.

A paradigm encompassing synthetic strategies, and often incorporating the use of solid-phase, was reported by Takahashi (Tokyo Institute of Technology). Takahashi’s group prepared a library of 122 derivatives of the anti-tumor compound macrophelide A using a method similar to split and mix, in which a radio frequency chip is employed to facilitate compound separation. Furthermore, the group prepared a library based on the anti-tumor antigen sphingolipid Lewis X using a solution/solid-phase hybrid strategy. Finally, the group presented the automated total synthesis of Taxol by the robot ChemKonzert.

The use of specially adapted microwave ovens for chemical synthesis was first reported by Kappe of the University of Graz. This method of transmitting energy can be applied to reactions in solution or on solid-phase, whether the resin serves as protecting group or reagent support. Microwave reactors are highly recommended for heterocycle synthesis and reactions involving transition metal catalysts or transpositions, and have recently been applied in peptide synthesis. Modern automated microwave ovens allow the successive treatment of a large number of reactions or can be used for work on a medium-scale. This technology improves yields and shortens reaction times, in many cases from hours to minutes. Lastly, microwave technology can be considered a green method, as it can be used to drive certain reactions in the absence of solvent.

Another example of green chemistry is the use of ionic liquids in synthesis and catalysis, described by Welton of the Imperial College. Owing to their highly reduced vapor pressure, these solvents can be used to manipulate chemical reactions by modifying factors such as reaction rate and selectivity.

Several new compounds in pharmaceutical development have chiral centers, hence strict synthetic control over stereochemistry is crucial. This area was tackled from different angles by Professors Reetz, of the Max Planck Institut, Barbas, of the Scripps Research Institute, and Palomo, of the Universidad del Pais Vasco. While Palomo focused on the use of chiral auxiliaries, Reetz and Barbas emphasized more bioorganic strategies, including enantioselective enzymes, catalytic antibodies and amino acids such as proline.

Yus, of the University of Alicante, presented novel methodology for a series of new arene-catalyzed lithiations. The presentation given by Roush from the Scripps Research Institute of Florida basically encompassed all of the new strategies outlined by the
other speakers. His talk highlighted the use of new techniques in the synthesis of structurally complex, biologically active natural products, such as 13-deoxytedanolide, amphidinol 3 and the amphidinolides C, E and F.

Presentations by Imperiali, of the Massachusetts Institute of Technology, and Mendoza, of the Institut Català d’Investigació Química, leaned more towards biochemistry. These talks comprised the use of chemical probes to study phosphorylation reactions, and molecular self-assembly as an alternative method for constructing supramolecular structures.

Audia, from Lilly Research Laboratories in Indianapolis, summarized the challenges currently facing pharmaceutical companies and proposed solutions to overcome them. According to Audia, these solutions can only stem from an interdisciplinary and multifaceted research philosophy that is well-rooted in knowledge, and in which properties such as activity and ADME-tox are studied at the earliest stages of a compound’s development. In closing his talk, he stressed that the changes occurring in modern drug development will yield opportunities on personal as well as commercial levels. Companies, which are now seeking professionals with more interdisciplinary backgrounds, are being forced to externalize operations in order to gain access to cutting edge technology. Small companies and academic groups must capitalize on this phenomenon if they are to play a more active role in the fascinating world of drug discovery.
In 1991, Richard A. Houghten and Kit S. Lam published the first two articles in the field of combinatorial chemistry, which takes its name from the corresponding term in mathematics. These authors described the concurrent synthesis of a multitude, or library, of compounds containing millions of chemical entities (in this case peptides), as well as the subsequent identification of those compounds capable of interacting with predetermined molecular targets. One year later, Jonathan A. Ellman published the first synthesis of a library of non-peptidic small organic molecules. The preparation of these 1,4-benzodiazepines marked the beginning of a new era in organic synthesis that would eventually find parallels in genomics, proteomics and metabolomics research. The aforementioned fields are providing an ever-widening array of therapeutic targets associated with various terminal diseases for which no drugs are currently available. The development of massive-scale biological assays, made possible by automation, has underscored the need for high-throughput synthesis (HTS) of new chemical entities for screening.

While combinatorial chemistry has undoubtedly revolutionized synthetic chemistry, it has also undergone an important evolution of its own. Combinatorial chemistry was initially envisioned as a strategy to maximize the chemical space covered by the products of a given synthesis and thereby amplify the probability of generating interesting new chemical entities. Preliminary work in the field comprised the preparation of libraries of thousands, or even millions, of compounds, primarily peptides, oligonucleotides and PNA’s. A library of pentapeptides containing every permutation of the 20 naturally occurring amino acids would contain 3,200,000 \((20^5)\) members. From a synthetic perspective, this library would have to be assembled on solid phase.

The solid-phase strategy developed by R. Bruce Merrifield in the 1960’s for peptide synthesis is based on the covalent linkage of the carboxylic acid of the first amino acid in a sequence to an insoluble polymer that acts as a protecting group. The resulting C-terminal component is insoluble in the solvents used for the synthesis. Excess reagents and most byproducts are ultimately removed by simple filtration and washing of the polymer that contains the growing peptide chain (Figure 1). This fact facilitates the use of large excesses of reagents, thus providing near-quantitative yields for many steps.

Biological screening of libraries of mixtures prepared on solid-phase are performed on the resin itself or once the desired compounds have been cleaved from the resin. In the latter case, deconvolution methods are used to identify active compounds.

On-resin biological screening requires libraries constructed by the split and mix method, as this method guarantees that each polymer bead contains only one compound. In this technique, also known as one-bead one-compound, the resin beads act as individual reactors, as illustrated in Figure 2.

In the split and mix assembly of trimers, a resin is divided into three portions, each of which is treated with a single monomer: A, B or C. The portions are then mixed and equally split among three reactors. Monomer D, E or F, is then added to each of the reactors, which are subsequently remixed, re-split and finally treated with monomer G, H or I. This process ensures that each resin bead contains exclusively one compound. The library of 27 compounds in this example of split and mix synthesis is prepared in only nine reactions, whereas classical methods (i.e., individual syntheses) would have required 81 \((27 \times 3)\) reactions.

Biological screening in solution of the aforementioned compounds upon their cleavage from the solid-support implies deconvolution, for which many methods exist. Deconvolution...
of the library described above via positional scanning would entail the preparation of nine sub-libraries, shown in Figure 3 (where X, Y and Z represent the following three component mixtures: A + B + C, D + E + F, and G + H + I, respectively). The sub-libraries are prepared on solid-phase incorporating the corresponding monomers. The best monomer for the first position of the trimers is determined by comparing the activity of the nine-component sub-libraries AYZ, BYZ and CYZ, which are identical in positions two and three (each containing mixtures of three monomers) and differ only in their first position. The best monomers for the remaining positions are then determined in an analogous fashion, ultimately providing the structure of the target trimer to be prepared.

![Figure 3. Sub-libraries prepared for biological screening in solution.](image)

While the utility of libraries of mixtures of small organic molecules has also been demonstrated, their preparation is complicated by differences in reactivity among members of a given class of organic reagents (e.g., acids, aldehydes, alcohols, amines, etc.) It should also be mentioned that screening mixtures of compounds is less reliable than screening individual compounds. These two facts have limited the use of mixtures of libraries to the screening of biomolecules (e.g., peptides, oligonucleotides, peptoids) for early stages of drug-discovery programs.

Combinatorial or high-throughput chemistry is now focused on the rapid and rational generation of libraries of approximately 150 compounds, in which each product is prepared on a scale of 5-25 mg, characterized by at least HPLC-MS and 1H-NMR, and greater than 95% pure. These libraries are assembled in parallel on solid-phase or in solution.

As previously mentioned, a polymer-support can act as a protecting group for peptide synthesis. Moreover, polymer-supports can be bound to myriad reagents, and the resulting functionalized resins can be used in solution-phase synthesis or purification. In the former, a reagent immobilized on solid-support is added to the reaction, and excess reagents or by-products are ultimately filtered off, whereas in the latter, a functionalized polymer, or scavenger resin, is added at the end of a reaction to bind to impurities and then removed by filtration. Solid-phase techniques allow the use and subsequent removal of excess reagents, often the key factor in optimizing the yield of a given reaction. Furthermore, solid-phase reagents facilitate the automation and parallel lay-out of many synthetic processes.
In enantioselective transition metal catalysis, the development of a single highly effective chiral catalyst requires the preparation and testing of a large number of ligands. Success depends on design, which is based on knowledge of the mechanism, intuition and molecular modeling. Often, trial and error also plays a role. Alternatively, biocatalysts can be used, but by nature the problem of substrate specificity persists.

A fundamentally different approach to the development of enantioselective catalysts is described, namely directed evolution as a method to stepwise increase the enantioselectivity of a given unselective enzyme. The underlying principle “evolution in the test tube” does not require any knowledge of the enzyme structure or of its catalytic mechanism. Proper molecular biological methods for random mutagenesis and expression of genes coupled with an efficient high-throughput screening system for the rapid identification of enantioselective mutants form the basis of our strategy.

Using our original UV/Vis-based system, which constitutes the first high-throughput ee-assay (allowing for 500-900 samples to be processed per day), we have applied the known methods of molecular biology to the directed evolution of enantioselective enzymes (specifically lipases) for use as catalysts in the hydrolytic kinetic resolution of ester 1. The original (wild-type) enzyme shows a selectivity factor of only $E = 1.1$ in slight favor of the $S$-acid (Scheme 2). The selectivity factor $E$ reflects the relative reaction rate of the $S$- with respect to the $R$-substrate. Several $S$-selective mutants were evolved ($E = 25 - 51$). Moreover, it was possible to invert the sense of enantioselectivity in favor $R$ ($E = 30$).

Sequencing of the best mutant showed six mutations, most of them occurring at remote positions. This surprising result was explained by a relay mechanism uncovered by MM/QM studies. One of the major challenges in synthetic organic chemistry concerns selective partial oxidation. Therefore, we have initiated projects pertaining to monooxygenases and P450-enzymes. For example, cyclohexanone monooxygenases can be used as catalysts in $O_2$-mediated Baeyer-Villiger reactions, but the degree of enantioselectivity is poor for many substrates. Directed evolution can be used to evolve highly enantioselective catalysts for a number of different substrates.

We have developed several high-throughput ee-assays. Examples include systems based on UV/Vis, CAE, ESI-MS, IR-thermography and most recently on NMR. Between 1000 and 20000 ee-determinations can be performed per day. Other authors such as K. Mikami, R. Kazlauskas, M.G. Finn, C.T. Seto, M.D. Shair and C. Miokowski have developed alternative ee-screens. No single screening system is universal.
In summary, directed evolution of enantioselective enzymes has emerged as a powerful new way of generating catalysts for asymmetric transformations. Several other academic and industrial groups have joined in these efforts, and further interesting perspectives are becoming visible.

The importance of chemical tools for studying complex biological systems is constantly expanding with the realization that such approaches can form powerful partnerships with traditional strategies based on genetic and immunologic approaches. A key advantage to chemical genetics-based methods lies in the unparalleled ability to control and monitor specific events in living cells in real time. Recent research has focused on the therapeutically important area of signal transduction. Due to the essential signaling role of protein kinase-mediated protein phosphorylation in all cellular processes, this central process has been adopted as a strategic target area for probe development.

Caged phosphopeptides and phosphoproteins

Caged compounds include a photolabile protecting group that masks an essential functionality. Thus, a caged biomolecule, such as the phosphopeptide illustrated in Figure 1A, would be biologically inactive, but rendered active only after unmasking by photolysis under mild and neutral conditions (Figure 1B).

Figure 1. A. Section of a peptide integrating caged phosphoserine. B. Chemical and biological generation of phosphoproteins

The most commonly implemented caging groups in biological studies are based on substituted o-nitrobenzyl systems, which can be uncaged at wavelengths >350 nm. The “caging” strategy allows for spatial and temporal control over the release of effector molecules in living systems. Until recently, access to caged phosphopeptides had been limited due to the absence of efficient synthetic strategies for the preparation of suitably-protected amino acid building blocks for use in solid phase peptide synthesis (SPPS). The key step in the building block synthesis is the phosphorylation of an appropriately-protected amino acid precursor followed by mild oxidation with t-butyl hydroperoxide or mCPBA and deprotection of the t-butyl ester as illustrated in Figure 2 for the synthesis of caged phosphoserine. An analogous strategy has also been applied to the synthesis of the other major eukaryotic protein phosphorylation targets - threonine and tyrosine.

Figure 2. Synthesis of a caged phosphoserine building block for SPPS

The caged phosphoamino acid analogs of serine, threonine and tyrosine have been incorporated into a number of polypeptides which were then used to study critical events in cell migration and cell cycle control. More recently, the synthetic strategy outlined in Figure 2 has been modified for the semi-synthesis of a transfer RNA (tRNA) that is charged with a caged phosphoamino acid. This tRNA was then used for the in vitro translation of a full-length caged phosphoprotein using the suppressor tRNA methodology. This major development in the semi-synthesis of caged phosphoproteins now provides access to a wide variety of caged biological molecules for the study of specific phosphoprotein effectors in signal transduction pathways.

Fluorescent probes of protein kinase activity

Fluorescence-based probes for monitoring protein phosphorylation and the spatial and temporal characteristics of protein kinase activities in cells provide the opportunity to understand the dynamics of cellular processes in healthy and transformed cells. The phosphorylation probes developed in the Imperiali group exploit novel amino acids with either environment-sensitive or chelation-enhanced fluorescent properties. The probes that


include environment-sensitive fluorophores, such as the 2-dimethylamino-6-naphthoyl or dimethylamino naphthalimide groups in the DANAv and 6-DMNvi amino acids respectively, can be used to monitor phosphorylation-induced binding of peptides and proteins to partner molecules as exemplified by the binding of phosphotyrosine-containing peptides to SH2 domains. Alternatively, the chelation-enhanced fluorophore in the Sox amino acid can be used to directly signal phosphorylation via a mechanism that exploits the enhanced affinities of phosphorylated peptides including Sox, for divalent magnesium. The Fmoc-Sox is prepared by the asymmetric alkylation of a glycine precursor using the Corey modification of the O’Donnell phase-transfer catalyzed reaction, which implements a modified cinchonidinium alkaloid as the chiral catalyst.

Figure 3. Asymmetric Synthesis of Fmoc-Sox

Chemosensor peptides, based on the Sox amino acid approach, have been developed for a number of physiologically important protein kinases including Akt, PKC, PKA and Abl. The kinase chemosensors are readily phosphorylated by recombinant target enzymes and undergo a severalfold increase in fluorescence signal upon phosphorylation. More recently, the aforementioned chemosensors have been demonstrated to show significant utility in unfractionated cell lysates. This latter development will be extremely valuable since it will enable the profiling of small molecule inhibitors of protein kinase activities in more native environments including physiological levels of ATP, which is not feasible with most of the assays currently available.

Conclusions

Recent developments in the design, synthesis, and implementation of new chemical probes for studying the roles of protein phosphorylation in cellular processes now provide powerful approaches for the study of complex biological systems.

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ix Shults, M. D.; Jones, K. A.; Lauffenburger, D. A.; Imperiali, B. Nature Methods

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NEW FRONTIERS IN ORGANIC SYNTHESIS
One of the ultimate goals in organic chemistry is the catalytic asymmetric assembly of simple and readily available precursor molecules into stereochemically complex products. As chemists, we often turn to nature for inspiration concerning stereochemically complex, diverse, and functional molecules. Indeed, the directed asymmetric assembly of simple achiral building blocks into stereochemically complex molecules like carbohydrates and polyketides has long been the purview of nature’s enzymes. Our approach to this problem began in 1997 when we embarked upon studies exploring the similarity between proline and a novel class of aldolase antibodies we had developed earlier. Recently, these studies have allowed us to describe the first direct organocatalytic asymmetric ketone and aldehyde additions in aldol, Michael, Mannich, and Diels-Alder reaction manifolds. Significantly, these studies were originally designed for antibody catalysis years before. This lecture will summarize the contributions of this laboratory to creating and converting enzymatic enamines, and in some cases imines, into a versatile catalytic asymmetric strategy powered by small organic molecules.

In recent years my laboratory has also focused on the development of diverse strategies for the development of therapeutics and the validation of molecular targets. Provided time, I will also introduce the concept of chemically programmed antibody therapeutics that utilize enamine chemistry to modify the pharmacokinetics and potency of small molecule drugs. The success of the antibody molecule as therapeutic agent is based on at least three properties; (i) an Fab moiety that permits antigen binding with high specificity and affinity, (ii) an Fc moiety that mediates effector functions, and (iii) a molecular weight of at least 150 kD that permits a circulatory half-life of up to 21 days. Although conventional therapeutic agents based on small organic molecules have been successful in many instances, they are clearly limited with respect to their short half-life in circulation and their inability to mediate effector functions. Proposing that a blend of these features will lead to therapeutic agents with superior properties, we have developed chemically programmed antibodies. In vitro and in vivo studies concerning unique antibody specificities that provide for the targeting of two angiogenic pathways combined with broader targeting of tumors themselves will be presented.
Figure 5. Chemically Programmed Antibodies: A New Immunotherapeutic Approach Powered by Chemistry.

REFERENCES:


The pharmaceutical industry is viewed by many as facing a productivity dilemma. Despite continued increases in research and development expenditures, no corresponding increases in new drug approvals have emerged.

In that context, an outline is provided for the discovery chemistry research and technology organization within Lilly Research Laboratories and some of the approaches taken in that group to substantially increase the productivity of Lilly's drug discovery efforts.

Central themes for this productivity enhancement include integration of new technologies in the appropriate context for drug discovery, shifting from probabilistic toward knowledge based lead generation, and aggressive multi-dimensional lead optimization.
A final element of this approach is the strategic utilization of outsourcing both for the introduction of new capabilities and technologies, but also for expansion of capacity in critical drug discovery activities.
ABSTRACT

The application of ionic liquids to synthesis and catalysis is the focus of increasing activity in both academic and industrial environments. This has resulted in an explosion of interest, with 470 papers appearing in the year 2003 with ionic liquid in the title compared to just 80 in 2000. Also in 2003, the first industrial application of ionic liquids, the BASF BASIL® process, was announced. In my own group, we have used ionic liquids as solvents for inorganic, organometallic, and transition metal catalysed transformations. Although much of the activity in the field has concentrated on ionic liquids as ‘green’ replacements for environmentally damaging organic solvents, it is the possibility of using them to change the outcomes (rates, selectivities etc.) of reactions that is the most exciting. It is on this latter subject that my research has been concentrated.

The application of ionic liquids as solvents for chemicals synthesis is still a young area. At first it was not even clear that ionic liquids would be able to be used in this role. Hence, there has been a phase of development that is best characterised as a scoping exercise. Most of these studies have involved taking a well-known reaction and trying it in a single ionic liquid to see if it “goes”, often without reference to the same reaction in molecular solvents. While this approach has fuelled the growing interest in ionic liquids it does little to explain how the use of ionic liquids can affect the reactions conducted in them, or how they might best be applied. Quantitative comparisons of the properties of the ionic liquids with other solvents, and between different ionic liquids, are the only way in which this can be achieved. There has been much speculation that the ionic nature of these liquids has a profound effect on processes carried out in them and in some cases tantalising initial results have been reported. Also, the much repeated proposition that ionic liquids are ‘designer solvents’ rests on the assumption that they are sufficiently different to each other to warrant this approach.

The aim of this talk is to introduce ionic liquids as potential reaction media for chemicals synthesis. I will show how ionic liquids can be described using empirical polarity scales, particularly the Kamlet-Taft system. I will then show examples of where the ionic liquids have affected the rates and selectivities of a number of stoichiometric organic reactions and use this as a basis to determine in which processes substitution of a molecular solvent with an ionic liquid.

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Recent studies on the development of new synthetic methodology will be presented, along with applications towards the total synthesis of stereochemically complex, biologically active natural products. The specific examples highlighted will be selected from our recent efforts on the total synthesis of 13-deoxytedanolide, amphidinol 3, and amphidinolides C, E and F.

William R Roush
Executive Director of Medicinal Chemistry, The Scripps Research Institute.
Florida, USA. roush@scripps.edu
New methodologies based on an arene-catalyzed lithiation

The arene-catalyzed lithiation has been shown to be a very efficient methodology for the lithiation of different substrates under very mild reaction conditions. The following four sections will explore recent representative applications of this procedure:

1. Preparation of organolithium compounds from non-halogenated materials

Different non-halogenated materials (such as ethers, thioethers, alcohols and their O-silyl derivatives, sulfonates, sulfates, nitrites, sulfoxides, sulfones, phosphates, esters, amides, carbonates, carbamates or ureas) are easily transformed into organolithium compounds using an arene-catalyzed lithiation (Scheme 1). In some cases it is necessary to perform the reaction in the presence of the electrophile (Barbier conditions) in order to avoid the decomposition of the in situ generated organolithium. The reaction has also been applied to the deprotection of different protected alcohols and amines (allyl, benzyl, sulfonyl or silyl derivatives).

2. Ring opening of heterocycles

The arene-catalyzed lithiation has been used for the reductive ring opening of different three-, four-, five-, six- and seven-membered saturated or benzofused oxygen-, sulphur- or nitrogen-containing heterocycles, so a series of functionalized organolithium compounds is easily accessible (Scheme 2). An interesting application of this methodology allows the selective functionalization of carbohydrates (glucose or fructose) and steroids (estrone or cholestanol) through the corresponding epoxides.

3. Dilithium synthons

Starting from dichlorinated materials, their arene-catalyzed lithiation in the presence of carbonyl compounds (Barbier reaction conditions) affords diols, which are easily transformed into interesting heterocyclic units, including perhydrofurofurans, perhydrofurans, and perhydroazepanes. These cyclic polyether moieties are extensively represented in naturally occurring compounds. The introduction of two different electrophilic fragments

\[ \text{Scheme 1} \]

\[ \text{Scheme 2} \]


is possible starting from the corresponding chloroethers, chlorothioethers or chloro bromo derivatives, simply controlling the lithiation conditions (by using either the stoichiometric or the catalytic version of the arene-promoted lithiation) (Scheme 3).

Scheme 3

4. Activation of other metals: Nickel

The NiCl₂·2H₂O/Li/arene(cat.) combination is an effective mixture to carry out hydrogenation processes without using molecular hydrogen. Thus, olefins or alkynes can be either fully hydrogenated to the corresponding alkanes or in the second case partially reduced to the corresponding cis-alkenes. Halogen or sulfonyloxy derivatives give the corresponding alkanes, whereas arenes or heteroarones can be partially hydrogenated (a Birch-type reaction) using the mentioned combination. Also nitrogen-nitrogen (in hydrazones, azo and azoxy compounds, and azides) or nitrogen-oxygen bonds (in amine N-oxide, nitrones and Weinreb amides) can be cleaved using the same reduction mixture (Scheme 4). When deuterium oxide was included in the nickel salt instead of water, the corresponding deuteriated products are obtained. On the other hand, the use of the anhydrous salt in the same process but employing molecular hydrogen at normal pressure results a reasonable alternative to Raney-Ni. Nickel nanoparticles are involved in all the mentioned processes.

Scheme 4

Acknowledgements

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The level and type of Diversity is affected by the different Drug Discovery Phases, and also by the new concepts of “Front Loading Discovery” and “Parallel Multi-Factorial Optimization” that the Pharma industry has incorporated few years ago. Thus, the philosophy behind combinatorial library design has changed drastically since the early days of huge chemistry driven libraries, targeting maximal diversity for general screening. Nowadays, focused libraries, with much more Medicinal Chemistry input, trying to hit a single or a family of targets are the main choice. These are result of multi-objective designs, which take into consideration drug- or lead-likeness, in silico ADMET profiling, diversity, etc. Nevertheless, the design and production of general screening libraries is still a hot field, where Nature is source of inspiration. Thus, there is a revival of natural products derived libraries, or Diversity Oriented Synthetic collections, which incorporate diversity features of the natural product space. The use of proprietary under-represented scaffolds and advanced building blocks for corporate library enrichment exercises should be also taken into account. General concepts regarding “scaffold-derived diversity vs. full combinatorial exploitation of a few” should be kept in mind. A balanced strategy, which incorporates these approaches into different levels of risk, timing, in-out source and investment is desirable. One step further, the principal aim of a “Hit to Lead” campaign is to test the potential of a particular hit (or hit series) to reach the lead criteria in terms of activity-selectivity, physicochemical and ADMET properties. Additionally, a consistent SAR/SPR, and establishment of correlations, which allow in silico predictions at the level of “early” LO is of high value. We, HTMC and MI at J&J-PRD-Toledo, are currently working in a platform, which targets the Multi-Factorial Optimization of hit/leads via systematic explorations. It is based in the key use of “SAR/SPR” sets of reagents, to sample the Med-Chem Space (Activity vs. Property) in a diverse manner. The results are then capitalized in further iterative focused design and finally in the implementation of “Local Models”. The platform has been tested with several applications and the preliminary results are promising, so far. This strategy is complemented, of course, with key “singleton type” modifications to have more meaningful outputs. These pre-defined sets of reagents have been optimized chemically with manual HTC protocols, which are now subject of automation, J&J-PRD-Beerse, with the aim of being of general use among medicinal chemistry teams in their discovery programs.

Round Table: High Throughput Synthesis

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NEW FRONTIERS IN ORGANIC SYNTHESIS

Multi-Factorial-Diversity
HTC-SAR/SPR Sets and Key Singletons

“Med-Chem Space”

ADMET

Optimization

Parallel-Iterative, In Silico-Vitro-Vivo Correlation

* HTC-Protocols and Automation
Combinatorial synthesis of natural products is developing to explore the analogue of lead compounds for drug discovery and recently to extend the construction of important chemical probes in the fields of chemical genetics, genomics and proteomics. Therefore, it is necessary to develop more efficient strategies for the high-speed synthesis of a natural product like libraries than those traditionally used for the synthesis of a single final product.

Macrosphelides A has received much attention as a lead compound for the development of new anti-cancer drugs. Herein we wish to report a highly convergent synthesis of a library of macrosphelide analogues on a solid-support utilizing radiofrequency encoded combinatorial (REC) chemistry by a split-and-pool method. In our strategy (Scheme-1) for the combinatorial synthesis of macrosphelides analogues (128-membered library), we chose a solid-phase synthesis utilizing the three synthetic building blocks A(four), B(four), and C(eight) as illustrated in scheme 1. The process involves: 1) attachment of the secondary alcohol in block A to a polymer-support, 2) esterification with block B, 3) chemoselective carbonylation of the vinyl iodide in A with alcohol C containing a vinyl bromide moiety, 4) carbonylative macrolactonization of the polymer-supported A,B,C by exploiting the rather less reactive vinyl bromide, and 5) cleavage from the polymer-support.

One-pot glycosylation, involving sequential activation of glycosyl donors in a single vessel, is effective not only for the high-speed synthesis of a single target oligosaccharide, but also for the parallel synthesis of oligosaccharide libraries. We have investigated the branched- and linear-type one-pot glycosylation (Scheme-2) based on the chemoselective activation of glycosyl donors attached with different leaving groups with appropriate activators.

As an application, we first investigated one-pot four-step synthesis of Lewis X sphingolipid (Scheme-3) which is an important tumor-associated antigen, and is composed of a b(1,3) and b(1,4) linked tetrasaccharide backbone attached with a(1,3) linked branching saccharides.

Based on the above methodology, we achieved an automated parallel solution-phase synthesis of a protected dimeric Lewis X library by one-pot glycosylation (Scheme-4).
We have been working on the development of laboratory automation in order to improve the quality, efficiency and importance of experimental works in organic synthesis. Recently, we have achieved a total synthesis of Baccatin III, in which the key intermediates were synthesized by performing reactions in ChemKonzert. In our synthetic strategy (Scheme-5), the B-ring is constructed by intramolecular cyanohydrin alkylation of 6 utilizing Microwave synthesizer. The A-ring 4 and the C-ring 5 were prepared from geraniol (1) by Ti-mediated radical cyclization⁵ of epoxyalkenes 2 and 3, respectively. The total 32 steps from geraniol to cyanohydrin 6 carried out by using automated synthesizer.

References

Control of the stereochemistry in a chemical transformation is a key issue in modern organic chemistry, which has led to the pre-eminence of asymmetric synthesis. One option for controlling stereochemistry—and thus for producing one of the stereoisomers over the other possible ones in a predictable fashion—is the use of a stoichiometric chiral auxiliary which is covalently attached to the prochiral substrate before chirality relay is performed. The auxiliary is removed for reuse once the new stereogenic center is built with the desired configuration. A more advanced and atom economic option for controlling stereochemistry relies on the use of a chiral catalyst, typically a transition metal complex, which is reversibly bound to the prochiral substrate in the catalytic cycle.

The understanding of how factors affect the stereochemical outcome of a given transformation and thus the identification of the key stereocontrolling elements seems more suitable when using the former option. As a result, it is not casual that observations made in stereoselective processes relying on covalently bound chiral auxiliaries may result extremely useful for the subsequent design of parallel processes that rely on chiral catalysis. A paradigmatic case is the development by Evans of amino acid-derived N-acyl oxazolidinone auxiliaries, wherein the metal-carbonyl double coordination is a key stereocontrol element, and the subsequent widespread use of N-acyl oxazolidinones as achiral templates in catalytic enantioselective processes.

In recent years we have explored the concept of metal- and proton-assisted chelation of the a-hydroxy carbonyl moiety as tool for substrate activation and reaction stereocontrol. In this context, several chiral a-hydroxy ketones (enones) have been developed for some fundamental C-C and C-heteroatom forming reactions, which has been subsequent-ly followed by the development of a family of simple, aqiral a-hydroxy enones as templates in a range of catalytic enantios-elective transformations.

**Chiral Auxiliary-Based Aldol and Mannich Reactions**

Initial studies led to the design of $\alpha'$-hydroxy ketone $\mathbf{5}$, which upon enolization affords a highly ordered chelate ready for an efficient chirality transfer event. We suc-ceeded in the application of this design reagent to aldol and Mannich reactions. Significantly, the reagent is readily available from acetylene and (1R)+(+)-camphor, two commodity chemicals available in bulk, and detachment of the camphor unit from adducts is straightforward.

**Chiral Auxiliary-Based Metal-Free Diels-Alder Reaction**

In subsequent experiments it was found that Brønsted acids are capable of activating $\alpha'$-hydroxy enones, presumably through an intermolecular hydrogen bond network. This principle was illus-trated in the context of Diels-Alder reaction by using a new family of camphor-based chiral enones, which upon catalytic action of either trifluoroacetic acid or triflic acid lead to cycloadducts with high chemical and stereochemical efficiency.
Catalytic Enantioselective Diels-Alder Reaction

Concurrent with these investigations it was also found that achiral α'-hydroxy enones upon combination with chiral Lewis acids provide a new platform for carrying out highly enantioselective catalytic reactions. For example, α'-hydroxy enones react with dienes in the presence of (S,S)-[Cu(tBu-box)](OTf)2 or (S,S)-[Cu(tBu-box)](SbF6)2 (2 to 10 mol %) to afford the corresponding Diels-Alder adducts in high yield and selectivity. Isomeric ratios (regioselectivity, endo/exo or cis/trans) of up to >99:1 and ee values of up to >99% are obtained. Significantly, difficult dienes such as isoprene, 2,3-dimethyl butadiene and piperylene behave satisfactorily. Subsequent oxidative cleavage of the ketol in the resulting cycloadducts by treatment with cerium ammonium nitrate (CAN) yields the corresponding enantiopure carboxylic acids. Alternatively, carbonyl addition and subsequent diol cleavage with CAN produces the corresponding ketone adducts.

Catalytic Enantioselective Conjugate Addition of Carbamates

The catalytic, enantioselective conjugate addition of carbamates has remained an elusive goal. For instance, the carbamate conjugate addition to typical bidentate templates such as N-enoyl oxazolidinones and related systems under the presence of some representative metal catalysts is completely unsuccessful. Hypothetically, the 1,4-metal arrangement resulting from the coordination of the α'-hydroxy enone templates with the catalyst is advantageous over the 1,5-metal binding pattern often operating in the above bidentate templates. Gratifyingly, it was found that carbamates indeed add to these hydroxyenones quite efficiently.

Catalytic Enantioselective Friedel-Crafts Reactions

A further demonstration of the potential scope of α'-hydroxy enones is shown in the Friedel-Crafts alkylation of pyroles and indoles where remarkably high and regular enantioselectivities are obtained.

The simple elaboration of adducts provides a route to enantioenriched aldehydes, carboxylic acids and ketones containing the pyrrole and indole frameworks. Moreover, in these transformations acetone is the only byproduct formed, an additional aspect of the approach that is of practical interest.

It is likely that this 1,4-metal-binding principle can provide similar levels of substrate activation and reaction stereoselectivity under a proper choice of metal catalyst in a yet uncovered wide type of transformations. Work in that direction is active in our laboratory.

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High-speed microwave synthesis has attracted a considerable amount of attention in recent years. Since the first reports on the use of microwave heating to accelerate organic chemical transformations by the groups of Gedye and Giguere/Majetic in 1986, more than 2000 articles have been published in the area of microwave-assisted organic synthesis (MAOS). The initial slow uptake of the technology in the late 1980s and early 1990s has been attributed to its lack of controllability and reproducibility, coupled with a general lack of understanding of the basics of microwave dielectric heating. The risks associated with the flammability of organic solvents in a microwave field and the lack of available systems for adequate temperature and pressure controls were major concerns. Although most of the early pioneering experiments in MAOS were performed in domestic, sometimes modified, kitchen microwave ovens, the current trend clearly is to use dedicated instruments for chemical synthesis which have become available only in the last few years. Since the late 1990s the number of publications related to MAOS has therefore increased dramatically to a point where it might be assumed that, in a few years, most chemists will probably use microwave energy to heat chemical reactions on a laboratory scale. Not only is direct microwave heating able to reduce chemical reaction times from hours to minutes, but it is also known to reduce side reactions, increase yields and improve reproducibility. Therefore, many academic and industrial research groups are already using MAOS as a forefront technology for rapid reaction optimization, for the efficient synthesis of new chemical entities, or for discovering and probing new chemical reactivity. A large number of review articles and several books provide extensive coverage of the subject.

Traditionally, organic synthesis is being carried out by conductive heating with an external heat source (i.e. an oil-bath). This is a comparatively slow and inefficient method for transferring energy into the system since it depends on the thermal conductivity of the various materials that must be penetrated, and results in the temperature of the reaction vessel being higher than that of the reaction mixture. In contrast, microwave irradiation produces efficient internal heating (in core volumetric heating) by direct coupling of microwave energy with the molecules (e.g. solvents, reagents, catalysts) that are present in the reaction mixture. Since the reaction vessels employed are typically made out of (nearly) microwave transparent materials such as borosilicate glass, quartz or Teflon, an inverted temperature gradient as compared to conventional thermal heating results (Figure 1). The very efficient internal heat transfer results in minimized wall effects (no hot vessel surface) which may lead to the observation of so-called specific microwave effects e.g. in the context of diminished catalyst deactivation.

Although many of the early pioneering experiments in microwave-assisted organic synthesis have been carried out in domestic microwave ovens, the current trend undoubtedly is to use dedicated instruments for chemical synthesis. All of today’s commercially available dedicated microwave reactors for synthesis feature built-in magnetic stirrers, direct temperature control of the reaction mixture with the aid of fiber-optic probes or IR sensors, and software that enables on-line temperature/pressure control by regulation of microwave power output (Figure 2). Currently two different philosophies with respect to microwave reactor design are emerging: multimode and monomode (also referred to as single mode) reactors. In the so-called multimode instruments (conceptually similar to a domestic oven), the microwaves that enter the cavity are being reflected by the walls and the load over the typically large cavity. In most instruments a mode stirrer ensures that the field distribution is as homogeneous as possible. In the much smaller monomode cavities, only
one mode is present and the electromagnetic irradiation is directed through an accurately designed rectangular or circular wave guide onto the reaction vessel mounted in a fixed distance from the radiation source, creating a standing wave. The key difference between the two types of reactor systems is that whereas in multimode cavities several reaction vessels can be irradiated simultaneously in multi-vessel rotors (parallel synthesis), in monomode systems only one vessel can be irradiated at the time. In the latter case high throughput can be achieved by integrated robotics that move individual reaction vessels in and out of the microwave cavity. Importantly, single-mode reactors processing comparatively small volumes also have a built-in cooling feature that allows for rapid cooling of the reaction mixture by compressed air after completion of the irradiation period (see Figure 2). The dedicated single-mode instruments available today can process volumes ranging from 0.2 to ca 50 mL under sealed vessel conditions (250 °C, ca 20 bar), and somewhat higher volumes (ca 150 mL) under open vessel reflux conditions. In the much larger multi-mode instruments several liters can be processed under both open and closed vessel conditions. For both single- and multimode cavities continuous flow reactors are nowadays available that already allow the preparation of kilograms of materials using microwave technology.

![Figure 2](image)

**Figure 2.** Temperature ($T$), pressure ($p$), and power ($P$) profile for a 3 mL sample of methanol heated under sealed vessel microwave irradiation conditions.

This lecture will highlight recent applications of controlled microwave heating technology from our laboratory, involving transition metal-mediated reactions, heterocycle synthesis, the use of solid-phase synthesis and of polymer-supported reagents. A variety of processing techniques as well as scale-up examples will be discussed.

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Self-Assembly, a Bio-Inspired Approach to Chemical Complexity

The chemical properties and behaviour of supramolecular entities are governed exclusively by the information stored in their molecular architectures. When two or more identical subunits possess geometrical and functional complementarity they may self-assemble to form a supermolecule held together by non-covalent contacts, such as hydrogen bonds or hydrophobic, electrostatic and van der Waals interactions. The most interesting examples of self-complementary dimers in solution occur when the molecular subunits have hemispherical or curved structures because the resulting assembly possesses a defined cavity which may encapsulate suitable guest molecules.

Cavitand and calixarene dimers

Since the advent in 1993 of the so-called tennis ball, a molecular capsule held by complementary hydrogen bonds, developments in the field have been impelled towards larger and more stable assemblies, like soft-balls and related cavities. Cavitands containing urea moieties reversibly self-assemble into cylindrical capsules allowing pair-wise complexation of suitable partners, such as a longer and a shorter carboxylic acid dimer, in an arrangement reminiscent of a “Russian doll” (dimers-inside-dimers) (Figure 1). On the other hand, dimeric capsules on this kind endowed with long hydrocarbon chains at the lower rim tend to self-organize into almost giant reverse vesicles in organic solvents. The vesicles are able to encapsulate dyes.

![Figure 1: Pair-wise encapsulation of carboxylic acid dimers in a cavitand dimeric capsule.](image)

Cone-shaped calixarenes endowed with urea functions at the wider rim have been extensively studied as self-assembling subunits, since they are semi-rigid and substantially preorganized. Extension of this dimerization process to the wider though more flexible calix[6]arenes resulted in rather stable triureidocalix[6]arene dimers (Figure 2a). Also, the array of urea-urea hydrogen bonds in tetraureidocalix[4]arenes can be further stabilized by an outer shell of additional hydrogen bonds by attachment of peptide fragments to the ureas (Figure 2b).

![Figure 2: Hydrogen-bonded urea dimers of calixarenes:](image)
aspartate or glutamate residues, or even protein surfaces. Recently, these oligomers have been employed as efficient non-peptidic cell penetrating agents, with selectivity for mitochondria. An example of a carboxylate (A) - guanidinium (B) ABBA polymeric self-assembly will be provided.

**Ureido-pyrimidinone scaffolds.**

Since hydrogen bonds are weak, for robust and extended assemblies a complex network of donors (D) and acceptors (A) is necessary, such as in the four-fold (DDAA-AADD) dimers of 2-ureido-4[1H]-pyrimidinones (UPy). UPy’s can be used to strongly link two calix[4]arenes (in 1,3-alt conformation) through a network of eight hydrogen bonds. The two dimeric UPy platforms, held together by the calixarenes, display syn-anti isomerism (Figure 3a). Attachment of two 2-ureido-4[1H]-pyrimidinones to a central spacer results in a cyclic array that could formally be described as a rosette (Figure 3b). However, depending on the nature and size of the R substituents the dimeric UPy surfaces can rotate about the spacer-urea bond so as the resulting cyclic oligomers are non-planar or tubular. The number of subunits in the main aggregate is strongly dependent on the angle between the two UPy subunits attached to the central spacer. Thus, for m-disubstituted benzenes (120°) the rosette is hexameric whereas for 1,3-disubstituted adamantane (109.5°) the self-assembly is a pentamer.

![Figure 3. Self-assembly based on ureido-pyrimidinone quadruple hydrogen-bonds: (a) 1,3-alt-calixarene dimers, (b) Rosettes and tubes](image)

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