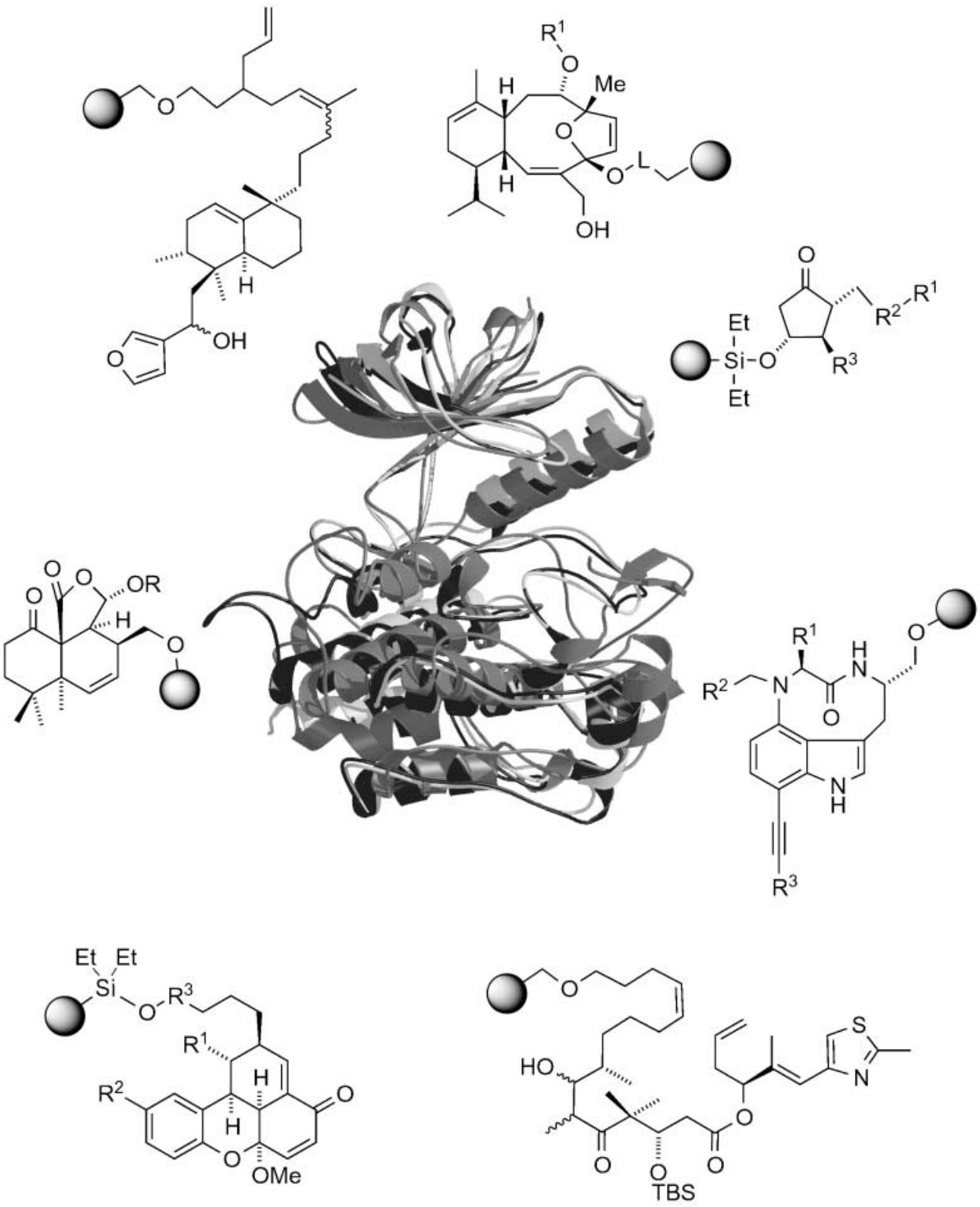
From Protein Domains to Drug Candidates



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From Protein Domains to Drug Candidates—Natural Products as Guiding Principles in the Design and Synthesis of Compound Libraries

Rolf Breinbauer, Ingrid R. Vetter, and Herbert Waldmann*

In the continuing effort to find small molecules that alter protein function and ultimately might lead to new drugs, combinatorial chemistry has emerged as a very powerful tool. Contrary to original expectations that large libraries would result in the discovery of many hit and lead structures, it has been recognized that the biological relevance, design, and diversity of the library are more important. As the universe of conceivable compounds is almost infinite, the question arises: where is a biologically validated starting point from which to build a combinatorial library? Nature itself might provide an answer: natural products have been evolved to bind to proteins. Recent results in structural biology and bioinformatics indicate that the number of distinct protein families and folds is fairly limited. Often the same structural domain is used by many proteins in a more or less modified form created by divergent evolution. Recent progress in solid-phase organic synthesis has enabled the synthesis of combinatorial libraries based on the structure of complex natural products. It can be envisioned that natural-product-based combinatorial synthesis may permit hit or lead compounds to be found with enhanced probability and quality.

Keywords: bioorganic chemistry · combinatorial chemistry · natural products · protein structures · solid-phase synthesis

1. Introduction

The completion of the Human-Genome Project has outlined the map for further expeditions towards providing a complete understanding of cellular processes at the molecular level. In particular, the nature, function, and amount of the proteins that are actually expressed in cells under particular conditions (proteomics), their interplay, and their interactions with supramolecular structures such as membranes and the cytoskeleton are of paramount interest. Recently, efforts have been initiated to determine the structures of all protein folds coded/expressed by the human genome by X-ray crystallography and NMR spectroscopy (structural genomics).[1] The structural determination is crucial for an understanding of the function of the proteins and will certainly help to achieve the ultimate goal: altering protein function by small molecules, which either selectively inhibit or activate a particular protein.[2] Combinatorial chemistry will become the method of choice to undertake this herculean task. Herein we propose and discuss the argument that in the almost indefinite space of conceivable chemical compounds,[3] natural products are viable, biologically validated starting points for library design. In this context, natural products are defined as all low-molecular-weight chemical compounds that are synthesized by biological organisms. Such natural-product-based libraries should permit hit or lead compounds to be found with enhanced probability and quality if these libraries are included in high-throughput screening.[4, 5]

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1.1. Disillusionment in Combinatorial Design

The hype of combinatorial chemistry as a tool in the pharmaceutical industry at the beginning of the 1990s has turned into a disappointment at the turn of the millennium. The original expectation that the synthesis of libraries that contain millions of compounds would produce as many or even more drug candidates as historical libraries of pharmaceutical companies and that it would thereby overcome the problem of efficient hit and lead finding has not been fulfilled.

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Even worse, some of the libraries contained hardly any hit structures. Clearly the underlying structure of the individual library members was not biologically relevant. It was soon recognized that the number game would not determine the quality of a library; the quality would be determined by its "diversity"[4] and its "drug-likeness"[6, 7]—qualitative parameters that are now being considered in current library design.

From this disillusioning experience, an intense and urgent demand was born to find efficient and new guiding principles for a significant improvement in library quality and the quest for library types that are biologically relevant and yield high hit and lead rates. In developing new concepts and logic for meeting this demand, the central and crucial tasks are to identify compound classes that represent already biologically validated starting points in structural space, to find a synthetic access to them that is amenable to combinatorial variation, and to design and synthesize combinatorial libraries centered on the identified underlying structural framework of these compound classes. In this Review we delineate arguments for a structure-based approach to this fundamental problem; the key feature of this approach is the use of the structural frameworks of biologically active natural products that have undergone evolutionary selection for binding to specific protein domains.

2. Protein Folding and Protein Function

Proteins can be regarded as modularly built biomolecules assembled from individual building blocks. These building


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Herbert Waldmann, born in 1957, received his Dr. rer. nat. in 1985 under the supervision of Prof. H. Kunz at the University of Mainz (Germany). After postdoctoral studies at Harvard University with Prof. G. Whitesides (1985–1986) and habilitation (1991, University of Mainz) he accepted a professorship at the University of Bonn (Germany) in 1991. In 1993 he moved to the University of Karlsruhe (Germany) as a Full Professor of Organic Chemistry. In 1999 he was appointed as Director at the Max-Planck-Institut für molekulare Physiologie, Dortmund (Department of Chemical Biology) and as Full Professor of Biochemistry at the University of Dortmund (Germany). Herbert Waldmann has been the recipient of the Friedrich Weygand Award for the advancement of peptide chemistry, of the Carl Duisberg Award of the Gesellschaft Deutscher Chemiker, the Otto Bayer Award, and the Steinhofer Award of the Steinhofer Foundation. His current research interests include bioorganic chemistry and natural-product synthesis as well as biocatalysis, stereoselective synthesis, and combinatorial chemistry. A major focus of the research activities is on the combination of organic chemistry, biophysics, and biology for the synthesis and biological evaluation of peptide and protein conjugates that are involved in biological signal-transduction processes. More recently, syntheses of natural products and natural-product-derived compound libraries on polymeric supports have been investigated (further information: http:\\www.mpi-dortmund.mpg.de).

Ingrid Vetter, born in 1963, studied biology in Frankfurt, Main (Germany). After completing her degree with Prof. H. Fasold/Prof. H. Rüterjans in 1987, she moved to Heidelberg (Germany) to join the group of Prof. P. Rösch. Her thesis describes NMR spectroscopic studies of adenylate kinase. In 1991, she started postdoctoral work in the field of crystallography in the group of Prof. D. Tsernoglou (EMBL, Heidelberg, Germany). After a 20-month postdoctoral stay in the group of Prof. B. Matthews (University of Oregon, Eugene, USA) she joined the Max-Planck-Institut für molekulare Physiologie, Dortmund (Germany) as a group leader in the department of Prof. A. Wittinghofer, where she is currently working. Her current research interests include the structural investigation of nuclear transport proteins and molecular modeling.

Rolf Breinbauer was born in Schärding (Austria) in 1970. He studied chemistry at the Technical University of Vienna (Austria) and the University of Heidelberg (Germany), where he completed his degree under the supervision of Prof. G. Helmchen in the field of asymmetric catalysis. During his doctoral studies (1995–1998) with Prof. M. T. Reetz at the Max-Planck-Institut für Kohlenforschung (coal research) in Mülheim an der Ruhr (Germany) his research interests focused on the synthesis and catalytic applications of transition-metal colloids. From 1998–1999 he worked as a Erwin Schrödinger fellow in the laboratory of Prof. E. N. Jacobsen at Harvard University (USA) where he studied cooperative effects in asymmetric catalysis. In 2000 he moved as a Liebig fellow to Dortmund where he leads a group in the Department of Chemical Biology at the Max-Planck-Institut für molekulare Physiologie and the Institute of Organic Chemistry at the University of Dortmund (Germany). His research interests include the development of new synthetic methods and its applications in solid-phase synthesis and combinatorial chemistry.

blocks are called "domains", parts of the proteins that fold independently from the rest of the structure to a compact arrangement of secondary structures (such as α-helices, β-sheets, or β-turns) and that are connected through more or less complex linker peptides. The term "domain family" in this Review refers to a family of related sequences that have a common ancestor and that have developed through divergent evolution. Different sequence families (i.e. domains) can have the same fold (Figure 1), which could be regarded either as convergence caused by functional and physical constraints or as a result of divergent evolution.[8] Domains are structurally conserved units, but can be genetically mobile.[9]

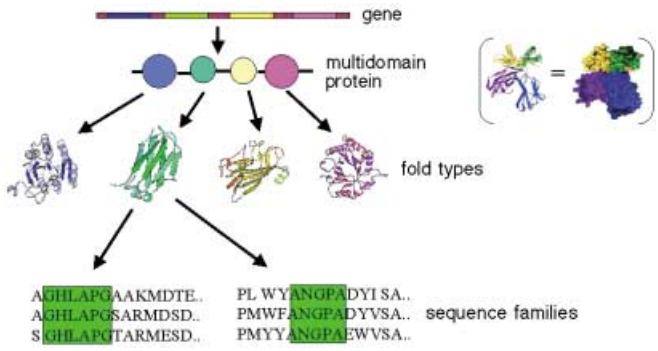


Figure 1. Modular build-up of multidomain proteins.

Although the estimate for the number of different proteins in humans range between 100000 and 450000, there is a common agreement that the number of domain families and—above all—of topologically distinct folds will be much smaller. At present approximately 600 fold types are known,[10] derived by classifying all structurally characterized proteins according to their three-dimensional structure.[11, 12] Data from the ongoing genome sequencing projects allow the number of existing folds and families in Nature to be estimated. Current estimates vary between 600 and 8000 distinct folds, and 4000 – 60000 sequence families.[12c, 13, 14]

There is an ongoing effort to understand the correlation between protein function and protein sequence.[9a, 15] Especially the increasing amount of data from genome-sequencing projects, evaluated with the tools of bioinformatics, allow a faster recognition of patterns than the more elaborate method of structural genomics (which tries to deduce the function from structure) would be able to do by itself.[9] Although we are still far from a deep and consistent understanding, and analysis is hampered by the small number of available X-ray structures of proteins with bound small-molecule binders,[16] some interesting observations have been made, and the following remarks should serve to give an overview about the diversity and evolutionary relationships of ligand-binding sites in proteins.[17]

2.1. Similar Folds Bind Similar Ligands—A Concept and Its Limitations

In many examples it has been confirmed that protein families can have similar folds even though they at first seem to have completely different sequences and/or catalyze quite different chemical reactions with a different arrangement of active-site residues. However, these protein families did evolve from the same ancestors and can still bind similar ligands.[18] Since the sequence homology is sometimes weak or not recognizable, the detection of these cases is not necessarily straightforward.

A striking example is leukotriene A4 hydrolase/aminopeptidase (LTA4H), a bifunctional enzyme whose aminopeptidase functionality is combined with an additional function, namely the vinylogous hydrolysis of the leukotriene epoxide LTA4 into LTB4. Both reactions are catalyzed in the same Zn-containing active site.[19a] In this case, the presence of some short conserved sequence elements was sufficient to prompt investigations of the relationship of this enzyme to metallopeptidases.[19e] The evolutionary relationship of the LTA4H fold (which was unknown at the time) to M1 metallopeptidases would have immediately suggested that peptidase inhibitors be investigated as potential ligands. Indeed, the aminopeptidase inhibitor bestatin also inhibits LTB4 biosynthesis.[19e] This result and the related observation that captopril, an inhibitor of the angiotensin-converting enzyme (which is also a metalloprotease), also inhibits LTA4H,[19e] have inspired the variation of these lead structures, which led to the syntheses of molecules that inhibit LTA4H in the nanomolar range.[19b–d] Subsequent determination of the structure of LTA4H and comparison with thermolysin, confirmed that both enzymes exhibit the same fold (Figure 2, Scheme 1). In fact, the fold structure of the catalytic domain of LTA4H is also adopted by the peptidases aureolysin, neprilysin, and elastase.

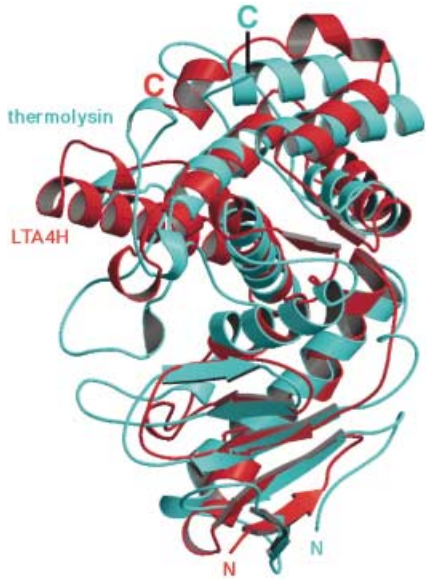
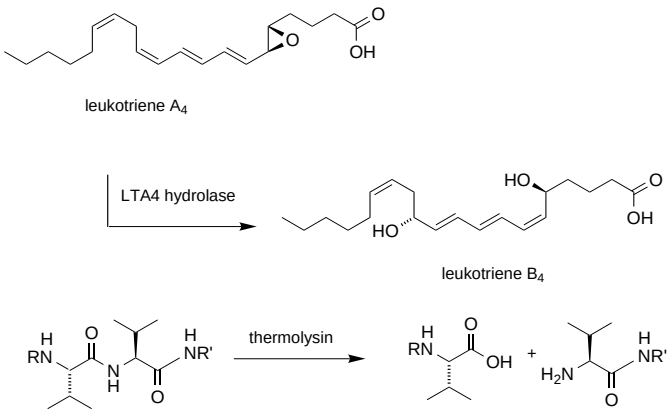


Figure 2. Overlay of the X-ray structure of LTA4H (red) and thermolysin (blue).

Empirical observations show that related sequences have similar folds. This does not mean, however, that the active sites have to be built up by sequentially homologous residues: a thorough comparative X-ray analysis of enzymes of the enolase superfamily (enolase, muconate lactonizing enzyme,



Scheme 1. LTA4 hydrolase and thermolysin exhibit the same fold and are both inhibited by bestatin, but catalyze two different reactions.

and mandelate racemase) by Petsko and co-workers led to the discovery of common features in the design of the active site. They found that the abstraction of the α proton of a carboxylic acid plays a crucial role in the overall reaction sequence.[20] Although the residues that contain the catalytic atoms are located at different points in the protein fold and their identity and roles in catalysis vary, the position of catalytic groups relative to the substrate remains constant. Indeed, the three enzymes share some significant sequence homologies, have a similar fold, and are thus assumed to belong to the same protein family. Although they would not have been classified as belonging to the same functional target family because they catalyze different reactions, their common evolutionary relationship revealed by the similar structures would have allowed a rational lead search for ligands that would bind to the active sites with their common features.

This approach based on sequence analysis promises that in the long run the process from gene identification to lead discovery may be shortened and accelerated significantly.[21] For instance, the analysis of a newly discovered gene by bioinformatic tools may suggest that the corresponding protein will be a multidomain protein composed of already known domains.[22] If ligands for these domains are already known, then these can serve as starting points for the design and synthesis of libraries that target the protein product of the desired gene (Figure 3). This inhibitor development could be initiated without further information on the three-dimensional structure of the protein product, which can usually be obtained by X-ray crystallography or NMR spectroscopy, and even without further knowledge about its biological functions, binding partners, etc., which are usually obtained by biochemical and cell biological techniques.

A case in which the "similar folds bind similar ligands" approach will fail is when divergent evolution has proceeded too far, so that the similarities in the binding site are completely obscured.[17a] However, even in those cases some similarities might remain, for example, the position of the binding site in a fold. This has led to the introduction of the term "supersites", for example, in the ferredoxin-like fold.[23] A potential supersite can also be observed in the cystatin-like fold (Figure 4). These similarities are thought to be the result of divergent evolution.[24] Even if the diversity in the binding

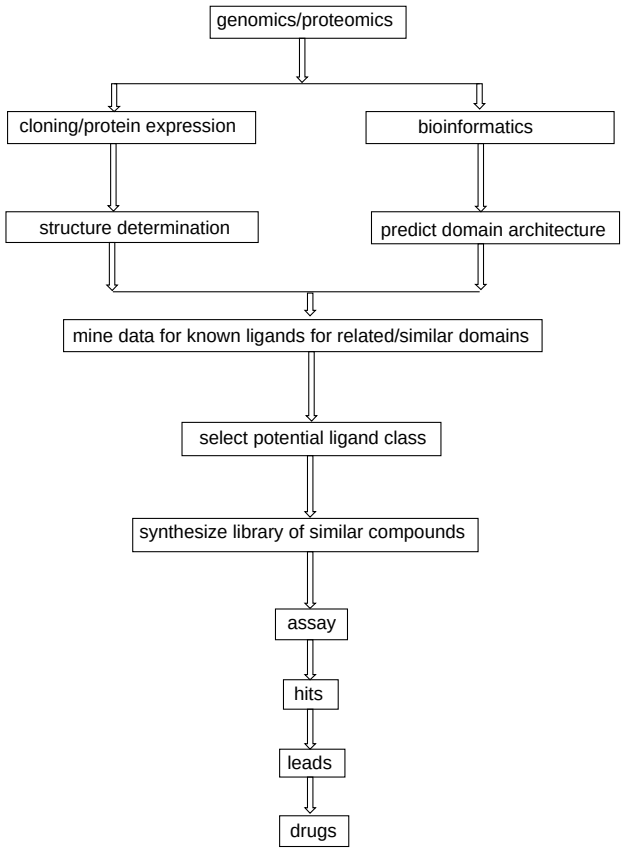


Figure 3. Influence of sequence analysis in the drug-finding process.

site cannot be addressed by a combinatorial library built around a single scaffold, the evolutionary relationship, if known, could provide hints about which other leads should be tested.

In summary, the advantage of the domain family classification over the conventional, functional classification of target families (kinases, proteases, phosphatases, etc.) is that the former can be solely derived from sequence information. This can be very helpful in the initial stages of screening when little is known about the protein. In this case, all existing ligands that bind to sequentially related proteins could be tried; in the example described above, the peptidase inhibitor bestatin could be tried against the leukotriene A4 hydrolase.

2.2. Different Folds Bind the Same Ligand—Privileged Structures

The term "privileged structure" was originally coined by the group of B. E. Evans at Merck who recognized in their pharmacological studies of benzodiazepines,[25] that derivatives within this compound class bind not only to benzodiazepine receptors of the central nervous system, but also to cholecystokinin receptors, although the natural substrates showed little resemblance to this compound class. A third class of unrelated receptors that bind benzodiazepines are the peripheral benzodiazepine receptors. As peptidomimetics, benzodiazepines can be assumed to have an intrinsic good

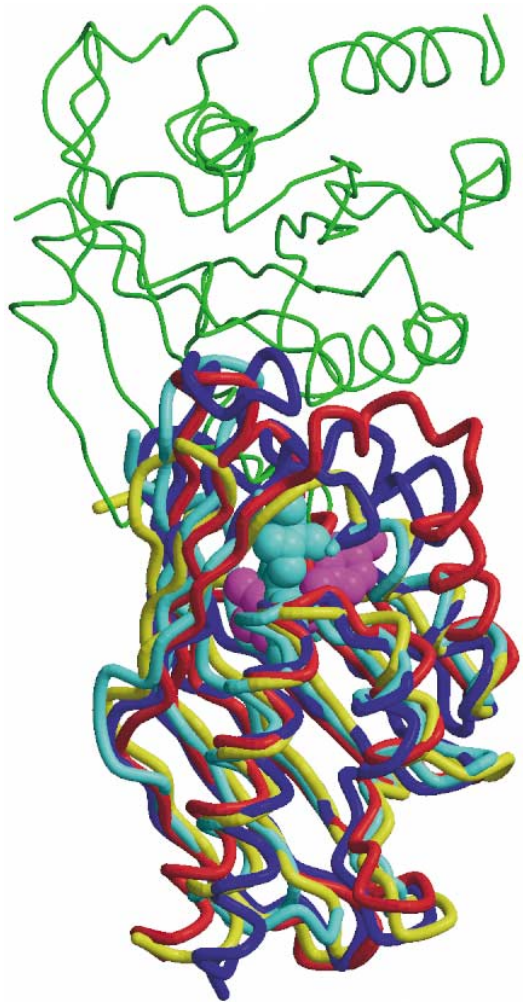


Figure 4. Proteins that embody the cystatin fold. Turquoise: Δ^5-3-ketosteroid isomerase complexed with the reaction intermediate equilenin (PDB code 4TSU); blue: scytalone dehydratase complexed with the inhibitor carpropamid (pink; PDB code 2STD); red: naphthalene 1,2-dioxygenase β-subunit (no bound ligand; PDB code 1EG9); yellow: NTF2 complexed with Ran (green; PDB code 1A2K).

binding affinity to several proteins that bind similar regions of peptides or other proteins. According to the definition of Evans, privileged structures are a compound class that can bind to various protein-receptor surfaces.

3. Natural Compounds and Privileged Structures

Natural products very often embody privileged structures according to the definition described in Section 2.2, since they are synthesized or modified by different proteins (and thus have to bind to them), and their function might involve binding to other proteins as well. Examples include nucleotide triphosphates (NTPs), which bind (among many other proteins) to P-loop-containing NTP hydrolases (the fourth most frequent domain in the current human genome database), to protein kinases (fifth most common), to the actin-like domain, and to many others. NTPs are thus bound by a plethora of different proteins that have either similar or different folds (Figures 5 and 6).[26] In the cases of different folds (e.g. the P-loop-containing NTP hydrolases and the protein kinases), the similar shape and properties of the binding pockets for the nucleotide in the different scaffolds are

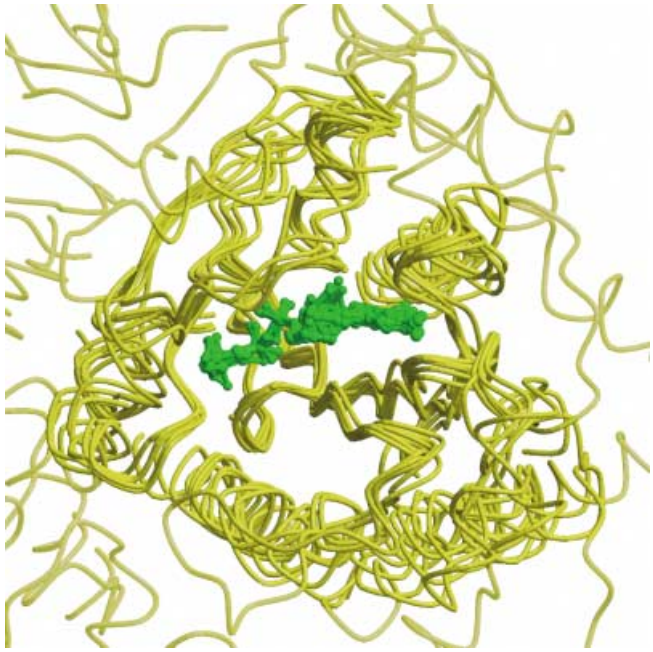


Figure 5. Overlay of the GTP-binding sites of some known X-ray structures of GTP-binding proteins, including small GTP-binding proteins, heterotrimeric G proteins, and the elongation factor Tu. The nucleotides are shown in green, the protein backbones in yellow.

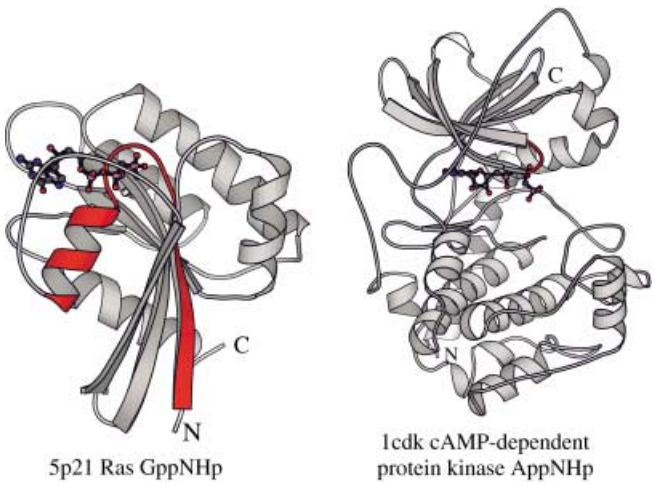


Figure 6. NTP-binding sites in two different folds: Ras (left) and protein kinase C (right).

most likely the result of convergent evolution (for instance the protein kinases and P-loop-containing NTPases both have a glycine-rich motif to bind the phosphates of the nucleotide, but have a different arrangement of glycine residues).[27]

It is thus likely that more exploitable privileged structures such as the peptidomimetic benzodiazepine might be found among the evolutionarily selected ligands that bind to

particular binding pockets or folds that have also been selected by Nature in its effort to use modular domains to render evolution more efficient.

When looking for privileged structures among natural substances the question arises as to why natural products from different organisms have (often unexpected) effects in humans and why the hit rates in high-throughput screens determined for natural-product collections are often dramatically higher than the rates found for large classical libraries obtained from combinatorial chemistry.[28] Two cases can be distinguished: natural products might bind by chance to particular human proteins or they were selected by evolution to bind to a protein similar to a human protein. The latter case is quite likely given the close relationship of the genomes of all organisms. Furthermore, one can assume that at least most of the substances synthesized have a purpose for the organism that produces it. Many plants synthesize alkaloids to protect themselves from being eaten by animals, especially mammals. Therefore, the alkaloids have evolved to bind to mammalian proteins and to be poisonous to the corresponding organisms. Such alkaloids include drugs such as morphine, nicotine, ephedrine, strychnine, quinine, and atropine. Other examples are the venom of snakes, frogs, or spiders which are designed to poison or to paralyze mammals. Among other targets, these poisons are active against ion channels in mammalian nerve-cell membranes.

Drugs such as cyclosporin, FK506, and rapamycin are probably synthesized for the chemical defense of the organisms that produce them.[29] There are proteins that are homologous to the human proteins involved in immunosuppression, for example, in yeast. Therefore, it is not accidental that those substances are active against human proteins, although with a quite different functions in the end.

A given scaffold can thus be privileged in a chemical sense, that is, a scaffold with the necessary compromise of flexibility and rigidity combined with the ability to present functional groups in a favorable arrangement (e.g. biphenyl compounds). Second, and maybe additionally, it may be privileged in a biological sense in that it mimics natural compounds, which very often bind to different protein domains. An example is the protein-kinase inhibitor genistein, which is a competitive inhibitor of ATP binding by protein tyrosine kinases (PTK), but also inhibits topoisomerase II and 1-phosphatidylinositol 4-kinase and interacts with the estrogen receptor.[30] A privileged core structure is a basis for finding good protein binders, but an optimization along this core scaffold will be necessary to improve both the binding affinity and selectivity. Not necessarily, but surprisingly often, certain natural substances exhibit already advantageous pharmacokinetic properties as well. This appears to be logical because in a biological environment (e.g. after production by a particular organism to defend itself) they must find their way to the final target protein inside other organisms. The importance of natural products in drug discovery and development is reflected by the fact that close to half of the best-selling pharmaceuticals are either natural products or derivatives thereof.[31–33] Their role is even more prominent in antibacterial, immunosupressant, and anticancer drugs.

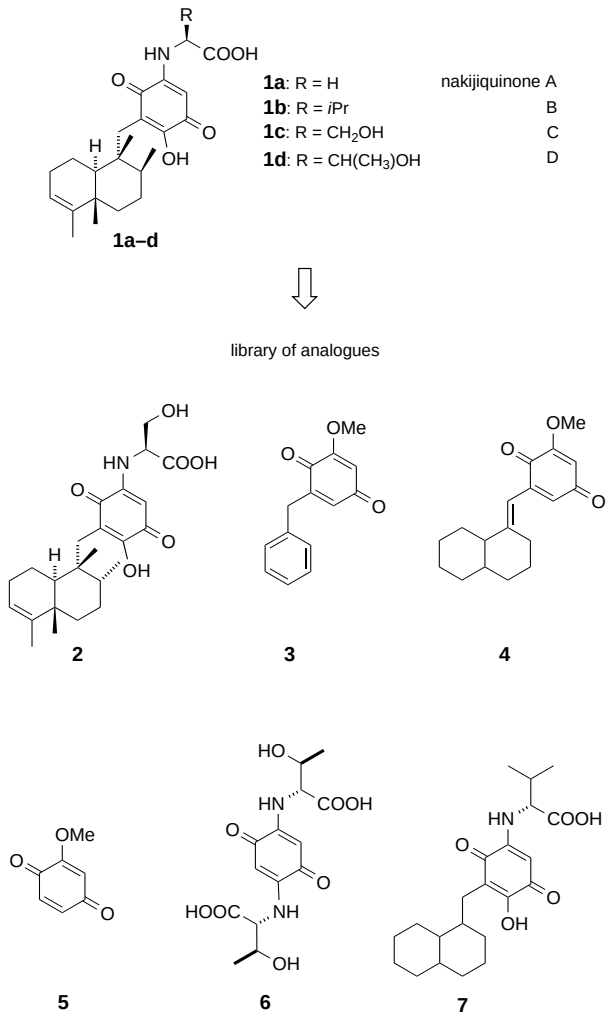
4. Biologically Validated Starting Points for Library Design

If ligand types or frameworks for certain domain families are already known from the investigation of evolutionarily related proteins, then the underlying structure of this ligand may be employed as the guiding principle for library development. Such ligands would provide targeted, biologically validated starting points in structural space for focused library development. As detailed above, natural products are such biologically validated structural entities.

Libraries designed and synthesized around the basic structure of natural products should yield modulators of protein activity with high hit rates with significantly reduced library size. Within a group of closely related protein domains, the details of the structure usually vary substantially, which provides the basis for selective binding and inhibition (e.g. in the ATP-binding sites of protein kinases). Therefore, the stereochemistry and substitution pattern of the chosen underlying framework should be varied. It is not necessary and probably even counterproductive to build up a given natural structure slavishly in every structural detail.

The structure-based concept delineated above differs in its fundamental reasoning from related approaches that focus on the creation of chemical diversity. It does not neglect this issue, however, and builds on the diversity created by Nature itself. This approach, therefore, offers the opportunity to identify new privileged structures from Nature, which may prove broadly usable for various applications in chemical biology and biomedicine.

The validity of this approach was demonstrated by Waldmann and Giannis and their co-workers.[34] Nakijiquinones **1a–d** (Scheme 2) are the only known naturally occurring inhibitors of the Her-2/Neu receptor tyrosine kinase, which is overexpressed in about 30% of primary breast, ovary, and gastric carcinomas. In light of the concept described above a library of 56 analogues of this lead structure was synthesized and screened for its inhibitory activity against other receptor tyrosine kinases involved in cell signaling and proliferation, such as the vascular endothelial growth-factor receptors (VEGFR1-3), the Tie-2-receptor, the insulin-like growth-factor 1 receptor (IGF1R), and the epidermal growth-factor receptor (ErbB-1). Whereas none of the natural products **1a–d** exhibited significant inhibitory activity against the new set of receptor kinases, six members of the library of analogues were identified as kinase inhibitors in the low micromolar range (Scheme 2, Figure 7, and Table 1). In particular, a structural pattern emerged that may allow the Tie-2 receptor kinase to be targeted selectively, which is of paramount importance in the regulation of blood-vessel formation and therefore in cancer development. This result stresses the importance of combinatorial libraries based on natural products, in contrast to using only the natural substances by themselves. If only natural substances had been screened, this inhibitor would have been missed.



Scheme 2. Compounds **2**–**7** of a 56-membered library of nakijiquinone analogues.

5. Solid-Phase Synthesis of Natural-Product Libraries

Paramount to the success of this library-design approach is that efficient and reliable methods and multistep sequences for the total synthesis of natural products and their analogues on polymeric supports are available. The corresponding transformations must proceed with a degree of selectivity and robustness typical of related classical solution-phase transformations, irrespective of the stringencies and differing demands imposed by the anchoring to the polymeric support.

The generation of libraries of natural-product analogues has received increasing attention during the last five years. As this subject has been reviewed just recently,[35] we specifically want to focus on solid-phase syntheses that involve the construction of entire molecular skeletons as opposed to subsequent derivatization of preformed and then immobilized natural-product cores. We present this field with a few selected examples, which represent the state of the art of this discipline and also mark the current frontiers.

Epothilones are natural products isolated from myxobacteria, which have been found to exhibit cytotoxic activity against paclitaxel-resistant tumor cell lines by inducing tubulin polymerization. The successful synthesis of epothilone A by Nicolaou and co-workers represents a landmark in the synthesis of complex molecules on solid supports.[36] A polymer-bound Wittig ylide **8** was further elaborated by olefination with **9**, aldol reaction with **10**, and esterification with **11** (Scheme 3). Following a very clever cyclo-release strategy, the acyclic precursor formed the macrocyle **12** through an olefin metathesis reaction mediated by the Grubbs catalyst. After two subsequent reactions in solution, epothilone A (**13**) was isolated. This example demonstrated for the first time the feasibility of multistep natural-product synthesis on solid supports. In a subsequent paper, Nicolaou et al. prepared a library of further analogues, which helped to establish structure–activity relationships of this compound class.[36b]

Prostaglandins play a prominent role in a wide variety of physiological processes and exhibit a very subtle structure–

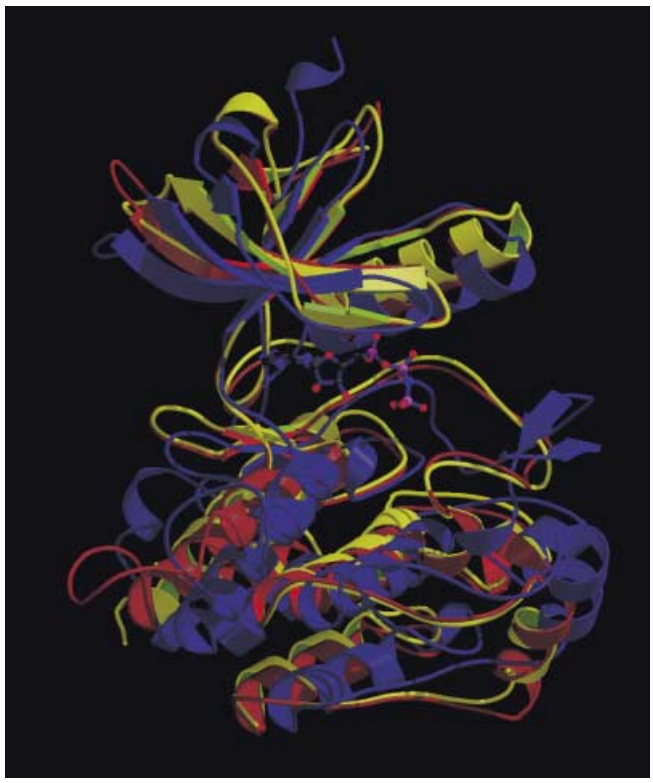
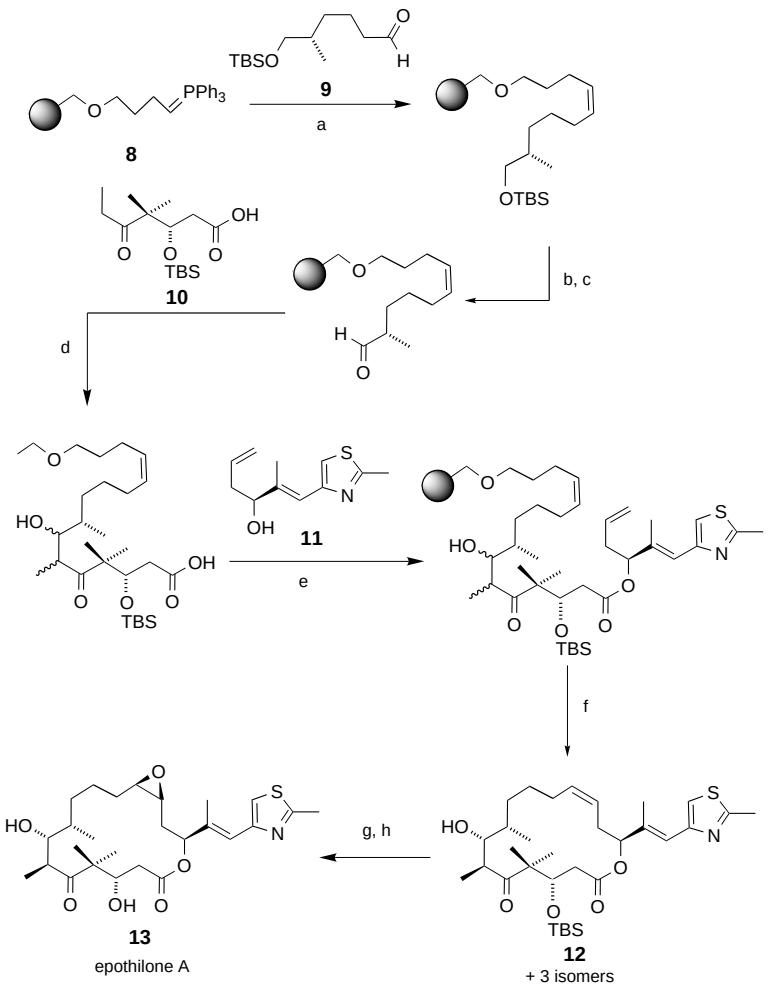


Figure 7. Overlay of receptor tyrosine kinases inhibited by the nakijiquinone-based library. Yellow: Tie-2 receptor kinase, red: VEGFR2, blue: IGF1R with a bound ATP analogue. (We thank Lars Kissau for preparing this figure.)

Table 1. Inhibition of different receptor tyrosine kinases by nakijiquinone analogues.

	IC_{50} [μM] for the receptors					
Compound	EGFR	ErbB-2	IGF1R	VEGFR-2	VEGFR-3	Tie-2
1a–d	–	–	–	–	–	–
2	–	–	–	21	–	–
3	–	–	–	–	–	18
4	–	–	–	–	–	14
5	–	29	–	8	–	–
6	–	–	6	–	3	5
7	–	–	0.5	–	–	9



Scheme 3. Total synthesis of epothilone A on a solid phase by Nicolaou et al.: a) **9**, THF, 0 °C, 3 h; b) 10% HF·pyridine in THF, 25 °C, 12 h; c) $(COCl)_2$, DMSO, Et_3N, −78 °C→RT; d) **10**, LDA, THF, −78 → −40 °C, 1 h; e) **11**, DCC, DMAP, room temperature, 15 h; f) Grubbs catalyst, CH_2Cl_2, room temperature, 48 h, 30% yield of the desired isomer; g) TFA in CH_2Cl_2 (20%); h) methyl(trifluoromethyl)dioxirane, CH_3CN, 0 °C, 2 h. DMSO = dimethylsulfoxide, LDA = lithium diisopropylamide, DCC = dicyclohexylcarbodiimide, DMAP = 4-dimethylaminopyridine, TFA = trifluoroacetic acid, TBS = *tert*-butyldimethylsilyl.

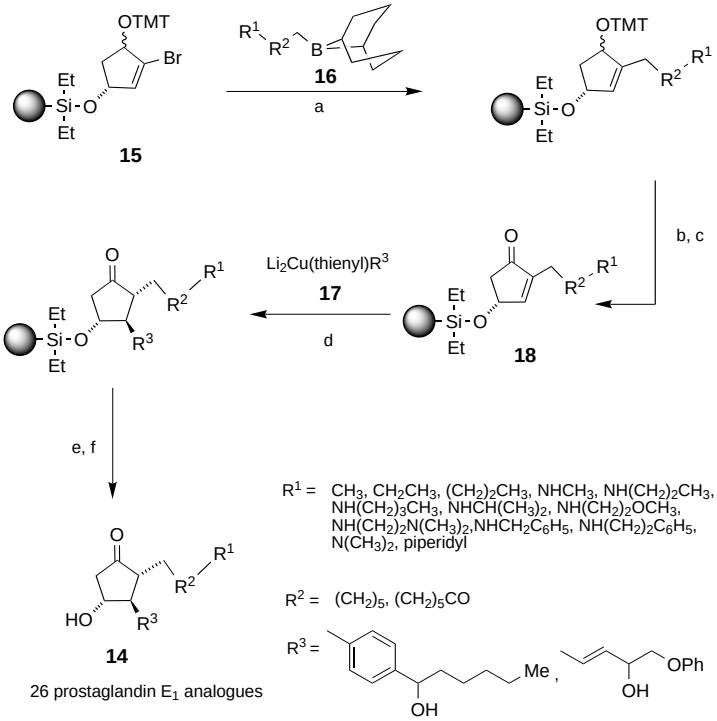
activity relationship, which make them a target for combinatorial chemistry of foremost interest and significance. Ellman and co-workers disclosed the solid-phase synthesis of a 26-membered library of prostaglandin E_1 analogues (**14**). After modification of the core structure **15** by a Suzuki coupling with building block **16**, the relative stereochemistry of the two carbon centers that bear the side chains was set by a diastereoselective Michael addition of the higher-order cuprate **17** across the enone **18** (Scheme 4).[37]

In an experimentally challenging effort, Shair and co-workers completed a biomimetic synthesis of carpanone-derived compounds **19** by a $PhI(OAc)_2$-mediated oxidative heterodimerization. In only three steps on a solid support, they were able to access a library of six different compounds of high structural complexity (Scheme 5). Shair and co-workers announced the preparation of a 100000-membered library and high-throughput biological screens, which will be received with great interest because of the biological activity of carpanone.[38]

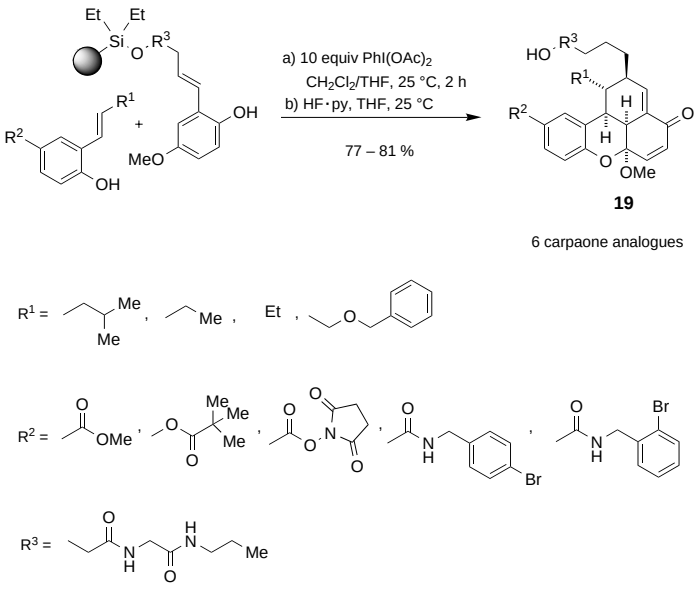
Sarcodictyins are a class of natural products that have received much attention by synthetic chemists recently owing to their structural complexity and especially because of their anticancer properties, originated by its paclitaxel-like mechanism of action in the tubulin–microtubulin interplay. Nicolaou et al. have built a library of sarcodictyin derivatives **20a–c**. The complexity of the natural product prompted a strategy in which they prepared the core structure **21** of the target compound in solution, attached it to a solid support through an acid-labile linker, and then carried out further modifications on three different reactive entities of the core structure (Scheme 6). In their library of 22 derivatives, they identified compounds with biological activities superior than those of the parent structure and they also established a structure–activity relationship.[39]

A similar approach of synthesizing the core structure in solution and then elaborating it on solid supports for further diversification was applied by Waldmann and co-workers in their synthesis of a 31-membered library of analogues **22** of the protein kinase C activator indolactam V (Scheme 7).[40]

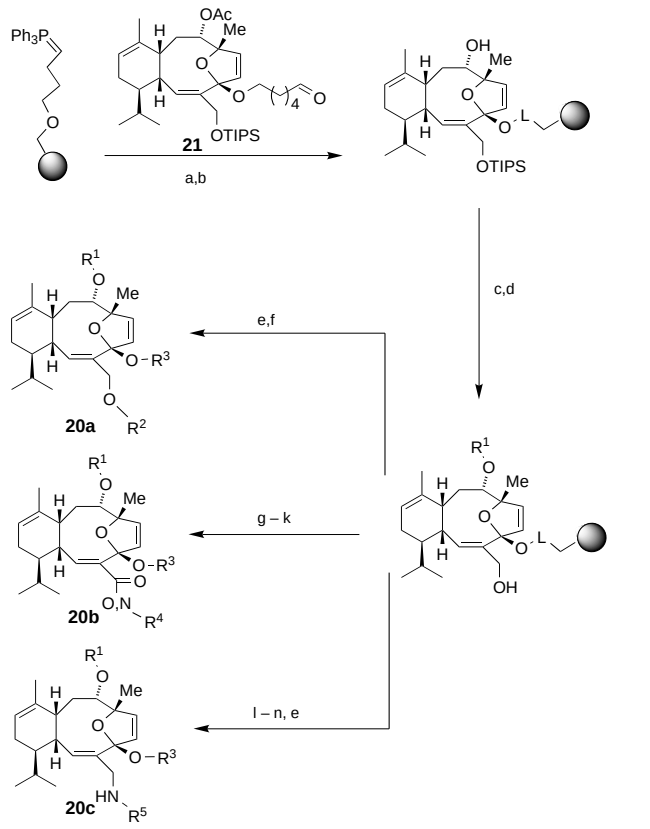
Waldmann and co-workers synthesized a library of analogues of the antitumor phosphatase inhibitor dysidiolide.[41] A notable feature of this 11-step solid-phase reaction sequence is that a wide range of transformations with widely differing requirements



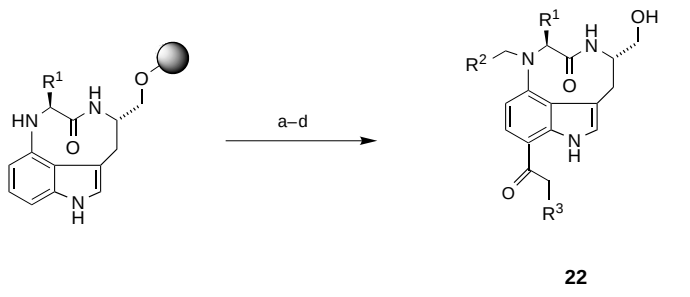
Scheme 4. Synthesis of a prostaglandine library by Ellman et al.: a) $[Pd(PPh_3)_4]$, Na_2CO_3, THF, 0 °C; b) HCOOH (1M), CH_2Cl_2, 5 min; c) Dess–Martin periodinane, CH_2Cl_2; d) THF, −78 →20 → −78 °C, then AcOH/THF (10%); e) HF/pyridine in THF; f) TMSOMe. TMS = trimethylsilyl, TMT = trimethoxytrityl.



Scheme 5. Synthesis of a library of carpanone analogues by Shair et al.



Scheme 6. Synthesis of a library of sarcodictyin analogues by Nicolaou et al.: a) THF, −78 °C→RT; b) NaOMe, MeOH; c) R^1X, Et_3N, DMF; d) TBAF, THF, 25 °C; e) R^2X, Et_3N, DMAP, CH_2Cl_2, room temperature; f) PPTS, MeOH, room temperature; g) Dess–Martin periodinane, $NaHCO_3$, pyridine, CH_2Cl_2, room temperature; h) $NaClO_2$, KH_2PO_4, 2-methyl-2-butene, THF, THF/*t*BuOH/H_2O (5:5:1), 25 °C; i) HOR^4, DEAD, Ph_3P, THF, 0→25 °C, 12 h; j) H_2NR^4, DCC, DMAP, DMF, room temperature, 20 h; k) CSA, HOR^3, room temperature, 15 h; l) $(PhO)_2PON_3$, DEAD, Ph_3P, THF, 0 °C→RT, 4 h; m) Ph_3P, H_2O, THF, room temperature, 8 h; n) XR^5, Et_3N, CH_2Cl_2, room temperature, 10 h. DMF = *N*,*N*-dimethylformamide, TBAF = tetrabutylammonium fluoride, PPTS = pyridinium *p*-toluenesulfonate, DEAD = diethylazodicarboxylate, CSA = camphorsulfonic acid, TIPS = triisopropylsilyl



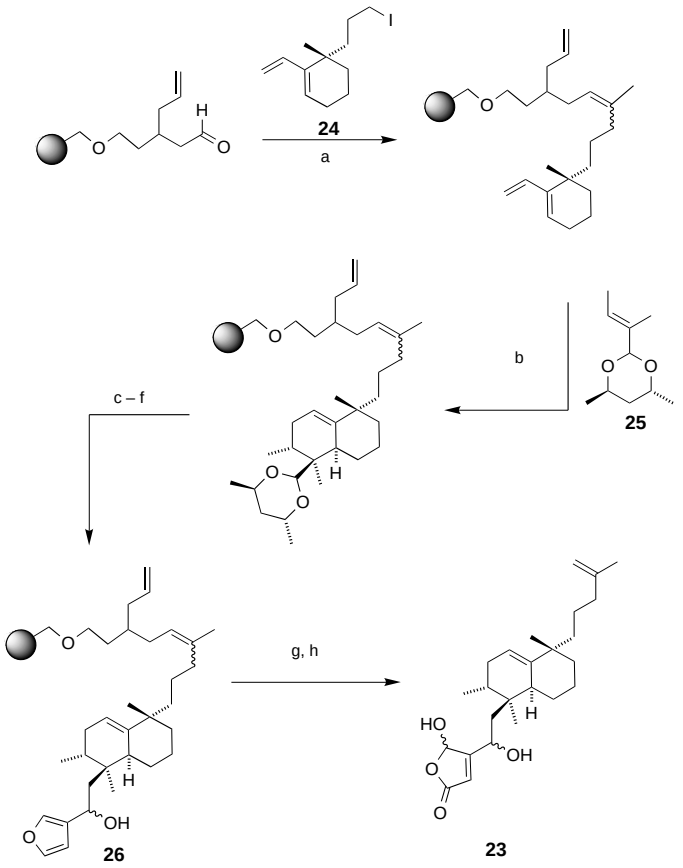
Scheme 7. Synthesis of a library of indolactam V analogues by Waldmann et al.: a) R^2CHO, $NaBH(OAc)_3$, DMF/AcOH (99:1), room temperature; b) I_2, pyridine/1,4-dioxane, 0 °C; c) R^3CCH, $[(PPh_3)_2PdCl_2]$, CuI, Et_3N, 1,4-dioxane, room temperature; d) TFA/H_2O (95:5).

could be developed successfully. Key transformations of the synthesis are an asymmetric Diels–Alder reaction with chiral dienophile **25**, oxidative elaboration with singlet oxygen of furan **26** on a solid support, and complete cleavage of the products from the solid support by means of olefin metathesis (Scheme 8). The sequence rapidly yielded access to nine analogues of the natural product and led to the identification of a potent inhibitor of the cell-cycle-controlling phosphatase cdc25c, which displays a very promising selectivity pattern. Contrary to the solid-phase synthesis of many other natural product, this synthesis builds up the natural-product framework on a solid phase in several steps, rather than immobilizing an advanced intermediate generated in solution as a scaffold and varying its substitution pattern on the solid support. Thus, it convincingly demonstrated that multistep total synthesis of natural products and close analogues on polymeric supports can be achieved.

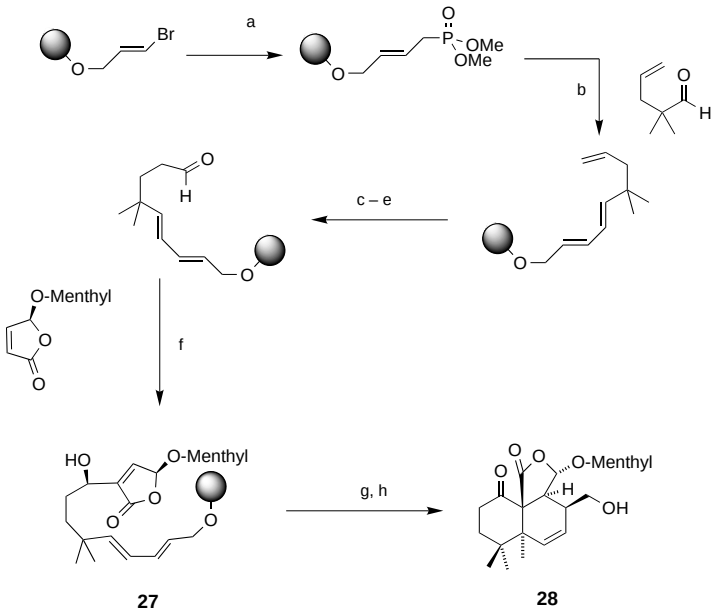
Reiser and Jauch have recently paved the way for a solid-phase library synthesis of mniopetals, a compound class that has been shown to contain inhibitors of HIV reverse transcriptase.[42] Notable features of their synthesis are the formation of adduct **27** through a Baylis–Hillman reaction and its further elaboration by an intramolecular Diels–Alder reaction into **28** (Scheme 9).

6. Alternative Approaches

The approach delineated in this article is only one new concept among others. For instance, in recent years in silico methods have gained much interest in the development of new and more efficient approaches to hit and lead identification.[43] Among the different concepts currently under evaluation, diversity-oriented synthesis introduced by Schreiber and co-workers is conceptually related to natural-product-based library design.[44] Diversity-oriented synthesis for chemical genetics aims at libraries of compounds with huge three-dimensional structural complexity, functionality, and diversity; in this sense, these substances can be regarded as "natural-product"-like.[45] Whereas natural-product libraries are motivated by a biologically validated starting point in the conformational and structural space of potential protein



Scheme 8. Synthesis of a library of 6-epi-dysidiolide analogues by Waldmann et al.: a) $EtPPh_3I$, *n*BuLi, **24**, THF, room temperature, 16 h, then *n*BuLi, 0 °C; b) TMSOTf, CH_2Cl_2, −78 °C, 5 h; c) PTSA, acetone, DCE, H_2O; c) $(Ph_3PCH_2OMe)Cl$, KO*t*Bu, THF, room temperature, 2 h; d) PPTS, THF, H_2O (1 %), reflux, 16 h; f) 3-bromofuran, *n*BuLi, THF, −78 °C, 5 h; g) O_2, Hünig base, bengal rose, *hν*, −78 °C, CH_2Cl_2; h) Grubbs catalyst (2 × 10 %), CH_2Cl_2, room temperature, 16 h. OTf = trifluoromethanesulfonate, PTSA = *p*-toluenesulfonic acid, DCE = 1,2-dichloroethane.



Scheme 9. Synthesis of the central building block of a mniopetal library by Jauch et al.: a) $P(OMe)_3$, 90 °C, 48 h; b) −80 → −10 °C, 12 h; c) 9-BBN (4 equiv), room temperature, 24 h; d) H_2O_2/NaOH, room temperature, 4 h; e) IBX, room temperature, 5 h; f) PhSeLi (5 equiv), −60 → −30 °C, 12 h; g) Dess–Martin periodinane, 2,6-lutidine, CH_2Cl_2, room temperature, 12 h; h) DDQ, room temperature, 40 min. IBX = *o*-iodoxybenzoic acid, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, 9-BBN = 9-boracyclo[3.3.1]nonane, Menthyl = (+)-menthyl.

binders, diversity-oriented synthesis addresses an area of compound diversity around a starting point that has been chosen by its mere chemical accessibility and "natural-product likeness", that is, structural complexity (Table 2). In Table 2, the extreme positions for both approaches are listed; however, these two approaches are intertwined and for each combinatorial library synthesized so far, elements of both can be found, with variations only in the degree of expression.

Protein binders that would be found by screening such diversity-oriented libraries will be of complementary interest, because they might be based on scaffolds different from the known natural products. This may lead to the identification of unprecedented new scaffolds, for example, as in the discovery of benzodiazepines.

7. Conclusion and Outlook

The recent success in the generation of natural-product libraries has convincingly proven that solid-phase organic chemistry has reached a level that allows its application in natural-product synthesis. It is now possible to tackle complex synthesis targets that require double-digit numbers of individual chemical steps. Although the overall yields of the presented syntheses are highly respectable, the demand for

Table 2. Comparison of "natural-product-guided combinatorial synthesis" with "diversity-oriented combinatorial synthesis".

Natural-product-guided	Diversity-oriented
• aims to achieve focused diversity around a biologically validated starting point in the chemistry "universe"—the diversity of these starting points is selected by nature	• aims to achieve maximum diversity around a spot in the chemistry "universe" chosen for its chemical accessibility and "natural-product likeness", that is, structural complexity
• synthetic methods are primarily chosen to lead to the target core structure, even if this goal requires a considerable amount of optimization	• synthesis route is driven by "powerful" chemical reactions (general, high-yielding, stereoselective, bond-constructing, functional-group-tolerant, etc.)
• retrosynthetic analysis will be also validated by its potential for diversity generation	• forward diversity-oriented synthetic design
• building blocks are commercially available or synthesized in solution	• building blocks should be commercially available in great diversity
• parallel or split-pool synthesis	• encoded split-pool synthesis is preferred method
• small to middle library size	• large library size

new, efficient, and selective reactions that can be applied in solid phase synthesis is greater than ever. It can be anticipated that the ongoing progress in methodology development will have an impact in the quest for structurally more complex and more diverse libraries. A further direction for future efforts will certainly be the creation of larger libraries in which the advantages of solid-phase synthesis over synthesis in solution will justify the initial cost of optimization.[5, 38b] The return on this additional investment in synthetic effort, which is necessary to generate combinatorial libraries of structurally diverse natural-product analogues, should result in higher and better hit rates and better leads with libraries of much smaller size than conventional random libraries.

As Nature has already identified natural products as lead structures of biological relevance in the almost infinite universe of chemical structures, there are good reasons to take the effort to design combinatorial libraries based on these scaffolds. Nature has been fairly restrictive in exploiting the infinite number of possible protein structures and has allowed itself access to only a limited number of folds and domains. However, the possible variations on these scaffolds by individual amino acid side chains will ensure that the search for small-molecule ligands for these proteins remains a challenge.

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