Pure Appl. Chem., Vol. 75, Nos. 2–3, pp. 143–155, 2003.

Inspirations from Nature. New reactions, therapeutic leads, and drug delivery systems*

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Abstract: Studies in our laboratory focus on problems in chemistry (new reactions and synthesis), biology (novel modes of action), and medicine (new therapeutic leads and drug delivery systems). These interconnected and often synergistic activities are inspired by an interest in novel structures, frequently from nature, that possess unique modes of action and significant clinical potential. Described herein are some examples of recent work from our laboratory that have led to new transition metal-catalyzed reactions, a new and remarkably potent therapeutic lead, and new drug delivery systems that are in clinical trials.

Research in our group focuses on novel structures with novel biological activities. These structures are selected because they provide new synthetic challenges and thereby new opportunities for advancing the frontiers of synthesis through the design and development of new reactions, reagents, and strategies. These structures are also selected because they exhibit unique activities, allowing for the investigation of new biochemical pathways that could lead to novel therapeutic candidates. Our studies are often inspired by structures found in nature—for example, Taxol® [1], phorbol [2], resiniferatoxin [3], apoptolidin [4], HIV-Tat [5], and bryostatin [6]. In the past, many such biologically active natural products were taken forward without modification into clinical trials because they were too difficult to modify or too little was known about how modifications would improve performance. While such unmodified natural products have occasionally succeeded as drugs, it is clear that many natural leads have failed because they are not "designed" for therapeutic use. The increasing effectiveness of synthesis coupled with our growing understanding of therapeutic pathways is now changing this situation. It has now become possible to examine a natural lead with an eye toward synthesis and biological function and design a potentially superior clinical candidate that can be synthesized in a practical fashion. Thus, while many natural products will continue to be beyond the reach of practical synthesis, insightful design could allow practical synthetic access to targets that possess similar, if not superior, activity. Representative examples of this philosophy are illustrated herein, along with the related role that this work has in inspiring the design of new reactions and synthetic strategies.

NEW THERAPEUTIC LEADS

The bryostatins are a structurally novel family of marine-derived macrocyclic lactones. Bryostatin 1 was first isolated by Pettit and coworkers from the sea sponge *Bugula neritina* in 1968 and structurally

Pure Appl. Chem. **75**, 141–419 (2003). An issue of reviews and research papers based on lectures presented at the 23rd IUPAC International Symposium on the Chemistry of Natural Products, Florence, Italy, 28 July–2 August 2002 on the theme of natural products.
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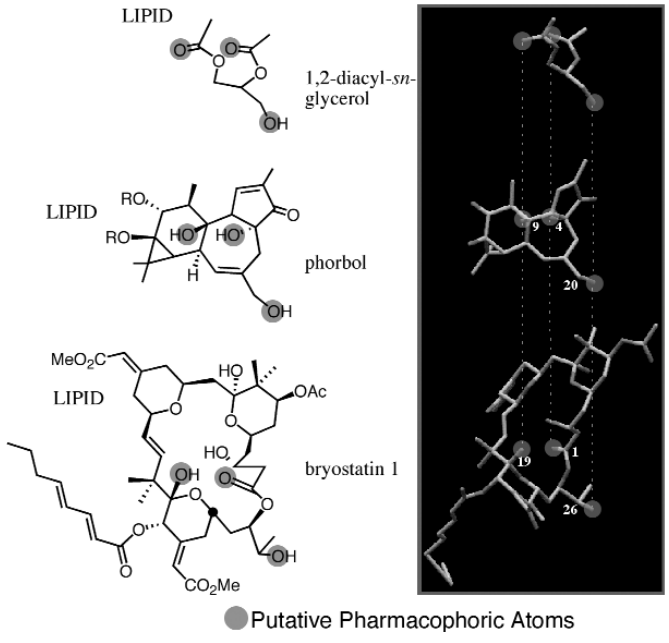
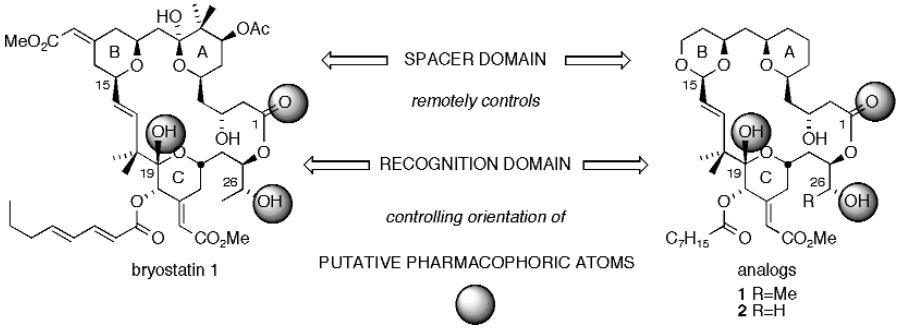
characterized in 1981 [7]. Since that time, a total of 18 naturally occurring bryostatins have been isolated [8]. Early work on the biological activity of the bryostatins demonstrated their remarkable potency against murine P388 lymphocytic leukemia in vivo and prompted an explosion of research toward the development of these compounds as human therapeutic agents [9]. These efforts have included three total syntheses (broystatin 2 [10], 3 [11], and 7 [12]), large-scale cGMP adherent isolation of bryostatin 1 (18 grams with an isolation yield of 1.4×10^{-4} %) [13], and phase I and II clinical trials of bryostatin 1, as both a single agent and in combination with existing therapeutics, to treat a variety of cancers [14]. It is thought that the mode of action of bryostatin 1 is mediated, at least in part, through its modulation of protein kinase C (PKC) activity, although other proteins with similar C1 domains could be involved [15]. While much remains to be done to elucidate its mode of action on the molecular level, the activities elicited by bryostatin are remarkable. In various studies, bryostatin has been shown to reverse multidrug resistance [16], restore apoptotic function [17], stimulate the immune system [18], act synergistically with other oncolytic agents [19], and protect cells from ionizing radiation [20].

The exceptional activity and potency of bryostatin 1 make it a potentially powerful agent for the treatment of human disease. However, its complexity and low natural abundance make it inaccessible in useful amounts and preclude access to derivatives that could contribute to our understanding of its mode of action and possibly lead to clinically superior agents. In order to address these issues and opportunities, we turned to the design of bryostatin analogs that would retain the activity and potency of the natural product and be accessible through chemical synthesis. Previous work in our group on the synthesis of phorbol esters [2], known modulators of PKC activity, had led to the hypothesis that the phorbol esters are structurally constrained analogs of the natural PKC activators, diacyl glycerols. Because the phorbol esters, diacyl glycerols, and bryostatins bind competitively to the same activator domain of PKC, we examined whether all three might share a similar arrangement of functionality for recognition by PKC. Computer modeling of these compounds indicated that all three possess a similar set of hydrogen bond donors and acceptors (Scheme 1), which, in the case of bryostatin, is the combination of the C1 carbonyl oxygen, the C19 hemiketal oxygen, and the C26 hydroxyl group [21]. In addition, all three possess a lipid domain, which, in bryostatin, is associated with the A and B rings and the



Scheme 1

fatty-acid side-chain. This analysis suggested that the A and B rings of bryostatin might serve principally to hold the key recognition elements in the correct orientation for binding to PKC. As a consequence, it would be possible to significantly simplify the structure of bryostatin to analogs such as **1** [6e] and retain biological function (Scheme 2). Computer modeling of **1** indicated that a low-energy conformation of the molecule overlays nearly perfectly with the crystal structure of bryostatin 1 and presents the proposed pharmacophoric atoms in the correct orientation.



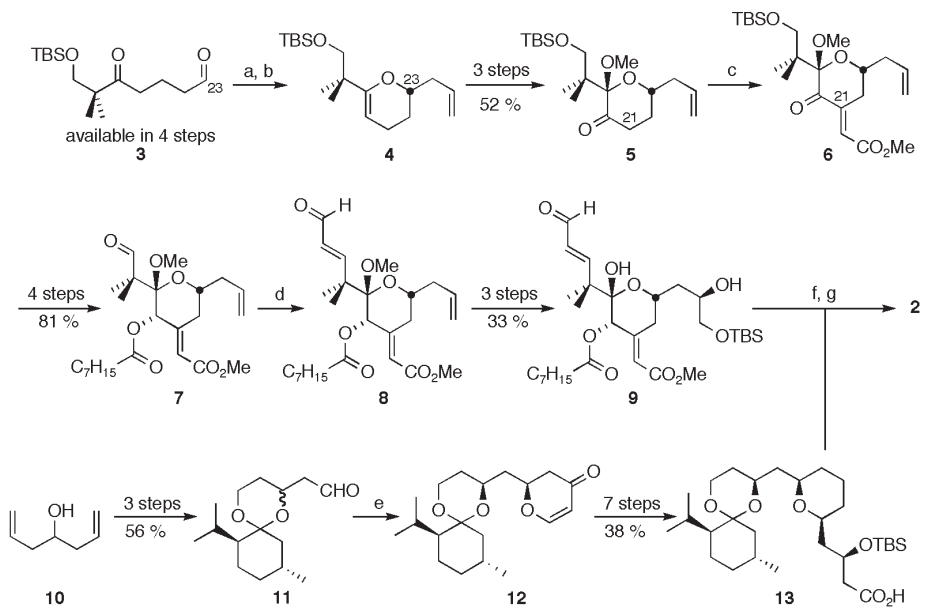
Scheme 2

Prompted by this favorable "in silico" assay, **1** was targeted for synthesis and ultimately prepared in 27 steps longest linear sequence (LLS) [6e]. With the observation that most PKC activators possess a primary hydroxyl group, a second-generation analog **2**, possessing a hydroxymethyl group, was also prepared by modification of a late-stage intermediate in the synthesis of **1**.

In agreement with the predictions from the computer modeling studies and the pharmacophore comparison, compound **1** exhibited low nanomolar binding affinity to PKC (K_i = 3.4 nM; bryostatin 1 K_i = 1.35 nM), and compound **2** exhibited picomolar binding affinity to PKC (K_i = 250 pM). When these new analogs were tested in vitro against various human cancer cell lines, **1** displayed greater potency than bryostatin 1 in 17 of 35 cell lines and **2** displayed greater potency than bryostatin 1 in 24 of 35 cell lines. In select cell lines, such as MOLT-4 and NCI-H460, **2** is 3 orders of magnitude more potent than bryostatin 1 at inhibiting cell growth [6a]. Preliminary in vivo studies have shown that **1** and **2** are also more potent than bryostatin 1 at inhibiting tumor growth in animal models.

The remarkable potency of our designed analogs prompted further interest in their development as novel therapeutic leads. While our original synthetic sequence was designed for speed and provided enough material to test our pharmacophore hypothesis, the significant activity exhibited by our analogs necessitated the development of a second-generation synthesis to produce the quantities of these candidates needed for clinical development. The challenge of designing a synthetic route to meet such material demands resulted in a step-economical second-generation sequence that offered greater scalability and efficiency (Scheme 3) [6a].

A marked improvement in the second-generation synthesis of the recognition domain was made in the enantioselective formation of the C23 stereocenter (92 % ee), which is now created through an asymmetric Keck allylation of ketoaldehyde **3** at room temperature [22]. Additionally, the C ring is formed in 6 total steps, compared to the 11 steps previously needed. A further improvement was realized in the procedure for introduction of the C21 unsaturated ester: treatment of ketone **5** with K_2CO_3 and methyl glyoxylate in MeOH provides enoate **6** in 72 % yield in a *single step* instead of the three steps required previously. Alternatively, the commercially available methyl 2-hydroxy-2-methoxyacetate can be used as the electrophile to generate **6** in 56 % yield under the same reaction conditions. Finally, a two-carbon homologation of aldehyde **7** to enal **8** is accomplished in a single operation and in excellent yield (90 %) by using the vinyl zincate derived from (*Z*)-1-bromo-2-ethoxyethene followed by in situ acid-catalyzed rearrangement of the intermediate vinyl ether. In contrast to the originally required four-step sequence, this single-step transformation is a significant late-stage improvement and



Scheme 3 (a) 10 mol % R-BINOL, 4 Å MS, 5 mol % $Ti(OiPr)_4$, $B(OMe)_3$, allyl-$SnBu_3$, CH_2Cl_2, rt, 77 %; (b) cat. *p*-TsOH·H_2O, 4 Å MS, MePh, rt, 85 %; (c) K_2CO_3, $OHCCO_2Me$, MeOH, rt, 72 %; (d) (i) (*Z*)-1-bromo-2-ethoxyethene, *t*-BuLi, Me_2Zn, then **7**, Et_2O, –78 °C; (ii) 1M HCl, –78 °C → rt, 90 %; (e) Danishefsky's diene, catalyst (vide infra), 4 Å MS, acetone, rt, then TFA, 88 %; (f) **13**, Et_3N, 2,4,6-trichlorobenzoyl chloride, then **9**, DMAP, MePh, rt, 87 %; (g) 70 % HF·pyridine, THF, rt, 82 %.

overcomes the sterically encumbered nature of the aldehyde without affecting the other reactive functionalities in the molecule.

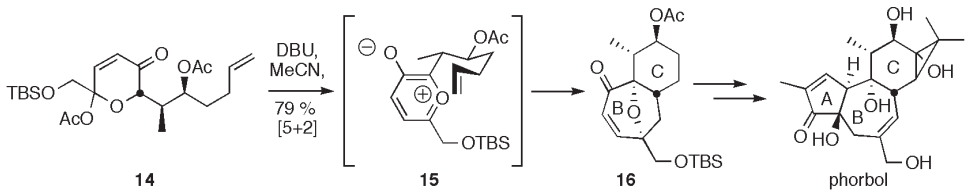
Several key changes were also made in the synthesis of the spacer domain. The synthesis now begins with the ozonolysis and in situ reduction of **10** (Scheme 3), which produces pentane-1,3,5-triol in a manner superior to our previous methods [23]. The triol is desymmetrized with (–)-menthone to give a mixture of diastereomeric aldehydes **11** (1.6β:1α). A hetero Diels–Alder cycloaddition between **11β** and Danishefsky's diene using Jacobsen's tridentate Cr(III) catalyst provides pyranone **12** with exceptional selectivity [24]. The diastereoselectivity (33:1) obtained with this catalyst has far exceeded that of others (1:2 to 4:1) and is unprecedented in overcoming the inherent bias (1:3.5) of substrate **11β**. Pyranone **12** is converted to **13** in 7 steps, providing the fully elaborated spacer domain in 11 steps and 11 % yield overall. Recently, a second-generation synthesis of the top piece was completed by our group, offering a two-fold increase in overall yield (20 %) and >95 % selectivity in a total of 10 steps [25].

One of the powerful advantages of designing targets for function is the ability to select for synthetic opportunities as well, as is found in the bryostatin analog design. The coupling protocol to bring together the spacer and recognition domains and generate the analog B ring (see **1** or **2** in Scheme 2) is an example of designed synthetic opportunity. In the design of the analogs, the tetrahydropyran B-ring of the bryostatins was modified to a dioxolane to explore a novel macrotransacetalization strategy for macrocycle formation. Toward this end, the spacer and recognition domain fragments (**9** and **13**) are first coupled using the Yamaguchi esterification protocol [26]. Macrotransacetalization, mediated by HF·pyridine, then allows for closure of the macrocycle, introduction of the C15 stereocenter under thermodynamic control, and removal of two silyl protecting groups to provide the completed analog **2** in a single operation.

At present, the synthesis of these analogs requires only 30 steps (LLS = 19 steps), which is less than half of that required currently for a total synthesis of bryostatin. Importantly, because of the potency of the analogs, this step count is within the requirements for clinical development. By designing a novel target for enhanced function, a new class of compounds has been realized that is functionally superior to the natural product in most assays performed thus far and is readily synthesized in a practical fashion.

NEW REACTIONS

One of the major justifications for complex molecule synthesis, whether the target is natural or designed, is that it allows advancement of the field by improving our understanding of reaction mechanisms and thereby improving reaction conditions, selectivities, and efficiencies. At the same time, complex molecule synthesis exposes deficiencies in our methodology. For example, our studies on the synthesis of phorbol esters, which led to our hypothesis about the pharmacophoric functionalities in bryostatin, were based on a novel intramolecular oxidopyrylium [5+2] cycloaddition (Scheme 4). While this reaction proved particularly effective in the assembly of the BC-bicyclic ring system found in phorbol and other daphnane and tigliane diterpene natural products [2,3], it represents one of the few cycloaddition reactions for the formation of seven-membered rings.



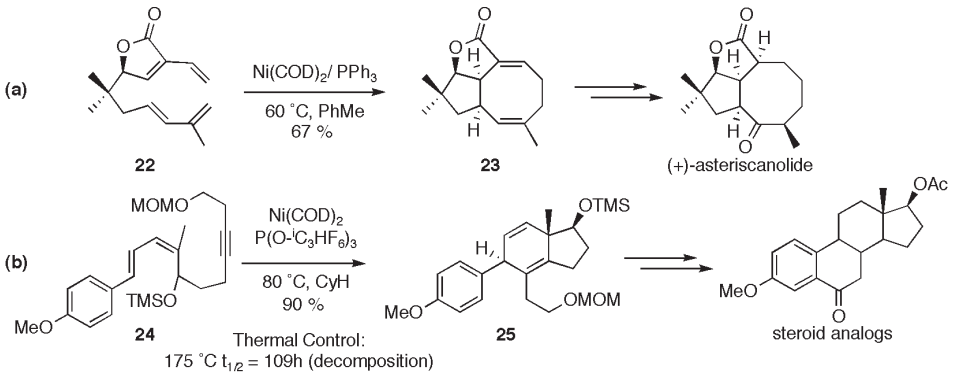
Scheme 4

Given the number of natural and non-natural targets that possess seven-membered rings and the profound biological activity that many of these exhibit, we sought to design a new reaction for seven-membered ring synthesis that would address these problems with the same generality and utility that the Diels–Alder reaction has delivered for six-membered ring synthesis (Scheme 5) [27]. In principle, a homolog of the Diels–Alder [4+2] cycloaddition, namely a [5+2] cycloaddition, could be realized through the reaction of a homolog of a diene **17** with dieneophile **18**. A vinylcyclopropane (VCP) **20** is an attractive five-atom homolog of a four-atom diene, due to its inherent strain-driven reactivity but kinetic stability.

Scheme 5

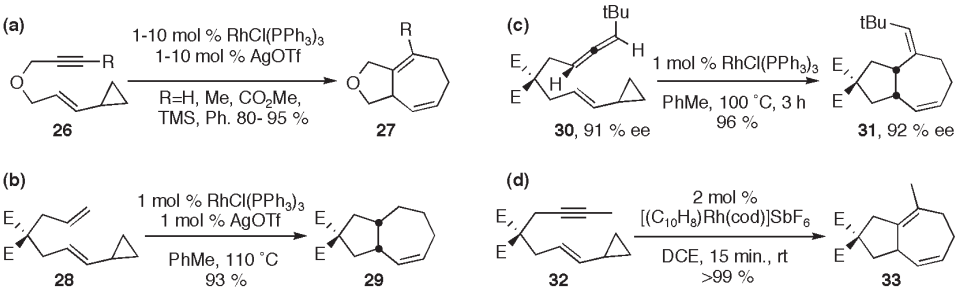
The literature reveals attempts to achieve this reaction thermally, but thus far such reactions are limited to specific substrates with constrained VCPs embedded in bicyclic networks [28]. This is partly due to the formidable activation energy required to cleave simple vinylcyclopropanes (~50 kcal/mol) [29]. In the early 1990s, we proposed a new approach to achieving a [5+2] cycloaddition of VCPs, in which a metal catalyst would be employed to facilitate cleavage and cycloaddition of the VCP. This approach further exemplifies our larger effort directed at the design and implementation of new transition metal-catalyzed reactions that would be impossible or difficult to achieve in the absence of catalyst [30]. For example, [4+4] cycloadditions of dienes are forbidden thermally, but, as seen in the pioneering work of the Reppe, Reed, and Wilke groups, such reactions can be achieved with simple dienes [31].

Unfortunately, this process does not work well with more complex dienes, as are needed in complex molecule synthesis. To address this problem, we explored whether these reactions could be achieved intramolecularly, even with complex substitution. This led to the first successful intramolecular [4+4] cycloadditions of bis-dienes as illustrated in the synthesis of asteriscanolide (Scheme 6a) [32]. Realizing that this approach could also be used to facilitate or enable otherwise difficult Diels–Alder cycloadditions (see thermal control), we were also able to use metal catalysts to effect the first intramolecular metal-catalyzed [4+2] cycloadditions of dienes with pi-systems, as illustrated in access to steroid analogs (Scheme 6b) [33].



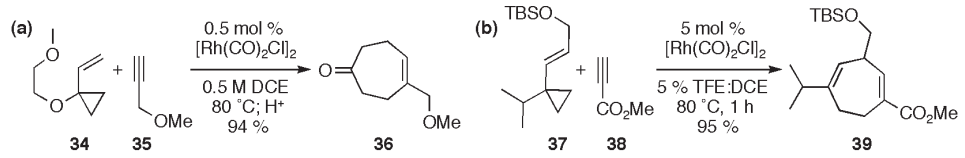
Scheme 6

In 1995, we reported the first metal-catalyzed [5+2] cycloadditions of VCPs and tethered alkynes (Scheme 7a) [34]. This reaction proceeds with impressive efficiency for a range of alkyne substituents, from R = H to electron-donating and -withdrawing groups (e.g., R = TMS, CO_2Me). Tethered alkenes are also superb 2-carbon components in this process (Scheme 7b) [35]. While the use of internal olefins in the reaction was problematic [36], allenes proved to be excellent partners (Scheme 7c) [37]. More recently, arene complexes of rhodium were shown to be highly effective catalysts for the [5+2] cycloaddition (Scheme 7d). These air-stable, readily prepared, and easily handled complexes effect reactions faster, at lower temperature, and in higher yields than previously investigated catalytic systems. Some substrates undergo cycloaddition *quantitatively in 15 min at room temperature* [38].



Scheme 7

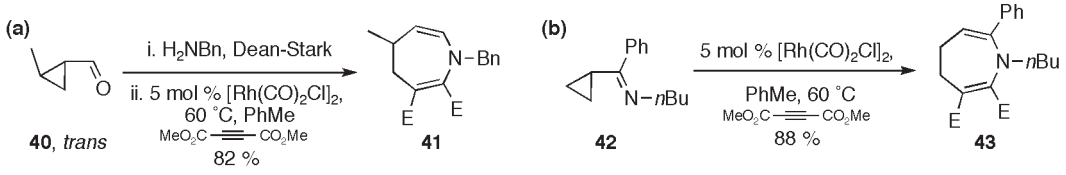
Given the ready commercial availability of alkynes, the development of an *intermolecular* [5+2] cycloaddition reaction, based on alkynes, offers a powerful and efficient method for the facile construction of simple substituted seven-membered ring systems (Scheme 8). For example, VCP **34** in the presence of $[Rh(CO)_2Cl]_2$ affords cycloheptenone **36** in 94 % yield upon hydrolysis of the initially formed enol ether [39]. Significantly, recent studies have demonstrated that oxygen activation of the



Scheme 8

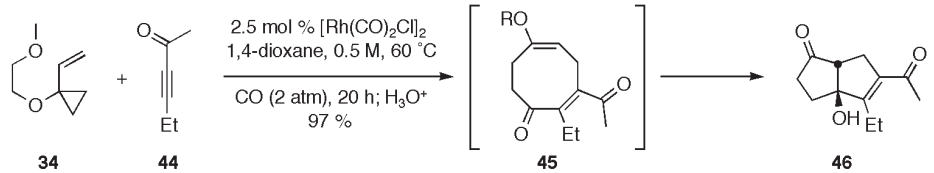
VCP is not necessary to effect the reaction, as even simple alkyl-substituted VCPs, such as **37**, react efficiently with alkynes [40]. Presumably, substitution of the VCP stabilizes the reactive *s-cis* conformation, allowing the reaction to proceed at a useful rate.

Building on the analogy of this new [5+2] cycloaddition to the Diels–Alder [4+2] cycloaddition, studies were recently initiated on hetero-[5+2] cycloadditions, in which the five-atom component includes a heteroatom (Scheme 9). Cyclopropyl imines, readily available five-atom components [41], were found to react efficiently with an alkyne in the presence of a Rh(I) catalyst [42], enabling a new route to dihydroazepines, a common substructure of many natural products. The reaction can be carried out as a serial process by initial in situ formation of the imine followed by addition of catalyst and alkyne to effect the cycloaddition (Scheme 9a). This new hetero-[5+2] cycloaddition works well with aldimines, ketimines, and with substituted cyclopropanes, affording the desired dihydroazepine products in excellent yields as single regioisomers.



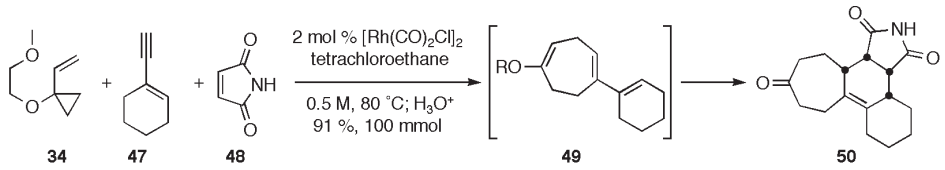
Scheme 9

The preceding discussion illustrates the interconnectedness of complex molecule synthesis (phorbol) and the invention of new reactions ([5+2]). New reactions in turn beget other new reactions, as illustrated by our subsequent success with the first [6+2] cycloadditions of vinylcyclobutanones with pi-systems [43] and the more recently introduced [5+2+1] cycloaddition [44]. The latter three-component process allows one to assemble complex cycloadducts from simple starting materials, producing richly functionalized building blocks for synthesis or scaffolds for combinatorial libraries (Scheme 10). Starting from a variety of carbonyl-substituted alkynes, bicyclo-[3.3.0]-octenone products **46**, resulting from a "bonus" in situ transannular closure of the initially formed eight-membered ring **45**, are produced. These [m+n+o] cycloadditions are reliable and efficient, leading to well-defined products in excellent yields.



Scheme 10

Linking reactions serially is another strategy to achieve greater complexity and thereby step-economical syntheses. For example, a serial [5+2]/[4+2] cycloaddition process allows the generation of complex polycyclic products in a single operation with two new rings, four carbon–carbon bonds, and up to four new stereocenters from readily available starting materials with excellent yields and selec-



Scheme 11

tivities (Scheme 11) [45]. These cycloadducts serve as excellent scaffolds for the generation of libraries for biological screening.

NEW DRUG DELIVERY SYSTEMS

In the first section of this presentation, we described how the naturally occurring bryostatins inspired the design and synthesis of simpler and more potent analogs and, in the second section, how that process was coupled to the invention of new reactions. We return to the theme of improving upon nature in this section in connection with the development of agents that enable or enhance drug delivery—a problem of global importance in chemotherapy and biochemistry.

The bioavailability of drugs or molecular probes with intracellular targets depends significantly on their being sufficiently polar to allow administration and distribution throughout the body and sufficiently nonpolar to enable passive diffusion through the cell membrane. As a result, most drugs are limited to a narrow range of physical properties (log *P*), and many promising drug candidates fail to advance clinically because they fall out of this range. Traditional approaches to address this problem include significant changes in formulation or extensive tuning of physical properties through the synthesis of dozens if not hundreds of modified analogs. Such efforts take time and, for certain therapeutics, the required optimization is not possible without an accompanying significant loss of activity.

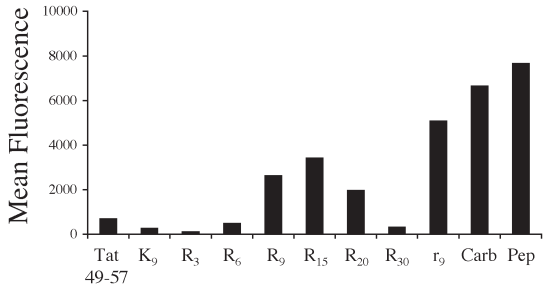
An alternative strategy arises from the observation that certain proteins such as HIV-Tat [46] (Scheme 12) and *Drosophila* Antennapedia [47] exhibit facilitated passage across the nonpolar cell membrane and that this ability can be attributed to a short, highly charged segment of the protein. It follows that a drug candidate or probe molecule that could not otherwise cross the cell membrane, could be attached to these natural transporters, thereby enabling cellular uptake and eliminating time-consuming iterative syntheses of analogs. For many applications, however, the performance of these transporters would need to be improved, as would their synthetic accessibility. Toward these ends, investigation of the HIV-$Tat_{49–57}$ 9-mer (Scheme 12, bold residues), the segment of Tat that enables uptake [48], revealed that all the basic amino acid residues are important for cellular uptake. Importantly, charge alone is not sufficient for enhancement of cellular uptake, as fluorescently labeled 9-mers of lysine (Scheme 13, K9) did not efficiently cross the cell membrane, while a 9-mer of arginine was internalized into cells more rapidly than the Tat 9-mer (cf. R9 and $Tat_{49–57}$). Length is also important, as uptake increased to a maximum at approximately 15 residues and then declined (Scheme 13). Inversion of the arginine stereochemistry (from L- to D-arginine) allowed for increased uptake (Scheme 13, r9), presumably due to the greater proteolytic stability of the D-isomer [5e]. Furthermore, rationally designed guanidine-rich peptoid [5e] and oligocarbamate [5a] 9-mers exhibited dramatically enhanced cellular uptake compared to the original $Tat_{49–57}$ 9-mer (Scheme 13, Pep and Carb, respectively).

MEPVDPNLEPWKHPGSQPRTACNNCYCKKCCFH

CQVCFTKKGLGISYG**RKKRRQRRR**PPQDSQTHQ

SSLSKQPTSQLRGDPTGPTESKKKVERETETDPVH

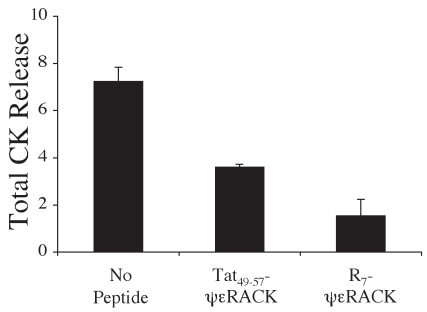
Scheme 12



Scheme 13

Drawing on these observations, we sought to utilize these transporter peptides to enable or enhance the delivery of medicinally relevant cargoes. Several cargo chemo-types were considered including small molecules, peptides, proteins, nucleic acids, probes, and metals. Our work on cargo type is exemplified by the delivery of biologically active peptides. As part of our program in developing agents that modulate PKC, we are interested in molecules that selectively regulate the various PKC isozymes. Some of the most promising isozyme-selective PKC agents are short peptides termed ψRACKs (receptors for activated C-kinase) [49]. While these peptides can selectively activate or inhibit various PKC isozymes upon microinjection into cells, their inability to cross the cell membrane preclude their clinical use [50]. Peptides, in general, are an important class of therapeutics that suffer from cell uptake problems and therefore represent a significant drug delivery opportunity.

To test transporter delivery of peptides, we targeted PKC-ε activation, which is implicated in cardiac preconditioning [51]. An active peptide agonist has been identified by our colleague, Prof. Daria Mochly-Rosen, and her coworkers. Unfortunately, this peptide alone does not enter cells and had to be delivered by microinjection. Efficient delivery of the cell-impermeable ψεRACK peptide, a PKC-ε agonist, would thus provide the basis for an effective therapeutic that mimics the effects of clinical cardiac preconditioning. Conjugates of this peptide and an arginine transporter were formed via a disulfide linkage that could be cleaved in vivo to liberate the active peptide. When administered to isolated cardiac myocytes (data not shown) and whole rat hearts (Scheme 14), these conjugates were rapidly taken up, releasing their active peptide cargo and providing significant cardioprotection (ca. 80 %), as evidenced by a decrease in the release of creatine phosphokinase (CK) [5d]. Furthermore, the R_7 conjugate was found to be a superior vehicle for delivery of the ψεRACK peptide compared to the naturally derived Tat delivery system. In summary, the oligoarginine transporter enables the delivery of the ψεRACK peptide into tissue and cells where it is released to act on its intracellular target. This bodes well for the general use of such transporters for the delivery of peptides and other cargoes into cells and tissue.



Scheme 14

In addition to crossing cell membranes efficiently, the oligoarginine transporters are able to penetrate tissue barriers such as skin. Skin is the primary barrier between an organism and the external envi-

ronment, and as such, represents a formidable obstacle to the delivery of therapeutics. Thus, local delivery would allow for the avoidance of systemic side effects associated with the oral administration of some drugs. For example, cyclosporine A can be used systemically to treat dermal disorders, but its oral use often results in irreversible organ damage. Unfortunately, cyclosporine A does not penetrate the skin when applied topically. However, when a conjugate of cyclosporine A with R_7 is applied to human skin, it penetrates efficiently and inhibits cutaneous inflammation [5f]. Conjugates of these arginine-rich transporters and cyclosporine A are currently in human clinical trials.

ACKNOWLEDGMENTS

Financial support for aspects of the work described herein was provided by grants from the National Institutes of Health (CA 31841, CA 31845), the National Science Foundation (CHE-0131944), Eli Lilly & Co., CellGate, Inc., and GPC Biotech. This work is a product of many exceptionally talented, creative, and dedicated investigators whose names appear as coauthors and in the references provided.

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