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PERSPECTIVE

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Macrocycles Are Great Cycles: Applications, Opportunities, and Challenges of Synthetic Macrocycles in Drug Discovery

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1. INTRODUCTION

Macrocycles occupy a unique segment of chemical space. In the past decade, their chemical diversity expanded significantly, supported by advances in bioinformatics and synthetic methodology. As a consequence, this structural type has now been successfully tested on most biological target classes. The goal of this article is to put into perspective the current applications, opportunities, and challenges associated with synthetic macrocycles in drug discovery.[1]

Historically, macrocyclic drug candidates have originated primarily from two sources. The first, natural products, provided unique drugs such as erythromycin, rapamycin, vancomycin, cyclosporin, and epothilone. Excellent reviews are dedicated to this class and how it inspired further synthetic and medicinal chemistry efforts; thus, it will not be covered here.[1−3] From a molecular evolution standpoint, the medicinal chemistry of macrocyclic natural products usually involved direct use as a therapeutic agent or functionalization of the natural product scaffold by hemisynthesis. It parallels significant advances in the total synthesis of macrocyclic natural products during the past 2 decades.[4,5]

The second traditional source of macrocycles stems from peptides, some of which are natural products and, hence, also belong to the first category. Macrocyclization was recognized early in peptide chemistry as an efficient way to restrict peptide conformation, reduce polarity, increase proteolytic stability, and consequently improve druggability.[6−10] Chemists accessed macrocyclic peptides with different geometries (head to tail, side chain to side chain, head to side chain), including the incorporation of nonpeptidic groups.[6,7,10] Compelling examples of macrocyclic scaffolding of peptides include the works on somatostatins, melanocortins, and integrins, among others.[11−14] Macrocyclic peptides generated several drugs from synthetic or natural sources, including octreotide, cyclosporine, eptifibatide, and caspofungin. Purely peptidic, depsipeptidic, and peptoid macrocycles will also not be covered in this article; the reader is instead referred to previous reviews.[6,15,16] It is well understood that the boundary between synthetic macrocycles and the above categories is not always clear-cut; as a result, examples presented in the following sections could occasionally belong to one of these categories. In these cases, they were selected owing to their relevance to the perspective.

Macrocycles are defined herein as molecules containing at least one large ring composed of 12 or more atoms. On the basis of standard molecular descriptors, macrocycles as a class are at the outskirts of the window generally considered optimal for good PK-ADME properties using these criteria.[17] Indeed, their molecular weights tend to be on the higher end (often in the 500−900 g·mol^{-1} range), their numbers of H-bond donors and acceptors, as well as their polar surface area (PSA), tend to be on the far side of the accepted druglike spectrum.[18] For an equal number of heavy atoms, macrocycles inherently possess a lower number of rotatable bonds than their acyclic analogues, a beneficial feature for oral bioavailability (in the following, "acyclic" will be used in the sense of "nonmacrocyclic").[18] As a result, macrocycles are more conformationally restricted than their acyclic analogues, which potentially can impart higher target binding and selectivity and improved oral bioavailability (in this assessment, endocyclic bonds are considered to be nonrotatable, which is only an approximation; see ref 18). For a systematic chemoinformatic analysis of biologically active macrocycles, the reader is referred to the recent review of Brandt et al.[19] Topologically, macrocycles have the unique ability to span large surface areas while remaining conformationally restricted compared to acyclic molecules of equivalent molecular weight. This characteristic makes them especially suited for targets displaying shallow surfaces, which can prove to be quite challenging for acyclic small molecules.

Medicinal chemistry relies strategically on robust synthetic methods capable of producing an acceptable chemical diversity to adequately interrogate the chemical space of a biological target. Macrocycles are often (and rightly so) perceived as difficult to synthesize and hence deterred many medicinal chemists because of the lack of versatile synthetic platforms. The macrocyclization step is regularly plagued by low yields and often requires high dilution conditions to counterbalance entropic loss. In other words, the reduction in entropy responsible for beneficial conformational restrictions to the final molecule comes at a price during synthesis: what goes around comes around.

Accordingly, the first part of this article is dedicated to the drug discovery aspects of macrocycles and highlights salient features of their medicinal chemistry. This section is organized by target class, a choice aimed at providing the reader an appreciation of the structural diversity generated for each class. To give the reader an appreciation of the tools available to construct macrocyclic scaffolds, the site and method of the pivotal macrocyclization step are indicated in the figures. Readers are referred to the source articles for further details. In the second part, the technologies and synthetic approaches that already have demonstrated utility or possess a high potential for macrocycle-based

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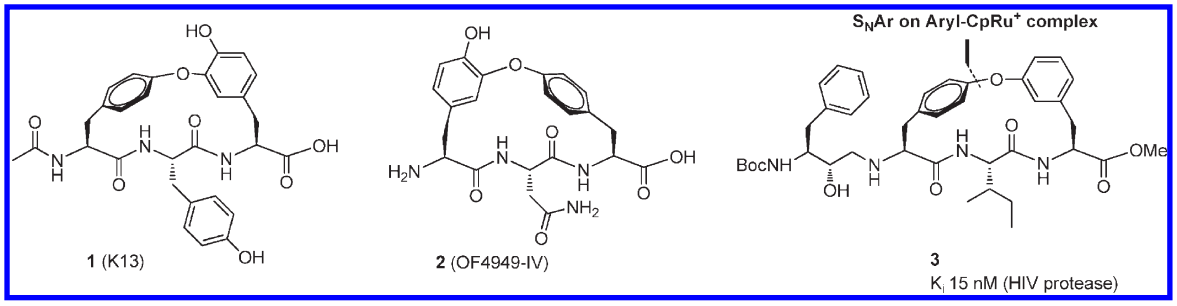

Figure 1. Macrocyclic biphenyl ether scaffold.

drug discovery are discussed. Finally, a perspective on the future of synthetic macrocycles in medicinal chemistry is offered.

2. APPLICATIONS OF SYNTHETIC MACROCYCLES IN DRUG DISCOVERY

Macrocycles have been used successfully on most pharmaceutical target classes. The largest number of reports comes from the protease field, a consequence of the development of rigidified peptide ligand mimics. Second, agonists and antagonists of G protein-coupled receptors (GPCRs) have been generated with macrocyclic structures. The third target class, and perhaps the one possessing the greatest growth potential given the high level of interest it attracted in the past few years, is the disruption of protein−protein interactions, which exploits the unique properties of the macrocyclic topology. Rather than reviewing extensively every macrocycle on each target class, we will focus on selected examples and put in perspective their characteristics, including advantages and limitations over acyclic analogues when available.

2.1. Macrocyclic Enzyme Inhibitors. Many programs aimed at identifying enzyme inhibitors started from X-ray structures of the enzyme's substrate or hydrolysis product in complex with the enzyme. In the protease field, macrocyclization proved to be an efficient way to bias the conformation of a lead compound toward a bound conformation while simultaneously increasing proteolytic stability and reducing polarity.[6,7,10]

Several X-ray structures revealed that proteases universally recognize their ligands in the β-strand conformation, biasing the rational design of inhibitors toward this specific secondary structure.[20,21] This mode of interaction is also observed in several naturally occurring macrocyclic protease inhibitors such as the ACE inhibitor **1** (K-13) and aminopeptidase B inhibitor **2** (OF4949-IV),[22,23] which inspired the design of several compounds including the HIV protease inhibitor **3** (Figure 1).[24] The different types of β-strand mimics incorporated in macrocycles have been reviewed by Glenn and Fairlie.[25] Macrocyclization was recognized in the 1980s as an efficient way to restrict peptidic transition state mimics into conformations closely resembling those found in enzyme−inhibitor complexes.[7] Pioneering works from several groups established the required features of macrocyclic β-strand mimics via the use of constrained side chain to backbone cyclization for aspartic and serine proteases.[24,26−28] This class of macrocyclic inhibitors was used extensively to counteract the cooperative nature of enzyme−inhibitor binding (induced fit),[29] a mutual recognition that can have short- or long-distance conformational repercussions and can impede the design of selective inhibitors.[30−32]

In the following sections, we have selected several examples of the optimization process leading to protease inhibitors from diverse chemical classes to give the reader a sense of the potential and challenges associated with macrocyclic protease inhibitors. Because of space limitations, all the published examples were not included.

2.1.1. Renin Inhibitors. Renin, a member of the aspartyl protease family, catalyzes the first and rate-determining step of the renin−angiotensin cascade and constitutes a validated target for the regulation of blood pressure. The only known substrate of this aspartyl protease is angiotensinogen (**4**, Figure 2), making this target a potentially very specific one.[33] Early macrocyclic inhibitors of renin (e.g., **5**−**7**) based on the P2−P1 macrocyclization of a bioactive angiotensinogen fragment[34,35] demonstrated the impact of cyclization and ring size on inhibition. **7** displayed good selectivity toward other aspartyl proteases such as pepsin and cathepsin D and was resistant to proteolytic cleavage by chymotrypsin. However, insufficient potency (micromolar range) precluded further development. On the other hand, P4−P2 macrocyclization led to more potent inhibitors such as **8** (IC_{50} = 0.29 nM).[36] Several groups exploited the spatial proximity of the P2 and P1′ sites of renin-bound angiotensinogen.[37−39] For example, Weber et al. reported **9** (IC_{50} = 590 nM) as a first generation compound. Further extension toward the P4 site delivered inhibitor **10** (IC_{50} = 56 nM), which was further evolved into potent inhibitor **11** (IC_{50} = 1.3 nM).[40] When administered iv to sodium-depleted rhesus monkeys at 0.05 mpk, **11** demonstrated a rapid and complete inhibition of PRA (plasma renin activity), along with a sharp decrease in blood pressure, which was unfortunately short-lived. Upon oral dosing at 15 mpk, the effect was sustained for 6 h, despite a low 1% bioavailability. Subsequent metabolism studies indicated cleavage of the serine ester bond of macrocycle **11** and extensive first pass hepatic extraction.

This collection of renin inhibitors exemplifies how enzyme inhibition can be tackled with macrocyclic peptidomimetics under different modes of cyclization. Both peptidic and transition state isosteres were tested in these efforts, leading ultimately to orally efficacious macrocycles like **11**.

2.1.2. Neutral Endopeptidase 24.11 Inhibitors. McPherson et al. reported macrocyclic inhibitor **12** (Figure 3) as a potent inhibitor of neutral endopeptidase (NEP) 24.11.[41] NEP is a zinc metalloprotease responsible for the first hydrolytic step of atrial natriuretic factor (ANF), a hormonal target for cardiovascular disorders due to its involvement in natriuresis, diuresis, and lowering of blood pressure. Design of the macrocycle was inspired by the X-ray structure of a linear transition state inhibitor of thermolysin, which revealed two contiguous hydrophobic pockets.[42] Macrocycle **12** was obtained after thorough optimization of the exocyclic amide substituent. It displayed subnanomolar inhibition of NEP 24.11. Its double prodrug **13** (CGS-25155)

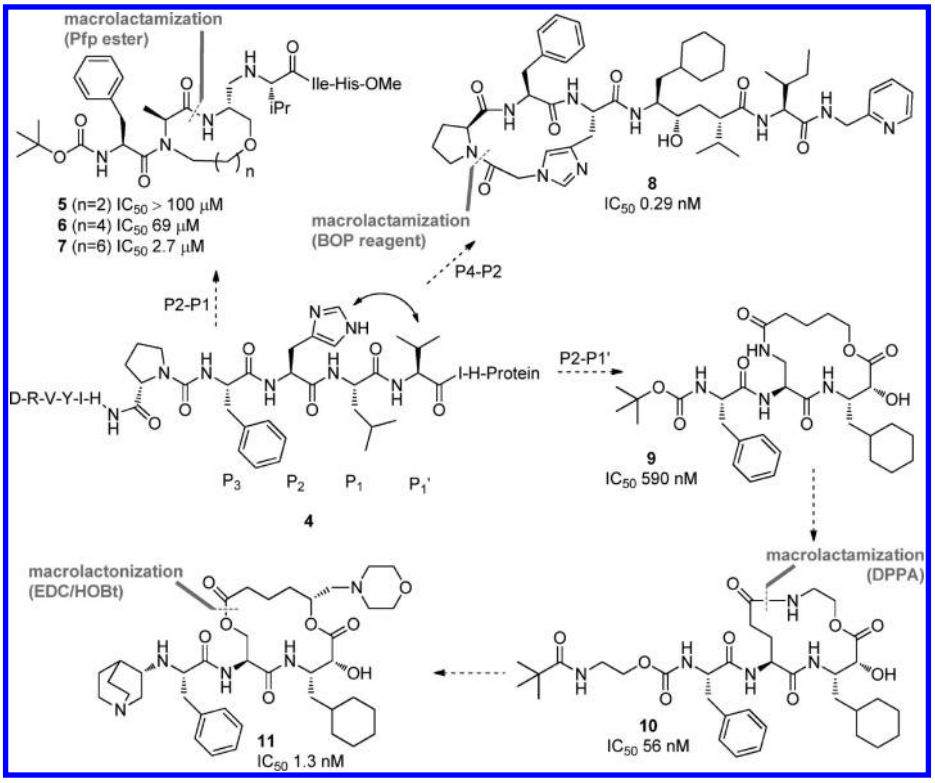

Figure 2. Macrocyclic renin inhibitors.

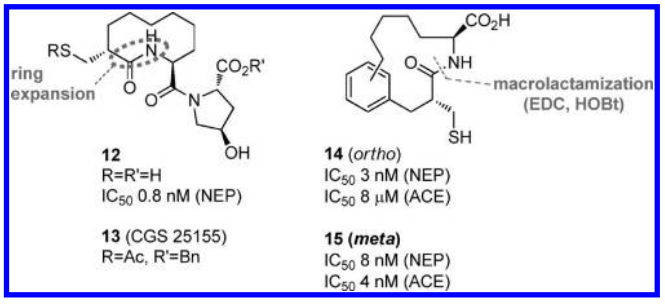

Figure 3. Macrocyclic NEP inhibitors.

provided a sustained decrease in mean arterial blood pressure of hypertensive rats after administration at 30 mpk, with an effect lasting up to 6 h. **12**, **13**, and analogues were obtained by two consecutive ring expansions from cyclooctanone. Ksander et al. reported two similar series of NEP inhibitors. The first, based on ortho-substituted macrocycle **14**, displayed potent NEP inhibition with a high selectivity for angiotensin-converting enzyme (ACE).[43,44] Computer-aided design led to meta-substituted analogue **15**, which possessed a dual NEP/ACE inhibitory profile. The stereochemistry of the thiol-bearing center was found to be important for enzyme inhibition. This is an excellent example of how the balance of conformational restriction and flexibility of similar macrocyclic scaffolds can be exploited to fine-tune selectivity.

2.1.3. Thrombin Inhibitors. Inhibitors of the serine protease thrombin have a well-recognized potential for the treatment of deep vein thrombosis, pulmonary embolism, and thromboembolic stroke. Examination of X-ray structures of thrombin in complex with the highly potent acyclic inhibitor **16** (Figure 4)[45] revealed the proximity of the P3 and P1 groups, leading Nantermet et al. to restrict conformational freedom of the molecule via macrocyclization.[46] Macrocycles **17** did not possess improved potency; however, it retained good selectivity (>2000-fold) vs trypsin and tissue plasminogen activator (tPa). Replacement of the proline moiety with a pyrazinone led to inhibitor **18** possessing outstanding potency (K_i = 0.09 nM) and selectivity against trypsin and tPa (23000- and 7100-fold, respectively). The secondary amine moiety of **18** was crucial for interaction with Glu[192] of thrombin, a residue replaced by Gln in both trypsin and tPa, leading to the increased potency and selectivity observed. To be noted, these macrocycles are quite different from the macrocyclic natural product cyclotheonamide A (**19**), which is also an inhibitor of thrombin (K_i = 0.18 μM).[47]

2.1.4. HIV Protease Inhibitors. The HIV protease is a clinically validated target for the management of HIV. As with other aspartyl proteases, its inhibition was successfully approached using the hydroxyethylamino transition state mimic. Inspired by the structure of natural products **1** and **2**, Janetka et al. reported biphenyl ether **3** (K_i = 15 nM, Figure 1) as an early inhibitor of HIV protease.[24] Macrocyclization imposed a conformationally restricted extended β-strand structure to its peptidic portion. The para−meta substitution pattern on the diphenyl ether was 60-fold more potent than the meta−para isomer, suggesting a better fit of the side chains in the protease active site. Macrocyclization was performed using S_NAr via a ruthenium π-aryl complex.[48] Additional inhibitors possessing an endocyclic phenoxy ether were also reported.[49]

The crystal structure of the HIV protease in complex with acyclic hydroxyethylene isostere inhibitor **20** (JG365)[50] led to the design of several macrocyclic inhibitors, exemplified by **21** (K_i = 12 nM, Figure 5).[51] These 15- to 17-membered macrocycles all possess the β-strand conformation.

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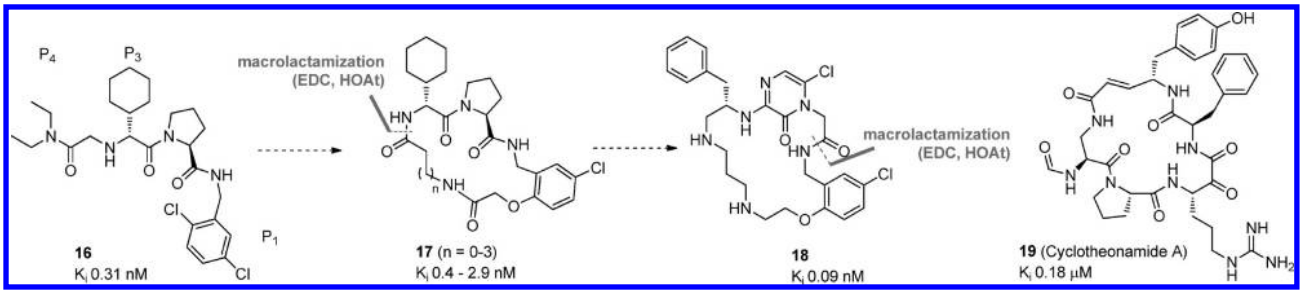

Figure 4. Macrocyclic thrombin inhibitors.

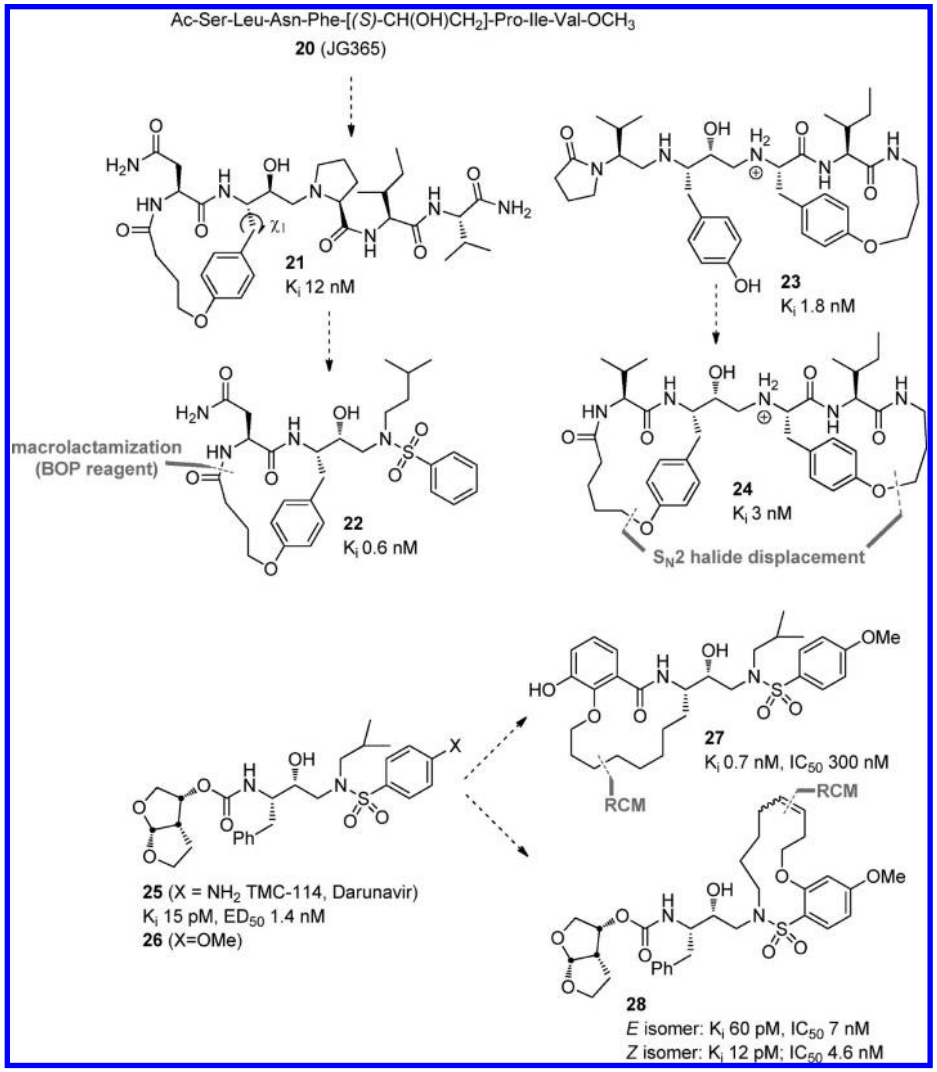

Figure 5. Macrocyclic HIV protease inhibitors.

Inhibitors **20** and **21** adopt similar binding modes, with the macrocyclic structure imposing a difference of 25° in the χ_1 dihedral angle of the endocyclic aromatic ring in **21**. Similar to their acyclic congeners, inhibitor **22** (K_i = 0.6 nM)[52] and analogues were subject to the known switching stereoisomer preference of HIV protease inhibitors,[53] imposed by the difference in hydrophobicity of the P1′ residue. Replacement of the Asn moiety of **22** by Val decreased binding by about 1 order of magnitude, due to the loss of an important H-bond.

On the basis of the same starting inhibitor (**20**), cyclization was investigated on the C-terminal end of the peptide, giving analogues such as **23** bearing a positive ammonium group. Compared to previous analogues displaying interactions of both hydroxyl groups with Asp^{25} and Asp^{125} in the catalytic site, the hydroxyl group of **23** interacted with Asp^{125} whereas its ammonium group interacted with Asp^{25} and the macrocycle possessed an extra atom. This change was accommodated by a translation of about 1.5 Å in the crystal structure. With both N- and C-terminal ends of the peptidomimetic amenable to macrocyclization

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(**22** and **23**), it was tempting to determine whether the enzyme's active site could simultaneously house a macrocyclic substructure at both ends of the inhibitor. The K_i value of 3 nM for inhibitor **24** positively answered this question; however, the presence of the two macrocycles did not bring a synergistic increase in binding.[54] Structurally, the binding mode of the C-terminal macrocyclic inhibitor **23** was imposed, with hydroxyl and ammonium groups simultaneously interacting with Asp^{125} and Asp^{25}, respectively. This class of macrocycles mimics tripeptide β-strand structures in water, a motif universally recognized by proteases.[21,55] Moreover, these macrocycles adopted a rigid topology (large $^3J_{NH-CH\alpha}$ of 9−10 Hz) with no intramolecular hydrogen bond associated with typical turn structures observed (all amide bond chemical shifts were temperature dependent, with $\Delta\delta/\Delta T$ values of 7−12 ppb/deg). This combination of structural features created an ideal template for proteases. Bismacrocycle **24** and its analogues were also used as probes to further elucidate the catalytic mechanism.[56]

The X-ray structure of highly potent HIV inhibitor **25** (FDA-approved darunavir, Figure 5) and its analogue **26** in complex with the HIV protease indicated a proximity between the P1 and P2 domains. On the basis of this observation, Ghosh et al.[57] incorporated the left-hand side of the molecule in a series of 13- to 15-membered macrocycles. Darunavir was initially designed to (1) inhibit HIV protease and (2) prevent the key step of HIV protease dimerization while minimizing interactions with the variable side chains of the protease and maximizing interactions with the less variable protease backbone. The most potent analogue of the series, **27**, displayed subnanomolar potency on the isolated enzyme. Ring size or the presence of endocyclic unsaturation had minimal impact on potency. Likewise, open-chain structures also displayed potencies in the low nanomolar level. In order to fill the S1′ and S2′ subsites, the authors investigated flexible macrocyclic analogues retaining polar backbone interactions while maximizing van der Waals interactions.[58] As a result, 13-membered macrocycles **28** displayed exquisite inhibitory activity in vitro and in cellulo. To be noted, 10- to 15-membered macrocycles were synthesized with potencies varying essentially within 1 log unit; the 10- and 13-membered analogues showed the best potencies and unsaturation generally proved beneficial. In contrast, acyclic derivatives possessed K_i between 0.1 and 17 nM and in cellulo IC_{50} above 300 nM, emphasizing the benefits of macrocyclization on cellular penetration. Of particular interest, these macrocycles possessed excellent activity profiles against several multidrug-resistant clinical isolates, an effect attributed to the combination of (1) direct binding to the less variable protease backbone domain and (2) occupancy of a hydrophobic pocket in the S1′−S2′ subsites. **28** possessed more van der Waals interactions with the HIV protease than **25** (Val^{84} and Ile^{82}).[59] In general, these macrocycles, as well as other members of the same family,[60] were synthesized using ring-closing metathesis (RCM). Similar macrocyclic scaffolds were successfully applied to generate inhibitors of other pharmaceutically relevant proteases, including calpain,[61] plasmepsins,[62] peptide deformylase,[63] matrix metalloproteases,[64−66] and TNF-α converting enzyme (TACE).[67,68]

2.1.5. HCV NS3 Protease Inhibitors. The hepatitis C virus (HCV) NS3 protease is an essential enzyme in the replication of the virus and a clinically validated target for the treatment of hepatitis C. The most advanced HCV protease inhibitors, **35** (telaprevir, Figure 7) and **36** (boceprevir, SCH503034), are currently in phase III clinical development.[69] Both are acyclic covalent transition state mimics featuring a reversible trap for the catalytic Ser^{139} residue on their C terminus. Structurally, the particularity of this serine protease is the presence of a shallow, solvent-exposed cleft as the S4−S1 substrate binding region, which proved challenging to target with small molecules.[70,71] Structural analysis of the target revealed that the S3−S1, S4−S2, and S3−S2 binding pockets were in proximity, providing the rationale for several modes of macrocyclization.

2.1.5.1. P3−P1 Inhibitors. The first macrocyclic inhibitors were designed based on the observation that the N-terminal cleavage products of the endogenous substrate possess inhibitory activity.[72,73] These products interact with the target in extended conformations with high affinities, yet their peptidic nature precluded further development for the intended indication.[74] Extensive NMR and molecular modeling based studies of NS3 interactions with its ligand and cofactor NS4A led to the understanding that ligand binding induces stabilization of the NS3 binding site. Binding of substrate-based inhibitor **29** in an extended conformation induced rigidification (Figure 6),[75,76] bringing the side chains of Nva in P1 and Val in P3 in proximity and providing the grounds for macrocyclization.[74,77] Combination with the potent heterocyclic proline substituent of lead series **30**[77,78] led ultimately to clinical candidate **32** (BILN 2061, ciluprevir).[79−81] Advantages of this transformation included preorganization of the molecule in a binding conformation, restriction of the P3−P2 proline amide bond in the trans geometry, and reduction of the inherent cis/trans equilibrium observed in linear peptides. This resulted in decreased binding entropy and a reduction in overall polarity. Macrocyclization was estimated to improve binding by 30- to 40-fold,[74] allowing the removal of solvent-exposed P6−P4 fragments and a significant reduction in total polar surface area (PSA) and number of rotatable bonds,[18] despite only a modest decrease in molecular weight (804−744 Da). Ring size and macrocycle topology were found to be crucial to maintain potency.[74] Although the pharmacokinetic (PK) profile of linear hexapeptide **29** has not been reported, it is expected to be poorly cell-permeable with low bioavailability. Clinical candidate **32** displayed high inhibition of the genotype 1a and 1b enzymes in vitro (K_i of 0.3 and 0.66 nM), as well as high activity in cellulo (EC_{50} of 3 and 4 nM), indicative of high cellular penetration. The PK profile demonstrated species-dependent oral bioavailability (7−36%) and half-life (0.8−7 h) with low clearance (1−14 mL/min/kg) and high liver/plasma ratio, a crucial parameter for target exposure. Macrocycle **32** was the first macrocyclic HCV NS3/4A protease inhibitor to enter clinical development and was well tolerated and efficacious in phase 1 and 2 clinical studies.[81,82] Clinical development of **32** was, however, halted because of cardiotoxicity observed in 4-week rhesus monkey studies.[82]

The story of **32** exemplifies the value of thorough biophysical studies in lead optimization. Although **32** did not reach the finish line, it certainly blazed the trail for numerous congeners.[83] Indeed, two P3−P1 macrocyclic analogues are currently in the clinic, **33** (TMC435350) and **34** (ITMN-191, danoprevir).[84,85] Compared to **32**, **33** involved the replacement of the hydroxyproline moiety of **32** by a cyclopentane core, as well as modifications to the exocyclic substituents.[85,86] It possessed lower cellular efficacy compared to **32**; however, it had improved oral bioavailability despite a higher clearance. Following once-daily administration in phase 1 clinical development, **33** was found to be safe and well tolerated, demonstrating a robust viral load decline.[85−87] The crystal structure of **33** in complex with the

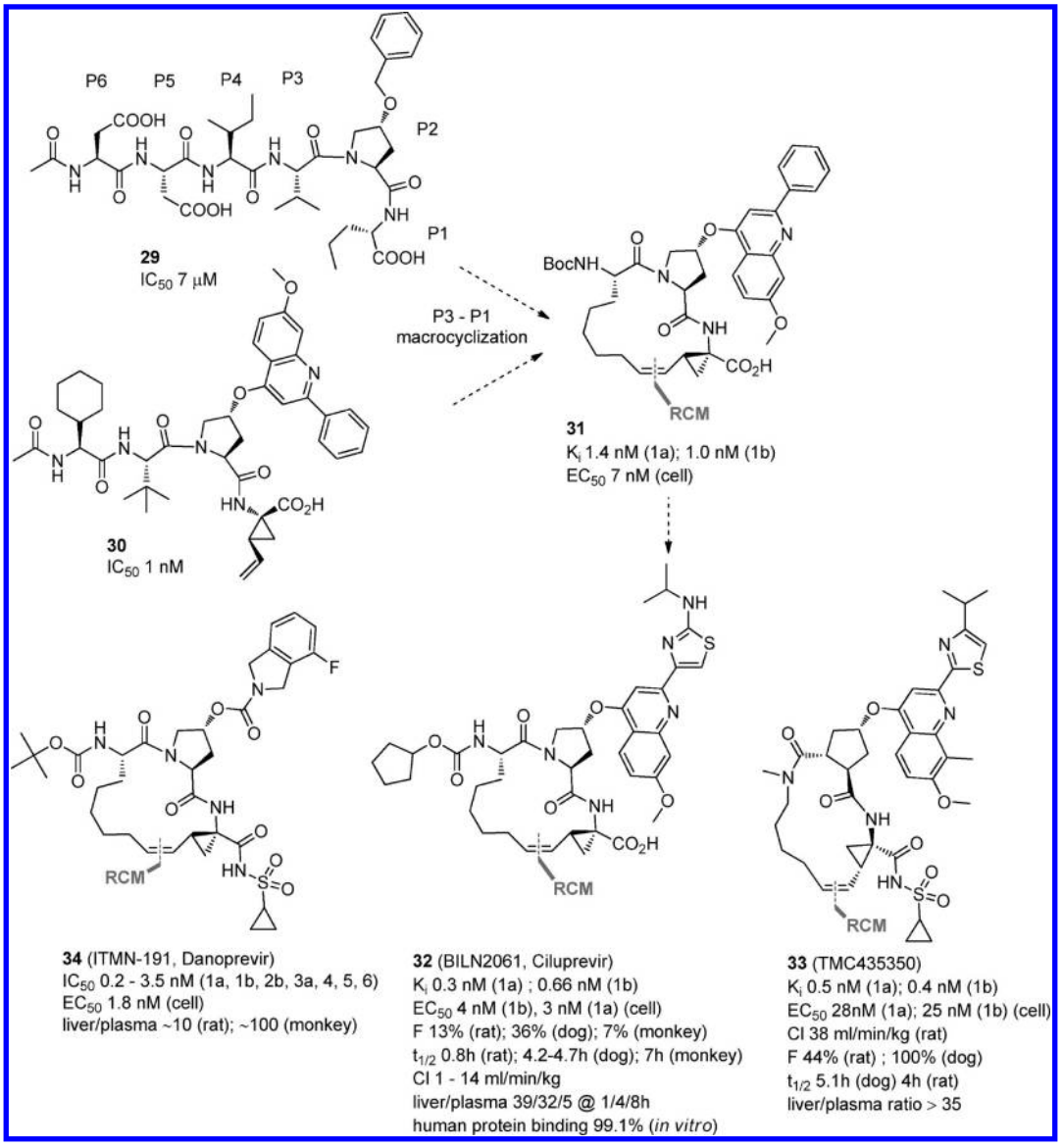

Figure 6. Molecular evolution toward macrocyclic P3−P1 HCV NS3 protease inhibitors.

HCV NS3/4A protease at a resolution of 2.4 Å revealed docking into an extended S2 subsite to accommodate a large P2 substituent, providing insights on the interface between the protease and its partner, the RNA helicase.[88] On the same scaffold, the hydroxyproline group was also replaced by a urea-linked hydroxyproline moiety, which improved PK-ADME properties while conserving strong inhibitory activities both in vitro and in cellulo.[89] Further optimization of the exocyclic heterocyclic P2 group was also used to modulate PK-ADME properties.[90]

34 conserved the macrocyclic core of **32**, combined with a fluoroisoindane proline exocyclic group and the ketosulfonamide P1′ group of **33**.[84,91] It possessed very high potency in cellulo, as well as a high liver/plasma ratio.

A second class of P3−P1 macrocycles (Figure 7) incorporated the reversible covalent serine trap found in linear inhibitor **36**.[69,92,93] Starting from the structure of **36**, cyclization between the P3 and P1 positions followed by further optimization led to **37**.[94] The presence of the ketoamide moiety, combined with an exocyclic extension into the P2′−P3′ (**38**) or the P4 region (**37**), conferred potent and slow binding kinetics. **37** possessed high binding potency in vitro (K_i^* = 3 nM) and 11-fold improved efficacy in the cellular replicon assay compared to **36** (30 nM vs 350 nM). It was also 2800-fold selective vs human neutrophil elastase. The chemical nature of the macrocyclic arm had a crucial impact on enzyme interaction. Compared to **36**, macrocyclization provided improved enzyme binding and cellular activity, likely due to increased cellular penetration, as well as higher elastase selectivity.[94] A distinct potent macrocyclic scaffold **39** was also reported from the same team.[95] Macrocyclization enhanced binding by 50- to 80-fold compared to linear congeners; however, the compound possessed low oral bioavailability in rats (<10%).[95] Finally, macrocycles **40** and **41** deserve attention.[96,97] Both feature a macrocyclization from the hydroxyproline to the P3 residue and possess the serine trap moiety of clinical candidate **35**. In order to increase potency in vitro and in cellulo, extension into the P1′−P2′ sites was necessary (**41**). However, this was done at the expense of PK. Indeed, analogue **40**, without this extension, despite much lower potency, displayed a remarkable 97% oral bioavailability and identical in vitro and in cellulo potencies, indicating excellent cell permeability.

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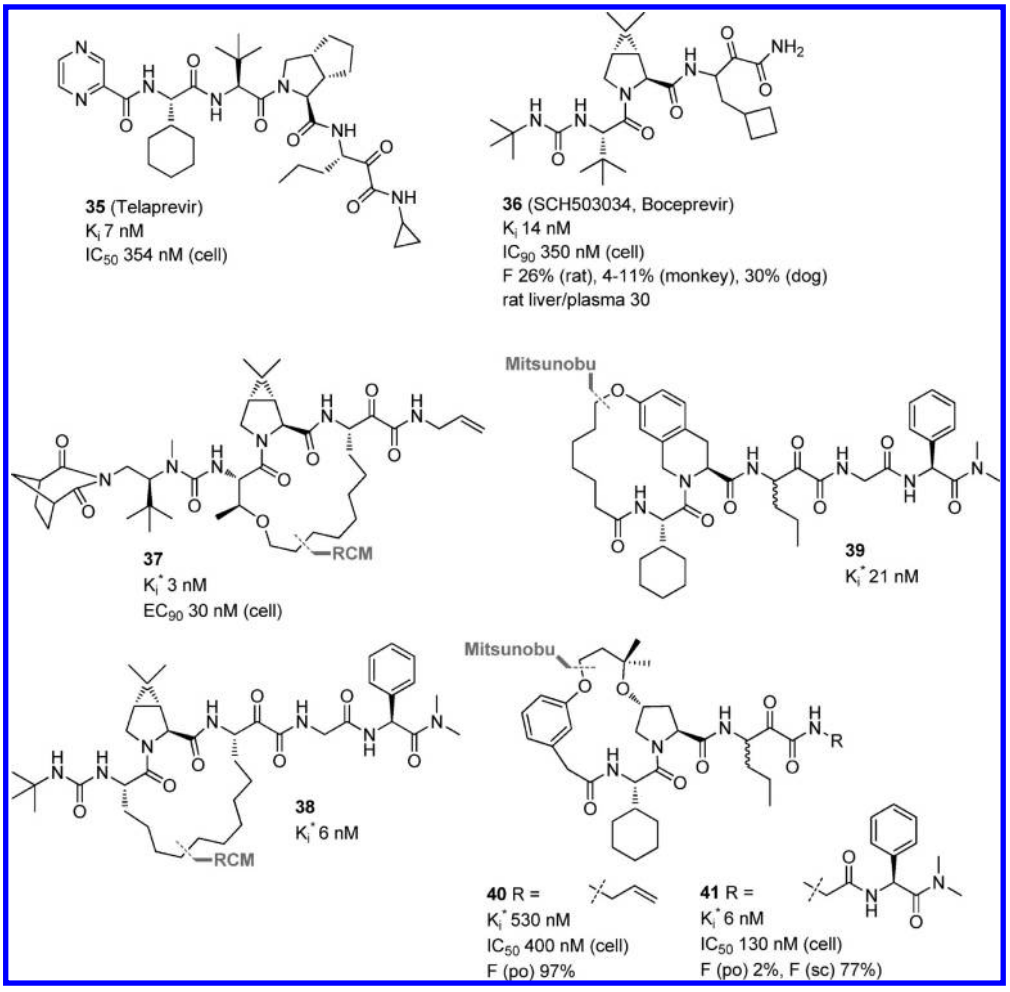

Figure 7. Macrocyclic covalent reversible HCV NS3 protease inhibitors.

Thus, the potency gained by a peptidic extension of **41** was detrimental to PK and cell penetration. This is in contrast with **37**, which did not bear the P1′−P2′ extension but instead protruded into the P4 region.

2.1.5.2. P4−P2 Inhibitors. Molecular modeling analysis of **32** and analogues in complex with the NS3 protease suggested that its thiazolylquinoline portion laid on a featureless enzyme surface, an observation hardly compatible with nonspecific interactions given that this part of the molecule was responsible for a dramatic improvement in potency (above 30000-fold).[98] Modeling studies based on the apoenzyme helicase domain supported the hypothesis of a direct docking of the heterocycle into a helicase pocket, leaving sufficient space for a direct linkage to the carbamate side chain. This provided arguments for ring closure between the heterocycle and the carbamate side chain, leading to macrocycle **42** (Figure 8).[98] The latter possessed subnanomolar binding and low nanomolar cellular efficacy, retaining high liver exposure. In contrast, its analogue **43**, differing only in the substitution pattern of the quinolyl group, was significantly more potent as an inhibitor but provided poor liver exposure. Further molecular evolution ultimately delivered clinical candidate **44** (MK-7009, vaniprevir).[99,100] Of note, ring size did not have a profound effect on SAR in this series. **44** possessed outstanding in vitro (IC_{50} = 50 pM) and excellent in cellulo potency (IC_{50} = 3−20 nM), combined with advantageous selectivity compared to several other NS3/4A inhibitors. It also possessed medium-to-high clearance, relatively short half-life (∼1 h), and low bioavailability (<15%) in rat, dog, and rhesus monkey. **44** appropriately displayed high liver concentrations at 24 h postdosing, particularly in chimpanzee (2400-fold cellular EC_{50}), ensuring high target exposure. On the basis of this latter observation, the compound was advanced into clinical development.[99]

The same research team also achieved the tantalizing synthesis of a doubly macrocyclic structure combining both P3−P1 and P4−P2 macrocyclization. The resulting compound **45** possessed exquisite potency against genotype 1b protease and a 5-fold increased potency against genotype 3a enzyme compared to its P3−P1 acyclic congener. The specific impact of double macrocyclization on the PK-ADME profile was not reported. Finally, Li et al. recently reported novel NS3 inhibitors exploiting the same scaffold as **44**, in which the serine trap was replaced by an aminoboronate, as exemplified by **46**.[101] This series of analogues reached submicromolar cellular inhibition of the NS3 protease. The authors demonstrated, based on X-ray analysis, that the boron atom indeed trapped the protease Ser^{139} moiety.

Inhibition of the HCV NS3/4A protease provides a compelling example of how macrocycles can make a significant difference in drug discovery. As amply demonstrated, multiple modes of macrocyclization were successfully applied to develop inhibitors of this protease, which otherwise turned out to be very difficult to tackle with traditional small molecules because of the shallow topology of its binding site. Comparison of the

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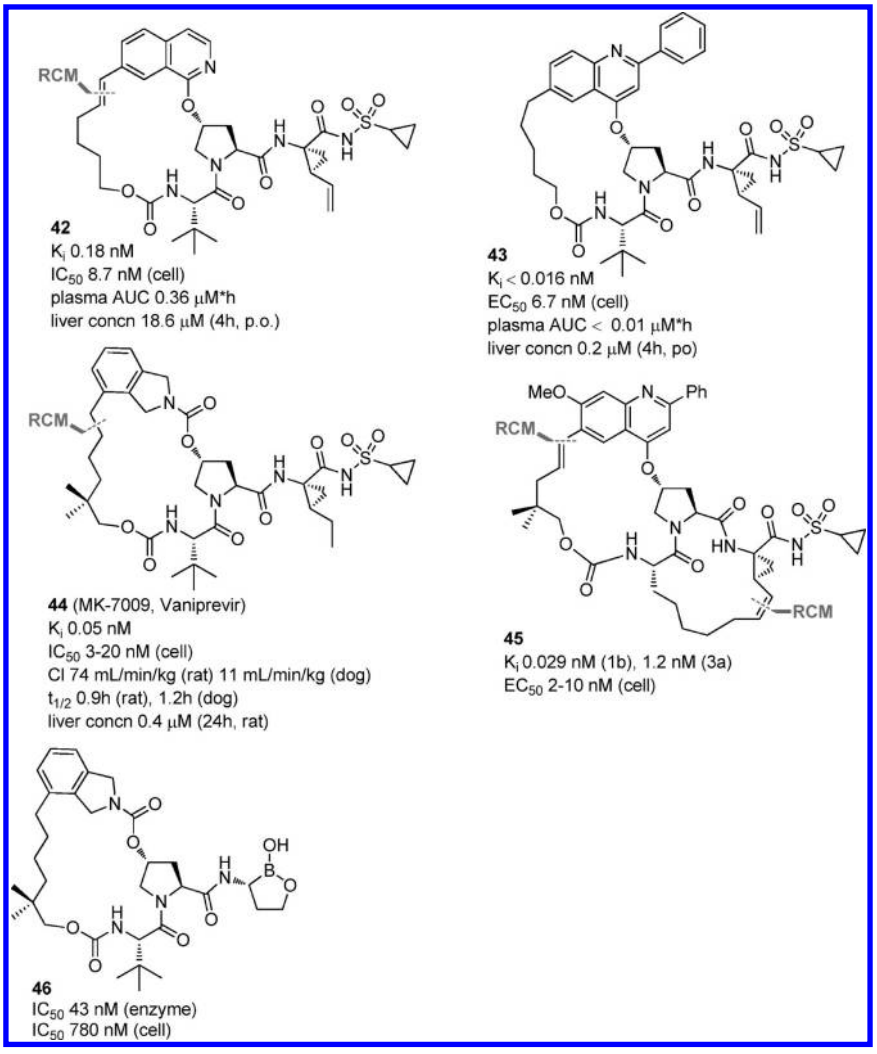

Figure 8. Macrocyclic P4−P2 HCV NS3 protease inhibitors.

pharmacodynamic and PK-ADME profile of acyclic and macrocyclic drug candidates currently in clinical development clearly indicated that both dimensions could be improved from the former to the latter chemical class, particularly regarding the critical parameter of cellular penetration. These examples also demonstrated the tremendous utility of incorporating biophysical and computational approaches in macrocyclic drug discovery. In this instance, target location in hepatocytes tolerated reasonably high lipophilicities, contributing to high hepatic accumulation (typical liver/plasma ratios varied between 30:1 and 100:1).

2.1.6. β-Secretase (BACE-1) Inhibitors. β-Amyloid cleaving enzyme (BACE-1, memapsin-2) is the principal β-secretase in neurons. Cleavage of the amyloid precursor protein (APP) by BACE-1 is the rate-limiting step in the production of the neurotoxic peptide fragment $A\beta_{1-42}$, the major component of the insoluble β-amyloid plaque and one of the pathological hallmarks of Alzheimer's disease. As a result, inhibition of BACE-1 is considered a promising therapeutic strategy for the treatment of this debilitating condition. Compared to HIV and HCV proteases, inhibitors of BACE-1 are faced with the additional challenge of crossing the blood−brain barrier (BBB) to reach their site of action, which generally requires compounds of acceptably lower polarity and molecular weight.[102]

X-ray structures of linear inhibitors **47** (OM99-2) and **48a** in complex with BACE-1 (Figure 9) revealed the proximity of the P3−P1 and P1−N2 portions of these peptidomimetics, as well as their positioning in a large P3−P1 lipophilic pocket.[103] On the basis of these observations, Rojo et al. reported a first series of macrocyclic BACE-1 inhibitors, exemplified by **48b** and **49**.[104] The P3−P1 cyclization, which removed a beneficial aromatic π−π interaction between the P1 side chain and the enzyme, led to 13-membered macrocycle **48b** possessing decreased enzymatic activity and no measurable cellular activity. In contrast, the P1−N2 cyclization, with the same deletion of a beneficial interaction, preserved enzymatic activity and increased cellular activity as demonstrated by the smaller 10-membered macrocycle **49**. Thus, it seemed that the lack of a major interaction in the P1 cavity was compensated for by the additional preorganization of the molecule and that a smaller macrocycle masking one amide bond was key to improving cellular penetration and cellular activity.

Transition state mimic **47** inspired the design of **50** as a minimal inhibitor with K_i = 1.4 μM and poor activity in cellulo. Analysis of the 2.0 Å resolution crystal structure of **50** in complex with BACE-1 revealed that the P3 and P1 side chains were within 3.8 Å and in a reasonably open area of the protease, making

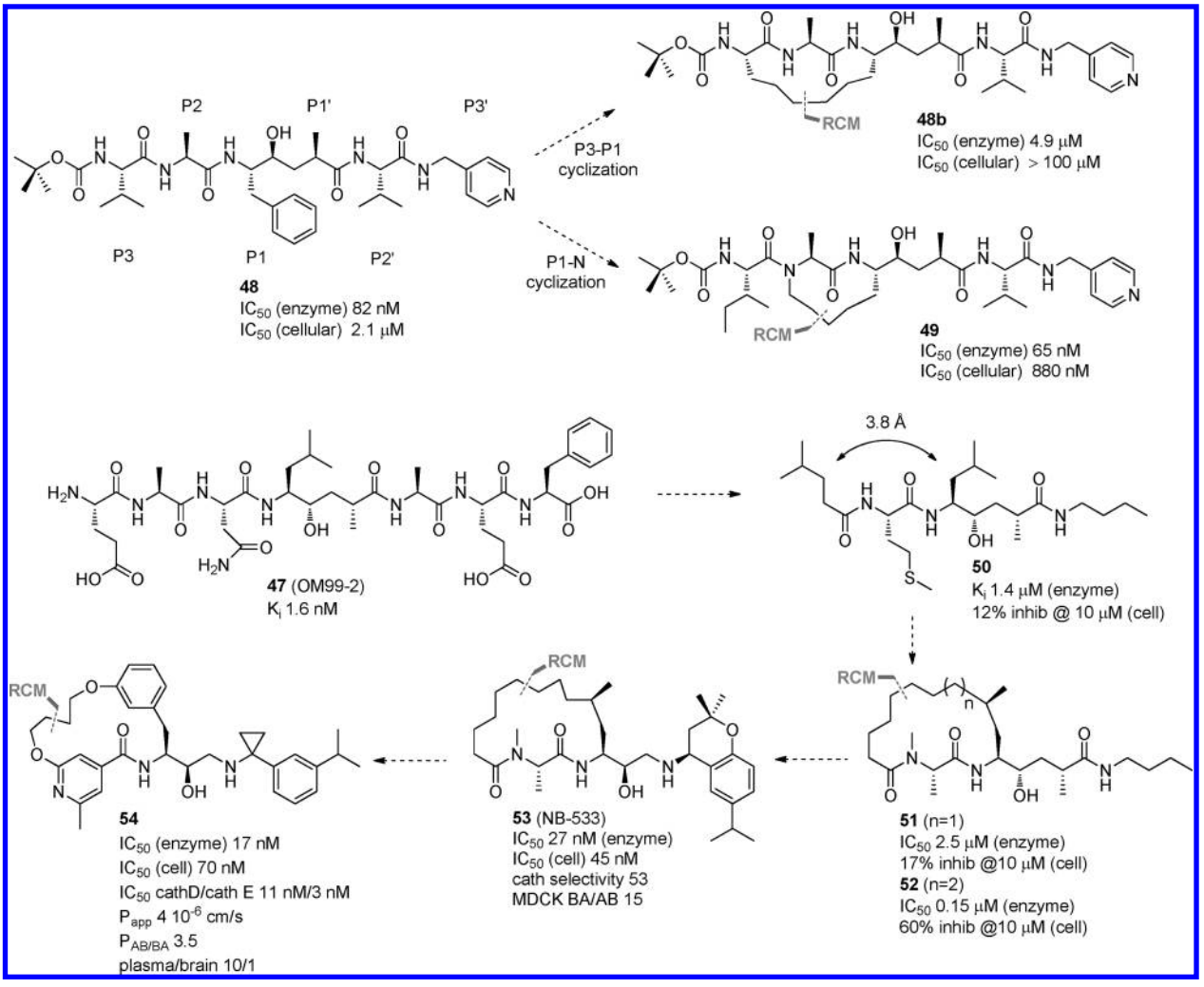

Figure 9. Molecular evolution toward macrocyclic semipeptidic BACE-1 inhibitors.

macrocyclization an attractive option. A first generation of analogues produced 15- and 16-membered rings **51** and **52**, with modestly improved enzymatic and cellular activities[105−107] and 50- to 100-fold selectivity vs cathepsin D. Further optimization delivered 16-membered macrocycle **53**, possessing IC_{50} of 27 nM in vitro and 45 nM in cellulo, testifying to a much improved cellular penetration,[106] with 53-fold selectivity for cathepsin D. Molecular evolution from **52** to **53** showed significant differences, notably a reversal in hydroxyl group stereochemistry and the introduction of a basic secondary amine and an aryl group in place of the butanamide. Overall, the reduction in peptidic character and introduction of a basic group translated into improved cellular penetration. After iv administration of **53** at 15 mpk, brain concentrations remained above 1 μM for a 5 h period, with a brain/plasma ratio of 4:1. A 14% decrease of $A\beta_{40}$ was observed after double dosing. Unfortunately, **53** was a P-glycoprotein (P-gp) substrate, as indicated by a BA/AB ratio of 15 in the MDCK permeation assay. Administration of **53** at 15 mpk in the presence of a P-gp inhibitor improved efficacy with a 29% decrease in $A\beta_{40}$ in the brain (55% decrease in CSF). **53** further demonstrated activity after oral administration (150 mpk), leading to a 31% decrease in $A\beta_{40}$ in the brain when coadministered with a P-gp inhibitor. Subsequent optimization provided lead macrocycle **54**,[108] distinct from its predecessors in several aspects. First, enzyme and cellular potency were preserved. Second, decreased basicity of the secondary amine translated into increased permeability and reduced efflux ratio in the MDR1-MDCK assay. On the other hand, lower pK_a reduced enzymatic activity. Striking a balance around $pK_a = 7-7.5$, cyclopropanated analogue **54** provided the best compromise, correlating with observations from the acyclic series.[109] When administered po at 33 mpk, **54** reached a brain concentration of 0.32 μM with a plasma/brain ratio of 10/1, reducing the proportion of $A\beta_{40}$ by 74% 4 h postdose. Unfortunately, this series of compounds possessed poor selectivity for cathepsins D and E and was not further advanced. Additional macrocyclic BACE-1 inhibitors involving an alternative P4−P2 cycle were reported by Ghosh et al.[110] and Barazza et al.[111]

BACE-1 inhibitor **55** (Figure 10) originated from high throughput screening followed by lead optimization. **55** was a potent BACE-1 inhibitor both in vitro and in cellulo (IC_{50} of 7 and 20 nM, respectively). However, it possessed a poor pharmacokinetic profile, being also a substrate for P-gp, which reduced its apparent permeability. **55** decreased $A\beta$ levels by 50−70% in murine models when administered intracranially but had no efficacy when delivered iv. To improve CNS penetration, macrocyclization was envisaged as a way to (1) reduce the peptidic character of **55**, (2) reduce molecular weight

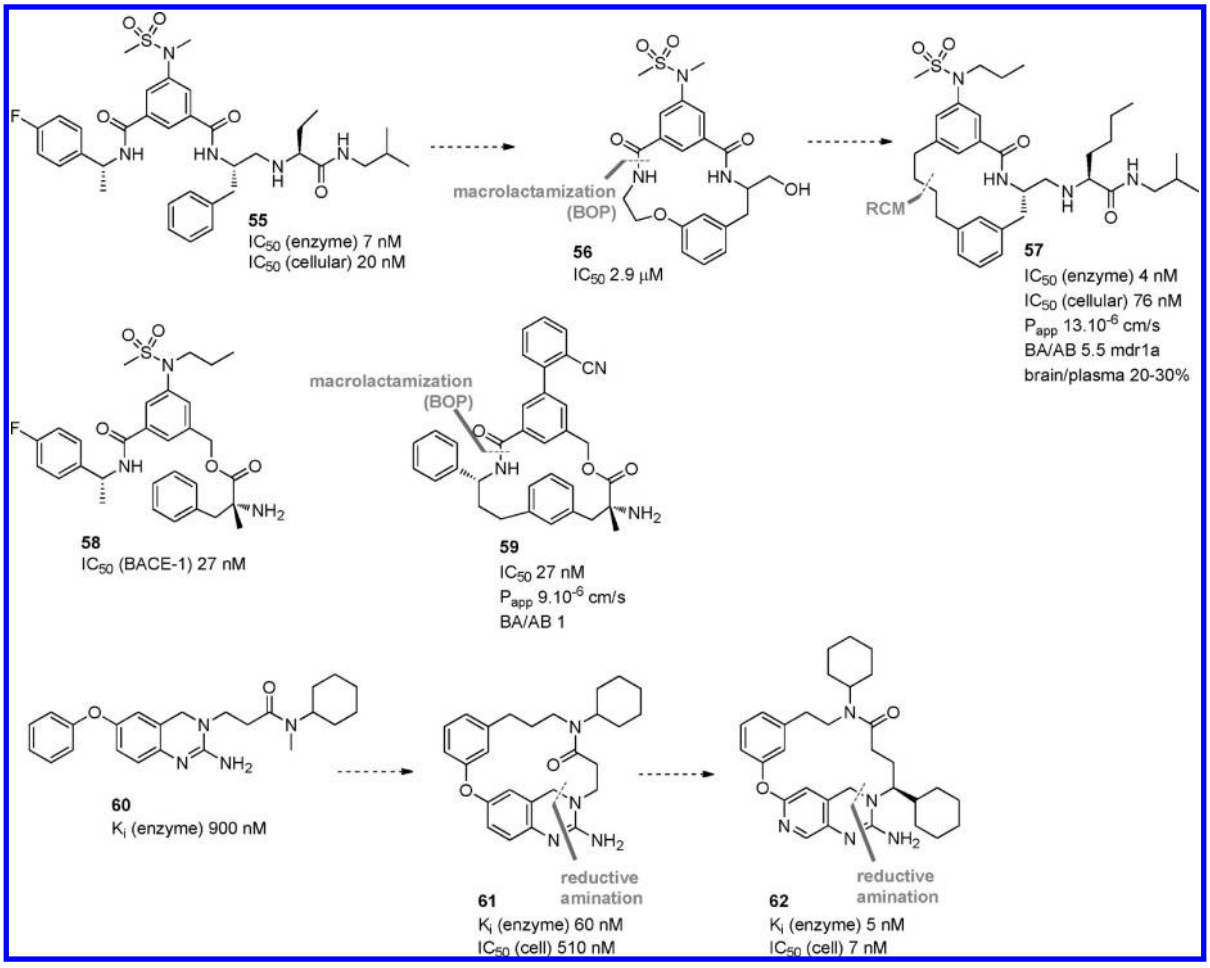

Figure 10. Nonpeptidic macrocyclic BACE-1 inhibitors.

via ligand preorganization, and (3) exploit the proximity between the P3 and P1 groups. This delivered the first macrocyclic scaffold **56** (IC_{50} = 2.9 μM).[112] Its acyclic analogue (cleaved at the ethanolamine junction) did not elicit any inhibition, confirming the importance of macrocyclization. Subsequent elongation into the P1′ space delivered 14-membered ring **57** with excellent enzymatic activity in vitro and in cellulo. More importantly, **57** possessed good cell permeability (P_{app} = 13 × 10^{-6} cm/s) and reduced P-gp susceptibility (BA/AB 5.5 mdr1a), as well as a brain/plasma ratio of 20−30%. **57** decreased Aβ levels by 25% when dosed iv at 100 mpk in APP-YAC mice, thus representing the first peripherally administered macrocyclic BACE-1 inhibitor exhibiting in vivo efficacy. Disappointingly, efficacy levels at 3 h decreased to 10%, suggesting limited target exposure. Linear carbinamines such as **58** offered the promise of retaining potent enzymatic activity while giving an opportunity to reduce both molecular weight and PSA given the absence of the exocyclic peptidic portion. Macrocyclization gave rise to a series of analogues exemplified by **59**. Despite numerous modifications to every polar moiety in the residue, reduction of P-gp efflux was often reached only at the price of reduced enzymatic activity and vice versa. So far, this last series has not yet yielded compounds possessing a suitable profile to warrant further development.[113]

A final series of macrocyclic BACE inhibitors was reported by Huang et al.,[114] starting with HTS hit **60**. Molecular modeling analysis of this compound docked in the enzyme's active site revealed a hairpin structure that provided the basis for exploring the macrocyclic chemical space. Macrocycle **61** showed good activity on the isolated enzyme but possessed poor cellular activity. Further optimization led to macrocycle **62**, a potent inhibitor in vitro and in cellulo. However, upon either oral or parenteral administration, the compound demonstrated some efficacy for the reduction of plasma Aβ, yet did not reduce brain Aβ in animals, suggesting poor CNS penetration. No subsequent work on this series has yet been reported.

BACE-1 is considered a highly promising target for the treatment of Alzheimer's disease. However, its location intracellularly and in the central nervous system makes it an extraordinarily challenging one. The macrocycles generated to date on this target generally possessed molecular weights above 500 g/mol and high cellular penetration. Several of these compounds, typically with reduced peptidic character and polarity, were able to demonstrate acute effects in vivo after iv or po administration. So far, to the best of our knowledge, none of these macrocycles have reached clinical development.

2.1.7. Other Enzyme Inhibitors

2.1.7.1. CDK Inhibitors. Protein kinases, responsible for the transfer of a phosphoryl group to acceptor proteins, are key intermediates in intracellular signaling of myriad processes, such as cell division and apoptosis. Deregulation of kinase signaling pathways is associated with multiple pathologies, including

cancer. Modified cyclin-dependent kinase (CDK) control, for example, is associated with several cancers resulting from alterations in the order of events in the cell division cycle, since CDKs control cell cycle checkpoints. Lücking et al. reported a novel series of macrocyclic CDK inhibitors (Figure 11).[115] Starting from moderately potent acyclic CDK1/CDK2 inhibitor **63**, macrocyclization was envisaged to stabilize a bioactive conformation. Even though the first cyclic analogue led to a decrease in inhibitor potency, subsequent functionalization of the macrocyclic ring gave analogue **64** possessing moderately improved in vitro potency but 20-fold increased antiproliferative activity against MCF-7 breast cancer cells. Interestingly, macrocycle **64** possessed a broader target profile, including beneficial inhibitory properties against the VEGF receptor. Macrocycle **64** demonstrated efficacy in a tumor xenograft model when dosed po at 50−100 mpk. Additional analogues with increased potency and alternative selectivity profiles were reported as well.

Kawanishi et al. also reported macrocyclic CDK inhibitors.[116] Examination of the X-ray structure of inhibitor **65** in complex with CDK2 in the ATP-binding hinge region identified an intramolecular hydrogen bond that brought the urea and pyridine moieties into coplanarity. Cyclization of this part of the molecule and bridging the extremities of the molecule to fill the furanose site of the ATP-binding pocket led to macrocyclic molecules, the most potent of which, **66**, displayed low nanomolar potency against CDK isozymes 1, 2, 4, and 6. Of note, **66** was highly selective for the CDK subfamily and possessed greater than 1000-fold selectivity against other kinase families. Further, **66** demonstrated robust suppression of tumor xenograft growth at low doses (infusion, 0.07 and 0.14 mpk/h).[117] Unfortunately, it also possessed immunosuppressive activity in rats at high doses.

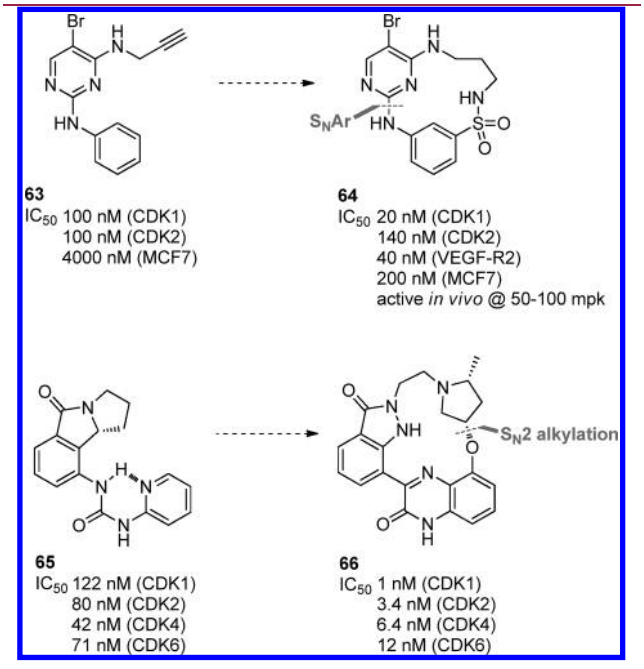

Figure 11. Macrocyclic CDK inhibitors.

2.1.7.2. HDAC Inhibitors. Acetylation and deacetylation of lysine residues on histones affect the compactness and dynamics of proteins and consequently chromatin structure, thereby playing an important role in gene transcription. Deacetylation is under the control of histone deacetylases (HDACs), the inhibition of which leads to cell differentiation and cell cycle arrest in multiple cancer cells. As a consequence, histone deacetylases are an important anticancer target class and several HDAC inhibitors are in clinical development or marketed drugs. Histone deacetylases are zinc metalloproteases that possess a long history with macrocycles, as reviewed by Mwakwari et al.,[118] since several macrocyclic natural products are inhibitors, such as **67** (FK228, romidepsin, Figure 12), largazole (**68**), FR901375, azumamides, spiruchostatins, and trapoxin B. Several of these have been the object of SAR studies to improve upon their metabolic stability. The most advanced macrocyclic HDAC inhibitor is **67**,[119] actually a bis-macrocyclic depsipeptide prodrug, since its disulfide bridge is reductively cleaved in the intracellular environment. Synthetic analogues of **67** were reported by Yurek-George et al., supporting mechanistic studies indicating that one of the thiols interacts with the zinc atom of the enzyme after intracellular cleavage.[120] Recently, Pirali et al. reported new nonpeptidic macrocyclic HDAC inhibitors designed based on an X-ray structure of two HDACs in complex with the linear natural product inhibitor trichostatin.[121] The authors reported that both macrocycles **69** and **70** possessed IC_{50} values of 6 μM in a cytotoxicity assay and HDAC inhibitory activity using a fluorimetric assay, with IC_{50} values of 4.3 and 6.1 μM, respectively. Compared to natural products currently in clinical development, macrocycles **69** and **70** are nonpeptidic, yet retain the necessary zinc-binding hydroxamic acid found in multiple HDAC inhibitors. For this series, ring size was found to significantly impact activity.

2.1.7.3. Farnesyltransferase Inhibitors. Farnesyltransferase (FTase) is responsible for the post-translational prenylation of proteins, playing a key role in cell proliferation. The inhibition of FTase has been pursued as an approach to fight cancer, leading to several clinical candidates. Using trNOE experiments,

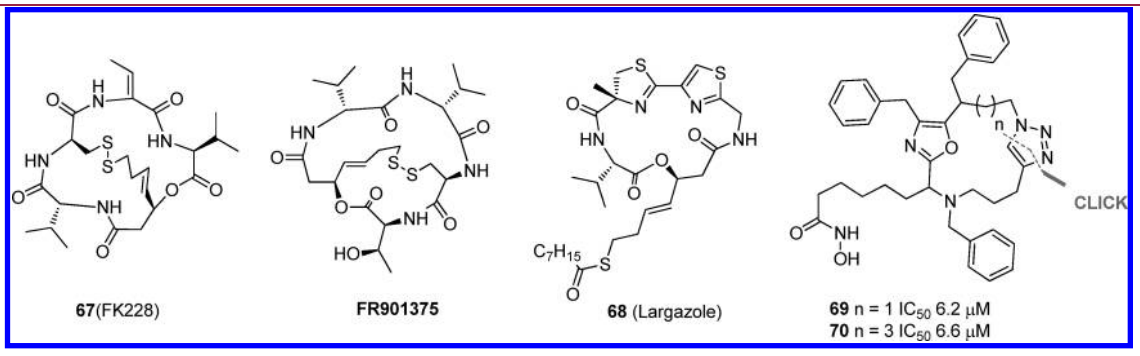

Figure 12. Macrocyclic HDAC inhibitors.

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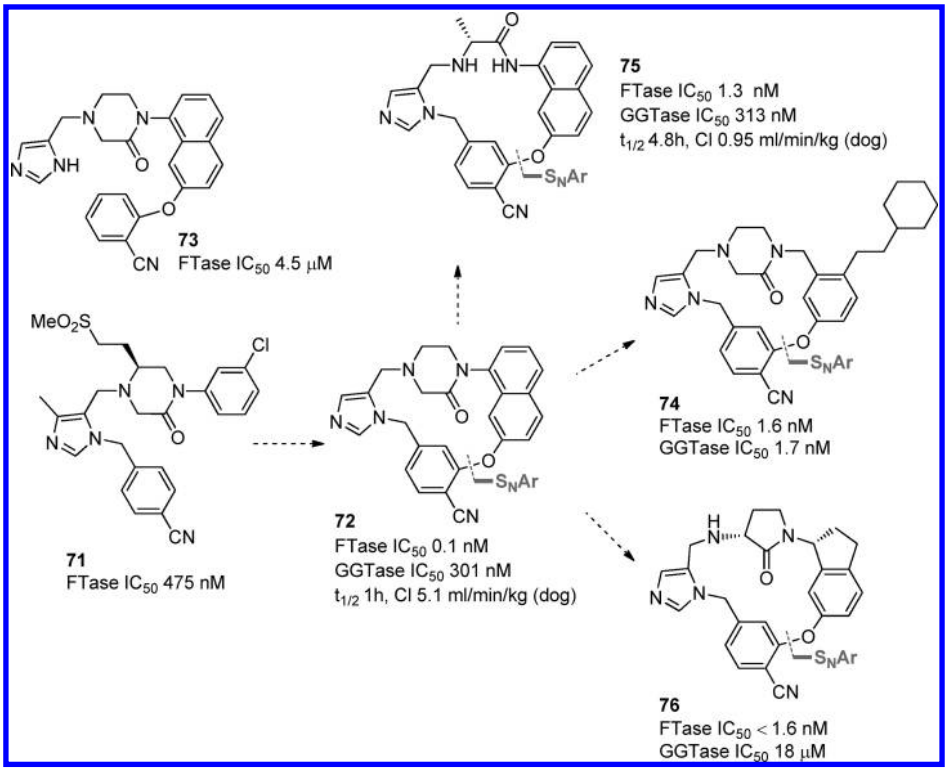

Figure 13. Macrocyclic farnesyltransferase inhibitors.

Dinsmore et al. observed that weak inhibitor **71** (Figure 13) underwent a conformational rearrangement upon binding to the enzyme, bringing the two extremities of the molecule into spatial proximity.[122] To lower this entropic penalty, the two extremities were linked into a macrocycle, providing **72**. This dramatically improved inhibitory activity from 475 to 0.1 nM. The direct impact of macrocyclization compared to its acyclic congener **73** was estimated to be over 40000-fold. In subsequent studies aimed at incorporating geranylgeranyltransferase I (GGTase) inhibitory activity into the same molecule, the authors reported lead **74**.[123] Whereas macrocycle **72** possessed only modest GGTase activity (IC_{50} = 301 nM), **74** possessed low nanomolar inhibition on both enzymes. In a more detailed cell culture assay measuring protein processing, **74** was shown to inhibit the prenylation of three key proteins (HDJ2, Rap1a, and K_i-Ras (EC_{50}) of 12, 140, and 1065 nM, respectively), whereas inhibitor **72** was able to inhibit the prenylation of HDJ2 but not Rap1a or K_i-Ras (EC_{50} of 2 nM, >10 μM, >10 μM). In this series, replacement of the piperazinone linker by amino acids provided compounds with improved PK profile compared to **72**, as exemplified by **75**.[124] A similar lead series represented by macrocycle **76** possessed very high FTase activity and very low GGTase activity together with an excellent PK profile, exhibiting clearances generally below 5 (mL/min)/kg and oral bioavailabilities of 47−92%.[125]

Enzyme targets have so far been the widest field of application for synthetic macrocycles. In the majority of cases, inhibitor design started from a combination of (1) acyclic substrates or inhibitors (peptidic in nature in the case of proteases) and (2) thorough biophysical analysis combining X-ray structure of ligand−enzyme complex, molecular modeling, and/or NMR studies. The rationale for macrocyclization was usually (1) the preorganization of the substrate in a preferred conformation such as a β-sheet, (2) a reduction in conformational flexibility to enhance beneficial interactions, (3) a decrease in polarity to impart onto the resulting molecule improved cellular penetration to reach the target and improved PK-ADME properties. As a consequence, several macrocyclic enzyme inhibitors have reached clinical development, the most eloquent example being undoubtedly the HCV NS3 protease inhibitors.

2.2. G Protein-Coupled Receptors. G Protein-coupled receptors (GPCRs) are the largest class of pharmaceutical targets representing 30−40% of current marketed drugs. The design of GPCR ligands does not benefit from the same degree of structural support compared to proteases, since only five GPCR X-ray structures have been reported to date as opposed to several thousand for proteases. Typically, molecular modeling of GPCRs is done by homology to the handful of receptors that have yielded to crystallization. Perhaps as a direct consequence of this difference, the identification of synthetic GPCR ligands has relied extensively on high throughput screening of compound libraries rather than rational design as widely applied to proteases. Localization on the cell surface is an advantage for GPCRs in terms of target accessibility compared to intracellular targets such as many proteases or protein−protein interactions. This difference is significant, since molecules aimed at GPCRs do not have to penetrate cells to reach their targets. Macrocyclic peptides have emerged as a class of "privileged structures" for GPCRs.[126−128] Although they will not be described in detail in this article, such structures often can provide a reasonable starting point for nonpeptidic ligand design.

2.2.1. CXCR4 Antagonists. Chemokine receptors CCR5 and CXCR4 are cofactors for the entry of HIV into host cells. Targeting this mechanism to prevent cellular entry of the virus is a new treatment option, complementing earlier approaches based on the inhibition of intracellular viral enzymes. Whereas

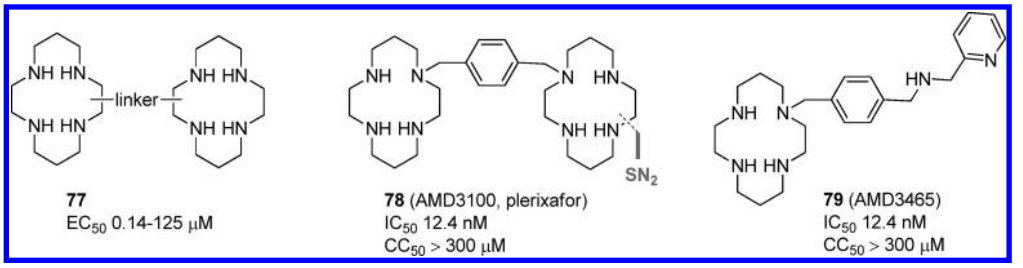

Figure 14. Macrocyclic CXCR4 antagonists.

the CCR5 receptor is typically associated with early phases of infection by HIV and is used by M-tropic viruses, the CXCR4 is generally used by the more pathogenic T-type viruses. The first macrocycles to be identified as CXCR4 inhibitors were bis-tetraazamacrocycles (bicyclams, generic structure **77**, Figure 14).[129] The class had no effect on either the CD4 receptor or gp120 glycoprotein, and at the time of its discovery its target was unknown. It was later demonstrated that **78** (AMD3100, plerixafor) prevents interaction of the CXCR4 receptor with its cognate ligand, the CXCL12 chemokine. The most potent macrocycle of this dimeric series inhibited HIV-1 replication at a concentration of 0.005 μg/mL, yet exhibited no cytotoxicity up to 500 μg/mL. The aryl linker was beneficial for biological activity.[130] Early QSAR analysis of a series of analogues demonstrated that both macrocycles were necessary for high antiviral activity and that ring size, distance between ring centroids, and lack of nitrogen substitution were important parameters for activity.[131] **78** lacked oral bioavailability and was administered iv, restricting its applicability at a time when many anti-HIV drugs are administered orally. **78** was approved recently as a new HIV treatment, yet also possesses potential for inflammatory diseases, cancer, and stem-cell mobilization.[132,133] The search for potent and orally bioavailable CXCR4 antagonists is ongoing.[134] Recently, analogue **79** (AMD3465) possessing a single cyclam moiety was reported to possess similar activity to **78** in vitro.[135] Additional reports by Bridger et al. thoroughly elucidated the importance of having one vs two rings and ring size, as well as the number of nitrogen atoms in each ring.[136]

2.2.2. Motilin Antagonists. Motilin is a 22-amino acid peptide hormone that interacts through a GPCR located predominantly in the gastrointestinal (GI) tract. Pulsatile release of motilin corresponds to phase 3 of the migrating motor complex (MMC, also called the GI "housekeeper"), a peristaltic contractile activity originating from the stomach, and traveling aborally and responsible for gut motility in the fasted state. As such, the motilin receptor is involved in the regulation of GI motor activity and a potential target for the treatment of GI motility disorders. Several agonists of the motilin receptor, almost all with macrolide core structures derived from erythromycin, have reached clinical development. Marsault et al. reported a novel class of motilin antagonists, following HTS of 10 000 macrocycles from a diversity-oriented library of macrocyclic peptidomimetics (section 3.3).[137] From this HTS campaign, motilin antagonist **80** (Figure 15) was identified, possessing a high level of potency from which to initiate a program (IC_{50} = 137 nM). Lead optimization studies led to multiple analogues with low nanomolar potency, including analogues possessing novel unnatural basic amino acids (generic structure **81**, IC_{50} = 1−20 nM).[138] The absence of receptor interaction with the corresponding linear ligand indicated that macrocyclization increased

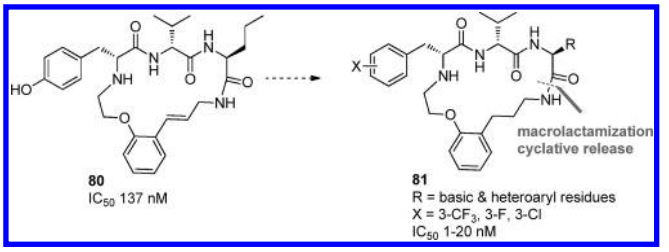

Figure 15. Macrocyclic motilin antagonists.

affinity by at least 4 orders of magnitude. Analogues were synthesized using two solid phase synthetic approaches, using macrolactamization or RCM, both in conjunction with a cyclative release step key to the delivery of crude products with acceptable purity. Ring size, chemical nature of the four fragments, and stereochemistry were systematically varied to confirm that the DDL stereochemistry for the amino acid components was clearly preferred. Animal studies in dogs demonstrated that lead JTZ2002 (undisclosed structure) was able to block the spontaneously occurring MMC and reduce fundic tone and that inhibition of the motilin receptor reduced both fasted and postprandial mechanical activity, properties that may be useful in the development of a drug for functional dyspepsia or the irritable bowel syndrome.[139] Further, the most advanced candidate of this macrocyclic series, TZP-201 (undisclosed structure), demonstrated efficacy in a dog model of chemotherapy-induced diarrhea provoked by irinotecan.[140] TZP-201 proved to be superior to current treatments, loperamide and octreotide, in this respect.

2.2.3. Ghrelin Agonists and Antagonists. Ghrelin is central to the gut−brain−energy axis and the only natural orexigenic gastrointestinal peptide. It possesses multiple physiological roles, including the release of growth hormone, regulation of glucose homeostasis, adipogenesis, cardioprotective effects, and increased gut motility and exerts these effects though interaction at a GPCR (GRLN, hGHS-R1a) located in the GI tract and other tissues. Among other uses, ghrelin agonists have been considered as a new therapeutic approach for GI dysmotility disorders. From an HTS campaign of the diversity-oriented macrocyclic library described in section 3.3, Hoveyda et al. identified macrocycle **82** (Figure 16) as a ghrelin agonist possessing EC_{50} = 68 nM, a high level of potency directly from the initial screen.[141] Further optimization led to macrocycle **83** (TZP-101, ulimorelin), a low nanomolar ghrelin agonist with suitable PK profile. In this instance again, acyclic analogues of **83** were devoid of receptor interaction, emphasizing the paramount importance of the macrocyclic structure. **83** possessed a good PK profile and dose-dependently restored normal gastric emptying in rats suffering from postoperative ileus, morphine-induced delayed gastric emptying, or the combination of both.[142,143] Interestingly,

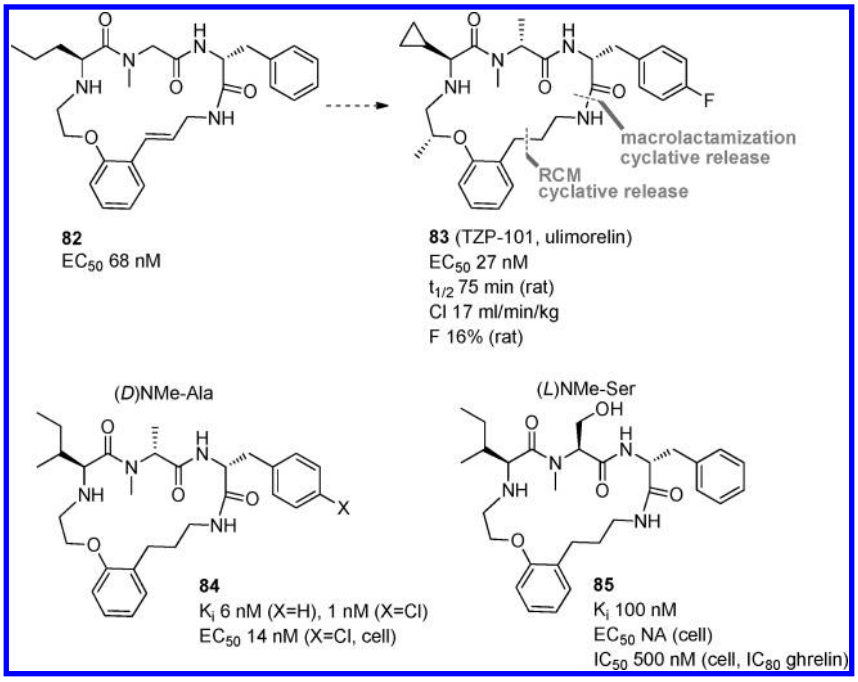

Figure 16. Macrocyclic ghrelin agonists and antagonists.

83 did not elicit growth hormone release after icv administration to rats.[144] In phase 1 clinical development, it was found to be safe and well tolerated following iv infusion at doses of 20 − 600 μg/kg.[145] Furthermore, the compound demonstrated efficacy in two phase 2 clinical studies when administered iv for the treatment of postoperative ileus and acute gastroparesis.[145−148] **83** has recently entered phase 3 clinical trials. At the higher doses in clinical phase 1, **83** was found to possess a nonlinear pharmacokinetic profile in humans at high doses, due to its strong association with α-acidic glycoprotein.[149] A second generation compound derived from **83**, TZP-102 (undisclosed structure), recently demonstrated efficacy when administered po in a phase 2 clinical study for the treatment of diabetic gastroparesis.

The replacement of the central amino acid in the agonist scaffold exemplified by **84** from a (D)NMe-Ala to an (L)NMe-Ser residue switched the profile of the resulting compounds from agonist (**84**, K_i = 1−6 nM, EC_{50} = 14 nM) to antagonist (**85**, K_i = 100 nM, no efficacy to elicit Ca^{2+} release, IC_{50} = 500 nM in the presence of EC_{80} ghrelin).[150] The use of ghrelin antagonists for the treatment of type 2 diabetes and obesity has been validated in animal models. Although the mechanistic basis for the result of such a switch remains unclear, it represents a remarkable example of how a minor structural change can significantly alter the efficacy profile of a lead candidate.

The ghrelin agonists and motilin antagonists reported in the two preceding sections demonstrate the value of technologies that expand macrocycle chemical diversity (section 3.3). Indeed, whereas most examples of macrocyclic protease inhibitors originated from biophysical studies of target−ligand interactions, the latter examples are among the few macrocycles that emanated from diversity-oriented collections focused on synthetic macrocycles. In these examples, high potency hits were obtained directly from HTS and the structures enabled rapid evolution toward clinical candidates.

In contrast to the previous examples of protease inhibitors, macrocyclic GPCR ligands have usually not been identified starting from rational drug design approaches. This is more a reflection of the modus operandi of GPCR vs protease drug discovery rather than a specificity of macrocycles.

2.3. Protein−Protein Interaction Inhibitors. Macrocycles appear particularly well-suited for the modulation of protein−protein interactions (PPI). Indeed, the interacting residues in PPI hot spots typically cover a surface area of several hundred Å^2.[151,152] Macrocycles cover topologically defined surface areas with restricted conformational flexibility and hence appear ideal to serve as potential mimics for interaction at such hot spots. Additionally, macrocyclization is an efficient way of increasing cellular penetration via the decrease in polarity of peptidic leads as amply demonstrated with proteases (section 2.1). Essentially, macrocycles seem to possess the attributes required for a privileged structure for the modulation of PPI.[128] A compelling example of the particular relevance of the macrocyclic structure in this target area is found in the multiple macrocyclic natural products and analogues thereof that display inhibition of Hsp90, which have been reviewed and will not be covered specifically here.[1,153]

2.3.1. Grb SH2 Modulators. The Grb2 family of SH2 domains is involved in the signaling of ErbB-2 and is linked to several breast cancers.[154] Phosphorylated tyrosine (pY) residues present on the consensus sequence pY-XNX exhibit high affinities for SH2 domains, with specificity orchestrated by the residues flanking these pivotal residues. SH2 domains themselves serve to mediate protein tyrosine kinase (PTK) dependent signal transduction. Examination of the 2.1 Å resolution crystal structure of the Grb2 SH2 domain in complex with the peptide KPF-pY-VNV revealed the presence of a β-turn conformation in the ligand, bringing the pY and Asn binding pockets into proximity.[155] On the basis of lead series **86** (Figure 17) preorganized in a β-turn conformation, the generation of macrocycle **88** (IC_{50} = 20 nM) proved to be a judicious choice, improving binding potency by 2 orders of magnitude compared to analogue **87** missing the pY α-amino group.[156−160] In whole

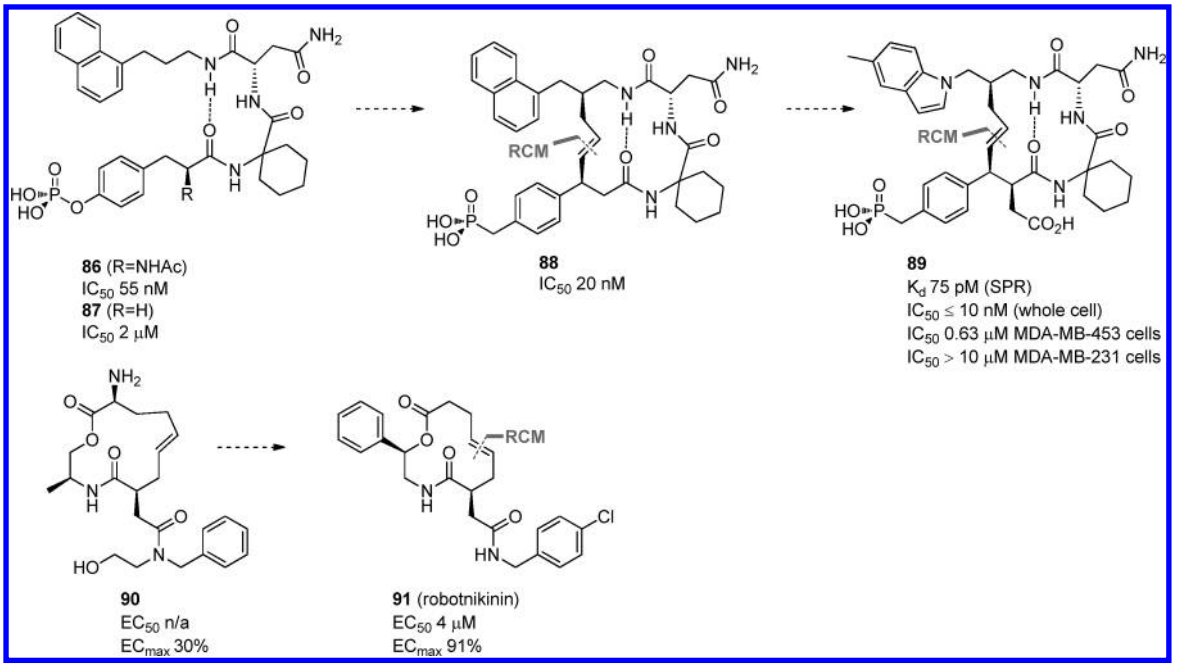

Figure 17. Macrocyclic Grb SH2 domain inhibitors and Sonic Hedgehog inhibitors.

cell assays, however, the potency of macrocycle **88** was weaker than its acyclic congeners, a difference consistently observed in a human breast cancer cell growth assay. Analysis of the solution structure of the Grb SH2 domain complexed with macrocycle **88** using a perdeuterated protein domain[161] led to exquisitely potent inhibitor **89** (K_d = 75 pM) bearing an acidic group in lieu of the acetamido side chain of pY and an electron-rich 5-methylindole group.[162] Importantly, this macrocycle was efficacious in whole cell assays, displaying robust antiproliferative properties in ErbB2-dependent MDA-MB-453 breast cancer cells. The specificity of the observed effect was confirmed by the lack of antiproliferative properties in MDA-MB-231 breast cancer cells that are independent of the Erb-B2 growth pathway. Subsequent optimization involved the replacement of the phosphonic acid by a malonate, which docks into the pY binding pocket.[163−165] Alternative macrocyclic peptidic scaffolds devoid of the pY residue were recently reported as well.[166]

2.3.2. Sonic Hedgehog Modulators. Exploiting the rich chemical diversity of polyketide synthase-derived macrolactones, a compound class that includes many biologically attractive molecules such as FK506, epothilones, erythromycin, and enterobactin, the Schreiber group synthesized a library of 2070 macrolactones of diverse ring size and stereochemistry, incorporating a 1,2-aminoalcohol template. Upon screening on the extracellular Sonic Hedgehog (Shh) protein, macrocycle **90** (Figure 17) was identified as a weak modulator.[167] The Sonic hedgehog pathway is implicated in several cancers and developmental disorders. Diversification around scaffold **90** led to macrocyclic inhibitor **91** (robotnikinin), which is able to fully inhibit the pathway with IC_{50} = 4 μM. **91** demonstrated concentration-dependent inhibition of Shh-induced pathway activation in the NIH-3T3 cell line, with no observable cytotoxicity. Furthermore, it reduced Gli1 and Gli2 transcription in primary human keratinocytes on isolated cells or in human-derived tissue while keeping tissue histologically normal. This constitutes an eloquent example of the wide potential of synthetic macrocycles inspired from natural sources in the disruption of protein−protein interactions.

2.3.3. Neurotrophin Mimics. On the basis of the macrocyclic peptidomimetic scaffold developed by Burgess and co-workers (section 3.3), Maliartchouk et al. reported macrocycle **92** (Figure 18) as an agonist of the TrkA receptor, which possessed high affinity for the neurotrophin nerve growth factor (NGF).[168] **92** was isolated from a small library of 60 macrocyclic peptidomimetics designed to mimic β-turn hot spots. Conceptually, **92** represents an attractive alternative to the therapeutic use of NGF. It selectively and concentration-dependently blocked mAb 5C3−TrkA interactions with IC_{50} = 4 μM and also potentiated TrkA-dependent NGF-stimulated DRG neuronal survival. This effect was not observed in the presence of epidermal growth factor (EGF), suggesting specificity for NGF. Subsequently, Pattarawarapan et al. reported macrocycle **93** based on the same scaffold.[169] **93**, a mimic of neurotrophin-3 (NT-3), demonstrated its ability to enhance the survival of TrkC-expressing NIH-3T3 cells exposed to suboptimal NT-3 doses (100 pM). The effect was specific for TrkC-expressing cells and was abolished in TrkA-expressing cells. Essentially, macrocycles **92** and **93** demonstrate the ability of small molecule macrocycles to mimic large protein partners.[170]

2.3.4. Additional Macrocyclic Strategies for the Disruption of Protein−Protein Interactions. Protein−protein interactions often involve large interacting surfaces and/or distant binding epitopes.

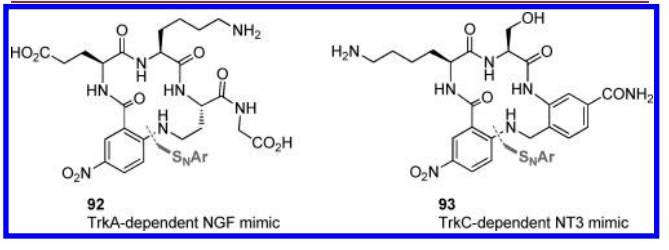

Figure 18. Macrocyclic NGF and NT-3 mimics.

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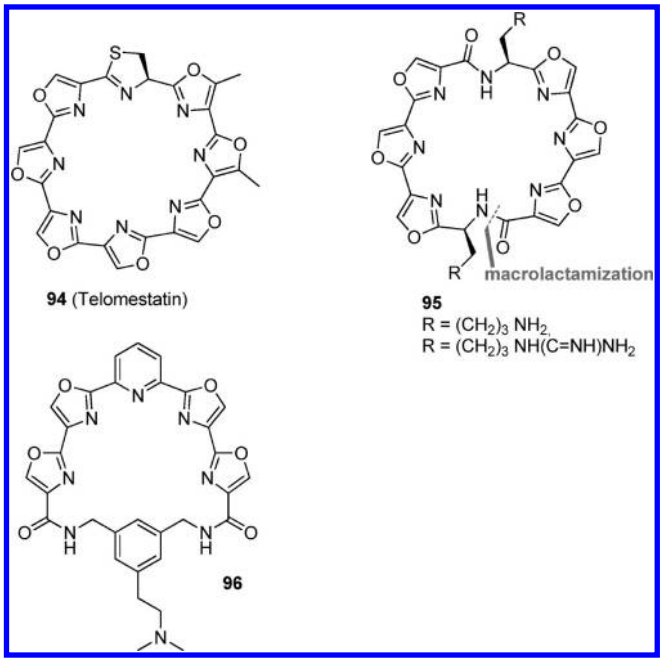


Figure 19. Macrocyclic DNA G-quadruplex ligands.

An efficient way to stabilize a peptidic secondary structure is to create a covalent linkage between otherwise more flexible secondary structures, such as helices. Although outside the scope of this Perspective, it is worthwhile mentioning that side chain to side chain covalent linkage formation through macrocyclization has been used as an efficient way to stabilize peptide secondary structure. Examples of "stapled peptides"[171] for the stabilization of α-helical structures have appeared with diverse applications, such as inhibition of the NOTCH complex,[172] p53 mimicry,[173] or PDZ domain ligands.[174,175] Additional examples include incorporation of heterocycles such as oxazoles and thiazoles to mimic the secondary structure of interhelical loops.[176]

2.4. Miscellaneous Targets. *2.4.1. DNA G-Quadruplex Recognition.* The DNA G-quadruplex is a secondary structure of DNA characterized by the ability of contiguous guanine residues to self-associate into planar G-quartets via Hoogsteen-type H-bonding. These quartets are in turn capable of stacking to form a secondary structure called the G-quadruplex.[177] The biological roles of G-quadruplexes include chromosome capping and subsequent protection of their integrity. G-Quadruplexes are involved in the structure and function of telomeres, in promoter gene regions of oncogenes, and in subsequent transcription. Stabilization of G-quadruplexes is a potential approach against cancer.[178,179] Hexaoxazole analogues of the natural product **94** (telomestatin, Figure 19)[180] were reported by Tera et al.[181,182] Analogue **95** bearing cationic side chains displayed potent DNA sequence selectivity for the telo24 sequence, in sharp contrast to their neutral side chain analogues that were devoid of activity. A similar heptaoxazole analogue bearing only one side chain also bound strongly and selectively with the ss-telo24 sequence and induced a conformational change in the antiparallel G-quadruplex structure.[183] Other variations on the theme were reported as well, indicating the possibility to fine-tune selectivity for the target sequence by modifications to the structure of the macrocycle.[178,179] On the basis of the same scaffold, Rzuczek et al. reported a series of pyridylpolyoxazoles possessing excellent selectivity for the stabilization of G-quadruplex DNA with no

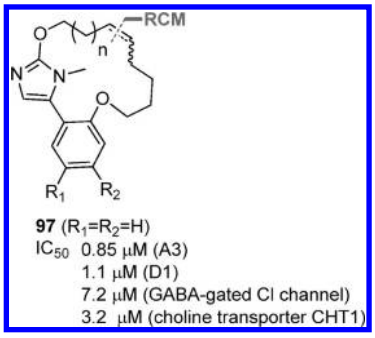


Figure 20. Multipotent macrocycles.

stabilization of duplex DNA or RNA.[184] The most potent of these (**96**) stabilized quadruplex mRNA encoding for the Aurora A kinase. Macrocycle **96** displayed robust cytotoxicity against human lymphoblastoma cells RPMI8402 (IC_{50} = 0.18 μM) and human epidermal carcinoma cells KB3-1 (IC_{50} = 0.04 μM). It was shown to be a substrate for the efflux tranporter MDR-1 but not the BCRP transporter. Finally, it possessed in vivo efficacy in athymic nude mice bearing a human breast cancer xenograft, displaying significant anticancer activity when administered ip at 25 and 42 mpk with no observable weight loss or other adverse effects.

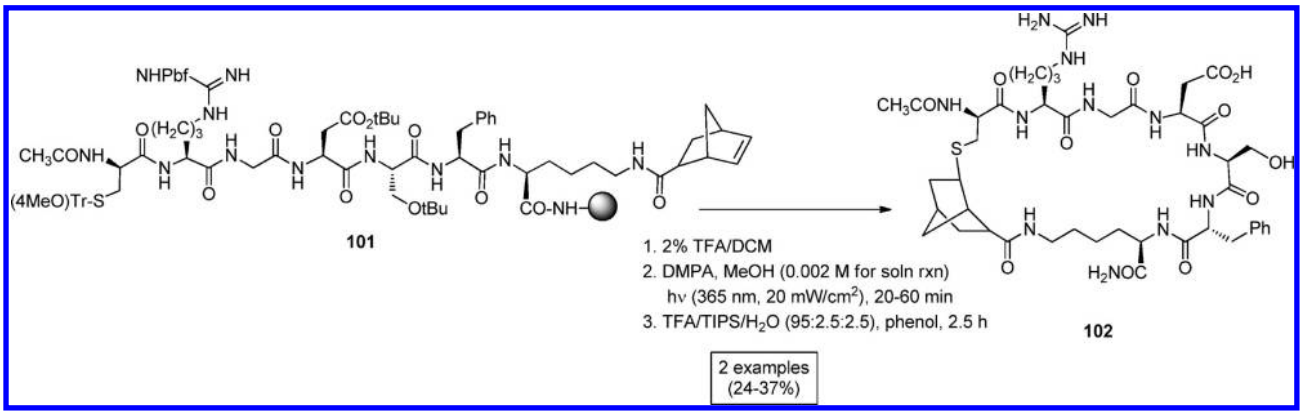
2.4.2. Multipotent Macrocycles. On the basis of the observation that 4(5)-aryl-1*H*-substituted imidazoles are found in many pharmacologically active compounds, Nshimyumukiza et al. reported the synthesis of six macrocycles incorporating this motif, as illustrated in Figure 20 (**97**, n = 1, 3; R_1, R_2 = H, F, Me, OMe).[185] The rationale behind this initiative was to introduce conformational restrictions, as well as to demonstrate how to increase chemical diversity around this scaffold. The authors assessed the pharmacological promiscuity of the scaffold via screening in a panel of 71 receptors and 16 enzymes and identified compound **97** (R_1 = R_2 = H, n = 1) with low micromolar activity on the adenosine A_3, the dopamine D_2, the GABA-gated chloride channel, and the CHT_1 choline transporter. Little difference in activity between acyclic and macrocyclic products was observed except for a 6-fold increase in potency at the D_2 receptor upon cyclization.

3. SYNTHETIC APPROACHES TO MACROCYCLES

3.1. Macrocyclization Reactions. One of the challenges associated with the exploration of the macrocyclic framework for drug discovery is the difficulty in synthesizing such structures, particularly as a series of molecules for SAR elucidation or to build screening libraries. As can be surmised from the variety of structures described in the first part, a number of viable synthetic routes have nonetheless been successfully developed. Certain strategies have proven more versatile than others, including amide and ester coupling, nucleophilic displacement chemistries, ring-closing metathesis (RCM), and "click" chemistry cycloadditions, as were indicated for many of the specific examples already presented.

Traditional methodologies such as lactonization and lactamization are among the most standard approaches to macrocycle formation.[5,186,187] The wide variety of coupling agents available from peptide chemistry provides the chemist with a significant armamentarium for macrolactamization.[188,189] Not surprisingly, such methodology is particularly advantageous in the assembly of macrocyclic peptidomimetics, including the range of protease

Scheme 1



inhibitors described earlier (section 2.1). In one noteworthy modification, macrolactamization can be effected on an activated solid support where cyclization occurs simultaneous with release from the resin, such as with an oxime linker,[190] which functions essentially as a leaving group in this instance. Such a cyclative release strategy can prove advantageous, as will be further discussed later (section 3.3).[191]

Numerous methods likewise have been developed for macrolactonization, the majority originating from the significant efforts directed toward the total synthesis of macrolide natural products.[5,192] These two approaches have multiple advantages: (1) well-established chemistries and methodologies; (2) a variety of readily available reagents; (3) utility for a wide range of ring sizes; (4) linear precursors easily synthesized with high diversity; (5) straightforward execution in either solid or solution phase. However, not all desired targets lend themselves readily to these methods, which typically require high dilution conditions to prevent formation of dimeric and higher order oligomeric side-products.

Two other standard chemical transformations, S_N2 and S_NAr displacements, were initially utilized for simple macrocyclic structures; however, these straightforward methodologies have proven to be applicable for more complex structures as well. Despite their simplicity, these displacement reactions can be incompatible with certain functional groups, require protection of reactive functionalities, are often complicated by side reactions such as elimination, must generally be performed under high dilution conditions for larger ring sizes to reduce the formation of dimers and oligomers, and are quite sensitive to the conformational population of the cyclization precursor. Of course, such approaches also possess attractive benefits: (1) multiple reaction conditions and reagents are available; (2) chemistry is typically well-established and easy to execute; (3) a range of sites of ring closure can be investigated (for S_N2 processes); and (4) reactions can be conducted readily in parallel or on solid supports for diversity generation. Indeed, the facility with which the S_NAr process, in particular, occurs on resin has led to its rather wide application for the construction of constrained macrocyclic peptidomimetics, including those designed to mimic β-turns.[193,194] S_NAr-based macrocyclization is another effective method for the synthesis of natural products, especially those containing a biaryl ether in analogy to vancomycin, teicoplanin, bouvardin, piperazinomycin, **1**, and **2**.[195] The approach is equally well-suited for the construction of non-natural product templates containing such moieties, as they are of interest as a privileged structural feature for new drug design. It is noted that microwave activation has been shown to facilitate the S_NAr process.[196]

On the basis of the pioneering work of Grubbs,[197] ring-closing metathesis (RCM) has become the most widely used reaction for the synthesis of macrocyclic structures, with the second generation ruthenium catalysts being the most popular choice.[4,85,198,199] Advantages of this highly versatile methodology are (1) broad scope and high tolerance of other chemical functionalities, (2) gentle reaction conditions with multiple catalysts of varying reactivity and selectivity readily available, and (3) adaptability to many ring sizes. On the other hand, certain limitations also exist: (1) the product is usually a mixture of olefin geometrical isomers; (2) it can display significant variability in yield based upon type and site of substrate substitution, steric factors, and the presence or absence of heteroatoms; (3) removal of the catalyst to the trace levels acceptable for pharmaceutical use can be technically problematic.

Another viable methodology is the "click chemistry" cycloaddition reaction between alkynes and azides, which has been utilized for several macrocyclic structural types, primarily peptidomimetic,[200] including a β-turn mimic,[201] although applications to nonpeptidic molecules have also been explored.[202,203] This strategy has provided routes to some novel macrocyclic structures; however, it can suffer from the significant formation of dimers or other oligomeric products, indeed sometimes in preference to the monomeric products. Nonetheless, a number of bioactive macrocyclic structures have been successfully accessed using this strategy.[202] This procedure was executed successfully on resin, demonstrating its potential for building diverse libraries.[204] It is noted that click chemistry is particularly tolerant of functional groups and is even one of the few biocompatible ligation techniques. Of course, a triazole has to be acceptable in the final product.

An alternative type of "click chemistry," the thiol−ene reaction,[205] which to date has seen greater application in the materials arena, was recently reported as a route to cyclic RGD peptide mimetics (Scheme 1).[206] The protected linear precursor **101** was assembled either on resin or in solution. Then cyclization executed either photochemically (solid phase) or thermally (solution) gave **102**. Although the potential for medicinal chemistry applications appears more limited than the azide−alkyne cycloaddition, the reaction does occur rapidly with comparable or better yields to other solid phase procedures.

In addition to the organometallic chemistry utilized for RCM and click cycloadditions, a number of other such approaches have

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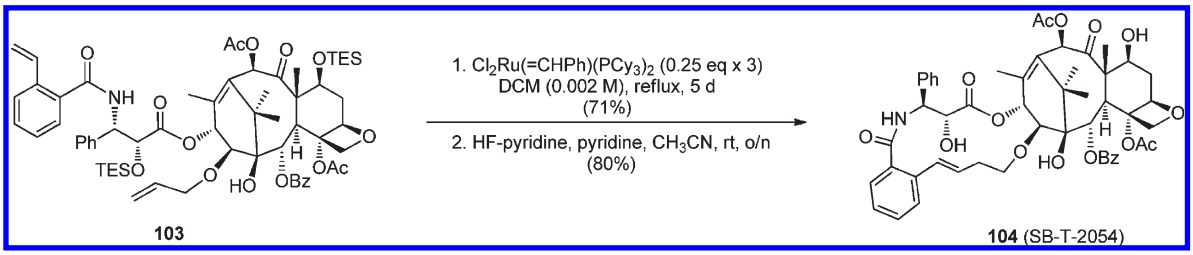
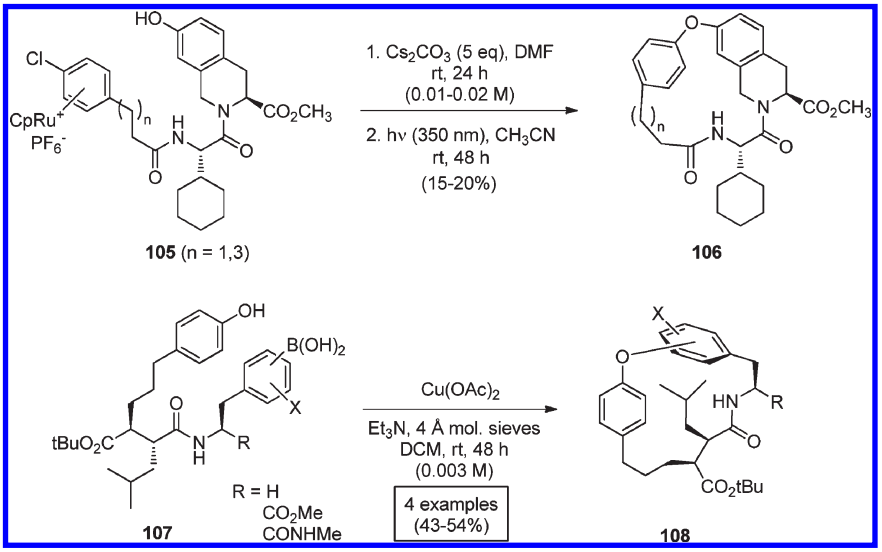
Scheme 2



Scheme 3



been developed to successfully access the macrocyclic framework. For example, an interesting alternative chemistry with ruthenium was observed in the construction of macrocyclic taxoids designed to mimic the bioactive conformation of paclitaxel.[207] In this case, the expected RCM of **103** did not proceed, but rather a diene-coupling reaction occurred to give the highly bioactive compound **104** (SB-T-2054, Scheme 2). However, the scope of this reaction was not further explored.

Ruthenium complexes were also instrumental in another approach to macrocyclic structures, in particular those containing a biaryl ether, complementary to the S_NAr methodology already discussed. Ru π-arene chemistry was exploited to efficiently form biaryl ether macrocycles for the preparation of inhibitors of the HCV NS3/4A and the HIV proteases.[24,48,208,209] In the typical, albeit low yielding, sequence presented in Scheme 3,[209] the linear precursor was converted to the Ru complex **105**. Then arylation was induced through treatment with an excess of strong base to generate macrocycle **106**. The ruthenium was subsequently efficiently removed via photolysis. In addition to the formation of biaryl ethers, this reaction can provide other biaryl systems and thioethers through facilitation of the S_NAr process.[210] Despite the drawbacks of this methodology, including the use of stoichiometric Ru and high dilution to limit oligomerization, it does proceed generally in good to very good yields for macrocyclization and provides easy access to a range of pharmaceutically attractive structures.

Similarly, a copper-assisted process was applied to the synthesis of biaryl ether containing macrocyclic metalloprotease inhibitors (Scheme 3).[211] The target molecules **108** were accessed through the intramolecular reaction of a phenol with a substituted aryl boronic acid **107**. Cyclization conditions were mild enough to tolerate additional functionalities such as amides and esters. However, the presence of an additional phenol proved detrimental and gave <5% of the expected product.

Another broad category of organometallic reactions, palladium-mediated chemistry, has proven quite versatile in accessing the macrocyclic framework. The use of the intramolecular Stille reaction for a variety of macrocyclic structures has been reviewed.[212] This chemistry has been effectively applied to the construction of macrocyclic trienes **110** possessing defined geometry, which were themselves precursors for subsequent transannular Diels−Alder reactions (Scheme 4).[213,214] In addition, Stille coupling provided the key step in a cyclization-release solid phase synthesis strategy directed toward macrocyclic natural products (**111** to **112**, Scheme 4).[215] The Sonogashira reaction, for its part, has proven less widely applicable to macrocycles to date, although it has been successfully utilized for a series of rigidified peptide mimetics as illustrated in Scheme 4 for the synthesis of tripeptidomimetics **114**.[216] This process was best conducted copper-free, as standard conditions resulted in the dimer as the sole product. The same process was applied to dipeptide and tetrapeptide analogues but with lower yield in the latter instance. The Sonogashira route for macrocycles was also effectively employed on resin and for larger rings, as demonstrated by the construction of the 65-membered mimetic of an immunoglobulin loop domain.[217]

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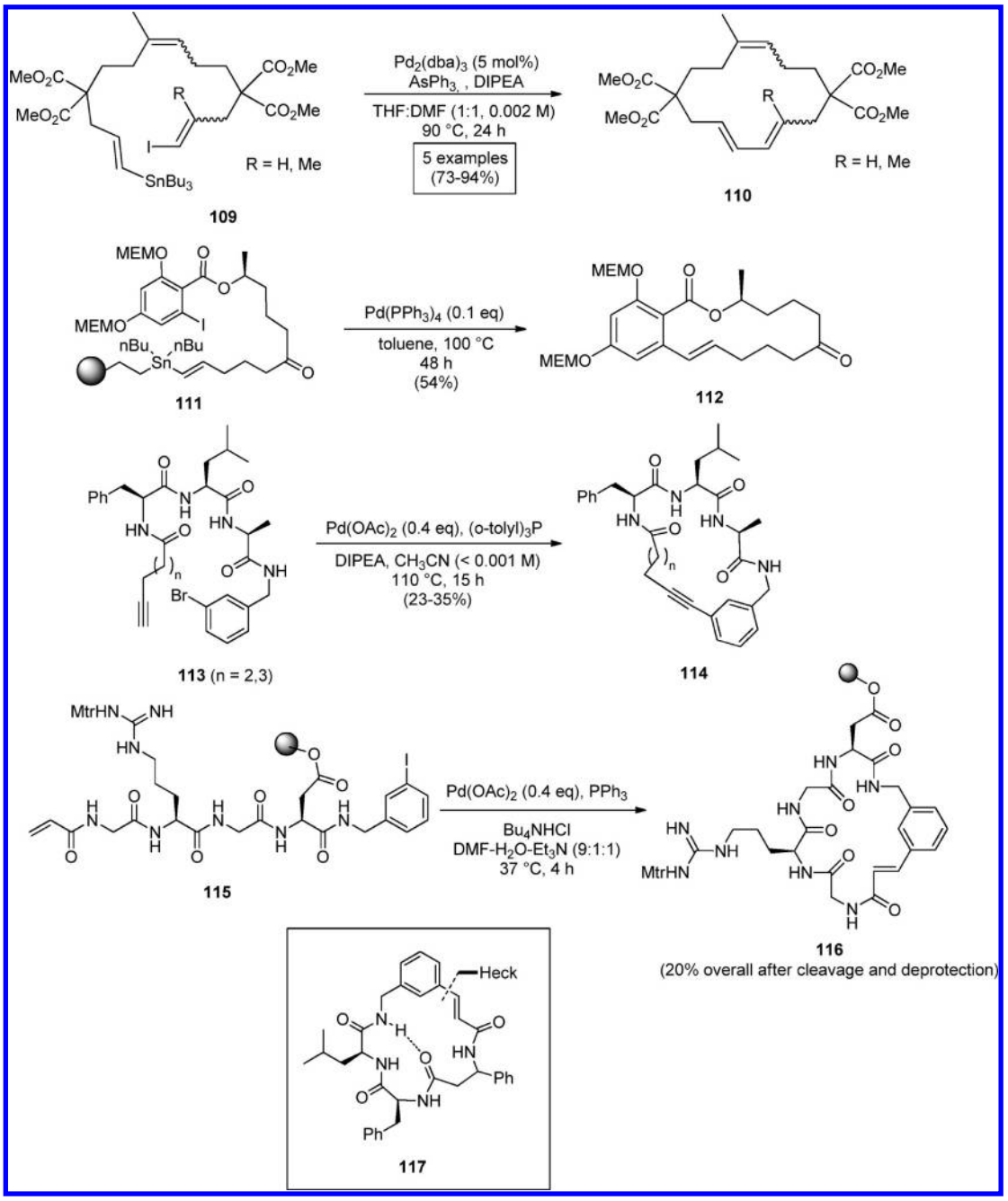
Scheme 4



Where functionalized aromatics are a component of the macrocyclic molecule, the Heck reaction has been found to be a very useful approach, although yields are often modest. For example, Heck methodology was employed in the solid phase construction of cyclic RGD peptidomimetics **116** (Scheme 4).[218] The analogous reaction in solution proceeded more slowly. This methodology was also used to prepare small mixture libraries of similar analogues. In addition, β-turn mimics **117** were constructed utilizing an intramolecular Heck reaction for formation of the macrocycle.[219] It was speculated that cyclization was facilitated by an internal hydrogen bond that preorganized the linear precursor. The use of microwave irradiation for the Heck transformation was shown to accelerate the synthesis of peptidomimetic macrocycles in both solution and solid phase.[220]

Heck reactions have been employed in the synthesis of HCV protease inhibitors[221] and macrocyclic taxoids as well as other natural products.[222,223] The latter applications demonstrated the high complexity of substrates that this process can accommodate. For taxoid structures, this route proved to be more successful than RCM, which was sluggish.[224]

In an even better illustration of the power of Pd-mediated processes, studies directed toward the total synthesis of complestatin relied on the use of an intramolecular Larock indole synthesis to effect macrocyclization (Scheme 5). Systematic investigations led to optimized conditions for this reaction,

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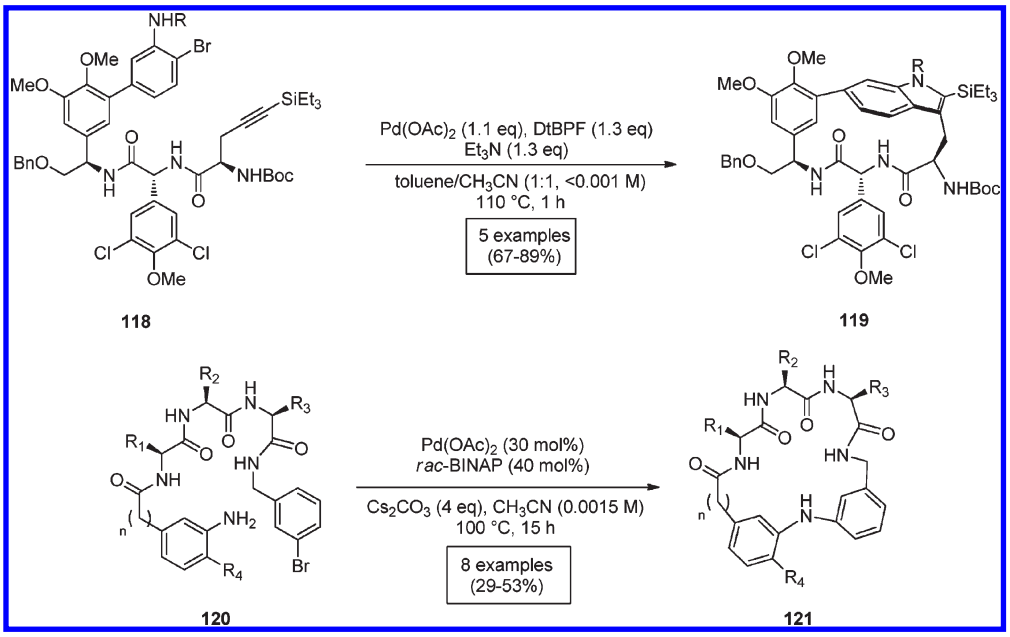
Scheme 5



opening up another avenue for the construction of complex ring systems utilizing palladium chemistry.[225]

Balraju and Iqbal employed the Buchwald−Hartwig C−N coupling reaction for macrocyclization in the construction of peptidomimetics **121** constrained with a diphenylamine linker (Scheme 5).[226] The same research group described the use of Trost's palladium-catalyzed enyne cycloisomerization as another route to macrocyclic structures of this general type (Scheme 6).[227] Application of this chemistry to the diene tripeptidomimetic structure **122** proceeded to give **123**. In contrast to the usual product with two exocyclic double bonds, a conjugated diene with one endocyclic and one exocyclic double bond was obtained. The *E*-stereochemistry and *s*-transoid form of the macrocyclic diene was exclusively formed regardless of the length of the linker on the alkyne portion of the substrate. Furthermore, modifications to the position of the reactive functionalities gave the same stereochemical result for **124**, despite the increased rigidity of the arylalkyne starting material. A similar reaction was executed with a dipeptidic starting molecule, albeit in lower yield (28%). The product dienes **123** were subjected to a Diels−Alder reaction for further structure elaboration.

Denmark et al. recently reported a systematic study on a combination RCM/Pd cross-coupling reaction as a new route to unsaturated macrolactones (Scheme 6).[228] A tandem sequence consisting of RCM to form siloxane **126**, followed by a Pd-catalyzed, Si-assisted cross-coupling cyclization, provided good yields of product **127** as a single stereoisomer. A detailed study of the second step of this process determined the optimal catalyst and solvent while also finding that hydrated fluoride ion was required as an activator for best results. Under the optimized conditions, good yields for the usually very difficult medium ring sizes of 11−14 atoms were obtained and high dilution conditions were not necessary (Scheme 6). This protocol was successfully extended to the more challenging synthesis of the benzo-fused macrocyclic lactones **128** as well.

Palladium π-allyl chemistry was employed as the key step of a multicomponent condensation/metal-catalyzed cycloetherification sequence to construct a series of conformationally restricted macrocycles such as **130**, designed as rigid peptidomimetics (Scheme 7).[229] This approach tolerated a variety of functionality, including alcohols, amides, thioethers, and selected heteroaromatics. The methodology was applied to assemble specific macrocycles found to promote neurogenesis.[230] This reaction sequence was also successfully demonstrated on solid support.

An interesting twist on the use of palladium catalysis in macrocyclization was recently disclosed by Barnickel and Schobert in model studies directed toward the total synthesis of macrocidins A and B (Scheme 7).[231] In a tandem deallylation−etherification sequence, tetramic acids **131** were treated under basic conditions in the presence of $Pd(PPh_3)_4$ to give macrocycles **132** in good yields. However, the transformation failed for the 20-membered ring ($n = 5$), indicating a possible limitation to the generality of this process. A similar transformation led to a closer analogue of macrocidins such as **134**. Palladium was required for ring closure, as reaction of the deallylated material did not proceed in its absence, although the precise mechanism is unknown.

Suzuki-type coupling is one of the most versatile reactions for the synthesis of aryl-containing complex organic structures. It is therefore not surprising that it has also been utilized fairly widely for macrocyclic molecules. In a representative example, side chain cyclization of **135** using typical Suzuki conditions gave the simplified ketomethylene analogue of proteasome inhibitor TMC-95A **136** (Scheme 8),[232] whereas macrolactamization failed to provide acceptable yields of the desired compound. Similarly, an intramolecular Suzuki−Miyaura reaction of tripeptidomimetic **137**, a model intermediate for the synthesis of the arylomycin signal peptidase inhibitors, was studied extensively with regard to catalyst, solvent, and reaction temperature to optimize the yield of the strained biphenyl 14-membered macrocycle **138** (Scheme 8).[233] An analogous transformation was observed to proceed in a highly atroposelective manner for the synthesis of the DEFG ring system of complestatin and

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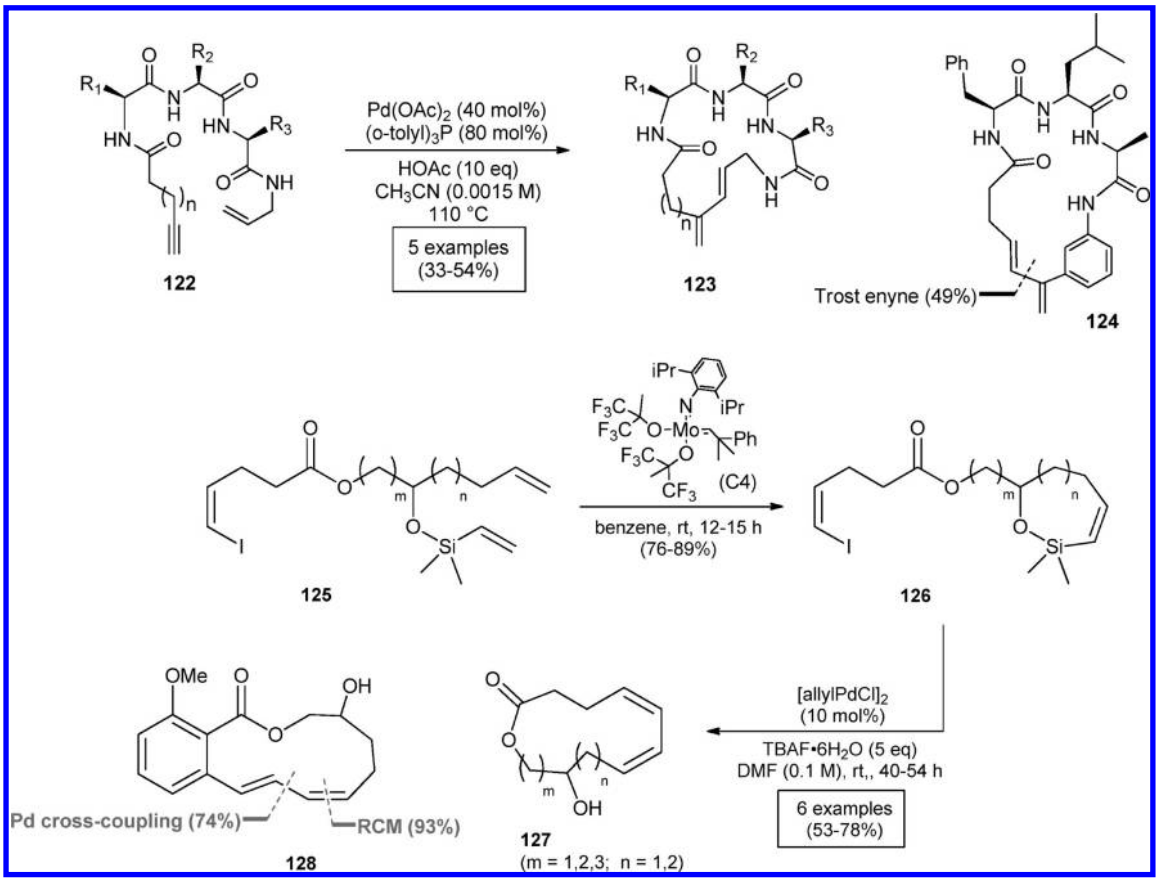
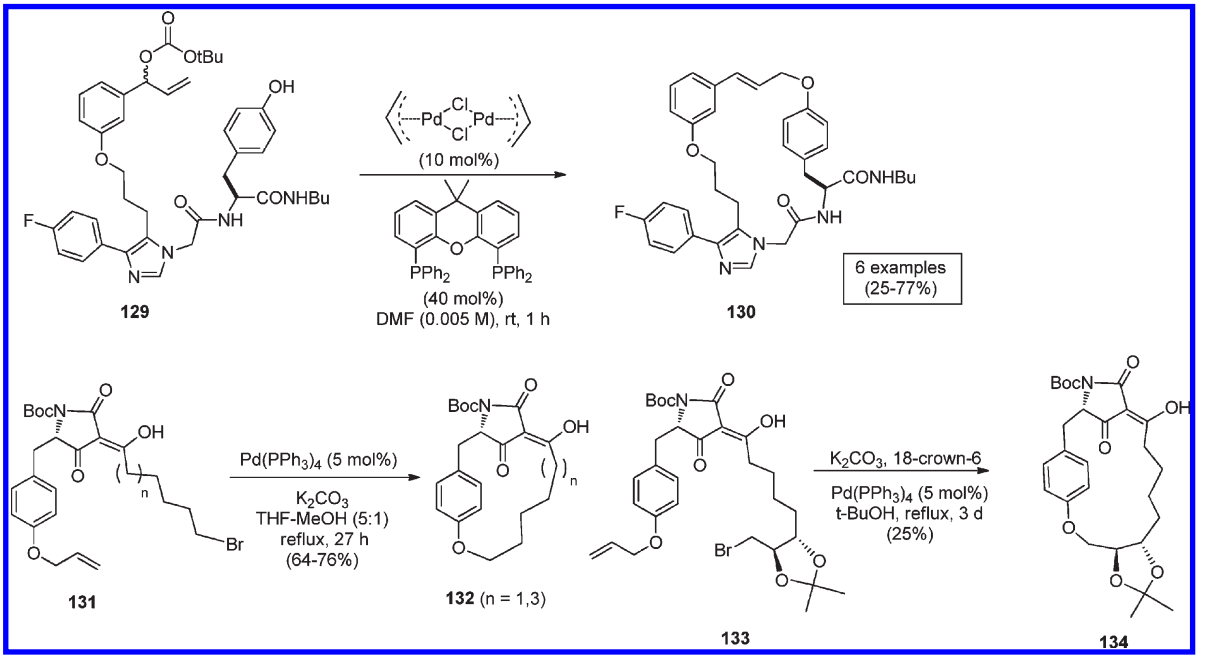
Scheme 6



Scheme 7



chloropeptin I.[234] An alternative rendition, the B-alkyl Suzuki reaction, provided a route to the tricyclic system **140** (Scheme 8) for which RCM failed[235] and also has seen wide application in total synthesis.

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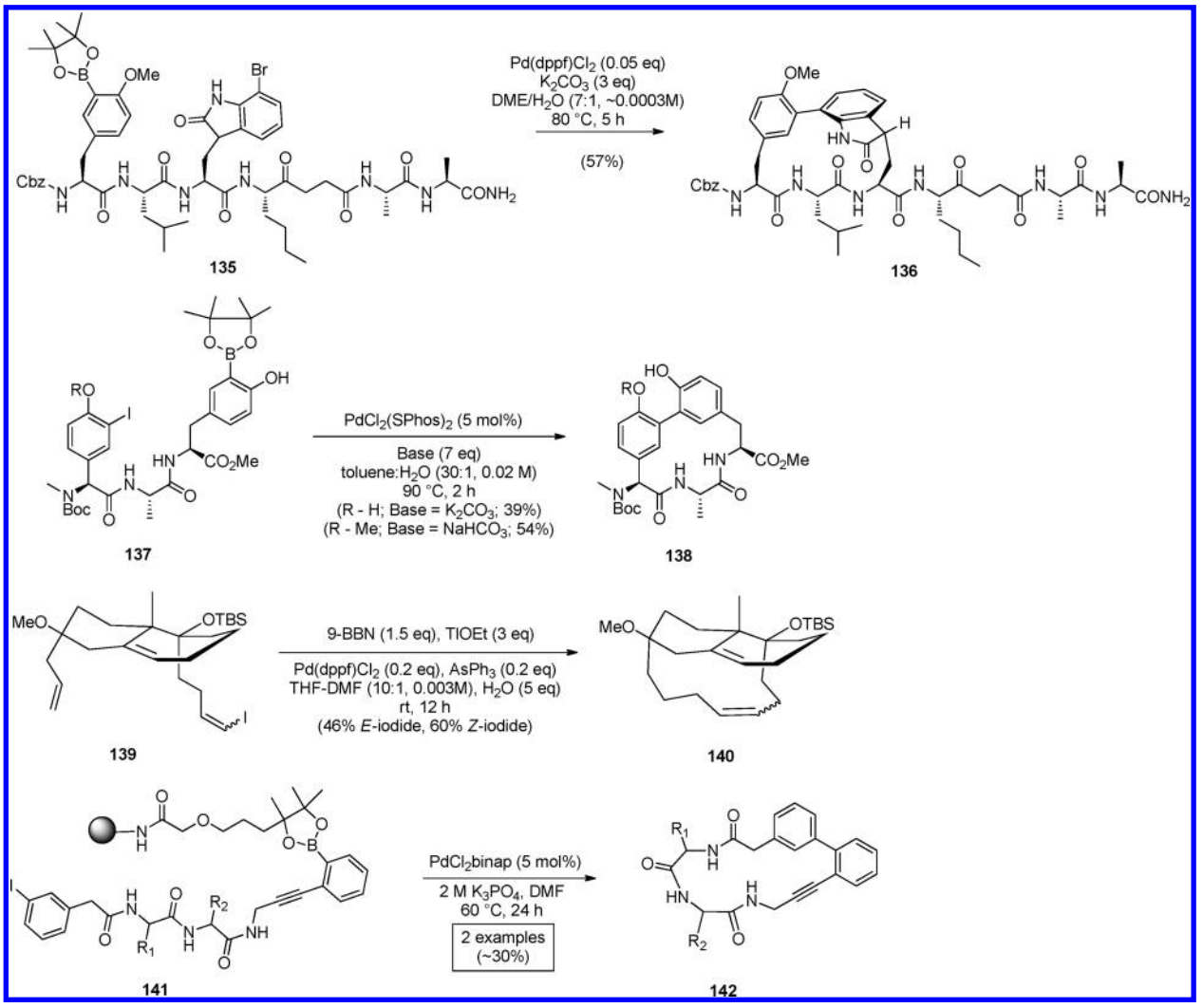
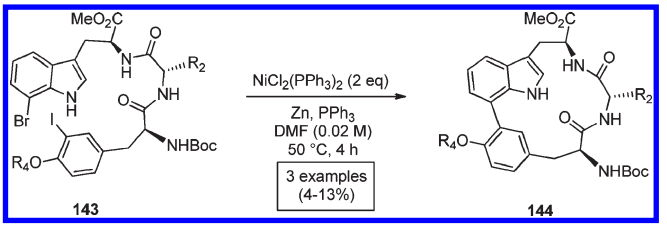
Scheme 8



As another route to β-turn mimetics, Li and Burgess reported solid phase linker **141** specifically for enabling a simultaneous Suzuki coupling, macrocyclization, and resin release process (Scheme 8). Although yields of the product **142** were modest, this does provide an alternative chemistry for construction of the biaryl macrocyclic structures.

In yet another demonstration of the use of organometallics in macrocyclization, again aimed at **136** and analogues, nickel(0)-mediated cyclization of **143** provided the biaryl target molecule **144** (Scheme 9).[236] This process proceeded in poor yield and failed to give product in certain cases. However, all attempts to apply a host of Suzuki coupling conditions for the same transformation were completely unsuccessful.

Scheme 9



Another potentially powerful methodology that has seen only limited application to the macrocyclic framework is the Wittig reaction and its variants. The construction of VCAM-VLA-4 antagonists, in which a carbon chain was substituted for a disulfide linkage in a previous generation of compounds, was enabled through ring closure between an aldehyde and a phosphonoglycine moiety (Scheme 10).[237] Precursor phosphonate **145** was oxidized with osmium tetroxide/sodium periodate, which gave cleaner conversion than ozone. Then the crude intermediate aldehyde was treated with DBU or another hindered base in methylene chloride to provide macrocycle **146**. Reduction of the double bond along with an appropriate deprotection sequence yielded the target structures. The Wittig reaction was also the cornerstone for a diversity-oriented approach to macrocyclic molecules (section 3.3).

Multicomponent reactions (MCR), such as the Ugi, Biginelli, Staudinger, and Passerini reactions, have experienced a renaissance as chemists continue to seek ways of rapidly building complex structures of potential pharmaceutical interest.[238,239] Applications to macrocyclic structures are, however, somewhat limited, although the MiB methodology described in section 3.3 is an exception. Dömling and colleagues have reported the

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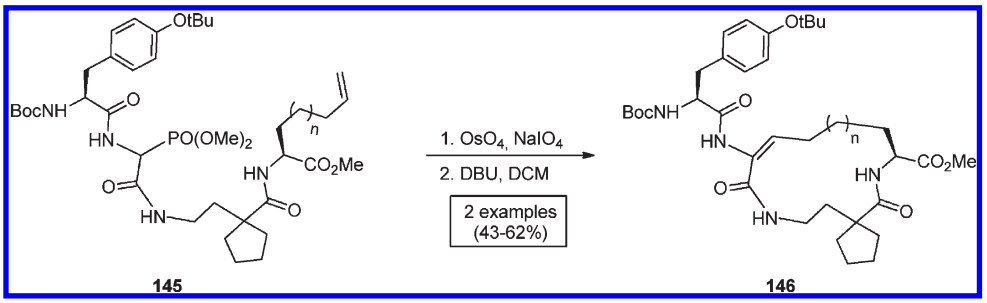
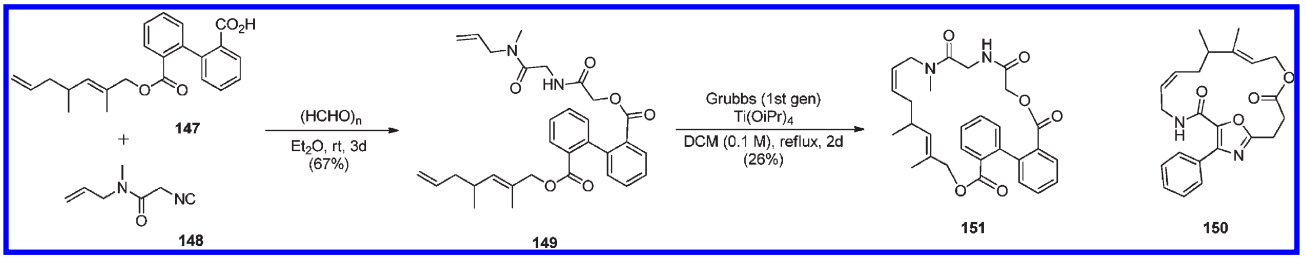
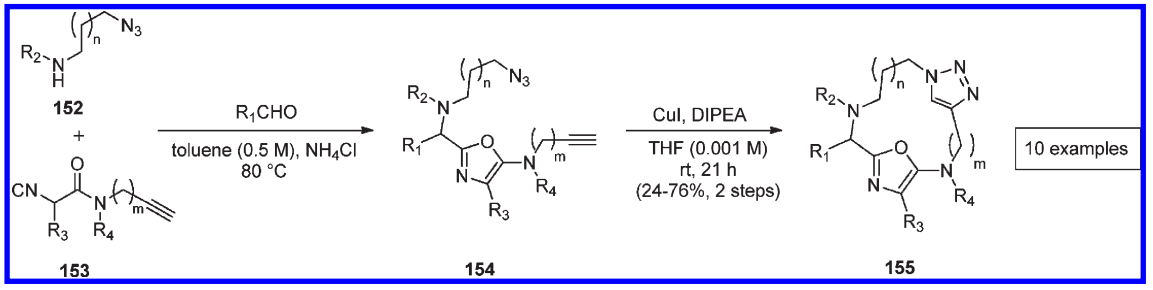
Scheme 10



Scheme 11



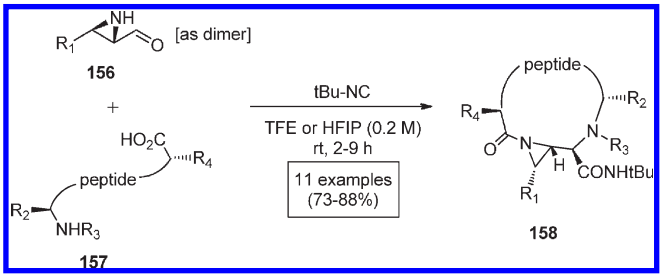
Scheme 12



combination of the Ugi and Passerini type MCR with RCM and described the potential applicability of this sequence for generating libraries of diverse macrocycles.[240] The approach for a representative compound is outlined in Scheme 11, wherein 22-membered macrocycle **151** was produced after the tandem sequence beginning from acid **147**, isocyanide **148**, and paraformaldehyde. In a similar manner, the 17-membered macrocycle **150** was accessed. Because of the limited availability of the acid and isocyanide components for this procedure, methods for their synthesis from more readily available starting materials were also developed.

Analogously, Zhu and co-workers reported a tandem three-component reaction with an azide−alkyne [3 + 2] cycloaddition as a straightforward route to macrocycles (Scheme 12).[241] The initial reaction between ω-azidoamines **152**, aldehydes, and alkynyl isocyanides **153** led to an intermediate 5-aminooxazole **154**, the rigidity of which predisposes the structure to undergo "click" ring closure. This was demonstrated for the formation of 14-, 15-, and 16-membered ring macrocycles **155** in 24−76% yields. It is noted that this methodology and the tandem MCR-RCM sequence above have the potential to be extended to larger numbers of compounds, assuming that an appropriate diversity of the requisite starting materials can be obtained.

Scheme 13



An intriguing application of the Ugi MCR was recently reported by Yudin (Scheme 13),[242] who used amphoteric aldehydes **156** as starting materials to promote a high-yielding process that formed modified peptidic macrocycles **158** with ring sizes varying between 9 and 18 atoms. The resulting molecules represent novel structures themselves or can be used as intermediates for further transformations, for example, aziridine opening with nucleophiles, to increase diversity. This methodology is noteworthy for its fidelity even for typically difficult to access medium size rings, relatively rapid reaction time, no necessity for high dilution, the excellent purity of products including the avoidance of oligomers, exceptional diastereoselectivity, and lack

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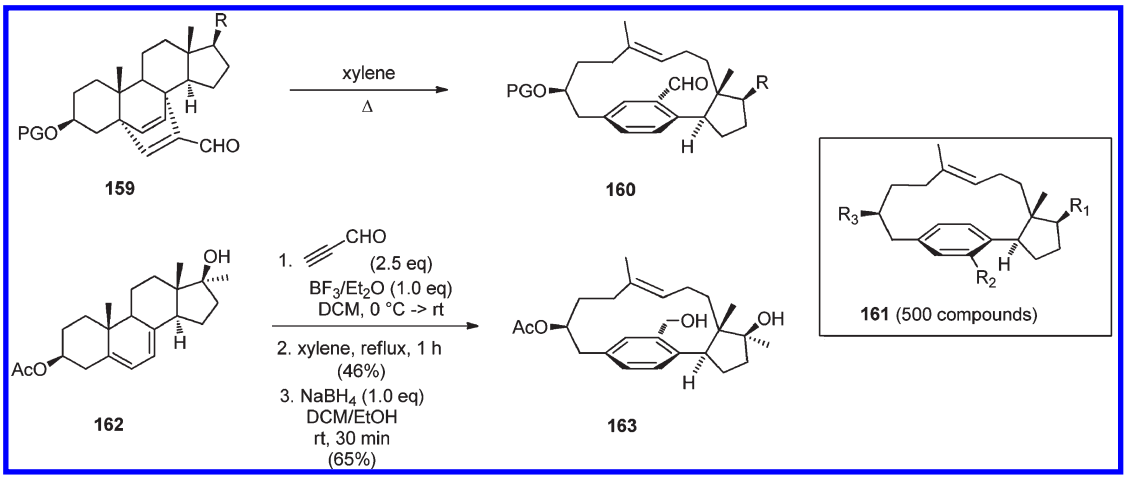
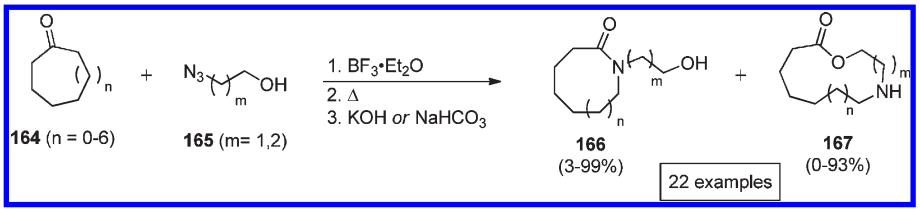
Scheme 14



Scheme 15



of observable racemization. Some limitations of the method appear to be the requirements to match the aziridine configuration with the N-terminal amino acid configuration (i.e., (*S*) with L or (*R*) with D) and for a turn-inducing Pro residue in the peptide chain to obtain the highest yields. However, with continued development, this process holds promise for the generation of libraries of unique biologically interesting compounds with significant diversity. As a step in this direction, the automation of this sequence in continuous flow on a digital microfluidics platform was recently described.[243]

An even more unusual approach provided access to a class of dopamine antagonists in which the reductive ring-opening of a multicyclic precursor led to the macrocyclic structure.[244] This procedure was successfully applied to a wide range of potent dopamine antagonists.[245] Applications of this strategy are naturally limited in scope to the few systems appropriate for such an internal bond-breaking mechanism and containing functionality stable to the strongly reductive conditions. Despite these potential limitations, an analogous strategy was employed to generate macrocyclic molecules starting from steroids (Scheme 14).[246] This scaffold rearrangement proceeded via a Diels−Alder/retro-Diels−Alder sequence to provide a single regioisomer and atropisomer of the product **160** despite the vigorous reaction conditions. About 500 compounds of type **161** were constructed employing this methodology. In addition, these materials can undergo additional conversions to further increase diversity. One example of an entire sequence from the steroid precursor **162** to the 14-membered ring-opened product **163** is illustrated in Scheme 14. This approach was applied to the synthesis of phosphatase Cdc25B inhibitors.[247]

Another route that is limited in terms of scope but is appropriate for certain macrocycles is ring expansion. For example, the synthesis of neutral endopeptidase 24.11 inhibitor **12** (Figure 3) was achieved utilizing two successive ring expansions from cyclooctanone.[41] In a similar vein, Forsee and Aubé reported a nitrogen insertion/ring expansion of cyclic ketones **164** that provided either larger ring lactams **166** or lactones **167** depending on the starting ring size and the base employed (Scheme 15). Starting materials with larger ring sizes and the use of a weaker base ($NaHCO_3$) favored the formation of **167**, although production of the lactam could not be completely suppressed. This has undoubtedly limited the potential applicability of this route, which does not appear to have been further investigated.[248]

The Mitsunobu reaction and its variants are among the most useful reactions in synthetic chemistry.[249] Not surprisingly, therefore, this mild and versatile chemistry has also found some utility for the construction of macrocyclic structures (Scheme 16). For example, two series of MMP inhibitors (**170**, **173**) were elaborated using this methodology.[64,250] In addition, this route was utilized for the macrocyclic portion of several HCV protease inhibitors as exemplified by **39** and **40**.[95,97]

The synthesis of the BACE-1 inhibitors **61** and **62** showcases application of another standard reaction transformation for cyclization in which an intramolecular reductive amination was used for ring closure (Scheme 17).[114] A similar strategy taken to an extreme has been employed in the construction of the G-quadruplex macrocyclic ligand **176** (BOQ1), whereby the cyclodimerization of a dialdehyde precursor and diethylenetriamine was accomplished via reductive amination on four aldehyde moieties.[251]

Free radical chemistry has also been successfully applied to the construction of macrocyclic structures. In the representative reaction shown in Scheme 18, the cyclic tripeptidomimetic **178** was accessed by treatment of the acryloyl bromide **177** under standard radical-generating conditions. The transformation proceeded in similar yields for dipeptidomimetics and in the

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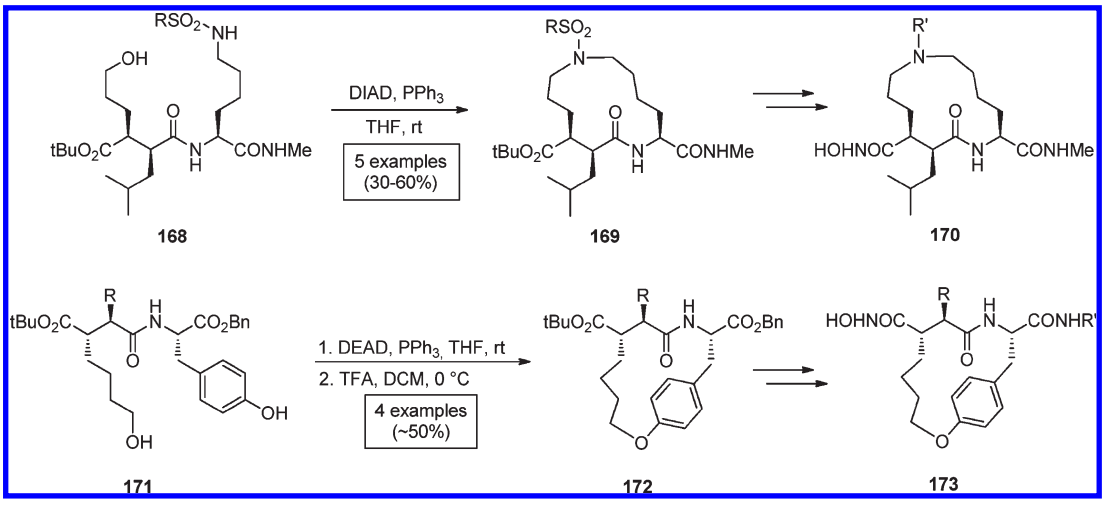
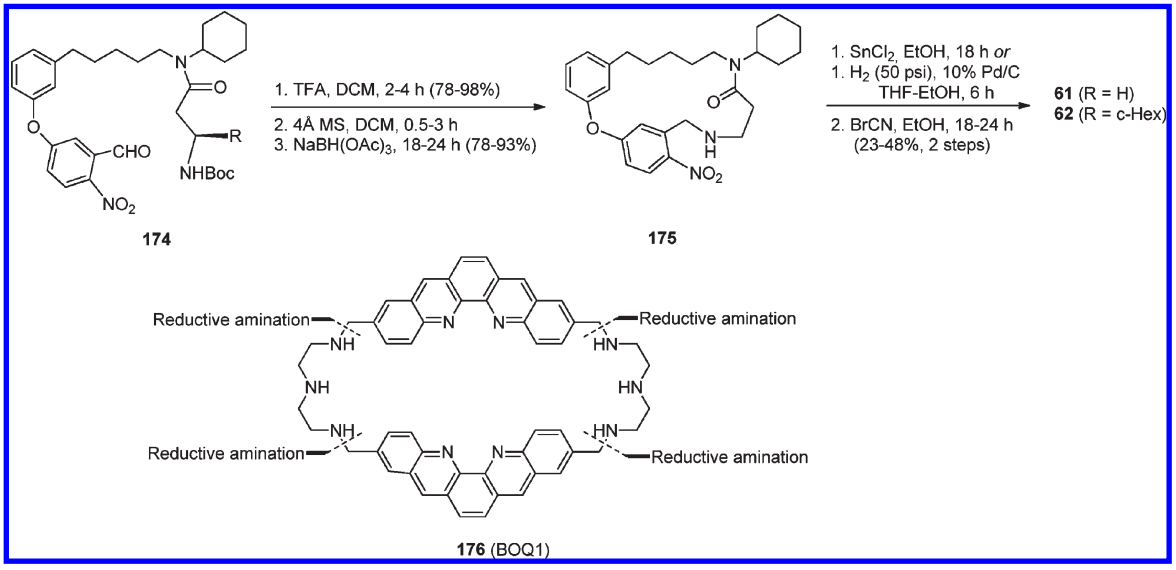
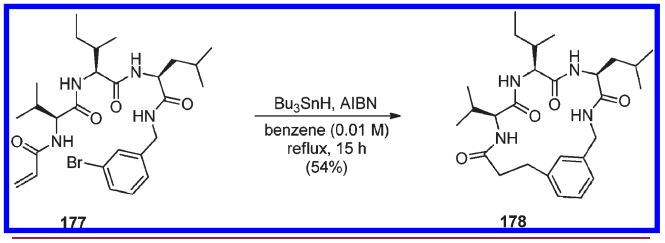
Scheme 16



Scheme 17



Scheme 18



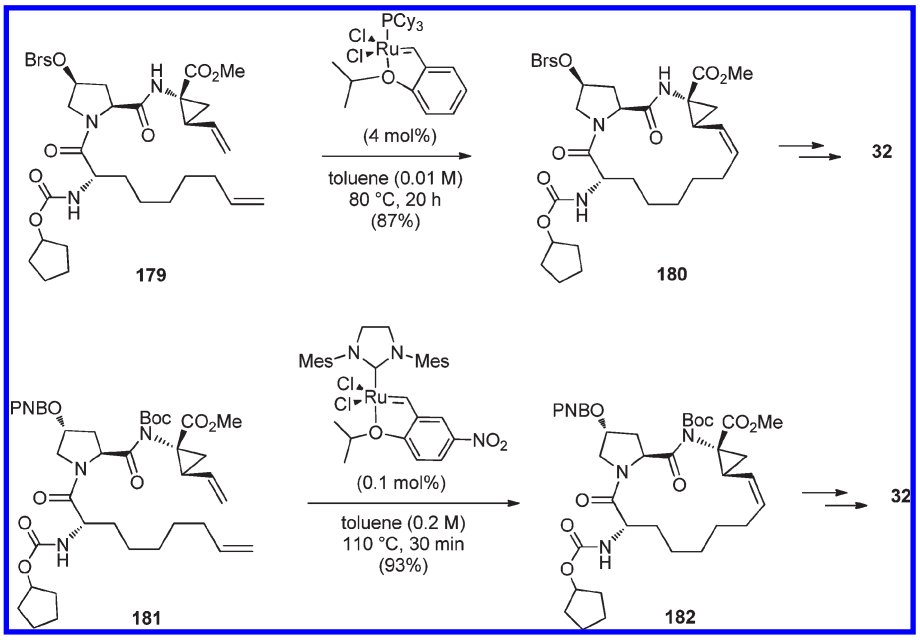
presence of additional substrate constraints, such as a proline residue.[252]

In addition to the examples described above, a number of interesting methods have been developed and exploited for macrocyclic structures in the context of natural product total synthesis.[2] Some of these reactions have been studied extensively or have provided access to structures with significant complexity, so they are noted here as leading references for the adventurous and innovative researcher to consider when approaching the construction of novel macrocyclic molecules. As a first strategy, the Nozaki−Hiyama−Kishi cyclization has been utilized for formation of medium-size rings in the construction of new epothilone analogues[253] and in a formal total synthesis of amphidinolactone A.[254] A Dieckmann-type cyclization was the key step in the assembly of the core of diazonamide A.[255] Multiple laboratories have explored the utility of the Prins cyclization, including for (−)-kendomycin.[256] Another method suited for medium-sized rings is the pinacol macrocyclization, which has been applied to the CDE ring portion of lancifodilactone G.[257]

As can be seen from the rich variety of processes just described that have been successfully applied to the macrocyclic structure, it appears highly likely that examples of bioactive macrocyclic molecules will continue to increase in number as more explorations in this area are conducted.

3.2. Large Scale Synthesis. Few examples of large scale syntheses of macrocyclic drug candidates have been reported, and this remains a clear and future challenge for such structures. Nonetheless, the potential for these structures to be accessed on

Scheme 19



the scales required for clinical development has been demonstrated and reported in at least two cases. For the HCV NS3/4A protease inhibitor **32**,[258] the key reaction in the sequence upon scale-up remained RCM (Scheme 19). A systematic study of alternative approaches and reaction conditions led to meaningful modifications and improvements of the route. Significant attention was devoted to the RCM step, with the best reaction conditions shown in Scheme 19. The result was the development of a new convergent process, which was scaled up to provide over 400 kg of the macrocyclic product.[258,259] However, certain aspects of the RCM step were identified for further optimization, specifically lower catalyst load, increase in reaction rate, and reaction concentration. These aspects were indeed all able to be significantly improved in the second generation process (Scheme 19), which included a change in the catalyst, leading to a 10-fold increase in reaction efficiency and 20-fold reduction in solvent consumption (from **181** to **182**).[260]

In the case of **83**, the kilo scale process involved several key differences from the stepwise linear construction utilized during medicinal chemistry investigations, which was a necessity because the initial compounds were obtained from solid phase synthesis. Indeed, an efficient convergent procedure was developed, involving the straightforward assembly of alkylated amino acid **183** with dipeptide **184**, selective deprotection and cyclization, followed by purification by crystallization to provide **83** in very good overall yield (Scheme 20).[261] For the latter chemical transformation, Goodman's DEPBT reagent proved to be the most effective in terms of yield,[262] giving no observable epimerization, and high dilution conditions were not required because the macrolactamization of **186** was performed at a concentration up to 0.06 M. **183** was in turn constructed from L-cyclopropylglycine and an electrophilic amino alcohol derivative via an S_N2 alkylation, replacing the Mitsunobu reaction of the original solid phase procedure, while **183** was obtained from standard coupling of the appropriate amino acid derivatives.

It can be expected that further examples of efficient large scale processes will become available as the bioactive compounds described in the first section of this Perspective advance in clinical evaluation.

3.3. Macrocycle-Based Library Technologies. Although the pharmaceutical potential of the macrocyclic structure is well-recognized, one of the reasons this molecular class has not been more widely explored is the difficulty in accessing libraries of such molecules for incorporation into the standard high throughput screening processes central to most modern drug discovery efforts. Only a handful of methodologies have been successfully demonstrated to date for the construction of large libraries of macrocyclic molecules. Each of these relies on a different fundamental underlying technology that permits the simultaneous synthesis of multiple macrocycles. However, a common feature is that they typically exploit the excellent variety available within the large pool of commercially available amino acid building blocks, both standard and unnatural, to generate diversity in the resulting macrocyclic structures. In addition, they typically take advantage of the benefits of synthesis on polymeric supports with respect to the ease of handling larger numbers of compounds and the pseudo-dilution effect of the resin effectively limiting formation of dimeric and oligomeric side products.

One of these successful methods targeted the synthesis of libraries of tripeptidomimetic macrocycles incorporating a non-peptidic tether component specifically designed to define the conformational population of the resulting cyclic molecules and improve druglikeness (Scheme 21).[263] A key step in the solid phase synthetic route was the connection of the tether moiety to the peptidic portion of the molecule via a Fukuyama−Mitsunobu reaction using the benzothiazolesulfonyl (Bts) moiety for activation. Also noteworthy in this process was the use of a cyclative release mechanism for macrolactamization for which yields of 16−75% were obtained. As could be expected, cyclization was highly sequence-dependent, and silver ion generally facilitated the more difficult cyclizations.[264] This methodology was used to generate a screening library of over 40 000 macrocyclic molecules, from which potent and selective motilin antagonists **80** and **81**,[137] ghrelin agonists **82**−**84**,[141] and ghrelin antagonist **85**

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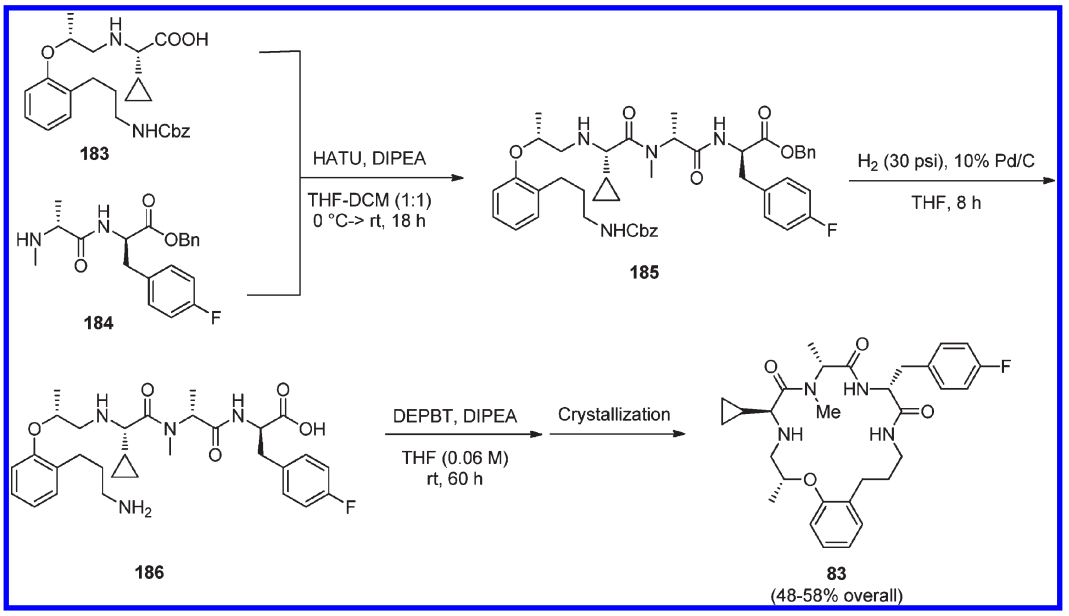
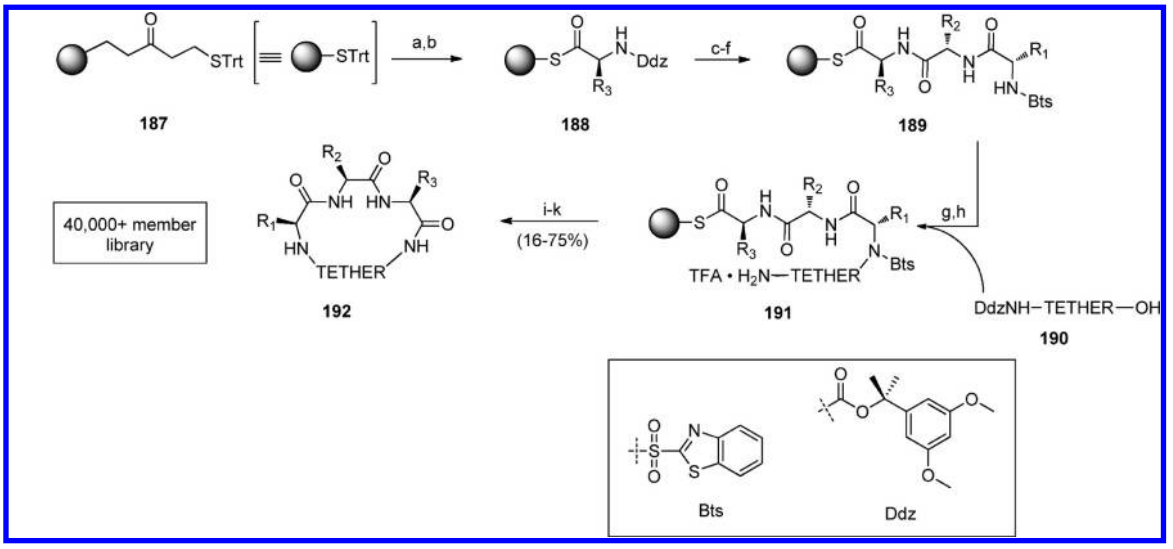
Scheme 20



Scheme 21[a]



[a] Reagents and conditions: (a) 10% TFA, Et_3SiH, DCM; (b) Ddz-AA_3-OH, PyBOP, DIPEA, NMP; (c) 1−2% TFA, Et_3SiH, DCM; (d) Ddz-AA_2-OH, HBTU, DIPEA, NMP; (e) 1−2% TFA, Et_3SiH, DCM; (f) Bts-AA_1-OH, HBTU, DIPEA, NMP; (g) **190**, PPh_3, DIAD, THF; (h) 1−2% TFA, Et_3SiH, DCM; (i) DIPEA, THF, MP-carbonate, $Ag(OCOCF_3)$ (optional); (j) PS-thiophenol, KOTMS, THF/EtOH; (k) 50% TFA, Et_3SiH, DCM.

originated. This approach to macrocycles is appealing in (1) its reaction simplicity, (2) the high fidelity of the macrocyclization, and (3) the ease and speed with which subsequent hit follow-up and lead optimization can be performed due to the ready availability of diverse components and the use of the same chemistry as employed for library construction. The use of a cyclative release mechanism (from **191** to **192**)[191] effectively improved the purity of the synthetic products, since materials that did not undergo macrocyclization remained bound to the resin. Difficult syntheses were therefore reflected in poor yields, yet acceptable purities. However, the use of less standard protection strategy (the highly acid sensitive Ddz group for the α-amino moieties) and the activated nature of the thioester group on the growing resin bound chain having the potential for premature loss from resin, as well as racemization of the chiral center of the C-terminal portion, are limitations in this approach. The use of other chemistries, including RCM, to construct these molecules has also been reported.[137]

Another approach for the preparation of libraries of macrocycles emanates from the work of Liu and co-workers on DNA-templated synthesis both to facilitate the ring-forming reaction and to track the monomer units used in the construction of the individual molecules (Scheme 22).[265] Watson−Crick base-pairing of complementary DNA strands organizes reaction substrates and

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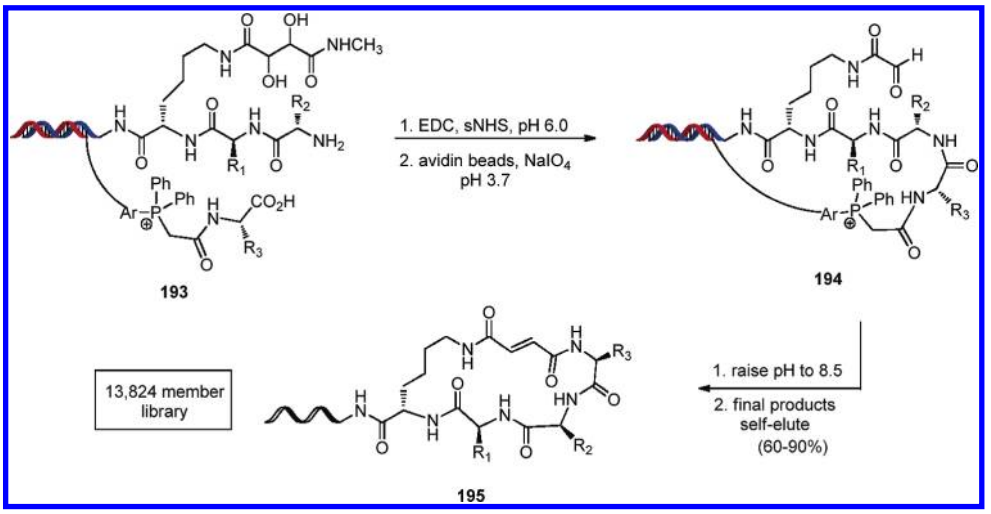
Scheme 22



increases the effective molar concentration of the reactants. In this manner, macrocyclization can be favored over other potential reaction pathways. The resulting DNA−macrocycle conjugates can then be subjected to in vitro selection for binding affinity to target proteins, which enriches the active components versus the more prevalent nonbinding ones in the mixture. This latter step permits the evaluation of the products for activity in lieu of traditional HTS. The DNA fragments act as well as bar codes for the identification of the individual reactants and, hence, the bound product compounds. Other noteworthy features of this strategy are the extremely low reactant concentrations (nanomolar) required and its compatibility with aqueous solvents. This latter point could prove to be a limitation on the types of chemistry that can be employed, although the process has been successfully extended to organic solvents as well.[266] Potentially applicable to a range of chemistries and structures, this strategy was first demonstrated on the assembly of a small library of 65 macrocycles involving a Wittig reaction for macrocyclization (Scheme 22).[267] Each step of the sequence was conducted using the DNA-templated methodology with the successive building blocks each conjugated to a 10-mer or 12-mer oligonucleotide. In this manner, the resulting individual library compound was connected to an encoding DNA sequence. The library was then subjected to in vitro selection for desired activity, with PCR amplification and sequencing for deconvolution and identification of binding molecules. As a demonstration of this latter process, macrocycles possessing high affinity for carbonic anhydrase were successfully selected from the library.

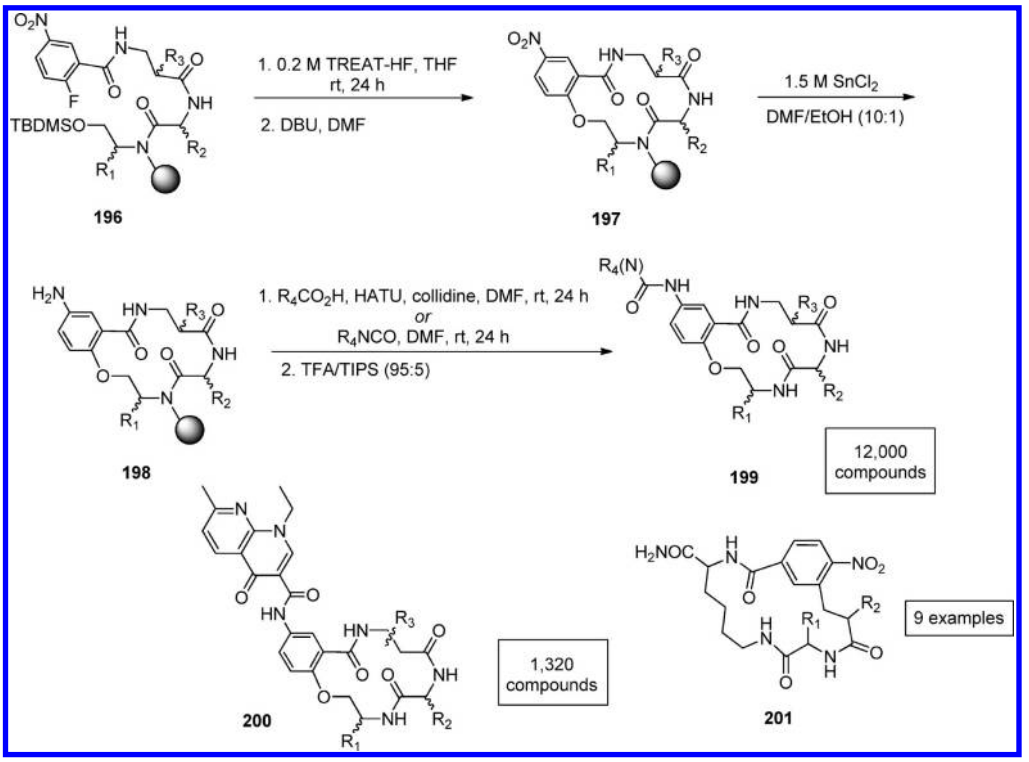
Some of the beneficial features of DNA-templated methodology for the construction of macrocyclic libraries are the following: (1) very large numbers of compounds can be accessed; (2) the size of the macrocyclic ring can span from medium to quite large; (3) a number of standard reaction types have been shown to be compatible with the requirements of the method; (4) it enables in vitro selection for the best affinity to a given target, as those entities that are found to bind can be amplified by PCR. However, the approach has its limitations as well: (1) not all desired chemistries can be performed due to incompatibility with the DNA strand; (2) it is necessary to develop additional chemistry for the hit-to-lead and lead optimization stages; (3) methods are not directly adaptable to scale-up. Nonetheless, this technology has been applied to the construction of a larger 13 824 member library,[268] which was recently shown to be a source of selective kinase inhibitors and activators.[269] The strategy was also applied to the identification of TNF-α inhibitors.[270] As another demonstration of the utility of DNA-templated technology, the discovery of a new palladium-catalyzed alkyne−alkene macrocyclization reaction was enabled through application of this approach.[271]

A reaction described earlier that has demonstrated its utility for the synthesis of a number of interesting macrocyclic structures, the S_NAr, was employed as a critical step in the construction of a combinatorial library of 12 000 macrocycles.[272] For these libraries, diversity was incorporated not only by the multiple amino acid components but also by postcyclization transformations (Scheme 23). Deconvolution led to the identification of inhibitors of bacterial protein synthesis. These researchers have also reported a smaller library of 1320 compounds containing a particular quinolone pharmacophore (**200**), from which other antibacterial lead compounds were identified.[273] An analogous approach was reported by Giulanotti and Nefzi who assembled structures of general type **201** through an intramolecular S_NAr process on resin.[274] Although clearly applicable to library construction, extension to this purpose was not described in detail.

S_NAr methodology was also utilized as a key step in the solid phase assembly of a small library of macrocyclic dipeptidomimetics **204** designed as mimics of the β-turn structure of neurotrophin growth factors. Further diversity was introduced by derivatization of the aromatic amine prior to cleavage of the compounds from the resin (Scheme 24). Both agonists and antagonists of the neurotrophin receptors TrkA and TrkC were identified from this targeted collection (**92** and **93**).[170]

In addition to these strategies, the literature is replete with elegant methodologies that have the potential to be applied to the construction of libraries of macrocyclic compounds. Given the ubiquitous use of RCM in macrocycle formation, it is not surprising that a systematic study of the reaction for diversity purposes has been described by Schreiber (Scheme 25).[275] Generally, the reaction proceeded quite well, although the appearance of dimers and stereoisomers was occasionally a confounding factor. It was envisioned that manipulation of the available functionality in **206** after macrocycle assembly

Scheme 23



would be performed in order to incorporate additional diversity. X-ray analyses of the resulting macrocyclic products provided guidance regarding the effects of substitution and stereochemistry on reaction outcome. Further, in preparation for the synthesis of larger numbers of target compounds, the feasibility of performing the optimized sequence on resin supports was confirmed. An analogous RCM process was used to prepare a 122-member library of fused macrocyclic carbohydrate molecules (**206a**, **206b**, **206c**). Again, preliminary investigations were conducted in solution, then transferred to solid phase.[276]

An analogous similar report on the diversity-oriented synthesis of macrolactones from the same lab included a detailed study of the factors involved in controlling the key macrocyclization step.[277] The desired chemical sequence originating from hydroxy acids was developed, as shown for the representative transformations in Scheme 25, then applied to the preparation of a small library on solid phase (**209**). In contrast to the stereochemical bias observed in the RCM work, the configuration of the linear precursors was not seen to have a significant effect on macrolactonization. Although some trends were evident in this limited series of compounds, the difficulties inherent in undertaking the synthesis of macrocycles in a library format are succinctly reflected in one of the final sentences of this report: "The rules for macrocyclization are fairly complex."

In addition, RCM was used to construct a library comprising 12-, 13-, and 14-membered macrocycles on solid phase (Scheme 26). Enantiomeric oxazolidinone moieties were employed to prepare either isomer of **210**, which after cleavage of the chiral auxiliary was subjected to sequential amide and ester couplings under standard conditions to provide **211**. RCM then yielded macrocycles **212**. This methodology was utilized to prepare a 2070 compound library from which an inhibitor of Sonic Hedgehog (**91**, robotnikinin) was identified.[278]

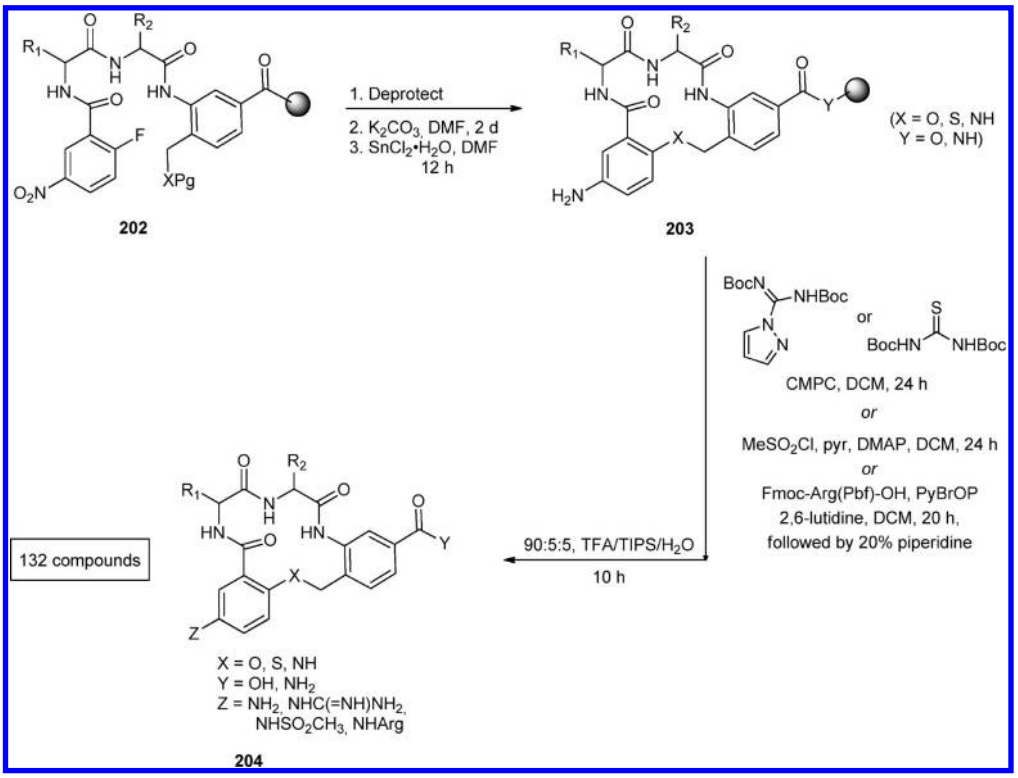
One last demonstration of the application of RCM worth mentioning in the context of libraries is the elegant diversity-oriented synthesis studies of Nelson on the use of fluorous tags and a small series of optimized reactions, including metathesis cascades, to create over 80 different natural product-like scaffolds. Not surprisingly, macrocycles such as **213** and **214** were included among the unique structures generated (Scheme 27).[279]

As another example of diversity inspired by natural products, small libraries of modified hapalosin analogues **216** were constructed in solution utilizing a variety of β-hydroxy-γ-amino acid components and a standard DPPA-mediated macrolactamization (Scheme 28). Investigation of a diversity of substituents at C3, C4, and C9 positions was reported.[280]

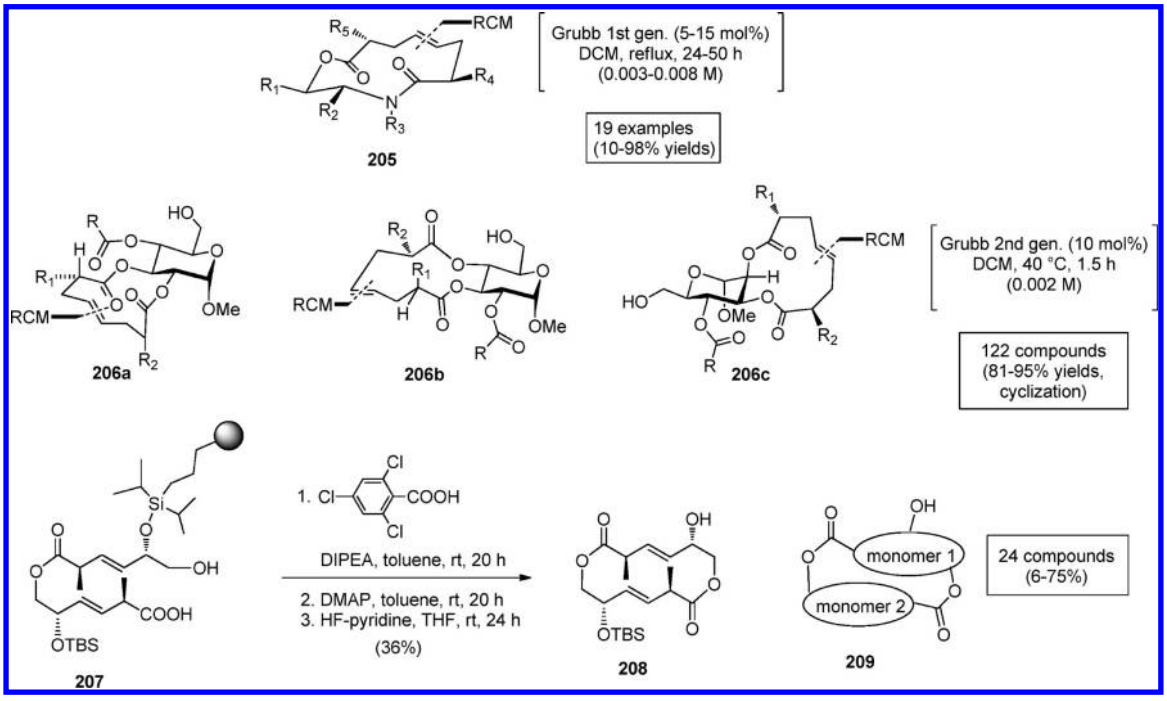
Macrocyclic dilactones (or macrodiolides) comprise a wellspring of bioactive natural products. Beeler et al. have described the homo- and heterodimerization of a series of chiral hydroxy ester substrates catalyzed by tin and using microwave irradiation to produce a small synthetic library within this structural class. Appropriate preliminary investigations led to the identification of a fluorous stannane as the best catalyst and chlorobenzene as the preferred solvent, as well as determination of the optimal microwave conditions and reaction concentration. A wide range of ring sizes (11−23) containing diverse functionalities was accessed successfully using this methodology (see Scheme 29 for a representative example), although larger ring sizes (>19) were disfavored. Initial biological studies on the library identified **221** as a novel κ-opioid receptor antagonist.[281]

As described earlier, Pd chemistry has been explored quite effectively for macrocyclic structures. One of the first reported

Scheme 24



Scheme 25



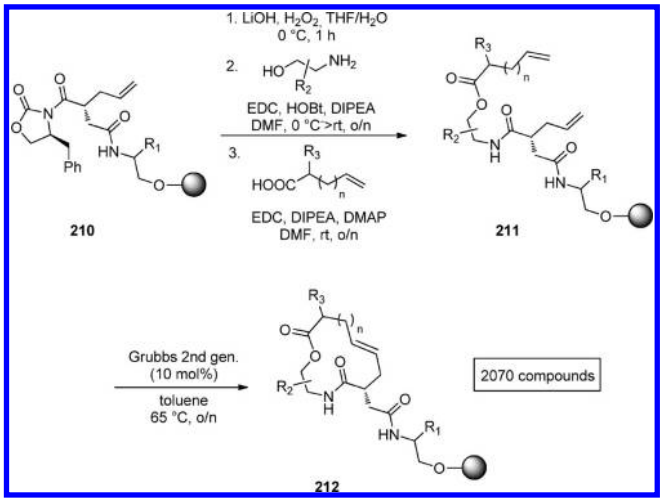
efforts on targeting diversity-oriented macrocyclic structures was the construction of a small library of 20−24 membered nonpeptide macrocycles (**223**) using a Heck reaction as the cornerstone for scaffold assembly (Scheme 30).[282]

Palladium-catalyzed cyclocarbonylation was utilized by Takahashi and co-workers for the solid phase construction of small libraries of both RGD mimic macrocycles (**225**, Scheme 31) and macrosphelide analogues (**227**). Intramolecular

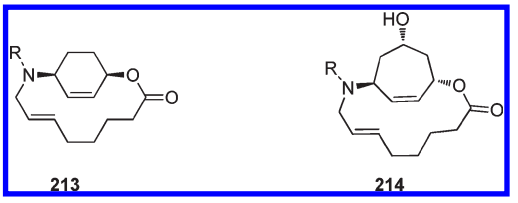
cyclization of a resin bound amine or alcohol (**224** and **227**, respectively) with an iodophenyl moiety in the presence of a Pd catalyst and carbon monoxide gas provided the target structures after cleavage from the polymer support (Scheme 31). For the RGD library, although reaction conditions for the key transformation were optimized for base, solvent, and temperature, yields varied widely depending on the stereochemistry of the Asp substituent.[283−285] This process tolerated a wide variety of functionalities, including halides, ethers, ketones, and esters, and so may enjoy broader application in the future.

Multicomponent reactions have proven to be very fruitful routes for the construction of libraries; however, as mentioned earlier, only limited attention has been directed toward their potential for accessing the macrocyclic target class.[238,239,286] One exception is multiple multicomponent macrocyclizations including bifunctional building blocks (MiB) strategy, which has been shown to be quite useful for a range of complex macrocyclic structures and has been exemplified with a number of different substrates and reaction modalities, including the generation of combinatorial libraries.[287] As with many MCR approaches, the benefits of MiB are the following: (1) chemistry development times for rather complex structures are shorter, as multiple steps occur in a single reaction vessel; (2) it is compatible with both traditional solution phase and solid phase techniques; (3) hit-to-lead investigations can utilize the same chemistry as library construction; (4) choice of appropriate building blocks can impart favorable physicochemical properties on the resulting molecules; (5) the process is adaptable to scale-up. The chemistry is typically conducted under pseudo-high-dilution conditions involving slow syringe pump addition of one of the components into the reaction mixture. Although yields in most cases are rather modest, the formation of a high number of bonds and the density of functionality that can be introduced in a single step are considerable advantages.

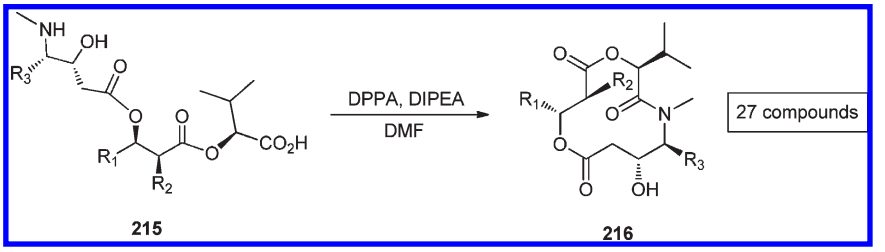
Scheme 26



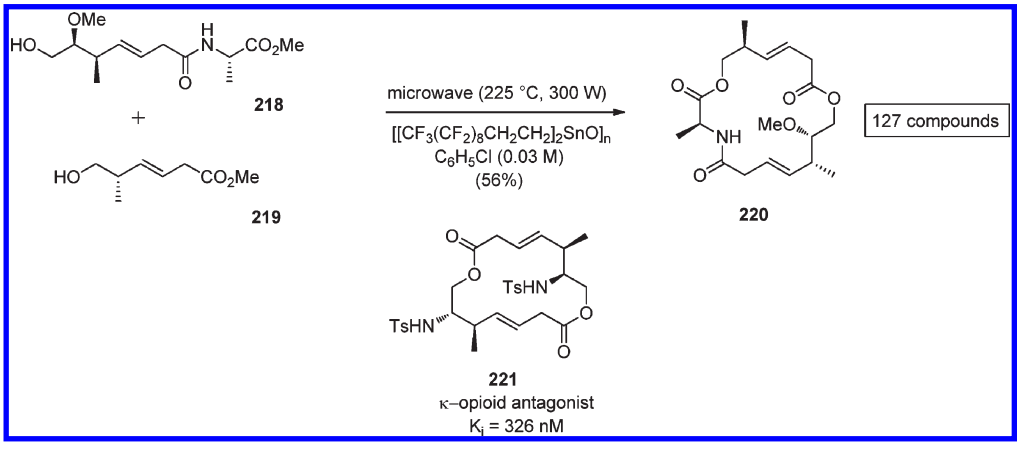
Scheme 27



Scheme 28



Scheme 29



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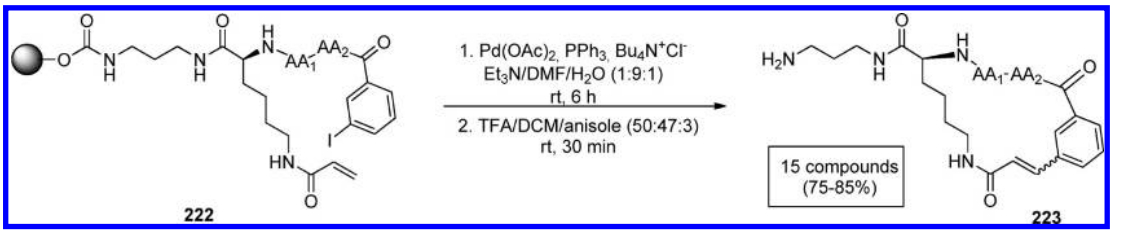
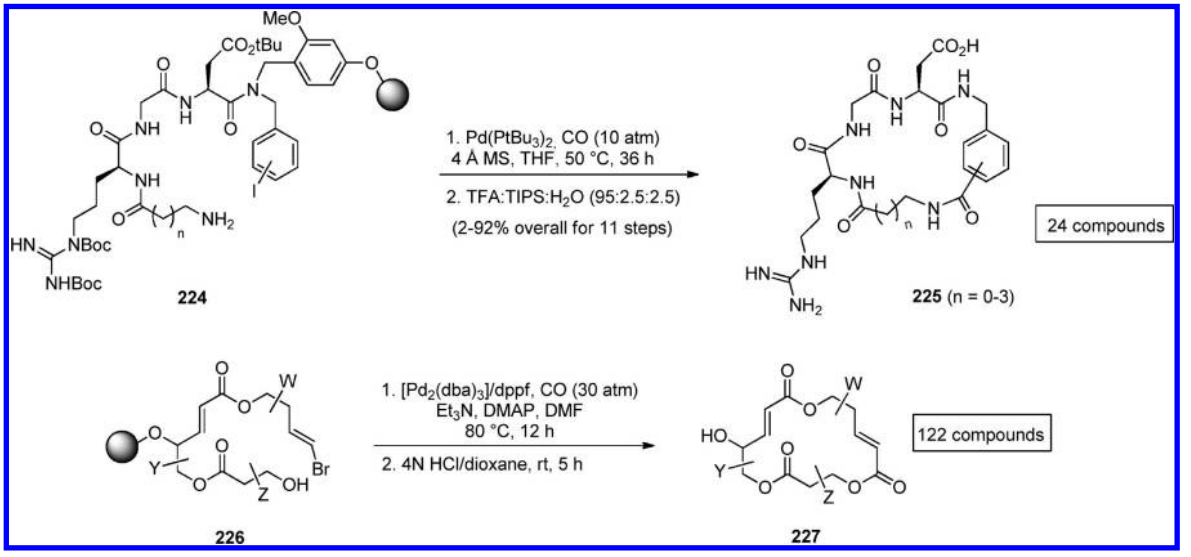
Scheme 30



Scheme 31



Most of the MiB approaches involve some type of Ugi MCR for macrocycle formation, such as that shown in Scheme 32,[288] which was successfully demonstrated for a range of diamines, diisocyanides, and all standard amino acids except Arg. Indeed, it is possible to sequentially conduct multiple MCRs, with the last one resulting in formation of the macrocycle. For example, three consecutive Ugi 4-CR were used to construct RGD analogues **229** and four tandem Ugi reactions were employed to assemble steroid−peptoid macrocycles, including some with ring sizes of 60 atoms.[289,290] Varying the nature of the individual components or the order of addition of the components can result in different product structures, providing significant enhancements to the diversity that can be derived from a given reaction sequence. The MiB strategies have been successfully employed for the construction of steroid−peptoid macrocycles,[290−292] steroid−biaryl ether macrocycles **230**,[293] which can in turn be subjected to additional MCR to form even larger 60- and 68-membered macrocycles, biaryl peptoids **231** with up to 50-membered rings,[294] and a variety of large peptoid structures and large macromulticycles.[295,296] The latter were designed as synthetic receptors but also represent complex structures displaying novel architecture of possible interest for materials chemistry.

In addition to the Ugi MCR, the Passerini and Staudinger 3-CR has been successfully applied in the MiB strategy (Scheme 33). Depsipeptide-like macrocycles **232** and **233** resulted from the Passerini and Passerini−Zhu (oxidative) procedures, respectively, while compounds containing multiple β-lactams **234** are formed from the Staudinger process. Potentially large libraries could be made using these MiB approaches, and the chemistry proved to be rather versatile, although except for some work directed toward very small mixture libraries, such an application has not yet been specifically reported. Further, the diversity accessible in this strategy, as with most MCRs, was limited by the availability of building blocks, particularly for the isocyanide component. Additionally, the symmetrical nature of the products does not lend itself readily to lead optimization efforts, as it is difficult to systematically modify the individual sites as would be desired in a typical medicinal chemistry effort. In such cases, alternative synthetic approaches then must be designed.

In another demonstration of the use of MCR in methods applicable to synthesizing macrocyclic libraries (Scheme 34), Zhu and co-workers described the 3CR between amines **235**, aldehydes **236**, and α,α-disubstituted α-isocyanoacetamides **237** in the presence of ammonium chloride to produce intermediate 5-iminoisoxazolines. These were converted by treatment with acid into macrodespipeptides (**238**) of 14−16 atom ring size.[297]

As one final example of the formation of libraries of macrocyclic compounds, Walsh et al. reported a chemoenzymatic approach to molecules that contain structural elements of both peptides and polyketides (Scheme 35).[298] The linear precursors **239** are cyclized in solution or on solid phase using an enzyme, TycC thioesterase, with a permissive substrate range to yield the hybrid target structures. Unlike many macrocyclization processes, this reaction proceeded well regardless of the stereochemistry of the chiral centers of **239**, with the primary side product (∼20%) resulting from hydrolysis. Although this process was demonstrated on only a few representative substrates such as **240**, it has applicability for the construction of unique diverse libraries.

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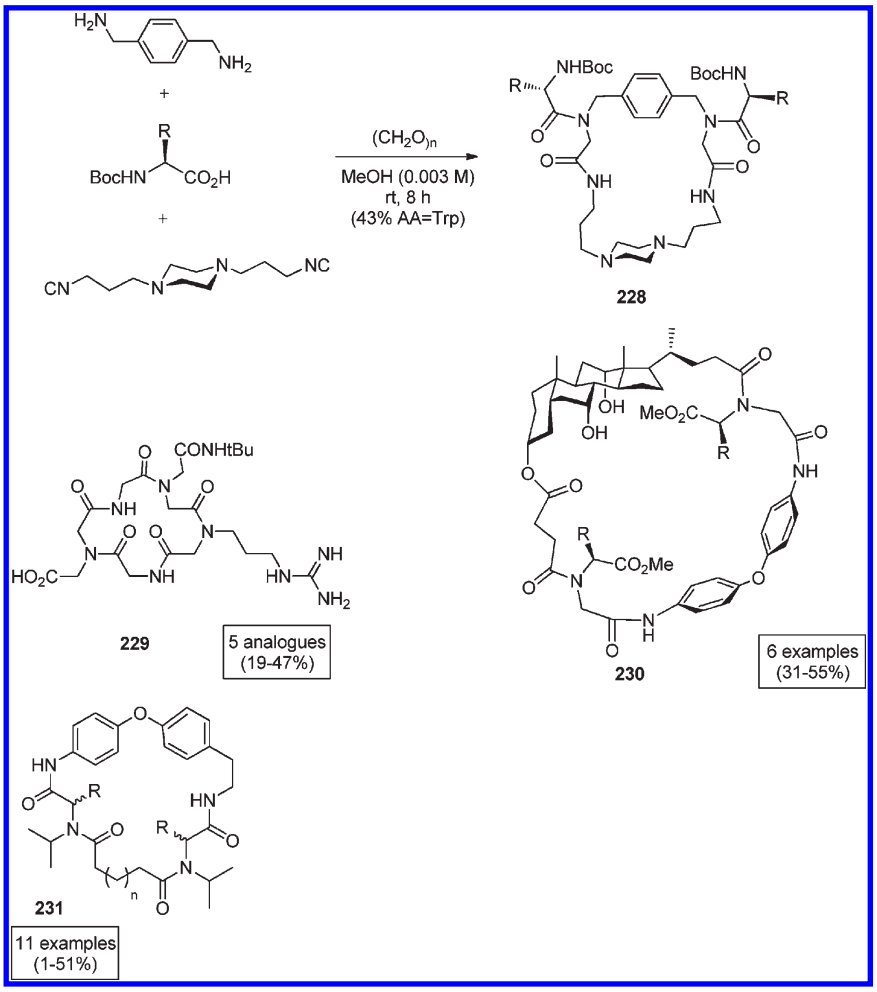
Scheme 32



With the growing number of methodologies that have either proven their ability to generate macrocyclic libraries or possess unrealized potential for such an effort, it is only a matter of time before the screening collections in pharmaceutical and biotechnology companies become populated with an increasing number of diverse macrocyclic scaffolds.

4. PERSPECTIVE

Macrocycles have been exploited on most classes of pharmaceutical targets. Whether originating from natural products or peptides, multiple drugs belonging to the macrocycle chemotype are currently used in therapy. Synthetic macrocycles represent the most recent subclass, yet as exemplified in the preceding sections, their chemical diversity will be limited only by our imagination. Synthetic macrocycles have already made significant contributions to drug discovery. On proteases, macrocyclization is a very advantageous way to preorganize a peptidomimetic ligand in the β-turn conformation suitable for binding to the active site of serine, aspartyl, cysteine, and metalloproteases. Thus, macrocyclization is an excellent way to lock out alternative conformations that may lead to liabilities such as side effects or poor bioavailability. Macrocyclization also reduced overall polarity, enhanced cellular penetration and increased bioavailability. Indeed, several of the above examples created molecules with good to excellent oral bioavailabilities. On proteases, it supported the transition from nondruglike peptidic leads to druggable clinical candidates. On other enzymes, it was found to be an efficient way to fine-tune the selectivity profile.

Topologically, macrocycles can cover a broad surface area in a conformationally restricted way, as opposed to acyclic small molecules of similar molecular weights. At an equal number of heavy atoms, this is a direct consequence of their lower number of rotatable bonds. We believe that this unique property of macrocycles makes for a behavior that is closer to small molecules than their molecular weight would actually predict. As a result, it seems that macrocyclization extends the acceptable range of molecular weight and polarity toward higher values. This is a direct effect of entropic control based on transannular interactions. A parallel can be drawn with synthetic chemistry observations pertaining to the Diels—Alder reaction: from an intermolecular to an intramolecular to a transannular Diels—Alder reaction, the level of control on the stereo- and regiochemical outcome of the reaction increased to near perfection, owing to transannular interactions.[299,300]

Macrocyclization offers two directions for diversity generation. First, it represents an efficient way to scan chemical space without adding molecular weight, which is often a problem with acyclic molecules. Examples where ring size and topology greatly affected biological activity and PK profile are numerous.

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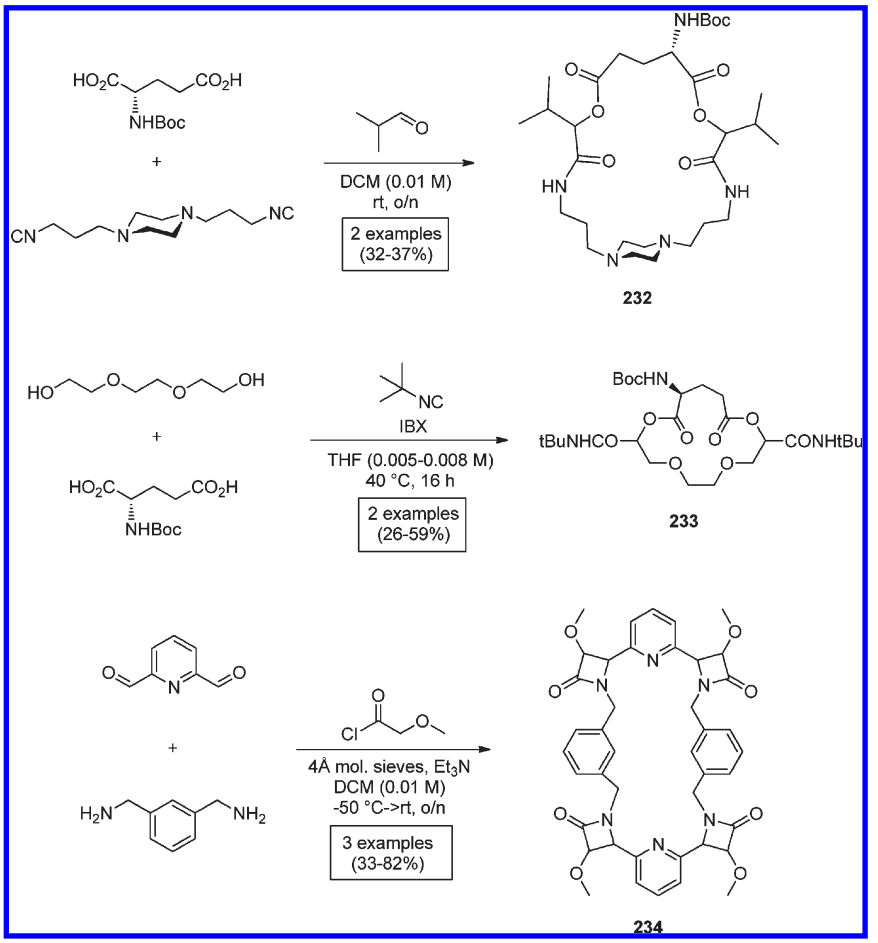
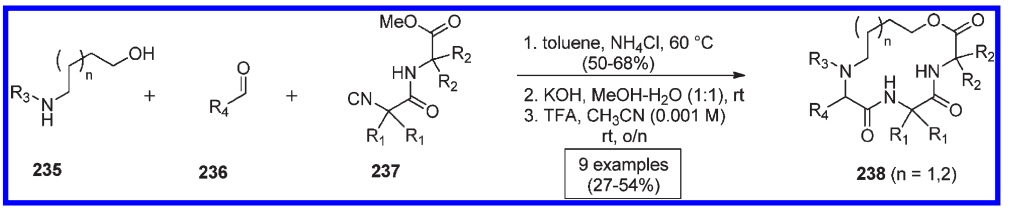
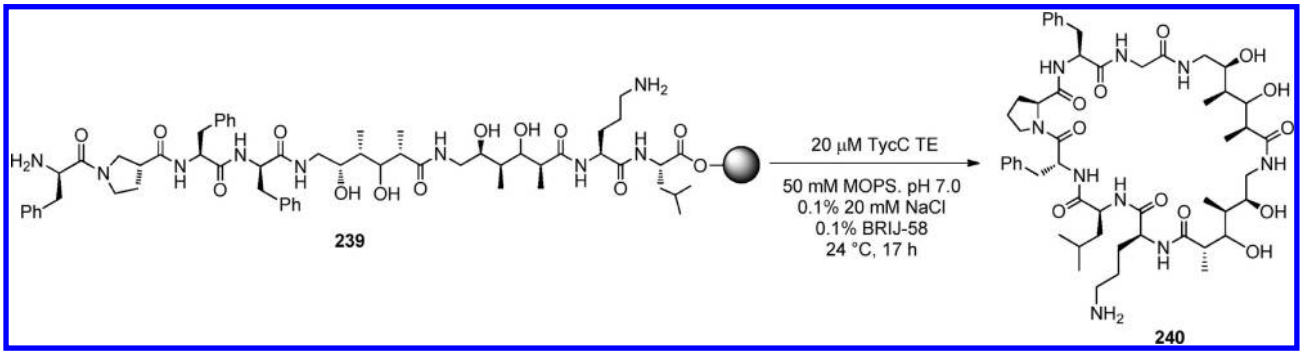
Scheme 33



Scheme 34



Scheme 35



Second, macrocycles can be used as templates for the restricted spatial display of pharmacophores. For those targets that require large interacting surface areas and/or potentially distant epitopes such as the shallow targets of protein−protein interactions, macrocycles appear to have an edge over their acyclic counterparts.

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One of the limiting factors to broader exploitation of the macrocycle chemotype remains their availability in sufficient chemical diversity. In our opinion, at a time when high throughput screening of large collections is the starting point of many discovery programs, the paucity of robust, general synthetic methods required for library construction is one of the main reasons that the compounds have not been more broadly used in medicinal chemistry. Natural products have proven to be a very reliable source of chemical diversity and delivered multiple macrocyclic drugs; however, their chemical complexity is often self-limiting. It is indeed very difficult to implement a medicinal chemistry effort if the basic scaffold is not accessible in large quantities and with a minimal number of chemical steps. Nevertheless, efforts invested in the synthesis of readily diversified natural product-inspired macrocycles demonstrated their potential, as exemplified by the identification of a macrolactone inhibitor of the Sonic Hedgehog pathway. On the other hand, large libraries of macrocyclic peptides and peptidomimetics are available and rely on robust methodologies. However, purely peptidic macrocycles, except for a few cases such as cyclosporine, are often too polar to become orally bioavailable drug candidates with acceptable PK-ADME properties. They constitute excellent entry points into a discovery program though, which can later evolve toward leads with lower peptidic character. Several approaches to the generation of diversified synthetic macrocycles have emerged recently. Two examples have already given interesting results: the diversity-oriented macrocyclic peptidomimetic technology, which delivered several preclinical candidates and two advanced clinical candidates on GPCR targets, and the DNA-templated technology, which delivered modulators of protein−protein interactions. The properties of the former category are definitely distant from peptidic drugs and closer to those of small molecules. With the broad variety of synthetic approaches that have been successfully applied to macrocycle synthesis, we believe that the application of such methods to the generation of large and chemically diverse libraries will permit investigation of this new, largely untapped chemical space that possesses unique properties for the discovery of new drugs on otherwise difficult targets.

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BIOGRAPHIES

Eric Marsault obtained undergraduate training at ESCOM and Université Pierre et Marie Curie (Paris), then a Ph.D. in Organic Chemistry at McGill University, Canada (with the late Dr. George Just). Following a 2-year stay with Sanofi (Italy), he joined Dr. Pierre Deslongchamps' lab in Sherbrooke (Québec, Canada) for postdoctoral studies, then the company Néokimia/Tranzyme Pharma in 2000 as a researcher, then group leader and director of medicinal chemistry. The work of this team established the macrocyclic drug discovery platform of the company, from which several preclinical candidates and two clinical candidates currently in advanced development were optimized. He joined Université de Sherbrooke, Canada, in 2009 to start a medicinal chemistry laboratory. His research interests are in the medicinal chemistry of GPCRs, the validation of emerging target classes, and targeted cell delivery.

Mark L. Peterson is currently Vice President, IP and Operations, at Tranzyme Pharma. He was previously with Monsanto and Advanced ChemTech, where he worked in a variety of research areas including structure-based design, solid phase organic chemistry, combinatorial libraries, synthetic automation, heterocycles, and peptides. With Tranzyme Pharma since 1999, Dr. Peterson led the chemistry research and development efforts during the technology development stage of the company and the initiation of its drug discovery programs. Dr. Peterson received his Ph.D. in Organic Chemistry from Washington State University (with D. Matteson) and conducted postdoctoral research at the University of Minnesota (with R. Vince). He is author or coauthor of over 80 publications and abstracted presentations plus one book chapter, as well as co-inventor on over 20 patents and patent applications.

ABBREVIATIONS USED

Aβ, amyloid β peptide; ACE, angiotensin-converting enzyme; ANF, atrial natriuretic factor; APP, amyloid precursor protein; BACE-1, β-amyloid cleaving enzyme 1; BBB, blood−brain barrier; Bts, benzothiazole-2-sulfonyl; CDK, cyclin-dependent kinase; CHT1, choline transporter 1; CNS, central nervous system; CSF, cerebrospinal fluid; DPPA, diphenylphosphorylazide; DRG, dorsal root ganglion; EGF, epidermal growth factor; FTase, farnesyltransferase; GGTase, geranylgeranyltransferase; GI, gastrointestinal; GPCRs, G-protein-coupled receptors; HCV, hepatitis C virus; HDAC, histone deacetylase; HIV, human immunodeficiency virus; HTS, high throughput screening; icv, intracerebroventricular; iv, intravenous; mAb, monoclonal antibody; MCR, multicomponent reaction; MDCK, Madin−Darby canine kidney cell line; MDR1, multidrug resistance 1; MiB, multicomponent macrocyclization including bifunctional building blocks; MMC, migrating motor complex; MMP, matrix metalloprotease; mpk, milligram per kilogram; NGF, nerve growth factor; NEP, neutral endopeptidase; NT, neurotrophin; PCR, polymerase chain reaction; P-gp, P-glycoprotein; PK-ADME, pharmacokinetics, absorption, distribution, metabolism, excretion; po, per os (orally); PPI, protein−protein interactions; PRA, plasma renin activity; PSA, polar surface area; PTK, protein tyrosine kinase; pY, phosphotyrosine; RCM, ring-closing metathesis; SAR, structure−activity relationship; TACE, TNF-α converting enzyme; tPa, tissue plasminogen activator; trNOE, transfer nuclear Overhauser Effect; VEGF, vascular endothelial growth factor

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