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Medicinal Chemistry

Advisory Board

N-Methylation of Peptides and Proteins: An Important Element for Modulating Biological Functions

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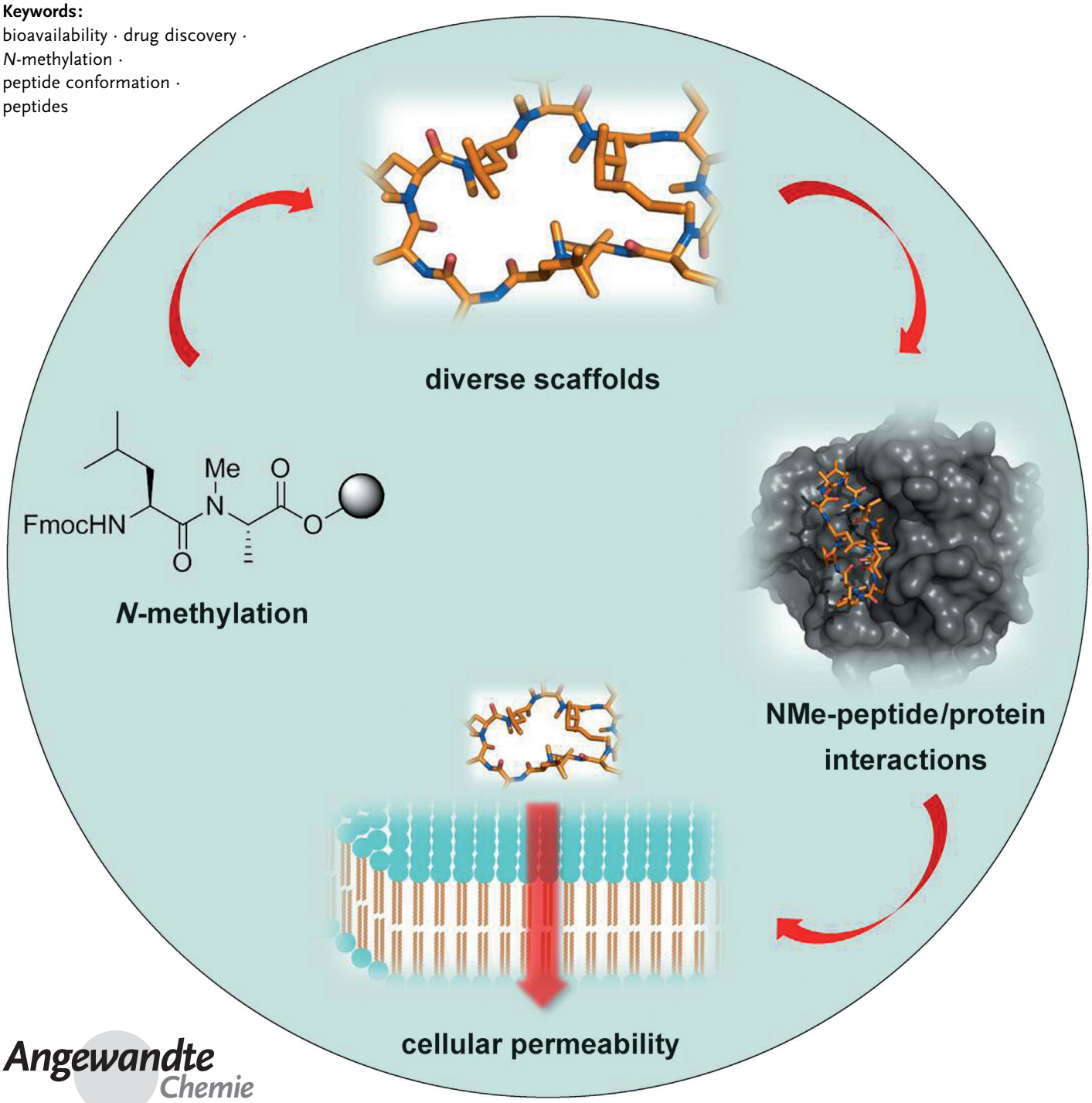

N-*Methylation is one of the simplest chemical modifications often occurring in peptides and proteins of prokaryotes and higher eukaryotes. Over years of evolution, nature has employed* N*-methylation of peptides as an ingenious technique to modulate biological function, often as a mode of survival through the production of antibiotics. This small structural change can not only mobilize large protein complexes (as in the histone methylation), but also inhibits the action of enzymes by selective recognition of protein–protein interaction surfaces. In recent years through the advancement in synthetic approaches, the potential of* N*-methylation has begun to be revealed, not only in modulating biological activity and selectivity as well as pharmacokinetic properties of peptides, but also in delivering novel drugs. Herein, we summarize the current knowledge of the versatility of* N*-methylation in modulating biological, structural, and pharmacokinetic properties of peptides.*

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1. Introduction

Methylation of nitrogen atoms is one of the most important chemical modifications to regulate biological functions. *N*-methylation especially plays a crucial role in epigenetics, which comprises all meiotically and mitotically heritable changes in gene expression for a cellular phenotype that are not coded in the Watson–Crick base pairing of DNA itself.[1,2] In this context, *N*-methylation of histones is deeply involved in the structural changes of chromatin and thus closely associated with its biological functions.[3] Among others, *N*-methylation is the reason why an identical genotype, that is, having the same DNA sequence, gives rise to many different phenotypes. Besides its central role in histone modification, DNA methylation is another well-established epigenetic marker which plays a critical role in the control of gene activity and the architecture of the cell nucleus.[4] The methylation of DNA occurs at the N^6 position of adenine and in the C^5 position of cytosines in CpG sites, and is brought about by various DNA methyltransferases.[2,5] The global and gene-specific hypomethylation and hypermethylation are the main types of DNA methylation.[6] Besides its important biological role, epigenetic methylation changes can cause several major human diseases including cancer, various syndromes, and mental retardation, which gave rise to the interest in investigating the exact mechanisms causing these phenomena.[2] In addition to its role in epigenetics, methylation is found in non-histone proteins in the nucleus, such as p53, Hsp90, or NF-κB.[7,8] However, in all these protein modifications the *N*-methylation occurs in side chains, whereas N^{α}-methylation at the N-terminus of proteins has been observed only in a few bacterial and eukaryotic proteins.[9] For this reason, very little is known about the biological function of the N^{α}-methylation; nevertheless it has been shown to be critically involved in the normal progression of the cell cycle playing a role in the accurate formation of the bipolar spindles and chromosome segregation.[10,11] Recently the first N^{α}-methyltransferase in yeast as well as in humans was discovered, which might be of importance for the further understanding of N^{α}-methylation.[9,12] To date, no example of *N*-methylation of a *peptide bond in mammalian proteins* has been described. It is surprising that nature has not used this modification for post-translational modification of proteins.

2. N*-Methylation of Histones*

The *N*-methylation of histones occurs in the lysine or arginine side chains and is crucially important for gene regulation.[13,14] Lysine *N*-methylations are carried out by a family of highly specific lysine methyltransferases and can induce activation as well as repression of transcription, depending on the methylation position.[15] Certain lysine residues can be methylated up to three times and, thus, lead to different methylated states for each lysine. The lysine methylation is a reversible process, in which demethylation is carried out by a family of lysine demethylases.[15] Like

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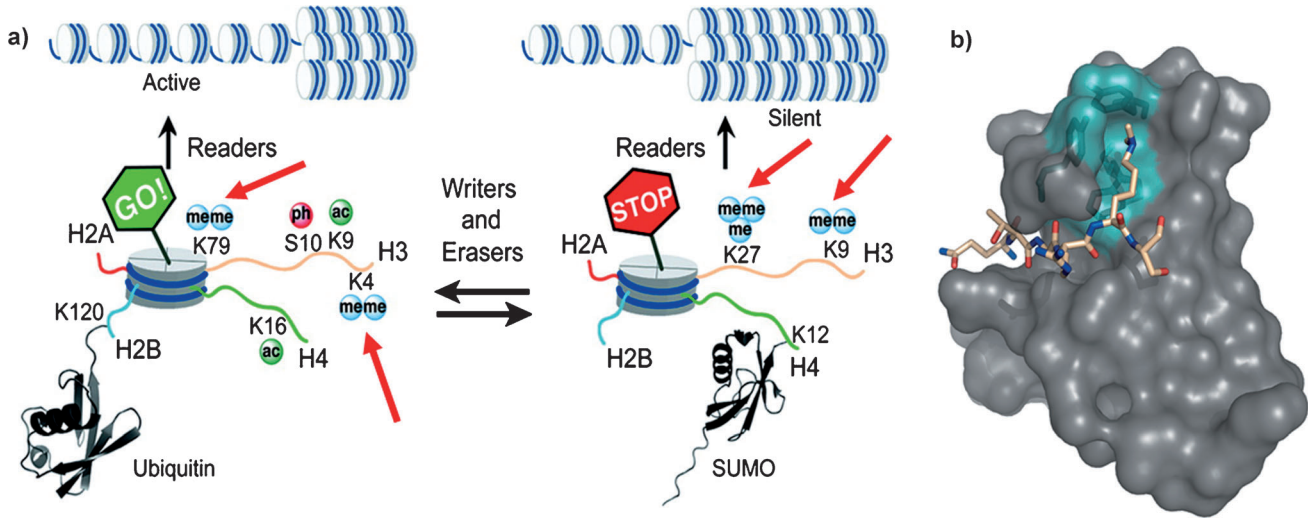

Figure 1. a) Histone modifications and their consequences. DNA is wrapped around an octameric complex consisting of two copies of the four core histones (H2A, H2B, H3, H4). Posttranslational modifications including single and multiple methylation (me, highlighted by red arrows), acetylation (ac), phosphorylation (ph), ubiquitinylation, and SUMOylation of specific positions in the histones can induce binding of non-histone proteins (Readers) and, thus, leading to either activation or suppression of gene transcription. Adapted with permission from Ref. [19], Copyright 2012 American Chemical Society. b) Crystal structure of Drosophila HP1 chromodomain (surface representation) and histone H3 tail peptide with methyllysine at residue 9 (sticks). The increased hydrophobicity of the lysine by *N*-methylation favors hydrophobic interactions with nonpolar groups and stabilizes the cation–π interaction of the methylammonium group with the aromatic cage formed by two tyrosines and a tryptophan (cyan) (protein data bank (PDB) code: 1KNA).[21]

lysines, the *N*-methylation of arginines can also lead to different *N*-methylation states, which can be either activating or repressive for transcription.[16] In 2007 the first arginine demethylase was described.[17] Since there are many different *N*-methylation patterns and histones may also be subject to other posttranslational modifications, this enormous variation yields a multiplicity of possible combinations of different modifications.[2] This situation lead to the hypothesis that there is as "histone code", which can be read by enzymes and proteins and thus translated into biological functions.[18] It has been reported that patterns and levels of histone methylation are closely associated with many biological processes, such as stem-cell maintenance and differentiation or DNA damage response, and are crucial for normal gene regulation.[13,15] Thus, *N*-methylation can dramatically alter the gene expression by turning the chromatin structure into an active form and as a consequence affecting the recruitment of non-histone proteins (Figure 1a).[14,19] It has been shown that errors in histone methylation can cause tumor initiation and are involved in the development of different human cancer types.[2,14] Recently it has been reported that changes in the *N*-methylation of histones can lead to drug resistance, driven by dynamic chromatin modifications, and can be reversed by chromatin-modifying drugs.[20] Thus it is interesting to note that such a small modification of only 14 Daltons by *N*-methylation remarkably alters the dynamic recognition pattern of large protein complexes (Figure 1b).[21]

3. N-*Methylated Peptides of Natural Origin*

As pointed out above, *N*-methylation of the amide bonds *in proteins* is not a posttranslational modification mark. However there are many *N*-methylated linear or cyclic peptides that have interesting biological functions. These peptides are synthesized enzymatically in nature to induce specific physical and biological properties. Several naturally occurring *N*-methylated cyclic and linear peptides, owing to their diverse origin, target affinity, and specificity, have demonstrated enormous potential as modulators of biomo-

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Florian Rechenmacher was born in Meran, Italy, and studied chemistry at the Technische Universität München. After completing his M.Sc. in 2009 in organic and biological chemistry, he joined the group of Prof. Kessler as a fellowship holder of the International Graduate School of Science and Engineering (IGSSE) at TUM. The main research interests are the functionalization of integrin subtype specific antagonists for various medicinal applications, and investigations of cyclic peptides for improvement of oral bioavailability.

lecular functions. Herein we summarize some of the *N*-methylated natural peptides targeted towards various subcellular components in mammalian cells that have either been studied extensively or show intriguing phenotypes when applied to cells. Nevertheless, it should be kept in mind that the few natural products listed below under no circumstances exemplify the diversity of the *N*-methylated peptidic natural products obtained from nature.

3.1. N-*Methylated Peptides Targeting the Cell Membrane*

Enniatins are a group of *N*-methylated cyclic depsipeptides[22] composed of trimeric esters of a dipeptidole monomer consisting of an *N*-methyl amino acid (Leu, Val, Ile) and a D-2-hydroxycarboxylic acid derived from these branched-chain amino acids (Figure 2).[23] Enniatins easily incorporate into

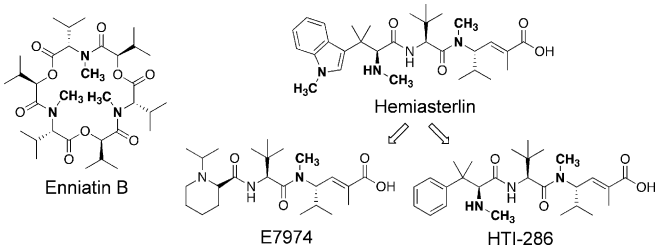

Figure 2. Some naturally occurring *N*-methylated peptides which target the cell membrane (Enniatin B) and microtubules (hemiasterlin and analogues).

the cell membrane where they form vertically stacked sandwich complexes with mono- and divalent cations. In this way they act as an ionophore and disrupt membrane potential.[24] It was demonstrated recently that enniatins exert profound p53-dependent cytostatic and p53-independent cytotoxic activities especially against human cancer cells, suggesting their potential as an anticancer drug.[25,26] Additionally it was also found that enniatin B strongly inhibits Pdr5p, which is one of the major multidrug efflux pumps whose overexpression confers multidrug resistance (MDR) in *Saccharomyces cerevisiae*. Enniatins specifically inhibit the function of Pdr5p in a manner similar to that of the common immunosuppressive drug FK506 (Tacrolimus).[27] Moreover, it was speculated that while FK506 acts as an inhibitor of P-glycoprotein and Pdr5p,[28,29] enniatins and their analogues could potentially bind to human multidrug resistance protein 1 (MDR 1) and inhibit the multidrug resistance in cancer cells.

3.2. N-*Methylated Peptides Targeting Microtubules*

Hemiasterlins comprises a small family of naturally occurring *N*-methylated tripeptides containing three highly modified amino acids, *tert*-leucine, 4-amino-2,5-dimethylhex-2-enoic acid, and *N*,*N*,β,β-tetramethyltryptophan (Figure 2).[30–32] Hemiasterlin is a potent cytotoxic peptide exerting its antiproliferative effects by binding to tubulin, preventing tubulin polymerization, and inducing mitotic arrest of cells in the G2-M phase of cell cycle, similar to vinblastine (an antitumor drug used to treat several kinds of cancer). As the anticancer therapeutic efficacy of hemiasterlin was associated with toxicity, this has fostered extensive research leading to the synthesis of several potent analogues of hemiasterlin.[33] One of the hemiasterlin analogues, HTI-286 (Figure 2) was a potent inhibitor of proliferation (mean IC_{50} of 1 nM in 18 human tumor cell lines) and had substantially less interaction with multidrug resistance protein 1 (MDR 1, "P-glycoprotein 1) than currently used antimicrotubule agents, including paclitaxel, docetaxel, vinorelbine, or vinblastine. Moreover, HTI-286 inhibited the growth of human tumor xenografts where paclitaxel and vincristine were ineffective because of inherent or acquired resistance associated with MDR 1.[34] E7974 (Figure 2) is yet another analogue of hemiasterlin that is currently being evaluated in clinical phase I against cancer.[35] E7974 inhibits polymerization of tubulin with an IC_{50} value similar to that of the tubulin polymerization inhibitor vinblastine. For the proper segregation of chromosomes during mitosis, attachment to microtubules is absolutely critical.[36] Thus, interference with tubulin polymerization by E7974 results in the inability of cells to complete mitosis. Immunofluorescence analyses of cells treated with E7974 show marked increases in the numbers of cells positive for phospho-histone H3, a marker for mitosis, as well as profoundly abnormal mitotic spindles (Figure 3) proving it to be an attractive candidate towards its development into a cancer chemotherapeutic.[33] At this point we would like to stress that the structures of E7974 and vinblastine, barely show any structural resemblance yet display remarkable similarity in bioactivity and potency to treat cancer.

Horst Kessler studied chemistry in Leipzig and Tübingen and was appointed as full professor in 1971 to J. W. Goethe University in Frankfurt. In 1989 he moved to the TU München. In 2008, he became the Carl von Linde Professor at the Institute for Advanced Study at the TUM. Since 2011 he is also Adjunct Professor at King Abdulaziz University in Jeddah, Saudi Arabia. His interests are drug development from peptides and peptidomimetics and NMR spectroscopy.

3.3. N-*Methylated Peptides Targeting DNA*

A handful of naturally occurring *N*-methylated peptides have been identified as potent DNA intercalators, their potential has been exploited in the study of important signaling pathways and to develop novel anticancer drugs. Echinomycin is a partially C_2 symmetric *N*-methylated cyclic depsipeptide (Figure 4) displaying an unusual disulfide bridge between cysteine residues. It consists of two quinoxaline-2-carboxylic acid moieties. Echinomycin was originally discovered as an antibiotic present in cultured filtrates of *Strepto-*

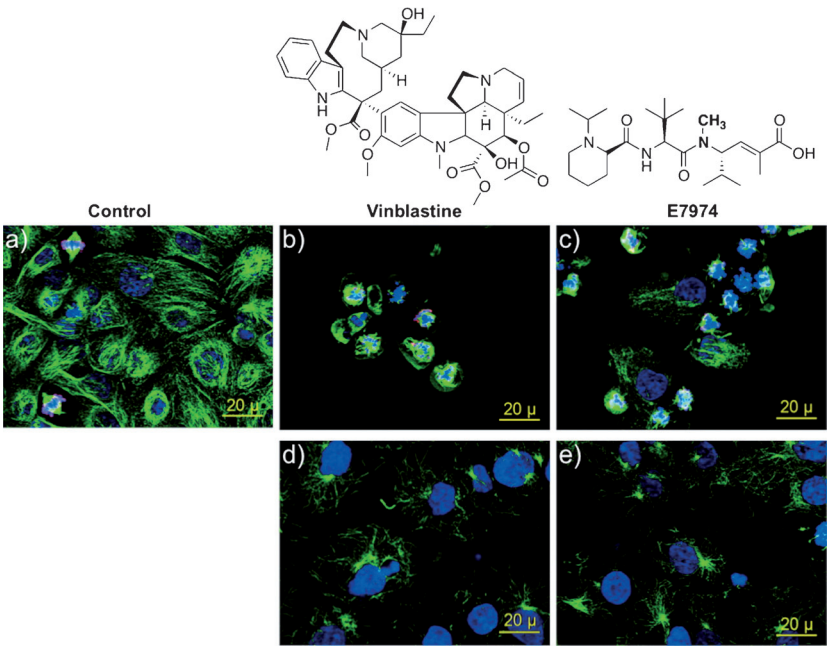

Figure 3. Vinblastine and E7974 block mitotic spindle formation and decrease microtubule density in DU 145 human prostate cancer cells. DU 145 human prostate cancer cells were treated for 18 h: a) control (DMSO); b) 8.4 nmol L^{-1} and d) 28 nmol L^{-1} of vinblastine; c) 19.5 nmol L^{-1} and e) 65 nmol L^{-1} of E7974. Cells were then fixed and stained with DAPI (blue), anti-β-tubulin (green), and anti-phospho-histone H3 (red; top row) or DAPI (blue) and anti-β-tubulin (green; bottom row). Adapted with permission from Ref. [33], Copyright: the American Association for Cancer Research.

myces echinatus.[37] It belongs to the class of compounds known as DNA bisintercalators,[38] where the compound binds to the minor groove of DNA in a sequence-specific fashion with a central two base-pair (bp) sequence 5′-CG-3′ and inserting its quinoxaline chromophores between the bases of the DNA by forming a two-base-pair sandwich (Figure 5). Echinomycin initiates apoptosis in cancer cells following the formation of the complex with DNA and subsequently inhibiting the transcription by blocking RNA polymerase.[39] Although echinomycin was brought to clinical trials as a cancer chemotherapeutic by the NCI several years ago, minimal or no antitumor activity was found in phase II clinical trials and it caused severe side effects, such as nausea, vomiting, reversible liver enzyme abnormalities.[40] However, rather recently in a screening of a 140 000 small-molecule library, echinomycin was found to be one of the most potent compounds in inhibiting the binding of hypoxia-inducible factor-1 (HIF-1) to DNA.[41] HIF-1 is a transcription factor that controls genes involved in glycolysis, angiogenesis, migration, and invasion, all of which are important for tumor progression and metastasis. Thus, a selective inhibitor of HIF-1 would now serve as ideal tool to probe the HIF-1 pathway and establish the therapeutic potential of HIF-1 inhibition, where several other tools, such as antisense or small interfering RNA strategies have failed.[42,43] It is interesting to note here that such medium-sized peptides like echinomycin are now also considered as "small-molecules" and are being employed in phenotype-based screens; although the molecular size is far beyond the 500 Da borderline of the Lipinski rules,[44] which is commonly used as selection criterion for drug development.

A structurally similar analogue of echinomycin is thiocoraline (Figure 4),[45] which contains thioester linkages instead of ester linkages in typical depsipeptide antibiotics. Thiocoraline shows a wide spectrum of anti-proliferative activity against various cancer cell lines in vitro[46] and in vivo.[47] Thiocoraline exerts its anti-proliferative activity by arresting cells in the G1 phase of the cell cycle and decreasing the rate of S phase progression towards G2/M phases by inhibiting DNA polymerase α activity by the formation of an enzyme–thiocoraline–DNA ternary complex.[46] It is worth noting that thiocoraline is highly unstable and requires the aid of delivery systems for its administration.[48] To avert this disadvantage, Albericio et al. envisioned the replacement of the thioester linkages with *N*-

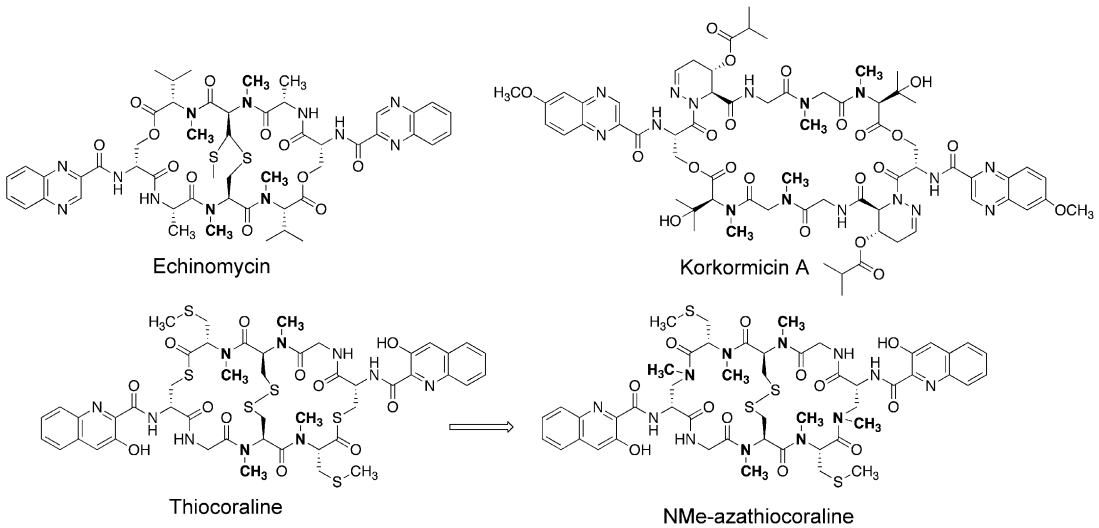

Figure 4. Naturally occurring *N*-methylated peptides targeting DNA.

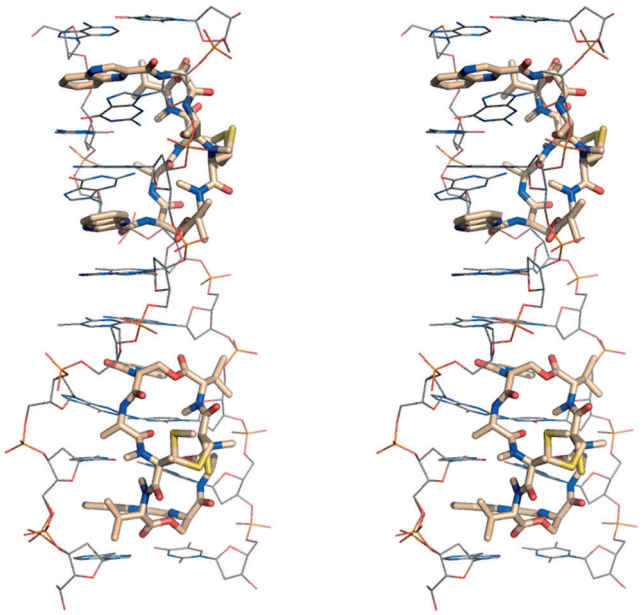

Figure 5. Stereoimage of the co-crystal structure of echinomycin (sticks) bound to a DNA d(ACGTACGT) duplex (lines), showing the stacking interactions (PDB code: 3GO3).[50]

methylated amide linkages in thioacoraline, which enhanced the half-life of *N*Me-azathiocoraline by a factor of two in human serum without affecting its bioactivity.[49]

Korkormicin A (Figure 4),[51] is yet another structurally similar DNA bisintercalator but with the absence of a disulfide bridge. It induces apoptosis in cancer cells by a novel mechanism, the induction of p53 phosphorylation, leading to inhibition of p53 degradation and activation of p53-dependent transcription, suggesting its promising potential as an antitumor drug.[52] Again, at this point we would like point out the chemical structures of these bisintercalators, which in spite of showing a comparable pattern of *N*-methylation, displays a subtle variation in the side-chain functionalities and the size of the macrocycle, dramatically altering their antitumor potencies. The structural diversity of these compounds points towards the possibility in modulating the bioactivity and pharmacology of peptide-based natural products by minor chemical changes. Additionally, it is surprising that although these compounds display a similar biological activity, there is a clear distinction between the biological pathways they affect, which can be harnessed towards the discovery of mechanism based therapeutics.

3.4. N-*Methylated Peptides Targeting Ribosomes*

Bouvardin is an *N*-methylated cyclic hexapeptide (Figure 6) isolated from the plant *Bouvardia ternifolia* (Rubiaceae), which was used by ancient Mexican Indians as a general medicine against dysentery and other diseases.[53] Bouvardin is structurally composed of two L-alanines, one D-alanine, and three modified *N*-methyl-L-tyrosines. The interesting feature is the constrained 14-membered ring structure formed by oxidative coupling of the phenolic hydroxy group

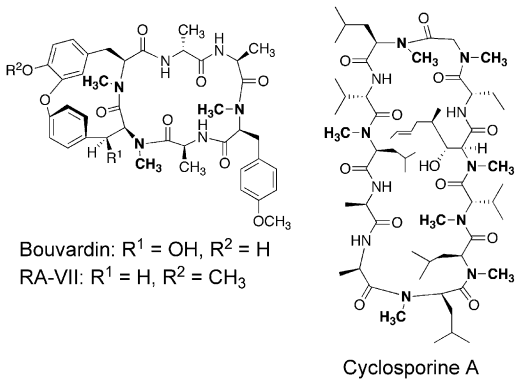

Figure 6. Naturally occurring *N*-methylated peptides targeting ribosomes (bouvardin and analogues) and protein–phosphatase (cyclosporine A).

of one tyrosine to the adjacent tyrosine. Bouvardin and its closely related analogue RA-VII, show potent antitumor activity by arresting cell progression uniformly throughout the cell cycle. They block protein synthesis[54] by interacting with eukaryotic 80S ribosome[55] and inhibit aminoacyl-*t*RNA binding and peptidyl-*t*RNA translocation. Studies of the properties of RA-VII have not only revealed efficacious antitumor activity but also demonstrated complete cures of solid tumor in colon adenocarcinoma.[56] In a recent conformation-activity study involving the *N*-methylation of D-Ala-1 and Ala-4 of RA-VII, the importance of the turn structures in mediating the cytotoxicity of RA-VII was demonstrated, pointing towards the importance and the requirement of conformational studies of peptides for a better understanding of their biological activity.[57]

3.5. N-*Methylated Peptides Targeting Protein Phosphatases*

The classic example of an *N*-methylated cyclic peptide drug with extensive use in medicine is cyclosporin A (CsA) (Figure 6). CsA is a cyclic undecapeptide with seven of its eleven amide bonds *N*-methylated, and was initially identified by screening of antibiotic materials produced by fungi.[58] It is used as an immunosuppressive drug to prevent graft rejection following organ transplantation. CsA exerts its therapeutic effect by inhibiting T-cell activation.[59] Inside cells, CsA forms a complex with the intracellular protein cyclophilin A (immunophilin), and this cyclophilin A-CsA complex eventually targets and inhibits the protein phosphatase calcineurin. The inhibition of calcineurin by the cyclophilin A-CsA complex fails to unmask the nuclear localization signal of the cytoplasmically located transcription factor NFAT, preventing its nuclear transport and subsequently blocking activation of IL-2 and related cytokines.[60] It is worth mentioning that NMR spectroscopy and X-ray crystallography played a crucial role in understanding not only the structure of CsA,[61–65] but also its mechanism of action through the formation of a transition-state mimic termed the "twisted amide surrogate" with cyclophilin, which is a *cis*–

trans peptidyl–prolyl isomerase.[66–72] The story of CsA is a striking evidence of the ability of *N*-methylated cyclic peptides to modulate protein–protein interactions.

3.6. N-*Methylated Peptides and Their Potential as Future Drugs*

To end this Section we mention a few *N*-methylated peptides that are currently being evaluated in clinical trials, displaying the promise of *N*-methylation in delivering next generation drugs (Figure 7).

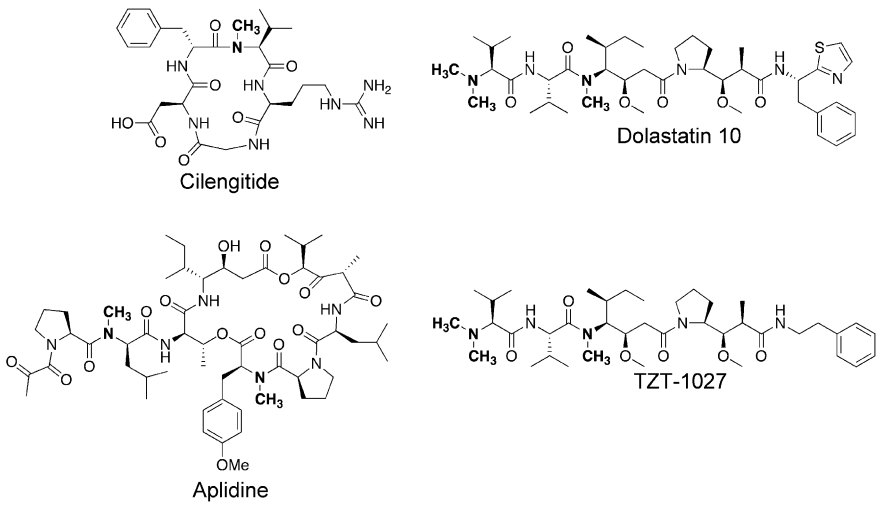

Figure 7. Structures of four *N*-methylated peptides that are currently undergoing clinical trials for treatment of various cancer types.

The integrin antagonist Cilengitide, a cyclic RGD pentapeptide [c(RGDf*N*MeV)] identified by spatial screening, is currently in clinical phase III trials for the treatment of glioblastoma and in phase II for several other tumors.[73] The closely related cyclic peptide c(RGDfV) was the first superactive αvβ3 integrin inhibitor with high selectivity against the platelet integrin αIIbβ3.[74] It is important to note that the incorporation of one D-amino acid next to the RGD sequence in the cycle is essential for the correct conformation. The phenyl ring of the Phe residue interacts with an aromatic residue in the β-subunit of the integrin receptor.[75,76] An *N*-methyl scan of this lead structure yielded Cilengitide,[73,77] which showed an even increased affinity for αvβ3, but also affinity in the low nanomolar range for the integrins αvβ5 and α5β1.[77] This highly active and selective cyclic pentapeptide was chosen by Merck KGaA for drug development and later named Cilengitide. It is important to mention that three parameters are responsible for its stability against enzymatic degradation: the cyclization, the D-amino acid in the ring, and the *N*-methylation.

Aplidine, a marine cyclodepsipetide isolated from *Aplidum albicans*, is another *N*-methylated peptide, that showed antitumor activity against a variety of human cancer cell lines such as breast, melanoma, and lung cancers.[78–80] It has been discovered that *Aplidine* induces apoptosis by inhibition of protein synthesis and might act as antiangiogenic drug through blocking VEGF secretion.[78,81] Aplidine showed antitumor activity in clinical phase I and has already been tested in clinical phase II for the treatment of non-small cell lung cancer, multiple myeloma, and several other cancer types.[82–87] Recently, it has entered clinical phase III trials for the treatment of relapsed/refractory multiple myeloma in combination with dexamethasone.[88]

Dolastatine 10 is a linear *N*-methylated pentapeptide isolated from the marine organism *Dolabella Auricularia*. The cytotoxic peptide showed antiproliferative activity against cancer cells and has been evaluated in clinical phase II trials for different cancer types.[89–94] Like Dolastatine, TZT-1027, a synthetic Dolastatine 10 derivative, acts as an inhibitor of microtubule assembly and tubulin polymerization. It was evaluated in clinical phase I trials and showed reduced cytotoxicity in patients with solid tumors compared to Dolastatine 10.[95] However, TZT-1027 showed no activity in treatment of patients with advanced or metastatic soft-tissue sarcomas as well as no response in treatment of patients with previously treated non-small cell lung cancer.[96,97]

N-methylation of the hexapeptidic sequence NFGAIL derived from the 37-residue islet amyloid polypeptide (IAPP) showed inhibition of amyloid formation and fibrillogenesis.[98,99] This is of interest since aggregation of IAPP leads to the cytotoxic pancreatic amyloid in type II diabetes patients.[98] Another derivative, the double *N*-methylated [*N*MeG^{24}, *N*MeI^{26}]-IAPP (IAPP-GI) full-length IAPP analogue, is highly soluble, non-amyloidogenic, non-cytotoxic, and blocks the IAPP self-assembly with activity in the low nanomolar range.[100] Furthermore it has been reported that IAPP-GI inhibits the cytotoxic oligomerization of the β-amyloid peptide, the major component of the Alzheimer's disease brain amyloid,[101] and can inhibit insulin aggregation with nanomolar affinity.[102] This result might suggest a molecular link between Alzheimer's disease and type II diabetes.[101] It is worth mentioning that the *N*-methylation was designed to successfully break the association of the β-sheet forming peptides by removing their ability to form hydrogen-bond bridged fibrillar structures. In other work, *N*-methylation of the β-sheet-breaking peptide Ac-LPFFD has been shown to increase its in vitro stability and in vivo half-life while maintaining its activity.[103]

4. *Biosynthesis of* N-*Methylated Peptides*

The naturally occurring *N*-methylated peptides are not released from the ribosome as proteins, but produced by large multifunctional enzymes in the so-called non-ribosomal peptide synthetases (NRPSs).[104,105] In contrast to the ribosomal machinery, NRPSs can insert hundreds of different building blocks into the peptidic chain leading to a huge variety of peptides or peptidomimetics with a broad spectrum of structural and biological activity.[105] The NRPS is a multi-

enzyme complex and simultaneously acts as template and biosynthetic machinery.[105,106] It consists of distinct sections defined as modules that are responsible for the insertion of amino acids and derivatives into the growing polypeptidic chain.[104] These modules are subdivided into different domains representing enzymatic units, which are responsible for substrate recognition, activation, binding, modification, elongation, and release (Figure 8).[105]

The adenylation domain (A domain) initiates the non-ribosomal peptide synthesis by activation of the substrate and controls its primary sequence through selection (Figure 8a). After building the aminoacyl adenylate, the intermediate is transferred onto a free thiol group of a cofactor (ppan), leading to an activated thioester (Figure 8b).[105] At exactly that stage, different modifications, such as *N*-methylation can occur. The *N*-methylation is carried out by an *N*-methylation domain, which is inserted into the A domain. The methyl group is transferred from *S*-adenosyl-L-methionine to the thioesterified amino acid, thereby releasing *S*-adenosyl-L-homocysteine.[105,107] After several peptide bond formations (Figure 8c) through interactions of two carrier domains with an elongation domain (elongation), the release at the final domain leads to linear or cyclic peptides (termination). Besides *N*-methylation, NRPSs are also able to introduce D-amino acids, heterocycles, and several other moieties that generate the gigantic pool of peptides produced by the non-ribosomal machinery.[107]

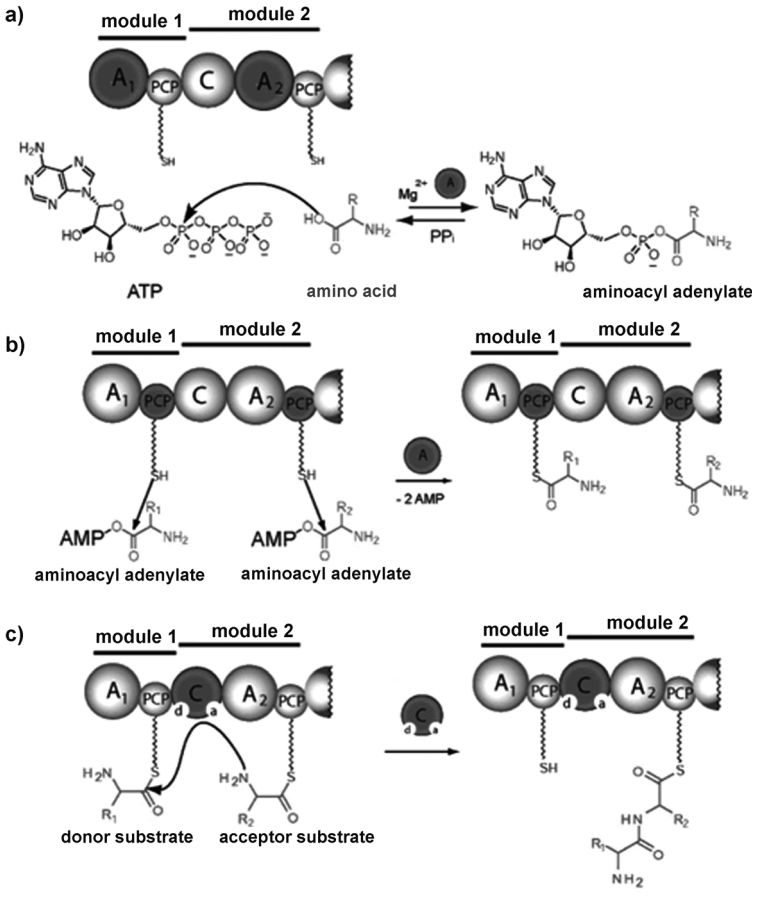

Figure 8. a) Activation of the amino acid through the A domain. b) Formation of thioester by transfer of activated amino acid to the cofactor ppan. At this stage *N*-methylation and other modifications can occur. c) Peptide bond formation and elongation. Reprinted with permission from Ref. [105]; Copyright 2005 American Chemical Society.

5. Chemical Synthesis of N-Methylated Peptides

Upon recognizing the potential of backbone *N*-methylation of peptides by nature in producing newer therapeutic agents with novel molecular architecture, there was an urgent need for robust and inexpensive synthetic strategies to efficiently synthesize *N*-methylated peptides.[108]

N-methyl amino acids were initially observed to undergo racemization under acidic and basic conditions,[109] which prompted the attention of organic chemists to develop racemization-free methods for their synthesis. Although several efficient methods were available for the synthesis of *N*-methylated amino acids,[110,111] the most commonly used conventional method was the one developed by Freidinger et al., involving the reductive cleavage of 5-oxazolidinones to obtain the enantiomerically pure *N*-methyl amino acids.[112] However, this method is not applicable for some side-chain functionalized amino acids, such as Trp, His, or Cys. It turned out that the base-catalyzed *N*-alkylation of *o*-nitrobenzenesulfonamide (*o*-NBS) protected α amino groups using dimethylsulfate as the methylating agent is a better alternative.[113] A further modification of this method employs a Mitsunobu reaction, which was optimized to obtain all possible *N*-methylated amino acids in solution[114] or on solid support.[115,116]

Furthermore, to obtain *N*-methylated analogues of a given lead peptide or bioactive sequence with the desired pharmacological properties, two approaches can be envisioned 1) a library approach: in which all the possible *N*-methylated congeners of the active sequence are synthesized, or 2) a structure-based design approach to *N*-methylate selected peptide bonds, in which the structural information regarding the bioactive conformation is available. Nevertheless, in either case to expedite the screening of the *N*-methylated analogues, a fast and efficient *N*-methylation procedure is desirable. Although, the above-mentioned strategies successfully yield the *N*-methylated amino acids in satisfactory amounts, the biggest challenge remains in their assembly into peptides. The subsequent coupling onto the sterically hindered *N*-methyl amino acids is cumbersome and often unsuccessful. The couplings mostly result in epimerization, formation of diketopiperazine during subsequent Fmoc deprotection,[117] and loss of peptide segments during final cleavage from the resin,[118] resulting in notoriously poor yields of the final peptide. Therefore, double or triple couplings are performed using either triphosgene,[119] 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo-[4,5-b]pyridinium hexafluoro-

phosphate 3-oxide (HATU)/1-hydroxy-7-azabenzotriazole (HOAt), (7-azabenzotriazol-1-yloxy) tris(pyrrolidino)phosphonium hexafluorophosphate (PyAOP), or PyBOP using a large excess of the amino acids on solid support.[117] Recently, Albericio et al. reported the utility of two additional comparatively inexpensive reagents (1-cyano-2-ethoxy-2-oxoethylidenaminooxy) dimethylamino morpholino carbenium hexafluorophosphate (COMU) and ethyl (hydroxyimino) cyanoacetate (oxyma) that showed a better efficiency of coupling *N*-methylated amino acids than HATU and HOAt. It should be noted here that COMU and oxyma have also been tested to be non-explosive in comparison to HOAt. The efficiency of these reagents is demonstrated by their ability to form a highly *N*-methylated cyclic peptide by the cyclization at sterically hindered *N*-methylated amino side chain (Figure 9a).[120] It is worth mentioning that the cyclization yields of peptides with *N*-methylated peptide bonds are often higher than for non-methylated peptides, as *N*-methylation induces a certain amount of *cis*-peptide bonds (turn-induction, see below) that brings the N- and C-termini close together facilitating the ring closure.

Hence, the utility of *N*-methyl peptides has recently spurred considerable interest[123] for the development of newer approaches towards the synthesis of *N*-methylated peptides. Towards this goal Danishefsky et al. recently reported an elegant epimerization-free isonitrile-mediated coupling (Figure 9b) to obtain *N*-methylated peptides. They synthesized cyclosporine A in moderate yield of 54% but with a clear demonstration of the robustness of their approach.[121]

6. Ribosomal Synthesis of N-methylated Peptides

As an alternative to the still troublesome synthesis of *N*-methylated peptides, Szostak et al. proposed an in vitro translation system consisting of purified recombinant factors derived from *Escherichia coli* with the aim of obtaining combinatorial libraries of *N*-methylated peptides. Using this simple and elegant method in which the *E. coli* translation system is supplemented with chemically synthesized *N*-methylated aminoacyl tRNA (Figure 9c and Figure 10), they could synthesize a triply *N*-methylated peptide.[124] However, one of the best examples is shown by Suga et al. who used a similar approach to Szostak et al. but employing in their system a ribozyme dubbed flexizyme,[125] to prepare the non-standard aminoacyl-tRNA. Recently they also reported the significant advantage of their in vitro translation machinery by coupling it to an in vitro display technique for selecting potent macrocyclic *N*-methylated peptides against ubiquitin ligase from a de novo library.[126] This example provided strong evidence for the possibility to generate macrocyclic *N*-methylated combinatorial libraries to analyze the potential of *N*-methylated peptides in interfering with novel protein–protein interactions. However, these ribosomal peptide syntheses are currently not applicable for all amino acids (e.g. with charged and β-branched side chains) and the yields are low compared to those obtained by chemically synthesis. Nonetheless, they allow for the exploration of structures that might then be synthesized in larger amounts by other methods if needed.

7. Proline as a "Mimic for N-Methylation" in Proteins

Proline is unique in its cyclic structure among the ribosomally encoded amino acids and is often found in turn structures of proteins.[127] The ability of tertiary amide bonds to adopt the *cis* conformer (see next Section) is exploited in proteins by the incorporation of a proline (not by *N*-methylated amino acids). Many peptide bonds on the N-

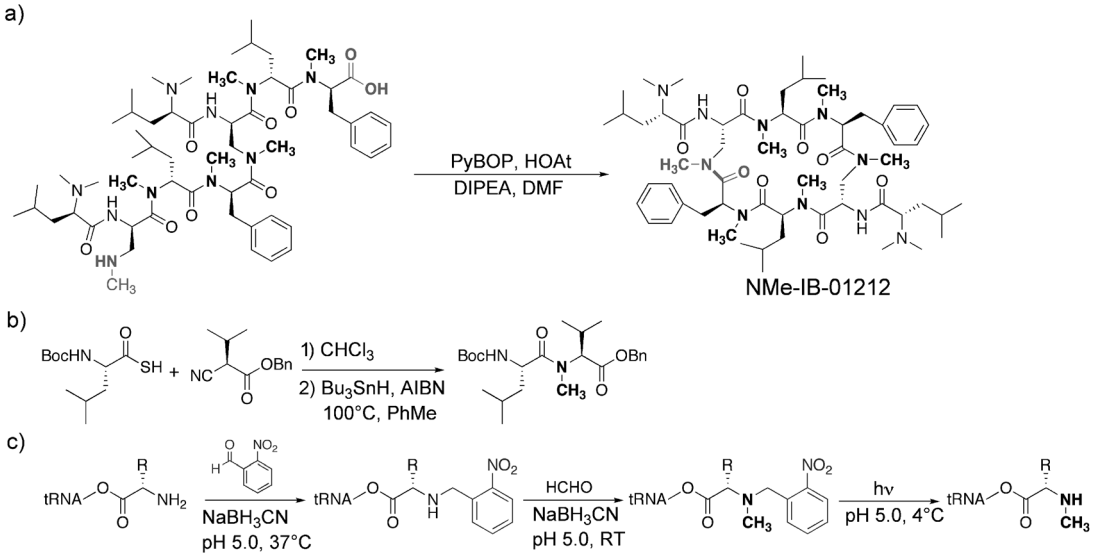

Figure 9. a) Cyclization of a highly *N*-methylated peptide at sterically hindered *N*-methyl side chain.[120] b) Isonitrile-mediated coupling reaction to obtain an *N*-methylated dipeptide by the formation of *N*-thioformyl amide.[121] c) Biocompatible *N*-methylation to obtain *N*-methyl aminoacyl tRNA.[122]

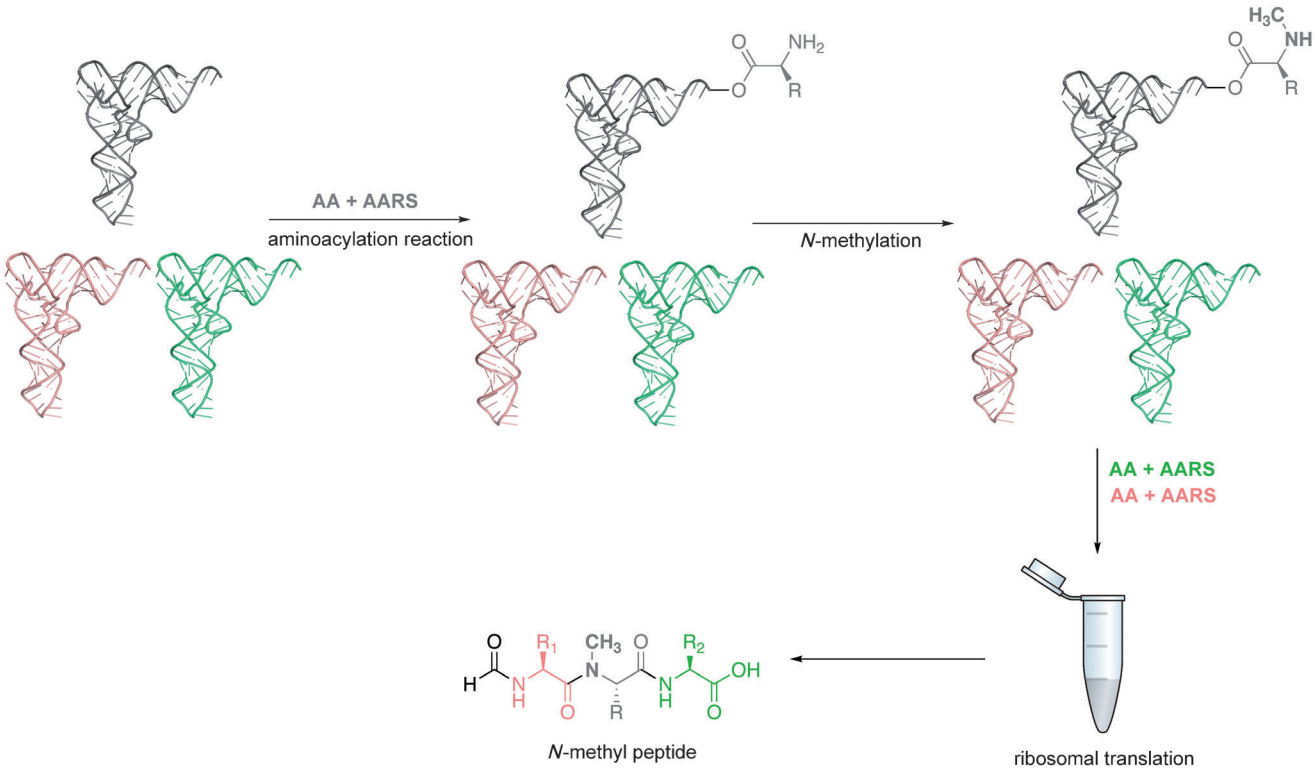

Figure 10. Schematic illustration of the ribosomal incorporation of *N*-methyl amino acids into peptides. Total tRNA is loaded with the desired amino acid, that has been chemically *N*-methylated, and then added to the in vitro translation system instead of the corresponding natural amino acid (AA) and aminoacyl–tRNA synthetase (AARS). The amino acids and AARS required for the other constituent amino acids of the desired *N*-methylated peptide are also present in the translation system. Reprinted with permission from Ref. [124]; Copyright 2008 American Chemical Society.

terminal site of proline in proteins are in the *cis* conformation and *cis–trans* isomerization may be used as a way to regulate functions.[128,129] Furthermore, the conformational influence exerted by proline in peptides can be very similar to that of *N*-methylated amino acids (see below).[130] Vice versa, proline-containing bioactive peptides may be modified by the synthetic incorporation of *N*-methylated amino acids.

8. Impact of N-methylation on Peptides

8.1. Conformational Modulation

Peptides in general are highly flexible because of the low rotational barriers about the two bonds adjacent to the amide bond (the bond between N and Cα, determined by the ϕ dihedral angle, and the bond between Cα and CO, given by the ψ dihedral angle). Steric effects and conformational restrictions due to secondary structure formation or cyclization restrict the conformational manifold, but small linear peptides usually do not prefer a single conformation. In that respect peptides differ from their "small molecule" drug-like counterparts which have less-rotatable bonds.[131] Conformational flexibility is usually an undesired property of a ligand, as ensembles of conformations due to entropic effects often result in lower affinity and selectivity towards receptor subtypes, such as the subtypes of G-protein coupled receptors (GPCRs). On the other hand, flexibility allows different shapes to be adopted, and there is thus a higher probability of detecting (low affinity) ligands, which can then be optimized to give rigid and selective ligands of higher binding affinities.

Despite the far-reaching consequences of peptide bond *N*-methylation in drug development, far-less attention has been paid towards the conformational investigation of *N*-methylated peptides. First of all, *N*-methylation increases the steric hindrance about the *N*-methylated peptide bond, which influences the *cis–trans* equilibrium of the *N*-methylated amide bond.[132] Additionally, the steric interactions between the *N*-methyl group and the amino acid side chains also strongly dictate the peptide conformation. Thus, *N*-methylation affects not only the conformation of the modified amino acid but also of the adjacent residues,[133] and thus has a long-range impact on the backbone of cyclic peptides. Secondly, the removal of the hydrogen-bonding capability by *N*-methylation disrupts the stabilizing secondary structural elements, that is, intermolecular hydrogen bonds. This effect is particularly pronounced in larger peptides; however, for smaller cyclic peptides, in which intramolecular hydrogen bonds do not play a major role in dictating the peptide conformation, *N*-methylation modulates their conformation mainly by steric effects. It is difficult to generalize the importance of these effects as they also depend on the specific sequence and chirality.

Besides steric hindrance, an additional important factor, which controls peptide conformations, is the preferred parallel orientation of the CO (i) and the CH ($i+1$) bond vectors.[134] These factors also eventually contribute to the deviation of the standard torsion angles of β-turns in *N*-methylated peptides. It should be kept in mind that in small *N*-methylated or non-*N*-methylated cyclic peptides the turn patterns are mainly governed by the chirality of the constituent amino acids and steric interactions (Figure 11) rather

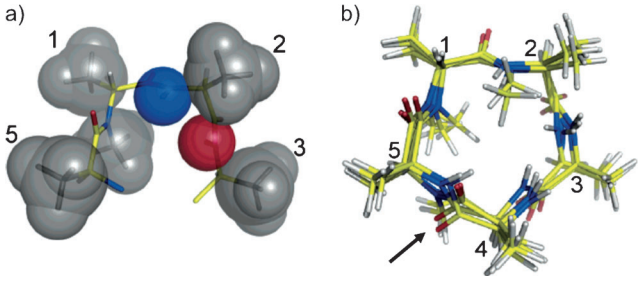

Figure 11. a) A cut-out of the conformation of cyclo(-*N*Me-D-Ala^1-Ala^2-Ala^3-Ala^4-Ala^5-) showing allowed (blue sphere) and disallowed (red sphere) sites where a *N*-methyl group can be incorporated without inducing a *cis*-peptide bond formation. b) Overlay of differentially *N*-methylated analogues of cyclo(-D-Ala-L-Ala$_4$-) showing the similar structural elements. Note the flip in the Ala^4–Ala^5 peptide bond (arrow), owing to *N*-methylation, to allow the *N*-methyl group to fit sterically.

than by the possibilities in the formation of intramolecular hydrogen bonds.[135]

Model studies on cyclic alanine penta- and hexapeptides carried out in our laboratory not only helped us in understanding the behavior of peptide backbone conformation on *N*-methylation, but also in the search for novel template structures,[136,137] which could be used for "spatial screening" of bioactive peptides.[138] In this approach the important functional groups of the amino acids (pharmacophores) are consecutively shifted in each position of a given conformational template and by subsequent assessment of the biological activity, the optimal conformation of the ligand can be identified. The major outcome of this study was the stabilization effect of the *N*-methylated D-residue that induces conformational homogenity in the N-methylated cyclic peptide. This was later confirmed by McAlpine et al.,[139] demonstrating its utility towards the rational design of *N*-methylated sansalvamide A analogues of higher potency than the currently marketed drugs used to treat drug resistant colon cancer.[140]

The importance of steric effects in determining the conformation and the *cis–trans* equilibrium is clearly exhibited by comparing the conformation of the three peptides c(-RGDfK-), c(-RGDf*N*MeK-) and c(-RGDf*N*MeV-) = *Cilengitide*. As expected, the non-*N*-methylated parent peptide exhibits only the all-*trans* conformation. The introduction of the *N*-methylation at the lysine residue introduces a 15% occurrence of the *N*-methylated *cis* peptide bond conformation, whereas in case of cilengitide with *N*MeVal, a sterically more demanding β-branched amino acid, no *cis* peptide bond was observed.[141] It was also shown that the conformational impact which *N*-methylated residues have on the cyclic peptide backbone is similar to that of proline when used at certain sites but also can differ in other positions.[130]

8.2. Prevention of Hydrogen Bonds

We have already pointed out above that *N*-methylation can be used to avoid aggregation of peptides into β-sheets (fibril formation) through the substitution of NH groups, which are involved in hydrogen bridges, by NCH_3 groups.[98–102] This effect was also exploited by Ghadiri et al. to inhibit the formation of nanotubes in cyclic D,L-peptides. The consecutive upward and downward orientations of the amide bonds induce stacking, which can be prevented by *N*-methylation of every second amide bond (Figure 12).[142–144]

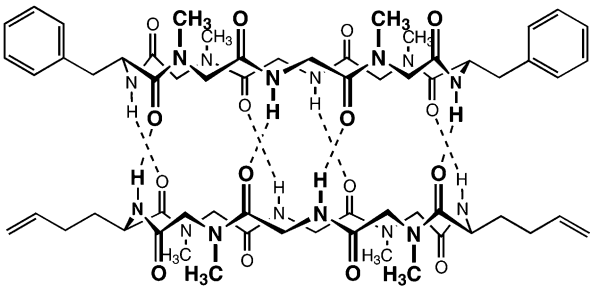

Figure 12. Schematic illustration of the selective assembly of dimeric *N*-methylated cyclic peptides. *N*-methylation prevents the association of further cyclic peptides on either face of the dimer by blocking the hydrogen-bond formation. The olefinic side chains were introduced to allow further chemical modification.[144]

8.3. Receptor Subtype Selectivity

Differentiation in receptors into subtypes by natural selection of mutants during evolution often results in the recognition of different ligand conformations. Flexible (linear) peptides can usually adapt to all these receptors, often resulting in only slightly different binding affinities. These differences in binding affinities to different receptor subtypes are usually explained by postulating additional binding sites (exosites). However, often these subtypes bind the ligands in different conformations. Accessibility of those conformations by different ligands may also determine subtype preferences. Suitable conformational restrictions by cyclizations, incorporation of rigid non-peptidic fragments (peptidomimetics),[145] or by *N*-methylation (Figure 13) can result in super-activity as well as selectivity for distinct receptor subtypes.[146–148] The cell adhesion sequence RGD[149] is a representative example: the glycine residue in the middle is a typical residue which can play a role as substitute for a D or an L amino acid and is used in nature as an element of flexibility. Restriction of the conformation of this peptide sequence in cyclic peptides resulted in high selectivity between integrin subtypes αvβ3, αvβ5, or α5β1 and the

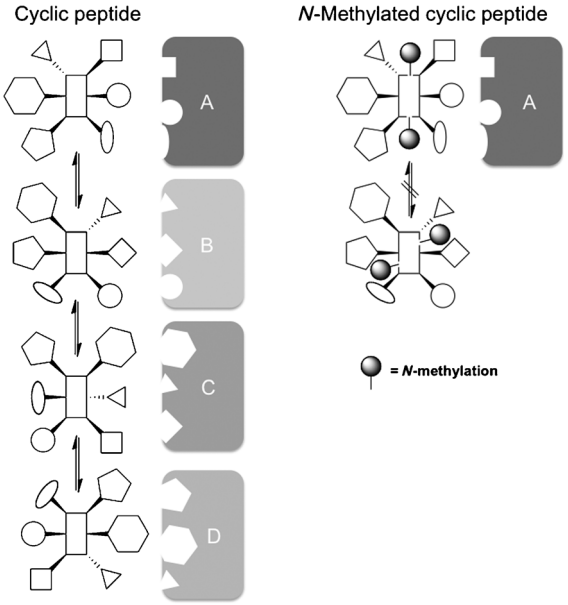

Figure 13. Schematic representation of the influence of *N*-methylation of cyclic peptides to achieve receptor subtype selectivity. A, B, C, and D represent receptor subtypes. Restricting the conformational freedom of cyclic peptides by sterically blocking their conformational interconversion allows then to fit only into receptor subtype A.

platelet integrin αIIbβ3.[74,150] In this case the incorporation of a D- amino acid was also used to induce a specific conformational preference of the backbone. Finally *N*-methylation resulted in the drug candidate Cilengitide.[73,77]

Thus, *N*-methylation has successfully resulted in receptor subtype selectivities being induced,[146–148] which is a critical factor in determining the efficacy of a drug and for precise study of signal-transduction networks.

8.4. Oral Bioavailability and Cell Permeability

The successful transfer of the biological response of an *N*-methylated peptide from in vitro studies to an in vivo or in living cell context requires the availability of the peptide at its target site in sufficient amounts. Therefore, the *N*-methylated peptides not only have to cross the biological membranes sequestering the protein/nucleic acid targets but also have to be sufficiently stable against the metabolic enzymes. Although *N*-methylation together with cyclization and/or incorporation of D-amino acids often conveys robust metabolic stability to peptides against enzymatic degradation, its influence on cellular and intestinal permeability of the peptides is not fully understood. Two different mechanisms, the paracellular[151] and transcellular[152] (passive, carrier-mediated, and vesicular), act synergistically in the transport of the peptides across the intestinal epithelium. On the other hand, the presence of efflux pumps,[153] for example, MDR 1 mediated efflux pumps, reduces the intracellular accumulation or the transcellular flux of a wide variety of drugs including peptides, making it difficult to accurately predict the modulation of the cellular permeability by *N*-methylation. Nevertheless, recent studies performed on *N*-methylated cyclic peptides, clearly suggest that *N*-methylation enhances the intestinal permeability of peptides,[154–156] thus resulting in better oral bioavailability. The increase in permeability of *N*-methylated peptides has shown to be affected by their overall hydrogen-bonding potential, conformation, charge, and size.[157–159] Lokey et al. identified orally bioavailable cyclic peptide templates, which contain almost exclusively very lipophilic amino acids, displaying, clearly defined turn structures with internal hydrogen bonds and externally oriented (solvent exposed) *N*-methylated peptide bonds. This observation further confirms that these factors are key determinants in enhancing bioavailability of peptides by rigidifying the cyclic peptide backbone conformation.[160] These results corroborated our earlier studies, in which we also observed similar template structures although with altered physicochemical properties, that displayed acceptable oral availability with enhanced intestinal permeability.[161] In that study we successfully converted a biologically active peptide into its tri-*N*-methylated variant that retained almost full activity towards two somatostatin receptor subtypes and was stable against proteolytic degradation in serum and gut. Additionally the *N*-methylated analogue showed an oral availability of 10% in rats, note that several marketed drugs display an oral availability in this range.

Recently in a model study involving a library of *N*-methylated cyclic alanine peptides, we elucidated the importance of preferred backbone conformations introduced by *N*-methylation in enhancing the intestinal permeability of the cyclic peptides.[156] Although the precise transport mode of the highly permeable *N*-methylated peptides could not be characterized, we could identify two crucial turn structures that were responsible for the rapid transport of the peptides across the Caco-2 membrane (Figure 14). Additionally, the effect of reducing the polarity of a peptide by *N*-methylation seems to be overestimated, as we failed to observe any correlation between decreasing polarity with the increasing

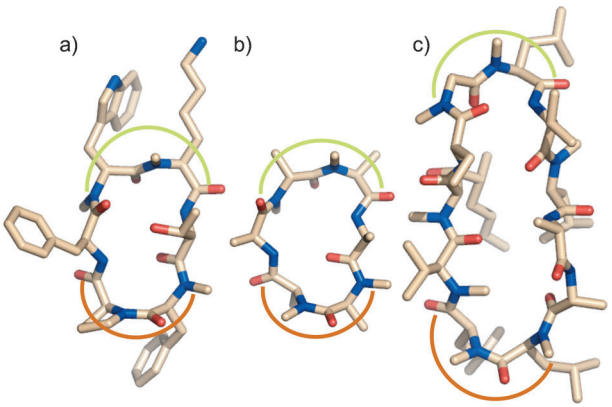

Figure 14. a) Conformation of the orally bioavailable somatostatin analogue cyclo(-*N*Me-D-Trp-NMeLys-Thr-*N*MePhe-Pro-Phe-). b) Conformation of a highly Caco-2 permeable *N*-methylated cyclic alanine hexapeptide cyclo(-*N*Me-D-Ala-*N*MeAla-Ala-*N*MeAla-*N*MeAla-Ala-). c) Conformation of cyclosporine A. Note the structural similarity in the turns at the terminals of all the three *N*-methylated cyclic peptides. The hydrogen atoms are omitted for clarity. The two crucial representative turn elements are highlighted.

number or position of *N*-methylations.[156] It is interesting to note that the cyclic peptide cyclosporine A described above which is about 19% orally available, displays a clearly defined turn structure with its externally oriented/solvent-exposed amide bonds *N*-methylated, a pattern observed in several of the alanine peptides that are Caco-2 membrane permeable.[156] These studies identified for the first time the importance of distinct backbone conformations for Caco-2 permeability. Nevertheless, to validate this hypothesis, extensive efforts should be devoted not only towards the synthesis of cyclic peptide libraries of diverse size, *N*-methylation, and side-chain functionalities but also to assess their oral availability in animal models.

9. Future perspectives

To date most peptidic drugs target receptors that are embedded in cellular membranes (e.g. GPCRs, integrins). In those cases "only" the intestinal barrier and the metabolic stability during the passage through the liver and in the blood serum need to be overcome by the peptide. But currently there is a significant interest in the development of small-molecules as tools to disrupt protein–protein interactions[162] or for the identification of novel interaction partners of proteins by phenotype-based cellular screens.[163] Although these molecules have proved to be great tools to perturb biological system and to study in depth the protein functions, most small molecules are not devoid of off-target bindings, they can interact with other biomolecules as a result of their lipophilicity.[164] Peptides, however, show remarkable target selectivity with their increased size. But, the utility of peptides in forward chemical genetic screens[165] has been limited because of their inherent lack of cell-permeability. Although, peptides are known to poorly permeate into cells, several *N*-methylated natural product peptides (as described above) elicit a phenotype only after penetrating the cell membrane and showing a cytoplasmic and/or nucleoplasmic distribution, which is yet another critical issue in tracking down intracellular targets with peptides.[166] However, much more data is required before it is possible to speculate about the mechanism of uptake and the influence of *N*-methylation on cellular permeability. It is an open question that needs to be addressed before the potential of *N*-methylated peptides can be fully exploited. Furthermore, peptides have long been recognized as attractive candidates for the design of ligands that bind to extracellular receptors, however, their utility as drugs have always been limited owing to their unfavorable pharmacokinetic properties. However, a simple modification of peptide backbones by *N*-methylation clearly conveyed favorable pharmacokinetic properties to natural peptides, holding great promise for the future drug-discovery programs. Although the advancement in the state of art technologies in "omics" science has afforded the study of a multitude of protein–protein, protein–DNA, and protein–RNA interactions, there has been a sharp decline in the development of new chemical entities as drugs, necessitating the development of novel molecules to expand the existing chemical space. Thus, peptides with their unique conformational features and functional diversity, which can even be enhanced by incorporating unnatural amino acids, *N*-methylation, and cyclization, offer the chance to expand the pool of bioactive and drug-like compounds.

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[1] A. D. Goldberg, C. D. Allis, E. Bernstein, *Cell* **2007**, *128*, 635–638.
[2] G. Egger, G. N. Liang, A. Aparicio, P. A. Jones, *Nature* **2004**, *429*, 457–463.
[3] T. Kouzarides, *Cell* **2007**, *128*, 693–705.
[4] M. Esteller, *N. Engl. J. Med.* **2008**, *358*, 1148–1159.
[5] M. G. Marinus, J. Casadesus, *FEMS Microbiol. Rev.* **2009**, *33*, 488–503.
[6] A. P. Feinberg, *Nature* **2007**, *447*, 433–440.
[7] S. Chuikov, J. K. Kurash, J. R. Wilson, B. Xiao, N. Justin, G. S. Ivanov, K. McKinney, P. Tempst, C. Prives, S. J. Gamblin, N. A. Barlev, D. Reinberg, *Nature* **2004**, *432*, 353–360.
[8] R. J. Sims III, D. Reinberg, *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 815–820.
[9] C. E. Schaner Tooley, J. J. Petkowski, T. L. Muratore-Schroeder, J. L. Balsbaugh, J. Shabanowitz, M. Sabat, W. Minor, D. F. Hunt, I. G. Maccara, *Nature* **2010**, *466*, 1125–1128.
[10] T. Chen, T. L. Muratore, C. E. Schaner-Tooley, J. Shabanowitz, D. F. Hunt, I. G. Macara, *Nat. Cell Biol.* **2007**, *9*, 596–603.
[11] Y. Hao, I. G. Macara, *J. Cell Biol.* **2008**, *182*, 827–836.
[12] K. J. Webb, R. S. Lipson, Q. Al-Hadid, J. P. Whitelegge, S. G. Clarke, *Biochemistry* **2010**, *49*, 5225–5235.
[13] A. P. Feinberg, B. Tycko, *Nat. Rev. Cancer* **2004**, *4*, 143–153.
[14] P. Chi, C. D. Allis, G. G. Wang, *Nat. Rev. Cancer* **2010**, *10*, 457–469.
[15] Y. Shi, J. R. Whetstine, *Mol. Cell* **2007**, *25*, 1–14.
[16] M. T. Bedford, S. G. Clarke, *Mol. Cell* **2009**, *33*, 1–13.
[17] B. Chang, Y. Chen, Y. Zhao, R. K. Bruick, *Science* **2007**, *318*, 444–447.
[18] T. Jenuwein, C. D. Allis, *Science* **2001**, *293*, 1074–1080.
[19] A. Dhall, C. Chatterjee, *ACS Chem. Biol.* **2011**, *6*, 987–999.
[20] S. V. Sharma, D. Y. Lee, B. H. Li, M. P. Quinlan, F. Takahashi, S. Maheswaran, U. McDermott, N. Azizian, L. Zou, M. A. Fischbach, K. K. Wong, K. Brandstetter, B. Wittner, S. Ramaswamy, M. Classon, J. Settleman, *Cell* **2010**, *141*, 69–80.
[21] S. A. Jacobs, S. Khorasanizadeh, *Science* **2002**, *295*, 2080–2083.
[22] A. E. Desjardins, R. H. Proctor, *Int. J. Food Microbiol.* **2007**, *119*, 47–50.
[23] P. A. Plattner, U. Nager, *Helv. Chim. Acta* **1948**, *31*, 665–671.
[24] M. Kamyar, P. Rawnduzi, C. R. Studenik, K. Kouri, R. Lemmens-Gruber, *Arch. Biochem. Biophys.* **2004**, *429*, 215–223.
[25] R. Dornetshuber, P. Heffeter, M. R. Kamyar, T. Peterbauer, W. Berger, R. Lemmens-Gruber, *Chem. Res. Toxicol.* **2007**, *20*, 465–473.
[26] U. Hyun, D. H. Lee, C. Lee, C. G. Shin, *Toxicon* **2009**, *53*, 723–728.
[27] K. Hiraga, S. Yamamoto, H. Fukuda, N. Hamanaka, K. Oda, *Biochem. Biophys. Res. Commun.* **2005**, *328*, 1119–1125.
[28] A. Kralli, K. R. Yamamoto, *J. Biol. Chem.* **1996**, *271*, 17152–17156.
[29] R. J. Arceci, K. Stieglitz, B. E. Bierer, *Blood* **1992**, *80*, 1528–1536.
[30] J. E. Coleman, E. D. de Silva, F. Kong, R. J. Andersen, T. M. Allen, *Tetrahedron* **1995**, *51*, 10653–10662.

[31] R. Talpir, Y. Benayahu, Y. Kashman, L. Pannell, M. Schleyer, *Tetrahedron Lett.* **1994**, *35*, 4453 – 4456.
[32] W. R. Gamble, N. A. Durso, R. W. Fuller, C. K. Westergaard, T. R. Johnson, D. L. Sackett, E. Hamel, J. H. Cardellina II, M. R. Boyd, *Bioorg. Med. Chem.* **1999**, *7*, 1611 – 1615.
[33] G. Kuznetsov, K. TenDyke, M. J. Towle, H. Cheng, J. Liu, J. P. Marsh, S. E. Schiller, M. R. Spyvee, H. Yang, B. M. Seletsky, C. J. Shaffer, V. Marceau, Y. Yao, E. M. Suh, S. Campagna, F. G. Fang, J. J. Kowalczyk, B. A. Littlefield, *Mol. Cancer Ther.* **2009**, *8*, 2852 – 2860.
[34] F. Loganzo, C. M. Discafani, T. Annable, C. Beyer, S. Musto, M. Hari, X. Tan, C. Hardy, R. Hernandez, M. Baxter, T. Singanallore, G. Khafizova, M. S. Poruchynsky, T. Fojo, J. A. Nieman, S. Ayral-Kaloustian, A. Zask, R. J. Andersen, L. M. Greenberger, *Cancer Res.* **2003**, *63*, 1838 – 1845.
[35] C. M. Rocha-Lima, S. Bayraktar, J. Macintyre, L. Raez, A. M. Flores, A. Ferrell, E. H. Rubin, E. A. Poplin, A. R. Tan, A. Lucarelli, N. Zojwalla, *Cancer* **2012**, DOI: 10.1002/cncr.27428.
[36] M. A. Jordan, L. Wilson, *Nat. Rev. Cancer* **2004**, *4*, 253 – 265.
[37] R. Corbaz, L. Ettlinger, E. Gäumann, W. Keller-Schierlein, F. Kradolfer, L. Neipp, V. Prelog, P. Reusser, H. Zähner, *Helv. Chim. Acta* **1957**, *40*, 199 – 204.
[38] S. Dawson, J. P. Malkinson, D. Paumier, M. Searcey, *Nat. Prod. Rep.* **2007**, *24*, 109 – 126.
[39] G. G. Gause, Jr., N. P. Loshkareva, I. B. Zbarsky, *Biochim. Biophys. Acta Nucleic Acids Protein Synth.* **1968**, *166*, 752 – 754.
[40] A. Y. Chang, K. Kim, H. Boucher, P. Bonomi, J. A. Stewart, D. D. Karp, R. H. Blum, *Cancer* **1998**, *82*, 292 – 300.
[41] D. Kong, E. J. Park, A. G. Stephen, M. Calvani, J. H. Cardellina, A. Monks, R. J. Fisher, R. H. Shoemaker, G. Melillo, *Cancer Res.* **2005**, *65*, 9047 – 9055.
[42] X. Sun, J. R. Kanwar, E. Leung, K. Lehnert, D. Wang, G. W. Krissansen, *Gene Ther.* **2001**, *8*, 638 – 645.
[43] Q. Zhang, Z. F. Zhang, J. Y. Rao, J. D. Sato, J. Brown, D. V. Messadi, A. D. Le, *Int. J. Cancer* **2004**, *111*, 849 – 857.
[44] C. A. Lipinski, F. Lombardo, B. W. Dominy, P. J. Feeney, *Adv. Drug Delivery Rev.* **2001**, *46*, 3 – 26.
[45] F. Romero, F. Espliego, J. Pérez Baz, T. García de Quesada, D. Grávalos, F. de La Calle, J. L. Fernández-Puentes, *J. Antibiot.* **1997**, *50*, 734 – 737.
[46] E. Erba, D. Bergamaschi, S. Ronzoni, M. Faretta, S. Taverna, M. Bonfanti, C. V. Catapano, G. Faircloth, J. Jimeno, M. D'Incalci, *Br. J. Cancer* **1999**, *80*, 971 – 980.
[47] D. Bergamaschi, M. Faretta, S. Ronzoni, S. Taverna, P. De Feudis, M. Bonfanti, G. Guidi, M. Faircloth, J. Jimeno, M. D'Incalci, E. Erba, *Eur. J. Histochem.* **1997**, *41*, 63 – 64.
[48] E. F. Brandon, R. W. Sparidans, I. Meijerman, I. Manzanares, J. H. Beijnen, J. H. Schellens, *Invest. New Drugs* **2004**, *22*, 241 – 251.
[49] J. Tulla-Puche, E. Marcucci, E. Prats-Alfonso, N. Bayó-Puxan, F. Albericio, *J. Med. Chem.* **2009**, *52*, 834 – 839.
[50] R. Pfoh, J. A. Cuesta-Seijo, G. M. Sheldrick, *Acta Crystallogr. Sect. F* **2009**, *65*, 660 – 664.
[51] K. S. Lam, D. R. Gustavson, G. A. Hesler, T. T. Dabrah, J. A. Matson, R. L. Berry, W. C. Rose, S. Forenza, *J. Ind. Microbiol.* **1995**, *15*, 60 – 65.
[52] J. Kitagaki, Y. Yang, *Biochem. Biophys. Res. Commun.* **2011**, *414*, 186 – 191.
[53] S. D. Jolad, J. J. Hoffmann, S. J. Torrance, R. M. Wiedhopf, J. R. Cole, S. K. Arora, R. B. Bates, R. L. Gargiulo, G. R. Kriek, *J. Am. Chem. Soc.* **1977**, *99*, 8040 – 8044.
[54] R. A. Tobey, D. J. Orlicky, L. L. Deaven, L. B. Rall, R. J. Kissane, *Cancer Res.* **1978**, *38*, 4415 – 4421.
[55] M. Zalacaín, E. Zaera, D. Vázquez, A. Jiménez, *FEBS Lett.* **1982**, *148*, 95 – 97.
[56] T. Kato, Y. Suzumura, S. Takamoto, K. Ota, *Anticancer Res.* **1987**, *7*, 329 – 334.
[57] Y. Hitotsuyanagi, J. E. Lee, S. Kato, I. H. Kim, H. Kohashi, H. Fukaya, K. Takeya, *Bioorg. Med. Chem.* **2011**, *19*, 2458 – 2463.
[58] M. Dreyfuss, E. Harri, H. Hoffmann, H. Kobel, W. Pache, H. Tscherter, *Eur. J. Appl. Microbiol.* **1976**, *3*, 125 – 133.
[59] J. F. Borel, C. Feurer, C. Magnée, H. Stähelin, *Immunology* **1977**, *32*, 1017 – 1025.
[60] J. Liu, J. D. Farmer, Jr., W. S. Lane, J. Friedman, I. Weissman, S. L. Schreiber, *Cell* **1991**, *66*, 807 – 815.
[61] H. Kessler, H. R. Loosli, H. Oschkinat, *Helv. Chim. Acta* **1985**, *68*, 661 – 681.
[62] H. R. Loosli, H. Kessler, H. Oschkinat, H. P. Weber, T. J. Petcher, A. Widmer, *Helv. Chim. Acta* **1985**, *68*, 682 – 704.
[63] H. Kessler, W. Bermel, C. Griesinger, *J. Am. Chem. Soc.* **1985**, *107*, 1083 – 1084.
[64] H. Kessler, M. Köck, T. Wein, M. Gehrke, *Helv. Chim. Acta* **1990**, *73*, 1818 – 1832.
[65] J. Klages, C. Neubauer, M. Coles, H. Kessler, B. Luy, *ChemBioChem* **2005**, *6*, 1672 – 1678.
[66] S. W. Fesik, R. T. Gampe, Jr., T. F. Holzman, D. A. Egan, R. Edalji, J. R. Luly, R. Simmer, R. Helfrich, V. Kishore, D. H. Rich, *Science* **1990**, *250*, 1406 – 1409.
[67] C. Weber, B. Wider, B. von Freyberg, R. Traber, W. Braun, H. Widmer, K. Wüthrich, *Biochemistry* **1991**, *30*, 6563 – 6574.
[68] S. W. Fesik, R. T. Gampe, Jr., H. L. Eaton, G. Gemmecker, E. T. Olejniczak, P. Neri, T. F. Holman, D. A. Egan, R. Edalji, R. Simmer, R. Helfrich, J. Hochlowski, M. Jackson, *Biochemistry* **1991**, *30*, 6574 – 6583.
[69] S. W. Fesik, P. Neri, R. Meadows, E. T. Olejniczak, G. Gemmecker, *J. Am. Chem. Soc.* **1992**, *114*, 3165 – 3166.
[70] C. Spitzfaden, H. P. Weber, W. Braun, J. Kallen, G. Wider, H. Widmer, M. D. Walkinshaw, K. Wüthrich, *FEBS Lett.* **1992**, *300*, 291 – 300.
[71] J. Kallen, C. Spitzfaden, M. G. M. Zurini, G. Wider, H. Widmer, K. Wüthrich, M. D. Walkinshaw, *Nature* **1991**, *353*, 276 – 279.
[72] G. Fischer, B. Wittmann-Liebold, K. Lang, T. Kiefhaber, F. X. Schmid, *Nature* **1989**, *337*, 476 – 478.
[73] C. Mas-Moruno, F. Rechenmacher, H. Kessler, *Anti-Cancer Agents Med. Chem.* **2010**, *10*, 753 – 768.
[74] M. Aumailley, M. Gurrath, G. Müller, J. Calvete, R. Timpl, H. Kessler, *FEBS Lett.* **1991**, *291*, 50 – 54.
[75] J. P. Xiong, T. Stehle, R. G. Zhang, A. Joachimiak, M. Frech, S. L. Goodman, M. A. Arnaout, *Science* **2002**, *296*, 151 – 155.
[76] L. Marinelli, A. Lavecchia, K.-E. Gottschalk, E. Novellino, H. Kessler, *J. Med. Chem.* **2003**, *46*, 4393 – 4404.
[77] M. A. Dechantsreiter, E. Planker, B. Mathä, E. Lohof, G. Hölzemann, A. Jonczyk, S. L. Goodman, H. Kessler, *J. Med. Chem.* **1999**, *42*, 3033 – 3040.
[78] M. Broggini, S. V. Marchini, E. Galliera, P. Borsotti, G. Taraboletti, E. Erba, M. Sironi, J. Jimeno, G. T. Faircloth, R. Giavazzi, M. D'Incalci, *Leukemia* **2003**, *17*, 52 – 59.
[79] G.-M. Suarez-Jimenez, A. Burgos-Hernandez, J.-M. Ezquerra-Brauer, *Mar. Drugs* **2012**, *10*, 963 – 986.
[80] G. Andavan, R. Lemmens-Gruber, *Mar. Drugs* **2010**, *8*, 810 – 834.
[81] L. F. García-Fernández, A. Losada, V. Alcaide, A. M. Álvarez, A. Cuadrado, L. González, K. Nakayama, K. I. Nakayama, J. M. Fernández-Sousa, A. Muñoz, J. M. Sánchez-Puelles, *Oncogene* **2002**, *21*, 7533 – 7544.
[82] C. Peschel, J. T. Hartmann, A. Schmittet, C. Bokemeyer, F. Schneller, U. Keilholz, D. Buchheidt, S. Millan, M. A. Izquierdo, R. D. Hofheinz, *Lung Cancer* **2008**, *60*, 374 – 380.
[83] M. V. Mateos, M. T. Cibeira, P. G. Richardson, F. Prosper, A. Oriol, J. de La Rubia, J. J. Lahuerta, R. Garcia-Sanz, S. Extremera, S. Szyldergemajn, C. Corrado, H. Singer, C. S. Mitsiades, K. C. Anderson, J. Blade, J. San Miguel, *Clin. Cancer Res.* **2010**, *16*, 3260 – 3269.

[84] E. Baudin, J. P. Droz, L. Paz-Ares, A. T. van Oosterom, M. Cullell-Young, M. Schlumberger, *Am. J. Clin. Oncol.* **2010**, *33*, 83 – 88.
[85] H. Dumez, E. Gallardo, S. Culine, J. C. Galceran, P. Schoffski, J. P. Droz, S. Extremera, S. Szyldergemajn, A. Flechon, *Mar. Drugs* **2009**, *7*, 451 – 463.
[86] T. Eisen, J. Thomas, W. H. Miller, M. Gore, P. Wolter, P. Kavan, J. A. L. Martin, P. Lardelli, *Melanoma Res.* **2009**, *19*, 185 – 192.
[87] P. Schöffski, V. Guillem, M. Garcia, F. Rivera, J. Tabernero, M. Cullell, J. A. Lopez-Martin, P. Pollard, H. Dumez, X. G. del Muro, L. Paz-Ares, *Mar. Drugs* **2009**, *7*, 57 – 70.
[88] http://clinicaltrials.gov/ct2/show/NCT01102426?term = aplidine&rank = 3.
[89] G. R. Pettit, E. J. Flahive, M. R. Boyd, R. Bai, E. Hamel, R. K. Pettit, J. M. Schmidt, *Anti-Cancer Drug Des.* **1998**, *13*, 47 – 66.
[90] L. M. Krug, V. A. Miller, G. P. Kalemkerian, M. J. Kraut, K. K. Ng, R. T. Heelan, B. A. Pizzo, W. Perez, N. McClean, M. G. Kris, *Ann. Oncol.* **2000**, *11*, 227 – 228.
[91] U. Vaishampayan, M. Glode, W. Du, A. Kraft, G. Hudes, J. Wright, M. Hussain, *Clin. Cancer Res.* **2000**, *11*, 4205 – 4208.
[92] E. D. Saad, E. H. Kraut, P. M. Hoff, D. F. Moore, D. Jones, R. Pazdur, J. L. Abbruzzese, *Am. J. Clin. Oncol.* **2002**, *25*, 451 – 453.
[93] E. A. Perez, D. W. Hillman, P. A. Fishkin, J. E. Krook, W. W. Tan, P. A. Kuriakose, S. R. Alberts, S. R. Dakhil, *Invest. New Drugs* **2005**, *23*, 257 – 261.
[94] H. L. Kindler, P. K. Tothy, R. Wolff, R. A. McCormack, J. L. Abbruzzese, S. Mani, K. T. Wade-Oliver, E. E. Vokes, *Invest. New Drugs* **2005**, *23*, 489 – 493.
[95] K. Tamura, K. Nakagawa, T. Kurata, T. Satoh, T. Nogami, K. Takeda, S. Mitsuoka, N. Yoshimura, S. Kudoh, S. Negoro, M. Fukuoka, *Cancer Chemother. Pharmacol.* **2007**, *60*, 285 – 293.
[96] S. Patel, M. L. Keohan, M. W. Saif, D. Rushing, L. Baez, K. Feit, R. DeJager, S. Anderson, *Cancer* **2006**, *107*, 2881 – 2887.
[97] G. J. Riely, S. Gadgeel, I. Rothman, B. Saidman, K. Sabbath, K. Feit, M. G. Kris, N. A. Rizvi, *Lung Cancer* **2007**, *55*, 181 – 185.
[98] A. Kapurniotu, A. Schmauder, K. Tenidis, *J. Mol. Biol.* **2002**, *315*, 339 – 350.
[99] M. Tatarek-Nossol, L. M. Yan, A. Schmauder, K. Tenidis, G. Westermark, A. Kapurniotu, *Chem. Biol.* **2005**, *12*, 797 – 809.
[100] L. M. Yan, M. Tatarek-Nossol, A. Velkova, A. Kazantzis, A. Kapurniotu, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 2046 – 2051.
[101] L. M. Yan, A. Velkova, M. Tatarek-Nossol, E. Andreetto, A. Kapurniotu, *Angew. Chem.* **2007**, *119*, 1268 – 1274; *Angew. Chem. Int. Ed.* **2007**, *46*, 1246 – 1252.
[102] A. Velkova, M. Tatarek-Nossol, E. Andreetto, A. Kapurniotu, *Angew. Chem.* **2008**, *120*, 7222 – 7227; *Angew. Chem. Int. Ed.* **2008**, *47*, 7114 – 7118.
[103] C. Adessi, M. J. Frossard, C. Boissard, S. Fraga, S. Bieler, T. Ruckle, F. Vilbois, S. M. Robinson, M. Mutter, W. A. Banks, C. Soto, *J. Biol. Chem.* **2003**, *278*, 13905 – 13911.
[104] R. Finking, M. A. Marahiel, *Annu. Rev. Microbiol.* **2004**, *58*, 453 – 488.
[105] S. A. Sieber, M. A. Marahiel, *Chem. Rev.* **2005**, *105*, 715 – 738.
[106] D. Schwarzer, R. Finking, M. A. Marahiel, *Nat. Prod. Rep.* **2003**, *20*, 275 – 287.
[107] H. von Döhren, U. Keller, J. Vater, R. Zocher, *Chem. Rev.* **1997**, *97*, 2675 – 2706.
[108] "Synthesis of *N*-Alkylated Peptides": C. Gilon, M. A. Dechantsreiter, F. Burkhart, A. Friedler, H. Kessler in *Houben-Weyl: Methods of Organic Chemistry. Vol. E22c* (Eds.: M. Goodman, A. Felix, L. Moroder, C. Toniolo), Thieme, Stuttgart, **2003**, pp. 215 – 271.
[109] J. R. McDermott, N. L. Benoiton, *Can. J. Chem.* **1973**, *51*, 2562 – 2570.
[110] L. Aurelio, R. T. Brownlee, A. B. Hughes, *Chem. Rev.* **2004**, *104*, 5823 – 5846.
[111] E. Marcucci, N. Bayó-Puxan, J. Tulla-Puche, J. Spengler, F. Albericio, *J. Comb. Chem.* **2008**, *10*, 69 – 78.
[112] R. M. Freidinger, J. S. Hinkle, D. S. Perlow, *J. Org. Chem.* **1983**, *48*, 77 – 81.
[113] S. C. Miller, T. S. Scanlan, *J. Am. Chem. Soc.* **1997**, *119*, 2301 – 2302.
[114] E. Biron, H. Kessler, *J. Org. Chem.* **2005**, *70*, 5183 – 5189.
[115] E. Biron, J. Chatterjee, H. Kessler, *J. Pept. Sci.* **2006**, *12*, 213 – 219.
[116] J. Chatterjee, B. Laufer, H. Kessler, *Nat. Protoc.* **2012**, *7*, 432 – 444.
[117] M. Teixidó, F. Albericio, E. Giralt, *J. Pept. Res.* **2005**, *65*, 153 – 166.
[118] W. J. Fang, M. A. Bennett, J. V. Aldrich, *Biopolymers* **2011**, *96*, 97 – 102.
[119] E. Falb, T. Yechezkel, Y. Salitra, C. Gilon, *J. Pept. Res.* **1999**, *53*, 507 – 517.
[120] E. Marcucci, J. Tulla-Puche, F. Albericio, *Org. Lett.* **2012**, *14*, 612 – 615.
[121] X. Wu, J. L. Stockdill, P. Wang, S. J. Danishefsky, *J. Am. Chem. Soc.* **2010**, *132*, 4098 – 4100.
[122] C. Merryman, R. Green, *Chem. Biol.* **2004**, *11*, 575 – 582.
[123] V. R. Pattabiraman, J. W. Bode, *Nature* **2011**, *480*, 471 – 479.
[124] A. O. Subtelny, M. C. Hartman, J. W. Szostak, *J. Am. Chem. Soc.* **2008**, *130*, 6131 – 6136.
[125] H. Murakami, A. Ohta, H. Ashigai, H. Suga, *Nat. Methods* **2006**, *3*, 357 – 359.
[126] Y. Yamagishi, I. Shoji, S. Miyagawa, T. Kawakami, T. Katoh, Y. Goto, H. Suga, *Chem. Biol.* **2011**, *18*, 1562 – 1570.
[127] G. Müller, M. Gurrath, M. Kurz, H. Kessler, *Proteins Struct. Funct. Genet.* **1993**, *15*, 235 – 251.
[128] M. J. Feige, S. Groscurth, M. Marcinowski, Z. T. Yew, V. Truffault, E. Paci, H. Kessler, J. Buchner, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 13373 – 13378.
[129] M. J. Feige, S. Groscurth, M. Marcinowski, Y. Shimizu, H. Kessler, L. M. Hendershot, J. Buchner, *Mol. Cell* **2009**, *34*, 569 – 579.
[130] B. Laufer, J. Chatterjee, A. O. Frank, H. Kessler, *J. Pept. Sci.* **2009**, *15*, 141 – 146.
[131] D. F. Veber, S. R. Johnson, H.-Y. Cheng, B. R. Smith, K. W. Ward, K. D. Kopple, *J. Med. Chem.* **2002**, *45*, 2615 – 2623.
[132] H. Kessler, *Angew. Chem.* **1970**, *82*, 237 – 253; *Angew. Chem. Int. Ed. Engl.* **1970**, *9*, 219 – 235.
[133] F. Piriou, K. Lintner, S. Fermandjian, P. Fromageot, M. C. Khosla, R. R. Smeby, F. M. Bumpus, *Proc. Natl. Acad. Sci. USA* **1980**, *77*, 82 – 86.
[134] M. Heller, M. Sukopp, N. Tsomaia, M. John, D. F. Mierke, B. Reif, H. Kessler, *J. Am. Chem. Soc.* **2006**, *128*, 13806 – 13814.
[135] J. P. Snyder, *J. Am. Chem. Soc.* **1984**, *106*, 2393 – 2400.
[136] J. Chatterjee, D. Mierke, H. Kessler, *J. Am. Chem. Soc.* **2006**, *128*, 15164 – 15172.
[137] J. Chatterjee, D. F. Mierke, H. Kessler, *Chem. Eur. J.* **2008**, *14*, 1508 – 1517.
[138] H. Kessler, R. Gratias, G. Hessler, M. Gurrath, G. Müller, *Pure Appl. Chem.* **1996**, *68*, 1201 – 1205.
[139] P. S. Pan, K. L. McGuire, S. R. McAlpine, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5072 – 5077.
[140] K. Otrubova, G. Lushington, D. Vander Velde, K. L. McGuire, S. R. McAlpine, *J. Med. Chem.* **2008**, *51*, 530 – 544.
[141] B. Laufer, A. O. Frank, J. Chatterjee, T. Neubauer, C. Mas-Moruno, G. Kummerlöwe, H. Kessler, *Chem. Eur. J.* **2010**, *16*, 5385 – 5390.
[142] K. Kobayashi, J. R. Granja, M. R. Ghadiri, *Angew. Chem.* **1995**, *107*, 79 – 81; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 95 – 98.
[143] T. D. Clark, M. R. Ghadiri, *J. Am. Chem. Soc.* **1995**, *117*, 12364 – 12365.

[144] D. T. Bong, T. D. Clark, J. R. Granja, M. R. Ghadiri, *Angew. Chem.* **2001**, *113*, 1016 – 1041; *Angew. Chem. Int. Ed.* **2001**, *40*, 988 – 1011.
[145] A. Giannis, *Angew. Chem.* **1993**, *105*, 1303 – 1326; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1244 – 1267.
[146] J. Chatterjee, O. Ovadia, G. Zahn, L. Marinelli, A. Hoffman, C. Gilon, H. Kessler, *J. Med. Chem.* **2007**, *50*, 5878 – 5881.
[147] L. Doedens, F. Opperer, M. Cai, J. G. Beck, M. Dedek, E. Palmer, V. J. Hruby, H. Kessler, *J. Am. Chem. Soc.* **2010**, *132*, 8115 – 8128.
[148] C. Mas-Moruno, J. G. Beck, L. Doedens, A. O. Frank, L. Marinelli, S. Cosconati, E. Novellino, H. Kessler, *Angew. Chem.* **2011**, *123*, 9668 – 9672; *Angew. Chem. Int. Ed.* **2011**, *50*, 9496 – 9500.
[149] M. D. Pierschbacher, E. Ruoslahti, *Nature* **1984**, *309*, 30 – 33.
[150] T. Weide, A. Modlinger, H. Kessler, *Top. Curr. Chem. Bioact. Conform.* **2007**, *272*, 1 – 50.
[151] M. S. Balda, L. Gonzalez-Mariscal, K. Matter, M. Cereijido, J. M. Anderson, *J. Cell Biol.* **1993**, *123*, 293 – 302.
[152] M. Fujikawa, R. Ano, K. Nakao, R. Shimizu, M. Akamatsu, *Bioorg. Med. Chem.* **2005**, *13*, 4721 – 4732.
[153] W. H. Peters, C. E. Boon, H. M. Roelofs, T. Wobbes, F. M. Nagengast, P. G. Kremers, *Gastroenterology* **1992**, *103*, 448 – 455.
[154] O. Ovadia, S. Greenberg, J. Chatterjee, B. Laufer, F. Opperer, H. Kessler, C. Gilon, A. Hoffman, *Mol. Pharm.* **2011**, *8*, 479 – 487.
[155] Q. G. Dong, Y. Zhang, M. S. Wang, J. Feng, H. H. Zhang, Y. G. Wu, T. J. Gu, X. H. Yu, C. L. Jiang, Y. Chen, W. Li, W. Kong, *Amino Acids* **2012**, DOI: 10.1007/s00726-012-1322-y.
[156] J. G. Beck, J. Chatterjee, B. Laufer, U. K. Marelli, A. O. Frank, S. Neubauer, O. Ovadia, S. Greenberg, C. Gilon, A. Hoffman, H. Kessler, *J. Am. Chem. Soc.* **2012**, *134*, 12125 – 12133.
[157] D. C. Kim, P. S. Burton, R. T. Borchardt, *Pharm. Res.* **1993**, *10*, 1710 – 1714.
[158] G. M. Pauletti, F. W. Okumu, R. T. Borchardt, *Pharm. Res.* **1997**, *14*, 164 – 168.
[159] G. T. Knipp, D. G. V. Velde, T. J. Siahaan, R. T. Borchardt, *Pharm. Res.* **1997**, *14*, 1332 – 1340.
[160] T. R. White, C. M. Renzelman, A. C. Rand, T. Rezai, C. M. McEwen, V. M. Gelev, R. A. Turner, R. G. Linington, S. S. Leung, A. S. Kalgutkar, J. N. Bauman, Y. Zhang, S. Liras, D. A. Price, A. M. Mathiowetz, M. P. Jacobson, R. S. Lokey, *Nat. Chem. Biol.* **2011**, *7*, 810 – 817.
[161] E. Biron, J. Chatterjee, O. Ovadia, D. Langenegger, J. Brueggen, D. Hoyer, H. A. Schmid, R. Jelinek, C. Gilon, A. Hoffman, H. Kessler, *Angew. Chem.* **2008**, *120*, 2633 – 2637; *Angew. Chem. Int. Ed.* **2008**, *47*, 2595 – 2599.
[162] J. A. Wells, C. L. McClendon, *Nature* **2007**, *450*, 1001 – 1009.
[163] B. R. Stockwell, *Nature* **2004**, *432*, 846 – 854.
[164] P. D. Leeson, B. Springthorpe, *Nat. Rev. Drug Discovery* **2007**, *6*, 881 – 890.
[165] B. R. Stockwell, *Nat. Rev. Genet.* **2000**, *1*, 116 – 125.
[166] M. Lindgren, M. Hällbrink, A. Prochiantz, U. Langel, *Trends Pharmacol. Sci.* **2000**, *21*, 99 – 103.