Molecular Diversity

DOI: 10.1002/ange.201405747

Synthesis of a Natural Product-Like Compound Collection through Oxidative Cleavage and Cyclization of Linear Peptides**

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Dedicated to Professor Klaus Bock on the occasion of his 70th birthday

Abstract: *Massive efforts in molecular library synthesis have strived for the development of synthesis methodology which systematically delivers natural product-like compounds of high spatial complexity. Herein, we present a conceptually simple approach that builds on the power of solid-phase peptide synthesis to assemble precursor peptides (oligomers) designed to undergo oxidative cascade reactions. By harnessing the structural side-chain diversity and inherent stereochemical features offered by readily available amino acids (monomers), a proof-of-concept collection of 54 skeletally and stereochemically diverse compounds was generated, and selected compounds were elaborated into isoform-selective metalloprotease inhibitors.*

High-throughput screening (HTS) of large compound collections remains a major avenue towards the identification of new molecular starting points in early drug and probe discovery.[1] It remains unquestionable that the size, design, and quality of compound libraries are key determinants for the outcome of HTS campaigns. However, it is also becoming increasingly accepted that available HTS collections generally lack the structural diversity needed to address more difficult targets, such as protein–protein interactions, transcription factor assemblies, and nucleic acid macromolecules.[2] The science of matching small molecules with macromolecular targets and biological functions is extremely complicated but several noteworthy strategies for the systematic synthesis of more optimal compound collections have been introduced to meet the challenges.

Some particularly successful strategies have emphasized structural complexity, frequently inspired by biologically "pre-validated" scaffolds and substructures of natural products,[3] and expanded molecular diversity[4] as optimal design parameters to access target-relevant chemical space.[5] To effectively cope with the inherent structural complexity of such compounds and provide smoother downstream elaboration of screening hits into selective modulators of biological targets, careful synthetic planning, and innovative synthesis pathways are in great demand.

Substantial differences in target modulation of individual stereoisomers are often observed, and the ability to conduct stereostructure–activity relationship studies on screening hits may provide unique structural insights about the target under investigation.[6] However, complete control of stereodiversity is a long-standing challenge for library design. Synthetic

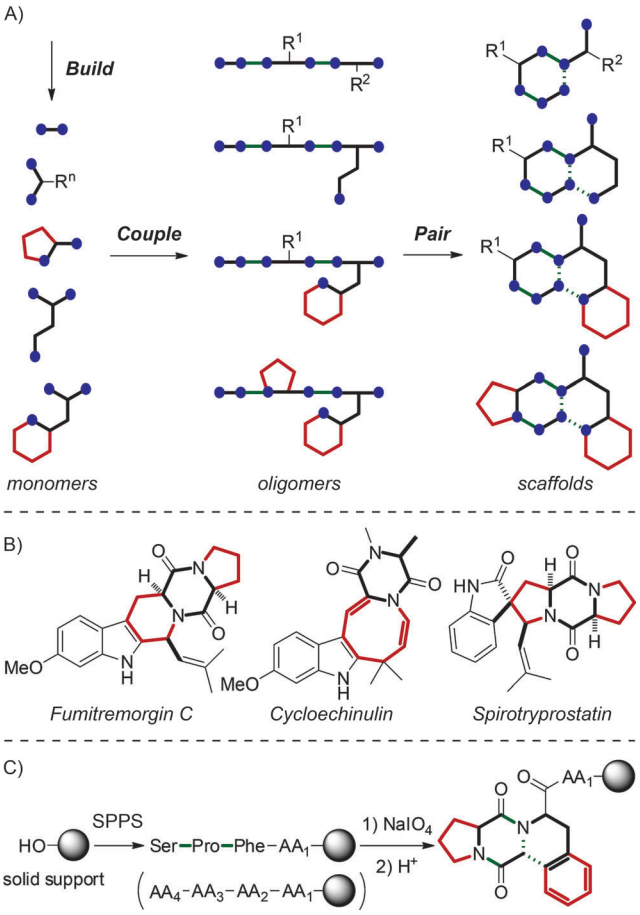

Figure 1. Synthesis of natural product-like fused diketopiperazines (DKPs): A) Outline of the B/C/P strategy. B) Natural product DKPs. C) Solid-phase peptide synthesis (SPPS) and cyclization cascade to fused natural product-like DKPs.

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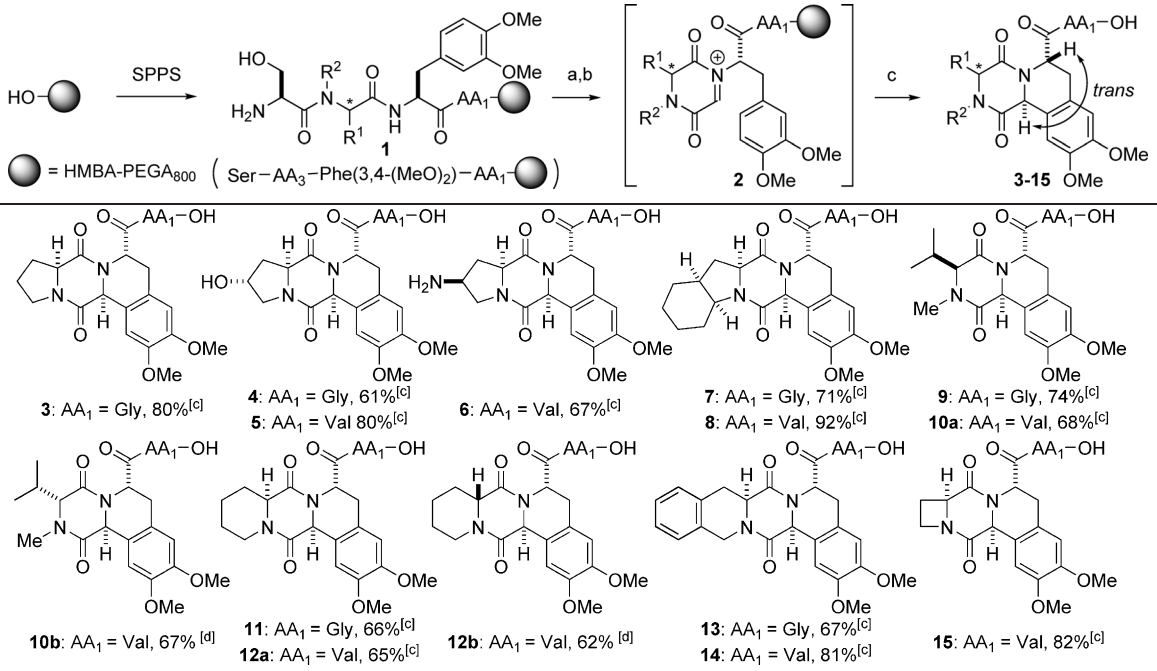
[**] The Technical University of Denmark, Danish Council for Independent Research, Lundbeck Foundation, Carlsberg Foundation, and DSF Center for Antimicrobial Research (Danish Council for Strategic Research) are gratefully acknowledged for financial support.

Supporting Information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201405747.

tactics referred to as Build/Couple/Pair (B/C/P) strategies may guide this process (Figure 1).[7] Herein we present a conceptually simple oligomer-based strategy[8] (Figure 1 A) which captures the synthetic benefits offered by solid-phase peptide synthesis (SPPS). Amino acids represent a class of densely functionalized building blocks (monomers) which are readily available in all stereoisomeric forms (Build). Decades of peptide coupling chemistry ensure that any amino acid sequence (oligomers) can be constructed quantitatively (Couple). We envisioned that carefully designed sequences with tailored functionalities might serve as precursors for natural product-like scaffolds, where stereocenters embedded in rings, junctions, and side-chains ideally are fully controlled by the choice of combinations of building blocks and modes of cyclization towards the final scaffold (Pair). Heterocyclic scaffolds of natural products are often derived from amino acids and short peptide strands, and such core structures would be logical targets in this context. It was decided to pursue the fusion of diketopiperazines (DKPs)[9] with other biologically relevant heterocyclic cores[10] to effectively generate a natural product-inspired compound collection (Figure 1 B). Commercial molecular libraries are normally prepared by attaching combinations of appendages to a limited number of common skeletons, which are typically flat and characterized by minimal stereochemical complexity, that is, few stereogenic units and a high proportion of sp^2-hybridized carbon atoms. In addition, it has been estimated that 83% of the core-ring scaffolds found in natural products are absent among commercially available compounds.[11] To effectively address these inadequacies we therefore sought to maximize spatial complexity by increasing the fraction of sp^3-hybridized carbon atoms and the number of stereogenic centers.[12]

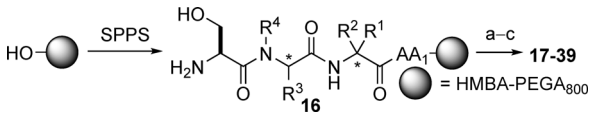
It was envisioned that the classical oxidation with periodate could be applied to a serine-terminated oligomeric peptide sequence to generate a terminal aldehyde which would undergo an intramolecular condensation reaction with the amide backbone to simultaneously form an *N*-acyliminium[13] intermediate/DKP capable of undergoing further cyclization by Pictet–Spengler-type reactions to form fused DKPs (Figure 1 C). This cascade was initially investigated with a Ser-AA_3-Phe(3,4-$(MeO)_2$)-AA_1 sequence, which was quickly assembled on an HMBA-functionalized PEGA resin (**1**, Table 1) by standard SPPS protocols. The aldehyde was liberated using $NaIO_4$ (aq),[14] and subsequent TFA treatment mediated the *N*-acyliminium cyclization with excellent purity of the products **3–10a** and **11, 12a**, and **13–15**, which were released using 0.1 M NaOH (aq). The broad applicability of the reaction sequence was evidenced by the successful incorporation of structurally diverse *N*-alkylated moieties at the AA_3 position. It was noted that whereas the Val residue (**1**; $R^1 = i$Pr, $R^2 = H$) is not compatible with the methodology, the corresponding *N*-Me-Val derivative leads to the desired compound **9**, presumably indicating the role of *N*-alkylation to promote a more rapid equilibration into the condensation-reactive cisoid amide bond. Hydroxy- and amino-substituted Pro residues could be introduced, thus affording the substituted tetracyclic scaffolds **4–6**, incorporating handles for appendage modifications. Skeletal diversity was conveniently introduced by exchanging Pro with other heterocyclic amino acids, thus providing a range of complex polycyclic products (**7–8**, **11–15**) in high product yields and excellent *trans*-diastereoselectivities between the H at the newly formed ring junction and the α-H of the AA_2 residue.

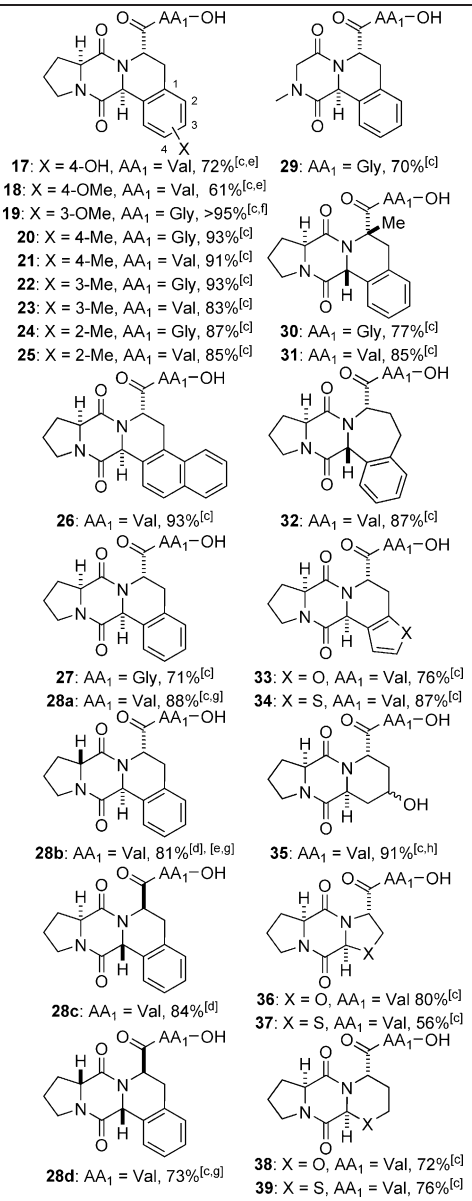
Table 1: Synthesis of fused natural product-like DKPs by intramolecular formation of *N*-acyliminium ions: Variation of the AA_3 residue.[a,b]



[a] Reagents and conditions: a) $NaIO_4$, H_2O; b) TFA (50% v/v), CH_2Cl_2; c) NaOH (aq) or HCl (aq). [b] Values represent purities (RP-HPLC). [c] Product released with 0.1 M NaOH (aq). [d] Product released with 4 M HCl (aq). TFA = trifluoroacetic acid.

Table 2: Synthesis of fused natural product-like DKPs by intramolecular formation of *N*-acyliminium ions: variation of the AA_2 residue.[a,b]





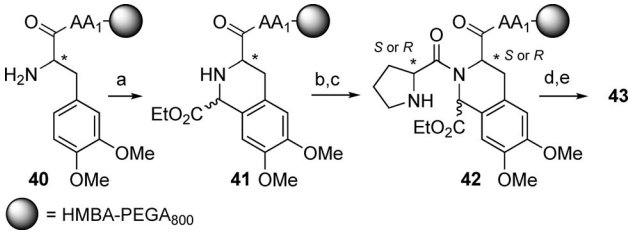
[a] Reagents and conditions: a) $NaIO_4$, H_2O; b) TFA (50% v/v), CH_2Cl_2; c) NaOH (aq) or HCl (aq). [b] Values represent purities (RP-HPLC). [c] Product released with 0.1 M NaOH (aq). [d] Product released with 4 M HCl (aq). [e] $BF_3{\cdot}OEt_2$ cyclization. [f] r.r. (regioisomeric ratio) 1:1. [g] Values represent conversions (RP-HPLC). [h] d.r. 1:1.

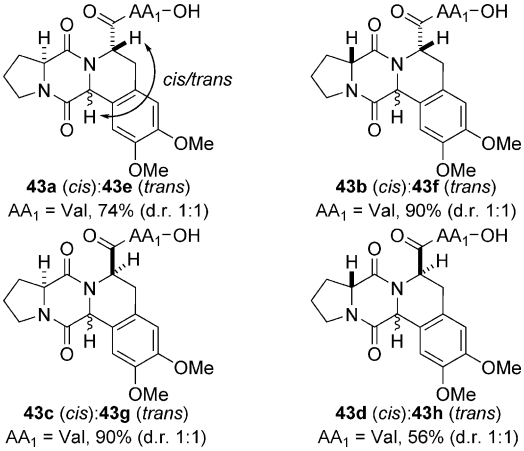
The scope of the scaffold diversity was further examined by incorporating various rings into the amino acid side-chain at the AA_2 position (Table 2). Importantly, the methodology allowed the formation of diverse scaffolds, including those resulting from reactions of less nucleophilic rings (**17**–**31**), heteroaromatics (**33**–**34**), alkene (**35**), and heteroatoms (**36**–**39**), in high yields and excellent *trans* diastereoselectivities (Table 2). Even α-methylation and an additional methylene in the side chain of AA_2 were tolerated, thus providing **30**–**31** and **32**, respectively. The possibility for controlling the stereochemical features of the scaffolds was investigated with combinations of L- and D-Phe and L- and D-Pro at the AA_2 and AA_3 positions, respectively. In all combinations, the excellent *trans*-diastereoselectivity was perfectly retained, thus giving the enantiopure compounds **28a**–**d** in high yields. When releasing **28b** from the solid support with 0.1 M NaOH (aq), we noted that epimerization at the α-position of the Pro residue occurred to give **28a**. The resulting product mixture (2:3) of **28b** and **28a** is clearly indicative of a proton exchange pathway, active under basic conditions.

Similar patterns (16–29% epimerization) were observed during cleavage of the diastereomeric product **28c** and D-*N*-Me amino acid derivative **10b** (Table 1) with 0.1 M NaOH (aq). Fortunately, this issue could be circumvented by effecting the cleavage with 4.0 M HCl (aq), which cleanly released the products without any detectable epimerization. With methodology at hand to easily access *trans*-diastereomeric scaffolds through intramolecular aldehyde–amide condensation, the synthesis of *cis*-diastereomeric products was then undertaken.

To keep in line with practical transformations on solid support for the scaffold being pursued, we anticipated that an intermolecular aldehyde–amine condensation pathway might provide an entry to some degree of *cis*-product formation. To

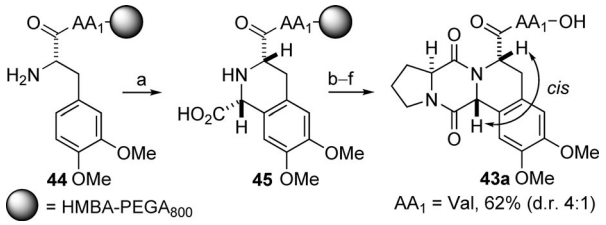
Table 3: Synthesis of tetrahydroisoquinolines (THIQs) and fused natural product-like DKPs by Pictet–Spengler reaction of ethyl glyoxylate.[a,b,c]





[a] Reagents and conditions: a) EtO_2CCHO, TFA (50% v/v), CH_2Cl_2; b) D- *or* L-Boc-Pro-OH, BTC, 2,4,6-collidine; c) TFA (95% v/v), H_2O; d) 0.1 M 4-pyrrolidinopyridine, DMF (30% v/v), H_2O, 3 °C; v) 4 M HCl (aq). [b] Values represent purities (RP-HPLC). [c] AA_1 = Val. Boc = *tert*-butoxycarbonyl, BTC = bis(trichloromethyl) carbonate, DMF = *N*,*N*-dimethylformamide.

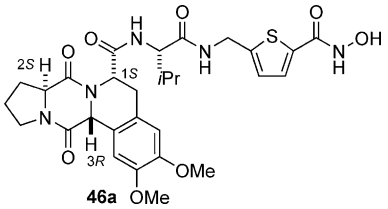
this end, we envisioned that the dipeptide **40** would undergo Pictet–Spengler reaction with a suitable glyoxylic acid derivative, followed by peptide coupling and final DKP formation (Table 3). Pictet–Spengler reaction of **40** with ethyl glyoxylate led to a 1:1 diastereomeric mixture of the tetrahydroisoquinolines (THIQs) **41**. Subsequent installation of D- and L-Pro residues provided the tripeptides **42**, and DKP formation was promoted by using 4-pyrrolidinopyridine as the acyl activating agent at low temperature.[15] Subsequent release with 4 M HCl (aq) gave all four pairs of the expected 1:1 diastereomeric mixtures (**43a–h**). In a final effort to develop a *cis*-selective pathway (Scheme 1), we were pleased



Scheme 1. *cis*-Selective synthesis of THIQ and fused natural product-like DKP by Pictet–Spengler reaction of glyoxylic acid. Reagents and conditions: a) $HO_2CCH(OH)_2$, TFA (50% v/v), CH_2Cl_2; b) FmocCl, iPr_2NEt, CH_2Cl_2; c) ClH_2N-Pro-OFm, TBTU, *N*-ethylmorpholine, DMF; d) piperidine (20% v/v), DMF; e) TBTU, *N*-ethylmorpholine, DMF; f) 4 M HCl (aq). AA_1 = Val, Fm = 9-fluorenylmethyl, TBTU = *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium tetrafluoroborate *N*-oxide.

to observe that the Pictet–Spengler reaction of **44** with glyoxylic acid indeed gave the THIQ **45** with good diastereoselectivity in favor of the *cis*-product. Subsequent conversion of **45** into **43a** (with **43e** as the minor side-product) was easily achieved in good yield, thus showcasing a methodology which is highly efficient for the stereoselective synthesis of all the diastereomers **43a–h**.

Table 4: Stereoisomeric matrix of fused natural product-like DKP hydroxamic acids and their histone deacetylase (HDAC) inhibitory activities.[a]



Compound	Configuration	IC_{50} HDAC 6 [μM]	IC_{50} HDAC 10 [μM]
SAHA	–	0.20	0.31
46a	(1*S*,2*S*,3*R*)	0.22	13
46b	(1*S*,2*R*,3*R*)	0.42	8.3
46c	(1*R*,2*S*,3*S*)	0.39	21
46d	(1*R*,2*R*,3*S*)	0.30	7.3
46e	(1*S*,2*S*,3*S*)	0.27	7.8
46f	(1*S*,2*R*,3*S*)	0.24	1.9
46g	(1*R*,2*S*,3*R*)	0.43	10
46h	(1*R*,2*R*,3*R*)	0.25	6.0

[a] See Supporting Information for experimental details.

For preliminary biological screening, we generated a full stereoisomeric matrix of fused natural product-like DKP-containing hydroxamic acids (**46a–h**; Table 4). The approved hydroxamic acid drug SAHA inhibits both isoforms of the class IIb histone deacetylases (HDACs 6 and 10), and much to our delight the synthesized hydroxamic acids **46a–h** show a marked selectivity against HDAC 6 over HDAC 10. An 11-fold difference in modulation activity against HDAC 10 was noted for the stereoisomers **46c** and **46f**, thus indicating the potential of the compound collection to identify important binding interactions remote from the active site through stereostructure–activity studies. In summary, we have developed a multistep methodology which allows the all-solid-phase assembly of natural product-like compounds in excellent overall yields (generally > 50%) using simple amino acid building blocks and common reagents for peptide synthesis. A proof-of-concept collection of 54 skeletally and stereochemically diverse compounds was prepared with excellent stereochemical control. The ability to conduct stereostructure–activity relationship studies using complete sets of stereoisomers enabled the development of isoform-selective HDAC inhibitors.

Received: May 29, 2014
Published online: September 11, 2014

Keywords: drug discovery · heterocycles · molecular diversity · peptides · solid-phase synthesis

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