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J. Org. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.joc.6b01150 • Publication Date (Web): 08 Jul 2016

Applications of Convertible Isonitriles in the Ligation and Macrocyclization of Multicomponent Reaction-Derived Peptides and Depsipeptides

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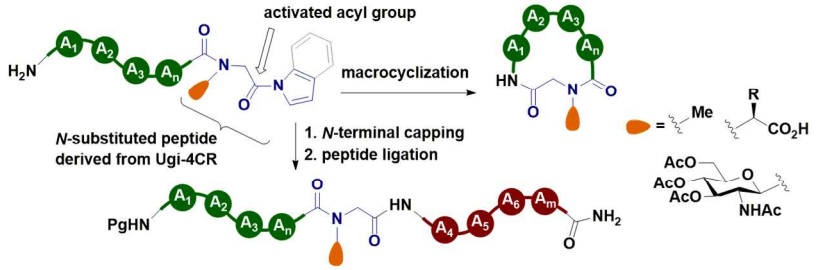
Graphical Abstract



Abstract

Peptide ligation and macrocyclization are among the most relevant approaches in the field of peptide chemistry. Whereas a variety of strategies relying on coupling reagents and native chemical ligation are available, there is a continuous need for efficient peptide ligation and cyclization methods. Herein we report on the utilization of convertible isonitriles as effective synthetic tools for the ligation and macrocyclization of peptides arising from isocyanide-based multicomponent reactions. The strategy relies on the use of convertible isonitriles – derived from Fukuyama amines – and peptide carboxylic acids in Ugi and Passerini reactions to afford *N*-alkylated peptides and depsipeptides, respectively, followed by conversion of the *C*-terminal amide onto either *N*-peptidoacyl indoles or pyrroles. Such activated peptides proved efficient in the ligation to peptidic, lipidic and fluorescently labeled amines and in macrocyclization protocols. As a result, a wide set of *N*-substituted peptides (with methyl, glycosyl and amino acids as *N*-substituents), cyclic *N*-methylated peptides and a depsipeptide were produced in good yields using conditions that involve either classical heating or microwave irradiation. This report improves the repertoire of peptide covalent modification methods by exploiting the synthetic potential of multicomponent reactions and convertible isonitriles.

Keywords

Peptide ligation, *N*-methyl peptides, multicomponent reactions, convertible isonitriles, fluorescent labeling, macrocyclization

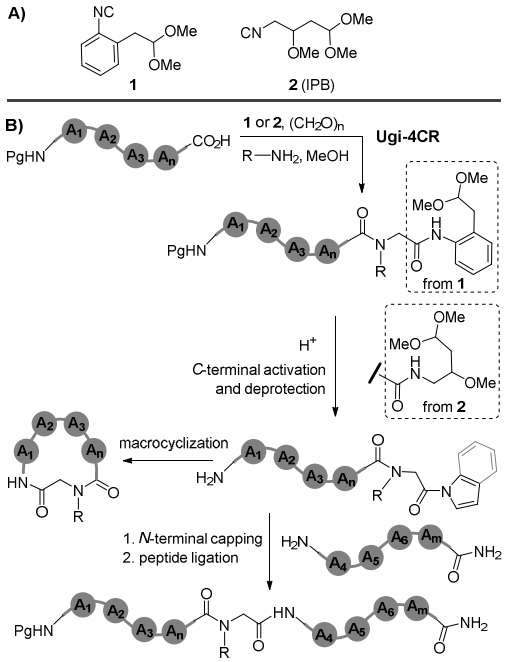
Introduction

Isonitrile-based multicomponent reactions (I-MCRs) have proven to be powerful tools for the synthesis and derivatization of peptides and peptidomimetics.[1] Among the I-MCRs, the Ugi four-component reaction[2] (Ugi-4CR) has the greatest applicability on this field,[1] not only because it utilizes amino and carboxylic groups but also due to its high chemical efficiency and diversity-generating character. A common application of the Ugi-4CR has been the ligation of – at least – two amino acids or peptide fragments to assemble *N*-substituted oligopeptidic skeletons. This Ugi-ligation strategy has enabled the preparation of naturally occurring peptides[3] as well as synthetic ones of medicinal,[4] catalytic[5] and biomimetic[6] importance. Another emerging application of this type of I-MCR is its utilization in the synthesis of cyclic peptides[7] and peptidomimetics,[8] by means of approaches using the multicomponent process for assembling the acyclic scaffold, for the ring closure, or both.[9]

In peptide and medicinal chemistry, a strategy comprising an Ugi-4CR followed by either an efficient macrolactamization or peptide ligation can be a useful tool for the construction of *N*-functionalized (e.g., methyl, dye-labeled) (cyclo)peptides. However, a drawback limiting the implementation of this strategy is the poor reactivity in acylation processes of the *C*-terminal secondary amide. A solution for this can be found in the development of convertible isonitriles,[10,11] reagents that upon participation in the Ugi-4CR – and eventual activation – generate reactive amides suitable for follow-up derivatization. The utilization of these reagents has enabled effective applications of I-MCRs in the synthesis of naturally occurring compounds and analogues.[3b,12]

The derivatizations reported so far for convertible isonitrile-derived amides include hydrolysis, methanolysis, intramolecular acylations to five and six-membered lactones and lactams, as well as acylation of aliphatic amines.[10,11,12] However, to our knowledge, applications in crucial approaches of peptide chemistry such as ligation of two peptide fragments and macrolactamization have remained

elusive so far. Herein we report on the utilization of convertible isonitriles for the derivatization of peptides by I-MCRs and their subsequent activation to enable either ligation to a second peptide or macrolactamization under dilution conditions. Of course, such a ligation is not limited to inter and intramolecular couplings, but can be extended to attaching lipids, labels and glycosidic moieties. To develop this strategy, we focused on the utilization of the structurally related and versatile convertible isocyanides **1** and **2** (scheme 1A). 1-Isocyano-2-(2,2-dimethoxyethyl)benzene (**1**) was developed independently by the groups of Wessjohann[11b] and Kobayashi[11c] in 2007, while 4-isocyanopermethylbutane-1,1,3-triol (IPB, **2**) was reported by Wessjohann and co-workers in 2012.[11a] Both isonitriles show excellent reactivity in I-MCRs and are available in multigram scale from amines previously introduced by Fukuyama *et. al.*[13]



Scheme 1. Strategy for the utilization of convertible isonitriles **1** and **2** in peptide ligation and macrocyclization.

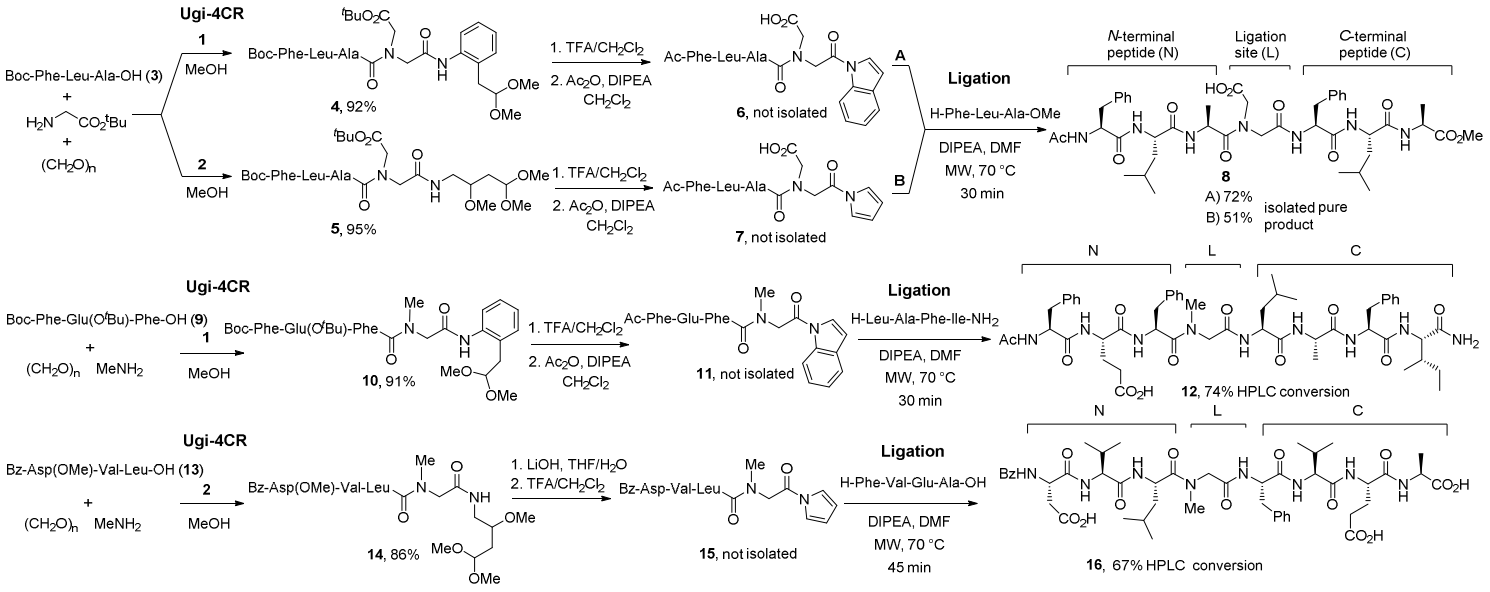
Results and Discussion

As depicted in scheme 1, the approach devised to exploit the potential of convertible isonitriles on peptide chemistry comprises their utilization in the Ugi-4CR by reaction with a peptidic carboxylic acid, a primary amine and an oxo-compound to produce a larger peptide incorporating an *N*-alkylated amino acid, in this case preferentially Gly by the use of paraformaldehyde. The internal *N*-alkylation of peptides, and especially *N*-methylation,[14] has proven to be a successful way to improve pharmacological properties such as metabolic stability, membrane permeability and pharmacokinetics, as compared with their non-*N*-alkylated congeners.[14] As mentioned before, the reactivity of the *C*-terminal secondary amides derived from other convertible isocyanides has enabled their conversion to terminal carboxylic acids and esters, but not their utilization either in the direct ligation to another peptide fragment or in macrolactamization processes. On the other hand, the activation mode of terminal amides derived from isonitriles **1** and **2** comprises the conversion – upon mild acidic treatment – to *N*-acyl indoles and pyrroles,[13] respectively. Both types of activated acyl groups are known to react readily with primary and secondary amines,[11a,b,13] thus paving the way for the development of both peptide ligation and intramolecular acylation of a peptidic amine, i.e., *N*-terminus or side chain.

As shown in scheme 1B, our strategy encompasses the implementation of the Ugi-4CR with Boc-protected peptides as the carboxylic acid component and either isonitrile **1** or **2**, thus enabling the simultaneous activation of the *C*-terminus – by formation of either *N*-peptidoacyl indole or pyrrole – and the deprotection of the *N*-terminal residue upon mild acidic treatment (also known as UDAC, Ugi-Deprotection-Activation-Cyclization/Condensation). Consequently, head-to-tail macrocyclization can be straightforward by setting up dilution conditions typically required to cyclize oligopeptides, while ligation to another peptide fragment ideally requires previous capping of the *N*-terminus, e.g., by acetylation or a *N*-terminal protection not cleaved upon activation. Thus, the Fmoc protecting group

may be installed at the *N*-terminus, while Lys side chains could be orthogonally protected with e.g., Cbz, if concomitant deprotection is not desired during acid-mediated *C*-terminal activation.

To prove the scope of this strategy, we decided to implement a ligation process wherein peptides having various carboxylic groups could be selectively ligated by the one taking part in the Ugi-4CR with a convertible isonitrile and being subsequently activated. Peptide synthesis was carried out either in solution using the Boc tactic or by a stepwise solid-phase Fmoc strategy on the Am-MBHA resin.[15] Oligopeptides used as substrates of the I-MCRs and of the ligation processes are either known compounds reported by our group or were prepared as described in the Supporting Information.



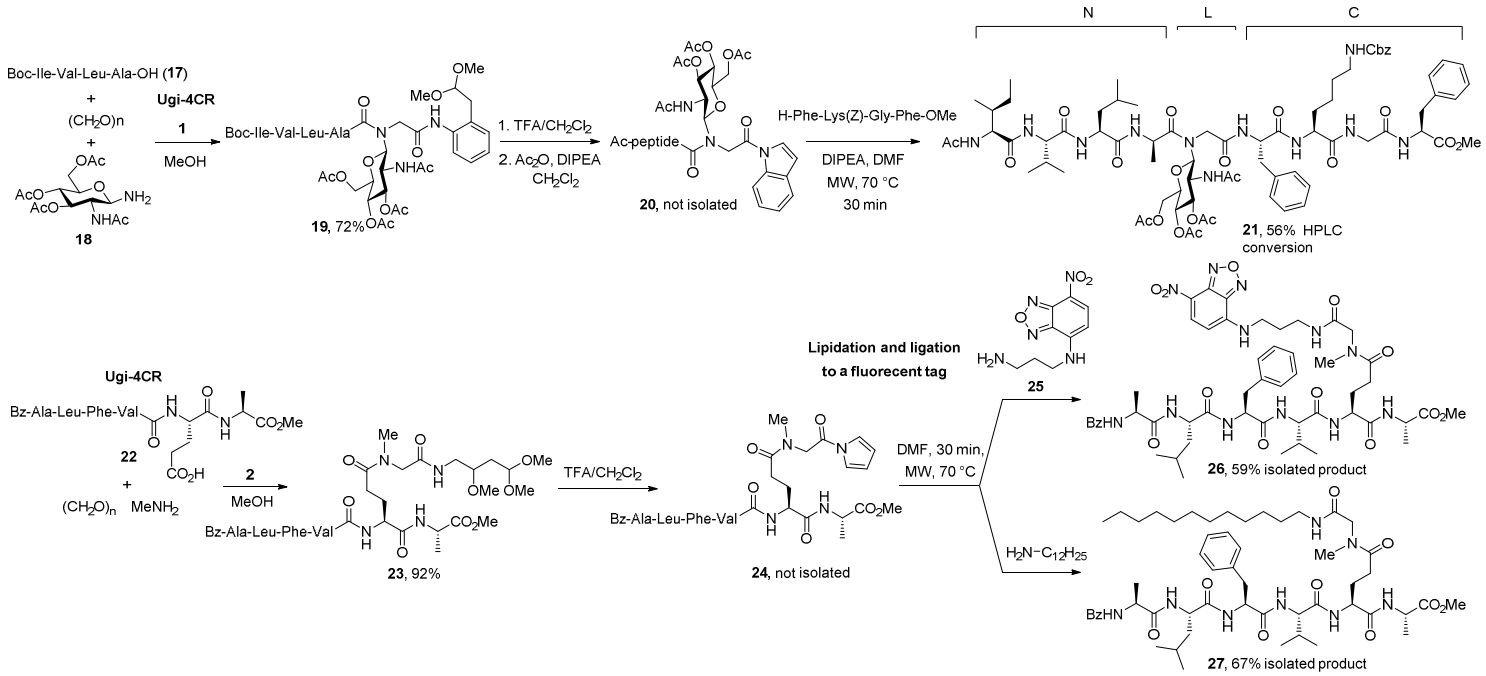
Scheme 2. Ligation of Ugi-modified peptides *via* *N*-peptidoacyl indoles and pyrroles.

As depicted in scheme 2, we initially carry out a comparison of the ligation efficiency of an *N*-acyl indole and *N*-acyl pyrrole having identical peptide sequences and reacting them with the same nucleophilic aminopeptide. For this, tripeptide **3** was reacted in parallel with glycine *t*-butyl ester, paraformaldehyde and isonitriles **1** and **2** to furnish the branched *N*-substituted peptides **4** and **5**, respectively, in excellent yield after 24 h. Both intermediates were subjected to *C*-terminal activation

and simultaneous Boc and *t*-butyl ester removal by treatment with 20% TFA in CH_2Cl_2, followed by acetylation of the *N*-terminus to render *N*-peptidoacyl indole **6** and pyrrole **7**, which were used without further purification. A variety of protocols were studied to assess the best conditions to ligate the *C*-activated peptides to the model tripeptide Phe-Leu-Ala-OMe. Tested conditions include stirring a solution of both peptides in either DMF or THF and either at room temperature, 50 °C or 70 °C during several hours. The best conversion was found with DMF as solvent at 70 °C for 24 h. A solution to shorten the ligation time was the use of microwave irradiation at 70 °C for 30 min, which proved effective in the ligation of the two peptide fragments similar to the traditional heating for 24 h. Several parallel experiments demonstrated that peptides functionalized at the *C*-terminus as *N*-acyl indole are more activated than those having the *N*-acyl pyrrole. This was consistent with the synthesis of peptide **8**, which was obtained as isolated pure product in 72% yield from **6** and only in 51% from **7**. HPLC monitoring of routes A and B shown in scheme 2 confirmed higher conversion into peptide **8** in the ligation process based on intermediate **6** than that involving **7** (see the Supporting Information).

In an endeavour to expand the scope of the peptide ligation, we turned to ligate peptides having unprotected Glu and Asp side chains. Peptide **9** was reacted with isonitrile **1**, methylamine and paraformaldehyde in the Ugi-4CR to produce ***N***-methylated peptide **10** in excellent yield. The *C*-terminal activation/deprotection procedure followed by *N*-terminal capping rendered *N*-peptidoacyl indole **11** bearing an unprotected Glu side chain and activated *C*-terminus. As before, the MW-assisted ligation process proved success in the conjugation to tetrapeptide Leu-Ala-Phe-Ile-NH_2 yielding the *N*-methylated octapeptide **12** with 74% of conversion after 30 min, as indicated by analytical RP-HPLC analysis. As shown in scheme 2, a third example of ligation was implemented with an *N*-peptidoacyl pyrrole as intermediate. For this, tripeptide **13** was combined with isonitrile **2** to render the *N*-methylated peptide **14**, which was subjected to deprotection of the Asp side chain by saponification

followed by *C*-terminal activation upon acidic treatment. Final ligation to tetrapeptide Phe-Val-Glu-Ala-OH was also undertaken under MW irradiation, albeit it required longer reaction time to achieve peptide **16** with a 67% conversion. With the last two examples, we demonstrated the success of the ligation of unprotected *N*-peptidoacyl indoles and pyrroles to aminopeptides bearing *C*-terminal methyl esters, carboxamides and free carboxylic acid groups. Finally, since peptide **13** was initially protected at the *N*-terminus with an acid stable benzoyl group, *N*-terminal capping between the Ugi-4CR and the *C*-terminal activation can be avoided, saving this extra step altogether.



Scheme 3. Ligation strategy to glycosylated, lipidated and fluorescently tagged peptides.

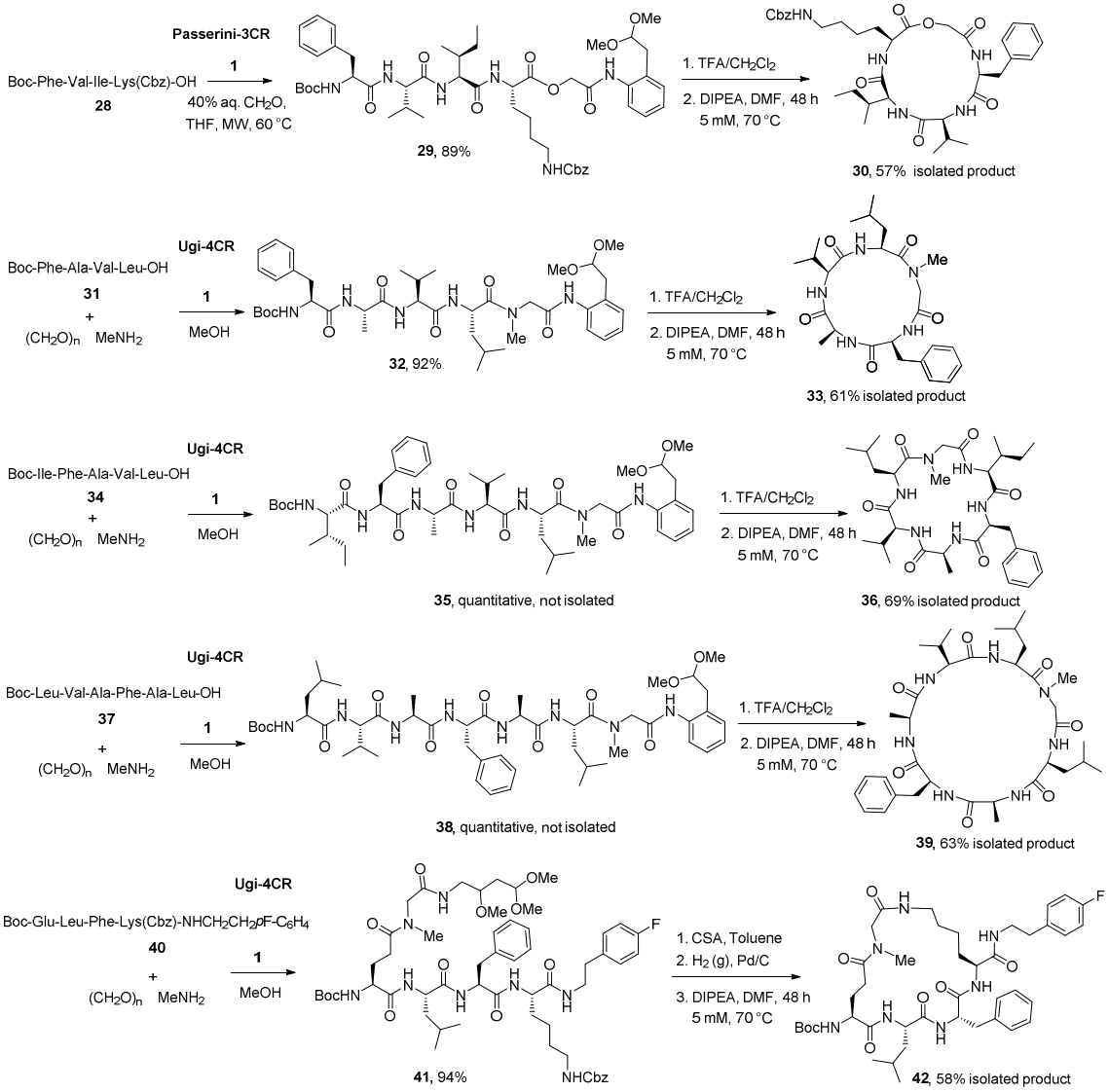
Next we turned to assess the scope of the ligation process to produce glyco-, lipo- and fluorescently labelled peptides. As depicted in scheme 3, an initial strategy relied on employing glycosyl amine **18** – derived from the biologically relevant *N*-acetyl glucosamine – in the Ugi-4CR with peptide **17** and isonitrile **1** to afford *N*-glycosylated peptide **19** in good yield. After acidic treatment to enable the *C*-terminal activation and Boc removal, followed by *N*-terminal acetylation to yield *N*-acyl indole **20**, this latter intermediate was ligated to tetrapeptide H-Phe-Lys(Cbz)-Gly-Phe-OMe to afford the remarkably

complex *N*-glycosylated nonapeptide **21**. HPLC monitoring of this reaction showed a moderate conversion of 56%, while a substantial amount of intermediate **20** remained unreacted after 45 min under MW irradiation. However, a longer reaction time was not considered, as decomposition of both compounds **20** and **21** becomes competitive after 1h.

Thus far, ligation at the *C*-termini of peptides was the focus, while such a type of derivatization is also possible at the side chains of peptides endowed with Asp and Glu. Scheme 3 illustrates the Ugi-4CRs of hexapeptide **22** – having an unprotected Asp side chain and protected *N* and *C*-termini – with isonitrile **2** and the methylamine/paraformaldehyde combination to afford the side chain-functionalized peptide **23** in excellent yield. Classic activation by acidic treatment rendered *N*-peptidoacyl pyrrole **24** in quantitative yield, which was subsequently used in the ligation protocol without further purification. Thus, **24** was reacted with the 7-nitrobenz-2-oxa-1,3-diazol-4-yl (NBD)-derived fluorescent amine **25**[16] and *n*-docecylamine to furnish the side-chain fluorescently-tagged peptide **26** and the lipidated version **27** in 59% and 67% yield, respectively, after column chromatography. It is worth-mentioning that first attempts to produce side-chain *N*-glycosides by reaction of activated peptide **24** with a glycosyl amine were thwarted by poor conversion and significant decomposition upon conjugation conditions with either MW or traditional heating.

After demonstrating the scope of convertible isonitriles in Ugi-4CR/activation/ligation protocols, we turned to demonstrate applications in the synthesis of both cyclic depsipeptides and *N*-methylated peptides. Cyclic depsipeptides are natural products composed by amino acids (or bioisosters) and at least one hydroxy acid, thus forming a lactone bond in the cyclopeptidic skeleton. Previously, I-MCRs have been employed to produce cyclic depsipeptide mimics,[17] though convertible isocyanides have not been used for such purposes yet. As shown in scheme 4, our strategy to cyclic depsipeptides comprised the implementation of the Passerini 3-component reaction (Passerini-3CR) between *N*-protected peptide

28, isonitrile **1** and aqueous formaldehyde in chloroform under MW irradiation to furnish depsipeptide **29** in excellent yield. Treatment of **29** with TFA in CH_2Cl_2 enabled the simultaneous *N*-terminal deprotection and the *C*-terminal activation (UDAC) by formation of the *N*-acyl indole, thus paving the way for the macrolactamization step. A variety of macrocyclization conditions were studied for this and other *C*-activated peptides (not including heating by MW irradiation), resulting in the selection of a 5 mM concentration in DMF under basic conditions at 70 °C as the most suitable one. The reaction time for such macrolactamizations was set to 48 hours, but in some cases longer reaction times might be required for higher yields. Under these conditions, the cyclic depsipeptide **30** was obtained in 57% yield of isolated pure product, while no byproducts derived from the feared cleavage of the lactone bond were detected by HPLC and ESI-MS analysis of the crude product.



Scheme 4. Macrocyclization strategy to a cyclic depsipeptide and *N*-methylated peptides.

A similar approach was utilized for the synthesis of *N*-methylated peptides endowed with different sequences and macrocyclic ring sizes, but relying on the Ugi-4CR for the installation of the *N*-methylated amide bond. Initially, *N*-protected tetrapeptide **31** was reacted with isonitrile **1**, methylamine and paraformaldehyde in the Ugi-4CR to produce *N*-methylated peptide **32** in excellent yield. The *C*-terminal activation/deprotection procedure rendered the corresponding *N*-peptidoacyl indole, which was subjected to the macrocyclization protocol to furnish cyclic pentapeptide **33** in good yield over two steps. The same UDAC sequence was employed for the synthesis of *N*-methylated cyclic hexapeptide

36 and heptapeptide **39** in 69% and 63% yield, respectively, over three steps. As shown in scheme 4, the experience gained in the previous protocols led us to implement the synthesis of **36** in **39** without chromatographic purification of any intermediate, which was possible mainly due the high reactivity of isonitrile **1** in combination with methylamine, paraformaldehyde and peptide carboxylic acids, enabling the Ugi-4CRs to proceed quantitatively in 24 h.

Besides of the head-to-tail cyclization, the method should also enable the side chain-to-side chain cyclization of peptides. For this, peptide **40**, having both termini capped and an unprotected Glu side chain, was submitted to the Ugi-4CR with methylamine, paraformaldehyde and isonitrile **2** to produce the side chain-functionalized peptide **41** in excellent yield. As compound **41** is protected at the *N*-terminus with Boc, camphor sulphonic acid (CSA, 0.1 equiv) and quinoline (0.1 equiv) in toluene were chosen for the *C*-terminal activation, conditions upon which the Boc group remained unaffected.[13a] Subsequently, the Cbz group of the Lys side chain was orthogonally cleaved by hydrogenation, and the resulting intermediate was cyclized under previously described conditions to furnish the side chain cross-linked tetrapeptide **42** in 58% yield over three steps. With this final example, we have proven that convertible isonitriles **1** and **2** are suitable reagents for the incorporation of either *N*-alkylated or depsipeptide moieties by means of Ugi-4CR and Passerini-3CR, respectively, while enabling the mild activation of either the peptide *C*-terminus or side chain for ligation and macrocyclization purposes.

Conclusions

We have demonstrated the feasibility of using convertible isonitriles – derived from Fukuyama amines – as synthetic means to enable peptide ligation and macrocyclization. The strategy comprises their utilization in I-MCRs such as the Ugi-4CR and the Passerini-3CR for the assembly of *N*-alkylated peptides and depsipeptides, respectively, followed by either side chain or *C*-terminal activation by acidic treatment to afford *N*-peptidoacyl indoles or pyrroles. The latter intermediates proved to be

properly activated to enable either the ligation to nucleophilic aminopeptides or macrolactamization under diluted conditions. Both ligation and macrocyclization protocols required either microwave irradiation or heating at 70 °C to proceed n reasonable time, thus providing a variety of *N*-substituted (cyclo)peptides and a cyclic depsipetide. These results provide further evidence of the potential of convertible isonitriles and I-MCRs as powerful synthetic tools in peptide chemistry.

Experimental Section

Convertible isonitriles **1** and **2** were synthesized according to references 11a and 11b. Peptides **3**, **9**, **17**, **28**, **31**, **34**, and **37** are known compounds and were produced according to references 6b and 18. Peptide synthesis grade DMF, CH_2Cl_2, *i*Pr_2EtN, TFA, and HPLC-grade acetonitrile were used. HPLC analysis was performed in a reverse-phase (RP) C18 column (4.6× 150 mm, 5μm). A linear gradient from 5% to 60% of solvent B in solvent A over 35 min at a flow rate of 0.8 mL/min was used (solvent A: 0.1% (*v*/*v*) TFA in water, solvent B: 0.05% (*v*/*v*) TFA in acetonitrile). Detection was accomplished at 226 or 254 nm. Flash column chromatography was performed on silica gel 60 (>230 mesh) and analytical thin layer chromatography (TLC) was performed using silica gel aluminum sheets. For peptides that were not purified to >95% by column chromatography on silica, purity was assessed by RP-HPLC and characterization of an analytical sample was made by electrospray ionization mass spectrometry (ESI-MS). The high resolution ESI mass spectra were obtained either from a 70e Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer equipped with an Infinity™ cell, a 7.0 Tesla superconducting magnet or from an Orbitrap Elite mass spectrometer equipped with an HESI electrospray ion source. Reactions involving microwave irradiation were performed in a Robotic Microwave Synthesizer (Biotage Emrys Personal Chemistry Optimizer Microwave Synthesizer). ^{1}H NMR and ^{13}C NMR spectra were recorded on a 400 spectrometer at 399.94 MHz and 100.57 MHz,

respectively. Chemical shifts (δ) are reported in ppm relative to TMS (^{1}H NMR) and to the solvent signal (^{13}C NMR).

General procedure for the Ugi-4CR: A solution of the amine (0.6 mmol, 1.2 equiv.) and paraformaldehyde (0.6 mmol, 1.2 equiv.) in MeOH/CH_2Cl_2 (5 mL, 5:1, *v*/*v*) is stirred for 1 h at room temperature. NEt_3 (0.6 mmol) is added when amine hydrochlorides were employed as amino components. The peptide carboxylic acid (0.5 mmol, 1 equiv.) and the convertible isocyanide (0.5 mmol, 1 equiv.) are then added and the reaction mixture is stirred at room temperature for 24 h. The volatiles are then concentrated under reduced pressure and the resulting crude product is dissolved in 50 mL of $CHCl_3$. The organic phase is washed sequentially with an aqueous saturated solution of citric acid (30 mL), aqueous 10% $NaHCO_3$ (30 mL), and brine (30 mL), and then dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product is purified by flash column chromatography (CH_2Cl_2/MeOH) on silica to afford the corresponding *N*-alkylated peptide.

General procedure for the simultaneous Boc/*t*Bu removal and *C*-terminal activation by conversion of Ugi-4CR-derived amides into *N*-peptidoacyl indoles and pyrroles: The Ugi-4CR-derived peptide (0.5 mmol) is dissolved in CH_2Cl_2 (5 mL) and treated with trifluoroacetic acid (1 mL) at 0 °C. The reaction mixture is allowed to reach room temperature and stirred for 2 h, then concentrated under reduced pressure. TFA is removed completely by repetitive addition and evaporation of CH_2Cl_2 to furnish the TFA salt of the *C*-activated peptide, which is used without further purification.

General acetylation procedure of the free *N*-terminal peptide: The peptide (0.5 mmol) is dissolved in 5 mL of CH_2Cl_2 and treated with *i*Pr_2EtN (0.52 mL, 3 mmol) and Ac_2O (0.28 μL, 3 mmol). The reaction mixture is stirred at room temperature for 2 h and then the volatiles are evaporated under reduced pressure. The product is dissolved in EtOAc (25 mL) and washed vigorously with aq. 10% HCl

(2×15 mL) and brine (15 mL). The organic phase is dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford the *N*-acetyl peptide.

General peptide ligation procedure under MW irradiation: The peptidyl or alkyl amine (0.25 mmol, 1 equiv.) and the *N*-peptidoacyl indole or pyrrole (0.25 mmol, 1 equiv.) are dissolved in 5 mL of DMF in a 10 mL glass tube. *i*Pr_2EtN (0.17 mL, 1 mmol, 4 equiv.) is added and the glass tube is sealed and introduced in the microwave reactor. The flask is irradiated for 30 min (150 W) under high-speed magnetic stirring at 70 °C, while the reaction course is monitored by TLC. Additional cycles of 15 min are applied in cases of poor consumption of the starting material. The volatiles are removed under reduced pressure and the reaction product is washed several times with frozen diethyl ether, then taken up in *ca.* 20-30 mL of MeOH and filtered through a pad of silica gel C18 to partially remove the indole or pyrrole derivatives. The resulting solution is concentrated to dryness and the crude product is purified either by flash column chromatography or analyzed by RP-HPLC and ESI-MS. In the latter case, the crude peptide is taken up in 2:1 acetonitrile/water and lyophilized prior to HPLC analysis and purification.

Peptide 4: HCl·Gly-O^{t}Bu (100 mg, 0.6 mmol), Et_3N (83µL, 0.6 mmol), paraformaldehyde (18 mg, 0.6 mmol), peptide **3** (225 mg, 0.5 mmol) and isonitrile **1** (95.5 mg, 0.5 mmol) were reacted in MeOH/CH_2Cl_2 (5 mL, 5:1, *v*/*v*) for 24 h according to the general Ugi-4CR procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 12:1) afforded peptide **4** (360 mg, 92%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.88 (s, 1H); 7.33 (m, 1H); 7.27 (m, 1H); 7.26-7.05 (m, 9H); 6.97 (m, 1H); 5.22 (m, 1H); 4.62 (t, 1H, *J*=5.7 Hz); 4.57 (m, 1H) 4.54-4.50 (m, 2H); 4.38-4.31 (m, 2H); 4.17-4.06 (m, 2H); 3.38 (s, 3H); 3.37 (s, 3H); 3.06 (dd, 1H, *J*=13.8/5.2 Hz); 3.01 (m, 1H); 2.91 (m, 2H); 1.80-1.76 (m, 1H); 1.67-1.61 (m, 2H); 1.40 (s, 9H); 1.38 (s, 9H); 1.36 (d, 3H, *J*=7.0 Hz); 0.89 (d, 3H, *J*=6.4 Hz), 0.87 (d, 6H, *J*=6.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 17.7, 22.6, 22.9, 25.1,

28.2, 28.4, 38.3, 40.8, 41.1, 47.7, 49.9, 51.6, 52.6, 55.3, 55.5, 80.5, 81.4, 104.5, 122.3, 126.6, 126.8, 128.6, 129.1, 129.3, 130.5, 131.2, 136.3, 136.5, 155.9, 166.7, 167.3, 171.5, 171.6, 171.9, 173.0. HRMS (ESI-FT-ICR) *m/z*: 806.4322 $[M+Na]^+$, calcd. for $C_{41}H_{61}O_{10}NaN_5$: 806.4316.

Peptide 5: HCl·Glu-O^{t}Bu (100 mg, 0.6 mmol), Et_3N (83μL, 0.6 mmol), paraformaldehyde (18 mg, 0.6 mmol), peptide **3** (225 mg, 0.5 mmol) and isonitrile **2** (87 mg, 0.5 mmol) were reacted in MeOH/CH_2Cl_2 (5 mL, 5:1, *v*/*v*) for 24 h according to the general Ugi-4CR procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 10:1) afforded peptide **5** (364 mg, 95%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.36 (d, 1H, *J*=9.2 Hz); 7.26-7.21 (m, 3H); 7.15 (m, 2H); 7.11 (m, 1H); 7.07 (m, 1H); 6.99 (m, 1H); 5.24 (m, 1H); 4.66 (m, 1H); 4.60-4.51 (m, 3H); 4.32-4.27 (m, 3H); 4.25 (dd, 1H, *J*=17.2/4.5 Hz); 4.12 (dd, 1H, *J*=17.1/4.6 Hz); 3.53 (m, 1H); 3.38 (s, 3H); 3.32 (s, 6H); 3.27 (m, 1H); 3.06 (dd, 1H, *J*=13.8/5.2 Hz); 2.96 (dd, 1H, *J*=13.9/7.9 Hz); 1.81-1.72 (m, 3H); 1.66-1.60 (m, 2H); 1.41 (s, 9H); 1.30 (s, 9H); 1.35 (d, 3H, *J*=7.2 Hz); 0.88 (d, 3H, *J*=6.4 Hz), 0.87 (d, 6H, *J*=6.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 17.9, 22.0, 22.3, 25.1, 28.2, 38.2, 40.7, 42.6, 41.3, 47.6, 49.1, 49.9, 51.4, 55.3, 55.4, 56.2, 76.2, 80.3, 81.0, 102.5, 126.9, 128.8, 129.2, 136.3, 155.9, 166.2, 170.0, 171.6, 171.9, 172.6, 173.3. HRMS (ESI-FT-ICR) *m/z*: 788.4427 $[M+Na]^+$, calcd. for $C_{38}H_{63}O_{11}NaN_5$:788.4422.

Peptide 8: Peptide **4** (235 mg, 0.3 mmol) was subjected to the general procedure for the simultaneous Boc/*t*Bu removal and *C*-terminal activation, followed by the general acetylation procedure to yield quantitatively the *N*-peptidoacyl indole **6**, which was used forward without previous purification. *C*-activated peptide **6** (151 mg, 0.25 mmol) was reacted with the hydrochloride salt of tripeptide H-Phe-Leu-Ala-OMe (100 mg, 0.25 mmol) for 30 minutes in the presence of *i*Pr_2EtN according to the general ligation procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 10:1) afforded peptide **8** (153 mg, 72%) as a white amorphous solid. In parallel, peptide **5** was subjected to the general procedure

for the simultaneous Boc/*t*Bu removal and *C*-terminal activation, followed by acetylation to give **7**, which was equally submitted to the ligation procedures to furnish **8** (108 mg, 51%) after column chromatography. Mixture of two conformers. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.27-7.16 (m, 10H); 5.13, 4.97 (2×m, 1H); 4.67-4.48 (m, 3H); 4.30-4.24 (m, 2H); 4.19 (d, *J*=18.8 Hz); 4.17 (d, *J*=17.2 Hz); 3.93 (d, *J*=17.2 Hz); 3.91 (d, *J*=17.2 Hz); 3.79 (d, *J*=17.6 Hz); 3.74 (s, 3H); 3.19-3.09 (m, 2H); 2.95-2.85 (m, 2H); 1.90 (s, 3H); 1.75-1.42 (m, 6H); 1.36 (d, 3H, *J*=6.8 Hz); 1.31 (d, 3H, *J*=6.6 Hz); 0.99 (d, 3H, *J*=6.6 Hz); 0.94 (d, 3H, *J*=6.2 Hz); 0.90 (d, 6H, *J*=6.4 Hz); 0.86 (d, 3H, *J*=6.4 Hz); 0.85 (d, 3H, *J*=6.4 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ = 17.7, 17.8, 22.0, 22.4, 22.8, 22.9, 23.0, 23.2, 23.3, 23.4, 25.6, 5.7, 25.8, 38.5, 39.0, 39.7, 41.6, 41.8, 42.2, 46.1, 46.2, 46.8, 47.0, 50.6, 52.7, 53.0, 53.4, 53.5, 55.1, 55.3, 56.0, 56.5, 59.3, 127.6, 127.8, 129.4, 129.5, 130.2, 130.4, 130.6, 138.2, 138.4, 171.0, 171.4, 172.0, 172.1, 172.6, 172.9, 173.1, 173.4, 173.5, 173.8, 174.4, 174.7, 174.8, 175.4. HRMS (ESI-FT-ICR) *m/z*: 874.4331 $[M+Na]^+$, calcd. for $C_{43}H_{61}O_{11}NaN_7$: 874.4327.

Peptide 10: $HCl{\cdot}MeNH_2$ (40 mg, 0.6 mmol), Et_3N (83μL, 0.6 mmol), paraformaldehyde (18 mg, 0.6 mmol), peptide **9** (334 mg, 0.5 mmol) and isonitrile **1** (95.5 mg, 0.5 mmol) were reacted in MeOH/CH_2Cl_2 (5 mL, 5:1, *v*/*v*) for 24 h according to the general Ugi-4CR procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 15:1) afforded peptide **10** (378 mg, 91%) as a pale yellow amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.07 (s, 1H); 7.30-7.07 (m, 14H); 6.79 (d, 1H, *J*=8.6 Hz); 6.76 (d, 1H, *J*=8.8 Hz); 5.73 (m, 1H); 5.07 (m, 1H); 4.94 (m, 1H); 4.69 (t, 1H, *J*=5.8 Hz); 4.42 (m, 1H); 4.37 (d, 1H, *J*=15.8 Hz); 4.08 (d, 1H, *J*=15.8 Hz); 3.38 (s, 3H); 3.37 (s, 3H); 3.24 (s, 3H); 3.21-3.19 (m, 2H); 3.03 (m, 1H); 3.01 (m, 1H); 2.90 (m, 2H); 2.38 (m, 2H); 2.19 (m, 2H); 1.41 (s, 9H); 1.39 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 26.9, 28.2, 28.4, 30.6, 35.3, 38.3, 38.5, 40.8, 51.6, 53.2, 53.6, 55.2, 55.9, 80.8, 81.3, 104.5, 122.3, 126.6, 127.7, 126.8, 127.0, 128.5, 128.6, 129.0, 129.1, 129.3, 130.8,

131.3, 132.8, 136.1, 136.3, 136.4, 157.9, 167.0, 170.7, 171.9, 172.1, 173.0. HRMS (ESI-FT-ICR) *m/z*: 854.4321 $[M+Na]^+$, calcd. for $C_{45}H_{61}O_{10}NaN_5$:854.4316.

Peptide 12: Peptide **10** (249 mg, 0.3 mmol) was subjected to the general procedure for the simultaneous Boc/*t*Bu removal and *C*-terminal activation, followed by the acetylation procedure to yield quantitatively the *N*-peptidoacyl indole **11**, which was used forward without previous purification. *C*-activated peptide **11** (163 mg, 0.25 mmol) was reacted with the trifluoroacetate salt of tetrapeptide H-Leu-Ala-Phe-Ile-NH_2 (144 mg, 0.25 mmol) for 30 minutes in the presence of *i*Pr_2EtN according to the general ligation procedure to afford peptide **12** (239 mg) as a pale yellow amorphous solid. RP-HPLC analysis of the crude product showed 74% of conversion. An analytical sample was purified by RP-HPLC to >95% purity for ESI-MS characterization. R_t = 20.7 min. HRMS (ESI-FT-ICR) *m/z*: 996.5190 $[M-H]^-$, calcd. for $C_{52}H_{70}O_{11}N_9$: 996.5200.

General peptide coupling procedure. The Boc-protected L-aminoacid (1.0 mmol, 1.0 equiv.), HOBt (168 mg, 1.1 mmol, 1.1 equiv.), EDC (210 mg, 1.1 mmol, 1.1 equiv.) and the L-aminoacid methyl ester hydrochloride are suspended in dry CH_2Cl_2 (15 mL). Et_3N (0.15 mL, 1.1 mmol, 1.1 equiv.) is syringed in one portion and the resulting solution is stirred at room temperature overnight (~12 h). The reaction mixture is then diluted with 100 mL EtOAc, transferred to a separatory funnel and sequentially washed with 0.5 M aqueous solution of citric acid (2×50 mL) and saturated aqueous suspension $NaHCO_3$ (2×50 mL). The organic phase is dried over $MgSO_4$, filtered and concentrated under reduced pressure.

General Boc removal procedure: The peptide is dissolved in a 4 M HCl solution in dioxane (2 mL) and the solution is stirred at room temperature. As the material dissolved, gas evolution could be detected and the pressure that built up inside the reaction flask is regularly relieved by opening the reaction flask. After 30 min, usually no starting material is detected by thin layer chromatography and the reaction is concentrated under a stream of dry N_2. The volatiles are then fully removed by

concentrating the resulting thick oily residue under reduced pressure in the rotary evaporator and then placing the flask under high vacuum for 2 h. The resulting salt was used forward assuming quantitative yield.

Peptide 13: *N*-Boc-Val-OH (217 mg, 1.0 mmol) was coupled to HCl·Leu-OBzl (257 mg, 1.0 mmol) according to the peptide coupling procedure, following by deprotection of the *N*-terminus by Boc removal. The same protocol was employed for the coupling of *N*-Asp(Me)-OH (247 mg, 1.0 mmol). Flash column chromatography purification (CH_2Cl_2/MeOH 15:1) furnished peptide Boc-Asp(OMe)-Val-Leu-OBzl (433 mg, 79%) as a white amorphous solid. This latter tripeptide (412 mg, 0.75 mmol) was subjected to deprotection of the *N*-terminus by the Boc removal procedure. The resulting rude peptide is dissolved in 10 mL of CH_2Cl_2 and treated with *i*Pr_2EtN (0.52 mL, 3 mmol) and BzCl (0.28 μL, 3 mmol). The reaction mixture was stirred at room temperature for 8 h and then the volatiles evaporated under reduced pressure. The crude product was dissolved in EtOAc (25 mL) and washed with aq. 10% HCl (2×10 mL) and brine (2×10 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford the *N*-benzoyl peptide. The resulting product was dissolved in MeOH (30 mL) and 10% Pd/C (80 mg) was added. The mixture was subjected successively to hydrogen atmosphere and vacuum and finally stirred under hydrogen atmosphere for 24 h. The catalyst was removed by filtration over a pad of Celite and the filtrate was evaporated under reduced pressure. Flash column chromatography purification (CH_2Cl_2/MeOH 15:1) furnished peptide **13** (268 mg, 77%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.95 (d, 1H, *J* = 8.1 Hz), 7.82 (m, 2H), 7.56 (m, 1H), 7.53 – 7.47 (m, 1H), 7.41 (m, 2H), 7.17 (d, 1H, *J* = 8.1 Hz), 5.09 (m, 1H), 4.61 – 4.50 (m, 1H), 4.31 (m, 1H), 3.66 (s, 3H), 2.94 (d, 2H, *J* = 6.1 Hz), 2.24 – 2.11 (m, 1H), 1.66 (m, 3H), 0.90 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 17.9, 19.2, 21.8, 23.0, 25.0, 30.5, 35.6, 40.8,

50.2, 51.2, 52.3, 59.3, 125.6, 127.5, 128.8, 132.2, 133.3, 167.8, 171.3, 171.6, 172.5, 175.6. HRMS (ESI-FT-ICR) *m/z*: 462.2245 $[M-H]^-$, calcd. for $C_{23}H_{32}O_7N_3$: 462.2246.

Peptide 14: $HCl{\cdot}MeNH_2$ (40 mg, 0.6 mmol), Et_3N (83µL, 0.6 mmol), paraformaldehyde (18 mg, 0.6 mmol), peptide **13** (232 mg, 0.5 mmol) and isonitrile **2** (87 mg, 0.5 mmol) were reacted in $MeOH/CH_2Cl_2$ (5 mL, 5:1, *v*/*v*) for 24 h according to the general Ugi-4CR procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 18:1) afforded peptide **14** (292 mg, 86%) as a white amorphous solid. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.83 (m, 2H); 7.58-7.51 (m, 1H); 7.46 (m, 2H); 5.04 (t, 1H, *J*=7.1 Hz); 4.87 (m, 1H); 4.52 (m, 1H); 4.28 (m, 1H); 4.11-3.97 (m, 2H), 3.69 (s, 3H); 3.45-3.39 (m, 1H); 3.37 (s, 3H); 3.34-3.30 (m, 6H); 3.27-3.14 (m, 3H); 3.02 (m, 1H); 2.92 (s, 1H); 2.86 (dd, 1H, *J*=16.6/7.7 Hz); 2.11 (m, 1H); 1.79-1.62 (m, 4H); 1.55 (m, 1H); 1.00-0.86 (m, 12H). ^{13}C NMR (100 MHz, CD_3OD): δ = 18.3, 19.8, 22.0, 23.7, 25.8, 32.1, 36.3, 36.7, 37.3, 41.3, 42.7, 51.7, 52.3, 52.5, 53.5, 53.7, 57.6, 57.7, 59.7, 77.6, 103.5, 128.5, 129.6, 133.0, 135.0, 170.2, 170.8, 172.8, 173.1, 173.3, 174.7. HRMS (ESI) *m/z*: 678.3715 $[M-H]^-$, calcd. for $C_{33}H_{52}O_{10}N_5$: 678.3720.

Peptide 16: Peptide **14** (204 mg, 0.3 mmol) was dissolved in THF/H_2O (2:1, 15 mL) and LiOH (50 mg, 1.2 mmol) is added at 0 °C. The mixture was stirred at 0 °C for 3 h and then acidified with aqueous 10% $NaHSO_4$ to pH 3. The resulting phases were separated and the aqueous phase was additionally extracted with EtOAc (2×20 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting crude was subjected to the general procedure for the *C*-terminal activation to yield quantitatively the *N*-peptidoacyl pyrrole **15**, which was used forward without previous purification. *C*-activated peptide **15** (146 mg, 0.25 mmol) was reacted with the trifluoroacetate salt of tetrapeptide H-Phe-Val-Glu-Ala-OH (145 mg, 0.25 mmol) for 45 minutes in the presence of *i*Pr_2EtN according to the general ligation procedure to afford peptide **16** (212 mg) as a white amorphous solid. RP-HPLC analysis of the crude product showed 67% purity. A sample was purified by RP-HPLC

to >95% purity for characterization. R_t = 9.72 min. ^{1}H NMR (600 MHz, CD_3OD): δ = 7.89 – 7.80 (m, 2H), 7.52 (m, 1H), 7.48 – 7.40 (m, 2H), 7.30 – 7.14 (m, 5H), 5.00 (t, 1H, *J* = 7.3 Hz), 4.77 – 4.69 (m, 1H), 4.65 (m, 1H), 4.49 – 4.39 (m, 1H), 4.32 (m, 1H), 4.27 (m, 1H), 4.24 – 4.15 (m, 1H), 4.08 (m, 1H), 4.04 – 3.89 (m, 2H), 3.21 – 3.15 (m, 1H), 3.11 – 3.03 (m, 2H), 3.01 – 2.88 (m, 3H), 2.86 – 2.79 (m, 1H), 2.58 – 2.47 (m, 1H), 2.46 – 2.36 (m, 2H), 2.15 – 2.02 (m, 3H), 1.98 – 1.89 (m, 1H), 1.71 – 1.59 (m, 2H), 1.59 – 1.48 (m, 1H), 1.45 – 1.40 (m, 1H), 1.40 – 1.34 (m, 3H), 1.30 (dd, 1H, *J* = 14.1, 6.6 Hz), 1.02 – 0.75 (m, 18H). ^{13}C NMR (151 MHz, CD_3OD): δ = 18.9, 19.8, 19.9, 22.1, 23.6, 25.8, 28.5, 30.2, 31.2, 36.3, 37.3, 38.7, 41.5, 52.1, 53.8, 56.1, 57.5, 57.6, 59.2, 60.2, 61.0, 127.7, 128.4, 128.5, 128.6, 129.3, 129.5, 129.6, 130.3, 130.4, 131.2, 134.1, 138.5, 166.7, 168.4, 171.4, 172.0, 172.2, 172.6, 173.2, 173.5, 174.5, 175.3, 180.4. HRMS (ESI) *m/z*: 965.4619 $[M-H]^-$, calcd. for $C_{47}H_{65}O_{14}N_8$: 965.4626.

Peptide 19: Glucosyl amine **18** (208 mg, 0.6 mmol), paraformaldehyde (18 mg, 0.6 mmol), peptide **17** (257 mg, 0.5 mmol) and isonitrile **1** (95.5 mg, 0.5 mmol) were reacted in MeOH/CH_2Cl_2 (10 mL, 5:1, *v*/*v*) for 36 h according to the general Ugi-4CR procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 12:1) afforded glycopeptide **19** (383 mg, 72%) as a pale yellow amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.12 (s, 1H); 7.62 (d, 1H, *J*=7.2 Hz); 7.48 (m, 1H); 7.24-7-08 (m, 4H); 5.62 (d, 1H, *J*=8.2 Hz); 5.50 (d, 1H, *J*=8.2 Hz); 5.39 (t, 1H, *J*=9.4 Hz); 5.00 (m, 1H); 4.87 (m, 1H); 4.75-4.64 (m, 3H); 4.58 (t, 1H, *J*=5.6 Hz); 4.44-4.40 (m, 1H); 4.36-4.30 (m, 3H); 4.14 (m, 1H); 4.06 (d, 1H, *J*=15.8 Hz); 3.87 (m, 1H); 3.39 (s, 3H); 3.37 (s, 3H); 2.90 (m, 2H); 2.01, 2.04, 2.05, 2.08 (4×s, 4×3H); 1.81 (m, 1H); 1.73-1.62 (m, 2H); 1.56-1.46 (m, 2H); 1.43 (s, 9H); 1.36 (d, 3H, *J*=7.0 Hz); 0.96 (d, 3H, *J*=6.0 Hz); 0.93-0.86 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 15.9, 17.4, 18.9, 20.7, 21.3, 21.4, 22.2, 22.7, 22.8, 24.6, 24.9, 28.3, 28.4, 30.5, 37.5, 40.0, 41.2, 43.5, 48.6, 51.4, 51.9, 52.5, 53.6, 57.2, 59.3, 62.8, 68.6, 69.7, 73.2, 79.7, 84.4, 104.5, 122.3, 126.9, 129.3, 130.5, 131.1, 136.1, 158.3,

1710.2, 171.4, 171.7, 171.9, 172.3, 172.5, 172.8. HRMS (ESI-FT-ICR) *m/z*: 1086.5590 $[M+Na]^+$, calcd. for $C_{51}H_{81}O_{17}NaN_7$: 1086.5587.

Peptide **21:** Peptide **19** (320 mg, 0.3 mmol) was subjected to the general procedure for the simultaneous Boc/*t*Bu removal and *C*-terminal activation, followed by the acetylation procedure to yield quantitatively the *N*-peptidoacyl indole **20**, which was used forward without previous purification. *C*-activated peptide **20** (235 mg, 0.25 mmol) was reacted with the hydrochloride salt of tetrapeptide H-Phe-Lys(Cbz)-Gly-Phe-OMe (170 mg, 0.25 mmol) for 30 minutes in the presence of iPr$_2$EtN according to the general ligation procedure to afford peptide **21** (257 mg) as a pale yellow amorphous solid. RP-HPLC analysis of the crude product showed 56% purity. An analytical sample was purified by RP-HPLC to >95% purity for ESI-MS characterization. R_t = 17.3 min. HRMS (ESI-FT-ICR) *m/z*: 1492.7234 $[M+Na]^+$, calcd. for $C_{73}H_{103}O_{21}N_{11}Na$: 1492.7228.

Bz-Ala-Leu-Phe-Val-Glu-Ala-OMe (22): *N*-Boc-Glu(OBzl)-OH (337 mg, 1.0 mmol) was coupled to HCl·Ala-OMe (139 mg, 1.0 mmol) according to the peptide coupling procedure, following by deprotection of the *N*-terminus by Boc removal. The same protocol was employed for the sequential coupling of *N*-Boc-Val-OH (217 mg, 1.0 mmol) and *N*-Boc-Phe-OH (265 mg, 1.0 mmol). Flash column chromatography purification (CH_2Cl_2/MeOH 15:1) furnished peptide Boc-Phe-Val-Glu(OBzl)-Ala-OMe (411 mg, 72%) as a white amorphous solid. This latter tetrapeptide (400 mg, 0.6 mmol) was subjected to deprotection of the *N*-terminus by the Boc removal procedure, followed by sequential coupling of *N*-Boc-Leu-OH (139 mg, 0.6 mmol) and *N*-Boc-Ala-OH (113 mg, 0.6 mmol). Flash column chromatography purification (CH_2Cl_2/MeOH 12:1) furnished hexapeptide Boc-Ala-Leu-Phe-Val-Glu(OBzl)-Ala-OMe (414 mg). This latter peptide was subjected to *N*-terminal deprotection by the Boc removal procedure, followed by benzoylation of the *N*-terminus and removal of the benzyl protecting group of the Glu side chain according to the procedures described for peptide **13**. Flash column

chromatography purification (CH_2Cl_2/MeOH 12:1) furnished the hexapeptide **22** (264 mg, 71%) as a white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.54 – 8.40 (m, 2H), 8.35 – 8.21 (m, 1H), 8.08 – 7.99 (m, 1H), 7.98 – 7.90 (m, 1H), 7.90 – 7.82 (m, 2H), 7.52 (m, 1H), 7.45 (m, 2H), 7.26 – 7.07 (m, 5H), 6.39 (m, 2H), 4.61 – 4.51 (m, 1H), 4.45 (m, 1H), 4.36 – 4.29 (m, 1H), 4.25 (m, 2H), 4.15 (m, 1H), 3.96 – 3.84 (m, 2H), 3.63 – 3.56 (m, 3H), 3.45 (s, 3H), 3.26 (s, 1H), 3.19 (m, 2H), 3.04 (dd, 1H, *J* = 14.0, 4.0 Hz), 2.95 (m, 2H), 2.83 – 2.74 (m, 2H), 2.45 – 2.40 (m, 2H), 2.39 – 2.32 (m, 1H), 2.01 – 1.91 (m, 1H), 1.84 (m, 4H), 1.66 (p, 1H, *J* = 5.4 Hz), 1.55 (m, 3H), 1.37 (t, 1H, *J* = 7.3 Hz), 1.27 (m, 6H), 1.21 – 1.13 (m, 1H), 0.76 (m, 9H), 0.71 (dd, 3H, *J* = 6.5, 3.6 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 16.7, 17.6, 18.1, 19.1, 21.5, 21.7, 22.9, 23.0, 24.7, 28.4, 29.2, 36.1, 36.2, 36.5, 37.1, 41.2, 44.9, 47.51, 48.5, 48.9, 51.8, 52.8, 53.3, 57.8, 99.2, 102.0, 126.1, 127.4, 127.5, 127.9, 128.2, 129.1, 129.3, 131.3, 134.0, 137.6, 137.7, 137.9, 144.6, 145.2, 166.0, 166.2, 168.4, 171.1, 171.0, 172.1, 172.2, 172.9. HRMS (ESI-FT-ICR) *m/z*: 765.3823 $[M-H]^-$, calcd. for $C_{39}H_{53}O_{10}N_6$: 765.3829.

Peptide 23: HCl·$MeNH_2$ (40 mg, 0.6 mmol), Et_3N (83μL, 0.6 mmol), paraformaldehyde (18 mg, 0.6 mmol), peptide **22** (383 mg, 0.5 mmol) and isonitrile **2** (87 mg, 0.5 mmol) were reacted in MeOH/CH_2Cl_2 (8 mL, 5:1, *v*/*v*) for 24 h according to the general Ugi-4CR procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 15:1) afforded peptide **23** (452 mg, 92%) as a white amorphous solid. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.88 (d, 2H, *J*=7.5 Hz); 7.59-7.50 (m, 1H); 7.46 (m, 2H); 7.24-7.10 (m, 5H); 6.92 (s, 1H); 4.68 (dd, 1H, *J*=9.9/4.8 Hz); 4.55-4.46 (m, 2H); 4.45-4.35 (m, 2H); 4.34-4.26 (m, 1H); 4.20 (dd, 1H, *J*=11.7/7.6 Hz); 4.16-4.06 (m, 1H); 4.02 (m, 1H); 3.70 (s, 3H); 3.39 (m, 1H); 3.38-3.34 (m, 3H); 3.31 (m, 9H); 3.20 (dd, 1H, *J* = 13.4/4.7 Hz); 3.05 (m, 2H); 3.02-2.94 (m, 1H); 2.93-2.89 (m, 1H); 2.58 (m, 1H); 2.48 (m, 1H); 2.15-2.07 (m, 2H); 2.01-1.92 (m, 1H); 1.72 (m, 2H); 1.60 (m, 1H); 1.45 (m, 4H); 1.40 (m, 6H); 0.98-0.79 (m, 12H). ^{13}C NMR (101 MHz, CD_3OD): δ = 17.3, 17.7, 19.8, 22.1, 23.4, 25.8, 28.7, 28.0, 30.9, 31.9, 35.4, 36.8, 37.4, 38.4, 42.8, 51.7, 52.2, 52.7,

53.1, 53.6, 53.7, 55.9, 56.5, 57.5, 57.7, 60.4, 77.7, 103.6, 126.1, 127.7, 128.6, 129.4, 129.5, 130.2, 130.3, 132.9, 135.0, 138.4, 138.6, 139.2, 170.1, 170.4, 171.3, 173.3, 173.7, 174.5, 175.2, 175.3, 175.5. HRMS (ESI) *m/z*: 981.5287 $[M-H]^-$, calcd. for $C_{49}H_{73}O_{13}N_8$: 981.5303.

Peptide 26: Peptide **23** (392 mg, 0.4 mmol) was subjected to the general procedure for the *C*-terminal activation to yield quantitatively the *N*-peptidoacyl pyrrole **24**, which was used forward without previous purification. *C*-activated peptide **24** (133 mg, 0.15 mmol) was reacted with the NBD-derived fluorescent amine **25** (48 mg, 0.20 mmol) for 30 minutes in the presence of *i*Pr_2EtN according to the general ligation procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 15:1) afforded pure peptide **26** (94 mg, 59%) as a white amorphous solid. R_t = 11.7 min. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.54-8.40 (m, 2H); 8.35-8.21 (m, 1H); 8.08-7.99 (m, 1H); 7.98-7.90 (m, 1H); 7.90-7.82 (m, 2H); 7.52 (m, 1H); 7.45 (m, 2H); 7.26-7.07 (m, 5H); 6.39 (m, 2H); 4.61-4.51 (m, 1H); 4.45 (m, 1H); 4.36-4.29 (m, 1H); 4.25 (m, 2H); 4.15 (m, 1H); 3.96-3.84 (m, 2H); 3.63-3.56 (m, 3H); 3.45 (s, 3H); 3.26 (s, 1H); 3.19 (m, 2H); 3.04 (dd, 1H, *J*=14.0/ 4.0 Hz); 2.95 (m, 2H); 2.83-2.74 (m, 2H); 2.45-2.40 (m, 2H); 2.39-2.32 (m, 1H); 2.01-1.91 (m, 1H); 1.84 (m, 4H); 1.66 (d, 1H, *J*=5.4 Hz); 1.55 (m, 3H); 1.37 (t, 1H, *J*=7.3 Hz); 1.27 (m, 6H); 1.21-1.13 (m, 1H); 0.76 (m, 9H); 0.71 (m, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 16.7, 17.6, 18.1, 19.1, 21.5, 21.7, 22.9, 23.0, 24.1, 28.4, 29.2, 36.1, 36.2, 36.5, 37.1, 41.2, 45.0, 47.5, 48.5, 48.9, 51.9, 52.8, 53.3, 57.8, 99.2, 102.0, 126.1, 127.4, 127.5, 127.9, 128.2, 129.1, 129.3, 131.3, 134.0, 137.6, 137.7, 137.90, 144.6, 145.2, 166.0, 166.2, 168.4, 171.0, 171.0, 172.1, 172.2, 172.9, 174.9. HRMS (ESI) *m/z*: 1055.4952 $[M-H]^-$, calcd. for $C_{51}H_{67}O_{13}N_{12}$: 1055.4956.

Peptide 27: *N*-peptidoacyl pyrrole **24** (133 mg, 0.15 mmol), obtained as describe above, was reacted with *n*-dodecylamine (100 mg, 0.20 mmol) for 30 minutes in the presence of *i*Pr_2EtN according to the general ligation procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 20:1) afforded pure peptide **27** (102 mg, 67%) as a white amorphous solid. R_t = 16.0 min. H NMR (400 MHz, DMSO-

d_6): δ = 8.07-7.94 (m, 1H); 7.93-7.84 (m, 2H); 7.56-7.50 (m, 1H); 7.49-7.40 (m, 2H); 7.29-7.09 (m, 5H); 4.57 (m, 1H); 4.47 (m, 1H); 4.31 (m, 1H); 4.25 (m, 2H); 4.20-4.08 (m, 1H); 3.93-3.80 (m, 2H); 3.63-3.56 (m, 3H); 3.35 (m, 6H); 3.07-2.99 (m, 3H); 2.95-2.90 (m, 2H); 2.79-2.72 (m, 2H); 2.42-2.32 (m, 1H); 2.07 (s, 1H); 2.03-1.92 (m, 1H); 1.88 (m, 1H); 1.78 (m, 1H); 1.60-1.49 (m, 1H); 1.41-1.33 (m, 2H); 1.30 (m, 2H); 1.28 (m, 2H); 1.27-1.25 (m, 2H); 1.25-1.20 (m, 18H); 0.87-0.69 (m, 15H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 14.0, 16.8, 17.0, 17.6, 18.1, 18.2, 19.2, 21.6, 21.7, 22.1, 22.9, 23.0, 24.1, 26.4, 27.7, 28.7, 29.0, 30.4, 31.3, 34.0, 34.1, 36.1, 37.1, 38.4, 41.0, 47.5, 49.1, 50.2, 50.7, 51.1, 51.9, 53.7, 57.8, 126.1, 126.2, 127.4, 127.5, 127.9, 127.9, 128.2, 129.2, 129.3, 131.3, 134.0, 138.0, 166.0, 167.6, 167.9, 170.6, 171.0, 171.9, 172.1, 172.7, 173.0. HRMS (ESI) *m/z*: 1003.6232 $[M-H]^-$, calcd. for $C_{54}H_{83}O_{10}N_9$: 1003.6238.

General macrocyclization procedure: The *C*-activated peptide (0.25 mmol, 1 equiv.) is dissolved in DMF (50 mL) and treated with *i*Pr_2EtN (0.17 mL, 1 mmol, 4 equiv.). The reaction mixture is stirred for 48 h at 70 °C and then concentrated under reduced pressure. The reaction product is taken up in *ca.* 20-30 mL of MeOH and filtered through a pad of silica gel C18 to partially remove the indole or pyrrole derivatives and the resulting solution is concentrated to dryness. The resulting crude cyclic peptide is purified by flash column chromatography (CH_2Cl_2/ MeOH).

Depsipeptide 29: Peptide **28** (222 mg, 0.3 mmol) was dissolved in 5 mL of THF a 10 mL glass tube and treated with 0.1 mL of 40% aqueous formaldehyde and isonitrile **1** (57 mg, 0.3 mmol). The flask was irradiated for 30 min (300 W) under high-speed magnetic stirring 60 °C and then the volatiles were evaporated under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH 20:1) to furnish depsipeptide **29** (256 mg, 89%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.18 (s, 1H); 7.74 (d, 1H, *J*=7.8 Hz); 7.29 (d, 1H, *J*=7.2 Hz); 7.34-7.18 (m, 10H); 7.25-7.20 (m, 2H); 7.14-7.08 (m, 2H); 6.84 (d, 1H, *J*=8.2 Hz); 5.60 (m, 1H); 5.15 (m, 1H); 5.08

(s, 2H); 4.85 (d, 1H, *J*=15.4 Hz); 4.67 (d, 1H, *J*=15.4 Hz); 4.61 (m, 1H); 4.43 (m, 2H); 4.22 (m, 1H); 3.40 (s, 3H); 3.38 (s, 3H, C*H*$_3$O); 3.24-3.14 (m, 2H); 3.04 (dd, 1H, *J*=14.0/5.2 Hz); 2.96-2.88 (m, 3H); 2.14 (m, 1H); 1.96 (m, 2H); 1.85 (m, 1H); 1.56-1.44 (m, 4H); 1.37 (s, 9H); 0.93-0.81 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.3, 15.4, 17.4, 19.2, 23.4, 22.7, 24.6, 28.1, 29.2, 30.2, 30.9, 36.2, 36.7, 37.9, 40.4, 42.1, 52.3, 54.8, 55.3, 56.0, 58.0, 59.0, 63.6, 66.4, 80.7, 107.0, 124.8, 125.6, 127.0, 127.5, 127.9, 128.4, 128.7, 129.1, 131.0, 135.5, 135.9, 136.6, 155.9, 156.9 165.4, 170.4, 170.9, 171.5, 172.1. HRMS (ESI-FT-ICR) *m/z*: 983.5105 $[M+Na]^+$, calcd. for $C_{51}H_{72}O_{12}NaN_6$: 983.5106.

Cyclic Depsipeptide 30: Depsipeptide **29** (240 mg, 0.25 mmol) was subjected to the procedure for the simultaneous Boc/*t*Bu removal and *C*-terminal activation to yield quantitatively the intermediate *N*-peptidoacyl indole, which was next submitted to the general macrocyclization procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 18:1) afforded the pure cyclic depsipeptide **30** (98 mg, 57%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.21 (d, 1H, *J*=7.8 Hz); 7.64 (d, 1H, *J*=7.4 Hz); 7.59 (d, 1H, *J*=7.0 Hz); 7.51 (d, 1H, *J*=7.1 Hz); 7.35-7.31 (m, 5H); 5.11 (s, 2H); 4.92 (d, 1H, *J*=15.8 Hz); 4.72 (d, 1H, *J*=15.7 Hz); 4.50 (m, 1H); 4.37 (m, 1H); 4.28 (m, 2H); 3.18-3.13 (m, 3H); 3.08 (dd, 1H, *J*=14.0/5.2 Hz); 2.09 (m, 1H); 1.86 (m, 2H); 1.72 (m, 1H); 1.52-1.49 (m, 4H); 0.93-0.84 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.1, 15.3, 17.6, 19.0, 24.2, 29.4, 30.3, 31.4, 36.5, 36.7, 39.6, 40.8, 53.4, 56.6, 58.7, 59.9, 63.4, 66.5, 126.9, 127.7, 127.9, 128.1, 128.5, 128.8, 129.1, 129.3, 136.3, 136.7, 157.8, 169.8, 170.9, 171.5, 172.2, 172.8. HRMS (ESI-FT-ICR) *m/z*: 702.3477 $[M+Na]^+$, calcd. for $C_{36}H_{49}O_8NaN_5$: 702.3479.

Peptide 32: $HCl{\cdot}MeNH_2$ (20 mg, 0.3 mmol), Et_3N (44μL, 0.3 mmol), paraformaldehyde (9 mg, 0.3 mmol), peptide **31** (137 mg, 0.25 mmol) and isonitrile **1** (48 mg, 0.25 mmol) were reacted in MeOH/CH_2Cl_2 (5 mL, 5:1, *v*/*v*) for 24 h according to the general Ugi-4CR procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 15:1) afforded peptide **32** (180 mg, 92%) as a pale yellow

amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.91 (s, 1H); 7.31 (m, 1H); 7.27 (m, 1H); 7.26-7.07 (m, 9H); 6.96 (m, 1H); 5.78 (m, 1H); 5.24 (m, 1H); 4.63 (t, 1H, *J*=5.6 Hz); 4.59-4.52 (m, 4H); 4.36 (m, 1H, *J*=15.8 Hz); 4.08 (m, 1H, *J*=15.9 Hz); 3.39 (s, 3H); 3.37 (s, 3H); 3.25 (s, 3H); 3.08 (dd, 1H, *J*=13.9/5.0 Hz); 3.08 (dd, 1H *J*=13.9/5.0 Hz); 2.96 (dd, 1H *J*=13.9/8.0 Hz); 2.90 (m, 2H); 1.81-1.74 (m, 1H); 1.66-1.60 (m, 1H); 1.55-1.42 (m, 2H); 1.38 (s, 9H); 1.37 (d, 3H, *J*=7.1 Hz); 0.96 (d, 3H, *J*=6.4 Hz); 0.94 (d, 3H, *J*=6.4 Hz); 0.87 (d, 6H, *J*=6.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 17.9, 18.8, 19.0, 21.9, 22.1, 25.1, 28.2, 38.3, 40.4, 40.7, 41.3, 47.6, 51.4, 51.7, 51.9, 55.3, 55.7, 80.4, 104.5, 122.3, 126.6, 126.9, 128.6, 129.2, 129.3, 130.5, 131.2, 136.2, 136.6, 155.8, 166.7, 171.5, 170.8, 171.9, 172.9. HRMS (ESI-FT-ICR) *m/z*: 805.4472 $[M+Na]^+$, calcd. for $C_{41}H_{62}O_9NaN_6$: 805.4476.

Cyclic peptide 33: Peptide **32** (156 mg, 0.2 mmol) was subjected to the procedure for the simultaneous Boc/*t*Bu removal and *C*-terminal activation to yield quantitatively the intermediate *N*-peptidoacyl indole, which was next submitted to the general macrocyclization procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 16:1) afforded the pure cyclic peptide **33** (101 mg, 61%) as a white amorphous solid. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.28-7.21 (m, 5H); 4.84 (dd, 1H, *J*=9.9/4.6 Hz); 4.55 (dd, 1H, *J*=11.0/3.8 Hz); 4.45 (q, 1H, *J*=6.9 Hz); 4.38 (d, 1H, *J*=14.1 Hz); 3.44 (d, 1H, *J*=10.1 Hz); 3.34 (m, 1H); 3.31 (m, 1H); 3.24 (d, 1H, *J*=14.1 Hz); 3.17 (s, 3H); 2.82 (dd, 1H, *J*=11.0/14.1 Hz); 2.51-2.43 (m, 1H); 1.73-1.62 (m, 1H); 1.53-1.46 (m, 2H); 1.39 (d, 3H, *J*=6.9 Hz); 1.01-0.95 (m, 12H). ^{13}C NMR (100 MHz, CD_3OD): δ = 17.0, 20.0, 21.7, 23.6, 26.2, 29.7, 37.8, 38.3, 41.9, 49.3, 51.0, 54.6, 56.6, 66.2, 127.8, 129.5, 129.9, 138.9, 172.1, 173.1, 174.5, 174.5, 175.2. HRMS (ESI-FT-ICR) *m/z*: 783.5354 $[M+H]^+$, calcd. for $C_{40}H_{73}O_{10}N_5$: 783.5357.

Cyclic peptide 36: $HCl{\cdot}MeNH_2$ (20 mg, 0.3 mmol), Et_3N (44μL, 0.3 mmol), paraformaldehyde (9 mg, 0.3 mmol), peptide **34** (165 mg, 0.25 mmol) and isonitrile **1** (48 mg, 0.25 mmol) were reacted in MeOH/CH_2Cl_2 (5 mL, 5:1, *v*/*v*) for 24 h according to the general Ugi-4CR procedure to afford

quantitatively peptide **35**. This latter peptide was subjected without further purification to the procedure for the simultaneous Boc removal and *C*-terminal activation to yield quantitatively the intermediate *N*-peptidoacyl indole, which was next submitted to the general macrocyclization procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 18:1) afforded the pure cyclic peptide **36** (106 mg, 69%) as a white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.54 (d, 1H, *J*=7.2 Hz); 7.27 (br. m, 5H); 4.84-4.74 (m, 4H); 4.62-4.58 (m, 2H); 4.46 (m, 1H); 4.14 (m, 1H); 4.04 (d, 1H, *J*=5.7 Hz); 3.97 (d, 1H, *J*=7.2 Hz); 3.42 (d, 1H, *J*=15.2 Hz); 3.40 (dd, 1H, *J*=14.0/4.4 Hz); 3.18 (s, 3H); 3.13 (m, 1H); 2.27 (m, 1H); 1.85-1.79 (m, 2H); 1.63 (m, 4H); 1.48 (d, 3H, *J*=7.2); 1.36-1.28 (m, 4H); 1.01-0.89 (m, 12H); 0.74 (d, 3H, *J*=8.4 Hz); 0.72 (d, 3H, *J*=9.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.0, 15.4, 15.9, 18.3, 19.6, 22.4, 22.9, 24.6, 25.0, 28.5, 29.7, 34.0, 36.3, 38.5, 40.7, 48.3, 54.6, 55.8, 55.9, 56.0, 56.1, 126.9, 128.6, 129.0, 137.0, 170.3, 172.0, 172.8, 173.0, 174.1, 174.3. HRMS (ESI-FT-ICR) *m/z*: 637.3685 $[M+Na]^+$, calcd. for $C_{32}H_{50}O_6NaN_6$: 637.3689.

Cyclic peptide 39: $HCl{\cdot}MeNH_2$ (20 mg, 0.3 mmol), Et_3N (44μL, 0.3 mmol), paraformaldehyde (9 mg, 0.3 mmol), peptide **37** (183 mg, 0.25 mmol) and isonitrile **1** (48 mg, 0.25 mmol) were reacted in MeOH/CH_2Cl_2 (5 mL, 5:1, *v*/*v*) for 24 h according to the general Ugi-4CR procedure to afford quantitatively peptide **38**. This latter peptide was subjected without further purification to the procedure for the simultaneous Boc removal and *C*-terminal activation to yield quantitatively the intermediate *N*-peptidoacyl indole, which was next submitted to the general macrocyclization procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 16:1) afforded the pure cyclic peptide **39** (108 mg, 63%) as a white amorphous solid. Mixture of the *S-cis* and *S-trans* isomers of the *N*-methylated amide bond. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.81, 8.45 (2×d, 1H, *J*=8.8 Hz); 8.41, 8.34 (2×d, 1H, *J*=6.2 Hz); 7.71 (d, 1H, *J*=9.5 Hz); 7.67, 7.47 (2×d, 1H, *J*=5.4 Hz); 7.55, 7.41 (2×br. s, 1H); 7.27 (br. m, 5H); 6.73, 6.60 (2×d, 1H, *J*=7.8 Hz); 4.70 (td, 1H, *J*=10.2/4.5 Hz); 4.60 (m, 1H); 4.54-4.45 (m, 2H); 4.27-4.12 (m, 4H);

3.30 (m, 1H); 3.29 (s, 3H, CH_3N); 3.20 (dd, 1H, *J*=14.0/4.4 Hz); 3.03 (dd, 1H, *J*=14.1/10.0 Hz); 1.91 (m, 2H); 1.69 (m, 2H); 1.62 (m, 1H); 1.42 (d, 3H, *J*=7.3, CH_3); 1.23 (d, 3H, *J*=7.0, CH_3); 1.01-0.84 (m, 18H, 6×CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 18.1, 18.9, 20.9, 21.4, 22.3, 22.9, 23.1, 23.4, 24.6, 24.8, 32.4, 33.9, 36.8, 40.2, 40.2, 49.1, 49.7, 51.4, 52.2, 52.6, 55.8, 57.7, 127.0, 128.5, 128.6, 128.7, 129.0, 137.0, 169.3, 171.3, 171.8, 172.3, 172.6, 173.0, 173.2. HRMS (ESI-FT-ICR) *m/z*: 708.4063 $[M+Na]^+$, calcd. for $C_{35}H_{55}O_7NaN_7$: 708.4060.

Boc-Glu-Leu-Phe-Lys(Cbz)-NHCH$_2$CH$_2$*p*F-C$_6$H$_4$ (40): N^{α}-Boc-Lys(Cbz)-OH (380 mg, 1.0 mmol) was coupled to 2-(4-fluorophenyl)ethan-1-amine (139 mg, 1.0 mmol) according to the peptide coupling procedure, following by deprotection of the *N*-terminus by Boc removal subsequent coupling of *N*-Boc-Phe-OH (265 mg, 1.0 mmol). Flash column chromatography purification (CH_2Cl_2/MeOH 18:1) furnished peptide Boc-Phe-Lys(Cbz)-NHCH$_2$CH$_2$*p*F-C$_6$H$_4$ (544 mg, 84%) as a white amorphous solid. This latter peptide (389 mg, 0.6 mmol) was subjected to deprotection of the *N*-terminus by the Boc removal procedure, followed by sequential coupling of *N*-Boc-Leu-OH (139 mg, 0.6 mmol) and *N*-Boc-Glu(OMe)-OH (157 mg, 0.6 mmol). The resulting cure peptide was dissolved in THF/H_2O (2:1, 10 mL) and LiOH (105 mg, 2.5 mmol) was added at 0 °C. The mixture was stirred at 0 °C for 3 h and then acidified with aquouse 10% $NaHSO_4$ to pH 3. The resulting phases are separated and the aqueous phase is additionally extracted with EtOAc (2×30 mL). The combined organic phases are dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to yield the crude deprotected peptide. Flash column chromatography purification (CH_2Cl_2/MeOH 15:1) furnished the peptide **40** (368 mg, 69%) as a white amorphous solid. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.32 (d, 4H, *J* = 4.4 Hz), 7.28 – 7.16 (m, 8H), 6.98 (t, 2H, *J* = 8.8 Hz), 5.05 (m, 2H), 4.58 (dt, 1H, *J* = 14.4/7.1 Hz), 4.24 (m, 2H), 4.11 – 3.99 (m, 1H), 3.36 (m, 2H), 3.11 (m, 3H), 2.97 (dd, 1H, *J* = 14.1/8.7 Hz), 2.75 (t, 2H, *J* = 7.3 Hz), 2.37 (t, 2H *J* = 7.5 Hz), 1.99 (m, 1H), 1.85 (m, 1H), 1.73 (m, 1H), 1.66 – 1.56 (m, 2H), 1.54 – 1.46 (m, 3H),

1.43 (s, 9H), 1.35 – 1.21 (m, 3H), 0.89 (d, 3H, *J* = 6.6 Hz), 0.85 (d, 3H, *J* = 6.5 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ = 22.1, 23.4, 24.1, 25.7, 28.1, 28.7, 30.4, 31.2, 32.7, 35.6, 38.2, 41.6, 41.9, 53.8, 54.9, 55.5, 56.1, 67.3, 80.8, 115.9, 116.2, 127.9, 128.8, 128.9, 129.4, 129.5, 130.3, 130.4, 131.5, 131.6, 136.3, 138.2, 138.4, 158.0, 158.8, 163.0 (d, 1JC-F = 242.7 Hz), 173.3, 173.9, 174.7, 174.8, 176.5. HRMS (ESI) *m/z*: 889.4509 $[M-H]^-$, calcd. for $[C_{47}H_{62}FN_6O_{10}]^-$ 889.4511.

Peptide 41: $HCl{\cdot}MeNH_2$ (20 mg, 0.3 mmol), Et_3N (44μL, 0.3 mmol), paraformaldehyde (9 mg, 0.3 mmol), peptide **40** (223 mg, 0.25 mmol) and isonitrile **2** (43.5 mg, 0.25 mmol) were reacted in $MeOH/CH_2Cl_2$ (5 mL, 5:1, *v*/*v*) for 24 h according to the general Ugi-4CR procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 15:1) afforded peptide **41** (260 mg, 94%) as a pale yellow amorphous solid. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.32 (d, 4H, *J*=4.2 Hz), 7.29–7.16 (m, 8H), 6.99 (m, 2H), 5.49 (m, 1H), 5.06 (m, 2H), 4.60–4.48 (m, 2H), 4.29–4.16 (m, 2H), 4.14–3.97 (m, 4H), 3.41–3.34 (m, 6H), 3.30 (m, 6H), 3.20–3.06 (m, 4H), 3.02 (m, 2H), 2.98–2.87 (m, 2H), 2.76 (t, 2H, *J*=7.3 Hz), 2.55-2.33 (m, 2H), 2.01 (m, 2H), 1.87 (m, 2H), 1.76-1.69 (m, 2H), 1.61 (m, 2H), 1.54-1.46 (m, 3H), 1.42 (s, 9H), 1.25 (m, 3H), 0.90 (d, 3H, *J*=6.4 Hz), 0.86 (m, 3H). ^{13}C NMR (100 MHz, CD_3OD): δ = 22.0, 23.4, 24.1, 25.7, 28.8, 30.5, 32.7, 35.6, 36.8, 37.3, 38.1, 41.6, 41.9, 42.8, 52.1, 53.6, 53.6, 53.6, 56.2, 57.7, 57.7, 67.3, 77.7, 80.8, 103.6, 116.0, 116.2, 127.8, 128.8, 128.9, 129.5, 129.6, 130.3, 131.5, 131.6, 136.3, 138.4, 138.5, 158.9, 163.0 (d, 1JC-F = 242.7 Hz), 170.8, 171.3, 173.3, 173.9, 174.9, 174.9, 175.2. HRMS (ESI) *m/z*: 1105.5985 $[M-H]^-$, calcd. for $C_{57}H_{82}FO_{13}N_8$: 1105.5991.

Cyclic peptide 42: Peptide **41** (221 mg, 0.2 mmol) was dissolved in toluene (10 mL) and 10-camphorsulfonic acid (4.6 mg, 0.02 mmol) and quinoline (2.6 mg, 0.02 mmol) were added. The reaction mixture was stirred at reflux for 30 min, then diluted with 30 mL of EtOAc and washed with aqueous 10% HCl (15 mL). The aqueous phase was additionally extracted with EtOAc (2×25 mL and the combined organic phases were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure

to yield the *C*-activated peptide. This latter compound was dissolved in EtOH (25 mL) and 10% Pd/C (60 mg) was added. The mixture was subjected successively to hydrogen atmosphere and vacuum and finally stirred under hydrogen atmosphere for 24 h. The catalyst was removed by filtration over a pad of Celite and the filtrate was evaporated under reduced pressure. The resulting *N*-deprotected and *C*-activated peptide was subjected to the general macrocyclization procedure. Flash column chromatography purification (CH_2Cl_2/MeOH 18:1) afforded the pure cyclic peptide **42** (94 mg, 58%) as a white amorphous solid. R_t = 11.9 min. ^{1}H NMR (400 MHz, CD_3OD): δ = 8.02 (m, 1H); 7.90-7.74 (m, 1H); 7.32-7.16 (m, 7H); 7.00 (m, 2H); 4.61-4.46 (m, 1H); 4.38 (m, 1H); 4.29-4.16 (m, 2H); 4.12-4.04 (m, 1H); 4.04-3.95 (m, 1H); 3.94-3.80 (m, 1H); 3.71-3.60 (m, 1H); 3.56-3.40 (m, 1H); 3.40-3.32 (m, 2H); 3.24-3.13 (m, 1H); 3.08 (m, 2H); 3.01 (m, 1H); 2.96-2.89 (m, 1H); 2.83-2.69 (m, 2H); 2.55-2.27 (m, 2H); 2.11-1.94 (m, 2H); 1.91-1.79 (m, 1H); 1.76-1.61 (m, 2H); 1.52 (m, 2H), 1.47-1.37 (m, 11H); 1.34-1.26 (m, 2H); 0.96-0.70 (m, 6H). ^{13}C NMR (100 MHz, CD_3OD): δ = 21.7, 23.5, 25.7, 28.7, 29.6, 30.8, 31.8, 32.1, 35.6, 37.2, 37.7, 40.0, 41.4, 41.9, 52.7, 53.7, 54.8, 56.3, 57.5, 80.7, 116.0, 116.1, 127.9, 129.6, 130.1, 130.3, 131.6, 136.3, 138.4, 157.8, 163.0 (d, 1JC-F = 242.7 Hz), 171.2, 173.5, 173.9, 174.9, 175.2. HRMS (ESI) *m/z*: 808.4416 $[M-H]^-$, calcd. for $C_{42}H_{59}O_8N_7F$: 808.4415.

Acknowledgment. D. G. Rivera is grateful to the Alexander von Humboldt Foundation for an Experienced Researcher Fellowship. We also thank O. Kreye and R. A. W. Neves Filho for experimental assistance on the preparation of the convertible isonitriles and Dr. Andrea Porzel and Dr. Jürgen Schmidt for assistance in the NMR and MS characterization, respectively.

Supporting Information: NMR spectra, ESI-MS and HPLC analysis of final peptides.

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