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A Sequential Ugi Multicomponent/Cu-Catalyzed Azide–Alkyne Cycloaddition Approach for the Continuous Flow Generation of Cyclic Peptoids

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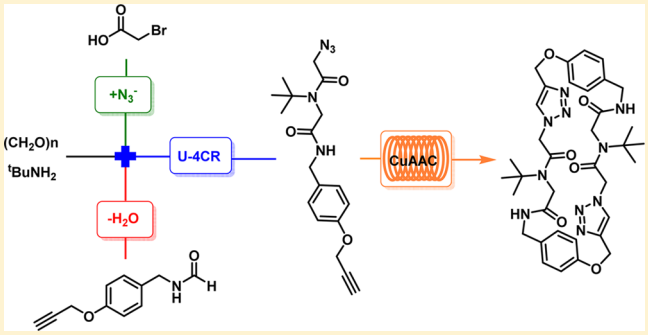
Supporting Information

ABSTRACT: The development of a continuous flow multistep strategy for the synthesis of linear peptoids and their subsequent macrocyclization via Click chemistry is described. The central transformation of this process is an Ugi four-component reaction generating the peptidomimetic core structure. In order to avoid exposure to the often toxic and malodorous isocyanide building blocks, the continuous approach was telescoped by the dehydration of the corresponding formamide. In a concurrent operation, the highly energetic azide moiety required for the subsequent intramolecular copper-catalyzed azide–alkyne cycloaddition (Click reaction) was installed by nucleophilic substitution from a bromide precursor. All steps yielding to the linear core structures can be conveniently coupled without the need for purification steps resulting in a single process generating the desired peptidomimetics in good to excellent yields within a 25 min reaction time. The following macrocyclization was realized in a coil reactor made of copper without any additional additive. A careful process intensification study demonstrated that this transformation occurs quantitatively within 25 min at 140 °C. Depending on the resulting ring strain, either a dimeric or a monomeric form of the cyclic product was obtained.



INTRODUCTION

Proteins and peptides are key building blocks for life and therefore omnipresent in humans, animals, plants, and microorganisms. These classes of biooligomers possess a vast diversity of different functions including catalysis (enzymes), transport and storage of atoms/molecules, communication between tissues and organs (hormones), or defending the body from antigens (antibodies).[1] A significant number of these tasks are regulated by protein–protein interactions. Consequently, abnormalities in these interactions can lead to a variety of diseases.[2] It is therefore apparent that a detailed understanding of the underlying mechanisms of protein–protein interactions is of importance for the development of potential therapies. Unfortunately, naturally occurring peptides are often not ideal candidates for drug discovery due to their low stability against proteolysis and their poor bioavailability. To circumvent these issues, so-called peptidomimetics are often used as non-natural alternatives. These compounds mimic natural occurring proteins having the ability to bind to the same target molecules resulting in identical biological effects.[1,3] Oligomers of *N*-substituted alkyl glycines (alias peptoids) are among the most promising examples to study the biological functions of peptides.[4] Structurally, peptoids are substituted at the amide nitrogen atom instead of the α-carbon like their natural occurring analogs.

Peptoids comprise a vast array of interesting non-natural oligomeric structures, which have a huge variety of biological activities and potential application for drug discovery.[4,5] This feature comes from the ability of this class of poly-*N*-substituted glycine compounds to mimic the primary structure of peptides, and their main advantages include ease of synthesis, long half-lives due to high proteolytic stability, and a much higher bioavailability compared to the natural peptides.[3a,5,6] Furthermore, the presence of tertiary amines lowers the number of intramolecular hydrogen bonds, increasing the membrane permeability by passive diffusion and, consequently, enhancing their pharmacological potential.[7] Analogously to peptides and proteins, peptoids bearing chiral side chains may form secondary structures assuming helicoidal conformations.[8]

The synthesis of peptoid motifs is traditionally carried out using submonomer solid-phase synthesis.[6b,9] A possibly more

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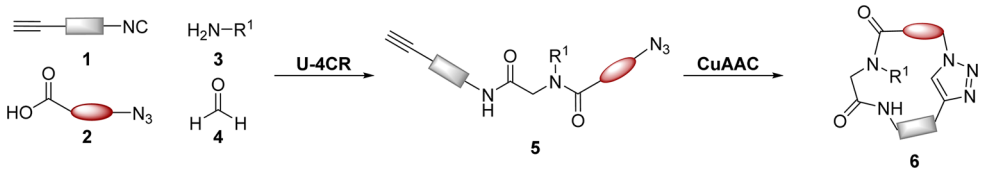
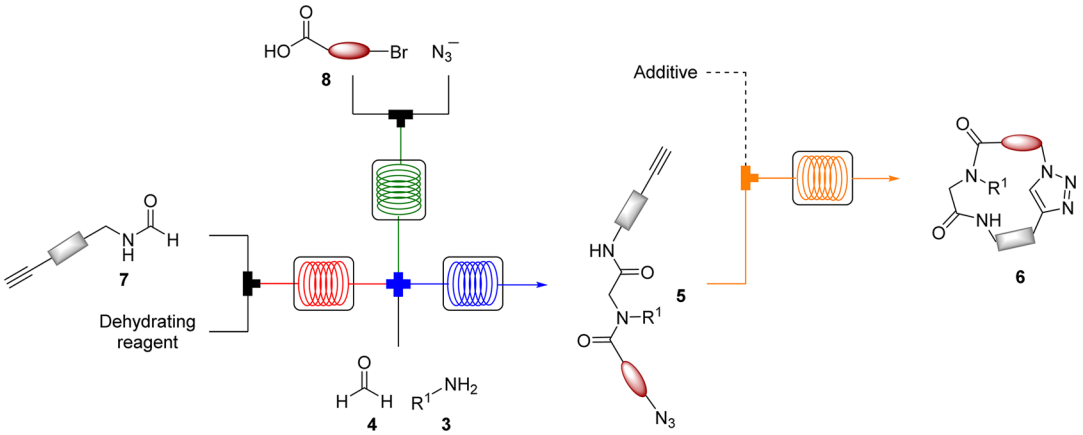
Scheme 1. General Synthetic Strategy for the Sequential Synthesis of Linear and Cyclic Peptidomimetic Scaffolds



Scheme 2. Continuous Strategy for the Modular Synthesis of Linear and Cyclic Peptoids Consisting of Isocyanide Synthesis (red), Azide Preparation (green), Ugi Reaction (blue), and Final CuAAC Macrocyclization (orange)



straightforward alternative for such sequential approaches is isocyanide-based multicomponent reactions (IMCRs).[10] Among these, the Ugi four-component reaction (U-4CR) is one of the most versatile examples for constructing peptoid scaffolds in a highly efficient manner.[11] This generally high yielding reaction has a high functional group tolerance allowing coverage of a broad range of chemical space.[11] It may combine efficient and environmentally benign techniques such as microwaves and continuous flow processing[11d,12] and has an intrinsically efficient ability to assess complex molecules in tandem processes.[11c,g,13]

Despite the advantages associated with the use of peptoids as peptidomimetics, the conformational flexibility and the lack of secondary interactions of linear peptoids decrease their capacity of interaction and molecular recognition with potential pharmaceutical targets.[14] To overcome this limitation, the peptoid backbone cyclization has been employed as a strategy to restrict conformations of linear peptoids, rigidifying their structure and allowing a selective molecular recognition.[15] In fact, the backbone cyclization in several cyclic molecules is considered as the most challenging aspect in the synthesis of this class of compounds.[15b]

Cyclic peptoids are resistant to protease degradation and show high conformational stability.[15b,16] For this reason, an increase in their affinity to the protein domains may occur due to the low entropy loss during the bonding process.[17] Thus, the structural stability may allow the design of compounds that present favorable interactions with the molecular target. In this sense, there has been a considerable interest in the development of this class of compounds, which can be confirmed by the recent increase in the number of publications dealing with the synthesis of cyclic peptoids.[14,18] Additionally, a recent study revealed that cyclic peptoids show complexation properties allowing their effective use in phase transfer catalysis.[19] In fact, the macrocyclization approach has been applied to the synthesis of numerous biologically active peptidomimetics.[20]

The most common ways to obtain a macrocycle from acyclic molecules involve lactonization and lactamization, but other strategies include ring closing metathesis (RCM), cycloadditions (mainly "click" chemistry), and nucleophilic displacement reactions.[3b] More recently, the intramolecular Ugi reaction has proven to be an efficient synthetic methodology to assess these compounds as well.[11c,g,18b] In particular, the multiple multicomponent macrocyclizations, including the bifunctional building blocks strategy (MiB), have proved to be very useful in the construction of macrocyclic diversity.[21]

Another appealing structural motif which is frequently applied in peptidomimetic chemistry is those of 1,2,3-triazoles having similar electronic and structural characteristics as peptides.[22] These heterocyclic scaffolds can be efficiently synthesized using so-called copper-catalyzed azide alkyne cycloadditions (CuAACs).[23] As one of the model reactions within the "Click Chemistry" concept, the CuAAC is an ideal chemical transformation in peptidomimetic chemistry.[24,25] Applications range from ligation of oligomers to foldamer generation, conjugation reactions, and macrocyclizations.[24] In the case of peptoid chemistry, the latter transformation results in cyclic scaffolds often forcing the molecule into a well-defined secondary structure.[25]

When an amide bond of a peptide or peptoid is substituted by a triazole ring, rigidity is introduced in the molecule, which can mimic the amide bond in either its *cis*- or *trans*-like configuration.[26] An interesting example of this feature is the synthesis of a macrocyclic peptidomimetic, which showed significant antibacterial activity against *Staphylococcus aureus*.[20b] Furthermore, a library of 16 stereoisomeric somatostatine analogues embedding a triazole unit were prepared and screened for binding activity with the human somatostatine-receptor subtypes with interesting results.[27]

In order to combine the above-mentioned synthetic strategies, we turned our attention to the development of U-4CR protocols generating linear peptoids of type **5** from an

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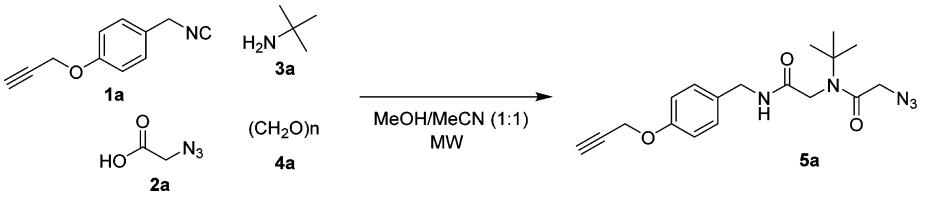
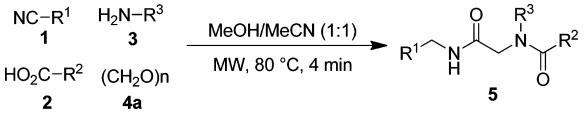
Table 1. Batch Microwave Optimization of the Peptoid Synthesis[a]

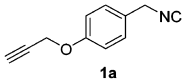
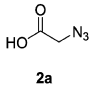
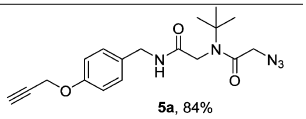
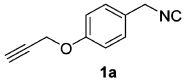
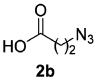
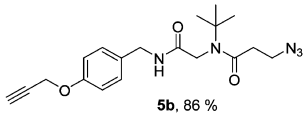
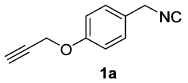
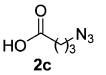
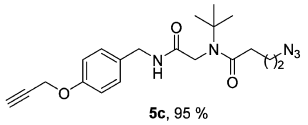
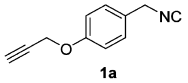
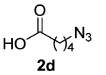
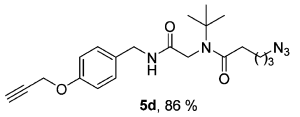
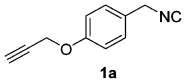
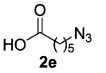
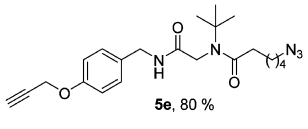
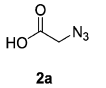
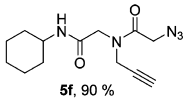
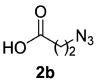
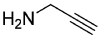
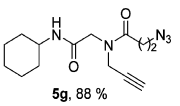
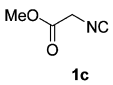
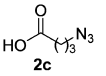
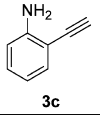
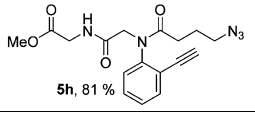


entry	stoichiometry [**1a**:**2a**:**3a**:**4a**]	T [°C]	t [min]	conversion [%][b]
1	1:1:1:1	70	30	12
2	1:1:1:1	80	30	54
3	1:1:1:1	100	30	38
4	1:1.5:1.5:1.5	80	30	68
5	1:2:2:2	80	30	>99
6	1:2:2:2	80	15	>99
7	1:2:2:2	80	4	>99(84)[c]

[a]Reactions were carried out using 0.5 mmol of isocyanide **1a** in 2 mL of MeCN/MeOH. [b]Determined based on isocyanide **1a** as HPLC peak area percent at 215 nm. [c]Isolated yield in parentheses.

Table 2. Scope of the Optimized U-4CR Peptoid Synthesis[a]



entry	isocyanide	Acid	amine	Peptoid
1	 **1a**	 **2a**	 **3a**	 **5a**, 84%
2	 **1a**	 **2b**	 **3a**	 **5b**, 86 %
3	 **1a**	 **2c**	 **3a**	 **5c**, 95 %
4	 **1a**	 **2d**	 **3a**	 **5d**, 86 %
5	 **1a**	 **2e**	 **3a**	 **5e**, 80 %
6	 **1b**	 **2a**	 **3b**	 **5f**, 90 %
7	 **1b**	 **2b**	 **3b**	 **5g**, 88 %
8	 **1c**	 **2c**	 **3c**	 **5h**, 81 %

[a]Conditions: isocyanide (**1**, 0.5 mmol), carboxylic acid (**2**, 1.0 mmol), amine (**3**, 1.0 mmol), and paraformaldehyde (**4a**, 1.0 mmol) in 2 mL of MeCN/MeOH (1:1).

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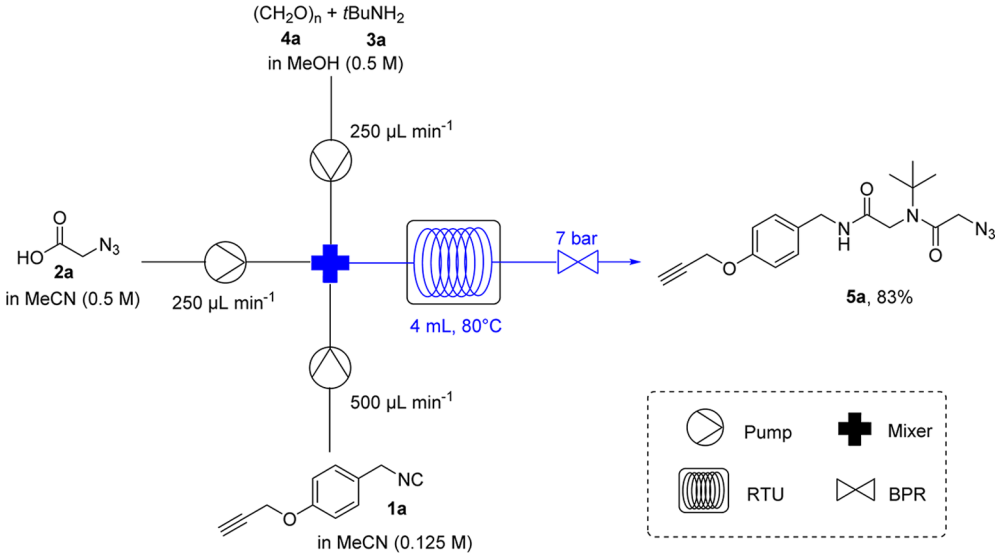
Scheme 3. Synthesis of Linear Peptoid 5a via U-4CR in Continuous Flow Mode



isocyanide (**1**), an acid (**2**), an amine (**3**), and an oxo-component (**4**), simultaneously installing an alkyne as well as an azide group for subsequent intramolecular CuAAC (Scheme 1). Similar strategies were already used for linear peptidomimetic scaffolds,[28] but applications for cyclic derivatives (**6**) are however scarce in the literature.[29]

Traditionally, such reactions are carried out batchwise using an iterative series of reaction and workup steps. An increasingly popular alternative for such habitual processes is performing organic reactions in continuous flow devices.[30,31] These methodologies offer the possibility to carry out multistep reaction sequences by employing several reactors in a linear arrangement circumventing tedious and unnecessary isolation procedures.[32] This is of particular importance when hazardous reagents or intermediates are involved, making the scale-up of such transformations difficult.[30−32]

Herein, we present the development of a multistep continuous flow procedure for the synthesis of linear and cyclic peptoids using the microwave-to-flow paradigm.[33] The described methodology includes the continuous formation of an isocyanide as well as of an azide-functionalized carboxylic acid, which are subsequently used in an U-4CR. The obtained linear peptoids can be further cyclized without the addition of any additive using a residence time unit made out of copper.

RESULTS AND DISCUSSION

The original concept of our continuous flow approach was based on four separate liquid feeds each delivering a unique building block for the U-4CR (Scheme 2). After mixing of the reagents, a heated residence time unit was to be used for the IMCR. The resulting peptoid can subsequently be converted into the cyclic isomer using an intramolecular version of the CuACC process. For the latter transformation we intended to use a flow reactor made out of copper, circumventing the necessity of adding the catalytically active Cu metal species separately.[34] As it is known that macrocyclizations of this type often require special ligands in order to achieve acceptable yields and selectivities,[35] we also considered the need for an additional feed. Since the multicomponent reaction involves isocyanides which are often characterized by an unpleasant odor and can be quite toxic, we additionally decided to telescope our original strategy by an upstream flow dehydration step of the respective formamide **7** (Scheme 2), thereby generating the required isonitrile **1** in situ.[12c] Similarly, the potentially hazardous azide building block **2** was also prepared in-line *via* an upstream nucleophilic substitution from readily available halide precursors **8** (Scheme 2).[36]

Peptoid Synthesis. Initially, we optimized the reaction parameters for the IMCR using microwave dielectric heating methodologies (Table 1).[37] To generate the peptoid scaffold and simultaneously install the alkyne and azide functionalities for the following intramolecular Click reaction, isocyanide **1a** and 2-azidoacetic acid (**2a**) were selected for the U-4CR. Paraformaldehyde (**4a**) as an oxo-component and *tert*-butyl amine (**3a**) completed the building blocks for the model reaction system resulting in peptoid **5a**.

An early temperature screening using equimolar amounts of all starting materials exhibited that the multicomponent reaction has an optimum temperature of ca. 80 °C (Table 1, entries 1−3).[38] A longer reaction time was not considered as a relatively high throughput is desirable for any continuous process. Thus, reagents **2a**−**4a** were added in excess to isocyanide **1a** in order to drive the reaction to completion. As expected, when 1.5 equiv were used, a significantly higher conversion was obtained (entry 4). A further increase to 2 equiv of the acid, the amine, and the aldehyde resulted in the total consumption of the limiting building block within 30 min (entry 5). Concurrently the reaction time could be dramatically reduced to 4 min maintaining a quantitative reaction of isocyanide **1a** and a high purity profile (entries 6 and 7). Workup of the crude reaction mixture by column chromatography resulted in 84% of the functionalized peptoid **5a**.

Encouraged by these promising results, we applied the optimized conditions to various combinations of starting materials as shown in Table 2. Several peptoids (**5a**−**5e**) with different chain lengths on the azide-containing carboxylic acids (**2a**−**2e**) were prepared without the need for any reoptimization (Table 2, entries 1−5). These compounds were specifically prepared for the subsequent copper-catalyzed Click reactions in order to examine the role of the chain length on the cyclization characteristics. Furthermore, we introduced the alkyne moiety by the amine building block, simultaneously varying the

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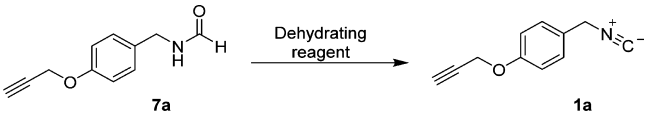
isocyanide for a more diverse set of peptidomimetic compounds (entries 6–8). The oxo-component was limited to paraformaldehyde (**4a**), as other aldehydes or ketones would not result in the desired peptoid scaffold. The resulting, hitherto undisclosed linear peptoids were isolated in good to excellent yields proving the robustness of the Ugi multicomponent reaction. The next step was to translate the optimized batch protocol into a continuous flow process. Thus, we assembled a flow reactor with three separate liquid feeds as shown in Scheme 3.[39] The number of required feeds resulted from the fact that our ultimate goal was to prepare the azide moiety as well as the isocyanide group prior to the IMCR in telescoped upstream flow transformations (Scheme 2).

Therefore, the amine- and oxo-building blocks were combined into one feed. In order to obtain a proper stoichiometry, a 0.125 M solution of the limiting isocyanide in acetonitrile was pumped with 0.5 mL min^{-1} flow rate and all other reagents with a concentration of 0.5 M were pumped with a flow rate of 0.25 mL min^{-1}. As a residence time unit (RTU), a 4 mL perfluoroalkoxy (PFA) coil with an inner diameter of 0.8 mm was used in order to allow a similar reaction time as in the microwave batch experiments. Since the optimum temperature of 80 °C is more or less equal to the boiling point of acetonitrile (82 °C) and above the boiling point of MeOH (65 °C), a 7 bar back pressure regulator (BPR) was applied to accurately control the residence time. Gratifyingly, the limiting isocyanide was quantitatively consumed resulting in a similar isolated yield as in the batch microwave experiment under identical processing conditions.[33]

Isocyanide Preparation. Having an optimized protocol for the peptoid formation in hand, we turned our attention to the upstream synthesis of isocyanide **1a** from the corresponding formamide **7a**. Using traditional batch techniques, several literature procedures for this dehydration were evaluated for their suitability in a continuous process (Table 3). When

Table 3. Screening of Dehydration Reagents for Isocyanide Formation



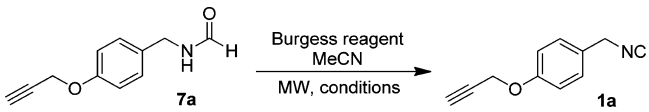
entry	experimental conditions	yield [%][a]
1	CCl_4, Et_3N, PPh_3, CH_2Cl_2, 12 h, reflux	34
2	TCT,[b] pyridine, CH_2Cl_2, 12 h, reflux	–[c]
3	TCT,[b] Et_3N, CH_2Cl_2, 12 h, reflux	–[c]
4	$POCl_3$, Et_3N, CH_2Cl_2, 12 h, −78 to 25 °C	76
5	Burgess reagent,[d] CH_2Cl_2, 80 min, reflux	67

[a]Isolated yield. [b]TCT = 2,4,6-trichloro[1,3,5]triazine. [c]No product formation observed. [d]Methyl *N*-(triethylammoniumsulfonyl)-carbamate.

triphenylphosphine[40] was used as a dehydration agent we observed low isolated yields, even after 12 h. In addition, the resulting triphenylphosphinoxide would likely cause problems in a continuous process due to its low solubility. No product formation could be observed in the case of 2,4,6-trichloro-[1,3,5]triazine (cyanuric chloride, TCT).[41] However, phosphoryl chloride[42] as well as the Burgess reagent (methyl *N*-(triethylammoniumsulfonyl)carbamate)[43] delivered promising isolated yields in this initial screening. We decided to carry out further experiments using the Burgess reagent, as its application is far more convenient and in contrast to the use of $POCl_3$ a totally homogeneous reaction was observed.

As displayed above, the majority of literature dehydration protocols are typically conducted in dichloromethane. Since the U-4CR was already successfully optimized using a polar solvent combination (MeOH/MeCN), we decided to switch to acetonitrile for the process intensification experiments (Table 4). An initial experiment employing 1 equiv of the dehydrating

Table 4. Batch Process Intensification for Formamide Dehydration Using Burgess Reagent[a]



entry	Burgess reagent [equiv]	T [°C]	t [min]	conversion [%][b]
1	1	50	10	50
2	1	80	10	51
3	1	100	10	53
4	1	50	25	64
5	1.5	50	15	77
6	1.5	50	20	81
7	2	50	20	>99(93)[c]
8	2	50	15	84

[a]Reactions were carried out using 2 mmol of formamide **7a** in 2 mL MeCN; Burgess reagent = methyl *N*-(triethylammoniumsulfonyl)-carbamate. [b]Determined as HPLC peak area percent at 215 nm. [c]Isolated yield in parentheses.

agent at 50 °C indicated that the reaction indeed also results in the desired isonitrile molecule using a polar solvent (Table 4, entry 1). Unfortunately, neither an elevated temperature nor a longer reaction time provided reasonable conversions of formamide **7a** (entries 2–4). By increasing the amount of the Burgess reagent stepwise to 2 equiv it was demonstrated that a quantitative dehydration can be achieved within 20 min at 50 °C (entry 7). These optimized conditions were subsequently tested in a continuous protocol keeping the optimized conditions of the continuous U-4CR in mind (Scheme 4).[39] Since the flow rate for the isocyanide feed in the multicomponent reaction was 0.5 mL min^{-1}, the continuous dehydration protocol needed to be adjusted in order to result in the same overall liquid flow. We thus decided to use the same flow rate for the formamide as well as the Burgess reagent feed (250 μL min^{-1}), controlling the stoichiometry by employing a 2-fold concentration of the latter reagent. In order to reach the necessary reaction time of 20 min, a 10 mL PFA coil was installed and heated in an oil bath to the desired temperature. To match the conditions of the peptoid synthesis, the same back pressure was used (7 bar). Gratifyingly, these reaction conditions resulted in full conversion of the isocyanide precursor and provided similar isolated yields for **1a** after column chromatography (Scheme 4).

Azide Formation. To complete our continuous concept for the synthesis of the linear peptidomimetics a continuous azide formation had to be developed. Due to their easy accessibility, we decided to synthesize these highly energetic compounds from their corresponding bromides. Usually such nucleophilic substitutions of halides are conducted using NaN_3 in DMSO or mixtures of H_2O and DMSO in flow mode.[36,44] To circumvent solubility issues throughout the overall continuous process using our solvent system (MeCN/MeOH), we decided to use

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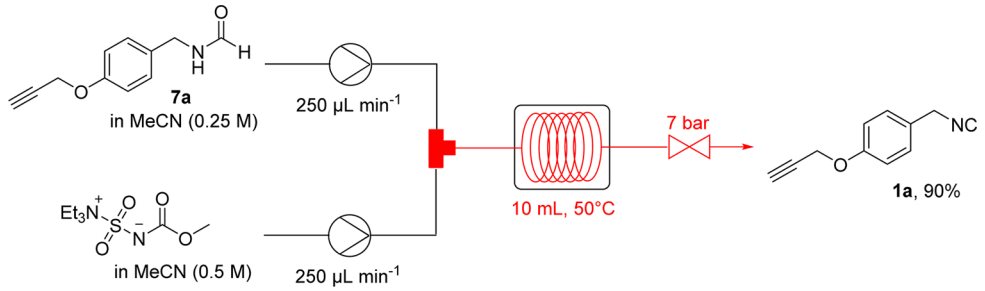
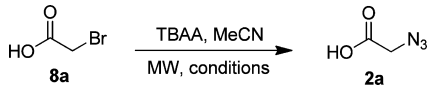
Scheme 4. Continuous Isocyanide Synthesis Employing Burgess Reagent



tetrabutylammonium azide (TBAA) instead of inorganic azide salts.[45] Optimization of all reaction parameters was once again carried out in acetonitrile employing batch microwave technology as demonstrated in Table 5. Bromoacetic acid

Table 5. Optimization of Azide Formation Using TBAA[a]



entry	TBAA [equiv]	T [°C]	t [min]	conversion [%][b]
1	1	80	5	72
2	1	80	15	83
3	1	80	20	81
4	1	100	15	84
5	1.5	100	15	>99(92)[c]
6	1.5	80	15	93
7	1.5	100	10	89

[a]Reactions were carried out using 1 mmol of bromoacetic acid (**8a**) in 2 mL of MeCN; TBAA = tetrabutylammonium azide. [b]Determined as HPLC peak area percent at 215 nm. [c]Isolated yield in parentheses.

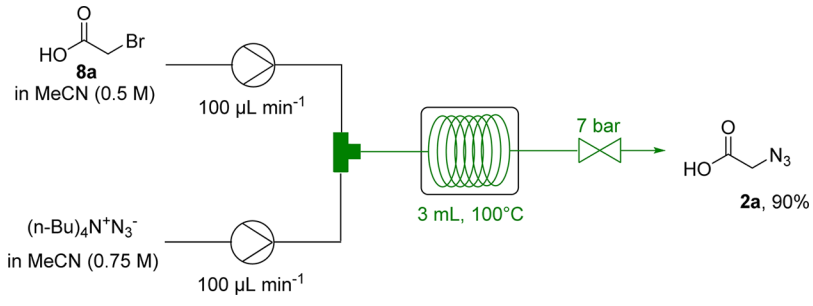
(**8a**) and 1 equiv of TBAA were dissolved in 2 mL of MeCN for a first set of experiments. A quantitative reaction would result in a 0.5 M solution of azide **2a** as used in the continuous U-4CR described above (Scheme 3). These initial experiments resulted in 72−84% of the desired product depending on the reaction time and temperature (Table 5, entries 1−4). By increasing the amount of the organic salt to 1.5 equiv a quantitative reaction was observed at 100 °C within 15 min (entry 5). A further reduction of both reaction and temperature resulted in incomplete reactions (entries 6−7).

Translation of the batch conditions to a continuous flow methodology was straightforward (Scheme 5).[39] A 100 μL min^{-1} stream of a 0.5 M bromide solution was mixed with a 100 μL min^{-1} stream of TBAA in MeCN (0.75 M) and pumped through a 3 mL PFA coil set at 100 °C. After exactly 15 min the reaction mixture left the heated reaction zone and was subsequently collected after passing a 7 bar BPR. Isolation afforded azidoacetic acid **2a** in excellent yield confirming the results obtained in batch.

Multistep Synthesis of Linear Peptoids in Continuous Flow. Having suitable flow processes for the continuous U-4CR, the upstream azide, and isocyanide formations in hand, we next aimed for combining all continuous protocols, resulting in a single flow process (Scheme 6). We employed the azide synthesis described above (Scheme 5) as the basis for calculating all flow rates and coil lengths in order to obtain the necessary stoichiometry and residence times. Thus, a 0.25 M solution of formamide **7a** in acetonitrile was pumped with 200 μL min^{-1} and mixed with the Burgess reagent (0.5 M in MeCN) resulting in an overall flow rate of 400 μL min^{-1} for the isocyanide formation. After passing an 8 mL RTU (20 min residence time) set at 50 °C, the stream was directly mixed with the outcome of the azide formation and a 0.5 M solution of paraformaldehyde (**4a**) and *tert*-butylamine (**3a**). The resulting output stream with an overall flow rate of 0.8 mL min^{-1} was then heated in a 4 mL PFA tubing (5 min residence time) to 80 °C executing the intensified U-4CR protocol. Analysis of the collected reaction mixture indicated a quantitative reaction with the limiting formaldehyde reagent **7a** being fully converted into peptoid **5a.** After purification, 80% of the target molecule was obtained which is in good agreement with the previous batch and flow experiments. Notably, no purification step was required when combining the different transformations and the fully continuous protocol afforded the peptidomimetic compound within an overall reaction time of only 25 min in a process that avoided the handling of potentially toxic and/or explosive intermediates.

CuAAC in Continuous Flow. Copper-catalyzed 1,3-dipolar cycloadditions resulting in the 1,4-substituted 1,2,3-triazole scaffold were carried out for the first time in a copper coil in 2009 by Bogdan and Sach,[46] and since then have been applied in a multitude of synthetic strategies.[34] Continuous prepara-

Scheme 5. Continuous Synthesis of 2-Azidoacetic Acid



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Scheme 6. Synthesis of Linear Peptoid 5a via Isocyanide Synthesis (red), Azide Preparation (green), and an U-4CR (blue) in a Single Continuous Operation

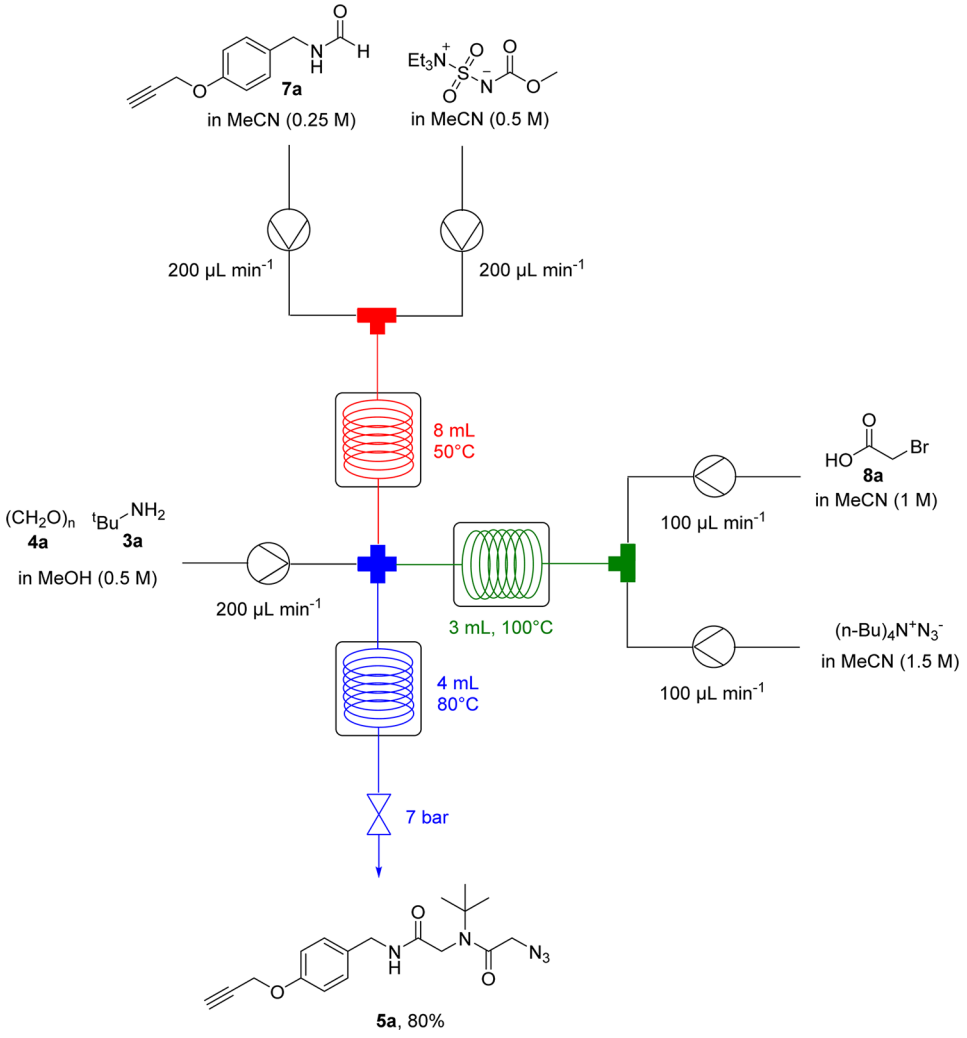
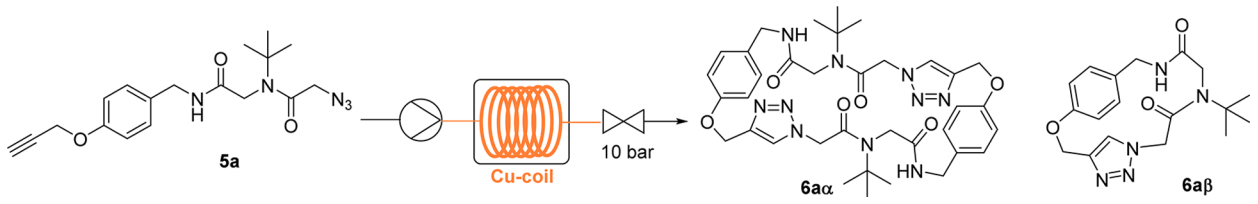


Table 6. Process Intensification for the Continuous Macrocyclization Using a Copper Coil[a]



entry	*c* [mM]	additive (10 mol %)	*T* [°C]	*t* [min]	**5a** [%][b]	***6aα*** [%][b]	***6aβ*** [%][b]
1[c]	62.5	TTTA	100	20	17	44	5
2[c]	62.5	TBTA	100	20	9	27	8
3	62.5	TTTA	100	20	12	59	9
4	2.0	TTTA	100	20	3	67	23
5	2.0	TTTA	120	20	2	70	19
6	2.0	–	120	20	10	72	5
7	2.0	–	140	20	0	83	3
8	2.0	–	140	16	2	74	7
9	2.0	–	140	25	0	86(74)[d]	2

[a]Reactions were performed on a 0.140 mmol scale in MeCN/MeOH (1:1). [b]Determined as peak area percent at 215 nm. [c]2 equiv of DIPEA were added to the reaction mixture. [d]Isolated yield in parentheses. TTTA = (tris((1-*tert*-butyl-1*H*-1,2,3-triazolyl)methyl)amine); TBTA = (tris(benzyltriazolylmethyl)amine).

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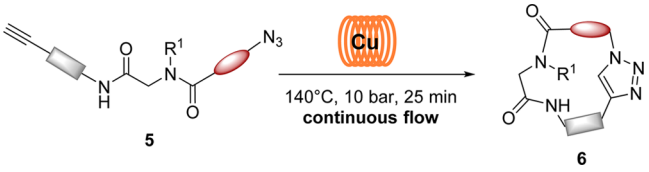
tions of macrocyclic compounds using the copper-catalyzed Click strategy were intensively studied by the groups of Collins[47,48] and James.[35,49,50] In the latter case, the use of flow reactors made out of elemental copper turned out to be highly beneficial, as the nature of the coil itself avoided the necessity to add additional catalytically active species. Earlier work from our laboratories revealed that these cycloadditions are not catalyzed by zerovalent copper but more likely by a surface layer of Cu_2O.[51] Thus, a homogeneous reaction mechanism was postulated resulting in leaching of the metal species when carried out continuously.[51,52]

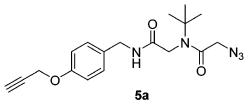
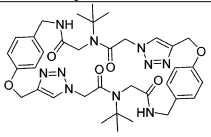
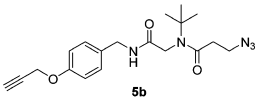
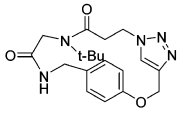
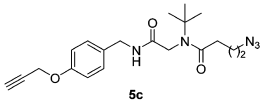
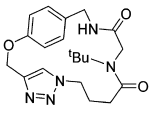
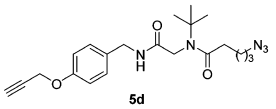
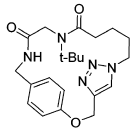
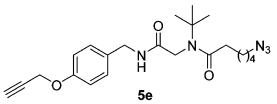
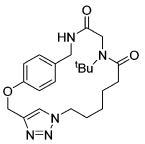
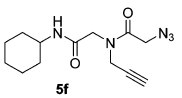
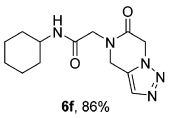
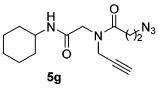
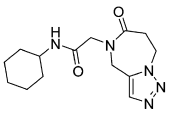
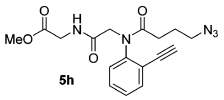
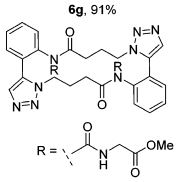
For the optimization study of the intermolecular CuAAC of linear peptoid **5a**, a simple continuous setup consisting of a syringe pump, a 20 mL copper coil, and an adjustable back pressure regulator was assembled (Table 6). Since literature conditions are typically carried out in the presence of tris[(1-*tert*-butyl-1*H*-1,2,3-triazolyl)methyl]amine (TTTA) as the ligand and *N*,*N*-diisopropylethylamine (DIPEA) as the base for better monomer-to-dimer ratios, we initiated our macrocyclization attempts using a similar approach (Table 6, entry 1). Unfortunately, these conditions resulted in dimer **6a*α*** as the main product in addition to the generation of various unidentified byproducts. These compounds are most likely related to higher oligomers or even polymeric material but could not be clearly identified.[53] The selectivity was even worse when we changed the ligand from TTTA to TBTA (tris-(benzyltriazolylmethyl)amine, entry 2). Interestingly, when the reaction using TTTA was carried out without the addition of the base, a better conversion to the CuAAC products **6a*α*** and **6a*β*** was observed (entry 3). We next decided to process more diluted reaction mixtures which demonstrated a slightly improved selectivity for monomer **6a*β*** (entries 4–5). Notably, when we carried out a control experiment in the absence of TTTA, only very small amounts of the desired molecule were detected by HPLC analysis, almost selectively forming **6a*α*** (entry 6). We concluded that a protocol selectively yielding monomer **6a*β*** with a suitable throughput would not be achievable and, thus, decided to intensify this additive-free process in order to fully consume the linear precursor **5a**. We anticipated that the resulting protocol would yield the desired monomeric scaffold for homologues **5b–e**, as the resulting ring structures may be less strained.[50] Increasing the temperature to 140 °C resulted in full consumption of linear peptoid **5a** yielding **6a*α*** almost selectively (entry 7). Since we realized that a slightly shorter residence time provided significantly inferior results, we decided to extend the residence time to 25 min to avoid incomplete cyclization procedures by using a flow rate of 0.8 mL min^{-1} (entries 8–9). Isolation using chromatographic techniques provided good isolated yields for the complex cyclic peptoid **6a*α***.

When the same conditions were employed for the CuAAC of linear peptoid **5b**, which only differs from **5a** by a single CH_2 group, our hypothesis was confirmed (Table 7, entry 2). Careful analysis revealed that the monomeric cyclopeptoid **6b** was selectively formed instead of the dimeric derivative. The same selectivity was obtained for linear peptoids **5c–5e** and the cyclic products **6c–6e** could be isolated in good to excellent yields (entries 3–5). The structure of compound **6e** was confirmed by X-ray crystallography.[54] Not surprisingly, the structurally different peptoids **5f** and **5g** smoothly cyclized leading to the desired 6- and 7-membered ring structures (entries 6–7). A high ring strain may also be the reason for compound **5h** to not form a monomeric cyclopeptoid.

Table 7. Continuous CuAAC Macrocyclization of Linear Peptoids Using a Copper Coil[a]



5 6

entry	substrate	product
1	 5a	 6aα, 74%
2	 5b	 6b, 81%
3	 5c	 6c, 81%
4	 5d	 6d, 80%
5	 5e	 6e, 83% [X-ray]
6	 5f	 6f, 86%
7	 5g	 6g, 91%
8	 5h	 R = 6h, 73%

[a]Reactions were carried out using 2 mM solutions of the linear peptoids **5a–h** in MeCN/MeOH (1:1).

However, also in this case reasonable amounts of the dimeric structure **6h** could be isolated.

CONCLUSION

In summary, we have developed an efficient fully continuous multistep strategy for the synthesis of linear peptoids and a subsequent copper-catalyzed dipolar cycloaddition resulting in their cyclic analogs. The whole continuous process is based on

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the synthesis of the peptoid scaffold using an U-4CR. The limiting isocyanide was synthesized in a continuous process by the dehydration of the corresponding amide using Burgess reagent. Since the following CuAAC cyclization requires a potentially hazardous azide functionality, we developed a flow protocol for its in-line synthesis by the nucleophilic substitution of a bromide precursor with tetrabutylammonium azide. The individual steps could be successfully coupled without the need to isolate any synthetic intermediate. The resulting convergent continuous synthesis is characterized by an overall processing time of ca. 25 min generating the desired peptidomimetics in good to excellent yields. The subsequent CuAAC macrocyclization was realized using a continuous flow reactor made of copper avoiding a separate addition of a catalytically active species. Depending on the nature of the linear precursor and the resulting ring strain, either a dimeric or a monomeric form of the cyclic product was obtained.

EXPERIMENTAL SECTION

General Remarks. All microwave-assisted reactions were carried out in a Biotage Initiator 2.5 instrument in Pyrex vessels (2−5 mL). Reaction temperature was controlled by an external IR sensor. For continuous flow experiments, commercially available syringe pumps, reagent injector units, and tube reactors were used (Asia Flow Chemistry modules, Syrris). The back pressure was controlled using either static or adjustable regulation units.[39] ^{1}H NMR and ^{13}C spectra were recorded on a 300 MHz instrument using $CDCl_3$ or DMSO-d_6 as solvent. Chemical shifts (δ) are expressed in ppm downfield from TMS as the internal standard. The letters s, d, t, q, qt, and m are used to indicate a singlet, doublet, triplet, quadruplet, quintuplet, and multiplet, respectively. Melting points were determined on a standard melting point apparatus. Analytical HPLC analysis was carried out on a C-18 reversed-phase (RP) analytical column (150 mm × 4.6 mm, particle size 5 μm) at 37 °C using a mobile phase A (water/acetonitrile 90:10 (v/v) + 0.1% TFA) and B (MeCN + 0.1% TFA) at a flow rate of 1.0 mL min^{-1}. The following gradient was applied: linear increase from solution 30% B to 100% B in 8 min, hold at 100% solution B for 2 min. HRMS experiments were performed on a TOF LC/MS instrument equipped with an ESI ion source (positive ionization mode). X-ray diffraction measurements were performed on a standard CCD diffractometer by using graphite monochromatized Mo Kα radiation. Column chromatography was carried out using an automated flash chromatography system using petroleum ether/ethyl acetate mixtures as the eluent. Preparative HPLC separations were carried on a C-18 reversed-phase column (250 mm × 16 mm, particle size 5 μm) at 25 °C using a mobile phase A (water/acetonitrile 90:10 (v/v)) and B (MeCN) at a flow rate of 8.0 mL min^{-1}. The following gradient was applied: linear increase from solution 30% B to 100% B in 18 min, hold at 100% solution B for 4 min. TLC analysis was performed on silica gel F_{254} plates.

All compounds and solvents were obtained from standard commercial vendors and used without further purification. Proof of purity was obtained by ^{1}H NMR and HPLC−UV spectroscopy.

Starting Materials. 4-(Prop-2-yn-1-yloxy)benzonitrile,[55] (4-(prop-2-yn-1-yloxy)phenyl)-methanamine,[56] 3-azidopropionic acid,[57] 4-azidobutanoic acid, 5-azidopentanoic acid,[58] and 6-azidohexanoic acid[59] were prepared and characterized according to literature procedures.

***N*-(4-(Prop-2-yn-1-yloxy)benzyl)formamide (7a).** A stirred mixture of 4-(prop-2-yn-1-yloxy)phenyl)methanamine (1.7 g, 10.6 mmol) in 5 mL of ethyl formate was refluxed overnight. Afterward, the solvent was removed under reduced pressure and the residue was purified by flash chromatography to give the title compound in 90% yield (1.8 g, 9.5 mmol) as a colorless solid, mp 93−94 °C. R_f: 0.42 (95% EtOAc/petroleum ether). HRMS (ESI): m/z: calcd for $C_{11}H_{12}NO_2$ $[M + H]^+$: 190.086255, found: 190.086639. ^{1}H NMR (300 MHz, $CDCl_3$, presence of rotamers) δ 8.21 (s, 0.9H), 8.15 (d, J = 12 Hz, 0.1H), 7.30−7.13 (m, 2H), 6.98−6.88 (m, 2H), 6.10 (brs, 1H), 4.80 and 4.70 (2 d, J = 2.5 Hz, 2H), 4.41 and 4.35 (2 d, J = 5.8 Hz, 2H), 2.53 (t, J = 2.5 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, presence of rotamers) 164.6, 161.1, 157.2, 157.0, 130.7, 130.5, 129.1, 128.3, 115.3, 115.1, 114.9, 78.4, 75.7, 58.9, 55.8, 45.1, 41.5; FT-IR (KBr, cm^{-1}) 3269, 3217, 3041, 2920, 2889, 2111, 1642, 1538, 1218, 1174, 1113, 1026, 812, 787, 601.

Microwave Assisted Synthesis of 1-(Isocyanomethyl)-4-(prop-2-yn-1-yloxy)benzene 1a. *N*-(4-(Prop-2-yn-1-yloxy)benzyl)-formamide (**7a**) (0.5 mmol, 94 mg) and methyl *N*-(triethylammoniumsulfonyl)carbamate (0.75 mmol, 179 mg) were dissolved in acetonitrile (2 mL) and heated to 50 °C for 20 min using microwave irradiation. The obtained yellow solution was concentrated in vacuum and purified by flash chromatography to give the title compound in 93% yield (79.0 mg, 0.46 mmol) as a colorless solid, mp 58−60 °C. R_f: 0.36 (10% EtOAc/petroleum ether). HRMS (APCI): m/z: calcd for $C_{10}H_9O$ $[M - NC + H]^+$: 145.064791, found: 145.064668; calcd for C_8H_9O [M-propargyl]$^-$: 132.045487, found: 132.045744; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (d, J = 8.7 Hz, 2H), 7.02 (d, J = 8.7 Hz, 2H), 4.73 (d, J = 2.4 Hz, 2H), 4.60 (brs, 2H), 2.55 (t, J = 2.4 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.5, 157.2, 128.1, 125.5, 115.4, 78.2, 75.8, 55.9, 44.9; FT-IR (KBr, cm^{-1}) 3249, 3071, 2963, 2922, 2865, 2159, 2123, 2039, 1613, 1588, 1509, 1453, 1184, 940, 778, 635.

Microwave Assisted Synthesis of 2-Azidoacetic Acid 2a. 2-Bromoacetic acid (1.0 mmol, 0.137 mg) and *N,N,N,N*-tetrabutylammonium azide (1.5 mmol, 0.426 mg) were dissolved in acetonitrile (2 mL) and heated to 100 °C for 15 min using microwave irradiation. After cooling to room temperature, the solvent was evaporated. The concentrate was dissolved in H_2O and acidified with HCl to pH = 1. The solution was extracted 3× with Et_2O, and the combined organic phases were dried over Na_2SO_4. Careful solvent evaporation under reduced pressure resulted in a 92% yield of the title compound (93 mg, 0.92 mmol). The data obtained by NMR are identical to those reported in literature.[60]

General Procedure for Preparation of Linear Peptoids by the U-4CR in Batch (Table 2). A sealed 2−5 mL microwave process vial containing a mixture of the isocyanide (**1**, 0.5 mmol), the corresponding acid (**2**, 1.0 mmol), the amine (**3**, 1.0 mmol), and paraformaldehyde (**4**, 1.0 mmol) in 2 mL of MeOH/MeCN (1:1) was kept for 4 min at 80 °C using a single mode microwave reactor. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure and purified by flash column chromatography using petroleum ether/ethyl acetate gradients to yield the corresponding linear peptoids.

2-Azido-*N*-(*tert*-butyl)-*N*-(2-oxo-2-((4-(prop-2-yn-1-yloxy)-benzyl)amino)ethyl)acetamide (5a). Prepared from paraformaldehyde (**4a**, 30 mg, 1.0 mmol), *tert*-butylamine (**3a**, 73 mg, 1.0 mmol), 2-azidoacetic acid (**2a**, 101 mg, 1.0 mmol), and isocyanide **1a** (85 mg, 0.5 mmol). Peptoid **5a** was isolated in 84% yield (150 mg, 0.42 mmol) as a colorless oil. R_f: 0.50 (50% EtOAc/petroleum ether). HRMS (ESI): m/z: calcd for $C_{18}H_{23}N_5O_3Na$ $[M + Na]^+$: 380.169311, found: 380.169070. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20 (d, J = 8.5 Hz, 2H), 6.93 (d, J = 8.5 Hz, 2H), 6.75−6.64 (m, 1H), 4.67 (d, J = 2.3 Hz, 2H), 4.36 (d, J = 5.6 Hz, 2H), 3.90 (s, 2H), 3.81 (s, 2H), 2.53 (t, J = 2.3 Hz, 1H), 1.38 (d, J = 11.6 Hz, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.8, 168.7, 157.1, 156.9, 130.7, 129.1, 115.2, 78.4, 77.5, 77.1, 76.7, 75.7, 75.7, 58.8, 55.8, 52.6, 47.8, 43.1, 28.4; FT-IR (KBr, cm^{-1}) 3294, 2102, 1654, 1509, 1396, 1363, 1215, 1190, 1023, 933, 829, 641.

3-Azido-*N*-(*tert*-butyl)-*N*-(2-oxo-2-((4-(prop-2-yn-1-loxy)-benzyl)amino)ethyl)propanamide (5b). Prepared from paraformaldehyde (**4a**, 30 mg, 1.0 mmol), *tert*-butylamine (**3a**, 73 mg, 1.0 mmol), 3-azidopropionic acid (**2b**, 115 mg, 1.0 mmol), and isocyanide **1a** (85 mg, 0.5 mmol). Peptoid **5b** was isolated in 86% yield (0.159 g, 0.43 mmol) as a colorless solid, mp 92−94 °C. R_f: 0.37 (50% EtOAc/petroleum ether). HRMS (ESI): m/z: calcd for $C_{19}H_{25}N_5O_3Na$ $[M + Na]^+$: 394.184961, found: 394.185216. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.21 (d, J = 8.4 Hz, 2H), 6.94 (d, J = 8.4 Hz, 2H), 6.46 (brt, J = 5.1 Hz, 1H), 4.68 (t, J = 2.4 Hz, 2H), 4.40 (d, J = 5.7 Hz, 2H), 3.98 (s, 2H), 3.60 (t, J = 6.2 Hz, 2H), 2.53 (t, J = 2.4 Hz, 1H), 2.46 (t, J = 6.2 Hz, 2H), 1.41 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.7, 169.1, 157.1,

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130.6, 129.1, 115.2, 78.4, 75.7, 58.3, 55.8, 49.0, 47.6, 43.2, 35.3, 28.6; FT-IR (KBr, cm^{-1}) 3292, 2973, 2928, 2098, 1649, 1586, 1543, 1509, 1405, 1362, 1328, 1297, 1113, 807, 731, 671.

4-Azido-*N*-(*tert*-butyl)-*N*-(2-oxo-2-((4-(prop-2-yn-1-yloxy)-benzyl)amino)ethyl)butanamide (5c). Prepared from paraformaldehyde (**4a**, 30 mg, 1.0 mmol), *tert*-butylamine (**3a**, 73 mg, 1.0 mmol), 4-azidobutanoic acid (**2c**, 129 mg, 1.0 mmol), and isocyanide **1a** (0.085 g, 0.5 mmol). Peptoid **5c** was isolated in 95% yield (181 mg, 0.47 mmol) as a colorless solid, mp 93−95 °C. R_f: 0.40 (50% EtOAc/petroleum ether). HRMS (ESI): *m*/*z*: calc. for $C_{20}H_{27}N_5O_3Na$ $[M + Na]^+$: 408.200611, found: 408.200814. ^1H NMR (300 MHz, $CDCl_3$) δ 7.15 (d, J = 8.6 Hz, 2H), 6.87 (d, J = 8.4 Hz, 2H), 6.44 (brt, J = 5.4 Hz, 1H), 4.61 (d, J = 2.4 Hz, 2H), 4.35 (d, J = 5.8 Hz, 2H), 3.91 (s, 2H), 3.17 (t, J = 6.4 Hz, 2H), 2.46 (t, J = 2.4 Hz, 1H), 2.22 (t, J = 6.8 Hz, 2H), 1.82 (qt, J = 6.6 Hz, 2H), 1.33 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.4, 169.5, 157.1, 130.8, 129.2, 115.2, 78.4, 75.7, 58.1, 55.8, 50.9, 49.1, 43.0, 32.8, 28.6, 24.4; FT-IR (KBr, cm^{-1}) 3317, 3255, 3072, 2968, 2908, 2087, 1663, 1643, 1512, 1266, 1240, 1193, 1179, 1116, 934, 813, 607, 553.

5-Azido-*N*-(*tert*-butyl)-*N*-(2-oxo-2-((4-(prop-2-yn-1-yloxy)-benzyl)amino)ethyl)pentanamide (5d). Prepared from paraformaldehyde (**4a**, 30 mg, 1.0 mmol), *tert*-butylamine (**3a**, 73 mg, 1.0 mmol), 5-azidopentanoic acid (**2d**, 143 mg, 1.0 mmol), and isocyanide **1a** (85 mg, 0.5 mmol). Peptoid **5d** was isolated in 86% yield (171 mg, 0.43 mmol) as a colorless solid, mp 72−74 °C. R_f: 0.42 (50% EtOAc/petroleum ether). HRMS (ESI): *m*/*z*: calcd for $C_{21}H_{29}N_5O_3Na$ $[M + Na]^+$: 422.216281, found: 422.216353. ^1H NMR (300 MHz, $CDCl_3$) δ 7.24−7.18 (m, 2H), 6.98−6.93 (m, 2H), 6.36 (brt, J = 5.5 Hz, 1H), 4.69 (d, J = 3.1 Hz, 2H), 4.43 (d, J = 5.8 Hz, 2H), 4.00 (s, 2H), 3.25 (t, J = 5.4 Hz, 2H), 2.53 (t, J = 2.4 Hz, 1H), 2.24 (t, J = 7.1 Hz, 2H), 1.70−1.60 (m, 2H), 1.58−1.48 (m, 2H), 1.41 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.0, 169.6, 157.2, 130.7, 129.1, 115.2, 78.3, 75.7, 58.0, 55.8, 51.2, 49.1, 43.1, 35.4, 28.8, 28.3, 22.4; FT-IR (KBr, cm^{-1}) 3281, 3068, 2928, 2871, 2091, 1724, 1678, 1611, 1585, 1454, 1359, 1211, 1177, 1024, 752, 655, 576.

6-Azido-*N*-(*tert*-butyl)-*N*-(2-oxo-2-((4-(prop-2-yn-1-yloxy)-benzyl)amino)ethyl)hexanamide (5e). Prepared from paraformaldehyde (**4a**, 30 mg, 1.0 mmol), *tert*-butylamine (**3a**, 73 mg, 1.0 mmol), 6-azidohexanoic acid (**2e**, 155 mg, 1.0 mmol) and isocyanide **1a** (85 mg, 0.5 mmol). Peptoid **5e** was isolated in 80% yield (165 mg, 0.40 mmol) as a colorless solid, mp 88−90 °C. R_f: 0.45 (50% EtOAc/petroleum ether). HRMS (ESI): *m*/*z*: calcd for $C_{22}H_{31}N_5O_3Na$ $[M + Na]^+$: 436.230574, found: 436.231116. ^1H NMR (300 MHz, $CDCl_3$) δ 7.23−7.14 (m, 2H), 6.97−6.88 (m, 2H), 6.38 (brt, J = 5.6 Hz, 1H), 4.67 (d, J = 2.4 Hz, 2H), 4.40 (d, J = 5.8 Hz, 2H), 3.97 (s, 2H), 3.23 (t, J = 6.8 Hz, 2H), 2.53 (t, J = 2.4 Hz, 1H), 2.19 (t, J = 7.3 Hz, 2H), 1.65−1.50 (m, 4H), 1.39 (s, 9H), 1.35−1.25 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.2, 169.6, 157.1, 130.7, 129.1, 115.2, 78.4, 75.7, 57.9, 55.8, 51.2, 49.1, 43.0, 35.9, 28.8, 26.3, 24.7. FT-IR (KBr, cm^{-1}) 3273, 3074, 2932, 2867, 2092, 1726, 1679, 1585, 1555, 1421, 1212, 1114, 1021, 751, 579, 552.

2-Azido-*N*-(2-(cyclohexylamino)-2-oxoethyl)-*N*-(prop-2-yn-1-yl)acetamide (5f). Prepared from paraformaldehyde (**4a**, 30 mg, 1.0 mmol), propargylamine (**3b**, 55 mg, 1.0 mmol), 2-azidoacetic acid (**2a**, 101 mg, 1.0 mmol), and cyclohexyl isocyanide (**1b**, 55 mg, 0.5 mmol). Peptoid **5f** was isolated in 90% yield (125 mg, 0.45 mmol) as a colorless solid, mp 125−127 °C. R_f: 0.42 (50% EtOAc/petroleum ether). HRMS (ESI): *m*/*z*: calcd for $C_{13}H_{20}N_5O_2$ $[M + H]^+$: 278.161151, found for $[M + H]^+$: 278.161152. ^1H NMR (300 MHz, $CDCl_3$ mixture of rotamers) ^1H NMR (300 MHz, $CDCl_3$) δ 6.01 and 5.98 (2 brs, 1H), 4.25 and 4.09 (2 brs, 2H), 4.04 and 4.01 (2 s, 2H), 3.93 and 3.87 (2 brs, 2H), 3.82−3.54 (m, 1H), 2.33 and 2.26 (2 brs, 1H), 1.88−1.77 (m, 2H), 1.72−1.47 (m, 3H), 1.40−1.17 (m, 2H), 1.19−0.96 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$ mixture of rotamers) δ 168.1, 166.8, 165.9, 74.1, 73.7, 50.4, 50.1, 50.1, 48.9, 48.5, 38.2, 36.6, 32.9, 25.4, 24.7; FT-IR (KBr, cm^{-1}) 3298, 3259, 2927, 2852, 2102, 1670, 1637, 1554, 1457, 1370, 1082, 891, 657, 559.

3-Azido-*N*-(2-(cyclohexylamino)-2-oxoethyl)-*N*-(prop-2-yn-1-yl)propanamide (5g). Prepared from paraformaldehyde (**4a**, 30 mg, 1.0 mmol), propargylamine (**3b**, 55 mg, 1.0 mmol), 3-azidopropionic acid (**2b**, 115 mg, 1.0 mmol), and cyclohexyl isocyanide (**1b**, 55 mg, 0.5 mmol). Peptoid **5g** was isolated in 88% yield (128 mg, 0.44 mmol) as a colorless solid, mp 109−111 °C. R_f: 0.30 (50% EtOAc/petroleum ether). HRMS (ESI): *m*/*z*: calcd for $C_{14}H_{22}N_5O_2$ $[M + H]^+$: 292.176801, found 292.176016. ^1H NMR (300 MHz, $CDCl_3$ mixture of rotamers) δ 5.97 (brs, 1H), 4.24 and 4.12 (2 d, J = 2.4 Hz, 2H), 4.00 and 3.91 (2 brs, 2H), 3.82−3.65 (m, 1H), 3.67−3.55 (m, 2H), 2.67 (t, J = 6.3 Hz, 1H), 2.45 (t, J = 6.2 Hz, 1H), 2.30 and 2.24 (2 t, J = 2.4 Hz, 1H), 1.90−1.75 (m, 2H), 1.72−1.48 (m, 3H), 1.39−1.19 (m, 2H), 1.17−0.96 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$ mixture of rotamers) δ 170.9, 170.7, 167.2, 166.4, 78.3, 73.7, 73.5, 51.3, 50.4, 48.8, 48.3, 47.1, 38.9, 36.6, 32.9, 32.8, 32.7, 32.5, 25.4, 25.3, 24.8, 24.7; FT-IR (KBr, cm^{-1}) 3316, 3239, 2942, 2925, 2855, 2114, 2084, 1649, 1549, 1463, 1433, 1237, 1053, 882, 720, 617.

Methyl-2-(2-(4-Azido-*N*-(2-ethynylphenyl)butanamido)-acetamido)acetate (5h). Prepared from paraformaldehyde (**4a**, 30 mg, 1.0 mmol), 2-ethynylaniline (**3c**, 117 mg, 1.0 mmol), 4-azidobutanoic acid (**2c**, 129 mg, 1.0 mmol), and methyl isocyanoacetate (**1c**, 50 mg, 0.5 mmol). Peptoid **5f** was isolated in 81% yield (146 mg, 0.41 mmol) as a brownish oil. R_f: 0.32 (50% EtOAc/petroleum ether). HRMS (ESI): *m*/*z*: calcd for $C_{17}H_{20}N_5O_4$ $[M + H]^+$: 358.150981, found 358.151069. ^1H NMR (300 MHz, $CDCl_3$ mixture of rotamers) δ 7.54 and 7.52 (2 brs, 1H), 7.43−7.37 (m, 2H), 7.35−7.27 (m, 1H), 6.95 (brs, 1H), 4.77 (d, J = 15.5 Hz, 1H), 4.11 (dd, J = 18.2, 6.0 Hz, 1H), 3.88 (dd, J = 18.2, 4.9 Hz, 1H), 3.76 (d, J = 15.5 Hz, 1H), 3.69 (s, 3H), 3.26 (s, 1H), 3.25−3.16 (m, 2H), 2.20−2.07 (m, 2H), 1.88−1.76 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$ mixture of rotamers) δ 173.3, 170.2, 168.9, 144.1, 134.1, 130.7, 129.1, 128.8, 121.3, 83.2, 79.4, 53.1, 52.4, 50.6, 41.1, 30.6, 24.3; FT-IR (KBr, cm^{-1}) 3288, 2951, 2094, 1746, 1654, 1532, 1485, 1448, 1435, 1252, 1020, 755, 657, 631.

Synthesis of Linear Peptoid 5a in a Continuous Multistep Approach (Scheme 5). *Isocyanide Generation.* A feed consisting of formamide **7a** (0.25 mmol) dissolved in acetonitrile (1 mL) was pumped with a flow rate of 200 μL min^{-1} and mixed in a T-shaped mixing unit with a second feed with an identical flow rate containing a 0.5 M solution of Burgess reagent (0.5 mmol in 1 mL acetonitrile). For a better control of the process, the reagents were each stored in a 1 mL sample loop connected via a 6-way valve and simultaneously injected into the flow system. After mixing, the resulting 400 μL min^{-1} stream was passed through a PFA coil (0.8 mm inner diameter; 8 mL reactor volume, 20 min residence time) set at 50 °C using an oil bath, theoretically resulting in an isocyanide stream of 0.05 mmol min^{-1}.

Azide Formation. A feed consisted of 2-bromoacetic acid **8a** (2.0 mmol) dissolved in acetonitrile (2 mL) was pumped with a flow rate of 100 μL min^{-1} and mixed in a T-shaped mixing unit with a second feed with an identical flow rate containing a 1.5 M solution of tetrabutylammonium azide (3.0 mmol in 2 mL acetonitrile). For better control of the process, the reagents were each stored in a 2 mL sample loop connected via a 6-way valve and simultaneously injected into the flow system. After mixing, the resulting 200 μL min^{-1} stream was passed through a PFA coil (0.8 mm inner diameter; 3 mL reactor volume, 15 min residence time) set at 100 °C using an oil bath, theoretically resulting in an azide stream of 0.1 mmol min^{-1} (2 equiv).

U-4CR. A solution of *tert*-butylamine **3a** (0.5 M) and paraformaldehyde **4a** (0.5 M) in methanol was constantly pumped with a flow rate of 200 μL min^{-1} (0.1 mmol min^{-1}, 2 equiv) and mixed with the outcome of the *isocyanide generation* and the *azide formation* in a cross shaped mixing device. The resulting reaction stream (800 μL min^{-1}) was heated at 80 °C in a PFA coil (0.8 mm inner diameter, 4 mL reactor volume, 5 min residence time). The reaction mixture was collected after depressurization by passing a static 7 bar backpressure regulator. Purification by flash column chromatography resulted in linear peptoid **5a** in 80% yield (71 mg, 0.2 mmol) as a colorless oil. Data obtained by mass spectrometry, ^1H NMR, and ^{13}C NMR are identical to those from the corresponding batch experiment (see above).

General Procedure for the Continuous CuAAC Using a Copper Coil. A solution of the respective linear peptoid **5a−h** in MeCN/MeOH (1:1; 2 mM) was pumped at 800 μL min^{-1} through a

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copper coil (2 mm inner diameter, 20 mL internal volume) heated at 140 °C in a GC oven. After passing a back pressure regulator set at 10 bar, the reaction mixture was collected and the solvent was removed under reduced pressure. Isolation by preparative HPLC resulted in the cyclic peptoids **6a**–**h** in analytical purity.

Cyclic Peptoid 6a. Prepared from peptoid **5a** (50 mg, 0.140 mmol) in 70 mL of MeCN/MeOH (1:1). Isolation afforded the title compound in 74% yield (37 mg, 0.052 mmol) as a colorless solid, mp 254–256 °C (dec.). HRMS (ESI): *m/z*: calcd for $C_{36}H_{47}N_{10}O_6$ $[M + H]^+$: 715.367456, found: 715.360619. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.59 (brs, 2H), 8.10 (s, 2H), 7.25 (d, J = 8.4 Hz, 4H), 7.03 (d, J = 8.4 Hz, 4H), 5.35 (s, 4H), 5.11 (s, 4H), 4.35–4.20 (m, 4H), 4.15 (s, 4H), 1.32 (s, 18H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.3, 167.1, 157.7, 142.7, 131.7, 129.2, 126.8, 115.0, 61.4, 58.2, 52.9, 47.2, 42.3, 28.4; FT-IR (KBr, cm^{-1}) 3303, 3075, 2977, 2931, 1658, 1612, 1585, 1543, 1509, 1463, 1435, 1364, 1218, 1191, 812, 586.

Cyclic Peptoid 6b. Prepared from peptoid **5b** (50 mg, 0.135 mmol) in 67.5 mL of MeCN/MeOH (1:1). Isolation afforded the title compound in 81% yield (39 mg, 0.11 mmol) as a colorless solid, mp 240–242 °C. HRMS (ESI): *m/z*: calcd for $C_{19}H_{26}N_5O_3$ $[M + H]^+$: 372.201679, found: 372.202446. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.43 (brs, 1H), 8.18 (s, 1H), 7.17 (d, J = 8.3 Hz, 2H), 6.97 (d, J = 8.3 Hz, 2H), 5.08 (s, 2H), 4.53 (t, J = 6.5 Hz, 2H), 4.23 (d, J = 4.9 Hz, 2H), 4.01 (s, 2H), 2.93 (d, J = 6.7 Hz, 2H), 1.31 (s, 9H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.1, 169.7, 157.5, 142.9, 131.9, 129.1, 125.2, 115.0, 61.5, 57.5, 48.0, 46.5, 42.2, 35.9, 28.6; FT-IR (KBr, cm^{-1}) 3318, 3131, 3076, 2970, 1676, 1637, 1606, 1509, 1294, 1093, 817, 596.

Cyclic Peptoid 6c. Prepared from peptoid **5c** (50 mg, 0.130 mmol) in 65 mL of MeCN/MeOH (1:1). Isolation afforded the title compound in 81% yield (41 mg, 0.105 mmol) as a colorless solid, mp 259–261 °C. HRMS (ESI): *m/z*: calcd for $C_{20}H_{28}N_5O_3$ $[M + H]^+$: 386.218666, found: 386.218942. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.26 (brs, 1H), 7.68 (s, 1H), 7.10 (d, J = 7.9 Hz, 2H), 6.81 (d, J = 7.9 Hz, 2H), 5.26 (s, 2H), 4.23 (s, 2H), 3.99 (brs, 2H), 3.75 (s, 2H), 1.91 (brs, 2H), 1.56 (brs, 2H), 1.29 (s, 9H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.7, 169.8, 157.5, 143.1, 132.0, 129.1, 128.9, 124.9, 115.0, 61.6, 57.3, 49.5, 48.1, 42.2, 32.3, 28.6, 26.4; FT-IR (KBr, cm^{-1}) 3293, 2962, 2929, 1645, 1585, 1508, 1458, 1421, 1394, 1334, 1216, 1176, 999, 807, 668, 509.

Cyclic Peptoid 6d. Prepared from peptoid **5d** (50 mg, 0.125 mmol) in 62.5 mL of MeCN/MeOH (1:1). Isolation afforded the title compound in 80% yield (40 mg, 0.10 mmol) as a colorless solid, mp 213–215 °C. HRMS (ESI): *m/z*: calcd for $C_{21}H_{30}N_5O_3$ $[M + H]^+$: 400.234316, found: 400.234235. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.39 (brs, 1H), 8.20 (s, 1H), 7.19 (d, J = 8.2 Hz, 2H), 6.98 (d, J = 8.3 Hz, 2H), 5.09 (s, 2H), 4.34 (t, J = 6.2 Hz, 2H), 4.23 (d, J = 4.9 Hz, 2H), 3.98 (s, 2H), 2.30–2.15 (m, 2H), 1.83–1.70 (m, 2H), 1.54–1.36 (m, 2H), 1.30 (s, 9H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.4, 170.0, 157.5, 143.1, 132.1, 129.1, 124.8, 115.0, 61.6, 57.1, 49.7, 48.1, 42.2, 34.7, 29.8, 28.7, 22.2; FT-IR (KBr, cm^{-1}) 3353, 3125, 3078, 2978, 2965, 2938, 1645, 1508, 1300, 1060, 1032, 993, 979, 881.

Cyclic Peptoid 6e. Prepared from peptoid **5e** (50 mg, 0.121 mmol) in 61 mL of MeCN/MeOH (1:1). Isolation afforded the title compound in 83% yield (41 mg, 0.10 mmol) as a colorless solid, mp 268–270 °C. HRMS (ESI): *m/z*: calcd for $C_{22}H_{32}N_5O_3$ $[M + H]^+$: 414.248629, found: 414.249174. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.42 (t, J = 6.2 Hz, 1H), 8.11 (s, 1H), 7.15 (d, J = 8.6 Hz, 2H), 6.86 (d, J = 8.6 Hz, 2H), 5.31 (s, 2H), 4.31 (brs, 2H), 4.10 (brs, 2H), 3.85 (s, 2H), 1.80–1.60 (m, 4H), 1.37 (s, 9H), 1.29–1.18 (m, 2H), 0.66–0.52 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.7, 170.0, 157.5, 143.0, 132.1, 129.1, 124.8, 114.9, 61.6, 57.0, 49.7, 48.1, 42.1, 35.2, 30.1, 28.7, 26.0, 24.6; FT-IR (KBr, cm^{-1}) 3348, 3130, 3077, 2940, 2873, 1508, 1465, 1438, 1418, 1170, 1089, 846, 704, 580.

Cyclic Peptoid 6f. Prepared from peptoid **5f** (50 mg, 0.180 mmol) in 90 mL of MeCN/MeOH (1:1). Isolation afforded the title compound in 86% yield (43 mg, 0.155 mmol) as a colorless solid, mp 236–238 °C (dec.). HRMS (ESI): *m/z*: calcd for $C_{13}H_{20}N_5O_2$ $[M + H]^+$: 278.161151, found: 278.160641. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.92 (d, J = 7.8 Hz, 1H), 7.71 (s, 1H), 5.13 (s, 2H), 4.73 (s, 2H), 4.08 (s, 2H), 3.61–3.45 (m, 1H), 1.75–1.60 (m, 4H), 1.60–1.50 (m, 1H), 1.33–1.01 (m, 5H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.4, 163.0, 129.2, 129.0, 49.2, 48.7, 48.2, 43.5, 32.9, 25.6, 25.0; FT-IR (KBr, cm^{-1}) 3294, 2927, 2855, 1643, 1556, 1494, 1444, 1422, 1410, 1375, 1348, 1304, 1275, 1252, 1098, 988, 828, 693.

Cyclic Peptoid 6g. Prepared from peptoid **5g** (50 mg, 0.172 mmol) in 86 mL of MeCN/MeOH (1:1). Isolation afforded the title compound in 91% yield (46 mg, 0.156 mmol) as a colorless solid, mp 151–153 °C. HRMS (ESI): *m/z*: calcd for $C_{14}H_{22}N_5O_2$ $[M]^+$: 292.176801, found: 292.176707. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (s, 1H), 5.87 (d, J = 7.8 Hz, 1H), 4.69 (s, 2H), 4.67–4.61 (m, 2H), 4.00 (s, 2H), 3.68–3.53 (m, 1H), 3.17–3.09 (m, 2H), 1.79–1.68 (m, 2H), 1.66–1.46 (m, 3H), 1.33–1.16 (m, 2H), 1.13–0.92 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.6, 166.7, 131.7, 131.5, 51.6, 48.5, 45.4, 43.0, 32.8, 32.6, 25.4, 24.7; FT-IR (KBr, cm^{-1}) 3297, 3067, 2930, 2854, 11646, 1540, 1449, 1292, 1239, 1133, 1047, 891, 726, 644.

Cyclic Peptoid 6h. Prepared from peptoid **5h** (50 mg, 0.14 mmol) in 70 mL of MeCN/MeOH (1:1). Isolation afforded the title compound in 71% yield (36 mg, 0.051 mmol) as a colorless solid, mp 236–238 °C (dec.). HRMS (ESI): *m/z*: calcd for $C_{34}H_{39}N_{10}O_8$ $[M + H]^+$: 715.294685, found: 715.294219. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.35 (t, J = 5.8 Hz, 2H), 8.06–8.00 (m, 2H), 7.80 (s, 2H), 7.63–7.55 (m, 2H), 7.53–7.35 (m, 4H), 4.89 and 4.84 (2 s, 2H), 4.63–4.51 (m, 2H), 4.32–4.17 (m, 2H), 3.95–3.79 (m, 4H), 3.63 (s, 6H), 2.30–1.78 (m, 8H), 1.31–1.12 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.5, 170.6, 168.7, 142.6, 138.8, 130.8, 129.6, 129.4, 128.7, 122.0, 52.2, 51.3, 48.6, 30.0, 25.4; FT-IR (KBr, cm^{-1}) 3282, 3083, 2932, 1750, 1658, 1552, 1487, 1372, 1175, 970, 759, 662, 552.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Detailed description of all continuous flow setups; ^{1}H and ^{13}C NMR spectra of all compounds, and X-ray crystallographic data for compound **6e**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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