Sequential Nucleophilic Substitution on Halogenated Triazines, Pyrimidines, and Purines: A Novel Approach to Cyclic Peptidomimetics

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A novel concept for the synthesis of macrocyclic peptidomimetics which incorporate heteroaromatic units is reported. The method involves sequential S_NAr reactions of orthogonally protected amino groups of peptides and other linear oligomers on halogenated heterocycles such as 2,4,6-trichloro-[1,3,5]triazine, 2,4,6-trichloropyrimidine, 4,6-dichloro-5-nitropyrimidine, and 2,6,8-trichloro-7-methylpurine. The scope of this novel solid-phase approach was systematically evaluated by means of the SPOT-synthesis methodology on planar cellulose membranes. Besides the question of the accessibility of different ring sizes and the compatibility with protecting groups of commonly used amino acids, the applicability of the technique toward different halogenated heteroaromatics and peptidomimetics was studied. It was found that the procedure is well suited to assemble a wide variety of cyclic peptidomimetics differing in both size (11- to 37-membered rings) and chemical nature of the assembled backbones.

Introduction

Peptidomimetic drug design has emerged as an important tool for medicinal chemists in both academia and industry.[1] Most of these efforts involve the development of compounds that show, compared to the parental peptide sequence, increased proteolytic stability, improved bioavailability, decreased side effects, and higher selectivity and potency.[2] Strategies for the design of such peptidomimetics has involved the synthesis of peptides containing nonnatural and conformationally restricted amino acids, the incorporation of amide-bond isosteres, template modification using pharmacophore models, and cyclization reactions.[3] Since the cyclization technique is especially suited for the stabilization of a putative bioactive conformation, numerous macrocyclization procedures have been developed including the incorporation of lactams, disulfides, urethanes, esters, lanthionines, and others as bridges in the assembly of cyclic peptidomimetics.[4] However, despite the topological constraints introduced in the cyclization process, such cyclic compounds may still possess remarkable flexibility.[5] In contrast, the incorporation of aromatic rings into the backbone of cyclic peptidomimetics was reported to impose severe conformational restrictions due to ring planarity.[3] Several recent reports have described procedures for the incorporation of aromatic rings such as benzylic and phenolic systems into cyclic peptides.[6−8] Usually, synthesis is performed via a two-step procedure involving incorporation of the aromatic system into the linear peptide chain and subsequent cyclization utilizing a second functionality of the corresponding arene-containing building block. Among the most widely used systems are aryl-containing amino acids such as 3-aminobenzoic acid[9] or tyrosine,[10] and bis-electrophilic aromatic moieties such as bis(bromomethyl)benzenes,[11] 2-(bromomethyl)benzoyl chloride,[12] 1,3-benzenedicarbonyl dichloride,[7] or 2-fluoro-5-nitrobenzoyl derivatives[8,13] as well as arylic building blocks bearing both electrophilic and

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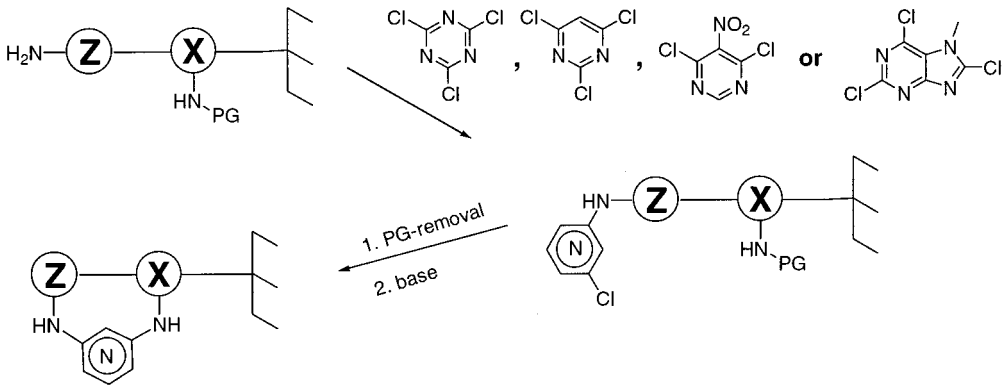
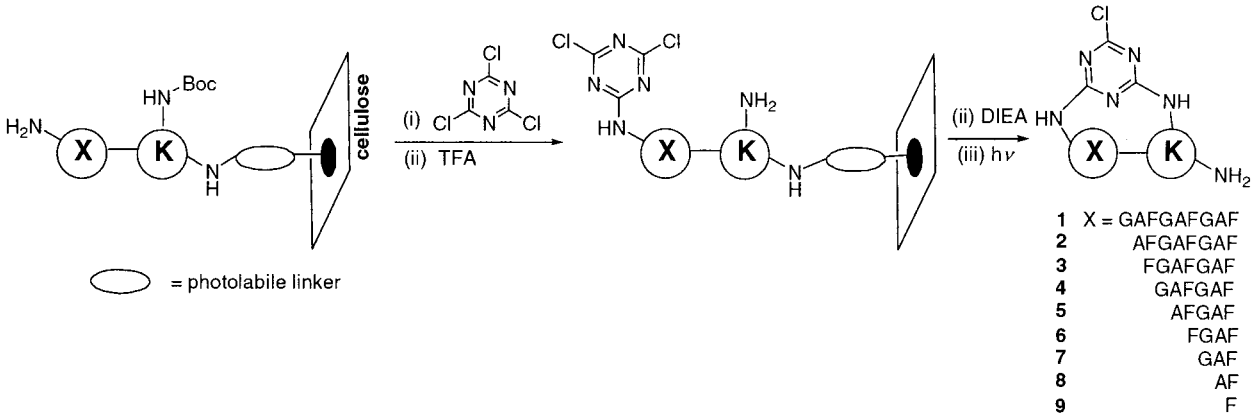


Figure 1. General cyclization procedure for linear oligomers Z−X bearing two orthogonally protected amino groups by S_NAr reactions at halogenated heteroaromatic systems.

Scheme 1. Cyclization of Di- to Decapeptides by Sequential Nucleophilic Substitution at 2,4,6-Trichloro-[1,3,5]triazine on Cellulose Membranes



nucleophilic functionalities.[14] However, the diversity of the accessible structures is limited, and only a few of the methods noted are applicable to modern solid-phase synthesis strategies. The present study describes an investigation of a new solid-phase cyclization procedure based on the sequential nucleophilic attack of two orthogonally protected amino functions of a linear peptide or peptidomimetic onto halogenated heteroaromatics such as 2,4,6-trichloro-[1,3,5]triazine,[15] 2,4,6-trichloropyrimidine, 4,6-dichloro-5-nitropyrimidine, and 2,6,8-trichloro-7-methylpurine to yield cyclic peptidomimetics (Figure 1).

Results and Discussion

The SPOT-synthesis methodology[16] was applied to chemically screen the scope of the solid-phase procedure with regard to the formation of different ring sizes, compatibility of the method with side-chain protecting groups of commonly used amino acids, and application to different peptidomimetics and various halogenated heteroaromatics.

Initially, the method was tested on the cyclization of peptides of varied chain length. Thus, the model peptide GAFGAFGAFK and the corresponding analogues systematically truncated by one to nine *N*-terminal amino acids were obtained by stepwise Fmoc-based solid-phase SPOT-synthesis on a photolinker-modified cellulose membrane.[17] Assembly was followed by S_NAr-type attachment of 2,4,6-trichloro-[1,3,5]triazine onto the *N*-terminus of the peptides followed by deblocking of the Boc protecting group at the Lys side-chain by trifluoroacetic acid (TFA). Cyclization was then achieved directly on the cellulose membrane via nucleophilic attack of the released amino function at the dichloro-[1,3,5]triazine moiety at room temperature within 30 min under basic conditions (20% *N*,*N*-diisopropylethylamine (DIEA) in *N*-methyl-2-pyrrolidinone (NMP)) (Scheme 1). Peptides were cleaved from the cellulose membrane by dry-state UV-irradiation of the planar carrier to yield compounds adhesively bound at the location of synthesis. The resulting SPOTs were punched out into the wells of a microtiter plate and dissolved to confirm their identity and purity by LC-MS. It was found that cyclization could be successfully achieved, and the main products corresponded in all cases

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Table 1. Cyclization of Model Peptides by S_NAr Reaction of the ϵ-Amino Group of the Lys-residue of the *N*-Terminus-Bound Dichloro-[1,3,5]triazine Unit

entry	sequence cyclized by 2,4,6-trichloro-[1,3,5]triazine		purity after cyclization [%]	*M* (calcd)	*M* (found) $[M + H]^+$
1	GAFGAFGAF**K**	**1**	89	1081.4	1082.5
2	AFGAFGAF**K**	**2**	71	1024.4	1025.4
3	FGAFGAF**K**	**3**	75	953.4	954.3
4	GAFGAF**K**	**4**	73	806.3	807.5
5	AFGAF**K**	**5**	76	749.3	750.5
6	FGAF**K**	**6**	65	678.3	679.3
7	GAF**K**	**7**	84	531.2	532.4
8	AF**K**	**8**	86	474.2	475.1
9	F**K**	**9**	76	403.1	404.2
10	K		n.d.[a]	256.1	-
11	GAFGAFGAF**K**		88	1081.4	1082.2
12	GAFGAFGA**K**F		86	1081.4	1082.3
13	GAFGAFG**K**AF		84	1081.4	1082.2
14	GAFGAF**K**GAF		81	1081.4	1082.2
15	GAFGA**K**FGAF		83	1081.4	1082.2
16	GAFG**K**AFGAF		81	1081.4	1082.2
17	GAF**K**GAFGAF		79	1081.4	1082.3
18	GA**K**FGAFGAF		71	1081.4	1082.1
19	G**K**AFGAFGAF		25	1081.4	1082.3
20	**K**GAFGAFGAF		n.d.[a]	1081.4	-

[a] No target molecule detected.

to the cyclic peptidomimetic having an incorporated monochloro triazine moiety (Table 1). In no case was cross-linking of two linear peptides by one triazine moiety observed.

In a subsequent experiment the ring sizes of peptides to be cyclized were systematically varied by a stepwise scan of one of the nucleophilic amino functions through the entire peptide. Again the peptide GAFGAFGAFK was used as model compound together with nine analogues in which the *C*-terminal Lys residue was shifted by one to nine positions in the *N*-terminal direction. Synthesis, cyclization, and analysis were performed as described above. LC-MS analysis of the crude products revealed again the high purity of the resulting cyclic peptidomimetics in most cases (Table 1). The low purity in the case of GKAFGAFGAF (Table 1, entry 19) is mainly related to impurities arising from the peptide synthesis step (incomplete incorporation of Lys). In no case (with exception of entries 10 and 20, Table 1) could the linear parental sequences with or without an attached triazine moiety be detected by LC-MS after the cyclization process. Interestingly, it was found that in both series the smallest ring size achievable in good purity was related to 13 atoms which corresponds to a cyclized dipeptide whereas ring closure of the Lys ϵ-amino function to the *N*-terminally attached dichloro-[1,3,5]triazine (10-membered ring) did not occur (Table 1, entries 10 and 20). In an effort to verify the minimal ring size accessible several compounds were synthesized with systematically reduced numbers of atoms in the rings obtained (Table 2). In agreement with the results summarized in Table 2 it could be shown that ring sizes between 11 and 16 atoms were achievable in good purities whereas 10-membered rings cannot be formed.

To evaluate the general applicability of the new cyclization approach with regard to side-chain compatibility of the commonly used amino acids 38 trimeric peptides based on Ala-Xxx-Lys and Xxx-Phe-Lys were synthesized, where Xxx corresponds to all proteinogenic amino acids except Cys (Table 2). The *C*-terminal Lys was incorporated using a 4-methyltrityl(Mtt)-side chain protecting group. Attachment of 2,4,6-trichloro-[1,3,5]triazine to the *N*-terminus and selective removal of the Mtt-group by 1% TFA in dichloromethane (DCM) containing 5% triisopropylsilane (TIPS) was followed by cyclization, deprotection of all other side-chains, and release of products from the cellulose membrane by UV-irradiation and analysis. Again all crude products were found to be sufficiently pure according to HPLC and MS (Table 3), demonstrating complete attachment of 2,4,6-trichloro-[1,3,5]triazine to the different *N*-termini as well as efficient cyclization of all amino acids used regardless of their position in the linear parental sequence.

Table 2. Determination of the Minimum Achievable Ring Size

entry	sequence	ring-size [no. atoms]	purity after cyclization/cleavage [%]
1	Phe-Lys	13	71
2	Phe-Orn	12	75
3	Phe-Dbu	11	60
4	Phe-Dpr	10	n.d.[a]
5	Ala-Phe-Lys	16	85
6	Ala-Phe-Orn	15	80
7	Ala-Phe-Dbu	14	80
8	Ala-Phe-Dpr	13	75

[a] No target molecule detected.

The remaining chloro substituent of the cyclic monochloro-[1,3,5]triazinyl peptides, not involved in the cyclization process, can be used for further modification at the aromatic unit. Accordingly, a set of 10 nucleophiles[18] was employed for substitution of the chlorine atom of the cyclic triazinyl-peptide **8** bound to the cellulose membrane. Reaction was supported by microwave irradiation, recently shown to have a rate-enhancing effect on nucleophilic substitution at solid support-bound monochloro triazines.[19] It was found that after attachment of the nucleophiles to the aromatic unit, all products were obtained in high purities,[18] enabling systematic modification of the molecular properties of the cyclic peptidomimetics.

To examine whether the new cyclization technique is applicable to oligomers other than peptides, a peptomer (peptide−peptoid hybrid oligomer)[20] and a urea-bearing system were synthesized by the SPOT technique on a modified cellulose membrane modified with a photolabile linker. Analogous to the peptide case, attachment of 2,4,6-trichloro-[1,3,5]triazine to the *N*-terminal amino function was followed by deprotection of a second amino group and cyclization under basic conditions. Cleavage of the cyclized peptidomimetics from the membrane surface by UV-irradiation and subsequent LC-MS analysis proved that the peptomer-derived ring (Figure 2, structure **10**) and the cyclic urea derivative (Figure 2, structure **11**)

(18) The following nucleophiles have been used under the conditions given in ref 19 (the number in brackets indicates the purity of the crude products obtained after cleavage): β-alanine-amide (79%), *p*-anisidine (81%), 1,5-diaminopentane (84%), 3-chlorobenzylamine (82%), *N*-methylpiperazine (92%), 4-phenoxyaniline (75%), 2-picolylamine (71%), piperidine (94%), tyramine (86%), and 4-fluorophenole as cesium salt (86%).

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Table 3. Purities of Tripeptides Cyclized via Sequential S_NAr Reactions on 2,4,6-Trichloro-[1,3,5]triazine

entry	sequence	side-chain protecting group	purity (crude) [%]	entry	sequence	side-chain protecting group	purity (crude) [%]
1	A−F−K	-	83	20	A−A-K	-	81
2	D−F−K	^{t}Bu	75	21	A−D-K	^{t}Bu	77
3	E−F−K	^{t}Bu	70	22	A−E-K	^{t}Bu	75
4	F−F−K	-	81	23	A−F−K	-	89
5	G−F−K	-	37	24	A−G-K	-	70
6	H−F−K	Trt	39	25	A−H−K	Trt	40
7	I−F−K	-	81	26	A−I−K	-	90
8	K−F−K	Boc	72[a]	27	A−K−K	Boc	80
9	L−F−K	-	83	28	A−L-K	-	88
10	M−F−K	-	77[b]	29	A−M-K	-	73[b]
11	N−F−K	Trt	76	30	A−N−K	Trt	82
12	P−F−K	-	83	31	A−P−K	-	39
13	Q−F−K	Trt	75	32	A−Q-K	Trt	81
14	R−F−K	Pmc	78	33	A−R−K	Pmc	85
15	S−F−K	^{t}Bu	73	34	A−S−K	^{t}Bu	79
16	T−F−K	^{t}Bu	75	35	A−T-K	^{t}Bu	80
17	V−F−K	-	81	36	A−V−K	-	91
18	W−F−K	Boc	82	37	A−W−K	Boc	86
19	Y−F−K	^{t}Bu	85	38	A−Y−K	^{t}Bu	88

[a] Summarizes HPLC peak areas of target molecule and trifluoroacetylated side product resulting from side chain deprotection by TFA. [b] Summarizes HPLC peak areas of sulfide and sulfoxide moiety.

Table 4. Optimization of the Cyclization Conditions for Selected Halogenated Heteroaromatics

entry	heteroaromatic	conditions[a]	ratio between linear and target molecule after cleavage
1	4,6-dichloro-5-nitropyrimidine	30 min room temp	1:20
2	4,6-dichloro-5-nitropyrimidine	1 h rt	1:50
3	2,4,6-trichloropyrimidine	1 h rt	19:1
4	2,4,6-trichloropyrimidine	12 h rt	19:1[b]
5	2,4,6-trichloropyrimidine	2 h 80 °C	2:3
6	2,4,6-trichloropyrimidine	5 h 80 °C	1:3[b]
7	2,4,6-trichloropyrimidine	2 × 3 min mw	1:50
8	2,6,8-trichloro-7-methylpurine	1 h rt	50:1
9	2,6,8-trichloro-7-methylpurine	14 h rt	19:1[b]
10	2,6,8-trichloro-7-methylpurine	2 h 80 °C	10:1[b]
11	2,6,8-trichloro-7-methylpurine	2 × 3 min mw	1:10
12	2,6,8-trichloro-7-methylpurine	3 × 3 min mw	1:50

[a] Solvent = 20% DIEA in NMP (v/v) rt = room temperature, mw = microwave irradiation [b] Partial decomposition of halogenated heteroaromatic was observed.

were as pure as the corresponding peptides having similar ring sizes.

Successful application of 2,4,6-trichloro-[1,3,5]triazine to the cyclization of peptides and peptidomimetics inspired additional studies to extend the cyclization procedure via sequential nucleophilic substitution to other halogenated heteroaromatic systems such as 2,4,6-trichloropyrimidine, 2,6,8-trichloro-7-methylpurine, and 4,6-dichloro-5-nitropyrimidine. It was shown that the model peptide **AFK** could be attached to all of these systems at the peptide *N*-terminus using slightly modified conditions (see Experimental Section). However, attachment of 2,4,6-trichloropyrimidine and 2,6,8-trichloro-7-methylpurine to the *N*-terminus of the **AFK** peptide resulted in mixtures of two products each having the same masses according to LC-MS. Since the parental peptide did not exhibit such a mixture, the resulting products were attributed to different regioisomers resulting from the nucleophilic substitution of the *N*-terminal amino function of the peptide at different positions on the heterocyclic ring. In the case of 2,4,6-trichloropyrimidine the two isomers displayed a ratio of 3:2 whereas in the case of 2,6,8-trichloro-7-methylpurine a ratio of 3:1 was found. In accordance with previous results,[21] the major product of the 2,4,6-trichloropyrimidine attachment was tentatively assigned to nucleophilic attack at the 4-position of the pyrimidine ring, whereas the main product resulting from the attachment of the purine derivative to the peptide was attributed to the attack at the 8 position.[22] Subsequent cyclization at room temperature under conditions applied for the triazine system gave only low cyclization yields of peptides incorporating pyrimidine and purine units. Prolonging the reaction time improved cyclization in the case of 4,6-dichloro-5-nitropyrimidine (Table 4 entry 2 and Figure 2, **14**), whereas with 2,4,6-trichloropyrimidine and 2,6,8-trichloro-7-methylpurine results were not significantly improved (Table 4, entries 3, 4, 8, and 9). Therefore, increased temperatures and microwave irradiation were examined. Short microwave irradiation (3 min 810 W two and three times for 2,4,6-trichloropyrimidine and 2,6,8-trichloro-7-methylpurine, respectively) resulted in products of higher purity (Table 4, entries 7 and 12) compared to cyclizations performed at increased temperatures for several hours (Table 4, entries 6 and 10). After cyclization the corresponding regioisomers could not be separated by HPLC. Since only small amounts of linear products were detected, when microwave irradiation was applied,

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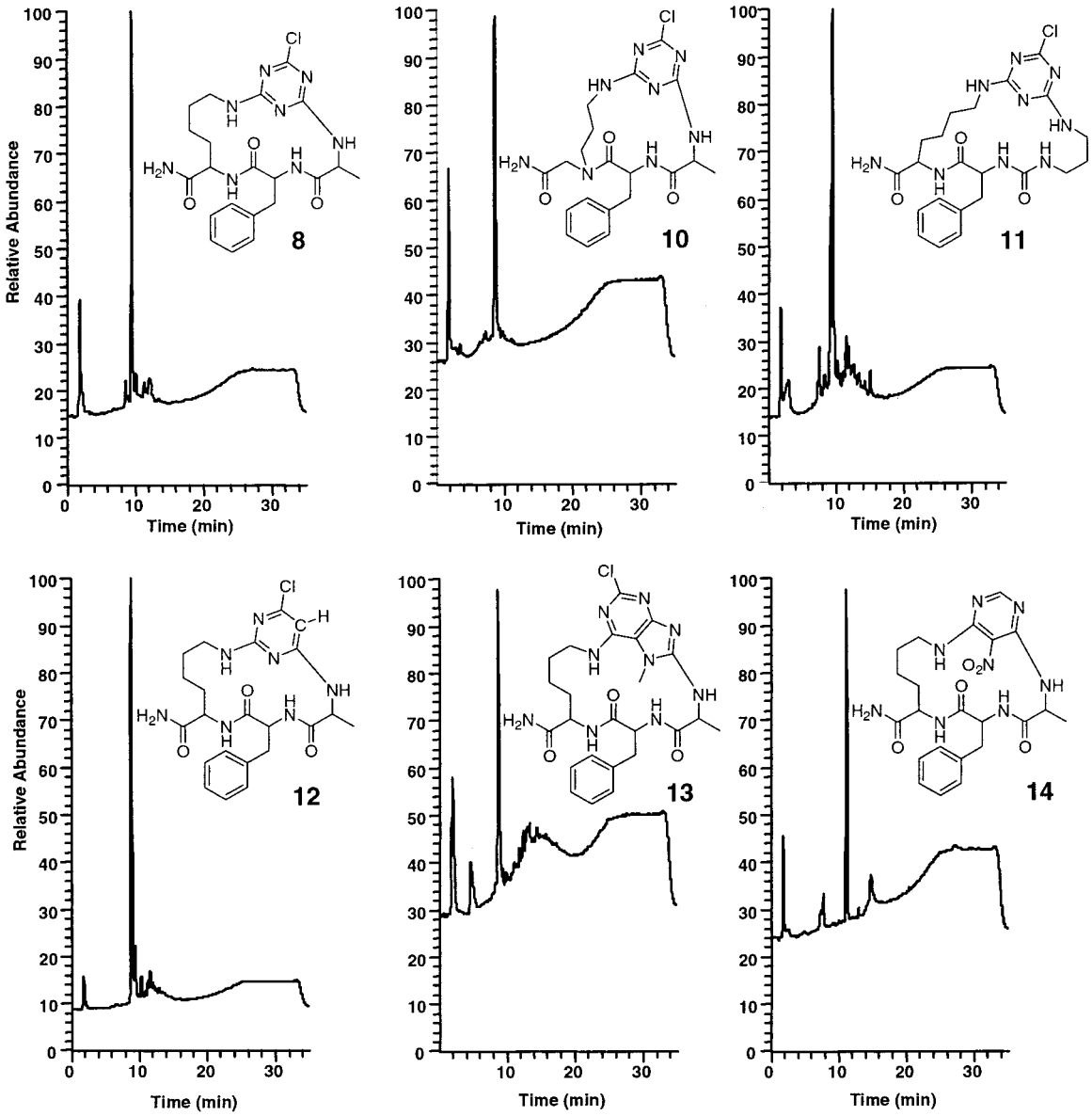

Figure 2. HPLC traces of cyclic oligomers obtained via sequential nucleophilic attack on various halogenated heterocyclic systems (structures **12** and **13** relate to the major isomer expected on the basis of the reactivity of the chlorine atoms of the corresponding scaffold).

the cyclization seems to proceed nearly quantitatively at both linear oligomers. The HPLC purities of the crude products obtained are shown in Figure 2 (**12** and **13**).

Conclusion

An efficient solid-phase approach was developed for the synthesis of cyclic peptidomimetics which incorporate aromatic heterocycles. Sequential nucleophilic substitution on 2,4,6-trichloro-[1,3,5]triazine by the orthogonally protected amino functions of a linear peptide or peptidomimetic was successfully applied to give cyclic modified peptides of various ring sizes (11- to 37-membered rings) and cyclic backbone structures. It was shown that the new cyclization method can be employed along with the commonly used side-chain protecting groups in peptide synthesis. Besides 2,4,6-trichloro-[1,3,5]triazine, a variety of other halogenated heteroaromatics such as 2,4,6-trichloropyrimidine, 4,6-dichloro-5-nitropyrimidine, and 2,6,8-trichloro-7-methylpurine can serve as the ring-closure scaffolds. SPOT-synthesis was found to be an efficent tool to chemically screen the scope of these new solid-phase reactions. Studies are in progess to extend this method to targets of biological relevance.

Experimental Section

General Methods. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Unity-plus 500 MHz spectrometer using TMS as internal standard. Chemical shifts are given in parts per million. HRMS were obtained on a MAT 95ST (Trap) equipped with a "Cs-gun" and on a Micromass AutoSpec by LSIMS-FAB measurement directly from the compounds synthesized on cellulose. A HP 1100 LC (Hewlett-Packard, Waldbronn, Germany) combined with an LCQ MS instrument (Finnigan MAT, Bremen, Germany) equipped with an ESI-source was used to follow all reactions. All HPLC experiments were carried out with a VYDAC C-18 column (15 × 0.21 cm) using a linear gradient: eluent A 0.05% TFA in water; eluent B 0.05% TFA in acetonitrile, λ = 220 nm; flow rate 0.3 mL/min.

Materials. Cellulose sheets (Whatman 50) were purchased from Whatman, Maidstone, UK. Reagents were obtained from

Sigma-Aldrich GmbH, Steinheim, Germany, and Lancaster Synthesis GmbH, Mühlheim/Main, Germany, except bulk solvents which were purchased from Merck KGaA, Darmstadt, Germany. Amino acid derivatives were provided by Calbiochem-Novabiochem, Bad Soden, GmbH, Germany.

Washing. If not otherwise noted, all membranes were washed in a stainless steel dish between each reaction step with DMF, MeOH, and DCM three times each.

Heating. For heating of the membranes a steel dish placed on a heating plate (80 °C) or a glass dish in a microwave oven (810 W, Eletronia, Selters, Germany) was used.

Cleavage. The air-dried cellulose-membranes were placed on a UV table (λ_{max} = 365 nm, 7 mW/cm^2,Vilber Lourmat TFX-20LC, Montpellier, France) for 120 min for each side. The SPOTs containing the adsorbed products were punched out into the wells of a microtiter plate to confirm identity and purity of the dissolved products by LC-MS

SPOT-Synthesis of Peptides.[23] A 2 μL amount of a 0.6 M solution of the corresponding Fmoc-protected amino acid pentafluorophenyl ester was pipetted onto the 4-[4-(1-aminoethyl)-2-methoxy-5-nitrophenoxy]butyramido-modified cellulose membrane[17] on a 1 × 1 cm grid, which was drawn on the cellulose with a soft pencil prior to the pipetting steps. After 20 min, the procedure was repeated twice followed by washing and drying in air. Capping of unreacted amino groups by acetylation was achieved by immersing the membrane in a 20% solution of acetic anhydride in DMF containing 10% DIEA (v/v/v) followed by washing and drying. After removal of the *N*-terminal Fmoc group by immersing the membrane in a 20% solution of piperidine in DMF (v/v) (2 × 20 min) followed by washing and drying in air, the procedure was repeated with the next amino acid until the desired sequence had been synthesized.

Peptides Cyclized with 2,4,6-Trichloro-[1,3,5]triazine (1−9). 2,4,6-Trichloro-[1,3,5]triazine was attached to the *N*-termini of the various peptides by immersing the membrane in a 4 M solution of 2,4,6-trichloro-[1,3,5]triazine in DCM for 20 min. After washing, the Boc-group at the ϵ-amino function of Lys was removed by treating the cellulose-bound dichlorotriazinyl peptides with 80% TFA in DCM for 1 h. Ring closure was achieved through nucleophilic substitution of the second chlorine atom at the *N*-terminally attached dichloro-[1,3,5]-triazines by the deprotected ϵ-amino group of Lys by immersing the cellulose membrane in a solution of 20% DIEA in NMP (v/v) for 30 min at room temperature. After being washed and dried, the compounds were cleaved from the membrane, dissolved in acetonitrile−water 1:1, and analyzed by LC-MS.

For NMR analysis, cyclized AFK **8** was resynthesized on a 5 × 5 cm cellulose membrane. Linker-attachment and peptide-synthesis was performed as previously described, except that larger amounts of building blocks were used (750 μL per coupling step). After triazine attachment, side-chain deprotection, cyclization, and cleavage, 1.6 mg (71%) of **8** was obtained. ^{1}H NMR (DMSO-d_6): δ 7.88 (d, J = 8 Hz, 1H), 7.77 (d, J = 6 Hz, 1H), 7.66 (d, J = 9 Hz, 1H), 7.3−7.1 (m, 6H), 6.92 (d, J = 28 Hz, 2H), 4.58 (t, J = 8 Hz, 1H), 4.10 (dd, J = 4 Hz, J = 9 Hz, 1H), 3.99 (m, 1H), 3.31 (m, 1H), 2.91 (d, J = 9 Hz, 2H), 2.18 (t, J = 8 Hz, 2H), 2.00 (m, 2H), 1.89 (m, 2H), 1.08 (d, J = 8 Hz, 3H). HRMS: [M + H] (calcd) = 475.1972, [M + H] (found) = 475.1976.

Lysine-Scan. The linear peptides were synthesized according to the general procedure outlined and cyclized with 2,4,6-trichloro-[1,3,5]triazine as described for peptides **1**−**9**.

Peptomer 10. The cellulose bound peptomer was synthesized according to the procedure given in ref 17 employing mono-Boc-1,3-diaminopropane. The [1,3,5]triazine moiety was attached to the *N*-terminus by immersing the membrane in a 4 M solution of 2,4,6-trichloro-[1,3,5]triazine in DCM for 20 min. After the membrane was washed, the Boc-group of the peptoid side-chain amino function was removed by treating the cellulose-bound dichlorotriazinyl peptoid with 80% TFA in DCM for 1 h. Ring-closure was achieved by nucleophilic substitution of the second chlorine atom of the *N*-terminally attached dichloro-[1,3,5]triazine by the deprotected amino side chain functionality of the peptoid unit by immersing the membrane in a 20% DIEA solution in NMP (v/v) for 30 min at room temperature followed by washing, drying, and cleavage.

For NMR analysis, **10** was resynthesized on 250 mg of Rink-Tentagel-resin. Synthesis was performed as described above by using larger amounts of the appropriate building blocks (2 mL per coupling step). After triazine attachment, side-chain deprotection and release from the solid support was achieved in parallel by TFA (95% in DCM). After removal of TFA cyclization of the peptide−triazine derivative was completed within 30 min in a 1 mM solution in acetonitrile containing 5% DIEA. Evaporation to dryness afforded 9.8 mg (96%) of crude **10**. The material was purifed by HPLC (t_R = 9.6 min) giving 7.9 mg. ^{1}H NMR (DMSO-d_6): δ 7.88 (d, J = 7 Hz, 1H), 7.58 (m, 2H), 7.46 (d, J = 8 Hz, 1H), 7,28−7.13 (m, 3H), 4,73 (d, J = 18 Hz, 1H), 4.28 (m, 1H), 4.31 (m, 1H), 3.8−3.5 (m, 6H), 2.97 (t, J = 8 Hz, 2H), 2.76 (d, J = 11 Hz, 2H), 1.72 (m, 1H), 1.61 (m, 1H), 1.10 (d, J = 8 Hz, 3H). ^{13}C NMR (DMSO-d_6): δ 173.86, 172.31, 170.67, 167.37, 165.31, 164.65, 139.14, 127.81, 125.96, 52.79, 49.89, 47.30, 35.20, 34.28, 18.06, and 16.85. HRMS: [M + H] (calcd) = 461.1816, [M + H] (found) = 461.1800.

Urea 11. FK(Boc) was synthesized according to the procedure described on a 4-[4-(1-aminoethyl)-2-methoxy-5-nitrophenoxy]butyramido-modified cellulose membrane.[17] After cleavage of the *N*-terminal Fmoc-group (20% piperidine in DMF (v/v), 2 × 20 min) and after being washed and dried in air, the membrane was immersed twice for 2 h each in a 1 M solution of *p*-nitrophenyl chloroformate in DCM containing 1% *N*-methylmorpholine (NMM). The activated carbamate was cleaved with 1,3-diaminopropane by immersing the cellulose membrane in a 5 M solution of the diamine in NMP at 60 °C for 4 h to give the corresponding urea. After the membrane was washed and dried in air, the 2,4,6-trichloro-[1,3,5]triazine was attached as previously described. The Boc group at the ϵ-amino function of Lys was cleaved, and cyclization was achieved as described above followed by washing, drying, and cleavage. HRMS: [M + H] (calcd) = 504.2238, [M + H] (found) = 504.2213.

AFK Cyclized with 2,4,6-Trichloropyrimidine (12). 2,4,6-Trichloropyrimidine was attached to the *N*-terminus of the cellulose-bound AFK(Boc) by immersing the membrane in a 50% solution in NMP containing 10% DIEA (v/v/v) at 80 °C for 2 h. After the membrane was washed and dried in air, the Boc-group of Lys was removed with 80% TFA in DCM for 1 h followed by washing and drying. Ring-closure was achieved under microwave irradiation (810W, 3 min, twice) of the membrane immersed in a 20% DIEA solution in NMP followed by washing, drying, and cleavage. HRMS: [M + H] (calcd) = 474.2020, [M + H] (found) = 474.2028.

AFK Cyclized with 2,6,8-Trichloro-7-methylpurine (13). 2,6,8-Trichloro-7-methylpurine was attached to the N-terminus of the cellulose-bound AFK(Boc) by immersing the membrane in a 50% solution in NMP containing 10% DIEA (v/v/v) at 80 °C for 2 h. After the membrane was washed and dried in air, the Boc-group of Lys was removed with 80% TFA in DCM for 1 h followed by washing and drying. Ring-closure was achieved under microwave irradiation (810W, 3 min, three times) of the membrane immersed in a 20% DIEA solution in NMP followed by washing, drying, and cleavage. HRMS: [M + H] (calcd) = 528.2238, [M + H] (found) = 528.2254.

AFK Cyclized with 4,6-Dichloro-5-nitropyrimidine (14). 4,6-Dichloro-5-nitropyrimidine was attached to the N-terminus of the cellulose-bound AFK(Boc) by pipetting a 2 M solution in NMP containing 1% DIEA (v/v) two times directly on the membrane at room-temperature, allowing a reaction time of 1 h each. After the membrane was washed and dried in air, the Boc-group of Lys was removed with 80% TFA in DCM for 1 h. Ring-closure was achieved through the deprotected ϵ-amino group of Lys by immersing the membrane in a solution of 20% DIEA in NMP (v/v) for 120 min at room

(23) Kramer, A.; Reineke, U.; Dong, L.; Hoffmann, B.; Hoffmüller, U.; Winkler, D.; Volkmer-Engert, R.; Schneider-Mergener, J. SPOT-synthesis: observations and optimizations. *J. Pept. Res.* **1999**, *54*, 319−327.

temperature followed by washing, drying, and cleavage. HRMS: [M + H] (calcd) = 484.2183, [M + H] (found) = 484.2187.

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Supporting Information Available: LC-MS data for cyclic peptides obtained from lysine-scan, truncation, Xxx-Phe-Lys and Ala-Xxx-Lys. This material is available free of charge via the Internet at http://pubs.acs.org.

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