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1,3,5-Triazepan-2,6-diones as Structurally Diverse and Conformationally Constrained Dipeptide Mimetics: Identification of Malaria Liver Stage Inhibitors from a Small Pilot Library

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Dedicated to the memory of the late Professor Murray Goodman

Abstract: The development of the 1,3,5-triazepan-2,6-dione system as a novel, conformationally restricted, and readily accessible class of dipeptidomimetics is reported. The synthesis of the densely functionalized 1,3,5-triazepan-2,6-dione skeleton was achieved in only four steps from a variety of simple linear dipeptide precursors. To extend the practical value of 1,3,5-triazepane-2,6-diones, a general polymer-assisted solution-phase synthesis approach amenable to library production in a multiparallel format was developed. The conformational preferences of the 1,3,5-triazepan-2,6-dione skeleton were investigated in detail by NMR spectroscopy and X-ray diffraction. The ring exhibits a characteristic folded conformation which was compared to that of related dipeptide-derived scaffolds including the more planar 2,5-diketopiperazine (DKP). Molecular and structural diversity was increased further through post-cyclization appending operations at urea nitrogens. Preliminary biological screens of a small collection of 1,3,5-triazepan-2,6-diones revealed inhibitors of the underexplored malaria liver stage and suggest strong potential for this dipeptide-derived scaffold to interfere with and to modulate biological pathways.

Keywords: heterocycles · molecular diversity · peptides · peptidomimetics · solid-phase synthesis

Introduction

The design and synthesis by combinatorial chemistry techniques of cyclic/polycyclic molecular frameworks that can efficiently distribute selected pharmacophores in the 3D space is an important method to identify small-molecules capable of modulating biological processes and for dissecting biological pathways.[1–3] In this context, molecules incorporating

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Supporting information for this article is available on the WWW under http://www.chemeurj.org/ or from the author and contains a ^{1}H NMR spectrum of crude TFA·H_2N-Phe-*g*Sar-COOSu (**3a**), an illustration of polymer-supported quenching for removal of electrophiles after alkylation of urea nitrogens, partial 500 MHz ROESY spectra of 1,3,5-triazepin-2,6-dione **4a**, **4i**, **4p**, and **10a**, stability of 1,3,5-triazepin-2,6-diones in [D_6]DMSO and H_2O, C_{18} RP-HPLC traces, FTIR, ^{1}H and ^{13}C NMR spectra for all compounds **4**–**13**, and toxicity of the different 1,3,5-triazepin-2,6-diones towards hepatocytes in vitro.

small or medium rings derived from peptides are of particular interest owing to their facile access, the chemical and stereochemical diversity of these peptide derivatives, and enhanced diversity resulting from post-cyclization appending operations. In addition, such molecules are potentially useful to constrain intrinsically short linear peptide chains and for mimicking local folded polypeptide structures, such as β-turns. The piperazin-2,5-dione or 2,5-diketopiperazine (DKP) **A** (Figure 1) is the archetype of a peptide-derived heterocycle and has been the subject of considerable interest and numerous studies.[4] This essentially planar head-to-tail cyclodipeptide structure characterizes the core of many natural products (for example, demethoxyfumitremorgin C, tryprostatins, brevianamides, (iso)roquefortine C, rosstratins A, and vertihemiptellides)[4b,5] and represents a versatile scaffold for new drug discovery.[6] Several routes suitable for library production have been developed, making the DKP scaffold readily available in combinatorial and multiparallel format for biological screens.[7] In recent years, elegant synthetic pathways amenable to polymer-supported chemistry have been explored to transform short peptide fragments into novel heterocyclic scaffolds through backbone cyclization.[8–10] However, the possibility to rapidly generate conformationally defined DKP-related dipeptidomimetics with enlarged ring and nonplanar geometry through simple synthetic manipulation of the dipeptide backbone has remained underused.[11]

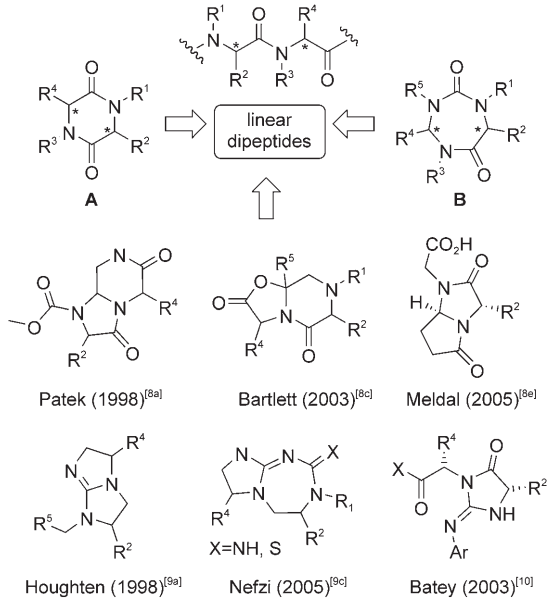
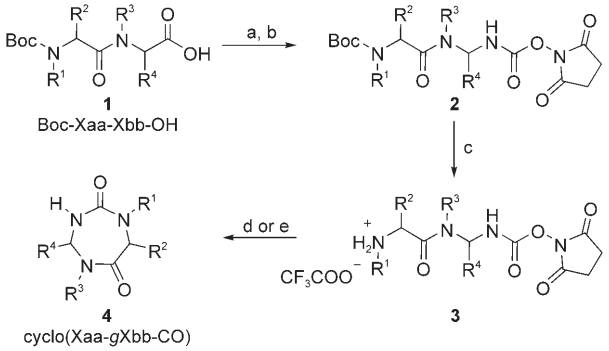


Figure 1. Skeletal diversity from simple dipeptide substrates.

We report a general synthetic approach leading to the densely functionalized (five points of diversity) seven-membered 1,3,5-triazepan-2,6-dione scaffold **B** that further expands the skeletal and structural diversity attainable with simple dipeptide substrates. Our interest in developing and evaluating this previously unreported scaffold stemmed from the remarkable biological activities exhibited by molecules with diazepine and triazepine skeletons. In particular, seven-membered cyclic ureas have attracted much attention with application in the development of HIV-protease[12] and reverse transcriptase[13] inhibitors, Factor Xa inhibitors,[14] β-lactamases inhibitors,[15] phospholipase C inhibitors,[16] and chemokine receptor antagonists.[17] A polymer-assisted solution-phase synthesis appropriate for library construction has been developed, and post-cyclization diversification at urea nitrogens examined. Conformational and structural features of the 1,3,5-triazepan-2,6-dione were investigated in detail with comparison to related peptide-derived heterocyclic systems and the utility of the scaffold was demonstrated by screening a small "prospecting" library against the malaria liver stage (LS) which holds great promise for drug targeting.

Results and Discussion

Solution-phase synthesis: As shown in Scheme 1, 1,3,5-triazepan-2,6-diones **4**[18] are constructed in only four steps by head-to-tail cyclization of simple activated dipeptide derivatives, and the approach benefits from the considerable diversity of commercial α-amino acids.[19] The general cyclization strategy parallels our previously reported approach to the preparation of enantiopure macrocyclic oligoureas.[20]



Scheme 1. Solution-phase synthesis of 1,3,5-triazepan-2,6-diones **4**. a) EtOCOCl, NMM, THF, −20 °C, then NaN_3 in H_2O; b) toluene, 65 °C, then HOSu, py; c) TFA, 30 min; d) DIEPA, MeCN; e) PS-DIPEA, CH_2Cl_2.

It involves a key linear diamino precursor activated at one end as a succinimidyl carbamate and protected at the other end by a Boc group. Succinimidyl carbamates **2** were prepared from Boc-dipeptides **1** as previously described for *N*-protected α- and β-amino acids.[21] Selective removal of the Boc group by treatment of crude **2** with trifluoroacetic acid (TFA) afforded **3**[23] which cyclized to **4** in the presence of diisopropylethylamine (DIPEA). The cyclization proceeded rapidly with concomitant release of *N*-hydroxysuccinimide (HOSu) and formation of the TFA salt of DIPEA. These byproducts were removed by either recrystallization of crude **4**, liquid/liquid extraction, or polymer supported se-

questration (PSS) with tris-(2-aminoethyl)amine polystyrene (PS-triamine).[23] Alternatively, polymer-supported DIPEA (PS-DIPEA) was found to be very effective in promoting cyclization and simultaneously removing byproducts (see Supporting Information).

A set of 18 monocyclic to tetracyclic 1,3,5-triazepin-2,6-diones **4a–s** were synthesized by this approach in moderate to high overall yield starting from a variety of dipeptide sequences (Table 1, Figure 2).

Table 1. Solution-phase synthesis of 1,3,5-triazepan-2,6-diones **4** from dipeptides **1**.

Entry	1	Xaa	Xbb	Yield **3** [%][a]	Yield **4** [%][b,c]
1	**a**	L-Phe	Sar	93	71[d](82[e])
2	**b**	D-Phe	Sar	78	74[d]
3	**c**	L-Val	Sar	95	67[f]
4	**d**	L-Leu	Sar	86	34[f]
5	**e**	L-Dap(Fmoc)	Sar	72	43[f]
6	**f**	L-Phg	Sar	58	41[f](67[e])
7	**g**	L-2-Nal	Sar	50	77[f]
8	**h**	L-Tic	Sar	59	68[d]
9	**i**	L-Phe	L-Pro	90	66[d]
10	**j**	L-2-Nal	L-Pro	82	78[d]
11	**k**	L-Tic	L-Pro	77	57[d]
12	**l**	L-Phe	L-*N*-MePhe	34	97[d]
13	**m**	L-Phe	L-Hyp(Bn)	73	99[f,g]
14	**o**	L-Ala	L-Tic	54	97[i]
15	**p**	D-Phe	L-Pro	80	66[h]
16	**q**	D-Phe	L-*N*-MeAla	60	48
17	**r**	L-Pro	L-Val	66	11[h,j,k]
18	**s**	L-Pro	L-Leu	58	7[h,j,l]

[a] Overall yields from **1**. [b] Yields from **3**. [c] Cyclizations performed by using DIPEA unless otherwise stated. [d] Purification by recrystallization. [e] PS-DIPEA used for cyclization. [f] Purification by flash-column chromatography (CH_2Cl_2/MeOH/AcOH 97:3:1). [g] Hydrogenation of the benzyl group in **4m** afforded triazepandione **4n** with a hydroxyl side chain in 70% yield. [h] Purification by C_{18} RP-HPLC. [i] Purification by using PS-triamine. [j] Cyclization performed with *N*-methyl morpholine at a concentration of 10^{-3} M. The formation of **4** was accompanied by the formation of the corresponding cyclodimer **5**. [k] **4r**/**5r** 54:46. **5r** isolated in 35% yield. [l] **4s**/**5s** 50:50. **5s** isolated in 10% yield. Abbreviations: Dap = Diaminopropanoic, Phg = Phenylglycine, 2-Nal = 2-naphtylphenylalanine, Tic = Tetrahydroisoquinoline-3-carboxylic acid, Hyp = *cis*-4-Hydroxyproline.

Carbamates **2a–m** and **2o** bearing a tertiary amide N-terminal to the *g*Xbb residue ($R^3 \neq H$) were expected to give an equilibrium mixture between *trans* and *cis* isomers (a ~1:1 ratio was observed for **3a** by ^1H NMR spectroscopy in CD_3CN, see the Supporting Information) and as a result readily cyclize to **4** (entries 1–14).

In the case of carbamates derived from sequences composed of heterochiral residues (entries 15 and 16), cyclization to **4** was also found to proceed readily. Minor epimerization (approximately 5–10%) was observed at the *gem*-diamino residue during cyclization of **3p**. In contrast, cyclization of precursors **3** with a secondary amide N-terminal to *g*Xbb ($R^3 = H$, entries 17 and 18) gave **4** together with the corresponding 14-membered ring cyclodimer **5**. The ratio of monomer versus dimer was found to be strongly influenced by the concentration and the nature of the base. A higher

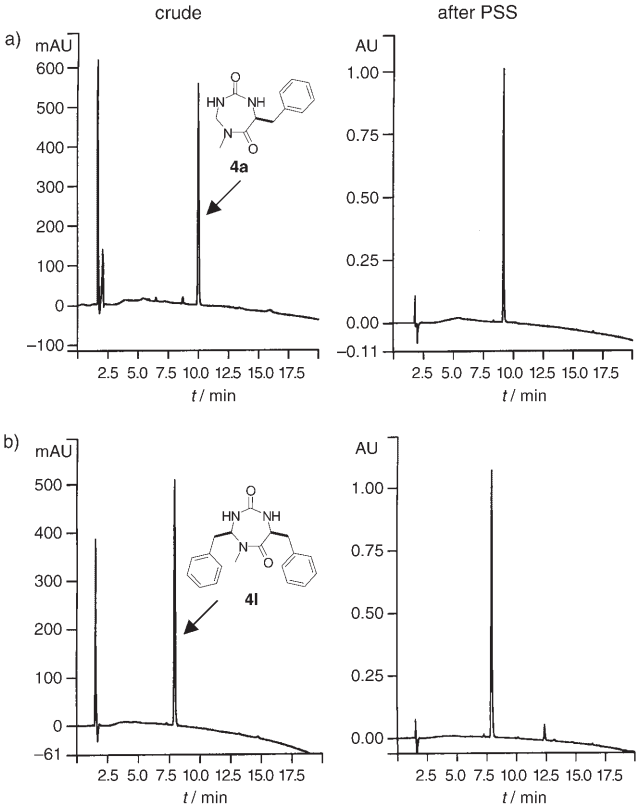
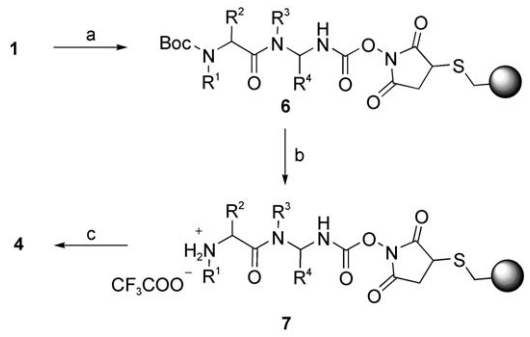


Figure 2. C_{18}-RP-HPLC traces of crude cyclization products before and after PSS with PS-triamine: a) cyclo(L-Phe-*g*Sar-CO) (**4a**) and b) cyclo(L-Phe-*gN*-MePhe-CO) (**4l**).

4:**5** ratio was obtained when cyclization was conducted at 10^{-3} M in the presence of *N*-methyl morpholine (NMM) instead of DIPEA as the base. Cyclooligomers of higher order were not observed under these conditions. The 1,3,5-triazepan-2,6-dione **4** and cyclodimer **5** were purified by preparative C_{18} RP-HPLC which may explain the somewhat low yield of recovered product.

Polymer-assisted synthesis: To extend the practical value of 1,3,5-triazepane-2,6-diones as dipeptide mimetics, a polymer-assisted solution-phase synthesis amenable to large scale library production was developed. The selected strategy which combines catch and release and cyclative cleavage[24] is outlined in Scheme 2. The key intermediate in this concise procedure is the polymer-bound carbamate **6** accessible in a one pot sequence from protected dipeptides. Several resins including polymer-supported tetrafluorophenol (PS-TFP), *N*-hydroxysuccinimide (PS-HOSu), and oxime (PS-NOH) resins were initially considered for the synthesis of the polymer-bound carbamate. PS-HOSu was finally chosen based on our solution-phase synthesis experience. PS-HOSu was prepared according to the method reported by Adamczyk and coworkers.[25] Treatment of (mercaptomethyl)polystyrene (PS-SH) (2 mmol g^{-1}) with *N*-hydroxymaleimide in presence of pyridine yielded PS-HOSu with quantitative capping (1.6 mmol g^{-1}). After the reaction, the resin



Scheme 2. Polymer-assisted synthesis of 1,3,5-triazepan-2,6-diones **4**. a) (i) DPPA (1.1 equiv), Et_3N (1.1 equiv), RT, 30 min; (ii) 70 °C for 1.5 h; (iii) PS-HOSu (0.3 equiv), 70 °C, 40 min; b) TFA/CH_2Cl_2 (1:1), 2×5 min, RT, washings; c) DIPEA (1.1 equiv), 40–60 °C, 4×30 min.

was controlled by IR spectroscopy. A characteristic stretch was present at 1720 cm^{-1} corresponding to ν(O=C−N−C=O).

Starting from the N-Boc-protected dipeptide **1**, the acyl azide was generated in situ by reaction with diphenylphosphoryl azide (DPPA) in the presence of Et_3N.[26] It was then rearranged under heating to the corresponding isocyanate which was trapped with PS-HOSu to give the polymer-supported carbamate **6** in a one pot process (Scheme 2). This approach enabled for easy removal of soluble impurities and excess reagents by filtration. Evidence for the formation of the polymer-bound carbamates **6** came from FTIR spectroscopic observation of characteristic stretches at 3300, 1740 and 1165 cm^{-1}. Removal of the Boc group by treatment with TFA, followed by neutralisation of the TFA salt **7** and slight heating afforded the desired product **4** in solution.

Optimized conditions for the synthesis of **4** were investigated by systematically varying the solvent in both the catch and release steps. Three solvents were evaluated for the preparation of polymer-bound carbamates **6a** and **6l** derived from Boc-Phe-Sar-OH (**1a**) and Boc-Phe-*N*-MePhe-OH (**1l**), namely ethyl acetate, toluene, and THF. Experiments were carried out in small flasks equipped with cooling systems, for both of the model compounds and the reactions were monitored by FTIR spectroscopy. Each sample was then divided into three portions and subjected to TFA treatment and cyclative cleavage. Again three solvents (CH_2Cl_2, THF, and MeCN) were investigated in the cyclorelease step. In CH_2Cl_2, cyclization was generally completed in less than 30 minutes at 40 °C. The best average purity (90% purity for **4a** and 84% purity for **4l**) was obtained when the catch and cyclorelease steps were conducted in THF and CH_2Cl_2, respectively (see Figure 3).

This optimized polymer-assisted three-step procedure developed for **4a** and **4l** should greatly facilitate the access to

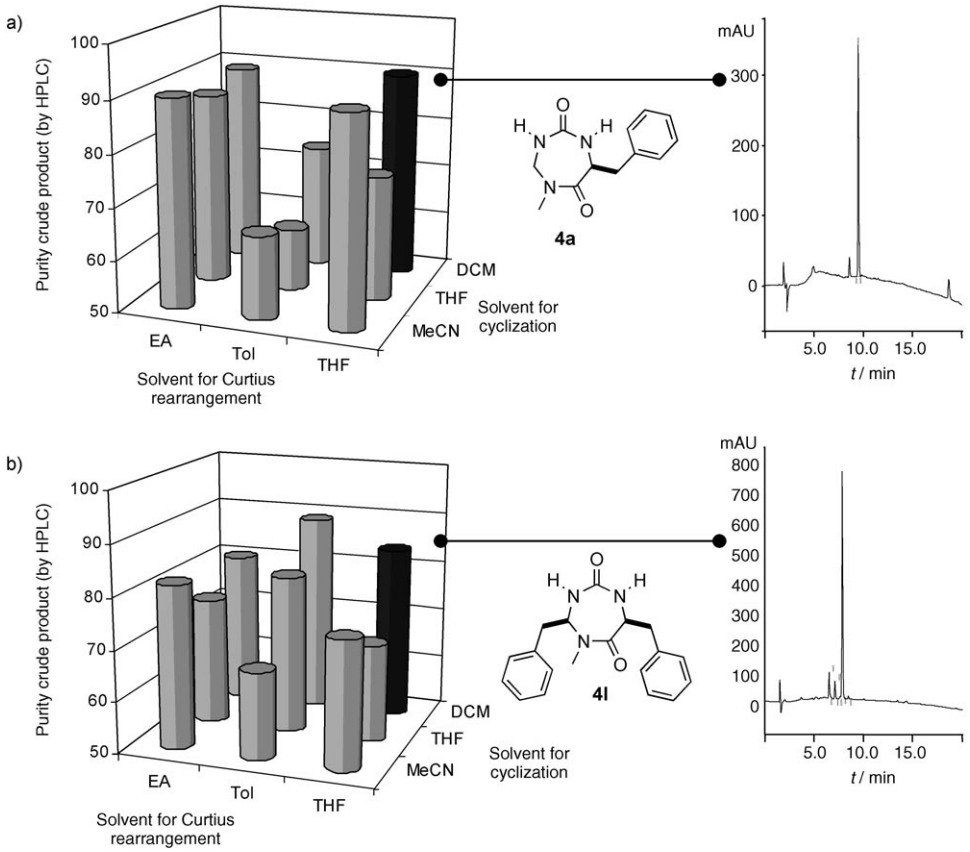
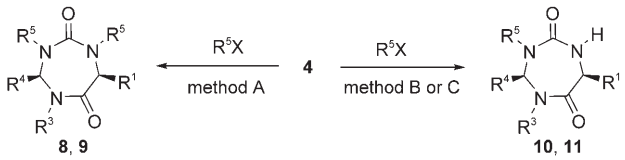


Figure 3. Solvent optimization in polymer-assisted synthesis of a) **4a** and b) **4l**.

Table 2. Diversification of parent scaffold **4** by di- and mono-N alkylation of urea.

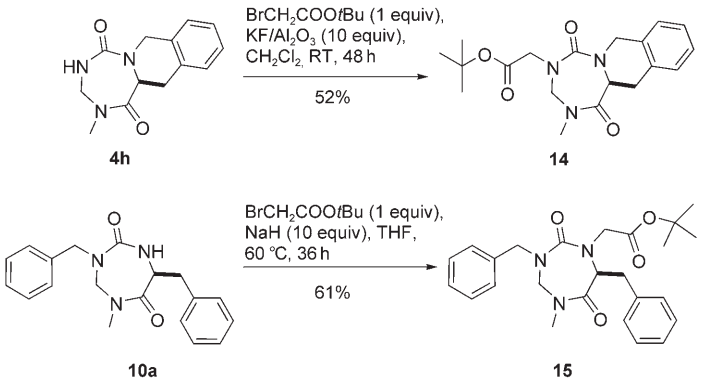


Entry	4	R^5X	Product	Method[a]	T [°C]	Yield [%][b]	Ratio[c] **10:8** or **11:9**
1	**a**	BnBr	**8a**	A	25	50	0:100
2	**i**	BnBr	**8i**	A	60	66	0:100
3	**a**	$BrCH_2COOtBu$	**9a**	A	25	82	0:100
4	**i**	$BrCH_2COOtBu$	**9i**	A	60	73	0:100
5	**a**	BnBr	**10a**	B	25	68	>99:1
6	**c**	BnBr	**10c**	B	25	94	>99:1
7	**i**	BnBr	**10i**	C	40	50	55:45
8	**a**	$BrCH_2COOtBu$	**11a**	B	25	65	>99:1
9	**c**	$BrCH_2COOtBu$	**11c**	B	25	92	>99:1
10	**f**	$BrCH_2COOtBu$	**11f**	B	25	79	97:3
11	**i**	$BrCH_2COOtBu$	**11i**	C	40	64	79:21

[a] Synthetic methods: method A: NaH (4 equiv), R^5X (4 equiv), THF, 48 h; method B: KF/Al_2O_3 40% wt (10 equiv), R^5X (1.5 equiv), CH_2Cl_2, 24–48 h; method C: NaH (2 equiv), R^5X (1.5 equiv), CH_2Cl_2, 24 h. [b] Yields calculated for pure compounds after purification by flash-column chromatography. [c] Ratio determined by C_{18} RP-HPLC of crude products. In all cases, there was no starting cycle **4** remaining.

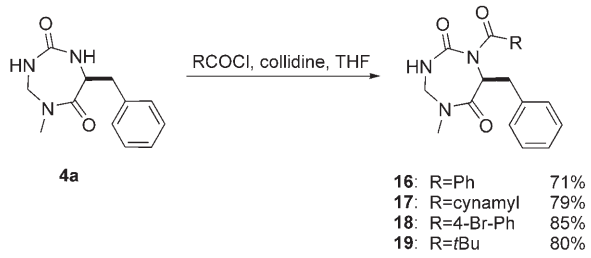
the 1,3,5-triazepan-2,6-dione skeleton in multiparallel library format.

Post-cyclization appending operations: The urea moiety in the 1,3,5-triazepan-2,6-dione ring offers one or two additional sites for post-cyclization appending operations. Further diversification of parent scaffold **4** was achieved by either alkylation (Table 2, Scheme 3) or acylation (Scheme 4) of the urea moiety.



Scheme 3. Synthesis of dissymmetrically N-alkylated 1,3,5-triazepan-2,6-diones.

Di-N-alkylation of seven-membered cycloureas (for example, 1,3-diazepan-2-diones) which has been used extensively in the preparation of C_2-symmetric HIV protease inhibitors generally proceeds in good yields.[27] Similarly, treatment of scaffold **4** with NaH (5 equiv) and various electrophiles (3 equiv) afforded dialkylated cycloureas (for example, **8** and **9**) in good yields and purities after purification by flash-column chromatography (Table 2, entries 1–4). Alternatively, liquid/liquid extraction followed by PSS of excess electrophile with *N*-(2-mercaptoethyl)aminomethyl-PS afforded pure compounds (Supporting Information). Desymmetrization of urea in 1,3,5-triazepan-2,6-diones was also investigated. Direct monoalkylation of C_2-symmetric cycloureas in the presence of one equivalent of electrophile is often inconsistent, proceeding in low yield due to both incomplete reaction and competing dialkylation.[28] Alternative methods include the formation of *O*-alkylated isourea derivatives,[29] the monodeprotection of symmetric bis-benzylated derivatives,[30] or the discrimination of urea nitrogens by selective introduction of an alkoxycarbonyl protecting group.[31] Interestingly, we found that direct alkylation of cyclo(Xaa-*g*Sar-CO) **4a**, **4c**, **4f**, and cyclo(Phe-*g*Pro-CO) **4i**, in the presence of KF/Al_2O_3 40% wt (10 equiv)[32] or NaH (2 equiv), respectively, reliably provided **10** and **11** monoalkylated at the *gem*-diamino urea nitrogen (Table 2, entries 5–11).[33] Mono-N-alkylation reaction occurred exclusively on the N3-atom. It is worth noting that excellent selectivities of mono versus dialkylated products (>99:1) were generally observed in KF/Al_2O_3-mediated alkylation (Table 2, entries 5, 6, and 8–10). 1,3,5-Triazepane-2,6-diones containing trisubstituted ureas with either N3H or N1H can be further manipulated to generate dissymmetrically N-alkylated rings as exemplified for **14** and **15** (Scheme 3).



Scheme 4. Monoacylation reactions of 1,3,5-triazepan-2,6-dione **4a**.

Conditions for acylation of triazepandiones **4** were also investigated. Again differential reactivity of urea nitrogens was observed. Treatment of **4a** with four equivalents of benzoyl chloride afforded **16** monoacylated at the N1 position with minor amounts of the corresponding diacylated derivative and no sign of acylation at N3. The optimized procedure for the acylation reaction entailed the use of collidine as the base and THF as the solvent. Under these conditions,

1,3,5-triazepan-2,6-dione **4a** was cleanly converted to monoacylated derivatives **16**–**19** in yields ranging from 71 to 85% (Scheme 4).

The use of other bases, such as pyridine, DMAP (DMAP = 4-dimethylaminopyridine), and 2,6-di-*tert*-butyl-4-methylpyridine proved less satisfactory with much slower conversion. Alternatively, inorganic bases (for example, K_2CO_3) were also found to be effective in promoting clean monobenzoylation of **4a**. Monoacylation thus offers another interesting possibility to desymmetrize the urea in 1,3,5-triazepan-2,6-diones and expands further the diversity accessible from post-cyclization operations.

Structural features and comparison with related dipeptide-derived skeletons: The conformational preferences of 1,3,5-triazepan-2,6-dione were investigated by detailed X-ray diffraction and ^{1}H NMR spectroscopy studies of representative members of the library. In the solid state, compounds **4** derived from homochiral sequences generally adopt a rigid nonplanar conformation with side chains R^2 and R^4 in the pseudoequatorial position (Figure 4).[34,35]

The "V-shape" of the molecules can be described by the measure of the angle (α) between the mean amide and urea plans (Table 3).

The angle α is either close to 120° (**4j**, **4k**(I), **4l**, **4o** and **4s**) or 160° (**4k**(II)) showing that 1,3,5-triazepane-2,6-diones **4** can adopt two different two-folded conformations in the solid state, A and B (vide infra), respectively. Examination of torsion angles T1, T2, and T5 (see Table 3) revealed that in all triazepanediones **4** the amide moiety is essentially planar and that the urea is nearly planar when α is close to 120°. In the crystal structure of tetracyclic cyclo(Tic-*g*Pro-CO) (**4k**), the two conformations A and B of the ring are coexisting. The conformation A adopted by **4k**(I) follows the general tendency discussed above with side chains R^2 and R^4 in an equatorial position. However, in the second molecule **4k**(II), the substituent R^2 adopts a pseudoaxial orientation thus forcing the urea to deviate from planarity (T1 = −52.8°, T2 = −26.7°) and leading to a higher α angle value (158°). This deviation is associated with a strong pyramidalization of N1 in **4k**(II) which is apparent when the sum of the angles formed by the substituents at-

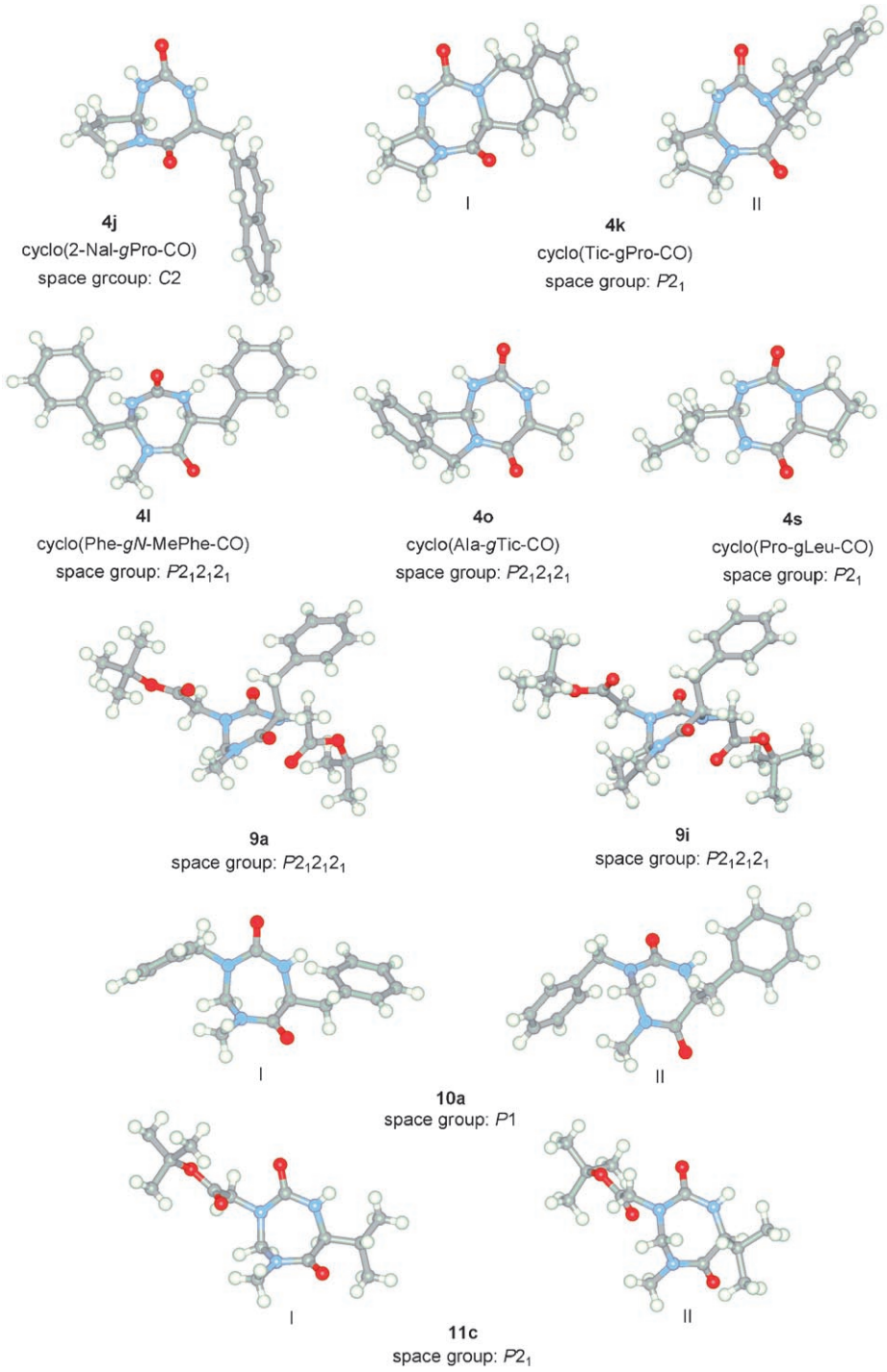


Figure 4. X-ray crystal structures of selected 1,3,5-triazepan-2,6-diones **4** and **9**–**11**.

Table 3. Torsion angles and geometrical data for 1,3,5-triazepan-2,6-diones.

Entry	Compound	Form	Space group	Crystallization Solvent	Torsion angle [°][a]							α [°][b]
					T1	T2	T3	T4	T5	T6	T7	
1	**4j**		*C*2	CH_2Cl_2/DIPE or dioxane	3.8	15.2	35.4	−64.9	4.5	69.6	−67.6	117.4
2	**4k**	I	$P2_1$	AE/Hex	3.3	−14.3	66.6	−59.6	−13.9	74.9	−52.7	120.0
		II			−52.8	−26.7	80.9	−47.5	3.9	−14.3	65.3	158.5
3	**4l**		$P2_12_12_1$	AE/Hex	−14.3	4.4	61.5	−72.1	−4.7	67.8	−44.1	115.9
4	**4o**		$P2_12_12_1$	CH_2Cl_2	5.8	0.8	50.8	−70.9	7.7	61.9	−60.9	119.5
5	**4s**		$P2_1$	CH_2Cl_2/DIPE	1.4	−13.6	65.8	−62.9	−12.6	69.2	−48.4	122
6	**9a**		$P2_12_12_1$	CH_2Cl_2/DIPE	−50.1	−33.3	88.5	−52.6	11.1	−25.4	74.6	170.9
7	**9i**		$P2_12_12_1$	CH_2Cl_2/DIPE	−52.0	−34.5	83.5	−41.6	0.7	−20.8	76.3	162.9
8	**10a**	I	*P*1	CH_2Cl_2/DIPE	−2.3	6.6	52.7	−75.7	5.9	62.8	−56.2	117.9
		II			18.6	5.2	−68.9	70.2	3.4	−53.0	25.1	125.5
9	**11c**	I	$P2_1$	CH_2Cl_2/DIPE	6.3	11.2	42.2	−71.9	8.2	67.2	−68.9	117.6
		II			21.2	2.0	−67.1	72.2	1.4	−52.5	25	124.4

[a] The dihedral angles T1–7 refer to the torsion angles of the sequence of atoms C7-N1-C2-N3, N1-C2-N3-C4, C2-N3-C4-N5, N3-C4-N5-C6, C4-N5-C6-C7, N5-C6-C7-N1, C6-C7-N1-C2, respectively (see Figure 5 for labeling of atoms). According to the IUPAC-IUB rules for the conformation of polypeptide chains, T7 can also be defined as φ_1, T6 as ψ_1, and T5 as ω. [b] α is measured between the mean urea and amide planes. Abbreviations: DIPE = diisopropylether.

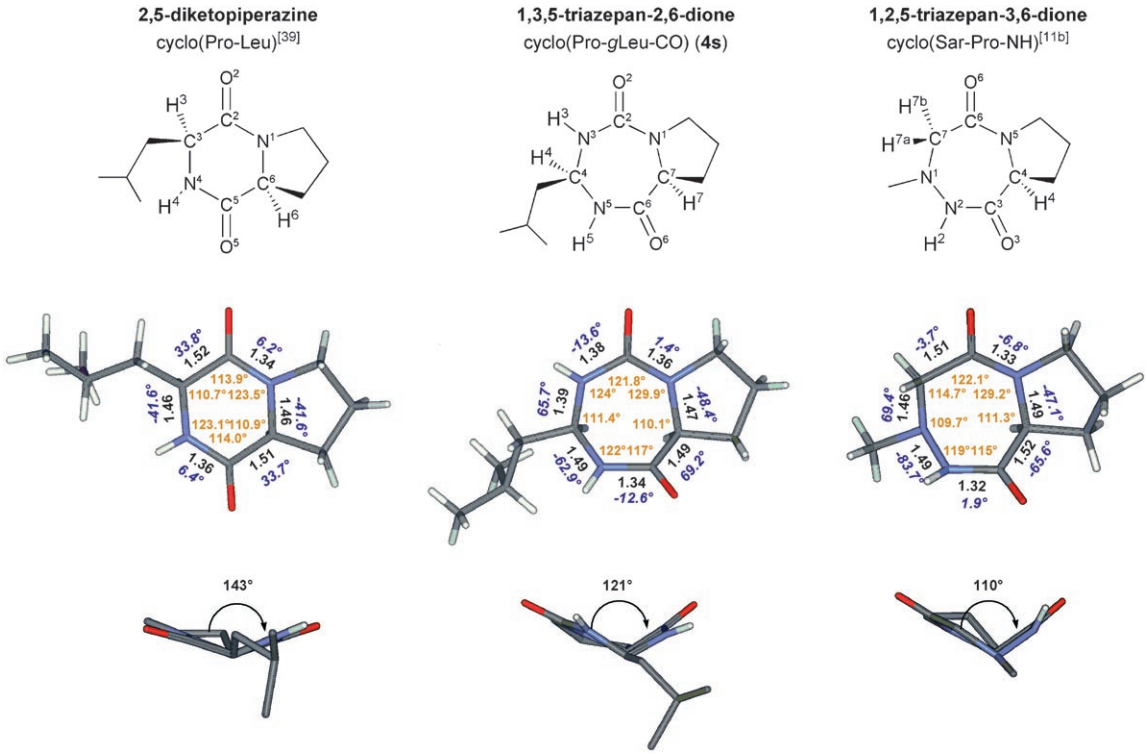


Figure 5. Structural comparison of 2,5-diketopiperazine (DKP), 1,3,5-triazepan-2,6-diones, and 1,2,5-triazepan-3,6-dione skeletons. For each skeleton, internal angles are shown in orange and bond lengths (Å) in black. Backbone torsion angles are in blue and italicized. The α angle between amide, amide/urea planes are indicated for the three molecules.

tached to N1 are compared: 358.7° for **4k**(I) versus 341.5° in **4k**(II). The folded conformation A induces an unusual close proximity of the hydrogens H4 and H7 (refer to Figure 5 for numbering of atoms). In **4l** the distance between H4 and H7 is 1.92 Å[36] which is less than the sum of the Van der Waals radii ($\Sigma = 2.4$ Å) for two hydrogen atoms. When comparing the two structures **4k**(I) and **4k**(II) discussed above, the distance between H4 and H7 changes from 2.14 to 3.71 Å as a result of the reorganization in the pseudoaxial orientation of the R^2 side chain. The conformation of **4a** and **4i** in solution was investigated by ^{1}H NMR spectroscopy in [D_6]DMSO. The observation of a strong ROE correlation in ROESY experiments between H4 and H7 suggests a short interproton distance characteristic of the folded conformation A with R^2 (phenylalanine) side chain in pseudoequatorial orientation (Supporting Information).

Structural features of 1,3,5-triazepane-2,6-dione **4** and DKP originating from a common dipeptide precursor (L-Pro-L-Leu) were compared (Figure 5).

The structures of DKPs in the solid state have been extensively studied and reviewed.[4c,37,38] Depending on the peptide sequence and stereochemistry, the DKP ring has been shown to adopt several conformations ranging from planar or nearly planar (for example, in cyclo(Gly-Gly) and cyclo(D-Ala-L-Ala) the α angle between amide planes is ~180°) to folded (twist boat) (for example, cyclo(L-Ala-L-Ala), α = ~151°). The substituents on the C^{α} atom have been observed to be either in the axial or most of the time in quasi-equatorial position depending on their nature and the stereochemistry. With α values as low as 117°, the 1,3,5-triazepan-2,6-dione ring is much more folded than the corresponding 2,5-diketopiperazine ring. In the Pro-Leu series, α values of 143 and 122° are observed, respectively, for the DKP[39] and the triazepanedione (Figure 5). The inter $C^{\alpha}H$ proton distance (*d*(H3,H6) of 2.81 Å in the DKP) is much larger than that observed in the corresponding triazepandione ring and reflects the more planar conformation. The quasi-equatorial position of the side chains on C^{α} carbons is common to both scaffolds.[40]

In 1996 Lenman et al. reported the design and synthesis of *cis*-peptidyl prolinamide mimetics based on a 4,5-fused 1,2,5-triazepane-3,6-dione skeleton that differ from the original *cis*-proline peptide by only two hydrogens.[11b] These molecules were obtained by cyclization of α-bromoacetyl proline hydrazide intermediates. Comparison of 1,2,5-triazepane-3,6-dione and 1,3,5-triazepane-2,6-dione-ring structures (cyclo(Pro-*g*Leu-CO) versus cyclo(Sar-Pro-NH) respectively, Figure 5) reveals a very close match with almost perfect superimposition of backbone traces, the urea nitrogen N3 in **4s** overlying the α-carbon C7 in cyclo(Sar-Pro-NH).

The 1,2,5-triazepan-3,6-diones thus exhibit the folded conformation A defined above with a α angle slightly lower (110°) than that observed in 1,3,5-triazepan-2,6-diones. The major difference in the ring geometry is observed between the bond angles (C4-N3-C2) in **4s** and (N1-C7-C6) in cyclo(*N*-MeSar-Pro-NH) which differ by approximately 10° (Figure 5). This similarity suggests that the 1,3,5-triazepan-2,6-dione scaffold alkylated on the N3 urea nitrogen could be used to design mimetics of *cis*-peptidyl prolinamides.

The crystal structures solved for several di- and mono-N-alkylated compounds (**9a**, **9i**, **10a**, and **11c**) further highlight the structural diversity among 1,3,5-triazepan-2,6-diones (Figure 4). The overall ring geometry undergoes important changes upon alkylation of urea nitrogen N3. Di-N-alkylated compounds **9a** and **9i** exhibit the characteristics of the folded conformation B defined above and observed for compound **4k**(II): the R^2 substituent (phenylalanine side chain for both **9a** and **9i**) adopts a quasi-axial orientation; the values of T1 and T2 torsion angles (approximately, −50 and −35°) show that the urea is no longer planar and that the geometry of the ring is highly distorted; the sums of the angles formed by the substituents attached to N1 (approximately 350°) and N3 (approximately 356°, Table 3) indicate some pyramidalization of the urea nitrogens. Both crystal structures of N3-alkylated 1,3,5-triazepane-2,6-diones **10a** and **11c** show two different conformations coexisting in the crystal cell. One molecule in the asymmetric unit (**10a**(I) and **11c**(I)) adopts the standard folded conformation A commonly found for compounds **4**, with the R^2 substituent in pseudoequatorial position and an α angle close to 120°. In the second conformation observed (**10a**(II) and **11c**(II)), the puckering of the ring is reversed thus keeping the α angle close to 120° but causing the R^2 substituent to adopt a quasi-axial orientation. This overall inversion of the ring geometry results in pulling apart the two hydrogens H4 and H7 which adopt quasi-equatorial positions. The *d*(H4,H7) increases from 1.98 in **11c**(I) to 4.67 Å in **11c**(II). The conformation of **10a** was investigated by NMR spectroscopy in [D_6]DMSO. The presence of a single set of sharp peaks and the strong ROE correlation observed between H4 and H7 may suggest that conformation A is mainly populated in solution.

First biological screens: Identification of Malaria liver stage inhibitors: Because they are structurally diverse and rapidly accessible in a library format from simple dipeptides, 1,3,5-triazepan-2,6-diones have a strong potential for use in biological screens. Here, we report a preliminary account of our screening efforts to identify malaria liver stage inhibitors.

Invasion of host hepatocytes by sporozoites represents an early step in the life cycle of the malaria parasite. Sporozoites develop in the liver and give rise to merozoites which subsequently invade erythrocytes after release into the bloodstream. This blood phase is responsible for the pathology of the malaria infection. Malaria liver stages (LS) hold great promise for drug targeting because 1) they possess a more complex and distinct metabolism than their blood stage counterpart and 2) they precede the pathogenic blood stage, thus offering prophylaxis possibilities against malaria. However, very few drugs against LS parasites are available and most of them like primaquine display severe side effects or loose efficiency due to the development of parasite resistance.[41] The discovery of new and specific inhibitors of the LS would lead eventually to the identification of novel biological targets, thus revealing new therapeutic horizons.

Seventeen different 1,3,5-triazepan-2,6-diones were randomly selected and were first tested at different doses for toxicity on primary mouse hepatocyte culture. From these, nine were shown to be toxic at all doses tested (6.25–100 mg mL^{-1}) and two at high doses (over 50 mg mL^{-1}, see Supporting Information).[42] The six remaining nontoxic molecules were thus tested to evaluate their effects on sporozoite invasion and development in hepatocytes. Four of these **4p**, **9a**, **5r**, and **13a** had no significant effect on liver parasites (Figure 5a, 5c and data not shown). In contrast, two molecules, **4b** and **10a**, were shown to inhibit significantly and repeatedly LS development (Figures 6a and b), **4b** being the most potent.

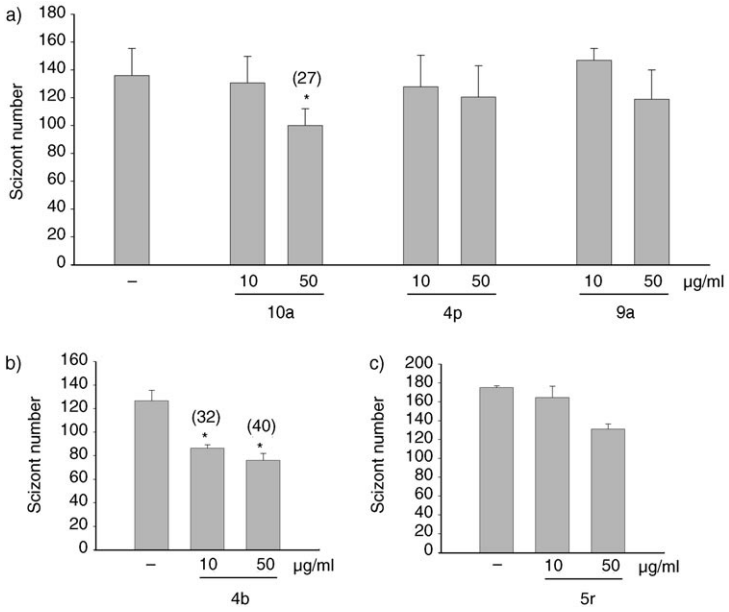


Figure 6. Effect of selected 1,3,5-triazepan-2,6-diones on malaria liver stage development. Compounds were added at the time of sporozoite invasion and during liver stage development. Schizont mber was estimated by counting this mature parasite forms in 48 h cultures. Results are expressed as the mean ±SD of 3 triplicate cultures. Data are representative of 2 to 6 experiments per compounds tested; * = $P < 0.05$, one-way analysis of variance, followed by the post-hoc Tukey test.

Conclusion

We have presented the design and construction of a structurally diverse small library of dipeptide-derived 1,3,5-triazepan-2,6-diones, which led to the identification of two molecules active against the malaria liver stages, which do not show any toxicity on mouse hepatocytes. Because of its simple and rapid elaboration, the 1,3,5-triazepan-2,6-dione system nicely complements the existing collection of skeletons accessible from small linear dipeptide precursors. Large-scale library construction by integrating a more comprehensive set of dipeptides (including side-chain and stereochemical diversity) and appending processes is ongoing and should help for the rapid identification of more potent molecules for use in the underexplored stages of malaria.

Experimental Section

General methods: THF was distilled from Na/benzophenone. CH_2Cl_2 and cyclohexane were distilled from CaH_2. TLC was performed on silica gel 60 F254 (Merck) with detection by UV light and charring with 1% w/w ninhydrin in ethanol followed by heating. Flash-column chromatography was carried out on silica gel (0.063–0.200 nm). HPLC analysis was performed on a Nucleosil C_{18} column (5 µm, 3.9 ′ 150 mm) by using a linear gradient of A (0.1% TFA in H_2O) and B (0.08% TFA in CH_3CN) at a flow rate of 1.2 mL min^{-1} with UV detection at 214 nm. Optical rotations were recorded with a Perkin–Elmer polarimeter. IR spectra were recorded on a Perkin–Elmer Spectrum One in four scans with a ATR (attenuated total reflection) accessory. ^{1}H and ^{13}C NMR spectra were recorded by using Bruker Advance DPX-300, DPX-400, and DPX-500 instruments. Mass spectra have been recorded by using a MALDI-TOF apparatus (BRUKER Protein-TOF) and an ESI-TOF apparatus (Bruker micro-TOF).

General procedure for the solution-phase synthesis of 1,3,5-triazepan-2,6-diones 4 (exemplified for 4a): *N*-methyl morpholine (NMM, 2.3 mL, 21.4 mmol, 1.2 equiv) was added to Boc-L-Phe-Sar-OH (**1a**, 6.0 g, 17.8 mmol) dissolved in dry THF (60 mL) under argon. The mixture was placed into a bath at −20 °C and ethyl chloroformate (2.05 mL, 21.4 mmol, 1.2 equiv) was added. The mixture was stirred at −20 °C for 25 min, then the bath was removed and a solution of NaN_3 (2.9 g, 44.6 mmol, 2.5 equiv) in water was added. The mixture was stirred at room temperature for 5 min. The reaction was quenched by addition of brine and extracted by ethyl acetate (1 × 240 mL). The organic layer was dried over Na_2SO_4 and concentrated under vacuum without any heating. The resulting residue was dissolved in dry toluene (40 mL) under argon and heated at 70 °C. The Curtius rearrangement was followed by gas evolution (bubbling). When gas emission decreased, HOSu (2.05 g, 17.8 mmol, 1 equiv) and pyridine (1 mL, 12.3 mmol, 0.7 equiv) were added. The reaction was stirred at 70 °C for 40 additional minutes and allowed to cool to room temperature. Solvents were concentrated under vacuum to give succinimidyl carbamate **2a** as a yellow foam which was used in the next step without purification. The crude succinimidyl carbamate **2a** was dissolved in trifluoroacetic acid (8 mL). After the reaction had been stirred at room temperature for 30 min, a mixture of cyclohexane and diethyl ether (1:4 v/v) was slowly added to the reaction to precipitate the TFA salt. This heterogeneous system was stored at −20 °C for 4 h, triturated, and sonicated prior to filtration. The precipitate was washed several times with a cyclohexane/diethyl ether mixture (1:4 v/v) and dried under high vacuum overnight. Compound **3a** was obtained as a pale yellow solid (7.6 g, 16.5 mmol, 93%). The TFA salt **3a** (4 g, 8.66 mmol) was dissolved in CH_3CN (200 mL) and the resulting solution was added dropwise to a solution of DIPEA (1.66 mL, 9.5 mmol, 1.1 equiv) in acetonitrile (400 mL). When the addition was completed, the mixture was stirred for an additional 30 minutes at room temperature and solvent was evaporated. The desired compound was recrystallized from a mixture of CH_2Cl_2 and DIPE. After filtration and drying under high vacuum, the filtrate was concentrated and the recrystallization process was repeated several times. Finally, cyclo(L-Phe-*g*Sar-CO) **4a** was obtained (2.72 g, 11.7 mmol, 71%).

Preparation of PS-HOSu:[25] PS-SH (5 g, 10 mmol) was swollen in dry DMF (150 mL) and *N*-hydroxymaleimide (2.26 g, 20 mmol) followed by pyridine (4.85 mL, 60 mmol) were added. The mixture was stirred for 24 h at room temperature and then heated at 55 °C for 3 h under an inert atmosphere. The resin was filtered and washed with DMF (×3), H_2O (×3), MeOH (×3), and CH_2Cl_2 (×4). Once the washings were complete, the resin was dried under high vacuum for 16 h (mass = 5.99 g, 98%). Based on mass recovery the loading of PS-HOSu was determined to be 1.60 mmol g^{-1}.

General procedure for the polymer-assisted synthesis of 1,3,5-triazepan-2,6-diones (exemplified for 4a): A round-bottomed flask (10 mL) equipped with cooling system was charged with Boc-L-Phe-Sar-OH (**1a**, 245 mg, 0.73 mmol) and THF (1.5 mL) under Ar. Et_3N (110 µL, 0.79 mmol) and DPPA (157 µL, 0.73 mmol) were added. The mixture was stirred for 30 min at RT, followed by heating at 70 °C for 1.5 h. Addition of PS-HOSu (150 mg, 0.24 mmol) was followed by gentle stirring at 70 °C for 40 min. The content of the flask was transferred into a polypropylene syringe equipped with a filter. Filtration and washes (dry DMF ×3, THF ×3, and CH_2Cl_2 ×3) afforded the substituted resin which was dried under high vacuum for 16 h. The reaction was monitored by using IR spectroscopy. Removal of the Boc group was performed with TFA/CH_2Cl_2 (1:1 v/v, 1.5 mL) for 5 min at RT. The resin was then washed with dry CH_2Cl_2 (×4). This step was repeated twice and the resin was swollen in dry CH_2Cl_2 (2 mL). DIPEA (45 µL, 0.26 mmol) was added and the syringe was vortexed at 40 °C for 1 h. The resin was filtered and rinsed with dry CH_2Cl_2 (2 × 1 mL). The filtrate was evaporated to give **4a** in 77% yield and 90% purity as determined by HPLC analysis.

Cyclo(L-Phe-*g*Sar-CO) (4a): White solid; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): t_R =

10.38 min; $[\alpha]_D^{20} = -41.3$ ($c = 1.1$ in MeOH); ^{1}H NMR (500 MHz, $CDCl_3$, 298 K): $\delta = 2.81$ (dd, $J = 8.9$, 14.4 Hz, 1H; CH_2Ph), 3.08 (s, 3H; NCH_3), 3.35 (dd, $J = 5.2$, 14.4 Hz, 1H; CH_2Ph), 4.00 (dd, $J = 7.0$, 15.6 Hz, 1H; NCH_2N), 4.62–4.65 (m, 1H; NCHCO), 4.87 (brs, 1H; NH), 5.20 (dd, $J = 5.1$, 15.5 Hz, 1H; NCH_2N), 6.97 (brs 1H; NH), 7.24–7.27 (m, 3H; H-Ar), 7.31–7.34 ppm (m, 2H; H-Ar); ^{13}C NMR (125 MHz, $CDCl_3$, 298 K): $\delta =$ 33.8 (CH_3), 37.3 (CH_2), 54.5 (CH), 56.0 (CH_2), 127.2, 129.0, 129.3 (CH-Ar), 136.2 (Cq-Ar), 157.8, 169.9 ppm (CO); IR (solid): $\tilde{\nu} = 3182$ (NH), 3049 (Csp^2), 2928 (Csp^3), 1651 cm^{-1} (CO); HRMS (ESI): m/z: calcd for $C_{12}H_{16}N_3O_2$: 234.1242; found: 234.1281.

Cyclo(D-Phe-*g*Sar-CO) (4b): White solid; $[\alpha]_D^{20} = 36.5$ ($c = 0.81$ in MeOH); other spectroscopic data corresponding to **4a**.

Cyclo(Val-*g*Sar-CO) (4c): White solid; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): $t_R =$ 8.50 min; $[\alpha]_D^{20} = -53.5$ ($c = 0.63$ in MeOH); ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): $\delta = 0.98$ (d, $J = 6.6$ Hz, 3H; CH_3), 1.02 (d, $J = 6.6$ Hz, 3H; CH_3), 2.03–2.14 (m, 1H; $CH(CH_3)_2$), 3.01 (s, 3H; NCH_3), 3.88 (dd, $J = 4.8$, 8.1 Hz, 1H; NCHCO), 4.25 (dd, $J = 6.3$, 15.6 Hz, 1H; CH_2NH), 4.91 (dd, $J = 5.1$, 15.6 Hz, 1H; NCH_2N), 5.59 (brs; NH), 7.46 ppm (brs; NH); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): $\delta = 18.4$, 19.8 (CH_3), 29.9 (CH), 33.7 (NCH_3), 56.0 (CH_2), 60.2 (CH), 158.9, 170.2 ppm (CO); IR (solid): $\tilde{\nu} =$ 3238 (NH), 2961, 2926, (Csp^3), 1652 cm^{-1} (CO); HRMS (ESI): m/z: calcd for $C_8H_{16}N_3O_2$: 186.1234; found: 186.1237.

Cyclo(Leu-gSar-CO) (4d): Pale yellow solid; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): $t_R = 9.37$ min; $[\alpha]_D^{20} = -29.2$ ($c = 0.85$ in MeOH); ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): $\delta = 0.94$ (d, $J = 2.7$ Hz, 3H; CH_3), 0.95 (d, $J = 2.7$ Hz, 3H; CH_3), 1.39–1.48 (m, 1H; $CH(CH_3)_2$), 1.70–1.88 (m, 2H; $CHCH_2CH$), 3.07 (s, 3H; NCH_3), 4.06 (dd, $J = 6.9$, 15.6 Hz, 1H; NCH_2N), 4.35–4.40 (m, 1H; NCHCO), 4.96 (brs; NH), 5.25 (dd, $J = 5.1$, 15.6 Hz, 1H; NCH_2N), 6.85 ppm (brs; NH); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): $\delta = 22.02$, 22.82 (CH_3), 24.38 (CH), 33.75 (CH_3), 39.97 (CH_2), 51.00 (CH), 56.17 ppm (CH_2); IR (solid): $\tilde{\nu} = 3579$, 3219 (NH), 2927 (Csp^3), 1676, 1646 cm^{-1} (CO); HRMS (ESI): calcd for $C_9H_{18}N_3O_2$: 200.1399; found: 200.1420.

Cyclo(Dap(Fmoc)-gSar-CO) (4e): White solid, C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0-100% B, 1.2 mL min^{-1}, 20 min): $t_R = 13.74$ min; ^{1}H NMR (400 MHz, CD_3CN, 298 K): $\delta =$ 2.95 (s, 3H; CH_3), 3.26–3.33 (m, 1H; $CHCH_2NH$), 3.44–3.51 (m, 1H; $CHCH_2NH$), 3.94–3.99 (m, 1H; NCH_2N), 4.19–4.22 (m, 1H; NCH_2N), 4.34 (s, 2H; CH-(Fmoc), CH_2Fmoc), 4.50 (s, 1H; CH_2Fmoc), 5.08–5.13 (m, 1H; NCHCO), 5.87 (brs, 1H; NH), 6.05 (brs, 2H; NH), 7.26–7.42 (m, 4H; CH-Ar), 7.57–7.64 (m, 2H; CH-Ar), 7.75–7.82 ppm (m, 2H; CH-Ar); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): $\delta = 33.24$ (CH_2), 42.51 (CH_3), 48.08, 54.25 (CH_2), 56.33, 67.29 (CH), 120.97, 126.15, 128.12, 128.69 (CH-Ar), 142.16, 145.10 (Cq-Ar), 158.33, 171.12 ppm (CO); HRMS (ESI): calcd for $C_{21}H_{23}N_4O_4$: 395.1714; found: 395.1704.

Cyclo(Phg-*g*Sar-CO) (4f): White solid; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): $t_R =$ 9.26 min; $[\alpha]_D^{20} = -86.9$ ($c = 0.64$ in MeOH); ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): $\delta = 3.02$ (s, 3H; CH_3N), 3.87 (dd, $J = 6.9$, 15.6 Hz, 1H; NCH_2N), 4.54 (dd, $J = 5.7$, 15.6 Hz, 1H; NCH_2N), 5.26 (d, $J = 6.9$ Hz, 1H; NCHCO), 6.29 (d, $J = 6.6$ Hz, 1H; CHN*H*), 6.99 (brs; NH), 7.32–7.44 ppm (m, 5H; H-Ar); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): $\delta = 33.2$ (NCH_3), 54.1 (CH_2), 61.2 (CH), 124.3, 127.4, 128.4 (CH-Ar), 137.2 (Cq-Ar), 158.2, 168.9 ppm (CO); IR (solid): $\tilde{\nu} = 3278$ (NH), 3108 (Csp^2), 2956, 2934 (Csp^3), 1674, 1639 cm^{-1} (CO); HRMS (ESI): m/z: calcd for $C_{11}H_{14}N_3O_2$: 220.1081; found: 220.1069.

Cyclo(2-Nal-*g*Sar-CO) (4g): White solid; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): $t_R =$ 12.69 min; $[\alpha]_D^{20} = -17.9$ ($c = 0.92$ in MeOH); ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): $\delta = 2.97$ (dd, $J = 8.7$, 14.4 Hz, 1H; CH_2-Nal), 3.09 (s, 3H; NCH_3), 3.51 (dd, $J = 5.1$, 14.4 Hz, 1H; CH_2-Nal), 4.00 (dd, $J = 6.9$, 15.6 Hz, 1H; NCH_2N), 4.71–4.76 (m, 1H; NCHCO), 4.82 (brs; NH), 5.21 (dd, $J = 5.1$, 15.6 Hz, 1H; NCH_2N), 6.57 (brs; NH), 7.36 (dd, $J = 1.5$, 8.4 Hz, 1H; H-Ar), 7.45–7.49 (m, 2H; H-Ar), 7.72 (s, 1H; H-Ar), 7.78–7.83 ppm (m, 3H; H-Ar); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): $\delta = 33.9$ (NCH_3), 37.6 (CH_2), 54.4(CH), 56.1 (CH_2), 125.9, 126.4, 126.9, 127.6, 127.6, 128.2, 128.8 (CH-Ar), 132.5, 133.5, 133.6 (Cq-Ar), 157.7, 169.9 ppm (CO); IR (solid): $\tilde{\nu} = 3402$, 3233 (NH), 3058 (Csp^2), 2921 (Csp^3), 1658 cm^{-1} (CO); HRMS (ESI): m/z: calcd for $C_{16}H_{17}N_3O_2$: 384.1394; found: 384.1382.

Cyclo(Tic-*g*Sar-CO) (4h): White solid, C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): $t_R =$ 10.91 min; $[\alpha]_D^{20} = -78.7$ ($c = 0.60$ g in MeOH); ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): $\delta = 3.02$–3.09 (m, 4H; NCH_3, CH_2CH), 3.34 (dd, $J = 11.1$, 15.6 Hz, 1H; CH_2CH), 4.37 (dd, $J = 6.9$, 15 Hz, 1H; NCH_2N), 4.47 (d, $J =$ 15.6 Hz, 1H; CH_2N), 4.53 (dd, $J = 4.2$, 11.1 Hz, 1H; CH), 4.72 (d, $J =$ 15.6 Hz, 1H; CH_2N), 4.83 (dd, $J = 5.7$, 15 Hz, 1H; NCH_2N), 7.08 (brs; NH), 7.14–7.24 ppm (m, 4H; H-Ar); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): $\delta = 32.4$ (CH_2), 34.1 (NCH_3) 46.5, 56.6 (CH_2) 57.7 (CH), 126.0, 126.7, 127.3, 127.6 (CH-Ar), 134.3, 134.4 (Cq-Ar), 159.1, 170.0 ppm (CO); IR (solid): $\tilde{\nu} = 3337$, 3297 (NH), 3078 (Csp^2), 2956, 2922 (Csp^3), 1651, 1641 cm^{-1} (CO); HRMS (ESI): m/z: calcd for $C_{13}H_{16}N_3O_2$: 246.1237; found: 246.1229.

Cyclo(Phe-*g*Pro-CO) (4i): White solid; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): $t_R =$ 10.91 min; $[\alpha]_D^{20} = -18.2$ ($c = 1.06$ in MeOH); ^{1}H NMR (500 MHz, [D_6]DMSO, 298 K): $\delta = 1.77$–2.01 (m, 4H; $2CH_2$), 2.71 (dd, $J = 7.2$, 14.1 Hz, 1H; CH_2Ph), 3.05 (dd, $J = 6.5$, 14.1 Hz, 1H; CH_2Ph), 3.22–3.27 (m, 1H; CH_2), 3.55–3.60 (m, 1H; CH_2), 4.65–4.68 (m, 1H; NCHCO), 5.56–5.58 (m, 1H; NCHN), 6.14 (brs; NH), 6.98 (brs; NH), 7.19–7.21 (m, 1H; H-Ar), 7.26–7.29 (m, 2H; H-Ar), 7.34–7.36 ppm (m, 2H; H-Ar); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): $\delta = 22.6$, 33.5, 36.5, 46.2 (CH_2), 55.00, 64.2 (CH), 127.2, 128.9, 129.3 (CH-Ar), 136.3 (Cq-Ar), 157.5, 167.8 ppm (CO); IR (solid): $\tilde{\nu} = 3196$ (NH), 3076 (Csp^2), 2961, 2890 (Csp^3), 1651 cm^{-1} (CO); HRMS (ESI): m/z: calcd for $C_{14}H_{18}N_3O_2$: 260.1399; found: 260.1394.

Cyclo(2-Nal-*g*Pro-CO) (4j): Pale yellow solid; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): $t_R = 13.29$ min; $[\alpha]_D^{20} = -4.0$ ($c = 0.99$ in MeOH); ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): $\delta = 1.87$–1.95 (m, 2H; CH_2), 2.01–2.25 (m, 2H; CH_2), 2.95 (dd, $J = 8.7$, 14.4 Hz, 1H; CH_2-Nal), 3.44–3.57 (m, 2H; CH_2), 3.77–3.85 (m, 1H; CH_2-Nal), 4.63–4.69 (m, 1H; NCHCO), 4.96 (brs; NH), 5.45 (d, $J = 4.8$ Hz, 1H; NCHN), 6.53 (brs; NH), 7.38 (dd, $J = 1.5$, 8.4 Hz, 1H; H-Ar), 7.45–4.49 (m, 2H; H-Ar), 7.74 (s, 1H; H-Ar), 7.79–7.81 ppm (m, 3H; H-Ar); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): $\delta = 22.6$, 33.3, 36.7, 42.2 (CH_2), 54.9, 64.3 (CH), 125.9, 126.4, 127.0, 127.7, 128.3, 128.7 (CH-Ar), 132.5, 133.5, 133.7 (Cq-Ar), 157.6, 167.7 ppm (CO); IR (solid): $\tilde{\nu} = 3269$ (NH), 3057 (Csp^2), 2951, 2926, 2886 (Csp^3), 1650 cm^{-1} (CO); HRMS (ESI): calcd for $C_{18}H_{20}N_3O_2$: 310.1550; found: 310.1556.

Cyclo(Tic-*g*Pro-CO) (4k): White solid; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): $t_R =$ 11.23 min; $[\alpha]_D^{20} = 17.5$ ($c = 0.51$ in MeOH); ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): $\delta = 1.72$–1.97 (m, 2H; CH_2(*g*Pro)), 2.02–2.13 (m, 1H; CH_2(*g*Pro)), 2.25–2.34 (m, 1H; CH_2(*g*Pro)), 3.08 (dd, $J = 5.1$, 15.7 Hz, 1H; $CHCH_2$Ph), 3.33 (dd, $J = 10.5$, 15.6 Hz, 1H; $CHCH_2$Ph), 3.52–3.60 (m, 1H; CH_2(*g*Pro)), 3.73–3.81 (m, 1H; CH_2(*g*Pro)), 4.36 (d, $J = 15.3$ Hz, 1H; NCH_2Ph), 4.56 (dd, $J = 5.1$, 10.5 Hz, 1H; NCHCO), 4.87 (d, $J = 15.2$ Hz, 1H; NCH_2Ph), 5.41–5.46 (m, 1H; NCHN), 5.61 (brs; NH), 7.18–7.25 ppm (m, 4H; CH-Ar); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): $\delta = 22.40$, 30.57, 34.08, 46.28 (CH_2), 56.00, 65.35 (CH), 125.83, 126.69, 127.40, 127.55 (CH-Ar), 137.77, 135.15 (Cq-Ar), 158.17, 167.80 ppm (CO); IR (solid): $\tilde{\nu} = 3265$, 3221 (NH), 3067 (Csp^2), 2966, 2916 (Csp^3), 1658, 1636 cm^{-1} (CO); HRMS (ESI): calcd for $C_{15}H_{18}N_3O_2$: 272.1399; found: 272.1405.

Cyclo(Phe-*g*N-MePhe-CO) (4l): White solid; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): $t_R = 14.06$ min; $[\alpha]_D^{20} = -18.8$ ($c = 0.73$ in MeOH); ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): $\delta = 2.80$ (dd, $J = 8.1$, 14.7 Hz, 1H; CH_2Ph), 2.96 (s, 3H; NCH_3), 2.98–3.09 (m, 2H; CH_2Ph), 3.38 (dd, $J = 5.1$, 14.4 Hz, 1H; CH_2Ph), 4.74–4.79 (m, 1H; NCHCO), 5.06 (brs; NH), 5.64–5.71 (m, 1H; NCHN), 7.13 (brs; NH), 7.19–7.35 ppm (m, 10H; CH-Ar); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): $\delta = 27.1$ (CH_3), 37.5, 37.9 (CH_2), 54.6, 63.9 (CH), 127.3, 127.7, 128.7, 129.1, 129.2, 129.3 (CH-Ar), 134.7, 136.2 (Cq-Ar), 156.8, 170.5 ppm (CO); IR (solid): $\tilde{\nu} = 3402$, 3301, 3224 (NH), 3064 (Csp^2), 2965 (Csp^3), 1652 cm^{-1} (CO); HRMS (ESI): m/z: calcd for $C_{19}H_{22}N_3O_2$: 324.1707; found: 324.1701.

Cyclo(Phe-*g*Hyp(Bn)-CO) (4m): Pale yellow solid; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): t_R = 14.05 min; $[\alpha]_D^{20}$ = −4.5 (c = 1.19 in MeOH); ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 1.97–2.03 (m, 1H; CHCH_2CH), 2.48–2.56 (m, 1H; CHCH_2CH), 2.79 (dd, J = 9.6, 14.7 Hz, 1H; CHCH_2Ph), 3.38 (dd, J = 9.6, 14.7 Hz, 1H; CHCH_2Ph), 3.71 (dd, J = 4.5, 12.6 Hz, 1H; CHCH_2N), 3.87–3.91 (m, 1H; CHCH_2N), 4.24 (m, 1H; OCH), 4.45–4.55 (m, 3H; PhCH_2O, NCHCO), 4.66 (brs; NH), 5.59–5.60 (m, 1H; NCHN), 6.62 (brs; NH), 7.25–7.32 ppm (m, 10H; CH-Ar); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 36.7, 39.6, 50.5 (CH_2), 54.5, 63.5 (CH), 71.4 (CH_2), 74.9 (CH), 127.2, 127.8, 128.0, 128.6, 129.0, 129.2 (CH-Ar), 136.1, 137.2 (Cq-Ar), 157.5, 168.4 ppm (CO); IR (solid): $\tilde{\nu}$ = 3207 (NH), 3064 (Csp^2), 2930 (Csp^3), 1651 (CO), 1091 cm^{-1} (C−O−C); HRMS (ESI): m/z: calcd for $C_{21}H_{24}N_3O_3$: 366.1817; found: 366.1839.

Cyclo(Ala-*g*Tic-CO) (4o): White solid; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): t_R = 10.43 min; $[\alpha]_D^{20}$ = 89.0 (c = 0.30 in MeOH); ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 1.38 (d, J = 6.6 Hz, 3H; CH_3), 2.87–2.94 (m, 1H; CH_2CH), 3.19–3.26 (m, 1H; CH_2CH), 4.53–4.56 (m, 1H; NCHCO), 4.59 (d, J = 15 Hz, 1H; CH_2N), 4.71 (d, J = 15 Hz, 1H; CH_2N), 5.04 (brs; NH), 5.73 (m, 2H; NCHN, NH), 7.21–7.29 ppm (m, 4H; H-Ar); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 16.7 (CH_3), 33.9 (CH_2N), 42.3 (CH_2), 48.8, 59.4 (CH), 126.4, 127.8, 127.9, 128.0 (CH-Ar), 131.2, 134.6 (Cq-Ar), 156.9, 169.7 ppm (CO); IR (solid): $\tilde{\nu}$ = 3456, 3394, 3237 (NH), 3053 (Csp^2), 2926 (Csp^3), 1641 cm^{-1} (CO); HRMS (ESI): m/z: calcd for $C_{13}H_{16}N_3O_2$: 246.1242; found: 246.1295.

Cyclo(D-Phe-*g*Pro-CO) (4p): White solid; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): t_R = 10.63 min; ^{1}H NMR (500 MHz, [D_6]DMSO, 298 K): δ = 1.63–1.67 (m, 1H; CH_2), 1.79–1.83 (m, 1H; CH_2), 1.88–1.90 (m, 1H; CH_2), 1.98–2.02 (m, 1H; CH_2), 2.99–3.08 (m, 2H; CH_2Ph), 3.23–3.28 (m, 1H; CH_2), 3.47–3.51 (m, 1H; CH_2), 3.95 (q, J = 6.6 Hz, 1H; NCHCO), 4.90–4.92 (m, 1H; NCHN), 6.29 (d, J = 5.7 Hz, 1H; NH), 7.16–7.18 (m, 2H; NH, H-Ar), 7.20–7.23 (m, 1H; H-Ar), 7.26–7.29 ppm (m, 3H; H-Ar); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 21.9, 32.7, 42.2, 46.7 (CH_2), 61.3, 63.8 (CH), 127.4, 128.8, 129.6 (CH-Ar), 136.1 (Cq-Ar), 160.7, 167.4 ppm (CO); IR (solid): ν = 3331, 3240 (NH), 2925, 2882 (Csp^3), 1669, 1639 cm^{-1} (CO); HRMS (ESI): m/z: calcd for $C_{14}H_{18}N_3O_2$: 260.1379; found: 260.1394.

Cyclo(D-Phe-*g*N-MeAla-CO) (4q): White solid; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): t_R = 11.34 min; ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 1.43–1.45 (d, J = 6.4 Hz, 3H; CH_3), 2.80 (dd, J = 8.4, 14.5 Hz, 1H; CH_2Ph), 2.96 (s, 3H; NCH_3), 3.39 (dd, J = 5.3, 14.5 Hz, 1H; CH_2Ph) 4.74–4.79 (m, 2H; NCHCO, NH), 5.62–5.66 (m, 1H; NCHN), 6.42–6.43 (brs; NH), 7.05–7.35 ppm (m, 5H; H-Ar); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 17.6, 26.4 (CH_3), 37.3 (CH_2), 54.3, 58.8 (CH), 127.2, 129.0, 129.3 (CH-Ar), 145.5 (Cq-Ar), 157.0, 170.3 ppm (CO); IR (solid): $\tilde{\nu}$ = 3301, 3188 (NH), 3069 (Csp^2), 2927 (Csp^3), 1652 cm^{-1} (CO); HRMS (ESI): m/z: calcd for $C_{13}H_{18}N_3O_2$: 248.1394; found: 248.1400.

Cyclo(Pro-*g*Val-CO) (4r): White solid; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): t_R = 7.76 min; ^{1}H NMR (500 MHz, [D_6]DMSO, 298 K): δ = 0.94 (d, J = 6.7 Hz, 3H; CH_3), 0.95 (d, J = 6.7 Hz, 3H; CH_3), 1.67–1.71 (m, CH_2, 3H; CH-(CH_3)$_2$), 1.79–1.89 (m, 2H; CH_2), 2.22–2.29 (m, 1H; CH_2), 4.70 (t, J = 7.1 Hz, 1H; CHCH$_2$), 4.82 (dd, J = 7.2, 13.1 Hz, 1H; CHCH), 6.72 (d, J = 5.6 Hz; NH), 8.34 ppm (d, J = 7.6 Hz; NH); HRMS (ESI): m/z: calcd for $C_{10}H_{18}N_3O_2$: 212.1393; found: 212.1394.

Cyclo(Pro-*g*Leu-CO) (4s): White solid; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): t_R = 8.74 min; ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 0.92 (d, J = 5.3 Hz, 3H; CH_3), 0.95 (d, J = 5.3 Hz, 3H; CH_3), 1.47–1.52 (t, J = 6.9 Hz, 2H; CHCH_2CH), 1.60–1.91 (m, 3H; (CH_3)$_2$CH, CH_2), 1.96–2.07 (m, 1H; CH_2), 2.44–2.56 (m, 1H; CH_2), 3.49–3.56 (m, 2H; CH_2), 4.57–4.61 (t, J = 6.9 Hz, 1H; NHCHCO), 5.09–5.13 (m, 1H; NHCHNH), 5.55 (d, J = 4.6 Hz; NH), 6.50 ppm (d, J = 7.3 Hz; NH); IR (solid): $\tilde{\nu}$ = 3260, 3207 (NH), 2957, 2925, 2871 (Csp^3), 1689, 1661, 1620 (CO), 1453 cm^{-1} (NH lactam); HRMS (ESI): m/z: calcd for $C_{11}H_{20}N_3O_2$: 226.1555; found: 226.1554.

Cyclo(Pro-*g*Val-CO-Pro-*g*Val-CO) (5r): White solid; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): t_R = 10.74 min; ^{1}H NMR (500 MHz, $CDCl_3$, 273 K; signals of rotamers are in italic): δ = 0.93 (d, J = 6.7 Hz, 3H; CH_3), 1.00 (d, J = 6.7 Hz, 3H; CH_3), 1.87–2.28 (m, 5H; CHMe$_2$, βCH$_2$ Pro, γCH$_2$ Pro), 3.35 (m, 1H; δCH Pro), 3.51, 3.64 (m, 1H; δCH Pro), 4.21, 4.29 ppm (dd, J = 2.7, 8.6 Hz, J = 5.5, 7.9 Hz, 1H; αCH Pro); HRMS (ESI): m/z: calcd for $C_{20}H_{35}N_6O_4$: 423.2720; found: 423.2754.

Cyclo(Pro-*g*Leu-CO-Pro-*g*Leu-CO) (5s): White solid; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): t_R = 9.86 min; ^{1}H NMR (300 MHz, CD_3CN, 298 K): δ = 0.91 (m, 3H; CH_3), 0.94 (m, 3H; CH_3), 1.49–1.65 (m, 3H; CH_2, CH$_2$CHMe$_2$), 1.77–2.02 (m, 3H; CH_2, CH$_2$CHMe$_2$), 2.14–2.21 (m, 1H; CH_2, CH$_2$CHMe$_2$), 3.22–3.29 (m, 1H; NCH_2), 3.55–3.63 (m, 1H; NCH_2), 4.14–4.18 (m, 1H; NCHCO), 5.28–5.36 (m, 1H; NCHN), 5.75 (d, J = 9.0 Hz, 1H; NH), 7.28–7.31 ppm (brs, 1H; NH); IR (solid): $\tilde{\nu}$ = 3313 (NH), 2957, 2870 (Csp^3), 1651 (CO), 1511 cm^{-1} (HN−CO); HRMS (ESI): m/z: calcd for $C_{22}H_{37}N_6O_4$: 451.3027; found: 451.3023.

1,3,7-Tribenzyl-5-methyl-[1,3,5]triazepane-2,6-dione (8a): BnBr (204 μL, 1.72 mmol) and NaH (95% wt, 42 mg, 1.72 mmol) were added to a solution of **4a** (100 mg, 0.43 mmol) in THF (8 mL) under Ar. The reaction was stirred at RT under Ar for 48 h. After this time, the reaction mixture was dissolved in ethyl acetate and washed with H_2O. The organic layer was dried over Na_2SO_4 and evaporated. The crude residue was dissolved in CH_2Cl_2 and purified by addition of *N*-(2-mercaptoethyl)aminomethyl-polystyrene (645 mg, 1.29 mmol, used as supplied). The mixture was gently stirred for 24 h. The resin was removed by filtration. The filtrate was evaporated and concentrated under vacuum. Purification by silica-gel chromatography (ethyl acetate/cyclohexane 1:1 v/v) yielded **8a** (89 mg, 50%) as a colorless oil. C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): t_R = 16.82 min; $[\alpha]_D^{20}$ = 2.4 (c = 0.75 in MeOH); ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 2.68 (s, 3H; NCH_3), 3.16–3.33 (m, 2H; CHCH_2Ph), 3.51 (d, J = 15.0 Hz, 1H; NCHN), 4.01 (d, J = 13.9 Hz, 1H; NCH_2Ph), 4.13–4.17 (m, 1H; NCHCO), 4.29 (d, J = 14.9 Hz, 1H; NCH_2Ph), 4.53 (d, J = 14.9 Hz, 1H; NCH_2Ph), 4.74 (d, J = 15.0, 1H; NCHN), 4.90 (d, J = 13.9 Hz, 1H; NCH_2Ph), 7.11–7.14 (m, 2H; H-Ar), 7.22–7.37 ppm (m, 13H; H-Ar); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 34.5 (CH_3), 39.7, 51.4, 52.5, 62.4 (CH_2), 66.7 (CH), 126.8, 127.3, 127.7, 127.9, 128.3, 129.5, 128.6, 129.8 (CH-Ar), 136.7, 137.3 (Cq-Ar), 163.2, 169.0 ppm (CO); IR (solid): $\tilde{\nu}$ = 3062, 3029 (Csp^2), 2926 (Csp^3), 1666, 1638 cm^{-1} (CO); HRMS (ESI): m/z: calcd for $C_{26}H_{28}N_3O_2$: 414.2176; found: 414.2176.

5,6,8-Tribenzyl-hexahydro-3a,6,8-triaza-azulene-4,7-dione (8i): Colorless oil; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): t_R = 17.30 min; $[\alpha]_D^{20}$ = 57.2 (c = 0.57 in MeOH); ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 1.65–2.12 (m, 4H; CH_2), 3.07 (dd, J = 8.6, 13.9 Hz, 1H; CHCH_2Ph), 3.30 (dd, J = 3.6, 13.9 Hz, 1H; CHCH_2Ph), 3.41 (d, J = 14.8 Hz, 1H; NCH_2Ph), 3.46–3.53 (m, 1H; CH_2), 3.69–3.77 (m, 1H; CH_2), 4.12 (dd, J = 3.6, 8.6 Hz, 1H; NCHCO), 4.35 (d, J = 16.6 Hz, 1H; NCH_2Ph), 4.50 (d, J = 16.6 Hz, 1H; NCH_2Ph), 4.65 (d, J = 14.9 Hz, 1H; NCH_2Ph), 5.57 (dd, J = 6.4, 9.0 Hz, 1H; NCHN), 7.10–7.12 (m, 2H; H-Ar), 7.20–7.35 ppm (m, 13H; H-Ar); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 21.7, 30.8, 39.7, 46.1, 47.6, 53.3 (CH_2), 66.2, 71.2 (CH), 1126.8, 126.9, 127.0, 127.4, 128.3, 128.4, 128.6, 128.6, 130.0 (CH-Ar), 137.5, 137.8, 139.0 (Cq-Ar), 165.0, 168.0 ppm (CO); IR (solid): $\tilde{\nu}$ = 3083, 3058, 3029 (Csp^2), 2951, 2926, 2881 (Csp^3), 1667, 1634 cm^{-1} (CO); HRMS (ESI): m/z: calcd for $C_{28}H_{30}N_3O_2$: 440.2333; found: 440.2342.

(7-Benzyl-3-*tert*-butoxycarbonylmethyl-5-methyl-2,6-dioxo-[1,3,5]triazepan-1-yl)acetic acid *tert*-butyl ester (9a): White solid; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): t_R = 16.55 min; $[\alpha]_D^{20}$ = −15.0 (c = 1.05 in MeOH); ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 1.39 (s, 9H; C(CH_3)$_3$), 1.47 (s, 9H; C(CH_3)), 2.91 (d, J = 17.7 Hz, 1H; CHCH_2Ph), 3.06 (s, 3H; NCH_3), 3.19–3.24 (m, 2H; CHCH_2Ph, NCH_2tBu), 3.92 (q, J = 17.4 Hz, 2H; NCH_2tBu), 4.02 (d, J = 17.7 Hz, 1H; NCH_2tBu), 4.08 (dd, J = 4.9, 8.4 Hz, 1H; NCHCO), 4.24 (d, J = 14.1 Hz, 1H; NCH_2N), 5.21 (d, J = 14.1 Hz, 1H; NCH_2N), 7.22–7.33 ppm (m, 5H; H-Ar); ^{13}C NMR (75 MHz, $CDCl_3$,

298 K): δ = 28.0, 28.0 (CH_3, 6C), 35.2 (NCH_3), 40.4, 51.2, 52.0, 65.2 (CH_2), 69.7 (CH), 81.8, 82.2 (Cq), 127.0, 128.6, 129.7 (CH-Ar), 137.2 (Cq-Ar), 162.0, 168.6, 168.8, 169.2 ppm (CO); IR (solid): $\tilde{\nu}$ = 3032 (Csp^2), 2976, 2921, 2850 (Csp^3), 1739, 1682, 1637 cm^{-1} (CO); HRMS (ESI): *m/z*: calcd for $C_{24}H_{36}N_3O_6$: 462.2599; found: 462.2593.

(5-Benzyl-8-*tert*-butoxycarbonylmethyl-4,7-dioxo-octahydro-3a,6,8-triaza-azulen-6-yl)acetic acid *tert*-butyl ester (9i) : Pale yellow solid; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): t_R = 17.00 min; $[\alpha]_D^{20}$ = −26.8 (*c* = 1.18 in MeOH); ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 1.37 (s, 9H; $C(CH_3)_3$), 1.46 (s, 9H; $C(CH_3)_3$), 1.80–1.99 (m, 3H; CH_2), 2.25–2.32 (m, 1H; CH_2), 2.78 (d, *J* = 17.7 Hz, 1H; NCH_2CO), 3.18 (dd, *J* = 9.6, 13.9 Hz, 1H; CH_2Ph), 3.28 (dd, *J* = 3.5, 13.9 Hz, 1H; CH_2Ph), 3.50–3.60 (m, 1H; CH_2), 3.76 (d, *J* = 17.9 Hz, 1H; NCH_2CO; m, 1H; CH_2), 3.97 (d, *J* = 17.9 Hz, 1H; NCH_2CO; m, 1H; NCHCO), 4.07 (d, *J* = 17.7 Hz; NCH_2CO), 5.70 (dd, *J* = 6.0, 8.7 Hz, 1H; NCHN), 7.20–7.32 ppm (m, 5H; H-Ar); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 21.4 (CH_2), 28.0 (CH_3, 6C), 30.7, 40.5, 46.4, 46.9, 52.7 (CH_2), 69.7, 71.6 (CH), 81.7, 81.9 (Cq), 126.9, 128.5, 129.8 (CH-Ar), 137.7 (Cq-Ar), 163.9, 167.7, 168.6, 169.2 ppm (CO); IR (solid): $\tilde{\nu}$ = 2977, 2956, 2922, 2853 (Csp^3), 1739, 1679, 1638 (CO), 1221, 1146 cm^{-1} (C−O); HRMS (ESI): *m/z*: calcd for $C_{26}H_{38}N_3O_6$: 488.2755; found: 488.2755.

3,7-Dibenzyl-5-methyl[1,3,5]triazepane-2,6-dione (10a): BnBr (80 μL, 0.67 mmol) and KF/Al_2O_3 40% wt (930 mg, 6.4 mmol) were added to a solution of **4a** (150 mg, 0.64 mmol) in CH_2Cl_2 (6 mL) under Ar. The mixture was stirred for 24 h at RT under Ar. After this time, the reaction mixture was diluted with CH_2Cl_2, washed with H_2O, dried over Na_2SO_4, and concentrated. The crude residue was purified by silica-gel chromatography (ethyl acetate/cyclohexane/AcOH 50:50:1 v/v/v) to yield **10a** (141 mg, 68%) as a white powder. C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): t_R = 14.07 min; $[\alpha]_D^{20}$ = −74.1 (*c* = 0.63 in MeOH); ^{1}H NMR (500 MHz, $CDCl_3$, 298 K): δ = 2.65 (s, 3H; NCH_3), 2.83 (dd, *J* = 9.1, 14.3 Hz, 1H; $CHCH_2Ph$), 3.36 (dd, *J* = 4.5, 14.3 Hz, 1H; $CHCH_2Ph$), 4.13 (d, *J* = 15.6 Hz, 1H; NCH_2N), 4.39 (d, *J* = 15.1 Hz, 1H; NCH_2Ph), 4.55–4.59 (m, 2H; NCHCO, NH), 4.79 (d, *J* = 15.1 Hz, 1H; NCH_2Ph), 5.15 (d, *J* = 15.6 Hz, 1H; NCH_2N), 7.26–7.36 ppm (m, 10H; H-Ar); IR (solid): $\tilde{\nu}$ = 3296, 3199 (NH), 3063 (Csp^2), 2956, 2923, 2853 (Csp^3), 1653 cm^{-1} (CO); HRMS (ESI): *m/z*: calcd for $C_{19}H_{22}N_3O_2$: 324.1707; found: 324.1702.

3-Benzyl-7-isopropyl-5-methyl[1,3,5]triazepane-2,6-dione (10c): White solid; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): t_R = 12.45 min; $[\alpha]_D^{20}$ = −54.4 (*c* = 0.82 in MeOH); ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 1.03 (d, *J* = 6.7 Hz, 3H; CH_3), 1.06 (d, *J* = 6.7 Hz, 3H; CH_3), 2.14–2.25 (m, 1H; $CH(CH_3)_2$), 2.62 (s, 3H; NCH_3), 3.93 (dd, *J* = 4.2, 6.8 Hz, 1H; NCHCO), 4.43 (d, *J* = 15.3 Hz, 1H; NCH_2Ph), 4.50 (d, *J* = 15.0 Hz, 1H; NCH_2N), 4.71 (d, *J* = 15.0 Hz, 1H; NCH_2N), 4.83–4.88 (m, 2H; NCH_2Ph, NH), 7.26–7.37 ppm (m, 5H; H-Ar); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 18.1, 19.8 (CH_3), 31.0 (CH), 34.2 (NCH_3), 51.5, 60.7 (CH_2), 127.9, 128.3, 128.9 (CH-Ar), 137.6 (Cq-Ar) 158.6, 170.0 ppm (CO); IR (solid): $\tilde{\nu}$ = 3393, 3199 (NH), 3063 (Csp^2), 2959, 2920, 2850 (Csp^3), 1647 cm^{-1} (CO); HRMS (ESI): *m/z*: calcd for $C_{15}H_{22}N_3O_2$: 276.1680; found: 276.1691.

5,8-Dibenzyl-hexahydro-3a,6,8-triaza-azulene-4,7-dione (10i): White solid; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): t_R = 14.57 min; ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 1.76–1.86 (m, 2H; CH_2), 1.96–2.06 (m, 1H; CH_2), 2.18–2.28 (m, 1H; CH_2), 2.84 (dd, *J* = 9.2, 14.2 Hz, 1H; $CHCH_2Ph$), 3.44–3.68 (m, 3H; CH_2, $CHCH_2Ph$), 4.38–4.44 (m, 1H; NCHCO), 4.54 (d, *J* = 16.8 Hz, 1H; NCH_2Ph), 4.80 (d, *J* = 16.8 Hz, 1H; NCH_2Ph), 5.12 (d, *J* = 2.4 Hz, 1H; NH), 5.69 (dd, *J* = 4.4, 6.8 Hz, 1H; NCHN), 7.19–7.35 ppm (m, 10H; CH-Ar); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 22.6, 30.6, 38.6, 45.7, 47.5 (CH_2), 58.0, 69.2 (CH), 126.3, 127.1, 127.1, 128.8, 128.8, 129.6 (CH-Ar), 136.8, 138.8 (Cq-Ar), 160.4, 163.4 ppm (CO); IR (solid): $\tilde{\nu}$ = 3211 (NH), 3029 (Csp^2), 2925 (Csp^3), 1663, 1635 cm^{-1} (CO); HRMS (ESI): *m/z*: calcd for $C_{21}H_{24}N_3O_2$: 350.1863; found: 350.1866.

(6-Benzyl-1-methyl-4,7-dioxo[1,3,5]triazepan-3-yl)acetic acid *tert*-butyl ester (11a): White solid; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): t_R = 13.82 min; $[\alpha]_D^{20}$ = −53.0 (*c* = 0.84 in MeOH); ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 1.45 (s, 9H; $C(CH_3)_3$), 2.81 (dd, *J* = 9.2, 14.3 Hz, 1H; CH_2Ph), 3.13 (s, 3H; NCH_3), 3.36 (dd, *J* = 4.5, 14.3 Hz, 1H; CH_2Ph), 3.86 (d, *J* = 17.7 Hz, 1H; NCH_2CO), 4.17 (d, *J* = 15.7 Hz, 1H; NCH_2N), 4.28 (d, *J* = 17.7 Hz, 1H; NCH_2CO), 4.58–4.63 (m, 2H; NCHCO, NH), 5.44 (d, *J* = 15.7 Hz, 1H; NCH_2N), 7.23–7.36 ppm (m, 5H; H-Ar); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 28.1 (CH_3, 3C), 34.7 (NCH_3), 38.2, 51.5 (CH_2), 56.0 (CH), 62.8 (CH_2), 82.4 (Cq), 127.3, 129.0, 129.4 (CH-Ar), 136.1 (Cq-Ar), 157.3, 168.9, 169.8 ppm (CO); IR (solid): $\tilde{\nu}$ = 3291, 3221 (NH), 3063 (Csp^2), 2975, 2925, 2850 (Csp^3), 1741, 1653 cm^{-1} (CO); HRMS (ESI): *m/z*: calcd for $C_{18}H_{26}N_3O_4$: 348.1918; found: 348.1917.

(6-Isopropyl-1-methyl-4,7-dioxo[1,3,5]triazepan-3-yl)acetic acid *tert*-butyl ester (11c): White solid; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): t_R = 12.18 min; $[\alpha]_D^{20}$ = −49.1 (*c* = 0.43 in MeOH); ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 1.02 (d, *J* = 7.0 Hz, 3H; CH_3), 1.04 (d, *J* = 7.0 Hz, 3H; CH_3), 1.43 (s, 9H; CH_3), 2.15–2.22 (m, 1H; $CH(CH_3)_2$), 3.07 (s, 3H; NCH_3), 3.91–3.97 (m, 2H; NCH_2CO, NCHCO), 4.19 (d, *J* = 17.7 Hz, 1H; NCH_2CO), 4.47 (d, *J* = 15.6 Hz, 1H; NCH_2N), 4.84 (d, *J* = 3.8 Hz, 1H; NH), 5.09 ppm (d, *J* = 15.6 Hz, 1H; NCH_2N); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 18.0, 19.7, 28.0 (CH_3), 30.6 (NCH_3), 34.7 (CH), 51.3 (CH_2), 61.4 (CH), 62.8 (CH_2), 83.1 (Cq), 157.9, 168.9, 170.0 ppm (CO); IR (solid): $\tilde{\nu}$ = 3392, 3296, 3226 (NH), 2921, 2850 (Csp^3), 1734, 1651 cm^{-1} (CO); HRMS (ESI): *m/z*: calcd for $C_{14}H_{26}N_3O_4$: 300.1918; found: 300.1907.

(5-Benzyl-4,7-dioxo-octahydro-3a,6,8-triaza-azulen-8-yl)acetic acid *tert*-butyl ester (11i): Colorless oil; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): t_R = 14.33 min; ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 1.45 (s, 9H; $C(CH_3)_3$), 1.91–2.21 (m, 4H; CH_2), 2.81 (dd, *J* = 9.7, 14.3 Hz, 1H; CH_2Ph), 3.44 (dd, *J* = 4.2, 14.3 Hz, 1H; CH_2Ph), 3.56–3.69 (m, 2H; CH_2), 3.74 (d, *J* = 18.1 Hz, 1H; NCH_2CO), 4.43 (d, *J* = 18.1 Hz, 1H; NCH_2CO; m, 1H; NCHCO), 4.57 (br s; NH), 5.76 (dd, *J* = 3.9, 6.3 Hz, 1H; NCHN), 7.22–7.37 ppm (m, 5H; H-Ar); IR (solid): $\tilde{\nu}$ = 3272 (NH), 3058 (Csp^2), 2927, 2874 (Csp^3), 1735, 1705, 1674 (CO), 1612 cm^{-1} (C=C); HRMS (ESI): *m/z*: calcd for $C_{20}H_{27}N_3O_4$: 374.2074; found: 374.2086.

(7-Benzyl-1-carboxymethyl-5-methyl-2,6-dioxo[1,3,5]triazepan-3-yl)acetic acid (12): Pale yellow solid; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): t_R = 11.74 min; ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 2.91 (s, 3H; CH_3), 3.10 (dd, *J* = 7.5, 13.6 Hz, 1H; CH_2Ph), 3.22 (dd, *J* = 6.9, 13.6 Hz, 1H; CH_2Ph), 3.52 (d, *J* = 17.5 Hz, 1H; NCH_2), 3.79 (d, *J* = 17.5 Hz, 1H; NCH_2), 3.92 (d, *J* = 17.6 Hz, 1H; NCH_2), 4.05 (d, *J* = 17.6 Hz, 1H; NCH_2), 4.15 (t, *J* = 7.2 Hz, 1H; NCHCO), 4.82 (q, *J* = 17.9 Hz, 1H; NCH_2N), 7.18–7.30 ppm (m, 5H; CH-Ar) ; ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): δ = 34.58 (CH_3), 39.6, 50.6, 50.8, 62.8 (CH_2), 68.8 (CH), 127.0, 128.6, 130.0 (CH-Ar), 137.5 (Cq-Ar), 160.3, 169.0, 171.5, 171.7 ppm (CO); IR (solid): $\tilde{\nu}$ = 3448 (OH), 2927 (Csp^3), 2622, 2531 (OH), 1713, 1918 cm^{-1} (CO); HRMS (ESI): *m/z*: calcd for $C_{16}H_{20}N_3O_6$: 350.1347; found: 350.1343.

(6-Benzyl-1-methyl-4,7-dioxo[1,3,5]triazepan-3-yl)acetic acid (13): White solid; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): t_R = 10.35 min; ^{1}H NMR (300 MHz, [D$_6$]DMSO, 298 K): δ = 2.78 (dd, *J* = 6.8, 14.1 Hz, 1H; CH_2Ph), 2.95, (s, 3H; NCH_3), 3.06 (dd, *J* = 6.6, 14.1 Hz, 1H; CH_2Ph), 4.03 (q, *J* = 17.6 Hz, 2H; NCH_2), 4.35 (d, *J* = 15.6 Hz, 1H; NCH_2N), 4.77–4.80 (m, 1H; NCHCO), 5.56 (d, *J* = 15.6 Hz, 1H; NCH_2N), 6.28 (s; NH), 7.16–7.32 ppm (m, 5H; H-Ar); ^{13}C NMR (75 MHz, [D$_6$]DMSO, 298 K): δ = 33.4 (CH_3), 36.2, 50.3 (CH_2), 54.1 (CH), 61.5 (CH_2), 126.2, 129.0, 129.4 (CH-Ar), 138.1 (Cq-Ar), 156.3, 170.5, 171.6 ppm (CO); IR (solid): $\tilde{\nu}$ = 3277 (NH), 2924 (Csp^3), 2455 (OH), 1702, 1672, 1608 cm^{-1} (CO); HRMS (ESI): *m/z*: calcd for $C_{14}H_{18}N_3O_4$: 292.1292; found: 292.1294.

(9-Methyl-6,10-dioxo-5,8,9,10,10a,11-hexahydro-5a,7,9-triazacyclohepta[b]naphthalen-7-yl)acetic acid *tert*-butyl ester (14): White solid; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): t_R = 14.92 min; ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 1.46 (s, 9H; CH_3), 3.11–3.31 (m, 3H; NCH_3, $CHCH_2Ph$), 4.07 (s, 2H; NCH_2Ph), 4.29–4.39 (m, 2H; NCH_2CO, NCHCO), 4.59 (d, *J* = 15.1 Hz, 1H; NCH_2N), 4.72 (d, *J* = 16.4 Hz, 1H; NCH_2CO), 4.98 (d, *J* = 15.1 Hz, 1H; NCH_2N), 7.12–7.20 ppm (m, 4H; H-Ar) ; ^{13}C NMR

(75 MHz, $CDCl_3$, 298 K): $\delta = 28.1$ (CH_3), 33.9 (CH_2), 35.3 (CH_3), 49.2, 51.3 (CH_2), 62.6 (CH), 63.5 (CH_2), 82.3 (Cq), 126.3, 126.6, 126.8, 128.3 (CH-Ar), 133.2, 133.5 (Cq-Ar), 160.7, 168.8, 170.1 ppm (CO); IR (solid): $\tilde{\nu} = 2980$, 2926 (Csp^3), 1747, 1673, 1629, 1617 cm^{-1} (CO); HRMS (ESI): m/z: calcd for $C_{19}H_{26}N_3O_4$: 360.1918; found: 360.1907.

(3,7-Dibenzyl-5-methyl-2,6-dioxo[1,3,5]triazepan-1-yl)acetic acid *tert*-butyl ester (15): White solid; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): $t_R = 16.63$ min; ^1H NMR (300 MHz, $CDCl_3$, 298 K): $\delta = 1.40$ (s, 9H; $(CH_3)_3$), 2.69 (s, 3H; NCH_3), 3.00 (d, $J = 17.6$ Hz, 1H; NCH_2CO), 3.16 (dd, $J = 8.6$, 13.8 Hz, 1H; CH_2Ph), 3.25 (dd, $J = 4.1$, 13.8 Hz, 1H; CH_2Ph), 4.03–4.10 (m, 3H; NCH_2CO, NCH_2N, NCHCO), 4.32 (d, $J = 15.0$ Hz, 1H; NCH_2Ph), 4.55 (d, $J = 15.0$ Hz, 1H; NCH_2Ph), 5.03 (d, $J = 13.9$ Hz, 1H; NCH_2N), 7.22–7.34 ppm (m, 10H; CH-Ar); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): $\delta = 28.0$, 34.7 (CH_3), 40.6, 51.7, 52.2, 62.7 (CH_2), 69.9 (CH), 81.8 (Cq), 127.1, 127.9, 128.5, 128.6, 128.8, 129.9 (CH-Ar), 136.9, 137.1 (Cq-Ar), 162.4, 168.9, 169.2 ppm (CO); IR (solid): $\tilde{\nu} = 3075$ (Csp^2), 2977, 2930 (Csp^3), 1736, 1672, 1641 cm^{-1} (CO); HRMS (ESI): m/z: calcd for $C_{25}H_{32}N_3O_4$: 438.2387; found: 438.2366.

1-Benzoyl-7-benzyl-5-methyl[1,3,5]triazepane-2,6-dione (16): Cyclo(L-Phe-*g*Sar-CO) (**4a**, 100 mg, 0.43 mmol) was dissolved in distilled THF (10 mL) and then 2,4,6-collidine (113 µL, 0.86 mmol) was added, followed by the dropwise addition of BzCl (100 µL, 0.86 mmol). The reaction was stirred at RT under inert atmosphere for 7 h. After this time, the solvent was evaporated and the crude residue was purified by flash-column chromatography (EtOAc/cyclohexane 3:2 v/v) to yield **16** (103 mg, 71%) as a white solid. C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): $t_R = 12.01$ min; $[\alpha]_D^{20} = 121.8$ ($c = 0.63$ in MeOH); ^1H NMR (300 MHz, $CDCl_3$, 298 K): $\delta = 3.02$ (s, 3H; NCH_3), 2.95–3.07 (m, 1H; $CHCH_2Ph$), 3.45 (dd, $J = 14.1$, 3.9 Hz, 1H; $CHCH_2Ph$), 4.10 (dd, $J = 14.1$, 7.5 Hz, 1H; NCHN), 5.15 (dd, $J = 14.1$, 5.4 Hz, 1H; NCHN) 5.69 (dd, $J = 9.6$, 3.9 Hz, 1H; NCH_2Ph), 6.68–6.75 (m, 1H; NH), 7.18–7.50 ppm (m, 10H; H-Ar); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): $\delta = 35.5$ (CH_3), 40.0 (CH_2), 57.0 (CH_2), 60.7 (CH), , 126.9, 127.0, 128.1, 128.3, 130.0, 131.3 (CH-Ar), 135.0, 136.5 (Cq-Ar), 158.4, 168.8, 170.0 ppm (CO); IR (solid): $\tilde{\nu} = 3261$ (NH), 2927 (Csp^2), 2851 (Csp^3), 1728, 1674, 1634 cm^{-1} (CO); HRMS (ESI): m/z: calcd for $C_{19}H_{20}N_3O_3$: 338.1499; found: 338.1497.

1-Benzoyl-7-benzyl-5-methyl[1,3,5]triazepane-2,6-dione (17): White solid; Yield: 123 mg, 79%; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): $t_R = 13.52$ min; $[\alpha]_D^{20} = 180.4$ ($c = 0.68$ in MeOH); ^1H NMR (300 MHz, $CDCl_3$, 298 K): $\delta = 3.06$ (s, 3H; NCH_3), 2.97–3.10 (m, 1H; $CHCH_2Ph$), 3.51 (dd, $J = 14.3$, 3.04 Hz, 1H; $CHCH_2Ph$), 4.13 (dd, $J = 14.1$, 6.9 Hz, 1H; NCH_2N), 5.08 (dd, $J = 14.1$, 5.1 Hz, 1H; NCHCO), 6.66–6.75 (m, 1H; NH), 6.77 (d, $J = 15.3$ Hz, 1H; $CHCHPh$), 7.15–7.49 (m, 10H; H-Ar), 7.62 ppm (d, $J = 15.$ Hz, 1H; $CHCHPh$); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): $\delta = 35.8$ (CH_3), 40.4, 57.4 (CH_2), 59.8 (CH), 117.7 (CH), 127.2, 128.5, 128.5, 128.6, 129.1, 129.1, 130.0, 130.0, 130.7 (CH-Ar), 134.7, 136.8 (Cq-Ar), 158.7, 165.7, 169.3 ppm (CO); IR (solid): $\tilde{\nu} = 3121$ (NH), 2926 (Csp^2), 2851 (Csp^3), 1719, 1679, 1620 cm^{-1} (CO); HRMS (ESI): m/z: calcd for $C_{21}H_{22}N_3O_3$: 364.1656; found: 364.1632.

7-Benzyl-1-(4-bromo-benzoyl)-5-methyl[1,3,5]triazepane-2,6-dione (18): In this case, 4-bromo-benzoyl chloride (3.5 equiv, 330 mg, 1.51 mmol) was used and the reaction was stirred for 30 h at 40°C. White solid; Yield: 152 mg, 85%; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): $t_R = 13.60$ min; $[\alpha]_D^{20} = 64.7$ ($c = 0.83$ in MeOH); ^1H NMR (300 MHz, $CDCl_3$, 298 K): $\delta = 3.07$ (s, 3H; NCH_3), 2.99–3.08 (m, 1H; $CHCH_2Ph$), 3.48 (dd, $J = 14.4$, 3.9 Hz, 1H; $CHCH_2Ph$), 4.17 (dd, $J = 14.1$, 7.2 Hz, 1H; NCHN), 5.16 (dd, $J = 14.4$, 5.4 Hz, 1H; NCHN) 5.70 (dd, $J = 9.6$, 3.9 Hz, 1H; NCH_2Ph), 6.63–6.74 (m, 1H; NH), 7.08–7.37 (m, 7H; H-Ar), 7.46 ppm (d, $J = 8.4$ Hz, 2H; H-ArBr); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): $\delta = 35.8$ (CH_3), 40.3, 57.3 (CH_2), 61.2 (CH), 126.3 (CH-Ar), 127.4 (C–Br), 128.5, 128.9, 130.4, 132.0, (CH-Ar), 134.2, 136.6 (Cq-Ar), 158.5, 168.9, 169.5 ppm (CO); IR (solid): $\tilde{\nu} = 3266$ (NH), 2925 (Csp^2), 2852 (Csp^3), 1726, 1674, 1633 cm^{-1} (CO); HRMS (ESI): m/z: calcd for $C_{19}H_{19}Br_1N_3O_3$: 416.0604; found: 416.0592; calcd for $C_{19}H_{19}Br_1N_3Na_1O_3$: 438.0424; found: 438.0415.

7-Benzyl-1-(2,2-dimethyl-propionyl)-5-methyl[1,3,5]triazepane-2,6-dione (19): In this case, trimetylacetyl chloride (4 equiv, 100 µL 1.72 mmol) was used and the reaction mixture was stirred for 30 h at 50°C. White solid; Yield: 109 mg, 80%; C_{18}-RP-HPLC (A: 0.1% TFA in H_2O, B: 0.08% TFA in MeCN, 0–100% B, 1.2 mL min^{-1}, 20 min): $t_R = 12.67$ min; $[\alpha]_D^{20} = 156.2$ ($c = 0.73$ in MeOH); ^1H NMR (300 MHz, $CDCl_3$, 298 K): $\delta = 1.16$ (s, 9H; $(CH_3)_3CCO$), 2.91 (dd, $J = 14.4$ Hz, 10.2 Hz, 1H; $CHCH_2Ph$), 3.04 (s, 3H; NCH_3), 3.43 (dd, $J = 14.1$ Hz, 3,9 Hz, 1H; $CHCH_2Ph$), 4.11 (dd, $J = 13.8$ Hz, 6.9 Hz, 1H; NCH_2N), 5.07 (dd, $J = 14.1$ Hz, 5.7 Hz, 1H; NCH_2N), 5.72 (dd, $J = 10.2$ Hz, 3,8 Hz, 1H; $CHCH_2Ph$), 6.67 (brt, $J = 6.3$ Hz, 1H; NH), 1.17–7.35 ppm (m, 5H; H-Ar); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K): $\delta = 27.8$, 35.4 (CH_3), 39.6 (CH_2), 42.0 (Cq), 56.9 (CH_2), 60.1 (CH), 126.6, 127.9, 129.8 (CH-Ar), 136.7 (Cq-Ar), 158.3, 169.2, 178.8 ppm (CO); IR (solid): $\tilde{\nu} = 3157$ (NH), 2927 (Csp^2), 2866 (Csp^3), 1735, 1675, 1615 cm^{-1} (CO); HRMS (ESI): m/z: calcd for $C_{17}H_{24}N_3O_3$: 318.1812; found: 318.1801.

Mice and parasites: BALB/c female mice were purchased from Harlan Laboratories (Orléans, France). All experiments and procedures conformed to the French Ministry of Agriculture regulations for animal experimentation (1987). Sporozoites of the uncloned line of the 265BY strain of *P. yoelii yoelii* were obtained by dissection of the salivary glands of infected *Anopheles stephensi* mosquitoes, 16 to 21 days after an infected blood meal.

Hepatocyte culture: Mouse hepatocytes were prepared as described, with minor modifications.[43] Cells were isolated by collagenase perfusion (Boehringer Mannheim) of liver fragments and were further purified over a 60% Percoll gradient (Pharmacia Biotech, Uppsala, Sweden). Hepatocyte purity and viability were >95% as assessed by Trypan blue dye exclusion. Cells were cultured in eight-chamber plastic Lab-Teck slides (Nunc, Naperville, IL) or in flat bottom 96 wells (Costar) in William's E medium (Life Technologies, Edinburgh, Scotland) supplemented with 5% FCS (Life Technologies), 100 UI ml^{-1} of penicillin-streptomycin (Life Technologies) and incubated at 37°C in 3.5% CO_2 for 24 h.

Evaluation of drug toxicity: Drugs were diluted first in DMSO then in incomplete medium. Toxicity of the drugs to primary culture of hepatocytes (20×10^3 I flat bottom 96 wells) was evaluated in duplicate by adding the drugs at decreasing concentrations. Medium was changed 3 and 24 h after with fresh medium containing the drug at the same concentration. After 48 h, 110 µl of a solution containing 10 µl of a MTT solution (5 mg mL^{-1}) and 100 µl of medium was added to dissolve formazan crystals. Optical density was read immediately at 570 nm with a reference wavelength at 630 nm.[44]

In vitro assay of sporozoite invasion of and development in hepatocytes: After removal of medium from the culture chambers, 10^4 sporozoites were added in 100 µl of fresh supplemented medium with the various drugs tested at different concentrations. Medium was replaced 3 and 24 h after with fresh medium containing the drug tested. Experimental determinations were performed in triplicate. Control cultures were incubated with medium containing 10% DMSO (the highest concentration used). Cultures were stopped 48 h after sporozoite infection, fixed with cold methanol and schizonts numbers were assessed by immunofluorescence by using a monoclonal antibody recognizing *P. yoelii* liver stages.[45,46]

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