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Due to their well-defined three-dimensional geometry, spiro compounds are widely utilized in drug research. From the central tetrahedral carbon atom, besides the regular structure, an inverted spiro connectivity may be envisioned. Here we disclose the synthesis of this molecule class that we have coined quasi[1]catenanes. Next to their fascinating and aesthetic shape, the higher compactness as compared to regular spiro bicycles is noteworthy. To enable synthetic access to compact entangled multimacrocyclic molecules, we have developed a new strategy. The key element is a template, which is covalently connected to the linear precursors, and spatially directs the sterically congested backfolding macrocyclizations that are required to give quasi[1]catenanes. Similarly, quasi[1]rotaxanes are made.
Enmeshed molecular architectures inspire synthetic chemists for creative endeavours and find application in nanotechnology and materials research.1 Striking natural examples are the lasso peptides2, and also catenane structures found in some proteins3 and DNA4. Synthetically, an impressive array of entangled molecules such as catenanes and rotaxanes have been made that are mainly applied in nanotechnology research1. Supramolecular three-dimensional templating via metal coordination, π-stacking or hydrogen bonding as developed by the respective Sauvage5/Leigh6, Stoddart7,8 and Hunter9/Vögtle10 groups gave access to the vast majority of the catenane and rotaxane series11. However, the prerequisite of moieties able to form non-covalent interactions limits the structural diversity of the entangled molecules that can be obtained. Remarkably, the first example of a non-statistical [2]catenane synthesis was reported back in 1964 by Schill and Lüttringhaus12 using a covalent approach13–15. Over the past years, there has been a comeback of covalent approaches towards entangled structures16–20. Currently, we are developing covalent template-directed synthetic concepts to disclose unknown or ‘impossible’20 entangled or mechanically interlocked molecular geometries with access to the natural [1]rotaxane-type lasso peptide series as the ultimate goal21. Here we report a synthetic concept based on template-directed backfolding macrocyclization of which the utility is demonstrated by the efficient synthesis of an uncommon spiro geometry22. The regular spiro geometry is obtained after connection of two rings to the same core tetrahedral carbon atom and can be considered as the molecular equivalent of the figure of eight23. The fascinating spirocyclic geometry is found in many natural compounds and due to their rigid and well-defined three-dimensional shape, characterized by a perpendicular arrangement of the two rings, spiro compounds find wide application in drug research24. Besides the regular geometry (as for structure 1, Fig. 1), an alternative connectivity may be envisioned by backfolding of the two rings at the central tetrahedral carbon atom to give an inverted spiro configuration (that is, 2, Fig. 1). An intermediate in the landmark [2]catenane synthesis by the Schill group already displayed a similar inverted spiro geometry12. Despite the fact that both spiro geometries do not contain any stereocentre, they are still interesting from a stereochemical point of view. Uniquely, in the spiro connectivity case, after inverting the centre-of-symmetry tetrahedral carbon, a diastereomer is obtained with different properties while retaining the C2 symmetry around the same axis. Although we realize that every name is a compromise because no mechanical bond is present, we coined the topologically trivial inverted spiro geometry a quasi-[1]catenane 2 (ref. 25). Similarly, a quasi-[1]rotaxane 3 can be drawn, of which unwinding to give conformer 4 is prevented by large stoppers (Fig. 1).

For steric reasons, the synthesis of the quasi-[1]catenane 2 and quasi-[1]rotaxane 3 architectures requires specifically tailored methodologies. We have tackled this challenge, as will be pointed out first for quasi-[1]catenane 2, by using a covalent template directing the required backfolding cyclization. Template 6 (Fig. 2, route B) will be temporarily connected to two of the four linear ring precursors in 5 and will eventually be part of the spirocyclic ring formed with the other precursor chains. Due to the tetrahedral geometry of the central carbon atom, cyclization of the first ring on the template in 7 will occur in a perpendicular and backfolded fashion over the linear precursor of the second ring to form a pseudo-quasi-[1]rotaxane 8 (ref. 26). Subsequent backfolding cyclization of the second ring to give the inverted spiro connectivity, followed by cleavage of the temporal scaffolding bonds provides bicyclic quasi-[1]catenane 2. Essentially, spiro quasi-[1]catenane 2 may be obtained from the tetrahedral precursor 5 in just four steps, of which three are macrocyclizations27. Without making the temporal connections to the template, the regular spiro bicycle 1 will be obtained (Fig. 2, routes B and C). An alternative route to the regular spiro bicycle 1 opens up after breaking the temporary bonds in 8 (Fig. 2, route D), initiating unwinding of the sterically congested quasi-thread fragment to give 10, followed by closure of the second ring. Capping the end-groups in pseudo-quasi-[1]rotaxane 8 by large stoppers (Fig. 2, route E) to give 12 prevents the unwinding process after temporal bond cleavage to give quasi-[1]rotaxane 3. Stopper attachment to 10 (Fig. 2, route F) gives the sterically relaxed conformer 4.

Results

Design of the building blocks. To avoid the requirement of protective groups and to facilitate the sterically difficult macrocyclizations, robust transformations were selected, that is, transesterification/lactonization28 for the scaffolding temporal connection to the template, Cu-catalysed azide alkyne cycloaddition29,30 (CuAAC) for the first ring closure and olefin metathesis31 for the second and final ring closure or introduction of the quasi-rotaxane step elements. For synthetic reasons and to ensure the perpendicular mutual arrangement of the two pairs of ring-precursor chains at precursor 5, we have chosen a spiro-linkage via the tetrahedral carbon atom of a 9H-fluorene moiety (Fig. 3)32. The design of the temporal covalent scaffolding tether at the central precursor 5 is based on amide-N-benzylic moieties containing phenolic hydroxyl groups that will be esterified to template 6 and may be removed from the final spiro or rotaxane compounds by consecutive transesterification and protolytic cleavage of the amide benzylic linkages33. As the template, 2,5-bis(pent-4-yn-1-ylxy)-1,4-benzenedicarboxylic ester 6 was chosen. The tether length in precursor 5 and template 6 gives access to spiro architectures of 27- and 31-membered rings, resulting from CuAAC and ring-closing metathesis (RCM)

**Figure 1 | Sketches of the quasi-[1]catenane and quasi-[1]rotaxane geometries.** These are, together with the two regular ring systems, all available from a single quaternary carbon precursor. By starting from compound 5 in which four linear-chain ring precursors are connected to the same central tetrahedral carbon atom, after closure of the perpendicularly arranged rings, two spiro geometries may be envisioned. Besides the regular spiro bicycle 1, after double-backfolding ring closure, the inverted spiro configuration is obtained giving quasi-[1]catenane 2. Alternatively, by installation of large stoppers at the end of the two linear chain fragments, next to the regular closure to give 4, cyclization via backfolding gives access to the mechanically locked quasi-[1]rotaxane 3 conformation.
We first followed the synthesis of quasi[1]catenane 2, please see the Supplementary Information.
monocycle 10 was obtained, although in a yield of 42% only (Fig. 4a, route C). It should be noted that this yield is significantly lower than the CuAAC reactions to 8 that both occurred in a template/scaffold-preorganized intramolecular fashion. As an elegant detour, monocycle 10 could be obtained quantitatively after methoxide-induced cleavage of the tether in pseudo-quasi[1]rotaxane 8 inducing immediate steric relaxation (Fig. 4a, route D). By lowering the temperature to 30 °C at high dilution, the RCM reaction proceeded cleanly and no truncated cycloalkene was observed. Protolytic removal of the 2-hydroxy-4-methoxybenzyl groups led to an unexpectedly stable adduct of TFA and 1, which could not be separated. However, treatment with methanolic 3 M HCl at 50 °C gave a clean removal of the benzyl groups and the resulting triazolium ions were neutralized with aqueous NaHCO₃ to give the regular spiro macrocycle 1 in a yield of 51%. Catalytic hydrogenation removed the RCM-derived olefin E/Z mixture to give 1-H₂₁. The different geometries of 1-H₂₁ and 2-H₂₁ are obvious from the ¹H NMR spectra (Fig. 5) showing almost no overlapping signals. In quasi[1]catenane 2 especially, the four pairs of methylene protons (Fig. 5, protons f–i) experience an upfield shift to the 0.5 p.p.m. region due to their presence in the shielding region above the plane of the aromatic fluorene phenyl groups. For the same reason, a dramatic upfield shift of over 3 p.p.m. of the NH triplets (Fig. 5, protons k) of 2-H₂₁ and 2.4 Å, respectively, show these hydrogen bonds in the solid...
state of 2. The different chromatographic retention times of the two spiro diastereomers visualizes the different physical properties showing a higher polarity for the densely packed quasi[1]catenane 2.

**Synthesis of the quasi[1]rotaxane.** The backfolded intermediate 8 also provides access to quasi[1]rotaxane 3 (Fig. 4c). Cross-metathesis of the terminal thread alkenes in 8 with the acrylamide-functionalized stopper 11, followed by tether detachment by consecutive transesterification and protolysis gave quasi[1]rotaxane 3 in a yield of 39% over three steps. The unthreaded conformation 4 was obtained via cross-metathesis-mediated stopper attachment to unwound intermediate 10 and protolytic debenzylation in a two-step yield of 28%. The structural dissimilarity between the quasi[1]rotaxane 3 and conformer 4 was reflected by comparing the $^1$H NMR spectra showing, as in the quasi[1]catenane series, remarkable shift differences, especially for the methylene protons in the thread fragment close to the fluorene moiety, the central amide NH’s and the triazole proton (Fig. 5). Quasi[1]rotaxane 3 and the unwound isomer 4 only differ in conformation of which interconversion is mechanically blocked by the stoppers. In contrast to the very facile unwinding of the pseudo-thread in 8 through the 31-membered ring to give 10 (Fig. 4a, route D), the tris(4-(tert-butyl)phenyl)methane stoppered quasi[1]rotaxane 3 combination was completely stable, even after heating in dimethylsulphoxide at 110°C for 18 h as was confirmed by $^1$H NMR spectroscopy.

**Discussion**

Generally, spiro compounds are obtained after two ring closures of the four linear-chain ring precursors connected to a central tetrahedral carbon atom. We have shown that after prior temporal covalent connection via a template of two of the four ring-precursor chains, ring closure of the second pair to the template is forced in a backfolded fashion. Final closure of the first pair of ring-precursor chains followed by cleavage of the temporary linkers to the template provides an inverted spiro geometry coined here as a quasi[1]catenane. Although the regular spiro and quasi[1]catenane geometries only differ by inversion of the configuration of the centre-of-symmetry tetrahedral carbon, they relate as diastereomers with inherently different physical properties as reflected by their contrasting $^1$H NMR spectra.

The longer-term goal is to apply the covalent tether-directed backfolding cyclization concept to synthetically disclose the natural [1]rotaxane-type lasso peptide series by incorporation of two amides of the thread section of the peptide backbone into a imidazolidin-4-one as the perpendicular and cleavable thread/ring connecting moiety. Covalent linking in a perpendicular fashion of the ring over thread fragment, as is the case with the backfolding cyclization concept, ensures the atom-precise mutual positioning of these components, which is a prerequisite for the eventual total synthesis of the lasso peptides.

**Methods**

**Macrolactonization of the tetrahedral precursor to template 6a.** 5 (1.94 g, 1.89 mmol), 6a (1.38 g, 2.08 mmol, 1.1 equiv), Cs$_2$CO$_3$ (2.45 g, 7.58 mmol, 4 equiv) and 4 Å molecular sieves (1.00 g) were dissolved in dry CH$_3$CN (750 ml) and the mixture was stirred overnight at 60°C under a N$_2$ atmosphere. The solvent was evaporated and the residue was taken in ca. 20 ml CH$_2$Cl$_2$ and filtered through a plug of Celite, which was washed with CH$_2$Cl$_2$. The organic layer was concentrated in vacuo and dry-loaded on silica and purified by column chromatography (petroleum ether (PE):EtOAc 4:1→3:1) to give 7 (1.72 g, 1.31 mmol, 69%) as a thick colourless oil.
Double CuAAC macrocyclizations towards cage 8. 7 (420 mg, 0.319 mmol) and TBD (42 mg, 0.080 mmol, 0.25 equiv) were dissolved in 65 ml dry CH₂Cl₂ and degassed with 5 vacuum/N₂ cycles, after which Cu(NH₃)₂BF₄ (25 mg, 0.080 mmol, 0.25 equiv) was added and the mixture was stirred overnight at reflux under N₂ atmosphere. The reaction mixture was concentrated in vacuo and dry-loaded on silica and purified by column chromatography (PE:EtOAc 1:2) to give 8 (333 mg, 0.253 mmol, 79%) as a colourless foam.

Ring-closing metathesis to the pseudo-quasi[1]catenane. 8 (79 mg, 0.060 mmol) was dissolved in dry CH₂Cl₂ (60 ml) and degassed with 5 vacuum/N₂ cycles. To the solution was added Grubbs second-generation catalyst (10 mg, 0.012 mmol, 0.2 equiv) and the mixture was stirred overnight at 40 °C. The mixture was concentrated in vacuo, dry-loaded on silica and purified by column chromatography (PE:EtOAc 1:2→1:0.2→0.1) to give 8 (333 mg, 0.253 mmol, 79%) as a colourless foam.

Temporary tether removal to liberate quasi[1]catenane 2. The pseudo-quasi[1]catenane (55 mg, 0.043 mmol) was dissolved in dry THF/CH₃OH (2 ml, 1:1) and anhydrous NaOCH₃ (12 mg, 0.21 mmol, 5 equiv) was added and the mixture was stirred at room temperature for 1 h. The reaction was quenched by addition of 2-H₂ compound Figs 5–71. CCDC 1517772 contains the supplementary crystallographic data for 8. CCDC 1517772 contains the supplementary crystallographic data for 8.

Data availability. Additional data supporting the findings reported in this article are available within the Supplementary Information file. For the experimental procedures and spectral data of all compounds described, see the Supplementary Methods. For 1H and 13C NMR spectra of all compounds, see Supplementary Figs 5–71. CCDC 1517772 contains the supplementary crystallographic data for compound 2-H₂. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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Author contributions

L.S. and M.J.W. carried out the experimental work. M.L. carried out the X-ray crystallographic analysis. H.H. was involved in important discussions. L.S. and H.J.V.M. designed the research and wrote the manuscript. H.J.V.M. directed the research. All the authors contributed to the analysis of the results and editing the manuscript.
Additional information
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