

REPORT

MOLECULAR KNOTS

Braiding a molecular knot with eight crossings

Jonathan J. Danon, Anneke Krüger, David A. Leigh,* Jean-François Lemonnier, Alexander J. Stephens, Iñigo J. Vitorica-Yrezabal, Steffen L. Woltering

Knots may ultimately prove just as versatile and useful at the nanoscale as at the macroscale. However, the lack of synthetic routes to all but the simplest molecular knots currently prevents systematic investigation of the influence of knotting at the molecular level. We found that it is possible to assemble four building blocks into three braided ligand strands. Octahedral iron(II) ions control the relative positions of the three strands at each crossing point in a circular triple helicate, while structural constraints on the ligands determine the braiding connections. This approach enables two-step assembly of a molecular 8_{19} knot featuring eight nonalternating crossings in a 192-atom closed loop ~20 nanometers in length. The resolved metal-free 8_{19} knot enantiomers have pronounced features in their circular dichroism spectra resulting solely from topological chirality.

Knots (1) form spontaneously in flexible polymer chains (2) and are found in circular DNA (3) and ~1% of proteins in the Protein Data Bank (PDB) (4). To date, only three nontrivial knot topologies (trefoil, figure-eight, and pentafoil) (5–16) out of more than 6 billion (17, 18) known prime knots have been synthesized with small molecules. Most of the molecular knots isolated to date have been unanticipated reaction products (6, 7, 10, 12, 14), but a few designed strategies for the construction of simple small-molecule knots have been successfully developed, including the use of linear (5) and cyclic (11) double helicates that twist two ligand strands about each other several times (13).

Every knot can be formed by braiding (19) strings and joining their ends together (Fig. 1) (20). However, the number of strings required for a particular knot depends on its topology. Twisting two strings about each other can generate only four of the 84 prime knots with up to nine crossings, whereas having three strings to braid makes nearly one-third of such knots accessible (21). Sauvage synthesized the first molecular trefoil knot (5), as well as a Solomon link (22), using linear double helicates in which two ligand strands are twisted about one another to generate the required number of crossings (three and four, respectively), followed by joining the termini of each strand along the length of the helicate (Fig. 1A). Recently, a Solomon link (23), pentafoil knots (11, 24) (five crossings), and a Star of David catenane (25) (six crossings) were prepared using double helicates that are cyclic, which reduces the distances needed to join the strands into closed loops (Fig. 1B). These circular helicates use Fe(II) ions to entwine two

ligand strands: Two bipyridine (bipy) chelating groups coordinated to the same metal ion are connected in the knot-forming reaction to form a strand that crosses that of the third bipy unit bound to the metal ion. However, the octahedral coordination geometry of these metal ions could, in principle, be used to position three bipy-containing strands with respect to each other. The key is to design ligands so that connections can only be made to bipy groups that are coordinated to adjacent metal ions. In this way the octahedral Fe(II) metal ions can control the relative position of three ligand strands at each crossing point (Fig. 1C).

The 8_{19} knot [Alexander-Briggs notation, where

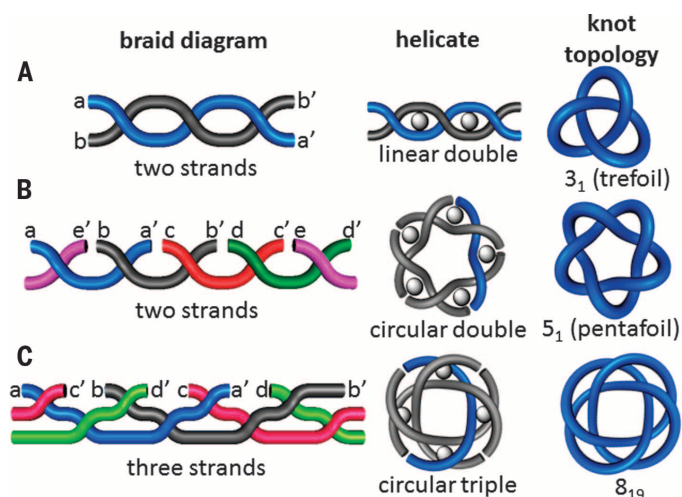
the main number denotes the number of crossings and the subscript differentiates the knot from others with the same number of crossings (20)] is one of 21 prime knots with eight crossings (20). Along with the 8_{20} and 8_{21} knots, the 8_{19} knot is the simplest nonalternating knot; it is absent from early knot tables (26) because it was not immediately recognized (27) [much less proven (28)] that not all knots can be written in a form that alternates crossings over-under around the closed loop. The 8_{19} knot is the T(3,4) torus knot, meaning that it can be represented by a closed loop that wraps around a torus (doughnut shape) three times longitudinally and four times meridionally without crossing itself (fig. S18) (20). Its most symmetrical form suggests a strategy to access it through a three-stranded braid using a four-fold repeat motif (Fig. 1C). Indeed, recent simulations showed an 8_{19} knot to be a favored structure in the theoretical assembly of four rigid helical building blocks with sticky ends (29). We envisaged employing a tetrameric circular triple helicate (30) within which each of four metal ions binds to bipy units from three different ligand strands. Rigid extensions from the bipy groups at both ends of each strand ensure that the terminal alkene groups are positioned such that they can only undergo olefin metathesis to groups coordinated to adjacent Fe(II) ions, producing the connections required to form an 8_{19} knot (Fig. 2).

Ligand strand 1 and FeCl₂ were heated at 130°C in *N,N*-dimethylformamide (DMF) for 24 hours (Fig. 2, step 1). Subsequent treatment with methanolic KPF₆ afforded a red precipitate, determined by electrospray ionization mass spectrometry (ESI-MS) to be a tetrameric complex [Fe₄L₄](PF₆)₈ (Fig. 2, step 2). Carrying out the assembly process in solvents other than DMF [e.g., ethylene glycol or dimethylsulfoxide (DMSO)] produced substantial quantities of a trimer, [Fe₃L₃](PF₆)₆—

Fig. 1. Tying molecular knots by twisting and braiding ligand strands.

(A) Sauvage's strategy for assembling trefoil knots from linear double helicates. Two metal ions control three crossings, and the knot is obtained by closing the oriented end groups a to b' and b to a'. (B) Pentafoil knots have been derived from cyclic pentameric double helicates. Five metal ions control five crossing points for five building blocks assembled into a circular double helix

through connections a to d', b to e', c to a', d to b', and e to c'. (C) An 8_{19} knot can be synthesized from a cyclic tetrameric triple helicate. Four metal ions control eight crossing points for four building blocks assembled into a circular triple helix with connections made from a to b', b to c', c to d', and d to a'. A feature of circular helicates is that the building blocks can be joined at different points along the braid to generate the desired number and sequence of crossings, rather than only at the braid ends.



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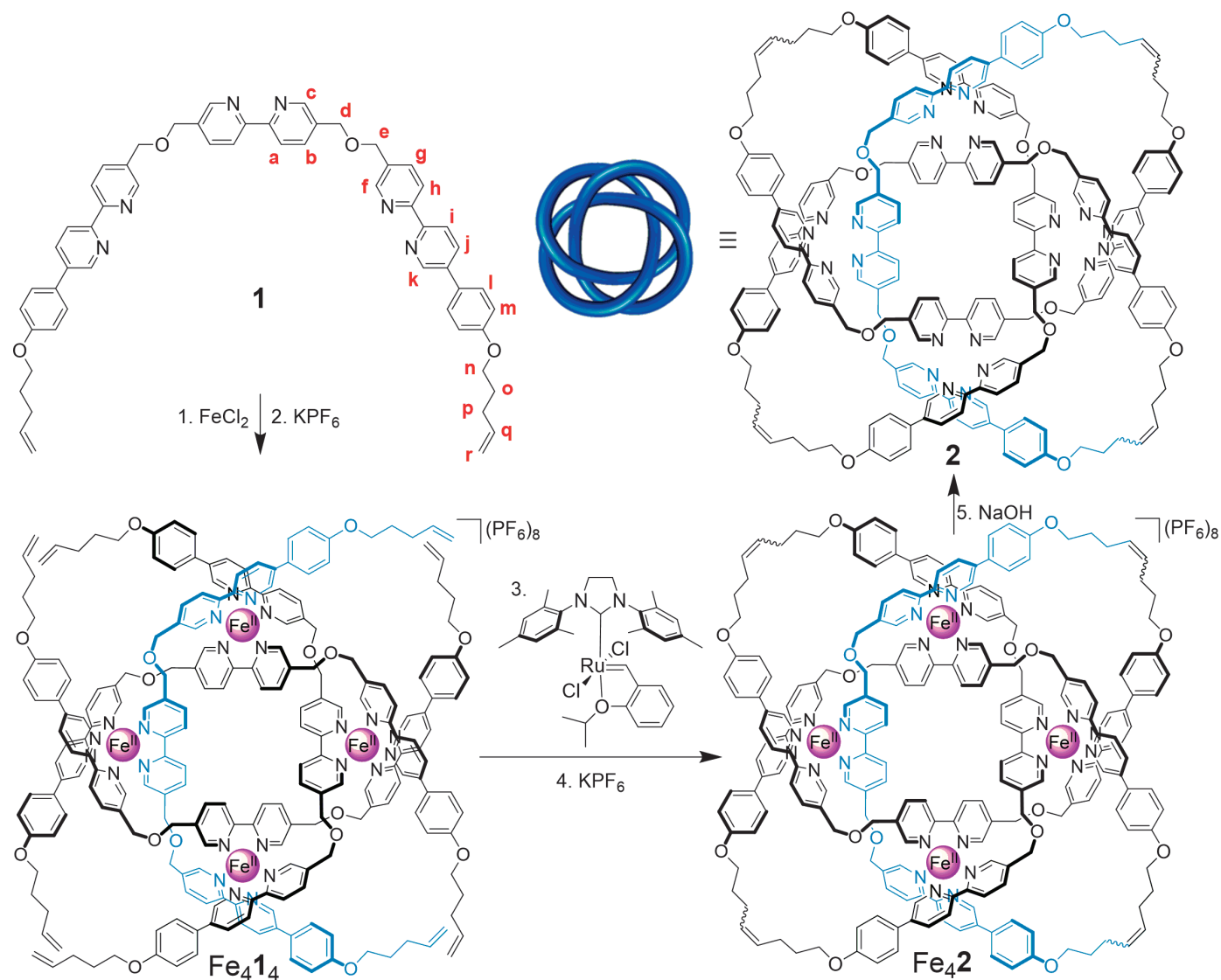


Fig. 2. Synthesis of a molecular 8_{19} knot, **2.** Reaction conditions: (1) **1** (1.0 equiv.), FeCl_2 (1.0 equiv.), *N,N*-dimethylformamide (DMF), 130°C, 24 hours; (2) KPF_6 , MeOH, 60% (two steps); (3) Hoveyda-Grubbs second-generation catalyst (25 mol % per olefin metathesis reaction), $\text{MeNO}_2/1,2$ -dichloroethane 1:1, 60°C, 24 hours; (4) KPF_6 , H_2O , 62% (two steps); (5) $\text{NaOH}_{(\text{aq})}$ (1 M)/MeCN 1:1, 70°C, 30 min, 38%.

presumed to be a linear triple helicate (**25**)—which could not easily be separated from the tetramer. The ^1H nuclear magnetic resonance (NMR) spectrum of $[\text{Fe}_4\mathbf{1}_4](\text{PF}_6)_8$ (Fig. 3B) showed all four ligands to be in the same symmetric environment, which is possible only when ligands are arranged cyclically, with the diastereotopic splitting of protons H_d and H_e indicative of a helical conformation.

Tetrameric complex $[\text{Fe}_4\mathbf{1}_4](\text{PF}_6)_8$ was treated with the Hoveyda-Grubbs second-generation catalyst (**3**) at 60°C in nitromethane/1,2-dichloroethane (1:1) (Fig. 2, step 3). After 24 hours, the reaction was quenched with ethyl vinyl ether and the product precipitated with aqueous KPF_6 in an effort to confer anion uniformity and to remove catalyst residues (Fig. 2, step 4), furnishing $[\text{Fe}_4\mathbf{2}](\text{PF}_6)_8$ in 62% yield over two steps. ESI-MS was consistent with the loss of four ethene molecules from $[\text{Fe}_4\mathbf{1}_4](\text{PF}_6)_8$ (fig. S7), and ^1H NMR spec-

troscopy confirmed the loss of the terminal olefin signals from the precursor complex (Fig. 3C). In some experiments, ESI-MS indicated incomplete anion exchange and contamination of the product with a small amount of $[\text{Fe}_4\mathbf{2}](\text{PF}_6)_7\text{Cl}$ (fig. S13).

Slow evaporation of a saturated acetonitrile solution of the $\text{Fe}_4\mathbf{2}$ knot complex produced red needles over several days suitable for single-crystal x-ray diffraction. The solid-state structure confirms the topology and D_4 symmetry of the molecular 8_{19} knot (Fig. 4, movie S1, and supplementary materials). The organic ligand weaves a continuous 192-atom path passing each of the four coplanar iron centers three times to form a circular triple helicate closed loop with eight non-alternating crossings. Both *E*- and *Z*-olefins are present in the chain, with some disorder, in approximately a 1:1 ratio. Each Fe(II) ion is bound to three bipy groups; the Fe-N distances are in

the range of 1.92 to 2.02 Å, as is typical (**32**) for $\text{Fe}(\text{bipy})_3^{2+}$ complexes. The metal ion octahedral coordination geometry is slightly distorted by the constraints of the tightly knotted ligand, with N-Fe-N angles within each bipyridyl moiety of $\sim 81^\circ$. The bipyridine groups define a cavity of diameter 3.30 ± 0.02 Å [versus 3.7 ± 0.1 Å for a related Solomon link (**33**)]. At the center, a chloride anion is bound by eight $\text{CH}\cdots\text{Cl}$ hydrogen bonds. Although the presence of the chloride ion is somewhat surprising given the anion exchange reaction with KPF_6 , it is reminiscent of the solid-state structures of anion template pentafoil knots (**11**, **24**) and, as with those knots, we found the $\text{Fe}_4\mathbf{2}$ knot complex to bind a single chloride anion strongly in solution (association constant $K_a = 2.6 \pm 0.1 \times 10^8 \text{ M}^{-1}$ in MeCN, 298 K; see supplementary materials).

Treatment of an acetonitrile solution of $[\text{Fe}_4\mathbf{2}](\text{PF}_6)_8$ with NaOH (1 M) at 70°C for 30 min

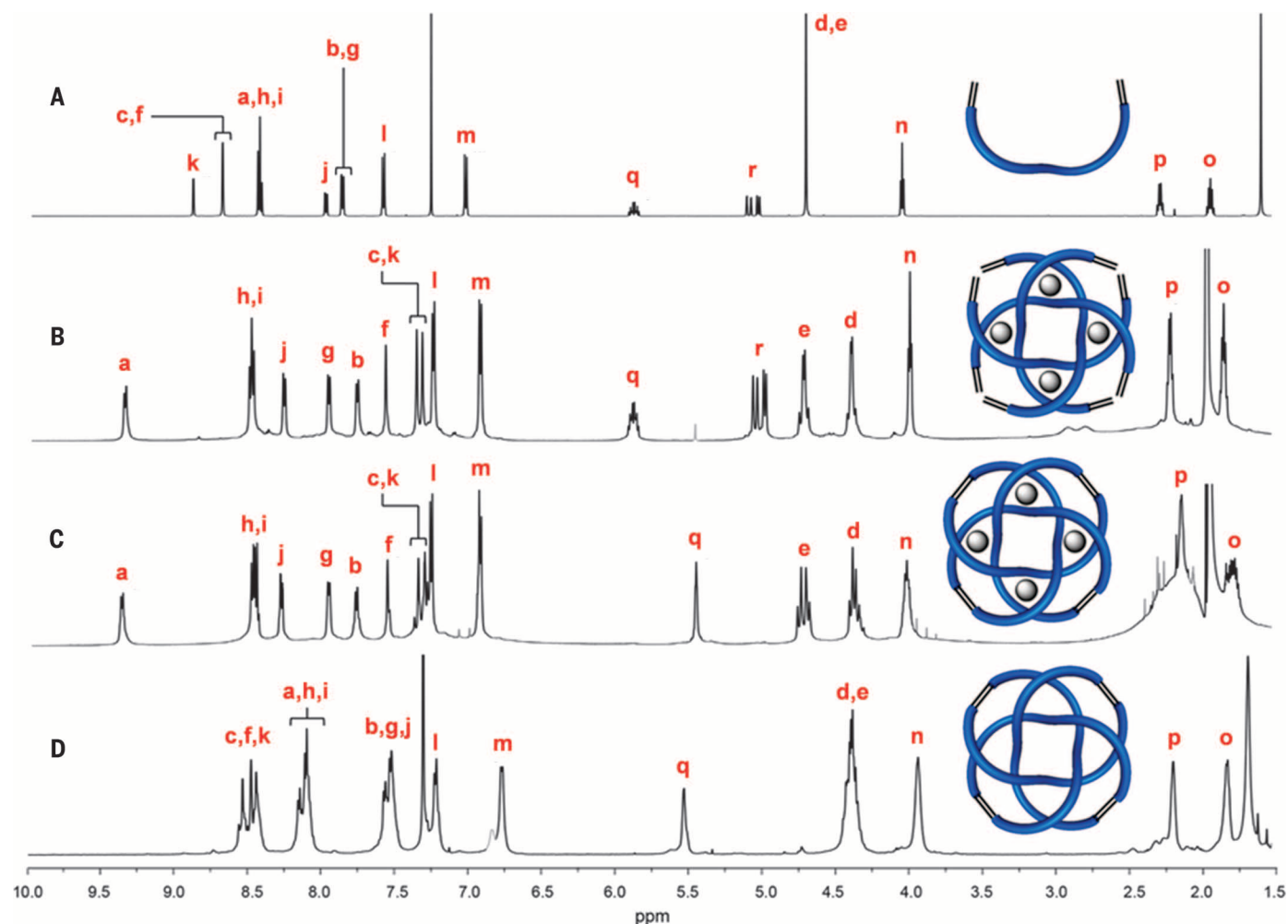


Fig. 3. ^1H NMR spectra (600 MHz, 298 K) of molecular 8_{19} knot **2** and its complexes and precursors. (A) Ligand strand **1** (solvent CDCl_3); (B) tetrameric circular helicate $[\text{Fe}_4\mathbf{1}_4](\text{PF}_6)_8$ (solvent CD_3CN); (C) 8_{19} knot complex $[\text{Fe}_4\mathbf{2}](\text{PF}_6)_8$ (solvent CD_3CN); (D) metal-free 8_{19} knot **2** (solvent CDCl_3). The lettering corresponds to the proton labeling in Fig. 2. Full spectral assignments are given in the supplementary materials.

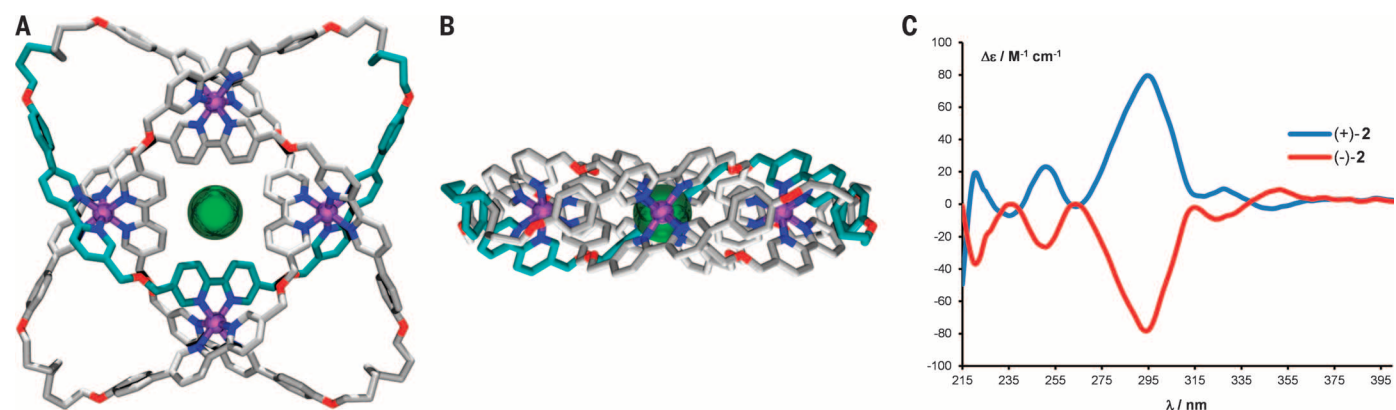


Fig. 4. X-ray crystal structure of molecular 8_{19} knot complex $[\text{Fe}_4\mathbf{2}](\text{PF}_6)_7\text{Cl}$ and circular dichroism (CD) spectra of each mirror image form of metal-free 8_{19} knot ligand **2**. (A) X-ray structure viewed perpendicular to the plane of the four Fe(II) ions. (B) Side view, in the plane of the four Fe(II) ions. C atoms are light gray (turquoise in one repeat unit of the knot); N, blue; O, red; Fe, purple. PF_6^- anions and hydrogen atoms omitted for clarity. The iron(II) centers in each molecular knot have the same coordination stereochemistry (Δ or Δ). Both enantiomers are present in each unit cell; the all- Δ enantiomer is shown here. (C) CD spectra of 8_{19} knot enantiomers (+)-**2** (blue) and (-)-**2** (red, 90% ee) in CH_2Cl_2 (1.76×10^{-4} M) at 298 K.

afforded the demetallated 8_{19} knot **2** in 38% yield (Fig. 2, step 5). Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) gave

an isotope distribution for the $[\mathbf{2}\cdot\text{Na}]^+$ molecular ion that matched the theoretical pattern and values (fig. S10). The ^1H NMR spectrum of **2** (Fig. 3D)

is relatively broad in comparison to both the metallated knot $[\text{Fe}_4\mathbf{2}](\text{PF}_6)_8$ (Fig. 3C) and building block **1** (Fig. 3A). Molecular modeling and

variable temperature ^1H NMR (fig. S16) suggest that **2** does not exist in a single well-defined conformation and that the broad features of the ^1H NMR spectrum are likely a consequence of reptation [the thermal motion of long, entangled polymer chains (34)] as the 192-atom-long knot backbone's motion is restricted when it passes through tight loops and traverses crossings. Furthermore, most of the signals in the ^1H NMR spectrum of **2** (Fig. 3D) are shielded with respect to that of building block **1** (Fig. 3A); this reflects the compact structure of the knot bringing each region of the loop into close proximity with aromatic rings during reptation.

The 8_{19} knot is intrinsically chiral by virtue of its topology. One enantiomer, (+)-**2** (sign determined by optical rotation), could be isolated by chiral high-performance liquid chromatography (see supplementary materials) and a sample of the other enantiomer obtained in highly enriched form [(-)-**2**, 90% enantiomeric excess (ee)] (35). The mirror-image 8_{19} knots have circular dichroism (CD) spectra of equal and opposite shape and sign (Fig. 4C) (35, 36). Despite the knots possessing no elements of Euclidean chirality, several features of the CD spectra (particularly the maxima and minima at 221, 250, 294, 327, and 351 nm) show that the chiral environment is well expressed despite the dynamics of the closed loop and the absence of well-defined conformations, presumably also a result of the tightness of the knotting.

The braiding of ligand strands should enable the synthesis of many more molecular knots [and links (25, 37)] of greater complexity than has previously been possible (fig. S19). The inherent chirality of many knots, and the tightness of the knotting possible through this synthetic approach, may prove useful when investigating

applications of knotting in molecular tools (24, 33, 38) and materials (39, 40).

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/355/6321/159/suppl/DC1
Materials and Methods
Figs. S1 to S18
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Movie S1
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Editor's Summary

Three strands ironed closely together

It is not uncommon when braiding hair or bread to intertwine three different strands. At the molecular level, however, synthetic knots have thus far been restricted to architectures accessible from two-strand braids. Danon *et al.* used iron ion coordination to guide three organic ligand strands to form a knot geometry with eight separate crossings.

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