DOI: 10.1002/ange.201404496

Helical Self-Assembly

Enantioselective Assembly of a Ruthenium(II) Polypyridyl Complex into a Double Helix**

Kristof Van Hecke, Thomas Cardinaels, Peter Nockemann, Jeroen Jacobs, Louis Vanpraet, Tatjana N. Parac-Vogt, Rik Van Deun, Koen Binnemans, and Luc Van Meervelt*

Abstract: *Evolution can increase the complexity of matter by self-organization into helical architectures, the best example being the DNA double helix. One common aspect, apparently shared by most of these architectures, is the presence of covalent bonds within the helix backbone. Here, we report the unprecedented crystal structures of a metal complex that self-organizes into a continuous double helical structure, assembled by non-covalent building blocks. Built up solely by weak stacking interactions, this alternating tread stairs-like double helical assembly mimics the DNA double helix structure. Starting from a racemic mixture in aqueous solution, the ruthenium(II) polypyridyl complex forms two polymorphic structures of a left-handed double helical assembly of only the Λ-enantiomer. The stacking of the helices is different in both polymorphs: a crossed woodpile structure versus a parallel columnar stacking.*

It is of prime importance for the understanding of the origins of life how simple molecules can self-organize into complex supramolecular structures such as double helices. The B-DNA double helical motif, for example, is directed by lateral hydrogen bonding of the bases, and stabilized by hydrophobic stacking interactions, perpendicular to the hydrogen bonds, with the covalently connected sugar-phosphate backbone adopting a right-handed double helix.[1]

Specific synthetic polymers with attached peripheral chiral groups are known to self-assemble into helical architectures.[2] A number of chiral supramolecular compounds feature helical structures, primarily governed by strong hydrogen bonds or built up from planar discotic molecules.[3] Even liquid-crystalline supramolecular polymers can exhibit helicity when induced by molecular chirality.[4] Interconversion between single and double molecular helices has been reported for oligomeric molecules with bent conformations, and pyridine-pyridazine heterocyclic oligomers tend to self-assemble into helical columns and fibres.[5] Helical self-organization and supramolecular chemistry in general lead to complex chemical systems and architectures.[6]

The hydrophobic effect is commonly believed to direct the self-assembly of many non-polar units in aqueous solutions and to be an important driving force behind protein folding and biological structures such as membranes, micelles and nucleic acid structures.[7]

The self-organized continuous helical structures reported so far, all feature either covalent backbones or large chiral molecules, which self-organize by strong hydrogen bonds, which steer the helical stacking. We have initiated crystallization experiments in aqueous solution using a racemic mixture of the Λ- and Δ-enantiomers of the ruthenium(II) polypyridyl complex $[Ru(bipy)_2(dppzCl_2)]Cl_2$, where bipy is 2,2′-bipyridine and $dppzCl_2$ is 7,8-dichloro-dipyrido[3,2-*a*:2′,3′-*c*]phenazine (Figure 1 A).

Two different polymorphs, both in two distinct shapes (block and rod shaped crystals, Supporting Information, Figure S1), were obtained and fully characterized by single-crystal X-ray diffraction (see Experimental Section and Table S1 in the Supporting Information). In both polymorphs the ruthenium(II) complex exhibits solely stacking interactions leading to self-organization into a complex double helical architecture. Although we started from a racemic mixture of the Λ- and Δ-enantiomers of the ruthenium(II) complex, both polymorphs only contain the Λ-enantiomer.[8] This might be attributed to Oswald ripening, leading to complete enantiopurity.[9] The presence of both enantiomers in the same helix is impossible due to steric clashes of the ancillary bipy ligands. Examining the preservation of the helices in non-crystalline state shows the absence of circular dichroism (CD) signals, which might indicate a mixture of left- and right-handed helices. Upon dissolving the crystals themselves, weak CD signals indicate inefficient racemisation of the Λ-enantiomer, which would require Ru^{II}–N bond breakage (Figures S4–S6). When using ethanol instead of an aqueous solution we previously observed a non-helical crystal structure consisting of racemic building blocks.[10]

Polymorph **1** crystallizes in the chiral space group $I4_122$ (no. 98), with the asymmetric unit consisting of four discrete

---

[*] Prof. K. Van Hecke, Prof. R. Van Deun
Department of Inorganic and Physical Chemistry, Ghent University
Krijgslaan 281-S3, 9000 Gent (Belgium)
E-mail: Kristof.VanHecke@UGent.be
Dr. T. Cardinaels,[+] Dr. P. Nockemann,[++] J. Jacobs, L. Vanpraet, Prof. T. N. Parac-Vogt, Prof. K. Binnemans, Prof. L. Van Meervelt
Department of Chemistry, KU Leuven
Celestijnenlaan 200F P.O. Box 2404, 3001 Leuven (Belgium)

[+] Present address: Institute for Nuclear Materials Science, SCK-CEN, Boeretang 200, 2400 Mol (Belgium)

[++] Present address: The QUILL Research Centre, School of Chemistry and Chemical Engineering, David Keir Building, Queen's University Belfast, Stranmillis Road, Belfast BT9 5AG (UK)

[**] The authors thank the staff of beamline PXIII of the Swiss Light Source (SLS) in Villigen for their support with the synchrotron experiments. K.V.H. and R.V.D. thank the Hercules Foundation (projects AUGE/11/029 "3D-SPACE: 3D Structural Platform Aiming for Chemical Excellence" and AUGE/09/024 "Advanced Luminescence Setup", respectively) for funding. K.V.H. thanks Roel Van Hecke for proofreading the manuscript.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201404496.

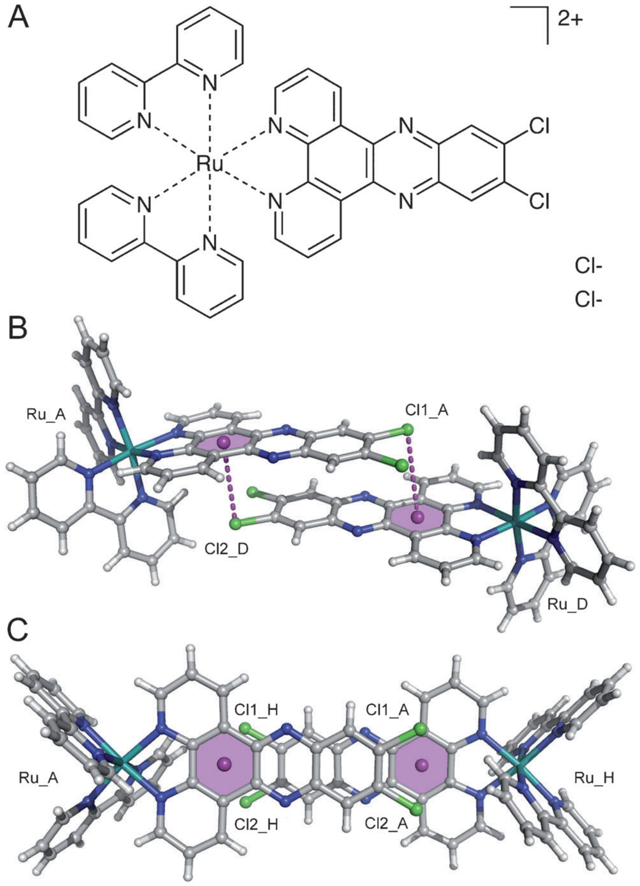

Figure 1. Helical assembly of ruthenium(II) complexes. A) Schematic drawing of the ruthenium(II) complex Λ-[Ru(bipy)$_2$(dppzCl$_2$)]Cl$_2$, where bipy is 2,2′-bipyridine and dppzCl$_2$ is 7,8-dichloro-dipyrido[3,2-*a*:2′,3′-*c*]phenazine. Two chloride anions are present as countercharges. B) Stacking interactions in a step showing strong overlap for polymorph **1**. The step shown is step 1, 2. Two Cl–π stacking interactions occur with distances to ring centroid of 3.43 Å for Cl2 and 3.72 Å for Cl1. C) Strong overlapping steps in polymorph **2** show almost no Cl–π interactions. The purple dots represent ring centroids.

Λ-[Ru(bipy)$_2$(dppzCl$_2$)]Cl$_2$ molecules. The crystal structure shows a continuous left-handed double helical assembly of non-covalently bonded Λ-[Ru(bipy)$_2$(dppzCl$_2$)]Cl$_2$ building blocks, with the outer Ru$^{\text{II}}$-bipy moieties representing the helical backbones, whereas the dppzCl$_2$ ligands are positioned on the inside of the helix (Figure 2 A). The complete double helical repeating unit is generated by two-fold crystallographic symmetry and is thus antiparallel and contains 16 molecular units. The stacking of the helices in the crystal lattice resembles a crossed alternating woodpile structure with the double helices in adjacent, parallel layers, rotated by 90° (Figure 3 A). Remarkably, polymorph **1** contains very large voids. When the water molecules are omitted, no less than 74.9 % of the unit cell is void (void volume of 150 904 Å^3). After inclusion of the water molecules, still 55.8 % of the unit cell is void (void volume of 112 389 Å^3). A view along the crystallographic *b*-axis illustrates this porosity by the presence of huge channels (Figure S2 A).

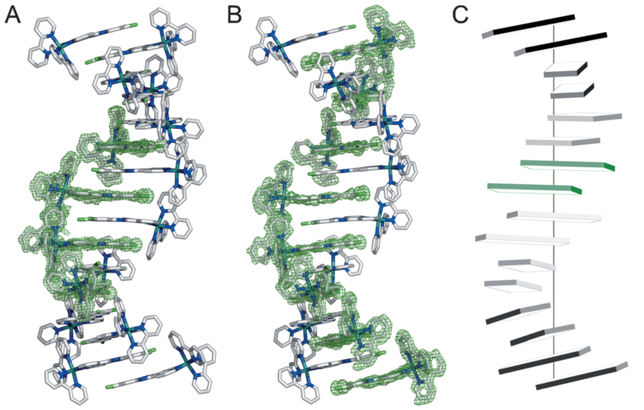

Figure 2. Continuous left-handed helical assembly of the Λ-[Ru(bipy)$_2$(dppzCl$_2$)]Cl$_2$ complex. A) Polymorph **1**. Final $(2F_o-F_c)$ electron-density map of the asymmetric unit is shown (four complexes in green), contoured at 1.0σ level. Hydrogen atoms are omitted for clarity. The second strand is generated by a twofold axis (symmetry operation $-x, -y, z$); hence the assembly is considered an antiparallel double helix. A complete double helical repeat is generated by two other twofold axes ($-x, y+1/2, -z+1/4$ and $x, -y+1/2, -z+1/4$), and contains 16 molecular units. B) Polymorph **2**. Final $(2F_o-F_c)$ electron-density map of the asymmetric unit is shown (eight complexes in green), contoured at 1.0σ level. The second strand is generated by a twofold axis ($-x, y, -z+1/2$), also leading to 16 molecular units in one double helical repeat. C) Schematic diagram of the left-handed helical assembly in polymorph **1**, with the dppzCl$_2$ ligands represented by blocks. The helical axis is indicated and one repeating unit is coloured green.

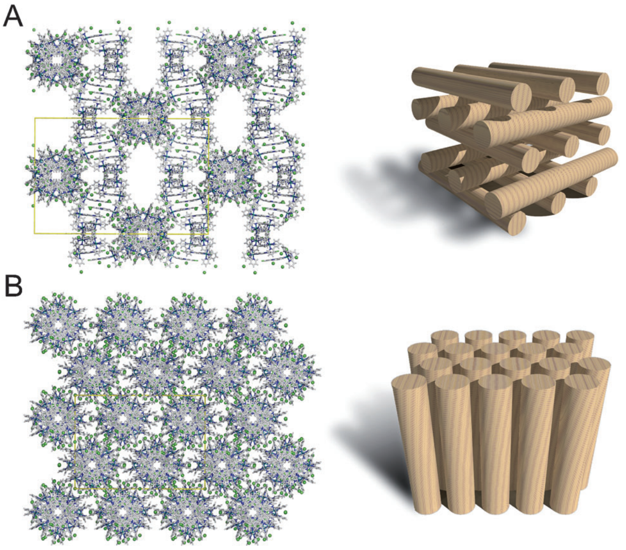

Figure 3. Packing diagrams of the Λ-[Ru(bipy)$_2$(dppzCl$_2$)]Cl$_2$ complex. A) Polymorph **1** in space group $I4_122$, showing an alternating crossed woodpile arrangement. B) Polymorph **2** in space group $C222_1$, showing a columnar parallel packing. Both projections are along the crystallographic *a*-axis.

Polymorph **2** crystallizes in the chiral space group $C222_1$ (no. 20), with eight molecules in the asymmetric unit. The second strand is generated by a crystallographic twofold axis, also leading to 16 molecular units in one antiparallel left-handed double helical repeat (Figure 2 B). The crystal lattice consists of double helices aligned in the crystallographic *a*-direction resulting in a parallel, alternating columnar stacking (Figure 3 B). The lattice of this polymorph contains large voids as well, that is, 58.1 % of the unit cell volume is void

(void volume of 70939 Å^3). When including the water molecules, 39.8% of the unit cell is void (void volume of 48577 Å^3). Large channels are present along the [111] direction (Figure S2B).

The antiparallel left-handed helices obtained here by self-assembly of ruthenium(II) complexes show clear analogy to the well known classical nucleic acid double helices. In fact, these kinds of complexes are expected to interact with nucleic acids,[11] for example, as luminescent probes and not to mimic their double helical structure (Supporting Information, luminescence study, Figures S7 and S8). For both polymorphs the repeating unit consists of two ruthenium(II) complexes from different strands kept together by hydrophobic stacking interactions, and not by hydrogen bonding as in nucleic acids. The complete helix contains eight such repeating units per turn. We have adopted the following numbering scheme for the molecular units in the helix. The complexes are sequentially numbered from top to bottom of the helix, from 1 to 16. The first repeating unit then consists of 1 and 2; the first strand of the odd complexes 1, 3, …, 13, 15; the second strand of the even complexes 2, 4, …, 14, 16. The average helical twist alters between higher and lower twist values of approximately −50° and −40° (Figure 2C and Table S2). The axial rise per unit is 6.4 Å (or 3.2 Å per ruthenium(II) complex) with a helical pitch of 51.2 Å. One helical turn is exactly completed after eight repeating units; hence the helical symmetry can be described by a 8-fold screw axis 8_1. For nucleic acids the screw axis depends on the family: 11_1, 10_1 and 12_1 for the A, B, and Z-family, respectively.

The average distance between the adjacent backbone ruthenium(II) ions is 9.6 Å for both polymorphs. The dppzCl$_2$ ligands are almost perpendicular, but displaced from the helical axis, leaving a small central channel. Antiparallel π–π stacking interactions are observed between the outer quinoxaline parts of dppzCl$_2$ ligands. This interaction between two subsequent dppzCl$_2$ moieties is not uniform along the helix but shows an alternation between stronger and weaker overlap. The stronger overlap occurs within steps where the long axes of both dppzCl$_2$ are almost parallel (e.g. the angle between the long axes is 6.7° and 1.6° for step 1, 2 in polymorph **1** and **2**, respectively). For polymorph **1** these steps show also two Cl–π stacking interactions (Figure 1B). In contrast, for polymorph **2**, the Cl–π stacking interactions between neighboring dppzCl$_2$ rings are rather an exception (Figure 1C). For the steps with weaker overlap the angles between the long axes of dppzCl$_2$ are higher (e.g. 52.5° and 43.1° for step 2, 3 in polymorph **1** and **2**, respectively). These characteristics, showing alternating helical steps, lead to an alternating tread stairs-like appearance.

In analogy with double helical nucleic acids, two grooves can be defined. The very broad and deep groove, also called major groove here, contains at its floor the Cl1 atoms of dppzCl$_2$. At the other side of the helix a narrow and shallow groove, called minor groove, contains atoms N4 of dppzCl$_2$ at its floor. In both cases the walls of the grooves consist of aromatic rings from the 2,2′-bipyridine ligands (Figure 4).

The Cl$^-$ anions do not have a specific binding site and always interact by C−H···Cl$^-$ contacts and with at least one water molecule. They do not play an important role in the helix formation, which is not the case for the Cl atoms present in the dppzCl$_2$ ligands. When these Cl atoms are absent, no supramolecular assembly occurs (will be published elsewhere).

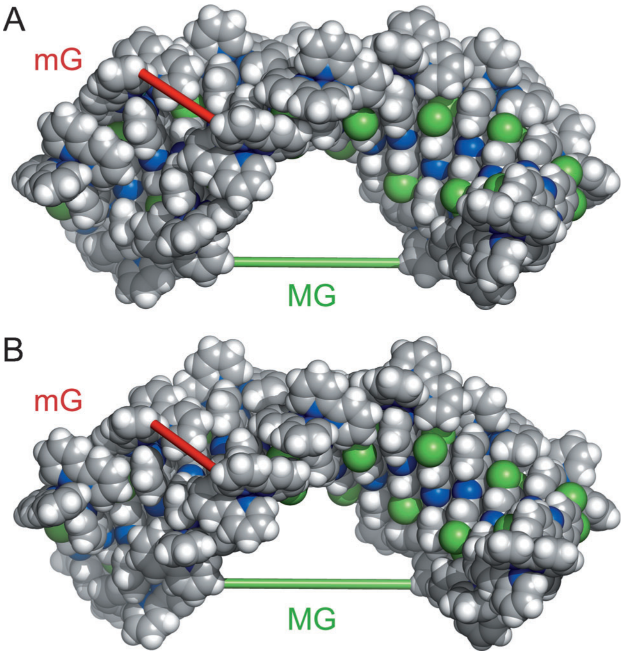

Figure 4. Major (MG) and minor (mG) grooves in the antiparallel double helices. A) For polymorph **1** the dimensions are 14.2 Å (MG, green stick) and 9.1 Å (mG, red stick). B) For polymorph **2** the dimensions are 19.9 Å (MG) and 11.0 Å (mG). The broad and deep MG contains at its floor the Cl1 atoms of dppzCl$_2$, the narrow and shallow mG contains atoms N4 of dppzCl$_2$ at its floor. In both cases the walls of the grooves consist of aromatic rings from the bipy ligands. MG/mG are calculated as the shortest distance (H21···H21/H37···H37) across the groove minus twice the Van der Waals radius of hydrogen.

Since the use of water as solvent seems to be essential for the self-organization, this process is likely driven by hydrophobic interactions, resulting in two very similar helical assemblies (Figure S3).

In conclusion, these structures now create a novel insight on the remarkable self-assembly capabilities of specific ruthenium(II) metal complexes. Apparently, weak stacking interactions are sufficient to construct left-handed double helices, with non-covalent building blocks, leading to an alternating tread stairs-like arrangement, which mimics the DNA double helix. Although initiated from a racemate, these helices only contain the Λ-enantiomer and finally pack in two different crystal polymorphs, a crossed woodpile versus a parallel columnar stacking.

Experimental Section

Crystallization conditions of the [Ru(bipy)$_2$(dppzCl$_2$)]Cl$_2$ complex were screened using Crystal Screen 1 and 2 (Hampton Research, Aliso Viejo, USA) by the sitting-drop vapor-diffusion method at 16°C, using a TECAN Freedom EVO crystallization robot and CrystalQuick 96 square-well plates (Greiner Bio-One, Wemmel, Belgium). Red-colored crystals, indicating the presence of ruthenium(II) (Figure S1), suitable for X-ray diffraction, appeared after approximately two days by mixing 1.5 μL of a solution of $[Ru(bipy)_2(dppzCl_2)]Cl_2$ in MilliQ water (15 mg mL^{-1}) with 1.5 μL of a screening condition, against a 100 μL reservoir of the same screening solution. Polymorph **1** crystallized from a screening condition containing 2.0 M sodium chloride and 0.1 M sodium acetate trihydrate, pH 4.6 and polymorph **2** crystallized from a screening condition containing 2.0 M sodium chloride and 10 % (w/v) PEG 6000. Block-, as well as rod-like crystal morphologies could be distinguished, however, these are not related to the different structural polymorphs. The crystals were cryoprotected with 20 % (v/v) glycerol in water and flash-frozen in liquid nitrogen prior to data collection.

Data were collected on a MAR225 CCD detector with wavelength of 1.00 Å, a ϕ range of 202.5°, an increment of 0.75° and crystal-to-detector distance of 90 mm for polymorph **1** and a ϕ range of 180°, an increment of 1.00° and crystal-to-detector distance of 100 mm for polymorph **2**, under a liquid-nitrogen vapor cryostream of 100 K at the beamline PXIII of the SLS synchrotron in Villigen, Switzerland. Data were processed with Mosflm 7.0.3,[12] and reduced with SCALA 3.3.16, as part of the CCP4 suite.[13] Although the resolution edge of the diffraction pattern was 1.14 Å for polymorph **1** and 1.21 Å for polymorph **2**, the data were truncated to 1.20 Å and 1.30 Å, respectively, as the R_{merge} at higher resolution increased significantly. Polymorph **1** and **2** crystallized in the chiral space groups $I4_122$ and $C222_1$ with unit cells $a = b = 51.21$, $c = 76.83$ Å and $a = 53.26$, $b = 56.41$, $c = 40.63$ Å, respectively.

The structure of polymorph **1** in space group $I4_122$ could be solved by phasing with SHELXC/D/E,[14] on the anomalous signal of the Ru^{II} atoms, whereas the structure of polymorph **2** in $C222_1$ was solved by molecular replacement, using the program Phaser 2.1.4,[15] with structure **1** as model. In total, diffraction data were collected for four different crystals in space group $I4_122$ and three crystals in $C222_1$, all showing the presence of only the Λ-entantiomers of $[Ru(bipy)_2(dppzCl_2)]Cl_2$ and left-handed helical assemblies. Polymorph **1** contains four complexes in the asymmetric unit, whereas polymorph **2** consists of eight complexes per asymmetric unit. Model building and refinement was done with the program Coot 0.5.2 and Phenix 1.8.1.[16] An additional dictionary restraint library was created, with bond distances and angles taken from the previously deposited $[Ru(bipy)_2(dppz(Cl_2))]Cl_2$ structure in the Cambridge Structural Database (CSD),[17] with CCDC 690425.[10] Torsion angles were not restrained. Non-hydrogen atoms were refined isotropically, except ruthenium(II) ions and the dipyrido phenazine (dppz) ligand chloride atoms, which were anisotropically refined, and hydrogen atoms in the riding mode with isotropic temperature factors fixed at 1.2 times the B factor of the parent atoms. For polymorph **1**, the likelihood-based refinement converged to an R_{work} and R_{free} value of 12.68 % and 14.53 %, respectively. For polymorph **2**, final R_{work} and R_{free} values of 13.72 % and 16.02 % were reached, respectively.

As the ruthenium(II) ions in the asymmetric units of both structures should be countercharged by negative charges, chloride ions were detected and placed in the electron density. For the structure of polymorph **1**, nine chloride ions (including one on a special position and one in half occupancy) and for the structure of polymorph **2**, 17 chloride ions (including one on a special position and one in half occupancy) were added to the models, providing a total neutral charge. Additionally, water molecules were included in the models (90 and 98 waters for structures **1** and **2**, respectively) by combination of the "Find waters" module in Coot 0.5.2 and the water-picking mode in the phenix.refine GUI.[16] The waters were within hydrogen-bonding distance of chemically reasonable groups, appeared in mF_o-DF_c maps contoured at 3.0 σ and had a B factor of less than 80 A^2. No sodium ions could be detected.

Data collection and refinement statistics are summarized in Table S1. The coordinates and structure factors have been deposited in the Cambridge Structural Database (CSD) (deposition numbers 966366 (**1**) and 966367 (**2**)).

Received: April 20, 2014
Revised: May 22, 2014

Keywords: double helix · enantiomer · polypyridyl · ruthenium · self-assembly

---

[1] J. D. Watson, F. H. C. Crick, *Nature* **1953**, *171*, 737 – 738.

[2] a) L. Brunsveld, E. W. Meijer, R. B. Prince, J. S. Moore, *J. Am. Chem. Soc.* **2001**, *123*, 7978 – 7984; b) L. Brunsveld, J. Vekemans, J. Hirschberg, R. P. Sijbesma, E. W. Meijer, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4977 – 4982.

[3] a) H. Y. Lee, J. Park, M. S. Lah, J.-I. Hong, *Chem. Commun.* **2007**, 5013 – 5015; b) C. Bao, R. Lu, M. Jin, P. Xue, C. Tan, T. Xu, G. Liu, Y. Zhao, *Chem. Eur. J.* **2006**, *12*, 3287 – 3294; c) L. Brunsveld, B. J. B. Folmer, E. W. Meijer, R. P. Sijbesma, *Chem. Rev.* **2001**, *101*, 4071 – 4097; d) L. Brunsveld, H. Zhang, M. Glasbeek, J. A. J. M. Vekemans, E. W. Meijer, *J. Am. Chem. Soc.* **2000**, *122*, 6175 – 6182; e) J. H. K. K. Hirschberg, L. Brunsveld, A. Ramzi, J. A. J. M. Vekemans, R. P. Sijbesma, E. W. Meijer, *Nature* **2000**, *407*, 167 – 170.

[4] T. Gulik-Krzywicki, C. Fouquey, J.-M. Lehn, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 163 – 167.

[5] a) V. Berl, I. Huc, R. G. Khoury, M. J. Krische, J.-M. Lehn, *Nature* **2000**, *407*, 720 – 723; b) L. A. Cuccia, J.-M. Lehn, J.-C. Homo, M. Schmutz, *Angew. Chem.* **2000**, *112*, 239 – 243; *Angew. Chem. Int. Ed.* **2000**, *39*, 233 – 237.

[6] a) J.-M. Lehn, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4763 – 4768; b) J.-M. Lehn, *Science* **2002**, *295*, 2400 – 2403.

[7] a) C. Tanford, *Science* **1978**, *200*, 1012 – 1018; b) D. Chandler, *Nature* **2005**, *437*, 640 – 647; c) S. Liu, B. C. Gibb, *Chem. Commun.* **2008**, 3709 – 3716.

[8] In contrast to the initial racemic mixture, containing both the Λ- and Δ-enantiomer of the ruthenium(II) complex, the observed helical assemblies only contain the Λ-enantiomer, which is referred to as an enantioselective assembly. However, the helices themselves are considered diastereo(iso)mers, assuming that also right-handed helices occur, built up by the same Λ-enantiomer.

[9] W. L. Noorduin, E. Vlieg, R. M. Kellogg, B. Kaptein, *Angew. Chem.* **2009**, *121*, 9778 – 9784; *Angew. Chem. Int. Ed.* **2009**, *48*, 9600 – 9606.

[10] T. Cardinaels, J. Ramaekers, K. Driesen, P. Nockemann, K. Van Hecke, L. Van Meervelt, B. Goderis, K. Binnemans, *Inorg. Chem.* **2009**, *48*, 2490 – 2499.

[11] a) H. Song, J. T. Kaiser, J. K. Barton, *Nat. Chem.* **2012**, *4*, 615 – 620; b) H. Niyazi, J. P. Hall, K. O'Sullivan, G. Winter, T. Sorensen, J. M. Kelly, C. J. Cardin, *Nat. Chem.* **2012**, *4*, 621 – 628.

[12] A. G. W. Leslie, H. R. Powell, *NATO Sci. Ser., Ser. II* **2007**, *245*, 41 – 51.

[13] a) P. R. Evans, *Acta Crystallogr. Sect. D* **2006**, *62*, 72 – 82; b) Collaborative Computational Project, Number 4. *Acta Crystallogr. Sect. D* **1994**, *50*, 760 – 763.

[14] G. M. Sheldrick, *Acta Crystallogr. Sect. A* **2008**, *64*, 112 – 122.

[15] A. J. McCoy, R. W. Grosse-Kunstleve, P. D. Adams, M. D. Winn, L. C. Storoni, R. J. Read, *J. Appl. Crystallogr.* **2007**, *40*, 658 – 674.

[16] a) P. Emsley, B. Lohkamp, W. G. Scott, K. Cowtan, *Acta Crystallogr. Sect. D* **2010**, *66*, 486 – 501; b) P. D. Adams, P. V. Afonine, G. Bunkoczi, V. B. Chen, I. W. Davis, N. Echols, J. J. Headd, L. W. Hung, G. J. Kapral, R. W. Grosse-Kunstleve, A. J. McCoy, N. W. Moriarty, R. Oeffner, R. J. Read, D. C. Richardson, J. S. Richardson, T. C. Terwilliger, P. H. Zwart, *Acta Crystallogr. Sect. D* **2010**, *66*, 213 – 221.

[17] F. H. Allen, *Acta Crystallogr. Sect. B* **2002**, *58*, 380 – 388.