SYNTHESIS OF A SMALL MOLECULE WALKER AND THE APPLICATION OF MECHANICALLY INTERLOCKED LIGANDS IN ASYMMETRIC CATALYSIS

A thesis submitted to The University of Manchester for the degree of
Doctor of Philosophy
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ABSTRACT

Synthesis of a small molecule walker and the application of mechanically interlocked ligands in asymmetric catalysis

A thesis submitted to The University of Manchester for the degree of Doctor of Philosophy
in the Faculty of Engineering and Physical Sciences

2015

Steven Hoekman
School of Chemistry

This thesis reports the synthesis of a novel synthetic small molecule walker, a chiral [2]rotaxane and a single-handed trefoil knot. The last two were employed as ligands for metal catalysed asymmetric reactions.

Chapter 1 explains what small molecule walkers are and their resemblance to nature’s walking proteins. The motor protein myosin is discussed in more detail, followed by a section about small molecules that diffuse along a surface and recent advances in dynamic covalent walker systems.

Chapter 2 shows the design and synthesis of a novel directional dynamic covalent walker. In the first section the concept is discussed. Afterwards, the synthesis of the walker and model system is described in detail.

Chapter 3 is a short introduction about the history of interlocked molecules and the strategies that are applied to make them. The chapter highlights the formation of rotaxanes via the active metal template strategy. Also some useful features of rotaxanes are reviewed.

Chapter 4 reports the use of a chiral macrocycle to construct a [2]rotaxane architecture via the Cu-catalysed Goldberg reaction. The threaded macrocycle also facilitates the Ni-catalysed asymmetric Michael addition of diethyl malonate and nitrostyrenes and gives improved enantioselectivity compared to a non-interlocked ligand.

Chapter 5 is an extension on enantioselective catalysis with mechanically interlocked ligands. This chapter reports the first results of the application of a molecular trefoil knot in the lanthanide-catalysed Mukaiyama aldol addition.
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GENERAL METHODS

Unless stated otherwise, reagents were obtained from commercial sources and used without purification. Anhydrous THF (HPLC grade, Fischer scientific), CH₂Cl₂ (HPLC grade, Fischer scientific), and PhMe (>99%, Fischer scientific) were obtained by passing the solvent through an activated alumina column on a Phoenix SDS (solvent drying system; JC Meyer Solvent Systems, CA, USA). DMF (Peptide synthesis grade, Merck) was used throughout. Cesium carbonate (99.5%, Acros Organics) was dried overnight at 150 °C in a standard laboratory oven. ¹H NMR spectra were recorded on a Bruker Avance III instrument with an Oxford AS600 magnet equipped with a cryoprobe [5mm CPDCH ¹³C-¹H/D] (600 MHz), a Bruker AV 400 or Bruker AV 500 (cryoprobe). Chemical shifts are reported in parts per million (ppm) from high to low frequency using the residual solvent peak as the internal reference (CDCl₃ = 7.26 ppm, CD₂Cl₂ = 5.32 ppm, CD₃OD = 3.31 ppm and C₆D₆ = 7.16 ppm).¹⁻⁵ All ¹H resonances are reported to the nearest 0.01 ppm. The multiplicity of ¹H signals are indicated as: s = singlet; d = doublet; t = triplet; quint = quintet; m = multiplet; br = broad; or combinations of thereof. Coupling constants (J) are quoted in Hz and reported to the nearest 0.1 Hz. Where appropriate, averages of the signals from peaks displaying multiplicity were used to calculate the value of the coupling constant. ¹³C NMR spectra were recorded on the same spectrometer with the central resonance of the solvent peak as the internal reference (CDCl₃ = 77.16 ppm, CD₂Cl₂ = 54.00 ppm, CD₃OD = 49.00 ppm and C₆D₆ = 128.06 ppm).¹⁻² All ¹³C resonances are reported to the nearest 0.01 ppm to aid in the differentiation of closely resolved signals. DEPT, COSY, HSQC and HMBC experiments were used to aid structural determination and spectral assignment. Where necessary, NOESY or ROESY spectra were used to aid the assignment of ¹H spectra. Fully characterized compounds were chromatographically homogeneous. Flash column chromatography³ was carried out using Silica 60 Å (particle size 40-63 μm, Sigma Aldrich, UK) as the stationary phase. Automated purification was performed using pre-packed silica columns (Reveleris® Flash Cartridges) on the Reveleris® automated Flash Chromatography System. Preparative TLC was performed using either PLC 20×20 cm, 60 F₂₅₄ Prep plates (Merck) or Silica Gel GF 20×20 cm, U₂₅₄ Prep plates (Analtech) of various thicknesses. TLC was performed on precoated silica gel plates (0.25 mm thick, 60 F₂₅₄, Merck, Germany) and visualized using both short and long waved ultraviolet light in combination with standard laboratory stains (acidic potassium permanganate, acidic ammonium molybdate and ninhydrin). Low resolution ESI mass spectrometry was performed with a Thermo Scientific LCQ Fleet Ion Trap Mass Spectrometer or an Agilent Technologies 1200 LC system with 6130 single quadrupole MS
detector. High-resolution mass spectrometry was carried out by the EPSRC National Mass Spectrometry Service Centre (Swansea, UK). Melting points (Mp) were determined using a Büchi M-565 apparatus and are corrected. Optical rotations were measured using a Rudolph Research Analytical Autopol I polarimeter with both AP Accuracy (±0.004°) and resolution upgrades with a built in thermoprobe for temperature measurement/control. Measurements were conducted using a sodium lamp (l 589 nm, D-line); [α]20D values were reported in 10 deg cm² g⁻¹, concentration (c) in g per 100 ml. Enantiomeric ratios were determined by HPLC on a Agilent 1260 Infinity system with UV detection at 210 or 254 nm. A Chiralpak IC or IA (5 μm Particle size, 250×4.6 mm, Diacel Corporation) column and hexane/2-propanol (95/5 or 9/1) as eluent (1 ml/min flow-rate) were used for separations unless otherwise indicated. Steady state emission spectra were recorded on an Edinburgh Instrument FP920 Phosphorescence Lifetime Spectrometer at 295 K equipped with a 450 watt xenon lamp and a red sensitive photomultiplier in peltier (air cooled) housing, (Hamamatsu R928P). Lifetime data were recorded following excitation into the ligand absorption bands with a 2 W xenon flash lamp (Edinburgh Instruments), using multichannel scaling. Lifetimes were obtained by tail fit on the data obtained, and quality of fit judged by minimization of reduced chi-squared and residuals squared. The inner sphere hydration numbers (q) were determined by recording lifetime data for the complexes in MeOH and MeOD using the Horrock’s equation.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
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<tbody>
<tr>
<td>AcOH</td>
<td>acetic acid</td>
</tr>
<tr>
<td>ADP</td>
<td>adenosine diphosphate</td>
</tr>
<tr>
<td>AMT</td>
<td>active metal template</td>
</tr>
<tr>
<td>AQ</td>
<td>anthraquinone</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>BHEAN</td>
<td>1,5-bis[2-(2-hydroxyethoxy)ethylamino]naphthalene</td>
</tr>
<tr>
<td>BHEEN</td>
<td>1,5-bis[2-(2-hydroxyethoxy)ethoxy]naphthalene</td>
</tr>
<tr>
<td>Boc</td>
<td>tert-butylcarbonyl</td>
</tr>
<tr>
<td>CBPQT</td>
<td>cyclobis(paraquat-p-phenylene)</td>
</tr>
<tr>
<td>CD</td>
<td>cyclodextrin</td>
</tr>
<tr>
<td>COSY</td>
<td>correlation spectroscopy</td>
</tr>
<tr>
<td>CPK</td>
<td>Corey-Pauling-Koltun space filling</td>
</tr>
<tr>
<td>d</td>
<td>days</td>
</tr>
<tr>
<td>DEPT</td>
<td>distortionless enhancement by polarization transfer</td>
</tr>
<tr>
<td>DFT</td>
<td>density functional theory</td>
</tr>
<tr>
<td>DIAD</td>
<td>diisopropyl azodicarboxylate</td>
</tr>
<tr>
<td>DIBALH</td>
<td>diisobutylaluminium hydride</td>
</tr>
<tr>
<td>DIPEA</td>
<td>N,N-diisopropylethylamine</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-(dimethylamino)pyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>dr</td>
<td>diastereomeric ratio</td>
</tr>
<tr>
<td>DTA</td>
<td>dithioanthracene</td>
</tr>
<tr>
<td>EDCI</td>
<td>N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>ee</td>
<td>enantiomeric excess</td>
</tr>
<tr>
<td>equiv.</td>
<td>equivalents</td>
</tr>
<tr>
<td>ESI</td>
<td>electrospray ionisation</td>
</tr>
<tr>
<td>et al.</td>
<td>et alia</td>
</tr>
<tr>
<td>Et₂O</td>
<td>diethyl ether</td>
</tr>
<tr>
<td>EtOH</td>
<td>ethanol</td>
</tr>
<tr>
<td>Et₃N</td>
<td>triethylamine</td>
</tr>
<tr>
<td>Et₃SiH</td>
<td>triethylsilane</td>
</tr>
</tbody>
</table>
EtOAc  ethyl acetate
EXSY  exchange spectroscopy
h  hour
HMBC  heteronuclear multiple-bond correlation spectroscopy
HOBt  1-hydroxybenzotriazole
HPLC  high performance liquid chromatography
HRMS  high resolution mass spectrometry
HSQC  heteronuclear single-quantum correlation spectroscopy
Hz  hertz
J  coupling constant
LAH  lithium aluminium hydride
LRMS  low resolution mass spectrometry
M  molar
m/z  mass-to-charge ratio
MALDI  matrix assisted laser desorption/ionisation
MeNO₂  nitromethane
MeOH  methanol
MHz  megahertz
min  minutes
mL  millilitre
mM  millimolar
mmol  millimol
NDI  naphthalene diimide
nm  nanometre
NMR  nuclear magnetic resonance
NOESY  nuclear Overhauser effect spectroscopy
OAc  acetate
OTf  triflate
p  para
PET  petroleum ether
PhMe  toluene
Pi  inorganic phosphate
ppm  part per million
pTSA  para-toluenesulfonic acid
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>PyBroP</td>
<td>bromotripyrrolidinophosphonium hexafluorophosphate</td>
</tr>
<tr>
<td>quant.</td>
<td>quantitative</td>
</tr>
<tr>
<td>ROESY</td>
<td>rotating frame nuclear Overhauser effect spectroscopy</td>
</tr>
<tr>
<td>rt</td>
<td>room temperature</td>
</tr>
<tr>
<td>STM</td>
<td>scanning tunnel microscopy</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
</tr>
<tr>
<td>Trt</td>
<td>trityl or triphenylmethyl</td>
</tr>
<tr>
<td>v/v</td>
<td>volume ratio</td>
</tr>
<tr>
<td>v/v%</td>
<td>volume percentage</td>
</tr>
<tr>
<td>δ</td>
<td>chemical shift</td>
</tr>
<tr>
<td>μL</td>
<td>microlitre</td>
</tr>
<tr>
<td>μm</td>
<td>micrometre</td>
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Synopsis: Inspired by nature’s walking proteins, several synthetic small molecule walkers have been reported to date. Despite being much smaller in size and far less complex, these small walking molecules are able to mimic their larger counterparts. Still, as the research topic is relatively young, much progress can be made. A synthetic small molecule walker that can transport a cargo in a forward motion along a polymeric track should be viable in the near future. For now, some small molecule walkers already exhibit characteristics that are arguably better than nature’s walking proteins.
Characteristics of a molecular walker

A molecular walker is described as microscopic object that is able to move along a track or surface. Chemical processes and/or interactions drive the motion of all molecular walkers. The irreversible consumption of a fuel allows the walker to do mechanical work (move to an endpoint or state that is not the walker’s initial state), thus it can be considered a molecular motor. Either covalent bonds or non-covalent (secondary) interactions bind the walker to its path. Ideally a walker molecule exhibits five fundamental characteristics.1 (i) Processivity: the walker remains attached to the track during its operation. (ii) Directional movement: the walker migrates to one desired end of the track and does not randomly take forward/backward steps. (iii) Progressive operation: the walker (motor) can be reset after each step (cycle) without undoing the work that had been done. (iv) Repetitive operation: the walker’s (motor’s) ability to take more than one step (perform more than one cycle). (v) Autonomous operation: the walker walks (motor functions) as long as there is a source of fuel.

A large diversity of walkers exists ranging from synthetic small molecule walkers to nature’s complex motor proteins that move along cytoskeletal polymers. Each architecture is unique and makes most walkers behave very differently in both operation and motion. Below various walker systems are described including motor proteins as well as small molecule walkers. Extensive reviews have been written about synthetic DNA-walkers.1,9 In these systems walking is commonly achieved through base pair interactions between the walker, track and fuel (fuel strands or enzymes). Although less complex than motor proteins they are often of similar size and arguably don’t fit in the realm of small molecule walkers. In the following chapters the design and synthetic efforts towards a new small molecule walker will be discussed.
Walkers in nature – Motor proteins that move along cytoskeletal fibres

Many processes in the cell require movement of protein complexes, chromosomes, cargo vesicles, organelles and mRNA towards very specific destinations. A class of proteins, commonly known as motor proteins, carries out this task. Especially interesting are the ones of the myosin, kinesin and dynein family. These so-called motor proteins move directionally along rigid tracks that are known as cytoskeletal polymers. This movement can be visualised as walking, since many of these proteins essentially have two legs that successively take a step forward along the polymer track. The motion is typically highly directional and processive i.e. the walker moves to one particular end and does not fully detach from the track over a series of steps.

Although the structures of the aforementioned motor proteins are highly similar, they perform very different and unique tasks. Myosin II for example plays a central role in muscle contraction by means of moving over an actin filament and thereby pulling it inwards. Other members of the myosin family like the well-studied myosin I and V function as vesicle carriers and operate in a slightly different manner and achieve high processivity.

There are two types of tracks these walkers walk on. Proteins of the myosin family move along microfilaments (actin filament or A tubule), whereas kinesins and dyneins move along microtubules. All known myosins to date move towards the (+) end (cell periphery) of the track with exception of myosin VI, which moves to the (-) end (cell centre) of the track. Most kinesins move towards the (+) end and dyneins are known to be (-) end directed. In order to deliver work a walker needs to consume fuel, hence the name motor protein. In the cell this fuel is present in the form of ATP (adenosine triphosphate), which is hydrolysed by an ATPase on the motor domain of the protein to give ADP (adenosine diphosphate) and Pi (inorganic phosphate). The consumption of ATP induces a conformational change in the protein and in short results in a directional stepwise movement.
Myosin motors are most commonly known to power muscle contraction (myosin II). However, this family of motor proteins operates in a wide array of other essential cellular processes, for instance vesicle transport (myosin V, VI), cell movement (myosin XIV), organisation/anchoring of the Golgi complex (myosin XVIII) and cytokinesis (myosin II). The structures of these myosins are fairly similar and overall consist of a motor domain, lever arm, dimerization domain and cargo binding domain (figure 1).

![Figure 1: Schematic illustration of myosin V.](image)

The motor domain can be seen as the foot of the walker, since it binds to (steps on) the track. This is also the domain where the fuel in the form of ATP is hydrolysed to ADP and Pi. An important subdomain in the motor region is the converter, which links the motor domain to the lever arm and translates or amplifies a conformational change in the motor unit to the lever arm. Further towards the C-terminus the dimerization domain comprising a coiled coil sequence can usually be found. The formation of a dimer gives the myosin its characteristic two-legged appearance, although one-legged motor protein monomers exist and can function as processive walkers too. Finally, the region at the C-terminus attaches myosin to a cargo (e.g. vesicles, organelles) and carries it to a destination as myosin moves along the actin filaments. From there the cargo can either be processed or taken over by another motor protein.
The exact mechanism of how these walkers move *i.e.* how they interact with ATP, the track and what conformations they adopt is not yet fully understood, since some structural information in the kinetic cycle is still missing. However, the general consensus is that myosins operate according to the hand-over-hand myosin lever arm hypothesis.\textsuperscript{3,4} The kinetic cycle that is described next is based on myosin V (figure 2). All myosin kinetic cycles are fairly similar, as the variation in the rates of phosphate and ADP release is the only major difference.

![Figure 2: Simplified representation of the kinetic cycle of myosin V.](image)

When the motor domain (trailing foot) is strongly bound to F-actin only (rigor-state, figure 2a) it rapidly dissociates (post-rigor state) from the actin filament upon binding of one molecule of ATP (figure 2b). Now myosin closes the ATP binding site, thereby making it catalytically active and primes the lever arm (recovery stroke).\textsuperscript{5,6} While the myosin motor domain is detached it is free to undergo Brownian motion (random movement). However, the conformational changes induced by ATP biases the motion and make it more likely for the motor to bind further towards the plus end of the actin filament (74 nm away), in front of the earlier leading foot (figure 2b).\textsuperscript{7,8} After the recovery-stroke ATP is hydrolysed to ADP and Pi, which stay bound to the motor domain and the motor now binds weakly to the actin filament (figure 2c). Another conformational change in the motor domain prompts the release of Pi and triggers a large rotation (power-stroke) of the myosin lever arm via the converter that connects the motor domain to the lever arm. The motor head now stays strongly bound to actin (the other foot starts his cycle) until ADP dissociates and ATP binds
again to complete the cycle (figure 2d). The duration of the strong binding state can be correlated to the processivity of the motor protein. Myosin V for example is a highly processive walker and during most of its cycle remains in a strong binding state. As a consequence, simultaneous detachment of both feet from the track is unlikely and therefore the walker does not come off the track. The ratio of strong binding state and weak+detached+strong states is known as the duty ratio\(^3\). A high duty ratio corresponds to a highly processive motor protein and a low duty ratio to a less processive motor protein. Low processivity does not imply a motor protein functions poorly. Myosin II, which powers muscle contraction, has a low duty ratio.\(^3\) However, it is the low duty ratio that makes it so adequate in its task. Myosin II in muscle tissue sarcomeres (functional domains in muscle tissue that are responsible for muscle contraction) forms large aggregates (thick filament) and is bound to actin (thin filament). Either end of the sarcomere has a filamentous network of proteins (Z-disk or Z-line) that serve as an anchoring point for actin. The myosin II aggregate consists of many motor domains. These feet operate simultaneously and walk unidirectionally over the thin filament, thereby pulling it to the centre of the sarcomere, since the thin filaments are fixed to the Z-disc. If the feet were mostly bound to the track it would just restrain the pulling process. The fact that individual myosin proteins don’t interact with actin for a period of time has no consequence, because a few other myosin feet in the aggregate will interact and retain the filaments.
Small molecule walkers

Inspired by the elegance and complexity of motor proteins, scientists, have tried to mimic the walking motion of these large molecular structures using much smaller and simpler molecules. First the motion of small molecule walkers over flat surfaces will be discussed. Dynamic covalent walkers that walk along a track and more closely resemble motor proteins will be reviewed after.

Motion through diffusion over flat surfaces

The group of Raval recently reported the stochastic motion of a bis(imidazolyl) walker along a monocrystalline Cu(110) surface (figure 3).¹⁰

![Figure 3: Random motion between fences.](image)

DFT (density functional theory) calculations showed that the walker (figure 4) adopts a horseshoe conformation, wherein the molecule stands up on its imidazolyl feet and interacts with the Cu surface through ligand metal interactions with its nitrogen atoms. The most favourable geometries were found when the feet were attached to the same row of the Cu grid and separated by three (contracted state) or four (extended state) Cu atoms. Orthogonal molecular porphyrin fences (figure 4) along the [001] surface direction physically confined the motion and theoretical modeling suggested the displacement is of the inchworm-type (one foot leads the other follows) in a one-dimensional direction.
Figure 4: Walker and fence.

Scanning tunnel microscopy (STM) measurements confirmed the perpendicular diffusion of the walker between the fences and the structure was individually imaged when trapped between fences in close proximity or at low temperature as a three-lobe structure with its feet three Cu atoms apart.

In 2005 Bartels and co-workers developed a system that achieved random motion of 9,10-dithioanthracene (DTA) along one direction on a high-symmetry Cu(111) surface (figure 5). The walkers were visualised by STM and showed that each molecule appeared with slightly elevated ends, while the two sulfur atoms could be unambiguously identified at each side of the centre as low protrusions. The anthracene moieties of the single molecules or clusters were always aligned with the substrate symmetry in the [110]-like direction. The diffusion of individual DTA molecules was monitored at 50-70 K and exclusively showed motion along the substrate high-symmetry direction, while the corresponding mono-thiol (9-thioanthracene) only rotated around its sulfur atom. Two energy minima were determined by DFT calculations. The first, wherein the aromatic core was aligned with the substrate high-symmetry, was in agreement with the STM data and had one sulfur atom located between the bridge and hexagonal close packed hollow site and the other close to an on-top site. The second found both sulfur atoms close to the energetically favourable near-bridge sites but had the anthracene functionality out of alignment with the substrate (figure 5).

Figure 5: Alternating rotation around the sulfur atoms makes the walker move.
The last minimum was not detected by STM, although it was possible to manipulate the molecules in this orientation at 10 K by STM tip. Linear diffusion of DTA would occur through alternating small rotations around the sulfur atoms between the two energy minima.

Two years later the same researchers were able to reversibly bind CO$_2$ as a cargo on anthraquinone (AQ) and transport it along a straight line across an isotropic Cu(111) surface. Similar to the system described above, linear diffusion was caused by alternating rotations around AQ’s carbonyl groups. With CO$_2$ bound to AQ they were now able to image the molecular orientations that correlated with the individual steps. They also showed that the diffusion velocity was decreased upon binding of the CO$_2$ load.

More recently, the group increased the variety of walkers by extending the aromatic backbone, the introduction of extra carbonyl groups (a quadruple system) and substitution of the aromatic core with terminal methyl groups.

In 2011 the group of Huskens reported the gradient-driven diffusion of divalent ligand guest molecules on a monolayer of β-cyclodextrin (CD) receptors. The guest molecules were loaded on the substrate as a fine line by microcontact printing and interacted with the CD receptors through its functionalised adamantate feet. They were labelled with lissamine rhodamine B for fluorescence detection. Upon addition of a solution of competing CD receptors, the guest molecules began to spread from their original contact areas (lines) to neighbouring vacant CD receptors and the resulting pattern was imaged by fluorescence microscopy. The measurements indicated multiple spreading mechanisms and the concentration of free CD in the solution determined the dominant type of diffusion. A walking (step to adjacent receptor) pathway was preferred in pure water, while at increasing concentrations of free CD so-called hopping (jump to nearby receptor) and flying (displacement to far receptor) became the main modes of diffusion.
Dynamic covalent motion

In 2009 Leigh et al. reported the first linear small molecule walker. This 21-atom two-legged walker was able to operate in a processive (37 steps) and directional fashion. In addition, the walker’s operation was repetitive (repeatedly performed the same cycle) and progressive (motion was stopped and continued without undoing any work). A unique feature of this system was the presence of two chemically dissimilar feet, whereas motor proteins in nature have an identical pair of feet. This property enabled the walker to detach one foot, while the other foot was kinetically locked and remained bound to the track. This particular small molecule walker had a hydrazide foot and was bound to the track as a hydrazone (figure 6). The other foot was a thiol and bound to the track as a disulfide. A stimulus of acid or base allowed the dynamic covalent bond (hydrazone or disulfide respectively) between the walker and the track to equilibrate with the other positions on the track and walking (taking one or zero steps) was achieved under thermodynamic control. A sequential stimulus of acid and base resulted in the walker reaching the track’s terminal position. As one foot is always bound to the track while the other is allowed to move walking proceeds via a hand-over-hand gait (if the motion is fully biased).

With one foot bound constantly excellent processivity is expected. However, outstanding processivity was not accomplished with this system. Although the walker did indeed not detach in a manner that it moved around freely and unbound, the system was free to move in solution and came occasionally in close proximity to another system. As a consequence, the foot of one system was able to bind with a foothold on the other system and thus bridge the tracks. If the subsequent stimulus was given the second foot would detach from the old track and most likely bind to a foothold on the new track. This phenomenon is known as intermolecular overstepping and can be avoided if the systems are immobilised or operated under high dilution conditions. After several dynamically controlled acid-base cycles the four-foothold system reached a steady-state (most walkers were on the 1,2- and 2,3-position) i.e. a step in the forward direction is as likely as a step backwards. Unfortunately,

Figure 6: An acid/base oscillation makes the walker walk.
these simple acid-base oscillations didn’t provide a net input of energy and the walker couldn’t be biased away from its thermodynamic minimum. To overcome this issue the base induced step (from the 2,3-position) was replaced with a kinetically controlled redox-mediated disulfide exchange reaction. As this kinetic acid-redox sequence provided a net input of energy a reasonable bias (the forward step is $1.5 \times$ more likely to occur than reconnecting to the original position) was achieved.

In a successive study the central triazole linker was replaced with a stilbene moiety (figure 7). The advantage of introducing this particular functionality was that it had the potential to isomerise ($E/Z$) under the influence of light. If the system remained in the $cis$-isomer it would more or less behave as the formerly described acid/base sequenced walker. However, when the walker was bound to the stilbene moiety ($Z$-2,3-position) and irradiated with visible light (500 nm) in the presence of $I_2$, isomerization to the $trans$-isomer occurred. The macrocycle now experienced ring-strain (thermodynamic minimum was raised) and preferred to bind to the next foothold in the consecutive step (acid), as opposed to remaining in an energetically unfavourable state.

Thus by sequentially adjusting thermodynamic minima via energy input in the form of light directionality can be accomplished. The alteration (raise) of a thermodynamic minimum (from $Z$-2,3-1 to $E$-2,3-1) to favour the movement to a new lower energy state ($E$-3,4-1) is described as the Brownian energy ratchet mechanism, whereas the former acid/redox system, were directionality is achieved by prohibiting backwards motion, corresponds to the so-called information type ratchet mechanism.
The same group recently reported the completion of a walker that mediates its movement along the track through Michael/retro-Michael reactions.\textsuperscript{17} The concept was based on the research conducted by Lawton and co-workers in the late 1970s on the dynamic cross-linking of biomolecules.\textsuperscript{18} No sequential external inputs were required to operate this system, hence the walker moved in an autonomous fashion. The track contained five amine footholds and a fluorescent anthracene moiety at the terminal position. The walker unit, α-methylene-4-nitrostyrene, attached to the first amine foothold was operated in DMSO and slowly moved to the adjacent amine foothold via the postulated seven-membered ring intermediate (figure 8).

\begin{center}
\begin{tabular}{c}
\textbf{Figure 8: Motion of the walker via a seven-membered ring intermediate.}
\end{tabular}
\end{center}

From this bridged intermediate the walker was able to either return to its initial state or move to the second amine foothold and so on. When the walker finally reached the last foothold the anthracene group’s fluorescence was quenched. This effect was directly measured and the experimental results indicated that the system reached equilibrium after 6.5 hours. \textsuperscript{1}H-NMR-studies were in agreement with this observation. The processivity of the exchange was determined by the addition of a different walkerless track under operation conditions. Mass spectrometry indicated that after six days less than 3\% of the walker had migrated to the other track meaning that a walker takes an average of 530 steps before being detached. It was also shown that the walker didn’t overstep footholds through the replacement of the central amine foothold by a carbon on a three-foothold track. In this system the walker was never able to reach the last foothold. Although this walker was autonomous and highly processive its motion was unbiased.

Two years later the same researchers published an improved design that allowed for a certain degree of directionality and incorporated an extended track of up to nine amine footholds (figure 9).\textsuperscript{19}
Figure 9: Directional bias on a nine-foothold track with a terminal naphtylmethylamine group as a thermodynamic minimum.

The key feature that biased the motion was the replacement of the anthracene group on the last foothold by a naphtylmethylamine moiety. As there was a small preference for the walker to reside on this foothold the dynamic equilibrium was biased more towards the end of the track. The system was synthesized as the amine TFA salt and the best results were found when the operation was conducted with a one equivalent excess of base. On a five-foothold system 46% of the walkers resided on the last foothold when the steady-state was reached after 48 hours. On the nine-footholds system 19% of the walkers resided on the naphtylmethylamine foothold after 90 hours.

The group of Lehn reported a conceptually similar walker at the same time as Leigh’s Michael walker. Molecular motion was realised through an internal exchange process of self-transimination. The walker unit, salicylaldehyde, was attached to an oligoamine track as an imine and moved to the adjacent amine foothold via an aminal intermediate in an inchworm-type fashion (figure 10).
Intramolecular exchange (motion) was measured by 2D-EXSY (exchange spectroscopy) $^1$H NMR and showed cross-peaks between the ends of the oligoamine moiety. As expected the exchange was fastest in the short diethylenetriamine track. No intermolecular exchange was observed in both diethylenetriamine and triethylenetetraamine, which showed the system was processive to a certain extent. Overstepping or terminal transfer of the aldehyde were ruled out when the two central amines of triethylenetetraamine were replaced by oxygens and only intermolecular exchange was observed. They found that the introduction of substituent on the aromatic ring had an effect on the rate of exchange and a fifty-fold increase in speed was measured when a 5-nitro group was introduced. The solvent system was also of great importance as the exchange in a mixture of CD$_3$CN/D$_2$O (1:1) was eighty times faster than in pure CD$_3$CN.

A follow-up paper was published wherein a pH-driven bidirectional displacement over an unsymmetrical track was described. The new walker, 2-formyl-3-hydroxybenzoic acid, was used to move along an asymmetric 4-foothold oligoamine track that contained a primary amine at one end and a hydroxylamine group at the other (figure 11).
When the assembly of the molecular machine was carried out with the sodium salt of the walker it exclusively attached to the primary amine terminus as an imine in a condensation reaction. To quantitatively attach the walker to the hydroxylamine terminus as an amino lactone the corresponding free acid was used. Rapid displacement to the primary amine terminus was observed when three equivalents of base were added starting from the walker on hydroxylamine end. Back-titration with three equivalents of TFA resulted in reverse motion to the hydroxylamine terminus. The motion was pH-driven and resulted in the thermodynamically most stable product. The primary amine acted as a thermodynamic minimum under basic conditions as did the hydroxylamine terminus under acidic conditions. Random motion took place on the central amines. The acid-base cycle could be repeated up to five times and no dissociation of the aldehyde from the track was observed when the acid was added in multiple portions.

In 2014 the group of Bayley was able to measure the motion of an individual organoarsenic walker within a protein nanoreactor.\textsuperscript{22} The walker was initially bound to two thiol ligands and moved along a cysteine residue track on a β-strand within an α-haemolysin protein pore. The thiol side chains of the five-foothold track all pointed into the lumen of the transmembrane β-barrel and motion was observed through changes in the flow of ionic currents when an Ar-S-bond was formed or broken. The proposed mechanism is illustrated below (figure 12).
The first cysteine residue on the track binds to the walker and displaces one of the walker’s thiol ligands. The adjacent residue now cyclises on the arsenic and the second ligand leaves. The cyclic is now prone to attack by a free ligand and can result in the mono adduct of either one of the two residues. From here on the walking motion continues or starts over from foothold 1 depending on how the cyclic adduct opened. The cycle is continuous and a weak bias towards the last foothold, probably because it is an environmentally induced thermodynamic minimum, was observed. About 7% of all walks resulted in a complete walk from foothold 1 to foothold 5 or vice versa at a speed of roughly 1 nm s\(^{-1}\). Dissociation rates depended on the concentration of the thiol ligand and the walker took an average of six steps under operation conditions.
Aim and objectives – Directional stepping

Various molecular walkers, from nature’s complex motor proteins that possess all five fundamental characteristics of a molecular walker to simple yet clever synthetic designs that allow the walker to exhibit at least some of those desired properties, have been discussed so far. Interestingly, synthetic systems such as the highly processive Michael walker sometimes surpass the abilities of biological walkers and/or are equally progressive, repetitive or autonomous. Still, true directional stepping has been difficult to achieve. Leigh’s two-legged stilbene walker that utilises light to accomplish directionality was only able exploit this feature in one of the two steps the walker had to take to reach the end of the track. Other systems, like Lehn’s pH-driven walker or Leigh’s second Michael walker, don’t really walk in a directional fashion but were able to reach a thermodynamic minimum at the track’s end through random stepping. The design of the former even permitted the walker to move in a bi-directional manner where the location of the thermodynamic minimum depended on the pH of the environment. Since directional stepping is an aspect that can be much improved upon, a novel walker system that could match the directionality of biological systems would be quite desirable. The following chapter describes and discusses the design of a system that combines the architecture of some of the previously described walkers with the incorporation of units that allow for intramolecular secondary interactions between the walker and the track in order to achieve this kind of directionality.
References


Synopsis: A small molecule walker that could walk directionally along a four-foothold track was designed. The walker’s hydrazide and thiol feet allow orthogonal stepping and a motional bias would be achieved by π-π stacking interactions between the walker and the track. A model compound wherein the walker is attached to a two-foothold track with both feet was synthesised. Afterwards, the desired system was constructed in 24 steps but unfortunately the walker was not able to attach its hydrazide foot to the second foothold. Intramolecular folding or other unfavourable conformations might have prevented the foot from reaching that foothold. As the system contained many functionalities a simplified design could be considered.

Acknowledgements: I’d like to thank dr. Victor Blanco-Suarez for getting me started on the project and his contribution to the synthesis of the model compound, dr. Fabien Cougnon for his contribution to the synthesis of the revised walker and diaminonaphthalene station and dr. Guzman Gil-Ramirez for helpful discussions and his contribution to the synthesis. I have worked on this project from the start until the end, have synthesised all compounds at least once and characterised all compounds.
The design of a $\pi$-$\pi$ stacking walker

Inspired by the work of Sanders$^{1,2}$ and Iverson$^3$ on aromatic electron donor-acceptor interactions a new potential walker, based on these $\pi$-$\pi$ stacking interactions and the Leigh group’s previously described two-legged hydrazone/disulfide walker, was proposed. Since we anticipated some complications with the first design, adjustments were made and the improved molecular machine is depicted below (figure 1).

![Fig. 1: Design of the $\pi$-$\pi$ stacking walker.](image)

The footholds are linked by aromatic stations and allow the walker to be at three positions. The walker is connected to foothold 1 and 2 (1,2-position) and would step to foothold 3 (2,3-position) and 4 (3,4-position) via a hand-over-hand mechanism (figure 2).

![Fig. 2: Schematic view of the walker on the track.](image)

A motional bias would be achieved by the dual role of base and acid stimuli in promoting stepping dynamics and secondary interactions between the walker and the track. The concept is illustrated in figure 3.
In the initial position a $\pi-\pi$ stacking interaction is established between the electron poor naphthalene diimide (NDI) acceptor of the walker unit and the electron rich hydroquinone donor of the track. Upon addition of base disulfide exchange allows the walker to either remain on its position or move towards foothold 3 (2,3-position). Since the hydroquinone group is a much poorer electron-donor than 1,5-diaminonaphtalene, the $\pi-\pi$ stacking interaction between the latter and the diimide is favoured. Consequently, the equilibrium is shifted to the 2,3-position. Successive addition of acid labialises the hydrazone bond and protonates the diaminonaphthalene functionality. The protonation of the central stacking station results in the loss of its strong electron donating properties and connects the walker to foothold 4 (3,4-position), where a donor-acceptor stacking interaction is established with the now relatively strong electron donating hydroquinone group.

The incorporation of these particular aromatic units, amide bonds and the ethylester functionalities at the ends of the track mainly result from an early study conducted in the group of Iverson.\textsuperscript{3} Herein, the solvent effects in aromatic stacking were investigated. They concluded that aromatic donor-acceptor (electron rich-poor) interactions are highly favored and acceptor-acceptor interactions are favored to some degree (figure 4). Donor-donor stacking barely occurred.
The experiments were conducted in a variety of solvents ranging from apolar to polar and aprotic to protic. Strong binding took place in polar protic solvents (MeOH, water) and was mainly caused by the hydrophobic effect. However, they postulate that not only the desolvation driving force but also electrostatic interactions play a role in the binding affinity, as the donor-acceptor interactions were found to be a few orders of magnitude stronger than acceptor-acceptor and donor-donor interactions in water (figure 5).

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$K_d$ (M$^{-1}$) (donor-donor)</th>
<th>$K_a$ (M$^{-1}$) (acceptor-acceptor)</th>
<th>$K_{da}$ (M$^{-1}$) (donor-acceptor)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDCl$_3$</td>
<td>(1)</td>
<td>(1)</td>
<td>2 ± &lt;0.5</td>
</tr>
<tr>
<td>Acetone-d$_6$</td>
<td>1 ± &lt;0.5</td>
<td>1 ± &lt;0.5</td>
<td>8 ± &lt;0.5</td>
</tr>
<tr>
<td>DMSO-d$_6$</td>
<td>1 ± 1</td>
<td>2 ± &lt;0.5</td>
<td>3 ± &lt;0.5</td>
</tr>
<tr>
<td>CD$_3$CN</td>
<td>1 ± 1</td>
<td>3 ± &lt;0.5</td>
<td>11 ± &lt;0.5</td>
</tr>
<tr>
<td>CD$_3$OD</td>
<td>1 ± &lt;0.5</td>
<td>8 ± &lt;0.5</td>
<td>30 ± &lt;0.5</td>
</tr>
<tr>
<td>CD$_3$OD/D$_2$O 3:1</td>
<td>1 ± &lt;0.5</td>
<td>15 ± &lt;0.5</td>
<td>63 ± 2</td>
</tr>
<tr>
<td>CD$_3$OD/D$_2$O 1:1</td>
<td>2 ± &lt;0.5</td>
<td>28 ± 2</td>
<td>254 ± 41</td>
</tr>
<tr>
<td>CD$_3$OD/D$_2$O 1:3</td>
<td>10 ± 2</td>
<td>101 ± 28</td>
<td>952 ± 64</td>
</tr>
<tr>
<td>D$_2$O</td>
<td>20 ± 4</td>
<td>245 ± 101</td>
<td>2045 ± 63</td>
</tr>
</tbody>
</table>

Figure 5: Binding constants.

Sanders et al. have been synthesizing catenanes based on similar motifs for over a decade. They find that catenane formation ($\pi$-$\pi$ stacking of the templates) predominantly occurs in a solvent of high ionic strength like water. In order to increase that ionic strength salts (NaCl, NaNO$_3$, KNO$_3$, Na$_2$SO$_4$ and K$_2$SO$_4$) were added, which aided the formation of catenanes.
These results indicate that the operation of the walker should be carried out in a polar solvent like MeOH, water or a combination of the two. A potential problem could be the solubility of the system. Some NDIs are known to be quite insoluble and not much can be said about the solubility of the track. The incorporation of amides would hopefully increase the solubility in polar solvents and the ethylester groups at the ends of the track not only function to make synthesis shorter (by symmetrising the system) but can also be further functionalised with polar moieties.

In the initial design the system didn’t have any of these features incorporated thus would most likely not be soluble in polar solvent systems (figure 6).

Another challenge was found in the synthesis of this first design. The parts were to be connected via aryl Mitsunobu reactions. This chemistry didn’t work or was low yielding at best. The unsymmetrical footholds complicated the construction even more by not allowing a short synthetic route.

As mentioned before the system will operate through a sequential input of acid and base. This chemistry has been extensively studied in our group and has proven to be a reliable method to consecutively move the two feet. Finally a more recent publication by Stoddart et al. supports the hypothesis that the protonated dianiminaphthalene functionality will likely result in a weakened π-π stacking interaction with the electron poor walker unit and drive the walker’s motion forward. The study described the utilization of 1,5-bis[2-(2-hydroxyethoxy)ethoxy]naphthalene (BHEEN) as a template to synthesize cyclobis(paraquat-p-phenylene) (CBPQT). For the purpose of obtaining CBPQT pure, addition of the electron rich 1,5-bis[2-(2-hydroxyethoxy)ethylamino]naphthalene (BHEAN) to a solution containing the BHEEN-CBPQT complex resulted in a displacement of BHEEN from the electron poor CBPQT pocket. The newly formed BHEAN-CBPQT complex was subsequently treated with concentrated acid, thereby protonating BHEAN. Consequently, BHEAN is driven out of its CBPQT pocket and CBPQT is obtained without a template (figure 7).
Figure 7: Template mediated synthesis of CBPQT.

These electron donors resemble the ones in the proposed π-π stacking walker and the results suggest that the diaminonaphthalene has a higher affinity to the walker than the hydroquinone functionality. That the protonated BHEAN repels CBPQT is encouraging and we hope to achieve a significantly weakened interaction between the walker and diaminonaphthalene station upon protonation.
Retrosynthesis of the molecular machine

A retrosynthetic scheme of the molecular machine is outlined below (figure 8).

Figure 8: Retrosynthetic scheme of the walker’s track.

The last step of the synthesis will involve the macrocyclisation of the walker with the track through hydrazone formation with the aldehyde of foothold 2 (figure 8 step a). In order to set the starting position of the walker on the track, the walker will be connected to foothold 1 first and the resulting assembly attached to the track containing footholds 2, 3 and 4.
(figure 8 step b). The different domains of the system will be connected via a series of amide bonds. These connections were chosen because amide bonds should enhance the solubility of the system in polar solvents and not interfere with the operation of the machine. In addition, amide bond formation by peptide coupling chemistry is versatile and well established. The synthesis of a model system that resembles the walker on foothold 1 and 2 (1,2-position) will also be described. It is essential to know if such a model can be made, as the molecular machine is complex and will take a considerable amount of effort the synthesise. A concern is that the walker’s foot might not be able to reach the aldehyde of foothold 2 and thus may not form the desired macrocycle (walker on 1,2-position). The length and flexibility of the legs play an important role. A rigid machine or a design wherein the walker has short feet might results in an unfavourable stacking orientation of the aromatic components or the inability of the feet to connect to a foothold. On the contrary, if there is too much flexibilty the feet might not affix that easily because they aren’t forced to be in close proximity to the foothold. A CPK (Corey-Pauling-Koltun space filling) model was built to investigate the walker’s ability to attach to the footholds and this proved to be the conceivable. The aromatic rings were also capable to stack in the right orientation. The synthesis of the walker is described first and is followed by the track footholds, hydroquinone stacking station, model compound and daminonaphthalene stacking station respectively.
Design and synthesis of the walker

The retrosynthesis of the first generation walker is outlined below (figure 9). The reasonably short C4 side-chains should keep the aromatic moieties close together, but still offer enough flexibility to facilitate a good stacking orientation.

The total synthesis of the walker is described next. Side chain 4 wasn’t commercially available and the synthetic route is depicted below (figure 10). This Trt (triphenylmethyl) protected thiol was obtained via two different pathways\(^6\)\(^8\) of which the shorter two-step route is preferred, despite its low yielding LAH reduction. Herein, partial cleavage of the Trt protecting group was observed and seemed to account for the poor yield. Milder reaction conditions (0 °C, or shorter reactions times) could prevent this and afford 4 in better yields.
With the desired thiol foot precursor in hand NDI 5 was synthesised in a one-pot reaction from 4, the commercially available 5-aminovaleric acid and 1,4,5,8-naphthalenetetracarboxylic dianhydride (figure 11).

The two-step reaction was conducted in the microwave\(^9\) and almost exclusively formed the mono-reacted species when the acid was added first. Subsequent addition of 4 afforded NDI 5 in a moderate yield. Formation of unsymmetrical NDIs via conventional methods (reflux several days) is not selective and would yield large quantities of both symmetrical species as side products. Acid 5 was easily converted to hydrazide 6 via a PyBroP (bromotripyrrolidinophosphonium hexafluorophosphate) mediated amide bond formation. Finally, walker precursor 6 was deprotected in a mixture of CH\(_2\)Cl\(_2\) and TFA (1:1 v/v) with Et\(_3\)SiH to give the desired walker (figure 12).
TLC (thin layer chromatography) analysis confirmed the full conversion of the starting material and a yellow solid was isolated. Unfortunately, this solid couldn’t be taken up in any solvent so further analysis was impossible. The amount of collected material corresponded to the theoretical yield, which seemed to indicate that the concurrent Boc (tert-butylcarbonyl) and Trt deprotection of 6 was successful. Due to the insoluble nature of NDIs the outcome was foreseen and an improved design that would significantly enhance the solubility of the walker was quickly proposed. The retrosynthesis of the second generation walker is outlined below (figure 13).

Improved solubility can be achieved by the introduction of a cysteine-functionality as one of the walker’s legs. The tertiary carbon of the cysteine leg inhibits self-stacking of the NDIs to some degree due to steric hindrance and so allow the material to be solubilised better. The
commercially available cysteine methyl ester was readily converted to Trt protected species 7 in the first step of the synthetic pathway (figure 14).  

Figure 14: Synthesis of cysteine incorporated NDI.

NDI 8 was acquired in a two-step procedure from the commercially available 1,4,5,8-naphthalentetracarboxylic dianhydride, 5-aminovaleric acid and cysteine methyl ester 7. An amide bond formation furnished 9 in a good yield if PyBroP was added at 0 °C. In the concluding step 9 was deprotected under the same conditions as described before and the now soluble walker 10 was acquired in a quantitative yield (figure 15). Rapid decomposition of the walker was observed by \(^1\)H NMR, hence the compound had to be stored cooled and under an inert atmosphere.

Figure 15: Hydrazide formation and subsequent deprotection.

Before NDI 10 could be coupled to the thiol foothold and connected to the track, the hydrazide functionality had to be reprotected. A typical Boc protection was attempted but
resulted in the decomposition of the already unstable starting material. To circumvent the entire stability issue a selective Trt deprotection of 9 was carried out in a solution of I₂ in MeOH (figure 16). This relatively clean reaction provided disulfide dimer 11, which was promptly formed from the corresponding free thiol under air, in a reasonable yield.

![Figure 16: Hydrazide reprotction.](image)

Disulfide 12 was furnished through thiol-disulfide exchange between 11 and thiol foothold 16 (figure 17).

![Figure 17: Attachement of the walker to the thiol foothold.](image)

Free thiol 16 (synthesis discussed in next section) initially breaks the NDI’s disulfide bond. By performing the reaction under oxidative conditions (air) eventually only a mixture of disulfides remained. Because an excess of the foothold was used most of dimer 11 was converted to 12. By reason of its labile nature compound 12 was stored cool and under an inert atmosphere. This assembly will later be attached to the remaining track via a peptide coupling.
Synthesis of the footholds

Thiol foothold 16 was acquired via two different pathways (figure 18).

In route 1 aldehyde foothold precursor 13 was easily formed from the commercially available diethyl (5-hydroxymethyl)isophthalate. Alcohol 13 was then refluxed in neat SOCl₂ and stirred in a mixture of water/acetone to give chloride 14. An S₂N₂ reaction with 14 and triphenylmethanethiol provided Trt protected thiol 15. Route 2 began with conversion of diethyl (5-hydroxymethyl)isophthalate to mesylate 17. A subsequent one-pot-two-step synthesis of 17 with triphenylmethanethiol and LiOH in a mixture of THF and water furnished 15 in a low yield. A lot of starting material and di-acid was retrieved conceivably because the organic and water layer didn’t mix. Since pathway 1 gave higher yields no further notice was given to pathway 2.
Synthesis and functionalization of the hydroquinone stacking station

Amine functionalised hydroquinone stacking station 19 was synthesised from commercially available hydroquinone in a Williamson ether synthesis with chloroacetonitrile, before being reduced to the diamine (figure 19).

The reduction of dinitrile 18 was troublesome at first. Various methods including LAH reduction, hydrogenation (Pd/C) and DIBALH reduction were screened. The first two resulted in the decomposition of the starting material, whereas the last had no effect and left the dinitrile intact. Finally a BH$_3$ reduction provided diamine 19 clean and in a high yield. From there, a Boc protection with one equivalent of Boc$_2$(O) furnished 20 in a moderate yield.
Synthesis of the model compound

As explained earlier the synthesis of a model compound will reveal if the desired macrocycle can be formed. A model resembling the walker on the 1,2-position can easily be constructed by the assembly of the preceding building blocks. The target molecule is illustrated in figure 20.

Figure 20: Disconnections of model 25.

Mono-protected diamine 20 was selectively coupled to foothold 13 with a peptide coupling (figure 21). The presence of the hydroxyl group on foothold 13 might have caused side reactions and product 21 was only acquired in a moderate yield.

Figure 21: Hydroquinone based stacking station 20 is connected to aldehyde foothold precursor 13.

Alcohol 21 is a key intermediate in the synthetic route of the track and can be readily customized to create various domains. The use of 21 as a building block for the track will be described later. Before compound 21 was linked to the walker assembly 12, the alcohol was
oxidized with activated MnO$_2$ to form corresponding aldehyde 22 and the Boc group removed to yield amine 23 (figure 22).

**Figure 22:** Component 23 was connected to walker-foothold assembly 12 to form open model 24.

Amine 23 was generated in a mixture of TFA and CH$_2$Cl$_2$. However, the presence of the aldehyde and amine functionality led to complications under these conditions, as the formation of imine oligomers was unavoidable. As a solution, the crude solid was taken up in THF and the addition of a drop of concentrated aqueous HCl broke up the oligomers promptly. A peptide coupling with walker-foothold assembly 12 gave open model system 24 in a fair yield. Next, 24 was taken up CH$_2$Cl$_2$ and acidified with TFA to not only deprotect the hydrazide group but also to promote hydrazone formation (figure 23).

**Figure 23:** Open model 24 was closed under high dilution (letters correspond to peak assignment in figure 24,25,27).
The ring-closure needed to be performed under high dilution to ensure no dimer- or oligomerisation took place. Hence, the reaction mixture was diluted with CHCl₃, after the acidified mixture had stirred for two hours. The remaining mixture was left to equilibrate overnight and the macrocycle was locked in place, once the somewhat acidic solution was neutralised with a mild base. ¹H NMR analysis confirmed the formation of the model, since it showed the hydrazone protons and also differentiated between the two protons that were attached to the carbon adjacent the hydrazone functionality. The macrocycle was present as both a cis- and trans-isomer due to slow exchange on the ¹H NMR timescale and the ratio was solvent depended (for a stackplot of 24 with 25 see figure 24). To further support this hypothesis it was shown that the peaks coalesced in a VT ¹H NMR (120 °C in DMSO) experiment (figure 25). An EXSY experiment revealed the correlation between the two peaks of the hydrazone protons in particular (figure 26) but also correlation between two sets of NDI protons (figure 27) was noticed. Unfortunately, a suitable sample for single-crystal X-ray diffraction couldn’t be procured.

Figure 24: ¹H NMR spectra of a) 24 600 MHz, Acetone, 295 K, b) 25 600 MHz, Acetone, 295 K. Dotted lines indicate hydrazone NH (green), Hw (blue), Hz (red) and Hp (black) shifts.
Figure 25: VT $^1$H NMR spectra of 25 at a) 600 MHz, DMSO, 295 K, b) 500 MHz, DMSO, 393 K. Dotted lines indicate coalescence of hydrazone NH (green), H$_w$ (blue), H$_z$ (red) and H$_p$ (black).

Figure 26: Partial EXSY (600 MHz, Acetone, 295 K) of 25 showing the hydrazone (NH) signals (green).
Figure 27: Partial EXSY (600 MHz, CD$_2$Cl$_2$, 295 K) of 25 showing the NDI signals $H_w$ in blue ($H_x$ signals are shown in purple).
Synthesis of the track domains

Encouraged by the formation of model system 25 synthesis and assembly of the track domains was carried further. First, hydrolysis or Boc deprotection of key intermediate 21 afforded acid 26 and amine 27 respectively in excellent yields (figure 28). These compounds together make up approximately half of the track and will be linked to the diaminonaphthalene stacking station. The synthesis of this station will be discussed now.

Figure 28: Synthesis of track domain 26 and 27.
Synthesis of the diaminonaphthalene stacking station

Commercially available 1,5-diaminonaphthalene was functionalised with Boc protected ethyleneamine chains via reductive amination to yield ethyleneamine 28. A following Boc deprotection afforded free amine 29 (figure 29).

![Chemical structure](image)

Figure 29: Synthesis of diaminonaphthalene based stacking station 29.

The electron rich 1,5-diaminonaphthalene is a very delicate substrate. Much effort was made to introduce the ethyleneamine functionalities. The compound is very sensitive to heating and 28 was exclusively obtained through reductive amination. The reactions only worked well on the scale described in the protocol and required the commercially available aldehyde reagent to be of high purity. The products 28 and 29 or any other molecule that contained diaminonaphthalene had to be kept out of light to prevent decomposition.
Track assembly and formation of the molecular machine

It wasn't possible to mono-functionalise the central stacking station by attaching only one chain or to desymmetrise 28 and 29 by selective Boc (de)protection. When 29 was stirred with either one equivalent of 26 or 15 the amine became more reactive once coupled and therefore always reacted twice to give mixture of symmetrical product and unreacted starting material. Alternatively, 29 was connected to the footholds in a three-component reaction as the only option to yield unsymmetrical product 30 (figure 30).

![Figure 30: Three-component peptide coupling.](image)

The reaction showed many side-products by TLC and was low yielding at first. A cumbersome work-up significantly diminished the yield due to precipitation of the product as a gel and consequently the reaction mixture was columned directly without a work-up. Optimised flash column chromatography eventually afforded the product in a yield range of 25-50%.

Before the next domain could be attached ester 30 was hydrolysed (figure 31). This saponification afforded the corresponding acid 31, albeit not in quantitative yield. Some side-products were observed and this was perhaps caused by the instability of the diaminonaphthalene functionality. Another peptide coupling furnished full track 32 in a reasonable yield.
The introduction of a disulfide placeholder and the deprotection of the primary amine are the only remaining steps before the walker can be attached to the track. The placeholder functions as the protecting group of the thiol foothold but can under basic conditions participate in disulfide exchange and allow the walker to step on that foothold. Cleavage of the Trt group will allow the introduction of the placeholder. In a first attempt the track was stirred in CH₂Cl₂/TFA (1:1) for a few hours, which would cleave both the Boc and Trt protecting groups. Sadly, after concentrating the solution on the rotary evaporator at 40 °C, ³¹H NMR analysis showed complete decomposition of the material. The selective removal of the Trt group with I₂ in MeOH, which was the preferred method in the synthesis of the walker unit, generated many by-products and no trace of product was isolated (figure 32).
When the initial method (CH$_2$Cl$_2$/TFA) was repeated and the solvent removed at room temperature *i.e.* not in a warm water bath, the desired free thiol 33 was obtained in quantitative yield (figure 33).

![Figure 32: Selective Trt cleavage.](image)

The track became quite temperature sensitive as soon as the Trt group was cleaved. Consequently, the track and compounds further down the route were not exposed to temperatures above 30 °C. The placeholder attachment was carried out in DMF with an excess of 2-mercaptoethanol. The solution was subsequently basified with a small amount of Et$_3$N and exposed to air (figure 34). The oxygen and base allowed disulfides to form and after

![Figure 33: One-pot Boc and Trt deprotection.](image)
stirring the mixture for three hours it was concentrated by blowing air over it. The excess placeholder was washed out and disulfide 34 was acquired in excellent yield.

![Chemical structure](image)

**Figure 34:** Placeholder introduction via disulfide formation.

With the complete track in hand a last peptide coupling will connect walker-foothold assembly 12 and nearly complete the synthesis of the molecular machine. Track 34 and assembly 12 were joined through amide bond formation to afford 35 in a relatively low yield (figure 35).
Figure 35: Walker-track assembly.

From here only two steps remained to finalise the small molecule walker machine. The first step involved a mild oxidation of the benzylic alcohols to provide the corresponding aldehyde footholds, the second the deprotection of the hydrazide moiety on the walker. The acidic conditions used in the deprotection step could further encourage the formation of the hydrazone and yield the end product.

Disulfide 35 was taken up in a small amount of DMF and thirty equivalents of activated MnO$_2$ were added (figure 36).
Figure 36: Oxidation of benzylic alcohols to generate the aldehyde footholds.

After the mixture had stirred for a few hours it was evident that some by-products had already formed. When the mixture was stirred for longer periods all the starting material was consumed, but no product could be detected. The starting material clearly wasn’t stable under these conditions. Various solvent systems, that included DMF/acetone DMF/CH₂Cl₂ and CH₂Cl₂/MeOH, were investigated but gave similar results. To determine the cause for these discouraging results additional experiments were carried out on the less complex diaminonaphthalene containing alcohol 30. Again, the starting material was decomposing quickly. The electron rich diaminonaphthalene was most likely getting oxidised instead and generated many fragments in the process. Other oxidizing agents like Dess-Martin didn’t work or involved the use of base, a sulfide or generate a sulfide, which wouldn’t be compatible with the walker’s disulfide bonds.
A new strategy

A new approach, wherein the aldehydes are formed before the diaminonaphthalene moiety is introduced, had to be considered. The benzylic alcohols can be oxidised in an earlier stage of the synthesis and then later connected to the diaminonaphthalene station. The chemistry of this new approach will not differ significantly from the conventional route as the elongation of the track with peptide couplings in the presence of aldehydes or acetal protected aldehydes should work.

We've shown that alcohol 21 can easily be transformed to corresponding aldehyde 22. A subsequent protection with ethylene glycol to form cyclic acetal 36 could avoid unnecessary side reactions further in the synthesis (figure 37).

![Chemical structure of aldehydes and acetal formation](image)

Figure 37: Formation of cyclic acetal.

Interestingly, the procedure yielded a significant amount of the non-cyclic diol. The reaction mixture was diluted by a factor ten and left to stir overnight to convert this to the cyclic acetal. Afterwards, acetal 36 was hydrolysed to acid analogue 37 and utilised in the three-component peptide coupling (figure 38).
Figure 38: Three-component peptide coupling with protected aldehyde 37.

The saponification of resulting ester 38 generated acid 39. These methods were established in the former route and provided the compounds in similar yields. Regrettably, the ethylene glycol group couldn’t be removed from either 38 or 39 under acidic conditions (TFA or HCl) at rt and the molecule and fell apart upon heating (figure 39).

Figure 39: Hydrolysis and cyclic acetal deprotection.
As a result, aldehyde 22 was used unprotected and hydrolysed to acid 40 in quantitative yield. The three-component peptide coupling afforded trackpart 41 in similar yield as the alcohol and protected aldehyde counterpart 30 and 38 (figure 40).

![Diagram showing reaction pathways for aldehyde 22 to acid 40, and peptide coupling to afford trackpart 41.]

Figure 40: Three-component peptide coupling with unprotected aldehyde 40.

The saponification of ester 41 with LiOH in a mixture of THF/water led to an unexpected destruction (as indicated by $^1$H NMR) of the material during the reaction. As a precaution the aldehyde group was protected with trimethyl orthoformate in dry MeOH to give dimethyl acetal 42 (figure 41). Now hydrolysis of the ester didn’t lead to decomposition, still purification by flash column chromatography resulted in a substantial loss of material and was therefore avoided. The crude of product 43 was taken forward.
Figure 41: Acetal protection and hydrolysis.

Note that the dimethyl acetal was cleaved during the acidic work-up of the saponification. Despite the undesired cleavage it was still more practical than the formerly used cyclic acetal, as the material can always be reprotected if necessary. Finally, aforementioned fragment 23 was attached to acid 43 by peptide coupling to furnish track 44 (figure 42).

Figure 42: Three-component peptide coupling to yield the dialdehyde track.
The new track that now contained two aldehyde functionalities was subjected to the deprotection conditions for track 32 to obtain the free thiol and amine. Upon analyzing the product, it became evident that former conditions couldn’t be applied to the new track as degradation of the material was observed. Et₃SiH, which is used as a scavenger for the Trt cation, was not compatible with the aldehyde component. An attempt to protect the track in situ under dry conditions as an acetal (dimethyl and ethylene), before the mixture was acidified enough to deprotect the Trt and Boc group, failed as well. Deprotection of the Trt group without the addition of a scavenger caused incomplete conversion and some formation of side-products. Since the Boc group on the amine did get cleaved and made the compound very polar it couldn’t be purified on standard and reverse phase preparative TLC plates or reverse phase HPLC due to solubility issues. Alcohols can also act as scavengers, although much less efficiently than Et₃SiH because they bind reversibly to the Trt cation. The replacement of Et₃SiH with 10 v/v% MeOH resulted in a much cleaner reaction but still left a substantial amount of protected thiol. To achieve full deprotection 2-(Pyridin-2-yldisulfanyl)ethanol (45) was added to the reaction mixture. These disulfides are generally known to react with free thiols under mild acidic or basic conditions to form a new disulfide and 2-pyridinethione. The free thiol that is formed during the deprotection could react with this disulfide and the reaction would be driven to completion because there wouldn’t be any free thiol left for the cation to reattach to. By choosing the right disulfide, in this case 45, the desired placeholder would be introduced at the same time (figure 43).

Figure 43: Only partial cleavage of Trt was achieved.
At first two equivalents of the disulfide were added to the reaction mixture. This had no noticeable effect therefore the amount was increased to a hundred equivalents. This method was successful and cleaved some of the Trt group. However, the resulting free thiol didn’t react with 45 to form desired disulfide 46 and the free thiol equivalent was not obtained pure, because there was still a good amount of starting material left. The role of 45 remains unknown but due to the large excess used and the presence of an alcohol group it might have acted as a reversible scavenger for the Trt cation. The crude material was taken through to see if the placeholder introduction was possible through another approach (neutral or basic conditions). Free thiol 47 (structure in figure 44, reaction not shown) was taken up in DMF and two equivalents of 45 were added. The reaction mixture was left to stir for two days. Unfortunately, only a trace of the wanted compound was found and most of the starting material retrieved. The experiment was conducted again under various conditions, which included the addition of a hundred equivalents of 45 in MeOH or DMF/benzene (1:1) and a neat reaction in 45 with a drop of MeOH or DMF to solubilize the material. There appeared to be a small improvement in conversion but still mostly starting material was retrieved. As a consequence, the aforesaid procedure to introduce the disulfide placeholder on alcohol track 33, where 2-mercaptoethanol and Et₃N are added to the solution in DMF and stirred under air, was attempted. The ¹H NMR of the reaction product indicated that the material had decomposed under these conditions. The procedure was repeated without the addition of Et₃N and now only starting material was retrieved. One of the samples that was subjected to these Et₃N-free conditions did however show some conversion to disulfide 46. The sample was analysed and showed contamination of disulfide 45 that was used in the preceding step. This suggested that the presence of this impurity aided the conversion to the desired disulfide (figure 44).
More reactions were carried out and addition of several portions of 45 to the reaction mixture resulted in near complete conversion of the unprotected starting material. Perhaps 45 acted as a base like Et$_3$N without causing the side-reactions. To obtain more of the material and to get a pure sample for analysis the last two reactions were repeated on a larger scale but several problems were encountered. It appeared that some of the deprotected track started to form imine oligomers, which was caused by the presence of the free amine in combination with the aldehydes in the molecule. These problems were observed in both the deprotection and placeholder introduction step. The oligomers were sometimes partially converted back to the free amine when the compound was dissolved in a mixture of THF and aqueous HCl. However, this didn’t always work and a new approach had to be investigated. Imine oligomer formation can be prevented by the addition of an excess of $n$-butylamine that competes with the free amine of the track. This strategy worked fairly well but didn’t solve all the troubles, since complete cleavage of the Trt protecting group was too challenging to achieve because of the lack of a good Trt cation scavenger. In order to reach full conversion to the free thiol the reaction had to be resubmitted so often that it ended in product degradation. Luckily, a solution was found. As described before (figure 43) an attempt to utilise 45 in order to protect the liberated thiol in situ failed, since the generated thiol didn’t react with 45 to yield the corresponding protected thiol 46 and 2-pyridinethione. Yet, when the much more reactive 2,2’-dipyridyl disulfide was used, the
reaction was driven to completion as the free thiol readily reacted with this disulfide dimer \textit{in situ} to give 48 (figure 45).

In the end addition of 2,2'-dipyridyl disulfide and n-butylamine to the reaction mixture allowed the full deprotection of Trt protected thiol in the presence of aldehydes without the use of a traditional scavenger like Et$_3$SiH and amine deprotection with no formation of imine oligomers. A minor concern was that this new method provided the track with the 2-mercaptopyridine and not the preferred 2-mercaptoethanol placeholder. Nevertheless, the 2-mercaptopyridine placeholder shouldn’t really interfere with the remaining synthesis or the machine’s operation.
With the placeholder introduced a concluding peptide coupling connected the walker to the track (figure 46).

A small amount of product 49 was collected after purification by preparative thin layer chromatography (7.5% EtOH in CH₂Cl₂). The following one-pot Boc deprotection and macrocyclisation with TFA/CH₂Cl₂ to yield the final product was conducted under similar conditions as for the model system. Again, the mixture was stirred at high dilution because more concentrated conditions can favour the formation of undesired track-track oligomers. Surprisingly, this time the ring-closure didn’t occur, although the Boc group was cleaved. The reaction was also not that clean and a milder method to cleave the Boc group was found.

Figure 46: Attachment of the walker and Boc deprotection of the walker’s hydrazide foot.
Compound 49 was dissolved in a mixture of 1.25 M HCl MeOH and CH₂Cl₂ (1:1) and stirred at high dilution (0.1 mM) for two days (figure 47). After the mixture was neutralised with a 1 M aqueous NaHCO₃ solution, sadly only deprotected product 50 and not the wanted macrocycle was isolated. As an alternative, the HCl salt of hydrazide 50 was precipitated with Et₂O/heptane (1:1) after 49 had stirred in 1.25 M HCl MeOH and CH₂Cl₂ (1:1). The advantage of this method is that the salt can be stirred under neutral conditions and allows more flexibility in the choice of solvents. The resulting precipitate was dissolved in CH₂Cl₂ or DMSO, diluted to 0.1 mM and stirred overnight, but once more no ring-closure was seen. Another solvent system to run the deprotection in was screened when Boc protected 49 was taken up in a solution of THF/1 M aqueous HCl (4:1) first, then diluted with THF to 0.1 mM and stirred overnight. No product was acquired and the method merely resulted in degradation of the material. Because the molecular machine couldn’t be formed directly, neutral intermediate 50 was taken up in a mixture of CH₂Cl₂/MeOH (8:2) and stirred for two days at high dilution (0.1 mM) with a drop of acetic acid to promote intramolecular hydrazone formation (figure 47).

Analysis of the sample indicated that no hydrazone formation had taken place and the starting material was retrieved. Also stirring hydrazide 50 with a drop of acetic acid in CHCl₃/MeOH (9:1), DMF or DMF/water gave no reaction. The addition of a few drops of 1 M
aqueous HCL to a solution of free hydrazide in THF only resulted in acetal hydrolysis. To be able to follow the ring-closure by $^1$H NMR the free hydrazide was dissolved in deuterated DMSO and a drop of TFA was added. Here too acetal hydrolysis and slow degradation over a period of a few weeks was detected. Heating the compound to 60 °C for two hours in CHCl$_3$ with a drop of acetic acid resulted in retrieval of starting material as well. Perhaps the best results were found when the free hydrazide was taken up in CH$_2$Cl$_2$ or a mixture of CH$_2$Cl$_2$/toluene with a catalytic amount of pTSA (para-toluenesulfonic acid). The hydrazone proton of what might be a trace amount of product was seen on $^1$H NMR but mainly the corresponding unclosed aldehyde was isolated.
Conclusion and discussion

Despite the successful formation of model macrocycle 25, the attachment of the walker’s second foot to the extended track couldn’t be accomplished. Since the marocyclisation from aldehyde 24 to hydrazone 25 worked so well, not being able to do the same with a similar analogue was truly disappointing. The many functionalities and size of these molecules required an elaborate synthetic route and made it nearly impossible to acquire enough material to screen for hydrazone formation conditions. The route, starting on a multigram scale, had been repeated multiple times to obtain only a few milligrams of 49. A larger scale-up was not possible due to restrictions in the experimental procedure of diaminonaphthalene 28 and 29. Furthermore, problems were encountered with the peptide couplings, deprotections and saponifications. Generally, these transformations were hard to reproduce and resulted in variable yields, sensitive products, decomposition or compounds that were challenging to purify. Most obstacles were found in molecules that contained the diaminonaphthalene functionality. Those had to be kept out of the light at all times, which complicated the synthesis even more, and likely contributed to the irreproducibility of the chemistry. Another notable issue was encountered when a minor change was made in the synthetic route. Unable to oxidise the benzylic alcohols in the second last step of the original synthetic route, the decision was made to perform the oxidation several steps before. Unfortunately, solving one problem resulted in the emergence of another. Working with free or protected aldehydes during the Trt/Boc deprotections or peptide couplings was not desirable, however we were able to work around those difficulties to some degree. In order to address the problems with the diaminonaphthalene functionality the replacement of this station with something more practical could be considered. Another solution to avoid a number of issues is to think of an alternative approach to assemble the molecular machine. For example, the macrocycle can be formed first and then coupled to the remaining track. Alternatively, the macrocycle could be connected to the central diaminonaphthalene station in a one-pot-three component reaction together with a fully functionalised hydroquinone station, much like the peptide couplings that are described before. This method advantageously allows the screening of several alternative central stations. The reason why such approaches haven’t been considered earlier is that they would extend the synthetic route by several more steps. Finally, the macrocycle could also be formed through disulfide formation if the walker had been attached to the track as a hydrazone. This would have required a drastic change in the synthetic route and was therefore avoided. Nevertheless, the inability to close the macrocycle through hydrazone formation might also suggest that
intramolecular folding or other unfavourable conformations prevented the hydrazide foot from reaching the aldehyde foothold. In that case, even if the molecular machine was constructed, the walker might have never stepped the way it was intended to and thus the entire design needs to be reconsidered.
Synthetic procedures and characterization details

\textit{N-(4-bromobutyl)phthalimide (1)}

Phthalamide (1.00 g, 6.80 mmol), K$_2$CO$_3$ (4.70 g, 34.0 mmol) and benzyltriethylammonium chloride (126 mg, 0.68 mmol) were suspended in acetone (20 mL) and 1,4-dibromobutane (7.34 g, 34.0 mmol) was added. The resulting mixture was stirred at rt overnight. Upon completion, the reaction mixture was concentrated under reduced pressure and the residue was taken up in water (15 mL). After the aqueous layer was extracted with CH$_2$Cl$_2$ (3 × 20 mL), the organic layers were combined, dried (MgSO$_4$), filtered and concentrated under reduced pressure to yield 1 (1.42 g, 74% yield) as white solid, which was carried forward without further purification. Analyses in agreement with the literature\textsuperscript{6}

\textit{N-(4-(Tritylmercapto))butyl)phthalimide (2)}

Triphenylmethanethiol (486 mg, 1.76 mmol) was dissolved in dry DMF (5 mL) and NaH (60%, 77 mg, 1.94 mmol) was carefully added at 0 °C. The reaction mixture was stirred at 0 °C for 30 min. Then a solution of \textit{N-(4-bromobutyl)phthalimide} 1 (547 mg, 1.94 mmol) in dry DMF (5 mL) was added dropwise and the reaction mixture was stirred at rt overnight. Upon completion, the reaction mixture was quenched with water (10 mL) and extracted with EtOAc (3 × 20 mL). The organic layers were combined, dried (MgSO$_4$), filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (PET/EtOAc, 100:0→70:30) to afford 2 (391 mg, 85% yield) as a white solid. Analyses in agreement with the literature\textsuperscript{7}

\textit{4-(tritylmercapto)butanenitrile (3)}

An aqueous solution of NaOH (3 N, 0.48 mL, 1.45 mmol) was added to a solution of triphenylmethanethiol (400 mg, 1.45 mmol) and 4-bromobutyronitrile (144 µL, 1.45 mmol) in EtOH/PhMe (4 mL, 3:1 v/v). After 10 min at 50 °C the mixture was quenched, with saturated aqueous NH$_4$Cl (5 mL) and the product was extracted with CH$_2$Cl$_2$ (3 × 10, mL). The organic layers were combined, dried (MgSO$_4$), filtered and concentrated under reduced pressure. The residue was purified by column chromatography (PET/EtOAc, 1:0→3:1) to afford 3, (455 mg, 92% yield) as a white solid. R$_f$ 0.50 (PET/EtOAc, 8:2). $^1$H NMR (400 MHz, CDCl$_3$): δ 7.44–7.39 (m, 6H, H$_d$), 7.33–7.27 (m, 6H, H$_d$), 7.25–7.20 (m, 3H, H$_d$), 2.33 (t, J = 7.1 Hz, 2H, H$_a$ or c), 2.26 (t, J = 7.2 Hz, 2H, H$_a$ or c), 1.60 (quint, J = 7.1 Hz, 2H, H$_b$).
4-(Tritylmercapto)butylamine (4)

From 3: A suspension of LiAlH₄ (108 mg, 2.86 mmol) in dry THF (10 mL) was cooled to 0 °C and a solution of 3 (313 mg, 1.06 mmol) in dry THF (10 mL) was added dropwise. The reaction mixture was stirred at rt for 30 min. After quenching with water (108 μL) the mixture was stirred for 30 min and 15% aqueous NaOH (108 μL) was added. The mixture was stirred for an additional 30 min and another portion of water (324 μL) was added. After stirring for 45 min the suspension was dried (Na₂SO₄), filtered and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (CH₂Cl₂/MeOH/Et₃N, 98:0:2→88:10:2) to afford 4 (148 mg, 37% yield) as a colourless oil.

From 2: Hydrazine monohydrate (273 μL, 5.63 mmol) was added to a suspension of 2 in EtOH/n-BuOH (30 mL, 5:1 v/v). The reaction mixture was stirred at 50 °C for 2 h. Subsequently, the mixture was allowed to cool to rt and the resulting precipitate was filtered off. The filtrate was collected and concentrated under reduced pressure. The residue was purified by flash column chromatography (CH₂Cl₂/MeOH/Et₃N, 98:0:2→90:8:2) to afford 4 (391 mg, 85% yield) as a colourless oil.

Analyses in agreement with the literature.⁷

5

To a pressure-tight microwave vial containing dry DMF (6.0 mL) were added 1,4,5,8-naphthalenetetracarboxylic dianhydride (114 mg, 0.426 mmol), 5-aminovaleric acid (50 mg, 0.426 mmol), and Et₃N (119 μL, 0.426 mmol). The suspension was sonicated until the mixture became homogeneous. After the reaction mixture was heated for 5 min at 140 °C under microwave irradiation, 4 (148 mg, 0.426 mmol) and another equivalent of Et₃N (119 μL, 0.426 mmol) were added. The reaction mixture was sonicated for 20 min and heated for an additional 5 min at 140 °C under microwave irradiation. The solvent was evaporated and the crude mixture was taken up in CHCl₃. The suspension was filtered and the filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography (CH₂Cl₂/MeOH, 100:0→97:3) to afford 5 (160 mg, 54% yield) as a brown solid. Rₛ 0.55 (CH₂Cl₂/MeOH, 9:1). Mp 151 °C. ¹H NMR (400 MHz, CDCl₃): δ 8.75 (d, J = 7.8 Hz, 2H, H₁ or r₁), 8.72 (d, J = 7.8 Hz, 2H, H₁ or r₁), 7.42 (d, J = 7.7 Hz, 6H, H₆), 7.27 (dd, J = 7.6, 7.6 Hz, 6H, H₆), 7.22–7.15 (m, 3H, H₃), 4.24 (t, J = 6.4 Hz, 2H, H₉), 4.10 (t, J = 7.1 Hz, 2H, H₈), 2.47 (t, J = 6.7 Hz, 2H, H₇), 2.23 (t, J = 7.1 Hz, 2H, H₆), 1.92–1.67 (m, 6H, H₉, H₉), 1.56–1.46 (m, 2H, H₈). ¹³C
NMR (126 MHz, CDCl₃): δ 178.97, 162.84, 162.72, 144.94, 131.05, 131.00, 129.62, 129.06, 128.25, 127.88, 126.64, 126.59, 126.51, 66.52, 40.49, 40.39, 33.62, 31.70, 27.57, 27.50, 26.16, 22.14. LRMS (ESI) m/z calc for C₆₈H₁₇N₄O₂S₂ [2M–H]⁺: 1391.45, found 1391.00. HRMS (ESI) m/z calc for C₄₂H₆₀N₃O₆S [M+NH₄]⁺: 714.2632, found 714.2619.

6

PyBroP (65 mg, 0.14 mmol) and tert-butyl carbazate (18 mg, 0.14 mmol) were added to a solution of 5 (65 mg, 0.093 mmol) in CH₂Cl₂ (0.5 mL). Subsequently, DIPEA was added and the solution was stirred at rt for 2 h under a nitrogen atmosphere. The reaction mixture was then diluted with CH₂Cl₂ (15 mL), washed with 1 M aqueous HCl (3 × 5 mL) and brine (5 mL), dried (MgSO₄) and concentrated under reduced pressure. The residue was purified by flash column chromatography (CH₂Cl₂/Acetone, 100:0→75:25) to afford 6 (63 mg, 84% yield) as a yellow oil. Rₜ 0.55 (CH₂Cl₂/MeOH, 9:1). ¹H NMR (400 MHz, CDCl₃): δ 8.73 (d, J = 7.7 Hz, 2H, H₁or₁), 8.71 (d, J = 7.9 Hz, 2H, H₁or₁), 7.93 (br s, 1H, NH), 7.43–7.38 (m, 6H, H₆), 7.30–7.24 (m, 6H, H₆), 7.21–7.16 (m, 3H, H₃), 6.76 (br s, 1H, NH), 4.22 (t, J = 6.6 Hz, 2H, H₃), 4.09 (t, J = 7.4 Hz, 2H, H₃), 2.37 (t, J = 6.6 Hz, 2H, H₃), 2.21 (t, J = 7.3 Hz, 2H, H₃), 1.88–1.78 (m, 4H, H₇–₉), 1.76–1.67 (m, 2H, H₅), 1.53–1.46 (m, 2H, H₇), 1.44 (s, 9H, H₉). ¹³C NMR (126 MHz, CDCl₃): δ 172.35, 162.97, 162.76, 155.62, 144.98, 131.12, 131.03, 129.66, 128.04, 128.00, 127.93, 126.67, 126.64, 126.56, 81.80, 66.56, 40.54, 40.14, 33.41, 31.73, 28.23, 27.61, 27.39, 26.20, 22.69. LRMS (ESI) m/z calc for C₄₂H₆₀N₃O₆S [M+NH₄]⁺: 711.26, found 711.53.

Methyl S-trityl-L-cysteinate TFA salt (7)

Cysteine methyl ester (500 mg, 2.92 mmol) and triphenyl methanol (758 mg, 2.92 mmol) were dissolved in TFA (5 mL). The red solution was stirred for one hour, after which the volatiles were removed under reduced pressure. The residue was taken up in Et₂O and upon addition of hexane precipitated 7 (1.10 g, 91 %) as a white solid. Analyses in agreement with the literature.¹⁰

8

To a pressure-tight microwave vial containing dry DMF (30 mL) were added 1,4,5,8-naphthalenetetracarboxylic dianhydride (614 mg, 2.29 mmol), 5-aminovaleric acid (268 mg, 2.29 mmol), and
Et₃N (960 μL, 6.87 mmol). The suspension was sonicated until the mixture became homogeneous. After the reaction mixture was heated for 5 min at 140 °C under microwave irradiation, 7 (1126 mg, 2.29 mmol) and another portion of Et₃N (960 μL, 6.87 mmol) were added. The reaction mixture was sonicated for 20 min and heated for an additional 5 min at 140 °C under microwave irradiation. The solvent was evaporated and the crude oil was sonicated in 1 M aqueous HCl. The yellow precipitate was isolated by filtration and taken up in CHCl₃ (100 mL). The resulting suspension was filtered and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (CH₂Cl₂/MeOH, 100:0→97:3) to afford 8 (899 mg, 54% yield) as a yellow solid. Rf 0.24 (CH₂Cl₂/MeOH, 95:5). Mp 95 °C. [α]D²⁰°=4.1 (c 0.67, CH₂Cl₂). ¹H NMR (600 MHz, CDCl₃): δ 8.77 (d, J = 7.6 Hz, 2H, H₁,α'), 8.72 (d, J = 7.6 Hz, 2H, H₁,α'), 7.33–7.32 (m, 6H, Hₖ), 7.20–7.17 (m, 6H, Hₖ), 7.15–7.13 (m, 3H, Hᵢ), 5.55 (dd, J = 10.1, 5.0 Hz, 1H, Hₑ), 4.24 (t, J = 7.2 Hz, 2H, Hₒ), 3.64 (s, 3H, Hₘ), 3.24 (dd, J = 13.5, 5.1 Hz, 1H, Hᵣ), 3.21 (dd, J = 13.5, 10.2 Hz, 1H, Hᵣ), 2.45 (t, J = 7.3 Hz, 2H, Hₒ), 1.85–1.76 (m, 4H, Hₐ,b). ¹³C NMR (151 MHz, CDCl₃): δ 178.65, 168.78, 162.91, 162.32, 144.41, 131.66, 131.13, 130.12, 129.71, 128.04, 127.08, 126.89, 126.83, 126.35, 67.56, 53.10, 52.94, 40.49, 33.56, 30.63, 27.56, 22.18. LRMS (ESI) m/z calc for C₃₆H₇₂N₂O₁₂S₂: 715.40, found 715.08 (100). C₂₄H₁₇N₂O₄S [M−H]−: 725.20, found 725.58 (92). HRMS (ESI) m/z calc for C₃₄H₆₅N₂O₈SNa [M+Na]^+: 749.1934, found: 749.1923.

9

PyBroP (0.56 g, 1.2 mmol), tert-butyl carbazate (0.16 g, 1.2 mmol) and 8 (0.58 g, 0.80 mmol) were dissolved in CH₂Cl₂ (3 mL) at 0 °C. After addition of DIPEA (0.42 mL, 2.4 mmol), the solution was left to warm up to rt and stirred for 10 min. Subsequently, the solvent was removed under reduced pressure. The crude oil was taken up in CHCl₃ (25 mL) and washed with 1 M aqueous HCl (20 mL) and brine (20 mL). The organic layers were dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (PhMe/EtOAc, 1:0→1:2) and afforded 9 (0.57 g, 85%) as a yellow solid. Rf 0.28 (CH₂Cl₂/MeOH, 95:5). Mp 153 °C. [α]D²⁰°=−1.5 (c 0.54, CH₂Cl₂). ¹H NMR (600 MHz, CDCl₃): δ 8.76 (d, J = 7.5 Hz, 2H, H₁,α), 8.71 (d, J = 7.5 Hz, 2H, H₁,α'), 7.49 (br s, 1H, NH), 7.33 (d J = 8.0 Hz, 6H, Hₖ), 7.20–7.13 (m, 9H, Hₖ), 6.48 (s, 1H, NH), 5.55 (dd, J = 10.1, 4.8 Hz, 1H, Hₑ), 4.24 (t, J = 6.9 Hz, 2H, Hₒ), 3.64 (s, 3H, Hₘ), 3.24 (dd, J = 13.5, 4.9 Hz, 1H, Hᵣ), 3.20 (dd, J = 13.5, 10.3 Hz, 1H, Hᵣ), 2.35 (t, J = 7.6 Hz, 2H, Hₒ), 1.86–1.80 (m, 4H, Hₐ,b), 1.43 (s, 9H, Hₐ). ¹³C NMR (151 MHz, CDCl₃) δ 171.05, 167.61, 161.84, 161.15, 154.28, 143.25, 130.48, 130.00, 128.96,
Subsequently, the resulting filtrate was concentrated under reduced pressure. The red mixture was diluted with CHCl₃ and changed to a red.

11

A round-bottom flask charged with 9 (284 mg, 0.338 mmol) and CH₂Cl₂ (1 mL), MeOH (9 mL) and I₂ (857 mg, 2.97 mmol) were added. After 1.5 h of stirring, saturated aqueous Na₂SO₃ (15 mL) was added and the resulting white slurry was extracted with CHCl₃ (3 × 50 mL). The organic layers were combined, dried (MgSO₄) and concentrated under reduced pressure. The crude solid was then taken up in a small amount of CHCl₃ and left to dry under air. The remaining solid was washed CH₂Cl₂ (3 × 15 mL) to afford dimer 11 (135 mg, 67% yield) as a yellow solid. Rf 0.21 (CH₂Cl₂/MeOH, 95:5). Mp 213 °C. [α]D²⁰ = −7.0 (c 0.58, DMF).

1H NMR (600 MHz, DMSO, 343K): δ 9.30 (s, 1H, NH), 8.85–8.45 (m, 4H, Hₖ₋₋), 8.36 (br s, 1H, NH), 5.95 (dd, J = 8.4, 5.5 Hz, 1H, Hₖ), 4.11 (t, J = 7.0 Hz, 2H, H₄), 3.68–3.64 (m, 4H, H₃₋₋), 3.42 (dd, J = 14.5, 8.6 Hz, 1H, H₃), 2.14 (t, J = 7.3 Hz, 2H, H₃), 1.73 (quint, J = 7.4 Hz, 2H, H₄), 1.63 (quint, J = 7.4 Hz, 2H, H₂), 1.36 (s, 9H, H₂). 13C NMR (151 MHz, DMSO): δ 171.57, 168.69, 162.57, 162.37, 155.28, 131.44, 130.50, 127.17, 126.39, 126.18, 125.04, 78.93, 52.72, 52.58, 40.06, 36.11, 32.91, 28.05, 27.20, 22.65. LRMS (ESI) measured isotopic distribution for C₅₆H₉₉N₁₀O₂₈S₂Na [M+Na]+: 1217.17 (100), 1218.17 (60), 1219.08 (33), 1220.08 (13). Calculated: 1217.32 (100), 1218.32 (66), 1219.32 (34), 1220.32 (13).

12

Dimer 11 (296 mg, 0.248 mmol) and thiol 16 (179 mg, 0.744 mmol) were dissolved in DMF (3 mL) and Et₃N (138 µL, 0.992 mmol) was added. The mixture turned dark brown upon addition of Et₃N and changed to a red-brown colour over time. After stirring for 3 h under air the reaction mixture was diluted with CHCl₃ (25 mL), washed with water (1 × 10 mL) and concentrated under reduced pressure. The crude solid was suspended in CHCl₃ (10 mL) and filtered. Subsequently, the resulting filtrate was concentrated under reduced pressure and purified by flash column chromatography (CH₂Cl₂/PhMe/MeOH, 1:1:0→2:2:1) to afford 12 (312 mg, 75% yield) as a yellow solid. Rf 0.13 (CH₂Cl₂/MeOH, 95:5). Mp 96 °C. [α]D²⁰ +0.2 (c 0.86,
CHCl₃). ¹H NMR (600 MHz, CD₂Cl₂): δ 8.77–8.69 (m, 4H, H₈/H₉), 8.47 (s, 1H, Hᵢ), 8.11 (s, 1H, Hᵢ), 7.99 (s, 1H, Hᵣ), 7.80 (br s, 1H, NH), 6.77 (br s 1H , NH), 6.02 (dd, J = 9.4, 3.8 Hz, 2H, H₆), 4.37 (q, J = 6.9 Hz, 2H, H₇), 4.20 (m, 2H, H₆), 4.01 (dd, J = 13.1 Hz, 1H, Hᵢ), 3.96 (d, J = 13.0 Hz, 1H, Hᵢ), 3.69 (s, 3H, H₄), 3.38 (dd, J = 14.6, 3.5 Hz, 1H, Hᵢ), 3.30 (dd, J = 14.6, 10.0 Hz, 1H, Hᵢ) 2.32 (m, 2H, H₆), 1.85–1.69 (m, 4H, H₆, H₇). ¹³C NMR (151 MHz, CD₂Cl₂) δ 173.05, 169.27, 169.27, 165.87, 163.39, 163.24, 155.96, 138.83, 134.82, 134.69, 132.00, 131.89, 131.39, 130.23, 127.12, 127.39, 127.24, 126.51, 81.89, 61.97, 53.76, 53.28, 42.71, 40.54, 36.65, 33.77, 28.35, 27.78, 23.06, 14.64. LRMS (ESI) m/z calc for C₉H₉N₄O₁₃S₂ [M–H]⁻: 835.20, found: 835.58 (100), C₇₈H₇₉N₄O₂₆S₄ [2M–H]⁻: 1671.40, found: 1670.92 (13). HRMS (ESI) m/z calcd for C₃₉H₄₀N₄NaO₁₃S₂ [M+Na⁺]: 859.1931, found: 859.1925.

**mono-Ethyl (5-hydroxymethyl)isophthalate (13)**

Water (5 mL) and LiOH·H₂O (0.8 g, 20 mmol) were added to a solution of diethyl (5-hydroxymethyl)isophthalate (5.0 g, 20 mmol) in EtOH (15 mL). The resulting mixture was stirred at rt for 2 d and concentrated under reduced pressure. The residue was redissolved in a mixture of water (10 mL) and EtOAc (20 mL). The aqueous layer was acidified with 1 M aqueous HCl (10 mL) and the product was extracted with EtOAc (3 × 50 mL). The organic layers were combined, dried (MgSO₄), filtered and concentrated under reduced pressure. The crude solid was thoroughly washed with warm water and PhMe to yield 13 (3.2 g, 71% yield) as a white solid. Rf 0.22 (CH₂Cl₂/acetone, 1:1). Mp 108 °C. ¹H NMR (500 MHz, MeOD): δ 8.53 (dd, J = 1.6, 1.6 Hz, 1H, H₈), 8.25–8.22 (m, 2H, H₆, H₇), 4.72 (s, 2H, Hᵢ), 4.40 (q, J = 7.1 Hz, 2H, H₆), 1.41 (t, J = 7.1 Hz, 3H, Hᵢ). ¹³C NMR (126 MHz, MeOD): δ 168.74, 167.20, 144.39, 133.16, 132.73, 132.69, 132.24, 130.36, 64.15, 62.49, 14.57. LRMS (ESI) m/z calc for C₁₃H₁₁O₃ [M–H]⁻: 223.1, found 223.1 (100), C₂₂H₂₃O₂₀ [2M–H]⁻: 447.1, found 447.1 (19). HRMS (ESI) m/z calcd for C₁₃H₁₁O₃ [M–H]⁻: 223.0601, found: 223.0604.

**mono-Ethyl (5-chloromethyl)isophthalate (14)**

A drop of DMF was added to a suspension of 13 (800 mg, 3.57 mmol) in neat SOCl₂ (5.0 mL). The reaction mixture was brought to reflux and stirred for 3 h. The solvent was evaporated and the white solid was taken up in a mixture of water (15 mL) and acetone (85 mL). The solution was stirred for 5 h, added to ice water (300 mL) and the resulting white precipitate was filtered to afford S₁₁ (806 mg, 93% yield) as a white solid. Rf 0.45 (CH₂Cl₂/acetone, 1:1). Mp 166 °C. ¹H NMR (600 MHz, Acetone): δ 8.60 (dd, J = 1.6, 1.6 Hz, 1H, H₈), 8.34 (dd, J = 1.7, 1.7 Hz, 1H, Hᵢ), 8.32 (dd, J = 1.7, 1.7 Hz, 1H, Hᵢ), 4.92 (s, 2H, Hᵢ),
4.41 (q, J = 7.1 Hz, 2H, Ht), 1.40 (t, J = 7.1 Hz, 3H, Hj). \(^{13}\)C NMR (151 MHz, Acetone): δ 166.51, 165.66, 140.28, 134.73, 134.31, 132.81, 132.45, 130.89, 62.06, 45.57, 14.54. LRMS (ESI) m/z calc for C\(_{11}\)H\(_{10}\)ClO\(_4\) [M–H]–: 241.0, found 241.1 (100), C\(_{22}\)H\(_{21}\)Cl\(_3\)O\(_8\) [2M–H]–: 483.1, found 483.0 (28). HRMS (ESI) m/z calcld for C\(_{21}\)H\(_{12}\)ClO\(_4\) [M+H]–: 243.0419, found: 243.0419.

**mono-Ethyl (5-(tritylmercaptomethyl))isophthalate (15)**

From 17: Mesylate 17 (8.62 g, 26.1 mmol) and triphenylmethylthiol (7.22 g, 26.1 mmol) were dissolved in THF (30 mL) and a solution of LiOH·H\(_2\)O (1.10 g, 26.1 mmol) in water (10 mL) was added. The reaction mixture was stirred for 1 h at rt. Another portion of LiOH·H\(_2\)O (1.10, 26.1 mmol) was added and the resulting mixture was stirred for 2 d. Upon completion, the mixture was concentrated, taken up in 1 M aqueous HCl (50 mL) and extracted with CH\(_2\)Cl\(_2\) (3 × 100 mL). The organic layers were combined, dried (MgSO\(_4\)) and concentrated under reduced pressure. The residue was purified by flash column chromatography (CH\(_2\)Cl\(_2\)/MeOH, 100:0→95:5) to afford 15 (890 mg, 7% yield) as a white solid.

From 14: Chloride 14 (797 mg, 3.28 mmol) and triphenylmethylthiol (908 mg, 3.28 mmol) were dissolved in DMF (10 mL) and Et\(_3\)N (916 \(\mu\)L, 6.57 mmol) was added. The reaction mixture was stirred at rt overnight. Upon completion, the reaction mixture was concentrated under reduced pressure and the residue was taken up in CH\(_2\)Cl\(_2\) (50 mL). The solution was poured into a separation funnel containing 1 M aqueous HCl (20 mL) and extracted with CH\(_2\)Cl\(_2\) (3 × 50 mL). The organic layers were combined, dried (MgSO\(_4\)), filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (CH\(_2\)Cl\(_2\)/MeOH, 100:0→95:5) to afford 15 (1.43 g, 90% yield) as a white solid. R\(_f\) 0.25 (CH\(_2\)Cl\(_2\)/MeOH, 95:5). Mp 62 °C. \(^1\)H NMR (600 MHz, CDCl\(_3\)): δ 8.58 (s, 1H, H\(_d\)), 8.01–7.98 (m, 2H, H\(_{e,f}\)), 7.47 (d, J = 7.5 Hz, 6H, H\(_a\)), 7.352 (dd, J = 7.6, 7.6 Hz, 6H, H\(_b\)), 7.26–7.22 (m, 3H, H\(_g\)), 4.41 (q, J = 7.1 Hz, 2H, H\(_t\)), 3.45 (s, 2H, H\(_d\)), 1.43 (t, J = 7.1 Hz, 3H, H\(_j\)). \(^{13}\)C NMR (151 MHz, CDCl\(_3\)): δ 170.87, 165.56, 144.45, 138.82, 135.36, 134.89, 131.35, 130.00, 129.86, 129.72, 128.19, 127.04, 67.94, 61.60, 36.39, 14.48. HRMS (ESI) m/z calcld for C\(_{30}\)H\(_{25}\)O\(_4\)S [M–H]–: 481.1474, found: 481.1460.

**Diethyl (5-mercaptomethyl)isophthalate (16)**

Acid 15 (200 mg, 0.414) was taken up in a mixture of CH\(_2\)Cl\(_2\)/TFA (4 mL, 1:1) and upon addition of Et\(_3\)SiH (0.2 mL) the yellow reaction mixture became colourless. The resulting mixture was stirred for another 1.5 h at rt and
concentrated under reduced pressure. The crude solid was washed with hexane to yield 16 (100 mg, quantitative yield) as a white solid. Rf 0.24 (CH₂Cl₂/MeOH, 95:5). Mp 160 °C. ¹H NMR (600 MHz, CDCl₃): δ 8.64 (s, 1H, H₄), 8.26 (m, 2H, H₆, J), 4.43 (q, J = 7.1 Hz, 2H, H₃), 3.85 (d, J = 7.9 Hz, 2H, H₅), 1.87 (t, J = 7.9 Hz, 1H, H₇), 1.43 (t, J = 7.1 Hz, 3H, H₈). ¹³C NMR (151 MHz, CDCl₃): δ 170.57, 165.55, 142.53, 134.47, 133.98, 131.77, 130.16, 130.16, 61.74, 28.51, 14.74. LRMS (ESI) m/z calcd for C₁₁H₁₁O₃SNa [M+Na]⁺: 353.0671, found: 353.0667.

**Diethyl (5-(methylsulfonyloxy)methyl)isophthalate (17)**

MsCl (829 μL, 5.95 mmol) was added to a solution of diethyl 5-(hydroxymethyl) isophthalate (1.00 g, 3.96 mmol) and Et₃N (460 μL, 5.95 mmol) in THF (50 mL) at rt over 10 min. After stirring for 45 min, the reaction mixture was concentrated, taken up in EtOAc (50 mL) and washed with cold 1M aqueous HCl (3 × 50 mL), saturated aqueous NaHCO₃ (50 mL) and brine (50 mL). The organic layer was dried over MgSO₄ and concentrated under reduced pressure. The resulting yellow residue was washed with Et₂O to yield 17 (1.24 g, 95% yield) as a white solid, which was carried forward without further purification. Rf 0.22 (CH₂Cl₂). Mp 76 °C. ¹H NMR (500 MHz, CDCl₃): δ 8.70 (t, J = 1.5 Hz, 1H, H₄), 8.26 (d, J = 1.5 Hz, 2H, H₆), 5.31 (s, 2H, H₇), 4.43 (q, J = 7.1 Hz, 4H, H₈), 3.03 (s, 3H, H₉), 1.43 (t, J = 7.1 Hz, 6H, H₁₀). ¹³C NMR (126 MHz, CDCl₃): δ 165.32, 134.59, 133.66, 131.93, 131.48, 69.71, 61.83, 38.40, 14.46. HRMS (ESI) m/z calcd for C₂₄H₂₃O₄SNa [M+Na]⁺: 479.1, found: 479.0. HRMS (ESI) m/z calcd for C₁₃H₁₂O₃SNa [M+Na]⁺: 353.0671, found: 353.0667.

**1,4-Phenylenedioxydiacetonitrile (18)**

Chloroacetonitrile (17.2 mL, 272 mmol) was added to a solution of hydroquinone (12.0 g, 109 mmol) and K₂CO₃ (37.6 g, 272 mmol) in acetonitrile (400 mL). The reaction mixture was brought to reflux and stirred for 2 d. Upon completion, K₂CO₃ was filtered off and the filtrate was concentrated under reduced pressure. The resulting black solid was taken up in a minimal amount of acetonitrile and poured into cold 1M aqueous HCl (300 mL). After stirring the mixture for 10 min the precipitate was filtered, washed with water and suspended in CHCl₃ (400 mL). The remaining solid was filtered once more and the filtrate was concentrated under reduced pressure. The resulting orange solid was recrystallized from CHCl₃ to yield 18 (13.9 g, 68%) as a yellowish solid. Rf 0.39 (CH₂Cl₂). Mp 90 °C. ¹H NMR (500 MHz, CDCl₃): δ 6.99 (s, 4H, H₆), 4.73 (s, 4H, H₈).
overnight at rt. Upon completion, the mixture was left to stir overnight. Upon completion, the residue was taken up in 5:2 v/v). The organic layers were combined, dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure. Filtration over silica (CH$_2$Cl$_2$/MeOH/Et$_3$N, 88:10:2) afforded 19, (8.80 g, 97% yield) as a white paste. R$_f$ 0.07 (CH$_2$Cl$_2$/MeOH/Et$_3$N, 93:5:2). $^1$H NMR (400 MHz, MeOD): δ 6.96 (s, $J = 9.4$ Hz, 4H, $H_a$), 4.03 (t, $J = 5.3$ Hz, 4H, $H_b$), 3.05 (t, $J = 5.3$ Hz, 4H, $H_c$). $^{13}$C NMR (126 MHz, MeOD): δ 154.34, 116.81, 66.16, 40.51. HRMS (ESI) m/z calcd for C$_{12}$H$_8$N$_2$O$_2$Na [M+Na]$^+$: 211.0483, found 211.0479.

2-[	ext{4-}(2-	ext{aminoethoxy} 	ext{phenoxy})	ext{ethanamine (19)}]

A 1 M solution of BH$_3$ in THF (320 mL) was slowly added to a solution of 18 (8.70 g, 46.2 mmol) in dry THF (320 mL). The reaction mixture was brought to reflux and stirred overnight. Upon completion, the mixture was cooled to rt, quenched with cold MeOH (50 mL) and concentrated under reduced pressure. The residue was taken up in 50% saturated aqueous K$_2$CO$_3$ (150 mL) and extracted with 2-propanol/CHCl$_3$ (3 × 250 mL, 1:3 v/v). The organic layers were combined, dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure. The residue was brought to reflux and stirred overnight. Upon completion, the mixture was cooled to rt, quenched with cold MeOH (50 mL) and concentrated under reduced pressure. The residue was taken up in 50% saturated aqueous K$_2$CO$_3$ (150 mL) and extracted with 2-propanol/CHCl$_3$ (3 × 250 mL, 1:3 v/v). The organic layers were combined, dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure. Filtration over silica (CH$_2$Cl$_2$/MeOH/Et$_3$N, 88:10:2) afforded 19, (8.80 g, 97% yield) as a white paste. R$_f$ 0.07 (CH$_2$Cl$_2$/MeOH/Et$_3$N, 93:5:2). $^1$H NMR (400 MHz, MeOD): δ 6.96 (s, $J = 9.4$ Hz, 4H, $H_a$), 4.03 (t, $J = 5.3$ Hz, 4H, $H_b$), 3.05 (t, $J = 5.3$ Hz, 4H, $H_c$). $^{13}$C NMR (126 MHz, MeOD): δ 154.34, 116.81, 66.16, 40.51. HRMS (ESI) m/z calcd for C$_{12}$H$_8$N$_2$O$_2$Na [M+Na]$^+$: 211.0483, found 211.0479.

20

A solution of (Boc)$_2$O (2.47 g, 11.3 mmol) in MeOH (10 mL) was added dropwise to a solution of 19 (2.22 g, 11.3 mmol) in MeOH/Et$_3$N (70 mL, 5:2 v/v). The reaction mixture was stirred at rt overnight and was concentrated under reduced pressure upon completion. The residue was purified by flash column chromatography (CH$_2$Cl$_2$/MeOH/Et$_3$N, 98:0:2→88:10:2) to afford 20 (1.19 g, 35% yield) as a white paste. R$_f$ 0.39 (CH$_2$Cl$_2$/MeOH/Et$_3$N, 93:5:2). $^1$H NMR (500 MHz, MeOD): δ 6.86 (br s, 4H, $H_a$), 3.96–3.91 (m, 4H, $H_b$), 3.49 (t, $J = 5.7$ Hz, 2H, $H_e$), 2.95 (t, $J = 5.3$ Hz, 2H, $H_f$), 1.44 (s, 9H, $H_d$). $^{13}$C NMR (126 MHz, MeOD): δ 158.51, 154.69, 154.56, 116.62, 116.56, 80.17, 71.07, 68.58, 41.98, 41.10, 28.75. HRMS (ESI) m/z calcd for C$_{18}$H$_{24}$N$_2$O$_4$ [M+H]$^+$: 297.1814, found: 297.1812.

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Amine 20 (2.18 g, 7.36 mmol), acid 13 (2.14 g, 9.54 mmol) and HOBr hydrate (1.29 g anhydrous basis, 9.54 mmol) were dissolved in DMF (15 mL) and EDCI-HCl (1.83 g, 9.54 mmol) and DIPEA (2.56 mL, 14.7 mmol) were added. The reaction mixture was left to stir overnight at rt. Upon completion, the mixture was diluted with EtOAc (150 mL) and washed
with 1M aqueous HCl (3 × 60 mL), 5% aqueous LiCl (3 × 60 mL), saturated aqueous NaHCO₃ (3 × 60 mL) and brine (60 mL). The organic layer was dried (Na₂SO₄) and concentrated under reduced pressure. The residue was purified by flash column chromatography (PhMe/EtOAc, 1:0→1:3) to afford 21 (1.68 g, 45% yield) as a white solid. Rᵣ 0.15 (PhMe/EtOAc, 1:1). Mp 71 °C. ¹H NMR (500 MHz, CDCl₃): δ 8.30 (m, 1H, Hₐ), 8.16 (m, 1H, Hₐ), 8.00 (m, 1H, Hₐ), 6.88–6.80 (m, 4H, Hₐ), 6.70 (t, J = 5.5 Hz, 1H, NH), 4.98 (br s, 1H, OH), 4.80 (s, 2H, Hₐ), 4.40 (q, J = 7.1 Hz, 2H, Hₐ), 4.12 (t, J = 5.0 Hz, 2H, H₈), 3.96 (t, J = 5.1 Hz, 2H, H₈), 3.87 (app dd, J = 10.4, 5.4 Hz, 2H, H₈), 3.54–3.44 (m, 2H, H₈), 1.45 (s, 9H, Hₙ₉), 1.41 (t, J = 7.1 Hz, 3H, H₇). ¹³C NMR (126 MHz, CDCl₃): δ 166.92, 165.95, 156.07, 153.25, 152.97, 142.35, 134.98, 131.20, 130.77, 129.92, 126.91, 115.72, 115.65, 79.70, 67.86, 67.44, 64.29, 61.59, 40.30, 39.96, 28.52, 14.44. LRMS (ESI) m/z calcd for C₂₆H₃₄N₂NaO₈ [M+Na]⁺: 525.2, found: 525.3. HRMS (ESI) m/z calcd for C₂₆H₃₅N₂NaO₈ [M+H]⁺: 503.2388, found: 503.2378.

22

Activated MnO₂ (260 mg, 2.99 mmol) was added to a solution of 21 (100 mg, 0.199 mmol) in THF (4 mL). The resulting suspension was stirred at rt for 2 d and subsequently filtered over celite. The filtrate was collected and concentrated under reduced pressure. The crude product was resubmitted and stirred under the same conditions for another 2 d to afford 22 (73 mg, 73% yield) as a white solid. Mp 56 °C. Rᵣ 0.53 (EtOAc/PhMe, 1:1). ¹H NMR (600 MHz, CDCl₃): δ 9.95 (s, 1H, Hₐ), 8.64 (s, 1H, Hₐ), 8.47 (s, 1H, Hₐ), 8.44 (s, 1H, Hₐ), 7.66 (m, 1H, NH), 6.70 (d, J = 9.1 Hz, 2H, H₁₉), 6.67 (d, J = 9.1 Hz, 2H, H₁₉), 5.20 (m, 1H, NH), 4.31 (q, J = 7.1 Hz, 2H, H₈), 4.03 (t, J = 5.1 Hz, 2H, H₈), 3.83 (t, J = 5.2, Hz, 2H, H₈), 3.79 (app dd, J = 10.3, 5.0 Hz, 2H, H₈), 3.43–3.33 (m, 2H, H₈), 1.35 (s, 9H, Hₙ₉), 1.31 (t, J = 7.1 Hz, 3H, H₇). ¹³C NMR (151 MHz, CDCl₃): δ: 190.76, 165.66, 164.70, 155.93, 152.88, 152.65, 136.51, 135.69, 133.40, 132.97, 131.78, 131.68, 115.34, 115.30, 79.29, 67.47, 66.89, 61.75, 40.01, 39.90, 28.27, 14.14. HRMS (ESI) m/z calcd for C₂₆H₃₅N₂NaO₈ [M+Na]⁺: 523.2051, found: 523.2043.

23

TFA (300 μL) was added to a solution of aldehyde 22 (150 mg, 0.300 mmol) in dry CH₂Cl₂ (600 μL). The resulting mixture was stirred for 5 min and concentrated under reduced pressure. The crude solid was taken up in THF (2 mL) and a drop of
concentrated aqueous HCl was added. The mixture was stirred for 10 min and concentrated under reduced pressure to yield 23 (131 mg, quantitative yield) as a yellow solid. Mp 136 °C. Rf 0.06 (CH₂Cl₂/MeOH, 19:1). ¹H NMR (600 MHz, DMSO): δ 10.15 (s, 1H, Hᵣ), 9.23 (t, J = 5.1 Hz, 1H, NH), 8.71 (s, 1H, H₈), 8.65 (s, 1H, Hᵣ), 8.58 (s, 1H, H₈), 8.27 (br s, 3H, NH), 6.97–6.90 (m, 4H, Hₖ), 4.40 (q, J = 7.1 Hz, 2H, Hₙ), 4.14–4.06 (m, 4H, H₉,₁₀), 3.68–3.62 (app dd, J = 11.2, 5.6 Hz, 2H, Hₗ), 3.19–3.12 (m, 2H, H₀), 1.37 (t, J = 7.1 Hz, 3H, Hₗ). ¹³C NMR (151 MHz, DMSO): δ 192.41, 164.64, 164.54, 152.93, 152.04, 135.63, 133.00, 132.47, 131.86, 131.10, 115.79, 115.48, 66.33, 64.84, 61.55, 39.18, 38.33, 14.17. HRMS (ESI) m/z calcld for C₂₁H₂₃N₂O₆ [M+H]⁺: 471.1707, found: 401.1696.

24

DIPEA (32 μL, 0.182 mmol), HOBt hydrate (7 mg anhydrous basis, 0.055 mmol) and EDCI-HCl (11 mg, 0.055 mmol) were added to a solution of acid 12 (30 mg, 0.036 mmol) and amine 23 (31 mg, 0.072 mmol) in DMF (400 μL). The reaction mixture was left to stir overnight at rt. The next day another portion of HOBt hydrate (7 mg anhydrous basis, 0.055 mmol) and EDCI-HCl (11 mg, 0.055 mmol) was added. After the mixture had stirred for another 2 h it was diluted with CH₂Cl₂ (10 mL) and washed with 0.5 M aqueous HCl (10 mL) and saturated aqueous NaHCO₃ (10 mL). The organic layer was dried (MgSO₄) and concentrated under reduced pressure. The residue was purified by preparative thin layer chromatography (CH₂Cl₂/MeOH, 93:7) to afford 24 (21 mg, 47% yield) as a red-brown solid. Rf 0.62 (CH₂Cl₂/MeOH, 9:1). Mp 84 °C. [c]D⁻¹⁰⁻³ (c 1.01, CH₂Cl₂). ¹H NMR (600 MHz, Acetone): δ 10.15 (s, 1H, Hᵣ), 8.81 (br s 1H, NH), 8.72 (d, J = 7.3 Hz, 2H, H₈), 8.72 (s, 1H, H₈), 8.68 (d, J = 7.5 Hz, 2H, H₉), 8.60 (s, 1H, H₈), 8.57 (s, 1H, H₈), 8.47 (t, J = 5.1 Hz, 1H, NH), 8.34 (s, 1H, H₈), 8.22 (t, J = 5.5 Hz, 1H, NH), 8.08 (s, 1H, H₈), 8.06 (s, 1H, H₈), 7.79 (br s, 1H, NH), 6.85–6.87 (m, 4H, Hₗ), 6.03 (dd, J = 9.1, 4.9 Hz, 1H, H₉), 4.40 (q, J = 7.1 Hz, 2H, Hₗ,₁₀), 4.35 (q, J = 7.1 Hz, 2H, Hₗ,₁₀), 4.16–4.05 (m, 8H, Hₗ,₁₀), 3.78 (app dd, J = 11.4, 5.9 Hz, 2H, Hₗ,₁₀), 3.75 (app dd, J = 1.5, 5.9 Hz, 2H, Hₗ,₁₀), 3.69 (s, 3H, H₂), 3.46 (dd, J = 14.7, 4.7 Hz, 1H, Hₗ), 3.33 (dd, J = 14.7, 9.3 Hz, 1H, Hₗ), 2.28 (t, J = 6.5 Hz, 2H, Hₗ), 1.83–1.67 (m, 4H, Hₗ,₁₀), 1.42–1.34 (m, 15H, Hₗ,₁₀). ¹³C NMR (151 MHz, Acetone) δ 192.08, 172.70, 169.48, 166.51, 165.99, 165.78, 165.40, 163.47, 163.44, 156.40, 153.94, 153.09, 139.46, 137.95, 136.99, 136.30, 133.89, 133.39, 133.36, 133.08, 132.64, 132.52, 132.17, 131.83, 131.34, 128.18, 127.82, 127.56, 127.49, 126.57, 116.28, 116.22, 80.21, 67.61, 67.50, 62.29, 61.88, 54.03, 53.05, 42.32, 40.90, 40.49, 40.39, 37.07, 33.95,
28.37, 28.32, 23.62, 14.60, 14.54. LRMS (ESI) measured isotopic distribution for C₆₀H₆₀N₆O₇S₂ [M+Na⁺]: 1241.25 (100), 1242.25 (69), 1243.17 (38), 1244.08 (14), 1245.25 (6). Calculated: 1241.35 (100), 1242.43 (71), 1243.35 (37), 1244.35 (15), 1245.35 (4). LRMS (ESI) measured isotopic distribution for C₆₀H₆₀N₆O₇S₂ [M(methyl hemicetal)+Na⁺]: 1273.25 (100), 1274.17 (68), 1275.25 (37), 1276.33 (13), 1277.25 (6). Calculated: 1273.37 (100), 1274.38 (72), 1275.38 (39), 1276.38 (15), 1277.38 (5).

TFA (1 mL) was added to a solution of 24 (20 mg, 0.0164 mmol) in CH₂Cl₂ (3 mL) and stirred for 2 h at rt. Subsequently, the reaction mixture was diluted with CHCl₃/MeOH (9:1, 160 mL) and left to stir overnight. The reaction was quenched with saturated aqueous NaHCO₃ (100 mL). The organic layer was collected, washed with brine (50 mL), dried (MgSO₄) and concentrated under reduced pressure. The crude solid was purified by preparative thin layer chromatography (CH₂Cl₂/MeOH, 95:5) to yield 25 (11 mg, 61% yield) as an orange solid. Rf 0.45 (CH₂Cl₂/MeOH, 95:5). Mp 236 °C. [α]D²⁰ = –3.1 (c 0.53, CH₂Cl₂/MeOH, 9:1). H NMR (600 MHz, CH₂Cl₂, anti/syn 9:2): δ 9.47 (s, 1H, NH), 8.60 (d, J = 7.5 Hz, 2H, H₆), 8.57 (d, J = 7.5 Hz, 2H, H₆), 8.53 (s, 1H, H₆), 8.46 (s, 1H, H₆), 8.29 (s, 1H, H₆), 8.13 (s, 1H, H₆), 8.03 (s, 1H, H₆), 7.97 (s, 1H, H₆), 7.86 (s, 1H, H₆), 7.17 (t, J = 5.2 Hz, 1H, NH), 7.13 (t, J = 5.0 Hz, 1H, NH), 6.61 (d, J = 8.9 Hz, 2H, H₄), 6.54 (d, J = 8.9 Hz, 2H, H₄), 5.81 (dd, J = 9.2, 5.2 Hz, 1H, H₄), 4.36 (app q, J = 7.1 Hz, 2H, H₄), 4.32–4.20 (m, 2H, H₄), 4.06–3.90 (m, 6H, H',H₂); 3.89–3.72 (m, 4H, H), 3.69 (s, 3H, H₃), 3.36 (dd, J = 14.9, 5.2 Hz, 1H, H₃), 3.21 (dd, J = 14.9, 9.3 Hz, 1H, H₄), 2.96–2.89 (m, 1H, H₄), 2.88–2.81 (m, 1H, H₃), 1.91–1.76 (m, 4H, H₅), 1.39 (app t, J = 7.1 Hz, 4H, H₆). C NMR (151 MHz, CDCl₃) δ 175.85, 169.09, 166.99, 166.56, 165.94, 165.70, 163.37, 163.33, 153.36, 153.23, 141.53, 138.79, 136.10, 136.09, 135.36, 133.46, 132.55, 132.37, 132.27, 131.95, 131.85, 131.27, 130.18, 127.84, 127.53, 127.42, 127.16, 127.08, 126.36, 115.93, 115.73, 75.73, 67.73, 62.14, 62.01, 54.09, 53.30, 42.75, 40.74, 40.36, 37.25, 32.45, 27.95, 22.71, 14.63, 14.61. LRMS (ESI) measured isotopic distribution for C₆₉H₅₂N₆O₁₇S₂ [M+Na⁺]: 1123.42 (100), 1124.42 (65), 1125.50 (35), 1126.50 (13). Calculated: 1123.28 (100), 1124.29 (65), 1125.29 (33), 1126.29 (12).
successively washed with 1M aqueous HCl, dilute aqueous NaHCO$_3$ reaction mixture was stirred for 1 h. The solution was filtered over celite and the filtrate was subsequently washed with 1M aqueous HCl, dilute aqueous NaHCO$_3$, and brine. After reduced pressure to furnish 26 (745 mg, 99% yield) as a white paste. $R_f$ 0.08 (CH$_2$Cl$_2$/MeOH/Et$_3$N, 93:5:2). $^1$H NMR (500 MHz, MeOD): $\delta$ 8.38 (s, 1H, $H_a$), 8.18 (s, 1H, $H_b$), 8.02 (s, 1H, $H_d$), 6.89–6.80 (m, 4H, $H_{g,k}$), 4.70 (s, 2H, $H_d$), 4.10 (t, $J = 5.7$ Hz, 2H, $H_i$), 3.91 (t, $J = 5.6$ Hz, 2H, $H_j$), 3.74 (t, $J = 5.6$ Hz, 2H, $H_i$), 3.37 (t, $J = 5.6$ Hz, 2H, $H_j$), 1.42 (s, 9H, $H_k$). $^{13}$C NMR (126 MHz, MeOD): $\delta$ 169.52, 168.89, 158.47, 154.53, 154.49, 144.11, 136.07, 132.52, 131.80, 130.91, 128.39, 116.64, 116.56, 80.18, 68.49, 67.93, 64.26, 41.05, 40.87, 28.72. HRMS (ESI) m/z calcd for C$_{24}$H$_{29}$N$_3$O$_8$ [M–H]$^-$: 473.1918, found: 473.1921.

A mixture of EtOH and 1 M aqueous HCl (20 mL, 4:1 v/v) was added to 21 (807 mg, 1.61 mmol). The resulting solution was stirred for 4 h and afterwards concentrated under reduced pressure to furnish 27 (705 mg, quantitative yield) as a yellow solid. $R_f$ 0.16 (CH$_2$Cl$_2$/MeOH/Et$_3$N, 93:5:2). Mp 156 °C. $^1$H NMR (500 MHz, MeOD): $\delta$ 8.38 (s, 1H, $H_a$), 8.18 (s, 1H, $H_b$), 8.04 (s, 1H, $H_d$), 6.94 (m, 4H, $H_{g,k}$), 4.72 (s, 2H, $H_d$), 4.40 (q, $J = 7.1$ Hz, 2H, $H_b$), 4.17 (t, $J = 4.7$ Hz, 2H, $H_j$), 4.14 (t, $J = 5.7$ Hz, 4H, $H_i$), 3.77 (t, $J = 5.6$ Hz, 2H, $H_k$), 3.34–3.30 (m, 2H, $H_j$), 1.41 (t, $J = 7.1$ Hz, 3H, $H_s$). $^{13}$C NMR (126 MHz, MeOD): $\delta$ 169.05, 166.99, 154.72, 153.47, 143.98, 135.75, 131.83, 131.27, 130.80, 127.94, 116.64, 116.52, 67.76, 65.77, 64.04, 62.37, 40.69, 40.49, 14.57. HRMS (ESI) m/z calcd for C$_{21}$H$_{23}$N$_2$O$_6$ [M+H]$^+$: 403.1869, found: 403.1865.

A mixture of 1,5-diaminonaphthalene (944 mg, 5.97 mmol), N-Boc-2-aminoacetaldehyde (2.38 g, 14.9 mmol) and glacial acetic acid (853 mL, 14.9 mmol) was stirred for 30 min in dry MeOH (25 mL) with molecular sieves (3 Å). Subsequently, NaBH$_3$CN (825 mg, 14.9 mmol) was added and the reaction mixture was stirred for 1 h. The solution was filtered over celite and the filtrate was successively washed with 1M aqueous HCl, dilute aqueous NaHCO$_3$, and brine. After
purification by flash column chromatography, 28 (1.98 g, 75%) was isolated as a red-brown powder. Rf 0.84 (CH$_2$Cl$_2$/MeOH/Et$_3$N, 93:5:2). Mp 189 °C. $^1$H NMR (600 MHz, DMSO): δ 7.26 (d, $J = 8.5$ Hz, 2H, H$_i$), 7.16 (dd, $J = 7.9, 7.9$ Hz, 2H, H$_a$), 7.08 (t, $J = 5.6$ Hz, 2H, NH), 6.50 (d, $J = 7.5$ Hz, 2H, H$_d$), 5.88 (m, 2H, NH), 3.28–3.17 (m, 8H, H$_{e,f}$), 1.40 (s, 18H, H$_j$). $^{13}$C NMR (151 MHz, DMSO): δ 156.22, 144.02, 124.88, 123.53, 108.88, 102.81, 77.87, 43.89, 38.85, 28.27. LRMS (ESI) m/z calcd for C$_{24}$H$_{32}$N$_4$O$_4$ [M+H]$^+$: 445.3, found: 445.3 (100), C$_{24}$H$_{30}$N$_4$NaO$_4$ [M+Na]$^+$: 467.3, found 467.3 (38). HRMS (ESI) m/z calcd for C$_{24}$H$_{32}$N$_4$O$_4$ [M+H]$^+$: 445.2809, found: 445.2803.

29

A mixture of TFA (10 mL) and CH$_2$Cl$_2$ (10 mL) was added to 28 (1.98 g, 4.45 mmol). The reaction mixture was stirred for 1 h at rt and the solvent was removed under reduced pressure. The residue was taken up in a small amount of ethanol and 29 (1.70 g, 80%) precipitated as a brown or grey powder upon addition of PET. Rf 0.01 (CH$_2$Cl$_2$/MeOH/Et$_3$N, 93:5:2). Mp 213 °C. $^1$H NMR (500 MHz, MeOD): δ 7.45 (d, $J = 8.6$ Hz, 2H, H$_i$), 7.30 (dd, $J = 8.3, 7.7$ Hz, 2H, H$_{a,b}$), 6.69 (d, $J = 7.6$ Hz, 2H, H$_d$), 3.60 (t, $J = 6.1$ Hz, 4H, H$_e$), 3.32–3.27 (m, 4H, H$_b$). $^{13}$C NMR (126 MHz, MeOD): δ 144.77, 126.30, 126.20, 111.83, 105.59, 42.39, 39.64. HRMS (ESI) m/z calcd for C$_{12}$H$_{21}$N$_4$ [M+H]$^+$: 245.1761, found: 245.1762.

30

DIPEA (514 μL, 2.95 mmol) was added to a solution of acid 26 (200 mg, 0.421 mmol), amine 29 (199 mg, 0.421 mmol) and acid 15 (203 mg, 0.421 mmol) in DMF (400 μL) and the reaction mixture was stirred for 30 min. Subsequently, HOBT hydrate (137 mg anhydrous basis, 1.01 mmol) and EDCI·HCl (194 mg, 1.01 mmol) were added. The reaction mixture was left to stir overnight at rt. Upon completion, the mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (CH$_2$Cl$_2$/MeOH/NH$_4$OH, 99:0:1→92:7:1) to afford 30 (236 mg, 48% yield) as a grey-purple solid. Rf 0.47 (CH$_2$Cl$_2$/MeOH, 9:1). Mp 116 °C. $^1$H NMR (600 MHz, Acetone/MeOD): δ 8.35 (s, 1H, H$_d$), 8.29 (s, 1H, H$_a$), 8.02 (s, 1H, H$_a$), 8.00 (s, 1H, H$_a$), 7.88 (s, 1H, H$_d$), 7.83 (s, 1H, H$_a$), 7.44 (d, $J = 7.6$ Hz, 6H, H$_a$), 7.35–7.30 (m, 8H, H$_{e,f}$), 7.27–7.19 (m, 5H, H$_{d,y}$), 6.89–6.82 (m, 4H, H$_a$), 6.61 (m, $J = 6.9$ Hz, 2H, H$_d$), 4.69 (s, 2H, H$_b$), 4.35 (q, $J =
7.1 Hz, 2H, Hα), 4.12 (t, J = 5.7 Hz, 2H, Hβ), 3.95 (t, J = 5.5 Hz, 2H, Hγ), 3.85–3.79 (m, 4H, Hε,ζ), 3.76 (t, J = 5.6 Hz, 2H, Hν), 3.53–3.48 (m, 4H, Hξ), 3.49 (s, 2H, Hβ), 3.40 (t, J = 5.5 Hz, 2H, Hδ), 1.39 (s, 9H, Hθ), 1.36 (t, J = 7.1 Hz, 4H, Hυ). 13C NMR (151 MHz, Acetone/MeOD): δ 168.30, 167.60, 167.38, 165.94, 156.72, 153.94, 153.89, 145.36, 145.14, 145.10, 144.02, 139.30, 136.30, 135.75, 133.08, 133.00, 131.74, 130.87, 130.33, 128.85, 128.77, 127.71, 127.53, 125.97, 125.97, 125.47, 124.93, 124.93, 116.23, 116.19, 109.85, 109.75, 104.02, 104.02, 78.78, 78.39, 68.06, 75.9, 75.9, 45.35, 45.24, 40.75, 40.32, 39.89, 39.77, 36.87, 28.60, 14.59. LRMS (ESI) measured isotopic distribution for C_{68}H_{72}N_{10}O_{10}S [M+Na]^+: 1187.33 (100), 1188.33 (79), 1189.33 (36), 1190.25 (12). Calculated: 1187.49 (100), 1188.50 (78), 1189.50 (37), 1190.50 (13).

A solution of 30 (115 mg, 0.099 mmol) in THF (3 mL) was added to a solution of LiOH·H_{2}O (8 mg, 0.197 mmol) in water (1 mL). The reaction mixture was stirred for 30 h and subsequently diluted with 1 M aqueous HCl (20 mL). The resulting suspension was extracted with CHCl_{3}/2-propanol, 3:1 (3 × 50 mL) and the organic layers were combined, dried (MgSO_{4}), filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (CH_{2}Cl_{2}/MeOH, 1:0→8:2) to yield 31 (95 mg, 84% yield) as a grey solid. Mp 175 °C. R_{f} 0.26 (CH_{2}Cl_{2}/MeOH, 9:1). 1H NMR (600 MHz, DMSO): δ 8.91–8.31 (m, 2H, NH), 8.84 (t, J = 5.4 Hz, 1H, NH), 8.31 (s, 1H, Hδ), 8.27 (m, 1H, Hγ), 7.98 (s, 1H, Hβ), 7.96 (s, 1H, Hδ), 7.80 (s, 1H, Hζ), 7.79 (s, 1H, Hθ), 7.40–7.34 (m, 12H, Hν), 7.32–7.30 (m, 2H, Hβ), 7.27–7.25 (m, 3H, Hδ), 7.22–7.18 (m, 2H, Hδ), 6.90 (t, J = 5.6 Hz, 1H, NH), 6.90–6.83 (m, 4H, Hβ), 6.61 (m, J = 6.8 Hz, 2H, Hδ), 6.14–6.05 (m, 2H, NH), 5.43 (br s, 1H, OH), 4.60 (s, 2H, Hζ), 4.06 (t, J = 6.0 Hz, 2H, Hζ), 3.87 (t, J = 5.9 Hz, 2H, Hθ), 3.62–3.57 (m, 6H, Hξ), 3.40 (s, 2H, Hθ), 3.39–3.35 (m, 4H, Hζ), 3.24 (t, J = 5.7 Hz, 2H, Hδ), 1.37 (s, 9H, Hθ). 13C NMR (151 MHz, DMSO) δ 166.57, 166.49, 166.22, 166.14, 155.69, 152.57, 152.52, 144.12, 144.00, 143.95, 143.09, 134.75, 134.52, 134.48, 134.38, 134.34, 132.28, 129.67, 129.13, 128.18, 127.85, 127.82, 126.93, 126.81, 124.93, 124.81, 124.67, 123.62, 123.56, 115.43, 115.43, 108.96, 108.96, 102.89, 102.89, 77.76, 67.12, 66.84, 66.40, 62.43, 43.31, 43.20, 39.03, 38.91, 38.40, 38.28, 35.86, 28.24. LRMS (ESI) m/z calcd for C_{68}H_{70}N_{9}O_{10}S (M−H)^−: 1135.46, found: 1135.58.
DIPEA (37 µL, 0.215 mmol) was added to a solution of acid 31 (70 mg, 0.062 mmol) and amine 27 (27 mg, 0.062 mmol) in DMF (300 µL) and the reaction mixture was stirred for 30 min. Subsequently, HOBT hydrate (10 mg anhydrous basis, 0.074 mmol) and EDCI-HCl (14 mg, 0.074 mmol) were added. The reaction mixture was left to stir overnight at rt. Upon completion, the mixture was diluted with CHCl₃/2-propanol 3:1 (50 mL) and washed with 1 M aqueous HCl (10 mL), 5% aqueous LiCl (10 mL), saturated aqueous NaHCO₃ (10 mL) and brine (10 mL). The organic layer was dried (Na₂SO₄) and concentrated under reduced pressure. The residue was purified by flash column chromatography (CH₂Cl₂/MeOH, 1:0→9:1) to afford 32 (70 mg, 75% yield) as a yellow solid. Rf 0.45 (CH₂Cl₂/MeOH, 9:1). Mp 127 °C. ¹H NMR (600 MHz, Acetone/MeOD): δ 8.38 (s, 1H, H₁), 8.36–8.29 (m, 3H, NH), 8.27 (s, 1H, H₄), 8.21 (s, 1H, H₃), 8.18 (t, J = 5.5 Hz, 1H, NH), 8.14 (t, J = 5.5 Hz, 1H, NH), 8.11 (s, 1H, H₄), 8.10 (s, 1H, H₃), 8.00 (s, 1H, H₂), 7.98 (s, 1H, H₆), 7.75 (s, 1H, H₅), 7.73 (s, 1H, H₆), 7.39 (d, J = 8.0 Hz, 6H, H₇), 7.30–7.27 (m, 8H, H₈), 7.20 (m, 3H, H₉), 7.16 (m, 2H, H₁₀), 6.82–6.76 (m, 8H, H₆), 6.57–6.52 (m, 2H, H₉), 6.20 (m, 1H, NH), 5.77 (m, 2H, NH), 4.71 (s, 2H, H₁₁ or e'), 4.65 (s, 2H, H₁₁ or e'), 4.32 (q, J = 7.1 Hz, 2H, H₁₂), 4.09–4.04 (m, 4H, H₁₂ or e), 4.03 (t, J = 5.6 Hz, 2H, H₁₂ or e), 3.91 (t, J = 5.7 Hz, 2H, H₁₂), 3.81–3.68 (m, 10H, H₁₂ or e'), 3.48–3.42 (m, 4H, H₁₂ or e'), 3.40–3.37 (app dd, J = 11.5, 5.7 Hz, 2H, H₁₂), 3.36 (s, 2H, H₁₂), 3.22 (br s, 2H, OH), 1.38 (s, 9H, H₁₂), 1.31 (t, J = 7.1 Hz, 4H, H₁₂). ¹³C NMR (151 MHz, Acetone): δ 168.35, 168.04, 167.47, 167.47, 167.25, 167.18, 166.35, 156.81, 153.95, 153.92, 153.89, 153.89, 145.37, 145.07, 145.06, 144.32, 144.01, 138.83, 136.05, 135.98, 135.90, 135.74, 135.71, 131.54, 131.33, 130.59, 130.46, 130.31, 128.86, 128.78, 128.78, 127.69, 127.48, 126.03, 126.00, 125.73, 125.53, 125.53, 124.92, 124.92, 116.24, 116.24, 116.20, 116.20, 109.84, 109.84, 104.10, 104.10, 104.10, 78.86, 68.22, 68.07, 67.58, 67.58, 67.55, 63.94, 63.76, 61.74, 45.20, 45.15, 40.77, 40.38, 40.27, 40.27, 39.93, 39.80, 37.06, 28.61, 14.60. LRMS (ESI) m/z calcld for C₈₂H₉₂N₁₄O₁₅S [M+Na]+: 1543.63, found: 1543.42.
TFA (500 μL), CH₂Cl₂ (500 μL) and Et₃SiH (50 μL, 0.313 mmol) were added to a round-bottom flask charged with 32 (53 mg, 0.035 mmol). After two hours of stirring, all the volatiles were removed. The residue was washed with Et₂O several times and dried under reduced pressure to give 33 (45 mg, quantitative yield). ¹H NMR (600 MHz, MeOD): δ 8.30 (m, 1H, Hₐ), 8.17 (m, 1H, Hₐ), 8.09 (m, 1H, Hₐ), 8.08 (m, 1H, Hₐ), 7.96 (m, 1H, Hₐ), 7.91 (m, 2H, Hₐ), 7.81 (m, 2H, Hₐ) 7.26–7.22 (m, 2H, Hₐ), 7.18–7.14 (m, 2H, Hₐ), 7.08–7.04 (m, 2H, Hₐ), 6.86–6.81 (m, 4H, Hₐ), 6.76–6.72 (m, 4H, Hₐ), 4.63 (m, 4H, Hₐ) 3.48 (m, 4H, Hₐ). LRMS (ESI) m/z calcd for C₆₃H₇₁N₉O₁₃S [M+H]⁺: 590.25, found: 590.25 (100), C₆₃H₇₁N₉O₁₃S [M+H]⁺: 1179.49, found: 1279.42 (36). LRMS (ESI) measured isotopic distribution for C₆₃H₇₁N₉O₁₃S [M+H]⁺: 1179.67 (100), 1180.50 (74), 1181.58 (33), 1182.50 (12). Calculated: 1179.49 (100), 1180.49 (74), 1181.49 (34), 1182.49 (12).

Thiol 33 (45 mg, 0.035 mmol) was taken up in DMF (1 mL) and 2-mercaptoethanol (25 μL, 0.35 mmol) and Et₃N (49 μL, 0.35 mmol) were added. The resulting mixture was stirred for 3 h under air and subsequently concentrated by blowing air over it. The crude solid was washed thoroughly with CHCl₃ to yield 34 (42 mg, 95% yield) as a purple solid and was used without further purification. ¹H NMR (600 MHz, DMSO): δ 8.31 (s, 1H, Hₐ), 8.28 (s, 1H, Hₐ),
8.24 (s, 1H, Hₙ), 8.06 (m, 2H, Hₜ), 8.0–7.93 (m, 4H, Hₘ), 7.31 (m, 2H, Hₜ), 7.21 (m, 2H, Hₙ), 6.95–6.85 (m, 8H, Hₜ), 6.62 (m, 2H, Hₘ), 6.09 (m, 2H, NH), 5.46 (t, J = 5.6 Hz, 1H, OH), 5.42 (t, J = 5.6 Hz, 1H, OH), 4.90 (t, J = 5.4 Hz, 1H, OH), 4.63–4.56 (m, 4H, Hₐ,e), 4.35 (q, J = 7.1 Hz, 2H, Hₙ), 4.11–4.00 (m, 8H, Hₐ,e,h,j), 3.66–3.55 (m, 12H, H_{c,c',l,f,f',n}), 3.40–3.35 (m, 4H, H_{d,d'}), 3.31 (s, 2H, Hₙ), 3.19 (m, 2H, Hₙ), 2.67 (t, J = 6.4 Hz, 2H, Hₘ), 1.34 (t, J = 7.0 Hz, 3H, Hₙ). LRMS (ESI) m/z calcd for C₆₅H₇₆N₈O₈S₂ [M+H]^+: 1255.48, found: 1255.58 (100), C₆₅H₇₆N₈NaO₈S₂ [M+Na]^+: 1277.47, found: 1277.58 (26).

DIPEA (17 µL, 0.096 mmol), HOBT hydrate (6 mg anhydrous basis, 0.042 mmol) and EDCI-HCl (8 mg, 0.042 mmol) were added to a solution of amine 34 (37 mg, 0.029 mmol) and acid 12 (27 mg, 0.032 mmol) in DMF (250 µL). The reaction mixture was left to stir overnight at rt. Upon completion, the mixture was concentrated and the crude solid was washed with water (5 mL) and MeOH (5 mL). The residue was purified by flash column chromatography (CH₂Cl₂/MeOH, 1:0→9:1) to afford 35 (19 mg, 32% yield) as a brown solid. Rₜ 0.30 (CH₂Cl₂/MeOH, 9:1). ¹H NMR (600 MHz, CDCl₃): δ 9.47 (s, 1H, NH), 8.93–8.89 (m, 2H, NH), 8.87–8.83 (m, 2H, NH), 8.83–8.78 (m, 2H, NH), 8.69 (d, J = 7.6 Hz, 2H, Hₙ), 8.65 (d, J = 7.5 Hz, 3H, NH, Hₙ), 8.32 (s, 1H, Hₙ), 8.31 (s, 1H, Hₙ), 8.27 (s, 1H, Hₙ), 8.23 (s, 1H, Hₙ), 8.07–8.05 (m, 2H, Hₙ), 8.04 (s, 1H, Hₙ), 8.02 (s, 1H, Hₙ), 7.99–7.97 (s, 2H, Hₙ), 7.96 (s, 1H, Hₙ), 7.95 (s, 1H, Hₙ), 7.27 (m, 2H, Hₙ), 7.20–7.16 (m, 2H, Hₙ), 6.91–6.86 (m, 8H, Hₙ), 6.58 (m, 2H, Hₙ), 6.06 (br s, 2H, NH), 5.97 (dd, J = 8.8, 5.1 Hz, 1H, Hₙ), 4.60 (s, 2H, H_{e,or,e'}), 4.59 (s, 2H, H_{e,or,e'}), 4.34 (app q, J = 7.1 Hz, 4H, H_{k,k'}), 4.11–4.01 (m, 12H, H_{b,b',x,x',h',x'}), 3.62–3.56 (m, 17H, H_{c,c',l,f,f',n,n,a}), 3.41–3.29 (m, 7H, H_{d,d',e',a}), 3.25 (dd, J = 14.6, 8.8 Hz, 1H, Hₐ), 2.67 (t, J = 6.5 Hz, 2H, Hₘ), 2.12 (t, J = 6.9 Hz, 2H, Hₙ), 1.71–1.65 (quint, J = 7.4 Hz, 2H, Hₙ), 1.62–1.55 (quint, J = 7.4 Hz, 2H, Hₙ), 1.37–1.32 (m, 15H, H_{f,f',a}). LRMS (ESI) m/z calcd for C₁₀₄H₁₃₂N₁₂O₂₆S₄ [M+Na]^+: 2095.66, found: 2095.27.
Molecular sieves (3 Å) and p-toluenesulfonic acid monohydrate (13 mg, 0.066 mmol) were added to a solution of 22 (330 mg, 0.659 mmol) in a mixture of dry THF/ethylene glycol (1:1, 1.5 mL). The reaction mixture was stirred for 1 h and subsequently diluted with dry THF (10 mL). After stirring overnight, the mixture was quenched with saturated aqueous NaHCO₃ (10 mL) and extracted with EtOAc (3 × 50 mL). The organic layers were combined and concentrated under reduced pressure. The residue was purified by flash column chromatography (EtOAc/PhMe, 1:1–3:1) to obtain 36 (305 mg, 85% yield) as a sticky white solid. Mp 44 °C. Rf 0.48 (EtOAc/PhMe, 1:1). ¹H NMR (600 MHz, CDCl₃): δ 8.37 (s, 1H, H₈), 8.15 (s, 1H, H₉), 8.06 (s, 1H, H₅), 7.35 (t, J = 5.6 Hz, 1H, NH), 6.75–6.65 (m, 4H, H₁₀–₁₃), 5.73 (s, 1H, H₄), 5.22 (m, 1H, NH), 4.28 (q, J = 7.1 Hz, 2H, H₆), 4.03–3.97 (m, 4H, H₇₉−₉₃), 3.96–3.91 (m, 2H, H₁₄), 3.84 (t, J = 5.2 Hz, 2H, H₂₅), 3.74 (app dd, J = 10.7, 5.3 Hz, 2H, H₂₆), 3.42–3.36 (m, 2H, H₁), 1.36 (s, 9H, H₁₆), 1.29 (t, J = 7.1 Hz, 3H, H₁₇) ¹³C NMR (151 MHz, CDCl₃): δ 166.47, 165.40, 155.88, 152.82, 152.69, 139.08, 134.81, 130.84, 130.29, 129.60, 128.62, 115.31, 115.26, 102.43, 79.20, 67.46, 66.95, 65.24, 61.28, 39.99, 39.68, 28.24, 14.14. HRMS (ESI) m/z calcd for C₂₈H₃₃N₂O₉ [M+H]: 545.2499, found: 545.2490.

Acetal 36 (162 mg, 0.297 mmol) was taken up in THF (6 mL) and a solution of LiOH·H₂O (25 mg, 0.596 mmol) in water (2 mL) was added. The resulting emulsion was left to stir overnight before it was quenched with aqueous HCl (0.5 N, 15 mL) and extracted with EtOAc (3 × 20 mL). The organic layers were combined and concentrated under reduced pressure to obtain 37 (138 mg, 90% yield) as a white solid. Mp 63 °C. Rf 0.32 (CH₃Cl₂/MeOH, 9:1). ¹H NMR (600 MHz, Acetone): δ 8.56 (s, 1H, H₈), 8.43 (m, 1H, NH), 8.25 (m, 2H, H₁₀–₁₁), 6.87 (d, J = 9.0 Hz, 2H, H₉₀–₉₁), 6.84 (d, J = 8.9 Hz, 2H, H₉₂–₉₃), 6.20 (m, 1H, NH), 5.85 (s, 1H, H₄), 4.15 (t, J = 5.6 Hz, 2H, H₁₀), 4.12–4.07 (m, 2H, H₁₁), 4.05–4.00 (m, 2H, H₁₂), 3.96 (t, J = 5.4 Hz, 2H, H₁₃), 3.81 (app q, J = 5.5 Hz, 2H, H₁₄), 3.42 (app q, J = 5.5 Hz, 2H, H₁₅), 1.40 (s, 9H, H₁₆). ¹³C NMR (151 MHz, Acetone): δ 167.00, 166.79, 156.75, 153.93, 153.89, 153.55, 136.06, 131.90, 131.04, 130.65, 129.68, 116.22, 116.18, 103.23, 78.83, 68.04, 67.51, 66.00, 40.73, 40.41, 28.56. LRMS (ESI) m/z calcd for C₂₈H₃₃N₂O₉ [M−H]⁺: 515.2, found: 515.2 (100), C₂₄H₂₁N₂O₇. [M−C₂H₅O]⁺: 471.2, found: 471.1 (10). HRMS (ESI) m/z calcd for C₂₆H₃₂N₂O₉ [M−H]⁺: 515.2035, found: 515.2011.
and acid 15 (463 mg, 0.960 mmol) in DMF (6.5 mL). The resulting solution stirred for 30 min. Subsequently, HOBT hydrate (454 mg anhydrous basis, 3.36 mmol) and EDCI·HCl (644 mg, 3.36 mmol) were added. The reaction mixture was left to stir overnight at rt. Upon completion, the mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (CH$_2$Cl$_2$/MeOH, 1:0→94:6) to afford 38 (374 mg, 32% yield) as a brown solid. R$_f$ 0.59 (CH$_2$Cl$_2$/MeOH, 9:1). Mp 108 °C. $^1$H NMR (600 MHz, Acetone): δ 8.43 (m, 1H, H$_a$), 8.41–8.38 (m, 2H, NH), 8.36 (m, 1H, H$_b$), 8.25 (t, $J$ = 5.6 Hz, 1H, NH), 8.12 (m, 1H, H$_c$), 8.10 (m, 1H, H$_d$), 7.89 (m, 1H, H$_e$), 7.82 (m, 1H, H$_f$), 7.43 (dd, $J$ = 8.4, 1.1 Hz, 6H, H$_g$), 7.34–7.29 (m, 8H, H$_{8-15}$), 7.25–7.22 (m, 3H, H$_{16}$), 7.21–7.18 (m, 2H, H$_{17}$), 6.85–6.79 (m, 4H, H$_{18}$), 6.59 (m, 2H, H$_{19}$), 6.16 (m, 1H, NH), 5.82–5.76 (m, 2H, NH), 5.77 (s, 1H, H$_{20}$), 4.33 (q, $J$ = 7.1 Hz, 2H, H$_{21}$), 4.09 (t, $J$ = 5.8 Hz, 2H, H$_{22}$), 4.05–4.00 (m, 2H, H$_{23}$), 3.98–3.95 (m, 2H, H$_{24}$), 3.93 (t, $J$ = 5.7 Hz, 2H, H$_{25}$), 3.85–3.79 (m, 4H, H$_{26-29}$), 3.76 (app q, $J$ = 5.7 Hz, 2H, H$_{30}$), 3.52–3.48 (m, 4H, H$_{31-34}$), 3.46 (s, 2H, H$_{35}$), 3.43–3.38 (app q, $J$ = 5.7 Hz, 2H, H$_{36}$), 1.40 (s, 9H, H$_{37}$), 1.34 (t, $J$ = 7.1 Hz, 3H, H$_{38}$). $^{13}$C NMR (151 MHz, Acetone) δ 167.83, 167.59, 166.96, 165.93, 156.70, 153.95, 153.89, 145.36, 145.11, 145.11, 140.29, 140.29, 139.29, 136.31, 135.99, 135.95, 133.08, 132.99, 131.74, 130.87, 130.33, 128.84, 127.71, 127.58, 127.52, 125.96, 125.96, 124.94, 124.46, 116.23, 116.19, 109.79, 109.79, 104.01, 104.01, 103.41, 78.77, 68.39, 68.07, 67.57, 65.95, 61.78, 45.25, 45.23, 40.75, 40.36, 39.91, 39.89, 36.87, 28.60, 14.59. LRMS (ESI) m/z calcd for C$_{70}$H$_{54}$NaO$_{12}$S [M+Na]$^+$: 1229.50, found: 1229.55.

A solution of 38 (318 mg, 0.263 mmol) in THF (3.5 mL) was added to a solution of LiOH·H$_2$O (17 mg, 0.396 mmol) in water (1.1 mL). The reaction mixture was stirred for 24 h and subsequently diluted with 0.5 M aqueous HCl (0.5 mL). The resulting suspension was extracted with CHCl$_3$/2-propanol, 3:1 (3 × 15 mL) and the organic layers were combined, dried (MgSO$_4$), filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography...
(CH₃Cl/MeOH, 1:0→8:2) to yield 39 (213 mg, 69% yield) as a light brown solid. Mp 177 °C. R₇ 0.35 (CH₃Cl/MeOH, 9:1). ¹H NMR (600 MHz, DMSO): δ 8.94 (t, J = 5.7 Hz, 1H, NH), 8.91 (t, J = 5.5 Hz, 1H, NH), 8.88 (t, J = 5.5 Hz, 1H, NH), 8.40 (s, 1H, H₈), 8.29 (s, 1H, H₉), 8.08–8.05 (m, 2H, H₅), 7.81–7.76 (m, 2H, H₆), 7.40–7.34 (m, 12H, H₉), 7.31 (m, 2H, H₈), 7.28–7.24 (m, 3H, H₆), 7.20 (m, 2H, H₇), 6.98 (t, J = 5.5 Hz, 1H, NH), 6.91–6.82 (m, 4H, H₈), 6.61 (m, 2H, H₉), 6.12–6.05 (m, 2H, NH), 5.85 (s, 1H, H₄), 4.11–4.07 (m, 2H, H₆), 4.06 (t, J = 5.9 Hz, 2H, H₇), 4.03–3.98 (m, 2H, H₅), 3.86 (t, J = 5.9 Hz, 2H, H₆), 3.64–3.56 (m, 6H, Hₓₓ‑ₙ), 3.40 (s, 2H, H₇), 3.39–3.35 (m, 4H, Hₓₓ,e), 3.26–3.21 (app dd, J = 11.6, 5.8 Hz, 2H, H₅), 1.37 (s, 9H, Hₓ). ¹³C NMR (151 MHz, DMSO) δ 166.08, 166.08, 165.76, 165.76, 155.68, 152.57, 152.51, 144.11, 143.99, 143.99, 138.81, 138.81, 134.81, 134.81, 134.64, 134.64, 132.26, 129.67, 129.14, 128.18, 127.98, 127.94, 127.04, 126.93, 126.72, 124.92, 124.92, 123.64, 123.63, 115.43, 115.43, 108.95, 108.95, 102.90, 102.90, 102.13, 77.76, 67.13, 66.83, 66.37, 64.98, 43.28, 43.20, 39.10, 39.10, 38.44, 38.39, 35.82, 28.24. LRMS (ESI) m/z calcd for CₙH₂₈N₄O₈ [M–H]⁻: 1177.47, found: 1177.80.

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Aldehyde 22 (605 mg, 1.21 mmol) was taken up in EtOH (21 mL) and a solution of LiOH·H₂O (101 mg, 2.42 mmol) in water (7 mL) was added. The resulting emulsion was left to stir overnight before it was quenched with 0.5 M aqueous HCl (20 mL) and extracted with EtOAc (1 × 80 mL, 2 × 20 mL). The organic layers were combined and concentrated under reduced pressure to obtain 40 (571 mg, quantitative yield) as a white solid. Mp 82 °C. R₇ 0.15 (CH₃Cl/MeOH, 9:1). ¹H NMR (600 MHz, DMSO): δ 13.61 (br s, 1H, OH), 10.14 (s, 1H, H₁), 9.14 (m, 1H, NH), 8.72 (s, 1H, H₄), 8.61 (s, 1H, H₅), 8.57 (s, 1H, H₆), 6.98 (m, 1H, NH), 6.89 (d, J = 8.4 Hz, H₆ or H₁), 6.85 (d, J = 8.7 Hz, H₁ or H₆), 4.08 (t, J = 5.5 Hz, 2H, H₄), 3.87 (t, J = 5.5 Hz, 2H, H₅), 3.69–3.60 (app dd, J = 10.3, 4.9 Hz, 2H, H₆), 3.28–3.20 (m, 2H, H₇), 1.37 (s, 9H, H₈). ¹³C NMR (151 MHz, DMSO): δ 192.53, 166.10, 164.75, 155.69, 152.60, 152.52, 136.62, 135.53, 133.17, 132.79, 132.13, 131.51, 115.46, 115.43, 77.77, 66.84, 66.34, 39.94, 39.94, 28.25. LRMS (ESI) m/z calcd for C₂₄H₂₇N₂O₈ [M+Na]⁺: 495.2, found: 495.1. HRMS (ESI) m/z calcd for C₂₄H₂₇N₂O₈ [M–H]⁻: 471.1762, found: 471.1770.
reduced pressure to yield MeOH/CH$_2$Cl$_2$ (16). Calculated: 1185.48 (100), 1186.48 (78), 1187.68 (46), 1188.18 (18). LRMS (ESI) measured isotopic distribution for C$_{66}$H$_{50}$N$_{8}$NaO$_{12}$S [M+Na]$^+$: 1185.09 (100), 1186.18 (76), 1187.09 (46), 1188.18 (16). Calculated: 1185.48 (100), 1186.48 (78), 1187.48 (37), 1188.18 (18).

Subsequently, HOBt hydrate (234 mg anhydrous basis, 1.73 mmol) and EDCI·HCl (332 mg, 1.73 mmol) were added. The reaction mixture was left to stir overnight at rt. The next day another portion of HOBt hydrate (49 mg anhydrous basis, 0.36 mmol) and EDCI·HCl (69 mg, 0.36 mmol) was added. The mixture was stirred for another 2 h and concentrated under reduced pressure. The residue was purified by flash column chromatography (CH$_2$Cl$_2$/MeOH, 1:0→95:5) to afford 41 (352 mg, 42% yield) as a yellow solid. R$_f$ 0.65 (CH$_2$Cl$_2$/MeOH, 9:1). Mp 100 °C. $^1$H NMR (400 MHz, Acetone): δ 9.99 (s, 1H, H$_{d}$), 8.75 (s, 1H, H$_{i}$), 8.65 (m, 1H, NH), 8.56 (m, 1H, NH), 8.53–8.49 (m, 2H, NH, H$_{i}$), 8.48 (s, 1H, H$_{i}$), 8.40 (s, 1H, H$_{i}$), 7.92 (s, 1H, H$_{i}$), 7.81 (s, 1H, H$_{j}$), 7.43 (d, $\delta$ = 7.7 Hz, 6H, H$_{b}$), 7.37–7.27 (m, 8H, H$_{d,i}$), 7.25–7.16 (m, 5H, H$_{d,i}$), 6.84–6.76 (m, 4H, H$_{i}$), 6.58 (m, 2H, H$_{i}$), 6.24 (t, $\delta$ = 5.3 Hz, 1H, NH), 5.91–5.67 (br s, 2H, NH), 4.32 (q, $\delta$ = 7.0 Hz, 2H, H$_{a}$), 4.09 (m, 2H, H$_{i}$), 3.93 (t, $\delta$ = 5.3 Hz, 2H, H$_{i}$), 3.90–3.75 (m, 6H, H$_{e,f,g}$), 3.60–3.47 (m, 4H, H$_{f,f'}$), 3.46–3.37 (m, 4H, H$_{c}$), 1.42 (s, 9H, H$_{1}$), 1.32 (t, $\delta$ = 7.1 Hz, 3H, H$_{b}$). $^{13}$C NMR (101 MHz, Acetone) δ 191.94, 167.64, 166.96, 166.27, 165.81, 156.64, 153.73, 153.62, 145.17, 144.88, 144.84, 139.10, 137.37, 136.45, 136.38, 136.38, 136.05, 133.00, 132.91, 132.21, 131.52, 131.04, 130.70, 130.16, 128.72, 127.57, 127.47, 125.88, 125.88, 124.77, 124.77, 116.04, 116.02, 109.76, 109.72, 104.03, 104.00, 78.74, 68.24, 67.90, 67.34, 61.70, 44.98, 44.83, 40.65, 40.45, 39.94, 39.85, 36.74, 28.56, 14.53. LRMS (ESI) measured isotopic distribution for C$_{66}$H$_{50}$N$_{8}$NaO$_{12}$S [M+Na]$^+$: 1185.09 (100), 1186.18 (76), 1187.09 (46), 1188.18 (16). Calculated: 1185.48 (100), 1186.48 (78), 1187.48 (37), 1188.18 (18).

Trimethyl orthoformate (100 µL) and 3 drops of TFA were added to a solution of 41 (340 mg, 0.29 mmol) in MeOH/CH$_2$Cl$_2$ (6 mL, 1:1). The resulting mixture was stirred under nitrogen overnight at rt. The next day the mixture was concentrated by blowing nitrogen over it and dried under reduced pressure to yield 42 (354 mg, quantitative yield) as an orange solid, which was used
without further purification. Mp 92 °C. Rf 0.65 (CH₂Cl₂/MeOH, 9:1). ¹H NMR (600 MHz, Acetone): δ 8.40 (s, 1H, Hₐ), 8.39 (t, J = 5.7 Hz, 1H, NH), 8.36–8.33 (m, 2H, Hₐ, NH), 8.23 (t, J = 5.4 Hz, 1H, NH), 8.10 (s, 1H, Hₐ), 8.08 (s, 1H, Hₐ), 7.89 (s, 1H, Hₐ), 7.83 (s, 1H, Hₐ), 7.45 (d, J = 7.9 Hz, 6H, Hₐ), 7.37–7.30 (m, 8H, Hₐ), 7.25 (m, 3H, Hₐ), 7.23–7.20 (m, 2H, Hₐ), 6.89–6.82 (m, 4H, Hₐ), 6.61 (m, 2H, Hₐ), 6.15 (m, 1H, NH), 5.85–5.78 (m, 2H, NH), 5.44 (s, 1H, Hₐ), 4.35 (q, J = 7.1 Hz, 2H, Hₐ), 4.12 (t, J = 5.8 Hz, 2H, Hₐ), 3.95 (t, J = 5.7 Hz, 2H, Hₐ), 3.86–3.80 (m, 4H, Hₐ, H), 3.79–3.74 (app q, J = 5.7 Hz, 2H, Hₐ), 3.27 (s, 6H, Hₐ, 1.40 (s, 9H, Hₐ), 1.36 (t, J = 7.1 Hz, 3H, Hₐ). ¹³C NMR (151 MHz, Acetone): δ 167.90, 167.52, 166.96, 165.96, 156.70, 154.03, 153.98, 145.42, 145.19, 145.15, 140.33, 139.37, 136.41, 135.92, 133.09, 133.02, 131.82, 130.93, 130.39, 129.07, 129.01, 128.89, 127.76, 127.53, 126.98, 125.98, 125.97, 124.98, 124.98, 116.27, 116.25, 109.80, 109.75, 103.99, 103.99, 103.12, 78.76, 68.44, 68.12, 67.62, 61.80, 53.00, 45.35, 45.24, 40.78, 40.34, 39.90, 39.88, 36.91, 28.59, 14.60. LRMS (ESI) measured isotopic distribution for C₇₀H₆₆N₆O₃S [M+Na]⁺: 1231.33 (100), 1232.33 (83), 1233.33 (41), 1234.33 (16). Calculated: 1232.52 (100), 1232.52 (80), 1233.52 (38), 1234.53 (14).

Acetal 42 (345 mg, 0.30 mmol) was taken up in THF (6 mL) and a solution of LiOH·H₂O (26 mg, 0.61 mmol) in water (2 mL) was added. The resulting emulsion was left to stir overnight before it was quenched with 0.5 M aqueous HCl (10 mL) and extracted with CHCl₃/2-propanol (3 × 25 mL). The organic layers were combined and concentrated under reduced pressure to obtain 43 (341 mg, quantitative yield) as an orange solid, which was used without further purification. Mp 183 °C. Rf 0.43 (CH₂Cl₂/MeOH, 9:1). ¹H NMR (600 MHz, DMSO): δ 10.13 (s, 1H, Hₐ), 9.15 (t, J = 5.5 Hz, 1H, NH), 9.12 (t, J = 5.4 Hz, 1H, NH), 8.93 (t, J = 5.5 Hz, 1H, NH), 8.74 (s, 1H, Hₐ), 8.55 (m, 2H, Hₐ), 8.30 (s, 1H, Hₐ), 7.85 (s, 1H, Hₐ), 7.77 (s, 1H, Hₐ), 7.44–7.16 (m, 19H, Hₐ), 7.00–6.95 (m, 1H, NH), 6.92–6.74 (m, 6H, Hₐ), 4.09 (t, J = 5.8 Hz, 2H, Hₐ), 3.87 (t, J = 5.8 Hz, 2H, Hₐ), 3.69–3.54 (m, 6H, Hₐ), 3.49–3.35 (m, 6H, Hₐ), 3.27–3.19 (m, 2H, Hₐ), 1.37 (s, 9H, Hₐ). LRMS (ESI) m/z calcd for C₆₆H₆₆N₆O₃S [M–H]⁻: 1133.45, found: 1133.67.
DIPEA (101 μL, 0.580 mmol), HOBr hydrate (59 anhydrous basis, 0.435 mmol) and EDCI-HCl (83 mg, 0.435 mmol) were added to a solution of 43 (329 mg, 0.290 mmol) and 23 (152 mg, 0.348 mmol) in DMF (1.5 mL). The reaction mixture was left to stir overnight at rt. The next day another portion of HOBr hydrate (20 mg anhydrous basis, 0.145 mmol) and EDCI-HCl (28 mg, 0.145 mmol) was added. After the mixture had stirred for another 2 h it was diluted with CHCl₃/2-propanol 3:1 (20 mL) and washed with 0.5 M aqueous HCl (10 mL) and 5% aqueous LiCl (10 mL). The organic layer was dried (MgSO₄) and concentrated under reduced pressure. The residue was purified by flash column chromatography (CH₂Cl₂/MeOH, 1:0→9:1) to afford 44 (165 mg, 37% yield) as a yellow solid. Rₜ 0.53 (CH₂Cl₂/MeOH, 9:1).Mp 121 °C. ¹H NMR (600 MHz, Acetone): δ 10.11 (s, 1H, Hₑor’e), 10.05 (s, 1H, Hₑor’e), 8.74 (s, 1H, Hₓ), 8.67 (s, 1H, Hₓ), 8.59 (s, 1H, Hₓ), 8.56 (s, 1H, Hₓ), 8.54–8.49 (m, 2H, NH), 8.49 (s, 1H, Hₓ), 8.47 (s, 1H, Hₓ), 8.39 (t, J = 5.5 Hz, 1H, NH), 8.28 (t, J = 5.7 Hz, 1H, NH), 8.22 (s, 1H, Hₓ), 8.10 (t, J = 5.5 Hz, 1H, NH), 7.76 (s, 1H, Hₓ), 7.74 (s, 1H, Hₓ), 7.40 (d, J = 7.40 Hz, 6H, Hₓ), 7.32–7.26 (m, 8H, Hₓ,y), 7.21 (m, 3H, Hₓ), 7.16 (m, 2H, Hₓ), 6.85–6.79 (m, 8H, Hₓ), 6.57 (d, J = 7.7 Hz, 1H, Hₓ), 6.54 (d, J = 7.6 Hz, 1H, Hₓ), 6.16 (m, 1H, NH), 5.81–5.71 (m, 2H, NH), 4.38 (q, J = 7.1 Hz, 2H, Hₓ), 4.12–4.08 (m, 4H, Hₑor’or’e’), 4.05 (t, J = 5.6 Hz, 2H, Hₑor’or’e’), 3.93 (t, J = 5.7 Hz, 2H, Hₓ), 3.86–3.83 (app dd, J = 11.5, 5.7 Hz, 2H, Hᵣᵣ’,ᵣ’,or’e’), 3.81–3.76 (m, 6H, Hᵣᵣ’,ᵣ’,or’e’), 3.74–3.69 (m, 2H, Hᵣᵣ’,ᵣ’,or’e’), 3.54–3.43 (m, 4H, H₄d,d’), 3.40 (app dd, J = 11.7, 5.9 Hz, 2H, Hₓ), 3.37 (s, 2H, Hₓ), 1.39 (s, 9H, Hₓ), 1.36 (t, J = 7.1 Hz, 3H, Hₓ). ¹³C NMR (151 MHz, Acetone) δ 191.31, 191.20, 167.13, 166.17, 166.16, 165.37, 165.10, 164.55, 155.86, 153.14, 153.10, 153.05, 153.03, 144.54, 144.27, 144.20, 137.94, 137.04, 136.84, 136.11, 135.92, 135.88, 135.27, 135.21, 133.07, 132.28, 131.76, 131.64, 131.42, 130.45, 130.34, 130.29, 129.47, 128.01, 126.85, 125.13, 125.13, 124.87, 124.87, 124.09, 124.07, 115.41, 115.41, 115.40, 115.36, 109.01, 108.92, 103.26, 103.17, 77.93, 67.37, 67.23, 66.76, 66.68, 66.65, 61.41, 44.48, 44.22, 39.91, 39.69, 39.65, 39.50, 39.19, 39.05, 36.22, 27.75, 13.69. LRMS (ESI) measured isotopic distribution for C₈₀H₇₆N₂O₁₅S [M−H]−: 1515.25 (100), 1516.33 (100), 1517.25 (57), 1518.17 (26), 1519.50 (9). Calculated: 1515.60 (100), 1516.60 (100), 1517.61 (57), 1518.61 (24), 1519.61 (8).
2-(pyridin-2-yl)disulfanyl)ethan-1-ol (45)

Under an atmosphere of nitrogen 2,2′-dipyridyl disulfide (9.42 g, 42.8 mmol) was dissolved in degassed MeOH (20 mL). Subsequently, 2-mercaptoethanol (1.00 mL, 14.2 mmol) was added dropwise. The solution turned yellow and was allowed to stir for 2 h at rt. The mixture was concentrated under reduced pressure and was purified by flash chromatography (PhMe/EtOac 1:0→1:2) to afford 45 (2120 mg, 80% yield) as a colourless oil.

Analyses in agreement with the literature.14

A spatula tip of 2,2′-dipyridyl disulfide and 5 drops of n-butylamine were added to a solution of aldehyde 44 (12.5 mg, 0.0082 mmol) in CH2Cl2 (2 mL). The mixture was put on ice and TFA (2 mL) was added carefully. After 10 min n-butanol (300 μL) was added and the reaction mixture was stirred for another 2 h at rt. Upon completion, the reaction was quenched with a small amount of n-butanol until the mixture became pale yellow or colourless. De resulting solution was then concentrated until approximately 2 mL of solvent remained. The product was precipitated from Et2O/heptane (1:1) and collected. The crude was washed with Et2O and water respectively before it was taken up in a small amount of THF containing 1 drop of 2M aq. HCl. The resulting solution was stirred for 5 min, concentrated and washed once more with water to yield 48 (9 mg, 83% yield) as a pale brown solid. 1H NMR (600 MHz, DMSO): δ 10.14 (s, 1H, H₆₋₁₇), 10.13 (s, 1H, H₆₋₁₇), 9.17 (t, J = 5.1 Hz, 1H, NH), 9.13–9.08 (m, 2H, NH), 8.83 (t, J = 5.5 Hz, 1H, NH), 8.79 (t, J = 5.1 Hz, 1H, NH), 8.70 (s, 1H, H₅), 8.69 (s, 1H, H₄), 8.63 (s, 1H, H₃), 8.58 (s, 1H, H₂), 8.55 (s, 1H, H₁), 8.53 (s, 1H, H₀), 8.36 (br d, J = 4.6 Hz, 1H, H₉), 8.23 (s, 1H, H₈), 7.96 (m, 2H, H₇), 7.94 (br s, 3H, NH), 7.68–7.64 (dd, J = 8.1, 7.2 Hz, 1H, H₆), 7.59 (d, J = 8.1 Hz, 1H, H₅), 7.35–7.29 (m, 2H, H₄), 7.23–7.18 (m, 2H, H₃), 7.13 (dd, J = 7.2, 4.9 Hz, 1H, H₂), 6.96–6.84 (m, 8H, H₁), 6.64–6.60 (m, 2H, H₀), 6.11 (br s, 2H, NH), 4.39 (q, J = 7.1 Hz, 2H, H₈), 4.22 (s, 2H, H₇), 4.11–4.01 (m, 8H, H₆₋₁₇), 3.68–3.55 (m, 10H, H₁₋₃, H⁵₋⁷), 3.42–3.34 (m, 4H, H₄₋₅), 3.18 (app dd, J = 10.2, 5.1 Hz, 2H, H₆), 1.36 (t, J = 7.1 Hz, 3H, H₇). 13C NMR (151 MHz, DMSO): δ 193.02, 192.82, 166.55, 166.20, 165.81, 165.58, 165.09, 164.98, 159.00, 153.41, 153.01, 152.96, 152.43, 149.88, 144.43, 144.36, 137.92, 137.85, 137.12,
136.81, 136.08, 135.99, 135.83, 135.20, 135.05, 133.41, 132.98, 132.21, 132.15, 131.55, 131.35, 130.95, 130.91, 125.69, 125.69, 125.39, 125.39, 124.11, 124.11, 121.60, 119.78, 116.18, 115.95, 115.91, 109.49, 109.49, 103.40, 103.40, 66.86, 66.82, 66.79, 65.35, 61.99, 43.72, 43.54, 42.24, 38.97, 38.93, 38.93, 38.86, 38.84, 14.61. LRMS (ESI) measured isotopic distribution for $\text{C}_{68}\text{H}_{70}\text{N}_{3}\text{O}_{13}\text{S}_{2}$ [M+H]$^+$: 1284.50 (100), 1285.33 (79), 1286.33 (42), 1287.33 (16), 1288.33 (6). Calculated: 1284.45 (100), 1285.46 (80), 1286.46 (43), 1287.46 (17), 1288.46 (6).

49

DIPEA (17 μL, 0.091 mmol), HOBt hydrate (6 mg anhydrous basis, 0.041 mmol) and EDCI-HCl (8 mg, 0.041 mmol) were added to a solution of 12 (34 mg, 0.0406 mmol) in DMF (150 μL). The reaction mixture was stirred for 1 h before a solution of 48 (40 mg, 0.030 mmol) in DMF (150 μL) was added dropwise. The reaction mixture was left to stir overnight at rt and was concentrated the next day. The resulting solid was taken up in a small amount of CH$_2$Cl$_2$ and the product precipitated from MeOH. The crude was purified by preparative thin layer chromatography (7.5% EtOH in CH$_2$Cl$_2$) to yield 49 (10 mg, 15% yield) as a pale brown solid.

$^3$H NMR (600 MHz, DMSO): δ 10.14 (s, 1H, H$_{e or e'}$), 10.12 (s, 1H, H$_{e or e'}$), 9.47 (br s, 1H, NH), 9.16 (t, J = 5.5 Hz, 1H, NH), 9.10–9.04 (m 2H, NH), 8.91 (t, J = 5.6 Hz, 1H, NH), 8.81 (t, J = 5.7 Hz, 1H, NH), 8.77 (t, J = 5.6 Hz, 1H, NH), 8.70–8.63 (m, 7H, NH, H$_{w,y}$), 8.62 (s, 1H, H$_{a}$), 8.57 (s, 1H, H$_{a}$), 8.53 (s, 1H, H$_{a}$), 8.52 (s, 1H, H$_{a}$), 8.36 (d, J = 4.5 Hz, 1H, H$_{m}$), 8.32 (s, 1H, H$_{b}$), 8.22 (s, 1H, H$_{b}$), 8.04 (s, 1H, H$_{b}$), 8.02 (s, 1H, H$_{b}$), 7.96 (s, 1H, H$_{b}$), 7.95 (s, 1H, H$_{b}$), 7.65 (app td, J = 7.7, 1.6 Hz, 1H, H$_{m}$), 7.58 (d, J = 7.8 Hz, 1H, H$_{m}$), 7.28–7.25 (m 2H, H$_{b}$), 7.20–7.16 (m, 2H, H$_{b}$), 7.12 (dd, J = 7.3, 5.0 Hz, 1H, H$_{m}$), 6.92–6.87 (m, 8H, H$_{b}$), 6.60–6.57 (m, 2H, H$_{b}$), 6.06 (m, 2H, NH), 5.97 (dd, J = 8.7, 5.1 Hz, 1H, H$_{a}$), 4.39 (q, J = 7.1 Hz, 2H, H$_{k or k'}$), 4.34 (q, J = 7.1 Hz, 2H, H$_{k or k'}$), 4.22 (s, 2H, H$_{b}$), 4.11–4.01 (m, 12H, H$_{e,g,i,g',i',h,r}$), 3.67–3.56 (m, 15H, H$_{c,f,r,r',r'',a}$), 3.45–3.31 (m, 5H, H$_{d,d',d''}$), 3.25 (dd, J = 14.6, 8.8 Hz, 1H, H$_{e}$), 2.11 (m, 2H, H$_{b}$), 1.71–1.64 (m, 2H, H$_{b}$), 1.61–1.55 (m, 2H, H$_{b}$), 1.38–1.31 (m, 15H, H$_{f,x}$). LRMS (MALDI) measured isotopic distribution for...
C_{107}H_{108}N_{13}O_{25}S_{4} [M+H]^+: 2103 (66), 2104 (100), 2105 (75), 2106 (49), 2107 (21), 2108 (9).
Calculated: 2103 (76), 2104 (100), 2105 (81), 2106 (50), 2107 (25), 2108 (10).

Figure 48: MALDI simulated (top) and found (bottom) isotopic distribution for 49.

51 (50)

Boc protected hydrazide 49 (1 mg, 0.475 μmol) was taken up in CH_{2}Cl_{2} (2.5 mL) and 1.25 M HCl MeOH (2.5 mL) was added. The reaction mixture was stirred at rt for 2 d, diluted with CH_{2}Cl_{2} (10 mL) and quenched with a 1 M aqueous NaHCO_{3} solution (10 mL). The organic layer was collected, dried (MgSO_{4}) and concentrated. The resulting solid was washed with water and Et_{2}O to yield hydrazide intermediate 50 (0.8 mg, 88% yield) as a pale gray solid. The hydrazide was then taken up in CH_{2}Cl_{2} (4 mL) and a catalytic amount of p-toluenesulfonic acid was added. The reaction mixture was stirred at rt for 2 d, diluted with CH_{2}Cl_{2} (5 mL) and quenched with a 1M aqueous NaHCO_{3} solution (5 mL). The organic layer was collected, dried (MgSO_{4}) and concentrated. The resulting solid was washed with water and Et_{2}O to yield dialdehyde 51 (0.6 mg, 78% yield) as a pale gray solid. ^{1}H NMR (600 MHz,
DMSO): δ 10.14 (s, 1H, H_{e or e'}), 10.12 (s, 1H, H_{e or e'}), 9.16 (t, J = 5.3 Hz, 1H, NH), 9.10–9.03 (m, 2H, NH), 8.90 (t, J = 5.3 Hz, 1H, NH), 8.81 (t, J = 5.6 Hz, 1H, NH), 8.77 (t, J = 5.3 Hz, 1H, NH), 8.71–8.61 (m, 7H, H_{a,x}), 8.57 (s, 1H, H_a), 8.53 (s, 1H, H_a), 8.52 (s, 1H, H_a), 8.36 (d, J = 3.8 Hz, 1H, H_m), 8.31 (s, 1H, H_a), 8.22 (s, 1H, H_a), 8.03 (s, 1H, H_a), 8.02 (s, 1H, H_a), 7.96 (s, 1H, H_a), 7.95 (s, 1H, H_a), 7.65 (app td, J = 8.0, 1.8 Hz, 1H, H_m), 7.58 (d, 1H, J = 8.1 Hz, 1H, H_m), 7.28–7.25 (m, 2H, H_p), 7.20 (br s, NH), 7.19–7.15 (m, 2H, H_p), 7.12 (dd, J = 7.3, 4.8 Hz, 1H, H_m), 6.92–6.84 (m, 8H, H_p), 6.69–6.64 (br s, NH), 6.60–6.57 (m, 2H, H_p), 6.05 (m, 2H, NH), 5.97 (dd, J = 8.8, 5.1 Hz, 1H, H_p), 4.39 (q, J = 7.1 Hz, 2H, H_{k or k'}), 4.34 (q, J = 7.1 Hz, 2H, H_{k or k'}), 4.22 (s, 2H, H_a), 4.11–3.98 (m, 12H, H_{a,d,e,e',h,i,l}), 3.67–3.57 (m, 15H, H_{c,c',f,f',h,i,q}), 3.42–3.29 (m, 5H, H_{d,e,o}), 3.25 (dd, J = 14.5, 8.9 Hz, 1H, H_o), 2.36 (t, J = 7.1 Hz, 2H, H_p), 1.67 (quint, J = 7.0 Hz, 2H, H_i), 1.60 (quint, J = 7.0 Hz, 2H, H_i), 1.38–1.31 (m, 6H, H_{i,f}).
References


4. DFT calculations were performed at the standard B3LYP/6-31G(d) level to calculate the ground state geometry and the energy of the molecules. The electrostatic potential maps have been computed setting the parameter IsoValue as 0.002.


Synopsis: Since the first mechanically interlocked molecule, a catenane, was reported many elegant methods have been developed to acquire these types of architectures. With the knowledge we have today even the most complex interlocked molecules can be made. Perhaps the most versatile interlocked architecture, the rotaxane, has been extensively used in molecular shuttles, (switchable) catalysts, protecting groups and molecular machines. The latest strategy to synthesise rotaxanes, the active metal template approach, is discussed in more detail.
Traditional methods for rotaxane synthesis

Since Wasserman reported\(^1\) the synthesis of the first mechanically interlocked molecule (a catenane) in 1960 many new and more elegant strategies to obtain these types of structures have been developed. Mechanically interlocked molecules can be divided in the group of catenanes and rotaxanes. The former can be described as a molecule containing two or more interlocked rings or macrocycles, whereas the latter consists of a macrocycle that is threaded by a linear molecular chain. These chains contain bulky groups or so called stoppers at both ends so that the macrocycle can’t slide off and the architecture and is truly mechanically interlocked. Schematic representations of both a catenane and rotaxane are depicted in figure 1.

![Figure 1: Schematic view of a catenane (left) and a rotaxane (right).](image)

Higher order catenanes and rotaxanes, wherein the architecture contains more than two interlocked components, also exist (figure 2).\(^2\)

![Figure 2: A double threaded macrocycle (left) and a thread enclosed by two macrocycles (right) are both [3]rotaxanes.](image)

In 1967 I. T. Harrison and S. Harrison reported the first rotaxane.\(^3\) Herein, the interlocked structure was formed by the treatment of a resin bound macrocycle with a solution of chain and stopper components. During the process the chain and stopper moieties would mostly react to form a non-interlocked stoppered chain, however a very small amount would by
chance react through the cavity of the macrocycle and yield a rotaxane. Because the macrocycle was bound to a resin the yield was improved by repeating this process seventy times and in the end 6% of the macrocycle was converted to rotaxane. This so-called ‘capping’ method, where the linear chain resides in the macrocycle before bulky stoppers react en trap the chain, is still widely applied today, albeit in a more elegant manner (figure 3).

Figure 3: Capping of the thread.

Nowadays secondary interactions \(^4,^5,^6\) between the macrocycle and thread such as, \(\pi-\pi\) stacking \(^7\), hydrogen-bonding \(^8,^9\), metal-ligand \(^10\), ionic \(^11\), cyclodextrin \(^12,^13\) and hydrophobic \(^14\) interactions can be used to successfully template the individual components to form a pseudo-rotaxane. These non-mechanically interlocked structures can then be capped with bulky stoppers to yield a true rotaxane (figure 4).

Figure 4: A secondary interaction templates unstoppered thread and macrocycle.

For example Stoddart used hydrogen-bonding interactions between a crown ether-like macrocycle and a thread comprising a protonated benzylic amine moiety to form a pseudo interlocked structure, which was then capped to yield a rotaxane based molecular shuttle (figure 5). \(^15\)
The thread in this molecular shuttle also included a bipyridinium functionality, which allowed the macrocycle to travel between the NH$_2^+$ and BIPY$^{2+}$ station upon treatment with acid or base. Hydrogen-bonding interactions between the macrocycle and NH$_2^+$ station were stronger but upon deprotonation of the NH$_2^+$ species ionic interactions between the macrocycle and bipyridinium functionality became more favourable and the macrocycle would move to the bipyridinium station. Alternating acid and base stimuli allowed the macrocycle to shuttle between the two stations. Later a more complex molecular elevator was constructed and operated via the same principle.$^{16}$

Metal-ligand interactions can also be used as a powerful tool to afford rotaxanes in high yields. In 2004 Leigh et al. used a selection of divalent metal ions (Cu$^{2+}$, Zn$^{2+}$, Co$^{2+}$ etc.) to template a bis-amine macrocycle with 2,6-diformylpyridine in the presence of an aniline stopper (figure 6).$^{17}$
Figure 6: Reversible imine formation yields the thermodynamically favoured rotaxane.

Through reversible imine formation the thermodynamically favoured octahedral metal-rotaxane complex was formed in moderate to excellent yields. This was the first example of rotaxane formation around an octahedral complex. Later the same group reported the synthesis of rotaxanes and catenanes with Co$^{3+}$, a more challenging trivalent transition metal ion.$^{18}$ Instead of a neutral tridentate system like the pyridine-2,6-diimino ligand, which is described above and very suitable for soft divalent metal ions, a hard trivalent metal ion chelated by a bis-anionic pyridine-2,6-dicarboxamido ligand was chosen to template the rotaxane formation (figure 7).
Figure 7: A bis-anionic pyridine-2,6-dicarboxamido ligand binds strongly to Co(III).

The thread and pseudo macrocycle (both holding the bis-anionic pyridine-2,6-dicarboxamide motif) were first arranged around a kinetically labile Co^{2+} centre, as ligand exchange with Co^{3+} was very slow. Subsequent exposure to air resulted in oxidation of Co^{2+} to Co^{3+} and locked the complex. The stable pseudo-rotaxane was isolated and closed (clipped) via ring closing metathesis to yield the mechanically interlocked structure (figure 8).

Figure 8: The macrocycle is mechanically locked via the clipping mechanism.
The active metal template (AMT) strategy

Despite the elegance and high yields of this so-called passive metal template strategy a few drawbacks exist. Stoichiometric amounts of metal salts are required and both components (thread and macrocycle) need to bear a recognition motif in order for system to assemble. In 2006 the group of Leigh developed a new approach named the active metal template (AMT) strategy (figure 9).19,20

Figure 9: The metal that catalyses the bond formation between half-threads resides in the pocket of the macrocycle.

Herein, a Cu(I)-catalysed 1,3-dipolar Huisgen cycloaddition facilitated the rotaxane formation. The reactions components included a pyridine based macrocycle and an azide and alkyne half-thread (stopper). Co-ordination of the Cu$^{1+}$ ion to the monodentate ligand encouraged the Cu(I)-catalysed catalysed cycloaddition between the terminal alkyne and azide half-threads to occur through the macrocycle’s cavity, thereby generating a mechanically captured 1,4-disubstituted triazole thread (figure 10).
Stoichiometric amounts of reactants afforded the rotaxane in 57% yield. The non-interlocked thread was formed in 41% yield, which indicated that the reaction could also take place at one face of and not through the cavity of the macrocycle. Optimal reaction conditions were found by using an excess of the less valuable stopper constituents. Adding a substoichiometric amount of the copper salt resulted in the reaction to stop after an equal amount of rotaxane was formed. This implied that the multidentate rotaxane captured the copper salt and made it unavailable for further reactions. Addition of a competing ligand like pyridine released the copper ion and allowed the catalyst to turn over. Ultimately 82% of rotaxane was obtained with only 20 mol% of the Cu(I) salt.

Further investigations showed that acyclic pyridine based ligands accelerated product formation by a factor two compared to the ligand-free Cu(I)-catalysed reaction. Interestingly, when a cyclic ligand i.e. the macrocycle was used the reaction became much slower. Since the azide-alkyne cycloaddition must happen through the cavity of the macrocycle they postulated that steric effects limited the freedom of movement or changes of geometry at the copper centre that are necessary for bond formation. Additionally, the complex could favour the reaction to proceed through a different but slower reactive
intermediate. A screening of various macrocycles including bi- and tridentate ligands showed that sterically unhindered monodentate pyridine ligands catalysed the reaction fastest and were highest yielding. The same publication also reported the unexpected formation of a [3]rotaxane (consisting of two macrocycles and one thread) at a high ratio of macrocycle/Cu(I). Molecular models suggested the reaction to occur through a bridged two copper atom intermediate.

Some years later Leigh applied the azide-alkyne cycloaddition to construct a molecular machine based on a [2]rotaxane architecture that was able to synthesis a hexapeptide via native chemical ligation.\(^{21}\)

In 2013 the group of Goldup used the Cu(I)-catalysed azide-alkyne 1,3-dipolar cycloaddition to synthesise a [2]rotaxane with a thread so short it only contained the triazole moiety (figure 11).\(^{22}\)

![Figure 11: A sterically hindered cavity protects the usually labile Cu(I) intermediate.](image)

Due to steric hindrance of the bulky substituents on the triazole group with the macrocycle, the Cu(I) triazolide intermediate was trapped and protected from reprotonation. The reaction was conducted in various solvents including EtOH and a mixture of THF/water to afford the Cu(I) triazolide in good yields. The intermediate was in fact so stable they were able to obtain a crystal structure and even when exposed to AcOH it only slowly converted to the corresponding organic rotaxane.

Shortly after the group of Leigh published their initial results on the active metal template approach Saito et al. reported the synthesis of two more rotaxanes via the same concept.\(^{23}\) Cu(I) bound to a phenanthroline macrocycle facilitated the oxidative homocoupling of an alkyne half-thread (Glaser coupling, 72% yield, C-C bond formation)\(^ {24}\) and a cross coupling reaction between an aryliodide and aliphatic thiol stopper (27% yield, C-S bond formation).\(^ {25}\)
Other metals have also been employed in AMT-synthesis. Not long after the first Cu(I) examples were presented Leigh and co-workers reported their findings in palladium catalysed approaches. Unable to get any encouraging results with Pd(0), possibly due to the poor binding of the metal to the ligand, Pd(II) chemistry was investigated. They found that Pd(OAc)$_2$ facilitated the formation of 2[rotaxanes] with a bipyridine macrocycle in an oxidative Heck-coupling quite efficiently (figure 12).

![Figure 12: Pd(II) catalysed oxidative Heck cross-coupling.](image)

The interlocked product was formed through β-H-elimination and the process generated a Pd(0) species. Oxidation of Pd(0) to Pd(II) with oxygen and benzoquinone regenerated the catalyst in situ and allowed the use of only 1 mol% of catalyst.

In another example a Pd(II)-complex was formed with a tridentate macrocycle and ethylcyanooacetate. The resulting complex was then reacted with two identical vinyl ketone stopper components via successive Michael additions to give the [2]rotaxane. The template motif was retained and allowed the macrocycle to act as a shuttle between the nitrile and ketone functionalities on the thread.

Usually shuttling of a macrocycle between two stations on an interlocked thread is slow due to the relatively strong binding motifs that originate from the strategy that is used to
assemble the rotaxane. To achieve faster switching kinetics the Leigh group constructed a rotaxane that didn’t leave strong intercomponent binding motifs.\textsuperscript{28} They applied the AMT-strategy to connect two different half-threads, one bearing an aniline motif and the other a DMAP functionality, in a Cadiot-Chodkiewicz reaction (figure 13).

![Rotaxane Diagram](image)

Figure 13: Acid/base or ion induced shuttling.

They postulate that in this case classic (passive) approaches wouldn’t have been sufficient to template the components and mechanically interlock them by capping or clipping due to the weak interaction between the bipyridine unit on the macrocycle and the aniline moiety on the thread. Shuttling from the aniline to the DMAP station was accomplished through protonation of the DMAP moiety or by addition of LiI. The macrocycle returned to its starting position when the solution was neutralised or the metal salt removed.

Another synthetic challenge was concluded when the same researchers reported the formation of a [2]rotaxane via a Lewis acid catalysed Diels-Alder reaction.\textsuperscript{29} An acryloyl imidazolidone and cyclopentadiene half-thread were reacted through a Cu(II)- or Zn(II)-bound macrocycle. Interestingly, the diene derivative existed as an almost equimolar mixture of 1- and 2-substituted isomers under the reaction conditions but only the 2-
substituted isomer reacted through the macrocycle’s cavity to yield the corresponding interlocked Diels-Alder adduct. Additionally, a significant amount of 1,4-isomer was found as opposed to the 1,3-isomer (9:1). When the reaction was carried out with an acyclic ligand all four isomers of the non-interlocked thread were isolated. By incorporating a pyridine unit in the acryloyl imidazolidone half-thread the macrocycle of the resulting [2]rotaxane was able to shuttle between the pyridine and imidazolidone moiety upon addition of PdCl$_2$ or Zn(OTf)$_2$ (figure 14).

Figure 14: Pd/Zn induced shuttling.

After Cu(I), Zn(II) and Pd(II) chemistry Leigh applied the AMT-strategy to furnish 1,4-diyne [2]rotaxanes via an unusual Ni/Cu bimetallic alkyne homocoupling.$^{30}$ A Li-acetylide was formed in situ and a solution of Ni(II)-macrocycle complex was added. Next, Cu(I) was added and the reaction mixture was heated to form the [2]rotaxane. The sequence of addition was
crucial and they found that leaving either copper or nickel out resulted in poor yields. Furthermore, in the absence of nickel low selectivity for the formation of [2]rotaxane over non-interlocked product was seen. A proposed mechanism is depicted in figure 15.

![Mechanism](image)

Figure 15: Simplified mechanism of Ni/Cu bimetallic alkyne homocoupling.

Shortly after, nickel was utilised in a combination with zinc to template the homocoupling of unactivated $sp^3$-$sp^3$ alkyl bromides (figure 16).
They postulated that Ni(0) bound to the macrocycle underwent oxidative addition of one alkylbromide half-threads to give the Ni(II)-complex. Reduction by Zn(0) then generated the intermediate Ni(I) species and ZnBr₂. Another oxidative addition of the bromide half-thread gave the Ni(III)-complex followed by a reductive elimination to furnish the desired interlocked molecule. To complete the catalytic cycle the remaining Ni(I) was reduced once more to Ni(0).
In 2013 Saito et al. developed a method to afford [2]rotaxanes by a Cu(I)-catalysed Sonogashira-type cross-coupling using a phenanthroline macrocycle. They noticed that the substitution pattern of the ester group on the aryl iodide component had a significant influence on the reaction rate, yield, free thread formation and homocoupling.

In 2012 Anderson and co-workers employed a phenanthroline macrocycle to capture a C8, C12 or C20 polyyne chain. When a rhenium-macrocyle complex was prepared normal luminescence behavior was detected. In contrast, the luminescence of the rhenium [2]rotaxane-complex was quenched as a result of the polyyne thread being in close proximity to the macrocyle-bound rhenium.

A few years later the same researchers synthesised two [9]cumulene rotaxanes by treating the corresponding polyyne rotaxanes with SnCl₂ and HCl (figure 17).
Cumulenes, especially the larger ones, are notoriously difficult to handle and in case of [9]cumulene stabilisation with bulky end-groups is not effective enough to allow a comprehensive analysis of the linear molecule. The enclosed [9]cumulene thread was found to be much more stable than its free counterpart and allowed analysis by quantitative UV/Vis spectroscopy, cyclic voltammetry and differential scanning calorimetry.
**Aim and objectives – New AMT approaches and functional rotaxanes**

Many metal catalysed reactions, such as alkyne homocouplings, palladium catalysed oxidative Heck cross-couplings, nickel catalysed sp$^1$-sp$^3$ homocouplings, Sonogashira cross-couplings and Diels-Alder reactions, have successfully been applied in the AMT synthesis of both rotaxanes and catenanes. Different reactions allow the synthesis of rotaxanes through different kinds of bond formations (C-C, C-S, triazole). Using a new reaction we are interested in making the first rotaxane through C-N bond formation. One of key selling points of the AMT strategy is that only the macrocycle requires a recognition motif to assemble the interlocked molecule. Consequently, only one idle functionality remains in the architecture. The fully exploit this remaining motif we are interested in using the rotaxane as a ligand for catalysis. If a chiral macrocycle is used to assemble the rotaxane it should be possible to employ it in metal-catalysed asymmetric catalysis. A few examples where rotaxanes are used as catalysts already exist. These typically utilise the thread-component as catalytic unit, where the macrocycle-component enhances the selectivity. In a rotaxane switchable catalyst system the macrocycle-component can function as a shuttle to (de)activate the catalyst or allow one catalytic reaction over the other by shuttling between the functional/catalytic sites on the thread. We aim to use the chiral macrocycle-component of the rotaxane as a ligand for a metal catalyst. The thread-component would function to induce additional stereoselectivity. This would then be the first example of a rotaxane ligand employed in asymmetric metal catalysis.
References


Synopsis: We report on the active template synthesis of a [2]rotaxane through a Goldberg copper-catalysed C–N bond forming reaction. A C2-symmetric cyclohexylidiamine macrocycle directs the assembly of the rotaxane, which can subsequently serve as a ligand for enantioselective nickel-catalysed conjugate addition reactions. Rotaxanes are a previously unexplored ligand architecture for asymmetric catalysis. We find that the rotaxane gives improved enantioselectivity compared to a non-interlocked ligand, at the expense of longer reaction times.


Acknowledgements: I’d like to thank all authors for their contribution and support. I’ve been involved in the project from the start until the end and have synthesised all compounds at least once.
Goldberg active template synthesis of a [2]rotaxane ligand for asymmetric transition-metal catalysis

The active metal template synthesis of rotaxanes utilizes both a metal ion’s preferred coordination geometry (to direct the assembly of a threaded intermediate) and its ability to catalyse chemical reactions (to covalently capture the interlocked molecular architecture). Advantages of this approach compared to classical ‘passive’ metal template synthesis include that (i) the traditionally separate ‘threading’ and ‘covalent capturing’ processes are combined in a single reaction, and (ii) the metal catalyst can often turn over, meaning that only sub-stoichiometric quantities of the template may be required. In addition, it is unnecessary to have a permanent ligand set on the axle of the rotaxane in active template synthesis, making it easier for rotaxanes to be designed that can bind metal ions without saturating all of their co-ordination sites. Furthermore, the orthogonal orientation of the threaded components should embed a co-ordinated metal ion within a binding pocket where the shape and surface topography is well defined in all three dimensions. In principle, these latter two features could be exploited to assemble well-expressed chiral environments for catalysis. Here we report on the synthesis and efficacy of the first rotaxane ligand employed in asymmetric transition metal catalysis.

To introduce asymmetry close to the metal center in a target rotaxane ligand we searched for reactions that might form rotaxanes with a chiral C2-symmetric trans-\(N,N\)'-dialkyl-1,2-cyclohexanediamine macrocycle, 1. Buchwald and co-workers have described an effective strategy for the amidation of aryl halides—the Goldberg reaction—employing a copper catalyst bound to a cyclohexyldiamine ligand, and we envisaged that this could be adapted for active template synthesis (Scheme 1).
In the proposed catalytic cycle, macrocycle 1 co-ordinates the copper ion endotopically to generate complex 2. Subsequently the anion is displaced by the stoppered nucleophile 3, generating 4. Oxidative insertion into the C–I bond of aryl iodide 5 should occur preferentially from the other face of the macrocycle to give threaded Cu(III) species 6. Reductive elimination should then liberate [2]rotaxane 7, concurrently regenerating Cu(I) allowing its re-entry into the catalytic cycle. Reactions catalysed by copper ions not bound to 2, or co-ordinated outside of the cavity, would produce the non-interlocked thread 8 instead.

Scheme 1: Proposed catalytic cycle for the Goldberg active metal template synthesis of [2]rotaxane 7 from 1, 3, and 5.
Macrocycle 1 was synthesised in 5 steps from the commercially available of 3-(4-methoxyphenyl)propionic acid (figure 1).

The low-yielding last step was the result of a difficult flash column purification. From common intermediate 17 half-thread 3 was obtained through a Mitsunobu reaction with 3-bromo-1-propanol followed by the Williamson ether synthesis with 4-hydroxbenzamide (figure 2). When 17 was reacted with 3-bromo-1-propanol in an $S_N2$ reaction the resulting alcohol was reacted with 4-iodobenzoic acid in an ester bond formation to give half-thread 5.
We started our investigation of the Goldberg active metal template rotaxane-forming reaction using conditions reported by Buchwald and co-workers. A screen of reaction parameters found toluene to be the optimal solvent when Cs₂CO₃ was employed as the base, and Cu(OAc)₂ was identified as the most effective copper source. We optimised these conditions for [2]rotaxane formation (Table 1).
Table 1: Optimisation of [2]rotaxane synthesis by active template Goldberg C–N bond formation.$^a$

<table>
<thead>
<tr>
<th>entry</th>
<th>Cu(OAc)$_2$ (equiv.)</th>
<th>time (h)</th>
<th>temp (°C)</th>
<th>conv. of 5 (%)</th>
<th>rotaxane 7 (conv. %)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>8</td>
<td>110</td>
<td>26</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>20</td>
<td>110</td>
<td>78</td>
<td>15</td>
</tr>
<tr>
<td>3$^c$</td>
<td>0.5</td>
<td>20</td>
<td>110</td>
<td>99</td>
<td>21</td>
</tr>
<tr>
<td>4$^c$</td>
<td>0.1</td>
<td>20</td>
<td>110</td>
<td>65</td>
<td>14</td>
</tr>
<tr>
<td>5$^c$</td>
<td>2</td>
<td>20</td>
<td>110</td>
<td>82</td>
<td>11</td>
</tr>
<tr>
<td>6$^{c,d}$</td>
<td>0.5</td>
<td>48</td>
<td>110</td>
<td>74</td>
<td>51</td>
</tr>
<tr>
<td>7$^{c,d}$</td>
<td>0.5</td>
<td>48</td>
<td>90</td>
<td>51</td>
<td>51</td>
</tr>
<tr>
<td>8$^{c,e}$</td>
<td>0.9</td>
<td>15</td>
<td>120</td>
<td>99</td>
<td>57 (50$^{f}$)</td>
</tr>
</tbody>
</table>

$^a$ Performed with macrocycle 1 (1.0 equiv.), aryl amide 3 (1.5 equiv.), aryl iodide 5 (1.0 equiv.) and Cs$_2$CO$_3$ (2 equiv.) in toluene (0.0725 M). $^b$ Conversion to rotaxane 7 determined by $^1$H NMR. $^c$ 0.15 M concentration. $^d$ Aryl amide 3 (4.5 equiv.), aryl iodide 5 (4.0 equiv.) and Cs$_2$CO$_3$ (8.0 equiv.). $^e$ Performed with aryl amide 3 (5.5 equiv.), aryl iodide 5 (5.0 equiv.) and Cs$_2$CO$_3$ (10 equiv.). $^f$ Isolated yield.

Extending the reaction time from 8 h to 20 h improved the yield of 7 from 6% to 15% (Table 1, entries 1 and 2). Increasing the reaction concentration to 0.15 M, close to the limit of solubility of 3, increased the rotaxane yield to 21% (Table 1, entry 3). While a decrease in the loading of the copper catalyst (from 0.5 to 0.1 equiv.) slowed the reaction rate (Table 1, entry 4), higher copper loadings reduced the amount of rotaxane 7 formed (Table 1, entry 5), probably by increasing the rate of the non-ligated-Cu(I)-catalyzed reaction to form 8.

Using a higher ratio of the axle components 3 and 5 to macrocycle 1 increased the yield of [2]rotaxane to a synthetically viable 50% (Table 1, entries 6–8).

The rotaxane architecture of 7 was confirmed by mass spectrometry and NMR spectroscopy. The $^1$H NMR spectra of [2]rotaxane 7 and its components (1 and 8) also provided insight into the interactions and relative positions of the components within the rotaxane (Figure 3).
Figure 3: $^1$H NMR spectra (600 MHz, C$_6$D$_6$, 298 K) of a) macrocycle 1, b) rotaxane 7, c) thread 8. The assignments correspond to the lettering shown in Scheme 1.

Resonances for protons associated with, or proximate to, the amide group of the axle (H$_k$, H$_l$ and H$_j$) are shifted dramatically downfield in the rotaxane (Figure 3b) compared to the non-interlocked thread (Figure 3c), with H$_k$ shifting nearly 3 ppm. This is indicative of the axle being held in position by intercomponent hydrogen bonding between the amide H-bond donor of the axle and the H-bond acceptor amines of the macrocycle. Several proton resonances for the macrocycle are doubled in the rotaxane (e.g. H$_{Go}$, Figure 3b), a consequence of the threaded unsymmetrical axle rendering the faces of the macrocycle inequivalent.

Having established that the Goldberg reaction could be used to prepare a chiral [2]rotaxane with an embedded metal ion binding pocket with vacant co-ordination sites, our attention turned to evaluating the efficacy of 7 as a ligand in enantioselective metal catalysis (Table 2).
Table 2: Ligand-nickel-catalysed enantioselective Michael-addition of diethyl malonate 9 and trans-b-nitrostyrene 10a.

<table>
<thead>
<tr>
<th>ligand</th>
<th>time (d)</th>
<th>conv. (%)</th>
<th>er (S,R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acyclic ligand 12</td>
<td>2</td>
<td>&gt;98</td>
<td>68:32</td>
</tr>
<tr>
<td>Macrocycle 1</td>
<td>2</td>
<td>&gt;98</td>
<td>85:15</td>
</tr>
<tr>
<td>Rotaxane 7</td>
<td>27</td>
<td>&gt;98</td>
<td>93:7</td>
</tr>
</tbody>
</table>

a Reaction conditions: 10a (1.0 equiv.), 9 (1.2 equiv.), ligand (10 mol%) and NiBr₂ (4.5 mol%) in toluene (1 M) at rt. b Reactions were run to full conversion as determined by ¹H NMR. c Enantiomeric ratios determined by HPLC using a Chiralpak IC column. d In the absence of NiBr₂, diamine 12 gives >98% conversion to 11a over 4 days with er 49:51 (consistent with the diamine acting as a base, as with other amines in this type of reaction) and the reaction with rotaxane 7 becomes 10× slower.

As a proof-of-concept, we examined the well-studied metal-catalysed enantioselective Michael addition of diethyl malonate (9) to trans-b-nitrostyrene (10a). Evans has demonstrated the utility of chiral cyclohexyldiamine ligands in NiBr₂-promoted variants of this reaction to afford 11a, and we compared the effectiveness of acyclic ligand 12 (Table 2, entry 1) and [2]rotaxane 7 (Table 2, entry 2) in this process. Pleasingly, both ligands catalysed the reaction between 9 and 10a. However, they exhibited significant differences in catalytic behavior and stereochemical outcome of the reaction. Although acyclic ligand 12 facilitated rapid formation of 11a, only modest enantiomeric enrichment (68:32 er) was observed. In contrast, despite more sluggish activity (10x longer reaction times than for 12), [2]rotaxane 7 afforded product 11a in good yield (>98% conversion) and enantioselectivity (93:7 er). This behavior is consistent with the rotaxane providing a much more structurally defined 3D pocket for the metal ion and substrate, improving the expression of chirality of the ligand and reducing the degrees of freedom (in terms of conformation and orientation) that the substrate can adopt upon binding to the catalytic center. However, burying the
metal ion deeper in the ligand structure apparently reduces its accessibility and availability for catalysis, increasing the reaction time needed for full conversion to product. The substrate scope of the rotaxane-nickel-catalysed reaction was investigated (Table 3), with high levels of enantiomeric enrichment obtained regardless of substitution pattern (11b-d) or electronic properties (11e,f) of the nitrostyrene employed.

Table 3: Scope of the rotaxane-nickel-catalysed enantioselective Michael-addition of diethyl malonate 9 and trans-b-nitrostyrenes 10a-f.a

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Conversion</th>
<th>Enantiomeric Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>11a–f</td>
<td>&gt;98%</td>
<td>93:7 er</td>
</tr>
<tr>
<td>11b–f</td>
<td>&gt;98%</td>
<td>93:7 er</td>
</tr>
<tr>
<td>11c–f</td>
<td>&gt;98%</td>
<td>92:8 er</td>
</tr>
</tbody>
</table>

aReaction conditions: 10a-f (1.0 equiv.), 9 (2.0 equiv.), 7 (10 mol%) and NiBr₂ (10 mol%) in toluene (0.2 M) at rt. b Reactions were performed under the conditions used for Table 2. c Conversions determined by ¹H NMR. d Enantiomeric ratios determined by HPLC using a Chiralpak IC column.

In conclusion, we have demonstrated that the Goldberg copper-catalyzed amidation of an aryl iodide provides an effective means of introducing a C₂-symmetric chiral cyclohexyldiamine macrocycle into a [2]rotaxane architecture. Once the rotaxane is assembled, the ligand remains active in promoting asymmetric transition-metal-catalyzed reactions, giving markedly higher enantioselectivities compared to a non-interlocked analogue, albeit at the expense of significantly longer reaction times.
Conclusion and discussion

Enzymes often perform asymmetric catalysis within deep binding pockets of well-defined shape and surface topography. It can be difficult to construct three-dimensional cavities in which the chirality is similarly well-expressed using conventional small molecule structures. The orthogonal arrangement of the mechanically interlocked components of rotaxanes, which can conveniently incorporate chiral C$_2$-symmetric (and other) macrocyclic ligands through active template synthesis, offers an intriguing way of assembling chiral three-dimensional binding pockets that have a ligated transition metal ion with accessible coordination sites at the core. Since the AMT strategy is a well-established approach for the synthesis of mechanically interlocked molecules it is often assumed that these complex structures are more easily acquired than expected. Unfortunately, this is usually not the case and the search for good conditions to obtain rotaxane 7 became very time consuming. Not only was it difficult to get consistently good conversions in the air and moisture sensitive Goldberg reaction, the rotaxane also proved to be susceptible to decomposition under air. As a result, the yields after purification by flash column chromatography only correlated well to conversion by $^1$H NMR when solvents were degassed and nitrogen was used for column chromatography. Likewise, the product was only obtained pure when care was taken during this process. As a first example of a rotaxane ligand for asymmetric metal catalysis we have demonstrated that the rotaxane architecture has some interesting properties compared to its non-interlocked analogues. Still, there is a lot to improve upon, for example reaction time and catalyst loading. Tuning of the structure of rotaxane ligand 7, by shortening the thread (to further restrict the freedom of movement of the interlocked components) and varying the axle constitution, might give a better understanding on how the rotaxane’s components affect the catalytic environment. That the mechanically captured unsymmetrical thread renders the C$_2$-symmetric macrocycle’s halves unequal (as indicated by NMR) is noteworthy as this may influence the stereoselectivity as well.
Synthetic procedures and characterization details

3-(4-methoxyphenyl)propanoyl chloride (13)

Thionyl chloride (22.9 mL, 315 mmol) and DMF (0.2 mL) were added to a stirred solution of 3-(4-methoxyphenyl)propionic acid (19.0 g, 105 mmol) in toluene (50 mL). The mixture was stirred for 3 h at 70 °C, after which all volatiles were removed in vacuo. The resulting yellow oil was used without further purification. $^1$H NMR (600 MHz, CD$_2$Cl$_2$): δ 7.14 (d, $J = 8.6$ Hz, 2H, H$_a$), 6.86 (d, $J = 8.6$ Hz, 2H, H$_d$), 3.78 (s, 3H, H$_e$), 3.20 (t, $J = 7.4$ Hz, 2H, H$_j$), 2.96 (t, $J = 7.4$ Hz, H$_b$). $^{13}$C NMR (151 MHz, CD$_2$Cl$_2$): δ 173.71 (C), 159.03 (C), 131.27 (C), 129.84 (CH), 114.51 (CH), 55.71 (CH$_3$), 49.32 (CH$_2$), 30.63 (CH$_3$).

$N,N'$-(1R,2R)-cyclohexane-1,2-diylbis(3-(4-methoxyphenyl)propanamide) (14)

$S_2$ was synthesized following a modified literature procedure.$^{10}$ 4 M aqueous NaOH (250 mL) was added to a stirred suspension of (1R,2R)-(+)-1,2-diaminocyclohexane L-tartrate (6.18 g, 23.4 mmol) in CH$_2$Cl$_2$ (310 mL). Acid chloride $S_1$ (18.6 g, 93.6 mmol) was added slowly and the mixture was stirred for 24 h. The mixture was diluted with water (100 mL) and extracted with CH$_2$Cl$_2$ (3 × 200 mL). The combined organic layers were washed with brine, dried over MgSO$_4$ and concentrated under reduced pressure. The residue was recrystallized from warm MeOH to give $S_2$ (10.3 g, quantitative yield) as a colorless solid. $R_f$ 0.56 (CH$_2$Cl$_2$/MeOH, 9:1). Mp 219 °C. [α]$^2_0$ +9.2 (c 1.00, CHCl$_3$). $^1$H NMR (600 MHz, CD$_2$Cl$_2$): δ 7.08 (d, $J = 8.5$ Hz, 4H, H$_b$), 6.78 (d, $J = 8.5$ Hz, 4H, H$_d$), 6.10–6.13 (br m, 2H, H$_d$), 3.73 (s, 6H, H$_e$), 3.60–3.54 (m, 2H, H$_j$), 2.84–2.75 (m, 4H, H$_h$), 2.33 (t, $J = 7.8$ Hz, 4H, H$_i$), 1.95–1.93 (m, 2H, H$_a$ eq), 1.74–1.67 (m, 2H, H$_a$ ax), 1.33–1.24 (m, 2H, H$_a$ ax), 1.22–1.12 (m, 2H, H$_b$ ax). $^{13}$C NMR (151 MHz, CD$_2$Cl$_2$): δ 173.07 (C), 158.56 (C), 133.55 (C), 129.71 (CH), 114.24 (CH), 55.66 (CH$_3$), 54.25 (CH), 39.04 (CH$_2$), 32.71 (CH$_2$), 31.27 (CH$_2$), 25.24 (CH$_2$). HRMS (ESI) m/z calcd for C$_{26}$H$_{35}$N$_2$O$_4$ [M+H]$^+$: 439.2591, found: 439.2589.

(1R,2R)-$N^1,N^2$-bis(3-(4-methoxyphenyl)propyl)cyclohexane-1,2-diamine (12)

A solution of LiAlH$_4$ (1.0 M in THF, 13.7 mL) was added dropwise to a solution containing $S_2$ (1.0 g, 2.28 mmol) in THF (20 mL) and heated to reflux. After 18 h, the suspension was diluted with CH$_2$Cl$_2$ (20 mL). The reaction mixture was carefully quenched by the addition of water (0.5 mL). After letting the mixture stir for 30 min, 4 M aqueous NaOH was added (0.5 mL) and the mixture was stirred for an additional 30 min. Finally, another
portion of water (1.5 mL) was added and the mixture was left to stir for 45 min. The formed suspension was filtered and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (CH\(_2\)Cl\(_2\)/MeOH, 9:1→CH\(_2\)Cl\(_2\)/Et\(_3\)N, 97:3) to obtain 12 (0.84 g, 90% yield) as a yellow oil. R\(_f\) 0.38 (CH\(_2\)Cl\(_2\)/MeOH, 9:1). \([\alpha]_D^{20}\) = −40.2 (c 0.92, CHCl\(_3\)). \(^1\)H NMR (600 MHz, CDCl\(_3\)): \(\delta\) 7.10 (d, \(J = 8.5\) Hz, 4H, \(H_d\)), 6.81 (d, \(J = 8.5\) Hz, 4H, \(H_l\)), 3.77 (s, 6H, \(H_e\)), 2.77 (dt, \(J = 11.5, 7.0\) Hz, 2H, \(H_c\)), 2.65–2.59 (t, \(J = 7.6\) Hz, 4H, \(H_g\)), 2.45 (dt, \(J = 11.4, 7.1\) Hz, 2H, \(H_f\)), 2.31 (br s, 2H, \(H_a\)), 2.15–2.09 (m, 2H, \(H_c\)), 2.09–2.04 (m, 4H, \(H_{b eq}\)), 1.77 (p, \(J = 7.3\) Hz, 4H, \(H_i\)), 1.73–1.66 (m, 2H, \(H_{a eq}\)), 1.24–1.16 (m, 2H, \(H_{ax}\)), 1.04–0.95 (m, 2H, \(H_b\)). \(^1\)C NMR (151 MHz, CDCl\(_3\)): \(\delta\) 157.75 (C), 134.25 (C), 129.34 (CH), 113.78 (CH), 61.65 (CH), 55.29 (CH\(_2\)), 46.29 (CH\(_2\)), 32.71 (CH\(_2\)), 32.32 (CH\(_2\)), 31.56 (CH\(_2\)), 25.11 (CH\(_2\)). HRMS (ESI) m/z calcd for C\(_{26}\)H\(_{35}\)N\(_2\)O\(_2\) [M+H]\(^+\): 411.3006, found: 411.3002.

**4,4′-(((1R,2R)-cyclohexane-1,2-diyl)bis(azanediyl))bis(propane-3,1-diyl)diphenol (15)**

Diamine 12 (841 mg, 2.05 mmol) was dissolved in dry CH\(_2\)Cl\(_2\) (16 mL) and cooled to −78 °C. A solution of BBr\(_3\) (1 M in CH\(_2\)Cl\(_2\), 16.4 mL) was added dropwise and the mixture was stirred for 1 h at −78 °C. After allowing the mixture to warm to room temperature, it was stirred for an additional hour. Excess BBr\(_3\) was quenched by carefully adding saturated aqueous NaHCO\(_3\) (40 mL). The precipitate was filtered and washed with water and CH\(_2\)Cl\(_2\).

The remaining solid was washed once more with saturated aqueous NaHCO\(_3\) and water to yield S3 (780 mg, quantitative yield) as a yellow solid, which was used without further purification. R\(_f\) 0.28 (CH\(_2\)Cl\(_2\)/MeOH, 9:1). Mp 76 °C. \([\alpha]_D^{20}\) = −42.5 (c 0.64, CHCl\(_3\)). \(^1\)H NMR (600 MHz, MeOD): \(\delta\) 7.07 (d, \(J = 8.4\) Hz, 4H, \(H_b\)), 6.72 (d, \(J = 8.5\) Hz, 4H, \(H_l\)), 3.47 (br s, 2H, \(H_e\)), 3.20 (dt, \(J = 11.3, 5.4\) Hz, 2H, \(H_c\)), 3.04 (dt, \(J = 11.1, 4.5\) Hz, 2H, \(H_c\)), 2.70–2.60 (m, 4H, \(H_d\)), 2.27–2.21 (m, 2H, \(H_{b eq}\)), 2.20–2.10 (m, 2H, \(H_f\)), 2.09–1.99 (m, 2H, \(H_{b eq}\)), 1.82–1.77 (m, 2H, \(H_{ax}\)), 1.64–1.56 (m, 2H, \(H_{ax}\)), 1.45–1.36 (m, 2H, \(H_{ax}\)). \(^1\)C NMR (151 MHz, MeOD): \(\delta\) 156.94 (C), 132.30 (C), 130.43 (CH), 116.32 (CH), 58.69 (br, CH), 46.73 (CH\(_2\)), 32.76 (CH\(_2\)), 29.47 (br CH\(_2\)), 27.30 (br CH\(_2\)), 23.33 (br CH\(_2\)). HMRS (ESI) m/z calcd for C\(_{26}\)H\(_{35}\)N\(_2\)O\(_2\) [M+H]\(^+\): 383.2693, found: 383.2696.
Macrocycle (1)

A solution of S3 (780 mg, 2.04 mmol) in DMF (25 mL) and 1,12-dibromododecane (670 mg, 2.04 mmol) in DMF (25 mL) were added simultaneously over 20 h to a stirred suspension of Cs2CO3 (10.3 mmol, 3.34 g) in DMF (800 mL) at 50 °C. When the addition was complete, the suspension was stirred for an additional 2 days at 50 °C. The mixture was filtered and concentrated under reduced pressure. The residue was taken up in CH2Cl2, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (CH2Cl2/MeOH, 1:0→85:15) to yield macrocycle 1 (190 mg, 17% yield) as a pale yellow or colorless solid. Rf 0.37 (CH2Cl2/MeOH, 9:1). Mp 86 °C. [α]D 20 +18.4 (c 1.08, CHCl3). 1H NMR (600 MHz, C6D6): δ 7.09 (d, J = 8.5 Hz, 4H, Ha), 6.86 (d, J = 8.6 Hz, 4H, Hb), 3.73 (t, J = 6.4 Hz, 4H, Hc), 2.72 (dt, J = 11.4, 6.8 Hz, 2H, H1), 2.68 (ddd, J = 13.6, 9.1, 6.7 Hz, 2H, H2), 2.60 (ddd, J = 13.6, 9.1, 6.7 Hz, 2H, H3), 2.43 (dt, J = 11.3, 6.7 Hz, 2H, H4), 2.13–2.07 (m, 2H, H5), 2.05–2.00 (m, 2H, Hb-ax), 1.81–1.72 (m, 4H, Hb-eq), 1.67–1.60 (m, 4H, Hc-eq), 1.39 (quint, J = 7.0 Hz, 4H, Hc), 1.29–1.24 (m, 12H, Hm, Hn, Ht), 1.18–1.12 (m, 2H, Ha-eq), 1.00–0.91 (m, 2H, Hb-ax).

13C NMR (151 MHz, C6D6): δ 157.93 (C), 134.76 (C), 129.71 (CH), 114.93 (CH), 67.52 (CH2), 62.16 (CH2), 46.56 (CH2), 33.62 (CH3), 33.26 (CH3), 32.24 (CH3), 29.44 (CH3), 29.22 (CH3), 28.98 (CH2), 28.78 (CH2), 25.98 (CH2), 25.60 (CH2). HRMS (ESI) m/z calcld for C36H57N2O2 [M+H][+] : 549.4415, found: 549.4399.

tris(4-(tert-butyl)phenyl)methanol (16)

To magnesium turnings (1.43 g, 59.1 mmol) was added dry THF (90 mL) and 1-bromo-4-tert-butylbenzene (12.0 g, 56.3 mmol) at 0 °C. The mixture was slowly warmed to room temperature when initiation was observed and stirred for 2 h. Subsequently diethyl carbonate (2.02 g, 17.1 mmol) in dry THF (40 mL) was added dropwise and the mixture was stirred for another 3 h. The reaction was quenched with MeOH (5 mL) and a saturated solution of aqueous NH4Cl (100 mL) was added. The layers were separated and the aqueous layer was extracted with toluene (3 × 100 mL). The combined organic layers were dried over MgSO4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (CHCl3) to yield alcohol S4 (4.23 g, 58% yield) as a colorless solid.

Analysis in agreement with the literature.7c
4-(tris(4-(tert-butyl)phenyl)methyl)phenol (17)

Alcohol S4 (1.99 g, 3.94 mmol) was dissolved in phenol (10 g) and concentrated HCl (37%, 0.35 mL) was added. The mixture was heated at 160 °C overnight. Upon completion toluene (30 mL) was added and the mixture was washed with 1M aqueous NaOH (3 × 30 mL). The organic layer was dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was boiled in hexane for 30 min. After cooling, the mixture was filtered and the residue was dried to S5 (1.77 g, 76% yield) as a colorless solid. Analysis in agreement with the literature.¹¹

4,4',4''-((4-(3-bromopropyloxy)phenyl)methanetriyl)tris(tert-butylbenzene) (18)

Phenol S5 (2.0 g, 4.0 mmol), 3-bromo-1-propanol (1.1 g, 7.9 mmol) and PPh₃ (2.1 g, 7.9 mmol) were dissolved in a minimal amount of THF. The mixture was cooled to 0 °C and DIAD (1.6 g, 7.9 mmol) was added dropwise. After stirring overnight at room temperature, the mixture was concentrated under reduced pressure. The residue was redissolved in CH₂Cl₂ (10 mL) and precipitated with MeOH (100 mL). The precipitate was filtered and dried to yield S6 (2.2 g, 89% yield) as a colorless solid. Analysis in agreement with the literature.²c

3-(4-(tris(4-(tert-butyl)phenyl)methyl)phenoxy)propan-1-ol (19)

Phenol S5 (2.00 g, 3.96 mmol) and 1-bromopropanol (0.52 mL, 6.00 mmol) were dissolved in butanone (160 mL) and K₂CO₃ (5.94, 39.6 mmol) was added. The resulting suspension was heated at 80 °C for 48 h. Upon completion, the reaction mixture was concentrated and taken up in CH₂Cl₂ (50 mL). The organic layer was washed with water (3 × 50 mL), dried (MgSO₄), filtered and concentrated under reduced pressure to afford alcohol S7 as a colorless solid (2.05 g, 92% yield). Analysis in agreement with the literature.²c
3-(4-(tris(4-(tert-butyl)phenyl)methyl)phenoxy)propyl 4-iodobenzoate (5)

Alcohol S7 (300 mg, 0.53 mmol), 4-iodobenzoic acid (246 mg, 1.07 mmol) and EDCI-HCl (117 mg, 0.61 mmol) were dissolved in CH₂Cl₂ (10 mL). Subsequently, DMAP (6.50 mg, 53.0 μmol) was added and the resulting solution was stirred for 24 h at rt. The reaction mixture was poured into water (10 mL) and extracted with CH₂Cl₂ (3 × 10 mL). The organic layers were combined, dried (MgSO₄) and concentrated under reduced pressure. Purification by flash column chromatography (hexane/EtOAc, 10:1) yielded iodide stopper 5 as a colorless solid (300 mg, 71% yield).

Analysis in agreement with the literature.²c

4-(3-(4-(tris(4-(tert-butyl)phenyl)methyl)phenoxy)propoxy)benzamide (3)

To a solution of bromide S6 (2.10 g, 3.36 mmol) and 4-hydroxybenzamide (0.92 g, 6.72 mmol) in butanone (150 mL) was added K₂CO₃ (4.6 g, 33.6 mmol). The suspension was heated overnight at 80 °C. After cooling to room temperature, the mixture was diluted with CH₂Cl₂ (100 mL) and washed with 1 M aqueous NaOH (3 × 100 mL) and water (100 mL). The organic layer was dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was redissolved in a minimal amount of CH₂Cl₂ (10 mL) and precipitated with MeOH. The precipitate was filtered, dried and purified by flash chromatography (CH₂Cl₂/MeOH, 100:0→99:5) to yield 3 (1.83 g, 80% yield) as a colorless solid. Rf 0.05 (CH₂Cl₂). Mp 221 °C.¹H NMR (600 MHz, CD₂Cl₂): δ 7.74 (d, J = 8.7 Hz, 2H, Hj), 7.26 (d, J = 8.5 Hz, 6H, Hc), 7.18–7.12 (m, 8H, Hb,d), 6.95 (d, J = 8.8 Hz, 2H, Hi), 6.79 (d, J = 8.8 Hz, 2H, He), 4.21 (t, J = 6.1 Hz, 2H, Hf or Hg), 4.14 (t, J = 6.0 Hz, 2H, Hh or Hf), 2.26 (quint, J = 6.1 Hz, 2H, Hg), 1.30 (s, 27H, Ha). ¹³C NMR (151 MHz, CD₂Cl₂): δ 168.75 (C), 162.49 (C), 157.21 (C), 148.93 (C), 145.03 (C), 140.36 (C), 132.44 (CH), 130.92 (CH), 129.73 (CH), 126.08 (C), 124.83 (CH), 114.74 (CH), 113.68 (CH), 65.30 (CH₂), 64.69 (CH₃), 63.63 (C), 34.74 (CH₃), 31.63 (CH₃), 29.74 (CH₃). LRMS (ESI) m/z calcd for C₄₇H₅₉N₂O₃ [M+Na]⁺: 704.4, found: 704.4, HRMS (ESI) m/z calcd for C₄₇H₅₉N₂O₃ [M+NH₄]⁺: 699.4520, found: 699.4515.
Active metal template rotaxane synthesis

To a dry microwave vial charged with macrocycle 1 (50 mg, 0.091 mmol), amide stopper 3 (342 mg, 0.501 mmol), iodide stopper 5 (384 mg, 0.456 mmol) and Cu(OAc)$_2$ (14.9 mg, 0.082 mmol) was added oven dried Cs$_2$CO$_3$ (297 mg, 0.911 mmol). The flask was immediately capped and flushed with argon. Degassed toluene (3.00 mL) was added and the reaction mixture was brought to 120 °C and stirred for 15 h under argon. Upon completion, the reaction mixture was poured in a flask containing CHCl$_3$ (15 mL) and a solution of saturated EDTA in 17.5% aqueous NH$_3$ (10 mL). The resulting mixture stirred until the suspension became homogeneous. The organic layer was collected, dried (Na$_2$SO$_4$) and concentrated under reduced pressure. The resulting pale brown or white solid was purified by flash chromatography (CH$_2$Cl$_2$) to give free thread 8 (485 mg) as a colorless solid or purified by flash chromatography (first CH$_2$Cl$_2$ then CH$_2$Cl$_2$/CH$_3$CN/MeOH, 5:5:1) to afford rotaxane 7 (86 mg, 50% yield) as a colorless solid. Since rotaxane 7 is sensitive to oxidation best results are obtained when flash column chromatography is performed using nitrogen (instead of compressed air) and degassed solvents.

Thread (8)

\[ R_f \ 0.46 \ (CH_2Cl_2). \text{Mp} \ 227 ^\circ C. \]

$^1$H NMR (600 MHz, C$_6$D$_6$): $\delta$

8.16 (d, $J = 8.6$ Hz, 2H, H$_{12}$),
7.62 (d, $J = 6.8$ Hz, 2H, H$_{18}$),
7.60 (d, $J = 6.8$ Hz, 2H, H$_{22}$),
7.46–7.44 (m, 12H, H$_{3,3d}$),
7.42 (d, $J = 8.9$ Hz, 2H, H$_{30}$),
7.40 (d, $J = 8.9$ Hz, 2H, H$_{9}$),
7.35 (s, 1H, NH),
7.21–7.20 (m, 12H, H$_{4,33}$),
6.73 (d, $J = 8.9$ Hz, 2H, H$_{29}$),
6.70 (d, $J = 8.9$ Hz, 2H, H$_{10}$),
6.68 (d, $J = 6.8$ Hz, 2H, H$_{23}$),
4.32 (t, $J = 6.3$ Hz, 2H, H$_{14}$),
3.76 (t, $J = 5.9$ Hz, 2H, H$_{27}$),
3.71–3.67 (m, 4H, H$_{25,32}$),
1.90–1.84 (m, 4H, H$_{26,13}$),
1.22 (s, 54H, H$_{1,38}$).

$^{13}$C NMR (151 MHz, C$_6$D$_6$): $\delta$

165.90 (C$_{15}$),
164.78 (C$_{20}$),
162.23 (C$_{24}$),
157.37 (C$_{11}$),
157.29 (C$_{28}$),
148.62 (C$_3$ or 36),
148.53 (C$_9$ or 30),
145.12 (C$_{6 or 33}$),
145.05 (C$_{6 or 33}$),
143.12 (C$_{19}$),
140.34 (C$_{31}$),
140.08 (C$_8$),
132.93 (C$_{9 or 30}$),
132.84 (C$_{6 or 33}$),
131.50 (C$_{5 or 34}$),
131.48 (C$_{5 or 34}$),
131.14 (C$_{17}$),
129.49 (C$_{22}$),
127.37 (C$_{21}$),
126.13 (C$_{16}$),
124.74 (C$_{6 or 33}$),
124.71 (C$_{4 or 35}$),
119.38 (C$_{18}$),
114.59 (C$_{23}$),
113.69 (C$_{10 or 29}$),
113.61 (C$_{10 or 29}$),
64.89 (C$_{12 or 25}$),
64.45 (C$_{12 or 25}$),
64.11 (C$_{27}$),
63.84 (C$_{7,32}$),
61.88 (C$_{14}$),
34.37 (C$_2$ or 37),
34.36 (C$_2$ or 37),
31.51 (C$_{1 or 38}$),
31.50 (C$_{1 or 38}$),
29.39 (C$_{26}$),
29.11 (C$_{13}$). HRMS (ESI) m/z calcd for C$_{94}$H$_{111}$N$_2$O$_6$ [M+NH$_4$]$^+$: 1364.8470, found: 1364.8468.
Rotaxane (7)

\[
\begin{align*}
R_f & \quad 0.60 \quad (\text{CH}_2\text{Cl}_2/ \\
& \quad \text{CH}_3\text{CN}/\text{MeOH}, \text{ 5:5:1}). \quad \text{Mp}
\end{align*}
\]

157 °C. \([\delta]_{D}^{10} -10.0 \text{ (c 0.87, toluene).}\) \(^1\text{H}\) NMR (600 MHz, C\(_6\text{D}_6\)): \(\delta\) 9.89 (s, 1H, NH), 8.41 (d, \(J = 8.4\) Hz, 2H, H\(_23\)), 8.32 (d, \(J = 8.5\) Hz, 2H, H\(_{18}\)), 8.08 (d, \(J = 8.7\) Hz, 2H, H\(_{13}\)), 7.47–7.44 (m, 12H, H\(_{3,34}\)), 7.40 (d, \(J = 8.9\) Hz, 2H, H\(_{30}\)), 7.38 (d, \(J = 8.9\) Hz, 2H, H\(_9\)), 7.21–7.20 (m, 12H, H\(_{4,35}\)), 6.97 (d, \(J = 8.4\) Hz, 2H, H\(_8\)), 6.94 (d, \(J = 8.4\) Hz, 2H, H\(_8\)), 6.81 (d, \(J = 8.8\) Hz, 2H, H\(_{23}\)), 6.78–6.75 (d, \(J = 8.4\) Hz, 4H, H\(_2\)), 6.71 (d, \(J = 8.9\) Hz, 2H, H\(_{29}\)), 6.69 (d, \(J = 8.9\) Hz, 2H, H\(_{10}\)), 4.31–4.22 (m, 2H, H\(_{14}\)), 3.80–3.72 (m, 6H, H\(_{16,27}\)), 3.68 (t, \(J = 6.2\) Hz, 2H, H\(_{11}\)), 3.63 (t, \(J = 6.1\) Hz, 2H, H\(_{25}\)), 2.66–2.59 (m, 2H, H\(_{d\text{ or }d'}\)), 2.42–2.34 (m, 4H, H\(_{f\text{ or }f',d\text{ or }d'}\)), 2.33–2.27 (m, 3H, H\(_{g\text{ or }g',c\text{ or }c'}\)), 2.15–2.07 (m, 1H, H\(_i\)), 1.90–1.83 (m, 6H, H\(_{13,26,2\text{ or }b}\)), 1.81–1.73 (m, 4H, H\(_9\)), 1.72–1.65 (m, 4H, H\(_j\)), 1.48–1.37 (m, 18H, H\(_{m,n,o,p,a\text{ or }b}\)), 1.22 (m, 54H, H\(_{1,38}\)), 1.17–1.07 (m, 2H, H\(_{a\text{ or }b}\)) 0.92–0.81 (m, 2H, H\(_{a\text{ or }b}\)). \(^{13}\text{C}\) NMR (151 MHz, C\(_6\text{D}_6\)): \(\delta\) 166.16 (C\(_{15}\)), 154.66 (C\(_{20}\)), 162.00 (C\(_{24}\)), 158.12 (C\(_{1\text{ or }j}\)), 158.06 (C\(_j\text{ or }j'\)), 157.36 (C\(_{11\text{ or }28}\)), 157.35 (C\(_{11\text{ or }28}\)), 148.59 (C\(_{4,36}\)), 145.09 (C\(_{6\text{ or }33}\)), 145.08 (C\(_{6\text{ or }33}\)), 144.76 (C\(_9\)), 140.13 (C\(_{8\text{ or }31}\)), 140.03 (C\(_{8\text{ or }31}\)), 133.13 (C\(_{g\text{ or }g'}\)), 132.83 (C\(_{8\text{ or }30}\)), 132.79 (C\(_9\text{ or }30\)), 131.48 (C\(_{5,34}\)), 130.83 (C\(_{17}\)), 130.54 (C\(_{22}\)), 129.51 (C\(_{h\text{ or }h'}\)), 129.45 (C\(_{h\text{ or }h'}\)), 127.62 (C\(_{21}\)), 125.09 (C\(_{16}\)), 124.72 (C\(_{3\text{ or }35}\)), 124.71 (C\(_{1\text{ or }33}\)), 120.07 (C\(_{18}\)), 115.01 (C\(_{1\text{ or }r}\)), 114.98 (C\(_{1\text{ or }r}\)), 114.48 (C\(_{23}\)), 113.66 (C\(_{10\text{ or }29}\)), 113.63 (C\(_{10\text{ or }29}\)), 67.55 (C\(_{k\text{ or }k'}\)), 67.53 (C\(_{k\text{ or }k'}\)), 64.67 (C\(_{25\text{ or }12\text{ or }27}\)), 64.49 (C\(_{25\text{ or }12\text{ or }27}\)), 64.36 (C\(_{25\text{ or }12\text{ or }27}\)), 63.84 (C\(_{7,32}\)), 61.58 (C\(_{14}\)), 60.54 (C\(_{c\text{ or }c'}\)), 59.59 (C\(_{c\text{ or }c'}\)), 45.94 (C\(_{d\text{ or }d'}\)), 45.51 (C\(_{d\text{ or }d'}\)), 34.37 (C\(_{2,37}\)), 32.88 (C\(_{f\text{ or }f'}\)), 32.65 (C\(_{f\text{ or }f'}\)), 32.11 (C\(_{e\text{ or }e'}\)), 31.51 (C\(_{1,38}\)), 31.22 (C\(_{e\text{ or }e'}\)), 30.25–29.12 (C\(_{m,n,o,p,a\text{ or }b,13,26}\)), 28.88 (C\(_{a\text{ or }b}\)), 26.21 (C\(_{m\text{ or }m'}\)), 26.18 (C\(_{m\text{ or }m'}\)), 24.61 (C\(_{a\text{ or }b}\)), 24.56 (C\(_{a\text{ or }b}\)). HRMS (ESI) m/z calcld for C\(_{130}H_{164}N_3O_8\) [M+H]\(^+\): 1896.2545, found: 1896.2512.
Figure 4: ESI found (top) and simulated (bottom) isotopic distribution of rotaxane 7.

(S)-Ethyl-2-carboethoxy-4-nitro-3-phenylbutyrate (11a)

A solution of the amine ligand (0.02 mmol), NiBr$_2$ (2.0 mg, 0.009 mmol), diethyl malonate (38.4 mg, 0.24 mmol) and trans-β-nitrostyrene (29.8 mg, 0.20 mmol) in toluene (200 μL) was stirred for 2 d at rt. Upon completion (>98% conversion as determined by $^1$H NMR), the mixture was purified by preparative chromatography (petroleum ether/EtOAc, 4:1) to afford Michael adduct 11a as a colorless oil. $^1$H NMR (600 MHz, CDCl$_3$): δ 7.33–7.29 (m, 2H, H$_g$), 7.28–7.25 (m, 1H, H$_h$), 7.25–7.22 (m, 2H, H$_f$), 4.92 (dd, J = 13.1, 4.7 Hz, 1H, H$_e$), 4.86 (dd, J = 13.1, 9.3 Hz, 1H, H$_e'$), 4.27–4.18 (m, 3H, H$_d$, b), 4.00 (q, J = 7.1 Hz, 2H, H$_b'$), 3.82 (dd, J = 9.4 Hz, 1H, H$_c$), 1.26 (t, J = 7.1 Hz, 3H, H$_a$), 1.04 (t, J = 7.1 Hz, 3H, H$_a'$). $^{13}$C NMR (151 MHz, CDCl$_3$): δ 167.58, 166.94, 136.31, 129.05, 128.47, 128.14, 77.77, 62.29, 62.02, 55.08, 43.08, 14.10, 13.86. HRMS (ESI) m/z calcd for C$_{15}$H$_{19}$ClNO$_6$Na [M+Na]$^+$: 332.1110, found: 332.1094.

When the reaction was performed with ligand 12 the product was isolated in 65% yield (40 mg, 0.13 mmol) and 35% ee. When the reaction was performed with ligand 1 the product was isolated in 95% yield (59 mg, 0.19 mmol) and 69% ee. The reaction was performed on a smaller scale with ligand 7 (0.005 mmol ligand, 0.5 mg NiBr$_2$, 9.6 mg diethyl malonate and 7.5 mg trans-β-nitrostyrene in 50 μL toluene) and stirred for 27 d to afford the product in 89% isolated yield (13.8 mg, 0.045 mmol) and 86% ee. [α]$^25_D$ +7.9 (c 0.25, CHCl$_3$).

Enantiomeric excess was determined by HPLC with a Chiralpak IC (5 μm Particle size, 250×4.6 mm, Diachel Corporation) column (hexane/2-propanol, 95:5, 1 mL/min, 210 nm); major enantiomer $t_r$ = 20.6 min, minor enantiomer $t_r$ = 30.8 min. The optical rotation was
compared to the literature value and assigned as (S). Lit: \([\alpha]^{25}_D +7.09 \) (c 1.00, CHCl\(_3\)), (S)-isomer (95% ee).\(^8a\)

(5)-Ethyl-2-carboethoxy-4-nitro-3-(2-chlorophenyl)butyrate (11b)\(^8a\)

To rotaxane 7 (1.4 mg, 0.75 \(\mu\)mol) and NiBr\(_2\) (0.15 mg, 0.67 \(\mu\)mol) was added a mixture of diethyl malonate (2.4 mg, 0.015 mmol) and trans-2-chloro-\(\beta\)-nitrostyrene (1.4 mg, 0.0075 mmol) in toluene (37 \(\mu\)L) from a stock solution (3.7 mL). The reaction mixture was stirred at rt and monitored by \(^1\)H NMR. After 35 days (>98% conversion as determined by \(^1\)H NMR) the reaction mixture was purified by thin layer chromatography (petroleum ether/EtOAc, 4:1) to afford Michael adduct 11b (86% ee) as a colorless oil. \(^1\)H NMR (600 MHz, CDCl\(_3\)): \(\delta\) 7.41–7.38 (m, 1H, H\(_d\)), 7.27–7.21 (m, 3H, H\(_{e1,6,7}\)), 5.10 (dd, \(J = 3.5, 8.5\) Hz, 1H, H\(_e\)), 4.94 (dd, \(J = 13.5, 4.4\) Hz, 1H, H\(_e\)), 4.74 (ddd, \(J = 8.6, 8.6, 4.4\) Hz, 1H, H\(_d\)), 4.25–4.15 (m, 2H, H\(_b\)), 4.08 (d, \(J = 8.8\) Hz, 1H, H\(_f\)), 4.07 (q, \(J = 7.1\) Hz, 2H, H\(_v\)), 1.23 (t, \(J = 7.1\) Hz, 3H, H\(_a\)), 1.10 (t, \(J = 7.1\) Hz, 3H, H\(_\beta\)). \(^13\)C NMR (151 MHz, CDCl\(_3\)): \(\delta\) 167.53, 166.94, 134.23, 133.82, 130.58, 129.61, 128.99, 127.38, 75.81, 62.28, 62.17, 53.21, 39.52, 14.07, 13.88. HRMS (ESI) m/z calcld for C\(_{15}\)H\(_{14}\)ClNO\(_3\)Na [M+Na]: 366.0720, found: 366.0726.

Enantiomeric excess was determined by HPLC with a Chiralpak IC (5 \(\mu\)m Particle size, 250×4.6 mm, Diacel Corporation) column (hexane/2-propanol, 95:5, 1 mL/min, 210 nm); major enantiomer \(t =\) 15.7 min, minor enantiomer \(t =\) 21.5 min. To determine the chirality of 11b another sample (91% ee) was produced with an alternative ligand on larger scale to give the same major enantiomer. \([\alpha]^{25}_D +10.3\) (c 1.83, CHCl\(_3\)). The isomer was assigned as (S) by comparison to the literature value. Lit: \([\alpha]^{25}_D +11.1\) (c 1.13, CHCl\(_3\)), (S)-isomer (92% ee).\(^8a\)

(5)-Ethyl-2-carboethoxy-4-nitro-3-(3-chlorophenyl)butyrate (11c)\(^13\)

The title compound was prepared as described above using trans-3-chloro-\(\beta\)-nitrostyrene (1.4 mg, 0.0075 mmol). The reaction mixture was stirred at rt for 35 days (>98% conversion as determined by \(^1\)H NMR) and purified by thin layer chromatography (petroleum ether/EtOAc, 4:1) to afford Michael adduct 11c (85% ee) as a colorless oil. \(^1\)H NMR (600 MHz, CDCl\(_3\)): \(\delta\) 7.27–7.23 (m, 3H, H\(_{e1,6,7}\)), 7.16–7.12 (m, 1H, H\(_e\)), 4.91 (dd, \(J = 13.4, 4.6\) Hz, 1H, H\(_e\)), 4.84 (dd, \(J = 13.4, 9.5\) Hz, 1H, H\(_e\)), 4.26–4.18 (m, 3H, H\(_{e1,6,7}\)), 4.04 (q, \(J = 7.1\) Hz, 2H, H\(_v\)), 3.78 (d, \(J = 9.1\) Hz, 1H, H\(_f\)), 1.25 (t, \(J = 7.1\) Hz, 3H, H\(_a\)), 1.08 (t, \(J = 7.1\) Hz, 3H, H\(_\beta\)). \(^13\)C NMR (151 MHz, CDCl\(_3\)): \(\delta\) 167.31, 166.70, 138.43, 134.83, 130.33, 128.72, 128.43, 126.37, 77.30, 62.42, 62.19, 54.79, 42.62, 14.06, 13.86. HRMS (ESI) m/z calcld for
C_{15}H_{18}ClNO_{3}Na [M+Na]^+: 366.0720, found: 366.0706. Analysis in agreement with the literature.\textsuperscript{13}

Enantiomeric excess was determined by HPLC with a Chiralpak IC (5 \( \mu \)m Particle size, 250×4.6 mm, Diacel Corporation) column (hexane/2-propanol, 95:5, 1 mL/min, 210 nm); major enantiomer \( t_r = 15.5 \) min, minor enantiomer \( t_r = 19.4 \) min. To determine the chirality of \textbf{11c} another sample (88% ee) was produced with an alternative ligand on larger scale to give the same major enantiomer. \([\alpha]_{D}^{25} +6.8 \) (c 2.1, CHCl\(_3\)). The isomer was assigned as (S) by analogy.

\textbf{(S)-Ethyl-2-carboethoxy-4-nitro-3-(4-chlorophenyl)butyrate (11d)}\textsuperscript{12}

The title compound was prepared as described above using \textit{trans}-4-chloro-\( \beta \)-nitrostyrene (1.4 mg, 0.0075 mmol). The reaction mixture was stirred at rt for 35 days (>98% conversion as determined by \(^1\)H NMR) and purified by thin layer chromatography (petroleum ether/EtOAc, 4:1) to afford Michael adduct \textbf{11d} (87% ee) as a colorless oil. \(^1\)H NMR (600 MHz, CDCl\(_3\)): \( \delta \) 7.29 (d, \( J = 8.4 \) Hz, 2H, \( H_{1\text{a},\text{b}} \)), 7.18 (d, \( J = 8.4 \) Hz, 2H, \( H_{1\text{a},\text{b}} \)), 4.90 (dd, \( J = 13.2 \), 4.6 Hz, 1H, \( H_3 \)), 4.82 (dd, \( J = 13.2 \), 9.5 Hz, 1H, \( H_e \)), 4.26–4.17 (m, 3H, \( H_{1\text{c},\text{d},\text{e}} \)), 4.02 (q, \( J = 7.1 \) Hz, 2H, \( H_g \)), 3.77 (d, \( J = 9.3 \) Hz, 1H, \( H_b \)), 1.25 (t, \( J = 7.2 \) Hz, 3H, \( H_s \)), 1.07 (t, \( J = 7.2 \) Hz, 3H, \( H_s \)). \(^1\)C NMR (151 MHz, CDCl\(_3\)): \( \delta \) 167.34, 166.73, 134.84, 134.40, 129.56, 129.25, 77.51, 62.40, 62.16, 54.83, 42.44, 14.07, 13.88. HRMS (ESI) m/z calcd for C\(_{25}\)H\(_{18}\)ClNO\(_3\)Na [M+Na]^+: 366.0720, found: 366.0713.

Enantiomeric excess was determined by HPLC with a Chiralpak IC (5 \( \mu \)m Particle size, 250×4.6 mm, Diacel Corporation) column (hexane/2-propanol, 95:5, 1 mL/min, 210 nm); major enantiomer \( t_r = 16.8 \) min, minor enantiomer \( t_r = 22.2 \) min. To determine the chirality of \textbf{11d} another sample (93% ee) was produced with an alternative ligand on larger scale to give the same major enantiomer. \([\alpha]_{D}^{25} +8.1 \) (c 2.43, CHCl\(_3\)). The isomer was assigned as (S) by comparison to the literature value. Lit: \([\alpha]_{D}^{25} +8.6 \) (c 1.0, CHCl\(_3\)), (S)-isomer (93% ee).\textsuperscript{15}

\textbf{(S)-Ethyl-2-carboethoxy-4-nitro-3-(4-methylphenyl)butyrate (11e)}\textsuperscript{8a}

The title compound was prepared as described above using \textit{trans}-4-Methyl-\( \beta \)-nitrostyrene (1.2 mg, 0.0075 mmol). The reaction mixture was stirred at rt for 42 days (96% conversion as determined by \(^1\)H NMR) and purified by thin layer chromatography (petroleum ether/EtOAc, 4:1) to afford Michael adduct \textbf{11e} (87% ee) as a colorless oil. \(^1\)H NMR (600 MHz, CDCl\(_3\)): \( \delta \) 7.11 (m, 4H, \( H_{1\text{a},\text{b},\text{c},\text{d}} \)), 4.89 (dd, \( J = 13.0 \), 4.7 Hz, 1H, \( H_e \)), 4.83 (dd, \( J = 13.0 \), 9.3 Hz, 1H, \( H_e \)), 4.26–4.16 (m, 3H, \( H_{1\text{c},\text{d},\text{e}} \)), 4.01 (q, \( J = 7.1 \) Hz, 3H, \( H_s \)), 1.26 (t, \( J = 7.2 \) Hz, 3H, \( H_s \)).
2H, H₂), 3.79 (d, J = 9.3 Hz, 1H, Hₕ), 2.29 (s, 3H, H₆), 1.26 (t, J = 7.1 Hz, 3H, H₇), 1.06 (t, J = 7.1 Hz, 3H, Hₘ). ¹³C NMR (151 MHz, CDCl₃): δ 167.64, 166.97, 138.18, 133.19, 129.71, 127.94, 77.90, 62.23, 61.97, 55.14, 42.73, 21.19, 14.09, 13.88. HRMS (ESI) m/z calcd for C₁₆H₁₂NO₆Na [M+Na]⁺: 346.1266, found: 346.1253.

Enantiomeric excess was determined by HPLC with a Chiralpak IC (5 μm Particle size, 250×4.6 mm, Diacel Corporation) column (hexane/2-propanol, 95:5, 1 mL/min, 210 nm); major enantiomer tᵣ = 21.6 min, minor enantiomer tᵣ = 28.7 min.

To determine the chirality of 11e another sample (95% ee) was produced with an alternative ligand on larger scale to give the same major enantiomer. [α]°D +6.8 (c 2.14, CHCl₃). The isomer was assigned as (S) by comparison to the literature value. Lit: [α]°D +6.25 (c 1.36, CHCl₃), (S)-isomer (95% ee).³⁸⁻⁹

(S)-Ethyl-2-carboethoxy-4-nitro-3-(4-methoxyphenyl)butyrate (11f)¹²

The title compound was prepared as described above using trans-4-Methoxy-β-nitrostyrene (1.3 mg, 0.0075 mmol). The reaction mixture was stirred at rt for 42 days (91% conversion as determined by ¹H NMR) and purified by thin layer chromatography (petroleum ether/EtOAc, 4:1) to afford Michael adduct 11f (87% ee) as a colorless oil. ¹H NMR (600 MHz, CDCl₃): δ 7.15 (d, J = 8.7 Hz, 2H, Hₕ), 6.83 (d, J = 8.6 Hz, 2H, Hₖ), 4.88 (dd, J = 12.9, 4.7 Hz, 1H, H₇), 4.80 (dd, J = 12.9, 9.4 Hz, 1H, Hₘ), 4.26–4.15 (m, 3H, Hₜₑ,₂,₃), 4.01 (q, J = 7.1 Hz, 2H, Hₜₐ), 3.78 (d, J = 9.5 Hz, 1H, H₅), 3.76 (s, 3H, H₆), 1.26 (t, J = 7.1 Hz, 3H, Hₗ), 1.06 (t, J = 7.1 Hz, 3H, Hₗ). ¹³C NMR (151 MHz, CDCl₃): δ 167.64, 166.98, 159.52, 129.36, 128.08, 114.39 78.03, 62.25, 61.98, 55.34, 55.21, 42.43, 14.11, 13.92. HRMS (ESI) m/z calcd for C₁₆H₁₂NO₆Na [M+Na]⁺: 362.1216, found: 362.1211.

Enantiomeric excess was determined by HPLC with a Chiralpak IC (5 μm Particle size, 250×4.6 mm, Diacel Corporation) column (hexane/2-propanol, 95:5, 1 mL/min, 210 nm); major enantiomer tᵣ = 33.1 min, minor enantiomer tᵣ = 41.6 min. To determine the chirality of 11f another sample (95% ee) was produced with an alternative ligand on larger scale to give the same major enantiomer. [α]°D +6.5 (c 1.98, CHCl₃). The isomer was assigned as (S) by comparison to the literature value. Lit: [α]°D +6.2 (c 1.3, CHCl₃), (S)-isomer (94% ee).¹²
References


3. The available metal co-ordination site in active template rotaxanes has been used to promote further threading to form higher order rotaxanes,\(^{2,6,7}\) or to control the dynamics,\(^{2,6,7}\) and position\(^{8,9}\) of the macrocycle within molecular shuttles and switches.

4. Chiral rotaxanes have been employed as organocatalysts [(a) Tachibana, Y.; Kihara, N.; Takata, T. Asymmetric benzo condensation catalyzed by chiral rotaxanes tethering a thiazolium salt moiety via the cooperation of the component: Can rotaxane be an effective reaction field. J. Am. Chem. Soc. 2004, 126, 3438–3439. (b) Tachibana, Y.; Kihara, N.; Nakazono, K.; Takata, T. Phosphorus, Sulfur, Silicon Relat. Elem. 2010, 185, 1182–1205; (c) Blanco, V.; Leigh, D. A.; Marcos, V.; Morales-Serna, J. A.; Nussbaumer, A. L. A switchable


9. The ability of **12** to confer enantioselectivity in the conjugate addition increases at reduced ligand stoichiometry (e.g. 1:1) and concentration. Rotaxane **7** is much less sensitive to changes in these reaction parameters.


**CHAPTER 5**

**SYNTHESIS OF AN ASYMMETRIC LANTHANIDE KNOT OF SINGLE HANDEDNESS FOR ENANTIOSELECTIVE CATALYSIS**

Synopsis: A molecular trefoil knot was employed as a novel ligand for the lanthanide catalysed asymmetric Mukaiyama aldol addition. Small variations in the ligand architecture were shown to significantly influence the efficacy of the catalyst, as did the choice of solvent. The accessibility of the lanthanide within the chiral pocket of the knot was also investigated and demonstrated that a single solvent molecule was capable of binding to the core.

Acknowledgements: I’d like to thank dr. Guzman Gil-Ramirez for the synthesis of knot 6 and helpful discussions, dr. Mattew Kitching for the measurements of the $q$ values, catalysis experiments, synthesis of knot 9 and all his help, and dr. Gen Zhang for his hard work, his idea to use an asymmetric knot, synthesis of knot 6 and catalysis experiments. I have contributed to the synthesis of knot 7, synthesis of the ligand, catalysis experiments and characterisations.
Synthesis of an asymmetric knot of single-handedness for enantioselective catalysis

Knots are ubiquitous in human history, underpinning the technological development of early man.\(^1\) Despite these ancient beginnings, nature has been tying knots for millennia.\(^2\) The natural world contains knots in a wide variety of biological polymers, from proteins to DNA and RNA.\(^2\) In comparison, the chemical synthesis of small molecular knots is relatively recent.\(^3\) Despite the success of the chemical community in the synthesis of knotted structures,\(^3\) they have yet to find useful applications in synthesis. Indeed, within natural proteins, the effects of knots are yet to be fully understood. One potential explanation for the prevalence of knots in proteins can be inferred from their presence in a number of active sites of enzymes,\(^4\) allowing for well-defined chiral pockets to be produced. Furthermore, computational studies suggest that these knots may change the activity and stability of protein structures that contain them.\(^5\) Inspired by our recent success employing rotaxanes to generate a well-defined chiral pocket for enantioselective catalysis\(^6\) – we sort to explore whether knots could offer similar effects.

Given the wide use of lanthanides in asymmetric catalysis,\(^7\) and our recent successes in the synthesis of racemic\(^8\) and chiral\(^9\) trefoil knots using a triskelion approach around a lanthanide template, we sought to examine whether chiral information contained within the knot could be transferred to the products of a lanthanide catalysed reaction to deliver enantioenriched materials. Inspired by Hunter’s linear approach in synthesising a chiral knot,\(^10\) we also wished to explore whether our lanthanide template could be extended to generate chiral knots in this fashion.

Ligand 4 was synthesised from bromide 3 and diol 1\(^{\text{a,b}}\) via a Williamson reaction. The resulting polydentate ligand was templated by Eu(OTf)\(_3\) to form pseudo-knot 5 and was then mechanically interlocked through ring-closing metathesis to give 6 (figure 1a, 1b).

![Figure 1a: Towards the synthesis of ligand 4.](image-url)
We envisioned that the asymmetry in the architecture might give interesting results in enantioselective catalysis compared to the previously reported symmetrical trefoil knots 8,9, their samarium analogue 7, and Eu precursor 10, and (figure 2).9

The Mukaiyama aldol addition was found to be a suitable reaction to explore lanthanide knot catalysis.11 At first four different lanthanide catalysts were screened (entry 1-4) and
only asymmetric knot 6 afforded the enantioenriched material (table 1). An exact conversion for entry 4 and a few other entries still needs to be determined.

Table 1. Optimisation of the Mukaiyama Aldol Addition.\textsuperscript{a}

<table>
<thead>
<tr>
<th>entry</th>
<th>catalyst</th>
<th>Solvent (5:2)</th>
<th>time (h)</th>
<th>conversion (%)\textsuperscript{b}</th>
<th>syn:anti\textsuperscript{b,c}</th>
<th>ee (%)\textsuperscript{c}</th>
<th>R</th>
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<td>8</td>
<td>MeOH/CH\textsubscript{3}CN</td>
<td>48</td>
<td>29</td>
<td>49:51</td>
<td>0</td>
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<td>96</td>
<td>41</td>
<td>52:48</td>
<td>0</td>
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</tr>
<tr>
<td>4</td>
<td>6</td>
<td>MeOH/CH\textsubscript{3}CN</td>
<td>96</td>
<td>ND\textsuperscript{i}</td>
<td>55:45</td>
<td>23</td>
<td>Me</td>
</tr>
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<td>-</td>
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<td>MeOH</td>
<td>96</td>
<td>ND\textsuperscript{i}</td>
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<td>CH\textsubscript{3}CN</td>
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<td>-</td>
<td>-</td>
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<td>&lt;5</td>
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<td>-</td>
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<td>MeOH/CH\textsubscript{3}CN</td>
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<td>ND\textsuperscript{i}</td>
<td>55:45</td>
<td>15</td>
<td>Me</td>
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</table>

\textsuperscript{a} Performed with 4-nitrobenzaldehyde (1.0 equiv.) and trimethyl(1-phenylpropenyloxy)silane (1.5 equiv.) at –10 °C. \textsuperscript{b} Determined by \textsuperscript{1}H NMR. \textsuperscript{c} Determined by HPLC. \textsuperscript{d} Ratio 1:1. \textsuperscript{f} 10 mol\% loading. \textsuperscript{g} Syn or anti configuration not unambiguously determined. \textsuperscript{h} Minor diastereoisomer. \textsuperscript{i} Conversion not accurately determined.

Entry 5 shows that there is a considerable background reaction. A solvent screen (entry 6-10) indicated that the optimal solvent system was already established in the first entries. Interestingly the ee was very low in pure MeOH and the reaction didn’t proceed in pure CH\textsubscript{3}CN, while a 1:1 mixture of MeOH/CH\textsubscript{3}CN (entry 10) gave a lower ee than a 5:2 mixture (entry 4). Furthermore, an increase in catalyst loading to 10 mol\% (entry 11) corresponded to a small increase in ee. Notably, the use of 10 mol\% 9 (entry 12) and non-interlocked complex 10 (entry 13) gave smaller ee’s than asymmetric knot 6.
In order to determine the accessibility of the lanthanide to co-ordination while bound within the chiral pocket of the knot, luminescence decay lifetimes of complexes 6, 9 and 10 were used to determine the number of bound solvent molecules to the lanthanide core.\textsuperscript{12} Taking advantage of the antenna effect,\textsuperscript{13} excitation of the bound pyridine-based ligands allows interrogation of metals bound to the organic framework of the knot. As N-H and O-H oscillators are known to promote the relaxation of excited state lanthanide species,\textsuperscript{12} comparison of the rates of luminescence decay in protic and deuterated solvents allows calculation of the number of bound solvent molecules in solution.\textsuperscript{12} Importantly, due to the slow exchange of the N-H protons of the amide ligand, additional correction values for these complexes are not required.\textsuperscript{14} Conducting these experiments in methanol and deuterated methanol demonstrated that for both knots 6 and 9, a single species was present in solution with a q value of 0.82 and 0.81 respectively – demonstrating a single solvent molecule is capable of binding to the core. In contrast, 10 proved to be more dynamic. Two species were observable in solution, with q values 0.82, and 3.28 respectively, which might suggest that without the formal covalent scaffold provided by a knot, the ligands are in dynamic exchange under the reaction conditions.
Conclusion and future work

The first results in the field of enantioselective knot catalysis are promising. Although the selectivity is not excellent the chemistry is novel and there is much room for improvement. Optimal reactions conditions have been found and a full substrate scope, including the missing conversions and the assignment of the absolute configurations of the aldol adducts, will be realised in the near future. A structural characterisation of 6 by single-crystal X-ray diffraction has revealed the catalyst’s geometry and it would be exciting to compare it to less selective catalysts 9 and 10. We have also shown that the knot has an available coordination site and that small differences in the ligand’s architecture have a great effect on the enantioselectivity. Given the differences in catalyst selectivities it can be concluded that it’s not just the chirality of the ligand but the architecture as a whole that is accountable for the observed selectivity. Furthermore, we would like to include a stackplot of the corresponding asymmetric Lu-knot with its unclosed precursor, as 6 gave broad $^1$H NMR signals. Future work may include the synthesis of knots with bulkier groups at the ligand’s chiral centre or the separation of enantiomers of a topologically racemic mixture of knots with no inherent chemical chirality. In search of high ee’s other reactions could also be investigated.
Synthetic procedures and characterization details

Trimethyl(1-phenylpropenyloxy)silane

Propiophenone (1.34 g, 9.99 mmol) in THF (20 mL) was added dropwise to a stirred solution of LiHMDS (1 M in THF, 15 mL) over a period of 30 min at rt. The resulting solution was stirred for another 15 min before the addition of chlorotrimethylsilane (1.62 g, 14.9 mmol) in THF (10 mL). The reaction mixture was concentrated and the residue taken up in CH$_2$Cl$_2$ (50 mL). The resulting suspension was filtered and the filtrate concentrated under reduced pressure to afford the silyl enol ether (1.85 g, 8.69 mmol) as a yellow oil in 87% yield. The product was stored in the freezer and used without further purification.\textsuperscript{15} Analysis is in agreement with the literature.\textsuperscript{16}

3-Hydroxy-2-methyl-3-(4-nitrophenyl)-1-phenylpropan-1-one

A solution of lanthanide catalyst (0.66 µmol) and 4-nitrobenzaldehyde (10 mg, 0.066 mmol) in a mixture of dry solvent (0.7 mL) was cooled to $-10$ °C. Trimethyl(1-phenylpropenyloxy)silane (20 mg, 0.099 mmol) was added dropwise and the reaction mixture was stirred at the same temperature for 4 d. The reaction was concentrated and the remaining solid taken up in CH$_2$Cl$_2$. The suspension was filtered and the solution was concentrated under reduced pressure. The crude product was purified by preparative thin layer chromatography (PET/EtOAc, 5:1) to afford the aldol adduct. The analysis is in agreement with the literature.\textsuperscript{17}

Enantiomeric excess was determined by HPLC with a Chiralpak IA (5 µm Particle size, 250×4.6 mm, Diacel Corporation) column (hexane/2-propanol, 9:1, 1 mL/min, 254 nm); syn diastereoisomer $t_r = 16.3$, 17.4 min, anti diastereoisomer $t_r = 19.0$, 31.3 min.

2

Diol 2 (500 mg, 0.99 mmol) was taken up in DMF (10 mL) and K$_2$CO$_3$ (410 mg, 2.97 mmol) was added. The reaction mixture was stirred at 80 °C for 2 h under a nitrogen atmosphere. The mixture was concentrated under reduced pressure and purified by flash column chromatography (CH$_2$Cl$_2$/EtOAc, 10:1→5:1) to afford the title compound (357 mg, 0.65 mmol) as a white solid in 66% yield. Mp 153 °C. [$\alpha$]$^2_D$=342.4 (c 1.15, MeOH). $^1$H NMR (600 MHz, CDCl$_3$): $\delta$ 8.25–8.21 (m, 2H, H$_b$), 8.04 (app t, $J =7.8$ Hz, 1H, H$_d$), 7.73–7.70 (m, 2H, H$_g$), 7.69–7.63 (m, 3H, H$_{j,h}$), 7.59 (d, $J =8.6$ Hz, 1H, H$_i$), 7.46 (d, $J =8.5$ Hz, 1H, H$_i$), 7.42 (d, $J =8.6$ Hz, 1H, H$_f$), 7.17–7.15 (m, 1H, H$_b$), 7.13–7.09 (m, 1H, H$_b$), 7.08–7.06
Calculated: distribution for 125.07, 125.02, 124.72, 124.72, 119.70, 119.66, 118.05, 107.00, 106.72, 71.40, 71.02, 70.75 MHz, CDCl₃ H Hz, 17.3, 1.6 Hz, 7.1. LRMS (ESI) m/z calc for C₃₄H₆₁N₃O₄ [M+Na]⁺: 568.2, found 568.2. LRMS (ESI) measured isotopic distribution for C₃₄H₆₀N₃O₄ [M–H]⁻: 544.2 (100), 545.2 (39), 546.2 (8).

Calculated: 544.3 (100), 545.3 (39), 546.3 (8). HRMS (ESI) m/z calc for C₃₄H₆₂N₃O₄ [M+H]⁺: 546.2387, found 546.2383.

3

DMF (5 mL) was added to a flask containing alcohol 2 (250 mg, 0.46 mmol), 1,2-bis(2-bromoethoxy)ethane (253 mg, 0.92 mmol) and K₂CO₃ (190 mg, 1.37 mmol). The resulting suspension was stirred at 80 °C for 3 h under a nitrogen atmosphere. The mixture was concentrated under reduced pressure and purified by flash column chromatography to give bromide 3 as a pale brown solid (249 mg, 0.34 mmol) in 73% yield. Mp 118 °C. [α]D²⁰ +165.0 (c 1.23, CHCl₃). ¹H NMR (600 MHz, CDCl₃): δ 8.36, (d, J = 7.8 Hz, 2H, Hₘ), 8.03 (app t, 1H, Hₙ), 7.89–7.85 (m, 2H, Hₜ), 7.74–7.71 (m, 2H, H₝), 7.70–7.67 (m, 2H, Hᵣ), 7.66–7.62 (m, 2H, Hₜ), 7.45–7.41 (m, 2H, H₝), 7.21–7.18 (m, 2H, Hₙ), 7.13–7.10 (m, 2H, Hᵣ), 6.13 (ddt, J = 17.3, 10.6, 5.3 Hz, 1H, Hₜ), 5.48 (dd, J = 17.3, 1.6 Hz, 1H, Hₘ), 5.46–5.41 (m, 2H, H₝), 5.33 (dd, J = 10.5, 1.5 Hz, 1H, Hₙ), 4.66 (d, J = 5.4 Hz, 2H, Hᵣ), 4.28–4.25 (m, 2H, Hₙ), 3.96–3.93 (m, 2H, H₝), 3.82 (t, 2H, H₝), 3.79–3.75 (m, 2H, H₈), 3.74–3.70 (m, 2H, H₉), (3.47 (t, J = 6.3 Hz, 2H, Hₚ), 1.67–1.64 (m, 6H, Hₙ). ¹³C NMR (151 MHz, CDCl₃): δ 162.69, 162.69, 157.13, 156.90, 148.93, 148.93, 139.23, 138.02, 138.02, 134.03, 134.01, 133.17, 129.54, 129.50, 128.98, 128.96, 127.70, 127.70, 125.31, 125.31, 125.07, 125.02, 124.72, 124.72, 119.70, 119.66, 118.05, 107.00, 106.72, 71.40, 71.02, 70.75, 69.69, 68.99, 67.60, 49.19, 49.19, 30.49, 21.79, 21.75. LRMS (ESI) measured isotopic distribution for C₄₀H₄₂Br₃N₃O₆ [M+Na]⁺: 762.3 (76), 763.3 (47), 764.3 (100), 765.3 (58).

Calculated: 762.2 (93), 763.2 (42), 764.2 (100), 765.2 (43). HRMS (ESI) m/z calc for C₄₀H₄₂Br₃N₃O₆ [M+H]⁺: 740.2330, found 740.2336.
Bromide 3 (40 mg, 0.054 mmol) and diol 1 (14 mg, 0.027 mmol) were dissolved in DMF (3 mL). The reaction mixture was heated to 80 °C and stirred under nitrogen for 3 h. Upon completion, the mixture was concentrated under reduced pressure and purified by flash column chromatography (CH₂Cl₂/MeOH, 15:1→10:1) to afford the asymmetric (R,R) ligand 4 as a white solid (32 mg, 0.018 mmol) in 68% yield. Mp 115 °C. [α]$_D^{20}$ = −192.7 (c 0.45, CHCl₃). $^1$H NMR (600 MHz, CDCl₃): δ 8.31–8.25, (m, 6H, Hₐ), 8.06–8.01 (m, 6H, H₈), 7.95–7.88 (m, 3H, Hₐ), 7.65–7.56 (m, 18H, H₈,m,h), 7.40–7.35 (m, 6H, Hₐ), 7.17–7.11 (m, 6H, Hₛ), 7.09–7.05 (m, 6H, Hₙ), 6.12 (ddt, $J = 17.3, 10.5, 5.3$ Hz, 2H, Hₘ), 5.47 (dd, $J = 17.3, 1.6$ Hz, 1H, Hₜ), 5.42–5.33 (m, 6H, Hₙ), 4.64 (d, $J = 5.3$ Hz, 4H, Hₙ), 4.24–4.29 (m, 8H, Hₚ), 3.95–3.89 (m, 8H, Hₜ), 3.79 (br s, 8H, Hₗ), 1.57 (d, $J = 6.8$ Hz, 6H, H₂), 1.54–1.48 (m, 12H, Hₜ,e′,e′). $^{13}$C NMR (151 MHz, CDCl₃): δ 162.80, 162.80, 162.80, 157.07, 157.07, 156.82, 148.87, 148.84, 148.84, 139.05, 139.05, 138.05, 138.05, 138.05, 133.93, 133.91, 133.91, 133.17, 129.45, 129.41, 129.39, 128.90, 128.88, 128.86, 127.62, 127.59, 127.58, 125.24, 125.24, 125.22, 125.16, 125.14, 124.61, 124.59, 124.59, 119.62, 119.62, 119.59, 118.02, 106.93, 106.75, 106.75, 71.07, 71.07, 69.93, 69.93, 68.95, 67.59, 67.59, 49.00, 48.98, 48.98, 48.98, 21.59, 21.53, 21.53.

LRMS (ESI) measured isotopic distribution for C$_{111}$H$_{109}$N$_9$O$_{16}$ [M+Na$^+$]: 1846.67 (80), 1847.58 (100), 1848.58 (65), 1849.58 (29), 1850.67 (12). Calculated: 1846.79 (80), 1847.79 (100), 1848.80 (65), 1849.80 (30), 1850.80 (11). HRMS (ESI) m/z calc for C$_{111}$H$_{110}$N$_9$O$_{16}$ [M+H$^+$]: 1825.8100, found 1825.8087.
**S1**

Dialkene (R,R)-**R1** ligand\(^{ab}\) (100 mg, 0.16 mmol) was dissolved in MeCN (10.0 mL) and treated with samarium(III) triflate (32 mg, 0.054 mmol). The reaction mixture was stirred under an inert atmosphere at rt for 2 h. The mixture was concentrated under reduced pressure and the resulting paste was suspended in CH\(_2\)Cl\(_2\). The formed precipitate was filtered off and washed with CH\(_2\)Cl\(_2\) (3 \times 10\ mL) to give complex **S1** as a grey powder (110 mg, 0.044 mmol) in 81% yield. Mp 259 °C. LRMS (ESI) m/z calc for C\(_{124}\)H\(_{129}\)N\(_9\)O\(_{15}\)SSm [M−2OTf\(^{2+}\)]: 1112.42, found 1113.08 (100), C\(_{123}\)H\(_{129}\)N\(_9\)O\(_{12}\)Sm [M−3OTf\(^{3+}\)]: 691.96, found 692.67 (31).

**5**

Prepared as described as above starting from 4 (45 mg, 0.0247 mmol) and europium(III) triflate (15 mg, 0.0247 mmol) to give complex 5 as a white powder (51 mg, 0.0210 mmol) in 85% yield. LRMS (ESI) m/z calc for C\(_{112}\)H\(_{119}\)EuF\(_3\)N\(_9\)O\(_{19}\)S [M−2OTf\(^{2+}\)]: 1062.84, found 1062.92 (100), C\(_{111}\)H\(_{109}\)EuN\(_{9}\)O\(_{16}\) [M−3OTf\(^{3+}\)]: 658.91, found 659.75 (51). HRMS (ESI) m/z calc for C\(_{111}\)H\(_{109}\)EuN\(_{9}\)O\(_{16}\) [M−3OTf\(^{3+}\)]: 658.9070, found 658.9054.\(^{19}\)

**6**

Prepared as described as above starting from complex 5 (24 mg, 0.0099 mmol) to give 6 knot as a white powder (22 mg, 0.0091 mmol) in 92% yield. LRMS (ESI) m/z calc for C\(_{110}\)H\(_{105}\)EuF\(_3\)N\(_9\)O\(_{19}\)S [M−2OTf\(^{2+}\)]: 1048.82, found 1048.92 (100), C\(_{109}\)H\(_{105}\)EuN\(_{9}\)O\(_{16}\) [M−3OTf\(^{3+}\)]: 649.56, found 649.75 (56). HRMS (ESI) m/z calc for C\(_{109}\)H\(_{105}\)EuN\(_{9}\)O\(_{16}\) [M−3OTf\(^{3+}\)]: 649.5631, found 649.5623.\(^{19}\)
Figure 3: ESI found (top) and simulated (bottom) isotopic distribution of 6.

A degassed mixture of CH₂Cl₂ and MeNO₂ (3:1, 200 mL) was added to a flask containing S1 (50 mg, 0.020 mmol). Hoveyda-Grubbs (2nd generation) catalyst (6.2 mg, 0.010 mmol) was added under nitrogen and the mixture was heated to 50 °C for 18 h. The solution was allowed to cool to rt and and quenched with ethyl vinyl ether (1.5 mL). The mixture was stirred for another 30 min (the solution turned brown), concentrated (to ca. 2 mL) and diluted with CH₂Cl₂ (20 mL) added. The precipitate was filtered off and washed with CH₂Cl₂ (3 x 5 mL) to give the crude product as a pale brown solid. The solid was taken up in DMF (4 mL) and aqueous solution of Na₅DTPA (40%, 200 µL) was added. The mixture was stirred at rt for 45 min, filtered off and the filtrate concentrated under reduced pressure. The solid was washed with CH₂Cl₂ (3 x 10 mL) to give knot 6 as pale brown solid (32 mg, 0.013 mmol) in 66% yield. Mp 268 °C. [α]D²⁰ +234.5 (c 0.50, MeOH). LRMS (ESI) m/z calc for C₁₁₈H₁₁₇F₃N₉O₁₅Sm [M−2OTf]⁺: 1070.38, found 1070.08 (100), C₁₁₈H₁₁₇N₉O₁₅Ssm [M−3OTf]⁺: 663.93, found 665.00 (74).
Prepared as described for S1 starting from dialkene (R,R)-R1 ligand (100 mg, 0.16 mmol) and europium(III) triflate (32 mg, 0.054 mmol). Complex 10 was obtained as a pale brown powder (111 mg, 0.045 mmol) in 83% yield.

The analysis is in agreement with the literature.9a
Figure 1: Spectroscopic characterisation of complex 10. A) Emission spectra of 10 recorded with irradiation at 260 nm in MeOH. B) Emission spectra of 10 recorded with irradiation at 260 nm in d₄-MeOD. C) Excitation/emission spectra of 10 in d₄-MeOD monitoring emission at 617 nm. D) UV-Vis absorption of 10 in MeOH. E) Calculation of the absorption co-efficient of 10 in MeOH at 260 nm. F) Lifetime decay profile of 10 in MeOH (λₑₓ = 260 nm, λₑₘ = 617 nm) at 298 K. G) Lifetime decay profile of 10 in d₄-MeOD (λₑₓ = 260 nm, λₑₘ = 617 nm) at 298 K. H) q Value calculations and summary data table.

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Figure 2: Spectroscopic characterisation of symmetric knot 9. A) Emission spectra of 9 recorded with irradiation at 260 nm in MeOH. B) Emission spectra of 9 recorded with irradiation at 260nm in $d_4$-MeOD. C) Excitation/emission spectra of 9 in $d_4$-MeOD monitoring emission at 617 nm. D) UV-Vis absorption of 9 in MeOH. E) Calculation of the absorption co-efficient of 9 in MeOH at 260 nm. F) Lifetime decay profile of 9 in MeOH ($\lambda_{ex} = 260$ nm, $\lambda_{em} = 617$ nm) at 298 K. G) Lifetime decay profile of 9 in $d_4$-MeOD ($\lambda_{ex} = 260$ nm, $\lambda_{em} = 617$ nm) at 298 K. H) $q$ Value calculations and summary data table.
Figure 3: Spectroscopic characterisation of asymmetric knot 6. A) Emission spectra of 6 recorded with irradiation at 260 nm in MeOH. B) Emission spectra of 6 recorded with irradiation at 260nm in d₄-MeOD. C) Excitation/emission spectra of 6 in d₄-MeOD monitoring emission at 617 nm. D) UV-Vis absorption of 6 in MeOH. E) Calculation of the absorption co-efficient of 6 in MeOH at 260 nm. F) Lifetime decay profile of 6 in MeOH (\( \lambda_{ex} = 260 \text{ nm} \), \( \lambda_{em} = 617 \text{ nm} \)) at 298 K. G) Lifetime decay profile of 6 in d₄-MeOD (\( \lambda_{ex} = 260 \text{ nm} \), \( \lambda_{em} = 617 \text{ nm} \)) at 298 K. H) q Value calculations and summary data table.
Crystallography

Data Collection: Synchrotron X-ray data were collected at beamline I19 (λ = 0.6889 Å) Diamond Light Source\textsuperscript{20} for 6 at temperature of 100 K. Data were measured using CrystalClear-SM Expert 2.0 r5 suite of programs.

Crystal structure determinations and refinements: X-ray data were processed and reduced using CrysAlisPro suite of programs. Absorption correction was performed using empirical methods based upon symmetry-equivalent reflections combined with measurements at different azimuthal angles.\textsuperscript{21} The crystal structure was solved and refined against all $F^2$ values using the SHELXTL suite of programs using Olex2.\textsuperscript{22} Atoms corresponding to the aliphatic chains were refined isotropically due to the high disorder of these moieties. Hydrogen atoms were placed in calculated positions refined using idealized geometries (riding model) and assigned fixed isotropic displacement parameters. The phenyl groups were restrained to have idealized geometries using AFIX commands. The C-C, C-O, C-F, C-S and S-O distances in the aliphatic chains and triflates ions were restrained using DFIX and SADI command. The atomic displacement parameters (adp) of the ligands have been restrained using RIGU, EADP and SIMU commands.

Compounds 6 present large voids filled with a lot of scattered electron density, the SQUEEZE protocol inside PLATON suites was used to account the void electron density.\textsuperscript{23} A large number B alerts were found due to high disorder found in the aliphatic chains.

CCDC 6 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax: (+44)1223-336-033; or deposit@ccdc.cam.ac.uk).
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\( ^a R_1(F) = \Sigma (|F_o| - |F_c|)/|F_o|; \) \( wR^2(F_o^2) = [\Sigma w(F_o^2 - F_c^2)^2]/\Sigma wF_o^4\); \( S(F_o^2) = [\Sigma w(F_o^2 - F_c^2)^2]/(n + r - p)\)
Figure 4: Stick representation of the X-ray crystal structure of 6.

Figure 4: ORTEP representation (ellipsoids at 10% probability) of the X-ray crystal structure of 6.
References


19. No NMR data is given because the europium-metal caused the spectra to appear very broad.


23. PLATON, A Multipurpose Crystallographic Tool (Utrecht University, Utrecht, The Netherlands, 2008).
OUTLOOK

A selection of topics in the field of supramolecular chemistry, including the synthesis of molecular machines, mechanically interlocked architectures and their applications, have been discussed. Perhaps the most interesting of all, the small molecule walkers, are not that often reported in the literature. A small molecule walker that is directional, processive, progressive, repetitive and autonomous is not easy to design or synthesise. As nature needs molecules that are much larger to achieve these goals we can wonder if we are ever able to accomplish the same with small synthetic molecules. Small is perhaps not the best word to define the synthetic walker that is described in this manuscript. Although nowhere near the size of kinesin or myosin, it is quite a synthetic effort to make. The difficulty lies in the amount of functional groups that are needed to accomplish the abovementioned five characteristics. Consequently, the target molecule is rather complex and difficult to manipulate. The sensitivity of the molecules required a cumbersome practical execution of the work. The experiments, for example, had been conducted in a dark environment for years. This affected not only me but also a few colleagues, who kindly allowed me to turn the lights in the bay off whenever I needed to. However, the main issue on this particular project was the reproducibility of the chemistry. On several occasions large quantities of material were lost near the end of the synthetic route. Reactions that were conducted successfully before had suddenly become problematic. Usually, these newly emerged difficulties were resolved but at the cost of restarting the synthetic pathway and losing months of progress. In the final two years of the project we had come very close to our target molecule. Being within reach of our goal encouraged me to keep the project going, despite the many drawbacks we experienced during those years. Nevertheless, in the end we decided that the chemistry was too complex and our energy was better invested in other more promising endeavours. From my personal experience and to have seen colleagues working on similar ideas I believe that these sizeable projects should only be considered when the design of the molecule is carefully chosen, the synthetic pathway is substantially shorter and more people are actively participating in it.

Nowadays methods to craft mechanically interlocked structures are well established. We have come to a point where the question is not to how to make such a molecule but what we can do with it. This manuscript described the use of a chiral rotaxane and molecular knot in asymmetric catalysis. The chiral rotaxane was formed using a new reaction in the AMT-approach. After, we successfully exploited the remaining recognition motif of the rotaxane’s macrocycle to employ the rotaxane as a ligand in asymmetric metal catalysis. I find the
ability of the macrocycle to facilitate rotaxane formation through endotopically bound copper and the use of the same recognition motif afterwards to co-ordinate nickel to participate in enantioselective Michael additions, very attractive. Equally appealing is the knot catalysis project, wherein a lanthanide is used to template the trefoil knot formation and employed again (co-ordinated to the molecular knot) in the Mukaiyama aldol addition. Interesting catalytic activities were already discovered in the early stage of this project, although many reactions were not found suitable due to the knot’s low solubility. In contrast to the rotaxane, molecular knots of the type we were interested in had already been made by some of my co-worker, which really accelerated the process and will allow us to complete the project within the next months. Of course there have also been complications. Both the rotaxane and molecular trefoil knot are complex architectures and acquiring a good amount to screen for catalysis conditions was very challenging. We often had to perform these reactions with quantities of a few milligrams due to the lack of enough chiral ligand. Luckily the reactions were clean and easy to monitor by $^1$H NMR and HPLC. So far only a few examples exist wherein the rotaxane architecture is used for asymmetric catalysis, whereas knot catalysis has never been explored. It will be very exciting to see how this field will develop and what advantages mechanically interlocked ligands provide compared to traditional catalysts.