Peptide rotaxanes as potential prodrugs

By
Stéphanie Potok

Degree of Doctor of Philosophy
Department of Chemistry
University of Edinburgh
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Abstract

Peptides are potential therapeutic agents involved in a wide range of biological processes. In principle, rotaxanes can be used as novel prodrug delivery systems to overcome the problems of peptide degradation *in vivo* and their poor membrane transport permeability. The presence of the macrocycle around the peptide thread acts as a protective shield against peptidases and modifies its cell membrane transport characteristics. However, the classical 'clipping' method of rotaxane formation is mainly limited to dipeptide sequences since the presence of intramolecular hydrogen bonds in longer peptide threads causes folding of the backbone, preventing good interactions with the precursor to the macrocycle. This thesis focused on the synthesis of short peptide rotaxane building blocks. Their elongation on both sides of the peptide backbone was then applied to the straightforward synthesis of oligopeptide [2] and [3]rotaxanes in very good yields.
Attended Lectures and Meetings

1. 41ème Semaine d'Etudes de Chimie Organique SECO, Sainte-Maxime - France – 23-29 May 2004
2. UK Macrocycles and Supramolecular Chemistry Meeting Sheffield – 8-9 January 2004
   Poster: “Nouvelle stratégie pour la synthèse de rotaxanes : Application à la synthèse d'un rotaxane de la Leu-enképhaline“
4. 226th ACS National Meeting New York City, NY - 7-11 September 2003
   Talk: “Synthesis and N-elongation of new rotaxane building blocks: towards new prodrug systems”
5. 40ème Semaine d'Etudes de Chimie Organique SECO, La Grande Motte - France – 11-17 May 2003
   Talk: “Synthèse de nouveaux synthons rotaxanes en série peptidique : vers de nouvelles prodrogues”
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List of Abbreviations

Chromatography Hydrophobicity Index: CHI
logP: octanol/water partition coefficient
CD cyclodextrin
DMSO dimethylsulphoxide
DMF $N,N'$-dimethylformamide
THF tetrahydrofuran
TFA trifluoroacetic acid
EDCI-HCl = 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride
DMAP 4-dimethylaminopyridine
NMR Nuclear Magnetic Resonance
ppm part per million
δ chemical shift
$E_{trans}$ isomer
$Z_{cis}$ isomer
m.p. melting point
TLC Thin Layer Chromatography
FAB Fast Atom Bombardment
rt room temperature
mL millilitres
g grams
HRMS High Resolution Mass Spectrometry
Calcd. Calculated
DIPEA N-ethyldiisopropyl amine
ADEPT antibody-directed enzyme prodrug therapy
General Remarks on Experimental Data

All the melting points (m.p.) were determined using a Electrotermal 9100 melting point apparatus and are uncorrected. $^1$H (400 MHz) and $^{13}$C (100 MHz) NMR spectra were recorded on a Bruker DPX 400 MHz spectrometer using dilute solution in CDCl$_3$, $d_6$-DMSO, C$_2$D$_2$Cl$_4$, d$_4$-MeOD) and the chemical shifts are reported in part per million (ppm) from low to high field. All the $^1$H and $^{13}$C NMR spectra were recorded at 298K unless otherwise stated. $^1$H NMR are reported as follows: br = broad, s = singlet, d = doublet, dd = doublet of doublets, t = triplet, dt doublet of triplets, q = quartet, m = multiplet, $J$(H,H) = coupling constant. H-H COSY and HMQC were also recorded for most of the compounds to enable more detailed assignement of $^1$H and $^{13}$C signals. Column chromatography was carried out using Kiesegel C60 (Merck) as stationary phase. TLC detection was performed on silica gel plates (0.25 mm thick, 60 F254, Merck, Germany). The TLC plates were observed under UV light or spotted using 0.2% ninhydrin in EtOH and successively heated with an heatgun. Mass spectrometry and HRMS analyses, using Masslynx software version 2.3, were performed by the University of Edinburgh mass spectrometry service using fast atom bombardment (FAB) from m-nitrobenzyl alcohol matrix unless otherwise stated. HPLC grade MeOH was obtained from Fischer. Solution of 0.1% TFA in MeOH was prepared. Luna 5u C$_{18}$(2) 100A column (250 mm x 2.00 mm, 5 μm) was purchased from Phenomenex. Reagents and anhydrous solvents used for the reactions were purchased from Aldrich and were in general used without further purification. Isophthaloyl dichloride was routinely recrystallized from hexane and para-xylylenediamine was distilled under reduced pressure.
Layout of this Thesis

The work presented in this thesis describes (i) the synthesis of rotaxane building blocks and their C- and N-elongation towards oligopeptide rotaxanes. A brief review of the literature is given in chapter one describing the background of hydrogen bondings as templates for rotaxane synthesis, from simple amides to elaborate peptides.

The remainder of the thesis discusses my own experiments in this area and is presented in the form of three chapters that are actually articles that have either already been published, are in press or have been prepared for submission to a peer-reviewed journal. No attempt has been made to rewrite the work out of context.

To my family
Chapter One

Introduction

Naturally occurring peptides:

Muses for the synthesis of interlocked architectures

1.1. Naturally occurring interlocked peptide architectures

Nature is a rich source of topologically complex molecular architectures with more and more examples being reported every year. In 1967, Vinograd described the first mitochondrial DNA dimers and higher oligomers as stable and isolable DNA molecules.\(^1\) They consist of independent, double stranded closed circles topologically interlocked as catenanes. Diverse enzymes have shown their ability to form knots and catenanes in DNA rings including \textit{Escherichia coli} DNA topoisomerase\(^1\) and a resolvase or recombination enzyme.\(^4\) The formation of such topological structures is of crucial importance as it occurs during the replication or recombination of the DNA.

It has also been known for many years that proteins often contain "random" disulfide bonds between cysteine groups of different loops, origin of knot formation. The first cystine knot was discovered in 1982 by Rees and Lipscomb in the potato inhibitor complex of carboxypeptidase A.\(^5\) This amazing structure, now known to be common in proteins, consists of a macrocycle, formed by two disulfide bonds and their interconnecting backbone segments, threaded by a third disulfide bond. Following this
cystine knot-containing bioactive peptides were discovered. Their compact structure, their remarkable high stability\textsuperscript{6} \textit{in vivo} due to high content in disulfide bridges and their “knotted” architecture make them very attractive scaffolds for drug design.\textsuperscript{7} Indeed they exhibit a broad range of biological properties including antiviral,\textsuperscript{8-10} antibiotic\textsuperscript{11} and insecticidal activities for example. They are classified into three sub-categories (Figure 1.1).

![Figure 1.1](image)

\textbf{Figure 1.1} The three classes of cystine knots. The beta-strands are represented as arrows, the cysteine residues are labelled I-VI from the \textit{N}- to \textit{C}-terminus and the disulfide bonds are drawn as red lines.

(i) The growth factor cystine knots are made of 60 to 4000 amino acids, with an 8 to 14 amino acid ring. There are more than 600 members known, most being hormones. (ii) The inhibitor cystine knots are made of 25 to 100 amino acid residues, with an 8 to 15 amino acid ring. There are more than 300 members known including cation-channel blockers, protease inhibitors, neurotoxins, antimicrobials, antitumour agents, insecticides. (iii) The cyclic cystine knots, or “cyclotides”, include one more degree of topological complexity as they combine both a head-to-tail cyclized backbone and the cystine knot. They are made of 28 to 38 amino acid residues, with an 8 amino acid ring. There are more than 40 members known including antivirals, neurotensin inhibitors, antimicrobials, antitumour agents, uterotonics (Figure 1.2.).
In the early 1990’s, knots in the backbone were unambiguously identified in proteins.\textsuperscript{14-18} The presence of a knot in the middle of the protein backbone of lyvel was recently confirmed by the use of a computer algorithm to “smooth” the folded structure.\textsuperscript{17}

Between 1994 and 1996, several “lasso” peptides were identified including RES-701-1,\textsuperscript{19} anantin,\textsuperscript{20} siamycin I and II,\textsuperscript{21} RP 719555\textsuperscript{22} and MS-271.\textsuperscript{23} Their common features are: (i) 16 to 21 amino acid residues including a ring of 8 or 9 amino acids, (ii) a “tail” passing through the “ring” region, (iii) a compact structure along with high \textit{in vivo} stability and high biological activity.

In 1998, Duda identified the bacteriophage HK97 capsid as a 42 kDa protein containing 420 monomers and a portal.\textsuperscript{24} This icosohedral-shaped viral capsid is made of 60 hexameric and 12 pentameric rings assembled into a pseudo-catenated supramolecular architecture which, after closure, give a “protein chainmail”: a network of topologically catenated protein macrocycles.\textsuperscript{25}
In 2003, three research groups simultaneously reported a seventh 3D “lasso” structure in Microcin J25 (MccJ25), a 21-amino acid peptide (Figure 1.3.). At one end of the peptide is a ring formed by an amide linkage between the N-terminus and the side group on the eighth amino acid residue, the linear end of the peptide loops back and threads through the cyclic part. This rotaxane-like secondary structure is stabilized by the lateral chains of the two bulky amino acids Phe19 and Tyr20, which prevent from “dethreading”.

![Figure 1.3. Microcin J25 (MccJ25), a 21 amino acid “lasso” peptide.](image)

Originally reported to be circular, MccJ25 has generated a widespread interest primarily because of its highly unusual mode of antibacterial action as well as its intriguing structural characteristics. MccJ25 was mainly shown to inhibit bacterial transcription by binding to RNA-polymerase and obstructing its secondary channel. MccJ25 also exhibits interesting pharmacological features: extreme stability to peptidases, unique modes of antimicrobial and antiviral action, impressive membrane transport and solubility characteristics, and unprecedented stability to thermal and chemical denaturing.
Recently, chemists have taken up the challenge proposed by nature by endeavoring the synthesis such interlocked molecules. In 2001 Dawson reported the synthesis of the first protein [2]catenane, via a folding-directed strategy. Taking advantage of the dimeric type structure of the tumor suppressor protein p53 and of its particular spatial conformation, a [2]catenane was obtained by joining the N-terminal of a cysteine residue with the C-terminus via native chemical ligation (Figure 1.4.).

![Image of p53 catenane synthesis]

**Figure 1.4.** Synthesis of the p53 catenane.

The Leigh group has pioneered the derivatization of peptides as rotaxanes as means of providing enzymatic resistance against proteases and modulating physical properties of the peptide - such as its solubility and lipophilicity - via the wrapping of a macrocycle along the backbone (Scheme 1.1.). My thesis took place with the aim of building hybrids of natural peptides using rotaxanes (See clipping strategy as part of the hydrogen-bonding interactions section).
In order to efficiently build such interlocked architectures, chemists have developed synthetic strategies using four different templating interactions for self-assembly. Hydrogen bonding will be examined more deeply in this short review as a key-feature for the synthesis of peptide rotaxanes. Previous work on their synthesis will be exposed along with their inherent limitations. Finally, an insight into rotaxanes and their future potential as drug delivery systems will be discussed.

1.2. Towards template directed synthesis of interlocked architectures

Interlocked architectures have generated a widespread interest because of their intrinsic properties: the individual components can move independently of each other, but cannot be separated without the cleavage of a covalent bond.

1.2.1. Definitions and synthetic strategies

The most studied and synthesized interlocked architectures are rotaxane, catenanes and knots (Figure 1.5.).
A catenane, from the Latin *catena*, meaning chain, is a molecular species formed by two, or more, mechanically interlocked macrocycles which can only be separated by breaking one or more covalent bonds. A rotaxane, from the Latin *rota* and *axis* meaning respectively wheel and axle, consists of one or more macrocycles threaded onto a linear molecule ("thread") which is kinetically stable due to the bulky groups ("stoppers") at both ends preventing the "slippage" of the macrocyclic unit. A rotaxane without stopper is referred to as a "pseudo-rotaxane". In all these systems, a prefix number in square bracket indicates the number of the interlocked components.

Three distinct strategies have been developed for the synthesis of rotaxanes (Figure 1.6.): i) "threading", where the equilibrium-dependent formation of a pseudo-rotaxane is trapped by a "capping" reaction with two bulky stoppers preventing the macrocycle from dethreading, ii) "slippage", where a macrocyclic unit passes over the stoppers of a thread, usually at elevated temperatures and iii) "clipping", where the...
rotaxane is obtained by the closure of an acyclic unit templated around a "stoppered" thread.

![Diagram showing three different synthetic strategies to obtain rotaxanes.]

**Figure 1.6.** Three different synthetic strategies to obtain rotaxanes.

1.2.2. Early attempts of synthesis via statistical methods

The first synthesis of a [2]rotaxane, 1.3, was reported by Harrison and Harrison in 1967. The synthesis used a statistical approach based on the random threading of a linear dialcohol 1.1 through a 30-membered ring loaded on a Merrifield resin 1.2. The resulting pseudo-rotaxane was "stoppered" by reaction with an excess of a trityl chloride to give the [2]rotaxane in 6% yield after repeating the reaction 70 times (Scheme 1.2.).
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Scheme 1.2. Synthesis of the first rotaxane 1.3 in 6% yield by a statistical method repeated 70 times. a)
(i) triphenylmethylchloride, pyridine, DMF, toluene, (ii) cleavage.

Shortly after this, Schill and co-workers reported another synthetic methodology consisting of the directed synthesis of a rotaxane precursor 1.4. Selective cleavage of covalent bonds afforded the desired interlocked molecule 1.5 (Scheme 1.3). This methodology did not resolve the major and limiting problem of extremely poor yields in the synthesis of such interlocked molecules.

Scheme 1.3. Synthesis of the first rotaxane 1.5 by "covalently" directed synthesis.

The low yields in the above examples highlight the problem of using statistical methods: as there is no thermodynamic driving force to facilitate the formation of interlocked species. Research aimed at improving the yields of interlocked species focused on the use of templating interactions.
1.2.3. Template directed synthesis

The advent of templates marked a new era for interlocked architectures. The use of non-covalent intermolecular interactions made it possible to form stable interlocked complexes in high yields.

1.2.3.1. Hydrophobic interactions

The most extensively studied type of rotaxanes formed by the use of hydrophobic interactions are those with a cyclodextrin-based structure. Cyclodextrins (CDs) are cyclic oligosaccharides consisting of six or more \( \alpha-1,4 \)-linked D-glucopyranose units. The conformation of CDs is a rigid cavity with a truncated cone shape. They have an hydrophilic exterior, due to the hydroxyl groups on the outside surface, and a hydrophobic core due to the hydrocarbon units of the interior. Such physicochemical properties make them both water-soluble and convenient hosts for complexing molecular guests in water.

Examples of cyclodextrin-based [2]rotaxanes, reflecting the diversity of possible structures, are shown in scheme 1.4.

Scheme 1.4. Examples of cyclodextrin-based [2]rotaxane 1.6 and 1.7.
1.2.3.2. *Metal template*

Another strong type of interaction used in the synthesis of interlocked molecules is the metal coordination of organic ligands. The synthesis and study of rotaxanes and catenanes based on these interactions have been carried out by the group of Sauvage since 1983, after the successful preparation of a [2]catenane.\(^4\) Once an interlocked molecule has been synthesized *via* a metal template, it can generally be demetallated, leaving the two interlocked parts free to move relative to each other, as illustrated in scheme 1.5.\(^3\)

![Scheme 1.5. Synthesis of rotaxane 1.12 via transition metal coordination, with \(-\text{Ar}_3=\text{C}(p\text{-t-Bu})_2\text{Ph.}\) a) \([\text{Cu}(\text{CH}_3\text{CN})_4]^+\text{BF}_4^-\) 1.10; b) (i) \(\text{I}(\text{CH}_2)_2\text{Ar}_3\), \(\text{K}_2\text{CO}_3\), (ii) Amberlite-CN.\(^3\)](image-url)
1.2.3.3. \( \pi-\pi \) stacking interactions

Stoddart's research group studied\(^{48}\) the inclusion complexes of electron-deficient paraquat with electron-rich crown ethers 1.13, and the analogous \( \pi \)-deficient tetracationic receptor with \( \pi \)-rich guests such as 1,4-dimethoxybenzene 1.14 (Scheme 1.6).

![Scheme 1.6. Inclusion complexes using aromatic \( \pi-\pi \) stacking interactions](image)

Stoddart used these \( \pi-\pi \) stacking interactions as a template for the synthesis of rotaxanes, such as rotaxane 1.15 (Scheme 1.7).\(^{49}\)

![Scheme 1.7. Example of rotaxane 1.15 using aromatic interactions.](image)
1.2.3.4. Hydrogen-bonding interactions

1.2.3.4.1. Threading

Vögtle et al. reported the first synthesis of an amide-based rotaxane by threading of an acid chloride through a preformed macrocycle that serves as hydrogen-bond donor (Scheme 1.8.). With the aim of studying the limits of the templating effect, a series of rotaxanes were synthesized using heterocyclic diamide threads threads. The threading process was found to be very tolerant of the different structural and electronic characteristics of the selected threads. The key-step of this molecular recognition mechanism is the formation of an amide bond between one stopper and the dichloride moiety through the macrocycle.

Expansion of the macrocycle by a sulfonamide$^{54,55}$ and methylene groups$^{56}$ enabled the use of a range of sterically hindered stoppers.$^{57-63}$
It is noteworthy that Vögtle reported the first synthesis of rotaxane by trapping of an anion-macrocycle complex in 1999.\textsuperscript{64} The phenolate anion 1.20 was bound to the tetralactam macrocycle 1.17 by hydrogen-bond interactions. This nucleophile then reacted with the electrophile 1.22 to form rotaxane 1.23 (Scheme 1.9).

\begin{center}
\textbf{Scheme 1.9. Anion-template synthesis of rotaxanes 1.22.}
\end{center}

\begin{enumerate}
\item \textbf{1.2.3.4.2. Slippage}

Vögtle synthesized rotaxanes by fast melting of the macrocycle and the thread.\textsuperscript{65} The slippage strategy allows for the synthesis of the “unobtainable” rotaxane 1.24 where no template interaction occurs between the macrocycle 1.17 and the thread 1.23 (Scheme 1.10.).\textsuperscript{56}
\end{enumerate}
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Scheme 1.10. Nontemplate synthesis of rotaxane 1.24 by slippage.

1.2.3.4.3. Clipping and preambule to the thesis

Leigh et al. reported the synthesis of the first benzylic amide rotaxane 1.28 in 28% by clipping around the isophthalamide thread 1.25 of an equimolar mixture of isophthaloyl dichloride 1.26 and para-xyylene diamine 1.27 in presence of Et$_3$N in CHCl$_3$ (Scheme 1.11.).

Scheme 1.11. Synthesis of rotaxane 1.28 via the clipping strategy.
The more rigid fumaric rotaxane 1.29 was synthesized in 97% yield. The success of the fumaramide template was explained by the spatial arrangement of the cooperative hydrogen bonding sites on the rigid thread, due to the E-isomery of its double bond, at an ideal distance apart to template the formation of the benzylic amide macrocycle through the intercomponent hydrogen bonding. Remarkably enough the substitution of one or both the amides of the highly preorganized thread by much weaker hydrogen bond acceptor groups as esters still templated the formation of rotaxanes 1.30 and 1.31 (Scheme 1.12.).

The mechanism of rotaxane formation is initiated by the directed assembly of the macrocycle around two transoid amide bonds. It is noteworthy that peptide sequences carry this structural arrangement leading to the synthesis of the first dipeptide rotaxane, GlyGly 1.32, in 62% yield. The thread template induces a conformational change of the open-chain precursor from syn-anti to syn-syn via cooperative multipoint hydrogen bonding interactions, bringing the terminal amine and acid chloride in close proximity in complex I to lead to rapid cyclization. During the crucial phase of the “wrapping”, the precursor to the macrocycle establishes a complicated pattern of weaker secondary interactions depending on the intermolecular binding with the two amide groups of the
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thread (Scheme 1.13.). The better the fit between the thread and the macrocycle precursor, the higher the yield in rotaxanes as illustrated by the series of dipeptide rotaxanes synthesized via clipping strategy (Scheme 1.13.).

```
\[
\text{Gly-Gly} \quad 1.32 \quad R_1 = H \quad R_2 = H \quad R_3 = H \quad R_4 = H \quad 62% \\
\text{Gly-Sar} \quad 1.33 \quad R_1 = H \quad R_2 = H \quad R_3 = Me \quad R_4 = H \quad 60% \\
\text{Gly-L-Trp} \quad 1.34 \quad R_1 = H \quad R_2 = H \quad R_3 = H \quad R_4 = 56% \\
\text{Gly-L-Ala} \quad 1.35 \quad R_1 = H \quad R_2 = H \quad R_3 = H \quad R_4 = CH_3 \quad 40% \\
\text{Gly-L-Leu} \quad 1.36 \quad R_1 = H \quad R_2 = H \quad R_3 = H \quad R_4 = CH_2CH(CH_3)_2 \quad 37% \\
\text{Gly-L-Met} \quad 1.37 \quad R_1 = H \quad R_2 = H \quad R_3 = H \quad R_4 = CH_2CH_2SMe \quad 36% \\
\text{Gly-L-Phe} \quad 1.38 \quad R_1 = H \quad R_2 = H \quad R_3 = H \quad R_4 = CH_2Ph \quad 32% \\
\text{Gly-L-Cys} \quad 1.39 \quad R_1 = H \quad R_2 = H \quad R_3 = H \quad R_4 = CH_2SCH_2Ph \quad 25% \\
\text{Sar-Gly} \quad 1.40 \quad R_1 = Me \quad R_2 = H \quad R_3 = H \quad R_4 = H \quad 22% \\
\text{Gly-L-Šer} \quad 1.41 \quad R_1 = H \quad R_2 = H \quad R_3 = H \quad R_4 = CH_2OCH_2Ph \quad 19% \\
\text{Gly-L-Asp} \quad 1.42 \quad R_1 = H \quad R_2 = H \quad R_3 = H \quad R_4 = CH_2CO_2CH_2Ph \quad 16% \\
\text{Gly-L-Tyr} \quad 1.43 \quad R_1 = H \quad R_2 = H \quad R_3 = H \quad R_4 = CHCH_3OCH_2Ph \quad 14% \\
\text{Gly-L-Thr} \quad 1.44 \quad R_1 = H \quad R_2 = H \quad R_3 = H \quad R_4 = H_2C-\text{OCH}_2\text{Ph} \quad 12% \\
\text{Gly-L-Pro} \quad 1.45 \quad R_1 = H \quad R_2 = H \quad R_3 = H \quad R_4 = 6% \\
\text{L-AlaGly} \quad 1.46 \quad R_1 = H \quad R_2 = CH_3 \quad R_3 = H \quad R_4 = H \quad 0% \\
```

**Scheme 1.13.** Rotaxane synthesis with flexible peptide-based hydrogen-bond templates.
These results showed that structural requirements for efficient dipeptide rotaxane synthesis via hydrogen bonding are: (i) an N-terminal glycine residue is necessary to avoid steric hindrance, (ii) the distance and the transoid arrangement of the two amide carbonyl moieties in the peptide backbone are suitable for the template effect between the peptide thread and the precursor of the macrocycle, (iii) the α-substituent on the second amino acid residue must not be too bulky to allow a correct hydrogen bonding site. The synthesis of the L-PheGlyGly and TyrGlyGlyPheLeu rotaxanes in 2.5 and 1% yield respectively highlighted that an internal template site in a longer peptide chain can satisfy the requirements for rotaxane formation. However the likely folding of the backbone by intramolecular hydrogen bonding is probably responsible for the dramatically low yields obtained, preventing from a good preorganisation of the precursor macrocycle around the thread.\textsuperscript{71}

Leigh et al. has shown\textsuperscript{71} that the presence of a macrocycle around a peptide thread protects against enzymatic degradation and can completely modify the cell membrane transport characteristics of the peptide. Derivatization of peptides into rotaxanes could allow the design of novel prodrug delivery systems as, despite of their involvement in a wide range of biological processes, peptides generally fail as therapeutic agents\textsuperscript{72} because of their degradation \textit{in vivo} and their poor membrane transport permeability and absorption properties.\textsuperscript{73} However, there has been recently a renewed interest to their development into human therapies as selective drug-delivery systems in treatments of infections, inflammation, immune system disorders, obesity, diabetes, cardiovascular diseases and cancer.\textsuperscript{74}
The main issue towards the use of peptide rotaxanes as prodrugs is their efficient synthesis in good yields. This thesis describes the synthesis of oligopeptide rotaxanes via extension of short rotaxane building blocks. In chapter two, the synthesis of a series of activated ester rotaxane building blocks is reported. Their elongation was carried out for the efficient synthesis of oligopeptide rotaxanes and especially of the rotaxane of a protected bioactive pentapeptide. Chapter three is dedicated to the synthesis of N-protected tripeptide rotaxane building blocks functionalized on their lateral chain by a labile bulky stopper and elongated after N-deprotection, via classical peptide couplings, to reach oligopeptide [2] and [3]rotaxanes in solution. Fusion of both types of building blocks leads to the synthesis of an N-protected tripeptide activated ester rotaxane building block extended via both the C- and N-terminal sides (chapter 4).
1.3. Properties modulation via encapsulation and potential applications towards drug delivery systems

Such interlocked architectures are interesting candidates for the design of potential drug delivery systems. A rotaxane made of a physiologically active macrocycle or thread could indeed be carried to a specific receptor and then be decomposed into its constituting components via stopper cleavage and release the active form of the prodrug. Little research has been done so far on this topic and investigations still remain at the in vitro step.

1.3.1. Rotaxane encapsulation: towards modulation of solubility, lipophilicity and enzymatic degradation

Leigh et al. showed that encapsulation of the thread within the macrocycle results in changes in the physical, chemical and biological properties of both components. The effect of encapsulation on lipophilicity was studied by measurement of the Chromatography Hydrophobicity Index (CHI log D values, comparable to log P) of a series of [2]rotaxanes of the glycyglycine thread 1.47 (Scheme 1.14.).

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The CHI log D values varied with the structure of the macrocycle. Pyridyl derivative 1.51 showed a high value in the CHI log D series, due to its exceptionally lipophilic nature (Table 1.1.).

<table>
<thead>
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<th>X</th>
<th>Y</th>
<th>peptide rotaxane derivatives</th>
<th>Yield %</th>
<th>CHI log D</th>
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<td>3.67</td>
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</tr>
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<tr>
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<td>NO₂</td>
<td>1.52</td>
<td>50</td>
<td>3.10</td>
</tr>
</tbody>
</table>

Table 1.1. Lipophilicity data for a family of functionalized [2]rotaxanes at pH 7.4.

Reagents: (i) 2,2'-N-methylmorpholine N-oxide, cat. OsO4, CH₂Cl₂, 97%.
Conditions: reverse phase HPLC - ODS2-1K5 Inertsil column - 150 x 4.6 x mm (Capital HPLC Ltd., Broxburn, Scotland), mobile phase: 50 mM ammonium acetate (7.0<pH<7.3)/acetonitrile, flow-rate: 1.00 mL/min.

Leigh et al. synthesized the rotaxane of the Leu-enkephalin (TyrGlyGlyPheLeu) 1.53, a bioactive pentapeptide, in order to evaluate the protective potential of the macrocycle towards enzymatic degradation (Scheme 1.15.).

Scheme 1.15. Synthesis of the Leu-enkephalin rotaxane 1.53.
α-chymotrypsin-catalyzed degradation showed the protective effect of the macrocycle on the peptide thread as no degradation of the debenzylated Leu-enkephalin rotaxane 1.55 could be detected even after 24 hours compared to a 35 minute half-life for the corresponding debenzylated thread 1.54 (Scheme 1.16).

Scheme 1.16. Biodegradation studies of the tBoc-protected Leu-enkephalin 1.54 and its corresponding rotaxane 1.55 by α-chymotrypsin. Conditions: 10% acetonitrile/water (0.05M tris buffer, pH 7.8) using a 0.01M solution of α-chymotrypsin.

These results indicate that encapsulation of peptide threads within a tetraamide macrocycle can be used to modulate lipophilicity and enzyme degradation of the supramolecular architecture, two properties of fundamental importance to successful peptide based drug design.
1.3.2. Host-[2]rotaxanes as guest transporters

Smithrud et al.'s research aims at using rotaxane architectures as mimetics for artificial receptors able to carry a guest through the many biological environments encountered during cell penetration. For this purpose, a series of host-[2]rotaxanes\textsuperscript{77} was synthesized. The convergent arrangement of the functional groups of the hydrophobic pocket and the macrocyclic amino acid residue was shown to be particularly efficient for binding amino acid type guests that have an aromatic ring and a negative charge (Scheme 1.17.).\textsuperscript{78}
Scheme 1.17. Host-[2]rotaxanes 1.56-1.58.\textsuperscript{79}
1.3.3. Biodegradable polyrotaxanes as potential drug delivery systems

Cyclodextrins (CDs) are of interest in medicinal chemistry due to their ability to form water-soluble and low-toxicity complexes with encapsulated guest molecules in their hydrophobic cavities. Yui and Ooya have proposed new types of drug carriers made of biodegradable cyclodextrin and PEG polyrotaxanes that can be dissociated via terminal hydrolysis of their bulky stoppers.

1.3.3.1. Characterization of biodegradable cyclodextrin polyrotaxanes

The drug-polyrotaxane conjugates are characterized by the supramolecular structure of the drug carrier and the drug-immobilized cyclodextrin (Figure 1.7.).

Specific drug delivery can be achieved by building conjugates with two distinct degradable moieties: (i) the endgroup stoppers cleaved by an extracellular enzyme and (ii) the drug-cyclodextrin spacers cleaved by an intracellular enzyme (Figure 1.8.).
1.3.3.2. Synthesis of drug-polyrotaxane conjugates

1.3.3.2.1. Example of the Theophylline-polyrotaxane conjugate

The theophylline-polyrotaxane conjugate 1.59 was the first example of drug-polyrotaxane conjugates synthesized by Ooya et al. (Scheme 1.18). 80

Efficient release of Theo-α-CDs from theophylline-polyrotaxane conjugate 1.59 was only observed in presence of papain or α-chymotrypsin. This indicates that the association
of the conjugates does not bring any steric hindrance but rather enhances the accessibility of the enzyme to the terminal peptide linkage.

1.3.3.2. Multivalent-ligand-polyrotaxane conjugates

Many ligand molecules have the ability to interact with surface binding proteins, especially in the intestine, which expose multivalent interactions on cell surfaces as multiple copies of recognition elements. Multivalent-ligand-polyrotaxane conjugates, including biotin and peptide-polyrotaxane conjugates, have been designed to temporally bind with transporters until dissociation via controlled terminal enzymatic hydrolysis leading to ligand-α-CD conjugate release (Figure 1.9.).

![Diagram](image)

**Figure 1.9.** Controlled-multivalent binding with the transporters using ligand-polyrotaxane conjugates for altering the transporter properties.

1.3.3.3 Polyrotaxanes as drug penetration enhancers
Transdermal drug delivery is an ideal form of drug administration as it maintains quasi-constant plasma level of drugs. However this requires the use of penetration enhancer formulations necessary to increase the skin permeability to the drug at rates sufficiently high for therapeutic efficacy. The use of polymeric enhancers may be a solution to increase drug penetration with no induction of physiological problems in subcutaneous tissues, such as acute and chronic inflammation. The mechanical aspects of their introduction into the cell membranes have been extensively discussed in relation to (i) changing the structure or dissolving skin lipids, (ii) altering the conformation or denaturing skin proteins, (iii) disrupting the water structure in skin and (iv) increasing membrane fluidity. Increased membrane fluidity can indeed accelerate two cellular functions: (i) the mobility of membrane proteins or enzymes, which are related to regulating cellular metabolism and (ii) the permeability of ions and drugs (Figure 1.10.).

![Diagram](image)

**Figure 1.10.** Effect of enhanced membrane fluidity on cellular functions.\(^{86}\)

Ooya’s and Yui’s polyrotaxanes have been shown (i) to increase plasma fluidity of red blood cells,\(^ {86}\) (ii) to decrease the bound water rate at the *stratum corneum*\(^ {87}\) at 37°C, indicating effective extraction of polar lipids or proteins, or the exchange of water in the

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polar lipids and (iii) to maintain the structure of the lipid bilayers in the *stratum corneum* even after treatment. Enhanced permeation of indomethacin, a drug used in the treatment of arthritis, through the skin was observed by treatment with polyrotaxanes.\textsuperscript{88}

Ooya and Yui have shown that biodegradable polyrotaxanes are promising drug delivery systems due to (i) their supramolecular dissociation *via* terminal hydrolysis and (ii) their rodlike structure. They are feasible drug-carriers and drug-penetration enhancers.
1.4. Conclusion and Outlook

Nowadays interlocked architectures are not only curiosities as this was the case in the 1960's: the creation of functional molecules has been proved to be feasible in the laboratory.

This is due to a better understanding of self-assembly and especially to the use of chemical templates for the synthesis of a large range of incredibly elaborate interlocked architectures.

Besides synthesizing these challenging molecules, their use as new therapeutic agents is getting more and more challenging. Much attention is focused on their potential as drug delivery systems including mimetics for artificial receptors in a host-guest molecular recognition process, permeation enhancers, enzyme resistant structures.

In vitro assays have shown their great potential and hopefully in vivo assays will be the next step towards their further development.
1.5. References


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Elongation of readily-accessible building blocks:

An efficient synthesis of a Leu-enkephalin rotaxane

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**Abstract:** The synthesis of new activated ester rotaxane building blocks, in peptidic and fumaric series, as new tools for making hard to reach molecules is reported. Their further elongation was carried out to efficiently give oligopeptide rotaxanes in very good yields. To overcome solubility problems and folding of the backbone, the method was then extended to the synthesis of a Leu-enkephalin rotaxane and a bistable molecular shuttle, otherwise isolated in poor yields via the conventional "clipping" strategy. This strategy leads easily to very large libraries of interlocked architectures.

**Keywords:** activated ester - peptides – building blocks – rotaxanes – hydrogen bonds – solubility - library.
2.1. Introduction

In the last few years, knots, then catenanes and finally rotaxanes of naturally occurring peptides and protein backbones have been unambiguously identified. Amongst the latter, a few naturally occurring “lassoed” type small peptides (16-21 amino acids) have been reported: Microcin J25(MccJ25), anantin, RES-701-01, RP 71955, syamicyn II, and MS-271. Unlike conventional peptides, these have shown very exciting properties such as unique modes of antimicrobial and antiviral actions, impressive membrane transport and solubility characteristics, unprecedented stability to thermal and chemical denaturing and, of greater relevance, an extreme stability to peptidases. Inspired by the impressive interlocked architectures that nature can build, we recently described the derivatization of dipeptides into rotaxanes incorporating benzylic 1,3-carbamide macrocycles, using a five-component hydrogen bond-directed assembly. Solubility in organic solvents and enzymatic resistance properties of the threads can be greatly modulated by the presence of the macrocycle, thus tailoring their cell membrane transport characteristics towards successful peptide based prodrug systems. However, encapsulation via the clipping strategy has been limited so far to short peptide sequences. Intramolecular hydrogen bonding and insufficient solubility of longer peptide threads in apolar solvents can induce the folding of the backbone, thus preventing a good preorganization between the peptide thread and the precursor of the macrocycle. As an example, the rotaxane formation of a Leu-enkephalin has previously been reported in a low yield of 1%.
In order to remedy this problem, we describe here an efficient and versatile synthesis of new activated ester rotaxane building blocks in peptidic and fumaric series. The choice of 2,6-diphenyl-\textit{para}-nitrophenyl as an activated ester was two-fold: (i) its bulkiness prevents the disassembly between the macrocycle and the thread, and (ii) its reactivity is controlled: despite the steric hindrance in the rotaxane building blocks, the ester was found to be sufficiently reactive to nucleophilic attack\textsuperscript{22} and of similar reactivity to \textit{para}-nitrophenyl ester,\textsuperscript{23} but still less reactive than isophthaloyl dichloride towards a large excess of \textit{para}-xylylene diamine during the rotaxane formation. Also these building blocks are stable enough to be purified by column chromatography and stored for many weeks at room temperature. Only two other examples of rotaxane building blocks used for elongation have been reported in the literature so far, however none has been applied to the preparation of oligopeptide rotaxanes.\textsuperscript{24,25}

2.2. Results and discussion

\textit{Synthesis of rotaxane building blocks in peptidic and fumaric series}

Three activated ester threads were prepared with the aim of synthesising rotaxane building blocks (Schemes 2.1. and 2.2.). They only differed in their templating moiety for rotaxane formation: (i) a glycylglycine subunit, the best template in dipeptide series,\textsuperscript{21} (ii) a fumaric amide ester and (iii) a fumaric diamide template, the latter having been reported as the most efficient hydrogen bonding motif in our interlocked systems.\textsuperscript{26} The dipeptide activated thread 2.4 was prepared in a three step sequence from the dipeptide glycylglycine derivative 2.1 (Scheme 2.1.) using standard BOP coupling peptide chemistry.\textsuperscript{27}
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Scheme 2.1. Synthesis of the dipeptide activated ester thread 2.4. a) Diphenylacetylchloride, Et$_3$N, CHCl$_3$, 99%; b) i) KOH, EtOH/H$_2$O, ii) HCl 94%; c) 2,6-diphenyl para-nitrophenol, BOP, Et$_3$N, CHCl$_3$, 60%.

The fumaric threads 2.8 and 2.11 were both synthesised from the fumaric acid monoethylester 2.5 in 3 and 5 step standard coupling syntheses respectively (Scheme 2.2.).

Scheme 2.2. Synthesis of the fumaramide ester and amide activated ester threads 2.8 and 2.11. a) Diphenylethyl amine, DMAP, EDCl, HCl, CHCl$_3$, 97%; b) i) NaOH, EtOH/H$_2$O, ii) HCl, 94%; c) 2,6-diphenyl para-nitrophenol, BOP, Et$_3$N, CHCl$_3$, 65%; d) HCL-H-Gly-OEt, BOP, Et$_3$N, CHCl$_3$, 81%; e) i) NaOH, EtOH/H$_2$O, ii) HCl, 90%; f) 2,6-diphenyl para-nitrophenol, BOP, Et$_3$N, CHCl$_3$, 68%.
Unlike our previous studies, the formation of rotaxanes from the three threads was then carried out by adding equimolar quantities of isophthaloyl dichloride in chloroform and a mixture of para-xylylene diamine and triethylamine in chloroform (Scheme 2.3.). The presence of a large excess of base mixed with the thread can indeed be responsible for the formation of an oxazolone, then leading to the dissociation of the macrocycle from the thread.

![Scheme 2.3. Synthesis of the rotaxane building blocks 2.12-2.14.](image)

It is noteworthy that the three rotaxane building blocks could be stored for many weeks at room temperature without observing any degradation. The yields of the pure isolated [2]rotaxanes are reported in table 2.1.
As previously observed in the fumaric series, the fumaric ester rotaxane 2.13 was obtained in the lowest yield of 37%. Ester carbonyls being poor hydrogen bond acceptors, their interaction with the amide NH of the precursors of the macrocycle are weaker than with the corresponding amide carbonyls. In the dipeptide series, the glycyglycine rotaxane 2.12 was obtained in 48% yield, lower than for the corresponding diphenylethyl ester stoppered rotaxane (62%), probably due to a partial nucleophilic side-attack of the para-xyllylene diamine on the activated ester.
Rotaxane building block solid state structures

Crystals of the building blocks were obtained from slow diffusion of methanol into solutions of rotaxanes 2.12 and 2.13 in chloroform (Figure 2.1.).

![Diagram of rotaxane building blocks](image)

Figure 2.1. X-ray crystallographic structures of the building blocks 2.12 and 2.13. Intramolecular hydrogen-bond lengths (Å) and angles as indicated. Carbon atoms of the macrocycle are shown in blue and those of the thread in yellow; oxygen atoms are red, nitrogen atoms dark blue and amide hydrogen atoms white. Non-amide hydrogen atoms are omitted for clarity.

In both structures, a set of hydrogen bonds, from weak to strong, is observed between the macrocycle and the thread. The crystal structure of the glycyglycine rotaxane 2.12 contains one pair of bifurcated intramolecular hydrogen bonds (NH—O 2.51-2.52 Å, O—HN 161.2°-161.7°) and one standard short intramolecular hydrogen bond (NH—O 2.04 Å, O—HN 166.5°). Similarly, the fumaramide ester 2.13 contains one set of bifurcated hydrogen bonds (NH—O 2.10-2.19 Å, O—HN 160.5°-166.9°) and one standard hydrogen bond (NH—O 2.30 Å, O—HN 142.9°). However, its hydrogen bonding pattern
differs to that of 2.12 since one amide NH of the thread interacts with one carbonyl of the macrocycle (NH-O 2.17 Å, O-HN 174.8°).

**Ready-accessibility to oligopeptide rotaxanes**

With the aim of making oligopeptide rotaxanes, the C-terminus elongation of the rotaxane 2.12 was investigated using various stoppered amino acids (Scheme 2.4.). Unlike the thread 2.4, the corresponding rotaxane did not react with the primary amine of stoppered amino acids at room temperature, due to the presence of the macrocycle which acts as a steric protective shield along the thread. In a first attempt, rotaxane 2.12 was heated at reflux in chloroform in the presence of an excess of triethylamine to free *in situ* the stoppered glycine derivative from its trifluoroacetate salt. The resulting tripeptide GlyGlyGly rotaxane 2.15 was then isolated in an average yield of 61%. In a typical procedure then, and to prevent this side reaction, a mixture of rotaxane 2.12 and at least two equivalents of mono- or tri-peptides as a free amine moiety in chloroform was heated at reflux and the reaction monitored by TLC (Table 2.2.). The appearance of a yellow color in the reaction crude indicated the release of the 2,6-diphenyl-para-nitrophenolate.

**Scheme 2.4.** Synthesis of oligopeptide rotaxanes 2.15-2.19 through the elongation of the dipeptide rotaxane 2.12.
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<table>
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<th>Peptide rotaxane derivative</th>
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<th>t (hours)</th>
<th>Yield %</th>
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<td>24</td>
<td>91</td>
</tr>
<tr>
<td>GlyGly-L-Ala 2.16</td>
<td>2</td>
<td>24</td>
<td>88</td>
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</tr>
<tr>
<td>GlyGly-L-AlaGlyGly 2.19</td>
<td>2</td>
<td>72</td>
<td>55</td>
</tr>
</tbody>
</table>

Table 2.2. Synthesis of oligopeptide rotaxanes 2.15-2.19.

The yields of isolated rotaxanes 2.15-2.19 varied from good to excellent depending on the bulkiness of the side-chain of the amino acid added.

The $^1$H NMR comparisons in CDCl$_3$ of the glycylglycine activated ester thread 2.4, its corresponding rotaxane building block 2.12, and the tripeptide glycylglycylglycine rotaxane 2.15 are reported in Figure 2.2. In spectra b and c, the asymmetry of the thread stoppers leads to two different dd signals for the enantiotopic benzylic hydrogens $H_F$ of the macrocycle. A shielding effect occurs on the hydrogens of the thread located in the cavity of the macrocycle. In spectrum b, the signals for $H_a$, $H_b$ and $H_c$ of the rotaxane building block 2.12 are shifted upfield compared to the corresponding signals in the thread 2.4 (spectrum a), indicative of the macrocycle being positioned over these protons. It can be assumed that the macrocycle interacts with the two amide carbonyls of the thread in CDCl$_3$, in accordance with the X-ray structure of 2.12 (Figure 2.1.). In the GlyGlyGly rotaxane 2.15 (spectrum c), $H_a$, $H_b$ and $H_c$ are now shifted downfield whereas $H_e$ is shifted upfield. The similar chemical shifts of $H_e$ and $H_e$ suggest that they experience
almost the same shielding effect of the macrocycle, thus indicating that the macrocycle in compound 2.15 is moving quickly in the NMR time scale.

**Figure 2.2.** Comparison of the $^1$H NMR spectra (400 MHz, CDCl$_3$) of a) the GlyGly activated thread 2.4, b) the activated GlyGly rotaxane building block 2.12 and c) the GlyGlyGly tripeptide elongated rotaxane 2.15.

**Rotaxane building blocks as new tools for overcoming solubility problems and folding of the backbone**

Previous work in our laboratory has evidenced that insufficient solubility and folding of long thread backbones lead to rotaxane formation in poor yield. This is mainly due to intramolecular hydrogen bonding in the thread preventing good preorganization with the precursor of the macrocycle. Rotaxane building blocks appear to be a very convenient
tool for making elaborate rotaxanes, not easily reachable by conventional encapsulation methods.

The synthesis of the rotaxane of a biologically active pentapeptide, the protected Leu-enkephalin, has been reported in a very low yield of 1% via a clipping strategy. In order to improve this result, the new methodology discussed in this chapter was successfully applied to the synthesis and elongation of a tyrosylglycylglycine rotaxane building block 2.24 (Scheme 2.5.). The Boc and benzyl protecting groups on the tyrosine residue and the side-chains of successive phenylalanine and leucine residues were found to be bulky enough to act as stoppers for the size of the benzylic 1,3-carbamide macrocycle. Thus, the tripeptide thread 2.23 was prepared in a three-step sequence in an overall yield of 59%. The rotaxane formation was carried out similarly to that for the building blocks 2.12-2.14, but using 16 equivalents of para-xyylene diamine and isophthaloyl dichloride. Rotaxane building block 2.24 was very easily purified via silica gel chromatography and isolated in 19% yield, then further elongated with H-Phe-Leu-OBn to give the protected Leu-enkephalin rotaxane 2.25 in 86% yield. This strategy allows the straightforward synthesis of a pentapeptide rotaxane in very good yield, purified via convenient silica gel chromatography and with no solubility problems.
The synthesis of a bistable molecular shuttle 2.28 has been previously reported\textsuperscript{31} in an overall 32\% yield via a clipping strategy around the Z-thread 2.26 followed by thermal isomerisation at 120°C in C\textsubscript{2}H\textsubscript{2}Cl\textsubscript{4} for 7 days. In fact, the E-thread could not be used for the rotaxane formation via a clipping strategy due to its very poor solubility in non hydrogen bonding disrupting solvent. With the aim of improving the feasibility and overcoming solubility problems, the new methodology was successfully applied to the straightforward elongation of the fumaric activated ester rotaxane building block 2.13 with \( N-(12\text{-aminododecyl})-N'(2, 2\text{-diphenylethyl})\) succinamide to give 2.28 in 71\% yield (Scheme 2.6.). Although it is similar to the clipping strategy in terms of reaction steps and yield, our methodology ensured a real improvement of the physical properties of these interlocked architectures: (i) purification via silica gel chromatography was made
very easy due to the large difference in polarity of 2.13 and 2.28 and (ii) no solubility problem was experienced at any step of the formation of the latter rotaxane derivatives.

Scheme 2.6. Synthesis of a bistable molecular shuttle 2.28. a) isophthaloyl dichloride, para-xyylene diamine, Et3N, CHCl3, 40%; b) C2H2Cl4 at 120°C for 7 days, 80%; c) NEI2(CH2)12CO(CH2)4CONHCH2CHPh2 (5 equivalents), CHCl3 reflux, 72 hours, 71%.

Compared to the direct encapsulation strategy, the synthesis of rotaxane building blocks, followed by elongation with nucleophiles appears to give much better results both in terms of yields and convenience, since the problems of solubility and folding of threads can be avoided. Moreover, the synthesis of a few rotaxane building blocks allows for the preparation of a wide range of elaborate interlocked architectures and could then be an extraordinary versatile tool for making libraries.
2.3. Conclusion

We have shown a versatile synthesis of activated ester rotaxane building blocks via standard coupling chemistry, in dipeptide and fumaric series, which can be isolated and stored for many weeks at room temperature with no degradation. They have then been efficiently used for the synthesis, in very good yield, of a wide range of oligopeptide rotaxanes, reached in very poor yield by the classical methods of encapsulation due to the folding of the peptide backbone. The syntheses of a bistable molecular shuttle and a protected Leu-enkephalin rotaxane in very good yields illustrate the ability of the strategy to reach, in only a few steps, non readily-accessible molecules by overcoming solubility and folding problems. It is noteworthy that, when encapsulated within a tetraamide macrocycle, oligopeptides present interesting changing properties: their solubility in organic solvents and their vulnerability against enzymes can indeed be incredibly modulated by the macrocycle acting as a protective sheath around the peptide backbone. These are very promising results for the design of successful peptide based drugs.
2.4. Experimental section

Method for the preparation of the dipeptide thread 2.4

N-diphenylacetylglutamylglycine ethyl ester 2.2

Triethylamine (7.700 g, 0.076 mmol) was added to a stirring suspension of glycyglycine ethyl ester hydrochloride (2.1, 5.000 g, 0.025 mmol) in anhydrous chloroform (200 mL). When no more material remained in suspension, diphenylacetyl chloride (7.050 g, 0.030 mmol) was added in small portions as a solid. After 2 hours of stirring, the reaction mixture was washed with water (3*200 mL) and the organic layer dried over MgSO₄. The solvent was then removed under reduced pressure and the crude material recrystallised from toluene to give the desired product as a white powder. Yield (8.750 g, 97%); mp 158°C; ¹H NMR (400 MHz, CDCl₃): δ = 8.27 (t, 1H, J = 6.0 Hz, CONHCH₂CO), 8.12 (t, 1H, J = 6.0 Hz, CONHCH₂CO), 7.21-7.10 (m, 10 H, Ar-H), 4.81 (s, 1 H, Ph₂CHCONH), 3.82 (q, 2H, J = 7.0 Hz, CO₂CH₂CH₃), 3.59 (d, 2H, J = 6.0 Hz, NHCH₂CO), 3.54 (d, 2H, J = 6.0 Hz, NHCH₂CO), 0.94 (t, 3H, J = 7.0 Hz, CO₂CH₂CH₃); ¹³C NMR (100 MHz, CDCl₃): δ = 173.02, 169.67, 169.34, 139.20, 129.36, 129.00, 128.86, 127.43, 61.55, 58.57, 43.35, 41.30, 14.25; FAB-MS m/z 355 [M+H⁺], calcd. for C₂₀H₂₂N₂O₄: C, 67.8, H 6.3, N 7.9, found C, 67.8, H 6.4, N 7.9.

N-diphenylacetylglutamylglycine 2.3

The (N-diphenylacetyl)glycyl glycine ethyl ester (2.2, 9.521 g, 26.900 mmol) was dissolved in 180 mL of absolute EtOH. A solution of KOH (3.130 g, 55.800 mmol) in 20 mL of water was added. The reaction mixture was stirred at RT during 3 hours. The solvent was removed under reduced pressure. HCl was added. The precipitate was
filtered and dried by evaporation under reduced pressure to afford a white powder that showed spectroscopic data according to literature.\textsuperscript{32} Yield (8.146 g, 94%).

**N-diphenylacetylglucylglycine 4-nitro-2,6-diphenylphenylester 2.4**

*N*-diphenylacetylglucylglycine (2.3, 1.046 g, 3.200 mmol), 4-nitro-2,6-diphenyl phenol (4.662 g, 16.000 mmol), NEt\textsubscript{3} (4.877 g, 48.000 mmol) and BOP (2.119 g, 4.800 mmol) were dissolved in 70 mL of CHCl\textsubscript{3}. The reaction mixture was stirred 4 hours at room temperature. The crude material was purified via silica gel chromatography (AcOEt/cyclohexane: 1/1) to afford a yellow powder. Yield (1.146 g, 60%); mp 171.4°C; \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): \(\delta = 8.20\) (s, 2 H, NO\textsubscript{2}-Ph-H), 7.20-7.10 (m, 20 H, Ar-H), 6.25-6.21 (br t, 2H, Gly CONHCH\textsubscript{2}CO), 4.81 (s, 1 H, Ph\textsubscript{2}CHCONH), 3.70 (d, 2H, J=5.3 Hz, NHCH\textsubscript{2}CO), 3.65 (d, 2H, J=5.3 Hz, NHCH\textsubscript{2}CO); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}): \(\delta = 172.72, 168.60, 166.86, 149.26, 146.06, 138.83, 137.44, 135.31, 128.86, 128.81, 128.73, 127.62, 127.42, 124.99, 58.68, 43.19, 40.96;\) FAB-HRMS for C\textsubscript{36}H\textsubscript{30}N\textsubscript{3}O\textsubscript{6} \(m/z [M+H^+]\), calcd. 600.21346, found 600.21254.

**Method for the preparation of the fumaramide ester thread 2.8**

2,2-diphenylethylaminofumarylethylester 2.6

Fumaric acid monoethyl ester (2.5, 3.320 g, 0.023 mol), 2,2-diphenylethylamine (5.000 g, 0.025 mol) and DMAP (2.810 g, 0.023 mol) were dissolved in 150 mL of CH\textsubscript{2}Cl\textsubscript{2}. The reaction mixture was cooled at 0°C. Then EDCI.HCl (4.410 g, 0.023 mol) was added. The reaction mixture was stirred one hour at 0°C and brought to room temperature and stirred overnight. The crude material was concentrated under reduced pressure. The organic phase was washed with 2M HCl and saturated NaHCO\textsubscript{3}. The organic phase was
dried over MgSO₄. The solvent was removed under reduced pressure to afford a pink solid. Yield (7.480 g, 91%); mp 128°C; ¹H NMR (400 MHz, CDCl₃): δ = 7.36-7.20 (m, 10H, Ar-H), 6.78 & 6.72 (2d, 2H, J = 15.6 Hz, COCH & CHCOO), 5.75 (br t, 1H, Ph₂CHCH₂NHCO), 4.25-4.17 (m, 3H, Ph₂CHCH₂ & COOCH₂CH₃), 3.99 (t, 2H, J = 8.0 Hz, Ph₂CHCH₂), 1.29 (t, 3H, J = 7.2 Hz, COOCH₂CH₃); ¹³C NMR (100 MHz, CDCl₃): δ = 165.52, 163.49, 141.40, 135.95, 130.63, 128.86, 127.98, 127.04, 61.20, 50.35, 44.07, 14.11; FAB-HRMS for C₂₀H₂₂NO₃ m/z [M+H⁺], calcd. 324.15997, found 324.16098.

2,2-diphenylethylaminofumaric acid 2.7

Following the experimental procedure leading to derivative 2.3.

Yield (0.958 g, 100%); mp 203°C; ¹H NMR (400 MHz, CDCl₃): δ = 8.50 (t, 1H, J = 6.6 Hz, Ph₂CHCH₂NHCO), 7.27-7.09 (m, 10H, Ar-H), 6.77 & 6.41 (2d, 2H, J = 15.4 Hz, COCH & CHCOO), 4.17 (t, 1H, J = 6.6 Hz, Ph₂CHCH₂), 3.76 (t, 2H, J = 6.6 Hz, Ph₂CHCH₂); ¹³C NMR (100 MHz, CDCl₃): δ = 167.17, 163.91, 143.30, 137.04, 130.81, 129.09, 128.45, 127.05, 50.54, 43.99; FAB-HRMS for C₁₉H₁₈NO₃ m/z [M+H⁺], calcd. 296.12867, found 296.12800.

2,2-diphenylethylaminofumaryl 4-nitro-2,6-diphenylphenylester 2.8

Following the experimental procedure leading to derivative 2.4.

Yield (1.081 g, 65%); mp 191°C; ¹H NMR (400 MHz, CDCl₃): δ = 8.29 (s, 2H, NO₂-Ph-H), 7.45-7.16 (m, 20H, Ar-H), 6.62 & 6.43 (2d, 2H, J = 15.2 Hz, COCH & CHCOO), 5.54 (br t, 1H, Ph₂CHCH₂NHCO), 4.15 (t, 1H, J = 8.2 Hz, Ph₂CHCH₂), 3.94
(dd, 2H, $J = 5.6 \& 8.2$ Hz, Ph$_2$CHCH$_2$); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 162.59, 158.38, 149.46, 145.94, 141.19, 137.45, 135.41, 135.41, 128.91, 128.76, 128.71, 128.65, 128.16, 127.92, 124.99, 50.26, 44.06; FAB-HRMS for C$_{36}$H$_{29}$N$_2$O$_5$ $m/z$ [M+H$^+$], calcd. 569.20765, found 569.20870.

**Method for the preparation of the fumaramide glycine thread 2.11**

**2,2-diphenylethylaminofumarylglutylester 2.9**

*Following the experimental procedure leading to derivative 2.4.*

Yield (0.600 g, 81%); mp 237°C; $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 7.36-7.19$ (m, 10H, Ar-H), 6.95 & 6.74 (2d, 2H, $J = 14.8$ Hz, COCH & CHCONH), 6.57 (br t, 1H, CONHCH$_2$CO), 5.87 (br t, 1H, Ph$_2$CHCH$_2$NHCO), 4.25-4.18 (m, 3H, Ph$_2$CHCH$_2$ & COOCH$_2$CH$_3$), 4.10 (d, 2H, $J = 4.8$ Hz, NHCH$_2$CO), 3.99 (dd, 2H, $J = 6.0 \& 8.0$ Hz, Ph$_2$CHCH$_2$), 1.28 (t, 3H, $J = 6.8$ Hz, COOCH$_2$CH$_3$); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 164.84, 163.77, 132.97, 131.73, 128.32, 127.42, 126.50, 61.23, 49.79, 43.53, 41.09, 13.57; FAB-HRMS for C$_{22}$H$_{25}$N$_2$O$_4$ $m/z$ [M+H$^+$], calcd. 381.18143, found 381.18135.

**2,2-diphenylethylaminofumarylglutycine 2.10**

*Following the experimental procedure leading to derivative 2.3.*

Yield (0.250 g, 90%); mp 214°C; $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 8.76$ (t, 1H, $J = 6.0$ Hz, CONH), 8.52 (t, 1H, $J = 5.2$ Hz, CONH), 7.35-7.16 (m, 10H, Ar-H), 6.86 & 6.78 (2d, 2H, $J = 15.2$ Hz, COCH & CHCONH), 4.23 (t, 1H, $J = 8.0$ Hz, Ph$_2$CHCH$_2$), 3.87-3.78 (m, 4H, Ph$_2$CHCH$_2$ & NHCH$_2$CO); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 168.75,
2,2-diphenylethylaminofumaryl-glycyl-4-nitro-2,6-diphenylphenylester 2.11

Following the experimental procedure leading to derivative 2.4.

Yield (0.190 g, 68%), $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 8.28 (s, 2H, NO$_2$-Ph-H), 7.47-7.15 (m, 20H, Ar-H), 6.73 & 6.63 (2d, 2H, $J$ = 14.8 Hz, COCH & CHCONH), 6.06 (brt, 1H, CONHCH$_2$CO), 5.83 (br t, 1H, Ph$_2$CHCH$_2$NHCO), 4.17 (t, 1H, $J$ = 8.0 Hz, Ph$_2$CHCH$_2$), 3.94 (t, 2H, $J$ = 6.4 Hz, Ph$_2$CHCH$_2$), 3.84 (d, 2H, $J$ = 7.4 Hz, NHCH$_2$CO); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ = 166.82, 164.58, 158.22, 141.36, 138.91, 137.39, 135.24, 133.78, 131.78, 128.89, 128.86, 128.82, 128.72, 127.94, 127.47, 127.06, 125.02, 50.32, 44.05, 41.33; FAB-HRMS for C$_{38}$H$_{32}$N$_3$O$_6$ m/z [M+H$^+$], calcd. 626.22911, found 626.23051.

Method for the preparation of the tripeptide activated ester thread 2.24, precursor of the Leu-enkephalin

$N$-terbutoxycarbonyl-$O$-benzyl-$l$-tyrosylglycylglycine ethyl ester 2.21

Following the experimental procedure leading to derivative 2.6.

Yield (2.905 g, 98%), $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 7.45-7.23 (m, 6H, Ar-H & Gly CONHCH$_2$CO), 7.11 (d, 2H, $J$ = 7.8 Hz, CH$_2$ArH$^+$OBn), 6.91 (d, 2H, $J$ = 7.8 Hz, CH$_2$ArH$^+$OBn), 6.74 (br t, 1H, Gly CONHCH$_2$CO), 5.10 (d, 1H, $J$ = 6.6 Hz, (CH$_3$)$_3$COCONH), 5.03 (s, 1H, OCH$_2$Ph), 4.28 (q, 1H, $J$ = 6.6 Hz, Tyr NHCHCO), 4.17 (q, 2H, $J$ = 7.6 Hz, COOCH$_2$CH$_3$), 4.04-3.86 (m, 4H, Gly NHCH$_2$CO), 3.06 (dd, 1H, $J$ = 6.8 Hz & 14.0 Hz, Tyr PhCH$_2$OArCH$^+$), 2.96 (dd, 1H, $J$ = 6.8 Hz & 14.0 Hz, Tyr PhCH$_2$OArCH$^+$), 2.96 (dd, 1H, $J$ = 6.8 Hz & 14.0 Hz, Tyr PhCH$_2$OArCH$^+$), 2.96 (dd, 1H, $J$ = 6.8 Hz & 14.0 Hz, Tyr PhCH$_2$OArCH$^+$), 2.96 (dd, 1H, $J$ =
**Chapter Two**

PhCH$_2$OArCHH'), 1.39 (s, 9H, (CH$_3$)$_3$COCO), 1.25 (t, 3H, COOCH$_2$CH$_3$); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ = 169.74, 167.24, 166.70, 155.57, 134.55, 127.95, 126.27, 126.21, 125.69, 125.14, 112.77, 78.26, 67.68, 59.16, 54.12, 40.62, 38.86, 34.84, 25.93; FAB-HRMS for C$_{27}$H$_{36}$N$_3$O$_7$ m/z [M+H$^+$], calcd. 514.25533, found 514.25639.

**N-terbutoxycarbonyl–O-benzyl-L-tyrosylglycylglycine 2.22**

*N-terbutoxycarbonyl–O-benzyl-L-tyrosylglycylglycine ethyl ester (2.21, 1.720 g, 3.350 mmol) was dissolved in 30 mL of a solution of 1 M KOH in MeOH. The reaction temperature was stirred 3 hours at RT and monitored by TLC. The solvent was removed under reduced pressure to afford a solid. Yield (1.400 g, 80%); $^1$H NMR (400 MHz, DMSO): $\delta$ = 8.54-8.43 (2 br s, 2H, Gly CONHCH$_2$), 7.54-7.43 (m, 5H, Ar-H), 7.26 (d, 2H, $J$ = 8.4 Hz, CH$_2$ArHH'OBn), 7.06 (d, 1H, $J$ = 8.0 Hz, (CH$_3$)$_3$COCONH), 6.96 (d, 2H, $J$ = 8.4 Hz, CH$_2$ArHH'OBn), 5.12 (s, 1H, PhCH$_2$O), 4.22-4.14 (m, 1H, $J$ = 6.6 Hz, Tyr NHCHCO), 3.92-3.65 (m, 4H, Gly NHCH$_2$CO), 3.09-2.99 (br t, 1H, PhCH$_2$OArCHH'), 2.78-2.69 (m, 1H, PhCH$_2$OArCHH'), 1.35 (s, 9H, (CH$_3$)$_3$COCO) (CH$_3$)$_3$COCO, $^{13}$C NMR (100 MHz, DMSO): $\delta$ = 172.16, 169.71, 167.41, 156.77, 155.26, 137.21, 130.52, 130.19, 128.35, 127.69, 127.59, 77.89, 69.05, 56.04, 44.03, 42.25, 40.06, 36.62, 28.10; FAB-HRMS for C$_{25}$H$_{31}$KN$_3$O$_7$ m/z [M+H$^+$], calcd. 524.17991, found 524.17971.

**N-terbutoxycarbonyl–O-benzyl-L-tyrosylglycylglycine 4-nitro-2, 6-diphenylphenyl ester 2.23**

*Following the experimental procedure leading to derivative 2.4.*
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Yield (0.825 g, 59%); $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 8.30$ (s, 2H, NO$_2$Ph-\text{H}), 7.52-7.27 (m, 16H, Ar-\text{H} & CONH), 7.09-7.06 (m, 2H, CH$_2$ArHH'-OBn), 6.91-6.88 (m, 2H, CH$_2$ArHH'-OBn), 6.71 (br s, 1H, CONH), 6.57 (br s, 1H, CONH), 5.04 (s, 2H, OCH$_3$Ph), 4.26-4.10 (m, 1H, Tyr NHCHCO), 3.90-3.69 (m, 4H, Gly NHCH$_2$CO), 3.05-2.97 (m, 1H, Tyr NHCH(CHH')CO), 3.05-2.97 (m, 1H, Tyr NHCH(CHH')CO), 1.35 (s, 9H, (CH$_3$)$_3$COCO), $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 172.16$, 169.02, 166.80, 157.96, 149.28, 145.99, 137.44, 136.82, 135.32, 130.23, 130.17, 128.89, 128.81, 128.75, 128.62, 128.25, 128.06, 127.49, 124.99, 115.14, 80.79, 70.01, 56.43, 42.67, 40.98, 36.99, 28.21, FAB-HRMS for C$_{43}$H$_{43}$N$_4$O$_9$ $m/z$ [M+H'], calcd. 759.30300, found 759.30444.


Thread 2.4, 2.8, 2.11 or 2.23 (1.910 mmol) was dissolved in 100 mL of CHCl$_3$ under nitrogen and stirred vigorously whilst solutions of the mixture of para-xylene (2.121 g, 15.600 mmol) and NEt$_3$ (3.965 g, 39.300 mmol) in 50 mL of CHCl$_3$ and the isophthaloyl dichloride (3.151 g, 15.500 mmol) in 50 mL of CHCl$_3$ were simultaneously added to the reaction mixture over a period of 3 hours using motor-driven syringe pumps. The reaction mixture was stirred 2 hours under nitrogen, then filtered on celite and the filtrate was evaporated under reduced pressure to afford a syrup which was purified via silica gel chromatography to yield, in order of elution, the unconsumed activated ester thread (AcOEt/cyclohexane: 1/1) and the rotaxane (CHCl$_3$/MeOH: 98/2).

Glycylglycine activated rotaxane 2.12
Chapter Two

Yield (1.047 g, 48%); m.p. 171°C; $^1$H NMR (400 MHz, CDCl$_3$): $\delta =$ 8.27 (s, 2 H, NO$_2$-Ph-H), 8.11 (d, 4H, $J =$ 7.6 Hz, isophthaloyl 4-H & 6-H), 7.88 (s, 2H, isophthaloyl 2-H), 7.59 (t, 2H, $J =$ 7.6 Hz, isophthaloyl 5-H), 7.48-6.90 (m, 21 H, thread Ar-H & CON$^\text{H}$), 7.22 (br t, 4H, macrocyclic CONH), 6.76 (s, 8H, macrocyclic para-xylylene, Ar-H), 5.18 (br t, 1H, Ph$_2$CHCONH), 4.41 (dd, 4H, $J =$ 4.8 Hz, $J =$ 13.8 Hz, macrocyclic NHCHH$'$), 4.18 (dd, 4H, $J =$ 4.4 Hz, $J =$ 13.8 Hz, macrocyclic NHCHH$'$), 3.95 (s, 1H, Ph$_2$CHCONH), 3.67 (d, 2H, $J =$ 5.2 Hz, Gly NHCH$_2$CO), 2.58 (br d, 2H, Ph$_2$CHCONHCH$_2$CO); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta =$ 172.15, 169.82, 166.66, 165.85, 148.94, 146.17, 138.17, 137.31, 135.17, 133.84, 131.57, 129.48, 128.76, 128.61, 128.17, 127.58, 125.01, 123.74, 58.07, 43.60, 41.70, 40.30; FAB-HRMS for C$_{68}$H$_{58}$N$_7$O$_{10}$ m/z [M+H$^+$], calcd. 1132.42452, found 1132.42500; $[\alpha]_D =$ 0 in CHCl$_3$.

Fumaryl activated rotaxane 2.13

Yield (0.428 g, 37%); m.p. 135°C; $^1$H NMR (400 MHz, CDCl$_3$): $\delta =$ 8.16 (d, 4H, $J =$ 8.0 Hz, isophthaloyl 4-H & 6-H), 8.13 (br s, 4H, isophthaloyl 2-H & NO$_2$-Ph-H), 7.59 (t, 2H, $J =$ 8.0 Hz, isophthaloyl 5-H), 7.50-6.92 (m, 24H, macrocyclic CONH & thread Ar-H & thread CONH), 6.51 (s, 8H, macrocyclic para-xylylene ArH), 5.88 & 5.30 (2d, 2H, $J =$ 15.2 Hz, COCH & CHCOO), 4.42 (dd, 4H, $J =$ 4.8 Hz, $J =$ 14.8 Hz, macrocyclic NHCHH$'$), 4.34 (dd, 4H, $J =$ 4.4 Hz, $J =$ 14.8 Hz, macrocyclic NHCHH$'$), 3.79 (t, 1H, $J =$ 8.0 Hz, Ph$_2$CHCH$_2$), 3.16 (m, 2H, Ph$_2$CHCH$_2$); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta =$ 166.78, 163.72, 162.44, 145.97, 141.63, 137.65, 137.44, 136.87, 135.40, 134.18, 134.11, 130.90, 129.33, 129.06, 128.90, 128.83, 128.76, 128.66, 128.21, 127.81,
126.93, 125.12, 125.08, 52.44, 44.86, 44.11; FAB-HRMS for \( \text{C}_{68}\text{H}_{57}\text{N}_{6}\text{O}_{9} \ m/z \ [\text{M+H}^+] \), calcd. 1101.41870, found 1101.41928.

**Fumarylglycine activated rotaxane 2.14**

Yield (0.176 g, 80%); m.p. 183°C; \(^1\text{H NMR (400 MHz, C}_2\text{D}_2\text{Cl}_4\text{):}\) \( \delta = 8.47 \) (s, 2H, \( \text{NO}_2\text{-Ph-H} \)), 8.19 (s, 2H, isophthaloyl 2-H), 8.16 (d, 4H, \( J = 7.6 \text{ Hz, isophthaloyl 4-H & 6-H} \)), 7.66 (t, 2H, \( J = 7.6 \text{ Hz, isophthaloyl 5-H} \)), 7.54-6.99 (m, 26H, macrocyclic \( \text{CONH} \) & thread \( \text{Ar-H} \) & thread \( \text{CONH} \)), 6.66 (s, 8H, macrocyclic \( \text{para-xylylene Ar-H} \)), 5.37 & 5.32 (2d, 2H, \( J = 14.8 \text{ Hz, COCH & CHCO} \)), 4.30-4.26 (m, 4H, macrocyclic \( \text{NHCHH'} \)), 4.15-4.12 (m, 4H, macrocyclic \( \text{NHCHH'} \)), 4.04 (t, 1H, \( J = 8.0 \text{ Hz, PhCHCH}_2 \)), 3.68-3.67 (m, 4H, \( \text{PhCHCH}_2 \) & \( \text{Gly NHCH}_2\text{CO} \)); \( \delta = 13\text{C NMR (100 MHz, C}_2\text{D}_2\text{Cl}_4\text{):}\) 167.06, 166.50, 166.28, 165.73, 165.30, 149.66, 147.47, 146.44, 142.08, 137.76, 137.28, 135.61, 134.04, 132.02, 130.01, 129.37, 129.17, 129.12, 129.09, 128.18, 127.66, 125.49, 124.68, 45.19, 43.96, 43.95, 39.09, 38.88; FAB-HRMS for \( \text{C}_{70}\text{H}_{60}\text{N}_{7}\text{O}_{10} \ m/z \ [\text{M+H}^+] \), calcd. 1158.44017, found 1158.43976.

**rBoc-1-tyrosylglycyl glycine activated rotaxane 2.24**

Yield (0.107 g, 19%); \(^1\text{H NMR (400 MHz, CDCl}_3\text{):}\) \( \delta = 8.26 \) (s, 2H, \( \text{NO}_2\text{-Ar-H} \)), 8.04 (d, 4H, \( J = 7.6 \text{ Hz, isophthaloyl 4-H & 6-H} \)), 7.97 (s, 2H, isophthaloyl 2-H), 7.54 (br s, 1H, macrocyclic \( \text{CONH} \)), 7.52 (t, 2H, \( J = 7.6 \text{ Hz, isophthaloyl 5-H} \)), 7.48-7.20 (m, 26H, isophthaloyl 5-H & thread \( \text{Ar-H} \) & thread \( \text{CONH} \) & macrocyclic \( \text{CONH} \)), 7.04 (s, 8H, macrocyclic \( \text{para-xylylene, Ar-H} \)), 6.83 (m, 4H, \( \text{CH}_2\text{ArHH'}\text{OBn} \) & \( \text{CH}_2\text{ArHH'}\text{OBn} \)), 5.81 (br s, 1H, \( \text{BocTyr(OBn)NHCH}_2\text{CO} \)), 5.01 (s, 2H, \( \text{OCH}_3\text{Ph} \)), 4.49-4.29 (m, 9H, macrocyclic \( \text{NHCHH'} \) & macrocyclic \( \text{NHCHH'} \) & Tyr \( \text{(CH}_3\text{)}_3\text{COCONH} \)), 3.89-3.61 (m,
3H, Tyr NHCHCO & Gly NHCHH'COO & Gly NHCHH'COO), 2.80-2.63 (m, 3H, Gly NHCHCO & Tyr NHCH(CHH')CO), 2.36-2.24 (m, 1H, Tyr NHCH(CHH')CO), 1.12 (s, 9H, (CH₃)₃COCO); ¹³C NMR (100 MHz, CDCl₃): δ = 171.69, 169.33, 166.94, 166.83, 166.23, 157.90, 149.05, 146.14, 137.44, 137.35, 137.29, 136.86, 135.27, 134.27, 131.39, 131.12, 130.02, 129.08, 128.95, 128.89-128.76, 128.70, 128.60, 128.32, 128.00, 127.39, 127.05, 124.28, 114.99, 80.95, 69.97, 55.06, 44.27, 44.05, 41.89, 40.78, 36.75, 27.90; FAB-HRMS for C₇₅H₇₀N₈O₁₃Na m/z [M+Na+], calcd. 1313.49604, found 1313.49817.

General procedure for the elongation of [2]rotaxane building blocks 2.12, 2.13 and 2.24

The [2]rotaxane building block 2.12, 2.13 or 2.24 (0.079 mmol) and a stoppered nucleophile (0.390 mmol but 0.980 mmol for 2.18) were dissolved in 20 mL of CHCl₃. The reaction mixture was then heated at reflux and monitored by TLC (CHCl₃/MeOH: 90/10). The solvent was removed under reduced pressure. The crude material was directly purified via silica gel chromatography (CHCl₃/MeOH: from 98/2 to 95/5) to afford in order of elution the 2,6-diphenyl-para-nitrophenol, and the elongated rotaxane as a white powder.

Glycylglycylglycine rotaxane 2.15

Yield (0.078 g, 91%); m.p. 123°C; ¹H NMR (400 MHz, CDCl₃): δ = 8.21 (s, 2H, isophthaloyl 2-H), 8.06 (d, 4H, J = 7.6 Hz, isophthaloyl 4-H & 6-H), 7.75 (br t, 4H, macrocyclic CONH), 7.51 (t, 2H, J = 7.6 Hz, isophthaloyl 5-H), 7.23-7.06 (m, 22 H,
thread Ar-H & thread Gly CONHCH₂CO), 6.95 (s, 8H, macrocyclic para-xylene, Ar-H), 6.28 (br s, 1H, Gly CONHCH₂CO), 4.59 (s, 1H, Ph₂CHCO), 4.45-4.14 (m, 11H, macrocyclic NHCHH' & NHCHH' & COOCHH'CHPh₂ & COOCHH'CHPh₂ & COOCHH'CHPh₂), 3.41 (s, 2H, Gly NHCH₂CO), 3.07 (s, 2H, Gly NHCH₂CO), 3.02 (s, 2H, Gly NHCH₂CO); ¹³C NMR (100 MHz, CDCl₃): δ = 172.88, 169.27, 168.79, 167.16, 167.09, 140.49, 138.71, 137.25, 134.03, 131.27, 129.17, 128.96, 128.69, 128.62, 128.53, 127.97, 127.49, 126.96, 124.62, 67.50, 58.17, 44.05, 43.92, 41.83, 40.65; FAB-HRMS for C₆₆H₆₂N₇O₉ m/z [M+H⁺], calcd. 1096.46090, found 1096.45996; [α]D = 0 in CHCl₃.

**Glycylglycyl-L-alanine rotaxane 2.16**

Yield (0.078 g, 88%); m.p. 134°C; ¹H NMR (400 MHz, CDCl₃): δ = 8.10 (s, 2H, isophthaloyl 2-H), 8.03 (d, 2H, J = 7.6 Hz, isophthaloyl 4-H), 8.02 (d, 2H, J = 7.6 Hz, isophthaloyl 6-H), 7.51-7.43 (m, 4H, macrocyclic CONH), 7.47 (t, 2H, J = 7.6 Hz, isophthaloyl 5-H), 7.27-7.02 (m, 22 H, thread Ar-H & CONH), 6.95 (d, 4H, J = 8.0 Hz, macrocyclic para-xylene, Ar-H), 6.92 (d, 4H, J = 8.0 Hz, macrocyclic para-xylene, Ar-H), 5.87 (br t, 1H, CONH), 4.62 (dd, 1H, J = 10.7 Hz, J = 8.3 Hz, COOCH₂CHPh₂), 4.50 (s, 1H, Ph₂CHCO), 4.41-4.17 (m, 10H, macrocyclic NHCHH' & NHCHH' & COOCHH'CHPh₂ & COOCHH'CHPh₂), 4.09 (quint., 1H, J = 6.8 Hz, Ala NHCH(CH₃)CO), 3.16 (dd, 1H, J = 4.8 Hz, J = 16.8 Hz, Gly NHCHH'CO), 3.09 (dd, 1H, J = 4.0 Hz, J = 16.8 Hz, Gly NHCHH'CO), 2.99 (br s, 2H, Gly NHCH₃CO), 0.98 (d, 3H, J = 6.8 Hz, Ala NHCH(CH₃)CO); ¹³C NMR (100 MHz, CDCl₃): δ = 172.61, 172.14, 169.26, 167.62, 166.89, 166.83, 138.62, 137.27, 137.21, 134.07, 134.01, 131.39, 131.31, 129.20, 129.09, 128.88, 128.80, 128.67, 128.62, 128.53, 128.15,
Glycylglycyl-L-leucine rotaxane 2.17

Yield (0.087 g, 78%); m.p. 143°C; ¹H NMR (400 MHz, CDCl₃): δ = 8.10 (s, 2H, isophthaloyl 2-H), 8.07-7.99 (m, 4H, isophthaloyl 4-H & 6-H), 7.51-7.43 (m, 6H, macrocyclic CONH, isophthaloyl 5-H), 7.20-7.06 (m, 22H, thread Ar-H & CONH), 6.95 (d, 4H, J = 8.4 Hz, macrocyclic para-xylylene, Ar-H), 6.94 (d, 4H, J = 8.4 Hz, macrocyclic para-xylylene, Ar-H), 5.90 (br t, 1H, CONH), 4.63 (dd, 1H, J = 8.8 Hz J = 10.8 Hz, COOCH₂CHPh₂), 4.52 (s, 1H, Ph₂CHCO), 4.44-4.22 (m, 10H, macrocyclic NHCH'H & NHCH'H & COOCH'HCHPh₂ & COOCH'HCHPh₂), 4.19-4.10 (m, 1H, Leu NHCH(CH₂CH(CH₃)₂)CO), 3.17 (br s, 2H, J = 2.8 Hz, Gly NHCH₂CO), 3.04 (br s, 2H, Gly NHCH₂CO), 1.25-1.04 (m, 3H, Leu NHCH(CH₂CH(CH₃)₂)CO & NHCH(CH₂CH(CH₃)₂)CO), 0.58 (d, 3H, J = 6.4 Hz, Leu NHCH(CH₂CH(CH₃)(CH₃'))CO), 0.56 (d, 3H, J = 6.0 Hz, Leu NHCH(CH₂CH(CH₃)(CH₃'))CO); ¹³C NMR (100 MHz, CDCl₃): δ = 172.68, 172.33, 169.31, 167.95, 166.84, 166.79, 140.54, 140.25, 138.62, 137.24, 137.22, 133.96, 131.35, 129.16, 129.08, 128.88, 128.79, 128.66, 128.61, 128.52, 128.11, 128.04, 127.58, 127.01, 126.97, 124.31, 67.45, 58.44, 52.41, 51.12, 49.69, 44.17, 42.49, 42.04, 40.79, 29.71, 24.67, 22.40, 21.69; FAB-HRMS for C₇₀H₇₀N₁₀O₉ m/z [M+H'], calcd. 1152.52350, found 1152.52267; [α]₀ = -5.1 in CHCl₃.

Glycylglycyl-L-phenylalanine rotaxane 2.18
Yield (0.063 g, 74%); m.p. 144°C; $^1$H NMR (400 MHz, CDCl$_3$): δ = 8.05 (s, 2H, isophthaloyl 2-H), 8.01 (d, 4H, $J = 8.0$ Hz, isophthaloyl 4-H & 6-H), 7.46 (t, 4H, $J = 7.6$ Hz, macrocyclic CONH), (7.36 (t, 2H, $J = 5.2$ Hz, isophthaloyl 5-H), 7.21-7.02 (m, 31H, thread Ar-H & CONH & macrocyclic para-xylylene Ar-H), 6.87 (d, 4H, $J = 3.8$ Hz, Phe Ar-H), 6.81 (d, 4H, $J = 3.8$ Hz, Phe Ar-H), 5.87 (br d, 1H, Phe CONH), 4.50 (s, 1H, Ph$_2$CHCO), 4.58 (dd, 1H, $J = 10.8$ Hz, $J = 8.4$ Hz, COOCH$_2$CHPh$_2$), 4.43-4.21 (m, 10H, macrocyclic NHCHH' & NHCHH' & COOCHH'CHPh$_2$ & COOCHH'CHPh$_2$), 4.14 (t, $J = 8.0$ Hz, 1H, Phe NHCH(CH$_2$Ph)CO), 3.06 (d, 2H, $J = 5.4$ Hz, Gly NHCH$_2$CO), 3.00 (d, 2H, $J = 5.4$ Hz, Gly NHCH$_2$CO), 2.69 (dd, 1H, $J = 6.6$ Hz, $J = 14.4$ Hz, Phe NHCH(CH$_2$Ph)CO), 2.57 (dd, 1H, $J = 6.6$ Hz, $J = 14.4$ Hz, Phe NHCH(CH$_2$Ph)CO); $^{13}$C NMR (100 MHz, CDCl$_3$): δ = 172.61, 170.98, 169.23, 168.05, 166.79, 166.70, 140.59, 140.34, 138.62, 137.25, 137.02, 135.97, 134.03, 133.87, 131.48, 131.25, 129.18, 129.12, 129.08, 129.02, 128.80, 128.66, 128.51, 128.13, 128.03, 127.61, 127.58, 127.32, 127.07, 124.31, 67.64, 58.49, 54.12, 49.53, 44.164, 42.40, 41.99, 37.24; FAB-HRMS for C$_73$H$_{68}$N$_7$O$_9$ m/z [M+H$^+$], calcd. 1186.50785, found 1186.50971; [α]$_D$ = -1.8 in CHCl$_3$.

**Glycylglycyl-L-alanylglycylglycine rotaxane 2.19**

Yield (0.074 g, 55%); m.p. 133°C; $^1$H NMR (400 MHz, C$_2$D$_2$Cl$_2$): δ = 8.17 (s, 2H, isophthaloyl 2-H), 8.01 (d, 2H, $J = 7.6$ Hz, isophthaloyl 4-H), 7.99 (d, 2H, $J = 7.6$ Hz, isophthaloyl 6-H), 7.58 (br s, 1H, macrocyclic CONH), 7.49 (t, 2H, $J = 7.6$ Hz, isophthaloyl 5-H), 7.30-7.03 (m, 23H, thread Ar-H & macrocyclic CONH), 7.01 (s, 8H, macrocyclic para-xylylene Ar-H), 6.92 (br s, 1H, CONH), 6.81 (br s, 1H, CONH), 6.64 (br s, 1H, CONH), 6.56 (br s, 1H, CONH), 6.51 (br s, 1H, CONH), 4.79 (s, 1H,
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Ph₂CHCO), 4.47-3.24 (m, 18 H, Ala NHCH(CH₃)CO & Gly NHCH₂CO & macrocyclic NHCH₂ & COOCHH'CHPh₂ & COOCHH'CHPh₂ & COOCHH'CHPh₂), 3.05-2.95 (m, 2H, Gly NHCH₂CO), 1.01 (d, 3H, J = 6.4 Hz, Ala NHCH(CH₃)CO); ¹³C NMR (100 MHz, C₂D₂Cl₂): δ = 169.88, 169.66, 169.30, 167.62, 167.43, 164.14, 162.75, 162.39, 141.05, 141.04, 139.30, 137.75, 129.71, 129.59, 129.47, 129.38, 129.36, 129.33, 129.29, 129.55, 128.05, 127.61, 62.56, 50.06, 47.74, 47.44, 46.47, 46.21, 45.17, 45.00, 44.77, 44.61; FAB-HRMS for C₇₁H₇₀N₉O₁₁ m/z [M+H⁺], calcd. 1224.51948, found 1224.51808; [α]₀ not measurable in CHCl₃.

¹Boc-Leu-Enkephalin-OBn rotaxane 2.25

Yield (0.040 g, 86%); ¹H NMR (400 MHz, CDCl₃): δ = 8.11 (s, 2H, isophthaloyl 2-H), 7.96 (d, 4H, J = 7.6 Hz, isophthaloyl 4-H & 6-H), 7.76 (br t, 1H, CONH), 7.67 (br s, 1H, CONH), 7.40 (t, 2H, J = 7.6 Hz, isophthaloyl 5-H), 7.34-6.97 (m, 26H, thread Ar-H & CONH & macrocyclic CONH& macrocyclic para-xylylene Ar-H), 6.91 (br t, 1H, Gly CONHCH₂CO), 6.83 (d, 2H, J = 8.4 Hz, CH₂ArH'H'OBn), 6.75 (d, 2H, J = 8.4 Hz, CH₂ArH'H'OBn), 6.42 (br d, 1H, CONH), 6.34 (br s, 1H, Leu CONHCH(CH₂CH(CH₃)₂)CO), 5.00 (s, 2H, PhCH₂COO), 4.95-4.86 (m, 3H, Tyr (CH₃)₃COCONH & OCH₃Ph), 4.48-4.31 (m, 10H, macrocyclic NHCHH' & macrocyclic NHCHH' & Phe NHCHCO & Leu NHCHCO), 3.99-3.94 (m, 1H, Tyr NHCHCO), 3.06-2.70 (m, 7H, Tyr NHCH(CHH'ArOBn)CO & Phe NHCH(CHH'Ph)CO & Phe NHCH(CHH'Ph)CO & Gly NHCH₂CO & Gly NHCH₂CO), 2.52-2.38 (m, 1H, Tyr NHCH(CHH'ArOBn)CO), 1.45-1.29 (m, 3H, Leu NHCH(CHCH₂CH(CH₃)₂)CO & NHCH(CHCH₂CH(CH₃)₂)CO), 1.15 (s, 9H, (CH₃)₃COCO), 0.71-0.69 (m, 6H, NHCH(CHCH₂CH(CH₃)₂)CO); ¹³C NMR (100 MHz, CDCl₃): δ = 172.55, 172.38,
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171.44, 169.07, 168.18, 167.31, 167.24, 157.62, 155.82, 137.18, 136.83, 136.43, 135.19, 134.05, 133.94, 131.28, 131.22, 130.20, 129.92, 129.24, 129.03, 128.68, 128.60, 128.54, 128.42, 128.16, 127.95, 127.42, 127.10, 125.01, 114.78, 80.48, 69.96, 67.03, 55.29, 54.68, 51.05, 44.15, 42.33, 41.73, 40.47, 38.19, 37.08, 27.99, 24.70, 22.47, 21.65; FAB-HRMS for C_{79}H_{86}N_{9}O_{13} m/z [M+H^+], calcd. 1368.63451, found 1368.63346.

**Bistable molecular shuttle rotaxane 2.28**

Yield (0.082 g, 71%); m.p. 194.7°C; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 8.40 (s, 2H, isophthaloyl 2-H), 8.11 (d, 4H, $J$ = 7.8 Hz, isophthaloyl 4-H & 6-H), 7.64 (br t, 4H, macrocyclic CONH), 7.55 (t, 2H, $J$ = 7.8 Hz, isophthaloyl 5-H), 7.32-7.14 (m, 21 H, thread Ar-H & thread CONH), 6.94 (s, 8H, macrocyclic para-xylylene, Ar-H), 6.87 (br s, 1H, CONH), 6.27 (br s, 1H, CONH), 6.13 (br s, 1H, CONH), 5.88 & 5.73 (2d, 2H, $J$ = 15.0 Hz, NHCOCH & CHCONH), 4.42 (br d, 8H, $J$ = 4.4 Hz, macrocyclic NHCH$_2$), 4.20 & 4.15 (2t, 2H, $J$ = 7.8 Hz & 7.6 Hz, Ph$_2$CHCH$_2$NHCOCH$_2$ & CHCONHCH$_2$CHPh$_2$), 3.89-3.79 (m, 4H, Ph$_2$CHCH$_2$NHCOCH$_2$ & CHCONHCH$_2$CHPh$_2$), 3.15-3.07 (m, 4H, CH$_2$NHCOCH & CH$_2$CONHCH$_3$), 2.13-2.02 (m, 4H, succinic COCH$_2$ & CH$_2$CO), 1.48-1.39 (m, 4H, CH$_2$CONHCH$_2$CH$_2$ & CH$_3$CH$_2$NHCOCH), 1.31-1.22 (m, 16H, alkyl CONHCH$_2$CH$_2$(CH$_3$)$_8$CH$_2$CH$_2$NHCO), $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ = 172.37, 172.22, 166.70, 165.63, 165.29, 141.86, 141.59, 136.91, 131.32, 129.10, 129.01, 128.86, 128.70, 128.00, 127.99, 127.88, 127.14, 126.84, 126.81, 124.55, 50.55, 50.41, 44.76, 43.93, 40.06, 39.56, 31.83, 31.74, 29.69, 29.47, 29.38, 29.31, 29.26, 29.25, 29.16, 29.07, 26.96, 26.76; FAB-HRMS for C$_{80}$H$_{89}$N$_8$O$_8$ m/z [M+H$^+$], calcd. 1289.68034, found: 1289.68031.
2.5. References


(8) Rosengren, K. J.; Clark, R. J.; Daly, N. L.; Göransson, U.; Jones, A.; Craik, D. J. *J. Am. Chem. Soc.* 2003, 125, 12464-12474.


To the best of our knowledge, the synthesis and the nucleophilic attack of 2,6-diphenyl-para-nitrophenyl ester has never been reported so far. Then tailoring the activation of an acid using bulky diphenyl substitution on a nitrophenyl ester and its further nucleophilic attack is a novelty.


A convenient synthesis of oligopeptide rotaxanes

via classical peptide coupling reactions

Abstract: The synthesis of three tripeptide rotaxane building blocks, N-Fmoc and -tBoc protected L-SerGlyGly and L-AspGlyGly functionalized on the lateral chain by ether and ester labile bulky stoppers respectively, is described. After N-deprotection, further elongation of the building blocks via classical peptide coupling synthesis was carried out to conveniently give oligopeptide rotaxanes. The N-Fmoc-L-SerGlyGly rotaxane building block gave the best results in terms of (i) feasibility and (ii) fast shuttling of the macrocycle along the peptide backbone. By reacting this N-deprotected building block, with our previously reported 2,6-diphenyl para-nitrophenyl activated ester dipeptide rotaxane building block,¹ the method was successfully applied to the synthesis in solution of oligopeptide [3]rotaxanes which could be purified via reverse phase HPLC. Such peptide rotaxane building blocks are very versatile and convenient tools for overcoming...
usual foldings of long peptide backbones and making libraries of oligopeptide rotaxanes in good yields, with no purification and solubility problems.


### 3.1. Introduction

DNA is well-known to exhibit a large range of topologies: linear, circular and catenated\(^2,3\) and knotted\(^4\). Until very recently, the case for naturally occurring interlocked peptides and secondary protein structures was not so clear. Dogma long held that proteins, even of complicated structures, contained mechanical links or knots only through disulfide bonds and cofactors.\(^5,6\) In the last few years, however, knots,\(^7-10\) catenanes\(^11,12\) and most recently rotaxanes\(^13-15\) have been clearly characterized in peptide and protein skeletons. A few naturally occurring small peptides (16-21 amino acids) including a rotaxane-like substructure has indeed been reported: Microcin J25(MccJ25),\(^13-15\) anantin,\(^16\) RES-701-01,\(^17\) RP 71955,\(^18\) syamicyn II,\(^19\) and MS-271.\(^20\) These unconventional peptides, with a "lasso"-like structure, exhibit intriguing properties ranging from unique modes of antimicrobial and antiviral action\(^21-23\) to remarkable membrane transport and solubility characteristics, impressive stability to thermal and chemical denaturing\(^24\) and, of higher significance, an extraordinary stability to peptidases. Efficient syntheses of such interlocked architectures rely upon non-covalent interactions between the components to direct the kinetically stable entanglement. We recently
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reported the synthesis of dipeptide rotaxanes incorporating benzylic 1,3-carbamide macrocycles, using a five-component hydrogen bond-directed assembly. However this approach is restricted to short peptide sequences only. In fact, in longer sequences the components will be largely self-satisfied in terms of hydrogen bonding through folding of the backbone, thus preventing a good preorganization between the peptide thread and the precursor of the macrocycle.

A method to overcome this problem is the recently reported synthesis of versatile activated ester rotaxane building blocks which can be elongated via their C-terminal side to promote the synthesis of oligopeptide rotaxanes in very good yields. A new strategy we describe herein is the synthesis of new tripeptide rotaxane building blocks which can be N-extended via classical peptide coupling reactions. The choice of N-Fmoc or -Boc protected L-Ser- or L-Asp-GlyGly sequences as a tripeptide was three-fold: (i) they contain the most efficient GlyGly dipeptide template, (ii) the lateral chain of the first amino acid residue, L-Ser or L-Asp, respectively, functionalized as an ether or an ester, acts as a labile bulky stopper for the macrocycle and (iii) N-terminal Fmoc or tBoc deprotection of L-Ser or L-Asp residues promotes peptide coupling reactions towards the synthesis of oligopeptide rotaxanes, followed by further deprotection of the labile bulky stopper, allowing shuttling of the macrocycle along the peptide backbone at a rate that is within the NMR time scale.
3.2. Results and discussion

Synthesis of tripeptide rotaxane building blocks

Three tripeptide threads were prepared as templates for rotaxane formation. The threads only differed in their first amino acid residue and their N-terminal and lateral protecting groups: (i) an N-Fmoc protected L-Ser residue made bulky on its lateral chain by a tert-butyldiphenylsilyl ether (TBDPS) and (ii) N-Fmoc- and N-tBoc-protected L-Asp residues with, respectively, a tert-butyl and a diphenylethyl ester on the lateral chain.

The N-Fmoc protected L-SerGlyGly tripeptide thread 3.3 was prepared in a two step sequence starting from the commercially available N-Fmoc-L-Ser-OH 3.1, using standard EDCI coupling peptide chemistry followed by TBDPS protection of the lateral alcohol (Scheme 3.1.).

\[
\text{Scheme 3.1. Synthesis of the N-Fmoc-L-serylglycylglycine thread 3.3. a) (i)H-GlyGly-NHCH}_2\text{CHPh}_2, DMAP, EDCI,HCl, CHCl}_3, 45\%, (ii) TBDPSCl, imidazole, DMF, 70\^\circ\text{C}, 67\%.
\]

The N-Fmoc protected L-AspGlyGly tripeptide thread 3.5 was prepared in one step from the commercially available N-Fmoc-L-Asp(OtBu)-OH 3.4 (Scheme 3.2.).
The N-tBoc protected L-AspGlyGly tripeptide thread 3.9, protected on the lateral chain as a diphenyl ethyl ester, was prepared in a three step synthesis (Scheme 3.3).

In a typical procedure for rotaxane formation, equimolar quantities of isophthaloyl dichloride in chloroform and a mixture of para-xylylene diamine and triethylamine in chloroform were added to a solution of the thread 3.3, 3.5 or 3.9 in chloroform (Scheme 3.4).
The yields of pure isolated rotaxane building blocks ranged from 11 to 19% (Table 1), similar to a previous result obtained in the tripeptide series. The yields were improved by using multiple rotaxanation (see overal yields).

<table>
<thead>
<tr>
<th>N-PG</th>
<th>X</th>
<th>PG</th>
<th>Peptide rotaxane derivative</th>
<th>Yield %</th>
<th>Overall yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fmoc</td>
<td>O</td>
<td>TBDPS</td>
<td>L-SerGlyGly 3.10</td>
<td>14</td>
<td>73⁸</td>
</tr>
<tr>
<td>Fmoc</td>
<td>CO₂</td>
<td>tBu</td>
<td>L-AspGlyGly 3.11</td>
<td>19</td>
<td>62⁹</td>
</tr>
<tr>
<td>tBoc</td>
<td>CO₂</td>
<td>CH₂CH₂Ph₂</td>
<td>L-AspGlyGly 3.12</td>
<td>11</td>
<td>49⁹</td>
</tr>
</tbody>
</table>

Table 3.1. Synthesis of the building blocks 3.10-3.12. * Overall yield respectively obtained after a) 4, b) 6 and c) 5 cycles of rotaxane formation. A cycle includes the synthesis of the rotaxane followed by its purification. The thread collected back from the column is then put on rotaxanation again.

The ¹H NMR comparison in CDCl₃ of the N-Fmoc-L-SerGlyGly thread 3.3 and its corresponding N-Fmoc protected and deprotected rotaxane building blocks 3.10 and 3.13, respectively, are reported in figure 3.1. In spectra b and c, two different dd signals are observed for the enantiotopic benzylic hydrogens H₅ of the macrocycle due to the asymmetry of the thread stoppers. A shielding effect on the protons of the thread located in the cavity of the macrocycle is observed. Thus in spectrum b, the signals for H₄, H₅ and the first Gly residue are shifted upfield compared with those spectrum a, indicating the average position of the macrocycle around these protons. The H₄ and H₅ protons are shielded in the deprotected rotaxane 3.13, compared with those in the building block 3.10, by δ = 0.54 and 0.20 ppm respectively. This shielding effect indicates the localization of the macrocycle mainly around the L-Ser residue, which hinders the lone pair of the free amine. The benzylic protons H₅ of the macrocycle are similar in both compounds.
Figure 3.1. Comparison of the $^1$H NMR spectra (400 MHz, CDCl$_3$) of a) the N-Fmoc protected tripeptide L-SerGlyGly thread 3, b) the N-Fmoc protected tripeptide L-SerGlyGly rotaxane 3.10 and c) the deprotected tripeptide L-SerGlyGly rotaxane 3.13. See scheme 3.5 for the proton assignment.

Straightforward synthesis of oligopeptide rotaxanes via classical peptide coupling reactions

In order to make oligopeptide rotaxanes, the $N$-terminal elongation of rotaxanes 3.10 and 3.12 was investigated using various stoppered amino acids (Scheme 3.5.) and classical peptide chemistry. In a typical procedure, rotaxane building block 3.10 was $N$-Fmoc deprotected using piperidine in chloroform. The resulting free amine rotaxane 3.13 was then reacted overnight at room temperature with 2 equivalents of stoppered mono- and di-peptides using BOP coupling peptide chemistry. The TFA salt obtained after $N$-$t$Boc deprotection of the rotaxane building block 3.12 was directly converted into the free
amine \textit{in situ} by using an excess of base, for further coupling with a stoppered amino acid. The resulting oligopeptide rotaxanes \textbf{3.14-3.17} and \textbf{3.18} were further deprotected on their lateral chain with a 1M TBAF solution in THF and potassium hydroxide in methanol, respectively, to give the deprotected oligopeptide rotaxanes \textbf{3.19-3.23} (Table 3.2.).

\textbf{Scheme 3.5.} Synthesis of oligopeptide rotaxanes promoted by coupling of rotaxane \textbf{3.10} with various stoppered amino acids (n=1, 2). a) piperidine/CHCl$_3$: 1/3, 99%; b) stoppered amino acid, BOP, Et$_3$N, CHCl$_3$; c) 1M TBAF in THF.

The yields of isolated rotaxanes \textbf{3.14-3.18} varied from good to excellent depending on the steric hindrance of the stoppered amino acids added (Table 3.2.).
Table 3.2. Synthesis of oligopeptide rotaxanes 3.14-3.18.

<table>
<thead>
<tr>
<th>N-PG</th>
<th>X</th>
<th>PG</th>
<th>n</th>
<th>Peptide rotaxane sequence</th>
<th>Coupling yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fmoc</td>
<td>O</td>
<td>TBDPS</td>
<td>1</td>
<td>Gly-L-SerGlyGly 3.14/ 3.19</td>
<td>90</td>
</tr>
<tr>
<td>Fmoc</td>
<td>O</td>
<td>TBDPS</td>
<td>2</td>
<td>GlyGly-L-SerGlyGly 3.15/ 3.20</td>
<td>77</td>
</tr>
<tr>
<td>Fmoc</td>
<td>O</td>
<td>TBDPS</td>
<td>1</td>
<td>L-Ala-L-SerGlyGly 3.16/ 3.21</td>
<td>69</td>
</tr>
<tr>
<td>Fmoc</td>
<td>O</td>
<td>TBDPS</td>
<td>1</td>
<td>L-Leu-L-SerGlyGly 3.17/ 3.22</td>
<td>47</td>
</tr>
<tr>
<td>tBoc</td>
<td>CO₂</td>
<td>CH₂CHPh₂</td>
<td>1</td>
<td>Gly-L-AspGlyGly 3.18/ 3.23</td>
<td>70</td>
</tr>
</tbody>
</table>

The $^1$H NMR comparisons in CDCl₃ of the TBDPS-protected and deprotected Gly-L-SerGlyGly rotaxanes 3.14 and 3.19, respectively, and the deprotected rotaxane GlyGly-L-SerGlyGly 3.20 are shown in figure 3.2. After deprotection of 3.14, a shielding effect on one set of Gly protons is observed, this indicates that the macrocycle is shuttling along the whole peptide backbone at a rate that is fast on the NMR timescale. The same behaviour is observed in the longer deprotected GlyGly-L-SerGlyGly rotaxane 3.20. It has been shown that such a shuttling of the macrocycle along the peptide thread allows for peptide resistance against enzyme biodegradation.²⁸
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Figure 3.2. Comparison of $^1$H NMR spectra (400 MHz, CDC$_3$) of a) the TBDPS-protected Gly-L-SerGlyGly tetrapeptide rotaxane 3.14, b) the deprotected Gly-L-SerGlyGly tetrapeptide rotaxane 3.19 at 313K and c) the deprotected GlyGly-L-SerGlyGly pentapeptide rotaxane 3.20 at 313K.

It is noteworthy that in the aspartic series, rotaxane 3.23 did not display any consequent shuttling of the macrocycle on the NMR time scale, even though examination of CPK models indicated that the carboxylate lateral chain of the aspartic residue should not act as a stopper for the benzylic 1,3-carbamide macrocycle.

The N-Fmoc protected L-SerGlyGly tripeptide template, functionalized as a TBDPS ether on its lateral chain, was selected for further investigations.
Tripeptide rotaxane building blocks as new tools for convenient synthesis of oligopeptide [3]rotaxanes in solution

Synthesis of oligopeptide [3]rotaxanes has never been reported so far in the literature. A convenient method for making such complex interlocked architectures is by “fusion” of the present and previously reported strategies: the 2,6 diphenyl para-nitrophenyl activated ester of the dipeptide rotaxane building block 3.28 is displaced by nucleophilic attack of an N-terminal free amine rotaxane building block. Both solvent polarity and steric hindrance of the nucleophile are parameters of importance. Therefore, the rotaxane building block 3.13 was extended by a Glyₙ spacer (n=1 and 2) and further N-Fmoc deprotected to give the free amine rotaxanes 3.26 and 3.27, respectively. The reaction was performed by dissolution of the activated rotaxane building block 3.28 and the building blocks 3.13, 3.26 or 3.27 either at reflux in CHCl₃ or in DMF at 65°C (Scheme 3.6).

The yields of [3]rotaxane formation are reported in table 3.3. With no Gly spacer, the lone pair of the rotaxane building block 3.13 is likely to be too sterically hindered by the close proximity of the macrocycle to induce any nucleophilic attack on 3.28 since only traces of [3]rotaxane 3.29 are detected by LC-MS after refluxing in chloroform for one
week. This is consistent with the appearance of the $^1$H NMR spectra obtained in CDCl$_3$ (Figure 3.1.). A second attempt was carried out in DMF, the highly polar solvent was expected to disrupt the hydrogen bonding between the macrocycle and the free amine peptide rotaxane 3.13, but again only traces of 3.29 were detected by LC-MS. When one or two Gly spacers is present in 3.26 and 3.27, respectively, as the lone pair of the N-terminal Gly residue is completely available for nucleophilic attack, oligopeptide [3]rotaxanes were synthesised in chloroform at reflux and isolated by preparative HPLC in about 50% yield. This strategy allows for the straightforward synthesis of oligopeptide [3]rotaxanes easily purified via preparative reverse phase HPLC (gradient H$_2$O/MeOH) and obtained in average yields.

<table>
<thead>
<tr>
<th>n</th>
<th>Peptide [3]rotaxane derivative</th>
<th>n$_{3.28}$</th>
<th>n$<em>{3.13}$, n$</em>{3.26}$ or n$_{3.27}$</th>
<th>Solvent reaction</th>
<th>Temperature °C</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>GlyGly-L-SerGlyGly 3.29</td>
<td>1</td>
<td>2</td>
<td>CHCl$_3$</td>
<td>reflux</td>
<td>traces</td>
</tr>
<tr>
<td>0</td>
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<td>~50</td>
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<td>CHCl$_3$</td>
<td>reflux</td>
<td>~50</td>
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</table>

3.3. Conclusion

We have described the synthesis of three tripeptide rotaxane building blocks, N-Fmoc and -tBoc protected L-SerGlyGly and L-AspGlyGly functionalized on their lateral chain by a labile bulky stopper. After N-deprotection using piperidine or TFA, further elongation of the building blocks via classical peptide coupling chemistry was carried out to easily give oligopeptide rotaxanes. The N-Fmoc protected L-SerGlyGly rotaxane building block was found to be the most efficient template, as the macrocycle displayed a fast shuttling along the peptide backbone after lateral chain deprotection. Reaction of the N-deprotected tripeptide building block with our previously described 2,6-diphenyl para-nitrophenyl activated ester dipeptide rotaxane successfully yielded oligopeptide [3]rotaxanes, which were purified via reverse phase HPLC. Such peptide rotaxane building blocks are very versatile and convenient tools for making wide libraries of peptide interlocked architectures without problems of purification and solubility.
3.4. Experimental section

Synthesis of oligopeptide rotaxanes by N-coupling with various stoppered amino acids

Method for the preparation of the tripeptide L-SerGlyGly thread 3.3

N-α-Fmoc-L-serylglycylglycine-2,2-diphenylethyl amide 3.2

N-α-Fmoc-L-serine (3.1, 1.815 g, 5.550 mmol), glycylglycine-2,2-diphenylethyl amide (1.721 g, 5.530 mmol) and DMAP (0.677 g, 5.54 mmol) were dissolved in 100 mL of CHCl₃. The reaction mixture was cooled at 0°C. Then EDCI.HCl (1.609 g, 8.390 mmol) was added. The reaction mixture was stirred one hour at 0°C and brought to room temperature and stirred overnight. The crude material was washed with 1M HCl and saturated NaHCO₃. The organic phase was dried over MgSO₄. The solvent was removed under reduced pressure. The purification via silica gel chromatography (CHCl₃/MeOH: 96/4) afforded a white powder. Yield (1.553 g, 45%); m.p. 90°C; ¹H NMR (400 MHz, CDCl₃): δ = 7.75 (d, 2H, J = 7.4 Hz, aromatic Ar-H Fmoc), 7.57 (d, 2H, J = 7.4 Hz, aromatic Ar-H Fmoc), 7.38 (t, 2H, J = 7.4 Hz, aromatic Ar-H Fmoc), 7.41-7.13 (m, 14H, aromatic Ar-H & aromatic Ar-H Fmoc & CONH), 6.26 (br t, 1H, CONBCH2CHPh2), 5.99 (d, 1H, J = 7.6 Hz, Ser OCONHCH(CH2OH)CO), 4.40 (d, 2H, J = 6.8 Hz, Fmoc CHCH2000NH), 4.27-4.21 (m, 1H, Ser NHCH(CH2OH)CO), 4.12 (t, 1H, J = 6.8 Hz, Fmoc CHCH2OCONH), 4.17 (t, 1H, J = 6.8 Hz, Fmoc CHCH2OCONH), 3.92 (dd, 1H, J = 5.2 Hz, J = 11.2 Hz, Ser NHCH(CHH’OH)CO), 3.89-3.84 (m, 2H, Gly NHCH₃CO), 3.81 (t, 2H, J = 7.4 Hz, CONHCH₃CHPh₂), 3.70 (d, 2H, J = 4.8 Hz, Gly NHCH₃CO), 3.62 (dd, 1H, J = 5.2 Hz, J = 11.2 Hz, Ser NHCH(CHH’OH)CO); ¹³C NMR (100 MHz, CDCl₃): δ = 171.81, 169.54, 169.00, 157.86, 143.59, 141.52, 141.31,
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128.75, 127.96, 127.83, 127.13, 126.92, 125.01, 120.06, 67.15, 62.98, 56.35, 50.28, 47.05, 43.89, 43.14, 42.73; FAB-HRMS for C_{36}H_{37}N_{4}O_{6} m/z [M+H^+], calcd.: 621.27131, found: 621.27105.

\textit{N-\alpha-Fmoc-O-(\textit{tert}-butyldiphenylsilyl)-L-serylglycylglycine-2,2-diphenylethyl amide 3.3}

\textit{N-\alpha-Fmoc-L-seryl glycylglycine-2,2-diphenylethyl amide (3.2, 0.819 g, 1.320 mmol)} and imidazole (0.245 g, 3.600 mmol) were dissolved in dry DMF (8 mL) under nitrogen. TBDPSCI (3.5 mL, 13.680 mmol) was added and the solution was stirred 24 hours at 70°C. The solvent was removed under reduced pressure. The purification via silica gel chromatography (CHCl₃/MeOH: 98/2 to 90/10) afforded a yellowish powder. Yield (0.763 g, 67%); m.p. 188°C; ^1H NMR (400 MHz, CDCl₃): δ = 7.76 (d, 2H, aromatic Ar-H Fmoc, J = 7.6 Hz), 7.63-7.12 (m, 26H, aromatic Ar-H & aromatic Ar-H Fmoc), 7.00 (br t, 1H, CONHCH₃), 6.88 (br t, 1H, CONHCH₃), 6.25 (br t, 1H, CONHCH₂), 5.54 (d, 1H, J = 6.0 Hz, Ser OCONHCH(CH₂OH)CO), 4.45-4.33 (m, 2H, Fmoc CHCH₂OCONH), 4.21-4.10 (m, 3H, Fmoc CHCH₂OCONH & Ser NHCH(CHH' (OTBDPS))CO & CONHCH₂CHPh₂), 3.96 (dd, 1H, J = 4.8 Hz, J = 13.2 Hz, Ser NHCH(CHH' (OTBDPS))CO), 3.91-3.75 (m, 6H, CONHCH₂CHPh₂ & Gly NHCH₂CO & Gly NHCH₂CO), 3.70 (dd, 1H, J = 4.8 Hz, J = 13.2 Hz, Ser NHCH(CHH' (OTBDPS))CO), 1.04 (s, 9H, OSi(CH₃)₃); ^13C NMR (100 MHz, CDCl₃): δ = 170.61, 168.71, 167.92, 157.85, 143.60, 143.58, 141.71, 141.37, 141.33, 135.44, 134.80, 132.48, 132.27, 130.18, 128.65, 128.07, 128.05, 128.02, 128.00, 127.85, 127.81, 127.71, 127.14, 127.09, 126.75, 125.01, 124.94, 120.07, 81.33, 67.26, 63.49,
Method for the preparation of the N-Fmoc protected tripeptide L-AspGlyGly thread 3.5

N-a-Fmoc-L-(tert-butyl ester)-asparticglycylglycine-2,2-diphenylethyl amide 3.5

Following the experimental procedure leading to derivative 3.2.

Yield (0.822 g, 91%); amorphous solid; 1H NMR (400 MHz, CDCl3): δ = 7.76 (d, 2H, J = 7.6 Hz, aromatic Ar-H Fmoc), 7.58 (d, 2H, J = 7.2 Hz, aromatic Ar-H Fmoc), 7.41 (td, 2H, J = 2.4 Hz, J = 7.6 Hz, aromatic Ar-H Fmoc), 7.34-7.16 (m, 12H, aromatic Ar-H & aromatic Ar-H Fmoc), 7.05 (br t, 1H, CONHCH2), 6.77 (br t, 1H, CONHCH2), 6.26 (t, 1H, J = 5.6 Hz, CONHCH2CHPh2), 5.66 (d, 1H, J = 8.8 Hz, Asp OCONHCH(CH2CO2-tBu)CO), 4.52 (d, 2H, J = 6.0 Hz, Fmoc CHCH2OCONH), 4.45-4.38 (m, 1H, Asp NHCH(CH2CO2-tBu)CO), 4.22 (t, 1H, J = 6.0 Hz, Fmoc CHCH2OCONH), 4.18 (t, 1H, J = 8.0 Hz, CONHCH2CHPh2), 3.94-3.71 (m, 6H, CONHCH2CHPh2 & Gly NHCH2CO), 2.90 (dd, 1H, J = 5.0 Hz, J = 17.2 Hz, Asp NHCH(CHH’CH2CO2-tBu)CO), 2.63 (dd, 1H, J = 5.0 Hz, J = 17.2 Hz, Asp CONHCH(CHH’CO2-tBu)CO), 1.41 (s, 9H, CO2C(CH3)3); 13C NMR (100 MHz, CDCl3): δ = 171.48, 171.45, 168.94, 168.73, 157.86, 143.50, 141.80, 141.37, 128.68, 128.67, 128.09, 127.88, 127.15, 127.12, 126.77, 124.92, 124.87, 120.12, 120.10, 82.62, 67.15, 51.37, 50.39, 47.18, 43.73, 43.30, 43.22, 37.23, 28.03; FAB-HRMS for C41H45N4O7 m/z [M+H⁺], calcd.: 705.32883, found: 705.32888.

Method for the preparation of the N-tBoc tripeptide L-AspGlyGly thread 3.9
N-terbutoxycarbonyl-L-(2,2-diphenyl ethyl ester)-aspartic benzylic ester 3.7

Following the experimental procedure leading to derivative 3.2.

Yield (5.978 g, 95%); m.p. 122°C; $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 7.39-7.15$ (m, 15H, aromatic Ar-H), 5.27 (d, 1H, $J = 8.4$ Hz, Asp OCONHCH(CH$_2$CO$_2$CH$_2$CHPh$_2$)CO), 5.10 (s, 2H, COOCH$_2$Ph$_2$), 4.63-4.49 (m, 3H, COOCH$_2$CHPh$_2$ & Asp NHCH(CH$_2$CO$_2$CH$_2$CHPh$_2$)CO), 4.26 (t, 1H, $J = 7.6$ Hz, COOCH$_2$CHPh$_2$), 2.95 (dd, 1H, $J = 4.4$ Hz, $J = 17.2$ Hz, Asp NHCH(CHH'CO$_2$CH$_2$CHPh$_2$)CO), 2.72 (dd, 1H, $J = 4.4$ Hz, $J = 17.2$ Hz, Asp NHCH(CHH'CO$_2$CH$_2$CHPh$_2$)CO), 1.42 (s, 9H, Boc Cl$_3$); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta =$ 170.77, 170.59, 155.36, 140.82, 140.80, 135.34, 128.66, 128.40, 128.26, 128.11, 126.92, 80.09, 67.16, 49.96, 49.67, 36.79, 28.31; FAB-HRMS for C$_{30}$H$_{34}$NO$_6$ m/z [M+H$^+$], calcd.: 504.23861, found: 504.23765.

N-terbutoxycarbonyl-L-(2,2-diphenylethylester)-aspartic acid 3.8

N-terbutoxycarbonyl-L-(2,2-diphenyl ethyl ester) aspartic benzylic ester (3.7, 5.766 g, 11.450 mmol) and catalytic palladium, 10% wt. on carbon powder were dissolved in 100 mL of EtOAc. The system was then placed under vacuum and hydrogen was introduced at atmospheric pressure for four hours. The reaction mixture was filtered over celite. The solvent was removed under reduced pressure to afford a white powder. Yield (4.626 g, 98%); m.p. 155°C; $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 7.36-7.18$ (m, 10H, aromatic Ar-H), 5.31 (d, 1H, $J = 8.4$ Hz, Asp OCONHCH(CH$_2$CO$_2$CH$_2$CHPh$_2$)CO), 4.66 (d, 2H, $J = 7.8$ Hz, COOCH$_2$CHPh$_2$), 4.54-4.52 (m, 1H, Asp NHCH(CH$_2$CO$_2$CH$_2$CHPh$_2$)CO), 4.35 (t, 1H, $J = 7.8$ Hz, CO$_2$CH$_2$CHPh$_2$), 2.95 (br dd, 1H, Asp NHCH(CHH'CO$_2$CH$_2$CHPh$_2$)CO), 2.61 (dd, 1H, $J = 4.8$ Hz, $J = 17.2$ Hz, Asp
NHCH(CH'HCO₂CH₂CHPh₂)CO), 1.45 (s, 9H, Boc CH₃); ¹³C NMR (100 MHz, CDCl₃): δ = 170.77, 155.58, 140.73, 140.70, 128.67, 128.66, 128.32, 128.16, 128.13, 126.95, 80.51, 67.33, 49.76, 49.69, 36.46, 28.30; FAB-HRMS for C₂₃H₂₈N₀₆ m/z [M+H⁺], calcd.: 414.19166, found: 414.19146.

N-terbutoxycarbonyl-L-(2,2-diphenylethylester)-asparticglycylglycine-2,2-diphenyl ethyl amide 3.9

Following the experimental procedure leading to derivatice 3.2.

Yield (3.280 g, 49%); m.p. 64°C; ¹H NMR (400 MHz, CDCl₃): δ = 7.33-7.16 (m, 20H, aromatic Ar-H), 7.06 (t, 1H, J =5.6 Hz, CONHCH₂CO), 6.82 (br t, 1H, CONHCH₂CHPh₂), 6.32 (t, 1H, J =5.6 Hz, CONHCH₂CO), 5.28 (d, 1H, J =8.4 Hz, Asp OCONHCH(CH₂CO₂CH₂CHPh₂)CO), 4.67 (dd, 1H, J = 7.6 Hz, J = 11.2 Hz, CO₂CHH'CHPh₂), 4.58 (dd, 1H, J = 7.6 Hz, J = 11.2 Hz, CO₂CHH'CHPh₂), 4.36-4.29 (m, 2H, Asp NHCH(CH₂CO₂CH₂CHPh₂)CO & Asp NHCH(CH₂CO₂CHH'CHPh₂)CO), 3.92-3.80 (m, 3H, Gly NHCHH'CO & Gly NHCHH₂CO), 3.75 (t, 2H, J =6.8 Hz, CONHCH₂CHPh₂), 3.64 (dd, 1H, J = 5.6 Hz, J = 16.8 Hz, Gly NHCHH'CO), 2.89 (dd, 1H, J = 5.2 Hz, J = 17.2 Hz, Asp NHCH(CH₂CO₂CH₂CHPh₂)CO), 2.61 (dd, 1H, J = 5.2 Hz, J = 17.2 Hz, Asp NHCH(CH₂CO₂CH₂CHPh₂)CO), 1.45 (s, 9H, Boc CH₃); ¹³C NMR (100 MHz, CDCl₃): δ = 171.57, 170.85, 168.77, 167.95, 158.11, 141.83, 140.74, 140.65, 139.82, 131.10, 130.57, 129.20, 128.68, 128.65, 128.12, 128.09, 127.29, 127.00, 126.74, 125.92, 79.48, 67.45, 50.40, 49.74, 49.92, 45.70, 43.74, 42.92, 42.42, 36.13, 28.30; FAB-HRMS for C₄₁H₄₇N₄O₇ m/z [M+H⁺], calcd.: 707.34448, found: 707.34508.
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Thread 3.3, 3.5, 3.9 (1.910 mmol) was dissolved in 100 mL of CHCl₃ under nitrogen and stirred vigorously whilst solutions of the mixture of para-xylylene diamine (2.121 g, 15.600 mmol) and NEt₃ (3.965 g, 39.300 mmol) in 50 mL of CHCl₃ and the isophthaloyl dichloride (3.151 g, 15.500 mmol) in 50 mL of CHCl₃ were simultaneously added to the reaction mixture over a period of 3 hours using motor-driven syringe pumps. The reaction mixture was stirred 2 hours under nitrogen, then filtered over celite and the filtrate was evaporated under reduced pressure to afford a syrup which was purified via silica gel chromatography to yield, in order of elution, the unconsumed activated ester thread (AcOEt/cyclohexane: 1/1) and the rotaxane (CHCl₃/MeOH: 98/2).

Fmoc-L-(O-(tert-butyldiphenylysilyl))-serylglycylglycine rotaxane 3.10

Yield (0.078 g, 14%); overall yield after 4 cycles of rotaxane formation (0.278 g, 73%); m.p. 137°C; ¹H NMR (400 MHz, CDCl₃): δ = 8.23 (s, 2H, isophthaloyl 2-H), 8.02 (d, 4H, J = 8.0 Hz, isophthaloyl 4-H & 6-H), 7.73 (d, 2H, aromatic Ar-H Fmoc, J = 7.6 Hz), 7.63-7.51 (m, 8H, aromatic Ar-H & aromatic Ar-H Fmoc & macrocyclic CONH), 7.47 (t, 2H, J = 7.6 Hz, isophthaloyl 5-H), 7.44-7.09 (m, 24 H, aromatic Ar-H), 7.01 (s, 8H, macrocyclic para-xylylene Ar-H), 6.89 (br t, 1H, CONHCH₂), 6.75 (br t, 1H, CONHCH₂), 6.68 (br t, 1H, Gly CONHCH₂CO), 5.42 (d, 1H, J = 7.2 Hz, Ser OCONHCH(CH₂(OTBDPS))CO), 4.66 (dd, 2H, J = 5.6 Hz, J = 14.0 Hz, macrocyclic NHCHH'), 4.53 (dd, 2H, J = 5.6 Hz, J = 14.0 Hz, macrocyclic NHCHH'), 4.30 (dd, 2H, J = 4.4 Hz, J = 14.0 Hz, macrocyclic NHCHH'), 4.25 (d, 2H, J = 3.2 Hz, Fmoc CHCH₂OCONH), 4.20 (dd, 2H, J = 4.4 Hz, J = 14.0 Hz, macrocyclic NHCHH'), 4.08-3.96 (m, 3H, Fmoc CHCH₂OCONH & Ser NHCH(CH₂(OTBDPS))CO &
CONHCH₂CHPh₂), 3.81 (dd, 1H, J = 5.0 Hz, J = 10.2 Hz, Gly NHCHH'CO), 3.67 (dd, 1H, J = 5.6 Hz, J = 10.2 Hz, Gly NHCHH'CO), 3.65-3.56 (m, 2H, CONHCH₂CHPh₂), 3.25 (dd, 1H, J = 5.2 Hz, J = 16.0 Hz, Ser NHCH(CHH'(OTBDPS))CO), 3.10 (br dd, 1H, Ser NHCH(CHH'(OTBDPS))CO), 2.77 (br d, 2H, Gly NHCHH'CO), 0.99 (s, 9H, OSi(CH₃)₃); ¹³C NMR (100 MHz, CDCl₃): δ = 168.86, 167.91, 166.99, 166.93, 157.89, 143.60, 143.40, 141.49, 141.28, 141.26, 137.35, 137.17, 135.42, 135.36, 134.18, 133.98, 132.44, 132.29, 131.06, 130.18, 129.03, 128.97, 128.75, 127.99, 127.82, 127.79, 127.09, 127.05, 126.95, 125.12, 124.95, 120.01, 81.33, 67.38, 63.03, 58.97, 56.49, 45.87, 44.42, 44.28, 44.15, 42.69, 42.00, 26.77; FAB-HRMS for C₈₄H₈₃N₈O₁₀Si m/z [M+H⁺], calcd.: 1391.60207, found: 1391.60250.

**Fmoc-L-((tert-butyl ester)-asparticglycylglycine rotaxane 3.11**

Yield (0.205 g, 19%); overall yield after 6 cycles of rotaxane formation (0.682 g, 62%); m.p. 115°C; ¹H NMR (400 MHz, CDCl₃): δ = 8.31 (s, 2H, isophthaloyl 2-H), 8.06 (d, 2H, J = 8.0 Hz, isophthaloyl 4-H & 6-H), 8.05 (d, 2H, J = 8.0 Hz, isophthaloyl 4-H & 6-H), 7.73 (d, 2H, J = 7.6 Hz, aromatic Ar-H Fmoc), 7.67 (br t, 2H, macrocyclic CONH), 7.55-7.48 (m, 2H, isophthaloyl 5-H), 7.48-7.43 (m, 2H, macrocyclic CONH), 7.37 (t, 2H, aromatic Ar-H Fmoc), 7.33-7.04 (m, 23H, aromatic Ar-H Fmoc & thread Ar-H & macrocyclic para-xylene Ar-H & Gly CONHCH₂CO), 6.70 (s, 1H, Gly CONHCH₂CO), 6.52 (s, 1H, CONHCH₂CHPh₂), 6.12 (d, 1H, J = 8.4 Hz, Asp OCONHCH(CH₂CO₂-tBu)CO), 4.97-4.85 (m, 2H, Fmoc CHCH₂OCONH), 4.48-4.39 (m, 4H, macrocyclic NHCHH'), 4.15-4.07 (m, 3H, Fmoc CHCH₂OCONH & Asp NHCH(CH₂CO₂-tBu)CO & CONHCH₂CHPh₂), 4.05-3.97 (m, 4H, macrocyclic NHCHH'), 3.71 (t, 2H, J = 6.8 Hz, CONHCH₂CHPh₂), 3.18 (br s, 2H, Gly NHCHH₂CO),
3.37 (dd, 1H, $J = 4.0$ Hz, $J = 17.2$ Hz, Gly NHCHH'CO), 2.84 (dd, 1H, $J = 4.0$ Hz, $J = 17.2$ Hz, Gly NHCHH'CO), 2.52 (dd, 1H, $J = 4.0$ Hz, $J = 16.8$ Hz, Asp NHCH(CHH'CO$_2$-tBu)CO), 2.36 (dd, 1H, $J = 4.0$ Hz, $J = 16.8$ Hz, Asp NHCH(CHH'CO$_2$-tBu)CO), 1.31 (s, 9H, CO$_2$C(C$_{113}$)$_3$);

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ = 171.40, 168.71, 167.63, 166.85, 166.76, 156.79, 143.26, 143.19, 141.40, 141.38, 141.35, 137.76, 136.76, 134.06, 133.48, 131.65, 131.31, 130.67, 129.17, 129.01, 128.75, 128.67, 128.61, 128.12, 128.06, 127.81, 127.05, 126.96, 125.01, 124.80, 124.37, 120.04, 82.08, 67.43, 50.51, 50.42, 46.84, 44.55, 44.00, 43.84, 42.80, 42.38, 35.33, 27.88; FAB-HRMS for C$_{73}$H$_{73}$N$_8$O$_{11}$ m/z [M+H$^+$], calcd.: 1237.53988, found: 1237.54031.

$t$Boc-L-(2,2-diphenylethylester)-asparticglycylglycine rotaxane 3.12

Yield (0.059 g, 11%); overall yield after 5 cycles of rotaxane formation (0.199 g, 49%); m.p. 147.5$^\circ$C; $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 8.25$ (s, 2H, isophthaloyl 2-H), 8.08 (d, 4H, $J = 4.4$ Hz, isophthaloyl 4-H & 6-H), 7.53-7.45 (m, 4H, macrocyclic CONH & isophthaloyl 5-H), 7.38 (br t, 2H, macrocyclic CONH$'$), 7.32-7.10 (m, 20 H, thread Ar-H), 7.09 (s, 8H, macrocyclic para-xylene Ar-H), 6.57 (br t, 1H, CONHCH$_2$), 6.47 (br t, 1H, CONHCH$_2$), 6.29 (br t, 1H, CONHCH$_2$), 5.29 (d, 1H, $J = 8.8$ Hz, Asp OCONHCH(CH$_2$CO$_2$CH$_2$CHPh$_2$)CO), 4.75-4.42 (m, 6H, macrocyclic NHCHH$'$ & Asp NHCH(CH$_2$CO$_2$CH$_2$CHPh$_2$)CO), 4.39-4.19 (m, 5H, macrocyclic NHCHH$'$ & Asp NHCH(CH$_2$CO$_2$CH$_2$CHPh$_2$)CO), 4.18-4.11 (m, 1H, Asp NHCH(CH$_2$CO$_2$CH$_2$CHPh$_2$)CO), 4.02 (t, 1H, $J = 8.0$ Hz, CONHCH$_2$CHPh$_2$), 3.65 (br d, 2H, CONHCH$_2$CHPh$_2$), 3.24 (dd, 1H, $J = 4.4$ Hz, $J = 17.2$ Hz, Gly NHCHH$'$CO), 3.08 (dd, 1H, $J = 5.2$ Hz, $J = 17.2$ Hz, Gly NHCHH$'$CO), 2.85 (s, 2H, Gly NHCH$_2$CO), 2.58
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(d, 1H, J = 4.8 Hz, J = 17.2 Hz, Asp NHCH(CH\text{H'}CO_2CH_2CHPh_2)CO), 2.39 (dd, 1H, J = 6.0 Hz, J = 17.2 Hz, Asp NHCH(CH\text{H'}CO_2CH_2CHPh_2)CO), 1.36 (s, 9H, Boc CH_3);

^{13}C\text{ NMR} (100\text{ MHz, CDCl}_3): \delta = 172.45, 172.37, 168.44, 166.84, 166.71, 158.00, 141.38, 140.74, 140.55, 137.56, 137.03, 134.02, 133.93, 131.42, 131.25, 129.18, 129.07, 128.80, 128.66, 128.61, 128.12, 128.05, 127.81, 127.02, 126.95, 124.69, 8023, 67.29, 50.39, 49.74, 44.62, 44.17, 44.10, 43.25, 42.70, 36.17, 28.22; FAB-HRMS for C_{73}H_{75}N_8O_{11}\text{ m/z [M+H']}\text{, calcd.}: 1239.55553, found: 1239.55403.

General procedure for N-Fmoc deprotection

N-Fmoc protected rotaxane (0.036 mmol) was dissolved in 2 mL piperidine/CHCl\textsubscript{3} (1/3) and was stirred at rt for 2 hours. The solvent was removed under reduced pressure. The crude material was purified via silica-gel column chromatography (CHCl\textsubscript{3}/MeOH: 96/4) to afford the free amine rotaxane.

L-(O-(tert-butyldiphenylsilyl))-serglyglycine rotaxane 3.13

Yield (0.042 g, 99%); $^1$H NMR (400 MHz, CDCl\textsubscript{3}): $\delta$ = 8.26 (s, 2H, isophthaloyl 2-H), 8.07 (d, 2H, $J$ = 7.4 Hz, isophthaloyl 4-H & 6-H), 8.03 (d, 2H, $J$ = 7.4 Hz, isophthaloyl 4-H & 6-H), 7.78 (br t, 1H, macrocyclic CONH), 7.73 (br t, 2H, macrocyclic CONH), 7.70 (br t, 1H, macrocyclic CONH), 7.51 (t, 2H, $J$ = 7.4 Hz, isophthaloyl 5-H), 7.44 (br t, 1H, CONHCH\textsubscript{2}CHPh\textsubscript{2}), 7.47-7.07 (m, 21H, aromatic Ar-H & Gly CONHCH\textsubscript{2}CO), 7.05 (s, 8H, macrocyclic para-xylylene Ar-H), 6.86 (br s, 1H, Gly CONHCH\textsubscript{2}CO), 4.65 (dd, 2H, $J$ = 6.4 Hz, $J$ = 14.4 Hz, macrocyclic NHCHH'), 4.51 (dd, 2H, $J$ = 4.8 Hz, $J$ = 93
14.4 Hz, macrocyclic NHCHH'), 4.37 (dd, 2H, J = 4.4 Hz, J = 14.0 Hz, macrocyclic NHCHH'), 4.23 (dd, 2H, J = 1.8 Hz, J = 14.0 Hz, macrocyclic NHCHH'), 4.12 (t, 1H, J = 7.6 Hz, CONHCH₂CHPh₂), 3.79 (t, 2H, J = 7.6 Hz, CONHCH₂CHPh₂), 3.57-3.42 (m, 3H, Gly NHCH₂CO & Ser NH₂CH(CH₂(OTBDPS)CO), 3.05 (dd, 1H, J = 4.8 Hz, J = 17.0 Hz, Ser NH₂CH(CHH'(OTBDPS))CO), 2.97 (br s, 2H, Gly NHCH₂CO), 2.90 (dd, 1H, J = 1.8 Hz, J = 17.0 Hz, Ser NH₂CH(CHH'(OTBDPS))CO), 2.13-1.49 (m, 2H, Ser NH₂CH(CH₂(OTBDPS)CO), 0.99 (s, 9H, OSiC(CF₃)₃); ¹³C NMR (100 MHz, CDCl₃): δ = 169.89, 167.92, 166.79, 166.72, 141.44, 141.38, 137.53, 137.01, 135.41, 135.36, 134.10, 133.56, 132.79, 132.66, 131.72, 131.29, 130.10, 130.06, 129.20, 129.10, 128.82, 127.88, 127.83, 127.06, 124.27, 80.35, 65.42, 56.19, 50.53, 44.36, 44.13, 43.00, 42.36, 36.37, 26.79; FAB-HRMS for C₆₉H₇₃N₈O₈Si m/z [M+H⁺], calcd.: 1169.53207, found: 1169.53017.

General procedure for the synthesis of protected oligopeptide rotaxanes 3.14-3.18

by N-terminal coupling with various stoppered amino acids

L-(O-(tert-butylidiphenylsilyl))-serylglycylglycine rotaxane (3.13, 0.042 g, 0.035 mmol), N-diphenylacetyl amino acid (0.019 g, 0.072 mmol) and NEt₃ (till pH 11) and BOP (0.024 g, 0.055 mmol) were dissolved in 5 mL of CHCl₃. The reaction mixture was stirred overnight at rt. The solvent was removed under reduced pressure. The crude material was purified via silica gel chromatography (CHCl₃/Methanol: 98/2 to 95/5).

Glycyl-L-(O-(tert-butylidiphenylsilyl))-serylglycylglycine rotaxane 3.14
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Yield (0.045 g, 90%); 

Proton NMR (400 MHz, CDCl₃): δ = 8.18 (s, 2H, isophthaloyl 2-H), 7.97 (d, 4H, J = 7.6 Hz, isophthaloyl 4-H & 6-H), 7.85 (br s, 4H, macrocyclic CONH), 7.63-6.95 (m, 43 H, aromatic Ar-H & isophthaloyl 5-H & macrocyclic para-xylylene Ar-H & CONHCH₂), 6.89 (br t, 1H, CONHCH₂), 6.63 (d, 1H, J = 7.2 Hz, CONHCH(CH₃(OTBDPS))CO), 5.05 (s, 1H, Ph₂CHCO), 4.57-4.45 (m, 4H, macrocyclic NHCH'), 4.34-4.18 (m, 5H, macrocyclic NHCHH' & Ser NHCH(CH₃(OTBDPS))CO), 4.00 (t, 1H, J = 8.0 Hz, CONHCH₂CHPh₂), 3.86 (dd, 1H, J = 4.0 Hz, J = 10.0 Hz, Ser NHCH(CHH'(OTBDPS))CO), 3.72 (dd, 1H, J = 6.0 Hz, J = 16.8 Hz, Gly NHCHH'CO), 3.63-3.45 (m, 4H, CONHCH₂CHPh₂ & Ser NHCH(CHH'(OTBDPS))CO & Gly NHCHH'CO), 3.34 (dd, 1H, J = 4.0 Hz, J = 16.8 Hz, Gly NHCHH'CO), 3.25 (dd, 1H, J = 4.0 Hz, J = 16.0 Hz, Gly NHCHH'CO), 2.58 (dd, 1H, J = 5.2 Hz, J = 15.8 Hz, Gly NHCHH'CO), 2.05 (br dd, 1H, J = 15.8 Hz, Gly NHCHH'CO), 1.00 (s, 9H, OSi(CH₃)₃); 

Carbon NMR (100 MHz, CDCl₃): δ = 173.81, 170.50, 169.30, 168.65, 168.06, 167.52, 167.37, 141.80, 141.73, 139.02, 137.10, 136.93, 135.45, 135.34, 134.60, 134.27, 132.69, 132.32, 130.85, 130.53, 130.13, 129.12, 128.92, 128.86, 128.75, 128.67, 128.61, 127.98, 127.86, 127.27, 126.73, 126.15, 80.24, 65.86, 58.19, 54.24, 50.38, 44.62, 44.56, 44.11, 43.73, 42.92, 41.19, 26.83; FAB-HRMS for C₈₅H₇₆N₉O₉Si m/z [M+H⁺], calcd.: 1420.62669, found: 1420.62491.

Glycylglycyl-L-((O-(tert-butyldiphenylsilyl))-serylglycylglycine rotaxane 3.15

Yield (0.019 g, 77%); 

Proton NMR (400 MHz, CDCl₃): δ = 8.19 (s, 2H, isophthaloyl 2-H), 8.00 (d, 4H, J = 6.8 Hz, isophthaloyl 4-H & 6-H), 7.59-6.96 (m, 49 H, aromatic Ar-H & isophthaloyl 5-H & macrocyclic para-xylylene Ar-H & macrocyclic CONH & Gly CONHCH₂CO), 6.68 (br s, 1H, CONH), 5.01 (s, 1H, Ph₂CHCO), 4.67-4.46 (m, 5H, 5H,
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macrocyclic NHCHH' & Ser NHCH(CH₂(OTBDPS))CO), 4.37-4.14 (m, 4H, macrocyclic NHCHH'), 4.05 (dd, 1H, J = 4.4 Hz, J = 16.8 Hz, Gly CONHCHH'CO), 3.99-3.88 (m, 2H, Ser NHCH(CHH'(OTBDPS))CO & CONHCH₂CHPh₂), 3.78 (dd, 1H, J = 4.8 Hz, J = 16.8 Hz, Gly NHCHH'CO), 3.70-3.57 (m, 2H, Ser NHCH(CHH'(OTBDPS))CO & Gly NHCHH'CO), 3.56-3.48 (m, 2H, CONHCH₂CHPh₂), 3.42-3.30 (m, 2H, Gly NHCHH'CO & Gly NHCHH'CO), 3.11 (dd, 1H, J = 5.0 Hz, J = 18.4 Hz, Gly NHCHH'CO), 2.69 (dd, 1H, J = 5.2 Hz, J = 15.2 Hz, Gly NHCHH'CO), 2.00 (dd, 1H, J = 6.0 Hz, J = 15.2 Hz, Gly NHCHH'CO), 0.98 (s, 9H, OSiC(CH₃)₃); ¹³C NMR (100 MHz, CDCl₃): δ = 172.85, 170.45, 169.77, 169.18, 168.09, 167.42, 167.28, 141.72, 141.65, 139.05, 138.99, 137.11, 137.05, 135.46, 135.35, 134.59, 134.47, 132.69, 132.37, 130.65, 130.09, 129.09, 128.88, 128.79, 128.70, 128.66, 128.61, 127.92, 127.84, 127.36, 127.32, 126.83, 126.24, 80.12, 64.69, 54.53, 52.30, 50.41, 44.61, 43.97, 43.43, 43.01, 42.70, 41.28, 26.75; FAB-HRMS for C₈₇H₈₉N₁₀O₁₁Si m/z [M+H⁺], calcd.: 1477.64816, found: 1477.64929.

L-alanyl-L-((O-(tert-butylidiphenylsilyl))-serylglycylglycine rotaxane 3.16

Yield (0.011 g, 69%); ¹H NMR (400 MHz, CDCl₃): δ = 8.24 (s, 2H, isophthaloyl 2-H), 8.02 (d, 4H, J = 7.6 Hz, isophthaloyl 4-H & 6-H), 7.84-7.74 (m, 4H, macrocyclic CONH), 7.60-7.00 (m, 35 H, aromatic Ar-H & isophthaloyl 5-H & thread CONH), 6.97 (d, 8H, J = 1.2 Hz, macrocyclic para-xylylene Ar-H), 6.36 (br t, 1H, Gly CONHCH₂CO), 6.06 (d, 1H, J = 3.6 Hz, Ala CONHCH(CH₃)CO), 4.84 (s, 1H, Ph₂CHCO), 4.79-4.62 (m, 4H, macrocyclic NHCHH⁺), 4.21-4.05 (m, 4H, macrocyclic NHCHH'), 4.00 (t, 2H, J = 7.4 Hz, CONHCH₂CHPh₂), 3.94-3.90 (m, 1H, Ser NHCH(CHH'(OTBDPS))CO), 3.83-3.79 (m, 1H, Ala NHCH(CH₃)CO), 3.68 (dd, 1H, J
= 5.6 Hz, \( J = 13.4 \) Hz, Gly NHCHH'CO), 3.56 (t, 2H, \( J = 7.4 \) Hz, CONCHH(CHPh2), 3.36 (dd, 1H, \( J = 5.0 \) Hz, Ser NHCH(\( \text{CH}^\text{H'} \text{(OTBDPS)} \))CO), 3.16 (dd, 1H, \( J = 5.0 \) Hz, Ser NHCH(\( \text{CH}^\text{H'} \text{(OTBDPS)} \))CO), 3.03 (dd, 1H, \( J = 5.6 \) Hz, \( J = 13.4 \) Hz, Gly NHCHH'CO), 2.56 (dd, 1H, \( J = 4.6 \) Hz, \( J = 16.6 \) Hz, Gly NHCHH'CO), 2.36 (dd, 1H, \( J = 4.6 \) Hz, \( J = 16.6 \) Hz, Gly NHCHH'CO), 1.19 (d, 3H, \( J = 7.2 \) Hz, Ala NHCH(CH3)CO), 1.06 (s, 9H, OSiC(CH3)3); 13C NMR (100 MHz, CDCl3): \( \delta = 174.75, 172.08, 171.88, 170.37, 169.79, 168.07, 141.62, 141.49, 141.42, 138.94, 138.30, 138.06, 137.12, 136.89, 136.89, 134.47, 132.35, 130.92, 130.86, 130.71, 130.27, 130.09, 129.26, 129.07, 128.97, 128.88, 128.80, 128.72, 128.67, 128.04, 127.95, 127.90, 127.69, 127.62, 127.44, 127.32, 126.93, 126.85, 125.95, 125.45, 79.92, 63.09, 58.42, 55.04, 51.85, 50.62, 44.50, 44.31, 44.11, 43.12, 42.05, 26.91, 16.92; FAB-HRMS for C_{86}H_{88}N_{9}O_{10}Si m/z [M+H'], calcd.: 1434.64235, found: 1434.64217.

**L-leucyl-L-(O-(tert-butyldiphenylsilyl))-serylglycylglycine rotaxane 3.17**

Yield (0.006 mg, 47%); \(^1\)H NMR (400 MHz, CDCl3): \( \delta = 8.21 \) (s, 2H, isophthaloyl 2-H), 8.03 (d, 2H, \( J = 7.8 \) Hz, isophthaloyl 4-H & 6-H), 7.99 (d, 2H, \( J = 7.8 \) Hz, isophthaloyl 4-H' & 6-H'), 7.81 (br t, 4H, macrocyclic CONH), 7.66-6.87 (m, 43 H, aromatic Ar-H & isophthaloyl 5-H & macrocyclic para-xyylene Ar-H & thread CONH), 6.82 (d, 1H, \( J = 8.4 \) Hz, Ser CONHCH(CH2(OTBDPS))CO), 6.09 (d, 1H, \( J = 5.2 \) Hz, Gly CONHCH2CO), 5.06 (s, 1H, Ph2CHCO), 4.68-4.63 (m, 2H, macrocyclic NHCHH'), 4.46-4.41 (m, 2H, macrocyclic NHCHH'), 4.27-4.21 (m, 4H, macrocyclic NHCHH'), 4.13-4.11 (m, 1H, Leu NHCH(CH2CH(CH3)2)CO), 3.91 (t, 2H, \( J = 8.4 \) Hz, CONHCH2CHPh2), 3.87-3.83 (m, 1H, Ser NHCH(CH2(OTBDPS))CO), 3.69 (dd, 1H, \( J = 6.0 \) Hz, \( J = 17.2 \) Hz, Gly NHCHH'CO), 3.42 (dd, 1H, \( J = 4.8 \) Hz, \( J = 16.0 \) Hz, Ser
NHCH(CHH'(OTBDPS))CO), 3.37-3.29 (m, 2H, CONHCH₂CHPh₂), 3.10 (dd, 1H, $J = 4.8$ Hz, $J = 16.0$ Hz, Ser NHCH(CHH'(OTBDPS))CO), 2.97 (dd, 1H, $J = 6.0$ Hz, $J = 17.2$ Hz, Gly NHCHH'CO), 2.41 (dd, 1H, $J = 4.4$ Hz, $J = 15.4$ Hz, Gly NHCHH'CO), 1.94 (dd, 1H, $J = 3.6$ Hz, $J = 15.4$ Hz, Gly NHCHH'CO), 1.36-1.18 (m, 3H, Leu NHCH(CH₂CH(CH₃)₂)CO & Leu NHCH(CH₂CH(CH₃)₂)CO), 0.98 (s, 9H, OSi(CH₃)₃), 0.86 (d, 6H, $J = 6.4$ Hz, Leu NHCH(CH₂CH(CH₃)₂)CO); $^{13}$C NMR (100 MHz, CDCl₃): δ = 174.90, 171.91, 169.74, 168.01, 167.50, 166.88, 141.66, 141.49, 139.21, 138.27, 137.11, 135.37, 134.46, 134.24, 133.98, 132.96, 132.40, 131.32, 130.90, 130.57, 130.25, 129.97, 129.29, 129.00, 128.91, 128.80, 128.71, 128.62, 128.03, 127.90, 127.84, 127.78, 127.65, 127.50, 127.30, 126.90, 126.82, 126.72, 126.24, 125.45, 80.02, 63.21, 58.56, 54.92, 54.70, 50.43, 44.58, 44.30, 44.01, 43.29, 41.37, 26.70, 24.62, 22.84, 22.54, 19.46; FAB-HRMS for C₈₉H₈₄N₉O₁₀Si m/z [M+H⁺], calcd.: 1476.68930, found: 1476.69098.

**Glycyl-L-(2,2-diphenylethylester)-asparticglyclylglycine rotaxane 3.18**

In this particular example, the tBoc protected rotaxane building block 3.12 was deprotected using TFA and the free amine was released in situ for the N-coupling reaction.

Yield (0.031 g, 70%); m.p. 145.6°C; $^1$H NMR (400 MHz, CDCl₃): δ = 8.24 (s, 2H, isophthaloyl 2-H), 8.01 (d, 4H, $J = 4.4$ Hz, isophthaloyl 4-H & 6-H), 7.77 (br t, 2H, macrocyclic CONH), 7.72 (br t, 2H, macrocyclic CONH'), 7.47 (t, 2H, $J = 7.6$ Hz, isophthaloyl 5-H), 7.35 (br t, 1H, Gly CONHCH₂CO), 7.31-7.11 (m, 31 H, thread Ar-H & CONHCH₂CHPh₂), 7.07 (d, 1H, $J = 8.4$ Hz, Asp CONHCH(CH₂CO₂CH₂CHPh₂)CO), 7.00 (s, 8H, macrocyclic para-xylylene Ar-H), 6.85 (br t, 1H, Gly')
Ph₂CHCONHCH₂CO), 6.56 (br t, 1H, GlyCONHCH₂CO), 5.02 (s, 1H, Ph₂CHCO), 
4.64-4.45 (m, 7H, macrocyclic NHCHH’ & Asp NHCH(CH₂CO₂CH₂CHPh₂)CO & Asp 
NHCH(CH₂CO₂CH₂CHPh₂)CO), 4.33-4.17 (m, 5H, macrocyclic NHCHH’ & Asp 
NHCH(CH₂CO₂CH₂CHPh₂)CO), 3.99 (t, 2H, J = 7.6 Hz, CONHCH₂CHPh₂), 3.57 (t, 
1H, J = 7.6 Hz, CONHCH₂CHPh₂), 3.47 (br s, 2H, Gly Ph₂CHCONHCH₂CO), 3.23 (dd, 
1H, J = 4.8 Hz, J = 16.6 Hz, Gly NHCHH’CO), 3.07 (dd, 1H, J = 6.0 Hz, J = 16.6 Hz, 
Gly NHCHH’CO), 2.50 (t, 1H, J = 4.0 Hz, Gly NHCH₂CO), 2.60 (dd, 1H, J = 5.2 Hz, J 
= 17.2 Hz, Asp NHCH(CHH’CO₂CH₂CHPh₂)CO), 2.41 (dd, 1H, J = 5.6 Hz, J = 17.2 
Hz, Asp NHCH(CHH’CO₂CH₂CHPh₂)CO), 13C NMR (100 MHz, CDCl₃): δ = 173.37, 
170.69, 170.15, 168.36, 168.13, 167.29, 166.50, 166.22, 140.73, 139.96, 139.83, 
138.03, 137.90, 136.28, 136.11, 133.46, 133.25, 130.33, 130.09, 128.27, 128.18, 
128.08, 128.00, 127.87, 127.83, 127.80, 127.31, 127.27, 126.99, 126.97, 126.68, 
126.61, 126.12, 126.07, 124.60, 57.45, 49.52, 48.78, 48.18, 43.58, 43.56, 43.34, 42.15, 
40.82; FAB-HRMS for C₆₄H₉₀N₁₁O₁₁ m/z [M+H⁺], calcd.: 1390.59773, found: 
1390.60005.

**General procedure for the TBDPS deprotection**

Protected oligopeptide rotaxanes 3.14-3.17 (0.019 mmol) were dissolved in 1 mL of 
THF and then treated with 0.1 mL of 1 mol.dm⁻³ solution of TBAF in THF. The reaction 
mixture was stirred at rt for one hour and then concentrated under reduced pressure. The 
crude material was purified via silica gel chromatography (CHCl₃/MeOH: 98/2 to 90/10).
**Glycyl-L-serglycylglycine rotaxane 3.19**

Yield (0.023 g, 100%); $^1$H NMR (400 MHz, 313K, CDCl$_3$): $\delta$ = 8.19 (s, 2H, isophthaloyl 2-H), 7.99 (d, 4H, $J$ = 7.6 Hz, isophthaloyl 4-H & 6-H), 7.65 (br s, 2H, macrocyclic CONH), 7.64 (br s, 2H, macrocyclic CONH$'$), 7.44 (t, 2H, $J$ = 7.6 Hz, isophthaloyl 5-H), 7.28-7.02 (m, 22 H, aromatic Ar-H & CONH), 6.98 (s, 8H, macrocyclic para-xylene Ar-H), 6.76 (br t, 1H, Gly CONHCH$_2$CO), 6.69 (br t, 1H, Gly CONHCH$_2$CO), 6.60 (br s, 1H, CONH), 4.80 (s, 1H, Ph$_2$CHCO), 4.47-4.43 (m, 4H, macrocyclic NHCH$'$), 4.30-4.26 (m, 4H, macrocyclic NHCHH$'$), 4.16-4.07 (m, 1H, Ser NHCH(CHOH)CO), 4.01 (t, 1H, $J$ = 7.6 Hz, CONHCH$_2$CHPh$_2$), 3.69-3.56 (m, 4H, Ser NHCH(CHOH)CO & Gly NHCHH$'$CO & Gly NHCH$_2$CO), 2.83 (br d, 1H, Gly NHCHH$'$CO), 2.75 (br d, 1H, Gly NHCHH$'$CO); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ = 169.47, 169.34, 168.92, 168.37, 167.35, 167.28, 141.66, 138.84, 136.99, 134.20, 130.96, 129.02, 128.92, 128.79, 128.75, 128.68, 127.87, 127.48, 126.85, 125.47, 62.10, 58.13, 54.99, 50.28, 44.34, 44.04, 43.08, 42.72, 42.65, 41.76; FAB-HRMS for C$_{69}$H$_{68}$N$_9$O$_{10}$ $m/z$ [M+H$^+$], calcd.: 1182.50892, found: 1182.50460.

**Glycylglycyl-L-serglycylglycine rotaxane 3.20**

Yield (0.019 g, 100%); $^1$H NMR (400 MHz, 313K, CDCl$_3$): $\delta$ = 8.21 (s, 2H, isophthaloyl 2-H), 8.01 (d, 4H, $J$ = 7.8 Hz, isophthaloyl 4-H & 6-H), 7.74-7.67 (m, 4H, macrocyclic CONH), 7.46 (t, 2H, $J$ = 7.8 Hz, isophthaloyl 5-H), 7.35-7.04 (m, 31 H, aromatic Ar-H & macrocyclic para-xylene Ar-H & CONH), 6.75 (br t, 1H, Gly CONHCH$_2$CO), 6.61-6.52 (m, 2H, CONH), 4.80 (s, 1H, Ph$_2$CHCO), 4.56-4.44 (m, 4H,
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macrocyclic NHCHH'), 4.34-4.23 (m, 4H, macrocyclic NHCHH'), 4.16-4.08 (m, 1H, Ser NHCH(CHOH)CO), 4.01 (t, 1H, J = 7.6 Hz, CONHCH2CHPh2), 3.68-3.54 (m, 3H, Ser NHCH(CHH'OH)CO & CONHCH2CHPh2), 3.53-3.24 (m, 6H, Ser NHCH(CHH'OH)CO & Gly NHCHH'CO & Gly NHCHH'CO & Gly NHCHH'CO & Gly NHCHH'CO), 2.98-2.81 (m, 3H, Gly NHCHH'CO & Gly NHCHH'CO); 13C NMR (100 MHz, CDCl3): δ = 169.51, 169.44, 168.31, 168.12, 167.85, 167.39, 167.25, 141.69, 138.21, 137.12, 134.37, 130.93, 129.19, 128.82, 128.76, 128.68, 128.41, 127.90, 127.56, 126.84, 125.17, 61.07, 58.14, 54.25, 49.78, 44.32, 44.01, 43.71, 43.11, 42.27, 41.12; FAB-HRMS for C71H71N10O11 m/z [M+H'], calcd.: 1239.53038, found: 1239.53367.

L-alanyl-L-serylglyclylglycine rotaxane 3.21

Yield (0.017 g, 100%); 1H NMR (400 MHz, 323K, CDCl3): δ = 8.28 (s, 2H, isophthaloyl 2-H), 8.11 (d, 4H, J = 7.8 Hz, isophthaloyl 4-H & 6-H), 7.62-7.45 (m, 28 H, aromatic Ar-H & macrocyclic CONH & isophthaloyl 5-H), 7.34-6.97 (m, 28 H, aromatic Ar-H & macrocyclic para-xylene Ar-H), 6.83 (t, 1H, Gly CONHCH2CO), 6.32-6.23 (m, 2H, CONHCH2CHPh2 & Gly CONHCH2CO), 6.16-6.10 (m, 2H, Ala CONHCH(CH3)CO & Ser CONHCH(CH2OH)CO), 4.92 (s, 1H, Ph2CHCO), 4.61 (dd, 2H, J = 5.2 Hz, J = 14.6 Hz, macrocyclic NHCHH'), 4.56 (dd, 2H, J = 5.2 Hz, J = 14.4 Hz, macrocyclic NHCHH'), 4.34 (dd, 2H, J = 5.2 Hz, J = 14.6 Hz, macrocyclic NHCHH'), 4.27 (dd, 2H, J = 5.2 Hz, J = 14.4 Hz, macrocyclic NHCHH'), 4.19-4.12 (m, 1H, Ala NHCH(CH3)CO), 4.06-4.00 (m, 1H, Ser NHCH(CHOH)CO), 3.96 (t, 1H, J = 7.2 Hz, CONHCH2CHPh2), 3.55-3.36 (m, 4H, CONHCH2CHPh2 & Ser NHCH(CHH'OH)CO & Gly NHCHH'CO), 3.08 (dd, 1H, J = 6.0 Hz, J = 17.2 Hz, Gly NHCHH'CO), 2.92 (dd,
1H, J = 5.2 Hz, J = 16.4 Hz, Ser NHCH(CHH'OH)CO), 2.63 (dd, 1H, J = 5.2 Hz, J = 18.4 Hz, Gly NHCHH'CO), 2.53 (dd, 1H, J = 5.6 Hz, J = 18.4 Hz, Gly NHCHH'CO), 1.49-1.43 (m, 1H, Ser NHCH(CHH'OH)CO), 1.14 (d, 3H, J = 7.6 Hz, Ala NHCH(CH₃)CO); ¹³C NMR (100 MHz, CDCl₃): δ = 169.44, 169.12, 168.57, 168.49, 168.12, 167.42, 167.24, 141.62, 141.52, 138.48, 137.10, 134.53, 134.16, 131.06, 130.76, 128.95, 128.91, 128.79, 128.73, 128.69, 127.83, 127.77, 127.62, 127.58, 126.87, 125.77, 61.50, 58.20, 55.21, 50.91, 50.16, 44.61, 44.68, 44.30, 44.13, 42.81, 16.89; FAB-HRMS for C₇₀H₇₀N₉O₁₀ m/z [M+H⁺], calcd.: 1196.52457, found: 1196.52447.

L-leucyl-L-serylglycylglycine rotaxane 3.22

Yield (0.012 g, 100%); ¹H NMR (400 MHz, 313K, CDCl₃): δ = 8.21 (s, 2H, isophthaloyl 2-H), 8.04 (d, 2H, J = 8.0 Hz, isophthaloyl 4-H & 6-H), 7.99 (d, 2H, J = 8.0 Hz, isophthaloyl 4-H & 6-H), 7.70 (br t, 2H, macrocyclic CONH), 7.60 (br t, 2H, macrocyclic CONH'), 7.51 (t, 2H, J = 7.6 Hz, isophthaloyl 5-H), 7.33-6.95 (m, 28H, aromatic Ar-H & macrocyclic para-xylylene Ar-H), 6.90 (d, 1H, J = 6.4 Hz, Ser CONHCH(CH₂OH)CO), 6.79 (br t, 1H, CONHCH₂CHPh₂), 6.50 (br t, 1H, Gly CONHCH₂CO), 6.42 (br t, 1H, Gly CONHCH₂CO), 6.11 (d, 1H, J = 5.6Hz, Leu CONHCH(CH₂CH(CH₃)₂)CO), 4.98 (s, 1H, Ph₂CHCO), 4.61 (dd, 2H, J = 6.0 Hz, J = 14.6 Hz, macrocyclic NHCHH'), 4.53 (dd, 2H, J = 5.6 Hz, J = 14.2 Hz, macrocyclic NHCHH'), 4.35 (dd, 2H, J = 5.6 Hz, J = 14.2 Hz, macrocyclic NHCHH'), 4.26 (dd, 2H, J = 6.0 Hz, J = 14.6 Hz, macrocyclic NHCHH'), 4.19-4.11 (m, 1H, Leu NHCH(CH₂CH(CH₃)₂)CO), 4.03-3.96 (m, 2H, Ser NHCH(CHH'OH)CO & CONHCH₂CHPh₂), 3.60-3.37 (m, 4H, Ser NHCH(CHH'OH)CO & CONHCH₂CHPh₂ &
Gly(3.04 (dd, 1H, J = 6.0 Hz, J = 11.6 Hz, Gly NHCHH’CO), 2.83 (dd, 1H, J = 5.2 Hz, J = 17.2 Hz, Ser NHCH(CHH’OH)CO), 2.39 (dd, 1H, J = 4.8 Hz, J = 16.4 Hz, Gly NHCHH’CO), 2.00 (dd, 1H, J = 4.8 Hz, J = 16.4 Hz, Gly NHCHH’CO), 1.58 (m, 4H, Ser NHCH(CH2OH)CO & Leu NHCH(CH2CH(CH3)2)CO & Leu NHCH(CH2(CH3)2)CO), 0.77-0.68 (m, 6H, Leu NHCH(CH2CH(CH3)2)CO); 13C NMR (100 MHz, CDCl3): δ = 174.05, 172.59, 171.08, 169.41, 168.09, 167.45, 167.28, 141.76, 139.25, 138.54, 137.19, 134.38, 131.09, 130.57, 129.08, 128.97, 128.82, 128.71, 128.59, 127.82, 127.62, 127.35, 126.84, 126.69, 125.49, 62.42, 54.57, 53.98, 53.80, 50.38, 44.35, 44.17, 42.96, 41.79, 40.98, 24.79, 22.82, 21.99; FAB-HRMS for C73H76N9O10 m/z [M+H]+, calcd.: 1238.57152, found: 1238.57192.

Glycyl-L-asparticglycylglycine rotaxane 3.23

Rotaxane (3.18, 0.021 g, 0.015 mmol) and KOH (0.001 g, 0.015 mmol) was dissolved in 3 mL of MeOH. The reaction mixture was stirred at RT during 3 hours. The solvent was removed under reduced pressure to afford a white powder.

Yield (0.027 g, 100%); 1H NMR (400 MHz, 323K, CDCl3): δ = 8.13 (s, 2H, isophthaloyl 2-H), 8.01 (d, 4H, J = 7.4 Hz, isophthaloyl 4-H & 6-H), 7.60 (br t, 4H, macrocyclic CONH), 7.47 (t, 2H, J = 7.4 Hz, isophthaloyl 5-H), 7.36-7.09 (m, 20 H, thread Ar-H), 7.01 (s, 8H, macrocyclic para-xylylene Ar-H), 6.96 & 6.92 & 6.53 & 6.40 & 5.98 (br s, 5H, CONH), 4.97 (s, 1H, Ph2CHCO), 4.57-4.12 (m, 9H, macrocyclic NHCH2 & Asp NHCH(CH2CO2K)CO), 4.02-3.92 (m, 1H, CONHCH2CHPh2), 3.84-3.75 (m, 2H, CONHCH2CHPh2), 3.62-3.48 (m, 2H, Gly Ph2CHCONHCH2CO), 3.21 (br dd, 1H, Gly NHCHH’CO), 2.98 (br dd, 1H, Gly NHCHH’CO), 2.78 (br dd, 1H, Asp NHCH(CHH’CO2K)CO), 2.68-2.59 (m, 2H, Gly NHCH2CO), 2.45 (br dd, 1H, Asp NHCH(CHH’CO2K)CO), 2.68-2.59 (m, 2H, Gly NHCH2CO), 2.45 (br dd, 1H, Asp

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NHCH(CHH'CO₂K)CO; FAB-HRMS for C₇₀H₆₇N₉O₁₁ m/z [M+H+K⁺], calcd.: 1248.45971, found: 1248.45931.

**General procedure for the synthesis of oligopeptide [3]rotaxanes in solution**

**Following the experimental procedure leading to oligopeptide rotaxanes 3.14-3.18**

**Fmoc-glycyl-L-(O-(tert-butyldiphenylsilyl))-serylglycylglycine rotaxane 3.24**

Yield (0.034 g, 86%); ¹H NMR (400 MHz, CDCl₃): δ = 8.24 (s, 2H, isophthaloyl 2-H), 8.08 (br t, 2H, macrocyclic CONH), 8.03 (d, 4H, J = 7.6 Hz, isophthaloyl 4-H & 6-H), 7.93 (br t, 2H, macrocyclic CONH'), 7.74 (d, 2H, J = 4.8 Hz, aromatic Ar-H Fmoc), 7.61 (d, 2H, J = 6.8 Hz, aromatic Ar-H Fmoc), 7.55-6.94 (m, 37H, aromatic Ar-H & aromatic Ar-H Fmoc & isophthaloyl 5-H & macrocyclic para-xylene Ar-H & CONH), 6.55 (br t, 1H, CONBCH₂CO), 4.63-4.52 (m, 5H, macrocyclic NHCHH' & OCONHCH₂CO), 4.39-4.27 (m, 6H, macrocyclic NHCHH' & Fmoc CHCH₂OCONH), 4.21-4.19 (m, 1H, Ser NHCH(CH₃(OTBDPS))CO), 4.17 (t, 1H, J = 7.6 Hz, Fmoc CHCH₂OCONH), 3.99 (t, 1H, J = 8.8 Hz, CONHCH₂CHPh₂), 3.93 (dd, J = 2.8 Hz, J = 10.2 Hz, Ser NHCH(CHH'(OTBDPS))CO), 3.74 (dd, J = 7.2 Hz, J = 17.2 Hz, Gly NHCHH'CO), 3.57 (dd, J = 5.2 Hz, J = 10.2 Hz, Ser NHCH(CHH'(OTBDPS))CO), 3.54-3.33 (m, 5H, Gly NHCHH'CO & Gly NHCHH'CO & Gly NHCHH'CO & CONHCH₂CHPh₂), 2.73 (dd, 1H, J = 7.2 Hz, J = 18.0 Hz, Gly NHCHH'CO), 2.11 (dd, 1H, J = 7.2 Hz, J = 18.0 Hz, Gly NHCHH'CO), 0.96 (s, 9H, OSiC(CH₃)₃); ¹³C NMR (100 MHz, CDCl₃): δ = 170.51, 169.19, 167.96, 167.55, 167.38, 157.45, 143.68, 143.58, 141.82, 141.70, 141.26, 141.22, 137.20, 136.99, 135.41, 135.30, 134.65.
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134.27, 132.42, 132.02, 130.85, 130.55, 130.13, 129.08, 128.81, 128.66, 128.60,
127.97, 127.88, 127.85, 127.80, 127.16, 127.13, 126.82, 126.70, 126.19, 125.13,
120.00, 67.54, 63.18, 54.22, 50.34, 46.96, 44.88, 44.67, 44.48, 44.03, 42.82, 41.30,
26.71; FAB-HRMS for C_{86}H_{86}N_{9}O_{11}Si m/z [M+H]^+. calcd.: 1448.62161, found:
1448.62124.

Fmoc-glycylglycyl-L-(O-(tert-butyldiphenylsilyl))-serylglycylglycine rotaxane 3.25

Yield (0.032 g, 78%); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta = 8.20\) (s, 2H, isophthaloyl 2-H),
7.98 (d, 4H, \(J = 7.6\) Hz, isophthaloyl 4-H & 6-H), 7.92-7.86 (m, 4H, macrocyclic
CONH), 7.72 (d, 2H, \(J = 7.2\) Hz, aromatic Ar-H Fmoc), 7.58-6.94 (m, 39H, aromatic
Ar-H & aromatic Ar-H Fmoc & isophthaloyl 5-H & macrocyclic para-xylylene Ar-H &
CONH), 6.86 (d, 1H, \(J = 6.8\) Hz, Ser CONHCH(CH\(_2\)(OTBDPS))CO), 6.13 (br t, 1H,
CONHCH\(_2\)CO), 4.59-4.50 (m, 5H, macrocyclic NHCH\(_2\) & OCONHCH\(_2\)CO), 4.43-
4.37 (m, 1H, Ser NHCH(CH\(_2\)(OTBDPS))CO), 4.35-4.22 (m, 4H, macrocyclic
NHCH\(_2\)H\(_2\) ), 4.15 (t, 1H, \(J = 6.8\) Hz, Fmoc CHCH\(_2\)OCONH), 3.98 (t, 1H, \(J = 7.6\)Hz,
CONHCH\(_2\)CHPh\(_2\)), 3.94-3.87 (m, 2H, Gly NHCH\(_2\)CO & Gly NHCH\(_2\)H\(_2\)CO), 3.75 (dd,
1H, \(J = 4.8\) Hz, \(J = 17.2\) Hz, Gly NHCH\(_2\)H\(_2\)CO), 3.69-3.62 (m, 1H, Ser
NHCH(CH\(_2\)(OTBDPS))CO), 3.56-3.47 (m, 2H, CONHCH\(_2\)CHPh\(_2\)), 3.47-3.36 (m, 2H,
Ser NHCH(CH\(_2\)(OTBDPS))CO & Gly NHCH\(_2\)H\(_2\)CO), 3.35-3.27 (m, 2H, Gly
NHCH\(_2\)CO), 2.67 (dd, 1H, \(J = 5.2\) Hz, \(J = 16.8\) Hz, Gly NHCH\(_2\)H\(_2\)CO), 2.12 (dd, 1H, \(J =
5.2\) Hz, \(J = 16.8\) Hz, Gly NHCH\(_2\)H\(_2\)CO), 0.98 (s, 9H, OSiC(CH\(_3\))\(_3\); \(^{13}\)C NMR (100 MHz,
CDCl\(_3\)): \(\delta = 170.55, 170.46, 169.27, 168.64, 168.03, 167.60, 167.45, 156.79, 143.67,
141.74, 141.65, 141.24, 137.02, 136.97, 135.44, 135.32, 134.58, 134.34, 132.60,
132.25, 130.73, 130.54, 130.11, 129.00, 128.84, 128.65, 128.60, 127.92, 127.86,
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127.83, 127.76, 127.12, 127.07, 126.82, 126.73, 126.27, 125.07, 120.00, 80.03, 67.23, 63.46, 54.64, 50.34, 47.01, 44.63, 44.57, 44.44, 44.04, 43.12, 42.81, 41.32, 26.74;
FAB-HRMS for C_{88}H_{89}N_{10}O_{12}Si m/z [M+H'], calcld.: 1505.64307, found: 1505.64504.

Following the general procedure for the N-Fmoc deprotection

Glycyl-L-(O-(tert-butyldiphenylsilyl))-serylglycylglycine rotaxane 3.26

Yield (0.012 g, 36%); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta = 8.26\) (s, 2H, isophthaloyl 2-H), 8.05 (d, 4H, \(J = 9.2\) Hz, isophthaloyl 4-H & 6-H), 7.86-7.75 (m, 4H, macrocyclic CONH), 7.63-7.09 (m, 24H, aromatic Ar-H & isophthaloyl 5-H & CONH), 7.04 (s, 8H, macrocyclic \(\text{para-xylene Ar-H}\)), 6.90-6.78 (m, 2H, CONH), 4.64 (dd, 2H, \(J = 6.4\) Hz, \(J = 14.4\) Hz, macrocyclic NHCHH'), 4.55 (dd, 2H, \(J = 5.2\) Hz, \(J = 13.6\) Hz, macrocyclic NHCHH'), 4.31 (dd, 2H, \(J = 4.0\) Hz, \(J = 14.4\) Hz, macrocyclic NHCHH'), 4.22-4.15 (m, 3H, macrocyclic NHCHH' & Ser NHCH(CH\(_2\)OTBDPS)CO), 4.02 (t, 1H, \(J = 8.0\) Hz, CONHCH\(_2\)CHPh\(_2\)), 3.94 (dd, \(J = 4.4\) Hz, \(J = 10.0\) Hz, Ser NHCH(CH\(_2\)OTBDPS)CO), 3.66 (dd, \(J = 4.8\) Hz, \(J = 10.0\) Hz, Ser NHCH(CH\(_2\)OTBDPS)CO), 3.62-3.57 (m, 2H, CONHCH\(_2\)CHPh\(_2\)), 3.10 (dd, 1H, \(J = 6.0\) Hz, \(J = 17.0\) Hz, Gly NHCHH'CO), 3.08 (d, 2H, \(J = 8.8\) Hz, Gly NHCH\(_3\)CO), 2.99 (dd, 1H, \(J = 6.0\) Hz, \(J = 17.0\) Hz, Gly NHCHH'CO), 2.85 (dd, 1H, \(J = 4.4\) Hz, \(J = 16.4\) Hz, Gly NHCHH'CO), 2.71 (dd, 1H, \(J = 4.0\) Hz, \(J = 16.4\) Hz, Gly NHCHH'CO), 1.86-1.62 (m, 2H, NH\(_2\)CH\(_2\)CO), 1.01 (s, 9H, OSi(C\(_3\)F\(_3\))\(_3\)); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta = 170.86, 169.02, 167.87, 167.13, 167.07, 141.45, 141.54, 137.27, 137.07, 135.43, 135.42, 134.29, 133.92, 132.46, 132.38, 131.17, 131.05, 130.14, 130.11, 129.08, 128.93, 128.72, 128.25, 127.92, 127.85, 126.90, 125.25, 80.14, 62.75, 53.98, 50.31,
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45.82, 44.36, 44.27, 44.06, 42.71, 41.91, 26.75; FAB-HRMS for C\textsubscript{71}H\textsubscript{76}N\textsubscript{9}O\textsubscript{9}Si \textit{m/z} [M+H\textsuperscript{+}], calcd.: 1226.55353, found: 1226.55231.

**Glycylglycyl-1-(O-(tert-butyldiphenylsilyl))-serylglycylglycine rotaxane 3.27**

Yield (0.023 g, 87%); \textsuperscript{1}H NMR (400 MHz, 323K, C\textsubscript{2}D\textsubscript{2}Cl\textsubscript{4}): \( \delta = 8.22 \) (s, 2H, isophthaloyl 2-H), 7.97 (d, 4H, \( J = 6.8 \) Hz, isophthaloyl 4-H & 6-H), 7.63 (br t, 4H, macrocyclic CONH), 7.51-7.04 (m, 24H, aromatic Ar-H & isophthaloyl 5-H & CONH), 6.96 (s, 8H, macrocyclic para-xylene Ar-H), 6.75 & 6.56 & 6.37 (br s, 3H, CONH), 4.5-4.37 (m, 4H, macrocyclic NHCHH\textsuperscript{t} & CONHCH\textsubscript{2}CHPh\textsubscript{2} & Gly NHCHH\textsuperscript{t}CO), 3.94-3.81 (m, 2H, Ser NHCH(CH\textsubscript{3})(OTBDPS))CO & Gly NHCHH\textsuperscript{t}CO), 3.66-3.53 (m, 2H, CONHCH\textsubscript{2}CHPh\textsubscript{2} & Ser NHCH(CH\textsubscript{3})(OTBDPS))CO), 3.48-3.39 (m, 1H, Ser NHCH(CH\textsubscript{3})(OTBDPS))CO), 3.37-3.29 (m, 2H, Gly NHCHH\textsuperscript{t}CO & Gly NHCHH\textsuperscript{t}CO), 3.24-3.10 (m, 2H, Gly NHCHH\textsuperscript{t}CO & Gly NHCHH\textsuperscript{t}CO), 2.55-2.35 (m, 1H, Gly NHCHH\textsuperscript{t}CO), 2.22-2.01 (m, 1H, Gly NHCHH\textsuperscript{t}CO), 1.28-1.11 (br t, 2H, Gly NH\textsubscript{2}CH\textsubscript{2}CO), 0.92 (s, 9H, OSiC(CH\textsubscript{3})\textsubscript{3}); \textsuperscript{13}C NMR (100 MHz, C\textsubscript{2}D\textsubscript{2}Cl\textsubscript{4}): \( \delta = 173.25, 171.14, 169.98, 167.85, 167.63, 168.27, 167.08, 144.94, 144.83, 144.15, 142.22, 140.91, 139.50, 137.49, 136.40, 135.89, 135.78, 133.99, 133.13, 132.82, 131.56, 130.74, 129.84, 129.66, 129.58, 129.57, 129.38, 129.28, 128.53, 128.48, 128.36, 68.75, 54.82, 50.62, 45.06, 44.99, 44.97, 44.94, 44.91, 44.88, 44.83, 27.22; FAB-HRMS for C\textsubscript{73}H\textsubscript{79}N\textsubscript{10}O\textsubscript{10}Si \textit{m/z} [M+H\textsuperscript{+}], calcd.: 1283.57499, found: 1283.57406.
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General procedure for the coupling of [2]rotaxanes 3.13, 3.26, 3.27 with the activated ester rotaxane 3.28

The [2]rotaxane 3.13, 3.26 or 3.27 (0.010 mmol) and the activated rotaxane 3.28 (0.010 mmol) were dissolved in 10 mL of CHCl₃. The reaction mixture was then heated at reflux for seven days and monitored by TLC (CHCl₃/MeOH: 90/10). The solvent was removed under reduced pressure. The crude material was directly purified via reverse phase preparative HPLC (gradient H₂O/MeOH: 95/5) using a Luna 5u C₁₈(2) 100A column (250 mm x 2.00 mm, 5 µm) purchased from Phenomenex.


Yield (0.010 g, 50%); ¹H NMR (400 MHz, 363K, C₂D₂Cl₄): δ = 8.16 (s, 4H, isophthaloyl 2-H & isophthaloyl 2-H'), 8.06 (d, 4H, J = 7.6 Hz, isophthaloyl 4-H & 6-H), 7.89 (d, 4H, J = 7.2 Hz, isophthaloyl 4-H' & 6-H'), 7.50-7.03 (m, 42H, aromatic Ar-H & isophthaloyl 5-H & 5-H', macrocyclic CONH & CONH'), 6.94 & 6.93 (2s, 16H, macrocyclic para-xylene Ar-H & Ar-H'), 6.81 & 6.49 & 6.43 & 6.27 & 6.21 & 6.08 & 5.69 (7 br s, 7H, macrocyclic NHCHH' & macrocyclic NHCHH' & Ph₂CHCO & Ser NHCH(CH₂(O-TBDPS))CO & CONHCH₂CHPh₃), 3.90-3.82 (m, 1H, Gly NHCHH'CO), 3.79-3.73 (m, 1H, CONHCHH'CHPh₂), 3.58-3.51 (m, 1H, CONHCHH'CHPh₂), 3.49-3.40 (m, 1H, Gly NHCHH'CO), 3.34-2.95 (m, 8H, Gly NHCH₂CO & Gly NHCH₂CO & Gly NHCH₂CO & Gly NHCH₂CO), 2.57 (br dd, 1H, Ser NHCH(CHH'(O-TBDPS))CO), 2.34 (br dd, 1H, Ser NHCH(CHH'(O-TBDPS))CO), 0.91 (s, 9H, OSiC(CH₃)₃); ¹³C NMR (100 MHz, C₂D₂Cl₄): δ = 172.95, 170.87, 170.79, 108
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170.71, 170.55, 170.32, 69.70, 169.61, 168.60, 142.14, 140.59, 139.01, 137.84, 137.39, 135.90, 135.87, 135.70, 135.07, 134.64, 133.15, 132.99, 132.73, 132.42, 131.97, 131.15, 130.77, 130.71, 130.66, 130.30, 129.77, 129.66, 129.28, 129.17, 129.04, 128.53, 128.46, 128.41, 128.30, 128.25, 128.16, 128.14, 127.79, 127.41, 127.30, 81.03, 63.77, 55.12, 54.55, 49.77, 45.20, 45.19, 45.14, 44.64, 44.62, 44.60, 44.53, 44.39, 44.35, 44.32, 27.24; FAB-HRMS for C_{121}H_{120}N_{15}O_{16}Si m/z [M+H^+] , calcd.: 2066.88068, found: 2066.87980.

Glycylglycylglycylglycyl-1-(O-(tert-butylphenylsilyl))-serylglycylglycine

[3]rotaxane 3.31

Yield (0.017 g, 50%); $^1$H NMR (400 MHz, 373K, C$_2$D$_2$Cl$_4$): $\delta = 8.14-8.07$ (m, 4H, isophthaloyl 2-H & 2-H'), 8.00-7.87 (m, 8H, isophthaloyl 4-H & 6-H & 4-H' & 6-H'), 7.38-7.03 (m, 42H, isophthaloyl 5-H & 5-H' & macrocyclic CONH &CONH', aromatic Ar-H), 6.90 & 6.85 (2s, 16H, macrocyclic para-xylene Ar-H & Ar-H'), 6.71 & 6.52 & 6.39 & 6.31 & 6.29 & 6.09 & 5.99 (8 br s, 7H, CONH), 4.70-4.54 (m, 1H, Ser NHCH(CH(OTBDPS))CO), 4.41-4.18 (m, 18H, macrocyclic NHCHH' & macrocyclic NHCHH' & Ph$_2$CHO & CONHCH$_2$CHPh$_2$), 3.73 (s, 2H, Gly NHCH$_2$CO), 3.41-3.18 (m, 12H, CONHCH$_2$CHPh$_2$ & Gly NHCH$_2$CO), 2.90-2.88 (m, 2H, Ser NHCH(CH(OTBDPS))CO), 1.15 (s, 9H, OSiC(CH$_3$)$_3$); $^{13}$C NMR (100 MHz, C$_2$D$_2$Cl$_4$): $\delta = 173.41, 171.73, 171.17, 170.23, 169.08, 166.34, 166.07, 165.91, 163.84, 163.61, 143.50, 142.15, 136.53, 136.48, 136.18, 135.75, 135.28, 135.08, 135.00, 134.94, 134.24, 132.96, 131.53, 131.42, 130.30, 129.94, 129.88, 129.86, 129.75, 129.41, 129.30, 129.22, 129.19, 129.17, 129.14, 128.89, 128.88, 128.86, 128.58, 128.43, 128.34, 128.07, 127.77, 127.71, 127.49, 127.44, 81.07, 52.07, 51.95, 51.37, 45.63,
Chapter Three

45.22, 44.94, 44.88, 44.05, 43.26, 42.52, 43.76, 43.42, 42.43, 42.00, 28.48; FAB-HRMS for C_{123}H_{123}N_{16}O_{17}Si m/z [M+H'], calcd: 2124.90842, found: 2124.91145.
3.5. References


(14) Rosengren, K. J.; Clark, R. J.; Daly, N. L.; Göransson, U.; Jones, A.; Craik, D. J. *J. Am. Chem. Soc.* 2003, 125, 12464-12474.


Abstract: The synthesis of an N-Fmoc protected tripeptide L-SerGlyGly rotaxane building block, functionalized on the lateral chain by an ether bulky stopper, is described. Its sequential extension on the C- and N-terminal sides was carried out to easily give oligopeptide rotaxanes in very good yields. The method was then applied to the synthesis of the first unprotected peptide rotaxane with free amine and carboxylic acid terminal functions. Such peptide rotaxane building blocks could be utilised to make large libraries of interlocked peptides without problems of purification and solubility.

4.1. Introduction

The synthesis of dipeptide rotaxanes incorporating benzylic 1,3-carbamide macrocycles, using a five-component hydrogen bond-directed assembly has been recently reported. However, such an approach is restricted to short peptide sequences due to the folding of the backbone by intramolecular hydrogen-bonding in the longer peptides.

Methods to overcome this problem and synthesise oligopeptide rotaxanes in very good yields have been developed recently: (i) the synthesis of versatile activated ester rotaxane building blocks which can be elongated via their C-terminal side and (ii) the synthesis of N-protected tripeptide rotaxane building blocks, functionalized on the lateral chain by labile bulky stoppers and extendable on their N-terminal side via classical peptide coupling reactions after N-deprotection. A combined strategy we describe herein is the synthesis of a new N-Fmoc protected L-SerGlyGly tripeptide rotaxane building block which can be both C- and N-extended via classical peptide coupling reactions. The choice of the N-Fmoc protected L-SerGlyGly activated ester sequence as a tripeptide was five-fold: (i) it contains the most efficient GlyGly dipeptide template, (ii) L-SerGlyGly was found to be a more efficient template than L-AspGlyGly as the macrocycle experienced a fast shuttling along the peptide backbone after coupling and lateral chain deprotection of the prior, (iii) a labile ether on the lateral chain of the L-Ser residue acts as a labile bulky stopper for the macrocycle, (iv) 2,6-diphenyl-para-nitrophenyl acts both as a bulky stopper and an activated ester, (v) N-terminal Fmoc deprotection of an L-Ser residue promotes peptide coupling reactions to give oligopeptide rotaxanes, followed by further deprotection of the labile bulky stopper.
4.2. Results and discussion

Synthesis of the tripeptide activated ester rotaxane building block

The N-Fmoc-L-SerGlyGly tripeptide activated ester thread 4.6 was prepared in a five step sequence by classical peptide coupling reaction (Scheme 4.1.). Rotaxane 4.7 was synthesised as described above, purified via silica gel chromatography and could be stored for many weeks at room temperature with no degradation.

Scheme 4.1. Synthesis of the two-side-extension tripeptide rotaxane building block 4.7. a) HOCH₂Ph, DMAP, EDCI.HCl, CHCl₃, 100%; b) (i) TFA, CHCl₃, (ii) DIPEA, CHCl₃, (iii) N-Fmoc-L-Ser-OH, DMAP, EDCI.HCl, CHCl₃, 57%; c) (i) TBDPSCl, imidazole, DMF, 73%, (ii) H₂, Pd/C, P atm, EtOAc, 100%, (iii) 2,6-diphenyl para-nitrophenol, BOP, Et₃N, CHCl₃, 53%; c) isophthaloyl dichloride, para-xylene diamine, Et₃N, CHCl₃, 14% (26% overall yield after 8 cycles of rotaxane formation).

The ¹H NMR spectra of the N-Fmoc-L-SerGlyGly activated ester thread 4.6 and its corresponding rotaxane 4.7 in CDCl₃ are reported in Figure 4.1. In spectrum b, the
asymmetry of the thread stoppers leads to two different dd signals for the enantiotopic benzylic hydrogens $H_E$ of the macrocycle. A shielding effect on the hydrogens of the thread located in the cavity of the macrocycle is observed. Thus in spectrum b, the signals for $H_4$ and $H_5$ of the rotaxane building block 4.7 are shifted upfield by $\delta = 0.54$ and 1.31 ppm, respectively, compared with the corresponding signals in the spectrum of the thread 4.6 (spectrum a), indicating the position of the macrocycle around these protons.

**Figure 4.1.** Comparison of the $^1$H NMR spectra (400 MHz, CDCl$_3$) of a) the N-Fmoc protected activated tripeptide L-SerGlyGly activated thread 4.6 and b) the N-Fmoc protected activated tripeptide L-SerGlyGly rotaxane building block 4.7.
Extensive oligopeptide synthesis via the two-side extension promoter

In a typical procedure, a mixture of the rotaxane building block 4.7 and five equivalents of a mono- or tri-peptide as a free amine moiety was heated at reflux in chloroform (Scheme 4.2.). The isolated resulting derivatives 4.8, 4.9 and 4.16 were then reacted following our present method. N-Fmoc deprotection using piperidine in chloroform, followed by further coupling with two equivalents of stoppered mono-, di- or tri-peptide using BOP and final lateral deprotection. It is noteworthy that the TBDPS deprotection using a 1M TBAF solution in THF was unsuccessful for the octa- and nonapeptide rotaxanes 4.15 and 4.19 respectively and required the use of pure TFA.

Scheme 4.2. Synthesis of oligopeptide rotaxanes 4.14-4.15 by elongation and coupling of the two-side-extension rotaxane promoter 4.7. a) Stoppered amino acid (n=1, 3), CHCl₃ reflux; b) (i) piperidine/CHCl₃: 1/3, (ii) stoppered amino acid (n'=1-3), BOP, Et₃N, CHCl₃; c) TBAF 1M in THF for 4.12 and TFA for 4.13.
The yields of oligopeptide rotaxanes were excellent as reported in table 4.1.

<table>
<thead>
<tr>
<th>Peptide rotaxane sequence</th>
<th>Yield % C-elongation</th>
<th>Yield % N-elongation</th>
</tr>
</thead>
<tbody>
<tr>
<td>TFA-H-L-Leu-L-PheGly-L-SerGlyGly-Gly-L-Phe-L-LeuOH 4.18/4.19</td>
<td>78/4.16</td>
<td>84/4.18</td>
</tr>
</tbody>
</table>

Table 4.1. Synthesis of oligopeptide rotaxanes from the two-side-extension rotaxane promoter 4.7.

It is noteworthy that rotaxane 4.19 is the first example ever reported of an unprotected peptide rotaxane with free amine and carboxylic acid functions on the N- and C-terminal sides of the peptide backbone, respectively (Scheme 4.3.). The successive lateral chains of the Leu and Phe residues are bulky enough to act as stoppers for the macrocycle.⁴

![Scheme 4.3. Nonapeptide rotaxane 4.19, stoppered by the lateral chains of the last two amino acid residues.](image)

Influence of the macrocycle on the nonapeptide conformation in solution in CD₃OH is under investigation by the biomolecular NMR group of Dr. D. Uhrin at the University of Edinburgh.
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The double extension of the rotaxane building block 4.7 is a key tool for the easy synthesis of libraries of unprotected oligopeptide rotaxanes (i) in very good yields, (ii) able to be purified via silica gel chromatography and (iii) without solubility problems in organic solvents. Studies into its incorporation in glucuronylated prodrugs for ADEPT strategy in cancer chemotherapy are ongoing, in collaboration with the Gesson group at the University of Poitiers - France.
4.3. Conclusion

We have described the synthesis of an N-Fmoc protected tripeptide L-SerGlyGly activated ester rotaxane building block functionalized on its lateral chain by a labile bulky stopper. It can be stored many weeks at room temperature with no degradation. It has been efficiently used for the synthesis of oligopeptide rotaxanes, in very good yields, by successive extension on its C- and N-terminal sides. Such a peptide rotaxane building block is potentially a very convenient tool for building wide libraries of peptide interlocked architectures as it reacts both with nucleophiles and electrophiles. Investigations towards its loading on solid support as a method for synthesising large libraries of polyrotaxanes are currently ongoing in our group.
4.4. Experimental section

Method for the preparation of the thread 4.6

N-terbutoxycarbonyl-glycylglycine benzyl ester 4.2

Boc-glycylglycine (4.1, 5.228 g, 22.500 mmol), benzylalcohol (2.5 mL, 24.200 mmol) and DMAP (8.258 g, 67.590 mmol) were dissolved in 100 mL of CHCl₃. The reaction mixture was cooled at 0°C. Then EDCI.HCl (6.477 g, 33.790 mmol) was added. The reaction mixture was stirred one hour at 0°C and brought to room temperature and stirred overnight. The crude material was washed with 1M HCl and saturated NaHCO₃. The organic phase was dried over MgSO₄. The solvent was removed under reduced pressure to afford a white powder. Yield (7.257 g, 100%); m.p. 74°C; ¹H NMR (400 MHz, CDCl₃): δ = 7.40-7.33 (m, 5H, aromatic Ar-H), 6.57 (br t, 1H, CONH₂CO), 5.19 (s, 2H, CO₂CH₂Ph), 5.10 (br s, 1H, Boc-CONHCH₂CO), 4.11 (d, 2H, J = 5.2 Hz, Gly NH₂CO), 3.85 (d, 2H, J = 5.2 Hz, Gly NH₂CO), 1.46 (s, 9H, Boc CH₃); ¹³C NMR (100 MHz, CDCl₃): δ = 169.84, 169.63, 156.09, 135.07, 128.66, 128.58, 128.38, 80.39, 67.28, 44.17, 41.23, 28.29; FAB-HRMS for C₁₆H₂₅N₂O₅ m/z [M+H⁺], calcd.: 323.16070, found: 323.16069.

N-α-Fmoc-L-serylglycylglycine benzyl ester 4.3

TFA was added to a solution of N-terbutoxycarbonyl-glycylglycine benzyl ester (4.2, 4.571 g, 14.180 mmol) in CHCl₃. The reaction was stirred for 4 hours and then concentrated under reduced pressure. To a solution of the residue in CHCl₃ was added DIPEA (2.427 mL, 14.180 mmol), followed by addition of N-α-Fmoc-L-serine (4.641 g, 14.180 mmol) and DMAP (0.677 g, 5.540 mmol) in 100 mL of CHCl₃. The reaction
mixture was cooled at 0°C. Then EDCI·HCl (4.083 g, 14.180 mmol) was added. The reaction mixture was stirred one hour at 0°C and brought to room temperature and stirred overnight. The crude material was washed with 1M HCl and saturated NaHCO₃. The organic phase was dried over MgSO₄. The solvent was removed under reduced pressure. The purification via silica gel chromatography (CHCl₃/MeOH: 98/2 to 92/8) afforded a white powder. Yield (4.309 g, 57%); m.p. 132°C; ¹H NMR (400 MHz, CDCl₃): δ = 7.73 (d, 2H, J = 7.6 Hz, aromatic Ar-H Fmoc), 7.57-7.24 (m, 13H, aromatic Ar-H & aromatic Ar-H Fmoc & CONH), 6.12 (d, 1H, J = 6.4 Hz, Ser OCONHCH₂OH), 5.12 (s, 2H, CO₂CH₂Ph), 4.38 (d, 2H, J = 7.0 Hz, Fmoc CHCH₂CONH), 4.33-4.26 (m, 1H, Ser NHCH₂OH), 4.16 (t, 1H, J = 7.0 Hz, Fmoc CHCH₂CONH), 4.05-3.91 (m, 5H, Ser NHCH₂OHCO & Gly NHCH₂CO & Gly NHCH₂CO), 3.70-3.63 (m, 1H, Ser NHCH₂OH), 2.04-1.93 (m, 1H, Ser NHCH₂OHCO); ¹³C NMR (100 MHz, CDCl₃): δ = 170.95, 170.12, 169.53, 158.00, 143.62, 141.29, 134.85, 128.66, 128.63, 128.35, 127.81, 127.13, 125.04, 120.05, 67.45, 67.24, 62.92, 56.33, 47.04, 43.05, 41.25; FAB-HRMS for C₂₉H₂₉N₃O₇Na m/z [M+Na⁺], calcd.: 554.19035, found: 554.19050.

**N-α-Fmoc-L-(O-(tert-butyldiphenylsilyl))-serylglycylglycine benzyl ester 4.4**

N-α-Fmoc-L-seryl glycylglycine benzyl ester (4.3, 1.890 g, 3.550 mmol) and imidazole (0.609 g, 8.950 mmol) were dissolved in dry DMF (5 mL) under nitrogen. TBDPSCI (4.5 mL, 17.580 mmol) was added and the solution was stirred 24 hours at rt. The solvent was removed under reduced pressure. The purification via silica gel chromatography (CHCl₃/MeOH: 98/2) afforded a white powder. Yield (2.060 g, 73%); m.p. 155°C; ¹H NMR (400 MHz, CDCl₃): δ = 7.76 (d, 2H, aromatic Ar-H Fmoc, J = 7.2 Hz), 7.64-7.23
(m, 2H, aromatic Ar-H & aromatic Ar-H Fmoc), 7.05 (br t, 1H, CONHCH₂CO), 6.97 (br t, 1H, CONHCH₂CO), 5.63 (d, 1H, J = 6.0 Hz, Ser OCONHCH(CH₂(OTBDPS))CO), 5.11 (s, 2H, CO₂CH₂Ph), 4.46-4.35 (m, 2H, Fmoc CHCH₂OCONH), 4.31-4.25 (m, 1H, Ser NHCH(CH₂(OTBDPS))CO), 4.19 (t, 1H, J = 6.8 Hz, Fmoc CHCH₂OCONH), 4.09-3.93 (m, 5H, Ser NHCH(CHH'(OTBDPS))CO & Gly NHCH₂CO & Gly NHCH₂CO), 3.84 (dd, 1H, J = 5.2 Hz, J = 10.4 Hz, Ser NHCH(CHH'(OTBDPS))CO), 1.05 (s, 9H, OSiC(C₁₃)₃); ¹³C NMR (100 MHz, CDCl₃): δ = 169.15, 169.03, 168.24, 157.95, 141.33, 135.48, 135.45, 134.80, 130.14, 128.64, 128.55, 128.37, 127.98, 127.79, 127.12, 125.08, 124.96, 120.05, 80.03, 68.01, 67.41, 67.20, 57.12, 56.15, 43.11, 41.18, 26.80; FAB-HRMS for C₄₅H₄₈N₃O₇Si m/z [M+H⁺], calcd.: 770.32616, found: 770.32591.

N-α-Fmoc-L-(O-(tert-butyldiphenylsilyl))-serylglycylglycine 4.5

N-α-Fmoc-L-(O-(tert-butyldiphenylsilyl))-serylglycylglycine benzyl ester (4.4, 2.338 g, 3.037 mmol) and catalytic palladium, 10% wt. on carbon powder were dissolved in 80 mL of AcOEt. The system was then placed under vacuum and hydrogen was introduced at atmospheric pressure for four hours. The reaction mixture was filtered over celite. The solvent was removed under reduced pressure to afford a white powder. Yield (2.065 g, 100%); m.p. 81°C; ¹H NMR (400 MHz, CDCl₃): δ = 7.73 (d, 2H, aromatic Ar-H Fmoc, J = 6.8 Hz), 7.58 (d, 2H, aromatic Ar-H Fmoc, J = 6.8 Hz), 7.42-7.24 (m, 15H, aromatic Ar-H & aromatic Ar-H Fmoc & CONH), 7.15 (br t, 1H, CONHCH₂CO), 5.71 (d, 1H, J = 6.4 Hz, Ser OCONHCH(CH₂(OTBDPS))CO), 4.39-4.31 (m, 3H, Fmoc CHCH₂OCONH & Ser NHCH(CH₂(OTBDPS))CO), 4.19-4.11 (m, 2H, Fmoc CHCH₂OCONH & Ser NHCH(CHH'(OTBDPS))CO), 3.99 (brd, 2H, Gly NHCH₂CO),
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3.93 (brd, 2H, Gly NH\textsubscript{2}CO), 3.82 (dd, 1H, $J = 4.8$ Hz, $J = 9.6$ Hz, Ser NHCH\textsubscript{(CH\textsubscript{2})\textprime(OTBDPS)}CO), 1.02 (s, 9H, OSi(CH\textsubscript{3})\textsubscript{3}); $^{13}$C NMR (100 MHz, CDCl\textsubscript{3}):

$\delta = 172.05, 170.82, 169.51, 156.57, 143.67, 143.48, 141.24, 135.43, 134.79, 132.38,$

130.06, 129.64, 127.93, 127.91, 127.79, 127.70, 127.12, 126.90, 125.07, 125.01,

120.01, 119.83, 80.12, 67.43, 63.81, 56.58, 46.95, 42.90, 41.22, 26.73; FAB-HRMS for

C\textsubscript{38}H\textsubscript{42}N\textsubscript{3}O\textsubscript{7}Si $m/z$ [M+H'], calcd.: 680.27921, found: 680.27965.

**N-α-Fmoc-L-(O-(tert-butyldiphenylsilyl))-serylglycylglycine-4-nitro-2,6-diphenylphenylester 4.6**

N-α-Fmoc-L-(O-(tert-butyldiphenylsilyl))-serylglycylglycine (4.5, 2.065 g, 3.040 mmol), 4-nitro-2,6-diphenyl phenol (1.770 g, 6.080 mmol), DIPEA (0.530 mL, 3.040 mmol) and BOP (2.017 g, 4.560 mmol) were dissolved in 60 mL of CHCl\textsubscript{3}. The reaction mixture was stirred overnight at room temperature. The crude material was purified via silica gel chromatography (AcOEt/cyclohexane: 1/2 to 1/1) to afford a yellow powder.

Yield (1.534 g, 53%); m.p. 104°C; $^{1}$H NMR (400 MHz, CDCl\textsubscript{3}): $\delta = 8.24$ (s, 2H, NO\textsubscript{2}-Ph-H), 7.76 (d, 2H, aromatic Ar-H Fmoc, $J = 7.6$ Hz), 7.61-7.22 (m, 26H, aromatic Ar-H & aromatic Ar-H Fmoc & CONH), 6.57 (br t, 1H, CONHCH\textsubscript{2}CO), 6.44 (br t, 1H, CONHCH\textsubscript{2}CO), 5.37 (d, 1H, $J = 5.2$ Hz, Ser OCONHCH\textsubscript{(CH\textsubscript{2})\textprime(OTBDPS)}CO), 4.38 (dd, 1H, $J = 6.4$ Hz, $J = 10.4$ Hz, Fmoc CHCH\textsubscript{2}OCONH), 4.30 (dd, 1H, $J = 6.4$ Hz, $J = 10.4$ Hz, Fmoc CHCH\textsubscript{2}OCONH), 4.18-4.10 (m, 2H, Fmoc CHCH\textsubscript{2}OCONH & Ser NHCH\textsubscript{(CH\textsubscript{2})\textprime(OTBDPS)}CO), 4.00 (dd, 1H, $J = 4.8$ Hz, $J = 10.8$ Hz, Ser NHCH\textsubscript{(CH\textsubscript{2})\textprime(OTBDPS)}CO), 3.88 (dd, 1H, $J = 5.2$ Hz, $J = 18.8$ Hz, Gly NHCH\textsubscript{H'}CO), 3.82-3.73 (m, 3H, Gly NHCH\textsubscript{2}CO & Ser NHCH\textsubscript{(CH\textsubscript{2})\textprime(OTBDPS)}CO), 3.69 (dd, 1H, $J = 4.8$ Hz, $J = 18.8$ Hz, Gly NHCH\textsubscript{H'}CO), 1.03 (s, 9H, OSi(CH\textsubscript{3})\textsubscript{3}); $^{13}$C
NMR (100 MHz, CDCl₃): \( \delta = 171.12, 167.78, 166.94, 157.71, 149.25, 145.95, 143.62, 141.30, 137.37, 135.46, 135.42, 135.26, 130.17, 128.84, 128.78, 128.68, 128.00, 127.98, 127.85, 127.81, 127.09, 127.05, 124.99, 124.93, 124.88, 120.07, 80.73, 67.25, 63.35, 56.89, 47.05, 42.84, 40.85, 26.77; FAB-HRMS for \( \text{C}_{56}\text{H}_{53}\text{N}_4\text{O}_9\text{Si} \) m/z \([\text{M}+\text{H}]^+\), calcd.: 953.35818, found: 953.35770.

\text{N-\(\alpha\)-Fmoc-L-(O-(tert-butylidiphenylsilyl))-serylglycylglycine activated ester rotaxane 4.7}

\( \text{N-\(\alpha\)-Fmoc-L-(O-(tert-butylidiphenylsilyl))-serylglycylglycine-4-nitro-2,6-diphenylphenylester} \ (4.6, \ 0.495, \ 0.520 \text{ mmol}) \) was dissolved in 20 mL of CHCl₃ under nitrogen and stirred vigorously whilst solutions of the mixture of \( \text{para-xylylene diamine} \) (1.135 g, 8.350 mmol) and \( \text{NEt}_3 \) (1.685 g, 16.680 mmol) in 35 mL of CHCl₃ and the isophthaloyl dichloride (1.694 g, 8.340 mmol) in 35 mL of CHCl₃ were simultaneously added to the reaction mixture over a period of 3 hours using motor-driven syringe pumps. The reaction mixture was stirred 2 hours under nitrogen, then filtered over celite and the filtrate was evaporated under reduced pressure to afford a syrup which was purified via silica gel chromatography to yield, in order of elution, the unconsumed activated ester thread (AcOEt/cyclohexane: 1/1) and the rotaxane (CHCl₃/MeOH: 98/2). Yield (0.108 g, 14%); Overall yield after 8 cycles of rotaxane formation (0.546 g, 26%); m.p. 144°C; \( ^1\text{H NMR} \) (400 MHz, CDCl₃): \( \delta = 8.23 \) (s, 2H, NO₂-Ph-H), 8.07 (s, 2H, isophthaloyl 2-H), 8.03 (d, 2H, \( J = 7.8 \text{ Hz} \), isophthaloyl 4-H & 6-H), 7.96 (d, 2H, \( J = 7.8 \text{ Hz} \), isophthaloyl 4-H & 6-H), 7.77-7.17 (m, 27H, aromatic Ar-H & aromatic Ar-H Fmoc & macrocyclic CONH, isophthaloyl 5-H, thread CONH), 6.94 (d, 4H, \( J = 8.2 \text{ Hz} \), macrocyclic \( \text{para-xylylene Ar-H} \)), 6.91 (d, 4H, \( J = 8.2 \text{ Hz} \), macrocyclic \( \text{para-xylylene} \),
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Ar-\(H^+\), 6.48 (br s, 1H, CONHCH\(_2\)CO), 5.22 (d, 1H, \(J = 6.0\) Hz, Ser OCONHCH(CH\(_2\)(OTBDPS)CO), 4.65 (dd, 2H, \(J = 6.0\) Hz, \(J = 13.6\) Hz, macrocyclic NHCH\(_H\)\'), 4.33 (dd, 2H, \(J = 4.4\) Hz, \(J = 14.2\) Hz, macrocyclic NHCH\(_H\)\'), 4.24 (dd, 2H, \(J = 4.8\) Hz, \(J = 14.2\) Hz, macrocyclic NHCH\(_H\)\') & Fmoc CHCH\(_2\)OCONH), 3.93 (t, 1H, \(J = 6.8\) Hz, Fmoc CHCH\(_2\)OCONH), 3.75 (dd, 1H, \(J = 5.6\) Hz, \(J = 18.4\) Hz, Gly NHCH\(_H\)\'CO), 3.67-3.53 (m, 4H, Ser NHCH(CH\(_2\)(OTBDPS)CO & Gly NHCH\(_H\)\'CO & Gly NHCH\(_H\)CO), 2.90 (dd, 1H, \(J = 3.2\) Hz, \(J = 17.0\) Hz, Ser NHCH(CH\(_H\)'(OTBDPS)CO), 2.36 (dd, 1H, \(J = 4.0\) Hz, \(J = 17.0\) Hz, Ser NHCH(CH\(_H\)'(OTBDPS)CO), 0.99 (s, 9H, OSiC(CH\(_3\))\(_3\)); \(^1\)C NMR (100 MHz, CDCl\(_3\)): \(\delta = 169.20, 168.12, 167.02, 166.86, 166.20, 158.12, 149.09, 146.10, 141.26, 137.39, 137.34, 137.10, 133.92, 132.41, 132.24, 131.27, 131.17, 130.87, 130.18, 129.22, 128.96, 128.87, 128.79, 128.69, 128.56, 127.98, 127.89, 127.83, 127.11, 127.04, 125.15, 125.04, 124.94, 120.01, 81.02, 67.58, 62.39, 52.44, 46.97, 44.32, 44.12, 42.72, 40.68, 26.74; FAB-HRMS for C\(_{88}\)H\(_{81}\)N\(_8\)O\(_{13}\)Si m/z [M+H\(^+\)], calcd.: 1485.56924, found: 1485.56038.


The [2]rotaxane building block 4.7 (0.079 mmol) and a stoppered nucleophile (0.395 mmol) were dissolved in 6 mL of CHCl\(_3\). The reaction mixture was then heated at reflux and monitored by TLC (CHCl\(_3\)/MeOH: 90/10). The solvent was removed under reduced pressure. The crude material was directly purified via silica gel chromatography.
(CHCl₃/Methanol: from 98/2 to 90/10) to afford in order of elution the 2,6-diphenyl-para-nitrophenol, and the elongated rotaxane as a white powder.

* N-α-Fmoc-L-(O-(tert-butyldiphenylsilyl))-serylglycylglycylglycine rotaxane 4.8 

Yield (0.062 g, 93%); ¹H NMR (400 MHz, CDCl₃): δ = 8.21 (s, 2H, isophthaloyl 2-H), 8.01 (d, 4H, J = 7.6 Hz, isophthaloyl 4-H & 6-H), 7.73 (d, 2H, J = 7.6 Hz, aromatic Ar-H Fmoc), 7.67-7.59 (br t, 4H, macrocyclic CONH), 7.58-7.06 (m, 36 H, aromatic Ar-H & aromatic Ar-H Fmoc & isophthaloyl 5-H & macrocyclic para-xylene Ar-H), 7.04-6.98 (m, 2H, Gly CONHCH₂CO), 6.93 (br t, 1H, Gly CONHCH₂CO), 5.50 (d, 1H, J = 7.2 Hz, Ser OCONHCH(CH₂(OTBDPS))CO), 4.59 (dd, 2H, J = 6.0 Hz, J = 14.0 Hz, macrocyclic NHCHH'), 4.52-4.43 (m, 4H, macrocyclic NHCHH' & CO₂CH₂CHPh₂), 4.35 (dd, 2H, J = 4.8 Hz, J = 14.0 Hz, macrocyclic NHCHH'), 4.29-4.22 (m, 3H, macrocyclic NHCHH' & Fmoc CHCHH'OCONH), 4.21-4.14 (m, 2H, Fmoc CHCHH'OCONH & CO₂CH₂CHPh₂), 4.08-3.99 (m, 2H, Ser NHCH(CH₂(OTBDPS))CO & Fmoc CHCH₂OCONH), 3.78 (dd, 1H, J = 5.2 Hz, J = 10.2 Hz, Ser NHCH(CHH'(OTBDPS))CO), 3.69 (dd, 1H, J = 4.8 Hz, J = 10.2 Hz, Ser NHCH(CHH'(OTBDPS))CO), 3.29 (br d, 2H, Gly NHCH₃CO), 3.24 (dd, 1H, J = 5.2 Hz, J = 16.8 Hz, Gly NHCHH'CO), 3.18-3.11 (m, 2H, Gly NHCHH'CO & Gly NHCHH'CO), 3.04 (dd, 1H, J = 4.8 Hz, J = 16.0 Hz, Gly* NHCHH'CO), 0.99 (s, 9H, OSiC(CH₃)₃); ¹³C NMR (100 MHz, CDCl₃): δ = 170.70, 169.32, 169.27, 168.85, 167.05, 156.63, 143.56, 143.39, 141.26, 141.22, 140.81, 140.47, 137.38, 137.22, 135.41, 135.37, 134.28, 134.10, 132.44, 132.28, 131.12, 131.02, 130.15, 129.05, 128.98, 128.69, 128.62, 128.29, 128.13, 128.00, 127.97, 127.92, 127.80, 127.09,
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126.91, 124.98, 124.94, 120.02, 80.72, 67.64, 67.09, 63.04, 56.20, 49.55, 46.87, 44.35, 44.21, 42.52, 42.38, 40.90, 26.75; FAB-FIRMS for C_{86}H_{85}N_{8}O_{12}Si m/z [M+H], calcd.: 1449.60563, found: 1449.60571.

*N-a-Fmoc-L-(O-(tert-butyldiphenylsilyl))-serylglycylglycyl-L-alanylglycylglycine rotaxane 4.9*

Yield (0.068 g, 94%); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta = 8.27\) (s, 2H, isophthaloyl 2-H), 8.02 (d, 2H, \(J = 6.6\) Hz, isophthaloyl 4-H & 6-H), 8.00 (d, 2H, \(J = 6.6\) Hz, isophthaloyl 4-H & 6-H), 7.86 (br t, 2H, macrocyclic CONH), 7.75 (br t, 2H, macrocyclic CONH'), 7.72 (d, 2H, \(J = 7.6\) Hz, aromatic Ar-H Fmoc), 7.61-7.13 (m, 32 H, aromatic Ar-H & isophthaloyl 5-H & aromatic Ar-H Fmoc & CONH), 7.08 (s, 8H, macrocyclic para-xylene Ar-H), 6.96 (br s, 1H, CONHCH\(_2\)CO), 5.69 (d, 1H, \(J = 5.6\) Hz, Ser OCONHCH(CH\(_3\)(OTBDPS))CO), 4.62 (dd, 2H, \(J = 4.4\) Hz, \(J = 18.0\) Hz, macrocyclic NHCHH'), 4.49-4.40 (m, 4H, macrocyclic NHCHH' & CO\(_2\)CH\(_2\)CHPh\(_2\)), 4.39-4.31 (m, 4H, macrocyclic NHCHH' & Fmoc CHCH\(_2\)OCONH), 4.24 (dd, 2H, \(J = 6.8\) Hz, \(J = 18.0\) Hz, macrocyclic NHCHH'), 4.17-4.13 (m, 2H, Ser NHCH(CH\(_3\)(OTBDPS))CO & CO\(_2\)CH\(_2\)CHPh\(_2\)), 4.06 (t, 1H, \(J = 6.8\) Hz, Fmoc CHCH\(_2\)OCONH), 4.03-3.97 (m, 1H, Ala NHCH(CH\(_3\))CO), 3.92 (dd, 1H, \(J = 6.0\) Hz, \(J = 11.0\) Hz, Ser NHCH(CH\(_3\))CO), 3.83 (dd, 1H, \(J = 4.8\) Hz, \(J = 11.0\) Hz, Ser NHCH(CH\(_3\))CO), 3.53 (dd, 1H, \(J = 5.6\) Hz, \(J = 17.2\) Hz, Gly NHCHH'CO), 3.46-3.42 (m, 3H, Gly NHCH\(_2\)CO & Gly NHCHH'CO), 3.38 (dd, 1H, \(J = 4.4\) Hz, \(J = 14.0\) Hz, Gly NHCHH'CO), 3.29 (dd, 1H, \(J = 4.8\) Hz, \(J = 17.2\) Hz, Gly NHCHH'CO), 3.17 (br dd, 1H, \(J = 15.6\) Hz, Gly NHCHH'CO), 3.02 (br dd, 1H, \(J = 15.6\) Hz, Gly NHCHH'CO), 1.11 (d, 3H, \(J = 6.8\) Hz, Ala NHCH(CH\(_3\))CO), 1.02 (s, 9H,
\[ \text{OSiC(CH}_3\text{)}_3; \] \[ ^{13}C \text{ NMR (100 MHz, CDCl}_3): \delta = 172.58, 171.16, 169.55, 169.37, 169.14, 167.31, 167.16, 156.80, 143.53, 143.40, 141.26, 141.22, 140.65, 140.48, 137.39, 137.07, 135.44, 135.41, 134.33, 134.16, 132.47, 132.32, 131.13, 130.99, 130.15, 129.86, 129.06, 128.94, 128.67, 128.10, 127.98, 127.84, 127.81, 127.66, 127.09, 127.00, 126.94, 125.22, 124.94, 120.05, 79.85, 67.58, 67.49, 63.29, 56.89, 49.50, 48.94, 46.91, 44.35, 44.17, 43.06, 42.54, 40.91, 26.77, 16.92; \]

\[ \text{FAB-RRMS for } C_{91}H_{93}N_{10}O_{14}Si \text{ m/z [M+H}], \text{ calcd.: 1577.66420, found: 1577.65004.} \]

\( N\text{-a-Fmoc-L-}(O\text{-tert-butyldiphenylsilyl})\text{-serylglycylglycylglycyl-L-phenylalanyl-L-leucine-tert-butyl ester rotaxane} \)

\text{4.16} \]

Yield (0.074 g, 98%); \( ^1H \) NMR (400 MHz, CDCl\(_3\)): \( \delta = 8.27 \text{ (s, 1H, isophthaloyl 2-H)}, 8.22 \text{ (s, 1H, isophthaloyl 2-H)}, 8.02 \text{ (d, 2H, } J = 7.8 \text{ Hz, isophthaloyl 4-H & 6-H)}, 7.98 \text{ (d, 2H, } J = 7.8 \text{ Hz, isophthaloyl 4-H & 6-H)}, 7.86 \text{ (br t, 2H, macrocyclic CONH)}, 7.79 \text{ (br t, 2H, macrocyclic CONH'), 7.74-7.15 \text{ (m, 29 H, aromatic Ar-H & isophthaloyl 5-H & aromatic Ar-H Fmoc & CONH)}, 7.10 \text{ (s, 4H, macrocyclic } \text{para-xylene Ar-H)}, 7.09 \text{ (s, 4H, macrocyclic } \text{para-xylene Ar-H)}, 6.56 \text{ (br s, 1H, CONH)}, 5.58 \text{ (d, 1H, } J = 6.8 \text{ Hz, Ser OCONHCH(CH}_3\text{(OTBDPS))CO)}, 4.62-4.43 \text{ (m, 5H, macrocyclic NHCHH' & NHCHH' & Phe NHCH(CH}_3\text{Ph)CO)}, 4.38-4.21 \text{ (m, 7H, macrocyclic NHCHH' & NHCHH' & Fmoc CHCHH'OCONH & Fmoc CHCHH'OCONH & Leu NHCH(CH}_2\text{CH(CH}_3\text{)}_2\text{CO)}, 4.19-3.97 \text{ (m, 2H, Fmoc CHCH}_2\text{OCONH & Ser NHCH(CH}_3\text{(OTBDPS))CO), 3.80 \text{ (dd, 1H, } J = 5.6 \text{ Hz, } J = 10.2 \text{ Hz, Ser NHCH(CHH'(OTBDPS))CO), 3.75 \text{ (dd, 1H, } J = 5.2 \text{ Hz, } J = 10.2 \text{ Hz, Ser NHCH(CHH'(OTBDPS))CO), 3.25 \text{ (dd, 1H, } J = 4.8 \text{ Hz, } J = 16.8 \text{ Hz, Gly NHCHH'CO), 3.17-3.05 (m, 2H, Gly NHCHH'CO & Gly NHCHH'CO), 2.99 (dd, 1H, } J \)
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= 4.0 Hz, \( J = 16.8 \) Hz, Gly NHCHH\(^\prime\)CO), 2.95-2.89 (m, 2H, Gly NHCHH\(^\prime\)CO & Phe NHCH(CHOH\(^\prime\)Ph)CO), 2.84 (dd, 1H, \( J = 7.6 \) Hz, \( J = 13.6 \) Hz, Phe NHCH(CHOH\(^\prime\)Ph)CO), 2.60 (dd, 1H, \( J = 4.4 \) Hz, \( J = 17.2 \) Hz, Gly NHCHH\(^\prime\)CO), 1.50-1.33 (m, 12H, Leu NHCH(CH\(_3\)CH(CH\(_3\))\(_2\))CO & Leu NHCH(CH\(_2\)CH(CH\(_3\))\(_2\))CO & CO\(_2\)(CH\(_3\))\(_3\)), 1.00 (s, 9H, OSiC(CH\(_3\))\(_3\)), 0.81 (d, 3H, \( J = 5.8 \) Hz, Leu NHCH(CH\(_2\)CH(CH\(_3\))\(_2\))CO & CH\(_3\)CO), 0.78(d, 3H, \( J = 5.8 \) Hz, Leu NHCH(CH\(_2\)CH(CH\(_3\))\(_2\))CO & CH\(_3\)CO); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta = 171.56, 170.56, 170.32, 170.16, 169.17, 168.18, 167.28, 167.16, 143.57, 141.26, 137.29, 136.29, 136.14, 135.43, 134.42, 134.17, 132.51, 132.40, 131.08, 130.99, 130.12, 129.36, 129.19, 129.03, 128.92, 128.83, 128.70, 128.65, 128.21, 127.96, 127.81, 127.13, 127.08, 125.71, 125.41, 124.99, 120.02, 82.11, 82.01, 67.34, 63.17, 56.46, 54.77, 51.74, 46.91, 44.41, 44.26, 42.75, 42.24, 41.77, 41.35, 38.43, 27.97, 26.76, 24.80, 22.51; FAB-HRMS for \( C_{91}H_{101}N_{10}O_{14}Si \) m/z \([M+H]^+\), calcd.: 1585.72680, found: 1585.73695

General procedure for N-Fmoc deprotection of rotaxane derivates 4.8, 4.9, 4.16

N-Fmoc protected rotaxane (0.036 mmol) was dissolved in 2 mL piperidine/CHCl\(_3\) (1/3) and was stirred at rt for 2 hours. The solvent was removed under reduced pressure. The crude material was purified \textit{via} silica-gel column chromatography (CHCl\(_3\)/MeOH: 96/4) to afford the free amine rotaxane.

\( L-\{(O-(tert-butyltriphenylsilyl))\text{-seryl\text{glycylglycylglycine rotaxane 4.10} \} \)
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Yield (0.035 g, 100%); $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 8.26$ (s, 2H, isophthaloyl 2-H), 8.09 (d, 2H, $J = 8.4$ Hz, isophthaloyl 4-H & 6-H), 8.06 (d, 2H, $J = 8.4$ Hz, isophthaloyl 4-H & 6-H), 7.65 (br t, 2H, macrocyclic CONH), 7.65 (br t, 2H, macrocyclic CONH'), 7.50 (t, 2H, $J = 8.4$ Hz, isophthaloyl 5-H), 7.45-7.09 (m, 30 H, aromatic Ar-H & macrocyclic para-xyylene Ar-H), 7.05 (br t, 1H, CONH), 4.62-4.49 (m, 6H, macrocyclic NHCH$_2$CO, NHCH$_2$CO & CO$_2$CH$_2$CHPh$_2$), 4.38 (dd, 2H, $J = 4.8$ Hz, $J = 14.0$ Hz, macrocyclic NHCHH'), 4.30 (dd, 2H, $J = 4.0$ Hz, $J = 14.0$ Hz, macrocyclic NHCHH'), 4.24 (t, 1H, $J = 7.6$ Hz, CO$_2$CH$_2$CHPh$_2$), 3.68 (d, 2H, $J = 5.2$ Hz, Gly NHCH$_2$CO), 3.52 (dd, 1H, $J = 4.8$ Hz, $J = 9.6$ Hz, Ser NH$_2$CH(CHH'(OTBDPS))CO), 3.47 (dd, 1H, $J = 5.0$ Hz, $J = 17.2$ Hz, Gly NHCHH'CO), 3.38 (dd, 1H, $J = 4.8$ Hz, $J = 9.6$ Hz, Ser NH$_2$CH(CHH'(OTBDPS))CO), 3.25 (dd, 1H, $J = 4.2$ Hz, $J = 17.0$ Hz, Gly NHCHH'CO), 3.15 (dd, 1H, $J = 4.2$ Hz, $J = 17.0$ Hz, Gly NHCHH'CO), 3.04 (dd, 1H, $J = 5.0$ Hz, $J = 17.2$ Hz, Gly NHCHH'CO), 2.96-2.90 (m, 1H, Ser NH$_2$CH(CHH'(OTBDPS))CO), 1.95-1.55 (m, 2H, Ser NH$_2$CH(CHH'(OTBDPS))CO), 0.98 (s, 9H, OSi(CH$_3$)$_3$); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 173.84$, 169.97, 169.09, 168.60, 166.79, 141.12, 140.48, 137.46, 137.20, 135.39, 135.34, 134.12, 133.64, 132.75, 132.65, 131.58, 131.26, 130.03, 129.50, 129.20, 129.09, 128.66, 128.30, 128.13, 128.02, 127.86, 127.00, 124.23, 80.01, 67.59, 65.40, 56.21, 49.63, 44.19, 44.10, 42.79, 42.38, 41.06, 26.77; FAB-HRMS for C$_{71}$H$_{75}$N$_8$O$_{10}$Si m/z [M+H$^+$], calcd.: 1227.53755, found: 1227.53756.

L-(O-(tert-butlydiphenylsilyl))-serylglycylglycyl-L-alanylglucylglycine rotaxane

4.11
Yield (0.042 g, 71%); $^1$H NMR (400 MHz, 313K, CDCl$_3$): $\delta$ = 8.28 (s, 2H, isophthaloyl 2-H), 8.09 (d, 4H, $J$ = 6.8 Hz, isophthaloyl 4-H & 6-H), 7.68 (br t, 4H, macrocyclic CONH), 7.58-7.01 (m, 34 H, isophthaloyl 5-H & aromatic Ar-H & CONH & macrocyclic para-xylylene Ar-H), 6.90 (br t, 1H, CONH), 4.62-4.46 (m, 6H, macrocyclic NHCHH' & macrocyclic NHCHH' & CO$_2$CH$_2$CPh$_2$), 4.45-4.38 (m, 3H, macrocyclic NHCHH' & Ser NH$_2$CH(CH$_2$(OTBDPS))CO), 4.31 (dd, 2H, $J$ = 4.0 Hz, $J$ = 13.6 Hz, macrocyclic NHCHH'), 4.20 (t, 1H, $J$ = 7.6 Hz, CO$_2$CH$_2$CPh$_2$), 4.15-4.08 (m, 1H, Ala NHCH(CH$_3$)CO), 3.74 (dd, 1H, $J$ = 3.6 Hz, $J$ = 10.8 Hz, Ser NH$_2$CH(CHH'(OTBDPS))CO), 3.58-3.15 (m, 9H, Ser NH$_2$CH(CHH'(OTBDPS))CO & Gly NHCH$_2$CO & Gly NHCH$_2$CO & Gly NHCH$_2$CO & Gly*NHCH$_2$CO), 2.46-2.12 (m, 2H, Ser NH$_2$CH(CH$_2$(OTBDPS))CO), 1.15 (d, 3H, $J$ = 6.4 Hz, Ala NHCH(CH$_3$)CO), 0.99 (s, 9H, OSiC(CH$_3$)$_3$); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ = 174.31, 172.75, 169.79, 169.49, 169.25, 168.92, 167.11, 140.54, 137.37, 137.28, 135.41, 134.23, 134.09, 132.71, 131.21, 130.05, 129.06, 128.93, 128.69, 128.03, 127.87, 127.02, 124.82, 79.85, 67.58, 65.49, 56.49, 49.56, 49.11, 44.27, 44.16, 42.79, 42.58, 42.32, 40.98, 26.79, 17.35; FAB- HRMS for C$_{76}$H$_{83}$N$_{10}$O$_{12}$S$_1$ m/z [M+H$^+$], calcd.: 1355.59612, found: 1355.59738.

L-(O-(tert-butyldiphenylsilyl))-serylglycylglycylglycyl-L-phenylalanyl-L-leucine-tert-butyl ester rotaxane 4.17

Yield (0.050 g, 78%); $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 8.30 (s, 2H, isophthaloyl 2-H), 8.10(d, 2H, $J$ = 8.6 Hz, isophthaloyl 4-H & 6-H), 8.07 (d, 2H, $J$ = 8.6 Hz, isophthaloyl 4-H & 6-H), 7.69-7.57 (m, 4H, macrocyclic CONH), 7.56-7.13 (m, 19 H, isophthaloyl 5-H & aromatic Ar-H & CONH) 7.09 (s, 8H, macrocyclic para-xylylene Ar-H), 6.92 (d, 1H,
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$J = 7.2 \text{ Hz, CONH}), 6.85 \text{ (br t, 1H, CONH), 6.46 (d, 1H, } J = 7.6 \text{ Hz, CONH)}$, 4.60-4.42 (m, 5H, macrocyclic NHCHH' & macrocyclic NHCHH' & Phe NHCH(CH$_2$Ph)CO), 4.41-4.25 (m, 5H, macrocyclic NHCHH' & macrocyclic NHCHH' & Leu NHCH(CH$_2$CH(CH$_3$)$_2$)CO), 3.72-3.63 (m, 1H, Ser NH$_2$CH(CH$_2$(OTBDPS))CO), 3.47-3.37 (m, 2H, Gly NHCHH'CO & Gly NHCHH'CO), 3.36-3.24 (m, 3H, Ser NH$_2$CH(CHH'(OTBDPS))CO & Phe NHCH(CHH'Ph)CO & Gly NHCHH'CO), 3.11 (dd, 1H, $J = 3.2 \text{ Hz, } J = 16.4 \text{ Hz, Phe NHCH(CHH'Ph)CO}$), 3.06-2.98 (m, 2H, Ser NH$_2$CH(CHH'(OTBDPS))CO & Gly NHCHH'CO), 2.97-2.84 (m, 2H, Gly NHCHH'CO & Gly NHCHH'CO), 1.86-1.65 (m, 2H, Ser NH$_2$CH(CH$_2$(OTBDPS))CO), 1.58-1.46 (m, 1H, Leu NHCH(CH$_2$CH(CH$_3$)(CH$_3$)CO), 1.45-1.33 (m, 1H, Leu NHCH(CH$_2$CH(CH$_3$)(CH$_3$)CO & CO$_2$(CH$_3$)$_2$), 1.00 (s, 9H, OSi(CH$_3$)$_3$), 0.85 (m, 6H, Leu NHCH(CH$_2$CH(CH$_3$)(CH$_3$)CO & Leu NHCH(CH$_2$CH(CH$_3$)(CH$_3$)CO), $^{13}$C NMR (100 MHz, CDC$_3$): $\delta = 171.99, 171.58, 170.27, 170.23, 169.89, 168.78, 167.98, 166.84, 137.45, 137.37, 136.22, 136.12, 135.41, 135.38, 133.88, 132.77, 131.41, 131.33, 130.05, 130.02, 129.27, 129.12, 129.00, 128.60, 128.53, 127.86, 127.07, 127.03, 124.66, 124.52, 82.03, 82.00, 65.59, 56.43, 54.52, 51.74, 44.16, 42.53, 42.41, 41.43, 41.19, 38.81, 38.58, 27.97, 26.80, 24.81, 22.52, 22.10; FAB-HRMS for C$_{76}$H$_{91}$N$_{10}$O$_{12}$Si $m/z$ [M+H$^+$], calcd.: 136365872, found: 1363.65512.

*N-terminal coupling of rotaxane derivatives 4.10, 4.11 and 4.17*

L-(O-(tert-butyldiphenylsilyl))-serglyglycine rotaxane (4.10, 4.11 or 4.17, 0.025 mmol), N-diphenylacetyl amino acid (0.050 mmol) and NEt$_3$ (till pH 11) and BOP (0.017 g, 0.038 mmol) were dissolved in 10 mL of CHCl$_3$. The reaction mixture was stirred
overnight at rt. The solvent was removed under reduced pressure. The crude material was purified via silica gel chromatography (CHCl₃/MeOH: 98/2 to 86/14).

**Glycyl-L-((O-(tert-butylidiphenylsilyl))-serylglycylglycylglycine rotaxane 4.12**

Yield (0.040 g, 96%); ¹H NMR (400 MHz, CDCl₃): δ = 8.20 (s, 2H, isophthaloyl 2-H), 8.04-7.99 (m, 8H, isophthaloyl 4-H & 6-H & macrocyclic CONH), 7.55-7.04 (m, 44H, aromatic Ar-H & isophthaloyl 5-H & macrocyclic para-xylylene Ar-H & CONH), 6.76 (d, 1H, J = 6.8 Hz, Ser CONHCH(CHOH(OTBDPS))CO), 5.05 (s, 1H, Ph₂CHCO), 4.52 (dd, 2H, J = 6.4 Hz, J = 14.4 Hz, macrocyclic NHCHH'), 4.47 (dd, 2H, J = 5.2 Hz, J = 14.0 Hz, macrocyclic NHCHH'), 4.40 (d, 2H, J = 7.6 Hz, CO₂CH₂CHPh₂), 4.33 (dd, 2H, J = 4.4 Hz, J = 14.4 Hz, macrocyclic NHCHH'), 4.29 (dd, 2H, J = 4.0 Hz, J = 14.0 Hz, macrocyclic NHCHH'), 4.24-4.22 (m, 1H, Ser NHCH(CH₂(OTBDPS))CO), 4.15 (t, 1H, J = 7.6 Hz, CO₂CH₂CHPh₂), 3.81-3.71 (m, 2H, Ser NHCH(CH₂(OTBDPS))CO & Gly NHCHH'CO), 3.56 (dd, 1H, J = 6.4 Hz, J = 10.4 Hz, Ser NHCH(CH₂(OTBDPS))CO), 3.51 (dd, 1H, J = 6.4 Hz, J = 17.2 Hz, Gly NHCHH'CO), 3.41 (dd, 1H, J = 4.4 Hz, J = 16.0 Hz, Gly NHCHH'CO), 3.30 (dd, 1H, J = 5.2 Hz, J = 17.2 Hz, Gly NHCHH'CO), 3.25-3.20 (m, 2H, Gly NHCHH'CO), 2.86 (dd, 1H, J = 5.6 Hz, J = 16.4 Hz, Gly NHCHH'CO), 2.63 (dd, 1H, J = 5.6 Hz, J = 16.4 Hz, Gly NHCHH'CO), 0.99 (s, 9H, OSiC(CH₃)₃); ¹³C NMR (100 MHz, CDCl₃): δ = 173.64, 170.47, 169.38, 169.29, 169.11, 169.06, 167.47, 167.33, 140.57, 140.55, 139.09, 139.06, 137.36, 137.06, 135.44, 135.35, 134.63, 134.32, 132.62, 132.29, 130.98, 130.66, 130.07, 129.15, 128.94, 128.84, 128.76, 128.74, 128.66, 128.63, 128.11, 128.01, 127.93, 127.86, 127.30, 127.22, 126.99, 125.80, 80.12, 67.48, 63.09, 58.05,
54.71, 49.52, 44.55, 44.42, 43.79, 42.75, 41.61, 40.75, 26.77; FAB-HRMS for C_{67}H_{88}N_{9}O_{12}Si m/z [M+H'], calcd.: 1478.63217, found: 1478.63873.

**Glycylglycyl-L-(O-(tert-butyldiphenylsilyl))-serylglycylglycyl-L-alanylglycylglycine rotaxane 4.13**

Yield (0.037 g, 91%); ^1^H NMR (400 MHz, 323K, CDCl$_3$): $\delta$ = 8.25 (s, 2H, isophthaloyl 2-H), 8.05 (d, 2H, $J$ = 5.2 Hz, isophthaloyl 4-H & 6-H), 8.04 (d, 2H, $J$ = 5.2 Hz, isophthaloyl 4-H & 6-H), 7.81 (br t, 2H, macrocyclic CONH), 7.66 (br t, 2H, macrocyclic CONH'), 7.61-6.98 (m, 46H, aromatic Ar-H & isophthaloyl 5-H & macrocyclic $para$-xylylene Ar-H & CONH), 6.87 (br t, 1H, CONH), 6.80 (br t, 1H, CONH), 4.96 (s, 1H, Ph$_2$CHCO), 4.59 (dd, 2H, $J$ = 6.4 Hz, $J$ = 13.6 Hz, macrocyclic NHCHH'), 4.47 (dd, 2H, $J$ = 4.8 Hz, $J$ = 14.8 Hz, macrocyclic NHCHH'), 4.41-4.33 (m, 4H, macrocyclic NHCHH' & CO$_2$CH$_2$CHPh$_2$), 4.28 (dd, 2H, $J$ = 4.0 Hz, $J$ = 13.6 Hz, macrocyclic NHCHH'), 4.13 (t, 1H, $J$ = 7.2 Hz, CO$_2$CH$_2$CHPh$_2$), 4.05-3.93 (m, 2H, Ala NHCH(CH$_3$)CO & Gly NHCHH'CO), 3.87 (dd, 1H, $J$ = 5.2 Hz, $J$ = 10.4 Hz, Gly NHCHH'CO), 3.83-3.78 (m, 1H, Gly NHCHH'CO), 3.73-3.61 (m, 2H, Ser NHCH(CH$_3$(OTBDPS))CO & Gly NHCHH'CO), 3.57 (dd, 1H, $J$ = 4.4 Hz, $J$ = 18.4 Hz, Ser NHCH(CHH'(OTBDPS))CO, 3.51-3.38 (m, 3H, Ser NHCH(CHH'(OTBDPS))CO & Gly NHCHH'CO), 3.30-3.19 (m, 3H, Gly NHCHH'CO & Gly NHCHH'CO), 3.18-3.06 (m, 2H, Gly NHCHH'CO & Gly NHCHH'CO), 2.95 (dd, 1H, $J$ = 4.4 Hz, $J$ = 16.4 Hz, Gly NHCHH'CO), 1.14 (d, 3H, $J$ = 7.2 Hz, Ala NHC(CH$_3$)CO), 1.02 (s, 9H, OSiC(CH$_3$)$_3$); ^1^C NMR (100 MHz, CDCl$_3$): $\delta$ = 173.52, 173.02, 172.97, 172.88, 171.23, 170.30, 169.79, 169.44, 169.33, 167.44, 167.34, 140.41, 138.98, 138.89, 137.38, 137.05, 135.40, 135.37, 134.18, 134.07, 132.42, 131.08, 130.99, 129.97, 136.
128.98, 128.86, 128.74, 128.70, 127.86, 127.81, 127.78, 127.26, 126.92, 125.27, 80.05, 67.53, 62.79, 58.13, 56.17, 51.47, 50.21, 49.46, 44.05, 43.98, 42.81, 42.75, 42.33, 41.83, 40.91, 40.59, 26.56, 16.65; FAB-HRMS for C_{94}H_{99}N_{12}O_{15}Si m/z [M+H^+], calcd.: 1663.71222, found: 1663.72154.

\textit{tBoc-L-leucyl-L-phenylalanylglycyl-L-(O-(tert-butyldiphenylsilyl))-serylglycylglycylglycyl-L-phenylalanyl-L-leucine-tert-butyl ester rotaxane 4.18}

Yield (0.054 g, 84%); \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): \( \delta = 8.30 \) (s, 2H, isophthaloyl 2-H), 8.06 (d, 4H, J = 7.2 Hz, isophthaloyl 4-H & 6-H), 7.90 (br t, 2H, macrocyclic CON\textsubscript{H}), 7.85 (br t, 2H, macrocyclic CON\textsubscript{H'}), 7.60-7.00 (m, 31H, aromatic Ar-H & isophthaloyl 5-H & CON\textsubscript{H} & macrocyclic para-xylene Ar-H & CON\textsubscript{H}), 6.90 (br t, 1H, CON\textsubscript{H}), 6.79 (d, 1H, J = 7.2 Hz, CON\textsubscript{H}), 6.63 (br d, 1H, CON\textsubscript{H}), 6.54 (br s, 1H, CON\textsubscript{H}), 6.47 (br s, 1H, CON\textsubscript{H}), 6.13 (d, 1H, J = 6.8 Hz, Leu CON\textsubscript{H}CH(CH\textsubscript{2}CH(CH\textsubscript{3})\textsubscript{2})CO), 5.92 (d, 1H, J = 8.0 Hz, N-terminal Gly CON\textsubscript{H}CH\textsubscript{2}CO), 4.92 (br d, 1H, Leu CON\textsubscript{H}CH(CH\textsubscript{2}CH(CH\textsubscript{3})\textsubscript{2})CO), 4.76-4.54 (m, 4H, macrocyclic NH\textsubscript{CH\textsuperscript{H}}' & NH\textsubscript{CH\textsuperscript{H}}'), 4.50-4.43 (m, 1H, N-terminal Phe NH\textsubscript{CH}CH(CH\textsubscript{2}Ph)CO), 4.39-4.14 (m, 8H, macrocyclic NH\textsubscript{CH\textsuperscript{H}}' & NH\textsubscript{CH\textsuperscript{H}}') & C-terminal Leu NH\textsubscript{CH}CH(CH\textsubscript{2}CH(CH\textsubscript{3})\textsubscript{2})CO & C-terminal Phe NH\textsubscript{CH}CH(CH\textsubscript{2}Ph)CO & Ser NH\textsubscript{CH}CH(CH\textsubscript{2}(OTBDPS))CO & N-terminal Gly NH\textsubscript{CH\textsuperscript{H}}'CO), 3.93-3.86 (m, 1H, N-terminal Leu NH\textsubscript{CH}CH(CH\textsubscript{2}CH(CH\textsubscript{3})\textsubscript{2})CO), 3.83-3.78 (m, 1H, N-terminal Gly NH\textsubscript{CH\textsuperscript{H}}'CO), 3.74-3.62 (m, 1H, C-terminal Gly NH\textsubscript{CH\textsuperscript{H}}'CO), 3.51-3.35 (m, 2H, C-terminal Gly NH\textsubscript{CH\textsuperscript{H}}'CO & C-terminal Gly NH\textsubscript{CH\textsuperscript{H}}'CO), 3.25 (dd, 1H, J = 6.8 Hz, J = 17.2 Hz, C-terminal Gly NH\textsubscript{CH\textsuperscript{H}}'CO), 3.20-3.14 (m, 1H, C-terminal Gly NH\textsubscript{CH\textsuperscript{H}}'CO), 3.10-2.96 (m, 3H, C-terminal Gly NH\textsubscript{CH\textsuperscript{H}}'CO & N-terminal Phe NH\textsubscript{CH}CH(CH\textsubscript{2}Ph)CO & Ser NH\textsubscript{CH}CH(CH\textsubscript{2}(OTBDPS))CO), 2.89-2.76 (m, 3H, Ser
NHCH(CHOH(OTBDPS))CO & C-terminal Phe CONHCH(CHOHPh)CO & C-terminal Phe NHCH(CHOHPh)CO, 2.65-2.62 (m, 1H, N-terminal Phe NHCH(CHOHPh)CO), 1.57-1.47 (m, 1H, Leu NHCH(CH2CH(CH3)(CH3)CO), 1.43-1.32 (m, 22H, Leu NHCH(CH2CH(CH3)(CH3)CO & CO2(CH3) & Boc CH3), 1.00 (s, 9H, OSi(CH3)3), 0.86-0.75 (m, 6H, Leu NHCH(CH2CH(CH3)(CH3)CO) & Leu NHCH(CH2CH(CH3)(CH3)CO); 13C NMR (100 MHz, CDCl3): δ = 173.19, 172.54, 171.73, 171.40, 170.76, 170.46, 170.12, 169.62, 167.84, 167.31, 167.08, 157.38, 137.36, 137.25, 136.15, 136.00, 135.52, 134.42, 134.13, 132.81, 132.60, 131.24, 131.01, 129.93, 129.30, 129.20, 128.97, 128.92, 128.82, 128.65, 127.78, 127.32, 127.27, 127.15, 127.00, 125.59, 81.91, 81.20, 81.03, 55.16, 54.78, 54.57, 54.40, 51.79, 51.59, 44.58, 44.38, 43.87, 42.76, 42.20, 41.27, 41.00, 40.34, 38.73, 36.72, 28.22, 27.98, 26.79, 24.80, 22.84, 22.49, 22.07, 21.91, 21.65; FAB-HRMS for C98H122N13O17Si m/z [M+H]+, calcld.: 1781.88845, found: 1781.88738.

TBDPS deprotection of rotaxane derivatives 4.12, 4.13 and 4.18

Glycyl-L-serylglycylglycylglycine rotaxane 4.14

Glycyl-L-(O-(tert-butyldiphenylsilyl))-serylglycylglycylglycine rotaxane 4.12 (0.028 g, 0.019 mmol) were dissolved in 1 mL of THF and then treated with 0.1 mL of 1 mol.dm−3 solution of TBAF in THF. The reaction mixture was stirred at rt for one hour and then concentrated under reduced pressure. The crude material was purified via silica gel chromatography (CHCl3/MeOH: 98/2 to 86/14). Yield (0.016 g, 71%); 1H NMR (400 MHz, 323K, CDCl3): δ = 8.20 (s, 2H, isophthaloyl 2-H), 8.00 (d, 4H, J = 7.6 Hz, isophthaloyl 4-H & 6-H), 7.61 (br t, 4H, macrocyclic CONH), 7.43 (d, 2H, J = 7.6 Hz,
isophthaloyl 5-H), 7.28-6.98 (m, 30H, aromatic Ar-H & macrocyclic \textit{para}-xylylene Ar-H & CONH), 6.94 (br t, 1H, CONH), 6.85 (br t, 1H, CONH), 6.59 (br t, 1H, CONH), 4.78 (s, 1H, Ph$_2$CHCO), 4.51-4.28 (m, 10H, macrocyclic NHCHH' & NHCHH' & CO$_2$CH$_2$CHPh$_2$), 4.20 (t, 1H, $J = 7.2$ Hz, CO$_2$CH$_2$CHPh$_2$), 4.13-4.06 (m, 1H, Ser NHCH(CH$_2$OH)CO), 3.66 (dd, 1H, $J = 3.2$ Hz, $J = 10.8$ Hz, Ser NHCH(CHH'OH)CO), 3.45-3.29 (m, 7H, Ser NHCH(CHH'OH)CO & Gly NHCH$_3$CO & Gly NHCH$_3$CO & Gly NHCH$_2$CO), 3.11 (br d, 2H, Gly NHCH$_2$CO), 2.14 (m, 1H, Ser NHCH(CHH'OH)CO); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 173.36, 170.85, 169.52, 169.35, 169.19, 167.42, 167.35, 167.21, 140.52, 138.80, 137.24, 134.18, 131.11, 130.99, 129.05, 128.94, 128.71, 128.65, 128.00, 127.42, 126.99, 125.14, 67.58, 61.76, 58.14, 54.98, 49.57, 44.26, 44.21, 44.13, 44.08, 42.86, 42.55; FAB-HRMS for C$_{71}$H$_{70}$N$_9$O$_{12}$Si m/z [M+H$^+$], calcd.: 1240.51439, found: 1240.51300.

\textbf{Glycylglycyl-L-serylglycylglycyl-L-alanylglycylglycine rotaxane 4.15}

Glycylglycyl-L-\((O$\text{-}(\text{tert}$-butyldiphenylsilyl})$\)-serylglycylglycyl-L-alanylglycylglycine rotaxane (4.13, 0.017 g, 0.010 mmol) and diphenylethanol (0.001 g, 0.050 mmol) were dissolved in 3 mL of TFA. The reaction mixture was stirred at room temperature for one hour and then concentrated under reduced pressure. The crude material was precipitated out of diethylether. Yield (0.014 g, 100%); $^1$H NMR (400 MHz, MeOD-$d_4$): $\delta = 8.27$ (s, 2H, isophthaloyl 2-H), 7.99 (d, 4H, $J = 7.8$ Hz, isophthaloyl 4-H & 6-H), 7.54 (t, 2H, $J = 7.8$ Hz, isophthaloyl 5-H), 7.22-6.87 (m, 28H, aromatic Ar-H & macrocyclic \textit{para}-xylylene Ar-H), 4.53 (s, 1H, Ph$_2$CHCO), 4.44-4.34 (m, 4H, macrocyclic NHCHH' & NHCHH'), 4.28-4.21 (m, 6H, macrocyclic NHCHH' & NHCHH' & CO$_2$CH$_2$CHPh$_2$), 4.19-4.13 (m, 3H, Ser NHCH(CH$_2$OH)CO & Ala NHCH(CH$_3$)CO & CO$_2$CH$_2$CHPh$_2$),
4.12-3.99 (m, 1H, Ser NHCH(CH₂OH)CO), 3.88-3.68 (m, 4H, Gly NHCH₂CO & Gly
NHCH₂CO), 3.62 (br d, 1H, Gly NHCHH'CO), 3.60-3.59 (m, 1H, Ser
NHCH(CHH'OH)CO), 3.58-3.56 (m, 2H, Gly NHCH₂CO), 3.54-3.51 (m, 2H, Ser
NHCH(CHH'OH)CO & Gly NHCHH'CO), 3.49 (br d, 1H, Gly NHCHH'CO), 3.47-3.44
(m, 3H, Gly NHCH₂CO & Gly NHCHH'CO), 1.19 (d, 3H, J = 7.2 Hz, Ala
NHCH(CH₃)CO); ¹³C NMR (100 MHz, MeOD): δ = 175.51, 174.06, 173.09, 172.15,
172.08, 171.02, 170.83, 270.50, 168.88, 165.28, 164.90, 142.35, 141.64, 138.67,
135.75, 135.72, 131.81, 130.09, 130.05, 130.00, 129.97, 129.63, 129.52, 129.26,
129.03, 128.87, 128.16, 127.85, 127.74, 67.32, 62.67, 45.70, 45.23, 45.14, 45.10, 45.08,
43.71, 43.41, 42.15, 41.54, 16.27; FAB-HRMS for C₇₈H₈₁N₁₂O₁₅ m/z [M+H⁺], calcd:
1425.59444, found: 1425.59569.

L-leucyl-L-phenylalanylglycyl-L-serylglycylglycylglycyl-L-phenylalanyl-L-leucine
rotaxane 4.19

tBoc-L-leucyl-L-phenylalanylglycyl-L-(O-(tert-butyldiphenylsilyl))-serylglycylglycylglycylglycyl-L-phenylalanyl-L-leucine-tert-butyl ester rotaxane (4.18, 0.019 g,
0.010 mmol) was dissolved in 6 mL of TFA. The reaction mixture was stirred overnight
at room temperature and then concentrated under reduced pressure. The crude material
was precipitated out of diethylether. Yield (0.014 g, 100%); ¹H NMR (400 MHz, MeOD-
δ): δ = 8.40 (s, 2H, isophthaloyl 2-H), 8.05 (d, 2H, J = 7.5 Hz, isophthaloyl 4-H & 6-
H), 8.03 (d, 2H, J = 7.5 Hz, isophthaloyl 4-H & 6-H), 7.61 (t, 2H, J = 7.5 Hz,
isophthaloyl 5-H), 7.41-7.18 (m, 1OH, aromatic Ar-H), 7.16 (s, 8H, macrocyclic para-
xylylene Ar-H), 4.59-4.49 (m, 3H, Ser NHCH(CH₂OH)CO & C- and N-terminal Phe
NHCH(CH₂Ph)CO), 4.47-4.39 (m, 8H, macrocyclic NHCHH' & NHCHH'), 4.37-4.35
Chapter Four

(m, 1H, Leu NHCH(CH₂CH(CH₃)₂)CO), 4.22 (dd, 1H, J = 2.8 Hz, J = 5.6 Hz, Gly NHCHH'CO), 3.91 (dd, 1H, J = 4.8 Hz, J = 10.4 Hz, Gly NHCHH'CO), 3.84-3.79 (m, 1H, Leu' NHCH(CH₂CH(CH₃)₂)CO), 3.72 (dd, 1H, J = 4.8 Hz, J = 10.4 Hz, Gly NHCHH'CO), 3.69-3.62 (m, 2H, Gly NHCHH'CO & Gly NHCHH'CO), 3.56-3.51 (m, 2H, Gly NHCH₂CO), 3.48 (dd, 1H, J = 4.8 Hz, J = 7.2 Hz, Gly NHCHH'CO), 3.15-3.06 & 2.99-2.90 & 2.81-2.71 (m, 6H, C- and N-terminal Phe NHCH(CH₃Ph)CO & Ser NHCH(CH₂OH)CO), 1.67-1.41 (m, 6H, C- and N-terminal Leu NHCH(CH₂CH(CH₃)₂)CO & Leu NHCH(CH₂CH(CH₃)₂)CO), 0.86-0.77 (m, 12H, C- and N-terminal Leu NHCH(CH₂CH(CH₃)₂)CO); $^{13}$C NMR (100 MHz, MeOD): $\delta =$ 175.66, 173.82, 173.52, 172.58, 172.20, 171.66, 171.14, 170.42, 170.23, 169.28, 169.09, 138.64, 138.60, 138.44, 138.17, 135.66, 135.61, 131.82, 131.75, 130.38, 130.27, 130.24, 130.09, 129.95, 129.59, 129.55, 128.87, 128.64, 128.06, 127.97, 127.88, 62.79, 56.79, 56.13, 56.00, 52.73, 52.21, 45.43, 45.32, 45.28, 43.37, 43.18, 42.44, 41.58, 41.38, 39.22, 38.10, 26.73, 25.95, 25.23, 23.34, 21.90, 21.53; FAB-HRMS for C₇₃H₈₈N₁₃O₁₅ m/z [M+H⁺], calcd.: 1386.65229, found: 1386.65029.
4.5. References


Conclusion and Outlook

Recently, dipeptides have been successfully derivatised into rotaxanes incorporating benzylic 1,3-carbamide macrocycles, using a five-component hydrogen bond-directed assembly.\textsuperscript{1,2} When encapsulated within a tetraamide macrocycle, oligopeptides present interesting changing properties: their solubility in organic solvents, their lipophilicity and their vulnerability against enzymes\textsuperscript{3} can be incredibly modulated by the macrocycle acting as a protective sheath around the peptide backbone. However, encapsulation via the clipping strategy has been limited so far to short peptide sequences since intramolecular hydrogen bonding and insufficient solubility of longer peptide threads in apolar solvents can induce the folding of the backbone, thus preventing a good preorganization between the peptide thread and the precursor of the macrocycle.\textsuperscript{4,5}

This thesis dealt with the design of new chemical tools able to overcome such problems. Short peptide rotaxane building blocks were synthesized and extended on their terminal sides to promote the synthesis of oligopeptide rotaxanes in very good yields (Scheme 5.1.).

In Chapter Two,\textsuperscript{6} activated ester rotaxane building blocks, in dipeptide and fumaric series, were synthesized. Their elongation on the C-terminal side was applied to the synthesis of a bistable molecular shuttle and a protected Leu-enkephalin rotaxane in very good yields.
In Chapter Three, we described the synthesis of three $N$-protected L-SerGlyGly and L-AspGlyGly tripeptide rotaxane building blocks, functionalized on the lateral chain by a labile bulky stopper. After $N$-deprotection, further elongation via classical peptide coupling chemistry was carried out to give oligopeptide [2] and [3]rotaxanes.

Chapter Four dealt with the fusion of the two previous strategies. An $N$-Fmoc protected tripeptide L-SerGlyGly activated ester rotaxane building block, functionalized on its lateral chain by a labile ether, was synthesized. Successive extension on its $C$- and $N$-terminal sides led to the synthesis of oligopeptide rotaxanes, in very good yields. This strategy was especially applied to the synthesis of the first oligopeptide rotaxane with free amine and carboxylic acid terminal sides.

We have demonstrated the feasibility of synthesizing model oligopeptide rotaxanes, in very good yields, avoiding problems of folding of the peptide backbone, solubility and purification.
Direct clipping of the macrocycle around the oligopeptide thread

Dipeptides: 6 to 62%
Tripeptides: 1-2.5%

Scheme 5.1. Extension of short peptide rotaxane building blocks for the synthesis of oligopeptide rotaxanes.
However even though such compounds have reached academic success, major problems still have to be overcome before industrialisation of any kind. The purification step - *one reaction, one column* – is indeed a critical point.

Key-steps towards successful peptide based prodrug systems are three-fold:

(i) **the delivery of the prodrug.** Water solubility and lipophilicity properties of rotaxanes need to be modulated in order to cross natural barriers (brain, cells…). Functionalisation of the macrocycle has led to promising results.³

(ii) **the drug release.** It can occur by cleavage of the macrocycle or stoppers which both need to be non toxic and excretable. For example the use of a cyclic peptide as macrocycle could be an interesting option and such studies are actually ongoing in the Leigh group. Cleavage would occur *via* a specific stimulus such as irradiation of a photosensitive group, enzymatic degradation with slipping out of the macrocycle... Previous studies in the group showed that carbohydrate-branched carbohydrates can be effectively cleaved allowing the slow release of the macrocycle.

(iii) **the design of specific drugs.** The strategies developed so far did not allow much freedom in terms of the peptide drug primary structure and the nature of the macrocycle. The use of a cleavable building block rotaxane able to promote the slipping of the macrocycle onto an added peptide backbone would be a really impressive tool towards the powerful synthesis of impossible peptide rotaxanes.

The tailoring of these three main topics is the key towards the use of peptide rotaxanes as powerful and universal drug delivery systems.
References


