1 *Article*

Stepwise, Protecting Group Free Synthesis of [4]rotaxanes.

4 **James E. M. Lewis,[1] Joby Winn[2] and Stephen M. Goldup[1]***

5 [1] Chemistry, University of Southampton, Highfield, Southampton, UK
6 [2] School of Biological and Chemical Sciences, Queen Mary University of London, Mile End Road, London,
7 UK
8 * Correspondence: s.goldup@soton.ac.uk

11 **Abstract:** Despite significant advances in the last three decades towards high yielding syntheses
12 of rotaxanes, the preparation of systems constructed from more than two components remains a
13 challenge. Herein we build upon our previous report of an active template CuAAC rotaxane
14 synthesis with a diyne in which, following the formation of the first mechanical bond, the steric
15 bulk of the macrocycle tempers the reactivity of the second alkyne unit. We have now extended this
16 approach to the use of 1,3,5-triethynylbenzene in order to successively prepare [2]-, [3]- and
17 [4]rotaxanes without the need for protecting group chemistry. Whilst the first two iterations
18 proceeded in good yield, the steric shielding that affords this selectivity also significantly reduces
19 the efficacy of the AT-CuAAC reaction of the third alkyne towards the preparation of [4]rotaxanes,
20 resulting in severely diminished yields.

23

1. Introduction

25   Although the first synthesis of a rotaxane was reported in 1967,[1] the development of
26 convenient and high yielding methods for preparing mechanically interlocked molecules (MIMs)
27 remains an active area of research due to their potential applications[2] in fields as diverse as
28 sensing,[3] catalysis,[4] medicine,[5] and as artificial molecular machines[6]. Following Sauvage's
29 ground-breaking use of metal ions as templating species for the synthesis of MIMs,[7] a variety of
30 interactions have been utilized to arrange components prior to formation of the final covalent bond
31 that establishes the mechanical bond.[8] These have included coordination bonds with a range of
32 main group and transition row metal ions,[9] anion-templation,[10] π-stacking interactions,[11]
33 H-bonding[12] and radical-radical interactions[13] amongst others. However, the synthesis of more
34 complex rotaxanes in which two or more macrocycles encircle a thread,[14] remains challenging.
35   Whilst the use of non-covalent interactions to direct the self-assembly of
36 oligo[n]pseudorotaxanes prior to formation of a permanent mechanical bond is the most
37 operationally simple method towards forming multi-component interlocked species,[15] this
38 generally offers little in the way of control over structural or sequence specificity. A limited number
39 of examples of self-sorting systems have been reported.[16] However, iterative approaches[17] tend
40 to offer the best opportunity for rapid and economical formation of precision designed
41 oligo[n]rotaxanes.
42   We have developed a *small macrocycle*[18] modification of Leigh's active template Cu-mediated
43 azide-alkyne cycloaddition (AT-CuAAC) methodology[19] that enables the preparation of rotaxanes
44 in very high yield, and removes the need for extraordinarily large stoppering units.[20] As part of a

preliminary investigation into the synthesis of oligo[n]rotaxanes through an iterative methodology, we recently reported that the steric crowding of the macrocycle around functional stoppering substituents modulates their reactivity; in this manner a diyne (**1**) was able to be reacted under AT-CuAAC conditions to give exclusive formation of a [2]rotaxane, rather than the anticipated statistical mixture of [2]- and [3]-rotaxanes, due to the formation of a stable Cu(I)-triazolide intermediate[21] that hindered access to the second alkyne moiety (Scheme 1). Following purification, the [2]rotaxane could be subjected to another iteration of the reaction conditions, resulting in formation of the [3]rotaxane architecture.[22]
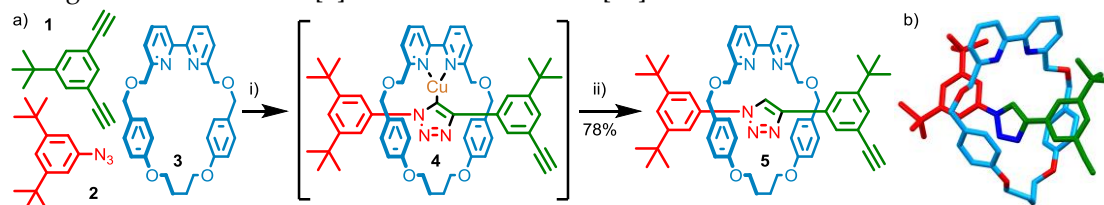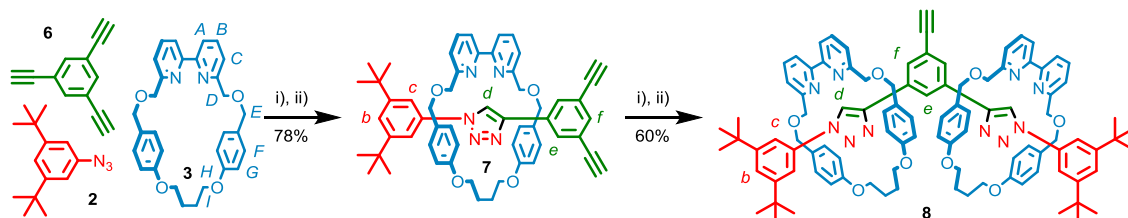

Scheme 1. a) Synthesis of [2]rotaxane **4** from diyne **1**. Reagents and conditions: i) 1 equiv. each of **1**, **2**, **3**, [Cu(CH₃CN)₄](PF₆) and *i*Pr₂Net, EtOH, 100 °C (μw), 2 h; ii) CH₂Cl₂, 100 °C (μw), 1 h. b) X-ray crystal structure of **4**.

With a view to extending this approach towards more complex dendritic-type systems,[23] we herein report the use of a triyne (**6**) in a similar manner, allowing the stepwise, high-yielding synthesis of [2]- and [3]rotaxanes. Disappointingly, due to the steric crowding which allows us to conduct this protecting-group free synthesis, the attempted formation of [4]rotaxanes proceeded in severely diminished yields. Through the use of larger, more flexible macrocycles and less sterically encumbered stoppering units the yield could be increased but failed to give the triply-interlocked species as the major product.

2. Results and Discussion

2.1 Stepwise Synthesis of [2]- and [3]rotaxanes

Scheme 2. Stepwise synthesis of [2]rotaxane **7**, and [3]rotaxane **8**. Reagents and conditions: i) 1 equiv. each of **6**, **2**, **3**, [Cu(CH₃CN)₄](PF₆) and *i*Pr₂Net, EtOH, μW, 100 °C, 2 h; ii) CH₂Cl₂, μW, 100 °C, 1 h.

1,3,5-Triethynylbenzene, **6**, was subjected to the optimized reaction conditions previously established for diyne **1** with the intention of exclusively forming [2]rotaxane **7**. As such **6** was reacted with one equivalent each of macrocycle **3**, azide **2**, [Cu(CH₃CN)₄](PF₆) and *i*Pr₂NEt in EtOH at 100 °C under microwave irradiation for 2 h, followed by a solvent swap to CH₂Cl₂ and an additional hour heating at 100 °C to effect protonolysis of the Cu-triazolide intermediate.[24] Pleasingly, analysis of the ¹H NMR spectrum of the crude reaction mixture revealed complete consumption of the starting materials, and almost exclusive formation of the desired [2]rotaxane. After purification by column chromatography, **7** was obtained in 78% isolated yield, and its identity confirmed by NMR (*vide infra*) and MS (*m/z* = 864.45 [M+H]⁺).

With **7** in hand the next step was to examine whether the [3]rotaxane **8** could be obtained under the same conditions. Pleasingly submission of **7** to another iteration of the reaction conditions with a further equivalent each of **2**, **3**, [Cu(CH₃CN)₄](PF₆) and *i*Pr₂NEt gave, following column chromatography, **8** (*m/z* = 1577.87 [M+H]⁺) in 60% isolated yield.

82   *2.2 Analysis of [2]- and [3]rotaxanes **7** and **8***

83   A comparison of the ^{1}H NMR spectra of **7** and **8** (Figure 1b and 1c, respectively) shows that, as
84   expected, the overall symmetry of the thread does not change following the second AT-CuAAC
85   reaction; however, some peaks were found to resonate at different chemical shifts. The triazole
86   proton (H$_d$) shifts 0.12 ppm further downfield, whilst those of the terminal stoppering units (H$_b$ and
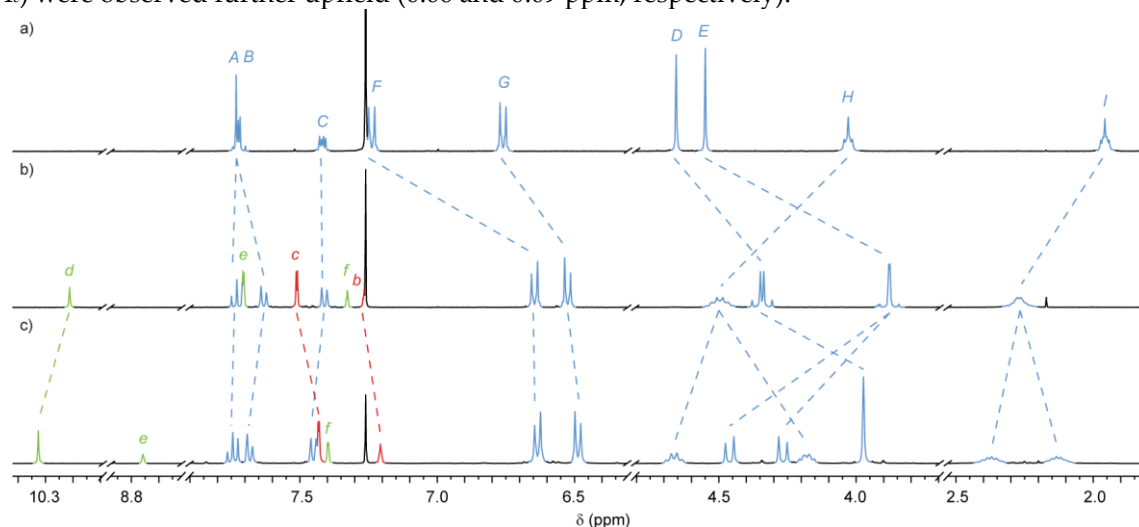87   H$_c$) were observed further upfield (0.06 and 0.09 ppm, respectively).

88
89   **Figure 1.** ^{1}H NMR spectra (400 MHz, CDCl$_3$, 298 K) of a) macrocycle **3**, b) [2]rotaxane **7**, and c)
90   [3]rotaxane **8**. For macrocycle and thread labeling see Scheme 2.
91

92   Most interestingly the diastereotopic protons of the methylene units of the macrocycle butyl
93   chain appear as distinct multiplets (Δδ = 0.48 and 0.23 ppm for H$_H$ and H$_I$, respectively). This is
94   presumably a result of the increased steric hindrance from the presence of two macrocycles in close
95   proximity, reducing flexibility of the butyl chain. H$_E$, the methylene unit adjacent to the flanking
96   aromatic moieties of the macrocycle, was also observed to split into two distinct doublets.

97   As we have previously found with similar [3]rotaxane structures,[22] proton H$_e$ – sandwiched
98   between the two triazole moieties on the thread – was found to resonate 1.05 ppm higher than the
99   corresponding proton (H$_e$) in the [2]rotaxane, indicative of a *syn-syn* arrangement of the two triazole
100   rings. X-ray quality crystals of **8** were obtained by vapour diffusion of pentane into a CH$_2$Cl$_2$
101   solution, allowing the solid state structure to be determined by crystallography (Figure 2). The
102   structure shows the expected hydrogen-bond interactions between the protons of the triazole rings
103   and the bipyridine moieties of the macrocycles (C-H⋯N distance 2.4 – 2.7 Å), and reveals the thread
104   to be in the *syn-syn* conformation suggested by the solution phase ^{1}H NMR data.
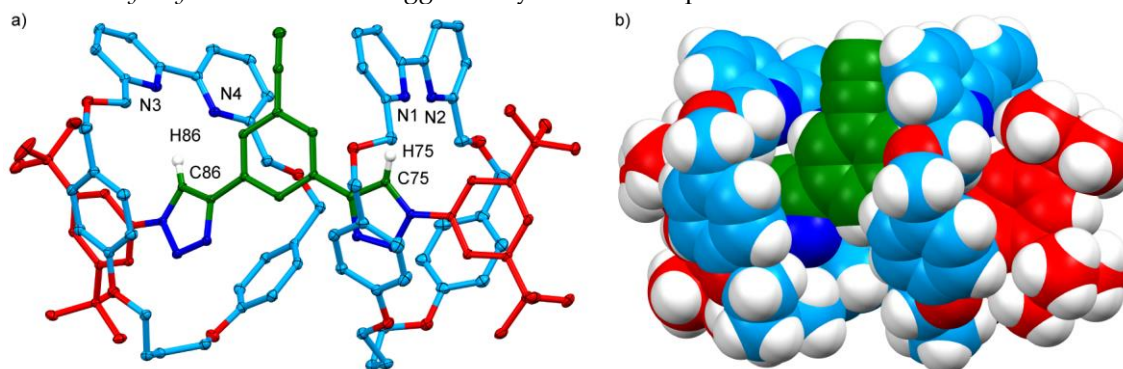

105
106   **Figure 2.** X-ray crystal structure of **8** shown as a) ellipsoid, and b) space-filling plots. Ellipsoids are
107   shown at 50% probability. Some hydrogen atoms have been omitted for clarity. Interactions lengths
108   (Å) and distance (°): H75⋯N1 2.392, H75⋯N2 2.688, C75-H75⋯N1 167.3, C75-H75⋯N2 126.0,
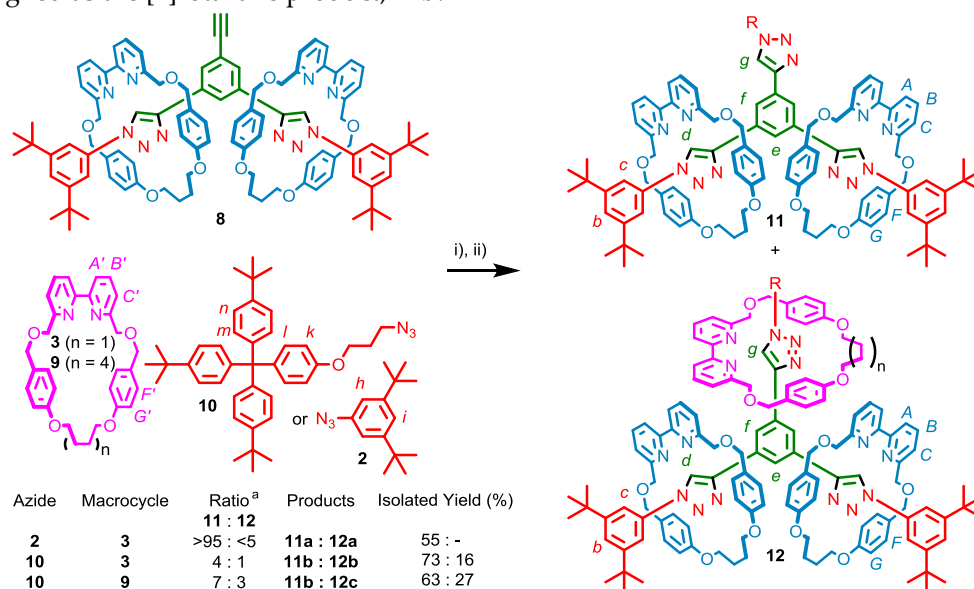109   H86⋯N3 2.541, H86⋯N4 2.534, C86-H86⋯N3 156.3, C86-H86⋯N4 135.6.

110 *2.3 Synthesis of [4]rotaxanes*

111   Having successfully prepared **8** in two steps in good yield (47% overall), a third AT-CuAAC
112 reaction was attempted to prepare the desired [4]rotaxane, **12a** (Scheme 3). Submission of **8** to
113 another iteration of the reaction conditions resulted in a crude NMR which appeared to show a
114 single species, with peaks shifted relative to **8**. However, following column chromatography,
115 macrocycle **3** was recovered, along with a new product, identified as the tris-triazole thread
116 encircled by two macrocycles (**11a**; m/z = 905.01 [M+2H]$^{2+}$).

117   Closer inspection of the ^{1}H NMR spectrum of this purified [3]rotaxane (Figure 3b) revealed a
118 small amount (<5%) of impurity to be present that had co-eluted with the major product
119 (re-examination of the crude reaction mixture showed a similar ratio of this impurity). Although a
120 weak signal consistent with the formulation of the desired [4]rotaxane could be observed by MS
121 (m/z = 1146 [M+2H]$^{2+}$; see Supporting Information), the identity of this species was unable to be
122 reliably confirmed, and as such it could not be determined conclusively whether any **12a** had
123 formed.

124   It was assumed that the steric bulk of the two existing macrocycles was hindering access to the
125 third alkyne moiety. To alleviate some of this crowding, the reaction was repeated using azide **10**
126 which incorporated an alkyl chain. The crude ^{1}H NMR of the reaction mixture revealed two
127 products, with the ratio of these estimated at 1:4 based on the integration values of two
128 downfield-shifted triazole proton resonances at 10.56 and 10.44 ppm, respectively (see Supporting
129 Information). Pleasingly, the products were able to be separated by column chromatography. The
130 major species, isolated in 73% yield, was identified as the product of the reaction between **8** and **10** –
131 without trapping of macrocycle **3** – by the presence of resonances consistent with the new
132 stoppering unit, but lacking any signals attributable to a third macrocycle (Figure 3c), with MS data
133 supporting this conclusion (m/z = 1082.13 [M+2H]$^{2+}$).

134   The minor species, isolated in 16% yield, showed additional peaks in the ^{1}H NMR spectrum
135 consistent with a mechanically trapped macrocycle in a different environment to the pre-existing
136 two (Figure 3d, *vide infra*). With additional evidence from MS (m/z = 1324.23 [M+2H]$^{2+}$), this species
137 was assigned as the [4]rotaxane product, **12b**.

138

Azide	Macrocycle	Ratio[a] 11 : 12	Products	Isolated Yield (%)
2	3	>95 : <5	**11a** : **12a**	55 : -
10	3	4 : 1	**11b** : **12b**	73 : 16
10	9	7 : 3	**11b** : **12c**	63 : 27

139   **Scheme 3.** Synthesis of [4]rotaxanes. Reagents and conditions: i) 1 equiv. each of **2** or **10**, **3** or **9**,
140 [Cu(CH$_3$CN)$_4$](PF$_6$) and ^{i}Pr$_2$Net, EtOH, μW, 100 °C, 2 h; ii) CH$_2$Cl$_2$, μW, 100 °C, 1 h. [a] Determined
141 by ^{1}H NMR.

142   Subsequently, in a further attempt to decrease steric crowding and increase the yield of the
143 [4]rotaxane product, the effect of increasing the size of the macrocycle was investigated by
144 employing the larger macrocycle **9**. The reaction between **8**, azide **10** and macrocycle **9** again yielded

145 two products, this time in a ratio of 3:7, as judged by ^{1}H NMR analysis of the crude reaction mixture
146 (see Supporting Information). The NMR of the major species was identical to the previously isolated
147 [3]rotaxane, **11b**, whilst the minor species again exhibited additional signals consistent with the
148 presence of a third macrocycle in a different chemical environment to the other two (Figure 3e).
149 Following column chromatography the major species was isolated in 63% yield, and its identity
150 confirmed as the [3]rotaxane **11b**. Isolated in 27% yield, the minor species was identified as the
151 anticipated [4]rotaxane by NMR (*vide infra*) and MS (m/z = 1366.27 [M+2H]$^{2+}$). As such, through
152 alleviation of the steric crowding around the incoming macrocycle and azide, the yield of the third
153 AT-CuAAC reaction was able to be increased from <5% to a respectable 27%.

154 *2.4 Analysis of [4]rotaxanes **12b** and **12c***

155 The ^{1}H NMR spectra of the [3]rotaxanes (**11a** and **11b**) and [4]rotaxanes (**12b** and **12c**) each
156 display characteristic peaks related to their structure and conformation in solution. The resonance
157 for the proton *ortho* to the two equivalent triazole rings (H$_e$) does not shift significantly in any of
158 these species, suggesting that the rotaxane maintains the *syn-syn* conformation observed in solution
159 and the solid state for [3]rotaxane **8**. The signal for the phenyl protons adjacent to the third alkyne
160 (H$_f$) was found to shift downfield by approximately 0.7 ppm ($\Delta\delta$ = 0.66 – 0.82) upon formation of the
161 third triazole ring in the [3]- and [4]rotaxanes, presumably similarly due to interactions between the
162 protons and the triazole ring; the observation of a single signal suggests free rotation of the
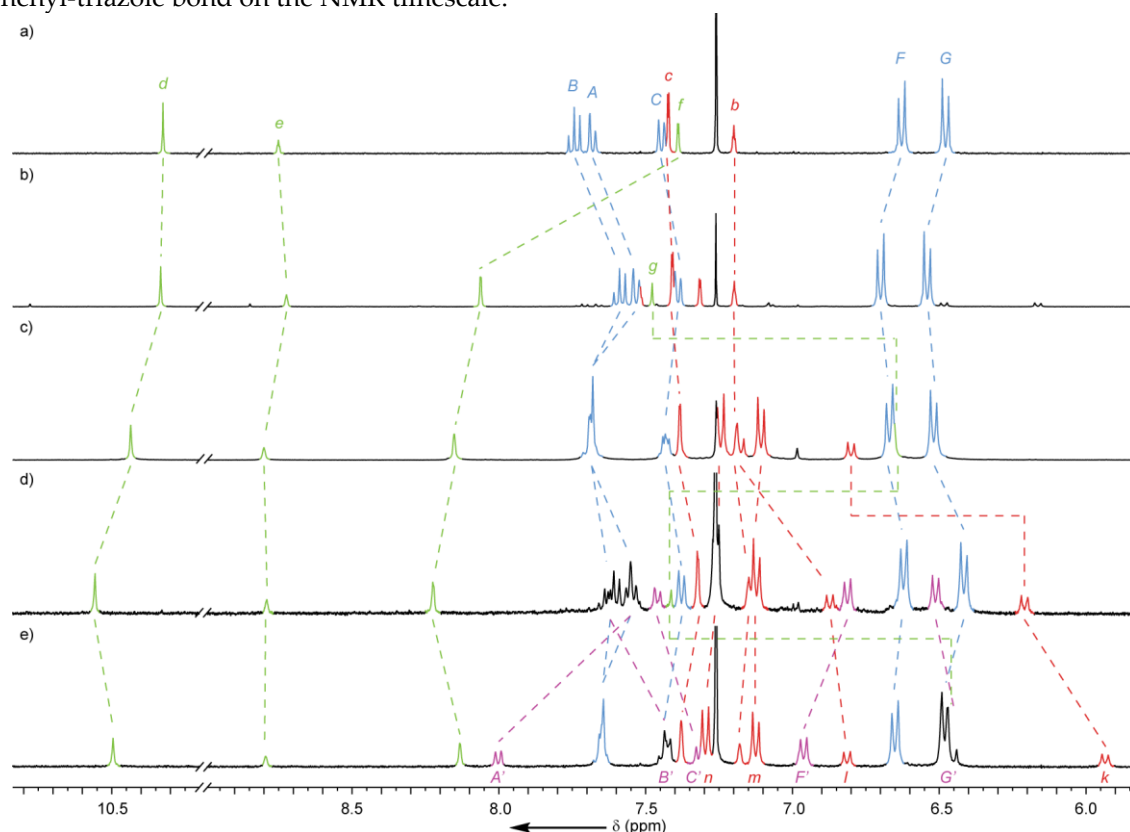163 phenyl-triazole bond on the NMR timescale.

164

Figure 3. Partial ^{1}H NMR spectra (CDCl$_3$, 400 MHz, 298 K) of [3]rotaxanes a) **8**, b) **11a**, c) **11b**, and
[4]rotaxanes d) **12b** and e) **12c**.

167 The ^{1}H NMR spectra of both [4]rotaxanes display some noticeable shifts relative to the
168 corresponding [3]rotaxane, **11b**, consistent with formation of an interlocked molecule. The aromatic
169 peaks of the trityl stoppering unit closest to the triazole, H$_k$ and H$_l$, shift significantly upfield in the
170 presence of macrocycles **3** ($\Delta\delta$ = 0.59 and 0.31, respectively) and **9** ($\Delta\delta$ = 0.86 and 0.36, respectively) in
171 **12b** and **12c**, respectively. Crucially the resonances of the third macrocycle were shifted relative to
172 the free component; particularly those associated with the flanking aromatic units of the macrocycles

173 (H$_{F'}$ and H$_{G'}$) were found to shift downfield relative to the free macrocycle, consistent with an
174 interlocked structure.

175   As previously reported,[20] the size of the third macrocycle greatly affects the hydrogen
176 bonding interaction between the bipyridine unit and the triazole proton of the thread (H$_g$), as
177 evidenced by the chemical shift of the latter. In the case of [4]rotaxane **12c** with the larger macrocycle
178 **9**, the triazole proton is observed at a similar chemical shift to the non-interlocked component of the
179 corresponding [3]rotaxane **11b** (6.44 vs. 6.65 ppm, respectively). Conversely, in the [4]rotaxane **12b**
180 with the smaller macrocycle, **3**, H$_g$ resonates further downfield at 7.41 ppm.

181 **3. Conclusions**

182   We have shown that the steric encumbrance of a macrocycle can be used in lieu of protecting
183 group chemistry to successively prepare [2]-, [3]- and [4]rotaxanes from a simple triethynylbenzene
184 unit.   Whilst the first two iterations proceed in good yield, the steric bulk of two macrocycles results
185 in severely diminished yields of the third AT-CuAAC reaction. However, through alleviation of
186 steric constraints by employing larger and more flexible macrocycles and stoppering units the yield
187 of this final iteration could be raised from <5% to 27%. We are currently investigating structures that
188 will allow the high yielding synthesis of [4]rotaxanes, whilst maintaining the selectivity for a single
189 AT-CuAAC reaction with each step.

198 **Author Contributions:** All authors contributed to conceiving and designing the experiments and analyzing the
199 data; JEML and JW performed the experiments. JEML and SMG wrote the paper.

200 **Conflicts of Interest:** The authors declare no conflict of interest.

201 **References**

1. Harrison, I. T.; Harrison, S. The Synthesis of a Stable Complex of a Macrocycle and a Threaded Chain. *J. Am. Chem. Soc.* **1967**, *89*, 5723-5724.
2. (a) Ma, X.; Tian, H. Bright functional rotaxanes. *Chem. Soc. Rev.* **2010**, *39*, 70–80. (b) Neal, E. A; Goldup, S. M. Chemical consequences of mechanical bonding in catenanes and rotaxanes: isomerism, modification, catalysis and molecular machines for synthesis. *Chem. Commun.* **2014**, *50*, 5128. (c) van Dongen, S. F. M.; Cantekin, S.; Elemans, J. A. A. W.; Rowan, A. E.; Nolte, R. J. M. Functional interlocked systems. *Chem. Soc. Rev.* **2014**, *43*, 99–122. (d) Xue, M.; Yang, Y.; Chi, X.; Yan, X.; Huang, F. Development of Pseudorotaxanes and Rotaxanes: From Synthesis to Stimuli-Responsive Motions to Applications. *Chem. Rev.* **2015**, *115*, 7398–7501. (e) Lewis, J. E. M.; Galli, M.; Goldup, S. M. Properties and emerging applications of mechanically interlocked ligands. *Chem. Commun.* DOI: 10.1039/C6CC07377H.
3. (a) Chmielewski, M. J.; Davis, J. J.; Beer, P. D. Interlocked hostrotaxane and catenane structures for sensing charged guest species via optical and electrochemical methodologies. *Org. Biomol. Chem.* **2009**, *7*, 415–424. (b) Caballero, A.; Zapata, F.; Beer, P. D. Interlocked host molecules for anion recognition and sensing. *Coord. Chem. Rev.* **2013**, *257*, 2434–2455. (c) Langton, M. J.; Beer, P. D. Rotaxane and Catenane Host Structures for Sensing Charged Guest Species. *Acc. Chem. Res.* **2014**, *47*, 1935–1949.
4. (a) Leigh, D. A.; Marcos, V.; Wilson, M. R. Rotaxane catalysts. *ACS Catal.* **2014**, *4*, 4490–4497. For recent examples of rotaxanes as ligands for metal-catalysed reactions see: (b) Hoekman, S.; Kitching, M. O.; Leigh, D. A.; Papmeyer, M.; Roke, D. Goldberg Active Template Synthesis of a [2]Rotaxane Ligand for

Asymmetric Transition-Metal Catalysis. *J. Am. Chem. Soc.* **2015**, *137*, 7656–7659. (c) Galli, M.; Lewis, J. E. M.; Goldup, S. M. A Stimuli-Responsive Rotaxane-Gold Catalyst: Regulation of Activity and Diastereoselectivity. *Angew. Chem. Int. Ed.* **2015**, *54*, 13545–13549.

5. (a) Cotí, K. K.; Belowich, M. E.; Liong, M.; Ambrogio, M. W.; Lau, Y. A; Khatib, H. A; Zink, J. I.; Khashab, N. M.; Stoddart, J. F. Mechanised nanoparticles for drug delivery. *Nanoscale* **2009**, *1*, 16-39. (b) Ambrogio, M. W.; Thomas, C. R.; Zhao, Y.-L.; Zink, J. I.; Stoddart, J. F. Mechanized Silica Nanoparticles: A New Frontier in Theranostic Nanomedicine. *Acc. Chem. Res.* **2011**, *44*, 903–913. (c) Pairault, N.; Barat, R.; Tranoy-Opalinski, I.; Renoux, B.; Thomas, M.; Papot, S. Rotaxane-based architectures for biological applications. *Comptes Rendus Chim.* **2016**, *19*, 103–112. For select recent examples of biologically active rotaxanes see (d) Fernandes, A.; Viterisi, A.; Coutrot, F.; Potok, S.; Leigh, D. A.; Aucagne, V.; Papot, S. Rotaxane-Based Propeptides: Protection and Enzymatic Release of a Bioactive Pentapeptide. *Angew. Chem. Int. Ed.* **2009**, *48*, 6443–6447. (e) Smithrud, D. B.; Wang, X.; Tarapore, P.; Ho, S. Crown Ether Host-Rotaxanes as Cytotoxic Agents. *ACS Med. Chem. Lett.* **2013**, *4*, 27–31. (f) Barat, R.; Legigan, T.; Tranoy-Opalinski, I.; Renoux, B.; Péraudeau, E.; Clarhaut, J.; Poinot, P.; Fernandes, A. E.; Aucagne, V.; Leigh, D. A.; Papot, S. A mechanically interlocked molecular system programmed for the delivery of an anticancer drug. *Chem. Sci.* **2015**, *6*, 2608–2613.

6. (a) Kay, E. R.; Leigh, D. A. Beyond switches: Rotaxane- and catenane-based synthetic molecular motors. *Pure Appl. Chem.* **2008**, *80*, 17–29. (b) Erbas-Cakmak, S.; Leigh, D. A.; McTernan, C. T.; Nussbaumer, A. L. Artificial Molecular Machines. *Chem. Rev.* **2015**, *115*, 10081–10206. (c) Coskun, A.; Banaszak, M.; Astumian, R. D.; Stoddart, J. F.; Grzybowski, B. A. Great expectations: can artificial molecular machines deliver on their promise? *Chem. Soc. Rev.* **2012**, *41*, 19–30.

7. (a) Dietrich-Buchecker, C. O.; Sauvage, J. P. Une nouvelle famille de molecules: les metallo-catenanes. *Tetrahedron Lett.*, 1983, **24**, 5095-5098. (b) Dietrich-Buchecker, C. O.; Sauvage, J. P.;. Kern, J. M. Templated synthesis of interlocked macrocyclic ligands: the catenands. *J. Am. Chem. Soc.*, 1984, **106**, 3043–3045.

8. (a) Ayme, J.-F.; Beves, J. E.; Campbell, C. J.; Leigh, D. A. Template synthesis of molecular knots. *Chem. Soc. Rev.* **2013**, *42*, 1700–1712. (b) Evans, N. H.; Beer, P. D. Progress in the synthesis and exploitation of catenanes since the Millennium. *Chem. Soc. Rev.* **2014**, *43*, 4658.

9. (a) Crowley, J. D.; Goldup, S. M.; Lee, A.-L.; Leigh, D. A; McBurney, R. T. Active metal template synthesis of rotaxanes, catenanes and molecular shuttles. *Chem. Soc. Rev.* **2009**, *38*, 1530–1541. (b) Beves, J. E.; Blight, B. A.; Campbell, C. J.; Leigh, D. A.; McBurney, R. T. Strategies and Tactics for the Metal-Directed Synthesis of Rotaxanes, Knots, Catenanes, and Higher Order Links. *Angew. Chem. Int. Ed.* **2011**, *50*, 9260–9327. (c) Chambron, J.-C.; Sauvage, J.-P. Topologically complex molecules obtained by transition metal templation: it is the presentation that determines the synthesis strategy. *New J. Chem.* **2013**, *37*, 49–57.

10. (a) Vickers, M. S.; Beer, P. D. Anion templated assembly of mechanically interlocked structures. *Chem. Soc. Rev.* **2007**, *36*, 211–225. (b) Mullen, K. M.; Beer, P. D. Sulfate anion templation of macrocycles, capsules, interpenetrated and interlocked structures. *Chem. Soc. Rev.* **2009**, *38*, 1701-1713. (c) Clegg, J. K.; Lindoy, L. F. Template Synthesis. In *Anion Coordination Chemistry*; Bowman-James, K., Bianchi, A., García-España; Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, 2011; pp. 289-320. (d) Spence, G. T.; Beer, P. D. Expanding the Scope of the Anion Templated Synthesis of Interlocked Structures. *Acc. Chem. Res.* **2013**, *46*, 571–586.

11. Barin, G.; Coskun, A.; Fouda, M. M. G.; Stoddart, J. F. Mechanically Interlocked Molecules Assembled by π-π Recognition. *ChemPlusChem* **2012**, *77*, 159–185.

12. (a) Schalley, C. A.; Weilandt, T.; Brüggemann, J.; Vögtle, F. Hydrogen-Bond-Mediated Template Synthesis of Rotaxanes, Catenanes, and Knotanes. *Top. Curr. Chem.* **2005**, *248*, 141–200. (b) Kay, E. R.; Leigh, D. A. Hydrogen Bond-Assembled Synthetic Molecular Motors and Machines. *Top. Curr. Chem.* **2005**, *262*, 133-177.

13. For a recent example of a radical-templated MIM syntheses see: Cheng, C.; Cheng, T.; Xiao, H.; Krzyaniak, M. D.; Wang, Y.; McGonigal, P. R.; Frasconi, M.; Barnes, J. C.; Fahrenbach, A. C.; Wasielewski, M. R.; Goddard, W. A.; Stoddart, J. F. Influence of Constitution and Charge on Radical Pairing Interactions in Tris-radical Tricationic Complexes. *J. Am. Chem. Soc.* **2016**, *138*, 8288–8300.

14. A number of examples of [n]rotaxanes in which [n-1] threads pass through a single macrocycle have also been reported: (a) Klotz, E. J. F.; Claridge, T. D. W.; Anderson, H. L. Homo- and Hetero-[3]Rotaxanes with Two π-Systems Clasped in a Single Macrocycle. *J. Am. Chem. Soc.* **2006**, *128*, 15374–15375. (b) Prikhod'ko, A. I.; Durola, F.; Sauvage, J. Iron(II)-Templated Synthesis of [3]Rotaxanes by Passing Two Threads through the Same Ring. *J. Am. Chem. Soc.* **2008**, *130*, 448–449. (c) Prikhod'ko, A. I.; Sauvage, J.-P. Passing Two Strings through the Same Ring Using an Octahedral Metal Center as Template: A New Synthesis of [3]Rotaxanes. *J. Am. Chem. Soc.* **2009**, *131*, 6794–6807. (d) Goldup, S. M.; Leigh, D. A.; McGonigal, P. R.; Ronaldson, V. E.; Slawin, A. M. Z. Two Axles Threaded Using a Single Template Site: Active Metal Template Macrobicyclic [3]Rotaxanes. *J. Am. Chem. Soc.* **2010**, *132*, 315–320. (e) Cheng, H. M.; Leigh, D. A.; Maffei, F.; McGonigal, P. R.; Slawin, A. M. Z.; Wu, J. En Route to a Molecular Sheaf: Active Metal Template Synthesis of a [3]Rotaxane with Two Axles Threaded through One Ring. *J. Am. Chem. Soc.* **2011**, *133*, 12298–12303. (f) Yamashita, Y.; Mutoh, Y.; Yamasaki, R.; Kasama, T.; Saito, S. Synthesis of [3]Rotaxanes that Utilize the Catalytic Activity of a Macrocyclic Phenanthroline-Cu Complex: Remarkable Effect of the Length of the Axle Precursor. *Chem. Eur. J.* **2015**, *21*, 2139–2145. (g) Hayashi, R.; Mutoh, Y.; Kasama, T.; Saito, S. Synthesis of [3]Rotaxanes by the Combination of Copper-Mediated Coupling Reaction and Metal-Template Approach. *J. Org. Chem.* **2015**, *80*, 7536–7546. (h) Movsisyan, L. D.; Franz, M.; Hampel, F.; Thompson, A. L.; Tykwinski, R. R.; Anderson, H. L. Polyyne Rotaxanes: Stabilization by Encapsulation. *J. Am. Chem. Soc.* **2016**, *138*, 1366–1376. (i) Danon, J. J.; Leigh, D. A.; McGonigal, P. R.; Ward, J. W.; Wu, J. Triply Threaded [4]Rotaxanes. *J. Am. Chem. Soc.* **2016**, *138*, 12643–12647.

15. For select recent reviews on oligo- and poly[n]rotaxanes see: (a) Takata, T. Polyrotaxane and Polyrotaxane Network: Supramolecular Architectures Based on the Concept of Dynamic Covalent Bond Chemistry. *Polym. J.* **2006**, *38*, 1–20. (b) Araki, J.; Ito, K. Recent advances in the preparation of cyclodextrin-based polyrotaxanes and their applications to soft materials. *Soft Matter* **2007**, *3*, 1456. (c) Harada, A.; Hashidzume, A.; Yamaguchi, H.; Takashima, Y. Polymeric Rotaxanes. *Chem. Rev.* **2009**, *109*, 5974–6023. (d) Arunachalam, M.; Gibson, H. W. Recent developments in polypseudorotaxanes and polyrotaxanes. *Prog. Polym. Sci.* **2014**, *39*, 1043–1073. (e) Wei, P.; Yan, X.; Huang, F. Supramolecular polymers constructed by orthogonal self-assembly based on host–guest and metal–ligand interactions. *Chem. Soc. Rev.* **2015**, *44*, 815–832. (f) Hou, X.; Ke, C.; Fraser Stoddart, J. Cooperative capture synthesis: yet another playground for copper-free click chemistry. *Chem. Soc. Rev.* **2016**, *45*, 3766–3780.

16. (a)Wilson, E. A.; Vermeulen, N. A.; McGonigal, P. R.; Avestro, A.-J.; Sarjeant, A. A.; Stern, C. L.; Stoddart, J. F. Formation of a hetero[3]rotaxane by a dynamic component-swapping strategy. *Chem. Commun.* **2014**, *50*, 9665. (b) Neal, E. A.; Goldup, S. M. A Kinetic Self-Sorting Approach to Heterocircuit [3]Rotaxanes. *Angew. Chem. Int. Ed.* **2016**, *55*, 12488–12493. (c) Araki, J.; Ito, K. Recent advances in the preparation of cyclodextrin-based polyrotaxanes and their applications to soft materials. *Soft Matter* **2007**, *3*, 1456. (d) Harada, A.; Hashidzume, A.; Yamaguchi, H.; Takashima, Y. Polymeric Rotaxanes. *Chem. Rev.* **2009**, *109*, 5974–6023. (e) Avestro, A.-J.; Belowich, M. E.; Stoddart, J. F. Cooperative self-assembly: producing synthetic polymers with precise and concise primary structures. *Chem. Soc. Rev.* **2012**, *41*, 5881. (f) Arunachalam, M.; Gibson, H. W. Recent developments in polypseudorotaxanes and polyrotaxanes. *Prog. Polym. Sci.* **2014**, *39*, 1043–1073. (g) Wei, P.; Yan, X.; Huang, F. Supramolecular polymers constructed by orthogonal self-assembly based on host–guest and metal–ligand interactions. *Chem. Soc. Rev.* **2015**, *44*, 815–832.

17. (a) Fuller, A.-M. L.; Leigh, D. A.; Lusby, P. J. One Template, Multiple Rings: Controlled Iterative Addition of Macrocycles onto a Single Binding Site Rotaxane Thread. *Angew. Chem. Int. Ed.* **2007**, *46*, 5015–5019. (b) Daniell, H. W.; Klotz, E. J. F.; Odell, B.; Claridge, T. D. W.; Anderson, H. L. Solid-Phase Synthesis of Oligo(phenylene ethynylene) Rotaxanes. *Angew. Chem. Int. Ed.* **2007**, *46*, 6845–6848. (c) Spruell, J. M.; Dichtel, W. R.; Heath, J. R.; Stoddart, J. F. A One-Pot Synthesis of Constitutionally Unsymmetrical Rotaxanes Using Sequential CuI-Catalyzed Azide–Alkyne Cycloadditions. *Chem. Eur. J.* **2008**, *14*, 4168–4177. (d) Fuller, A.-M. L.; Leigh, D. A.; Lusby, P. J. Sequence Isomerism in [3]Rotaxanes. *J. Am. Chem. Soc.* **2010**, *132*, 4954–4959. (e) Langton, M. J.; Matichak, J. D.; Thompson, A. L.; Anderson, H. L. Template-directed synthesis of π-conjugated porphyrin [2]rotaxanes and a [4]catenane based on a six-porphyrin nanoring. *Chem. Sci.* **2011**, *2*, 1897. (f) Yamada, Y.; Okada, M.;

Tanaka, K. Repetitive stepwise rotaxane formation toward programmable molecular arrays. *Chem. Commun.* **2013**, *49*, 11053. (g) Wang, W.; Chen, L.-J.; Wang, X.-Q.; Sun, B.; Li, X.; Zhang, Y.; Shi, J.; Yu, Y.; Zhang, L.; Liu, M.; Yang, H.-B. Organometallic rotaxane dendrimers with fourth-generation mechanically interlocked branches. *Proc. Natl. Acad. Sci.* **2015**, *112*, 5597–5601.

18. Lewis, J. E. M.; Bordoli, R. J.; Denis, M.; Fletcher, C. J.; Galli, M.; Neal, E. A.; Rochette, E. M.; Goldup, S. M. High yielding synthesis of 2,2`-bipyridine macrocycles, versatile intermediates in the synthesis of rotaxanes. *Chem. Sci.* **2016**, *7*, 3154–3161.

19. Aucagne, V.; Hänni, K. D.; Leigh, D. A.; Lusby, P. J.; Walker, D. B. Catalytic "Click" Rotaxanes: A Substoichiometric Metal-Template Pathway to Mechanically Interlocked Architectures. *J. Am. Chem. Soc.* **2006**, *128*, 2186–2187.

20. Lahlali, H.; Jobe, K.; Watkinson, M.; Goldup, S. M. Macrocycle Size Matters: "Small" Functionalized Rotaxanes in Excellent Yield Using the CuAAC Active Template Approach. *Angew. Chem. Int. Ed.* **2011**, *50*, 4151–4155.

21. Winn, J.; Pinczewska, A.; Goldup, S. M. Synthesis of a Rotaxane Cu[I] Triazolide under Aqueous Conditions. *J. Am. Chem. Soc.* **2013**, *135*, 13318–13321.

22. Lewis, J. E. M.; Winn, J.; Cera, L.; Goldup, S. M. Iterative Synthesis of Oligo[*n*]rotaxanes in Excellent Yield. *J. Am. Chem. Soc.* DOI: 10.1021/jacs.6b08958.

23. (a) Lee, J. W.; Kim, K. Rotaxane dendrimers. *Top. Curr. Chem.* **2003**, *228*, 111–140. (b) Ho, W. K.-W.; Lee, S.-F.; Wong, C.-H.; Zhu, X.-M.; Kwan, C.-S.; Chak, C.-P.; Mendes, P. M.; Cheng, C. H. K.; Leung, K. C.-F. Type III-B rotaxane dendrimers. *Chem. Commun.* **2013**, *49*, 10781. (c) Wang, W.; Chen, L.-J.; Wang, X.-Q.; Sun, B.; Li, X.; Zhang, Y.; Shi, J.; Yu, Y.; Zhang, L.; Liu, M.; Yang, H.-B. Organometallic rotaxane dendrimers with fourth-generation mechanically interlocked branches. *Proc. Natl. Acad. Sci.* **2015**, *112*, 5597–5601.

24. Repeating the reaction at room temperature allowed examination of each step by ^{1}H NMR (see Supporting Information), confirming that following the reaction in EtOH predominantly a single interlocked species was formed, assigned as the triazolide intermediate of the AT-CuAAC reaction, with MS supporting this conclusion. After heating in CH_2Cl_2 and washing with EDTA-NH_3 solution to abstract Cu ions present the main species observed was the [2]rotaxane product, **7**.