How to cite: Angew. Chem. Int. Ed. 2024, e202400344
doi.org/10.1002/anie.202400344

Rotaxanes

# A Platform Approach to Cleavable Macrocycles for the Controlled Disassembly of Mechanically Caged Molecules

*Abed Saady, Georgia K. Malcolm, Matthew P. Fitzpatrick, Noel Pairault, Graham J. Tizzard, Soran Mohammed, Ali Tavassoli, and Stephen M. Goldup**

**Abstract:** Inspired by interlocked oligonucleotides, peptides and knotted proteins, synthetic systems where a macrocycle cages a bioactive species that is "switched on" by breaking the mechanical bond have been reported. However, to date, each example uses a bespoke chemical design. Here we present a platform approach to mechanically caged structures wherein a single macrocycle precursor is diversified at a late stage to include a range of trigger units that control ring opening in response to enzymatic, chemical, or photochemical stimuli. We also demonstrate that our approach is applicable to other classes of macrocycles suitable for rotaxane and catenane formation.

Synthetic mechanically interlocked molecules[1] have been demonstrated in a range of prototypical applications including catalysis,[2] sensing,[2a,3] materials[4] and as molecular machines.[5] The underpinning principle behind many of these applications is that the mechanical bond gives rise to properties that are not found in the individual covalent subcomponents, from the trivial but practically important (e.g., changes in solubility[6]) to the fundamental (e.g., unusual stereochemistry[7]). Although synthetic interlocked molecules were discussed as early as 1912,[8] as with many things, these concepts were already in use in biological systems;[9] catenated DNA was observed for the first time in 1967,[10] around the same time as the first synthetic catenanes were characterized.[11] Later, the threaded structure of microcin J25, a "lasso peptide"[12] was identified.[13] In both cases, the role of the mechanical bond is thought to be to stabilize the biomolecule by protecting it from the action of nucleases and proteases respectively.

[*] Dr. A. Saady, G. K. Malcolm, Prof. S. M. Goldup
School of Chemistry, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK
E-mail: s.m.goldup@bham.ac.uk
Dr. M. P. Fitzpatrick, Dr. N. Pairault, Dr. G. J. Tizzard, Dr. S. Mohammed, Prof. A. Tavassoli
School of Chemistry, University of Southampton, University Road, Southampton, SO17 1BJ, UK

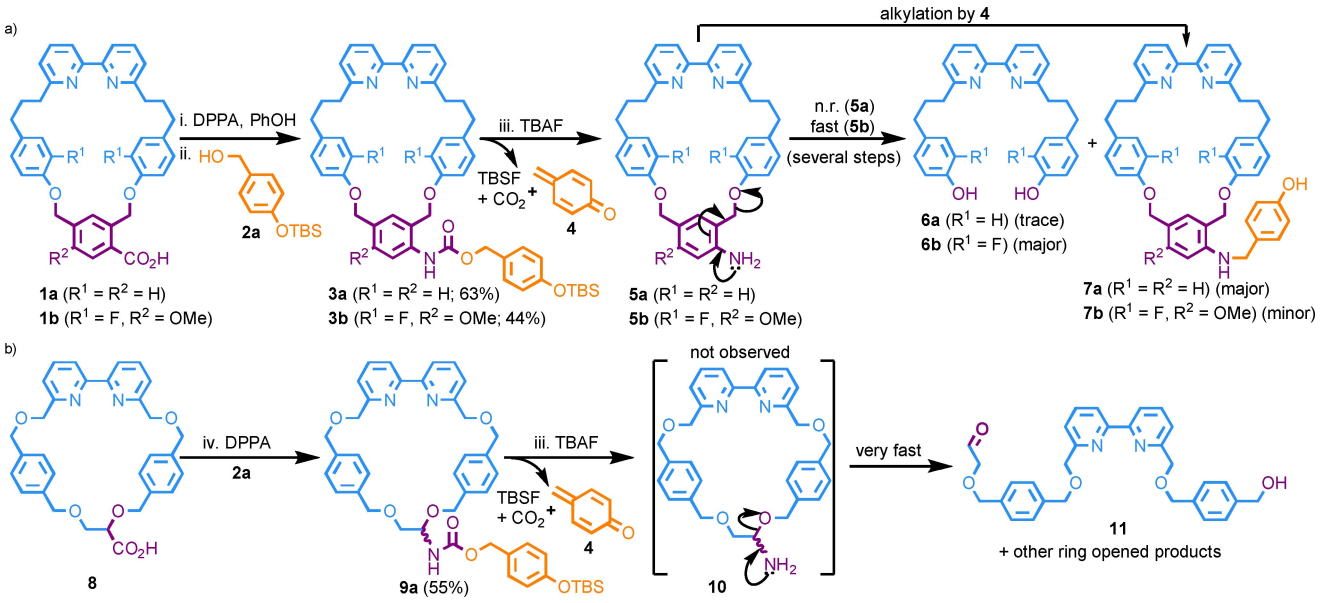


The "caging" of molecules with a desirable function by including self-immolative[14] units in their structure that are made labile in response to enzymatic, chemical or photochemical bond cleavage[15] is a well-established concept in prodrug chemistry chemical biology, and soft materials.[16] More recently examples have appeared in which a mechanical bond in a rotaxane or catenane is used to cage chemical or biological activity. In 2009, Papot, Leigh and co-workers reported a mechanically caged peptidic prodrug in which a macrocycle encircling an axle protects the peptide from protease-mediated degradation and inhibits its biological activity. This activity can be restored when the axle is removed from the macrocycle by esterase-mediated cleavage of the stoppering unit.[17] A second generation of this system responds to β-galactosidase through the cleavage of the macrocyclic component to release a paclitaxel-based prodrug.[18] More recently, Lewis, Vilar and co-workers reported a mechanically caged quadraplex binding Pt complex whose activity can be restored by esterase-mediated or photo-cleavage of the axle.[19] The same concept has also been demonstrated in the context of catalysis; Chiu and co-workers reported a mechanically interlocked Cu complex in which the mechanical bond inhibits the catalytic behavior of the metal ion,[20,21] which can be restored by cleavage of the macrocycle. Papot and co-workers recently reported a catenane that achieves a similar function.[22]

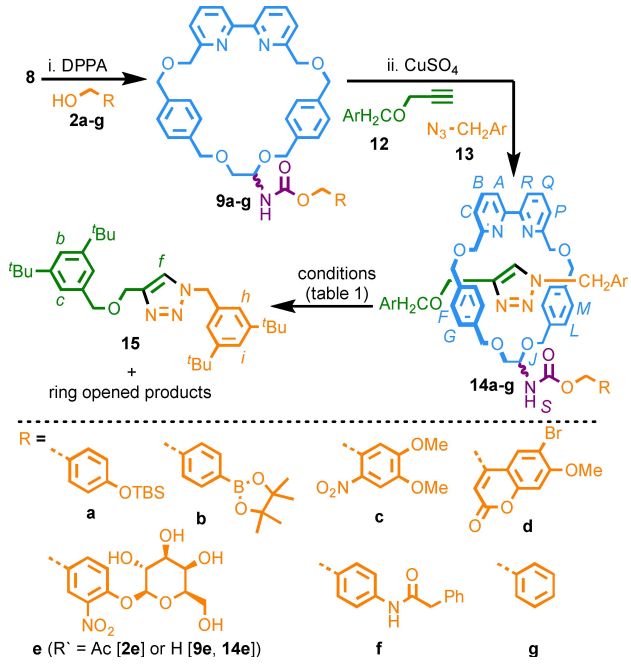
Despite the success of the mechanical caging approach, a drawback of the reported systems is that they are typically bespoke to a specific application or target. Here we present a platform approach for the preparation of mechanically caged structures in which the stimuli responsive unit can be varied at a late stage in the synthesis. To achieve this, we applied a Curtius rearrangement strategy to incorporate the cleavable unit into a common advanced intermediate, and a self-immolative linker based on a methylene alkoxy carbamate self-immolative unit[23] to ensure that the mechanical bond is cleaved rapidly without side reactions that lead to inactive byproducts.

We initially set out to incorporate an *o*-benzylic ether-carbamate unit, as previously used by Papot and co-workers,[18] in a bipyridine-containing macrocycle suitable for the active template[24] Cu-mediated alkyne-azide cycloaddition (AT-CuAAC[25]) reaction[26] such that late stage attachment of the trigger unit was possible. Accordingly, we synthesized carboxylic acid macrocycle **1a** using a concise Ni-mediated strategy.[27] Subsequent reaction with diphenyl phosphoryl azide (DPPA, Scheme 1a) converted **1a** to the corresponding acyl azide that underwent a Curtius




***Scheme 1.*** Synthesis by Curtius rearrangement and ring opening of a) first-generation macrocycles **3** based on an established self-immolative motif (carbamoyl aniline bearing an ortho leaving group); b) second-generation cleavable macrocycle **9** containing a carbamoyl hemiaminal ether self-immolative motif. Reagents and conditions: i. DPPA, $NEt_3$, PhMe, 80 °C; ii. **2a**, DMAP, $NEt_3$, THF, 50 °C; iii. TBAF (3 equiv.), THF–$H_2O$ (3 : 1), 37 °C; iv. **2a**, DPPA, $K_2CO_3$, $CH_2Cl_2$, rt.




***Scheme 2.*** Synthesis of macrocycles **9**, their conversion to rotaxanes **14** and their subsequent axle-releasing reactions (Ar = 3,5-di-$^t$Bu–$C_6H_3$). Reagents and conditions: i. **2**, DPPA, $K_2CO_3$, $CH_2Cl_2$, rt;[31] ii. $CuSO_4$, sodium ascorbate, THF–$H_2O$ (10 : 1), rt, 16 h.[32]

rearrangement[28] in the presence of phenol to give an activated carbamate. This was then reacted with alcohol **2a** to obtain macrocycle **3a**. Treatment of **3a** with a fluoride source was expected to remove the trigger unit[29] to give aniline **5a** that would then ring open to produce an ortho-quinone methide.[30]

However, neither **3a** nor the corresponding rotaxane (see ESI) underwent significant ring opening; only trace amounts of ring opened products were observed by LCMS analysis. Instead, treatment with TBAF resulted in rapid conversion to the corresponding aniline (e.g., **5a**), which persisted and underwent alkylation by *p*-quinone methide **4** to give benzyl substituted aniline **7a**. These results indicated that aniline **5a** is kinetically stable with respect to ring opening, either due to the weak electron donating effect of the aniline unit or the poor leaving group ability of the phenolate. Thus, we synthesized macrocycle **3b**, in which the aniline unit is more electron rich, and the leaving group ability of the phenolate unit is increased. Pleasingly, treatment of **3b** or the corresponding rotaxane (see ESI) with TBAF led to ring opening to give **6b** and other ring opened products as the major species. However, corresponding alkylated byproduct **7b** was also observed.

Although electronically activated macrocycle **3b** underwent ring opening, it is inefficient both from an application (alkylated aniline **7b** was still observed) and a synthetic (9 linear steps from commercial materials, 10 % overall yield) perspective. Thus, we considered other self-immolative linkers and identified the recently reported methylene alkoxy carbamate unit[23] that undergoes cleavage via the corresponding hemi-aminal ether. Thus, we synthesized carboxylic acid macrocycle **8** and successfully applied the Curtius rearrangement strategy to access macrocycle **9a**. Pleasingly, treatment of **9a** with TBAF led to rapid disappearance of the macrocycle and the appearance of ring opened products (e.g., **11**).

***Table 1:*** Summary of the isolated yields for macrocycles **9** and rotaxanes **14**, the stimuli they respond to and their observed reactivity.

| Entry | **9** (yield) | **14** (yield) | Stimulus | **9**[a] | **14**[a] |
|---|---|---|---|---|---|
| 1 | **9a** (55%) | **14a** (72%) | TBAF[29] | ✓ | ✓ |
| 2 | **9b** (42%) | **14b** (57%)[32] | $H_2O_2$[33] | ✓ | ✓ |
| 3 | **9c** (41%) | **14c** (60%) | UV (365 nm)[34] | ✓ | ✓ |
| 4 | **9d** (39%) | **14d** (74%) | UV (365 nm)[35] | ✓ | ✓ |
| 5 | **9e** (45%)[31] | **14e** (77%) | β-galactosidase[36] | ✓ | ✓ |
| 6 | **9f** (48%) | **14f** (86%) | penicillin G-amidase[37] | ✓ | ✗ |
| 7 | **9g** (48%) | **14g** (75%) | TBAF or UV (365 nm) | ✗ | ✗ |

[a] Indicates whether cleavage was observed under previously reported conditions (see ESI section S7) for the corresponding trigger.

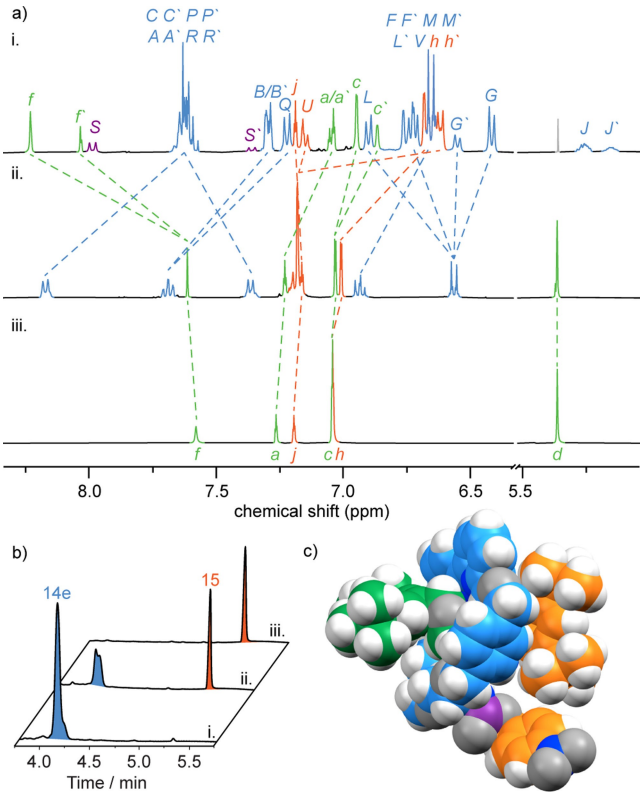
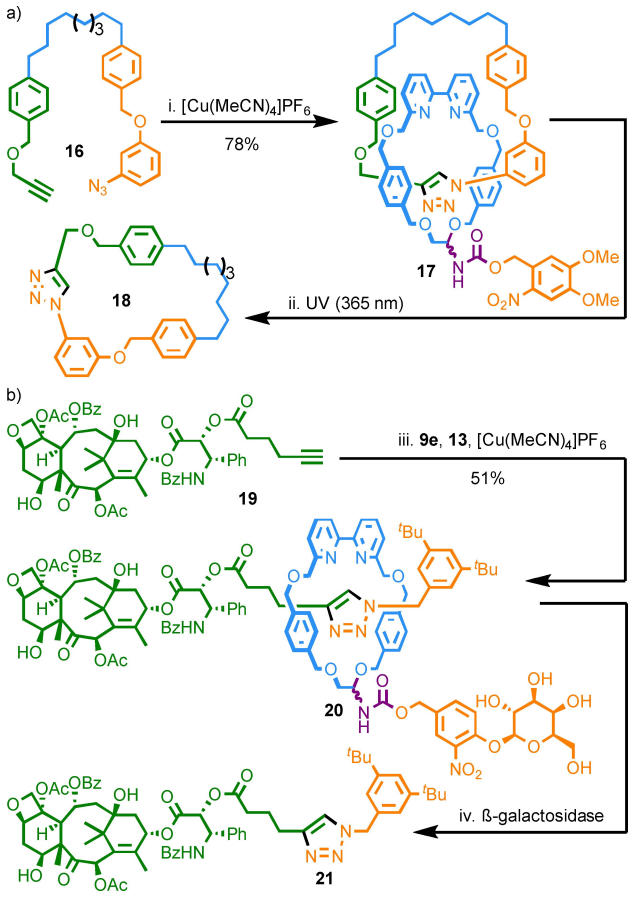
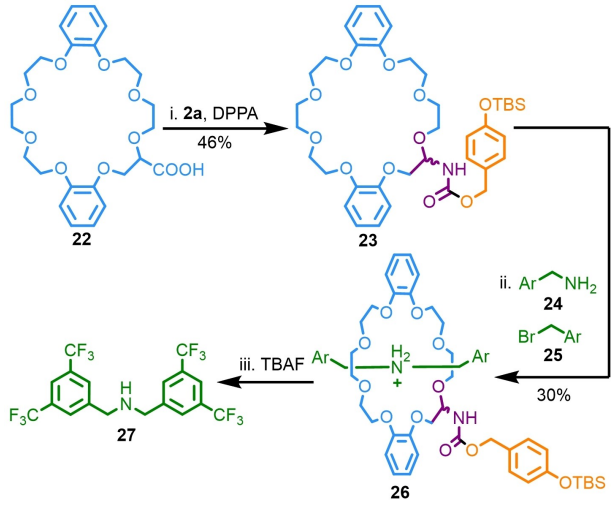



***Figure 1.*** a) $^1$H NMR (THF-$d_8$–$D_2O$ [3:1], 500 MHz, 298 K) analysis of the cleavage of rotaxane **14a**: i. rotaxane **14a** (‘ is used to signify signals arising from the minor diastereomer); ii. rotaxane **14a** + TBAF (3 equiv.) after 1 h; iii. axle **15** (labels and colors as in Scheme 2,. b) LCMS analysis of the cleavage of rotaxane **14e**: i. rotaxane **14e**; ii. rotaxane **14e** + β-galactosidase after 24 h; iii. axle **15**. c) Solid state structure of $NO_2$-derivative of rotaxane **14g** (R = *p*$NO_2$–$C_6H_4$) highlighting the steric crowding caused by the interlocked structure. Colors as in Scheme 2, except O (grey), N (dark blue), H (white).




***Scheme 3.*** a) Synthesis and photochemical cleavage of catenane **17**. b) Synthesis and enzymatic cleavage of paclitaxel-derived rotaxane **20**. Reagents and conditions: i. $[Cu(MeCN)_4]PF_6$, $N^iPr_2Et$, $CHCl_3$–EtOH (1:1), 60 °C, 4 h (pseudo high dilution); ii. Irradiation (UVA, $\lambda_{max}$ = 365 nm), $CH_3CN$–$H_2O$ (9:1), rt; iii. $[Cu(MeCN)_4]PF_6$, $N^iPr_2Et$, $CH_2Cl_2$, rt, 3 h; iv. β-Galactosidase, phosphate buffer-DMSO (9:1), 37 °C, 24 h.

Carboxylic acid macrocycle **8**, which is synthesized in 5 steps from readily available starting materials in reasonable yield (30% overall, see ESI), can be converted to carbamate macrocycles **9** in a single step, making it an excellent platform for developing cleavable macrocycles for mechanically caged molecules. To demonstrate the power of this approach, we synthesized a family of macrocycles **9b–f** containing previously reported trigger units that respond to chemical (**9b**[33]), photochemical (**9c**,[34]**d**[35]) and enzymatic (**9e**,[36]**f**[37]) stimuli (Scheme 2), all of which underwent the expected ring opening reactions (Table 1). We also synthesized benzyl carbamate macrocycle **9g**, which failed to ring




***Scheme 4.*** Synthesis of cleavable crown ether macrocycle, **23** and its conversion to rotaxane **26** (Ar = 3,5-di-$CF_3$–$C_6H_3$). Reagents and conditions: i. DPPA, $K_2CO_3$, $CHCl_3$, 70 °C, 16 h; ii. PhMe, rt; iii. TBAF (3 equiv.), THF, rt, 24 h.

open in response to chemical or photochemical stimuli and was recovered unchanged after treatment under basic (0.1 M NaOH, 24 h) or acidic (0.1 M HCl, 24 h) conditions, confirming that the carbamoyl hemi-aminal ether unit is robust and thus that ring opening of macrocycles **9a–f** takes place via removal of the trigger.

Pleasingly, when rotaxanes **14**, synthesized in good yield (Scheme 2, Table 1) by reaction of macrocycles **9** with alkyne **12** and azide **13** under AT-CuAAC conditions,[26] were subjected to their corresponding uncaging stimulus we were able to observe the release of axle **15** and the appearance of the acyclic products of macrocycle ring opening by $^1$H NMR (e.g., Figure 1a) or LCMS (e.g., Figure 1b)[38] analysis as appropriate. Once again, rotaxane **14g** was robust to either chemical or photochemical stimuli.

$^1$H NMR analysis (Figure 1ai) also highlights the unusual stereochemistry of these molecules; the stereogenic hemi-aminal ether center renders the macrocycle both chiral (synthesized as a racemate) and oriented and so the corresponding rotaxane displays both covalent and mechanical stereochemistry.[7] Thus, rotaxanes **14** are formed as racemic pair of mechanical epimers (~2:1 ratio),[39–41] somewhat complicating their characterization by NMR as because two sets of signals were observed for many resonances (e.g., triazole proton $H_f$), which is exacerbated further in the case of **14e** due to the enantiopure galactose unit.

Unfortunately, in the case of **14f**, we were unable to observe the desired ring opening behavior; no ring opened products were detected by LCMS when **14f** was exposed to penicilin G-amidase under conditions that successfully cleaved **9f**. We tentatively attribute the failure of rotaxane **14f** to undergo enzymatic cleavage, despite the confirmed lability of parent macrocycle, to the steric bulk of the rotaxane moiety preventing substrate access to the enzyme active site. Indeed, the solid-state structure[42] of an analogue of rotaxane **14g** bearing a nitro group (R = *p*$NO_2$–$C_6H_4$, Figure 1c) highlights the globular nature of the interlocked region of these molecules. This suggests that previously reported enzymatic trigger units may require re-optimization for application in interlocked structures.

Bipyridine macrocycles of the form of **9** are versatile intermediates for the synthesis of interlocked molecules.[43] Thus, when alkyne/azide pre-macrocycle **16** was used in place of half-axle components under pseudo-high dilution conditions (Scheme 3a),[44] catenane **17** was produced from macrocycle **9c** in good yield (74 %). As with rotaxane **14c**, irradiation of **17** led to rapid ring opening to release triazole-containing macrocycle **18**. Furthermore, the AT–CuAAC reaction tolerates a wide variety of functionalized substrates. To demonstrate this, we synthesized rotaxane **20** (Scheme 3b), a direct analogue of a caged paclitaxel derivative previously reported by Papot and co-workers,[18] using macrocycle **9e** and paclitaxel-derived alkyne **19**[18] under mild conditions in reasonable yield (51 %). Treatment of **20** with β-galactosidase *in vitro* resulted in ring opening to release axle **21** (See ESI, section S7).[45]

Finally, given that many macrocycles for the synthesis of interlocked structures contain $sp^3$ C–X bonds (X=O or N) within the macrocycle framework,[1] our late stage Curtius rearrangement method for installing a cleavable linker has the potential to be applied in a wide range of contexts. To demonstrate this, we synthesized crown ether **22**[46] that contains a carboxylic acid moiety and converted it to TBAF-cleavable macrocycle **23** (Scheme 4). Macrocycle **23** could be converted to rotaxane **26** under organocatalytic active template conditions[47] in reasonable yield and, as expected, underwent rapid ring opening upon treatment with TBAF to release axle **27**.

In conclusion, we have demonstrated a simple approach to cleavable macrocycles that can be used as a platform for the synthesis of mechanically caged molecules. The key feature of our platform is a late stage Curtius rearrangement that allows us to install the trigger moiety and create a carbamoyl hemiaminal ether self-immolative unit simultaneously. Using this approach, we were able to demonstrate photo-, enzyme- and chemically-cleaved macrocycles, and rotaxanes and catenanes derived from them, by making use of previously reported trigger moieties. Furthermore, we showed that our platform concept can be extended to different types of macrocycles such as crown ethers. Macrocycles **9** and **20** represent an excellent new resource for the preparation of mechanically caged molecules. Furthermore, our Curtius rearrangement approach to install a carbomoyl hemi-aminal ether self-immolative linker can be extended to many other macrocycles for the synthesis of interlocked structures.

## Acknowledgements

SMG thanks the ERC (Agreement no. 724987) and Leverhulme Trust (RPG-2020-399) for funding and the Royal Society for a Wolfson Research Fellowship (RSWF \FT\180010). A. Saady thanks the Council for Higher Education-Israel for a personal fellowship.

## Conflict of Interest

The authors declare no conflict of interest.

## Data Availability Statement

Data (raw characterization data for reported compounds) is available from the UBIRA eData repository at https://doi.org/10.25500/eData.bham.00001053.

**Keywords:** mechanical bonds · rotaxanes · macrocycles · crown ethers · prodrugs


[1] C. J. Bruns, J. F. Stoddart, *The Nature of the Mechanical Bond: From Molecules to Machines*, Wiley **2016**.

[2] a) N. Pairault, J. Niemeyer, *Synlett* **2018**, *29*, 689; b) A. Martinez-Cuezva, A. Saura-Sanmartin, M. Alajarin, J. Berna, *ACS Catal.* **2020**, *10*, 7719; c) R. W. Heard, J. M. Suarez, S. M. Goldup, *Nat. Chem. Rev.* **2022**, *6*, 182.

[3] K. M. Bąk, K. Porfyrakis, J. J. Davis, P. D. Beer, *Mater. Chem. Front.* **2020**, *4*, 1052.

[4] a) S. Mena-Hernando, E. M. Perez, *Chem. Soc. Rev.* **2019**, *48*, 5016; b) B. H. Wilson, S. J. Loeb, *Chem* **2020**, *6*, 1604; c) L. F. Hart, J. E. Hertzog, P. M. Rauscher, B. W. Rawe, M. M. Tranquilli, S. J. Rowan, *Nat. Rev. Mater.* **2021**, *6*, 508.

[5] S. Erbas-Cakmak, D. A. Leigh, C. T. McTernan, A. L. Nussbaumer, *Chem. Rev.* **2015**, *115*, 10081.

[6] M. L. Frampton, G. Sforazzini, S. Brovelli, G. Latini, E. Townsend, C. C. Williams, A. Charas, L. Zalewski, N. S. Kaka, M. Sirish, L. J. Parrott, J. S. Wilson, F. Cacialli, H. L. Anderson, *Adv. Funct. Mater.* **2008**, *18*, 3367.

[7] E. M. G. Jamieson, F. Modicom, S. M. Goldup, *Chem. Soc. Rev.* **2018**, *47*, 5266.

[8] H. L. Frisch, E. Wasserman, *J. Am. Chem. Soc.* **1961**, *83*, 3789.

[9] a) N. Pairault, R. Barat, I. Tranoy-Opalinski, B. Renoux, M. Thomas, S. Papot, *Compt. Rend. Chim.* **2016**, *19*, 103; b) S. R. Beeren, C. T. McTernan, F. Schaufelberger, *Chem* **2023**, *9*, 1378.

[10] B. Hudson, J. Vinograd, *Nature* **1967**, *216*, 647.

[11] a) E. Wasserman, *J. Am. Chem. Soc.* **1960**, *82*, 4433; b) A. S. Baluna, A. Galan, D. A. Leigh, G. D. Smith, J. T. J. Spence, D. J. Tetlow, I. J. Vitorica-Yrezabal, M. Zhang, *J. Am. Chem. Soc.* **2023**, *145*, 9825.

[12] J. D. Hegemann, M. Zimmermann, X. Xie, M. A. Marahiel, *Acc. Chem. Res.* **2015**, *48*, 1909.

[13] a) M. J. Bayro, J. Mukhopadhyay, G. V. Swapna, J. Y. Huang, L. C. Ma, E. Sineva, P. E. Dawson, G. T. Montelione, R. H. Ebright, *J. Am. Chem. Soc.* **2003**, *125*, 12382; b) K. J. Rosengren, R. J. Clark, N. L. Daly, U. Goransson, A. Jones, D. J. Craik, *J. Am. Chem. Soc.* **2003**, *125*, 12464; c) K. A. Wilson, M. Kalkum, J. Ottesen, J. Yuzenkova, B. T. Chait, R. Landick, T. Muir, K. Severinov, S. A. Darst, *J. Am. Chem. Soc.* **2003**, *125*, 12475.

[14] P. L. Carl, P. K. Chakravarty, J. A. Katzenellenbogen, *J. Med. Chem.* **1981**, *24*, 479.

[15] A. Alouane, R. Labruere, T. Le Saux, F. Schmidt, L. Jullien, *Angew. Chem. Int. Ed.* **2015**, *54*, 7492.

[16] Selected recent examples and reviews: a) J. Delahousse, C. Skarbek, A. Paci, *Cancer Chemother. Pharmacol.* **2019**, *84*, 937; b) A. Dal Corso, L. Pignataro, L. Belvisi, C. Gennari, *Chem. Eur. J.* **2019**, *25*, 14740; c) A. Dal Corso, V. Borlandelli, C. Corno, P. Perego, L. Belvisi, L. Pignataro, C. Gennari, *Angew. Chem. Int. Ed.* **2020**, *59*, 4176; d) R. V. Gonzaga, L. A. do Nascimento, S. S. Santos, B. A. Machado Sanches, J. Giarolla, E. I. Ferreira, *J. Pharm. Sci.* **2020**, *109*, 3262; e) I. C. Sun, H. Y. Yoon, D. K. Lim, K. Kim, *Bioconjugate Chem.* **2020**, *31*, 1012; f) Y. Xiao, X. Tan, Z. Li, K. Zhang, *J. Mater. Chem. B* **2020**, *8*, 6697; g) O. Shelef, S. Gnaim, D. Shabat, *J. Am. Chem. Soc.* **2021**, *143*, 21177; h) K. Darrah, J. Wesalo, B. Lukasak, M. Tsang, J. K. Chen, A. Deiters, *J. Am. Chem. Soc.* **2021**, *143*, 18665; i) Y. Xue, H. Bai, B. Peng, B. Fang, J. Baell, L. Li, W. Huang, N. H. Voelcker, *Chem. Soc. Rev.* **2021**, *50*, 4872; j) D. A. Rose, J. W. Treacy, Z. J. Yang, J. H. Ko, K. N. Houk, H. D. Maynard, *J. Am. Chem. Soc.* **2022**, *144*, 6050; k) F. Seidi, Y. Zhong, H. Xiao, Y. Jin, D. Crespy, *Chem. Soc. Rev.* **2022**, *51*, 6652; l) A. G. Gavriel, M. R. Sambrook, A. T. Russell, W. Hayes, *Polym. Chem.* **2022**, *13*, 3188; m) K. An, X. Deng, H. Chi, Y. Zhang, Y. Li, M. Cheng, Z. Ni, Z. Yang, C. Wang, J. Chen, J. Bai, C. Ran, Y. Wei, J. Li, P. Zhang, F. Xu, W. Tan, *Angew. Chem. Int. Ed.* **2023**, *62*, e202306824; n) L. Madegard, M. Girard, B. R. Yaw, K. Porte, D. Audisio, S. Papot, F. Taran, *Chem. Eur. J.* **2023**, *29*, e202301359.

[17] A. Fernandes, A. Viterisi, F. Coutrot, S. Potok, D. A. Leigh, V. Aucagne, S. Papot, *Angew. Chem. Int. Ed.* **2009**, *48*, 6443.

[18] R. Barat, T. Legigan, I. Tranoy-Opalinski, B. Renoux, E. Peraudeau, J. Clarhaut, P. Poinot, A. E. Fernandes, V. Aucagne, D. A. Leigh, S. Papot, *Chem. Sci.* **2015**, *6*, 2608.

[19] T. Kench, P. A. Summers, M. K. Kuimova, J. E. Lewis, R. Vilar, *Angew. Chem. Int. Ed.* **2021**, *60*, 10928.

[20] A. Bessaguet, Q. Blancart-Remaury, P. Poinot, I. Opalinski, S. Papot, *Angew. Chem. Int. Ed.* **2023**, *62*, e202216787.

[21] The ability of the mechanical bond to kinetically stabilize a bound metal ion was termed the "catenand effect" by Sauvage and co-workers (A. M. Albrecht-Gary, Z. Saad, C. O. Dietrich-Buchecker, J. P. Sauvage, *J. Am. Chem. Soc.* **1985**, *107*, 3205). In the example cited, the mechanical bond cages the catalytic activity of the Cu ion. Recently, examples have been reported where the caging of the metal ion reveals hidden reactivity: a) F. Modicom, E. M. G. Jamieson, E. Rochette, S. M. Goldup, *Angew. Chem. Int. Ed.* **2019**, *58*, 3875; b) B. Leforestier, M. R. Gyton, A. B. Chaplin, *Angew. Chem. Int. Ed.* **2020**, *59*, 23500.

[22] A. Bessaguet, Q. Blancart-Remaury, P. Poinot, I. Opalinski, S. Papot, *Angew. Chem. Int. Ed.* **2023**, *62*, e202216787.

[23] a) R. A. Mosey, P. E. Floreancig, *Org. Biomol. Chem.* **2012**, *10*, 7980; b) R. V. Kolakowski, K. T. Haelsig, K. K. Emmerton, C. I. Leiske, J. B. Miyamoto, J. H. Cochran, R. P. Lyon, P. D. Senter, S. C. Jeffrey, *Angew. Chem. Int. Ed.* **2016**, *55*, 7948.

[24] M. Denis, S. M. Goldup, *Nat Rev Chem* **2017**, *1*, 0061.

[25] A. Saady, S. M. Goldup, *Chem* **2023**, *9*, 2110.

[26] a) V. Aucagne, K. D. Hanni, D. A. Leigh, P. J. Lusby, D. B. Walker, *J. Am. Chem. Soc.* **2006**, *128*, 2186; b) H. Lahlali, K. Jobe, M. Watkinson, S. M. Goldup, *Angew. Chem. Int. Ed.* **2011**, *50*, 4151; c) S. M. Goldup, D. A. Leigh, T. Long, P. R. McGonigal, M. D. Symes, J. Wu, *J. Am. Chem. Soc.* **2009**, *131*, 15924.

[27] J. Lewis, R. Bordoli, M. Denis, C. Fletcher, M. Galli, E. Neal, E. Rochette, S. Goldup, *Chem. Sci.* **2016**, *7*, 3154.

[28] V. V. Sureshbabu, G. Chennakrishnareddy, N. Narendra, *Tetrahedron Lett.* **2008**, *49*, 1408.

[29] S. Y. Kim, J. I. Hong, *Org. Lett.* **2007**, *9*, 3109.

[30] S. Gnaim, D. Shabat, *Acc. Chem. Res.* **2014**, *47*, 2970.

[31] Macrocycle **9e** was subjected to hydrolysis (MeONa, THF-MeOH-$H_2O$ [1:1:1, 9 mL], rt, 10 min) prior to isolation.

[32] Rotaxane **14b** was isolated as a mixture of the pinacol boronate ester and the corresponding boronic acid (~4:1 ratio). The isolated yield given is based on the molecular weight of the boronate ester and thus represents a lower limit.

[33] L. C. Lo, C. Y. Chu, *Chem. Commun.* **2003**, 2728.

[34] A. Patchornik, B. Amit, R. B. Woodward, *J. Am. Chem. Soc.* **2002**, *92*, 6333.

[35] T. Furuta, S. S. Wang, J. L. Dantzker, T. M. Dore, W. J. Bybee, E. M. Callaway, W. Denk, R. Y. Tsien, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 1193.
[36] T. Legigan, J. Clarhaut, I. Tranoy-Opalinski, A. Monvoisin, B. Renoux, M. Thomas, A. Le Pape, S. Lerondel, S. Papot, *Angew. Chem. Int. Ed.* **2012**, *51*, 11606.
[37] Y. Meyer, J. A. Richard, B. Delest, P. Noack, P. Y. Renard, A. Romieu, *Org. Biomol. Chem.* **2010**, *8*, 1777.
[38] Although axle release was observed and the intensity of the peak corresponding to **14e** decreased significantly, the reaction did not go to completion over 24 h.
[39] We note that the absolute stereochemistry of rotaxanes **14** and catenane **17** can be fully described using two of three possible stereolabels (covalent, mechanical axial[40] and mechanical planar). We prefer the covalent and mechanical planar description as this highlights that, in the case of catenane **17** the mechanical stereogenic unit can be topological[41] in nature, as it is here. For related recent examples see: a) H. V. Schroder, Y. Zhang, A. J. Link, *Nat. Chem.* **2021**, *13*, 850; b) C. Lopez-Leonardo, A. Saura-Sanmartin, M. Marin-Luna, M. Alajarin, A. Martinez-Cuezva, J. Berna, *Angew. Chem. Int. Ed.* **2022**, *61*, e202209904; c) A. Savoini, P. R. Gallagher, A. Saady, S. M. Goldup, *J. Am. Chem. Soc.* **2024**, DOI: 10.1021/jacs.3c14594.
[40] a) J. R. J. Maynard, P. Gallagher, D. Lozano, P. Butler, S. M. Goldup, *Nat. Chem.* **2022**, *14*, 1038; b) P. R. Gallagher, A. Savoini, A. Saady, J. R. J. Maynard, P. W. V. Butler, G. J. Tizzard, S. M. Goldup, *J. Am. Chem. Soc.* **2024**, DOI: 10.1021/jacs.3c14329.
[41] N. Pairault, F. Rizzi, D. Lozano, E. M. G. Jamieson, G. J. Tizzard, S. M. Goldup, *Nat. Chem.* **2023**, *15*, 781.
[42] Deposition Numbers 2314458 (for **9b**), 2314459 (for **14g**) contain the supplementary crystallographic data for this paper. These data are provided free of charge by the joint Cambridge Crystallographic Data Centre and Fachinformationszentrum Karlsruhe Access Structures service.
[43] a) M. Denis, J. Pancholi, K. Jobe, M. Watkinson, S. M. Goldup, *Angew. Chem. Int. Ed.* **2018**, *57*, 5310; b) M. Denis, L. Qin, P. Turner, K. A. Jolliffe, S. M. Goldup, *Angew. Chem. Int. Ed.* **2018**, *57*, 5315; c) A. W. Heard, S. M. Goldup, *Chem* **2020**, *6*, 994; d) A. Acevedo-Jake, A. T. Ball, M. Galli, M. Kukwikila, M. Denis, D. G. Singleton, A. Tavassoli, S. M. Goldup, *J. Am. Chem. Soc.* **2020**, *142*, 5985.
[44] J. E. M. Lewis, F. Modicom, S. M. Goldup, *J. Am. Chem. Soc.* **2018**, *140*, 4787.
[45] In Papot's original report,[18] the mechanical bond protected the ester unit of the axle such that the rotaxane was biologically inactive. Upon mechanical bond cleavage, the ester was hydrolysed, and the active paclitaxel drug released. Unfortunately, testing of rotaxane **20** in MCF-7 cells with and without stimulated production of β-galactosidase resulted in no significant difference in activity between rotaxane **20** and axle **21**, suggesting that in this case, the ester unit is labile despite the presence of the mechanical bond (see ESI section S8). Work is ongoing to understand the key factors that allow the mechanical bond to protect labile axle functional groups.
[46] Y. Makita, N. Kihara, T. Takata, *Supramol. Chem.* **2021**, *33*, 1.
[47] a) S. D. P. Fielden, D. A. Leigh, C. T. McTernan, B. Perez-Saavedra, I. J. Vitorica-Yrezabal, *J. Am. Chem. Soc.* **2018**, *140*, 6049; b) C. Tian, S. D. P. Fielden, B. Perez-Saavedra, I. J. Vitorica-Yrezabal, D. A. Leigh, *J. Am. Chem. Soc.* **2020**, *142*, 9803; c) C. Tian, S. D. P. Fielden, G. F. S. Whitehead, I. J. Vitorica-Yrezabal, D. A. Leigh, *Nat. Commun.* **2020**, *11*, 744; d) M. J. Power, D. T. J. Morris, I. J. Vitorica-Yrezabal, D. A. Leigh, *J. Am. Chem. Soc.* **2023**, *145*, 8593.

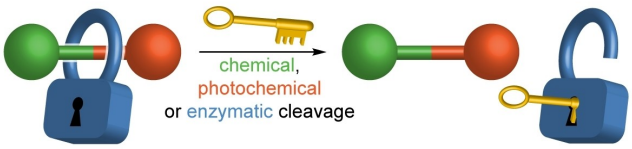
Manuscript received: January 5, 2024
Accepted manuscript online: January 26, 2024
Version of record online: ■■, ■■

# Communications

**Rotaxanes**

A. Saady, G. K. Malcolm, M. P. Fitzpatrick, N. Pairault, G. J. Tizzard, S. Mohammed, A. Tavassoli, S. M. Goldup* _ **e202400344**

A Platform Approach to Cleavable Macrocycles for the Controlled Disassembly of Mechanically Caged Molecules




**A mechanical lock with many keys**. We show that a single macrocyclic precursor can be converted to self-immolative macrocycles that open in response to enzymatic, chemical, or photochemical stimuli. Rotaxanes and catenanes based on these rings can be triggered to release their interlocked partner. This simple macrocycle platform can be used to accelerate the development of mechanically caged molecules.