nature nanotechnology

LETTERS

https://doi.org/10.1038/s41565-018-0105-3

An artificial molecular machine that builds an asymmetric catalyst

Guillaume De Bo[1], Malcolm A. Y. Gall[1], Sonja Kuschel[1], Julien De Winter[2], Pascal Gerbaux[2] and David A. Leigh[1*]

Biomolecular machines perform types of complex molecular-level tasks that artificial molecular machines can aspire to. The ribosome, for example, translates information from the polymer track it traverses (messenger RNA) to the new polymer it constructs (a polypeptide)[1]. The sequence and number of codons read determines the sequence and number of building blocks incorporated into the biomachine-synthesized polymer. However, neither control of sequence[2,3] nor the transfer of length information from one polymer to another (which to date has only been accomplished in man-made systems through template synthesis)[4] is easily achieved in the synthesis of artificial macromolecules. Rotaxane-based molecular machines[5–7] have been developed that successively add amino acids[8–10] (including β-amino acids[10]) to a growing peptide chain by the action of a macrocycle moving along a mono-dispersed oligomeric track derivatized with amino-acid phenol esters. The threaded macrocycle picks up groups that block its path and links them through successive native chemical ligation reactions[11] to form a peptide sequence corresponding to the order of the building blocks on the track. Here, we show that as an alternative to translating sequence information, a rotaxane molecular machine can transfer the narrow polydispersity of a leucine-ester-derivatized polystyrene chain synthesized by atom transfer radical polymerization[12] to a molecular-machine-made homo-leucine oligomer. The resulting narrow-molecular-weight oligomer folds to an α-helical secondary structure[13] that acts as an asymmetric catalyst for the Juliá–Colonna epoxidation[14,15] of chalcones.

To broaden the scope and potential utility of ribosome-inspired rotaxane synthesizing machines[8–10,16,17], we decided to investigate translating length information from a parent polymer to a molecular-machine-synthesized product, with the aim of making a functional molecular object (Fig. 1). Rotaxane-based machines that connect different building blocks in a particular order for sequence-specific synthesis require tracks built through multi-step synthesis[8–10]. This is unnecessary for translating length information from a parent track to a new homo-polymer and so, for this application, we chose for the thread polystyrene bearing leucine ester groups with a narrow molecular weight distribution achieved through atom transfer radical polymerization (ATRP) of styrene with *p*-leucyloxystyrene[12]. A polystyrene-based track should be inert under the molecular machine operation conditions and is relatively rigid compared to other common polyolefins. Corey–Pauling–Koltun (CPK) models suggest that the macrocycle should slide along styrene regions of the track without significant hindrance but that a pendant leucine ester is sufficiently large to act as a barrier towards shuttling. Oligomers of leucine of sufficient length fold to a secondary structure that, like some other short peptides[18], have efficacy in asymmetric catalysis.

Machine **1** was assembled by extending[9,10] one-barrier [2]rotaxane **2** with the leucine-bearing track **3** (Fig. 2a). The synthesis of alkyne-terminated polymer **3** was accomplished through copolymerization of styrene (**5**) and *p*-leucyloxystyrene (**6**) by ATRP[12] (Fig. 2b). The reactivity ratio of styrene and a similar styrene ester, *p*-acetoxystyrene, is close to 1 ($r_1 = 0.89$ and $r_2 = 1.22$; $r_{1(2)}$ is the probability of a chain terminated with monomer 1(2) adding a unit of monomer 1(2) rather than 2(1))[19], and so we anticipated that a styrene/*p*-leucyloxystyrene couple would lead to a random copolymer. We targeted a monomer ratio of 11:1, representing an average distance of ~22 Å (unperturbed dimension)[20] between adjacent ester groups.

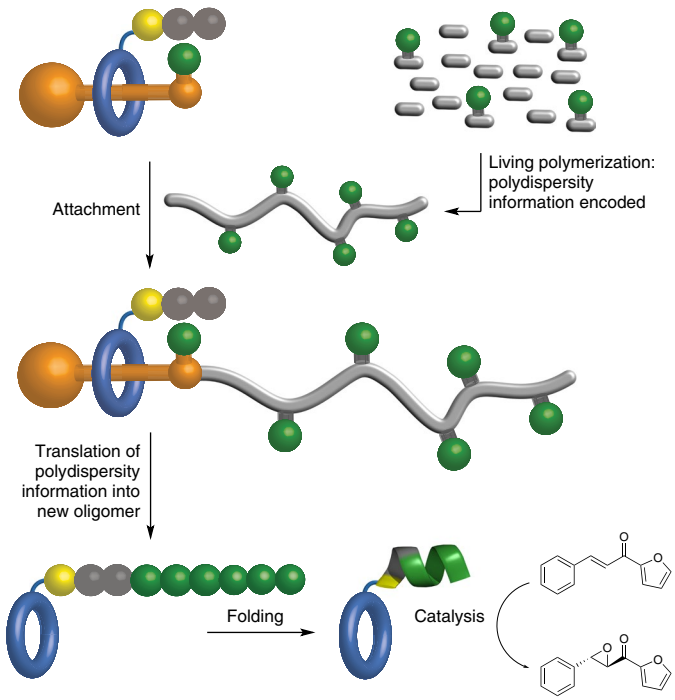

Fig. 1 | Assembly of an artificial molecular machine system that builds an asymmetric catalyst. Translating polydispersity from a parent polymer to a molecular-machine-built oligomer, which folds to a secondary structure that catalyses asymmetric epoxidation of a substrate.

[1]School of Chemistry, University of Manchester, Manchester, UK. [2]Organic Synthesis and Mass Spectrometry Laboratory, Interdisciplinary Center for Mass Spectrometry (CISMa), University of Mons, Mons, Belgium. *e-mail: david.leigh@manchester.ac.uk

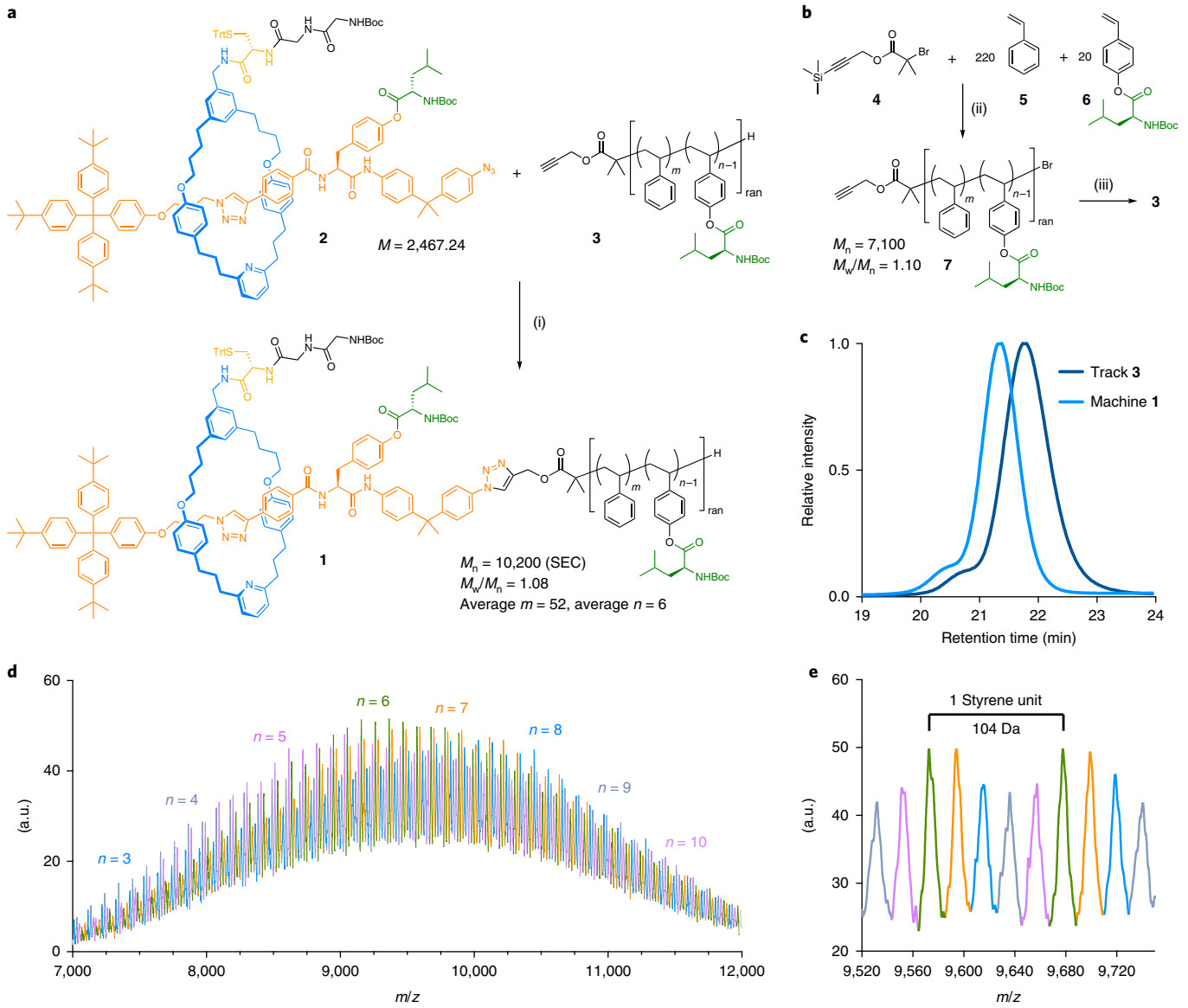

Fig. 2 | Assembly of molecular machine–track conjugate 1 by elongation of rotaxane 2 with polymer 3. a, Synthesis of **1**. (i) $Cu(CH_3CN)_4{\cdot}PF_6$, CH_2Cl_2:^{t}BuOH (4:1), room temperature (RT), 36 h, 96%. **b**, Synthesis of polystyrene **3** by ATRP. (ii) CuBr (1 equiv.), 4,4′-dinonyl-2,2′-dipyridyl (2.1 equiv.), anisole (20 wt%), 90 °C, 24 h, stopped after 22% monomer conversion. (iii) Bu_3SnH (3 equiv.), toluene, 85 °C, 1 h, 80%. **c**, SEC traces of track **3** and machine **1** (THF, 1 ml min^{-1}, refractive index detector, normalized intensities). **d**, MALDI-TOF-MS (positive ion detection) of machine **1** ($[M+Na]^+$) with peaks colour-coded according to the number of leucine residues present. **e**, Expansion of the region between *m/z* 9,520 and 9,750. Adjacent similarly coloured signals correspond to machine–track conjugates that differ in length by a single styrene residue. Adjacent differently coloured signals correspond to machine–track conjugates with different numbers of leucine residues. ran, random co-monomer distribution.

This spacing is similar to the distance (~20 Å) between the building blocks in other rotaxane peptide-synthesizing molecular machines[8–10], which proved to be a good balance between the inter-barrier distance and the overall track length. ATRP polymerization of monomers **5** (220 equiv.) and **6** (20 equiv.) was carried out from the trimethylsilyl(TMS)-alkyne-terminated initiator **4**, stopping at 22% conversion of the monomers, to afford polymer **7** in a process that concomitantly cleaved the silyl group. The resulting polymer had a narrow molar mass dispersity ($M_w/M_n = 1.10$; M_w = mass average molecular weight, M_n = number average molecular weight) and an average molecular weight of 7,100 Da (Fig. 2c). Its composition was in close agreement with the feeding ratio of **5** and **6**, confirming the similar reactivities of the co-monomers. So as to prevent any undesired reaction of the vestigial benzylic bromide during operation of the machine, polymer **7** was subjected to radical debromination mediated by Bu_3SnH to form **3** (Fig. 2b)[21]. Polymer track **3** was appended to [2]rotaxane **2** via copper(I)-catalysed azide-alkyne cycloaddition (CuAAC) with $Cu(CH_3CN)_4{\cdot}PF_6$ to afford the elongated rotaxane **1** in 96% yield (Fig. 2a). The composition of the machine–track composite **1** was determined by size-exclusion chromatography (SEC) (Fig. 2c), matrix-assisted laser desorption ionization–time of flight (MALDI–TOF) mass spectrometry (MS) (Fig. 2d) and ^{1}H and ^{13}C NMR spectroscopy (Supplementary Section 1).

Due to the molar mass dispersity of the polystyrene, machine–track composite **1** consists of a range of track lengths that also contain different numbers of leucine barriers. The ^{1}H NMR spectrum confirms an average of six leucine residues per machine, with a more detailed picture of the chain compositions revealed by MALDI and SEC (Fig. 2c–e). The MALDI profile follows a Gaussian distribution with a median value around 9.6 kDa (Fig. 2d). In Fig. 2d,e the

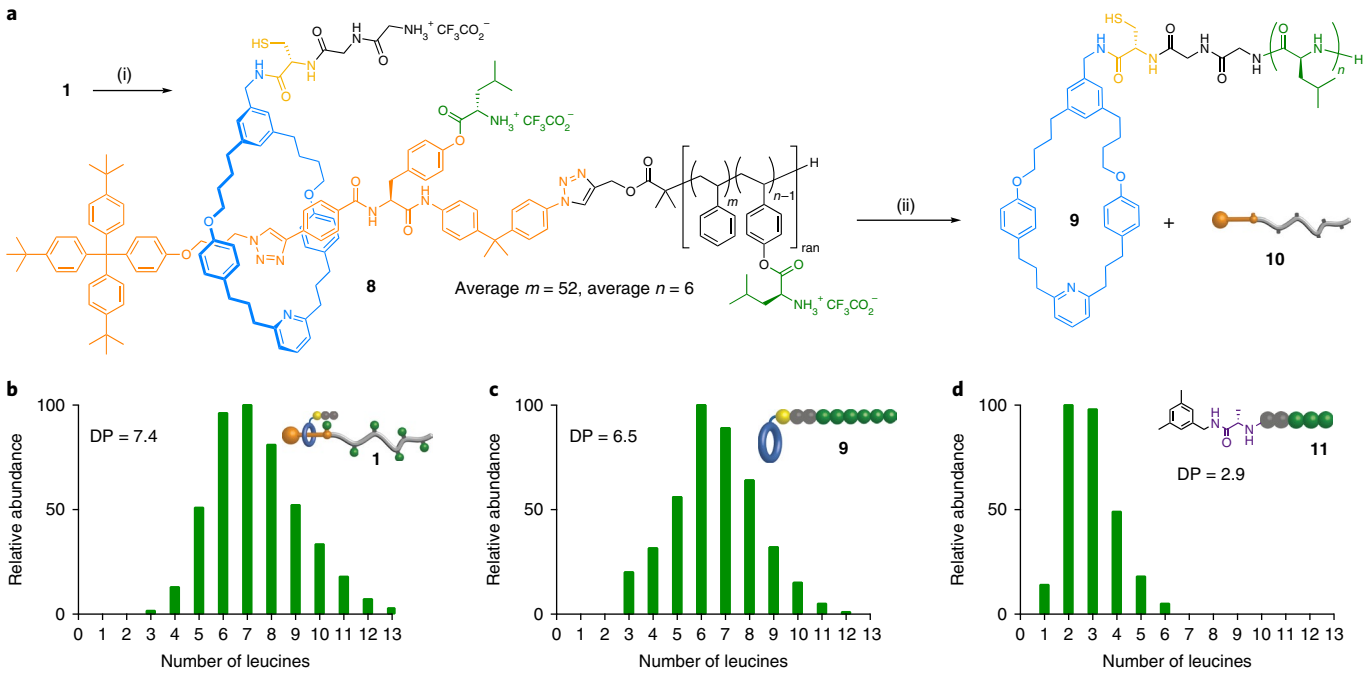

Fig. 3 | Operation of machine–track conjugate 1. a, Operation of machine **1**. (i) CH_2Cl_2:CF_3CO_2H (4:1), $^iPr_3SiH$ (25 equiv.), RT, 2 h, quantitative. (ii) Et_3N (50 equiv.), Ph_3P (3 equiv.), 1 mM in DMF-d_7, 65 °C, 96 h, 50%. **b**, Theoretical composition of the operation product based on Fig. 2c. **c**, Observed composition of the operation product. **d**, Composition of leucine oligomer **11** obtained from a non-machine-promoted polymerization. (i) **S15** (1 equiv.), leucine cresol ester **S12** (6 equiv.), CH_2Cl_2:CF_3CO_2H (4:1), RT, 45 min, quantitative. (ii) 0.5 mM in DMF, Et_3N (50 equiv.), 65 °C, 96 h, 62%. ran, random co-monomer distribution; DP, degree of polymerization in terms of leucine residues.

peaks are colour-coded according to the number of leucine units incorporated into each machine–track conjugate. Determination of the relative abundances is complicated by the overlap of populations separated by five *p*-leucyloxystyrene units. However, the relative abundance of each population can be determined by combining the SEC data with the average composition obtained by NMR (Supplementary Section 1.4), revealing that **1** is composed of molecular machines bearing 3–13 (detected by MALDI) leucine residues, with the most abundant populations carrying 6–7 units.

Machine–track conjugate **1** was deprotected under acidic conditions to reveal the amines and thiol necessary for reaction. Active machine **8** was operated at 65 °C in dimethylformamide (DMF)-d_7 for 4 days in the presence of triethylamine and triphenylphosphine (to minimize disulfide formation[8–10]), affording oligoleucine **9** (Fig. 3a). Following a native chemical ligation (NCL) mechanism, the thiolate reacts with the first leucine phenolic ester that blocks the macrocycle's path on the track to form a thioester. The amino acid residue is then transferred to the glycylglycine amine group, regenerating the catalytic thiolate group in the process (Supplementary Section 2.6). The composition profile of the operation product, determined from the positive ion electrospray ionization (+ESI) mass spectrum, reveals a narrow distribution of leucine oligomers ranging from 3 to 12 units and centred around 6 units (Fig. 3c). These values are in close agreement with the theoretical composition of the operation product calculated from machine–track conjugate **1** (Fig. 3b). The M_w/M_n (1.02) and degree of polymerization (DP) (6.5) values found for **9** closely match the theoretical values ($M_w/M_n = 1.02$, DP = 7.4) determined from Fig. 3b (Supplementary Section 1.5). This contrasts to the distribution obtained from a non-interlocked system (Fig. 3d), where a dummy initiator was reacted in similar conditions with 6 equiv. of a barrier mimic (leucine cresol ester), which furnished only a short oligomer **11** (Supplementary Section 2.8). The results indicate that the molecular machine operates processively and that the polydispersity of the track is translated into the product with high fidelity by the action of the artificial molecular machine.

The room-temperature circular dichroism (CD) spectrum of machine product **9** in methanol has a maximum around 190 nm and two minima at 205 and 220 nm indicative of appreciable α-helix character within the random coil conformation typical of short peptides[13] (Fig. 4d, yellow trace)[22]. Homo-leucine oligomers of sufficient length to form an α-helix have been shown to act as effective asymmetric catalysts in the Juliá–Colonna epoxidation of chalcones[23]. Under similar conditions (Fig. 4c), both the short-chain peptide **11** obtained from the dummy initiator (~2–3 leucine residues attached to AlaGlyGly) and machine product **9** (~6–7 leucine residues attached to CysGlyGly) gave good conversions of furyl chalcone **13** to the corresponding epoxide **14** (100 and 93%, respectively), but with modest enantiomeric excesses (e.e.) (16 and 26%, respectively).

Oxidation of the thiol of **9** to the corresponding sulfonic acid occurs under the conditions of the Juliá–Colonna epoxidation, disrupting the α-helix hydrogen-bonding network. We therefore carried out reductive radical desulfurization of **9** to form the corresponding alanine derivative **12** (Fig. 4a)[24]. The CD spectrum of the desulfurized product, **12**, showed similar α-helical secondary structure to **9** (Fig. 4d, purple trace) and, pleasingly, in the Juliá–Colonna **12** afforded epoxide **14** with quantitative conversion and excellent enantioselectivity (92% e.e.) in 18 h at room temperature (Fig. 4a) (higher conversion and similar asymmetric control to polyleucine itself[23]).

An artificial molecular machine has been developed that traverses a 50+ monomer unit long polystyrene track, picking up and connecting pendant building blocks to construct a new molecular chain with polydispersity information translated from the parent polymer. The time the machine takes to add six to seven leucine residues from this track (96 h) is significantly longer than the 36 h required to add three amino-acid residues in a previous peptide-synthesizing

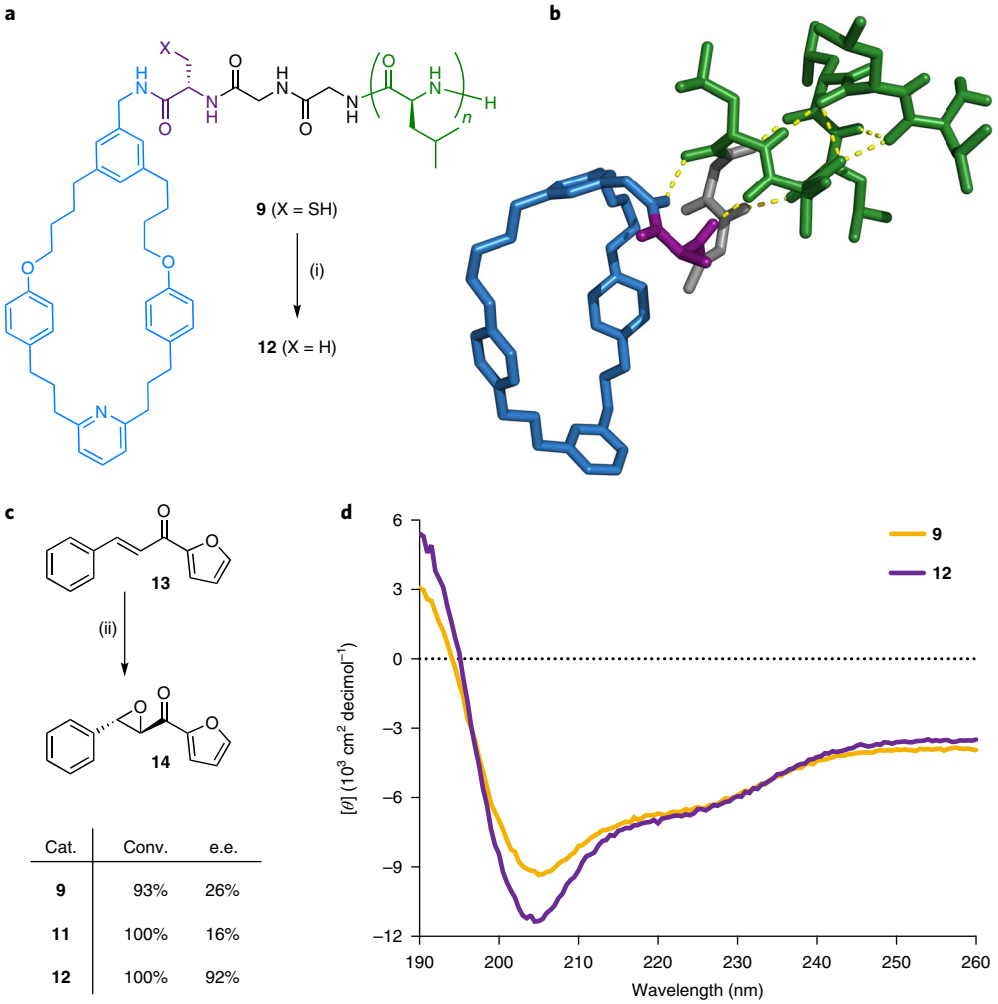

Fig. 4 | α-Helicity of operation product oligoleucines before (9) and after (12) post-operational modification and their asymmetric Juliá–Colonna epoxidation of furyl chalcone 13. a, Reductive desulfurization of **9**. (i) 2,2′-Azobis(2-methylpropionamidine)dihydrochloride (V-50), ^{t}BuSH, Et_3N, tris(2-carboxyethyl)phosphine (TCEP)·HCl, DMF, RT, 22 h, 77%. **b**, Energy-minimized structure of seven-leucine **12** (DFT calculation, B3LYP 6-31*G) featuring a hydrogen-bond-stabilized α-helix (non-amide hydrogen atoms not shown for clarity). **c**, Epoxidation of furyl chalcone **13** using **9**, **11** or **12** as catalyst. Conversions determined by ^{1}H NMR and e.e. by chiral HPLC. (ii) **9** (15 mol%), **11** (10 mol%) or **12** (10 mol%), urea·H_2O_2, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), tetrahydrofuran (THF), RT, 4 d (**9**) or 18 h (**11**, **12**). **d**, CD spectra (298 K, MeOH) of **9** (0.137 mM, yellow trace) and **12** (0.140 mM, purple trace).

machine[8], as a consequence of both the size of the cyclic transition state and the length of track the macrocycle can access increasing with every amino acid addition. Biological synthesizing machines such as the ribosome[1] overcome the second issue through ratcheting[5], a feature introduced in other synthetic molecular machines[25–28] that might be usefully incorporated into future molecular synthesizer designs. Unlike shorter oligomers, the product of the oligoleucine synthesizing molecular machine folds to a persistent secondary structure, an α-helix, that enables it to act as an effective asymmetric catalyst. The synthesis of a folded oligopeptide catalyst by a molecular machine that moves along a track is reminiscent of the way enzymes are produced in biology. Learning how to mimic aspects of the tasks performed by biomolecular machines with much simpler structures should prove useful in understanding how to design and build increasingly complex small-molecule machines[29–31], in terms of both mechanism and what they can achieve.

Methods

Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41565-018-0105-3.

Received: 7 January 2018; Accepted: 23 February 2018;
Published online: 02 April 2018

References

1. Yonath, A. Hibernating bears, antibiotics, and the evolving ribosome (Nobel Lecture). *Angew. Chem. Int. Ed.* **49**, 4340–4354 (2010).
2. Lutz, J.-F., Ouchi, M., Liu, D. R. & Sawamoto, M. Sequence-controlled polymers. *Science* **341**, 1238149 (2013).
3. ten Brummelhuis, N. Controlling monomer-sequence using supramolecular templates. *Polym. Chem.* **6**, 654–667 (2015).
4. Polowinski, S. Template polymerisation and co-polymerisation. *Prog. Polym. Sci.* **27**, 537–577 (2002).
5. Erbas-Cakmak, S., Leigh, D. A., McTernan, C. T. & Nussbaumer, A. L. Artificial molecular machines. *Chem. Rev.* **115**, 10081–10206 (2015).
6. Sauvage, J.-P. From chemical topology to molecular machines (Nobel Lecture). *Angew. Chem. Int. Ed.* **56**, 11080–11093 (2017).
7. Stoddart, J. F. Mechanically interlocked molecules (MIMs)—molecular shuttles, switches, and machines (Nobel Lecture). *Angew. Chem. Int. Ed.* **56**, 11094–11125 (2017).
8. Lewandowski, B. et al. Sequence-specific peptide synthesis by an artificial small-molecule machine. *Science* **339**, 189–193 (2013).
9. De Bo, G. et al. Efficient assembly of threaded molecular machines for sequence-specific synthesis. *J. Am. Chem. Soc.* **136**, 5811–5814 (2014).

10. De Bo, G. et al. Sequence-specific β-peptide synthesis by a rotaxane-based molecular machine. *J. Am. Chem. Soc.* **139**, 10875–10879 (2017).
11. Dawson, P. E., Muir, T. W., Clark-Lewis, I. & Kent, S. B. Synthesis of proteins by native chemical ligation. *Science* **266**, 776–779 (1994).
12. Matyjaszewski, K. & Tsarevsky, N. V. Macromolecular engineering by atom transfer radical polymerization. *J. Am. Chem. Soc.* **136**, 6513–6533 (2014).
13. Andrews, M. J. I. & Tabor, A. B. Forming stable helical peptides using natural and artificial amino acids. *Tetrahedron* **55**, 11711–11743 (1999).
14. Juliá, S., Masana, J. & Vega, J. C. 'Synthetic enzymes'. Highly stereoselective epoxidation of chalcone in a triphasic toluene-water-poly[(S)-alanine] system. *Angew. Chem. Int. Ed.* **19**, 929–931 (1980).
15. Juliá, S. et al. Synthetic enzymes. Part 2. Catalytic asymmetric epoxidation by means of polyamino-acids in a triphase system. *J. Chem. Soc., Perkin Trans.* **1**, 1317–1324 (1982).
16. Thordarson, P., Bijsterveld, E. J. A., Rowan, A. E. & Nolte, R. J. M. Epoxidation of polybutadiene by a topologically linked catalyst. *Nature* **424**, 915–918 (2003).
17. van Dongen, S. F. M. et al. A clamp-like biohybrid catalyst for DNA oxidation. *Nat. Chem.* **5**, 945–951 (2013).
18. Lewandowski, B. & Wennemers, H. Asymmetric catalysis with short-chain peptides. *Curr. Opin. Chem. Biol.* **22**, 40–46 (2014).
19. Braun, D. et al. Analysis of the linear methods for determining copolymerization reactivity ratios, VII. A critical reexamination of radical copolymerizations of styrene. *Angew. Makromol. Chem.* **125**, 161–205 (1984).
20. Young, R. J. & Lovell, P. A. *Introduction to Polymers* 3rd edn (CRC Press, Boca Raton, 2011).
21. Coessens, V. & Matyjaszewski, K. Dehalogenation of polymers prepared by atom transfer radical polymerization. *Macromol. Rapid Comm.* **20**, 66–70 (1999).
22. Beychok, S. Circular dichroism of biological macromolecules. *Science* **154**, 1288–1299 (1966).
23. Flood, R. W. et al. Efficient asymmetric epoxidation of α,β-unsaturated ketones using a soluble triblock polyethylene glycol–polyamino acid catalyst. *Org. Lett.* **3**, 683–686 (2001).
24. Wan, Q. & Danishefsky, S. J. Free-radical-based, specific desulfurization of cysteine: a powerful advance in the synthesis of polypeptides and glycopolypeptides. *Angew. Chem. Int. Ed.* **46**, 9248–9252 (2007).
25. Hernández, J. V., Kay, E. R. & Leigh, D. A. A reversible synthetic rotary molecular motor. *Science* **306**, 1532–1537 (2004).
26. Chatterjee, M. N., Kay, E. R. & Leigh, D. A. Beyond switches: ratcheting a particle energetically uphill with a compartmentalized molecular machine. *J. Am. Chem. Soc.* **128**, 4058–4073 (2006).
27. Serreli, V., Lee, C.-F., Kay, E. R. & Leigh, D. A. A molecular information ratchet. *Nature* **445**, 523–527 (2007).
28. Wilson, M. R. et al. An autonomous chemically fuelled small-molecule motor. *Nature* **534**, 235–240 (2016).
29. von Delius, M., Geertsema, E. M. & Leigh, D. A. A synthetic small molecule that can walk down a track. *Nat. Chem.* **2**, 96–101 (2010).
30. Kassem, S., Lee, A. T. L., Leigh, D. A., Markevicius, A. & Solà, J. Pick-up, transport and release of a molecular cargo using a small-molecule robotic arm. *Nat. Chem.* **8**, 138–143 (2015).
31. Kassem, S. et al. Stereodivergent synthesis with a programmable molecular machine. *Nature* **549**, 374–378 (2017).

Acknowledgements

The authors thank M. Turner and J. Behrendt for assistance with the SEC instrumentation, J. Clayden and M. De Poli for assistance with CD measurements and G. Smith for MALDI analysis of earlier related systems. The UMONS MS laboratory acknowledges the Fonds National de la Recherche Scientifique (FRS-FNRS) for its contribution to acquisition of the Waters QToF Premier and Synapt G2-Si mass spectrometers and for continuing support. This research was funded by the Engineering and Physical Sciences Research Council (EP/P027067/1). The authors thank the Royal Society for a University Research Fellowship (to G.D.B.) and a Research Professorship (to D.A.L.).

Author contributions

G.D.B., M.A.Y.G. and S.K. planned and carried out the experimental work. J.D.W. and P.G. performed the MS analysis of polymers **1** and **3**. D.A.L. directed the research. All authors contributed to the analysis of the results and the writing of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41565-018-0105-3.

Reprints and permissions information is available at www.nature.com/reprints.

Correspondence and requests for materials should be addressed to D.A.L.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Methods

Deprotection of machine 1. A solution of machine **1** (10 mg, 1.06 µmol, 1 equiv.) and $^{i}Pr_3SiH$ (5.5 µl, 26.40 µmol, 25 equiv.) in CH_2Cl_2 (400 µl) and CF_3CO_2H (100 µl) was stirred for 2 h at room temperature. The solvent was removed by azeotropic distillation with toluene (2 × 2 ml). The solid residue was dried for 30 min under high vacuum, then washed with Et_2O (5 × 1 ml). The residual crude solid (**8**) was dried for 30 min under high vacuum, dissolved in DMF-d_7 (deuterated for ease of analysis) and operated without further purification.

Operation of deprotected machine 8. A solution of freshly deprotected machine **8** (1.06 µmol, 1 equiv.) in DMF-d_7 (1 ml) was added to a capped 5 ml Biotage vial loaded with PPh_3 (0.8 mg, 3.2 µmol, 3 equiv.) and purged with five vacuum/nitrogen cycles. The resulting solution was degassed via nitrogen sparging for 5 min. NEt_3 (7.5 µl, 53.0 µmol, 50 equiv.) was added and the solution was stirred at 65 °C for 96 h. An aliquot was removed for analysis (100 µl) and diluted with liquid chromatography–mass spectrometry (LC-MS) grade MeOH (1 ml). A precipitate formed. The solution was filtered over a 0.45 µm polytetrafluoroethylene (PTFE) membrane. MS analysis of the filtrate confirmed formation of the expected product, while ^{1}H NMR of the precipitate indicated it consisted of the building-block-free thread. The filtrate was concentrated to dryness and purified by preparative thin layer chromatography (TLC) (Merck, 500 µm, CH_2Cl_2:MeOH:$NH_{3(aq)}$ (100:10:0.5), 1 elution). The fraction at retention factor (R_f) = 0.5 was selected. The recovered solid was washed with MeOH (5 × 1 ml). The combined MeOH fractions were concentrated under reduced pressure to afford **9** as a colourless solid (0.7 mg, 50%).

Data availability. The data that support the findings of this study are available within the paper and its Supplementary Information, or are available from the Mendeley data repository (https://data.mendeley.com/) under doi 10.17632/3bybgz2jhz.1.