

## Durham Research Online

---

### Deposited in DRO:

18 September 2017

### Version of attached file:

Accepted Version

### Peer-review status of attached file:

Peer-reviewed

### Citation for published item:

De Bo, Guillaume and Gall, Malcolm A. Y. and Kitching, Matthew O. and Kuschel, Sonja and Leigh, David A. and Tetlow, Daniel J. and Ward, John W. (2017) 'Sequence-specific -peptide synthesis by a rotaxane-based molecular machine.', *Journal of the American Chemical Society.*, 139 (31). pp. 10875-10879.

### Further information on publisher's website:

<https://doi.org/10.1021/jacs.7b05850>

### Publisher's copyright statement:

This document is the Accepted Manuscript version of a Published Work that appeared in final form in *Journal of the American Chemical Society*, copyright © American Chemical Society after peer review and technical editing by the publisher. To access the final edited and published work see <https://doi.org/10.1021/jacs.7b05850>.

### Additional information:

## Use policy

---

The full-text may be used and/or reproduced, and given to third parties in any format or medium, without prior permission or charge, for personal research or study, educational, or not-for-profit purposes provided that:

- a full bibliographic reference is made to the original source
- a [link](#) is made to the metadata record in DRO
- the full-text is not changed in any way

The full-text must not be sold in any format or medium without the formal permission of the copyright holders.

Please consult the [full DRO policy](#) for further details.

# Sequence-Specific Beta-Peptide Synthesis by a Rotaxane-Based Molecular Machine

Guillaume De Bo, Malcolm A. Y. Gall, Matthew O. Kitching, Sonja Kuschel, David A. Leigh,\* Daniel J. Tetlow, and John W. Ward

School of Chemistry, University of Manchester, Oxford Road, Manchester, M13 9PL, United Kingdom.

*Supporting Information Placeholder*

---

**ABSTRACT:** We report on the synthesis and operation of a three-barrier, rotaxane-based, artificial molecular machine capable of sequence-specific beta-*homo* ( $\beta^3$ ) peptide synthesis. The machine utilizes non-proteinogenic  $\beta^3$ -amino acids, a class of amino acids not generally accepted by the ribosome, particularly consecutively. Successful operation of the machine *via* native chemical ligation (NCL) demonstrates that even challenging 15- and 19-membered ligation transition states are suitable for information translation using this artificial molecular machine. The peptide-bond-forming catalyst region can be removed from the transcribed peptide by peptidases; artificial and biomachines working in concert to generate a product that cannot be made by either machine alone.

---

## INTRODUCTION

Biomolecular machinery often carries out molecular construction with high efficiency and exquisite selectivity.<sup>1</sup> A stand-out example is the ribosome,<sup>2</sup> which translates genetic information encoded in an mRNA strand *via* the sequence-specific assembly of tRNA-loaded amino acid building blocks, generally beginning with the start-codon AUG which encodes for methionine.<sup>3</sup> However, the ribosome is incapable of the consecutive addition of unnatural D-amino acids,<sup>4,5</sup> even when they are functionalized with natural tRNA codons. Although bioengineering can go some way to addressing these limitations, modest changes to substrate or machine often lead to a loss of function in a largely unpredictable manner.<sup>6</sup>

Chemical routes to sequence-defined polymers are being developed,<sup>7</sup> including the use of artificial small-molecule machines<sup>8</sup> and DNA-based<sup>9</sup> templates and machines. Determining the structural tolerance and versatility of such systems is important. A rotaxane-based machine<sup>10</sup> that sequentially adds successive  $\alpha$ -amino acids to a growing peptide chain has previously been described.<sup>8</sup> The design uses native chemical ligation<sup>11</sup> (NCL) to translate sequence information from the molecular machine thread into the newly synthesized oligomer. The synthesis of non-proteinogenic  $\beta^3$ -peptides is an intriguing target for artificial molecular machines, as  $\beta^3$ -peptides are not normally made by natural ribosomes<sup>12</sup> and they exhibit enhanced biostability relative to their  $\alpha$ -peptide congeners.<sup>13</sup> Here we report on the modular synthesis and sequence-specific operation of a rotaxane-based molecular machine bearing three  $\beta^3$ -amino acid building blocks. Operating through challengingly sized NCL transition

states, the molecular device assembles a mixed  $\alpha\beta^3$ -hexapeptide of defined sequence. Furthermore, the inherent selectivity of peptidases allows the peptide sequence to be selectively hydrolyzed at the C-terminus  $\alpha$ -amino acid region to liberate the  $\beta^3$ -peptide fragment from the artificial translation machinery through which it was assembled.

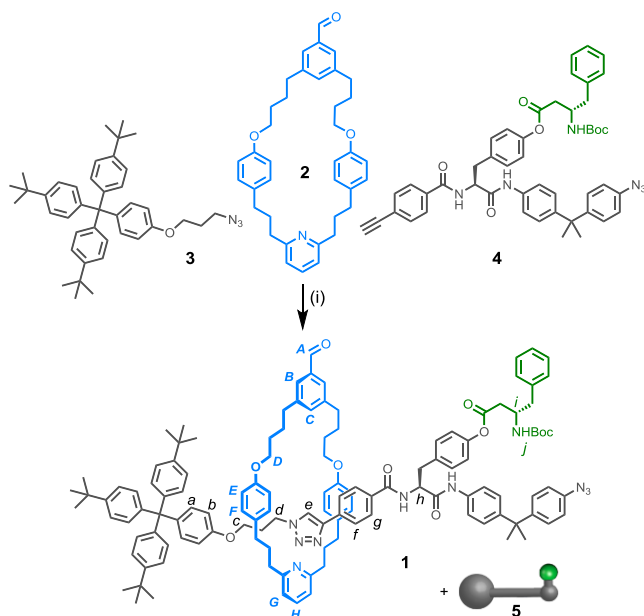
## RESULTS AND DISCUSSION

In order to construct the full molecular machine from a single barrier [2]rotaxane synthon,<sup>8c</sup> and to confirm that the extra carbon atom in  $\beta^3$ -amino acids does not prevent them acting as barriers to macrocycle shuttling along the rotaxane thread, we prepared rotaxane **1** (Scheme 1). Copper-catalyzed active metal template<sup>14</sup> synthesis of [2]rotaxane **1**, bearing a  $\beta^3$ -Phe amino acid barrier, was carried out using minor modifications to a previously reported protocol for an  $\alpha$ -phenylalanine blocking group (Scheme 1).<sup>8c</sup> Treatment of macrocycle **2** with  $\text{CuPF}_6 \cdot (\text{MeCN})_4$ , followed by addition of bulky azide **3** and  $\beta^3$ -amino acid-bearing group **4** afforded rotaxane **1** in 40 % yield, accompanied by 7 % of free thread **5**.

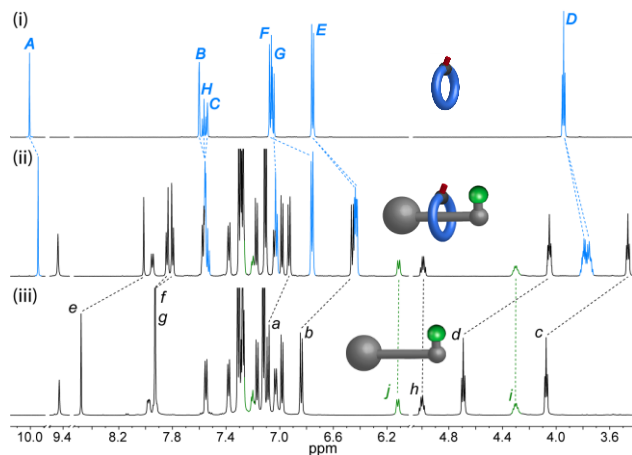
The mechanically interlocked structure of **1** was confirmed by <sup>1</sup>H NMR spectroscopy and mass spectrometry (Figure 1 and Supporting Information). In comparison with non-interlocked components **2** and **5**, rotaxane **1** features significant upfield shifts of proton resonances on both the triazole ( $H_c$ ) and the *n*-propyl tether ( $H_d$  and  $H_e$ ), consistent with shielding from the aromatic rings of the macrocycle. Similar shielding effects occur for several macrocycle protons (e.g.  $H_D$ ,  $H_E$  and  $H_F$ ), in addition to diastereotopic splitting of  $H_E$  and  $H_D$  caused by the unsymmetrical threaded axle. Protons  $H_h$ ,  $H_i$  and  $H_j$  experi-

ence negligible shifts, indicating that the macrocycle resides over a distal region of the thread. Rotaxane **1** did not dethread in solution over several weeks.

### Scheme 1. Active Metal Template Synthesis of Rotaxane Synthons **1**<sup>a</sup>



<sup>a</sup>Reagents and conditions: (i) **2** (1.5 equiv.), CuPF<sub>6</sub>(MeCN)<sub>4</sub> (0.5 equiv.), **3** (6 equiv.), **4** (1 equiv.), CH<sub>2</sub>Cl<sub>2</sub>:<sup>t</sup>BuOH (6:1), r.t., 16 h, 40 % of **1**, 7 % of **5**.



**Figure 1.** <sup>1</sup>H NMR spectra (600 MHz, *d*<sub>6</sub>-acetone, 298 K) of (i) macrocycle **2**, (ii) rotaxane **1**, (iii) free thread **5**. The assignments correspond to the lettering shown in Scheme 1.

Having established the structure and stability of the one-barrier rotaxane synthons, our attention turned to the assembly of three-barrier molecular machine **6** (Scheme 2). Rotaxane **1** and two-barrier extension unit **7** were joined by a CuAAC reaction in close-to-quantitative yield (Scheme 2, step (i)), followed by hydrazone formation *via* the direct condensation of Boc-Gly-Gly-Cys(Trt)-NHNH<sub>2</sub>,

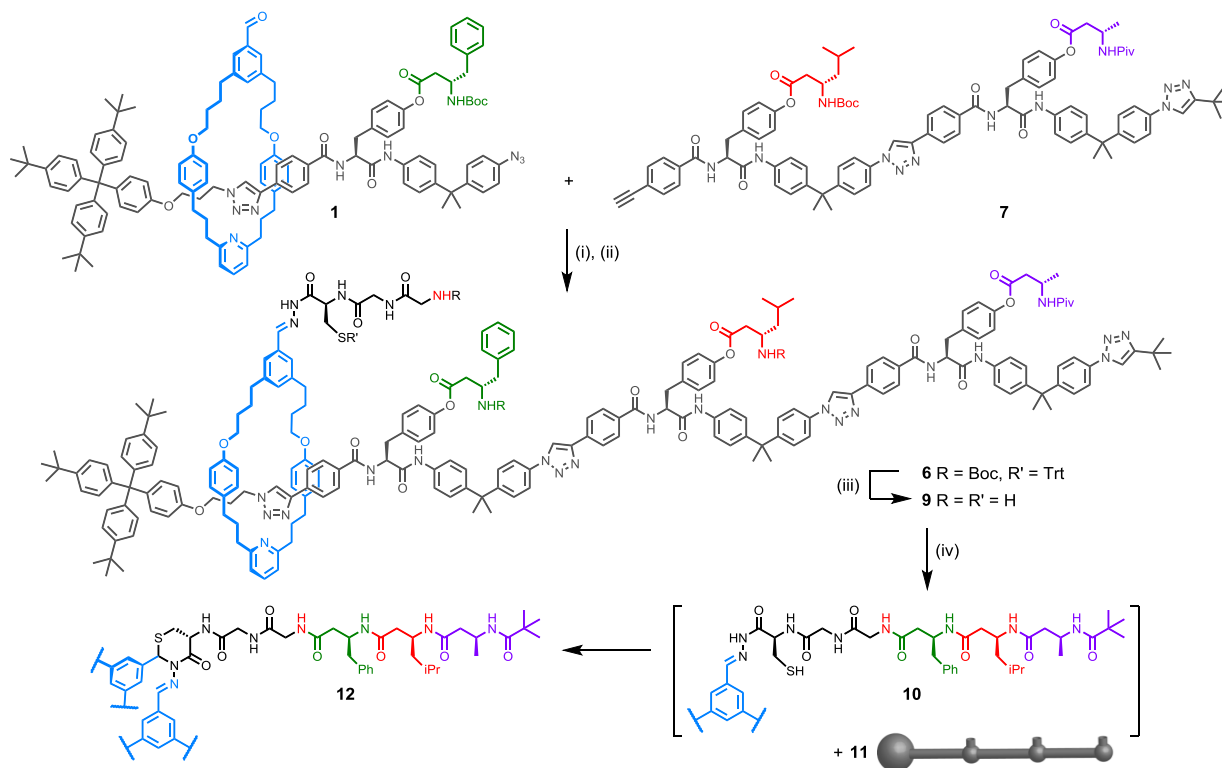
$\alpha$ -peptide catalytic unit hydrazide **8** (Scheme 2, step (ii)). This formed **6**, a rotaxane-based molecular machine bearing three different non-proteinogenic amino acid building blocks appropriate for the sequence-specific assembly of a  $\beta^3$ -peptide.

Following cleavage of the acid-labile Boc and trityl groups of **6** with trifluoroacetic acid (TFA) and triisopropylsilane (TIPSH), molecular machine **9** was operated for 7 days in the presence of triethylamine (Scheme 2). In successive NCL iterations, the macrocycle removes each amino acid barrier in turn, each cleavage event allowing the ring to move further along the length of the rotaxane axle in order to access the next barrier.<sup>8</sup> Reaction with barriers out of sequence is prevented by the compartmentalization of the macrocycle and the rigidity of the thread, which holds non-proximal barriers out of reach of the cysteine 'arm'. Following the transfer of all the amino acid groups from the track to the elongation site tethered to the macrocycle, the ring dethreads from the end of the track into the bulk medium.

We found that the  $\beta^3$ -Phe and  $\beta^3$ -Leu residues attached to the track *via* phenolic ester linkages were more prone to hydrolysis during the Boc and trityl deprotection stage of **6** than the  $\alpha$ -amino acids used in previous rotaxane machine systems,<sup>8</sup> which led us to optimize the conditions for step (iii), Scheme 2, by adding TIPSH. Pivaloyl protection of  $\beta^3$ -Ala renders the phenolic ester stable to the deprotection conditions. Notably, when partial loss of  $\beta^3$ -Phe and/or  $\beta^3$ -Leu occurred during the deprotection step, subsequent operation of the machine translated the sequence of amino acids that remained, including sequences missing hydrolyzed residues, with high fidelity into the corresponding sequence-specific operation products (see Supplementary Information for details). This provides further confirmation that incorrect sequences produced by these types of molecular machines are readily detected during the analysis of the operation products.

After operation of molecular machine **9** under the optimized conditions shown in Scheme 2, mass spectral analysis showed complete consumption of **9** and the formation of **10** and amino-acid-free track **11**. Following solvent removal and purification of the crude mixture by preparative TLC, **10** was converted to *S,N*-acetal **12** in line with previous systems.<sup>8</sup>

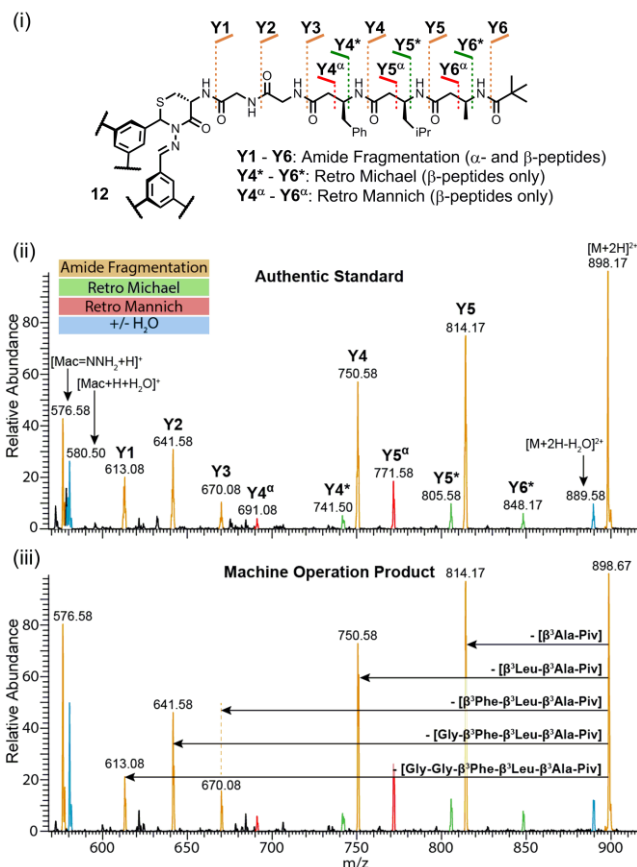
## Scheme 2: Synthesis and Operation of a Three-Barrier Machine Bearing Non-Proteinogenic $\beta^3$ -Amino Acids<sup>a</sup>



<sup>a</sup>Reagents and conditions: (i) CuPF<sub>6</sub>·(MeCN)<sub>4</sub> (1.5 equiv.), Tentagel™ TBTA resin, CH<sub>2</sub>Cl<sub>2</sub>:<sup>t</sup>BuOH (4:1), r.t., 48 h, 97 %. (ii) Boc-Gly-Gly-Cys(Trt)-NHNH<sub>2</sub> (**8**), aniline, CH<sub>2</sub>Cl<sub>2</sub>, r.t., 48 h, 72 %. (iii) TFA, TIPSH, CH<sub>2</sub>Cl<sub>2</sub>, r.t., 1 h. (iv) Et<sub>3</sub>N (44 equiv.), PPh<sub>3</sub> (3.5 equiv.), DMF, 60 °C, 7 d, 29 % over 2 steps (58 % based on macrocycle).

The sequence fidelity of the peptidic product, **12**, from the operation of machine **9** was confirmed by tandem mass spectrometry (MS-MS, Figure 2). The most intense MS-MS fragmentation profile was achieved with the [12+2H]<sup>2+</sup> mass-ion. Fragmentation showed iterative cleavage of the amide bonds of the assembled  $\alpha\beta^3$ -peptide backbone down to the parent macrocycle, consistent with the expected sequence. Seebach has shown that peptide oligomers containing  $\beta^3$ -amino acids undergo additional fragmentation to their  $\alpha$ -amino acid congeners.<sup>15</sup> Analysis of the MS-MS spectrum of [12+2H]<sup>2+</sup> showed peaks consistent with dehydration<sup>16</sup> (Figure 2, shown in blue), retro-Michael (Figure 2, shown in green, Y\*) and retro-Mannich (Figure 2, shown in red, Y<sup>α</sup>) fragmentation pathways, further confirmation of the successful sequence translation.

The mass spectrometry results were corroborated by comparison with authentic material, prepared unambiguously by conventional peptide synthesis. When operating pristine **9**, obtained from **6** under conditions where hydrolysis does not occur, no fragment ions indicative of peptides of incorrect sequence were observed.



**Figure 2.** Tandem mass-spectrometry sequencing of **12**. Analysis of doubly-charged  $[\mathbf{12}+2\text{H}]^{2+}$  mass ions bearing the translated Piv- $\beta^3$ Ala- $\beta^3$ Leu- $\beta^3$ Phe-Gly-Gly-Cys peptide sequence. (i) Fragmentation modes of  $\alpha$ - and  $\beta^3$ -peptides. (ii) MS-MS spectrum of authentic standard of the anticipated product of operation. (iii) MS-MS spectrum of isolated product of machine operation. Orange peaks indicate amide bond N-C fragmentation; green peaks indicate  $\beta^3$ -peptide retro-Michael fragmentation; red peaks indicate  $\beta^3$ -peptide retro-Mannich fragmentation; blue peaks indicate addition or loss of water.

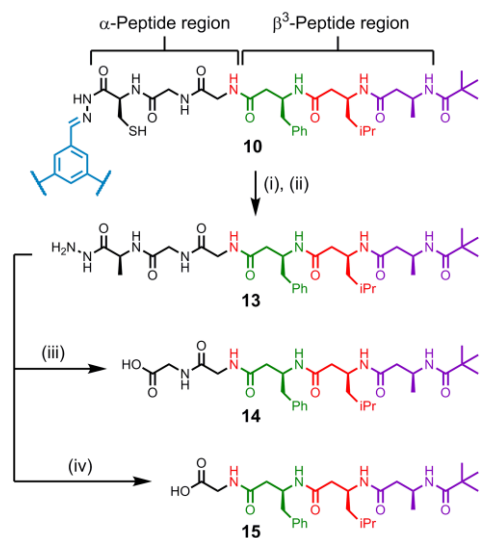
A feature of the homologated ( $\beta^3$ ) amino acids used in **9** is that the size of the NCL [S-N] trans-acyl shift transition states differs from those used in the previously reported  $\alpha$ -peptide-forming rotaxane machines.<sup>8</sup> Katritzky found that NCLs requiring 15- or 19-membered [S-N] acyl-transfer transition states, the size employed in the operation of **9**, are significantly slower than NCLs involving 11-, 14- and 17-membered transition states (which featured in the operation of the  $\alpha$ -peptide-forming machines<sup>8</sup>).<sup>17</sup> We attribute the longer reaction time necessary for complete operation of **9** (7 days cf. 3 days for  $\alpha$ -peptide-forming machines) to these less favorable transition state sizes. The successful *in machina* acyl-transfer of these challenging transition states, without sequence erosion, illustrates the robustness and structural tolerance of this type of molecular machine-promoted synthesis.

Following the formation of peptide **10** from molecular machine **6**, we investigated methods for cleaving the cysteine NCL catalyst unit, and adjacent GlyGly elongation site, from the translated amino acid sequence. We envis-

aged that the action of an endotopic protease could result in the selective digestion of the  $\alpha$ -region<sup>18d</sup> of the fused  $\alpha\beta^3$ -operation product **10**, thereby liberating the translated  $\beta^3$ -region. The basis for the anticipated selectivity stems from the high stability of  $\beta$ -peptides to both *in vitro*<sup>18</sup> and *in vivo*<sup>13b,19</sup> enzymatic degradation.

Initial studies indicated that removal of the cysteine thiol would ameliorate manipulation of **10**, preventing both conversion to **12** and disulfide formation. Furthermore, removal of the macrocycle should increase compatibility of the peptide with the aqueous conditions required for proteolysis. Machine product **10** was subjected to a sequence of reductive desulfurization mediated by radical initiator V-044,<sup>20</sup> followed by macrocycle removal with methoxyamine-HCL to give alanine hydrazide derivative **13** (Scheme 3). Hydrazide **13** was submitted to proteolysis with subtilisin Carlsberg, a broad scope serine endopeptidase that exhibits high stability and activity at elevated temperatures and optimal activity at pH 7-8.<sup>21</sup> After a five-day incubation period at 55 °C, ESI(-)-MS analysis indicated the selective cleavage of the Ala-Gly amide bond to afford **14** in high yield. Longer incubation times and refreshment of the enzyme did not lead to the cleavage of other peptide residues. All of the  $\beta^3$ -peptide links proved inert to enzymatic cleavage, as did the bridging  $\alpha$ - $\beta$  junction, consistent with previous observations on the action of peptidases on  $\alpha\beta^3$ -peptides.<sup>13c,21b</sup> The Gly-Gly link was also unaffected by subtilisin Carlsberg, presumably due to its proximity to the  $\beta$ -peptide region.<sup>21b,22</sup> In contrast, incubation of **13** with Pronase, a mixture of broad scope *endo*- and *exo*peptidases isolated from *Streptomyces griseus*,<sup>21b</sup> cleaved all of the amide bonds linking  $\alpha$ -amino acids to give **15**, leaving only the bridging  $\alpha$ - $\beta$  junction and the  $\beta$ - $\beta$  peptide bonds intact. The selective cleavage of amino acid residues from the residual catalytic arm of operation product **10** to give post-operationally modified  $\alpha\beta^3$ -peptides **14** or **15** is reminiscent of the deletion of N-terminal methionine residues (installed by the start-codon) from ribosomal proteins by methionine aminopeptidases in eukaryotic post-translational modification.<sup>23</sup>

### Scheme 3. Enzymatic Liberation of the $\beta^3$ -Peptide Region of the Machine Product from the NCL Catalyst Site.<sup>a</sup>



<sup>a</sup>Reagents and conditions: (i) V-044 (0.2 equiv.), TCEP·HCl (10 equiv.), NEt<sub>3</sub> (15 equiv.), <sup>t</sup>BuSH (10 equiv.), DMF:H<sub>2</sub>O (10:1), r.t., 65 h, 64 %. (ii) Methoxyamine·HCl, CHCl<sub>3</sub>/MeOH, reflux, 1 h, 75 %. (iii) Subtilisin Carlsberg (1 mg/mL), phosphate buffer (pH 8, 0.1 M), 50 °C, 5 days. (iv) Pronase (1 mg/mL), H<sub>2</sub>O, 37 °C, 4 days.

### CONCLUSIONS

An artificial rotaxane-based small-molecule machine can successfully carry out the autonomous sequential synthesis of peptides containing  $\beta^3$ -amino acids. Operating through  $\pi$ - and particularly challenging 15- and 19-membered NCL transition states, sequence information encoded into the molecular machine is transcribed into peptide products without detectable sequence scrambling. The structural tolerance of the small-molecule machine contrasts with that of natural ribosomes. Treatment of the small-molecule machine product with peptidases cleaves the NCL catalyst residue from the  $\alpha$ -peptide region, biological and artificial molecular machines working in concert to produce  $\beta^3$ -peptide products inaccessible through either strategy alone.

### ASSOCIATED CONTENT

#### Supporting Information

Detailed descriptions of synthetic procedures, characterization of new compounds, and spectroscopic data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

### AUTHOR INFORMATION

#### Corresponding Author

\* david.leigh@manchester.ac.uk

#### Notes

The authors declare no competing financial interest.

### ACKNOWLEDGMENTS

This research was funded by the Engineering and Physical Sciences Research Council (EP/H021620/1&2) and the ERC (Advanced Grant no. 339019). We thank the EPSRC National Mass Spectrometry Centre (Swansea, U.K.) for high-resolution mass spectrometry and the Royal Society for a University Research Fellowship (to G.D.B.) and Research Professorship (to D.A.L.).

### REFERENCES

- (1) Berg, J. M.; Tymoczko, J. L.; Stryer, L. *Biochemistry*; W. H. Freeman: New York, 6th edition, 2006.
- (2) (a) Yonath, A. *Angew. Chem., Int. Ed.* **2010**, *49*, 4340–4354. (b) Ramakrishnan, V. *Angew. Chem., Int. Ed.* **2010**, *49*, 4355–4380. (c) Steitz, T. A. *Angew. Chem., Int. Ed.* **2010**, *49*, 4381–4398.
- (3) (a) Ban, N.; Nissen, P.; Hansen, J.; Moore, P. B.; Steitz, T. A. *Science* **2000**, *289*, 905–920. (b) Zarivach, R.; Bashan, A.; Berisio, R.; Harms, J.; Auerbach, T.; Schlutzenzen, F.; Bartels, H.; Baram, D.; Pyetan, E.; Sittner, A.; Amit, M.; Hansen, H. A. S.; Kessler, M.; Liebe, C.; Wolff, A.; Agmon, I.; Yonath, A. *J. Phys. Org. Chem.* **2004**, *17*, 901–912. (c) Agmon, I.; Bashan, A.; Zarivach, R.; Yonath, A. *Biol. Chem.* **2005**, *386*, 833–844. (d) Blaha, G.; Stanley, R. E.; Steitz, T. A. *Science* **2009**, *325*, 966–970. (e) Lobanov, A. V.; Turanov, A. A.; Hatfield, D. L.; Gladyshev, V. N. *Crit. Rev. Biochem. Mol. Biol.* **2010**, *45*, 257–265.
- (4) (a) Heckler, T. G.; Roesser, J. R.; Xu, C.; Hang, P.-I.; Hecht, S. M. *Biochemistry* **1988**, *27*, 7254–7262. (b) Tan, Z.; Forster, A. C.; Blacklow, S. C.; Cornish, V. W. *J. Am. Chem. Soc.* **2004**, *126*, 12752–12753.
- (5) (a) Yamane, T.; Miller, D. L.; Hopfield, J. J. *Biochemistry* **1981**, *20*, 7059–7064. (b) Englander, M. T.; Avins, J. L.; Fleisher, R. C.; Bo Liu, B.; Effraim, P. R.; Wang, J.; Schulten, K.; Leyh, T. S.; Gonzalez Jr., R. L.; W. Cornish, V. W. *Proc. Natl. Acad. Sci. U.S.A.* **2015**, *112*, 6038–6043. (c) Fujino, T.; Goto, Y.; Suga, H.; Murakami, H. *J. Am. Chem. Soc.* **2013**, *135*, 1830–1837.
- (6) (a) Starck, S. R.; Qi, X.; Olsen, B. N.; Roberts, R. W. *J. Am. Chem. Soc.* **2003**, *125*, 8090–8091. (b) Josephson, K.; Hartman, M. C. T.; Szostak, J. W. *J. Am. Chem. Soc.* **2005**, *127*, 11727–11735. (c) Young, T. S.; Schultz, P. G. *J. Biol. Chem.* **2010**, *285*, 11039–11044. (d) Freed, E. F.; Bleichert, F.; Dutca, L. M.; Baserga, S. J. *Mol. Biosyst.* **2010**, *6*, 481–493. (e) Maini, R.; Chowdhury, S. R.; Dedkova, L. M.; Roy, B.; Daskalova, S. M.; Paul, R.; Chen, S.; Hecht, S. M. *Biochemistry* **2015**, *54*, 3694–3706. (f) Terasaka, N.; Iwane, Y.; Geiermann, A.-S.; Goto, Y.; Suga, H. *Int. J. Mol. Sci.* **2015**, *16*, 6513–6531. (g) Fujino, T.; Goto, Y.; Suga, H.; Murakami, H. *J. Am. Chem. Soc.* **2016**, *138*, 1962–1969.
- (7) (a) Badi, N.; Lutz, J.-F. *Chem. Soc. Rev.* **2009**, *38*, 3383–3390. (b) Ouchi, M.; Badi, N.; Lutz, J.-F.; Sawamoto, M. *Nat. Chem.* **2011**, *3*, 917–924. (c) Lutz, J.-F.; Ouchi, M.; Liu, D. R.; Sawamoto, M. *Science* **2013**, *341*, 1238149. (d) ten Brummelhuis, N. *Polym. Chem.* **2015**, *6*, 654–667. (e) Lutz, J.-F.; Lehn, J.-M.; Meijer, E. W.; Matyjaszewski, K. *Nat. Rev. Mater.* **2016**, 16024.
- (8) (a) Lewandowski, B.; De Bo, G.; Ward, J. W.; Papmeyer, M.; Kuschel, S.; Aldegunde, M. J.; Gramlich, P. M. E.; Heckmann, D.; Goldup, S. M.; D'Souza, D. M.; Fernandes, A. E.; Leigh, D. A. *Science* **2013**, *339*, 189–193. (b) McGonigal, P. R.; Stoddart, J. F. *Nat. Chem.* **2013**, *5*, 260–262. (c) Bertran-Vicente, J.; Hackenberger, C. P. R. *Angew.*

- Chem. Int. Ed.* **2013**, *52*, 6140–6142. (d) Wilson, C. M.; Gualandi, A.; Cozzi, P. G. *ChemBioChem* **2013**, *14*, 1185–1187. (e) De Bo, G.; Kuschel, S.; Leigh, D. A.; Pappmeyer, M.; Ward, J. W. *J. Am. Chem. Soc.* **2014**, *136*, 5811–5814.
- (9) (a) Gartner, Z. J.; Kanan, M. W.; Liu, D. R. *J. Am. Chem. Soc.* **2002**, *124*, 10304–10306. (b) Li, X.; Gartner, Z. J.; Tse, B. N.; Liu, D. R. *J. Am. Chem. Soc.* **2004**, *126*, 5090–5092. (c) Gartner, Z. J.; Tse, B. N.; Grubina, R.; Doyon, J. B.; Snyder, T. M.; Liu, D. R. *Science* **2004**, *305*, 1601–1605. (d) Liao, S.; Seeman, N. C. *Science* **2004**, *306*, 2072–2074. (e) Snyder, T. M.; Liu, D. R. *Angew. Chem. Int. Ed.* **2005**, *44*, 7379–82. (f) He, Y.; Liu, D. R. *Nat. Nanotechnol.* **2010**, *5*, 778–782. (g) McKee, M. L.; Milnes, P. J.; Bath, J.; Stulz, E.; Turberfield, A. J.; O'Reilly, R. K. *Angew. Chem. Int. Ed.* **2010**, *49*, 7948–7951. (h) Gu, H.; Chao, J.; Xiao, S.-J.; Seeman, N. C. *Nature* **2010**, *465*, 202–205. (i) He, Y.; Liu, D. R. *J. Am. Chem. Soc.* **2011**, *133*, 9972–9975. (j) Milnes, P. J.; McKee, M. L.; Bath, J.; Song, L.; Stulz, E.; Turberfield, A. J.; O'Reilly, R. K. *Chem. Commun.* **2012**, *48*, 5614–5616. (k) McKee, M. L.; Milnes, P. J.; Bath, J.; Stulz, E.; O'Reilly, R. K.; Turberfield, A. J. *J. Am. Chem. Soc.* **2012**, *134*, 1446–1449. (l) Niu, J.; Hili, R.; Liu, D. R. *Nat. Chem.* **2013**, *5*, 282–292.
- (10) For some recent examples of rotaxane-based molecular machines, see: (a) Wen, H.; Li, W.; Chen, J.; He, G.; Li, L.; Olson, M. A.; Sue, A. C.-H.; Stoddart, J. F.; Guo, X. *Sci. Adv.* **2016**, *2*, e160113. (b) Waelès, P.; Riss-Yaw, B.; Coutrot, F. *Chem. Eur. J.* **2016**, *22*, 6837–6845. (c) Legigan, T.; Riss-Yaw, B.; Clavel, C.; Coutrot, F. *Chem. Eur. J.* **2016**, *22*, 8835–8847. (d) Schäfer, C.; Ragazzon, G.; Colasson, B.; La Rosa, M.; Silvi, S.; Credi, A. *ChemistryOpen* **2016**, *5*, 120–124. (e) Zhu, K.; Vukotic, V. N.; Loeb, S. J. *Chem. Asian J.* **2016**, *11*, 3258–3266. (f) Vidonne, A.; Kosikova, T.; Philp, D. *Chem. Sci.* **2016**, *7*, 2592–2603. (g) Ma, X.; Zhang, J.; Cao, J.; Yao, X.; Cao, T.; Gong, Y.; Zhao, C.; Tian, H. *Chem. Sci.* **2016**, *7*, 4582–4588. (h) Meng, Z.; Xiang, J.-F.; Chen, C.-F. *J. Am. Chem. Soc.* **2016**, *138*, 5652–5658. (i) Wang, W.-K.; Xu, Z.-Y.; Zhang, Y.-C.; Wang, H.; Zhang, D.-W.; Liu, Y.; Li, Z.-T. *Chem. Commun.* **2016**, *52*, 7490–7493. (j) Qiao, B.; Liu, Y.; Lee, S.; Pink, M.; Flood, A. H. *Chem. Commun.* **2016**, *52*, 13675–13678. (k) Martínez-Periñán, E.; de Juan, A.; Pouillon, Y.; Schierl, C.; Strauss, V.; Martín, N.; Rubio, Á.; Guldi, D. M.; Lorenzo, E.; Pérez, E. M. *Nanoscale* **2016**, *8*, 9254–9264. (l) Benson, C. R.; Share, A. I.; Marzo, M. G.; Flood, A. H. *Inorg. Chem.* **2016**, *55*, 3767–3776. (m) Ragazzon, G.; Credi, A.; Colasson, B. *Chem. Eur. J.* **2017**, *23*, 2149–2156. (n) Lim, J. Y. C.; Bunchuay, T.; Beer, P. D. *Chem. Eur. J.* **2017**, *23*, 4700–4707. (o) Lim, J. Y. C.; Marques, I.; Thompson, A. L.; Christensen, K. E.; Félix, V.; Beer, P. D. *J. Am. Chem. Soc.* **2017**, *139*, 3122–3133. (p) Martínez-Cuevas, A.; Saura-Sanmartín, A.; Nicolás-García, T.; Navarro, C.; Orenes, R.-A.; Alajarin, M.; Berna, J. *Chem. Sci.* **2017**, *8*, 3775–3780. (q) Lewis, J. E. M.; Galli, M.; Goldup, S. M. *Chem. Commun.* **2017**, *53*, 298–312. (r) Yang, L.-P.; Jia, F.; Cui, J.-S.; Lu, S.-B.; Jiang, W. *Org. Lett.* **2017**, *19*, 2945–2948. (s) Pezzato, C.; Nguyen, M. T.; Cheng, C.; Kim, D. J.; Otley, M. T.; Stoddart, J. F. *Tetrahedron* **2017**, doi: 10.1016/j.tet.2017.05.087.
- (11) (a) Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. H. *Science* **1994**, *266*, 776–778. (b) Kent, S. B. H. *Chem. Soc. Rev.* **2009**, *38*, 338–351.
- (12) (a) Hartman, M. C. T.; Josephson, K.; Lin, C.-W.; Szostak, J. W. *PLoS ONE*, **2007**, *2*, e972. For the ribosomal synthesis of peptides with multiple, but non-consecutive,  $\beta$ -amino acids using a cell-free translation system with a reprogrammed genetic code, see: (b) Fujino, T.; Goto, Y.; Suga, H.; Murakami, H. *J. Am. Chem. Soc.* **2016**, *138*, 1962–1969.
- (13) (a) Schreiber, J. V.; Frackenhohl, J.; Moser, F.; Fleischmann, T.; Kohler, H.-P. E.; Seebach, D. *ChemBioChem* **2002**, *3*, 424–432. (b) Lind, R.; Greenhow, D.; Perry, S.; Kimmerlin, T.; Seebach, D. *Chem. Biodivers.* **2004**, *1*, 1391–1400. (c) Seebach, D.; Beck, A. K.; Bierbaum, D. J. *Chem. Biodivers.* **2004**, *1*, 1111–1239.
- (14) Crowley, J. D.; Goldup, S. M.; Lee, A.-L.; Leigh, D. A.; McBurney, R. T. *Chem. Soc. Rev.* **2009**, *38*, 1530–1541.
- (15) Schreiber, J. V.; Quadroni, M.; Seebach, D. *Chimia* **1999**, *53*, 621–626.
- (16) Ballard, K. D.; Gaskell, S. J. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 477–481.
- (17) (a) Katritzky, A. R.; Abo-Dya, N. E.; Tala, S. R.; Abdel-Samii, Z. K. *Org. Biomol. Chem.* **2010**, *8*, 2316–2319. (b) Hansen, F. K.; Ha, K.; Todadze, E.; Lillicotch, A.; Frey, A.; Katritzky, A. R. *Org. Biomol. Chem.* **2011**, *9*, 7162–7167. (c) Katritzky, A. R.; Tala, S. R.; Abo-Dya, N. E.; Ibrahim, T. S.; El-Feky, S. A.; Gyanda, K.; Pandya, K. M. *J. Org. Chem.* **2011**, *76*, 85–96. (d) Monbaliu, J.-C. M.; Katritzky, A. R. *Chem. Commun.* **2012**, *48*, 11601–11622. (e) Panda, S. S.; El-Nachef, C.; Bajaj, K.; Al-Youbi, A. O.; Oliferenko, A.; Katritzky, A. R. *Chem. Bio. Drug Des.* **2012**, *80*, 821–827. (f) Bol'shakov, O.; Kovacs, J.; Chahar, M.; Ha, K.; Khelashvili, L.; Katritzky, A. R. *J. Pept. Sci.* **2012**, *18*, 704–709. (g) Monbaliu, J.-C. M.; Dive, G.; Stevens, C. V.; Katritzky, A. R. *J. Chem. Theory Comput.* **2013**, *9*, 927–934. (h) Popov, V.; Panda, S. S.; Katritzky, A. R. *J. Org. Chem.*, **2013**, *78*, 7455–7461. (i) Panda, S. S.; Elagawany, M.; Marwani, H. M.; Çalışkan, E.; El-Katib, M.; Oliferenko, A.; Alamry, K. A.; Katritzky, A. R. *ARKIVOC* **2014**, *4*, 91–106. (j) Panda, S. S.; Dennis Hall, C.; Oliferenko, A.; Katritzky, A. R. *Acc. Chem. Res.* **2014**, *47*, 1076–1087. (k) Biswas, S.; Kayaleh, R.; Pillai, G. G.; Seon, C.; Roberts, I.; Popov, V.; Alamry, K. A.; Katritzky, A. R. *Chem. Eur. J.* **2014**, *20*, 8189–8198.
- (18) (a) Seebach, D.; Overhand, M.; Kohnle, F. N. M.; Martinoni, B.; Oberer, L.; Hommel, U.; Widmer, H.; *Helv. Chim. Acta* **1996**, *79*, 913–941. (b) Frackenhohl, J.; Arvidsson, P. I.; Schreiber, J. V.; Seebach, D. *ChemBioChem* **2001**, *2*, 445–455. (c) Gopi, H. N.; Ravindra, G.; Pal, P. P.; Pattanaik, P.; Balaram, H.; Balaram, P. *FEBS Lett.* **2003**, *535*, 175–178. (d) Hook, D. F.; Gessier, F.; Noti, C.; Kast, P.; Seebach, D. *ChemBioChem* **2004**, *5*, 691–706. (e) Geueke, B.; Kohler, H.-P. E. *Appl. Microbiol. Biotechnol.* **2007**, *74*, 1197–1204.
- (19) (a) Wiegand, H.; Wirz, B.; Schweitzer, A.; Camenisch, G. P.; Rodriguez Perez, M. I.; Gross, G.; Woessner, R.; Voges, R.; Arvidsson, P. I.; Frackenhohl, J.; Seebach, D. *Biopharm. Drug Dispos.* **2002**, *23*, 251–262. (b) Wiegand, H.; Wirz, B.; Schweitzer, A.; Gross, G.; Rodriguez Perez, M. I.; Andres, H.; Kimmerlin, T.; Rueping, M.; Seebach, D. *Chem. Biodivers.* **2004**, *1*, 1812–1828.
- (20) Wan, Q.; Danishefsky, S. J. *Angew. Chem. Int. Ed.* **2007**, *46*, 9248–9252.

(21) (a) Voordouw, G.; Milo, C.; Roche, R. S. *Biochemistry* **1976**, *15*, 3716–3724. (b) Hook, D. F.; Bindschädler, P.; Mahajan, Y. R.; Šebesta, R.; Kast, P.; Seebach, D. *Chem. Biodivers.* **2005**, *2*, 591–632.

(22) The vast majority of research into the proteolytic cleavage of amide bonds in mixed  $\alpha\beta^3$ -peptides has been carried out on systems with  $\alpha$ -peptides at the *N*-terminus of the  $\beta$ -peptide fragment, rather than at the *C*-terminus explored here.

(23) (a) Giglione, C.; Boularot, A.; Meinel, T. *Cell. Mol. Life Sci.* **2004**, *61*, 1455–1474. (b) Varland, S.; Osberg, C.; Arnesen, T. *Proteomics* **2015**, *15*, 2385–2401.

## Table of Contents Entry

