

Illuminating the Dark Conformational Space of Macrocycles Using Dominant Rotors

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Macrocycles can target complex protein interfaces that are not readily tractable through traditional small-molecule approaches. “Perhaps the most significant and enabling feature of macrocyclic drugs and biological probes, in contrast to their small molecule counterparts, is their intricate three-dimensional shape, or conformation,” said Professor Andrei Yudin, from the University of Toronto (Canada): “Apart from relatively small rings such as cyclohexane, deciphering conformation–activity relationships in larger molecules is substantially more complicated.¹ As a result, there is substantial interest in technologies that allow systematic studies of the effect of macrocyclic conformational changes on biological activity.”

In a recent paper, Professor Yudin et al. used the term ‘dark conformational space’ to describe the metastable states in peptide structures observed by NMR and X-ray crystallography. “The concept of dark space becomes intuitive when a parallel is made to less structurally complex small molecules,” explained Professor Yudin, who continued: “The twist-boat conformation in cyclohexane is approximately 5.5 kcal mol⁻¹ higher in energy than the chair form, making it challenging to detect and directly study its physicochemical properties. Interestingly, the replacement of four hydrogens in cyclohexane with cyclohexyl groups destabilizes the chair conformation significantly to exclusively reveal the twist-boat form.²”

Due to coupled bond rotations, macrocycles belong to a class of challenging structures in which slight alterations in dihedral angles can propagate through the ring and trigger conformational changes at distal position(s). “In the case of lorlatinib, an anticancer drug developed by Pfizer,” said Professor Yudin, “a methyl group five atoms away from the biaryl linkage results in severe allylic 1,3-strain and effectively destabilizes the non-bioactive conformer by 8.5 kcal mol⁻¹.³ Remarkably, a single methyl group in a 12-membered ring provides more stabilization than the *tert*-butyl group on cyclohexane (Figure 1A). Removing the methyl group in lorlatinib leads to a macrocyclic structure that exists as a mixture of two atropisomers separated by a conformational interconversion barrier of 24.6 kcal mol⁻¹.”

The authors reasoned that coupling among bond rotations within a macrocycle might be controlled by a ‘dominant rotor’,

whose rotational barrier is substantially higher than the rest of the system (Figure 1B). Professor Yudin said: “We hypothesized that this feature might remodel the accessible conformational space of a macrocycle into two non-interconverting ensembles we refer to as a two-well system. Rotors originating from amino acid amides generally display a *cis*–*trans* amide rotation barrier of about 18–20 kcal mol⁻¹, which is too low of a barrier for maintaining control of a given system under ambient conditions. To explore the energetic landscape of constrained peptides, we speculated that the dominant rotor candidates should possess a rotational energy barrier of at least 25 kcal mol⁻¹.”

The group evaluated a collection of N-protected biaryl amino acids and uncovered a particular class of phenylene–triazolyl rotors to meet their energetic requirements (Figure 1B). “We prepared the tunable atropisomeric building blocks 1–8 that were predicted to span a range of rotational barriers and introduced them into cyclic peptides,” said Professor Yudin. He continued: “This simple approach has allowed us to correlate conformational changes in a wide range of rings with the nature of the dominant rotor and led to the creation of two-well systems with controlled conformational behavior. Figure 2A depicts a representative two-well macrocycle (**9**) isolated. What is especially significant is that the two atropodiastereomers have completely different conformations, evidenced by different hydrogen-bond networks and differences in solubility.”

“Ordinarily, conformational isomers such as *S_c*-**9** and *R_c*-**9** cannot be isolated because the barrier to interconversion is easily surmountable at room temperature. This result underscores that staying away from equilibrium offers a fascinating possibility to control complex molecules,” remarked Professor Yudin, who added: “We also considered larger macrocycles that are composed mainly of amino acid residues and showed that higher-energy conformations could be enforced using dominant rotors. These units effectively remodel the conformational landscape of 16- to 22-membered rings and enable observation of peptide conformations that are uncommon because of their irregular geometrical features (Figure 2B).”

According to Professor Yudin, control over conformation in macrocycles is an unsolved problem. “Had mastery of this kind been possible, one could easily design a potent and selec-

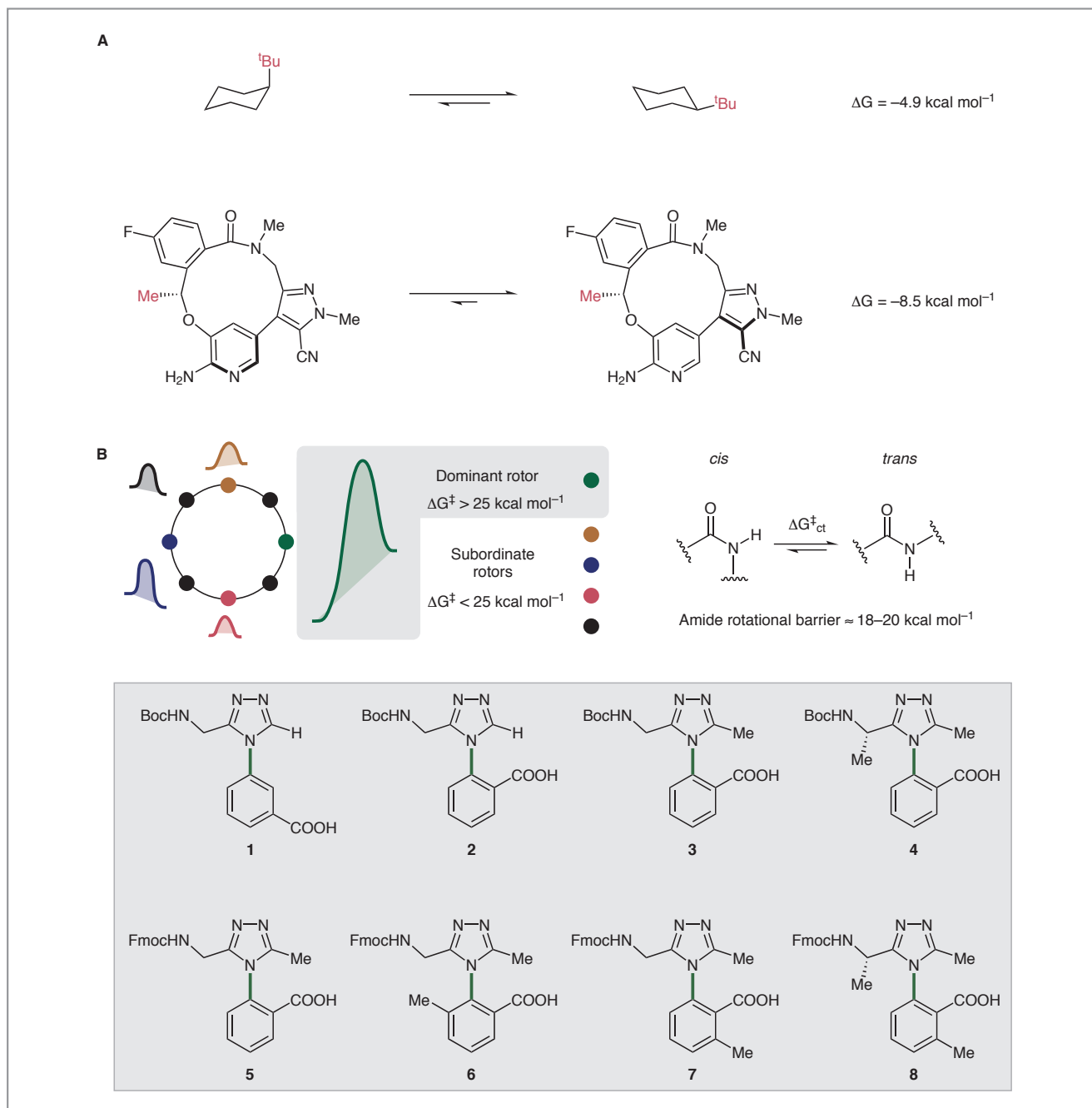


Figure 1 A. A methyl group in the appropriate environment of lorlatinib provides more stabilization than a *tert*-butyl group on cyclohexane. Both cyclic structures represent conformationally biased systems with a strong preference for one conformer. B. Tunable atropisomeric constituents with relatively high barriers termed 'dominant rotors' as a means to control complex energy landscapes of constrained chains of 'subordinate rotors'.

tive binder to any protein surface. While RNA and phage display technologies have contributed to the discovery of bioactive macrocycles, they have not advanced our understanding of the conformational factors that drive recognition at biological interfaces,” remarked Professor Yudin, who continued: “In the area of polypeptides, it is known which amino acid sequences result in the formation of an α -helix, a β -turn, and β -sheet structures. Other than formation of canonical motifs, there is little understanding of how to induce the formation of unusual conformations and irregular turn motifs in peptide macrocycles.⁴” Professor Yudin concluded: “The dominant

rotor method should now allow exploration of a wide range of structures, opening the tantalizing possibility that some molecular properties might have remained veiled because of the difficulties in generating and stabilizing conformational ensembles that are energetically unfavorable. We also recognize that a drastic increase in the rate of synthesis is needed to facilitate exploration of the dark conformational space. Current efforts are directed toward addressing this challenge.”

Professor Giorgio Colombo, an expert in biomolecular simulation and computational chemistry from the University of Pavia (Italy) commented: “The paper by Yudin and co-

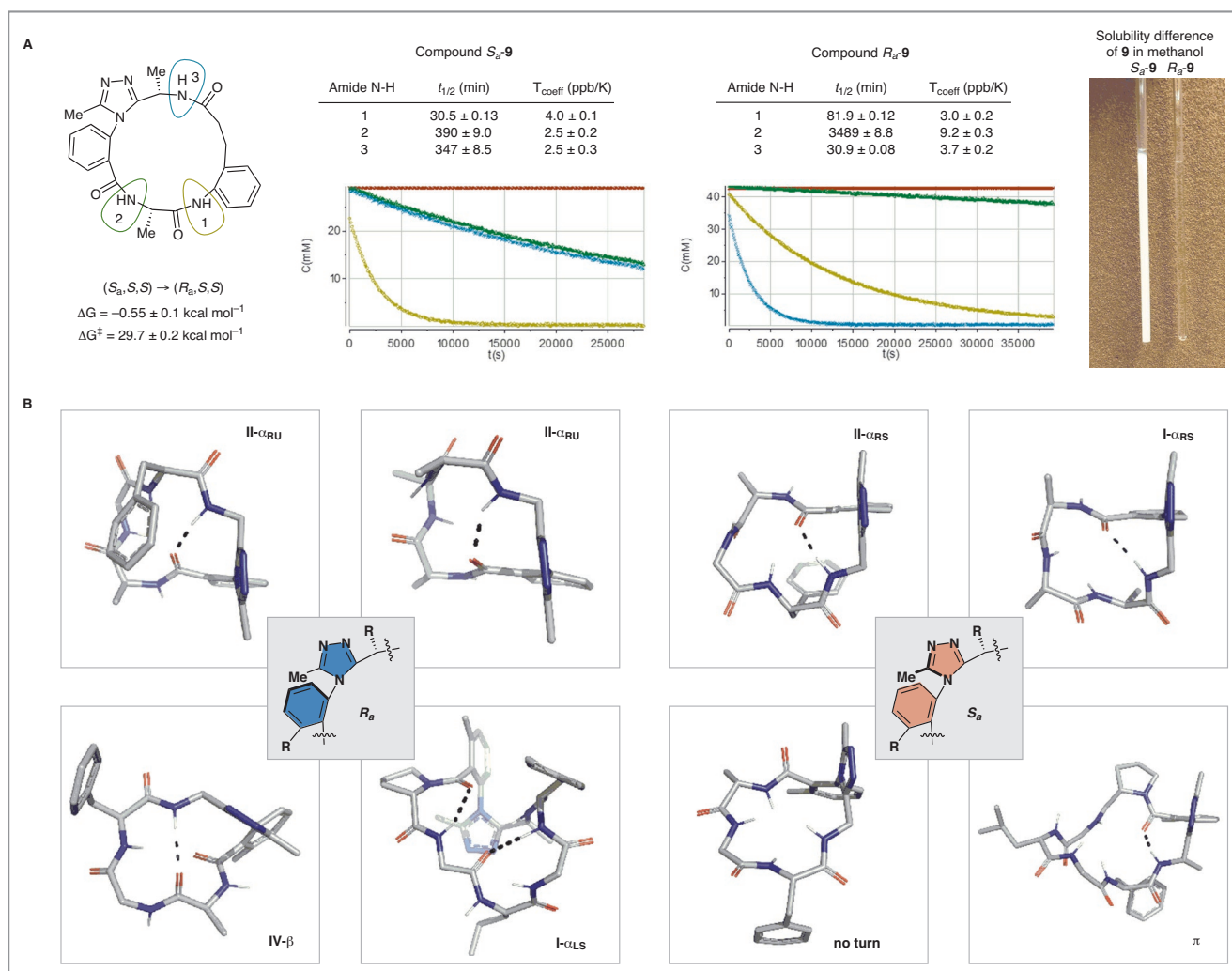


Figure 2 A. Differences in amide NH hydrogen–deuterium exchange rates and chemical shift/temperature coefficients show that dominant rotors can stabilize distinct solution conformations. The solubility of dominant rotor peptide R_{α} -9 in methanol at 20 mM suggests a more compact conformation with reduced polar surface area and higher lipophilicity relative to S_{α} -9. B. Examples of dominant rotor-containing macrocycles with amino acid residues forced into rare turn motifs not observed in their linear counterparts or homodetic rings with all-natural connectivity.

workers represents an interesting breakthrough in a difficult and problematic field, such as that of cyclic peptides. The possibility given by the dominant rotors method to unveil transient hidden conformations opens up new possibilities for the design of new active biomolecules. In this context, one of the main hurdles has often been that active conformations are not the dominant ones observed in solution. This new approach has the potential to enrich our design arsenal of an important new weapon to generate new structures with observable activities in a rational manner.”

Anastasia Fomale

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Andrei K. Yudin is a Professor of Chemistry at the University of Toronto (Canada). He received his B.Sc. from Moscow State University (Russia) in 1992 and a Ph.D. from the University of Southern California (USA) in 1996. After a postdoctoral stay at The Scripps Research Institute (USA) between 1996 and 1998, he joined the faculty at the University of Toronto, where he and his students have been working on fundamental problems of organic chemistry.



Dr. D. B. Diaz

Diego B. Diaz is currently an NSERC Postdoctoral Fellow at Harvard University (USA). He received an H.B.Sc. degree from the University of Toronto Mississauga (Canada) in 2014 and a Ph.D. from the University of Toronto (Canada) in 2020. Diego made numerous contributions during his Ph.D. under the supervision of Professor Andrei K. Yudin, from investigating boron's chameleonic behavior in both synthetic and biological settings to developing methods for controlling the conformation of complex peptides.

Solomon Appavoo received his B.Sc. degree in chemistry from Carleton University (Ottawa, Canada) in 2016, where he carried out research with Professor Jeffrey Manthorpe on the design of kinase inhibitors. In 2016 he joined the research group of Professor Andrei K. Yudin at the University of Toronto (Canada) where he is currently pursuing a Ph.D. degree in chemistry. His research interests involve the synthesis and conformational analysis of medicinally relevant organic molecules.



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Anastasia F. Bogdanchikova received an H.B.Sc. degree in chemistry from the University of Toronto (Canada) in 2017. She then joined *Encycle Therapeutics Inc.* and worked on macrocycle synthesis to target integrin alpha-4-beta-7, which is involved in the pathogenesis of inflammatory bowel disease. In 2019, Anastasia joined Professor Andrei K. Yudin at the University of Toronto to explore the application of atropisomeric building blocks in peptide-based macrocycles.

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Dr. Y. Lebedev

Yury Lebedev studied chemistry at Ivanovo State University of Chemistry and Technology (Russia). His Ph.D. research on organometallic synthesis was done with magna cum laude at the University of Bonn (Germany) under the supervision of Professor A. C. Filippou. This was followed by postdoctoral stays at RWTH Aachen University (Germany) and King Abdullah University of Science and Technology (Saudi Arabia) under the guidance of Professor Magnus Rueping. His other postdoctoral appointments were held at the University of Toronto (Canada) with Professor Andrei K. Yudin and the University of Vienna (Austria) with Professor Nuno Maulide, where he received the Lise Meitner scholarship. Since 2021 he occupies the position of discovery scientist at the biotech startup *Solgate* (Austria).



T. J. McTiernan

Timothy J. McTiernan received his B.Sc. from the University of Guelph (Canada) in 2019, where he focused on the synthesis of several Gal-(1,4)-GlcNAc disaccharide backbones of the Lewis B carbohydrate antigen under the guidance of Professor France-Isabelle Auzanneau. In 2019, Timothy joined Professor Andrei K. Yudin's research group at the University of Toronto to pursue a Ph.D. in chemistry. He currently holds an Ontario Graduate Scholarship with research interests in cyclic peptides, particularly on the effects of structural modification and conformational restriction on their properties.



Dr. G. P. Gomes

Gabriel dos Passos Gomes (Gabe) received his B.Sc. in chemistry from the Federal University of Rio de Janeiro (Brazil), under the supervision of Professor Pierre Mothè Esteves, in 2013. He earned his Ph.D. in 2018 from Florida State University (USA), under the guidance of Professor Igor V. Alabugin, where he also was awarded the LASER Fellowship in 2014 and the 2016–2017 IBM Ph.D. Scholarship. At FSU, Gabe's research was centered on the relationship between molecular structure and reactivity, focusing on the development and applications of stereoelectronic effects. For his work at FSU, Gabe was awarded: the FSU's Graduate Student Research and Creativity Award for his work in Computational Chemistry; the ACS COMP Chemical Computing Group Excellence Award for his work on the mechanism of the Gold-Catalyzed Bergman Cyclization; selected for the CAS Sci-Finder Future Leaders Program. In 2019, Gabe joined the University of Toronto (Canada) as a Postdoctoral Research Fellow in the Matter Lab, led by Professor Alán Aspuru-Guzik. In 2020, Gabe was awarded the prestigious NSERC Banting Postdoctoral Fellowship with the project „*Designing Catalysts with Artificial Intelligence*“ and was featured on the „*Next Great Impossible*“ series by Merck/Millipore-Sigma. Gabe joined the *Journal of Chemical Information and Modeling* as an Early Career Board member in 2021. His research rests at the interface between machine learning and organic chemistry, where he aims to develop new platforms for reaction discovery, with emphasis on catalysis.