

Article

Scaffolding-induced property modulation of chemical space

Jingyao Li, Vincenzo Di Lorenzo, Pravin Patil, Angel J. Ruiz Moreno, Katarzyna Kurpiewska, Justyna Kalinowska-Tu#cik, Marco A. Velasco-Velázquez, and Alexander Dömling

ACS Comb. Sci., **Just Accepted Manuscript** • DOI: 10.1021/acscombsci.0c00072 • Publication Date (Web): 22 May 2020

Downloaded from pubs.acs.org on May 24, 2020

Just Accepted

"Just Accepted" manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides "Just Accepted" as a service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. "Just Accepted" manuscripts appear in full in PDF format accompanied by an HTML abstract. "Just Accepted" manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are citable by the Digital Object Identifier (DOI®). "Just Accepted" is an optional service offered to authors. Therefore, the "Just Accepted" Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the "Just Accepted" Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these "Just Accepted" manuscripts.

Scaffolding-induced property modulation of chemical space

Jingyao Li,[†] Vincenzo Di Lorenzo,^{§,†} Pravin Patil,[†] Angel J. Ruiz Moreno^{||,†}, Katarzyna Kurpiewska[‡], Justyna Kalinowska-Thušcik[‡], Marco A. Velasco Velázquez^{||} and Alexander Dömling^{†*}

[†] University of Groningen, Department of Drug Design, A. Deusinglaan 1, 9713 AV Groningen, The Netherlands

[§] Università degli studi di Napoli Federico II, Department of Pharmacy, Via Domenico Montesano 49, 80131 Napoli, Italy

^{||} Universidad Nacional Autónoma de México, Departamento de Farmacología, Unidad Periférica de Investigación en Biomedicina Traslacional, Facultad de Medicina y Programa de Doctorado en Ciencias Biomédicas, C.P. 04510, Ciudad de México, México.

[‡] Jagiellonian University, Faculty of Chemistry, Gronostajowa 2, 30-387 Krakow, Poland

*e-mail: a.s.s.domling@rug.nl, www.drugdesign.nl

Supporting Information Placeholder

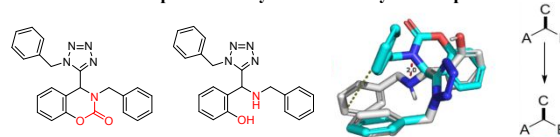
ABSTRACT: Physicochemical property switching of chemical space is of great importance for optimization of compounds, for example for biological activity. Cyclization is a key method to control 3D and other properties. A two-step approach, which involves a multicomponent reaction followed by cyclization, is reported to achieve the transition from basic moieties to charge neutral cyclic derivatives. A series of multi-substituted oxazolidinones, oxazinanones, oxazepanones as well as their thio- and sulfur-derivatives are synthesized from readily available building blocks with mild conditions and high yields. Like few other methods, MCR and cyclization, MCR and cyclization allow for the collective transformation of a large chemical space into a related one with different properties.

KEYWORDS: multicomponent reaction, cyclic carbamate, Ugi reaction, cyclization, tetrazole, scaffolds diversity

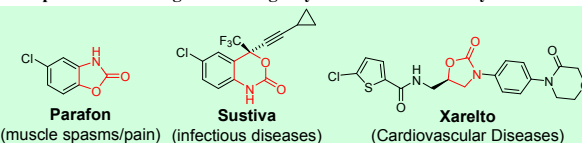
■ INTRODUCTION

The property design of organic compounds is of uttermost importance during the process of optimization to obtain compounds with perfect performance. Properties such as charges or neutrality, 3D distribution of lipophilic or hydrogen donor/acceptor moieties are introduced into molecules by an often lengthy, stepwise and sequential pathway. The principles of multicomponent reaction chemistry (MCR) allow for an orthogonally different approach.¹ In an intellectually and operationally easily building block approach, complex molecules are assembled in one-step from a very large number of available building blocks.^{2,3,4,5} Amongst, derivatization which often changes the properties dramatically are cyclizations. In the context of medicinal chemistry, cyclizations are often introduced to rigidify and generate a conformation similar to the receptor-bound structure, and also to modify drug-like properties such as stability to metabolism or increasing permeability.⁶ By overlapping the 3D structure of cyclic and non-cyclic compounds, stabilization via cyclization and a shift of the terminal moiety was observed (**Figure 1A**). While the number of primary MCRs with useful synthetic properties such as great scope, ease of performance and large number of building blocks (and thus chemical space) is limited, the number of secondary transformations and especially cyclizations is shire infinitely. Hulme and others introduced the very useful concept of UDC (Ugi-deprotection-cyclization) resulting in Epelsiban and Retosiban, which are currently tested in advanced clinical trials.^{7,8}

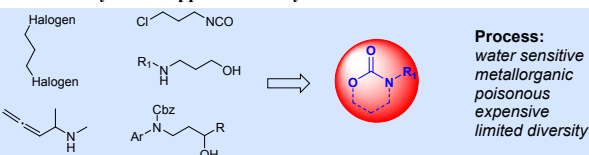
A: 3D structure comparison of cyclic and non-cyclic compounds



B: Representative Drugs containing a cyclic carbamate moiety



C: Previous synthetic approaches to cyclic carbamates



D: MCR/cyclization sequence (our work)

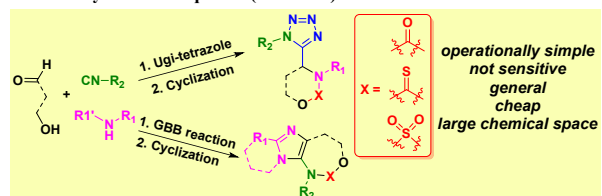


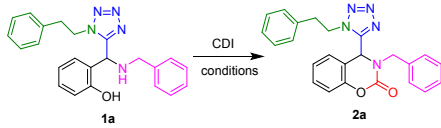
Figure 1. Cyclization strategies in chemistry.

Carbamate containing heterocycles are abundantly present as dominant moieties in plenty of valuable chemicals and therapeutic agents in modern drug discovery and material science development.⁹ Cyclic carbamate moieties are not only present in many drugs (**Figure 1B**), but also play important roles in several chemicals progresses as chiral auxiliaries^{10,11}, and in the preparation of hyper branched polymers^{12,13}. In particular, 6-membered (2-oxazinanone) cyclic carbamates has cumulatively been regarded as privileged scaffolds in drug discovery due to excellent chemical stability and cell membranes permeability. Owing to the prominent medicinal and industrial applicability of cyclic carbamates, various synthetic routes have been exploited (**Figure 1C**)¹⁴. The classical approaches generally involve either phosgene and its derivatives^{15,16}, alkyl halide chemistry¹⁷, or sacrificial reagents¹⁸, such as urea and organic carbonates. Shortly afterward, several other synthetic approaches have been developed to supersede these dissipative, costly and environment hazardous methods. Nevertheless, the following reactions confront various defects, for instance, harsh reaction conditions (0°C or heating)¹⁹, catalyst requirement²⁰, difficult to access starting materials^{21,22}, long reaction time²³, multiple steps²⁴, low yields²⁵, and limited scope²⁶.

Our strategy for a cyclization-induced property change based on MCR chemistry was to use bifunctional orthogonal amino and hydroxyl aldehydes in specific variants of the Ugi reaction, GBB-3CR and UT-4CR, followed by a secondary cyclization on the intermediates to yield (thio) carbamates, ureas and amino sulfonic acid esters of 6- and 7-membered ring size (**Figure 1D**).

RESULTS AND DISCUSSION

Table 1. Optimization of reaction conditions^a



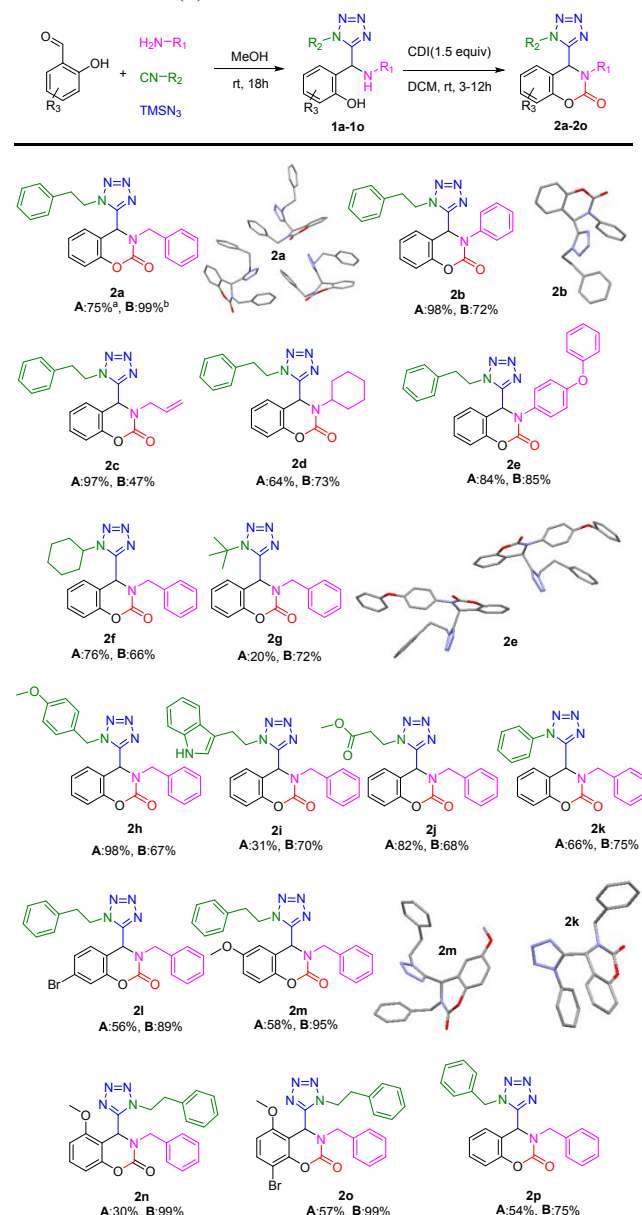
entry	equiv. of CDI	base	time (h)	<i>t</i> (°C)	yield (%) ^b
1	1	-	3	rt	76
2	1	-	3	50	83
3	2	-	3	rt	93
4	2	-	3	50	88
5	2	TEA	12	rt	61
6	2	DIPEA	12	rt	71
7	2	NaHCO ₃	12	rt	48
8	1.2	-	3	rt	85
9	1.5	-	3	rt	99

^aThe CDI conducted reaction was carried out in DCM with 1M concentration; ^bIsolated yields.

The azido-Ugi product **1a**, which features a secondary amine, was chosen as the model substrate to verify this hypothesis. Salicylaldehyde aldehyde was selected in this azido-Ugi reaction as the supplier of the free hydroxyl group. 1,1'-Carbonyldiimidazole (CDI) is a coupling reagent mainly used for the synthesis of amides, peptides, carbamates as well as ureas.^{27,28} Therefore, we envisioned that CDI could enable the desired cyclic carbamate formation by affording the carbonyl group to the MCR product. Having synthesized the

corresponding Ugi-tetrazole in hand, the optimization started with 1 equivalent of CDI in DCM at room temperature, giving a moderate yield of 76% (**Table 1, entry 1**) after 3 h. Further optimization was conducted by increasing either temperature or the equivalent of CDI. As expected, both conditions performed better yields of 83% (**Table 1, entry 2**) and 93% (**Table 1, entry 3**), respectively. Several researches indicated that a catalytic amount of base in CDI reaction will accelerate the formation of the corresponding product.²⁹ However, subsequent attempts have shown that the utilization of base (**Table 1, entry 5-6**) restrict the reaction process, even with longer reaction time. To our delight, superior conditions were found with shorter reaction time without heating. In the optimal protocol, 1.5 equivalent of CDI was added to a solution of azido-Ugi product in DCM at room temperature under air, and the corresponding oxazinanone was formed with nearly quantitative yield in a few hours (**Table 1, entry 9**).

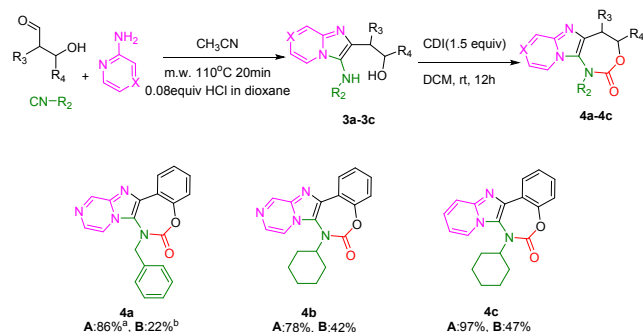
Scheme 1. Yields of the Ugi Products (**1**) and cyclized 1,3-oxazinan-2-one (**2**)



^aIsolated yield of **1**; ^bIsolated yield of **2**

Under optimized conditions, we next examined the substrate scope of cyclization with various amines, isocyanides, and salicylaldehydes with diverse substitutions (**Scheme 1**). The majority of the corresponding oxazinanes resulted in moderate to good yields over two steps. First of all, amino substituents have obvious influences on synthetic conversion. Compounds synthesized by aromatic amines exhibited excellent overall yields, including phenox phenyl substituted **2e** despite the possible steric hindrance. On the contrary, aliphatic amine substitutions reduced the yield of the cyclization. For instance, compound **2c** with allyl substitution affords only 47% yield. In addition, changes in the isocyanide components were well-tolerated. Most of the isolated products have approximately 70% yield. It is noteworthy that the desired compound **2i** was obtained in high yield in the presence of a competitive amino group in the indole ring. Furthermore, aldehydes with methoxyl and halogen substituents on ortho- or meta- positions were evaluated in the scope as well. Surprisingly, most of the cyclization products with either mono- or multi- substituents on the salicylaldehydes gave extraordinary quantitative yields.

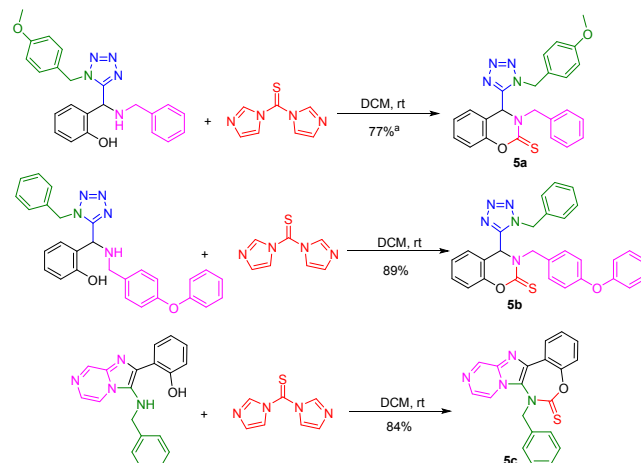
Scheme 2. Yields of the GBB Products (**3**) and cyclized oxazepanones (**4**)



^aIsolated yield of **3**; ^bIsolated yield of **4**

Encouraged by the initial results, we investigated the potential of the cyclization strategy based on Groebke-Blackburn-Bienaymé reactions (GBB reactions) which could afford secondary amines on imidazole heterobicyclic rings (**Scheme 2**).^{2,3,4} Equimolar amounts of aldehyde, amino amine and isocyanide, as well as 0.08 equivalent of HCl in dioxane, were combined in CH_3CN (1 M) in microwave at 110°C for 20 min. The corresponding imidazole-heterobicyclic product **3** was isolated by column chromatography with excellent yields (for example, **3c**, 97%). The identical cyclization approach as above was employed. Not surprisingly, the carbamate formation of the 7-membered 1,3-oxazepan-2-one appeared more difficult than the 6-membered 1,3-oxazin-2-ones. The overall yields of the cyclized GBB products are below 50%. A large amount of imidazole-1-carboxylate intermediates were observed even with longer reaction time.

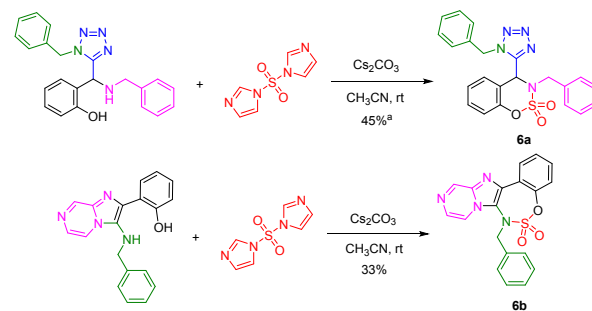
Scheme 3. Substrate Scope of cyclized 1,3-oxazinane-2-thiones and 1,3-oxazepane-2-thiones (**5**)



^aIsolated yield of cyclic thiocarbamate **5**.

In light of the aforementioned results, we next explored the synthesis of thiocarbamate derivatives (**Scheme 3**). 1,1'-Thiocarbonyldiimidazole (TCDI), the sulfur analog of CDI, was employed as the thiocarbonyl donor. Accordingly, the reaction was conducted with 2 equivalents of TCDI in room temperature for 12h in DCM. To our delight, the overall cyclization exhibited good to excellent yields. Positively, enhanced conversion to the thiocarbamate compounds was observed in the Ugi products, compared with the carbamate formation. Furthermore, it is noteworthy that the thiocarbamate GBB product (**5c**, 84%) went better than carbamate GBB (**4a**, 22%), effectively comparable with the Ugi product yields (**5a**, 77%; **5b**, 89%).

Scheme 4. Substrate Scope of cyclized 1,2,3-oxathiazinane-2,2-dioxides and 1,2,3-oxathiazepane-2,2-dioxides (**6**)

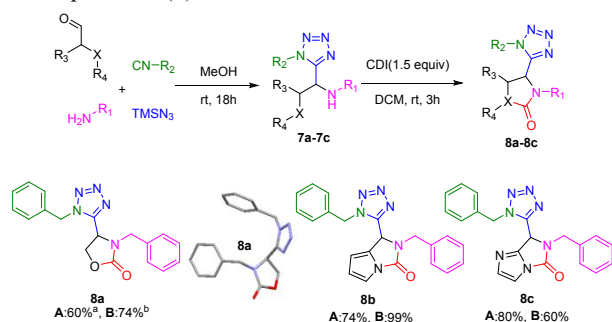


^aIsolated yield of cyclic thiocarbamate **6**.

Next, we attempted to use the uniform approach with the sulfonyl donor 1,1'-Sulfonyldiimidazole (SDI), for the synthesis of sulfamate derivatives (**Scheme 4**). However, under the previous conditions, the reaction remained at the stage of the imidazole-1-sulfonyl intermediate instead of cyclization to the sulfamate product. In order to push the reaction to the desired cyclization, excess amount of Cs_2CO_3 was added to the reaction solution. Under base-catalysis, 6-membered and 7-

membered cyclized sulfamate derivatives were then synthesized in 45% (**6a**) and 33% (**6b**) yield, respectively.

Scheme 5. Substrate Scope of 5-membered CDI cyclization of MCR products (**8**)



^aIsolated yield of **7**; ^bIsolated yield of **8**

As an application of this methodology, we next investigated the scope of 5-membered carbamate cyclization and urea cyclization with CDI as the carbonyl donor (**Scheme 5**). The main innovation of 5-membered ring formation is the employment of various aldehydes. Glycolaldehyde dimer, providing a free hydroxyl group, was used in the azido-Ugi reaction. CDI afforded oxazolidin-2-one **8a** with 74% yield. Pyrrole-2-carboxaldehyde and 2-imidazolecarboxaldehyde, which provided secondary amines, were employed to the formation of heterobicyclic ureas with 99% (**8b**) and 60% (**8c**) yield, respectively. For better yields, a catalytic amount of DIPEA was added in the urea formation.

Additionally, five oxazinanone derivatives (**2a**, **2b**, **2e**, **2k**, **2m**) and one oxazolidinone derivative (**8a**) have been confirmed by X-ray single-crystal analysis (**Scheme 1**, **Scheme 5** and **Supporting Information**).

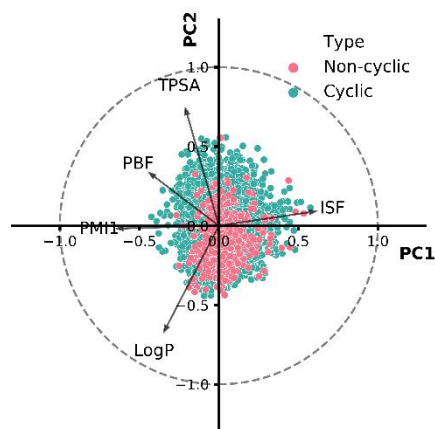


Figure 2 Normalized PC1 vs PC2 plot of cyclic and non-cyclic molecules. Cyclic molecules (green) showed a different distribution against non-cyclic molecules (pink).

To exemplify the scaffolding induced property modulation, we compared 14 physicochemical properties of each 1000 compound virtual libraries^{30,31} of the cyclized and non-cyclized structures (**Figure 2** and **Supporting Information**). PCA of five non-redundant physicochemical properties included 3D descriptors allowed us to identify interesting differences between non-cyclic and cyclic molecules. Topological polar surface area (TPSA) and logP, which are important values in

medicinal chemistry, were identified as the most relevant descriptors (black arrows) to explain the variance among the cyclic and non-cyclic molecules, indicating drug-likeness of all herein described cyclic scaffolds.

CONCLUSION

In summary, a MCR-based synthesis of 5-membered, 6-membered and 7-membered cyclic carbamate derivatives with at least 4 substitutions has been developed with the purpose to modify physicochemical properties. Both the azido-Ugi reaction and the GBB reaction are instrumental in this approach, leading to potentially bioactive bis-heterocyclic or multi-heterocyclic scaffold constructs. Furthermore, their thio- and sulfur- scaffolds are investigated along with the achievement of extraordinary scaffold diversity. The cheminformatics analysis clearly shows we are addressing a drug-like chemical space. Our protocol, utilizing mild condition and readily available building blocks, is of excellent maneuverability, scalability, and efficiency. It will add to a growing body in the development of material and organic synthesis, as well as medicinal chemistry.

ASSOCIATED CONTENT

Supporting Information

NMR spectra, crystal structure determinations and chemical space exploration are included as Supporting Information. This material is available free of charge via the Internet at <http://pubs.acs.org>.

AUTHOR INFORMATION

Corresponding Author

* a.s.s.domling@rug.nl, www.drugdesign.nl

ORCID

Jingyao Li: 0000-0002-9357-9896

Pravin Patil: 0000-0002-0903-8174

Angel J. Ruiz Moreno: 0000-0002-2798-5386

Alexander Dömling: 0000-0002-9923-8873

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research has been supported to (AD) by the National Institute of Health (NIH) (2R01GM097082-05), the European Lead Factory (IMI) under grant agreement number 115489, the Qatar National Research Foundation (NPRP6-065-3-012). Moreover, funding was received through ITN “Accelerated Early stage drug dIScovery” (AEGIS, grant agreement No

675555) and COFUND ALERT (grant agreement No 665250), Hartstichting (ESCAPE-HF, 2018B012) and KWF Kankerbestrijding grant (grant agreement No 10504). The author thanks Markella Konstantinidou (University of Groningen) for critical reading and careful revision of the manuscript, and Marcel de Vries (University of Groningen) for his help in HRMS analysis. Jingyao Li acknowledged the China Scholarship Council for financial support.

■ REFERENCES

- (1) Cioc, R. C.; Ruijter, E.; Orru, R. V. Multicomponent reactions: advanced tools for sustainable organic synthesis. *Green Chem.* **2014**, 16(6), 2958-75.
- (2) Dömling, A. Recent Developments in Isocyanide Based Multicomponent Reactions in Applied Chemistry. *Chem. Rev.* **2006**, 106(1), 17-89.
- (3) Dömling, A.; Ugi, I. Multicomponent Reactions with Isocyanides. *Angew. Chemie* **2000**, 39(18), 3168-3210.
- (4) Boltjes, A.; Dömling, A. The Groebke - Blackburn - Bienaymé reaction. *Eur. J. Org. Chem.* **2019**, 42, 7007-7049.
- (5) Ugi, I.; Werner, B.; Dömling, A.. The chemistry of isocyanides, their multicomponent reactions and their libraries. *Molecules.* **2003**, 8(1), 53-66.
- (6) Di, L.; Kerns, E. H. Drug-like properties: concepts, structure design and methods from ADME to toxicity optimization. Academic press. **2015**, Dec 17.
- (7) Hulme, C.; Gore, V. Multi-component Reactions: Emerging Chemistry in Drug Discovery "From Xylocain to Crixivan". *Curr. Med. Chem.* **2003**, 10(1), 51-80.
- (8) Borthwick, A. D.; Liddle, J.; Davies, D. E.; Exall, A. M.; Hamlett, C.; Hickey, D. M.; Mason, A. M.; Smith, I. E.; Nerozzi, F.; Peace, S.; Pollard, D. Pyridyl-2, 5-diketopiperazines as potent, selective, and orally bioavailable oxytocin antagonists: synthesis, pharmacokinetics, and in vivo potency. *J. Med. Chem.* **2012**, 55(2), 783-96.
- (9) Ghosh, A. K.; Brindisi, M. Organic Carbamates in Drug Design and Medicinal Chemistry. *J. Med. Chem.* **2015**, 58(7), 2895-2940.
- (10) Nicolaou, K. C.; Winssinger, N.; Pastor, J.; Murphy, F. Solid-Phase Synthesis of Macrocyclic Systems by a Cyclorelease Strategy: Application of the Stille Coupling to a Synthesis of (S)-Zearalenone. *Angew. Chemie - Int. Ed.* **1998**, 37(18), 2534-2537.
- (11) Evans, D. A.; Bartroli, J.; Shih, T. L. Enantioselective Aldol Condensations. 2. Erythro-Selective Chiral Aldol Condensations via Boron Enolates. *J. Am. Chem. Soc.* **1981**, 103(8), 2127-2129.
- (12) Voit, B. I.; Lederer, A. Hyperbranched and Highly Branched Polymer Architectures-Synthetic Strategies and Major Characterization Aspects. *Chem. Rev.* **2009**, 109(11), 5924-5973.
- (13) Kreye, O.; Mutlu, H.; Meier, M. A. R. Sustainable Routes to Polyurethane Precursors. *Green Chem.* **2013**, 15(6), 1431-1455.
- (14) Aricò, F.; Bravo, S.; Crisma, M.; Tundo, P. 1,3-Oxazinan-2-Ones via Carbonate Chemistry: A Facile, High Yielding Synthetic Approach. *In Pure and Applied Chemistry*; **2016**, 88(3), 227-237.
- (15) Murdock, K. C. 2-Oxazolidinones from an N-Dealkylation Reaction of Phosgene with Dialkylaminoalkanols. The Isolation and Reactivities of an N-Acyl Quaternary Ammonium Intermediate. *J. Org. Chem.* **1968**, 33(4), 1367-1371.
- (16) Dao Thi, H.; Le Nhat Thuy, G.; Catak, S.; Van Speybroeck, V.; Van Nguyen, T.; D'Hooghe, M. Use of 3-Hydroxy-4-(Trifluoromethyl)Azetidin-2-Ones as Building Blocks for the Preparation of Trifluoromethyl-Containing Aminopropanes, 1,3-Oxazinan-2-Ones, Aziridines, and 1,4-Dioxan-2-Ones. *Synth.* **2018**, 50(07), 1439-1456.
- (17) Trifunović, S.; Dimitrijević, D.; Vasić, G.; Radulović, N.; Vukićević, M.; Heinemann, F. W.; Vukićević, R. D. New Simple Synthesis of N-Substituted 1,3-Oxazinan-2-Ones. *Synth.* **2010**, 2010(06), 943-946.
- (18) Kim, Y. J.; Varma, R. S. Microwave-Assisted Preparation of Cyclic Ureas from Diamines in the Presence of ZnO. *Tetrahedron Lett.* **2004**, 45(39), 7205-7208.
- (19) Rice, G. T.; White, M. C. Allylic C-H Amination for the Preparation of Syn-1,3-Amino Alcohol Motifs. *J. Am. Chem. Soc.* **2009**, 131(33), 11707-11711.
- (20) Nahra, F.; Liron, F.; Prestat, G.; Mealli, C.; Messaoudi, A.; Poli, G. Striking AcOH Acceleration in Direct Intramolecular Allylic Amination Reactions. *Chem. - A Eur. J.* **2009**, 15(42), 11078-11082.
- (21) Fujita, M.; Kitagawa, O.; Suzuki, T.; Taguchi, T. Regiocontrolled Iodoaminocyclization Reaction of an Ambident Nucleophile Mediated by Basic Metallic Reagent. *J. Org. Chem.* **1997**, 62(21), 7330-7335.
- (22) Quinodoz, P.; Quelhas, A.; Wright, K.; Drouillard, B.; Marrot, J.; Couty, F. Iodocarbamation of N-Homopropargyl Carbamates: Mild and Stereoselective Entry to Functionalized Oxazinan-2-Ones. *European J. Org. Chem.* **2017**, 18, 2621-2626.
- (23) Mangelinckx, S.; Nural, Y.; Dondas, H. A.; Denolf, B.; Sillanpää, R.; De Kimpe, N. Diastereoselective Synthesis of 6-Functionalized 4-Aryl-1,3-Oxazinan-2-Ones and Their Application in the Synthesis of 3-Aryl-1,3-Aminoalcohols and 6-Arylpiperidine-2,4-Diones. *Tetrahedron* **2010**, 66(23), 4115-4124.
- (24) Ella-Menye, J. R.; Sharma, V.; Wang, G. New Synthesis of Chiral 1,3-Oxazinan-2-Ones from Carbohydrate Derivatives. *J. Org. Chem.* **2005**, 70(2), 463-469.
- (25) Kayaki, Y.; Mori, N.; Ikariya, T. Palladium-Catalyzed Carboxylate Cyclization of α -Allenyl Amines in Dense Carbon Dioxide. *Tetrahedron Lett.* **2009**, 50(47), 6491-6493.
- (26) Niemi, T.; Repo, T. Antibiotics from Carbon Dioxide: Sustainable Pathways to Pharmaceutically Relevant Cyclic Carbamates. *European J. Org. Chem.* **2019**, 6, 1180-1188.
- (27) Staab, H. A. New Methods of Preparative Organic Chemistry IV. Syntheses Using Heterocyclic Amides (Azolides). *Angew. Chemie Int. Ed. English* **1962**, 1(7), 351-367.
- (28) Paul, R.; Anderson, G. W. N,N'-Carbonyldiimidazole, a New Peptide Forming Reagent. *J. Am. Chem. Soc.* **1960**, 82(17), 4596-4600.
- (29) Larrivé-Aboussafy, C.; Jones, B. P.; Price, K. E.; Hardink, M. A.; McLaughlin, R. W.; Lillie, B. M., ... & Vaidyanathan, R. DBU catalysis of N, N'-carbonyldiimidazole-mediated amidations. *Org. Lett.* **2009**, 12(2), 324-327.
- (30) Instant JChem was used for structure database management, search and prediction, Instant JChem 19.4, 2019, ChemAxon (<http://www.chemaxon.com>).
- (31) Reactor was used for enumeration and reaction modeling, JChem 19.4, 2019, ChemAxon

(<http://www.chemaxon.com>).

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60