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Peptide to Peptoid Substitutions Increase Cell Permeability in Cyclic Hexapeptides

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S *Supporting Information*

ABSTRACT: The effect of peptide-to-peptoid substitutions on the passive membrane permeability of an *N*-methylated cyclic hexapeptide is examined. In general, substitutions maintained permeability but increased conformational heterogeneity. Diversification with nonproteinogenic side chains increased permeability up to 3-fold. Additionally, the conformational impact of peptoid substitutions within a *β*-turn are explored. Based on these results, the strategic incorporation of peptoid residues into cyclic peptides can maintain or improve cell permeability, while increasing access to diverse side-chain functionality.
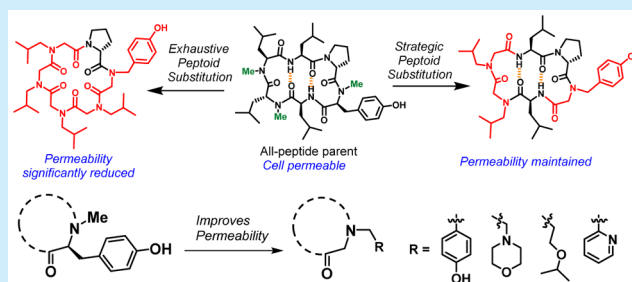
As drug discovery efforts increasingly focus on challenging intracellular targets such as protein–protein interactions (PPIs) and other types of relatively "flat" binding sites, finding highly potent inhibitors among classically drug-like molecules has become increasingly challenging.[1,2] Macrocycles occupy a middle ground in chemical space between biologics and typical small molecule drugs, with the complexity (and potential for binding flat surfaces) approaching that of the former, and the potential for cell permeability and oral bioavailability of the latter.[3−7] Their modular synthesis, combined with the favorable impact of macrocyclization on metabolic stability and cell permeability, makes cyclic peptides ideal macrocyclic scaffolds for exploring this chemical space. Furthermore, one-bead-one-compound (OBOC), DNA-encoded and DNA-programmed synthesis methodologies allow large (10^5) to very large (10^{12}) libraries of cyclic peptides to be generated and screened,[8−10] while structural diversity and complexity can be achieved by permuting backbone elements such as stereochemistry[11] and backbone *N*-methylation,[12] variables that can be modulated at the monomer level. Although solid phase peptide synthesis (SPPS) has been thoroughly developed since its first implementation over 50 years ago,[13] achieving side chain diversity in peptide libraries requires access to diverse amino acid building blocks, of which relatively few are commercially available.

Oligomers of N-substituted glycine units ("peptoids") were originally conceived as synthetic peptide derivatives that possess the modularity and protein binding characteristics of peptides, but avoid the pitfalls associated with the notoriously poor metabolic stability of peptides. Since then, peptoids have found a number of uses including metal chelation,[14] *in vitro* gene delivery,[15] and antifouling coatings.[16] Unlike peptides, which are generated (both chemically and biosynthetically) by coupling amino acids sequentially to a growing peptide chain, peptoids can be generated by the "submonomer method" in which the terminal amine is capped with a chloro- or bromoacetamide followed by introduction of the peptoid side chain by S_N2 substitution with an incoming primary amine.[17] In contrast to the relatively small pool of commercially available amino acids, the pool of commercially available primary amines numbers in the thousands.

Large peptoid libraries have yielded potent hits in phenotypic screens[18,19] and against a variety of protein targets, including VEGF,[20,21] the proteasome,[22] p53-MDM2,[23] and MMP14,[24]

among others.[25] Kodakek et al. recently reported that libraries of cyclic peptoids generated more high affinity hits over their linear counterparts[26] while Ovadia et al. found peptide to peptoid substitutions in a cyclic melanocortin agonist improved both metabolic stability and paracellular permeability.[27] A number of studies have evaluated the effect of peptide to peptoid substitution on the potency of bioactive cyclic peptides,[28−31] and their conformational preferences have been extensively investigated.[32,33] There is evidence that peptoids are more membrane permeable than peptides;[27,34] however, most of the peptoid ligands discovered to date have either been directed at extracellular targets[35,36] or have shown modest activity against intracellular targets.[22,37] To date there has been no direct comparison between cyclic peptides and peptide-peptoid hybrids with respect to passive transcellular permeability.

Previously our laboratories investigated the influence of backbone stereochemistry and N-methylation on the permeability and metabolic stability of cyclic hexapeptides. One permeable scaffold identified by our laboratory, cyclo[Leu1-D(NMe)Leu2-(NMe)Leu3-Leu4-DPro5-(NMe)Tyr6] (**1NMe3**)[38] (Figure 1), has been studied as a model cyclic

for the systematic investigation of the physiochemical properties imparted by N-methyl amino acid-to-peptoid substitutions.

To this end, we systematically replaced every N-methylated residue of **1NMe3** with the equivalent N-substituted glycine (peptoid) residue (Figure 1). In addition, the Leu at position 1 was replaced with N-isobutyl glycine (**L1N**) and all residues but Pro were replaced with peptoid residues (**L1-4Y6N**). Peptoid residues were accessed through the introduction of bromoacetic acid followed by nucleophilic displacement with the desired amine.[17] Interestingly, a number of these compounds, notably **L1N** and **L1-4Y6N**, displayed mixtures on the NMR and even chromatographic time scales (Supporting Information (SI) pp 9−21, 31−45; SI Table 8), highlighting the propensity for N-substituted glycine residues to adopt the cis-amide conformation.[45] To further explore the properties imparted by these peptoid substitutions, this small series was subjected to the MDCK-LE[46] (Madin-Darby Canine Kidney epithelial cells — Low Efflux) trans-well assay, an in vitro assessment of compound permeation across a monolayer of epithelial cells selected for low efflux pump expression with results reported as a one-dimensional diffusion rate ($\times$ 10^{-6} cm/s) across the cell monolayer (Figure 1).

Peptoid substitutions generally maintained the cell permeability of the parent compound. The single peptoid substitutions at Leu2 and Tyr6 (**L2N** and **Y6N**, respectively) led to a nearly 50% increase in cellular permeability. The bis-substitution Leu2, Tyr6 (**L2Y6N**) exhibited a similar increase in cell permeability. The exception to this trend was the pentapeptide substitution **L1-4Y6N**, which showed drastically reduced permeability. Notably, all compounds containing a Leu3-to-peptoid (**L3N**, **L2L3N**, **L3Y6N**, **L2L3Y6N**, and **L1-4Y6N**) substitution exhibited lower cell permeability than the parent, while all compounds in which Leu3 remained an N-methyl amino acid (**L1N**, **L2N**, **Y6N**, and **L2Y6N**) exhibited increased cell permeability. Since Leu2 and Leu3 represent i + 1 and i + 2 positions of a β-turn, it was hypothesized that positionally dependent modulation of this key structural element was the source of the augmented cellular permeability between the peptide−peptoid hybrids in this series.

In order to understand the relationship between peptoid position and cell permeability we sought to investigate and compare the low dielectric solution structures of **L2N** and **L3N**. NMR experiments were conducted in CDCl$_3$, which approximates the internal dielectric of a phospholipid bilayer.[47] Peak assignments were made using COSY, TOCSY, HSQC, and HMBC experiments, and ROESY-derived interproton distances were calculated based on crosspeak volumes. $^3J_{H-H}$ couplings were also used to provide dihedral information where available. Conformers were generated using molecular dynamics (MD) simulations, and ensembles were fit to the distance and dihedral restraints using the program DISCON.[48,49]

Of particular interest is how the β-turn is affected by the introduction of a peptoid residue in a cyclic system, a topic explored previously in linear di- and-tetrapeptide model systems.[50,51] In the structure of **1NMe3**, both the dLeu2-Leu3 and dPro5-Tyr6 β-turns adopt a type II′ β-turn, enforced by the D-residue at the i + 1 position.[38] Both **L2N** and **L3N** also adopt a double β-turn structure (SI Tables 6 and 7). Interestingly, the β-turn encompassing positions 2 and 3 in compound **L2N** has inverted to a type II β-turn, likely due to the loss of D chirality at the i + 1 position. **L3N** maintains the chirality at position 2 but, in contrast to the parent **1NMe3**, fails to achieve a tight β-turn about positions 2 and 3 with H−O H-bond distances of 3.9 and 1.9 Å respectively. This is likely due to the steric constraints
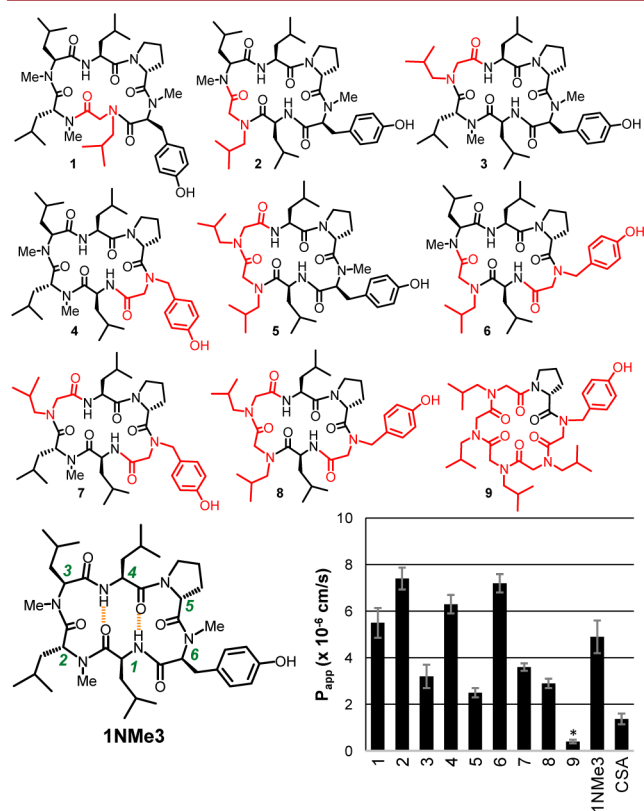

Figure 1. Peptoid series and MDCK-LE apparent cell permeability (average of 3 replicates, * denotes 2 replicates only).

peptide in recent publications from our group and others.[39−43] This compound's defining structural characteristics are a double type II′ β-turn structure, engaging both non-N-methylated amide protons in intramolecular H-bonds. β-Turn mimicry may represent an accessible pharmacological niche for small cyclic peptides, as a number of protein−protein interactions are mediated by loops containing this structural motif.[44] Because of its favorable ADME/PK properties, double β-turn structure, and multiple N-alkylated residues, **1NMe3** provided a model system

imposed by the increased size of the *N*-alkyl group leading to increased solvent exposure of the carbonyl of Leu1 in the more expanded structure of **L3N** (Figure 2e and f). Using the
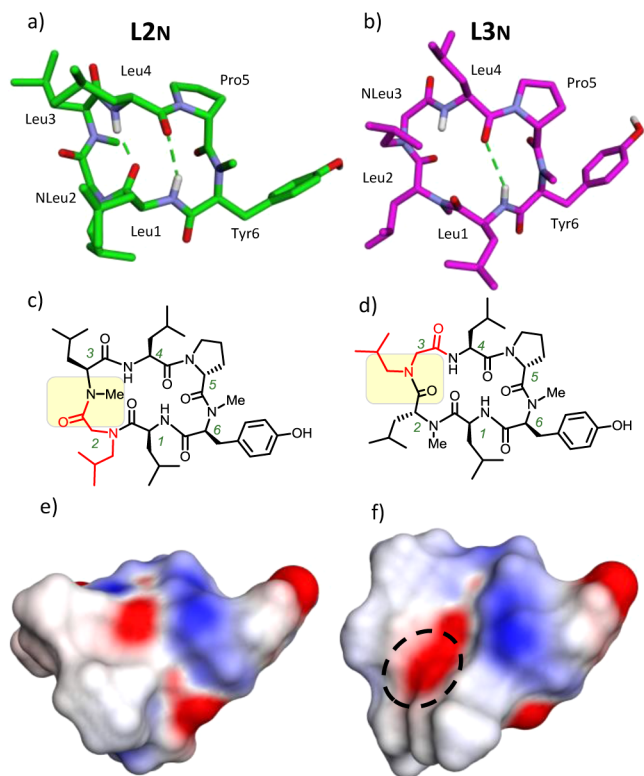

Figure 2. (a,b) NMR restrained solution conformations of **L2N** and **L3N**, respectively. (c,d) 2D representation of the conformations of **L2N** and **L3N**, respectively, with the amide between positions 2 and 3 highlighted in yellow, peptoid residues highlighted in red. (e, f) Electrostatic surfaces of the **L2N** and **L3N**, with the solvent exposed carbonyl of Leucine 1 highlighted with a dashed circle.

aforementioned NMR structures the expanded conformation of **L3N** has a higher computationally derived solvent exposed polar surface area (101 Å^2) than either **L2N** (78 Å^2) or **1NMe3** (87.2 Å^2) employing the method developed by Ertl et al.[52] and used extensively by Guimaraes et al.[53] to analyze a large library of permeable macrocycles. However, both **L2N** and **L3N** have minor conformations that are stable on the NMR time scale (most likely cis−trans rotamers about the peptoid and/or N-Me amide bonds). Therefore, the observed difference in permeability between these compounds cannot be attributed solely to differences in solvent exposure between the major conformers alone. Future NMR studies should provide insight into the contribution of minor conformers to the permeability of the ensemble.

Finally, since a benefit of peptoid residues is the potential for facile diversification, we sought to test whether the favorable cell permeability properties of **Y6N** tolerated the introduction of more diverse chemical functionality. To this end, we synthesized a small series of cyclic peptide peptoid hybrids encompassing chemical space not easily obtainable in the pool of commercially available, protected amino acids (non-natural heterocycles, trifluoromethylation, and ether linkages) and subjected these to the same MDCK-LE assay as compounds **1−9**.

Compound **10** exhibited increased permeability over **1−9** while **12** and **13** showed a modest increase (Figure 3). The
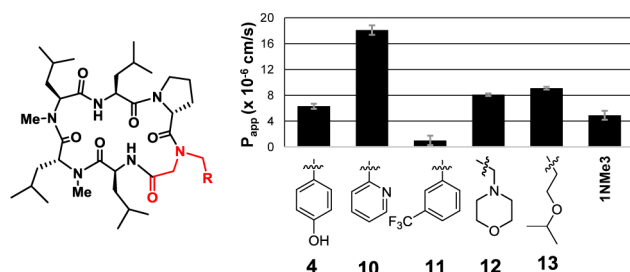

Figure 3. Peptoid series and MDCK-LE apparent cell permeability (average of 3 replicates).

increased permeability of **10**, **12**, and **13** over **Y6N** may be due to the removal of the H-bond donating phenolic −OH. Despite the mild basicity of **12** (pK_a ~7.4), it shows comparable permeability to the parent compound. The low permeability of compound **11** is likely due to low recovery (40.5%) in the MDCK-LE assay, which is not surprising given its exceedingly high lipophilicity. Compounds **Y6N**, **10**, **12**, and **13** have calculated (2D) ALogP[54] values of <4.0 while **11** has an ALogP of 5.2. Additionally, the replacement of the phenolic side chain (present in **1−9**) with one lacking a H-bond donor (**10−13**) greatly decreased the EPSA[55] for those peptides: 76−82 vs 53−58 Å^2 respectively (SI Table 9 and Figure 1). The EPSA value is determined through a chromatographic technique designed to experimentally assess the polar surface area of a molecule in a low dielectric environment. This highlights the permeability penalty incurred by the presence of unsatisfied H-bond donors in the side chains.

The series of compounds **1−13** demonstrate promise for designing peptide−peptoid hybrid macrocycles with favorable ADME properties. Interestingly **L1N** exhibits permeability in the top 50% of those observed in the study, higher than that of **1NMe3**. The replacement of the amide hydrogen of L1 with an isobutyl would completely prevent any *β*-turn about this position due to the removal of a H-bond donor and the increased steric bulk. A complex mixture on the NMR time scale in chloroform (SI p 40; SI Table 8) prevented a solution structure from being obtained, but it has likely found another or multiple lipophilic conformations other than the double *β*-turned structure.

In the cases of **L2N** and **L3N** the use of NMR and molecular dynamics offers a potential explanation into the source of their observed differences in permeability. These results suggest that cyclic peptide peptoid hybrids combine the chemical diversity achievable using amine-derived peptoids with the stereochemical complexity and intramolecular H-bonding of peptides to allow access to novel, versatile, and cell permeable macrocycles.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

These data include synthetic and computational details as well as thorough characterization of all compounds synthesized. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01162.

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Notes

The authors declare the following competing financial interest(s): M.P.J. is a consultant to Schrödinger LLC and Pfizer Inc. R.S.L. and M.P.J. are founders of Circle Pharma.

■ REFERENCES

(1) Hopkins, A. L.; Groom, C. R. *Nat. Rev. Drug Discovery* **2002**, *1*, 727.

(2) Raj, M.; Bullock, B. N.; Arora, P. S. *Bioorg. Med. Chem.* **2013**, *21*, 4051.

(3) Driggers, E. M.; Hale, S. P.; Lee, J.; Terrett, N. K. *Nat. Rev. Drug Discovery* **2008**, *7*, 608.

(4) Villar, E. A.; Beglov, D.; Chennamadhavuni, S.; Porco, J. A.; Kozakov, D.; Vajda, S.; Whitty, A. *Nat. Chem. Biol.* **2014**, *10*, 723.

(5) Biron, E.; Chatterjee, J.; Ovadia, O.; Langenegger, D.; Brueggen, J.; Hoyer, D.; Schmid, H. A.; Jelinek, R.; Gilon, C.; Hoffman, A.; Kessler, H. *Angew. Chem., Int. Ed.* **2008**, *47*, 2595.

(6) Beck, J. G.; Chatterjee, J.; Laufer, B.; Kiran, M. U.; Frank, A. O.; Neubauer, S.; Ovadia, O.; Greenberg, S.; Gilon, C.; Hoffman, A.; Kessler, H. *J. Am. Chem. Soc.* **2012**, *134*, 12125.

(7) Nielsen, D. S.; Hoang, H. N.; Lohman, R.-J.; Diness, F.; Fairlie, D. P. *Org. Lett.* **2012**, *14*, 5720.

(8) Liu, T.; Joo, S. H.; Voorhees, J. L.; Brooks, C. L.; Pei, D. *Bioorg. Med. Chem.* **2009**, *17*, 1026.

(9) Georghiou, G.; Kleiner, R. E.; Pulkoski-Gross, M.; Liu, D. R.; Seeliger, M. A. *Nat. Chem. Biol.* **2012**, *8*, 366.

(10) Yamagishi, Y.; Shoji, I.; Miyagawa, S.; Kawakami, T.; Katoh, T.; Goto, Y.; Suga, H. *Chem. Biol.* **2011**, *18*, 1562.

(11) Rezai, T.; Bock, J. E.; Zhou, M. V.; Kalyanaraman, C.; Lokey, R. S.; Jacobson, M. P. *J. Am. Chem. Soc.* **2006**, *128*, 14073.

(12) Chatterjee, J.; Mierke, D. F.; Kessler, H. *Chem.—Eur. J.* **2008**, *14*, 1508.

(13) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149.

(14) Knight, A. S.; Zhou, E. Y.; Pelton, J. G.; Francis, M. B. *J. Am. Chem. Soc.* **2013**, *135*, 17488.

(15) Murphy, J. E.; Uno, T.; Hamer, J. D.; Cohen, F. E.; Dwarki, V.; Zuckermann, R. N. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 1517.

(16) Van Zoelen, W.; Buss, H. G.; Ellebracht, N. C.; Lynd, N. A.; Fischer, D. A.; Finlay, J.; Hill, S.; Callow, M. E.; Callow, J. A.; Kramer, E. J.; Zuckermann, R. N.; Segalman, R. A. *ACS Macro Lett.* **2014**, *3*, 364.

(17) Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. *J. Am. Chem. Soc.* **1992**, *114*, 10646.

(18) Montoliu, C.; Humet, M.; Canales, J.-J.; Burda, J.; Planells-Cases, R.; Sánchez-Baeza, F.; Carbonell, T.; Pérez-Payá, E.; Messeguer, A.; Ferrer-Montiel, A.; Felipo, V. *J. Pharmacol. Exp. Ther.* **2002**, *301*, 29.

(19) Mas-Moruno, C.; Cruz, L. J.; Mora, P.; Francesch, A.; Messeguer, A.; Pérez-Payá, E.; Albericio, F. *J. Med. Chem.* **2007**, *50*, 2443.

(20) De León-Rodríguez, L. M.; Lubag, A.; Udugamasooriya, D. G.; Proneth, B.; Brekken, R. A.; Sun, X.; Kodadek, T.; Dean Sherry, A. *J. Am. Chem. Soc.* **2010**, *132*, 12829.

(21) Lynn, K. D.; Udugamasooriya, D. G.; Roland, C. L.; Castrillon, D. H.; Kodadek, T. J.; Brekken, R. A. *BMC Cancer* **2010**, *10*, 397.

(22) Lim, H.-S.; Archer, C. T.; Kim, Y.-C.; Hutchens, T.; Kodadek, T. *Chem. Commun.* **2008**, 1064.

(23) Hara, T.; Durell, S. R.; Myers, M. C.; Appella, D. H. *J. Am. Chem. Soc.* **2006**, *128*, 1995.

(24) Gao, Y.; Amar, S.; Pahwa, S.; Fields, G.; Kodadek, T. *ACS Comb. Sci.* **2015**, *17*, 49.

(25) Zuckermann, R. N.; Kodadek, T. *Curr. Opin. Mol. Ther.* **2009**, *11*, 299.

(26) Gao, Y.; Kodadek, T. *ACS Comb. Sci.* **2015**, *17*, 190.

(27) Ovadia, O.; Linde, Y.; Haskell-Luevano, C.; Dirain, M. L.; Sheynis, T.; Jelinek, R.; Gilon, C.; Hoffman, A. *Bioorg. Med. Chem.* **2010**, *18*, 580.

(28) Demmer, O.; Frank, A. O.; Hagn, F.; Schottelius, M.; Marinelli, L.; Cosconati, S.; Brack-Werner, R.; Kremb, S.; Wester, H.-J.; Kessler, H. *Angew. Chem., Int. Ed.* **2012**, *51*, 8110.

(29) Shankaramma, S. C.; Moehle, K.; James, S.; Vrijbloed, J. W.; Obrecht, D.; Robinson, J. A. *Chem. Commun.* **2003**, 1842.

(30) Fowler, S. A.; Stacy, D. M.; Blackwell, H. E. *Org. Lett.* **2008**, *10*, 2329.

(31) Olsen, C. A.; Montero, A.; Leman, L. J.; Ghadiri, M. R. *ACS Med. Chem. Lett.* **2012**, *3*, 749.

(32) Renfrew, P. D.; Craven, T. W.; Butterfoss, G. L.; Kirshenbaum, K.; Bonneau, R. *J. Am. Chem. Soc.* **2014**, *136*, 8772.

(33) Fowler, S. A.; Blackwell, H. E. *Org. Biomol. Chem.* **2009**, *7*, 1508.

(34) Tan, N. C.; Yu, P.; Kwon, Y. U.; Kodadek, T. *Bioorg. Med. Chem.* **2008**, *16*, 5853.

(35) Udugamasooriya, D. G.; Ritchie, C.; Brekken, R. A.; Kodadek, T. *Bioorg. Med. Chem.* **2008**, *16*, 6338.

(36) Udugamasooriya, D. G.; Dineen, S. P.; Brekken, R. A.; Kodadek, T. *J. Am. Chem. Soc.* **2008**, *130*, 5744.

(37) Lim, H.-S.; Archer, C. T.; Kodadek, T. *J. Am. Chem. Soc.* **2007**, *129*, 7750.

(38) White, T. R.; Renzelman, C. M.; Rand, A. C.; Rezai, T.; McEwen, C. M.; Gelev, V. M.; Turner, R. A.; Linington, R. G.; Leung, S. S. F.; Kalgutkar, A. S.; Bauman, J. N.; Zhang, Y.; Liras, S.; Price, D. A.; Mathiowetz, A. M.; Jacobson, M. P.; Lokey, R. S. *Nat. Chem. Biol.* **2011**, *7*, 810.

(39) Wang, C. K.; Northfield, S. E.; Colless, B.; Chaousis, S.; Hamernig, I.; Lohman, R.-J.; Nielsen, D. S.; Schroeder, C. I.; Liras, S.; Price, D. A.; Fairlie, D. P.; Craik, D. J. *Proc. Natl. Acad. Sci. U.S.A.* **2014**, *111*, 17504.

(40) Rand, A. C.; Leung, S. S. F.; Eng, H.; Rotter, C. J.; Sharma, R.; Kalgutkar, A. S.; Zhang, Y.; Varma, M. V.; Farley, K. A.; Khunte, B.; Limberakis, C.; Price, D. A.; Liras, S.; Mathiowetz, A. M.; Jacobson, M. P.; Lokey, R. S. *MedChemComm* **2012**, *3*, 1282.

(41) Lewis, I.; Schaefer, M.; Wagner, T.; Oberer, L.; Sager, E.; Wipfli, P.; Vorherr, T. *Int. J. Pept. Res. Ther.* **2015**, *21*, 205.

(42) Thansandote, P.; Harris, R. M.; Dexter, H. L.; Simpson, G. L.; Pal, S.; Upton, R. J.; Valko, K. *Bioorg. Med. Chem.* **2015**, *23*, 322.

(43) Wang, C. K.; Northfield, S. E.; Swedberg, J. E.; Colless, B.; Chaousis, S.; Price, D. A.; Liras, S.; Craik, D. J. *Eur. J. Med. Chem.* **2015**, *97*, 202.

(44) Gavenonis, J.; Sheneman, B. A.; Siegert, T. R.; Eshelman, M. R.; Kritzer, J. A. *Nat. Chem. Biol.* **2014**, *10*, 716.

(45) Sui, Q.; Borchardt, D.; Rabenstein, D. L. *J. Am. Chem. Soc.* **2007**, *129*, 12042.

(46) Di, L.; Whitney-Pickett, C.; Umland, J. P.; Zhang, H.; Zhang, X.; Gebhard, D. F.; Lai, Y.; Federico, J. J.; Davidson, R. E.; Smith, R.; Reyner, E. L.; Lee, C.; Feng, B.; Rotter, C.; Varma, M. V.; Kempshall, S.; Fenner, K.; El-Kattan, A. F.; Liston, T. E.; Troutman, M. D. *J. Pharm. Sci.* **2011**, *100*, 4974.

(47) Koehorst, R. B. M.; Spruijt, R. B.; Vergeldt, F. J.; Hemminga, M. A. *Biophys. J.* **2004**, *87*, 1445.

(48) Atasoylu, O.; Furst, G.; Risatti, C.; Smith, A. B., III. *Org. Lett.* **2010**, *12*, 1788.

(49) Evans, D. A.; Bodkin, M. J.; Baker, S. R.; Sharman, G. J. *Magn. Reson. Chem.* **2007**, *45*, 595.

(50) Rainaldi, M.; Moretto, V.; Crisma, M.; Peggion, E.; Mammi, S.; Toniolo, C.; Cavicchioni, G. *J. Pept. Sci.* **2002**, *8*, 241.

(51) Butterfoss, G. L.; Drew, K.; Renfrew, P. D.; Kirshenbaum, K.; Bonneau, R. *Biopolymers* **2014**, *102*, 369.

(52) Ertl, P.; Rohde, B.; Selzer, P. *J. Med. Chem.* **2000**, *43*, 3714.

(53) Guimaraes, C. R. W.; Mathiowetz, A. M.; Shalaeva, M.; Goetz, G.; Liras, S. *J. Chem. Inf. Model.* **2012**, *52*, 882.

(54) Ghose, A. K.; Crippen, G. M. *J. Comput. Chem.* **1986**, *7*, 565.

(55) Goetz, G. H.; Philippe, L.; Shapiro, M. J. *ACS Med. Chem. Lett.* **2014**, *5*, 1167.