JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Communication

pubs.acs.org/JACS

Site-Specific Integration of Amino Acid Fragments into Cyclic Peptides

Christopher J. White, Jennifer L. Hickey, Conor C. G. Scully, and Andrei K. Yudin*

Davenport Research Laboratories, Department of Chemistry, University of Toronto, 80 St. George Street, Toronto, Ontario M5S 3H6, Canada

Supporting Information

ABSTRACT: The concept of site-specific integration of fragments into macrocyclic entities has not yet found application in the realm of synthetic chemistry. Here we show that the reduced amidicity of aziridine amide bonds provides an entry point for the site-specific integration of amino acids and peptide fragments into the homodetic cyclic peptide architecture. This new synthetic operation improves both the convergence and divergence of cyclic peptide synthesis.

Cyclic peptides have been gaining traction in both drug discovery and biological probe design.[1] Significant promise lies in the structural attributes of these molecules.[2] Their large surface area, capacity to "wrap" polar functionalities,[3] and resistance to proteolytic degradation[4] make macrocycles attractive tools for the interrogation of challenging biological targets such as protein−protein interactions.[5] Although a number of natural products with large ring structures are known, macrocycles are still underrepresented in the screening collections of both the pharmaceutical industry and academic centers. This paucity of accessible structures has fueled interest in developing new methods for peptide cyclization. Although great strides in devising ways to close the ring have been made,[6] there exist few approaches that allow for selective modification of the macrocycle core itself.[7]

Our recent studies in peptide macrocyclization using aziridine aldehyde dimers[8] prompted us to explore the stereoelectronic features of aziridine amides in other contexts. *N*-Acyl aziridines stand out as direct precursors to both natural and unnatural amino acid residues in homodetic cyclic peptides through the use of the nonstandard amino acid aziridine-2-carboxylic acid (Azy) and its derivatives.[9a,b] Gin and van der Donk showed that ring opening of Azy residues with thiol nucleophiles provides a direct route to backbone-modified peptides.[10] A disadvantage of *N*-acyl aziridines, however, is their susceptibility toward amide hydrolysis.[11] Here we show that this susceptibility can be turned into an enabling tool for site-specific integration of molecular fragments into cyclic peptides (Figure 1). There exist several examples showcasing function-driven integration of "foreign" fragments into existing biological molecules. Thus, the retroviral enzyme integrase binds both termini of viral DNA and inserts them into a host-cell chromosome.[12] Although site-specific integration is well-known in nature, this concept has yet to find application in the realm of synthetic chemistry.

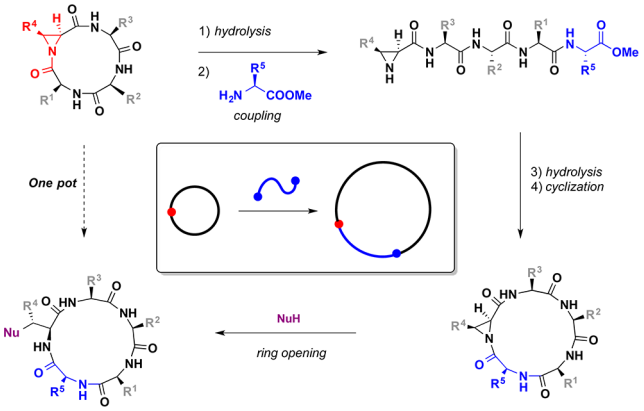


Figure 1. General synthetic strategy for the integration of molecular fragments into cyclic peptides. Box: concept of integration by site-specific fragment incorporation.

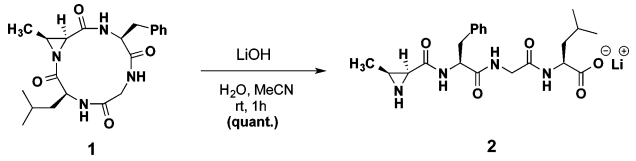
Distorted amides exhibit fast transacylation kinetics, often comparable to that of activated esters or even acyl halides.[13] This is in part due to significant amide bond distortion as a result of the nitrogen lone-pair pyramidalization. Because aziridine amides can be chemoselectively hydrolyzed,[14] we set out to explore this property as a synthetic means to realize the concept of site-specific integration. If site-specific linearization of an Azy-containing macrocycle followed by coupling with the desired integration fragment and macrolactamization could be performed in the same pot, the resulting synthetic sequence would exemplify such a process. A final aziridine ring-opening reaction would then generate the native peptide bond (Figure 1). In target-oriented applications, such a method could improve the overall convergence of the synthesis, whereas in the diversity-oriented domain of synthesis, this method could allow for split/pool applications and enable integration of rare and unnatural fragments that may not be amenable to activation/coupling strategies.

We used our recently reported protocol[9b] to synthesize the Azy-containing cyclic tetrapeptide template **1**. With aqueous LiOH, the aziridine amide bond of **1** was site-selectively cleaved within 1 h to generate the linear tetrapeptide **2** (Scheme 1). *N*-H Azy-containing peptides with a free carboxylic acid have been reported to be unstable toward purification and storage,[15] yet we found the linearized material **2** to be stable toward handling and prolonged storage *as long as the C-terminal carboxylic acid exists in*

Received: December 2, 2013
Published: February 17, 2014

   dx.doi.org/10.1021/ja412256f | *J. Am. Chem. Soc.* 2014, 136, 3728−3731

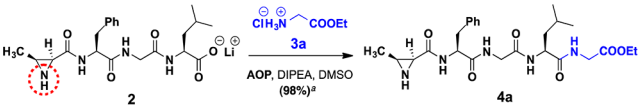
Scheme 1. Hydrolysis of *cyclo*[Leu-3-MeAzy-Phe-Gly]



the form of a carboxylate salt.[16] From here, we were ready to couple the integration fragment.[17] Since the aziridine nitrogen remains unprotected upon linearization, we feared that acylation of the *N*-H aziridine would compete with the acylation of the desired amine during the ligation step. The *N*-H aziridine is indeed a competent nucleophile, for when we stirred **2** with a coupling reagent in the absence of an external amine nucleophile, we observed the formation of a mixture of oligomeric products of **2**.

We chose H-Gly-OEt **3a** as our integration fragment and screened various coupling reagents in hopes of maximizing the selectivity for the formation of pentapeptide **4a** (Scheme 2). The

Scheme 2. Chemoselective Ligation of Unprotected Azy-tetrapeptide 2



[a]HPLC yield

coupling reagent (7-azabenzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (AOP) was found to be superior in this reaction (see the Supporting Information). The success of this chemoselective coupling is rooted in the difference between the rates of acylation of primary amines (pK_{aH} = 9−11) and aziridines (pK_{aH} = 7.9). With this reagent, we have coupled a variety of different amino acid- or peptide-based fragments in high yields with no detectable epimerization (see Table 1). Minor oligomeric byproducts formed through the combination of multiple molecules of **2** and one molecule of the integration fragment **3** were observed in varying amounts, depending on the reaction conditions and coupling partner. However, these byproducts are not a dead end in our synthetic scheme, as we can leverage the reduced amidicity of the aziridine amide during the subsequent saponification, which simultaneously cleaves the *C*-terminal alkyl ester and the internal aziridine amide of the oligomeric byproducts, liberating one molecule of the desired product **4′** and *n* molecules of the starting material **2** (Figure 2).[18]

Saponification of **4a** with LiOH yielded **4a′** as a carboxylate salt. A subsequent lactamization was performed[19] with AOP.[20a,b] The desired cyclic pentapeptide was formed with no detectable oligomerization by HPLC. Using our strategy, we were able to site-specifically insert a variety of amino acid residues into **1** (Table 1, entries 1 and 3−8). Critically, there was no evidence for oxazoline formation, a well-recognized challenge with linear acyl aziridines.[20c] We next sought to insert larger peptide fragments into **1**. Integration of the dipeptides Gly-Gly and Phe-D-Pro furnished the corresponding cyclic hexapeptides, whereas integration of the tripeptides Gly-Phe-D-Pro and Ala-Phe-D-Pro gave the corresponding cyclic heptapeptides (entries 12−15). Unnatural amino acids can also be used as integration fragments (entries 2, 9, and 10). Of particular interest is the use of azetidine-

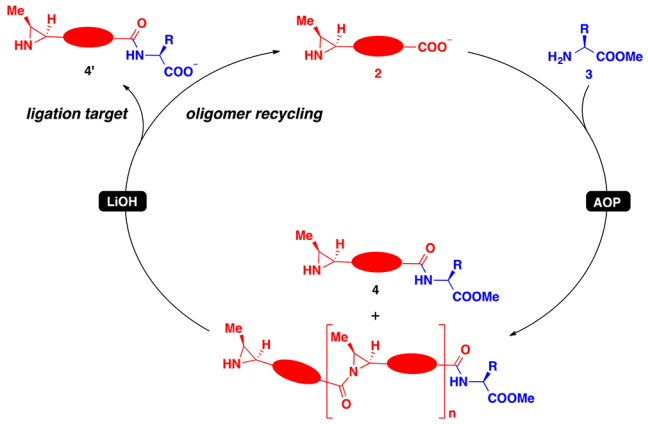


Figure 2. Oligomer recycling: a solution to undesired polymerization.

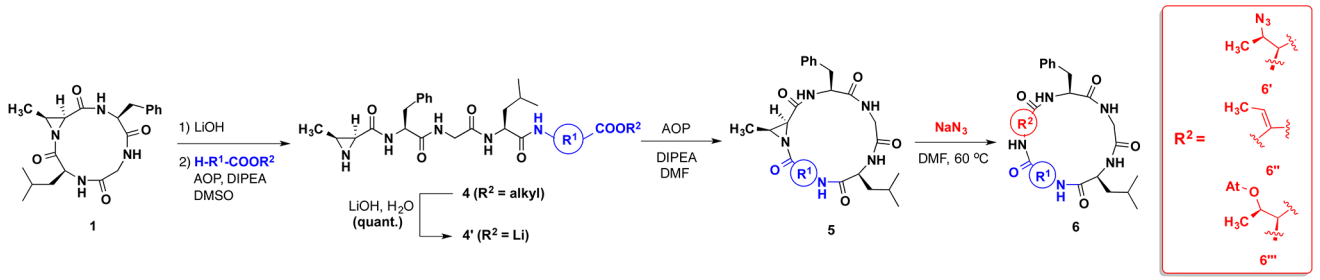
2-carboxylic acid (Aze) (entry 9). This four-membered ring analogue of proline possesses significant ring strain (25.4 kcal/mol) comparable to that of its cousin Azy (27 kcal/mol), yet it survives our synthetic sequence of fragment integration.

We then sought to develop a telescopic transformation to further diversify the *N*-acyl aziridines **5** in a site-specific fashion by way of nucleophilic opening of their aziridine rings. We turned to azide as a nucleophile.[21] It is well-known that azide can function as a leaving group or as a precursor to an amine or a tetrazole which leads to further site-specific functionalization.[22] When the cyclization to furnish **5** was complete, the reaction mixture was treated with sodium azide. For substrates **5a**−**p**, the azide anion attacked the β-carbon of the aziridine in a regioselective fashion, generating the corresponding azido-functionalized α-amino acid-containing cyclic peptide **6′**. A second product **6″**, corresponding to hydrazoic acid elimination, was formed to a varying extent. In several cases, a third product **6‴**, in which HOAt opened the aziridine ring, was observed in varying amounts. This occurred as a result of HOAt being present from the previous macrocyclization step with AOP. *Importantly, treatment with DBU at 60 °C was shown to effect the full elimination of both the azide and HOAt-containing macrocyles forming* **6″**. Our late-stage, one-pot aziridine ring opening/azide elimination should find application in the design of electrophilic biological probes inspired by microcystin.[23]

We also considered the integration of more exotic amino acid-containing fragments. Starting from **1**, we were able to successfully ligate the unnatural amino acid fragments **3p** and **3q** (Figure 3a).[24] After saponification of the *C*-terminal ester and subsequent cyclization with AOP, we were able to isolate the aziridine-containing macrocycles **5p** and **5q** in moderate yields over a four-step sequence (Figure 3a). We then investigated aziridine ring-opening reactions of these scaffolds with sodium azide. When **5p** was treated with excess sodium azide, results similar to those for cyclic peptides **6j**−**o** were observed (Figure 3a). A highly regioselective attack of the azide anion on the *N*-acyl aziridine took place at the β-carbon to furnish **6p′**, whose formation was accompanied by minor amount of the elimination product **6p″**. In contrast, the regioselectivity of azide attack on **5q** was very different from that of **5p**. Here, the regioisomer in which azide attacks the α-carbon of the aziridine ring, **6q**, was formed as the major product in the reaction, and the opposite regioisomer **6q′** was formed as a minor product. The observed macrocycle-dependent regioselectivity of aziridine ring-opening speaks to the adoption of different reactive conformations of the *N*-acyl aziridine depending on the neighboring residues and ring size.

dx.doi.org/10.1021/ja412256f | J. Am. Chem. Soc. 2014, 136, 3728−3731

Table 1. Integration Substrate Scope



	integration fragment		ligation		cyclization		Azy ring-opening	
entry	R^1	R^2	product	yield (%)[a]	product	yield (%)[a]	product ratio (**6′**:**6″**:**6‴**)[b]	yield (%)[c]
1	Gly	Et	**4a**	98	**5a**	96	74:13:13	30
2	Sar	Me	**4b**	77	**5b**	84	51:49:0	14
3	Ala	Me	**4c**	96	**5c**	91[d]	78:6:16	17
4	Leu	Me	**4d**	94	**5d**	94[d]	65:4:31	10
5	Val	Me	**4e**	95	**5e**	88[d]	69:19:12	17
6	Phe	Me	**4f**	85	**5f**	88[d]	67:26:7	35
7	Tyr	Me	**4g**	87	**5g**	90[d]	67:13:20	36
8	Trp	Me	**4h**	83	**5h**	86[d]	70:11:19	18
9	Aze	Me	**4i**	97	**5i**	92	66[e]:18:16	10
10	*β*-Ala	Me	**4j**	95	**5j**	95	51:49:0	14
11	*Nα*-Ac-Lys	Me	**4k**	95	**5k**	94[d]	61:34:5	48
12	Gly-Gly	Me	**4l**	99	**5l**	86	43:57:0	31
13	Phe-D-Pro	Me	**4m**	92	**5m**	97	41:58:1	19
14	Gly-Phe-D-Pro	Me	**4n**	92	**5n**	96	33:67:0	17
15	Ala-Phe-D-Pro	Me	**4o**	92	**5o**	93	40:60:0	40

[a]HPLC yields. [b]Determined by peak integration in crude HPLC traces. [c]Isolated yields of combined **6′**, **6″**, and **6‴** (after HPLC purification) with respect to **1** after five steps. [d]Cα-epimerization of the integration fragment was detected during the cyclization step. For the extent of epimerization, see the Supporting Information. [e]The two regioisomers of azide attack were formed in a 43:57 ratio (HPLC).

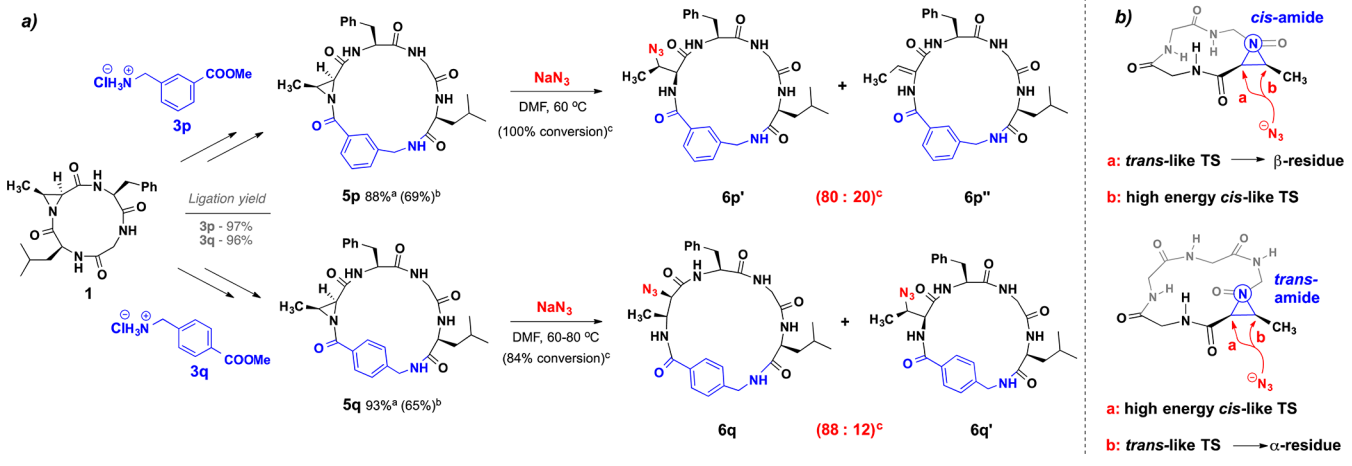


Figure 3. (a) Site-specific integration of unnatural amino acids. Notes: [a]HPLC yield of the cyclization step; [b]isolated yield over four steps; [c]determined by peak integration of crude HPLC traces. (b) Proposed model for regioselectivity in Azy ring openings of cyclic peptides.

The so-called "perpendicular" conformation that is stereoelectronically favored for *N*-acyl aziridines[9c] is expected to be the dominant contributor to the transition state for ring opening. We reason that the conformation of the aziridine amide plays a significant role in rationalizing our observed regioselectivity, as this factor is translated into the transition state for aziridine ring opening (buildup of $A^{1,3}$ strain) (Figure 3b). When the adaptive aziridine amide[9d] adopts a *cis* conformation and azide attacks the *α*-carbon of Azy, a *trans*-like amide forms in the transition state. Alternatively, attack at the *β*-carbon of Azy leads to the development of a less favorable *cis*-like amide bond. The observed regiochemistry for the reaction with **1** is in agreement with this hypothesis.[9b] The larger Azy-containing macrocycles **5a**–**p** and linear Azy-containing peptides[10] have additional flexibility compared with **1** and thus would accommodate an aziridine amide bond in its *trans* conformation. In this case our transition-state model is reversed, and attack of azide at the *β*-carbon leads to the development of a more favorable *trans*-like amide bond in the transition state. We note that this is the major isomer observed with macrocycles **5a**–**p** as well as with the ring opening of linear Azy-containing peptides with thiol-based nucleophiles.[10,2s]

dx.doi.org/10.1021/ja412256f | J. Am. Chem. Soc. 2014, 136, 3728–3731

In closing, we have capitalized on the susceptibility of *N*-acyl aziridines to amide hydrolysis, which is often considered to be their "Achilles' heel", to develop a tool for site-specific incorporation of molecular fragments into homodetic cyclic peptides. The reduced amidicity of the *N*-acyl aziridine linkage facilitated site-selective hydrolysis and was important in two additional embodiments: it enabled us to avoid the well-known problem of diketopiperazine formation,[16] and it made the problem of oligomerization a correctible mistake. By stressing the reversibility of *N*-acyl aziridine formation under mild conditions, our study paves a way to thermodynamically controlled applications of active amides in dynamic combinatorial chemistry.[26] In the course of our study, we also discovered the peculiar stereoelectronic consequences of placing *N*-acyl aziridine units into homodetic cyclic peptides of different sizes. The products of this integrative chemistry are useful building blocks that can be employed for the late-stage installation of unnatural side chains. This technique should be readily adaptable to solid-phase synthesis, be extended to split and pool protocols using molecular fragments of varying diversity, and inspire non-amide-bond-forming approaches to fragment integration.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details, optimization data, characterization data, HPLC traces, and ^{1}H and ^{13}C NMR spectra of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

ayudin@chem.utoronto.ca

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank NSERC and CIHR for financial support.

■ REFERENCES

(1) (a) Driggers, E. M.; Hale, S. P.; Lee, J.; Terrett, N. K. *Nat. Rev. Drug Discovery* **2008**, *7*, 608. (b) Marsault, E.; Peterson, M. L. *J. Med. Chem.* **2011**, *54*, 1961.

(2) Glenn, M. P.; Kelso, M. J.; Tyndall, J. D. A.; Fairlie, D. P. *J. Am. Chem. Soc.* **2003**, *125*, 640.

(3) (a) Rezai, T.; Yu, B.; Millhauser, G. L.; Jacobson, M. P.; Lokey, R. S. *J. Am. Chem. Soc.* **2006**, *128*, 2510. (b) Fernández, A. *Nat. Biotechnol.* **2004**, *22*, 1081.

(4) Tyndall, J. D. A.; Nall, T.; Fairlie, D. P. *Chem. Rev.* **2005**, *105*, 973.

(5) (a) Robinson, J. A. *ChemBioChem* **2009**, *10*, 971. (b) Burgess, K. *Acc. Chem. Res.* **2001**, *34*, 826. (c) Whitby, L. R.; Boger, D. L. *Acc. Chem. Res.* **2012**, *45*, 1698. (d) Bullock, B. N.; Jochim, A. L.; Arora, P. S. *J. Am. Chem. Soc.* **2011**, *133*, 14220. (e) Nowisk, J. S. *Acc. Chem. Res.* **2008**, *41*, 1319. (f) Robinson, J. A. *Acc. Chem. Res.* **2008**, *41*, 1278. (g) Angelini, A.; Cendron, L.; Chen, S.; Touati, J.; Winter, G.; Zanotti, G.; Heinis, C. *ACS Chem. Biol.* **2012**, *7*, 817.

(6) White, C. J.; Yudin, A. K. *Nature Chem.* **2011**, *3*, 509.

(7) (a) Seebach, D.; Beck, A. K.; Bossler, H. G.; Gerber, C.; Ko, S. Y.; Murtiashaw, W.; Naef, R.; Shoda, S.-I.; Thaler, A.; Krieger, M.; Wenger, R. *Helv. Chim. Acta* **1993**, *76*, 1564. (b) 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.

(8) Hili, R.; Rai, V.; Yudin, A. K. *J. Am. Chem. Soc.* **2010**, *132*, 2889.

(9) (a) Nakajima, K.; Takai, F.; Tanaka, T.; Okawa, K. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 1577. (b) White, C. J.; Yudin, A. K. *Org. Lett.* **2012**, *14*, 2898. (c) Rademacher, P.; Würthwein, E.-U. *J. Mol. Struct.* **1986**, *139*, 315. (d) Falkenstein, R.; Mall, T.; Speth, D.; Stamm, H. *J. Org. Chem.* **1993**, *58*, 7377.

(10) (a) Galonić, D. P.; van der Donk, W. A.; Gin, D. Y. *J. Am. Chem. Soc.* **2004**, *126*, 12712. (b) Galonić, D. P.; Ide, N. D.; van der Donk, W. A.; Gin, D. Y. *J. Am. Chem. Soc.* **2005**, *127*, 7359.

(11) Lygo, B. *Tetrahedron* **1995**, *51*, 12859.

(12) Lewinski, M. K.; Bushman, F. D. *Adv. Genet.* **2005**, *55*, 147.

(13) (a) Blackburn, G. M.; Skaife, C. J.; Kay, I. T. *J. Chem. Res., Miniprint* **1980**, 3650. (b) Somayaji, V.; Brown, R. S. *J. Org. Chem.* **1986**, *51*, 2676.

(14) (a) Ślebacka-Tilk, H.; Brown, R. S. *J. Org. Chem.* **1987**, *52*, 805. (b) Bennet, A. J.; Wang, Q. P.; Ślebacka-Tilk, H.; Somayaji, V.; Brown, R. S.; Santarsiero, B. D. *J. Am. Chem. Soc.* **1990**, *112*, 6383.

(15) Korn, A.; Rudolph-Böhner, S.; Moroder, L. *Tetrahedron* **1994**, *50*, 1717.

(16) In peptide chemistry, the attack of the *N*-terminal amine at the (*i* + 1) peptide bond is a commonly observed problem that leads to the formation of unwanted diketopiperazines. We do not observe this undesired side process in our system.

(17) For a recent example showcasing amide couplings with carboxylate salts, see: Goodreid, J. D.; Duspara, P. A.; Bosch, C.; Batey, R. A. *J. Org. Chem.* **2014**, *79*, 943.

(18) The yields of the desired products **4** and thus **4′** increased upon the addition of water to the oligomeric mixture. We performed a control experiment with H-Sar-O^{t}Bu to quantify this effect. See the Supporting Information for details.

(19) Malesevic, M.; Strijowski, U.; Bächle, D.; Sewald, N. *J. Biotechnol.* **2004**, *112*, 73.

(20) (a) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397. (b) Ehrlich, A.; Heyne, H.-U.; Winter, R.; Beyermann, M.; Haber, H.; Carpino, L. A.; Bienert, M. *J. Org. Chem.* **1996**, *61*, 8831. (c) Cardillo, G.; Gentilucci, L.; Tolomelli, A.; Tomasini, C. *Tetrahedron Lett.* **1997**, *38*, 6953.

(21) (a) Legters, J.; Willems, J. G. H.; Thijs, L.; Zwanenburg, B. *Recl. Trav. Chim. Pays-Bas* **1992**, *111*, 59. (b) Bucciarelli, M.; Forni, A.; Moretti, I.; Prati, F.; Torre, G. *Tetrahedron: Asymmetry* **1995**, *6*, 2073.

(22) (a) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004. (b) Tornøe, C. W.; Christensen, C.; Meldal, M. J. *J. Org. Chem.* **2002**, *67*, 3057.

(23) Rosenker, C. J.; Krenske, E. H.; Houk, K. N.; Wipf, P. *Org. Lett.* **2013**, *15*, 1076.

(24) Previous examples incorporating nonpeptidic fragments within larger peptide macrocycles include: (a) Smith, J. M.; Vitali, F.; Archer, S. A.; Fasan, R. *Angew. Chem., Int. Ed.* **2011**, *50*, 5075. (b) Chen, S.; Morales-Sanfrutos, J.; Angelini, A.; Cutting, B.; Heinis, C. *ChemBioChem* **2012**, *13*, 1032. (c) Wu, X.; Wang, L.; Han, Y.; Regan, N.; Li, P.-K.; Villalona, M. A.; Hu, X.; Briesewitz, R.; Pei, D. *ACS Comb. Sci.* **2011**, *13*, 486.

(25) We have also shown that thiol nucleophiles are amenable to our methodology. PhSH was shown to react with **5o** to yield two regioisomeric products. See the Supporting Information for details.

(26) Ramström, O.; Lehn, J.-M. *Nat. Rev. Drug Discovery* **2002**, *1*, 26.