JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Communication
pubs.acs.org/JACS

Site-Selective Bromination of Vancomycin

Tejas P. Pathak and Scott J. Miller*

Department of Chemistry, Yale University, 225 Prospect Street, New Haven, Connecticut 06520-8107, United States

Supporting Information

ABSTRACT: We report the site-selective bromination of vancomycin to produce, with substantial efficiency, previously unknown monobromovancomycins, a dibromovancomycin, and a tribromovancomycin. We document the inherent reactivity of native vancomycin toward *N*-bromophthalimide. We then demonstrate significant rate acceleration and perturbation of the inherent product distribution in the presence of a rationally designed peptide-based promoter. Alternative site selectivity is observed as a function of solvent and replacement of the peptide with guanidine.

The selective chemical modification of complex molecules represents a long-standing challenge for chemical synthesis, due in part to the presence of multiple functional groups that often exhibit comparable reactivity. The application of catalysts to natural product derivatization provides a powerful way forward, provided that substantial selectivity can be achieved for different products. In recent years, both enzymes[1] and small-molecule-based catalysts and reagents[2,3] have been studied in this context. A critical challenge in the field is the identification of new strategies that alter the inherent selectivity profiles of complex, polyfunctional scaffolds.[3]

In this context, we wondered about the possible connection between recently described enantioselective arene functionalization reactions[4] and selective derivatization of complex molecules such as vancomycin (**4**). For example, peptide-based catalyst **1** can effect enantioselective bromination reactions of racemic biaryl compounds such as **2** to deliver stereodefined biaryl compounds such as **3** (Figure 1a). These observations prompted us to assess whether catalysts might also

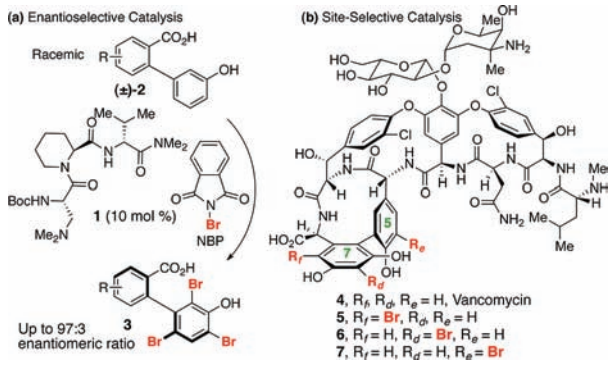

Figure 1. (a) Atroposelective tribromination of biaryls. (b) Three possible sites (in red) for site-selective bromination of **4**.

be found that could deliver selectively the various regioisomers of monobromovancomycin (Figure 1b). **4** is a critical tool in the treatment of bacterial infections, yet resistance to it has stimulated numerous studies of analogues of **4** as alternatives.[5] Total synthesis,[6] biosynthetic manipulation,[7] and chemical derivatization[8] have all contributed to the epic study of **4** as a tool in the fight against antibiotic resistance.

4 represents an intriguing scaffold for the study of site-selective modifications. Our goal was to establish whether catalysis could enable the synthesis of alternative isomers of bromovancomycins, which themselves could prove to be candidates for further functionalization to yield new analogues.[9] While there are many candidate sites for bromination of **4**, we speculated that the most likely sites would be the R_f position of arene ring 7 (**5**), the R_d position of the same ring (**6**) and the R_e position of ring 5 (**7**). In addition, multiple brominated derivatives of **4** might result if the reactive sites are of comparable reactivity. Indeed, at the outset of this study, the inherent reactivity of **4** toward generic brominating agents had not to our knowledge been described.

Our studies began with a delineation of the inherent reactivity of **4** toward *N*-bromophthalimide (NBP). Initially, our studies were undertaken with the guiding principle that reactions would be conducted in H_2O on native **4** without the use of protecting groups. These decisions offered the potential for increased utility. As shown in Figure 2, exposure of **4** to 2.0

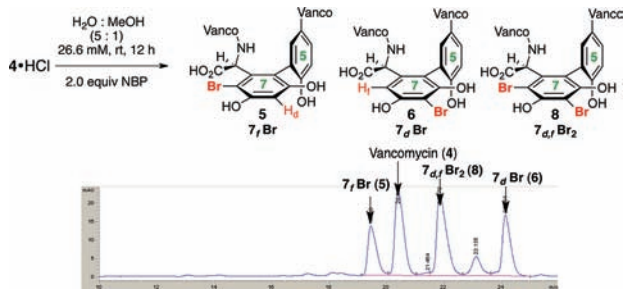

Figure 2. HPLC trace (at 280 nm) for the uncatalyzed reaction of **4**.

equiv of NBP produces a mixture of products, with unreacted **4** as a major component. LC−MS analysis and extensive preparative HPLC purification allowed the major constituents to be isolated, albeit in modest quantities.

LC−MS and 2D NMR methods enabled structural assignments. Recording the spectra in D_2O (500 MHz) gave excellent chemical shift resolution of diagnostic signals, and comparisons

Received: February 16, 2012
Published: March 30, 2012

dx.doi.org/10.1021/ja301566t | J. Am. Chem. Soc. 2012, 134, 6120−6123

of the brominated derivatives to native **4** were particularly instructive. Figure 3 shows overlays of the decisive region of

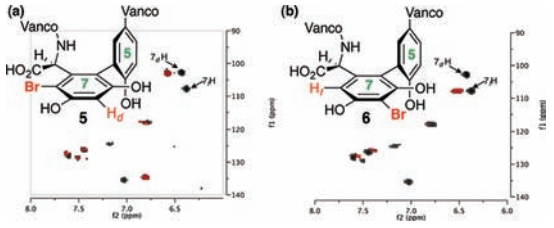

Figure 3. Overlay of characteristic changes in the HSQC spectrum for brominated vancomycins (**4** in gray, **5** and **6** in red).

HSQC spectra for compounds **5** and **6** (cross-peaks in red) with that obtained for **4** (cross-peaks in gray). In each case, we noted the absence of the indicated Ar−H cross-peak where Br had putatively been installed. Perturbation of chemical shifts elsewhere in the structures proved to be minimal.[10a] Of critical significance, the uncatalyzed bromination of **4** delivers an essentially 1:1 ratio of the monobromovancomycins **5** and **6** along with a very similar quantity of the dibromovancomycin **8** (71% conv.; Figure 2).

We then considered how catalysts might perturb the intrinsic product distribution exhibited by **4** (Figure 2). Given the well-known binding of **4** to DAla-DAla segments of the bacterial cell wall as part of its biological mode of action (Figure 4a),[11] we

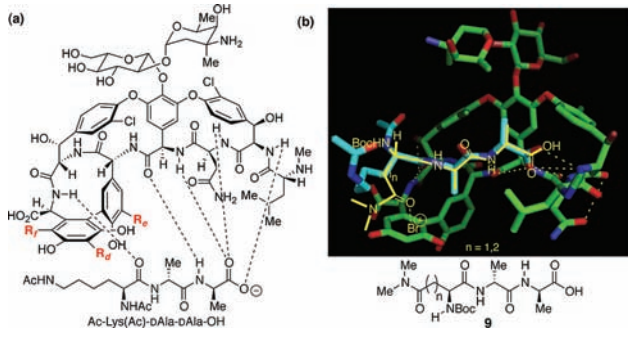

Figure 4. (a) Binding of **4** to Ac-Lys(Ac)-DAla-DAla-OH (adapted from ref 11b). (b) Overlay of proposed peptide scaffold (yellow) with that of Ac-Lys(Ac)-DAla-DAla-OH (light-blue) in the vancomycin binding pocket (PDB entry 1FVM).

designed catalysts based on this molecular recognition motif. For example, we targeted catalysts that would retain the DXaa-DXaa dipeptide motif as part of a binding domain between the catalyst and the substrate. In addition, we incorporated *N,N*-dimethylamide functionality, which we suspected would accelerate the bromination reactions.[12] Thus, peptides related to **9** (Figure 4b) emerged as our leads for study.

The peptides we evaluated are presented in Table 1. As in our assessment of the intrinsic reactivity of **4**, we first conducted all of the reactions in H_2O. In addition, we adopted a first-order analysis of the HPLC traces of the reactions, assessing relative peak areas without a rigorous determination of response factors for individual components of a given reaction. Even with these approximations, we were able to observe immediately that the impact of the peptide-based promoters could be quite significant.

The most striking observation throughout the study was the profound rate acceleration in the presence of peptides

Table 1. Optimization of Peptide Scaffold and Reaction Conditions for the conversion of 4•HCl to 5, 6 and 8[a]

Entry	Catalyst	mol%	t (h)	% conv.	5 : 6 : 8 7_fBr : 7_dBr : $7_{d,f}Br_2$
1	No catalyst	NA	12	71	1.0 : 1.0 : 1.3[b]
2a	*N,N*-dimethylacetamide	100	12	68	1.0 : 1.0 : 1.8[b]
2b	*N,N*-dimethylacetamide	7100	16	64	1.0 : 1.5 : 2.9[b]
3	Boc-Asn(Me_2)-DAla-DAla-OH (**10**)	100	2	97	1.0 : 6.8 : 2.6[b]
4	Boc-DAsn(Me_2)-DAla-DAla-OH (**11**)	100	2	90	1.0 : 4.9 : 1.8[b]
5	Boc-Gln(Me_2)-DAla-DAla-OH (**12**)	100	2	99	1.0 : 4.0 : 9.3[b]
6	Boc-DGln(Me_2)-DAla-DAla-OH (**13**)	100	2	99	1.0 : 3.0 : 3.4[b]
7	Boc-Leu-DAla-DAla-OH (**14**)	100	12	60	1.0 : 5.7 : 2.5[b]
8a	Boc-Asn(Me_2)-DAla-DAla-OH (**10**)	100	1.5	98	**1.0 : 14.6** : 2.8[c]
8b		200	1.5	99	1.0 : 19.0 : 6.0[c]
8c		50	2	97	1.0 : 3.4 : 1.1[c]
8d		25	1.5	83	1.0 : 1.9 : 0.7[c]
9	Boc-Leu-DAsn(Me_2)-DAla-OH (**15**)	200	12	96	1.0 : 6.5 : 2.7[c]
10	Boc-Leu-DAla-DAsn(Me_2)-OH (**16**)	200	12	85	1.0 : 3.5 : 1.0[c]
11	Boc-Asn(Me_2)-DAla-DAla-OH (**10**)	100	1.5	16	1.0 : 10.5 : 0.9[d]
12	Boc-Leu-DAla-DAsn(Me_2)-OH (**16**)	100	1.5	12	1.0 : 3.5 : 1.6[d]

[a]Ratios were measured by HPLC at λ = 280 nm. [b]2.0 equiv of NBP, 250 μL of water, 50 μL of MeOH, 8 μmol of **4**. [c]2.0 equiv of NBP, 1000 μL of water, 200 μL of MeOH, 8 μmol of **4**. [d]50 mol% NBP, 0.033 mmol of **4**.

containing the *N,N*-dimethylamide functionality at a particular position. As noted in Figure 3, the uncatalyzed reaction delivers essentially equal quantities of **5** and **6** (Table 1, entry 1). The exploration of *N,N*-dimethylacetamide itself as a promoter (in various concentrations; entries 2a and 2b) provided neither a noticeable rate acceleration nor a significant perturbation of the **5**:**6** ratio, although slightly more **8** was observed. However, when peptides containing the DAla-DAla sequence along with the *N,N*-dimethylamido side chain were examined, essentially all of the starting material (**4**) was consumed within 2 h (entries 3−6). These results stand in stark contrast to the rate of consumption of **4** in our control experiments. Moreover, the product distribution responded to structural changes in the peptide. For example, employing peptide **10**, with a Asn(Me_2)-DAla-DAla structure, we observed 7_d-Br derivative **6** as the major product with a 6.8:1 preference over monobromide **5**, with the dibromide **8** also formed in considerable quantity (entry 3). Altering the stereochemistry of the DAsn(Me_2) residue (peptide **11**; entry 4) also delivered **6** as the major product, but with a lower **6**:**5** ratio (4.9:1). We are cautious about overinterpreting these differences in selectivity because of the tendency of polyfunctionalization reactions to exhibit different product distributions as a function of conversion [e.g., as monofunctionalized products (e.g., **5** and **6**) are depleted and polyfunctionalized products (e.g., **8**) are formed].[13] In any event, we also observed that exchange of the Asn residue to Gln(Me_2) (catalyst **12**; entry 5) also contributed to efficient consumption of **4**, with the dibromide **8** emerging as the dominant product of the reaction. However, this effect was attenuated with the epimeric Gln(Me_2) structure **13** (entry 6). Notably, when the *N,N*-dimethylamide moiety was excised from the peptide structure (peptide **14**; entry 7), much lower conversion was observed. Even so, in this case a preference for **6** was observed.

The peptide concentration and stoichiometry appear to influence substantially both the rate and product distribution. Lowering the concentration led to a significant improvement in the **6**:**5** ratio (14.6:1; entries 3 and 8a) without an appreciable rate decrease. Furthermore, increasing the peptide loading to 200 mol% led to a modest improvement in the **6**:**5** ratio (19:1; entry 8a vs 8b). On the other hand, lowering the peptide loading to 50 mol% gave a lower **6**:**5** ratio (3.4:1; entry 8a vs 8c). We noted that with 50 mol% peptide, full consumption of

dx.doi.org/10.1021/ja301566t | *J. Am. Chem. Soc.* 2012, 134, 6120−6123

4 was still observed within 2 h. However, reduction of the peptide loading to 25 mol% led to a further erosion of the selectivity relative to the results observed with higher peptide amounts (1.9:1.0; entry 8d). These results imply that while the peptide-based promoters exhibit rate acceleration, a principal hallmark of catalysis, the turnover rates do not appear to be high in the present case. This latter phenomenon may be due to the high affinity of **4** for the DAla-DAla motif.[14]

Furthermore, the effect of relocating the Asn(Me_2) residue within the tripeptide also appears to influence both the rate and product distribution (cf. entries 8b, 9, and 10). When the residue is disposed closer to the C-terminal position, the peptide appears to be a less effective promoter, as the rate diminishes and the selectivity for **6** decreases. These facts may reveal the importance of situating the putative directing functional group at the right locus. Finally, when a limiting quantity (0.5 equiv) of NBP is used, the selectivity trends are preserved and the quantity of dibromide **8** is reduced (entries 11 and 12). The observations in these two experiments suggest that the preference for monobromide **6** is not simply a function of overconversion and depletion of **5**.

With encouraging data regarding the use of peptide **10**,[14] we explored its use for preparative reactions of particular brominated vancomycins. Under the optimized reaction conditions, treatment of 100 mg of **4** provided 43.1 mg (41% yield) of analytically pure 7_d-Br derivative **6** in a single experiment. By way of comparison, 100 mg of **4** under the uncatalyzed reaction conditions shown in Figure 2 delivered only 11.6 mg (11% yield) of **6**, and a quite tedious purification was required. Figure 5 shows the HPLC traces for the peptide-

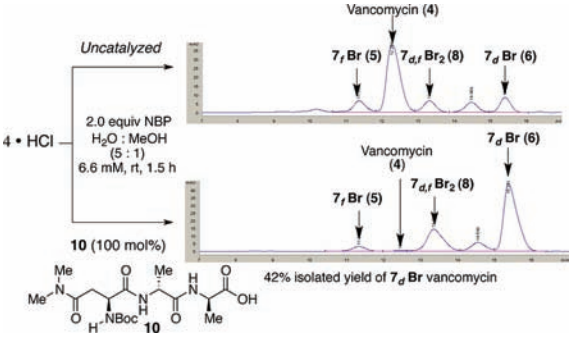

Figure 5. Reversed-phase HPLC traces for the (top) uncatalyzed and (bottom) peptide-catalyzed brominations of **4**.

mediated reaction of **4** with NBP (bottom trace) with that of the corresponding uncatalyzed reaction (top trace) under identical conditions for direct comparison.

Next, we addressed the efficient synthesis of dibromovancomycin **8**. This particular derivative is generally the major product in the uncatalyzed bromination of **4** with excess brominating reagent. However, as noted, these reactions are generally unselective, which makes product isolation difficult. On the other hand, treatment of **4** with NBP (3 equiv) in the presence of 100 mol% **10** provides the desired product with excellent efficiency (Figure 6 bottom). The control reaction provides **8** as a major product, but as a constituent along with significant quantities of the other brominated vancomycins (Figure 6 top). The treatment of 100 mg of **4** under peptide-promoted reaction conditions provided 60.2 mg (55% yield) of analytically pure **8** in a single experiment.

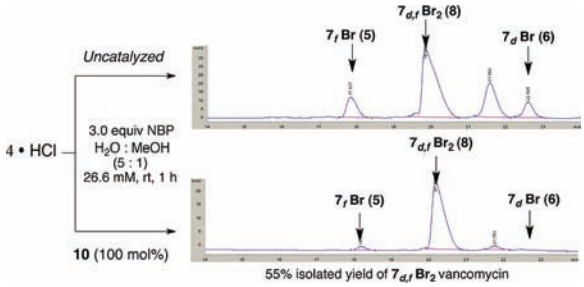

Figure 6. Reversed-phase HPLC traces of the (top) uncatalyzed and (bottom) peptide-catalyzed dibrominations of **4**.

Our explorations of the peptide-based mediators for the bromination of **4** also delivered some additional outcomes and new design opportunities. For example, during a study of the peptide-dependent capacity to deliver highly brominated derivatives, we observed a hint of a new compound (**17**; Figure 7 top), which proved to be a tribrominated analogue.

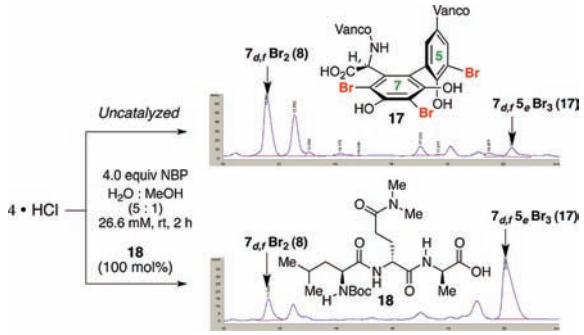

Figure 7. Reversed-phase HPLC traces of the (top) uncatalyzed and (bottom) peptide-catalyzed tribrominations of **4**.

Intriguingly, treatment of **4** with NBP in the presence of 100 mol% **10** under otherwise identical conditions enabled the observation of **8** and **17**,[10a] although with ratios no better than ~1:1. Characterization of **17**[10a] allowed its assignment as the illustrated tribromovancomycin, wherein the phenolic moiety of residue **5** was also brominated. This structural assignment stimulated the exploration of catalyst **18**, wherein the central Gln(Me_2) residue was postulated to place the dimethylamido moiety in proximity to this previously recalcitrant bromination site. When **4** was exposed to NBP under the reaction conditions in the presence of catalyst **18**, tribromide **17** was produced in significant quantity preferentially over the dibromide (**17**:**8** = 3−4:1; Figure 7 bottom) in a substantially cleaner reaction. This observation demonstrates the power of a peptide catalyst to provide a new brominated vancomycin that is otherwise difficult to acquire. In a preparative mode, 49.0 mg of **4** led to the isolation of 20.2 mg (35% yield) of **17**.

We then turned our attention to the question of reversal of the selectivity exhibited by peptide **10**, which preferentially delivers monobromide **6**. A preliminary examination of DAla−DAla-based peptides did not unveil a **5**-selective catalyst. However, in the course of these studies, we made the surprising observation that the reaction medium had a substantial effect on the site-selectivity of the initial bromination. When **4** is exposed to 2 equiv of NBP in either H_2O:MeOH (5:1) or MeOH alone, sluggish reactions occur, and the ratio of **5**:**6** is near unity (Table 2, entries 1 and 2). Yet, when a larger quantity (4 equiv of NBP) is employed, in the absence of a

dx.doi.org/10.1021/ja301566t | J. Am. Chem. Soc. 2012, 134, 6120−6123

Table 2. Optimization of the Reaction Conditions Aiming at Analogue 5[a]

Entry	Additive	Solvent	NBP (equiv)	t (h)	% conv.	5 7_fBr : 6 7_dBr : 8 $7_{d,f}Br_2$
1	No catalyst	H_2O:MeOH (5:1)	2	12	71	1.0 : 1.0 : 1.3[b]
2	No catalyst	MeOH	2	2	17	1.0 : 1.0 : 3.0[b]
3	No catalyst	MeOH	4	1	92	14.3 : 1.0 : 53.8[c]
4	10	MeOH	4	1	31	0.5 : 1.0 : 1.3[b]
5	Guanidine•HCl	MeOH	4	1	85	12.7 : 1.0 : 10.8[c]
6	Guanidine•HCl	MeOH	4	1	92	11.8 : 1.0 : 11.4[d]

[a]Ratios were measured by HPLC at λ = 280 nm. [b]8 μmol of **4**, 6.6 mM. [c]16 μmol of **4**, 3.3 mM, 18 equiv of guanidine·HCl. [d]similar to (c) except with 6 equiv of guanidine·HCl.

catalyst, a surprising preference for **5** is observed, with a substantial increase in the observed quantity of dibromide **8** (entry 3). Peptide **10** maintains its capacity to favor **6** in MeOH solvent, although the selectivity is strongly attenuated (entry 4).

We then chose to examine guanidine as an additive because of its propensity to accelerate bromination reactions.[15] Additionally, guanidine possesses the capacity to bind carboxylate under a variety of conditions.[16] Thus, we hypothesized that guanidine might effectively target the acid of **4** while simultaneously delivering bromide to the proximal 7_f site of **4** (Figure 8 in Supporting Information). The assessment of this hypothesis provides a striking result. As illustrated in entries 5 and 6, guanidine not only provides **5** with good selectivity over **6** but also significantly improves the ratio of monobromide (**5**) to dibromide (**8**), making viable the isolation of **5** in good quantities (entry 3 vs 5 and 6). On a preparative scale, the reaction of 100 mg of **4** with 4 equiv of NBP in the presence of guanidine provided 23.3 mg (21% yield) of analytically pure **5**, which is otherwise very difficult to isolate cleanly in significant quantities (Figure 2). These results are consistent with the hypothesis that guanidine may associate with the acid of **4** and simultaneously deliver the bromide ion.[17,18] This effect may lead to kinetically favored formation of **5** and also the formation of **8** via depletion of **6**. Additional studies are needed to establish definitively the putative mechanism shown in Figure 8 in Supporting Information.

In conclusion, we have developed simple and efficient site-selective brominations of vancomycin. The approach, in a single step, provides peptide-based-promoter- and additive-dependent product ratios and access to compounds that are otherwise not easily accessible. The concepts are based on the storied DAla-DAla binding motif exploited by natural systems as well as chemically grounded ideas about carboxylate targeting. The introduction of Br into complex, bioactive structures has the potential to modulate their biological activity.[10b] Moreover, from a more chemical standpoint, the Ar−Br moiety may also serve as a platform for further functionalization of glycopeptide antibiotics.[9] Studies of this type are current topics of interest in our lab.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures for all experiments, characterization data, and biological activity data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

scott.miller@yale.edu

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Dr. Jeffery L. Gustafson for initial experiments. We are grateful to the NIH NIGMS (GM-068649) for support and Eli Lilly for initial donations of **4**.

■ REFERENCES

(1) (a) *Natural Products via Enzymatic Reactions*; Piel, J.; Ed.; Topics in Current Chemistry, Vol. *297*; Springer: Berlin, 2010. (b) Steffan, N.; Grundmann, A.; Yin, W. B.; Kremer, A.; Li, S. M. *Curr. Med. Chem.* **2009**, *16*, 218. (c) Gatto, G. J.; Boyne, M. T.; Kelleher, N. L.; Walsh, C. T. *J. Am. Chem. Soc.* **2006**, *128*, 3838.

(2) (a) Lewis, C. A.; Miller, S. J. *Angew. Chem., Int. Ed.* **2006**, *45*, 5616. (b) Lewis, C. A.; Longcore, K. E.; Miller, S. J.; Wender, P. A. *J. Nat. Prod.* **2009**, *72*, 1864. (c) Jordan, P. A.; Miller, S. J. *Angew. Chem., Int. Ed.* **2012**, *51*, 2907.

(3) (a) Chen, M. S.; White, M. C. *Science* **2007**, *318*, 783. (b) Brückl, T.; Baxter, R. D.; Ishihara, Y.; Baran, P. S. *Acc. Chem. Res.* **2011**, DOI: 10.1021/ar200194b. (c) Chamni, S.; He, Q.-L.; Dang, Y.; Bhat, S.; Liu, J. O.; Romo, D. *ACS Chem. Biol.* **2011**, *6*, 1175. (d) Snyder, S. A.; Gollner, A.; Chiriac, M. I. *Nature* **2011**, *474*, 461.

(4) Gustafson, J. L.; Lim, D.; Miller, S. J. *Science* **2010**, *328*, 1251.

(5) (a) Crane, C. M.; Pierce, J. G.; Leung, S. S. F.; Tirado-Rives, J.; Jorgensen, W. L.; Boger, D. L. *J. Med. Chem.* **2010**, *53*, 7229. (b) Weist, S.; Kittel, C.; Bischoff, D.; Bister, B.; Pfeifer, V.; Nicholson, G. J.; Wohlleben, W.; Sussmuth, R. D. *J. Am. Chem. Soc.* **2004**, *126*, 5942. (c) Xie, J.; Pierce, J. G.; James, R. C.; Okano, A.; Boger, D. L. *J. Am. Chem. Soc.* **2011**, *133*, 13946. (d) For a recent review, see: Ashford, P.-A.; Bew, S. P. *Chem. Soc. Rev.* **2012**, *41*, 957.

(6) For example, see ref 5c and: (a) Nicolaou, K. C.; Boddy, C. N. C.; Brase, S.; Winnssinger, N. *Angew. Chem., Int. Ed.* **1999**, *38*, 2096. (b) Evans, D. A.; Wood, M. R.; Trotter, B. W.; Richardson, T. I.; Barrow, J. C.; Katz, J. L. *Angew. Chem., Int. Ed.* **1998**, *37*, 2700.

(7) For example, see: Losey, H. C.; Peczuh, M. W.; Chen, Z.; Eggert, U. S.; Dong, S. D.; Pelczer, I.; Kahne, D.; Walsh, C. T. *Biochemistry* **2001**, *40*, 4745.

(8) Nicolaou, K. C.; Hughes, R.; Cho, S. Y.; Winssinger, N.; Smethurst, C.; Labischinski, H.; Endermann, R. *Angew. Chem., Int. Ed.* **2000**, *39*, 3823. Also see ref 5d.

(9) Nakama, Y.; Yoshida, O.; Yoda, M.; Araki, K.; Sawada, Y.; Nakamura, J.; Xu, S.; Miura, K.; Maki, H.; Arimoto, H. *J. Med. Chem.* **2010**, *53*, 2528.

(10) (a) See the Supporting Information (SI) for details. (b) MICs for all new derivatives were measured and found to be no better than that for **4** (see the SI).

(11) (a) For a review, see: Perkins, H. R. *Pharmacol. Ther.* **1982**, *16*, 181. (b) McComas, C. C.; Crowley, B. M.; Boger, D. L. *J. Am. Chem. Soc.* **2003**, *125*, 9314. (c) For crystal structure data, see: Nitanai, Y.; Kikuchi, T.; Kakoi, K.; Hanamaki, S.; Fujisawa, I.; Aoki, K. *J. Mol. Biol.* **2009**, *385*, 1422.

(12) Mitchell, R. H.; Lai, Y.-H.; Williams, R. V. *J. Org. Chem.* **1979**, *44*, 4733. (b) Denmark, S. E.; Burk, M. T. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 20655.

(13) Schreiber, S. L.; Schreiber, T. S.; Smith, D. B. *J. Am. Chem. Soc.* **1987**, *109*, 1525.

(14) Several other brominating agents were examined and gave comparable results (see the SI for details).

(15) Fu, X.; Tan, C.-H. *Chem. Commun.* **2011**, *47*, 8210.

(16) Blondeau, P.; Segura, M.; Pérez-Fernández, R.; de Mendoza, J. *Chem. Soc. Rev.* **2007**, *36*, 198.

(17) We prepared the corresponding methyl ester of **4** and found that the analogous reactions with NBP in the presence or absence of guanidine delivered similar results; neither reaction produces **4**-derived monobromides cleanly (see the SI).

(18) Other additives, such as simple urea-based compounds, also exhibited this effect (see the SI).