Total Synthesis of Bastadins 2, 3, and 6

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A chemoenzymatic strategy has been developed for the synthesis of bastadins 2, 3, and 6. The requisite dimeric dityrosine and isodityrosine building blocks were successfully prepared by oxidative C−C and C−O phenolic coupling of mono- and dihalogenated derivatives of tyrosine and tyramine using horseradish and soybean peroxidases. By carefully controlling the experimental parameters, the requisite synthons may now be prepared in synthetically useful yields without the exhaustive protection and deprotection of the sensitive functional groups.

Introduction

The bastadins are a family of modified macrocyclic tetrapeptides isolated from the marine sponges *Ianthella basta*[1] and *Psammaplysilla purpurea*.[2] They are biogenetically derived from tyrosines via oxidative phenolic coupling of two tyramine-tyrosine units.[3] The first seven members of this family were described in 1981 by Kazlauskas et al.[1] Since then more than a dozen additional members have been isolated.[2,3] Bastadins 1−7 exhibited moderate antibacterial activity, whereas other bastadins have been shown to possess cytotoxic activity against human tumor cell lines or antiinflammatory activity.[2,3a] More recently, bastadin 5 was shown to increase the ryanodine binding capacity of SR membranes by stabilizing the high affinity conformation of RyR-1 for ryanodine without shifting the affinity of the activator site for Ca^{2+} or altering the response to caffeine or adenine nucleotides.[4] Most binding and Ca^{2+} transport are antagonized by the immunosuppressant FK 506.

Unlike FK 506, bastadin 5 does not directly promote the dissociation of FKBP 12 from the RyR-1 membrane complex, but it markedly enhances the release of FKBP 12[5] induced by FK 506. Thus, bastadins represent a new class of chemical probes for studying the functional significance of immunophilin/ryanodine-sensitive Ca^{2+} channel interactions in skeletal muscle. In view of their limited supply from natural sources and their interesting biological activities,[2,3] bastadins serve as an attractive total synthetic target. To date, Yamamura's group[6] has reported the syntheses of bastadins 1, 2, 3, and 6 using a method, in which the aryl ethers were synthesized via thallium trinitrate (TTN) oxidation. However, the synthetic routes required extensive protection and deprotection of functional groups which resulted in low yields of bastadins.

Recently, we reported the successful oxidative C−O phenolic coupling of dihalogenated tyrosine and tyramine derivatives to form the isodityrosine framework using horseradish peroxidase.[7] As an extension of these investigations, we have applied this oxidative coupling technology to the synthesis of bastadins. Herein we report a chemoenzymatic strategy for the synthesis of bastadins 2, 3, and 6.

Retrosynthetic Analysis

Retrosynthetic analysis of the bastadin 6 molecule reveals that the macrocycle may be dissected into two units constituting the eastern and western segments joined to each other via amide bonds. The eastern unit is composed of two brominated tyrosine derivatives (α-hydroxyimino group) bonded to each other by an aryl ether linkage whereas the western unit is composed of two brominated tyramines linked similarly to each other via a diaryl ether bond. Our synthetic strategy entails the use of enzymes to catalyze the oxidative coupling of the respective monomers to form the diaryl ether bond in the eastern and western units. The amide bonds of the bastarane carbon skeleton are generated by joining the dimeric units using conventional chemical methods. The acyclic bastadin 2 molecule may be synthesized by the condensation of the eastern segment with 2 equiv of 3-bromotyramine. Bastadin 3 may be constructed in a similar fashion but, in this case, the requisite dimeric synthon must be prepared via the oxidative C−C bond coupling of two brominated tyrosine derivatives using peroxidases (Scheme 1).

Enzymatic Oxidative Phenolic Coupling

The synthesis of bastadin 6 was first investigated with the attempted oxidative coupling of 3,5-dibromotyramine (**1a**) using horseradish peroxidase (HRP) with a view to preparing the western hemibastadin segment. When the enzymatic reaction was conducted in NaOAc−HOAc buffer at pH 4.0, the major product formed was **4a**, which

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(2) Carney, J. R.; Scheuer, P. J.; Kelly-Borges, M. *J. Nat. Prod.* **1993**, *56*, 153−157.
(3) (a) Pordesimo, E. O.; Schmitz, F. J. *J. Org. Chem.* **1990**, *55*, 4704−4709. (b) Miao, S.; Andersen, R. J. *J. Nat. Prod.* **1990**, *53*, 1441−1446. (c) Dexter, A. F.; Garscon, M. J.; Hemling, M. E. *Ibid.* **1993**, *56*, 782−786. (d) Butler, M. S.; Lim, T. K.; Capon, R. J. *Aust. J. Chem.* **1991**, *44*, 287−296. (e) Gulavita, N. K.; Wright, A. E.; McCarthy, P. J.; Pomponi, S. A.; Kelly-Borges, M.; Chin, M.; Sills, M. A. *J. Nat. Prod.* **1993**, *56*, 1613−1617.
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(5) Schreiber, S. L. *Science* **1991**, *251*, 283−287.
(6) (a) Nishiyama, S.; Yamamura, S. *Tetrahedron Lett.* **1982**, *23*, 1281−1284. (b) Nishiyama, S.; Suzuki, T.; Yamamura, S. *Tetrahedron Lett.* **1982**, *23*, 3699−3702. (c) Nishiyama, S.; Suzuki, T.; Yamamura, S. *Chem. Lett. (Chem. Soc. Jpn.)* **1982**, 1851−1852.
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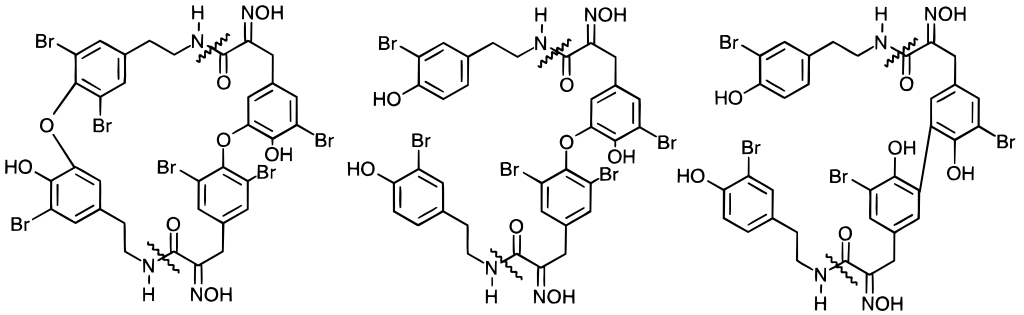
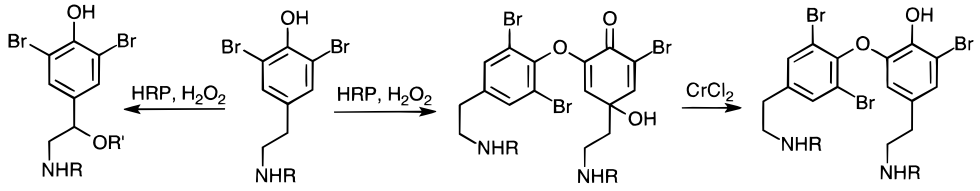
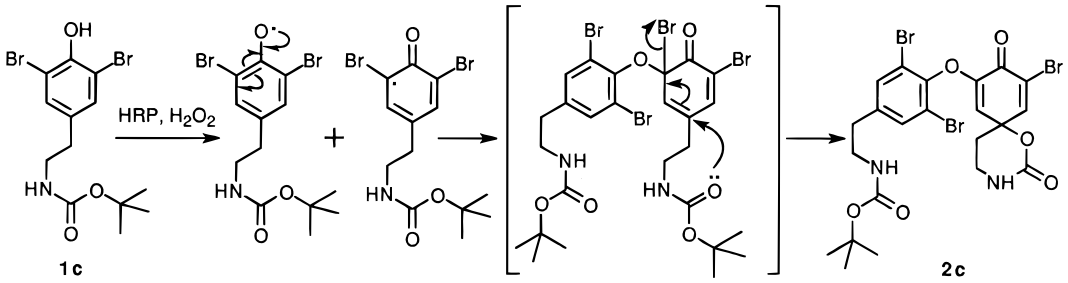
Scheme 1



Scheme 2



Scheme 3



was isolated in 30% yield along with another product (20%), not characterized, but it afforded a very polar compound upon reduction with $CrCl_2$. When the reaction was carried out in Na_2HPO_4−citrate buffer at pH 4.0 or 6.0, a complex mixture of products were obtained. However, when *N*-acetyl-3,5-dibromotyramine (**1b**) was exposed to HRP at pH 5.0, three products were isolated and identified to be **2b** (10%), **3b** (15%), **4b** (24%), and recovered **1b** (35%). By carrying out the oxidative coupling at pH 4.0 in sodium acetate buffer, the quantity of **4b** was markedly reduced. Further, isolation of the products could be simplified considerably by in situ treatment of the reaction mixture with $CrCl_2$. Although **3b** was obtained in 65% yield, it did not possess the requisite regiochemical differentiation of the amino functional groups necessary for further synthetic elaborations. When **3b** was exposed to the action of several proteases [α-chymotrypsin (Sigma type II); Subtilisin Carlsberg (Sigma type VIII); Protease 2A and Prozyme 6 (Amano)], the hydrolytic reaction could not be efficiently terminated at the monoamine stage. Hence the regioselectivity of hydrolysis was not further examined (Scheme 2).

On the other hand, N-(*tert*-butoxycarbonyl)-3,5-dibromotyramine (**1c**) was found to be an excellent substrate for the heme peroxidases. More importantly, the oxygen atom of the *tert*-butoxycarbonyl group reacted at the *ipso* position of the aromatic ring to form a cyclic carbamate (**2c**) with the concomitant generation of the cyclohexadienone ring system as shown. Under optimum conditions, **2c** was obtained in 53% yield using soybean peroxidase (SPO) as the catalyst (Scheme 3).

As shown in Table 1, several experimental parameters, which are interrelated, have to be carefully controlled and defined to obtain optimum yield of the product. These included the amount of H_2O_2 and organic solvent, pH, enzyme-to-substrate ratio, and reaction time.

Like other heme peroxidases, HRP exhibits two catalytically active intermediates, compound I and II. When HRP reacts with H_2O_2, compound I (Fe^{V}), which is two oxidizing equivalents above the ferric state, is formed. Compound I is converted into compound II (Fe^{IV}) by one-electron reduction. Further reduction of compound II to native enzyme (Fe^{III}) is often rate-limiting in the overall catalytic cycle.[8]

In most of our experiments, a molar ratio of 1.2 of H_2O_2 to substrate (2.4 equiv of H_2O_2) was used. Higher concentrations (5 equiv) led to the inhibition of enzyme activity, which may be due to the accumulation of compound III (Fe^{III}) during turnover since compound III is not involved in the peroxidase reaction cycle. Moreover, excess H_2O_2 causes the oxidative destruction of the porphyrin ring of HRP.

In general, C−O coupling reactions proceeded efficiently in the pH range of 4−6. The reaction rate was

(8) Walsh, C. *Enzymatic Reaction Mechanisms*; W. H. Freeman Co.: San Francisco, 1979; pp 464−500.

Table 1. Effect of Reaction Conditions on Peroxidase−Catalyzed C−O Coupling of 1c[a]

pH	acetonitrile (%)	enzyme/**1c** (unit/μmol)	reaction time (min)	yield (%, by HPLC) **2c**	yield (%, by HPLC) remaining **1c**
6	35	40 HRP	10	33	0
4	35	40 HRP	20	61	0
4	35	20 HRP	20	62	0
4	35	20 HRP	20	46[b]	
4	35	10 HRP	20	35	18
8	35	6 SPO	10	0	18
7	35	3 SPO	20	0	39
6	35	3 SPO	20	46	7
5	35	6 SPO	20	42	3
5	25	5 SPO	20	50	5
5	35	5 SPO	20	53[c]	
5	35	3 SPO	20	39	16
4	40	40 SPO	10	35	0
4	40	10 SPO	10	50	6

[a] The experiments were carried out at 24 °C, at a scale of 10 μmol of **1c**, and 5 mM of substrate concentration. The reaction mixtures were analyzed by RP-HPLC (8 × 100 mm cartridge, 1.5 mL/min, solvent B from 20% to 80% in 12 min). The retention times of **1c** and **2c** were 11.6 and 11.9 min, respectively. HRP and SOP were abbreviations of horseradish peroxidase and soybean peroxidase, respectively. [b] Isolated yield at a scale of 5 mmol of substrate **1c**. [c] Isolated yield at a scale of 3 mmol of substrate **1c**.

slower at lower pH, but more byproducts were formed at neutral to alkaline pH. For example, there was a marked difference in the product profiles between pH 6 and 7. This observation is consistent with the published report that the reactivity of compound II was pH dependent due to changes in the bond length of the sixth coordinated ligand of HRP compound II with pH.[10] At pH 3.0, SPO was more active and stable than HRP but the reaction rate was too slow to be useful in most cases. At pH 5−6, the reactivities of the two enzymes were virtually identical. The concentrations of substrates were in the range of 5 to 20 mM, which depended on their solubilities.

Higher concentrations of substrates were used for intermolecular coupling reactions, but the tendencies to form polymers were also enhanced. The amount of organic solvent used depended on the solubility of substrate in buffer of defined pH. Under acidic conditions, most of the substrates, except 3,5-dibromotyramine, cannot be dissolved in aqueous buffer without the addition of a cosolvent. It is known that addition of cosolvents may prevent the inactivation of heme peroxidases during catalytic turnover. For example, *tert*-butyl alcohol is known to protect the deactivation of chloroperoxidase during its oxidation of indole.[9] Of the organic solvents (dioxane, acetone, methanol, DMSO, DMF, and acetonitrile) tested, acetonitrile was found to be the best cosolvent for it gave less byproducts. At a 10% acetonitrile concentration the peroxidases were inhibited only slightly, but at higher concentrations the inhibition became very pronounced and more enzyme must be added to compensate the reaction rate. In most cases, a clear solution of 5 to 10 mM substrate could be obtained in aqueous buffer containing 10% acetonitrile. At pH 3, more dilute substrate solution was used or more acetonitrile was added to ensure a clear reaction mixture. Acetonitrile concentrations as high as 35% could be used as in the case of N-(*tert*-butoxycarbonyl)-3,5-dibromotyramine to solubilize a 5 mM substrate solution.

The enzyme−substrate ratio was also an important factor and this ratio varied for each substrate and depended on other factors as well, such as pH and cosolvent concentration. This phenomenon has been observed in other peroxidase-catalyzed oxidations of aromatic substrates and may thus be rationalized similarly. For example, in the oxidation of indole-3-acetate by peroxidase, a high enzyme−substrate ratio resulted in the formation of peroxidase compound II, and 3-methyleneoxindole was obtained as the major end product.[11] On the other hand, when indole-3-acetate and peroxidase were mixed in stoichiometric amounts, only ferroperoxidase and compound III were formed, and indole-3-aldehyde became the major product of oxidation. In general, the reaction time for C−O coupling should be completed within a period of 5 to 20 min under optimum conditions.

When a low enzyme−substrate ratio was used, longer reaction times were required which resulted in more side reactions. On the other hand, when too much enzyme was used, nonhomogeneous polymers were formed as evidenced by the appearance of broad HPLC peaks. When in situ reduction was employed, some residual substrate was always recovered. However, this did not necessarily mean that the reaction did not go to completion but rather monomeric quinol derivatives, such as **6** and **7**, were reduced back to the starting material.

The requisite eastern segment, **8**, was prepared using 3-(3,5-dibromo-4-hydroxyphenyl)-2-(hydroxyimino)propionic acid (**5**) as the starting material.[12] After much experimentation, we found that the optimum condition for C−O coupling occurred at pH 4.0 in buffer containing 10% acetonitrile. Under these conditions, **8** was obtained in 46% yield accompanied by two monomeric side products, **6** and **7**. Under mildly acidic conditions, **8** was found to undergo isomerization to yield **9**. The availability of **2c** and **8** provided us with the requisite synthons for the synthesis of bastadin 6. Reduction of **8** gave the key intermediate **10** for the synthesis of bastadin 2. Under optimized conditions **10** was secured in 49% overall yield by the in situ treatment of the reaction mixture with $NaHSO_3$ prior to workup (Scheme 4).

For the synthesis of bastadin 3, it was necessary to prepare the dimeric synthon **11b**. The dimerization of the monomer **12a** by means of C−C ortho phenolic coupling, catalyzed by HRP, was achieved at pH 9.0 to give **11a** in 20% yield. It was transformed into **11b** by reaction with dioxane−bromine complex. Alternatively, **11b** was obtained in 30% yield by the exposure of **12b** to HRP at pH 9.0 (Scheme 5).

In general the oxidative phenolic C−C coupling of unhalogenated or monohalognated tyrosine derivatives was best achieved in the pH range of 8 to 10. Although the reaction rate was faster at lower pH, more polymeric products were formed. As a rule, less enzyme was needed for C−C than for C−O coupling reactions. The availability of the requisite synthons, successfully prepared by enzymatic oxidative C−C and C−O couplings, allowed us to proceed with the synthesis of the proposed targets via amide bond formation using conventional methods.

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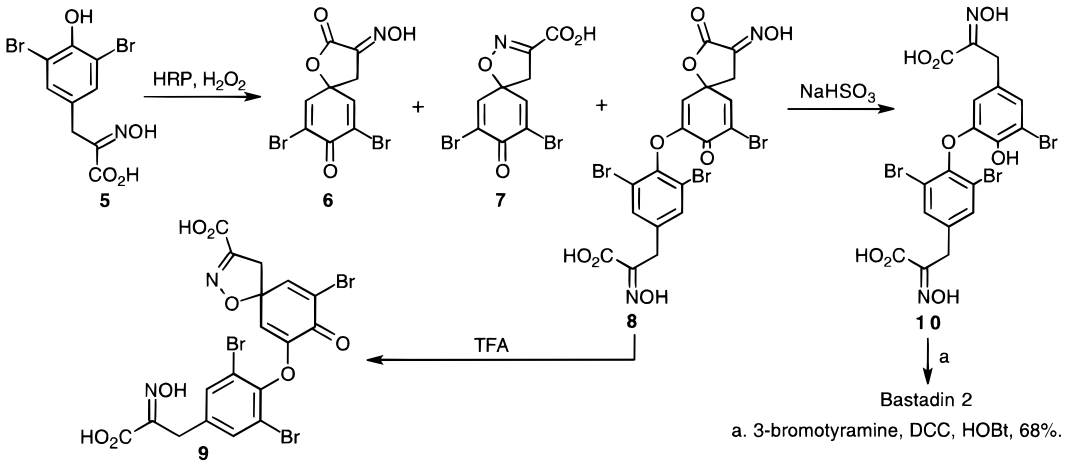
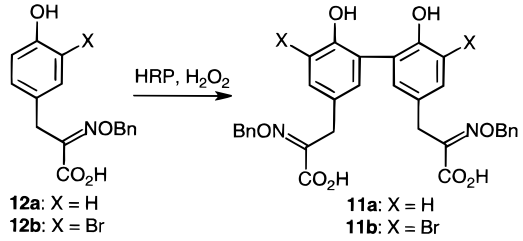
(12) Compound **5** was prepared from 4-hydroxybenzaldehyde. (a) Forrester, A. R.; Thomson, R. H.; Woo, S. O. *J. Chem. Soc., Perkin Trans. 1* **1975**, 2340−2348. (b) Shiba, T.; Cahnman, H. J. *J. Org. Chem.* **1964**, *29*, 3061−3063.

Scheme 4



Scheme 5



Completion of the Synthesis of Bastadins

To complete the synthesis of bastadin 6, the cyclic carbamate **2c** was reduced with $CrCl_2$ to give **13** in 98% yield. Surprisingly, treatment of **2c** with $NaHSO_3$ afforded a complex mixture of products. Reaction of **13** with **8** in the presence of DCC and l-hydroxybenzotriazole (HOBt) gave **14** in 72% yield, which upon reduction of the cyclohexadienone with $NaHSO_3$/NaOH at pH 8 followed by the removal of the *t*-BOC group with TFA gave **16** (95%). The formation of the macrocycle was accomplished by the treatment of **16** with DCC/HOBt giving bastadin 6 in 64% yield, whose physical properties (^{1}H NMR) were identical to reported values.[1] The structure of the synthetic bastadin 6 was further confirmed by converting it to the tetramethyl derivative, **17**, using methyl iodide. No tetramethyl isobastadine 6[1,13] was detected by ^{1}H NMR analysis (Scheme 6). Bastadin 2 was prepared by the condensation of **10** with an excess of 3-bromotyramine in the presence of DCC/HOBt to afford bastadin 2 in 68% yield (Scheme 4). It is worthy of note that when extra base such as diisopropylethylamine or triethylamine were added to the reaction mixture, lower yield of the product was obtained. Instead a large amount of a less polar byproduct appeared, whose structure was not identified.

Two methods were used for the synthesis of bastadin 3. The first of these entailed the condensation of **11b** with 3-bromotyramine to give the bastadin 3 derivative **18** in 57% yield. After debenzylation with BBr_3, bastadin 3 was obtained in 80% yield. Alternatively, **11b** was first hydrogenated to remove the benzyl protecting group (74%), and the resulting intermediate **19** was then transformed into bastadin 3 by reaction with excess 3-bromotyramine, which proceeded in 73% yield (Scheme 7).

(13) Franklin, M. A.; Penn, S. G.; Lebrilla, C. B.; Lam, T. H.; Pessah, I. N.; Molinski, T. F. *J. Nat. Prod.* **1996**, *59*, 1121−1127.

Conclusion

In summary, we have achieved the synthesis of the proposed bastadin targets using a chemoenzymatic approach, which avoids the extensive protection and deprotection of functional groups thereby demonstrating the usefulness of peroxidases for the synthesis of the diaryl ether bonds. To our knowledge, this approach represents a markedly improved method for the preparation of macrocyclic bastadins. In this connection it is also noteworthy that this mode of oxidative coupling may represent the biogenetic route for bastadin formation.[1] That is, the isodityrosine and isodityramine frameworks are formed via the coupling of dihalogenated tyrosine and tyramine derivatives respectively rather than oxidative coupling of tyrosine and tyramine followed by bromination. Although one cytochrome P450 enzyme of plant origin was reported to catalyze diaryl ether formation of phenolic compounds,[14] it is, unfortunately, not readily accessible for synthetic use. Until now, the yields of C−O coupling of tyrosine derivatives assisted by peroxidases were extremely low. We have shown that by carefully controlling the experimental parameters, isodityrosine derivatives can now be prepared in synthetically useful yields without the exhaustive protection and deprotection of the sensitive functional groups. Further application of this oxidative enzyme technology to the synthesis of complex natural products are currently in progress.

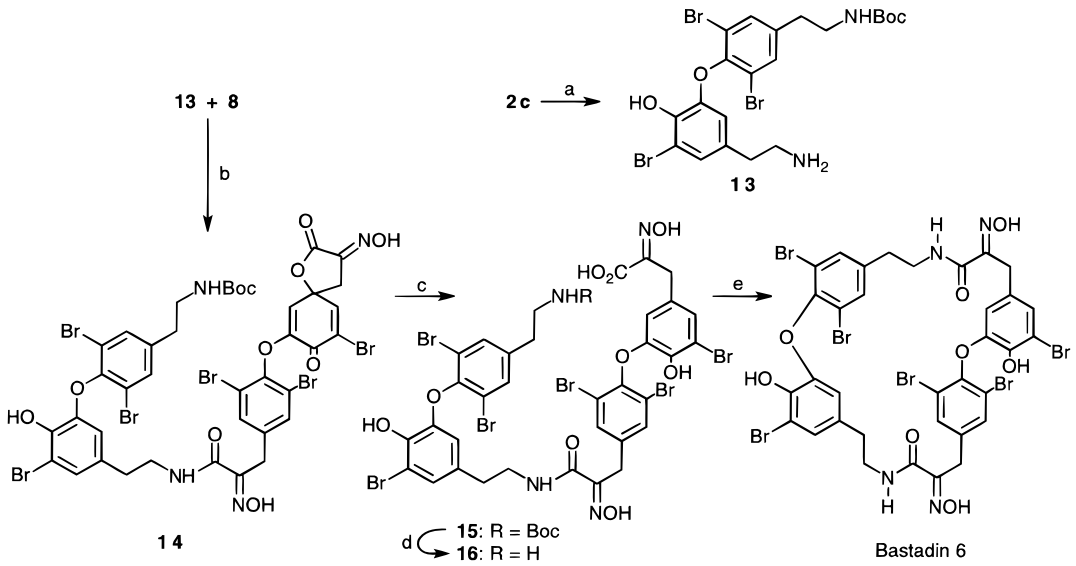
Experimental Section

General experimental techniques and analytical measurements were applied as previously described.[15] Horseradish peroxidase (HRP, type 1) was purchased from Sigma Chemical Co. Soybean peroxidase (SPO) was a product of Enzymol International, Inc. The following aqueous buffers were used in this work unless otherwise stated: 0.1 M sodium borate−boric acid at pH 9 to 10; 0.2 M Na_2HPO_4−NaH_2PO_4 at pH 6 to 8; 0.2 M Na_2HPO_4−0.1 M citric acid at pH 3 to 5. All NMR spectra were recorded on a spectrometer operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C, in a solvent as indicated. Reverse phase high performance liquid chromatography (RP-HPLC) was carried out on a Waters Nova-Pak C18 cartridge (8 × 100 mm) or a Prep Nova-Pak HR C18 column (19 × 300 mm, preparative column) using an elution gradient from solvent A

(14) (a) Zenk, M. H.; Gerardy, R.; Stadler, R. *J. Chem. Soc., Chem. Commun.* **1989**, 1725−1727. (b) Stadler, R.; Zenk, M. H. *J. Biol. Chem.* **1993**, *268*, 823−831.

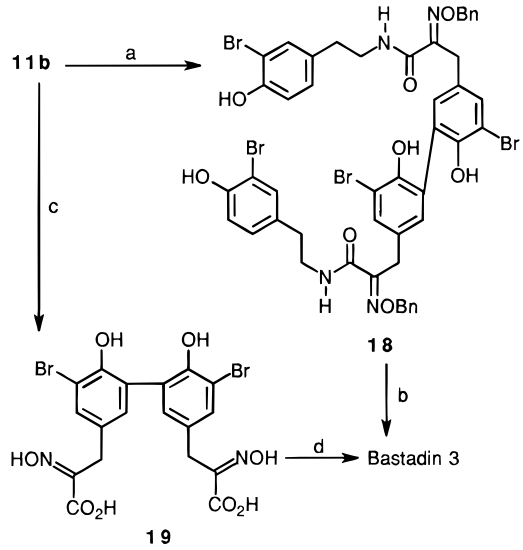
(15) Salamonczyk, G. M.; Han, K.; Guo, Z. W.; Sih, C. J. *J. Org. Chem.* **1996**, *61*, 6893−6900.

Scheme 6



a. $CrCl_2$, 98%; b. DCC, HOBt, 72%; c. $NaHSO_3$, NaOH, 99%; d. TFA, 95%; e. DCC,HOBt, 64%.

Scheme 7



a. 3-bromotyramine, HOBt, EDCI, 57%; b. BBr_3, 80%;
c. Pd/C, EtOH, 74%; d. 3-bromotyramine, HOBt, EDCI, 73%.

(0.1% TFA in water) to solvent B (0.1% TFA in 90% aqueous acetonitrile). Baker silica gel (40 μm) was used for flash chromatography.

***N*-(*tert*-Butoxycarbonyl)-3,5-dibromotyramine (1c). 1c** was prepared from tyramine. NMR ($CDCl_3$): δ ^{1}H 7.22 (s, 2H), 6.01 (brs, 1H), 4.60 (brs, 1H), 3.29 (brq, 2H), 2.67 (t, 6.9, 2H), 1.42 (s, 9H) ppm; δ ^{13}C 155.9, 148.1, 133.7, 132.3 (2C), 109.9 (2C), 79.54, 41.61, 34.73, 28.39 (3C) ppm; MS FAB *m*/*z* (relative intensity) 394/396/398 [80/100/48, (M + H)].

10-Bromo-8-[4-[2-(*tert*-butoxycarbonylamino)ethyl]-2,6-dibromophenoxy]-2,9-dioxo-3-aza-1-oxaspiro[5.5]-undeca-7,10-diene (2c). To a clear solution of **1c** (1185 mg, 3 mmol) in 340 mL of pH 5.0 buffer and 210 mL of acetonitrile was added 50 mL of SPO (300 unit/mL) followed by 3.6 mL of 1 M H_2O_2. The resulting mixture was stirred at 24 °C for 20 min, quenched with 10 mL of 1 M $KHSO_4$, and extracted with ethyl acetate (3 × 300 mL). The combined organic extracts were washed with brine (150 mL), dried over $MgSO_4$, and concentrated to dryness under reduced pressure. The residue was subjected to flash chromatography (hexanes−ethyl acetate 2:1 to 0:1) to give **2c** (520 mg, 53%). NMR ($CDCl_3$−DMSO-d_6 4:1): δ ^{1}H 7.52 (d, 2.6, 1H), 7.48 (s, 2H), 5.63 (d, 2.6, 1H), 3.51−3.42 (m, 1H), 3.38−3.29 (m, 1H), 3.33 (t, 7.0, 2H), 2.80 (t, 7.0, 2H), 2.19−2.02 (m, 2H), 1.44 (s, 9H) ppm; δ ^{13}C 172.0, 156.0, 151.5, 146.3, 146.1, 145.5, 140.8, 133.6 (2C), 124.3 (2C), 118.4, 116.7, 78.94, 76.91, 41.07, 37.15, 34.99, 30.99, 28.41 (3C) ppm; MS FAB *m*/*z* (relative intensity) 649/651/653/655 [52/98/100/54, (M + H)]. Similarly, **2c** was obtained in 46% yield at a larger scale (5 mmol of **1c**) with HRP at pH 4.0.

3-[2-(Acetylamino)ethyl]-5-[4-(2-acetylaminoethyl)-2,6-dibromophenoxy]-1-bromo-3-hydroxy-6-oxo-1,4-cyclohexadiene (2b); *N*-Aectyl-3-[4-[2-(acetylamino)ethyl]-2,6-dibromophenoxy]-5-bromotyramine (3b); and *N*-Acetyl-2-(3,5-dibromo-4-hydroxyphenyl)-2-(hydroxyethyl)-amine (4b). To a clear solution of *N*-acetyl-3,5-dibromotyramine (**1b**, 169 mg, 0.5 mmol) in 80 mL of pH 5.0 buffer and 20 mL of acetonitrile was added 0.5 mL of HRP (1000 unit/ml) followed by 0.6 mL of 1 M H_2O_2. The resulting reaction mixture was stirred at 24 °C for 15 min, adjusted to pH 3 with 0.5 mL of 1 M $NaHSO_3$ and 2 mL of 1 M HCl, and then concentrated to dryness under reduced pressure. The residue was extracted with 20 mL of dichloromethane−acetone (1:3), and the solids were removed by filtration. The organic solution was concentrated to dryness under reduced pressure. The residue was then dissolved in 10 mL of 30% aqueous acetonitrile. After centrifugation, the supernatant was analyzed, and the products were isolated by RP-HPLC (8 × 100 mm cartridge, 1.5 mL/min, solvent B from 20% to 60% in 12 min). The retention times and yields were as follows: **4b** (3.8 min, 23%), recovered **1b** (6.4 min, 30%), **2b** (7.6 min, 10%), **3b** (9.8 min, 15%). Compound **2b**, NMR (acetone-d_6−DMSO-d_6 9:1) δ ^{1}H: 7.62 (s, 2H), 7.50 (d, 1.8, 1H), 5.65 (d, 1.8, 1H), 3.44 (m, 1H), 3.32 (m, 1H), 2.82 (t, 7.2, 1H), 1.94 (m, 1H), 1.88 (s, 3H), 1.86 (s, 3H) ppm; δ ^{13}C: 173.4, 171.4, 171.1, 154.2, 146.8, 146.0, 141.9, 134.4 (2C), 124.6, 121.8, 117.6 (2C), 71.89, 41.21, 40.64, 35.05 (2C), 22.83, 22.71 ppm; MS FAB *m*/*z* (relative intensity) 607/609/611/613 [39/98/100/34, (M + H)]. **3b**, NMR (acetone-d_6−DMSO-d_6 9:1) δ ^{1}H: 7.66 (s, 2H), 7.10 (d, 1.9, 1H), 6.23 (d, 1.9, 1H), 3.45 (t, 6.9, 1H), 3.23 (t, 7.2, 1H), 2.85 (t, 6.9, 1H), 2.60 (t, 7.2, 1H), 1.89 (s, 3H), 1.83 (s, 3H) ppm; δ ^{13}C: 171.6, 171.5, 147.6, 145.7, 142.9, 141.6, 134.4 (2C), 132.5, 127.3, 118.3 (2C), 113.8, 110.8, 41.15, 40.84, 35.02, 34.75, 22.78 (2C) ppm; MS FAB *m*/*z* (relative intensity) 591/593/595/597 [38/100/92/34, (M + H)]. **4b**, NMR (acetone-d_6) δ ^{1}H: 7.52 (s, 2H), 4.70 (dd, 7.6, 4.4, 1H), 3.45 (m, 1H), 3.28 (m, 1H), 1.90 (s, 3H).

Reduction of 2b. Compound **2b** (12 mg, 0.02 mmol) was dissolved in 2 mL of methanol and 2 mL of pH 3 buffer. To this mixture was added $CrCl_2$ (20 mg, 0.16 mmol), and the contents were stirred at 24 °C for 10 min and analyzed, and **3b** (10 mg, 83%) was isolated by RP-HPLC, whose physical

data were identical to a sample isolated directly from the enzymatic reaction mixture.

Enzymatic Oxidative C−O Coupling of 1b Followed by *in situ* Reduction. To a clear solution of **1b** (337 mg, 1 mmol) in 160 mL of pH 4.0, 0.2 M NaOAc−HOAc buffer and 40 mL of acetonitrile was added 5 mL of HRP (1000 unit/mL) followed by 1.2 mL of 1 M H_2O_2. The resulting reaction mixture was stirred at 24 °C for 5 min and quenched with 1 mL of 1 M $NaHSO_3$, and $CrCl_2$ (984 mg, 8 mmol) was then added. The pH of the mixture was adjusted to 3 with 1 M HCl, stirred for additional 20 min, and then extracted with ethyl acetate (3 × 200 mL). The extracts were concentrated to dryness under reduced pressure. The residue was extracted with acetonitrile (2 × 50 mL), and the solid salt was removed by filtration. The acetonitrile solution was concentrated to dryness to give 310 mg of solids, which were stirred in 2 mL of water for 20 min. The remaining solids were collected by filtration, washed with another 1 mL of water, and dried under reduced pressure to give **3b** (192 mg, 65% overall), whose ^{1}H NMR spectrum was identical to a sample isolated by RP-HPLC in the previous experiment.

2-Acetoxy-2-(3,5-dibromo-4-hydroxyphenyl)ethylamine (4a). To a clear solution of 3,5-dibromotyramine (**1a**, 30 mg, 0.1 mmol) in 5 mL of pH 4.0, 0.2 M NaOAc−HOAc buffer was added 0.1 mL of HRP (1000 unit/mL) followed by 0.12 mL of 1 M H_2O_2. The resulting reaction mixture was stirred at 24 °C for 10 min and quenched with 0.2 mL of 1 M $NaHSO_3$ and 1 mL of acetonitrile. The pH of the mixture was then adjusted to 3 with 1 M HCl and was then analyzed by RP-HPLC (8 × 100 mm cartridge, 1.0 mL/min, from solvent A to B in 20 min). The elution profile showed three major peaks: peak 1 (25%, 11.8 min, the same retention time as **1a**), peak 2 (30%, 12.3 min), peak 3 (20%, 13.9 min). After the addition of $CrCl_2$ (98 mg, 0.8 mmol), the mixture was stirred for 10 min and analyzed, and the products were isolated by RP-HPLC. Peaks 1 and 2 remained unchanged, whereas peak 3 was transformed into a new peak with a retention time of 3.2 min, whose chemical identity was not determined. ^{1}H NMR analysis confirmed peak 1 was recovered **1a**. The structure of peak 2 was established as **4a**, NMR (D_2O): δ ^{1}H 7.60 (s, 2H), 5.83 (dd, 8.8, 4.1, 1H), 3.43 (dd, 14.2, 8.8, 1H), 3.36 (dd, 14.2, 4.1, 1H), 2.20 (s, 3H) ppm; δ ^{13}C 176.8, 154.0, 134.1 (2C), 133.9, 114.8 (2C), 75.0, 47.2, 24.2 ppm; MS FAB *m*/*z* (relative intensity) 352/354/356 [60/100/55, (M + H)].

7,9-Dibromo-2,8-dioxo-3-(hydroxyimino)-1-oxaspiro-[4.5]deca-6,9-diene (6); 7,9-Dibromo-3-carboxy-8-oxo-2-aza-1-oxaspiro[4.5]deca-2,6,9-triene (7); and 7-Bromo-2,8-dioxo-3-(hydroxyimino)-9-[2,6-dibromo-4-[2-carboxy-2-(hydroxyimino)ethyl]phenoxy]-1-oxaspiro[4.5]deca-6,9-diene (8). To a clear solution of 3-(3,5-dibromo-4-hydroxyphenyl)-2-(hydroxyimino)propionic acid (**5**, 2120 mg, 6 mmol) in 420 mL of pH 4.0 buffer and 60 mL of acetonitrile was added 120 mL of SPO (250 unit/mL) followed by 7.2 mL of 1 M H_2O_2. The resulting reaction mixture was stirred at 24 °C for 10 min and extracted with ethyl acetate (3 × 500 mL). The combined organic extracts were washed with brine (300 mL), dried over $MgSO_4$, and concentrated to dryness under reduced pressure. The residue was subjected to flash chromatography (hexanes−ethyl acetate−acetone 1:1:0 to 0:4:1) to give **6** (42 mg, 4%), NMR ($CDCl_3$−acetone-d_6 4:1): δ ^{1}H 12.18 (brs, 1H, NOH), 7.60 (s, 2H), 3.39 (s, 2H) ppm; δ ^{13}C 171.9, 164.1, 147.2 (2C), 144.8, 123.6 (2C), 79.33, 33.59 ppm, **7** (28 mg, 3%), NMR ($CDCl_3$−acetone-d_6 1:1): δ ^{1}H 7.64 (s, 2H), 3.65 (s, 2H) ppm; δ ^{13}C 171.5, 160.0, 152.7, 145.7 (2C), 122.8 (2C), 86.45, 42.96 ppm, and **8** (860 mg, 46%), NMR (DMSO-d_6) δ ^{1}H:12.83 (s, 1H, NOH), 12.55 (s, 1H, NOH), 7.94 (d, 2.2, 1H), 7.56 (s, 2H), 6.33 (d, 2.2, 1H), 3.84 (s, 2H), 3.23 (d, 19.1, 1H), 2.93 (d, 19.1, 1H) ppm; NMR (acetone-d_6): δ ^{1}H 7.80 (d, 2.6, 1H), 7.64 (s, 2H), 6.23 (d, 2.6, 1H), 3.93 (s, 2H), 3.44 (d, 19.5, 1H), 3.21 (d, 19.5, 1H); δ ^{13}C 172.6, 165.3, 164.7, 149.7, 148.3, 146.5 (2C), 145.5, 139.0, 134.5 (2C), 123.9, 120.3, 117.4 (2C), 78.99, 34.21, 29.46 ppm; MS FAB *m*/*z* (relative intensity) 621/623/625/627 [35/92/100/44, (M + H)]. Compound **8** was also obtained in 47% yield under the same reaction conditions except HRP was used instead of SPO on a smaller scale (1 mmol of **5**).

7-Bromo-3-carboxy-8-oxo-9-[2,6-dibromo-4-[2-carboxy-2-(hydroxyimino)ethyl]phenoxy]-2-aza-1-oxa-spiro[4.5]-deca-2,6,9-triene (9). A clear solution of 50 mg of **8** in 9 mL of dichloromethane and 1 mL of TFA was stirred at 24 °C. The reaction progress was monitored by RP-HPLC (8 × 100 mm cartridge, 1.5 mL/min, solvent B from 20% to 80% in 12 min). The retention times of **8** and **9** were 10.5 and 10.2 min, respectively. The conversion was 35% at 6 h, and the isomerization was complete after 24 h. The mixture was concentrated to dryness under reduced pressure, and the residue was recrystallized from hexanes−ethyl acetate to give **9** (40 mg, 80%), NMR (DMSO-d_6): δ ^{1}H 12.55 (s, 1H, NOH), 7.82 (d, 2.4, 1H), 7.56 (s, 2H), 6.10 (d, 2.4, 1H), 3.85 (s, 2H), 3.51 (d, 17.6, 1H), 3.32 (d, 17.6, 1H) ppm; δ ^{13}C 172.2, 165.0, 160.6, 153.5, 149.0, 147.2, 145.2, 145.1, 138.0, 133.2 (2C), 122.3, 118.7, 116.5 (2C), 85.09, 43.19, 28.80 ppm.

3-[3-Bromo-5-[2,6-dibromo-4-[2-carboxy-2-(hydroxyimino)ethyl]phenoxy]-4-hydroxyphenyl]-2-(hydroxyimino)propionic Acid (10). To a clear solution of **5** (353 mg, 1 mmol) in 90 mL of pH 5.0 buffer and 10 mL of acetonitrile was added 5 mL of HRP (1000 unit/mL) followed by 1.2 mL of 1 M H_2O_2. The resulting reaction mixture was stirred at 24 °C for 10 min, quenched with 1 M $NaHSO_3$ (3.0 mL), adjusted to pH 7.5 with 1 M NaOH, stirred for 10 min, acidified to pH 3.0 with $KHSO_4$ (3 g), and then extracted with ethyl acetate (2 × 120 mL). The combined organic extracts were washed with brine (2 × 50 mL), dried over $MgSO_4$, and concentrated to dryness under reduced pressure. The residue was subjected to flash chromatography (dichloromethane−acetone−acetic acid 4:1:0.1 to 0:5:0.1) to give recovered **5** (28 mg, 8%) and product **10** (153 mg, 49%), NMR (acetone-d_6): δ ^{1}H 7.65 (s, 2H), 7.16 (d, 1.8, 1H). 6.42 (d, 1.8, 1H), 3.99 (s, 2H), 3.70 (s, 2H) ppm; δ ^{13}C 164.9, 164.7, 150.8, 150.3, 147.8, 145.3, 143.2, 138.4, 134.4 (2C), 129.5, 127.6, 118.3 (2C), 114.5, 110.3, 29.52, 29.39 ppm; MS FAB *m*/*z* (relative intensity) 623/625/627/629 [42/100/100/40, (M + H)].

2-(Benzyloxyimino)-3-[3-[5-[2-(benzyloxyimino)-2-carboxyethyl]-2-hydroxyphenyl]-4-hydroxyphenyl]propionic Acid (11a). To a clear solution of 2-(benyloxyimino)-3-(4-hydroxyphenyl)propionic acid (**12a**, 286 mg) in 52 mL of pH 9.0 buffer and 6 mL of dioxane was added 1430 units of HRP followed by 136 μL of 30% H_2O_2. The reaction mixture was stirred at 24 °C for 10 min, quenched and adjusted to pH 2 with 5 M HCl, and extracted with 50 mL of ethyl acetate. The organic extracts were washed with 30 mL of water and concentrated to dryness under reduced pressure. The residue was subjected to flash chromatography (dichloromethane−acetone 100:0 to 7:1) to afford **11a** (58 mg, 20%), NMR ($CDCl_3$): δ ^{1}H 7.20−7.16 (m, 12H), 7.09 (d, 8.1, 2H), 6.81 (dd, 8.1, 2.0, 2H), 5.16 (s, 4H), 3.76 (s, 4H) ppm; δ ^{13}C 164.3 (2C), 151.7 (2C), 150.3 (2C), 135.9 (2C), 132.3 (2C), 130.3 (2C), 128.6 (4C), 128.5 (2C), 128.3 (4C), 128.0 (2C), 124.9 (2C), 117.0 (2C), 78.25 (2C), 29.75 (2C) ppm; MS FAB *m*/*z* 569 (M + H).

2-(Benzyloxyimino)-3-[3-bromo-5-[5-[2-(benzyloxyimino)-2-carboxyethyl]-3-bromo-2-hydroxyphenyl]-4-hydroxyphenyl]propionic Acid (11b). To a clear solution of 2-(benzyloxyimino)-3-(3-bromo-4-hydroxyphenyl)propionic acid (**12b**, 510 mg) in 92 mL of pH 9.0 buffer and 10 mL of dioxane was added 1275 units of HRP followed by 190 μL of 30% H_2O_2. The reaction mixture was stirred at 24 °C for 5 min, quenched with 60 mL of 5% citric acid aqueous solution, and extracted with 100 mL of ethyl acetate. The organic extracts were washed with 40 mL of water and concentrated to dryness under reduced pressure. The residue was subjected to flash chromatography (dichloromethane−acetone 100:0 to 5:1) to afford **11b** (155 mg, 30%). NMR: δ ^{1}H (acetone-d_6−D_2O 9:1) 7.44 (s, 2H), 7.33−7.26 (m, 10H), 7.04 (s, 2H), 5.29 (s, 4H), 3.84 (s, 4H) ppm; δ ^{13}C ($CDCl_3$−acetone-d_6 1:2) 164.8 (2C), 151.3 (2C), 150.4 (2C), 137.4 (2C), 133.7 (2C), 132.4 (2C), 129.8 (2C), 129.2 (4C), 129.1 (4C), 128.9 (2C), 127.4 (2C), 111.7 (2C), 78.25 (2C), 30.21 (2C) ppm; MS FAB *m*/*z* (relative intensity) 725/727/729 [50/100/56, (M + H)].

3-Bromo-5-[2,6-dibromo-4-[2-(*tert*-butoxycarbonylamino)ethyl]phenoxy]tyramine (13). To a clear solution of **2c** (326 mg, 0.5 mmol) in methanol−dichloromethane (1:1, 50 mL)

at 24 °C was added $CrCl_2$ (492 mg, 4 mmol). The resulting reaction mixture was stirred at the same temperature for 30 min. Water (50 mL) was added to the mixture after the solvent was partially removed under reduced pressure. The mixture was extracted with ethyl acetate (3 × 80 mL). The combined organic extracts were washed with brine (150 mL), dried over $MgSO_4$, and concentrated to dryness under reduced pressure. The residue was mixed with 10 mL of water and stored at 0 °C overnight after the pH was adjusted to 7.0 with 1 M NaOH. The precipitate was filtered and washed with 2 mL of water and dried under reduced pressure to give **13** (298 mg, 98%). NMR (DMSO-d_6−acetone-d_6−D_2O 8:1:1): δ ^{1}H 7.55 (s, 2H), 7.12 (d, 1.9, 1H), 6.18 (d, 1.9, 1H), 3.20 (t, 6.6, 2H), 2.91 (t, 8.1, 2H), 2.71 (t, 6.6, 2H), 2.64 (t, 8.1, 2H), 1.29 (s, 9H) ppm; δ ^{13}C 157.2, 146.7, 145.5, 142.8, 141.3, 134.1 (2C), 129.6, 126.9, 117.7 (2C), 113.6, 111.2, 79.49, 41.10, 40.59, 34.89, 32.22, 28.60 (3C) ppm.

7-Bromo-2,8-dioxo-3-(hydroxyimino)-9-[2,6-dibromo-4-[2-(hydroxyimino)-2-[2-[3-bromo-4-hydroxy-5-[2,6-dibromo-4-[2-(*tert*-butoxycarbonylamino)ethyl]phenoxy]-phenyl]ethylaminocarbonyl]ethyl]phenoxy]-1-oxaspiro[4.5]deca-6,9-diene (14). To a solution of **8** (62 mg, 0.1 mmol), **13** (61 mg, 0.1 mmol), and 1-hydroxybenzotriazole (HOBt, 14 mg, 0.1 mmol) in dioxane (8 mL) and DMF (1 mL) at 0 °C was added a solution of DCC (31 mg, 0.15 mmol) in 1 mL of dichloromethane. The resulting reaction mixture was stirred at 24 °C for 24 h. Ethyl acetate (40 mL) was added to the mixture after the solvent was partially removed under reduced pressure. The organic solution was washed with 1 M $KHSO_4$ (20 mL) and brine (2 × 20 mL), dried over $MgSO_4$, and concentrated to dryness under reduced pressure. The residue was subjected to flash chromatography (dichloromethane−acetone 19:1 to 4:1) to give **14** (86 mg, 72%). NMR ($CDCl_3$−acetone-d_6 1:1): δ ^{1}H 12.17 (s, 1H), 11.23 (s, 1H), 8.34 (s, 1H), 7.61 (d, 2.6, 1H), 7.59 (s, 2H), 7.50 (s, 2H), 7.29 (t, 5.8, 1H), 7.06 (d, 1.8, 1H), 6.16 (d, 1.8, 1H), 5.90 (d, 2.6, 1H), 3.87 (s, 2H), 3.46−3.32 (m, 5 H), 3.17 (d, 19.7, 1H), 2.85 (brt, 2H), 2.64 (t, 6.8, 2H), 1.41 (s, 9H) ppm; δ ^{13}C 172.5, 164.4, 163.5, 156.9, 151.3, 147.8, 147.5, 146.7, 146.4, 145.5, 145.3, 142.9, 141.2, 138.9, 134.7 (2C), 134.3 (2C), 131.9, 127.3, 124.4, 119.6, 118.3 (2C), 117.4 (2C), 113.7, 110.7, 79.29, 78.92, 42.09, 41.17, 35.73, 35.15, 34.61, 28.90 (3C), 28.83 ppm.

3-[3-Bromo-4-hydroxy-5-[2,6-dibromo-4-[2-(hydroxyimino)-2-[2-[3-bromo-4-hydroxy-5-[2,6-dibromo-4-[2-(*tert*-butoxycarbonylamino)ethyl]phenoxy]phenyl]ethylaminocarbonyl]ethyl]phenoxy]phenyl]-2-(hydroxyimino)propionic Acid (15). To a clear solution of **14** (81 mg, 0.068 mmol) in acetonitrile (10 mL) and water (5 mL) was added 0.27 mL of 1 M $NaHSO_3$ followed by 1 mL of pH 8.0 buffer. The mixture was stirred at 24 °C for 10 min after the pH was adjusted to 8.0 with a few drops of 1 M NaOH. It was then acidified to pH 3.5 with 1 M $KHSO_4$, extracted with ethyl acetate (3 × 30 mL), washed with brine (2 × 30 mL), dried over $MgSO_4$, and concentrated to dryness under reduced pressure. The solid residue was washed with 2 mL of hexane and dried again to give **15** (80 mg, 99%), NMR (acetone-d_6): δ ^{1}H 7.64 (s, 2H), 7.60 (s, 2H), 7.48 (t, 6.0, 1H), 7.15 (d, 1.8, 1H), 7.11 (d, 1.9, 1H), 6.39 (d, 1.8, 1H), 6.25 (d, 1.9, 1H), 3.94 (s, 2H), 3.70 (s, 2H), 3.46−3.34 (m, 4H), 2.86 (t, 6.8, 2H), 2.66 (t, 7.3, 2H), 1.39 (s, 9H) ppm; δ ^{13}C 164.8, 164.5, 156.6, 151.9, 150.9, 147.6, 147.4, 145.5, 145.4, 143.2, 143.1, 141.7, 138.8, 134.5 (2C), 134.4 (2C), 132.3, 129.5, 127.6, 127.3, 118.2 (4C), 114.5, 113.7, 110.6, 110.3, 78.68, 41.85, 41.19, 35.48, 34.96, 29.41, 28.82, 28.53 (3C) ppm; MS FAB *m/z* (relative intensity) 1211/1213/1215/1217/1219/1221/1223 [30/42/74/100/72/40/28, (M + H)].

3-[3-Bromo-4-hydroxy-5-[2,6-dibromo-4-[2-(hydroxyimino)-2-[2-[3-bromo-4-hydroxy-5-[2,6-dibromo-4-(2-aminoethyl)phenoxy]phenyl]ethylaminocarbonyl]ethyl]-phenoxy]phenyl]-2-(hydroxyimino)propionic Acid (16). To a mixture of **15** (60 mg, 0.05 mmol) in dichloromethane (2 mL) at 0 °C was added TFA (2 mL). The reaction mixture was stirred at 0 °C for 5 min and then at 24 °C for 30 min and concentrated to dryness under reduced pressure. The residue was triturated in 2 mL of water and 0.5 mL of pH 7.0 buffer. The mixture was adjusted to pH 7.0 with 1 M NaOH and stirred for 5 min. The solids were collected by filtration, washed with water (3 × 1 mL), and dried under reduced pressure to give **16** (52 mg, 95%), which was used directly for bastadin 6 synthesis.

Bastadin 6. To a solution of **16** (50 mg, 0.045 mmol) and HOBt (7 mg, 0.054 mmol) in dioxane (16 mL) and DMF (2 mL) at 0 °C was added a solution of DCC (19 mg, 0.09 mmol) in 2 mL of dichloromethane. The resulting mixture was stirred at 24 °C for 8 h. Ethyl acetate (50 mL) was added to the mixture after the solvent was partially removed under reduced pressure. The organic solution was washed with 1 M $KHSO_4$ (20 mL) and brine (2 × 20 mL), dried over $MgSO_4$, and concentrated to dryness under reduced pressure. The residue was subjected to flash chromatography (dichloromethane−acetone 19:1) to give white solid bastadin 6 (32 mg, 64%), NMR ^{1}H 1D (DMSO-d_6) and COSY (DMSO-d_6−D_2O−acetone-d_6 8:1:1) spectra were in accord with the literature data[1]; NMR (DMSO-d_6): δ ^{13}C 163.3, 163.1, 156.6, 151.4, 150.5, 146.1, 144.8, 144.7, 141.9, 141.6, 140.2, 137.7, 133.7 (2C), 133.3 (2C), 130.8, 128.2, 127.0, 126.3, 117.6 (2C), 117.2 (2C), 112.7, 111.7, 110.2, 109.8, 40.48, 38.33, 33.95, 32.75, 28.76, 27.36 ppm; MS FAB *m/z* (peak maximum mass) 1099 (M + H).

Tetramethyl Bastadin 6 (17). The structure of the synthetic bastadin 6 was further confirmed by converting it to the tetramethyl derivative **17** with methyl iodide. Bastadin 6 (20 mg) was stirred in DMF (5 mL) with K_2CO_3 (0.3 g) and methyl iodide (0.3 mL) at 24 °C for 20 h. The reaction mixture was diluted with dichloromethane (30 mL) and filtered. The filtrate was washed with 1 M HCl (15 mL), 5% $NaHCO_3$ (15 mL), 1 M HCl (15 mL), brine (15 mL), dried over $MgSO_4$, and concentrated to dryness under reduced pressure. The residue was subjected to flash chromatography (dichloromethane−acetone 19:1) to give a colorless gum **17** (10 mg). The structural analysis of **17** was in accord with the literature data;[1] NMR ($CDCl_3$): δ ^{1}H 7.53 (s, 2H), 7.46 (s, 2H), 7.19 (d, 1.8, 1H), 7.08 (d, 1.8, 1H), 6.8−6.7 (m, 2H), 6.25, (d, 1.8, 1H), 6.23 (d, 1.8, 1H), 4.06 (s, 3H), 4.03 (s, 3H), 4.02 (s, 3H), 3.85 (s, 2H), 3.72 (s, 2H), 3.61 (s, 3H), 3.6−3.4 (m, 4H), 3.0−2.6 (m, 4H) ppm; no methoxy singlet at δ 3.92 of tetramethylisobastadin 6 was detected;[1,10] MS FAB *m/z* (relative intensity) 1149/1151/1153/1155/1157/1159/1161 [22/42/80/100/82/52/20, (M + H)].

Bastadin 2. To a solution of **10** (31 mg, 0.05 mmol), 3-bromotyramine (26 mg, 0.12 mmol), and HOBt (14 mg, 0.1 mmol) in dioxane (8 mL) and DMF (1 mL) at 0 °C was added a solution of DCC (31 mg, 0.15 mmol) in 1 mL of dichloromethane. The resulting reaction mixture was stirred at 24 °C for 24 h. Ethyl acetate (40 mL) was added to the mixture after the solvent was partially removed under reduced pressure. The organic solution was washed successively with 1 M $KHSO_4$ (2 × 10 mL) and brine (2 × 10 mL), dried over $MgSO_4$, and concentrated to dryness under reduced pressure. The residue was subjected to flash chromatography (dichloromethane−acetone 19:1 to 4:1) to give a colorless gum, which was triturated in 2 mL of hexane to afford bastadin 2 (35 mg, 68%). NMR (DMSO-d_6) ^{1}H 1D and COSY spectra were in accord with the literature data[1]; δ ^{13}C 162.8, 162.7, 152.2 (2C), 151.3, 150.8, 146.2, 144.5, 141.9, 137.6, 133.2 (2C), 132.6 (2C), 131.4, 131.3, 128.7 (2C), 128.3, 126.1, 117.2 (2C), 116.1 (2C), 113.3, 110.0, 109.0 (2C), 40.35 (2C), 33.50, 33.26, 28.01, 27.70 ppm; it should be noted that the signal at δ 117.2 was not reported in the literature, which corresponds to the carbons 28 and 30 according to the nomenclature proposed by Kazlauskas[1]; MS FAB *m/z* (relative intensity) 1018/1020/1022/1024/1026/1028 [26/64/80/100/64/26, (M + H)].

Dibenzylbastadin 3 (18). To a solution of **11b** (100 mg) in dichloromethane were added HOBt (41 mg) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI HCl, 63 mg) at 0 °C, followed by 3-bromotyramine hydrochloride (77 mg), diisopropylethylamine (39 mg), and 2 mL of DMF. The reaction mixture was stirred at the same temperature for 2 h, quenched with 15 mL of water, and then extracted with 20 mL of dichloromethane. The organic extracts were washed with 20 mL of 5% $NaHCO_3$, 20 mL of

5% HCl, and 20 mL of water twice, dried over $MgSO_4$, and concentrated to dryness under reduced pressure. The residue was subjected to flash chromatography (dichloromethane−acetone 100:0 to 5:1) to afford **18** (88 mg, 57%). NMR ($CDCl_3$): δ ^{1}H 7.39 (d, 1.8, 2H), 7.20−7.18 (m, 12H), 7.01 (d, 1.8, 2H), 6.82−6.76 (m, 4H), 6.65 (t, 6.6, 2H), 5.08 (s, 4H), 3.78 (s, 4H), 3.38 (brq, 4H), 2.62, (t, 6.8, 4H) ppm; δ ^{13}C 162.4 (2C), 151.9 (2C), 151.3 (2C), 148.1 (2C), 136.4 (2C), 133.1 (2C), 132.2 (2C), 132.1 (2C), 132.0 (2C), 129.7 (2C), 129.4 (2C), 128.6 (4C), 128.3 (2C), 128.2 (4C), 125.2(2C), 116.2 (2C), 111.2 (2C), 110.1 (2C), 77.5 (2C), 40.72 (2C), 34.36 (2C), 28.80 (2C) ppm; MS FAB *m*/*z* (relative intensity) 1119/1121/1123/1125/1127 [53/67/100/82/44, (M + H)].

3-[3-Bromo-4-hydroxy-5-[3-bromo-2-hydroxy-5-[2-carboxy-2-(hydroxyimino)ethyl]phenyl]phenyl]-2-(hydroxyimino)propionic Acid (19). To a clear solution of **11b** (10 mg) in 4 mL of ethanol was added 10 mg of 10% Pd/C. The reaction mixture was stirred under an atmospheric pressure of hydrogen gas at 24 °C for 30 min. The catalyst was removed by filtration and washed with methanol. The combined filtrate was concentrated to dryness under reduced pressure to afford **19** (5.6 mg, 74%), which was used directly for bastadin 3 synthesis.

Bastadin 3 from 18. To a solution of **18** (38 mg) in 3.8 mL of thioanisole was added 812 μL of 1 M boron tribromide solution in dichloromethane. The reaction mixture was stirred at 24 °C for 30 min, quenched with 10 mL of 0.5 M HCl, and extracted with 20 mL of ethyl acetate. The organic extracts were washed with 10 mL of water twice, dried over $MgSO_4$, and concentrated to dryness under reduced pressure. The residue was triturated in hexane to afford a precipitate, which was subjected to flash chromatography (dichloromethane−acetone 100:0 to 5:1) to afford bastadin 3 (25.4 mg, 80%), NMR data (^{1}H and ^{13}C) were in accord with the literature data[1]; MS FAB *m*/*z* (relative intensity) 939/941/943/945/947 [32/78/100/78/35, (M + H)].

Bastadin 3 from 19. To a solution of **19** (5.4 mg) in 2 mL of dichloromethane were added HOBt (3.5 mg) and EDCI HCl (4.5 mg) at 0 °C, followed by 3-bromotyramine hydrochloride (5.5 mg), diisopropyl ethylamine (2.8 mg), and 0.1 mL of DMF. The reaction mixture was stirred at the same temperature for 4 h, quenched with 2 mL of water, and extracted with 5 mL of ethyl acetate. The organic extracts were successively washed with 2 mL of 5% $NaHCO_3$, 2 mL of 5% HCl, and 2 mL of water twice, dried over $MgSO_4$, and concentrated to dryness under reduced pressure. The residue was subjected to flash chromatography (dichloromethane−acetone 100:0 to 5:1) to afford bastadin 3 (6.8 mg, 73%); the analytical data were identical to the sample prepared from **18**.

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Supporting Information Available: NMR (^{1}H, ^{1}H−^{1}H COSY, ^{13}C, DEPT) and MS spectra of compounds **1b**, **2c**, **2b**, **3b**, **4b**, **4a**, **6**, **7**, **8**, **9**, **10**, **11a**, **11b**, **13**, **14**, **15**, and **18** (78 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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