Cationic Reduction of Bastadin-4 to Bastadin-5. Preparation of 5-[^{2}H]-Bastadin-5 by Site-Specific Isotopic Labeling

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A chemoselective conversion of bastadin-4 to the important Ca^{2+} channel modulator bastadin-5 (**1a**) has been achieved using cationic hydrogenation (Et_3SiH, TFA, 60%). Specifically deuterated bastadin-5 (**1b**, >95 at. %) was prepared following this method and the simplified ^{1}H NMR H-5/H_2-6 spin system of **1b** exploited to study temperature-dependent macrocyclic ring dynamics.

The natural product bastadin-5 (**1a**) first isolated from a marine sponge of *Ianthella basta* Pallas, 1766 (Ianthellidae), collected on the Great Barrier Reef,[1] is a potent agonist of the RyR1 calcium channel.[2] Compound **1a** induces opening of the Ca^{2+} channel and mobilization of Ca^{2+} stores from the sarcoplasmic reticulum (SR) at micromolar concentrations as measured by binding of [^{3}H]-ryanodine to the open state of the channel (ED_{50} ~2.3 μM). Some congeners of **1a**, but not all, also stimulate opening of the SR Ca^{2+} channel; however, the electrophysiological characteristics are dramatically different. For example, bastadin-4 (**2**, 5,6-dehydrobastadin-5, EC_{50} 14.7 μM) is somewhat less efficacious than **1a**, while bastadin-6 (**3**, EC_{50} 2.6 μM), containing six bromine atoms instead of five, has comparable activity.[3] The constitutional isomer of **1a**, bastadin-19 (**4**),[2] which arises through an alternate phenolic coupling that places both catechol units in the same hemisphere, is essentially inert (EC_{50} >100 μM). Of particular interest is bastadin-10 (**5**[4]), which mobilizes Ca^{2+} from SR stores (EC_{50} 5.8 μM) in the absence of threshold levels of Ca^{2+} (~μM),[5] thus obviating the absolute Ca^{2+} requirement for native channel opening.

Our interest in the mechanistic and electrophysiological aspects of bastadin-promoted Ca^{2+} channel gating led to a requirement for specifically tritiated bastadin-5 (**1c**). Radiolabeled **1c** would enable study of the kinetic and thermodynamic details of channel gating in finer detail. The most direct approach to **1c** appeared to be tritiation by catalytic hydrogenation of naturally occurring **2** which co-occurs in some extracts of *I. basta*. Unfortunately, the molecular structure of **2** is replete with multiple functional groups including aryl Br and ketoximes that are intolerant to hydrogenation conditions. In fact, heterogeneous catalytic hydrogenation of **2** (Pd−C, H_2, 1 atm) resulted in extensive hydrogenolysis of all aryl-Br groups and reduction of the oximes to the corresponding primary amines.[6] Even short exposures (Pd−C or Rh_2O_3 or PtO_2, 1 atm H_2 in MeOH, EtOH, or *i*-PrOH, 5−20 min) gave mainly over-reduced products or irreproducible yields of **1a**. Heterogeneous catalytic tritiation or deuteriation has drawbacks due to the possibility of "scrambling" of label or unpredictable levels of ^{3}H incorporation due to label "wash-out" in protic solvents. In fact, attempted deuteriation of **2** (D_2, 1 atm, PtO_2) gave only low yields of **1a** (<30%) with *no detectable incorporation* of deuterium.[6] Attempted homogeneous catalytic hydrogenation of **2** or the corresponding tetra-*O*-TBS ether with Wilkinson's catalyst [$(Ph_3P)_3RhCl$, H_2][7] gave no reaction, even at high pressure (>1000 psi H_2) presumably due to steric congestion at the 5,6-*trans*-double bond within the macrocycle.[6]

In anticipation of these problems we recently developed an efficient procedure for the cationic reduction of *N*-styrenyl carbamates using triethylsilane in the presence of TFA.[8] Yields for the reduction *N*-styrenyl carbamates were consistently >90%. Isotopic labeling experiments with Et_3SiD and TFA-*d* showed that the hydride equivalent was delivered exclusively from the silane to the *N*-substituted carbon of the enamine with no evidence of isotope scrambling (>99 at. % with Et_3SiD).[8] The reaction also worked with $NaBH_4$ in TFA, albeit with lower yield; however, we reasoned that the oxime groups in **1a** would be more resistant to cationic hydride reduction conditions compared with conventional metal hydride reagents. We now report an efficient conversion of **2** to **1a** using cationic reduction and its adaptation to the synthesis of 5-^{2}H-bastadin-5 (**1b**). The latter compound was prepared in >90 at. % isotopic yield and complete regioselectivity.

Samples of bastadin-4 (>95% pure) were prepared by chromatographic separation−purification of MeOH/CH_2Cl_2 extracts of *Ianthella basta*, collected in Guam, using a modification of a previously described procedure.[9] Trial reductions were carried out to determine optimum conditions for conversion and minimization of over-reduction (Table 1). Bastadin-5 dissolves readily in TFA, but Et_3SiH and TFA are immiscible liquids and the reaction proceeds under two-phase conditions. We found that the method of mixing had a critical bearing on the outcome of the reaction. Optimum conditions (entry 3, Et_3SiH, 10 equiv, vortexing) gave smooth reduction of **2** to **1a** in 20 min at room temperature on an analytical scale (69% yield of **1a**, 29% of unreacted **2**). Use of excess Et_3SiH (100 equiv) did not significantly improve the rate of reaction or yield. The rate of reduction of **2** was slower than that of more electrophilic α,β-unsaturated carbamates[8] under the same conditions. Extended reaction times resulted in lower yields and byproducts arising, presumably, by reduction of the oxime groups.

The method was applicable to milligram-scale preparation of **1a**. Thus, 4.0 mg of **2** was converted to **1a** (60% yield, 30% recovered **2**). The ^{1}H NMR spectrum and MS data (ESI negative ion, *m*/*z* 1012.6, $C_{34}H_{26}O_8N_4{}^{79}Br_5$, [M − H]$^-$) of the product were identical with those of an authentic sample of **1a** isolated from *I. basta*. Specific deuteriation of **2** was achieved under essentially the same conditions using Et_3SiD (10 equiv) to provide pure **1b** (40%). The MS and ^{1}H NMR spectra of **1b** verified incorporation of deuterium (ESIMS *m*/*z* 1013.8, [M − H]$^-$, >95 at. %).

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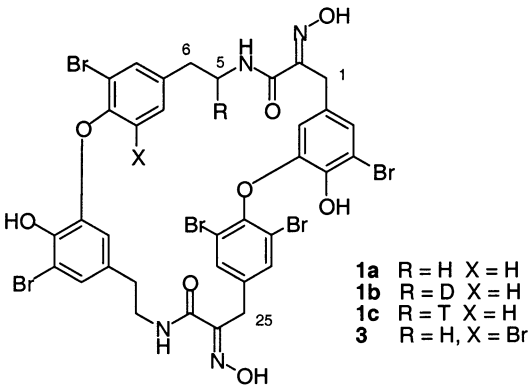
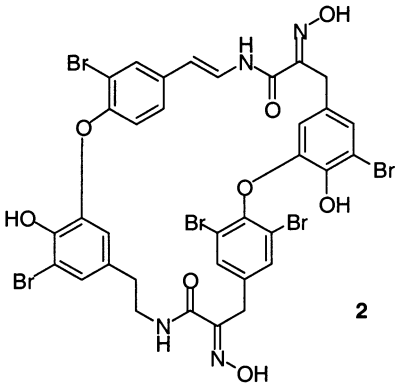


1a R = H X = H
1b R = D X = H
1c R = T X = H
3 R = H, X = Br



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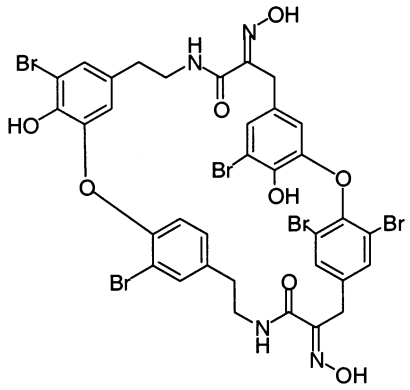
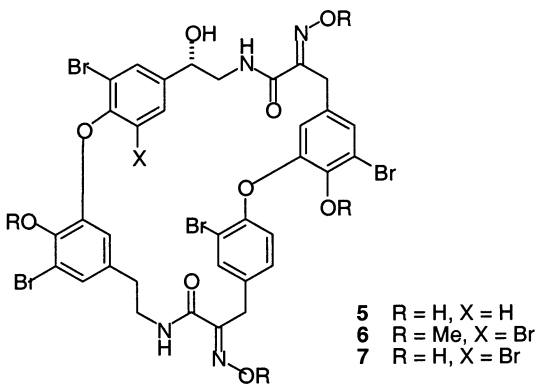


4



5 R = H, X = H
6 R = Me, X = Br
7 R = H, X = Br

Analysis of the ^{1}H NMR spectrum ($CDCl_3$)[10] of **1b** (Figure 1) confirmed the presence of one deuterium at C-5. The methylene signal, which appeared as a triplet in **1a** (δ 2.83, t, J = 5.8 Hz), had now collapsed to a doublet in **1b** (2.82, d, J = 6 Hz), while the upfield amide NH signal changed

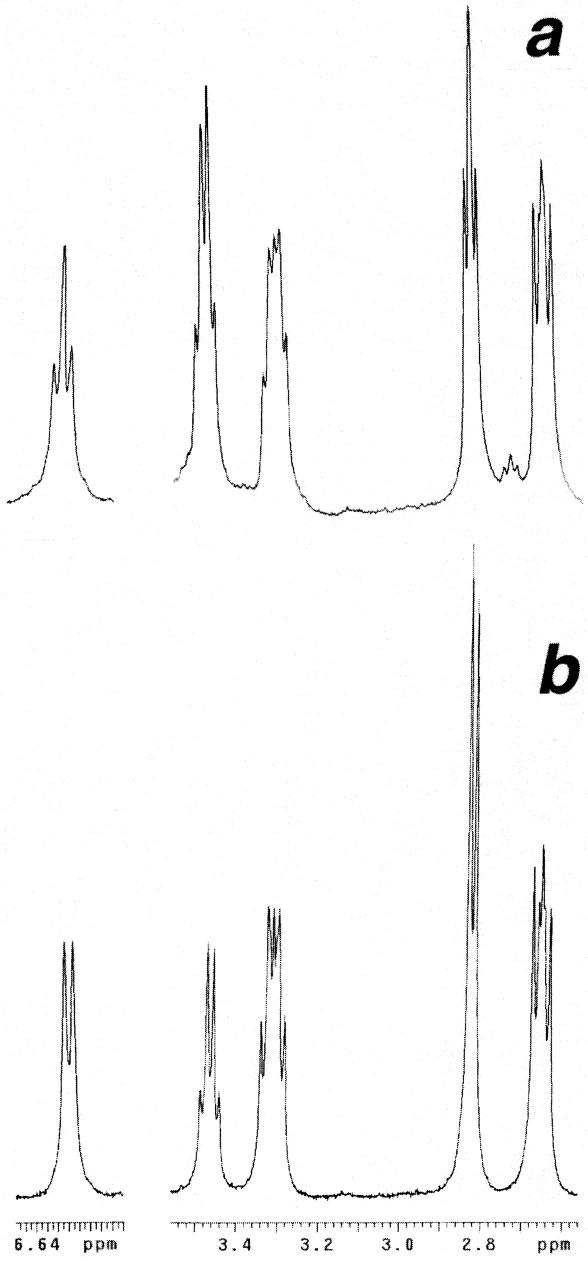


Figure 1. Partial ^{1}H NMR spectra of **1a** (a) and **1b** (b), $CDCl_3$, 23 °C.

from a triplet to a doublet (δ 6.57, d, J = 6.4 Hz), as expected. The regiospecific incorporation of only one deuterium atom at C-5 in **1b** is consistent with a mechanism that involves initial protonation at the more electron-rich C-6 (cf. Figure 1 in ref 8).

The ^{1}H NMR spectrum of **1b** displays a simplified ^{1}H NMR spin system at C-5 and C-6, which is useful in examining conformational dynamics of the bastarane macrolactam ring. Using Pople notation, the spin network at H-5/H_2-6 in **1b** approaches an A_2X system if conformational freedom allows averaging of the diastereotopic proton signals due to H_2-6, or ABX if conformational rigidity imposes a barrier to inversion of the bastadin macrocycle. The ^{1}H NMR spectrum of **1b** (CD_3OD) at room temperature revealed the expected coupling pattern for H-5/H_2-6 corresponding to rapid dynamic interconversion between two or more *gauche* rotamers. At lower temperatures (T = −30 °C) the spectrum became broader, but H-5/H_2-6 did not separate into the ABX pattern expected from restricted conformational mobility.[11] The persistence of the pattern,

Table 1. Trial Cationic Hydrogenations of **1a** with Et_3SiH and TFA

entry	Et_3SiH (equiv)	conc of **2** (mM)	temp (°C)	method of mixing[a]	time (min)	unreacted **2** (%)	yield of **1**[a] (%)
1	100	6	25	A	10	48	48[b]
2	100	5	25	A	20	26	68[b]
3	10	4	25	A	20	29[c]	69[d]
4	100	3	0	B	480	32	27[e]
5	10	5	30	C	60	0	0[f]
6	100	5	30	C	60	0	5[f]

[a] Method A: vortex solution. B: magnetic stirring, only. C: sonication. Yields were determined by HPLC analysis and are normalized against calibration standards of **1a** and **2**. Ratios of **1** to **2** were verified by ^{1}H NMR (integration). [b] Shoulder on peak for **1a** (~20%). [c] Shoulder on peak for **2** (~50%). [d] Shoulder on peak for **1a** (~2%). [e] Unidentified "shoulder" (~50%). [f] Main peak, unidentified

even at −50 °C, shows that conformational mobility is maintained in contrast to **6**, the *O*-tetramethyl derivative of bastadin-12 (**7**), where atropisomerism emerges at −30 °C.[12]

In summary, chemoselective reduction of **2** with Et_3SiH or Et_3SiD delivers **1** in good yield and recovery. Since tritiated triethylsilane (Et_3SiT) is readily obtained by reduction of Et_3SiCl with $LiBT_4$,[13,14] the foregoing reaction provides a practicable route to the preparation of useful radiolabeled **1** and its analogues from **2** and other labeled bastadins from naturally occurring 5,6-dehydro analogues such as bastadins-7[1] and -14.[15]

Experimental Section

General Experimental Procedures. Et_3SiH, Et_3SiD, and TFA were obtained from Sigma-Aldrich and used as received. ^{1}H NMR spectra were recorded at 400 MHz and referenced to residual solvent peaks (δ 7.24 and 3.30 ppm for $CDCl_3$ and CD_3OD, respectively). Trial reductions were monitored by an analytical HPLC equipped with an HP 1040 diode array detector (4.6 × 250 mm RP C_{18}, 1.0 mL/min, 68:32 MeOH/H_2O−0.05% TFA). Mass spectra were recorded on a Thermofinnigan LC Deca in ESI mode by direct infusion as solutions in MeOH/0.1% HCO_2H.

Isolation of Bastadin-5 (1a), Bastadin-4 (2), and Bastadin-6 (3) from *Ianthella basta* (ex Guam). Samples of *I. basta* (Pallas, 1766) (Ianthellidae), collected in Guam, were soaked in 1:1 $CHCl_2$/MeOH, and the filtered extracts were concentrated to a red gum (1.00 g). The residue was redissolved in MeOH, diluted with H_2O (final concentration, 10% v/v H_2O), and partitioned against *n*-hexane. The aqueous MeOH-soluble fraction (860 mg) was applied to a silica column and eluted with a gradient of solvents (EtOAc, CH_2Cl_2, MeOH/CH_2Cl_2 mixtures, and 9:6:1 CH_2Cl_2/MeOH/NH_4OH). Fractions that eluted with 10% MeOH/CH_2Cl_2 were shown to contain **1a** and **2** (TLC, ^{1}H NMR) and further purified by preparative HPLC (C_{18} reversed phase, 10 × 250 mm, 59:41 MeOH/H_2O−0.5% TFA, 3.0 mL/min) to provide pure **1a**[1] (4 mg, 0.47% of extract, rt 22 min), **2**[1] (12 mg, 1.4%, rt 19 min), and **3**[1] (2 mg, 0.2%, rt 26 min) as colorless solids. Their identities were established by comparison of their ^{1}H NMR and MS spectra with those of literature values.[1,8]

Preparation of Bastadin-5 (1a) from Bastadin-4 (2). Bastadin-4 (**2**, 4.0 mg, 3.93 μmol) was added to a vial (1 dram) fitted with a magnetic micro stir-bar, rubber septum, and a nitrogen line. Triethylsilane (6.25 μL, 39.3 μmol) and then TFA (1.0 mL) were added to the vial by syringe and the contents rapidly agitated under an atmosphere of nitrogen. The septum was replaced with a Teflon-lined cap, and the vial then vortexed for 20 min. The volatiles were removed under a stream of nitrogen with constant stirring. Toluene (1 mL) was added and removed under vacuum. This procedure was repeated twice to remove residual TFA. The vial contents were dried under high vacuum overnight to obtain the crude product (4.0 mg). Integration of the ^{1}H NMR spectrum of the product revealed a 3:1 ratio of **1a** to **2**. The product was purified by HPLC (C_{18} reversed phase, 10 × 250 mm, 63:37 MeOH/H_2O−0.5% TFA, 3.0 mL/min) to afford pure **1a** (2.4 mg, 60%) and recovered **2** (1.2 mg, 30%). ESIMS *m/z* (negative ion): $[M - H]^-$ 1012.6, 1014.7, 1016.8, 1018.7, 1020.7, 1022.7, calcd $C_{34}H_{26}O_8N_4Br_5$ 1012.77.

5-[^{2}H]-Bastadin-5 (1b). The reduction was repeated as described above except for substitution of Et_3SiD for Et_3SiH. Purification in the usual way provided labeled **1b** (40%). The ^{1}H NMR of **1b** ($CDCl_3$) is identical to that of **1a**, except for the multiplicities of signals due to NH-4, H-5, and H-6 and integration of H-5 (see Figure 1). ESIMS (negative ion): $[M - H]^-$ *m/z* 1013.8, 1015.7, 1017.7, 1019.7, 1021.7, 1023.7, calcd for $C_{34}H_{25}DO_8N_4Br_5$ 1013.7730

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(10) Bastadins, including **1a**, are only sparingly soluble in $CDCl_3$ but very soluble in CD_3OD and DMSO-d_6.
(11) A slightly different phenomenon prevailed when the temperature-dependent spectra of **1b** were measured in $CDCl_3$. At −50 °C the H-5/6 proton signals broadened slightly, but the pair of enantiotopic isolated methylene signals due to H_2-1 and H_2-25 broadened into the baseline while the aryl signals remained sharp. This is consistent with expected changes in the ^{1}H NMR spectrum at or near the coalescence temperature as the A_2 pattern for each of the latter CH_2 signals transforms into a diastereotopic AB spin system (Friebolin, H. *Basic One- and Two-Dimensional NMR Spectroscopy*; VCH: Weinheim, 1991; pp 344). While this is consistent with slowed macrolactam dynamic exchange, we cannot discount other processes (e.g., aggregation).
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