An Efficient Protocol for the Synthesis of Unsymmetrical Pyrazines. Total Synthesis of Dihydrocephalostatin 1[1]

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Cephalostatin 1 (**1**)[2] is a potent member of a family of thirty trisdecacyclic pyrazines isolated from the marine tube worm *Cephalodiscus gilchristi*, by Pettit at Arizona State University, and from the tunicate *Ritterella tokioka*, by Fusetani at the University of Tokyo.[3] Cephalostatin 1 (**1**, Figure 1) has been forwarded for clinical trials in Europe, but testing has been stalled because of severe difficulty in harvesting this scarce material using SCUBA operations at 60−80 m in the white shark-infested waters off East Africa.[4]

Recently, we reported the first total synthesis of cephalostatin 7,[5] an unsymmetrical steroidal pyrazine, via a biomimetic approach in which the pyrazine ring was constructed by a statistical coupling of north and south α-amino ketosteroids (produced *in situ* from the corresponding α-azido ketosteroids). While our synthesis was useful in probing several biological questions, the strategy adopted was intrinsically incapable of providing an efficient source of the unsymmetrical (north−south) coupling product since both the alternative modes (north−north and south−south) of coupling also occurred. In principle, the solution to this problem already exists, since Smith and Heathcock[6] have demonstrated that heating of α-amino methoximes **5** with α-acetoxy ketones **6** in two stages at 90 °C (24 h) and 145 °C (24 h) provides unsymmetrical pyrazines **11**−**2** and **11**−**3** in 29 and 43%, respectively. In practice, however, this strategy is compromised both by the yields for preparation of the acceptor ketone **6** and by those for the key coupling step. Nevertheless, the Berkeley group's concept of using α-amino methoxime **5** as an imine progenitor, which fosters the aromatization in the absence of an additional oxidation, constitutes an important contribution (Scheme 1).[7]

As can be seen from Scheme 2, the seemingly trivial substitution of α-azido ketone **4** in place of α-acetoxy ketone **6** as the acceptor partner for imine formation has two important consequences. The first of these simply relates to better overall yield. We have recently found that tetramethylguanidinium

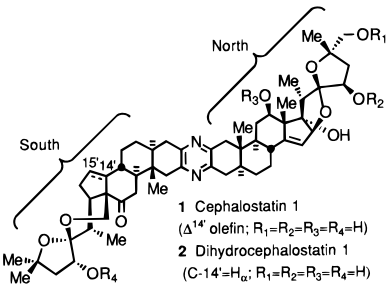
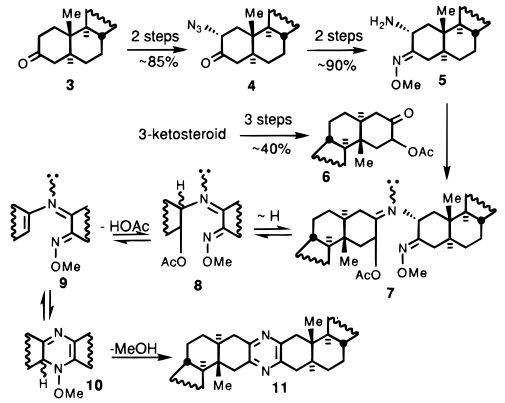
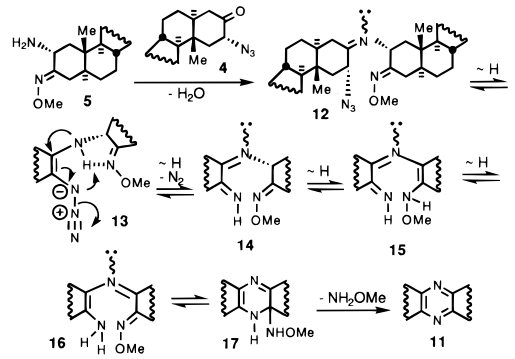


Figure 1.

Scheme 1



Scheme 2



azide (TMGA) in nitromethane[5,8] effects transformation of α-bromo ketones to α-azido ketones without the complication of competitive base-catalyzed decomposition to α-amino enones. *The more important difference pertains to an expected change of mechanism in the key coupling reaction.* Assuming initial formation of intermediate imine **12**, prototropic equilibration would provide enamine **13** which is exquisitely suited for fragmentation to bis-imine **14**. Conversion of **14** to the unsymmetrical pyrazine **11** is illustrated with intermediates **14**−**17** although many variations of this theme are possible (Scheme 2). While the mechanism is currently under investigation, preliminary observations indicate that the reaction produces N_2 gas and the medium becomes basic through the production of methoxyamine. If the azido moiety of **12** was simply serving

(1) Cephalostatin synthesis 10. For additional syntheses of cephalostatin-related pyrazines, see: (a) Pan, Y.; Merriman, R. L.; Tanzer, L. R.; Fuchs, P. L. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 967. (b) Kramer, A.; Ullmann, U.; Winterfeldt, E. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2865. (c) Reference 6.

(2) Pettit, G. R.; Inoue, M.; Kamano, Y.; Herald, D. L.; Arm, C.; Dufresne, C.; Christie, N. D.; Schmidt, J. M.; Doubek, D. L.; Krupa, T. S. *J. Am. Chem. Soc.* **1988**, *110*, 2006.

(3) (a) Pettit, G. R.; Xu, J.; Schmidt, J. M.; Boyd, M. R. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2027 and references cited therein. (b) Fukuzawa, S.; Matsunaga, S.; Fusetani, N. *Tetrahedron* **1995**, *51*, 6707 and references cited therein.

(4) Pettit reports that ~0.5 ton of the organisms yielded approximately 100 mg of cephalostatin 1 (**1**); ~1 g of material is required for the initial phases of the trials. Professor G. R. Pettit, personal communication, 1994.

(5) Jeong, J. U.; Sutton, S. C.; Kim, S.; Fuchs, P. L. *J. Am. Chem. Soc.* **1995**, *117*, 10157.

(6) (a) Smith, S. C.; Heathcock, C. H. *J. Org. Chem.* **1992**, *57*, 6379. (b) Heathcock, C. H.; Smith, S. C. *J. Org. Chem.* **1994**, *59*, 6828.

(7) It should be noted that many varients of the mechanism are possible and one shown by the Purdue group simply represents a working hypothesis inspired by the Weinreb pyridine synthesis. (a) Subramanyam, C.; Noguchi, M.; Weinreb, S. M. *J. Org. Chem.* **1989**, *54*, 5580. (b) Levin, J. I.; Weinreb, S. M. *J. Org. Chem.* **1984**, *49*, 4325.

(8) (a) Li, C.; Arasappan, A.; Fuchs, P. L. *Tetrahedron Lett.* **1993**, *34*, 3535. (b) Li, C.; Shih, T.-L.; Jeong, J. U.; Arasappan, A.; Fuchs, P. L. *Tetrahedron Lett.* **1994**, *35*, 2645.

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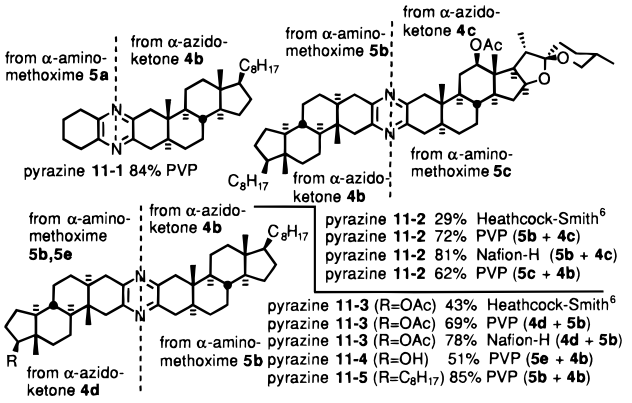


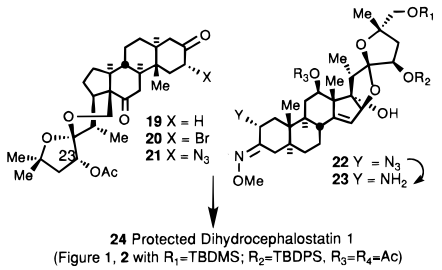
Figure 2.

as a leaving group as in the Heathcock−Smith synthesis, then hydrazoic acid would be produced and the solution would become *acidic*.

In the event, an equimolar mixture of α-azido ketones **4** and α-amino methoximes **5** along with an equal mass of either polyvinylpyridine (PVP) or the fluorinated sulfonic acid resin Nafion-H in a 0.02 M benzene solution containing 10 mol % of dibutyltin dichloride[9] was set for azeotropic distillation for 7−12 h to provide the unsymmetrical pyrazines **11**−**(1**−**5)** in the yields shown in Figure 2. Reactions without the indicated additives produced the target pyrazines in much inferior yields. Since both halves of the unsymmetrical pyrazines are ultimately generated from α-azido ketones, two independent coupling reactions are easily tested for each target. For example, it can be seen that the synthesis of pyrazine **11**−**2** is accomplished in better yield using the combination of α-amino methoxime **5b** and α-azido ketone **4c** rather than α-amino methoxime **5c** and α-azido ketone **4b**.

The above protocol was successfully employed to synthesize dihydrocephalostatin 1 (**2**). The known south hexacyclic diketone C_{23} alcohol **18** (not shown)[10] was converted to acetate **19** (cat. DMAP (4-(dimethylamino)pyridine), Ac_2O, NEt_3, CH_2Cl_2, 25 °C, 1 h, 99%) followed by treatment with phenyltrimethylammonium tribromide (1.1 equiv) in THF (6 min, 25 °C) to afford α-bromo ketone **20** in 70% yield. Subsequent reaction of **20** with TMGA in nitromethane[5,8] provided α-azido ketone **21** in 78% yield. Construction of the north α-amino methoxime **23** was accomplished in two steps by reaction of the north α-azido ketone (not shown)[5] with methoxyamine hydrochloride (2 equiv in 1:10 C_5H_5N/CH_2Cl_2, 0 to 25 °C, 4 h) to give α-azido methoxime **22** (99%) followed by reduction with triphenylphosphine (2 equiv in 3% aqueous THF for 24 h at 25 °C) to provide α-amino methoxime **23** (80% yield). The key coupling reaction involved heating an equimolar mixture of **21** (12 mg) and **23** (20 mg) in benzene in the presence of 10 mol % of dibutyltin dichloride and 32 mg of PVP at 0.02 M in a flask equipped with a Dean−Stark trap set for azeotropic distillation.[11] The reaction was stopped after 5 h to provide a 51% yield of **24** (75% based on recovered **23**). A 0.01 M

Scheme 3



solution of **24** in THF with 2.3 equiv of TBAF (tetrabutylammonium fluoride) was heated at reflux for 2 h to effect cleavage of the two silyl groups. The THF was evaporated, and the residue heated for 0.5 h in 8:1 methanol/water in the presence of 4 equiv of K_2CO_3 to afford dihydrocephalostatin 1 (**2**, 83%). The proton and carbon NMR spectra of **2**[12] closely resembled those for cephalostatin 1 (**1**)[2], with the north portion essentially superimposable and the South unit consistent with its dihydro functionality (Scheme 3).

Dihydrocephalostatin 1 (**2**) and natural cephalostatin 1 (**1**) were comparatively evaluated in the U.S. National Cancer Institute (NCI)'s *in vitro* human tumor cell line screen.[13,14] Each compound was tested in triplicate at each of three different concentration ranges (10^{-5}, 10^{-6}, and 10^{-7} M upper limits; five $\log_{10}$-spaced concentrations in each range) against the entire group of 60 cell lines comprising the NCI screening panel. The synthetic compound **2** produced a highly characteristic "cephalostatin-like" differential cytotoxicity profile[15] essentially indistinguishable from that of the natural reference compound **1** (*compare* correlation coefficients[14] ≥0.9). Moreover, the panel-averaged cytotoxic potency of **2** closely approximated that of **1** (mean panel GI_{50} values[14] of (4.09 ± 2.22) and (2.02 ± 0.81) × 10^{-9} M, respectively), placing it among the most potent cephalostatins known thus far. The synthetic accessibility and potent *in vitro* biological activity of **2** make it a potential candidate for *in vivo* antitumor evaluation. Contrary to earlier presumptions, by us[16] and by others,[6b] about the need of the south D-ring olefin moiety for the biological activity of **1**, it is clearly not a prerequisite for high activity. Whether such an assumption is valid for the north D-ring olefin functionality remains to be proven.

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Supporting Information Available: Key experimentals and ^1H and ^{13}C NMR of all new compounds (32 pages). See any current masthead page for ordering and Internet access instructions.

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(9) Dibutyltin dichloride is known to promote imine formation: Stetin, C.; de Jeso, B.; Pommier, J. C. *Synth. Commun.* **1982**, *12*, 495.
(10) Bhandaru, S.; Fuchs, P. L. *Tetrahedron Lett.* **1995**, *36*, 8351.
(11) In the synthesis of **2**, we elected to employ PVP as the additive since the north and south spiroketals are sensitive to acid.
(12) Silica gel R_f = 0.22 in 7% MeOH/CH_2Cl_2; $[\alpha]_D$ = +80° (*c* 0.04, MeOH); see Supporting Information for spectra.
(13) Boyd, M. R. In *Current Therapy in Oncology*; Neiderhuber, J. E., Ed.; B. C. Decker: Philadelphia, PA, 1993; pp 11−22.
(14) Boyd, M. R.; Paull, K. D. *Drug Dev. Res.* **1995**, *34*, 91.
(15) Pettit, G. R.; Xu, J.; Williams, M. D.; Christie, N. D.; Doubek, D. L.; Schmidt, J. M.; Boyd, M. R. *J. Nat. Prod.* **1994**, *57*, 52.
(16) Bhandaru, S.; Fuchs, P. L. *Tetrahedron Lett.* **1995**, *36*, 8347.