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Synthesis of (±)-Eusynstyelamide A

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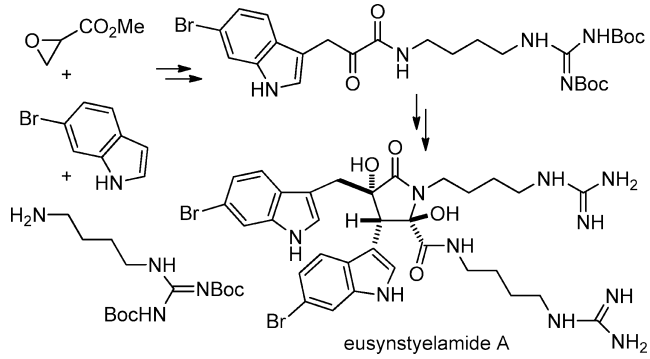
ABSTRACT



The synthesis of (±)-eusynstyelamide A has been accomplished in six steps in 13% overall yield from 6-bromoindole, methyl glycidate, and Boc-protected agmatine. If oxygen is carefully excluded from the reaction, the key NaOH-catalyzed aldol dimerization of the α-ketoamide proceeded efficiently to give Boc-protected eusynstyelamide A.

Tapiolas and co-workers recently reported the isolation of eusynstyelamides A (**1**), B (**2**), and C (**3**) from the Great Barrier Reef ascidian *Eusynstyela latericius* and assigned their structures from analysis of the spectral data (see Figure 1).[1] The spectral data for **1** are virtually identical to those reported for eusynstyelamide (**4**), isolated from *E. misakiensis*,[2] indicating that the structure of **4** should be reassigned as **1**, rather than the acyclic ketone dihydrate. Eusynstyelamides A−C (**1**−**3**) inhibit neuronal nitric oxide synthase (nNOS), with IC_{50} values of 41.7, 4.3, and 5.8 μM, respectively, and show slight antibiotic activity against *Staphylococcus aureus*.

We were intrigued by these structures because the dihydroxybutyrolactam core is identical to that of anchinopeptolide D (**7b**), which we synthesized several years ago (see Scheme 1).[3] α-Ketoamide **5** underwent an aldol dimerization on treatment with KOH in THF/MeOH. The amide nitrogen of the initially formed aldol adduct cyclized to the remaining ketone to form a dihydroxybutyrolactam. The phenol acetate was also hydrolyzed under these reaction conditions. We isolated three of the four possible diastereomeric products. The major product was anchinopeptolide D precursor **7a**, which was formed in 58% yield. Lactam **6a**, which was isolated in 19% yield, has the opposite stereochemistry at the hemiaminal center, but was formed from the same aldol adduct **10** (see Scheme 2) as **7a**. The third product **8a**, which was isolated in <5% yield, was formed from the diastereomeric aldol adduct. Cleavage of the four Boc groups of **6a** and **7a** in 1:1 TFA/CH_2Cl_2 for 1 h at 25 °C afforded **6b** (94%) and anchinopeptolide D (**7b**, 91%), respectively.

The stereochemistry of the major aldol adduct **10** can be rationalized by consideration of a chelated transition state for the aldol reaction. Enolization should give the *Z*-enolate to avoid steric interactions between the amide and the R^1 substituent. Transition state **9**, which leads to aldol adduct

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(2) Swersey, J. C.; Ireland, C. M.; Cornell, L. M.; Peterson, R. W. *J. Nat. Prod.* **1994**, *57*, 842–845.

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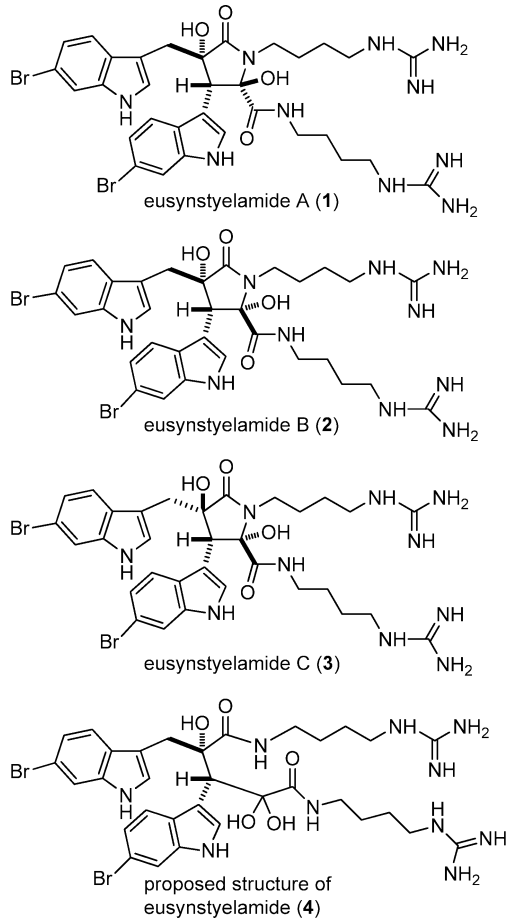


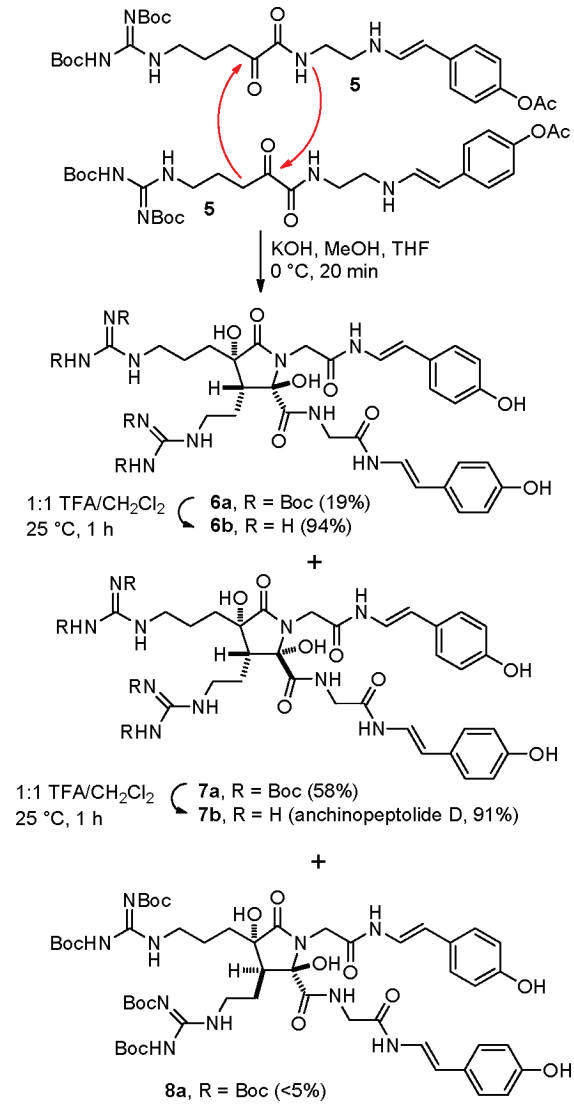
Figure 1. Structures of eusynstyelamides A (**1**), B (**2**), and C (**3**) and the proposed structure of eusynstyelamide (**4**).

10, might be favored for the aldol reaction since the metal can bind to all four oxygens. The amide nitrogen of adduct **10** can then add to either face of the ketone carbonyl group to give products **6** or **7**.

As expected, equilibration occurred readily at the hemiaminal center. Heating either **6a** or **7a** at reflux in CD_3OD for 1 h afforded an equilibrium 2:1 mixture of **6a** and **7a**, presumably by ring opening to give the aldol adduct **10**. Equilibration of either **6a** or **7a** with KOH in THF/MeOH for 17 h at 25 °C afforded a 6:3:1 mixture of **6a**, **7a**, and **8a**, respectively. Isomer **8a** can be formed from **6a** and **7a** by a retro-aldol/aldol sequence or by base-catalyzed epimerization of **10**.

The synthesis of eusynstyelamide A (**1**) is shown in Scheme 3. 6-Bromoindole (**11**) was prepared by the Batcho−Leimgruber indole synthesis from 4-bromo-2-nitrotoluene.[4] Coupling of **11** with methyl glycidate (**12**)[5] by

Scheme 1. Synthesis of Anchinopeptolide D (**7b**)



the procedure of Ōmura[6] using 0.16 equiv of $Yb(OTf)_3$ at 100 °C for 15 min in a microwave oven or at reflux for 1.5 h afforded the desired indolepropionate ester **13a** in 32% yield (56% based on recovered **11**) in only one step. Ōmura reported that reaction of indole, methyl glycidate, and $Yb(OTf)_3$ (0.1 equiv) in 1,2-dichloroethane at 80 °C for 24 h afforded 52% of the hydroxyester. The reaction with 6-bromoindole (**11**) proceeded in lower yield, even with more $Yb(OTf)_3$, because the bromine is electron withdrawing and makes the indole less nucleophilic.[7] Hydrolysis of the ester of **13a** with LiOH in 3:1:1 MeOH/THF/H_2O afforded

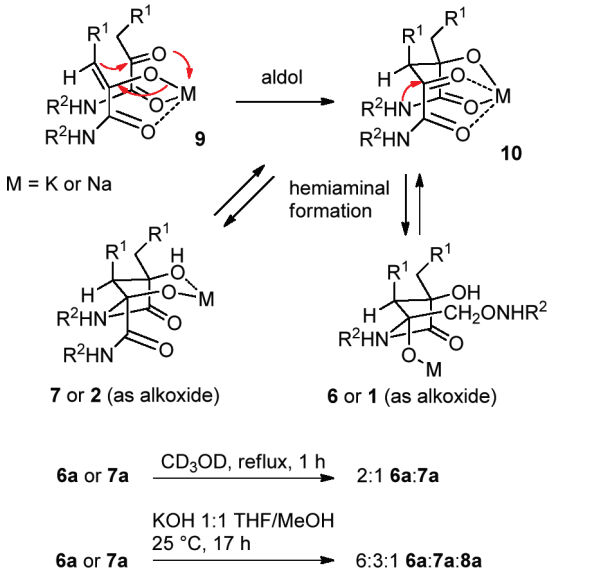
(4) (a) Schumacher, R. W.; Davidson, B. S. *Tetrahedron* **1999**, *55*, 935–942. (b) Konda-Yamada, Y.; Okada, C.; Yoshida, K.; Umeda, Y.; Arima, S.; Sato, N.; Kai, T.; Takayanagi, H.; Harigaya, Y. *Tetrahedron* **2002**, *58*, 7851–7861.

(5) Stevenson, C. P.; Nielsen, L. P. C.; Jacobsen, E. N. *Org. Synth.* **2006**, *83*, 162–169.

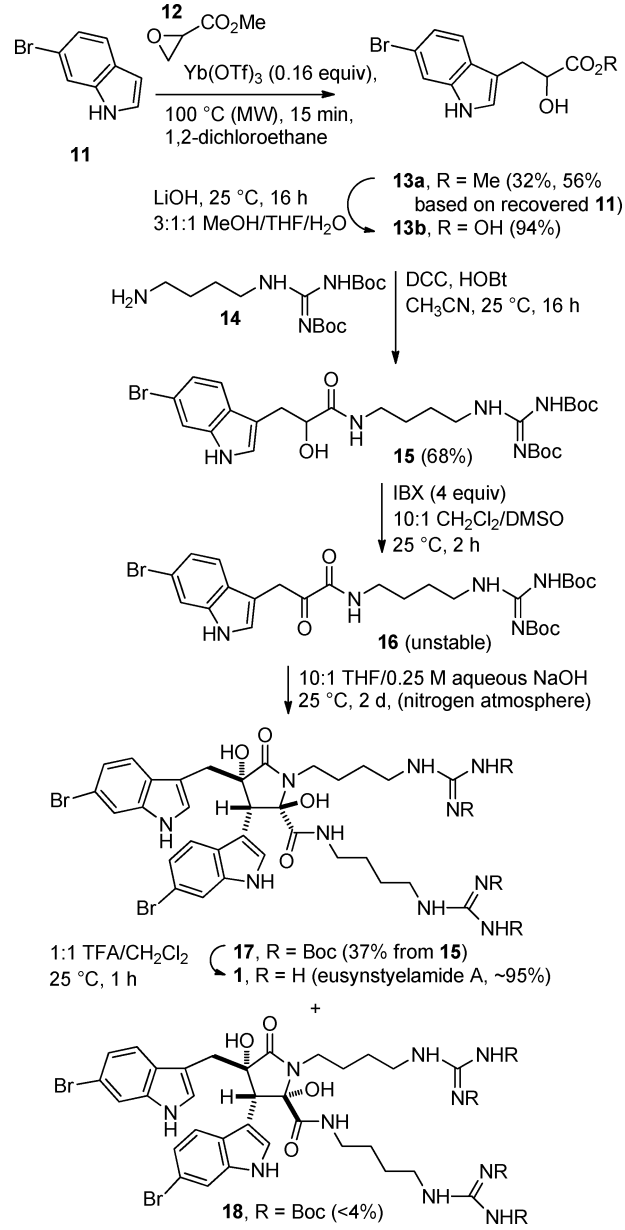
(6) (a) Sunazuka, T.; Shirahata, T.; Tsuchiya, S.; Hirose, T.; Mori, R.; Harigaya, Y.; Kuwajima, I.; Ōmura, S. *Org. Lett.* **2005**, *7*, 941–943. (b) Tsuchiya, S.; Sunazuka, T.; Shirahata, T.; Hirose, T.; Kaji, E.; Ōmura, S. *Heterocycles* **2007**, *72*, 91–94.

(7) Indole (with a nucleophilicity $N = 5.55$) reacted with styrene oxide in trifluoroethanol at 80 °C for 4 h to give the alkylation product β-phenyl-1*H*-indole-3-ethanol in 67% yield. Under the same conditions, 5-bromoindole ($N = 4.38$) gave the alkylation product in only 45% yield after 72 h. The reaction of 6-bromoindole was not reported. See: Westermaier, M.; Mayr, H. *Chem.—Eur. J.* **2008**, *14*, 1638–1647.

Scheme 2. Mechanistic Considerations and Equilibration Studies



Scheme 3. Synthesis of Eusynstyelamide A (**1**)



hydroxyacid **13b** in 94% yield. Coupling of acid **13b** with protected agmatine **14**[8] using DCC and HOBt in CH_3CN for 16 h at 25 °C afforded hydroxyamide **15** in 68% yield. Removal of dicyclohexylurea from **15** was difficult, but initial attempts at amide formation with either EDCI and HOBt or HATU and Et_3N were not promising. α-Ketoamide **5** was prepared by oxidation of the hydroxyamide with Dess−Martin reagent. This procedure did not work well for the conversion of **15** to **16**. Use of PCC or TEMPO and NaOCl was also unsuccessful. Finally, we found that oxidation[9] of **15** with IBX[10] in 10:1 CH_2Cl_2/DMSO afforded **16** cleanly. α-Ketoamide **16** is surprisingly unstable, decomposing on flash chromatography, in contrast to α-ketoamide **5** which is stable.

Initial attempts at carrying out the aldol dimerization of **16** with K_2CO_3 in acetone,[11] DBU in DMF, or KOH in THF or MeOH were unsuccessful, again in contrast to the facile aldol dimerization of **5**. There are significant differences between arylpyruvic acid derivatives such as **16** and saturated α-ketoacid derivatives such as **5**. Saturated α-ketoacid derivatives exist only in the keto form. Conjugation with the aryl group stabilizes the enol form of arylpyruvic acid derivatives. Methyl indol-3-ylpyruvate exists as the keto form in CD_3OD and mainly as the enol in $CDCl_3$.[12] Methyl phenylpyruvate exists as the enol in $CDCl_3$ and as mixture of enol and keto forms in CD_3CN.[13] Other methyl arylpyruvates exist mainly in the enol form in MeOH.[14] Simpkins reported that amide formation from an indol-3-ylpyruvic acid was presumably thwarted by the intervention of the enol form of the α-keto acid.[15] In contrast, α-ketoamide **16** exists in the keto form, as has been previously noted for other arylpyruvamides,[16] possibly because intramolecular hydrogen bonding between the ketone and amide NH stabilizes the keto form **16K** (see Scheme 4).

(8) Sarabia, F.; Sánchez-Ruiz, A.; Chammaa, S. *Bioorg. Med. Chem.* **2005**, *13*, 1691–1705.
(9) (a) Frigerio, M.; Santagostino, M. *Tetrahedron Lett.* **1994**, *35*, 8019–8022. (b) Frigerio, M.; Santagostino, M.; Sputore, S.; Palmisano, G. *J. Org. Chem.* **1995**, *60*, 7272–7276. (c) De Munari, S.; Frigerio, M.; Santagostino, M. *J. Org. Chem.* **1996**, *61*, 9272–9279.
(10) Frigerio, M.; Santagostino, M.; Sputore, S. *J. Org. Chem.* **1999**, *64*, 4537–4538.
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(12) Bachmann, S.; Knudsen, K. R.; Jørgensen, K. A. *Org. Biomol. Chem.* **2004**, *2*, 2044–2049.
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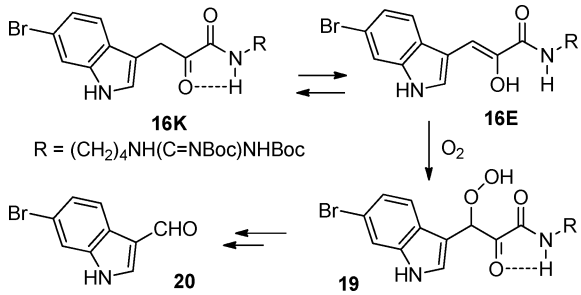
(15) Frebault, F.; Simpkins, N. S.; Fenwick, A. *J. Am. Chem. Soc.* **2009**, *131*, 4214–4215.
(16) González, J. F.; de la Cuesta, E.; Avendaño, C. *Tetrahedron* **2008**, *64*, 2762–2771.

Scheme 4. Autoxidation of α-Ketoamide **16**



However, the enol form **16E** is readily accessible, and it, or the enolate, is easily oxidized by oxygen to 3-hydroperoxy-3-(indol-3-yl)pyruvamide **19**, especially under the basic conditions needed for the aldol dimerization. The mass spectrum of fresh **16** shows a strong M^+. After storage, the sample showed an M^+ and an MO_2^+ for the hydroperoxide of equal intensity. There is limited precedent for this facile autoxidation in the literature. Autoxidation of 3-(4-hydroxy-3-iodophenyl)pyruvic acid gave 3-(4-hydroxy-3-iodophenyl)-2-hydroxy-3-hydroperoxycinnamic acid (the enol tautomer of the 3-hydroperoxyarylpyruvic acid).[17] Radical-initiated autoxidation of the enol tautomer of 3-(4-hydroxyphenyl)pyruvic acid provided 3-hydroperoxy-3-(4-hydroxyphenyl)pyruvic acid.[18] Hydroperoxide **19** decomposed to give a complex mixture of products including 6-bromoindole-3-carboxaldehyde (**20**), a natural product[19] that may be derived by a similar mechanism from 6-bromotryptophan.

Having established that the unexpected sensitivity of **16** to oxygen was causing side reactions, especially under basic conditions, we were able to successfully carry out the desired aldol dimerization under carefully controlled oxygen-free conditions. A solution of **16** in THF was carefully degassed, and aqueous 0.25 M NaOH solution was added. The solution was stirred for 2 days under nitrogen to give Boc-protected eusynstyelamide A **17** in 37% yield from **15** and impure Boc-protected eusynstyelamide B **18** in <4% yield from **15**. Cleavage of the four Boc groups of **17** in 1:1 TFA/CH_2Cl_2 for 1 h at 25 °C afforded eusynstyelamide A (**1**) in ∼95% yield. The ^1H and ^{13}C NMR spectra of synthetic **1** in CD_3OD and the ^1H NMR spectrum in DMSO-d_6 are identical to those reported for the natural product.[1]

Protected anchinopeptolide D **7a**, which has the same stereochemistry as eusynstyelamide B (**2**), readily equilibrates with **6a**, which has the same stereochemistry as eusynstyelamide A (**1**), in CD_3OD at reflux for 1 h (see Scheme 2). In contrast, Tapiolas and co-workers reported that eusynstyelamide A (**1**) does not equilibrate on heating in CD_3OD or during prolonged storage. They also isolated mixtures of all three isomers **1**−**3** from some samples of *E. latericius*, but only eusynstyelamide A (**1**) from other samples. Ireland reported the isolation of only a single compound, to which he assigned the structure eusynstyelamide (**4**), but which is actually eusynstyelamide A (**1**).[1] Therefore, the formation of eusynstyelamide A precursor **17** as the major isomer from **16** is consistent with the isolation of **1** as the sole isomer from some samples of *E. latericius* and from *E. misakiensis*. Eusynstyelamide A (**1**) does not equilibrate under the strongly acidic conditions needed to cleave the Boc groups. It is also does not equilibrate on storage in CD_3OD. Extensive decomposition occurs in basic (NaOD) CD_3OD.

Protected anchinopeptolide D **7a** and **6a** equilibrate readily, but eusynstyelamide A (**1**) and B (**2**) do not. The aldol dimerization of both **16** and **5** gave adduct **10** with high selectivity. However, hemiaminal formation afforded mainly eusynstyelamide A precursor **17** from **16** and mainly anchinopeptolide D precursor **7a**, which has the same stereochemistry as eusynstyelamide B (**2**), from **5**. The different side chains must be responsible for the differences in both the rates of equilibration and the stereochemistry of hemiaminal formation, but it is not clear why.

In conclusion, we have developed an efficient six-step route to eusynstyelamide A (**1**) from 6-bromoindole (**11**), methyl glycidate (**12**), and protected agmatine **14** in 13% overall yield. If oxygen is carefully excluded from the reaction, the key NaOH-catalyzed aldol dimerization of **16** proceeded efficiently to give Boc-protected eusynstyelamide A **17**.

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Supporting Information Available: Complete experimental procedures, comparison of the NMR spectral data of natural and synthetic eusynstyelamide A, and copies of ^1H and ^{13}C NMR spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

OL100896N

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