

DOI: 10.1002/ange.200602050

Stereocontrolled Total Synthesis of Bengazole A: A Marine Bisoxazole Natural Product Displaying Potent Antifungal Properties***James A. Bull, Emily P. Balskus, Richard A. J. Horan, Martin Langner, and Steven V. Ley**

Numerous oxazole-containing natural products have been isolated from marine sources in recent years^[1] and have been the subject of extensive synthetic investigation.^[2] A significant subsection of these natural products are bisoxazoles. In these cases, the two oxazole rings may be well separated, for example in phorboxazole^[3] and the disorazoles,^[4] or are directly linked as bisoxazole cores, such as in the hennoxazoles,^[5] the diazonamides,^[6] and in muscoride A.^[7]

The bengazoles, on the other hand, are unique in that the two oxazole rings flank a single carbon atom. In bengazole A (**1**), this C10 position carries a myristate ester unit, which creates a sensitive isolated stereogenic center (Scheme 1). Moreover, the left hand oxazole ring is a biogenically unusual 5-monosubstituted ring while the central 2,4-disubstituted oxazole ring links to a stereochemically dense tetraol side chain.

Bengazole A was originally isolated in 1988 by Crews and co-workers from a sponge of the genus *Jaspis*,^[8] and to date the bengazole family consists of 22 members.^[9,10] The structure and absolute stereochemistry of this highly functionalized molecule was determined by a combination of NMR studies, analysis of degradation products, and circular dichroism.^[8,10] The compound displays potent ergosterol-dependant antifungal activity comparable to that of amphotericin B, against *Candida albicans*,^[11,12] and is active against fluconazole-resistant *Candida* strains.^[13] Furthermore, bengazole A shows full anthelmintic activity against nematode *Nippostrongylus braziliensis* at just 50 $\mu\text{g mL}^{-1}$.^[8]

There has been one reported previous synthesis of bengazole A (**1**), by Molinski and co-workers,^[13] which used a regioselective metalation/addition strategy starting from 1,3-oxazole.^[14,15] However, this gave a 1:1 mixture of the C10

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[**] We would like to thank Novartis for a Novartis Research Fellowship (S.V.L.), the EPSRC for a studentship (J.A.B.) and a postdoctoral fellowship (R.A.J.H.), the Winston Churchill Foundation of the USA for a scholarship (E.P.B.), and the DAAD for a postdoctoral fellowship (M.L.).



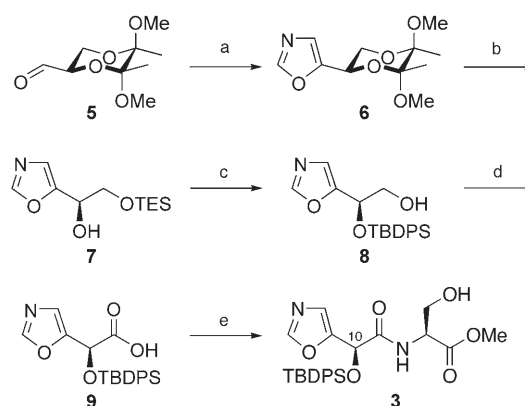
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epimers, and once the resulting bisoxazole unit was in place, the C10 epimers could not be separated at any stage of the synthesis. Shioiri and co-workers achieved a synthesis of deacylbengazole^[16] that was stereochemically enriched at the C10 position through an enantioselective reduction of a bisoxazole ketone which resulted in *ee* values up to 68%.^[17]

Owing to the interesting biological profile and the challenging structural components, we devised a synthetic route to **1** that would provide both a single stereoisomer of the natural product and also allow for analogues to be prepared. We envisaged installing the C10 stereogenic center early in the synthesis, prior to construction of the bisoxazole unit. This somewhat risky strategy would require retention of stereochemical integrity at the C10 center during the formation of the central oxazole ring and on to the end of the synthesis. The desired bisoxazole unit would be derived from the diastereomerically pure serine amide **3** (Scheme 1), which in turn would be converted into a suitable precursor for a diastereoselective nitrile oxide 1,3-dipolar cycloaddition with alkene **4**, which contains a diol protected as a butane-2,3-diacetal (BDA). Cycloaddition product **2**, which contains the complete carbon backbone of **1**, would then be elaborated to reveal the tetraol side chain to complete the total synthesis.

BDA-protected diols have been used extensively by our research group as stable chiral building blocks.^[18] The synthesis of amide fragment **3** relied upon stereocontrol derived from using our BDA-protected glyceraldehyde derivative **5**^[18d] as a substrate for a Schöllkopf-type oxazole synthesis.^[19] Treatment of aldehyde **5** with TosMIC and K₂CO₃ provided the desired 5-substituted oxazole **6** in excellent yield (Scheme 2). Oxazole **6** was isolated exclusively as the equatorially substituted diastereomer, hence efficiently establishing the desired stereochemistry at the C10 center for the natural product. Removal of the BDA group and manipulation of silyl protecting groups afforded the mono-TBDPS-protected diol **8**, with all reactions proceeding in excellent yields. Oxidation of the primary alcohol **8** proved to be difficult and was only achieved on using the Jones reagent, which provided carboxylic acid **9** in moderate yield. Subsequent amide coupling performed in DMSO gave the desired amide **3** as a single diastereomer, thus showing there was no loss of stereochemical integrity during the preceding steps.^[20]

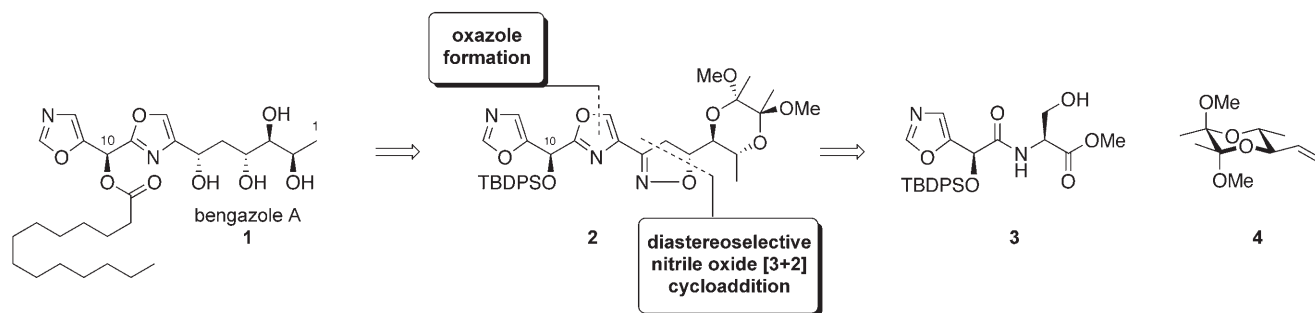
Having gained rapid access to amide **3** as a single diastereomer, the key challenge in this synthesis was the construction of the central 2,4-substituted oxazole ring under



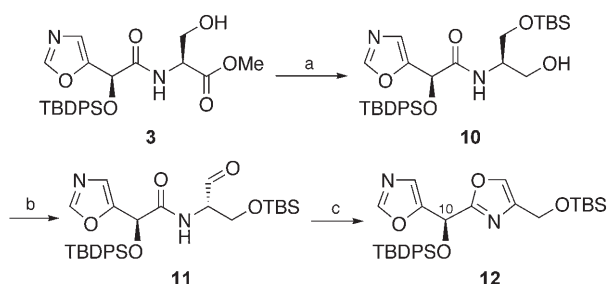
Scheme 2. Synthesis of amide fragment **3**. a) TosMIC, K₂CO₃, MeOH, reflux, 82%; b) 1. TFA, H₂O, RT; 2. TESCl, *i*Pr₂NEt, CH₂Cl₂, –78 °C, 94% over 2 steps; c) 1. TBDPSCI, Et₃N, DMAP, CH₂Cl₂, RT; 2. PPTS MeOH, RT, 96% over two steps; d) Jones reagent, acetone, 0 °C, 49%; e) TBTU, H-(L)-Ser-OMe-HCl, *i*Pr₂NEt, DMSO, RT, 64%. TosMIC = toluene-4-sulfonylmethylisocyanide, TFA = trifluoroacetic acid, TES = triethylsilyl, DMAP = 4-dimethylaminopyridine, PPTS = pyridinium-*p*-toluenesulfonate, TBTU = 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate, DMSO = dimethylsulfoxide.

mild conditions in the presence of the sensitive C10 stereogenic center. Initial attempts to form this oxazole ring from serine amide **3** included: 1) oxazoline formation^[21] followed by oxidation,^[22] and 2) using conditions modified from those reported by Shin and co-workers^[23] and involving dehydrative elimination followed by activation using *N*-bromosuccinimide, cyclization, and elimination. Although these approaches afforded the desired product, they proceeded in poor yields and caused significant racemization of the bisoxazole product.

We found that serine amide **3** could be converted into primary alcohol **10** in two steps (Scheme 3). Oxidation using buffered Dess–Martin periodinane^[24] provided aldehyde **11**, which was subjected to Robinson–Gabriel-type oxazole formation under conditions reported by Panek and Beresis,^[25] similar to those described previously by Wipf et al.^[26] Treating the crude aldehyde **11** with PPh₃, 2,6-di-*tert*-butylpyridine, and dibromotetrachloroethane in CH₂Cl₂ to form the intermediate bromooxazoline, followed by the addition of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to eliminate HBr,



Scheme 1. Structure and retrosynthetic analysis of bengazole A (**1**). TBDPS = *tert*-butyldiphenylsilyl.

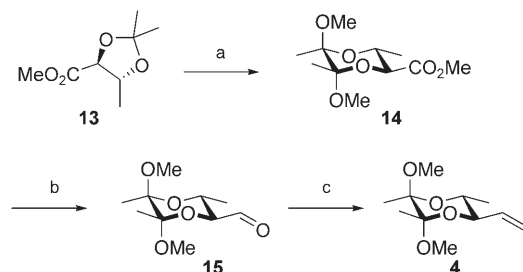


Scheme 3. Synthesis of bisoxazole **12**: a) 1. TBSCl, imidazole, DMAP, DMF, RT, 90%; 2. NaBH₄, LiCl, THF, MeOH, 0°C to RT, 80%; b) Dess–Martin periodinane, NaHCO₃, CH₂Cl₂, 0°C; c) PPh₃, C₂Br₂Cl₄, CH₂Cl₂, 2,6-di-*tert*-butylpyridine, 0°C, then Et₃N, MeCN, 0°C to RT, 89% over 2 steps. TBS = *tert*-butyldimethylsilyl.

provided the desired 2,4-disubstituted oxazole **12** in 72% yield over the two steps.

However, the product obtained from this process still displayed significant racemization (33–66% *ee*)^[27] at the crucial C10 center. The cause of this was identified as the DBU, used to eliminate the proposed bromooxazoline intermediate.^[26b] Replacing DBU with Et₃N completely prevented this racemization and also improved the yield over the two steps from alcohol **10** to an excellent 89%. Bisoxazole **12** was thus obtained with greater than 98% *ee* and the process was amenable to scale-up.^[28,29] We believe this to be a potentially useful general modification for the formation of oxazole rings adjacent to highly epimerizable stereogenic centers.

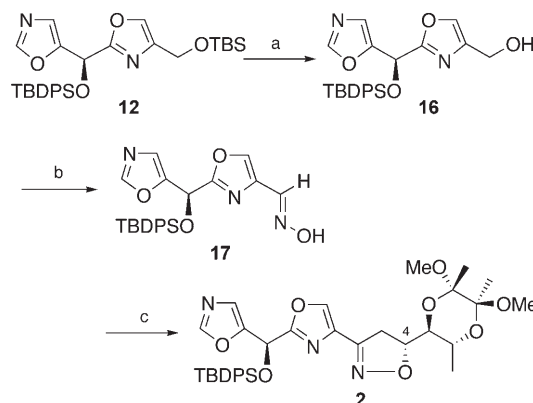
With the enantiopure bisoxazole unit in hand, we turned our attention to the alkene fragment. Alkene **4** was readily synthesized in 71% yield over four steps from the commercially available acetonide **13** (Scheme 4). Transacetalization using CSA and 2,3-butanedione in methanol at reflux introduced the BDA protecting group as a 6:1 mixture of anomerically and non-anomerically stabilized products, which were then converted exclusively into the fully stabilized isomer **14** by using BF₃·THF. Reduction of ester **14** with DIBAL-H provided aldehyde **15** in 86% yield as a crystalline solid, and Wittig methylenation formed the required alkene **4** in quantitative yield. This synthesis was readily scalable through easily handled crystalline intermediates, which con-



Scheme 4. Synthesis of alkene **4**: a) 1. 2,3-butanedione, CSA, trimethylorthoformate, MeOH, reflux; 2. BF₃·THF, CH₂Cl₂, RT, 83% over two steps; b) DIBAL-H, CH₂Cl₂, –78°C, 86%; c) Ph₃PCH₃Br, KHMS, THF, –78°C to RT, quant. CSA = camphorsulfonic acid, DIBAL-H = diisobutylaluminum hydride, KHMS = potassium hexamethyldisilazide.

trasts significantly with the synthesis of the simple acetonide-protected alkene derived from ester **13**; the known intermediate five-ring acetonide aldehyde^[30] is prone to polymerization and the corresponding alkene is difficult to handle because of its volatility. Furthermore, we hoped the embedded chirality and rigidity of alkene **4** would provide enhanced side-chain diastereoselectivity in the planned dipolar cycloaddition.

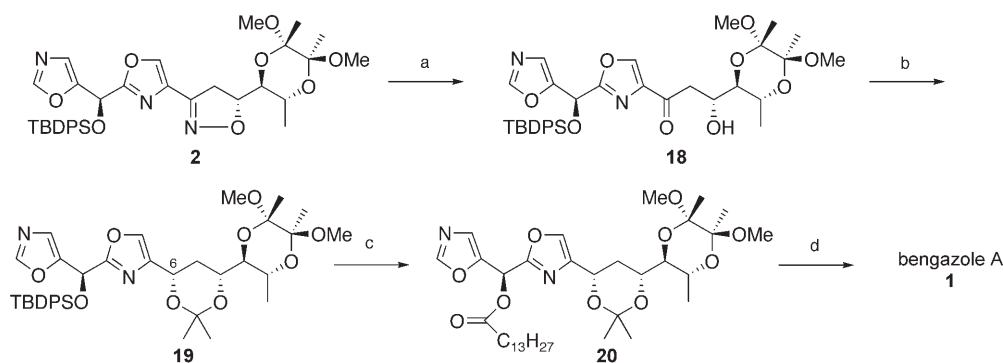
Bisoxazole **12** was next converted into oxime **17** in readiness for the 1,3-dipolar cycloaddition (Scheme 5). This



Scheme 5. Synthesis of isoxazoline **2**: a) PPTS, MeOH, RT, 93%; b) 1. Dess–Martin periodinane, NaHCO₃, CH₂Cl₂, 0°C; 2. NH₂OH·HCl, K₂CO₃, MeOH, H₂O, RT, 93% over two steps; c) 1. NCS, CH₂Cl₂, pyridine, RT, 2. alkene **4**, Cs₂CO₃, DME, RT, 77% over two steps, (d.r. 7:2). NCS = *N*-chlorosuccinimide, DME = 1,2-dimethoxyethane.

transformation proceeded in three steps, under mild conditions, with an average yield of 95%, and with complete retention of stereochemical integrity. The required nitrile oxide was generated from oxime **17** in two stages. Firstly, the oxime was chlorinated by treatment with NCS, and then Cs₂CO₃ was added to a solution of the chlorooxamic acid in DME in the presence of alkene **4**. The resulting 1,3-dipolar cycloaddition provided isoxazoline **2** in 60% yield of the major diastereomer.^[31] The stereochemistry at C4 of the major cycloadduct is that found in the natural product and the result is consistent with that predicted by the “inside alkoxy” model reported by Houk et al.^[32] The minor diastereomer (isolated in 17% yield) was easily removed by column chromatography and will be used in the studies towards analogues of **1**.

With the carbon skeleton complete, cycloadduct **2** was then elaborated to the natural product. Reductive cleavage of isoxazoline **2** using Raney nickel and boric acid in ethanol/water^[33] gave β-hydroxy ketone **18** in 61% yield, which was subjected to a *syn*-selective reduction using diethylmethoxyborane and sodium borohydride (Scheme 6). Protection of the resulting diol (d.r. 14:1 by NMR spectroscopy) as acetonide **19** occurred in 89% yield over the two steps, with the minor C6 diastereomer being removed at this stage. Removal of the TBDPS group and installation of the myristoyl side chain proceeded in excellent yield to afford the acetal-protected bengazole A **20**. Acidic deprotection



Scheme 6. Completion of the synthesis of bengazole A (**1**): a) Raney nickel, H₂, B(OH)₃, EtOH, H₂O, 61%; b) 1. Et₃BOMe, NaBH₄, THF, −78 °C, (d.r. 14:1); 2. 2,2-dimethoxypropane, TsOH, 89% over 2 steps; c) 1. TBAF, THF, 0 °C, quant.; 2. myristoyl chloride, NEt₃, CH₂Cl₂, 0 °C, 85%; d) TFA, H₂O, RT, 95%. Ts = toluene-4-sulfonic acid, TBAF = tetrabutylammonium fluoride.

using TFA/water removed both the acetonide and the BDA groups from **20** in 95 % yield to complete the total synthesis of bengazole A (**1**) in an overall yield of 3.4 %. The data for our synthetic material were consistent with those reported for the natural product, and ¹HNMR spectroscopic analysis confirmed our product to be a single C10 epimer.^[8,13,34–36]

In conclusion, we have completed a stereocontrolled total synthesis of bengazole A by using an oxazole synthesis under mild conditions and a diastereoselective 1,3-dipolar cycloaddition. Our route is the first to provide a single stereoisomer of the product and relies upon BDA building blocks to establish the stereogenic centers. Ongoing work in our laboratories is directed toward the synthesis of other members of the bengazole family and toward the synthesis of analogues for studies on structure–activity relationships.

Received: May 23, 2006

Revised: July 31, 2006

Published online: September 20, 2006

Keywords: antifungal agents · cycloaddition · heterocycles · natural products · total synthesis

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- [20] The yield of the amide coupling to form amide **3** is highly dependent on the quality of carboxylic acid **9**, which is unstable and decomposes on storage. A more practical route on a large scale generates acid **9** through hydrolysis of the equivalent non-enantiopure methyl ester. The coupling with the enantiopure serine methyl ester resolves the acid into diastereomers, which are easily separated by column chromatography to provide amide **3** as a single stereoisomer.
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- [28] Using Et₃N in place of 2,6-di-*tert*-butylpyridine from the start of the reaction again provided bisoxazole **12** as a single enantiomer. However, these conditions gave a yield of only 47% and a reduced reaction time, thus suggesting a different reaction pathway.
- [29] The *ee* value was determined by supercritical fluid chromatography on a chiral stationary phase following conversion into the primary alcohol **16** (Berger Minigram, Chiralcel AS column with 7% MeOH/CO₂, flow rate = 3 mL min⁻¹ at 100 bar, 35 °C, λ = 210 nm, retention times: (*S*)-C10 isomer 8.66 min, (*R*)-C10 isomer 9.08 min).
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- [34] Molinski and co-workers report that the ¹H NMR spectra of the C10 epimers are very similar apart from the C15 (α -CH₂ on ester) and C1 proton signals (see reference [13]). These signals show no such splitting in the ¹H NMR spectra of our synthetic material.
- [35] ¹H NMR (600 MHz, CD₃OD): δ = 0.89 (3H, t, *J* = 7.4 Hz, H27), 1.15 (3H, d, *J* = 6.4 Hz, H1), 1.28 (20H, m, H17 to H26), 1.63 (2H, t, *J* = 7.0 Hz, H16), 1.90 (1H, ddd, *J* = 14.1, 9.2, 7.1 Hz, H5'), 2.23 (1H, ddd, *J* = 14.1, 6.9, 2.5 Hz, H5), 2.43 (2H, t, *J* = 7.3 Hz, H15), 3.18 (1H, dd, *J* = 6.8, 3.4 Hz, H3), 3.67 (1H, ddd, *J* = 9.2, 6.8, 2.5 Hz, H4), 3.92 (1H, dq, *J* = 6.4, 3.4 Hz, H2), 4.91 (1H, dd, *J* = 7.1, 6.9 Hz, H6), 7.11 (1H, s, H10), 7.31 (1H, s, H12), 7.85 (1H, s, H8), 8.25 ppm (1H, s, H13); ¹³C NMR (150 MHz, CD₃OD): 14.4 (C27), 19.9 (C1), 23.7 (C26), 25.8 (C16), 30.8–30.0, 33.1 (C17 to C25), 34.5 (C15), 40.5 (C5), 62.9 (C10), 66.3 (C6), 67.8 (C2), 71.2 (C4), 78.8 (C3), 127.5 (C12), 138.0 (C8), 145.7 (C7), 147.7 (C11), 154.4 (C13), 159.7 (C9), δ = 173.4 ppm (C14); $[\alpha]_D^{25}$ = +6 (*c* = 0.175, CH₃OH); HRMS (ESI) for C₂₇H₄₅N₂O₈: *m/z* calcd for $[M+H]^+$: 525.3176, found: 525.3163.
- [36] We observed no racemization at the C10 atom on storage of synthetic benzazole A under argon at –20 °C over a period of one month.