ORGANIC LETTERS

2010
Vol. 12, No. 3
420−423

Total Synthesis and Absolute Configuration of (−)-Berkeleyamide A

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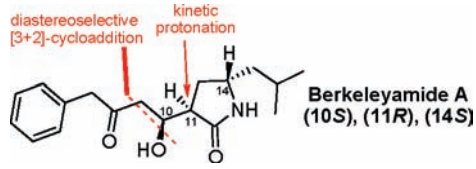
Received November 1, 2009

ABSTRACT

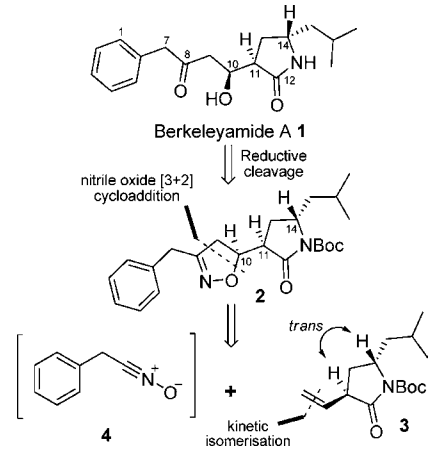


A chiral-pool approach to (−)-berkeleyamide A 1 based on a diastereoselective nitrile oxide [3 + 2]-cycloaddition completes the first total synthesis establishing the absolute stereochemistry of the natural product.

The Berkeley Pit Lake was formed in 1982 when mining in the Berkeley open-pit copper mine in Butte, Montana was abandoned. At present, the 1.5 mile long and 1800-foot deep lake is part of the largest United States EPA Superfund site in North America and consists of over 30 billion gallons of acidic (pH 2.0), metal-laden water.[1] The lake has subsequently proven to be a rich source of biota that thrive under hostile conditions, and the secondary metabolites[2] derived from these extremophilic organisms provide an unusual source of bioactive molecules with medicinal potential.[3]

One such molecule is berkeleyamide A **1**, a novel metabolite recently isolated from a *Penicillium rubrum* Stoll species from a water sample taken from a depth of 885 feet.[4] Berkeleyamide A **1** was subsequently shown to possess low micromolar activity against caspase-1[5] and matrix metalloproteinase-3 (MMP-3).[6] A total synthesis of berkeleyamide A is essential due to its medicinal potential and for structure confirmation; the absolute stereochemistry was not unambiguously determined in the isolation report.[4] Furthermore, the planned bioremediation of Berkeley Pit Lake would eliminate the natural source of this compound.

Scheme 1. Proposed Synthesis of Berkeleyamide A **1**



(1) http://www.mbmg.mtech.edu/env/env-berkeley.asp (last accessed 10/09).

(2) For the previous isolation of secondary metabolites from this lake, see: (a) Stierle, A. A.; Stierle, D. B.; Parker, K.; Goldstein, E.; Bugni, T.; Baarson, C.; Gress, J.; Blake, D. *J. Nat. Prod.* **2003**, *66*, 1097. (b) Stierle, D. B.; Stierle, A. A.; Hobbs, J. D.; Stokken, J.; Clardy, J. *Org. Lett.* **2004**, *6*, 1049. (c) Stierle, A. A.; Stierle, D. B.; Kemp, K. *J. Nat. Prod.* **2004**, *67*, 1392. (d) Stierle, A. A.; Stierle, D. B.; Kelly, K. *J. Org. Chem.* **2006**, *71*, 5357. (e) Stierle, D. B.; Stierle, A. A.; Patacini, B. *J. Nat. Prod.* **2007**, *70*, 1820.

(3) Wilson, Z. E.; Brimble, M. A. *Nat. Prod. Rep.* **2009**, *26*, 44.

(4) Stierle, A. A.; Stierle, D. B.; Patacini, B. *J. Nat. Prod.* **2008**, *71*, 856.

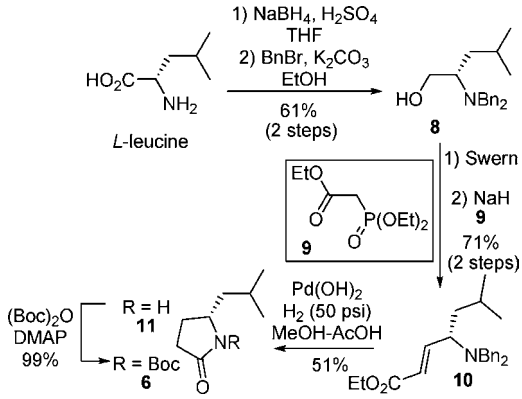
(5) (a) Matter, H.; Schudok, M. *Curr. Opin. Drug Discovery Dev.* **2004**, *7*, 513. (b) Whittaker, M.; Floyd, C. D.; Brown, P.; Gearing, A. J. H. *Chem. Rev.* **1999**, *99*, 2735. (c) Overall, C. M.; Kleifeld, O. *Nat. Rev. Cancer* **2006**, *6*, 227.

10.1021/ol902525k
Published on Web 12/23/2009

Our initial retrosynthetic analysis of berkeleyamide A assumed that the natural product originates from L-leucine. The synthesis itself hinges on a diastereoselective [3 + 2]-cycloaddition between chiral alkene **3** and nitrile oxide **4**.

Equipped with the knowledge that the dipolarophile component dictates the stereochemical course of [3 + 2]-cycloadditions,[7] it was anticipated that the chiral alkene **3** (C11) would exert significant stereocontrol upon the newly formed C10 stereocenter. Therefore, successful cycloaddition would afford an isoxazoline **2** which upon reductive cleavage would deliver the 1,3-ketoalcohol present in the natural product. This convergent strategy relies on the L-leucine stereocenter providing an anchor on which the absolute stereochemistry of the entire structure is established (Scheme 1).

Scheme 2. Synthesis of Lactam **6**



Our first consideration was to devise a scalable route to lactam **6**. Thus, $NaBH_4-H_2SO_4$ mediated reduction[8] of L-leucine followed by dibenzylation delivered **8**,[9] which was subjected to Swern oxidation followed by Horner-Wadsworth-Emmons olefination with phosphonate **9** affording ester **10**.[10] Next, hydrogenation of **10** under acidic conditions effected lactamisation, delivering the 2-pyrrolidinone **11**[11] in moderate yield. Smooth Boc-protection then afforded the key lactam **6**[12] (Scheme 2).

Initial thoughts for the synthesis of alkene **3** from lactam **6** were straightforward; it was assumed formylation of **6** followed by Wittig olefination would provide a facile route to alkene **3**. However, upon surveying the literature it became apparent that 2-pyrrolidinone-3-carbaldehydes often exist as mixtures of keto−enol tautomers, and reports detailing

(6) Caspase-I is a cysteine protease with an essential mediator role in inflammation, see: Ghayur, T.; Banerjee, S.; Hugunin, M.; Butler, D.; Herzog, L.; Carter, A.; Quintal, L.; Sekut, L.; Talanian, R.; Paskind, M.; Wong, W.; Kamen, R.; Tracey, D.; Allen, H. *Nature* **1997**, *386*, 619.
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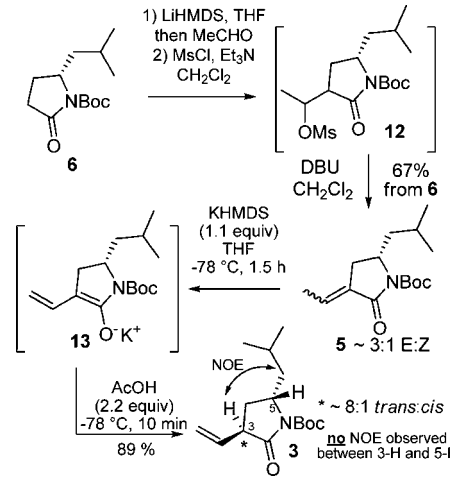
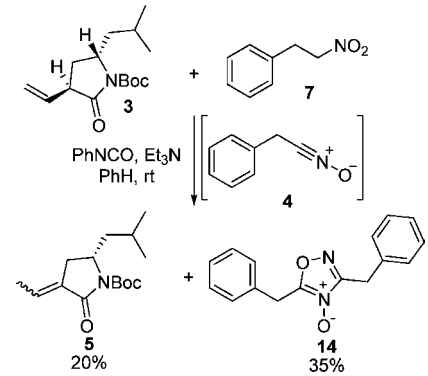
(10) Concellón, J. M.; Méjica, C. *Eur. J. Org. Chem.* **2007**, 5250.
(11) Craven, A. P.; Dyke, H. J.; Thomas, E. J. *Tetrahedron* **1989**, *45*, 2417.
(12) Smrcina, M.; Majer, P.; Majerovà, E.; Guerassina, T. A.; Eissenstat, M. A. *Tetrahedron* **1997**, *53*, 12867.

Scheme 3. Kinetic Protonation



difficulties in their manipulation are not uncommon.[13] A kinetic isomerization route[14] was therefore deemed more practical (Scheme 3). Thus, generation of the enolate of lactam **6** with lithium hexamethyldisilazide followed by alkyation with acetaldehyde and subsequent mesylation delivered the diastereomeric mesylates **12**. Elimination with DBU afforded the conjugated internal alkenes **5** as a 3:1 mixture of regioisomers. The key kinetic protonation was then effected using potassium hexamethyldisilazide followed by careful quenching of the extended enolate **13** with acetic acid as the sterically unencumbered proton source. Gratifyingly, the terminal alkene **3** was isolated as an 8:1 mixture of *trans*:*cis* isomers, as determined by a clear absence of NOE correlation between 3-H and 5-H and a strong NOE correlation between 3-H and the CH_2 of the leucine side chain (Scheme 3, also see Supporting Information).

Scheme 4. Failed Cycloaddition



With the alkene **3** in hand, attention turned to the key cycloaddition step. Initially, we sought to employ the dehydration of a nitroalkane precursor **7** to generate the nitrile oxide **4**,[15] based on a similar strategy to that reported by Kozlowski during recent studies toward the spiroacetal

Scheme 5. Cycloaddition

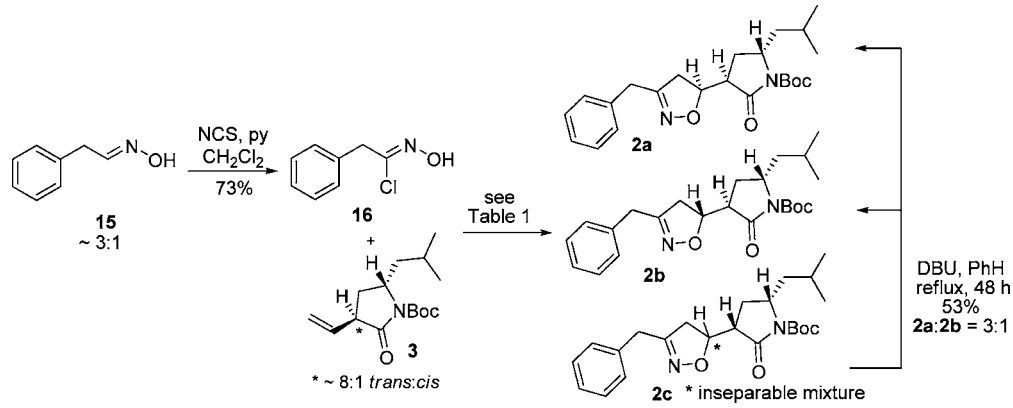


Table 1. Cycloaddition Conditions

entry	conditions	reaction time[a] (h)	temp	solvent	*dr* (**2a**:**2b**:**2c**)	yield (%)
1	**16** (2 equiv) and **3**, add Et_3N (2 equiv) over 15 min	7	−78 °C	CH_2Cl_2	1.9:1:0	23
2	**16** (2 equiv) and **3**, add Et_3N (2 equiv) over 2 h	7	−78 °C	CH_2Cl_2	2.3:1:0	27
3	**3** and Et_3N (2 equiv), add **16** (2 equiv) over 2 h	5	−78 °C	CH_2Cl_2	2.1:1:0	25
4	**3** and Et_3N (2 equiv), add **16** (2 equiv) over 4 h	3	−78 °C	CH_2Cl_2	1.5:1:0	12
5	**3** and Et_3N (2 equiv), add **16** (2 equiv) over 10 min	5	−45 °C	CH_2Cl_2	2:1:1.2	49
6	**16** (2 equiv) and **3**, add Et_3N (2 equiv) over 3 h	2	−45 °C	CH_2Cl_2	3:1.5:1	48
7	**16 (3 equiv) and 3, add Et_3N (3 equiv) over 3 h**	**2**	**−45 °C**	**CH_2Cl_2**	**2.1:1:1**	**53**
8	**16** (3 equiv) and **3**, add Et_3N (3 equiv) over 3 h	2	−45 °C	toluene	2.5:1:1	35
9	**16** (2 equiv) and **3**, add $(Bu_3Sn)_2O$ (1 equiv) over 5 min	5	−45 °C	CH_2Cl_2	3:1:0	17
10	**16** (3 equiv) and **3**, add $(Bu_3Sn)_2O$ (1.5 equiv) over 2 h	7	−45 °C	CH_2Cl_2	2.5:1:1.5	22

[a] After addition of final reactant described in 'conditions'.

moiety of the rubromycins.[16] However, treatment of a solution of nitroalkane[17] **7** and alkene **3** with phenyl isocyanate and catalytic quantities of triethylamine disappointingly effected isomerization of the alkene **3** and rapid homodimerization of the nitrile oxide to the novel oxadiazole *N*-oxide **14**. Inverse addition of the base and varying the equivalents of the reagents had no effect on the outcome of this reaction (Scheme 4).

From these preliminary studies it became clear that there were two problems—the nitrile oxide **4** was undergoing facile homodimerization at room temperature and the alkene **3** was undergoing rapid isomerization, even in the presence of catalytic quantities of base. It was therefore decided to generate the nitrile oxide **4** by dehydrohalogenation of a hydroxyiminoyl halide,[18] a transformation that can be conducted at lower temperatures hopefully suppressing homodimerization as well as the undesired isomerization of alkene **3**.

Thus, the revised cycloaddition was conducted as follows (Scheme 5). Treatment of oxime **15**[19] with *N*-chlorosuccinimide and a catalytic quantity of pyridine afforded the relatively unstable iminoyl chloride **16** after a short silica gel column.[20] Addition of freshly prepared alkene **3** and triethylamine to **16** at −78 °C gratifyingly afforded a mixture of two separable diastereomeric adducts **2a** and **2b**, albeit in poor yield (Entry 1, Table 1).

It was assumed that the cycloadducts **2a** and **2b** predominantly adopt a conformation wherein the C_{10}-O bond of the isoxazoline ring is antiperiplanar to the carbonyl group of the pyrrolidine ring thereby minimizing unfavorable dipole−dipole interactions. A strong NOE correlation between the H10 and H11 in cycloadduct **2b** clearly established the H10−H11 *syn* stereochemistry whereas the absence of a similar NOE correlation in cycloadduct **2a** established the H10−H11 *anti* stereochemistry in this case. The difference

(13) (a) Battersby, A. R.; Westwood, S. W. *J. Chem. Soc., Perkin Trans. 1* **1987**, 1679. (b) Tamura, N.; Matsushita, Y.; Iwama, T.; Harada, S.; Kishimoto, S.; Itoh, K. *Chem. Pharm Bull.* **1991**, *39*, 1199. (c) Katoh, T.; Nagata, Y.; Kobayashi, Y.; Arai, K.; Minami, J.; Terashima, S. *Tetrahedron* **1994**, *50*, 6221.

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(16) (a) Waters, S. P.; Fennie, M. W.; Kozlowski, M. C. *Tetrahedron Lett.* **2006**, *47*, 5409. (b) Waters, S. P.; Fennie, M. W.; Kozlowski, M. C. *Org. Lett.* **2006**, *8*, 3243.

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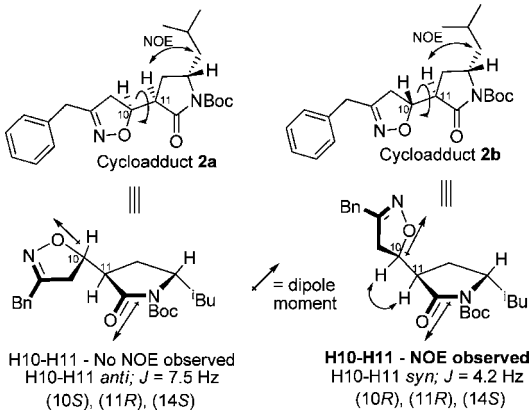
(18) Kozikowski, A. P.; Adamczyk, M. *J. Org. Chem.* **1983**, *48*, 366.

(19) Kabalka, J. W.; Gaudgaon, N. M. *Synth. Commun.* **1988**, *18*, 693.

(20) Quadrelli, P.; Mella, M.; Ivernizzi, A. G.; Caramella, P. *Tetrahedron* **1999**, *55*, 10497.

in magnitude of the observed vicinal $J_{10,11}$ coupling constants (7.5 Hz in **2a**, 4.2 Hz in **2b**) also supported this stereochemical assignment of the newly formed isoxazoline ring, with NOE also supporting the H11−H14 *trans*-relationship across the lactam (Scheme 6, also see SI).

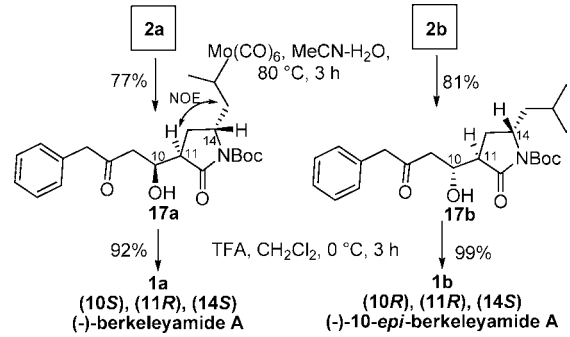
Scheme 6. NOE Correlations in Cycloadducts **2a** and **2b**



Slow addition of the alkene **3** had a negligible effect on yield and diastereoselectivity (entry 2), as did addition of the iminoyl chloride **16** to the alkene **3** and base (entries 3 and 4). Increasing the reaction temperature to −45 °C increased the yield significantly as well as producing an inseparable, third set of diastereomers **2c** (entries 5−7), presumably arising from successful cycloaddition of the minor *cis*-lactam obtained during the isomerization step. Nonetheless, epimerization of the diastereomers **2c** with DBU in boiling benzene delivered a further batch of **2a** and **2b** in moderate yield as a 3:1 mixture. Overall, the best results were obtained from the slow addition of alkene **3** and iminoyl chloride **16** at −45 °C, giving a 53% yield of the cycloadducts **2a**−**c** (entry 7). Conducting the reaction in toluene resulted in a reduction in yield (entry 8), as did the use of bis(tributyltin) oxide[21] as the dehalogenating agent (entries 9 and 10).

Next, the key reductive cleavage was conducted. A suspension of $Mo(CO)_6$ and **2a** in wet acetonitrile[22] was heated to 80 °C for 3 h, delivering the desired keto alcohol **17a** in good yield. Cycloadduct **2b** succumbed to the same reaction conditions delivering keto alcohol **17b**, also in good yield. The Boc group was removed from **17a** and **17b** with TFA delivering **1a** and **1b**, respectively (Scheme 7).

Scheme 7. Synthesis of (−)-Berkeleyamide A **1a** and (−)-10-*epi*-Berkeleyamide A **1b**



Comparison of the ^{1}H and ^{13}C NMR spectra of **1a** and **1b** with the spectroscopic data provided[4] for natural berkeleyamide A clearly showed that **1a** was identical in all aspects, whereas **1b** showed several significant differences (also see Supporting Information). This comparison conclusively established the previously unassigned relative stereochemistry of berkeleyamide A.

Next, we set out to assign the absolute stereochemistry of berkeleyamide A **1** using an authentic sample of the natural product. Co-injection of synthetic **1a** and the natural sample resulted in a single peak being observed by chiral-HPLC (see Supporting Information). Also, in our hands the optical rotations of natural $[\alpha]_D^{20}$ −15.2 (*c* 0.11, MeOH) and synthetic $[\alpha]_D^{20}$ −15.5 (*c* 0.11, MeOH) samples were in excellent agreement. We therefore conclude that the absolute stereochemistry of berkeleyamide A **1** is (10*S*), (11*R*), (14*S*) and **1** is thus derived from L-leucine.

In conclusion, we have completed a chiral-pool based total synthesis of (-)-berkeleyamide A **1** using a diastereoselective [3 + 2]-cycloaddition, thus assigning the absolute stereochemistry of the natural product.

Acknowledgment. We thank the Royal Society of New Zealand Marsden fund for financial support and Donald and Andrea Stierle (University of Montana) for providing an authentic sample of **1**.

Supporting Information Available: Characterization data and HPLC traces. This material is available free of charge via the Internet at http://pubs.acs.org.

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