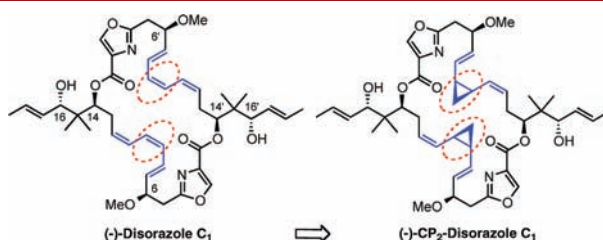# Total Synthesis of (−)-CP₂-Disorazole C₁

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ABSTRACT

The total synthesis of a bis-cyclopropane analog of the antimitotic natural product (−)-disorazole C₁ was accomplished in 23 steps and 1.1% overall yield. A vinyl cyclopropane cross-metathesis reaction generated a key (*E*)-alkene segment of the target molecule. IC₅₀ determinations of (−)-CP₂-disorazole C₁ in human colon cancer cell lines indicated low nanomolar cytotoxic properties. Accordingly, this synthetic bioisostere represents the first biologically active disorazole analog not containing a conjugated diene or polyene substructure element.

The disorazoles compromise a family of ∼30 closely related polyketide microtubule disruptors isolated since 1994 from the fermentation broth of the myxobacterium *Sorangium cellulosum* by Jansen, Reichenbach, Höfle and co-workers.[1,2] Members of the disorazole class have displayed anticancer activity in the nano- and picomolar range against a variety of transformed cell lines, including multidrug resistant cells.[3] To date, only (−)-disorazole C₁ has yielded to total synthesis,[4] although several simplified analogs and segments have been reported.[5] As part of our investigations of structure−activity relationships (SAR) of biologically active natural products,[2d,6] we designed a

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(1) Jansen, R.; Irschik, H.; Reichenbach, H.; Wray, V.; Höfle, G. *Liebigs Ann. Chem.* **1994**, 759.

(2) For reviews of disorazole chemistry and biology, see: (a) Hopkins, C. D.; Wipf, P. *Nat. Prod. Rep.* **2009**, *26*, 585. (b) Weissman, K. J.; Mueller, R. *Nat. Prod. Rep.* **2010**, *27*, 1276. (c) Kretschmer, M.; Menche, D. *Synlett* **2010**, 2989. (d) Wipf, P.; Graham, T. H.; Xiao, J. *Pure Appl. Chem.* **2007**, *79*, 753.

(3) (a) Lazo, J. S.; Reese, C. E.; Vogt, A.; Vollmer, L. L.; Kitchens, C. A.; Günther, E.; Graham, T. H.; Hopkins, C. D.; Wipf, P. *J. Pharmacol. Exp. Ther.* **2010**, *332*, 906. (b) Xu, F. L.; Rbaibi, Y.; Kiselyov, K.; Lazo, J. S.; Wipf, P.; Saunders, W. S. *BMC Chem. Biol.* **2010**, *10*, 1. (c) Tierno, M. B.; Kitchens, C. A.; Petrik, B.; Graham, T. H.; Wipf, P.; Xu, F. L.; Saunders, W. S.; Raccor, B. S.; Balachandran, R.; Day, B. W.; Stout, J. R.; Walczak, C. E.; Ducruet, A. P.; Reese, C. E.; Lazo, J. S. *J. Pharm. Exp. Ther.* **2009**, *328*, 715. (d) Wipf, P.; Graham, T. H.; Vogt, A.; Sikorski, R. P.; Ducruet, A. P.; Lazo, J. S. *Chem. Biol. Drug Des.* **2006**, *67*, 66. (e) Hearn, B. R.; Arslanian, R. L.; Fu, H.; Liu, F.; Gramajo, H.; Myles, D. C. *J. Nat. Prod.* **2006**, *69*, 148. (f) Elnakady, Y. A.; Sasse, F.; Lünsdorf, H.; Reichenbach, H. *Biochem. Pharmacol.* **2004**, *67*, 927.

(4) Wipf, P.; Graham, T. H. *J. Am. Chem. Soc.* **2004**, *126*, 15346.

(5) (a) Schaeckel, R.; Hinkelmann, B.; Sasse, F.; Kalesse, M. *Angew. Chem., Int. Ed.* **2010**, *49*, 1619. (b) Niess, B.; Hartung, I. V.; Haustedt, L. O.; Hoffmann, H. M. R. *Eur. J. Org. Chem.* **2006**, 1132. (c) Hearn, B. R.; Arslanian, R. L.; Fu, H.; Liu, F.; Gramajo, H.; Myles, D. C. *J. Nat. Prod.* **2006**, *69*, 148. (d) Haustedt, L. O.; Panicker, S. B.; Kleinert, M.; Hartung, I. V.; Eggert, U.; Niess, B.; Hoffmann, H. M. R. *Tetrahedron* **2003**, *59*, 6967. (e) Hartung, I. V.; Eggert, U.; Haustedt, L. O.; Niess, B.; Schaefer, P. M.; Hoffmann, H. M. R. *Synthesis* **2003**, 1844. (f) Hartung, I. V.; Niess, B.; Haustedt, L. O.; Hoffmann, H. M. R. *Org. Lett.* **2002**, *4*, 3239. (g) Hillier, M. C.; Price, A. T.; Meyers, A. I. *J. Org. Chem.* **2001**, *66*, 6037.

(6) (a) Wang, Z.; McPherson, P. A.; Raccor, B. S.; Balachandran, R.; Zhu, G.; Day, B. W.; Vogt, A.; Wipf, P. *Chem. Biol. Drug Des.* **2007**, *70*, 75. (b) Powis, G.; Wipf, P.; Lynch, S. M.; Birmingham, A.; Kirkpatrick, D. L. *Mol. Cancer Ther.* **2006**, *5*, 630. (c) Wipf, P.; Minion, D. J.; Halter, R. J.; Berggren, M. I.; Ho, C. B.; Chiang, G. G.; Kirkpatrick, L.; Abraham, R.; Powis, G. *Org. Biomol. Chem.* **2004**, *2*, 1911. (d) Wipf, P.; Fritch, P. C.; Geib, S. J.; Sefler, A. M. *J. Am. Chem. Soc.* **1998**, *120*, 4105.

(7) Examples documenting the lability of trienes include: (a) Burke, C. P.; Haq, N.; Boger, D. L. *J. Am. Chem. Soc.* **2010**, *132*, 2157. (b) Hicks, J. D.; Roush, W. R. *Org. Lett.* **2008**, *10*, 681. (c) Trost, B. M.; Frederiksen, M. U.; Papillon, J. P. N.; Harrington, P. E.; Shin, S.; Shireman, B. T. *J. Am. Chem. Soc.* **2005**, *127*, 3666. (d) Fuwa, H.; Sasaki, M.; Satake, M.; Tachibana, K. *Org. Lett.* **2002**, *4*, 2981.

cyclopropane analog of (−)-disorazole C_1 (i.e., CP_2-disorazole C_1), with the goal to replace the labile[7] (E,Z,Z)-triene subunit with an isosteric and biologically similarly effective moiety. The choice of absolute configuration for the *cis*-cyclopropanes in CP_2-disorazole C_1 reflected the stereochemistry observed for the epoxide present in disorazole A_1.[2a]

**Scheme 1.** Retrosynthetic Approach for CP_2-Disorazole C_1 **1**
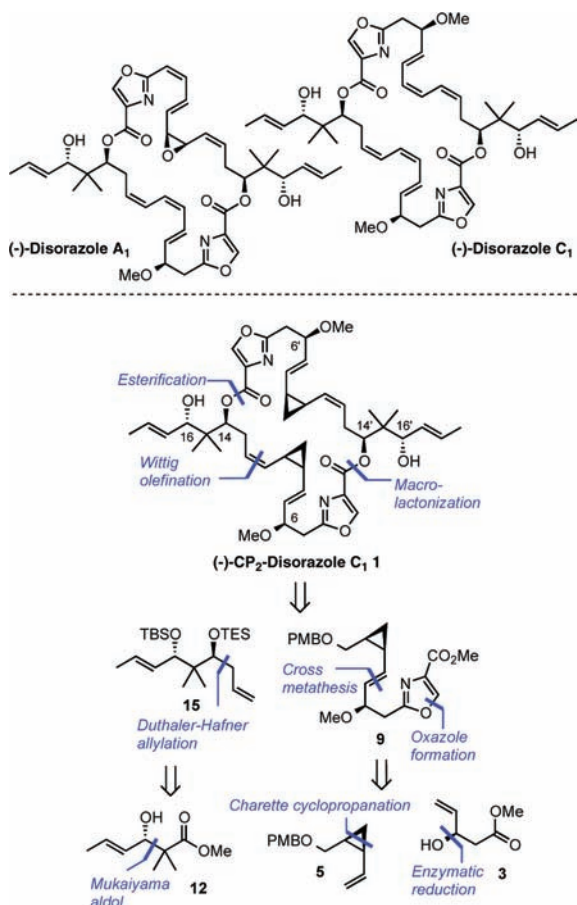
Retrosynthetically, the C_2-symmetrical macrodiolide CP_2-disorazole C_1 (**1**) could be constructed from two key fragments, **15** and **9** (Scheme 1). Diene **15** is derived from β-hydroxy ester **12** which can be obtained from an asymmetric Mukaiyama aldol reaction of crotonaldehyde. By taking advantage of a vinyl cyclopropane cross-metathesis reaction,[8] our plan was to access alkene **9** from the readily available cyclopropane **5** and the oxazole precursor **3**. This approach was realized in excellent yields beginning with the straightforward preparation of ester **3** in two steps from the known[9] alkyne **2** (Scheme 2).

Scheme 2. Synthesis of Ester **3**
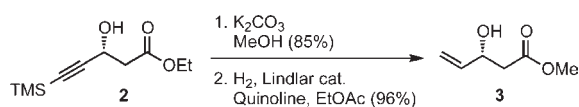
The vinyl cyclopropane **5** was prepared in 74% yield in a Wittig condensation from known aldehyde **4**[5d] (Scheme 3).

Scheme 3. Cross-Metathesis and Synthesis of Aldehyde **10**
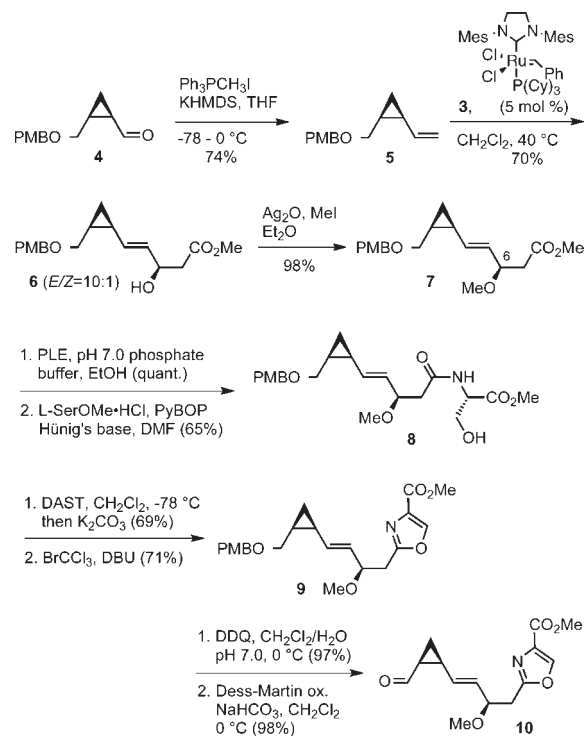
Cross-metathesis reaction of alkenes **5** and **3**[10] in the presence of a second generation ruthenium catalyst[11] provided alkene **6** in 70% yield as an inseparable 10:1 mixture of E/Z-stereoisomers. O-Methylation of **6** with silver oxide[12] and methyl iodide, followed by enzymatic hydrolysis[13] with pig liver esterase (PLE) and subsequent coupling of the carboxylic acid with serine methyl ester, yielded amide **8**. A two-step cyclodehydration/oxidation procedure[14] led to oxazole **9**. Oxidative deprotection of the PMB ether followed by further oxidation of the resulting primary alcohol under Dess−Martin conditions afforded the desired aldehyde **10** in excellent yield.

After considerable experimentation, we found that Kiyooka's conditions were best suited for the asymmetric Mukaiyama aldol reaction between the sterically

(8) Nicola, T.; Brenner, M.; Donsbach, K.; Kreye, P. *Org. Process Res. Dev.* **2005**, *9*, 513.

(9) (a) Salit, A.-F.; Meyer, C.; Cossy, J.; Delouvrié, B.; Hennequin, L. *Tetrahedron* **2008**, *64*, 6684. (b) Salit, A.-F.; Meyer, C.; Cossy, J. *Synlett* **2007**, 934.

(10) Attempts at performing the cross-metathesis of **5** with the methyl ether of **3** or the oxazole analog of **3** afforded complex mixtures.

(11) Trnka, T. M.; Grubbs, R. H. *Acc. Chem. Res.* **2001**, *34*, 18.

(12) Stronger bases led to a rapid β-elimination of the C6 methyl ether.

(13) Mahato, T. K.; Babu, S.; Basak, A. *Biotechnol. Lett.* **1993**, *15*, 1147. Alternative conditions, including LiOH, KOH, TMSOK, $(Bu_3Sn)_2O$, and Me_3SnOH, led to significant β-elimination at C6.

(14) Phillips, A. J.; Uto, Y.; Wipf, P.; Reno, M. J.; Williams, D. R. *Org. Lett.* **2000**, *2*, 1165.
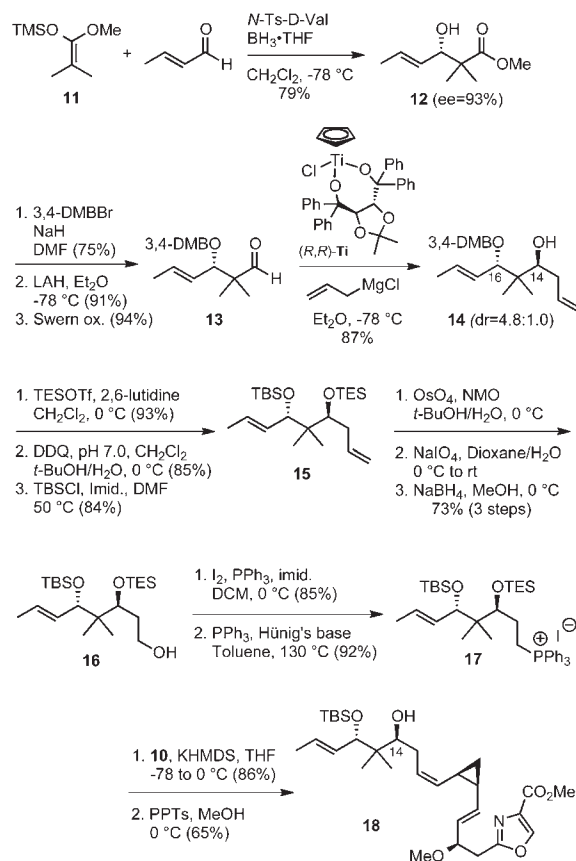
hindered silyl ketene acetal **11** and crotonaldehyde (Scheme 4).[5a,15]

The α,α-dimethylated aldol product **12** was obtained in 79% yield and 93% *ee* as determined by chiral HPLC analysis. Protection of the secondary alcohol in **12** with 3,4-dimethoxybenzyl (DMB) bromide[16] followed by ester reduction with LAH and oxidation of the primary alcohol under Swern conditions provided aldehyde **13**. Among contemporary allylation protocols,[17] we found that the Duthaler−Hafner allylation[18] of aldehyde **13** gave alcohol **14** in the highest (87%) yield and with a satisfactory *dr* = 4.8:1.0, favoring the desired *anti*-configuration of the 1, 3-diol. The minor isomer was present in diminishing amounts in subsequent intermediates until it was finally completely removed in the Wittig reaction of **17** and **10**.

At first, the advancement of homoallylic alcohol **14** to the desired phosphonium salt **17** proved problematic. Upon formation of the alkyl iodide at C12, an *in situ* S$_N$2-displacement of the iodide by the electron-rich DMB ether at C16 afforded the corresponding tetrahydropyran as the major product.[19] Although small quantities of the desired alkyl iodide were isolated, immediate formation of the tetrahydropyran occurred upon heating. Consequently, TES protection of the alcohol at C14 followed by the exchange of the DMB with a TBS ether at C16 was used to access the orthogonally silyl-protected diol **15** (Scheme 4). Stepwise Johnson−Lemieux oxidation followed by NaBH$_4$ reduction afforded the primary alcohol **16**. Iodination and phosphonium salt generation at elevated temperature in a sealed vessel proceeded now without incident to furnish **17** in good overall yield. The Wittig condensation of the freshly formed phosphonium salt with aldehyde **10** in a vigorously degassed THF solution yielded 86% of the desired *Z*-alkene as a single stereoisomer by ^{1}H NMR analysis. Finally, selective TES deprotection with PPTs in the presence of the allylic TBS ether proceeded in 65% yield to afford the advanced segment **18**.[20]

Originally, our end game strategy had envisioned the direct *cyclo*-dimerization of the carboxylic acid corresponding to **18**. However, all efforts to realize this *cyclo*-dimerization resulted in the exclusive formation of the

(15) Kiyooka, S.; Kaneko, Y.; Komura, M.; Matsuo, H.; Nakano, M. *J. Org. Chem.* **1991**, *56*, 2276.

(16) Use of the more electron rich 3,4-DMB ether suppressed overoxidation of the allylic alcohol at C16 during DDQ deprotection.
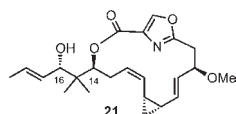
(17) Lu, Z.; Ma, S. *Angew. Chem., Int. Ed.* **2008**, *47*, 258.

(18) (a) Hafner, A.; Duthaler, R. O.; Marti, R.; Rihs, G.; Rothe-Streit, P.; Schwarzenbach, F. *J. Am. Chem. Soc.* **1992**, *114*, 2321. (b) Cossy, J.; Willis, C.; Bellosta, V.; BouzBouz, S. *J. Org. Chem.* **2002**, *67*, 1982.

(19) Jarosz, S.; Gajewska, A.; Luboradzki, R. *Tetrahedron: Asymmetry* **2008**, *19*, 1385.

(20) NOE analysis confirmed the *cis*-cyclopropane stereochemistry.

(21) Varying mixtures of the *cyclo*-monomer **21** and its corresponding C16 TBS-protected derivative were isolated, and their structures confirmed by MALDI-TOF MS. Even upon attempted stepwise coupling of the two monomers, **21** was formed by an apparent *in situ* deprotection of the TES group of the acid component.

Scheme 4. Synthesis of Hydroxy Ester Segment **18**

15-membered macrolactone arising from the direct lactonization at the C14 hydroxyl group, i.e. the formation of the cyclic monomer.[21] To circumvent this undesired reaction, a stepwise approach was adopted (Scheme 5). Hydrolysis of ester **9** followed by DCC-mediated coupling to the secondary alcohol **18** provided the bis-cyclopropane **19** in 89% yield. Oxidative PMB deprotection with DDQ and subsequent Dess−Martin oxidation of the resulting primary alcohol led to the requisite cyclopropyl aldehyde in good yield. Condensation of this aldehyde and the ylide generated from phosphonium salt **17** after vigorous degassing afforded the *seco*-dimer **20** in 74% yield as a single alkene stereoisomer.

Selective TES ether deprotection of **20** revealed the secondary alcohol at C14′ (Scheme 5). With all functional groups properly installed, the stage was now set for the final lactone formation. Selective saponification of the methyl ester in the presence of the internal ester linkage of **20** with barium hydroxide followed by Shiina macrolactonization[22] with 2-methyl-6-nitrobenzoic anhydride (MNBA) and DMAP provided the desired 30-membered macrocycle in 55% yield over the two steps. Finally, double TBS ether deprotection in the presence of HF-pyridine proceeded in 64% yield to the desired (−)-CP$_2$-disorazole C$_1$.

(22) (a) Shiina, I.; Kubota, M.; Oshiumi, H.; Hashizume, M. *J. Org. Chem.* **2004**, *69*, 1822. (b) Schweitzer, D.; Kane, J. J.; Strand, D.; McHenry, P.; Tenniswood, M.; Helquist, P. *Org. Lett.* **2007**, *9*, 4619.

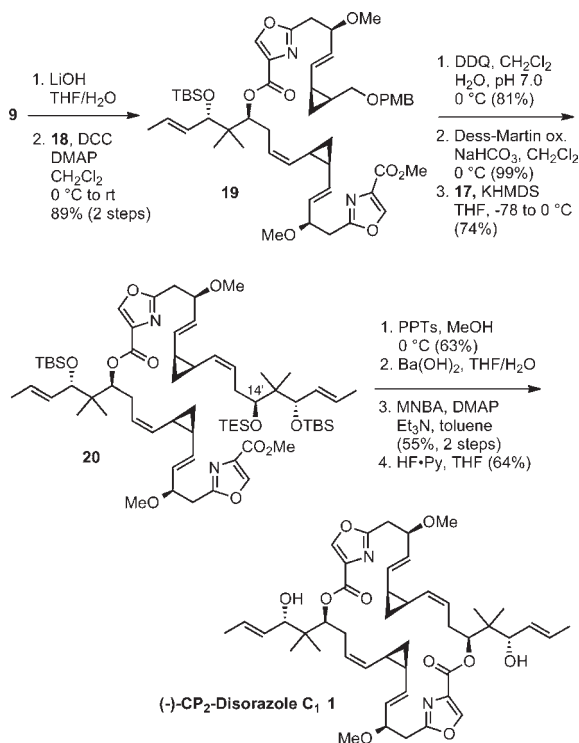**Scheme 5.** Segment Condensation and Shiina Macrolactonization

Table 1 illustrates our preliminary analysis of the antiproliferative properties of this novel disorazole analog. In three human colon cancer cell lines, **1** displayed low IC_{50} values ranging from 25 to 50 nM and, thus, proved to be only slightly less active than its parent, $(-)$-disorazole C_1.[3a–d,23] Also, **1** was equipotent to the clinically used anticancer vinca alkaloid vincristine in these colon cancer cells. In contrast, cyclomonomer **21** was inactive at concentrations up to 50 μM in all three cell lines, thereby serving as a negative control and confirming the importance of the 3-D architecture of the disorazoles.[3d]

Table 1. Cytotoxic Activity of **1** and Cyclic Monomer **21** in Human Colon Cancer Cell Lines[a,b]

cell line	**1** IC_{50} [nM][c]	**21** IC_{50} [μM][c]	vincristine IC_{50} [nM][c]
RKO	28.0 ± 9.2	>50	18.6 ± 7.6
HCT116	28.3 ± 11.6	>50	35.2 ± 11.9
H630	49.5 ± 25.0	>50	68.0 ± 16.3

[a] Vincristine is used as a reference compound.[23] [b] Cell proliferation and viability were quantified by the WST-1 assay (Roche Applied Science; Indianapolis, IN). [c] Values represent the mean $\pm$ SD from 4 to 5 independent determinations.

In summary, we have developed an efficient route to $(-)$-CP2-disorazole C_1 **1**, a bis-cyclopropane analog of the antimitotic natural product disorazole C_1.[24] The target agent was obtained in 23 steps and 1.1% overall yield for the longest linear sequence. It contains 10 stereogenic carbons, 4 more than disorazole C_1, and 6 double bonds, 2 less than the natural product. Most importantly, by replacing the central *cis*-alkenes at C9–C10/C9'-C10' of disorazole C_1 with *cis*-cyclopropane rings, **1** no longer has a labile conjugated (E,Z,Z)-triene; yet, it shows nearly the same potent level of low nanomolar *in vitro* cytotoxicity in human colon cancer cell lines as the natural myxobacterium metabolite. Further biological studies of **1** will be reported in due course.

Supporting Information Available. Experimental procedures and spectral data for all new compounds, including copies of ^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

(23) Disorazole C_1 was not available for a direct comparison in these preliminary assays. Ref. 3c reports the IC_{50} of disorazole C_1 and vincristine in HCT116 cells as 1.09 ± 0.41 and 5.62 ± 0.33 nM, respectively. Unsurprisingly, there is some variability in the common standard vincristine in these two assays. However, in both batches of HCT116 cells, disorazole C_1 and **1** are more potent than vincristine by a factor of 5 and 1.2, respectively, making disorazole C_1 ca. 4-fold more active than its cyclopropane analog **1**.

(24) For cyclopropane analogs of natural products, see: (a) Cachoux, F.; Isarno, T.; Wartmann, M.; Altmann, K.-H. *Synlett* **2006**, 1384. (b) Wipf, P.; Reeves, J. T.; Balachandran, R.; Day, B. W. *J. Med. Chem.* **2002**, *45*, 1901.