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Synthesis of 3,3′-Di-*O*-methyl Ardimerin and Exploration of Its DNA Binding Properties

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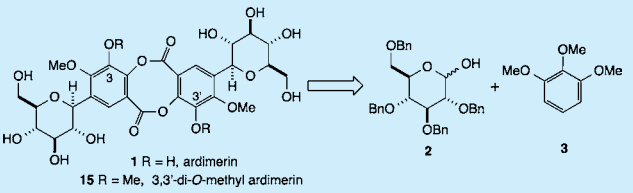
Supporting Information

ABSTRACT: The 3,3′-di-*O*-methyl derivative (**15**) of the bis-*C*-aryl glycoside natural product ardimerin (**1**) has been synthesized in 11 steps from 2,3,4,6-tetrabenzylglucose (**2**) and 1,2,3-trimethoxybenzene (**3**). Key steps in the synthesis involve a Lewis acid mediated Friedel−Crafts type glycosylation and a Yamaguchi lactonization under Yonemitsu conditions. 3,3′-Di-*O*-methyl ardimerin aggregates in aqueous solutions at concentrations greater than 1 μM, and both UV and fluorescence binding studies indicate that **15** has a low affinity for duplex DNA.



Plants used in traditional Chinese medicine have yielded a wealth of chemical constituents with important biological activities.[1] Ardimerin (**1a**, Figure 1), a dimeric lactone with

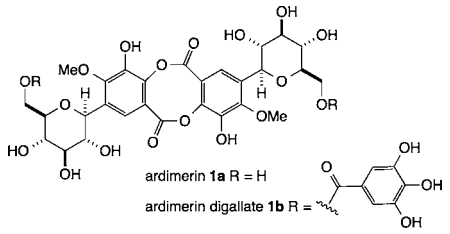


Figure 1. Structures of ardimerin and ardimerin digallate.

radical scavenging activity, was isolated from *Ardisia japonica* by Ryu et al. in 2002.[2] Subsequently, ardimerin digallate (**1b**) was isolated from the same species, along with the flavonoid quercitrin and the terpenoids friedelin, epifriedelinol, baurenol, and baurenyl acetate.[3] The digallate derivative of ardimerin was shown to inhibit HIV-1 and HIV-2 RNase H *in vitro* with IC_{50} values of 1.5 and 1.1 μM, respectively.

C-Aryl glycosides are an important class of naturally occurring compounds endowed with remarkable stability toward acid and enzymatic hydrolysis;[4] this affords them a sufficient intracellular lifetime to allow trafficking to the nucleus, where they bind DNA to form stable complexes.[5] The bis-*C*-aryl glycoside altromycin B has been shown by NMR studies to associate with DNA via a helix-threading mode of binding, with carbohydrate moieties positioned in opposite grooves of the duplex.[5e] Given that ardimerin is a symmetrical bis-*C*-aryl glycoside, we envisioned that, despite the nonplanarity of its aglycone,[6] it might also be capable of the recognition of nucleic acids by a threading mode of intercalation, with the glucosyl substituents positioned in both the major and minor grooves of DNA. To assess this possibility, we decided to undertake its synthesis and investigate its DNA binding properties.

We envisioned (Figure 2) that the C−C linkage between carbohydrate and aromatic moieties could be fashioned by a

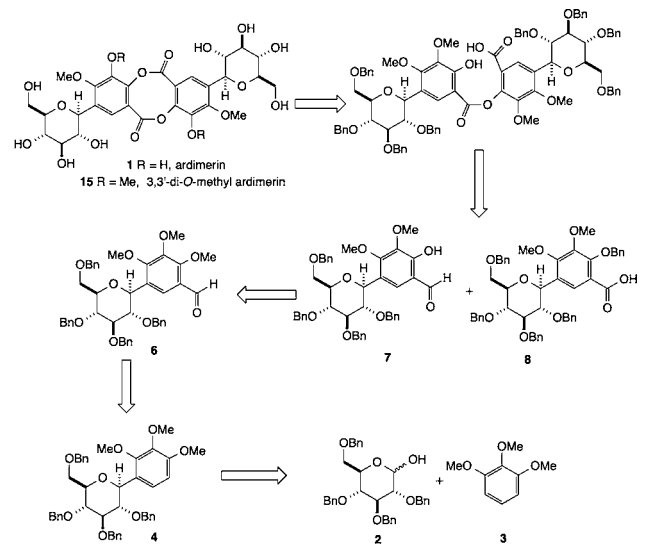


Figure 2. Retrosynthetic analysis of ardimerin.

Lewis acid mediated Friedel−Crafts type *C*-glycosylation reaction between protected glucose **2** and 1,2,3-trimethoxybenzene (**3**).[9] Aromatic ring carbonylation and selective *ortho* methoxy group deprotection would then provide **7**, a crucial substrate for esterification with the derived carboxylic acid monomer **8**. Oxidation, macrocyclization, and protecting group removal would then provide the natural product.

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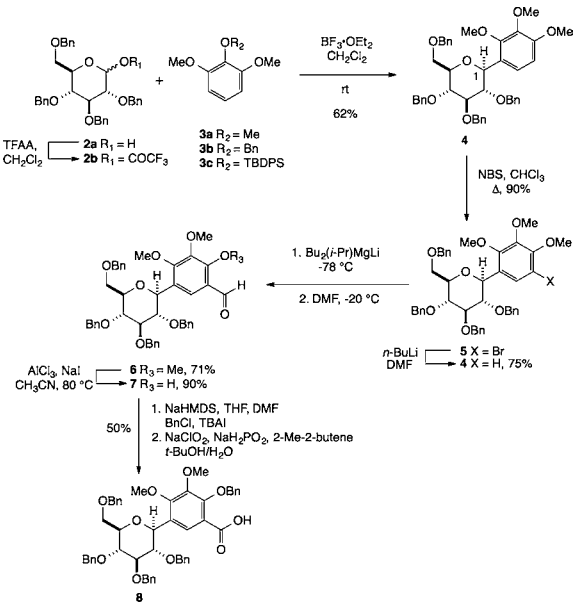
The carbohydrate coupling partner (**2**) required for the *C*-glycosylation reaction may be prepared in 72% overall yield from dextrose as previously described.[7] Treatment of **2a** with a 1:1 solution of trifluoroacetic anhydride and CH_2Cl_2 for 30 min, followed by evaporation and combination with commercially available 1,2,3-trimethoxybenzene (1.5 equiv) and $BF_3{\cdot}OEt_2$ (1.1 equiv) in CH_2Cl_2 at room temperature for 30 min, afforded coupled product **4** (>20:1 β:α at C.1) in 62% yield (Scheme 1).[8] Interestingly, 2-*O*-benzyl-1,3-dimethoxybenzene

Scheme 1. Synthesis of *C*-Glycoside Monomers 7 and 8



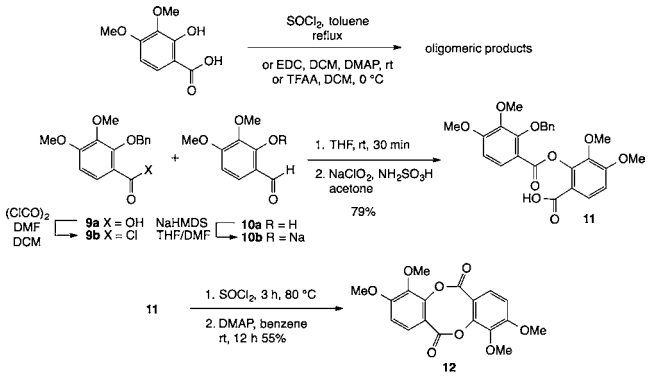
(**3b**) was not a suitable partner for the *C*-glycosylation reaction, undergoing rapid decomposition in the presence of either $BF_3{\cdot}OEt_2$ or TMSOTf. However, the 2-*O*-*tert*-butyldiphenylsilyl derivative **3c** coupled efficiently with **2b** in the presence of TMSOTf as a Lewis acid promoter to provide the *C*-aryl glycoside product in 72% yield.

Several methods were explored to introduce the aldehyde moiety on the aromatic ring. Attempted Vilsmeier formylation (DMF, $POCl_3$, toluene, 100 °C) of **4** resulted in minimal conversion, even after an extended reaction time (24 h).[9] Directed *ortho*-lithiation[10] with *n*-BuLi/TMEDA and trapping with DMF gave only low (<10%) yields of carbonyl-containing products, likely due to intramolecular protonation of the aryllithium species by the benzyl ether protecting groups on the carbohydrate.[11] Bromination (NBS, $CHCl_3$, reflux) of **4** resulted in the formation of aryl bromide **5** in 90% yield. Lithium−halogen exchange with *n*-BuLi, followed by rapid quenching with DMF, again led to a hydrodebrominated product arising from the aforementioned intramolecular proton transfer process. However, magnesiate formation according to the protocol of Oshima (*i*-PrMgCl, 2 equiv of *n*-BuLi, THF, 0 °C to −78 °C, then **5**) followed by quenching with DMF led to a 71% yield of the desired aldehyde **6**.[12]

Selective deprotection of the methoxy group *ortho* to the aldehyde initially proved to be problematic. Treatment of **6** with 1−3 equiv of BCl_3 in CH_2Cl_2 at −60 °C (1 h) or room temperature (overnight) led to significant substrate decomposition. The combination of **6** with $AlCl_3$ in benzene at 80 °C or in CH_2Cl_2 at room temperature gave only poor yields of the desired hydroxyl aldehyde. Finally it was discovered that treatment of **6** with 1.1 equiv of $AlCl_3$ and 1.5 equiv of NaI in CH_3CN (0.25 M) at 80 °C for 1 h gave hydroxy aldehyde **7** in 90% yield. Subsequent benzyl ether formation and oxidation with $NaClO_2$ gave a 50% overall yield of carboxylic acid **8**. Interestingly, all attempts to cleave the methoxy group *meta* to the aldehyde of **7** (corresponding to the C.3/C.3′ position of the natural product) by extended exposure to $AlCl_3$/NaI (80 °C) resulted in substrate decomposition.

With both **7** and **8** in hand, we set out to identify conditions for the construction of the eight-membered diolide (Scheme 2).

Scheme 2. Model Study: Synthesis of Diolide 12



Attempts to directly dimerize the model compound 2,3-dimethoxysalicylic acid ($SOCl_2$, dilute toluene, reflux; DCC or EDC, DCM, rt; TFAA, DCM, 0 °C) failed, producing only uncharacterized oligomers in low yields. In line with literature precendent,[13] coupling of the known acid **9a**[14] and aldehyde **10a**[15] was accomplished via direct addition of sodium alkoxide **10b** to acid chloride **9b** in THF at room temperature; subsequent oxidation ($NaClO_2$, NH_2SO_3H, acetone/water) afforded carboxylic acid **11**. Harris has demonstrated[13] that subjection of *ortho*-benzyl protected carboxylic acid substrates similar to **11** to refluxing thionyl chloride leads directly to eight-membered diolides of the type **12**, arising from acid chloride formation, *in situ* benzyl ether cleavage, and macrolactonization of the hydroxy acid chloride; however, we observed that refluxing **11** in $SOCl_2$ for 3 h led only to the intermediate hydroxy acid chloride, which was sufficiently stable to survive aqueous reaction workup. Instead, the acid chloride intermediate was diluted in benzene or toluene (0.01 M) and treated with 3 equiv of DMAP and stirred at room temperature overnight. In this way, diolide **12** could be secured in 50−60% yield.

With a method to prepare the diolide core of ardimerin in hand, we proceeded to explore the similar union of aldehyde **7** and carboxylic acid **8** (Scheme 3). Treatment of compound **8** with oxalyl chloride in the presence of catalytic quantities of DMF gave rise to the corresponding acid chloride, which was added to the potassium salt of **7** in THF at 0 °C; the resultant crude aldehyde **13a** was immediately oxidized under Pinnick−Lindgren−Kraus conditions[16] to afford the stable carboxylic acid **13b**. Refluxing **13b** in $SOCl_2$ for 3 h gave rise to the corresponding benzyloxy acid chloride and not the desired hydroxyl acid chloride; further heating in $SOCl_2$ overnight led only to extensive substrate decomposition. To effect removal of the benzyl ether before conversion to the acid chloride, compound **13b** was treated with a 1:1 mixture of TFA and toluene at room temperature for 5 min.[17] The intermediate

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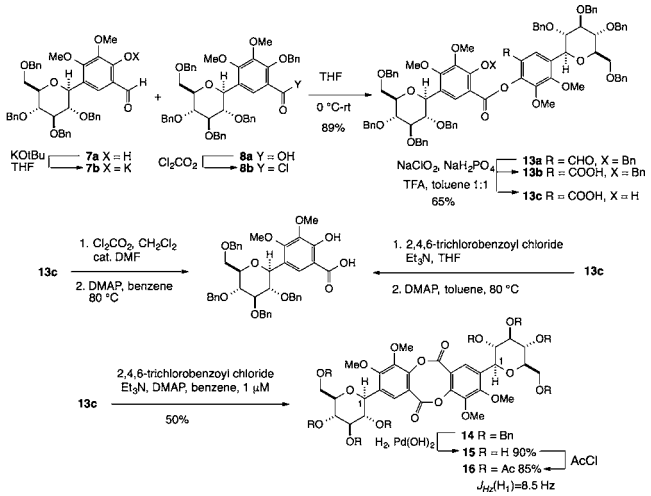
Scheme 3. Seco-acid Macrolactonization



hydroxy acid was then treated with oxalyl chloride (cat. DMF, CH_2Cl_2), and a dilute solution (0.1 M) of the resulting acid chloride was then added dropwise to a refluxing solution of DMAP (3 equiv) in benzene. However, this condition gave rise to the hydroxyacid monomer resulting from DMAP-induced cleavage of the ester linkage. Standard Yamaguchi lactonization conditions,[18] involving slow addition of the mixed anhydride (seco acid, 2,4,6-trichlorobenzoyl chloride, Et_3N, THF, rt) to a refluxing solution of DMAP in toluene, gave the same result. Gratifyingly, attempted macrolactonization under Yonemitsu conditions (2,4,6-trichlorobenzoyl chloride, Et_3N, DMAP, and the seco acid in benzene, 1×10^{-3} M)[19] at room temperature gave rise to the desired diolide **14** in 50% yield. Hydrogenation of **14** over Pearlman's catalyst gave 3,3′-di-*O*-methyl ardimerin **15** in 90% yield. Interestingly, attempted acylation of **15** (Ac_2O, Pyr, rt, 16 h or Ac_2O, *i*-Pr_2NEt, DMAP)[20] led to none of the desired peracetate and the production of numereous side products. Ultimately, it was found that stirring **15** in neat acetyl chloride[21] overnight led to formation of peracetate **16** in 85% yield. The β-stereochemistry of the glucosyl moieties was indicated by the 8.5 Hz coupling constant of the C.1 proton, and the connectivity of the molecule was verified by $^1H-^1H$ COSY and NOESY experiments (see Supporting Information).

In our attempts to access ardimerin by selective cleavage of the C.3 and C.3′ methyl ethers, treatment of **14** with BCl_3/CH_2Cl_2 (rt, overnight), $AlCl_3$/NaI (80 °C, CH_3CN, 3 h), or MgI_2 (50−80 °C, toluene)[22] initially led to no starting material conversion, but after a prolonged reaction and an increase in the number of equivalents of Lewis acid, extensive decomposition products, arising from diolide cleavage, were formed. Similarly, use of sodium ethanethiolate in DMF (100 °C, 2 h)[23] also led to dissolution of the bislactone moeity. These data indicate that removal of the requisite methyl ethers is likely to be successful only on substrates prior to formation of the diolide core of the natural product.

The binding of **15** to duplex DNA was explored by UV and fluorescence spectroscopies. A concentration-dependent red shift in the absorption at λ_{max} = 214 nm in the ultraviolet spectrum of **15** suggested that self-association/aggregation was occurring in an aqueous buffer solution (10 mM Tris-EDTA) at concentrations >1 μM (Figure 3).[24] Thermal denaturation studies showed no significant shift in the T_M (68 °C) of salmon testes DNA in the presence of **15** at low ligand/DNA ratios.[25]

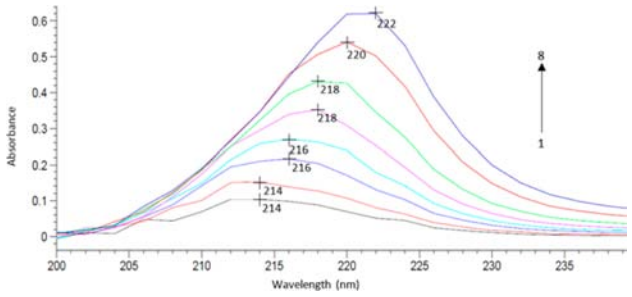


Figure 3. UV absorption spectra of **15** (10 mM Tris-EDTA) at varying concentrations; [**15**] = (0.79, 1.18, 1.95, 2.70, 3.80, 5.56, 8.81, and 11.76) $\times 10^{-6}$ mol L^{-1} for curves 1−8, respectively.

Furthermore, compound **15** displayed relatively limited ability to displace bound ethidium bromide from calf thymus DNA as compared to control compound daunorubicin over the same concentration range (1×10^{-9} M to 4×10^{-7} M; see Supporting Information).[26] These data suggest that **15** has a low affinity for duplex DNA, perhaps indicative of the difficulty in accommodating the bulky chromophore-linked *C*-glycosyl moieties in the narrow minor groove, the initial site of small-molecule binding to DNA.[27]

In summary, we have developed an 11 step synthesis of the 3,3′-di-*O*-methyl derivative of the natural product ardimerin and have shown that this substance readily aggregates in aqueous solution and has a low apparent affinity for duplex DNA. Current efforts toward the completion of the synthesis of ardimerin are centered around deprotection of the C.3 methyl ether of aldehyde **7**.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures including spectroscopic and analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) (a) Fukuyama, Y.; Kiriyama, Y.; Okino, J.; Kodama, M.; Iwaki, H.; Hosozawa, S.; Matsui, K. *Chem. Pharm. Bull.* **1993**, *41*, 561. (b) Jansakul, C.; Baumann, H.; Kenne, L.; Samuelsson, G. *Planta Med.* **1987**, *53*, 405.

(2) Ryu, G.; Lee, S. Y.; Kim, B. S.; Ryu, S. Y.; Hwang, H. J.; Choi, B. W.; Lee, B. H.; Jung, D. S. *Nat. Prod. Sci.* **2002**, *8*, 108.

(3) Dat, N. T.; Bae, K. H.; Wamiru, A.; McMahon, J. B.; Le Brice, S. F. J.; Bona, M.; Bentler, J. A.; Kim, Y. H. *J. Nat. Prod.* **2007**, *70*, 839.

(4) (a) Hansen, M. R.; Hurley, L. H. *Acc. Chem. Res.* **1996**, *29*, 249. (b) Hacksell, U.; Daves, G. D. *Prog. Med. Chem.* **1985**, *22*, 1.

(5) (a) Owen, E. A.; Burley, G. A.; Carver, J. A.; Wickham, G.; Keniry, M. A. *Biochem. Biophys. Res. Commun.* **2002**, *290*, 1602. (b) Pavlopoulos, S.; Bicknell, W.; Wickham, G.; Craik, D. J. *J. Mol. Recognit.* **1999**, *12*, 346. (c) Pavlopoulos, S.; Bicknell, W.; Craik, D. J.;

Wickham, G. *Biochemistry* **1996**, *35*, 9314. (d) Hansen, M.; Yun, S.; Hurley, L. *Chem. Biol.* **1995**, *2*, 229. (e) Hansen, M. R.; Hurley, L. *J. Am. Chem. Soc.* **1995**, *117*, 2421.
(6) The recent characterization of the natural product leinamycin as a DNA intercalator despite the absence of a typical intercalating moiety (ie, polycyclic aromatic unit) in the molecule raises the possibility that structures with relatively limited planar π-surfaces (a *Z,E*-penta-2,4-dienone moeity in the case of leinamycin) can bind nucleic acids by intercalation: Fekry, M. I.; Szekely, J.; Dutta, S.; Breydo, L.; Zang, H.; Gates, K. S. *J. Am. Chem. Soc.* **2011**, *133*, 17641 and references therein.
(7) Yepremyan, A.; Minehan, T. G. *Org. Biomol. Chem.* **2012**, *10*, 5194.
(8) Wendelin, F.; Schmidt, R. R. *Carbohydr. Res.* **1991**, *209*, 101.
(9) (a) Yepremyan, A.; Salehani, B.; Minehan, T. G. *Org. Lett.* **2010**, *12*, 1580. (b) Minehan, T. G.; Kishi, Y. *Tetrahedron Lett.* **1997**, *38*, 6815.
(10) Snieckus, V. *Chem. Rev.* **1990**, *90*, 879.
(11) For similar problems in total synthesis, see: Brimble, M. A.; Brenstrum, T. J. *J. Chem. Soc., Perkin Trans. 1* **2001**, 1612.
(12) Inoue, A.; Kitagawa, K.; Shinokubo, H.; Oshima, K. *J. Org. Chem.* **2001**, *66*, 4333.
(13) Harris, T. D.; Oruganti, S. R.; Davis, L. M.; Keehn, P. M.; Green, B. S. *Tetrahedron* **1987**, *43*, 1519.
(14) Chan, B. K.; Ciufolini, M. A. *J. Org. Chem.* **2007**, *72*, 8489.
(15) Fodor, G.; Bruckner, V.; Kiss, J.; Kovacs, J. *J. Am. Chem. Soc.* **1949**, *71*, 3694.
(16) (a) Lindgren, B. O.; Nilsson, T.; Husebye, S.; Mikalsen, O.; Leander, K.; Swahn, C.-G. *Acta Chem. Scand.* **1973**, *27*, 888. (b) Kraus, G. A.; Roth, B. *J. Org. Chem.* **1980**, *45*, 4825. (c) Kraus, G. A.; Taschner, M. J. *J. Org. Chem.* **1980**, *45*, 1175. (d) Bal, B. S.; Childers, W. E.; Pinnick, H. W. *Tetrahedron* **1981**, *37*, 2091.
(17) Fletcher, S.; Gunning, P. T. *Tetrahedron Lett.* **2008**, *49*, 4817.
(18) Inanagana, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989.
(19) (a) Hikota, M.; Sakurai, Y.; Horita, K.; Yonemitsu, O. *Tetrahedron Lett.* **1990**, *31*, 6367. (b) Hikota, M.; Tone, H.; Horita, K.; Yonemitsu, O. *J. Org. Chem.* **1990**, *55*, 7.
(20) Sakakura, A.; Kawajiri, K.; Ohkubo, T.; Kosugi, Y.; Ishihara, K. *J. Am. Chem. Soc.* **2007**, *129*, 14775.
(21) Horton, D. *Org. Synth., Collect. Vol. V* **1973**, 1.
(22) Bao, K.; Fan, A.; Dai, Y.; Zhang, L.; Zhang, W.; Cheng, M.; Yao, X. *Org. Biomol Chem.* **2009**, *7*, 5084.
(23) Kende, A. S.; Rizzi, J. P. *Tetrahedron Lett.* **1981**, *22*, 1779.
(24) Aguiar, M.; Akcelrud, L.; Pinto, M. R.; Atvars, T. D. Z.; Karasz, F. E.; Saltiel, J. *J. Photoscience* **2003**, *10*, 149.
(25) Due to the self-association/aggregation of **15**, low ligand/DNA ratios (0.05−0.01) were used in the thermal denaturation experiment: Shi, X.; Chaires, J. B. *Nucleic Acids Res.* **2006**, *34*, e14.
(26) Addition of a DNA-binding compound to DNA with pre-bound ethidium bromide results in a decrease in fluorescence due to displacement of the bound intercalator. See: Tse, W. C.; Boger, D. L. *Acc. Chem. Res.* **2004**, *37*, 61.
(27) McConnaughie, A. W.; Spychala, J.; Zhao, M.; Boykin, D.; Wilson, W. D. *J. Med. Chem.* **1994**, *37*, 1063.

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