ORGANIC LETTERS

2005
Vol. 7, No. 16
3429−3432

An Efficient Synthesis of Gougerotin and Related Analogues Using Solid- and Solution-Phase Methodology

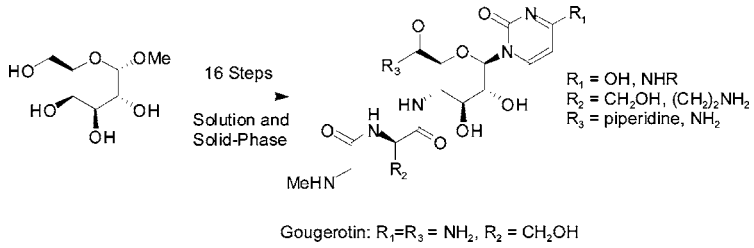
Michael T. Migawa,* Lisa M. Risen, Richard H. Griffey, and Eric E. Swayze

Ibis Therapeutics, a Division of Isis Pharmaceuticals, Inc., 1896 Rutherford Avenue, Carlsbad, California 52008

mmigawa@isisph.com

Received April 5, 2005 *(Revised Manuscript Received June 22, 2005)*

ABSTRACT



The first solid-phase synthesis of the natural product gougerotin has been accomplished. The synthetic route is versatile and allows for diversification at position C-4 of the heterocycle, C-6′ of the sugar ring, and both residues of the peptidic moiety at N-4′ in a parallel fashion.

Natural products that target RNA include the macrolides (e.g., erythromycin, clarythromycin, and azithromycin)[1] and the aminoglycosides (e.g., tobramycin and gentamicin).[2−4] Each of these has a remarkable ability to efficiently inhibit protein synthesis, resulting in excellent antibacterial activity, and has been the subject of many reports. However, the hexopyranosyl cytosines, a large class of RNA-binding natural products, have been less well explored. Members of this class are structurally related, containing a cytidine base attached to a six-membered ring sugar/s and often a modified peptidic moiety. Compounds in this class include bagougeramine A, blasticidin S, and gougerotin (Figure 1).[5−7] Experiments have suggested a common binding site for each compound which is located near the peptidal transferase region of the large subunit of rRNA.[8,9] Common points of contact of these compounds with the structured rRNA have been previously noted.[10]

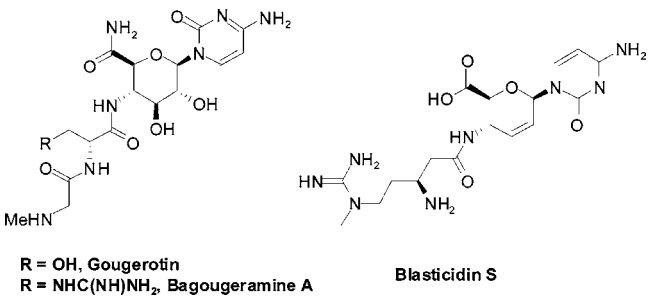
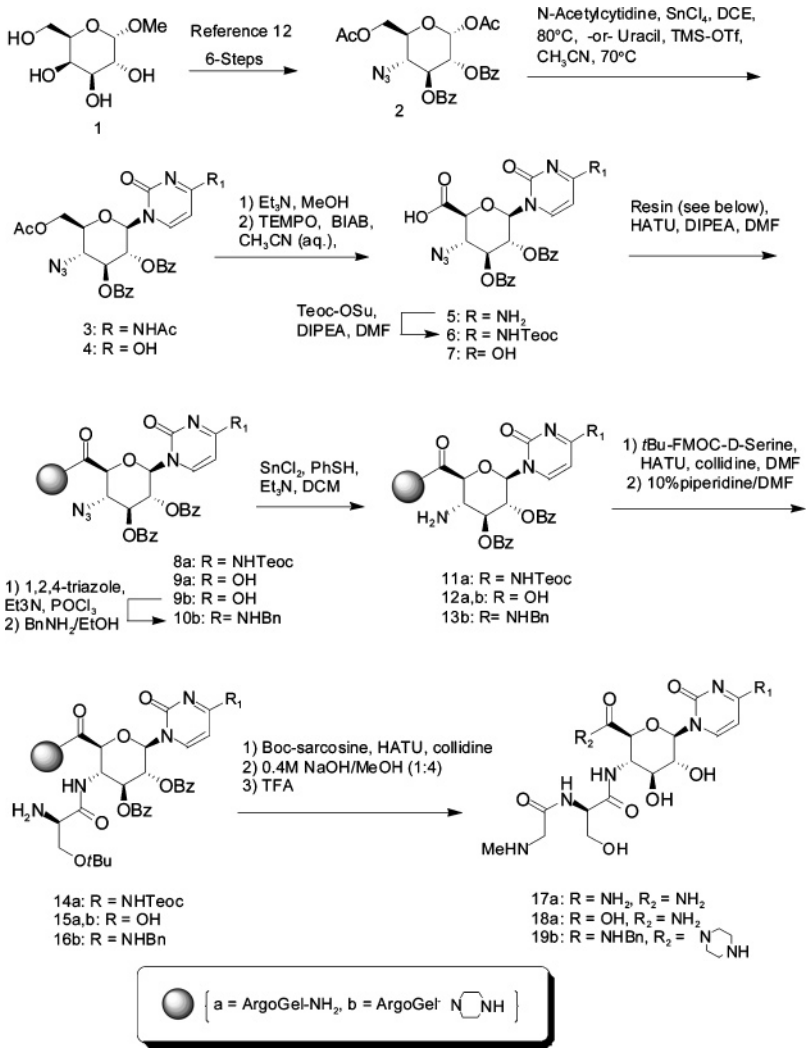


Figure 1. Several hexopyranosyl cytosines.

(1) Zhanel, G. G.; Dueck, M.; Hoban, D. J.; Vercaigne, L. M.; Embil, J. M.; Gin, A. S.; Karlowsky, J. A. *Drugs* **2001**, *61*, 443.
(2) Alper, P. B.; Hendrix, M.; Sears, P.; Wong, C.-H. *J. Am. Chem. Soc.* **1998**, *120*, 1965.
(3) Haddad, J.; Kotra, L. P.; Llano-Sotelo, B.; Kim, C.; Azucena, E. F., Jr.; Liu, M.; Vakulenko, S. B.; Chow, C. S.; Mobashery, S. *J. Am. Chem. Soc.* **2002**, *124*, 3229.
(4) Sucheck, S. J.; Wong, A. L.; Koeller, K. M.; Boehr, D. D.; Draker, K.-a.; Sears, P.; Wright, G. D.; Wong, C.-H. *J. Am. Chem. Soc.* **2000**, *122*, 5230.
(5) Takahashi, A.; Ikeda, D.; Naganawa, H.; Okami, Y.; Umezawa, H. *J. Antibiot.* **1986**, *39*, 1041.
(6) Tsukada, K. *Seitai no Kagaku* **1984**, *35*, 557.
(7) Lichtenthaler, F. W.; Morino, T.; Winterfeldt, W. *Nucleic Acids Res., Spec. Publ.* **1975**, *1*, S33.
(8) Vazquez, D.; Barbacid, M.; Carrasco, L. *Haematol. Bluttransfus.* **1974**, *14*, 327.
(9) Barbacid, M.; Vazquez, D. *Eur. J. Biochem.* **1974**, *44*, 445.

10.1021/ol0507322 CCC: $30.25 © 2005 American Chemical Society
Published on Web 07/13/2005

Scheme 1. Gougerotin Synthesis



The most frequently studied hexopyranosyl cytosine, gougerotin, has been obtained either by isolation from the fermentation broth of *Streptomyces gougeroti*[11] or through total synthesis.[12,13] No semisynthetic analogues have been reported in the literature, and no more than 30 analogues based on the total synthesis have been reported.[7,14−16] Additionally, compounds with improved activity relative to the parent compound have not been identified, and literature reports of additional synthetic work on gougerotin have remained absent since the 1970s. As part of our program to discover novel classes of compounds that bind RNA, we were intrigued by the fact that a synthesis of gougerotin appeared to be compatible with current solid-phase methodology, which would overcome the previous obstacle to advanced structure−activity relationship studies; i.e., the solution phase synthesis is too complex to be practical for the preparation of a large number of analogues for biological studies. A successful adaptation of the synthesis of gougerotin to solid-phase would allow us unprecedented access into a vast array of analogues and we could rapidly evaluate the hexopyranosyl cytosine class for biological activity.

Therefore, we initiated studies toward a solid-phase total synthesis of gougerotin that would not only be amenable to high-throughput analogue production but would allow us to access previously unexplored positions (e.g., N-4, C-5).

In light of the previous difficulties in obtaining analogues synthetically, our initial efforts were directed toward the total synthesis of gougerotin with a focus on key steps initially developed by Fox and Watanabe.[12,16−24] Using literature methods (Scheme 1), we were able to prepare the starting glycosyl donor **2** from commercially available galactoside **1** on a multigram basis. This compound was then coupled to give **3** and deacetylated under the conditions developed

(10) Menzel, H. M.; Lichtenthaler, F. W. *Nucleic Acids Res., Spec. Publ.* **1975**, *1*, S155.
(11) Clark, J. M., Jr. *Antibiotica* **1967**, *1*, 278.
(12) Fox, J. J.; Watanabe, K. A. *Pure Appl. Chem.* **1971**, *28*, 475.
(13) Lichtenthaler, F. W.; Morino, T.; Winterfeldt, W.; Sanemitsu, Y. *Tetrahedron Lett.* **1975**, 3527.
(14) Coutsogeorgopoulos, C.; Bloch, A.; Watanabe, K. A.; Fox, J. J. *J. Med. Chem.* **1975**, *18*, 771.
(15) Lichtenthaler, F. W.; Morino, T.; Menzel, H. M. *Tetrahedron Lett.* **1975**, 665.
(16) Watanabe, K. A.; Falco, E. A.; Fox, J. J. *J. Am. Chem. Soc.* **1972**, *94*, 3272.

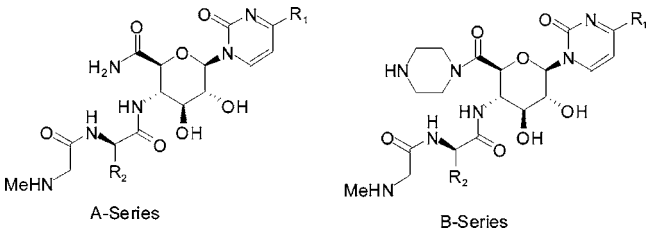
by Fox and Watanabe. A one-step oxidation using TEMPO/bis(acetoxy)iodobenzene (BIAB)[25] was then carried out in CH_3CN (aq) to give the carboxylic acid **5**, in good yield. Protection of the N-4 exocyclic amino group was accomplished to give the Teoc-protected[26] precursor **6**. Our precursor was then coupled with HATU to ArgoGel Rink resin to give the resin-bound precursor **8a**. Reduction of the azido group with Sn(II) chloride liberated the 4′-amino group to give amine **11a**. A subsequent HATU coupling with OtBu-Fmoc-D-serine followed by Fmoc removal with 10% piperidine/DMF gave amine **14a**. A second HATU-mediated coupling with Boc-sarcosine gave the fully protected resin-bound gougerotin. Compound **14a** was treated sequentially with 0.4 M NaOH/MeOH (1:5) to remove the benzoates and Teoc carbamate,[27] and then TFA to give gougerotin (**17a**) as its TFA salt. The structure of gougerotin was determined by 1- and 2-D, ^1H NMR, and ^{13}C NMR, and LCMS was used to determine purity (>90%).

Next, we coupled uracil to our glycosyl donor **2** under modified Vorbruggen conditions[28] using 3 equiv of TMS-OTf under refluxing conditions to give an 80% yield of compound **4**. In contrast, the $SnCl_4$ conditions gave only a small amount of product. After deacetylation and oxidation using the conditions developed for the protected cytidine, we were able to obtain our precursor **7**. A HATU-mediated coupling then gave our resin-bound uracil derivative **9a**.

Nucleophilic displacement of an appropriately installed leaving group on N-4 was then envisaged as a route to those analogues. Treatment of uracil **9a** with a preformed triazolating reagent[29] (i.e., 1,2,4-triazole, $POCl_3$, and Et_3N in CH_3CN) for 4 h followed by nucleophilic aromatic substitution with $BnNH_2$ failed to give any desired product. This was, however, believed to be due to either the primary amide or homogeneous nature of the reagent, as this reaction had never been reported on the solid phase. Therefore, compound **7** was coupled to ArgoGel-MBCHO[10] resin, functionalized as a piperazine, to give compound **9b**. Subjecting this compound with the preformed triazolating reagent followed by treatment with benzylamine in ethanol gave the putative intermediate **10b**. A small amount of this resin was then deprotected with TFA to give the desired product (LCMS [M + H] = 679.2 *m/z*, >95% purity). The remainder of the sequence was then affected (coupling both fragments and deprotection) to give the N-substituted gougerotin precursor **19b**. A similar sequence was carried out on the unfunctionalized uracil derivative **9a** and **9b** to give the uracil derivatives of gougerotin, **18a** and **18b**, respectively. A similar sequence was carried out on compound **6** using the piperazine-resin to give compound **17b**. In all cases, purities of 85 to >95% were achieved after cleavage from the resin, as determined by LC/MS, and these compounds could be assesed for biological activity without the need for any further purification.

A small test library of 12 compounds was prepared and screened for biological activity (Table 1) by appropriate substitutions of the procedure outlined in Scheme 1 (i.e., D-serine to D-diaminobutyric acid, benzylamine to phenethylamine). The results were very encouraging. Small changes in structure were found to have a dramatic impact on the activity of inhibition of peptide bond formation (i.e., inhibition of transcription/translation, T/T) and against pathogenic *E. coli* and *C. albicans*. The presence of a nitrogen at position-4 was absolutely required for activity. Additionally, substitutions at the amide portion of the sugar (series B), the presence of an alkylaryl group on N-4, and the D-diaminobutyric acid substitution at the first peptidic moiety all had positive effects on activity. For example, compound **22b** showed an approximate 4-fold increase over the parent against pathogenic *E. coli* (the MIC for gougerotin is >200

Table 1. Functionalized Gougerotin Analogues



compd	R_1	R_2	series	T/T[a] IC_{50} (μM)	MIC[b] *E. coli* (μM)	MIC[b] *C. albicans* (μM)
17a[c]	NH_2	CH_2OH	A	0.5	>200	50−100
18a	OH	CH_2OH	A	>100	>100	>100
20a	NH_2	$CH_2CH_2NH_2$	A	0.2	>100	>100
21a	OH	$CH_2CH_2NH_2$	A	>100	>100	>100
17b	NH_2	CH_2OH	B	0.4	>100	>100
18b	OH	CH_2OH	B	>100	>100	>100
19b	NHBn	CH_2OH	B	0.5	>100	>100
20b	NH_2	$CH_2CH_2NH_2$	B	0.3	50−100	1−3
21b	OH	$CH_2CH_2NH_2$	B	>100	>100	>100
22b	$NHCH_2Bn$	$CH_2CH_2NH_2$	B	0.5	25−50	NT[d]
23b	$NHCH_2Bn$	CH_2OH	B	2.3	>100	NT[d]
24b	NHBn	$CH_2CH_2NH_2$	B	0.4	50−100	6−12

[a] T/T = inhibition of the transcription/translation sequence in procaryotic system. [b] Minimum inhibitory concentration. [c] Gougerotin. [d] Not tested.

(17) Fox, J. J.; Kuwada, Y.; Watanabe, K. A. *Tetrahedron Lett.* **1968**, 6029.
(18) Kotick, M. P.; Klein, R. S.; Watanabe, K. A.; Fox, J. J. *Carbohydr. Res.* **1969**, *11*, 369.
(19) Watanabe, K. A.; Kotick, M. P.; Fox, J. J. *Chem. Pharm. Bull. (Tokyo)* **1969**, *17*, 416.
(20) Watanabe, K. A.; Kotick, M. P.; Fox, J. J. *J. Org. Chem.* **1970**, *35*, 231.
(21) Watanabe, K. A.; Fox, J. J. *Chem. Pharm. Bull.* **1973**, *21*, 2213.
(22) Chiu, T. M. K.; Warnock, D. H.; Watanabe, K. A.; Fox, J. J. *J. Heterocycl. Chem.* **1973**, *10*, 607.
(23) Watanabe, K. A.; Falco, E. A.; Fox, J. J. *J. Org. Chem.* **1972**, *37*, 1198.
(24) Chiu, T. M. K.; Watanabe, K. A.; Fox, J. J. *Carbohydr. Res.* **1974**, *32*, 211.
(25) Epp, J. B.; Widlanski, T. S. *J. Org. Chem.* **1999**, *64*, 293.
(26) Shute, R. E.; Rich, D. H. *Synthesis* **1987**, 346.
(27) Treatment of a small sample of compound **6** with 0.4M NaOH/MeOH (1:5) rapidly gives compound **5**, indicating that the Teoc comes off in the presence of base. Bjoerkman, S.; Chattopadhyaya, J. *Chem. Scr.* **1982**, *20*, 201.
(28) Vorbrueggen, H. *Acc. Chem. Res.* **1995**, *28*, 509.
(29) Krawczyk, S.; Migawa, M. T.; Drach, J. C.; Townsend, L. B. *Nucleosides, Nucleotides Nucleic Acids* **2000**, *19*, 39.

μM). Most interestingly, compound **20b** showed <3 μM MIC against *C. albicans*, which demonstrates a potential new class of antifungal therapeutic.

In conclusion, we have developed a solid-phase synthesis of the natural product gougerotin and prepared a small test library demonstrating this methodology. Positions amenable to substitution include N-4, the 6′-amide, and the first and second peptide residues and should be amenable to substitutions at C-5 and C-6. The purity of our analogues allows us to assess our compounds for biological activity without the need for chromatography or other purification. Most encouraging though, is the new and improved biological activities discovered using only a very small subset of the potential analogues this methodology makes available. We are currently seeking to prepare several hundred analogues and further expand our methodology.

Acknowledgment. We acknowledge USAMRID DAMD717-02-2-0023 for financial support. The U.S. Army Medical Research Acquisition Activity, 820 Chandler Street, Fort Detrick, MD 21702-5014, is the awarding and administering office.

Supporting Information Available: Experimental procedures and spectral data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0507322