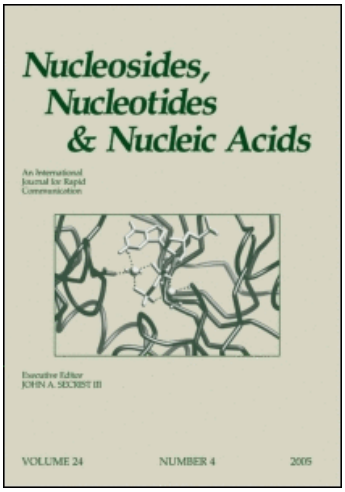

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Patrick J. Hrdlicka[a]; Jan S. Jepsen[b]; Jesper Wengel[a]
[a] Nucleic Acid Center, Department of Chemistry, University of Southern Denmark, Odense M, Denmark [b] Department of Tumor Endocrinology, Danish Cancer Society, Institute of Cancer Biology, Copenhagen, Denmark

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SYNTHESIS AND BIOLOGICAL EVALUATION OF CONFORMATIONALLY RESTRICTED AND NUCLEOBASE-MODIFIED ANALOGS OF THE ANTICANCER COMPOUND 3′-*C*-ETHYNYLCYTIDINE (ECYD)

Patrick J. Hrdlicka ▫ *Nucleic Acid Center, Department of Chemistry, University of Southern Denmark, Odense M, Denmark*

Jan S. Jepsen ▫ *Department of Tumor Endocrinology, Danish Cancer Society, Institute of Cancer Biology, Copenhagen, Denmark*

Jesper Wengel ▫ *Nucleic Acid Center, Department of Chemistry, University of Southern Denmark, Odense M, Denmark*

▫ *A series of conformationally restricted and nucleobase-modified analogs of the anticancer compound 3′-C-ethynylcytidine (ECyd) and its uracil analog (EUrd) have been synthesized. While none of ,the conformationally restricted analogs displayed anticancer activity, 5-iodo-EUrd and 5-bromo-EUrd displayed potent anticancer activity with IC_{50} values of 35 nM and 0.73 μM.*

INTRODUCTION

The clinically evaluated 3′-C-ethynylcytidine (ECyd, **1b**) and its uracil congener (EUrd, **2b**) (Scheme 1) display highly potent antitumor activity against a variety of massive tumors in vitro as well as in animal models with excellent selectivity.[1] To generate EUrd/ECyd analogs that can potentially overcome the crucial uridine-cytidine kinase (UCK) catalyzed phosphorylation step,[2] we synthesized EUrd/ECyd analogs **3b–12b** (Scheme 1) containing modified nucleobase moieties that are known to be tolerated by UCK in non-modified ribonucleosides.[3] Furthermore, since conformational restriction of the otherwise flexible fhranose ring has been a successful strategy to probe for conformational preferences of enzymes involved in nucleoside metabolism and nucleotide polymerization,[4] we synthesized

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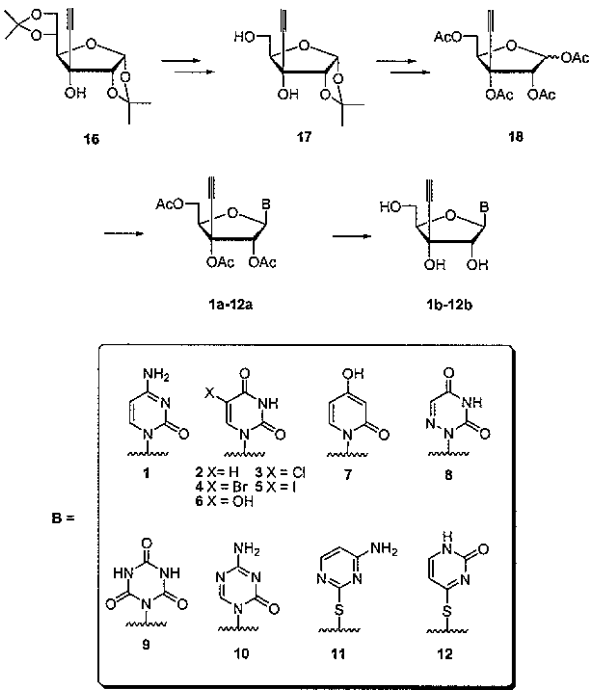
Address correspondence to Patrick J. Hrdlicka, Nucleic Acid Center, Department of Chemistry, University of Southern Denmark, Odense M DK-5230, Denmark; Fax: +45-66158780; E-mail: pjh@chem.sdu.dk



SCHEME 1

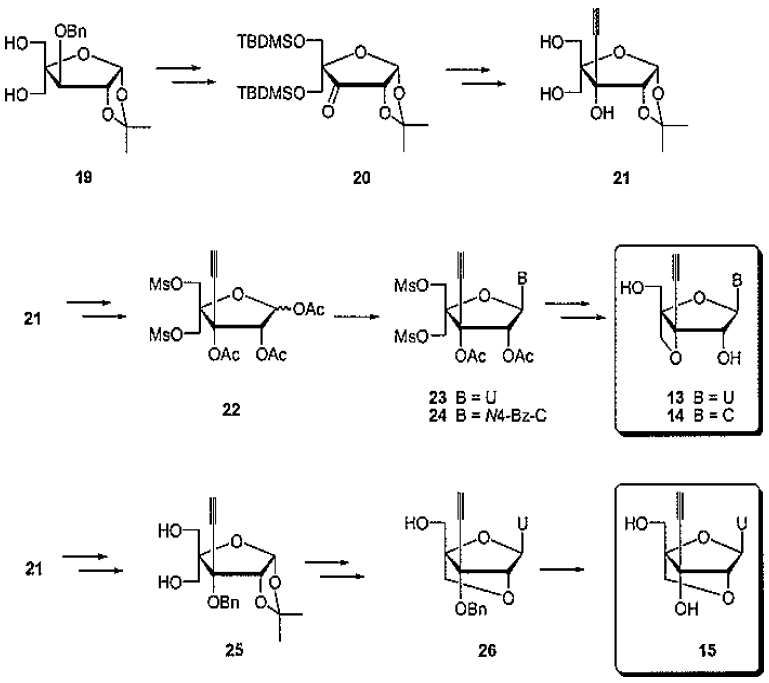
nucleosides **13–15**, which are chimeras of *S*-type 3′-*O*,4′-C-methylene ribonucleoside[5] or N-type LNA nucleosides[6] and EUrd/ECyd (Scheme 2).

RESULTS AND DISCUSSION

Synthesis of ECyd **1b**, EUrd **2b**, and nucleobase-modified analogs hereof **3b–12b** was initiated from furanose 16,[7] which was converted to diol **17** via a three-step sequence involving 5,6-*O*-isopropylidene cleavage, $NaIO_4$-mediated cleavage, and NaBHd-reduction. Further conversion hereof using standard methods gave glycosyl donor **18** (Scheme 1). Subsequent glycosylation of **18** with a series of persilylated nucleobases according to the Vorbrtiggen technique afforded nucleosides **1a–10a**, while reaction with persilyiated 2-thiocytosine or 4-thiouracil furnished S-linked ribosides **11a** and **12a**. Deacylation of **la–12a** mmished nucleosides **1b–12b**.

Synthesis of the conformationally restricted EUrd/ECyd analogs **13–15** initiated from 4′-C-hydroxymethyl pentofuranose **19**,[8] which, after protecting group manipulations and TEMPOIBAIB-mediated oxidation, afforded 3-ulose 20 (Scheme 2).

Subsequent nucleophilic addition of LiC ≡ CTMS and desilylation gave triol **21**, which, upon O5/O5′-mesylation, 1,2-*O*-isopropylidene cleavage, Lewis acid–catalyzed peracetylation (Ac_2O, TMSOTf, CH_2Cl_2, 0°C),[9] and glycosylation of furanose **22** furnished nucleosides **23** and **24**. Treatment of nucleosides **23** and



SCHEME 2

24 resulted in tandem deacylation and selective 3′-*O*,4′-C ring closure (most likely due to preorganization of **23** and **24** in *S*-type conformations), which, after mesylate exchange with benzoate and debenzoylation, afforded the bicyclic nucleosides **13** and **14**.

The synthesis of EUrd-LNA-type analog **15** was initiated from triol **21**, which was converted to furanose **25** via O5/O5′-DMT-protection, O3-benzylation, and detritylation (Scheme 2). Furanose **25** was then converted into nucleoside **26** in a sequence of steps similar to those described for bicyclic nucleosides **13** and **14**. Debenzylation of nucleoside **26** with BCl_3 afforded the desired nucleoside **15**.

Although less active than the reference compounds ECyd **1b** and EUrd **2b** (IC_{50} = 2.2 nM and 2.5 nM, respectively), the 5-iodomidine derivative **5b** displayed very potent anticancer activity (IC_{50} = 35 nM) against a human adenocarcinoma breast cancer (MCF-7) cell line. Decreasing the size of the 5-substituent as in nucleosides **3b** and **4b** resulted in a systematic drop in activity (IC_{50} = 0.73 μM and >25 μM, respectively). 5-Azacytidine **10b** and 6-azauridine **8b** derivatives were only marginally active (IC_{50} = 4.7 μM and 35 μM, respectively) and the remaining compounds inactive. We are currently investigating if the presented compounds are substrates of UCK.

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