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Synthesis of 2',3'-Fused (3.3.0) γ-Butyrolactone-Nucleosides and Coupling with Amino-Nucleosides To Give Amide-Linked Nucleotide-Dimer Analogues[1]

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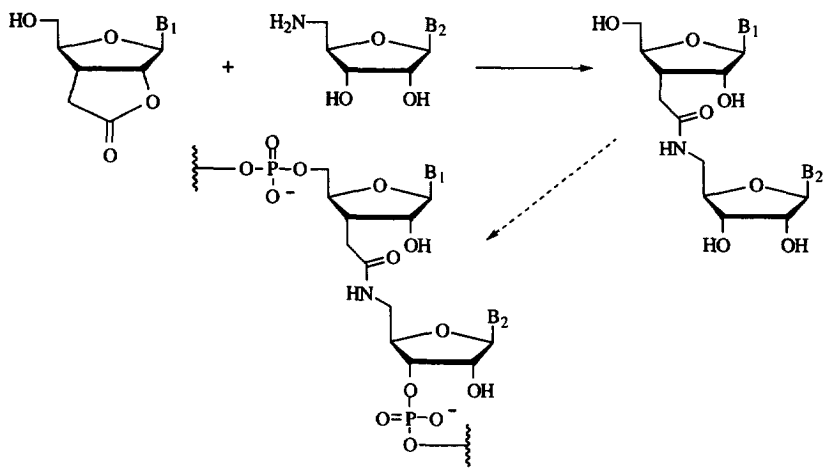
Abstract: Stereoselective hydrogenation of Wittig products obtained readily (via the 3'-ketones) from 2',5'-bis-*O*-(*tert*-butyldimethylsilyl)nucleosides provides efficient access to 2',3'-fused γ-butyrolactone-nucleosides that can be coupled with 5'-amino-nucleosides (2-hydroxypyridine catalysis) to give amide-linked nucleotide-dimer analogues.

Interest in 2',3'-fused (3.3.0) γ-butyrolactone-nucleoside systems has been stimulated by the possibility of branched-chain nucleoside analogues functioning as antitumor and antiviral agents, the reactivity of amine nucleophiles with such strained lactones, and general interest in the development of free radical-mediated routes to fused systems.[2-5] We had considered[3] that the enhanced reactivity of these lactones might allow synthesis of amide-linked nucleotide-analogues directly, without additional protection/deprotection and purification steps required for conventional peptide-bond formation with carboxylic acids and coupling reagents and/or activated esters (Scheme 1). Intensive recent effort has been expended in the preparation of oligonucleotide analogues with modified backbone structures designed to circumvent physical and biological limitations of the natural phosphodiester linkage.[6] Among various replacements investigated, amide linkages have been shown to possess favorable properties including increased duplex stability, increased resistance to nucleolytic degradation, and enhanced membrane permeability owing to the decreased backbone charge.[7]

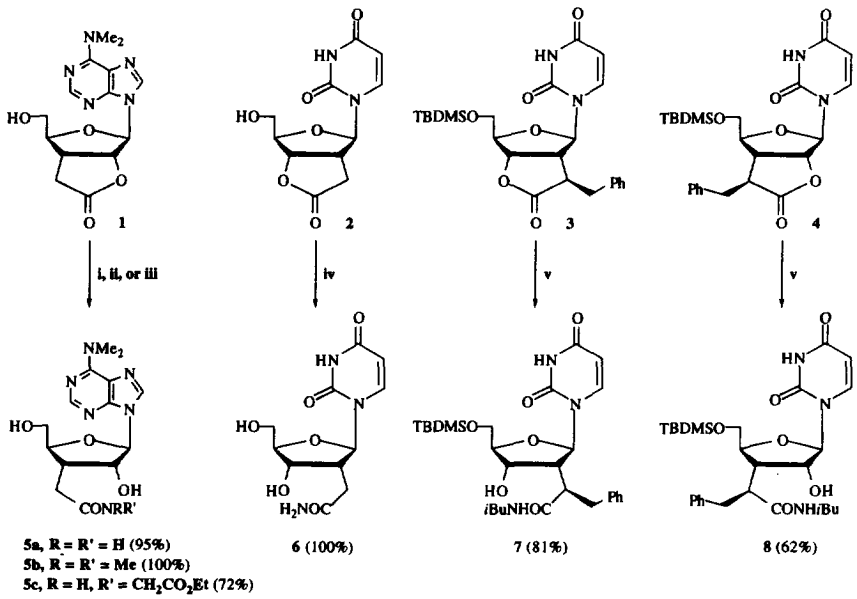
Scheme 1. Amide-Linked Oligoribonucleotide Analogues



Major progress in the synthesis of oligodeoxynucleotide analogues has been achieved and a variety of "antisense" oligomers have been prepared and evaluated biologically. Much less work has been reported with oligoribonucleotide analogues owing to enhanced difficulties with a 2'-hydroxyl function present. However, it has been shown that oligomers with substituents at C2' which confer the "ribo-like" rather than "deoxy-like" conformation exhibit enhanced binding with RNA, additional nuclease stability, and other favorable properties.[8]

The first γ-butyrolactone-nucleoside **1** was prepared by coupling a carbohydrate precursor with a purine.[2] Both 2',3'- and 3',2'-fused types (**2-4**) have been reported recently.[3-5] The reactivity of lactone-nucleosides with ammonia and certain amines (Scheme 2) supported possible applications for the synthesis of amide-linked ribonucleotide-analogue dimers that could be incorporated into nuclease-resistant oligoribonucleotides.

Scheme 2. Prior Studies on Ring Opening of Nucleoside Lactones with Ammonia or Amines[a]

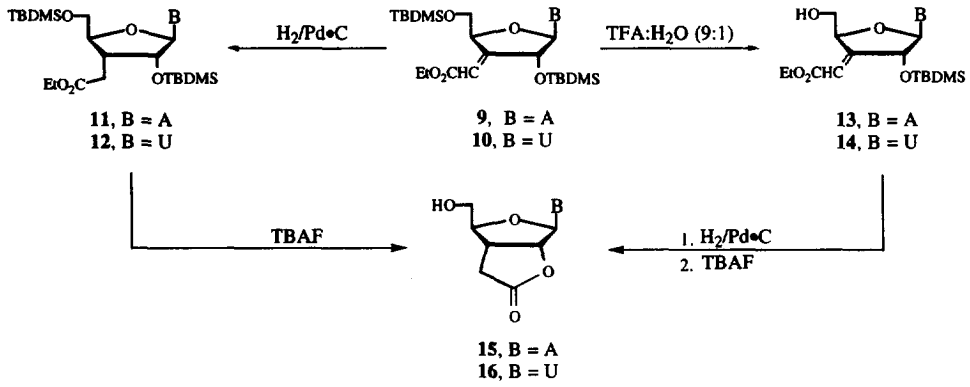


[a](i) NH_3 (liq)/6 h.[2] (ii) $HNMe_2$/0 °C/4 h.[2] (iii) $H_2NCH_2CO_2Et$/DMF/30 h.[2] (iv) NH_3/H_2O.[5] (v) *i*-$BuNH_2/AlCl_3$/16 h.[4]

We synthesized[9] the γ-butyrolactone-nucleosides **15** and **16** from precursors **9**[10] and **10**[3] (Scheme 3). The readily available 2',5'-bis-*O*-(*tert*-butyldimethylsilyl)-3'-keto-(adenosine and uridine)[11] were treated with [(ethoxycarbonyl)methylene]triphenylphosphorane in dichloromethane at reflux to give the Wittig adducts **9**[10] and **10**[3] as single diastereomers (80-90%). No attempts were made to determine the configurations of these intermediates since stereoselective hydrogenation of **9** to **11** had been demonstrated.[12] Analogous reduction of **10** (10% Pd•C/MeOH/5 psi/3 days/ambient temperature) was less stereoselective (≥4:1) than with the adenosine analogue. Structure **12** was indicated by difference NOE experiments (6% enhancement of the 3'-proton resonance upon irradiation of the H2' signal) for the major diastereomer and this assignment was confirmed by

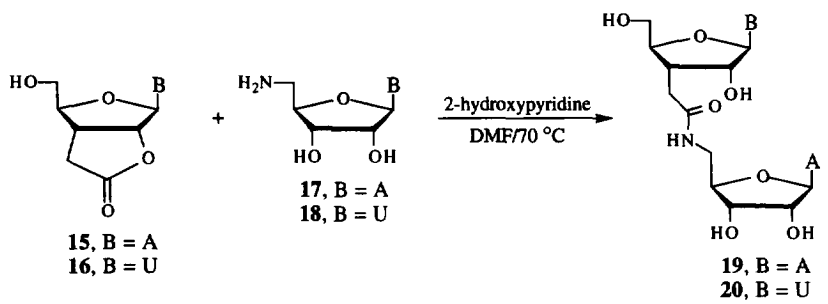
conversion of **12** to the lactone (Scheme 3). The 5'-*O*-TBDMS group was removed selectively from **9** and **10** with 90% aqueous trifluoroacetic acid for 20 min at 0 °C.[13] Catalytic hydrogenation of **13** and **14** and treatment of the products with Bu_4NF/THF gave the γ-butyrolactone-nucleosides **15** and **16** (67 and 77%, respectively). Treatment of **11** and **12** with TBAF/THF gave **15** and **16** (72 and 85%, respectively) which confirmed stereoselective hydrogenation from the β face in all cases.

Scheme 3. Synthesis of Nucleoside Lactones



Aqueous solutions containing lactones **15** or **16** and 5'-amino-5'-deoxyadenosine (**17**) or 5'-amino-5'-deoxyuridine (**18**) at various pH values were stirred at elevated temperatures, and other solvents and reaction conditions were investigated, without success. Similar lack of reactivity of γ-butyrolactone-nucleosides **3** and **4** with isobutylamine had been noted, and aluminum(III) chloride catalysis was required to promote formation of amides.[4] We examined several "acylation promoters"[14] including 1-hydroxybenzotriazole (HOBT), pyrazole, 1,2,4-triazole, and 2-hydroxypyridine, and the latter proved effective. A solution of **15**, 5'-amino-5'-deoxyadenosine (**17**, 5 equiv.), and 2-hydroxypyridine (2 equiv.) in DMF was stirred for 24 h at 70 °C to effect lactone ring opening. The amide-linked nucleotide-analogue dimer **19** was obtained in 60% yield (plus starting materials and decomposition products from the amino-nucleoside **17**). Analogous treatment of **16** with **17** (5 equiv.) and 2-hydroxypyridine (2 equiv.) in DMF for 30 h at 70 °C gave **20** (82%).

Scheme 4. Synthesis of Amide-Linked Dinucleotide Analogues



In summary, readily available 2',5'-bis-*O*-TBDMS nucleosides have been efficiently converted into 2',3'-fused (3.3.0) γ-butyrolactone-nucleosides in gram-scale quantities. These lactones are resistant to ring opening with 5'-amino-5'-deoxynucleosides. However, they can be induced to undergo coupling to give amide-linked nucleotide-analogue dimers in good yields with an excess of the 5'-amino-5'-deoxynucleoside in the presence of 2-hydroxypyridine. A sequence of ester saponification, activation of the carboxylic acid, coupling with the amino-nucleosides, and selective deprotection/protection is under investigation to give amide dimers suitable for synthesizer-mediated incorporation into oligomers of defined sequence.

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