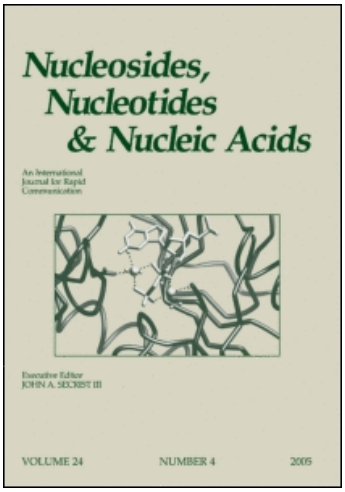

Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis and Antifungal Activity of 3'-Deoxyribonucleosides

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To cite this Article Kumar, Anil , Khan, Shoeb I. , Manglani, Anita , Khan, Z. K. and Katti, S. B.(1994) 'Synthesis and Antifungal Activity of 3'-Deoxyribonucleosides', Nucleosides, Nucleotides and Nucleic Acids, 13: 5, 1049 — 1058
To link to this Article: DOI: 10.1080/15257779408011878
URL: http://dx.doi.org/10.1080/15257779408011878

NUCLEOSIDES & NUCLEOTIDES, 13(5), 1049-1058 (1994)

SYNTHESIS AND ANTIFUNGAL ACTIVITY OF 3'-DEOXYRIBONUCLEOSIDES+

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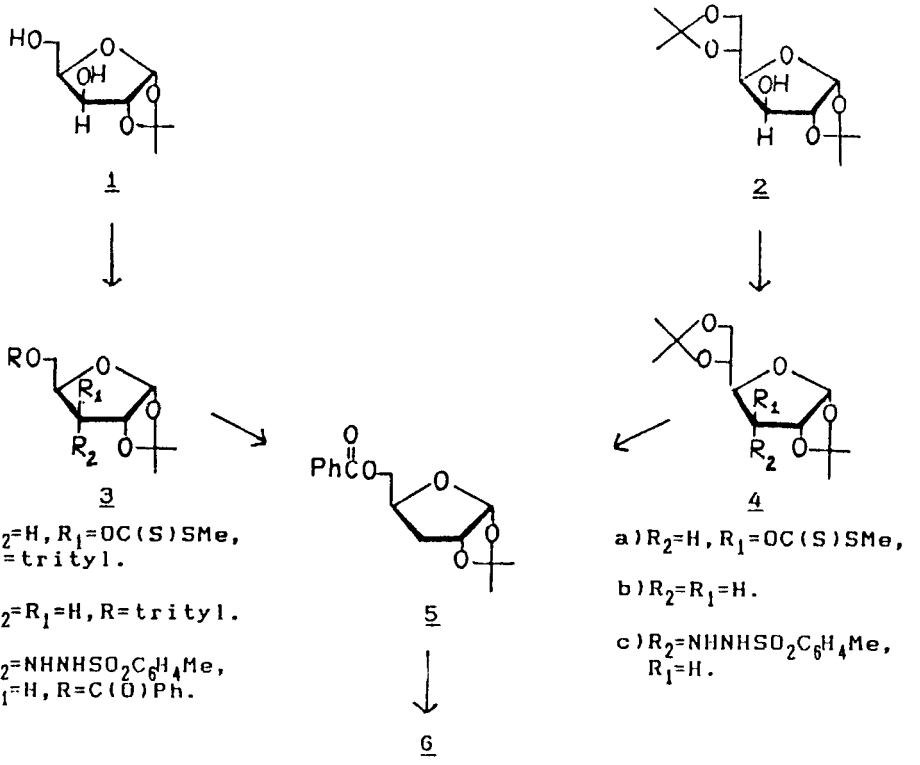
Abstract : Synthesis and antifungal activity of 3'-deoxyribonucleosides containing naturally occurring pyrimidine and purine bases are reported.

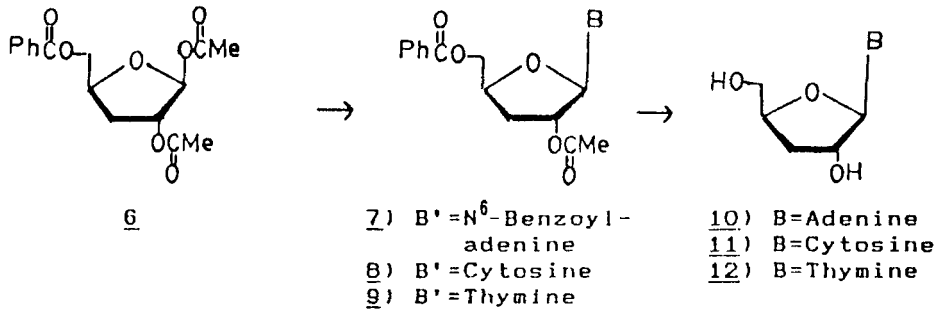
3'-Deoxyribonucleosides exhibit a number of interesting biological activities *viz.*, antibacterial[1], antiparasitic[2] and anticancer[3]. Another important application of these nucleosides is in the direct sequencing of RNA[4]. Recently using these nucleosides modified oligonucleotides have been synthesized to better understand the problems central to biology[5,6]. The wide spectrum of activity shown by these compounds is due to lack of 3' hydroxy group resulting in the inhibition of RNA synthesis. A literature survey of these compounds show that major efforts have gone into the synthesis of 3'-deoxyadenosine[7] (Cordycepin), a potent antibiotic isolated from Cordyceps militaris. However, the 3'-deoxyribonucleosides containing other naturally occurring purine and pyrimidine bases have received limited attention[8-12]. In view of their diverse biological activities, we thought that it would be appropriate to develop a general method for their preparative scale synthesis. Herein we report synthesis of

+ CDRI Communication Number 5203.

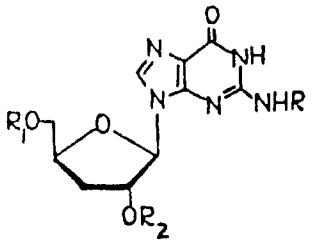
3'-deoxyribonucleosides along with their antifungal activities *in vitro* against some opportunistic fungi.

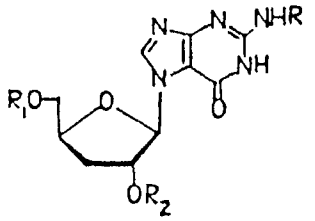
The existing methods for 3'-deoxyribonucleoside synthesis are based on selective deoxygenation of ribonucleosides[7,14] or glycosidation of the heterocyclic bases with the derivatized 3-deoxysugar[8-13]. Our procedure involves the synthesis of key intermediate 5 starting from D(+)-xylose or D(+)-glucose (SCHEME 1). The intermediate 5 was prepared in six steps from D(+)-xylose with an overall yield of 54%. Treatment of compound 3a with Bu_3SnH and α,α'-azobis isobutyronitrile (AIBN)[15] in refluxing toluene furnished the deoxysugar 3b in 80% yield[16]. Removal of the trityl protecting group followed by protection of the free 5-OH with benzoyl group gave the compound 5 in quantitative yield. This compound was also obtained by the same deoxygenation method from D(+)-glucose in seven steps with an overall yield of 23%. We have also synthesized this key intermediate 5 via tosylhydrazone route[17] by selecting the appropriate blocking groups (SCHEME 1). By this route the compound 5 was obtained in overall yield of 57% and 34% starting from D(+)-xylose and D(+)-glucose respectively. It is apparent from the above data and in view of the toxicity associated with organotin compounds, the tosylhydrazone route is a superior method for obtaining 3-deoxysugar derivative 5. The identity of 1,2-*O*-isopropylidene-5-*O*-benzoyl-3-deoxy-ribofuranose (5) was confirmed by ^1H NMR in which methylene protons (H-3) appeared as multiplets at δ 1.7 and δ 2.12. Two singlets at δ 1.28 and δ 1.48 were assigned to the methyl groups of the isopropylidene moiety. Other protons gave signals at the expected chemical shift values. In the FAB-MS spectrum of 5, the molecular ion peak did not appear. Instead a peak at m/z 263 $[M-15]^+$ appeared as a major peak. Acetolysis of compound 5 in our reaction conditions gave 1,2-di-*O*-acetyl-5- *O*-benzoyl-3-deoxyribofuranose (6). After the usual workup and silica gel column chromatography, the compound 6 was obtained in 65% yield. The diacetoxy compound 6 was found to be homogeneous on tlc in different solvent





(SCHEME 1)





13a) $R=R_2=C(O)Me, R_1=C(O)Ph$
14a) $R=C(O)CHMe_2, R_1=R_2=H$
15a) $R=R_1=R_2=H$

13b) $R=R_2=C(O)Me, R_1=C(O)Ph$
14b) $R=C(O)CHMe_2, R_1=R_2=H$
15b) $R=R_1=R_2=H$

(FIG. 1)

systems. The anomeric proton appeared as a singlet at δ 6.1 thereby suggesting that the compound 6 has β-configuration at C-1.

The second objective was to synthesize 3'-deoxy ribonucleosides of all the four naturally occuring heterocyclic bases. The glycosidation was carried out by the modified Hilbert-Johnson method[18]. The compound 6 was condensed with the different silylated bases using $SnCl_4$ as a lewis acid. The crude products 7-9, after the usual workup were purified by silica gel column chromatography. In all the cases, major compound was found to be the required product having β-configuration. The protected nucleosides were subjected to methanolic ammonia treatment to obtain completely deblocked nucleosides 10-12 in quantitative yields. In case of protected 3'-deoxyguanosine, we were unable to separate *N*-9 (13a) and *N*-7 (13b) isomers after nucleosidation (FIG. 1). Subsequently we found that deblocking of the protecting groups of the crude mixture followed by selective isobutyrylation of the exocyclic amino group by the transient protection method[19], the isomeric mixture (14a and 14b) was separable by silica gel column chromatography (using a linear gradient of 0 --> 10% MeOH in $CHCl_3$). The faster moving product 14a (R_f 0.43 in 10% MeOH in $CHCl_3$), after

TABLE 1 : *In vitro* activity of nucleosides by microbroth technique.

Compound (µg/ml)	MIC (µg/ml) against the pathogenic fungi					MFC
	1	2	3	4	5	5
10	50	50	50	25	50	50
11	-	50	50	25	50	50
12	50	50	50	25	50	25
15a	50	50	-	25	-	25
Tolciclate	ND	ND	ND	0.39	ND	0.78

1= Candida albicans, 2= Cryptococcus neoformans, 3= Sporothrix schenckii, 4= Trichophyton mentagrophytes, 5= Aspergillus fumigatus
ND= Not done.

deblocking gave the required *N*-9 isomer 15a as characterised by UV and ^{1}H NMR data. The other product (R_f 0.27 in 10% MeOH in $CHCl_3$) was characterized as *N*-7 isomer 15b[8] .

Biological Activity : All the 3'-deoxyribonucleosides (10-12 and 15a) were evaluated *in vitro* for antifungal activity against five pathogenic fungi (TABLE 1). The minimum inhibitory concentration (MIC) was determined by the microbroth technique[20]. From these studies it was apparent that

Trichophyton mentagrophytes was most sensitive to these compounds at MIC 25 µg/mL. Therefore, additional experiments were carried out only against T. mentagrophytes in order to evaluate the minimum fungicidal concentration (MFC)[21]. The MFC refers to the lowest concentration of the compound which yields less than five colonies per plate. Only two compounds 12 and 15a showed MFC at 25 µg/mL compared to that of tolciclate (anti-dermatophyte).

The synthetic approaches presented above have sufficient generality that can be used to synthesize other 3'-deoxyribonucleosides. Although antifungal activity of these compounds is far less than that of tolciclate, yet they offer an

interesting class of compounds for further exploration. To the best of our knowledge, this is the first report of antifungal activity of the 3'-deoxyribonucleosides. Since completion of this work using these nucleosides we have successfully synthesized 2',5'-oligo-3'-deoxyribonucleotides for some biophysical studies and the results will be reported elsewhere.

EXPERIMENTAL

All capillary melting points were determined on Toshniwal melting point apparatus and the values reported are uncorrected. The tlc was performed on Polygram SIL G/UV_{254} readymade plates in solvent systems, A: 2% Me_2CO in $CHCl_3$ and B: 20% MeOH in $CHCl_3$. Optical rotations were recorded on a Perkin Elmer 241 polarimeter at 30°C. Ultraviolet spectra were recorded on a Perkin Elmer Lambda 15 UV/VIS spectrophotometer. ^1H NMR spectra were recorded on a Perkin Elmer R-30 (90 MHz) or a Bruker WM-400 MHz spectrometers. FAB mass spectra were recorded on an SX-102/DA-6000.

All solvents and reagents used were freshly distilled and dried. Column chromatography was performed using silica gel (230-400 Mesh) supplied by Acme Synthetic Chemicals.

1,2-Di-*O*-acetyl-5-*O*-benzoyl-3-deoxy-D-ribofuranose (6) :

To a solution of 1,2-*O*-isopropylidene-5-*O*-benzoyl-3-deoxyribofuranose[13] (1.1 g, 4 mmol) in glacial AcOH (15 mL) and freshly distilled Ac_2O (1.8 mL, 20 mmol), cooled at 0°C was added dropwise conc. H_2SO_4 (1 mL) and stirred at room temp for 4 h. The reaction mixture was poured in chilled water and the product was extracted with $CHCl_3$. The organic layer was successively washed with H_2O, aq $NaHCO_3$, H_2O and brine. The organic layer was dried over anhyd Na_2SO_4 and concentrated to give 6 as colourless oil (0.83 g, 65 %); Rf 0.53 (A); $[\alpha]_D$ -27.2° (c 1.25, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.0 (m, 2H, Ar-H), 7.4-7.53 (m, 3H, Ar-H), 6.14 (s, 1H, H-1), 5.18 (m, 1H, H-2), 4.28-4.69 (m, 3H, H-4, H-5, H-5), 2.2 (m, 2H, H-3), 2.05 (s,

3H, O-Ac), 1.91 (s, 3H, O-Ac); FAB-MS (m/z) 345 $[M+23]^+$ 321$[M-H]^+$, 279$[M-Ac]^+$, 263$[M-AcOH]^+$.

General method for Glycosidation : Suitably derivatized heterocyclic base (3.72 mmol) was silylated by refluxing in dry MeCN (5 mL) and HMDS (5 mL) for 3 h. The clear solution obtained was concentrated under reduced pressure to dryness. Silylated base thus obtained was dissolved in dry MeCN (10 mL) and added to a solution of 6 in dry MeCN (10 mL) followed by addition of freshly distilled $SnCl_4$ (0.68 mL, 3.72 mmol) and the reaction mixture stirred at room temp for 45 min to 2 h. After completion of reaction, it was concentrated under reduced pressure to 5 mL. This was taken in $CHCl_3$ and successively washed with aq $NaHCO_3$, H_2O and brine. The organic layer was dried over anhyd Na_2SO_4 and concentrated. The product thus obtained was purified by silica gel column chromatography to give 7,8,9 and (13a and 13b) in 55%, 60%, 85% and 57% isolated yields, respectively. The compounds thus obtained were deblocked by methanolic ammonia at room temp to give the corresponding desired nucleosides 10, 11, 12 and (15a and 15b).

9-(3-Deoxy-ß-D-ribofuranosyl)adenine[7] (10)

mp 222°C; Rf 0.44 (B); $[\alpha]_D$ -45.9° (c 0.37, MeOH); UV(H_2O) λ_{max} 256 nm (pH 1), 256.8 nm (pH 7), 257.6 nm (pH 13); ^1H NMR (DMSO-d_6) δ 8.35 (s, 1H, H-8), 8.14 (s, 1H, H-2), 7.27 (s, 2H, NH_2), 5.86 (d, $J_{1',2'}$=2.9 Hz, 1H, H-1'),5.66(d, $J_{2',2'OH}$=4.7 Hz, 1H, 2'-OH), 5.16 (t, $J_{5',5'-OH}$=5.4 Hz, 1H, 5'-OH), 4.57 (brs, 1H, H-2'), 4.34 (brs, 1H, H-4'), 3.69 (m,1H,H-5'), 3.52 (m, 1H, H-5'), 2.25 (m, 1H, H-3'), 1.92 (m, 1H, H-3'); FAB-MS (m/z) 252 $[M+H]^+$.

1-(3-Deoxy-ß-D-ribofuranosyl)cytosine[10] (11) :

mp 220°C; Rf 0.23 (B); $[\alpha]_D$ +45.8° (c 0.72, MeOH); UV (H_2O) λ_{max} 279.6 nm (pH 1), 270.4 nm (pH 7), 272.8 nm (pH 13); ^1H NMR (DMSO-d_6) δ 7.92 (d, $J_{5,6}$ = 7.9 Hz, 1H, H-6), 7.12 (br s, 1H, NH_2), 7.02 (brs, 1H, NH_2), 5.67 (s, 1H, H-1'),

5.64 (d, $J_{6,5}$ = 5.5 Hz, 1H, H-5), 5.48 (d, $J_{2',2'\text{-OH}}$ = 4.7 Hz, 1H, 2'-OH), 5.04 (t, $J_{5', 5'OH}$ = 5.9 Hz, 1H, 5'OH), 4.26 (m, 1H, H-2), 4.08 (s, 1H, H-4'), 3.74 (m, 1H, H-5'), 3.54 (m, 1H, H5'), 1.86 (m, 1H, H-3'), 1.7 (m, 1H, H-3'); FAB-MS (m/z) 228[M+H]$^+$.

1-(3-Deoxy-β-D-ribofuranosyl)thymine[8] (12)

mp 96°C; Rf 0.46 (B); $[\alpha]_D$ -1.84° (c 2.17, MeOH); UV (H_2O) λ_{max} 266.8 nm (pH 1), 267.2 nm (pH 7), 268 nm (pH 13); ^{1}H NMR (DMSO-d_6) δ 7.83 (s, 1H, H-6), 5.65 (d, $J_{1',2'}$ = 2.7 Hz, 1H, H-1'), 5.5 (d, $J_{2',2'\text{-OH}}$ = 4.6 Hz, 1H, 2'-OH), 5.09 (t, $J_{5',5'\text{-OH}}$ = 5.5 Hz, 1H, 5'-OH), 4.25 (m, 1H, H-2'), 4.18 (brs, 1H, H-4'), 3.73 (m, 1H, H-5'), 3.53 (m, 1H, H-5'), 2.0 (m, 1H, H-3'), 1.75 (m, 1H, H-3'); FAB-MS (m/z) 243 [M+H]$^+$.

9-(3-Deoxy-β-D-ribofuranosyl)guanine[10] (15a)

mp 280°C; Rf 0.26 (B); $[\alpha]_D$ -40° (c 0.25, 1:1 MeOH-H_2O); UV (1:1 MeOH-H_2O) λ_{max} 248 nm (pH 1), 247 (pH 7), 272 (pH 13); ^{1}H NMR (DMSO-d_6) δ 8.28 (s, 1H, H-8), 6.16 (s, 2H, NH_2), 6.0 (s, 1H, H-1'), 5.54 (d, $J_{2',2'\text{-OH}}$ = 4.7 Hz, 1H, 2'-OH), 5.01 (t, $J_{5',5'\text{-OH}}$ = 5.9 Hz, 1H, 5'-OH), 4.66 (s, 1H, H-2'), 4.32 (m, 1H, H-5'), 2.1 (m, 1H, H-3'), 1.8 (m, 1H, H-3'); FAB-MS (m/z) 268 [M+H]$^+$.

Biological activity : Microtitre plate with 96(12x8) wells were filled with microbroth (270 µL per well in the first row and 150 µL per well in the subsequent rows). The test samples were dissolved in DMSO (stock solution 1 mg/mL) and 30 µL of this was added separately to the individual wells in the first row. The dilutions were made serially (transferred 150 µL) with a multichannel eppendorf pipette. The test inoculum (10^5 cells/mL) was added (20 µL per well) separately to each well. Appropriate controls were set accordingly. The observations were made after 72 h (at 28±1°C) by automated ELISA reader (Flow Labs. Scotland) based upon optical density (492 nm, matrix 0.2). The last well with no growth of the

fungi was taken to represent the MIC of the test compound. The MFC was determined from the concentration of compounds in microtitre wells. The dilutions (100 µL) were plated on to the SDA and colony forming units (CFU) determined after 48-72 h post incubation (28 ± 1°C).

Acknowledgement : Grateful acknowledgement is made to RSIC, CDRI, for providing UV, NMR and FABMS data. The authors thank Dr. K.B. Mathur, Deputy Director and Head, Division of Biopolymers, CDRI for his encouragement and help.

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Received 10/12/93
Accepted 1/4/94