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Synthesis and Antiviral Activity of Novel D- and L-2′-Azido-2′,3′-dideoxyribofuranosyl-4′-thiopyrimidines and Purines

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Abstract—Novel D- and L-2′-azido-2′,3′-dideoxyribofuranosyl-4′-thiopyrimidines and purines have been synthesized starting from L-xylose and D-xylose, respectively. Among synthesized compounds tested against several viruses such as HIV-1, HSV-1, HSV-2, and HCMV, D-β-N^6-methyladenine (**ent-22a**) and D-α-N^6-methyladenine (**ent-22b**) analogues were found to exhibit significant anti-HCMV activity.

Introduction

Since 3′-azido-3′-deoxythymidine (AZT, zidovudine)[1] has been identified as a potent anti-HIV agent, a number of 2′,3′-dideoxynucleosides having a substituent like azide or fluorine on their 2′ or 3′ position have been synthesized and many of them exhibited potent antiviral activities.[2] Based on these findings, bioisosteric 4′-thionucleosides of the above-mentioned nucleosides have been paid great attention by medicinal chemists for the development of new antiviral agents, but their synthetic difficulties hindered them from being studied for SARs.

With the development of efficient syntheses of 4-thiosugars in the 1990s, several groups published improved syntheses of D-2′-substituted-4′-thionucleosides and their biological activities such as antiviral and antitumor activities,[3–5] but these nucleosides were studied as 2′-deoxy-4′-thio analogues modified only at the 2′-position.

Since 2′,3′-dideoxy-4′-thionucleosides with various substituents on their 2′ or 3′ position have rarely been reported, we have been interested in the synthesis of these classes of 4′-thionucleosides as a part of our ongoing effort for the development of new antiviral agents. Several groups[6] had reported the synthesis of 3′-azido-3′-deoxy-4′-thiothymidine (**1**) with no anti-HIV activity and Marquez and Jeong[7,8] had reported the synthesis and conformations of 2′,3′-dideoxy-2′- or 3′-fluoro-4′-thiouridines and cytidines (**2a** and **2b**) in which 2′-'down'-fluoro cytidine analogue showed weak anti-HIV activity in CEM cells (Fig. 1). From the X-ray crystallographic analyses, conformations of **2a** and **2b** were found to be the same as those of the corresponding 4′-oxonucleosides.[7,8] Therefore, since the synthesis of 2′,3′-dideoxy-4′-thionucleosides in which the 2′-position was substituted with azide has never been reported so far, it is interesting to synthesize this class of nucleosides and to evaluate them for antiviral activity. It is also interesting to synthesize D- and L-nucleosides and to compare their antiviral activities. Here, we report the first synthesis and antiviral activity of D- and L-2′-'down'-azido-2′,3′-dideoxy-4′-thionucleosides (**3** and **ent-3**) utilizing our versatile intermediate, 4′-thioarabitol derivative[9] developed by our laboratory.

Results and Discussion

Our synthetic strategy was first to synthesize the glycosyl donor and then to condense with nucleosidic bases. Synthesis of the glycosyl donor **9** is shown in Scheme 1.

D-Xylose was converted to the 4-thioarabitol derivative **4** according to the efficient synthetic procedure developed by our laboratory.[9] Benzyl protecting group in **4** was removed by treating it with boron trichloride at

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−78 °C in 84% yield to give the diol which was selectively silylated with TBDPSCl to afford monosilylate **5**. To remove the hydroxyl group at the C3 position of **5**, compound **5** was treated with phenyl chlorothionoformate to give the thiocarbonate, which, without purification, was reacted with *n*-tributyltin hydride and triethyl borane to give the deoxygenated product **6** in 70% yield. Compound **6** was treated with methanolic ammonia to give **7**. To introduce the azido group at the C2 position of **7**, Mitsunobu conditions (DPPA, DEAD, PPh_3) were employed, but were unsuccessful. Therefore, mesylation (99%) of **7** followed by the treatment of the resulting mesylate with sodium azide in DMF at 40 °C, afforded the desired azide **8** (84%). As reported by our laboratory[10] and Yoshimura and his co-workers,[5b] azidation was proceeded with pure S_N2 type without participation of ring sulfur unlike DAST fluorination. Oxidation of **8** with *m*CPBA at −78 °C followed by refluxing with acetic anhydride gave the L-glycosyl donor **9**.

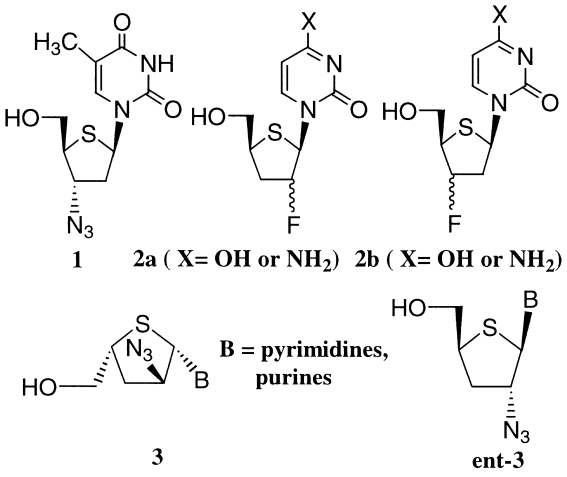
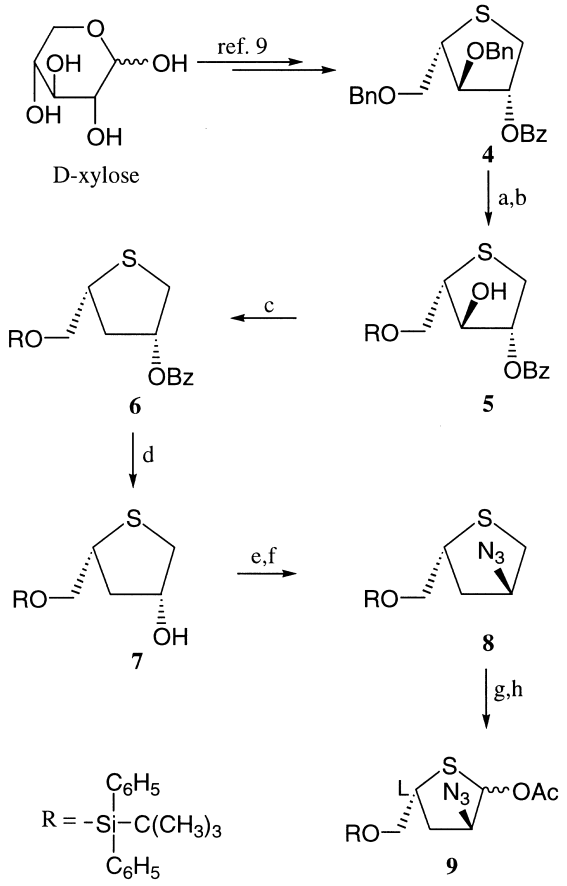
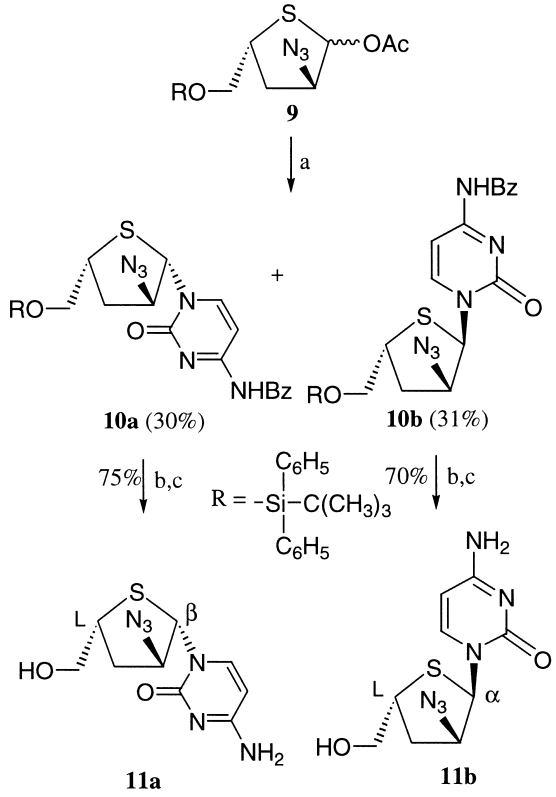


Figure 1. Rationale for the design of the target compounds.



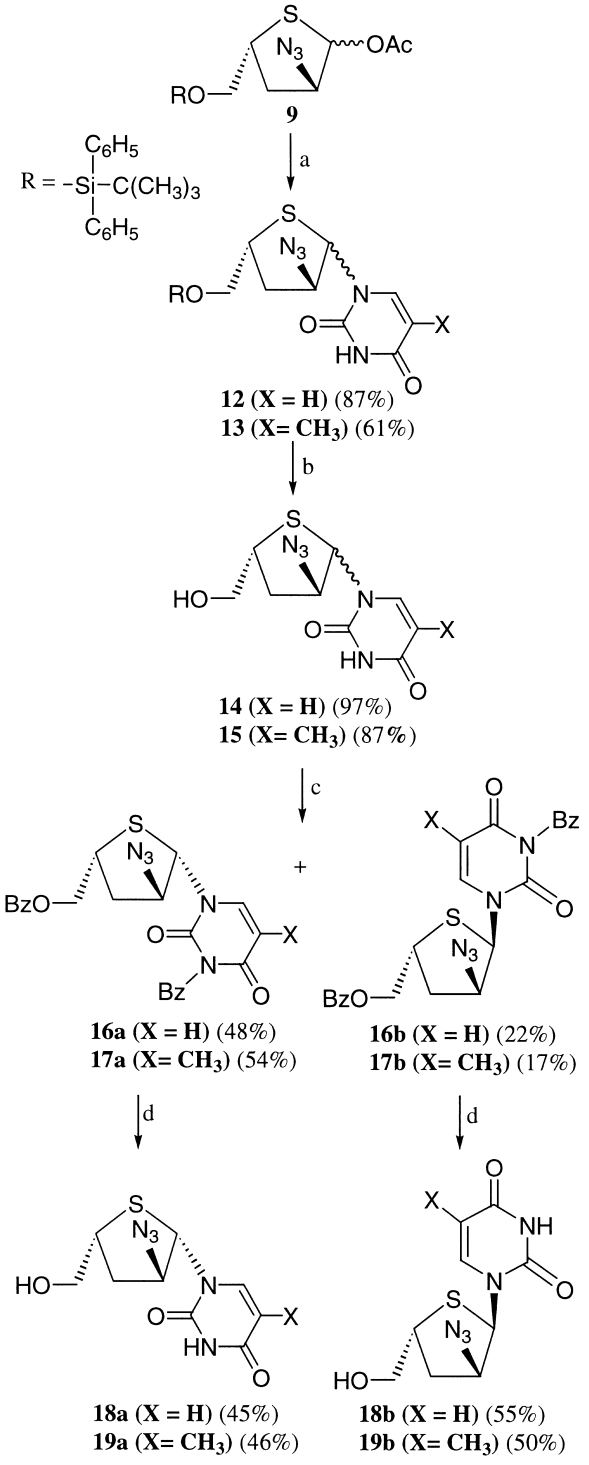
Scheme 1. Reagents and conditions: (a) BCl_3, CH_2Cl_2, −78 °C, 4 h, 84%; (b) RCl, imidazole, DMF, rt, 1.5 h, 76%; (c) i. PhOC(S)Cl, DMAP, CH_3CN, rt, 24 h. ii. Et_3B, *n*-Bu_3SnH, C_6H_6, 0 °C, 70%; (d) NH_3, MeOH, rt, 24 h, 78%; (e) MsCl, Et_3N, DMAP, CH_2Cl_2, 40 °C, 24 h, 99%; (f) NaN_3, DMF, 40 °C, 72 h, 84%; (g) *m*CPBA, CH_2Cl_2, −78 °C, 3 h, 98%; (h) Ac_2O, 100 °C, 24 h, 84%.

For the synthesis of the L-cytosine analogues (**11a** and **11b**) illustrated in Scheme 2, L-acetate **9** was condensed with silylated N^4-benzoylcytosine in the presence of TMSOTf in 1,2-dichloroethane to give L-β-anomer **10a** (30%) and L-α-anomer **10b** (31%) after separation by silica gel column chromatography. Anomeric configurations of **10a** and **10b** were assigned by ^{1}H NOE experiment and the comparison of chemical shifts and coupling constants of their ^{1}H NMRs. Irradiation of 2′-H of compound **10a** gave small NOE effect (7.1%) on its 1′-H, indicating β-anomer, while larger NOE effect (9.1%) was observed in the same experiment in the case of compound **10b**, resulting in α-anomer. Besides NOE effect, similar ^{1}H NMR patterns of **10a** and **10b** were observed with those of 2′-substituted-2′,3′-dideoxy-4′-oxonucleosides.[11] For example, coupling constant (J_{trans} = 1.2 Hz) of 1′-H in **10a** was smaller than that (J_{cis} = 4.9 Hz) of 1′-H in **10b** and



Scheme 2. Reagents and conditions: (a) silylated N^4-benzoylcytosine, TMSOTf, $ClCH_2Cl_2Cl$, rt, 24 h; (b) NH_3, MeOH, rt, 24 h; (c) *n*-Bu_4NF, THF, rt, 1 h.

chemical shift of the β-anomeric proton was upfield relative to the α-anomeric proton. Furthermore, the 4′-proton of α-anomer **10b** appeared downfield from that observed for β-anomer **10a** because of the deshielding effect by cytosine and the 5′-protons of **10a** appeared downfield from those observed for **10b** due to the same deshielding effect. This typical ^{1}H NMR pattern was maintained in the case of other pyrimidine and purine nucleoside derivatives which are shown in Scheme 3 and

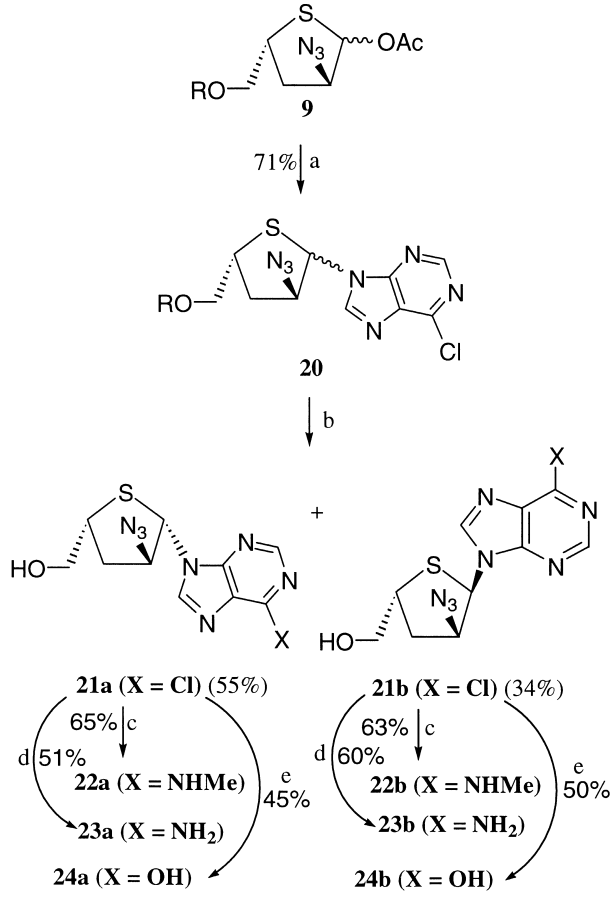


Scheme 3. Reagents and conditions: (a) silylated uracil or thymine, TMSOTf, $ClCH_2Cl_2Cl$, rt, 24 h; (b) *n*-Bu_4NF, THF, rt, 1 h; (c) BzCl, pyridine, 70 °C, 24 h; (d) NH_3, MeOH, rt, 24 h.

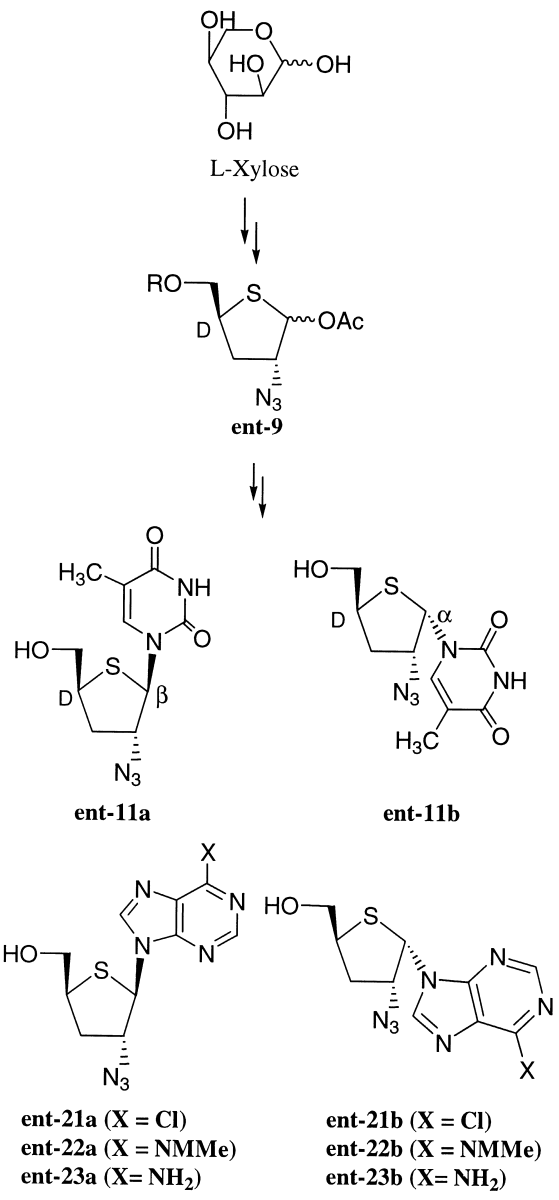
4. Compounds **10a** and **10b** were treated with methanolic ammonia followed by treating with *n*-tetrabutylammonium fluoride to afford the final L-cytosine analogues **11a** and **11b**, respectively.[12]

Synthesis of the L-uracil derivatives (**18a** and **18b**) and L-thymine derivatives (**19a** and **19b**) is shown in Scheme 3. L-Acetate **9** was condensed with silylated uracil and thymine using TMSOTf as a Lewis catalyst to give the inseparable α/β mixtures **12** and **13**, respectively. Desilylation of **12** and **13** with *n*-tetrabutylammonium fluoride also resulted in inseparable α/β mixtures **14** and **15**, respectively. In order to separate the inseparable anomeric mixture by silica gel column chromatography, α/β mixture **14** was perbenzoylated at 70 °C to afford separable L-β-anomer **16a** and L-α-anomer **16b**, which were deblocked separately using methanolic ammonia to give the final L-β-anomer **18a** and L-α-anomer **18b**, respectively. According to the similar procedure used in the preparation of L-uracil analogues **18a** and **18b**, L-thymine analogues **19a** and **19b** were synthesized from **15** via benzoylates **17a** and **17b**, respectively.

Synthesis of the L-purine derivatives was accomplished from the condensation of acetate **9** with silylated 6-chloropurine at 60 °C for 15 h to give inseparable α/β mixture **20** as illustrated in Scheme 4. As reported pre-



Scheme 4. Reagents and conditions: (a) silylated 6-chloropurine, TMSOTf, $ClCH_2Cl_2Cl$, rt to 60 °C, 24 h; (b) *n*-Bu_4NF, THF, rt, 1 h; (c) $MeNH_2$, MeOH, 80 °C, 15 h; (d) NH_3, MeOH, 80 °C, 15 h; (e) 1 M NaOMe, $HSCH_2CH_2OH$, MeOH, reflux, 24 h.



Scheme 5.

viously,[13] it was found that initially formed N^3-isomer cleanly migrated to N^9-isomer on heating. After inseparable α/β mixture **20** was desilylated with *n*-tetrabutylammonium fluoride, the mixture could be separated by silica gel column chromatography to give L-β-anomer **21a** (55%) and L-α-anomer **21b** (34%). L-β-6-Chloropurine derivative **21a** was converted to the L-β-N^6-methyladenine derivative **22a** (65%) and L-β-adenine derivative **23a** (51%) by treating with methylamine and ammonia in methanol at 80 °C for 15 h, respectively. Compound **21a** was also converted to the L-β-hypoxanthine derivative **24a** (45%) by refluxing with mercaptoethanol and sodium methoxide. L-α-6-Chloropurine derivative **21b** was also converted to the L-α-N^6-methyladenine derivative **22b** (63%), L-α-adenine derivative **23b** (60%) and L-α-hypoxanthine derivative **24b** (50%) according to the same procedure used in the synthesis of L-β-purine nucleoside derivatives.

The D-pyrimidine and purine nucleosides (**ent-11a, ent-11b, ent-21a–23a** and **ent-21b–23b**) were synthesized according to the same procedure used in the preparation of the corresponding L-pyrimidine and purine nucleosides (Scheme 5). Antiviral assays for the synthesized D- and L-nucleosides were performed against HIV-1 (MT-4 cells), HSV-1 (CCL81 cells), HSV-2 (CCL81 cells), and HCMV (HEL299 cells). All tested compounds did not exhibit significant antiviral activities except against HCMV. Among compounds tested, D-α-N^6-methyladenine analogue (**ent-22b**) was found to be the most potent in AD-169 (EC_{50} = 19.15 μg/mL) and Davis (EC_{50} = 25.79 μg/mL) infected cell lines without cytotoxicity up to 100 μg/mL when compared to positive control, ganciclovir (EC_{50} = 0.98 μg/mL, in AD-169; EC_{50} = 4.07 μg/mL in Davis) or phosphonoformate (EC_{50} = 9.79 μg/mL, in AD-169; EC_{50} = 86.54 μg/mL in Davis). It is not surprising that D-α-isomer **ent-22b** showed anti-HCMV activity in view that D-α-xylofuranosyl-4′-thiopurine nucleosides also exhibited potent anti-HCMV activity as reported by Secrist and co-workers.[6] D-β-N^6-Methyladenine analogue (**ent-22a**) was also found to show anti-HCMV activity in AD-169 (EC_{50} = 26.09 μg/mL) and Davis (EC_{50} = 90.21 μg/mL) infected cell lines without cytotoxicity up to 100 μg/mL. L-β-6-Chloropurine (**21a**), D-β-6-chloropurine (**ent-21a**), and D-α-6-chloropurine (**ent-21a**) were found to exhibit toxicity-dependent anti-HCMV activity. Other D- and L-nucleosides showed neither anti-HCMV activity nor cytotoxicity.

In summary, we have accomplished the first synthesis of D- and L-2′-'down'-azido-2′,3′-dideoxy-4′-thiopyrimidine and purine nucleosides. Although the tested compounds did not exhibit excellent antiviral activity, it is believed that observation of significant anti-HCMV activity allows this class of compounds to be the new template for the development of new antiviral agents.

Acknowledgements

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12. Compound **11a** (β-isomer): mp 123.8 °C; UV (MeOH) λ_{max} 274 nm; ^{1}H NMR (DMSO-d_6) δ 8.04 (d, 1H, J = 4.5 Hz, H-6), 7.19 (br s, 2H, NH_2, D_2O exchangeable), 5.94 (d, 1H, J = 2.4 Hz, 1′-H), 5.72 (d, 1H, J = 4.5 Hz, H-5), 5.19 (br s, 1H, OH, D_2O exchangeable), 4.35 (m, 1H, 2′-H), 3.66–3.49 (m, 3H, 4′-H and 5′-H), 2.03–1.86 (m, 2H, 3′-H). Anal. calcd for $C_9H_{12}N_6O_2S$: C, 40.29; H, 4.51; N, 31.32. Found: C, 40.69; H, 4.39; N, 30.98. Compound **11b** (α-isomer): mp 181.8 °C; UV (MeOH) λ_{max} 275 nm; ^{1}H NMR (DMSO-d_6) δ 7.66 (d, 1H, J = 4.6 Hz, H-6), 7.00 (br d, 2H, NH_2, D_2O exchangeable), 6.14 (d, 1H, J = 3.4 Hz, 1′-H), 5.54 (d, 1H, J = 4.6 Hz, H-5), 4.87 (t, 1H, J = 3.4 Hz, OH, D_2O exchangeable), 4.35 (m, 1H, 2′-H), 3.51–3.19 (m, 3H, 4′-H and 5′-H), 1.85–1.80 (m, 2H, 3′-H). Anal. calcd for $C_9H_{12}N_6O_2S$: C, 40.29; H, 4.51; N, 31.32. Found: C, 40.19; H, 4.04; N, 31.28.

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