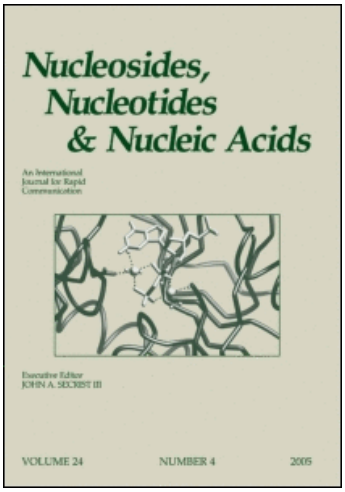

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SYNTHESIS AND ACTIVITY AGAINST HBV OF NOVEL *TETRA*-SECONUCLEOSIDE ANALOGUES OF DYPHLLINE HAVING THE ACYCLIC CHAINS OF ACV AND HBG

El Sayed H. El Ashry, Laila F. Awad, Nagwa Rashed, Adel AbdelRahman, and Hana A. Rasheed

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□ *Selective alkylation of dyphylline (**1**) with (2-acetoxyethoxy)methyl bromide (**2a**) or 4-acetoxybutyl bromide (**2b**) afforded 3′-O-[(acetoxyethoxy)methyl]dyphylline (**3a**) and 3′-O-(4-acetoxybutyl)-dyphylline (**3b**), respectively. A trans esterification process rather than alkylation of the dihydroxypropyl side chain in **1** had taken place during the reaction with 2-p-toluoyloxy)ethyl chloride (**5**) to afford the respective 3′-toluoyloxy derivative **7** and not the anticipated 3′-O-[(p-toluoyloxy)ethyl]-dyphylline (**6**). Deacylation of **3a,b** and **7** afforded **4a,b** and **1**, respectively. Viral screening of selected compounds against HBV has been investigated.*

Keywords Dyphylline; *Tetraseco*-Nucleosides; Acylonucleosides; ACV; HBG

INTRODUCTION

Considerable interest in the synthesis of nucleoside analogues resulted from their pronounced antiviral and antitumoral activities. In recent years major efforts have been directed toward the synthesis of *seco* nucleosides with various side chains and aglycons with the hope to discover new and effective leads as antiviral agents.[1–6] The skeletal modification of the heterocyclic portion and/or alkanol side chain of acyclo nucleosides have provided numerous *seco* nucleoside analogs possessing a wide variety of biological activities. Structure-activity relationship studies of acyclo nucleosides bearing a hydroxypropyl side chain in particular (*S*)-9-(2,3-dihydroxypropyl)adenine,[7] 3-(adenin-2-yl)-2-hydroxypropanoic acid (AHPA)[8,9] and a group of eritadenines[10] attracted our attention. All

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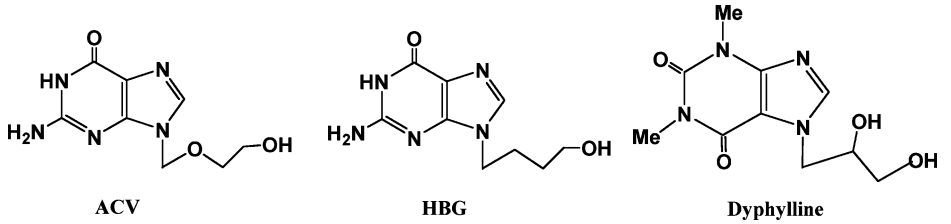


FIGURE 1

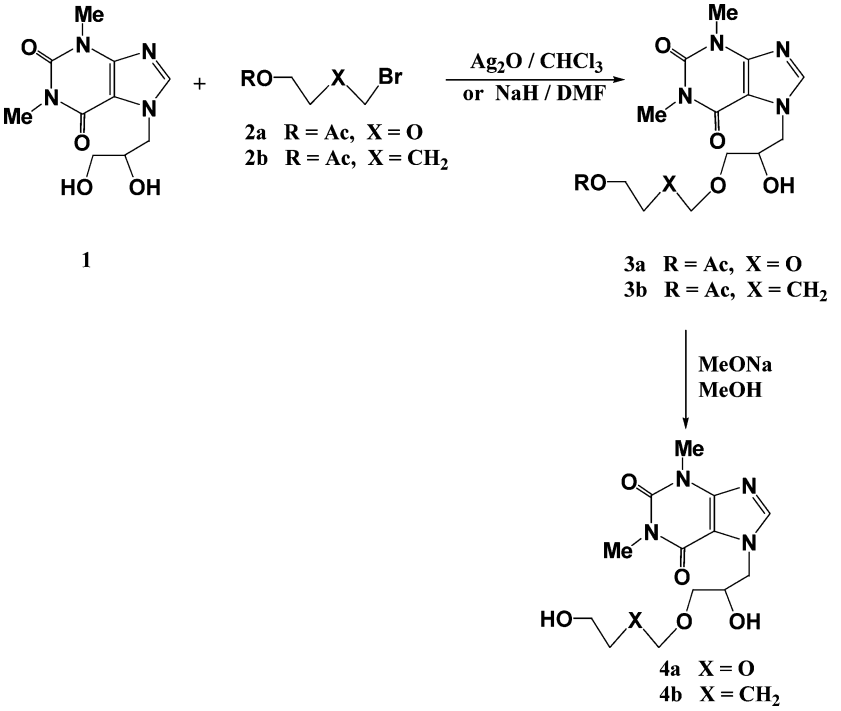
these compounds inhibit SAH hydrolases[11] and hence exert effect on proliferative processes. In the course of our ongoing research program devoted to the synthesis of carboacyclonucleosides containing 2,3-dihydroxypropyl chain,[12,13] novel acyclonucleoside analogs were synthesized by modification of the hydroxypropyl moiety.

Dyphlline (**1**) is effective in treatment bronchial asthma and we had modified, in an earlier publication, the glycerolyl side chain via its periodate oxidation.[13] Attracted by the discovery of acyclovir (**I**) and the carboacyclic analogue HBG **(II)**,[14,15] as potent and selective antiherpetic agents, the insertion of the (2-hydroxyethoxy)methyl and the 4-hydroxybutyl moieties, which are the side chains in ACV and HBG, respectively, to the 3′-*O*-position of dyphylline became our objective in this work. Moreover, selected compounds were found to exhibit interesting ant-HBV activity.

RESULTS AND DISCUSSIONS

Alkylation of dyphylline (**1**) with either (2-acetoxyethoxy)methyl bromide (**2a**) or 4-acetoxybutyl bromide (**2b)** in the presence of silver oxide as catalyst and chloroform as solvent resulted in a selective introduction of the (2-acetoxyethoxy)methyl and 4-acetoxybutyl moieties on the 3′-hydroxy group of **1** to give 3′-O-(acetoxyethoxy)methyl- and 3′-O-(4-acetoxybutyl)dyphyllines (**3a)** and (**3b)** in 58 and 60% yield, respectively (Scheme 1). On the other hand an attempt to perform the reaction using sodium hydride in DMF did not lead to a significant improvement in the yield. The structures of both compounds were assigned from their elemental analyses and ^{1}H NMR spectra. The introduction of the acyclic residue was confirmed by the presence of signals at δ 2.08 or 2.14 assigned for one acetyl group in each of **3a** and **3b**, respectively (Scheme 1).

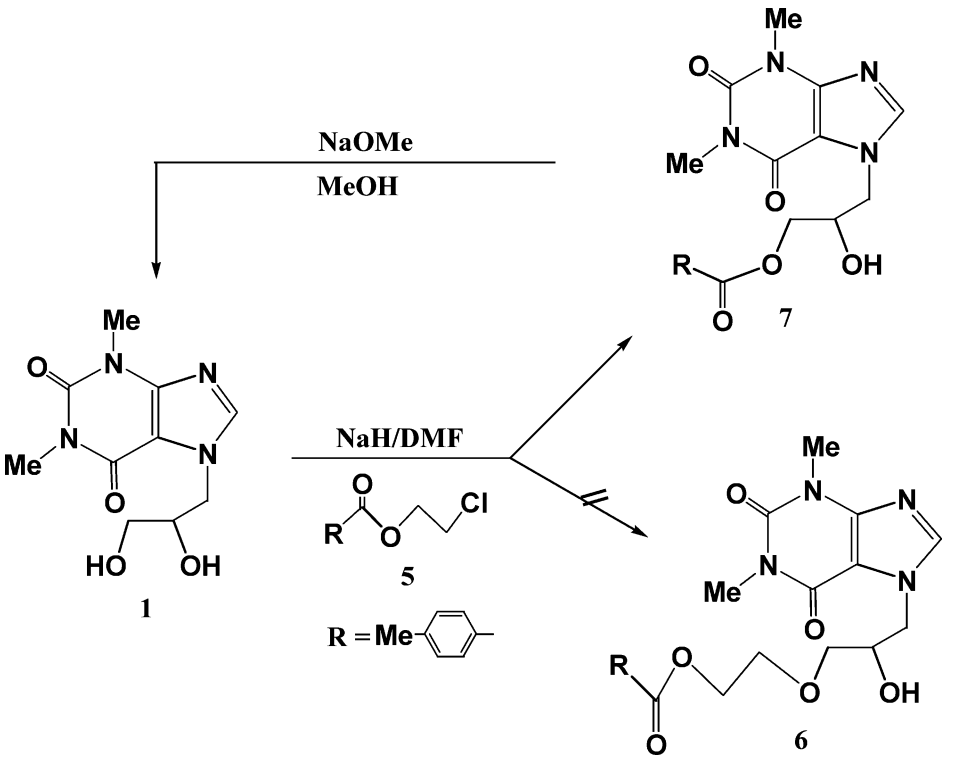
The methylene protons signals of the acyclic ethoxy moiety of **3a** were assigned as a doublet of doublet at δ 3.66 ($AcOCH_2$-$\underline{CH_2}O$) while the second one was appeared under a multiplet in the range of δ 4.16–4.36 together with both of H-2′ and H-3″ of dyphylline. The singlet corresponding to OCH_2O protons was assigned at δ 4.76. The methylene protons of the butyl moiety of **3b** were resonated as three multiplets at δ 1.26–1.44, 1.71–1.75,



SCHEME 1

and 4.15–4.35. These data confirmed the introduction of only one alkyl moiety on **1**. The deprotected products **4a** and **4b** were obtained in good yield by treatment of **3a** and **3b**, respectively with sodium methoxide in methanol. The structures of both compounds were based on their elemental analyses and spectral data which showed the absence of the acetyl signals.

On the other hand, attempted alkylation of **1** with 2-(*p*-toluoyloxy)ethyl chloride (**5)** in the presence of sodium hydride in DMF gave 3′-O-(*p*-toluoyloxy)ethyl derivative **6**, the 3′-O-*p*-toluoyl derivative **7**. Combustion analysis and mass spectrum of the product (Scheme 2) were characteristic for the structure of **7** which showed a molecular ion peak at m/z 372 in agreement with the molecular formula $C_{18}H_{20}N_4O_5$. The ^{1}H NMR spectrum of **7** indicated the absence of signals due to the methylene protons of the ethyl moiety anticipated in **6** and the presence of a tolyl group which appeared as a singlet (δ 2.40) and two doublets (δ 7.23 and 7.89) corresponding to the methyl and aromatic protons, respectively. These data confirmed the introduction of one *p*-toluoyl group on **1** rather than the *p*-toluoyloxyethyl group. The location of the *p*-toluoyl on the O-C-3′ was indicated by the appearance of the signals of protons of C-3′ (δ 4.10 and 4.37) in a downfield region compared to that of **1** (δ 3.36–3.45). On the other hand, carrying out the same reaction using silver oxide as catalyst to avoid such unexpected result led to the recovery of the starting material **1**.



SCHEME 2

The formation of the ester **7** could be attributed to the high electrophilicity of the carbonyl carbon of **5** rather than that of the methylene group linked to the chlorine atom. This led to its preferential attack by the anion generated from **1** and sodium hydride to give **7**. Thus, a trans esterification process proceeded more likely than the alkylation one. Deacylation of **7** with sodium methoxide in methanol afforded the starting dyphylline (**1**) which also unequivocally proved the structure of the ester **7**; otherwise **6** would afford a different compound than **1**.

BIOLOGICAL EVALUATION

The results of the viral screening against HBV of the selected compounds **3a**,**b** and **4a**,**b** indicated that compound **4b** showed moderate viral replication inhibition and mild cytotoxicity with selective index >227.3. On the other hand, the effective concentration of compounds **3a**, **3b** and **4a** was 10.0 μM which showed weak antiviral activity with low cytotoxicity with selective index >10.50, >12.50 and >140.80, respectively. These results indicated that the unacetylated derivatives showed better inhibition than the respective acetyl derivatives. Moreover, the presence of oxygen in the side

TABLE 1 Cytotoxic effect (CC_{50}), inhibitory concentration (IC_{50}) and selective index (SI) of compounds **3** and **4**

Compdound No.	HBV DNA IC_{50} (μM)	Hep G2 2.2.15 CC_{50} (μM)	SI
Lamivudine	<0.10	>100	>1000
3a	9.50	>100	>10.50
3b	8.00	>100	>12.50
4a	0.71	>100	>140.8
4b	0.44	>100	>227.3

chain of **4a** which is a characteristic feature in ACV is not necessary in this series of compounds where the carbo analogue showed a better inhibition.

EXPERIMENTAL

Melting points were determined on a Mel-Temp apparatus and are uncorrected. IR spectra were recorded with a unicam SP1025 spectrometer. EI mass spectra were recorded on a Varian MAT 311A spectrometer. ^{1}H NMR spectra were recorded with a Bruker AC 300 NHz. The chemical shifts are expressed on the δ scale using Me_4Si as a standard. J-values are given in Hz. TLC was performed on Merck Silica Gel 60 F254 with detection by UV light. Microanalyses were performed in the unit of Microanalysis at the Faculty of Science, Cairo University.

(±)-3′-O-Substituted-dyphylline (3a,b)

Method A

A mixture of compound **1** (0.25 g, 1.0 mmol), silver oxide (1.0 g) and molecular sieves (1.0 g) was stirred for 1 hour. in dry chloroform (10 ml) at room temperature and then a solution of **2a** (0.197 g, 1.0 mmol) or **2b** (0.15 g, 1.0 mmol) in dry chloroform (5 ml) was added over 2 hours. The stirring was continued for overnight. The mixture was filtered, washed with chloroform, and the filtrate was evaporated under reduced pressure. The residue was purified by flash column chromatography using petroleum ether **:** ethyl acetate (5**:**1) to afford **3a** (58% yield) and **3b** (60% yield).

Method B

A stirred solution of **1** (0.25 g, 1.0 mmol) in dry DMF (5 ml) was treated with sodium hydride (0.024 g, 1.0 mmol). After complete evolution of hydrogen gas, the mixture was heated at 80°C for 1 hour and then treated with compound **2a** or **2b** (1.0 mmol) with stirring at room temperature for overnight. The reaction mixture was diluted with cold water (20 ml) and extracted with ethyl acetate (3 × 20 ml). The organic layers were collected, washed with water, dried and evaporated under reduced pressure. The

residue was purified by column chromatography using petroleum ether : ethyl acetate (5:1) to give **3a** (55% yield) and **3b** (29% yield). They were identical with those obtained from method a.

(±)-3′-O-[(2-Acetoxyethoxy)methyl]dyphylline (3a)

Colorless syrup; R_f 0.15 (petroleum ether : ethyl acetate 1:1); ^{1}H NMR ($CDCl_3$, 300 NHz) δ_H: 2.08 (s, 3 H, OAc), 3.40, 3.59 (2 s, 6 H, 2 NMe), 3.66 (dd, 2 H, *J* 4.1 Hz, *J* 6.1 Hz, CH_2-CH_2-O), 3.80 (t, 1 H, $J_{2',3'}$ 8.0 Hz, H-3′), 4.16–4.36 (m, 4 H, H-2′, H-3″, CH_2-OAc), 4.59 (dd, 1 H, $J_{1',2'}$ 3.3 Hz, H-1′), 4.68 (t, 1 H, $J_{1',1''}$ 11.3 Hz, H-1″), 4.76 (s, 2 H, OCH_2O), 4.83 (bs, 1 H, D_2O exchangeable, OH), 7.69 (s, 1 H, H-8). Anal. calcd. For $C_{15}H_{22}N_4O_7$ (370.15)**:** C, 48.64; H, 5.99; N, 15.13; found**:** C, 48.92; H, 6.21; N, 15.44.

(±)-3′-O-(4-Acetoxybutyl)dyphylline (3b)

Yellow syrup; R_f 0.32 (petroleum ether **:** ethyl acetate 1**:**1); ^{1}H NMR ($CDCl_3$, 300 NHz) δ_H: 1.26–1.44, 1.71–1.75 (2 m, 4 H, $2CH_2$), 2.14 (s, 3 H, OAc), 3.40, 3.59 (2 s, 6 H, 2 NMe), 3.69 (d, 1 H, D_2O exchangeable, $J_{2',OH}$ 4.1 Hz, OH), 4.15–4.35 (m, 8 H, H-1′, H-2′, H-3′, H-3″, 2 CH_2), 4.58 (dd, 1 H, $J_{1',2'}$ 2.2 Hz, $J_{1',1''}$ 11.4 Hz, H-1″), 7.66 (s, 1 H, H-8), Anal. calcd. for $C_{16}H_{24}N_4O_6$ (368.17): C, 52.17; H, 6.57; N, 15.21; found: C, 52.36; H, 6.63; N, 15.51.

(±)-Deprotection of the acetylated nucleosides 3a,b

General Method

Compounds **3a,b** (0.30 mmol) in absolute methanol (3 ml) were treated with sodium methoxide in methanol (0.15 M, 3 ml) with stirring at room temperature for 2 hours (TLC). The reaction mixture was neutralized with Amberlite IR-120(H^+) resin, filtered, and the resin was washed with methanol. The combined filtrates were evaporated under reduced pressure and the residue was purified by column chromatography using petroleum ether **:** ethyl acetate (1**:**1) to afford **4a** and **4b**.

(±)-3′-O-[(2-Hydroxyethoxy)methyl]dyphylline (4a)

Colorless syrup (94% yield); ^{1}H NMR (CD_3OD+D_2O) δ_H: 3.33, 3.57 (2 s, 6 H, 2 NMe), 3.66–3.70 (m, 4 H, OCH_2CH_2O), 4.13–4.16 (m, 1 H, H-2′), 4.30 (m, 2 H, H-3′,3″), 4.59 (m, 2 H, H-1′,1″), 4.77 (s, 2 H, O-CH_2-O), 7.91 (s, 1 H, H-8). Anal. calcd. for $C_{13}H_{20}N_4O_6$ (328.14): C, 47.56; H, 6.14; N, 17.06; found: C, 48.22; H, 5.98; N, 16.96.

(±)-3′-O-(4-Hydroxybutyl)dyphylline (4b)

Yellow syrup (81% yield); ^{1}H NMR (CD_3OD) δ_H: 1.27–1.36 (m, 4 H, $2CH_2$), 3.21 (s, 3 H, NMe), 3.29–3.31 (m, 4 H, $2CH_2$), 3.41(s, 3 H, NMe), 3.80 (bs, 1 H, H-2), 4.03–4.13 (m, 2 H, H-3′,3″), 4.44 (dd, 2 H, $J_{1',2'}$ 3.2 Hz, $J_{1',1''}$ 13.5 Hz, H-1′,1″), 4.78 (bs, 1 H, D_2O exchangeable, OH), 5.03 (d, 1 H, D_2O exchangeable, OH), 7.96 (s, 1 H, H-8). Anal. calcd. for $C_{14}H_{22}N_4O_5$ (326.16): C, 51.52; H, 6.79; N, 17.17; found: C, 51.32; H, 6.32; N, 16.87.

(±)-3′-O-(p-Toluoyl)dyphylline (7)

To a stirred solution of **1** (0.25 g, 1.0 mmol) in dry DMF (5 ml), NaH (0.024 g, 1.0 mmol) was added. After complete evolution of hydrogen gas, the mixture was heated at 80°C for 1 hour, then compound **5** (0.19 g, 1.0 mmol) was added. The reaction mixture was stirred for overnight at room temperature and then diluted with cold water (20 ml) whereby a white precipitate was formed. It was filtered and recrystallized from ethanol to afford **7** (0.29 g, 70% yield) as white crystals mp.198–200°C; ^{1}HNMR ($CDCl_3$, 300 NHz) δ_H: 2.40 (s, 3 H, Me), 3.35, 3.52 (2 s, 6 H, 2 NMe), 4.10 (bs, 1 H, H-3′), 4.37–4.39 (m, 4 H, H-3″, H-2′, H-1′, OH), 4.63 (d, 1 H, $J_{1,1}$ 11.1 Hz, H-1), 7.23 (d, 2 H, J 8.1 Hz, Ar-H), 7.68 (s, 1 H, H-8), 7.89 (d, 2 H, J 8.1 Hz, Ar-H).MS : m/z (%): 372 ($M^{+\bullet}$, 15), 236($M^{+\bullet}$-p-$CH_3C_6H_4CO_2H$, 13), 194 (7-methyltheophyline$^+$, 60), 119 (p-$CH_3C_6H_4CO^\bullet$, 76) and 91 ($CH_3C_6H^{4\bullet}$, 48).

(±)-Treatment of 7 with sodium methoxide, dyphylline (1)

Compound **7** (0.1 g, 0.27 mmol) was treated with sodium methoxide in absolute methanol (3ml, 0.43 mmol). The reaction mixture was processed as in the general method for deprotection to afford **1** (0.062 g, 86% yield) as white crystals, mp 160°C, lit mp 162°C [16]; ^{1}H NMR (DMSO-d_6, 300 NHz) δ_H: 3.28 (s, 3 H, NMe), 3.36–3.45 (m, 2 H, H-3′, H-3″), 3.48 (s, 3 H, NMe), 3.87–3.90 (m, 1 H, H-2′), 4.17 (dd,1 H, $J_{1',2'}$ 8.4 Hz, $J_{1',1''}$13.5 Hz, H-1′), 4.50 (dd, 1 H, $J_{1'',2'}$ 3.6 Hz, $J_{1',1''}$13.5 Hz, H-1″), 4.80 (t,1 H, D_2O exchangeable,OH), 5.05 (d, 1 H, D_2O exchangeable, OH), 8.01 (s,1 H, H-8).

Preparation and culture of Hep G2 2.2.15 cells

The required cell line was made by transfection of Hep G2-cells with a plasmid containing multiple tandem copies of HBV genome (subtype ayw).[17] The 2.2.15 cell line was maintained in RPMI-1640 (Glutamax) culture media containing 100 IU/ml nystatin +380 μg/ml G418 (geneticin). The transferred HEP G2-2.2.15 cell line was kept in tissue culture flask at 37°C + 5% CO_2. Subcultures were set up after a week by aspiration of

the media from culture flask and washing the cells twice by PBS. A 10% versene/trypsin was added and the cells were incubated for 1 minutes at 37 °C.

The drug Lamivudine which is a potent selective inhibitor of HBV replication [18] has been used as a standard for the compartive studies.

PCR-ELLISA

The PCR reaction mixture was 14 μL extracted supernatant, 4 mmol/L $MgCl_2$, 10 μmol/L DIG-11-dUTP, 190 μmol/L dTTP, 200 μmol/L dATP, dGTP, dCTP, 1.5 U Taq polymerase, 20 mmol/L HCI (pH 8.4), 50 mmol/L KCI, 1 μmol/L HCID-1 primer (5′ GGA AAG AAG TCA GAA GGC A3), and 1 μmol/L HCID-2 (5TTG GGG GAG GAG ATT AGG TT3), in total volume 50 μL. PCR reaction conditions were 32 cycles of 1 minute at 94°C, 30 seconds at 58°C and 30 seconds at 72°C +3 seconds for each cycle in a thermal circler as described in literature.[19]

Cytotoxicity Assay

A colorimetric assay for living cells should utilize a colorless substrate that is modified to a colored product by any living cells, but not by dead cells or tissue culture medium. 3-(3,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) is the candidate for this purpose. The cytotoxic effect of the compounds was accessed by culturing the Hep G2-2.2.15 cells in the presence of compounds using a MTT-assay.[20]

Calculation of IC_{50}, CC_{50} and SI

The 50% inhibitory concentration of antiviral drugs (IC_{50}) was determined by interpolation from the plots of the amounts of DNA copies versus antiviral drug concentrations. The 50% cytotoxic effect (CC_{50}) was calculated from the average viability of the cells with concentration of drugs. The selective index (SI) could be calculated as CC_{50}/IC_{50}.

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