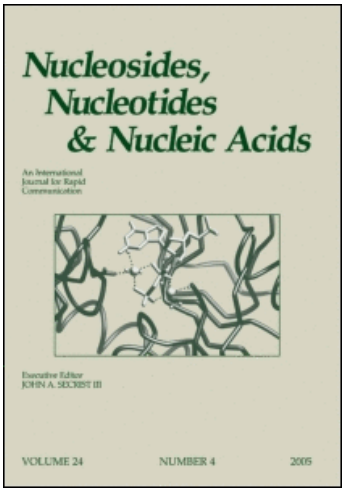

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Guzaev, Andrey P.(1992) 'An Efficient Synthesis of N^2-Isobutyryl-O^6-[2-(4-nitrophenyl)ethyl]-5'-O-Dimethoxytrityl-2'-deoxyguanosine', Nucleosides, Nucleotides and Nucleic Acids, 11: 8, 1463 — 1466
To link to this Article: DOI: 10.1080/07328319208021188
URL: http://dx.doi.org/10.1080/07328319208021188

NUCLEOSIDES & NUCLEOTIDES, 11(8), 1463-1466 (1992)

AN EFFICIENT SYNTHESIS OF N^2-ISOBUTYRYL-O^6-[2-(4-NITROPHENYL)ETHYL]-5'-O-DIMETHOXYTRITYL--2'-DEOXYGUANOSINE

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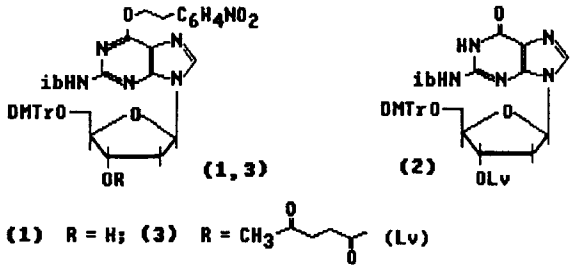
ABSTRACT: An efficient one-flask synthesis of N^2-isobutyryl-O^6-[2-(4-nitrophenyl)ethyl]-5'-O-dimethoxytrityl-2'-deoxyguanosine by alkylation of N^2-isobutyryl-3'-O-levulinyl-5'-O-dimethoxytrityl-2'-deoxyguanosine with 4-nitrophenylethanol in the presence of Ph_3P, diethyl azodicarboxylate, and N-ethyl-N,N-diisopropylamine followed by 3'-O-deprotection with hydrazine is described.

Recent developments in the oligonucleotide synthesis have demonstrated that efficient chain assembly of deoxyguanosine-enriched oligonucleotides requires protection of 2'-deoxyguanosine at the O^6-position [1-3], often with the 4-nitrophenylethyl (npe) blocking group [4]. Thus protected reactive monomeric intermediates in phosphite-triester [1,5] and H-phosphonate [6] approach were prepared using N^2-isobutyryl-O^6-[2-(4-nitrophenyl)ethyl]-5'-O-dimethoxytrityl-2'-deoxyguanosine **1** as a starting compound.

Jones et al. [7] synthesized **1** by activation of the O^6-function of compound **2** with 2,4,6-triisopropylbenzenesulphonyl chloride, followed by conversion to the trimethyl ammonium salt and displacement with 4-nitrophenylethanol (npe-OH).



It was found, that O^6-npe-deoxyguanosine derivatives can be prepared more easily by the Mitsunobu reaction [8]: alkylation of protected deoxyguanosine with npe-OH in the presence of diethyl azodicarboxylate (DEAD) and triphenylphosphine. Conditions of this reaction determinate a deoxyguanosine blocking strathegie. Not only 5'-O-protection but also 3'-O-

protection of starting compounds is strongly desired because N^3-3' anhydro derivative is formed from 3'-O-unprotected deoxyguanosine [9]. At the same time N^2-protection of starting compounds is not necessary [10]. Within this method N^2,3'-O-,5'-O-triisobutyryl- [4], 3',5'-di-O-acetyl- [10], and N^2-isobutyryl-3',5'-di-O-(trimethylsilyl)- [5,9] 2'-deoxyguanosines were used as a starting compounds. However, in order to obtain the desired **1**, the most suitable are 3'-O-protected N^2-isobutyryl-5'-O-dimethoxytrityl-2'-deoxyguanosines [1,9,11]. Although the mildly removable trimethylsilyl 3'-O-protection was reported to find application [9], the more successful is the method of Ogilvie et al. [1] using the 3'-O-acetylated nucleoside and giving after deacetylation (1 M NH_4OH/MeOH; 2.5 h / room temperature) target compound **1** in 85% total yield.

Though the reported methods were sufficiently effective, this relatively costly synthesis may be improved by choosing the more proper protection groups and reaction conditions. In this paper we report the efficient one-flask synthesis of the title compounds.

We have used as a starting compound N^2-isobutyryl-3'-O-levulinyl-5'-O-dimethoxytrityl-2'-deoxyguanosine (**2**) prepared according to published procedure [7b]. We found, that 3'-O-protective levulinyl group had a high reliability in Mitsunobu alkylation. However, some unexpected troubles complicated the reaction.

We observed that the alkylation of **2** was accompanied by the partial detritylation of all dimethoxytritylated compounds. Detritylation started immediatelly after the addition of npe-OH and Ph_3P to solution of **2** in dioxane. Treatment of mixture with DEAD caused the exothermic reaction, and detritylation increased with elevation of temperature. These undesirable effects can be eliminated: (1) by using N-ethyl-N,N-diisopropylamine to prevent the acidification of the reaction mixture, and (2) by cooling the reaction mixture while the alkylation reagents are added (npe-OH, Ph_3P, and DEAD). This mode makes it possible to achieve the 96-98% yields of isolated alkylation product **3**.

The second step, 3'-O-deprotection was traditionally carried out as a separate reaction using chromatography-purified **3** and its analogues [1,11,12]. In the case of 3'-O-levulinyl protection the reaction is performed by addition of 0.5 M hydrazine-hydrate in pyridine/acetic acid mixture to the **3** [12]. These removing conditions are compatible with the presence of any other guanosine protecting groups. 3'-O-Deblocking of **3** is completed within the 30-min time period, yielding an almost homogeneous **1** [12]. We found that hydrazine-containing solution used for deprotection can be added not only to the pure **3**, but also to the Mitsunobu reaction mixture after the alkylation of **2** had been completed. Similarly to the standard scheme, only **1** was formed from **3** without any side-products. Consequently, employment of levulinyl protecting group allows one omit the isolation and purification of **3**. The alkylation of **2** and deprotection of **3** can be performed sequentially, by one-flask procedure.

Thus, the application of the simple method mentioned above allows one prepare the desired compound **1** starting from **2** with the total yield 94% (5-25 mmol scale). In addition, the reaction sequence can be carried out using a one-flask procedure including chromatographic purification of target compound during one-day period.

EXPERIMENTAL

N^2-isobutyryl-O^6-[2-(4-nitrophenyl)ethyl]-5'-O-dimethoxytrityl-2'-deoxyguanosine (1). Compound **2** (7.38 g, 10.0 mmol) [7b], was dried by coevaporation with dioxane (2x30 ml), and was dissolved in 30 ml of dry dioxan and N-ethyl-N,N-diisopropylamine (0.6 ml). 4-Nitrophenylethanol (1.67 g, 12.0 mmol) and Ph_3P (2.62 g, 12.0 mmol) were added to a stirred solution at 10-15°C. The mixture was stirred until the components dissolved. A solution of diethyl azodicarboxylate (1.74 g, 12.0 mmol) in 10 ml of dry dioxan was added dropwise at 10-15°C. The mixture was stirred for 1.5 h at room temperature, and the reaction was judged complete by TLC (Kieselgel 60 F_{254}; $CHCl_3$-MeOH 20:1). The solvent was evaporated to one-half, pyridine (20 ml) was added to the residue. The solution was cooled to 10-15°C, and 40.0 ml of 0.5 M hydrazine-hydrate in pyridine-acetic acid (4:1) were added. The mixture was stirred then for 25 min. The solution was diluted with ethyl acetate (200 ml), washed with H_2O (50 ml), 5% $NaHCO_3/H_2O$ (2x200 ml), and dried (Na_2SO_4). The solvent was evaporated, the residue was coevaporated with toluene (2x50 ml). The product **1** was purified by chromatography (silica gel column, 40x100 mm), eluting first with 100 ml of $Et_2O/CHCl_3$ (2:1), then with 100 ml $Et_2O/CHCl_3$ (1:2), 300 ml of $CHCl_3$, 150 ml of 2% EtOH/$CHCl_3$, 300 ml of 3% EtOH/$CHCl_3$, 150 ml of 4% EtOH/$CHCl_3$, and 150 ml of 5% EtOH/$CHCl_3$. The fractions containing the product were pooled and evaporated to dryness, and purified again to give total 7.41 g (94% from **2**) of **1** as a white foam. TLC (Kieselgel 60 F_{254}; $CHCl_3$-MeOH 9:1): R_f = 0.65. UV (EtOH): max = 236, 272 nm; min = 248 nm (reported max = 241, 272 nm; min = 250 nm [1]; max = 235, 270 nm; min = 248 nm [12]). ^{1}H-NMR (250 MHz, $CDCl_3$): 1.20 (t, J = 7 Hz, 6H, $(CH_3)_2C\underline{H}$); 2.50 (m, 1H, C_2-H); 2.76 (m, 2H, C_2-H, $(CH_3)_2C$); 3.34 (t, J = 7 Hz, 2H, CH_2-$C_6H_4NO_2$); 3.4 (m, 2H, $C_{5'}$-H_2); 3.76 (s, 6H, $2CH_3O$); 4.14 (m, 1H, $C_{4'}$-H); 4.70 (m, 1H, $C_{3'}$-H); 4.84 (t, J = 7 Hz, 2H, C_6-OCH_2); 6.50 (m, 1H, $C_{1'}$-H); 6.7-8.3 (m, 18H, Ar, C_8-H).

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Received 7/26/91
Accepted 6/17/92