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N-4 Alkyl Cytosine Derivatives Synthesis: A New Approach

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Abstract: The selective *N*-4 alkylation of cytosine plays a critical role in the synthesis of biologically active molecules. This work focuses on the development of practical reaction conditions toward a regioselective synthesis of *N*-4-alkyl cytosine derivatives. The sequence includes a direct and selective sulfonylation at the *N*-1 site of the cytosine, followed by the alkylation of the amino site using KHMDS in CH_2Cl_2/THF mixture, providing a fast and efficient approach consistent with pyrimidine-based drug design.

Keywords: alkylation; biologically active molecules; cytosine; regioselectivity

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1. Introduction

Integrase (IN) catalyzes the insertion of viral DNA [1] into the genome of infected cells and acts as a cofactor for reverse transcription [2].

In the context of HIV-1 infection, IN was successfully targeted for drug development [3]. Raltegravir (MK-0518) [4,5] was approved by the *Food and Drug Administration* in 2007, and other integrase inhibitors (INI), including Elvitegravir (GS-9137) [6,7], are progressing through clinical development [8]. The breakthrough of INI has produced a great impulse in the use of multiple drugs that act on different viral targets, known as *Highly Active Antiretroviral Therapy* (HAART) [9]. Important examples of this class are the lens epithelium-derived growth factor (LEDGF) inhibitors [10–12] (Figure 1).

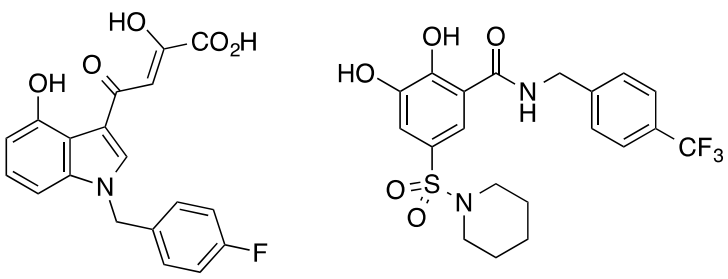


Figure 1. Small molecule inhibitors of the LEDGF/p75-IN interaction.

Unfortunately, the development of resistance is a constant and inevitable threat to the application of therapies; there is always a need for new antiviral drugs with high activity and low cytotoxicity to assist and sometimes also substitute previously utilized drugs.

Molecules acting on the IN HIV-1 are not immune to this problem [13]. This has prompted the research of more efficient and inexpensive new drugs. In this context is the design and synthesis of new cytosine-based antiretroviral (ARV) compounds, which are able to inhibit IN HIV-1.

Current studies of structure–activity relationships (SAR) on the above mentioned INI structures have identified two common regions [14]: a region with two metal-binding motifs critical to all members of this class of active site binders and a region with a hydrophobic site that requires a substituted benzyl group [15,16].

Taking into consideration these findings, we exploited the commercially available cytosine scaffold to synthetize new integrase strand transfer inhibitors (INSTIs) [1,3,4,17,18]. In detail, starting from a preliminary docking analysis [19], which clarified that chelation motif *N*-(aryl/alkyl sulfonyl) amide could selectively fill the binding site, we set out to investigate an original and efficient strategy for the synthesis of type **1** nucleobases (Figure 2).

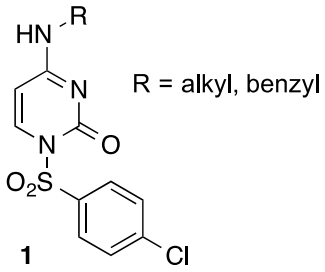


Figure 2. Nucleobase structure.

Cytosine derivatives are versatile intermediates in the synthesis of biologically and pharmaceutically active molecules [20–27] and are widely used as antineoplastic [6], antiviral [24], and anti-AIDS agents [27]. Some groups have very recently focused their attention on *N*-4 alkyl analogue, which improves uptake and bioavailability of gemcitabine, a worldwide chemotherapeutic cytidine analogue [20].

The reaction to obtain *N*-1 substituted cytosines has been intensively investigated [28–33]; nevertheless, to date, only a few examples describe *N*-4 alkyl derivatives [34–39]. One of the most useful examples involves a sodium bisulfate catalyzed transamination experiencing careful control of the pH, which is sometimes incompatible with the chemical stability of biological groups [39].

Likewise, the Borch reductive alkylation method [36,37] and the titanium (IV), which also catalyzed [35], required an excess of amine to favor the formation of the iminium intermediates, thereby hampering the dissolution in the solvent that was usually used.

The methodology described herein shows the regioselective formation of our new compounds under conditions consistent with the stability of future drug moieties.

2. Materials and Methods

All reagents (Aldrich, St. Louis, MA, USA and Merck, KGaA, Darmstadt, Germany) were acquired at the highest purity available and used without further purification. Thin-layer chromatographies were performed with silica gel plates Merck 60 F254, and the display of the products on TLC was performed with a lighting UV lamp, solutions of ninhydrin (0.2% in CH_3OH mol), and molecular iodine. The column chromatographies were carried out using silica gel 70–230 mesh (Merck, KGaA, Darmstadt, Germany). Elemental analyses were performed on a FlashSmart V Elemental Analyzer (ThermoFisher Scientific, Waltham, MA, USA). The ^1H and ^{13}C NMR spectra were recorded on spectrometers: Bruker DRX (400 MHz) and Varian Inova Marker (500 MHz) in $CDCl_3$ solution unless otherwise specified. The chemical shifts are reported in ppm (δ) and the *J* in Hz.

*2.1. Synthesis of 4-amino-1-((4-chlorophenyl)sulfonyl) pyrimidin-2(1H)-one (**2**)*

Sodium hydride (118 mg; 4.9 mmol) at 0 °C under nitrogen atmosphere was added to a stirring solution of cytosine (500 mg, 4.5 mmol) in dry DMF (38 mL). After 2 h, 4-chlorobenzenesulfonyl chloride (1.4 g, 6.8 mmol) was added and stirring was continued over a period of 30 min. The resulting solution was then allowed to warm to room temperature. After 1.5 h, the reaction was quenched with methanol (0.60 mL). The solvent was evaporated under reduced pressure, replaced with chloroform and washed with brine, and then dried (Na_2SO_4). The evaporation of the solvent under reduced pressure gave a crude mixture that was purified by column chromatography ($CHCl_3$/MeOH 95:5) to yield compound **2** (0.96 g, 75%). ^{1}H NMR (400 MHz; DMSO-d6): δ 8.08 (d, *J* 7.8 Hz, 1H), 7.97 (d, *J* 8.5 Hz, 2H), 7.93 (s, 2H), 7.71 (d, *J* 8.5 Hz, 2H), 5.95 (d, *J* 7.8 Hz, 1H); ^{13}C NMR (125 MHz; DMSO-d6); δ 166.5, 151.3, 140.0, 139.7, 136.5, 131.1, 129.7, 98.1. Anal. Calcd. for $C_{10}H_8ClN_3O_3S$ (285.70): C, 42.04%; H, 2.82%; N, 14.71%; found C, 42.15%; H, 2.73%; N, 14.59%.

2.2. General Procedure Synthesis of N-4 Alkyl Cytosine Derivatives

Derivative **2** (0.5 eq) was dissolved in dry CH_2Cl_2:THF (1:1, 5 mL), followed by the addition of 0.5 M KHMDS in THF (0.75 eq) at −40 °C under nitrogen atmosphere. After 1 h, electrophile (0.6 eq) was added and the reaction was allowed to warm to 5 °C within 24 h. TLC monitored the progress of the reaction. The mixture was then treated with methanol (0.5 mL) and further stirred for 10 min at rt. The solvent was evaporated under reduced pressure, replaced with ethyl acetate and washed with brine, and then dried (Na_2SO_4). The evaporation of the solvent under reduced pressure gave a crude mixture that was purified by PLC (1:1 Hexane/Ethyl Acetate) to yield the pairs **1a–3a**, **1b–3b**, **1e–3e**, and **1h–3h**.

1a. ^{1}H NMR (400 MHz): δ 8.05 (m, 3H), 7.51 (d, *J* 7.2 Hz, 2H), 7.36-7.22 (m, 5H), 5.69 (d, *J* 7.5 Hz, 1H), 5.47 (bs, NH), 4.64 (s, 2H); ^{13}C NMR (100 MHz, $(CD_3)_2CO$): δ 166.0, 156.9, 147.6, 139.4, 139.3, 129.9, 129.5, 128.9, 128.6, 92.6, 53.1. Anal. Calcd. for $C_{17}H_{14}ClN_3O_3S$ (375.83): C, 54.33%; H, 3.75%; N, 11.18%; found C, 54.35%; H, 3.83%; N, 11.03%.

3a. ^{1}H NMR (500 MHz): δ 8.07 (d, *J* = 8.6 Hz, 2H), 8.05 (d, *J* 8.1 Hz, 1H), 7.51 (d, *J* 8.5 Hz, 2H), 7.36–7.26 (m, 8H), 7.09 (d, *J* 7.3 Hz, 2H), 5.93 (d, *J* 8.1 Hz, 1H), 4.96 (s, 2H), 4.54 (s, 2H); ^{13}C NMR (125 MHz): δ 164.1, 151.3, 141.5, 140.0, 136.0, 135.1, 135.0, 131.4, 129.3, 129.2, 128.8, 128.7, 128.1, 128.0, 126.2, 94.5, 50.9, 50.8. Anal. Calcd. for $C_{24}H_{20}ClN_3O_3S$ (465.95): C, 61.87%; H, 4.33%; N, 9.02%; found C, 61.90%; H, 4.28%; N, 9.01%.

1b. ^{1}H NMR (400 MHz): δ 8.07–8.01 (m, 3H), 7.51 (d, *J* 8.6 Hz, 2H), 7.30–7.20 (m, 2H), 7.00 (t, *J* 8.6 Hz, 2H), 5.70 (d, *J* 7.9 Hz, 1H), 4.60 (d, *J* 5.5 Hz, 2H). ^{13}C NMR (100 MHz) δ 164.4, 160.3, 156.8, 141.4, 139.8, 135.7, 134.8, 131.3, 129.3, 127.9, 115.8, 94.3, 50.8. Anal. Calcd. for $C_{17}H_{13}ClFN_3O_3S$ (393.82): C, 51.85%; H, 3.33%; N, 10.67%; found C, 51.96%; H, 3.35%; N, 10.76%.

3b. ^{1}H NMR (500 MHz): δ 8.16–8.05 (m, 3H), 7.55 (d, *J* 8.8 Hz, 2H), 7.28 (m, 2H), 7.07–6.97 (m, 6H), 5.94 (d, *J* 8.2 Hz, 1H), 4.90 (s, 2H), 4.52 (s, 2H). ^{13}C NMR (100 MHz): 164.0, 163.7, 161.2, 151.0, 141.4, 140.1, 135.0, 131.6, 131.3, 130.4, 130.3, 129.3, 129.0, 127.8, 115.7, 115.5, 94.0. 50.2, 49.9. Anal. Calcd. for $C_{24}H_{18}ClF_2N_3O_3S$ (501.93): C, 57.43%; H, 3.61%; N, 8.37%; found C, 57.37%; H, 3.67%; N, 8.35%.

1c. ^{1}H NMR (400 MHz, $(CD_3)_2CO$): δ 8.13–8.08 (m, 3H), 8.00 (bs, 1H, NH), 7.68 (d, *J* 8.6 Hz, 2H), 7.35 (m, 1H), 7.17 (d, *J* 7.6 Hz, 1H), 7.12 (m, 1H), 7.00 (m, 1H), 6.13 (d, *J* 7.9 Hz, 1H), 4.62 (d, *J* 4.9 Hz, 2H). ^{13}C NMR (100 MHz, $(CD_3)_2CO$): δ 169.9, 164.0, 140.9, 140.1, 138.6, 136.4, 131.0, 130.1, 128.9, 123.5, 114.4, 113.7, 97.6, 43.4. Anal. Calcd. for $C_{17}H_{13}ClFN_3O_3S$ (393.82): C, 51.85%; H, 3.33%; N, 10.67%; found C, 51.87%; H, 3.25%; N, 10.63%.

3c. ^{1}H NMR (400 MHz): δ 8.14–8.04 (m, 3H), 7.53 (d, *J* 8.4 Hz, 2H), 7.39–7.23 (m, 3H), 7.08–6.75 (m, 5H), 5.91 (d, *J* 8.1 Hz, 1H), 4.94 (s, 2H), 4.53 (s, 2H). ^{13}C NMR (100 MHz): 167.8, 164.7, 159.8, 153.6, 145.2, 145.0, 144.4, 133.7, 131.9, 131.3, 128.2, 127.9, 116.7, 116.6, 112.5, 93.2, 58.6, 57.2. Anal. Calcd. for $C_{24}H_{18}ClF_2N_3O_3S$ (501.93): C, 57.43%; H, 3.61%; N, 8.37%; found C, 57.44%; H, 3.59%; N, 8.39%.

1d. ^{1}H NMR (400MHz, DMSO-d6): δ 8.16–8.07 (m, 3H), 7.67 (d, *J* 10.0 Hz, 2H), 7.41 (m, 1H), 7.31 (m, 1H), 7.14–7.06 (m, 2H), 6.12 (d, *J* 10.0 Hz, 1H), 4.64 (d, *J* 6.5 Hz, 2H). ^{13}C

NMR (100MHz, $(CD_3)_2CO$): δ 163.9, 161.9, 154.7, 140.1, 138.5, 136.3, 131.0, 130.3, 129.4, 129.3, 128.9, 124.2, 115.1, 97.5, 37.9. Anal. Calcd. for $C_{17}H_{13}ClFN_3O_3S$ (393.82): C, 51.85%; H, 3.33%; N, 10.67%; found C, 51.80%; H, 3.31%; N, 10.59%.

3d. ^{1}H NMR (400 MHz): δ 8.06 (d, *J* 8.5 Hz, 2H), 7.55–7.43 (m, 3H), 7.34–7.20 (m, 2H), 7.14–6.95 (m, 6H), 5.96 (d, *J* 8.2 Hz, 1H), 4.99 (s, 2H), 4.66 (s, 2H). ^{13}C NMR (100 MHz): 164.1, 163.3, 160.9, 151.3, 141.5, 140.0, 136.0, 135.1, 135.0, 131.4, 129.2, 129.1, 128.8, 128.6, 128.1, 128.0, 126.1, 115.2, 115.1, 94.5, 50.9, 50.7. Anal. Calcd. for $C_{24}H_{18}ClF_2N_3O_3S$ (501.93): C, 57.43%; H, 3.61%; N, 8.37%; found C, 57.45%; H, 3.63%; N, 8.38%.

1e. ^{1}H NMR (400MHz, $(CD_3)_2CO$): δ 8.10–8.05 (m, 3H), 7.68 (d, *J* 8.8 Hz, 2H), 7.20 (d, *J* 8.0 Hz, 2H), 7.10 (d, *J* 8.0 Hz, 2H), 6.07 (d, *J* 7.9 Hz, 1H), 4.52 (d, *J* 5.7 Hz, 2H), 2.27 (s, 3H). ^{13}C NMR (100 MHz, $(CD_3)_2CO$): δ 165.3, 152.3, 141.4, 139.9, 138.8, 135.7, 134.1, 132.0, 131.7, 130.9, 100.7, 46.9, 25.5. Anal. Calcd. for $C_{18}H_{16}ClN_3O_3S$ (389.85): C, 55.46%; H, 4.14%; N, 10.78%; found C, 55.50%; H, 4.13%; N, 10.65%.

3e. ^{1}H NMR (400 MHz; $(CD_3)_2CO$): δ 8.16 (d, *J* 8.2 Hz, 1H), 8.12 (d, *J* 8.8 Hz, 2H), 7.83 (d, *J* 8.7 Hz, 1H), 7.68 (d, *J* 8.8 Hz, 2H), 7.59 (d, *J* 8.7 Hz, 1H), 7.24-7.06 (m, 6H), 6.31 (d, *J* 8.2 Hz, 1H), 4.87 (s, 2H), 4.67 (s, 2H), 2.31 (s, 3H), 2.28 (s, 3H). ^{13}C NMR (100 MHz; $(CD_3)_2CO$): δ 164.6, 154.3, 140.1, 139.2, 138.8, 138.4, 131.4, 131.2, 131.1, 129.1, 129.0, 128.7, 128.6, 127.9, 127.8, 97.8, 44.3, 43.9, 32.4, 31.7. Anal. Calcd. for $C_{26}H_{24}ClN_3O_3S$ (494.01): C, 63.22%; H, 4.90%; N, 8.51%; found C, 63.26%; H, 4.87%; N, 8.46%.

1f. ^{1}H NMR (400MHz): δ 8.05 (d, *J* 8.8 Hz, 2H), 8.02 (d, *J* 7.9 Hz, 1H), 7.51 (d, *J* 8.8 Hz, 2H), 7.23 (m, 1H), 7.14-7.03 (m, 3H), 5.72 (d, *J* 7.9 Hz, 1H), 5.66 (m, 1H, NH), 4.60 (d, *J* 5.4 Hz, 2H), 2.33 (s, 3H). ^{13}C NMR (100 MHz): δ 163.1, 151.7, 142.7, 140.7, 138.6, 136.5, 135.1, 134.8, 131.1, 129.0, 128.7, 125.2, 123.7, 97.3, 45.3, 21.2. Anal. Calcd. for $C_{18}H_{16}ClN_3O_3S$ (389.85): C, 55.46%; H, 4.14%; N, 10.78%; found C, 55.53%; H, 4.17%; N, 10.70%.

3f. ^{1}H NMR (400 MHz): δ 8.10 (d, *J* 8.4 Hz, 2H), 8.05 (d, *J* 8.02 Hz, 1H), 7.53 (d, *J* 8.4 Hz, 2H), 7.25–7.17 (m, 2H), 7.15–7.05 (m, 4H), 6.92–6.86 (m, 2H), 5.94 (d, *J* 8.2 Hz, 1H), 4.94 (s, 2H), 4.50 (s, 2H), 2.4 (s, 3H), 2.3 (s, 3H). ^{13}C NMR (100 MHz): 163.1, 151.9, 141.4, 141.2, 140.7, 138.6, 136.5, 135.1, 134.8, 131.1, 129.0, 128.7, 125.2, 123.7, 97.3, 46.1, 45.3, 30.8, 29.6. Anal. Calcd. for $C_{26}H_{24}ClN_3O_3S$ (494.01): C, 63.22%; H, 4.90%; N, 8.51%; found C, 63.26%; H, 4.87%; N, 8.46%.

1g. ^{1}H NMR (400 MHz, DMSO-d6): δ 8.12–8.06 (m, 3H), 7.76 (m, 1H), 7.69 (d, *J* 8.7 Hz, 2H), 7.27 (d, *J* 6.9 Hz, 1H), 7.19-7.12 (m, 3H), 6.13 (d, *J* 7.9 Hz, 1H), 4.58 (d, *J* 5.3 Hz, 2H), 2.31 (s, 3H). ^{13}C NMR (125 MHz, $(CD_3)_2CO$): δ 164.9, 151.9, 141.0, 139.3, 137.5, 137.3, 136.4, 132.0, 131.1, 129.9, 128.5, 126.9, 98.6, 79.2, 43.3, 23.3. Anal. Calcd. for $C_{18}H_{16}ClN_3O_3S$ (389.85): C, 55.46%; H, 4.14%; N, 10.78%; found C, 55.44%; H, 4.13%; N, 10.79%.

3g. ^{1}H NMR (400 MHz): δ 8.08 (d, *J* = 8.7 Hz, 2H), 8.04 (d, *J* 8.2 Hz, 1H), 7.51 (d, *J* 8.7 Hz, 2H), 7.25–7.10 (m, 6H), 7.00 (d, *J* 7.9 Hz, 1H), 6.94 (d, *J* 6.8 Hz, 1H), 5.84 (d, *J* 8.2 Hz, 1H), 4.98 (s, 2H), 4.44 (s, 2H), 2.18 (s, 3H), 2.16 (s, 3H). ^{13}C NMR (100 MHz): 162.5, 152.7, 141.4, 139.7, 136.5, 135.0, 134.9, 133.4, 132.3, 131.3, 130.7, 130.5, 129.0, 128.4, 127.7, 127.5, 126.6, 126.1, 124.5, 94.2, 52.4, 48.5, 23.2, 22.7. Anal. Calcd. for $C_{26}H_{24}ClN_3O_3S$ (494.01): C, 63.22%; H, 4.90%; N, 8.51%; found C, 63.27%; H, 4.93%; N, 8.50%.

1h. ^{1}H NMR (400MHz, DMSO-d6): δ 8.18 (d, *J* 8.7 Hz, 2H), 8.13 (d, *J* 7.9 Hz, 1H), 8.09 (d, *J* 8.6 Hz, 2H), 7.68 (d, *J* 8.6 Hz, 2H), 7.61 (d, *J* 8.7 Hz, 2H), 6.16 (d, *J* 7.9 Hz, 1H), 4.77 (d, *J* 6.0 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d6): 164.2, 150.9, 147.1, 145.9, 140.2, 138.7, 136.3, 131.1, 128.9, 128.5, 123.3, 97.5, 43.3. Anal. Calcd. for $C_{17}H_{13}ClN_4O_5S$ (420.82): C, 48.52%; H, 3.11%; N, 13.31%; found C, 48.53%; H, 3.16%; N, 13.25%.

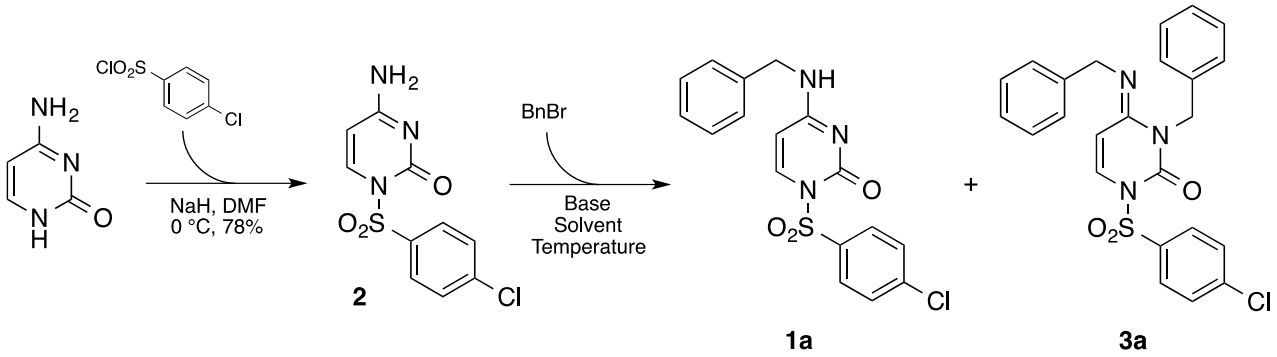
3h. (4%). ^{1}H NMR (400 MHz, DMSO-d6): δ 8.25–8.09 (m, 7H), 7.70 (d, *J* 8.7 Hz, 2H), 7.62 (d, *J* 8.6 Hz, 2H), 7.56 (d, *J* 8.6 Hz, 2H), 6.38 (d, *J* 8.2 Hz, 1H), 5.14 (s, 2H), 5.08 (s, 2H). ^{13}C NMR (125 MHz, DMSO-d6): δ 163.6, 156.6, 156.0, 149.7, 147.9, 147.8, 139.9, 139.1, 138.1, 129.8, 129.7, 128.5, 128.4, 126.8, 126.7, 95.2, 58.2, 57.4. Anal. Calcd. for $C_{26}H_{18}ClN_5O_7S$ (555.95): C, 51.85%; H, 3.26%; N, 12.60%; found C, 51.90%; H, 3.21%; N, 12.59%.

1i. ^{1}H NMR (400 MHz): δ 8.00 (d, *J* 8.7 Hz, 2H), 7.74 (d, *J* 8.6 Hz, 1H), 7.57 (d, *J* 8.7 Hz, 2H), 6.52 (d, *J* 8.6 Hz, 1H), 2.98 (bs, 3H). ^{13}C NMR (100 MHz, DMSO-d6): 163.3, 156.1, 140.0,

138.3, 137.0, 130.0, 128.9, 94.7, 28.1. Anal. Calcd. for $C_{11}H_{10}ClN_3O_3S$ (299.73): C, 44.08%; H, 3.36%; N, 14.02%; found C, 44.12%; H, 3.33%; N, 14.07%.

3. Results and Discussion

We started with the synthesis of the suitable precursor of our target, namely the cytosine sulfonylate derivate **2**, obtained by exploiting the well-known good reactivity of the *N*-1 site [31,32,40–42]. Indeed, as shown in Scheme 1, the commercially available cytosine was selectively sulfonylated with 4-chlorobenzene-1-sulfonyl chloride in DMF, the solvent required to overcome the known low solubility of the starting material. It is noteworthy that a temperature of 0 °C was mandatory to avoid a competitive reaction in favor of the exocyclic amine group. Under these conditions, compound **2** was obtained with 78% yield, as confirmed by NMR.



Scheme 1. *N*-4 alkyl model reaction.

Then, in our exploratory studies, we experimented with the representative *N*-4 alkylation (Scheme 1) with benzyl bromide as an electrophile under different conditions in terms of base, time, solvent, and temperature.

Firstly, the mixture of **2** and benzyl bromide was dissolved in DMSO and left at 25 °C for 4 h, then it was allowed to reach 80 °C and was kept under these conditions for a further 20 h, but no reaction took place and the starting materials remained completely unconsumed (Table 1, entry 1). Next, the exploitation of non-nucleophilic bases was investigated. In detail, pyridine (Pyr) and triethylamine (TEA) were found to be ineffective (Table 1, entries 2–4) and only a trace of the desired product was achieved when bicyclic amide (1,8-diazabiciclo(5.4.0)undec-7ene, (DBU)) [43,44]—which is able to form a charge transfer complex—was employed. The low nucleophilicity of the nitrogen atom, as well as the steric hindrance on the same nitrogen, resulting in a stalled reaction, could be clarified by the supposed complex reported in Figure 3.

Table 1. Reaction condition screening.

Entry	Base [1]	Solvent	T (°C)	1a (%)	3a (%)	Time (h) [2]
1		DMSO	25→80	ND	ND	24
2	Pyr	DMSO	25→80	<1	ND	24
3	Pyr	DMF	25→80	<1	ND	24
4	TEA	DMF	25→80	<1	ND	24
5	DBU	DMF	25	<1	Trace	24
6	LDA	DMF	−40	5	3	24
7	LiHMDS	DMF	−40	3.5	2.5	4
8	KHMDS	DMF	0	16	9	4
9	KHMDS	DMF	−23	30	18	4
10	KHMDS	DMF	−40	40	30	4
11	KHMDS	DMF	−60	20	15	4

[1] Reactions were performed using cytosine sulfonylate **2** (1 eq), bases (1.5 eq), and benzyl bromide (1.2 eq). [2] TLC monitored the progress of the reaction.

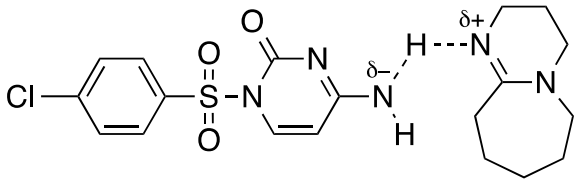


Figure 3. DBU Complex.

When the concept of strongest base (lithium diisopropylamide (LDA), hexamethyldisilazane lithium (LiHMDS), and hexamethyldisilazane potassium (KHMDS)) was explored, positive results were produced.

Remarkably, the *N*-1 substituted cytosines that participated as an acidic compound (with pKa lower than that of KHMDS) reacted with the base. Thus, the formed anion of the substrate could act as a nucleophile in reaction with benzyl halides. In fact, as reported in Table 1, when using KHMDS in DMF at −40 °C (entry 10) the reaction was completed within 4 h and workup afforded the expected monobenzylated product **1a** as the major compound with 40% yield, together with a minor side-product **3a** (with 30% yield, Scheme 1). As is well known for enolates, our products increased the separation of the metal cation from the anion with the larger alkali metals, which leads to a more reactive but less stable anionic intermediate.

Attempts to optimize the reaction through modification of the ratio between **2** and BnBr proved unsuccessful, and we did not find any effects of the ratio between **2** and bases on the reaction in terms of yield.

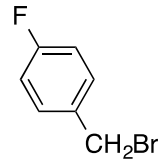
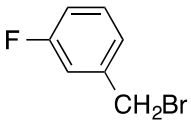
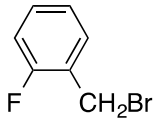
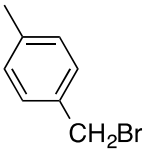
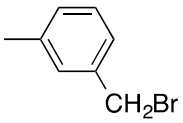
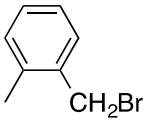
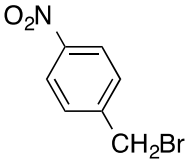
Therefore, the promising approach of the protocol prompted us to evaluate the substrate scope. As shown in Table 2 (entries **b**–**h**), a wide range of benzyl bromides containing both electron-donating (EDG) and electron-withdrawing (EWG) substituents were well tolerated with good conversion. However, at this stage, the results are difficult to rationalize. In relation to entry **i**, the reactivity of bromomethane is definitely higher compared to that of primary alkyl bromides, and the fact that there is more than one nucleophilic center on the cytosine substrate results in byproduct formation that is not valuable.

However, as in the model reaction, a mixture of two different *N*-alkylated products, namely **1** and **3**, were obtained. The mono/di-alkylation ratio ranged from 6:4 to 7:3, as determined by the integration of characteristic protons for each product in the ^{1}HNMR spectra of the concentrated reaction mixtures.

A combination of homo- and heteronuclear 2D NMR experiments (DQF-COSY, ^{13}C-^{1}H HSQC, and HMBC, NOESY) were used to assign all the spin systems of **1a** and **3a**. In detail, the proton resonances of all systems were obtained by the COSY technique and were used to assign the carbon resonance in the HSQC spectra. The ^{13}C-^{1}H HMBC spectrum of **3a** (see Supplementary Materials) shows a correlation between CH_2 at δ = 4.54 ppm and the nitrogen-bearing carbon C4 signal at δ = 164.1 ppm, as well as a comparable correlation between CH_2 at δ = 4.96 ppm and the same C4. However, the first CH_2 is also correlated to carbons at δ = 126.2 ppm and at δ = 135.0 ppm, whereas the second CH_2 shows a correlation to carbons at δ = 128.7 ppm and δ = 136.0 ppm. These values, together with the NOE contact, are diagnostic of benzyl groups on different nitrogen atoms, as depicted in the structure of **3a**.

Based on the entire experimental outcome and the reported literature [45–47], we postulated that the undesired dibenzylated byproduct **3a** might be due to the competitive pathway illustrated [47] in Scheme 2, where the nucleophilic substitution of benzyl bromide first occurs by the NH_2(*N*4) group and then by the cytosine *N*3 site of the bidentate nucleophile.

Table 2. Substrate scope.

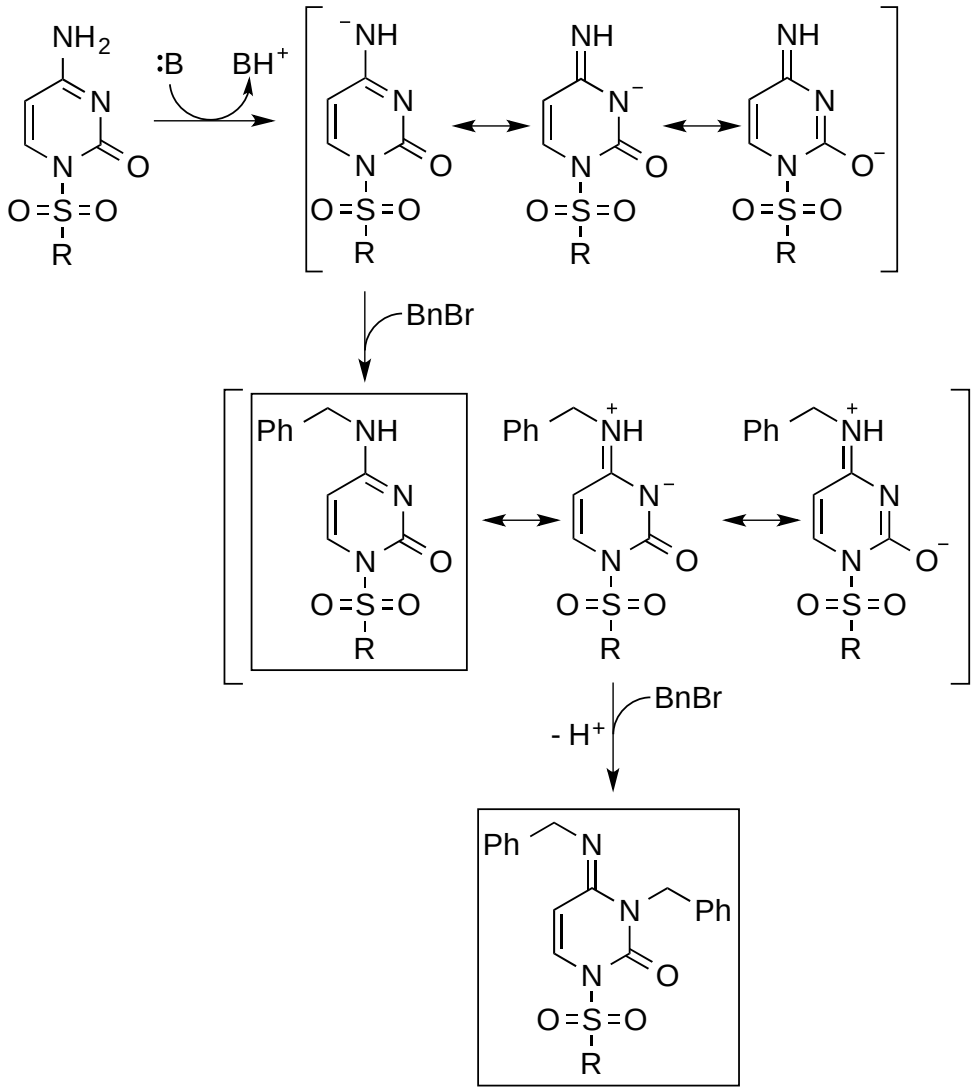
Entry	Electrophile [1]	Product 1 (%)	Product 3 (%)	Ratio 1:3
b		41	26	6:4
c		32	20	6:4
d		32	25	6:4
e		23	8	7:3
f		32	21	6:4
g		31	19	6:4
h		13	5	7:3
i	CH_3Br	20	ND	—

[1] Reactions were performed using cytosine sulfonylate **2** (1 eq), 0.5 M KHMDS in THF (1.5 eq), and electrophile (1.2 eq) in DMF at −40 °C for 4 h.

DMSO and DMF have large dielectric constants (47.24 and 38.25, respectively), large dipole moments (3.96 and 3.82 D, respectively), and they do not participate in hydrogen bonding. Their high polarity allows them to dissolve charged species such as various anions used as nucleophiles. The lack of hydrogen bonding in the solvent means that the latter is relatively "free" in the solution, making it more reactive.

Moreover, THF and CH_2Cl_2 as borderline polar aprotic solvents have moderately higher dielectric constants (7.52 and 8.93, respectively) and small dipole moments (1.75 and 1.60 D, respectively). The intermediate polarity makes them good "general purpose" solvents for a wide range of reactions where they serve only as the medium (for example, in the Grignard reaction and for enolate formation).

Thus, to reduce the reactivity of the nitrogen ring in an attempt to increase the efficiency of the behavior, the model reaction was carried out using DMF in a 1:1 mixture with THF as co-solvent [48,49]; we obtained an interesting result, which drove us to perform the reaction in a new 1:1 mixture of CH_2Cl_2/THF. As we postulated, these conditions led to very efficient results (Table 3, entry 4).



Scheme 2. Postulated mechanism.

Table 3. Solvent effects.

Entry	Base [1]	Solvent (1:1)	T (°C)	1a (%)	3a (%)	Time (h)
1	KHMDS	DMF/THF	−40	39	26	4
2	KHMDS	CH_2Cl_2/THF	−40	4	1	4
3	KHMDS	CH_2Cl_2/THF	−40→5	9	2	4
4	KHMDS	CH_2Cl_2/THF	−40→5	77	17	24

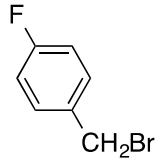
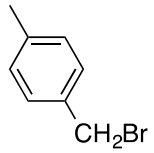
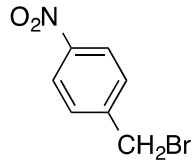
[1] Reactions were performed using cytosine sulfonylate **2** (1 eq), bases (1.5 eq), and benzyl bromide (1.2 eq).

In our mind, the CH_2Cl_2-THF mixture could synergistically ensure the solubility of our polar substrate, resulting in the best interaction of the latter with the base as well as the electrophile.

CH_2Cl_2 did not change the regiochemical outcome (*N*-enamine type versus *N*-imine type) of mono-alkylation.

Under the optimized reaction conditions, the scope of various para-substituted benzyl bromides was again investigated, and the results are summarized in Table 4.

Table 4. Optimized reaction conditions on different substituted benzyl bromides.

Entry	Electrophile [1]	Product 1 (%)	Product 3 (%)	Ratio 1:3
1		57	14	8:2
2		47	5	9:1
3		40	4	9:1

[1] Reactions were performed using cytosine sulfonylate **2** (1 eq), 0.5 M KHMDS in THF (1.5 eq), and electrophile (1.2 eq) in CH_2Cl_2:THF (1:1) at −40→5 °C for 24 h.

4. Conclusions

Cytosine derivatives have recently gained great interest as bioactive molecules. This work explores the synthesis and characterization of new cytosine-based potential ARV compounds, exploiting a route via the new *N*-1 sulfonylate precursor **2**. The latter, containing one of the groups useful for the targeted application, avoids any expensive protection–deprotection steps. Moreover, it prevents the use of excess reactant. The scope of the approach was explored, which provided a good tolerance and satisfactory yields. Further studies are currently underway in order to exploit the new methodology for other nucleoside analogues.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/reactions3010014/s1.

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