A New Laboratory Scale Synthesis for the Anticancer Drug 3′-*C*-Ethynylcytidine

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Abstract: A new synthetic route for the preparation of larger quantities of the anticancer nucleoside analogue 3′-*C*-ethynylcytidine is described. Starting from cytidine which was orthogonally protected in three steps, the ketonucleoside analogue as the key intermediate was obtained through oxidation of the unprotected 3′-hydroxy group. Stereoselective addition of the trimethylsilyl-protected acetylide residue at the 3′-carbonyl group followed by a complete deprotection afforded 3′-*C*-ethynylcytidine in an overall yield of 24% in seven steps.

Key words: drugs, nucleosides, stereoselectivity, ketones, oganometallic ragents

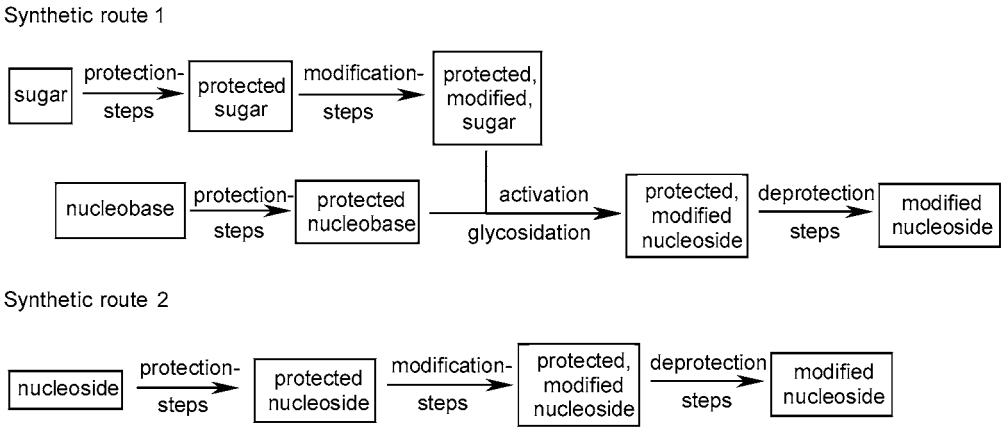
Ethynylated ribonucleoside analogues have shown their in vitro and in vivo antitumor potential against serveral different cell lines as well as tumor models.[1] These test results suggested 1-(3-*C*-ethynyl-β-D-ribopentafuranosyl)cytosine (3′-*C*-ethynylcytidine, ECyd) as the most promising representative of this new drug class which entered Phase I[2] clinical trials in patients with solid tumors. Due to its antitumor potential[1] and unique mechanism of action,[3,4] we consider ECyd as a possible new key structure for newly developed anticancer drugs with optimized antitumor potential obtained by derivatization of this parent nucleoside analogue. This motivates the need for a facile synthetic route to ECyd in preparative amounts with high yields.

The preparation of sugar-modified nucleoside analogues can be achieved as visualized in Scheme 1 through two main synthetic routes.

Synthetic route 1 chooses an appropriate sugar as starting material that is monofunctionalized through the introduction of protection groups, followed by the desired modification affording the modified sugar ready for the N-glycosidation. Meanwhile the appropriate nucleobase is protected and after activation both starting materials are condensed by building the *N*-glycoside bond to the protected, modified nucleoside analogue, which is deprotected subsequently to the desired nucleoside analogue. This reaction route requires a minimum of five reaction steps.

Synthetic route 2 starts with the naturally occurring nucleoside in which during the first two reaction steps protecting groups are introduced into the nucleobase and subsequently into the sugar residue. The protected nucleoside is modified in the desired way affording the fully protected modified nucleoside analogue, which is deprotected yielding the desired product. This reaction route requires a minimum of four reaction steps.

The advantages of synthetic route 1 lie in its increased flexibility: – The reaction route can be designed to afford the α- as well as the β-anomer. – Carbocyclic nucleoside analogues are available. – Sugar residues with strong modifications can be used. Moreover the reaction condi-



Scheme 1 Possible approaches to sugar-modified nucleoside analogues

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tions during the modification steps are more flexible since no cleavage of the *N*-glycoside bond can occur.

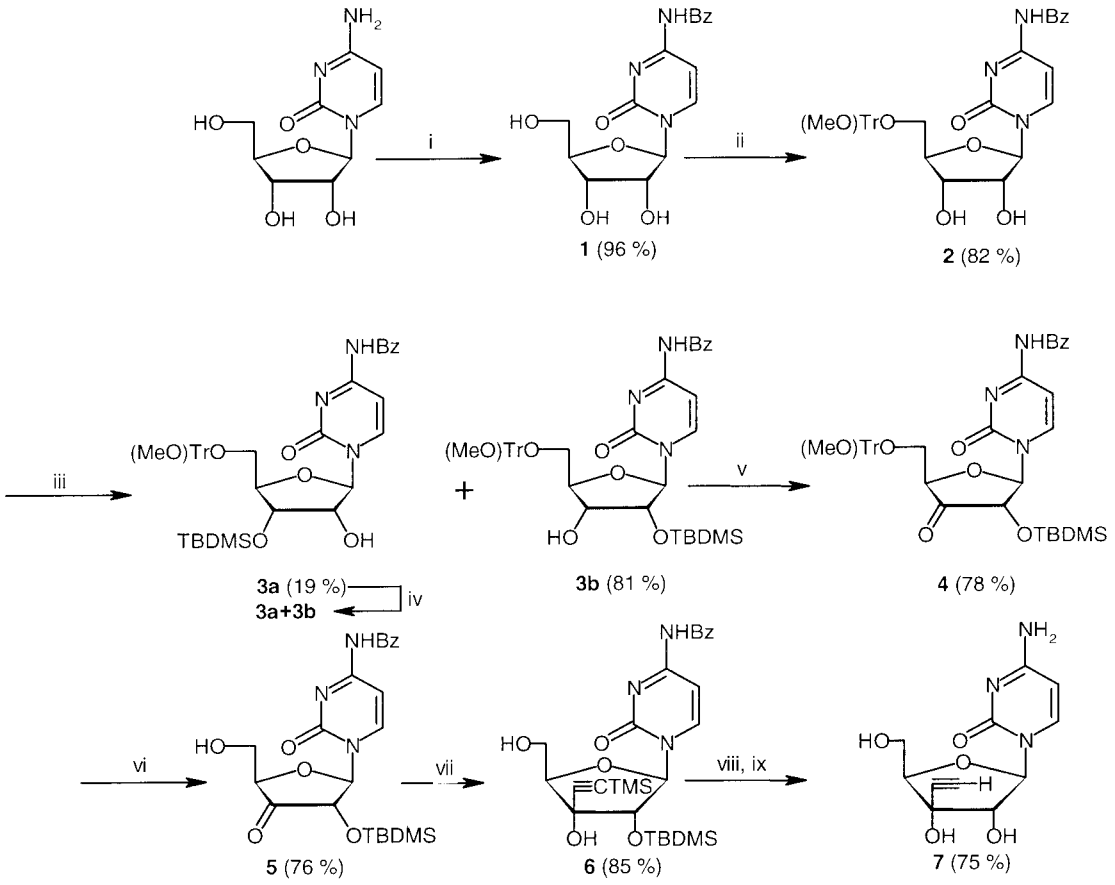
Synthetic route 2 is preferable if a short route to a sugar-modified β-nucleoside is needed. Since no glycosidation is executed after this route the obtained β-nucleosides contain no α-anomeric form as a byproduct.

Both synthetic routes were applied in literature to the preparation of sugar-modified nucleoside analogues which is illustrated by the following examples dealing with synthetic approaches to antiviral as well as antitumor nucleosides with modified sugar residues: 3′-azido-3′-deoxythymidine (Zidovudine, AZT),[5,6] 2′,3′-didehydro-2′,3′-dideoxythymidine (Stauvudine, Zerit, D4T),[7,8] 1-β-D-arabinofuranosylcytosine (Cytarabine, araC),[9,10] 2′,3′-dideoxycytidine (Zalcitabine, ddC).[11]

As we started our experimental work on ECyd, the only available synthetic approach to this nucleoside analogue was the initial preparation method[1] through which ECyd could be synthesized in 12 steps in a sub-gram scale. Meanwhile the initial preparation method using route 1 was adapted and optimized for large scale preparation[12] of ECyd. In this paper we present a new alternative for the laboratory scale preparation of the title compound using synthetic route 2 starting from cytidine as nucleoside.

ECyd was prepared as visualized in Scheme 2 following synthetic route 2 in seven reaction steps in an overall yield of 24% starting from cytidine which was initially selectively amino-protected by benzoylation with benzoic anhydride. The change of the solvent from MeOH to dioxane–H_2O increased the published yield[13] from 76% to a nearly quantitative reaction (96%). N^4-Benzoylcytidine (**1**) that was obtained as a white solid, was subsequently tritylated at the 5′-hydroxy group using 4-monomethoxytrityl chloride in pyridine. This reaction afforded the 5′-*O*-(4-monomethoxytrityl) compound **2** which was described as an amorphous solid.[14]

After purification by flash chromatography on silica gel we could crystallize N^4-benzoyl-5′-*O*-(4-monomethoxytrityl)cytidine (**2**) from Et_2O in 82% yield. Compound **2** was subsequently treated with *tert*-butyldimethylsilyl (TBDMS) chloride and silver nitrate in pyridine–THF[15] to afford a mixture of both possible isomeric forms (2′-*O*-TBDMS **3b** and 3′-*O*-TBDMS **3a**) with a slight excess of the 2′-isomer **3b** (ca. 60%). The use of silver perchlorate instead of silver nitrate which was proposed by



Scheme 2 *Reagents and conditions:* i) benzoic anhydride, dioxane, H_2O, 90 °C, 4 h; ii) 4-methoxytrityl chloride, pyridine, r.t., 18 h; iii) *tert*-butyldimethylsilyl chloride, $AgNO_3$, pyridine, THF, r.t., 3 h; iv) pyridine, MeOH, 70 °C, 12 h; v) CrO_3, Ac_2O, pyridine, CH_2Cl_2, r.t., 1 h; vi) *p*-toluenesulfonic acid (2%), $CHCl_3$, MeOH, r.t., 5 min; vii) ethynyltrimethylsilane, BuLi, hexane, $CeCl_3$, THF, –65 °C, 3 h; viii) ammonia, MeOH, r.t., 24 h; ix) Bu_4NF, THF, r.t., 4 h

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Hakimelahi[15] did not lead to further improved yields of the 2′-TBDMS-isomer **3b**. We achieved a higher yield of the 2′-isomer **3b** by transforming the undesired **3a**, which was obtained during the purification procedure of the reaction mixture in the silylation step, into the 2′-isomer by an isomerization step using induced base-catalyzed migration[16] of the TBDMS-protecting group in pyridine–MeOH. Compound **3b** was obtained in an overall yield of 81% including one isomerization step. The orthogonally protected cytidine **3b** was then oxidized to the 3′-ketonucleoside **4** in a yield of 78% with chromium oxide/acetic anhydride/pyridine in CH_2Cl_2. The yield could be slightly improved (82–85%) if the Dess–Martin[17,18] periodinane reagent in CH_2Cl_2 was used for the oxidation. The complex two-step preparation and an additionally high sensitivity to moisture of this reagent lead us to use chromium oxide as oxidation agent. 5′-Deprotection of **4** with *p*-toluenesulfonic acid (2%) in $CHCl_3$–MeOH (1:1) afforded N^4-benzoyl-2′-*O*-(*tert*-butyldimethylsilyl)-3′-ketocytidine (**5**) (76%), which was purified on silica gel and crystallized from Et_2O. The 5′-unprotected ketonucleoside **5** was stable for about 2 weeks when kept sealed at –25 °C. Storage at room temperature or in solution resulted in the loss of the nucleobase by β-elimination. Therefore **5** was converted as fast as possible to the ethynylated, fully protected ribonucleoside **6** by addition of the organocerium reagent[19,20] that was prepared from anhydrous cerium(III) chloride and trimethylsilyl acetylide to the ketonucleoside. This stereoselective addition afforded N^4-benzoyl-2′-*O*-(*tert*-butyldimethylsilyl)-3′-*C*-(trimethylsilylethynyl)cytidine (**6**) in 85% yield after purification on silica gel. Compound **6** was completely unprotected by treatment with methanolic ammonia for 24 h followed by the removal of the TBDMS protection group with tetrabutylammonium fluoride in THF affording analytically pure ECyd (**7**) in 75% yield after purification by flash chromatography and crystallization from aqueous MeOH.

After the successful preparation of ECyd we tried this synthetic route for the preparation of 3′-*C*-ethynyl-2′-deoxycytidine (EdCyd). Therefore, commercially available 2′-deoxycytidine hydrochloride was orthogonally protected in three steps yielding N^4-benzoyl-5′-*O*-(4-monomethoxytrityl)-2′-deoxycytidine.[11] Oxidation of this protected deoxycytidine resulted in the formation of an unstable ketonucleoside that quantitatively lost its nucleobase by β-elimination. The loss of the nucleobase occurred during the oxidation with the chromium oxide reagent as well as during oxidation with the mild Dess–Martin periodinane reagent. This is confirmed by Hansske et al.[21] who found the 3′-keto-5′-*O*-trityl-2′-deoxycytidine and N^4-acetyl-3′-keto-5′-*O*-trityl-2′-deoxycytidine to be unstable. Bender et al.[22] reported the nearly quantitative (>90%) formation of N^4-benzoyl-5′-(*O*-*tert*-butyldiphenylsilyl)-3′-keto-2′-deoxycytidine out of the protected 2′-deoxycytidine under these reaction conditions. These results indicate an influence of the 5′-protection group on the stability of the formed 3′-keto-2′-deoxycytidines. In contrast to the *tert*-butyldiphenylsilyl (TBDPS) protection group used by Bender et al.,[22] the trityl protection groups used by Hansske et al.[21] and by us seem to enhance the β-elimination of the nucleobase out of the intermediary formed protected 3′-keto-2′-deoxycytidine.

The comparison of both synthesis approaches leads to the following results: The preparation of ECyd following synthetic route 1[12] affords ECyd in an overall yield of 25% referred to xylose as starting material in 8 reaction steps. Since the *N*-glycoside bond is formed during the preparation, the α-anomer that is obtained as a by-product has to be removed completely to afford the desired β-anomer. All reaction products are obtained through crystallization, which is undoubtedly a very elegant procedure which, however, can be too time consuming in the case of slow crystallizing products.

This new preparation method following synthetic route 2 affords ECyd in an overall yield of 24% referred to cytidine as starting material in 7 reaction steps. We use flash chromatography as main purification procedure, which is necessary for the removal of traces of the toxic chromium oxidation reagent as well as for the separation of the orthogonally protected isomers **3a** and **3b**. This method yields the desired product in all cases within a few hours including the final crystallization that is carried out under sonication.

Both alternatives afford ECyd in nearly identical yields based on the starting material xylose and cytidine.

Benzoic anhydride (≥98%) and *tert*-butyldimethylsilyl chloride (97%) were obtained from Lancaster (Mülheim, Germany); BuLi (1.6 M in hexane), 4-monomethoxytrityl chloride (≥97%), tetrabutylammonium fluoride trihydrate (≥97%) and *p*-toluenesulfonic acid monohydrate (≥98%) were obtained from Fluka (Buchs, Switzerland); cerium(III) chloride heptahydrate (99%) was obtained from ABCR (Karlsruhe, Germany); cytidine was obtained from Pharma-Waldhof (Düsseldorf, Germany); silica gel 60 (0.04–0.063 mm) was obtained from Merck (Darmstadt, Germany). All reagents were used as obtained except that CH_2Cl_2 was dried and stored over molecular sieves (4 Å). Dioxane and THF were dried with sodium (benzophenone), distilled and stored over molecular sieves (5 Å and 4 Å). Pyridine was refluxed over KOH, distilled and stored over molecular sieves (4 Å). $AgNO_3$ was dried in vacuum over P_2O_5 prior to reaction. The TBAF cleaving solution (1 M) was prepared by dissolving tetrabutylammonium fluoride trihydrate (157.8 g) in anhyd THF (500 mL). Methanolic ammonia was prepared by saturating MeOH with gaseous ammonia at r.t. Methanolic *p*-toluenesulfonic acid solution (4%) was prepared by dissolving *p*-toluenesulfonic acid monohydrate (20 g) in MeOH (500 mL). Ethynyltrimethylsilane was synthesized as described.[23]

Reactions were monitored by TLC, carried out on precoated silica gel 60 F_{254} plates (0.25 mm, Merck, Darmstadt- Germany) using UV light as visualizing agent and $HClO_4$ (60%) and heat as developing agents. Multi step Flash chromatography was carried out on dry packed silica gel 60 (0.040–0.063 mm) columns using binary solvent mixtures prepared by volume ratios (v/v) as eluent. NMR spectra were recorded on a Bruker AMX 250 (250 MHz) instrument and calibrated using DMSO-d_6 as internal standard. 2D-Cosy and Dept135 spectra were used if necessary to determine the chemical shifts of the sugar carbons and protons. FD mass spectra were recorded on a Finnigan MAT 711 A spectrometer. Elementary analyses were performed for C, H, N. Melting points (not corrected) were

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determined in a Stuart Scientific SMP3 capillary melting point apparatus.

All reactions were performed at r.t. if not stated. The concentration of the reaction mixtures, solutions, organic layers and eluated fractions was done in vacuum at a bath temperature of 45 °C.

N^4-Benzoylcytidine (1)

To a suspension of cytidine (100 g, 412 mmol) in H_2O (240 mL) was added a solution of benzoic anhydride (140 g, 618 mmol) in anhyd dioxane (2 L) and the resulting mixture was heated to 90 °C for 4 h. After cooling to r.t., the precipitated product was collected by filtration, washed twice with dioxane (300 mL) and dried in vacuum, yielding 137 g (96%) N^4-benzoyl-cytidine (**1**) as a white powder; mp 238–240 °C;

R_f 0.3 ($CHCl_3$–MeOH, 9:1).

^{1}H NMR (250 MHz, DMSO-d_6): δ = 3.60–3.81 (m, 2 H, H-5′, H-5″), 3.93–4.04 (m, 3 H, H-2′, H-3′, H-4′), 5.09 (br s, 1 H, 3′-OH), 5.21 (t, *J* = 3.7 Hz, 1 H, 5′-OH), 5.54 (m, 1 H, 2′-OH), 5.83 (d, *J* = 2.5 Hz, 1 H, H-1′), 7.33 (d, *J* = 7.46 Hz, 1 H, H-5), 7.47–8.10 (m, 5 H, C_6H_5), 8.51 (d, *J* = 7.53 Hz, 1 H, H-6), 11.27 (s, 1 H, NH).

^{13}C NMR (68 MHz, DMSO-d_6): δ = 59.99 (C-5′), 68.74 (C-3′), 74.59 (C-2′), 84.32 (C-4′), 90.28 (C-1′), 96.11 (C-5), 128.43, 128.58, 132.70, 133.22 (arom-C), 145.24 (C-6), 154.55 (C-2), 162.94 (C-4), 167.50 (*C*OC_6H_5).

MS: *m*/*z* = 348.1 $[M + H]^+$.

Anal. Calcd for $C_{16}H_{17}N_3O_6$: C, 55.33; H, 4.93; N, 12.10. Found: C, 55.15; H, 4.73; N, 12.11.

N^4-Benzoyl-5′-*O*-(4-monomethoxytrityl)cytidine (2)

To a solution of **1** (137 g, 197 mmol) in anhyd pyridine (1 L) was added 4-monomethoxytrityl chloride (146.2 g, 237 mmol) under the exclusion of moisture. The reaction vessel was sealed air tight and the mixture was shaken for 18 h resulting in a clear solution to which MeOH (60 mL) was added under cooling. After 10 min of shaking, the mixture was concentrated to a syrup that was coevaporated twice with toluene (500 mL) before being dissolved in $CHCl_3$ (1 L). The obtained solution was extracted three times with a sat. aq solution of $NaHCO_3$ (300 mL). The organic layer was concentrated to a syrup that was coevaporated with toluene (500 mL) and was crystallized from Et_2O (1 L) yielding the crude product which was collected by filtration and washed with Et_2O (300 mL). The crude product was extracted once with hot Et_2O (1 L) and was dried in vacuum. To remove traces of impurities, the crude product was dissolved in $CHCl_3$ (350 mL) and flash chromatographed on a silica gel column (25 × 10 cm) using four steps of binary $CHCl_3$–MeOH mixtures; first: 100:0 (6 L); second: 99:1 (6 L); third: 98:2 (6 L) and fourth: 95:5 (8 L). The fractions containing the desired product were pooled, concentrated and crystallized from Et_2O yielding 200.4 g (82%) of the title compound **2** as colorless crystals; mp 138–140 °C; R_f 0.29 ($CHCl_3$–MeOH, 98:2).

^{1}H NMR (250 MHz, DMSO-d_6): δ = 3.33–3.48 (m, 2 H, H-5′,H-5″), 3.76 (s, 3 H, OCH_3), 4.08–4.11 (m, 2 H, H-4, H-2′), 4.20–4.28 (m, 1 H, H-3′), 5.17 (d, *J* = 6.92 Hz, 1 H, 3′-OH), 5.72 (d, *J* = 4.73 Hz, 1 H, 2′-OH), 5.84 (d, *J* = 1.21 Hz, 1 H, H-1′), 6.92–8.04 (m, 19 H_{arom}), 7.18 (d, *J* = 6.95 Hz, 1 H, H-5), 8.37 (d, *J* = 7.47 Hz, 1 H, H-6), 11.28 (s, 1 H, NH).

^{13}C NMR (68 MHz, DMSO-d_6): δ = 54.99 (CH_3O), 62.17 (C-5′), 68.60 (C-3′), 74.35 (C-2′), 81.82 (C-4′), 86.23 [*C*(C_6H_5)$_3$], 91.03 (C-1′), 96.02 (C-5), 113.30, 127.02, 127.96, 128.00, 128.09, 128.40, 129.98, 132.67, 133.14, 134.88, 143.78, 144.03, 158.26 (arom-C), 144.66 (C-6), 154.39 (C-2), 163.05 (C-4), 167.26 (*C*OC_6H_5).

MS: *m*/*z* = 618.9 $[M]^+$.

Anal. Calcd for $C_{36}H_{33}N_3O_7$: C, 69.78; H, 5.37; N, 6.78. Found: C, 69.62; H, 5.18; N, 6.52.

N^4-Benzoyl-2′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(4-monomethoxytrityl)cytidine (3b)

To a solution of $AgNO_3$ (65.8 g, 388 mmol) in anhyd pyridine (800 mL) was added a solution of **2** (200 g, 322 mmol) in anhyd THF (2 L) and the mixture was stirred under the exclusion of moisture for 15 min before a solution of *tert*-butyldimethylsilyl chloride (63.2 g, 420 mmol) in anhyd THF (400 mL) was added. The reaction vessel was sealed air tight and the mixture was stirred for 3 h and then added to a sat. aq solution of $NaHCO_3$ (1.7 L). After stirring for 10 min, the precipitate was collected by filtration (G3) and was washed twice with THF (200 mL) and discarded. To the filtrate a sat. solution of NaCl (2.2 L) was added resulting in the separation of two layers. The aqueous layer was extracted twice with THF (300 mL) and the combined organic layers were concentrated to a syrup that was dissolved in $CHCl_3$ (800 mL). The aqueous layer was separated and discarded. The organic layer was concentrated to a syrup which was coevaporated twice with toluene (500 mL) and then dissolved in $CHCl_3$ (1 L) and flash chromatographed on a silica gel column (25 × 10 cm) using Et_2O (10 L) as the eluent. The fractions containing the 2′-*O*-TBDMS-isomer that was eluted first were pooled and concentrated to a syrup which was crystallized from Et_2O. The fractions containing the 3′-*O*-TBDMS-product which was eluted subsequently and the fractions containing both isomers were pooled and concentrated to a syrup that was isomerized to a mixture (~1:1) of both isomers as follows: After dissolving the syrup in a mixture of pyridine–MeOH (1:4, 500 mL), the solution was stirred at 70 °C for 12 h, then concentrated and coevaporated twice with toluene. The isomeric mixture was rechromatographed on a silica gel column as described above. The title compound **3b** (192 g, 81%) was obtained as colorless crystals; mp 164–166 °C; R_f 0.71 (Et_2O).

^{1}H NMR (250 MHz, DMSO-d_6): δ = 0.12, 0.14 (s, 3 H each, $SiCH_3$), 0.89 [s, 9 H, $SiC(CH_3)_3$], 3.36–3.48 (m, 2 H, H-5′, H-5″), 3.75 (s, 3 H, OCH_3), 4.10–4.21 (m, 3 H, H-2′, H-3′, H-4′), 5.05 (d, *J* = 5.40 Hz, 1 H, 3′-OH), 5.80 (d, *J* = 1.2 Hz, 1 H, H-1′), 7.17 (d, *J* = 6.85 Hz, 1 H, H-5), 6.93–8.04 (m, 19 H_{arom}), 8.38 (d, *J* = 7.51 Hz, 1 H, H-6), 11.28 (s, 1 H, NH).

^{13}C NMR (68 MHz, DMSO-d_6): δ = –4.72 (CH_3Si), 18.00 [Si*C*(CH_3)$_3$], 25.77 [SiC(*C*H_3)$_3$], 55.03 (CH_3O), 62.03 (C-5′), 68.38 (C-3′), 76.41 (C-2′), 81.70 (C-4′), 86.34 [*C*(C_6H_5)$_3$], 90.88 (C-1′), 96.12 (C-5), 113.35, 127.07, 127.99, 128.14, 128.42, 130.04, 132.68, 133.25, 134.86, 143.79, 144.03, 158.34 (arom-C), 144.33 (C-6), 154.09 (C-2), 163.00 (C-4), 167.21 (*C*OC_6H_5).

MS: *m*/*z* = 733.1 $[M]^+$.

Anal. Calcd for $C_{42}H_{47}N_3O_7Si$: C, 68.73; H, 6.45; N, 5.73. Found: C, 68.59; H, 6.56; N 5.78.

N^4-Benzoyl-2′-*O*-(*tert*-butyldimethylsilyl)-3′-keto-5′-*O*-(4-monomethoxytrityl)cytidine (4)

To a suspension of CrO_3 (78.6 g, 786 mmol) in anhyd CH_2Cl_2 (2 L) were added pyridine (140 mL) and Ac_2O (74 mL, 785 mmol) under cooling. At a temperature of 15 °C was added **3b** (192 g, 262 mmol) and the reaction mixture was stirred for 1 h. The dark brown solution was then poured into EtOAc (6 L) and the resulting suspension was filtered through silica gel (700 g). The silica gel layer was thoroughly washed with EtOAc and the combined organic phases were concentrated to a syrup which was dissolved in $CHCl_3$ and purified by flash chromatography on a silica gel column (25 × 10 cm) using $CHCl_3$ (16 L) as the eluent. The fractions containing the desired product were pooled, concentrated to a syrup which was crystallized from Et_2O (700 mL) at –25 °C, yielding 149.4 g (78%) of **4** as colorless crystals; mp 145–147 °C; R_f 0.67 ($CHCl_3$–MeOH, 98:2).

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^{1}H NMR (250 MHz, DMSO-d_6): δ = 0.07, 0.12 (s, 3 H each, $SiCH_3$), 0.88 [s, 9 H, $SiC(CH_3)_3$], 3.13 (m, 1 H, H-5″), 3.57 (m, 1 H, H-5′), 3.78 (s, 3 H, OCH_3), 4.63–4.77 (m, 3 H, H-2′, H-3′, H-4′), 6.27 (d, *J* = 5.26 Hz, 1 H, H-1′), 6.89 (d, *J* = 7.40 Hz, 1 H, H-5), 7.23–8.07 (m, 19 H_{arom}), 8.35 (d, *J* = 7.51 Hz, 1 H, H-6), 11.45 (br s, 1 H, NH).

^{13}C NMR (68 MHz, DMSO-d_6): δ = –4.95(CH_3Si), 17.87 [Si*C*$(CH_3)_3$], 25.38 [SiC(*C*$H_3)_3$], 55.03 (CH_3O), 63.91 (C-5′), 75.69 (C-2′), 82.65 (C-4′), 86.16 [*C*$(C_6H_5)_3$], 88.31 (C-1′), 97.25 (C-5), 113.28, 126.99, 127.89, 128.46, 128.55, 130.01, 132.86, 134.47, 137.59, 143.89, 144.01, 158.35 (arom-C), 145.90 (C-4), 154.74 (C-2), 163.66 (C-4), 167.50 (*C*OC_6H_5), 207.46 (C-3′).

MS: *m*/*z* = 732.3 $[M]^+$.

Anal. Calcd for $C_{42}H_{45}N_3O_7Si$: C, 68.92; H, 6.20; N, 5.74. Found: C, 68.82; H, 6.23; N 5.78.

N^4-Benzoyl-2′-*O*-(*tert*-butyldimethylsilyl)-3′-ketocytidine (5)

To a solution of **4** (140 g, 192 mmol) in $CHCl_3$ (1.4 L) was added a methanolic solution of *p*-toluenesulfonic acid (4%, 1.4 L) and the resulting mixture was stirred for 5 min. The reaction mixture was then poured into a half sat. aq solution (2 L) of $NaHCO_3$. The organic layer was separated and concentrated (bath temperature <40 °C) to a syrup which was dissolved in acetone (600 mL). The precipitate was collected by filtration and discarded. The filtrate was concentrated (bath temperature <40 °C) to a syrup that was dissolved in $CHCl_3$ (600 mL) and flash chromatographed on a silica gel column (20 × 10 cm) using a two step $CHCl_3$–acetone gradient; first: 100:0 (6 L); second: 80:20 (6 L). The fractions containing the desired product were pooled, concentrated and crystallized from Et_2O (700 mL) at –25 °C. The precipitate was collected by filtration, washed with ice cold Et_2O (200 mL, –25 °C) and dried ($CaCl_2$), yielding 66.6 g (76%) of **5** as colorless crystals; mp 205 °C; R_f 0.46 ($CHCl_3$–acetone, 8:2).

^{1}H NMR (250 MHz, DMSO-d_6): δ = –0.04, 0.01 (s, 3 H each, $SiCH_3$), 0.85 [s, 9 H, $SiC(CH_3)_3$], 3.75 (br s, 2 H, H-5′, H-5′), 4.42–4.44 (m, 1 H, H-4′), 4.55 (d, *J* = 7.46 Hz, 1 H, H-2′), 5.36 (br s, 1 H, 5′-OH), 6.33 (d, *J* = 7.46 Hz, 1 H, H-1′), 7.48–8.08 (m, 6 H_{arom}, H-5), 8.48 (d, *J* = 7.57 Hz, 1 H, H-6), 11.43 (br s, 1 H, NH).

^{13}C NMR (68 MHz, DMSO-d_6): δ = –5.40, –4.94 (CH_3Si), 17.82 [Si*C*$(CH_3)_3$], 25.33 [SiC(*C*$H_3)_3$], 60.67 (C-5′), 76.73 (C-2′), 82.56 (C-4′), 86.43 (C-1′), 97.37 (C-5), 128.45, 128.53, 132.86 (arom-C), 145.72 (C-6), 154.71 (C-2), 163.45 (C-4), 167.40 (*C*OC_6H_5), 208.99 (C-3′).

MS: *m*/*z* = 460.2 $[M + H]^+$, 402.3 $[M – C_4H_9]^+$, 214.8 [*N*^{4} – benzoylcytosine – H]$^+$, 187.0 [M – *N*^{4} – benzoylcytosine – C_4H_9]$^+$.

Anal. Calcd for $C_{22}H_{29}N_3O_6Si$: C, 57.50; H, 6.36; N, 9.14. Found: C, 57.38; H, 6.02; N 8.92.

N^4-Benzoyl-2′-*O*-(*tert*-butyldimethylsilyl)-3′-*C*-(trimethylsilylethynyl)cytidine (6)

First a suspension of anhydrous cerium(III) chloride in THF was prepared as follows: Cerium(III) chloride heptahydrate (324 g, 869 mmol) was dried under stirring in vacuum (oil pump) at 140 °C for 3 h. The dry cerium(III) chloride was then suspended in anhyd THF (1 L) and stirred overnight under argon. The reaction was carried out under argon at –78 °C unless otherwise indicated. To a stirred solution of ethynyltrimethylsilane (120.6 mL, 869 mmol) in anhyd THF (300 mL) was added a solution of BuLi in hexane (544 mL, 869 mmol) keeping the temperature of the reaction mixture below –50 °C. After stirring for 30 min, the precooled (< –50 °C) suspension of cerium(III) chloride (214 g, 869 mmol) in anhyd THF (1 L) was added and the mixture was stirred for 2 h, and then a precooled solution (< –50 °C) of **5** (66.6 g, 145 mmol) in anhyd THF (260 mL) was added. After stirring at –65 °C for 3 h, the mixture was then quenched with an 1 M aq solution of NH_4Cl (900 mL). The mixture was brought to r.t. under stirring and diluted by the addition of H_2O (2 L) and filtered. The filtrate was extracted three times with EtOAc (600 mL). The combined organic layers were concentrated to a syrup that was dissolved in Et_2O (600 mL) and flash chromatographed on silica gel (25 × 10 cm), using Et_2O (8 L) as the eluent. The fractions containing the desired product were collected, pooled, concentrated and dried to a foam, yielding 68.6 g (85%) of **6**; R_f 0.57 ($CHCl_3$–acetone, 8:2).

^{1}H NMR (250 MHz, DMSO-d_6): δ = –0.02, 0.04 (s, 3 H each, $SiCH_3$), 0.10 [s, 9 H, $Si(CH_3)_3$], 0.85 [s, 9 H, $SiC(CH_3)_3$], 3.75–3.82 (m, 2 H, H-5′, H-5″), 4.03 (t, *J* = 4.43 Hz, 1 H, H-4′), 4.37 (d, *J* = 5.63 Hz, 1 H, H-2′), 5.10 (t, *J* = 4.75 Hz, 1 H, 5′-OH), 5.88 (s, 1 H, 3′-OH), 5.93 (d, *J* = 5.63 Hz, 1 H, H-1′), 7.38–8.01 (m, 6 H_{arom}, H-5), 8.43 (d, *J* = 7.58 Hz, 1 H, H-6), 11.29 (br s, 1 H, NH).

^{13}C NMR (68 MHz, DMSO-d_6): δ = –5.03, –4.41 (CH_3Si), –0.60 [$Si(CH_3)_2$], 17.79 [Si*C*$(CH_3)_3$], 25.63 [SiC(*C*$H_3)_3$], 61.50 (C-5′), 72.90 (C-3′), 74.78 (*C*≡CTMS), 80.35 (C-2′), 86.90 (C-4′), 88.51 (C-1′), 91.21 (C≡*C*TMS), 96.55 (C-5), 128.45, 132.78, 133.07 (arom-C), 145.76 (C-6), 154.79 (C-2), 163.18 (C-4), 167.37 (*C*OC_6H_5).

MS: *m*/*z* = 558.6 $[M + H]^+$, 500.3 $[M – C_4H_9]^+$.

Anal. Calcd for $C_{27}H_{39}N_3O_6Si_2$: C, 58.14; H, 7.05; N, 7.53. Found: C, 57.92; H, 6.87; N 7.38.

3′-*C*-Ethynylcytidine (7)

Compound **6** (68.6 g, 123 mmol) was suspended in methanolic ammonia (1.2 L) and the resulting solution kept sealed at r.t. for 24 h and then concentrated to a syrup that was coevaporated twice with toluene (400 mL). This syrup was dissolved in anhyd THF (700 mL) and the TBAF solution (240 mL) was added under exclusion of moisture. The reaction mixture was stirred for 4 h followed by concentration to a syrup that was dissolved in $CHCl_3$ (200 mL) and flash chromatographical purification on a silica gel column (16 × 9 cm) using a three step $CHCl_3$–MeOH gradient. First: 95:5 (4 L); second: 80:20 (4 L) and third: 60:40 (3 L). The fractions containing the desired product were pooled, concentrated to a syrup which was crystallized from MeOH–H_2O (8:2), yielding 24.6 g (75%) of 3′-*C*-ethynylcytidine (**7**) as colorless crystals; mp 233–235 °C; R_f 0.39 ($CHCl_3$–MeOH, 7:3); $[\alpha]_D^{20}$ +68 (*c* = 1.0, H_2O).

^{1}H NMR (250 MHz, DMSO-d_6): δ = 3.52 (s, 1 H, C≡CH), 3.67–3.75 (m, 2 H, H-5′, H-5″), 3.85–3.88 (m, 1 H, H-4′), 4.12 (t, *J* = 6.55 Hz, H-2′), 5.05 (t, *J* = 4.76 Hz, 1 H, 5′-OH), 5.74–5.83 (m, 3 H, 3′-OH, 2′-OH, H-5), 5.89 (d, *J* = 6.40 Hz, 1 H, H-1′), 7.26 (br s, 2 H, NH_2), 7.83 (d, *J* = 7.49 Hz, 1 H, H-6).

^{13}C NMR (68 MHz, DMSO-d_6): δ = 61.57 (C-5′), 72.56 (C-3′), 77.05 (*C*≡CH), 78.35 (C-2′), 83.03 (C≡*C*H), 86.08 (C-4′), 87.64 (C-1′), 94.28 (C-5), 141.80 (C-6), 155.56 (C-2), 165.49 (C-4).

MS: *m*/*z* = 267.9 $[M]^+$.

Anal. Calcd for $C_{11}H_{14}N_3O_5$: C, 49.25; H, 5.26; N, 15.66. Found: C, 49.03; H, 4.98; N, 15.48.

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