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Synthesis of 4-Aminophthalimide and 2,4-Diaminopyrimidine C-Nucleosides as Isosteric Fluorescent DNA Base Substitutes

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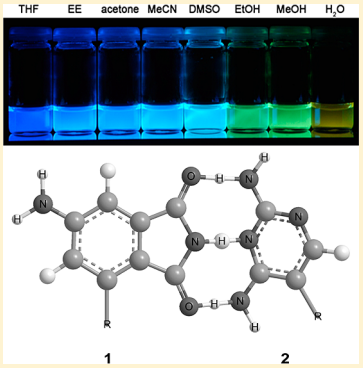
Supporting Information

ABSTRACT: The 4-aminophthalimide C-nucleoside **1** was designed as an isosteric DNA base surrogate, and a synthetic route to nucleoside **1** together with the 2,4-diaminopyrimidine-C-nucleoside **2** as a potential counterbase was worked out. The key steps in both synthetic routes represent a stereoselective Heck-type palladium-catalyzed cross-coupling with 2′-deoxyribofuranoside glycal followed by stereoselective reduction with $NaBH(OAc)_3$. The nucleoside **1** shows a solvatofluorochromic behavior and significantly red-shifted fluorescence in solvents of high polarity and with hydrogen bonding capabilities. Both nucleosides **1** and **2** can be further processed to the corresponding phosphoramidite as DNA building blocks that allow incorporation of these chromophores as artificial DNA bases by automated DNA synthesis. The combination of the poor stacking properties of **1** and the hydrogen bonding interface at the phthalimide functionality that does not fit to any of natural DNA bases in the counterstrand yields destabilization of the duplex by 4−11 °C. The fluorescence of **1** in a representative double stranded DNA is characterized by a large Stokes' shift and a quantum yield of approximately 12%. These are remarkable optical properties considering the very small size of the chromophore and indicate a high potential of these nucleoside analogues for fluorescent DNA analytics and imaging.



INTRODUCTION

The preparation of fluorescent DNA and RNA conjugates is well established but still of significant interest for chemical bioanalytics in assays and on arrays. The ways to covalently attach fluorophores have been studied for decades.[1−3] In the meantime, imaging of live cells attracts growing attention since biological processes can be observed in their native environment.[4] In this research field, the isomorphic or isosteric replacement of natural DNA bases by fluorophores attracts special interest since such labeled oligonucleotides, at least in principle, do not significantly exceed the size of natural B-form DNA.[3,5,6] This structural feature is required especially for the investigation of DNA−protein interactions inside cells and for DNA polymerase-assisted replication of fluorescent oligonucleotides by primer extension or PCR.[2,7] The most prominent and probably longest known example is 2-aminopurine as the fluorescent analogue of adenine[6,8,9] which has been extensively used, e.g., to study RNA conformation equilibria[10] and DNA−protein interactions.[11] The major disadvantages of this fluorescent DNA base surrogate are the excitation in the UV-B range (near DNA absorption range at 305 nm), the low extinction coefficient (ε = 6,000 M^{-1} cm^{-1}), the emission in the UV-A range (370 nm), and most importantly, fluorescence quenching inside double-stranded DNA due to charge-transfer processes. Alternatively, pteridines have an absorption that is better separated from the DNA absorption (330−350 nm) and emission occurs in the visible range (430 nm), but quenching inside DNA is still a problem.[3,12] Other promising and interesting structures that follow the concept of best possible isosteric DNA base replacements include ε-ethenoadenine,[13] pyrrolo-fused cytosine,[14] cyclized 4-*N*-carbamoyl-2′-deoxycytidine derivatives,[15] and thieno[3,4-*d*]-2′-deoxyuridine.[16] Although some of them are commercially available as DNA building blocks, fluorescence inside duplex DNA is accompanied typically by low quantum yields that limit their applicability. Wilhelmsson et al. published artificial nucleosides based on 1,3-diaza-2-oxophenoxazine and 1,3-diaza-2-oxophenothiazine as fluorescent substitutes with the recognition pattern typical for cytosine. The absorption is well separated from that of DNA (365−375 nm), and emission occurs in the visible range (455−500 nm) with good quantum yields (ca. 20%).[17] Compared to purines, the size of these nucleosides, however, is extended by at least one benzene ring that is a fused part of the phenoxazine or phenothiazine chromophore structure. In a more general approach, Castellano et al. developed push−pull systems that are based on purines as a lead structure.[18] To our knowledge, however, these purine-like nucleosides have not yet been incorporated into oligonucleotides. Finally, Hirao et al. attached bithienyl groups to artificial

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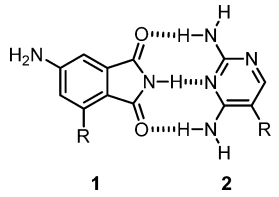
base pairs yielding fluorescent base analogues for the expansion of the genetic alphabet.[19]

4-Aminopthalimide is a well-known, highly fluorescent, and solvatofluorochromic dye[20] that has been applied in biopolymers[21] but only once for oligonucleotides.[22,23] The dye is excitable at the border between UV-A and the visible range (370−410 nm) and shows fluorescence in the visible range. The apparent Stokes' shift is large (up to 115 nm)[24] and depends highly on the environment, namely, the solvent polarity and the ability for H-bonding.[25,26] The time evolution of the shift ("time-resolved dynamic Stokes' shift") reflects the orientational dynamics of the surrounding medium. Accordingly, 4-aminophthalimides were used as solvatofluorochromic probes in cyclodextrines,[27] polymers,[28] membranes, micelles and enzymes complexes.[29] With respect to nucleic acids Hocek et al. published most recently a synthetic protocol that allows incorporating 4-aminophthalimide as covalent base modification in oligonucleotides using a polymerase-assisted preparation strategy.[22] Except for this publication, we are not aware of other attempts to prepare 4-aminophthalimide−nucleic acid conjugates.

In principle, 4-aminophthalimide resembles the size of natural purines and should therefore be an isosteric DNA base replacement in oligonucleotides. Moreover, the phthalimide functionality should be able to form hydrogen bonds with counterparts of pyrimidine size that are placed in the opposite position as part of the complementary strand. A completely new, bioorthogonal, and fluorescent DNA base pair with fully matching H-bonding pattern can be envisioned with 2,4-diaminopyrimidine[26] as opposite base (Figure 1).



1 2

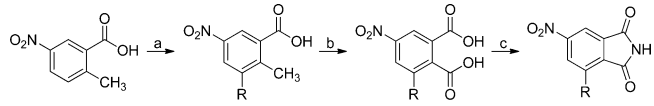
Figure 1. Base pair surrogate consisting of 4-aminophthalimide nucleoside **1** and 2,4-diaminopyrimidine nucleoside **2**; R = 2′-deoxyribofuranoside.

Herein we present the synthesis of the 4-aminophthalimide *C*-nucleoside **1** and the 2,4-diaminopyrimidine *C*-nucleoside **2** as artificial and fluorescent DNA base surrogates, their incorporation into oligonucleotides by automated phosphoramidite chemistry, and the optical properties of **1** inside double-stranded DNA with different opposing bases, including **2**. In particular, the synthesis of oligonucleotides modified with aminophthalimide **1** is a challenge due to the hydrolytic instability of the imide functionality in this small chromophore which requires special oligonucleotide synthesis and workup conditions.

RESULTS AND DISCUSSION

Synthesis. The synthesis of the aminophthalimide *C*-nucleoside **1** (Scheme 1) started with commercially available 2-methyl-5-nitrobenzoic acid (**3**) that was brominated with NBS or iodinated with NIS to the corresponding halogenated intermediates **4** and **5** in nearly quantitative yields. The nitro group which forms the amino group later in the synthetic procedure plays an important role here since it directs the

Scheme 1. Synthetic Steps to the Phthalimide Aglycon Precursors 8 and 9[a]



3 R = Br: **4** = I: **5** R = Br: **6** = I: **7** R = Br: **8** = I: **9**

[a]Key: (a) NBS or NIS, H_2SO_4, 60 °C, 2 h, 94% (NBS) or 97% (NIS); (b) $KMnO_4$, NaOH, H_2O, 100 °C, o.n.; (c) $CO(NH_2)_2$, *o*-xylene, 160 °C, 4 h, 32% or 35%, respectively.

halogenation into the desired position. Subsequently, the methyl group of **4** and **5** is oxidized under standard conditions ($KMnO_4$ under basis conditions at 100 °C) to give **6** and **7**, respectively. Finally the phthalimide functionality was built by condensation of urea to give **8** and **9** in 32% and 35% yield, respectively (over the last two steps).

The phthalimides **8** and **9** represent important intermediates since they already contain the necessary functional groups in the right substitution pattern. They bear, most importantly, a halogen atom to apply palladium-catalyzed cross-coupling methodology to form a glycosidic bond to the 2′-deoxyribofuranoside. The necessary sugar precursor for this type of glycosidic coupling represents glycal **10** that has been applied successfully for the *C*-nucleoside synthesis in the past[24] and can be synthesized in three steps from thymidine.[30]

The Heck-type coupling of the aminophthalimide precursors **8** and **9** to glycal **10** forms selectively the β-anomeric bond in acceptable yields (ca. 33%, Scheme 2). The stereoselectivity of these reactions is the result of substrate control because the lower face of glycal **10** is shielded effectively by the sterically demanding TBDMS group in the 3′-position. Interestingly, the expected silylenol ether **11** was isolated only in traces. Instead, the 3′-deprotected ketone **12** could be purified and subsequently reduced to nucleoside **13** by $NaBH(OAc)_3$. The stereoselectivity of the latter reaction is controlled by the 5′-hydroxy group that binds the boron atom and let the hydride attack the C═O bond from the top. Finally the reduction of the nitro group of **13** by NaSH gives the 4-aminophthalimide *C*-nucleoside **1** in 88% yield.

In order to incorporate nucleoside **1** into oligonucleotides by automated phosphoramidite chemistry a corresponding DNA building block has to be prepared. This means that the exocyclic amino group of **1** needs to be protected, and DMT has to be attached to the 5′-position and the phosphityl amide group to the 3′-position. With respect to the known base instability of the phthalimide functionality, we decided to use the phenoxyacetyl (Pac) protecting group for the exocyclic amino function. The attempts to protect the amino group in the 4-position of nucleoside **1** with in situ protection of the competing 3′- and 5′-hydroxyl groups with TMS failed completely. Alternatively, the nitro-substituted *C*-nucleoside **13** was protected at the 3′- and 5′-positions with TBDMS groups (Scheme 3). Subsequently, the nitro group of **14** was reduced by NaSH in 70% yield and the Pac protecting group could be attached. Finally, the two TBDMS groups of **15** were removed and the Pac-protected nucleoside **16** was obtained in 55% yields (over two steps). In the last two synthetic steps toward the DNA building block **18** the DMT and the phosphoramidite group were introduced by standard procedures. Preliminary experiments to prepare **DNA1** (Table 1) by automated synthesis using building block **18** failed, presumably

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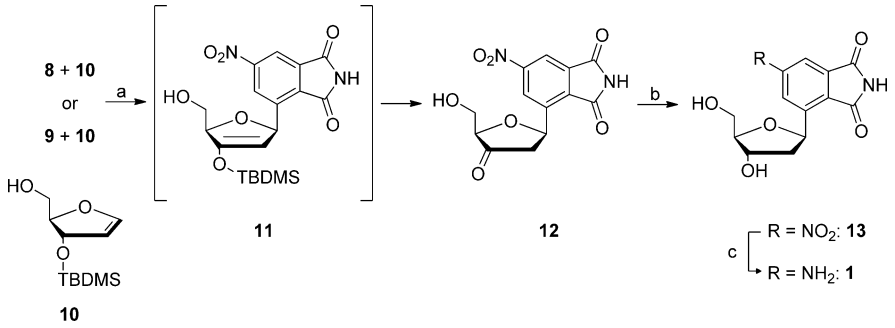
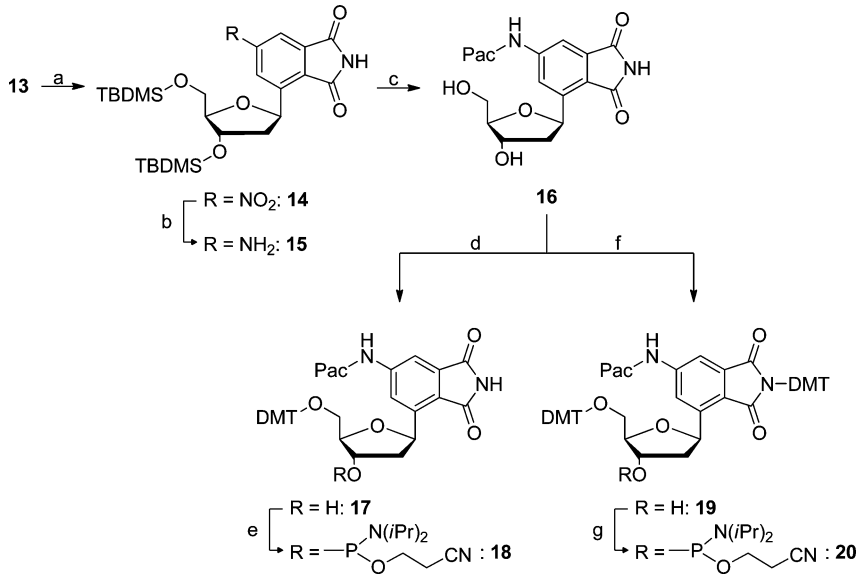
Scheme 2. Heck-Type Coupling of 8 or 9 to 10 and Further Steps to the Aminophthalimide-*C*-nucleoside 1[a]



[a]Key: (a) $P(PhF_5)_3$, $Pd(OAc)_2$, Et_3N, MeCN, 82 °C, o.n., 33%; (b) $NaBH(OAc)_3$, HOAc/MeCN 2:1, 0 °C, 1 h, 93%; (c) NaSH, H_2O, EtOH, 78 °C, 1 h, 88%.

Scheme 3. Syntheses of Aminophthalimide DNA Building Blocks 18 and 20[a]



[a]Key: (a) TBDMSCl, DMF, rt, o.n. 57%; (b) NaSH, H_2O, EtOH, 78 °C, 1 h, 70%; (c) (i) Pac_2O, pyridine, rt, o.n., (ii) $Et_3N{\cdot}3HF$, THF, rt, o.n., 55%; (d) DMTCl, pyridine, rt, 42 h, 52%; (e) 2-cyanoethoxy-*N*,*N*-diisopropylaminophosphanyl chloride, DIPEA, DCM, rt, 6 h, 59%; (f) DMTCl, Et_3N, pyridine, 30 °C, o.n., 64%; (g) 2-cyanoethoxy-*N*,*N*-diisopropylaminophosphanyl chloride, DIPEA, DCM, rt, 4 h, 67%.

Table 1. Melting Temperatures (T_m) of DNA1-X, DNA2-X, and DNA1-2 (X = A, G, T, C): 2.5 μM Duplex in 10 mM Na−Pi Buffer, 250 mM NaCl, pH 7.0, λ = 260 nm, 20−90 °C, Interval 0.7 C/min

duplex[a]	T_m (°C)	ΔT_m[b] (°C)	duplex[c]	T_m (°C)	ΔT_m[b] (°C)
DNA1-A	56.0	−4.3	**DNA2-A**	55.3	−5.0
DNA1-G	53.5	−11.4	**DNA2-G**	52.3	−12.6
DNA1-T	53.0	−9.1	**DNA2-T**	55.2	−6.9
DNA1-C	51.2	−9.9	**DNA2-C**	53.2	−7.9
DNA1−2	53.6				

[a]Sequence of **DNA1**: 5′-GCT-GCA-**1**AC-GTC-G-3′. Counter strands are fully complementary but vary at the base opposite to **1**. [b]In comparison to corresponding unmodified duplexes. [c]Sequence of **DNA2**: 5′-CGA-CGT-**2**TG-CAG-C-3′. Counter strands are fully complementary but vary at the base opposite to **2**.

due to the nucleophilicity of the phthalimide nitrogen. Hence, a second DMT group was introduced at this position of **16** and subsequent phosphitylation of **19** gave the fully protected DNA building block **20**.

With respect to the various synthetic detours and the increased number of synthetic steps in the procedure described above, an alternative synthetic route to the Pac-protected nucleoside **16** was worked out (Scheme 4). It starts with key intermediate **9**. The nitro group was reduced with NaSH in 56% yield and the resulting amino group of **21** was directly protected by Pac yielding **22** nearly quantitatively. As described above, this nucleoside precursor was coupled to glycal **10** by the Heck-type reaction to obtain the ketone **24**, again in low but still acceptable yield (27%). Stereoselective reduction with $NaBH(OAc)_3$ finishes in good yield this synthetic shortcut to the Pac-protected nucleoside **16**.

Because of the well-known lability of the phthalimide functionality toward bases and nucleophiles the solid-phase methodology had to be changed to the so-called ultramild conditions.[31] The corresponding DNA building blocks are commercially available and bear phenoxyacetyl, isopropylphenoxyacetyl, and acetyl protecting groups at the DNA bases instead of benzoyl and isobutyryl in conventional phosphoramidites. Accordingly, the use of concd aq ammonia can be avoided. Alternatively, deprotection is achieved with 0.05 M

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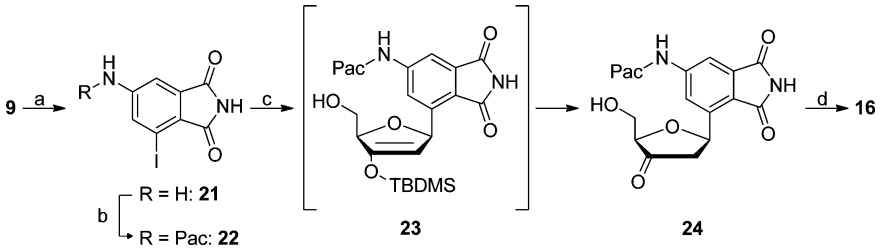
Scheme 4. Shorter Alternative Route to the Pac-Protected Aminophthalimide Nucleoside 16[a]

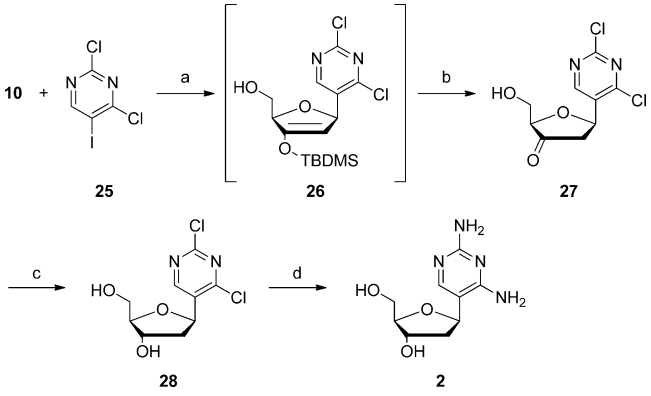


[a]Key: (a) NaSH, H_2O, EtOH, 78 °C, 1 h, 56%; (b) PacCl, imidazole, THF, pyridine, rt, 4 h, 95%; (c) **10**, $P(PhF_5)_3$, $Pd(OAc)_2$, Et_3N, MeCN, 82 °C, o.n., 27%; (d) $NaBH(OAc)_3$, HOAc/MeCN 2:1, 0 °C, 1 h, 94%.

K_2CO_3 in MeOH, typically within 4 h at rt. The first attempts to deprotect **DNA1** that was synthesized automatically using the phosphoramidite **20** were not successful. Mass spectrometry revealed that the protecting groups, especially the Pac groups at the adenines, could be cleaved off; however, the Pac group at the 4-aminophthalimide could not. Hence, the deprotection conditions were varied. This included additionally Cs_2CO_3 and K-*t*-BuO, each in MeOH or *i*-PrOH, NH_3 in H_2O, $MeNH_2$ in EtOH (1:1), and NaOH in MeOH (4:1). Several temperatures from 30 to 80 °C and incubation times from 2 to 24 h were tried. These experiments revealed that only the use of 0.05 M K_2CO_3 in MeOH, and an incubation time of 48 h at 65 °C gave successfully the completely deprotected oligonucleotide **DNA1**.

For the synthesis of the 2,4-diaminopyrimidine *C*-nucleoside **2** a synthetic route developed by Kubelka et al.[32] was adopted (Scheme 5). To this end 3-TBDMS-protected glycal **10** was

Scheme 5. Synthetic Route to 2,4-Diaminopyrimidine-*C*-nucleoside 2 Developed by Kubelka[32a]



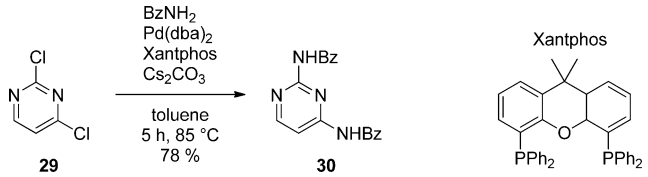
[a]Key: (a) $Pd(OAc)_2$, $P(PhF_5)_3$, Ag_2CO_3, $CHCl_3$, 12 h, 70 °C; (b) AcOH, TBAF, THF, 1 h, 0 °C, 34% overall yield; (c) $NaBH(OAc)_3$, AcOH, CH_3CN, 30 min, 0 °C, 92%; (d) NH_3, MeOH, 12 h, 120 °C, 73%.

reacted with 2,4-dichloro-5-iodopyrimidine **25** in the presence of palladium acetate and tris(pentafluorophenyl)phosphine. Since the silylenol ether **26** already starts to hydrolyze to 3′-ketoriboside **27** under these reaction conditions, the crude silylenol ether was directly converted to **27** by addition of acetic acid and TBAF. The β ketoriboside **27** was isolated in 34% overall yield with high stereoselectivity. The α-anomer could not be detected via TLC nor NMR. Stereoselective reduction of the ketone **27** by $NaBH(OAc)_3$ proceeded in nearly quantitative yields. Subsequent substitution of chlorine in **28** by ammonia yielded **2** in 73% yield.

With the unprotected diaminopyrimidine nucleoside **2** in hand, we tried to synthesize the corresponding phosphoramidite for later oligonucleotide preparation. Accordingly, the exocyclic amino functions were protected as phenoxyacetamides and the primary 5′-hydroxy-function by DMT group. The phenoxyacetamide group was selected to accomplish a faster cleavage by ammonia under milder conditions compared to benzamide. A first one-pot approach, in situ TMS protection of the hydroxy groups and subsequent introduction of the phenoxyacetyl moiety, led to an inseparable mixture of diverse protected nucleosides in low yields.

These results forced us to use another synthetic route to the protected phosphoramidite. Starting with the unprotected 2,4-dichloropyrimidine nucleoside **28**, the direct amination should be realized by introduction of benzamides[33] via a palladium-catalyzed Buchwald–Hartwig reaction. In a first series, we tested 2,4-dichloropyrimidine **29** as model substrate to find the optimal reaction conditions (working catalyst, etc.). Using a combination of $Pd(dba)_2$ and Xantphos as catalyst, 2,4-bis(benzoylamino)pyrimidine **30** was isolated in 78% yield (Scheme 6).

Scheme 6. Buchwald–Hartwig Amination of Model Substrate 2,4-Dichloropyrimidine 29



Upon transfer of these conditions to the unprotected 2,4-dichloropyrimidine nucleoside **28**, a mixture of starting material, monosubstituted product, and monosubstituted pyrimidine nucleoside in its pyranoside form was obtained. The low conversion might be due to the low solubility of **28** in toluene. We therefore turned to the more polar 1,4-dioxane and increased the amount of catalyst. By increasing the temperature to 100 °C, we were able to force the full substitution. At these elevated temperatures the formation of the pyranoside form is very likely, so we had to block the 5′-hydroxy group. The protection as DMT-ether seemed to be obvious, but the DMT-group is not stable under amination conditions.

As a result of this optimization work the following protocol was elaborated (Scheme 7). Initial protection of the 5′-hydroxy group of **28** was accomplished with TBDMSCl and imidazole in 85% yield. Subsequent Buchwald–Hartwig amination of **31**

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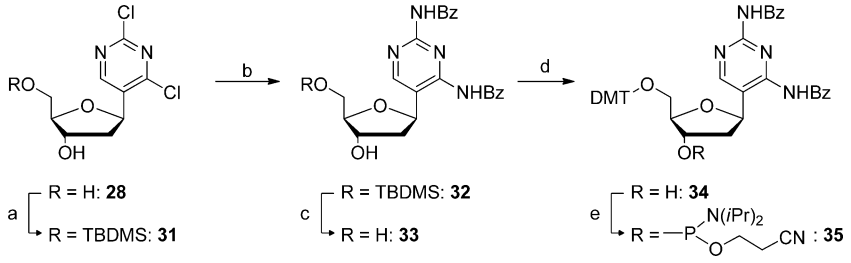
Scheme 7. Synthesis of 2,4-Bis(benzylamino)pyrimidine Nucleoside 33 and the Corresponding Protected Phosphoramidite 35[a]



[a]Key: (a) TBDMSCl, imidazole, DMF, 16 h, rt, 85%; (b) $BzNH_2$, $Pd_2(dba)_3$, Xantphos, $CsCO_3$, dioxane, 10 h, 100 °C, 82%; (c) $NEt_3 \cdot 3HF$, THF, 2 h, rt, 70%;)d) DMTCl, pyridine, 2 d, rt, 86%; (e) 2-cyanoethoxy-*N*,*N*-diisopropylaminophosphanyl chloride, DIPEA, CH_3CN, 2 h, rt, 60%.

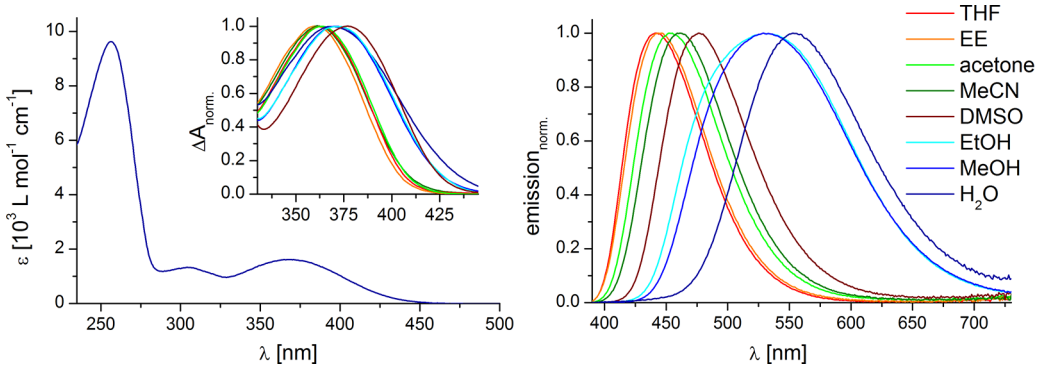


Figure 2. Solvatochromism (left) and solvatofluorochromism (right) of aminophthalimide nucleoside **1**. λ_{exc} = 385 nm.

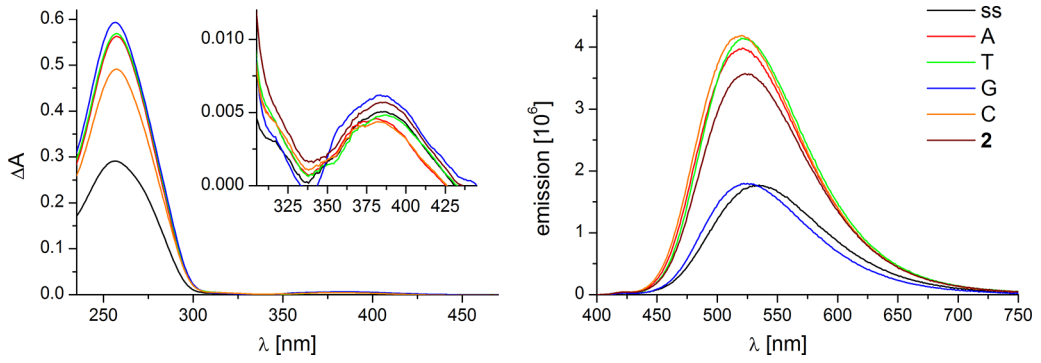


Figure 3. UV/vis absorption and fluorescence of modified single-stranded **DNA1**, double-stranded **DNA1A**–**DNA1C** and **DNA1-2**. 2.5 μM with 250 mM NaCl in 10 mM sodium phosphate buffer pH = 7.0, λ_{exc} = 385 nm.

with benzamide gave TBDMS-protected 2,4-bis(benzoylamino)pyrimidine nucleoside **32** in 82% yield. The TBDMS-group of **32** was removed by $NEt_3 \cdot 3HF$ in THF in 70% yield.

The bis(benzoylamino)pyrimidine nucleoside **33** was then protected with DMTCl at the 5′-hydroxy group and finally converted to phosporamidite **34** by 2-cyanoethoxy-*N*,*N*-diisopropylaminophosphanyl chloride. The DNA building block **35** was subjected to automated oligonucleotide synthesis. **DNA2** was deprotected and removed from the CPG by aqueous ammonia and subsequently purified by preparative HPLC.

Steady-State Optical Spectroscopy. The aminophthalimide *C*-nucleoside **1** was characterized both as monomer and as modification in **DNA1** by UV/vis absorption and steady-state fluorescence spectroscopy (Figure 2). The isolated nucleoside exhibits a solvatochromic behavior that is similar to the chromophore alone, including a very pronounced red-shift of absorption and emission with increasing solvent polarity. The fluorescence is additionally red-shifted in solvents with hydrogen-bonding capabilities. The quantum yield was determined to be 0.033 which was higher than the literature value of 0.022.[20] Overall, the sugar modification has only minor influence on the optical properties of 4-aminophthalimide in *C*-nucleoside **1**.

DNA1 was hybridized with five different complementary oligonucleotides as counter strands to five different double-stranded samples. The only difference represents the base (A, G, T, C, **2**) that was opposite to the aminophthalimide DNA base surrogate (Table 1). The determination of the melting temperatures of **DNA1-X** (X = A, G, T, C) revealed strong destabilization compared to the corresponding nonmodified duplexes. It became clear that the combination of the poor stacking properties of the 4-aminophthalimide chromophore as artificial DNA base and the hydrogen bonding interface at the phthalimide functionality that does not fit to any of natural DNA bases as counter bases yields the observed effect. Similar

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destabilizing effects were obtained with **DNA2-X** (X = A, G, T, C) again varying in the base that is opposite to the 2,4-diaminopyrimidine DNA base surrogate. Finally **DNA1** and **DNA2** were hybridized to duplex **DNA1-2**. Since they are fully complementary to each other the aminophthalimide **1** is placed opposite to the 2,4-diaminopyrimidine **2**. We assume that these two base surrogates form a base pair by their complementary hydrogen-bonding interface which is orthogonal to the natural base pairs. The T_m measurements do not reflect this base pair although it should be mentioned that the destabilizing effect is not doubled due to the presence of two different modifications in **DNA1-2**. NMR studies are currently performed to support this interpretation.

The UV/vis absorption spectra of single-stranded **DNA1**, double-stranded **DNA1-X** (X = A, G, T, C), and **DNA1-2** show the presence of the aminophthalimide chromophore by an additional absorption band between 350 and 420 nm that is well separated from the absorption range of the nucleic acid and allows selective excitation (Figure 3). When excited at 385 nm, fluorescence of single stranded **DNA1** shows a maximum at approximately 535 nm which shifts to 525 nm upon hybridization with the counter strands. Hence, the interior of the DNA base stack resembles the polarity and properties of MeOH. The Stokes' shift is remarkably large (140 nm). Single-stranded **DNA1** exhibits a quantum yield of 0.060 which represents a ca. 1.8 fold increase compared to the nucleoside monomer. The fluorescence is further increased to at least approximately double intensity in case of **DNA1-A**, **DNA1-T**, **DNA1-C** and **DNA1-2** but not **DNA1-G**. **DNA1-2** exhibits a quantum yield of 0.114. Time-resolved fluorescence studies will reveal the details of the photophysical interactions of 4-aminophthalimide in DNA.

CONCLUSIONS

A synthetic route to the 4-aminophthalimide *C*-nucleoside **1** and its incorporation into an oligonucleotide has been successfully worked out. The key steps represent a stereoselective Heck-type palladium-catalyzed cross-coupling of the halogenated precursors **8**, **9**, or **22** of the 4-aminophthalimide chromophore and glycal **10** followed by stereoselective reduction with $NaBH(OAc)_3$. The nucleoside **1** shows a solvatofluorochromic behavior and significantly red-shifted fluorescence in solvents of high polarity and with hydrogen bonding capabilities. Nucleoside **1** can be further processed to the corresponding phosphoramidite as DNA building block that allows incorporating the aminophthalimide as an artificial DNA base by automated DNA synthesis. It turned out that only the combination of a Pac-protecting group at the 4-amino function and a DMT group at the phthalimide function of phosphoramidite **20**, the so-called ultramild conditions for oligonucleotide synthesis and perfectly tuned deprotection conditions yielded the 4-aminophthalimide-modified oligonucleotide **DNA1**.

The 2,4-diaminopyrimidine-*C*-nucleoside **2** was obtained by palladium-catalyzed Heck-type coupling of 2,4-dichloro-5-iodopyrimidine **25** with glycal **10** and subsequent aminolysis of the chlorine atoms. The protected 2,4-bis(benzylamino)-pyrimidine-*C*-nucleoside phosphoramidite **35** was synthesized by Buchwald–Hartwig amination of **31** with benzamide. The DNA building block **35** was successfully incorporated into DNA by automated phosphoramidite synthesis yielding **DNA2**, the completely matched counter strand to **DNA1**.

The fluorescence of **1** in double-stranded DNA is characterized by a large Stokes' shift (excitation at 385 nm, emission at 525 nm) and a quantum yield of approximately 12%. These are remarkable optical properties considering the very small size of the chromophore. The 4-aminophthalimide nucleoside is an isosteric surrogate for purine nucleosides in DNA. The small steric demand of this fluorescent nucleoside represents an ideal prerequisite that 4-aminophthalimide-modified DNA does not significantly exceed the size of natural B-form duplexes. This structural feature is especially interesting for the investigation of DNA–protein interactions inside cells. Additionally it should allow the DNA polymerase-assisted replication of fluorescent oligonucleotides by primer extension or PCR. Moreover, together with 2,4-diaminopyrimidine as counter base a completely new, bioorthogonal and fluorescent DNA base pair will be created for chemical biology with nucleic acids, especially with high potential for live cell imaging.

EXPERIMENTAL SECTION

General Experimental Methods. Chemicals and dry solvents were purchased from commercial suppliers and were used without further purification unless otherwise mentioned. TLC was performed on silica gel F_{254} coated aluminum foil. Flash chromatography was carried out with silica gel (43–60 μm). Optical–spectroscopic measurements were recorded in Na–Pi buffer solution (10 mM, pH 7) or in the mentioned solvents using quartz glass cuvettes (l = 10 mm). Mass spectra were measured in the central analytical facility of the institute. HRMS were measured using ESI with TOF analyzer (marked with ESI-TOF) or ESI with FT-ICR analyzer (marked with ESI-FT-ICR). NMR spectra were recorded on a 300 or 600 MHz spectrometer at 300 K in deuterated solvents. Chemical shifts are given in ppm relative to TMS. Complete assignment of all NMR signals was performed using a combination of 2D-NMR (H,H–COSY, H,C-HSQC, H,C-HMBC, and NOESY) experiments. Absorption spectra and melting temperatures (2.5 μM DNA, 20–90 °C, 0.7 °C/min, step width 0.5 °C) were recorded on a UV/vis spectrometer equipped with a 6 × 6 cell changer unit. Fluorescence was measured on a fluorimeter with a step width of 1 nm an integration time of 0.2 s. All spectra were recorded with an excitation and emission bypass of 5 nm and are corrected for Raman emission of the buffer solution

3-Bromo-2-methyl-5-nitrobenzoic Acid (4). 2-Methyl-5-nitrobenzoic acid (**3**, 2.00 g, 11.0 mmol) was dissolved in concd sulfuric acid (10 mL) and heated to 60 °C. *N*-Bromosuccinimide (2.36 g, 13.3 mmol) was added in three portions over 30 min. The reaction mixture was stirred at 60 °C for an additional 2 h and then poured onto ice (24 g). The precipitate was filtered off and washed with cold water and hexane affording **4** (2.70 g, 10.4 mmol, 94%) as a white solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.53 (d, J = 2.5 Hz, 1H, H-4), 8.44 (d, J = 2.5 Hz, 1H, H-6), 2.65 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.7 (COOH), 145.6, 145.1, 134.4, 129.2 (C-4), 126.6, 123.4 (C-6), 20.8 (CH_3); HRMS (ESI-TOF) m/z calcd for $C_8H_5BrNO_4$ $[M-H]^-$ 257.9407, found 257.9398.

3-Iodo-2-methyl-5-nitrobenzoic Acid (5). 2-Methyl-5-nitrobenzoic acid (**3**, 2.02 g, 11.1 mmol) was dissolved in concd sulfuric acid (10 mL) and heated to 60 °C. *N*-Iodosuccinimide (2.93 g, 13.0 mmol) was added in three portions over 30 min. The reaction mixture was stirred at 60 °C for an additional 2 h and then poured onto ice (24 g). The precipitate was filtered off and washed with cold water and hexane to afford **5** (3.31 g, 10.8 mmol, 97%) as a white solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.68 (d, J = 2.5 Hz, 1H, H-4), 8.43 (d, J = 2.5 Hz, 1H, H-6), 2.67 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.9 (COOH), 148.1, 145.3, 135.3 (C-4), 133.2, 123.9 (C-6), 104.4, 26.3 (CH_3); HRMS (ESI-TOF) m/z calcd for $C_8H_5INO_4$ $[M-H]^-$ 305.9269, found 305.9256.

3-Brom-5-nitrophthalimide (8). Compound **4** (2.60 g, 10.0 mmol) was dissolved in water (78 mL) and 2 M NaOH solution (5.2 mL) and heated to 60 °C. Potassium permanganate (6.32 g, 40.0 mmol) was added, and the reaction was stirred under reflux overnight.

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The reaction mixture was filtered using Celite and washed with hot water. The solution was brought to pH 1 with hydrochloric acid and the solvent was evaporated to dryness. The residue containing **6** was taken up in *o*-xylene (116 mL), urea (1.80 g, 30.0 mmol) was added, and the reaction mixture was stirred under reflux for 4 h. The solution was filtered and the solvent was removed under reduced pressure. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/EtOAc = 30:1) to obtain **8** (864 mg, 3.19 mmol, 32%) as a yellow solid: R_f 0.75(CH_2Cl_2/EtOAc = 5:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.97 (s, 1H, NH), 8.73 (d, J = 1.8 Hz, 1H, H-4), 8.39 (d, J = 1.8 Hz, 1H, H-6); ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.0 (C═O), 165.8 (C═O), 151.3, 136.0, 134.7, 133.2 (C-4), 117.7, 116.8 (C-6); HRMS (ESI-TOF) m/z calcd for $C_8H_2BrN_2O_4$ $[M-H]^-$ 268.9203, found 268.9215.

3-Iodo-5-nitrophthalimide (9). Compound **5** (2.80 g, 9.12 mmol) was dissolved in water (84 mL) and 2 M NaOH solution (5.6 mL) and heated to 60 °C. Potassium permanganate (5.78 g, 36.6 mmol) was added, and the reaction was stirred under reflux overnight. The reaction mixture was filtered using Celite and washed with hot water. The solution was brought to pH 1 with hydrochloric acid, and the solvent was evaporated to dryness. The residue containing **7** was taken up in *o*-xylene (123 mL), urea (1.65 g, 27.5 mmol) was added, and the reaction mixture was stirred under reflux for 4 h. The solution was filtered and the solvent was removed under reduced pressure. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/EtOAc = 30:1) to obtain **9** (1.02 g, 3.21 mmol, 35%) as a yellow solid: R_f 0.76 (CH_2Cl_2/EtOAc = 5:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.94 (s, 1H, N*H*), 8.86 (d, J = 1.9 Hz, 1H, H-4), 8.39 (d, J = 1.9 Hz, 1H, H-6); ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.9 (C═O), 165.4 (C═O), 150.7, 139.0 (C-4), 137.7, 135.2, 117.1 (C-6), 90.3; HRMS (ESI-TOF) m/z calcd for $C_8H_2IN_2O_4$ $[M-H]^-$ 316.9065, found 316.9084.

5-Amino-3-iodophthalimide (21). A solution of **9** (1.03 g, 3.29 mmol) in ethanol (40 mL) was brought to boiling, and an aqueous sodium hydrogen sulfide solution (1.82 M, 3.0 mL, 5.46 mmol) was added dropwise. The reaction mixture was stirred under reflux for 1 h. The solvent was removed to dryness, and the residue was purified by flash chromatography on silica gel (EtOAc) to afford **21** (522 mg, 1.82 mmol, 56%) as a light yellow solid: R_f 0.27 (CH_2Cl_2/acetone = 9:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.92 (s, 1H, N*H*), 7.21 (d, J = 2.0 Hz, 1H), 6.87 (d, J = 2.0 Hz, 1H), 6.55 (s, 2H, NH_2); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.0 (C═O), 167.4 (C═O), 155.0, 136.6, 126.4 (C-4), 117.9, 107.1 (C-6), 90.8; HR-MS (ESI-TOF) m/z calcd for $C_8H_6IN_2O_2$ $[M + H]^+$ 288.9468, found 288.9490.

5-(*N*-Phenoxyacetylamino)-3-iodophthalimide (22). Under Ar atmosphere, **21** (300 mg, 1.04 mmol) and imidazole (510 mg, 7.50 mmol were dissolved in anhyd THF (25 mL) and anhyd pyridine (10 mL). Phenoxyacetyl chloride (865 μL, 6.25 mmol) was added dropwise, and the reaction mixture was stirred for 4 h at rt. Ethanol (1 mL) was added, and the solvents were removed under reduced pressure. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/acetone 1:0 to 15:1) to afford **22** (417 mg, 0.99 mmol, 95%) as a white solid: R_f 0.55(CH_2Cl_2/acetone = 9:1); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.42 (s, 1H, Imid-N*H*), 10.66 (s, 1H, Amid-N*H*), 8.45 (d, J = 1.8 Hz, 1H, H-4), 8.15 (d, J = 1.7 Hz, 1H, H-6), 7.35–7.30 (m, 2H, Aryl-H), 7.04–6.95 (m, 3H, Aryl-H), 4.77 (s, 2H, CH_2); ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.8 (C═O), 167.7 (C═O), 166.9 (C═O), 157.6, 143.8, 135.4, 133.6, 129.6 (C-4), 127.3, 121.4, 114.7 (C-6), 113.1, 89.9, 67.1 (CH_2); HR-MS (ESI-TOF) m/z calcd for $C_{16}H_{12}IN_2O_4$ $[M + H]^+$ 422.9836, found 422.9867.

1-(5-Nitrophthalimid-3-yl)-1,2,3-trideoxy-3-oxo-β-D-ribofuranose (12). Under Ar atmosphere, **10** (325 mg, 1.41 mmol) was dissolved in dry acetonitrile (29 mL). Compound **9** (408 mg, 1.28 mmol), tris(pentafluorophenyl)phosphine (273 mg, 0.51 mmol), palladium(II) acetate (60 mg, 0.27 mmol), and Et_3N (355 μL, 2.56 mmol) were added, and the reaction was stirred under reflux overnight. The solvent was removed to dryness, and the residue was purified by flash chromatography on silica gel (CH_2Cl_2/acetone = 9:1) to obtain **12** (128 mg, 0.42 mmol, 33%) as a white solid: R_f 0.31 (hexane/EtOAc = 1:1); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.87 (s, 1H, N*H*), 8.93 (d, J = 2.0 Hz, 1H, H-4), 8.38 (d, J = 2.0 Hz, 1H, H-6), 6.04 (dd, J = 10.3, 6.4 Hz, 1H, H-1′), 5.16 (t, J = 5.3 Hz, 1H, O*H*-5′), 4.19 (t, J = 3.0 Hz, 1H, H-4′), 3.83–3.70 (m, 2H, H-5′), 3.04 (dd, J = 18.0, 6.5 Hz, 1H, H-2′), 2.34 (dd, J = 18.0, 10.3 Hz, 1H, H-2′); ^{13}C NMR (126 MHz, DMSO-d_6) δ 213.0 (C-3′), 167.9 (C═O), 167.1 (C═O), 151.7, 142.3, 134.4, 132.8, 126.0 (C-4), 116.6 (C-6), 82.6 (C-4′), 71.2 (C-1′), 60.4 (C-5′), 44.2 (C-2′); HRMS (ESI-TOF) m/z calcd for $C_{13}H_9N_2O_7$ $[M-H]^-$ 305.0415, found 305.0414.

1-(5-Nitrophthalimid-3-yl)-1,2-dideoxy-β-D-ribofuranose (13). In a degassed mixture of acetic acid and acetonitrile (2:1, 5 mL), **12** (80 mg, 0.26 mmol) was dissolved under Ar atmosphere at 0 °C. Sodium triacetoxyborohydride (83 mg, 0.39 mmol) was added, and the reaction mixture was stirred at 0 °C for 1 h. A mixture of water and ethanol (1:1, 3.3 mL) was added, and the solvents were removed to dryness. The residue was purified by flash chromatography on silica gel ($CHCl_3$/MeOH 1:0 to 4:1) yielding **13** (75 mg, 0.24 mmol, 93%) as a white solid: R_f 0.24 ($CHCl_3$/MeOH = 9:1); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.80 (s, 1H, N*H*), 8.75 (dd, J = 1.9, 0.7 Hz, 1H, H-4), 8.30 (d, J = 2.0 Hz, 1H, H-6), 5.84 (dd, J = 9.7, 6.0 Hz, 1H, H-1′), 5.18 (d, J = 3.9 Hz, 1H, O*H*-3′), 4.91 (t, J = 5.3 Hz, 1H, O*H*-5′), 4.25–4.19 (m, 1H, H-3′), 3.90 (td, J = 4.7, 2.5 Hz, 1H, H-4′), 3.57 (td, J = 4.9, 1.9 Hz, 1H, H-5′), 2.37 (ddd, J = 12.8, 6.1, 2.1 Hz, 1H, H-2′), 1.77 (ddd, J = 12.7, 9.8, 5.7 Hz, 1H, H-2′); ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.0 (C═O), 167.2 (C═O), 151.5, 144.8, 134.5, 132.3, 125.7 (C-4), 116.0 (C-6), 88.1 (C-4′), 74.2 (C-1′), 72.1 (C-3′), 62.0 (C-5′), 42.7 (C-2′); HRMS (ESI-TOF) m/z calcd for $C_{13}H_{11}N_2O_7$ $[M-H]^-$ 307.0572, found 307.0570.

1-(5-Aminophthalimid-3-yl)-1,2-dideoxy-β-D-ribofuranose (1). A solution of **13** (110 mg, 0.36 mmol) in ethanol (5.5 mL) was brought to boiling, and an aqueous sodium hydrogen sulfide solution (1.82 M, 330 μL, 0.60 mmol) was added dropwise. The reaction mixture was stirred under reflux for 1 h. The solvent was removed to dryness, and the residue was purified by flash chromatography on silica gel ($CHCl_3$/EtOH 1:0 to 3:2) to afford **1** (87 mg, 0.31 mmol, 88%) as a light yellow solid: R_f 0.19 ($CHCl_3$/EtOH = 5:1); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.66 (s, 1H Imid-N*H*), 6.97 (d, J = 2.0 Hz, 1H, H-4), 6.74 (d, J = 2.0 Hz, 1H, H-6), 6.38 (s, 2H, NH_2), 5.60 (dd, J = 10.0, 5.8 Hz, 1H, H-1′), 5.07 (d, J = 3.9 Hz, 1H, O*H*-3′), 4.75 (t, J = 5.6 Hz, 1H, O*H*-5′), 4.17–4.10 (m, 1H, H-3′), 3.80 (ddd, J = 6.2, 5.2, 2.5 Hz, 1H, H-4′), 3.54 (dt, J = 10.8, 5.3 Hz, 1H, H-5′), 3.41 (dt, J = 11.5, 6.0 Hz, 1H, H-5′), 2.30 (ddd, J = 12.7, 5.9, 1.8 Hz, 1H, H-1′), 1.60 (ddd, J = 12.7, 10.0, 5.8 Hz, 1H, H1′); ^{13}C NMR (126 MHz, DMSO-d_6) δ (ppm) = 169.5 (C═O), 169.3 (C═O), 154.8, 144.5, 135.8, 113.2, 112.6 (C-4), 105.6 (C-6), 87.6 (C-4′), 74.7 (C-1′), 72.3 (C-3′), 62.5 (C-5′), 42.4 (C-2′); HRMS (ESI-TOF) m/z calcd for $C_{13}H_{15}N_2O_5$ $[M + H]^+$ 279.0975, found 279.1000.

1-(5-Nitrophthalimid-3-yl)-3,5-di-*O*-*tert*-butyldimethylsilyl-1,2-dideoxy-β-D-ribofuranose (14). Under Ar atmosphere, **13** (158 mg, 0.51 mmol) and imidazole (162 mg, 1.07 mmol) were dissolved in anhyd DMF (3.5 mL), and TBDMS-Cl (162 mg, 1.07 mmol) was added. The reaction mixture was stirred at rt overnight. Water (3.5 mL) was added, and the solution was extracted with hexane (3 × 10 mL). The combined organic layers were dried (Na_2SO_4), and the solvent was removed under reduced pressure yielding **14** (158 mg, 0.29 mmol, 57%) as a white solid: R_f 0.6 (CH_2Cl_2/EtOAc = 15:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.79 (s, 1H, N*H*), 8.63 (dd, J = 2.0, 0.8 Hz, 1H, H-4), 8.32 (d, J = 2.0 Hz, 1H, H-6), 5.86 (dd, J = 9.9, 5.7 Hz, 1H, H-1′), 4.43–4.34 (m, 1H, H-3′), 3.99–3.90 (m, 1H, H-4′), 3.80–3.74 (m, 2H, H-5′), 2.40 (ddd, J = 12.7, 5.7, 1.6 Hz, 1H, H-2′), 1.82 (ddd, J = 12.5, 10.0, 5.5 Hz, 1H, H-2′), 0.91 [s, 9H, C(CH_3)$_3$], 0.85 [s, 9H, C(CH_3)$_3$], 0.11 (s, 3H, CH_3), 0.09 (s, 9H, 3 × CH_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.9 (C═O), 167.1 (C═O), 151.5, 144.1, 134.5, 132.3, 125.7 (C-4), 116.0 (C-6), 88.1 (C-4′), 74.2 (C-1′), 72.1 (C-3′), 62.0 (C-5′), 42.7 (C-2′), 25.8 [C(CH_3)$_3$], 25.7 [C(CH_3)$_3$], 17.8 [C(CH_3)$_3$], 17.8 [C(CH_3)$_3$], −3.2 (CH_3), −4.8 (CH_3); HRMS (ESI-TOF) m/z calcd for $C_{25}H_{41}N_2O_7Si_2$ $[M + H]^+$ 537.2447, found 537.2477.

1-(5-Aminophthalimid-3-yl)-3,5-di-*O*-*tert*-butyldimethylsilyl-1,2-dideoxy-β-D-ribofuranose (15). A solution of **14** (401 mg, 0.75 mmol) in ethanol (11.5 mL) was brought to boiling, and an aqueous

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sodium hydrogen sulfide solution (1.82 M, 690 μL, 1.26 mmol) was added dropwise. The reaction mixture was stirred under reflux for 1 h. The solvent was removed to dryness, and the residue was purified by flash chromatography on silica gel (CH_2Cl_2/EtOAc 1:0 to 9:1) to afford **15** (264 mg, 0.52 mmol, 70%) as a light yellow solid: R_f 0.14 (CH_2Cl_2/EtOAc = 15:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.65 (s, 1H, Imid-N*H*), 6.89 (d, *J* = 1.9 Hz, 1H, H-4), 6.74 (d, *J* = 2.0 Hz, 1H, H-6), 6.39 (s, 2H, NH_2), 5.60 (dd, *J* = 10.3, 5.2 Hz, 1H, H-1′), 4.32 (d, *J* = 4.8 Hz, 1H, H-3′), 3.90−3.79 (m, 1H, H-4′), 3.73 (dd, *J* = 10.5, 4.5 Hz, 1H, H-5′), 3.49 (dd, *J* = 10.4, 7.5 Hz, 1H, H-5′), 2.35 (dd, *J* = 12.8, 5.2 Hz, 1H, H-2′), 1.65 (ddd, *J* = 12.6, 10.5, 5.3 Hz, 1H, H-2′), 0.90 [s, 9H, C(CH_3)$_3$], 0.87 [s, 9H, C(CH_3)$_3$], 0.10 (s, 3H, CH_3), 0.09−0.07 (m, 9H, 3x CH_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.5 (C═O), 169.2 (C═O), 154.9, 143.6, 135.9, 113.2, 112.2 (C-4), 105.6 (C-6), 87.5 (C-4′), 75.1 (C-1′), 74.5 (C-3′), 63.6 (C-5′), 41.9 (C-2′), 25.8 [C(CH_3)$_3$], 25.7 [C(CH_3)$_3$], 18.0 [C(CH$_3$)$_3$], 17.8 [C(CH$_3$)$_3$], −4.7 (CH_3), −4.8 (CH_3), −5.35 (CH_3), −5.39 (CH_3); HRMS (ESI-TOF) *m*/*z* calcd for $C_{25}H_{43}N_2O_5Si_2$ [M + H]$^+$ 507.2705, found 507.2718.

1-(5-(*N*-Phenoxyacetyl)aminophthalimid-3-yl)-1,2,3-trideoxy-3-oxo-β-D-ribofuranose (24). Under Ar atmosphere, **22** (735 mg, 1.74 mmol) was dissolved in dry acetonitrile (22 mL). Compound **10** (441 mg, 1.91 mmol), tris(pentafluorophenyl)-phosphine (371 mg, 0.70 mmol), palladium(II) acetate (78 mg, 0.35 mmol), and Et_3N (483 μL, 3.48 mmol) were added, and the reaction was stirred under reflux overnight. The solvent was removed to dryness, and the residue was purified by flash chromatography on silica gel (hexane/EtOAc 1:0 to 3:2) to obtain **24** (190 mg, 0.46 mmol, 27%) as a white solid: R_f 0.14 (hexane/EtOAc = 1:1); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.28 (s, 1H, Imid-N*H*), 10.73 (s, 1H, Amid-N*H*), 8.27 (d, *J* = 1.8 Hz, 1H, H-6), 8.12 (d, *J* = 1.3 Hz, 1H, H-4), 7.40−7.25 (m, 2H, Aryl-H), 7.05−6.90 (m, 3H, Aryl-H), 5.88 (dd, *J* = 10.6, 6.1 Hz, 1H, H-1′), 4.91 (t, *J* = 5.3 Hz, 1H, O*H*-5′), 4.78 (s, 2H, CH_2), 4.15 (dd, *J* = 4.1, 2.6 Hz, 1H, H-4′), 3.83−3.64 (m, 2H, H-5′), 3.01 (dd, *J* = 17.8, 6.3 Hz, 1H, H-2′), 2.27 (dd, *J* = 17.9, 10.6 Hz, 1H, H-2′); ^{13}C NMR (126 MHz, DMSO-d_6) δ 213.3 (C-3′), 168.9 (C═O), 168.7 (C═O), 167.6 (C═O), 157.7, 144.0, 141.1, 134.5, 129.6 (Aryl-*C*H), 122.6, 121.3 (Aryl-*C*H), 120.6 (C-4), 114.6 (Aryl-*C*H), 112.4 (C-6), 82.7 (C-4′), 71.8 (C-1′), 67.0 (CH_2), 60.5 (C-5′), 44.3 (C-2′); HRMS (ESI-TOF) *m*/*z* calcd for $C_{21}H_{17}N_2O_7$ [M − H]$^-$ 409.1041, found 409.1029.

1-(5-(*N*-Phenoxyacetyl)aminophthalimid-3-yl)-1,2-dideoxy-β-D-ribofuranose (16). Method 1. Under Ar atmosphere, **15** (264 mg, 0.52 mmol) was dissolved in dry pyridine (5.3 mL). Phenoxyacetic anhydride (224 mg, 0.78 mmol) was added, and the reaction mixture was stirred at rt overnight. Water (5.3 mL) was added, and the mixture was extracted first with hexane (2 × 25 mL, 1 × 30 mL) and then with CH_2Cl_2 (1 × 20 mL). The organic layers were combined and evaporated to dryness. The residue was taken up in anhyd THF (5.3 mL) under Ar atmosphere, and Et_3N·3HF (679 μL, 4.17 mmol) was added. The reaction mixture was stirred at rt overnight. A few drops of ethanol were added, and THF was evaporated under reduced pressure. The crude reaction mixture was purified by flash chromatography on silica gel ($CHCl_3$/EtOH = 5:1) to afford **16** (118 mg, 0.29 mmol, 55%) as a white solid. **Method 2.** In a degassed mixture of acetic acid and acetonitrile (2:1, 2.6 mL), **24** (56 mg, 0.13 mmol) was dissolved under Ar atmosphere at 0 °C. Sodium triacetoxyborohydride (43 mg, 0.20 mmol) was added, and the reaction mixture was stirred at 0 °C for 1 h. A mixture of water and ethanol (1:1, 1.7 mL) was added and the solvents were removed to dryness. The residue was purified by flash chromatography on silica gel ($CHCl_3$/EtOH 1:0 to 11:1) yielding **16** (53 mg, 0.13 mmol, 94%) as a white solid: R_f 0.49 ($CHCl_3$/EtOH = 5:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.22 (s, 1H, Imid-N*H*), 10.68 (s, 1H, Amid-N*H*), 8.17 (d, *J* = 1.7 Hz, 1H, H-6), 7.99 (d, *J* = 1.7 Hz, 1H, H-4), 7.37−7.27 (m, 2H, Aryl-H), 7.04−6.94 (m, 3H, Aryl-H), 5.74 (dd, *J* = 9.9, 5.7 Hz, 1H, H-1′), 5.15 (d, *J* = 3.7 Hz, 1H, O*H*-3′), 4.78−4.75 (m, 3H, O*H*-5′ + CH_2), 4.22−4.15 (m, 1H, H-3′), 3.90−3.80 (m, 1H, H-4′), 3.59 (dt, *J* = 9.9, 4.7 Hz, 1H, H-5′), 3.43 (dt, *J* = 11.2, 5.6 Hz, 1H, H-5′), 2.34 (dd, *J* = 12.3, 5.7 Hz, 1H, H-2′), 1.66 (ddd, *J* = 12.7, 10.0, 5.6 Hz, 1H, H-2′); ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.0 (C═O), 168.8 (C═O), 167.6 (C═O), 157.7, 143.8, 134.4, 129.6 (Aryl-*C*H), 122.1, 121.3 (Aryl-*C*H), 120.2 (C-4), 114.6 (Aryl-*C*H), 111.8 (C-6), 87.8 (C-4′), 74.4 (C-1′), 72.4 (C-3′), 67.0 (CH_2), 62.5 (C-5′), 42.4 (C-2′); HRMS (ESI-TOF) *m*/*z* calcd for $C_{21}H_{21}N_2O_7$ [M + H]$^+$ 413.1343, found 413.1364.

1-(5-(*N*-Phenoxyacetyl)amino-*N*-(dimethoxytrityl)-phthalimid-3-yl)-5-*O*-(dimethoxytrityl)-1,2-dideoxy-β-D-ribofuranose (19). Under Ar atmosphere, **16** (118 mg, 0.29 mmol) and DMT-Cl (291 mg, 0.86 mmol) were dissolved in anhyd pyridine, and Et_3N (139 μL, 1.00 mmol) was added. The reaction mixture was stirred at 30 °C overnight. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography on silica gel (hexane/EtOAc = 2:3, + 0.1% Et_3N) yielding **19** (187 mg, 0.18 mmol, 64%) as a white solid: R_f 0.48 (hexane/EtOAc = 1:1); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.60 (s, 1H, Amid-N*H*), 8.06 (d, *J* = 1.7 Hz, 1H, H-4), 7.97 (d, *J* = 1.8 Hz, 1H, H-6), 7.41−7.34 (m, 7H, Aryl-H), 7.31−7.24 (m, 8H, Aryl-H), 7.23−7.17 (m, 4H, Aryl-H), 7.15−7.10 (m, 2H, Aryl-H), 6.99−6.95 (m, 3H, Aryl-H), 6.88 (d, *J* = 8.8 Hz, 3H, Aryl-H), 6.82 (d, *J* = 8.9 Hz, 4H, Aryl-H), 5.61 (dd, *J* = 9.7, 5.8 Hz, 1H, H-1′), 5.18 (d, *J* = 4.1 Hz, 1H, O*H*-3′), 4.70 (s, 2H, CH_2), 4.10−4.04 (m, 1H, H-3′), 3.94 (td, *J* = 5.5, 2.8 Hz, 1H, H-4′), 3.73−3.70 (2 × s, 12H, OCH_3), 3.25 (dd, *J* = 9.9, 5.4 Hz, 1H, H-5′), 3.04 (dd, *J* = 9.8, 5.7 Hz, 1H, H-5′), 2.26 (ddd, *J* = 12.1, 5.7, 1.5 Hz, 1H, H-2′), 1.58 (ddd, *J* = 12.6, 10.0, 5.9 Hz, 1H, H-2′); ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.3 (C═O), 167.2 (C═O), 167.1 (C═O), 158.0, 157.6, 157.4, 144.9, 143.8, 142.9, 135.6, 135.0, 133.3, 129.7, 129.6, 129.5, 127.8, 127.7, 127.4, 126.6, 125.7, 121.3 (C-4), 114.6, 113.2, 112.8 (C-6), 85.8 (C-4′), 85.5, 74.5 (C-1′), 72.6 (C-3′), 72.0, 70.0 (CH_2), 64.1 (C-5′), 55.0 (OCH_3), 54.9 (OCH_3), 42.2 (C-2′); HRMS (ESI-TOF) *m*/*z* calcd for $C_{63}H_{55}N_2O_{11}$ [M − H]$^-$ 1015.3811, found 1015.3820.

1-(5-(*N*-Phenoxyacetyl)amino-*N*-(dimethoxytrityl)-phthalimid-3-yl)-3-*O*-[(2-cyanoethoxy)-diisopropylaminophosphanyl]-5-*O*-(dimethoxytrityl)-1,2-dideoxy-β-D-ribofuranose (20). Under Ar atmosphere, **19** (187 mg, 0.18 mmol) was dissolved in anhyd CH_2Cl_2 (10.5 mL) and *i*-Pr_2NEt (110 μL, 0.60 mmol) was added. 2-Cyanoethoxy-*N*,*N*-di-*iso*-propylaminophosphanyl chloride (103 μL, 0.46 mmol) was added, and the reaction mixture was stirred at rt for 4 h. The mixture was directly transferred on a silica gel column for purification (hexane/EtOAc = 3:2, + 0.1% Et_3N). Flash chromatography afforded **20** (150 mg, 0.12 mmol, 67%) as a white solid: R_f 0.52, 0.55 (hexane/EtOAc 3:2); ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.68−10.63 (2 × s, 1H, Amid-N*H*), 8.13−8.05 (2 × d, *J* = 1.5 Hz, 1H, H-4), 7.99−7.94 (2 × d, *J* = 1.8 Hz, 1H, H-6), 7.41−6.77 (m, 31H, Aryl-H), 5.61 (ddd, *J* = 16.2, 10.1, 5.6 Hz, 1H, H-1′), 4.72−4.70 (2 × s, 2H, CH_2), 4.38−4.28 (m, 1H, H-3′), 4.08−3.99 (m, 1H, H-4′), 3.71−3.69 (4 × s, 12H, OCH_3), 3.67−3.58 (m, 2H, CH_2), 3.55−3.46 [m, 2H, C*H*(CH$_3$)$_2$], 3.29−3.21 (m, 1H, H-5′), 3.16−3.11 (m, 1H, H-5′), 2.66 (t, *J* = 5.9 Hz, 1H, CH_2), 2.52 (t, *J* = 1.8 Hz, 1H, CH_2), 2.48−2.39 (m, 1H, H-2′), 1.72−1.62 (m, 1H, H-2′), 1.12−1.08 [4 × s, 6H, CH(CH_3)$_2$)], 1.00−0.98 [4 × s, 6H, CH(CH_3)$_2$]; ^{31}P NMR (243 MHz, DMSO-d_6) δ 147.46, 147.02; HRMS (ESI-TOF) *m*/*z* calcd for $C_{72}H_{74}N_4O_{12}P$ [M + H]$^+$ 1217.5035, found 1217.5126.

1β-(2,4-Dichloropyrimidin-5-yl)-1,2,3-trideoxy-3-oxo-D-ribofuranose (27). Dry $CHCl_3$ (40 mL) was added to an argon-purged, dried Schlenk flask containing 0.575 g (2.56 mmol, 0.10 equiv) Pd(OAc)$_2$ and 2.73 g (5.12 mmol, 0.20 equiv) P(PhF$_5$)$_3$. The mixture was stirred for 1 h at room temperature before 5.90 g (25.6 mmol, 1.00 equiv) of 3-*O*-(*tert*-butyldimethylsilyl)-1,2-dideoxy-1,2-didehydro-D-ribofuranose (**10**), 8.45 g (30.7 mmol, 1.20 equiv) of 2,4-dichloro-5-iodopyrimidine (**28**), and 7.06 g (25.6 mmol, 1.00 equiv) of Ag_2CO_3 were added. The reaction mixture was stirred for 10 h at 70 °C. After being cooled to rt, the reaction mixture was filtered on a pad of Celite and eluted with $CHCl_3$. Volatile compounds were removed under reduced pressure, and the remaining brown oil was dissolved in 150 mL THF. At 0 °C, 0.5 mL of AcOH and 2 mL of a 1 M TBAF solution in THF were added. After 1 h, the solvent was removed under reduced pressure hexanes−EtOAc 2:1 to 1:1). Title compound **27** (2.26 g, 8.59 mmol, 34% yield) was obtained as a yellow oil: ^{1}H NMR (500 MHz,

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$CDCl_3$) δ 8.96 (s, 1H, H6), 5.46 (ddd, J = 10.5, 6.4, 0.8 Hz, 1H, H1′), 4.11 (t, J = 3.0 Hz, 1H, H4′), 4.02 (dd, J = 12.1, 2.9 Hz, 1H, H5′a), 4.00 (dd, J = 12.1, 3.2 Hz, 1H, H5′b), 3.16 (dd, J = 18.1, 6.4 Hz, 1H, H2′a), 2.37 (dd, J = 18.1, 10.5 Hz, 1H, H2′b); ^{13}C NMR (126 MHz, $CDCl_3$) δ 211.7 (C3′), 160.0 (C2/C4), 159.9 (C2/C4), 158.5 (C6), 132.0 (C5), 82.3 (C4′), 72.3 (C1′), 61.5 (C5′), 43.5 (C2′); HRMS (ESI-FT-ICR) m/z calcd for $C_9H_7Cl_2N_2O_3^-$ $[M - H]^+$ 260.9839, found 260.9853.

1β-(2,4-Dichloropyrimidin-5-yl)-1,2-dideoxy-D-ribofuranose (28). A 2.26 g (8.59 mmol, 1.00 equiv) portion of 1β-(2,4-dichloropyrimidin-5-yl)-1,2-dideoxy-D-ribofuranose **27** was dissolved in 200 mL of CH_3CN and 30 mL of AcOH. At 0 °C, 2.73 g (12.9 mmol, 1.50 equiv) of sodium triacetoxyborohydride was added slowly. The reaction was monitored by TLC, and after 10 min, all starting material was consumed. Thirty milliliters of 50 vol % aqueous EtOH was added. Volatiles were removed under reduced pressure, and the residue was purified by flash chromatography on silica (CH_2Cl_2/MeOH 95:5). Nucleoside **28** (2.10 g, 7.92 mmol, 92% yield) was isolated as a white foam: ^{1}H NMR (500 MHz, CD_3OD) δ 8.92 (d, J = 0.9 Hz, 1H, H6), 5.36 (dd, J = 10.0, 5.8 Hz, 1H, H1′), 4.37 (ddd, J = 6.5, 3.7, 1.6 Hz, 1H, H3′), 4.00 (td, J = 4.4, 2.6 Hz, 1H, H4′), 3.74 (dd, J = 11.9, 4.1 Hz, 1H, H5′a), 3.71 (dd, J = 11.9, 4.8 Hz, 1H, H5′b), 2.50 (ddd, J = 13.0, 5.8, 2.1 Hz, 1H, H2′a), 1.90 (ddd, J = 13.0, 10.0, 5.9 Hz, 1H, H2′b); ^{13}C NMR (126 MHz, CD_3OD) δ 160.7 (C2/C4), 159.9 (C6), 159.9 (C2/C4), 135.1 (C5), 89.4 (C4′), 76.0 (C1′), 74.0 (C3′), 63.5 (C5′), 42.7 (C2′); HRMS (ESI-FT-ICR) m/z calcd for $C_9H_{11}Cl_2N_2O_3^+$ $[M + H]^+$ 265.0141, found 265.0139.

1β-(2,4-Diaminopyrimidin-5-yl)-1,2-dideoxy-D-ribofuranose (2). At −78 °C, ammonia was condensed into 40 mL of dry methanol. 1β-(2,4-dichloropyrimidin-5-yl)-1,2-dideoxy-D-ribofuranose (**28**) (0.670 g, 2.53 mmol) was added, and the solution was transferred to a glass reactor. The sealed reactor was heated to 120 °C for 12 h. After the mixture was cooled to rt, volatiles were removed under reduced pressure, and the crude product was purified by flash chromatography on silica (gradient: CH_2Cl_2/MeOH 9:1 to 65:35, + 5% H_2O). Title compound **2** (0.414 g, 1.83 mmol, 72% yield) was isolated as a yellow foam: ^{1}H NMR (300 MHz, CD_3OD) δ 7.64 (s, 1H, H6), 5.00−4.72 (m, 1H, H1′), 4.34 (dt, J = 6.2, 1.7 Hz, 1H, H3′), 3.88 (q, J = 2.6 Hz, 1H, H4′), 3.75−3.64 (m, 1H, H5′a), 3.70−3.58 (m, 1H, H5′b), 2.13 (ddd, J = 13.1, 11.0, 6.0 Hz, 1H, H2′a), 1.95 (ddd, J = 13.2, 5.5, 1.4 Hz, 1H, H2′b); ^{13}C NMR (75 MHz, CD_3OD) δ 164.7 (C2/C4), 156.9 (C2/C4), 141.7 (C6), 109.5 (C5), 89.5 (C4′), 77.8 (C1′), 74.3 (C3′), 62.9 (C5′), 40.9 (C2′); HRMS (ESI-FT-ICR) m/z calcd for $C_9H_{15}N_4O_3^+$ $[M + H]^+$ 227.1139, found 227.1137.

1β-(2,4-Dichloropyrimidin-5-yl)-1,2-dideoxy-5-*O*-(*tert*-butyldimethylsilyl)-D-ribofuranose (31). An argon-purged, dried Schlenk flask was charged with 20 mL of dry DMF, 404 mg (5.94 mmol, 2.50 equiv) of imidazole, and 630 mg (2.38 mmol, 1.00 equiv) of 1β-(2,4-dichloropyrimidin-5-yl)-1,2-dideoxy-D-ribofuranose (**28**). To that solution was slowly added 430 mg (2.85 mmol, 1.20 equiv) of TBDMSCl. The reaction mixture was stirred at rt for 4 h, and then 50 mL water was added. The aqueous phase was washed three times with 100 mL of ethyl acetate and dried over $MgSO_4$, and volatile compounds were removed under reduced pressure. The remaining crude product was purified by flash chromatography on silica (hexanes/EtOAc 3:1). Title compound **31** (770 mg, 2.00 mmol, 85% yield) was isolated as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.82 (s, 1H, H6), 5.37 (dd, J = 9.9, 6.0 Hz, 1H, H1′), 4.49 (ddt, J = 5.7, 2.2, 1.1 Hz, 1H, H3′), 4.07 (ddd, J = 4.4, 3.2, 2.3 Hz, 1H, H4′), 3.83 (dd, J = 10.8, 3.3 Hz, 1H, H5′a), 3.77 (dd, J = 10.8, 4.4 Hz, 1H, H5′b), 2.52 (ddd, J = 13.0, 5.9, 2.0 Hz, 1H, H2′a), 1.88 (ddd, J = 13.0, 9.9, 5.7 Hz, 1H, H2′b), 0.86 (s, 9H, $C(CH_3)_3$), 0.07 (s, 6H, Si-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.6 (C2/C4), 159.3 (C2/C4), 158.6 (C6), 133.6 (C5), 87.8 (C4′), 75.0 (C1′), 74.4 (C3′), 63.8 (C5′), 42.2 (C2′), 26.0 ($C(CH_3)_3$), 18.4 ($C(CH_3)_3$), −5.3 (Si-CH_3), −5.4 (Si-CH_3); HRMS (ESI-FT-ICR) m/z calcd for $C_{15}H_{25}Cl_2N_2O_3Si^+$ $[M + H]^+$ 379.1006, found 379.1007.

1β-[2,4-Bis(benzoylamino)pyrimidin-5-yl]-1,2-dideoxy-5-*O*-(*tert*-butyldimethylsilyl)-D-ribofuranose (32). An argon-purged, dried Schlenk flask was charged with 20 mL of dry 1,4-dioxane, 74 mg (0.081 mmol, 0.04 equiv) of $Pd_2(dba)_3$, 141 mg (0.244 mmol, 0.12 equiv) of Xantphos, 492 mg (4.06 mmol, 2.00 equiv) of benzamide, 1.32 g (4.06 mmol, 2.00 equiv) of Cs_2CO_3, and 770 mg (2.03 mmol, 1.00 equiv) of 1β-(2,4-dichloropyrimidin-5-yl)-1,2-dideoxy-5-*O*-(*tert*-butyldimethylsilyl)-D-ribofuranose **31**. The mixture was stirred for 24 h at 100 °C. After being cooled to rt, the reaction mixture was filtered on a pad of Celite and eluted with MeOH. Volatiles were removed from the filtrate under reduced pressure and the remaining crude product was purified by flash chromatography on silica (gradient: hexanes-acetone 1:1 to 2:3). Title compound **32** (905 mg, 1.65 mmol, 81%) was isolated as a colorless solid: ^{1}H NMR (500 MHz, CD_3OD) δ 8.56 (s, 1H, H6), 8.13−8.05 (m, 2H), 8.05−7.98 (m, 2H), 7.72−7.62 (m, 2H), 7.63−7.51 (m, 4H), 5.41 (dd, J = 10.5, 5.2 Hz, 1H, H1′), 4.40 (dt, J = 5.8, 1.7 Hz, 1H, H3′), 4.13 (td, J = 3.8, 2.0 Hz, 1H, H4′), 3.76 (s, 1H, H5′a), 3.76 (s, 1H, H5′b), 2.45 (ddd, J = 12.9, 5.3, 1.6 Hz, 1H, H2′a), 2.29−2.19 (m, 1H, H2′b), 0.82 (s, 9H, $C(CH_3)_3$), −0.00 (s, 3H, Si-CH_3), −0.08 (s, 3H, Si-CH_3); ^{13}C NMR (126 MHz, CD_3OD) δ 167.4 (C_{amide}), 167.1 (C_{amide}), 158.1 (C2/C4), 158.0 (C2/C4), 157.8 (C6), 135.4 (C_q), 135.1 (C_q), 134.2, 133.7, 130.1, 129.8, 128.9, 128.9 (CH_{Ar}), 120.1 (C5), 89.8 (C4′), 77.4 (C1′), 73.8 (C3′), 64.8 (C5′), 42.2 (C2′), 26.3 ($C(CH_3)_3$), 18.2 ($C(CH_3)_3$), −5.3 (Si-CH_3), −5.5 (Si-CH_3);); HRMS (ESI-FT-ICR) m/z calcd for $C_{29}H_{35}N_4O_5Si^-$ $[M - H]^+$ 547.2382, found 547.2381.

1β-[2,4-Bis(benzoylamino)pyrimidin-5-yl]-1,2-dideoxy-D-ribofuranose (33). 1β-[2,4-Bis(benzoylamino)pyrimidin-5-yl]-1,2-dideoxy-5-*O*-(*tert*-butyldimethylsilyl)-D-ribofuranose (**32**) (0.87 g, 1.55 mmol, 1.00 equiv) was dissolved in 25 mL of THF. NEt_3·3HF (0.60 mL, 4.00 mmol, 2.60 equiv) was added dropwise. After 2 h, volatiles were removed under reduced pressure, and the remaining solid was purified by flash chromatography on silica (CH_2Cl_2/MeOH 9:1). 466 mg (1.07 mmol, 70% yield) of the title compound **33** were isolated as white solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.06 (s, 1H, NH), 10.87 (s, 1H, NH), 8.91 (s, 1H), 8.15−7.86 (m, 4H), 7.72−7.35 (m, 6H), 5.12 (dd, J = 10.1, 5.6 Hz, 1H), 5.05 (d, J = 3.9 Hz, 1H), 4.85 (t, J = 5.6 Hz, 1H), 4.23−4.14 (m, 1H), 3.75 (td, J = 4.7, 2.3 Hz, 1H), 3.49 (m, 2H, H5′a + H5′b), 2.21 (ddd, J = 12.8, 5.7, 1.7 Hz, 1H, H2′a), 1.89 (ddd, J = 12.7, 10.1, 5.8 Hz, 1H, H2′b); ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.1 (C_{amide}), 165.6 (C_{amide}), 158.3 (C6), 157.1 (C2/C4), 156.4 (C2/C4), 134.1 (C_q), 133.0 (C_q), 132.5, 132.1 (CH_{Ar}), 128.6, 128.4, 128.2, 128.1 (CH_{Ar}), 126.2 (C_q), 109.6 (C5), 87.6 (C4′), 74.0 (C1′), 72.1 (C3′), 62.1 (C5′), 41.6 (C2′); HRMS (ESI-FT-ICR) m/z calcd for $C_{23}H_{22}N_4NaO_5^+$ $[M + Na]^+$ 457.1482, found 457.1485.

1β-[2,4-Bis(benzoylamino)pyrimidin-5-yl]-1,2-dideoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-D-ribofuranose (34). 1β-[2,4-Bis(benzoylamino)pyrimidin-5-yl]-1,2-dideoxy-D-ribofuranose (**33**) (434 mg, 1.00 mmol, 1.00 equiv) was dissolved in 12 mL of dry pyridine in an argon-purged, dried Schlenk flask. 4,4′-Dimethoxytrityl chloride (510 mg, 1.50 mmol, 1.50 equiv) was added slowly, and the resulting solution was stirred for 2 d at rt The reaction was quenched with 5 mL of MeOH, and volatile compounds were removed under reduced pressure. The remaining crude product was purified by flash chromatography on silica (hexanes/acetone 2:3, + 0.1% ethyldimethylamine) yielding 690 mg (0.93 mmol, 93% yield) of the title compound **34** as yellow foam: ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.55 (s, 1H, NH), 9.91 (s, 1H, NH), 8.42 (s, 1H, H6), 8.04−7.91 (m, 2H), 7.89−7.72 (m, 2H), 7.63−7.27 (m, 8H), 7.24−7.08 (m, 7H), 6.85−6.58 (m, 4H), 5.27 (dd, J = 10.4, 4.9 Hz, 1H, H1′), 4.58 (br s, 1H, OH), 4.42 (br s, 1H, H3′), 4.33 (t, J = 3.1 Hz, 1H, H4′), 3.69 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 3.25 (dd, J = 10.2, 5.2 Hz, 1H, H5′a), 3.21 (dd, J = 10.2, 4.5 Hz, 1H, H5′b), 2.53 (dd, J = 12.9, 5.1 Hz, 1H, H2′a), 2.37−2.23 (m, 1H, H2′b); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.4 (C_{amide}), 165.0 (C_{amide}), 158.6 (C_{Ar}-O), 157.5 (C2/C4), 157.2 (C2/C4), 156.4 (C6), 144.5, 135.7, 133.8 (C_q), 133.0, 132.4, 130.0, 129.1, 128.7, 127.6, 113.2 (CH_{Ar}), 110.1 (C5), 87.5 (C4′), 86.4 (C_q), 76.1 (C1′), 73.5 (C3′), 64.4 (C5′), 55.3 (OCH_3), 40.0 (C2′); HRMS (ESI-FT-ICR) m/z calcd for $C_{44}H_{40}N_4NaO_7^+$ $[M + Na]^+$ 759.2789, found 759.2790.

1β-[2,4-Bis(benzoylamino)pyrimidin-5-yl]-1,2-dideoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-D-ribofuranose-3-[(2-

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cyanoethyl)(*N*,*N*-diisopropyl)]phosphoramidite (35). 1β-[2,4-Bis(benzoylamino)pyrimidin-5-yl]-1,2-dideoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-D-ribofuranose (**34**) (170 mg, 0.23 mmol, 1.00 equiv) and 0.12 mL (0.69 mmol, 3.00 equiv) of diisopropylethylamine were dissolved in 5 mL of dry CH_2Cl_2 in an argon-purged, dried Schlenk flask. 2-Cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.08 mL, 0.35 mmol, 1.50 equiv) was added and the solution stirred for 2 h. Volatiles were removed under reduced pressure, and the crude product was purified by flash chromatography on silica (hexanes/acetone 2:1, + 0.1% ethyldimethylamine). Title compound **35** (130 mg, 0.14 mmol, 60% yield) was isolated as a colorless solid: ^{31}P NMR (121 MHz, DMSO-d_6) δ 147.7, 147.2; HRMS (ESI-FT-ICR) m/z calcd for $C_{53}H_{58}N_6O_8P^+$ $[M + H]^+$ 937.4048, found 937.4048.

Oligonucleotides (DNA1, DNA2). **DNA1** was synthesized on a DNA synthesizer with standard phosphoramidite protocols for the ultramild synthesis method using CPGs (1 μmol) with longer coupling times of 625 s and 0.1 M concentration of phosphoramidite **20**. A special capping mix (THF/pyridine/Pac_2O) for ultramild synthesis was applied. The chemicals for the DNA synthesis were purchased. After preparation, the trityl-off oligonucleotide was cleaved off the resin and deprotected with 1 mL of 0.05 M K_2CO_3 in MeOH at 65 °C for 48 h. After cooling, 6 μL of acetic acid was added, and the oligonucleotide was dried and purified by reversed-phase HPLC using the following conditions: A = NH_4OAc buffer (50 mM), pH = 6.5; B = MeCN; gradient = 0−12% B over 45 min. Oligonucleotide **DNA2** was synthesized and purified by a company starting from phosphoramidite **35**. Deprotection was achieved under standard conditions using concd ammonia at elevated temperature. Unmodified oligonucleotides were purchased. The oligonucleotides were lyophilized and quantified by their absorbance at 260 nm on a spectrophotometer. Duplexes were prepared by heating the modified oligonucleotide in presence of 1.05 equiv. of unmodified counter strand to 90 °C, followed by cooling to room temperature. All melting temperatures were determined by the change of absorbance at 260 nm (**1**: ε_{260} = 9,300 M^{-1} cm^{-1}, **2**: ε_{260} = 2,200 M^{-1} cm^{-1}) using the following conditions: 2.5 μM DNA, 10 mM Na−Pi buffer, pH = 7.0, 250 mM NaCl; heating/cooling rate 0.7 °C/min, data interval 0.5 °C.

■ ASSOCIATED CONTENT

Supporting Information

Images of NMR spectra and MS analysis of compounds; HPLC and MS analysis of modified oligonucleotides. This material is available free of charge via the Internet at http://pubs.acs.org/

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) (a) Wojczewski, C.; Stolze, K.; Engels, J. W. *Synlett* **1999**, 1667−1678. (b) Kolpashchikov, D. M. *Chem. Rev.* **2010**, *110*, 4709−4723. (c) Armitage, B. A. *Curr. Opin. Chem. Biol.* **2011**, *15*, 806−812.

(2) (a) Nakatani, K. *ChemBioChem* **2004**, *5*, 1623−1633. (b) Ranasinghe, R. T.; Brown, T. *Chem. Commun.* **2005**, 5487−5502. (c) Strerath, M.; Marx, A. *Angew. Chem., Int. Ed.* **2005**, *44*, 7842−7849.

(3) Sinkeldam, R. W.; Greco, N. J.; Tor, Y. *Chem. Rev.* **2010**, *110*, 2579−2619.

(4) (a) Berezin, M. Y.; Achiledu, S. *Chem. Rev.* **2010**, *110*, 2641−2684. (b) Koboyashi, H.; Ogawa, M.; Alford, R.; Choyke, P. L.; Urano, Y. *Chem. Rev.* **2010**, *110*, 2620−2640. (c) Patterson, G.; Davidson, M.; Manley, S.; Lippincott-Schwartz, J. *Annu. Rev. Phys. Chem.* **2010**, *61*, 345−367. (d) Chang, P. V.; Bertozzi, C. R. *Chem. Commun.* **2012**, *48*, 8864−8879.

(5) Krueger, A. T.; Lu, H.; Lee, A. H. F.; Kool, E. T. *Acc. Chem. Res.* **2007**, *40*, 141−150.

(6) Rist, M. J.; Marino, J. P. *Curr. Org. Chem.* **2002**, *6*, 775−793.

(7) Hall, L. M.; Gerowska, M.; Brown, T. *Nucleic Acids Res.* **2012**, *40*, e108.

(8) (a) Larsen, O. F. A.; van Stokkum, I. H. M.; Gobets, B.; van Grondelle, R.; van Amerongen, H. *Biophys. J.* **2001**, *81*, 1115−1126. (b) Kawai, M.; Lee, M. J.; Evans, K. O.; Nordlund, T. M. *J. Fluoresc.* **2001**, *11*, 23−3. (c) Rist, M.; Wagenknecht, H.-A.; Fiebig, T. *ChemPhysChem* **2002**, *3*, 704−707. (d) Neely, R. K.; Maginnis, S. W.; Dryden, D. T. F.; Jones, A. C. *J. Phys. Chem. B* **2004**, *108*, 17606−17610.

(9) Dallmann, A.; Daniel, L.; Peters, T.; Mügge, C.; Griesinger, C.; Tuma, J.; Ernsting, N. P. *Angew. Chem., Int. Ed.* **2010**, *49*, 5989−5992.

(10) Soulière, M.; Haller, A.; Rieder, R.; Micura, R. *J. Am. Chem. Soc.* **2011**, *133*, 16161−16167.

(11) (a) McCullough, A. K.; Dodson, M. L.; Schärer, O. D.; Lloyd, R. S. *J. Biol. Chem.* **1997**, *272*, 2710−27217. (b) Holz, B.; Klimasauska, S.; Serva, S.; Weinhold, E. *Nucleic Acids Res.* **1998**, *26*, 1076−1083. (c) Allan, B. W.; Beechem, J. M.; Lindstrom, W. M.; Reich, N. O. *J. Biol. Chem.* **1998**, *273*, 2368−2373. (d) Neely, R. K.; Daujotyte, D. D.; Grazulis, S.; Magennis, S. W.; Dryden, D. T. F.; Klimasauskas, S.; Jones, A. C. *Nucleic Acids Res.* **2005**, *33*, 6953−6960.

(12) Wilhelmsson, L. M. *Q. Rev. Biophys.* **2010**, *43*, 159−183.

(13) (a) Secrist, J. A.; Weber, G.; Leonard, N. J.; Barrio, J. R. *Biochemistry* **1972**, *11*, 3499−3506. (b) Secrist, J. A.; Barrio, J. R.; Leonard, N. J. *Science* **1972**, *175*, 646−647.

(14) Berry, D. A.; Jung, K. Y.; Wise, D. S.; Sercel, A. D.; Pearson, W. H.; Mackie, H.; Randolph, J. B.; Somers, R. L. *Tetrahedron Lett.* **2004**, *45*, 2457−2461.

(15) Miyata, K.; Tamamushi, R.; Ohkubo, A.; Taguchi, H.; Seio, K.; Santa, T.; Sekine, M. *Org. Lett.* **2006**, *8*, 1545−1548.

(16) Srivatsan, S. G.; Weizman, H.; Tor, Y. *Org. Biomol. Chem.* **2008**, *6*, 1334−1338.

(17) (a) Sandin, P.; Wilhelmsson, L. M.; Lincoln, P.; Powers, V. E. C.; Brown, T.; Albinsson, B. *Nucleic Acids Res.* **2005**, *33*, 5019−5025. (b) Sandin, P.; Lincoln, P.; Brown, T.; Wilhelmsson, L. M. *Nature Protocols* **2007**, *2*, 615−623. (c) Sandin, P.; Stengel, G.; Ljungdahl, T.; Börjesson, K.; Macao, B.; Wilhelmsson, L. M. *Nucleic Acids Res.* **2009**, *37*, 3924−3933. (d) Preus, S.; Wilhelmsson, L. M. *ChemBioChem* **2012**, *13*, 1990−2001.

(18) (a) Butler, R. S.; Myers, A. K.; Bellarmine, P.; Abboud, K. A.; Castellano, R. K. *J. Mater. Chem.* **2007**, *17*, 1863−1865. (b) Butler, R. S.; Cohn, P.; Tenzel, P.; Abboud, K. A.; Castellano, R. K. *J. Am. Chem. Soc.* **2009**, *131*, 623−633.

(19) (a) Kimoto, M.; Mitsui, T.; Yokoyama, S.; Hirao, I. *J. Am. Chem. Soc.* **2010**, *132*, 4988−4989. (b) Kimoto, M.; Mitusi, T.; Yamashige, R.; Sato, A.; Yokoyama, S.; Hirao, I. *J. Am. Chem. Soc.* **2010**, *132*, 15418−15426.

(20) Reichardt, C. *Chem. Rev.* **1994**, *94*, 2319−2358.

(21) (a) Soujanya, T.; Fessenden, R. W.; Samanta, A. *J. Phys. Chem.* **1996**, *100*, 3507−3512. (b) Vazquez, M. E.; Rothman, D. M.; Imperiali, B. *Org. Biomol. Chem.* **2004**, *2*, 1965−1966. (c) Sharma, V.; Lawrence, D. S. *Angew. Chem., Int. Ed.* **2009**, *48*, 7290−7293. (d) Mandal, D.; Sen, S.; Sukul, D.; Bhattacharyya, K.; Mandal, A. K.; Banerjee, R.; Roy, S. *J. Phys. Chem. B* **2002**, *106*, 10741−10747.

(22) Riedl, J.; Pohl, R.; Ernsting, N. P.; Orsag, P.; Fojta, M.; Hocek, M. *Chem. Sci.* **2012**, *3*, 2797−2806.

(23) Stambasky, J.; Hocek, M.; Kocovsky, P. *Chem. Rev.* **2009**, *109*, 6729−6764.

(24) Saroja, G.; Soujanya, T.; Ramachandram, B.; Samanta, A. *J. Fluoresc.* **1998**, *8*, 405−410.
(25) (a) Krystkowiak, E.; Dobek, K.; Maciejewski, A. *J. Photoch. Photobio. A* **2006**, *184*, 250−264. (b) Maciejewski, A.; Krystkowiak, E.; Koput, J.; Dobek, K. *ChemPhysChem* **2011**, *12*, 322−332.
(26) Sajadi, M.; Obernhuber, T.; Kovalenko, S. A.; Mosquera, M.; Dick, B.; Ernsting, N. P. *J. Phys. Chem. A* **2009**, *113*, 44−55.
(27) Soujanya, T.; Krishna, T. S. R.; Samanta, A. *J. Phys. Chem.* **1992**, *96*, 8544−8548.
(28) (a) Barja, B. C.; Chesta, C.; Atvars, T. D. Z.; Aramendía, P. F. *J. Phys. Chem. B* **2005**, *109*, 16180−16187. (b) Bucsiová, L. u.; Hrdlovič, P. *J. Macromol. Sci. A* **2007**, *44*, 1047−1053.
(29) Saroja, G.; Soujanya, T.; Ramachandram, B.; Samanta, A. *J. Fluoresc.* **1998**, *8*, 405−410.
(30) (a) Larsen, E.; Jørgensen, P. T.; Sofan, M. A.; Pedersen, E. B. *Synthesis* **1994**, *1994*, 1037−1038. (b) Cameron, M. A.; Cush, S. B.; Hammer, R. P. *J. Org. Chem.* **1997**, *62*, 9065−9069. (c) Joubert, N.; Pohl, R.; Klepetářová, B.; Hocek, M. *J. Org. Chem.* **2007**, *72*, 6797−6805.
(31) Schulhof, J. C.; Molko, D.; Teoule, R. *Nucleic Acids Res.* **1987**, *15*, 397−416.
(32) Kubelka, T.; Slavětínská, L.; Klepetářová, B.; Hocek, M. *Eur. J. Org. Chem.* **2010**, *2010*, 2666.
(33) We turned to the benzamide group because we found during an oligonucleotide synthesis containing a similar Pac-protected nucleoside that the Pac-group showed a high stability toward hydrolysis by ammonia and complete cleavage could only be achieved after 24 h at 65 °C.