A New Short and Efficient Synthetic Route to C8-*N*-Acetylarylamine 2′-Deoxyguanosine Phosphoramidites

Nicolas Böge, Sarah Krüger, Marcus Schröder, Chris Meier*

Institute of Organic Chemistry, Department of Chemistry, Faculty of Science, University of Hamburg, Martin-Luther-King Platz 6, 20146 Hamburg, Germany
Fax +49(40)428382495; E-mail: chris.meier@chemie.uni-hamburg.de
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Abstract: In addition to their C8-*N*H-arylamine-dG counterparts, C8-*N*-acetylarylamine adducts of 2′-deoxyguanosine (2′-dG) play an important role in the possible induction of chemical carcinogenesis. A new synthetic pathway of this adduct type using different aromatic amines has been developed following most probably an electrophilic amination reaction. These adducts can be converted into the corresponding phosphoramidites for incorporation into oligonucleotides.

Key words: arylamines, DNA damage, electrophilic addition, C8-*N*-Ac-dG adducts

Introduction

Exposure to chemical carcinogens can occur from environmental or work conditions, diet, smoking, and endogenous processes. Poly- and monocyclic aromatic amines belong to the class of chemical carcinogens that form covalently bonded adducts with DNA. Covalent alteration of DNA (by electrophiles) may be the reason for the induction of chemical carcinogenesis.[1] If these covalently bonded modifications are not repaired,[2] they may compromise the fidelity of DNA replication, leading to mutations and possibly cancer.[3,4] Prior to adduct formation, the aromatic amines undergo a metabolic activation that runs in parallel to the detoxification process. Ring oxidation and *N*-acetylation normally leads to detoxification. However, *N*-oxidation catalyzed by the monooxygenase cytochrome P450 leads to the formation of *N*-arylhydroxylamines. If these are esterified e.g. by acetyl-CoA to yield *N*-acetoxyarylamines, highly electrophilic reagents are formed that react with bionucleophiles, e.g. with the DNA base guanine.[5] Thus, the latter metabolite can act as a so-called ultimate carcinogen.[6] Additionally, *N*-arylamides follow this pathway and initially *N*-arylhydroxamic acids are formed that are transformed into *N*-acetoxy-*N*-acetylarylamines, which then react with guanine in an electrophilic amination reaction. Several covalent adducts have been identified in vivo.[7]

So far, the most extensively studied arylamine adducts are derived from *N*-acetyl-9*H*-fluoren-2-amine (2-AAF) **1a** and 9*H*-fluoren-2-amine (2-AF) **2a**,[8] as well as the corresponding derivatives of 4-aminobiphenyl **1b** and **2b** (Figure 1). Although 9*H*-fluoren-2-amine (2-AF) **2a** and *N*-acetyl-9*H*-fluoren-2-amine (2-AAF) **1a** differ only by one acetyl group, they have very different physicochemical properties and biological effects. The acetylated form **1a** causes a much more severe local distortion within the DNA double helix than the nonacetylated form **2a**.[9,10] Also the 2-AAF adduct **1a** is a more potent blocker of replication and transcription; however, it is also a better substrate for DNA-repair enzymes.[11,12]

Due to the importance of these two C8-dG adducts, an easy and straightforward synthesis of the corresponding phosphoramidites would be of great interest as these adducts could be incorporated site-specifically into DNA using standard automated solid-phase synthesis.

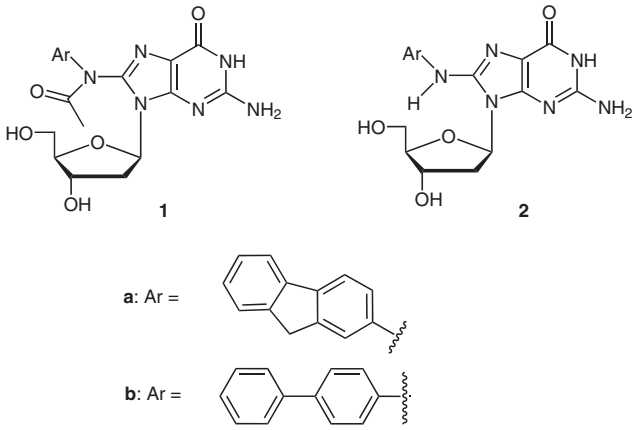

Figure 1 C8-Arylamine adducts of 2′-deoxyguanosine formed by metabolites of the strong carcinogens 9*H*-fluoren-2-amine [C8-(2-AAF)dG, **1a**; C8-(2-AF)dG, **2a**] and 4-aminobiphenyl [C8-(4-AAB)dG, **1b**; C8-(4-AB)dG, **2b**]

Results and Discussion

Our interest is related to DNA lesions caused by monocyclic aromatic amines that act as so-called borderline carcinogens like toluidine and anisidine.[13,14] In contrast to polycyclic *N*-arylamines, these are often used, for example, in pharmacophores or in dyes. In contrast, for example, to the strong liver carcinogens 2-AF and 2-AAF, their biological effects are only sparsely understood. Most importantly, it is not understood why these lesions of monocyclic aromatics do not lead to the same biological

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consequences as the strong carcinogens, although they form the same type of adducts in the same amounts in biomimetic studies.[15]

In our earlier work we focussed on the synthesis of the C8-*N*H-arylamine adducts of 2′-deoxyguanosine (2′-dG) and 2′-deoxyadenosine. In 2002 we published the first synthesis of C8-arylamine–dG adducts **5** using a palladium cross-coupling reaction of the protected 8-bromo-dG derivative **4**, its conversion into the corresponding phosphoramidites **7** via intermediates **6**, and the site-specific

Biographical Sketches

Nicolas Böge was born in Hamburg, Germany in 1981. He studied chemistry at the University of Hamburg, Germany and received his diploma under the guidance of Prof. Chris Meier and joined the research group as a Ph.D. student in 2005. In the same year he was invited to the Criegee-Gedenkveranstaltung in Karlsruhe, Germany. In 2006 he received a fellowship from the Gesellschaft Deutscher Chemiker for the participation at the Roundtable on Nucleosides, Nucleotides, & Nucleic Acids in Bern, Switzerland. At present he is working on arylamine-modified oligonucleotides.

Sarah Krüger was born in Zeven, Germany in 1983. She studied chemistry at the University of Hamburg, Germany and joined the research group of Prof. Chris Meier in 2007.

Marcus Schröder was born in Hamburg, Germany in 1982. Since 2003, he has studied chemistry at the University of Hamburg, Germany. In 2006 he spent three months at the University of Oslo, Norway, supported by the Erasmus programme.

Chris Meier was born in 1962 in Berlin, Germany. He studied Chemistry at the University of Marburg/Lahn, where he received his diploma in 1987 and his Ph.D. in 1989 with Professor G. Boche. During that time he became interested in bioorganic chemistry. During his postdoctoral studies at the Pasteur Institute in Paris, France he became involved in the chemistry of nucleosides, oligonucleotides, and prodrugs. In 1991 he returned to the University of Frankfurt/Main and finished his habilitation in 1996. In 1994 he received the Adolf Messer Award for interdisciplinary research. In 1997 he became Associate Professor of Organic Chemistry at the University of Würzburg and 1999 he moved to the University of Hamburg as a Full Professor of Organic Chemistry. In 2007 he received the William Prusoff Young Investigator Award of the Society for Antiviral Research. In 2005 he became Head of Department of the Chemistry Department of the University of Hamburg. His research interests: DNA damage induced by arylamine carcinogens, pronucleotide design for antiviral nucleoside analogues, stereoselective synthesis of carbocyclic nucleoside analogues, and the development of new synthesis pathways for important phosphorylated biomolecules.

incorporation into oligonucleotides.[16,17] Recently, we extended the studies to rarely investigated C8-arylamine-dA adducts that were site-specifically incorporated into oligonucleotides via automated phosphoramidite chemistry.[18]

Since then we have improved the synthesis by introducing the N^2-formamidine, instead of the isobutyryl, group (Scheme 1).[19] Using this mild deprotection strategy the cleavage was achieved in four hours at 40 °C instead of eight hours at 55 °C. Thus, the yield of adducted oligonucleotides was four- to fivefold higher than using the isobutyryl strategy.

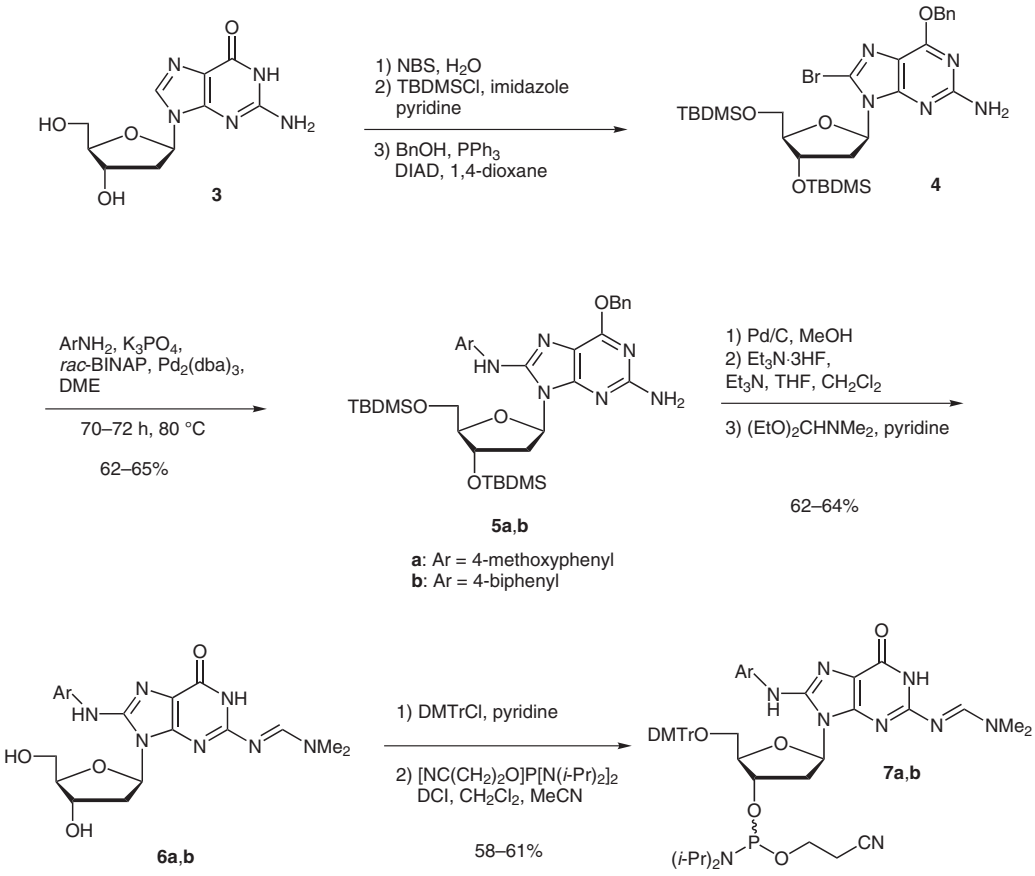
With the synthesized modified oligonucleotides (30mer) we investigated the effect of the modification concerning the thermal stability (Tm value) and the conformation of the DNA strand (CD spectra). For oligonucleotides modified with a strong carcinogen, such as 2-AF, a decrease of the Tm value was observed, whereas the modification with borderline carcinogens like aniline had no influence on the stability as compared to the unmodified strand.[18] To study the overall conformation of the damaged DNA hybrids, the CD spectra were recorded. The reference oligonucleotide as well as oligonucleotides bearing the lesions showed the typical spectra of a B-type DNA conformation; thus no strong conformational change of the C8-*N*H-arylamine 'damage' was induced.

Recently, we performed initial experiments concerning the action of such lesions on DNA polymerases. We observed that the interaction with DNA polymerases depends very much on the DNA polymerase that is used and the chemical structure of the adduct. In contrast to the expectation, the highest mutation incident was found when a high-fidelity DNA polymerase promoted nucleotide insertion opposite the lesion of the borderline carcinogen 4-anisidine instead of a 4-aminobiphenyl lesion.

In addition to these C8-*N*H-arylamine adducts **2**, their *N*-acetylated counterparts **1** were found in in vivo studies and thus, obviously, may also play an important role in arylamine-induced carcinogenesis. The acetyl group is most probably introduced in a very early step of the biotransformation catalyzed by two *N*-acetyl transferases NAT1 or NAT2.[20] This acetylation of the arylamine into an arylamide should promote the detoxification because the amide is less susceptible to *N*-oxidation.

Former synthesis of the C8-*N*-acetylarylamine adducts of dG were based on 'damaging' nucleosides or oligonucleotides post-synthetically with *N*-acetoxy-*N*-acetylarylamines or *N*-acetoxyarylamines and subsequent acetylation.[21] However, this strategy resulted in poor chemical yields and only damaged oligonucleotides containing one guanine base were accessible.[22]

A major aspect for the development of an efficient synthesis is the base lability of the N^8-acetyl function. To ensure



Scheme 1 Improved synthesis of the C8-arylamine adducts of dG and conversion into their phosphoramidites

that the acetyl group of dG-AAF is stable under the conditions for the deprotection step after oligonucleotide synthesis, very labile protecting groups are required. The first attempts to solve this problem were made by Zhou and Romano.[22,23] They used the 9-fluorenylmethoxycarbonyl (Fmoc) protecting-group strategy and mild deprotection conditions that ensured the stability of AAF-dG. However, a limiting factor of this strategy is that Fmoc-protected nucleotide phosphoramidites are not commercially available.

In a further strategy, Schärer and Gillet[24] used a transient 4,4′-dimethoxytrityl (DMTr) protecting group for the N^2-position of an 8-Br-dG derivative. After a palladium-catalyzed cross-coupling reaction of 8-Br-dG with the arylamine, the products were subsequently acetylated at the N^8-position. Finally, in two further steps the N^2-DMTr group was replaced by the N^2-isopropylphenoxyacetyl (*i*-PrPac) group, which yielded monomers suitable for DNA synthesis. In 2005 they reported the successful conversion of these adducts into the corresponding phosphoramidite (12 steps overall) and site-specific incorporation into oligonucleotides. It was proven that the N^8-acetyl group was not cleaved during the final deprotection.[25]

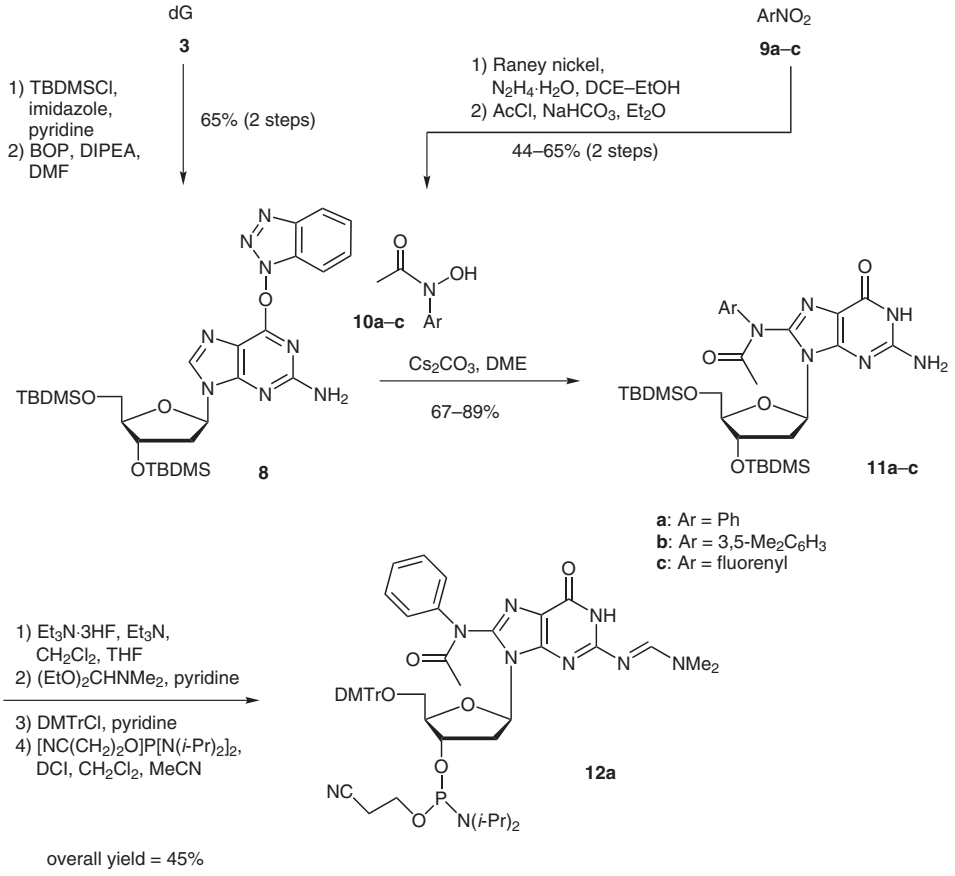
Despite these advances, an efficient method and short synthetic route for the synthesis of N^8-acetylarylamine adducts of dG is still needed. Here, we disclose our approach for a new synthesis of this adduct type without using the Buchwald–Hartwig cross-coupling and a complex transient protecting-group strategy.

The key step of this unprecedented reaction is the reaction between O^6-(1*H*-benzotriazol-1-yl)-dG **8** and *N*-arylacetohydroxamic acids that led to the C8-*N*-acetylated adducts. Due to the mild reaction conditions it is possible to introduce a very labile protecting group suitable for automated DNA synthesis at the N^2-position after the adduct formation.

Thus, after protection of the 5′- and 3′-hydroxy groups of dG **3** using *tert*-butyldimethylsilyl chloride, the benzotriazole moiety was introduced into the O^6-position using (1*H*-benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) (Scheme 2).[26]

The dG derivative **8** was reacted with an *N*-arylacetohydroxyamic acid **10a–c**, prepared by reduction of nitroarylamine compounds **9a–c** to the corresponding *N*-arylhydroxylamines and subsequent reaction with acetyl chloride to give *N*-arylacetohydroxamic acids **10a–c** in 44–65% yield (Scheme 2).[27,28]

The coupling reaction was performed in 1,2-dimethoxyethane using cesium carbonate as base and the C8-*N*-acetylaryl adducts **11a–c** were isolated in 67–89% yield; the formation of **11** in this way is previously unknown. The reaction mechanism for the formation of the C8 adduct remains unclear, but it is assumed that the *N*-arylacetohydroxamic acid reacts with **8** in a nucleophilic



Scheme 2 Preparation of *N*-acetylated adducts **11a–c** and the C8-[acetyl(phenyl)amino]-2′-dG phosphoramidite **12a**

displacement at the nitrogen atom with formation of the protected dG and the benzotriazolylated hydroxamic acid. The latter could form an arylnitrenium ion, which undergoes electrophilic amination at C8 (presumably at N7 and subsequent rearrangement to C8). The prior formation of the O^6-benzotriazolyl derivative **8** is essential; in a separate experiment the pre-incubation of the *N*-arylacetohydroxamic acids **10a–c** with the BOP reagent and subsequent addition of TBDMS-protected dG did not give **11a–c**.

For the conversion of **11a** into the corresponding phosphoramidite **12a**, the hydroxy groups were first deprotected ($Et_3N{\cdot}3HF$, Et_3N) to yield the *N*-acetylated dG adduct; the sensitive N^8-acetyl group was found to be stable under these conditions. For oligonucleotide synthesis, the N^2-position was selectively blocked using the formamidine group (mild deprotection strategy). The formamidine group has been used previously[29] and we have recently used this group successfully for C8-*N*H-arylamine-dG-oligonucleotides.[17] We have shown that this protecting group can be cleaved from **11a** and from oligonucleotides at 45 °C within only four hours using ammonia solution. Recently, Schärer et al. reported that the *i*-PrPac group can be cleaved from an oligonucleotide using the same cleavage conditions without losing the *N*-acetyl group.[25]

After N^2-protection, the 5′-hydroxy function was blocked with DMTr and the corresponding phosphoramidite was synthesized using previously described conditions.[16,18] We have shown that these phosphoramidites can be successfully incorporated into a 12mer-oligonucleotide by automated DNA synthesis.

For all *N*-acetylated compounds, a line-broadening in the ^{1}H NMR at room temperature was observed; similar behavior was previously described by Beland et al.[30] They suggested that the line-broadening was most likely due to a hindered rotation of the *N*-acetyl group in 2-AAF because of partial double-bond character in analogy to peptide bonds. Therefore, we performed high-temperature NMR studies with C8-acetylaniline adduct **11a** to confirm this assumption. As expected, in these experiments the resolution of the signals increased while heating the sample, and the determination of individual coupling constants from the NMR spectra was possible at 333 K (55 °C).

In conclusion, we have developed a new synthesis for C8-[acetyl(aryl)amine]-2′-dG adducts. With this much shorter route it is possible to introduce variable protecting groups for the exocyclic N^2-amino function of dG. Moreover, the expensive and time-consuming cross-coupling reaction for the formation of the C8-*N*H-arylamine adduct, which has to be acetylated in a separate step, can be circumvented. According to our new approach, this type of DNA damage was synthesized using *N*-arylacetohydroxamic acids within seven steps starting from dG to yield the corresponding phosphoramidite.

Due to the facile access to many nitroaryl compounds, this newly developed route for the preparation of 2′-dG–arylamine adducts should be useful for the synthesis of various C8-(acetylamino) adducts of mono- and polycyclic aromatic amines. Work along these lines is currently ongoing.

All reactions (except the synthesis of the hydroxylamines and hydroxamic acids) were performed in flame-dried glassware under an N_2 atmosphere. Commercial solvents and reagents were used without further purification with the following exceptions: pyridine, CH_2Cl_2, MeCN were distilled from CaH_2, DME and Et_2O were dried with potassium and distilled under N_2. H_2O was purified on a Milli-Q Water System. ^{1}H NMR and ^{13}C NMR spectra were recorded on AMX 400 MHz or DMX 500 MHZ Bruker spectrometer. NMR spectra were reported relative to the NMR solvent peaks [^{1}H NMR: δ = 2.50 (DMSO-d_6) and 7.16 (C_6D_6); ^{13}C NMR: δ = 39.52 (DMSO-d_6) and 128.06 (C_6D_6)]. HRMS analysis were performed on an analytical VG/70-250 F using FAB or Finnigan MAT 95 XL Thermo Quest spectrometer using ESI. IR spectra were recorded on an Avatar 370 FT-IR spectrophotometer using KBr plates. Melting points were measured on an apotec capillary melting point apparatus. TLC was performed on aluminum sheets coated with silica gel 60 F_{254} from Merck.

The synthesis of 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-dG was performed as previously described.[31]

O^6-(1*H*-Benzotriazolyl)-3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (8)

In a dried flask, 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (6.60 g, 13.3 mmol) and BOP (12.12 g, 27.54 mmol) were diluted in anhyd DMF (120 mL) and DIPEA (4.80 mL, 27.6 mmol) was added. The mixture was stirred at r.t. for 72 h and then the solvent was removed in vacuo. The residue was diluted with EtOAc, extracted with H_2O, and dried (Na_2SO_4). The solvent was removed under reduced pressure. Column chromatography (33% EtOAc–hexanes) gave the product as a yellow solid; yield: 5.45 g (67%); mp 88–90 °C.

$[\alpha]_{546}^{20}$ +27 (*c* 0.22, $CHCl_3$).

IR (KBr): 3204, 2954, 2928, 2884, 1514, 1445, 1280, 1227, 1190, 946, 882, 780, 742 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.30 (s, 1 H, H8), 8.11 (d, *J* = 8.4 Hz, 1 H, H14), 7.75 (d, *J* = 8.3 Hz, 1 H, H11), 7.64 (t, *J* = 7.2, 7.2 Hz, 1 H, H12), 7.51–7.55 (m, 1 H, H13), 6.74 (br s, 2 H, NH_2), 6.25 (dd, *J* = 6.7, 6.7 Hz, 1 H, H1′), 4.54 (ddd, *J* = 3.4, 3.4, 8.6 Hz, 1 H, H3′), 3.83–3.86 (m, 1 H, H4′), 3.70 (dddd, *J* = 5.2, 11.1, 15.6 Hz, 2 H, H5′a, H5′b), 2.75–2.82 (m, 1 H, H2′a), 2.31 (ddd, *J* = 3.6, 6.1, 13.2 Hz, 1 H, H2′b), 0.89 [s, 9 H, $SiC(CH_3)_3$], 0.86 [s, 9 H, $SiC(CH_3)_3$], 0.11 [s, 6 H, $Si(CH_3)_2$], 0.04, 0.03 [2 × s, 2 × 3 H, $Si(CH_3)_2$].

^{13}C NMR (101 MHz, DMSO-d_6): δ = 159.1, 158.9, 156.7, 142.6, 139.7, 129.2, 128.9, 124.9, 119.3, 111.8, 109.0, 86.9, 82.6, 71.8, 62.4, 38.8, 26.1, 24.6, 17.9, 17.5, –5.1, –6.5.

HRMS-FAB: *m/z* $[M]^+$ calcd for $C_{28}H_{44}N_8O_4Si_2$: 612.3024; found: 613.3102 $[M + H]^+$.

Arylhydroxylamines; General Procedure 1

In a flask, the nitroaryl compound (1 equiv) was dissolved in EtOH–DCE (1:1), Raney nickel was added and the mixture was cooled to 0 °C. After the addition of hydrazine hydrate (10 equiv), the soln was stirred for 1 h and filtered. The solvent was removed in vacuo and the product was recrystallized (toluene).

N-Phenylhydroxylamine
Following general procedure 1, **9a** (12.32 g, 100.07 mmol) and hydrazine hydrate (31.25 mL, 1 mol) gave the product as a white solid; yield: 7.62 g (70%); mp 92.3 °C.

IR (KBr): 3246, 2847, 2019, 1957, 1935, 1875, 1854, 1784, 1725, 1602, 1521, 1494, 1470, 1421, 1321, 1305, 1236, 1221, 1156, 1106, 1088, 1058, 1024, 925, 897, 833, 795, 749, 692, 595, 501, 433 cm^{-1}.

^1H NMR (400 MHz, DMSO-d_6): δ = 8.32 (d, *J* = 2.2 Hz, 1 H, NH), 8.25 (br s, 1 H, OH), 7.19 (dd, *J* = 7.4, 8.4 Hz, 2 H, H3), 6.87 (d, *J* = 7.9 Hz, 1 H, H2), 6.78 (t, *J* = 7.3, 7.3 Hz, 1 H, H4).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 152.0, 128.7, 119.2, 120.9, 113.0.

HRMS-FAB: *m*/*z* $[M]^+$ calcd for C_6H_7NO: 109.0528; found: 109.0753.

N-(3,5-Dimethylphenyl)hydroxylamine
Following general procedure 1, **9b** (5 g, 33 mmol) and hydrazine hydrate (17.0 mL, 350.5 mmol) gave the product as a white solid; yield: 3.86 g (85%); mp 105.2 °C.

IR (KBr): 3238, 3136, 3014, 2913, 2848, 1617, 1602, 1520, 1469, 1414, 1373, 1305, 1090, 1062, 1037, 949, 893, 868, 859, 839, 812, 695, 643 cm^{-1}.

^1H NMR (400 MHz, DMSO-d_6): δ = 8.17 (d, *J* = 2.2 Hz, 1 H, NH), 8.07–8.09 (m, 1 H, OH), 6.44 (s, 2 H, H2, H6), 6.37 (s, 1 H, H4), 2.17 (s, 6 H, 2 Me).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 152.0, 137.1, 120.9, 110.7, 21.1.

HRMS-FAB: *m*/*z* $[M]^+$ calcd for $C_8H_{11}NO$: 137.0919; found: 137.0828 $[M]^+$.

N-(9*H*-Fluoren-2-yl)hydroxylamine
Following general procedure 1, **9c** (6.36 g, 30.00 mmol) and hydrazine hydrate (15 mL, 309 mmol) gave the product as a yellow solid; yield: 3.90 g (66%); mp 211.3 °C.

IR (KBr): 3446, 3360, 3254, 2920, 2349, 2284, 1950, 1615, 1585, 1480, 1454, 1418, 1401, 1349, 1316, 1304, 1278, 1264, 1211, 1194, 1177, 1156, 1136, 1122, 1099, 1050, 1025, 1000, 951, 903, 870, 859, 827, 814, 764, 731, 711, 691, 573, 418 cm^{-1}.

^1H NMR (400 MHz, DMSO-d_6): δ = 8.38–8.39 (m, 2 H, NH, OH), 7.65–7.71 (m, 2 H, H9, H12), 7.48 (d, *J* = 7.4 Hz, 1 H, H6), 7.29 (t, *J* = 7.4, 7.4 Hz, 1 H, H8), 7.18 (t, *J* = 7.4, 7.4 Hz, 1 H, H7), 7.07 (s, 1 H, H2), 6.85 (dd, *J* = 1.5, 8.2 Hz, 1 H, H13), 3.82 (s, 2 H, CH_2).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 151.6, 143.8, 141.9, 141.6, 132.8, 126.4, 124.9, 124.7, 119.9, 118.5, 111.8, 109.5, 36.2.

HRMS-FAB: *m*/*z* $[M]^+$ calcd for $C_{13}H_{11}NO$: 197.0919; found: 197.0841 $[M]^+$.

N-Arylacetohydroxamic Acids 10a–c; General Procedure 2
In a flask, arylhydroxylamine (1 equiv) and sat. $NaHCO_3$ soln were suspended in Et_2O and stirred at 0 °C. After the addition of AcCl (1.1 equiv), the mixture was stirred at r.t. for 1 h. The layers were separated and the organic layer was extracted with 1 M NaOH (3 ×). Then the aqueous layer was extracted with Et_2O (3 ×). 3 M HCl was added until the aqueous layer was acidic and it was then extracted with Et_2O (3 ×). The combined organic layers were dried (Na_2SO_4) and the solvent was removed in vacuo.

N-Phenylacetohydroxamic Acid (10a)
Following general procedure 2, *N*-phenylhydroxylamine (3 g, 27.49 mmol), AcCl (2.3 mL, 32.35 mmol), and sat. $NaHCO_3$ soln (40.5 mL) gave the product as a brown solid; yield: 3.51 g (84%); mp 120 °C.

IR (KBr): 2901, 1641, 1593, 1545, 1308, 1177, 1101, 1034, 907, 691 cm^{-1}.

^1H NMR (400 MHz, DMSO-d_6): δ = 10.62 (s, 1 H, OH), 7.61 (d, *J* = 7.9 Hz, 2 H, H2), 7.34–7.38 (m, 2 H, H3), 7.14 (t, *J* = 7.3, 7.3 Hz, 1 H, H4), 2.20 (s, 3 H, H6).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 170.1, 142.0, 128.7, 123.3, 119.3, 22.8.

HRMS-FAB: *m*/*z* $[M]^+$ calcd for $C_8H_9NO_2$: 151.0633; found: 152.0659 $[M + H]^+$.

N-(3,5-Dimethylphenyl)acetohydroxamic Acid (10b)
Following general procedure 2, *N*-(3,5-dimethylphenyl)hydroxylamine (450 mg, 3.29 mmol), AcCl (0.23 mL, 3.51 mmol), and sat. $NaHCO_3$ soln (4.05 mL) gave the product as a slightly yellow solid; yield: 547.8 mg (93%); mp 49.0 °C.

IR (KBr): 3851, 3646, 3141, 2915, 1627, 1478, 1381, 1297, 1270, 1181, 1115, 1037, 999, 943, 890, 843, 811, 688, 584 cm^{-1}.

^1H NMR (400 MHz, DMSO-d_6): δ = 10.49 (s, 1 H, OH), 7.23 (s, 2 H, H2, H6), 6.79 (s, 1 H, H4), 2.26 (s, 6 H, 2 Me), 2.17 (s, 3 H, Ac-Me).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 141.5, 137.3, 126.1, 22.4, 21.0.

HRMS-FAB: *m*/*z* $[M]^+$ calcd for $C_{10}H_{13}NO_2$: 179.0946; found: 180.1045 $[M + H]^+$.

N-(9*H*-Fluoren-2-yl)acetohydroxamic Acid (10c)
Following general procedure 2, *N*-(9*H*-fluoren-2-yl)hydroxylamine (100 mg, 0.51 mmol), AcCl (0.04 mL, 0.56 mmol), and sat. $NaHCO_3$ soln (0.9 mL) gave the product as a yellow solid; yield: 94.6 mg (78%); mp 153.6 °C.

IR (KBr): 3149, 2848, 1620, 1484, 1453, 1428, 1395, 1304, 1286, 1252, 1215, 1179, 1138, 1090, 1029, 1002, 945, 870, 832, 764, 727, 622 cm^{-1}.

^1H NMR (400 MHz, DMSO-d_6): δ = 10.68 (s, 1 H, OH), 7.87–7.88 (m, 3 H, H2, H9, H12), 7.62–7.64 (m, 1 H, H13), 7.56–7.58 (m, 1 H, H6), 7.36–7.39 (m, 1 H, H8), 7.27–7.31 (m, 1 H, H7), 3.93 (s, 2 H, CH_2), 2.22 (s, 3 H, Ac-Me).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 143.2, 143.0, 140.6, 140.5, 126.7, 126.4, 126.3, 125.0, 119.7, 119.6, 36.5, 36.4, 22.4.

HRMS-FAB: *m*/*z* $[M + H]^+$ calcd for $C_{15}H_{14}NO_2$: 240.1025; found: 240.1030.

C8-*N*-Acetylarylamine Adducts 11a–c; General Procedure 3
In a dried flask, **8** (1 equiv), Cs_2CO_3 (10 equiv), and *N*-arylacetohydroxamic acid **10a–c** (10 equiv) were suspended in anhyd DME and stirred under a N_2 atmosphere at r.t.. When the reaction was complete H_2O and CH_2Cl_2 were added and the aqueous layer was extracted with CH_2Cl_2 (3 ×). The combined organic layers were dried (Na_2SO_4) and the solvent was removed in vacuo. The product was isolated by column chromatography (10% MeOH–CH_2Cl_2).

8-[Acetyl(phenyl)amino]-3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (11a)
Following general procedure 3, **8** (321 mg, 520 μmol) and **10a** (157 mg, 1.04 mmol) gave the product as a slightly brown solid; yield: 246 mg (73%); mp 227–229 °C (dec.).

$[\alpha]_{546}^{20}$ +90 (*c* 0.17, $CHCl_3$).

IR (KBr): 2954, 2886, 1704, 1602, 1542, 1431, 1330, 1112, 1031, 812, 779, 691 cm^{-1}.

^1H NMR (400 MHz, DMSO-d_6): δ = 10.65 (s, 1 H, H1), 7.60–7.62 (m, 2 H, H11), 7.33–7.36 (m, 2 H, H12), 7.12–7.15 (m, 1 H, H13), 6.40 (br s, 2 H, NH_2), 5.90–6.09 (m, 1 H, H1′), 4.49–4.58 (m, 1 H,

H3′), 3.66–3.75 (m, 4 H, H2′a, H4′, H5′a, H5′b), 3.06–3.17 (m, 1 H, H2′b), 2.19 (s, 3 H, H15), 0.86 [s, 9 H, $SiC(CH_3)_3$], 0.80 [s, 9 H, $SiC(CH_3)_3$], 0.07 [m, 6 H, $Si(CH_3)_2$], –0.04 [s, 6 H, $Si(CH_3)_2$].

^{13}C NMR (101 MHz, DMSO-d_6): δ = 170.2, 156.2, 153.5, 141.6, 129.1, 128.6, 128.3, 122.9, 118.9, 118.7, 115.1, 87.3, 83.2, 72.2, 63.8, 25.6, 25.5, 22.4, 17.8, 17.5, –5.4, –5.5.

HRMS-FAB: *m*/*z* $[M]^+$ calcd for $C_{30}H_{48}N_6O_5Si_2$: 628.3225; found: 629.3303 $[M + H]^+$.

8-[Acetyl(3,5-dimethylphenyl)amino]-3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (11b)

Following general procedure 3, **8** (4.00 g, 6.53 mmol) and **10b** (2.33 g, 13.1 mmol) gave the product as a slightly brown solid; yield: 2.89 g (67%); mp >200 °C (dec.).

$[\alpha]_{546}^{20}$ –3.3 (*c* 0.5, $CHCl_3$).

IR (KBr): 3337, 3162, 2954, 2929, 2857, 1699, 1628, 1596, 1542, 1472, 1434, 1368, 1314, 1257, 1110, 1082, 1032, 1006, 941, 837, 779, 714, 675 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.83 (s, 1 H, NH), 6.94 (s, 3 H, H2, H4, H6), 6.37 (s, 2 H, NH_2), 5.99 (s, 1 H, H1′), 4.54 (s, 1 H, H3′), 3.60–3.76 (m, 5 H, H4′, H5′a, H5′b, H2′a, H2′b), 2.25 (s, 6 H, 2 Me), 2.00 (s, 3 H, Ac-Me), 0.87 [s, 9 H, $SiC(CH_3)_3$], 0.81 [s, 9 H, $SiC(CH_3)_3$], 0.09 [s, 6 H, $Si(CH_3)_2$], –0.03 [s, 6 H, $Si(CH_3)_2$].

^{13}C NMR (101 MHz, DMSO-d_6): δ = 156.2, 140.6, 138.4, 115.0, 87.6, 87.3, 83.4, 72.5, 63.1, 25.7, 22.5, 21.0, 17.8, –5.5, –5.6.

HRMS-FAB: *m*/*z* $[M]^+$ calcd for $C_{32}H_{52}N_6O_5Si_2$: 656.3538; found: 657.3612 $[M + H]^+$.

8-[Acetyl(9*H*-fluoren-2-yl)amino]-3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (11c)

Following general procedure 3, **8** (25.61 mg, 41.82 μmol) and **10c** (20 mg, 83.65 μmol) gave the product as a slightly brown solid; yield: 26.6 mg (89%); mp 154.6 °C.

$[\alpha]_{546}^{20}$ –7.2 (*c* 0.5, $CHCl_3$).

IR (KBr): 3385, 3214, 2954, 2929, 2857, 1685, 1592, 1535, 1471, 1405, 1362, 1256, 1176, 1112, 1029, 1006, 939, 837, 814, 779, 734, 671 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.25 (s, 1 H, NH), 7.84–7.92 (m, 3 H, H2, H9, H12), 7.63–7.65 (m, 1 H, H13), 7.56–7.58 (m, 1 H, H6), 7.35–7.39 (m, 1 H, H8), 7.27–7.32 (m, 1 H, H7), 6.76 (s, 2 H, NH_2), 6.10 (dd, *J* = 6.1, 7.6 Hz, 1 H, H1′), 4.48 (q, *J* = 2.9, 2.9, 5.5 Hz, 1 H, H3′), 3.92 (s, 2 H, CH_2), 3.79–3.82 (m, 1 H, H4′), 3.60–3.72 (m, 2 H, H5′a, H5′b), 2.61–2.67 (m, 1 H, H2′a), 2.20–2.23 (m, 1 H, H2′b), 2.04 (s, 3 H, Ac-Me), 0.89 [s, 9 H, $SiC(CH_3)_3$], 0.87 [s, 9 H, $SiC(CH_3)_3$], 0.10 [s, 6 H, $Si(CH_3)_2$], 0.04 [s, 6 H, $Si(CH_3)_2$].

^{13}C NMR (101 MHz, DMSO-d_6): δ = 156.6, 140.6, 127.5, 125.5, 87.7, 72.7, 36.8, 26.1.

HRMS-FAB: *m*/*z* $[M]^+$ calcd for $C_{37}H_{52}N_6O_5Si_2$: 628.3225; found: 629.3303 $[M + H]^+$.

8-[Acetyl(phenyl)amino]-2′-deoxyguanosine

In a flask, **11a** (140 mg, 222 μmol) was dissolved in CH_2Cl_2–THF (1:1; 12 mL). After the addition of Et_3N (0.30 mL, 0.22 g, 2.2 mmol) and $Et_3N{\cdot}3HF$ (0.90 mL, (5.5 mmol), the mixture was stirred at r.t. for 16 h. It was then evaporated to dryness and the product was isolated by column chromatography (CH_2Cl_2–MeOH, 0–50%) as a yellow solid; yield: 85 mg (96%); mp 138–140 °C.

$[\alpha]_{546}^{20}$ +55 (*c* 0.29, CH_2Cl_2–MeOH, 1:1).

IR (KBr): 2937, 1718, 1680, 1671, 1647, 1576, 1369, 842 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.83 (s, 1 H, H1), 7.35–7.50 (m, 5 H, H11, H12, H13), 6.44 (br s, 2 H, NH_2), 6.04–6.10 (m, 1 H, H1′), 5.24 (br s, 1 H, OH), 4.96 (br s, 1 H, OH), 4.35–4.40 (m, 1 H, H3′), 3.80–3.83 (m, 1 H, H4′), 3.53–3.67 (m, 2 H, H5′a, H5′b), 3.06–3.12 (m, 2 H, H2′a, H2′b), 2.02 (s, 3 H, H15).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 128.9, 126.1, 125.0, 87.9, 83.6, 71.3, 62.3, 45.5, 22.3.

HRMS-FAB: *m*/*z* $[M]^+$ calcd for $C_{18}H_{20}N_6O_5$: 400.1589; found: 401.1621 $[M + H]^+$.

8-[Acetyl(phenyl)amino]-2′-deoxy-N^2-[(dimethylamino)methylene]guanosine

In a dried flask, 8-[acetyl(phenyl)amino]-2′-deoxyguanosine (75 mg, 0.18 mmol) was diluted in anhyd pyridine (4 mL) and *N*,*N*-dimethylformamide diethyl acetal (0.40 mL, 2.30 mmol) were added. After stirring the mixture at r.t. for 17 h, the solvent was removed in vacuo and the product was isolated by column chromatography (CH_2Cl_2–MeOH, 0–20%) as a yellow solid; yield: 65 mg (80%); mp 153–155 °C.

$[\alpha]_{546}^{20}$ +59 (*c* 0.29, CH_2Cl_2–MeOH, 1:1).

IR (KBr): 2931, 1734, 1653, 1594, 1569, 1491, 1436, 1357, 1286, 1055, 844 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 11.48 (s, 1 H, H1), 8.47 (s, 1 H, H16), 7.33–7.45 (m, 5 H, H11, H12, H13), 6.11–6.15 (m, 1 H, H1′), 5.30–5.33 (m, 1 H, H3′), 4.87–4.90 (m, 1 H, H5′a), 4.42–4.47 (m, 1 H, H5′b), 3.80–3.86 (m, 1 H, H4′), 3.52–3.65 (m, 2 H, H2′a, H2′b), 3.13 (s, 3 H, H17a), 3.03 (s, 3 H, H17b), 2.03 (s, 3 H, H15).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 158.4, 129.0, 126.7, 87.8, 83.9, 70.8, 61.4, 40.7, 34.5, 22.1.

HRMS-FAB: *m*/*z* $[M]^+$ calcd for $C_{21}H_{25}N_7O_5$: 455.1917; found: 456.1977 $[M + H]^+$.

8-[Acetyl(phenyl)amino]-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-N^2-[(dimethylamino)methylene]guanosine

In a dried flask, 8-[acetyl(phenyl)amino]-2′-deoxy-N^2-[(dimethylamino)methylene]guanosine (45 mg, 98 μmol) were diluted in anhyd pyridine (4 mL) and DMTrCl (72 mg, 0.21 mmol) were added. The mixture was stirred at r.t. for 16 h. CH_2Cl_2 and sat. $NaHCO_3$ soln were added and the aqueous layer extracted with CH_2Cl_2 (3 ×) The combined organic layers were dried (Na_2SO_4) and the solvent removed in vacuo. The product was isolated by column chromatography (CH_2Cl_2–MeOH, 0–20%) as a slightly brown solid; yield: 60 mg (80%); mp 174–176 °C.

$[\alpha]_{546}^{20}$ +44 (*c* 0.5, $CHCl_3$).

IR (KBr): 2931, 1734, 1653, 1594, 1569, 1491, 1436, 1357, 1286, 1055, 844 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.48 (s, 1 H, H1), 8.19 (s, 1 H, H16), 7.11–7.41 (m, 14 H, H11, H12, H13, arom. H/DMTr), 6.67–6.74 (m, 4 H, H11, H12, H13, arom. H/DMTr), 6.17–6.23 (m, 1 H, H1′), 5.31–5.39 (m, 1 H, H3′), 4.58–4.62 (m, 1 H, H4′), 3.89–3.94 (m, 1 H, H5′a), 3.68–3.70 (m, 7 H, OCH_3-DMTr, H5′b), 3.33–3.34 (m, 1 H, H2′a), 3.00 (s, 3 H, H17a), 2.94 (s, 3 H, H17b), 2.49–2.50 (m, 1 H, H2′b), 2.04 (s, 3 H, H15).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 156.9, 129.4, 128.9, 127.2, 126.6, 112.6, 112.4, 85.5, 82.9, 70.5, 54.6, 40.6, 34.4, 22.4.

HRMS-FAB: *m*/*z* $[M]^+$ calcd for $C_{42}H_{43}N_7O_7$: 757.3224; found: 758.3336 $[M + H]^+$.

8-[Acetyl(phenyl)amino]-3′-*O*-[(2-cyanoethyl)(diisopropylamino)phosphino]-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-N^2-[(dimethylamino)methylene]guanosine (12a)

In a dried flask, 8-[acetyl(phenyl)amino]-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-N^2-[(dimethylamino)methylene]guanosine (39 mg, 51 μmol) were diluted in anhyd CH_2Cl_2–anhyd MeCN (1:1, 6 mL) and 0.25 M DCI activator soln in THF (0.6 mL) and (2-cyanoethyl)bis(diisopropylamino)phosphine (0.10 mL, 0.32 mmol)

were added and the mixture was stirred at r.t. for 1 h. The reaction was quenched by addition of sat. $NaHCO_3$ soln. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 ×). The combined organic layers were dried (Na_2SO_4) and the solvent was removed in vacuo. Purification of the residue by chromatography (alumina) gave the desired product as a colorless solid, which was obtained as a fine powder after lyophilization from benzene; yield: 34 mg (68%); mp 68–70 °C.

$[\alpha]_{546}^{20}$ +74 (*c* 0.06, $CHCl_3$).

IR (KBr): 2968, 2932, 1700, 1696, 1680, 1672, 1629, 1554, 1517, 1493, 1368, 1326, 1116, 1079, 979 cm^{-1}.

^1H NMR (400 MHz, benzene-d_6): δ = 11.29 [s, 2 H, NH (I + II)], 8.36 [s, 1 H, H16 (I)], 8.23 [s, 1 H, H16 (II)], 7.62–6.62 [m, 36 H, DMTr-H (I + II) + aniline-H (I + II)], 6.41 [dd, *J* = 6.1, 6.1 Hz, 1 H, H1′ (I)], 6.31 [dd, *J* = 6.3, 6.3 Hz, 1 H, H1′ (II)], 4.85–4.82 [m, 2 H, H3′ (I + II)], 4.38–4.35 [m, 2 H, H4′ (I + II)], 3.56–3.30 [m, 38 H, DMTr-CH_3 (I + II) + *i*-Pr-H (I + II) + H2a′ (I + II) + H5′a + b (I + II) + aHa + b (I + II) + H17 + H18 (I + II)], 2.78–2.75 [m, 1 H, H2b′ (II)], 2.59–2.56 [m, 2 H, H2b′ (I) + bHa (I)], 2.23 [ddd, 1 H, bHb (I)], 2.04 [s, 6 H, H15 (I + II)], 1.85 [dd, *J* = 6.1, 6.1 Hz, 2 H, β′Ha (II) + bHb (II)], 1.15–0.96 [m, 24 H, *i*-Pr-CH_3 (I + II)].

^{13}C NMR (101 MHz, benzene-d_6): δ = 178.7, 159.3, 159.2, 158.2, 147.6, 147.5, 145.3, 145.3, 136.3, 133.2, 130.9, 130.8, 130.7, 130.6, 130.5, 129.3, 128.8, 128.7, 128.6, 128.4, 118.4, 118.2, 114.6, 113.7, 113.6, 87.1, 86.6, 85.6, 75.0, 74.8, 74.0, 73.8, 64.1, 64.0, 58.7, 58.6, 58.5, 58.5, 55.1, 54.9, 54.8, 43.6, 43.6, 43.5, 43.5, 38.6, 38.4, 37.7, 30.2, 24.8, 24.7, 24.6, 20.6, 20.5, 20.3.

^{31}P NMR (162 MHz, benzene-d_6): δ = 147.4, 147.1.

HRMS-ESI: *m/z* $[M]^+$ calcd for $C_{51}H_{60}N_9O_7P$: 957.4302; found: 980.4199 $[M + Na]^+$.

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